CLAIMS

Claim 1. A polypeptide composition analysis method based on M2MspA-N91H nanopore, comprising the following steps:

S1 Mixing an exopeptidase with a polypeptide to be tested uniformly and reacting to obtain a hydrolysis product; wherein the exopeptidase includes aminopeptidase and/or carboxypeptidase;

S2 Adding the hydrolysis product to a nanopore system, wherein the nanopore system comprises an M2MspA-N91H nanopore, an insulating membrane, a first medium, and a second medium, wherein the M2MspA-N91H nanopore is embedded in the insulating membrane, the M2MspA-N91H nanopore provides a channel that connects the first medium and the second medium, and the hydrolysis product is added to the first medium;

S3 Applying a driving force between the first medium and the second medium, a free amino acid in the hydrolysis product passes through the M2MspA-N91H nanopore and generates an electrical signal; wherein the free amino acid includes a natural amino acid and/or a non-natural amino acid; wherein the natural amino acid includes one or more of glycine, alanine, valine, leucine, isoleucine, phenylalanine, tryptophan, tyrosine, aspartic acid, histidine, asparagine, glutamic acid, lysine, glutamine, methionine, arginine, serine, threonine, cysteine, and proline; the non-natural amino acid includes one or more of citrulline, L-norvaline, and ibotenic acid;

S4 Data-analyzing the electrical signal to obtain information on amino acid type of the free amino acid;

S5 Comparing the information on the amino acid type of the free amino acid with expected sequence information of the polypeptide to be tested, to determine whether there is a difference between actual sequence and the expected sequence of the polypeptide to be tested; or

S5 Comparing the information on the amino acid type of the free amino acid combined with information on the exopeptidase with polypeptide sequence in database, to predict actual sequence of the polypeptide to be tested.

Claim 2. The method according to claim 1, wherein before adding the hydrolysis product to the nanopore system, the M2MspA-N91H nanopore is combined with a copper ion.

Claim 3. The method according to claim 1, wherein pH value of the first medium and the second medium includes 6.5 - 7.5.

Claim 4. The method according to claim 1, wherein final concentration of solution of the polypeptide to be tested is greater than 10 μM.

Claim 5. The method according to claim 1, wherein step of the data-analyzing in the S4 further includes:

i) Establishing a standard signal database;

ii) Setting a signal filtering condition;

iii) Excluding part of the electrical signal to obtain a retained electrical signal;

iv) Determining the amino acid type corresponding to the retained electrical signal based on blockage rate of a standard signal in the standard signal database;

v) Obtaining information on the amino acid type of the free amino acid.

Claim 6. The method according to claim 5, wherein the signal filtering condition is to exclude an electrical signal with an standard deviation (SD) value greater than an SD threshold, wherein the SD value of the electrical signal is calculated according to the following formula:

$$\mathrm{SD} = \sqrt{\frac{\sum(x_i - x)^2}{n-1}}$$

Wherein, x_i is current value of each sampling point of the signal, x is mean current value of the signal, and n is number of sampling point of the signal.