Lecture 19: Biosensors (continued)

Last time: biosensor device classes
Gene array biosensors

Today: detection methods

Detection Elements

- Readout
  - Macroscopic fluorescence, diffraction, or interference
    - what
  - Optical bar-coding[4]
    - Example: quantum dot-loaded microsphere capture agents[5]
      - QDs show size-dependent luminescence
        - Narrow emission bands from a common excitation wavelength
        - Stable against photobleaching
      - Approach:
        - Load polymer microspheres with different amounts of several colors of QDs to obtain a unique fluorescence signature
          - 6 colors at 10 possible intensities allows for > 10^6 possible 'codes'
        - Capture molecule on surface of beads grabs labeled analyte

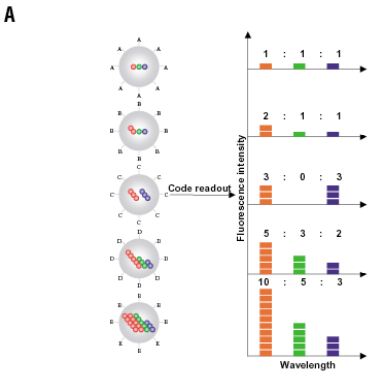


Figure 1. (A) Schematic illustration of optical coding based on wavelength and intensity multiplexing. Large spheres represent polymer microbeads, in which small colored spheres (multicolor quantum dots) are embedded according to predetermined intensity ratios. Molecular probes (A–E) are attached to the bead surface for biological binding and recognition, such as DNA–DNA hybridization and antibody–antigen/ligand–receptor interactions. The numbers of colored spheres (red, green, and blue) do not represent individual QDs, but are used to illustrate the fluorescence intensity levels. Optical readout is accomplished by measuring the fluorescence spectra of single beads. Both absolute intensities and relative intensity ratios at different wavelengths are used for coding purposes; for example (1:1:1) (2:2:2), and (2:1:1) are distinguishable codes. (B) Ten distinguishable emission colors of ZnS-capped CdSe QDs excited with a near-UV lamp. From left to right (blue to red), the emission maxima are located at 443, 473, 491, 500, 518, 543, 565, 587, 610, and 655 nm.

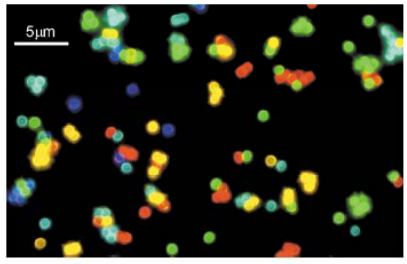
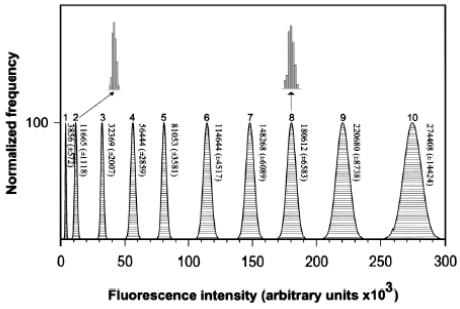
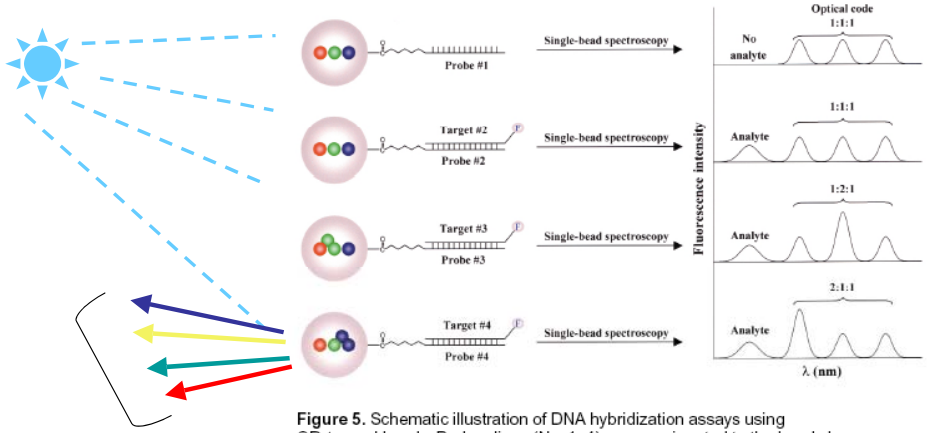


Figure 2. Fluorescence micrograph of a mixture of CdSe/ZnS QD-tagged beads emitting single-color signals at 484, 508, 547, 575, and 611 nm. The beads were spread and immobilized on a polylysine-coated glass slide, which caused a slight clustering effect. See Experimental Protocol for detailed conditions.



(Han et al, 2001)

Excitation of bar-code and target fluorochrome by same wavelength



Microscope-based spectrophotometer for detection of emission spectra from individual beads

Figure 5. Schematic illustration of DNA hybridization assays using QD-tagged beads. Probe oligos (No. 1–4) were conjugated to the beads by cross-linking, and target oligos (No. 1–4) were detected with a blue fluorescent dye such as Cascade Blue. After hybridization, nonspecific molecules and excess reagents were removed by washing. For multiplexed assays, the oligo lengths and sequences were optimized so that all probes had similar melting temperatures (T_m = 66°–99°C) and hybridization kinetics (30 min). See legend in Figure 6 for the sequences used.

- Optical absorption (colorimetric)
  - what
- Surface plasmon resonance and SPR arrays
  - Developed commercially later 1980's (Cooper 2002)
  - Typically, receptor is immobilized and free ligand is passed over sensor chip
    - Both ways possible, small ligands simply interfere with binding if immobilized

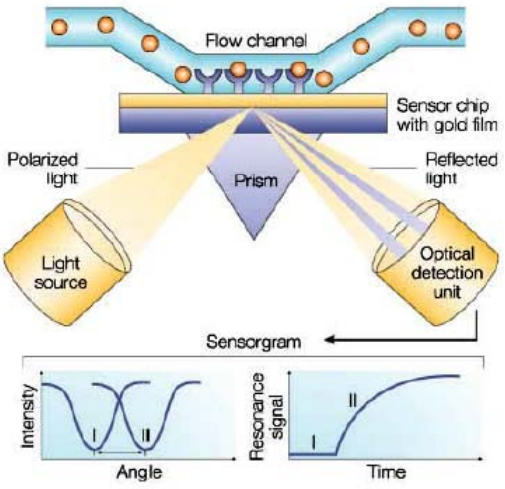
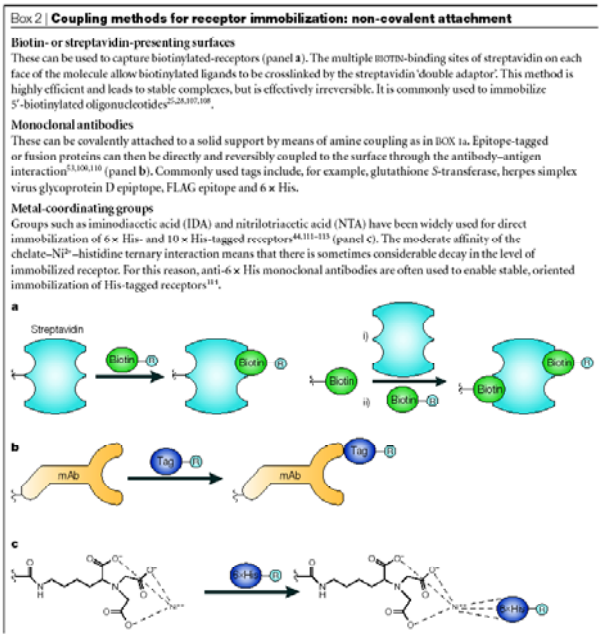
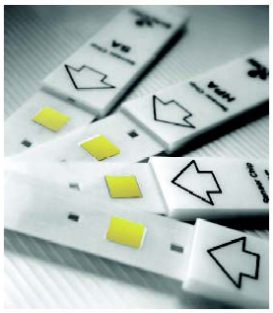


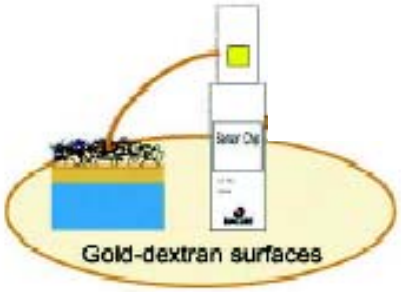
Figure 2 | **Typical set-up for an SPR biosensor.** Surface plasmon resonance (SPR) detects changes in the refractive index in the immediate vicinity of the surface layer of a sensor chip. SPR is observed as a sharp shadow in the reflected light from the surface at an angle that is dependent on the mass of material at the surface. The SPR angle shifts (from I to II in the lower left-hand diagram) when biomolecules bind to the surface and change the mass of the surface layer. This change in resonant angle can be monitored non-invasively in real time as a plot of resonance signal (proportional to mass change) versus time.



(Cooper 2002)



Biacore sensor chips

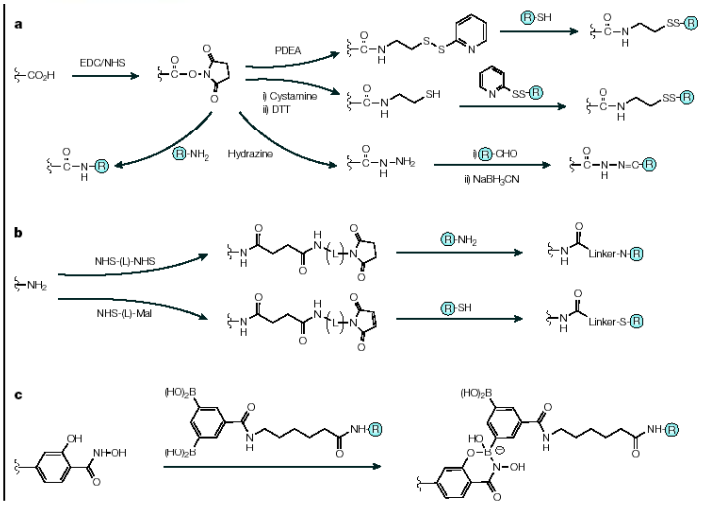


Box 1 | Coupling methods for receptor immobilization: covalent attachment

Immobilization of a receptor to the sensor surface is of central importance to the design of a successful biosensor assay[55]. The coupling method must be efficient, produce a highly stable association (to prevent signal drift) and allow control of the amount of receptor that is immobilized. Amine coupling (for example, to the amino terminus or surface lysine residues on a protein) will lead to a heterogeneous population of receptors with random orientation on the surface. Affinity-capture (BOX 2) and sulphydryl couplings can be used to produce a more homogeneous population of oriented receptors on the surface[102–104]. Strategies that are commonly used to covalently attach a receptor to a surface include:

- Water-soluble EDC-mediated activation of a carboxymethylated support, such as dextran or hyaluronic acid (panel **a**). The resultant reactive NHS ester can then be coupled directly with available amino moieties of a receptor (R) to form a stable amide linkage. Acidic receptors (with an isoelectric point (pI) < 3.5) are difficult to immobilize by amine coupling, as the low pH that is required for electrostatic pre-concentration to the sensor surface protonates the primary amino groups and reduces the coupling efficiency. Further derivatization with sulphydryl-reactive reagents (for example, PDEA or SPDP) allows reaction with free surface thiols (for example, cysteine or methionine) to form a reversible disulphide linkage. In a similar manner, stable thioether bonds can be formed using maleimide coupling reagents, such as sulpho-SMCC and GMBS. The surface can also be derivatized with cystamine to effect coupling with disulphide-activated receptors. Finally, treatment with hydrazine followed by a reductive amination allows coupling with aldheydes. The aldehyde groups could be native to the receptor or formed by mild oxidation of any *cis* diols that are present.
- Amino-presenting surfaces[45,105] can be treated with commercially available bifunctional linking reagents to effect coupling with free amino or sulphydryl groups on the receptor (panel **b**).
- Surfaces that are derivatized with SHA can be used to produce reversible complexes with receptors that have been activated with PDBA[106] (panel **c**):

DTT, dithiothreitol; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; GMBS, *N*-(γ-maleimidobutyrloxy) sulphosuccinimide ester; L, linker; Mal, maleimide; NHS, *N*-hydroxysuccinamidyl; PDBA, phenyldiboronic acid; PDEA, pyridinyldithioethanamine; SHA, salicylhydroxamic acid; SPDP, 3-(2-pyridinyldithio)propioic acid *N*-hydroxy-succinimide ester); sulpho-SMCC, sulphosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexanecarboxylate.



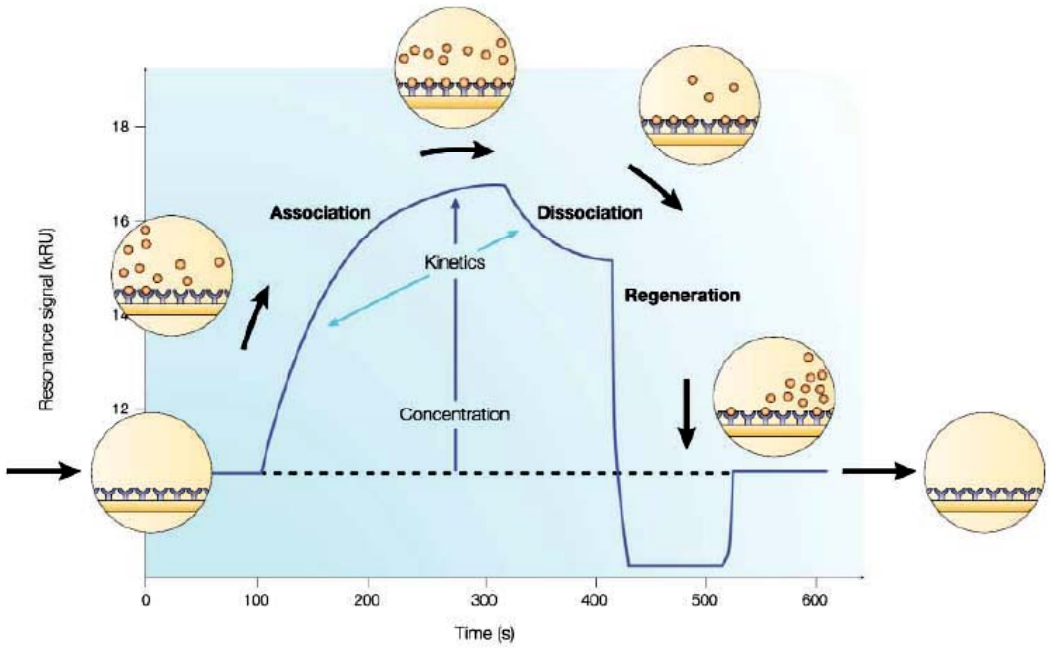


Figure 3 | **A typical binding cycle observed with an optical biosensor.** A molecule is immobilized on the sensor surface with appropriate coupling chemistry. At $t = 0$ s, buffer is contacted with the receptor through a microfluidic flow cell, or in some commercial instruments, through a cuvette. At $t = 100$ s, a solution of analyte in the running buffer is passed over the receptor. As the analyte binds to the surface, the refractive index of the medium adjacent to the sensor surface increases, which leads to an increase in the resonance signal. Analysis of this part of the binding curve gives the observed association rate (k_{obs}). If the concentration of the analyte is known, then the association rate constant of the interaction (k_{on}) can be determined. At equilibrium, by definition, the amount of analyte that is associating and dissociating with the receptor is equal. The response level at equilibrium is related to the concentration of active analyte in the sample. At $t = 320$ s, the analyte solution is replaced by buffer, and the receptor–analyte complex is allowed to dissociate. Analysis of these data gives the dissociation rate constant (k_{off}) for the interaction. Many complexes in biology have considerable half-lives, so a pulse of a regeneration solution (for example, high salt or low pH) is used at $t = 420$ s to disrupt binding and regenerate the free receptor. The entire binding cycle is normally repeated several times at varying concentrations of analyte to generate a robust data set for global fitting to an appropriate binding algorithm. The affinity of the interaction can be calculated from the ratio of the rate constants ($K_a = 1/K_d = k_{on}/k_{off}$) or by a linear or nonlinear fitting of the response at equilibrium at varying concentrations of analyte. In addition to determining the interaction affinities and kinetics, a thermodynamic analysis of a biomolecular interaction is also possible. This is done by applying van't Hoff's equations to the interaction affinities and kinetics of an interaction obtained at various different temperatures[16,17].

(Cooper 2002)

- Optical fiber-based
  - Single cell analysis optical fiber probes[6]

Advantages/disadvantages

- Pros
  - Fast measurements
  - Sensitive
- Cons
  - Cannot perform detection on turbid solutions

Electrochemical

Electrochemical readouts[7]

- Conductometric
  - Measure changes in the conductance of the biological component arising between a pair of metal electrodes due to e.g. metabolism
- Potentiometric
  - Measure electrical potential difference between a sample and reference electrode
  - Monitor the accumulation of charge at zero current created by selective binding at the electrode surface

- Electrode may be selective for certain ions or gases
  - E.g. F-, I-, CN-, Na+, K+, Ca2+, H+, NH4+
  - CO2, NH3
- Amperometric
  - Measure current generated by electrochemical oxidation or reduction of electroactive species at a constant applied potential

Electrochemical detection:

ISFET - ion-sensitive field-effect transistors

Different Types of ISFET

Detectable ions	Ionsensitive surface	References
K^+	Polymeric membrane/ionophore	3
Na^+	Polymeric membrane/ionophore	3
Ag^+	SiO_2 membrane	4
Ca^{2+}	PVC/ionophore	5
NH^{4+}	Polymeric membrane/ionophore	6
Cu^{2+}	As_2Se_3/Cu membrane	7
Ni^{2+}	As_2Se_3/Ni membrane	7
Pb^{2+}	Polymeric membrane/ionophore	8
Cd^{2+}	As_2S_3/Ag_2S/CdS membrane	9
NO^{3-}	Magnesium phosphate glasses	10
Cl^-	Polymeric membrane/ionophore	11
Br^-	AgBr	12
F^-	LaF_3	13
H^+	Si_3N_4/SiO_2/Si	14

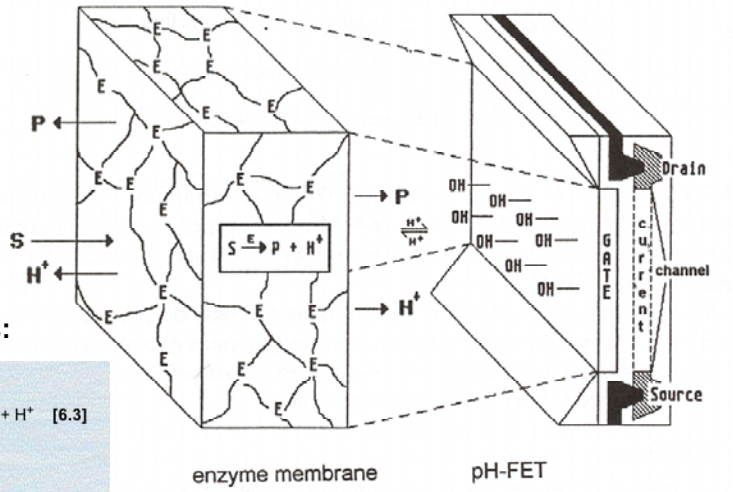


Fig. 2. Biosensor based on a pH-sensitive FET.

Common pH-modifying enzymatic reactions:

glucose oxidase
D-glucose + O_2 → D-glucono-1,5-lactone + H_2O_2 → D-gluconate + H^+ **[6.3]**

penicillinase
penicillin → penicilloic acid + H^+ **[6.4]**

urease (pH 6.0)[a]
$H_2NCONH_2 + H_2O + 2H^+ \rightarrow 2NH_4^+ + CO_2$ **[6.5]**

urease (pH 9.5)[b]
$H_2NCONH_2 + 2H_2O \rightarrow 2NH_3 + HCO_3^- + H^+$ **[6.6]**

lipase
neutral lipids + H_2O → glycerol + fatty acids + H^+ **[6.7]**

(Mulchandani and Rogers, 1998)

Advantages/disadvantages

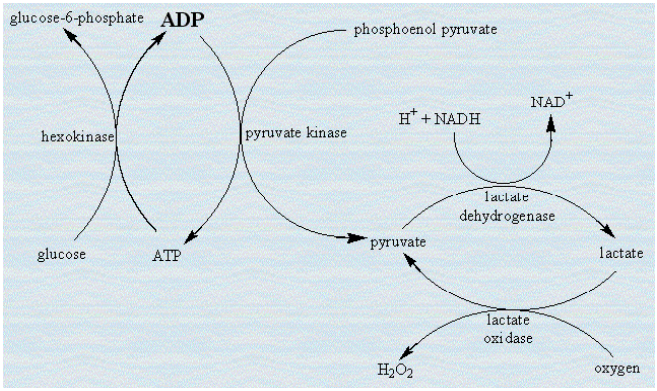
- Pros
  - Fast measurements
  - Sensitive
    - Low detection limits typically ~ 10^{-9} M
  - Ability to perform measurements on turbid/opaque solutions
- Cons
  - PH-sensing mechanisms require weakly buffered or non-buffered solutions

Calorimetric

Calorimetric readouts

- Measurement of heat generated by an enzymatic reaction
  - Typically utilize thermistors to transform heat into an electrical signal

Calorimetric detection:



http://www.sbu.ac.uk/biology/enztech/calorimetric.html

Schematic diagram of a calorimetric biosensor. The sample stream (a) passes through the outer insulated box (b) to the heat exchanger (c) within an aluminium block (d). From there, it flows past the reference thermistor (e) and into the packed bed bioreactor (f, 1ml volume), containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor (g) and the solution passed to waste (h). External electronics (I) determines the difference in the resistance, and hence temperature, between the thermistors.

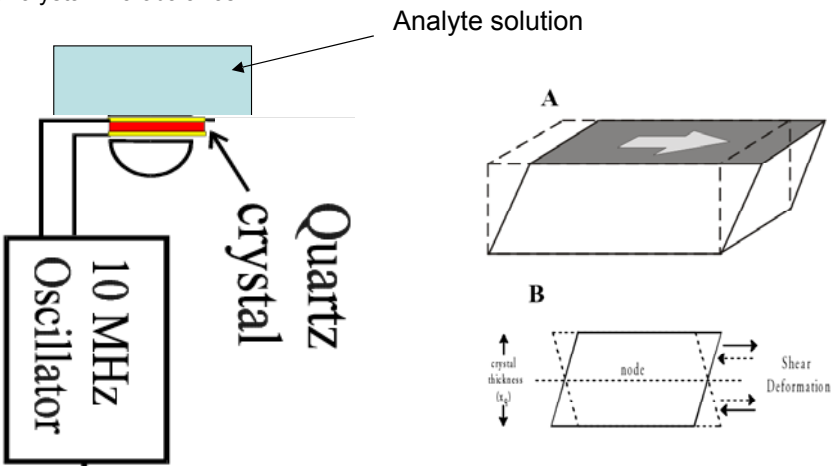
Piezoelectric[8,9]

- Based on quartz crystal microbalance detection
  - Crystal is oscillated at a defined frequency by an oscillating applied voltage
    - Shear deformation induced as dipoles in crystal seek to align with direction of electric field
    - Deformation typically 10-100 nm for AT-cut crystals operating in freq. range of 1-10 MHz
  - Binding of analyte to surface changes mass of crystal and alter oscillation frequency

- Figure below from : www-bond.chem.monash.edu.au/theses/ Graeme%20Snook/Chapter1.pdf

Piezoelectric detection:

Quartz crystal microbalance



http://www.q-sense.com/main.qcmd_tech.html

Piezoelectric detection:

Quartz crystal microbalance

Detecting HIV virions:

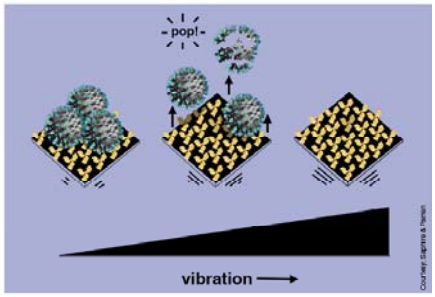


Figure 1. Listening to virus detachment by rupture event scanning. Virions are captured by specific antibodies immobilized onto the surface of a quartz crystal. The quartz crystal is subjected to an electrical field inducing a vibrational motion in the crystal with increasing amplitude. At a specific surface acceleration, the viruses suddenly detach from the surface, producing an abrupt noise of which the intensity is proportional to the number of virus particles involved.

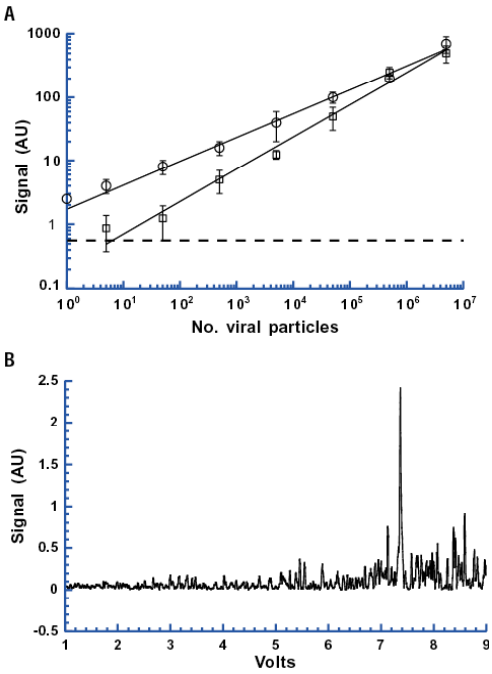


Figure 3. Signal linearity with particle numbers. (A) Serial 10-fold dilutions of HSV1 gD⁺ in PBS. A sample volume of either 1 µl (○) or 40 µl (□) was incubated for 40 min at room temperature on a QCM disk coated with anti-gD IgG mAb. The dashed line represents the noise floor. (B) Representative REVS spectrum corresponding to dissociation of a single virion in PBS from an anti-gD IgG mAb-coated QCM disk. The peak can be seen at –7.4 V.

(Cooper et al. 2001)

SPR Arrays[10]

- External analysis/detection
- Optical method

Cell- and tissue-based biosensors (Stenger 2001, Gross 1997)

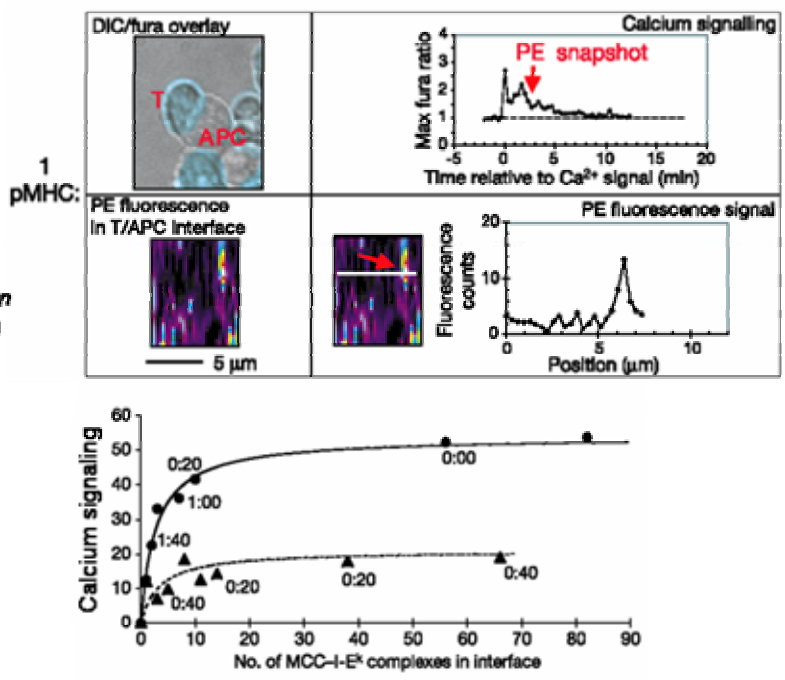
General concepts

- Why cell-based biosensors?
  - **Known ultrasensitivity of cells:**
    - Olfactory neurons respond to single odorant molecules
    - Retinal neurons triggered by single photons
    - T cells triggered by single antigenic peptides (Irvine 2002)

•Potential for single-molecule sensitivity

(e.g. T cell recognition of foreign peptide shown at right)

•Complex 'evaluation' of agents



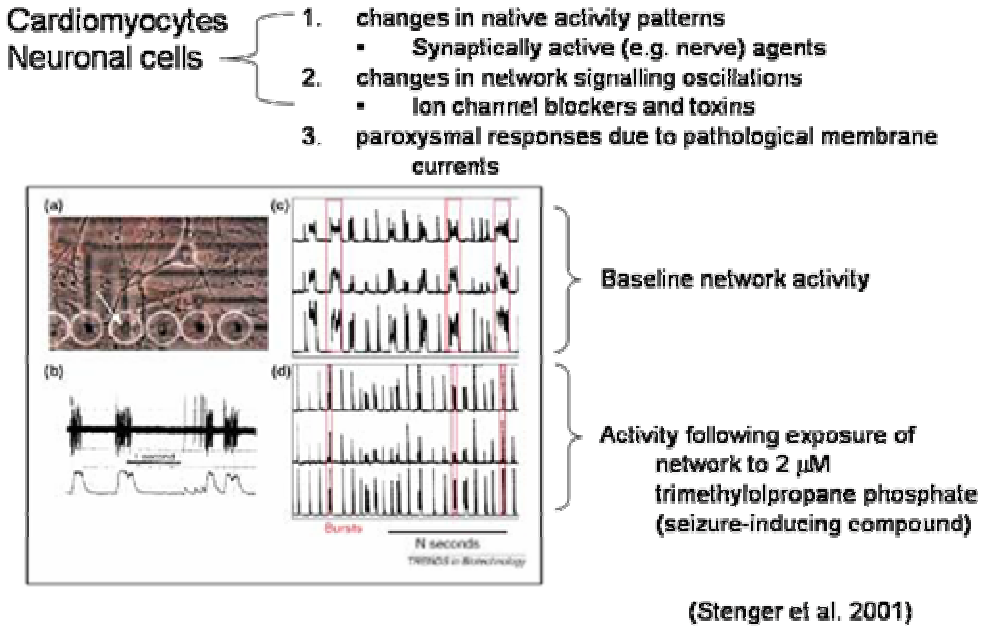
(Irvine et al. 2002)

  - **Ability to 'integrate' cellular or tissue response to compounds**
    - Detect functionality of compound in addition to its chemical presence
      - i.e. tell the difference between a dead and live virus

- Cell-based biosensors are based on a primary transducer (the cell) and secondary transducer (device which converts cellular/biochemical response into a detectable signal)
  - Secondary transducer may be electrical or optical
- Detection of arbitrary targets
  - Transfect cells with receptors to introduce responsiveness of e.g. neuronal cells to a chosen compound
- Basis of electrical secondary transducers
  - Electrically-excitable cells
    - Example cell types
      - Neurons

- Non-sensory neurons grown in culture outside of normal homeostasis and the insulation of the blood-brain barrier behave in a 'sensory' manner (Gross 1997)

- Cardiomyocytes
  - Generate electric signals in a substance-specific and concentration-dependent manner
  - Signals generated can be monitored by microelectrodes



(Stenger et al. 2001)

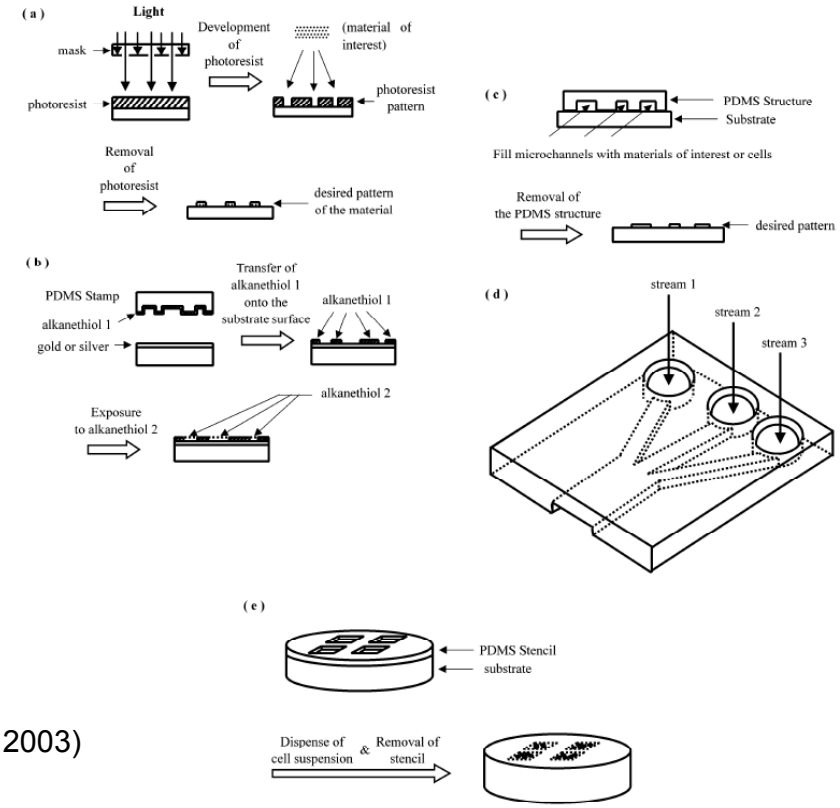
- Microphysiometer[11,12]
  - Measures changes in extracellular acidification rate: pH changes associated with alterations in ATP consumption by cells (metabolism)
  - Extremely sensitive readout of changes in cell metabolism
  - EXAMPLE OF HARDING MCCONNELL'S WORK WITH T CELLS

Relative advantages and disadvantages of cell-based sensors

- Pros
  - Cell-based sensors may utilize the ability of cells to respond to complex mixtures of signals in a unique way
  - May provide alternatives to animal testing in the future
- Cons
  - Issues of maintaining cell viability and reproducibility in measurements

Patterning cells for sensing[13]

- Techniques used:
  - Photolithography
  - Microcontact printing (soft lithography)
  - Microfluidic patterning
  - Membrane lift-off



(Park and Shuler, 2003)

Figure 1. Schematics of the processes of micropatterning: (a) photolithography, (b) microcontact printing, (c) microfluidic patterning using microchannels, (d) laminar flow patterning, (e) stencil patterning.

soft lithography and self-assembled monolayers

- Techniques based on the formation of gold (or other metal)-thiol bonds and spontaneous assembly of close-packed alkyl chain structures on a surface

Tissue analogs

- Any papers out on the liver chip? GRIFFITH LAB

In vitro toxicology studies: tissue biosensors

- Shown below is a model of the pharmacology of naphthalene[14]
  - Tissue distribution and toxic chemistry outlined is a multi-organ, multi-compartment phenomenon
- Potential methodology: Animal-on-a-chip
  - 2 cm x 2 cm Si chip
  - designed to have ratio of organ compartment size and liquid residence times physiologically realistic
  - minimum 10K cells per compartment to facilitate analysis of chemicals and enzyme activity
  - physiologic hydrodynamic shear stress values

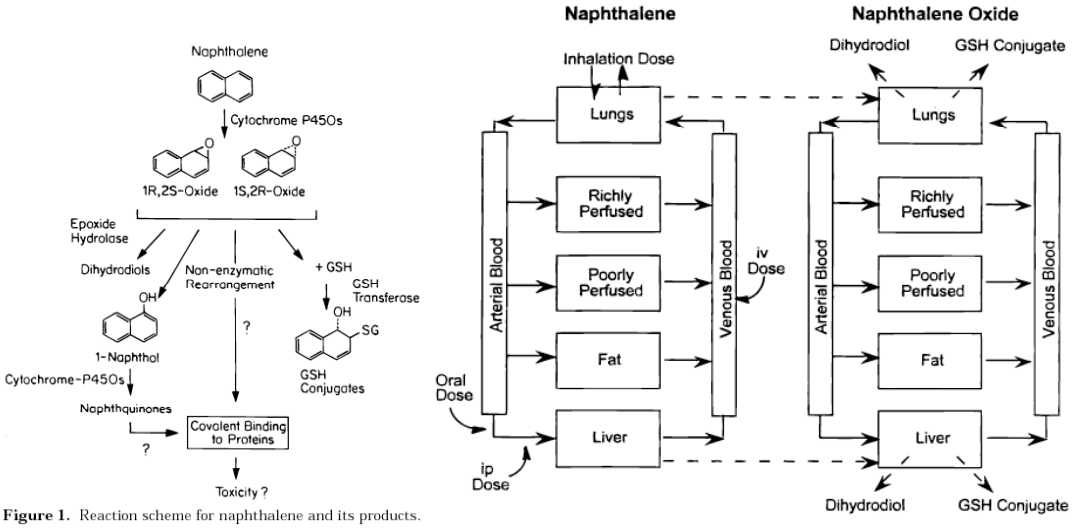
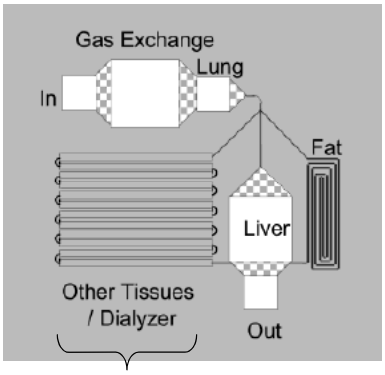


Figure 1. Reaction scheme for naphthalene and its products.

(Quick and Shuler 1999)



Models retention of chemical in blood and interstitial fluid

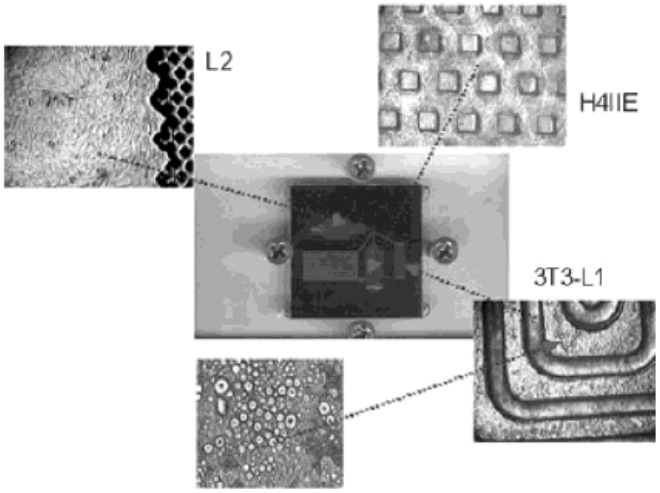


Figure 4. (a) Microscopic CCA system with four chambers. The dimensions (w × l × d) of the chambers are: lung 2 mm × 2 mm × 20 μm; liver 3.5 mm × 4.6 mm × 20 μm; other tissue 0.4 mm × 109 mm × 100 μm; fat 0.42 mm × 50.6 mm × 100 μm. Cells are cultured as monolayers on the silicon surfaces modified by adsorption of polylysine and collagen (b).

(Park and Shuler 2003)

In vivo detection

- Biofouling typically limits lifetime of *in vivo* measurements to 1-2 days
  - Inflammation
  - Fibrosis
  - Loss of vasculature

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