

LECTURE-31

Protein Interaction Analysis using SPR and SPRi

Handout

PREAMBLE

Study of protein-protein interactions is very important to understand the biological systems since these interactions play central role in vital physiological pathways, cellular communications and host-pathogen interactions (Berggård et al., 2007). Over the last few decades quite a few popular approaches, including two-hybrid system, co-immunoprecipitation and affinity chromatography have been routinely used for studying protein-protein interactions as well as interaction of proteins with other biomolecules and small ligands. In recent years, protein and antibody microarrays, and SPR-based sensing approaches have emerged for studying multiple protein-protein interactions simultaneously. SPR and SPRi-based biosensors are one of the most potential approaches for studying protein-protein interactions in a high-throughput manner (Yuk et al., 2006; Ding et al., 2009). In this lecture, we will discuss the application of SPR and SPRi-based biosensors for studying protein-protein interactions.

OUTLINE OF LECTURE

- I. Surface plasmon resonance (SPR) and SPR Imaging (SPRi); comparative analysis
- II. Study of protein-protein interactions using SPR and SPRi
- III. Data processing and analysis
- IV. Conclusions

Box 1: Terminology

SPR: Surface plasmon resonance (SPR) is a label-free method that measures variations in refractive index of the dielectric layer adjoining to the sensor surface as a result of the adsorption or desorption of molecules.

SPRi: SPR imaging (SPRi) is an advanced and high throughput platform of SPR, where the complete biochip surface is illuminated using a broad beam, monochromatic, polarized light and a CCD camera is used for simultaneous capturing of reflected light from each spot.

Sensorgram: Sensorgram is graphical representation of SPR data, which indicates the binding response in reflection intensity versus time.

Protein-protein interaction: Interaction/binding between two/more proteins, which often regulate the activity of the proteins. Interactions between proteins play crucial roles in vital physiological pathways, cellular communications and host-pathogen interactions.

Kinetics: Parameters indicating the rates of forward and reverse reactions.

Affinity: Affinity indicates the strength of interaction, which is measured quantitatively by dissociation constant.

Dissociation constant (KD): It is the ratio of on rate (K_a) and off rate (K_d), which reflects the strength of interaction.

On rate (K_a): Also known as association rate; indicates the rate of forward reaction leading towards product formation.

Off rate (K_d): Also known as dissociation rate; indicates the rate of reverse reaction leading to the dissociation of the complex.

I. SURFACE PLASMON RESONANCE (SPR) AND SPR IMAGING (SPRi); COMPARATIVE ANALYSIS

SPR-based sensing approaches depend on measurement of changes in the refractive index of medium directly in contact with sensor surface. In SPR measurement the sensorgram indicates the changes in reflection intensity with respect to incident angle when the target molecule binds to the sensor surface (Fig 1). Alteration in the refractive angle is directly proportional to the mass bound at the surface. Although SPR and SPRi follow the same working principle for bio-sensing, there are following major differences in SPRi instrumental set-ups:

- In SPRi a CCD camera is used for instantaneous capturing of reflected light from each spot of sensor surface, which allows instantaneous analysis of binding events on all spots.
- SPRi is more suitable for HT analysis compared to SPR

Both SPR and SPRi have the following advantages regarding analysis of protein-protein interactions:

- Allow label-free detection without need of any secondary reactants and extensive labeling process
- Real-time measurements
- Provide information regarding binding kinetics (rates of association and dissociation)
- Suitable for studying multiple biomolecular interactions simultaneously
- Provide both quantitative and qualitative measurements

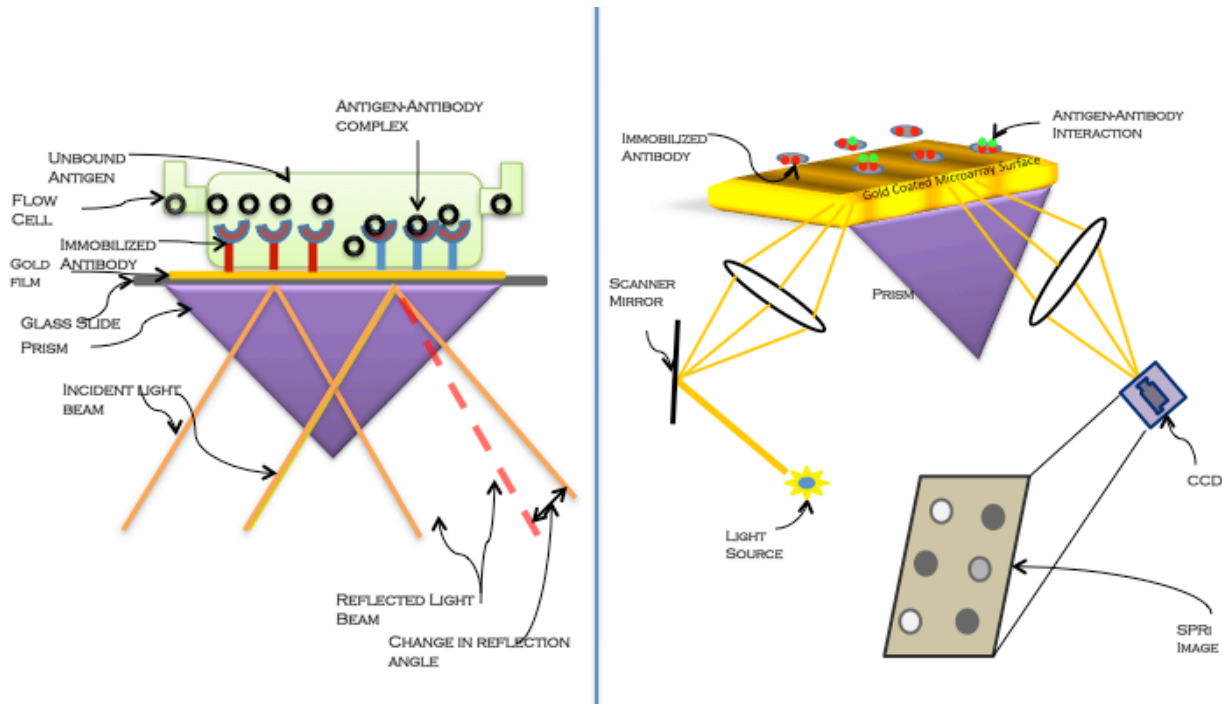


Fig 1. The basic working principle of SPR (A) and SPRi (B) for monitoring protein-protein interactions. In this label-free detection method one of the interaction partners is immobilized on the sensor surface and the second protein is allowed to pass-through. Interaction between the interacting protein partners are monitored by measuring changes in the refractive angle, which is inversely proportional to the mass bound at the sensor surface.

Illustration: Surface Plasmon Resonance Imaging Interactivity

Place the shapes in their correct positions by clicking on purple cylinder (light source), which must result in the emission of the light rays as shown, followed by appearance of the final grey surface at the bottom.

II. STUDY OF PROTEIN-PROTEIN INTERACTIONS USING SPR AND SPRi

SPR-based sensing approaches have been successfully used in studying protein-protein and other biomolecular interactions (Table 1) (Torreri et al., 2005; Huber et al., 2006). In order to analyze interactions between two proteins using SPR-based sensing approaches; one of the interaction partners is immobilized on the sensor surface, while the other one is injected in solution (Willander and Al-Hilli 2009). Binding of second partner on the sensor surface due to biomolecular interactions leads to changes in the refractive angle, which is inversely proportional to the mass bound at the sensor surface. The quantity of bound materials is continuously measured as a function of time. After monitoring the interactions for certain period of time, the buffer solution is changed to stop the interaction to monitor the dissociation of the complex formation (Fig 2).

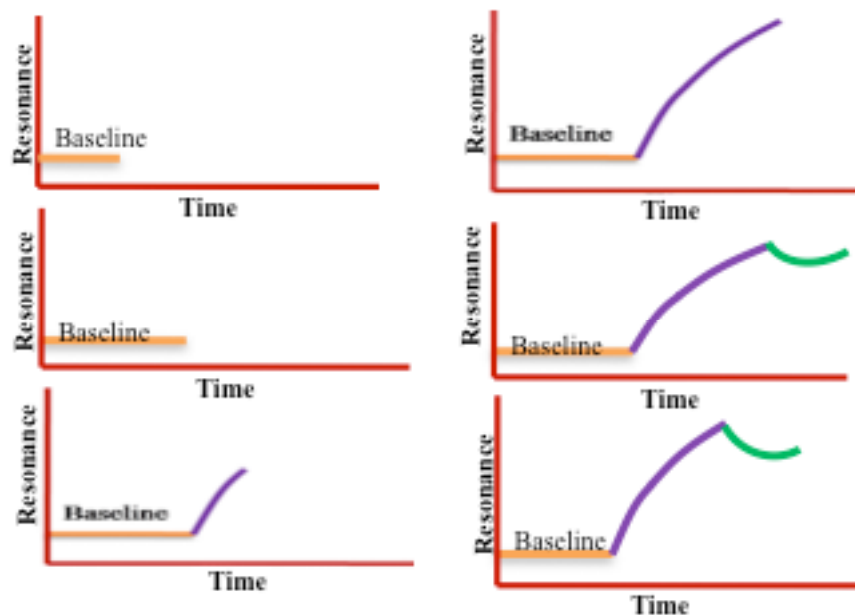
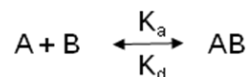


Fig 2. Determination of association and dissociation kinetics of biomolecular interaction using SPR. Different steps involved in biomolecular interaction analysis using SPR

sensor. The interaction between the molecules is measured by plotting the sensorgram as shown in panels.

SPR provides real-time measurements of interactions; for that reason kinetics of interactions [on rate (K_a) and off rate (K_d)] can be calculated.



Association rate constant of interaction can be calculated from the association period data, if the concentration of interacting partners is known. To measure the dissociation rate constant(s) exponential decay to the dissociation data are used (Berggård et al., 2007).

There are several published studies where SPR/SPRi has been used for measuring protein-protein or protein-small molecule interactions; few studies are described in tabulated formats (Table 1).

Table 1: Application of SPR and SPRi for studying protein-protein or protein-small molecule interactions

Study	Detection technique	Description
Antigen-antibody (IL6 and anti-IL6) interactions (Rispen et al., 2011)	SPR	Interaction between anti-interleukin 6 (IL6) antibodies, anti-IL6.16 and anti-IL6.8 with interleukin 6 (chips coated with either anti-IL6.16 or anti-IL6.8). Time point analysis was performed using different concentrations of IL6 and two antibodies (anti-IL6.16 and anti-IL6.8). Higher on-rate and smaller off-rate was found for anti-IL6.8 than anti-IL6.16, indicating higher affinity of anti-IL6.8 for interleukin 6.
Studying association or dissociation kinetics of diverse Fab and hK1 interaction (Wassaf et al., 2006)	SPR in combination with microarray	The overall aim of this study was detection of antibodies that bind at the active site of human tissue kallikrein 1 (hK1) and consequently inhibit the protease activity of hK1. Simultaneous analysis of kinetic constants (k_{on} and k_{off}) for 96 different F_{ab} fragments using array format. F_{abs} were categorized on basis of their capacity to recognize an apparent active site epitope. Immobilization of Fab was performed using specific capture surfaces (anti-cMyc or protein A).
IgG and Protein A interaction (Natarajan et al., 2008)	SPRi in combination with continuous-flow microfluidics (CFM)	This study demonstrated that coupling of continuous-flow microfluidics with SPRi could improve printing process. It allows immobilization of purified proteins on gold surface at a very low concentration. Low sample consumption and multiplexing capability are major advantages of this combined technology.
Clinically related protein-peptide	SPRi	Interactions of three peptides (C 20–40, C 131–150, and Ova 273–288) with rabbit anti-C 20–40 and anti-

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interactions (Cherif et al., 2006)		C 131–150 immune sera (1:100 diluted). Detection of such weak clinically relevant interactions is promising for clinical research.
Antibody-antigen binding (Nogues et al., 2010)	SPRi in combination with peptide microarrays	65kDa isoform of human glutamate decarboxylase (GAD65) and a human monoclonal antibody. Specific bindings of Rac1 and RhoA antibodies to their antigens immobilized on protein arrays were monitored by spectral SPR imaging. Kinetic parameters of the interaction were measured more efficiently than ELISA/RIA methods.
Interactions of GST-fusion proteins with their antibodies (Yuk et al., 2006)	SPRi (Wavelength interrogation-based self-constructed with Kretschmann-Raether geometry)	Interactions of glutathione S-transferase-fusion proteins with their antibodies; anti-Rac1 and anti-RhoA to Rac1 and RhoA have been studied. Quartz tungsten halogen lamp was applied as a light source. Protein arrays were prepared by immobilizing glutathione S-transferase (GST) fusion proteins on the glutathione surfaces. Protein arrays were analyzed by two-dimensional images.

III. DATA PROCESSING AND ANALYSIS

Processing of SPR raw data prior to analysis is essential to obtain superior interpretation of data generated during the association/ dissociation phase of SPR-based analysis of protein-protein interactions. After obtaining raw-data from experimental process Y-axis transformation is performed to fit the baselines of different time points and concentration variations (Fig 3). There are different commercially available software to fit curves and obtain apparent rate constants. More often presence of background spot intensity significantly reduces artifacts and improves the S/N ratio, and thereby quality of the data. To obtain sensorgram for kinetics analysis subtraction of background spots are essential (Fig 4). Response from reference surface and response of buffer injection are subtracted before analysis of SPR datasets. Considering a bidirectional interaction between two interacting partners the value for K_D (ratio of K_{on}/K_{off}) is generally calculated using a nonlinear least-squares analysis (O'Shannessy et al., 1993 & 1994).

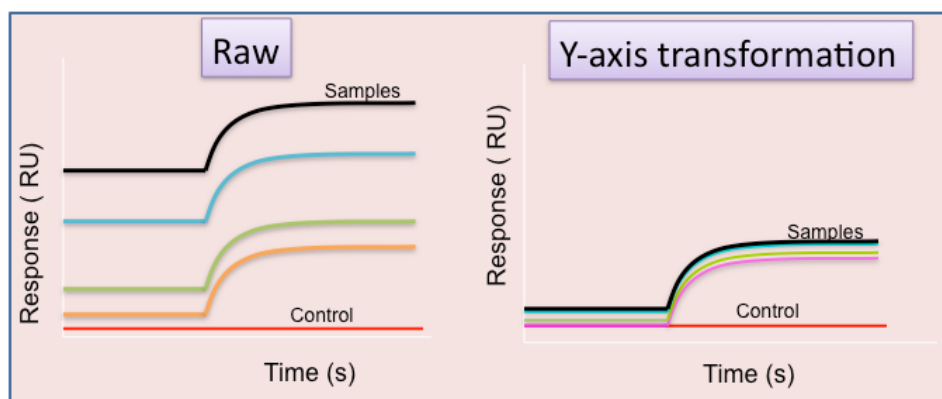


Fig 3. Y-axis transformation of SPR sensorgram to fit data

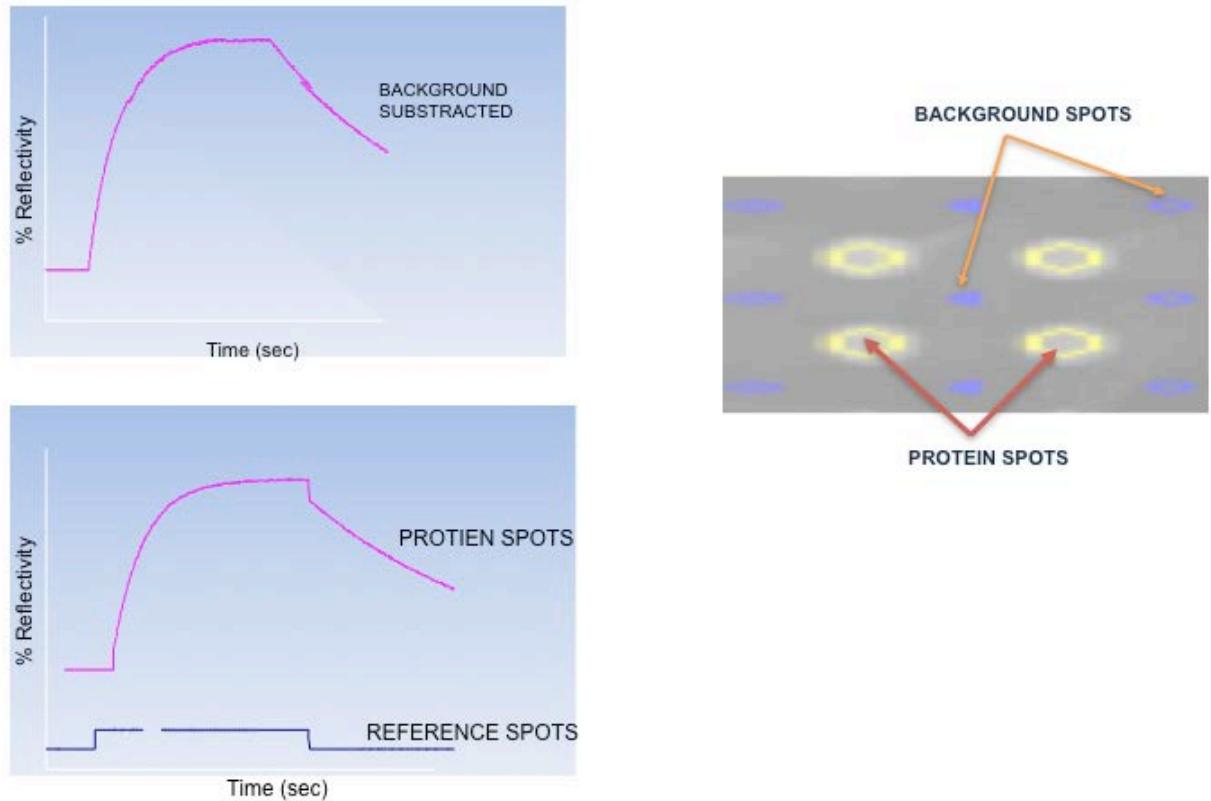


Fig 4. Subtraction of background reference spot intensity

To obtain kinetic constants various binding models, like simple Langmuir binding models are applied. Controls are prepared using empty surface for baseline checking. While determination of kinetics of reactions between two interacting partners, one partner is immobilized and multiple concentrations of second analyte are used and interactions are analyzed. Quality of the obtained data can be evaluated by verifying the K_{on} and K_{off} values.

Kinetics of binding is very crucial while selection of drug targets and screening of potential drug molecules. In SPR and SPRi the major advantage is that multiple interactions can be monitored simultaneously, which allows detection of interactions of two interacting partners using different concentration of ligands. The interaction of multiple analytes to same ligand can also be monitored and binding affinities can be compared. Even if multiple molecules have same affinity, they may have different K_{on} and K_{off} , and specific candidates can be selected according to the need of the investigator.

IV. CONCLUSIONS

Although SPR and SPRi-based biosensors are suitable for diverse types of applications, maximum use of those label-free sensing approaches are found to be in analysis of biomolecular interactions, since it provides real-time information regarding equilibrium binding constants, kinetic rate constants and thermodynamic parameters. Over the last decade large number of studies have proved the utility of SPR and SPRi-based biosensors for protein-protein and protein-small molecule interactions. Additionally, interactions of proteins with other types of biomolecules including protein-carbohydrate interactions (Yuk et al., 2006; Linman et al., 2008), DNA-protein interaction (Zhu et al., 2000) etc. are also vividly studied by SPR and SPRi. In spite of multiple advantages, SPR-based approaches have not yet gained extreme popularity in routine clinical use, mostly due to the detection cost associated with requirement of sophisticated instrumentation and restriction to gold/silver surfaces. To extend the applications of SPR-based techniques, now diverse amalgamated technological approaches have been developed such as SPR-MS, electrochemical SPR and surface plasmon fluorescence spectroscopy, which have successfully circumvented some of the basic limitations associated with SPR.

REFERENCES FOR FURTHER READING

- Torrieri P, Ceccarini M, Macioce P, Petrucci TC. Biomolecular interactions by Surface Plasmon Resonance technology. *Ann Ist Super Sanita*. 2005; 41(4):437-41.
- Huber W, Mueller F. Biomolecular interaction analysis in drug discovery using surface plasmon resonance technology. *Curr Pharm Des*. 2006; 12(31):3999-4021.
- Reddy PJ, Sadhu S, Ray S, Srivastava S. Cancer Biomarker Detection by Surface Plasmon Resonance Biosensors *Clin Lab Med*. 2012, 32(1), 47-72.
- Willander M, Al-Hilli S. Analysis of biomolecules using surface plasmons. *Methods Mol Biol*. 2009; 544:201-29.
- Ding X, Liu F, Yu X. Surface plasmon resonance biosensor for biomolecular interaction analysis based on spatial modulation phase detection. *Methods Mol Biol*. 2009; 503:21-35.
- Yuk, J. S., Kim, H. S., Jung, J. W., Jung, S. H. *et al.*, Analysis of protein interactions on protein arrays by a novel spectral surface plasmon resonance imaging. *Biosens. Bioelectron*. 2006, 21, 1521–1528.
- Berggård T, Linse S, James P. Methods for the detection and analysis of protein-protein interactions. *Proteomics*. 2007; 7(16):2833-42.

- O'Shannessy DJ, Brigham-Burke M, Soneson KK, Hensley P, Brooks I. Determination of rate and equilibrium binding constants for macromolecular interactions using surface plasmon resonance: use of nonlinear least squares analysis methods. *Anal Biochem.* 1993; 212(2):457-68.
- O'Shannessy DJ, Brigham-Burke M, Soneson KK, Hensley P, Brooks I. Determination of rate and equilibrium binding constants for macromolecular interactions by surface plasmon resonance. *Methods Enzymol.* 1994; 240:323-49.
- Rispens T, Te Velthuis H, Hemker P, Speijer H, Hermens W, Aarden L. Label-free assessment of high-affinity antibody-antigen binding constants. Comparison of bioassay, SPR, and PEIA-ellipsometry. *J Immunol Methods.* 2011; 365(1-2):50-7.
- Wassaf, D., Kuang, G., Kopacz, K., Wu, Q. L. *et al.*, Highthroughput affinity ranking of antibodies using surface plasmon resonance microarrays. *Anal. Biochem.* 2006, 351, 241–253.
- Natarajan, S., Katsamba, P. S., Miles, A., Eckman, J. *et al.*, Continuous-flow microfluidic printing of proteins for arraybased applications including surface plasmon resonance imaging. *Anal. Biochem.* 2008, 373, 141–146.
- Nogues C, Leh H, Langendorf CG, Law RH, Buckle AM, Buckle M. Characterisation of peptide microarrays for studying antibody-antigen binding using surface plasmon resonance imagery. *PLoS One.* 2010; 5(8):e12152.

- Garcia BH 2nd, Goodman RM. Use of surface plasmon resonance imaging to study viral RNA: protein interactions. *J Virol Methods*. 2008; 147(1):18-25.
- Cherif B, Roget A, Villiers CL, Calemczuk R, Leroy V, et al., Clinically related protein-peptide interactions monitored in real time on novel peptide chips by surface plasmon resonance imaging. *Clin Chem*. 2006; 52(2):255-62.