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Proteomics in Drug Discovery

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ABSTRACT

Drug discovery is a lengthy and highly expensive process that uses a variety of tools from diverse fields. To facilitate the process, several biotechnologies, including genomics, proteomics, cellular and organismic methodologies have been developed. The present review aims to provide a basic understanding of proteomics research by discussing the methods used to study large numbers of proteins and by reviewing the application of proteomics methods to identify biomarkers, to identify drug target and to conduct drug's mode of action and toxicology studies. It is expected that this will lead to important new insights into disease mechanisms and improved drug discovery strategies to produce novel therapeutics.

Keywords: Proteomics, Drug Discovery, Biomarker, Target Identification, Lead identification, Pharmacoproteomics.

INTRODUCTION

'Proteomics' is the study of the proteome and involves the technology used to identify and quantify the various proteins, protein-protein and protein-nucleic acid interactions within the proteome, as well as the post-translational modifications that affect protein activity (Hewick *et al.*, 2003). Proteomic technologies with computational methods have been advanced recently over many other complementary techniques. This enables scientists to screen large numbers of proteins within clinically distinct samples that helps to discover disease biomarkers, identify and validate drug targets, design more effective drugs, assessment of drug efficacy and patient response, i.e., to interfere with almost every steps in modern drug discovery process (Ahn *et al.*, 2008). Proteomic approach of drug discovery includes finding an unstable protein that is causing an undesirable affect and then usage of a molecule to modify its effect (Veenstra, 2006). Proteomics combines aspects of biology, chemistry, engineering and information science and apply them to all areas of drug discovery. Introduction of safer, more effective and more cost-effective drugs will be the ultimate outcome of improvement of this technology (Burbaum *et al.*, 2002).

The development of proteomics will require the simultaneous advancement of a number of techniques, because the challenges that face proteomics technologies are far reaching.

Both two-dimensional gel electrophoresis and Mass Spectrometry play a major role in proteomics; however, they are not the only technologies available and necessary. Some commonly used technologies are shown in the *Figure 1*:

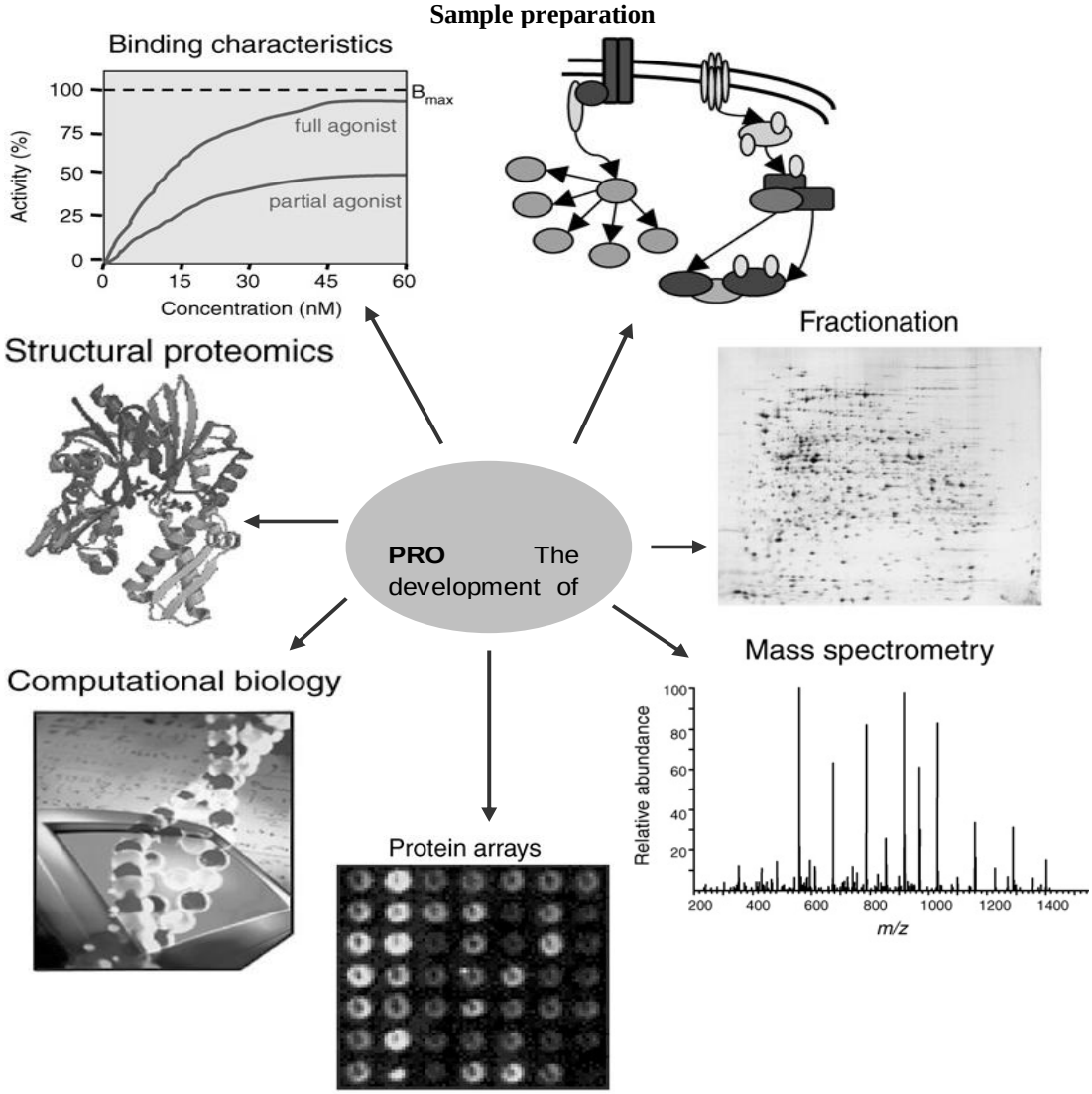

Fig. 1: A partial view of various proteomic technologies important in drug discovery. (redrawn from veenstra, 2006)

USE OF PROTEOMICS TO IDENTIFY BIOMARKERS

Goal of biomarker discovery

Biomarkers are biological parameters that can be measured and quantified as indicators for normal health and physiology-related assessments, such as pathogenic processes, environmental exposure, or pharmacologic responses to a drug (Seibert *et al.*, 2005).

Proteomes represent the net result of interactions between genetic background and environmental factors and may be considered as the signature of a disease, involving small circulating proteins or peptides from degraded molecules in various disease state (Meuwis *et al.*, 2007). Proteomics can identify alterations in post-translational modifications, cellular trafficking, and even total expression levels that may not be detected by RNA-based expression studies (Ornstein *et al.*, 2006).

Among many excellent outcome of biomarker discovery, some are listed below and simplified in Figure 2:

It can provide specific information about presence of disease and/or disease stage that enable early disease diagnosis (Bonney *et al.*, 2008; Choe *et al.*, 2006; Ornstein *et al.*, 2006; Rifai *et al.*, 2006).

Proteome analysis during preclinical or clinical development may allow the discovery of candidate markers for the prediction of drugs efficacy (Kelloff *et al.*, 2005).

Chemical alterations of well-defined biological systems or disease models can provide information on up- or down-regulation of mRNA or proteins (Blackwell, *et al.*, 2001; Clemons, *et al.*, 2001). Safer pre-clinical & clinical trials and precise clinical evaluation of treatment regimens can be achieved via consistent, validated toxicity biomarker study (Collings *et al.*, 2008).

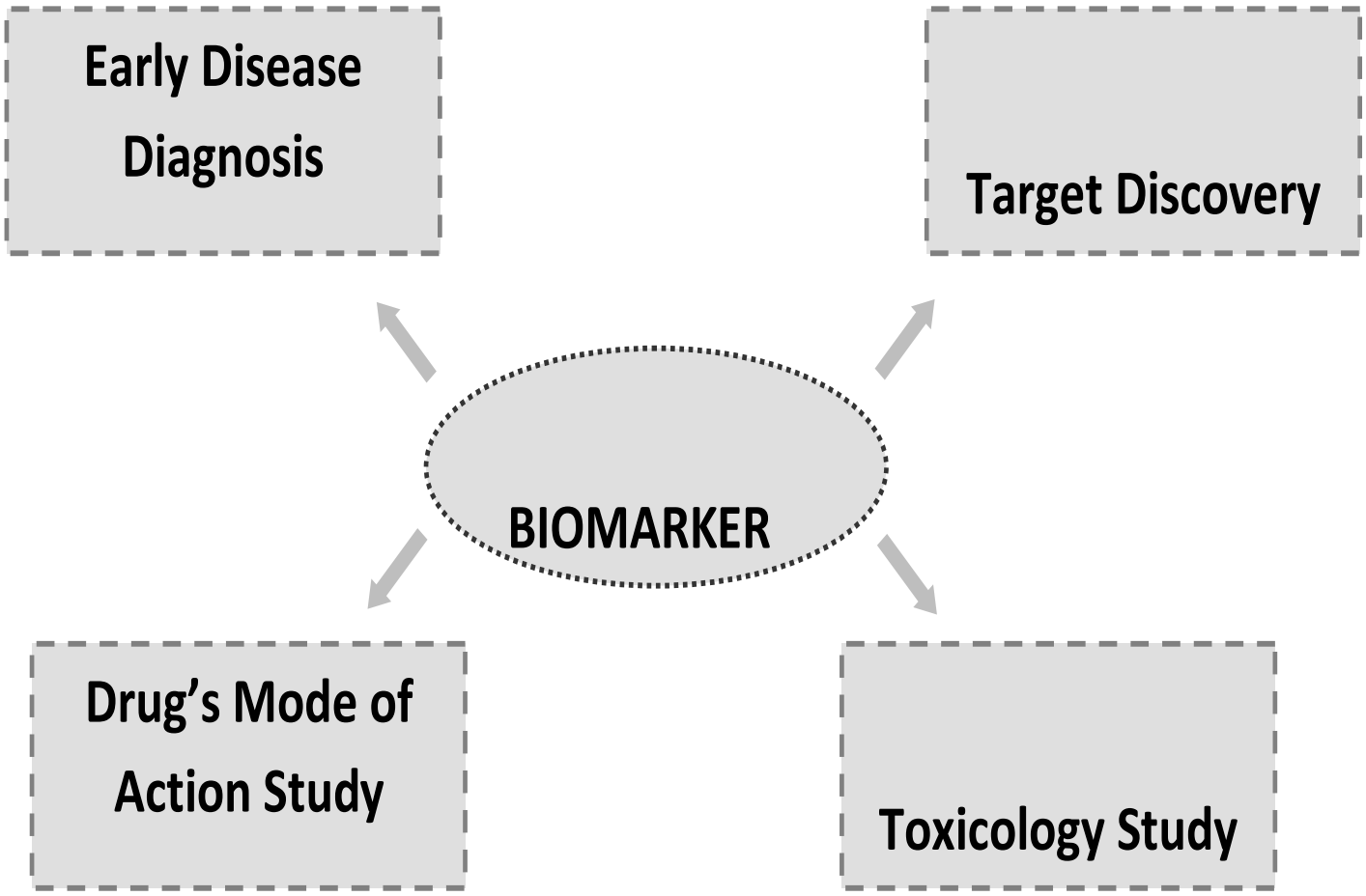

Fig. 2: potential use of biomarker.

Chung *et al.*, 2007 analyzed various organ specific tumour and normal tissues to search for differentially expressed proteins (biomarkers), as well as protein modifications such as phosphorylation of signalling receptors. Correlations between disease and gene aberrations or transcription levels of proteins help to understand the latent structures of pathophysiology (Gottfries *et al.*, 1995; Gottfries *et al.*, 2001) and highlight possible targets or metabolic pathways of interest (Masumarra *et al*., 2001) and trigger search for potential new treatments (Bonney *et al.*, 2008). Biomarker discovery is currently an exciting topic for the research scientists. The number of publications based on biomarker discovery is soaring day by day. Recently, a few promising biomarkers like annexin, PF4, Hpa2, FIBA, IKK-beta and MRP8 have been identified by various scientists (Chen *et al.*, 2004; Meuwis *et al.*, 2007; Ornstein *et al.*, 2006). Recent publications show a number of other biomarkers that are listed in the *Table 1*.

Table. 1: List of biomarkers identified for diagnosis of several diseases (Chen *et al.*, 2004; Meuwis *et al*., 2007; Ornstein *et al.*, 2006; Sinha *et al.*, 2007).

Disease	Clinical Biomarker
Alzheimer's Disease	Sulfatide, amyloid precursor, glycerophosphocholine and Tau proteins in CSF; Cystatin C and peptic fragment of the neurosecretory protein VGF Protein kinase C in red blood cells
Multiple Sclerosis	CSF cystatin C and matrix metalloproteinases in serum
Traumatic Brain Injury	C-tau, hyperphosphorylated axonal neuro-filment protein and serum S100B
Breast Cancers	HER-2/neu oncoprotein and tumor-specific glycoproteins
Gefitinib Resistance	Hypoxia-inducible factor-1 in head and neck cancer, epithelial membrane protein-1
Advanced Breast Cancer	Cdk6 and serum CA 15-3 for prognosis
Metastasic Breast Cancer	Protein kinase C
Gliomas	Receptor protein tyrosine phosphatase-B
Stroke	Lipoprotein associated phospholipase-A2, intracellular adhesion molecule 1, PARK7 and nucleoside diphosphate kinase-A
Ischemic Heart Disease	Troponin, natriuretic peptide, creatine kinase, myoglobin and fatty acid binding protein
Congestive Heart Failure	G protein-coupled receptor kinase-2
Artherosclerotic Heart Disease	Adipocyte-enhancer binding protein, lipid-modified proteins and lipid-phospholipase-A2
Prostate Cancer	Annexin
Inflammatory Bowl Disease	PF4, Hpa2, FIBA, MRP8
Tumour Hypoxia	IKK-beta

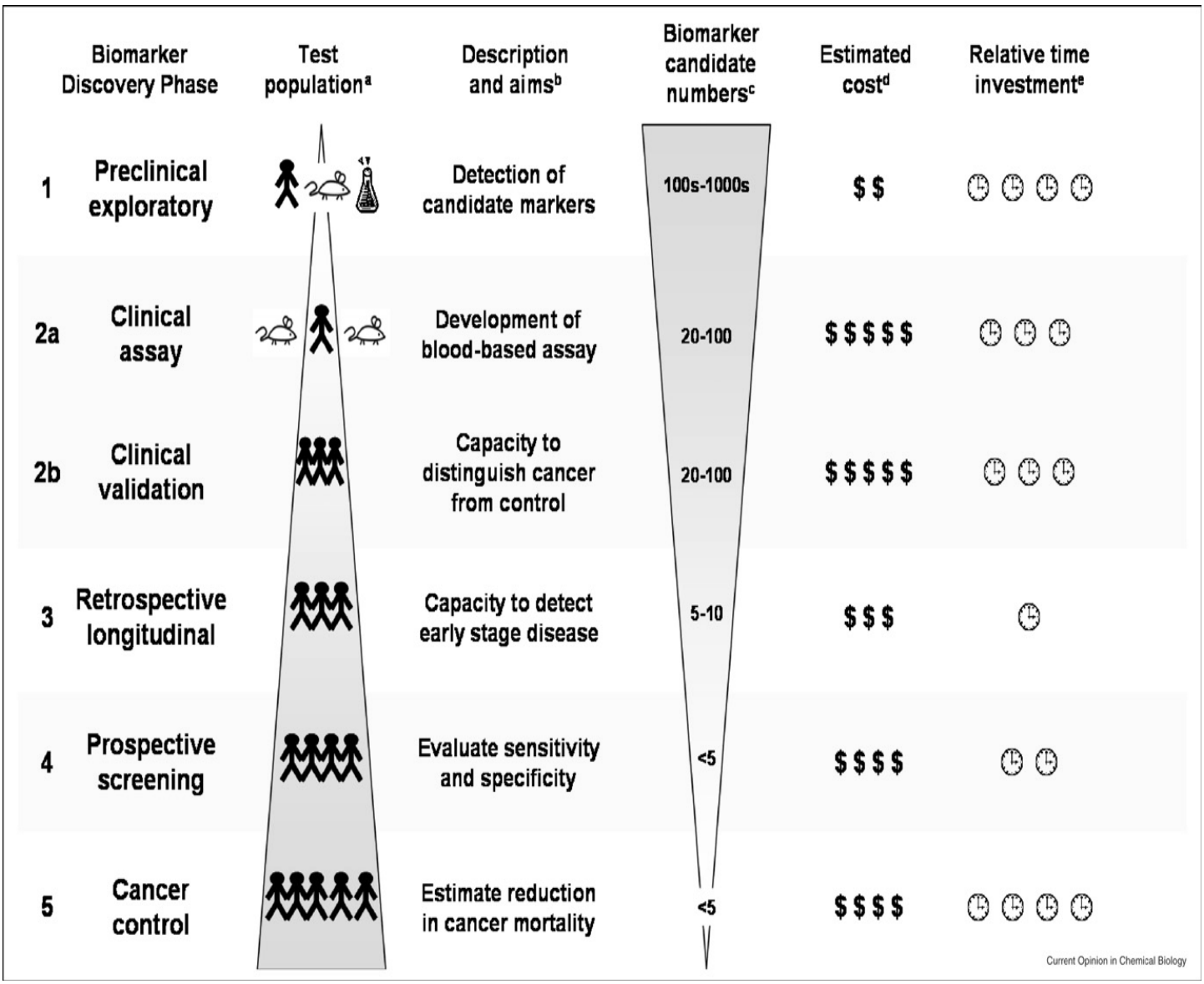

Fig. 3: pipeline for discovery and validation of biomarker candidates (Rifai *et al.,* 2006).

Process of biomarker discovery

Biomarker discovery process usually has five consecutive phases, from biomarker discovery to verification, assay optimization, validation, and finally to take discovery to the clinic. Every step has a selective goal that can be exploited in drug discovery (Rifai *et al.*, 2006). The following figure (*Figure 3*) illustrates the phases well:

There are several sources of biomarker, although blood is the most-used biomarker discovery matrix to date (Collings *et al.*, 2008; Omenn *et al.*, 2006). Other bio-specimens are also in use to overcome the shortcomings of profiling proteins in blood. These alternative specimens include:

- Tumor biopsy tissue (Sitek *et al.*, 2005),
- Cancer cell lines such as cathepsin D for prostate (Sardana *et al.*, 2007) and colon cancer (Volmer *et al.*, 2005),
- Soluble-secreted proteins and shed membrane proteins from tumour cells (Ahn *et al.*, 2007),
- Saliva for cancer, auto immune disease (Seibert *et al.*, 2005),
- Bile for biliary malignancy (Bonney *et al.*, 2008),
- Ventricular CSF for Neurologic disease (Choe *et al.*, 2006),
- Urine (Collings *et al.*, 2008; Kelloff *et al.*, 2005).

Proteome profiling is a method of biomarker discovery that generated great interest among the scientists (Bonney *et al.*, 2008; Seibert *et al.*, 2005) in the recent years (*Figure 4*). Analyzing the proteome content of blood or several other body fluids over the course of disease progression could reveal potential biomarkers indicative of specific disease status that may be used extensively in future medical diagnostics.

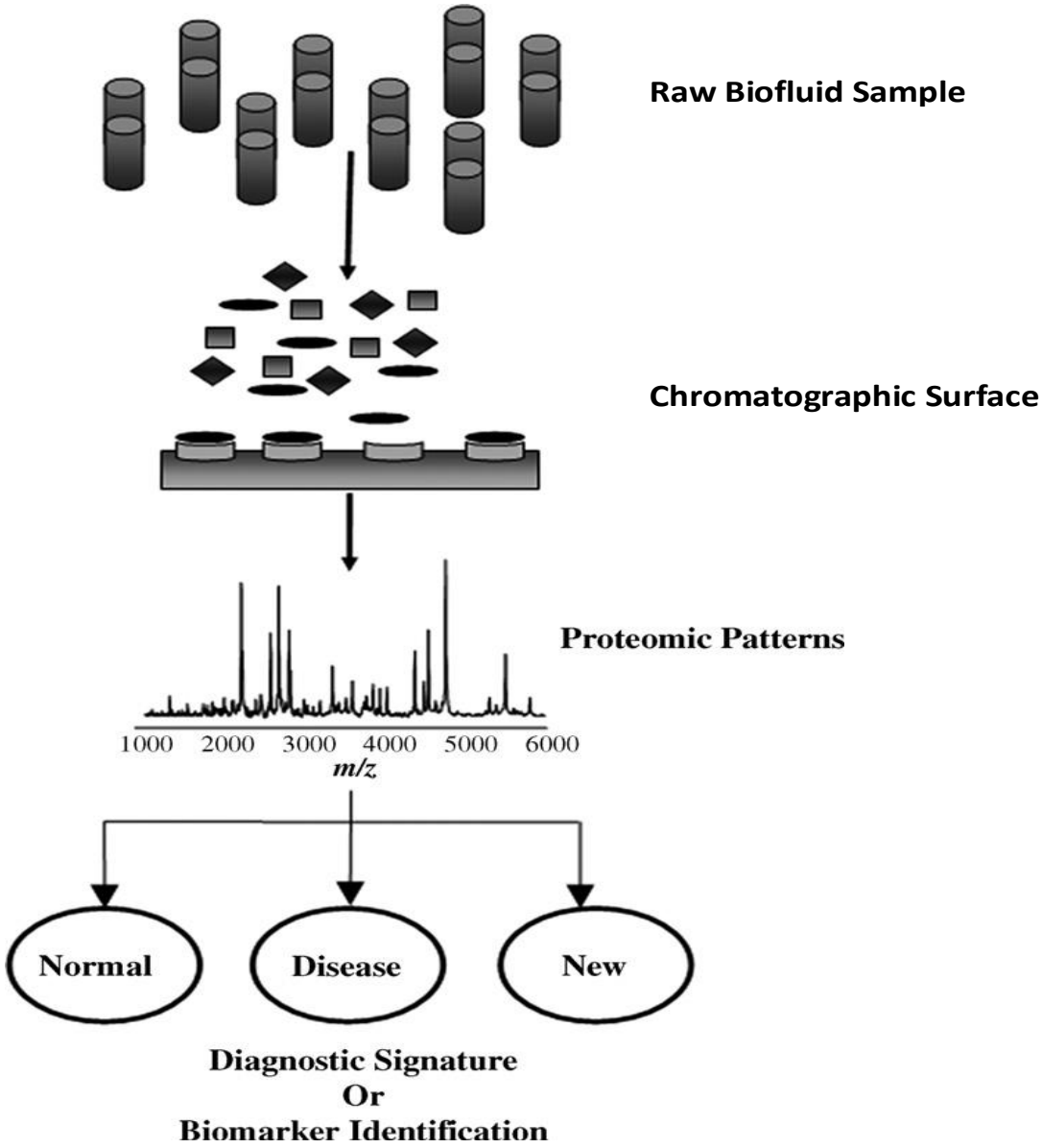

Fig. 4: proteome profiling using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF/MS)(Veenstra, 2007).

The process of proteome profiling can be briefly described as below (Bonney *et al.*, 2008; Veenstra, 2007):

1. Samples are selected to address the clinical question.
2. Proteins separated from raw bio-fluid sample by applying to a chip made up of a specific chromatographic surface. Proteins are allowed to bind to the surface, which is then washed to remove non-binding species.
3. The mass spectra of the several hundreds proteins bound to the chip spot are then recorded using a simple time-of-flight mass spectrometer from disease-affected patients and healthy controls.
4. Data are analysed bioinformatically to identify potential differentially expressed proteins or biomarkers compared to the healthy controls and classify the samples as coming from diseased, healthy or from an unknown condition.
5. Suitable assays are developed, results are validated.

This method of proteome profiling is a high-throughput process and has a great advantage of capability of analyzing and comparing hundreds of bio-fluid samples in a matter of days. Many individual studies showed great results in the ability to correctly classify the sources of bio-fluid samples from either healthy or diseased individuals (Veenstra, 2007).

IDENTIFICATION AND ASSIGNMENT OF CANDIDATE TARGET

Drug targets are proteins or signal transduction pathways in which proteins are involved.

Therapeutic relevancy of the chosen target must be proven first prior to initiating any other processes in drug discovery (Michael *et al.*, 2004). Some diseases and potential targets are listed below *(Table 2)*.

Table. 2: Some disease conditions with corresponding targets. (Katayama *et al.*, 2007; Kopec *et al.*, 2005).

Disease	***Potential Target***
Cancer	*Maleate dehydrogenase (Primary target)*
Tumour vascularization	*Tyrosin kinase receptor (PDGFR, VEGFR2, FGFR1), Arora kinases and TANK-binding kinase-1*
Malaria	*Aldehyde dehydrogenase-1 and quinine reductase-2*
Inflammation	*RICK (Rip-like interacting kinase), CLARP (caspase-like apoptosis-regulatory protein kinase), GAK (cyclin-G associated kinase) and CK1α*

Recombinant Protein Microarray and Computational Drug Design are the two unique techniques serving the purpose of identifying drug targets, target validation and 3D structure elucidation upon which a new drug molecule is being searched against the chosen target that usually involves high-throughput screening, wherein large libraries of chemicals are tested to determine their ability to modify the target.

Recombinant Protein Microarray

Recombinant protein arrays consist of purified active recombinant proteins. They enable the investigation of multiple proteins simultaneously (Schofield *et al.*, 2004). These arrays help to find out interactions formed between the protein and other molecules, including proteins, DNA, RNA and ligands (*Figure 5*), thus offering a unique assay system for studying protein function.

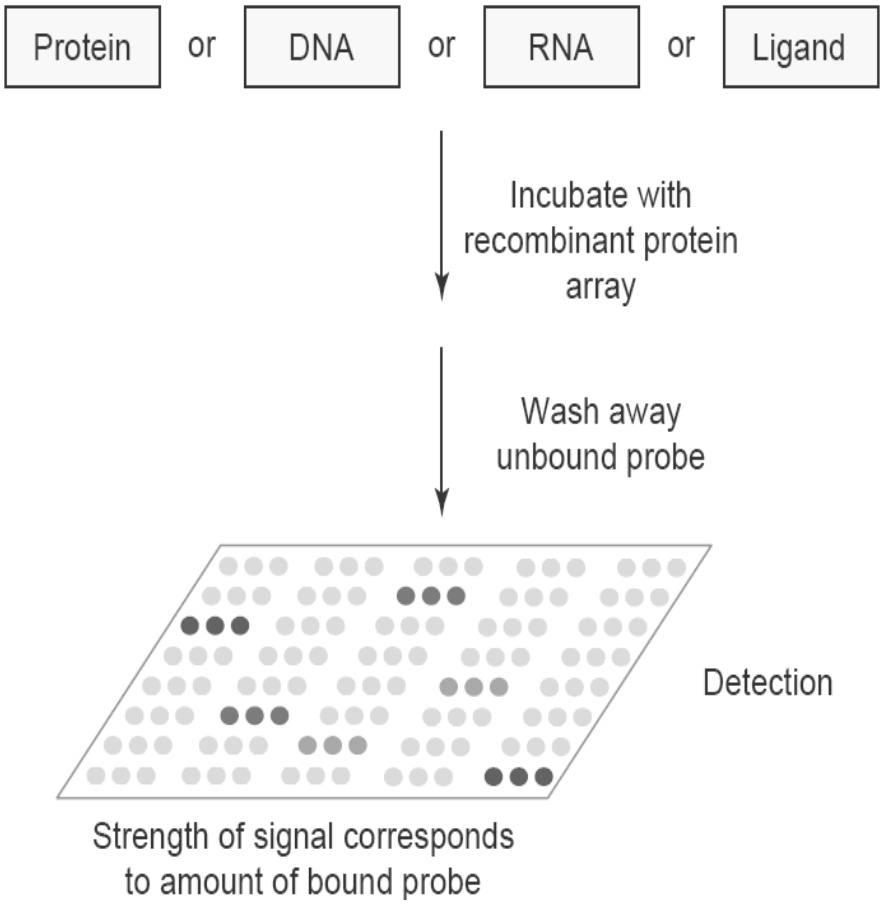

Fig. 5: the principle of recombinant protein array (sch ofield *et al., 2004*)

The recombinant protein array provides (Schofield *et al.*, 2004):

- Prospective uses in the area of drug target identification and validation.
- A high throughput screening platform to identify and subsequently to validate, protein targeted molecules as potential drug candidates.
- Screening of existing drug candidates against recombinant protein arrays to measure the specificity of the drug molecules.
- Elimination of pessimistic candidates from further development that can cause harmful side-effects through interaction with non-target molecules.

Computational Drug Design

Drug discovery and development are very time and resource consuming processes (Recanatini *et al.*, 2004). To minimize the cost and time, computational techniques are being used to significantly minimize time and resource requirements of chemical synthesis and biological testing (*Figure 6*).

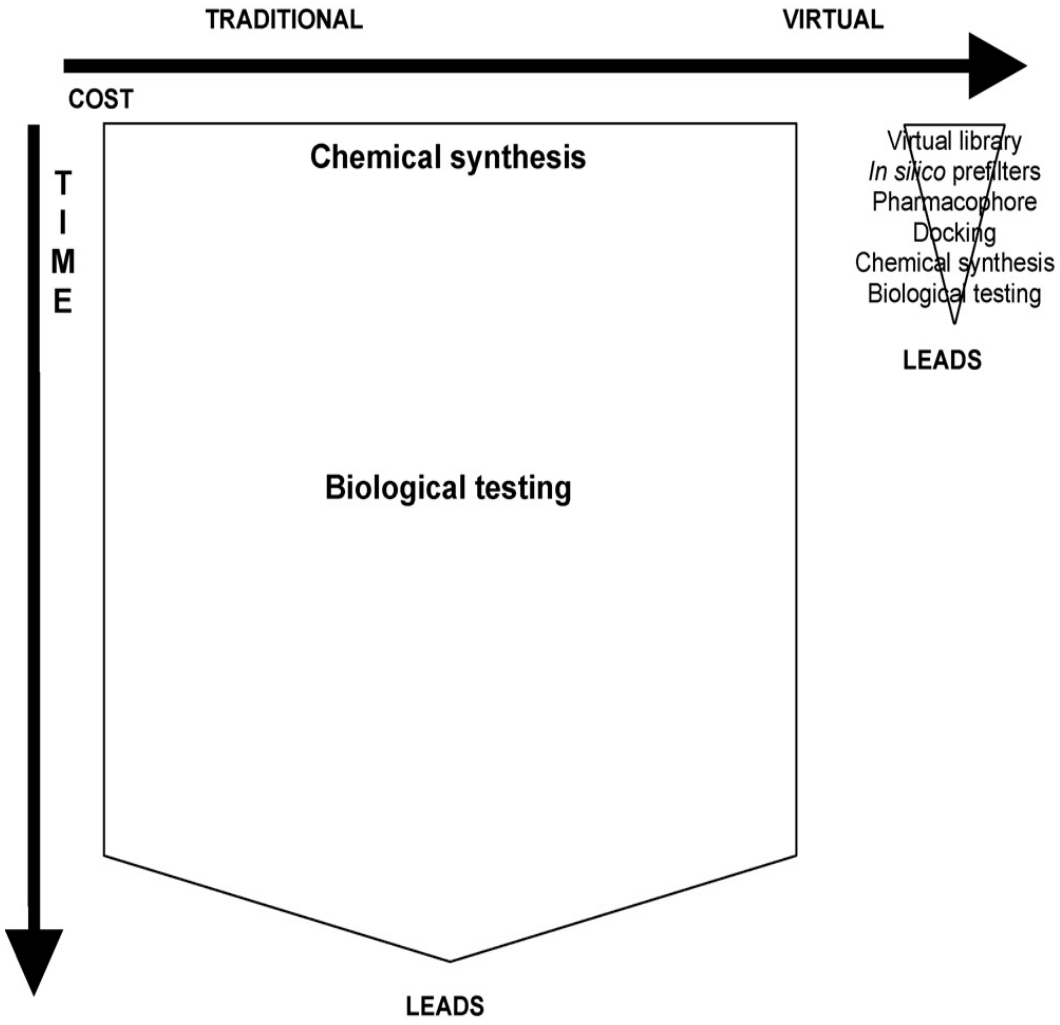

Fig. 6: comparison of traditional and virtual screening in terms of expected cost and time requirements (Kapetanovic, 2008).

Proteomic technique with the help of computer software can figure out the target for the disease which will ultimately help to discover the right drug. All the computer modelling processes involve few general objects (Kapetanovic, 2008):

- Use of computing power to simplify drug discovery and○ development process.
- Utilization of chemical and biological information about ligands and/or targets to identify and optimize new drugs.
- Design of in silico filters to eliminate compounds with○ undesirable properties (poor activity and/or poor absorption, distribution, metabolism, excretion and toxicity, ADMET) and select the most promising candidates.

Rapid development of this area has been made possible by recent improvement in computer software and acceleration and sophistication in computational power (better hardware). The publicly available target protein structures (database) are increasing rapidly day-by-day that helps the identification of molecular targets. (Kim *et al.*, 2008; Luzhkov *et al.*, 2007; Oh *et al.*, 2004; The Worldwide Protein Data Bank (wwPDB); Wang *et al.*, 2008).Some interesting examples of reported successful application of computer aided drug designing are as follows:

Kim *et al.*, 2008 have discovered 12 novel PRL-3 inhibitors by means of a computer-aided drug design protocol involving homology modelling of the target protein and the virtual screening with docking simulations.

Wang *et al.*, 2008 described the discovery of inhibitors, through virtual screening; those specifically act on SecA ATPase, which is a critical member of the Sec system. These are the very first inhibitors reported for intrinsic SecA ATPase.

Luzhkov *et al.*, 2007 reported high-throughput structure-based virtual screening of putative *Flavivirus* 2′-*O*-methyltransferase inhibitors together with results from subsequent bioassay tests of selected compounds.

Use of Proteomics In Drug's Mode-Of-Action And Toxicology Studies

Mode of action and toxicology study is important to obtain a new safe and effective drug molecule from the lead compound (Ornstein *et al.*, 2006). The process is pretty straight-forward and obviously time consuming. From target identification to the mode of action and toxicology studies can be well understood by the following workflow illustration (Figure 7):

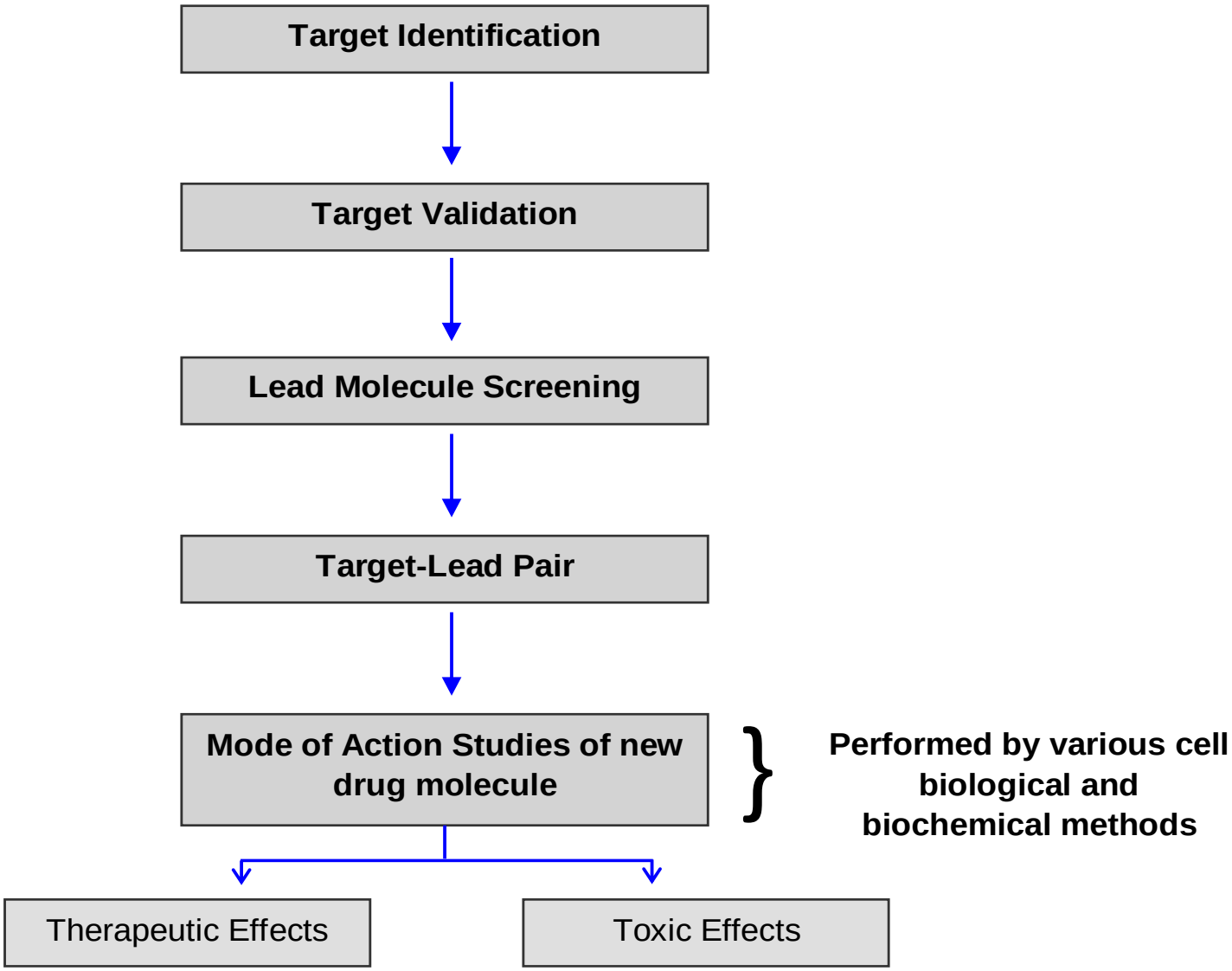

Fig. 7: Position of the mode of action study in the field of drug discovery (Williams, 2003).

In the past history of drug discovery, many chemical compounds with promising pharmacological activity had failed to see the face of light due to lack of *in vitro* and *in vivo* correlation in their overwhelming pharmacokinetic and toxicity profiles (Hu *et al.*, 2007). It is always desirable to launch a new molecule in the existing drug market as soon as possible because there is a direct monetary relationship between patent expiration and business potential of that new drug. The ability to achieve fast and accurate predictions of efficacy and toxicity within an *in vivo* setting would represent a big step forward in accelerating any drug discovery programme (FDA, 2004).

Various sets of criteria were developed to define the types and extent of mechanistic data required to determine the mode of action for a chemical or groups of chemicals that share a common mode of action, such as, physiological target, toxic intermediates, pharmacokinetics, detoxification pathways, dose addition etc. (US EPA, 2000; US EPA, 2005).

Proteins whose levels are modified in response to drug administration could provide vital clues with respect to drug effectiveness and toxicity. These proteins will serve as efficacy or toxicity biomarkers to guide clinical trial studies. Similarly, analyses of protein profiles before and after pharmacological treatments could also confirm the mechanism of drug action and provide insight for new drug discovery (Sung *et al.*, 2006). A biomarker consortium was launched in 2006 (Wagner *et al.*, 2007), in response to FDA (Food & Drug Administration, USA)'s Critical Path Initiative published in March 2004 reinforcing the requirement for additional biomarkers to predict drug toxicity in preclinical studies, specifically biomarkers that can act as surrogate endpoints and/or aid in making efficacious and cost-saving decisions or terminating drug development more quickly (Collings *et al.*, 2008; FDA, 2004). Lee *et al*., 2008 have been successfully implicated proteomics to understand the effects of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a toxic environmental pollutant and a potent liver carcinogen in various diseases. To understand the mechanisms of TCDD toxicities they analysed of the glycoproteins and phosphoproteins in Chang cells. Using 2-DE and MS, several candidate biomarkers that are potentially involved TCDD toxicities were identified. There are several other examples of toxicity biomarkers that are given in *Table 3*.

Table . 3: Biomarkers for the diagnosis of the effect of chemicals (Sinha *et al.*, 2007).

Biomarkers	Toxicants
Glial fibrillary acid protein	Neurotoxins
Transthyretin, sarcolectin and haptoglobin	Automobile emission and waste incineration
Glutathione S-transferase, testis-specific heat shock protein 70-2, glyceraldehyde phosphate dehydrogenase, phosphotidylethanolamine-binding protein	Ethylene glycol monomethyl ether, cyclophosphamide, sulfasalazine and 2,5-hexanedione
Glucose and lipid metabolizing enzymes and oxidative stress related proteins	Hydrazine exposure
Fumarylacetoacetate hydrolase, a toxicity-associated plasma protein	Aminophenol (4-AP) and D-serine, rodent nephrotoxins
Pyruvate dehydrogenase, phenylalanine hydroxylase and 2-oxoisovalerate dehydrogenase, down regulation of sulfite oxidase, chaperone-like protein, glucose-regulated protein 78, serum paraoxonase, serum albumin, and peroxiredoxin IV	Steasis causing hepatotoxins
Urinary parvalbumin-alpha	Skeletal muscle toxicity

A number of new technologies have evolved with the evolution of proteomics and proved their ability to understand the mode of action of a drug to the target and its toxicity (like chemical proteomics, pharmacoproteomics etc.). They will be discussed here:

Chemical Proteomics

It has been mentioned previously in this section that mode of action studies for the new drug molecules are performed by various cell biological and biochemical methods. One of the new and widely used methods is chemical proteomics. This technique uses small, drug-like molecules either bound to a resin or exposed to protein chips. Proteins binding the ligand are then viewed as potential drug targets. In addition to providing insights into the selectivity and mechanism of action of a compound, chemical proteomics can also identify previously unknown protein targets for a compound that may aid in the identification of potential side effects (Kopec *et al.*, 2005).

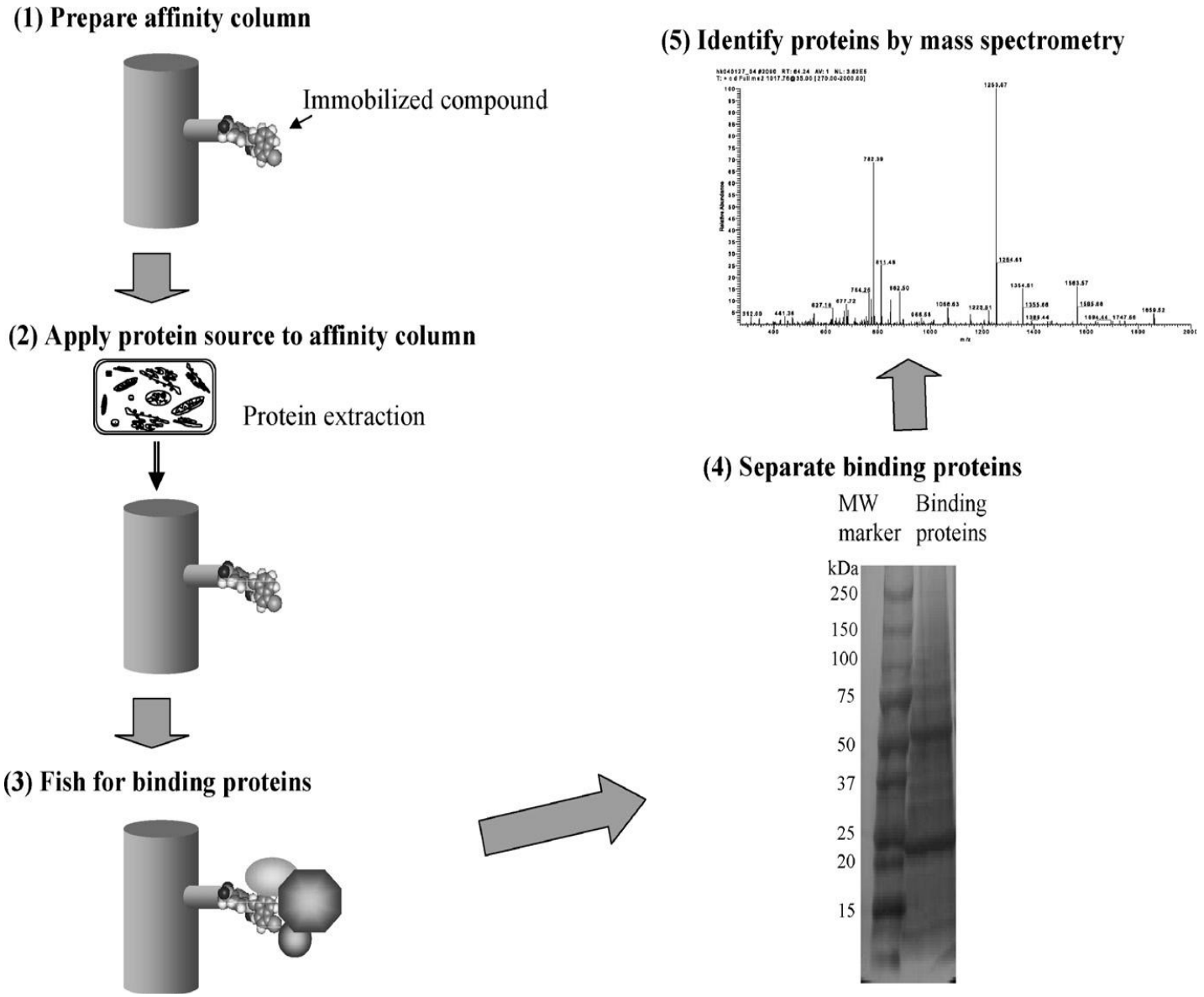

Fig. 8: Schematic illustration of chemical proteomics (Katayama *et al.*, 2007). To identify compound-binding proteins, the compound needs to be immobilized on a solid-support (1) before or after complex formation (2) Washing the support enriches binding proteins (3), which are then separated, e.g., by SDS-PAGE (4) Finally, binding proteins are identified by mass spectrometry (5).

Daub *et al., 2003* employed chemical proteomics to examine the selectivity of the mitogen-activated protein kinase, p38 inhibitor, which is widely used as pharmacological tool to examine the role of p38 in inflammation and other disease states.

Pharmacoproteomics

This new branch of proteomics has a potential role because proteins are the primary effectors of drug action and proteomic analysis represents a global approach to monitoring protein alterations in response to drug administration (Hu *et al.*, 2007). It can be used in drug industry almost in every case, including target identification and validation, discovery of efficacy and toxicity biomarkers and investigations into mechanisms of drug action or chemo-resistance. In pharmacoproteomics, high concentration of an experimental drug can be given to the test subject over time. Consecutive proteome analyses will be performed by collecting serum sample. Individual dose related markers can be analysed that correlate with the efficacy and severity of toxicity. The huge impact of the biomarkers can be summarised as follows (*Figure 9*):

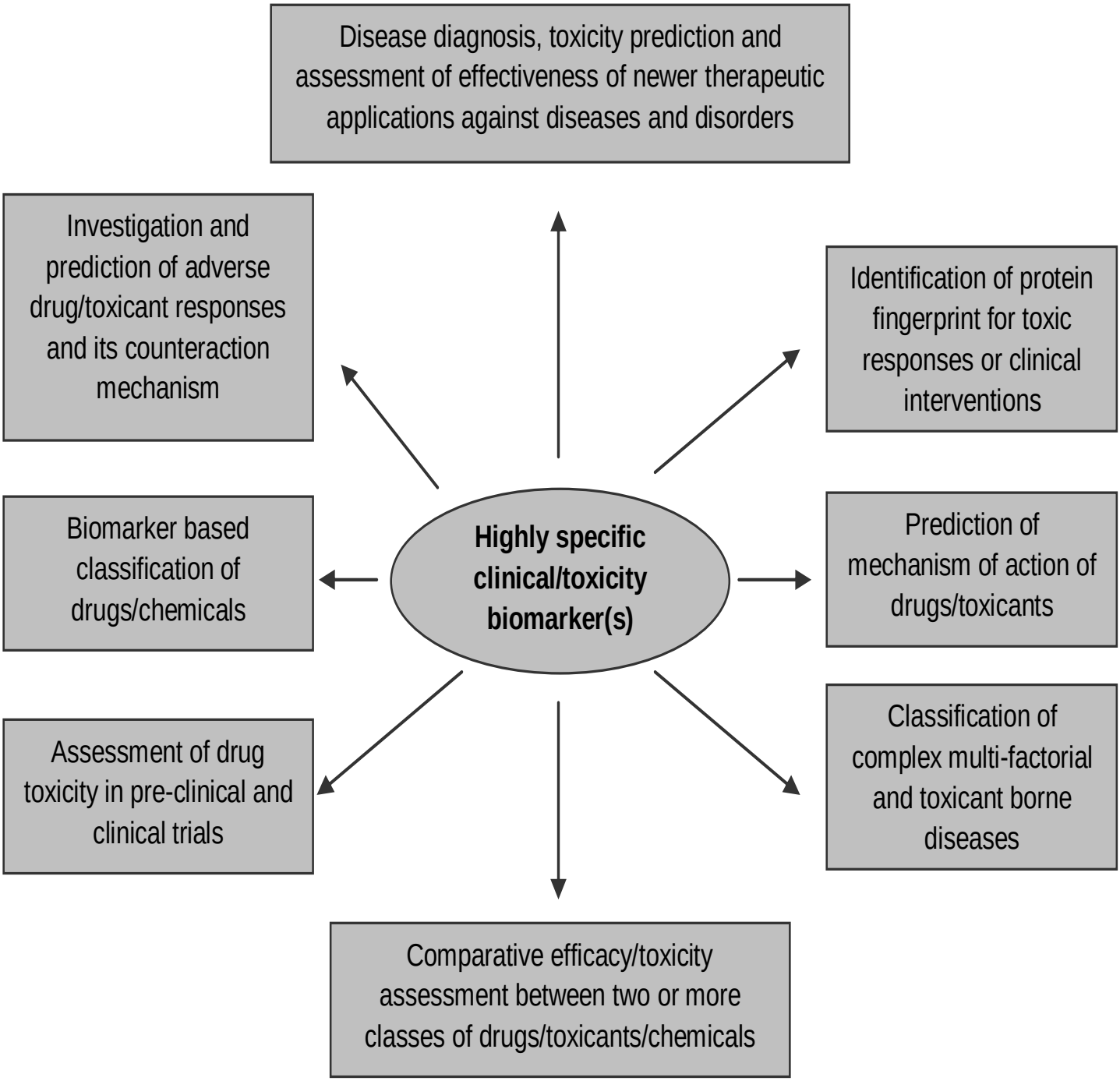

Fig. 9: Summary of role played by proteomics based biomarkers in toxicology and clinical interventions. (Redrawn from Sinha *et al.*, 2007)

Apart from these, this technique can classify patient subgroups and eventually lead to personalized therapy customizing therapeutic strategies for specific patients. Thus pharmacoproteomics potentially reduce the time and cost of clinical research, increase patient safety and reduce the risk associated with the development of new therapies (Hu *et al.*, 2007).

CONCLUSION

The drug discovery process is not a predefined series of steps. Modern approaches include target-based drug discovery in which researchers need to survey proteins like never before. The two most important needs for this type of technology are to find more effective biomarkers for disease detection and discover proteins to which therapeutic drugs can be targeted. It is well-known that the risks are high in drug discovery process and there are long timelines to be passed before it is known whether a candidate drug will succeed or fail. At each step of the drug discovery process there is often scope for flexibility in interpretation. Making accurate decisions within an accelerated process is the key to success to the pharmaceutical companies. Genomics revolution had a very positive impact upon these issues and now proteomics is in the field as a powerful new partner of genomics. The ability to analyse proteins from a very wide diversity of biological systems in a high-throughput way and in a systematic manner will add a significant new dimension to drug discovery. Each step of the process from target discovery to clinical trials is accessible to proteomics. Scientists are able to see every dimension of their biological focus, from genes, mRNA, proteins and their subcellular localization. This will greatly assist our understanding of the fundamental mechanistic basis of human disease and will allow discovery of improved, speedier, less toxic and hopefully, inexpensive drugs.

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