

CLAIMS

1. A method of generating an antibody to a protein, said method comprising:
 - 5 (i) identifying an antigenic epitope in said protein by exposing the protein to limited or restricted proteolysis by contacting the protein with at least one protease to form at least one digested, deconstructed or truncated version of the protein and at least one surface-exposed peptide that is cleaved off from the protein by the action of said protease and generating an antigenic epitope
 - 10 based on said surface-exposed peptide; and
 - (ii) raising an antibody against the antigenic epitope.

2. A method of generating an antibody to a protein, said method comprising:
 - (i) exposing the protein to limited or restricted proteolysis by contacting the protein
 - 15 with at least one protease to form at least one digested, deconstructed or truncated version of the protein and at least one surface-exposed peptide that is cleaved off from the protein by the action of said protease; and
 - (ii) identifying an antigenic epitope by identifying a surface-exposed epitope among the at least one surface-exposed peptide that is present in a region of
 - 20 the protein that results in a lack of, or significantly altered, biological function of the protein when the peptide is cleaved off or removed from the protein during the limited or restricted proteolysis; or
 - selecting at least one target region within the protein based on bioinformatics and/or known data of biological function of the protein and identifying an
 - 25 antigenic epitope by identifying a surface-exposed epitope among the at least one surface-exposed peptide present in said at least one target region; and
 - (iii) raising an antibody against the antigenic epitope.

 3. The method of claim 1 or claim 2, wherein said at least one protease is used under
 - 30 conditions which result in at most 8 or at most 7 or at most 5 surface exposed peptides, or at most 8 or at most 7 or at most 5 unique surface exposed peptides, being cleaved off from the protein by the action of said protease in a sample of the proteolytically digested material, and optionally multiple samples are taken or run in sequence or in parallel, optionally at different periods of time and/or at different concentrations of said protease.

4. The method of any one of claims 1 to 3, wherein said at least one protease is used under conditions which result in at most 8 or at most 7 or at most 5 surface exposed peptides being cleaved off from the protein by the action of said protease.
- 5 5. The method of any one of claims 1-4, wherein the kinetic activity of said at least one protease is slowed down so much that said surface exposed peptides are cleaved off one at a time or at most a few at a time, for example at most 8 or at most 7 or at most 5 at a time in a sample, and optionally multiple samples are taken or run in sequence or in parallel.
- 10 6. The method of any one of claims 1-5, wherein said cleaved off surface-exposed peptides are ranked based on order of appearance after being contacted with said at least one protease, wherein the surface exposed peptides that are cleaved off first or at the lowest concentration of said protease are given a high rank and the surface exposed peptides that are cleaved off late or at the highest concentration of said protease are given a low rank,
15 and optionally surface exposed peptides that are cleaved off in between may be ranked in order of their appearance.
7. The method of claim 6, wherein said method comprises picking a surface-exposed peptide having a high rank for antigenic epitope development and raising an antibody
20 against said antigenic epitope.
8. The method of claim 6, wherein said method comprises picking a surface-exposed peptide having a high rank, constructing an antigenic epitope based on said surface-exposed peptide and raising an antibody against said antigenic epitope.
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9. The method of claim 6, wherein said method comprises picking a surface-exposed peptide having a high rank, correlating said surface exposed-peptide with a defined biological property of the protein, constructing an antigenic epitope based on said surface-exposed peptide and raising an antibody against said antigenic epitope.
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10. The method of any one of claims 1-9, wherein a single protease is used to digest, deconstruct and/or truncate said protein.
11. The method of any one of claims 1-9, wherein multiple proteases are used to
35 digest, deconstruct and/or truncate said protein.

12. The method of claim 11, wherein the multiple proteases are used sequentially one at a time, are used in parallel, or are used in a single cocktail of multiple proteases.
13. The method of claim 11 or claim 12, wherein said multiple proteases are used to
5 identify overlapping, complementary, or unique surface-exposed peptides.
14. The method of any one of claim 1-13, wherein said protease is selected from the group consisting of: trypsin, Arg-C proteinase, Asp-N endopeptidase, Clostripain, Glutamyl endopeptidase, Lys-C, Lys-N, Chymotrypsin, Proteinase K, Thermolysin, Pepsin, Caspase
10 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, Enterokinase, Factor Xa, GranzymeB, Neutrophil elastase, Proline-endopeptidase, Staphylococcal peptidase I, and Thrombin.
15. The method of any one of claims 1-14, wherein said protease is trypsin.
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16. The method of any one of claims 1-15, wherein said protein is a membrane protein that is present in a proteoliposome derived from cells.
17. The method of claim 16, wherein said proteoliposome is immobilized in a flow cell to
20 create a stationary phase of membrane proteins.
18. The method of any one of claims 1-15, wherein said protein is in a protein-containing lipid vesicle that is surface-bound or suspended in a solution.
- 25 19. The method of any one of claims 1-15, wherein said protein is in an intact cell that is surface-bound or suspended in a solution.
20. The method of any one of claims 1-15, wherein said protein is in a solution.
- 30 21. The method of any one of claims 1-20, wherein said protein is any protein of the human proteome.
22. The method of any one of claims 1-21, wherein said protein is a membrane bound protein, a soluble protein, an extracellular protein or an intracellular protein.
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23. The method of any one of claims 1-22, wherein said protein is a membrane or

a membrane associated protein.

24. The method of any one of claims 1-23, wherein said protein is an ion channel in the TRP superfamily.

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25. The method of claim 24, wherein said protein is TRPV1 or TRPV2.

26. The method of any one of claims 1-23, wherein said protein is an excitatory amino acid receptor.

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27. The method of claim 26, wherein said protein is the NMDA receptor or a G-protein.

28. The method of any one of claims 1-23, wherein said protein is an oncogenic protein.

15 29. The method of claim 28, wherein said protein is an oncogenic small GTPase selected from the group consisting of KRAS, NRAS and HRAS.

30. The method of any one of claims 1-23, wherein said protein is an immunomodulatory protein.

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31. The method of claim 30, wherein said protein is selected from the group consisting of PD1, PDL1, CD40, OX40, VISTA, LAG-3, TIM-3, GITR and CD20.

25 32. The method of any one of claims 1-31, wherein said cleaved-off peptides are identified with mass spectrometry.

33. The method of claim 24, wherein said cleaved-off peptides are identified with LC-MS/MS.

30 34. The method of any one of claims 2-33, wherein said biological function is selected from the group consisting of capability of said protein to bind to a target such as a ligand or receptor, enzymatic activity of said protein, ion channel activity, transporter activity, and release such as insulin release and uptake machinery.

35. The method of any one of claims 1-34, wherein raising an antibody against an antigenic epitope is performed by hybridoma technology, phage display technology, or by immunizing an animal with said antigenic epitope.
- 5 36. The method according to any one of claims 1-35, wherein said antibody is monoclonal or polyclonal.