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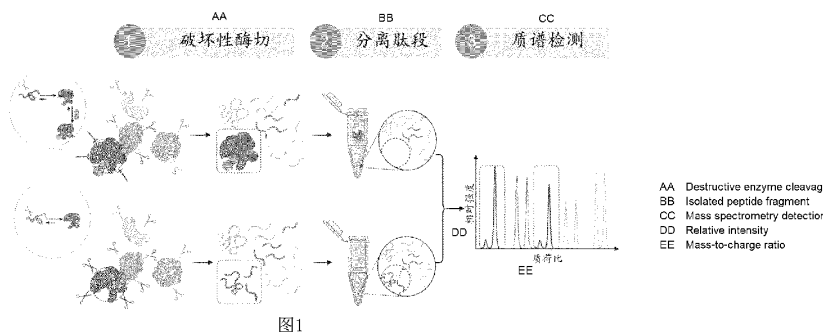
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(54) **Title:** METHOD FOR DETECTING PROTEIN HAVING CHANGES IN ENERGY STATE, OR AFFINITY OF LIGAND TO PROTEIN

(54) 发明名称: 探测能量状态发生变化的蛋白或配体与蛋白亲和力的方法



(57) **Abstract:** Disclosed in the present invention is a method for detecting a protein having changes in an energy state, and affinity of a ligand to a protein. Specifically, after the energy state of a protein changes, the tolerance to enzyme cleavage destruction changes, the structure of the protein in a low-energy state is also destroyed under a non-denaturation condition by using a large amount of enzymes, and a small peptide fragment which has a molecular weight of less than 5 KDa and can be directly used for bottom-top mass spectrometry analysis is directly generated. The method has extremely high sensitivity, and quantitative proteomics is used to find enzyme cleavage differential peptide fragments, and proteins to which the differential peptide fragments belong and the positions in the proteins are analyzed, so that a protein having changes in an energy state, and a change region can be determined in the whole proteome range. If the energy state of the protein changes due to addition of a ligand, the method can determine a binding protein and a binding region of the ligand; and the output of a quantitative result on the peptide fragment level further enables the method to determine the local affinity of binding of the ligand to the protein.

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本国际公布：
— 包括国际检索报告(条约第21条(3))。

(57) 摘要: 本发明公开了一种探测能量状态发生变化的蛋白及配体与蛋白亲和力的方法，具体涉及蛋白质能量状态发生变化之后对酶切破坏的耐受程度发生变化，采用较大量的酶在非变性条件下，使蛋白质的处于低能量状态的结构也被破坏，直接产生分子量小于 **5KDa**、直接可以用于自下而上质谱分析的小肽段。该方法具有极高的灵敏度，使用定量蛋白质组学找到酶切差异肽段，分析差异肽段所属蛋白以及位于蛋白质的位置，从而能够在整个蛋白质组范围内确定能量状态发生变化的蛋白及变化区域。若加入配体导致蛋白质能量状态发生变化，该方法能确定配体的结合蛋白及结合区域；肽段层次上定量结果的输出还使该方法能够测定配体与蛋白结合的局部亲和力。