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

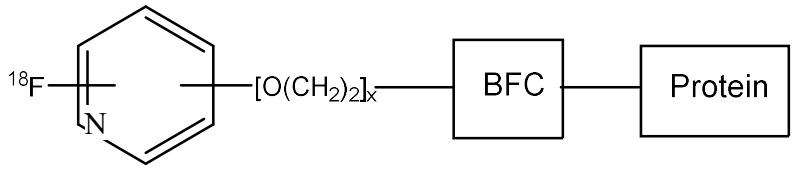
acid, Fc, Fc fragment, transferrin, serum albumin, a serum albumin binding protein, and a serum immunoglobulin binding protein.

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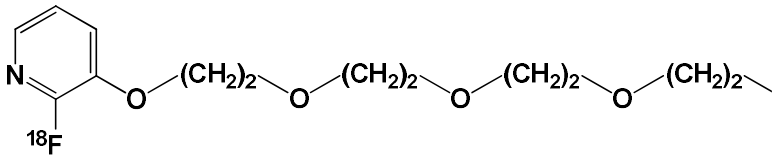
wherein the ^{18}F is ortho to the N atom, x is an integer from 1 to 8, and the protein comprises a polypeptide of claim 1.

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



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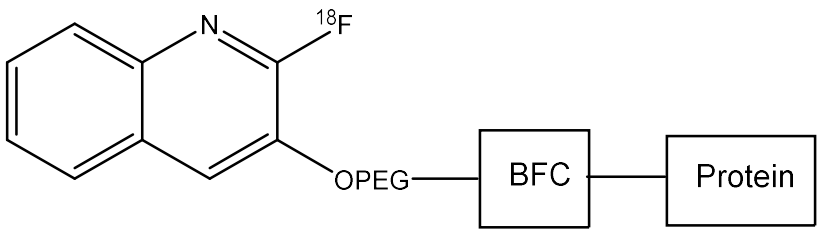
9. An imaging agent comprising a protein, an ^{18}F-radiolabeled prosthetic group, and a bifunctional conjugating (BFC) moiety, wherein the imaging agent has the following structure:



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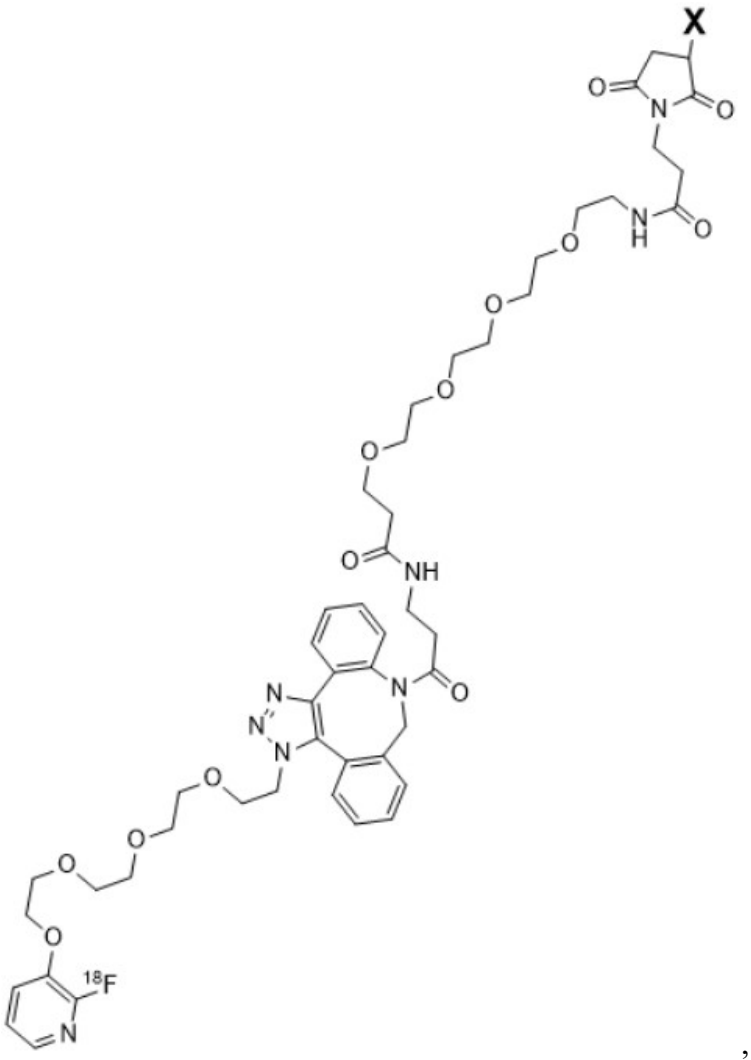
wherein "OPEG" is $[O(CH_2)_2]_x$, x is an integer from 1 to 8, and the protein comprises a polypeptide of claim 1.

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.

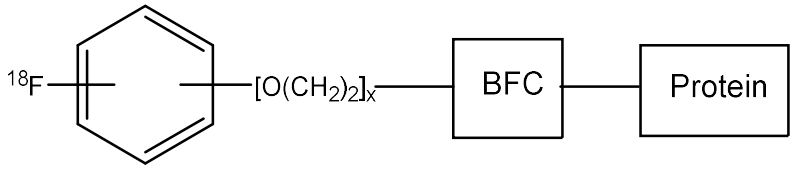
22. An imaging agent comprising the polypeptide of any one of claims 14-17, and a detectable label.

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,

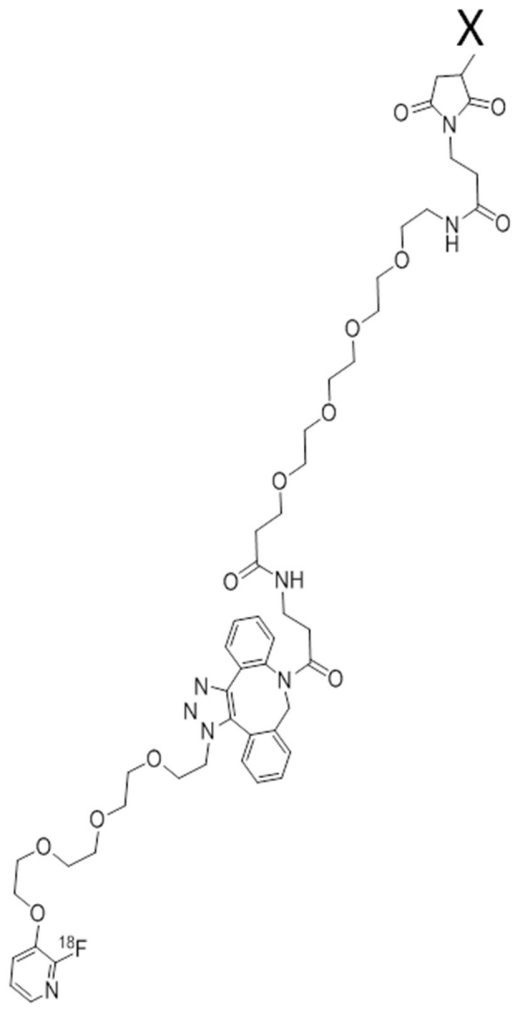


or a pharmaceutically acceptable salt thereof, wherein
(a) the ^{18}F is ortho to the N atom, x is an integer from 1 to 8, from 2 to 6, from 3 to 5 or x is 4;
(b) the BFC moiety is a cyclooctyne comprising (i) a reactive group that forms a covalent bond with an amine, carboxyl, carbonyl or thiol functional 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.