

Claims

1. An artificial nanopore comprising a multimeric assembly of subunits, each subunit comprising:
 - (i) the transmembrane (TM) sequence of a β -barrel or α -helical pore forming protein fused to the amino acid sequence of (ii) a subunit of a ring-forming protein which controls the transport of a polypeptide or polynucleotide across the TM region of the assembly.
2. Artificial nanopore according to claim 1, comprising the TM sequence of an α -helical pore forming protein, preferably the TM sequence of FraC, ClyA, AhlB or Wza (translocon for *E. coli* capsular polysaccharides).
3. Artificial nanopore according to claim 1, comprising the TM sequence of a β -barrel pore forming protein, preferably the TM sequence of α -hemolysin, aerolysin or anthrax protective antigen (PA).
4. Artificial nanopore according to claim 3, wherein the TM sequence comprises or consists of the amino acid sequence
VHGNAEVHASFFDIGGSVSAGF.
5. Artificial nanopore according to any one of claims 1-4, wherein the TM sequence is N- or C-terminally fused to the subunit of a ring-forming protein.
6. Artificial nanopore according to any one of claims 1-4, wherein the TM sequence is inserted within the sequence of the subunit of a ring-forming protein.
7. Artificial nanopore according to any one of claims 1-5, wherein the TM sequence is flanked on the N- and/or C-terminal side by a flexible linker of at least 3, preferably at least 5, amino acids, more preferably

wherein the N-terminal linker comprises or consists of the sequence GSS and/or wherein the C-terminal linker comprises or consists of the sequence SSG.

8. Artificial nanopore according to any one of claims 1-7, wherein the ring-forming protein is a heptameric protein.

9. Artificial nanopore according to claim 8, wherein the ring-forming heptameric protein controls the transport of a polynucleotide across the TM region.

10. Artificial nanopore according to claim 9, wherein the heptameric protein is an ATPase, preferably *A. aeolicus* ATPase or a homolog or functional equivalent thereof.

11. Artificial nanopore according to claim 8, wherein the ring-forming heptameric protein controls the transport of a polypeptide across the TM region.

12. Artificial nanopore according to claim 11, wherein the heptameric protein is proteasome activator PA28, PA26, or a homolog or functional equivalent thereof.

13. Artificial nanopore according to any one of claims 1-12, wherein the C-terminus of the subunit of the ring-forming protein comprising the TM sequence is genetically fused to the N-terminus of a proteasome α -subunit.

14. Artificial nanopore according to any one of claims 1-12, wherein the N-terminus of the subunit of the ring-forming protein comprising the TM sequence is genetically fused to the C-terminus of a Clp protease (ClpP) subunit.

15. A multi-protein nanopore sensor complex, comprising (i) an artificial nanopore according to any one of claims 1-14, (ii) one or two rings

composed of proteasome α -subunits and optionally (iii) one or two rings composed of proteasome β -subunits.

16. A multi-protein nanopore sensor complex according to claim 15, wherein the proteasome α -subunit lacks at least 5 amino acids at its N-terminus.

17. Multi-protein nanopore sensor complex according to claim 15 or 16, wherein the ring composed of proteasome β -subunits is engineered to provide a distinct type of protease activity.

18. Multi-protein nanopore sensor complex according to any one of claims 15-17, further comprising a protein translocase which can bind, unfold, and translocate a polynucleotide or polypeptide through the nanopore sensor complex in a sequential order.

19. Multi-protein nanopore sensor complex according to claim 18, wherein the protein translocase is an NTP-driven unfoldase, preferably an AAA+ unfoldase, more preferably wherein the protein translocase is selected from ClpX, VAT, PAN, AMA, 854, MBA and SAMP.

20. An analytical system comprising a hydrophobic membrane separating a fluid chamber into a *cis* side and a *trans* side, said membrane comprising an artificial nanopore according to any one of claims 1-14, or a multiprotein nanopore sensor complex according to any one of claims 15-19.

21. A method for single molecule analysis, preferably for identification and/or sequencing of a biopolymer, more preferably for single molecule polypeptide or polynucleotide sequencing, comprising adding a biopolymer to be analyzed to the chamber of an analytical system according to claim 20 and allowing the biopolymer to contact the pore.

22. The use of an analytical system according to claim 20, for single molecule analysis, preferably for identification and/or sequencing of a

biopolymer, more preferably for single molecule polypeptide or polynucleotide sequencing.

23. A nucleic acid molecule encoding a subunit of an artificial nanopore according to any one of claims 1-14.

24. An expression vector comprising a nucleic acid molecule according to claim 23.

25. A host cell comprising an expression vector according to claim 24, optionally further comprising a distinct expression vector encoding a proteasome beta-subunit and/or a proteasome alpha-subunit.