SPRing SCREENING

Surface plasmon resonance is increasingly useful in the study of biomolecular associations.

BY STEFAN LÖFÅS

FROM THE EARLY STAGES OF RESEARCH into disease mechanisms to the lead optimization process that helps select the best drug for a target, the importance of functional data for a compound should never be underestimated. Whereas genomics might identify which genes code for which proteins, it is not until researchers have elucidated protein interactions and functions that they can determine the proteins' roles in health and disease.

Surface plasmon resonance (SPR) technology can provide detailed functional characterization of biomolecular interactions across the drug discovery and development spectrum—serving not only to identify novel drug targets but also to give researchers the opportunity to make yes-or-no decisions on a candidate early in the drug discovery process. The unique functional data provided by SPR is being used for a wide variety of life science and drug discovery applications, from proteomics to neurobiology to cancer research. With its application in preclinical, clinical, and quality assurance/quality control, SPR offers advantages in quality, reliability, and throughput of data from the earliest stages of research to the strict regulatory environment of drug manufacture.

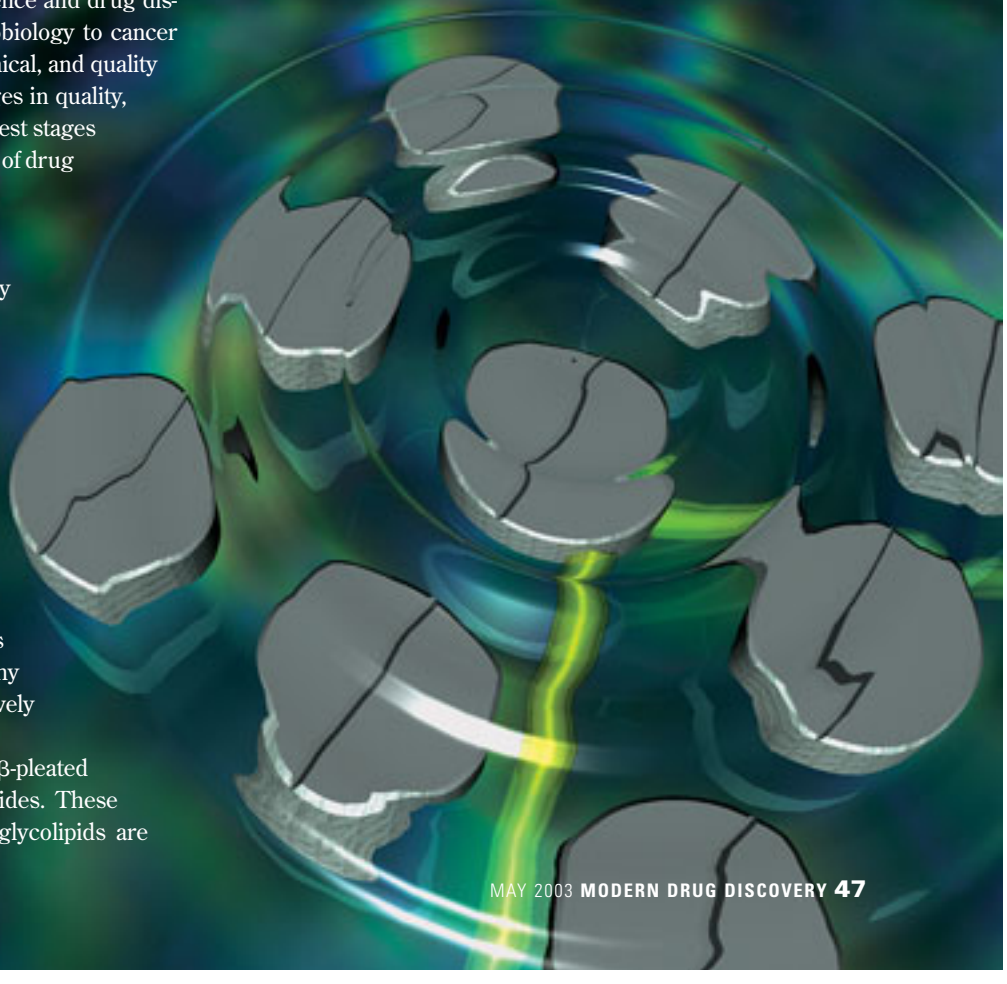
NEUROSCIENCE APPLICATIONS

The functional data provided by SPR is already assisting researchers in neurobiology. The technology has been used to investigate strategies for preventing neurodegenerative disorders such as Alzheimer's disease (AD), and it might therefore form the basis of novel therapeutic strategies.

AD is associated with the deposition of polymerized amyloid β (Aβ) peptide in lesions in the brain parenchyma. However, Aβ is expressed by almost all nucleated cells and is not secreted at elevated levels in healthy subjects as it is in AD patients. It is not known, however, why the protein is deposited to form lesions exclusively in tissues of the central nervous system.

In AD, Aβ is transformed into a neurotoxic β-pleated configuration in a process involving gangliosides. These ubiquitously expressed, membrane-spanning glycolipids are also associated with other neuropathies. For this reason, researchers are interested in producing potentially therapeutic antiganglioside antibodies.

Phase I clinical trials, in which low-immunogenicity, chimeric antiganglioside antibodies were tested in children with neuroblastomas, have shown that a host immune response is still induced to the murine component of the antibody. For this reason, K. Nakamura and colleagues at the Kyowa Hakko Kogyo Co. Ltd. (Machida-shi, Japan) engineered humanized antibodies that retain the murine specificity-defining complementarity-determining regions, but whose flanking V-region sequences have been replaced with human counterparts (*1*). In theory, this produces a less immunogenic protein because the murine component is reduced from 30% to 10%.



Nakamura's group based the characterization of the interaction between these humanized antibodies and immobilized gangliosides GD2 and GD3 on SPR experiments, which revealed that humanization changed the kinetic characteristics of the antibodies in ways that are difficult if not impossible to predict (Figure 1). This gave the researchers information about the molecular mechanisms underlying functional changes in humanized antibodies, a critical part of the development of human immunotherapy.

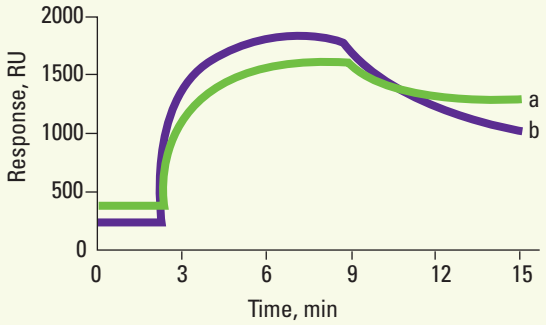


Figure 1. A (hu)man or a mouse? The SPR sensorgram compares the interactions between ganglioside GD2 and a chimeric (a) or humanized (b) antibody.

Optimizing tumor markers

SPR has also played an important role in cancer research. A common feature of solid tumors is their reliance on the formation of new blood vessels (angiogenesis) to supply rapidly proliferating tumor cells with nutrients. Angiogenesis depends on an intricate network of signals between tumor cells, vascular endothelial cells, and the surrounding environment.

Antibodies to tumor-specific markers are potentially useful for diagnostic imaging and targeted human cancer therapy. Human antibody fragments might be even more attractive for such applications, because they would be expected to show better bioavailability than whole antibodies. Dario Neri and colleagues at several institutes (*2*) used SPR technology to select optimal antibody fragments for diagnostic imaging. These might form the basis of future therapeutic strategies targeted at the tumor vasculature.

A variant of fibronectin (a ubiquitous extracellular matrix protein), B-FN, contains a novel splice-generated domain (ED-B). This isoform is found in fetal and neoplastic tissues and in the walls of developing neoplastic blood vessels, but it is absent in normal adult tissues. An antibody fragment directed against the ED-B domain should therefore be specific to tumors. Neri's group designed a "generic" tumor-specific antibody fragment against ED-B. In addition to the single-chain anti-ED-B fragment, they generated several single-chain and dimeric variants to select an optimal fragment for clinical trials.

The researchers used SPR technology to screen antibody fragment libraries and to compare detailed binding properties (affinity and kinetics) to immobilized fibronectin ED-B. The affinities of the antibody fragments varied by 100-fold (K_d values were 1.1–110 nM), with the improvements compared to the initial fragment deriving mainly from slower off-rates. The authors then tested the capability of these fragments to target tumors in vivo using real-time photodetection of fluorescently labeled antibodies and showed that the efficiency of the labeled antibody fragments correlated closely with the slower off-rates of ED-B binding.

The hit-to-lead process

Beyond providing functional data for initial research and target identification, SPR generates invaluable information for the selection process downstream of high-throughput screening (HTS) assays, streamlining the hit-to-lead process. The latest and most advanced SPR-based systems have been designed to monitor low-molecular-weight compound binding interactions and to provide critical data at key points in the hit-to-lead selection process, including rapid confirmation of HTS hits, comprehensive kinetic characterization of lead compounds, early absorption-distribution-metabolism-excretion (ADME) analysis, and detailed binding-kinetics-based quantitative structure–activity relationships (QSARs). This level

What is SPR?

SPR is a phenomenon that occurs in a thin metal film at an optical interface under conditions of total internal reflection, and it is observed as a decrease or dip in the reflected light intensity at a specific angle. This system uses polarized light and can detect subtle changes in optical resonance that occur when molecules bind to or dissociate from an immobilized target molecule. The technique is label-free, with no fluorescence or radioactive markers needed to recognize the binding event.

SPR allows investigation of the functional nature of an interaction and provides detailed kinetic information across a wide molecular weight range, including small molecules. It can therefore provide invaluable information for improved target selection and characterization as well as comprehensive analysis of the binding of small molecules to therapeutic targets.

In chip-based optical biosensor systems, the most widely used binding matrix is carboxymethyldextran. Optimized methods have been developed to facilitate covalent immobilization of the target molecules of interest. A range of derivatized surfaces is also now available to enable various immobilization techniques and minimize nonspecific binding. The surface can also help to ensure that, as far as possible, the integrity and function of the bound target molecules are maintained. Once a target molecule has been immobilized, the analyte solution is passed over the sensor chip. Any binding to the target molecule can be detected in real time.

SPR provides high sensitivity because it can detect subfemtomole amounts of protein bound to the sensor chip surface from complex fluids and can test a range of analytes—from 100-Da molecules to intact cells. As analyte is injected over the functionalized surface of a chip, a binding activity profile or sensorgram is derived, indicating the association and dissociation characteristics of the interaction.

of analysis accelerates the selection of compounds with optimum therapeutic potential for clinical evaluation.

For example, work involving a series of thrombin inhibitors highlighted the ability to rank and characterize compounds using a direct binding assay for thrombin (*3*). In addition, compound specificity was evaluated against carbonic anhydrase, while plasma-protein-binding studies were carried out simultaneously against human serum albumin (HSA) and α_1-acid glycoprotein (AGP).

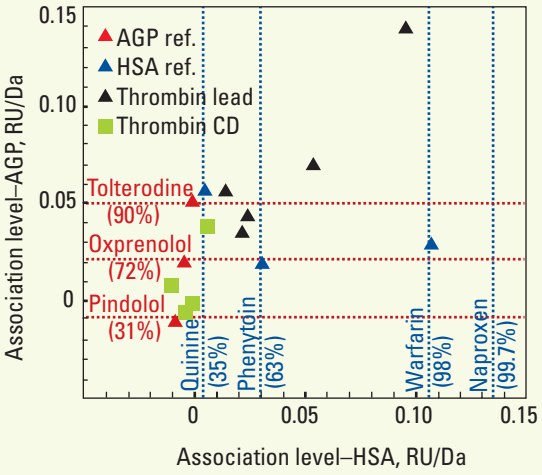


Figure 2. Bound by the data. Thrombin inhibitors and reference drugs interact with HSA and AGP. Drugs with known plasma-protein-binding properties were used to classify the properties of the unknown compounds.

Once compounds have been identified as a result of HTS assays, SPR can be used to further categorize them by rapidly providing detailed information on binding level and stability. Using a simple single-concentration secondary screen provides a higher level of data than standard yes-or-no assays. Compounds can be ranked on the basis of their association and dissociation as well as activity.

Specific early ADME applications can also be addressed. The kinetic profiling of small-molecule binders to defined plasma proteins, combined with affinity ranking, allows researchers to develop a complete picture of a compound series' interaction with plasma proteins. Researchers performed protein-binding tests for the thrombin inhibitors against HSA and AGP in a single experiment, giving rapid and detailed ranking of compound–protein interactions (Figure 2).

Kinetics-based SAR and QSAR

By combining high-quality SPR characterization of small-molecule/target interactions with compound structural information, kinetics-based SAR and QSAR become possible. Helena Danielson and colleagues at Uppsala University and Biacore AB (Uppsala, Sweden) recently demonstrated how structural changes affect not only compound activity but also association and dissociation rates (*4*). They evaluated a series of structural modifications to an HIV-1 protease inhibitor lead series and noted that although single R-group modifications might have a minimal effect on affinity, they can increase target recognition 100-fold while reducing binding stability by a similar margin. A simple affinity or activity measurement would miss the fact that the R-group modification completely changed the compound's binding profile. SPR therefore enables a kinetics-based structural and functional activity relationship to be determined, enhancing and increasing the efficiency of lead optimization.

Karl Andersson and colleagues at Biacore mutated amino acids at three positions in a peptide that bound to an antibody (*5*). To define the interactions by QSAR analysis, they gave the peptides mathematical descriptors and identified a model that related structure and kinetic values. The researchers noted that the on-rate was controlled by amino acids that induce helix formation at position 142 and by charged amino acids at position 145, whereas the off-rate was mainly controlled by helix-inducing amino acids at the same position.

Quality control

While improving the speed of their target identification and lead optimization, companies need to maintain strict compliance with Good Laboratory Practice/Good Manufacturing Practice (GLP/GMP). This means that the validation of analytical systems and procedures must be streamlined so that it does not become yet another bottleneck in the process. SPR technology also has application here, and specific systems have been developed to meet regulatory requirements. This can reduce time for biomolecular analysis while meeting the highest demands for accuracy, sensitivity, and reproducibility. SPR-based systems have been optimized for fully automated concentration analysis with advanced data evaluation and automatic report capabilities. These provide improved quantitative and qualitative concentration measurements while ensuring compliance with GLP/GMP regulations.

The bottom line

Efficient screening is essential for gathering information needed to identify drug candidates, and the more information a method can provide, the faster scientists can select molecules that have real potential for development. SPR technology can gather high-quality functional data on the biomolecular interactions of candidate molecules—information that is essential to understanding a drug's therapeutic profile.

SPR can be applied across the drug discovery and development process to provide invaluable information, from initial "blue-sky" research that identifies novel candidates for drug targets to comprehensive characterization of molecules and lead optimization. In this context, SPR has the potential to provide the pharmaceutical industry with the time and cost savings it needs to stay profitable.

References

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