(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 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 antigenic epitope by identifying a surface-exposed epitope among the at least one surface-exposed peptide present in said at least one target region.

In one aspect, the present disclosure refers to a method of identifying an epitope on a protein that can be bound by an antibody, said method comprising:

(i) performing limited or restricted proteolysis on said protein using a single first protease or a combination of first proteases;

(ii) performing non-limited proteolysis or performing limited or restricted proteolysis on said protein using a single second protease or a combination of second proteases, wherein the specificity(ies) of said second protease(s) are all different from the specificity(ies) of the protease(s) used in step (i);

(iii) analysing peptides which are released from said protein in step (ii) to identify peptides in which one end has been cut by a said first protease and the other end has been cut by a said second protease;

(iv) probing one or more epitopes in a region of the protein containing or flanking a cut site for a said first protease as identified in step (iii) with one or more antibodies directed to said epitopes, thereby identifying one or more epitopes on the protein that can be bound by an antibody.

In another example, there is provided an epitope identified by the method as described herein.

In another example, there is provided an antibody that binds to an epitope as described herein.

The present disclosure relates to methods of detecting and identifying amino acid sequences in proteins where said amino acid sequences are well-exposed, and functionally relevant, at least they are well-exposed. These amino acid sequences which we refer to as "hot spots", thus, may be utilized as antigenic epitopes that guides antibody targeting, discovery, and development. Furthermore, these amino acid sequences can be ranked based on their appearance after proteolytic digestion, and based on functional relevance from already known bioinformatic data or from functional/pharmacological testing. Thus, from

a list of several amino acid sequences resulting from a proteolytic digestion, the best suited amino acid sequences (based on functional and structural arguments) can be picked for antigenic epitope discovery and development. The proteolytic digestion is performed under limiting conditions, i.e. the activity of the protease or several proteases is very low such that just one or a few surface-exposed peptides are cleaved off from the target protein at a time. The proteases are thus used as druggability probes for antibody binding to a target protein.

In an example, the antibodies are pharmacologically active. In another example, the antibodies are pharmacologically active and developed for therapeutic usage. More specifically, such methods include proteomic tools to reveal hot spot epitopes of target proteins.

In an example of the disclosure, a protein is digested, deconstructed and/or truncated through protease action and all well-exposed amino acid sequences are used for antigenic epitope generation, and antibodies developed based on said antigenic epitopes are tried for potency, efficacy, pharmacological profiling, and other testing as customary in antibody discovery used in the pharmaceutical industry.

In an example of the disclosure, a protein is digested, deconstructed and/or truncated through protease action and in parallel probed by a functional assay on the digested, deconstructed

and/or truncated protein to delineate functionally important regions of the protein. The relevant protein is sometimes denoted target protein herein.

In an example, the digestion, deconstruction and/or truncation of the target protein is performed in parallel by a functional assay to delineate functionally important regions of the target protein to guide epitope selection for antibody generation.

In an example, the digestion, deconstruction and/or truncation, and functional assay of digested, deconstructed and/or truncated protein and native target protein are combined with other bioinformatic and otherwise known facts about protein function to delineate functionally important regions of the target protein to guide epitope selection for antibody generation.

In an example, a single protease may be used to digest, deconstruct and/or truncate the target protein. In another example, multiple proteases may be used to digest, deconstruct and/or truncate the target protein, sequentially one at a time or in parallel. Such proteases are exemplified but not limited to Arg-C proteinase, Asp-N endopeptidase, Clostripain, Glutamyl endopeptidase, Lys-C, Lys-N, Trypsin, Chymotrypsin, Proteinase K and Thermolysin. A region that is easily digested by several proteases should be located in an exposed region of the protein and a region that is only digested by a single protease is probably located in a more hidden region. Alternatively, the protease has unique cleaving specificity or/and physicochemical properties or/and structural features such that it can identify surface-exposed peptides on a target protein that other proteases cannot. Thus, the usage of multiple proteases is preferable, and each different protease can yield complementary or unique information about surface-exposed peptides suitability as antigenic epitopes.

The examples enable new methodology/technology for rapid and precise development of pharmacologically active antibodies that can be used for pharmacological studies, e.g. they can be used as a tool for detecting biological compounds in e.g. cell or in vitro assays. More importantly, said antibodies may be used to treat a medical condition in humans and animals. The examples can be applied to all proteins, soluble or membrane bound, extracellular or intracellular. The examples can furthermore be exploited to yield new fundamental understanding of protein function.

The present disclosure also provides an antibody generated by a method of the present disclosure.

CLAIMS

1. A method of identifying an epitope on a protein that can be bound by an antibody, said method comprising:

(i) performing limited or restricted proteolysis on said protein using a single first protease or a combination of first proteases;

(ii) performing non-limited proteolysis or performing limited or restricted proteolysis on said protein using a single second protease or a combination of second proteases, wherein the specificity(ies) of said second protease(s) are all different from the specificity(ies) of the protease(s) used in step (i);

(iii) analysing peptides which are released from said protein in step (ii) to identify peptides in which one end has been cut by a said first protease and the other end has been cut by a said second protease;

(iv) probing one or more epitopes in a region of the protein containing or flanking a cut site for a said first protease as identified in step (iii) with one or more antibodies directed to said epitopes, thereby identifying one or more epitopes on the protein that can be bound by an antibody.

2. The method of claim 1, wherein in step (ii) non-limited proteolysis is performed.

3. The method of claim 1 or claim 2, wherein a single first protease is used in step (i).

4. The method of any one of claims 1 to 3, wherein a single second protease is used in step (ii).

5. The method of any one of claims 1 to 4, wherein in step (ii) non-limited proteolysis is performed and wherein said method further comprises, subsequent to step (i), but prior to step (ii), an additional step of performing limited or restricted proteolysis on said protein using said single second protease or a combination of second proteases that is used in step (ii).

6. The method of any one of claims 1 to 5, wherein said single first protease or said combination of first proteases is selected from the group consisting of Trypsin, Arg-C, Lys-C and Lys-N.

7. The method of any one of claims 1 to 5, wherein said single first protease or said combination of first proteases is selected from the group consisting of pepsin, chymotrypsin and Glu-C.

8. The method of any one of claims 1 to 6, wherein said single second protease or said combination of second proteases is selected from the group consisting of pepsin, chymotrypsin and Glu-C.

9. The method of any one of claims 1 to 5 or 7, wherein said single second protease or said combination of second proteases is selected from the group consisting of Trypsin, Arg-C, Lys-C and Lys-N.

10. The method of any one of claims 1 to 9, wherein said method further comprises a denaturation step that is carried out before, during or after step (ii).

11. The method of any one of claims 1 to 10, wherein said peptides are analysed using mass spectrometry.

12. The method of any one of claims 1 to 11, wherein a plurality of epitopes is probed.

13. The method of any one of claims 1 to 12, wherein said method further comprises a step prior to step (iv) of generating one or more isolated epitopes having sequences that correspond to one or more epitopes on said protein that are in a region of the protein containing or flanking a cut site for a said first protease as identified in step (iii), and generating antibodies that are directed to said isolated epitopes, and using said antibodies in step (iv) for probing one or more epitopes on said protein.

14. The method of any one of claims 1 to 13, wherein said epitope is within 20 amino acids of said cut site for a said first protease.

15. The method of any one of claims 1 to 14, wherein a plurality of epitopes is probed and wherein said plurality of epitopes is a set of epitopes wherein the sequence of each epitope in the set is offset from another epitope in the set by 1, 2 or 3 amino acids.

16. The method of any one of claims 1 to 15, wherein the proteolysis in steps (i) and (ii) is performed on a protein that is present in a proteoliposome that is derived from cells.

17. The method of claim 16, wherein said proteoliposome is immobilized in a flow cell to create a stationary phase of the protein.

18. The method of any one of claims 1 to 17, wherein step (iii) comprises analysing peptides which are released from said protein in step (i) and step (ii).

19. The method of any one of claims 1 to 18, wherein said method further comprises a step of producing an antibody against an epitope identified by any one of claims 1 to 18.