Surface Plasmon Resonance: antigen-antibody interactions

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Analytical Techniques for Biomaterial Characterization

- ***Scanning Probe Microscopy (SPM):***
  - image changes at surface
- ***Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR):***
  - study conformational changes at solid-aqueous interfaces, although lacks sensitivity
- ***Spectral Ellipsometry:***
  - determination of thickness and refractive index of adsorbed layer
- ***Surface Plasmon Resonance (SPR):***
  - rapidly monitor dynamic processes to a wide range of biomedically relevant interfaces.

What is SPR?

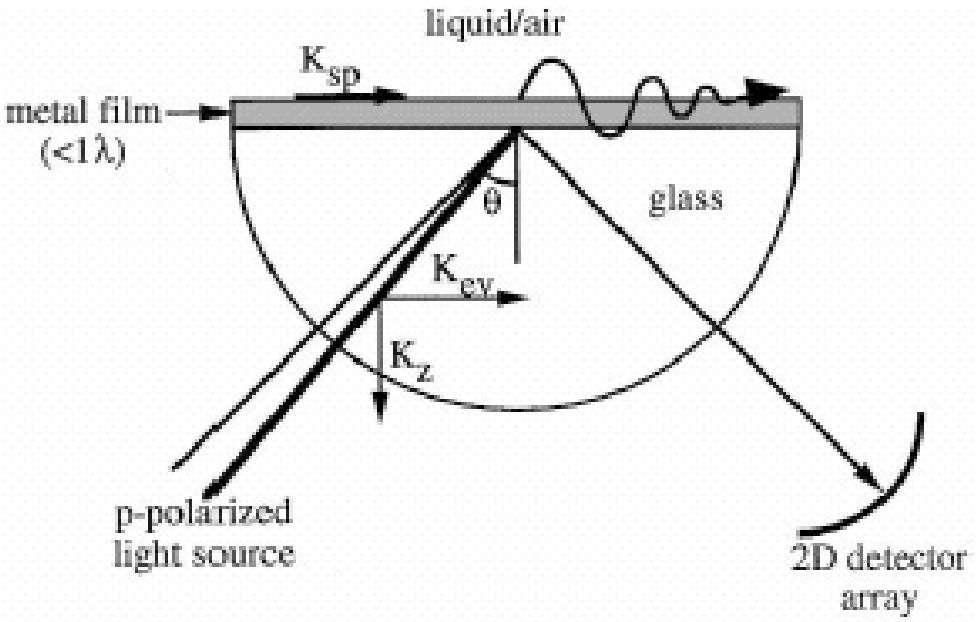
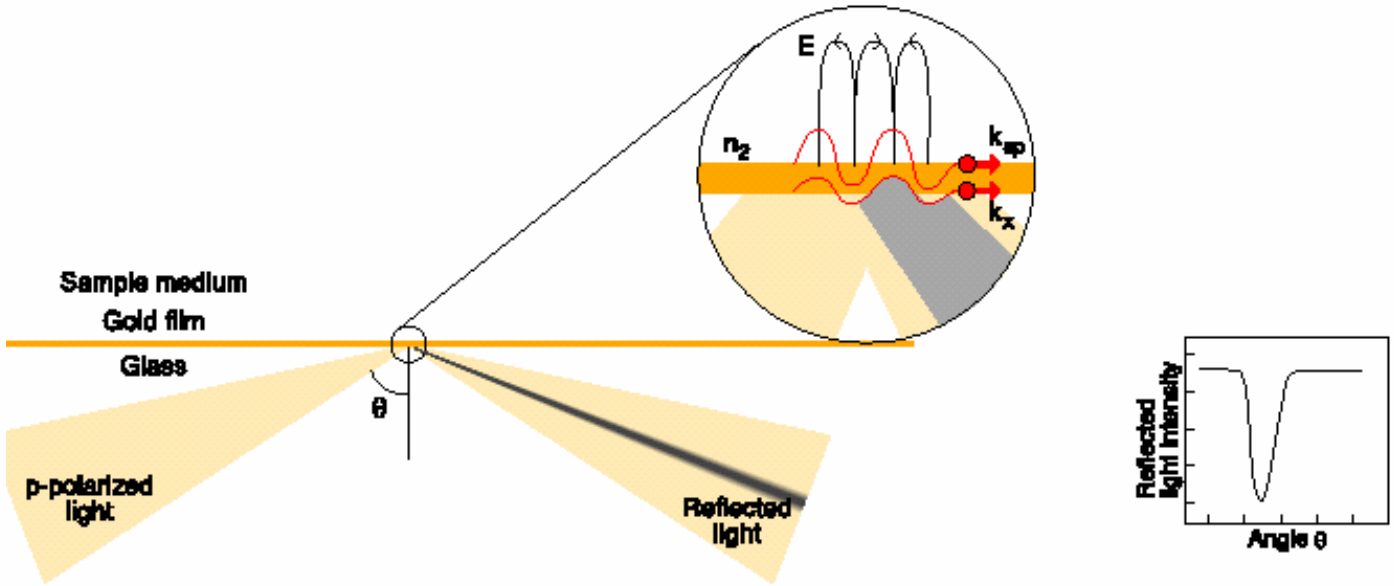


Fig. 1. The Kretchmann configuration for SPR. Resonance of a surface plasmon is excited at the metal/air interface when the angle of incidence of light is such that the evanescent component of its wave vector (K_{ev}) is equal to the wave vector of the propagating surface plasmon (K_{sp}).

- ***Surface Plasmon:*** Longitudinal charge density wave along the interface of two media, where one is metal and other is dielectric

SPR Principle

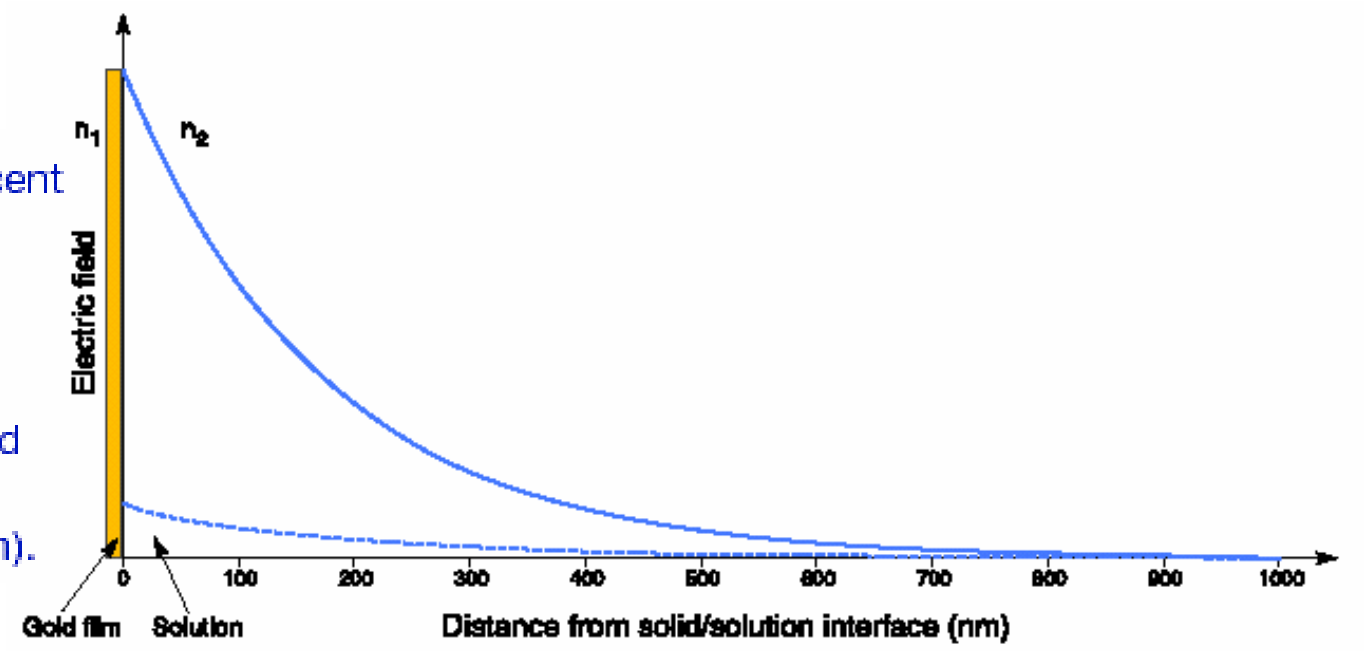
Figure 2. SPR is excited by p-polarized totally internally reflected light at a glass/metal film interface, the surface plasmon enhancing the evanescent field amplitude, E. In Biacore systems which use a sensor chip, this interface takes the form of an exchangeable gold-coated glass slide. SPR is observed as a dip in the reflected light intensity at a specific angle of reflection.



- Linear relationship is found between resonance energy and mass concentration of biochemically relevant molecules.

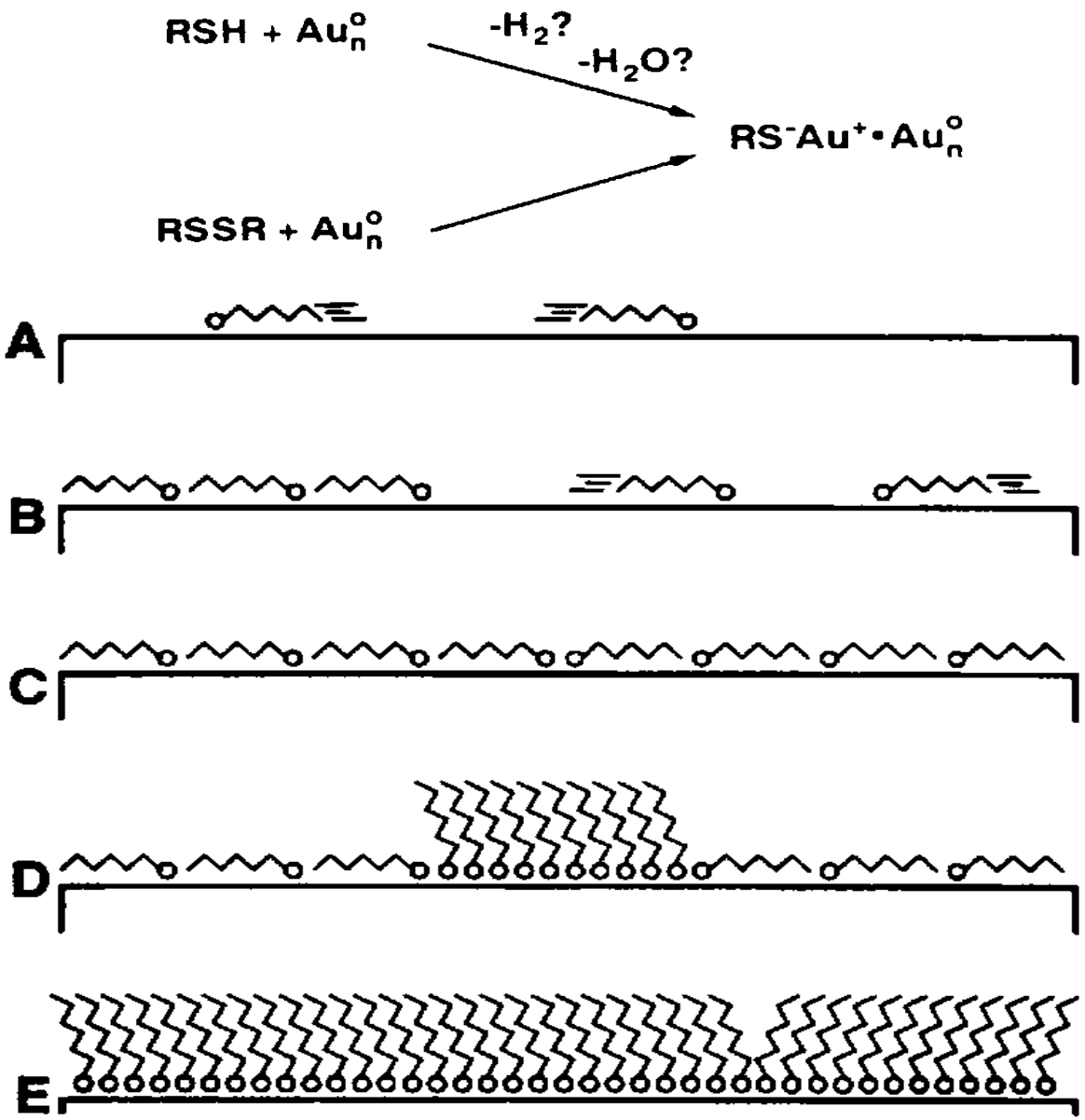
Advantage of using gold film

Figure 4. Relative evanescent electric field amplitude (E) versus distance to solid/ solution interface (nm). Continuous line for SPR-evanescent wave (gold film), dashed line for non-absorbing TIR (no gold film).

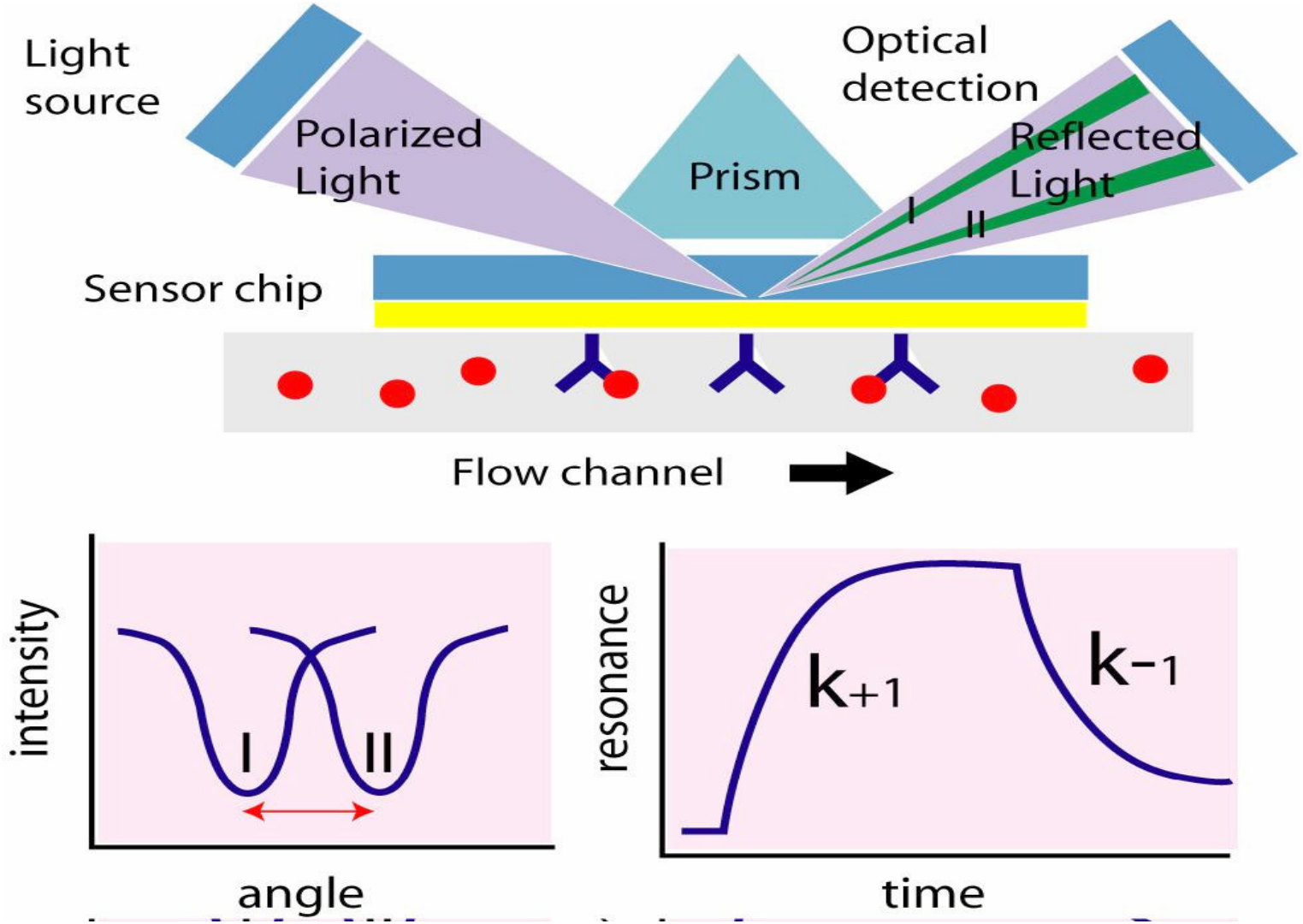


- ***Gold:*** Non-magnetic, surface plasmon wave is p-polarized, and due to its electromagnetic and surface propagating nature, creates enhanced evanescent wave

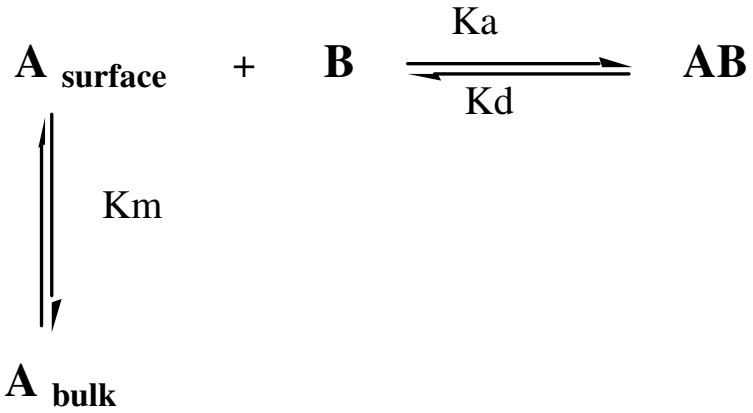
Gold-thiol Chemistry



Typical SPR Signal



SPR: Kinetics of Association phase



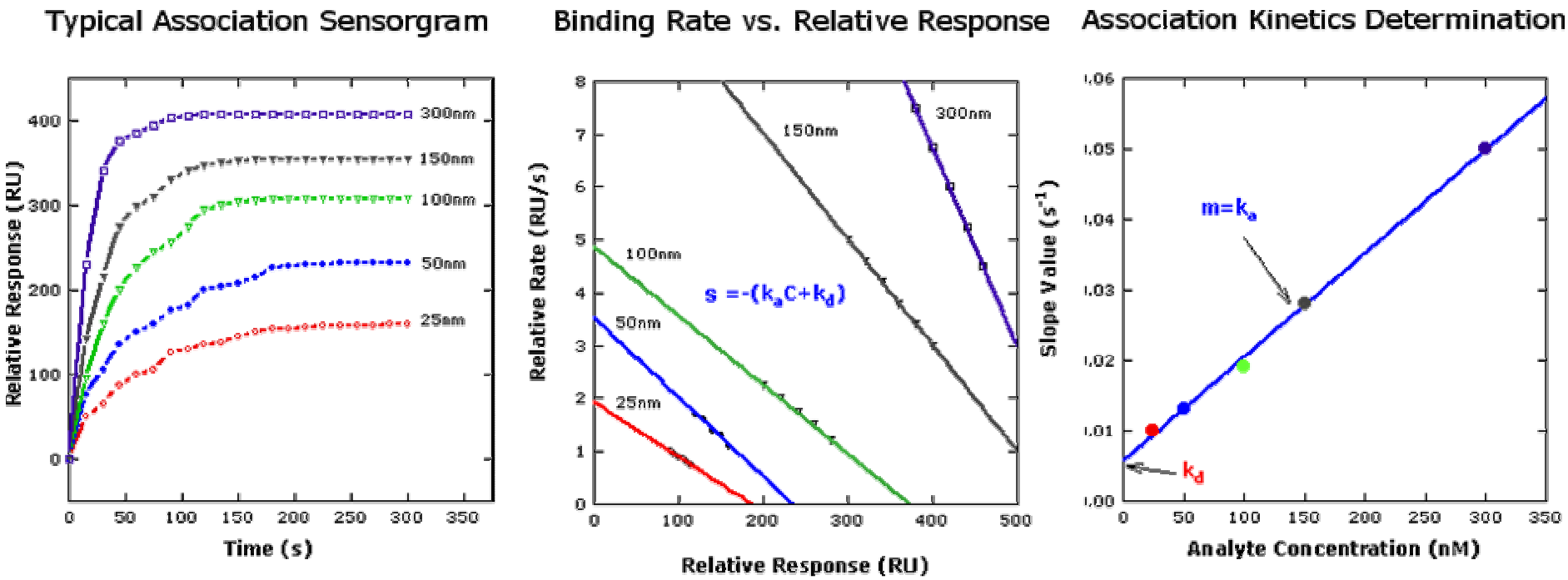
$$dR/dt = K_a C (R_{max} - R) - K_d R$$

or

$$dR/dt = K_a C R_{max} - (K_d + K_a C) R$$

- C= Concentration of analyte
- Rmax = maximum analyte binding capacity of the surface in RU
- R = SPR signal at time t in RU

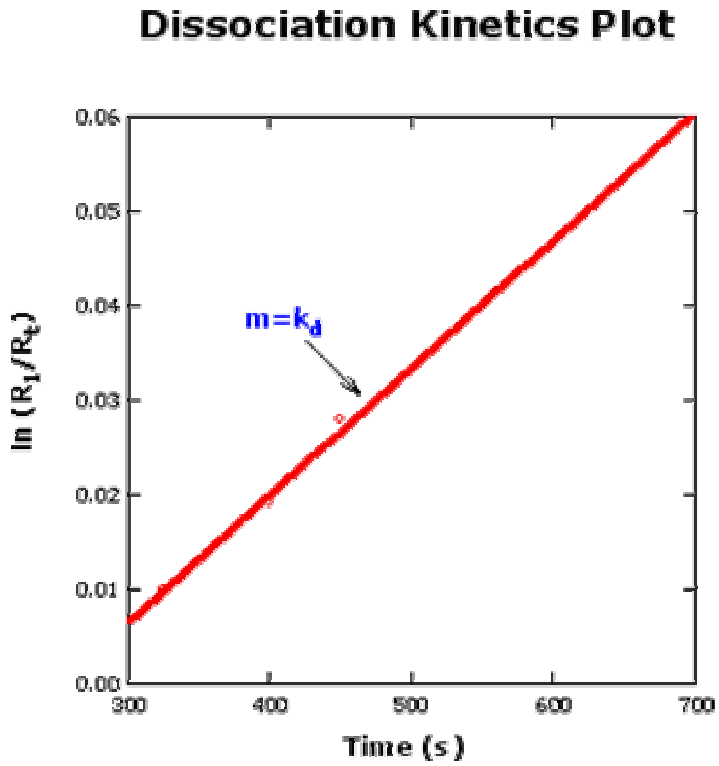
SPR: Kinetics of Association phase



- K_d is not very reliable as $K_aC >> K_d$

SPR: Kinetics of Dissociation phase

$$dR/dt = -K_d R$$



After integration and logarithm we have

$$\ln (R_0/R_t) = K_d (t-t_0)$$

- R_t is response at time t in RU
- R_0 is response at an arbitrary starting point

Applications of SPR

- ***Physical applications***: measure dielectric properties, adsorption processes, surface degradation or hydration of
  - Thin organic monolayers or bilayers
  - Polymer films
- ***Biological applications:*** as biosensors for specific biological interactions including adsorption and desorption kinetics, antigen-antibody binding and epitope mapping for determination of
  - Biomolecular structure and interactions of proteins, DNA & Viruses
  - Lipid Bilayers
  - Non-specific biomolecular interactions-bio-compatibility
  - Tissue engineering

SPR: Physical applications

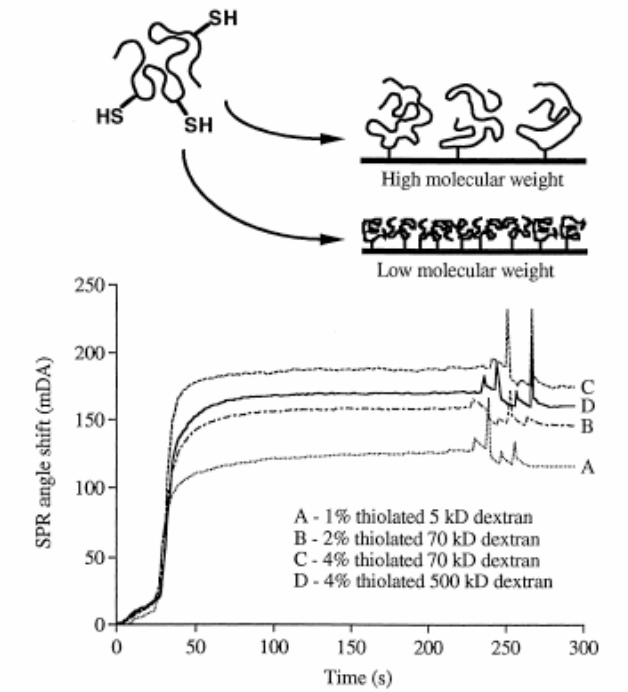


Fig. 4. The chemisorption of thiolated dextrans of varying molecular weight and thiol substitutions from solution onto a gold surface. The SPR angle shifts show a smaller range than might be expected when considering the 10-fold difference between the highest and lowest dextran molecular weights. Indeed, chemisorption of the highest molecular weight dextran does not lead to the highest SPR angle shift. This anomaly suggests differences in the structures of the dextran layers as depicted schematically. High molecular weight dextrans form more diffuse surface layers, while lower molecular weight dextrans form more compact layers.

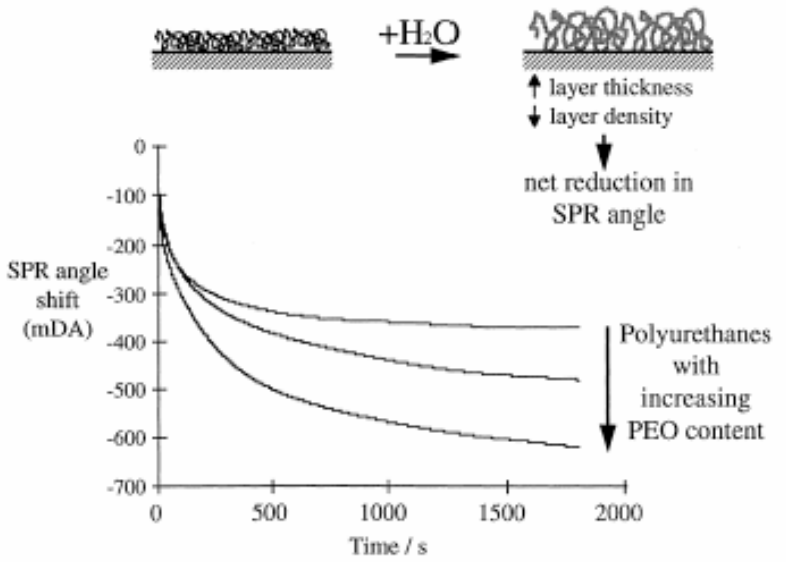


Fig. 5. The SPR hydration profiles for a series of polyurethanes of varying PEO content. The extent of hydration is shown to increase with increasing PEO content of the polymer film. A downward shift in the SPR angle is observed upon hydration, due to a reduction of the dielectric properties of the polymer film as water is incorporated into its structure. So, while the layer thickness increases, the layer density is decreased to yield a net reduction in the SPR angle.

Thin organic monolayers or bilayers

Polymer films

SPR: Biological applications

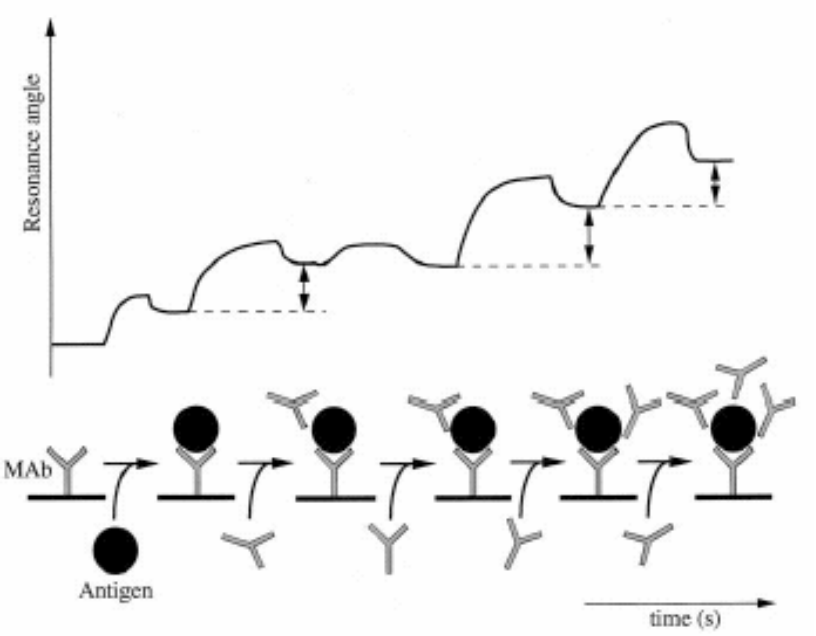
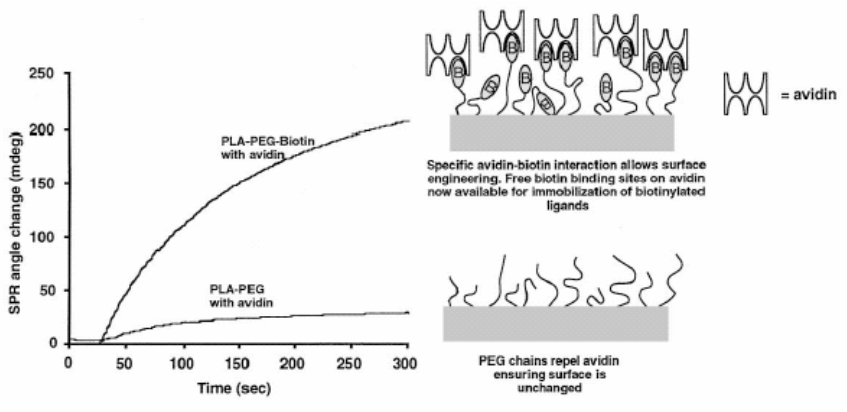


Fig. 7. Epitope mapping by SPR to test the possibility of several different monoclonal antibodies (MAbs) binding simultaneously to an antigen. A MAb is immobilized in a matrix and then the antigen and a sequence of MAbs are injected in turn. A complete epitope map is obtained by changing the sequence of antibodies until all combinations have been tested.

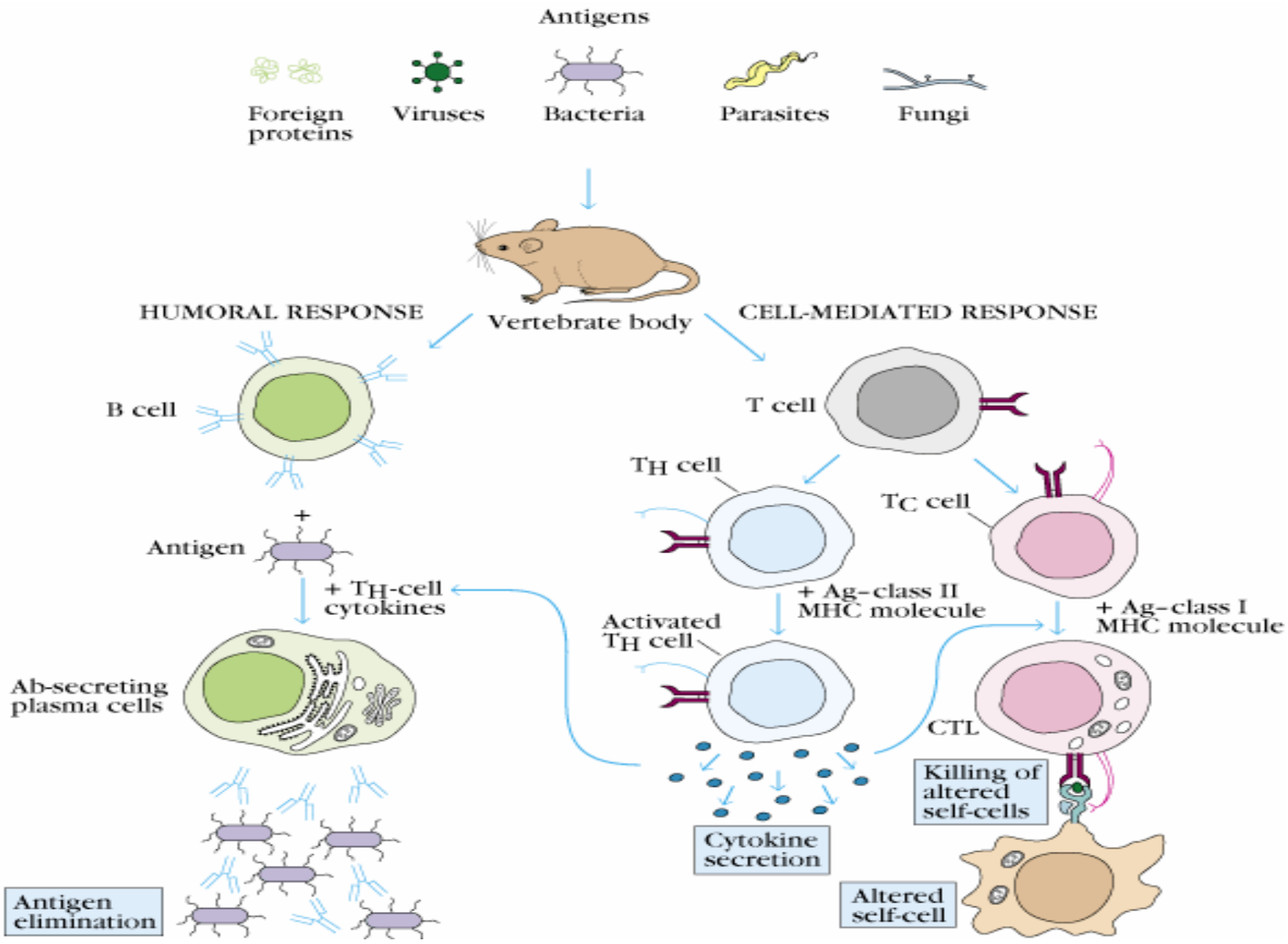
Epitope mapping



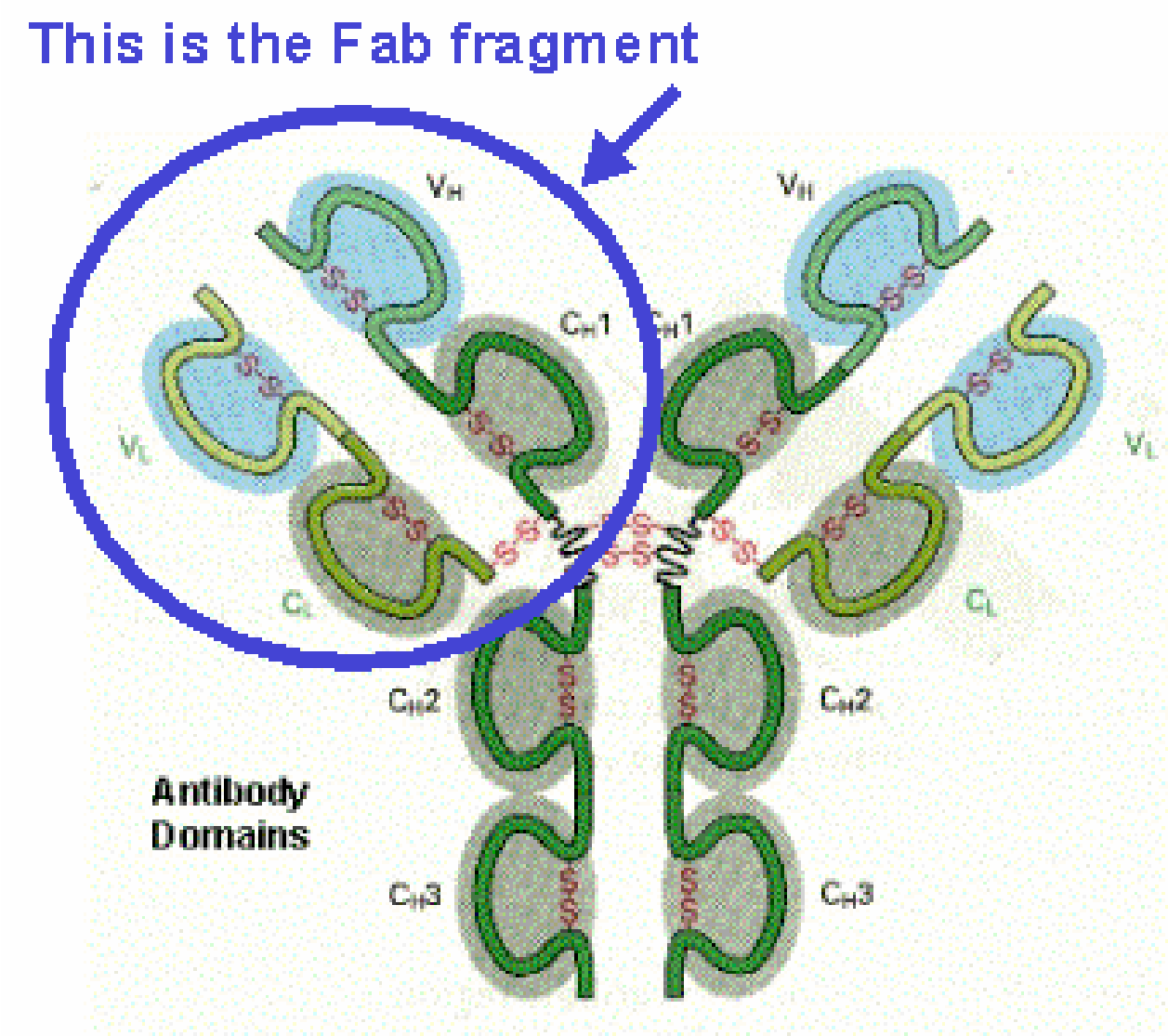
Surface engineering of a biotinylated polymer studied by SPR. Avidin specifically binds only to the biotinylated polymer.

Tissue engineering

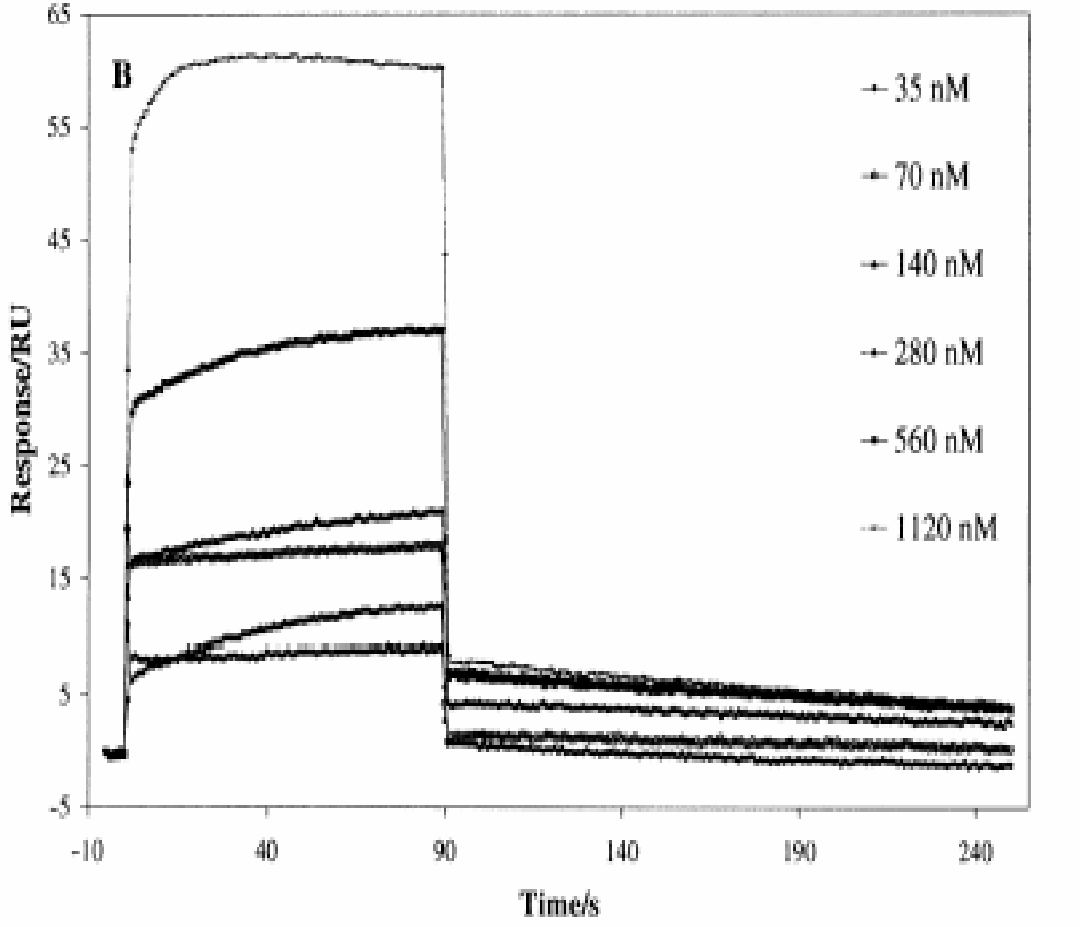
Immune response to antigen



Antibody structure



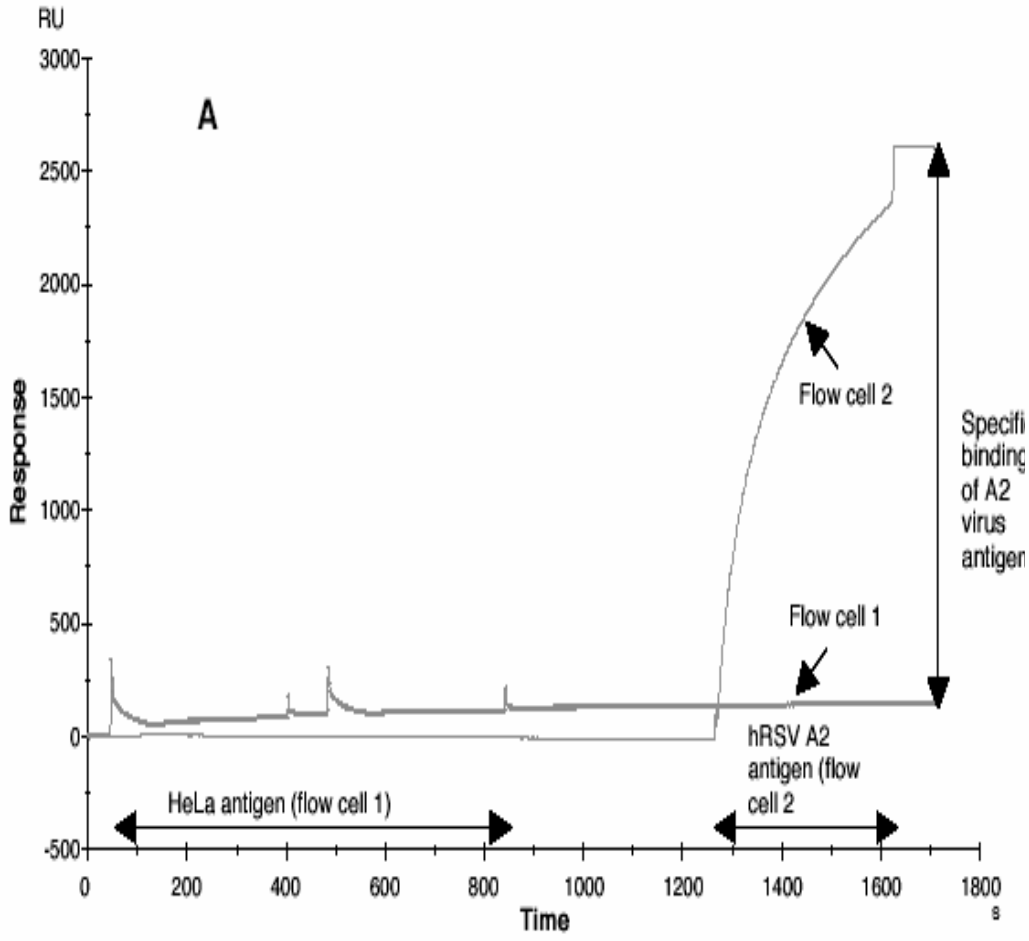
Single step analysis: FMDV antigen-antibody interactions



$K_a = 9.0 * 10^4\ M^{-1}\ S^{-1}$

$K_d = 1.2 * 10^{-3}\ S^{-1}$

Analysis of the antigen of different strains binding to antibody: single step

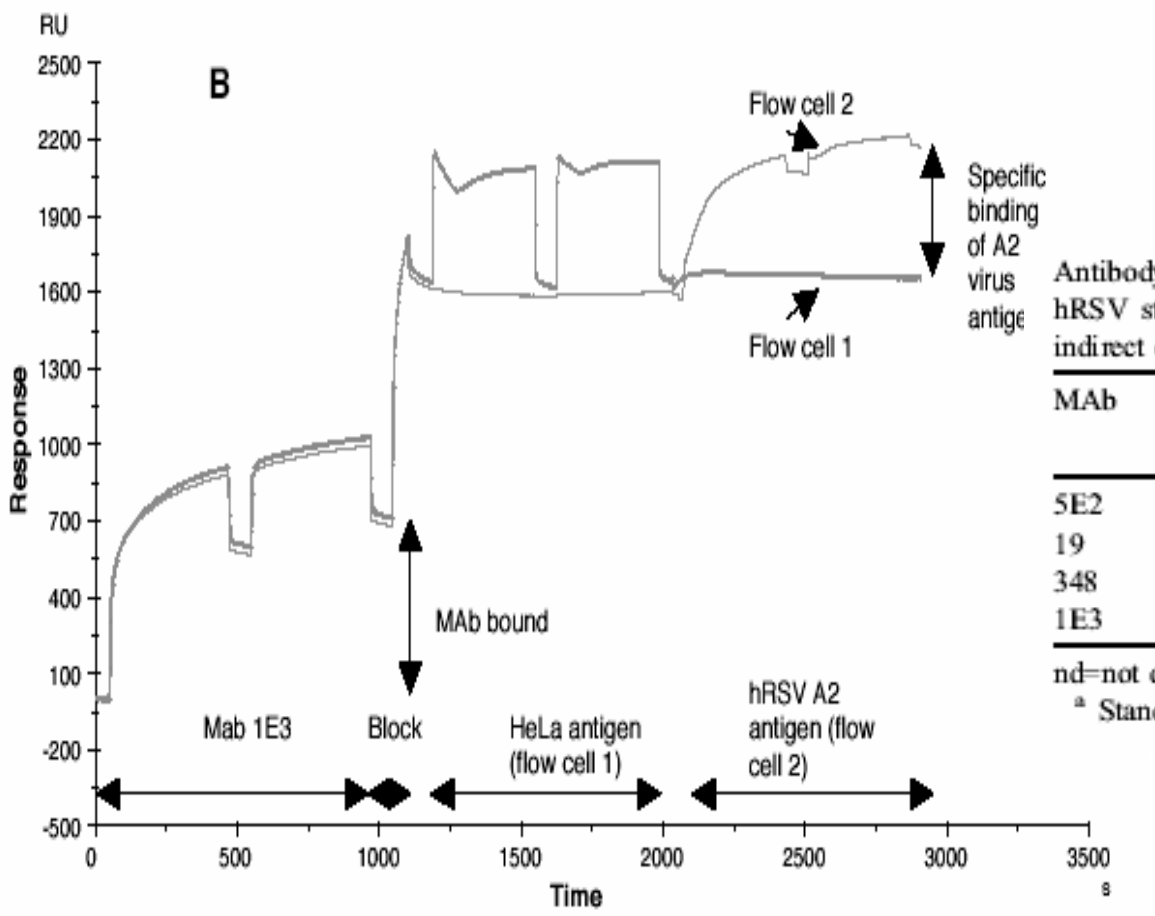


Binding ratios (AbBR) of anti-G MAbs to the indicated virus strain in the G glycoprotein specific BIAcore™ direct assay

MAb	Virus antigen strain				
	A2	GA5	GA7	GA3	SAA1
29	1.1	1.0	1.15	0.9	0.8
30	NB[a]	NB	NB	NB	NB
31	NB	NB	NB	NB	NB
38	0.9	NB	0.2	0.12	NB
3F4	NB	NB	NB	NB	NB
4G4	1.2	NB	NB	NB	NB

[a] NB indicates no significant binding was observed between the indicated MAb and the virus strain.

Analysis of the antigen of different strains binding to antibody: single step



Antibody binding ratios (AbBR) of MAb's to the F glycoprotein of hRSV strains of different lineages captured on MAb 1E3 in the indirect capture assay

MAb	Virus antigen strain				
	A2	GA3	GA7	GA5	SAA1
5E2	1.5[a]	1.1	1.1	1.1	1.1
19	1.4	1.0	1.1	1.0	1.0
348	0.17	nd	nd	nd	nd
1E3	0.18	nd	nd	nd	nd

nd=not done.

[a] Standard deviations ranged from 0.02 to 0.08.

Advantage of SPR

- Ability to perform real-time measurement:
  - Insight to dynamic nature of binding system and layer formation
- Use of selective slides to study binding events:
  - Eliminate the need for labeled reactants
- Exceptional sensitivity:
  - Small quantities of purified reagents are required

Disadavantages

- Disadvantage of SPR:
  - Lack of sensitivity when monitoring low molecular weight adsorbates
  - Rate limiting factor of mass transport-affecting kinetic analysis
- Methods to improve sensitivity:
  - Coupling to AFM
  - Coupling with Mass-spectrometry

References

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