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Applications of Protein Arrays for Small Molecule Drug Discovery and Characterization

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Introduction

The profiling of large chemical libraries produced using combinatorial chemistry techniques in high throughput assays has been considered to offer great promise in generating both more 'druggable' targets – i.e. targets responsive to drugs – and lead compounds for treating disease. However, to date, this promise has not been realized. In many cases, high throughput screens were conducted with 'lipophilic' small molecule libraries that failed to produce leads that finally made it to the clinic. This is perhaps unsurprising, since the task of developing a small molecule drug to combat a specific disease is a highly complicated, expensive, and time-consuming process, associated with a very high rate of failure. As a consequence, more attention is paid to *in silico*-based approaches during the early phase of drug discovery for both decreasing the costs and increasing the likelihood of producing so-called 'blockbuster' drugs (van de Waterbeemd and Gifford, 2003). This review will summarize some of the challenges faced by the pharmaceutical industry, and will address how functional protein arrays can be used to address these challenges.

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Abbreviations: ADMET, adsorption, distribution, metabolism, excretion, and toxicology; ATM, ataxia telangiectasia; CML, chronic myeloid leukaemia; COX, cyclooxygenase; ELISA, enzyme-linked immunoabsorbant assays; FDA, Food and Drug Administration; FKBP, FK506 binding protein; FRET, fluorescence resonance energy transfer; GSK, GlaxoSmithKline; HSV, herpes simplex virus; ICAM-1, intracellular adhesion molecule; LFA-1, leukocyte function-associated antigen-1; PLK, polo-like kinase; SCID, severe combined immunodeficiency; SMIR, small molecule inhibitor of rapamycin; SPA, scintillation proximity assays; STK, serine/threonine kinase; TK, thymidine kinase.

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Protein targets in disease for small molecule therapeutics

The completion of the human genome project has led to the prediction that there are about 20–25 000 genes in humans for which greater than 50% of the proteins are of unknown function (International Human Genome Sequencing Consortium, 2004; Ota *et al.*, 2004). It has been estimated that somewhere between 3000 and 10 000 disease-modifying genes may exist, raising the question of how many of these gene products can be successfully targeted by therapeutics (Hopkins and Groom, 2002). Hopkins and Groom argue that the probability of developing a small molecule therapeutic against a protein target is likely to be much higher for proteins that are predicted to naturally interact with small molecules inside cells (*Table 10.1*). By comparing the overlap of proteins predicted to interact with small molecules with the list of disease-modifying genes, they argue that only 600–1500 proteins are likely to be small molecule druggable targets. This view is generally accepted by private industry, and screens for compounds are typically focused against defined protein classes in pharmaceutical companies.

Most of the drugs on the market are directed against single targets, and the majority of these therapeutic targets fall within 130 protein families (Hopkins and Groom, 2002). The two largest protein classes for which targeting by small molecules is most effective are the protein kinases and G-protein coupled receptors (*Table 10.1*). Both protein classes interact with small molecules. Protein kinases are enzymes that transfer the γ-phosphate of ATP to acceptor proteins. G-protein coupled receptors are membrane proteins which, through interactions with ligands on their extracellular domain, initiate intracellular signalling pathways important for cellular growth, differentiation, and proliferation.

Only a very small percentage of small molecules that have potential to be drugs – as exhibited by binding to selected targets in high throughput screens or by displaying some desirable phenotype in cell-based or animal model screens – actually make it to the clinic. This proves a very expensive endeavour for those molecules that make it through this lengthy process as, on average, it takes about 15 years and usually costs in excess of $800 million (diMasi *et al.*, 2003). Yet those molecules that receive FDA approval can become multi-billion dollar per year revenue generators, such as the anti-depressant drug, Prozac (Eli Lilly), and the erectile dysfunction drug, Viagra (Pfizer) (Booth and Zemmel, 2003). Therefore, pharmaceutical companies believe the high rewards are certainly worth the risk.

Successful orally administered drugs are usually compounds that have high grades for ADMET parameters (adsorption, distribution, metabolism, excretion, and toxicology) (Kubinyi, 2003). Small molecules that bind to unexpected or unknown targets (so called 'off-targets') are likely to be candidates with increased toxicity. Many drugs fail during clinical trials because of unanticipated toxic side effects that were neither revealed in toxicity screens in animal model systems nor predicted using *in silico* modelling techniques. Therefore, there is clearly a need for more empirically-based comprehensive screens to identify these molecules and their off-targets prior to entering clinical trials (Pritchard *et al.*, 2003). In addition to specificity, a successful orally administered drug must be properly absorbed into the body. Lipinski has proposed rules for compounds, and compliance with these guidelines is thought to be necessary for small molecules to be properly absorbed in the body

Table 10.1. The 'druggable' proteome

Protein families	Percentage of druggable proteome	Examples of marketed drugs
Serine-Threonine/ Tyrosine protein kinases	22	Gleevec (Novartis)
G-protein coupled receptors	15	Inderal (Wyeth-Ayerst), Losartan (Merck), Imitrex & Zantac (GlaxoSmithKline)
Ion Channels	5	Norvasc (Pfizer), Ambien (Sanofi-Synthelabo), Xanax (Pharmacia & Upjohn)
Protein phosphatases	4	in development
Serine proteases	4	in development
Short-chain dehydrogenases/ reductases	2	in development
Metallopeptidases	2	in development
Nuclear hormone receptors	2	in development
Cytochrome P450	2	Arimidex (AstraZeneca)
γ-carboxylases	2	in development

The 10 largest protein classes of the druggable proteome (modified from Hopkins and Groom, 2002). For each protein class, the number of proteins as a percentage of the total number (3051) of druggable targets. Drugs on the market directed against proteins within each class are listed, along with the manufacturer. 'In development' refers to small molecules that are currently not marketed.

(Lipinski *et al.*, 2001). The 'rule-of-five' analysis by Lipinski and co-workers suggests that small molecules are likely to have poor absorption if there are more than five hydrogen bond donors, molecular mass is greater than 500 Daltons, the lipophilicity is high (cLogP >5), and the sum of nitrogen and oxygen atoms is more than ten (Lipinski *et al.*, 2001). Currently, approximately 400 proteins are targets of 'rule-of-five' compliant small molecules (Hopkins and Groom, 2002). Unsurprisingly, many biotech and pharmaceutical companies devote an extensive amount of time and resources to enriching chemical libraries for this rule-of-five compliance and to improving drug leads so that they will be better absorbed by the body. Compounds can also be metabolized quickly to inactive compounds. Thus, identifying the enzymes responsible for drug modification is a necessary step if a drug is to last within the body and have good bioavailabilty for the intended targets. By identifying the enzyme(s) responsible for drug metabolism, structural studies can be employed to identify regions of the small molecule that cause it to be metabolized: this can facilitate the introduction of modifications that will either eliminate or lessen the extent of drug metabolism by the body (Stout *et al.*, 2004). Therefore, orally administered drugs must satisfy certain minimal criteria for absorption, specificity/ toxicity, and metabolism.

Mechanism of inhibition by small molecules

The development of small molecule therapeutics has been the mainstay of drug development in pharmaceutical companies, and is clearly the preferred approach. Small molecules can exert their effects through a variety of mechanisms. The most common mechanism is through competition with an endogenous molecule for a site on a protein or enzyme. The drug AZT, first shown by GlaxoSmithKline to be effective for suppressing the growth of the HIV virus, does so by competing with

nucleotide analogues for the active site of DNA polymerase. Utilization of AZT by DNA polymerase inhibits DNA replication, and thus growth of the virus. Alternatively, small molecules can bind directly to proteins and inactivate them. Gleevec, the blockbuster drug developed by Novartis to treat chronic myeloid leukaemia (CML), is a small molecule that targets the Abl protein kinase. In CML, a gene translocation event results in aberrant activity for Abl. Gleevec ameliorates CML by binding to Abl and keeping the protein in a catalytically inactive state (Noble *et al.*, 2004). For some drugs, modifications have been introduced such that after non-covalent interaction of the drug with its target, an appended cross-reactive moiety covalently attaches the small molecule to the target and locks the protein in an inactive conformation. In other instances, drug metabolism by cellular enzymes produces metabolites, which in fact are the mediators of inhibition of a cellular enzyme. Zovirax (acyclovir, GSK) is a classic example of this class of inhibitor. This is used to treat herpes simplex virus (HSV) infected cells and turns out to be selectively modified by a thymidine kinase (TK) encoded by HSV. The modified drug is then further processed by the cellular machinery, and the resulting metabolite inhibits growth of the cell by inhibiting DNA replication.

Although more difficult to develop, small molecules have also been made that specifically interfere with protein–protein interactions. The interaction of the intracellular adhesion molecule (ICAM-1) with the leukocyte function-associated antigen-1 (LFA-1) regulates signalling pathways important for T cell activation. Small molecule inhibitors, in addition to peptide drugs, have been developed to interfere with this interaction. Such drugs have been useful for regulating autoimmune diseases (Anderson and Siahaan, 2003).

Lead identification and characterization

There are two general approaches to the production of lead drugs in the pharmaceutical industry. The first approach assumes that little or nothing is known about the intended target(s). For this reason, screens are performed in cell-based assays and/or animal models with very large chemical libraries, with the purpose of identifying those small molecules that produce a desirable effect. The results of these screens usually generate a list of molecules that vary considerably for drug potential. The decision as to which molecules to pursue further can be facilitated by the identification of the drug target(s). A common method for identifying targets for a small molecule is affinity purification. This approach usually relies on coupling the molecule either to a resin, or to tags (e.g. Biotin), which allows the subsequent identification of proteins from extracts that bind to the small molecule. This process, although sometimes successful, has several limitations. The likelihood of identifying targets is predicted to correlate with the amount of protein target in the extract. If a sufficient amount of target is not present, the process of identifying proteins, usually by mass spectrometry or protein sequencing, can be extremely difficult. In addition, if the affinity of the molecule for the target is weak, the interaction is not likely to be maintained during the assay and is unlikely to be detected. These issues have been partly addressed by designing inhibitors that contain both affinity purification tags and also covalently modify their protein targets.

A second approach is based on the assumption that some information is available

about the intended targets, and small molecule screens are thus conducted with defined protein classes. These assays can be divided into two types: homogeneous and separation-based assays (Walters and Namchuk, 2003). Fluorescence polarization, fluorescence resonance energy transfer (FRET), and scintillation proximity assays (SPA) are some examples of homogeneous assays used to identify and characterize small molecule–protein interactions. Typical separation-based assays are filter binding assays and enzyme-linked immunoabsorbant assays (ELISA). In most cases, these assays are typically performed in multi-well (96 384 or 1536) plates with the assistance of robotics, and have been used to study protein kinases, phosphatases, proteases, polymerases, and receptor–ligand interactions. The sensitivity and speed of these assays makes these approaches very attractive. The major downsides to these approaches are related to cost – these screens require considerable amounts of materials and comprehensiveness – screens are typically not conducted with all of the proteins for a particular class.

Functional protein microarrays

Surface plasmon resonance technology has been a technique used for many years to characterize interactions of proteins with molecules. This technology has been marketed by Biacore, and has been considered the gold standard in the protein microarray industry. The primary benefit of this technology is that kinetic measurements can be performed in real time to characterize on and off rates. In addition, the detection of molecular interactions does not require the labelling of molecules with fluorescent dyes or other tags commonly used in other high throughput screening assays. Therefore, lead compounds obtained from high throughput screening assays can be further characterized quickly. But, the technology is limited by an ability to address only a few molecular interactions at any one time and thus generally has not been a useful screening technology. This problem has been partly addressed by recent advances in instrumentation that now can process up to 400 molecular interactions in parallel (Baggio *et al.*, 2005). But, given that the number of proteins for most microorganisms greatly exceeds 400, high-density protein arrays containing thousands of proteins will provide invaluable tools for the identification and characterization of targets of small molecules.

Within the past five years, the use of protein arrays for research and development and drug discovery has been well documented (Predki, 2004). Protein arrays can be classified into either capture arrays, which typically contain antibodies printed on a glass slide, or functional protein arrays (Zhu and Snyder, 2001). Antibody capture arrays have been used mainly to profile the levels of protein in various samples in diagnostic assays and in biomarker discovery (Knezevic *et al.*, 2001). Functional protein arrays, in contrast to capture arrays, can be used for a variety of applications. Molecular interactions of proteins with DNA, RNA, lipid, sugars, peptides, proteins, antibodies, and small molecules (*Figure 10.1*) have all been demonstrated on protein arrays (MacBeath and Schreiber, 2000; Zhu *et al.*, 2001; Michaud *et al.*, 2003; Newman and Keating, 2003; Schweitzer *et al.*, 2003; Hall *et al.*, 2004; Predki, 2004). In addition, enzyme activities, even pathways, have been reconstituted on solid supports (Mirzabekov and Kolchinsky, 2002; Jung and Stephanopoulos, 2004). Therefore, the data from these proof-of-principle experiments strongly

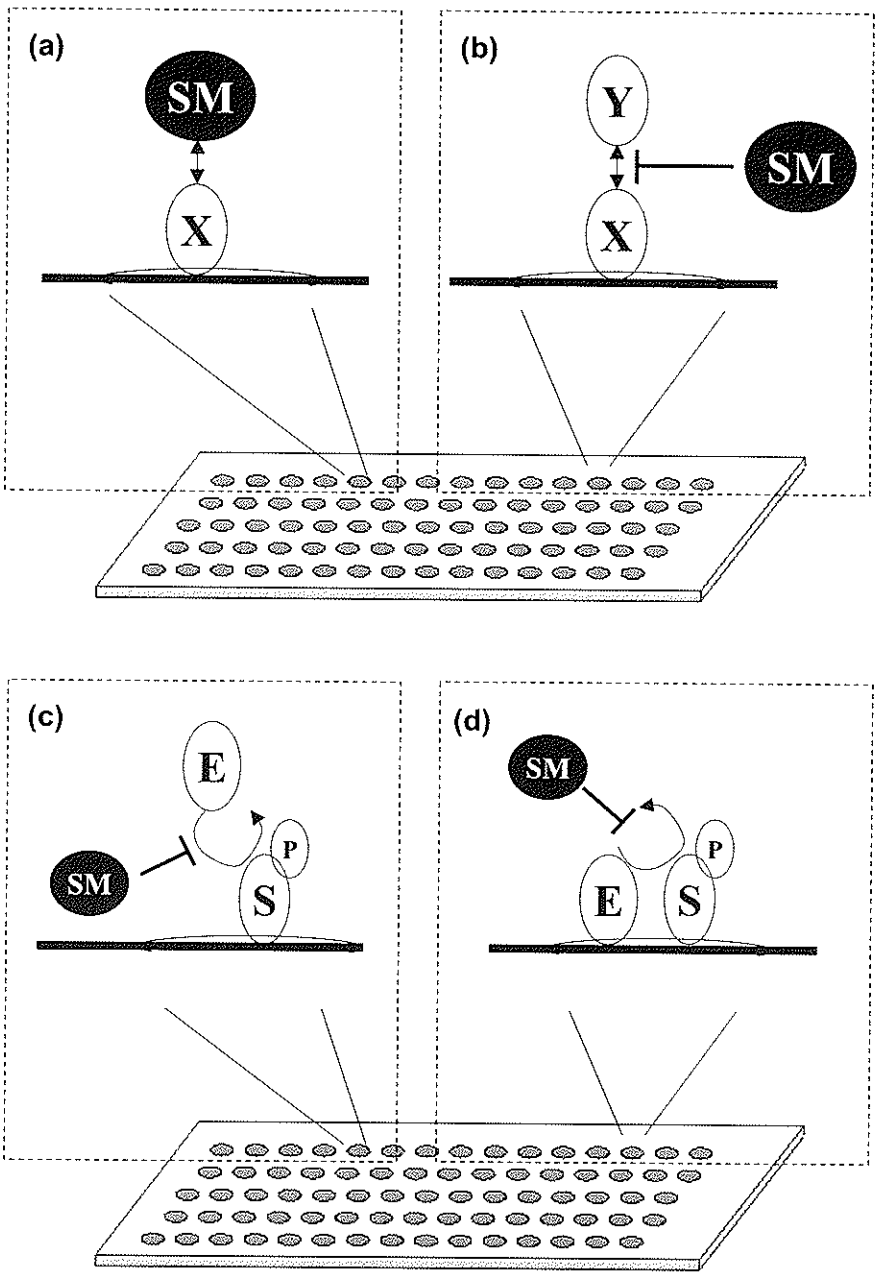

Figure 10.1. Profiling small molecules on protein arrays. Small molecules (SM) can be used for: a) target identification; b) inhibition of protein–protein interactions; c) inhibition of exogenously added enzymes against substrates on the array; and d) inhibition of enzymes on the array. X and Y are proteins, E is an enzyme, and in (c) and (d), the example represents a protein kinase that transfers a phosphate (P) to a substrate (S).

implies that proteins are, in fact, functional when immobilized on glass slides, and provides the basis for an alternative platform for the investigation of the molecular interactions of proteins with a variety of molecules. Below, we discuss how functional protein microarrays have been used to characterize small molecule–protein interactions.

Small molecule–protein interactions on protein arrays

INTERACTIONS WITH TARGET PROTEINS

The first demonstration of a small molecule–protein interaction on a modified glass slide protein microarray was reported by MacBeath and Schreiber (2000). After direct immobilization of the FK506 binding protein (FKBP12) on a glass slide, they were able to demonstrate interactions with three different fluorescently-labelled ligands for which the disassociation constants ranged from 10 nM to 3 uM (MacBeath and Schreiber, 2000). Schweitzer and co-workers were able to empirically determine a disassociation constant for an interaction of a GTP binding protein with radiolabelled GTP on glass slides (Schweitzer *et al.*, 2003). The calculated disassociation constant of 10 nM is in good agreement with other values reported in the literature. Other examples of small molecules binding to G-protein coupled receptors and nuclear hormone receptors have been documented (Ge, 2000; Fang *et al.*, 2002).

Another example of target binding is illustrated in *Figure 10.2*. To assess whether protein kinases retain activity and function on a glass support, protein kinases were immobilized on a modified glass slide. Two protein kinases, the serine/threonine kinase 16 (STK16) and the polo-like kinase (PLK), were tested for their ability to interact with staurosporine, a general protein kinase inhibitor (Takahashi *et al.*, 1987). To do so, these slides were probed with staurosporine that was conjugated to a fluorescent dye (Staurosporine-OG488), washed, and images acquired with a standard fluorescent microarray scanner. We observed that Staurosporine-OG488 interacted with STK16 and not with PLK (*Figure 10.2b, c*). It is worth noting that we estimate that approximately 8 pg of STK16 have been immobilized in one pair of spots, for which significant signal (approximately 3.5 fold over background) could be detected, implying that this approach can be an extremely sensitive approach for profiling molecular interactions. The observation that STK16 tightly binds Staurosporine-OG 488 and not PLK is consistent with solution measurements for these kinases (data not shown).

Huang and co-workers have recently used protein arrays to identify targets for small molecules that suppress a chemical-induced growth phenotype in the yeast *S. cerevisiae* (Huang *et al.*, 2004). The small molecule rapamycin is known to influence the TOR signalling pathway in yeast and in mammalian cells (Chan *et al.*, 2000). There is considerable interest in further characterizing this pathway because members of the TOR protein family are mutated in diseases such as severe combined immunodeficiency (SCID) and ataxia telangiectasia (ATM) (Huang *et al.*, 2004). To do so, a chemical genetics screen was utilized to identify six small molecule inhibitors of rapamycin (SMIRs) that reverse the inhibition of growth induced by rapamycin. To identify targets for these molecules, a biotin moiety was attached to

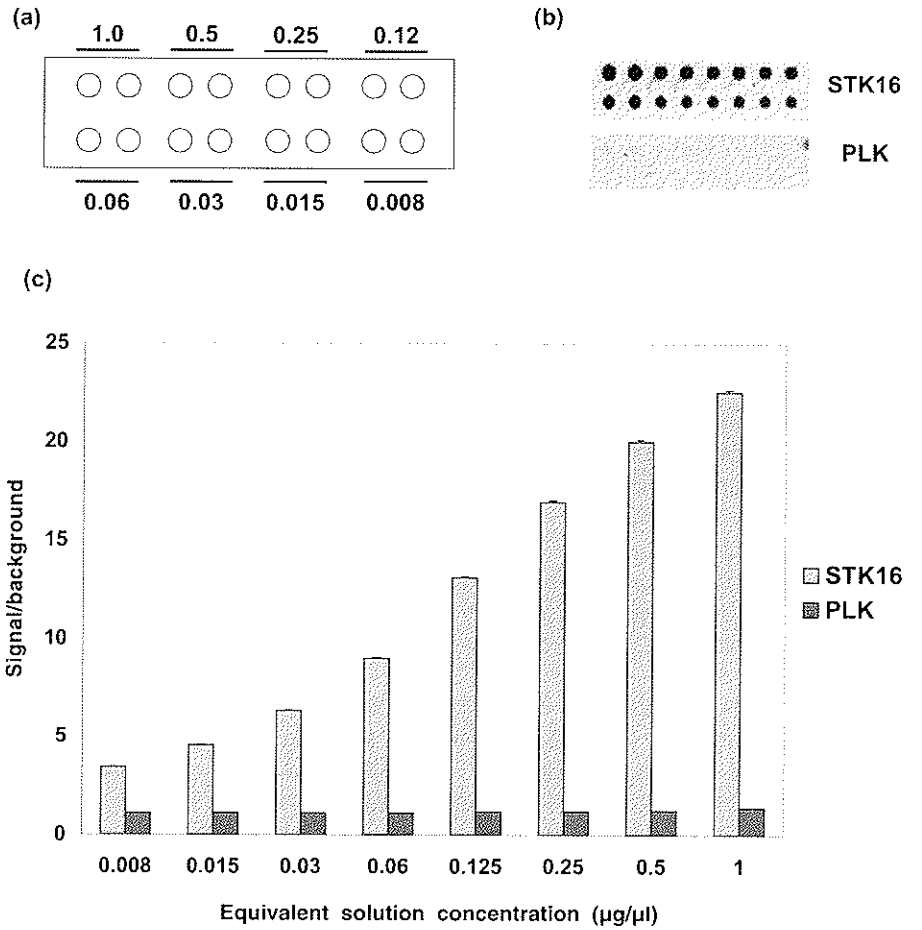

Figure 10.2. Staurosporine-Oregon green 488 interaction with protein kinases. a) A concentration gradient (1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015, 0.008 µg/µl) for the protein kinases, STK16 and PLK, was prepared and arrayed in duplicate on to a modified glass slide (UltraGaps, Corning). To these slides, 1 µM Staurosporine-Oregon green 488 was added, incubated at 4°C, the slides washed, and scanned with a fluorescent microarray scanner. Data were acquired with GenePix Pro Software (Molecular Devices) and processed in Microsoft Excel. b) Images for binding of Staurosporine-OG488 to STK16 and PLK. c) The signal/background ratios (*y*-axis) for each kinase are plotted against the equivalent solution protein concentration.

two molecules, SMIR3 and SMIR4, and these molecules were probed against yeast proteome protein microarrays. An analysis of the results produced eight hits for SMIR3 and thirty hits for SMIR4. Interestingly, one of the SMIR4 targets, Ybr077C, a protein of unknown function, was pursued further because a deletion of the Ybr077C gene from yeast produced a hypersensitive rapamycin phenotype. Therefore, the candidate target protein is involved, apparently positively, in the regulation of the TOR signalling pathway. The exact role of Ybr077C in yeast and those of the other SMIR targets remains to be revealed. Several studies are now under way, in our

lab as well as others, using high-content protein arrays to identify the protein targets for a variety of biological and synthetic small molecules.

INTERACTIONS WITH OFF-TARGET PROTEINS

Several notable examples of drug toxicity in recent years have resulted in the withdrawal of multi-billion dollar/year drugs from the market, the most recent of which was the voluntary withdrawal from the market of Vioxx, an anti-inflammatory medicine, by Merck (Couzin, 2004). Vioxx is a small molecule inhibitor of a class of enzymes known as the COX2 enzymes. The product of the COX2 gene encodes a cyclooxygenase, an enzyme important in inflammation (Weinberg, 2000). In addition to Vioxx, several other companies have COX2 inhibitors either currently approved for use in the US or in Europe: Pfizer (Celebrex and Bextra), Novartis (Prexige), and Merck (Arcoxia). The increased specificity of these drugs for COX2 over COX1 (a homologous cyclooxygenase that is the primary target for the drugs aspirin and naproxen) was hailed because COX2 inhibitors prevent undesirable side effects in the stomach. However, the recently published results of a long-term study have shown that patients taking Vioxx had an increased risk of heart attacks and strokes (Mamdani *et al.*, 2004). More importantly, the molecular mechanism by which Vioxx brings about these complications is still a subject of much debate and needs to be resolved in order to address the general safety of COX2 inhibitors for treatment of pain/inflammation.

A protein microarray containing all of the proteins encoded by the human proteome would present a good platform for addressing the toxicity of drugs. By immobilizing all human proteins on a glass slide and screening the slide for interactions with the small molecule of interest, one can possibly identify interactions with proteins that may provide clues to the toxicity observed in clinical trials. As an example of the potential of this approach, we have screened a human protein array containing approximately 2500 human proteins with a kinase inhibitor conjugated to a fluorescent dye. In addition to expected interactions with several kinases on the slide, a significant interaction was observed with a glutathione S-transferase (*Figure 10.3*).

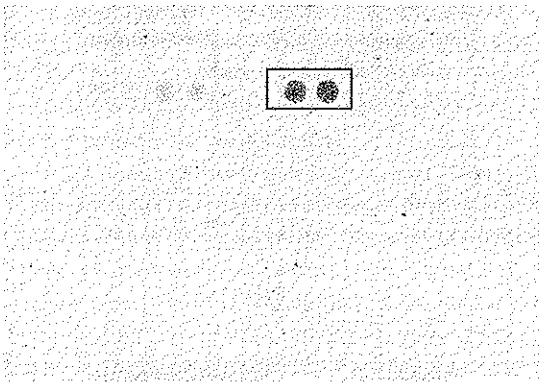

Figure 10.3. Small molecule profiling of human protein array. A kinase inhibitor conjugated to a fluorescent dye was probed against a human protein array containing approximately 2500 human proteins. One unexpected interaction was observed with glutathione S-transferase A4 (boxed).

The interaction of a kinase inhibitor with a glutathione S-transferase may reflect an interaction inside cells because it has been proposed that one mechanism for resistance to anti-cancer drugs is through the conjugation of cellular glutathione to drugs, and by so doing, inactivating them (Calvert *et al.*, 1998; Mattern *et al.*, 2002).

Biochemical arrays for characterizing small molecule protein inhibitors

In addition to protein kinases, as already mentioned, several other enzyme classes are targets for small molecule therapeutics, such as phosphatases, proteases, and reductases. Another means of assessing small molecule interactions with proteins is to determine if small molecules can interfere with the catalytic activity of enzymes on protein arrays (*Figure 10.1c/d*). Chen and co-workers were the first to demonstrate that proteases, phosphatases, and hydrolases are indeed active on arrays (Chen *et al.*, 2003). By covalently attaching fluorescent dyes to suicide inhibitors, specific interactions of known class-specific inhibitors with their target enzymes were demonstrated by these researchers. Control assays were performed to address the requirement for enzyme activity on glass slides for covalent attachment of inhibitors to enzymes. As suggested by Chen and co-workers, this strategy could be used to rapidly assess drugs as candidates for target-specific inactivation of proteins.

To show the utility of protein arrays for characterizing reversible small molecule inhibitors on protein arrays, we have tested, in recent work, the activities of protein kinases on arrays in the presence of known protein kinase inhibitors (M. Murtha, unpublished results). As shown in *Figure 10.4a*, protein kinases immobilized on glass slides can retain their ability to autophosphorylate. The five protein kinases, ABL, ARG, PKA, PKCδ, and PKCη, when incubated with ^{33}P-γ-ATP, transfer the gamma phosphate of ATP to either serine/threonine or tyrosine residues, bringing about radioactive signals on the array (*Figure 10.4a*). Staurosporine is a protein kinase inhibitor that effectively competes with ATP for the ATP-binding site on most kinases, and is thus a general inhibitor. To test for the effect of staurosporine on protein kinase activity on glass slides, a mixture of staurosporine and ^{33}P-γ-ATP was incubated with the arrays, the slides washed, and the data acquired. It is clear that staurosporine inhibits the activities of all five protein kinases, *Figure 10.4a/c*. The per cent inhibition is approximately 92–97% for the serine-threonine kinases PKA, PKCδ, and PKCη, and approximately 50% for the tyrosine kinases, Abl and Arg. To demonstrate small molecule target specificity, we added the known tyrosine kinase specific inhibitor, PP2 (Li *et al.*, 2000). As shown in *Figure 10.4a/c*, this inhibitor specifically inhibits both the Abl and Arg tyrosine kinases, 36 and 54% inhibition, respectively. The serine/threonine kinases PKA, PKCδ, and PKCη are not inhibited by PP2, illustrating that selective inhibition of enzymes indeed occurs on protein arrays.

It has been estimated that the human proteome encodes over 500 protein kinases (Manning *et al.*, 2002), and the majority of these kinases either have not been characterized or their functions are largely unknown. One way to address protein kinase function is to identify their substrates. In many examples, peptide arrays have been used to predict consensus protein sites for several protein kinases (Yaffe and Smerdon, 2004). Peptide arrays are valuable, but their utility is limited in discovery because of the inability to use such data to accurately predict *in vivo* substrates. An

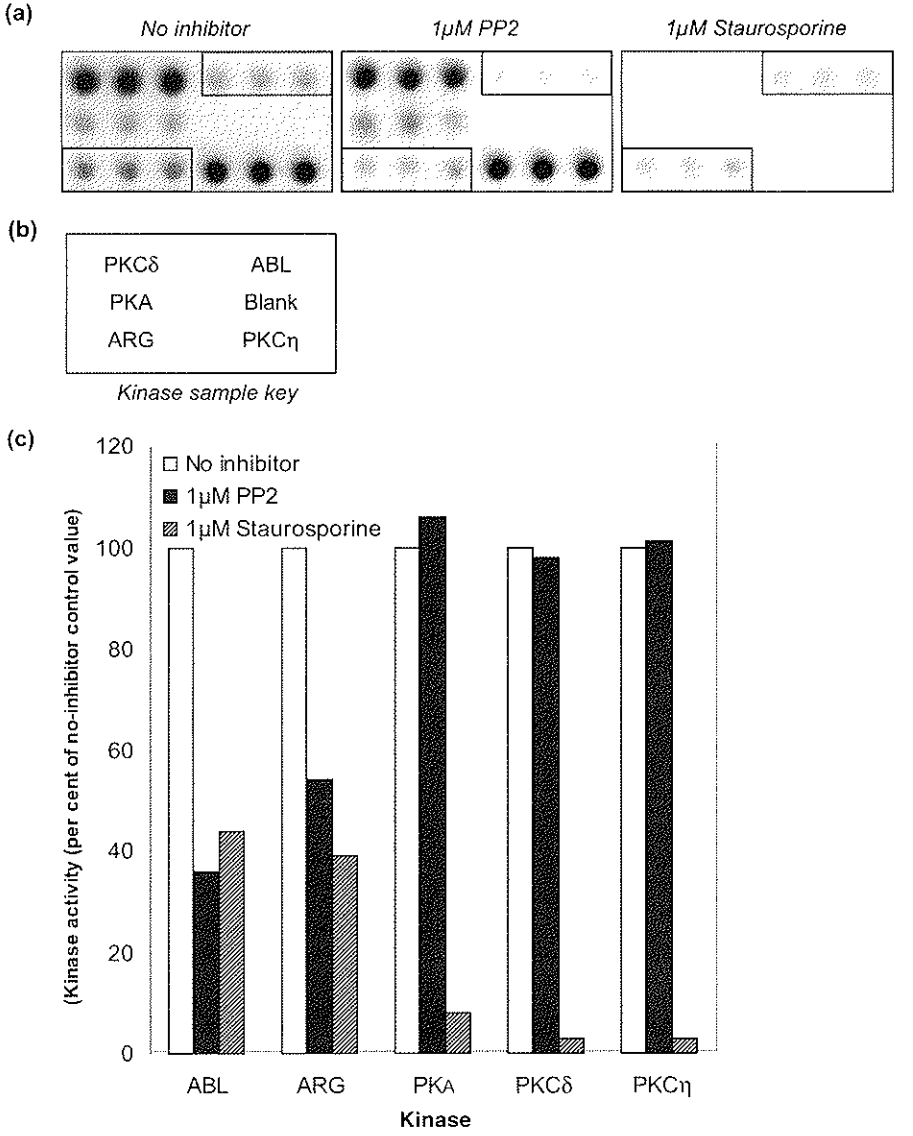

Figure 10.4. Specific inhibition of kinase activity on protein arrays. a) Phosphorimages from microarrays showing triplicate spots for 6 samples (5 kinases, PKCδ, PKCη, PKA, ABL and ARG, and a blank control). Kinase assays were performed in the presence of 1 mM specific inhibitor compound or no inhibitor (reference control), as indicated. Spot triplicates for the tyrosine kinases, ARG and ABL, are outlined for further reference. b) Key showing the location of each different kinase spot triplicate. c) Histogram showing the per cent inhibition of kinase activity for each compound. All values are given as a percentage of the reference control value.

alternative to this approach is to use full-length protein arrays. Invitrogen has developed a technology for the rapid identification of protein kinase substrates on both yeast and human protein arrays (*Figure 10.1c*). This same technology has also been used to characterize the effects of small molecule inhibitors on kinase substrate

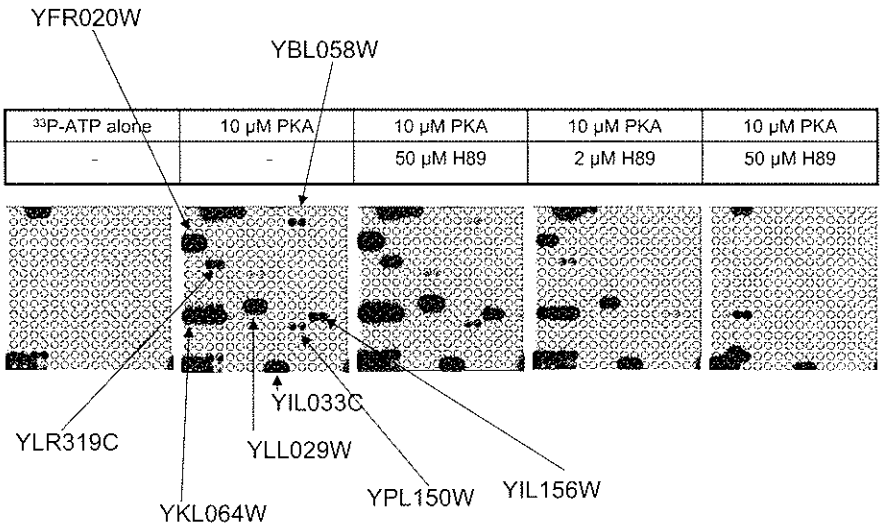

Figure 10.5. Specific inhibition of kinase activity against protein arrays. A high content protein array was treated with ^{33}P-γ-ATP in the presence of buffer, protein kinase A (PKA), PKA + 50 nM H89, PKA + 2 µM H89, and PKA + 50 µM H89. The phosphorimages show a dose-dependent inhibition of PKA by the kinase inhibitor H89.

phosphorylation. In *Figure 10.5*, the addition of protein kinase A results in the phosphorylation of expected (YIL033C) and novel substrates. A titration of the protein kinase inhibitor, H89, in the presence of protein kinase A results in a dose-dependent decrease in substrate phosphorylation on the array. H89 is a known potent inhibitor of protein kinase A (Davies *et al.*, 2000). In this example, the mode of inhibition, like that for staurosporine, is competitive for ATP. We anticipate that this assay can be used to characterize other types of inhibitors for their effects on substrate phosphorylation by protein kinases.

Future challenges of protein arrays in small molecule characterization

Protein arrays have been successfully used to reconstitute most of the interactions and enzymatic activities of proteins that occur inside cells. An ideal format for probing protein function for any given microorganism utilizing protein array technology would be to have proteome content immobilized on a glass slide suitable for a variety of applications. There are many challenges ahead before this goal can be reached. Multiple factors ultimately influence the activities of proteins on slides. The strategies employed for cloning, expression, purification, and the manufacturing of protein arrays will all have dramatic ramifications on protein activity. Certainly, advances in surface chemistry and techniques for coupling of proteins to slides to maximize protein function will be an area of extensive research. Further advances in both application development and detection technologies, either label-dependent or label-less, will certainly increase the ability to probe the roles of proteins inside cells and how these functions can be controlled specifically by drugs. Even with that said, it is likely that protein arrays will have to be tailored for specific applications.

It must be emphasized that high content protein arrays are ideally suited for screening as there is currently no alternative technology available that can allow a researcher to probe a molecule for interactions with thousands of proteins in a miniaturized format and identify both known and novel molecular interactions within a standard working day. The leads generated from screens on protein arrays, like any screening technology, should be cautiously viewed until such molecular interactions can be validated by other traditional or widely accepted methods. In summary, we envisage that protein array technology for profiling small molecules on functional protein arrays will have enormous potential for accelerating the pace of drug discovery and development because of the speed, sensitivity, and wealth of information that can be obtained in any single experiment.

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