

CLAIMS

We claim:

1. An engineered peroxidase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 4 and/or 6, or a functional fragment thereof, wherein said engineered peroxidase 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: 4 and/or 6.

2. The engineered peroxidase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 4, and wherein said engineered peroxidase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 393, 25/29/43, 25/29/65/82/92, 26/29, 80/128/306, 90, 127/168, 127/301, 128/307, 143, 160, 160/188, 160/307, 164, 168, 171/260, 185, 185/307, 189/190/226, 191, 191/260, 191/469, 202, 206, 208, 221, 222, 224, 224/260, 226, 228, 228/260, 241/270/283/306, 260/307, 290, 301, 306, 307, 311, 332, and 367, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

3. The engineered peroxidase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 6, and wherein said engineered peroxidase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 393, 25/29/43, 25/29/65/82/92, 80/128/306, 90, 127/301, 128/307, 143, 160, 160/188, 160/307, 164, 168, 171/260, 185, 185/307, 191, 191/260, 191/469, 202, 206, 208, 221, 222, 224, 224/260, 228, 228/260, 241/270/283/306, 260/307, 301, 306, 307, 311, 332, and 367, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6.

4. The engineered peroxidase of Claim 1, wherein said engineered peroxidase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered peroxidase variant set forth in Table 5.1 and/or 6.1

5. The engineered peroxidase of Claim 1, wherein said engineered peroxidase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered peroxidase variant set forth in SEQ ID NOS: 4 and/or 6.

6. The engineered peroxidase of Claim 1, wherein said engineered peroxidase is a variant engineered peroxidase set forth in SEQ ID NOS: 4 and/or 6.
7. The engineered peroxidase of Claim 1, wherein said engineered peroxidase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered peroxidase variant set forth in the even numbered sequences of SEQ ID NOS: 6-290.
8. The engineered peroxidase of Claim 1, wherein said engineered peroxidase comprises a polypeptide sequence forth in the even numbered sequences of SEQ ID NOS: 6-290.
9. The engineered peroxidase of any of Claims 1-8, wherein said engineered peroxidase comprises at least one improved property compared to wild-type *Anabaena (Nostoc) sp.* peroxidase.
10. The engineered peroxidase of Claim 9, wherein said improved property comprises improved activity on a substrate.
11. The engineered peroxidase of Claim 10, wherein said substrate comprises 10-acetyl-3,7-dihydroxyphenoxazine.
12. The engineered peroxidase of any of Claims 1-11, wherein said engineered peroxidase is purified.
13. A composition comprising at least one engineered peroxidase of any of Claims 1-12.
14. A polynucleotide sequence encoding at least one engineered peroxidase of any of Claims 1-13.
15. A polynucleotide sequence encoding at least one engineered peroxidase, wherein said polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOS: 3 and/or 5, wherein the polynucleotide sequence of said engineered peroxidase comprises at least one substitution at one or more positions.

16. A polynucleotide sequence encoding at least one engineered peroxidase comprising at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOS: 3 and/or 5, or a functional fragment thereof.

17. The polynucleotide sequence of Claims 15 or 16, wherein said polynucleotide sequence is operably linked to a control sequence.

18. The polynucleotide sequence of any of Claims 15-17, wherein said polynucleotide sequence is codon optimized.

19. The polynucleotide sequence of any of Claims 15-18, wherein said polynucleotide comprises an odd-numbered sequence of SEQ ID NOS: 5-289.

20. An expression vector comprising at least one polynucleotide sequence of any of Claims 15-19.

21. A host cell comprising at least one expression vector of Claim 20.

22. A host cell comprising at least one polynucleotide sequence of any of Claims 15-19.

23. A method of producing an engineered peroxidase in a host cell, comprising culturing the host cell of Claim 21 and/or 22, under suitable conditions, such that at least one engineered peroxidase is produced.

24. The method of Claim 23, further comprising recovering at least one engineered peroxidase from the culture and/or host cell.

25. The method of Claim 23 and/or 24, further comprising the step of purifying said at least one engineered peroxidase.