CLAIMS

1. A method of loading a motor protein onto a polynucleotide adapter, the method comprising:

    i) providing a polynucleotide adapter comprising a spacer;

    ii) contacting the polynucleotide adapter with a motor protein; and

    iii) positioning the motor protein on the spacer;

    wherein a blocking moiety bound to the polynucleotide adapter is bound adjacent to the spacer

    wherein the blocking moiety prevents the movement of the motor protein in the direction opposite to that in which the motor protein naturally processes a polynucleotide;

    wherein the blocking moiety prevents the motor protein from moving off the spacer; and

    wherein the blocking moiety is a physical or chemical blocking moiety.

2. A method according to claim 1, comprising:

    i) providing the polynucleotide adapter comprising the spacer and a blocking moiety bound to the polynucleotide adapter;

    ii) contacting the polynucleotide adapter with the motor protein; and

    iii) positioning the motor protein on the spacer;

    wherein the blocking moiety prevents the motor protein from moving off the spacer.

3. A method of loading a motor protein onto a polynucleotide adapter, the method comprising:

    i) providing a polynucleotide adapter comprising a spacer;

    ii) contacting the polynucleotide adapter with a motor protein;

    iii) causing the motor protein to progress onto the spacer; and

    iv) binding a blocking moiety to the polynucleotide adapter;

        wherein the blocking moiety is bound to the spacer adjacent to the end that is in the opposite direction from the directionality of the motor protein so that the blocking moiety prevents the motor protein from moving off the spacer;

    wherein the blocking moiety is a physical or chemical blocking moiety.

4. A method according to claim 3, comprising:

i) providing the polynucleotide adapter comprising a loading site connected to a spacer;

ii) contacting the loading site with the motor protein;

iii) causing the motor protein to progress from the loading site onto the spacer; and

iv) binding the blocking moiety to the polynucleotide adapter, wherein the blocking moiety prevents the motor protein from moving off the spacer and onto the loading site.

5. A method according to claim 4, wherein:

step (ii) comprises contacting the loading site with the motor protein, wherein the motor protein engages with the loading site; and

step (iv) comprises binding the blocking moiety to the polynucleotide adapter,

wherein the blocking moiety prevents the motor protein from moving off the spacer and re-engaging with the loading site.

6. A method according to any one of the preceding claims, wherein preventing the motor protein from moving off the spacer reduces the rate at which the motor protein turns over fuel molecules compared to when the motor protein is bound to a polynucleotide.

7. A method according to any one of claims 4 to 6 wherein the polynucleotide adapter comprises the loading site connected to the spacer and wherein the motor protein binds to the loading site of the polynucleotide adapter.

8. A method according to any one of claims 3 to 7, wherein causing the motor protein to progress onto the spacer comprises applying a physical or chemical force to the motor protein.

9. A method according to any one of claims 3 to 8, wherein causing the motor protein to progress onto the spacer comprises contacting the motor protein with one or more fuel molecules.

10. A method according to any one of claims 1 or 3 to 9, wherein the polynucleotide adapter comprises the loading site connected to the spacer; the motor protein is a first motor protein and causing the first motor protein to progress from the loading site onto the spacer comprises loading a second motor protein onto the loading site and causing the second motor protein to progress from the loading site towards the spacer, wherein the second motor protein forces the first motor protein onto the spacer.

11. A method according to any one of claims 2 to 10, wherein binding the blocking moiety to the polynucleotide adapter forces the motor protein onto the spacer.

12. A method according to any one of the preceding claims, wherein the polynucleotide adapter comprises the loading site connected to the spacer and the blocking moiety binds to the loading site.

13. A method according to claim 12, wherein the loading site is contiguous with the spacer and the blocking moiety binds to the loading site immediately adjacent to the spacer.

14. A method according to any one of the preceding claims, wherein step (iii) comprises causing the motor protein to progress onto the spacer such that the spacer occupies the active site of the motor protein.

15. A method according to any one of the preceding claims, wherein the polynucleotide adapter comprises the loading site connected to the spacer and the loading site comprises a single-stranded or non-hybridised polynucleotide.

16. A method according to claim 15, wherein the loading site comprises a single-stranded or non-hybridised polynucleotide having a length of between 2 and 1000 nucleotide units.

17. A method according to any one of claims 2 to 16, wherein binding the blocking moiety to the polynucleotide adapter sterically prevents the movement of the motor protein off the spacer.

18. A method according to any one of claims 2 to 17, wherein binding the blocking moiety to the polynucleotide adapter introduces a chemical group which prevents movement of the motor protein off the spacer.

19. A method according to any one of the preceding claims, wherein (i) the loading site comprises a single-stranded or non-hybridised polynucleotide and the blocking moiety comprises a single-stranded or non-hybridised polynucleotide; and (ii) binding the blocking moiety to the loading site comprises hybridising the blocking moiety to the loading site.

20. A method according to any one of the preceding claims wherein the blocking moiety comprises a single-stranded or non-hybridised polynucleotide having a length of between 2 and 1000 nucleotide units.

21. A method according to any one of the preceding claims, wherein the spacer comprises:

    i) one or more nitroindoles, one or more inosines, one or more acridines, one or more 2-aminopurines, one or more 2-6-diaminopurines, one or more 5-bromo-deoxyuridines, one or more inverted thymidines (inverted dTs), one or more inverted dideoxy-thymi dines (ddTs), one or more dideoxy-cyti dines (ddCs), one or more 5-m ethyl cyti dines, one or more 5-hydroxymethylcytidines, one or more 2'-O-Methyl RNA bases, one or more Iso-deoxycytidines (Iso-dCs), one or more Iso-deoxyguanosines (Iso-dGs), one or more C3 ($OC_3H_6OPO_3$) groups, one or more photo-cleavable (PC) [OC_3H_6-C(o)$NHCH_2$-$C_6H_3NO_2$-$CH(CH_3)OPO_3$] groups, one or more hexandiol groups, one or more spacer 9 (iSp9) [$(OCH_2CH_2)_3OPO_3$] groups, or one or more spacer 18 (iSp18) [$(OCH_2CH_{26}OPO_3$] groups;

    ii) one or more thiol connections;

    iii) one or more abasic nucleotides;

    iv) one or more nucleotides of different backbone structure to the loading site;

v) one or more chemical groups which cause the one or more motor proteins to stall; and/or

vi) a polymer, optionally wherein said polymer is a polypeptide or a polyethylene glycol (PEG).

22. A method according to any one of the preceding claims, wherein the spacer comprises one or more nucleotides, preferably wherein the spacer comprises one or more nucleotide islands.

23. A method according to claim 22, wherein the spacer comprises one or more moieties selected from:

-S-N-S-N-S-N-S-; -S-N-N-S-N-N-S-N-N-S-; -S-S-S-S-S-S-N-N-S-S-;

-S-S-S-S-S-N-N-S-N-N-S-S-S-; -S-S-S-N-N-S-N-N-S-S-N-N-S-;

-S-S-S-S-S-N-N-S-N-N-S-S-N-N-S-; -S-S-S-N-N-S-N-N-S-N-N-S-,

-N-N-S-S-N-N-S-S-S-S-S-; -S-N-N-S-S-N-N-S-S-S-S-;

-S-S-N-N-S-S-N-N-S-S-S-; -S-S-S-S-N-N-S-S-N-N-S-; -N-N-S-N-N-S-S-S-S-S-S-;

-S-N-N-S-N-N-S-S-S-S-S-; -S-S-N-N-S-N-N-S-S-S-S-; -S-S-S-N-N-S-N-N-S-S-S-:

-S-S-S-S-N-N-S-N-N-S-S-; and -S-S-S-N-N-S-S-N-N-S-;

wherein each S is a spacer unit and each N is a nucleotide.

24. A method according any one of the preceding claims, wherein the motor protein is a helicase, a polymerase, an exonuclease, a topoisomerase.

25. A method according to any one of the preceding claims, wherein the or each motor protein is a helicase independently selected from a Hel308 helicase, a RecD helicase, a Tral helicase, a TrwC helicase, an XPD helicase, and a Dda helicase.

26. A method according to any one of the preceding claims, further comprising the step of:

v) removing excess motor protein molecules which are not located on the spacer.

27. A method of controlling the movement of a target polynucleotide with respect to a transmembrane nanopore, comprising:

i) providing (A) a target polynucleotide; (B) a polynucleotide adapter comprising a spacer; and (C) a motor protein;

ii) carrying out a method according to any one of the preceding claims thereby stalling the motor protein on the spacer of the polynucleotide adapter;

iii) contacting the target polynucleotide and the stalled motor protein on the spacer of the polynucleotide adapter with the nanopore; and

iv) applying a potential across the transmembrane nanopore thereby causing the motor protein to move past the spacer onto the target polynucleotide thereby controlling the movement of the target polynucleotide with respect to the nanopore.

28. A method according to claim 27, wherein the motor protein is stalled on the polynucleotide adapter before the polynucleotide adapter is attached to the target polynucleotide.

29. A method according to claim 27, wherein the polynucleotide adapter is attached to the target polynucleotide before the motor protein is stalled on the polynucleotide adapter.

30. A method of controlling the movement of a target polynucleotide with respect to a transmembrane nanopore, comprising:

i) providing a target polynucleotide;

ii) providing a polynucleotide adapter comprising a spacer, loaded with a motor protein according to the method of any one of claims 1 to 26, wherein the motor protein is stalled thereon;

iii) contacting the target polynucleotide and the polynucleotide adapter with the nanopore; and

iv) applying a potential across the transmembrane nanopore thereby causing the motor protein to move past the spacer onto the target polynucleotide thereby controlling the movement of the target polynucleotide with respect to the nanopore.

31. A method of characterising a target polynucleotide, comprising:

i) carrying out the method of any one of claims 27 to 30; and

ii) taking one or more measurements as the target polynucleotide moves with respect to the nanopore, wherein the one or more measurements are indicative of one or more characteristics of the target polynucleotide, and thereby characterising the target polynucleotide as it moves with respect to the nanopore.

32. A polynucleotide adapter comprising (i) a spacer; (ii) a motor protein stalled on the spacer, wherein the active site of the motor protein is occupied by the spacer; and (iii) a blocking moiety bound to the adapter adjacent to the spacer, wherein the blocking moiety prevents the motor protein from moving off the spacer.

33. A polynucleotide adapter according to claim 32, wherein (i) the adapter comprises a loading site connected to the spacer and the blocking moiety is bound to the loading site; and (ii) the blocking moiety prevents the motor protein from engaging with the loading site.

34. A polynucleotide adapter according to claim 33 comprising:

i) $\{L_B\text{-}S\text{-}D\}_n$ or $\{D\text{-}S\text{-}L_B\}_n$ in the 5' to 3' direction; wherein L_B is a blocked loading site; S is a spacer; D is a double-stranded polynucleotide; and n is an integer, optionally an integer from 1 to 20; and

ii) one or more motor proteins stalled on the spacer (S);

wherein the or each L_B moiety prevents the or each motor protein from moving off the spacer (S) in the direction away from the double-stranded polynucleotide (D).

35. A polynucleotide adapter according to claim 33 or claim 34 comprising:

i) $\{L_B\text{-}S\text{-}D\}_n$ or $(D\text{-}S\text{-}L_B\}_n$ in the 5' to 3' direction; wherein L_B is a first double-stranded polynucleotide; S is a spacer; D is a second double-stranded polynucleotide; and n is an integer, optionally an integer from 1 to 20; and wherein the first double-stranded polynucleotide (L_B) is contiguous with the spacer (S) and the spacer (S) is contiguous with the second double-stranded polynucleotide (D); and

ii) one or more motor proteins is stalled on the spacer (S).

36. A kit for modifying a target polynucleotide, comprising:

i) a polynucleotide adapter comprising a spacer;

ii) a motor protein configured for controlling the movement of the target polynucleotide; and

iii) a blocking moiety for binding to the polynucleotide adapter adjacent to the spacer so that when the motor protein is located on the spacer of the polynucleotide adapter and the active site of the motor protein is occupied by the spacer, the motor protein is prevented from moving off the spacer;

wherein the polynucleotide adapter comprises a loading site and when in use the blocking moiety binds to the polynucleotide adapter so that the motor protein is prevented from engaging with the loading site.

37. A kit for modifying a target polynucleotide, comprising:

i) a polynucleotide adapter comprising a spacer and a blocking moiety bound to the polynucleotide adapter adjacent to the spacer; and

ii) a motor protein capable of controlling the movement of the target polynucleotide;

wherein when the motor protein is bound to the adapter, the blocking moiety prevents the motor protein from moving off the spacer.

38. A polynucleotide adapter or kit according to any one of claims 32 to 36, wherein:

the loading site, blocking moiety and/or spacer are each independently as defined in any one of claims 12 to 23; and/or

the motor protein is as defined in claim 24 or claim 25.