EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

FACULTY OF
SCIENCE
Institute for Physical and Theoretical Chemistry



Laboratory Techniques and Medical Device Approvals

Part Gauglitz: Novel Bioanalytical Approaches
Part II: Biosensors, Assays and BIA

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Biosensors

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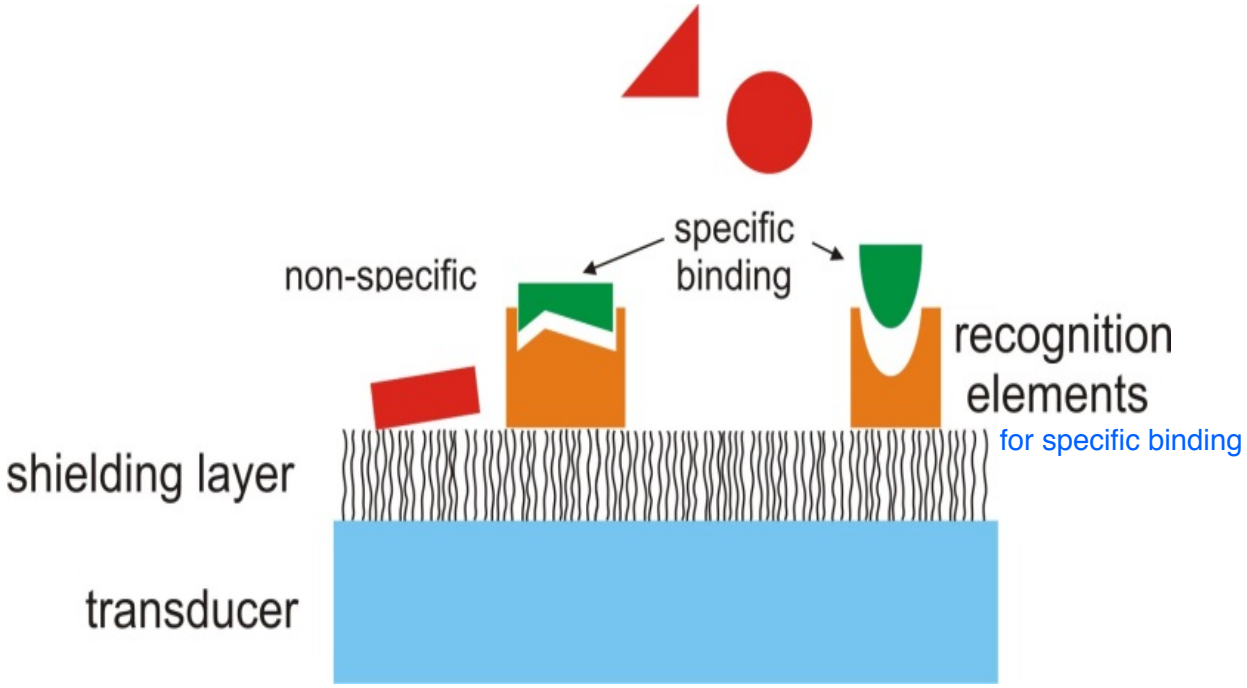
Biosensor principle

- ***Physical Sensor***
  temperature, pressure, light barrier
- ***Chemical or biochemical Sensor***
  ion sensitive electrode, optode



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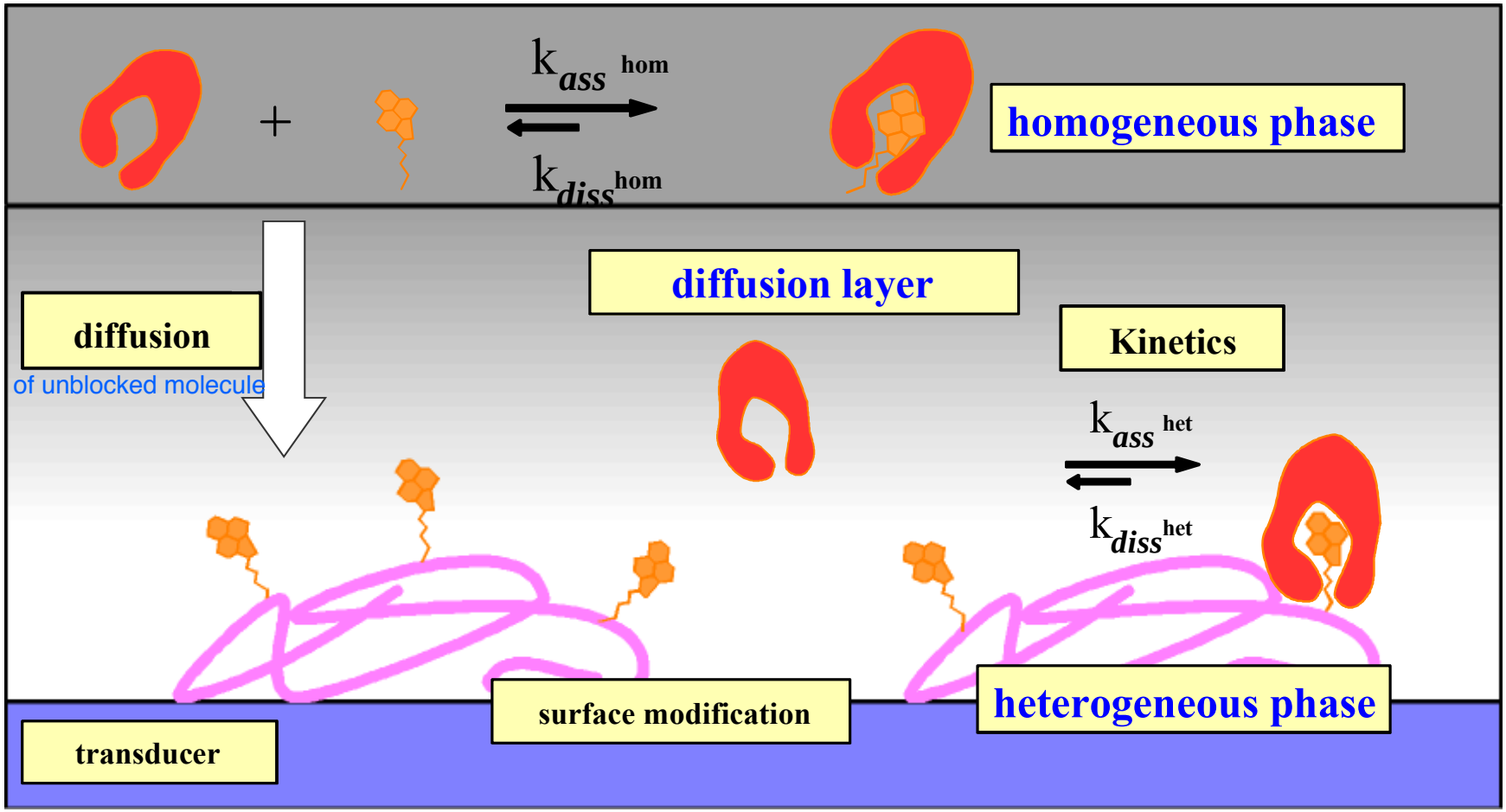
Biomolecular interaction



the two equilibirums determine the quality! sensitivity etc

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Time resolved and label free measurements

Qualitative Measurements

→ **binding / no binding**

Quantitative Measurements

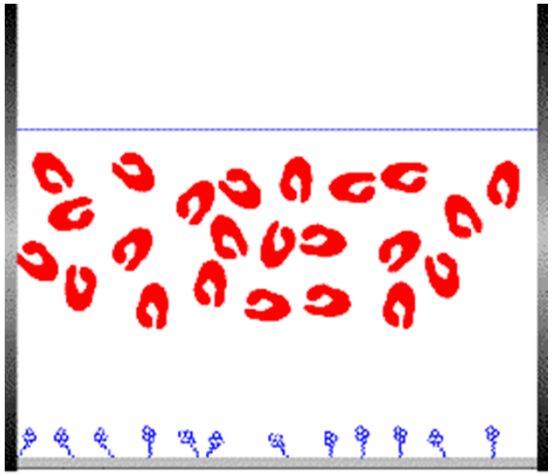
concentration

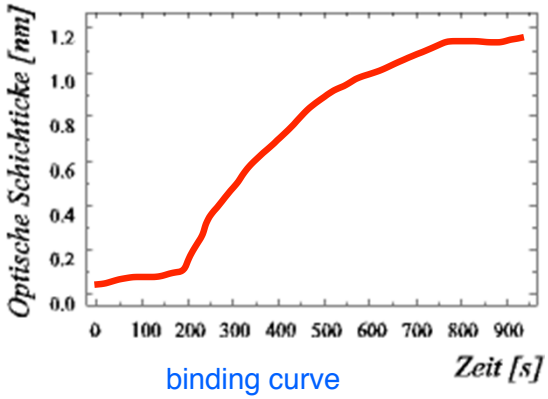
kinetics

association- und dissociation rate constants

thermodynamics

affinity and binding constants





binding curve

→ surface with low and high loading

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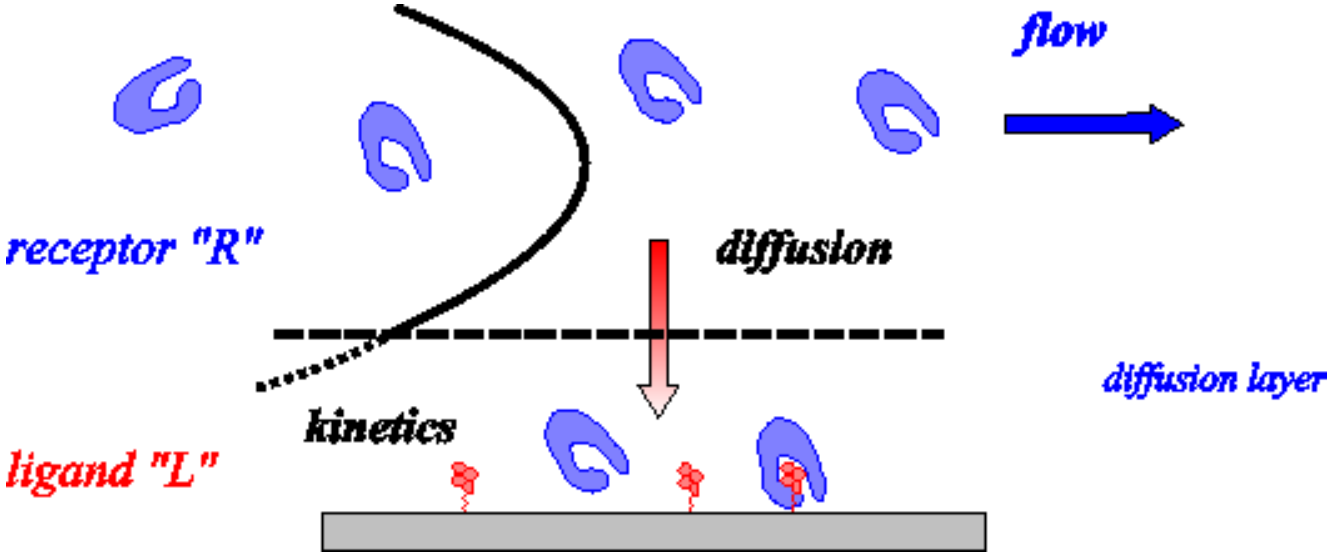
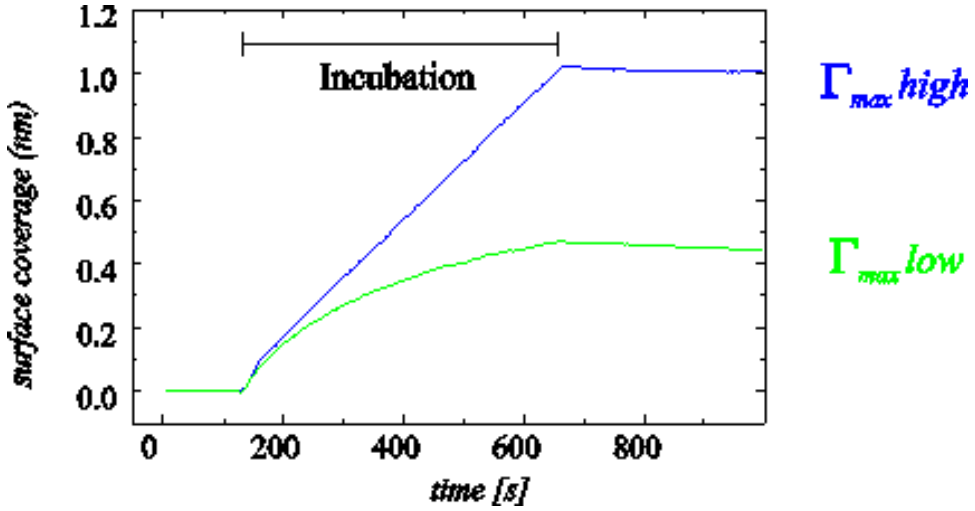
Interaction kinetics





Eddowes Biosensors 3 (1987/88) 1-15
O'Shannessy Anal. Biotechn. **5** (1994) 65-71

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Assays

- Homogeneous
- Heterogeneous

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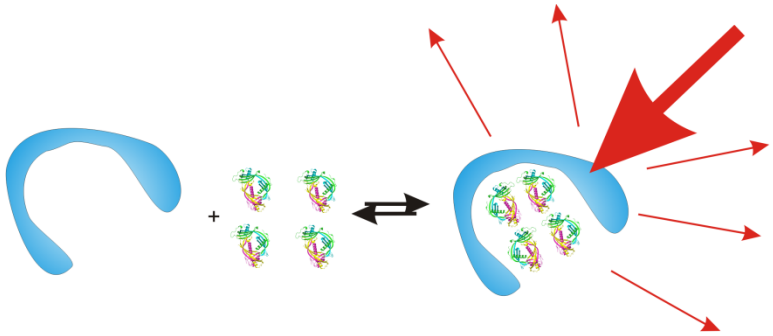
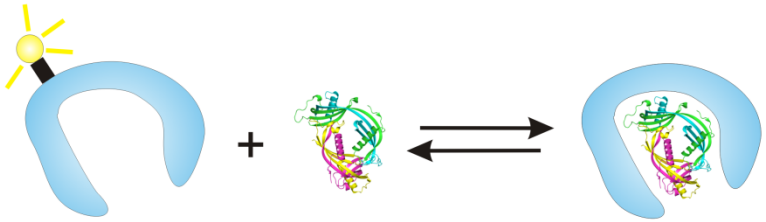
Homogeneous phase

no transducer needed
but detection is a problem

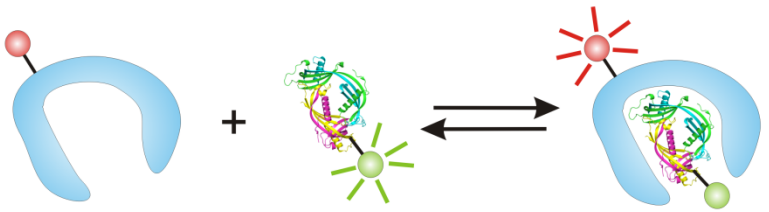
(a)



Scattering



(b) Quenching



(c) Fluorescence Resonance Energy Transfer (FRET)

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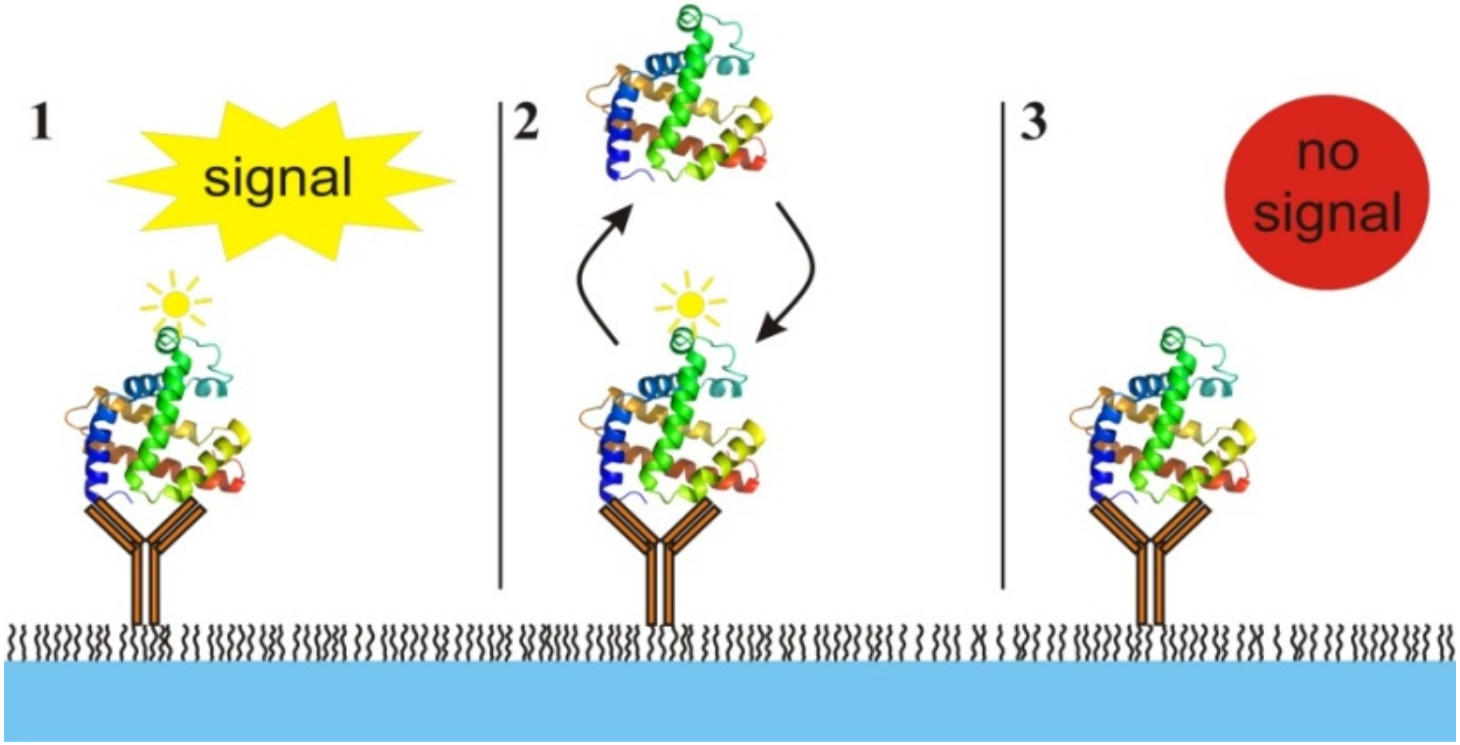
competitive assay



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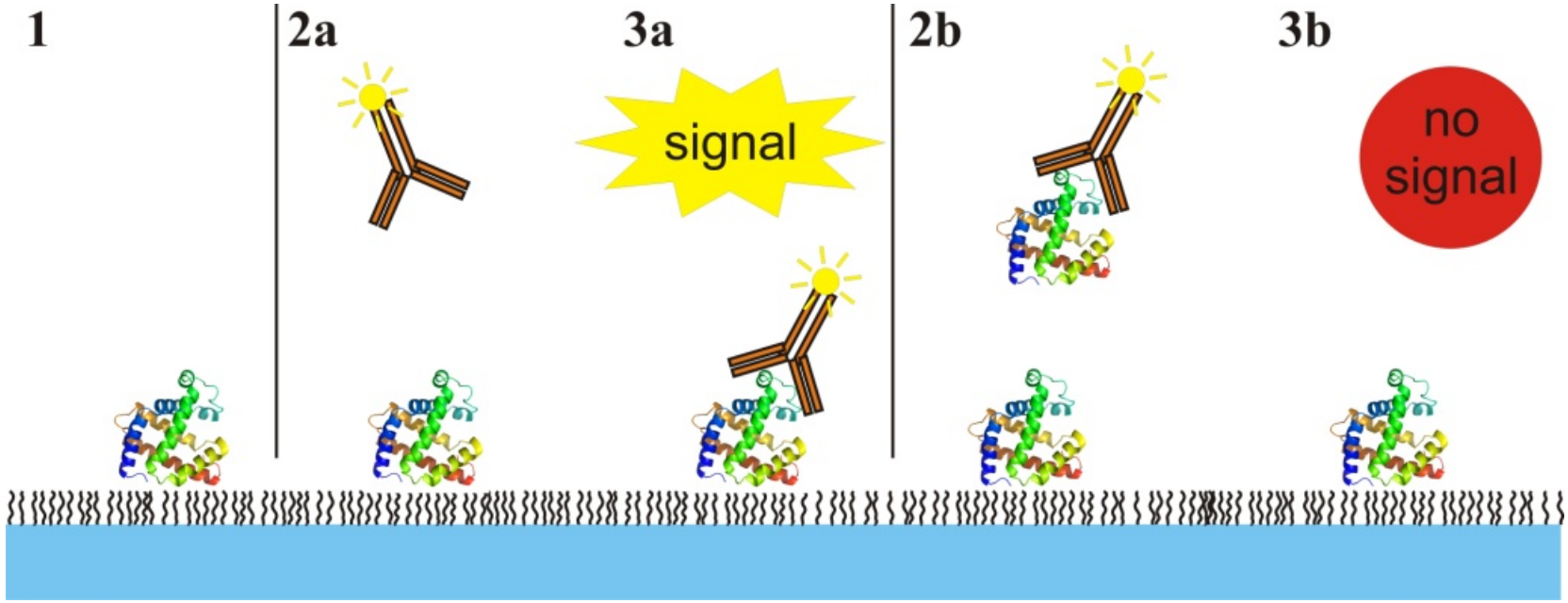
Binding inhibition assay



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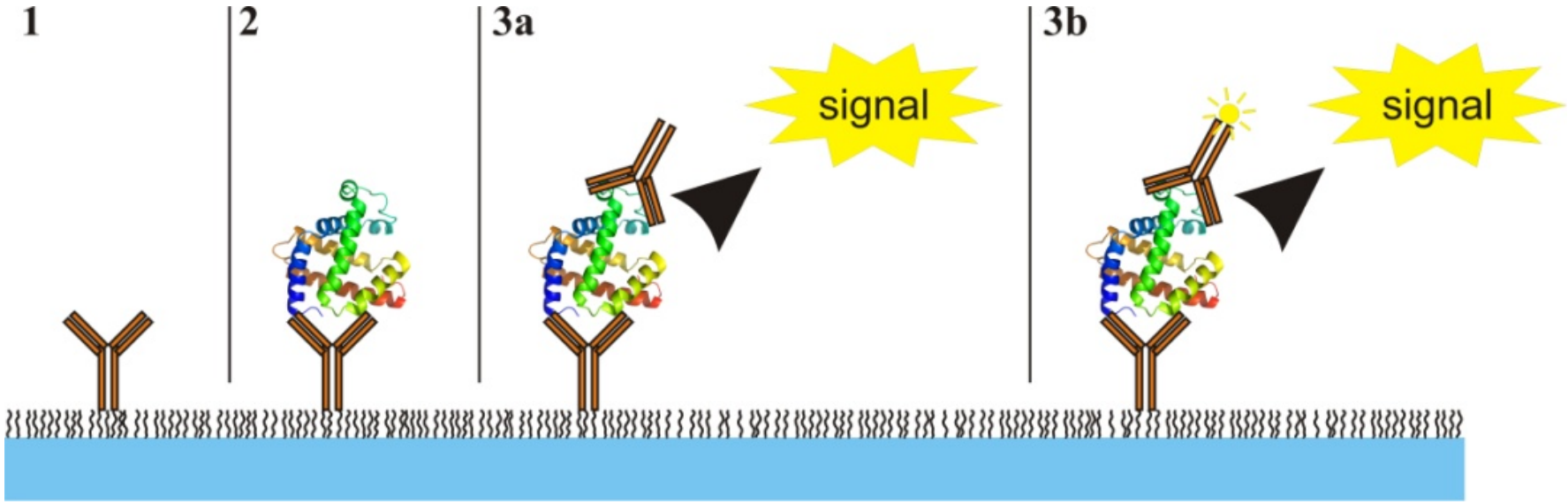
Sandwich assay



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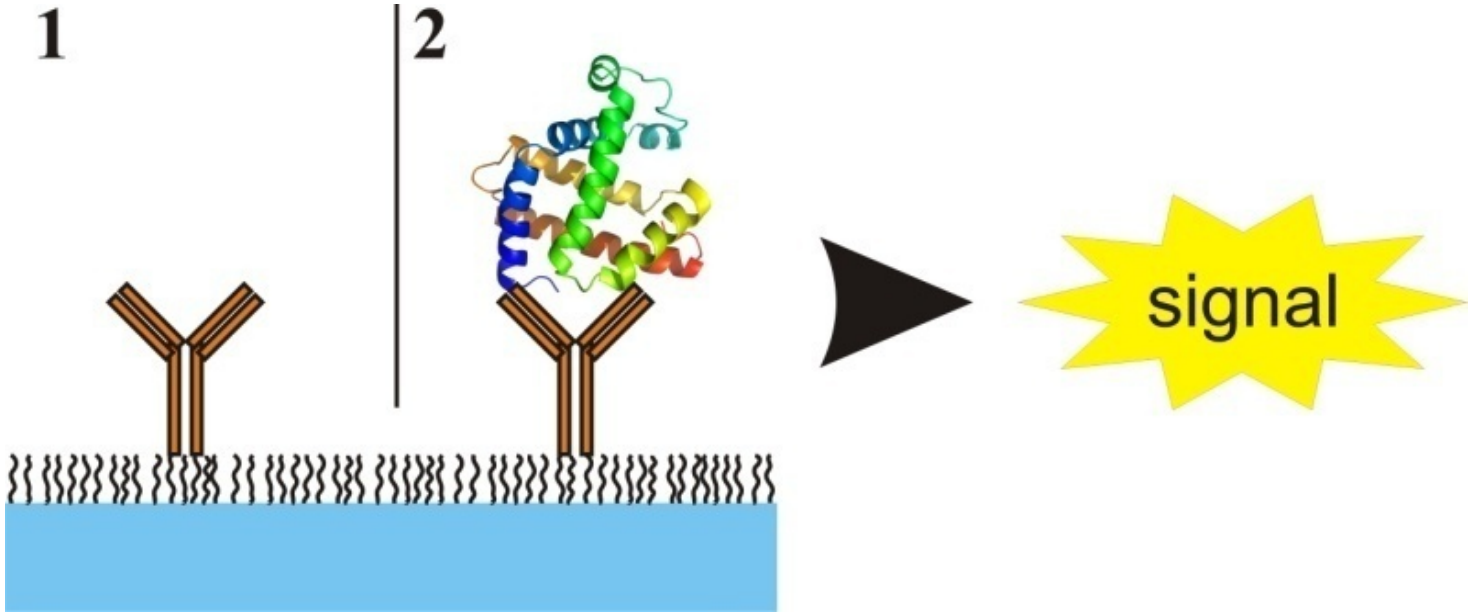
Direct assay



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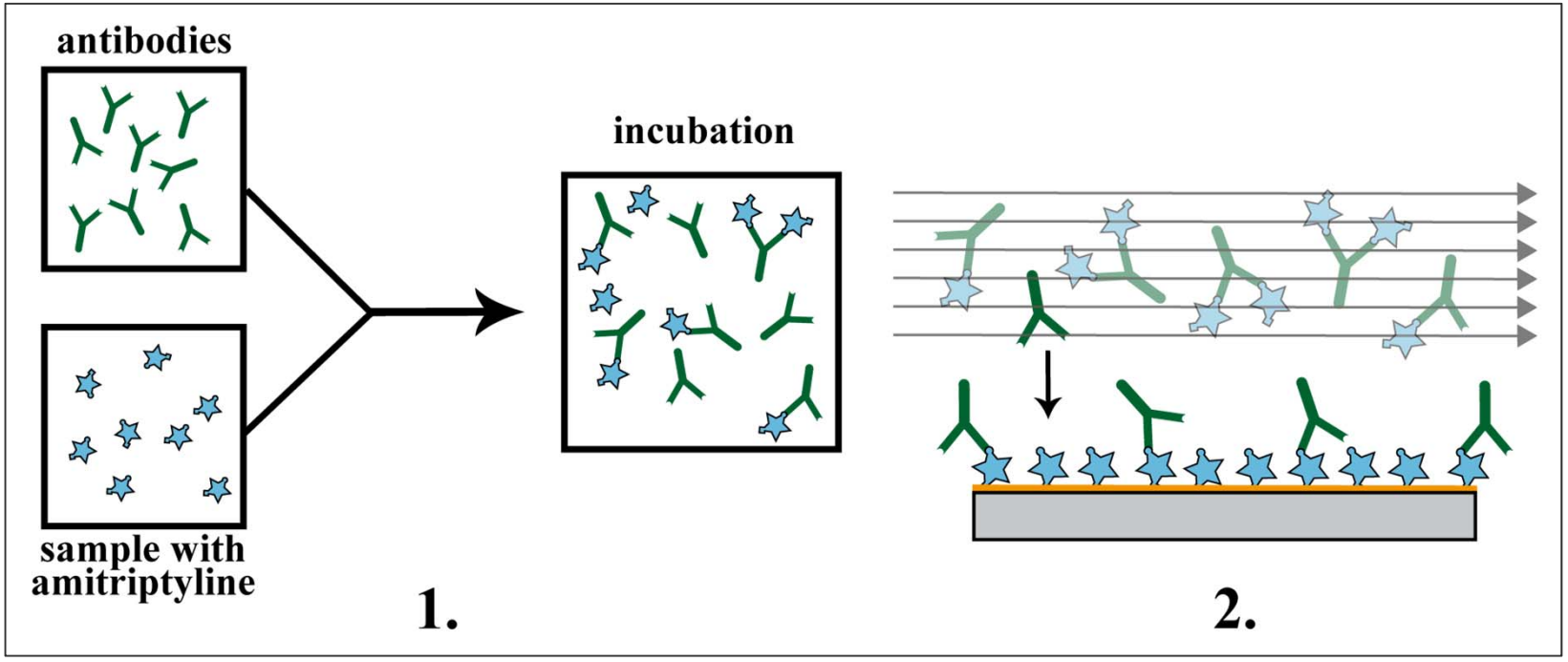
Test Format: Binding Inhibition

1. Sample and a defined antibody solution are mixed
   → incubation
2. Only non-blocked antibodies bind to the sensor surface



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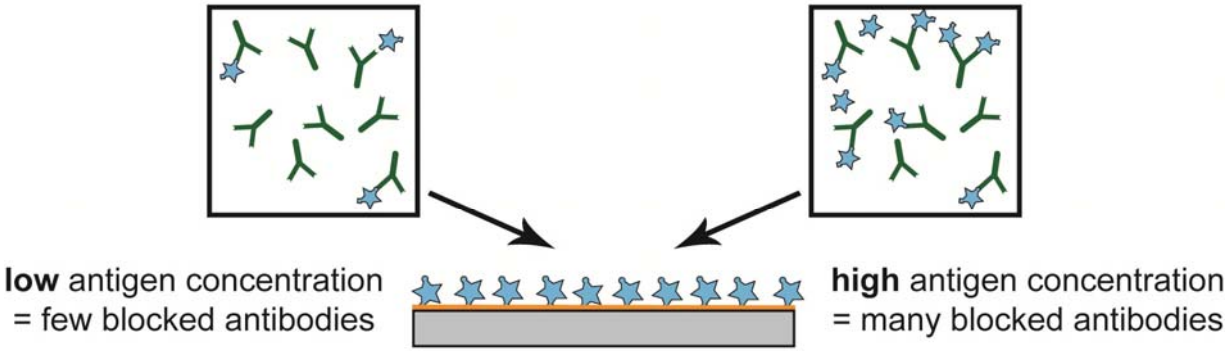
Test Format

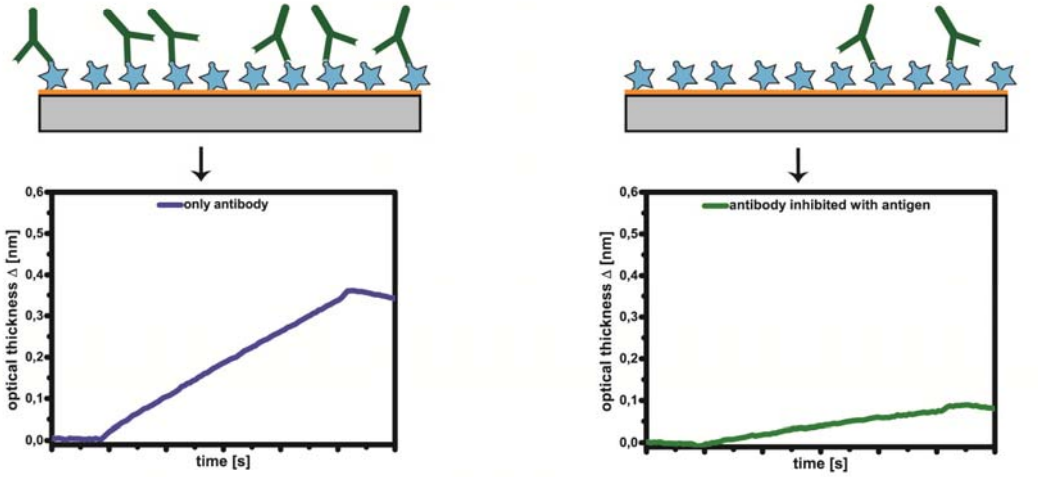
1. Incubation of antigen with defined amount of antibodies



low antigen concentration
= few blocked antibodies

high antigen concentration
= many blocked antibodies

2. Incubated solution is put on the transducer surface



high increase of optical thickness
low analyte concentration

low increase of optical thickness
high analyte concentration

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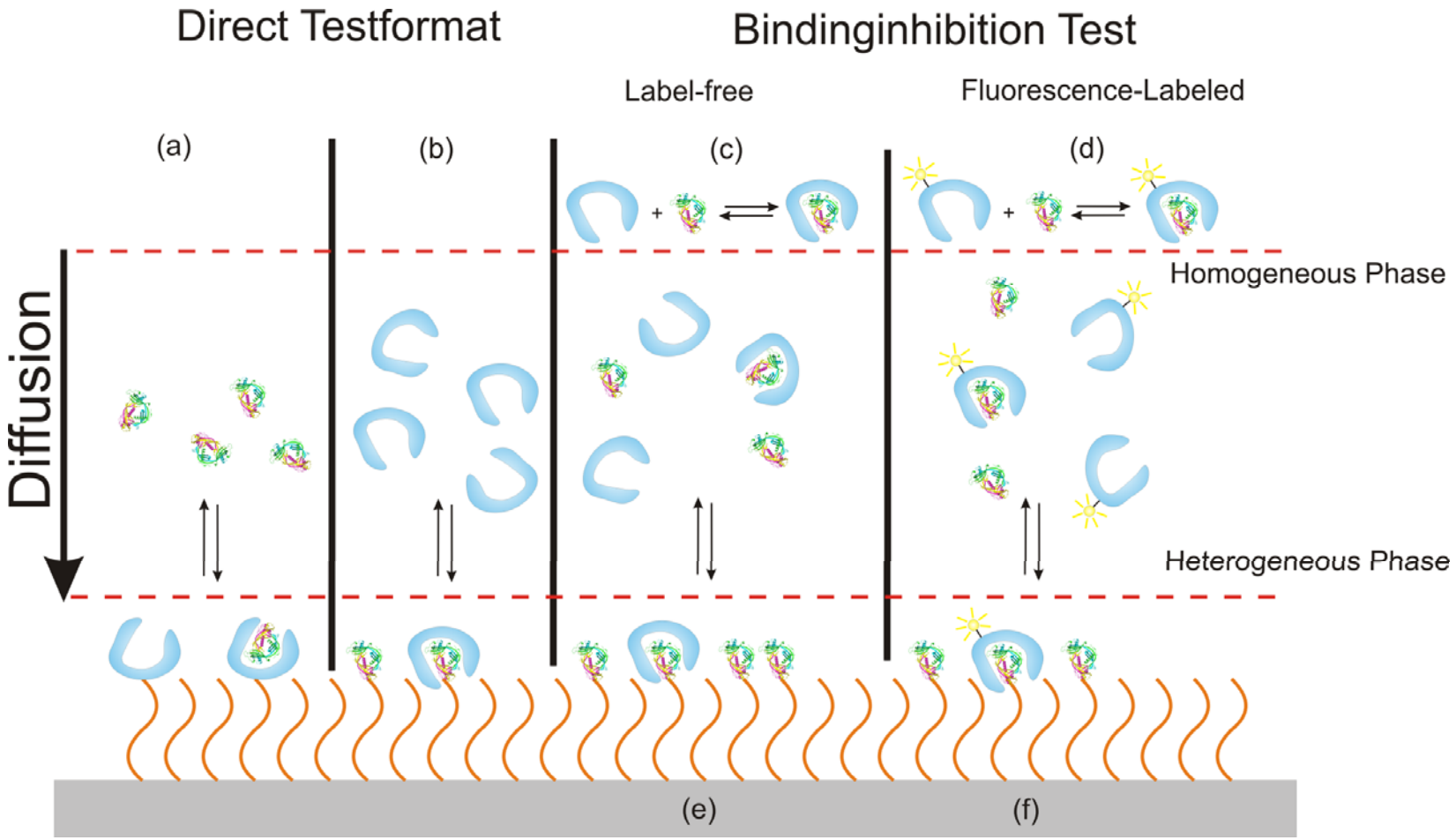
Binding at heterogeneous phase



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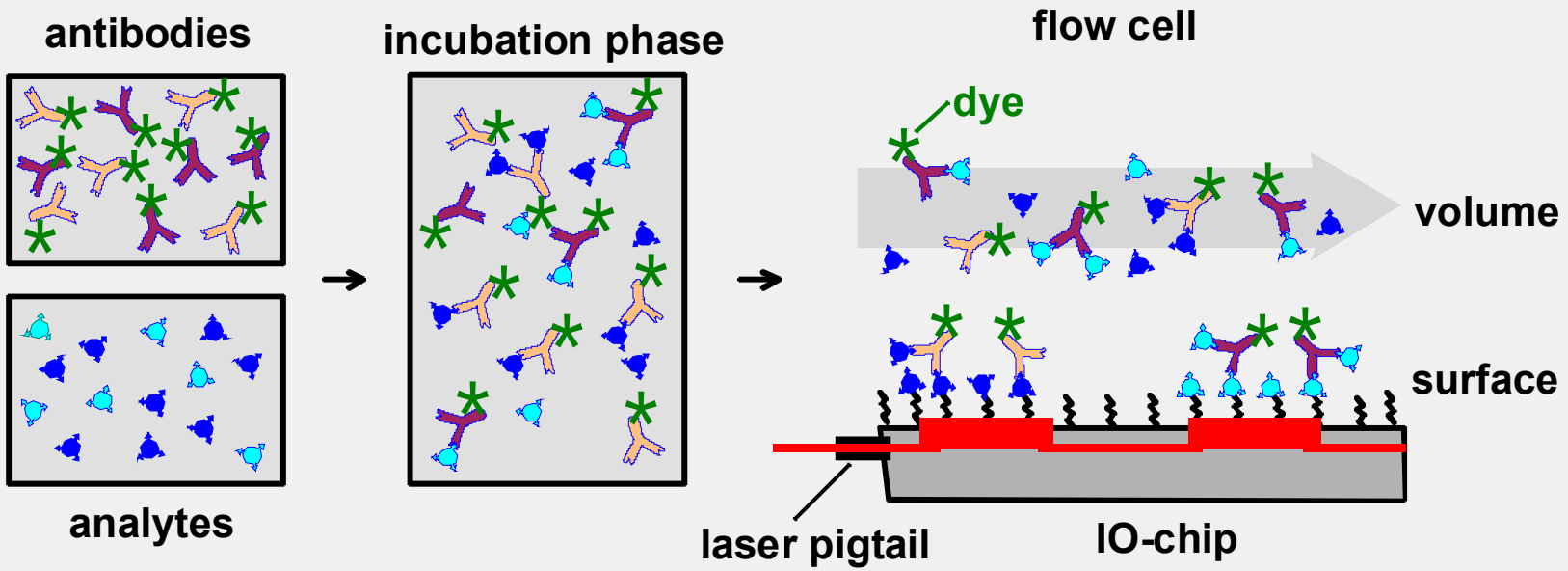
Antigen-Antibody-Interaction

- Binding inhibition assay
- Specific antibodies which are labelled with a fluorescence dye (Cy5.5™) recognise organic compounds in aqueous samples.
- Detection based on TIRF



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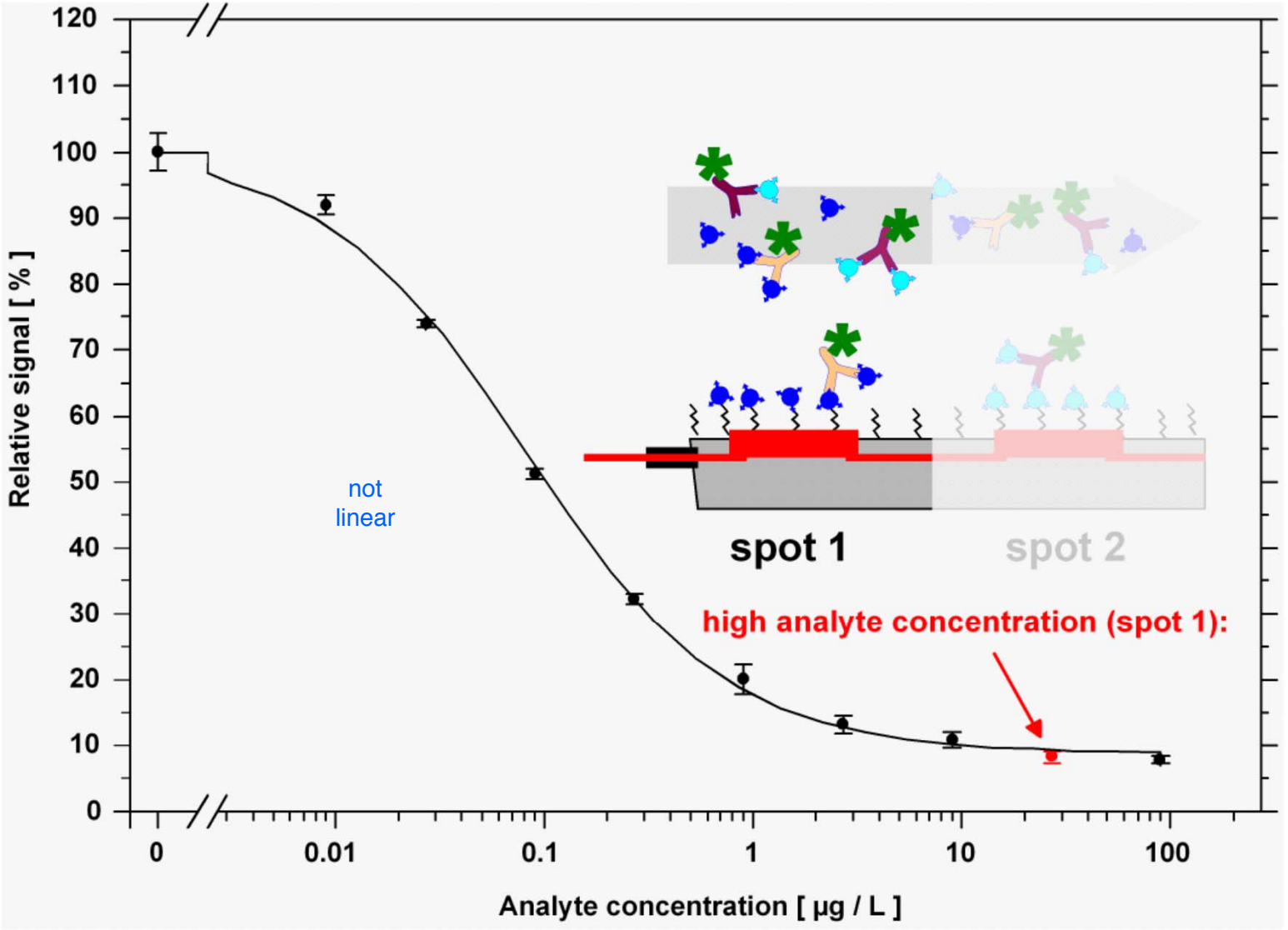
Measurement



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Recognition Elements and Surface Chemistry

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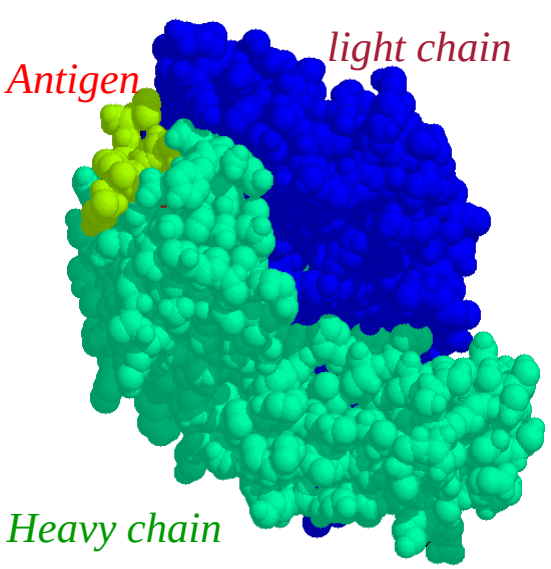
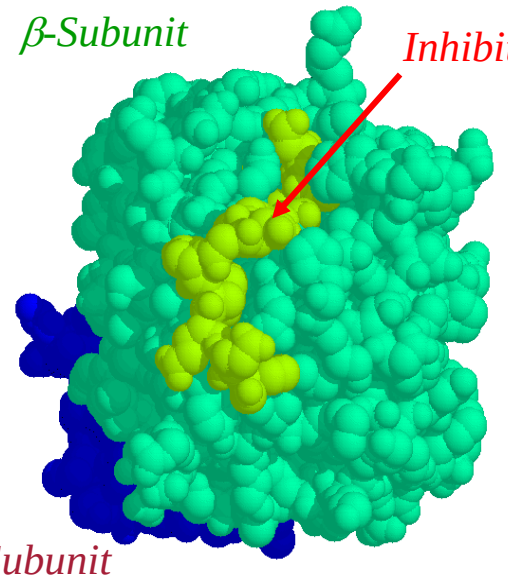
Biomolecular interactions

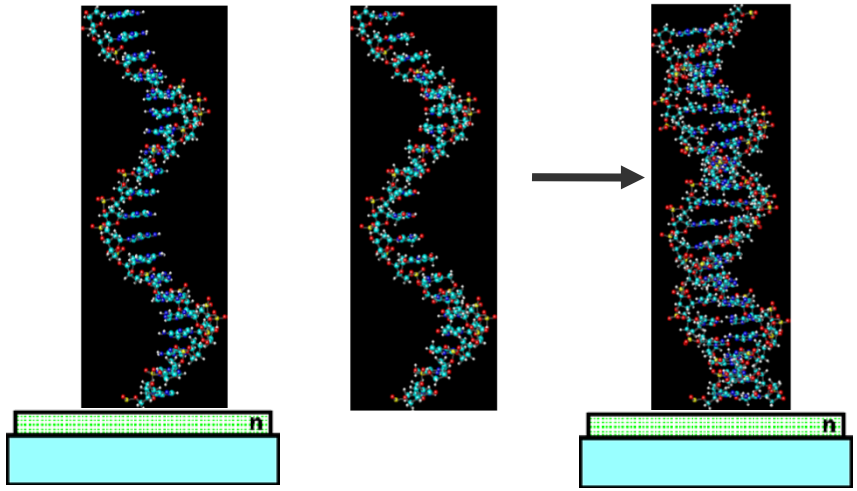




Churchill M.E.A. and Wilson I.A. 1996 PDB-Data base
Tulinsky A. and Krishnan R. 1996 PDB-data base

Biomolecular interaction by:

- *Electrostatic, hydrophobic or Van der Waals interaction*
- *Highly ordered arrangement of partners*



- *DNA immobilisation and on-line monitoring of hybridisation*
- *Reduction of affinity with increasing number of non-maching bases*

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Optimum surface

- Supplies
  - Possibility of high loading
  - Avoiding non-specific binding
  - High stability

- Surface modification
- Multi layer
- Localised spots
- Multiple regenerations

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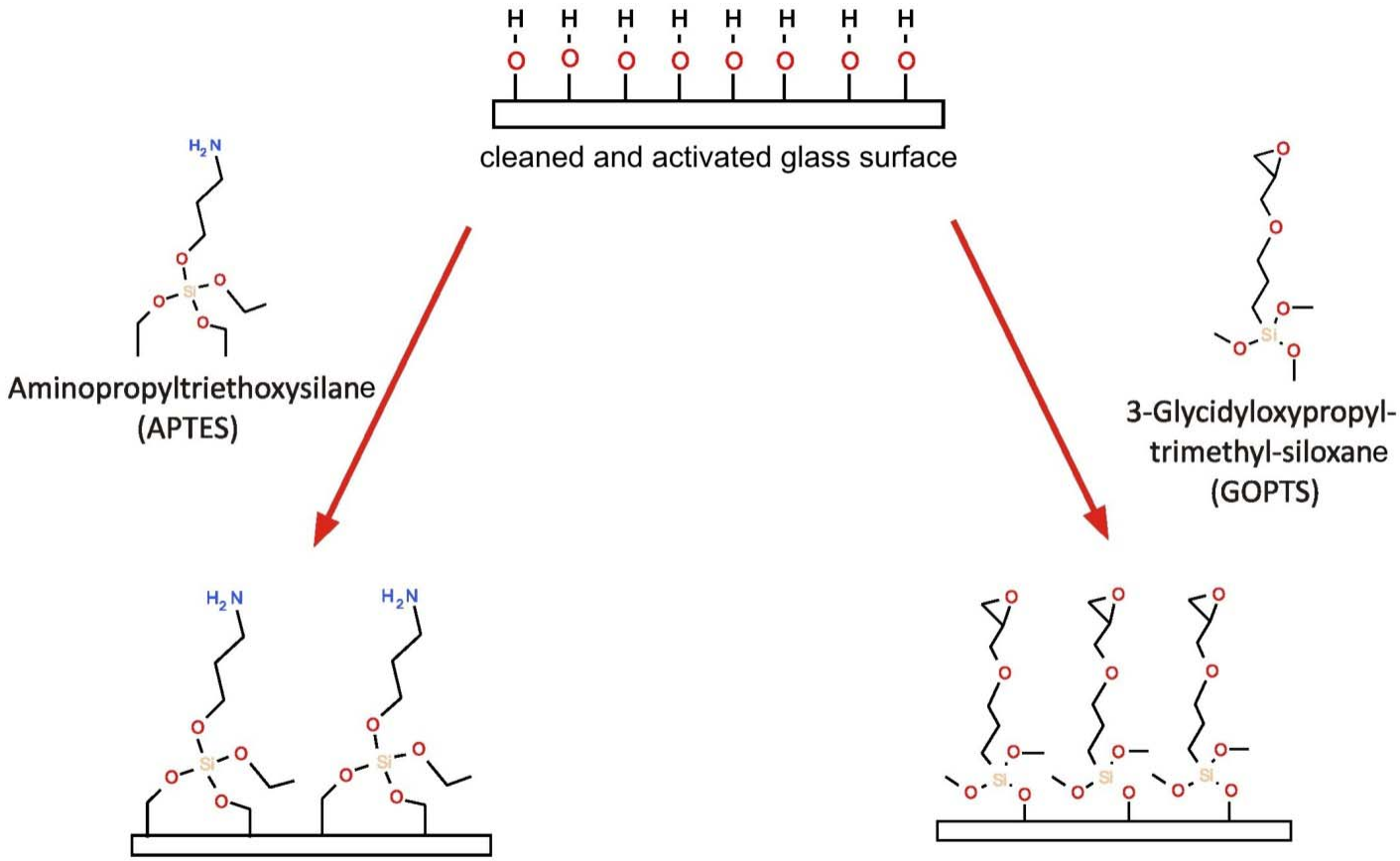
Surface chemistry

Silanes



Providing functional groups for direct immobilisation of recognition elements or further functionalized layers (e.g. biopolymers).

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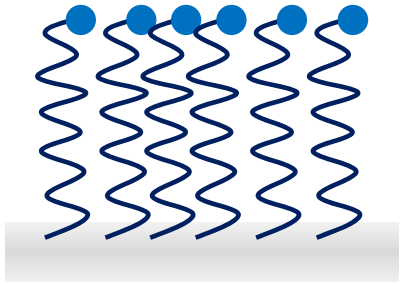
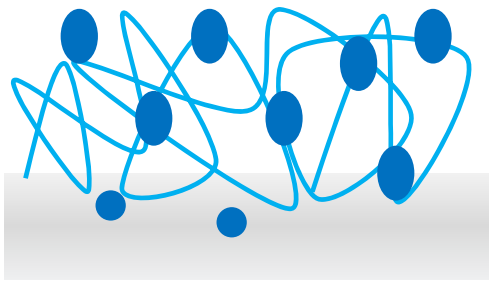
Functionalized surface layer

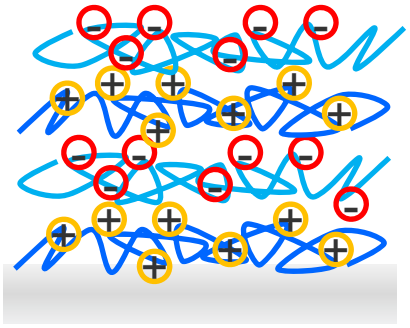
Polyethylenglycol



Dextran



Polyionic-multilayers



Characteristics:

- low binding capacity maximum: 1 monolayer (5-6 ng/mm^2 protein)
- NH_2 or COOH functionalised polymer

- flexible hydrogel
- high binding capacity up to 20 ng/mm^2 protein
- NH_2 or COOH functionalised polymer

- simple immobilisation of charged macro molecules
- regeneration by washing

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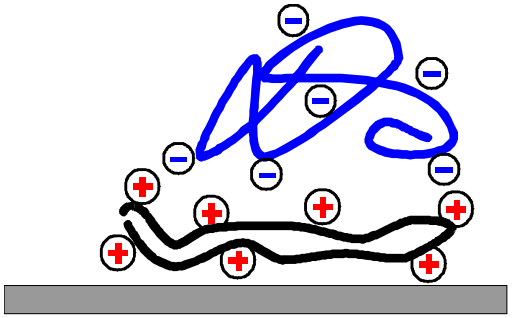
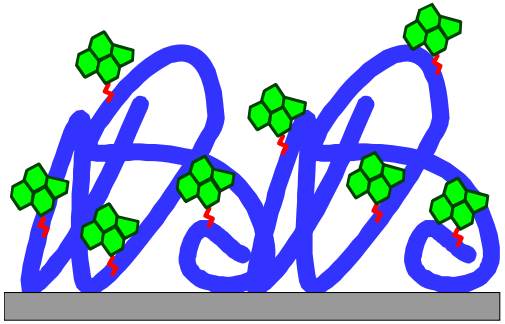
Surface chemistry



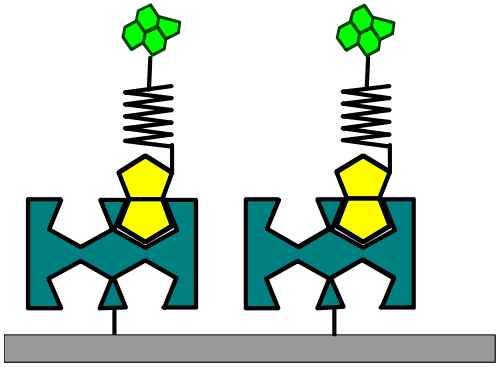
Polyionic

Simple immobilisation of charged macro molecules
Regeneration by washing



Dextran

flexible hydrogel
High binding capacity up to
20 ng/mm^2 protein
NH_2 or COOH functionalised



Streptavidin

Immobilisation by affinity binding
biotinylated subtances
can be immobilised
(not reversible)

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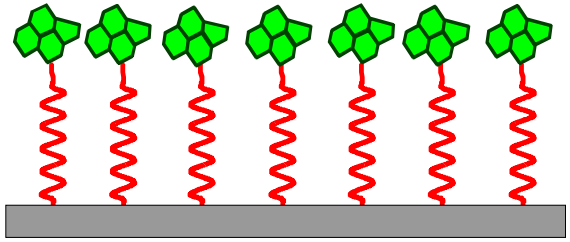
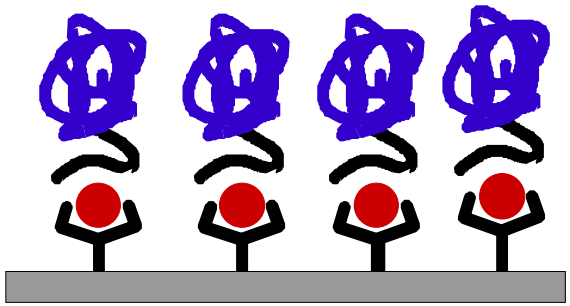
Surface chemistry



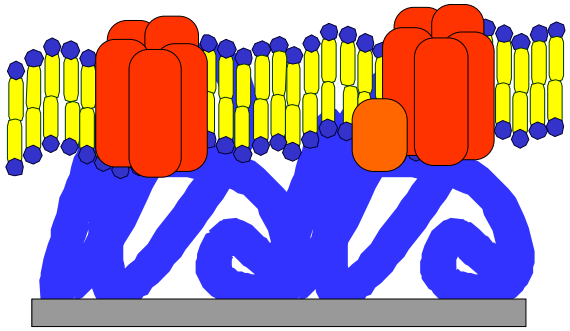
Polyethylen-glycol

low binding capacity
maximum: 1 monolayer
(5-6 ng/mm^2 protein)
amino- and carboxy-functionalised



His-Tag

Ni^{2+} surface
Proteins with a His-Tag can be immobilised (reversible)



Membrane-proteins

Surface for membrane-proteins
Immobilised using hydrogel

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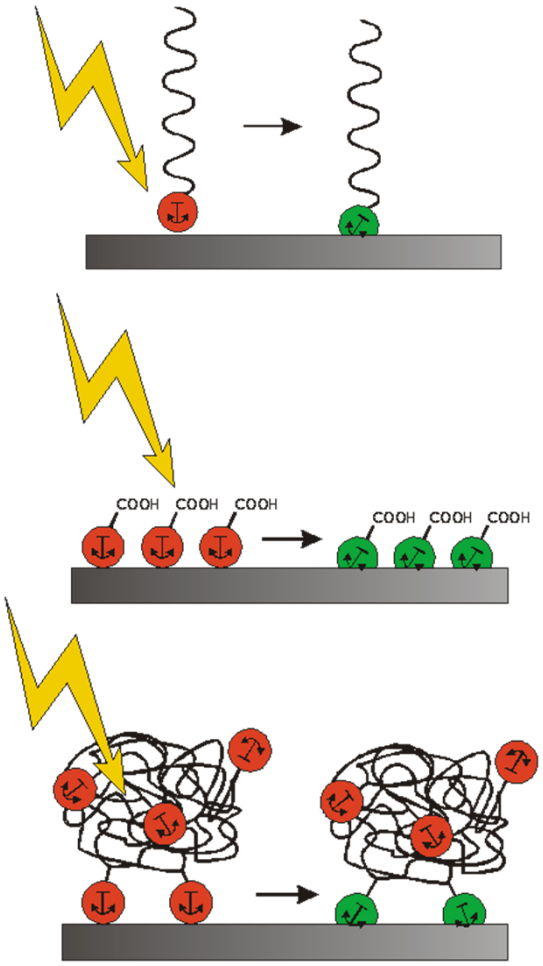
Possibilities of photolinking

- direct photoimmobilisation of biomolecules
  (DNA, „Photobiotin“)
- photofunctionalisation of polymer surfaces
- indirect photo-immobilisation using hydrogeles („Photodextran“)



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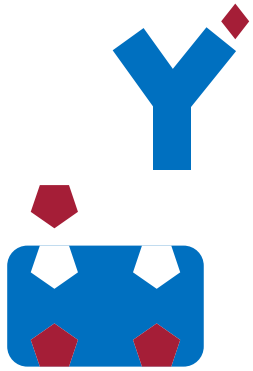
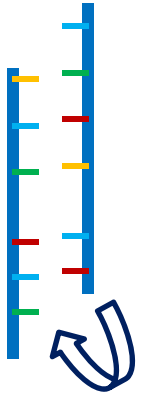
Recognition elements

- Polymers
- Functionalised polymers
- Molecular imprinted polymers
- **Biolayers as recognition structures**
  - Antigen/antibody
  - DNA/DNA
  - Peptide/protein
  - Protein/protein
  - Small ligands/protein



- Scaffolds
- Aptameres
- Membrane like structures
- Membranes



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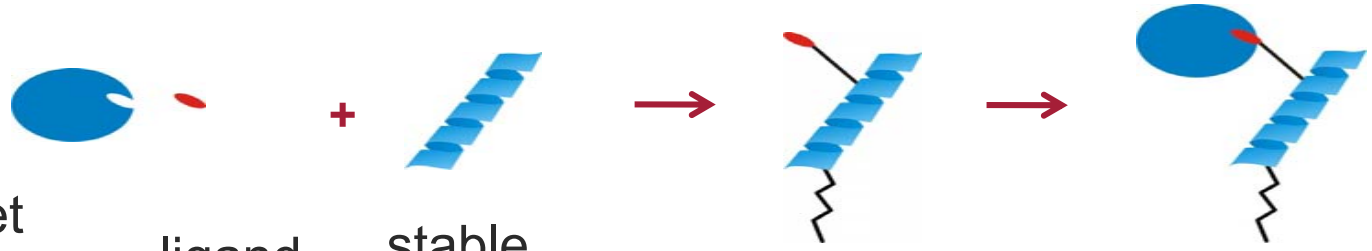
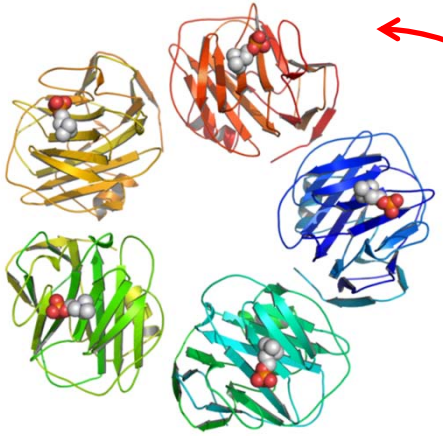
Scaffolds

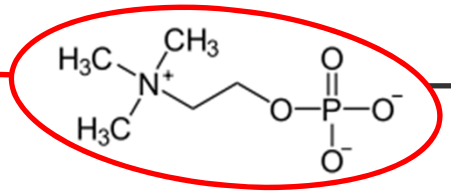
Principle of binder scaffolds



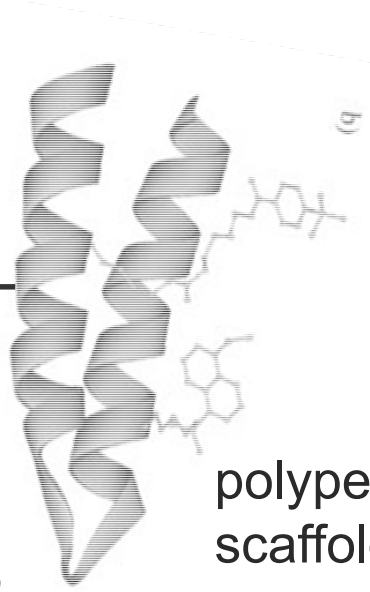
Example: Polypeptide scaffold for CRP



C-reactive protein (CRP)



phosphocholine



polypeptide scaffold

Lars Baltzer, Uppsala

Patent no. WO/2003/080653

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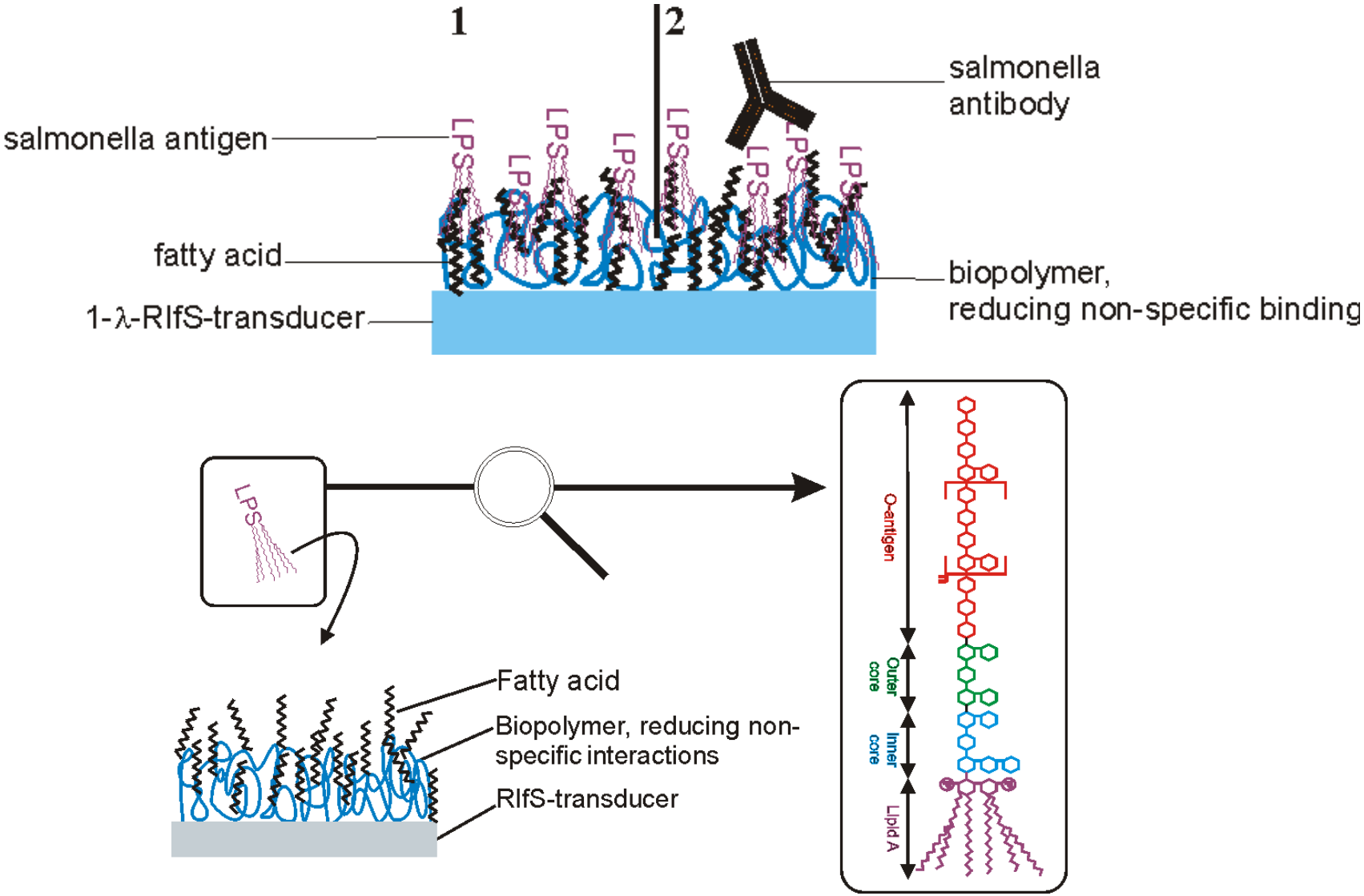
Membrane like recognition layer



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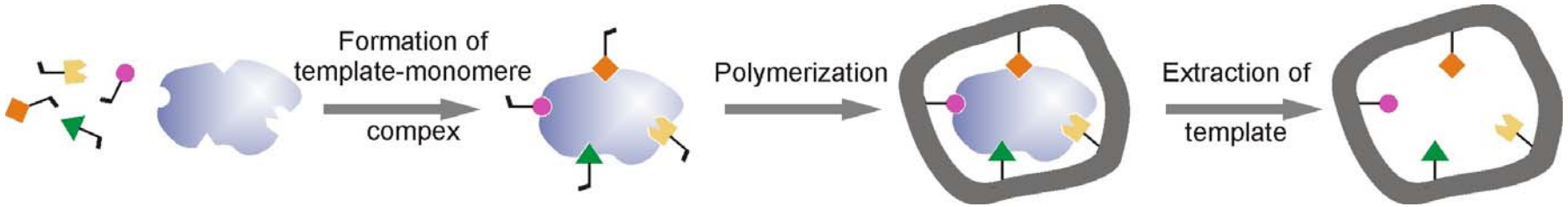
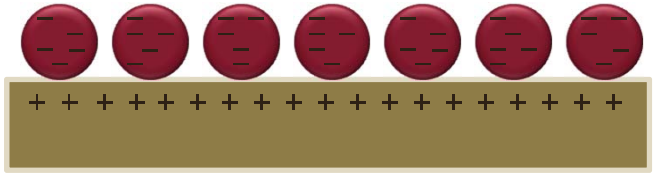
Molecular imprinted Polymers: MIP



Immobilization: Electrostatic adsorption of particles

Particles: negatively charged

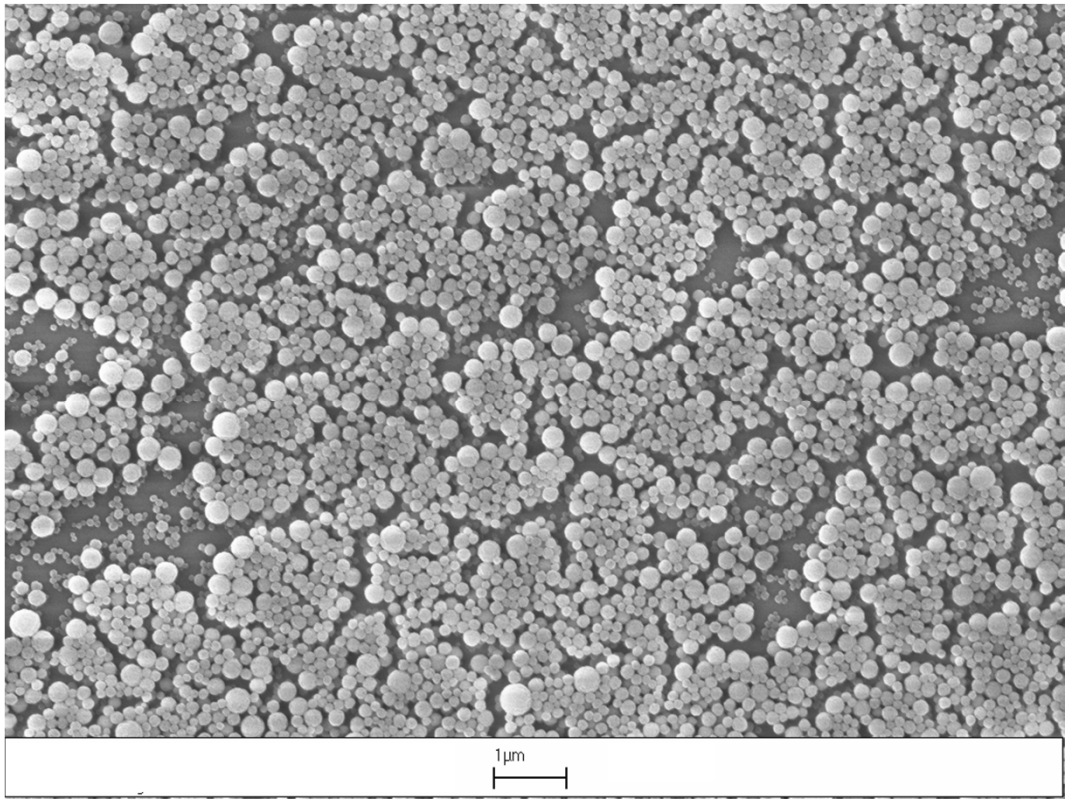


Surface with Amino groups
(positively charged)

Advantage of particles:

- Many recognition sites
- Fast diffusion

problems with response time -> small beads with cavities, large surface (in total)



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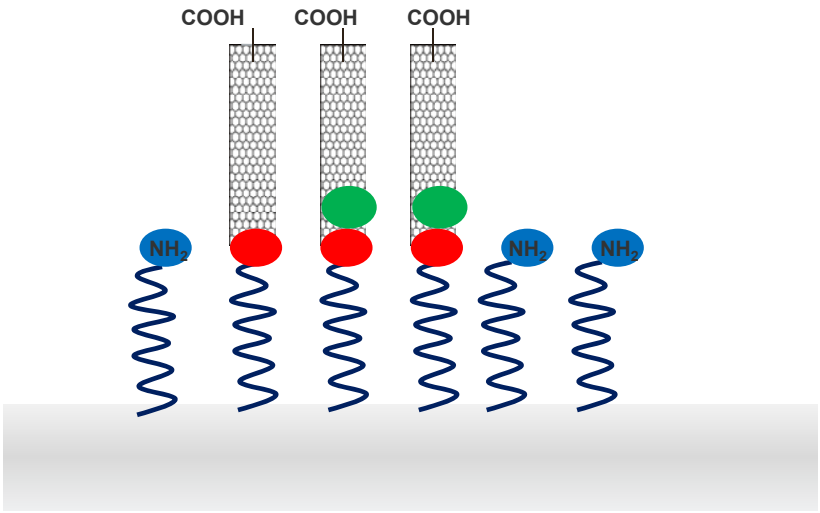
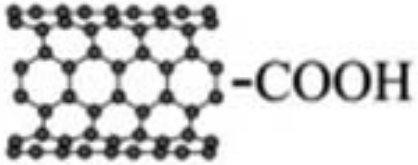
Recognition Element: Nanotubes





Example:
Carbon Nanotubes carboxy functionalised (-COOH) on Aminopolyethylenglycol

Nanoparticle can interact with nanotubes

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Biomolecular Interaction
BIA

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RIfS-Bio: time resolved and label free measurements

Qualitative Measurements

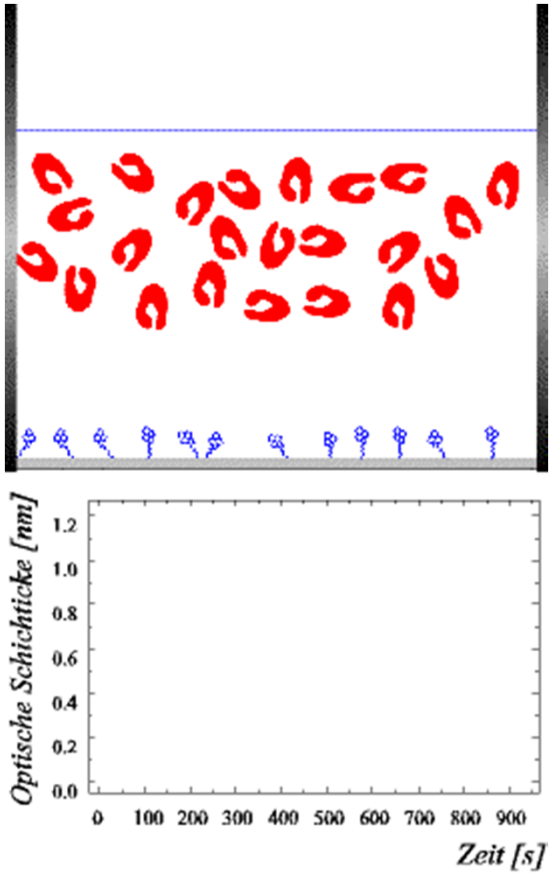
→ **binding / no binding**

Quantitative Measurements

concentration

kinetics
association- und dissociation rate constants

thermodynamics
affinity and binding constants



→ surface with low and high loading

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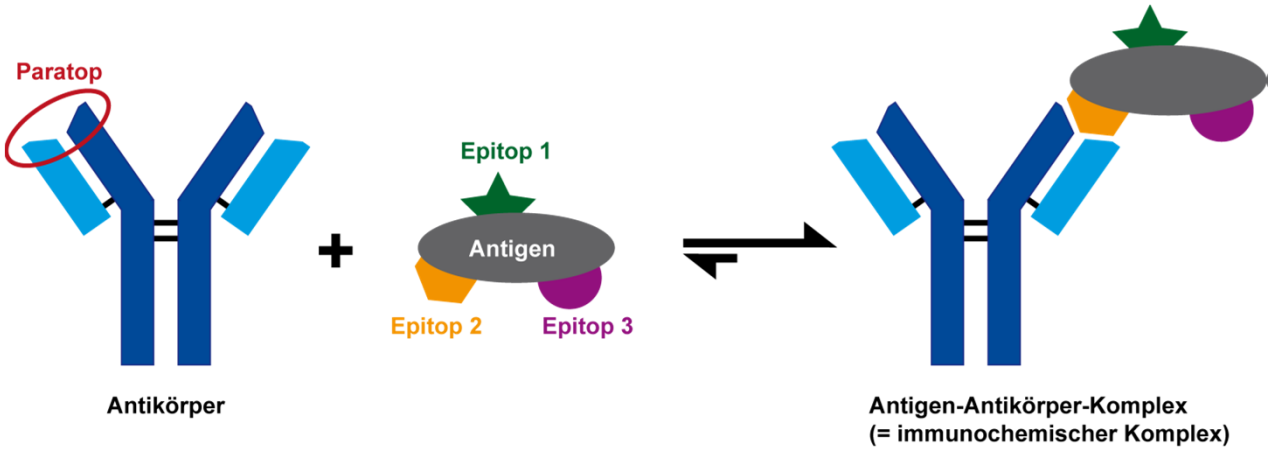
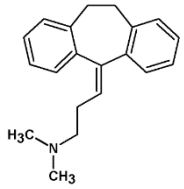
Immuno reactions

Analyte-detection with antibodies



Possible antigenes



small molecules



proteines

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Coverage at surface depending on concentration

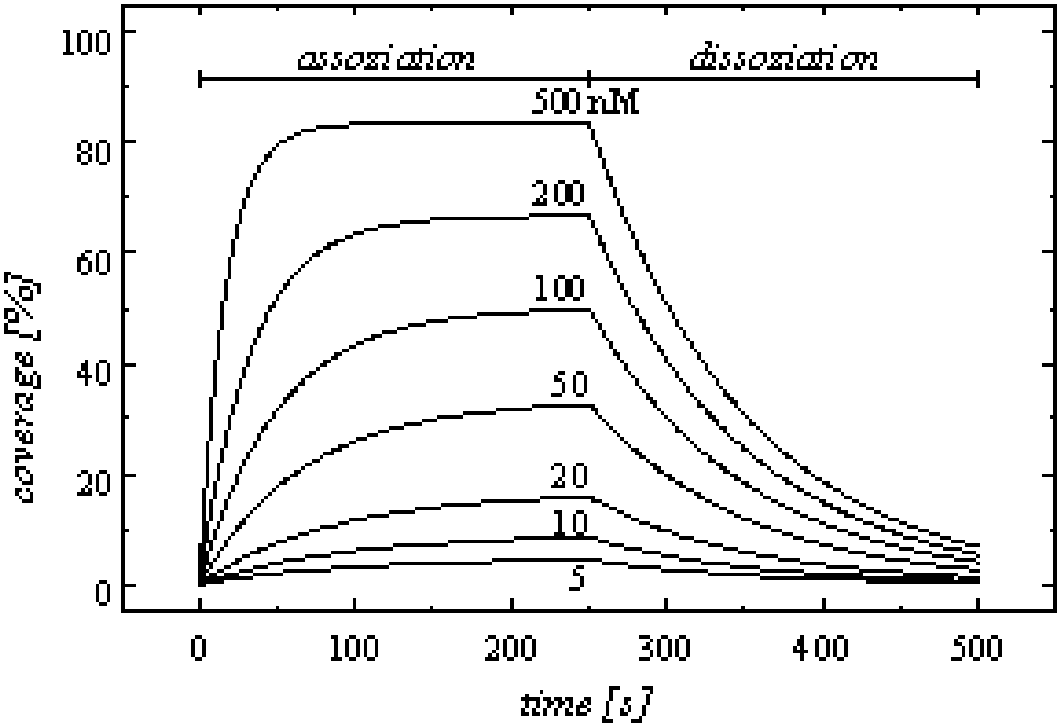
$$\frac{d\Gamma(t)}{dt} = k_a \cdot c \cdot (\Gamma_{max} - \Gamma(t)) - k_d \Gamma(t) \qquad \frac{d\Gamma(t)}{dt} = k_a \cdot c \cdot \Gamma_{max} - (k_a \cdot c + k_d) \cdot \Gamma(t)$$

$$\Gamma(t) = \Gamma_{GG} \cdot \left(1 - e^{-k_s \cdot t}\right) \qquad k_s = k_a \cdot c + k_d$$

formulas not important

$$\Gamma_{GG} = \Gamma_{max} \frac{K \cdot c}{1 + K \cdot c}$$

- Rate constant k_s *is curvature of binding curve*
- *Includes rate of dissoziation*
- *No information on assoziation rate*
- *The larger dissoziation rate the early equilibrium loading*



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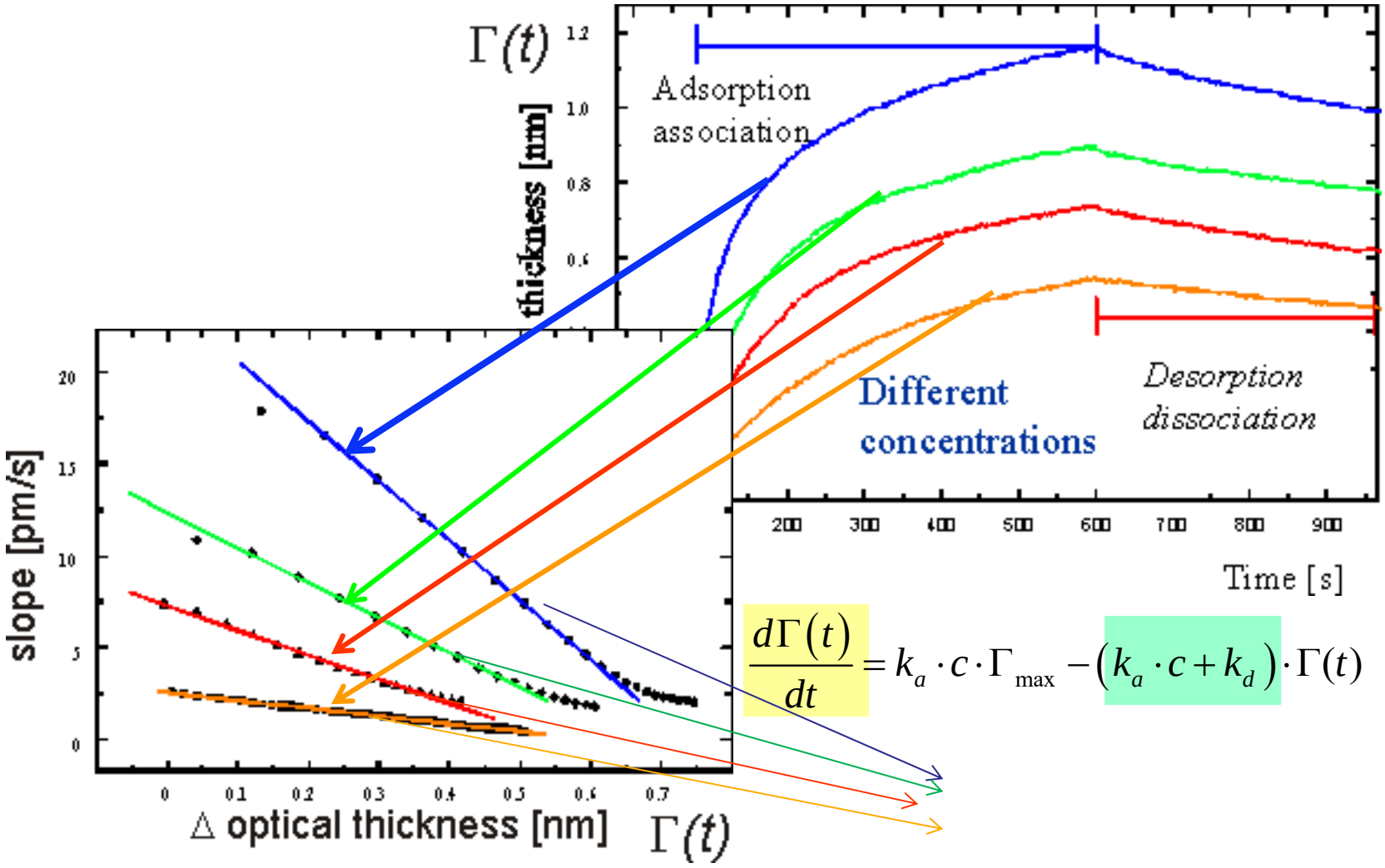
Deterination of k_s IMPORTANT



$$\frac{d\Gamma(t)}{dt} = k_a \cdot c \cdot \Gamma_{max} - \left(k_a \cdot c + k_d\right) \cdot \Gamma(t)$$

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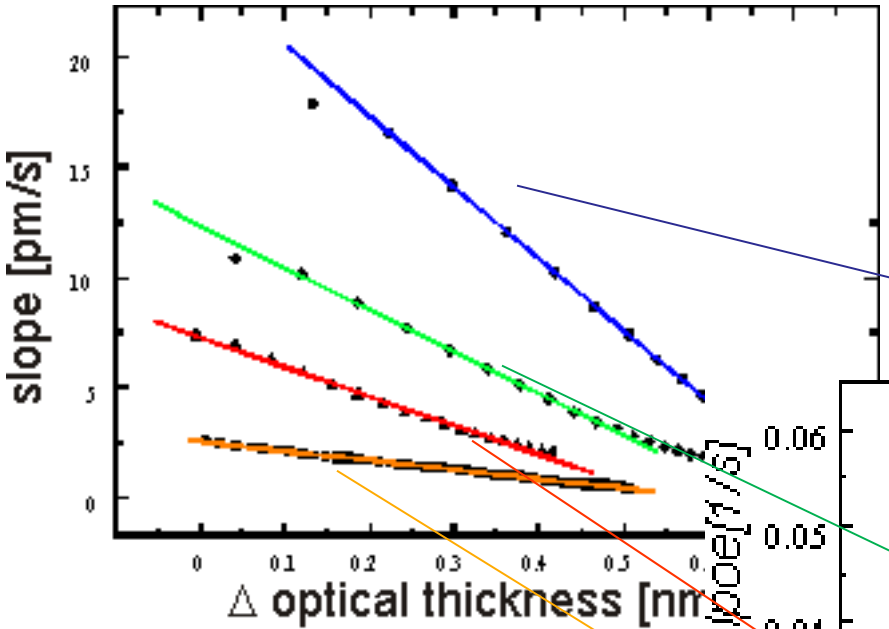
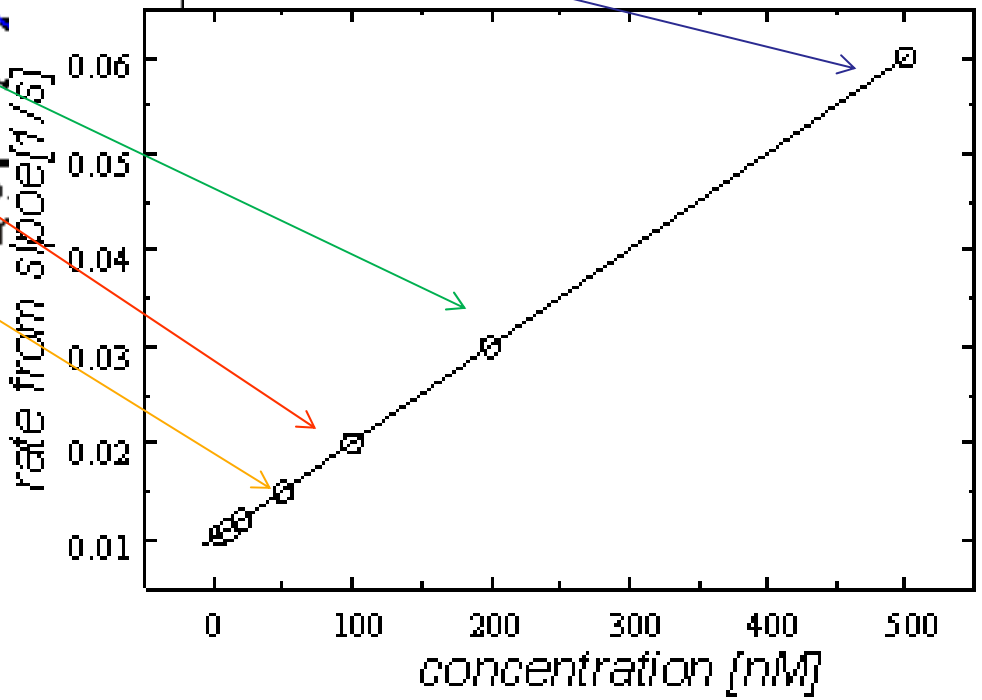
Determination of k_a

IMPORTANT



$$\frac{d\Gamma(t)}{dt}$$

$$k_s = k_a \cdot c + k_d$$



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Overcoming problems IMPORTANT

- Nonspecific binding

  Sophisticated surface chemisty
  - PEG
  - Hydrogels
  - Artificial membranes

- Limit of detection

  High loading

- High throughput

  Imaging techniques

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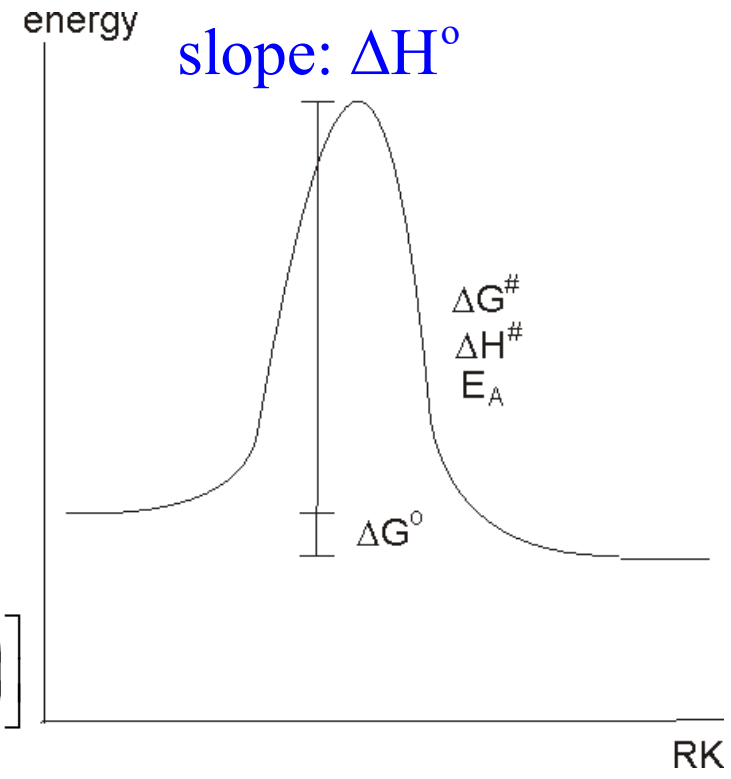
Thermodynamics of binding

$$\Delta G = \Delta G^\circ + RT \ln\left(\frac{[AB]}{[A][B]}\right) = -RT \ln K_a = RT \ln K_d$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K_a$$

$\ln K_a$ graphed vs. 1/T

slope: ΔH°



For protein/protein interactions T-dependence

$$\Delta H^\circ = \Delta H^\circ(T^\circ) + \Delta C_p\left(T - T^\circ\right)$$

$$\Delta S^\circ = \Delta S^\circ(T^\circ) + \Delta C_p \ln(T/T^\circ)$$

$$\Delta C_p = const.$$

$$\ln K_A = \frac{-\Delta H^\circ(T^\circ)}{RT} + \frac{\Delta S^\circ(T^\circ)}{R} + \frac{\Delta C_p}{R}\left[\left(\frac{T - T^\circ}{T}\right) - \ln\left(\frac{T}{T^\circ}\right)\right]$$

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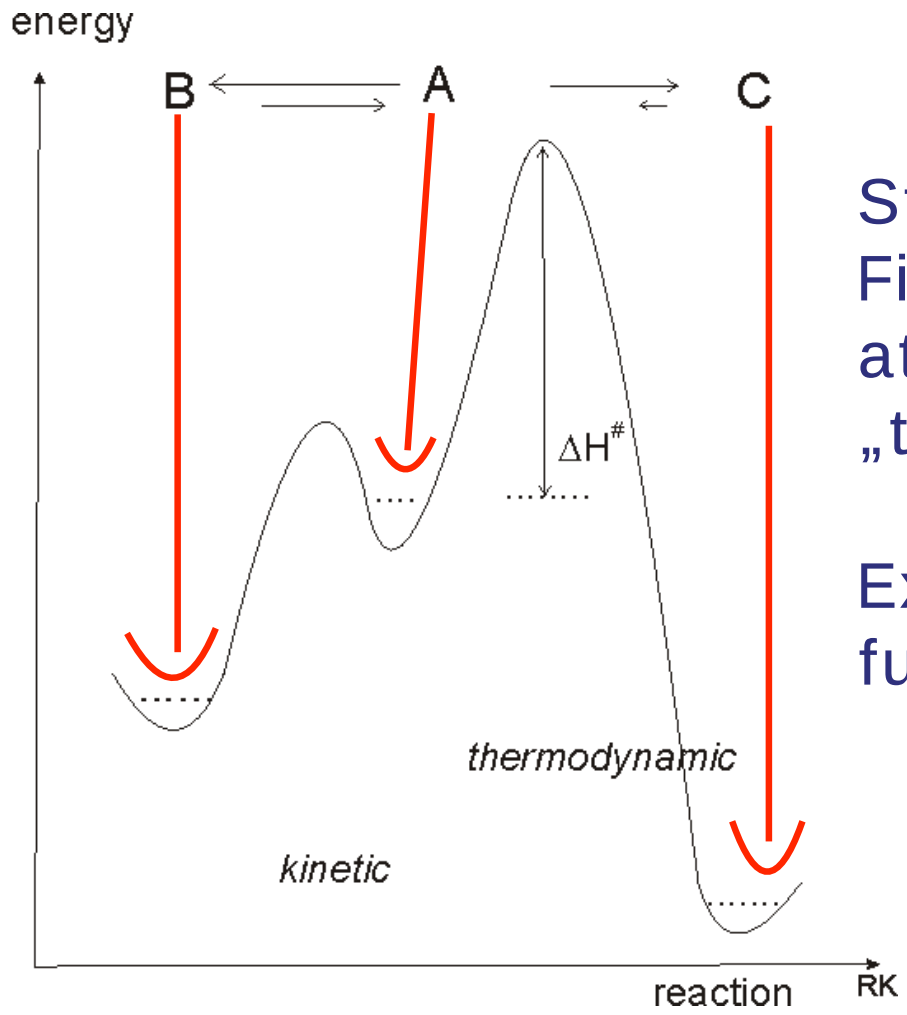
Competitive reactions



Starting from A
First more B ist formed
at the end most B is
„transformed“ to C

Except B is part of
further fast reaction

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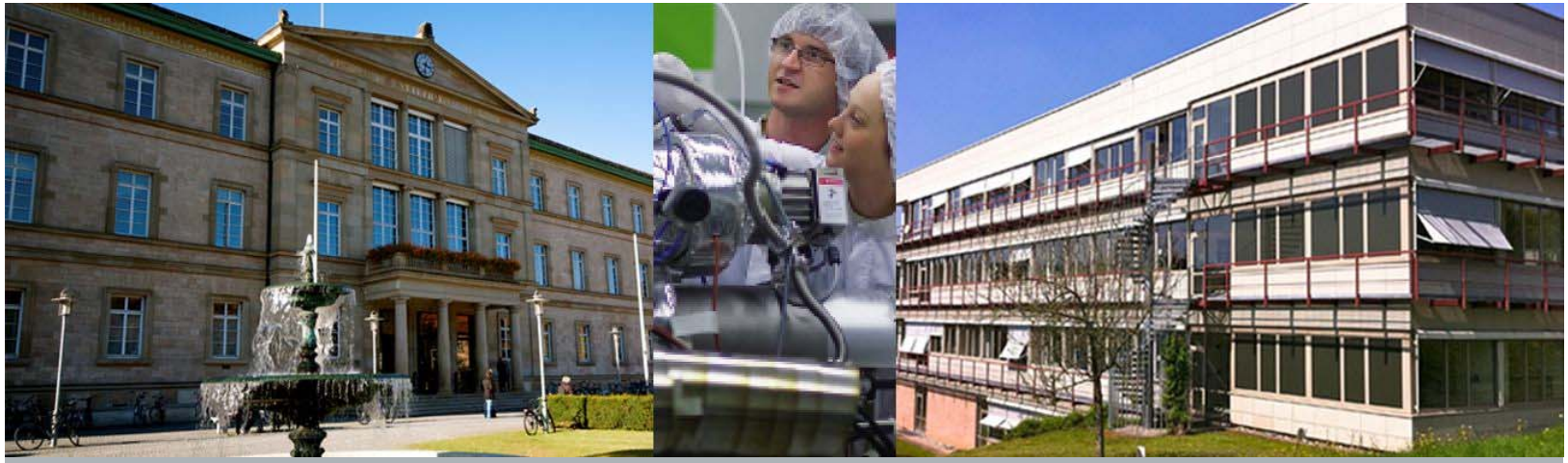

Laboratory Techniques and Medical Device Approvals

Gauglitz Part II: Biosensor, Assays and BIA

End

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