CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of characterizing a conformation state or mechanistic movement of a protein in a nanopore system comprising a nanopore disposed in a membrane that separates a first conductive liquid medium from a second conductive liquid medium, wherein the nanopore comprises an opening and a tunnel that provides liquid communication between the first conductive liquid medium and the second conductive liquid medium, and wherein the protein is physically associated with a polymer in the first conductive liquid medium, the method comprising:

(a) applying an electrical potential between the first conductive liquid medium and the second conductive liquid medium to cause the polymer to interact with the nanopore tunnel, wherein at least one dimension of the protein exceeds a diameter of the nanopore tunnel thereby preventing the protein from passing through the nanopore opening;

(b) measuring an ion current through the nanopore during the interaction of the polymer with the nanopore tunnel to provide a current pattern;

(c) determining a position and/or movement of at least one polymer subunit in the nanopore tunnel from the current pattern; and

(d) associating the position and/or movement of the at least one polymer subunit with the conformation state or mechanistic movement of the protein.

2. The method of Claim 1, wherein the polymer is a nucleic acid, PNA, or a combination thereof.

3. The method of Claim 2, wherein the nucleic acid is DNA, RNA, or a combination thereof.

4. The method of Claim 2, wherein the nucleic acid comprises an abasic residue.

5. The method of Claim 2, wherein the nucleic acid is not a homopolymer.

6. The method of Claim 1, wherein the protein is an enzyme.

7. The method of Claim 6, wherein the enzyme is a molecular motor.

8. The method of Claim 7, wherein the molecular motor is a translocase, a polymerase, a helicase, an exonuclease, a viral packaging motor, or a topoisomerase.

9. The method of Claim 6, wherein the enzyme is a Brownian motor, Brownian ratchet ribosome, myosin, or kinesin.

10. The method of Claim 1, wherein the protein is a mutant protein or fusion protein.

11. The method of Claim 1, wherein the protein comprises two or more domains capable of mutual interaction.

12. The method of Claim 1, wherein the protein is covalently coupled to the polymer.

13. The method of Claim 1, wherein the position and/or movement of the at least one polymer subunit can be resolved to about 35 pm.

14. The method of Claim 1, wherein the position of the at least one polymer subunit is associated with a conformational state of the protein.

15. The method of Claim 7, wherein the movement of the at least one polymer subunit is associated with a length of a discrete translocation step of the polymer within the nanopore tunnel that is conferred by the molecular motor.

16. The method of Claim 7, wherein the movement of the at least one polymer subunit is associated with a temporal duration of a discrete translocation step of the polymer within the nanopore tunnel that is conferred by the molecular motor.

17. The method of Claim 16, wherein the temporal duration can be resolved to about 800 ns.

18. The method of Claim 7, wherein the movement of the at least one polymer subunit is associated with an incidence rate of polymer translocation missteps committed by the molecular motor.

19. The method of Claim 6, wherein the conformation state or mechanistic movement of the enzyme correlates with a presence of a reaction condition or putative agonist, antagonist, or co-factor.

20. The method of Claim 1, wherein the nanopore is a solid-state nanopore, a protein nanopore, a hybrid solid state-protein nanopore, a biologically adapted solid-state nanopore, or a DNA origami nanopore.

21. The method of Claim 20, wherein the protein nanopore is alpha-hemolysin, leukocidin, *Mycobacterium smegmatis* porin A (MspA), outer membrane porin F (OmpF), outer membrane porin G (OmpG), outer membrane phospholipase A, *Neisseria* autotransporter lipoprotein (NalP), WZA, *Nocardia farcinica* NfpA/NfpB cationic selective channel, lysenin or a homolog or variant thereof.

22. The method of Claim 20, wherein the protein nanopore sequence is modified to contain at least one amino acid substitution, deletion, or addition.

23. The method of Claim 22, wherein the at least one amino acid substitution, deletion, or addition results in a net charge change in the nanopore.

24. The method of Claim 20, wherein the protein nanopore has a constriction zone with a non-negative charge.

25. The method of Claim 1, wherein the electrical potential applied is between 10 mV and 1 V or between -10 mV and -1 V.

26. A method of characterizing a conformation state or mechanistic movement of a protein in a nanopore system comprising a nanopore disposed in a membrane that separates a first conductive liquid medium from a second conductive liquid medium, wherein the nanopore comprises an opening and a tunnel that provides liquid communication between the first conductive liquid medium and the second conductive liquid medium, and wherein the protein is physically associated with a polymer in the first conductive liquid medium, the method comprising:

(a) applying an electrical potential between the first conductive liquid medium and the second conductive liquid medium to cause the polymer to interact with the

nanopore tunnel, wherein at least one dimension of the protein exceeds a diameter of the nanopore tunnel thereby preventing the protein from passing through the nanopore opening;

(b) measuring an ion current through the nanopore during the interaction of the polymer with the nanopore tunnel to provide a first current pattern;

(c) comparing the first current pattern to a reference current pattern;

(d) determining a change in position and/or movement of at least one polymer subunit in the nanopore tunnel from the position and/or movement of at least one polymer subunit in the nanopore tunnel determined from the reference current pattern; and

(e) associating the change in position and/or movement of the at least one polymer subunit in the nanopore tunnel with the conformation state or mechanistic movement of the protein.

27. The method of Claim 26, wherein the nanopore system has a presence or absence of a putative protein agonist, antagonist, or co-factor in the first conductive medium that is different from the nanopore system used to generate the reference current pattern.

28. The method of Claim 26, wherein the nanopore system has a concentration of a putative protein agonist, antagonist, or co-factor in the first conductive medium that is different from the nanopore system used to generate the reference current pattern.

29. The method of claim 27 or claim 28, wherein the conformation state or mechanistic movement correlates with a presence or degree of modulation of protein activity or conformation conferred by the presence, absence, or concentration of the putative agonist, antagonist, or co-factor.

30. The method of Claim 26, wherein the the nanopore system comprises at least one amino acid difference in the amino acid sequence of the protein compared to the amino acid protein sequence in the protein of the nanopore system used to generate the reference current pattern, wherein the conformation state or mechanistic movement correlates with the amino acid difference in the amino acid sequence.

31. The method of Claim 26, further comprising generating a reference current pattern.