embodiment the polypeptide in the samples is reduced and the free sulfhydryl residues are carboxymethylated prior to the incubation with the protease.

The samples are all treated separately, i.e. individually and not as mixture, in the steps according to the methods as reported herein. In one embodiment the samples are treated individually in the reducing step, the incubating step, and the analyzing step. In one embodiment the method is a method for high throughput determination.

In one embodiment the samples are incubated for 16 hours to 18 hours with the protease and immediately thereafter formic acid or trifluoro acetic acid are added. In a further embodiment the samples are incubated for 4 hours with the protease and immediately thereafter formic acid or trifluoro acetic acid are added.

One aspect as reported herein is a method for determining a polypeptide with a mutant amino acid sequence, characterized in comprising the following steps:

a) providing at least two samples of the polypeptide,
b) incubating the samples each with the same protease,
c) analyzing the incubated samples by a two dimensional data analysis using a combination of a reversed phase liquid chromatography separation and a mass spectrometry analysis and/or MS/MS analysis,
d) defining the data set obtained with one sample in step c) as reference sample and comparing the data sets obtained with the other samples in step c) with the data set of the reference sample, whereby every amino acid sequence difference with a ratio of more than 3 of the intensity of the sample mass spectrum signal to the intensity of the reference mass spectrum signal is an amino acid sequence mutation of the polypeptide and thereby determining a polypeptide with a mutant amino acid sequence, wherein the m/z frame width is 1.6 or more for pattern comparison~~determined is an amino acid sequence mutation of the polypeptide and thereby determining a polypeptide with a mutant amino acid sequence~~.

~~Another aspect as reported herein is a method for producing a polypeptide comprising the following step: selecting a cell producing a polypeptide, whereby the polypeptide comprises the smallest number of amino acid sequence mutations of all processed samples or with respect to a reference sample or predetermined amino acid sequence determined with a method as defined herein.~~

In one embodiment the provided cell is selected from a transiently transfected cell, or a stable transfected cell, or an immune cell obtained from an animal after immunization.

~~Another aspect as reported herein is a method for producing an immunoglobulin comprising the steps of:~~

~~a) providing at least two cells comprising a nucleic acid encoding the immunoglobulin,~~
~~b) single depositing and cultivating the cells,~~
~~c) performing a method according to any one of claims 1 to 14,~~
~~d) selecting a cell producing an immunoglobulin, whereby the immunoglobulin comprises the smallest number of amino acid sequence mutations with respect to a reference sample,~~
~~e) cultivating the cell,~~
~~f) producing the polypeptide by recovering the polypeptide from the cell or the cultivation medium.~~

Patent Claims

1. A method for determining a polypeptide with a mutant amino acid sequence, characterized in comprising the following steps:
   a) providing at least two samples of the polypeptide,
   b) incubating the samples each with the same protease,
   c) analyzing the incubated samples by a two dimensional data analysis using a combination of a reversed phase liquid chromatography separation and a mass spectrometry analysis and/or MS/MS analysis,
   d) defining the data set obtained with one sample in step c) as reference sample and comparing the data sets obtained with the other samples in step c) with the data set of the reference sample, whereby every amino acid sequence difference ~~determined is an amino acid sequence mutation of the polypeptide and thereby determining a polypeptide with a mutant amino acid sequence.~~

~~2. The method according to claim 1, characterized in that every amino acid sequence difference~~ with a ratio of more than 3 of the intensity of the sample mass spectrum signal to the intensity of the reference mass spectrum signal is an amino acid sequence mutation of the polypeptide and thereby determining a polypeptide with a mutant amino acid sequence~~.~~,

~~3. The method according to any one of the preceding claims, characterized in that~~ wherein the m/z frame width is 1.6 or more for pattern comparison.

4~~2~~. The method according to ~~any one of the preceding~~ claim~~s~~ 1 further comprising a step:
   e) determining the identity and position of the amino acid mutations in the amino acid sequence by MS/MS analysis.

5~~3~~. The method according to ~~any one of the preceding~~ claim~~s~~ 1 or 2, characterized in that a further sample is provided which comprises the polypeptide spiked with the polypeptide with a known amino acid sequence mutation and the further sample is incubated and analyzed and compared in addition to the provided samples.

6~~4~~. The method according to any one of the preceding claims, characterized in that the analyzing is performed at a pH value of less than pH 8.0 and at a temperature of less than 40 °C.

~~7~~5. The method according to any one of the preceding claims, characterized in that the samples are provided in a tris (hydroxymethyl) aminomethane buffer.

~~8~~6. The method according to any one of the preceding claims, characterized in that the incubating of the samples with a protease is a cleavage of the polypeptide by the protease in amino acid sequence fragments of from 3 to 60 amino acid residues in length.

~~9~~7. The method according to any one of the preceding claims, characterized in that the comparing in step d) is performed with the data of one or some or all MS charge states.

~~10~~8. The method according to any one of the preceding claims, characterized in that the polypeptide is an immunoglobulin, immunoglobulin fragment or immunoglobulin conjugate.

~~11~~9. The method according to any one of the preceding claims, characterized in that the samples are incubated for 16 hours to 18 hours with the protease and thereafter formic acid or trifluoro acetic acid are added.

~~12~~10. The method according to any one of claims 1 to ~~10~~8, characterized in that the samples are incubated for 4 hours with the protease and thereafter formic acid or trifluoro acetic acid are added.

~~13~~11. The method according to any one of the preceding claims, characterized in that the comparing comprises overlaying of the mass spectrometric total ion chromatogram (MS-TIC) of the reference sample and each of the other samples to be analyzed,
whereby the intensity ratio of all overlapped and aligned masses is calculated, whereby peaks with a ratio of more than 10 are evaluated for being an amino acid sequence mutation.

~~14~~12. The method according to any one of the preceding claims, characterized in that the comparing comprises in addition comparing the DNA translated proteolytic fragment peptide pattern of the theoretical amino acid sequence and the total mass spectrometry ion chromatogram (MS-TIC) of the sample to be analyzed, and amino acid sequence mutations are identified by adding to or substracting from, respectively, each calculated theoretical mass of the theoretical amino acid sequence the mass differences resulting from a nucleic

acid mutation, deletion, or insertion in a base triplet (codon) with an amino acid change.

~~15. A method for producing a polypeptide comprising the following step:~~

~~selecting a cell producing a polypeptide, whereby the polypeptide comprises the smallest number of amino acid sequence mutations of all processed samples or with respect to a reference sample or predetermined amino acid sequence determined with a method according to any one of claims 1 to 14.~~

~~16. A method for producing an immunoglobulin comprising the steps of:~~

~~a) providing at least two cells comprising a nucleic acid encoding the immunoglobulin,~~

~~b) single depositing and cultivating the cells,~~

~~c) performing a method according to any one of claims 1 to 14,~~

~~d) selecting a cell producing an immunoglobulin, whereby the immunoglobulin comprises the smallest number of amino acid sequence mutations with respect to a reference sample,~~

~~e) cultivating the cell,~~

~~f) producing the polypeptide by recovering the polypeptide from the cell or the cultivation medium.~~