NOVEL PD-L1 BINDING POLYPEPTIDES FOR IMAGING

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application No. 62/084,298, entitled "Novel PD-L1 Binding Polypeptides for Imaging" filed November 25, 2014, the contents of which are hereby incorporated by reference.

BACKGROUND

[0002] Programmed Death Ligand-1 (PD-L1) is a surface glycoprotein ligand for PD-1, a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression, which is found on both antigen-presenting cells and human cancers and downregulaes T cell activation and cytokine secretion by binding to PD-1 (Freeman et al., 2000; Latchman et al, 2001). Inhibition of the PD-L1/PD-1 interaction allows for potent anti-tumor activity in preclinical models, and antibodies that disrupt this interaction have entered clinical trials for the treatment of cancer (U.S. Patent Nos. 8,008,449 and 7,943,743; Brahmer et al., 2010; Topalian et al., 2012b; Brahmer et al., 2012; Flies et al., 2011; Pardoll, 2012; Hamid and Carvajal, 2013).

[0003] PET, or Positron Emission Tomography, is a non-invasive, nuclear medicine technique that produces a three-dimensional image of various molecular processes within the body, or the location of proteins associated with disease pathology. The methodology detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer) introduced into the body on a biologically active molecule. PET imaging tools have a wide variety of uses for drug development and have a unique translational medicine advantage, in that the same tool could be used both preclinically and clinically. Examples include direct visualization of in vivo saturation of targets; monitoring uptake in normal tissues to anticipate toxicity or patient to patient variation; quantifying diseased tissue; tumor metastasis; monitoring drug efficacy over time, or resistance over time, and more.

[0004] Described herein are novel anti-PD-L1 Adnectins suitable for use as diagnostic/imaging agents, for example, for use in positron emission tomography.

SUMMARY

[0005] The present invention is based, at least in part, on the discovery of new anti-human PD-L1 Adnectins which are useful as diagnostic/imaging agents, for example, for use in positron emission tomography. These agents are useful in, e.g., the differentiation of PD-L1

expressing cells from non-PD-L1 expressing cells, e.g., tumor cells, and the differentiation of PD-L1 expressing tissue from non-PD-L1 expressing tissue, e.g., cancer tissue.

[0006] In one aspect, provided herein is a polypeptide comprising a fibronectin type III tenth domain (^{10}Fn3), wherein (a) the ^{10}Fn3 domain comprises AB, BC, CD, DE, EF, and FG loops, (b) the ^{10}Fn3 has at least one loop selected from loop BC, DE, and FG with an altered amino acid sequence relative to the sequence of the corresponding loop of the human ^{10}Fn3 domain (SEQ ID NO: 1), and (c) the polypeptide specifically binds to PD-L1. In certain embodiments, the polypeptide binds to PD-L1 with a K_D of 500 mM or less, for example, 100 mM or less.

[0007] In certain embodiments, the ^{10}Fn3 domain comprises BC, DE, and FG loops comprising the amino acid sequences of:

(1) SEQ ID NOs: 6, 7, and 8, respectively;
(2) SEQ ID NOs: 21, 22, and 23, respectively;
(3) SEQ ID NOs: 36, 37, and 38, respectively;
(4) SEQ ID NOs: 51, 52, and 53, respectively;
(5) SEQ ID NOs: 66, 67, and 68, respectively;
(6) SEQ ID NOs: 81, 82, and 83, respectively;
(7) SEQ ID NOs: 97, 98, and 99, respectively;
(8) SEQ ID NOs: 113, 114, and 115, respectively;
(9) SEQ ID NOs: 124, 125 and 126, respectively;
(10) SEQ ID NOs: 135, 136 and 137, respectively;
(11) SEQ ID NOs: 146, 147 and 148, respectively;
(12) SEQ ID NOs: 157, 158 and 159, respectively;
(13) SEQ ID NOs: 168, 169 and 170, respectively;
(14) SEQ ID NOs: 179, 180 and 181, respectively;
(15) SEQ ID NOs: 190, 191 and 192, respectively;
(16) SEQ ID NOs: 201, 202 and 203, respectively;
(17) SEQ ID NOs: 212, 213 and 214, respectively;
(18) SEQ ID NOs: 223, 224 and 225, respectively;
(19) SEQ ID NOs: 234, 235, and 236, respectively;
(20) SEQ ID NOs: 245, 246 and 247, respectively;
(21) SEQ ID NOs: 256, 257 and 258, respectively;
(22) SEQ ID NOs: 267, 268 and 269, respectively;

(23) SEQ ID NOs: 278, 279 and 280, respectively;

(24) SEQ ID NOs: 289, 290 and 291, respectively;

(25) SEQ ID NOs: 300, 301 and 302, respectively;

(26) SEQ ID NOs: 311, 312 and 313, respectively;

(27) SEQ ID NOs: 322, 323 and 324, respectively;

(28) SEQ ID NOs: 333, 334 and 335, respectively;

(29) SEQ ID NOs: 344, 345 and 346, respectively;

(30) SEQ ID NOs: 355, 356 and 357, respectively;

(31) SEQ ID NOs: 366, 367 and 368, respectively;

(32) SEQ ID NOs: 377, 378 and 379, respectively;

(33) SEQ ID NOs: 388, 389 and 390 respectively;

(34) SEQ ID NOs: 399, 400 and 401, respectively;

(35) SEQ ID NOs: 410, 411 and 412, respectively;

(36) SEQ ID NOs: 421, 422 and 423, respectively;

(37) SEQ ID NOs: 432, 433 and 434respectively;

(38) SEQ ID NOs: 443, 444 and 445, respectively;

(39) SEQ ID NOs: 454, 455 and 456, respectively;

(40) SEQ ID NOs: 465, 466 and 467, respectively;

(41) SEQ ID NOs: 476, 477 and 478, respectively;

(42) SEQ ID NOs: 487, 488 and 489, respectively;

(43) SEQ ID NOs: 498, 499 and 500, respectively;

(44) SEQ ID NOs: 509, 510 and 511, respectively;

(45) SEQ ID NOs: 520, 521 and 522, respectively;

(46) SEQ ID NOs: 531, 530 and 531, respectively;

(47) SEQ ID NOs: 542, 543 and 544, respectively;

(48) SEQ ID NOs: 553, 554 and 555, respectively; or

(49) SEQ ID NOs: 564, 565 and 566, respectively.

[008] In certain embodiments, the polypeptide comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to a sequence set forth in Table 3, e.g, any one of SEQ ID NO: 5, 20, 35, 50, 65, 80,96, 112, 123, 134, 145, 156, 167, 178, 189, 200, 211, 222. 233, 244, 255, 266, 277, 288, 299, 310, 321, 332, 343, 354, 365, 376, 387, 398, 409, 420, 431, 442, 453, 464, 475, 486, 497, 508, 519, 530, 541, 552 and 563. In certain embodiments, the polypeptide comprises an amino acid sequence at least 80%, 85%, 90%,

95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of SEQ ID NO: 5, 20, 35, 50, 65, 80, 96, 112, 123, 134, 145, 156, 167, 178, 189, 200, 211, 222. 233, 244, 255, 266, 277, 288, 299, 310, 321, 332, 343, 354, 365, 376, 387, 398, 409, 420, 431, 442, 453, 464, 475, 486, 497, 508, 519, 530, 541, 552 or 563. In certain embodiments, the polypeptide comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of: SEQ ID NOs: 9-15, 24-30, 39-45, 54-60, 69-75, 84-91, 100-107, 116-122, 127-133, 138-144, 150-155, 160-166, 171-177, 182-188, 193-199, 204-210, 215-221, 227-232, 237-243, 248-254, 259-265, 271-276, 291-287, 292-298, 303-309, 314-320, 325-331, 337-342, 347-353, 358-364, 369-375, 380-386, 391-397, 402-408, 413-419, 424-430, 435-441, 446-452, 457-463, 468-474, 479-485, 490-496, 501-507, 512-518, 523-529, 534-540, 545-551, and 556-562. In certain embodiments, the polypeptide comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of an amino acid sequence selected from the group consisting of: SEQ ID NOs: 9-15, 24-30, 39-45, 54-60, 69-75, 84-91, 100-107, 116-122, 127-133, 138-144, 150-155, 160-166, 171-177, 182-188, 193-199, 204-210, 215-221, 227-232, 237-243, 248-254, 259-265, 271-276, 291-287, 292-298, 303-309, 314-320, 325-331, 337-342, 347-353, 358-364, 369-375, 380-386, 391-397, 402-408, 413-419, 424-430, 435-441, 446-452, 457-463, 468-474, 479-485, 490-496, 501-507, 512-518, 523-529, 534-540, 545-551, and 556-562.

[009] In certain embodiments, the polypeptide comprises an N-terminal leader selected from the group consisting of SEQ ID NOs: 574-583, and/or a C-terminal tail selected from the group consisting of SEQ ID NOs: 584-618 or PmCn, wherein P is proline, and wherein m is an integer that is at least 0 (e.g., 0, 1 or 2) and n is an integer of at least 1 (e.g., 1 or 2).

[0010] In certain embodiments, the polypeptide comprises one or more pharmacokinetic (PK) moieties selected from the group consisting of polyethylene glycol, sialic acid, Fc, Fc fragment, transferrin, serum albumin, a serum albumin binding protein, and a serum immunoglobulin binding protein. In certain embodiments, the PK moiety and the polypeptide are linked via at least one disulfide bond, a peptide bond, a polypeptide, a polymeric sugar or a polyethylene glycol moiety. In certain embodiments, the PK moiety and the polypeptide are linked via a linker with an amino acid sequence selected from the group consisting of SEQ ID NOs: 629-678.

[0011] Provided herein are nucleic acids encoding the polypeptides, as well as vectors and cells comprising the nucleic acids, described herein. In certain embodiments, the nucleic acid

comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 16-19, 31-34, 46-49, 61-64, 76-79, 92-95, and 108-111.

[0012] Provided herein are compositions comprising the polypeptides described herein, and a carrier. For example, the compositions described herein comprise a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 9-15, 20, 24-30, 35, 39-45, 50, 54-60, 65, 69-75, 80, 84-91, 96, 100-107, 112, 116-122, 123, 127-133, 134, 138-144, 145, 150-155, 156, 160-166, 167, 171-177, 178, 182-188, 189, 193-199, 200, 204-210, 211, 215-221, 222, 227-232, 233, 237-243, 244, 248-254, 255, 259-265, 266, 271-276, 277, 291-287, 288, 292-298, 299, 303-309, 310, 314-320, 321, 325-331, 332, 337-342, 343, 347-353, 354, 358-364, 365, 369-375, 376, 380-386, 387, 391-397, 398, 402-408, 409, 413-419, 420, 424-430, 431, 435-441, 442, 446-452, 453, 457-463, 464, 468-474, 475, 479-485, 486, 490-496, 497, 501-507, 508, 512-518, 519, 523-529, 530, 534-540, 541, 545-551, 552, and 556-562, and a carrier.

[0013] Provided herein are imaging agents comprising the polypeptide disclosed herein. In certain embodiments, the imaging agent comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 9-15, 20, 24-30, 35, 39-45, 50, 54-60, 65, 69-75, 80, 84-91, 96, 100-107, 112, 116-122, 123, 127-133, 134, 138-144, 145, 150-155, 156, 160-166, 167, 171-177, 178, 182-188, 189, 193-199, 200, 204-210, 211, 215-221, 222, 227-232, 233, 237-243, 244, 248-254, 255, 259-265, 266, 271-276, 277, 291-287, 288, 292-298, 299, 303-309, 310, 314-320, 321, 325-331, 332, 337-342, 343, 347-353, 354, 358-364, 365, 369-375, 376, 380-386, 387, 391-397, 398, 402-408, 409, 413-419, 420, 424-430, 431, 435-441, 442, 446-452, 453, 457-463, 464, 468-474, 475, 479-485, 486, 490-496, 497, 501-507, 508, 512-518, 519, 523-529, 530, 534-540, 541, 545-551, 552, and 556-562.

[0014] In certain embodiments, the imaging agent comprises a detectable label. In certain embodiments, the imaging agent comprises a polypeptide disclosed herein, a chelating agent, and a detectable label. In certain embodiments, the imaging agent comprises a polypeptide disclosed herein, a bifunctional chelator or conjugating (BFC) moiety and a detectable label. In certain embodiments, the detectable label is a prosthetic group containing a radionuclide. In certain embodiments, the detectable label is detectable by positron emission tomography.

[0015] In certain embodiments, the chelating agent and/or bifunctional chelator or conjugating (BFC) moiety is selected from the group consisting of DFO, DOTA, CB-DO2A, 3p-C-DEPA, TCMC, DBCO, DIBO, BARAC, DIMAC, Oxo-DO3A, TE2A, CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, DM-TE2A, diamsar, NODASA, NODAGA, NOTA, NETA,

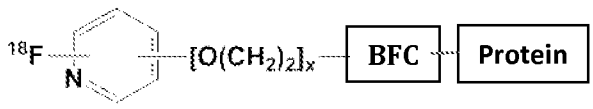
TACN-TM, DTPA, 1B4M-DTPA, CHX-A"-DTPA, TRAP, NOPO, AAZTA, DATA, H_2dedpa, H_4octapa, H_2azapa, H_5decapa, H_6phospa, HBED, SHBED, BPCA, CP256, PCTA, HEHA, PEPA, EDTA, TETA, and TRITA.

[0016] In certain embodiments, the detectable label is a radionuclide, for example,

[0017] ^{64}Cu, ^{124}I, $^{76/77}$Br, ^{86}Y, ^{89}Zr, ^{68}Ga, ^{18}F, ^{11}C, ^{125}I, ^{124}I, ^{131}I, ^{123}I, ^{131}I, ^{123}I, ^{32}Cl, ^{33}Cl, ^{34}Cl, ^{68}Ga, ^{74}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{78}Br, ^{89}Zr, ^{186}Re, ^{188}Re, ^{90}Y, ^{177}Lu, ^{99}Tc, or ^{153}Sm.

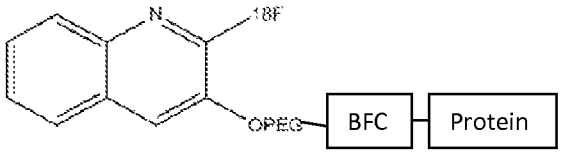
[0018] In certain embodiments, the chelating agent is NODAGA and the radionuclide is ^{64}Cu. In certain embodiments, the imaging agent comprises an anti-PD-L1 polypeptide (e.g., an anti-PD-L1 Adnectin described herein, e.g., an anti-PD-L1 Adnectin comprising the amino acid sequence set forth in SEQ ID NO: 80 or 96), the chelating agent NODAGA, and the radionuclide ^{64}Cu.

[0019] In certain embodiments, the imaging agent comprises an anti-PD-L1 polypeptide (e.g., an anti-PD-L1 Adnectin described herein, e.g., an anti-PD-L1 Adnectin comprising the amino acid sequence set forth in SEQ ID NO: 80 or 96), a bifunctional chelator or conjugating (BFC) moiety , and a prosthetic group comprising the radionuclide ^{18}F. In certain embodiments, the imaging agent has the following structure:



or a pharmaceutically acceptable salt thereof.

[0020] In certain embodiments, the imaging agent has the following structure:

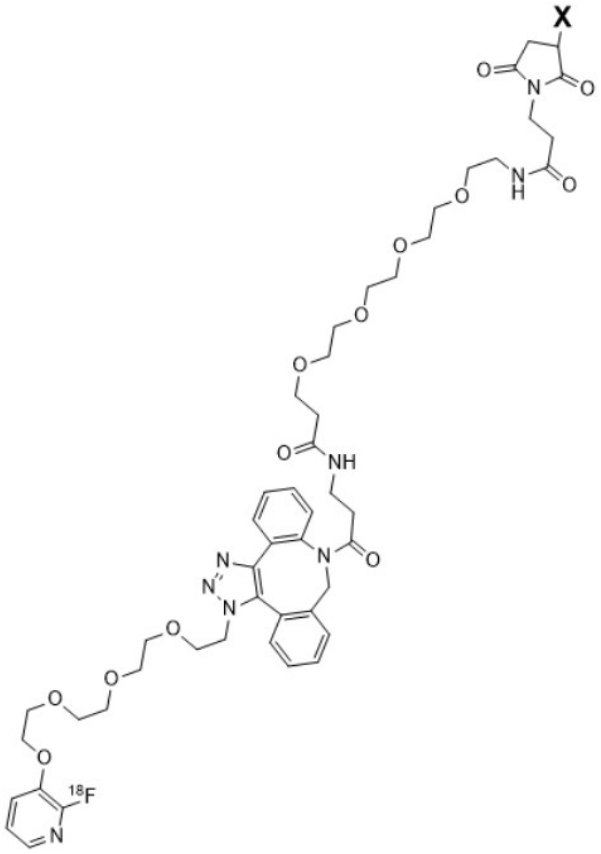


[0021] In some embodiments, the BFC is a cyclooctyne comprising a reactive group that forms a covalent bond with an amine, carboxyl, carbonyl or thiol functional group on the protein. In some embodiments, the cyclooctyne is selected from the group consisting of dibenzocyclooctyne (DIBO), biarylazacyclooctynone (BARAC), dimethoxyazacyclooctyne (DIMAC) and dibenzocyclooctyne (DBCO). In some embodiments, the cyclooctyne is DBCO.

[0022] In some embodiments, the BFC is DBCO-PEG4-NHS-Ester, DBCO-*Sulfo*-NHS-Ester, DBCO-PEG4-Acid, DBCO-PEG4-Amine or DBCO-PEG4-Maleimide. In some embodiments, the BFC is DBCO-PEG4-Maleimide.

[0023] In certain embodiments, the imaging agent has the structure:

a



[0024] wherein X is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 13, 28, 43, 58, 73, 88, 104, 120, 131, 142, 153, 164, 175, 186, 197, 208, 219, 230, 241, 252, 263, 274, 285, 296, 307, 318, 329, 340, 351, 362, 373, 384, 395, 406, 417, 428, 439, 450, 461, 472, 483, 494, 505, 516, 527, 538, 549, 560 and 571. In certain embodiments, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 88. In certain embodiments, thepolypeptide comprises the amino acid sequence set forth in SEQ ID NO: 104.

[0025] Provided herein are kits comprising an anti-PD-L1 Adnectin composition and/or imaging agent described herein, and instructions for use.

[0026] Provided herein is a method of detecting PD-L1 in a sample, the method comprising contacting the sample with an anti-PD-L1 Adnectin, and detecting PD-L1.

[0027] Provided herein is a method of detecting PD-L1 positive cells in a subject comprising administering to the subject an imaging agent comprising an anti-PD-L1 Adnectin, and detecting the imaging agent, the detected imaging agent defining the location of the PD-L1 positive cells in the subject.

[0028] Provided herein is a method of detecting PD-L1-expressing tumors in a subject comprising administering to the subject an imaging agent comprising an anti-PD-L1 Adnectin, and detecting the imaging agent, the detected imaging agent defining the location of the tumor in the subject. In certain embodiments, the imaging agent is detected by positron emission tomography.

[0029] Provided herein is a method of obtaining an image of an imaging agent comprising an anti-PD-L1 Adnectin, the method comprising,

a) administering the imaging agent to a subject; and
b) imaging *in vivo* the distribution of the imaging agent by positron emission tomography.

[0030] Provided herein is a method of obtaining a quantitative image of tissues or cells expressing PD-L1, the method comprising contacting the cells or tissue with an imaging agent comprising an anti-PD-L1 Adnectin, and detecting or quantifying the tissue expressing PD-L1 using positron emission tomography.

[0031] Provided herein is a method for detecting a PD-L1-expressing tumor comprising administering an imaging-effective amount of an imaging agent comprising an anti-PD-L1 Adnectin to a subject having a PD-L1-expressing tumor, and detecting the radioactive emissions of said imaging agent in the tumor using positron emission tomography, wherein the radioactive emissions are detected in the tumor.

[0032] Provided herein is a method of diagnosing the presence of a PD-L1-expressing tumor in a subject, the method comprising

(a) administering to a subject in need thereof an imaging agent comprising an anti-PD-L1 Adnectin; and

(b) obtaining an radio-image of at least a portion of the subject to detect the presence or absence of the imaging agent;

wherein the presence and location of the imaging agent above background is indicative of the presence and location of the disease.

[0033] Provided herein is a method of monitoring the progress of an anti-tumor therapy against PD-L1-expressing tumors in a subject, the method comprising

(a) administering to a subject in need thereof an imaging agent comprising an anti-PD-L1 Adnectin at a first time point and obtaining an image of at least a portion of the subject to determine the size of the tumor;

(b) administering an anti-tumor therapy to the subject;

(c) administering to the subject the imaging agent at one or more subsequent time points and obtaining an image of at least a portion of the subject at each time point; wherein the dimension and location of the tumor at each time point is indicative of the progress of the disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] Figure 1 is a schematic of the core amino acid sequences of exemplary anti-PD-L1 Adnectins described herein. The BC, DE, and FG loops are underlined. Wildtype (WT) (SEQ ID NO: 2), ATI-968 (SEQ ID NO: 5), ATI-964 (SEQ ID NO: 20), ATI-967 (SEQ ID NO: 65), A02 (SEQ ID NO: 80), E01 (SEQ ID NO: 96), ATI-965 (SEQ ID NO: 35), and ATI-966 (SEQ ID NO: 50).

[0035] Figure 2 is a schematic for the chemical synthesis of [^{18}F]-E01-4PEG-DBCO-FPPEGA. The E01 portion of the molecule has the sequence set forth in SEQ ID NO: 104.

[0036] Figure 3 is a graph demonstrating discrimination of hPD-L1-positive L2987 cells from hPD-L1-negative HT-29 cells with the ^{64}Cu-E01 anti-PD-L1 Adnectin (with a NODAGA chelator). Specificity was confirmed by the reduction of cell-associated ^{64}Cu -E01 when co-incubated with excess cold (unlabeled) E01 Adnectin.

[0037] Figure 4A is a PET image depicting the discrimination of hPD-L1 (+) from hPD-L1 (-) tumors in bilateral xenograft mice with a NODAGA-^{64}Cu-labeled A02 anti-PD-L1 Adnectin. Shown are summed 0 to 2 hour images showing areas of probe residence. Bright areas are tissues of greatest occupancy during exposure.

[0038] Figure 4B is a graph depicting a time course of tumor labeling in hPD-L1 (+) [L2987] tumors. hPD-L1(-) [HT29] tumors and pulse chase experiment in hPD-L1(+) systems [L2987 block] show the specificity of the labelling.

[0039] Figure 5 is a graph depicting tissue distribution of the ^{18}F-labeled A02 anti-PD-L1 Adnectin radiotracer in mice bearing bilateral hPD-L1(+) L2987 and hPD-L1(-) HT-29 xenografts as measured *ex vivo* by gamma counter.

[0040] Figure 6 is a composite image of ^{18}F-labeled E01 anti-PD-L1 Adnectin distribution in cynomolgus monkey.

[0041] Figure 7 is an image depicting *in vitro* autoradiography of xenograft and human lung tissues labelled with the 0.25 nM ^{18}F-DBCO-A02 anti-PD-L1 Adnectin co-incubated with the indicated concentrations of cold A02 Adnectin.

[0042] Figure 8 depicts immunohistochemistry images of xenograft and human lung tumor specimens labelled with anti-PD-L1 Adnectins to demonstrate tumor expression of hPD-L1.

[0043] Figure 9 shows a reaction scheme for synthesizing [^{18}F]-A02-4PEG-DBCO-FPPEGA. The same reaction scheme was used to label the E01 adnectin.

[0044] Figure 10 is a schematic of the GE TRACERlab FX2 N Synthesis module for automated synthesis of [^{18}F]-FPPEGA.

[0045] Figure 11 is a schematic of the Synthera Synthesis module (IBA) for automated synthesis of [^{18}F]-FPPEGA.

[0046] Figure 12 depicts exemplary competition curves of anti-PD-L1 Adnectins.

DETAILED DESCRIPTION

Definitions

[0047] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by the skilled artisan. Although any methods and compositions similar or equivalent to those described herein can be used in practice or testing of the present invention, the preferred methods and compositions are described herein.

[0048] "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD- 1 (the other being PD-L2) that downregulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-Ll), variants, isoforms, and species homologs of hPD-Ll, and analogs having at least one common epitope with hPD-Ll. The complete hPD-Ll sequence can be found under GenBank Accession No. Q9NZQ7. PD-L1 is also referred to as CD274, B7-H, B7H1, PDCD1L1, and PDCD1LG1.

[0049] "Polypeptide" as used herein refers to any sequence of two or more amino acids, regardless of length, post-translation modification, or function. "Polypeptide," "peptide," and "protein" are used interchangeably herein. Polypeptides can include natural amino acids and non-natural amino acids such as those described in U.S. Pat. No. 6,559,126, incorporated herein by reference. Polypeptides can also be modified in any of a variety of standard

chemical ways (e.g., an amino acid can be modified with a protecting group; the carboxy-terminal amino acid can be made into a terminal amide group; the amino-terminal residue can be modified with groups to, e.g., enhance lipophilicity; or the polypeptide can be chemically glycosylated or otherwise modified to increase stability or in vivo half-life). Polypeptide modifications can include the attachment of another structure such as a cyclic compound or other molecule to the polypeptide and can also include polypeptides that contain one or more amino acids in an altered configuration (i.e., R or S; or, L or D). The peptides described herein are proteins derived from the tenth type III domain of fibronectin that have been modified to bind specifically to PD-L1 and are referred to herein as, "anti-PD-L1 Adnectin" or "PD-L1 Adnectin."

[0050] A "polypeptide chain," as used herein, refers to a polypeptide wherein each of the domains thereof is joined to other domain(s) by peptide bond(s), as opposed to non-covalent interactions or disulfide bonds.

[0051] An "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to greater than 95% by weight of polypeptide as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing condition using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

[0052] A "region" of a ^{10}Fn3 domain as used herein refers to either a loop (AB, BC, CD, DE, EF and FG), a β-strand (A, B, C, D, E, F and G), the N-terminus (corresponding to amino acid residues 1-7 of SEQ ID NO: 1), or the C-terminus (corresponding to amino acid residues 93-94 of SEQ ID NO: 1) of the human ^{10}Fn3 domain.

[0053] A "scaffold region" refers to any non-loop region of a human ^{10}Fn3 domain. The scaffold region includes the A, B, C, D, E, F and G β-strands as well as the N-terminal region (amino acids corresponding to residues 1-7 of SEQ ID NO: 1) and the C-terminal region

(amino acids corresponding to residues 93-94 of SEQ ID NO: 1 and optionally comprising the 7 amino acids constituting the natural linker between the 10th and the 11th repeat of the Fn3 domain in human fibronectin).

[0054] "Percent (%) amino acid sequence identity" herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a selected sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR™) software. Those skilled in the art can readily determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

[0055] As used herein, the term “Adnectin binding site” refers to the site or portion of a protein (e.g., PD-L1) that interacts or binds to a particular Adnectin. Adnectin binding sites can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Adnectin binding sites formed by contiguous amino acids are typically retained on exposure to denaturing solvents, whereas Adnectin binding sites formed by tertiary folding are typically lost on treatment of denaturing solvents.

[0056] An Adnectin binding site for an anti-PD-L1 Adnectin described herein may be determined by application of standard techniques typically used for epitope mapping of antibodies including, but not limited to protease mapping and mutational analysis. Alternatively, an Adnectin binding site can be determined by competition assay using a reference Adnectin or antibody which binds to the same polypeptide, e.g., PD-L1 (as further

described *infra* in the section "Cross-Competing Adnectins and/or Adnectins that Bind to the Same Adnectin Binding Site." If the test Adnectin and reference molecule (e.g., another Adnectin or antibody) compete, then they bind to the same Adnectin binding site or to Adnectin binding sites sufficiently proximal such that binding of one molecule interferes with the other.

[0057] The terms "specifically binds," "specific binding," "selective binding, and "selectively binds," as used interchangeably herein in the context of Adnectins binding to PD-L1 refers to an Adnectin that exhibits affinity for PD-L1, but does not significantly bind (e.g., less than about 10% binding) to a different polypeptides as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (e.g., competition ELISA, BIACORE assay). The term is also applicable where e.g., a binding domain of an Adnectin described herein is specific for PD-L1.

[0058] The term "preferentially binds" as used herein in the context of Adnectins binding to PD-L1 refers to the situation in which an Adnectin described herein binds PD-L1 at least about 20% greater than it binds a different polypeptide as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (e.g., competition ELISA, BIACORE assay).

[0059] As used herein in the context of Adnectins, the term "cross-reactivity" refers to an Adnectin which binds to more than one distinct protein having identical or very similar Adnectin binding sites.

[0060] The term "K_D," as used herein in the context of Adnectins binding to PD-L1, is intended to refer to the dissociation equilibrium constant of a particular Adnectin-protein (e.g., PD-L1) interaction or the affinity of an Adnectin for a protein (e.g., PD-L1), as measured using a surface plasmon resonance assay or a cell binding assay. A "desired K_D," as used herein, refers to a K_D of an Adnectin that is sufficient for the purposes contemplated. For example, a desired K_D may refer to the K_D of an Adnectin required to elicit a functional effect in an in vitro assay, e.g., a cell-based luciferase assay.

[0061] The term "k_a", as used herein in the context of Adnectins binding to a protein, is intended to refer to the association rate constant for the association of an Adnectin into the Adnectin/protein complex.

[0062] The term "k_d", as used herein in the context of Adnectins binding to a protein, is intended to refer to the dissociation rate constant for the dissociation of an Adnectin from the Adnectin/protein complex.

[0063] The term "IC_{50}", as used herein in the context of Adnectins, refers to the concentration of an Adnectin that inhibits a response, either in an *in vitro* or an *in vivo* assay, to a level that is 50% of the maximal inhibitory response, *i.e.*, halfway between the maximal inhibitory response and the untreated response.

[0064] The term "PK" is an acronym for "pharmacokinetic" and encompasses properties of a compound including, by way of example, absorption, distribution, metabolism, and elimination by a subject. A "PK modulation protein" or "PK moiety" as used herein refers to any protein, peptide, or moiety that affects the pharmacokinetic properties of a biologically active molecule when fused to or administered together with the biologically active molecule. Examples of a PK modulation protein or PK moiety include PEG, human serum albumin (HSA) binders (as disclosed in U.S. Publication Nos. 2005/0287153 and 2007/0003549, PCT Publication Nos. WO 2009/083804 and WO 2009/133208), human serum albumin and variants thereof, transferrin and variants thereof, Fc or Fc fragments and variants thereof, and sugars (e.g., sialic acid).

[0065] The "serum half-life" of a protein or compound is the time taken for the serum concentration of the polypeptide to be reduced by 50%, in vivo, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The half-life can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering to a subject a suitable dose of the amino acid sequence or compound described herein; collecting blood samples or other samples from the subject at regular intervals; determining the level or concentration of the amino acid sequence or compound described herein in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence or compound described herein has been reduced by 50% compared to the initial level upon dosing. Reference is, for example, made to the standard handbooks, such as Kenneth, A. et al., Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al., Pharmacokinete Analysis: A Practical Approach (1996). Reference is also made to Gibaldi, M. et al., Pharmacokinetics, 2nd Rev. Edition, Marcel Dekker (1982).

[0066] Half-life can be expressed using parameters such as the $t_{1/2}$-alpha, $t_{1/2}$-beta, HL_Lambda_z, and the area under the curve (AUC). In the present specification, an "increase in half-life" refers to an increase in any one of these parameters, any two of these parameters,

any three of these parameters or all four of these parameters. An "increase in half-life" in particular refers to an increase in the $t_{1/2}$-beta, and/or HL_Lambda_z, either with or without an increase in the $t_{1/2}$-alpha and/or the AUC or both.

[0067] The term "detectable" refers to the ability to detect a signal over the background signal. The term "detectable signal" is a signal derived from non-invasive imaging techniques such as, but not limited to, positron emission tomography (PET). The detectable signal is detectable and distinguishable from other background signals that may be generated from the subject. In other words, there is a measurable and statistically significant difference (e.g., a statistically significant difference is enough of a difference to distinguish among the detectable signal and the background, such as about 0.1%, 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, or 40% or more difference between the detectable signal and the background) between the detectable signal and the background. Standards and/or calibration curves can be used to determine the relative intensity of the detectable signal and/or the background.

[0068] A "detectably effective amount" of a composition comprising an imaging agent described herein is defined as an amount sufficient to yield an acceptable image using equipment that is available for clinical use. A detectably effective amount of an imaging agent provided herein may be administered in more than one injection. The detectably effective amount can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the like. Detectably effective amounts of imaging compositions can also vary according to instrument and methodologies used. Optimization of such factors is well within the level of skill in the art. In certain embodiments, a PD-L1 imaging agent, e.g., those described herein, provides a differentiation factor (i.e., specific signal to background signal) of 2 or more, e.g., 3, 4, 5 or more.

[0069] The term "bioorthogonal chemistry" refers to any chemical reaction that can occur inside of living systems without interfering with native biochemical processes. The term includes chemical reactions that are chemical reactions that occur *in vitro* at physiological pH in, or in the presence of water. To be considered bioorthogonol, the reactions are selective and avoid side-reactions with other functional groups found in the starting compounds. In addition, the resulting covalent bond between the reaction partners should be strong and chemically inert to biological reactions and should not affect the biological activity of the desired molecule.

[0070] The term "click chemistry" refers to a set of reliable and selective bioorthogonal reactions for the rapid synthesis of new compounds and combinatorial libraries. Properties of for click reactions include modularity, wideness in scope, high yielding, stereospecificity and simple product isolation (separation from inert by-products by non-chromatographic methods) to produce compounds that are stable under physiological conditions. In radiochemistry and radiopharmacy, click chemisty is a generic term for a set of labeling reactions which make use of selective and modular building blocks and enable chemoselective ligations to radiolabel biologically relevant compounds in the absence of catalysts. A “click reaction” can be with copper, or it can be a copper-free click reaction.

[0071] The term "prosthetic group" or "bifunctional labeling agent" refers to a small organic molecule containing a radionulide (*e.g.,* ^{18}F) that is capable of being linked to peptides or proteins.

[0072] The term "chelator ligand" as used herein with respect to radiopharmaceutical chemistry refers to a bifunctional chelator or conjugating (BFC) moiety, which are used interchangeably herein, that covalently links a radiolabeled prosthetic group to a biologically active targeting molecule (*e.g.,* peptide or protein). BFCs utilize functional groups such as carboxylic acids or activated esters for amide couplings, isothiocyanates for thiourea couplings and maleimides for thiol couplings.

[0073] The terms "individual," "subject," and "patient," used interchangeably herein, refer to an animal, preferably a mammal (including a nonprimate and a primate), e.g., a human. In certain embodiments, a subject has a disease or disorder or condition that would benefit from a decreased level or decreased bioactivity of PD-L1. In certain embodiments, a subject is at risk of developing a disorder, disease or condition that would benefit from a decreased level of PD-L1or a decreased bioactivity of PD-L1.

[0074] A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth divide and grow results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream.

[0075] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body

of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0076] An "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

[0077] The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

[0078] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

[0079] “Administration” or “administering,” as used herein in the context of anti-PD-L1 Adnectins, refers to introducing a PD-L1 Adnectin or PD-L1 Adnectin-based probe or a labeled probe (also referred to as the “imaging agent”) described herein into a subject. Any route of administration is suitable, such as intravenous, oral, topical, subcutaneous, peritoneal, intra-arterial, inhalation, vaginal, rectal, nasal, introduction into the cerebrospinal fluid, or instillation into body compartments can be used.

[0080] The term "therapeutically effective amount" refers to at least the minimal dose, but less than a toxic dose, of an agent which is necessary to impart a therapeutic benefit to a subject.

[0081] As used herein, an "effective amount" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result.

[0082] As used herein, a "sufficient amount" refers to an amount sufficient to achieve the desired result.

[0083] As used herein, “positron emission tomography” or “PET” refers to a non-invasive, nuclear medicine technique that produces a three-dimensional image of tracer location in the body. The method detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. PET imaging tools have a wide variety of uses and aid in drug development both preclinically and clinically. Exemplary applications include direct visualization of in vivo saturation of targets; monitoring uptake in normal tissues to anticipate toxicity or patient to patient variation; quantifying diseased tissue; tumor metastasis; and monitoring drug efficacy over time, or resistance over time.

Overview

[0084] Provided herein are polypeptides that bind to human PD-L1 and can be coupled to heterologous molecule(s), such as a radiolabel. Such polypeptides are useful, for example, for detecting PD-L1 in a sample or tissue (e.g., a tissue, such as a cancer tissue that selectively expresses PD-L1) for diagnostic assays.

[0085] The invention is based on the development of a non-invasive clinical imaging agent that allows for whole body visualization of a patient’s PD-L1 expression. In certain embodiments, single day *“virtual biopsies”* of a patient’s whole body are performed to monitor and localize PD-L1 expression levels. PD-L1 imaging agents described herein may be used to provide a high contrast whole-body virtual biopsy in a single day.

I. Fibronectin-based Scaffolds

[0086] Fn3 refers to a type III domain from fibronectin. An Fn3 domain is small, monomeric, soluble, and stable. It lacks disulfide bonds and, therefore, is stable under reducing conditions. The overall structure of Fn3 resembles the immunoglobulin fold. Fn3 domains comprise, in order from N-terminus to C-terminus, a beta or beta-like strand, A; a loop, AB; a beta or beta-like strand, B; a loop, BC; a beta or beta-like strand, C; a loop, CD; a beta or beta-like strand, D; a loop, DE; a beta or beta-like strand, E; a loop, EF; a beta or beta-like strand, F; a loop, FG; and a beta or beta-like strand, G. The seven antiparallel β-strands are arranged as two beta sheets that form a stable core, while creating two "faces" composed of the loops that connect the beta or beta-like strands. Loops AB, CD, and EF are located at one face ("the south pole") and loops BC, DE, and FG are located on the opposing face ("the north pole").

There are at least 15 different Fn3 modules in human Fibronectin, and while the sequence homology between the modules is low, they all share a high similarity in tertiary structure.

[0087] Described herein are anti-PD-L1 Adnectins comprising an Fn3 domain in which one or more of the solvent accessible loops has been randomized or mutated. In certain embodiments, the Fn3 domain is an Fn3 domain derived from the wild-type tenth module of the human fibronectin type III domain (^{10}Fn3):

VSDVPRDLEVVAATPTSLLISW**DAPAVTVRY**YRITYGETGGNSPVQEFTV**PGSKST**A TISGLKPGVDYTITVYAV**TGRGDSPASSKP**ISINYRT (SEQ ID NO: 1) (94 amino acids; AB, CD, and EF loops are underlined; the core ^{10}Fn3 domain begins with amino acid 9 ("E") and ends with amino acid 94 ("T") and corresponds to an 86 amino acid polypeptide). The core wild-type human ^{10}Fn3 domain is set forth in SEQ ID NO: 2.

[0088] In certain embodiments, the non-ligand binding sequences of ^{10}Fn3, i.e., the "^{10}Fn3 scaffold", may be altered provided that the ^{10}Fn3 retains ligand binding function and/or structural stability. A variety of mutant ^{10}Fn3 scaffolds have been reported. In one aspect, one or more of Asp 7, Glu 9, and Asp 23 is replaced by another amino acid, such as, for example, a non-negatively charged amino acid residue (e.g., Asn, Lys, etc.). A variety of additional alterations in the ^{10}Fn3 scaffold that are either beneficial or neutral have been disclosed. See, for example, Batori et al., Protein Eng., 15(12):1015-1020 (December 2002); Koide et al., Biochemistry, 40(34):10326-10333 (Aug. 28, 2001).

[0089] Both variant and wild-type ^{10}Fn3 proteins are characterized by the same structure, namely seven beta-strand domain sequences designated A through G and six loop regions (AB loop, BC loop, CD loop, DE loop, EF loop, and FG loop) which connect the seven beta-strand domain sequences. The beta strands positioned closest to the N- and C-termini may adopt a beta-like conformation in solution. In SEQ ID NO: 1, the AB loop corresponds to residues 14-17, the BC loop corresponds to residues 23-31, the CD loop corresponds to residues 37-47, the DE loop corresponds to residues 51-56, the EF loop corresponds to residues 63-67, and the FG loop corresponds to residues 76-87.

[0090] Accordingly, in certain embodiments, the anti-PD-L1 Adnectin described herein is an ^{10}Fn3 polypeptide that is at least 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% identical to the human ^{10}Fn3 domain, shown in SEQ ID NO: 1, or its core sequence, as shown in SEQ ID NO: 2. Much of the variability will generally occur in one or more of the loops or one or more of the beta strands or N- or C-terminal regions. Each of the beta or beta-like strands of a ^{10}Fn3 polypeptide may consist essentially of an amino acid sequence that is at

least 80%, 85%, 90%, 95% or 100% identical to the sequence of a corresponding beta or beta-like strand of SEQ ID NO: 1 or 2, provided that such variation does not disrupt the stability of the polypeptide in physiological conditions.

[0091] In certain embodiments, the invention provides an anti-human PD-L1 Adnectin comprising a tenth fibronectin type III (^{10}Fn3) domain, wherein the ^{10}Fn3 domain comprises a loop, AB; a loop, BC; a loop, CD; a loop, DE; a loop EF; and a loop FG; and has at least one loop selected from loop BC, DE, and FG with an altered amino acid sequence relative to the sequence of the corresponding loop of the human ^{10}Fn3 domain. In some embodiments, the anti-PD-L1 Adnectins described herein comprise a ^{10}Fn3 domain comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the non-loop regions of SEQ ID NO: 1 or 2, wherein at least one loop selected from BC, DE, and FG is altered. In certain embodiments, the BC and FG loops are altered, in certain embodiments, the BC and DE loops are altered, in certain embodiments, the DE and FG loops are altered, and in certain embodiments, the BC, DE, and FG loops are altered, i.e., the ^{10}Fn3 domains comprise non-naturally occurring loops. In certain embodiments, the AB, CD and/or the EF loops are altered. By "altered" is meant one or more amino acid sequence alterations relative to a template sequence (corresponding human fibronectin domain) and includes amino acid additions, deletions, substitutions or a combination thereof. Altering an amino acid sequence may be accomplished through intentional, blind, or spontaneous sequence variation, generally of a nucleic acid coding sequence, and may occur by any technique, for example, PCR, error-prone PCR, or chemical DNA synthesis.

[0092] In certain embodiments, one or more loops selected from BC, DE, and FG may be extended or shortened in length relative to the corresponding human fibronectin loop. In some embodiments, the length of the loop may be extended by 2-25 amino acids. In some embodiments, the length of the loop may be decreased by 1-11 amino acids. To optimize antigen binding, therefore, the length of a loop of ^{10}Fn3 may be altered in length as well as in sequence to obtain the greatest possible flexibility and affinity in antigen binding.

[0093] In certain embodiments, the polypeptide comprises a Fn3 domain that comprises an amino acid sequence of the non-loop regions that is at least 80, 85, 90, 95, 98, 99, or 100% identical to the non-loop regions of SEQ ID NO: 1 or 2, wherein at least one loop selected from BC, DE, and FG is altered. In some embodiments, the altered BC loop has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, up to 1, 2, 3, or 4 amino acid deletions, up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid insertions, or a combination thereof.

[0094] In some embodiments, one or more residues of the integrin-binding motif "arginine-glycine-aspartic acid" (RGD) (amino acids 78-80 of SEQ ID NO: 1) may be substituted so as to disrupt integrin binding. In some embodiments, the FG loop of the polypeptides provided herein does not contain an RGD integrin binding site. In one embodiment, the RGD sequence is replaced by a polar amino acid-neutral amino acid-acidic amino acid sequence (in the N-terminal to C-terminal direction). In some embodiments, the RGD sequence is replaced with SGE. In one embodiment, the RGD sequence is replaced with RGE.

[0095] In certain embodiments, the fibronectin based scaffold protein comprises a ^{10}Fn3 domain that is defined generally by following the sequence:

E**V**VA**A**$(Z)_a$**LLISW**$(Z)_x$**YRITY**$(Z)_b$**FTV**$(Z)_y$**ATISGL**$(Z)_c$**YTITVYA**$(Z)_z$**ISINY**RT (SEQ ID NO: 3)

wherein the AB loop is represented by $(Z)_a$, the CD loop is represented by $(Z)_b$, the EF loop is represented by$(Z)_c$, the BC loop is represented by $(Z)_x$, the DE loop is represented by $(Z)_y$, and the FG loop is represented by $(Z)_z$. Z represents any amino acid and the subscript following the Z represents an integer of the number of amino acids. In particular, a may be anywhere from 1-15, 2-15, 1-10, 2-10, 1-8, 2-8, 1-5, 2-5, 1-4, 2-4, 1-3, 2-3, or 1-2 amino acids; and b, c, x, y and z may each independently be anywhere from 2-20, 2-15, 2-10, 2-8, 5-20, 5-15, 5-10, 5-8, 6-20, 6-15, 6-10, 6-8, 2-7, 5-7, or 6-7 amino acids. The sequences of the beta strands may have anywhere from 0 to 10, from 0 to 8, from 0 to 6, from 0 to 5, from 0 to 4, from 0 to 3, from 0 to 2, or from 0 to 1 substitutions, deletions or additions across all 7 scaffold regions relative to the corresponding amino acids shown in SEQ ID NO: 1 or 2. In certain embodiments, the sequences of the beta strands may have anywhere from 0 to 10, from 0 to 8, from 0 to 6, from 0 to 5, from 0 to 4, from 0 to 3, from 0 to 2, or from 0 to 1 conservative substitutions across all 7 scaffold regions relative to the corresponding amino acids shown in SEQ ID NO: 1 or 2. In certain embodiments, the core amino acid residues are fixed and any substitutions, conservative substitutions, deletions or additions occur at residues other than the core amino acid residues.

[0096] In certain embodiments, the anti-PD-L1 Adnectins described herein are based on a ^{10}Fn3 scaffold and are defined generally by the sequence:

EVVAATPTSLLISW$(Z)_x$YRITYGETGGNSPVQEFTV$(Z)_y$ATISGLKPGVDYTITVYA$(Z)_z$ ISINYRT (SEQ ID NO: 4).

wherein the BC loop is represented by $(Z)_x$, the DE loop is represented by $(Z)_y$, and the FG loop is represented by $(Z)_z$. Z represents any amino acid and the subscript following the Z represents an integer of the number of amino acids. In particular, x, y and z may each independently be anywhere from 2-20, 2-15, 2-10, 2-8, 5-20, 5-15, 5-10, 5-8, 6-20, 6-15, 6-10, 6-8, 2-7, 5-7, or 6-7 amino acids. In preferred embodiments, x is 11 amino acids, y is 6 amino acids, and z is 12 amino acids. The sequences of the beta strands may have anywhere from 0 to 10, from 0 to 8, from 0 to 6, from 0 to 5, from 0 to 4, from 0 to 3, from 0 to 2, or from 0 to 1 substitutions, deletions or additions across all 7 scaffold regions relative to the corresponding amino acids shown in SEQ ID NO: 1 or 2. In certain embodiments, the sequences of the beta strands may have anywhere from 0 to 10, from 0 to 8, from 0 to 6, from 0 to 5, from 0 to 4, from 0 to 3, from 0 to 2, or from 0 to 1 conservative substitutions across all 7 scaffold regions relative to the corresponding amino acids shown in SEQ ID NO: 1 or 2. In certain embodiments, the core amino acid residues, e.g., outside one or more loops, are fixed and any substitutions, conservative substitutions, deletions or additions occur at residues other than the core amino acid residues.

[0097] In certain embodiments, an anti-PD-L1 Adnectin may comprise the sequence as set forth in SEQ ID NO: 3 or 4, wherein at least one of BC, DE, and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, are altered. As described above, amino acid residues corresponding to residues 23-31, 51-56, and 76-87 of SEQ ID NO: 1 define the BC, DE, and FG loops, respectively. However, it should be understood that not every residue within the loop region needs to be modified in order to achieve a ^{10}Fn3 binder having strong affinity for a desired target (e.g., PD-L1).

[0098] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 21, 22, and 23, respectively.

[0099] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 21, 22, and 23, respectively.

[00100] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$,

respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 36, 37, and 38, respectively.

[00101] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 36, 37, and 38, respectively.

[00102] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 51, 52, and 53, respectively.

[00103] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 51, 52, and 53, respectively.

[00104] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 66, 67, and 68, respectively.

[00105] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 66, 67, and 68, respectively.

[00106] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 6, 7, and 8, respectively.

[00107] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$,

respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 6, 7, and 8, respectively.

[00108] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 81, 82, and 83, respectively.

[00109] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 81, 82, and 83, respectively.

[00110] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, have amino acid sequences at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the BC, DE or FG loop sequences set forth in SEQ ID NOs: 97, 98, and 99, respectively. In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 97, 98, and 99, respectively.

[00111] In certain embodiments, an anti-PD-L1 Adnectin comprises the sequence set forth in SEQ ID NO: 3 or 4, wherein BC, DE and FG loops as represented by $(Z)_x$, $(Z)_y$, and $(Z)_z$, respectively, comprise BC, DE, and FG loops having the amino acid sequences of SEQ ID NOs: 113, 114, and 115, respectively; SEQ ID NOs: 124, 125 and 126, respectively; SEQ ID NOs: 135, 136 and 137, respectively; SEQ ID NOs: 146, 147 and 148, respectively; SEQ ID NOs: 157, 158 and 159, respectively; SEQ ID NOs: 168, 169 and 170, respectively; SEQ ID NOs: 179, 180 and 181, respectively; SEQ ID NOs: 190, 191 and 192, respectively; SEQ ID NOs: 201, 202 and 203, respectively; SEQ ID NOs: 212, 213 and 214, respectively; SEQ ID NOs: 223, 224 and 225, respectively; SEQ ID NOs: 234, 235, and 236, respectively; SEQ ID NOs: 245, 246 and 247, respectively; SEQ ID NOs: 256, 257 and 258, respectively; SEQ ID NOs: 267, 268 and 269, respectively; SEQ ID NOs: 278, 279 and 280, respectively; SEQ ID NOs: 289, 290 and 291, respectively; SEQ ID NOs: 300, 301 and 302, respectively; SEQ ID NOs: 311, 312 and 313, respectively; SEQ ID NOs: 322, 323 and 324, respectively; SEQ ID NOs: 333, 334 and 335, respectively; SEQ ID NOs: 344, 345 and 346, respectively; SEQ ID

NOs: 355, 356 and 357, respectively; SEQ ID NOs: 366, 367 and 368, respectively; SEQ ID NOs: 377, 378 and 379, respectively; SEQ ID NOs: 388, 389 and 390 respectively; SEQ ID NOs: 399, 400 and 401, respectively; SEQ ID NOs: 410, 411 and 412, respectively; SEQ ID NOs: 421, 422 and 423, respectively; SEQ ID NOs: 432, 433 and 434respectively; SEQ ID NOs: 443, 444 and 445, respectively; SEQ ID NOs: 454, 455 and 456, respectively; SEQ ID NOs: 465, 466 and 467, respectively; SEQ ID NOs: 476, 477 and 478, respectively; SEQ ID NOs: 487, 488 and 489, respectively; SEQ ID NOs: 498, 499 and 500, respectively; SEQ ID NOs: 509, 510 and 511, respectively; SEQ ID NOs: 520, 521 and 522, respectively; SEQ ID NOs: 531, 530 and 531, respectively; SEQ ID NOs: 542, 543 and 544, respectively; SEQ ID NOs: 553, 554 and 555, respectively; or SEQ ID NOs: 564, 565 and 566, respectively. The scaffold regions of such anti-PD-L1 Adnectins may comprise anywhere from 0 to 20, from 0 to 15, from 0 to 10, from 0 to 8, from 0 to 6, from 0 to 5, from 0 to 4, from 0 to 3, from 0 to 2, or from 0 to 1 substitutions, conservative substitutions, deletions or additions relative to the scaffold amino acids residues of SEQ ID NO: 4. Such scaffold modifications may be made, so long as the anti-PD-L1 Adnectin is capable of binding PD-L1 with a desired K_D.

[00112] In certain embodiments, the BC loop of the anti-PD-L1 Adnectin comprises an amino acid sequence selected from the group consisting of: 6, 21, 36, 51, 66, 81, and 97.

[00113] In certain embodiments, the DE loop of the anti-PD-L1 Adnectin comprises an amino acid sequence selected from the group consisting of: 7, 22, 37, 52, 67, 82, and 98.

[00114] In certain embodiments, the FG loop of the anti-PD-L1 Adnectin comprises an amino acid sequence selected from the group consisting of: 8, 23, 38, 53, 68, 83, and 99.

[00115] In certain embodiments, the anti-PD-L1 Adnectin comprises a BC, DE and FG loop amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to any one of SEQ ID NOs: 6, 21, 36, 51, 66, 81, and 97; 7, 22, 37, 52, 67, 82, and 98; and 8, 23, 38, 53, 68, 83, and 99, respectively.

[00116] In certain embodiments, the anti-PD-L1 Adnectin comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 5, 20, 35, 50, 65, 80, 96, 112, 123, 134, 145, 156, 167, 178, 189, 200, 211, 222, 233, 244, 255, 266, 277, 288, 299, 310, 321, 332, 343, 354, 365, 376, 387, 398, 409, 420, 431, 442, 453, 464, 475, 486, 497, 508, 519, 530, 541, 552 and 563.

[00117] In certain embodiments, the anti-PD-L1 Adnectins described herein comprise an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of SEQ ID NOs: 5, 20, 35, 50, 65, 80, or 96.

[00118] In certain embodiments, the anti-PD-L1 Adnectin comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to any one of SEQ ID NOs: 9-15, 24-30, 39-45, 54-60, 6975, 84-91, 100-107, 116-122, 127-133, 138-144, 150-155, 160-166, 171-177, 182-188, 193-199, 204-210, 215-221, 227-232, 237-243, 248-254, 259-265, 271-276, 291-287, 292-298, 303-309, 314-320, 325-331, 337-342, 347-353, 358-364, 369-375, 380-386, 391-397, 402-408, 413-419, 424-430, 435-441, 446-452, 457-463, 468-474, 479-485, 490-496, 501-507, 512-518, 523-529, 534-540, 545-551, and 556-562. In certain embodiments, the anti-PD-L1 Adnectins described herein comprise an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of any one of SEQ ID NOs: 9-15, 24-30, 39-45, 54-60, 6975, 84-91, and 100-107.

[00119] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 6, 7, and 8, respectively.

[00120] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 21, 22, and 23, respectively.

[00121] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 36, 37, and 38, respectively.

[00122] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 51, 52, and 53, respectively.

[00123] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 66, 67, and 68, respectively.

[00124] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 81, 82, and 83, respectively.

[00125] In certain embodiments, the anti-PD-L1 Adnectin comprises BC, DE, and FG loops as set forth in SEQ ID NOs: 97, 98, and 99, respectively.

[00126] In certain embodiments, BC, DE and/or FG loop amino acid sequences described herein (e.g., SEQ ID NOs: 6, 21, 36, 51, 66, 81, and 97; 7, 22, 37, 52, 67, 82, and 98; and 8, 23, 38, 53, 68, 83, and 99, respectively) are grafted into non-^{10}Fn3 domain protein scaffolds. For instance, one or more loop amino acid sequences is exchanged for or inserted into one or

more CDR loops of an antibody heavy or light chain or fragment thereof. In some embodiments, the protein domain into which one or more amino acid loop sequences are exchanged or inserted includes, but is not limited to, consensus Fn3 domains (Centocor ,US), ankyrin repeat proteins (Molecular Partners AG, Zurich Switzerland), domain antibodies (Domantis, Ltd, Cambridge, MA), single domain camelid nanobodies (Ablynx, Belgium), lipocalins (e.g., anticalins; Pieris Proteolab AG, Freising, Germany), Avimers (Amgen, CA), affibodies (Affibody AG, Sweden), ubiquitin (e.g., affilins; Scil Proteins GmbH, Halle, Germany), protein epitope mimetics (Polyphor Ltd, Allschwil, Switzerland), helical bundle scaffolds (e.g. alphabodies, Complix, Belgium), Fyn SH3 domains (Covagen AG, Switzerland), or atrimers (Anaphor, Inc., CA).

[00127] In certain embodiments, the amino acid sequences of the N-terminal and/or C-terminal regions of the polypeptides provided herein may be modified by deletion, substitution or insertion relative to the amino acid sequences of the corresponding regions of the wild-type human ^{10}Fn3 domain (SEQ ID NO: 1 or 2). The ^{10}Fn3 domains generally begin with amino acid number 1 of SEQ ID NO: 1. However, domains with amino acid deletions are also encompassed by the invention. Additional sequences may also be added to the N- or C-terminus of a ^{10}Fn3 domain having the amino acid sequence of SEQ ID NO: 1 or 2. For example, in some embodiments, the N-terminal extension consists of an amino acid sequence selected from the group consisting of: M, MG, and G. In certain embodiments, an MG sequence may be placed at the N-terminus of the ^{10}Fn3 defined by SEQ ID NO: 1. The M will usually be cleaved off, leaving a G at the N-terminus. In addition, an M, G or MG may also be placed N-terminal to any of the N-terminal extensions shown in Table 3.

[00128] In exemplary embodiments, an alternative N-terminal region having from 1-20, 1-15, 1-10, 1-8, 1-5, 1-4, 1-3, 1-2, or 1 amino acids in length can be added to the N-terminal region of SEQ ID NO: 1 or 2 or any adnectin set forth in Table 3. Exemplary alternative N-terminal regions include (represented by the single letter amino acid code) M, MG, G, MGVSDVPRDL (SEQ ID NO: 574) and GVSDVPRDL (SEQ ID NO: 575). Other suitable alternative N-terminal regions, which may be linked, e.g., to the N-terminus of an adnectin core sequence, include, for example, X_nSDVPRDL (SEQ ID NO: 576), X_nDVPRDL (SEQ ID NO: 577), X_nVPRDL (SEQ ID NO: 578), X_nPRDL (SEQ ID NO: 579) X_nRDL (SEQ ID NO: 580), X_nDL (SEQ ID NO: 581), or X_nL, wherein n = 0, 1 or 2 amino acids, wherein when n = 1, X is Met or Gly, and when n = 2, X is Met-Gly. When a Met-Gly sequence is added to the N-terminus of a ^{10}Fn3 domain, the M will usually be cleaved off, leaving a G at

the N-terminus. In some embodiments, the alternative N-terminal region comprises the amino acid sequence MASTSG (SEQ ID NO: 582).

[00129] In exemplary embodiments, an alternative C-terminal region having from 1-20, 1-15, 1-10, 1-8, 1-5, 1-4, 1-3, 1-2, or 1 amino acids in length can be added to the C-terminal region of SEQ ID NO: 1 or 2 or any adnectin set forth in Table 3. Specific examples of alternative C-terminal region sequences include, for example, polypeptides comprising, consisting essentially of, or consisting of, EIEK (SEQ ID NO: 584), EGSGC (SEQ ID NO: 585), EIEKPCQ (SEQ ID NO: 586), EIEKPSQ (SEQ ID NO: 587), EIEKP (SEQ ID NO: 588), EIEKPS (SEQ ID NO: 589), or EIEKPC (SEQ ID NO: 590). In some embodiments, the alternative C-terminal region comprises EIDK (SEQ ID NO: 591), and in particular embodiments, the alternative C-terminal region is either EIDKPCQ (SEQ ID NO: 592) or EIDKPSQ (SEQ ID NO: 593). Additional suitable alternative C-terminal regions are set forth in SEQ ID NOs: 594-618.

[00130] In certain embodiments, an Adnectin is linked to a C-terminal extension sequence that comprises E and D residues, and may be between 8 and 50, 10 and 30, 10 and 20, 5 and 10, and 2 and 4 amino acids in length. In some embodiments, tail sequences include ED-based linkers in which the sequence comprises tandem repeats of ED. In exemplary embodiments, the tail sequence comprises 2-10, 2-7, 2-5, 3-10, 3-7, 3-5, 3, 4 or 5 ED repeats. In certain embodiments, the ED-based tail sequences may also include additional amino acid residues, such as, for example: EI, EID, ES, EC, EGS, and EGC. Such sequences are based, in part, on known Adnectin tail sequences, such as EIDKPSQ (SEQ ID NO: 593), in which residues D and K have been removed. In exemplary embodiments, the ED-based tail comprises an E, I or E1 residues before the ED repeats.

[00131] In certain embodiments, the N- or C-terminal extension sequences are linked to the anti-PD-L1 Adnectin sequences with known linker sequences (e.g., SEQ ID NOs: 629-678 in Table 3). In some embodiments, sequences may be placed at the C-terminus of the ^{10}Fn3 domain to facilitate attachment of a pharmacokinetic moiety. For example, a cysteine containing linker such as GSGC (SEQ ID NO: 638) may be added to the C-terminus to facilitate site directed PEGylation on the cysteine residue.

[00132] In certain embodiments, an alternative C-terminal moiety, which can be linked to the C-terminal amino acids RT (i.e., amino acid 94) comprises the amino acids P_mX_n, wherein P is proline, X is any amino acid, m is an integer that is at least 1 and n is 0 or an interger that is at least 1. In certain embodiments, the alternative C-terminal moiety comprises the amino

acids PC. In certain embodiments, the alternative C-terminal moiety comprises the amino acids PI, PC, PID, PIE, PIDK (SEQ ID NO: 605), PIEK (SEQ ID NO: 606), PIDKP (SEQ ID NO: 607), PIEKP (SEQ ID NO: 608), PIDKPS (SEQ ID NO: 609), PIEKPS (SEQ ID NO: 610), PIDKPC (SEQ ID NO: 611), PIEKPC (SEQ ID NO: 612), PIDKPSQ (SEQ ID NO: 613), PIEKPSQ (SEQ ID NO: 614), PIDKPCQ (SEQ ID NO: 615), PIEKPCQ (SEQ ID NO: 616), PHHHHHH (SEQ ID NO: 617), and PCHHHHHH (SEQ ID NO: 618). Exemplary anti-PD-L1 Adnectins having PC at their C-terminus are provided in the Examples and Table 3.

[00133] In certain embodiments, the Adnectins described herein have a 6X his tail (SEQ ID NO: 619).

[00134] In certain embodiments, the fibronectin based scaffold proteins comprise a ^{10}Fn3 domain having both an alternative N-terminal region sequence and an alternative C-terminal region sequence, and optionally a 6X his tail.

II. Biological properties of anti-PD-L1 adnectins

[00135] Provided herein are adnectins that bind to human PD-L1 with a KD of 10 nM, 1 nM, 0.5 nM, 0.1nM or less, as determined, e.g., by SPR (Biacore) and exhibit one or more of the following properties:

1. Inhibition of the interaction between human PD-L1 and human PD-1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., by flow cytometry, e.g., using a human PD-1Fc protein and human PD-L1 positive cells, such as L2987 cells;
2. Inhibition of the binding of human CD80 (B7-1) to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
3. Inhibition of the binding of the anti-PD-L1 antibody 12A4 (described, e.g,. in U.S. Patent No. 7,943,743) to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore); and
4. Inhibit cell proliferation in a mixed lymphocyte reaction (MLR).

[00136] In certain embodiments, an anti-PD-L1 adnectin binds to human PD-L1 with a KD of 1 nM or less and exhibits each one of properties 1-4. In certain embodiments, an anti-PD-L1 adnectin binds to human PD-L1 with a KD of 0.1 nM or less and exhibits each one of properties 1-4.

[00137] Provided herein are adnectins that comprise an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), bind to human PD-L1 with a KD of 10 nM, 1 nM, 0.5 nM, 0.1nM or less, as determined, e.g., by SPR (Biacore) and exhibit one or more of the following properties:

1. Inhibition of the interaction between human PD-L1 and human PD-1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., by flow cytometry, e.g., using a human PD-1Fc protein and human PD-L1 positive cells, such as L2987 cells;
2. Inhibition of the binding of human CD80 (B7-1) to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
3. Inhibition of the binding of the anti-PD-L1 antibody 12A4 to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore); and
4. Inhibit cell proliferation in a mixed lymphocyte reaction (MLR).

[00138] In certain embodiments, an anti-PD-L1 adnectin comprises an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), binds to human PD-L1 with a KD of 1 nM or less and exhibits each one of properties 1-4. In certain embodiments, an anti-PD-L1 adnectin comprises an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), binds to human PD-L1 with a KD of 0.1 nM or less and exhibits each one of properties 1-4.

[00139] In certain embodiments, the anti-PD-L1 Adnectins compete (e.g., cross-compete) for binding to PD-L1 with the particular anti-PD-L1 Adnectins described herein. Such competing Adnectins can be identified based on their ability to competitively inhibit binding to PD-L1 of Adnectins described herein in standard PD-L1 binding assays. For example, standard ELISA assays can be used in which a recombinant PD-L1 protein is immobilized on the plate, one of the Adnectins is fluorescently labeled and the ability of non-labeled Adnectins to compete off the binding of the labeled Adnectin is evaluated.

[00140] In certain embodiments, a competitive ELISA format can be performed to determine whether two anti-PD-L1 Adnectins bind overlapping Adnectin binding sites on PD-L1. In one format, Adnectin #1 is coated on a plate, which is then blocked and washed. To this plate

is added either PD-L1 alone, or PD-L1 pre-incubated with a saturating concentration of Adnectin #2. After a suitable incubation period, the plate is washed and probed with a polyclonal anti-PD-L1 antibody, such as a biotinylated anti-PD-L1 polyclonal antibody, followed by detection with streptavidin-HRP conjugate and standard tetramethylbenzidine development procedures. If the OD signal is the same with or without preincubation with Adnectin #2, then the two Adnectins bind independently of one another, and their Adnectin binding sites do not overlap. If, however, the OD signal for wells that received PD-L1/Adnectin#2 mixtures is lower than for those that received PD-L1 alone, then binding of Adnectin #2 is confirmed to block binding of Adnectin #1 to PD-L1.

[00141] Alternatively, a similar experiment is conducted by surface plasmon resonance (SPR, e.g., BIAcore). Adnectin #1 is immobilized on an SPR chip surface, followed by injections of either PD-L1 alone or PD-L1 pre-incubated with a saturating concentration of Adnectin #2. If the binding signal for PD-L1/Adnectin#2 mixtures is the same or higher than that of PD-L1 alone, then the two Adnectins bind independently of one another, and their Adnectin binding sites do not overlap. If, however, the binding signal for PD-L1/Adnectin#2 mixtures is lower than the binding signal for PD-L1 alone, then binding of Adnectin #2 is confirmed to block binding of Adnectin #1 to PD-L1. A feature of these experiments is the use of saturating concentrations of Adnectin #2. If PD-L1 is not saturated with Adnectin #2, then the conclusions above do not hold. Similar experiments can be used to determine if any two PD-L1 binding proteins bind to overlapping Adnectin binding sites.

[00142] Both assays exemplified above may also be performed in the reverse order where Adnectin#2 is immobilized and PD-L1–Adnectin#1 are added to the plate. Alternatively, Adnectin #1 and/or #2 can be replaced with a monoclonal antibody and/or soluble receptor-Fc fusion protein.

[00143] In certain embodiments, competition can be determined using a HTRF sandwich assay.

[00144] In certain embodiments, the competing Adnectin is an Adnectin that binds to the same Adnectin binding site on PD-L1 as a particular anti-PD-L1 Adnectin described herein. Standard mapping techniques, such as protease mapping, mutational analysis, HDX-MS, x-ray crystallography and 2-dimensional nuclear magnetic resonance, can be used to determine whether an Adnectin binds to the same Adnectin binding site or epitope as a reference Adnectin (see, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, G. E. Morris, Ed. (1996)). An epitope is defined by the method used to locate it. For example,

in certain embodiments, a PD-L1 adnectin or antibody binds to the same epitope as that of one of the PD-L1 adnectins described herein, as determined by HDX-MS or as determined by X-ray crystallography.

[00145] Candidate competing anti-PD-L1 Adnectins can inhibit the binding of anti-PD-L1 Adnectins described herein to PD-L1 by at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% and/or their binding is inhibited by at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% by anti-PD-L1 Adnectins. The % competition can be determined using the methods described above.

[00146] Provided herein are adnectins that bind to human PD-L1 with a KD of 10 nM, 1 nM, 0.5 nM, 0.1nM or less, as determined, e.g., by SPR (Biacore) and exhibit one or more of the following properties:

1. Inhibition of the interaction between human PD-L1 and human PD-1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., by flow cytometry, e.g., using a human PD-1Fc protein and human PD-L1 positive cells, such as L2987 cells;
2. Inhibition of the binding of human CD80 (B7-1) to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
3. Inhibition of the binding of the anti-PD-L1 antibody 12A4 to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
4. Inhibit cell proliferation in a mixed lymphocyte reaction (MLR); and
5. Compete with an anti-PD-L1 antibody described herein for binding to human PD-L1.

[00147] In certain embodiments, an anti-PD-L1 adnectin binds to human PD-L1 with a KD of 1 nM or less and exhibits each one of properties 1-5. In certain embodiments, an anti-PD-L1 adnectin binds to human PD-L1 with a KD of 0.1 nM or less and exhibits each one of properties 1-5.

[00148] Provided herein are adnectins that comprise an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), bind to human PD-L1 with a KD of 10 nM, 1 nM, 0.5 nM, 0.1nM or less, as determined, e.g., by SPR (Biacore) and exhibit one or more of the following properties:

1. Inhibition of the interaction between human PD-L1 and human PD-1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., by flow cytometry, e.g., using a human PD-1Fc protein and human PD-L1 positive cells, such as L2987 cells;
2. Inhibition of the binding of human CD80 (B7-1) to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
3. Inhibition of the binding of the anti-PD-L1 antibody 12A4 to human PD-L1 by at least 50%, 70%, 80%, 90% or more, as determined, e.g., in an ELISA assay or by SPR (Biacore);
4. Inhibit cell proliferation in a mixed lymphocyte reaction (MLR); and
5. Compete with an anti-PD-L1 antibody described herein for binding to human PD-L1.

[00149] In certain embodiments, an anti-PD-L1 adnectin comprises an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), binds to human PD-L1 with a KD of 1 nM or less and exhibits each one of properties 1-5. In certain embodiments, an anti-PD-L1 adnectin comprises an amino acid sequence that is at least 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to an anti-PD-L1 adnectin described herein or a portion thereof (e.g., the BC, DE and FG loops), binds to human PD-L1 with a KD of 0.1 nM or less and exhibits each one of properties 1-5.

III. Fusions, including pharmacokinetic moieties

[00150] In certain embodiments, the anti-PD-L1 Adnectins desirably have a short half-life, for example, when used in diagnostic imaging.

[00151] Alternatively, e.g., for therapeutic purposes, the anti-PD-L1 Adnectins described herein further comprise a pharmacokinetic (PK) moiety. Improved pharmacokinetics may be assessed according to the perceived therapeutic need. Often it is desirable to increase bioavailability and/or increase the time between doses, possibly by increasing the time that a protein remains available in the serum after dosing. In some instances, it is desirable to improve the continuity of the serum concentration of the protein over time (e.g., decrease the difference in serum concentration of the protein shortly after administration and shortly before the next administration). The anti-PD-L1 Adnectin may be attached to a moiety that reduces the clearance rate of the polypeptide in a mammal (e.g., mouse, rat, or human) by greater than two-fold, greater than three-fold, greater than four-fold or greater than five-fold relative to the unmodified anti-PD-L1 Adnectin. Other measures of improved

pharmacokinetics may include serum half-life, which is often divided into an alpha phase and a beta phase. Either or both phases may be improved significantly by addition of an appropriate moiety. For example, the PK moiety may increase the serum half-life of the polypeptide by more than 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 200, 400, 600, 800, 1000% or more relative to the Fn3 domain alone.

[00152] Moieties that slow clearance of a protein from the blood, herein referred to as "PK moieties", include polyoxyalkylene moieties (e.g., polyethylene glycol), sugars (e.g., sialic acid), and well-tolerated protein moieties (e.g., Fc and fragments and variants thereof, transferrin, or serum albumin). The anti-PD-L1 Adnectin may also be fused to albumin or a fragment (portion) or variant of albumin as described in U.S. Publication No. 2007/0048282, or may be fused to one or more serum albumin binding Adnectin, as described herein.

[00153] Other PK moieties that can be used in the invention include those described in Kontermann et al., (*Current Opinion in Biotechnology* 2011;22:868-76), herein incorporated by reference. Such PK moieties include, but are not limited to, human serum albumin fusions, human serum albumin conjugates, human serum albumin binders (e.g., Adnectin PKE, AlbudAb, ABD), XTEN fusions, PAS fusions (i.e., recombinant PEG mimetics based on the three amino acids proline, alanine, and serine), carbohydrate conjugates (e.g., hydroxyethyl starch (HES)), glycosylation, polysialic acid conjugates, and fatty acid conjugates.

[00154] Accordingly, in some embodiments the invention provides an anti-PD-L1 Adnectin fused to a PK moiety that is a polymeric sugar. In some embodiments, the PK moiety is a polyethylene glycol moiety or an Fc region. In some embodiments the PK moiety is a serum albumin binding protein such as those described in U.S. Publication Nos. 2007/0178082 and 2007/0269422. In some embodiments the PK moiety is human serum albumin. In some embodiments, the PK moiety is transferrin.

[00155] In some embodiments, the PK moiety is linked to the anti-PD-L1 Adnectin via a polypeptide linker. Exemplary polypeptide linkers include polypeptides having from 1-20, 1-15, 1-10, 1-8, 1-5, 1-4, 1-3, or 1-2 amino acids. Suitable linkers for joining the Fn3 domains are those which allow the separate domains to fold independently of each other forming a three dimensional structure that permits high affinity binding to a target molecule. Specific examples of suitable linkers include glycine-serine based linkers, glycine-proline based linkers, proline-alanine based linkers as well as any other linkers described herein. In some embodiments, the linker is a glycine-proline based linker. These linkers comprise glycine and proline residues and may be between 3 and 30, 10 and 30, and 3 and 20 amino acids in length.

Examples of such linkers include GPG, GPGPGPG (SEQ ID NO: 672) and GPGPGPGPGPG (SEQ ID NO: 673). In some embodiments, the linker is a proline-alanine based linker. These linkers comprise proline and alanine residues and may be between 3 and 30, 10 and 30, 3 and 20 and 6 and 18 amino acids in length. Examples of such linkers include PAPAPA (SEQ ID NO: 674), PAPAPAPAPAPA (SEQ ID NO: 675) and PAPAPAPAPAPAPAPAPA (SEQ ID NO: 676). In some embodiments, the linker is a glycine-serine based linker. These linkers comprise glycine and serine residues and may be between 8 and 50, 10 and 30, and 10 and 20 amino acids in length. Examples of such linkers include GSGSGSGSGS ($(GS)_5$; SEQ ID NO: 662), GSGSGSGSGSGS ($(GS)_6$; SEQ ID NO: 663), GSGSGSGSGSGSGSGSGSGS ($(GS)_{10}$; SEQ ID NO: 677), GGGGSGGGGSGGGGSGGGGS ($(G_4S)_4$; SEQ ID NO: 678), GGGGSGGGGSGGGGSGGGGSGGGGS ($(G_4S)_5$; SEQ ID NO: 670), and GGGGSGGGGSGGGSG (SEQ ID NO: 671). In exemplary embodiments, the linker does not contain any Asp-Lys (DK) pairs. A list of suitable linkers is provided in Table 3.

[00156] Optimal linker length and amino acid composition may be determined by routine experimentation in view of the teachings provided herein. In some embodiments, an anti-PD-L1 Adnectin is linked, for example, to an anti-HSA Adnectin via a polypeptide linker having a protease site that is cleavable by a protease in the blood or target tissue. Such embodiments can be used to release an anti-PD-L1 Adnectin for better delivery or therapeutic properties or more efficient production.

[00157] Additional linkers or spacers, may be introduced at the N-terminus or C-terminus of a Fn3 domain between the Fn3 domain and the polypeptide linker.

Polyethylene glycol

[00158] In some embodiments, the anti-PD-L1 Adnectin comprises polyethylene glycol (PEG). PEG is a well-known, water soluble polymer that is commercially available or can be prepared by ring-opening polymerization of ethylene glycol according to methods well known in the art (Sandler and Karo, Polymer Synthesis, Academic Press, New York, Vol. 3, pages 138-161). The term "PEG" is used broadly to encompass any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented by the formula: $X—O(CH_2CH_2O)_{n-1}CH_2CH_2OH$, where n is 20 to 2300 and X is H or a terminal modification, e.g., a C_{1-4} alkyl. PEG can contain further chemical groups which are necessary for binding reactions, which result from the chemical synthesis of the molecule; or which act as a spacer for optimal distance of parts of the molecule. In addition, such a PEG can consist of one or more PEG side-chains which are linked together. PEGs with

more than one PEG chain are called multiarmed or branched PEGs. Branched PEGs are described in, for example, European Published Application No. 473084A and U.S. Pat. No. 5,932,462.

[00159] One or more PEG molecules may be attached at different positions on the protein, and such attachment may be achieved by reaction with amines, thiols or other suitable reactive groups. The amine moiety may be, for example, a primary amine found at the N-terminus of a polypeptide or an amine group present in an amino acid, such as lysine or arginine. In some embodiments, the PEG moiety is attached at a position on the polypeptide selected from the group consisting of: a) the N-terminus; b) between the N-terminus and the most N-terminal beta strand or beta-like strand; c) a loop positioned on a face of the polypeptide opposite the target-binding site; d) between the C-terminus and the most C-terminal beta strand or beta-like strand; and e) at the C-terminus.

[00160] PEGylation may be achieved by site-directed PEGylation, wherein a suitable reactive group is introduced into the protein to create a site where PEGylation preferentially occurs. In some embodiments, the protein is modified to introduce a cysteine residue at a desired position, permitting site-directed PEGylation on the cysteine. Mutations may be introduced into a protein coding sequence to generate cysteine residues. This might be achieved, for example, by mutating one or more amino acid residues to cysteine. Preferred amino acids for mutating to a cysteine residue include serine, threonine, alanine and other hydrophilic residues. Preferably, the residue to be mutated to cysteine is a surface-exposed residue. Algorithms are well-known in the art for predicting surface accessibility of residues based on primary sequence or a protein. Alternatively, surface residues may be predicted by comparing the amino acid sequences of binding polypeptides, given that the crystal structure of the framework, based on which binding polypeptides are designed and evolved, has been solved (see Himanen et al., *Nature* 2001;414:933-8) and thus the surface-exposed residues identified. PEGylation of cysteine residues may be carried out using, for example, PEG-maleimide, PEG-vinylsulfone, PEG-iodoacetamide, or PEG-orthopyridyl disulfide. In certain embodiments, a PEG is linked to a C-terminal cysteine, e.g., a cysteine that has been added to an anti-PD-L1 Adnectin, such as in the form of a “PC” extension, as further described herein.

[00161] The PEG is typically activated with a suitable activating group appropriate for coupling to a desired site on the polypeptide. PEGylation methods are well-known in the art and further described in Zalipsky, S., et al., "Use of Functionalized Poly(Ethylene Glycols) for Modification of Polypeptides" in Polyethylene Glycol Chemistry: Biotechnical and

Biomedical Applications, J. M. Harris, Plenus Press, New York (1992), and in Zalipsky (1995) *Advanced Drug Reviews* 16: 157-182.

[00162] PEG may vary widely in molecular weight and may be branched or linear. Typically, the weight-average molecular weight of PEG is from about 100 Daltons to about 150,000 Daltons. Exemplary weight-average molecular weights for PEG include about 5,000 Daltons, about 10,000 Daltons, about 20,000 Daltons, about 40,000 Daltons, about 60,000 Daltons and about 80,000 Daltons. In certain embodiments, the molecular weight of PEG is 40,000 Daltons. Branched versions of PEG having a total molecular weight of any of the foregoing can also be used. In some embodiments, the PEG has two branches. In some embodiments, the PEG has four branches. In some embodiments, the PEG is a bis-PEG (NOF Corporation, DE-200MA), in which two Adnectins are conjugated (see, e.g., Example 1 and ATI-1341 of Table 5).

[00163] Conventional separation and purification techniques known in the art can be used to purify PEGylated anti-PD-L1 Adnectins, such as size exclusion (e.g., gel filtration) and ion exchange chromatography. Products may also be separated using SDS-PAGE. Products that may be separated include mono-, di-, tri-, poly- and un-PEGylated Adnectins, as well as free PEG. The percentage of mono-PEG conjugates can be controlled by pooling broader fractions around the elution peak to increase the percentage of mono-PEG in the composition. About 90% mono-PEG conjugates represent a good balance of yield and activity.

[00164] In some embodiments, the PEGylated anti-PD-L1 Adnectins will preferably retain at least about 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or 100% of the biological activity associated with the unmodified anti-PD-L1 Adnectin. In some embodiments, biological activity refers to its ability to bind to PD-L1, as assessed by K_D, k_{on}, or k_{off}. In some embodiments, the PEGylated anti-PD-L1 Adnectin shows an increase in binding to PD-L1 relative to unPEGylated anti-PD-L1 Adnectin.

Immunoglobulin Fc domain (and fragments)

[00165] In certain embodiments, the anti-PD-L1 Adnectin is fused to an immunoglobulin Fc domain, or a fragment or variant thereof. As used herein, a "functional Fc region" is an Fc domain or fragment thereof which retains the ability to bind FcRn. In some embodiments, a functional Fc region binds to FcRn, bud does not possess effector function. The ability of the Fc region or fragment thereof to bind to FcRn can be determined by standard binding assays known in the art. In some embodiments, the Fc region or fragment thereof binds to FcRn and possesses at least one “effector function” of a native Fc region. Exemplary "effector

functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an anti-PD-L1 Adnectin) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[00166] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95% sequence identity therewith.

[00167] In an exemplary embodiment, the Fc domain is derived from an IgG1 subclass, however, other subclasses (e.g., IgG2, IgG3, and IgG4) may also be used. Shown below is the sequence of a human IgG1 immunoglobulin Fc domain:

<u>DKTHTCPPCPAPELLG</u>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 620)

[00168] The core hinge sequence is underlined, and the CH2 and CH3 regions are in regular text. It should be understood that the C-terminal lysine is optional. Allotypes and mutants of this sequence may also be used. As is known in the art, mutants can be designed to modulate a variety of properties of the Fc, e.g., ADCC, CDC or half-life.

[00169] In certain embodiments, the Fc region used in the anti-PD-L1 Adnectin fusion comprises a CH1 region. In certain embodiments, the Fc region used in the anti-PD-L1 Adnectin fusion comprises CH2 and CH3 regions. In certain embodiments, the Fc region

used in the anti-PD-L1 Adnectin fusion comprises a CH2, CH3, and hinge region (e.g., as shown in SEQ ID NO: 620).

[00170] In certain embodiments, the "hinge" region comprises the core hinge residues spanning positions 1-16 of SEQ ID NO: 620 (DKTHTCPPCPAPELLG; SEQ ID NO: 621) of the IgG1 Fc region. In certain embodiments, the anti-PD-L1 Adnectin-Fc fusion adopts a multimeric structure (e.g., dimer) owing, in part, to the cysteine residues at positions 6 and 9 of SEQ ID NO: 620 within the hinge region. Other suitable exemplary hinge regions are set forth in SEQ ID NOs: 622-626.

Adnectins

[00171] In certain embodiments the PK moiety is another Adnectin specific, for example, to a serum protein (e.g., human serum albumin), as described in US 2012/0094909, herein incorporated by reference in its entirety. Other PK moieties that may be used with the Adnectins described herein are described in Kontermann et al. (*Current Opinion in Biotechnology* 2011;22:868-76), as discussed *supra*.

[00172] In some embodiments, an anti-PD-L1 Adnectin may be directly or indirectly linked for example, to an anti-HSA Adnectin via a polymeric linker. Polymeric linkers can be used to optimally vary the distance between each component of the fusion to create a protein fusion with one or more of the following characteristics: 1) reduced or increased steric hindrance of binding of one or more protein domains when binding to a protein of interest, 2) increased protein stability or solubility, 3) decreased protein aggregation, and 4) increased overall avidity or affinity of the protein.

[00173] In some embodiments, an anti-PD-L1 Adnectin is linked, for example, to an anti-HSA Adnectin, via a biocompatible polymer such as a polymeric sugar. The polymeric sugar can include an enzymatic cleavage site that is cleavable by an enzyme in the blood or target tissue. Such embodiments can be used to release an anti-PD-L1 Adnectin for better delivery or therapeutic properties or more efficient production.

IV. Nucleic Acid-Protein Fusion Technology

[00174] In one aspect, the invention provides an Adnectin comprising fibronectin type III domains that binds PD-L1. One way to rapidly make and test Fn3 domains with specific binding properties is the nucleic acid-protein fusion technology of Adnexus, a Bristol-Myers Squibb R&D Company. This disclosure utilizes the *in vitro* expression and tagging technology, termed 'PROfusion' which exploits nucleic acid-protein fusions (RNA- and

DNA-protein fusions) to identify novel polypeptides and amino acid motifs that are important for binding to proteins. Nucleic acid-protein fusion technology is a technology that covalently couples a protein to its encoding genetic information. For a detailed description of the RNA-protein fusion technology and fibronectin-based scaffold protein library screening methods see Szostak et al., U.S. Pat. Nos. 6,258,558, 6,261,804, 6,214,553, 6,281,344, 6,207,446, 6,518,018 and 6,818,418; Roberts et al., *Proc. Natl. Acad. Sci.*, 1997;94:12297-12302; and Kurz et al., *Molecules*, 2000;5:1259-64, all of which are herein incorporated by reference.

V. Vectors and Polynucleotides

[00175] Also included in the present disclosure are nucleic acid sequences encoding any of the proteins described herein. As appreciated by those skilled in the art, because of third base degeneracy, almost every amino acid can be represented by more than one triplet codon in a coding nucleotide sequence. In addition, minor base pair changes may result in a conservative substitution in the amino acid sequence encoded but are not expected to substantially alter the biological activity of the gene product. Therefore, a nucleic acid sequence encoding a protein described herein may be modified slightly in sequence and yet still encode its respective gene product. Certain exemplary nucleic acids encoding the anti-PD-L1 Adnectins and their fusions described herein include nucleic acids having the sequences set forth in SEQ ID NOs: 16-19, 31-34, 46-49, 61-64, 76-79, 92-95, and 108-111.

[00176] Also contemplated are nucleic acid sequences that are at least 50%, such as at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NOs: 16-19, 31-34, 46-49, 61-64, 76-79, 92-95, and 108-111, and encode a protein that binds to PD-L1. In some embodiments, nucleotide substitutions are introduced so as not to alter the resulting translated amino acid sequence.

[00177] Nucleic acids encoding any of the various proteins or polypeptides described herein may be synthesized chemically. Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the cell type selected. Specialized codon usage patterns have been developed for *E. coli* and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. See for example: Mayfield et al., *Proc. Natl. Acad. Sci. USA*, 100(2):438-442 (Jan. 21, 2003); Sinclair et al., *Protein Expr. Purif.*, 26(I):96-105 (October 2002); Connell, N.D., *Curr. Opin. Biotechnol.*, 12(5):446-449 (October 2001); Makrides et al.,

Microbiol. Rev., 60(3):512-538 (September 1996); and Sharp et al., *Yeast*, 7(7):657-678 (October 1991).

[00178] General techniques for nucleic acid manipulation are described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Vols. 1-3, Cold Spring Harbor Laboratory Press (1989), or Ausubel, F. et al., Current Protocols in Molecular Biology, Green Publishing and Wiley-Interscience, New York (1987) and periodic updates, herein incorporated by reference. Generally, the DNA encoding the polypeptide is operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, viral, or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding site, and sequences that control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants is additionally incorporated.

[00179] The proteins described herein may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. An exemplary N-terminal leader sequence for production of polypeptides in a mammalian system is: METDTLLLWVLLLWVPGSTG (SEQ ID NO: 583), which is removed by the host cell following expression.

[00180] For prokaryotic host cells that do not recognize and process a native signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, 1 pp, or heat-stable enterotoxin II leaders.

[00181] For yeast secretion the native signal sequence may be substituted by, e.g., a yeast invertase leader, a factor leader (including Saccharomyces and Kluyveromyces alpha-factor leaders), or acid phosphatase leader, the C. albicans glucoamylase leader, or the signal sequence described in U.S. Pat. No. 5,631,144. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such precursor regions may be ligated in reading frame to DNA encoding the protein.

[00182] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

[00183] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli.

[00184] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the protein described herein, e.g., a fibronectin-based scaffold protein. Promoters suitable for use with prokaryotic hosts include the phoA promoter, beta-lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system, and hybrid promoters such as the tan promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the protein described herein. Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

[00185] Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase,

phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[00186] Transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[00187] Transcription of a DNA encoding protein described herein by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the peptide-encoding sequence, but is preferably located at a site 5' from the promoter.

[00188] Expression vectors used in eukaryotic host cells (e.g., yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of mRNA encoding the protein described herein. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO 94/11026 and the expression vector disclosed therein.

[00189] The recombinant DNA can also include any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include, but are not limited to, a histidine tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be

found in Cloning Vectors: A Laboratory Manual, (Elsevier, New York (1985)), the relevant disclosure of which is hereby incorporated by reference.

[00190] The expression construct is introduced into the host cell using a method appropriate to the host cell, as will be apparent to one of skill in the art. A variety of methods for introducing nucleic acids into host cells are known in the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent).

[00191] Suitable host cells include prokaryotes, yeast, mammalian cells, or bacterial cells. Suitable bacteria include gram negative or gram positive organisms, for example, *E. coli* or Bacillus spp. Yeast, preferably from the Saccharomyces species, such as *S. cerevisiae*, may also be used for production of polypeptides. Various mammalian or insect cell culture systems can also be employed to express recombinant proteins. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow et al. (Bio/Technology, 6:47 (1988)). Examples of suitable mammalian host cell lines include endothelial cells, COS-7 monkey kidney cells, CV-1, L cells, C127, 3T3, Chinese hamster ovary (CHO), human embryonic kidney cells, HeLa, 293, 293T, and BHK cell lines. Purified polypeptides are prepared by culturing suitable host/vector systems to express the recombinant proteins. For many applications, the small size of many of the polypeptides described herein would make expression in *E. coli* as the preferred method for expression. The protein is then purified from culture media or cell extracts.

VI. Protein Production

[00192] Also described herein are cell lines that express an anti-PD-L1 Adnectin or fusion polypeptide thereof. Creation and isolation of cell lines producing an anti-PD-L1 Adnectin can be accomplished using standard techniques known in the art, such as those described herein.

[00193] Host cells are transformed with the herein-described expression or cloning vectors for protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[00194] Adnectins of the present invention can also be obtained in aglycosylated form by producing the Adnectins in, e.g., prokaryotic cells (e.g., *E. coli*). Notably, aglycosylated

forms of the Adnectins described herein exhibit the same affinity, potency, and mechanism of action as glycosylated Adnectins when tested *in vitro*.

[00195] The host cells used to produce the proteins of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma)) are suitable for culturing the host cells. In addition, many of the media described in Ham et al., Meth. Enzymol., 58:44 (1979), Barites et al., Anal. Biochem., 102:255 (1980), U.S. Pat. Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655, 5,122,469, 6,048,728, 5,672,502, or U.S. Pat. No. RE 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as Gentamycin drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00196] Proteins described herein can also be produced using cell-free translation systems. For such purposes the nucleic acids encoding the polypeptide must be modified to allow *in vitro* transcription to produce mRNA and to allow cell-free translation of the mRNA in the particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free translation system or prokaryotic such as a bacterial cell-free translation system).

[00197] Proteins described herein can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd Edition, The Pierce Chemical Co., Rockford, Ill. (1984)). Modifications to the protein can also be produced by chemical synthesis.

[00198] The proteins of the present invention can be purified by isolation/purification methods for proteins generally known in the field of protein chemistry. Non-limiting examples include extraction, recrystallization, salting out (e.g., with ammonium sulfate or sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion exchange chromatography, hydrophobic chromatography, normal phase chromatography, reversed-phase chromatography, get filtration, gel permeation chromatography, affinity

chromatography, electrophoresis, countercurrent distribution or any combinations of these. After purification, polypeptides may be exchanged into different buffers and/or concentrated by any of a variety of methods known to the art, including, but not limited to, filtration and dialysis.

[00199] The purified polypeptide is preferably at least 85% pure, or preferably at least 95% pure, and most preferably at least 98% pure. Regardless of the exact numerical value of the purity, the polypeptide is sufficiently pure for use as a pharmaceutical product.

High Throughput Protein Production (HTPP)

[00200] Selected binders cloned into the PET9d vector upstream of a HIS_6tag and are transformed into *E.coli* BL21 DE3 plysS cells and inoculated in 5 ml LB medium containing 50 μg/mL kanamycin in a 24-well format and grown at 37°C overnight. Fresh 5 ml LB medium (50 μg/mL kanamycin) cultures are prepared for inducible expression by aspiration of 200 μl from the overnight culture and dispensing it into the appropriate well. The cultures are grown at 37°C until A_{600} 0.6-0.9. After induction with 1 mM isopropyl-β-thiogalactoside (IPTG), the culture is expressed for 6 hours at 30°C and harvested by centrifugation for 10 minutes at 2750 g at 4°C.

[00201] Cell pellets (in 24-well format) are lysed by resuspension in 450μl of Lysis buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 1x Complete ™ Protease Inhibitor Cocktail-EDTA free (Roche), 1 mM PMSF, 10 mM CHAPS, 40 mM imidazole, 1 mg/ml lysozyme, 30 μg/ml DNAse, 2 μg/ml aprotonin, pH 8.0) and shaken at room temperature for 1-3 hours. Lysates are cleared and re-racked into a 96-well format by transfer into a 96-well Whatman GF/D Unifilter fitted with a 96-well, 1.2 ml catch plate and filtered by positive pressure. The cleared lysates are transferred to a 96-well Nickel or Cobalt-Chelating Plate that had been equilibrated with equilibration buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 40 mM imidazole, pH 8.0) and are incubated for 5 min. Unbound material is removed by positive pressure. The resin is washed twice with 0.3 ml/well with Wash buffer #1 (50 mM NaH_2PO_4, 0.5 M NaCl, 5 mM CHAPS, 40 mM imidazole, pH 8.0). Each wash is removed by positive pressure. Prior to elution, each well is washed with 50 μl Elution buffer (PBS + 20 mM EDTA), incubated for 5 min, and this wash is discarded by positive pressure. Protein is eluted by applying an additional 100 μl of Elution buffer to each well. After a 30 minute incubation at room temperature, the plate(s) are centrifuged for 5 minutes at 200 g and eluted protein collected in 96-well catch plates containing 5μl of 0.5 M $MgCl_2$ added to the bottom of elution catch plate

prior to elution. Eluted protein is quantified using a total protein assay with wild-type ^{10}Fn3 domain as the protein standard.

Midscale Expression and Purification of Insoluble Fibronectin-Based Scaffold Protein Binders

[00202] For expression of insoluble clones, the clone(s), followed by the HIS_6tag, are cloned into a pET9d (EMD Bioscience, San Diego, CA) vector and are expressed in E. coli HMS174 cells. Twenty ml of an inoculum culture (generated from a single plated colony) is used to inoculate 1 liter of LB medium containing 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol. The culture is grown at 37 °C until A_{600} 0.6-1.0. After induction with 1mM isopropyl-β-thiogalactoside (IPTG) the culture is grown for 4 hours at 30 °C and is harvested by centrifugation for 30 minutes at > 10,000 g at 4 °C. Cell pellets are frozen at -80 °C. The cell pellet is resuspended in 25 ml of lysis buffer (20mM $aH2P0_4$, 0.5 M NaCl, lx Complete Protease Inhibitor Cocktail-EDTA free (Roche), ImM PMSF, pH 7.4) using an ULTRA-TURRAX® homogenizer (IKA works) on ice. Cell lysis is achieved by high pressure homogenization (>18,000 psi) using a Model M-l 10S MICROFLUIDIZER® (Microfluidics). The insoluble fraction is separated by centrifugation for 30 minutes at 23,300 g at 4 °C. The insoluble pellet recovered from centrifugation of the lysate is washed with 20 mM sodiumphosphate/500 mM NaCl, pH7.4. The pellet is resolubilized in 6.0M guanidine hydrochloride in 20 mM sodium phosphate/500M NaCl pH 7.4 with sonication followed by incubation at 37 degrees for 1- 2 hours. The resolubilized pellet is filtered to 0.45 μm and loaded onto a Histrap column equilibrated with the 20mM sodium phosphate/500 M NaCl/6.0 M guanidine pH 7.4 buffer. After loading, the column is washed for an additional 25 CV with the same buffer. Bound protein is eluted with 50mM Imidazole in 20mM sodium phosphate/500 mM NaCl/6.0 M guan-HCl pH7.4. The purified protein is refolded by dialysis against 50 mM sodium acetate/150 mM NaCl pH 4.5.

Midscale Expression and Purification of Soluble Fibronectin-Base Scaffold Protein Binders

[00203] For expression of soluble clones, the clone(s), followed by the HIS_6tag, are cloned into a pET9d (EMD Bioscience, San Diego, CA) vector and expressed in E. coli HMS174 cells. Twenty ml of an inoculum culture (generated from a single plated colony) is used to inoculate 1 liter of LB medium containing 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol. The culture is grown at 37 °C until A_{600} 0.6-1.0. After induction with 1 mM isopropyl-β-thiogalactoside (IPTG), the culture is grown for 4 hours at 30 °C and harvested

by centrifugation for 30 minutes at >10,000 g at 4 °C. Cell pellets are frozen at -80 °C. The cell pellet is resuspended in 25 ml of lysis buffer (20mM NaH_2P0_4, 0.5 M NaCl, lx Complete Protease Inhibitor Cocktail-EDTA free (Roche), ImM PMSF, pH 7.4) using an ULTRA-TURRAX® homogenizer (IKA works) on ice. Cell lysis is achieved by high pressure homogenization (> 18,000 psi) using a Model M-1 10S MICROFLUIDIZER® (Microfluidics). The soluble fraction is separated by centrifugation for 30 minutes at 23,300 g at 4 °C. The supernatant is clarified via 0.45 μm filter. The clarified lysate is loaded onto a Histrap column (GE) pre-equilibrated with the 20 mM sodium phosphate/500M NaCl pH 7.4. The column is then washed with 25 column volumes of the same buffer, followed by 20 column volumes of 20mM sodium phosphate/500 M NaCl/ 25 mM Imidazole, pH 7.4 and then 35 column volumes of 20mM sodium phosphate/500 M NaCl/40 mM Imidazole, pH 7.4. Protein is eluted with 15 column volumes of 20 mM sodium phosphate/500 M NaCl/500 mM Imidazole, pH 7.4, fractions are pooled based on absorbance at A_2so and dialyzed against lXPBS, 50 mM Tris, 150mM NaCl; pH 8.5 or 50 mM NaOAc; 150 mM NaCl; pH4.5. Any precipitate is removed by filtering at 0.22 μm.

VII. Compositions

[00204] The present invention further provides compositions, such as pharmaceutical compositions and radiopharmaceutical compositions, comprising an anti-PD-L1 Adnectin or fusion proteins thereof described herein, wherein the composition is essentially endotoxin free, or at least contain no more than acceptable levels of endotoxins as determined by the appropriate regulatory agency (e.g., FDA).

[00205] Compositions of the present invention can be in the form of a pill, tablet, capsule, liquid, or sustained release tablet for oral administration; a liquid for intravenous, subcutaneous or parenteral administration; or a gel, lotion, ointment, cream, or a polymer or other sustained release vehicle for local administration.

[00206] Methods well known in the art for making compositions are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A. R. Gennaro AR., 2000, Lippincott Williams & Wilkins, Philadelphia, Pa.). Compositions for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the

compounds. Nanoparticulate compositions (e.g., biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the compounds. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The concentration of the compound in the composition varies depending upon a number of factors, including the dosage of the drug to be administered, the route of administration, and the purpose of the composition (e.g., prophylactic, therapeutic, diagnostic).

[00207] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as Tween, PLURONIC™ or polyethylene glycol (PEG).

[00208] The polypeptides of the present invention may be optionally administered as a pharmaceutically acceptable salt, such as non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. In one example, the polypeptide is formulated in the presence of sodium acetate to increase thermal stability.

[00209] The active ingredients may also be entrapped in a microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin

microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[00210] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the proteins described herein, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. While encapsulated proteins described herein may remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S—S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[00211] Compositions of the present invention for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose and sorbitol), lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Compositions for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium.

[00212] The pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior

to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[00213] Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

[00214] The compositions herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

VIII. Biophysical and Biochemical Characterization

[00215] Binding of an anti-PD-L1 Adnectin described herein to PD-L1 may be assessed in terms of equilibrium constants (e.g., dissociation, K_D) and in terms of kinetic constants (e.g., on-rate constant, k_{on} and off-rate constant, k_{off}). An Adnectin will generally bind to a target molecule with a K_D of less than 500 nM, 100 nM, 10 nM, 1 nM, 500 pM, 200 pM, or 100 pM, although higher K_D values may be tolerated where the k_{off} is sufficiently low or the k_{on}, is sufficiently high.

In Vitro Assays for Binding Affinity

[00216] An Anti-PD-L1 Adnectin that binds to and antagonizes PD-L1 can be identified using various in vitro assays. In certain embodiments, the assays are high-throughput assays that allow for screening multiple candidate Adnectins simultaneously.

[00217] Exemplary assays for determining the binding affinity of an anti-PD-L1 Adnectin includes, but is not limited to, solution phase methods such as the kinetic exclusion assay (KinExA) (Blake et al., *JBC* 1996; 271:27677-85; Drake et al., *Anal Biochem* 2004; 328:35-43), surface plasmon resonance (SPR) with the Biacore system (Uppsala, Sweden) (Welford et al., *Opt. Quant. Elect* 1991; 23:1; Morton and Myszka, *Methods in Enzymology* 1998; 295:268) and homogeneous time resolved fluorescence (HTRF) assays (Newton et al., *J Biomol Screen* 2008; 13:674-82; Patel et al., *Assay Drug Dev Technol* 2008; 6:55-68).

[00218] In certain embodiments, biomolecular interactions can be monitored in real time with the Biacore system, which uses SPR to detect changes in the resonance angle of light at the surface of a thin gold film on a glass support due to changes in the refractive index of the surface up to 300 nm away. Biacore analysis generates association rate constants, dissociation rate constants, equilibrium dissociation constants, and affinity constants. Binding affinity is obtained by assessing the association and dissociation rate constants using a Biacore surface plasmon resonance system (Biacore, Inc.). A biosensor chip is activated for covalent coupling of the target. The target is then diluted and injected over the chip to obtain a signal in response units of immobilized material. Since the signal in resonance units (RU) is proportional to the mass of immobilized material, this represents a range of immobilized target densities on the matrix. Association and dissociation data are fit simultaneously in a global analysis to solve the net rate expression for a 1:1 bimolecular interaction, yielding best fit values for k_{on}, k_{off} and R_{max} (maximal response at saturation). Equilibrium dissociation constants for binding, K_D's are calculated from SPR measurements as k_{off}/k_{on}.

[00219] In some embodiments, the anti-PD-L1 Adnectins described herein exhibit a K_D in the SPR affinity assay described in Example 2 of 500 nM or less, 400 nM or less, 300 nM or less, 200 nM or less, 150 nM or less, 100 nM or less, 90 nM or less, 80 nM or less, 70 nM or less, 60 nM or less, 50 nM or less, 40 nM or less, 30 nM or less, 20 nM or less, 15 nM or less, 10 nM or less, 5 nM or less, or 1 nM or less.

[00220] It should be understood that the assays described herein above are exemplary, and that any method known in the art for determining the binding affinity between proteins (e.g., fluorescence based-transfer (FRET), enzyme-linked immunosorbent assay, and competitive binding assays (e.g., radioimmunoassays)) can be used to assess the binding affinities of the anti-PD-L1 Adnectins described herein.

IX. *In vivo* imaging with anti-PD-L1 Adnectins

Imaging Agents

[00221] The anti-PD-L1 Adnectins described herein also are useful in a variety of diagnostic and imaging applications. In certain embodiments, an anti-PD-L1 Adnectin is labelled with a moiety that is detectable *in vivo* and such labelled Adnectins may be used as *in vivo* imaging agents, e.g., for whole body imaging. For example, in one embodiment, a method for detecting a PD-L1 positive tumor in a subject comprises administering to the subject an anti-

PD-L1 Adnectin linked to a detectable label, and following an appropriate time, detecting the label in the subject.

[00222] An anti-PD-L1 Adnectin imaging agent may be used to diagnose a disorder or disease associated with increased levels of PD-L1, for example, a cancer in which a tumor selectively overexpresses PD-L1. In a similar manner, an anti-PD-L1 Adnectin can be used to monitor PD-L1 levels in a subject, e.g., a subject that is being treated to reduce PD-L1 levels and/or PD-L1 positive cells (e.g., tumor cells). The anti-PD-L1 Adnectins may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a detectable moiety.

[00223] Detectable moieties that may be used include radioactive agents, such as: radioactive heavy metals such as iron chelates, radioactive chelates of gadolinium or manganese, positron emitters of oxygen, nitrogen, iron, carbon, or gallium, ^{18}F, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{124}I, ^{86}Y, ^{89}Zr, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{44}Sc, ^{47}Sc, ^{11}C, ^{111}In, ^{114m}In, ^{114}In, ^{125}I, ^{124}I, ^{131}I, ^{123}I, ^{131}I, ^{123}I, ^{32}Cl, ^{33}Cl, ^{34}Cl, ^{74}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{78}Br, ^{89}Zr, ^{186}Re, ^{188}Re, ^{86}Y, ^{90}Y, ^{177}Lu, ^{99}Tc, ^{212}Bi, ^{213}Bi, ^{212}Pb, ^{225}Ac, or ^{153}Sm.

[00224] In certain embodiments, the radioactive agent is conjugated to the Adnectin at one or more amino acid residues. In certain embodiments, one or more, such as two or more, three or more, four or more, or a greater number of radionuclides can be present in the labelled probe. In certain embodiments, the radionuclide is attached directly to the Adnectin by a chelating agent (e.g., see U.S. Patent 8,808,665). In certain embodiments, the radionuclide is present in a prosthetic group conjugated to the Adnectin by a bifunctional chelator or conjugating (BFC) moieity. In certain embodiments, the radionuclide chelating agent and/or conjugating moiety is DFO, DOTA and its derivatives (CB-DO2A, 3p-C-DEPA, TCMC, Oxo-DO3A), DBCO, TE2A, CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, DM-TE2A, diamsar and derivatives, NODASA, NODAGA, NOTA, NETA, TACN-TM, DTPA, 1B4M-DTPA, CHX-A"-DTPA, TRAP (PRP9), NOPO, AAZTA and derivatives (DATA), H_2dedpa, H_4octapa, H_2azapa, H_5decapa, H_6phospa, HBED, SHBED, BPCA, CP256, PCTA, HEHA, PEPA, EDTA, TETA, and TRITA based chelating agents, and close analogs and derivatives thereof.

[00225] In certain embodiments, the radionuclide chelating or conjugating (BFC) moiety is maleamide-NODAGA or maleamide-DBCO, whichcan be attached covalently to a polypeptide via cysteine residues near the C-terminus of the polypeptide. In certain embodiments, an anti-PD-L1 Adnectin is modified at its C-terminus by the addition of a

cysteine. For example, PxCy may be linked C-terminal to the amino acid residues NYRT, wherein P is proline, C is cysteine, and x and y are integrers that are at least 1. Exemplary anti-PD-L1 Adnectins having the amino acid residues PC at their C-terminus are set forth in the Examples. Maleimide-NODAGA or maleimide-DBCO can be reacted with the cysteine, to yield Adnectin-NODAGA or Adnectin-DBCO, respectively.

[00226] In certain embodiments, the radionuclide chelating agent is DFO, which can be attached, e.g., at random surface lysines.

[00227] In certain embodiments, the chelator for ^{64}Cu is DOTA, NOTA, EDTA, Df, DTPA, or TETA. Suitable combinations of chelating agents and radionuclides are extensively reviewed in Price et al., *Chem Soc Rev* 2014;43:260-90.

[00228] In certain embodiments, an anti-PD-L1 Adnectin is labelled with the PET tracer ^{18}F. ^{18}F is an attractive PET radionuclide with a 1.8 hour radioactive half life, which provides a same day imaging tool, where the PET radionuclide better matches the Adnectin's biological half-life, resulting in excellent images with less radiation exposure to the patient. A PD-L1 Adnectin may be labelled with a prosthetic group, such as [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine ([^{18}F]-FFPEGA), as further described in the Examples. As further shown in the Examples, an ^{18}F-labelled anti-PD-L1 Adnectin specifically and efficiently labelled human PD-L1 positive tumors in mice and PD-L1 positive tumors in cynomolgus monkeys. Specific details on the labelling method is provided below and in the Examples.

[00229] In certain embodiments, a PD-L1 imaging agent is an anti-PD-L1 Adnectin that is labelled with ^{64}Cu, e.g., as described in the Examples. ^{64}Cu may be linked to an Adnectin with a chelating agent, such as NODAGA. As further shown in the Examples, a ^{64}Cu -labelled anti-PD-L1 Adnectin specifically and efficiently labelled human PD-L1 positive tumors in mice and PD-L1 positive tumors in cyno.

[00230] Other art-recognized methods for labelling polypeptides with radionuclides such as ^{64}Cu and ^{18}F for synthesizing the anti-PD-L1 Adnectin-based imaging agents described herein may also be used. See, e.g., US2014/0271467; Gill et al., *Nature Protocols* 2011;6:1718-25; Berndt et al. *Nuclear Medicine and Biology* 2007;34:5-15, Inkster et al., *Bioorganic & Medicinal Chemistry Letters* 2013;23:3920-6, the contents of which are herein incorporated by reference in their entirety.

[00231] In certain embodiments, a PD-L1 imaging agent comprises a PEG molecule (e.g., 5KDa PEG, 6KDa PEG, 7KDa PEG, 8KDa PEG, 9KDa PEG, or 10KDa PEG) to increase the blood PK of the imaging agent by small increments to enhance the imaging contrast or increase avidity of the anti-PD-L1 Adnectin based imaging agent.

Administration and Imaging

[00232] In certain embodiments, the labeled anti-PD-L1 Adnectins can be used to image PD-L1-positive cells or tissues, e.g., PD-L1 expressing tumors. For example, the labeled anti-PD-L1 Adnectin is administered to a subject in an amount sufficient to uptake the labeled Adnectin into the tissue of interest (e.g., the PD-L1-expressing tumor). The subject is then imaged using an imaging system such as PET for an amount of time appropriate for the particular radionuclide being used. The labeled anti-PD-L1 Adnectin-bound PD-L1-expressing cells or tissues, e.g., PD-L1-expressing tumors, are then detected by the imaging system.

[00233] PET imaging with a PD-L1 imaging agent may be used to qualitatively or quantitatively detect PD-L1. A PD-L1 imaging agent may be used as a biomarker, and the presence or absence of a PD-L1 positive signal in a subject may be indicative that, e.g., the subject would be responsive to a given therapy, e.g., a cancer therapy, or that the subject is responding or not to a therapy.

[00234] In certain embodiments, the progression or regression of disease (e.g., tumor) can be imaged as a function of time or treatment. For instance, the size of the tumor can be monitored in a subject undergoing cancer therapy (e.g., chemotherapy, radiotherapy) and the extent of regression of the tumor can be monitored in real-time based on detection of the labeled anti-PD-L1 Adnectin.

[00235] The amount effective to result in uptake of the imaging agent (e.g., ^{18}F-Adnectin imaging agent, ^{64}Cu-Adnectin imaging agent) into the cells or tissue of interest (e.g., tumors) may depend upon a variety of factors, including for example, the age, body weight, general health, sex, and diet of the host; the time of administration; the route of administration; the rate of excretion of the specific probe employed; the duration of the treatment; the existence of other drugs used in combination or coincidental with the specific composition employed; and other factors.

[00236] In certain embodiments, imaging of tissues expressing PD-L1 is effected before, during, and after administration of the labeled anti-PD-L1 Adnectin.

[00237] In certain embodiments, the subject is a mammal, for example, a human, dog, cat, ape, monkey, rat, or mouse.

[00238] In certain embodiments, the anti-PD-L1 Adnectins described herein are useful for PET imaging of lungs, heart, kidneys, liver, and skin, and other organs, or tumors associated with these organs which express PD-L1.

[00239] In certain embodiments, the anti-PD-L1 imaging agents provide a contrast of at least 50%, 75%, 2, 3, 4, 5 or more. The Examples show that all anti-PD-L1 Adnectins that were used provided a PET contrast of 2 or more, and that the affinity of the Adnectins was not important.

[00240] When used for imaging (e.g., PET) with short half-life radionuclides (e.g., ^{18}F), the radiolabeled anti-PD-L1 Adnectins are preferably administrered intravenously. Other routes of administration are also suitable and depend on the half-life of the radionuclides used.

[00241] In certain embodiments, the anti-PD-L1 imaging agents described herein are used to detect PD-L1 positive cells in a subject by administering to the subject an anti-PD-L1 imaging agent disclosed herein, and detecting the imaging agent, the detected imaging agent defining the location of the PD-L1 positive cells in the subject. In certain embodiments, the imaging agent is detected by positron emission tomography.

[00242] In certain embodiments, the anti-PD-L1 imaging agents described herein are used to detect PD-L1 expressing tumors in a subject by administering to the subject an anti-PD-L1 imaging agent disclosed herein, and detecting the imaging agent, the detected imaging agent defining the location of the tumor in the subject. In certain embodiments, the imaging agent is detected by positron emission tomography.

[00243] In certain embodiments, an image of an anti-PD-L1 imaging agent described herein is obtained by administering the imaging agent to a subject and imaging in vivo the distribution of the imaging agent by positron emission tomography.

[00244] Disclosed herein are methods of obtaining a quantitative image of tissues or cells expressing PD-L1, the method comprising contacting the cells or tissue with an anti-PD-L1 imaging agent described herein and detecting or quantifying the tissue expressing PD-L1 using positron emission tomography.

[00245] Also disclosed herein are methods of detecting a PD-L1-expressing tumor comprising administering an imaging-effective amount of an anti-PD-L1 imaging agent described herein to a subject having a PD-L1-expressing tumor, and detecting the radioactive

emissions of said imaging agent in the tumor using positron emission tomography, wherein the radioactive emissions are detected in the tumor.

[00246] Also disclosed herein are methods of diagnosing the presence of a PD-L1-expressing tumor in a subject, the method comprising

(a) administering to a subject in need thereof an anti-PD-L1 imaging agent described herein; and

(b) obtaining an radio-image of at least a portion of the subject to detect the presence or absence of the imaging agent;

wherein the presence and location of the imaging agent above background is indicative of the presence and location of the disease.

[00247] Also disclosed herein are methods of monitoring the progress of an anti-tumor therapy against PD-L1-expressing tumors in a subject, the method comprising

(a) administering to a subject in need thereof an anti-PD-L1 imaging agent described herein at a first time point and obtaining an image of at least a portion of the subject to determine the size of the tumor;

(b) administering an anti-tumor therapy to the subject;

(c) administering to the subject the imaging agent at one or more subsequent time points and obtaining an image of at least a portion of the subject at each time point;

wherein the dimension and location of the tumor at each time point is indicative of the progress of the disease.

PET Imaging

[00248] Typically, for PET imaging purposes it is desirable to provide the recipient with a dosage of Adnectin that is in the range of from about 1 mg to 200 mg as a single intravenous infusion, although a lower or higher dosage also may be administered as circumstances dictate. Typically, it is desirable to provide the recipient with a dosage that is in the range of from about 1 mg to 10 mg per square meter of body surface area of the protein or peptide for the typical adult, although a lower or higher dosage also may be administered as circumstances dictate. Examples of dosages proteins or peptides that may be administered to a human subject for imaging purposes are about 1 to 200 mg, about 1 to 70 mg, about 1 to 20 mg, and about 1 to 10 mg, although higher or lower doses may be used.

[00249] In certain embodiments, administration occurs in an amount of radiolabeled Adnectin of between about 0.005μg/kg of body weight to about 50μg/kg of body weight per day, usually between 0.02μg/kg of body weight to about 3μg/kg of body weight. The mass

associated with a PET tracer is in the form of the natural isotope (e.g., ^{19}F for a ^{18}F PET tracer). A particular analytical dosage for the instant composition includes from about 0.5μg to about 100μg of a radiolabeled protein. The dosage will usually be from about 1μg to about 50μg of a radiolabeled protein.

[00250] Dosage regimens are adjusted to provide the optimum detectable amount for obtaining a clear image of the tissue or cells which uptake the radiolabeled Adnectin. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to which the radiolabeled Adnectin is to be administered. The specification for the dosage unit forms described herein are dictated by and directly dependent on (a) the unique characteristics of the targeting portion of the radiolabeled Adnectin; (b) the tissue or cells to be targeted; (c) the limitations inherent in the imaging technology used.

[00251] For administration of the radiolabeled Adnectin, the dosage used will depend upon the disease type, targeting compound used, the age, physical condition, and gender of the subject, the degree of the disease, the site to be examined, and others. In particular, sufficient care has to be taken about exposure doses to a subject. Preferably, a saturating dose of radiolabel (*e.g.,* ^{18}F or ^{64}Cu) is administered to the patient. For example, the amount of radioactivity of ^{18}F-labeled Adnectin usually ranges from 3.7 megabecquerels to 3.7 gigabecquerels, and preferably from 18 megabecquerels to 740 megabecquerels. Alternatively, the dosage may be measured by millicuries, for example. In some embodiments, the amount of ^{18}F imaging administered for imaging studies is 5 to 10 mCi. In some embodiments, an effective amount will be the amount of compound sufficient to produce emissions in the range of from about 1-5 mCi.

[00252] Actual dosage levels of the active ingredients in the pharmaceutical compositions described herein may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired uptake of the radiolabeled Adnectin in the cells or tissues of a particular patient, composition, and mode of administration, without being toxic to the patient. It will be understood, however, that the total daily usage of the radiolabeled Adnectin of the present disclosure will be decided by the attending physician or other attending professional within the scope of sound medical judgment. The specific effective dose level for any particular subject will depend upon a variety of factors, including for example, the activity of the specific composition employed; the specific composition

employed; the age, body weight, general health, sex, and diet of the host; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. In certain embodiments, the amount of radiolabeled Adnectin administered into a human subject required for imaging will be determined by the prescribing physician with the dosage generally varying according to the quantity of emission from the radionuclide.

[00253] A composition described herein can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for radiolabeled Adnectin described herein include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[00254] Alternatively, a radiolabeled Adnectin described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[00255] In certain embodiments, the radiolabeled Adnectin described herein can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. Agents may cross the BBB by formulating them, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies

(P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994).

Exemplary PET procedure

[00256] The following illustrative procedure may be utilized when performing PET imaging studies on patients in the clinic. A 20 G two-inch venous catheter is inserted into the contralateral ulnar vein for radiotracer administration. Administration of the PET tracer is often timed to coincide with time of maximum (T max) or minimum (T min) of the anti-PD-L1 Adnectin concentration in the blood.

[00257] The patient is positioned in the PET camera and a tracer dose of the PET tracer of radiolabeled anti-PD-L1 Adnectin such as [^{18}F]-ADX_5417_E01 (<20 mCi) is administered via i.v. catheter. Either arterial or venous blood samples are taken at 15 appropriate time intervals throughout the PET scan in order to analyze and quantitate the fraction of unmetabolized PET tracer of [^{18}F]-ADX_5417_E01 in plasma. Images are acquired for up to 120 min. Within ten minutes of the injection of radiotracer and at the end of the imaging session, 1 ml blood samples are obtained for determining the plasma concentration of any unlabeled anti-PD-L1 Adnectin which may have been administered before the PET tracer.

[00258] Tomographic images are obtained through image reconstruction. For determining the distribution of radiotracer, regions of interest (ROIs) are drawn on the reconstructed image including, but not limited to, the lungs, liver, heart, kidney, skin, or other organs and tissue (e.g., cancer tissue). Radiotracer uptakes over time in these regions are used to generate time activity curves (TAC) obtained in the absence of any intervention or in the presence of the unlabeled anti-PD-L1 Adnectin at the various dosing paradigms examined. Data are expressed as radioactivity per unit time per unit volume (μci/cc/mCi injected dose).

X. Detection of PD-L1 with anti-PD-L1 Adnectins

[00259] In addition to detecting PD-L1 in vivo, anti-PDL1 Adnectins, such as those described herein, may be used for detecting a target molecule in a sample. A method may comprise contacting the sample with an anti-PD-L1 Adnectins described herein, wherein said contacting is carried out under conditions that allow anti-PD-L1 Adnectin-target complex formation; and detecting said complex, thereby detecting said target in said sample. Detection may be carried out using any art-recognized technique, such as, e.g., radiography,

immunological assay, fluorescence detection, mass spectroscopy, or surface plasmon resonance. The sample may be from a human or other mammal. For diagnostic purposes, appropriate agents are detectable labels that include radioisotopes, for whole body imaging, and radioisotopes, enzymes, fluorescent labels and other suitable antibody tags for sample testing.

[00260] The detectable labels can be any of the various types used currently in the field of *in vitro* diagnostics, including particulate labels including metal sols such as colloidal gold, isotopes such as I^{125} or Tc^{99} presented for instance with a peptidic chelating agent of the N_2S_2, N_3S or N_4 type, chromophores including fluorescent markers, biotin, luminescent markers, phosphorescent markers and the like, as well as enzyme labels that convert a given substrate to a detectable marker, and polynucleotide tags that are revealed following amplification such as by polymerase chain reaction. A biotinylated antibody would then be detectable by avidin or streptavidin binding. Suitable enzyme labels include horseradish peroxidase, alkaline phosphatase and the like. For instance, the label can be the enzyme alkaline phosphatase, detected by measuring the presence or formation of chemiluminescence following conversion of 1,2 dioxetane substrates such as adamantyl methoxy phosphoryloxy phenyl dioxetane (AMPPD), disodium 3-(4-(methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo{3.3.1.1 3,7}decan}-4-yl) phenyl phosphate (CSPD), as well as CDP and CDP-star® or other luminescent substrates well-known to those in the art, for example the chelates of suitable lanthanides such as Terbium(III) and Europium(III). Other labels include those set forth above in the imaging section. The detection means is determined by the chosen label. Appearance of the label or its reaction products can be achieved using the naked eye, in the case where the label is particulate and accumulates at appropriate levels, or using instruments such as a spectrophotometer, a luminometer, a fluorimeter, and the like, all in accordance with standard practice.

[00261] In certain embodiments, conjugation methods result in linkages which are substantially (or nearly) non-immunogenic, e.g., peptide- (i.e. amide-), sulfide-, (sterically hindered), disulfide-, hydrazone-, and ether linkages. These linkages are nearly non-immunogenic and show reasonable stability within serum (see e.g. Senter, P. D., Curr. Opin. Chem. Biol. 13 (2009) 235-244; WO 2009/059278; WO 95/17886).

[00262] Depending on the biochemical nature of the moiety and Adnectin, different conjugation strategies can be employed. In case the moiety is naturally occurring or recombinant polypeptide of between 50 to 500 amino acids, there are standard procedures in

textbooks describing the chemistry for synthesis of protein conjugates, which can be easily followed by the skilled artisan (see e.g. Hackenberger, C. P. R., and Schwarzer, D., Angew. Chem. Int. Ed. Engl. 47 (2008) 10030-10074). In one embodiment the reaction of a maleinimido moiety with a cysteine residue within the Adnectin or the moiety is used. Alternatively, coupling to the C-terminal end of the Adnectin is performed. C-terminal modification of a protein can be performed as described in, e.g., Sunbul, M. and Yin, J., Org. Biomol. Chem. 7 (2009) 3361-3371). When the moiety is a peptide or polypeptide, the Adnectin and moiety can be fused by standard genetic fusion, optionally with a linker disclosed herein.

[00263] In general, site specific reaction and covalent coupling is based on transforming a natural amino acid into an amino acid with a reactivity which is orthogonal to the reactivity of the other functional groups present. For example, a specific cysteine within a rare sequence context can be enzymatically converted in an aldehyde (see Frese, M. A., and Dierks, T., ChemBioChem. 10 (2009) 425-427). It is also possible to obtain a desired amino acid modification by utilizing the specific enzymatic reactivity of certain enzymes with a natural amino acid in a given sequence context (see, e.g., Taki, M. et al., Prot. Eng. Des. Sel. 17 (2004) 119-126; Gautier, A. et al. Chem. Biol. 15 (2008) 128-136. Protease-catalyzed formation of C--N bonds is described at Bordusa, F., Highlights in Bioorganic Chemistry (2004) 389-403.

[00264] Site specific reaction and covalent coupling can also be achieved by the selective reaction of terminal amino acids with appropriate modifying reagents. The reactivity of an N-terminal cysteine with benzonitrils (see Ren, H. et al., Angew. Chem. Int. Ed. Engl. 48 (2009) 9658-9662) can be used to achieve a site-specific covalent coupling. Native chemical ligation can also rely on C-terminal cysteine residues (Taylor, E. Vogel; Imperiali, B, Nucleic Acids and Molecular Biology (2009), 22 (Protein Engineering), 65-96). EP 1 074 563 describes a conjugation method which is based on the faster reaction of a cysteine within a stretch of negatively charged amino acids than a cysteine located in a stretch of positively charged amino acids.

[00265] The moiety may also be a synthetic peptide or peptide mimic. In case a polypeptide is chemically synthesized, amino acids with orthogonal chemical reactivity can be incorporated during such synthesis (see e.g. de Graaf, A. J. et al., Bioconjug. Chem. 20 (2009) 1281-1295). Since a great variety of orthogonal functional groups is at stake and can

be introduced into a synthetic peptide, conjugation of such peptide to a linker is standard chemistry.

[00266] In order to obtain a mono-labeled polypeptide the conjugate with 1:1 stoichiometry may be separated by chromatography from other conjugation side-products. This procedure can be facilitated by using a dye labeled binding pair member and a charged linker. By using this kind of labeled and highly negatively charged binding pair member, mono conjugated polypeptides are easily separated from non-labeled polypeptides and polypeptides which carry more than one linker, since the difference in charge and molecular weight can be used for separation. The fluorescent dye can be useful for purifying the complex from un-bound components, like a labeled monovalent binder.

XI. Synthesis of ^{18}F-labeled anti-PD-L1 Adnectins

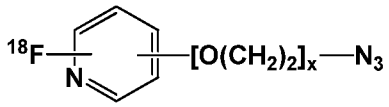
[00267] ^{18}F-labeled anti-PD-L1 Adnectins may be synthesized by first preparing an ^{18}F radiolabeled prosthetic group, linking an Adnectin to a bifunctional chelating agent, and then combining these two reagents (see, e.g., Figure 9).

^{18}F Radiolabeled Prosthetic Groups

[00268] In one aspect, provided herein is an ^{18}F-radiolabeled compound containing a prosthetic group for use in a bioorthogonal reaction involving 1,3-dipolar cycloaddition between an azide and a cyclooctyne which proceeds selectively under water tolerant conditions. The ^{18}F-radiolabeled prosthetic groups disclosed herein are soluble in 100% aqueous, and there is no need for an organic phase to link the prosthetic groups to the anti-PD-L1 Adnectins disclosed herein. This feature is particularly advantageous as there is no need for an organic phase to link the prosthetic group to the anti-PD-L1 Adnectins, which cannot withstand even small amounts of organic solvents, given degradation and aggregation issues.

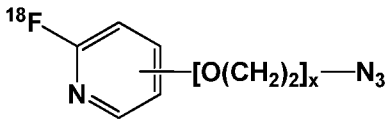
[00269] Additionally, unlike aliphatic prosthetic groups, the ^{18}F fluorination reaction can be monitored with UV, and the ^{18}F-radiolabeled prosthetic groups described herein are not volatile. Moreover, the ^{18}F-radiolabeled prosthetic groups can be incorporated into the anti-PD-L1 Adnectins using a copper free click chemistry, *e.g.,* as described in the Examples, thus avoiding the stability issues observed in some biologics when copper mediated click chemistry is used.

[00270] In one aspect, provided herein is a PEGylated ^{18}F-pyridine covalently bound to an azide with the following structure,



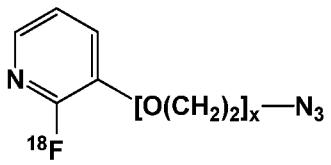
wherein x is an integer from 1 to 8. In certain embodiments, x is an integer from 2 to 6. In some embodiments x is an integer from 3 to 5. In certain embodiments, x is 4. In certain embodiments, ^{18}F is attached to the pyridine ortho to the N atom. In certain embodiments, the $[O(CH_2)_2]_x$ moiety is present in the 1-3 configuration relative to the nitrogen on the pyridine ring. In certain embodiments, the $[O(CH_2)_2]_x$ moiety is present in the 1-2 configuration relative to the nitrogen on the pyridine ring. In certain embodiments, the $[O(CH_2)_2]_x$ moiety is present in the 1-4 configuration relative to the nitrogen on the pyridine ring.

[00271] In certain embodiments, the ^{18}F-radiolabeled compound has the structure



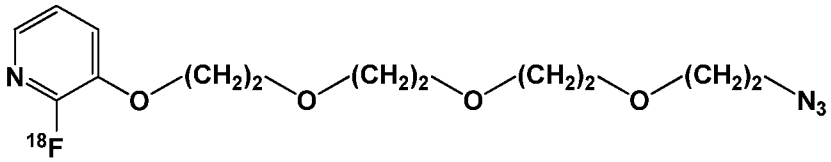
wherein x is an integer from 1 to 8. In certain embodiments, x is an integer from 2 to 6. In some embodiments x is an integer from 3 to 5. In certain embodiments, x is 4.

[00272] In certain embodiments, the ^{18}F-radiolabeled compound has the structure



wherein x is an integer from 1 to 8. In certain embodiments, x is an integer from 2 to 6. In certain embodiments x is an integer from 3 to 5. In certain embodiments, x is 4.

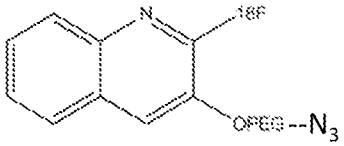
[00273] In certain embodiments, the ^{18}F-radiolabeled compound is [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine (^{18}F-FPPEGA) and has the structure



[00274] In certain embodiments, the ^{18}F-radiolabeled prosthetic group may contain additional groups on the pyridine ring which do not interfere with the fluorination reaction. In certain

embodiments, additions to the pyridine ring include C_{1-6} alkyl groups, for example methyl, ethyl and propyl.

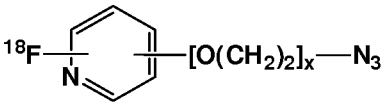
[00275] In certain embodiments, the ^{18}F-radiolabeled prosthetic group is a fused ring system with the following structure:



wherein "OPEG" is $[O(CH_2)_2]_x$, and x is an integer from 1 to 8. In certain embodiments, x is an integer from 2 to 6. In certain embodiments x is an integer from 3 to 5. In certain embodiments, x is 4.

[00276] The ^{18}F-radiolabeled prosthetic groups described herein may be produced using chemical reactions described in the Examples herein.

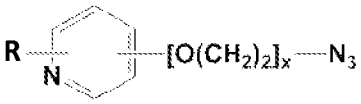
[00277] Also provided herein is a method of preparing a PEGylated ^{18}F-pyridine covalently bound to an azide with the following structure,

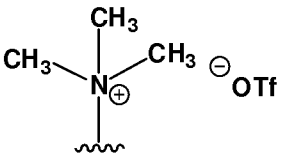


wherein x is an integer from 1 to 8, the method comprising the steps of

(a) providing a solution of a compound **a** with the following structure:

a



wherein x is an integer from 1 to 8, and R is NO_2, Br, F or  and is ortho to the N atom of the pyridine ring;

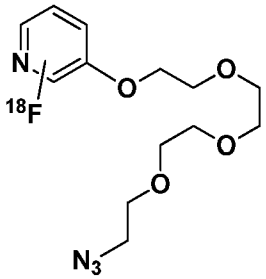
(b) providing a mixture of ^{18}F in ^{18}Owater, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane and a weak base;

c) drying the mixture from step b) to form a solid; and

d) reacting the solution from step a) with the solid from step c) to form the ^{18}F-labeled compound.

[00278] In certain embodiments, the method produces a ^{18}F-pyridine prosthetic group with the following structure **b**

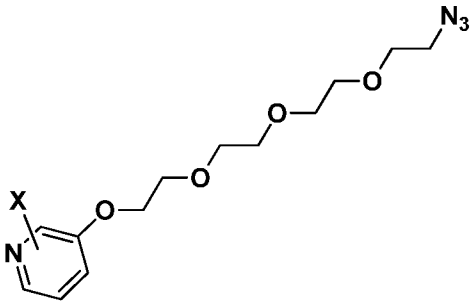
b

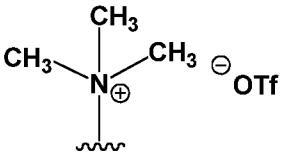


(where ^{18}F is ortho to the N atom), and includes the steps of

a) providing a solution of the compound of the structure

c



(where X is ortho to the N atom) where X is NO_2, Br or 

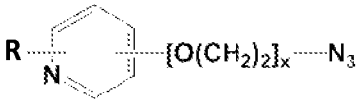
;

b) providing a mixture of ^{18}F in ^{18}Owater, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane and weak base, such as K_2CO_3;

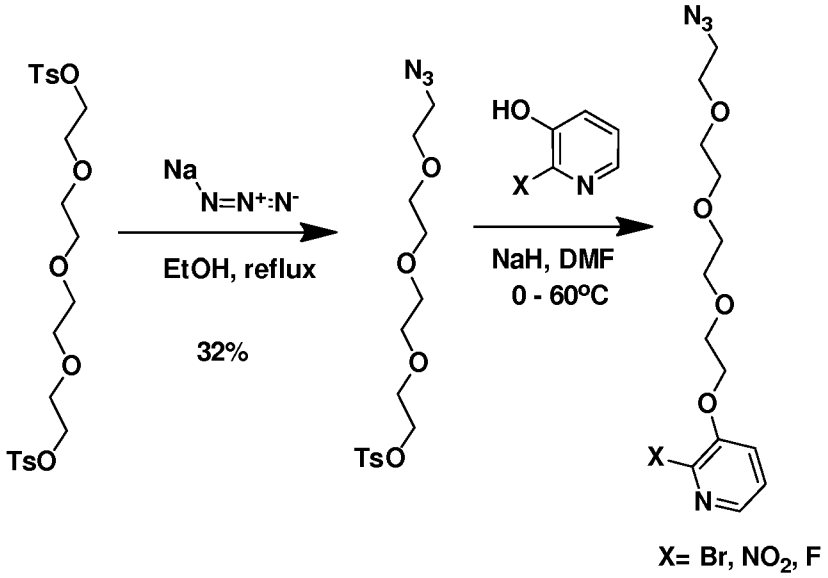
c) drying the mixture from step b) to form a solid; and

d) reacting the solution from step a) with the solid from step c) to form the ^{18}F-labeled compound.

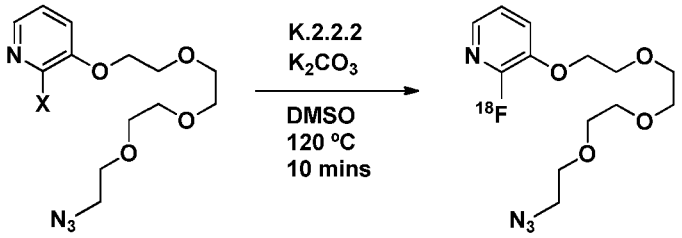
[00279] In certain embodiments, the method further comprises the step of producing a compound with the following structure **a**



according to the Scheme I shown below:



[00280] In certain embodiments, the method comprises producing ^{18}F-pyridine prosthetic group is [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine (^{18}F-FPPEGA), **e,** from **d** according to the following reaction conditions:

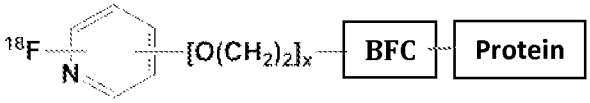


d **e**

([^{18}F]-FPPEGA)

^{18}F-radiolabeled PD-L1 Adnectins

[00281] In some aspects, provided herein are ^{18}F-radiolabeled probes or agents with the following structure,



wherein the Protein is a PD-L1 Adnectin and x is an integer from 1 to 8. In certain embodiments, x is an integer from 2 to 6. In certain embodiments x is an integer from 3 to 5. In some embodiments, x is 4.

BFC

[00282] Bifunctional chelating or conjugating (BFC) moietieswhich can be used in the ^{18}F-radiolabled compositions disclosed herein are commercially available (*e.g.,* Sigma Aldrich; Click Chemistry Tools), or may be synthesized according to well-known chemical reactions.

[00283] In certain embodiments, the BFC is selected from cyclooctyne based chelating agents (e.g., DBCO, DIBO), DFO, DOTA and its derivatives (CB-DO2A, 3p-C-DEPA, TCMC, Oxo-DO3A), TE2A, CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, DM-TE2A, diamsar and derivatives, NODASA, NODAGA, NOTA, NETA, TACN-TM, DTPA, 1B4M-DTPA, CHX-A"-DTPA, TRAP (PRP9), NOPO, AAZTA and derivatives (DATA), H_2dedpa, H_4octapa, H_2azapa, H_5decapa, H_6phospa, HBED, SHBED, BPCA, CP256, PCTA, HEHA, PEPA, EDTA, TETA, and TRITA based chelating agents, and close analogs and derivatives thereof. Suitable combinations of chelating agents and radionuclides are extensively described in Price et al., *Chem Soc Rev* 2014;43:260-90.

[00284] In certain embodiments, the BFC is a cyclooctyne comprising a reactive group that forms a covalent bond with an amine, carboxyl, carbonyl or thiol functional group on the targeting protein or peptide. Reactive groups on the cyclooctyne include esters, acids, hydroxyl groups, aminooxy groups, malaiemides, α-halogenketones and α-halogenacetamides.

[00285] In certain embodiments, the BFC is a cyclooctyne is dibenzocyclooctyne (DIBO), biarylazacyclooctynone (BARAC), dimethoxyazacyclooctyne (DIMAC) and dibenzocyclooctyne (DBCO). In certain embodiments, the cyclootyne is DBCO.

[00286] In certain embodiments, the cyclooctyne comprises a hydrophilic polyethylene glycol $(PEG)_y$ spacer arm, wherein y is an integer from 1 to 8. In certain embodiments, y is an integer from 2 to 6. In certain embodiments, y is 4 or 5.

[00287] In certain embodiments, the BFC is DBCO-PEG4-NHS-Ester or DBCO-*Sulfo*-NHS-Ester which react specifically and efficiently with a primary amine (e.g., side chain of lysine residues or aminosilane-coated surfaces). In certain embodiments, the BFC is DBCO-PEG4-Acid with terminal carboxylic acid (–COOH) that can be reacted with primary or secondary amine groups in the presence activators (e.g. EDC) forming a stable amide bond. In certain

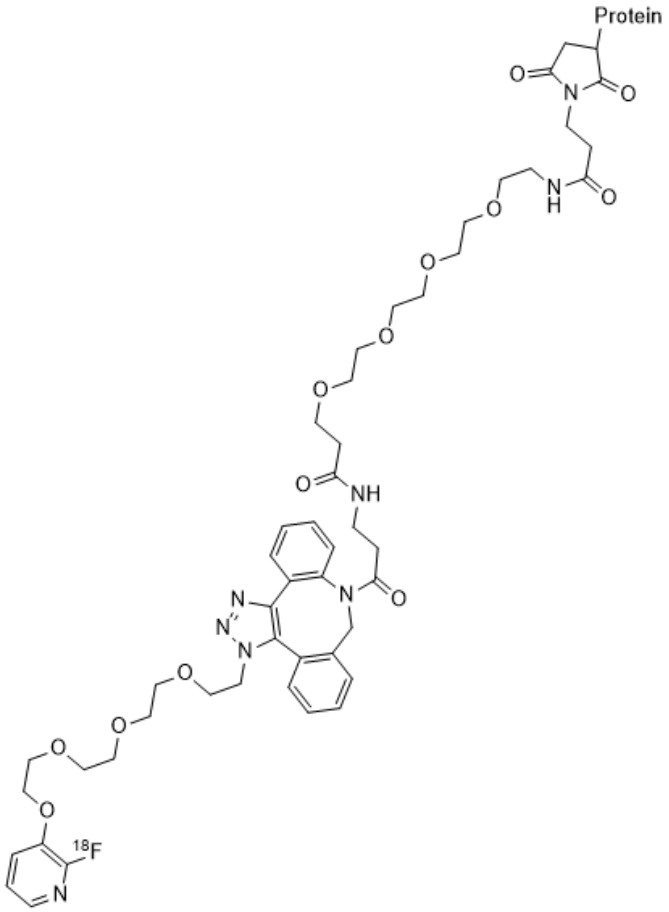
embodiments, the BFC is DBCO-PEG4-Amine which reacts with carboxyl groups in the presence of activators (e.g. EDC, or DCC) or with activated esters (e.g. NHS esters) forming stable amide bonds.

[00288] In certain embodiments, the BFC is DBCO-PEG4-Maleimide which reacts with sulfhydryl groups on cysteine residues, e.g., at or near the C-terminus of the polypeptide.

[00289] In certain embodiments, the polypeptide is modified at its C-terminus by the addition of a cysteine. For example, P_mC_n may be linked to the C-terminal amino acid residue of the polypeptide, wherein P is proline, C is cysteine, m is an integer that at least 0 and n is an integer that is at least 1. Methods for making such modifications are well-known in the art.

[00290] In certain embodiments, the ^{18}F-radiolabeled probe or agent has the following structure **a**,

a



wherein the BFC is conjugated to the protein (e.g., an anti-PD-L1 Adnectin) at a cysteine residue.

[00291] The ^{18}F-radiolabeled targeting agents described herein are produced using bioorthogonal, metal free click chemistry in medium suitable for direct use *in vivo* (e.g., saline) according to the procedures described herein.

XII. Therapeutic Methods

Immunotherapy of cancer patients using an anti-PD-Ll Adnectin

[00292] PD-L1 is the primary PD-1 ligand up-regulated within solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1-positive, tumor-infiltrating $CD4^+$ and $CD8^+$ T-cells, respectively (Dong et al, 2002; Hino et al, 2010; Taube et al, 2012). These properties make PD-L1 a promising target for cancer immunotherapy. For example, clinical trials with anti-PD-L1 immunotherapy, as described in WO2013/173223, which is herein incorporated by reference in its entirety, demonstrate that mAb blockade of the immune inhibitory ligand, PD-L1, produces both durable tumor regression and prolonged (> 24 weeks) disease stabilization in patients with metastatic NSCLC, MEL, RCC and OV, including those with extensive prior therapy. Accordingly, targeting PD-L1, e.g., using the PD-L1 Adnectins, are suitable for eliciting an anti-tumor response.

[00293] Based on the clinical data disclosed in WO2013/173223, described herein are methods for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of a PD-L1 Adnectin described herein. The disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a PD-L1 Adnectin described herein. In certain embodiments, the subject is a human.

[00294] Detailed methods for treating a subject with cancer by targeting PD-L1 are described in WO2013/173223.

[00295] In certain embodiments, the PD-L1 Adnectins described herein, which are suitable for use in the methods described herein, have one or more of the following features: high affinity for human PD-L1, increases T-cell proliferation, increases IL-2 secretion, e.g., from T cells, increases interferon-γ production, e.g., from T cells, inhibits the binding of PD-L1 to PD-1, and reverses the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells.

Adnectin drug conjugates (Adnectin-DCs)

[00296] Adnectins described herein may be conjugated through a cysteine, e.g., a C-terminal cysteine, to a therapeutic agent to form an immunoconjugate such as an Adnectin-drug conjugate ("Adnectin-DC").

[00297] In an Adnectin-DC, the Adnectin is conjugated to a drug, with the Adnectin-DC functioning as a targeting agent for directing the Adnectin to a target cell expressing its antigen, such as a cancer cell. Preferably, the antigen is a tumor associated antigen, *i.e.*, one that is uniquely expressed or over-expressed by the cancer cell. Once there, the drug is released, either inside the target cell or in its vicinity, to act as a therapeutic agent. For a review on the mechanism of action and use of drug conjugates as used with antibodies, e.g., in cancer therapy, see Schrama *et al.*, *Nature Rev. Drug Disc.* 2006, 5, 147.

[00298] Suitable therapeutic agents for use in drug conjugates include antimetabolites, alkylating agents, DNA minor groove binders, DNA intercalators, DNA crosslinkers, histone deacetylase inhibitors, nuclear export inhibitors, proteasome inhibitors, topoisomerase I or II inhibitors, heat shock protein inhibitors, tyrosine kinase inhibitors, antibiotics, and anti-mitotic agents. In an Adnectin-DC, the Adnectin and therapeutic agent preferably are conjugated via a linker cleavable such as a peptidyl, disulfide, or hydrazone linker. More preferably, the linker is a peptidyl linker such as Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val (SEQ ID NO: 169), Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, or Glu. The Adnectin-DCs can be prepared according to methods similar to those described in U.S. Pat. Nos. 7,087,600; 6,989,452; and 7,129,261; PCT Publications WO 02/096910; WO 07/038658; WO 07/051081; WO 07/059404; WO 08/083312; and WO 08/103693; U.S. Patent Publications 20060024317; 20060004081; and 20060247295; the disclosures of which are incorporated herein by reference. A linker can itself be linked, e.g., covalently linked, e.g., using maleimide chemistry, to a cysteine of the polypeptide, e.g., a cysteine at or near the C-terminus of the polypeptide, e.g., a cysteine of a P_mC_n moiety that is attached to the C-terminal amino acid residue of the polypeptide, wherein m is an integer that is at least 0 (e.g., 0, 1 or 2) and n is an integer that is at least 1 (e.g., 1 or 2). For example, a linker can be covalently linked to a cysteine, such as a cysteine in the C-terminal region of an Adnectin, e.g., a C-terminal cysteine in PC of an Adnectin-DC-P_mC_n, wherein m and n are independently an integer that is at least one. For example, a linker can be linked to an Adnectin-DC-PmCn, wherein P is a proline, C is a cysteine, and m and n are integers that are at least 1, e.g., 1-3. In certain embodiments, m is zero, i.e., the cysteine is not preceded by a

proline. Ligation to a cysteine can be performed as known in the art using maleimide chemistry (e.g., Taylor, E. Vogel; Imperiali, B, Nucleic Acids and Molecular Biology (2009), 22 (Protein Engineering), 65-96). For attaching a linker to a cysteine on an Adnectin, the linker may, e.g. comprise a maleinimido moiety, which moiety then reacts with the cysteine to form a covalent bond. In certain embodiments, the amino acids surrounding the cysteine are optimized to facilitate the chemical reaction. For example, a cysteine may be surrounded by negatively charged amino acid for a faster reaction relative to a cysteine that is surrounded by a stretch of positively charged amino acids (EP 1 074 563).

[00299] For cancer treatment, the drug preferably is a cytotoxic drug that causes death of the targeted cancer cell. Cytotoxic drugs that can be used in Adnectin-DCs include the following types of compounds and their analogs and derivatives:

(a) enediynes such as calicheamicin (see, *e.g.*, Lee *et al.*, *J. Am. Chem. Soc.* 1987, 109, 3464 and 3466) and uncialamycin (see, *e.g.*, Davies *et al.*, WO 2007/038868 A2 (2007) and Chowdari *et al.*, US 8,709,431 B2 (2012));

(b) tubulysins (see, *e.g.*, Domling *et al.*, US 7,778,814 B2 (2010); Cheng *et al.*, US 8,394,922 B2 (2013); and Cong *et al.*, US 2014/0227295 A1;

(c) CC-1065 and duocarmycin (see, *e.g.*, Boger, US 6,5458,530 B1 (2003); Sufi *et al.*, US 8,461,117 B2 (2013); and Zhang *et al.*, US 2012/0301490 A1 (2012));

(d) epothilones (see, *e.g.*, Vite *et al.*, US 2007/0275904 A1 (2007) and US RE42930 E (2011));

(e) auristatins (see, *e.g.*, Senter *et al.*, US 6,844,869 B2 (2005) and Doronina *et al.*, US 7,498,298 B2 (2009));

(f) pyrrolobezodiazepine (PBD) dimers (see, *e.g.*, Howard *et al.*, US 2013/0059800 A1(2013); US 2013/0028919 A1 (2013); and WO 2013/041606 A1 (2013)); and

(g) maytansinoids such as DM1 and DM4 (see, *e.g.*, Chari *et al.*, US 5,208,020 (1993) and Amphlett *et al.*, US 7,374,762 B2 (2008)).

Exemplary cancers treatable by PD-L1 Adnectins

[00300] The PD-L1 Adnectins described herein may be suitable for use in the treatment of a broad range of cancers, including treatment-refractory metastatic NSCLC, that are generally not considered to be immune-responsive. Exemplary cancers that may be treated using the PD-L1 Adnectins described herein include MEL (e.g., metastatic malignant melanoma), RCC,

squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC), and carcinoma of the esophagus. Additionally, the PD-L1 Adnectins described herein are suitable for use in treating refractory or recurrent malignancies.

[00301] Non-limiting examples of cancers that may be treated using the PD-L1 Adnectins, based on the indications of very broad applicability of anti-PD-L1 immunotherapy disclosed in WO2013/173223, include bone cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, castration-resistant prostate cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, carcinomas of the ovary, gastrointestinal tract and breast, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The PD-L1 Adnectins are also applicable to the treatment of metastatic cancers.

Combination therapy with PD-L1 Adnectins

[00302] In certain embodiments, the PD-L1 Adnectins described herein may be combined with an immunogenic agent, for example a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, and cells transfected with genes encoding immune stimulating cytokines (He et ah, 2004). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gpl00, MAGE antigens, Trp-2, MARTI and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF. PD-L1 blockade may also be effectively

combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic antibody (e.g., an anti-PD-1, anti-CTLA-4, and anti-LAG-3 Ab).

Medicaments and uses of anti-PD-L1 Adnectins

[00303] anti-PD-L1 Adnectin described herein may be used for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway so as to thereby potentiate an endogenous immune response in a subject afflicted with cancer. This disclosure also provides the use of any anti-PD-L1 Adnectin described herein for the preparation of a medicament for immunotherapy of a subject afflicted with cancer. The disclosure provides medical uses of any anti-PD-L1 Adnectin described herein corresponding to all the embodiments of the methods of treatment employing a PD-L1 Adnectin described herein.

[00304] Also described herein are anti-PD-L1 Adnectins for use in treating a subject afflicted with cancer comprising potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The disclosure further provides anti-PD-L1 Adnectins for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These anti-PD-L1 Adnectins may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers described herein. In certain embodiments, the cancers include MEL (e.g., metastatic malignant MEL), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC), and carcinoma of the esophagus.

Infectious Diseases

[00305] Also described herein are methods for treating patients that have been exposed to particular toxins or pathogens. For example, in certain aspects, this disclosure provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD-L1 Adnectin described herein such that the subject is treated for the infectious disease.

[00306] Similar to its application to tumors as discussed above, Adnectin-mediated PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to potentiate an immune response to pathogens, toxins, and/or self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than

completely effective. These include, but are not limited to HIV, Hepatitis (A, B, and C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of an infection. Novel epitopes on these antigens are recognized as foreign at the time of anti-PD-L1 Adnectin administration, thus provoking a strong T cell response that is not dampened by negative signals through the PD-1/PD-L1 pathway.

[00307] In the above methods, PD-L1 blockade can be combined with other forms of immunotherapy known in the art, such as cytokine treatment (e.g., administration of interferons, GM- CSF, G-CSF or IL-2).

XIII. Kits and Articles of Manufacture

[00308] The anti-PD-L1 Adnectins described herein can be provided in a kit, a packaged combination of reagents in predetermined amounts with instructions for use in the therapeutic or diagnostic methods described herein.

[00309] For example, in certain embodiments, an article of manufacture containing materials useful for the treatment or prevention of the disorders or conditions described herein, or for use in the methods of detection described herein, are provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container may hold a composition described herein for *in vivo* imaging, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is an anti-PD-L1 Adnectin described herein. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[00310] In certain embodiments, a kit comprises one or more reagents necessary for forming an ^{18}F labelled anti-PD-L1 Adnectin *in vivo* imaging agent, such as a PD-L1 Adnectin-PEG4-DBCO-^{18}F, as further described herein. For example, a kit may comprise a first vial comprising anti-PD-L1 Adnectin-PEG-4-DBCO and a second vial comprising [^{18}F]FPPEGA. A kit may comprise a first vial comprising anti-PD-L1 Adnectin-PEG-4-DBCO, a second vial

comprising 4-PEG-tosyl-azide and a third vial comprising ^{18}F in O^{18} water. The kits may further comprise vials, solutions and optionally additional reagents necessary for the manufacture of PD-L1 Adnectin-PEG4-DBCO-^{18}F. Similarly, kits may comprise the reagents necessary for forming a ^{64}Cu labelled anti-PD-L1 Adnectin, such as the reagents described herein.

Incorporation by Reference

[00311] All documents and references, including patent documents and websites, described herein are individually incorporated by reference to into this document to the same extent as if there were written in this document in full or in part.

[00312] The invention is now described by reference to the following examples, which are illustrative only, and are not intended to limit the present invention. While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one of skill in the art that various changes and modifications can be made thereto without departing from the spirit and scope thereof.

EXAMPLES

Example 1: Identification of PD-L1 binding Adnectins

[00313] Anti-PD-L1 Adnectins were isolated from an Adnectin library screened with a human PD-L1 protein, or were affinity matured by PROfusion from clones identified in the library.The full length sequences, core sequences, BC, DE, and FG loop sequences of these Adnectins, as well as variants with a "PC" modified C-terminus, are presented in Figure 1 and Table 3.

[00314] For example, the high-affinity, anti-PD-L1 Adnectin, ADX_5322_A02 (“A02”), was obtained by affinity-maturing the ATI-964 Adnectin. The gene encoding ATI-964 was re-diversified by introducing a small fraction of non-wild-type nucleotides at each nucleotide position that encoded a residue in loop BC, DE, or FG. The resulting library of Adnectin sequences related to ATI-964 was then subjected to in vitro selection by PROfusion (mRNA display) for binding to human PD-L1 under high-stringency conditions. The clones enriched after completed selection were sequenced, expressed in HTPP format, and screened for their ability to bind PD-L1 and for their fraction of monomericity. The clone with the best combination of affinity for PD-L1 and robust biophysical properties was mutated to include a

C-terminal Cysteine, first with the C-terminal sequence NYRTPCH6 (the form identified as ADX_5322_A02), and later with the C-terminal sequence NYRTPC.

[00315] The same process was followed to affinity-mature ATI-967, resulting in the Adnectin ADX_5417_E01. Similarly, affinity matured ATI_1760_C02, ATI_1760_E01 ("E01") and ATI_1760_F01 were obtained by affinity maturation of ATI_1422_G05.

[00316] Additional anti-human PD-L1 adnectins were isolated. Their sequences are set forth in Table 3.

Expression and purification of his-tagged anti-PD-L1 Adnectins

[00317] All DNA constructs contained an N-terminal his tag followed by a TVMV recognition sequence. The expression plasmids (pET-28 NM vector) for the anti-PD-L1 Adnectins described *supra* were transformed into BL21(DE3) cells (New England Biolabs). Cells were grown in Overnight Express Autoinduction media (Novagen) in 1L shake flasks at 37°C for 6 hours followed by 20°C for 16 hours at 220 RPM. Cells were harvested by centrifugation and suspended in PBS pH 7.2. Cells were lysed mechanically, then clarified by centrifugation. Soluble fractions were bound by gravity feed to Ni-NTA Agarose resin (Qiagen), washed in 20mM Tris + 10 mM Imidazole pH 8.0, followed by 20mM Tris + 40 mM Imidazole pH 8.0, and eluted with 20mM Tris + 400 mM Imidazole pH 8.0. Nickel eluates were spiked with TVMV protease at 1:23-fold molar excess of Adnectin. The TVMV-Adnectin eluate mixtures were dialyzed against 20mM Tris pH 8.0 at 4°C for 16 hours. To separate the TVMV protease and cleaved his tag fragments, samples were loaded onto a 10mL HisTrap FF column (GE Healthcare) and flow through fractions were collected.

Example 2: Biophysical assessment of PD-L1 Adnectins

[00318] The binding properties of ATI-1420D05, ATI-1420D05, AT1-1421E04 and ATI-1422G05, ATI_1760_C02, ATI_1760_E01 and ATI_1760_E01 were assessed.

Table 1: Binding properties of anti-PD-L1 Adnectins

Sequence Name	SEQ ID	K_D(nM)
ATI-1420B09		2.5
ATI-1420D05		9.5
AT1-1421E04		5.6

[00319] Binding properties of the purified ATI-964, ATI-967, ATI-968, ADX_5322_A02, and ADX_5417_E01 Adnectins to human or cyno PD-L1, as determined by Biacore, are shown in Table 2. Cell binding was determined by measuring binding to human PD-L1 positive cells L2987.

Table 2: Biophysical properties of anti-PD-L1 Adnectins

Sequence Name	SEQ ID	Binding Kinetics				Melting Transition Midpoint (Tm)°C	Cell Binding EC_{50}
		k_a (1/Ms)	k_d (s)	K_D (M)	K_D (M) for cynoPD-L1	N.D.	N.D.
ATI-964	30	3.6×10^5	7.7×10^{-5}	2.1×10^{-10}	1.4×10^{-10}	N.D.	4.4
ATI-965	45	4.6×10^5	3.3×10^{-4}	7.1×10^{-10}	8×10^{-10}	N.D.	4.9
ATI-966	60	2.5×10^6	6.9×10^{-5}	2.8×10^{-11}	3.8×10^{-11}	N.D.	2.3
ATI-967	75	2.1×10^6	6.7×10^{-5}	3.2×10^{-11}	6×10^{-11}	N.D.	N.D.
ATI-968	15	7.5×10^5	1.2×10^{-4}	1.6×10^{-10}	1.1×10^{-10}	60	3.4
ADX_5322_A02	91	2.5×10^6	5.7×10^{-4}	$2.28 \times 10\text{-}^{10}$	N.D.	82	0.43 nM (Cys-Capped)
ADX_5417_E01	107	2.0×10^7	2.6×10^{-4}	$1.3 \times 10\text{-}^{11}$	N.D.	73	N.D.

[00320] The binding data indicates that the affinity matured anti-human PD-L1 adnectins bind to human PD-L1 with affinities that are less than 1nM or even less than 0.1nM. Exemplary inhibition curves are shown in Figure 12.

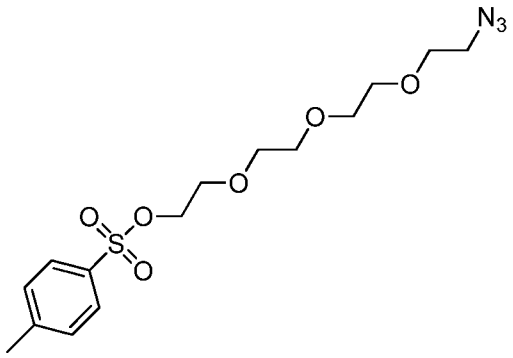
[00321] Anti-PD-L1 adnectins have the following additional characteristics:

- Inhibiting the binding of human PD-1 to human PD-L1, as determined by measuring inhibition of binding of human PD-1Fc to the PD-L1 positive cells L2987 by flow cytometry, and shown, e.g., for adnectins ATI-964, ATI-965, ATI-966, ATI-967, ATI-968, A02 and E01;
- Inhibiting the binding of human CD80 (B7-1) to human PD-L1, as determined by ELISA. For example, ATI-964 inhibits binding with an EC50 of 41pM; ATI-965 inhibits binding with an EC50 of 210 pM; ATI-966 inhibits binding with an EC50 of 28 pM; and ATI-968 inhibits binding with an EC50 of 56 pM;
- Inhibiting the binding of the anti-PD-L1 antibody 12A4 to human PD-L1, as determined by ELISA.

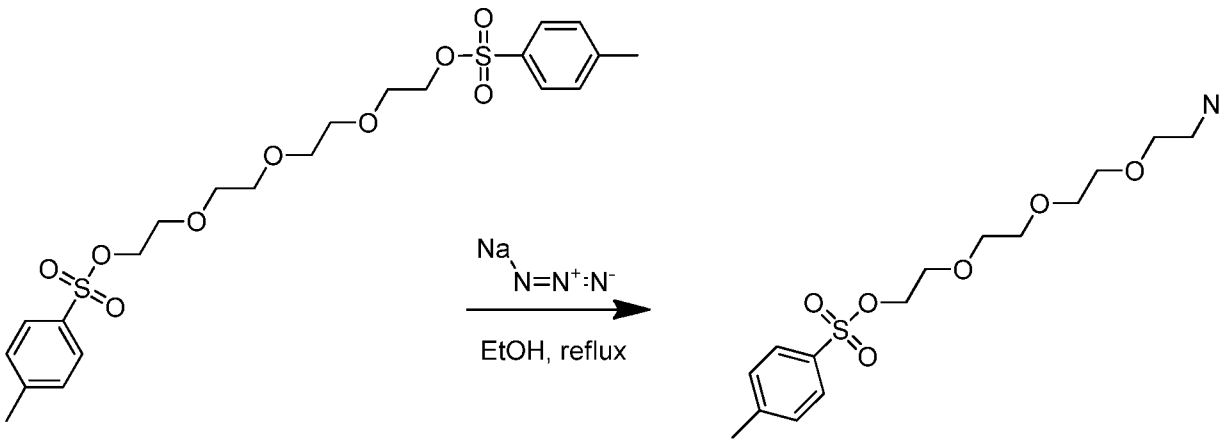
[00322] Anti-PD-L1 antibodies were also tested in a mixed lymphocyte reaction (MLR): ATI-964, ATI-965, and ATI-968 were active in an MLR, whereas ATI-966 and ATI-967 were not active in an MLR.

[00323] The following examples relate to the labeling of anti-PD-L1 Adnectins with ^{18}F and ^{64}Cu.

Example 3: Preparation of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate



2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate

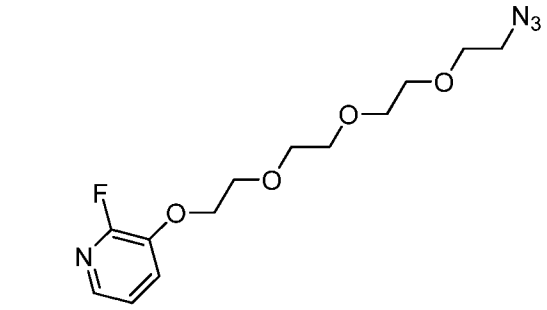


2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl methylbenzenesulfonate

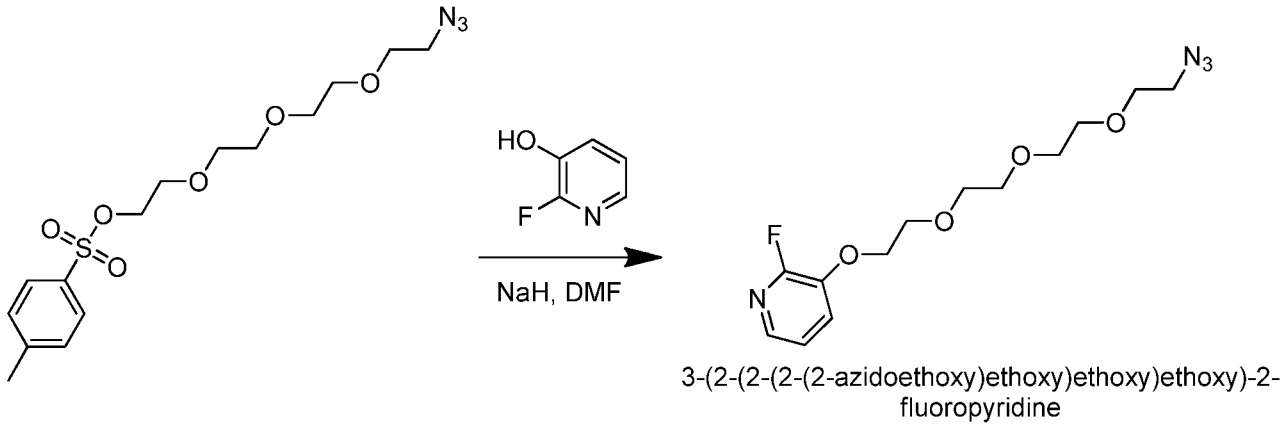
[00324] A mixture of ((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (5 g, 9.95 mmol) and sodium azide (0.647 g, 9.95 mmol) were dissolved in ethanol (50 mL) and the reaction was refluxed at 90 °C over a 17 hour period. The solvent was removed using partial vacuum and then loaded onto a 40 gram silica

cartridge and purified using flash chromatography (IscoCombiFlash - eluted using a linear gradient method starting from 10% ethyl acetate in hexanes going to a 90% ethyl acetate in hexanes over a 45 minute period). The pooled fractions were checked by TLC and combined to give 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate as a colorless oil. Due to the reactive nature of the 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate product this material was used "as is" without any further characterizations.

Example 4: Preparation of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine



3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine

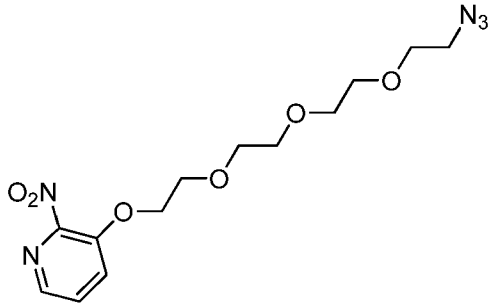


3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine

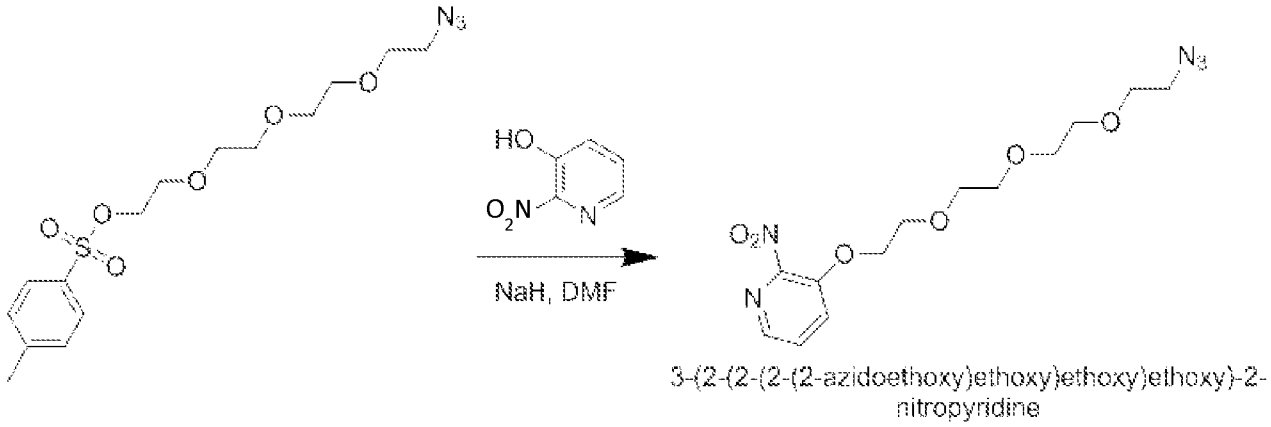
[00325] To a suspension of sodium hydride (0.129 g, 3.21 mmol) in DMF (10 mL) at 0 °C was dropwise added a stirring solution of 2-fluoropyridin-3-ol (0.363 g, 3.21 mmol) in DMF (5 mL), then followed by the dropwise addition of the solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (1.00 g, 2.68 mmol) in DMF (5 mL). The suspension was held at 0 °C for 10 min, then brought to ambient temperature for 1 hour, followed by additional heating at 60 °C for 4 hours. The solvent was removed in vacuo.

100 ml of ethyl acetate was added followed by 3 separate wash extractions with concentrated brine solution. The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude material was purified using flash chromatography (IscoCombiFlash - eluted with 10 - 50% EtOAc in Hex) to give a colorless oil. 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine (702 mg, 2.233 mmol, 83 % yield) was isolated as a clear oil. ^{1}H NMR (400MHz, CHLOROFORM-d) δ 7.75 (dt, J=4.9, 1.6 Hz, 1H), 7.33 (ddd, J=10.0, 8.1, 1.5 Hz, 1H), 7.10 (ddd, J=7.9, 4.9, 0.7 Hz, 1H), 4.30 - 4.16 (m, 2H), 3.95 - 3.83 (m, 2H), 3.80 - 3.61 (m, 10H), 3.38 (t, J=5.1 Hz, 2H) 13C NMR (101MHz, CHLOROFORM-d) d 142.3, 137.7, 137.5, 123.4, 123.4, 121.7, 121.6, 77.3, 76.7, 70.9, 70.7, 70.6, 70.0, 69.4, 69.0, 50.6 19F NMR (400MHz, CHLOROFORM-d) δ -83.55. HRMS (ESI) Theory:C13H20FN4O4+ m/z 315.464; found 315.1463.

Example 5: Preparation of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine



3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine

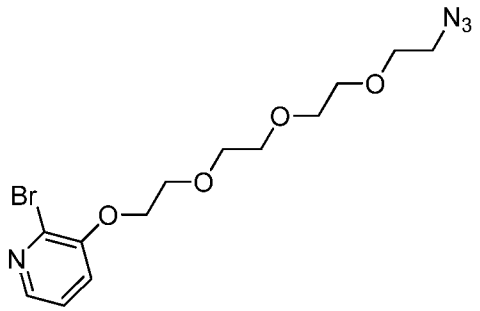


3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine

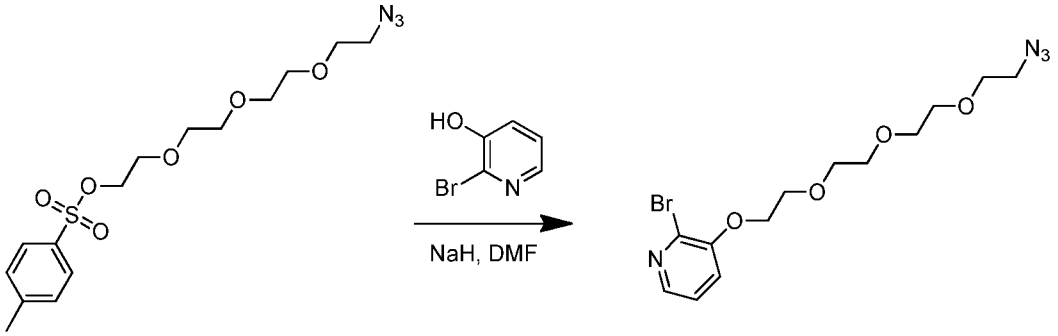
[00326] Sodium hydride (0.121 g, 3.01 mmol) (60% suspension in oil) was dissolved in DMF (7.0 mL) and the resulting suspension was cooled to 0 °C. A solution of 2-nitropyridin-3-ol (0.384 g, 2.74 mmol) in DMF (1.5 mL) was added slowly, followed by the dropwise addition

of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (1.023 g, 2.74 mmol) in DMF (1.5 mL). The suspension was held at 0 °C for 10 minutes, then brought to ambient temperature for 2 hours followed by heating 60 °C for a 72 hour period. The reaction was quenched with 10 ml of DI water, followed by ethyl acetate extraction (3 x 10 mL). Pooled EtOAc extracts were washed with a concentrated brine solution (10 mL), dried over sodium sulfate, filtered, and evaporated under reduced pressure to give a light yellow oil. The crude was purified by flash chromatography. 24 g silica cartridge, 25 mL/min, starting from 10% ethyl acetate in hexanes, followed by a linear change to 50% ethyl acetate in hexanes over a 25 minute period. After this time, the gradient was held at this solvent composition for 10 minutes then changed to 100% ethyl acetate over a 10 minute period. 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine was eluted between the 30-40 minute portion of the chromatogram and the pooled fractions were evaporated under reduced pressure, then under vacuum for 2 hours to give 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine (687 mg, 1.973 mmol, 72.0% yield) as a light yellow oil. ^{1}H NMR (400MHz, CHLOROFORM-d) δ 8.11 (dt, J=4.9, 1.6 Hz, 1H), 7.60 (ddd, J=10.0, 8.1, 1.5 Hz, 1H), 7.52 (ddd, J=7.9, 4.9, 0.7 Hz, 1H), 4.30 - 4.16 (m, 2H), 3.95 - 3.83 (m, 2H), 3.80 - 3.61 (m, 10H), 3.38 (t, J=5.1 Hz, 2H) 13C NMR (101MHz, CHLOROFORM-d) d 147.3, 139.5, 128.4, 124.4. 71.1, 70.7, 70.6,70.0, 69.9, 69.3, 50.7. HRMS (ESI) Theory:C13H20N5O6+ m/z 342.1408; found 342.1409

Example 6: Synthesis of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-bromopyridine

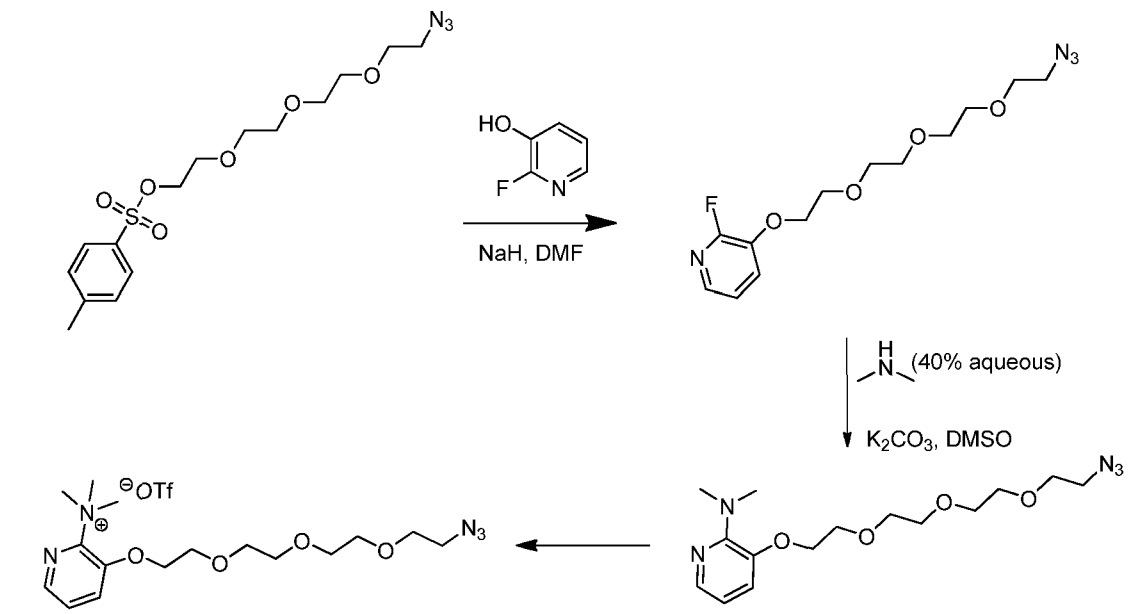


3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-bromopyridine

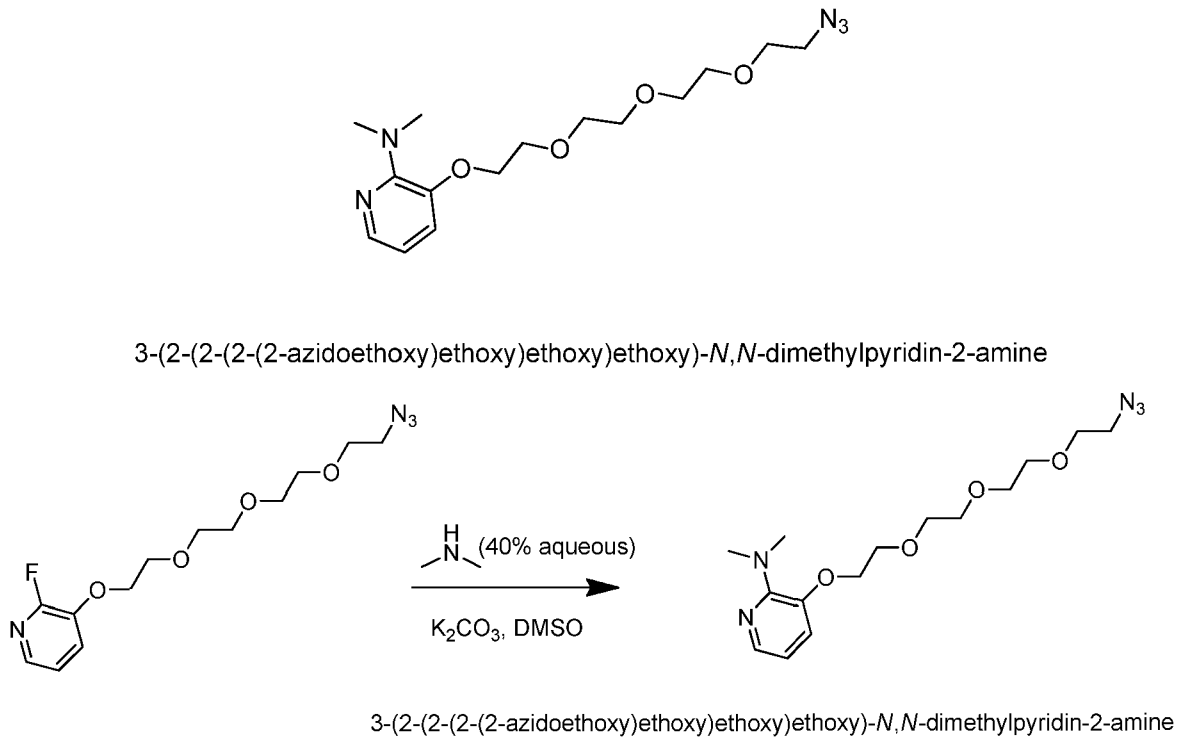


[00327] To the suspension of sodium hydride (NaH, 25.7 mg, 0.643 mmol) in dimethylformamide (DMF, 5 mL) at 0 °C was dropwise added a solution of 2-bromopyridin-3-ol (112 mg, 0.643 mmol) in DMF (1 mL), followed by the dropwise addition of the solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (200 mg, 0.536 mmol) in DMF (1 mL). The suspension was held at 0 °C for 10 minutes, then brought to ambient temperature and held for 1 hour, followed by heating to 60 °C for 4 hours. Upon completion of heating, the solvent of the crude reaction mixture was removed in vacuo. The crude reaction was reconstituted in 50 mL of ethyl acetate, washed with 2 x 50 mL of a aqueous brine solution, and the organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude reaction was purified using reverse-phase HPLC to give3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-bromopyridine, TFA (112 mg, 0.229 mmol, 42.7 % yield) as a light yellow oil. HRMS ESI m/z (M+H), Theory C13H20BrN4O4 375.0664 found 375.0662 ; ^{1}H NMR (400MHz, DMSO-d_6) δ 7.97 (dd, J=4.6, 1.5 Hz, 1H), 7.54 (dd, J=8.2, 1.6 Hz, 1H), 7.40 (dd, J=8.1, 4.6 Hz, 1H), 4.24 (dd, J=5.3, 3.9 Hz,2H), 3.85 - 3.78 (m, 2H), 3.68 - 3.62 (m, 2H), 3.62 - 3.52 (m, 8H), 3.42 - 3.34 (m, 2H).

Example 7: Scheme for synthesis of trimethylanilium compound



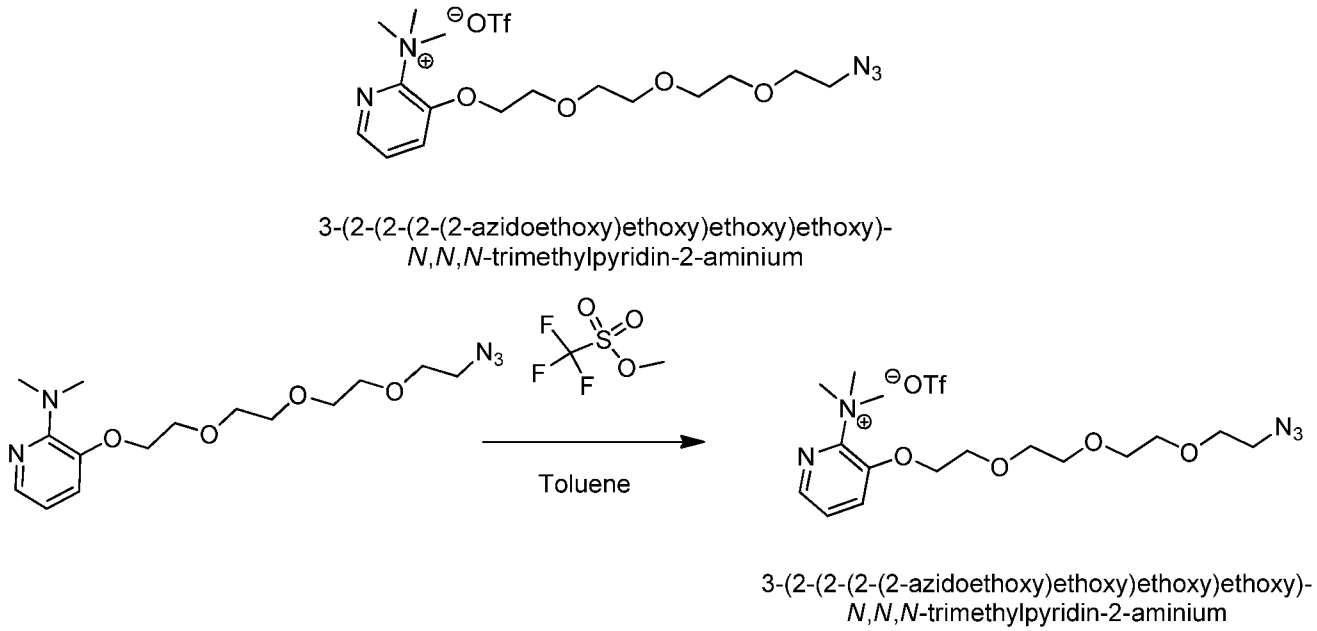
Example 8: Synthesis of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N-dimethylpyridin-2-amine



[00328] A mixture of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine

(160 mg, 0.509 mmol), potassium carbonate (K_2CO_3, 84 mg, 0.611 mmol), and dimethylamine (40% in water, 0.097 mL, 0.764 mmol) in dimethylsulfoxide (DMSO, 2.5 mL) were heated in a sealed pressure-proof vessel at 110 °C for 14 hours. Upon completion of heating, the solvent of the crude reaction mixture was removed in vacuo. The crude reaction was reconstituted in 50 mL of ethyl acetate, washed with 2 x 50 mL of a aqueous brine solution, and the organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude reaction was purified using normal-phase chromatography to give 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N-dimethylpyridin-2-amine (140 mg, 0.413 mmol, 81 % yield) as a colorless oil. ^{1}H NMR (400MHz, CHLOROFORM-d) δ 7.86 (dd, *J*=4.9, 1.5 Hz, 1H), 7.02 (dd, *J*=7.8, 1.5 Hz, 1H), 6.73 (dd, *J*=7.8, 4.9 Hz, 1H), 4.20 - 4.07 (m, 2H), 3.98 - 3.86 (m, 2H), 3.81 - 3.61 (m, 9H), 3.38 (t, *J*=5.1 Hz, 2H), 3.13 - 2.94 (m, 6H), 1.69 (s, 2H). HRMS (ESI) Theory:C15H26N5O4+ m/z 340.1980; found 340.1979.

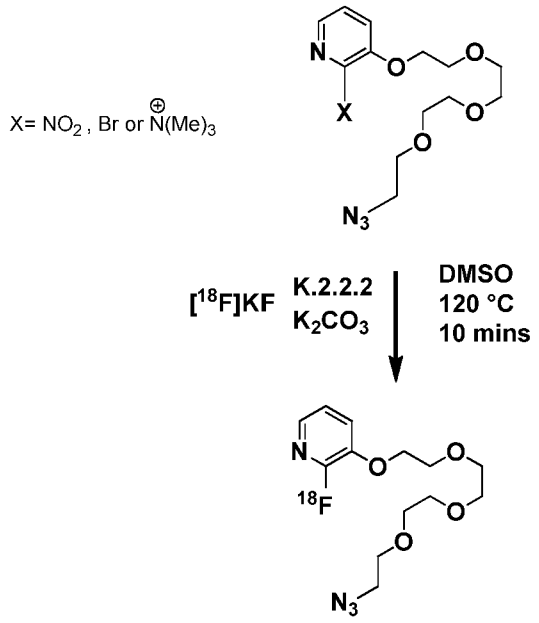
Example 9: Synthesis of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N,N-trimethylpyridin-2-aminium



[00329] Methyl trifluoromethanesufonate (0.065 mL, 0.589 mmol) was added to the solution of 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N-dimethylpyridin-2-amine (40 mg, 0.118 mmol) in toluene (1.5 mL) in a sealed container under a steady stream of nitrogen. The reaction mixture was stirred at room temperature over a 14 hour period. The solvent was removed and the resultant residue was washed with 2x 10 ml of ether, azeotropically dried with 2 x 1 ml of dichloromethane, and dried under high-pressure vacuumn overnight to give

3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N,N-trimethylpyridin-2-aminium, trifluoromethanesulfonate salt in quantitative yield as a thick colorless oil. LCMS m/z 354.33; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.24 - 8.17 (m, 1H), 7.98 (d, J=8.3 Hz, 1H), 7.75 (ddd, J=8.2, 4.6, 3.2 Hz, 1H), 4.44 (br. s., 2H), 3.88 (d, J=3.9 Hz, 2H), 3.69 - 3.45 (m, 21H).

Example 10: Synthesis of [^{18F}]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine using 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N,N-trimethylpyridin-2-aminium, trifluoromethanesulfonate salt



Synthesis of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine

[00330] An aqueous [^{18}F]-Fluoride solution (2.0 ml,33.3 GBq/ 900 mCi) was purchased from P.E.T. Net® Pharmaceuticals in West Point PA and directly transferred to a Sep-Pak light QMA [The Sep-Pak light QMA cartridge was pre-conditioned sequentially with 5ml of 0.5 M potassium bicarbonate, 5 ml of deionized water, and 5 ml of MeCN before use.] Upon completion of this transfer, the aqueous [^{18}F] fluoride was released from the QMA Sep-Pak by the sequential addition of potassium carbonate (15 mg/ml; 0.1ml) followed by a mixture of potassium carbonate (30 mg/ml, 0.1 ml), 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (15 mg, 0.04 mmol) and 1.2 ml of MeCN. The solvent was evaporated under a gentle stream of nitrogen at 90°C and vacuum. Azeotropic drying was repeated twice with 1 ml portions of acetonitrile to generate the anhydrous K.2.2.2/K[^{18}F]F complex. 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-N,N,N-trimethylpyridin-2-aminium, trifluoromethanesulfonate salt (2 mg, 5.6 μmol) was dissolved in 500 microliters of

DMSO and added to the dried cryptand. This solution was heated at 120°C for 10 minutes. After this time, the crude reaction mixture was diluted with 3 ml of DI water. The entire contents of the crude reaction mixture was then transferred, loaded, and purified using reverse phase HPLC under the following conditions: HPLC Column: Luna C18 250 x 10 Solvent A: 0.1% TFA in DI water; solvent B: 0.1% TFA in acetonitrile at a flow rate of 4.6 ml/minute using isocratic method 32% B while the UV was monitored at 280 nm. [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was isolated at the 24 min mark of the chromatogram and collect over a 2 minute period. This product was collected into a 100 ml flask that contained 10 ml of DI water and the entire contents were delivered to a Sep-Pak Vac tC18 6 cc 1g sep pack from Waters. 6.1 GBq/164 mCi of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was isolated from this reaction. This was released from the sep-pak using 3 ml of ethanol and this solution was reduced with 98°C heat source, a gentle stream of nitrogen, and vacuum over a 15 minute period until only a film remained in the vial. The final product was reconstituted in 100% 1x PBS buffer and was stable in this media for over 1 hour at 37°C.

[00331] The [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine may be used to generate ^{18}F-labeled biologic products (e.g., ^{18}F-labeled anti-PD-L1 Adnectins, as described below) by taking advantage of "click" azide-alkyne reaction with the appropriate biologic containing an alkynes.

Example 11: Production of ^{18}F-radiolabeled protein using "Click Chemistry"

A. Fluorination of the 4-PEG-tosyl-azide Precursor to Form [^{18}F]-FPPEGA

[00332] 900 mCi of ^{18}F in ^{18}O water (3ml) activity (purchased from IBA Molecular) was transferred directly into a micro vial (no QMA) that contained 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (2.8 mg, 7.44 µmol) and potassium carbonate (1.7 mg, 0.012 mmol). An additional 2.0 ml of acetonitrile was transferred into this crude reaction mixture and the entire mixture was azeotropically dried. This was completed by evaporating the solution using a 98 °C oil bath, and applying a gentle stream of N_2 and partial vacuum. The solution's volume was reduced to about 2 ml. An additional 2 ml of acetonitrile was added and the process was repeated 3 times over a 40 minute period. When the volume of the liquid was reduced to less than 0.3 ml, a 0.7 ml aliquot of acetonitrile was added and the solution reduced by further azeotropic distillation until the volume was ~0.1 ml. An additional 0.9 ml of acetonitrile was added and this process was completed until a white solid

was formed. This process took ~55 minutes. During the final procedure, the vial was removed from the oil bath before the solution had gone to dryness and the residue in the vial was placed under full vacuum (no N_2 flow) at room temperature for 20 minutes. Total time for transfer and drying of [^{18}F]-FPPEGA cryptand mixture was 65 min.

[00333] To the dried [^{18}F]-FPPEGA cryptand mixture was added 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine (2 mg, 5.86 μmol) dissolved in 500 microliters of DMSO and this mixture was heated at 120 ºC for 10 minutes. After this time the crude reaction mixture was diluted with 3 ml of DI water and the entire contents were then transferred and loaded onto the following HPLC column and conditions: HPLC Column: Luna C18 250 x 10 mm; Solvent A: 0.1 % TFA in DI water; Solvent B: 0.1 % TFA in acetonitrile; flow rate 4.6 ml/min; pressure 1820 PSI; isocratic method 32% B; UV - 280 nm. The [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine ([^{18}F]-FPPEGA) product was isolated at the 24 minute mark of the chromatogram and was collect over a 2 minute period. This product was collected into a 100 ml flask that contained 15 ml of DI water and the entire contents were delivered to a Sep PakVac tC18 6 cc 1g sep pack. PN WAT036795. The [^{18}F]-FPPEGA was released from the Sep Pak using 2.5 ml of ethanol and this solution was reduced with 98 ºC N_2 and vacuum over a 15 minute period until dryness. This compound was dissolved in 0.1 ml 1 X PBS (phosphate buffered saline). This product was analyzed using a Varian HPLC HPLC Column Luna C18 (2) 4.6 x 150 mm Solvent A: 0.1 % TFA in DI water; Solvent B: 0.1 % TFA in acetonitrile; flow rate 1.0 ml/min; gradient method 0 min 90% A 10% B; 15 mins 30% A 70% B; 17 mins 30% A 70% B; 18 mins 90% A 10% B; 20 mins 90% A 10% B; UV - 280 nm. 220 mCi of [^{18}F]-FPPEGA was isolated.

B. Preparation of E01-4PEG-DBCO

[00334] This Example describes the linking of the E01 anti-PD-L1 Adnectin to PEG4-DBCO.

[00335] As maleimide chemistry is used to link the Adnectin to PEG4-DBCO, the E01 Adnectin was first modified by adding a proline followed by a cysteine at its C-terminus. The amino acid sequence of this modified E01 Adnectin is provided in SEQ ID NO: 104. The cysteine is used to link the Adnectin to PEG4-DBCO.

[00336] A 4-fold molar excess of Maleimide-PEG4-DBCO (Click Chemistry Tools) was dissolved in DMSO and added to the purified modified E01 Adnectin in the presence of 1mM TCEP. Final DMSO concentrations did not exceed 5% in the conjugation mixtures. The conjugation mixture was left at room temperature for one hour before mass spec

analysis. After MS confirmation of conjugation, the sample was purified by size-exclusion chromatography using a HiLoad 26/60 Superdex 75 column (GE Healthcare) equilibrated in PBS pH 7.2.

C. Coupling of [^{18}F]-FPPEGA to Adnectin

[00337] A schematic for synthesizing [^{18}F]-E01-4PEG-DBCO-FPPEGA is shown in **Figures 2 and 9**.

[00338] 0.2 ml of a 5.4 mg/ml solution of the E01-4PEG-DBCO Adnectin solution (prepared as described in Section B) was incubated with 200 mCi of 0.1 ml of [^{18}F]-FPPEGA (Example 4) in 1 x PBS buffer. The solution was gently mixed by pipetting the crude reaction up and down several times and was incubated together for 45 minutes at 45°C or room temperature. The contents of this crude reaction mixture were purified using a SEC column. Superdex 200 0.5 ml/min 1 X PBS buffer and the [^{18}F]-E01-4PEG-DBCO-FPPEGA product was isolated at the 37 min mark of the chromatogram over a 2 minute period.

[00339] [^{18}F]-E01-4PEG-DBCO-FPPEGA was analyzed via SEC with co-injection of non-radioactive standard, RP HPLC using a PLRPS column and gel electrophoresis.

[00340] Size Exclusion Chromatography (SEC) was performed with the following parameters:

Superdex 200 column; Solvent 100% 1X PBS buffer; 0.5 ml/min 280 UV;
Reverse phase HPLC
Column: PLRPS 8 micron 1000 A 4.6 x 250 mm
Solvent A: 0.1% formic acid in DI water
Solvent B: Acetonitrile
Flow rate: 1 ml/min
Pressure: 1351 PSI
Gradient:

0 min 90% A 10% B
30 min 45 %A 55% B
32 min 25% A 75% B
36 min 25% A 75% B
50 min 90% A 10% B

[00341] 15 mCi [^{18}F]-E01-4PEG-DBCO-FPPEGA was isolated with a radiochemical purity (RCP) of >99% via both SEC and RP HPLC calculations, and with a specific activity of 0.6

mCi/nmol, when the reaction was conducted at 45°C. When conducting the reaction at room temperature, 5.72 mCi was obtained. Specific activity of the [^{18}F]-FPPEGA was 0.512 mCi/nmol and RCP of 85.7% 3 hours post the end of its synthesis, when conducting the reaction at 45°C or at room temperature, respectively.Specific activity was measured via Nanodrop (see www.nanodrop.com). The product co-eluted with non-radioactive standard on both SEC and PLRPS. Gel electrophoresis confirmed an ^{18}F product consistent with an 11 kDa molecular weight standard.

[00342] The ^{18}F-radiolabeled E01-4PEG-DBCO can be used in a variety of *in vitro* and/or *in vivo* imaging applications, including diagnostic imaging, basic research, and radiotherapeutic applications. Specific examples of possible diagnostic imaging and radiotherapeutic applications, include determining the location, the relative activity and/or quantifying of PD-L1 positive tumors, radioimmunoassay of PD-L1 positive tumors, and autoradiography to determine the distribution of PD-L1 positive tumors in a mammal or an organ or tissue sample thereof.

[00343] In particular, the The ^{18}F-radiolabeled E01-4PEG-DBCO is useful for positron emission tomographic (PET) imaging of PD-L1 positive tumors in the lung, heart, kidneys, liver and skin and other organs of humans and experimental animals. PET imaging using the ^{18}F-radiolabeled E01-4PEG-DBCO can be used to obtain the following information: relationship between level of tissue occupancy by candidate PD-L1 tumor-treating medicaments and clinical efficacy in patients; dose selection for clinical trials of PD-L1 tumor-treating medicaments prior to initiation of long term clinical studies; comparative potencies of structurally novel PD-L1 tumor-treating medicaments; investigating the influence of PD-L1 tumor-treating medicaments on *in vivo* transporter affinity and density during the treatment of clinical targets with PD-L1 tumor-treating medicaments; changes in the density and distribution of PD-L1 positive tumors during effective and ineffective treatment.

Example 12: Linking of PD-L1 Adnectins to NODAGA to generate NODAGA-PD-L1 Adnectins

[00344] This Example describes the linking of the E01 and A02 anti-PD-L1 Adnectins to NODAGA. As maleimide chemistry is used to link the Adnectins to NODAGA, both Adnectins used a proline followed by a cysteine at their C-terminus (as described for E01 above). The amino acid sequences of the modified E01 and A02 Adnectins are provided in SEQ ID NOs: 104 and 88, respectively. The cysteine will be used for linking the Adnectins

to NODAGA. For ^{64}Cu labeling of the Adnectins, a 50-fold molar excess of maleimide-NODAGA (CheMatech) was dissolved in PBS pH 7.4 and added to the purified Adnectins in the presence of 1 mM TCEP. Final DMSO concentrations did not exceed 5% in the conjugation mixtures. Conjugation mixtures were left at room temperature for one hour before mass spec analysis. After MS confirmation of conjugation, the samples were purified by size-exclusion chromatography using a HiLoad 26/60 Superdex 75 column (GE Healthcare) equilibrated in PBS pH 7.2.

Example 13: Synthesis of ^{64}Cu-based anti-PD-L1 Adnectin probes

Synthesis of ^{64}Cu-A02-NODAGA

[00345] [^{64}Cu]-Copper chloride ($^{64}CuCl_2$) in 0.1N hydrochloric acid solution was neutralized with 0.8 mL of 0.1N sodium acetate (NaOAc) aqueous solution for 4 minutes at ambient temperature. 1 mL of the ^{64}Cu/NaOAc solution was added to A02-NODAGA (30 μL of 1.6 mg/mL) and the crude reaction was gently pippetted to allow mixing followed by resting at ambient temperature for 30 minutes. The contents of crude reaction mixture were transferred to a PD-10 desalting column that was pre-activated with 20 mL of 1x phosphate buffered saline (PBS, pH 7.4) buffer prior to loading of sample. An additional 1.5 mL of 1XPBS was added to the column, followed by an additional 0.8 ml 1XPBS solution and these fractions were discarded. [^{64}Cu]-A02-NODAGA was then collected after a 1.2 mL elution of the PD-10 column to give 10.79 mCi as the desired product. Quality control was measured using a reverse phase HPLC system using an Agilent PLRP-S HPLC column Size: 250 x 4.60 mm, 8 μm, 280 nm and a mobile phase of 0.1% Formic Acid in distilled water and acetonitrile. A gradient method was used where the percentage of acetonitrile was increased linearly from 10% to 45% over a 30 minute time frame. [^{64}Cu]-A02-NODAGA co-eluted with reference standard at the 22 minute mark of the HPLC chromatogram. Radiochemical purity was measured to be 96% using this method. [^{64}Cu]-A02-NODAGA also co-eluted with reference material at the 20 minute mark using a size exclusion chromatography, (SEC) Column: GE Superdex 200 GL Size: 10 x 300 mm, 280 nm. The calculated specific activity was 956.8 mCi/ μmol based on Nanodrop protein concentration and isolated radioactivity of the purified sample.

Procedure for the synthesis of ^{64}Cu-E01-NODAGA

[00346] [^{64}Cu]-Copper chloride ([^{64}Cu]$CuCl_2$) in 0.1N hydrochloric acid solution (20 mCi in 0.25 mL) was pH adjusted with 1.10 mL of 0.1N ammonium acetate buffer, then mixed and

incubated with E01-NODAGA Adnectin in a 1XPBS aqueous solution (40 μL of 1.2 mg/mL, 4.62 nmol) for 30 minutes at ambient temperature. After 30 minutes of incubation, the reaction mixture (~1250 μL) was transferred to the PD-10 desalting column (GE Healthcare Life Science, Sephadex G25 Medium, 14.5 x 50 mm – equilibrated with 40 mL of 1X PBS), and the sample was allowed to enter the column completely by gravity and followed with 1.1 mL of 1X PBS. After the liquid completely passed through the column, the product was collected via elution in 1 mL increments with 1X PBS per sample vial. The ^{64}Cu-E01-NODAGA was isolated in the second 1 ml fraction and measured to be 9.26 mCi in 1 ml of 1XPBS. This sample was analyzed using an analytical size exclusion HPLC method using an Agilent HPLC system equipped with a UV/vis detector (λ = 280 nm), a posi-ram detector and Superdex 200 10/300 GL size-exclusion column (GE Healthecare Life Science, pore size 13 μm). The flow rate was 0.5 mL/min, and the aqueous mobile phase was isocratic with 1X PBS with 0.02% NaN_3 for 60 minutes. The radiochemical purity was 99% using this system, and the product co-eluted with non-radioactive reference standard. Specific activity was calculated based on the equation of the 3-points calibratio curve (y = 656978x). About 100 μL of the product solution from vial 3 was injected onto the Superdex -200 size exclusion column. Product peak was collected and measured to be 0.74 mCi, UV counts of product peak was 156367 unit, and specific activity was 3.1 mCi/nmol.

Example 14: *In vitro* Differentiation of PD-L1-positive cells from PD-L1-negative cells with an anti-PD-L1 Adnectin imaging agent

[00347] In this experiment, the ^{64}Cu-E01 anti-PD-L1 Adnectin (NODAGA was used as a chelator) was tested for its ability to discriminate between hPD-L1-positive cells and hPD-L1-negative cells *in vitro*. Cell labeling was specific, as evidenced by differential association of ^{64}Cu -E01 with hPD-L1-positive L2987 cells compared to hPD-L1-negative HT-29 cells (cell associated radioactivity was 44.6× higher in hPD-L1-positive L2987 cells). Specificity was further confirmed as evidenced by a marked reduction in cell-associated ^{64}Cu -E01 when co-incubated with excess 450 nM cold (unlabeled) E01 Adnectin (99.6% reduction). Cell associated ^{18}F-E01 was minimally reduced (9.9% reduction, not significant) when cells were co-incubated with 450 nM of a cold (unlabeled) non-PD-L1 binding Adnectin (**Figure 3**).

[00348] 1×10^6 hPD-L1-positive L2987 human lung carcinoma cells or hPD-L1-negative HT-29 human colorectal adenocarcinoma cells were placed into 5 mL culture tubes (n=3 tubes per condition). ^{64}Cu-E01 Adnectin solution was prepared in PBS + 0.5% BSA at a concentration of 300 nCi/200 μL. Portions of this solution were supplemented with either

cold (unlabeled) E01 Adnectin or cold (unlabeled) non-PD-L1 binding Adnectin to a final concentration of 450 nM. Cell samples were centrifuged for 5 min at 200×g and then resuspended in 200 μL of the appropriate ^{64}Cu -E01 Adnectin solution and incubated on ice for 1 hour. After the incubation period, cell samples were centrifuged at 200×g and the supernatant was discarded. Cell pellets were resuspended in 1 mL PBS + 0.5% BSA and the wash procedure repeated for a total of 3 washes. Following the final wash, cells were again centrifuged at 200×g and the supernatant was discarded. The radioactivity of the remaining cell pellets was then measured by gamma counter.

[00349] Taken together, these results demonstrate the ability of the ^{64}Cu-E01 Adnectin to differentiate PD-L1(+) vs. PD-L1(-) cells *in vitro*. Specificity was further demonstrated by a marked reduction in cell-associated radiotracer in samples co-incubated with 450 nM unlabeled anti-PD-L1 E01 Adnectin (and only a statistically insignificant reduction when co-incubated with 450 nM of a non-PD-L1 binding Adnectin). Similar experiments using different Adnectin variants as well as ^{18}F as the radionuclide were conducted, with similar results.

Example 15: Distinguishing PD-L1-positive tumors from PD-L1-negative tumors with an anti-PD-L1 Adnectin imaging agent

[00350] For PET imaging, rapid blood clearance rates provide an advantage over more slowly clearing proteins, such as antibodies, by minimizing the amount of time needed for "background" probe signals to deplete from non-relevant tissue. In the clinic, long blood half-life antibody-based-PET tracers may require several days of waiting post injection before images can be collected. Rapid clearing probes open the door to high contrast images that can be collected on the same day the probe is injected, and very importantly, they can also serve to reduce overall radiation exposure to the animals studied or patients examined.

[00351] In this experiment, the ^{64}Cu-A01 anti-PD-L1 Adnectin (NODAGA was used as the chelator), produced as described in the above Examples, was tested for its ability to discriminate between hPD-L1-positive tumors and hPD-L1-negative tumors in mice.

[00352] Mice bearing bilateral xenograft tumors were produced by introducing 1×10^6 hPD-L1(+) L2987 human lung carcinoma cells and 1.5×10^6 hPD-L1(-) HT-29 human colon carcinoma cells subcutaneously on opposite sides of the mouse. Once tumors reached approximately 300 mm^3 (approximately 2-3 weeks after cell implantation), animals were selected for imaging. For imaging, animals were placed under anesthesia with 2% isoflurane

and tail vein catheters were installed. Mice were then placed into a custom animal holder with capacity for 4 animals, where they remained under anesthesia for the duration of the study. The animal holder was transferred to the microPET® F120™ scanner (Siemens Preclinical Solutions, Knoxville, TN). The axial field of view of this instrument is 7.6 cm. With this limitation, animals were positioned such that the scanning region was from immediately in front of the eyes to approximately the base of the tail.

[00353] A 10-minute transmission image was first acquired using a ^{57}Co point source for the purpose of attenuation correction of the final PET images. Following the transmission scan, radiotracer solutions were administered via the previously installed tail vein catheters and a 2 hour emission image was acquired. Injected radiotracer solutions consisted of either approximately 200 μCi ^{64}Cu-A02 (with a NODAGA chelator) or 200μCi ^{64}Cu-A02 supplemented with 3 mg/kg final concentration of cold, unlabeled A02 Adnectin (based on individual animal weight). All injections were formulated in 200 μL saline prior to injection. Exact injected doses were calculated by taking direct measurements of the formulated dose and subtracting the radioactivity remaining in the syringe and the tail vein catheter.

[00354] Images were reconstructed using a maxiumum a posteriori (MAP) algorithm with attenuation correction using the collected transmission images and corrected for radioisotope decay. In the final images, regions of interest (ROIs) were drawn around the tumor boundary using ASIPro software (Siemens Preclinical Solutions). Time-activity curves were calculated for each ROI to yield a quantitative view of radiotracer within the tumor volume over the course of the 2 hour emission image. For final comparison, individual time-activity curves were normalized based on the injected radiotracer dose for each specific animal. Radiotracer uptake was compared across tumors using the final 10 minutes of each time-activity curve (1 hour 50 minutes – 2h post-radiotracer injection). Using this methodology, radiotracer uptake in hPD-L1(+) L2987 xenografts was 3.05× that seen hPD-L1(-) HT-29 xenografts in animals receiving only the ^{64}Cu-A02 radiotracer. In animals co-injected with the ^{64}Cu-A02 radiotracer and 3 mg/kg unlabeled A02 Adnectin uptake in the hPD-L1(+) L2987 xenografts was only 1.04× that seen in hPD-L1(-) HT-29 xenografts (**Figures 4A** and **4B**).

[00355] For some studies, animals were sacrificed via cervical dislocation immediately following imaging. Necropsy was then performed on the animals, and individual tissues were collected (blood, heart, lung, liver, spleen, kidney, muscle, stomach, bone, L2987 tumor, and HT-29 tumor) into pre-weighed tubes. All tissues were then weighed again to determine the weight of each tissue. The radioactivity in each tissue was then directly measured ex vivo

using a Perkin-Elmer Wizard3 gamma counter. For all tissues, measured values in counts per minute (CPM) were normalized to the injected radioactive dose for the individual animals and corrected for radioactive decay. These results were then plotted to show the biodistribution of the radiotracer. An example of this analysis for the ^{18}F-A02 Adnectin radiotracer is shown in **Figure 5**. These results demonstrate clear differential uptake of the radiotracer in hPD-L1(+) L2987 xenografts compared to hPD-L1(-) HT-29 xenografts. Furthermore, the only tissue with higher PD-L1 uptake was the kidney, which is expected as clearance of the ^{18}F-A02 Adnectin is expected to be via kidney filtration based on the molecular weight of the molecule.

[00356] Taken together, these results provide direct visualization of differentiation of hPD-L1(+) versus hPD-L1(-) xenograft tumors *in vivo*. Specificity was further demonstrated by co-injection of 3 mg/kg unlabeled anti-PD-L1 A02 Adnectin, resulting in a reduction of radiotracer uptake in hPD-L1(+) tumors to the level of hPD-L1(-) xenografts. This further validates the use of anti-PD-L1 Adnectins for visualization of PD-L1 tissue expression using PET imaging. Similar experiments using ^{18}F as the radionuclide were conducted in mice, and similar results were obtained, reaching a maximum radiotracer uptake ratio of 3.53:1 in hPD-L1(+) L2987 xenografts vs. hPD-L1(-) HT-29 xenografts using the ^{18}F-A02 Adnectin radiotracer.

[00357] The anti-PD-L1 Adnecin-based imaging agents also showed similar results when performed in cynomolgus monkeys. In these studies, the ^{18}F-E01 anti-PD-L1 Adnectin, produced as described in the above Examples, was tested for its ability to produce high-contrast images in cynomolgus monkeys. The anti-PD-L1 Adnectins described here maintain high affinity for cynomolgus PD-L1 (but have low affinity for rodent PD-L1). Furthermore, as cynomolgus monkeys do not contain PD-L1(+) tumors as in mouse models, imaging performance was assessed primarily on the background levels measured in the images in the context of endogenous PD-L1 expression (with low background enabling the potential for high-sensitivity detection of PD-L1(+) tissues). In these studies, background levels in the resulting PET images were very low, with notable radiotracer accumulation noted mainly in the kidneys, spleen, and bladder.

[00358] Cynomolgus male monkeys with a previously installed vascular access port (VAP) were anesthetized with 0.02 mg/kg atropine, 5 mg/kg Telazol and 0.01 mg/kg buprenorphine I.M. (all drawn into a single syringe). An i.v. catheter is then placed in the cephalic vessel for fluid administration during the imaging procedure to maintain hydration. Animals were

intubated with an endotracheal tube - usually 3.0 mm and transferred to the imaging bed of a microPET® F220™ PET instrument (Siemens Preclinical Solutions, Knoxville, TN). Anesthesia was maintained with isoflurane and oxygen and I.V. fluids (LRS) were administered at a rate of 6 ml/kg/hr during the imaging procedure. As the axial field of view of the microPET® F220™ instrument is only 7.6 cm, images over 5 distinct bed positions were acquired to create a composite image of the animals from just above the heart through approximately the pelvis.

[00359] For each field of view, a 10 minute transmission image was first acquired using a ^{57}Co point source for the purpose of attenuation correction of the final PET images. Once transmission images were acquired for all bed positions, approximately 1.5 mCi (approximately 0.015 mg/kg) of the ^{18}F-E01 Adnectin radiotracer was administered via the installed VAP. 5 minute duration emission scans were then sequentially acquired for each bed position, beginning at position 1 centered aproximately at the heart and moving toward the pelvis of the animal. Once images were acquired at each position (1 through 5), the imaging bed was moved back to bed position 1 and the process was repeated. Using this procedure, a total of 5 distinct images were acquired for each bed position over the duration of the imaging study.

[00360] Individual images were reconstructed using a filtered back projection (FBP) algorithm with attenuation correction using the collected transmission images and corrected for radioisotope decay. Final composite images were then produced by aligning images from all 5 bed positions obtained from a single pass (i.e. a single composite image was produced from each set of sequential images from bed positions 1 through 5) covering the duration of the imaging study. Final images were visually inspected to note areas of visible radiotracer uptake (i.e. spleen, kidney, bladder) and background tissue (muscle) (**Figure 6**). Background accumulation of ^{18}F-E01 Adnectin was very low, with little signal visible in background tissues such as muscle. Additionally, uptake was verified in the spleen, which is believed to be PD-L1(+) based on mRNA expression. Thus, studies in cynomolgus monkeys demonstrate the potential for high-sensitivity PD-L1 imaging in the context of endogenous PD-L1.

[00361] In aggregate, PET studies in rodent and cynomolgus monkey show that ^{64}Cu and ^{18}F labeled anti-human PD-L1 Adnectins provide strong and specific probes for *in vivo* labeling of PD-L1 positive tissues with the potential for high-sensitivity detection of tissues with low level PD-L1 expression.

[00362] *In vivo* imaging experiments were also conducted with an anti-PD-L1 antibody, and the areas that this imaging agent detected were the same areas that were detected with the PD-L1 imaging agent, therefore confirming that anti-PD-L1 Adnectin imaging agents successfully detect PD-L1 positive cells *in vivo.*

Example 16: *In vitro* autoradiography of human and xenograft tissue with [^{18}F]-E01 anti-PD-L1 adnectin

[00363] Human lung tumor tissues were embedded in OCT and chilled in 2-methylbutane for 2-5 minutes until frozen. Samples were stored in -80 °C degree freezer until use. Human xenograft tissues were also included in the assay. Mice bearing bilateral xenografts were produced by introducing 4×10^6 hPD-L1(+) L2987 cells and 1.5×10^6 hPD-L1(-) HT-29 t cells subcutaneously into opposite flanks of nu/nu mice. Once resulting xenograft tumors reached appropriate size (approx. 200-300 mm^3), mice were anesthetized with 2% isoflurane and sacrificed via cervical dislocation. Fresh tumor tissues were excised, immersed into OCT and chilled in 2-methylbutane for 2-5 minutes until frozen. The tissues were then wrapped in foil/ZIPLOC® bag and stored at -80 °C until use. For all tissues (human lung tumor and xenografts) sections of 5 µm thickness (collected as 2 sections/slide) were cut using a cryostat, thaw-mounted on glass microscope slides, and allowed to air dry for approximately 30 minutes.

[00364] Blocking studies with cold (unlabeled) A02 Adnectin at 0.025 nM, 0.25 nM, 2.5 nM and 25 nM respectively and 25 nM non-PD-L1 binding Adnectin were conducted using the following conditions. The individual slides, 1 slide per concentration, were placed in plastic slide cassettes and pre-incubated in Dako serum-free protein block solution for 30 minutes. Slides were then transferred to glass slide incubation chambers for further incubation. Separately, a stock solution of 0.25 nM ^{18}F-A02 Adnectin was produced by diluting 10.6 µl of the original stock radioligand solution (7064 nM at the time of experiment) with 300 ml of PBS + 0.5% BSA. From this stock solution, 40 ml was added to each incubation chamber. One of these chambers contained only the radioligand buffer solution, which is referred to as the total binding section. Other incubation chambers received 40 ml of this stock solution along with the relevant concentration of blocking compound (unlabeled A02 Adnectin at 0.025 nM, 0.25 nM, 2.5 nM, or 25 nM or unlabeled non-PD-L1 binding Adnectin at 25 nM). Slides were incubated in the individual buffer solutions for 1 hour at room temperature to reach maximum binding. After incubation, slides from each treatment group were removed from the incubation solutions and placed in an ice-cold wash buffer (PBS + 0.5% BSA) for 3

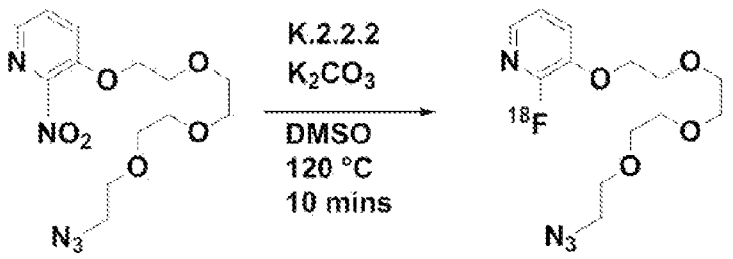
minutes and rinsed 4 separate times. Slides were then dried under a stream of cold air for approximately 30 minutes. The air-dried slides were exposed by placing the slides onto an imaging plate (BAS-SR 3545S) overnight at room temperature. The imaging plate was scanned using the bioimaging analyzer (Fujifilm Fluorescent Image Analyzer, FLA-9000). The pixel size of the autoradiogram images was 100 µm. Image analysis was performed using the Multi-Gauge software. The regions of interest (ROIs) were drawn to surround the entire tumor tissue in all study groups. Autogradiography signal from tissue-associated radioactivity was quantified from these ROIs.

[00365] The apparent displacement of the ^{18}F-A02 Adnectin radioligand when compared to the total binding sections was determined for 4 different concentrations (0.025 nM, 0.25 nM, 2.5 nM and 25 nM) of unlabeled A02 Adnectin in both human lung tumor sections as well as human xenograft sections. A dose dependent displacement of ^{18}F-A02 was seen in all tissue sections with the addition of unlabeled A02 Adnectin, while 25 nM non-PD-L1 binding Adnectin showed minimal blockade in all tissues compared to total binding (**Figure 7**).

[00366] Serial 5 µm tissue sections from each tissue were subjected to an anti-human-PD-L1 immunohistochemical procedure to verify the level of PD-L1 antigen expression in the samples (**Figure 8**).

[00367] Taken together, these results provide direct visualization of PD-L1 in both human lung tumor samples as well as human xenograft tissues. The level of radioligand binding in the individual tissues corresponds with the intensity of PD-L1 staining of frozen sections by IHC. In addition, the dose dependent blockade of the receptor with unlabeled anti-PD-L1 A02 Adnectin (and lack of blockade with unlabeled non-PD-L1 binding Adnectin), further validates the use of ^{18}F-A02 for visualization of PD-L1 tissue expression using PET imaging.

Example 17: Automated preparation of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine according to the general procedure for radiosynthesis using commercial GE TRACERlab FX2 N synthesis unit



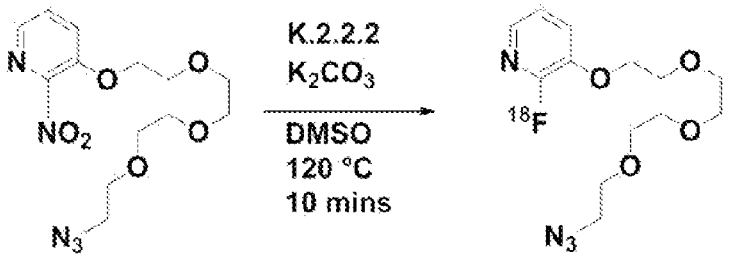
Procedure:

[00368] The automated synthesis of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was carried out using a non-cassette type GE TRACERlab FX2 N Synthesis module. The setup of the synthesis unit is summarized in Table 4. The aqueous [^{18}F]-Fluoride solution (2.0 ml, 29.6 GBq/ 800 mCi) was delivered to a Sep-Pak light QMA [The Sep-Pak light QMA cartridge was pre-conditioned sequentially with 5ml of 0.5 M potassium bicarbonate, 5 ml of deionized water, and 5 ml of acetonitrile before use.] Upon completion of this transfer, the aqueous [^{18}F] fluoride was released from the QMA Sep-Pak by the addition of the elution mixture (from "V1") into the reactor. The solvent was evaporated under a gentle stream of nitrogen and vacuum. The solution of precursor (from "V3") was added to the dried cryptand residue and this reaction mixture was heated 120 °C for 10 minutes. Then 4 ml of distilled water (from "V4") was added to the crude reaction mixture in the reactor and the mixture was transferred to the 5 ml sample injection loop of the semi-preparative HPLC via a liquid sensor which controls the end of the loading. The mixture was loaded onto the semi-preparative HPLC column (Luna C18(2). 250x10mm, Phenomenex). A mixture of 35% acetonitrile in an aqueous 0.1% trifluoroacetic acid solution was flushed through the column at a rate of 4.6 ml per minute. The product was collected from this HPLC column into the dilution flask which contained 15 ml distilled water and its entire contents were transferred to a tC18 1 gram, solid phase extraction cartridge. 352 mCi (13 GBq) of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was released from this cartridge (from "V14") with 3 ml of ethanol and may be used to generate ^{18}F labeled biologic products by taking advantage of "click" azide-alkyne reaction with the appropriate biologic containing an alkynes.

Table 4

Vial 1 (V1)	16 mg K.2.2.2, 3 mg Potassium carbonate, dissolved in 0.1 ml of distilled water and 1.4 ml of acetonitrile
Vial 3 (V3)	2 mg 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine in 0.5 ml DMSO
Vial 4 (V4)	4 ml of distilled water
Vial 14 (V14)	3 ml of 100% ethanol
Dilution Flask	15 ml of distilled water
Cartridge 1 (C1)	tC18 6 cc 1g sep pack
HPLC Column	Luna C18(2), 250x10mm, 5 □m, Phenomenex
HPLC Solvent	35% acetonitrile in an aqueous 0.1% trifluoroacetitic acid solution
HPLC flow	4.6 ml/min

Example 18 Automated preparation of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine according to the general procedure for radiosynthesis on IBA Synthera synthesis unit



Procedure:

[00369] The automated synthesis of [^{18}F]-3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was carried out using a cassette type IBA Synthera Synthesis module and an appropriately assembled integrator fluidic processor kit. The integrator fluidic processor (IFP) kit was loaded with appropriate precursors for this synthesis and is summarized in Table 5. The purification was performed on an Varian HPLC unit. The filling of the injection loop of the HPLC was controlled by a steady stream of nitrogen on the HPLC unit. The setup of both automates are summarized in Table 2. The aqueous [^{18}F]-Fluoride solution (2.0 ml, 29.6 GBq/ 800 mCi) was delivered to a Sep-Pak light QMA [The Sep-Pak light QMA cartridge was pre-conditioned sequentially with 5ml of 0.5 M potassium bicarbonate, 5 ml of deionized water, and 5 ml of acetonitrile before use.] Upon completion of this transfer, the aqueous [^{18}F] fluoride was released from the QMA Sep-Pak by the addition of the elution mixture (from "V1") into the reactor. The solvent was evaporated under a gentle stream of nitrogen and vacuum. The solution of precursor (from "V2") was added to the dried cryptand residue and this reaction mixture was heated 120 °C for 10 minutes. Then 3 ml of distilled water (from "V4") was added to the crude reaction mixture in the reactor and the mixture is transferred to the 5 ml sample injection loop of the semi-preparative HPLC via a liquid sensor which controls the end of the loading. The mixture was loaded onto the semi-preparative HPLC column (Luna C18(2). 250x10mm, Phenomenex). A mixture of 35% acetonitrile in an aqueous 0.1% trifluoroacetic acid solution was flushed through the column at a rate of 4.6 ml per minute. The product was collected from this HPLC column into the dilution flask which contained 15 ml distilled water and its entire contents were transferred to a tC18 1 gram, solid phase extraction cartridge. 325 mCi (12 GBq) of [^{18}F]-3-(2-(2-(2-(2-

azidoethoxy)ethoxy)ethoxy)ethoxy)-2-fluoropyridine was released from this cartridge with 3 ml of ethanol and may be used to generate ^{18}F labeled biologic products by taking advantage of "click" azide-alkyne reaction with the appropriate biologic containing an alkynes.

Table 5

Vial 1 (V1)	22 mg K.2.2.2, 4 mg Potassium carbonate, dissolved in 0.3 ml of distilled water and 0.3 ml of acetonitrile
Vial 2 (V2)	2 mg 3-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)-2-nitropyridine in 0.5 ml DMSO
Vial 4 (V4)	3 ml of distilled water
Dilution Flask	15 ml of distilled water
Cartridge 1 (C1)	tC18 6 cc 1g sep pack
HPLC Column	Luna C18(2), 250x10mm, 5 □m, Phenomenex
HPLC Solvent	35% acetonitrile in an aqueous 0.1% trifluoroacetitic acid solution
HPLC flow	4.6 ml/min

Example 19: Synthesis of ^{68}Ga-based anti-PD-L1 Adnectin probes

Synthesis of ^{68}Ga-E01-NODAGA

[00370] [^{68}Ga]-Gallium chloride in 0.1N hydrochloric acid solution was neutralized with 32 mg of sodium acetate (NaOAc) for 4 minutes at ambient temperature, the resultant solution was stirred to ensure the entire volume was properly mixed. This solution was then added to E01-NODAGA (15μL of 1.3 mg/mL) solution and the crude reaction was gently pipetted to allow mixing followed by resting at ambient temperature for 15 minutes. The contents of crude reaction mixture were transferred to a PD-10 desalting column that was pre-activated with 20 mL of 1x phosphate buffered saline (PBS, pH 7.4) buffer prior to loading of sample. An additional 1.5 mL of 1XPBS was added to the column, followed by an additional 0.8 ml 1XPBS solution and these fractions were discarded. [^{68}Ga]-E01-NODAGA was then collected after a 1.4 mL elution of the PD-10 column to give 5.78 mCi (214 MBq) as the desired product. Quality control was measured using a reverse phase HPLC system using an Agilent PLRP-S HPLC column Size: 250 x 4.60 mm, 8 μm, 280 nm and a mobile phase of 0.1% Formic Acid in distilled water and acetonitrile. A gradient method was used where the percentage of acetonitrile was increased linearly from 10% to 45% over a 30 minute time frame. [^{68}Ga]-E01-NODAGA co-eluted with reference standard at the 22 minute mark of the HPLC chromatogram. Radiochemical purity was measured to be 98% using this method. [^{68}Ga]-E01-NODAGA also co-eluted with reference material at the 20 minute mark using a size exclusion chromatography, (SEC) Column: GE Superdex 200 GL Size: 10 x 300 mm,

280 nm.

1 MGVSD VPRDL EVVAA TPTSL LISWR AQLSP SFYYR ITYGE

41 TGGNS PVQEF TVPND VMTAT ISGLK PGVDY TITVY AVTTH

81 GVYFY SPISI NYRTP CHHHH HH

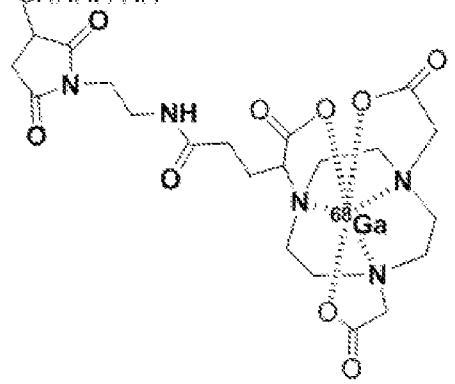


Table 3: SEQUENCE LISTING

SEQ ID	DESCRIPTION	SEQUENCE
1	Full length wild-type human ^{10}Fn3 domain	VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT
2	Core wild-type human ^{10}Fn3 domain	EVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT
3	Core ^{10}Fn3-based scaffold with variable AB, BC, CD, DE, EF, and FG loops	EVVAA(Z)$_a$LLISW(Z)$_x$YRITY(Z)$_b$FTV(Z)$_y$ATISGL(Z)$_c$YTITVYA(Z)$_z$ISINYRT
4	Core ^{10}Fn3-based scaffold with variable BC, DE, and FG loops	EVVAATPTSLLISW(Z)$_x$YRITYGETGGNSPVQEFTV(Z)$_y$ATISGLKPGVDYTITVYA(Z)$_z$ISINYRT
5	ATI-968 core (aka ADX_1760_C01)	EVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRT
6	ATI-968 BC loop	IAPFYNVIY
7	ATI-968 DE loop	PGTGYT
8	ATI-968 FG loop	VTDGASIASYAFP
9	ATI-968 w/ N leader	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRT
10	ATI-968 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTHHHHHH
11	ATI-968 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTEIDKPSQ
12	ATI-968 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTEIDKPSQHHHHHH
13	ATI-968 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTPC
14	ATI-968 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTPCHHHHHH
15	ATI-968 – full length	MGVSDVPRDLEVVAATPTSLLISWIAPFYNVIYYRITYGETGGNSPVQEFTVPGTGYTATISGLKPGVDYTITVYAVTDGASIASYAFPISINYRTEIDKPSQHHHHHH
16	ATI-968 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGATCGCTCCGTTCTACAATGTCATCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTGGTACTGGTTATACAGCTACAATCAGCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGTCACTGATGGAGCATCCATTGCTTCATACGCGTTTCCAATTTCCATTAATTACCGCACA
17	ATI-968 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGATCGCTCCGTTCTACAATGTCATCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTGGTACTGGTTATACAGCTACAATCAGCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGTCACTGATGGAGCATCCATTGCTTCATACGCGTTTCCAATTTCCATTAATTACCGCACA
18	ATI-968 w/ N leader and	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA

	modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	CCCCCACCAGCCTGCTGATCAGCTGGATCGCTCCGTTCTACAATGT CATCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTGGTACTGGTTATACAGCTACAATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTGATGGAGCATCCATTGCTTCATACGCGTTTCCAATTTCCATT AATTACCGCACACCGTGC
19	ATI-968 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGATCGCTCCGTTCTACAATGT CATCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTGGTACTGGTTATACAGCTACAATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTGATGGAGCATCCATTGCTTCATACGCGTTTCCAATTTCCATT AATTACCGCACAGAAATTGACAAACCATCCCAGCACCATCACCACC ACCACTGA
20	ATI-964 core (parent of ADX_5322_A02)	EVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPVQEFTVPPDQ KTATISGLKPGVDYTITVYAVRLEEAHYYRESPISINYRT
21	ATI-964 BC loop	SYDGSIERY
22	ATI-964 DE loop	PPDQKT
23	ATI-964 FG loop	VRLEEAHYYRESP
24	ATI-964 w/ N leader	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRT
25	ATI-964 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRTHHHHHH
26	ATI-964 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRTEIDKPSQ
27	ATI-964 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRTEIDKPSQHHHHHH
28	ATI-964 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRTPC
29	ATI-964 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISIN YRTPCHHHHHH
30	ATI-964 – full length	MGVSDVPRDLEVVAATPTSLLISWSYDGSIERYYRITYGETGGNSP VQEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYYRESPISI NYRTEIDKPSQHHHHHH
31	ATI-964 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGTCTT ACGACGGTTCGATTGAACGTTATTACCGCATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTCCGGATCAG AAGACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCAGGCTGGAAGAAGCTCATTACTATCGAGA GTCTCCAATTTCCATTAATTACCGCACA
32	ATI-964 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGACGGTTCGATTGA ACGTTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CAGGCTGGAAGAAGCTCATTACTATCGAGAGTCTCCAATTTCCATT AATTACCGCACA
33	ATI-964 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGACGGTTCGATTGA ACGTTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT

	methionine)	CAGGCTGGAAGAAGCTCATTACTATCGAGAGTCTCCAATTTCCATT AATTACCGCACACCGTGC
34	ATI-964 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGACGGTTCGATTGA ACGTTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CAGGCTGGAAGAAGCTCATTACTATCGAGAGTCTCCAATTTCCATT AATTACCGCACAGAAATTGACAAACCATCCCAGCACCATCACCACC ACCACTGA
35	ATI-965 core	EVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPVQEFTVGPRH HTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISINYRT
36	ATI-965 BC loop	TAYDSVDKY
37	ATI-965 DE loop	GPRHHT
38	ATI-965 FG loop	VYHTEPGYHAHMP
39	ATI-965 w/ N leader	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRT
40	ATI-965 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRTHHHHHH
41	ATI-965 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRTEIDKPSQ
42	ATI-965 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRTEIDKPSQHHHHHH
43	ATI-965 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRTPC
44	ATI-965 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGETGGNSPV QEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHAHMPISIN YRTPCHHHHHH
45	ATI-965 – full length	MGVSDVPRDLEVVAATPTSLLISWTAYDSVDKYYRITYGE TGGNSPVQEFTVGPRHHTATISGLKPGVDYTITVYAVYHTEPGYHA HMPISINYRTEIDKPSQHHHHHH
46	ATI-965 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGACTG CATACGACTCTGTTGACAAATATTACCGCATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGGGCCCTAGACAT CACACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCTATCACACTGAACCGGGCTATCATGCTCA TATGCCAATTTCCATTAATTACCGCACA
47	ATI-965 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGACTGCATACGACTCTGTTGA CAAATATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGGCCCTAGACATCACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CTATCACACTGAACCGGGCTATCATGCTCATATGCCAATTTCCATT AATTACCGCACA
48	ATI-965 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGACTGCATACGACTCTGTTGA CAAATATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGGCCCTAGACATCACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CTATCACACTGAACCGGGCTATCATGCTCATATGCCAATTTCCATT AATTACCGCACACCGTGC
49	ATI-965 w/ N leader and C tail + his tag (nucleotide	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGACTGCATACGACTCTGTTGA

	sequence with N-terminal methionine)	CAAATATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGGCCCTAGACATCACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CTATCACACTGAACCGGGCTATCATGCTCATATGCCAATTTCCATT AATTACCGCACAGAAATTGACAAACCATCCCAGCACCATCACCACC ACCACTGA
50	ATI-966 core	EVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPVQEFTVAGSV NTATISGLKPGVDYTITVYAVTIHNVSFPISINYRT
51	ATI-966 BC loop	HRFSSIMAY
52	ATI-966 DE loop	AGSVNT
53	ATI-966 FG loop	VTIHNVSFP
54	ATI-966 w/ N leader	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRT
55	ATI-966 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRTH HHHHH
56	ATI-966 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRTE IDKPSQ
57	ATI-966 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRTE IDKPSQHHHHHH
58	ATI-966 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRTP C
59	ATI-966 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSPV QEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRTP CHHHHHH
60	ATI-966 – full length	MGVSDVPRDLEVVAATPTSLLISWHRFSSIMAYYRITYGETGGNSP VQEFTVAGSVNTATISGLKPGVDYTITVYAVTIHNVSFPISINYRT EIDKPSQHHHHHH
61	ATI-966 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGCATA GGTTCTCTTCTATCATGGCGTATTACCGCATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGGCTGGCTCTGTT AACACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCACGATCCATAACGTTTCTTTCCCAATTTC CATTAATTACCGCACA
62	ATI-966w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCATAGGTTCTCTTCTATCAT GGCGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGCTGGCTCTGTTAACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACGATCCATAACGTTTCTTTCCCAATTTCCATTAATTACCGCACA
63	ATI-966 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCATAGGTTCTCTTCTATCAT GGCGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGCTGGCTCTGTTAACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACGATCCATAACGTTTCTTTCCCAATTTCCATTAATTACCGCACA CCGTGC
64	ATI-966 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCATAGGTTCTCTTCTATCAT GGCGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGGCTGGCTCTGTTAACACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACGATCCATAACGTTTCTTTCCCAATTTCCATTAATTACCGCACA GAAATTGACAAACCATCCCAGCACCATCACCACCACCACTGA
65	ATI-967 core (parent of	EVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPVQEFTVPVAS

	ADX_5417_E01)	GTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYRT
66	ATI-967 BC loop	QGQLSPSFY
67	ATI-967 DE loop	PVASGT
68	ATI-967 FG loop	VTSHGIYFYAP
69	ATI-967 w/ N leader	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR T
70	ATI-967 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR THHHHHH
71	ATI-967 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR TEIDKPSQ
72	ATI-967 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR TEIDKPSQHHHHHH
73	ATI-967 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR TPC
74	ATI-967 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSPV QEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINYR TPCHHHHHH
75	ATI-967 – full length	MGVSDVPRDLEVVAATPTSLLISWQGQLSPSFYYRITYGETGGNSP VQEFTVPVASGTATISGLKPGVDYTITVYAVTSHGIYFYAPISINY RTEIDKPSQHHHHHH
76	ATI-967 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGCAGG GACAGCTGTCTCCGTCTTTCTATTACCGAATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTGTTGCTAGT GGGACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCACTTCTCATGGCATATACTTCTACGCTCC AATTTCCATTAATTACCGCACA
77	ATI-967 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCAGGGACAGCTGTCTCCGTC TTTCTATTACCGAATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTGTTGCTAGTGGGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTTCTCATGGCATATACTTCTACGCTCCAATTTCCATTAATTAC CGCACA
78	ATI-967 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCAGGGACAGCTGTCTCCGTC TTTCTATTACCGAATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTGTTGCTAGTGGGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTTCTCATGGCATATACTTCTACGCTCCAATTTCCATTAATTAC CGCACACCGTGC
79	ATI-967 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGCAGGGACAGCTGTCTCCGTC TTTCTATTACCGAATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTGTTGCTAGTGGGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTTCTCATGGCATATACTTCTACGCTCCAATTTCCATTAATTAC CGCACAGAAATTGACAAACCATCCCAGCACCATCACCACCACCACT GAT
80	ADX_5322_A02 core	EVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPVQEFTVPPDQ KTATISGLKPGVDYTITVYAVRLEEAHYNREFPISINYRT
81	ADX_5322_A02 BC loop	SYDGPIDRY
82	ADX_5322_A02 DE loop	PPDQKT

83	ADX_5322_A02 FG loop	VRLEEAHYNREFP
84	ADX_5322_A02 w/ N leader	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRT
85	ADX_5322_A02 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTHHHHHH
86	ADX_5322_A02 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTEIDKPSQ
87	ADX_5322_A02 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTEIDKPSQHHHHHH
88	ADX_5322_A02 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTPC
89	ADX_5322_A02 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTPCHHHHHH
90	ADX_5322_A02-Mal-DBCO-FFPF18	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTPC-[Maleamide-DBCO-FFP(18F)]
91	ADX_5322_A02 full length	MGVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSP VQEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISI NYRTPCHHHHHH
92	ADX_5322_A02 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGTCTT ACGATGGCCCAATTGACCGGTATTACCGCATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTCCGGATCAG AAGACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCCGGCTGGAAGAAGCTCATTACAATCGAGA GTTTCCAATTTCCATTAATTACCGCACA
93	ADX_5322_A02 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGATGGCCCAATTGA CCGGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CCGGCTGGAAGAAGCTCATTACAATCGAGAGTTTCCAATTTCCATT AATTACCGCACA
94	ADX_5322_A02 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGATGGCCCAATTGA CCGGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CCGGCTGGAAGAAGCTCATTACAATCGAGAGTTTCCAATTTCCATT AATTACCGCACACCGTGC
95	ADX_5322_A02 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGTCTTACGATGGCCCAATTGA CCGGTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTCCGGATCAGAAGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CCGGCTGGAAGAAGCTCATTACAATCGAGAGTTTCCAATTTCCATT AATTACCGCACACCGTGCCACCATCACCACCACCACTGA
96	ADX_5417_E01 core	EVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPVQEFTVPNDV MTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYRT
97	ADX_5417_E01 BC loop	RAQLSPSFY
98	ADX_5417_E01 DE loop	PNDVMT
99	ADX_5417_E01 FG loop	VTTHGVYFYSP

100	ADX_5417_E01 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR T
101	ADX_5417_E01 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR THHHHHH
102	ADX_5417_E01 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR TEIDKPSQ
103	ADX_5417_E01 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR TEIDKPSQHHHHHH
104	ADX_5417_E01 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR TPC
105	ADX_5417_E01 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSPV QEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINYR TPCHHHHHH
106	ADX-5417_E01 –Mal-DBCO-FFPF18	GVSDVPRDLEVVAATPTSLLISWSYDGPIDRYYRITYGETGGNSPV QEFTVPPDQKTATISGLKPGVDYTITVYAVRLEEAHYNREFPISIN YRTPC-[Maleamide-DBCO-FFP(18F)]
107	ADX-5417_E01 full length	MGVSDVPRDLEVVAATPTSLLISWRAQLSPSFYYRITYGETGGNSP VQEFTVPNDVMTATISGLKPGVDYTITVYAVTTHGVYFYSPISINY RTPCHHHHHH
108	ADX-5417_E01 – core (nucleotide sequence)	GAAGTGGTTGCTGCCACCCCCACCAGCCTGCTGATCAGCTGGAGGG CTCAGCTGTCTCCGTCTTTCTATTACCGCATCACTTACGGCGAAAC AGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTAATGATGTA ATGACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCA TCACTGTGTATGCTGTCACTACTCATGGTGTTTATTTCTACTCACC AATTTCCATTAATTACCGCACA
109	ADX_5417_E01 w/ N leader (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGAGGGCTCAGCTGTCTCCGTC TTTCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTAATGATGTAATGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTACTCATGGTGTTTATTTCTACTCACCAATTTCCATTAATTAC CGCACA
110	ADX_5417_E01 w/ N leader and modified C-terminus including PC (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGAGGGCTCAGCTGTCTCCGTC TTTCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTAATGATGTAATGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTACTCATGGTGTTTATTTCTACTCACCAATTTCCATTAATTAC CGCACACCGTGC
111	ADX_5417_E01 w/ N leader and C tail + his tag (nucleotide sequence with N-terminal methionine)	ATGGGAGTTTCTGATGTGCCGCGCGACCTGGAAGTGGTTGCTGCCA CCCCCACCAGCCTGCTGATCAGCTGGAGGGCTCAGCTGTCTCCGTC TTTCTATTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCT GTCCAGGAGTTCACTGTGCCTAATGATGTAATGACAGCTACCATCA GCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATGCTGT CACTACTCATGGTGTTTATTTCTACTCACCAATTTCCATTAATTAC CGCACACCGTGCCACCATCACCACCACCACTGA
112	ATI_1420_A10 core	EVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKA TISGLKPGVDYTITVYAIRHPGMLEFGISINYRT
113	ATI_1420_A10 BC loop	PYPSYYIE
114	ATI_1420_A10 DE loop	IRHPGMLEFG
115	ATI_1420_A10 FG loop	VTDGASIASYAFP
116	ATI_1420_A10 w/ N	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQ

	leader	EFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRT
117	ATI_1420_A10 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTHHHHHH
118	ATI_1420_A10 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTEIDKPSQ
119	ATI_1420_A10w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTEIDKPSQHHHHHH
120	ATI_1420_A10w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTPC
121	ATI_1420_A10 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTPCHHHHHH
122	ATI_1420_A10– full length	MGVSDVPRDLEVVAATPTSLLISWPYPSYYIEYRITYGETGGNSPVQEFTVQSMKATISGLKPGVDYTITVYAIRHPGMLEFGISINYRTEIDKPSQHHHHHH
123	ATI_1420_B09 core	EVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRT
124	ATI_1420_B09 BC loop	HKFSSLMS
125	ATI_1420_B09 DE loop	GSVN
126	ATI_1420_B09 FG loop	IHNVGF
127	ATI_1420_B09 w/ N leader	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRT
128	ATI_1420_B09 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTHHHHHH
129	AATI_1420_B09 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTEIDKPSQ
130	ATI_1420_B09 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTEIDKPSQHHHHHH
131	ATI_1420_B09 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTPC
132	ATI_1420_B09 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTPCHHHHHH
133	ATI_1420_B09– full length	MGVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTEIDKPSQHHHHHH
134	ATI_1420_C02 core	EVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRT
135	ATI_1420_C02 BC loop	RIKSYYA
136	ATI_1420_C02 DE loop	RQHV
137	ATI_1420_C02 FG loop	RLGDVELVYE
138	ATI_1420_C02 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRT
139	ATI_1420_C02 w/ N	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSP

	leader + his tag	VQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTHHHHHH
140	ATI_1420_C02 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRTEIDKPSQ
141	ATI_1420_C02 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRTEIDKPSQHHHHHH
142	ATI_1420_C02 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRTPC
143	ATI_1420_C02 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWHKFSSLMSYRITYGETGGNSPVQEFTVGSVNATISGLKPGVDYTITVYAIHNVGFISINYRTPCHHHHHH
144	ATI_1420_C02– full length	MGVSDVPRDLEVVAATPTSLLISWRIKSYYAYRITYGETGGNSPVQEFTVRQHVATISGLKPGVDYTITVYARLGDVELVYEISINYRTEIDKPSQHHHHHH
145	ATI_1420_C11 core	EVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRT
146	ATI_1420_C11 BC loop	MYPLKSVP
147	ATI_1420_C11 DE loop	YSSG
148	ATI_1420_C11 FG loop	MSYSTYHAFM
149	ATI_1420_C11 w/ N leader	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRT
150	ATI_1420_C11 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTHHHHHH
151	ATI_1420_C11 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTEIDKPSQ
152	ATI_1420_C11 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTEIDKPSQHHHHHH
153	ATI_1420_C11 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTPC
154	ATI_1420_C11 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTPCHHHHHH
155	AATI_1420_C11– full length	MGVSDVPRDLEVVAATPTSLLISWMYPLKSVPYRITYGETGGNSPVQEFTVYSSGATISGLKPGVDYTITVYAMSYSTYHAFMISINYRTEIDKPSQHHHHHH
156	ATI_1420_D01 core	EVVAATPTSLLISWRTVPETDYRITYGETGGNSPVQEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT
157	ATI_1420_D01 BC loop	RTVPETD
158	ATI_1420_D01 DE loop	PDNT
159	ATI_1420_D01 FG loop	LETAHYNRDY
160	ATI_1420_D01 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPVQEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT
161	ATI_1420_D01 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPVQEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRTHHHHHH

162	ATI_1420_D01 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPV QEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT EIDKPSQ
163	ATI_1420_D01 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPV QEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT EIDKPSQHHHHHH
164	ATI_1420_D01 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPV QEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT PC
165	ATI_1420_D01 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSPV QEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYRT PCHHHHHH
166	ATI_1420_D01– full length	MGVSDVPRDLEVVAATPTSLLISWRTVPETDYRITYGETGGNSP VQEFTVPDNTATISGLKPGVDYTITVYALETAHYNRDYISINYR TEIDKPSQHHHHHH
167	ATI_1420_D05 core	EVVAATPTSLLISWTAYYSTIKYRITYGETGGNSPVQEFTVGPK HHATISGLKPGVDYTITVYAYNTKPGYHAHQISINYRT
168	ATI_1420_D05 BC loop	TAYYSTIK
169	ATI_1420_D05 DE loop	GPKHH
170	ATI_1420_D05 FG loop	YNTKPGYHAHQ
171	ATI_1420_D05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRT
172	ATI_1420_D05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRTHHHHHH
173	ATI_1420_D05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRTEIDKPSQ
174	ATI_1420_D05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRTEIDKPSQHHHHHH
175	ATI_1420_D05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRTPC
176	ATI_1420_D05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNSP VQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISIN YRTPCHHHHHH
177	ATI_1420_D05– full length	MGVSDVPRDLEVVAATPTSLLISWTAYYSTIKYRITYGETGGNS PVQEFTVGPKHHATISGLKPGVDYTITVYAYNTKPGYHAHQISI NYRTEIDKPSQHHHHHH
178	ATI_1420_D10 core	EVVAATPTSLLISWRIPSYHIQYRITYGETGGNSPVQEFTVYQK YATISGLKPGVDYTITVYAVSPPKQLRFGISINYRT
179	ATI_1420_D10 BC loop	RIPSYHIQ
180	ATI_1420_D10 DE loop	YQKY
181	ATI_1420_D10 FG loop	VSPPKQLRFG
182	ATI_1420_D10 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR T
183	ATI_1420_D10 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR THHHHHH

184	ATI_1420_D10 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR TEIDKPSQ
185	ATI_1420_D10 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR TEIDKPSQHHHHHH
186	ATI_1420_D10 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR TPC
187	ATI_1420_D10 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNSP VQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINYR TPCHHHHHH
188	ATI_1420_D010– full length	MGVSDVPRDLEVVAATPTSLLISWRIPSYHIQYRITYGETGGNS PVQEFTVYQKYATISGLKPGVDYTITVYAVSPPKQLRFGISINY RTEIDKPSQHHHHHH
189	ATI_1420_F10_core	EVVAATPTSLLISWPAPPSYVFYRITYGETGGNSPVQEFTVYPY MATISGLKPGVDYTITVYAYTSGFSISINYRT
190	ATI_1420_F10 BC loop	PAPPSYVF
191	ATI_1420_F10 DE loop	YPYM
192	ATI_1420_F10 FG loop	YTSGFS
193	ATI_1420_F10 w/ N leader	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRT
194	ATI_1420_F10 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTHHH HHH
195	ATI_1420_F10 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTEID KPSQ
196	ATI_1420_F10 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTEID KPSQHHHHHH
197	ATI_1420_F10 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTPC
198	ATI_1420_F10 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNSP VQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTPCH HHHHH
199	ATI_1420_F10– full length	MGVSDVPRDLEVVAATPTSLLISWPAPPSYVFYRITYGETGGNS PVQEFTVYPYMATISGLKPGVDYTITVYAYTSGFSISINYRTEI DKPSQHHHHHH
200	ATI_1421_C05_core	EVVAATPTSLLISWYMDHKSKYRITYGETGGNSPVQEFTVPDQR ATISGLKPGVDYTITVYALSEAHYLRDKISINYRT
201	ATI_1421_C05 BC loop	YMDHKSK
202	ATI_1421_C05 DE loop	PDQR
203	ATI_1421_C05 FG loop	LSEAHYLRDK
204	ATI_1421_C05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT
205	ATI_1421_C05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT HHHHHH
206	ATI_1421_C05 w/ N	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT

	leader and C tail	EIDKPSQ
207	ATI_1421_C05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT EIDKPSQHHHHHH
208	ATI_1421_C05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT PC
209	ATI_1421_C05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSPV QEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYRT PCHHHHHH
210	ATI_1421_C05– full length	MGVSDVPRDLEVVAATPTSLLISWYMDHKSKYRITYGETGGNSP VQEFTVPDQRATISGLKPGVDYTITVYALSEAHYLRDKISINYR TEIDKPSQHHHHHH
211	ATI_1421_C06 core	EVVAATPTSLLISWENLASYQYRITYGETGGNSPVQEFTVPDQA ATISGLKPGVDYTITVYALQTAHYYRQHISINYRT
212	ATI_1421_C06 BC loop	ENLASYQ
213	ATI_1421_C06DE loop	PDQA
214	ATI_1421_C06FG loop	LQTAHYYRQH
215	ATI_1421_C06 w/ N leader	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT
216	ATI_1421_C06 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT HHHHHH
217	ATI_1421_C06w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT EIDKPSQ
218	ATI_1421_C06 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT EIDKPSQHHHHHH
219	ATI_1421_C06 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT PC
220	ATI_1421_C06 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYRT PCHHHHHH
221	ATI_1421_C06– full length	MGVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSP VQEFTVPDQAATISGLKPGVDYTITVYALQTAHYYRQHISINYR TEIDKPSQHHHHHH
222	ATI 1421_D05 core	EVVAATPTSLLISWYYVQYNDYRITYGETGGNSPVQEFTVPDQS ATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
223	ATI 1421_D05 BC loop	YYVQYND
224	ATI 1421_D05 DE loop	PDQS
225	ATI 1421_D05 FG loop	LEKAHYYRQN
226	ATI 1421_D05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
227	ATI 1421_D05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT HHHHHH
228	ATI 1421_D05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQ

229	ATI 1421_D05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQHHHHHH
230	ATI 1421_D05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PC
231	ATI 1421_D05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PCHHHHHH
232	ATI 1421_D05 – full length	MGVSDVPRDLEVVAATPTSLLISWYYVQYNDYRITYGETGGNSP VQEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYR TEIDKPSQHHHHHH
233	ATI_1421_D06 core	EVVAATPTSLLISWGHNYDDEYRITYGETGGNSPVQEFTVPDQY ATISGLKPGVDYTITVYALAEAHVRKNHISINYRT
234	ATI_1421_D06 BC loop	GHNYDDE
235	ATI_1421_D06 DE loop	PDQY
236	ATI_1421_D06 FG loop	LAEAHVRKNH
237	ATI_1421_D06 w/ N leader	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT
238	ATI_1421_D06 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT HHHHHH
239	ATI_1421_D06 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT EIDKPSQ
240	ATI_1421_D06 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT EIDKPSQHHHHHH
241	ATI_1421_D06 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT PC
242	ATI_1421_D06 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSPV QEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYRT PCHHHHHH
243	ATI_1421_D06 – full length	MGVSDVPRDLEVVAATPTSLLISWGHNYDDEYRITYGETGGNSP VQEFTVPDQYATISGLKPGVDYTITVYALAEAHVRKNHISINYR TEIDKPSQHHHHHH
244	ATI_1421_E03 core	EVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQK ATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
245	ATI_1421_E03 BC loop	VYHYDAQ
246	ATI_1421_E03 DE loop	PDQK
247	ATI_1421_E03 FG loop	LSEAHHKRDS
248	ATI_1421_E03 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT HHHHHH
249	ATI_1421_E03w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQ
250	ATI_1421_E03w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQHHHHHH
251	ATI_1421_E03 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT

		PC
252	ATI_1421_E03 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT PCHHHHHH
253	ATI_1421_E03 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
254	ATI_1421_E03 – full length	MGVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSP VQEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYR TEIDKPSQHHHHHH
255	ATI_1421_E04 core	EVVAATPTSLLISWSYNGPIEYRITYGETGGNSPVQEFTVPDQQ ATISGLKPGVDYTITVYALEEAHYSRQSISINYRT
256	ATI_1421_E04 BC loop	SYNGPIE
257	ATI_1421_E04 DE loop	PDQQ
258	ATI_1421_E04 FG loop	LEEAHYSRQS
259	ATI_1421_E04 w/ N leader	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT
260	ATI_1421_E04 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT HHHHHH
261	ATI_1421_E04 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT EIDKPSQ
262	ATI_1421_E04 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT EIDKPSQHHHHHH
263	ATI_1421_E04 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT PC
264	ATI_1421_E04 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSPV QEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYRT PCHHHHHH
265	ATI_1421_E04– full length	MGVSDVPRDLEVVAATPTSLLISWSYNGPIEYRITYGETGGNSP VQEFTVPDQQATISGLKPGVDYTITVYALEEAHYSRQSISINYR TEIDKPSQHHHHHH
266	ATI_1421_F03 core	EVVAATPTSLLISWISVQTYDYRITYGETGGNSPVQEFTVPDQS ATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
267	ATI_1421_F03 BC loop	ISVQTYD
268	ATI_1421_F03 DE loop	PDQS
269	ATI_1421_F03 FG loop	LEKAHYYRQN
270	ATI_1421_F03 w/ N leader	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
271	ATI_1421_F03 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT HHHHHH
272	ATI_1421_F03 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQ
273	ATI_1421_F03 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQHHHHHH
274	ATI_1421_F03 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PC

275	ATI_1421_F03 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PCHHHHHH
276	ATI_1421_F03– full length	MGVSDVPRDLEVVAATPTSLLISWISVQTYDYRITYGETGGNSP VQEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYR TEIDKPSQHHHHHH
277	ATI_1421_F05 core	EVVAATPTSLLISWLARHDARYRITYGETGGNSPVQEFTVPDRM ATISGLKPGVDYTITVYALEQAHYYRLYISINYRT
278	ATI_1421_F05 BC loop	LARHDAR
279	ATI_1421_F05 DE loop	PDRM
280	ATI_1421_F05 FG loop	LEQAHYYRLY
281	ATI_1421_F05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT
282	ATI_1421_F05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT HHHHHH
283	ATI_1421_F05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT EIDKPSQ
284	ATI_1421_F05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT EIDKPSQHHHHHH
285	ATI_1421_F05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT PC
286	ATI_1421_F05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSPV QEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYRT PCHHHHHH
287	ATI_1421_F05– full length	MGVSDVPRDLEVVAATPTSLLISWLARHDARYRITYGETGGNSP VQEFTVPDRMATISGLKPGVDYTITVYALEQAHYYRLYISINYR TEIDKPSQHHHHHH
288	ATI_1421_G07 core	EVVAATPTSLLISWHSPTSGITYRITYGETGGNSPVQEFTVPYD PSATISGLKPGVDYTITVYAPYGSQYYPGYHISINYRT
289	ATI_1421_G07 BC loop	HSPTSGIT
290	ATI_1421_G07 DE loop	PYDPS
291	ATI_1421_G07 FG loop	PYGSQYYPGYH
292	ATI_1421_G07 w/ N leader	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRT
293	ATI_1421_G07 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRTHHHHHH
294	ATI_1421_G07 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRTEIDKPSQ
295	ATI_1421_G07 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRTEIDKPSQHHHHHH
296	ATI_1421_G07 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRTPC
297	ATI_1421_G07 w/ N leader and modified C-terminus including PC +	GVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSP VQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISIN YRTPCHHHHHH

	his tag	
298	ATI_1421_G07– full length	MGVSDVPRDLEVVAATPTSLLISWHSPTSGITYRITYGETGGNSPVQEFTVPYDPSATISGLKPGVDYTITVYAPYGSQYYPGYHISINYRTEIDKPSQHHHHHH
299	ATI_1421_H03 core	EVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRT
300	ATI_1421_H03 BC loop	VYHYDAQ
301	ATI_1421_H03 DE loop	PDSS
302	ATI_1421_H03 FG loop	LEQAHIDRTT
303	ATI_1421_H03 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRT
304	ATI_1421_H03 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTHHHHHH
305	ATI_1421_H03 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTEIDKPSQ
306	ATI_1421_H03 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTEIDKPSQHHHHHH
307	ATI_1421_H03 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTPC
308	ATI_1421_H03 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTPCHHHHHH
309	ATI_1421_H03 – full length	MGVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDSSATISGLKPGVDYTITVYALEQAHIDRTTISINYRTEIDKPSQHHHHHH
310	ATI_1421_H05 core	EVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRT
311	ATI_1421_H05 BC loop	TSVLLKD
312	ATI_1421_H05 DE loop	PDQH
313	ATI_1421_H05 FG loop	LQNAHHERLY
314	ATI_1421_H05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRT
315	ATI_1421_H05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTHHHHHH
316	ATI_1421_H05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTEIDKPSQ
317	ATI_1421_H05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTEIDKPSQHHHHHH
318	ATI_1421_H05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTPC
319	ATI_1421_H05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTPCHHHHHH

320	ATI_1421_H05– full length	MGVSDVPRDLEVVAATPTSLLISWTSVLLKDYRITYGETGGNSPVQEFTVPDQHATISGLKPGVDYTITVYALQNAHHERLYISINYRTEIDKPSQHHHHHH
321	ATI_1422_E06 core	EVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRT
322	ATI_1422_E06 BC loop	LPSYYIT
323	ATI_1422_E06 DE loop	SKDL
324	ATI_1422_E06 FG loop	FNGSSYYTFG
325	ATI_1422_E06 w/ N leader	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRT
326	ATI_1422_E06 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRTEIDKPSQHHHHHH
327	ATI_1422_E06 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRTEIDKPSQHHHHHH
328	ATI_1422_E06 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRTPC
329	ATI_1422_E06 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRTPCHHHHHH
330	ATI_1422_E06 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRTEIDKPSQHHHHHH
331	ATI_1422_E06– full length	MGVSDVPRDLEVVAATPTSLLISWLPSYYITYRITYGETGGNSPVQEFTVSKDLATISGLKPGVDYTITVYAFNGSSYYTFGISINYRT
332	ATI_1422_F04 core	EVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRT
333	ATI_1422_F04 BC loop	SIPSYFIS
334	ATI_1422_F04 DE loop	YKNY
335	ATI_1422_F04 FG loop	SEGIMFYN
336	ATI_1422_F04w/ N leader	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRT
337	ATI_1422_F04w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTHHHHHH
338	ATI_1422_F04 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTEIDKPSQ
339	ATI_1422_F04 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTEIDKPSQHHHHHH
340	ATI_1422_F04 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTPC
341	ATI_1422_F04 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTPCHHHHHH
342	ATI_1422_F04– full length	MGVSDVPRDLEVVAATPTSLLISWSIPSYFISYRITYGETGGNSPVQEFTVYKNYATISGLKPGVDYTITVYASEGIMFYNISINYRTEIDKPSQHHHHHH
343	ATI_1422_F05 core	EVVAATPTSLLISWPYPRGPYVFYRITYGETGGNSPVQEFTVYP

		GQATISGLKPGVDYTITVYAYTSGYVISINYRT
344	ATI_1422_F05 BC loop	PYPRGPYVF
345	ATI_1422_F05 DE loop	YPGQ
346	ATI_1422_F05 FG loop	YTSGYV
347	ATI_1422_F05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRT
348	ATI_1422_F05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTEI DKPSQHHHHHH
349	ATI_1422_F05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTEI DKPSQ
350	ATI_1422_F05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTEI DKPSQHHHHHH
351	ATI_1422_F05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTPC
352	ATI_1422_F05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGNS PVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTPC HHHHHH
353	ATI_1422_F05– full length	MGVSDVPRDLEVVAATPTSLLISWPYPRGPYVFYRITYGETGGN SPVQEFTVYPGQATISGLKPGVDYTITVYAYTSGYVISINYRTE IDKPSQHHHHHH
354	ATI_1422_H04 core	EVVAATPTSLLISWYLPSYYVQYRITYGETGGNSPVQEFTVKSY NATISGLKPGVDYTITVYARMGVYYLSYSISINYRT
355	ATI_1422_H04 BC loop	YLPSYYVQ
356	ATI_1422_H04 DE loop	KSYN
357	ATI_1422_H04 FG loop	RMGVYYLSYS
358	ATI_1422_H04 w/ N leader	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR T
359	ATI_1422_H04 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR THHHHHH
360	ATI_1422_H04 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR TEIDKPSQ
361	ATI_1422_H04 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR TEIDKPSQHHHHHH
362	ATI_1422_H04 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR TPC
363	ATI_1422_H04 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNSP VQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINYR TPCHHHHHH
364	ATI_1422_H04 – full length	MGVSDVPRDLEVVAATPTSLLISWYLPSYYVQYRITYGETGGNS PVQEFTVKSYNATISGLKPGVDYTITVYARMGVYYLSYSISINY RTEIDKPSQHHHHHH
365	ATI_1422_H05 core	EVVAATPTSLLISWQGQLSPSFYRITYGETGGNSPVQEFTVVAG MATISGLKPGVDYTITVYATSDVYFYSISINYRT
366	ATI_1422_H05 BC loop	QGQLSPSF

367	ATI_1422_H05 DE loop	VAGM
368	ATI_1422_H05 FG loop	TSDVYFYS
369	ATI_1422_H05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRT
370	ATI_1422_H05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRTH HHHHH
371	ATI_1422_H05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRTE IDKPSQ
372	ATI_1422_H05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRTE IDKPSQHHHHHH
373	ATI_1422_H05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRTP C
374	ATI_1422_H05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNSP VQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRTP CHHHHHH
375	– full length	MGVSDVPRDLEVVAATPTSLLISWQGQLSPSFYRITYGETGGNS PVQEFTVVAGMATISGLKPGVDYTITVYATSDVYFYSISINYRT EIDKPSQHHHHHH
376	ATI_1422_G05 core	EVVAATPTSLLISWIAPYYSVIYRITYGETGGNSPVQEFTVTGS GYATISGLKPGVDYTITVYATYCASVASYAFISINYRT
377	ATI_1422_G05 BC loop	IAPYYSVI
378	ATI_1422_G05 DE loop	TGSGY
379	ATI_1422_G05 FG loop	TYCASVASYAF
380	ATI_1422_G05 w/ N leader	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRT
381	ATI_1422_G05 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRTHHHHHH
382	ATI_1422_G05 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRTEIDKPSQ
383	ATI_1422_G05 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRTEIDKPSQHHHHHH
384	ATI_1422_G05 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRTPC
385	ATI_1422_G05 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISIN YRTPCHHHHHH
386	ATI_1422_G05– full length	MGVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNS PVQEFTVTGSGYATISGLKPGVDYTITVYATYCASVASYAFISI NYRTEIDKPSQHHHHHH
387	ATI_1760_C02 core	EVVAATPTSLLISWIAPYYSVIYRITYGETGGNSPVQEFTVPGS AYATISGLKPGVDYTITVYASSGASIAAYAFISINYRT
388	ATI_1760_C02 BC loop	IAPYYSVI
389	ATI_1760_C02 DE loop	PGSAY

390	ATI_1760_C02 FG loop	SSGASIAAYAF
391	ATI_1760_C02 w/ N leader	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRT
392	ATI_1760_C02 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRTHHHHHH
393	ATI_1760_C02 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRTEIDKPSQ
394	ATI_1760_C02 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRTEIDKPSQHHHHHH
395	ATI_1760_C02 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRTPC
396	ATI_1760_C02 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNSP VQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISIN YRTPCHHHHHH
397	ATI_1760_C02– full length	MGVSDVPRDLEVVAATPTSLLISWIAPYYSVIYRITYGETGGNS PVQEFTVPGSAYATISGLKPGVDYTITVYASSGASIAAYAFISI NYRTEIDKPSQHHHHHH
398	ATI_1760_E01 core	EVVAATPTSLLISWIAPYYSVKYRITYGETGGNSPVQEFTVAGA DYATISGLKPGVDYTITVYATYGASIASYAFISINYRT
399	ATI_1760_E01 BC loop	IAPYYSVK
400	ATI_1760_E01 DE loop	AGADY
401	ATI_1760_E01 FG loop	TYGASIASYAF
402	ATI_1760_E01 w/ N leader	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRT
403	ATI_1760_E01 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRTHHHHHH
404	ATI_1760_E01 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRTEIDKPSQ
405	ATI_1760_E01 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRTEIDKPSQHHHHHH
406	ATI_1760_E01 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRTPC
407	ATI_1760_E01 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNSP VQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISIN YRTPCHHHHHH
408	ATI_1760_E01– full length	MGVSDVPRDLEVVAATPTSLLISWIAPYYSVKYRITYGETGGNS PVQEFTVAGADYATISGLKPGVDYTITVYATYGASIASYAFISI NYRTEIDKPSQHHHHHH
409	ATI_1760_F01 core	EVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGG GYATISGLKPGVDYTITVYATGGASIAAYAFISINYRT
410	ATI_1760_F01 BC loop	IAPYYAVM
411	ATI_1760_F01 DE loop	PGGGY
412	ATI_1760_F01 FG loop	TGGASIAAYAF

413	ATI_1760_F01 w/ N leader	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRT
414	ATI_1760_F01 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTHHHHHH
415	ATI_1760_F01 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTEIDKPSQ
416	ATI_1760_F01 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTEIDKPSQHHHHHH
417	ATI_1760_F01 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTPC
418	ATI_1760_F01 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTPCHHHHHH
419	ATI_1760_F01– full length	MGVSDVPRDLEVVAATPTSLLISWIAPYYAVMYRITYGETGGNSPVQEFTVPGGGYATISGLKPGVDYTITVYATGGASIAAYAFISINYRTEIDKPSQHHHHHH
420	ATI_1494_D03 core	EVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRT
421	ATI_1494_D03 BC loop	SYPSYHL
422	ATI_1494_D03 DE loop	HIDY
423	ATI_1494_D03 FG loop	QSPPYDIYYE
424	ATI_1494_D03 w/ N leader	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRT
425	ATI_1494_D03 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTHHHHHH
426	ATI_1494_D03 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTEIDKPSQ
427	ATI_1494_D03 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTEIDKPSQHHHHHH
428	ATI_1494_D03 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTPC
429	ATI_1494_D03 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTPCHHHHHH
430	ATI_1494_D03– full length	MGVSDVPRDLEVVAATPTSLLISWSYPSYHLYRITYGETGGNSPVQEFTVHIDYATISGLKPGVDYTITVYAQSPPYDIYYEISINYRTEIDKPSQHHHHHH
431	ATI_1494_D04 core	EVVAATPTSLLISWMESSSNSYRITYGETGGNSPVQEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT
432	ATI_1494_D04 BC loop	MESSSNS
433	ATI_1494_D04 DE loop	PDQL
434	ATI_1494_D04 FG loop	LANAHYMRVG
435	ATI_1494_D04 w/ N leader	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPVQEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT

436	ATI_1494_D04 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPV QEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT HHHHHH
437	ATI_1494_D04 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPV QEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT EIDKPSQ
438	ATI_1494_D04 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPV QEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT EIDKPSQHHHHHH
439	ATI_1494_D04 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPV QEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT PC
440	ATI_1494_D04 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSPV QEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYRT PCHHHHHH
441	ATI_1494_D04– full length	MGVSDVPRDLEVVAATPTSLLISWMESSSNSYRITYGETGGNSP VQEFTVPDQLATISGLKPGVDYTITVYALANAHYMRVGISINYR TEIDKPSQHHHHHH
442	ATI_1523_A08 core	EVVAATPTSLLISWISVQTYXYRITYGETGGNSPVQEFTVPDQS ATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
443	ATI_1523_A08 BC loop	ISVQTYX
444	ATI_1523_A08 DE loop	PDQS
445	ATI_1523_A08 FG loop	LEKAHYYRQN
446	ATI_1523_A08 w/ N leader	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
447	ATI_1523_A08 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT HHHHHH
448	ATI_1523_A08 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQ
449	ATI_1523_A08 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQHHHHHH
450	ATI_1523_A08 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PC
451	ATI_1523_A08 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PCHHHHHH
452	ATI_1523_A08– full length	MGVSDVPRDLEVVAATPTSLLISWISVQTYXYRITYGETGGNSP VQEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYR TEIDKPSQHHHHHH
453	ATI_1523_B10 core	EVVAATPTSLLISWVYHYDXQYRITYGETGGNSPVQEFTVPDQK ATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
454	ATI_1523_B10 BC loop	VYHYDXQ
455	ATI_1523_B10 DE loop	PDQK
456	ATI_1523_B10 FG loop	LSEAHHKRDS
457	ATI_1523_B10 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
458	ATI_1523_B10 w/ N	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV

	leader + his tag	QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT HHHHHH
459	ATI_1523_B10 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQ
460	ATI_1523_B10 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQHHHHHH
461	ATI_1523_B10 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT PC
462	ATI_1523_B10 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT PCHHHHHH
463	ATI_1523_B10 – full length	MGVSDVPRDLEVVAATPTSLLISWVYHYDXQYRITYGETGGNSP VQEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYR TEIDKPSQHHHHHH
464	ATI_1523_C07 core	EVVAATPTSLLISWRMHTDPDYRITYGETGGNSPVQEFTVPDQE ATISGLKPGVDYTITVYAIQTAHYYRINISINYRT
465	ATI_1523_C07 BC loop	RMHTDPD
466	ATI_1523_C07 DE loop	PDQE
467	ATI_1523_C07 FG loop	IQTAHYYRIN
468	ATI_1523_C07 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT
469	ATI_1523_C07 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT HHHHHH
470	ATI_1523_C07 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT EIDKPSQ
471	ATI_1523_C07 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT EIDKPSQHHHHHH
472	ATI_1523_C07 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT PC
473	ATI_1523_C07 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSPV QEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYRT PCHHHHHH
474	ATI_1523_C07– full length	MGVSDVPRDLEVVAATPTSLLISWRMHTDPDYRITYGETGGNSP VQEFTVPDQEATISGLKPGVDYTITVYAIQTAHYYRINISINYR TEIDKPSQHHHHHH
475	ATI_1523_D07 core	EVVAATPTSLLISWENLASYQYRITYGETGGNSPVQEFTVPDVQ ATISGLKPGVDYTITVYALPYIHMKQRVISINYRT
476	ATI_1523_D07 BC loop	ENLASYQ
477	ATI_1523_D07 DE loop	PDVQ
478	ATI_1523_D07 FG loop	LPYIHMKQRV
479	ATI_1523_D07 w/ N leader	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT
480	ATI_1523_D07 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT

		HHHHHH
481	ATI_1523_D07 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT EIDKPSQ
482	ATI_1523_D07 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT EIDKPSQHHHHHH
483	ATI_1523_D07 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT PC
484	ATI_1523_D07 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSPV QEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYRT PCHHHHHH
485	ATI_1523_D07 – full length	MGVSDVPRDLEVVAATPTSLLISWENLASYQYRITYGETGGNSP VQEFTVPDVQATISGLKPGVDYTITVYALPYIHMKQRVISINYR TEIDKPSQHHHHHH
486	ATI_1523_D08 core	EVVAATPTSLLISWMRYYDAYYRITYGETGGNSPVQEFTVPDQS ATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
487	ATI_1523_D08 BC loop	MRYYDAY
488	ATI_1523_D08 DE loop	PDQS
489	ATI_1523_D08 FG loop	LEKAHYYRQN
490	ATI_1523_D08 w/ N leader	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT
491	ATI_1523_D08 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT HHHHHH
492	ATI_1523_D08 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQ
493	ATI_1523_D08 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT EIDKPSQHHHHHH
494	ATI_1523_D08 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PC
495	ATI_1523_D08 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSPV QEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYRT PCHHHHHH
496	ATI_1523_D08– full length	MGVSDVPRDLEVVAATPTSLLISWMRYYDAYYRITYGETGGNSP VQEFTVPDQSATISGLKPGVDYTITVYALEKAHYYRQNISINYR TEIDKPSQHHHHHH
497	ATI_1523_E08 core	EVVAATPTSLLISWHHYQHYEYRITYGETGGNSPVQEFTVPDMG ATISGLKPGVDYTITVYALEEAHSDRSSISINYRT
498	ATI_1523_E08 BC loop	HHYQHYE
499	ATI_1523_E08 DE loop	PDMG
500	ATI_1523_E08 FG loop	LEEAHSDRSS
501	ATI_1523_E08 w/ N leader	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT
502	ATI_1523_E08 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT HHHHHH
503	ATI_1523_E08 w/ N leader	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV

	and C tail	QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT EIDKPSQ
504	ATI_1523_E08 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT EIDKPSQHHHHHH
505	ATI_1523_E08 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT PC
506	ATI_1523_E08 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSPV QEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYRT PCHHHHHH
507	ATI_1523_E08– full length	MGVSDVPRDLEVVAATPTSLLISWHHYQHYEYRITYGETGGNSP VQEFTVPDMGATISGLKPGVDYTITVYALEEAHSDRSSISINYR TEIDKPSQHHHHHH
508	ATI_1523_F01 core	EVVAATPTSLLISWYKPSTIVTYRITYGETGGNSPVQEFTVYGY NATISGLKPGVDYTITVYAVHGVRFISINYRT
509	ATI_1523_F01 BC loop	YKPSTIVT
510	ATI_1523_F01 DE loop	YGYN
511	ATI_1523_F01 FG loop	VHGVRF
512	ATI_1523_F01 w/ N leader	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRT
513	ATI_1523_F01 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTHHH HHH
514	ATI_1523_F01 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTEID KPSQ
515	ATI_1523_F01 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTEID KPSQHHHHHH
516	ATI_1523_F01 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTPC
517	ATI_1523_F01 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNSP VQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTPCH HHHHH
518	ATI_1523_F01– full length	MGVSDVPRDLEVVAATPTSLLISWYKPSTIVTYRITYGETGGNS PVQEFTVYGYNATISGLKPGVDYTITVYAVHGVRFISINYRTEI DKPSQHHHHHH
519	ATI_1523_F04 core	EVVAATPTSLLISWGGSLSPTFYRITYGETGGNSPVQEFTVTYQ GATISGLKPGVDYTITVYATEGIVYYQISINYRT
520	ATI_1523_F04 BC loop	GGSLSPTF
521	ATI_1523_F04 DE loop	TYQG
522	ATI_1523_F04 FG loop	TEGIVYYQ
523	ATI_1523_F04 w/ N leader	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSP VQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRT
524	ATI_1523_F04 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSP VQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTH HHHHH
525	ATI_1523_F04 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSP VQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTE IDKPSQ
526	ATI_1523_F04 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSP VQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTE IDKPSQHHHHHH

527	ATI_1523_F04 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSPVQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTPC
528	ATI_1523_F04 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSPVQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTPCHHHHHH
529	ATI_1523_F04– full length	MGVSDVPRDLEVVAATPTSLLISWGGSLSPTFYRITYGETGGNSPVQEFTVTYQGATISGLKPGVDYTITVYATEGIVYYQISINYRTEIDKPSQHHHHHH
530	ATI_1523_F08 core	EVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRT
531	ATI_1523_F08 BC loop	VYHYDAQ
532	ATI_1523_F08 DE loop	PDQK
533	ATI_1523_F08 FG loop	LPRAHMDRSH
534	ATI_1523_F08 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRT
535	ATI_1523_F08 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTHHHHHH
536	ATI_1523_F08 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTEIDKPSQ
537	ATI_1523_F08 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTEIDKPSQHHHHHH
538	ATI_1523_F08 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTPC
539	ATI_1523_F08 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTPCHHHHHH
540	ATI_1523_F08– full length	MGVSDVPRDLEVVAATPTSLLISWVYHYDAQYRITYGETGGNSPVQEFTVPDQKATISGLKPGVDYTITVYALPRAHMDRSHISINYRTEIDKPSQHHHHHH
541	ATI_1523_G06 core	EVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRT
542	ATI_1523_G06 BC loop	RIKSYHK
543	ATI_1523_G06 DE loop	RSYA
544	ATI_1523_G06 FG loop	IMEETHLAYA
545	ATI_1523_G06 w/ N leader	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRT
546	ATI_1523_G06 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRTHHHHHH
547	ATI_1523_G06 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRTEIDKPSQ
548	ATI_1523_G06 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRTEIDKPSQHHHHHH
549	ATI_1523_G06 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPVQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRTPC
550	ATI_1523_G06 w/ N	GVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSPV

	leader and modified C-terminus including PC + his tag	QEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYRT PCHHHHHH
551	ATI_1523_G06– full length	MGVSDVPRDLEVVAATPTSLLISWRIKSYHKYRITYGETGGNSP VQEFTVRSYAATISGLKPGVDYTITVYAIMEETHLAYAISINYR TEIDKPSQHHHHHH
552	ATI_1523_G07 core	EVVAATPTSLLISWVYPQADDYRITYGETGGNSPVQEFTVPDQN ATISGLKPGVDYTITVYALAEAHLVRIYISINYRT
553	ATI_1523_G07 BC loop	VYPQADD
554	ATI_1523_G07 DE loop	PDQN
555	ATI_1523_G07 FG loop	LAEAHLVRIY
556	ATI_1523_G07 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT
557	ATI_1523_G07 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT HHHHHH
558	ATI_1523_G07 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT EIDKPSQ
559	ATI_1523_G07 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT EIDKPSQHHHHHH
560	ATI_1523_G07 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT PC
561	ATI_1523_G07 w/ N leader and modified C-terminus including PC + his tag	GVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSPV QEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYRT PCHHHHHH
562	ATI_1523_G07– full length	MGVSDVPRDLEVVAATPTSLLISWVYPQADDYRITYGETGGNSP VQEFTVPDQNATISGLKPGVDYTITVYALAEAHLVRIYISINYR TEIDKPSQHHHHHH
563	ATI_1523_H07 core	EVVAATPTSLLISWVYHYDAXYRITYGETGGNSPVQEFTVPDQK ATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
564	ATI_1523_H07 BC loop	VYHYDAX
565	ATI_1523_H07 DE loop	PDQK
566	ATI_1523_H07 FG loop	LSEAHHKRDS
567	ATI_1523_H07 w/ N leader	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT
568	ATI_1523_H07 w/ N leader + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT HHHHHH
569	ATI_1523_H07 w/ N leader and C tail	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQ
570	ATI_1523_H07 w/ N leader and C tail + his tag	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQHHHHHH
571	ATI_1523_H07 w/ N leader and modified C-terminus including PC	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT PC
572	ATI_1523_H07 w/ N leader and modified C-	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT PCHHHHHH

	terminus including PC + his tag	
573	ATI_1523_H07– full length	GVSDVPRDLEVVAATPTSLLISWVYHYDAXYRITYGETGGNSPV QEFTVPDQKATISGLKPGVDYTITVYALSEAHHKRDSISINYRT EIDKPSQHHHHHH
574	N-terminal leader	MGVSDVPRDL
575	N-terminal leader	GVSDVPRDL
576	N-terminal leader	X_nSDVPRDL
577	N-terminal leader	X_nDVPRDL
578	N-terminal leader	X_nVPRDL
579	N-terminal leader	X_nPRDL
580	N-terminal leader	X_nRDL
581	N-terminal leader	X_nDL
582	N-terminal leader	MASTSG
583	N-terminal leader	METDTLLLWVLLLWVPGSTG
584	C-terminal tail	EIEK
585	C-terminal tail	EGSGC
586	C-terminal tail	EIEKPCQ
587	C-terminal tail	EIEKPSQ
588	C-terminal tail	EIEKP
589	C-terminal tail	EIEKPS
590	C-terminal tail	EIEKPC
591	C-terminal tail	EIDK
592	C-terminal tail	EIDKPCQ
593	C-terminal tail	EIDKPSQ
594	C-terminal tail	EIEPKSS
595	C-terminal tail	EIDKPC
596	C-terminal tail	EIDKP
597	C-terminal tail	EIDKPS
598	C-terminal tail	EIDKPSQLE
599	C-terminal tail	EIEDEDEDEDED
600	C-terminal tail	EGSGS
601	C-terminal tail	EIDKPCQLE
602	C-terminal tail	EIDKPSQHHHHHH
603	C-terminal tail	GSGCHHHHHH
604	C-terminal tail	EGSGCHHHHHH
605	C-terminal tail	PIDK
606	C-terminal tail	PIEK
607	C-terminal tail	PIDKP
608	C-terminal tail	PIEKP
609	C-terminal tail	PIDKPS
610	C-terminal tail	PIEKPS
611	C-terminal tail	PIDKPC
612	C-terminal tail	PIEKPC
613	C-terminal tail	PIDKPSQ
614	C-terminal tail	PIEKPSQ
615	C-terminal tail	PIDKPCQ
616	C-terminal tail	PIEKPCQ
617	C-terminal tail	PHHHHHH

618	C-terminal tail	PCHHHHHH
619	6X-His tag	HHHHHH
620	Human IgG1 Fc domain	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
621	Core hinge region of Fc	DKTHTCPPCPAPELLG
622	Exemplary hinge sequence	EPKSSDKTHTCPPCPAPELLGGPS
623	Exemplary hinge sequence	EPKSSDKTHTCPPCPAPELLGGSS
624	Exemplary hinge sequence	EPKSSGSTHTCPPCPAPELLGGSS
625	Exemplary hinge sequence	DKTHTCPPCPAPELLGGPS
626	Exemplary hinge sequence	DKTHTCPPCPAPELLGGSS
627	Fc with CH2 and CH3 regions of IgG1 for Adnectin-hinge-Fc construct	VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK
628	Fc with CH2 and CH3 regions of IgG1 for Fc-hinge-Adnectin construct	VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSP
629	Linker 1	GAGGGGSG
630	Linker 2	EPKSSD
631	Linker 3	PVGVV
632	Linker 4	ESPKAQASSVPTAQPQAEGLA
633	Linker 5	ELQLEESAAEAQDGELD
634	Linker 6	GQPDEPGGS
635	Linker 7	GGSGSGSGSGSGS
636	Linker 8	ELQLEESAAEAQEGELE
637	Linker 9	GSGSG
638	Linker 10	GSGC
639	Linker 11	AGGGGSG
640	Linker 12	GSGS
641	Linker 13	QPDEPGGS
642	Linker 14	GSGSGS
643	Linker 15	TVAAPS
644	Linker 16	KAGGGGSG
645	Linker 17	KGSGSGSGSGSGS
646	Linker 18	KQPDEPGGS
647	Linker 19	KELQLEESAAEAQDGELD
648	Linker 20	KTVAAPS
649	Linker 21	KAGGGGSGG
650	Linker 22	KGSGSGSGSGSGSG
651	Linker 23	KQPDEPGGSG
652	Linker 24	KELQLEESAAEAQDGELDG
653	Linker 25	KTVAAPSG
654	Linker 26	AGGGGSGG
655	Linker 27	AGGGGSG
656	Linker 28	GSGSGSGSGSGSG

657	Linker 29	QPDEPGGSG
658	Linker 30	TVAAPSG
659	Linker 31	PSTSTST
660	Linker 32	EIDKPSQ
661	Linker 33	GSGSGSGS
662	Linker 34	GSGSGSGSGS
663	Linker 35	GSGSGSGSGSGS
664	Linker 36	GSGSGSGSGSGSGS
665	Linker 37	GGSGSGSGSGSGS
666	Linker 38	GGSGSGSGSGSGSGSG
667	Linker 39	GSEGSEGSEGSEGSE
668	Linker 40	GGSEGGSE
669	Linker 41	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
670	Linker 42	GGGGSGGGGSGGGGSGGGGSGGGGS
671	Linker 43	GGGGSGGGGSGGGGSG
672	Linker 44	GPGPGPG
673	Linker 45	GPGPGPGPGPG
674	Linker 46	PAPAPA
675	Linker 47	PAPAPAPAPAPA
676	Linker 48	PAPAPAPAPAPAPAPAPA
677	Linker 49	GSGSGSGSGSGSGSGSGSGS
678	Linker 50	GGGGSGGGGSGGGGSGGGGS

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

1. A polypeptide comprising a modified human fibronectin type III tenth (^{10}Fn3) domain, wherein the polypeptide specifically binds to human Programmed Death Ligand-1 (PD-L1), and wherein the BC, DE, and FG loops of the modified ^{10}Fn3 domain comprise the amino acid sequences set forth in:

(a) SEQ ID NOs: 6, 7 and 8, respectively;
(b) SEQ ID NOs: 21, 22 and 23, respectively;
(c) SEQ ID NOs: 36, 37 and 38, respectively;
(d) SEQ ID NOs: 51, 52 and 53, respectively;
(e) SEQ ID NOs: 66, 67 and 68, respectively;
(f) SEQ ID NOs: 81, 82 and 83, respectively;
(g) SEQ ID NOs: 97, 98 and 99, respectively;
(h) SEQ ID NOs: 113, 114 and 115, respectively;
(i) SEQ ID NOs: 124, 125 and 126, respectively;
(j) SEQ ID NOs: 135, 136 and 137, respectively;
(k) SEQ ID NOs: 146, 147 and 148, respectively;
(l) SEQ ID NOs: 157, 158 and 159, respectively;
(m) SEQ ID NOs: 168, 169 and 170, respectively;
(n) SEQ ID NOs: 179, 180 and 181, respectively;
(o) SEQ ID NOs: 190, 191 and 192, respectively;
(p) SEQ ID NOs: 201, 202 and 203, respectively;
(q) SEQ ID NOs: 212, 213 and 214, respectively;
(r) SEQ ID NOs: 223, 224 and 225, respectively;
(s) SEQ ID NOs: 234, 235 and 236, respectively;
(t) SEQ ID NOs: 245, 246 and 247, respectively;
(u) SEQ ID NOs: 256, 257 and 258, respectively;
(v) SEQ ID NOs: 267, 268 and 269, respectively;
(w) SEQ ID NOs: 278, 279 and 280, respectively;
(x) SEQ ID NOs: 289, 290 and 291, respectively;
(y) SEQ ID NOs: 300, 301 and 302, respectively;

(z) SEQ ID NOs: 311, 312 and 313, respectively;
(aa) SEQ ID NOs: 322, 323 and 324, respectively;
(bb) SEQ ID NOs: 333, 334 and 335, respectively;
(cc) SEQ ID NOs: 344, 345 and 346, respectively;
(dd) SEQ ID NOs: 355, 356 and 357, respectively;
(ee) SEQ ID NOs: 366, 367 and 368, respectively;
(ff) SEQ ID NOs: 377, 378 and 379, respectively;
(gg) SEQ ID NOs: 388, 389 and 390, respectively;
(hh) SEQ ID NOs: 399, 400 and 401, respectively;
(ii) SEQ ID NOs: 410, 411 and 412, respectively;
(jj) SEQ ID NOs: 421, 422 and 423, respectively;
(kk) SEQ ID NOs: 432, 433 and 434, respectively;
(ll) SEQ ID NOs: 443, 444 and 445, respectively;
(mm) SEQ ID NOs: 454, 455 and 456, respectively;
(nn) SEQ ID NOs: 465, 466 and 467, respectively;
(oo) SEQ ID NOs: 476, 477 and 478, respectively;
(pp) SEQ ID NOs: 487, 488 and 489, respectively;
(qq) SEQ ID NOs: 498, 499 and 500, respectively;
(rr) SEQ ID NOs: 509, 510 and 511, respectively;
(ss) SEQ ID NOs: 520, 521 and 522, respectively;
(tt) SEQ ID NOs: 531, 532 and 533, respectively;
(uu) SEQ ID NOs: 542, 543 and 544, respectively;
(vv) SEQ ID NOs: 553, 554 and 555, respectively; or
(ww) SEQ ID NOs: 564, 565 and 566, respectively.

2. The polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs: 5, 20, 35, 50, 65, 80, 96, 112, 123, 134, 145, 156, 167, 178, 189, 200, 211, 222, 233, 244, 255, 266, 277, 288, 299, 310, 321, 332, 343, 354, 365, 376, 387, 398, 409, 420, 431, 442, 453, 464, 475, 486, 497, 508, 519, 530, 541, 552 and 563.

3. The polypeptide of claim 1, wherein the polypeptide further comprises one or more pharmacokinetic (PK) moieties selected from the group consisting of polyethylene glycol, sialic

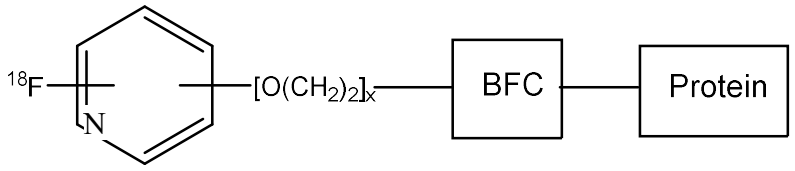
acid, Fc, Fc fragment, transferrin, serum albumin, a serum albumin binding protein, and a serum immunoglobulin binding protein.

4. A composition comprising the polypeptide of claim 1, and a carrier.

5. An imaging agent comprising the polypeptide of claim 1, and a detectable label.

6. The imaging agent of claim 5, wherein the detectable label is a radionuclide.

7. An imaging agent comprising a protein, an ^{18}F-radiolabeled prosthetic group, and a bifunctional conjugating (BFC) moiety, wherein the imaging agent has the following structure:



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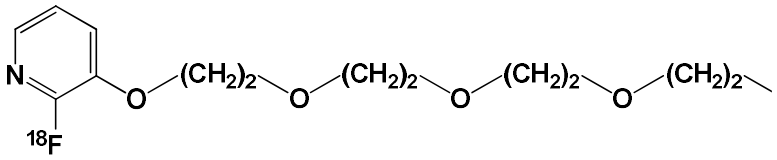
wherein the ^{18}F is ortho to the N atom, x is an integer from 1 to 8, and the protein comprises a polypeptide of claim 1.

8. The imaging agent of claim 7, wherein the ^{18}F-radiolabeled prosthetic group has the structure:



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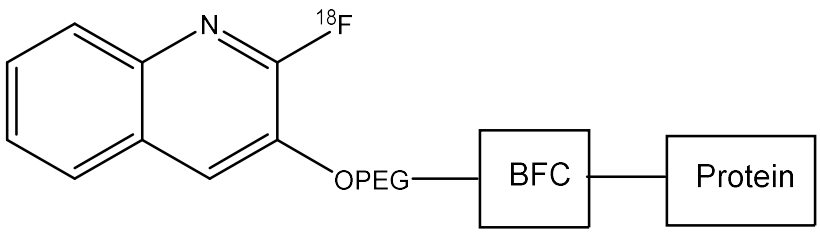
9. An imaging agent comprising a protein, an ^{18}F-radiolabeled prosthetic group, and a bifunctional conjugating (BFC) moiety, wherein the imaging agent has the following structure:



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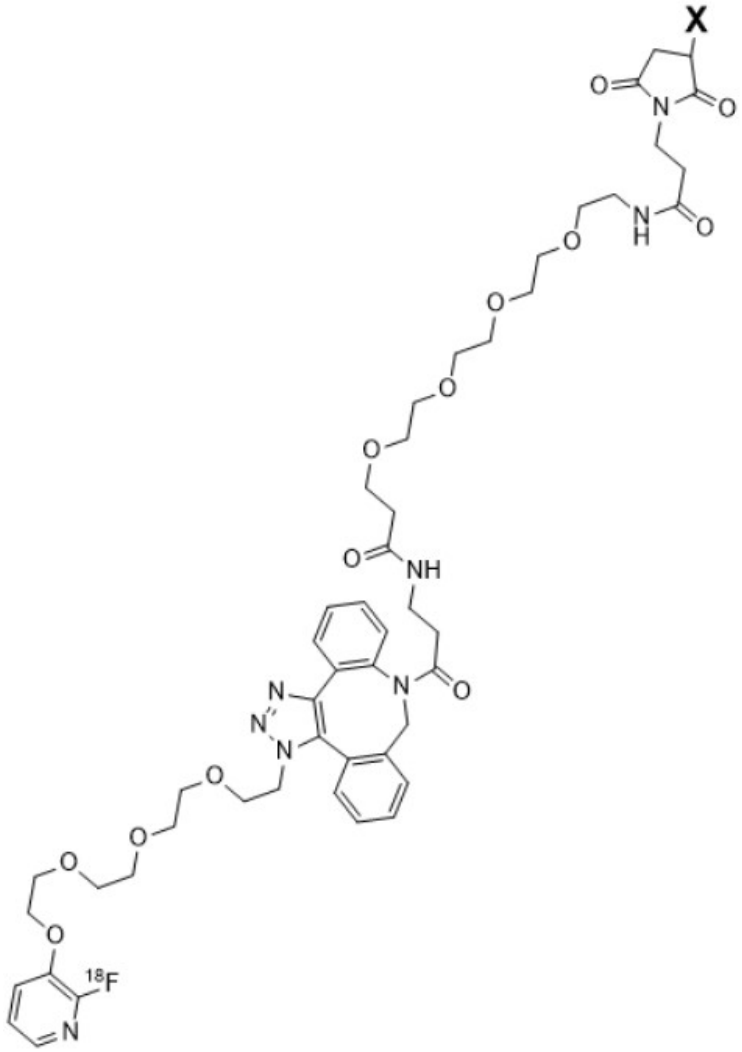
wherein "OPEG" is $[O(CH_2)_2]_x$, x is an integer from 1 to 8, and the protein comprises a polypeptide of claim 1.

10. The imaging agent of claim 7, wherein the BFC is dibenzocyclooctyne-PEG4-N-hydroxysuccinimidyl ester (DBCO-PEG4-NHS-Ester), dibenzocyclooctyne-*Sulfo*-N-hydroxysuccinimidyl ester (DBCO-*Sulfo*-NHS-Ester), dibenzocyclooctyne-PEG4-acid (DBCO-PEG4-Acid), dibenzocyclooctyne-PEG4-amine (DBCO-PEG4-Amine) or dibenzocyclooctyne-PEG4-maleimide (DBCO-PEG4-Maleimide).

11. An imaging agent comprising the structure:



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wherein X is a polypeptide of claim 1.

12. A kit comprising the polypeptide of claim 1, and instructions for use.

13. The polypeptide of claim 1, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain comprise the amino acid sequences set forth in:

(a) SEQ ID NOs: 6, 7 and 8, respectively;

(b) SEQ ID NOs: 21, 22 and 23, respectively;

(c) SEQ ID NOs: 36, 37 and 38, respectively;

(d) SEQ ID NOs: 51, 52 and 53, respectively;

(e) SEQ ID NOs: 66, 67 and 68, respectively;

(f) SEQ ID NOs: 81, 82 and 83, respectively; or
(g) SEQ ID NOs: 97, 98 and 99, respectively.

14. The polypeptide of claim 13, wherein the polypeptide specifically binds to PD-L1 with a KD of 10 nM or less.

15. The polypeptide of claim 14, wherein the ^{10}Fn3 domain comprises an amino acid sequence at least 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 20, 35, 50, 65, 80, and 96.

16. The polypeptide of claim 15, wherein the ^{10}Fn3 domain comprises an amino acid sequence at least 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of an amino acid sequence selected from the group consisting of: SEQ ID NOs: 9-15, 24-30, 39-45, 54-60, 69-75, 84-91, and 100-107.

17. The polypeptide of any one of claims 14-16, wherein the polypeptide comprises one or more pharmacokinetic (PK) moieties selected from the group consisting of polyethylene glycol, sialic acid, Fc, Fc fragment, transferrin, serum albumin, a serum albumin binding protein, and a serum immunoglobulin binding protein.

18. A nucleic acid encoding the polypeptide of any one of claims 14-16.

19. The nucleic acid of claim 18, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 16-19, 31-34, 46-49, 61-64, 76-79, 92-95, and 108-111.

20. A vector comprising the nucleic acid of claim 18 or claim 19.

21. A cell comprising the nucleic acid of claim 18 or claim 19.

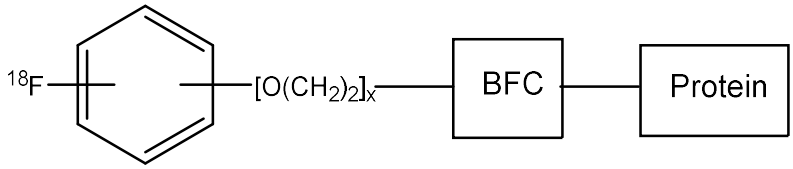
22. An imaging agent comprising the polypeptide of any one of claims 14-17, and a detectable label.

23. The imaging agent of claim 22, wherein the detectable label is a radionuclide selected from the group consisting of: ^{64}Cu, ^{124}I, $^{76/77}Br$, ^{86}Y, ^{89}Zr, ^{68}Ga, ^{18}F, ^{11}C, ^{125}I, ^{124}I, ^{131}I, ^{123}I, ^{131}I, ^{123}I, ^{32}Cl, ^{33}Cl, ^{34}Cl, ^{68}Ga, ^{74}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{78}Br, ^{89}Zr, ^{186}Re, ^{188}Re, ^{90}Y, ^{177}Lu, ^{99}Tc, or ^{153}Sm.

24. The imaging agent of claim 22 or 23, wherein the detectable label is conjugated to the polypeptide by a conjugating moiety selected from the group consisting of DFO, DOTA, CB-DO2A, 3p-C-DEPA, TCMC, DBCO, DIBO, BARAC, DIMAC, Oxo-DO3A, TE2A, CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, DM-TE2A, diamsar, NODASA, NODAGA, NOTA, NETA, TACN-TM, DTPA, 1B4M-DTPA, CHX-A"-DTPA, TRAP, NOPO, AAZTA, DATA, H_2dedpa, H_4octapa, H_2azapa, H_5decapa, H_6phospa, HBED, SHBED, BPCA, CP256, PCTA, HEHA, PEPA, EDTA, TETA, and TRITA.

25. The imaging agent of claim 24, wherein the conjugating moiety is NODAGA and the radionuclide is ^{64}Cu.

26. The imaging agent of claim 23, comprising a 18F-radiolabeled prosthetic group and a bifunctional conjugating (BFC) moiety, wherein the imaging agent has the following structure,

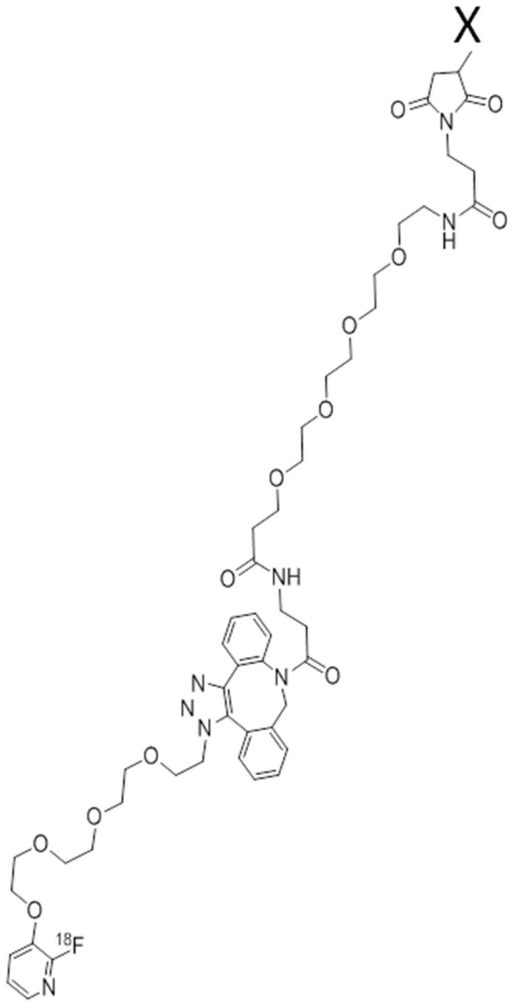


or a pharmaceutically acceptable salt thereof, wherein
(a) the ^{18}F is ortho to the N atom, x is an integer from 1 to 8, from 2 to 6, from 3 to 5 or x is 4;
(b) the BFC moiety is a cyclooctyne comprising (i) a reactive group that forms a covalent bond with an amine, carboxyl, carbonyl or thiol functional group on the protein, and (ii) a polyethylene glycol (PEG)y spacer arm wherein y is an integer from 1 to 8, from 2 to 6, or y is 4 or 5; and
(c) the Protein is a polypeptide of any one of claims 14-17.

27. The imaging agent of claim 26, wherein the cyclooctyne is selected from the group consisting of dibenzocyclooctyne (DIBO), biarylazacyclooctynone (BARAC), dimethoxyazacyclooctyne (DIMAC) and dibenzocyclooctyne (DBCO).

28. The imaging agent of claim 26, wherein the BFC is DBCO-PEG4-NHS-Ester, DBCO-*Sulfo*-NHS-Ester, DBCO-PEG4-Acid, DBCO-PEG4-Amine or DBCO-PEG4-Maleimide.

29. The imaging agent of claim 26, wherein the imaging agent has the structure:



wherein X is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 13, 28, 43, 58, 73, 88, and 104, and wherein the maleimide group of the BFC is covalently linked to the thiol group on a cysteine residue of the polypeptide.

30. The imaging agent of claim 29, wherein the maleimide group of the BFC is covalently linked to the thiol group on a C-terminal cysteine residue of the polypeptide.

31. The imaging agent of any one of claims 22-29, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 88 or SEQ ID NO: 104.

32. A pharmaceutical composition comprising the polypeptide of any one of claims 14-17, or the imaging agent of any one of claims 22-31.

33. Use of the imaging agent of any one of claims 23-31 in a method to detect the presence of a PD-L1-expressing tumor in a subject, the method comprising
(a) administering the imaging agent to the subject; and

(b) obtaining a radio-image of at least a portion of the subject to detect the presence or absence of the imaging agent; wherein the presence and location of the imaging agent above background is indicative of the presence and location of the PD-L1 expressing tumor.

34. The polypeptide of claim 13, wherein the modified human ^{10}Fn3 domain comprises the amino acid sequence set forth in any one of SEQ ID NOs: 5, 20, 35, 65, 80 and 96.

35. The polypeptide of claim 1, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain comprise the amino acid sequences set forth in SEQ ID NOs: 81, 82 and 83, respectively.

36. The polypeptide of claim 35, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 80.

37. The polypeptide of claim 36, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 84.

38. The polypeptide of claim 36, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 88.

39. The polypeptide of claim 1, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain comprise the amino acid sequences set forth in SEQ ID NOs: 97, 98 and 99, respectively.

40. The polypeptide of claim 39, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 96.

41. The polypeptide of claim 39, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 100.

42. The polypeptide of claim 39, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 104.

43. The imaging agent of claim 7, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain comprise the amino acid sequences set forth in SEQ ID NOs: 81, 82 and 83, respectively.

44. The imaging agent of claim 43, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 80.

45. The imaging agent of claim 7, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain comprise the amino acid sequences set forth in SEQ ID NOs: 97, 98 and 99, respectively.

46. The imaging agent of claim 45, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 96.

47. The imaging agent of claim 11, wherein X is a polypeptide comprising the amino acid sequence set forth in any one of SEQ ID NOs: 13, 28, 43, 58, 73, 88 and 104.

48. The imaging agent of claim 11, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain in X comprise the amino acid sequences set forth in SEQ ID NOs: 81, 82 and 83, respectively.

49. The imaging agent of claim 48, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 80.

50. The imaging agent of claim 48, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 88.

51. The imaging agent of claim 11, wherein the BC, DE and FG loops of the modified human ^{10}Fn3 domain in X comprise the amino acid sequences set forth in SEQ ID NOs: 97, 98 and 99, respectively.

52. The imaging agent of claim 51, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 96.

53. The imaging agent of claim 51, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 104.

54. The imaging agent of claim 50, wherein the C-terminal cysteine residue of SEQ ID NO: 88 is covalently bound to the maleimide moiety.

55. The imaging agent of claim 53, wherein the C-terminal cysteine residue of SEQ ID NO: 104 is covalently bound to the maleimide moiety.