Surface Plasmon Resonance for Immunoassays

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Chem 395

Instructor: Prof.Rusling

Outline

- Introduction
- Understanding the Basics
- SPR- Instrumental components
- Applications in Immunoassays
- Advantages / Disadvantages
- Summary

What is SPR?

- **Surface sensitive optical detection method–interactions between biomolecules**

  protein-protein

  protein-ligand

  protein-DNA

  protein-membrane

- **Phenomenon that occurs when light is reflected off thin metal films.**
- **Light energy interacts with the delocalized e^-s in the metal surface – reduced reflected light intensity.**
- **Identification and Quantification (association, dissociation and equilibrium constants, and energetics) of these interactions.**

Understanding the Basics of SPR

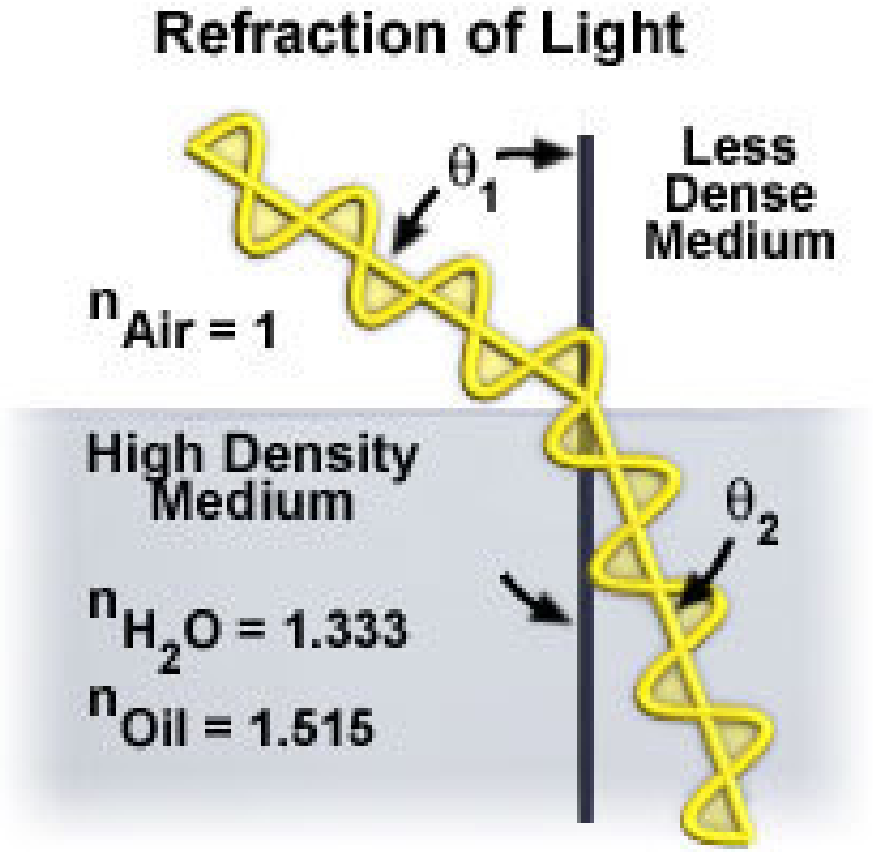
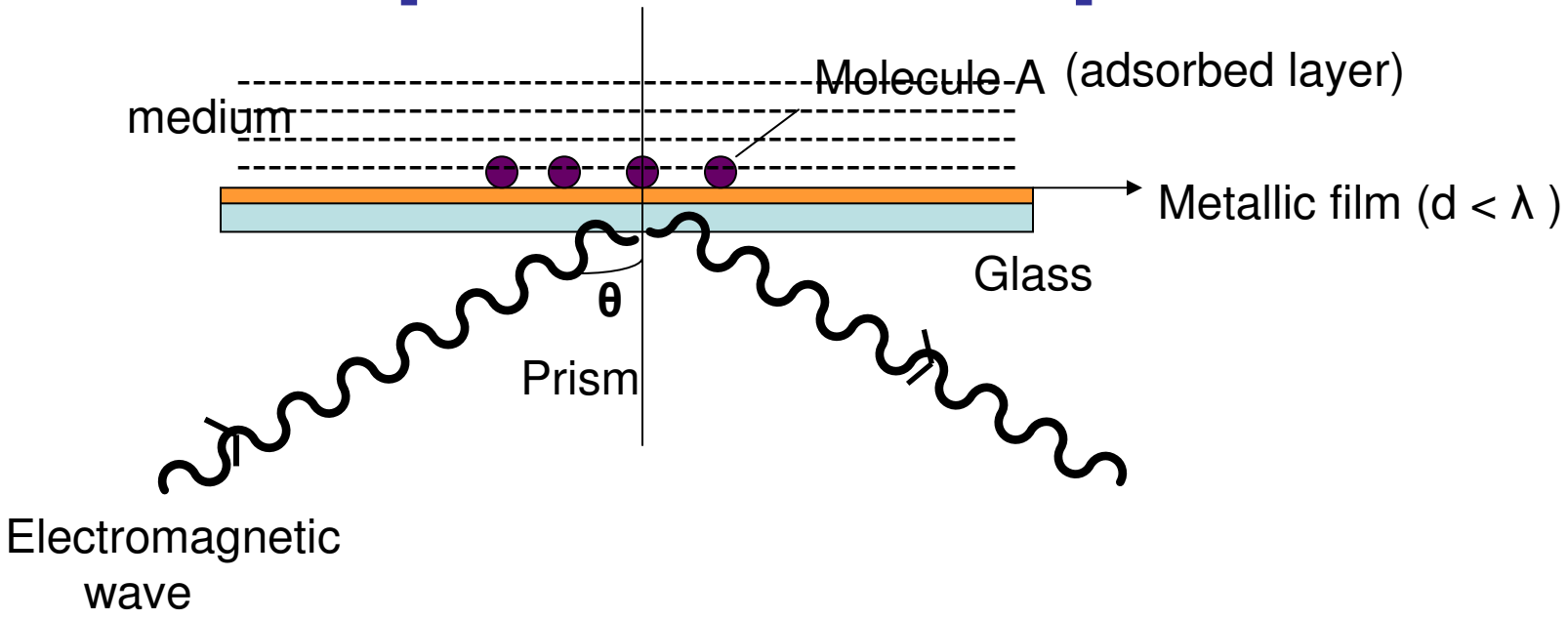


Figure 1

Refractive Index (RI) = Ratio of speed of light in vacuum to that in a denser medium

Principle of SPR operation



- **Tunneling of Electromagnetic field into interior side of surface (forming evanescent wave) and reflection .**

- **Photon energy absorbed by electrons on the surface when their momentum are equal (resonance condition).**

- **Oscillating electrons on the surface at resonance called "plasmons" hence the name "surface plasmon resonance" and the angle θ_{SPR} .**

Evanescent wave and θ_{SPR}

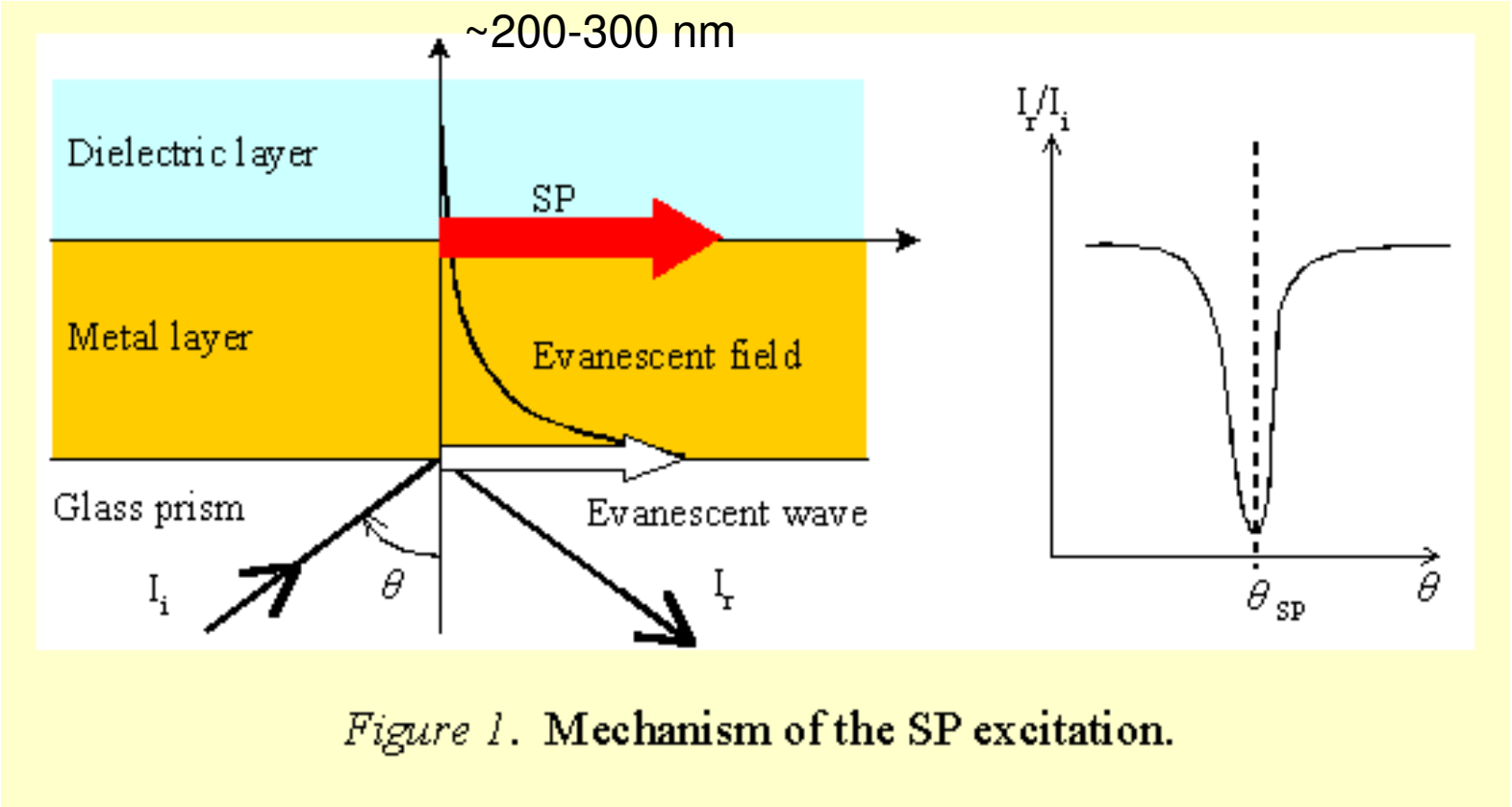


Figure 1. **Mechanism of the SP excitation.**

- **At θ_{SPR} , the reflected light intensity decreases and this difference is measured in SPR.**
- **When a molecule B interacts with immobilized A, shift in θ_{SPR} (or λ_{SPR}) can be observed due to change in refractive index.**

www.sys.eng.shizuoka.ac.jp/~j-kondoh/SP1.GIF

Refractive index change and permittivity

- **Refractive index change is related to the permittivity of the medium and adsorbed layer as below.**

$$n_{prism}.\sin(\theta) = \sqrt{\frac{\varepsilon_m . \varepsilon_{ad\ layer}}{\varepsilon_m + \varepsilon_{ad\ layer}}}$$

wave vector of radiation

$$\varepsilon_m = 1 - \frac{\lambda^2 \lambda_c}{\lambda_c(\lambda + i\lambda_c)}$$

$$\lambda = wavelength\ of\ resonance$$

$$\lambda_c = wavelength\ of\ plasmon$$

$$Hence, \varepsilon_m\ depends\ on\ \lambda$$

Two ways: either keep λ constant and vary θ or vice-versa and observe the change in the light intensity.

Summary-1

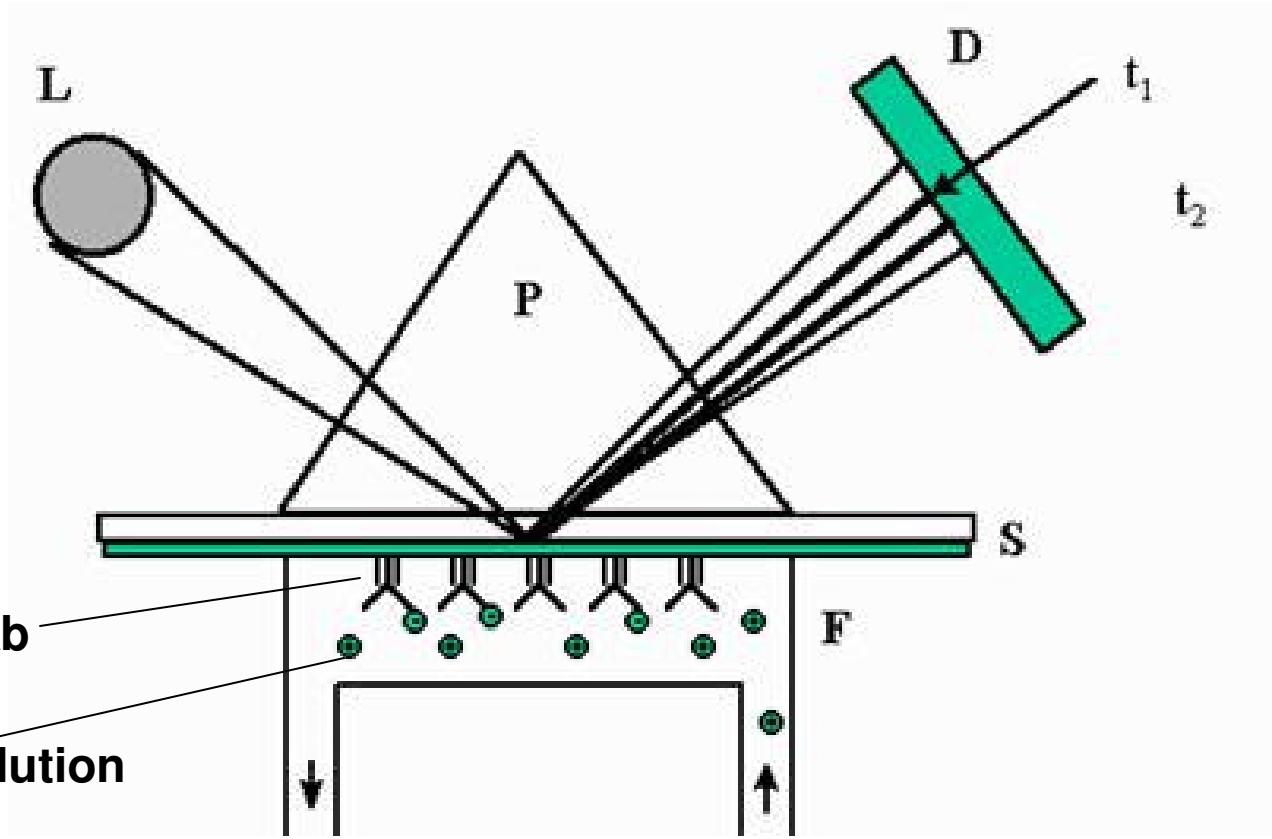
We have understood some basic concepts in SPR and interaction of molecules related to the SPR response.

-Let us know about immunoassays (remember Jim's lecture on Immunosensors).

Immunoassays

- A biochemical test-measures levels of a particular molecule in biological samples- e.g. serum, urine – uses antibody reaction to its antigen (specific binding).
- Clinically important in identifying pathogens. e.g. Prostate specific antigen, highly specific biomarker for prostate cancer.
- Monoclonal Antibody – binds only to one site of a particular antigen, hence specific and accurate.
- Polyclonal antibody – heterogeneous mixture of antibodies against different epitopes of the antigen.

SPR for Immunoassays

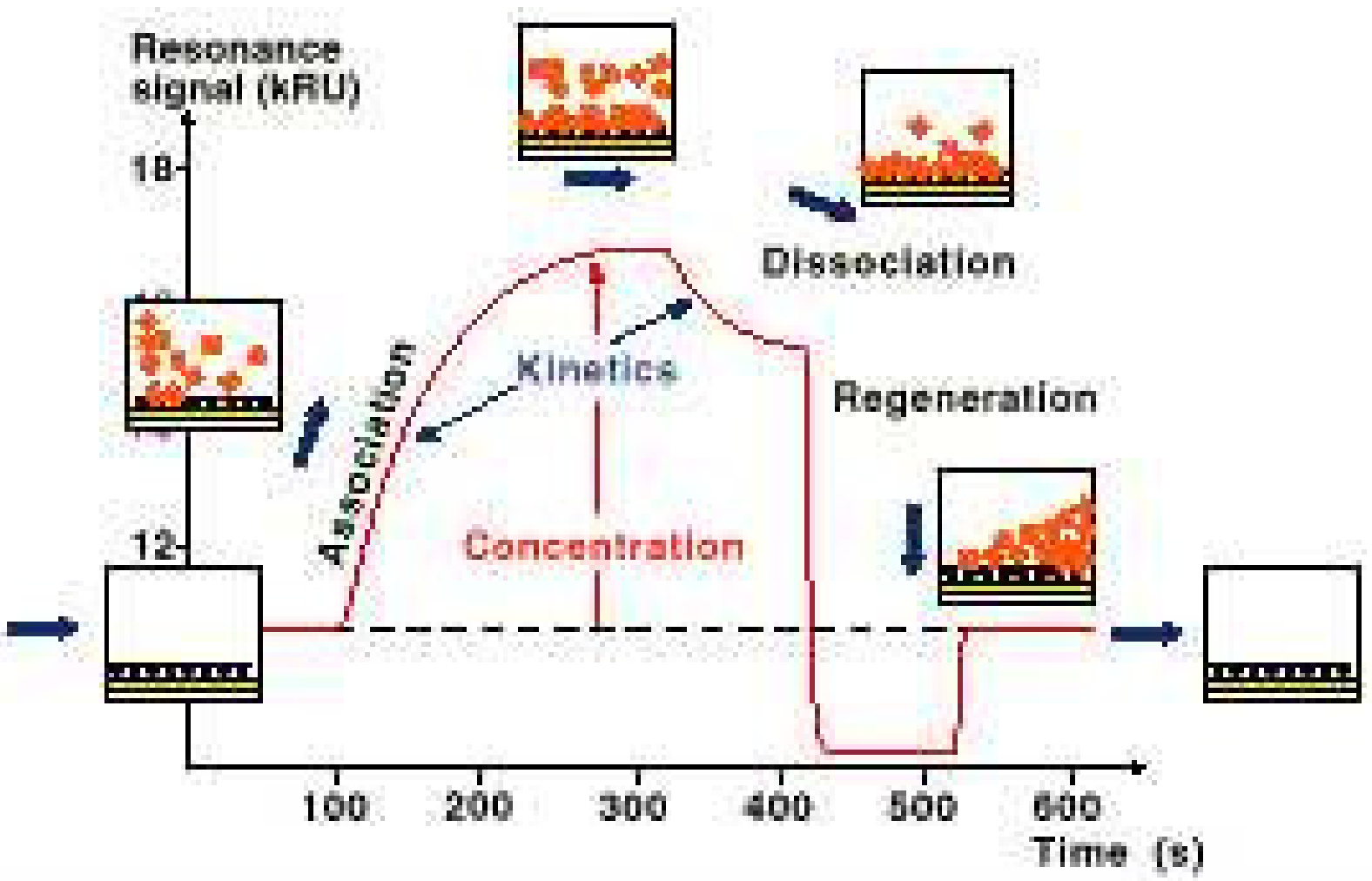


L - light source, P - prism, S – sensor surface, D– photodiode array, F- flow cell, Light intensity drop at times t_1 and t_2.

t_1 = before binding the antigen;
t_2 = resonance position after binding the antigen.

www.astbury.leeds.ac.uk/facil/SPR
Biacore SPR

SPR Sensogram



www.astbury.leeds.ac.uk/facil/SPR
Biacore SPR

Kinetics- Analysis of Experimental SPR Curves

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} A\text{-}B \text{ complex}, \quad K = \frac{k_a}{k_d} = \frac{[A.B]}{[A][B]}$$

Fit the experimental curve into various reaction models* and get the kinetic parameters from the best fit.

(1)Pseudo first-order reaction model

(2)Mass transport limitation model

(3)Inhomogeneous ligand model

(4)Inhomogeneous analyte model

*J. Luo *et al. J. Biochem.* 130, 553-559 (2001).

Kinetic analysis of a high-affinity antibody/antigen interaction performed by multiple Biacore users

Phinikoula S. Katsamba [a], Iva Navratilova [a], Maria Calderon-Cacia [b], Linsey Fan [c], Kevin Thornton [c], Mingde Zhu [c], Tim Vanden Bos [d], Carla Forte [d], Della Friend [d], Ite Laird-Offringa [e], Gisele Tavares, John Whatley, Ergang Shi [f], Angela Widom [g], Kevin C. Lindquist [h], Scott Klakamp [i], Andrew Drake [i], David Bohmann [j], Marina Roell [j], Larry Rose [j], Jill Dorocke [k], Bruce Roth [l], Béatrice Luginbühl [m], David G. Myszka [a,*]

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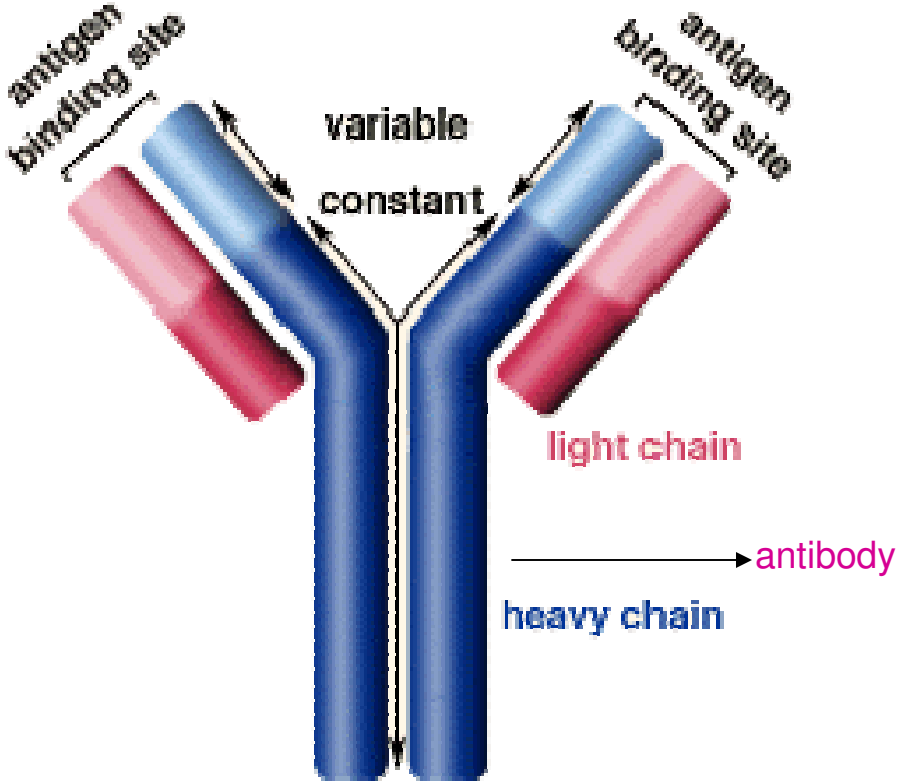
Prostate specific Antigen (PSA) binding to monoclonal antibody (mAb)



http://www.biology.arizona.edu/IMMUNOLOGY/tutorials/antibody/structure.html

Outline of the paper

- PSA- 30 kDa protein - routinely used marker in the diagnosis of prostate cancer.
- In this study, 22 participants measured the binding of PSA to a mAb by SPR.
- mAb-immobilized on carboxymethyl dextran surface-amine-coupling chemistry using EDC and NHS.
- Three different densities of mAb immobilized-varying contact times and dilution.
- [PSA] used in 2.5-600 nM range for k_a calculation.
- [PSA] of 600 nM for k_d experiment.
- Global fitting of data using 1:1 interaction model.

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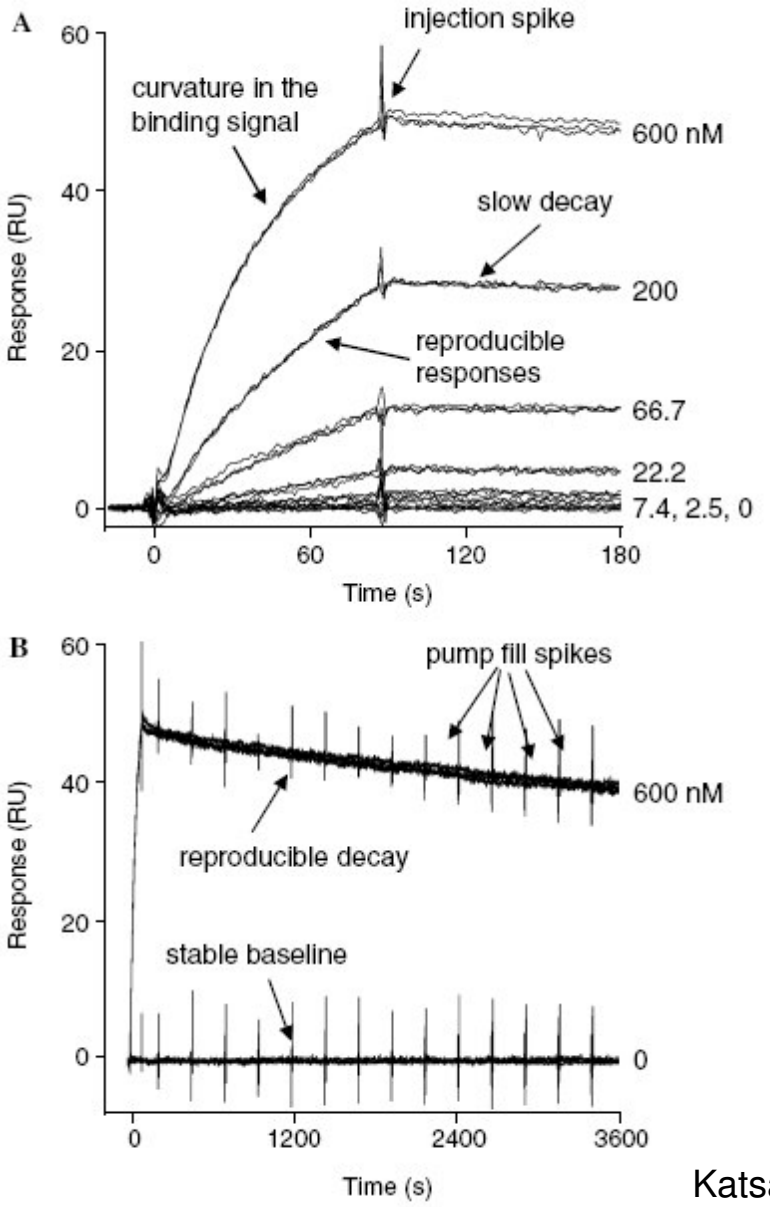
PSA/mAb – association and dissociation kinetics



➢**Association phase of the PSA/mAb interaction over a PSA concentration range is reproducible.**

➢**Dissociation phase of PSA/mAb interaction at [PSA]=600nM is reproducible. Spikes are artifacts arising from the filling of syringes.**

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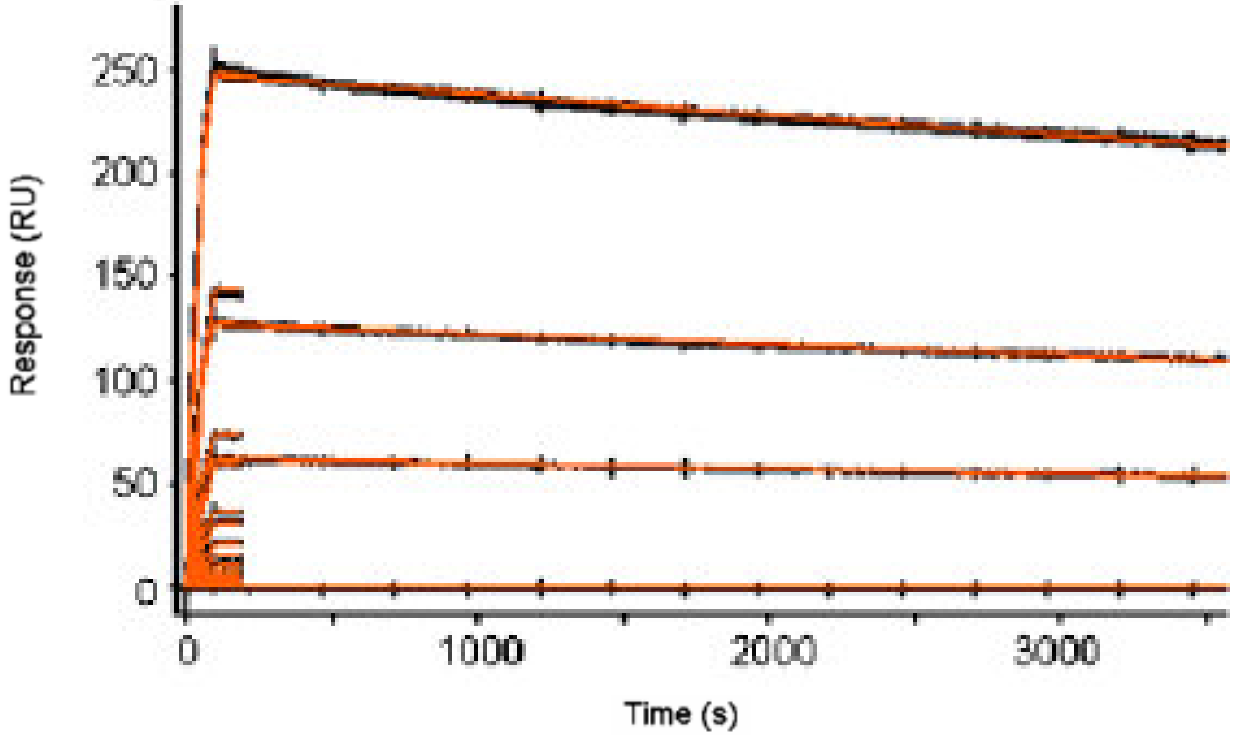
Analysis: 1:1 interaction model (A+B=AB), Scrubber software



Black lines = experimental (increasing concentrations of PSA)
Orange lines = model fit

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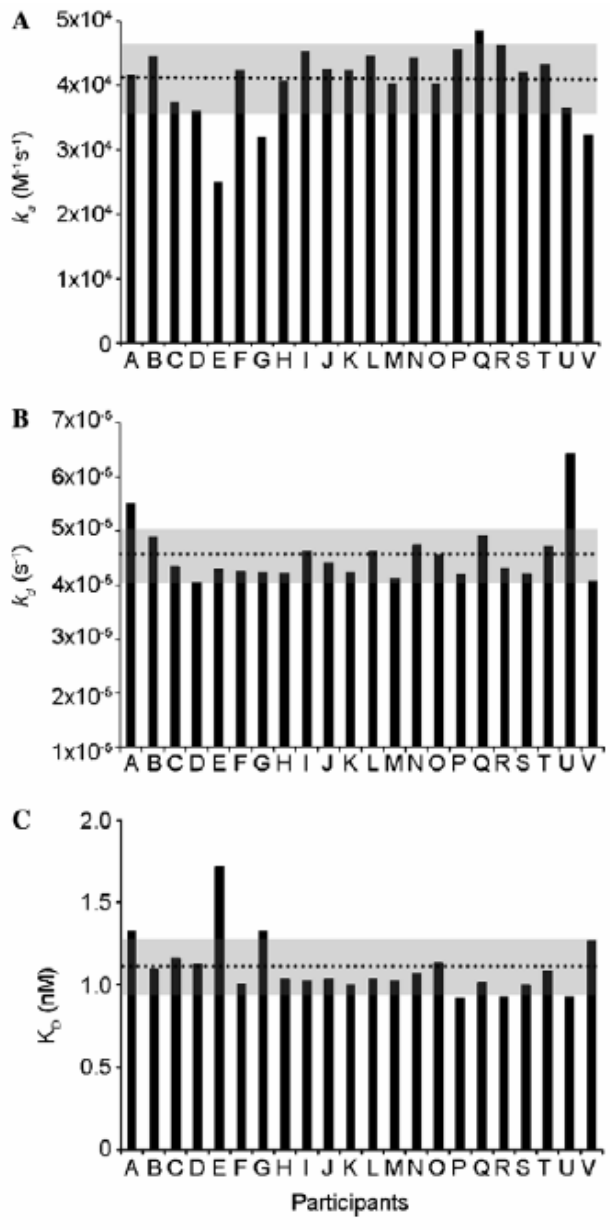
Distribution of kinetic rates & equilibrium binding constants among 22 users



$$k_a = (4.1 \pm 0.6) \times 10^4\ M^{-1}\ s^{-1}$$

$$k_d = (4.5 \pm 0.6) \times 10^{-5}\ s^{-1}$$

Equilibrium Binding constant,

$$K_D = \frac{k_d}{k_a} = 1.1 \pm 0.2\ nM$$

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Advantages

- Real time analysis & Label free technique – No need for radioactive, fluorescent or any other labelling.
- The Change in SPR signal - specific to the binding event - no need for purified sample – antigen in extracts can be used.
- Highly sensitive (RI changes $<10^{-5}$ with time resolution of few seconds) and simple construction.

Disadvantages

- **Mass transport can affect kinetic analysis.**
- **Any artifactual RI change other than from the interaction can also give signal.**
- **One of the interacting molecules should be immobilized on the surface.**
- **Thickness of the metal film (thin film is preferred).**

Summary-2

- **Surface plasmon resonance has been shown to be a powerful technique in studying real-time kinetics of immunoassays.**

- **Its advantages over other techniques can be understood.**

Acknowledgements

- Prof. Rusling
- Prof.Kumar
- Chem 395 class

Thank You

Questions?

Thickness and Surface concentration from SPR

$$d = (I_d / 2)(R / R_{\max}) = (I_d / 2)\{R / [m(\eta_a - \eta_s)\}$$

d =thickness of the adsorbed layer
l_d = decay length of evanescent wave
R = change in bulk index of refraction
m= slope of R vs $\Delta\eta$ plot
η_a = refractive index of adsorbed layer
η_s = refractive index of bulk solution.

$$N(in\,molecules/cm^3) = \frac{\theta(in\,molecules/cm^2)}{d(in\,cm)}$$

*J. Luo *et al. J. Biochem.* 130, 553-559 (2001).

Adsorption kinetics- anti-transferrin binding to staphylococcal protein A

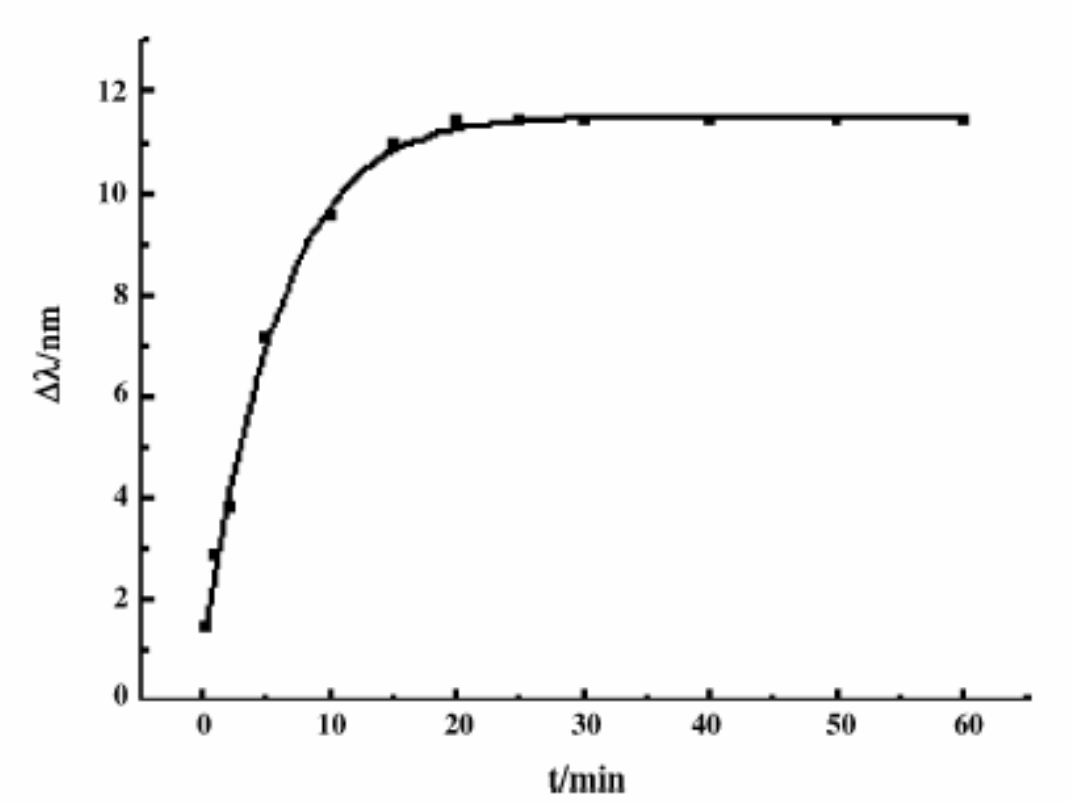

Fig. 2. The kinetic adsorption curve of anti-transferrin on the SPA monolayer in the directly immune assays.

*J. Luo *et al. J. Biochem.* 130, 553-559 (2001).

Differential rate equations of various reaction models*

Pseudo-first-order reaction model

$$d[B]/dt = -(k_a \cdot [A] \cdot [B] - k_d \cdot [AB])$$

$$d[AB]/dt = (k_a \cdot [A] \cdot [B] - k_d \cdot [AB])$$

$$R = [AB] + RI$$

Mass transport limitation model

$$d[A_{surr}]/dt = k_t \cdot ([A_{bulk}] - [A_{surr}]) - (k_a \cdot [A_{surr}] \cdot [B] - k_d \cdot [AB])$$

$$d[B]/dt = -(k_a \cdot [A_{surr}] \cdot [B] - k_d \cdot [AB])$$

$$d[AB]/dt = (k_a \cdot [A_{surr}] \cdot [B] - k_d \cdot [AB])$$

$$R = [AB] + RI$$

Inhomogeneous ligand model

$$d[B]/dt = -(k_{a1} \cdot [A] \cdot [B] - k_{d1} \cdot [AB])$$

$$d[B']/dt = -(k_{a2} \cdot [A] \cdot [B'] - k_{d2} \cdot [AB'])$$

$$d[AB]/dt = (k_{a1} \cdot [A] \cdot [B] - k_{d1} \cdot [AB])$$

$$d[AB']/dt = (k_{a2} \cdot [A] \cdot [B'] - k_{d2} \cdot [AB'])$$

$$R = [AB] + [AB'] + RI$$

Inhomogeneous analyte model

$$d[B]/dt = -(k_{a1} \cdot [A] \cdot MW \cdot [B] - k_{d1} \cdot [AB]) / MW - (2 \cdot k_{a2} \cdot [A_2] \cdot MW \cdot [B] - k_{d2} \cdot [A_2B]) / (2 \cdot MW)$$

$$d[AB]/dt = (k_{a1} \cdot [A] \cdot MW \cdot [B] - k_{d1} \cdot [AB])$$

$$d[A_2B]/dt = (2 \cdot k_{a2} \cdot [A_2] \cdot MW \cdot [B] - k_{d2} \cdot [A_2B])$$

$$R = [AB] + [A_2B] + RI$$

*J. Luo *et al. J. Biochem.* 130, 553-559 (2001).