Claims

1. A method of preparing a site-specifically radiolabeled tracer, the method comprising providing

   i) a peptidyl asparaginyl ligase (PAL);

   ii) a first peptide comprising a P1-P1'-P2' tripeptide PAL motif at the C-terminus, wherein P1 is Asn or Asp, P1' is Gln or any non-proline amino acid, and P2′ is a hydrophobic amino acid such as Val or Leu or Ile or a β-branched amino acid;

   iii) a second peptide comprising a P1″-P2″ motif at the N-terminus, wherein P1″ is Gly and P2″ is a hydrophobic amino acid such as Val or Leu or Ile, or a β-branched amino acid, wherein the second peptide is modified with a functional moiety to form a functionalized peptide;

   iv) contacting the peptidyl asparaginyl ligase (PAL) with said first peptide and said functionalized peptide to form a conjugate of said first peptide-functionalized peptide;

   wherein the method further comprises

      (a) radiolabeling the conjugate, or

      (b) radiolabeling the functionalized peptide prior to contacting with the PAL.

2. The method of claim 1, wherein the first peptide is an antibody, a scaffold protein, an antibody mimetic, or a functional fragment thereof, preferably an antibody or a functional fragment thereof, more preferably a single-domain antibody (sdAb), and most preferably hPD-L1-targeting sdAb or a CEA-targeting sdAb.

3. The method of claim 1 or 2, wherein P2' and/or P2" is a hydrophobic amino acid selected from the group consisting of Val, Leu and Ile.

4. The method of any one of the preceding claims, wherein:

   the P1-P1'-P2' tripeptide PAL motif is a Asn-X-Leu motif, wherein X is Gln (Q), Gly (G) or Glu (E); or

   the P1-P1'-P2' tripeptide PAL motif is Asn-Gln-Leu (NQL) or Asn-Gly-Leu (NGL).

5. The method of any one of the preceding claims, wherein:

the first peptide comprises a His_6 tag at its C-terminus or a -NGL-His_6 or a -NQL-His_6 tag at its C-terminus, preferably the first peptide is selected from the group consisting of (hPD-L1)-NGL-His_6, (hPD-L1)-NQL-His_6, and (CEA)- NQL-His_6 sdAb.

6. The method of any one of the preceding claims, wherein the P1″-P2″ motif is a -G-I motif.

7. The method of any one of the preceding claims, wherein the functionalized peptide comprises a –GIGK-, -GIGG-, or –GIGGGK- motif at its N-terminus, preferably the functionalized peptide is selected from the group consisting of GIGK-NOTA, GIGK-DOTA, GIGK-MeTz, GIGGGK-DOTA, GIGK-NOTA-[^{18}F]AlF, GIGK-Py-DABCO, Ac-GIGK-Py-DABCO, GIGK-Py-NMe_3, Ac-GIGK-$PyNMe_3$, and GIGK-FPy.

8. The method of any one of the preceding claims, wherein the PAL is selected from the group comprising VyPAL2 comprising the amino acid sequence set forth in SEQ ID NO:32, butelase1 comprising the amino acid sequence set forth in SEQ ID NO: 33, butelase-2 comprising the amino acid sequence set forth in SEQ ID NO: 34 or 35, VyPAL3 comprising the amino acid sequence set forth in SEQ ID NO: 36, OaAEP1b-C247A comprising the amino acid sequence set forth in SEQ ID NO: 37, HeAEP3 comprising the amino acid sequence set forth in SEQ ID NO: 38, AtLEGγ comprising the amino acid sequence set forth in SEQ ID NO: 39, VuPAL1 comprising the amino acid sequence set forth in SEQ ID NO: 40, HaPAL1 comprising the amino acid sequence set forth in SEQ ID NO: 41, OaAEP1b comprising the amino acid sequence set forth in SEQ ID NO: 42 and a functional fragment or a variant thereof, optionally wherein the PAL is VyPAL2 comprising the amino acid sequence set forth in SEQ ID NO: 32, or a functional fragment or variant thereof.

9. The method of any one of the preceding claims, wherein the contacting step is carried out in the presence of a glutaminyl cyclase (QC), preferably the PAL:QC stoichiometry is from 1:0.1 to 1:1, preferably 1:0.1 or 1:0.5.

10. The method of claim 9, wherein the QC is is selected from the group comprising Human glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 43, Mouse glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 44, Drosophila glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 45, Arabidopsis glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 46, Conus glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 47, Sistrurus glutaminyl cyclase comprising the

amino acid sequence set forth in SEQ ID NO: 48, Bacterial glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 49 and a functional fragment or a variant thereof, optionally wherein the QC is human glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 43, or a functional fragment or variant thereof.

11. The method of any one of the preceding claims, wherein the functionalized peptide is functionalized with a chelator or a tetrazine moiety, optionally wherein:

(a) the chelator is selected from the group consisting of 1,4,7-triazacyclononanetriacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid (NODAGA), further optionally wherein the chelator is further functionalized with a bicyclononyne; or

(b) the tetrazine moiety is – MeTz.

12. The method of claim 11, wherein the tetrazine-functionalized peptide forms a conjugate of said first peptide-(tetrazine-functionalized peptide), and the conjugate is further complexed with a bicyclononyne-functionalized chelator prior to radiolabeling, preferably the bicyclononyne-functionalized chelator comprises a chelator selected from the group consisting of 1,4,7-triazacyclononanetriacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid (NODAGA).

13. The method of any one of the preceding claims, wherein the conjugate or the functionalized peptide is radiolabeled with a radionuclide selected from the group consisting of Gallium-68 (^{68}Ga), Gallium-67 (^{67}Ga), Lutetium-177 (^{177}Lu), and Fluorine-18 (^{18}F), preferably the radionuclide is Gallium-68 (^{68}Ga), or Fluorine-18 (^{18}F) in the form of [^{18}F]AlF.

14. A site-specifically radiolabeled tracer prepared by the method of any one of claims 1 to 13, preferably selected from the group consisting of [^{68}Ga]Ga-NOTA-KGIG-(hPD-L1) sdAb, [^{18}F]AlF-NOTA-KGIG-(hPD-L1) sdAb, [^{18}F]F-NODAGA-BCN-MeTz-KGIG-(hPD-L1), and [^{177}Lu]Lu-DOTA-NHCS-KGIG-CEA sdAb.

15. A site-specifically radiolabeled tracer according to claim 14 for use as a nuclear imaging agent or for use in the treatment of a disease in a subject.