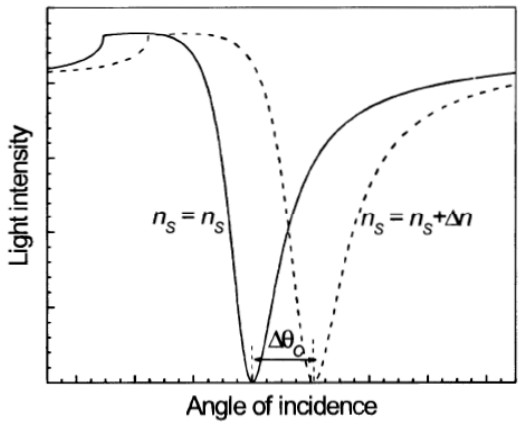
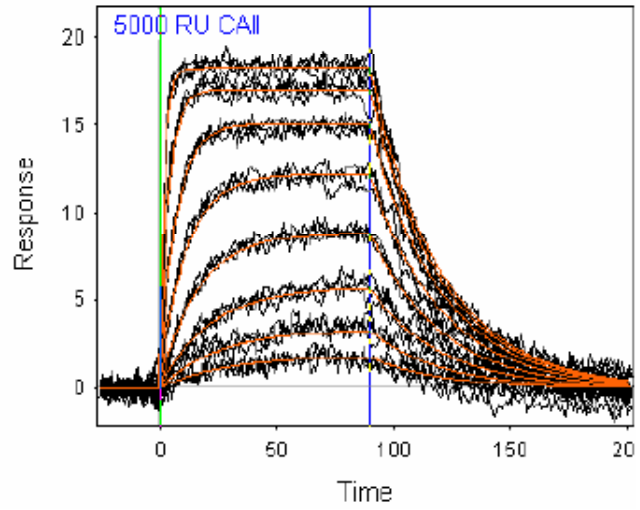
Lecture 8

SPR: immobilization strategies

The Aim of the Lecture

In the last lecture:





Quantitative parameters of a binding reaction: **concentration** of an analyte, **reaction rates**, **affinity** etc.

specific for a particular sensor type

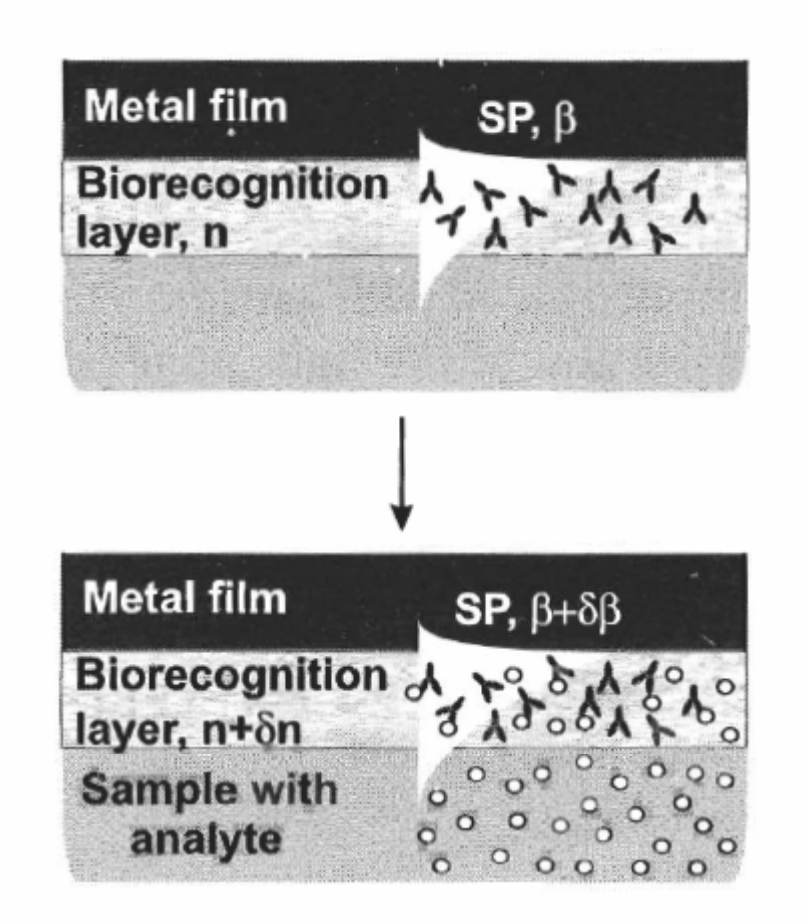
specific for a particular reaction and geometry

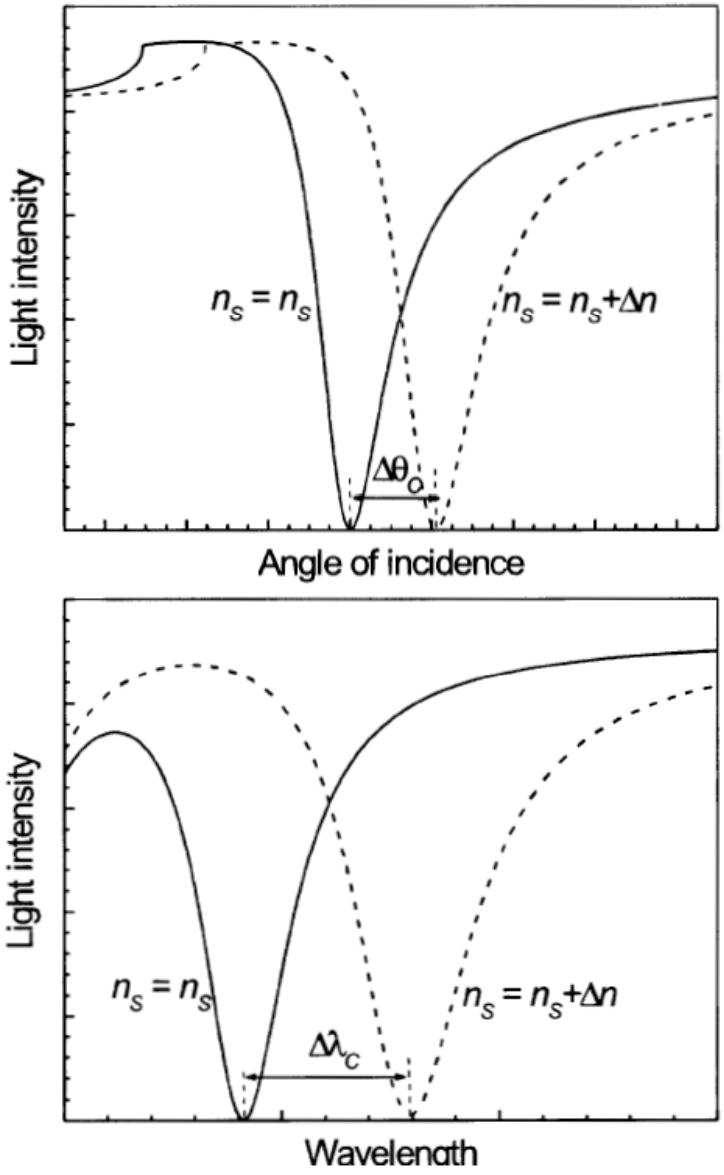
In this lecture:

- How to make our SPR sensor selective?

Surface plasmon sensor

- Principle of affinity SP biosensor

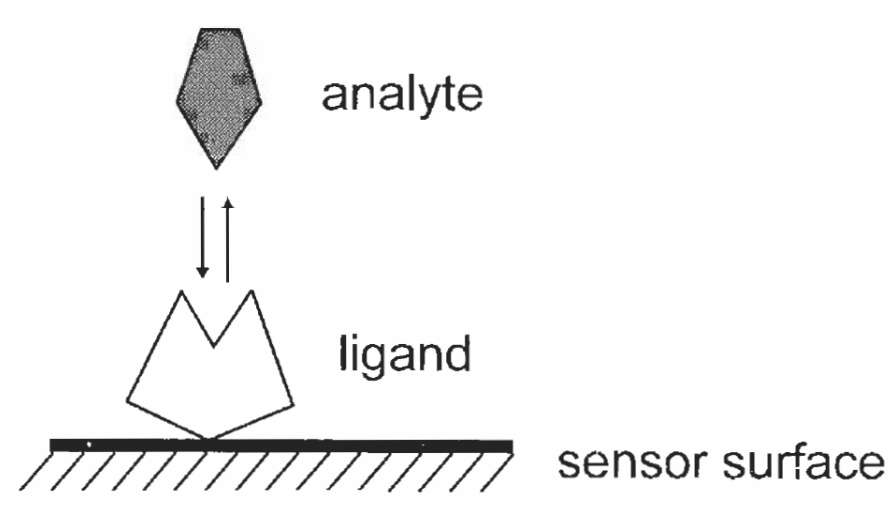




Immobilization techniques

Different applications might require specific binding techniques, however there are general requirements:

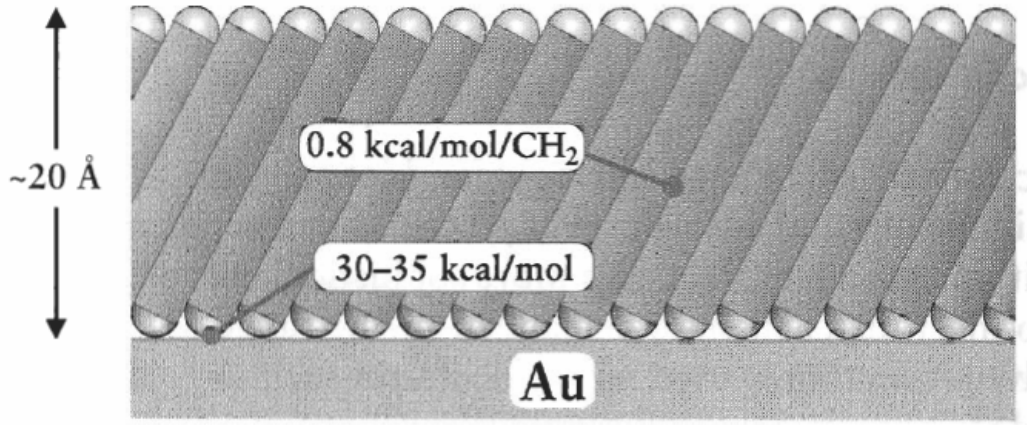
- successful label-free measurement of a specific binding event requires the best possible activity of the ligand
- conformation and orientation should be close to the *in vivo* situation
- non-specific binding should be kept low



Surface modification

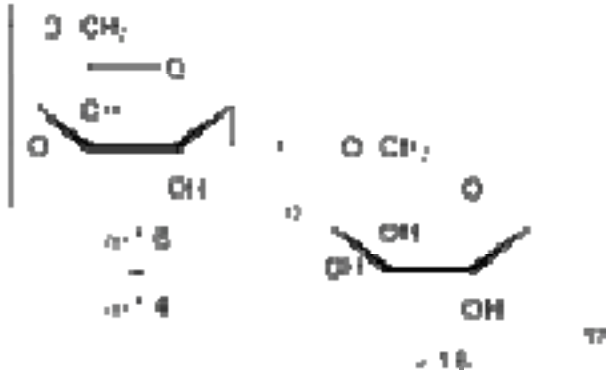
- physisorption
  - (partial) unfolding of a protein on the surface
  - uncontrolled exchange of molecules between the surface and the solution

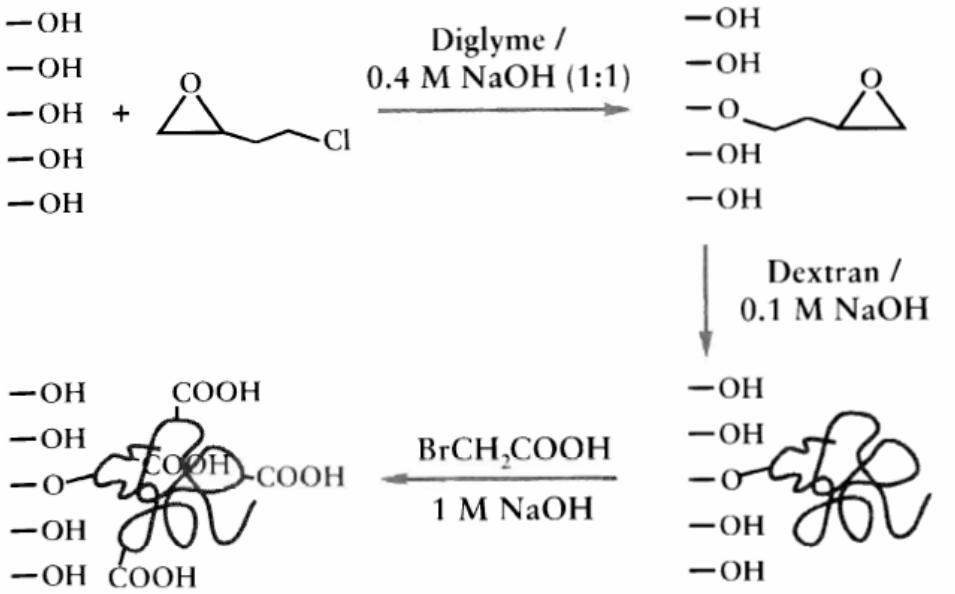
- Self-Assembled Monolayers (SAM) of thiols and disulfide molecules
  + densely packed and stable structure,
  + flexibility of the end-group chemistry (usually carboxyl but other can be used
  - still fairly rigid and flat that might reduce activity or denature proteins
  - doesn't use the whole volume penetrated by the field



Surface modification

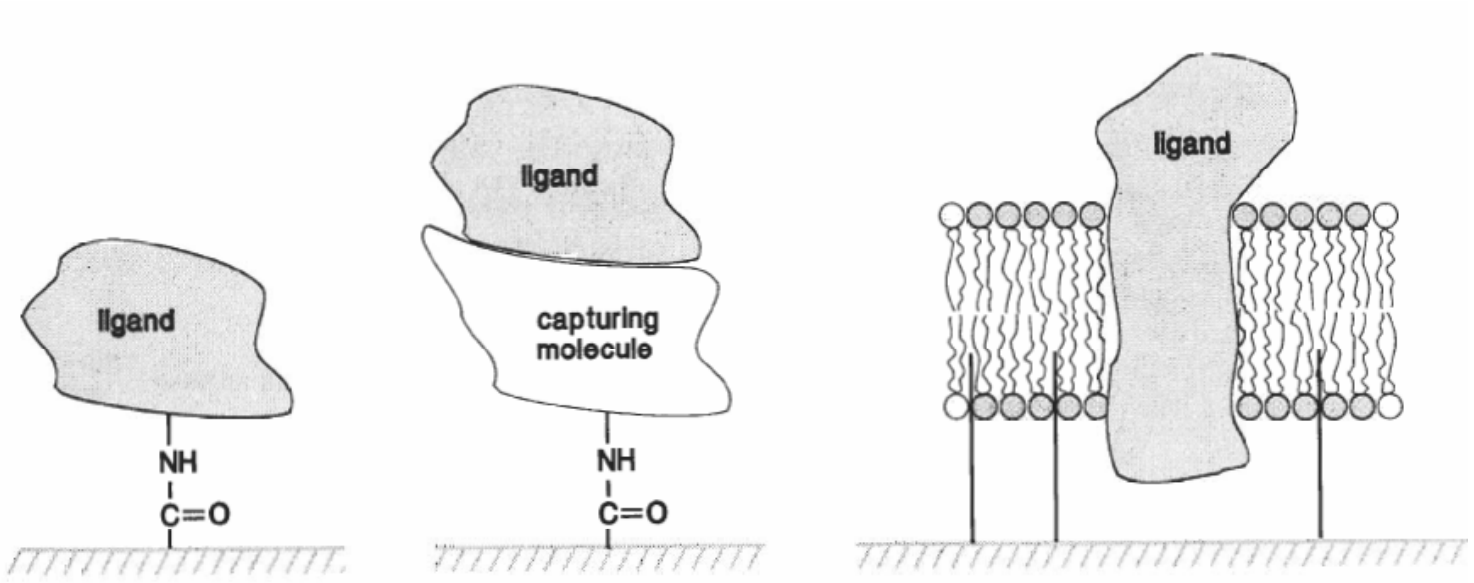
- **Dextran hydrogel**:
  thin hydrophilic (hydrogel) layer based on highly flexible mainly unbranched dextran molecules





- Advantages:
  - + highly hydrophilic environment with non-cross-linked structure (keeps molecules in a solution-like state)
  - + increased binding capacity
  - + thickness of the layer can match the penetration depth
  - + can be tailored using various molecular weight (10k – 1M) and different chemistry
- Other chemistries: Polyvinyl alcohol, polyacrylic acid, poly-L-lysine

Immobilisation strategies

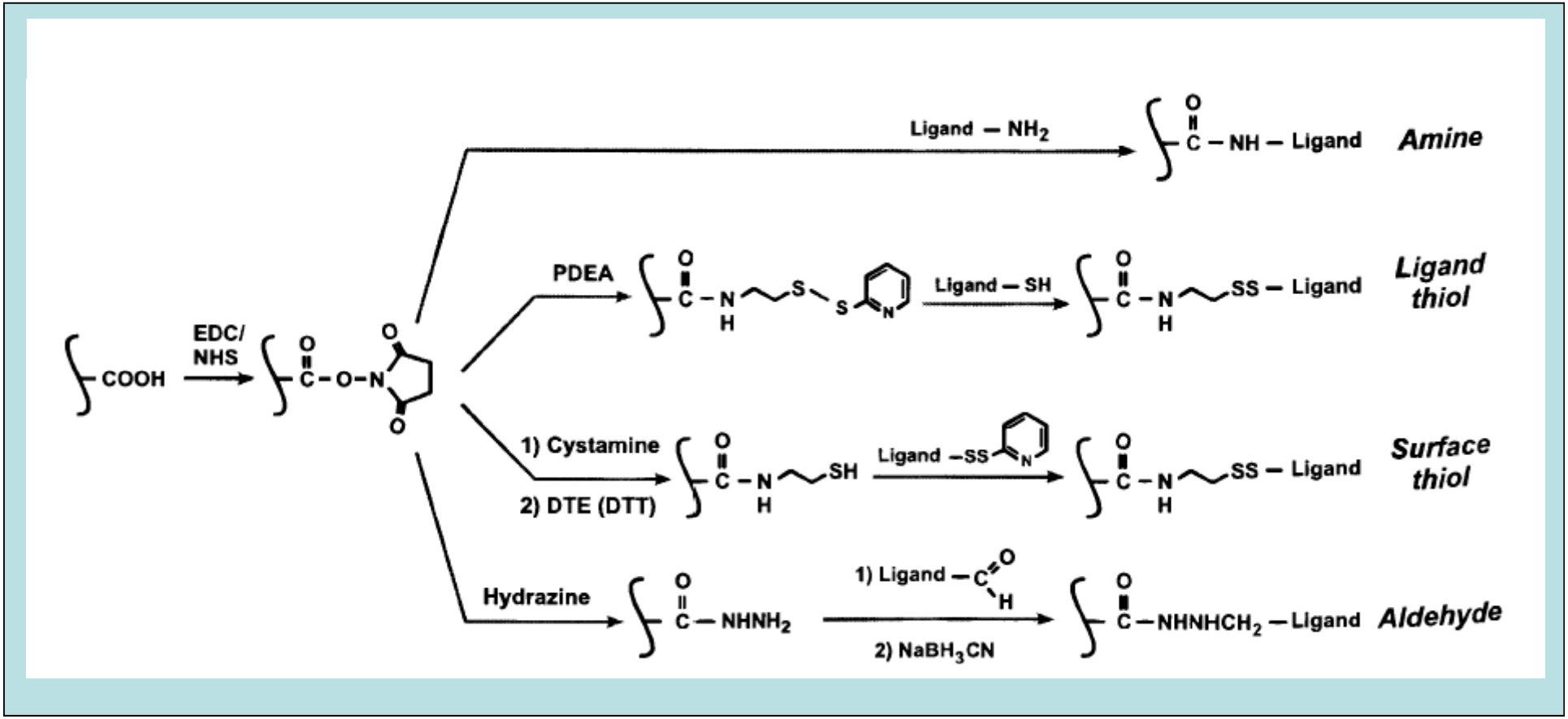


covalent

capture

Hydrophobic (supported bilayer)

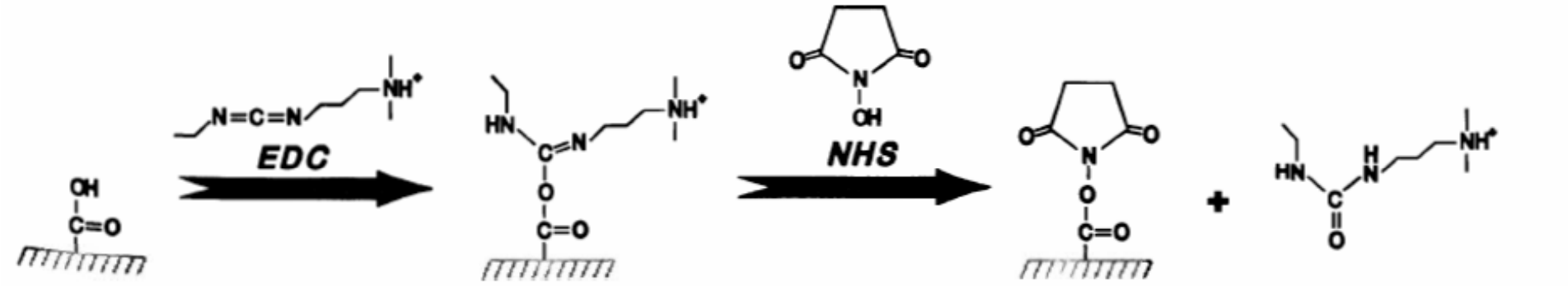
Covalent immobilisation



Main requirement: Covalent immobilization chemistry should run in **aqueous environment under mild conditions**

Amine coupling

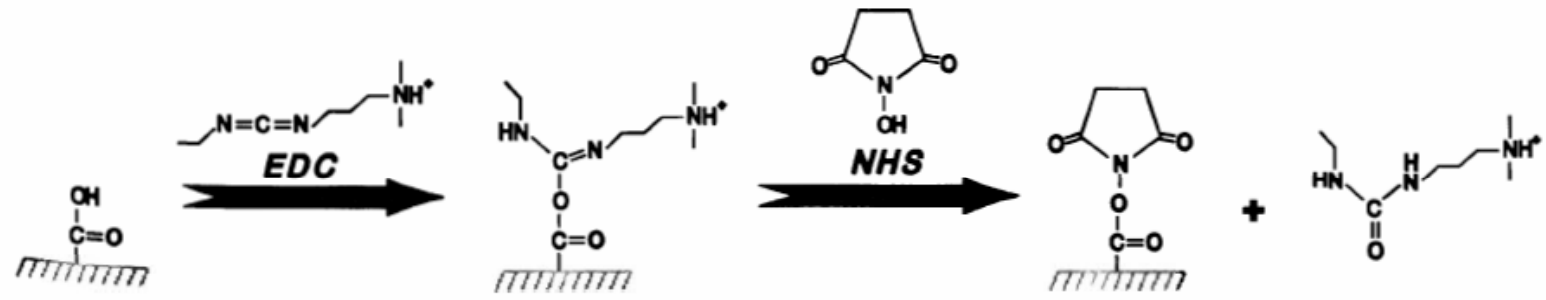
- Coupling to carboxylic groups: the most widely used strategy
- Carboxylic groups can be obtained:
  - SAM of mercaptoacid on gold
  - carboxilated dextrane (e.g. reaction of with haloacetic acids), normally purchased with given degree of carboxylation



very unstable, hydrolyses quickly

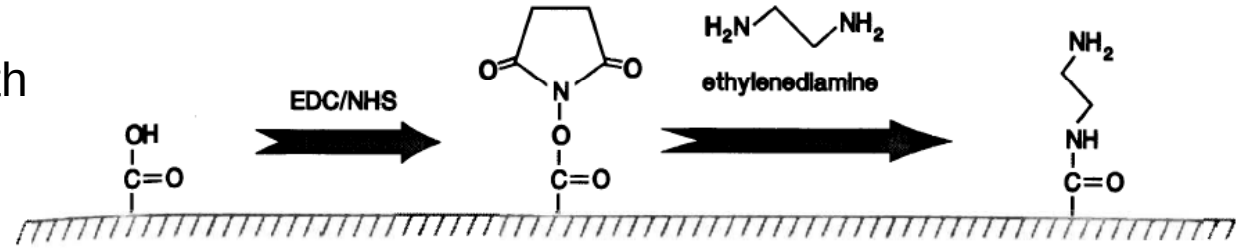
stable for several minutes to hours

Amine coupling



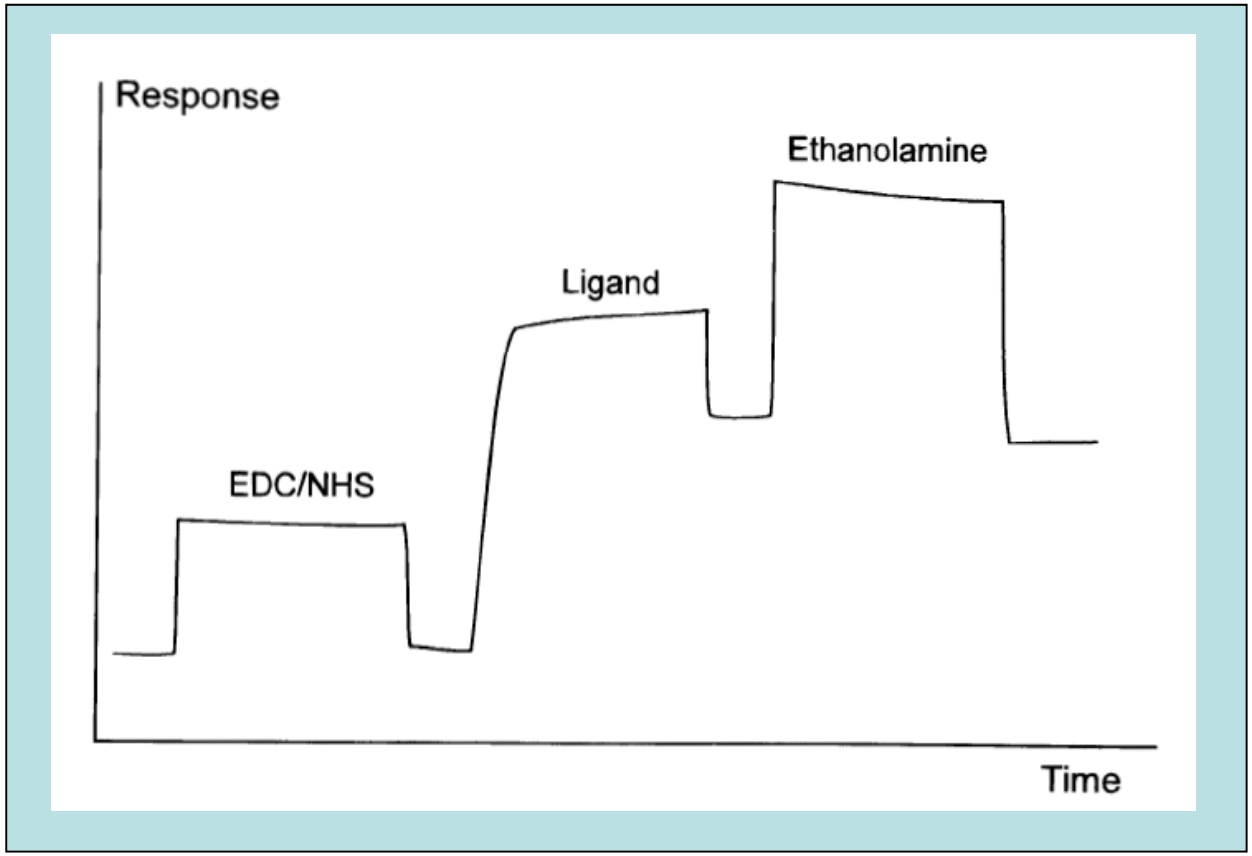
- **Typical protocol:**
  - injection of EDC mixed with NHS in water, pH=4-6 (natural buffering)
  - injection of a protein in acetate buffer pH=4-6, low ionic strength. Positively charged protein will be attracted to the negatively charged hydrogel (“pre-concentration”, fraction of carboxylic groups should remain unreacted), 1-10min.
- Protein concentration up to 50 ng/mm^2 (several monolayers) can be obtained
- Coupling under mild conditions, very few immobilization points, activity is largely preserved (e.g. IgG usually retains of approx. 75% of activity)

surface modification with amino groups



Amine coupling

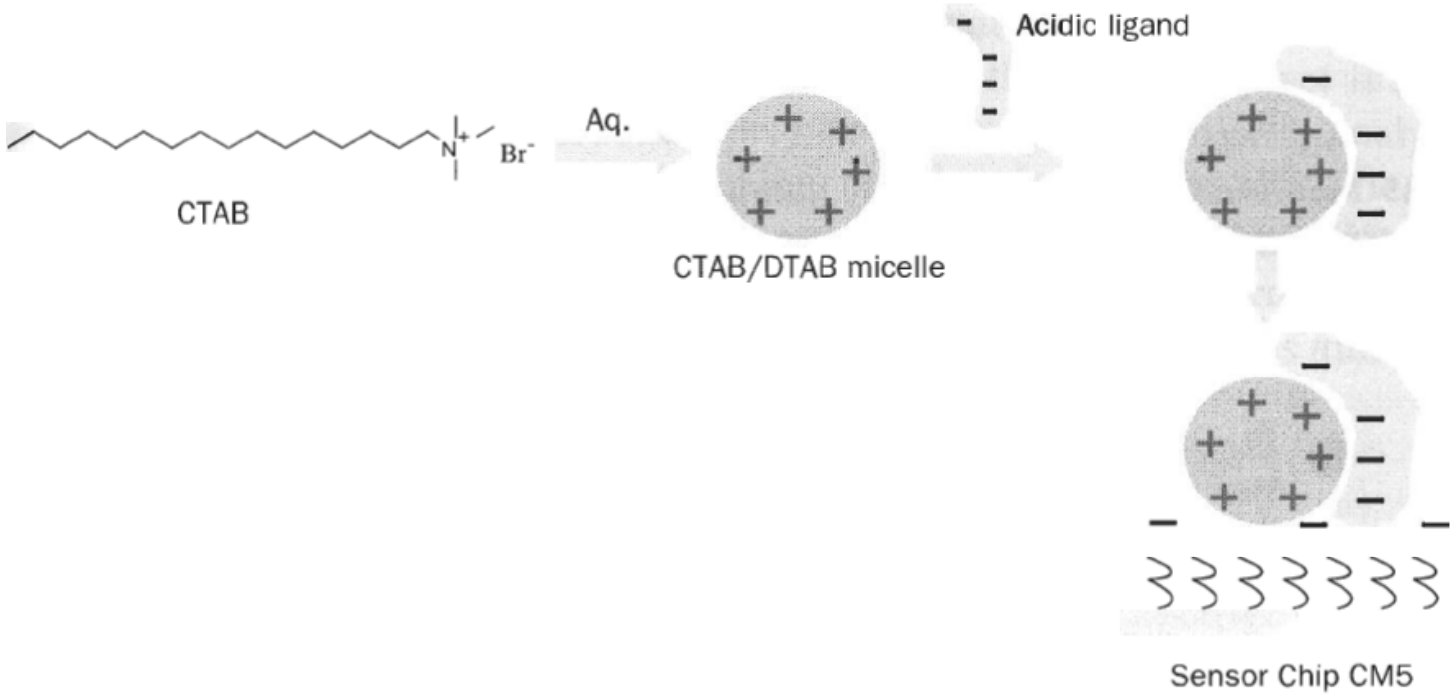
- typical sensogram for amine coupling



Proteins with pI>3.5 can be effectively pre-concentrated at pH~5. However acidic proteins will be repelled from the layer

Amine coupling

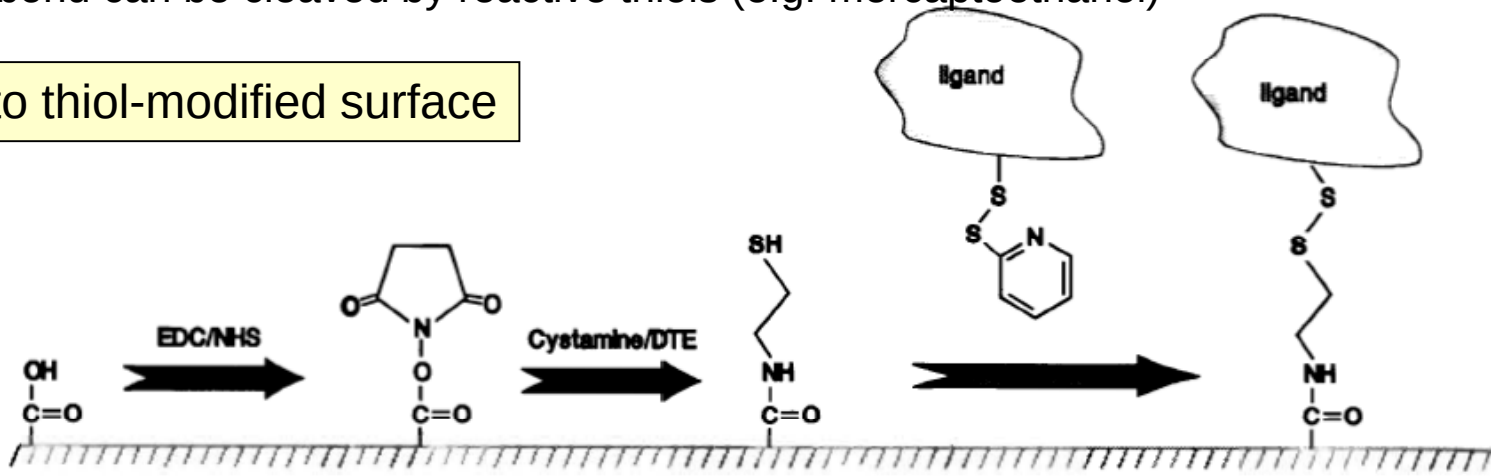
- micelle mediated immobilization of negatively charged proteins



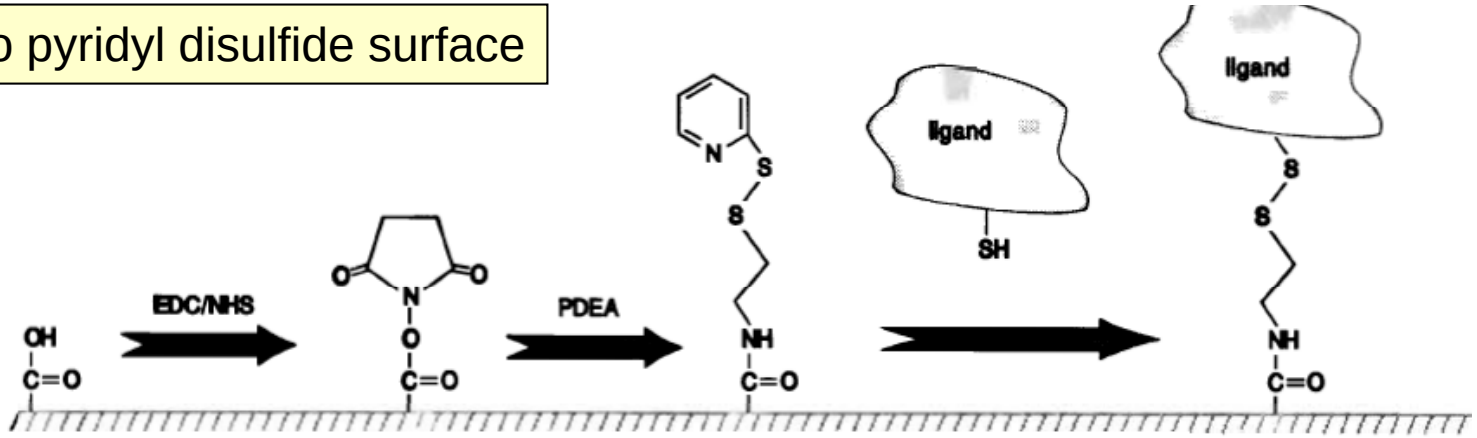
Thiol-based coouppling

- Coupling to thiol groups
  - mainly used in the situations when the ligand lacks amino groups or they are located close to the active center
  - can be introduced on the sensor surface or on the molecule
  - very specific
  - disulfide bond can be cleaved by reactive thiols (e.g. mercaptoethanol)

coupling to thiol-modified surface

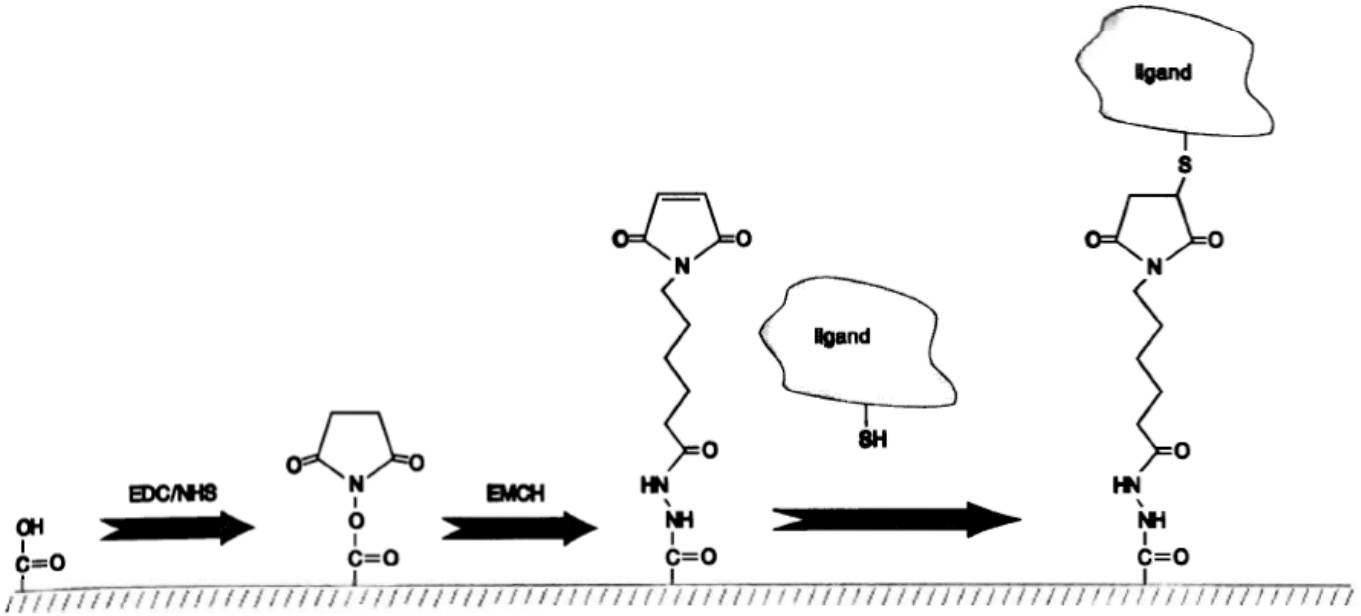


coupling to pyridyl disulfide surface



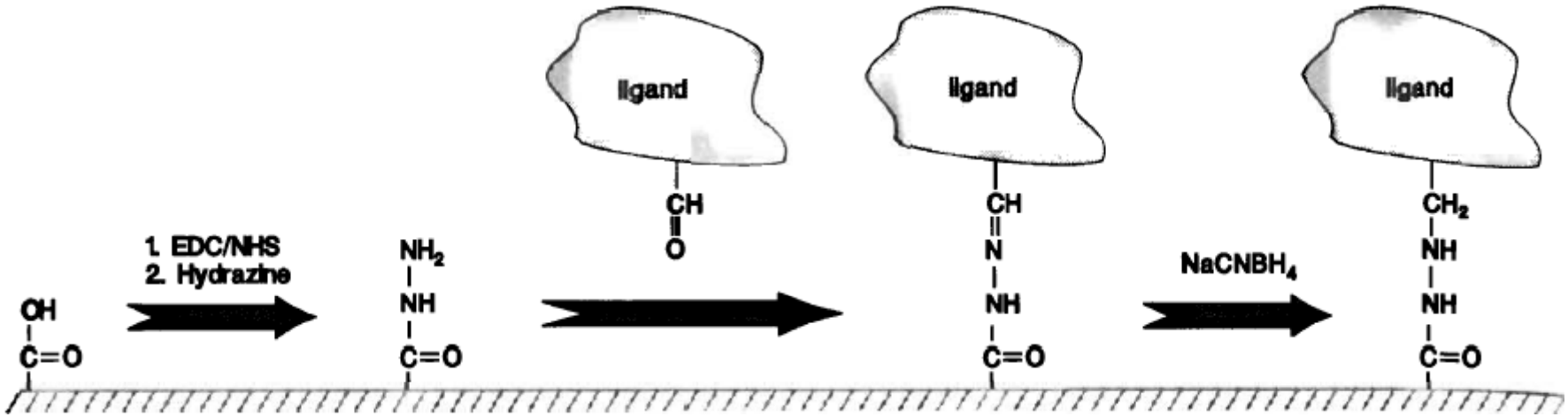
Thiol-based coouppling

- coupling thiolated binding partner to maleimide-modified sensor surface



Covalent immobilization

- **coupling to aldehyde groups**
  - based on the generation of aldehyde functionality by oxidation of carbohydrate residues
  - normally not located near the active site, therefore activity is well preserved
  - antibodies are particularly well suited for this type of immobilizaation



Capture-based coupling

- can be indispensable when
  - covalent immobilization might impair activity of the binding site
  - particular molecule should be captured from e.g. cell lysate
- analyte can be removed
- requirement: affinity should be high

- Commonly used pairs:
  - **avidin-biotin** bond: very robust, $k_d=(10^{-12}\text{-}10^{-15})$M, as strong as covalent bond
  - **His-tagged protein – nitrilotriacetic acid (NTA)+Ni^{2+}**. Can be easily broken by a chelating agent e.g. EDTA
  - **Antibody – antigen**

Coupling via Lipid layer

- Important for
  - targeting membrane protein with drugs (drug screening)
  - targeting lipid membranes (e.g. AMPs)

bilayer capture:

included in the vesicles

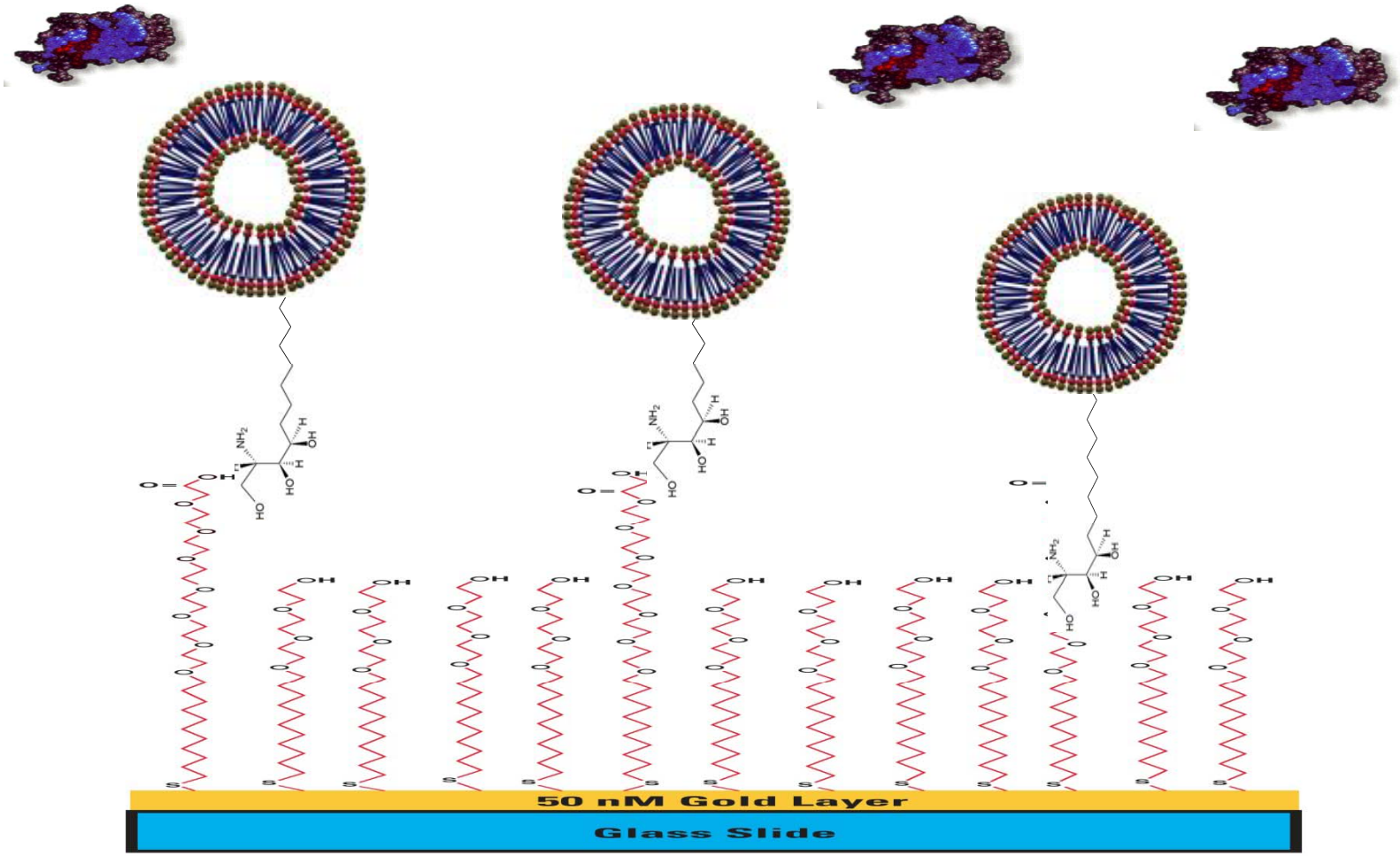
- phospholipids modified with a thiol group
- histidine modified lipids
- oligonucleotide modified lipids

included in the layer

- hydrophobic groups attached to CM dextran
- membrane protein attached to CM dextran

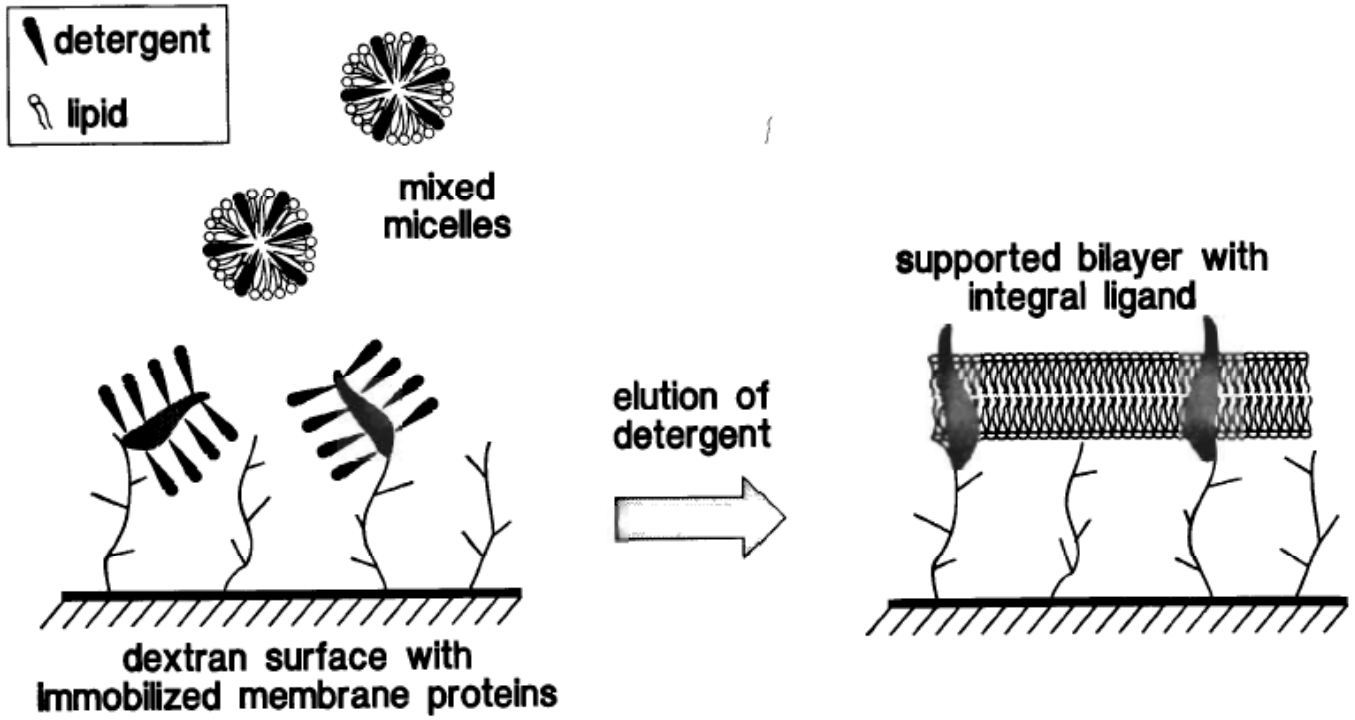
Coupling via Lipid layer

- adsorption of lipid vesicles



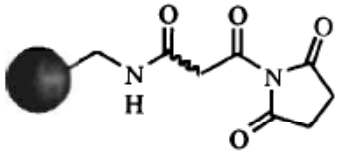
Coupling via Lipid layer

- lipid-detergent method:
  1. detergent solubilized membrane proteins are immobilized on the sensor surface with amino coupling
  2. mixed micelles (with detergent) are injected and captured by proteins
  3. Detergent is washed away, lipids form membrane-like bilayer

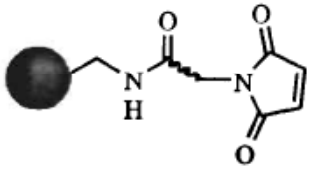


Protein modification in solution

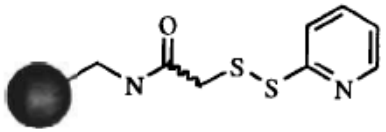
- can be considered:
  - to create functional groups for immobilisation in a particular region of protein
  - to tune pI of the protein



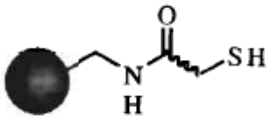
NHS-ester



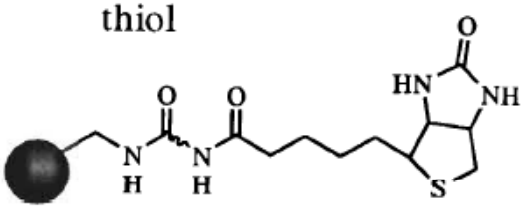
Maleimide



reactive disulphide



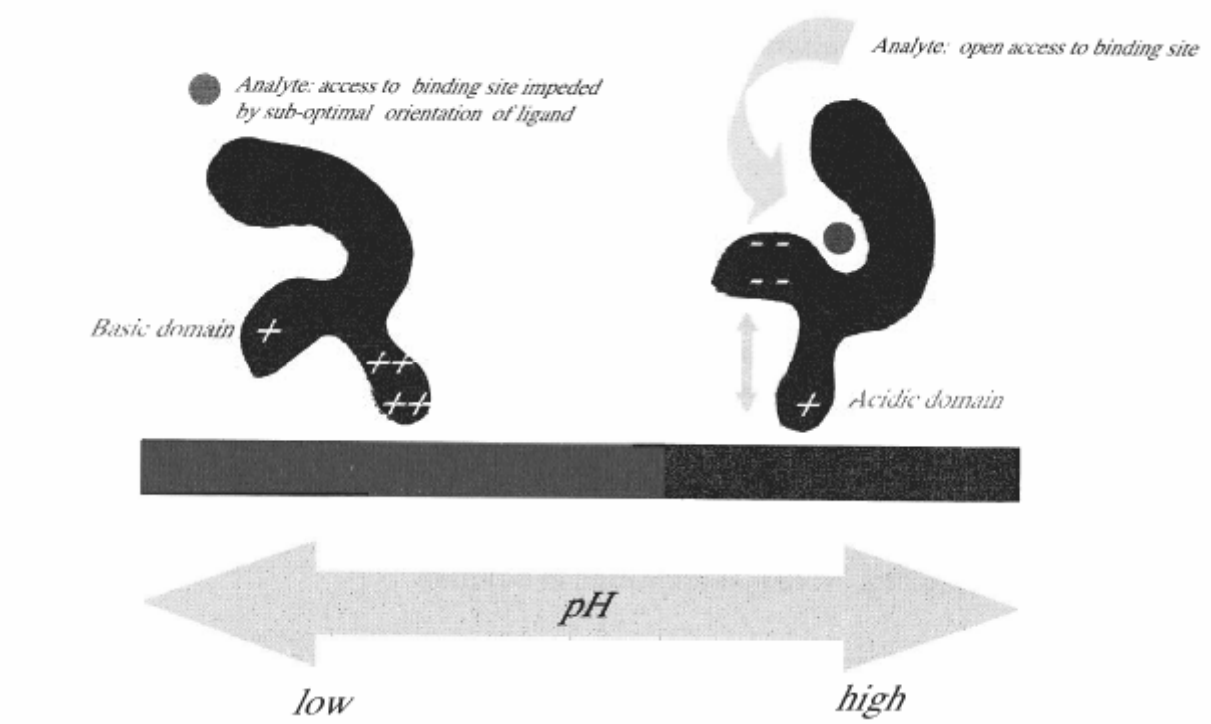
thiol



Biotin

Protein immobilization

- choice of pH might affect a conformation of protein during binding



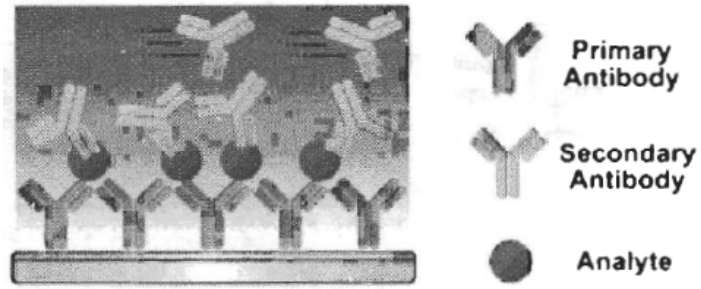
- it might be beneficial to **protect the active site** of the protein by having analyte present during immobilisation
- additional partial EDC/NHS **cross-linking** might be important to prevent a complex protein from dissociation into monomers

Immobilization of other molecules

- Oligonucleotides:
  - binding of thiolated derivatives to gold
  - use of biotinylated derivatives, binding at neutral pH
  - EDC/NHS crosslinking of amino-modified nucleotide in the presence of CTAB

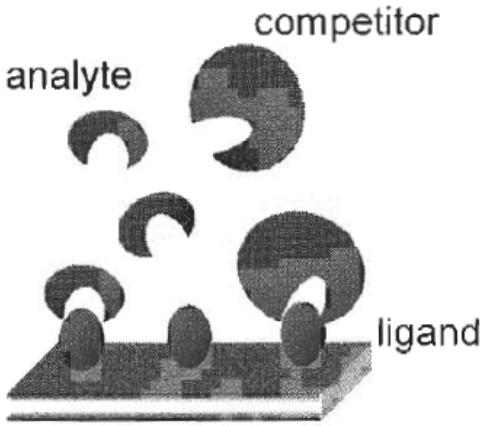
How to improve signal for small analytes

- **Sandwich assay**

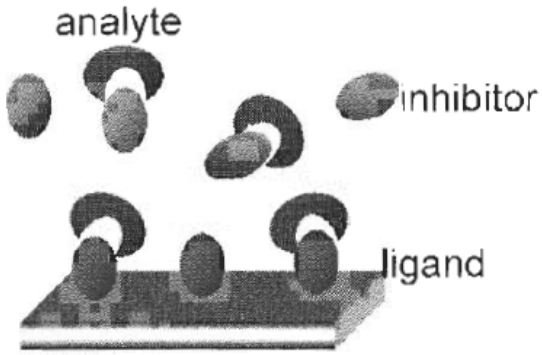


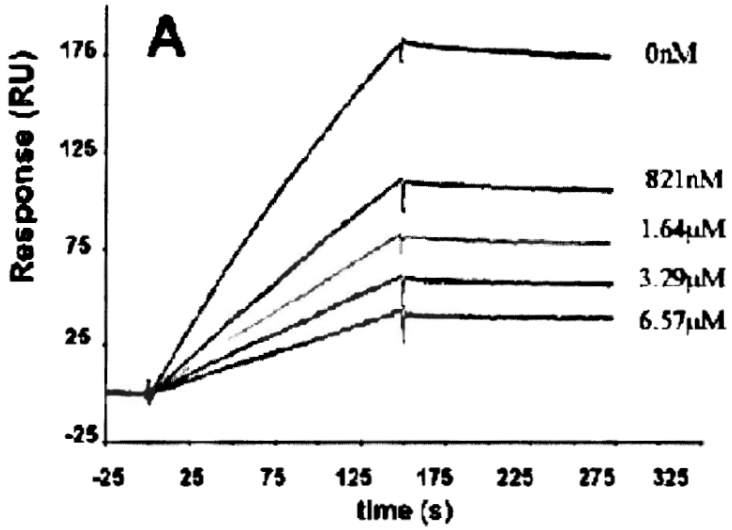
- **Competition** or **inhibition** analysis can be used for interaction that are difficult to analyse directly (e.g. due to low molecular mass of analyte).

Competition



Inhibition



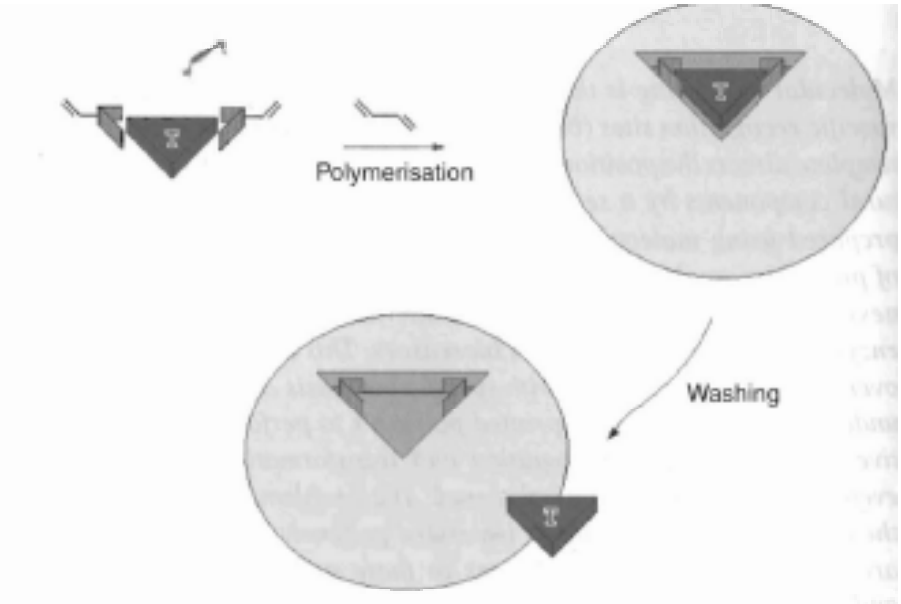


peptide 12p1 inhibition of gp120 YU2 binding to MoAb

Molecular imprinting polymers

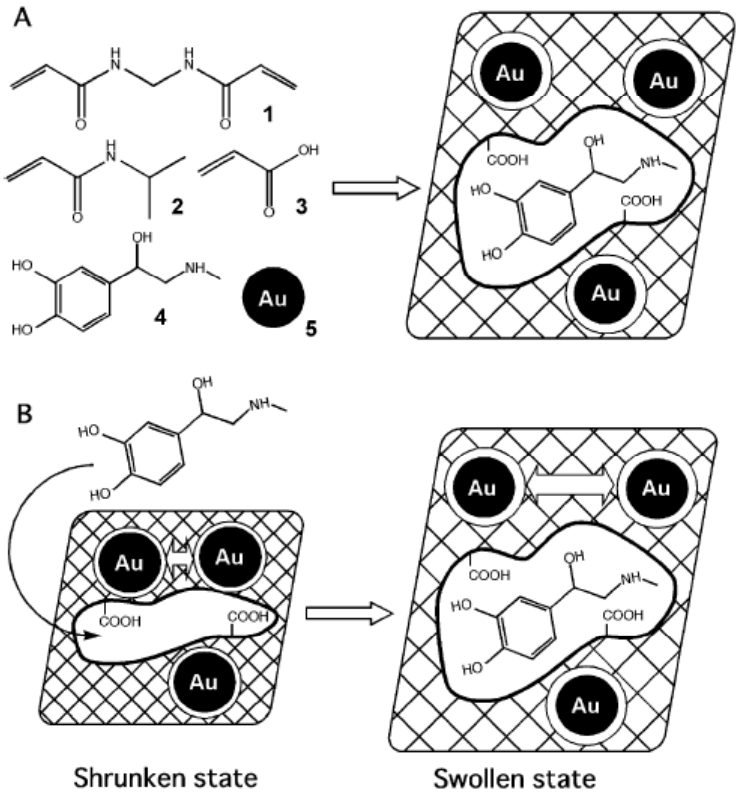
- Molecular imprinting – process of template-induced formation of specific recognition sites, binding or catalytic. Following removal of the template binding sites are left in polymer.

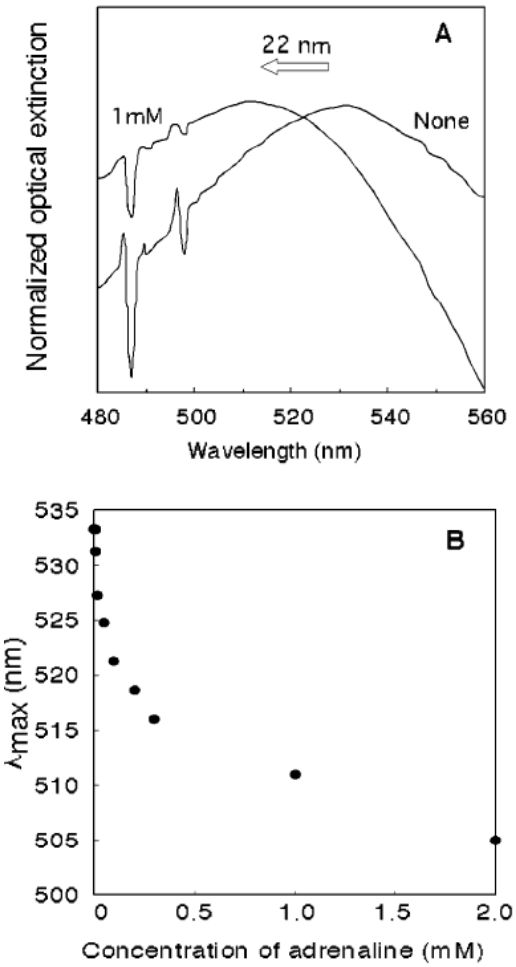
- Advantages
  - high stability
  - low cost
  - unlimited spectrum of analytes.
  - easy processing and integration into sensor design



Molecular imprinted polymers

- Detection of adrenaline using gold NP and MIP

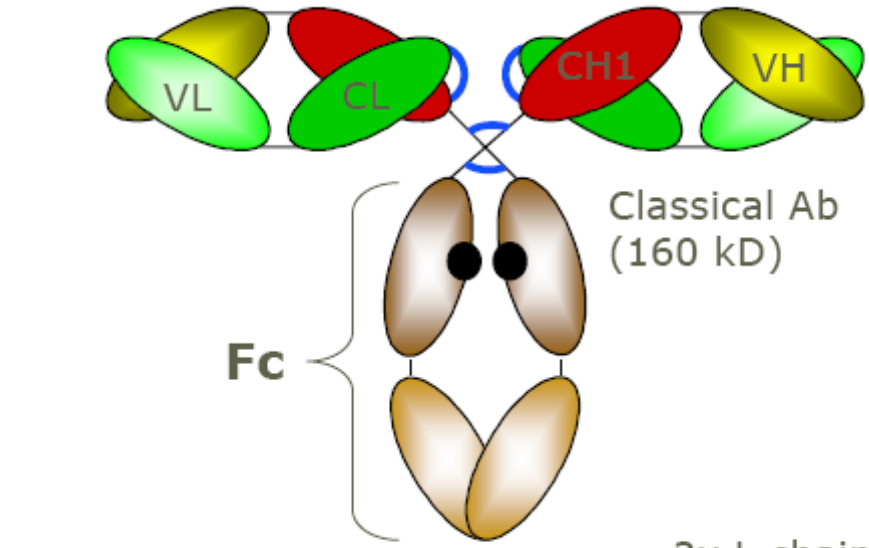


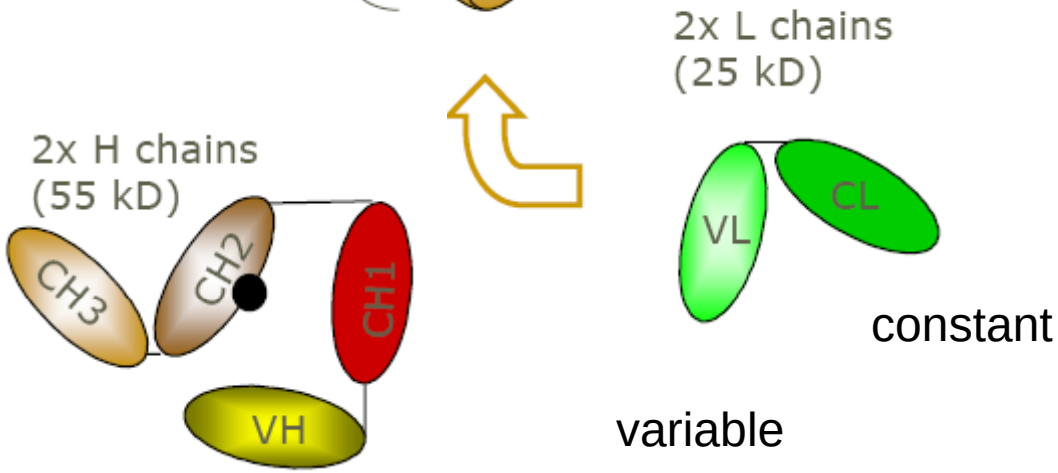


Matsui et al, Anal.Chem 76, 1310 (2004)

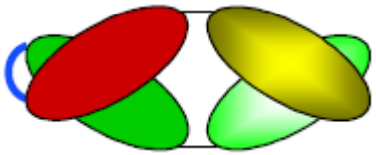
Antibodies

- Most of immunoassays relay on monoclonal or polyclonal antibodies





Immunoglobulin fragments



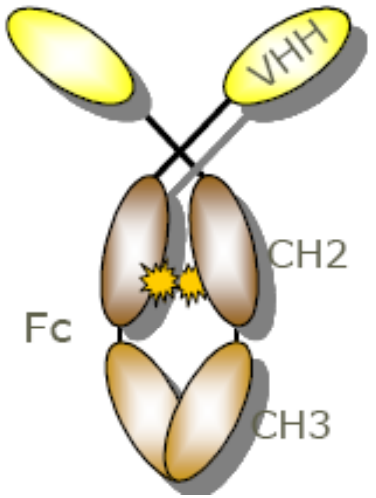
Fab

scFv

minimal antigen binding construct

Single domain Antibodies (sdAb)

- In addition to standard antibodies, certain animals such as sharks, llamas and camels contain single heavy chain antibodies



single-domain fragment 15kDa
diameter 2.4nm
length 4nm

Advantages:

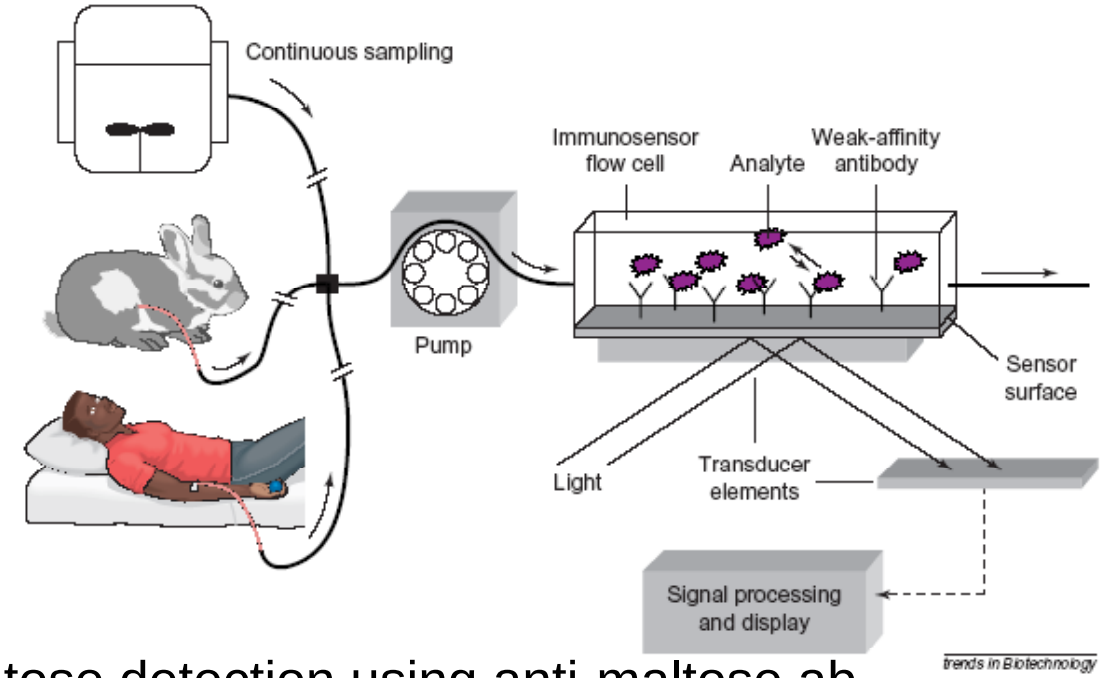
- can inhibit enzymes at active sites due to smaller finger size
- stable against high temperature (up to 90^0C), chemical denaturation, can refold after denaturation

SPR Biosensors for Medical diagnostics

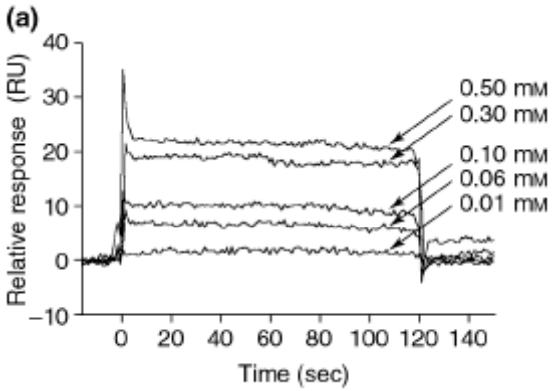
- diagnostic methods based on monitoring of concentration of disease biomarkers (e.g. PSA) are gaining popularity
- Desirable:
  - test directly on bodily fluid
  - sufficient throughput
  - continuous monitoring
- SPR has a potential to meet those requirements

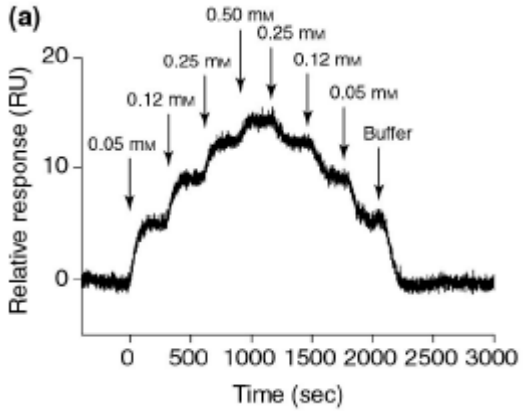
SPR detection for continous measurements

- Molecular detection format: continuous detection with weak affinity antibodies.



- Example: maltose detection using anti-maltose ab.





SPR Biosensors for Medical diagnostics

- cancer biomarker (12 approved by FDA)
  - PSA
  - carcinoembrionic antigen (CEA): colorectal& breast cancer
  - cancer antigen CA15-3 (breast cancer)
  - CA125 (ovarian cancer)
  - carbohydrate antigen CA19-9 (pancreas, colon, stomach cancer)
  - alpha-fetoprotein AFP (liver&testicular cancer)

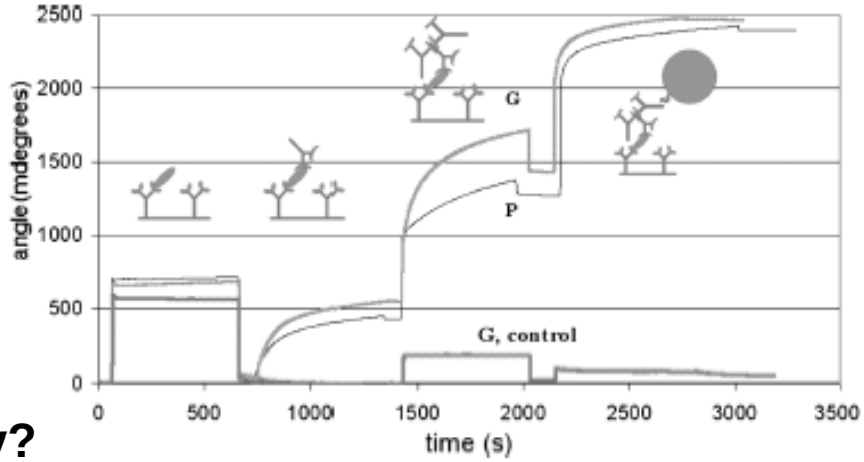
SPR Biosensors for Medical diagnostics

- PSA detection:
  - current clinical assays: ELISA (enzyme-linked immunosorbent), 0.1ng/mL
  - SPR sandwiched assay: 0.15 ng/mL on planar surfaces, 2.4ng/mL on hydrogel

1. mouse monoclonal anti-PSA immobilized
2. sample injection
3. amplification with polyclonal rabbit anti-PSA
4. amplification with biotinylated anti-rabbit
5. streptavidin coated latex spheres

   or

4. anti-rabbit coated colloidal gold



Why planar surfaces are better for this assay?

Besselink et al, Anal. Biochem 333, p. 165 (2004)

SPR Biosensors for Medical diagnostics

- Heart attack markers:
  Troponin I (TnI), troponin T (TnT), Troponin C (TnC), myoglobin, fatty acid binding protein

SPR experiment:

1. biotinylated cTnI antibodies immobilized on avidin layer on SAM
2. direct measurement (2.5-40ng/mL)

   or

   sandwiched assaywith detection limit 0.25ng/mL and range 0.5-20ng/mL

SPR Biosensors for Medical diagnostics

- Antibody based assays
  - type I diabetes diagnostics using anti-glutamic acid decarboxylase ab (GAD) (GAD immobilized on the surface)
  - detection of antibodies against cholera toxin (cholera toxin immobilized on the surface)
  - hepatitus C
  - anti-adenoviral antibodies
- Hormone based assays
  - direct detection of hCG (human chorionic gonadotropin, pregnance marker) using anti-hCG immobilized with biotinylated oligos. Detection limit below 0.5ng/mL
  - detection of estrone and estradiol using inhibition format
- Drug detection
  - coumarin (anticoagulant, 7-OHC): competition assay with 7-OHc on the surface and antibodies injected in a mixture with serum
  - warfarin (anticoagulant)
  - morphine and morphine metabolites

SPR for food safety

- Bacteria:
  - difficult to detect due to large size. Most of the time have to be destroyed by heat, ethanol or detergents
- E. coli O157:H7
  - direct detection using immobilized antibodies. Detection limit down to 100 cells/mL
  - detection of PCR products of E.coli genome
  - detection of enterotoxin
- Salmonella
- Listeria
- Campylobacter Jejuni (leading cause of diarrea)

SPR for food safety

- Proteins:
  - secreted by infectious bacteria, toxic in low doses, have low molecular weight 5kDa- 150kDa

- Staphylococcal enterotoxins
  - direct detection using immobilized antibodies. Detection limit down to 0.5ng/mL with secondary antibody amplification
- Botulinum neurotoxins
  - Detection limit down to 0.5ng/mL with sandwich assay with polyclonal antibodies.

SPR for food safety

- Low molecular weight compounds
  - large diffusion rate but low molecular weight doesn't produce significant change in refractive index
- Domoic acid: neurotoxin originated from algae
  - detection using molecularly imprinted polymer. Detection range 2ng/mL – 3.3 ug/mL
  - inhibition assay. Detection limit 0.1 ng/mL
- Mycotoxins (produced by Aspergillus, Penicillum and Fusarium)
  - Detection limit down to 0.5ng/mL with sandwich assay with polyclonal antibodies.