Claims

1. A Mmup monomer variant, characterized in that the Mmup monomer variant comprises an amino acid sequence with amino acid mutations of D91K and A99K based on SEQ ID NO: 1.

2. The Mmup monomer variant according to claim 1, characterized in that the Mmup monomer variant comprises an amino acid sequence of SEQ ID NO:2.

3. The Mmup monomer variant according to any one of claims 1-2, the Mmup monomer variant also comprise a mutation that introduces cysteine in order to link with the molecules for sequencing.

4. The Mmup monomer variant according to claim 3, the molecules for sequencing comprise the nucleic acid binding protein.

5. A construct comprising at least one Mmup monomer variant of any one of claims 1-4.

6. The construct according to claim 5, characterized in that the construct also comprises a wild-type Mmup monomer.

7. The construct according to any one of claims 5-6, characterized in that the construct comprises 1 to 20 Mmup monomer variants, wherein the Mmup monomer variants are identical or different.

8. The construct according to any one of claims 5-7, characterized in that the construct comprises 1 to 20 wild-type Mmup monomers.

9. The construct according to claim 6, characterized in that the Mmup monomer variant and the Mmup monomer variant, the wild-type Mmup monomer and the wild-type Mmup monomer, as well as the Mmup monomer variant and the wild-type Mmup monomer are covalently attached.

10. The construct according to any one of claims 5-7, characterized in that the construct comprises 4 to 10 identical or different Mmup monomer variants.

Claims

11. A porin comprising at least one Mmup monomer variant, characterized in that the Mmup monomer variant comprises an amino acid sequence with amino acid mutations of D91K and A99K based on SEQ ID NO: 1.

12. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the Mmup monomer variant comprises an amino acid sequence of SEQ ID NO:2.

13. The porin comprising at least one Mmup monomer variant according to any one of claims 11-12, the Mmup monomer variant also comprise a mutation that introduces cysteine in order to link with the molecules for sequencing.

14. The porin comprising at least one Mmup monomer variant according to claim 13, the molecules for sequencing comprise the nucleic acid binding protein.

15. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the porin comprising at least one Mmup monomer variant comprises at least 1 to 20 Mmup monomer variants, wherein the Mmup monomer variants are identical or different.

16. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the porin comprising at least one Mmup monomer variant comprises the wild-type Mmup monomer.

17. The porin comprising at least one Mmup monomer variant according to claim 16, characterized in that the porin comprising at least one Mmup monomer variant comprises 1 to 20 wild-type Mmup monomers.

18. The porin comprising at least one Mmup monomer variant according to any one of claims 11-17, characterized in that the porin comprising at least one Mmup monomer variant comprises 4 to 10 identical or different Mmup monomer variants.

19. The porin comprising at least one Mmup monomer variant according to claim 16,

characterized in that the Mmup monomer variant and the Mmup monomer variant, the wild-type Mmup monomer and the wild-type Mmup monomer, as well as the Mmup monomer variant and the wild-type Mmup monomer are covalently attached.

20. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the porin comprising at least one Mmup monomer variant comprises a constriction and a loops region.

21. The porin comprising at least one Mmup monomer variant according to claim 16, characterized in that the pore channel diameter of the constriction of the porin comprising at least one Mmup monomer variant is less than the pore channel diameter of the constriction of the porin comprising the wild-type Mmup monomer.

22. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the pore channel diameter of the constriction of the porin comprising at least one Mmup monomer variant is less than 15.5Å.

23. The porin comprising at least one Mmup monomer variant according to claim 11, characterized in that the pore channel diameter of the constriction of the porin comprising at least one Mmup monomer variant is less than 13.5Å.

24. The porin comprising at least one Mmup monomer variant according to claim 22, characterized in that the porin comprising at least one Mmup monomer variant also comprises a cap forming region and/or a barrel forming region.

25. A nucleotide sequence encoding the Mmup monomer variant of any one of claims 1-4, the construct of any one of claims 5-10, or the porin comprising at least one Mmup monomer variant of any one of claims 11-24.

26. A vector comprising the nucleotide sequence of claim 25.

27. The vector according to claim 26, characterized in that the vector comprises a promoter operably linked with the nucleotide sequence encoding the Mmup monomer variant of any one of claims 1-4, the construct of any one of claims 5-10, or the porin comprising at least one

Mmup monomer variant of any one of claims 11-24.

28. The vector according to claim 27, characterized in that the promoter is an inducible promoter or a constitutive promoter, wherein the inducible promoter is an acetamide inducible promoter.

29. The vector according to claim 26, characterized in that the nucleotide sequence encoding the porin comprising at least one Mmup monomer variant comprises at least one nucleotide sequence encoding the Mmup monomer variant.

30. The vector according to claim 29, characterized in that the nucleotide sequence encoding the porin comprising at least one Mmup monomer variant also comprises at least one nucleotide sequence encoding the wild-type Mmup monomer.

31. The vector according to claim 29, characterized in that the nucleotide sequence encoding the Mmup monomer variant and the nucleotide sequence encoding the Mmup monomer variant, the nucleotide sequence encoding the Mmup monomer variant and the nucleotide sequence encoding the wild-type Mmup monomer, or, the nucleotide sequence encoding the wild-type Mmup monomer and the nucleotide sequence encoding the wild-type Mmup monomer are linked by sequences encoding amino acid linker.

32. A mutant bacterium expressing the Mmup monomer variants of any one of claims 1-4, the construct of any one of claims 5-10, or the porin comprising at least one Mmup monomer variant of any one of claims 11-24.

33. A method of producing the Mmup porin, characterized in that the method includes: transforming the mutant bacterium of claim 32 with vector of any one of claims 26-31, and inducing the mutant bacterium to express the Mmup porin.

34. A method of characterizing a target polynucleotide, characterized in that method includes:

(a) Contacting the target polynucleotide with porin comprising at least one Mmup monomer variant of any one of claims 11-24, so that the target polynucleotide sequence passes

through the porin comprising at least one Mmup monomer variant; and

(b) Obtaining one or more characteristics of the interaction between the nucleotide and the porin comprising at least one Mmup monomer variant when the target polynucleotide passes through the porin comprising at least one Mmup monomer variant to characterize the target polynucleotide.

35. The method according to claim 34, characterized in that the step (a) also includes a step of contacting the target polynucleotide with one or a combination of two or more of the nucleic acid binding protein, helicase, exonuclease, telomerase, topoisomerase, reverse transcriptase, translocase and/or the polymerases, which makes the translocation speed of the target polynucleotide sequence through the porin less than the translocation speed in the absence of the nucleic acid binding protein, helicase, exonuclease, telomerase, topoisomerase, reverse transcriptase, translocase, and/or polymerase.

36. The method according to claim 34, characterized in that the method also includes a step of applying a potential difference across the porin contacted with the target polynucleotide.

37. An application in characterizing the target polynucleotide of the porin comprising at least one Mmup monomer variant of any one of claims 11-24, the Mmup monomer variant of any one of claims 1-4, the construct of any one of claims 5-10, the nucleotide sequence of claim 25, the vector of any one of claims 26-31, or the mutant bacterium of claim 32.

38. A kit for characterizing the target polynucleotide, characterized in that the kit comprises porin comprising at least one Mmup monomer variant of any one of claims 11-24, the Mmup monomer variant of any one of claims 1-4, the construct of any one of claims 5-10, the nucleotide sequence of claim 25, the vector of any one of claims 26-31, or the mutant bacterium of claim 32.

39. A device for characterizing the target polynucleotide, characterized in that the device comprises the porin comprising at least one Mmup monomer variant of any one of claims 11-24, the Mmup monomer variant of any one of claims 1-4, the construct of any one of claims 5-10, the nucleotide sequence of claim 25, the vector of any one of claims 26-31, or the mutant bacterium of claim 32.