CLAIMS

1. A method of making an enucleated erythroid cell functionalized with an exogenous polypeptide, the method comprising:

(a) providing an activated cell comprising an enucleated erythroid cell covalently bound to a first click handle, wherein the activated cell was made using a process that does not comprise contacting an enucleated erythroid cell or a precursor thereof with a sugar comprising the first click handle,

(b) providing an activated agent comprising the exogenous polypeptide covalently bound to a second click handle capable of reacting with the first click handle, and

(c) contacting the activated cell with the activated agent, thereby producing the enucleated erythroid cell functionalized with the exogenous polypeptide,

wherein one or both of the activated cell and the enucleated erythroid cell functionalized with the exogenous polypeptide lack(s) a sortase transfer signature; and

wherein reaction between the first click handle and the second click handle in step (c) does not require a copper ion catalyst.

2. The method of claim 1, wherein the exogenous polypeptide is greater than about 30 kilodaltons.

3. The method of claim 1, wherein the exogenous polypeptide is greater than about 500 amino acids in length.

4. The method of claim 1, wherein the process results in covalent linking of the activated agent to an amino acid side chain of an endogenous protein at the surface of the enucleated erythroid cell functionalized with the exogenous polypeptide.

5. The method of claim 1, wherein the method further comprises contacting an enucleated erythroid cell with a first coupling reagent comprising the first click handle, thereby producing the activated cell.

6. The method of claim 5, wherein the method further comprises:

(d) contacting the activated cell with a second coupling reagent comprising a third click handle, and

(e) contacting the activated cell with a second activated agent comprising a second exogenous polypeptide covalently bound to a fourth click handle capable of reacting with the third click handle.

7. The method of claim 5, wherein the first click handle comprises an azide or an alkyne.

8. The method of claim 7, wherein the first click handle is an azide, and wherein the azide comprises 3-azidopropionic acid sulfo-NHS ester or 6-azidohexanoic acid sulfo-NHS ester.

9. The method of claim 7, wherein the first click handle is an alkyne, and wherein the alkyne comprises DBCO-sulfo-NHS ester or DBCO-PEG-NHS ester.

10. The method of claim 9, wherein the alkyne comprises DBCO-PEG-NHS ester, and the DBCO-PEG-NHS ester is either DBCO-PEG4-NHS ester or DBCO-PEG5-NHS ester.

11. The method of claim 5, wherein the first coupling reagent comprises a linker.

12. The method of claim 1, wherein the method further comprises contacting the activated cell with an activated agent comprising a second exogenous polypeptide covalently bound to the second click handle capable of reacting with the first click handle.

13. The method of claim 1, wherein the enucleated erythroid cell functionalized with the exogenous polypeptide comprises at least 5,000 copies of the exogenous polypeptide.

14. The method of claim 1, wherein the method further comprises contacting the exogenous polypeptide with a second coupling reagent comprising the second click handle, thereby producing the activated agent.

15. The method of claim 14, wherein the second click handle comprises an azide or an alkyne.

16. The method of claim 15, wherein the second click handle is an azide, and wherein the azide comprises 3-azidopropionic acid sulfo-NHS ester or 6-azidohexanoic acid sulfo-NHS ester.

17. The method of claim 15, wherein the second click handle comprises an alkyne, and wherein the alkyne comprises DBCO-sulfo-NHS ester or DBCO-PEG-NHS ester.

18. The method of claim 17, wherein the alkyne comprises DBCO-PEG-NHS ester, and the DBCO-PEG-NHS ester is either DBCO-PEG4-NHS ester or DBCO-PEG5-NHS ester.

19. The method of claim 14, wherein the second coupling reagent comprises a linker.

20. The method of claim 1, wherein the exogenous polypeptide comprises an enzyme, an antigen, an antibody, an antibody-like molecule, a growth factor, a transporter, a cytokine, a chemokine, a growth factor receptor, a cytokine receptor, a chemokine receptor, a DNA binding protein, or an RNA binding protein.

21. The method of claim 1, wherein the activated cell lacks a sortase transfer signature.

22. The method of claim 1, wherein the enucleated erythroid cell functionalized with the exogenous polypeptide lacks a sortase transfer signature.

23. The method of claim 1, wherein both of the activated cell and the enucleated erythroid cell functionalized with the exogenous polypeptide lack a sortase transfer signature.

24. The method of claim 1, wherein the method does not comprise a sortagging step.

25. A method of making an enucleated erythroid cell functionalized with an exogenous polypeptide, the method comprising:

(a) providing an activated cell comprising an enucleated erythroid cell covalently bound to a first click handle, wherein the activated cell was made using a process that does not comprise contacting an enucleated erythroid cell or a precursor thereof with a sugar comprising the first click handle,

(b) providing an activated agent comprising the exogenous polypeptide covalently bound to a second click handle capable of reacting with the first click handle, and

(c) contacting the activated cell with the activated agent, thereby producing the enucleated erythroid cell functionalized with the exogenous polypeptide,

wherein one or both of the activated cell and the enucleated erythroid cell functionalized with the exogenous polypeptide lack(s) a sortase transfer signature, and

wherein:

(i) the first click handle comprises a cyclooctyne and the second click handle comprises and azide;

(ii) the first click handle comprises an azide and the second click handle comprises a cyclooctyne;

(iii) the first click handle comprises a transcycloalkene and the second click handle comprises a tetrazine; or

(iv) the first click handle comprises a tetrazine and the second click handle comprises a transcycloalkene.

26. The method of claim 25, wherein the exogenous polypeptide is greater than about 30 kilodaltons.

27. The method of claim 25, wherein the exogenous polypeptide is greater than about 500 amino acids in length.

28. The method of claim 25, wherein the process results in covalent linking of the activated agent to an amino acid side chain of an endogenous protein at the surface of the enucleated erythroid cell functionalized with the exogenous polypeptide.

29. The method of claim 25, wherein the method further comprises contacting an enucleated erythroid cell with a first coupling reagent comprising the first click handle, thereby producing the activated cell.

30. The method of claim 29, wherein the first coupling reagent comprises a linker.

31. The method of claim 25, wherein the method further comprises contacting the activated cell with an activated agent comprising a second exogenous polypeptide covalently bound to the second click handle capable of reacting with the first click handle.

32. The method of claim 29, wherein the method further comprises:

(d) contacting the activated cell with a second coupling reagent comprising a third click handle, and

(e) contacting the activated cell with a second activated agent comprising a second exogenous polypeptide covalently bound to a fourth click handle capable of reacting with the third click handle.

33. The method of claim 25, wherein the enucleated erythroid cell functionalized with the exogenous polypeptide comprises at least 5,000 copies of the exogenous polypeptide.

34. The method of claim 25, wherein the method further comprises contacting the exogenous polypeptide with a second coupling reagent comprising the second click handle, thereby producing the activated agent.

35. The method of claim 34, wherein the second coupling reagent comprises a linker.

36. The method of claim 25, wherein the exogenous polypeptide comprises an enzyme, an antigen, an antibody, an antibody-like molecule, a growth factor, a transporter, a cytokine, a chemokine, a growth factor receptor, a cytokine receptor, a chemokine receptor, a DNA binding protein, or an RNA binding protein.

37. The method of claim 25, wherein the activated cell lacks a sortase transfer signature.

38. The method of claim 25, wherein the enucleated erythroid cell functionalized with the exogenous polypeptide lacks a sortase transfer signature.

39. The method of claim 25, wherein both of the activated cell and the enucleated erythroid cell functionalized with the exogenous polypeptide lack a sortase transfer signature.

40. The method of claim 25, wherein the method does not comprise a sortagging step.

41. The method of claim 25, wherein the first click handle comprises a cyclooctyne and the second click handle comprises and azide.

42. The method of claim 25, wherein the first click handle comprises an azide and the second click handle comprises a cyclooctyne.

43. The method of claim 25, wherein the first click handle comprises a transcycloalkene and the second click handle comprises a tetrazine.

44. The method of claim 25, wherein the first click handle comprises a tetrazine and the second click handle comprises a transcycloalkene.