

Claims

- 5 1. A composition comprising a plurality of subsets of multimeric major histocompatibility complexes (multimeric MHC),

wherein each subset of multimeric MHC comprises i) two or more MHC molecules linked to a backbone molecule, and ii) at least one nucleic acid molecule linked to said backbone, said at least one nucleic acid molecule comprising a 5' first primer region, a central region (barcode region), and a 3'

10 second primer region, wherein said barcode serves as a specific label for a given peptide-MHC molecule,

wherein each subset of multimeric MHC has a different peptide decisive for T cell recognition and an associated unique nucleic acid molecule comprising a barcode region.
- 15 2. The composition according to claim 1, wherein each barcode in each subset of multimeric MHC have shared amplification ends, enabling amplification of all barcodes simultaneously in a PCR reaction.
- 20 3. The composition according to any of the preceding claims, wherein the backbone molecule is selected from the group consisting of polysaccharides, including glucans such as dextran, a streptavidin or a streptamer multimer.
- 25 4. The composition according to any of the preceding claims, wherein

 - a. the two or more MHC molecules are coupled to the backbone through a streptavidin-biotin binding and/or streptavidin-avidin and/or via the MHC heavy chain and/or via light chain (B2M), and/or
 - b. the at least one nucleic acid molecule is coupled to the backbone via a streptavidin-biotin binding and/or a streptavidin-avidin binding.
- 30 5. The composition according to any of the preceding claims, wherein said multimeric MHC comprises at least four MHC molecules, such as at least eight, such as at least ten, such as 2-30, such as 2-20, such as 2-10, such as 4-10 or such as 15-30 MHC molecules.
- 35 6. The composition according to any of the preceding claims, wherein each of the at least one nucleic acid molecule(s) has a length of 20-200 nucleotides, such

as 20-150, such as 20-100, such as 30-100, such as 30-80, such as 30-50 nucleotides.

- 5 7. The composition according to any of the preceding claims, wherein the at least one nucleic acid molecule comprises or consists of DNA, RNA, and/or artificial nucleotides such as PNA or LNA.
- 10 8. The composition according to any of the preceding claims, wherein the MHC is selected from the group consisting of a class I MHC, a class II MHC, a CD1, and other MHC-like molecules.
- 15 9. The composition according to any of the preceding claims, wherein the backbone further comprises one or more labels selected from the group consisting of fluorescent labels, His-tags, metal-ion tags, and other selectable tags or labels.
- 20 10. A method for detecting antigen responsive cells, such as multiple antigen responsive cells, in a sample comprising:

 - i) providing a composition comprising a plurality of subsets of multimeric MHC according to any of claims 1-9;
 - ii) contacting said composition with said sample; and
 - iii) detecting binding of each of the multimeric MHC to said antigen responsive cells, thereby detecting cells responsive to an antigen present in each subset of multimeric MHCs,

25 wherein said binding is detected by amplifying the barcode regions of said nucleic acid molecules linked to the one or more multimeric MHCs.
- 30 11. The method according to claim 10, wherein the sample is selected from the group consisting of a blood sample, such as a peripheral blood sample; a blood derived sample; a tissue sample, and a body fluid, such as spinal fluid or saliva.
- 35 12. A method for determining the affinity and/or binding motif of a given T-cell TCR on a single cell surface comprising:

 - i) providing a composition comprising a plurality of subsets of multimeric MHC according to any of claims 1-9;
 - ii) contacting said composition with said single cell; and

- iii) detecting binding of each of the multimeric MHC to said single cell, thereby detecting interaction of several different peptide-MHC multimers on a single cell surface, wherein said binding is detected by amplifying the barcode regions of said nucleic acid molecules linked to the one or more multimeric MHCs.

13. A method for determining the TCR antigen-specificity of given T-cell comprising:

- i) providing a multimeric major histocompatibility complex comprising i) two or more MHC molecules linked to a backbone molecule, and ii) at least one nucleic acid molecule linked to said backbone, said at least one nucleic acid molecule comprising a 5' first primer region, a central region (barcode region), and a 3' second primer region, wherein said barcode serves as a specific label for a given peptide-MHC molecule,;
- ii) contacting said multimeric MHC with said T-cell; and
- iii) detecting binding of multimeric MHC to said T-cell by amplifying the barcode region of said nucleic acid molecule linked to the multimeric MHCs, and
- iv) determining TCR identity by TCR sequencing.

14. The method according to any of claims 10-13, wherein the method further comprises one or more steps of cell selection by a method selected from the group consisting of flow cytometry such as FACS; magnetic-bead based selection; size-exclusion; gradient centrifugation; column attachment and gel-filtration.

15. The method according to any of claims 10-14, wherein the amplified barcode regions of each of said nucleic acid molecules linked to each subset of multimeric MHCs are detected by sequencing of said barcode regions, such as by deep sequencing, high-throughput sequencing, next generation sequencing, or by detection of said barcode region by PCR such as QPCR.

16. A method of diagnosis of a disease or condition, preferably cancer and/or infectious diseases, said method comprising:

- i) providing a sample obtained from a mammal,
- ii) providing a multimeric major histocompatibility complex comprising i) two or more MHC molecules linked to a backbone molecule, and ii) at least one nucleic acid molecule linked to said backbone, said at least

one nucleic acid molecule comprising a 5' first primer region, a central region (barcode region), and a 3' second primer region, wherein said barcode serves as a specific label for a given peptide-MHC molecule; or a composition according to claims 1-9,

- 5 iii) contacting said multimeric MHC or said composition with said sample;
 and
- iv) detecting binding of the multimeric MHC to antigen responsive cells in
 said sample, wherein said binding is detected by amplifying the barcode
10 regions of said nucleic acid molecules linked to the one or more
 multimeric MHCs, thereby diagnosing said disease or condition.