BIOSENOSRS

BIO 580

Optical Biosensors- theory part 2

WEEK-8

Fall Semester

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Topics that will be covered in the course

- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
  - ✓Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - ✓Design considerations for use of recognition elements in biosensors
  - ✓Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
  - ✓Covalent modification of surfaces using surface chemistry
  - ✓Self Assembled Monolayers (SAM) and adsorptions
  - ✓Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
  - ✓Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - ✓Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
  - ✓Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - ✓Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
  - ✓Inter and Intra-assays and Coefficient of variation (CV)
  - ✓Signal to noise ratio
  - ✓Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Electromagnetic Field

➢The electromagnetic field is a physical field produced by electrically charged objects.

➢Light is the electromagnetic field in a certain frequency range.

➢At lower frequencies the electromagnetic field may be radio waves or infrared light, while at higher frequencies it may be UV light or x-rays, among others.

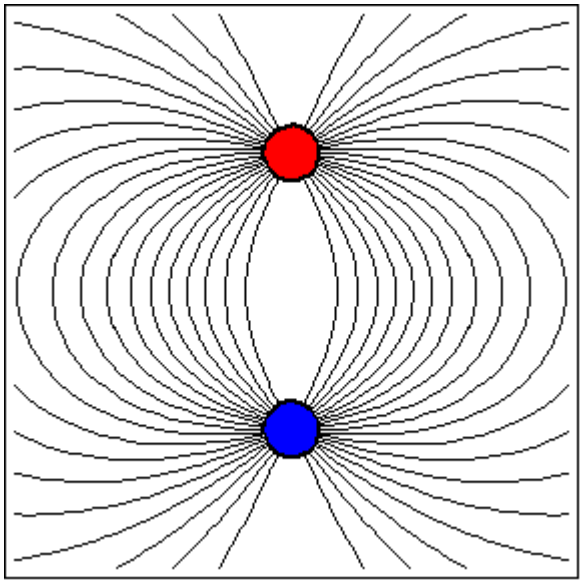
✓The electromagnetic field extends indefinitely throughout space and describes the electromagnetic interaction. It is one of the four fundamental forces of nature (the others are gravitation, the weak interaction, and the strong interaction).

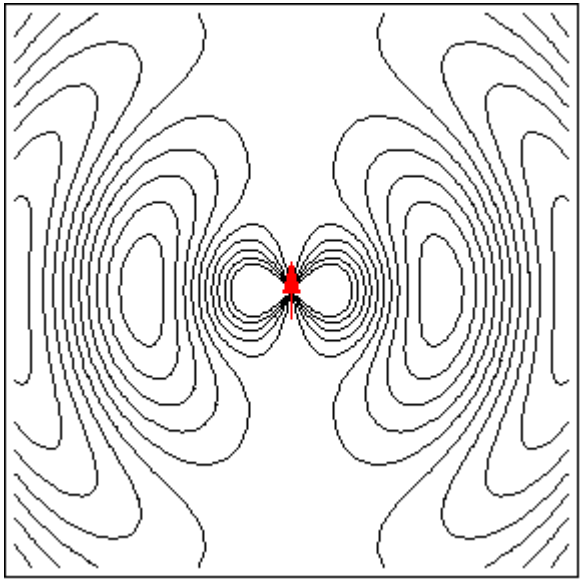
✓The field propagates by electromagnetic radiation; in order of increasing energy (decreasing wavelength) electromagnetic radiation comprises: radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays.

✓The field can be viewed as the combination of an electric field and a magnetic field.

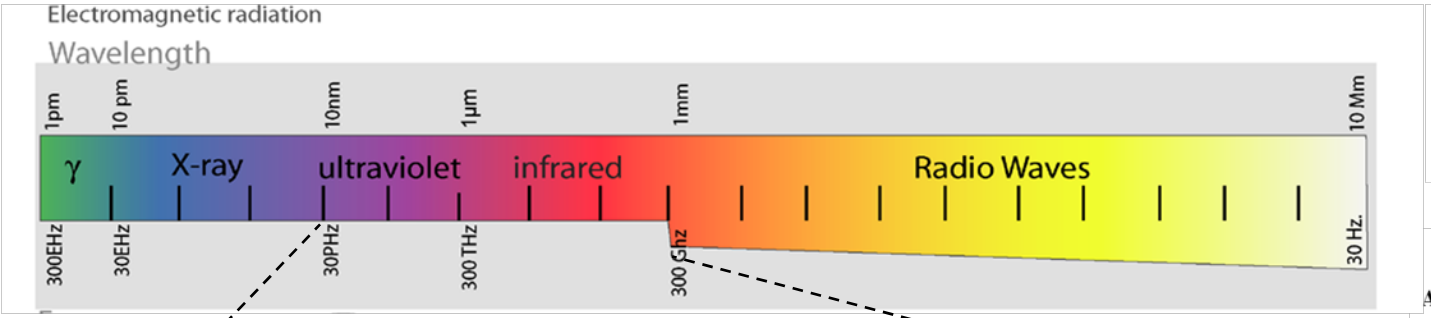
✓The electric field is produced by stationary charges, and the magnetic field by moving charges (currents); these two are often described as the sources of the field.

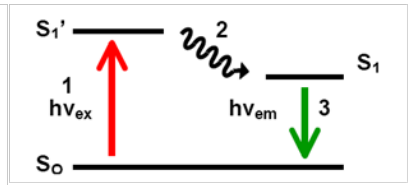
✓The way in which charges and currents interact with the electromagnetic field is described by Maxwell's equations and the Lorentz force law.

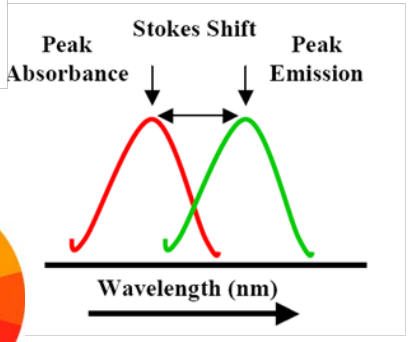


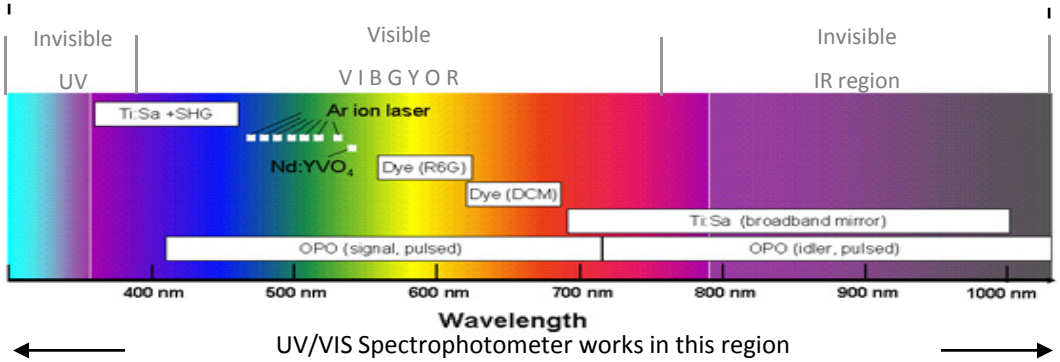


Spectrum, absorption and emission

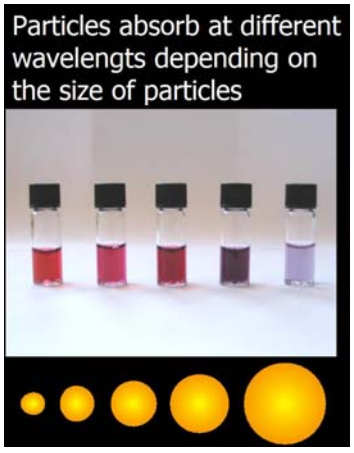


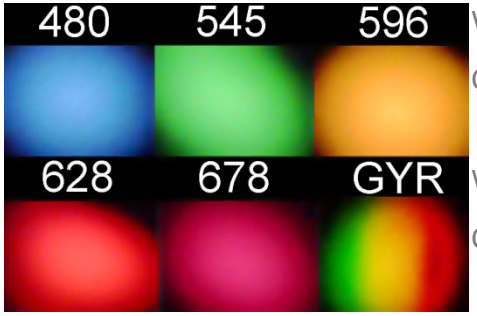






UV/VIS Spectrophotometer works in this region





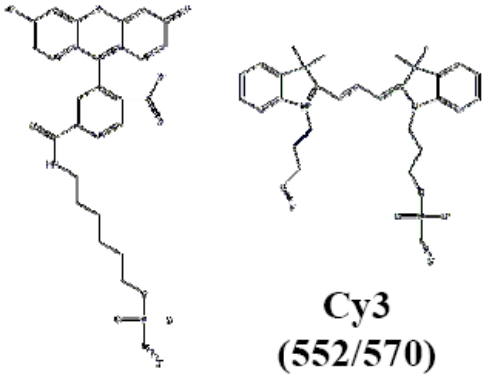
Wavelength (nm)

Color

Wavelength (nm)

Color

Examples



Brief Background

OPTICAL BIOSENSORS- an introduction

All molecules have the capability to interact with electromagnetic fields that pass through them because they contain atomic nuclei and a variety of electrons in various orbital states

Most fundamentally, electrons within molecules experience a force when they are exposed to the oscillating electromagnetic fields associated with the propagation of light. (Light is the electromagnetic field in a certain frequency range)

Molecules with an abundance of free electrons will become polarized by exposure to the light's electromagnetic field (one side of the molecule will be temporarily more negatively charged than the opposite side – resulting in the formation of an electric dipole)

The extent of the polarization may be different for any particular molecule depending on its size, shape, and orientation with respect to the electric field.

Polarizability of molecules

A constant known as the *electric susceptibility,* χ_e *, quantifies the extent* of a molecule's "polarizability," and molecules with greater χ_e *are more easily* polarized.

When a polarizable molecule is placed in an electric field, the induced electrical dipole produces a secondary electric field such that the resulting electric field (i.e., the sum of the originally applied field and the secondary field) is of lower magnitude than the applied field.

Because an electromagnetic field associated with light is time-varying, the electrons within the molecule will experience a time-varying force so that electrons will oscillate within the molecule.

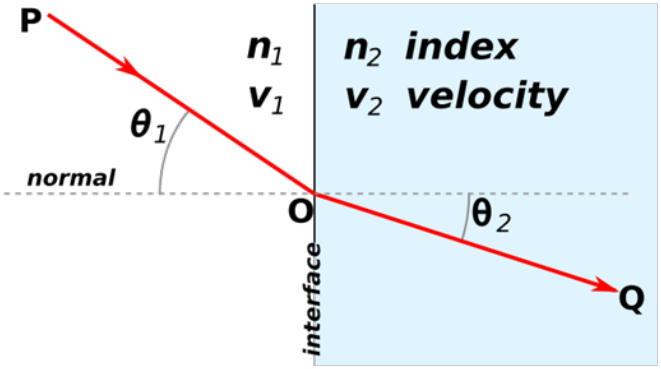
Moving electrons, by definition, produce an electrical current, so the molecule actually experiences a "polarization current" as a result of this electron motion.

The result of the polarization current is that light travels more slowly through the molecule than it would through free space.

The refractive index

Though free space has a permittivity defined by the constant ε_0, (*where* $\varepsilon_0 = 8.85 \times 10^{-12} F/m$), *the permittivity of a dielectric* material containing molecules is given by $\varepsilon = \varepsilon_r \varepsilon_0$, *where* εr *is known as the relative permittivity of the dielectric constant of the molecule.* ε_r *is directly related to* the polarizability of the molecule because it is mathematically defined as $\varepsilon_r = 1 + \chi_e$.

Many people are more familiar with the term *refractive index (n) to describe a* dielectric material. In an ordinary dielectric material at optical wavelengths, *n is* defined as $n = \sqrt{\varepsilon_r}$, *so the refractive index is directly related to the polarizability* of the molecules within a dielectric material.



Refraction of light at the interface between two m refractive indices, with $n_2 > n_1$. Since the phase vel second medium ($v_2 < v_1$), the angle of refraction θ_2 angle of incidence θ_1; that is, the ray in the higher- closer to the normal. (refractive index -Measure of refraction of a beam of light on entering a denser r

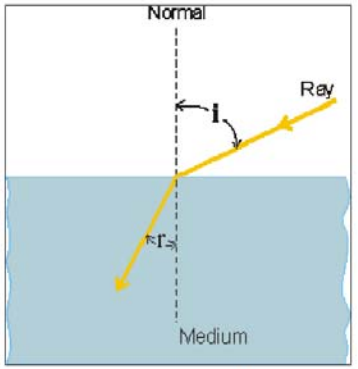


Figure 1: Refraction of light.

Generally, the refractive index is a quantity defined for a bulk material, while electric susceptibility and dielectric permittivity can apply to individual molecules such as those adsorbed to optical biosensor surfaces.

Optical Biosensor

The key behind optical biosensors' ability to detect biological analytes is that biological molecules, including proteins, cells, and DNA, all have dielectric permittivity greater than that of air and water.

Therefore, these materials all possess the intrinsic ability to reduce the propagation velocity of electromagnetic fields that pass through them.

Optical biosensors are designed to translate changes in the propagation speed of light through a medium that contains biological material into a quantifiable signal proportional to the amount of biological material present on the sensor surface.

In the design of optical biosensors, the detected biological material is often modeled as a thin film with a finite refractive index, although this is a simplification.

Several studies have been performed to characterize the dielectric properties of representative molecular monolayer films.

Therefore, if a biosensor transducer surface is covered with water, and if biological molecules can adsorb to the transducer surface, a small quantity of water molecules is displaced and replaced with a molecule that is more easily polarized by electromagnetic fields associated with light.

Therefore, the design goal for all optical biosensors is to provide a transducer with some externally measurable characteristic that is modified by changes in dielectric permittivity on its surface.

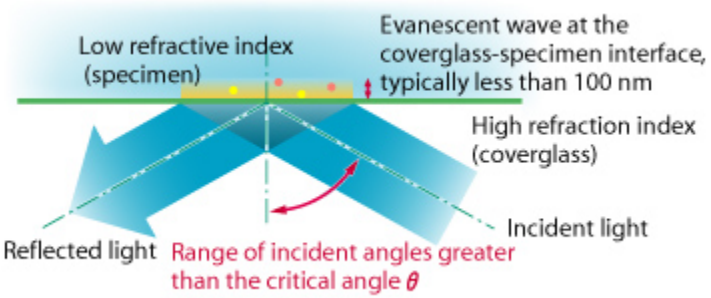
In this way, optical biosensors do not measure the mass of adsorbed material (as sometimes stated), although often the mass of deposited material is often related to the change in dielectric permittivity.

EVANESCENT-WAVE PHENOMENON

Total internal reflection of light at a surface–solution interface produces an electromagnetic field, or evanescent wave, that extends a short distance (100–200 nm) into the solution.

SPR is an evanescent- wave phenomenon that occurs at certain metallic surfaces.

Evanescent wave



Evanescent Wave Illumination method
Certain objects make it possible to introduce laser illumination at incident angles greater than the critical angle (θc) resulting in an evanescent wave immediately adjacent to the coverglass/metal-specimen interface.

The evanescent wave reaches maximally a few hundred nanometers into the specimen and its energy drops off exponentially.
Nikon's laser TIRF system utilizes this evanescent wave to excite single molecules in the thin section in contact with the coverglass. Because the specimen is not excited beyond the evanescent wave, this imaging system can produce fluorescence images with an extremely high signal-to-noise (S/N) ratio.

www.nikon.com/.../application/tirf2/index.htm

Evanescent wave, Surface Plamon Resonance, and θ_{SPR}

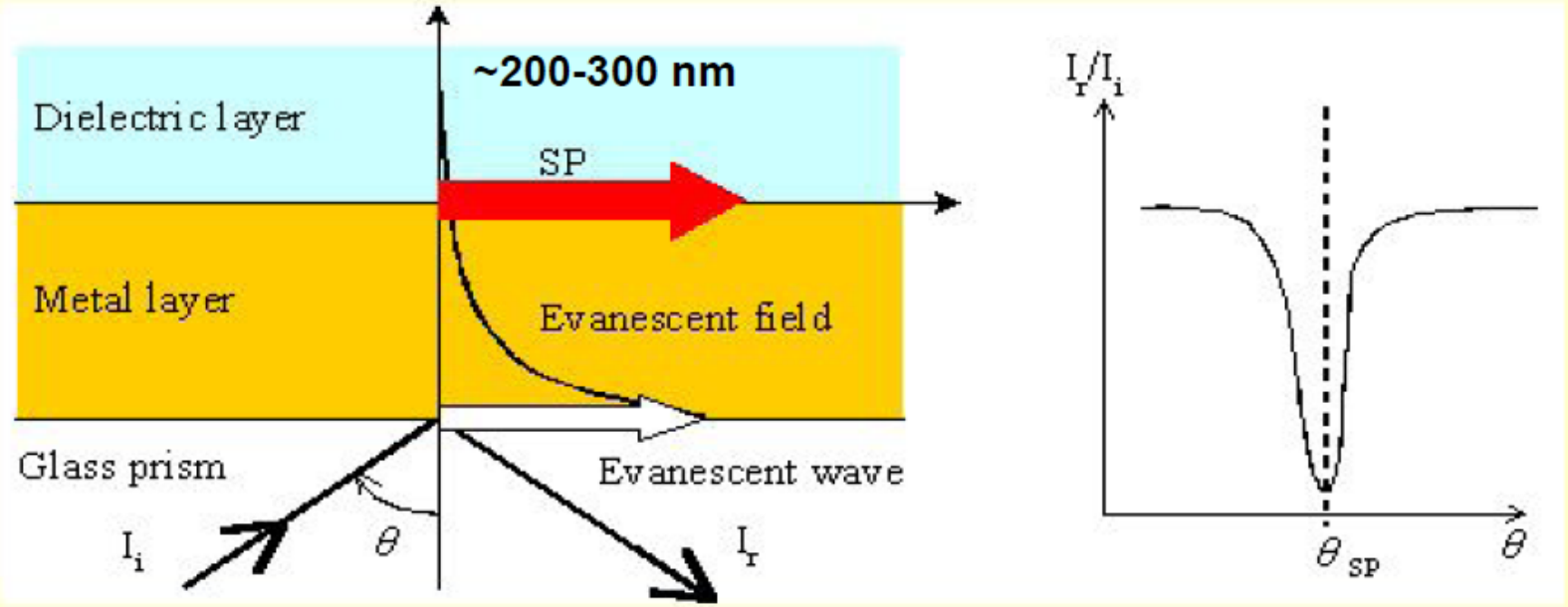
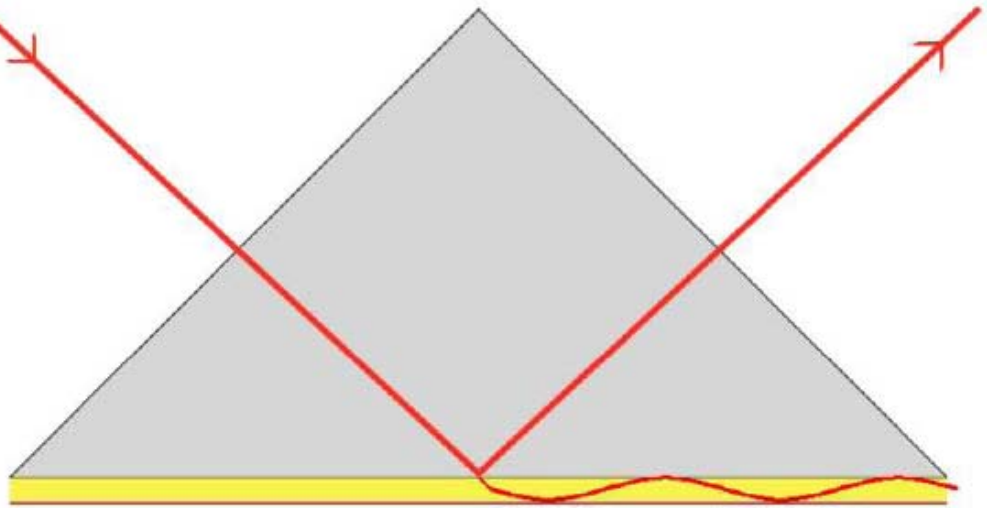


Figure 1. **Mechanism of the SP excitation.**

θ_{SPR} : a specific incident angle where SPR phenomenon occurs

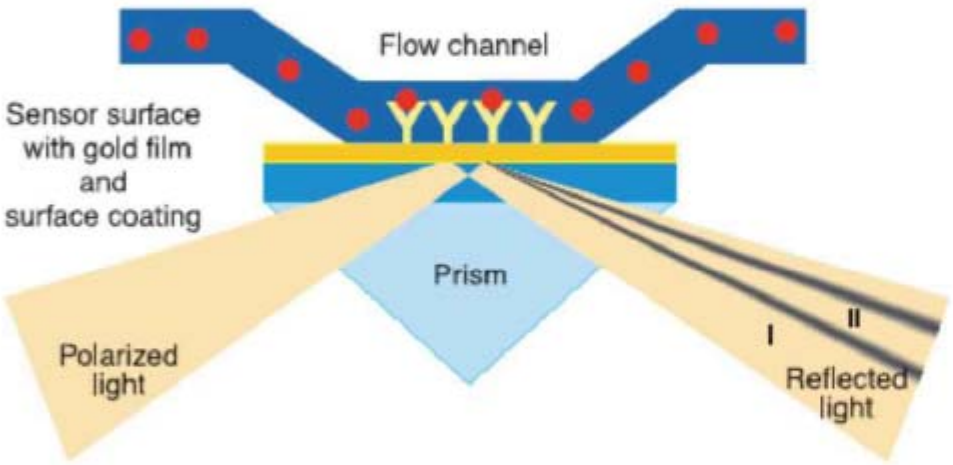
www.sys.eng.shizuoka.ac.jp/~j-kondoh/SP1.GIF

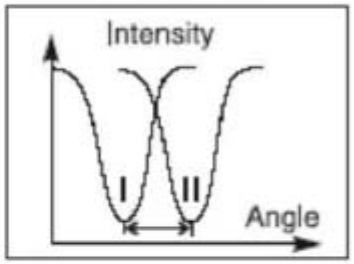
SPR (Surface Plasmon Resonance)



- SPR causes a reduction in intensity of incident light at a specific "resonance angle".
- The resonance angle depends on the local refractive index (n2)
- n2 depends on the local mass density of the sample medium surface

How Does SPR Detect Molecular Interactions





- Resonance angel changes upon ligand-analyte interaction
  (local mass density change→refractive index change→ resonance angel change)

Surface Plasmon Resonance (SPR)

- SPR – optical phenomenon based on evanescent field
- Evanescent wave is sensitive to refractive index (RI) in close proximity to the sensor surface
- Change in RI α solute concentration
- Advantages
  - Label-free technique
  - Highly sensitive to RI change, ~ 3×10^{-7} RU
  - Works in turbid or opaque samples

I. Surface Plasmon Resonance(SPR) Biosensor

A fraction of the light energy incident at a sharply defined angle can interact with the delocalised electrons in the metal film (plasmon) thus reducing the reflected light intensity. The precise angle of incidence at which this occurs is determined by a number of factors.

In biosensor applications the principal determinant becomes the refractive index close to the backside of the metal film. Target molecules are immobilised and addressed by ligands.

If binding occurs to the immobilized target the local refractive index changes, leading to a change in SPR angle, which can be monitored in real-time by detecting changes in the intensity of the reflected light. These changes can be used to determine affinity constants.

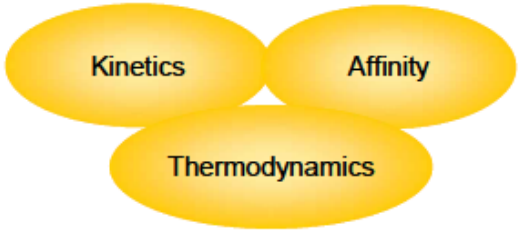
Where are SPR Biosensors used?

- **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.**
- **Identification and Quantification (association, dissociation and equilibrium constants, and energetics) of these interactions.**

What SPR Biosensors Measures



How fast, strong & why…

Is the binding of a lead compound

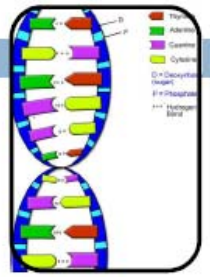
How specific & selective...

Is this drug binding to its receptor?

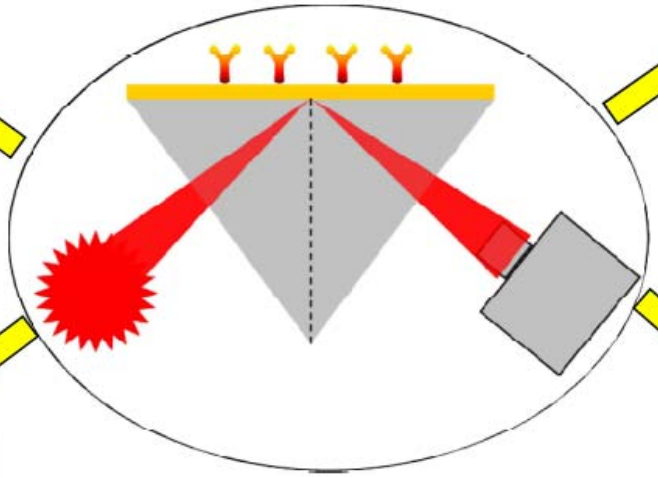
How much...

Biologically active compound is in a production batch?

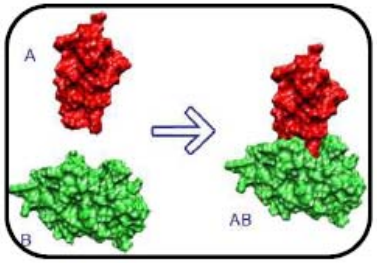
Biological Applications of SPR



DNA-DNA
or
DNA-RNA

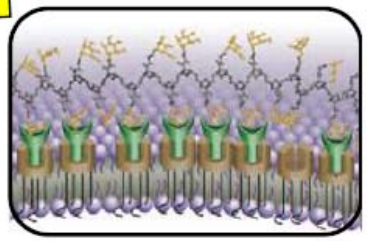


Antigen-Antibody



Protein-
protein

- Ligand - Receptor interaction
- RNA - Protein interaction
- Cell - Protein interaction
- Drug Screening



Carbohydrates

SPR detection

Principle

SPR detects refractive index changes close to the surface

E.g. accumulation of 1 pg/mm^2 gives a change of 1 µRIU or 1 RU

All biomolecules have refractive properties, so no labeling required

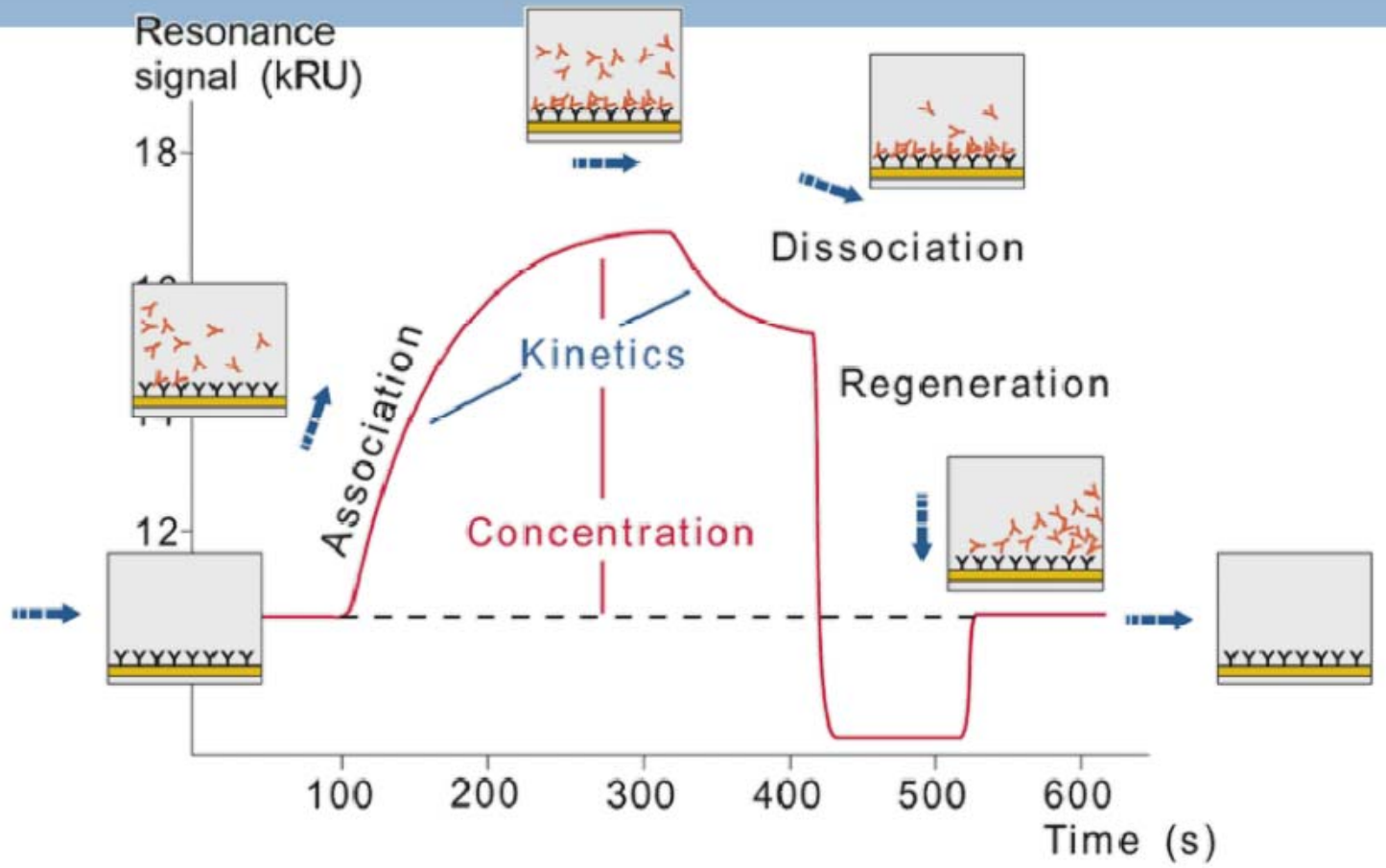
Result

No need to separate bound from free

This facilitates real-time measurements as a basis for taking kinetic data

Work with un-altered analytes possible

SPR Sensogram



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

Analysis of SPR with examples

- Specificity
- Concentration Assays
- Affinity Analysis

Specificity Analysis

- **Do two molecules interact with each other? Yes/No Answer**

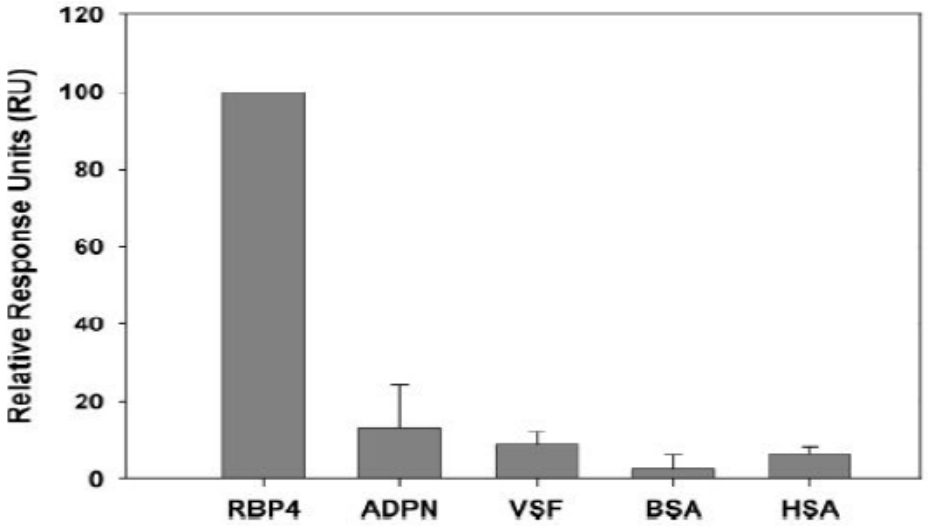


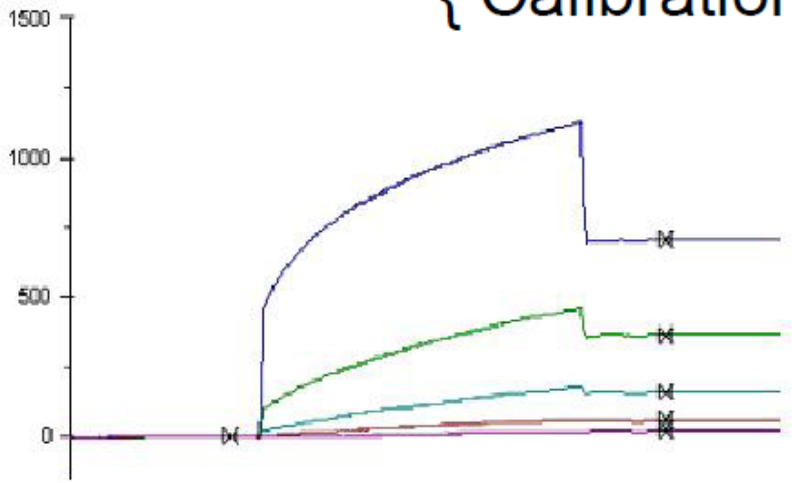
Figure 4. Specificity of aptamer no. 38 to RBP4. Specificity of the aptamer was determined with SPR analysis using a constant amount of ssDNA immobilized on the gold chip and by varying the concentrations of retinol binding protein 4 (RBP4), adiponectin (ADPN), visfatin (VSF), bovine serum albumin (BSA), or human serum albumin (HSA). The response units (RU) were obtained with 1 μM protein solutions at 25 °C and are shown relative with the RBP4 results.

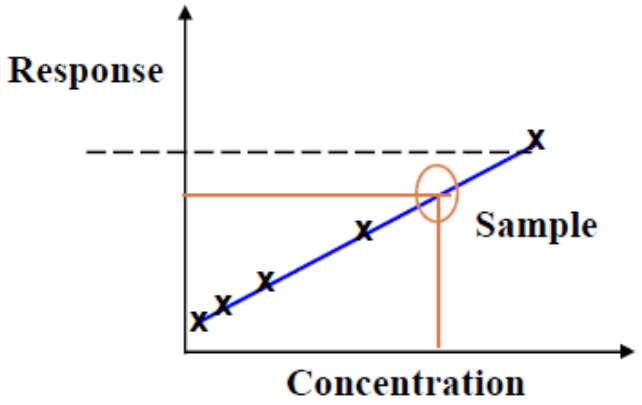
Lee et al., *Anal Chem*, 80, 2008

Concentration Assays

- Concentration based on biological activity
- All concentration assays require a calibration curve
- Concentrations of unknowns samples are calculated from this 4 - 7 concentrations in duplicate
- Direct binding formats

{ Calibration Curves }





Sample matrix for calibration curve

=

Sample matrix for unknown samples

Affinity Analysis

Equilibrium and Kinetic Constants are related

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

» Association rate: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

» Dissociation rate: $-\frac{d[AB]}{dt} = k_d \cdot [AB]$

» At equilibrium: Association = Dissociation

$$k_a \cdot [A] \cdot [B] = k_d \cdot [AB]$$

» The equilibrium constant:

$$K_A = \frac{[AB]}{[A] \cdot [B]} = \frac{k_a}{k_d} \qquad K_D = \frac{[A] \cdot [B]}{[AB]} = \frac{k_d}{k_a}$$

Rate Constants

	Association rate constant k_a	Dissociation rate constant k_d
Definition	$A + B \xrightarrow{k_a} AB$	$AB \xrightarrow{k_d} A + B$
Unit	$[M^{-1}s^{-1}]$	$[s^{-1}]$
Describes	Rate of complex formation, i.e. the number of AB formed per second in a 1 molar solution of A and B	Stability of the complex i.e. the fraction of complexes that decays per second.
Typical range	$1x10^{-3} – 1x10^{7}$	$1x10^{-1} – 5x10^{-6}$

Equilibrium Constants

	Equilibrium dissociation constant K_D	Equilibrium association constant K_A
Definition	$\frac{(A).(B)}{(AB)} = \frac{k_d}{k_a}$	$\frac{(AB)}{(A).(B)} = \frac{k_a}{k_d}$
Unit	[M]	[M^{-1}]
Describes	Dissociation tendency High K_D = low affinity	Association tendency High K_A = high affinity
Typical range	$1x10^{-5} – 1x10^{-12}$	$1x10^{5} – 1x10^{12}$

Kinetics- Analysis of Experimental SPR Curves

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

Fit the experimental curve into various reaction models* (non-linear regression model) 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).

Reaction rate equations

ZERO ORDER REACTION: A **0-order reaction** has a rate which is independent of the concentration of the reactant(s).

$$r = k$$

where r is the reaction rate, and k is the reaction rate coefficient with units of concentration/time.

$$2NH_3(g) \rightarrow 3H_2(g) + N_2(g)$$

FIRST ORDER REACTION: A **first-order reaction** depends on the concentration of only one reactant (a **unimolecular reaction**). Other reactants can be present, but each will be zero-order. The rate law for an elementary reaction that is first order with respect to a reactant A is $r = -\frac{d[A]}{dt} = k[A]$

$$\mathrm{H_2O_2}(l) \rightarrow \mathrm{H_2O}(l) + \frac{1}{2}\mathrm{O_2}(g)$$

$$\mathrm{2N_2O_5}(g) \rightarrow \mathrm{4NO_2}(g) + \mathrm{O_2}(g)$$

$$\mathrm{SO_2Cl_2}(l) \rightarrow \mathrm{SO_2}(g) + \mathrm{Cl_2}(g)$$

SECOND ORDER REACTION: A **second-order reaction** depends on the concentrations of one second-order reactant, or two first-order reactants.

For a second order reaction, its reaction rate is given by: either of the following 3 eqns.

$r = k[A]^2$ or $r = k[A][B]$ or $r = k[B]^2$

$$\mathrm{2NO_2}(g) \rightarrow \mathrm{2NO}(g) + \mathrm{O_2}(g)$$

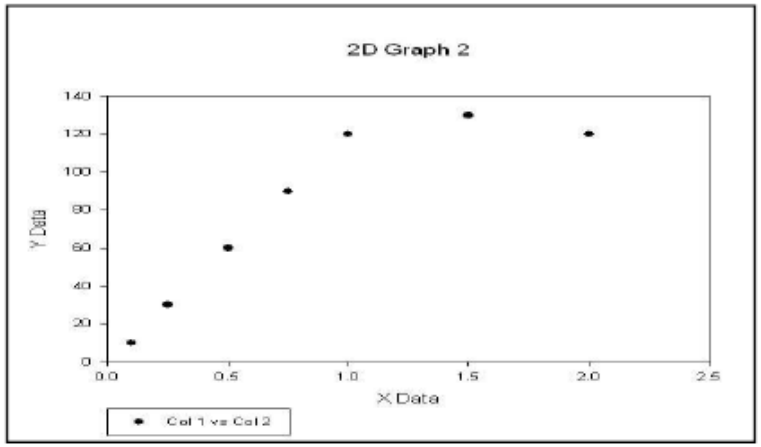
PSEUDO FIRST ORDER REACTION: Measuring a second order reaction rate can be problematic: the concentrations of the two reactants must be followed simultaneously, which is more difficult; or measure one of them and calculate the other as a difference, which is less precise. A common solution for that problem is the **pseudo first order approximation**

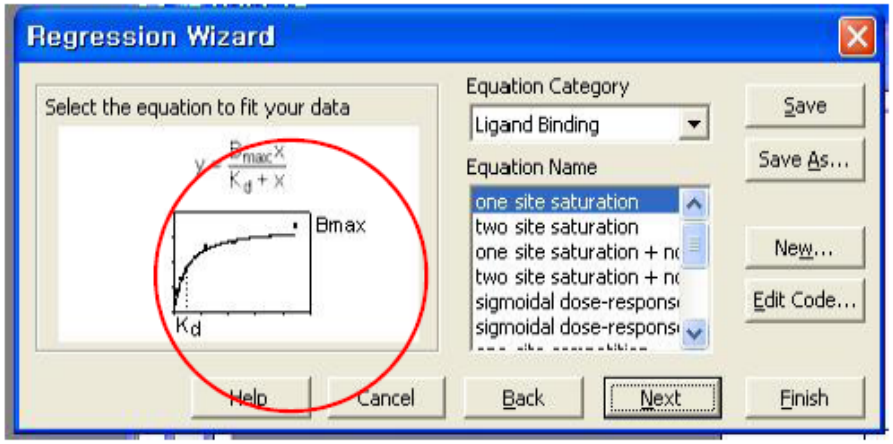
If *either* [A] or [B] remain constant as the reaction proceeds, then the reaction can be considered **pseudo first order** because in fact it only depends on the concentration of one reactant. If for example [B] remains constant then:

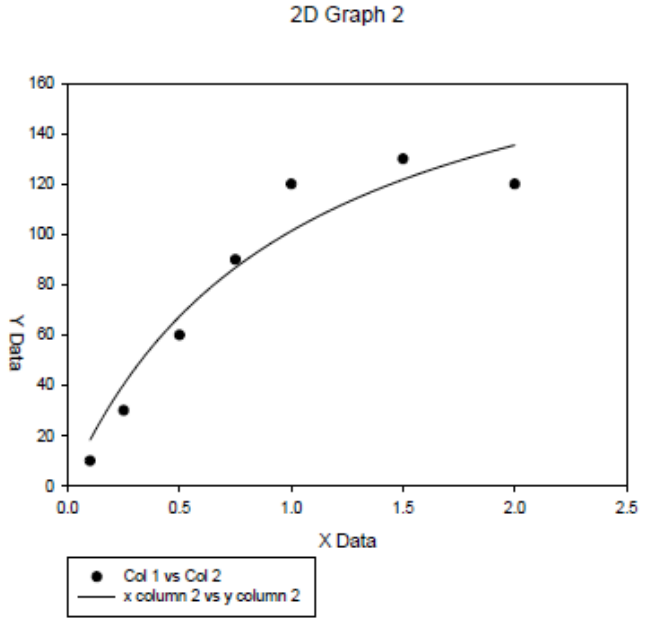
$$r = k[A][B] = k'[A]$$

Data 1*

	uM	RU
	1	2
1	0.1000	10.0000
2	0.2500	30.0000
3	0.5000	60.0000
4	0.7500	90.0000
5	1.0000	120.0000
6	1.5000	130.0000
7	2.0000	120.0000

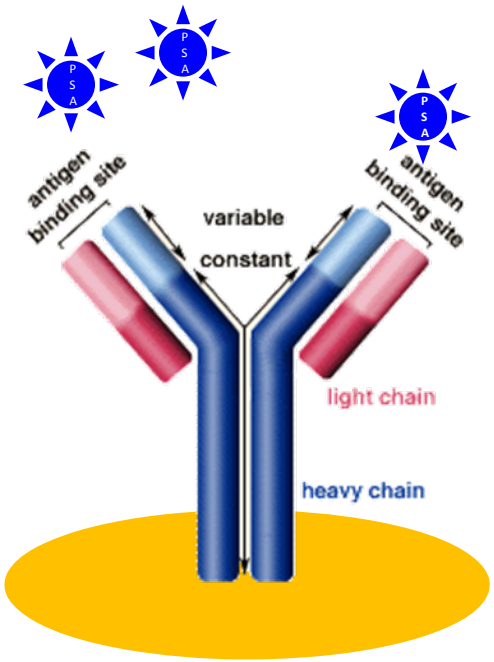






Use of Sigma Plot

Eg., PROSTATE SPECIFIC ANTIGEN (PSA) BINDING TO MONOCLONAL ANTIBODY (MAB)



- 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.

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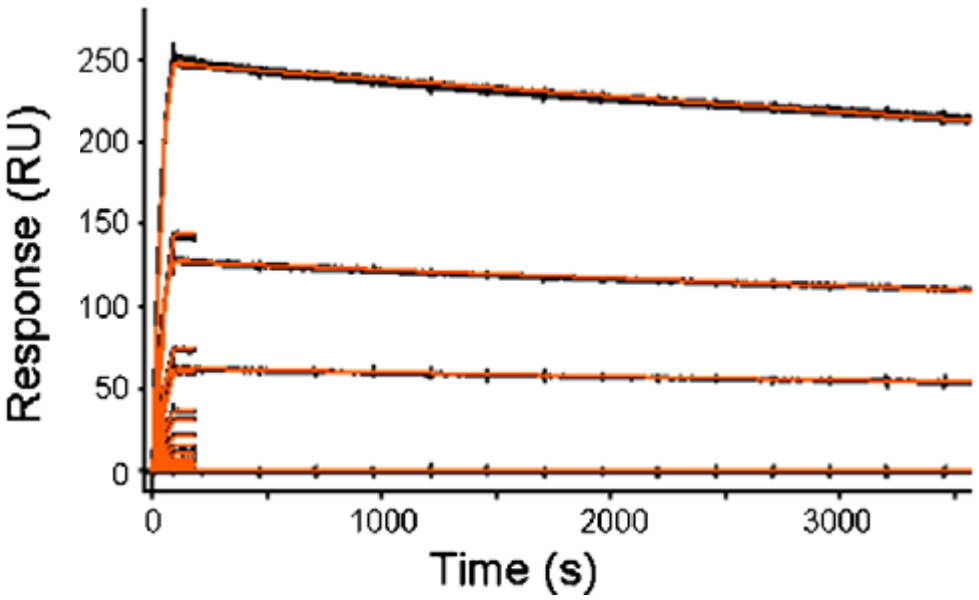
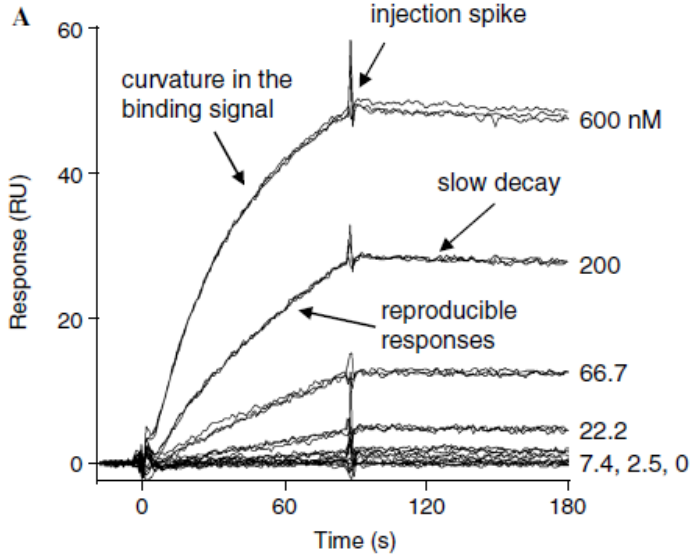
- [PSA] of 600 nM for k_d experiment.
- Global fitting of data using 1:1 interaction model.

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

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





Black lines = experimental (increasing concentrations of PSA)

Orange lines = model fit

Katsamba et al. Analytical Biochemistry 352 (2006) 208–221

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

- Surface plasmon resonance detects binding events as changes in mass at the chip surface
- Real-time kinetic measurements
- Qualitative rankings
- Measurement of concentrations
- Information about structure-activity relationships
- No labeling and low volumes samples needed