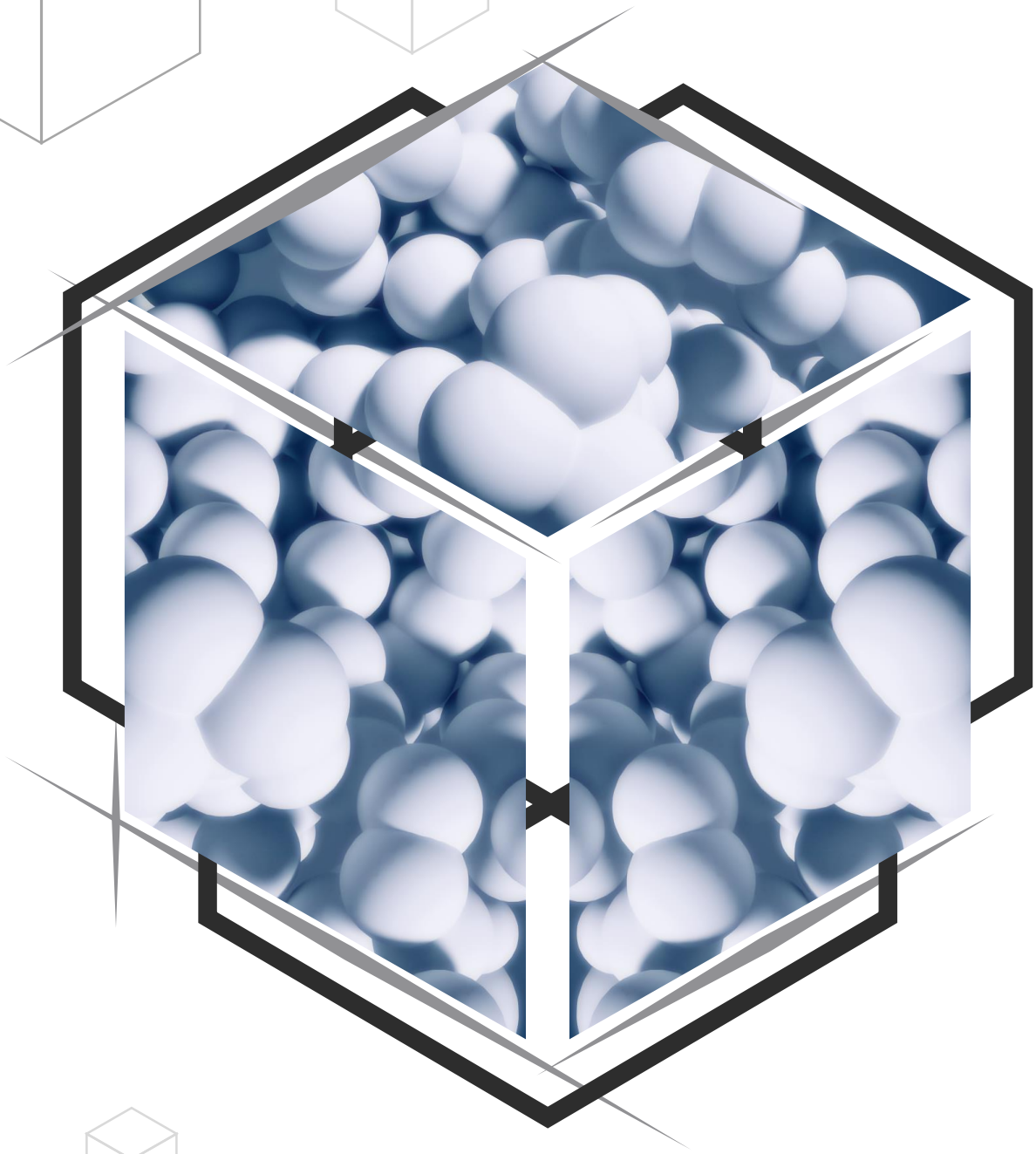
Creative Proteomics



Apply Proteomic Approaches to Biomarker Discovery

Why is it important to discover biomarkers?

Biomarkers are biological substances that can be measured and quantified as indicators for objective assessment of physiological processes, therapeutic outcomes, environmental exposure, or pharmacologic responses to a certain drug. Biomarker discovery has many excellent outcomes:

- Provides specific information about the presence of a certain disease or disease stage.
- Allows researchers to evaluate the safety or efficacy of a particular drug in development.
- Allows clinicians to predict or monitor drug efficacy.
- Confirms a drug's pharmacological or biological mechanism of action and helps minimize the safety risks.

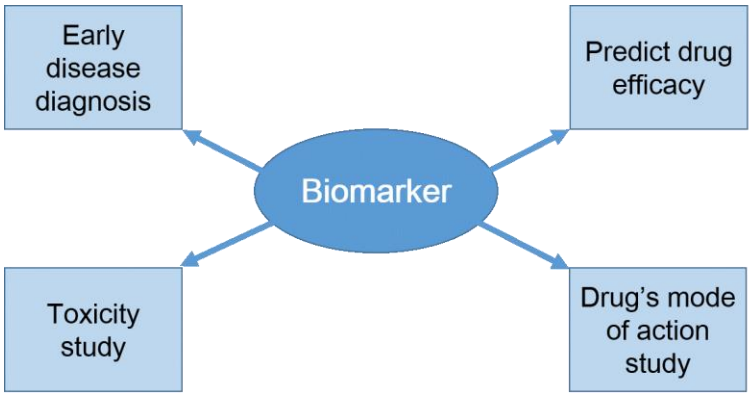


Figure 1. Potential use of biomarkers.

Proteomics in biomarker discovery

Typical molecular biomarkers include proteins, genetic mutations, aberrant methylation patterns, abnormal transcripts, miRNAs, and other biological molecules. Protein biomarkers are considered to be reliable indicators of the disease state and clinical outcome as they are the endpoint of biological processes. Remarkable innovations in proteomic technologies in the last years have greatly accelerated the process of biomarker discovery.

Proteomes and proteomics

Proteomes represent the network of interactions between genetic background and environmental factors. They may be considered as molecular signatures of disease, involving small circulating proteins or peptide chains from degraded molecules in various disease states. Proteomics is the study of the proteome including protein identification and quantification, and detection of protein-protein or protein-nucleic acid interactions, and post-translational modifications. Coupled with powerful computational methods, proteomics enables scientists to screen a number of proteins within clinically distinct samples that help discover, validate, identify, and quantify disease biomarkers.

Proteomics approaches for biomarker discovery

The process of biomarker discovery to clinical implementation consists of three consecutive phases, from biomarker discovery to verification and validation. The proteomic technologies exert a powerful influence in nearly every phase, especially in the initial discovery phase (Figure 2) through the identification of alterations in post-translational modifications (PTMs), total expression levels, and cellular trafficking.

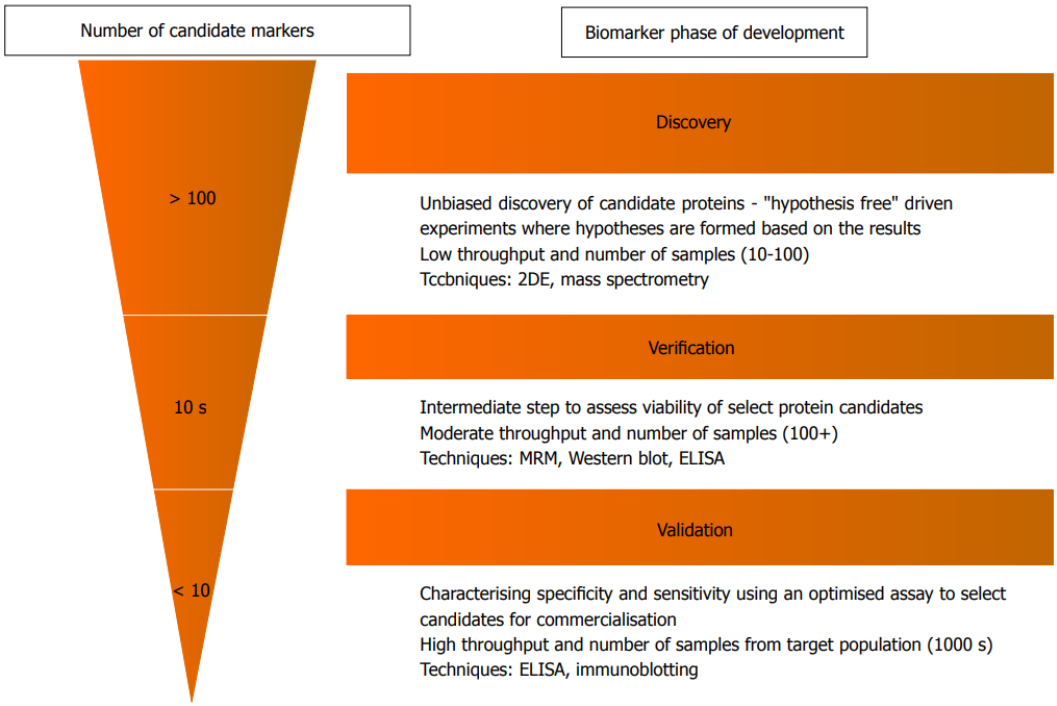


Figure 2. Pipeline for the discovery and validation of biomarker candidates (Chan *et al.* 2016).

There are three common proteomics study designs for biomarker discovery with dominant differences in the proteome coverage, sensitivity, and throughput (Figure 3):

- **Discovery (shotgun) proteomics** approaches have high coverage of proteome (up to 10,000 proteins in a single sample), but at the expense of throughput. High-resolution mass spectrometry (Orbitrap or time-of-flight instruments) is the representative technology platform of shotgun proteomics experiments.
- **Targeted discovery** approaches focus on a panel of high-potential proteins in sufficient numbers of samples. The coverage depends on the size of the panel or array used. The available technology platforms include antibody-based protein arrays, aptamer-based protein array, data-independent acquisition mass spectrometry, *etc*.
- **Targeted proteomics** is the most sensitive approach that allows the detection of low-abundance markers such as interleukin (IL)-6, but is limited in coverage and throughput. The available technology platforms include ELISA panels, multiple-reaction monitoring mass spectrometry (triple-quadruple instruments), *etc*.

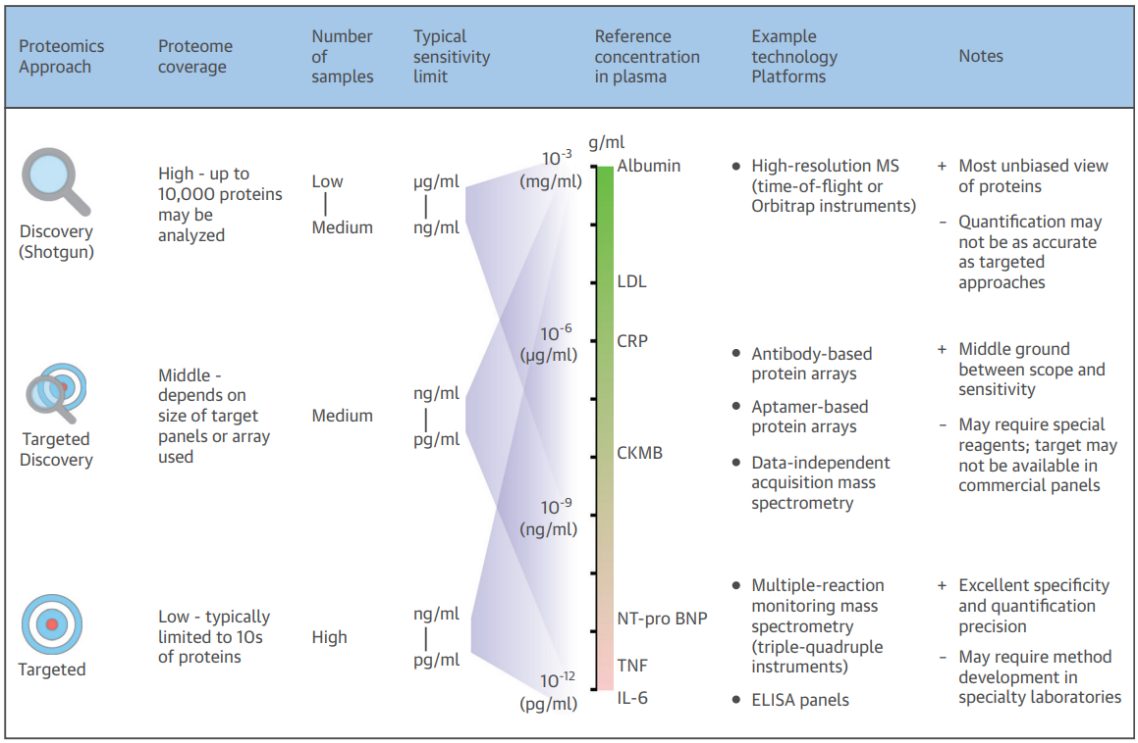


Figure 3. Sensitivity and Coverage of Various Proteomics Study Designs (Maggie *et al.* 2016).

The process of proteome profiling can be briefly described as below:

1. **Sample collection**. Samples (from serum, plasma, urine, cerebrospinal fluid, etc.) are selected from both healthy and diseased groups to address the clinical question.
2. **Protein separation**. Proteins are separated from the raw bio-fluid sample and are then purified.
3. **Mass spectrometry (MS), protein arrays, or ELISA panels**. This step is to identify, quantify, and characterize (targeted) proteins for downstream analysis.
4. **Bioinformatics analysis**. Data are analyzed using computational methods to identify potential differentially expressed proteins, so as to discover and characterize biomarkers.
5. **Evaluation and validation of biomarkers**. Suitable assays are developed to assess the sensitivity and specificity of biomarkers, paving the way to clinical use.

Our comprehensive proteomics service

At Creative Proteomics, we offer a comprehensive range of proteomics services from sample preparation, protein separation, to protein characterization and bioinformatics analysis. In order to help you discover, detect, quantify, and characterize biomarkers in a broad array of samples, we offer both discovery proteomics and targeted proteomics (including parallel reaction monitoring (PRM) and selected/multiple reaction monitoring (SRM/MRM)) approaches.

Our technical platforms include state-of-the-art NMR, GC-MS, LC-MS, LC-MS/MS, HPLC-UV/FD, UHPLC instruments, *etc*. Our technicians will work closely with you from the experimental design to report delivery. Please feel free to contact us for questions or ordering.

References:

1. Jhanker Y M, Kadir M F, Khan R I, *et al*. Proteomics in drug discovery. *Journal of Applied Pharmaceutical Science*, 2012, 2(08): 01-12.
2. Yeakuty Marzan Jhanker, *et al*. Proteomics in Drug Discovery. *Journal of Applied Pharmaceutical Science* 02 (08); 2012: 01-12
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4. Lam M P Y, Ping P, Murphy E. Proteomics research in cardiovascular medicine and biomarker discovery. *Journal of the American College of Cardiology*, 2016, 68(25): 2819-2830.