

CLAIMS

We claim:

1. A device for sequencing nucleic acids, comprising:
at least a first electrode and a second electrode, the first and second electrode are separated by a gap; and
a polymerase comprising two attachment sites, a first attachment site for attachment of the polymerase to the first electrode and a second attachment site for attachment of the polymerase to the second electrode, wherein the two attachment sites are separated by a distance of at least about 1 nm and when the polymerase is attached to the first and second electrodes, the first attachment site and the second attachment site do not undergo movement relative to each other that exceeds 10% of their separation when the polymerase undergoes open-to-closed conformational changes.
2. The device of claim 1, wherein the two attachment sites of the polymerase are in an inactive region of the polymerase.
3. The device of claim 1 or claim 2, wherein one of the two attachment sites is located on an n-terminus of the polymerase.
4. The device of any one of claims 1-3, wherein at least the first attachment site of the polymerase is specifically chemically modified for attachment to the first electrode.
5. The device of any one of claims 1-4, wherein the polymerase is a DNA polymerase.
6. The device of claim 5, wherein the DNA polymerase is phi29 polymerase.
7. The device of any one of claims 1-4, wherein the polymerase is an RNA polymerase.
8. The device of claim 7, wherein the RNA polymerase is a polio virus 3D RNA dependent RNA polymerase.

9. The device of any one of claims 1-8, wherein the polymerase is modified to incorporate a ligand-binding site.
10. The device of any one of claims 1-9, wherein the polymerase is a biotinylated polymerase.
11. The device of claim 10, wherein the polymerase is a biotinylated polymerase and is attached to the first electrode and/or second electrode via streptavidin.
12. The device of any one of claims 1-11, wherein the polymerase is modified to incorporate a non-canonical amino acid residue that allows for click-chemistry attachment of one or more other chemical groups to the first electrode and/or second electrode.
13. The device of claim 12, wherein the non-canonical amino acid is 4-Azido-L-phenylalanine.
14. The device of any one of claims 1-13, wherein atomic coordinates for the first and second attachment points are the same to within 10% when the polymerase is in an open or closed conformation.
15. The device of any one of claims 1-14, wherein the first electrode and second electrode comprise a metal selected from the group consisting of gold, platinum, palladium, and ruthenium or alloy thereof.
16. The device of any one of claims 1-15, wherein the first electrode and second electrode are co-planar.
17. The device of any one of claims 1-15, further comprising a dielectric substrate, wherein the first electrode is disposed on the dielectric substrate and an insulating dielectric layer disposed on the first electrode and the second electrode is disposed on the insulating dielectric layer; and a passivation layer disposed on the second electrode.
18. A system, comprising the device of any one of claims 1-17 and a nucleic acid template.

19. The system of claim 18, wherein the nucleic acid template is a double stranded DNA template comprising at least one nick of more than one nucleotide.

20. The system of claim 19, wherein the at least one nick of more than one nucleotide is of nine or more nucleotides.

21. The system of claim 18, wherein the nucleic acid template is a circular DNA comprising a double stranded region.

22. The system of claim 18, wherein the nucleic acid template is a primed single-stranded RNA.

23. A method of determining the number of nucleic acids repeated in a nucleic acid template sequence comprising:

- (a) introducing a solution comprising a nucleic acid template to a device of any one of claims 1-17;
- (b) measuring a first current generated when a bias is applied to the device;
- (c) introducing a solution comprising a dNTP to the device under conditions that allow for incorporation of the dNTP complementary to the nucleic acid template;
- (d) measuring a second current generated in step (c);
- (e) removing the solution comprising the unincorporated dNTP;
- (f) repeating steps (c) to (e);
- (g) obtaining a signal stream from the current generated in step (b) and step (d), wherein the signal stream comprises at least one pause and at least one burst;
- (h) identifying sections of current signal that fluctuate between two levels of current an amount of time of greater than about 40 ms; and
- (i) determining that the nucleotide has been repeated in the nucleic acid template sequence if the signal stream comprises a pause that is about twice the length of a plurality of pauses in the signals stream.

24. A method of determining the identity of a nucleotide being incorporated into a nucleic acid template sequence, the method comprising:

- (a) introducing a solution comprising a nucleic acid template to a device of any one of claims 1-17;
- (b) measuring a first current generated when a bias is applied to the device;
- (c) introducing a solution comprising a dNTP to the device under conditions that allow for incorporation of the dNTP complementary to the nucleic acid template;
- (d) measuring a second current generated in step (c);
- (e) removing the solution comprising the unincorporated dNTP;
- (f) repeating steps (c) to (e);
- (g) obtaining a signal stream from the current generated in step (b) and step (d), wherein the signal stream comprises at least one pause and at least one burst;
- (h) determining the identity of the incorporated nucleotide from the burst signal.

25. A method of sequencing a nucleic acid, comprising:

- (a) introducing a solution comprising a nucleic acid template to a device of any one of claims 1-17, wherein the nucleic acid template comprises all possible 16 combinations of two sequential bases in the sequence;
- (b) introducing a solution comprising a dNTP to the device under conditions that allow for incorporation of the dNTP complementary to the nucleic acid template;
- (c) obtaining a signal stream from repeat sequences in the nucleic acid template, wherein the signal stream comprises at least one pause and at least one burst;
- (d) using a signal analysis to characterize the burst signal of each incorporated dNTP; and
- (e) applying the signal analysis for sequencing a nucleic acid of unknown sequence.

26. The method of any one of claims 23-25, wherein fluctuations that signal an active polymerase activity are those 25% or more of the background current whereas fluctuations up to 10% characterize an inactive polymerase.

27. The method of any one of claims 23-26, wherein the nucleic acid template is a double stranded DNA template comprising at least one nick of more than one nucleotide.

28. The method of claim 27, wherein the at least one nick of more than one nucleotide is of nine or more nucleotides and the polymerase is phi29 DNA polymerase.
29. The method of any one of claims 23-26, wherein the nucleic acid template is a circular DNA comprising a double stranded region.
30. The method of any one of claims 23-26, wherein the nucleic acid template is a primed single-stranded RNA and the polymerase is an RNA polymerase, such as a polio virus 3D RNA dependent RNA polymerase.
31. The method of claim 30, wherein the primed single-stranded RNA is a hairpin primed single stranded RNA.