

CENTER FOR BIOMOLECULAR INTERACTION ANALYSIS

Reference list August 18, 2001

Reviews, Methodology and Theory

31. Surface plasmon resonance characterization of drug-liposome interactions
Baird CL, Courtenay ES & Myszka DG
(in preparation)

In the drug development process, optimizing a promising candidate to be absorbed across the intestinal mucosa remains a difficult process. New methods need to be developed to quickly and reliably predict the intestinal permeability of a drug. Surface plasmon resonance offers a high resolution way to measure the passive transport of a drug across a synthetic lipid membrane. An experimental protocol and test experimental conditions were developed to examine in detail the interactions of a panel of well characterized drugs with lipid surfaces immobilized on a Biacore L1 sensor chip. Liposome immobilization on the chip is reproducible and stable under a variety of conditions including pH, temperature, lipid content, cholesterol content, and DMSO concentration. Results show that surface plasmon resonance can be used as a method to predict how a drug will interact with a biological membrane. In the process of optimizing intestinal permeability of a promising drug candidate, this technique should be ideal in that it is rapid and yields high resolution lipid interaction data.

30. Direct comparison of equilibrium, thermodynamic, and kinetic rate constants determined by surface- and solution-based biophysical methods
Day YSN, Baird CL, Rich RL & Myszka DG
Biochemistry (submitted)

The binding interactions of small molecules with carbonic anhydrase II were used as model systems to compare the reaction constants determined from surface- and solution-based biophysical methods. Interaction data were collected for two arylsulfonamide compounds, 4-carboxybenzenesulfonamide (CBS) and 5-dimethyl-amino-1-naphthalene-sulfonamide (DNSA) binding to the enzyme using surface plasmon resonance, isothermal titration calorimetry, and stopped-flow fluorescence. We demonstrate that when the surface plasmon resonance biosensor experiments are performed with care the equilibrium, thermodynamic, and kinetic constants determined from this surface-based technique match those acquired in solution. These results validate the use of biosensor technology to collect reliable data on small molecules binding to immobilized macromolecular targets. Binding kinetics were shown to provide more detailed information about complex formation than equilibrium constants alone. For example, while carbonic anhydrase II bound DNSA with two-fold higher affinity than CBS, kinetic analysis revealed that CBS actually formed a more stable complex by displaying a four-fold slower dissociation rate. Analysis of the binding and transition state thermodynamics also revealed significant differences in the enthalpy and entropy of complex formation. The lack of labeling requirements, high information content, and high throughput of surface plasmon resonance biosensors will make this technology an important tool for characterizing the interactions of small molecules.

29. Surface plasmon resonance biosensors
Homola J, Yee, SS & Myszka DG
Optical Biosensors: Present and Future (in press) (Ed. Frances Ligler)

Surface plasmon resonance (SPR) biosensors exploit special electromagnetic waves – surface plasmon-polaritons - to probe changes in refractive index of solvents near metal surfaces. SPR biosensors can therefore be used to monitor the interaction between an analyte in solution and its biospecific partner immobilized on the metal surface without the use of labels. Major applications involve the ability to detect the amount of a bioanalyte in crude solutions as well as to monitor the rates of biomolecular interactions. In the past ten years, SPR biosensor technology has been commercialized and biosensors have become a central tool for characterizing and quantifying biomolecular interactions both in life science and pharmaceutical research.

28. Applying BIACORE in drug bioavailability studies

Rich RL & Myszka DG
BIAJOURNAL, (IN PRESS)

BIACORE has become a standard tool in the pharmaceutical industry for target characterization and lead optimization. Emerging applications in the area of drug bioavailability now have the technology poised to play an even larger role in the drug discovery process.

27. Current and emerging optical biosensors

Baird CL & Myszka DG
J. Mol. Recog. (in press)

The field of commercial optical biosensors is rapidly evolving, with new systems and detection methods being developed each year. This review outlines the currently available biosensor hardware and highlights unique features of each platform. Affinity-based biosensor technology, with its high sensitivity, wide versatility, and high throughput, is playing a significant role in basic research, pharmaceutical development, and the food and environmental sciences. Likewise, the increasing popularity of biosensors is prompting manufacturers to develop new instrumentation for dedicated applications. We provide a preview of some of the emerging commercial systems that are dedicated to drug discovery, proteomics, clinical diagnostics, and routine biomolecular interaction analysis.

26. High-resolution and high-throughput protocols for measuring drug/human serum albumin interactions using BIACORE

Rich RL, Day YSN, Morton TA & Myszka DG
Anal. Biochem. (in press)

Characterizing how chemical compounds bind to human serum albumin (HSA) is essential in evaluating drug candidates. Using warfarin as a test system, we validate the application of BIACORE SPR biosensors to reliably determine binding constants for drug/HSA interactions. The binding responses for warfarin over HSA surfaces were extremely reproducible even though warfarin is small compared to the size of the immobilized protein. At high concentrations, warfarin bound at more than one site on HSA, which is consistent with its known binding properties. The affinity we determined for the high-affinity site ($K_D^{25^\circ\text{C}} = 3.7 \pm 1.2 \mu\text{M}$), as well as the dissociation rate constant ($k_d^{25^\circ\text{C}} = 1.2 \text{ s}^{-1}$), are also consistent with binding constants determined previously. These results validate the biosensor technology and illustrate how BIACORE can be used to study drug/HSA interactions in a high-resolution mode. Using a set of ten test compounds, we present a protocol for determining equilibrium dissociation constants for HSA in a high-throughput mode.

Our method involves working at low compound concentrations and fitting the equilibrium data for all compounds simultaneously. We show that the % bound values determined by SPR correlate with the values determined by solution-based methods. The ability to examine directly the binding of small molecules (130-800 Da), coupled with minimal sample requirements and automated instrumentation, makes BIACORE technology applicable for evaluating drug/HSA interactions.

25. Survey of the year 2000 commercial optical biosensor literature
Rich RL & Myszka DG
J. Mol. Recog. (in press)

We have compiled a comprehensive list of the articles published in the year 2000 that describe work employing commercial optical biosensors. Selected reviews of interest for the general biosensor user are highlighted. Emerging applications in areas of drug discovery, clinical support, food and environment monitoring, and cell membrane biology, are emphasized. In addition, the experimental design and data processing steps necessary to achieve high-quality biosensor data are described and examples of well-performed kinetic analysis are provided.

24. BIACORE J: a new platform for routine biosensor analysis
Rich RL & Myszka DG
J. Mol. Recog. (2001) 14: 223-228

SPR biosensor technology continues to evolve. The recently released platform from Biacore AB (Uppsala, Sweden), BIACORE J, is designed for the routine analysis of biomolecular interactions. Using an antibody-protein A and a ligand-receptor system, we demonstrate the utility of BIACORE J in determining active concentration and binding affinities. The results from these studies illustrate the high sensitivity of the instrument and its ability to generate reproducible binding responses. The BIACORE J is easy to operate and useful in diverse applications, making SPR technology widely accessible as a research tool.

23. Recent developments in SPR biosensor technology: Application of BIACORE J
Rich RL & Myszka DG
Chemical Sensors (Chinese) (in press)

22. Survey of the 1999 surface plasmon resonance biosensor literature
Rich RL & Myszka DG
J. Mol. Recog. (2000) 13: 388-407

The application of surface plasmon resonance biosensors in life sciences and pharmaceutical research continues to increase. This review provides a comprehensive list of the commercial 1999 SPR biosensor literature and highlights emerging applications that are of general interest to users of the technology. Given the variability in the quality of published biosensor data, we present some general guidelines to help increase confidence in the results reported from biosensor analyses.

21. Implementing surface plasmon resonance biosensors in drug discovery
Myszka DG & Rich RL
Pharmaceutical Science & Technology Today (2000) 3: 310-317

Recent improvements in instrument hardware, experimental design and data processing have made it possible to use surface plasmon resonance (SPR) biosensor technology in the discovery and development of small-molecule drugs. The key features of SPR biosensors (i.e. real-time binding analysis and lack of labeling requirements) make this technology suitable for a wide range of applications. Current instruments have a throughput of approximately 100-400 assays per day, providing a complement to secondary screening. The ability to collect kinetic data on compounds binding to therapeutic targets yields new information for lead optimization. Small-molecule analysis and emerging applications in the areas of ADME (adsorption, distribution, metabolism and excretion) and proteomics have SPR biosensors poised to play a significant role in the pharmaceutical industry.

20. Evaluating the energetics of erythropoietin ligand binding to the homodimerized receptor extracellular domain
Hensley P, Doyle ML, Myszka DG, Woody RW, Brigham-Burke MR, Erickson-Miller CL, Griffin CA, Jones CS, McNulty DE, O'Brien SP, Amegadzie BY, MacKenzie L, Ryan MD & Young PR
Methods in Enzymology (2000) 323: 177-207

In contrast to the structures of macromolecules, which can in principle be defined by application of either X-ray crystallography or nuclear magnetic resonance (NMR), a description of their function requires the application of multiple technologies. When function is defined in terms of reversible molecular association, the mechanism, thermodynamics and kinetics of the interaction must all be elucidated. Consequently, approaches such as analytical ultracentrifugation (AUC), isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR) need to be coordinately employed.

19. Kinetic, equilibrium, and thermodynamic analysis on BIACORE
Myszka DG
Methods in Enzymology (2000) 323: 325-340

The popularity of using biosensors to characterize biomolecular interactions continues to grow. These instruments are capable of providing detailed information about the energetics of macromolecular interactions without labeling. While commercial instruments such as BIACORE are relatively easy to use, extracting accurate rate constants for a reaction requires careful experimental technique and robust methods of data analysis. Previous chapters in this series have described methods for improving the experimental design and qualitative methods for analyzing polymerization reactions. This chapter illustrates how biosensors can be used as a biophysical tool to determine equilibrium constants and kinetic rate constants for a reaction. In addition, by measuring reactions at different temperatures, it is possible to extract thermodynamic information about the system. The model system used in this study involves the interaction of IL-2 ligand with the alpha subunit of its receptor.

18. Sorting needles from haystacks
Hensley P & Myszka DG
Curr. Opin. Biotech. (2000) 11: 9-12

In the pharmaceutical industry, the ability to make and screen very large numbers of chemical entities is raising new questions regarding the approaches investigators and drug companies use to identify drug-like molecules. From the 1970s through the early 1990s, brute force screening

methods were the mode and successful drug identification was dominated by large companies. There were two reasons for this. First, drug companies had the high-throughput screening (HTS) approaches in place. Second, they had large proprietary chemical libraries (100,000–300,000 compounds). These were the accumulated chemical wealth of the oldest and biggest companies.

17. Advances in surface plasmon resonance biosensor analysis

Rich RL & Myszka DG

Curr. Opin. Biotech. (2000) 11: 54-61

The number and diversity of surface plasmon resonance (SPR) biosensor applications continue to increase. Evolutions in instrument and sensor chip technology, experimental methodology, and data analysis are making it possible to examine a wider variety of biomolecular interactions in greater mechanistic detail. SPR biosensors are poised to make a significant impact in basic research and pharmaceutical discovery.

16. A biosensor assay for studying ligand-membrane receptor interactions: binding of antibodies and HIV-1 Env to chemokine receptors

Hoffman TL, Canziani G, Jia L, Rucker J & Doms RW

Proc. Natl. Acad. Sci. U. S. A. (2000) 97:11215-11220

The HIV envelope (Env) protein mediates entry into cells by binding CD4 and an appropriate coreceptor, which triggers structural changes in Env that lead to fusion between the viral and cellular membranes. The major HIV-1 coreceptors are the seven transmembrane domain chemokine receptors CCR5 and CXCR4. The type of coreceptor used by a virus strain is an important determinant of viral tropism and pathogenesis, and virus-receptor interactions can be therapeutic targets. However, Envs from many virus strains interact with CXCR4 and CCR5 with low affinity such that direct study of this important interaction is difficult if not impossible using standard cell-surface binding techniques. We have developed an approach that makes it possible to study ligand binding to membrane proteins, including Env-coreceptor interactions, using an optical biosensor. CCR5, CXCR4, and other membrane proteins were incorporated into retrovirus particles, which were purified and attached to the biosensor surface. Binding of conformationally sensitive antibodies as well as Env to these receptors was readily detected. The equilibrium dissociation constant for the interaction between an Env derived from the prototype HIV-1 strain IIIB for CXCR4 was approximately 500 nM, explaining the difficulty in measuring this interaction using standard equilibrium binding techniques. Retroviral pseudotypes represent easily produced, stable, homogenous structures that can be used to present a wide array of single and multiple membrane-spanning proteins in a native lipid environment for biosensor studies, thus avoiding the need for detergent solubilization, purification, and reconstitution. The approach should have general applicability and can be used to correlate Env-receptor binding constants to viral tropism and pathogenesis.

15. Exploring biomolecular recognition using optical biosensors

Canziani G, Zhang W, Cines D, Rux A, Willis S, Cohen G, Eisenberg R & Chaiken I

Methods (1999) 19:253-69

Understanding the basic forces that determine molecular recognition helps to elucidate mechanisms of biological processes and facilitates discovery of innovative biotechnological methods and materials for therapeutics, diagnostics, and separation science. The ability to measure interaction properties of biological macromolecules quantitatively across a wide range of

affinity, size, and purity is a growing need of studies aimed at characterizing biomolecular interactions and the structural elements that drive them. Optical biosensors have provided an increasingly impactful technology for such biomolecular interaction analyses. These biosensors record the binding and dissociation of macromolecules in real time by transducing the accumulation of mass of an analyte molecule at the sensor surface coated with ligand molecule into an optical signal. Interactions of analytes and ligands can be analyzed at a microscale and without the need to label either interactant. Sensors enable the detection of bimolecular interaction as well as multimolecular assembly. Most notably, the method is quantitative and kinetic, enabling determination of both steady-state and dynamic parameters of interaction. This article describes the basic methodology of optical biosensors and presents several examples of its use to investigate such biomolecular systems as cytokine growth factor-receptor recognition, coagulation factor assembly, and virus-cell docking.

14. Survey of 1998 optical biosensor literature
Myszka DG
J. Mol. Recog. (1999) 12: 390-408

The utilization of optical biosensors to study molecular interactions continues to expand. In 1998, 384 articles relating to the use of commercial biosensors were published in 130 different journals. While significant strides in new applications and methodology were made, a majority of the biosensor literature is of rather poor quality. Basic information about experimental conditions is often not presented and many publications fail to display the experimental data, bringing into question the credibility of the results. This review provides suggestions on how to collect, analyze and report biosensor data.

13. Analysis of fibril elongation using a surface plasmon resonance biosensor
Myszka DG, Wood S & Biere AL
Methods in Enzymology (1999) 309: 386-402

Commercially available surface plasmon resonance (SPR) biosensors, such as BIACORE, have revolutionized the characterization of macromolecular interactions. These instruments can be used to monitor binding events in real-time without labeling, making them convenient for studying a wide variety of biomolecular systems. Biosensors are routinely used to provide insight into the reaction kinetics and thermodynamics for saturable bimolecular reactions such as ligand-receptor or antigen-antibody interactions. However, biosensors have the ability to provide detailed information on polymerization and aggregation reactions as well. There is a growing interest in kinetic analysis of fiber formation, mainly due to the increasing body of evidence implicating the involvement of peptide or protein fibers in human diseases, one prominent example being Alzheimer's disease. One of the pathological criteria for Alzheimer's disease are neuritic plaques, which predominantly consist of fibrillar amyloid β -peptide (A β), a 40-42 amino acid peptide. Unfortunately, in the case of A β , kinetic data regarding fibrillogenesis is still very limited. This is partly due to the lack of adequate techniques and partly to the difficulties in handling peptides that readily aggregate. Using A β as a model system, we illustrate how biosensors have the potential to provide detailed information on aggregation and polymerization processes. Because this represents a new application for biosensors, the chapter focuses on the experimental methods that are useful for monitoring fibril elongation.

12. Improving biosensor analysis
Myszka DG
J. Mol. Recog. (1999) 12: 1-6

While commercial biosensors are simple to operate, accurately interpreting binding reactions is not always straightforward. Since the majority of published biosensor data do not fit a simple bimolecular interaction model ($A + B = AB$), many investigators are concerned about the validity of biosensor analysis. However, the inability to fit data to a simple model is often a result of how the experiments are run and not a flaw in the technology. Many researchers collect data under conditions that are not suitable for measuring binding kinetics. There are a number of experimental artifacts that can complicate biosensor analysis including surface imposed heterogeneity, mass transport, aggregation, avidity, crowding, matrix effects and nonspecific binding. Improving the design of biosensor experiments, as well as improving the way binding data are collected and processed, can eliminate most of these artifacts. By improving the quality of the sensor data, we have described a number of reactions with simple interaction models. This article highlights the key steps required to improve the quality of data when the goal is to interpret the binding kinetics recorded on biosensors

11. Equilibrium analysis of high affinity interactions using BIACORE
Myszka DG, Jonsen MD & Graves BJ
Anal. Biochem. (1998) 265: 326-330

BIACORE biosensors are useful for measuring reaction kinetics and calculating affinity constants for macromolecular interactions without labeling requirements. However, one drawback with the flow system used in these instruments is that the standard injection procedures limit the amount of time available to collect association phase data. This is especially problematic during equilibrium analysis of high affinity interactions. Using protein-DNA interactions as a model system, we demonstrate a simple method for overcoming this limitation. By placing the analyte directly into the running buffer we were able to deliver a continuous supply of protein to the sensor surfaces for greater than 12 hr at a time. Complete equilibrium binding profiles were generated by changing the concentration of analyte and allowing the surface reactions to reequilibrate. Analyte concentrations were also decreased to demonstrate that the binding reactions were fully reversible. This method of analysis is a simple and convenient way of directly measuring equilibrium dissociation constants for very high affinity interactions.

10. Interpreting kinetic rate constants from optical biosensor data recorded on a decaying surface
Joss L, Morton TA, Doyle ML & Myszka DG
Anal. Biochem. (1998) 261: 203-210

A capturing assay was used to monitor a Fab-antigen interaction using a BIACORE optical biosensor. The antigen, a truncated single-site mutant (F43V) version of the CD4 receptor, was captured onto the sensor surface using an immobilized non-neutralizing monoclonal antibody. While this assay design created an oriented antigen surface, the antigen slowly dissociated during subsequent binding of the Fab, thus complicating the binding responses. In this paper, we illustrate how binding events occurring on a decaying surface can be accurately described by globally fitting the response data to a model that accounts for the background surface decay. Support for the method was obtained by showing the equilibrium dissociation constant calculated from the kinetic rate constants ($K_d = 2.20 \pm 0.01$ nM) was similar to the value measured in solution using titration

calorimetry ($K_d = 2.6 \pm 0.5$ nM). The ability to interpret rate constants from decaying surfaces significantly extends the types of experimental systems that can be quantitatively studied on optical biosensors.

9. CLAMP: a biosensor kinetic data analysis program

Myszka DG & Morton TA
TIBS (1998) 23: 149-150

CLAMP is a data analysis program designed to interpret reaction kinetics by fitting data with models based on hypothetical mechanisms. The program combines modeling and fitting routines in an easy to use format. An important feature of the program is its capacity to simultaneously analyze response data from different experiments. Grouping data sets that share common parameter values provides a better test for reaction models and improves the statistical behavior of the parameter estimates. Other advantages of simultaneous or "global analysis" are that it is easy to visualize how well a model directly fits all of the primary data and an entire data set can be analyzed very quickly. Typical data sets take less than one minute to analyze on a desktop personal computer. Global analysis, does however, require experimental data of very high quality, if simple models are to be used to describe binding reactions. It is, therefore, essential to design the experiment properly and minimize experimental artifacts such as bulk refractive index changes, nonspecific binding and instrument drift.

8. Extending the range of Rate Constants Available from BIACORE: Interpreting Mass Transport Influenced Binding Data

Myszka DG, Xiaoyi H, Bembo, M, Morton TA & Goldstein
Biophysical Journal (1998) 75: 583-594

Surface-based binding assays are often influenced by the transport of analyte to the sensor surface. Using simulated data sets, we test a simple two-compartment model to see if its description of transport and binding is sufficient to accurately analyze BIACORE data. First we present a computer model that can generate realistic BIACORE data. This model calculates the laminar flow of analyte within the flow cell, its diffusion both perpendicular and parallel to the sensor surface, and the reversible chemical reaction between analyte and immobilized reactant. We use this computer model to generate binding data under a variety of conditions. An analysis of these data sets with the two-compartment model demonstrates that good estimates of the intrinsic reaction rate constants are recovered even when mass transport influences the binding reaction. We also discuss the conditions under which the two-compartment model can be used to determine the diffusion coefficient of the analyte. Our results illustrate that this model can significantly extend the range of association rate constants that can be accurately determined from BIACORE.

7. Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors

Morton TA & Myszka DG
Methods in Enzymology (1998) 295 268-294

Optical biosensors are emerging as important tools for characterizing the interactions of biological macromolecules. Biosensors can provide qualitative information on macromolecular assembly processes under a variety of conditions. Quantitative information, in the form of affinity constants for complex formation, can be obtained in a manner similar to conventional solid phase assays. The major advantage of biosensors over other interaction technologies is that the formation and

breakdown of complexes can be monitored in real-time. This offers the possibility of determining the mechanism and kinetic rate constants associated with a binding event. This information is essential for understanding how biological systems function at the molecular level. There are several commercially available optical biosensors that are convenient to use. However, accurate interpretation of biosensor data is not always straightforward. The purpose of this chapter is to illustrate how to maximize the possibility of obtaining reliable information about reaction kinetics.

6. Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors

Myszka DG

Curr. Opin. Biotech. (1997) 8: 50-57

Surface plasmon resonance-based biosensors are being used to define the kinetics of a wide variety of macromolecular interactions. As the popularity of this approach grows, experimental design and data analysis methods continue to evolve. These advances are making it possible to accurately define the assembly mechanisms and rate constants associated with macromolecular interactions.

Specific interactions of macromolecules, such as proteins, oligonucleotides and oligosaccharides, provide a chemical foundation for all cellular processes. Quantitating how macromolecules assemble into complexes and break down over time is required to define the mechanism of binding. This kinetic information is pivotal in relating the structure of biological macromolecules to their function. Previously, kinetic analyses were restricted to only select systems with unique spectral properties. Fortunately, with the development of surface plasmon resonance biosensors (SPR), the kinetics of most macromolecular interactions are now routinely accessible.

5. Kinetic analysis of a protein antigen-antibody interaction limited by mass transport on an optical biosensor

Myszka DG, Morton TA, Doyle M & Chaiken IM

Biophys. Chem. (1997) 64: 127-137

Using BIACORE™ technology, we determined the rate constants for a protein antigen-antibody interaction that was mass transport limited on the optical biosensor. The antigen consisted of a soluble form of the human T-cell receptor CD4 (two amino terminal domains, D1D2) and the antibody was an anti-CD4 monoclonal from monkey engineered with the constant domains from human IgG1. High quality response data were obtained for this interaction by orienting the attachment of the antibody on the sensor surface and correcting for instrument artifacts with control experiments. Using numerical integration and global fitting, we demonstrate that a mass transport limited reaction was the only model of those tested that described well D1D2 binding to three different surface densities of the antibody. Statistical profiling techniques showed that the error space and correlation for the parameters in the nonlinear model were essentially linear, but only when the model was simultaneously fitted to data from multiple surface densities. The on and off rate constants ($1.2 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ and $2.9 \times 10^{-4} \text{ s}^{-1}$) determined from the kinetic analysis predict an equilibrium dissociation constant ($K_D = 0.24 \pm 0.01 \text{ nM}$) that agrees with the value measured in solution by titration calorimetry ($K_D = 0.2 \pm 0.1 \text{ nM}$). The results indicate that, although the D1D2-antibody reaction is partially controlled by mass transport on the optical biosensor, by optimizing the experimental design and analyzing data from multiple surface densities it is possible to determine accurate estimates of the intrinsic equilibrium and kinetic rate constants.

4. Global analysis of a macromolecular interaction recorded on BIAcore
Roden LD & Myszka DG
Biochem. Biophys. Res. Commun. (1996) 225: 1073-1077

We demonstrate that the interaction between myoglobin and an immobilized anti-myoglobin antibody measured on BIACORE 2000 can be described by a simple bimolecular reaction mechanism. We improved the quality of the sensor data by correcting for refractive index changes and nonspecific binding using a blank sensor surface. Applying nonlinear least squares analysis, we simultaneously fitted the association and dissociation phase data generated for a range of myoglobin concentrations injected across the antibody surface. The ability to globally fit these data to a simple binding model indicates that effects related to the sensor surface, like mass transport and the dextran matrix, did not complicate the observed binding responses. These results illustrate the potential of BIACORE to monitor macromolecular interactions in real-time and the utility of global analysis to resolve the reaction kinetics.

3. Molecular interaction analysis in ligand design using kinetic and thermodynamic methods
Doyle M, Myszka DG & Chaiken IM
J. Mol. Recog. (1996) 9: 65-74

Ligand design in biotechnology is underpinned by the control of molecular affinity. Hence, measuring binding interactions is a key component in designing ligands for such uses as therapeutics, diagnostics, biomaterials and separation science. Mass transport, kinetic and thermodynamic methods have been used for macromolecular interaction analysis but also have potential applicability as direct methods for measuring small molecular interactions. They can enhance the ligand design process by providing the ability to choose ligands based on both their kinetic and thermodynamic binding properties.

2. New opportunities for using immobilized ligands to characterize macromolecular recognition and design recognition molecules
Chaiken IM, Myszka DG & Morton TA
Advances in Molecular and Cellular Biology, JAI Press. (1996) 15B: 551-566

Characterization of protein interactions and interaction sites can provide a means both to learn about molecular recognition and assembly processes in biology and to identify and evaluate new recognition molecules of practical use in biotechnology. We have had a long-standing interest in using immobilized ligands as analytical tools for characterizing recognition mechanisms of proteins and other biological macromolecules. This interest started with analytical affinity chromatography (AAC). Recently, a new comer among analytical technologies using immobilized ligands has appeared, namely the surface plasmon resonance (SPR) biosensor. The SPR biosensor, as AAC, enables detection and measurement of non covalent interaction of soluble macromolecule with a solid phase containing covalently attached ligand. Importantly, the biosensor offers several unique feature including access to kinetics (hence deeper mechanistic understanding), ability to analyze molecules in mixtures (hence access to more biologically relevant conditions), and real-time observation of the interaction process (hence ability to observe interacting molecules as they form and are added). Recent results with HIV proteins including p24 self assembly and CD4-gp120 interactions, as well as with Interleukin 5 and its receptor reflect some of the growing uses of both AAC and SPR biosensor as macromolecular recognition tools. Overall, the advent of the SPR biosensor and the likely follow-up development of other automated devices promise to stimulate

evolution of the analytical use of immobilized ligands that started with AAC into a broad-based analytical solid phase science for the field of biomolecular recognition.

1. Interpreting complex binding kinetics from optical biosensors: a comparison of linear analysis, the integrated rate equation and numerical integration

Morton TA, Myszka DG & Chaiken IM

Anal. Biochem. (1995) 227: 176-185

The binding kinetics recorded for many interactions using BIAcore and IAsys optical biosensors do not fit a simple bimolecular interaction model ($A + B = AB$). Three methods of analysis have been used to derive estimates for kinetic constants from such data: linearization, curve fitting using the integrated rate equation, and curve fitting using numerical integration. To test how well these methods could interpret complex binding kinetics, we generated and analyzed simulated data for two systems, one involving a two-state conformational change ($A + B = AB = (AB)^*$) and a second involving surface heterogeneity ($A + B = AB$ and $A + B^* = AB^*$). The linearization method assumed a simple bimolecular interaction and was inadequate at interpreting these systems as both produced complex kinetics in the association and dissociation phases. The sum of two integrated rate equations correctly modeled surface heterogeneity; but, when applied non-globally, it fit the data from the conformational change system equally well and thus provided misleading results. Numerical integration allowed a choice of model for analysis and was therefore the only method capable of returning accurate estimates of rate constants for both complex systems. Global analysis, in combination with numerical integration, provided a stringent test of the assumed model. However, this stringency suggests that its application to experimental systems will require high-quality biosensor data.