Creative Proteomics

Proteomics—A Major New Technology for Drug Discovery

Proteomics and pharmacoproteomics

Proteomics is a discipline that analyzes the dynamics of protein components, including expression levels and modification states from a holistic perspective, understands the interactions and connections between proteins, reveals the function of proteins and the laws of cell life, and studies all proteins in cells and their behaviors. The concept of proteomics has been used in the field of pharmaceutical research, thereby developing pharmacoproteomics. This field includes: discovery of all possible drug targets and all possible compounds for these targets; study of drug action mechanisms and toxicology; drug screening. It is also possible to classify patients according to protein profiles, to provide individualized treatment, and to predict drug efficacy. Nowadays, the pharmaceutical proteomics has penetrated into all aspects of drug discovery and clinical application.

Proteomics accelerate the drug discovery process

The drug discovery process includes target identification, target validation, lead recognition, small molecule optimization, and preclinical/clinical development.

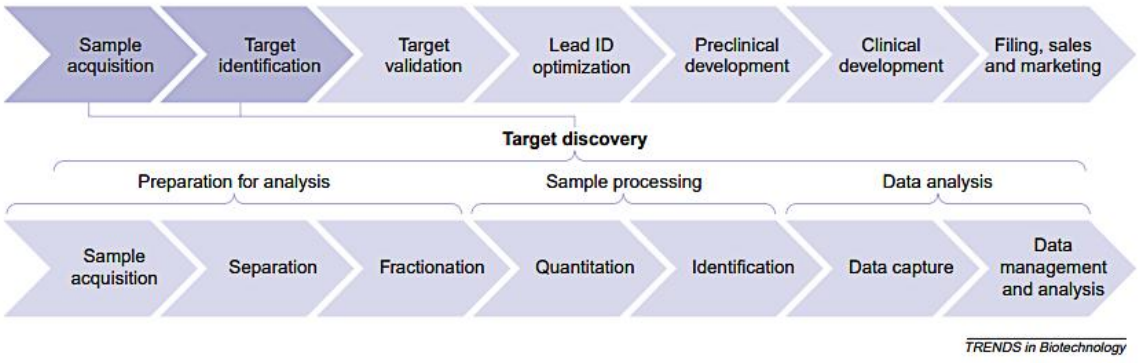

Figure 1. Workflow for large-scale proteomics approach for target discovery within a pharmaceutical setting (Ryan *et al.* 2002)

Proteomics for drug target recognition

Finding effective drugs and drug targets is one of the most widely used applications of proteomics. Proteomics can provide abundant protein expression in cells or tissues. Find differentially expressed proteins by comparing divergences in protein expression profiles between healthy and diseased tissues, cells, or body fluids, which may be potential biomarkers or drug targets.

Proteomics for drug target validation

The detection of only disease-associated proteins (targets) is not sufficient to begin the drug screening. Verifying the function of these proteins, and determining the role of proteins in the pathogenesis of the disease are critical to the process of drug discovery. Proteomics can be used in target validation to detect potential efficacy of drug candidates, to evaluate structure-activity relationships of drug analogues in combination with combinatorial chemistry, to study protein interactions, and to explore phenotypic changes when protein expression is excessive or inhibited.

Proteomics for the recognition and optimization of lead compounds

Proteomics technology can provide a high-throughput method for identifying and optimizing suitable lead compounds. For example, the identification of protein-protein interactions can be used to screen for lead compounds based on in vivo physiological responses, i.e., activity interference. Functional protein microarray can be used in vitro to detect protein-protein interactions, in the presence or absence of protein lead compounds, and to quickly identify molecules that prevent proteins from binding normally, which can change significantly by interfering with protein interactions in living cells. The same strategy can also be used to optimize lead compounds when suitable lead compounds are identified. In this case, protein interactions can be used to determine the presence of a chemical derivative of the lead compound to identify the most likely affected protein.

Proteomics technologies in drug discovery

1. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2DE) is the main means of protein separation and currently the most commonly applied technique for studying proteomics, which first separates proteins by isoelectric point and molecular weight by 2D polypropylene gel electrophoresis, and then analyzes the formed 2D gel electropherogram by software. The desired protein spots are then cleaved from the gel and subjected to mass spectrometry (MS) after enzymatic digestion. The 2DE has been able to isolate more than one thousand protein spots after several years of development.

However, it still has certain defects. For example, quantitative comparisons are not allowed between samples with extremely small minimal proteins, very basic acid proteins, hydrophobic proteins, and poorly isolated proteins with low abundance. While, it is estimated that more than 50% of the proteins in the cells are low abundance. Furthermore, it is time-consuming, labor-consuming, and the reproducibility is not satisfactory. Therefore, the next step is to optimize the technology or to find innovative ways to measure protein abundance and activity.

2. Mass spectrometry

Mass spectrometry is the fastest developing and most potential technique for protein identification at present, with the characteristics of high sensitivity, high accuracy and automation. The most commonly adopted ones are matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization tandem time-of-flight mass spectrometry (ESI-TOF-MS). In addition, new methods such as shotgun mass spectrometry (Shotgun-MS) and capillary electrophoresis-mass spectrometry (CE-MS) have been developed for direct identification of protein hydrolysates. In recent years, tandem mass spectrometry (TMS) has been used for protein sequencing and identification.

In addition, high performance liquid chromatography (HPLC) separation combined with mass spectrometry (MS) identification can effectively make up for the deficiency of 2DE, with a wide range of fixed phases and applications. Multidimensional protein identification (MUDPIT) and isotope affinity labeling (ICAT) are two technologies developed on the basis of LC-MS-MS. MUDPIT is suitable for large-scale protein isolation and identification, and it can detect low-abundance proteins. ICAT is suitable for detecting and quantitating low abundance proteins.

3. Protein microarray

The principle of the protein microarray is to arrange various purified proteins in an orderly manner on a filter or a slide, and then use a fluorescently labeled protein or small molecule as a probe to incubate the protein microarray, rinse to remove the unbound probe, and detect fluorescence signals. Protein microarray is a high-throughput screening method similar to gene microarray. Its applications in drug development mainly include: (i). screening of lead compounds for drug targets; (ii). detection of substances binding to small molecules (such as drugs, lead compounds); (iii). study of interactions between small molecules and proteins.

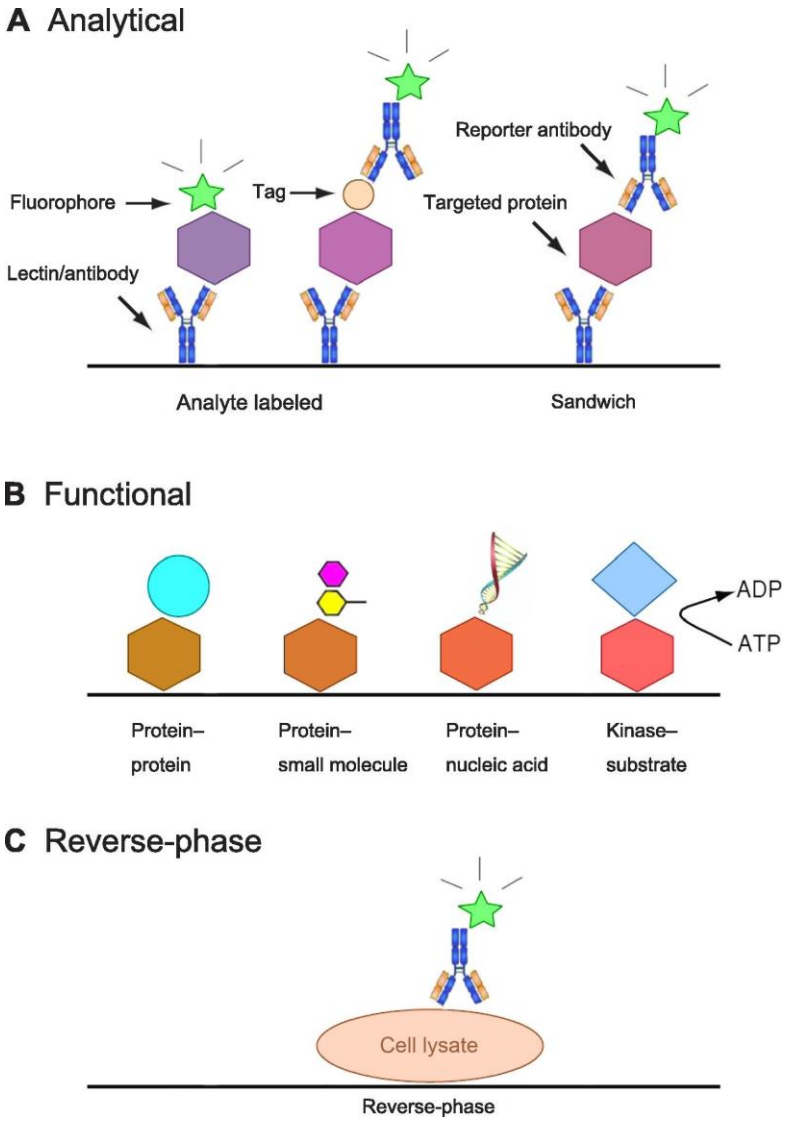

Figure 2. Protein microarray technologies in drug discovery (Huang *et al*, 2017)

4. Yeast two-hybrid system

The yeast two-hybrid system is one of the most powerful methods for analyzing protein interactions, which can be used not only to test protein interactions in vivo, but also to discover new proteins that interact with each other in gene libraries. The principle is to fuse the DNA domain (DB) and transcriptional activation domain (AD) of the transcriptional activator with a pair of proteins to be detected (referred to as "bait" and "prey", respectively), and examine the expression of the reporter gene.

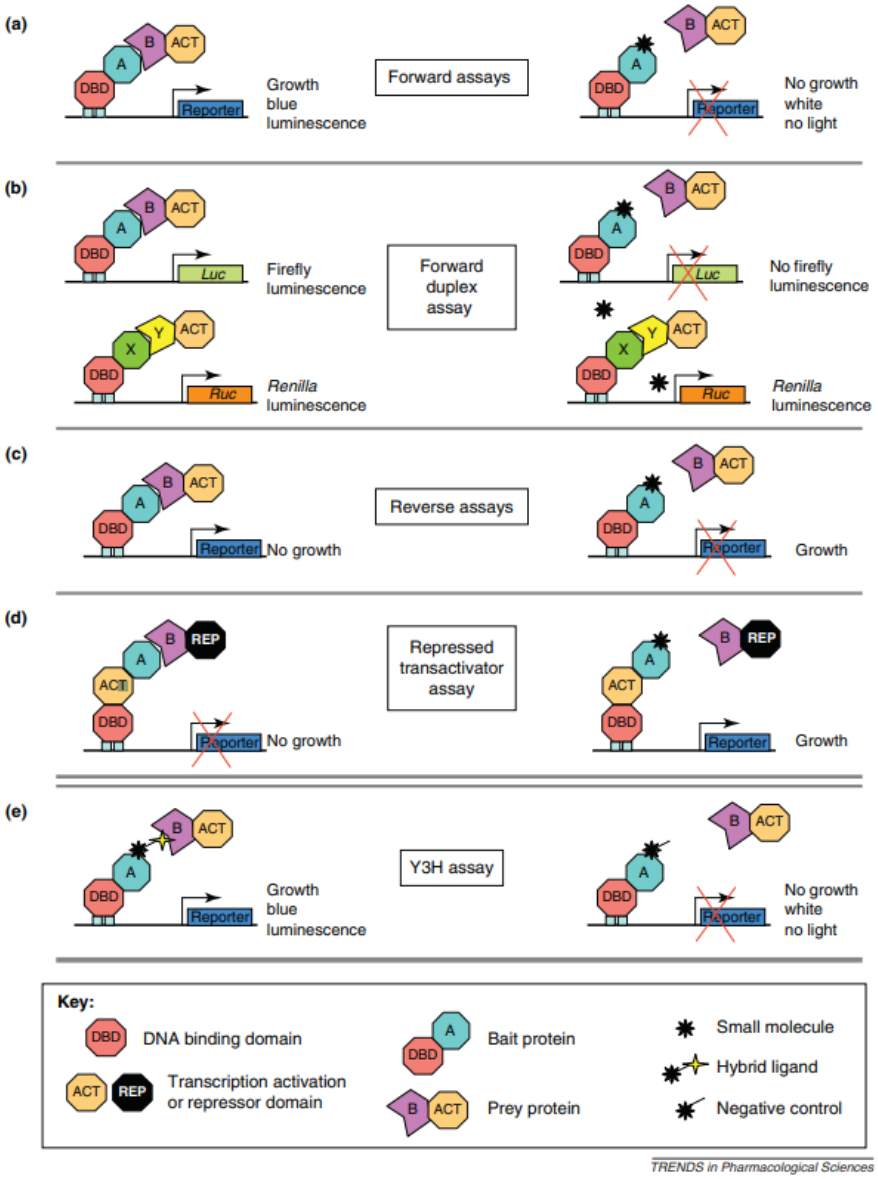

Figure 3. Yeast two-hybrid methods and their applications in drug discovery (Hamdi *et al*, 2012)

Our comprehensive proteomics services and products

Creative Proteomics can provide a comprehensive range of proteomics services to help you better conduct research in the drug discovery process, which include: protein gel and imaging analysis, protein identification, protein quantification, top-down proteomics, peptidomics, post-translational modification analysis, and protein-protein interaction. Our technical staff will work closely with you from experimental design to report delivery. If you have any questions or order, please feel free to contact us.

References:

1. Schirle M, Bantscheff M, Kuster B. Mass spectrometry-based proteomics in preclinical drug discovery. *Chemistry & biology*, 2012, 19(1): 72-84.
2. Burbaum J, Tobal G M. Proteomics in drug discovery. *Current Opinion in Chemical Biology*, 2002, 6(4): 427-433.
3. Wang J H, Hewick R M. Proteomics in drug discovery. *Drug discovery today*, 1999, 4(3): 129-133.
4. Grey J L, Thompson D H. Challenges and opportunities for new protein crystallization strategies in structure-based drug design. *Expert opinion on drug discovery*, 2010, 5(11): 1039-1045.
5. Chambliss A B, Chan D W. Precision medicine: from pharmacogenomics to pharmacoproteomics. *Clinical proteomics*, 2016, 13(1): 25.
6. Hamdi A, Colas P. Yeast two-hybrid methods and their applications in drug discovery. *Trends in pharmacological sciences*, 2012, 33(2): 109-118.