

complex, wherein the digestion is a partial proteolysis reaction, such that the parts of the protein that are not bound to the polymer are separated from the complex with the polymer; and (iii) isolating the peptide:polymer complex; and optionally (iv) sequencing the peptide; wherein the peptide corresponds to a binding site for a binding partner.

In another aspect, the present disclosure refers to the use of a molecularly imprinted polymer (MIP) for producing a composition suitable for determining, on a protein, a novel binding site for a binding partner, optionally wherein the binding site is an epitope to which an antibody binds, wherein the use comprises: (i) forming a protein:MIP complex; (ii) digesting the protein in the complex to produce a peptide:MIP complex, wherein the digestion is a partial proteolysis reaction, such that the parts of the protein that are not bound to the polymer are separated from the complex with the polymer; and (iii) isolating the peptide:MIP complex, wherein the peptide corresponds to a binding site for a binding partner.

In yet another aspect, the present disclosure refers to use of a kit for determining, on a protein, a binding site for a binding partner, the kit comprising: (i) a plurality of monomers suitable for contacting a target protein and polymerising the monomers to create a protein:polymer complex; and (ii) one or more protein cleaving agent and (iii) magnetic nanoparticles.

DETAILED DESCRIPTION

The invention provides methods, compositions, and kits for the characterisation and analysis of proteins.

Hence, in a first aspect of the invention, there is provided a method for determining, on a protein, a binding site for a binding partner, comprising:

- contacting the protein with a plurality of monomers, and polymerising the monomers to create a protein:polymer complex;
- digesting the protein in the complex to produce a peptide:polymer complex; and
- isolating the peptide:polymer complex; and optionally sequencing the peptide, wherein the peptide corresponds to a binding site for a binding partner.

Advantageously, the method of the first aspect does not require information about protein structure or the availability of antibodies for experimental identification of peptide sequences which are binding site candidates. The characterisation and analysis may be for obtaining information related to amino acid sequences exposed on a protein surface. The information derived is useful for the identification of binding sites upon a protein, which may be suitable for the binding of binding partners, such as antibodies, aptamers, affimers, small drug molecules, MIPs or other natural or synthetic receptors. The information about these sequences may be obtained using molecular imprinting and sequencing techniques. Knowledge about these amino acid sequences may be used for generating binding partners, such as high affinity antibodies or other natural or synthetic receptors, for research, diagnostic, or therapeutic applications.

Claims

1. A method for determining, on a protein, a binding site for a binding partner, comprising:
 - (i) contacting the protein with a plurality of monomers, and polymerising the monomers to create a protein:polymer complex;
 - (ii) digesting the protein in the complex to produce a peptide:polymer complex, wherein the digestion is a partial proteolysis reaction, such that the parts of the protein that are not bound to the polymer are separated from the complex with the polymer; and
 - (iii) isolating the peptide:polymer complex; and optionally
 - (iv) sequencing the peptide;
 wherein the peptide corresponds to a binding site for a binding partner.
2. A method according to claim 1, wherein the protein is a folded protein and/or wherein the binding site is a novel binding site, optionally wherein the binding site is an epitope to which an antibody binds.
3. A method according to either claim 1 or claim 2, wherein the protein is a complex with an enzyme inhibitor, drug molecular or other protein.
4. A method according to any preceding claim, wherein a complex between the protein and a molecularly imprinted polymer (MIP) is created by step (i).
5. A method according to any preceding claim, wherein the plurality of monomers comprises cross-linking monomers.
6. A method according to any preceding claim, wherein the plurality of monomers further comprises magnetic nanoparticles.
7. A method according to any preceding claim, wherein step (i) comprises contacting a protein with a plurality of monomers, wherein the protein is in solution or immobilised on a solid surface.
8. A method according to claim 7, wherein the solid surface is selected from a surface comprising polysaccharide, silica, organic or inorganic polymer, metal, or a combination thereof; and/or

wherein the form of the solid surface is selected from beads, magnetic beads, arrays, the surface of waveguides, fibres, membranes, or capillaries.

9. A method according to any preceding claim, wherein polymerisation is initiated
5 chemically, thermally, or optically.
10. A method according to any preceding claim, wherein the polymer product:
 - (a) has a magnetic core;
 - (b) is linear or cross-linked;
 - 10 (c) is covalently linked to the target protein; and/or
 - (d) is in a soluble or colloidal form.
11. A method according to any preceding claim, further comprising the step:
isolating the protein:polymer complex.
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12. A method according to claim 11, wherein the isolation of the protein:polymer
complex comprises the use of ultrafiltration, ultracentrifugation, electrophoresis,
sonication, chromatographic separation, washing, adding urea, guanidine, magnetic
forces, or any combination thereof; and/or
20 wherein the protein or polymer is linked to a solid support to facilitate the
isolation of the protein:polymer complex.
13. A method according to any preceding claim, wherein digestion step (ii)
comprises the use of one or more protein cleaving agent.
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14. A method according to any preceding claim, wherein step (iii) comprises the
separation of the peptide:polymer complex from unbound protein and/or unbound
peptide, optionally wherein comprising the use of ultrafiltration, ultracentrifugation,
electrophoresis, sonication, chromatographic separation, washing, adding urea,
30 guanidine, magnetic forces, or any combination thereof.
15. A method according to any preceding claim, further comprising the step:
releasing bound peptide from the peptide:polymer complex.
- 35 16. A method according to any preceding claim, wherein step (iv) comprises the use
of Edman degradation, mass spectrometry, mass spectrometry using matrix assisted

laser desorption ionization (MALDI), electrospray ionization (ESI) sources, atmospheric pressure ionisation techniques or tandem mass spectrometric analysis (MS/MS), or any combination thereof.

- 5 17. Use of a molecularly imprinted polymer (MIP) for producing a composition suitable for determining, on a protein, a novel binding site for a binding partner, optionally wherein the binding site is an epitope to which an antibody binds, wherein the use comprises:
- (i) forming a protein:MIP complex;
 - 10 (ii) digesting the protein in the complex to produce a peptide:MIP complex, wherein the digestion is a partial proteolysis reaction, such that the parts of the protein that are not bound to the polymer are separated from the complex with the polymer; and
 - (iii) isolating the peptide:MIP complex
- 15 wherein the peptide corresponds to a binding site for a binding partner.
18. The use according to claim 17, wherein the complex is formed between a pre-formed MIP and the protein.
- 20 19. The use according to claim 17, wherein the protein:MIP complex is formed by the contacting of the protein with a plurality of monomers, and the initiation of polymerisation of the monomers.
20. The use according to any of claims 17 to 19, comprising:
- 25 isolating the protein:MIP complex before step (ii).
21. The use according to any of claims 17 to 20, wherein the digestion of the protein in the protein:MIP complex comprises the partial proteolysis of the protein in the complex, such that the parts of the protein that are not bound to the polymer are
- 30 separated from the complex with the polymer, optionally wherein the digestion comprises the use of one or more protein cleaving agent, such as a protease.
22. The use according to any of claims 17 to 21, comprising:
- releasing the bound peptide from the peptide:MIP complex.
- 35 23. The use according to any of claims 17 to 22, comprising:

determining the sequence of the bound peptide.

24. Use of a kit for determining on a protein, a binding site for a binding partner, the kit comprising:

- 5 (i) a plurality of monomers suitable for contacting a target protein and polymerising the monomers to create a protein:polymer complex;
 (ii) one or more protein cleaving agent; and
 (iii) magnetic nanoparticles.

10 25. The use according to claim 24, wherein the plurality of monomers are suitable for forming a molecularly imprinted polymer (MIP) in complex with a target protein.

26. The use according to any of claims 24 to 25, further comprising one or more initiators, suitable for initiating polymerisation.

15 27. The use according to any of claims 24 to 26, further comprising materials comprising a solid surface suitable for the immobilisation of a target protein.

28. The use according to any of claims 24 to 27, further comprising materials for
 20 use in the isolation of a protein:polymer complex or for use in the isolation of a peptide:polymer complex, optionally wherein the materials are for ultrafiltration and/or centrifugation.

29. The use according to any of claims 24 to 28, wherein the one or more protein
 25 cleaving agent comprises an enzyme, optionally a protease.

30. The use according to any of claims 24 to 29, further comprising one or more buffers.

30 31. The method of any of claims 1 to 16, the use of claim 19, the use of any of claims 20 to 23 when dependent on claim 19, or use of any of claims 24 to 30, wherein the plurality of monomers comprises one or more monomer selected from the group consisting of: vinyl monomers, allyl monomers, acetylenes, acrylates, methacrylates, acrylamides, methacrylamides, chloroacrylates, itaconates, trifluoromethylacrylates,
 35 nucleosides, nucleotides, carbohydrates, ethylene glycol dimethacrylate, trimethylolpropane trimethacrylate, divinylbenzene, methylene bisacrylamide, ethylene

bisacrylamide and N,N'-bisacryloylpiperazine, N-Isopropylacrylamide (NIPAM), tert-butylacrylamide, acrylic acid, 3-aminopropyl methacrylate, methacrylic acid, or any combination thereof.