Welcome to Lecture 8

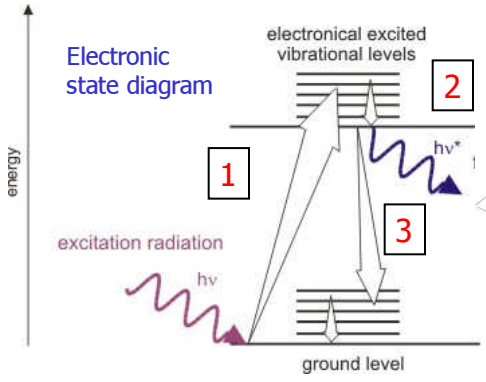
Last time:

Fluorescence

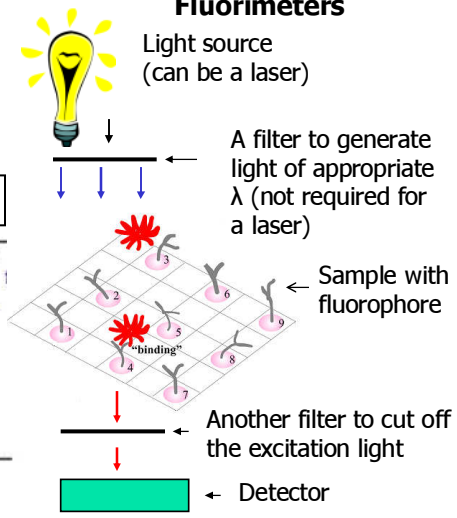
Excitation: $S_0 + h\nu \rightarrow S_1$

Relaxation: $S_1 + h\nu \rightarrow S_0$

Electronic state diagram

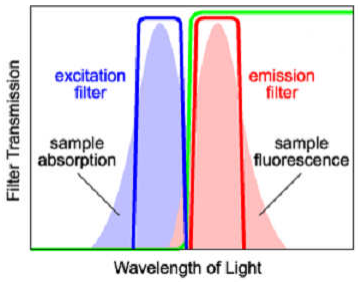


Fluorimeters



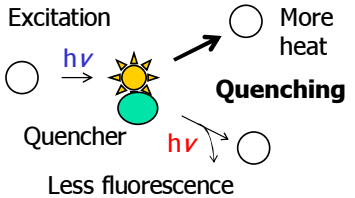
At the last lecture

Stokes shift and filters:

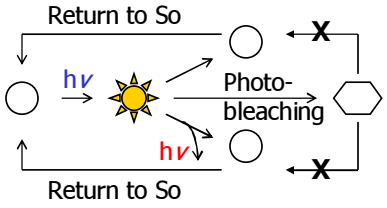


Quantum Yield:

$$\Phi = \frac{\text{N photons emitted}}{\text{N photons adsorbed}}$$



Photobleaching

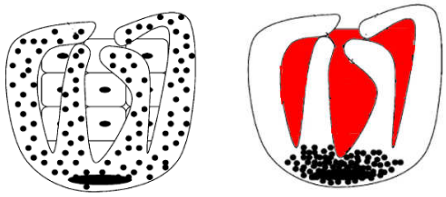


Cool sensors and materials

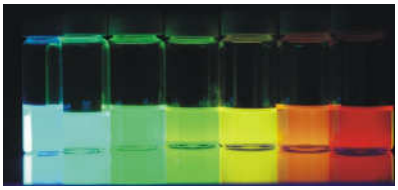
Antrax immunosensor

LMW **dyes**:

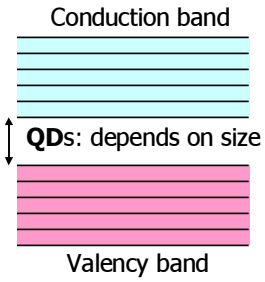
Melanophores on fluorescent **beads**



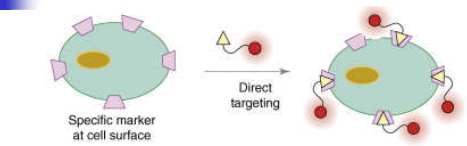
2.3nm ⟶ 5.5nm

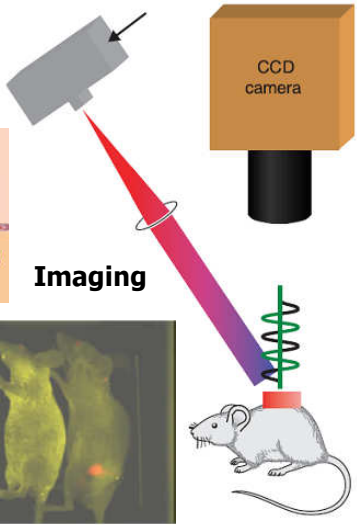


UV-excited CdSe **quantum dots**

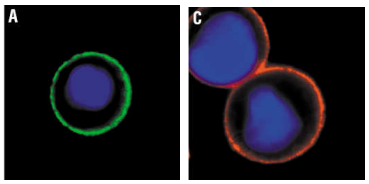


QDs in sensor and imaging

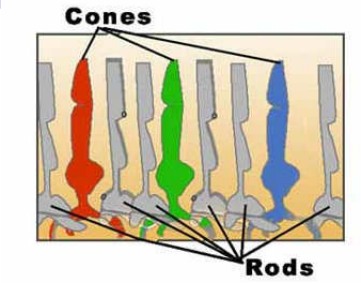


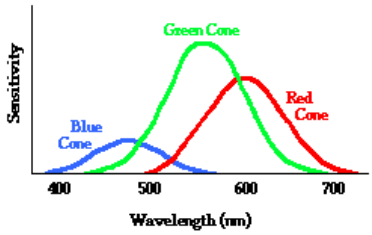


Ab-QD conjugates in cancer detection

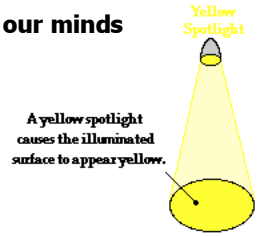


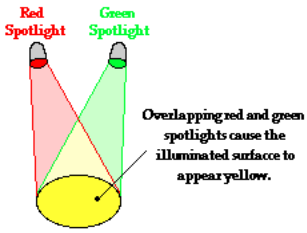
How colors work



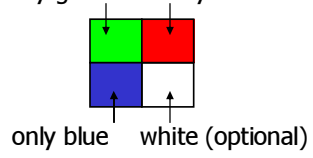


It's all in our minds



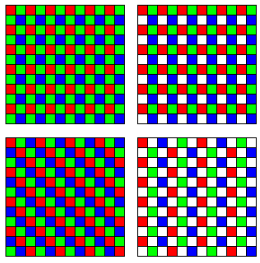


How CCDs detect colors

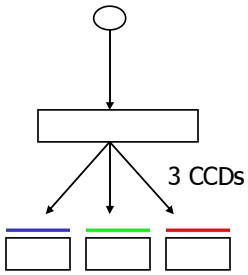


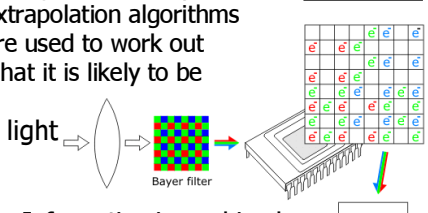
The green pixel doesn't know what the red is seeing; hence complex extrapolation algorithms are used to work out what it is likely to be

Bayer filters



Or you have to do it three times





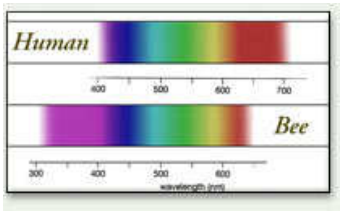
Information is combined to obtain an image

Animal vision

BIRDS: the inner segment contains a colored oil droplet at the base of the outer segment – natural filter through which light must pass before reaching rhodopsin

- The oil droplets are of several colors, due to the presence of different carotenoids
- Also, birds have 4 chromatic channels (some 5) i.e. they are tetra- or penta-chromatic

BEES and BUTTERFLIES:. The range of vision extends well into UV, presumbaly because many flowers they pollinate have special ultraviolet patterns that guide the insects deep into the flower



In dogs the central portion of retina is primarily composed of rods, they see well in the dark and probably detect motion and flickering much better. Also dogs have dichromatic vision - they only have two types of cones. Dogs are red-green color blind; occurs in ~4% of humans too

Human vision

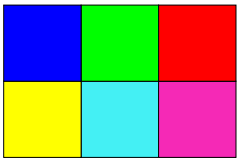
We are also different from machines

Suppose a human and a robot look at the same colored picture

What would a robot like the Terminator "see" when looking at a colored object?

Human vs Terminator

Human



Difference in signal detection:

R + G = Y R + B = M G + B = C R + G + B = W

The Terminator

A nm	B nm	C nm
X nm	Y nm	Z nm

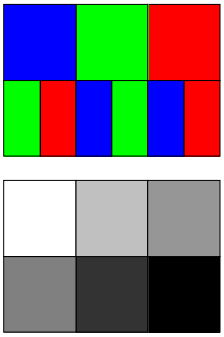
← Spectrum

255	204	153
102	51	0

← Light intensity

Do robots appreciate paintings? ☺

Human interpretation



Immobilization

Goals:

- To bring the recognition (sensing) biomolecules in close contact with transducer to facilitate signal transduction
- To stabilize biomolecules with the aim of increasing reusability and shelf-life of the biosensor device

Bioreceptor groups:

- Cells (including bacteria) and tissues
- Proteins (e.g. enzymes, antibodies, receptors)
- Nucleic acids (DNA and RNA)

Each group has its own requirements such as acceptable matrix, chemical treatment, etc

Creating recognition interface

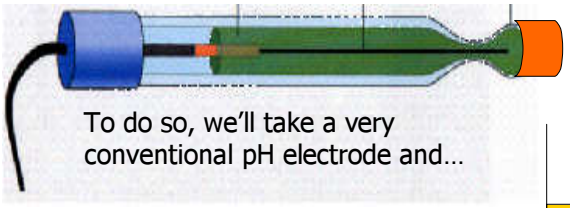
Means localization or attachment of a receptor on (or near to) the transducer surface

Many approaches:

- Physical entrapment near the surface e.g. the use of membranes or polymer gels
- Direct physical adsorption to the surface
- Covalent chemical coupling directly to the transducer or to an intermediate (e.g. polymer or a monolayer) on the transducer surface
- Non-covalent "capture" of the receptor, primarily using a highly specific bio-recognition system

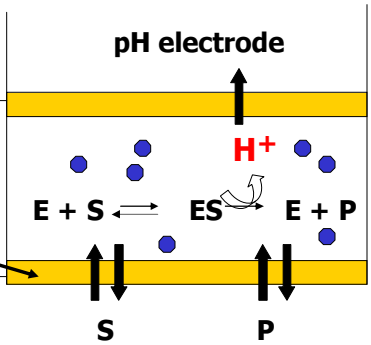
Whatever it is the biological activity must be preserved

Enzyme electrode



… add a little extra

To do so, we'll take a very conventional pH electrode and…



Biocatalytic layer (sensor) is located between two membranes & contains free or immobilized enzyme

- glucose oxidase or other enzyme (E)

Membrane permeable to S and P but not to E

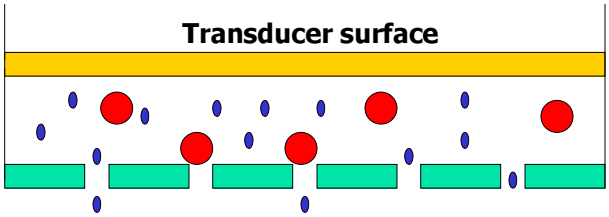
S - glucose or other analyte

P – gluconic acid or other product

Sample solution

Membrane entrapment

Entrapment behind semi-permeable membranes is simple method of retaining bioreceptors in a compartment adjacent to the transducer



Small analyte can go in but the large enzyme cannot get out

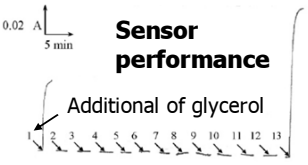
Plenty of inexpensive commercially available membranes: e.g. cellulose dialysis tubing – MW cut-off ~5-10KDa would keep most proteins in and allow small analytes diffuse in and out

Glycerol biosensors

Integrated multienzyme electrochemical biosensors for the determination of glycerol in wines

- Glycerol normally formed in fermentation (~1:10 ratio to EtOH); determination of glycerol is important for industrial quality control e.g. fermentation indicator, detection of possible adulteration
- Design: Enz immobilization of gold electrode + **dialysis membrane**

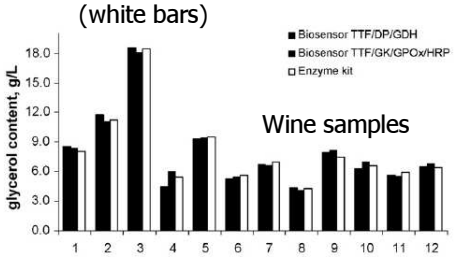
Two amperometric sensors: (i) glycerol dehydrogenase and (ii) glycerol kinase, both coupled to other enzymes to get a signal



Sensor performance

and 10 potential interferents EtOH, various sugars and acids

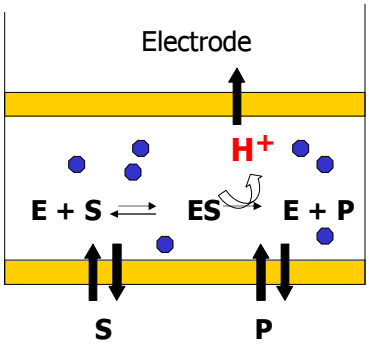
Comparison of the two biosensors with conventional enzyme-based assay (white bars)



Analytica Chimica Acta 609 (2008) 201–209

Optical biosensor

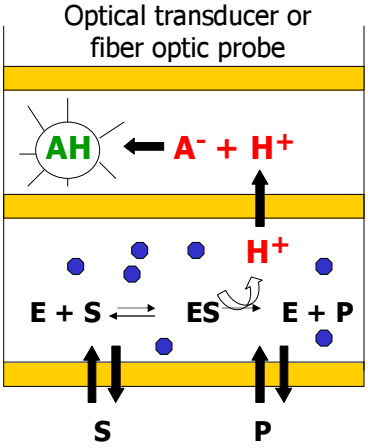
Electrochemical sensor



A^- can be a highly colored substance (e.g. pH indicator) or a fluorescent dye, to measure absorbance (OD) or fluorescence, respectively

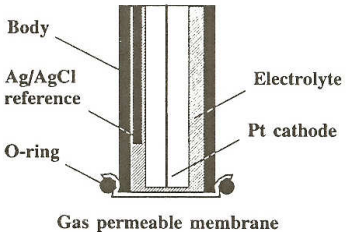
Optical biosensor

Optical transducer or fiber optic probe

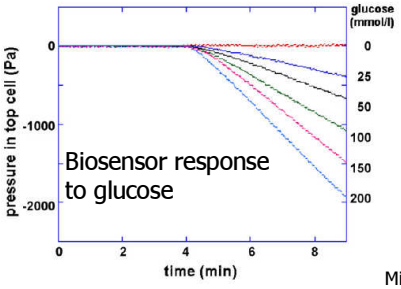


Sample solution

And another way

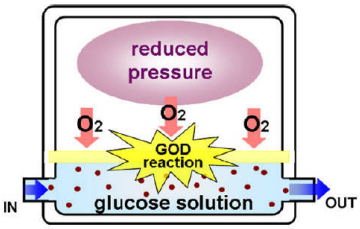


Gas permeable membrane

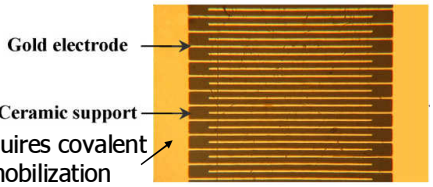


Biosensor response to glucose

Schematic diagram of chemo-mechanical glucose sensors



Membrane stuck can be used too



Requires covalent immobilization

Mitsubayashi et al (2008) Biosensors and Bioelectronics

Membrane entrapment

Advantages:

- Very simple and broadly applicable system for a wide range of receptors
- Mild conditions used – no chemicals, no procedures that can cause damage: the loss of activity or viability
- The bioreceptor can be maintained hydrated at suitable conditions (temp, pH) throughout
- The amount of volume is physically defined; hence control over size; excess of bioreceptor, if required

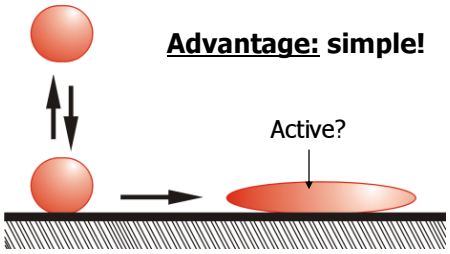
Disadvantages:

- Limited to certain type of transducers (e.g. optical, electrochemical) but no go with others (e.g. SPR)
- Often difficult to transfer to mass production (e.g. sealing wet membranes over devices)

Physical adsorption

Proteins and nucleic acids can be attached to a variety of surfaces by simple physical adsorption

- Generally proteins adsorb strongly to hydrophobic surfaces
- This adsorption is often followed by slow unfolding of the protein structure
- The process is thermodynamically driven to maximize the interaction between the protein core and the surface



Advantage: simple!

Disadvantages:

- Possible loss of activity due to denaturation
- Non-specific binding: any protein will stick to the surface too ☹

Physical adsorption

The surface does not have to be hydrophobic:

Proteins and nucleic acids typically carry a net charge at neural pH and can be attached to a surface with opposite charge

Does it solve the problem? **Not, entirely**

- Denaturation may no longer be an issue, but non-specific binding (surface "fouling") can still be…
- Nevertheless physical adsorption can be used advantageously in some circumstances e.g.

(iii) Adsorption of enzymes on carbon paste - electrode

(i) antibodies are relatively resistant to unfolding at hydrophobic interfaces due to the rigid *tert* structure

(ii) adsorption of DNA to positively charged membranes

Bottom line:

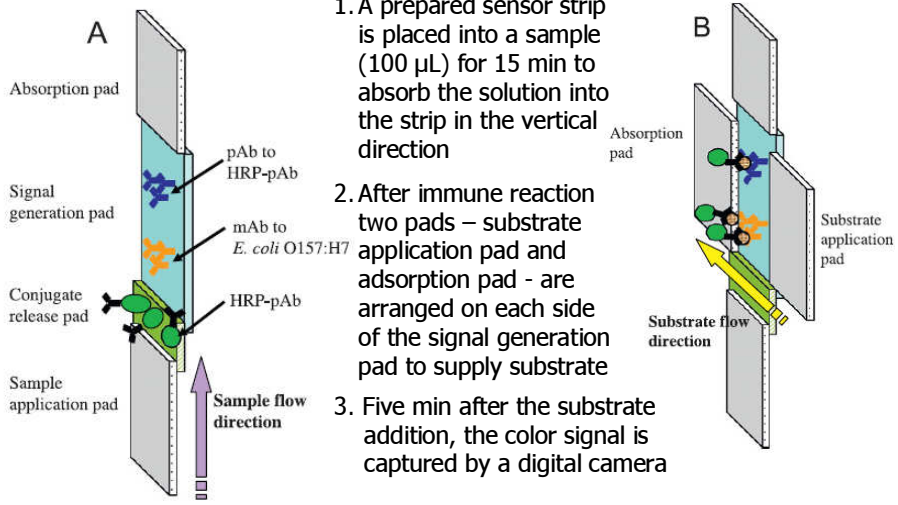
Physical adsorption often works best for single use applications

Immuno-strip biosensor

Enzyme-linked immuno-strip biosensor to detect Escherichia coli O157:H7

- About 90% of food-borne illnesses are caused by pathogenic microorganisms with *Escherichia coli* O157 being one of the most harmful - estimated 73,000 infection and 61 deaths in the U.S. each year
- ELISA is the most widely used method to detect and quantify bacteria but it takes relatively long time and appropriate equipment is required
- A membrane strip is a simple tool for rapid, on-site analysis - colorimetric immuno-sensor based on sandwich ELISA
- Colorimetric signal can be easily quantified, if required, by using a digital camera and simple image analysis software

Cross-flow strip design

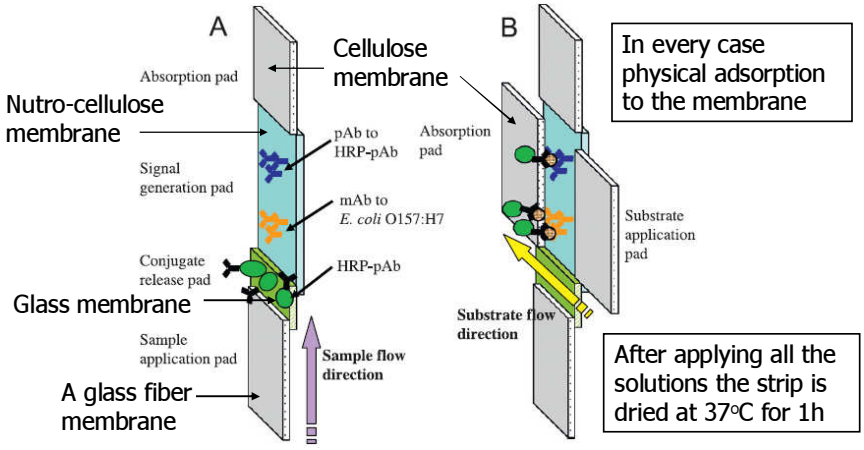


1. A prepared sensor strip is placed into a sample (100 µL) for 15 min to absorb the solution into the strip in the vertical direction
2. After immune reaction two pads – substrate application pad and adsorption pad - are arranged on each side of the signal generation pad to supply substrate
3. Five min after the substrate addition, the color signal is captured by a digital camera

The analytical procedure: (A) immuno-reaction in vertical direction and (B) enzymatic reaction in horizontal direction

Adsorption on membranes

Four different membranes were used for preparation

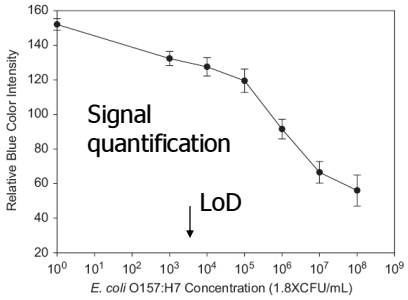


In every case physical adsorption to the membrane

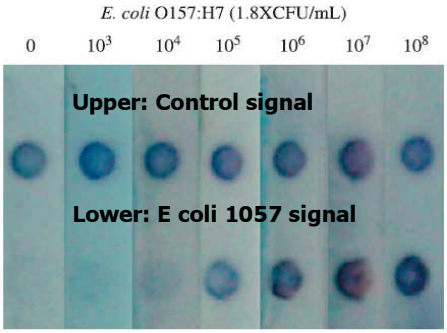
After applying all the solutions the strip is dried at 37°C for 1h

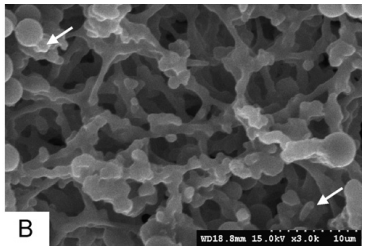
Ultramicroscopy 108 (2008) 1348– 1351

Biosensor performance



Colorimetric signal produced by Horse Reddish Perodxidase at two specific positions at the signal generation pad

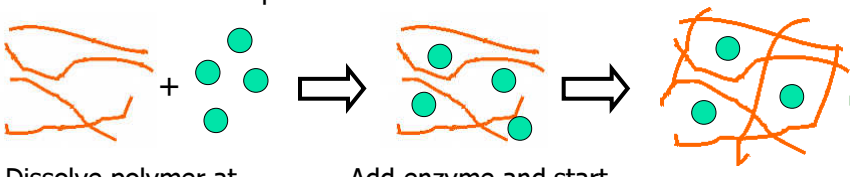




SEM images of the nitrocellulose membrane (signal detection pad) with E. coli O157 (white arrows) bound to immobilized mAb

Entrapment in hydrogels

A simple alternative to membranes



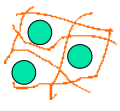
Dissolve polymer at elevated temperature

Add enzyme and start cooling down

A gel forms

Remember gelatin?

- Polymer hydrogels can be dried into a film to better preserve bioreceptors on storage or between usage
- More important, hydrogels can by covalently cross-linked to improve stability of receptors



drying

Hydrogels

Water swollen cross-linked polymers

Cross-linking is achieved by:

- Non-covalent interactions e.g. hydrogen bonds, charge, van der Waals forces
- Covalent chemical reaction in the presence of a suitable cross-linking agent:

Don't confuse with Abs !

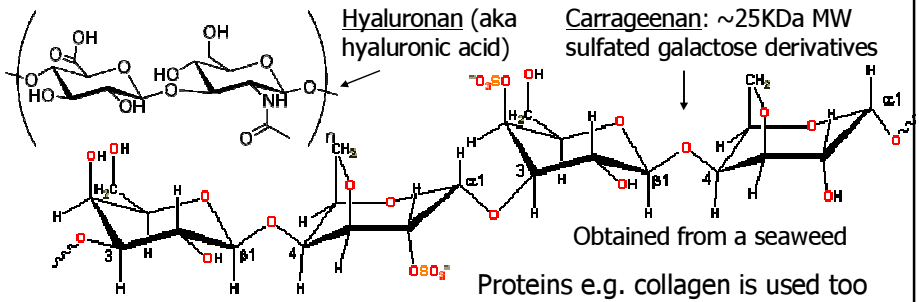
- small MW agents that links two chains together by reacting with its functional groups, e.g. cross-linking natural polysaccharides with bi-functional cross-linkers that react with -OH, -NH_2 or -COOH groups present
- copolymerization-crosslinking reactions between the monomers and a multifunctional monomer that is present in small quantities e.g. synthesis of polyacrylamide gels

Natural hydrogels

A variety of materials are available

Natural: e.g. Agarose, Alginate, Carrageenan

- Complex oligosaccharides derived from natural sources
- Gelation can be triggered by various factors e.g. temp (agarose), or addition of Ca ions (alginate); low melting points variants e.g. agarose gel at 40°C



Hyaluronan (aka hyaluronic acid)

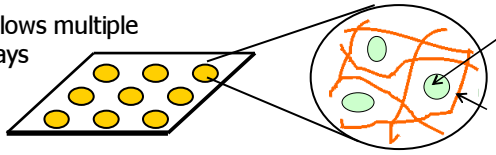
Carrageenan: ~25KDa MW sulfated galactose derivatives

Obtained from a seaweed

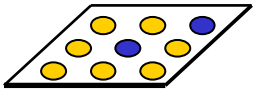
Proteins e.g. collagen is used too

Lymphocytes sense toxins

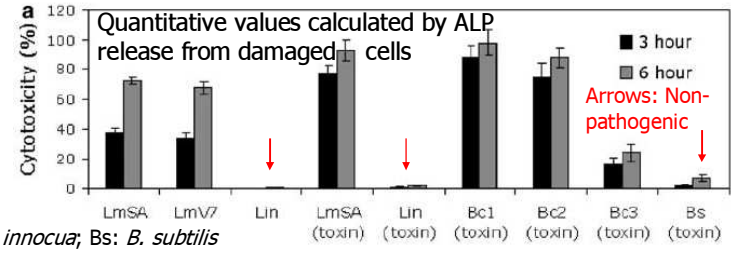
This setup allows multiple parallels assays



Exposure to hazardous bacteria/toxins



- Detects whether a toxin or bacteria have "broken" the lymphocytes
- If damaged, lymphocytes release alkaline phosphatase, which can be detected by a color reaction



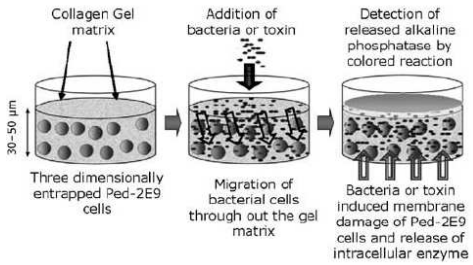
Lin: *L. innocua*; Bs: *B. subtilis*

Natural hydrogels in sensors

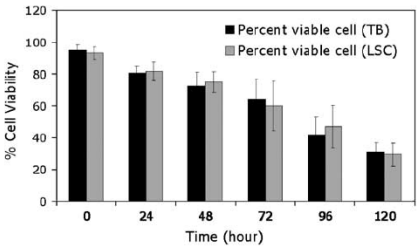
A novel and simple cell-based detection system with a collagen-encapsulated B-lymphocyte cell line as a biosensor for rapid detection of pathogens and toxins

Pratik Banerjee[1], Dominik Lenz[2], Joseph Paul Robinson[2], Jenna L Rickus[3] and Arun K Bhunia[1]

Strategy for cell-based biosensing using collagen-encapsulated cells and the assay procedure



Viability of sensor cells in collagen microenvironment



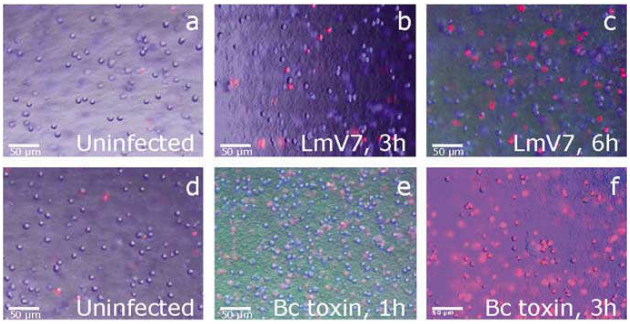
How do you access cells viability in a gel?

Laboratory Investigation (2008) 88, 196–206

Fluorescence

Laser scanning cytometry of the collagen matrix with entrapped cells

Also used as verification of the AP-based assay

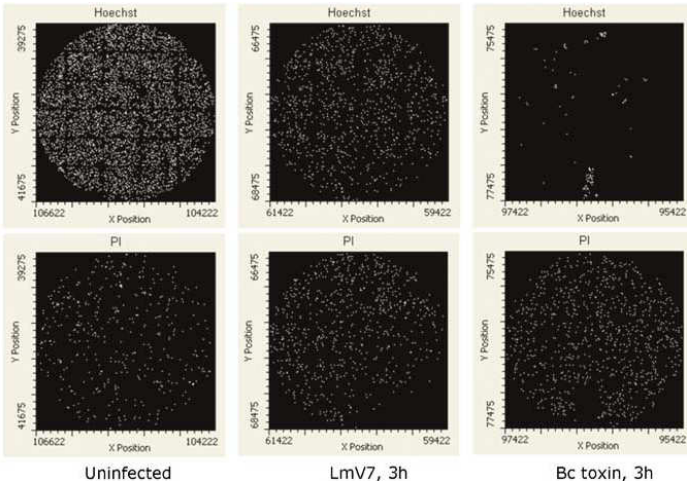


The images (a–f) are shown as merged and virtually colored images of blue (Hoechst 33342, 405 excitation line) and red (Propidium iodide, 488 excitation line) as obtained by the photomultiplier of the iCys LSC

The blue cells were counted as "live", the red cells - as "dead" (a) Control, uninfected cells; (b) and (c) infected with L. monocytogene and toxin from B. cereus

Can be quantified too

Image analysis of the data as in the previous slide



AP detection is simpler and cheaper ☺

Laboratory Investigation (2008) 88, 196–206

Synthetic hydrogels

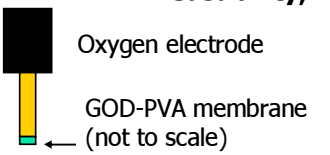
Synthetic: e.g. poly(vyinyl) alcohol (PVA) makes excellent dry film and polyacrylamide allows for well controlled cross-linking (important to minimize leaching out!)

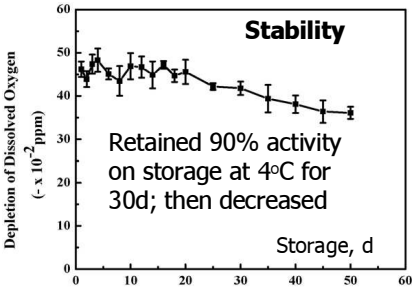
Entrapment within conducting polymers: Many redox enzymes can be directly "wired" into electrodes and the polymer can play a key role in the signal transduction mechanism. Pyrrole is a typical example. Polymerization can be carried out under mild conditions

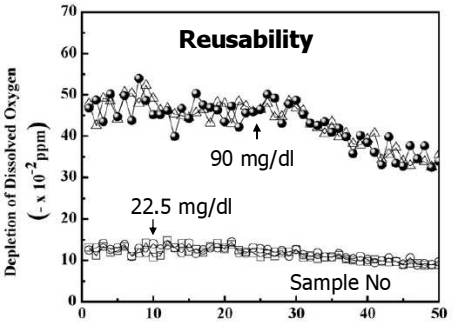
Entrapment within sol-gel glasses: Porous silicate network is formed under mild aqueous conditions. Sol gels are optically transparent (hence, a great choice for optical sensors) and provide excellent stabilization to bioreceptors. However, small pore size makes it diffucult to use sol-gels with large analyte targets e.g. proteins

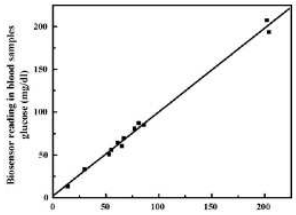
GOD-PVA with O_2 electrode

Reusability, reproducibility and stability







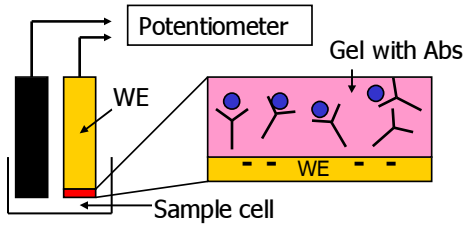


Correlation with standard hospital analysis

Kumar and D'Souza (2008) Talanta 75, 183–188

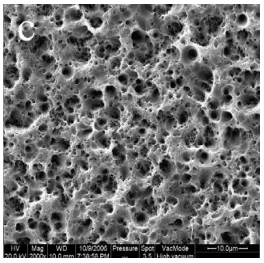
X-linked polymeric networks

Potentiometric Hepatitis B surface antigen (HBsAg) immunosensor



The signal is due to the increase of the electric charge density on the electrode surface after the antigen binding to the Ab

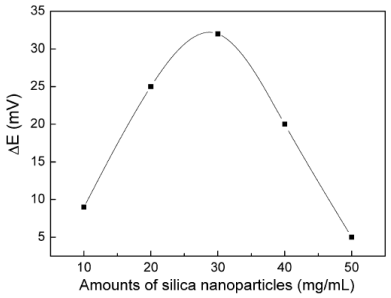
- Silica nanoparticles (d~100nm) are suspended in solution of chitosan and deposited on the electrode surface
- Treated with glutaraldehyde to obtain a 3D network (X-linking) and create extra CHO
- Silica particles removed by dissolution is aq HF
- Excess of aldehyde groups used to covalently immobilized Hepatatis B Abs (HBsA)



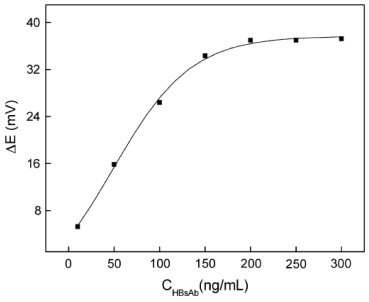
SEM of the resulting porous chitosan film

J Colloid Interface Sci 320 (2008) 125–131

Extensive optimization



1) Effect of the amount of silica nanoparticles in chitosan solution on potentiometric response in the presence of 100 ngmL^{-1} HBsAg



2) Effect of the amount of immobilized HBsAb on potentiometric response in the presence of 100 ngmL^{-1} HBsAg

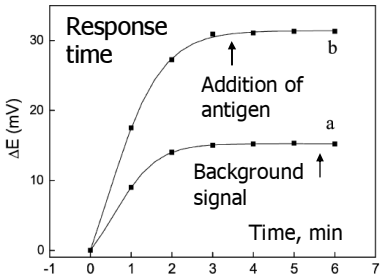
3) Further biochemical and electrochemical optimization too…

Detection limit: 3.89 ngmL^{-1} HBsAg and linear range: 7-700 ngmL^{-1}

J Colloid Interface Sci 320 (2008) 125–131

Sensor performance

- Hepatitis B surface antigen (HBsAg) is a protein antigen produced by HBV
- HBsAgis the earliest indicator of acute hepatitis B infection
- Often it can be detected before symptoms appear; hence used for early diagnostics



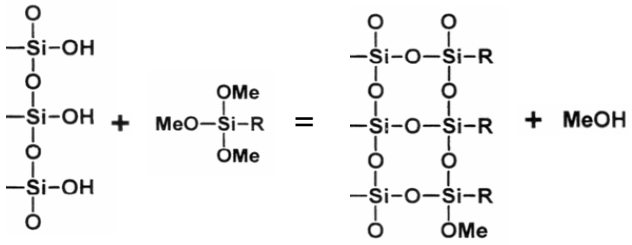
Comparison of the biosensor with conventional ELISA assay

Potentiometric responses of clinical serum samples

Sample number	ΔE (mV)	This method (ng mL^{-1})	ELISA (ng mL^{-1})
1	19.2	15.3	14.9
2	38.8	163.4	162.3
3	43.3	270.6	270.9
4	45.6	355.4	354.7
5	48.7	561.2	561.8

Sol-gel chemistry

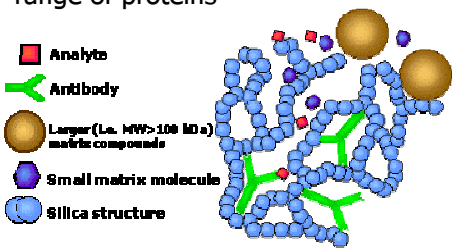
Organotrialkoxysilanes, $RSi(OR')_3$ are sol-gel monomers and coupling agents. They are commonly used to modify surfaces and to prepare coatings for microelectronics and photonics applications



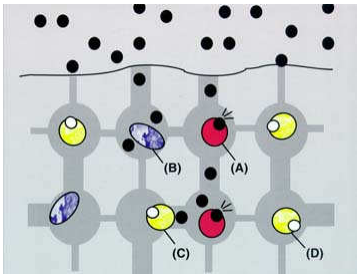
- Sol-gels are frequently used for entrapment of biomolecules
- In the absence of "interfering" molecules a very regular polymer network is obtained

Immobilization in sol-gels

Suitable for entrapment of a wide range of proteins

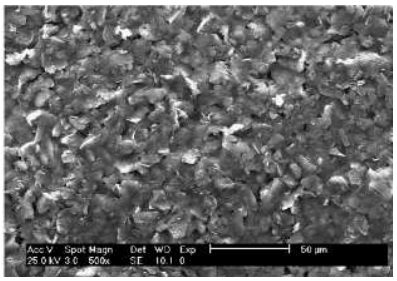


Drawbacks: accessibility, some inactivation, mass transfer



- Sol gels are optically transparent and provide excellent stabilization to biomolecules
- This chemistry is widely used for surface modification of certain type of transducers: coating thickness typically <5μm and often < 1μm

A sensor we discussed



SEM of AChE/Al2O3 sol-gel-carbon electrode - granular (porous) structure due to presence of AChE

AChE
Ac-choline + H_2O → thiocholine + CH_3COOH

anodic oxidation
Thiocholine → dithio-bischoline + $2H^+ + 2e^-$

Electrodes prepared in the same batch demonstrated a standard deviation of 1.4% and retain 98% of initial current response after 20 assays

Note:

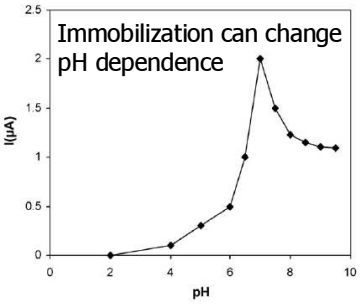


Table 1
Comparison between the three pesticides, 0.2 M phosphate buffer, pH 7.0, applied potential: 210 mV, 1 mM acetylthiocholine

Pesticide	Limit of detection
Paraoxon (M)	7.5×10^{-9}
Dichlorvos (M)	5.0×10^{-10}
Chlorpyrifos-ethyl-oxon (M)	2.5×10^{-10}

Zejli et al Talanta 77 (2008) 217–221

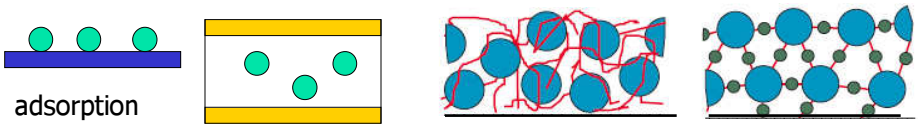
Membranes vs gels

Entrapment	Advantages	Disadvantages
Membrane	• Simple and universal • Very mild conditions • Often long shelf life	• Diffusion limitations can be significant • Difficult to mass produce
Polymer gels	• Much easier to mass produce • Highly efficient solution for many particular applications	• Higher possibility of denaturation • Special handling may be required

The rule of thumb: typically mild conditions e.g. temperature-(agarose) or ion-induced x-linking (alginate) are used for immobilizing cells and very labile macromolecules, while chemical x-linking is preferred for many enzymes and ABs (more tolerant to conditions)

Let's summarize

So far we looked at several methods:



adsorption

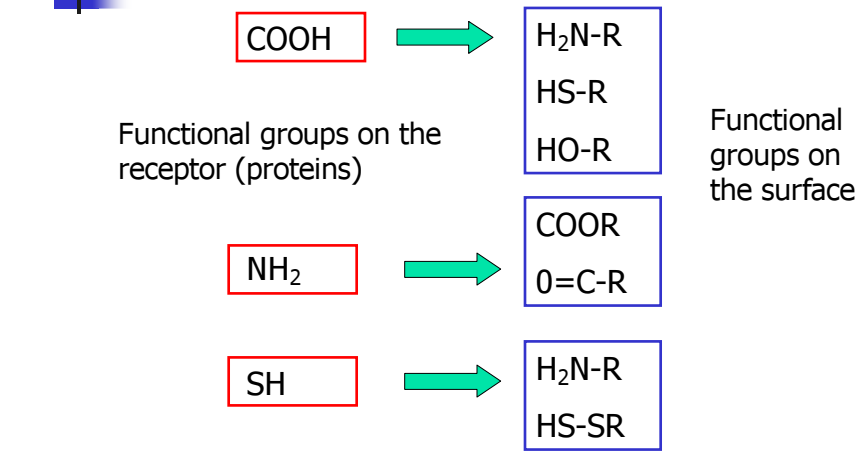
membrane entrapment gel entrapment and X-linking

The common feature: the biosensing layer is created by physical attachment to the transducer. **Drawbacks** – leaching, desorption

Covalent attachment: Biomolecules cannot escape but suitably reactive surface is required – appropriate functionality is everything

Functional groups are specific groups of atoms within molecules responsible for the characteristic chemical reactions of these molecules. Typically, the same functional group will undergo the same or similar chemical reaction(s) regardless of the size of the molecule it is a part of

Coupling chemistry



Other functional groups e.g. OH are used too but less frequently
e.g. Serine is less reactive; Tyrosine is often in the protein core

Covalent coupling

Relative usefulness of commonly used AA residues

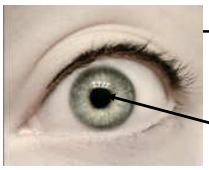
Residue	Content	Exposure	Reactivity	Stability	Use
Aspartate	+	++	+	+	+
Arginine	+	++	-	±	-
Cysteine	-	±	++	-	-
Glutamate	+	++	+	+	+
Histidine	±	++	+	+	±
Lysine	++	++	++	++	++
Serine	++	+	±	+	±
Threonine	++	±	±	+	±
Tyrosine	+	-	+	±	±
C terminus	-	++	+	+	+
N terminus	-	++	++	++	+

The good news

I am not going to discuss available reagents, reaction conditions and mechanisms – this chemistry is well developed and widely used

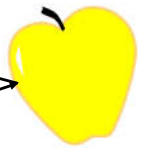
The bad news: There is a book chapter on immobilization methods that you have to read (required). Pick up a hard copy when you hand in the quiz

Quiz time



4) What color apple will you see?

Explain how you arrived to your answer



Blue light source

Typical surfaces

A very wide range of different materials is used

Some important types:

- Nobel metals
- Glass and silica
- Metal oxides
- POLYMERS (vast range)
- Carbon

Chemical attachment can be accomplished by covalent linkage, non-covalent interactions or a combination of the two

Either way functional groups ("handles") must be introduced

Gold

In biosensor research gold is the most widely used noble metal: eletrochemical and acoustic, most SPR devices

Key features:

- Inert (e.g. does not oxidize in air)
- Compatible with many semi-conductor manufacturing processes (e.g. evaporation coating)
- Thin films maintain a degree of transparency

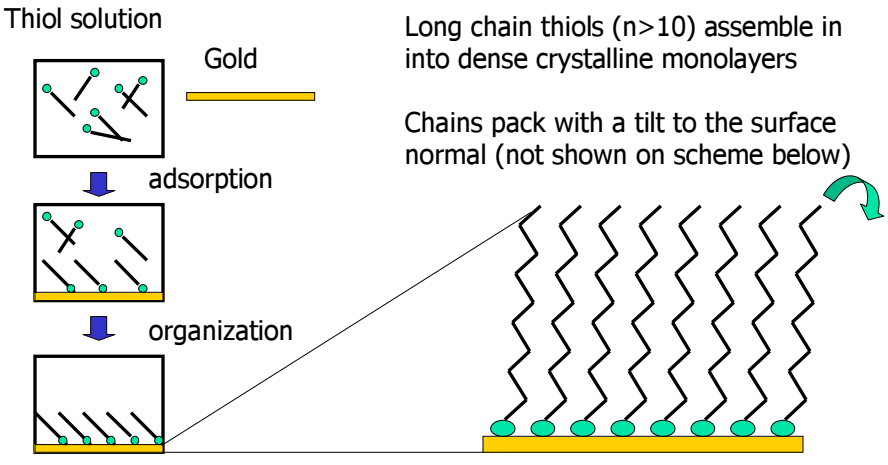
For biosensors research – a great method for controlled immobilization of bioreceptors:

SAMs – Self-Assembled Monolayers

Thiol SAMs on gold

Thiols spontaneously form monolayer on gold surface

$$\text{R-SH} + \text{Au} \longrightarrow \text{R-S-Au}$$

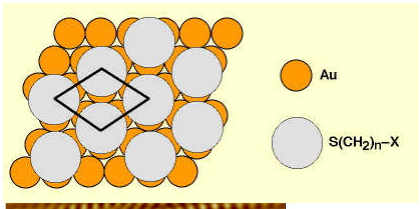


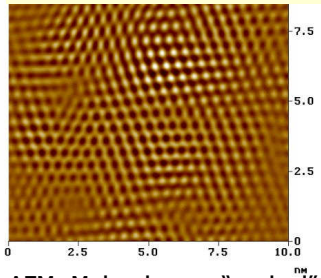
Long chain thiols (n>10) assemble in into dense crystalline monolayers

Chains pack with a tilt to the surface normal (not shown on scheme below)

Thiols on gold

Au-thiolate bond is 44 kcal/mol





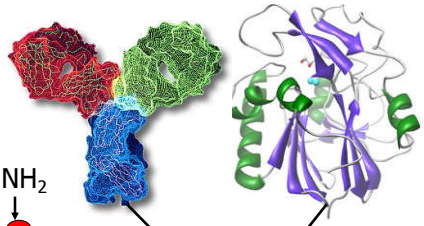
AFM: Molecules are "packed" together like in a crystal

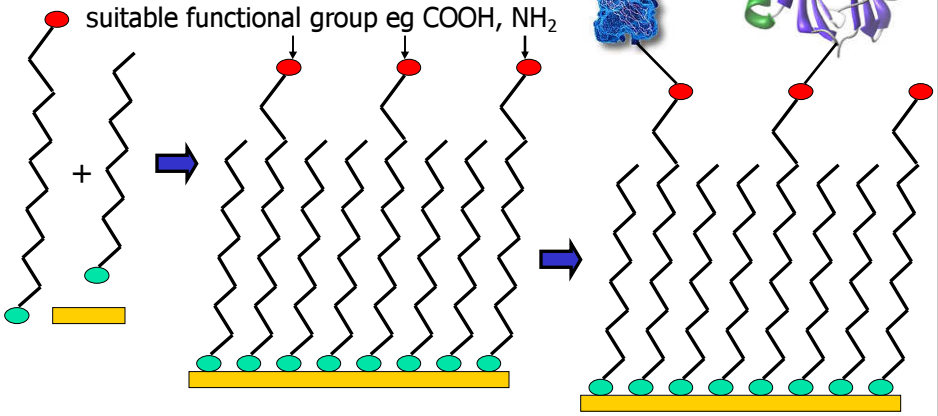
Important characteristics:

- Bond are very strong and show little tendency for dissociation or lateral migration under normal conditions
- Adsorption is random and statistical with the composition of the monolayer reflecting the concentration of different thiols in solution
- This means that mixed mono-layers can be formed with chains of different length or different end functionality

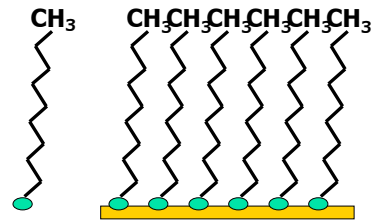
And now the exciting part

If we dilute simple alkyl thiols with suitably end-functionalized ones and self-assemble the mixture

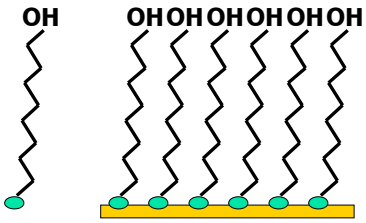




One more touch…



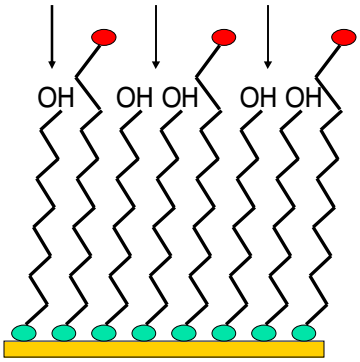
This is very hydrophobic surface



but this one is hydrophilic!

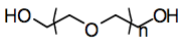
Remember the discussion of hydrophobicity and stickiness?

Hence, we REALLY want this arrangement on the surface

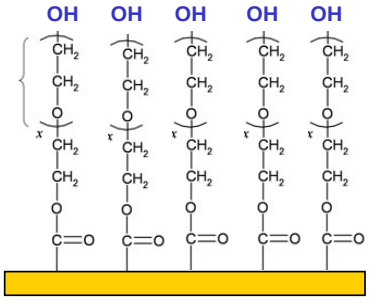


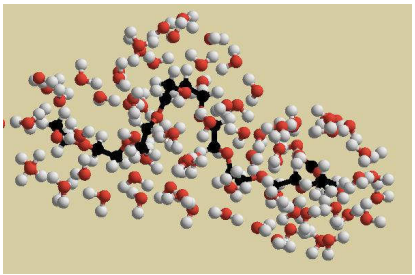
Making surface hydrophilic

Poly(ethylene glycol) is polymerized ethylene oxide



PEG is the most widely used polymer for surface modification





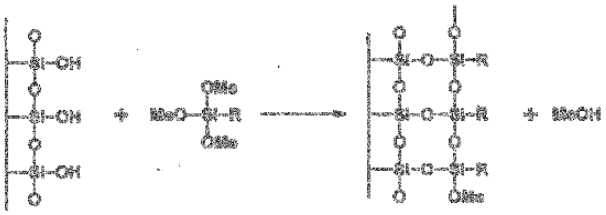
Modeling of PEG interactions with H_2O

The helical conformation of PEG chains in aqueous solution in such that water molecules fit perfectly into the "grove"

Glass silica and metal oxides

Main features:

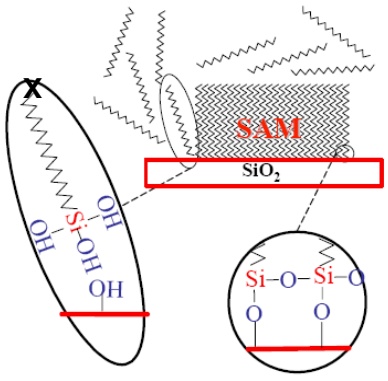
- Contain a mixture of oxygen bridged atoms (Si and Me) and OH groups
- Provide a surface which is relatively easy to further modify
- Glass and silica are ideal for optical applications (transparency)
- Silicon dioxide is used in field effect transducers; other metal oxides are also used/compatible with semi-conductor manufacturing process



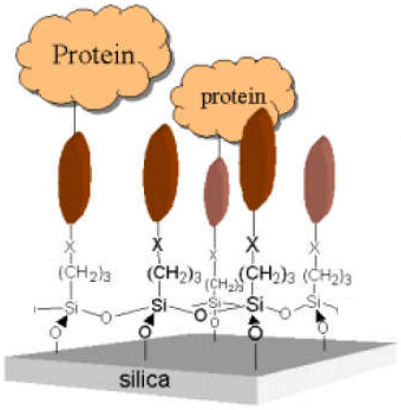
R is a suitable functionality e.g. NH_2-group to enable receptor immobilization

Siloxane monolayers

Same principle – different chemistry



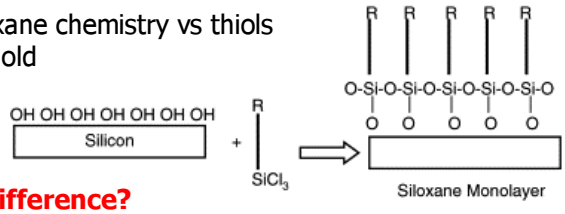
Formation of alkyl-siloxane monolayers



Protein immobilized on silica support

Different SAMs

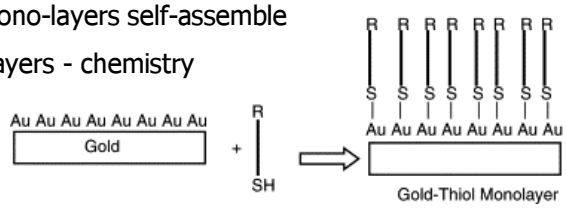
Siloxane chemistry vs thiols on gold



What is the difference?

Thiol on gold mono-layers self-assemble

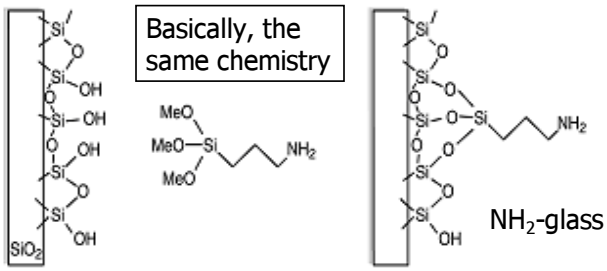
Siloxane monolayers - chemistry



Which one is better ☺?

Functionalization of glass

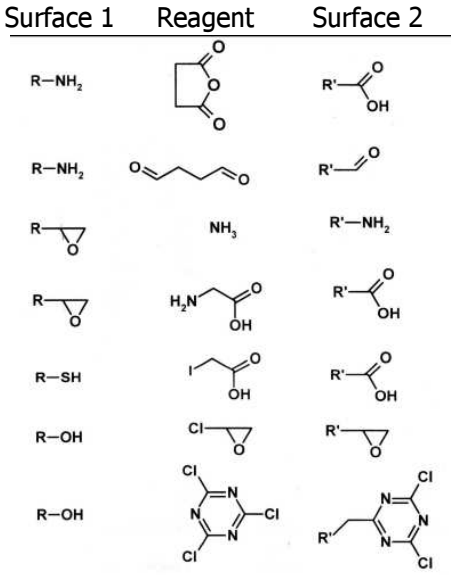
Glass (or silica) is subjected to harsh hydrolysis to maximize the number of OH-groups on the surface



Treatment with 3-Aminopropyltriethoxysilane (APTES)

- Other reagents are available for introducing other surface functionality e.g. COOH-groups, etc
- Once we got the functionality – we can do chemistry

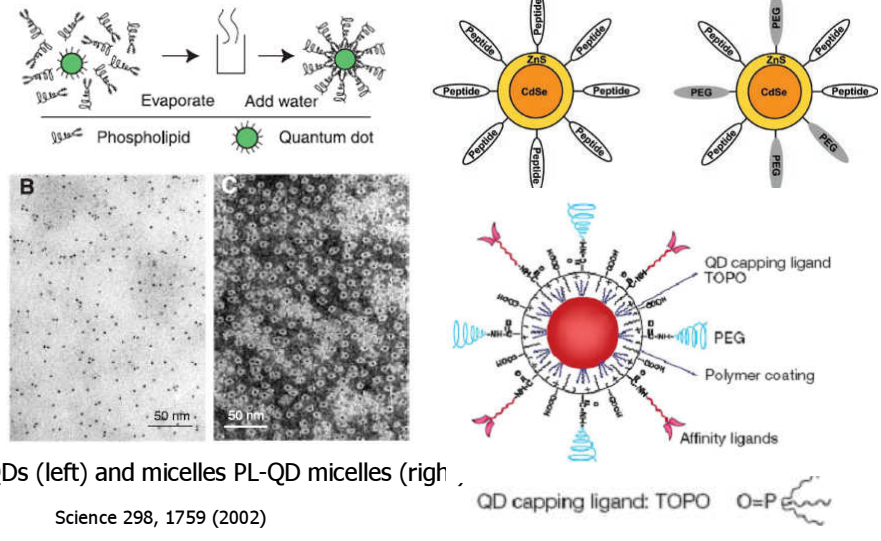
Changing surface functionality



Points to note:

- Commercial reagents and conditions are available to switch from surface with ease
- Functional groups used are often the same as present in proteins e.g. NH_2, OH, COOH, SH
- Often (but not always!) the same reagents are used for surface modification and for protein coupling to the surface, albeit under different conditions

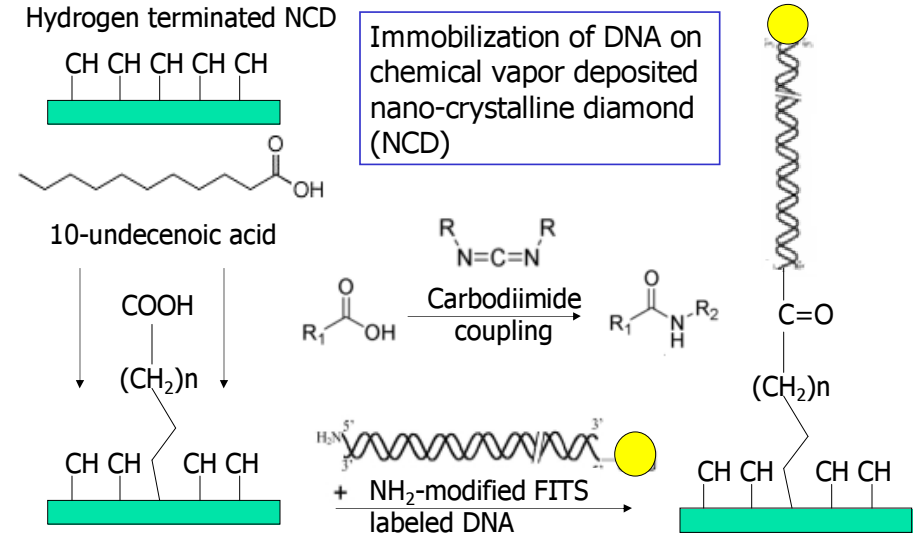
Surface modification of QDs



QDs (left) and micelles PL-QD micelles (righ

Science 298, 1759 (2002)

Carbon

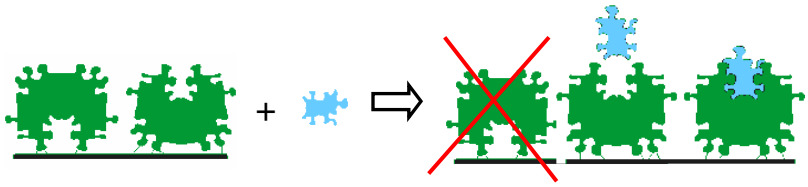


Polymers

The easiest proposition in terms of chemistry

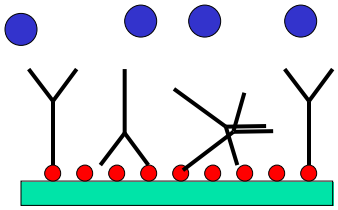
- Modern polymer technology enables the synthesis of polymers with practically any functionality
- Cheap and versatile, but physical properties (conductivity, transparency, stability of very thin films) are often inferior

We have looked at several methods of introducing chemical functionality on or near the surface of the transducer with the aim of attaching biomolecules. However, sometimes there is a problem – just chemistry may not be good enough….

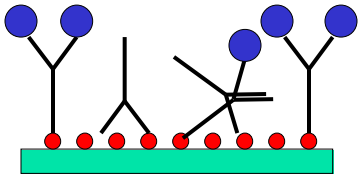


Non-covalent capture

A number of methods are available for immobilizing bioreceptors in non-random conformation

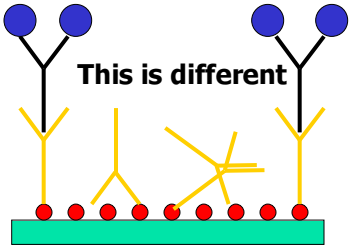


Immobilized Abs + analyte



Binding to "productive" sites

Not all the Abs are active but more importantly some sites are hindered – hence, different binding characteristics e.g. lower sensitivity

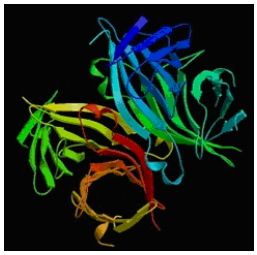


Immobilize Ab to Ab first

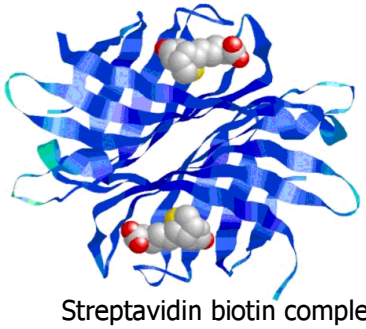
Other agents e.g. protein A

Streptavidin - biotin

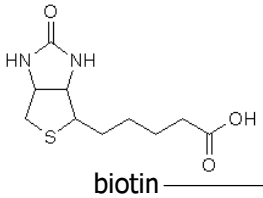
Streptavidin is a tetrameric protein which binds vitamin B7 biotin with Kd about 10^{-14}



streptavidin

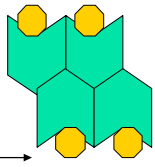


Streptavidin biotin complex



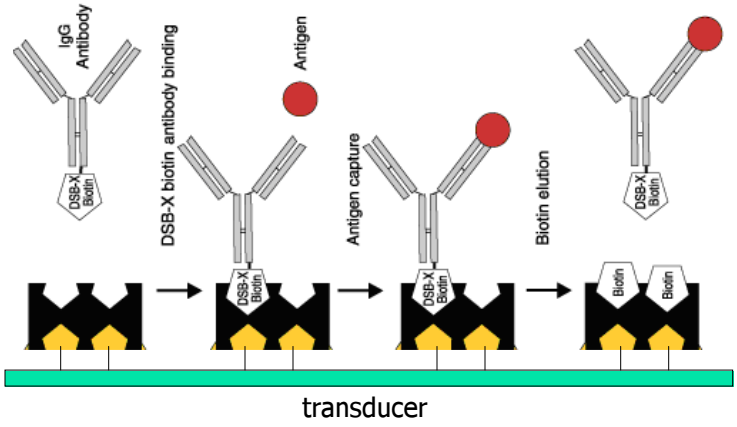
biotin

- Binding sites are on the opposite side of the globule



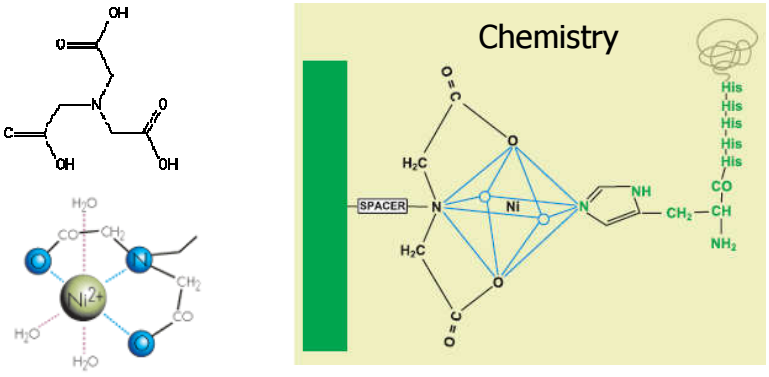
Biotynylated surface

Antibody capture on the biotynylated surface of a transducer



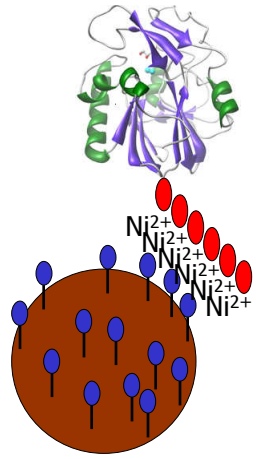
Histidine tags

Approach used for the purification of proteins and their attachment and orientation on the surface. It relies on the ability of histidine to complex metals



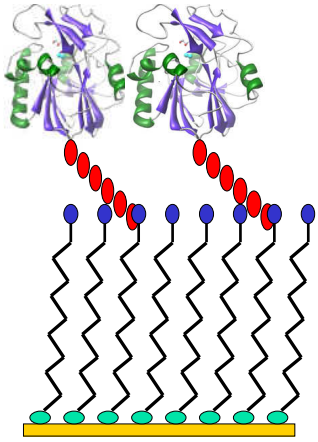
Nitriloacetic acid, its complex with Ni^{2+} and a *tert* complex with His

The use of histidine tags



A (poly)histidine-tag is an amino acid sequence of at least 6 histidine residues, typically attached to the N- or C-terminus of proteins

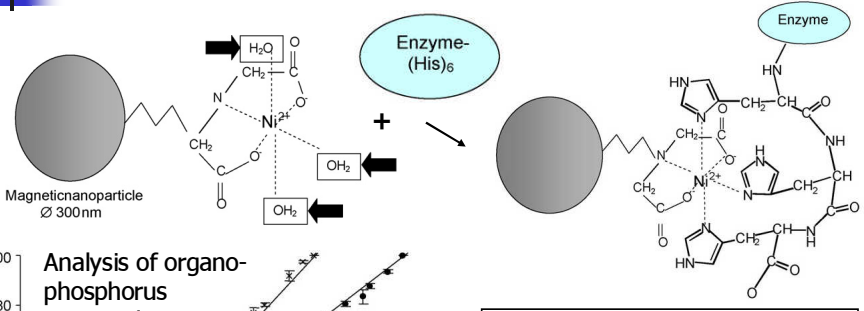
Simple and widely used technology

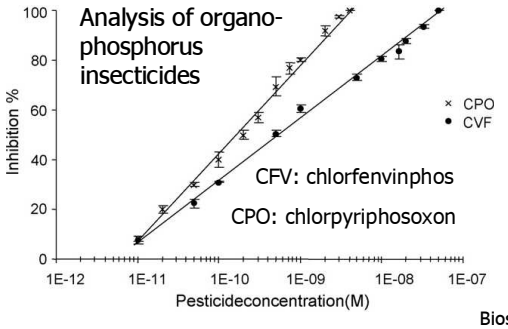


Surface capture

Purification of the target protein by Metal Chelate Affinity Chromatography

His-tag AChE on magnetic beads





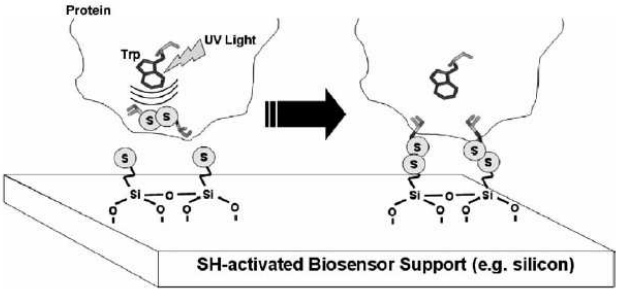
The biosensor was fabricated by placing 1 µL of the enzyme functionalised beads suspension on the surface of the working electrode, fitted with a 4 mm-diameter magnet on the back of the electrode

Biosensors and Bioelectronics 23 (2007) 506–512

Photonic activation

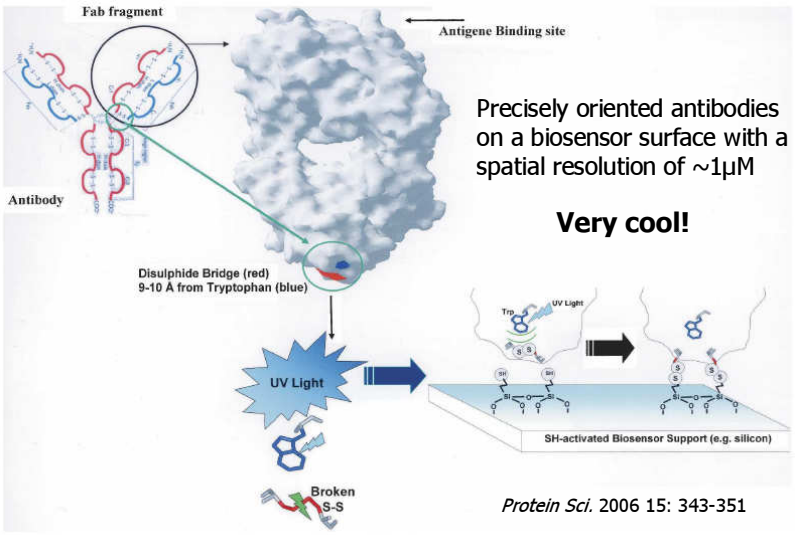
Light induced opening of a specific disulphide bridge which is a close proximity of aromatic amino acids in the protein structure

The reduced disulphides leave free thiols that can anchor covalently to a thiol coated surface



Advantages: (i) avoids chemicals in the reduction and (ii) modern laser technology allows focal spots with dimensions of <1µm – great for miniaturization and making arrays

Was used with Abs



Precisely oriented antibodies on a biosensor surface with a spatial resolution of ~1µM

Very cool!

Protein Sci. 2006 15: 343-351

Figure 1. UV light-induced immobilization illustrated with an immunoglobulin Fab fragment onto a thiol-derivatized surface. The disulfide bridge (red) located near a Tryptophan residue (blue) is located far away from the antigen binding site.

Covalent vs non-covalent

Covalent immobilization:

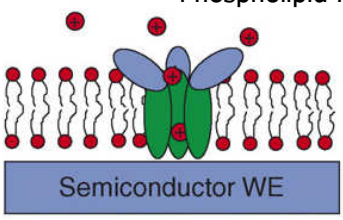
- Attached proteins do not leach, important for applications that require extensive washing and for continuous flow analysis
- Analytes can be stripped, allowing for further analysis
- Proteins are attached to surface directly and often non-specifically; often results in higher density

Non-covalent (capture) immobilization:

- No chemical inactivation due the nature of chemical reagents/ reaction conditions used
- No loss of biological activity due to the lack of orientation on the surface and steric hindrance

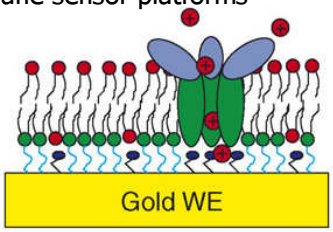
Membrane receptors

Phospholipid membrane sensor platforms



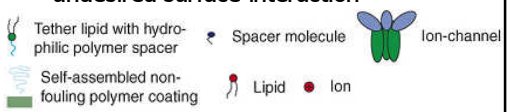
Typically a semiconductor or oxide substrate is used for direct assembly of a supported lipid bilayer on the surface, acting as a working electrode (WE) for electrochemical measurements

Drawback: little space between the lipid membrane and the solid support to accommodate hydrophilic domains of the integrated protein

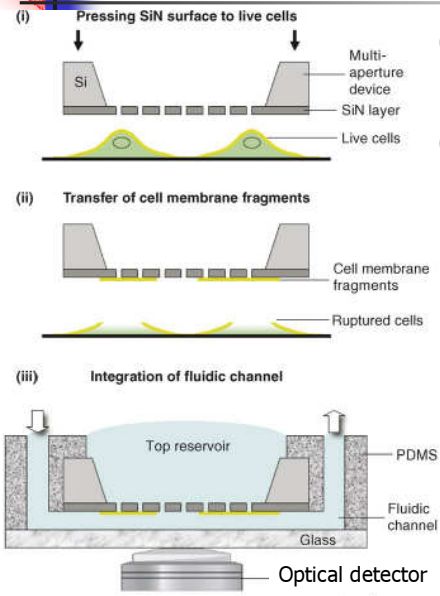


A supported lipid bilayer with a linker (often derived from lipids) to tether the lipid membrane to a gold

The increased space of a few nanometers allows integration of membrane proteins without undesired surface interaction

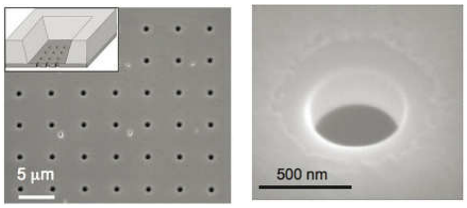


Nanoscale array



(a) Native membrane fragments are transferred onto the nanopore arrays by steps (i)–(iii)

(b) Scanning electron micrographs of 5 mm lattice array (top) of 500 nm holes fabricated in a 500 nm thick silicon nitride film (bottom). The inset shows a scheme of the 3D structure of the silicon chip

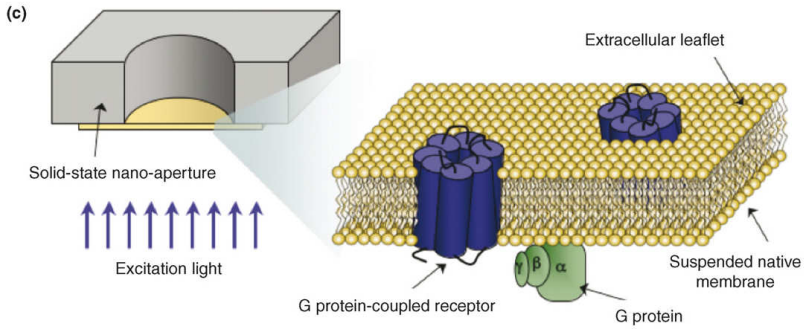


Reimhult, Trends Biotechnol, 2008

Why it is interesting

About 50% of drug target are extracellular membrane proteins

Schematic view of a membrane covering a nanoaperture



Membrane proteins e.g. GPCRs can be used with the native cell membrane, or partially purified and incorporated into an artificial phospholipid membrane

Drawbacks: Stability, stability and stability

The choice of method

depends on many parameters:

- Type of sensor is being produced
- Requirements for long-term stability e.g. will it be regenerated and re-used or is it a single use sensor that will be disposed
- The nature of the receptor e.g. how sensitive it is to handling (cells must be kept alive, enzymes active, etc)
- The nature of the transducer surface and the mechanism of signal generation (e.g. it's pointless to have a thick biolayer, if the transducer only "sees" a small fraction of it)
- Research tool *vs* commercial product (issues such cost per unit, feasibility of mass production, safety concerns)

There are no hard rules – arriving to the right decision is often a matter of experience (and trial & error ☺)

In conclusion

We have looked at immobilization of biomolecules at the surface

What you need to clear about:

- Physical vs chemical attachment
- Monolayers vs multiple layers/pads
- Covalent vs non-covalent
- Orientation vs random