Claims:

1. A microfluidic device comprising:

an enclosure comprising a base, a cover, and microfluidic circuit material defining a fluidic circuit therein,

wherein at least one inner surface of the base, the cover and the microfluidic circuit material has a plurality of first covalently bound surface modifications, each comprising

a first linking group, and

a first moiety, wherein the first moiety is a first surface contact moiety or a first reactive moiety;

wherein at least one inner surface of the base, the cover and the microfluidic circuit material has a plurality of second covalently bound surface modifications, each comprising

a second linking group, and

a second moiety, wherein the second moiety is a second surface contact moiety or second reactive moiety, and

wherein the first linking group and the second linking group are different from each other and/or the first moiety is different from the second moiety.

2. The microfluidic device of claim 1, wherein the first moiety and the second moiety are each covalently bound to the surface via a linking group LG independently selected from -W-Si(OZ)$_2$O- and -OP(O)$_2$O-, wherein W is O, S, or N, and wherein Z is a bond to a silicon atom in an adjacent linking group LG or is a bond to the surface.

3. The microfluidic device of claim 1 or 2, wherein the first surface contact moiety comprises one or more of an alkyl, fluoroalkyl, monosaccharide, polysaccharide, alcohol, polyalcohol, alkylene ether, polyelectrolytes, amino, carboxylic acid, phosphonic acid, sulfonate anion, carboxybetaines, sulfobetaine, sulfamic acid, amino acid moiety, or cleavable moiety; and/or

wherein the second surface contact moiety comprises one or more of an alkyl, fluoroalkyl, monosaccharide, polysaccharide, alcohol, polyalcohol, alkylene ether, polyelectrolytes, amino, carboxylic acid, phosphonic acid, sulfonate anion, carboxybetaines, sulfobetaine, sulfamic acid, amino acid moiety, or cleavable moiety.

4. The microfluidic device of claim 1 or 2, wherein the first surface contact moiety comprises a polyethylene glycol moiety, a dextran moiety, a proteinaceous moiety, a poly carboxylic acid, a polylysine moiety, or any combination thereof; and/or

wherein the second surface contact moiety comprises a polyethylene glycol moiety, a dextran moiety, a proteinaceous moiety, a poly carboxylic acid, a polylysine moiety, or any combination thereof.

5. The microfluidic device of any one of claims 1 to 4, wherein the first reactive moiety is an alkyne moiety, an azide moiety, a carboxylic acid moiety, an amine moiety, an olefinic moiety, a tetrazinyl moiety, a trans-cyclooctenyl moiety, a thiol moiety, a maleimide moiety, a biotin moiety, a streptavidin moiety, a halide moiety, a cyano moiety, isocyanate moiety, an epoxide moiety, a hydroxyamine moiety, or a sulfonyl fluoride moiety; and/or

wherein the second reactive moiety is an alkyne moiety, an azide moiety, a carboxylic acid moiety, an amine moiety, an olefinic moiety, a tetrazinyl moiety, a trans-cyclooctenyl moiety, a thiol moiety, a maleimide moiety, a biotin moiety, a streptavidin moiety, a halide moiety, a cyano moiety, isocyanate moiety, an epoxide moiety, a hydroxyamine moiety, or a sulfonyl fluoride moiety.

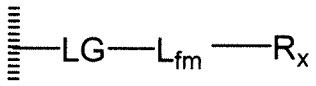
6. The microfluidic device of any one of claims 1 to 5, wherein each first covalently bound surface modification comprises a linker, wherein the linker comprises 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms; and/or

wherein each second covalently bound surface modification comprises a linker, wherein the linker comprises 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms.

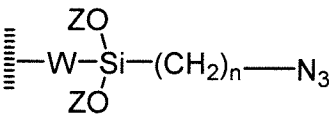
7. The microfluidic device of claim 6, wherein the linker of the first covalently bound surface modifications further comprises one or two coupling group CG moieties; and/or

wherein the linker of the second covalently bound surface modifications further comprises one or two coupling group CG moieties.

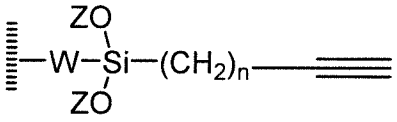
8. The microfluidic device of claim 1, wherein the first covalently bound surface modifications have a structure selected from Formula XXX, Formula V, Formula VII, Formula XXXI, Formula VIII, and Formula IX:



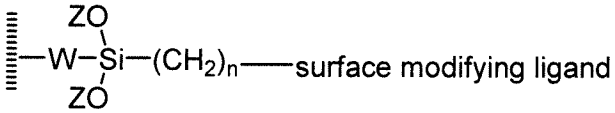
Formula XXX;



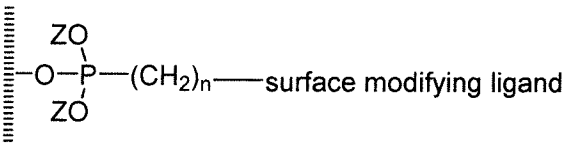
Formula V;



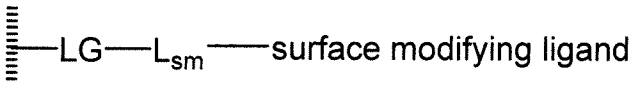
Formula VII;



Formula VIII;



Formula IX;



Formula XXXI;

wherein:

LG is -W-Si(OZ)$_2$O- or -OP(O)$_2$O-;

L_{fm} is a linker comprising 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms and further comprises 0 or 1 coupling groups CG;

R_x is a reactive moiety;

W is O, S, or N;

Z is a bond to an adjacent silicon atom or is a bond to the surface;

n is an integer of 3 to 21;

L_{sm} is a linker comprising 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms and further comprises 0, 1, 2, or 3 coupling groups CG; and

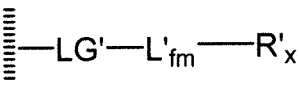
 is the surface.

9. The microfluidic device of claim 8, wherein LG is -W-Si(OZ)$_2$O-, and wherein W is O.

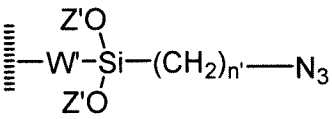
10. The microfluidic device of claim 8 or 9, wherein n is 7 to 21.

11. The microfluidic device of any one of claims 8 to 10, wherein the reactive moiety R_x is alkyne, azide, amine, carboxylic acid, biotin, or streptavidin.

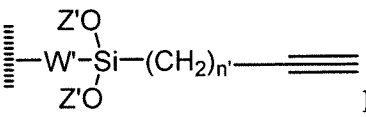
12. The microfluidic device of any one of claims 1 or 8 to 11, wherein the second covalently bound surface modifications have a structure selected from Formula XXX', Formula V', Formula VII', Formula XXXI', Formula VIII', and Formula IX':



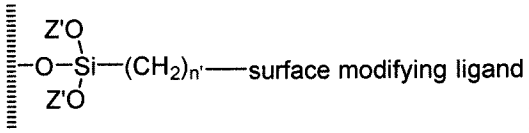
Formula XXX';



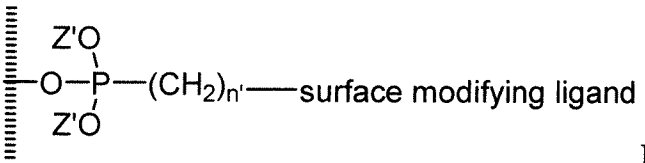
Formula V';



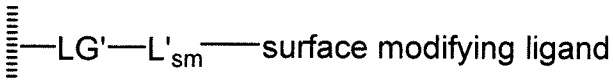
Formula VII';



Formula VIII';



Formula IX';



Formula XXXI';

wherein:

LG' is -W'-Si(OZ')$_2$O- or -OP(O)$_2$O-;

L'$_{fm}$ is a linker comprising 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms and further comprises 0 or 1 coupling groups CG;

R'$_x$ is a reactive moiety;

W' is O, S, or N;

Z' is a bond to an adjacent silicon atom or is a bond to the surface;

n' is an integer of 3 to 21;

L'$_{sm}$ is a linker comprising 1 to 200 non-hydrogen atoms selected from any combination of silicon, carbon, nitrogen, oxygen, sulfur and phosphorus atoms and further comprises 0, 1, 2, or 3 coupling groups CG; and

 is the surface.

13. The microfluidic device of claim 12, wherein LG' is -W'-Si(OZ')$_2$O-, and wherein W' is O.

14. The microfluidic device of claim 12 or 13, wherein n' is 7 to 21.

15. The microfluidic device of any one of claims 12 to 14, wherein the reactive moiety R'$_x$ is alkyne, azide, amine, carboxylic acid, biotin, or streptavidin.

16. The microfluidic device of any one of claims 1 to 15, wherein the first moiety is different from the second moiety.

17. The microfluidic device of any one of claims 12 to 15, wherein the first covalently bound surface modifications have a structure selected from Formula XXX, Formula V, and Formula VII, wherein the second covalently bound surface modifications have a structure selected from Formula XXX', Formula V', and Formula VII', wherein the first covalently bound surface modifications are different from the second covalently bound surface modifications, and wherein the reactive moiety of the first covalently bound surface modifications does not react with the reactive moiety of the second covalently bound surface modifications.

18. The microfluidic device of claim 17, wherein the first covalently bound surface modifications and the second covalently bound surface modifications are on a common inner surface of the base, the cover, and/or the microfluidic circuit material.

19. The microfluidic device of claim 18, wherein the common inner surface comprises a first region comprising the first covalently bound surface modifications and a second region comprising the second covalently bound surface modifications, and wherein the first region is adjacent to the second region.

20. The microfluidic device of claim 18, wherein the common inner surface comprises a plurality of first regions comprising the first covalently bound surface modifications and a second region comprising the second covalently bound surface modifications, wherein the first regions of the plurality are separated from each other by the second region.

21. The microfluidic device of any one of claims 1 to 20, wherein the fluidic circuit comprises a flow region and a sequestration pen, wherein the sequestration pen comprises an isolation region and a connection region, wherein the connection region comprises a proximal opening to the flow region and fluidically connects the isolation region to the flow region.

22. The microfluidic device of claim 21, wherein at least one surface of the flow region is modified with the first covalently bound surface modifications, wherein at least one surface of the sequestration pen is modified with the second covalently bound surface modifications.

23. The microfluidic device of claim 22, wherein the second covalently bound surface modifications comprises a surface contact moiety configured to anchor adherent cells.

24. The microfluidic device of claim 22 or 23, wherein the first covalently bound surface modifications comprises a surface contact moiety configured to inhibit migration of motile cells out of the sequestration pen.

25. The microfluidic device of any one of claims 21 to 24, wherein the fluidic circuit further comprises a plurality of sequestration pens each having at least one inner surface modified with the first and/or second covalently bound surface modifications.

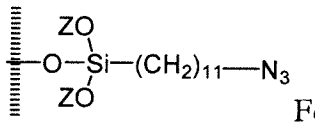
26. The microfluidic device of claim 1, wherein the first covalently bound surface modifications and/or the second covalently bound surface modifications form a monolayer.

27. The microfluidic device of claim 1, wherein the inner surface of the base and/or the inner surface of the cover of the enclosure comprises glass, silicon, silicon oxide, hafnium oxide, indium tantalum oxide, or aluminum oxide.

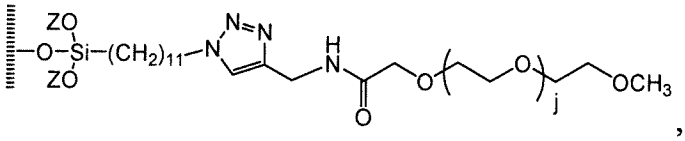
28. The microfluidic device of claim 1, wherein the inner surface of the microfluidic circuit material comprises polydimethylsiloxane (PDMS) or photopatternable silicone (PPS).

29. The microfluidic device of claim 1, wherein substantially all of the inner surfaces of the enclosure are covalently modified.

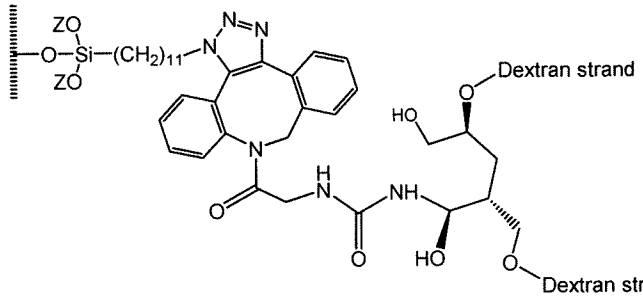
30. The microfluidic device of any one of claims 1 to 29, wherein the first or the second covalently bound surface modification has a structure of one of the following formulae:



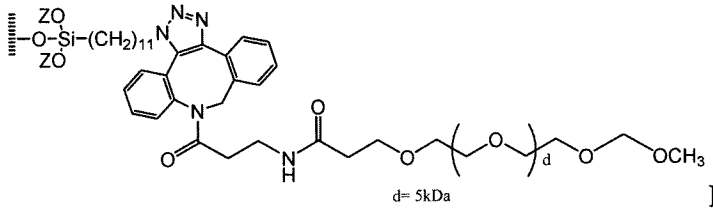
Formula XV;



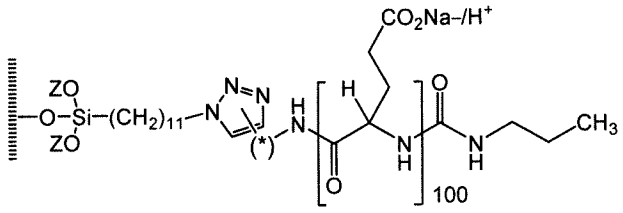
, j is 5kDa Formula XVI;



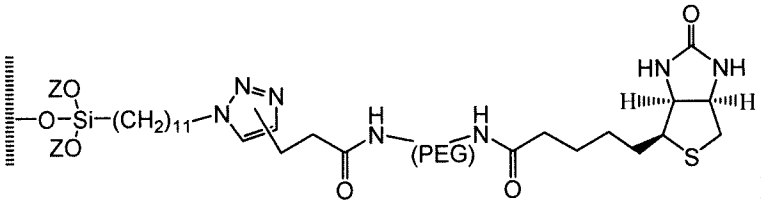
Formula XVII;



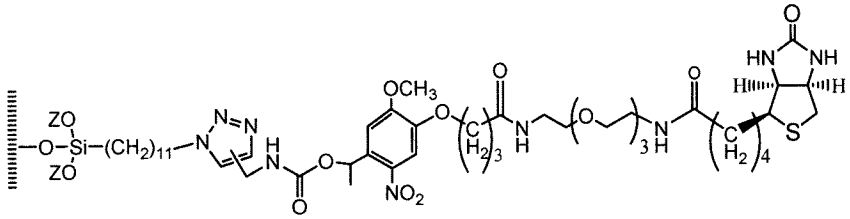
Formula XVIII;



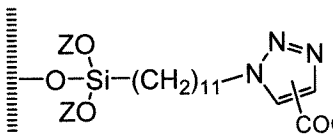
Formula XIX;



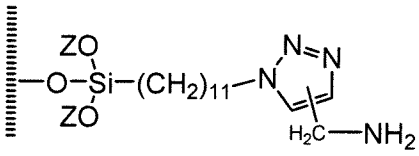
Formula XX;



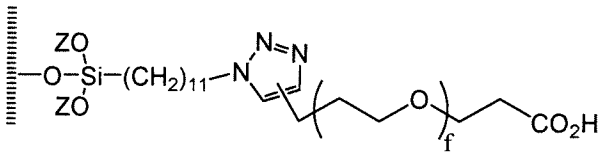
Formula XXI;



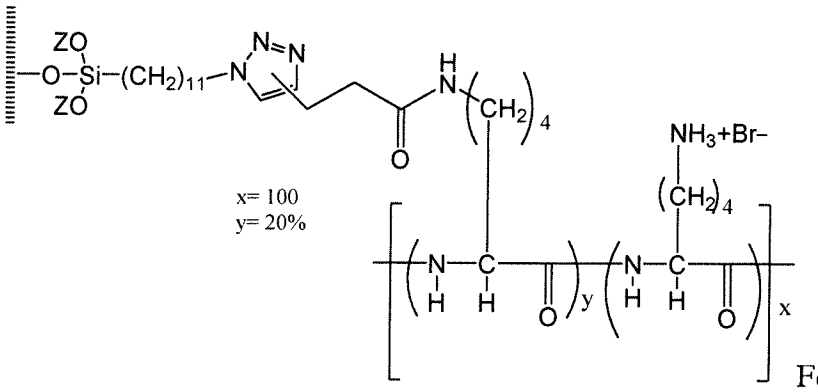
Formula XXII;



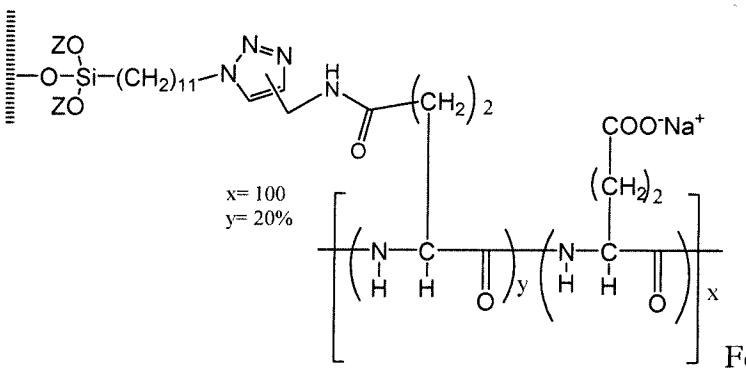
Formula XXIII;



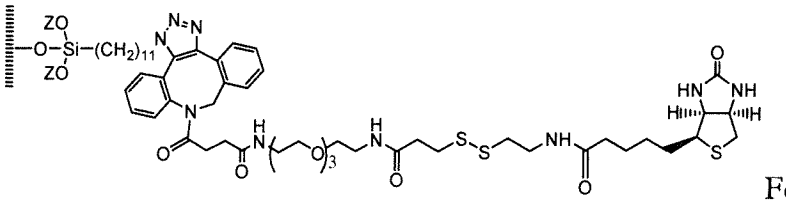
Formula XXIV (f =5000Da);



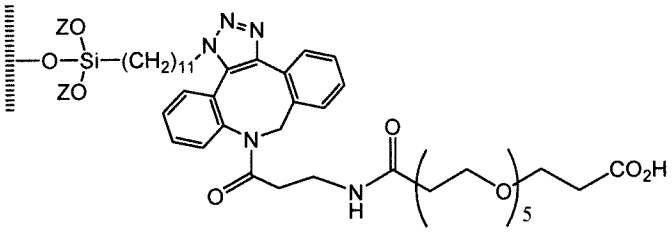
Formula XXV;



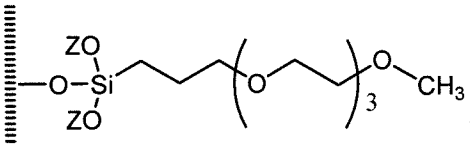
Formula XXVI;



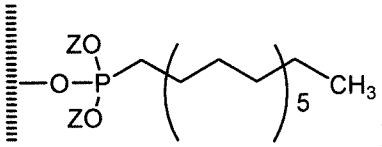
Formula XXVII;



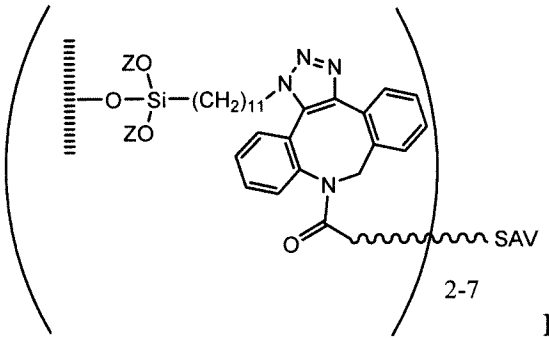
Formula XXVIII;



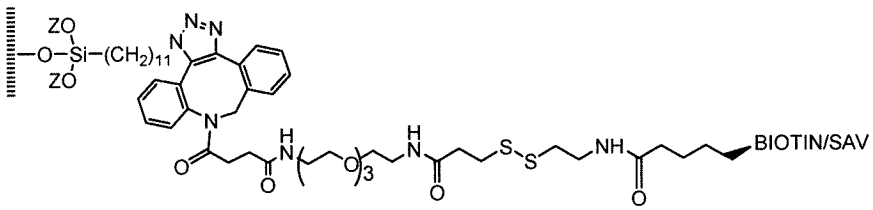
Formula XXIX;



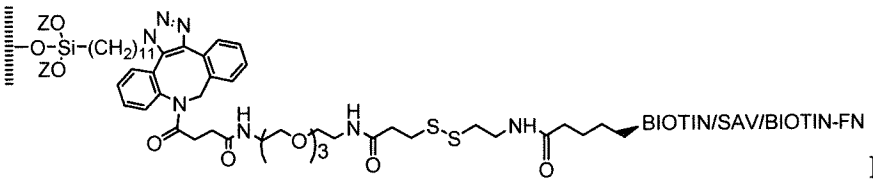
Formula XXXVI;



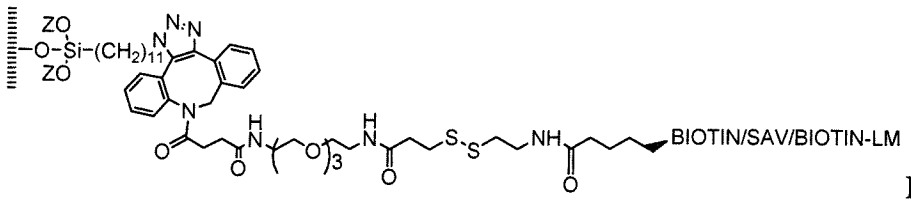
Formula XXXVII;



Formula XXXVIII;



Formula XXXIX;



Formula XL.

31. A method of forming different covalently modified surfaces in a regioselective manner within a microfluidic device, wherein the microfluidic device comprises an enclosure having a base, a cover, and a microfluidic circuit material defining a microfluidic circuit therein, wherein the microfluidic circuit comprises a flow region and a sequestration pen, and wherein the sequestration pen comprises an isolation region and a connection region, the connection region comprising a proximal opening to the flow region and fluidically connecting the isolation region to the flow region, the method comprising:

flowing a first modifying reagent through the flow region under conditions such that the first modifying reagent does not enter the isolation region of the sequestration pen;

reacting the first modifying reagent with nucleophilic moieties on at least one surface of the flow region, thereby forming a first modified surface within the flow region, wherein the first modified surface does not extend into the isolation region of the sequestration pen;

flowing a second modifying reagent through the flow region under conditions such that the second modifying reagent enters into the isolation region of the sequestration pen; and

reacting the second modifying reagent with nucleophilic moieties on at least one surface of the isolation region of the sequestration pen, thereby forming a second modified surface within the isolation region of the sequestration pen,

wherein the first modifying reagent does not have the same structure as the second modifying reagent.

32. The method of claim 31, wherein the conditions for flowing the first modifying reagent through the flow region comprise applying a negative pressure to the flow region.

33. The method of claim 32, wherein flowing the first modifying reagent comprises flowing a solution that comprises the first modifying reagent through the flow region at a rate of about 10 mm/sec or higher.

34. The method of claim 31, wherein the conditions for flowing the first modifying reagent through the flow region comprise applying a positive pressure to the flow region.

35. The method of claim 34, wherein flowing the first modifying reagent comprises flowing a solution that comprises the first modifying reagent through the flow region at a rate of about 2 mm/sec or less.

36. The method of any one of claims 30 to 35, wherein the second modifying reagent does not substantially react with moieties on the surfaces of the flow region.