

Claims:

1. A method comprising:

(a) contacting a Red Blood Cell (RBC) with a peptide in the presence of a ligase, under suitable conditions and for sufficient time to allow ligation of the peptide to the RBC to form an RBC-peptide conjugate;

wherein the peptide comprises a C-terminal ligase recognition sequence.

2. The method of claim 1 wherein the ligase is OaAEP1 ligase, preferably OaAEP1-Cys247Ala.

3. The method of claim 1 or claim 2 wherein the peptide is an effector molecule.

4. The method of claim 3 wherein the effector molecule has a C-terminal ligase recognition sequence selected from NGL, NDL, NPL, NAL, NCL, NEL, NFL, NHL, NKL, NIL, NLL, NQL, NRL, NSL, NTL, NVL, NWL, NYL, NSLD, NSLAN or NG, preferably NGL, NPL or NDL.

5. The method of claim 1 or claim 2 wherein the peptide is a linker peptide.

6. The method of claim 5 wherein the linker peptide comprises a C-terminal ligase recognition sequence and a motif for conjugation to an effector molecule, where the motif for conjugation to another molecule is an N-terminal ligase recognition sequence, a click chemistry functional group, or a biotin moiety.

7. The method of claim 6 wherein the RBC-peptide conjugate is a RBC-linker peptide conjugate, and wherein the method further comprises:

(b) contacting the RBC-linker peptide conjugate with an effector molecule under suitable conditions and for sufficient time for conjugation of the effector molecule to the RBC-linker peptide to form an RBC-linker-effector molecule conjugate.

8. The method of claim 7 wherein the linker peptide comprises an N-terminal ligase recognition sequence and wherein the C-terminal ligase recognition sequence is Xaa₁GG, wherein Xaa₁ is any amino acid except G or has the sequence NG or NCL.

9. The method of claim 8 wherein the N-terminal ligase recognition sequence is G, GG, GL, GGG, GLG or GGL.

10. The method of claim 8 or claim 9 wherein the effector molecule comprises a C-terminal ligase recognition sequence selected from the group consisting of NGL, NDL, NPL, NAL, NCL, NEL, NFL, NHL, NKL, NIL, NLL, NQL, NRL, NSL, NTL, NVL, NWL, NYL, NSLD, NSLAN or NG, preferably NGL, NPL or NDL.

11. The method of any one of claims 8-10 wherein the RBC-linker peptide conjugate is contacted with the effector molecule under suitable conditions and for sufficient time for ligation of the effector molecule to the RBC-linker peptide to form an RBC-linker-effector molecule conjugate.
12. The method of claim 11 wherein the ligase in (a) is the same as the ligase in (b), preferably OaAEP1 ligase, more preferably OaAEP1-Cys247Ala.
13. The method of claim 6 wherein the linker comprises a click chemistry functional group and a C-terminal ligase recognition sequence selected from the group consisting NGL, NDL, NPL, NAL, NCL, NEL, NFL, NHL, NKL, NIL, NLL, NQL, NRL, NSL, NTL, NVL, NWL, NYL, NSLD, NSLAN or NG, preferably NGL, NPL or NDL.
14. The method of claim 13 wherein the click chemistry functional group comprises azide moiety, a tetrazine moiety, a methyl tetrazine moiety a diarylcytooctyne (DBCO) moiety or a Transcyclooctyne (TCO) moiety.
15. The method of claim 14 wherein the effector molecule comprises a complementary click chemistry functional group.
16. The method of claim 13 to claim 15 wherein the RBC-linker conjugate is contacted with the effector molecule under suitable conditions and for sufficient time for conjugation of the effector molecule to the RBC-linker conjugate by Copper-free click chemistry.
17. The method of claim 6 wherein the RBC-peptide conjugate is a RBC-linker conjugate, wherein the linker comprises a biotin moiety, and wherein the method further comprises:
 - (b) contacting the RBC-linker peptide conjugate with streptavidin under suitable conditions and for sufficient time for conjugation of the biotin moiety of the RBC-linker peptide to streptavidin to form a RBC-linker-streptavidin conjugate; and
 - (c) contacting the RBC-linker-streptavidin conjugate with a biotinylated effector molecule under suitable conditions and for sufficient time for conjugation of the biotin moiety of the effector molecule to the streptavidin of the RBC-linker-streptavidin conjugate, thereby forming a RBC-linker-streptavidin-effector molecule conjugate.
18. The method according to any one of the preceding claims further comprising a step of washing the RBC-peptide conjugate, the RBC-linker-effector molecule conjugate or the RBC-linker-streptavidin-effector molecule conjugate.
19. The method according to any one of claims 5 to 18 wherein linker peptide comprises a linker body sequence, the linker body sequence:
 - (a) comprises or consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acids;
 - (b) comprises an α -helical peptide sequence

- (c) comprises repeats of the sequence EAAAK; or
 (d) comprises the sequence EQKLISEEDL.

20. The method of claim 19 wherein the linker consists of a sequence selected from the group consisting:

5

GLGEQKLISEEDLGLPETGG;
 DBCO-EAAAKEAAAKEAAKNGL;
 Azide-GSSGSGGEQKLISEEDLGGSGGSGSGNGL;
 GLGEQKLISEEDLGLPETGG;
 GGGEQKLISEEDLGLPETGG;
 10 GLGEQKLISEEDLGNGL;
 GGGEQKLISEEDLGNGL; and
 GLG(EAAAK)₅LPETGG.

21. The method according to any one of the preceding claims wherein the method RBC is deglycosylated.

15

22. A modified Red Blood Cell (RBC) produced by the method of any one of claims 1 to 21.

23. A modified RBC comprising, on its exterior surface, a peptide, wherein the peptide is conjugated to a native red blood cell protein.

20

24. The modified RBC according to claim 23 wherein the peptide is an effector molecule.

25. The modified of RBC according to claim 23 wherein the peptide is a linker protein.

25

26. The modified RBC according to claim 25 wherein the linker protein is further conjugated to an effector molecule.

27. The modified RBC according to any one of claims 23 to 26 wherein the RBC is deglycosylated.

30

28. A modified RBC according to any one of claims 22 to 27 for use in therapy.

29. Use of a modified RBC according to any one of claims 22 to 27 in the manufacture of a medicament for treatment.

35

30. The use according to claim 29 wherein the treatment is a treatment for an enzyme deficiency, metabolic disease, immune-related disorder, blood disorder, or cancer.