The ability to observe the mechanistic functioning of complex bio-molecules directly, and not just via the input and output of bulk assays, can accelerate health care and address how biological systems really work. However, notwithstanding the advances of single-molecule techniques, a need remains for inexpensive and facile techniques that can address mechanistic movements and conformation states of proteins at improved spatial and temporal resolutions.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

Disclosed is a method of characterizing a protein in a nanopore system. The nanopore system comprises a nanopore disposed in a membrane that separates a first conductive liquid medium from a second conductive liquid medium, wherein the nanopore comprises 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 comprises:

(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;

(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 a characteristic of the protein.

In one aspect, the present invention provides 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 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;

(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.

In one example, the polymer is a nucleic acid, PNA, or a combination thereof. In one example, the nucleic acid is DNA, RNA, or a combination thereof. In one example, the nucleic acid comprises an abasic residue. In one example, the nucleic acid is not a homopolymer.

In one example, the protein is an enzyme. In one example, the enzyme is a molecular motor. In one example, the molecular motor is a translocase, a polymerase, a helicase, an exonuclease, a viral packaging motor, or a topoisomerase. In one example, the enzyme is a Brownian motor, Brownian ratchet ribosome, myosin, or kinesin. In one example, the protein is a mutant protein or fusion protein. In one example, the protein comprises two or more domains capable of mutual interaction. In one example, the protein is covalently coupled to the polymer.

In one example, the position and/or movement of the at least one polymer subunit can be resolved to about 35 pm. In one example, the position of the at least one polymer subunit is associated with a conformational state of the protein. In one example, 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. In one example, 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. The temporal duration can be resolved to about 800 ns. In one example, the movement of the at least one polymer subunit is associated with an incidence rate of polymer translocation missteps committed by the molecular motor. In one example, the characteristic of the enzyme is a presence or degree of modulation of enzyme activity conferred by a reaction condition or putative agonist, antagonist, or co-factor.

In one example, 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. In one example, 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. In one example, the protein nanopore sequence is modified to contain at least one amino acid substitution, deletion, or addition. In one example, the at least one amino acid substitution, deletion, or addition results in a net charge change in the nanopore. In one example, the protein nanopore has a constriction zone with a non-negative charge.

In one example, the electrical potential applied is between 10 mV and 1 V or between -10 mV and -1 V.

Also disclosed is a method of characterizing a protein in a nanopore system. The comprises a nanopore disposed in a membrane that separates a first conductive liquid medium from a second conductive liquid medium, wherein the nanopore comprises 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 comprises:

(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;

(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 a characteristic of the enzyme.

In another aspect, the present invention provides 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 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;

(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.

In one example, the nanopore system comprises a difference from the nanopore system used to generate the reference current pattern. In one example, the difference is the presence or absence of a putative protein agonist, antagonist, or co-factor in the first conductive medium. In one example, the difference is a difference concentration of a putative protein agonist, antagonist, or co-factor in the first conductive medium. In one example, the characteristic is a presence or degree of modulation of protein activity or conformation conferred by the putative agonist, antagonist, or co-factor. In one example, the difference is at least one amino acid difference in the amino acid sequence of the protein compared to the amino acid protein sequence in the nanopore system used to generate the reference current pattern. In one example, the characteristic is a presence or degree of modulation of protein activity or conformation conferred by the amino acid difference in the amino acid sequence. In one example, the method further comprises generating a reference current pattern.

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 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;

(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 19, 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 19, wherein the protein nanopore sequence is modified to contain at least one amino acid substitution, deletion, or addition.

23. The method of Claim 21, 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 19, 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 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;

(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.