

Claims:

1. A kit for generating an antigen-presenting surface, the kit comprising:

~~(a)~~ a covalently functionalized synthetic surface;

~~(b)~~ a primary activating molecule that includes a major histocompatibility complex (MHC) molecule configured to bind to a T cell receptor (TCR), and a first reactive moiety configured to react with or bind to the covalently functionalized surface;

and

~~(c)~~ an initial peptide bound to the MHC molecule, wherein the initial peptide is non-immunogenic in the organism from which the MHC molecule is originated; and

a plurality of co-activating molecules presenting on the covalently functionalized synthetic surface, wherein each co-activating molecule is selected from a TCR co-activating molecule and an adjunct TCR activating molecule; further wherein the ratio of the TCR co-activating molecules to the adjunct TCR activating molecule is 20:1 to 1:20.

2. The kit of claim 1, further comprising an exchange factor, wherein the exchange factor is provided separately from the primary activating molecule.

3. The kit of claim 2 further comprising one or more of:

~~at least one co-activating molecule that includes a second reactive moiety configured to react with or bind to the covalently functionalized surface, wherein each co-activating molecule is selected from a TCR co-activating molecule and an adjunct TCR activating molecule;~~

a surface-blocking molecule capable of covalently binding to the covalently functionalized synthetic surface;

a buffer suitable for performing an exchange reaction; and

instructions for performing an exchange reaction wherein a peptide antigen displaces the exchange factor.

4. The kit of any one of claims 1-3, wherein the initial peptide comprises at least 4 or 5 amino acid residues.

5. The kit of any one of claims 1-4, wherein the initial peptide comprises a lysine as the fourth or fifth amino acid residue.

6. The kit of any one of claims 1-5, wherein the initial peptide binds the MHC molecule with a half-life of at least about 4 hours.

7. The kit of any one of claims 1-6, wherein the covalently functionalized synthetic surface presents a plurality of azido groups; and/or a plurality of biotin-binding agents, and wherein the first reactive moieties are configured to specifically bind to the biotin-binding agent.

8. The kit of any one of claims 2-7, wherein the exchange factor comprises Leu, Phe, Val, Arg, Met, Lys, Ile, homoleucine, cyclohexylalanine, or Norleucine as its C-terminal amino acid residue.

9. The kit of any one of claims 2-8, wherein the exchange factor comprises Gly, Ala, Ser, or Cys as its penultimate C-terminal residue.

10. The kit of any one of claims 2-9, wherein the exchange factor is 2 amino acid residues in length.

11. The kit of any one of claims 1-10, wherein the covalently functionalized synthetic surface further comprises at least one plurality of surface-blocking molecular ligands covalently attached to the surface, and further wherein:

(i) each of the plurality of surface-blocking molecular ligands includes a hydrophilic moiety, an amphiphilic moiety, a zwitterionic moiety, and/or a negatively charged moiety;

(ii) each of the plurality of surface-blocking molecular ligands includes a linker and a terminal surface-blocking group;

(iii) each of the plurality of surface-blocking molecular ligands includes a linker and a terminal surface-blocking group, wherein the terminal surface-blocking group comprises a hydrophilic moiety, amphiphilic moiety, zwitterionic moiety, and/or negatively charged moiety;

(iv) each of the plurality of surface-blocking molecular ligands is covalently bound to the covalently functionalized synthetic surface ~~or the proto-antigen-presenting surface~~; and/or

(v) the plurality of the surface-blocking molecular ligands may include 2, 3, or 4 different surface-blocking groups and/or 2, 3, 4, or more different lengths of linkers, chosen in any combination.

12. The kit of any one of claims 1-11, wherein the covalently functionalized synthetic surface ~~or the proto-antigen-presenting surface~~ is a wafer, an inner surface of a tube, an inner surface of a microfluidic device, or a bead.

13. The kit of claim 12, wherein the inner surface of the microfluidic device is within a chamber of the microfluidic device; and further wherein the chamber is a sequestration pen and the microfluidic device further comprises a flow region for containing a flow of a first fluidic medium; and the sequestration pen comprises an isolation region for containing a second fluidic medium and a connection region fluidically connecting the isolation region to the flow region; and further wherein the isolation region has a single opening and is an unswept region of the microfluidic device.

14. The kit of claim 13, wherein the microfluidic device comprises a microfluidic channel comprising at least a portion of the flow region, and the connection region comprises a proximal opening into the microfluidic channel having a width W_{con} ranging from ~~about~~ 20 microns to ~~about~~ 100 microns and a distal opening into the isolation region, and wherein a length L_{con} of the connection region from the proximal opening to the distal opening is at least 1.0 times a width W_{con} of the proximal opening of the connection region.

15. The kit of claim 14, wherein the length L_{con} of the connection region from the proximal opening to the distal opening is at least 1.5 times the width W_{con} of the proximal opening of the connection region, or wherein

the length L_{con} of the connection region from the proximal opening to the distal opening is at least 2.0 times the width W_{con} of the proximal opening of the connection region.

16. The kit of claim 15, wherein the proximal opening of the connection region is parallel to a direction of the flow of the first medium in the flow region.

17. The kit of claim 12, further comprising a wellplate, wherein the wafer or the beads are configured to be disposed within a well of the wellplate.

18. The kit of any one of claims 3-17, wherein the TCR co-activating molecule includes a CD28 binding protein or a fragment thereof which retains binding ability with CD28.

19. The kit of any one of claims 3-17, wherein the adjunct TCR activating molecule is configured to provide adhesion stimulation and/or the adjunct TCR activating molecular ligand includes a CD2 binding protein, anti-CD2 antibody, or a fragment thereof, wherein the fragment of the CD2 binding protein or anti-CD2 antibody retains binding ability with CD2.

20. The kit of any one of claims 1-19, wherein the initial peptide is GMGQKDSYV (SEQ ID NO: 1), RMQKEITAL (SEQ ID NO: 2), QLAKLMATL (SEQ ID NO: 3), or LMYAKRAFV (SEQ ID NO: 4).

21. A method of forming a proto-antigen-presenting surface, the method comprising:

~~forming a plurality of complexes, each comprising a major histocompatibility complex (MHC) molecule and an initial peptide, wherein the initial peptide is non-immunogenic; and~~

~~forming the proto-antigen presenting surface by reacting a plurality of primary activating molecules comprising the plurality of complexes with a first plurality of binding moieties disposed on a covalently functionalized synthetic surface, wherein each of the plurality of primary activating molecules comprises a reactive moiety attached to the MHC molecule or wherein each of the plurality of primary activating molecules is modified to introduce a reactive moiety to the MHC molecule of the complex prior to reacting with a respective binding moiety of the covalently functionalized synthetic surface.~~

synthesizing a plurality of major histocompatibility complex (MHC) molecules in the presence of initial peptide, thereby forming a plurality of complexes, each comprises an MHC molecule and an initial peptide, wherein the initial peptide is non-immunogenic in the organism from which the MHC molecule is originated;

preparing a plurality of primary activating molecules comprising the MHC molecules and first reactive moieties;

reacting the first reactive moieties of the plurality of primary activating molecules with a first plurality of binding moieties disposed on a covalently functionalized synthetic surface; and

reacting a plurality of co-activating molecules with a second plurality of binding moieties of the covalently functionalized synthetic surface thereby forming the proto-antigen-presenting surface;

wherein each of the plurality of co-activating molecular ligands comprises a TCR co-activating molecule or an adjunct TCR activating molecule, and further wherein the ratio of the TCR co-activating molecules to the adjunct TCR activating molecule is 20:1 to 1:20.

22. The method of claim 21 further comprising reacting the plurality of MHC molecules synthesized in the presence of the initial peptide with an exchange factor and a peptide antigen.

23. The method of claim 21 or 22, wherein the initial peptide comprises at least 4 or 5 amino acid residues; or has a length of about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 amino acid residues.

24. The method of any one of claims 21-23,, wherein the initial peptide comprises a lysine as the fourth or fifth amino acid residue.

25. The method of any one of claims 21-24, wherein the initial peptide binds the MHC molecule with a half-life of at least about 4 hours.

26. The method of any one of claims 21-25, wherein the covalently functionalized synthetic surface presents a plurality of azido groups; and/or a plurality of biotin-binding agents, and wherein the first reactive moieties are configured to specifically bind to the biotin-binding agent.

27. The method of any one of claims 21-26, wherein a ratio of the primary activating molecular ligands to the co-activating molecular ligands present on the antigen-presenting surface is 1:10 to 2:1, 1:5 to 2:1, 1:2 to 2:1, 1:10 to 1:1, 1:5 to 1:1, 1:1 to 2:1, or 1:2 to 1:1~~further comprising reacting a plurality of co-activating molecules with a second plurality of binding moieties of the covalently functionalized synthetic surface configured for binding the second reactive moieties; wherein each of the plurality of co-activating molecular ligands comprises a TCR co-activating molecule or an adjunct TCR activating molecule.~~

28. The method of ~~any one of claims 21-27~~claim 22, wherein the exchange factor comprises Leu, Phe, Val, Arg, Met, Lys, Ile, homoleucine, cyclohexylalanine, or Norleucine as its C-terminal amino acid residue.

29. The method of ~~any one of claims 21-28~~claim 22 or claim 28, wherein the exchange factor comprises Gly, Ala, Ser, or Cys as its penultimate C-terminal residue.

30. The method of any one of claims ~~21-29~~22, 28 and 29, wherein the exchange factor is 2 amino acid residues in length.

31. The method of any one of claims 21-30, wherein the covalently functionalized synthetic surface or the proto-antigen-presenting surface further comprises at least one plurality of surface-blocking molecular ligands covalently attached to the surface:

- (i) each of the plurality of surface-blocking molecular ligands includes a hydrophilic moiety, an amphiphilic moiety, a zwitterionic moiety, and/or a negatively charged moiety;
- (ii) each of the plurality of surface-blocking molecular ligands includes a linker and a terminal surface-blocking group;
- (iii) each of the plurality of surface-blocking molecular ligands includes a linker and a terminal surface-blocking group, wherein the terminal surface-blocking group comprises a hydrophilic moiety, amphiphilic moiety, zwitterionic moiety, and/or negatively charged moiety;
- (iv) each of the plurality of surface-blocking molecular ligands is covalently bound to the covalently

functionalized synthetic surface or the proto-antigen-presenting surface and/or

(v) the plurality of the surface-blocking molecular ligands and may include 2, 3, or 4 different surface-blocking groups and/or 2, 3, 4, or more different lengths of linkers, chosen in any combination.

32. The method of any one of claims 21-31, wherein the covalently functionalized synthetic surface or the proto-antigen-presenting surface is a wafer, an inner surface of a tube, an inner surface of a microfluidic device, or a bead.

33. The method of claim 32, wherein the inner surface of the microfluidic device is within a chamber of the microfluidic device; and further wherein the chamber is a sequestration pen and the microfluidic device further comprises a flow region for containing a flow of a first fluidic medium; and the sequestration pen comprises an isolation region for containing a second fluidic medium and a connection region fluidically connecting the isolation region to the flow region; and further wherein the isolation region has a single opening and is an unswept region of the microfluidic device.

34. The method of claim 33, wherein the microfluidic device comprises a microfluidic channel comprising at least a portion of the flow region, and the connection region comprises a proximal opening into the microfluidic channel having a width W_{con} ranging from ~~about~~ 20 microns to ~~about~~ 100 microns and a distal opening into the isolation region, and wherein a length L_{con} of the connection region from the proximal opening to the distal opening is at least 1.0 times a width W_{con} of the proximal opening of the connection region.

35. The method of claim 34, wherein the length L_{con} of the connection region from the proximal opening to the distal opening is at least 1.5 times the width W_{con} of the proximal opening of the connection region; or the length L_{con} of the connection region from the proximal opening to the distal opening is at least 2.0 times the width W_{con} of the proximal opening of the connection region.

36. The method of claims 35, wherein the proximal opening of the connection region is parallel to a direction of the flow of the first medium in the flow region.

37. The method of claim 32, wherein the wafer or the beads are configured to be disposed within a well of a wellplate.

38. The method of any one of claims 21-37, wherein the initial peptide is GMGQKDSYV (SEQ ID NO: 1), RMQKEITAL (SEQ ID NO: 2), QLAKLMATL (SEQ ID NO: 3), or LMYAKRAFV (SEQ ID NO: 4).