CLAIMS

What is claimed is:

1. An engineered glucose dehydrogenase polypeptide comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14, or a functional fragment thereof, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

2. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said glucose dehydrogenase polypeptide comprises at least one mutation as provided in Table 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 11.1 and/ or 12.1.

3. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:14, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 17, 95/96, 95/96/118, 95/96/118/156/200/253, 95/96/147, 95/96/147/200, 95/96/147/200/253, 95/96/155/156, 95/96/155/195/200, 95/96/159/195/253, 95/155/156, 95/155/159/200, 95/155/200, 96, 96/147/195/200/253, 96/155, 96/155/159, 96/155/159/195, 96/155/159/200, 96/156/159/195, 96/156/159/195/200, 96/195, 147, 147/155, 147/155/156, 147/155/156/200, 147/155/159, 147/195/200/253, 155, 155/156, 155/156/195/200/253, 155/159/200, and 253, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

4. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:14, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 17, 17/21, 17/21/42, 17/21/42/155/260, 17/21/49, 17/21/49/51, 17/21/49/51/96, 17/21/49/51/260, 17/21/49/96/155/260, 17/21/49/260, 17/21/51/96, 17/21/51/195, 17/21/96, 17/21/96/155, 17/21/96/195/260, 17/21/96/253, 17/21/147/155/253, 17/21/155/253, 17/21/195, 17/21/253, 17/42, 17/42/49/195/260, 17/42/96, 17/44, 17/44/47/51/96, 17/44/47/51/96/114/147/195/199/253, 17/44/47/51/96/114/147/199, 17/44/47/51/96/114/195/199, 17/44/47/51/96/147/253, 17/44/47/51/178/195, 17/44/47/195/199, 17/44/51/114/195/253, 17/46/47/51/96/147, 17/46/47/51/96/195/253, 17/46/47/51/155, 17/46/47/51/155/195/199, 17/46/47/96/195, 17/46/47/178, 17/46/96/253, 17/47, 17/47/51, 17/47/51/96/114/147/195,

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5. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14, wherein said engineered glucose dehydrogenase

polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 21/42/96/178/195/253, 21/42/96/195, 21/96, 21/96/147/195, 21/96/149, 21/96/155/195/253, 21/96/167, 21/96/167/195, 21/96/178, 21/96/179/195/251/253, 21/96/195, 21/147/149, 21/147/253, 21/149/178/195, 21/155/178/253/256, 21/178/195/256, 42/96/149/253/256, 94, 96, 96/114/155/198/218/253/256/257, 96/114/155/252/253/257, 96/114/155/253, 96/114/196/214/253/256, 96/114/214/253/256, 96/147, 96/147/149, 96/147/149/155, 96/147/167/195, 96/149/155/167/178/253, 96/149/155/195, 96/149/178/179/251/256, 96/149/178/253, 96/149/198/252/253/257, 96/155, 96/155/167, 96/155/167/178, 96/155/195, 96/155/196/218/253/256/257, 96/155/196/252/253, 96/155/198, 96/155/252/253, 96/167, 96/178, 96/178/179/195, 96/195, 96/196, 96/196/198, 96/196/201/256, 96/251/253/256, 96/253/257, 96/256/257, 98, 103, 114/253/256/257, 147, 147/149, 149/167, 149/195, 149/218, 149/253, 149/257, 155/167, 155/178/195, 155/178/195/251/253, 155/195/251/253/256, 155/195/253/256, 155/196/198/253, 167, 167/195, 167/195/251/253, 196, 196/253/256/257, 198/253/256, 198/256, 205, 214/253/256, 251, and 257, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

6. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 21/47/96/114/155/251, 21/47/149/257, 21/47/155/178/257, 21/47/178, 21/47/178/251/256/257, 21/96/114/155/178, 21/114/155/251/257, 21/114/178/179, 21/155/178/209/256, 25/155, 25/178/256, 42/47/155/178, 47/114/149/178/251, 47/114/178/257, 47/149, 47/178, 47/178/179/251/257, 47/178/251/257, 96/114/155/198, 96/114/155/253/257, 96/253, 114/149/155/251/256, 114/149/178/256/257, 114/155/195/198/256, 114/209/256/257, 149, 149/256/257, 155, 155/178/179, 155/178/198/252/253, 155/195/198, 155/195/198/253/256/257, 155/256/257, 178/253, 178/256, 179/256/257, 251, and 253, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

7. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 21/42/149, 21/42/149/214/253, 21/42/179/214/244/253, 21/42/253/260/261, 21/149, 21/149/179, 21/149/179/253, 21/149/179/253/260, 21/149/179/253/260/261, 21/149/179/261, 21/149/260/261, 21/149/261, 21/179/214/253, 21/179/253, 21/179/261, 21/214/253, 21/253, 40, 42/149/179/253/260, 42/149/179/260/261, 42/149/214/253, 42/179/253/261, 42/253, 104, 111, 149, 149/179, 149/179/253, 149/179/253/260/261, 149/179/260, 149/179/260/261, 149/179/261, 149/253,

149/261, 160, 178, 179/214/253, 179/253, 179/253/260, 179/253/260/261, 179/253/261, 179/260/261, 179/261, 253, 253/260/261, 253/261, and 255, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

8. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14 wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 3, 21/104/160/178/179, 21/104/160/255/258, 21/104/178/179/255, 21/104/178/255/258, 21/104/255, 21/104/255/258, 21/178/179/255/260, 21/255/260, 31, 32, 40, 40/104/178/179, 40/160/178, 46/82/259, 103, 104/149/255/258/260, 104/149/258, 104/160, 104/160/178, 104/160/178/179/255, 104/160/178/179/260, 104/160/178/255/260, 104/160/179, 104/178/179/255/258, 104/178/255/258/260, 104/178/255/260, 104/179, 104/255/260, 104/258/260, 111/149/255/258, 111/149/255/260, 111/160/178/179/255/258/260, 111/160/255/260, 154, 156, 160, 160/178/179, 174, 194, 197, 205, 205/229, 222, 253, 257, 258/260, and 260, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

9. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said polypeptide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14, wherein said engineered glucose dehydrogenase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 3/31/154/205/257, 3/31/205, 3/154/205, 31/55/154/205, 31/55/205, 31/154/205, 31/154/205/257, 31/205, 31/205/222, 40/55/205, 40/154/205, 40/154/205/257, 40/174/205/229, 40/205/229, 54/205/253, 63, 63/149/154/174/178/253, 63/149/154/174/205/253, 63/149/156/178/205/253, 63/149/205, 63/149/205/229/253, 63/149/205/253, 63/154, 63/154/178/205/253, 63/154/205, 63/154/205/229, 63/154/205/229/253, 63/154/205/253, 63/156, 63/156/205, 63/205, 63/205/253, 63/253, 67, 98, 149/154, 149/154/205, 149/156/253, 149/205, 149/205/253, 154, 154/160, 154/160/205, 154/160/205/222/257, 154/160/253, 154/174, 154/174/178/205, 154/174/205/229, 154/205, 154/205/222, 154/205/222/257, 154/205/229, 154/205/253, 154/205/257, 154/229, 154/253, 156/205, 156/205/253, 156/229/253, 160, 160/205, 160/205/229, 194, 205, 205/222, 205/229, 205/229/253, 205/253, 208, 226, and 253, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 14.

10. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said engineered glucose dehydrogenase polypeptide comprises a polypeptide sequence that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 14, or a functional fragment thereof.

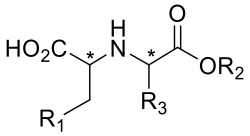
11. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said engineered glucose dehydrogenase polypeptide comprises SEQ ID NO: 14, or a functional fragment thereof.

12. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said engineered glucose dehydrogenase polypeptide comprises an amino acid sequence with at least 80% sequence identity to any even-numbered sequence set forth in any one of SEQ ID NO:6 to SEQ ID NO:1384.

13. The engineered glucose dehydrogenase polypeptide of Claim 1, wherein said engineered glucose dehydrogenase polypeptide comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOs: 6-1384.

14. The engineered glucose dehydrogenase polypeptide of any one of Claims 1-13, wherein said engineered glucose dehydrogenase polypeptide comprises a polypeptide sequence that exhibits at least one improved property compared to the engineered glucose dehydrogenase polypeptide of SEQ ID NO:4.

15. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved production of a compound of structural Formula **(IV)**



(IV)

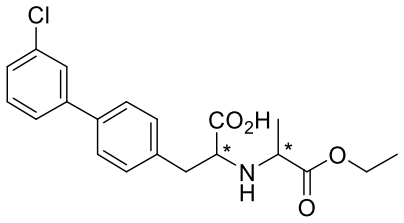
wherein

R_1 is selected from a hydrogen atom, or optionally substituted alkyl, alkenyl, alkynyl, alkoxy, arylalkoxy, hydroxyalkyl, heteroalkyl, heteroalkenyl, heteroalkynyl, haloalkyl, alkylthioalkyl, cycloalkyl, aryl, arylalkyl, heterocycloalkyl, heteroaryl, and heteroarylalkyl; and wherein

R_2 is independently selected from alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxy, aminocarbonyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carboxyalkyl, alkylamino, haloalkyl, alkylthioalkyl, cycloalkyl, aryl, arylalkyl, heterocycloalkyl, heteroaryl, and heteroarylalkyl; and wherein

R_3 is independently selected from methyl, d3-methyl and ethyl.

16. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved production of compound **(1)**



(1)

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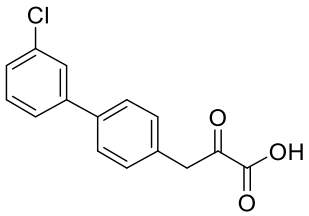
17. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved utilization of compound **(2)**



(2)

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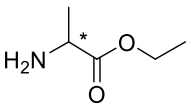
18. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved utilization of compound **(3)**



(3)

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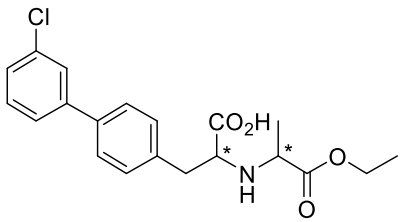
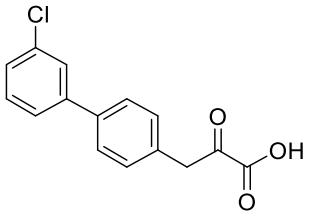
19. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved production of compound **(1)**



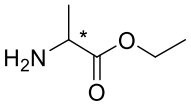
(1)

from compound **(2)**



(2)

and compound **(3)**



(3)

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20. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved enantioselectivity.

21. The engineered glucose dehydrogenase polypeptide of Claim 14, wherein said improved property comprises improved stability.

22. The engineered glucose dehydrogenase polypeptide of any one of Claims 1-21, wherein said engineered polypeptide is purified.

23. A composition comprising at least one glucose dehydrogenase polypeptide provided in any one of Claims 1-22.

24. An engineered polynucleotide encoding at least one engineered glucose dehydrogenase polypeptide of any one of Claims 1-22.

25. An engineered polynucleotide sequence encoding at least one engineered glucose dehydrogenase polypeptide, wherein said polynucleotide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 13, wherein the polynucleotide sequence of said glucose dehydrogenase polypeptide comprises at least one substitution at one or more positions.

26. The engineered polynucleotide sequence of Claim 24 or 25, wherein said polynucleotide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 13.

27. The engineered polynucleotide sequence of any one of Claims 24-26, wherein said polynucleotide sequence comprises SEQ ID NO: 13.

28. An engineered polynucleotide comprising the odd-numbered sequences set forth in any one of SEQ ID NO:5 to SEQ ID NO:1383.

29. A vector comprising the engineered polynucleotide of any one of Claims 24 to 28.

30. The vector of Claim 29, further comprising at least one control sequence.

31. An *in vitro* host cell comprising the vector of Claim 29 or 30.

32. The host cell of Claim 31, wherein said host cell produces at least one engineered glucose dehydrogenase polypeptide of any one of Claims 1-22.

33. A method of producing an engineered glucose dehydrogenase polypeptide in a host cell, comprising culturing the host cell of Claim 31 or 32, in a culture medium under suitable conditions, such that at least one engineered glucose dehydrogenase polypeptide is produced.

34. The method of Claim 33, further comprising the step of recovering said engineered glucose dehydrogenase polypeptide.

35. The method of Claim 33 or 34, further comprising the step of purifying said at least one engineered glucose dehydrogenase polypeptide.