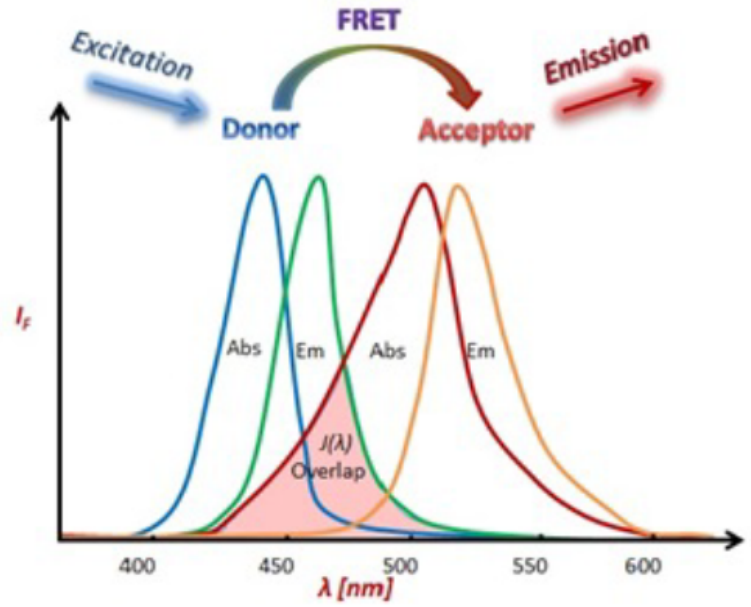
Optical Transducers

FRET: fluorescence (Förster) resonance energy transfer

- Energy transfer between two chromophores
- The efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor, making FRET extremely sensitive to small changes in distance



- FRET applications are used in cell biology efficiently and involves the fusion of two fluorescent proteins to the ends of an environmentally sensitive protein or peptide to act as a **biosensor** of specific cellular functions.

- By creatively fusing FRET-capable pairs of fluorescent proteins, intracellular biosensors useful for optical live-cell imaging is developped

- E.g. calcium induction, cyclic nucleotide messenger effects, pH, membrane potential fluctuations, phosphorylation, and intracellular protease action

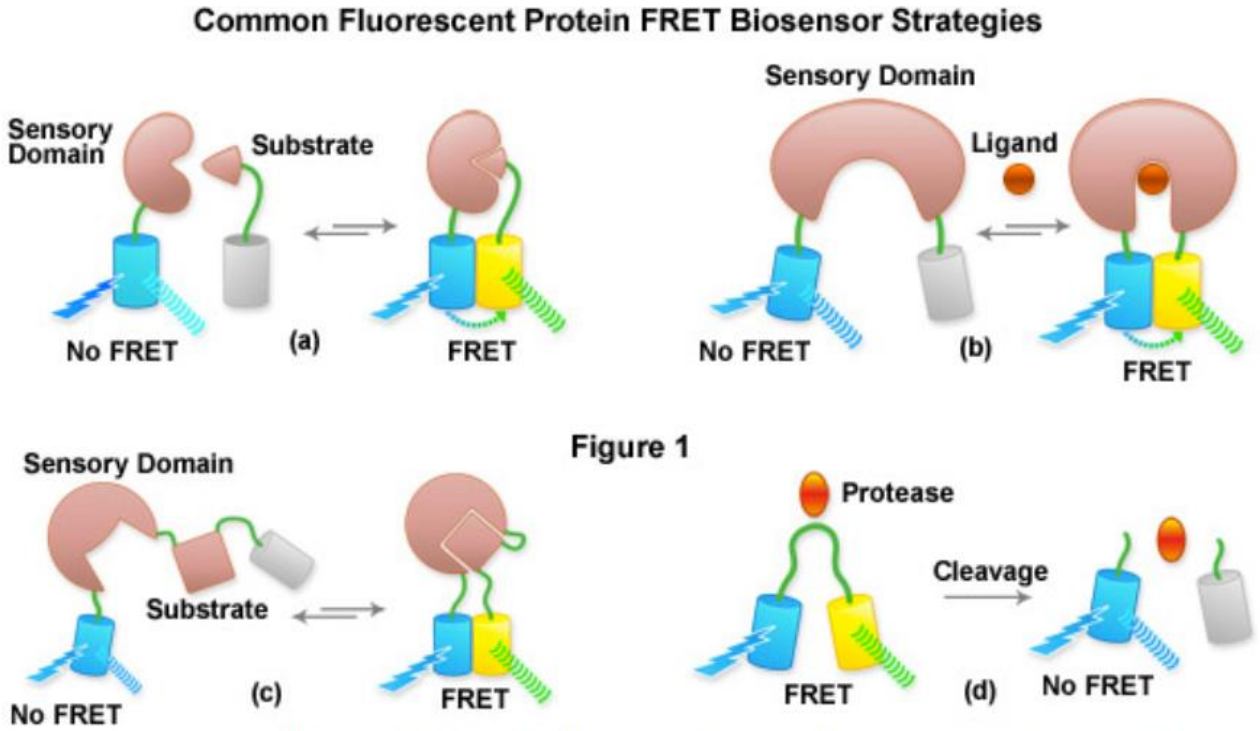
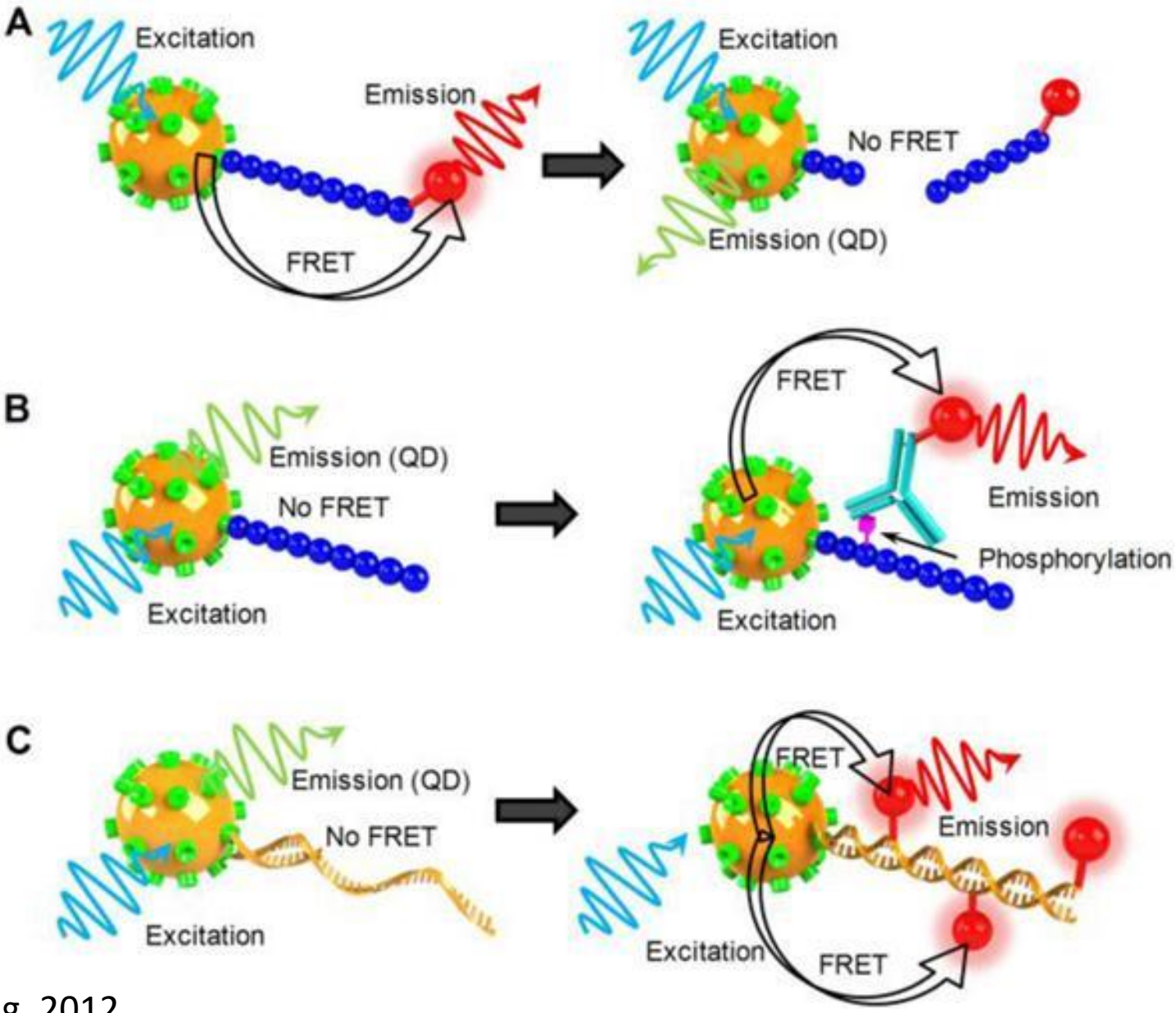


Figure 1

Presented in Figure 1 is a cartoon illustrating common fluorescent protein biosensor construction strategies. Cyan cylinders represent CFPs, gray cylinders represent YFPs without FRET, and yellow cylinders represent YFPs with FRET. Blue lightning bolts indicate excitation at 450 nanometers and parallel waves are fluorescence emission (at 475 nanometers, cyan; or 530 nanometers, yellow). Sensory domains are flesh-colored and effector agents are spheres or ellipses. Figure 1(a) shows intermolecular FRET between a separate sensory domain and substrate fused to CFP and YFP, respectively, while Figure 1(b) depicts a fluorescent protein biosensor featuring a single sensory domain and effector ligand. Binding of the substrate to the sensory domain in Figure 1(a) produces FRET whereas binding of the ligand in Figure 1(b) induces a conformational change in the sensory domain to bring the fluorescent proteins into the correct proximity for energy transfer. In some cases, the substrate and sensory protein domains are linked (Figure 1(c)) to create a single genetic expression unit for intramolecular FRET as opposed to the situation in Figure 1(a). A fluorescent protein biosensor that detects substrate cleavage (Figure 1 and Figure 1(d)) operates through the elimination of FRET after the appropriate protease has been induced. Protease cleavage biosensors are popular indicators of apoptosis.

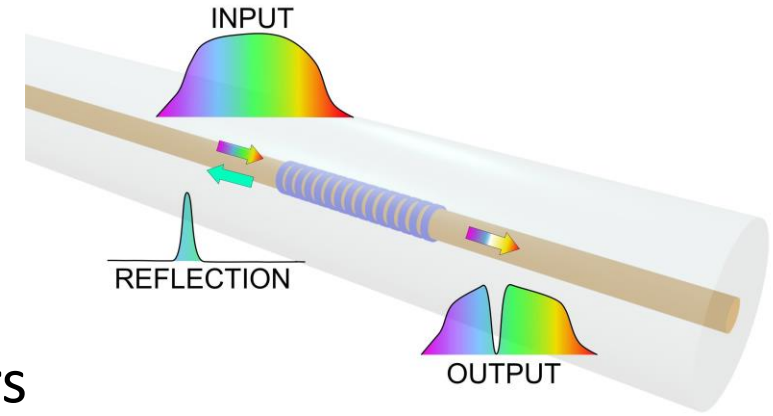
http://zeiss-campus.magnet.fsu.edu/tutorials/spectralimaging/fretbiosensors/indexflash.html



Zhang and Wang, 2012

Optical Transducers-Fiber Optics

A flexible, transparent fiber made of high quality extruded glass (silica) or plastic functioning as a waveguide, or “light pipe”



☺ Non-contact so non-invasive

☺ Highly sensitive and accurate

☺ Fast

☺ Microscale (3-9 μm) diameters

☺ Fexibility

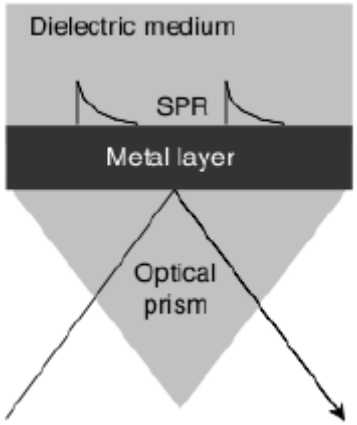
☺ Free of external references

☺ Multiparameter measurement is possible

Optical Transducers
Surface Plasmon Resonance (SPR)

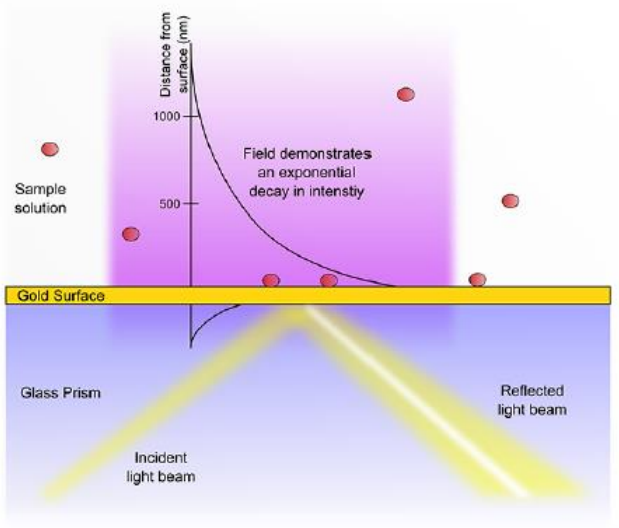
- The SPR is an optical phenomenon due to a charge density oscillation at the interface of a metal and a dielectric, which have dielectric constants of opposite signs

- *Kretschmann configuration*: prisms coated with a thin film of metal, usually gold or silver (~55-nm thick), was used. A light wave passes through the high-refractive-index prism
- Above a certain incidence angle, all of the light is reflected. This phenomenon is called Total Internal Reflection (TIR)



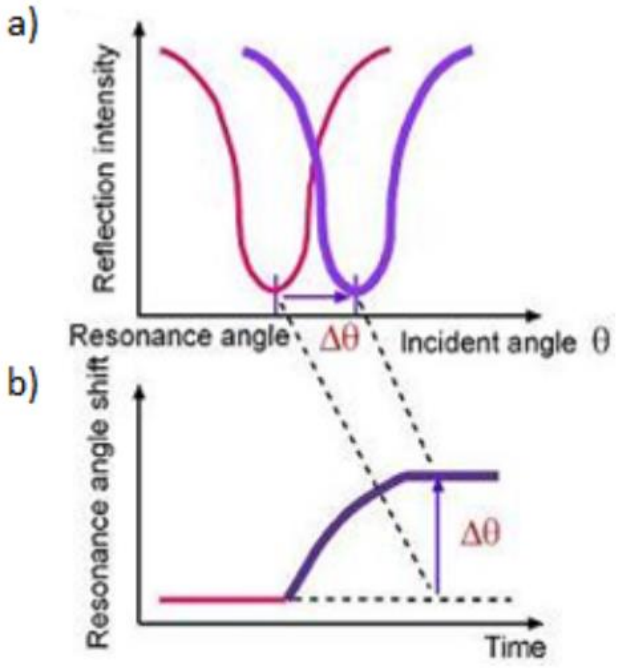
Optical Transducers
Surface Plasmon Resonance (SPR)

- In conducting metals, such as Au, the free conduction electrons form periodic oscillations, called plasma waves (plasmon).
- Surface plasmons are those plasmons that are confined to the surface of the metal.
- These plasmons create an electric field that extends about 100 nm both into the buffer solution and into the Au film and glass prism.
- This electrical field is called an evanescent wave, because it decays exponentially with distance



Optical Transducers
Surface Plasmon Resonance (SPR)

- When the incident light beam has the correct incidence angle, surface plasmon resonance occurs.
- Any change in surface, such as the binding of target, will alter the momentum of the surface plasmons, and their associated evanescent wave.
- As a consequence, SPR no longer occurs at the previous incidence angle, and a SPR shift takes place.
- The shift in the resonance angle is directly proportional to the change in mass at the Au surface.



a) Scan mode. b) Kinetic mode

Optical Transducers
Surface Plasmon Resonance (SPR)

Label-free and sensitive analysis (in pg/mL)

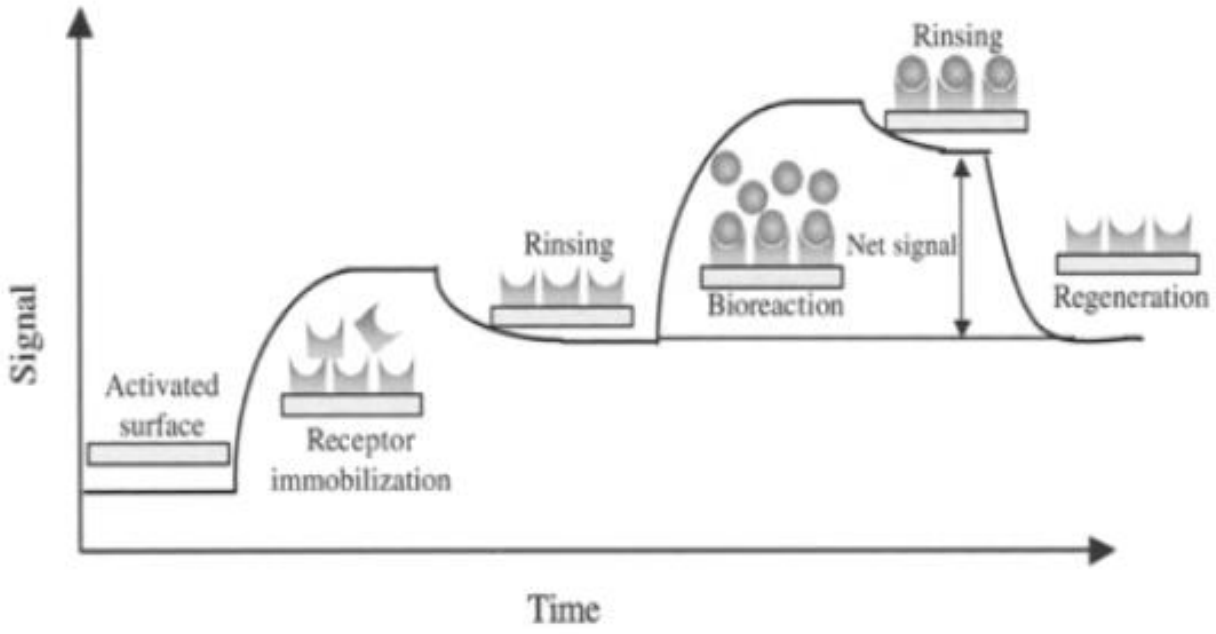
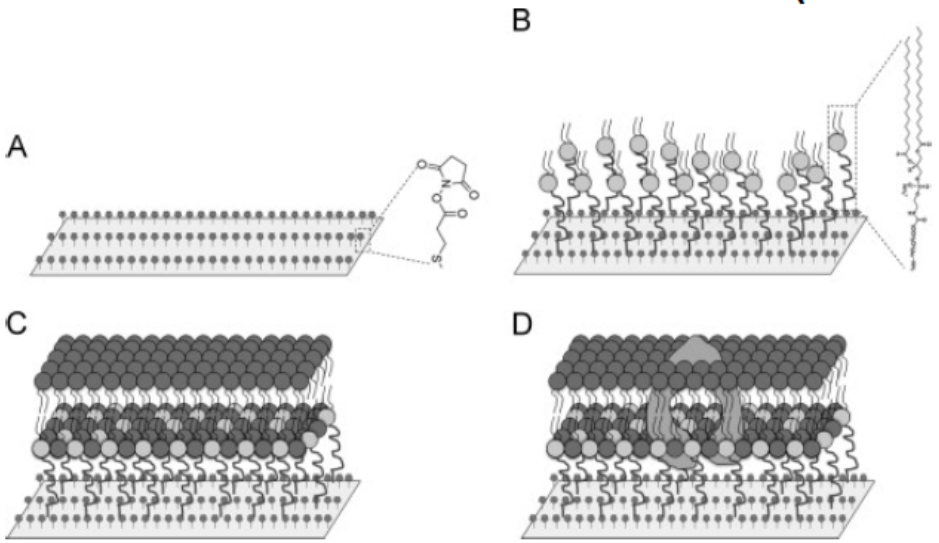
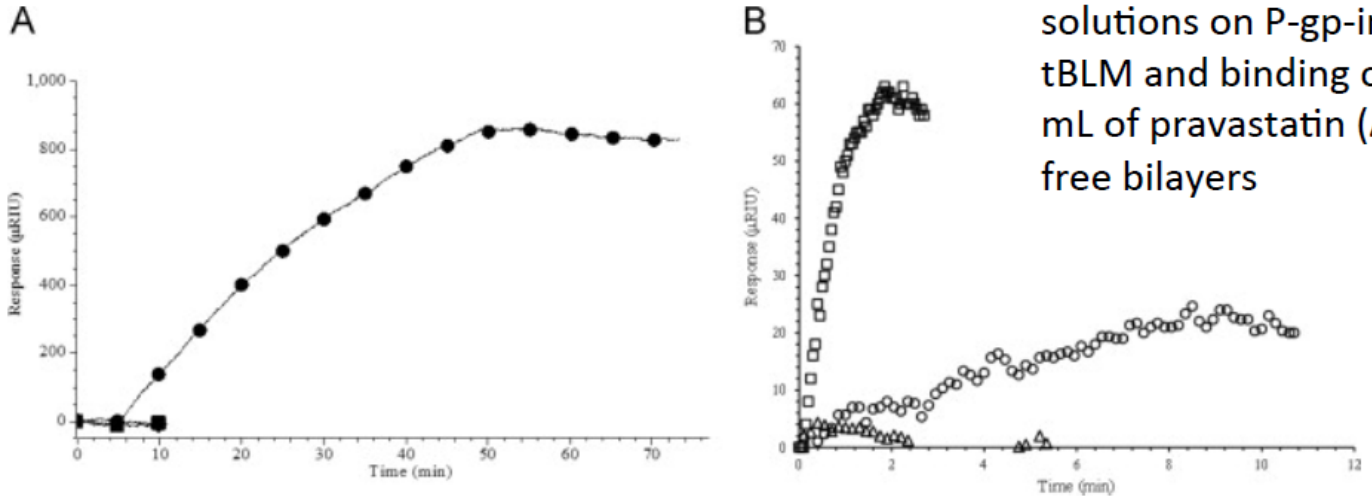


Fig. 5.3. The basic response curve in real time for any evanescent wave biosensor.

P-glycoprotein incorporated tethered lipid bilayer membranes (Inci et al, 2015)





Antibody and pravastatin binding on P-gp-incorporated tBLM by SPR.

(A) (●) anti-p-gp Mab on P-gp-incorporated tBLM. (■) anti-P-gp antibody on P-gp-free tBLM. (▲) anti-Pin-1 (G8) mouse MAb on P-gp-incorporated tBLM.

(B) Binding result of (○) 0.05 mg/mL and (□) 0.01 mg/mL pravastatin solutions on P-gp-incorporated tBLM and binding of 0.05 mg/mL of pravastatin (Δ) on P-gp-free bilayers

Calorimetric

- Based on measurement of the heat produced by the reaction and the amount of heat produced is correlated to the reactant concentration ($\Delta T = n\Delta H/c_p$)
- Enzyme-catalysed reactions → Considerable heat evolution (5-100 kJ/mol).
- Thus, calorimetric transducers are universally applicable in enzyme sensors.

Enzyme	Substrate	$-\Delta H$ (kJ/mol)
Catalase	Hydrogen peroxide	100.4
Cholesterol oxidase	*Cholesterol*	*52.9*
Glucose oxidase	***Glucose***	***80.0***
Hexokinase	*Glucose*	*27.6*

Mass sensitive

Quartz crystal microbalance (QCM)

- A piezoelectric quartz crystal that utilizes the Converse Piezoelectric Effect to determine mass changes as a result of frequency change of the crystal.

- When a mechanical stress was carried out to a piezoelectric material (e.g., quartz crystal), a voltage proportional to the stress was generated. In 1959, Sauerbrey proved that quartz crystal oscillation frequency depended on the mass change at the sensor surface, and he coined the term as quartz crystal microbalance

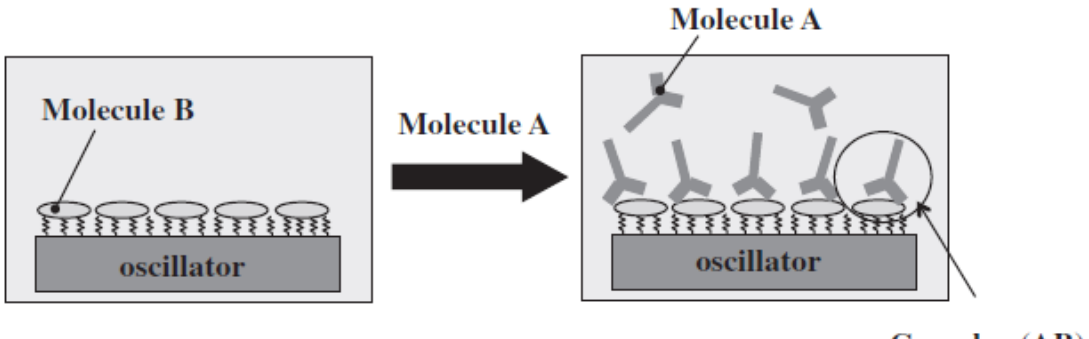
Mass sensitive-QCM

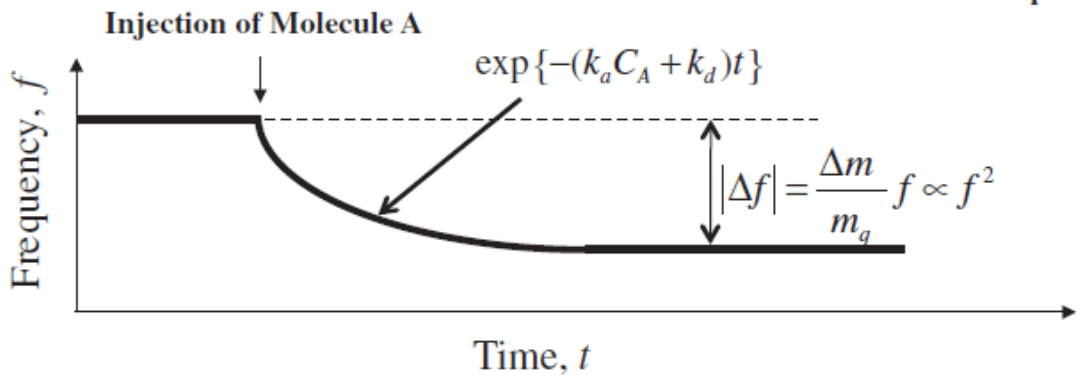
- QCM's are piezoelectric devices fabricated of a thin plate of quartz, with gold electrodes affixed to each side of the plate.
- Mass accumulated on the surface lowers the crystal frequency, the amount being directly related to its mass (ng).
- Quantification could be done by Sauerbrey eqn
  - Δf0 – Resonant frequency (Hz)
  - Δf – Frequency change (Hz)
  - Δm – Mass change (g)
  - A – Piezoelectrically active crystal area (cm2)
  - rho – Density of quartz (2.648 g/cm3)
  - μ – Shear modulus of quartz for AT-cut crystal (2.947x1011 g·cm-1·s-2)

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m$$

Mass sensitive-QCM

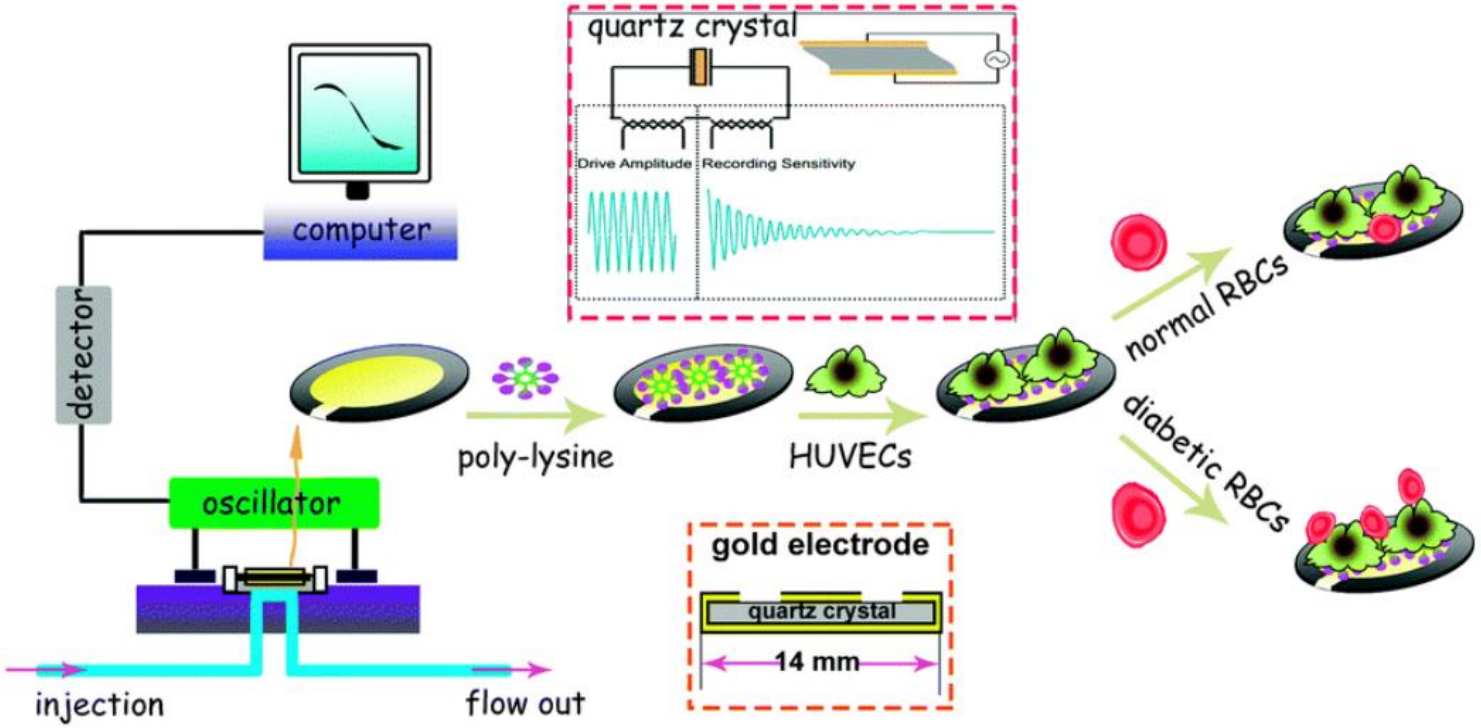
$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$





QCM-D: cell-cell interactions

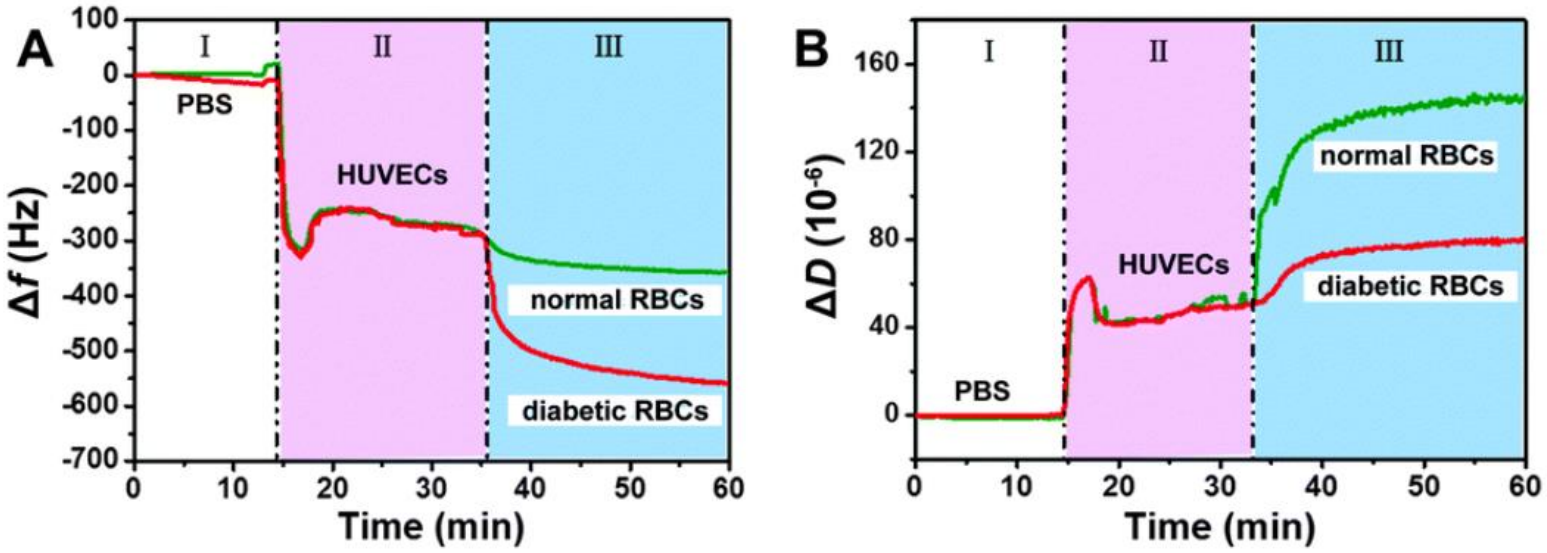
Zhang S et al, Chem. Commun., 2015



QCM-D: cell-cell interactions

Zhang S et al, Chem. Commun., 2015

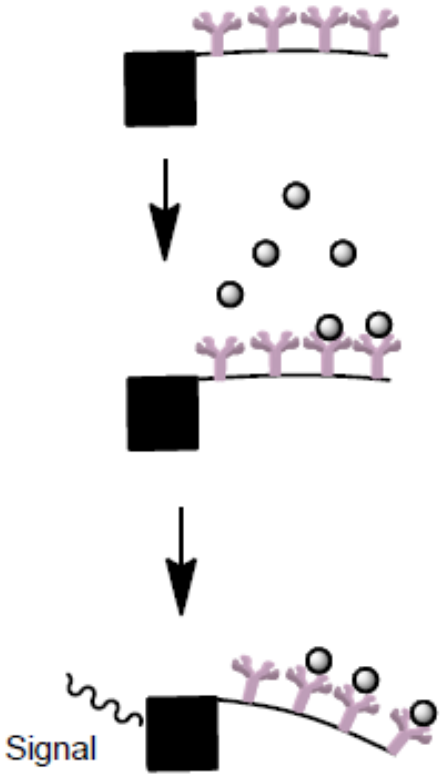
- The interactions between red blood cells and blood vessels are essential in inflammatory situations, infectious and thrombotic diseases.
- Abnormal RBC adhesion to human umbilical vein endothelial cells (HUVECs) has been correlated with vascular complications in diseases such as diabetes, sickle cell anemia and malaria.



Mass sensitive

Cantilever- nanomechanical biosensors

- Cantilevers are an example of label free biosensors which offer a simple, rapid, reliable, minimal cost and low limit of analyte detection.
- Due to its label free detection principle and small size, this type of biosensor has applicable advantages
- The surface is coated with recognition elements and once specific binding occurs, the cantilever will bend



Mass sensitive

Cantilever- nanomechanical biosensors