between sequencing runs, up to the point where binding the first polynucleotide is so limiting that it is unfeasible.

The limits of nanopore detection have been estimated for various analytes. Capture of a 92-nucleotide synthetic piece of single strand DNA (ssDNA) by a protein nanopore (hemolysin) was determined to be at a frequency of $3.0\pm0.2\ s^{-1}\ uM^{-1}$ (Maglia, Restrepo et al. 2008, Proc Natl Acad Sci U S A **105**(50): 19720-5). Capture could be increased ~10 fold by the addition of a ring of positive charges at the entrance to the hemolysin barrel ($23.0\pm2\ s^{-1}\ uM^{-1}$). To put this into context, 1 uM of 92 nucleotide ssDNA is equivalent to 31 ug of DNA required per single channel recording, assuming a cis chamber volume of 1 ml. The market leading genomic DNA purification kit from human blood (Qiagen's PAXgene Blood DNA Kit) currently gives expected yields of between 150 – 500 ug of genomic from 8.5 ml of human whole blood. Therefore, this disclosed increase in analyte detection is still well short of the step change required for ultra-sensitive detection and delivery.

Summary

In one aspect, the present disclosure refers to a method for the presence, absence or characteristics of an analyte, comprising (a) coupling the analyte to a membrane comprising a detector wherein the analyte is not coupled to the membrane via the detector and (b) allowing the analyte to interact with the detector present in the membrane and thereby determining the presence, absence or characteristics of the analyte.

In another aspect, the present disclosure refers to a method of sequencing an analyte which is a target polynucleotide, comprising:

(a) carrying out the method as disclosed herein wherein the detector comprises a transmembrane pore, such that the target polynucleotide moves through the pore; and

(b) measuring the current passing through the pore as the target polynucleotide moves with respect to the pore and thereby determining the sequence of the target polynucleotide.

In yet another aspect, the present disclosure refers to a kit for sequencing an analyte which is a target polynucleotide comprising (a) nucleic acid handling enzyme that is capable of controlling the movement of the target polynucleotide through a transmembrane pore and (b) hydrophobic anchor to couple the target polynucleotide to a membrane comprising a transmembrane pore.

In a further aspect, the present disclosure refers to an apparatus for sequencing an analyte which is a target polynucleotide, comprising (a) a membrane, (b) a plurality of transmembrane pores in the membrane, (c) a plurality of nucleic acid handling enzymes that are capable of

controlling the movement of the target polynucleotide through a transmembrane pore and (d) a plurality of target polynucleotides coupled to the membrane using a hydrophobic anchor.

The inventors have surprisingly demonstrated ultra low concentration analyte delivery by coupling the analyte to a membrane in which the relevant detector is present. This lowers by several orders of magnitude the amount of analyte required in order to be detected. The extent to which the amount of analyte needed is reduced could not have been predicted.

In particular, the inventors surprisingly report an increase in the capture of single stranded DNA by ~4 orders of magnitude over that previously reported. As both the detector and analyte are now on the same plane, then ~10^3 M s^{-1} more interactions occur per second, as diffusion of both molecules is in two dimensions rather than three dimensions. This has dramatic implications on the sample preparation requirements that are of key concern for diagnostic devices such as next-generation sequencing systems.

In addition, coupling the analyte to a membrane has added advantages for various nanopore-enzyme sequencing applications. In Exonuclease Sequencing, when the DNA analyte is introduced the pore may become blocked permanently or temporarily, preventing the detection of individual nucleotides. When one end of the DNA analyte is localised away from the pore, for example by coupling or tethering to the membrane, surprisingly it was found that this temporary or permanent blocking is no longer observed. By occupying one end of the DNA by coupling it to the membrane it also acts to effectively increase the analyte concentration over the detector and so increase the sequencing systems duty cycle. This is discussed in more detail below.

Accordingly, the disclosure provides a method for determining the presence, absence or characteristics of an analyte, comprising (a) coupling the analyte to a membrane and (b) allowing the analyte to interact with a detector present in the membrane and thereby determining the presence, absence or characteristics of the analyte.

The disclosure also provides:

- a method of sequencing an analyte which is a target polynucleotide, comprising:

  (a) coupling the target polynucleotide to a membrane;

  (b) allowing the target polynucleotide to interact with a detector present in the membrane, wherein the detector comprises a transmembrane pore and an exonuclease, such that the exonuclease digests an individual nucleotide from one end of the target polynucleotide;

  (c) allowing the nucleotide to interact with the pore;

  (d) measuring the current passing through the pore during the interaction and thereby determining the identity of the nucleotide; and

  (e) repeating steps (b) to (d) at the same end of the target polynucleotide and thereby determining the sequence of the target polynucleotide;

- a method of sequencing an analyte which is a target polynucleotide, comprising:

  (a) coupling the target polynucleotide to a membrane;

  (b) allowing the target polynucleotide to interact with a detector present in the membrane, wherein the detector comprises a transmembrane pore, such that the target polynucleotide moves through the pore; and

  (c) measuring the current passing through the pore as the target polynucleotide moves with respect to the pore and thereby determining the sequence of the target polynucleotide;

- a kit for sequencing an analyte which is a target polynucleotide comprising (a) a transmembrane pore, (b) a polynucleotide binding protein and (c) means to couple the target polynucleotide to a membrane; and

- an apparatus for sequencing an analyte which is a target polynucleotide, comprising (a) a membrane, (b) a plurality of transmembrane pores in the membrane, (c) a plurality of polynucleotide binding proteins and (d) a plurality of target polynucleotides coupled to the membrane.

Description of the Figures

Fig. 1 shows nanopore sensing of an analyte. A) Shows a nanopore with the direction of the current flow indicated by grey arrows. A predicted current trace is shown below. B) Shows a

CLAIMS

1. A method for determining the presence, absence or characteristics of an analyte, comprising (a) coupling the analyte to a membrane comprising a detector wherein the analyte is not coupled to the membrane via the detector and (b) allowing the analyte to interact with the detector present in the membrane and thereby determining the presence, absence or characteristics of the analyte.

2. A method according to claim 1, wherein the membrane is an amphiphilic layer or a solid state layer.

3. A method according to claim 1 or 2, wherein the analyte is present at a concentration of from about 0.001 pM to about 1 nM, such as less than 0.01 pM, less than 0.1 pM, less than 1 pM, less than 10 pM or less than 100 pM.

4. A method according to any one of the preceding claims, wherein the analyte is coupled to the membrane via a polypeptide or a hydrophobic anchor.

5. A method according to claim 4, wherein the hydrophobic anchor is a lipid, fatty acid, sterol, carbon nanotube or amino acid.

6. A method according to any one of the preceding claims, wherein the analyte is coupled to the membrane via a linker.

7. A method according to any one of the preceding claims, wherein the detector comprises a transmembrane pore or a transmembrane protein pore.

8. A method according to claim 7, wherein the transmembrane protein pore is derived from Msp or α-hemolysin (α-HL).

9. A method according to claim 7 or 8, wherein the method comprises:
(a) allowing the analyte to interact with the detector; and

(b) measuring the current passing through the pore during the interaction and thereby determining the presence, absence or characteristics of the analyte.

10. A method according to any one of the preceding claims, wherein the method is for (a) identifying the analyte or (b) estimating the sequence of or sequencing a target polynucleotide.

11. A method of sequencing an analyte which is a target polynucleotide, comprising:

(a) carrying out the method of any one of claims 1 to 10 wherein the detector comprises a transmembrane pore, such that the target polynucleotide moves through the pore; and

(b) measuring the current passing through the pore as the target polynucleotide moves with respect to the pore and thereby determining the sequence of the target polynucleotide.

12. A method according to claim 11, wherein the method comprises:

(a) coupling the target polynucleotide to a membrane;

(b) allowing the target polynucleotide to interact with the transmembrane pore and a nucleic acid handling enzyme, such that the enzyme controls the movement of the target polynucleotide through the pore and nucleotides in the target polynucleotide interact with the pore; and

(c) measuring the current passing through the pore as the target polynucleotide moves with respect to the pore and thereby determining the sequence of the target polynucleotide.

13. A kit for sequencing an analyte which is a target polynucleotide comprising (a) nucleic acid handling enzyme that is capable of controlling the movement of the target polynucleotide through a transmembrane pore and (b) hydrophobic anchor to couple the target polynucleotide to a membrane comprising a transmembrane pore.

14. A kit according to claim 13, wherein the hydrophobic anchor is a cholesterol, palmitate or tocopherol.

15. An apparatus for sequencing an analyte which is a target polynucleotide, comprising (a) a membrane, (b) a plurality of transmembrane pores in the membrane, (c) a plurality of nucleic acid handling enzymes that are capable of controlling the movement of the target polynucleotide through a transmembrane pore and (d) a plurality of target polynucleotides coupled to the membrane using a hydrophobic anchor.

16. An apparatus according to claim 15, wherein the analysis apparatus comprises:
a sensor device that is capable of supporting the membrane and plurality of pores and being operable to perform polynucleotide sequencing using the pores; and

at least one reservoir for holding material for performing the sequencing.