

CLAIMS

What is claimed is:

1. A method for sequencing a polynucleotide using a bioelectronic device, the method comprising:
 - (a) introducing a template polynucleotide to a bioelectronic device, wherein the bioelectronic device comprises a polymerase functionally coupled to at least one of a first electrode and a second electrode;
 - (b) introducing a solution comprising four nucleotidepolyphosphate monomers to the device comprising the template polynucleotide, wherein at least three of the four nucleotidepolyphosphate monomers present in the solution comprises a distinctive charge relative to its corresponding standard nucleotidetriphosphate monomer; and
 - (c) obtaining a bioelectronic signature of polymerase activity based on current fluctuations as each complementary nucleotidepolyphosphate monomer is incorporated into the template polynucleotide;wherein at least one characteristic of the bioelectronic signature identifies each of the complementary nucleotidepolyphosphate monomers incorporated into the template polynucleotide.
2. The method of claim 1, wherein the bioelectronic signature comprises a closed period corresponding to the polymerase being in a closed state.
3. The method of claim 1 or claim 2, wherein the at least one characteristic of the bioelectronic signature comprises current amplitude level.
4. The method of any of claims 1 to 3, wherein at least one of the nucleotidepolyphosphate monomers present in the solution comprises a distinctive negative charge relative to its corresponding standard nucleotidetriphosphate monomer.
5. The method of any of claims 1 to 3, wherein at least two of the nucleotidepolyphosphate monomers present in the solution comprise distinctive negative charges relative to their corresponding standard nucleotidetriphosphate monomer.

6. The method of any of claims 1 to 3, wherein at least three of the nucleotidetriphosphate monomers present in the solution comprise distinctive negative charges relative to their corresponding standard nucleotidetriphosphate monomer.
7. The method of any of claims 4 to 6, wherein the negative charge is conferred by at least one additional phosphate group relative to its corresponding standard nucleotidetriphosphate monomer.
8. The method of any of claims 4 to 6, wherein the negative charge is conferred by 1 to 20 additional phosphate groups.
9. The method of any of claims 1 to 8, wherein at least one of the nucleotidetriphosphate monomers present in the solution comprises a distinctive positive charge relative to its corresponding standard nucleotidetriphosphate monomer.
10. The method of any of claims 3 to 9, wherein a distinctive negative charge on the at least one nucleotidetriphosphate monomer corresponds to an increased or decreased current amplitude level relative to the current amplitude level of its corresponding standard nucleotidetriphosphate monomer.
11. The method of any of claims 3 to 9, wherein a distinctive positive charge on the at least one nucleotidetriphosphate monomer corresponds to an increased or decreased current amplitude level relative to the current amplitude level of its corresponding standard nucleotidetriphosphate monomer.
12. The method of any of claims 1 to 11, wherein the template polynucleotide is DNA.
13. The method of any of claims 1 to 12, wherein the exonuclease activity of the polymerase is disabled.
14. The method of any of claims 1 to 13, wherein the polymerase is functionally coupled to the first and second electrodes using a linker comprising thio-streptavidin.

15. The method of claim 14, wherein linker is attached to a region of the polymerase that is inactive.
16. The method of any of claims 1 to 15, wherein the method comprises applying a voltage bias between the first and second electrodes that is 100mV or less.
17. The method of any of claims 1 to 16, wherein the standard nucleotidetriphosphate monomers comprise adenine (dATP), cytosine (dCTP), guanine (dGTP), and thymine (dTTP).
18. The method of any one of claims 1 to 17, wherein the first and/or the second electrode comprises gold, palladium, platinum, silver, copper, or any alloys thereof.
19. The method of any of claims 1 to 18, wherein the device comprises a dielectric layer at least partially covering a top surface of the first and/or second electrode.
20. The method of any of claims 1 to 19, wherein the first electrode and second electrode are positioned so that about a 1 nm to about a 50 nm gap is formed between the two electrodes.
21. A bioelectronic device comprising:
 - a first electrode and a second electrode separated by a gap; and
 - a protein attached to the first and second electrodes via a linker comprising a distinctive electrical charge;wherein the distinctive electrical charge modulates conductance through the protein.
22. The device of claim 21, wherein the linker comprises a peptide or polypeptide.
23. The device of claim 21 or claim 22, wherein the linker comprises streptavidin.
24. The device of any of claims 21 to claim 23, wherein the protein is biotinylated.
25. The device of any of claims 21 to claim 24, wherein the linker comprises a distinctive negative charge.

26. The device of claim 25, wherein the distinctive negative charge is conferred by addition of a glutamate moiety, an aspartate moiety, or a combination thereof, coupled to the streptavidin.
27. The device of claim 25 or claim 26, wherein the distinctive negative charge increases the conductance through the protein.
28. The device of any of claims 21 to 24, wherein the linker comprises a distinctive positive charge.
29. The device of claim 28, wherein the distinctive positive charge is conferred by addition of an arginine moiety, a histidine moiety, a lysine moiety, or a combination thereof, coupled to the streptavidin.
30. The device of claim 28 or claim 29, wherein the distinctive positive charge increases or decreases the conductance through the protein.
31. The device of any of claims 21 to 30, wherein the first and/or the second electrode comprises gold, palladium, platinum, silver, copper, or any alloys thereof.
32. The device of any of claims 21 to 31, wherein the protein is selected from the group consisting of a polymerase, a nuclease, a proteasome, a glycopeptidase, a glycosidase, a kinase and an endonuclease.
33. The device of any of claims 21 to 32, wherein the linker is attached to an inactive region of the protein.
34. The device of any of claim 21 to 33, wherein the linker comprises streptavidin coupled to a polyglutamate moiety, the protein comprises a polymerase, and the first and second electrodes comprise palladium, platinum, or any alloys thereof.
35. A method of modulating electronic conductance through a protein using any of the devices of claims 21 to 34.