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Optical Biosensors: Surface Plasmon Resonance Biosensors

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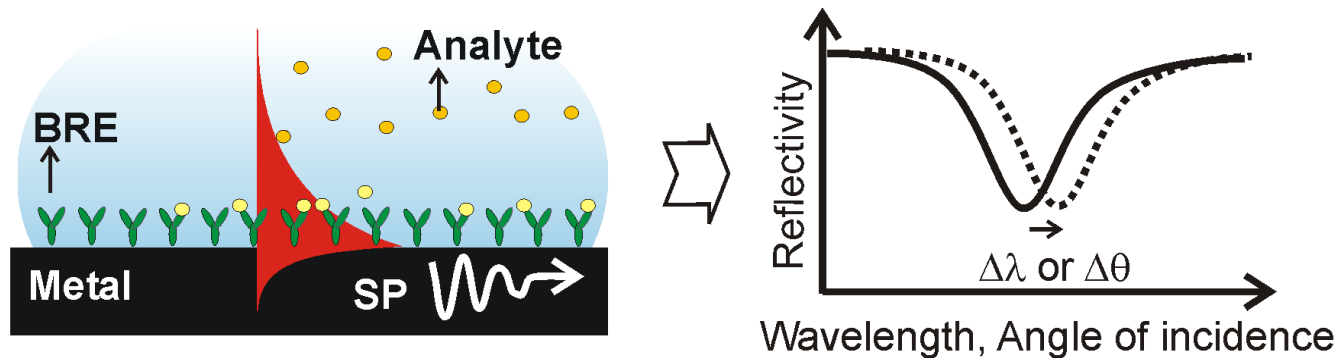
Web: <http://www.fzu.cz/> | <https://www.dpu-research-list.at/> | <http://www.jakubdostalek.cz>

Content

- Principle of SPR biosensors.
- Direct, competitive, inhibition assay format.
- Biorecognition elements.
- Propagating surface plasmons (PSPs) on metallic films - optical configurations for the coupling (ATR, grating).
- Localized surface plasmons on metallic nanostructures attached to solid substrates.
- Angular and wavelength interrogation of SPR.
- Surface mass density measurements with SPR biosensors.
- Common implementations of SPR biosensors and commercial instruments.

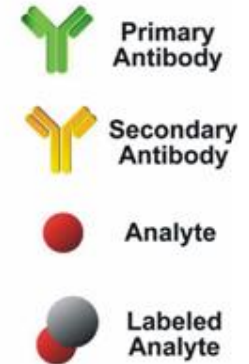
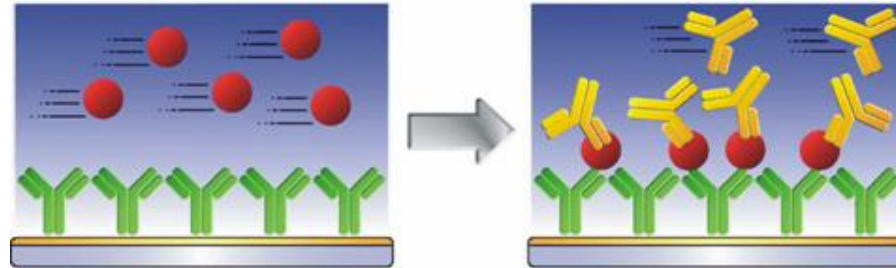
Probing of Affinity Binding Events

The capture of target analyte on the sensor surface forms an additional layer with increased refractive index δn_d which is probed by surface plasmon resonance:

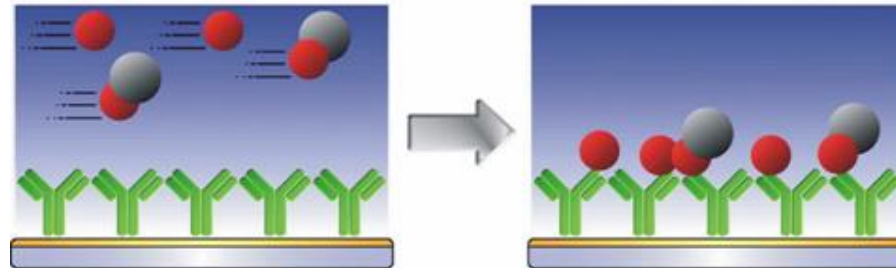


Heterogeneous Assays

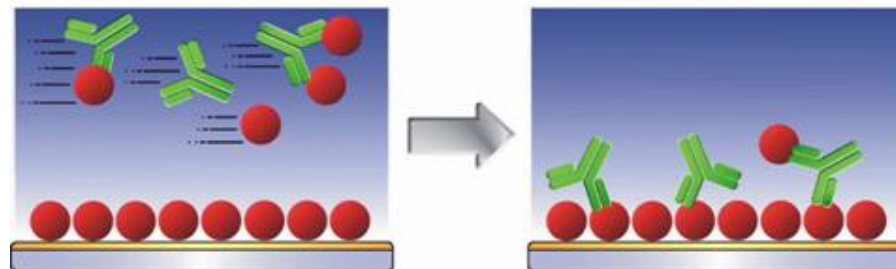
Direct/
Sandwich



Competitive



Inhibition

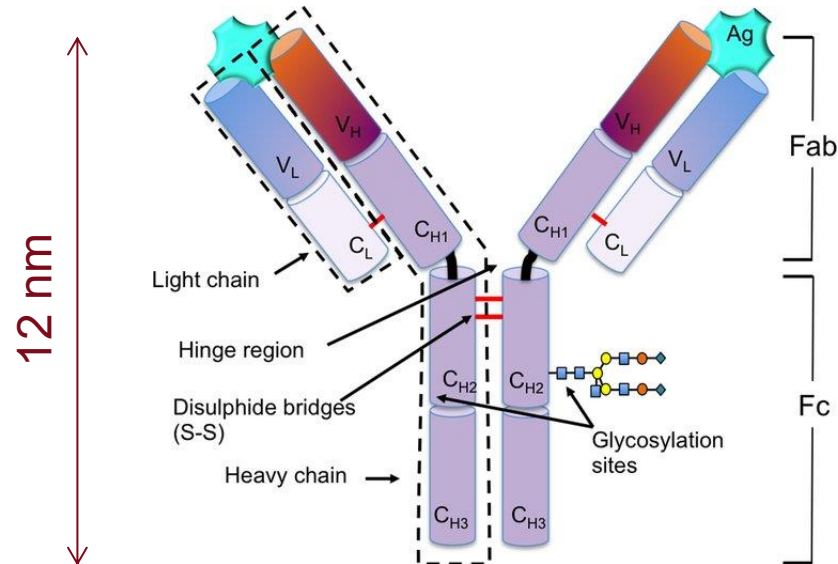


J. Homola (editor): Surface Plasmon Resonance Based Sensors, Springer, 2006.

Polyclonal Antibodies

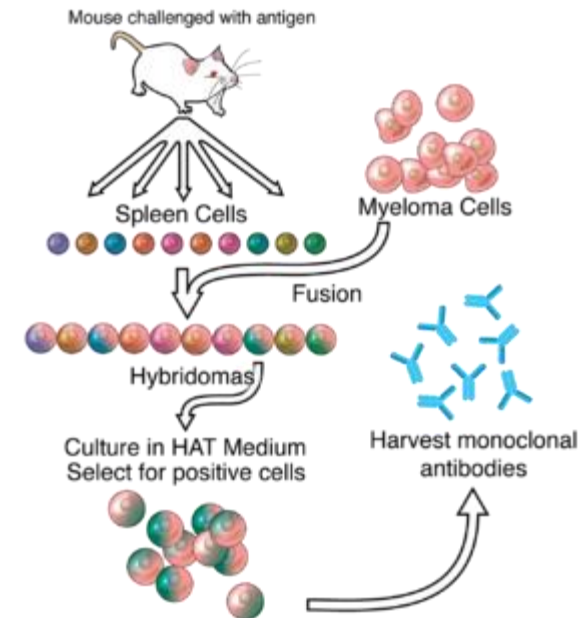
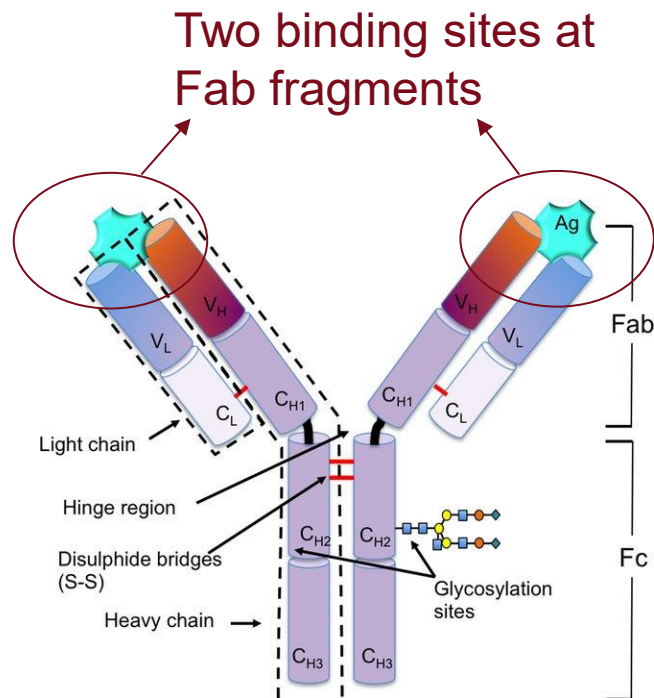
Immunoglobulin G
antibody with MW of
about 150 kDa.

Hydrodynamic radius
of about 7.5 nm



- ➔ Polyclonal antibodies: mixture of different antibodies expressed by an animal (mice, goats,...) that are specific to (different sides of) an antigen. Extracted from blood.

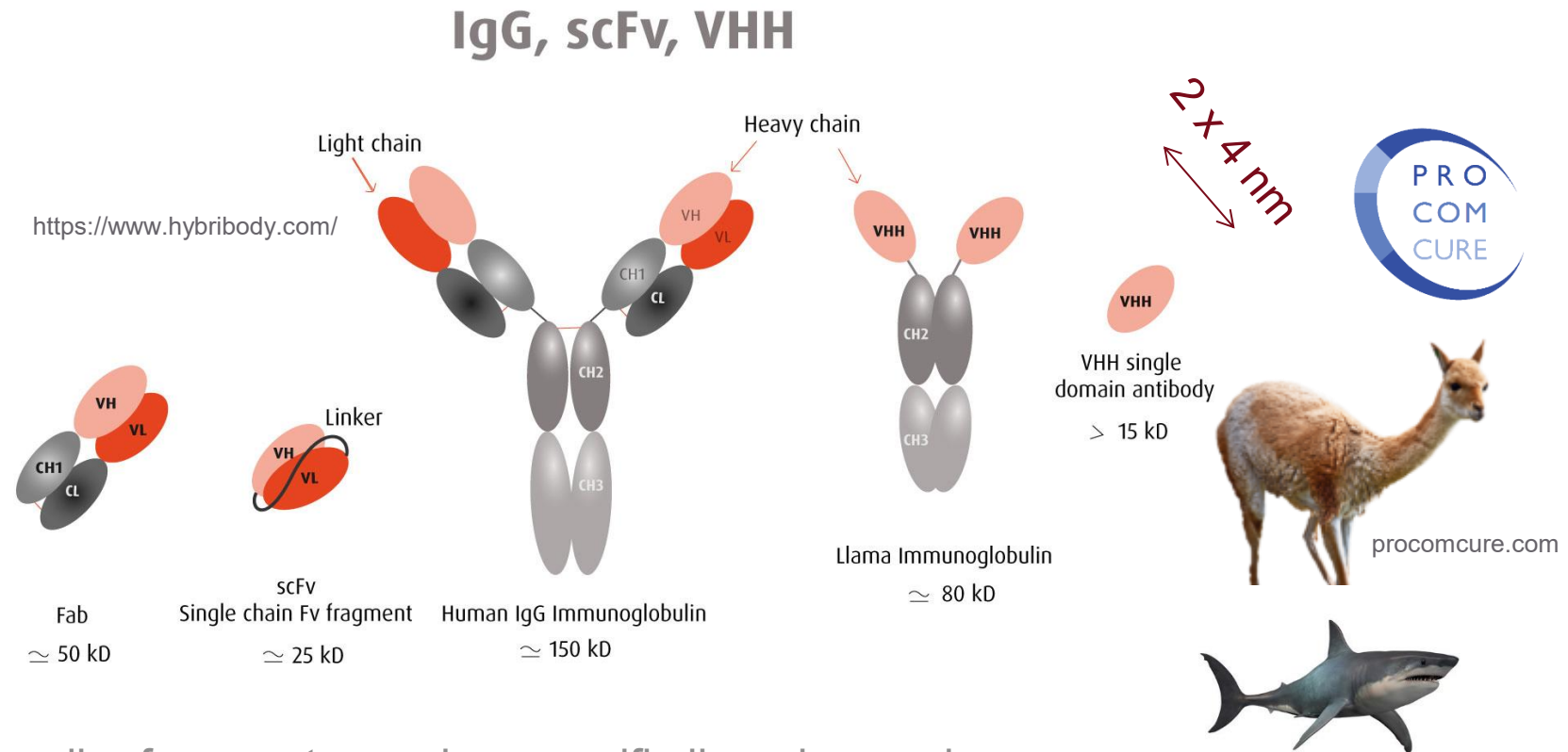
Monoclonal Antibodies



https://en.wikipedia.org/wiki/Monoclonal_antibody

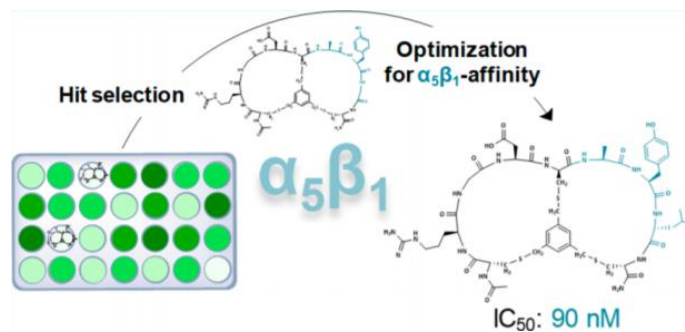
- ➔ Monoclonal antibodies: identical molecules that are specific only to one part of the antigen (epitope). Prepared from cell lines and typically provide higher affinity than polyclonal antibodies

Antibody Fragments - Nanobodies



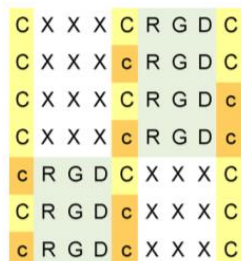
- ➔ Smaller fragments used as specific ligands, e.g. in therapeutic use. Accessibility of hidden epitopes and in biosensors more recognition sites can be attached to their surface.

Peptides



Molecular
weigh of
> 1 kDa

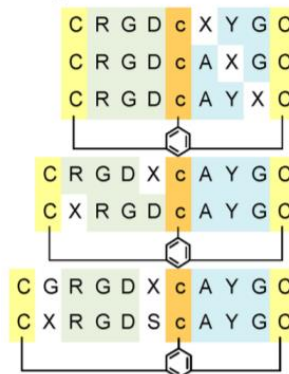
Design of 1st generation libraries



in total 672 peptides

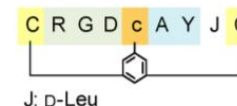
1. Screening
2. IC₅₀ determination
for best binders
3. Lead selection
AYG

Design of 2nd generation libraries



1. Screening
2. IC₅₀ determination
for best binders

$\alpha_5\beta_1$ -binder



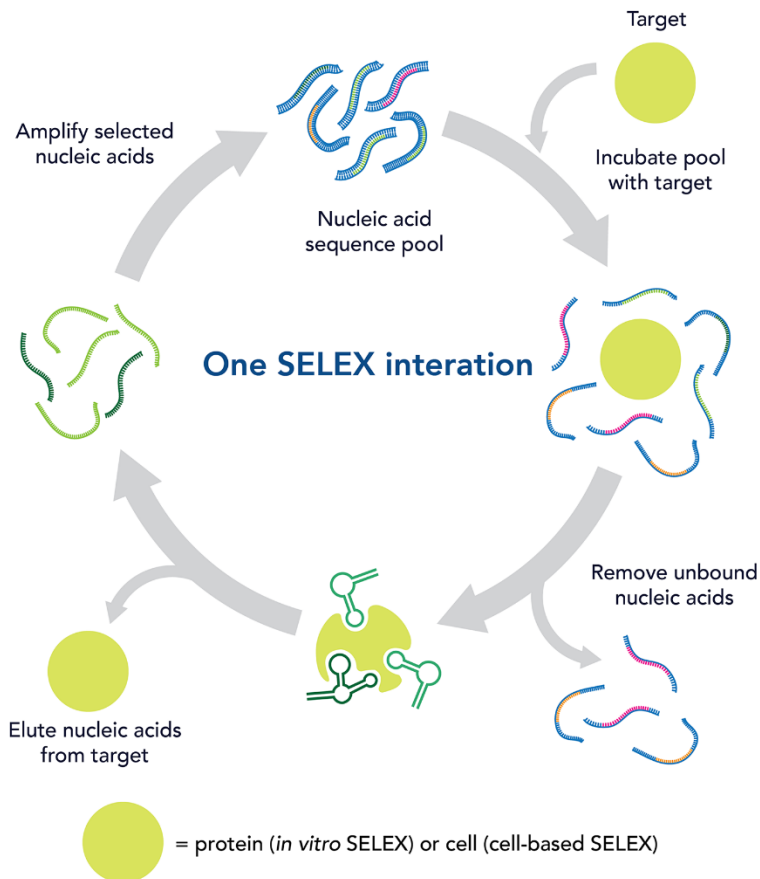
3. K_d determination
4. Selectivity test

<https://doi.org/10.1021/acscombsci.9b00081>



- ➔ Short peptide chains, selection for affinity to target analyte.
- ➔ More stable, cheaper production, straightforward conjugation as prepared by purely synthetic means.

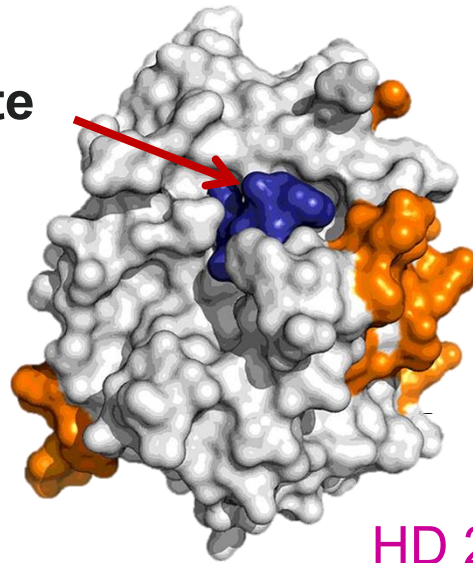
Aptamers



- ➔ Short oligonucleotide chains, selection for affinity to target analyte.
- ➔ Systematic evolution of ligands by exponential enrichment (SELEX)
- ➔ Similar advantages as peptide ligands, arguably more efficient selection process powered by PCR (which does not exist for peptides / proteins...)

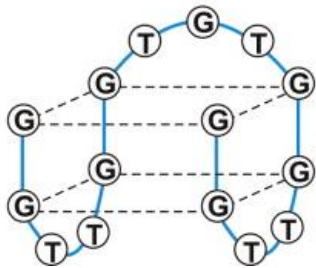
Thrombin Aptamer

Active site

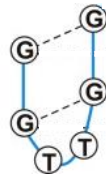


Exosite I

HD 1



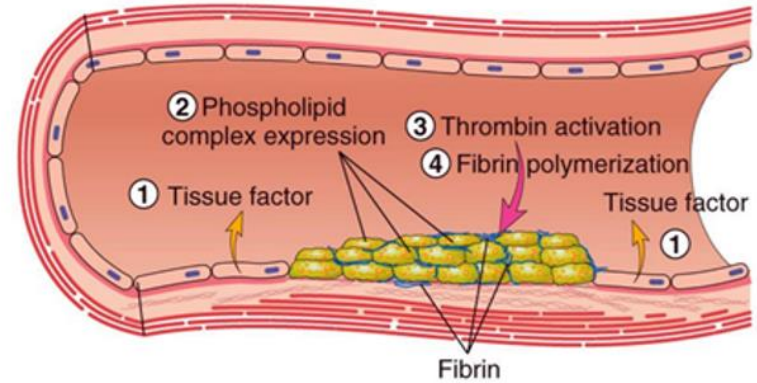
HD 1 short



HD 22



Exosite II



Periyah MH, Halim AS, Saad AZM. Mechanism action of platelets and crucial blood coagulation pathways in Hemostasis. *Int J Hematol Stem Cell Res.* 2017;11(4):319–27.

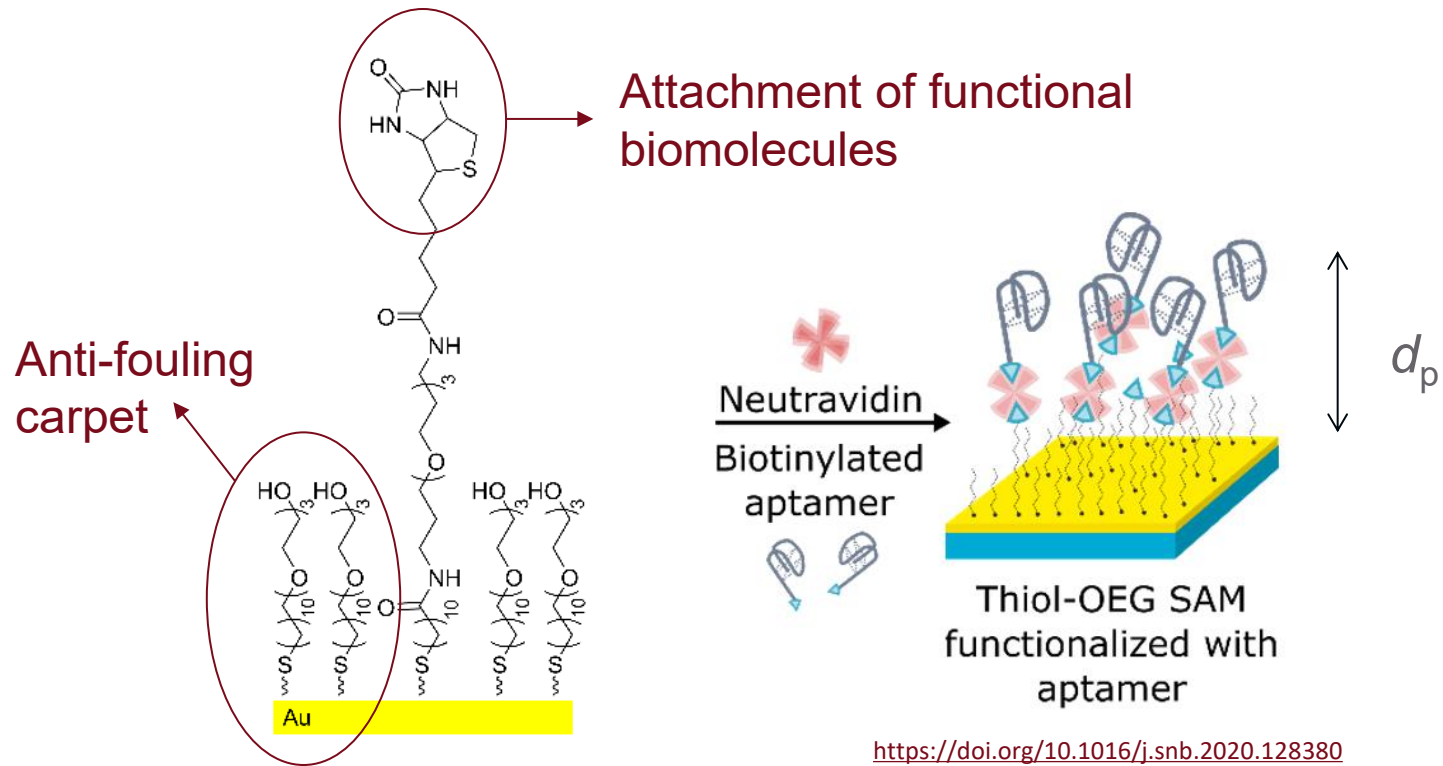


A set of aptamers specifically recognizing thrombin with nM K_d constant discovered

<https://doi.org/10.1016/j.snb.2020.128380>

<https://doi.org/10.1021/acssensors.9b00827>

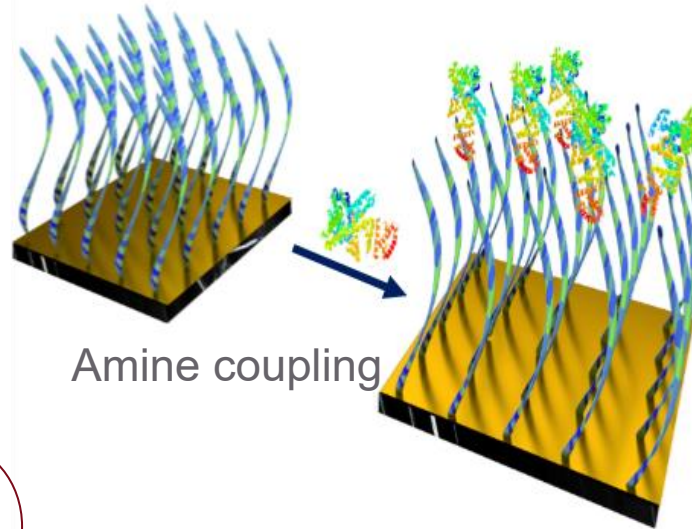
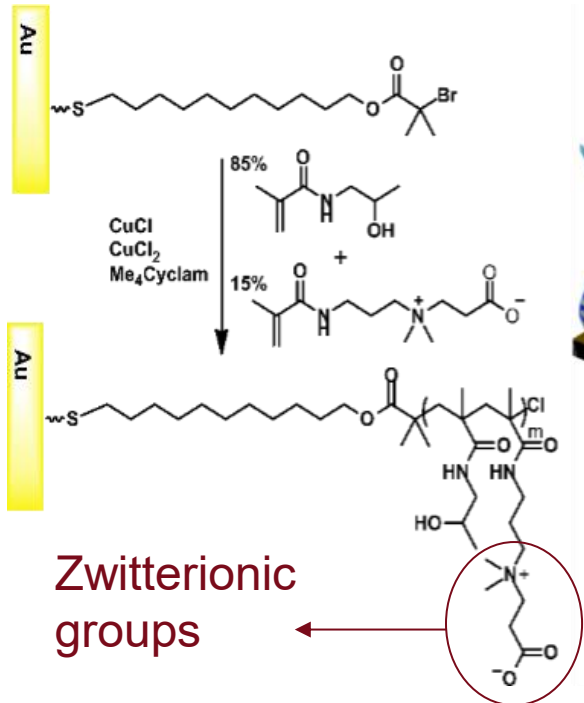
Mixed Thiol SAM



➔ Mixed thiol SAM allows combining different functionality.

Antifouling Brushes

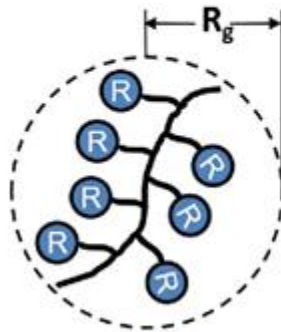
DWI Leibniz-Institut
für Interaktive Materialien



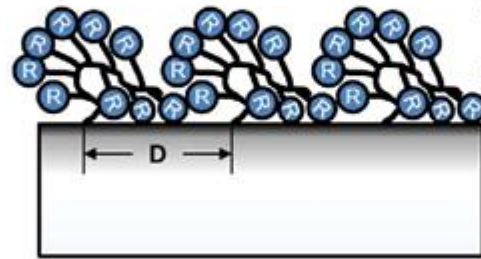
Dr. Riedel
Dr. Emmenegger
Dr. Lisalova

- ➔ Brushes based on poly[(N-(2-hydroxypropyl) methacrylamide)-co-(carboxybetaine methacrylamide)] - (poly (HPMA-co-CBMAA))
- ➔ Dense brushes that are 'grafted from' and can be designed to provide repelling of unspecific sorption from complex samples such as blood serum, plasma, and whole blood.

Polymer Brushes – Key Parameters

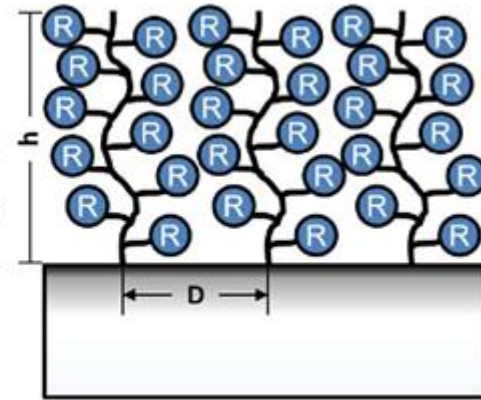


Gyration radius
 R_g



$h \sim R_g$

Mushroom
conformation



$h \gg R_g$

Brush
conformation

[10.1021/acsami.6b09068](https://doi.org/10.1021/acsami.6b09068)

$$R_g = A_0 N^\nu$$

R_g – average distance between center and circle of a molecule

N – number of units

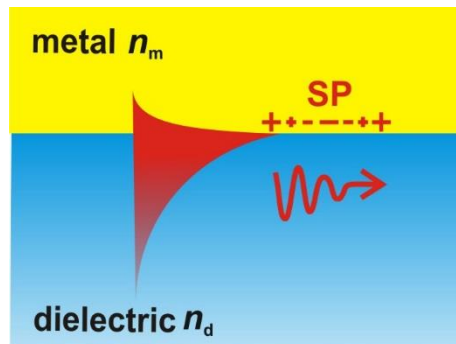
A_0 – distance between units (e.g. ~ 3 Å for dsDNA)

ν – takes into account rigidity of the chain (fluffy - 0.5, stiff - 1).

Propagating Surface Plasmons (PSP)

Surface plasmons (SPs) or also called surface plasmon polaritons (SPPs) are waves originating from coupled oscillations of electron plasma density and associated electromagnetic field on a metal – dielectric interface.

They travel along single interface which serves a waveguide.



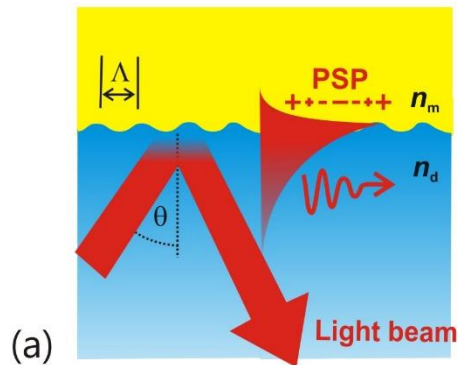
Propagation constant β can be analytically expressed as:

$$\beta = \frac{2\pi}{\lambda} \sqrt{\frac{n_m^2 n_d^2}{n_m^2 + n_d^2}}$$

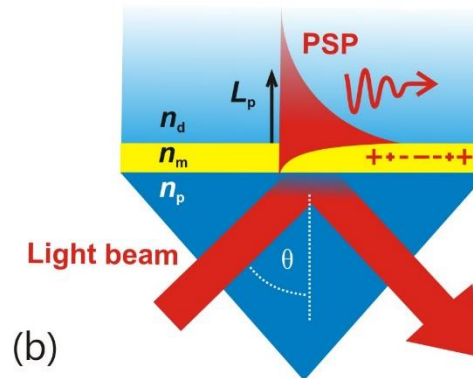
- ➡ SPs allows for tight confinement of electromagnetic field at the interface.
- ➡ For visible near infrared wavelength typically gold and silver is used where the $\text{Re}\{n_m^2\} < 0$.
- ➡ Majority of the field is probing the dielectric n_d .

Coupling to PSPs

Grating coupling



Prism coupling



Phase-matching condition:

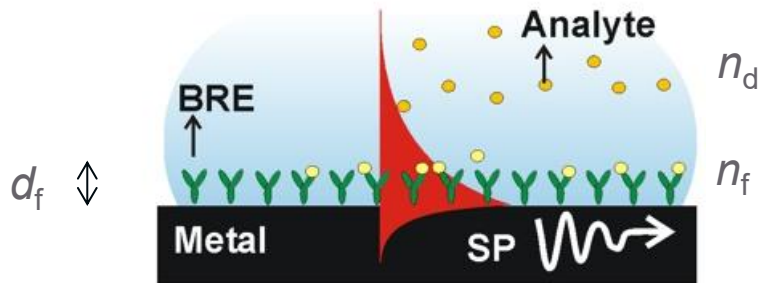
$$\beta = \frac{2\pi}{\lambda} \sqrt{\frac{n_m^2 n_d^2}{n_m^2 + n_d^2}}$$

$$\text{a) } \text{Re}\{\beta\} = \frac{2\pi}{\lambda} n_d \sin(\theta) \pm p \frac{2\pi}{\Lambda}$$

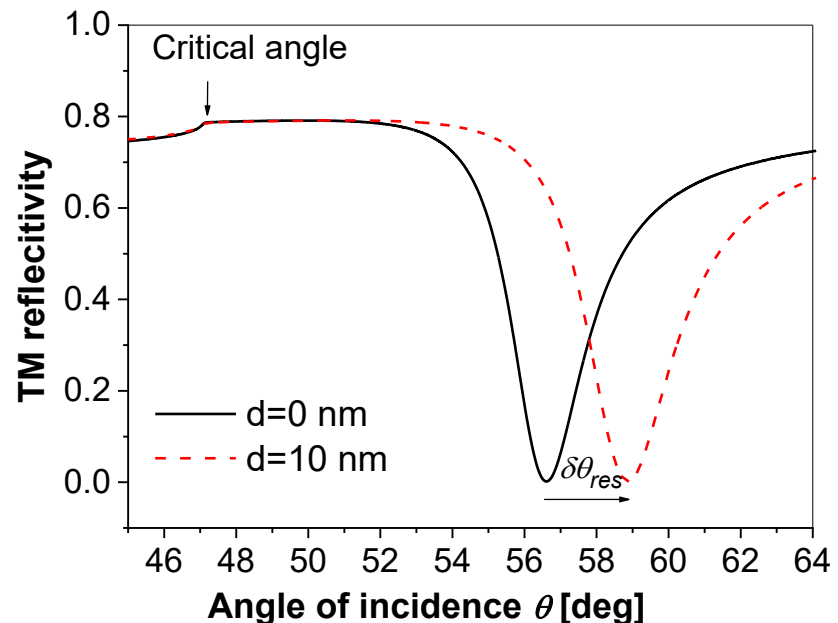
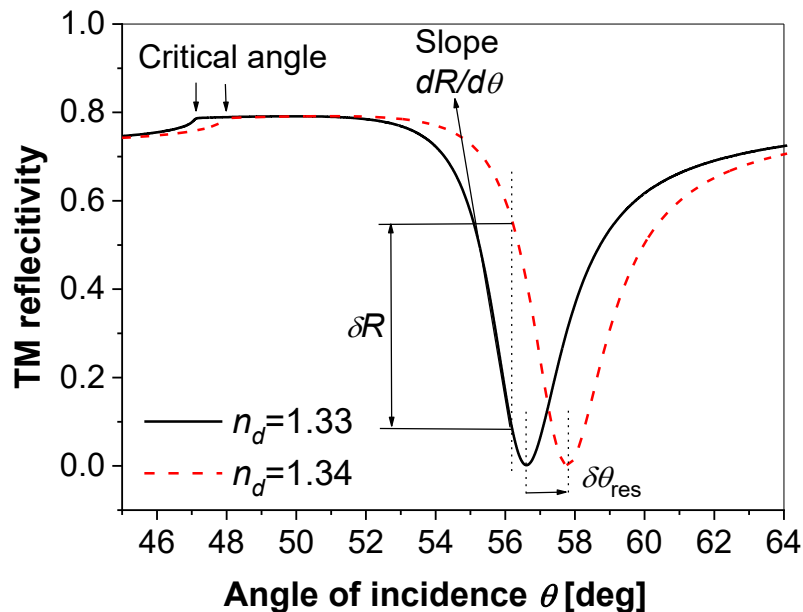
$$\text{b) } \text{Re}\{\beta\} = \frac{2\pi}{\lambda} n_p \sin(\theta)$$

- ➡ Resonant effect, coupling occurs only for certain combinations of λ and θ , where the phase-matching is fulfilled.

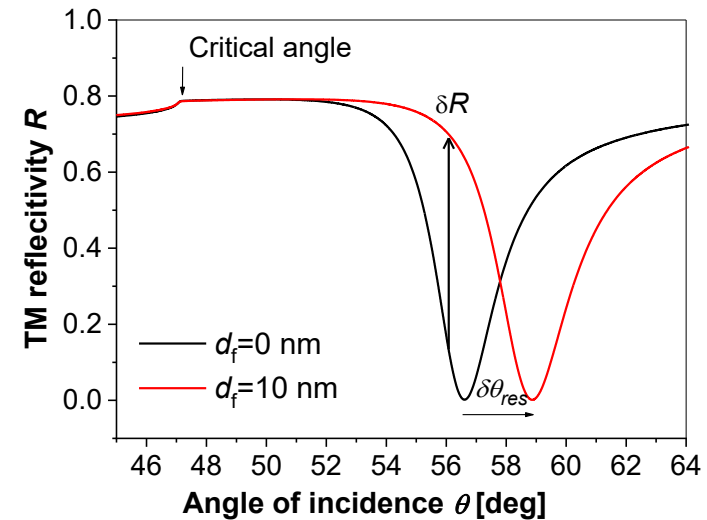
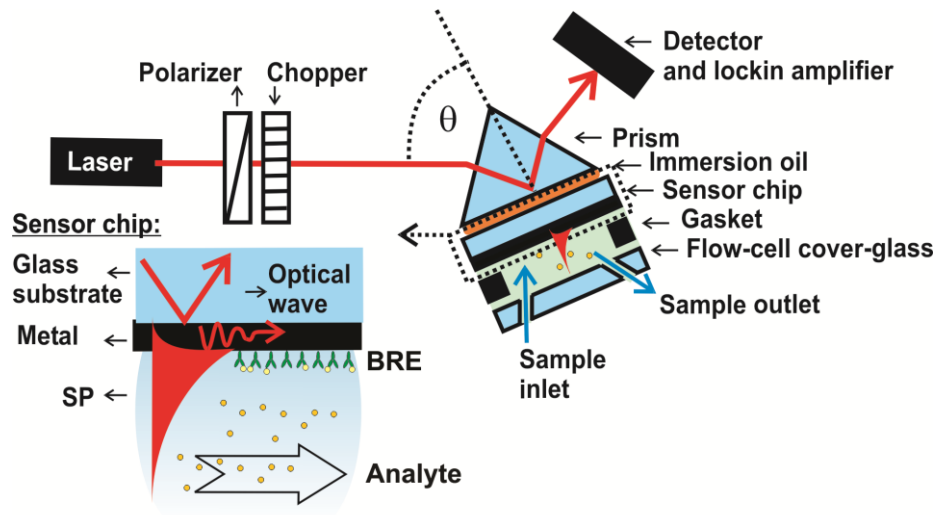
Refractive Index Sensitivity – Angular Interrogation



➔ SPR changes with both changes in the bulk n_d and surface refractive index changes.

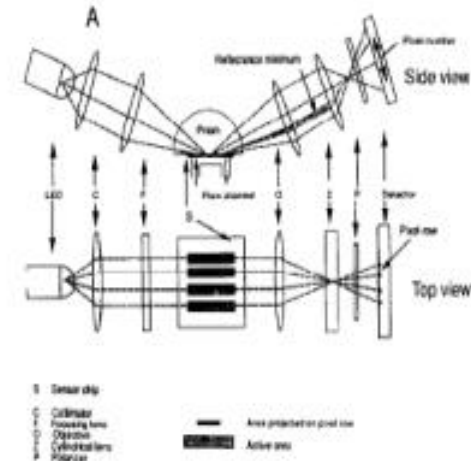
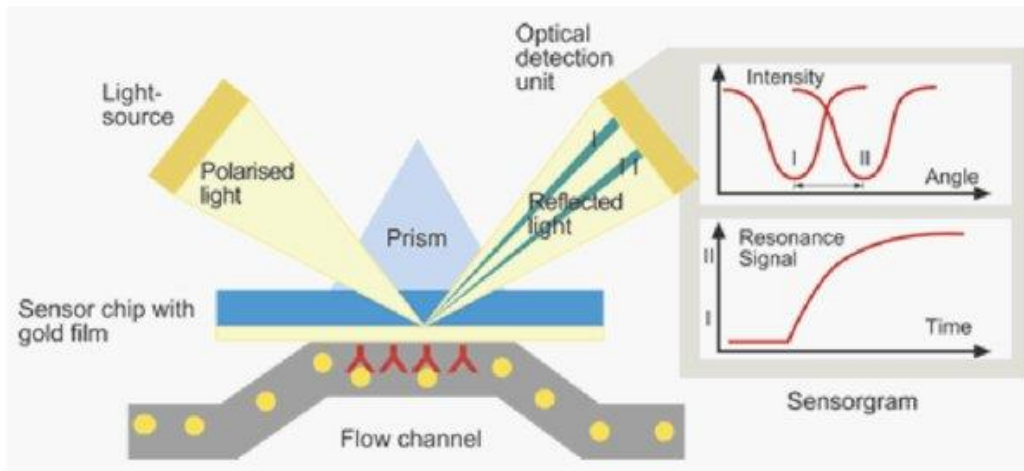


Intensity Modulation



- ➡ Angle of incidence θ and wavelength λ are fixed at the edge of the SPR dip, where slope $\delta R/\delta\theta$ is highest.
- ➡ Simple implementation that allows for fast measurements of SPR changes
- ➡ Drawback is the small dynamic range, where δR shifts linearly with the measured changes (represented as a thickness of biolayer d_f).
- ➡ Typically the accuracy of the measurement is low (resolution 10^{-5} RIU).

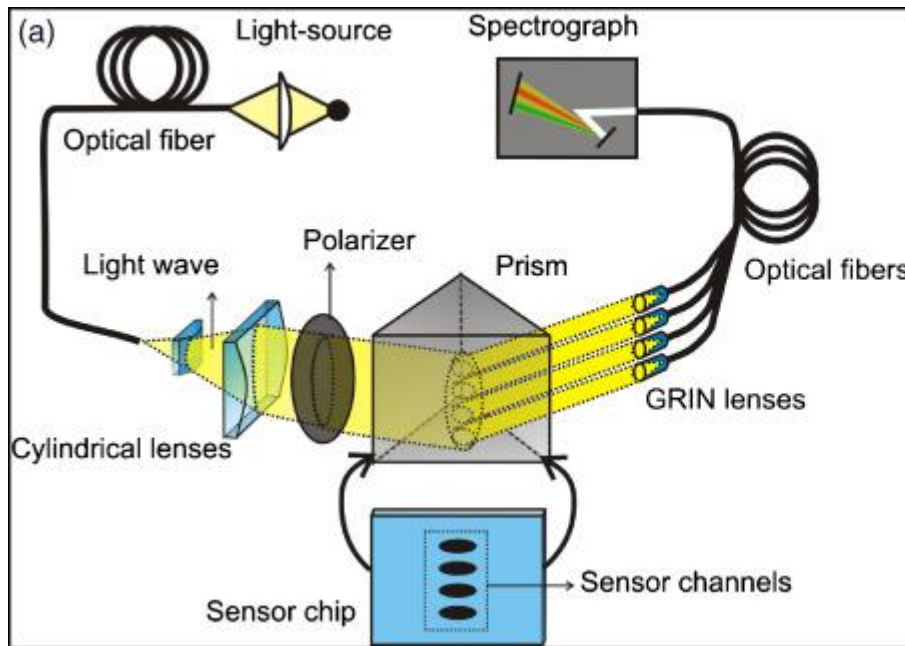
Angular Modulation of SPR



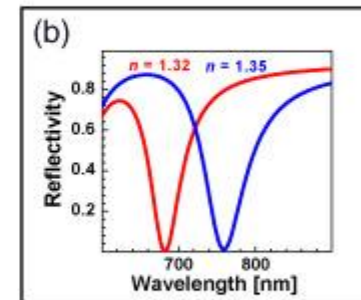
Liedberg, B., C. Nylander and I. Lundstrom Biosensing with surface plasmon resonance - how it all started. *Biosens.Bioelectron.* 10: i-ix; (1995)

- ➔ Measuring the whole reflectivity spectrum $R(\theta)$ by using a converging monochromatic optical beam (fixed λ) that is projected to a CCD detector (no movable parts). Introduced in early 1990ties by a Swedish company Farmacia Bioacore.
- ➔ Typically not versatile and build for specific refractive index range.
- ➔ Fast measurement of whole reflectivity spectrum allows for tracking of refractive index changes with high accuracy (resolution 10^{-7} RIU).

Wavelength Modulation of SPR



<https://doi.org/10.1016/j.snb.2008.09.006>



J. Homola, J. Dostalek, S. Chen, A. Rasooly, S. Jiang, S. S. Yee: Spectral Surface Plasmon Resonance Biosensor for Detection of Staphylococcal Enterotoxin B (SEB) in Milk, *Journal of Microbiology*, 75, (2002) 61-69.

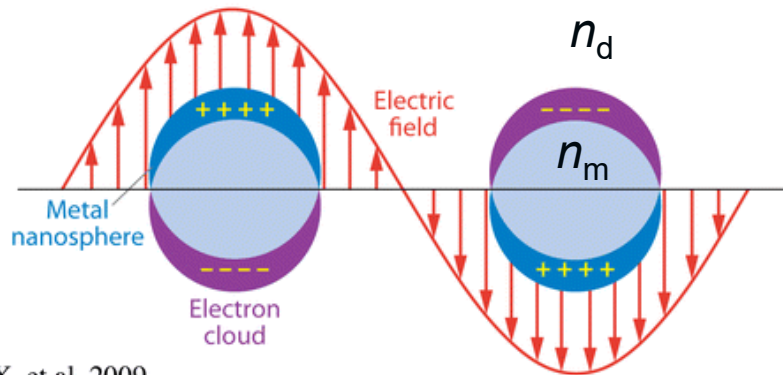
- ➔ Measuring wavelength reflectivity spectrum $R(\lambda)$ for a fixed angle of incidence θ . The spectrum is analysed by a spectrometer that changes in resonant wavelength are tracked in time.
- ➔ No movable parts and high accuracy (10^{-7} RIU).
- ➔ The sensitivity is nonlinearly changing with wavelength λ .

Localized Surface Plasmons (LSPs)

Localized surface plasmons (LSPs) are associated with electron plasma density oscillations on metallic nanoparticles. Provides unique optical / plasmonic characteristics.

Resonant effect, e.g. for spherical metallic nanoparticle with $d \ll \lambda$ the resonance wavelength λ_{LSPR} obeys:

$$\text{Re}\{n_m^2(\lambda)\} + 2n_d(\lambda) = 0$$

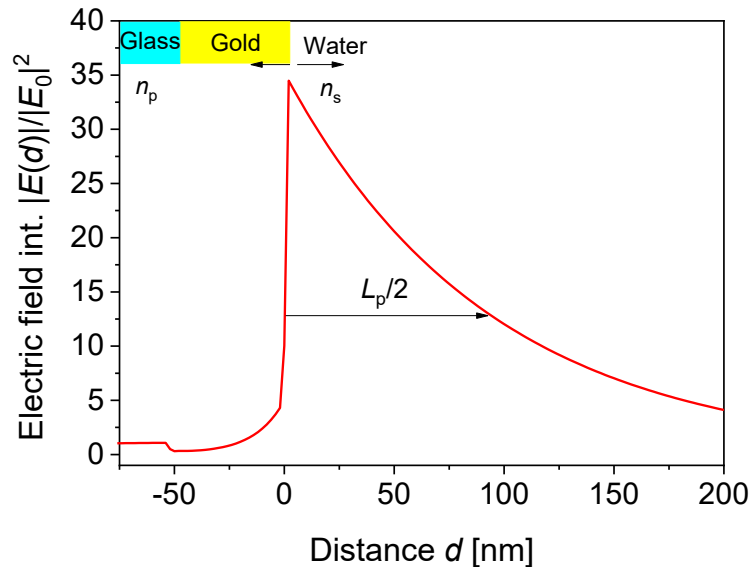
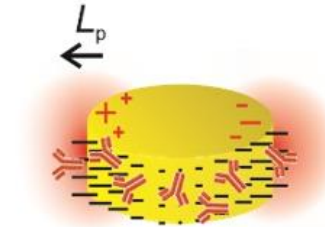
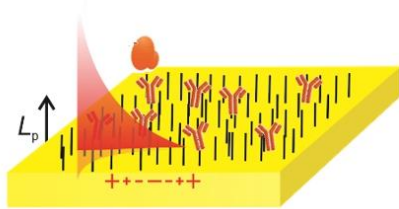


Localized surface plasmon resonance is associated with strong:

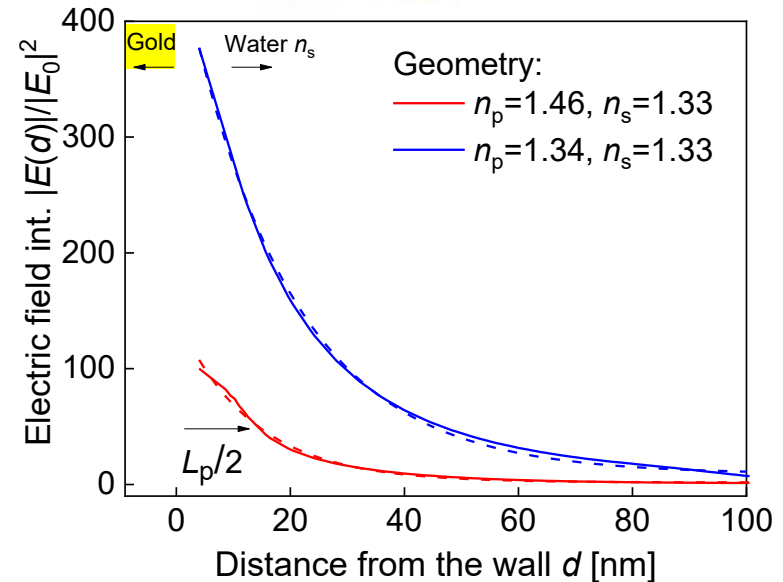
- ➔ Absorption
- ➔ Scattering
- ➔ Field confinement and enhancement

Lu X, et al. 2009.
Annu. Rev. Phys. Chem. 60:167–92

Probing Depth – LSP and PSP

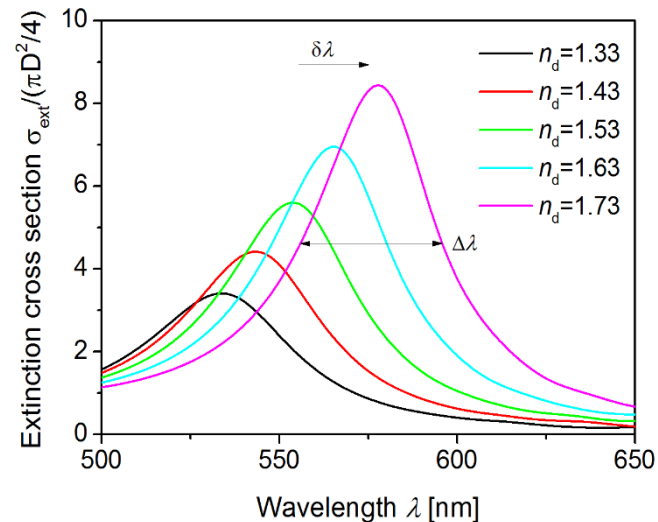
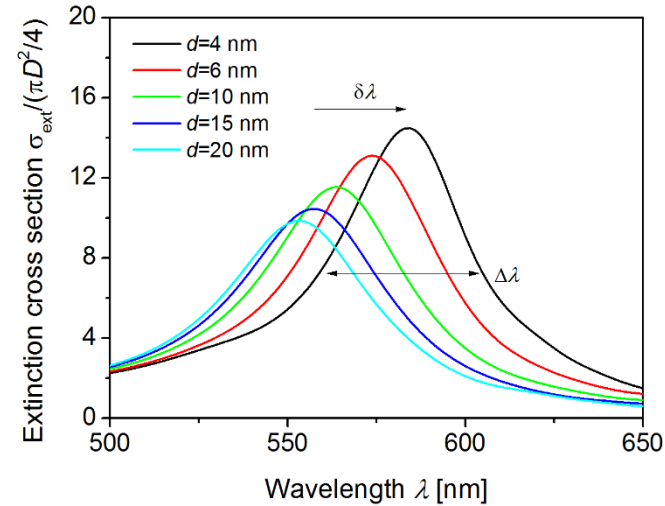
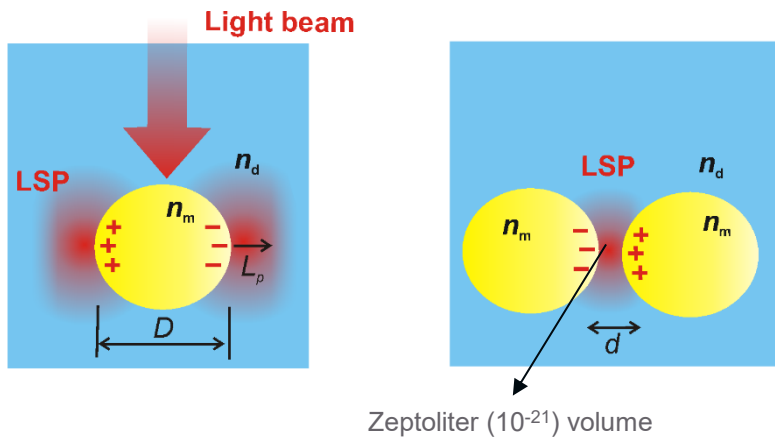


$L_p/2=93$ nm



$L_p/2=13-18$ nm

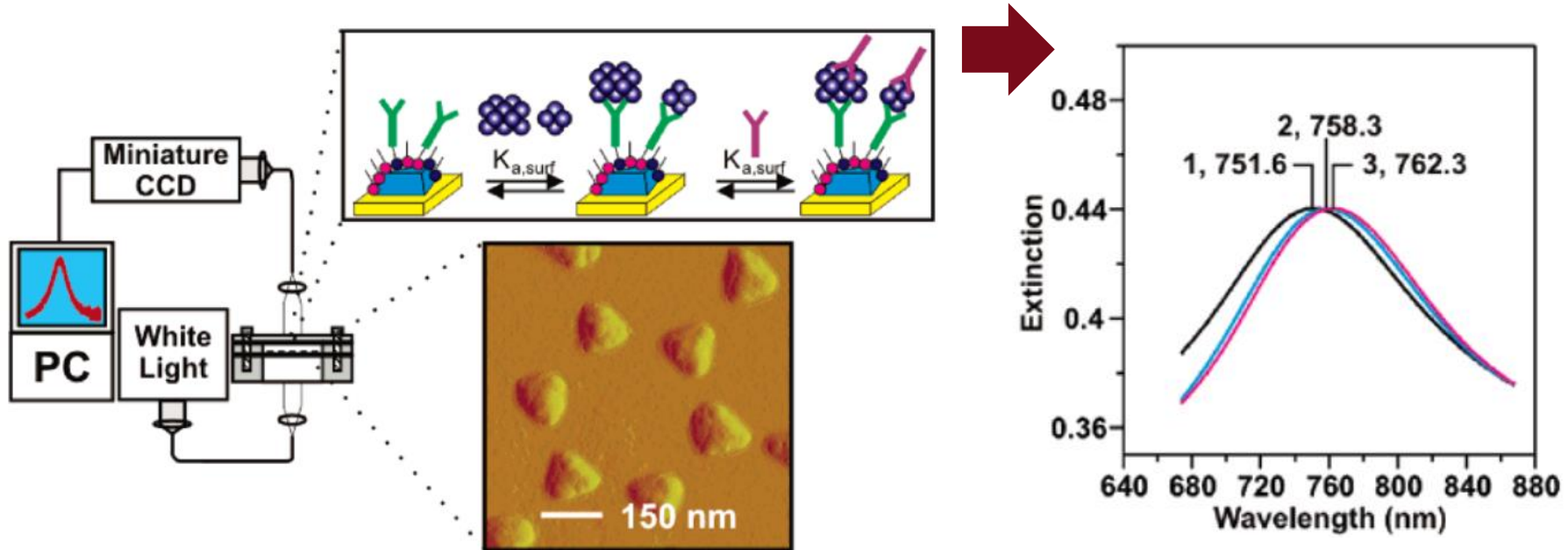
Localized Surface Plasmon Resonance (LSPs)



- ➔ Resonant effect, e.g. for spherical metallic nanoparticle with $D \ll \lambda$ the resonance wavelength λ_{LSPR} .
- ➔ Confined electromagnetic field intensity, $L_p \sim D$.

Implementations of LSPR Biosensor

LSPR transmission

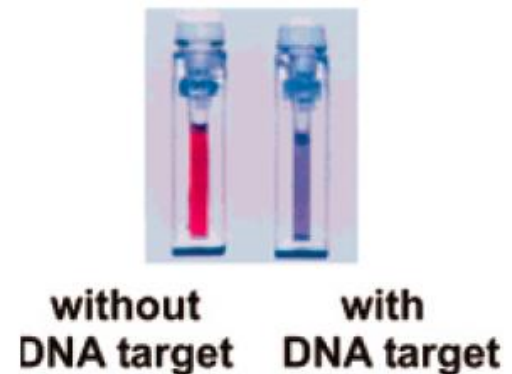
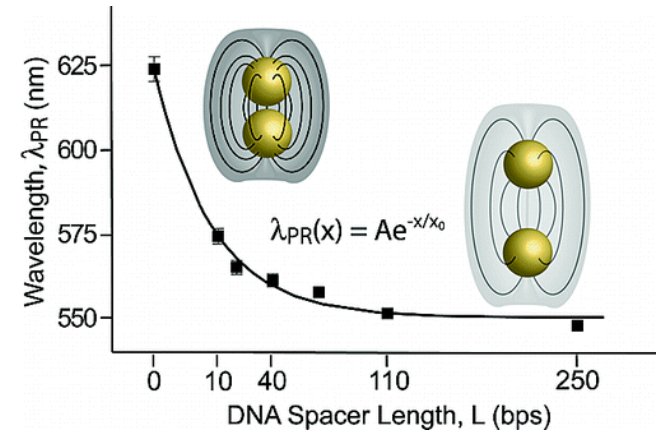
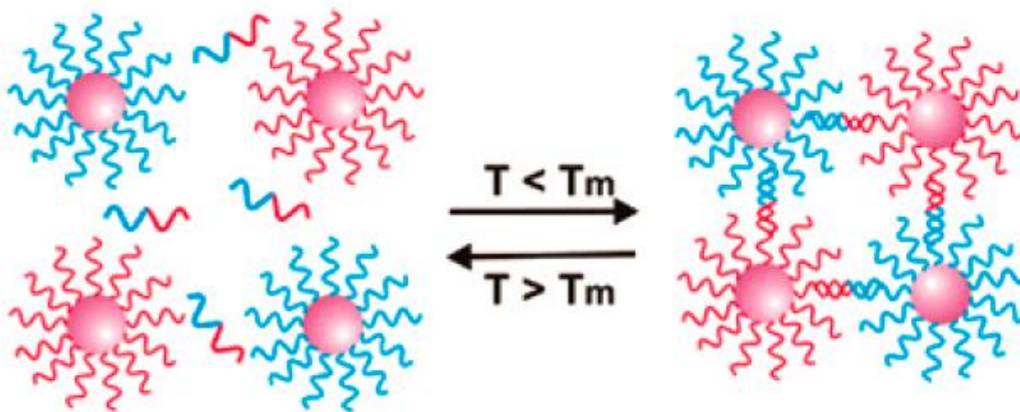


Example of first implementation of LSPR for analysis of biomarkers.

Tracking of LSPR
wavelength λ_{LSPR}

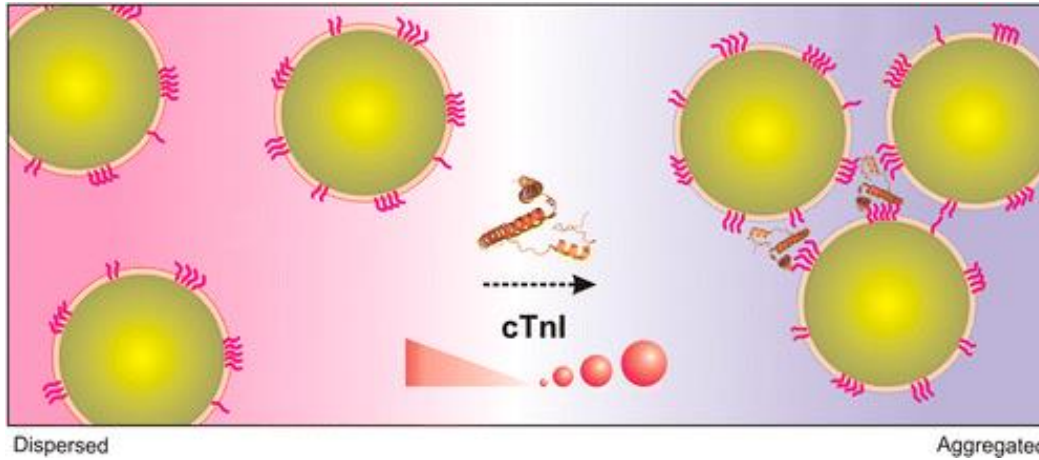
LSPR Homogenous Assays

Concept of plasmonic ruler:

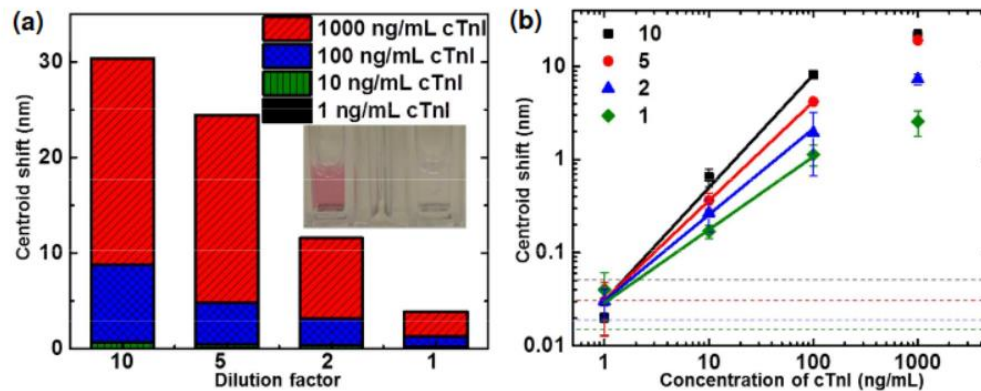


- ➡ In the presence of complementary target DNA, oligonucleotide-functionalized Au NPs will aggregate (left), resulting in a change in the color of the solution from red to blue (right).

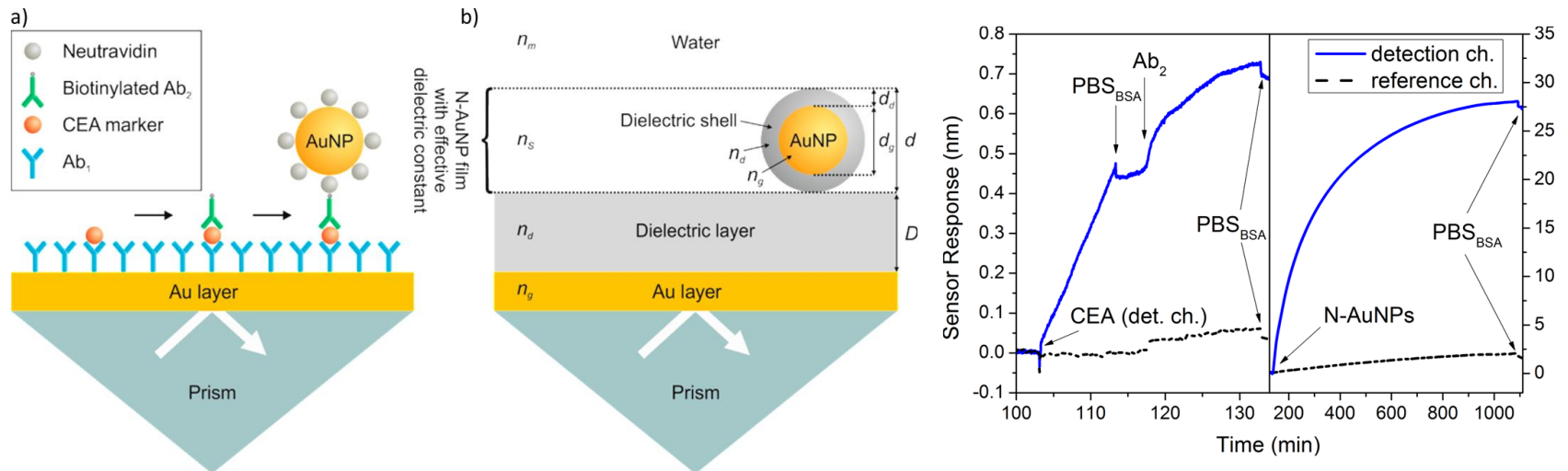
LSPR Homogenous Assays



- ➔ Aggregation assays were developed for protein analytes such as cardiac marker troponin.
- ➔ Using of low molecular weight ligands such as peptides allows achieving stronger effect due to shorted distances between NPs.



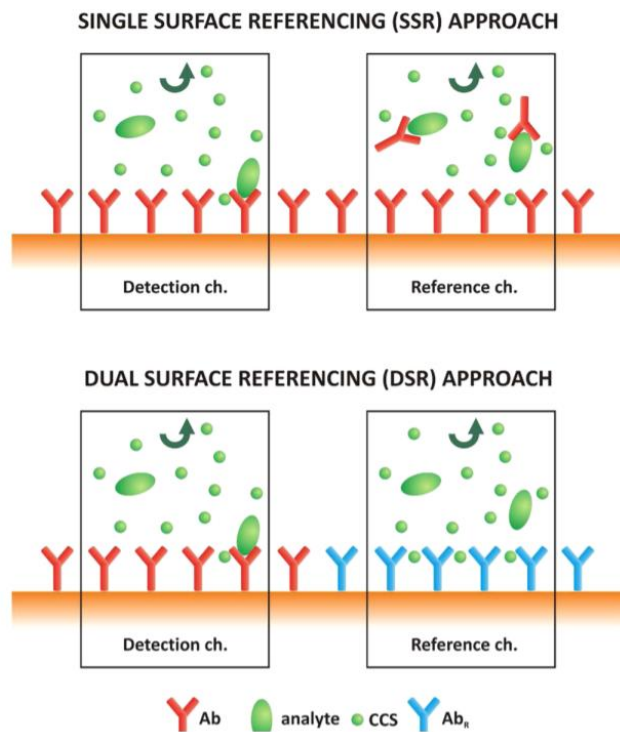
Sandwich Assay Enhanced with Metallic Nanoparticles



[dx.doi.org/10.1021/ac502637u](https://doi.org/10.1021/ac502637u)

- ➡ Sandwich assay with the use of Au NP labels allows to enhance the surface mass density change associated to molecular binding event.
- ➡ Respectively, limit of detection can be enhanced. The size of metallic NP matters as smaller ones generates weaker enhancement in equilibrium, but diffuse slower.

Reference Channel – Compensation for False Unspecific Signal



➔ Compensating for unspecific sorption and bulk refractive index change

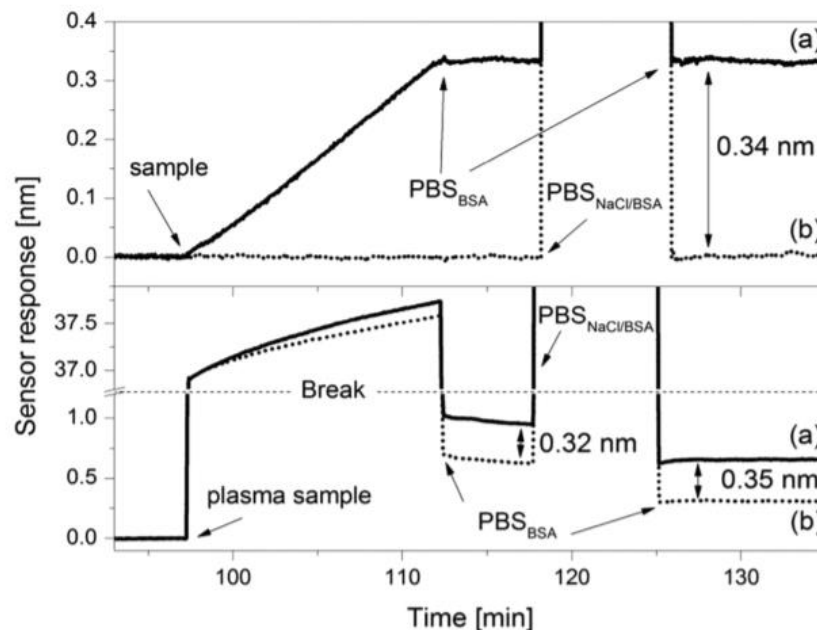
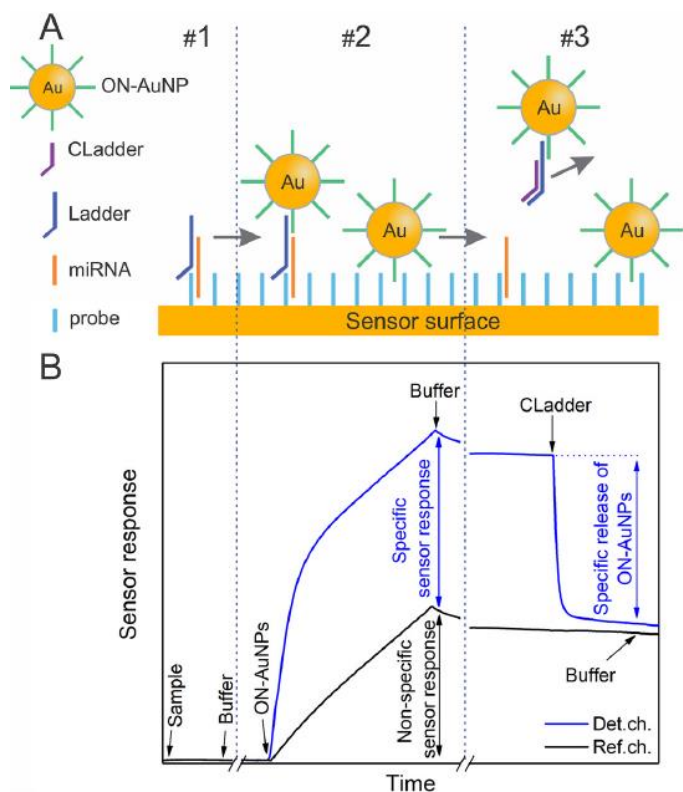


Figure 2. Reference-compensated detection of CEA in PBS_{BSA} (upper graph) and 50% human blood plasma P_1 (lower graph) for the SSR approach. Curve (a) denotes the response to the concentration of CEA of 500 ng/mL (detection channel). Curve (b) presents the sensor response to the mixture of 500 ng/mL CEA with an addition of 5000 ng/mL Ab_{CEA} (reference channel).

Displacement Referencing



➔ Amplified specific response retrieved based on displacement.

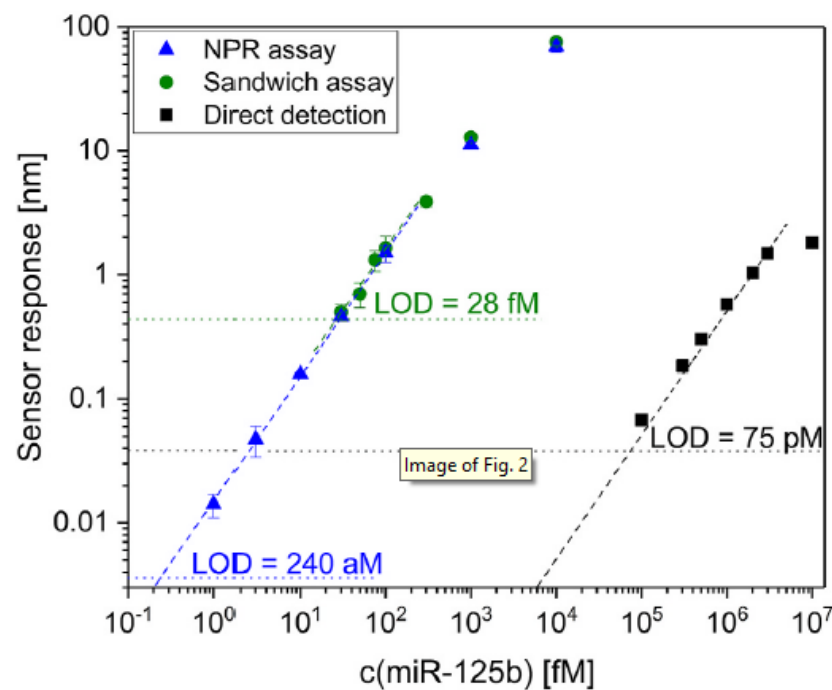
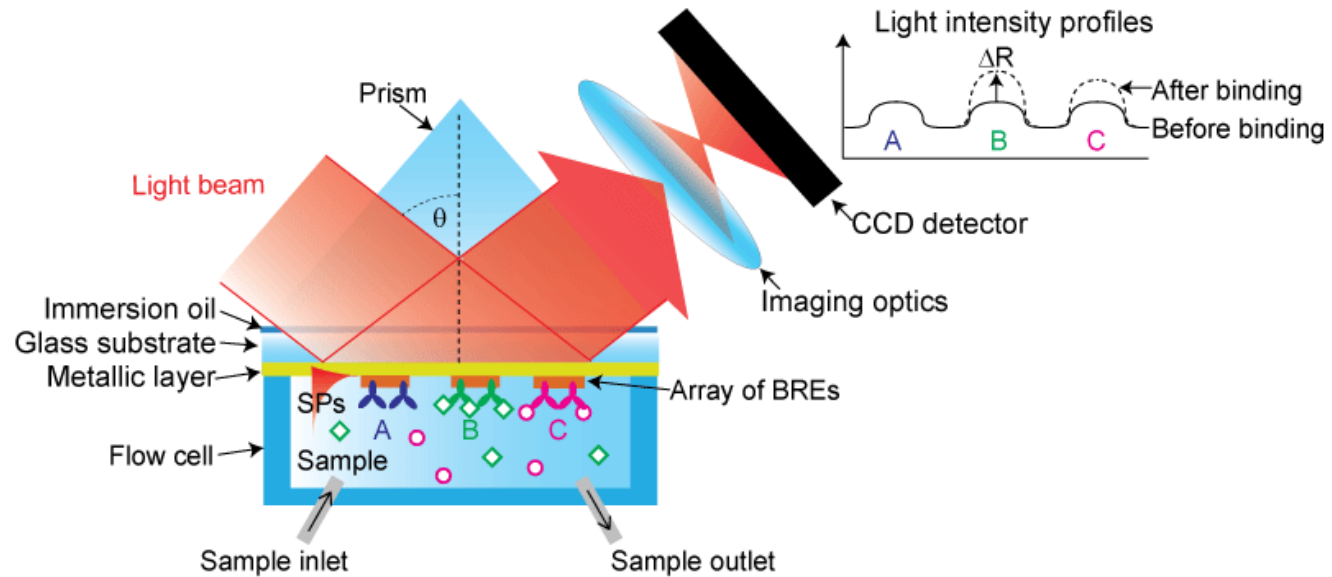


Fig. 3. Calibration curves for the detection of miR-125b in buffer. Dashed lines represent the linear fits of the calibration curves for concentrations of miR-125b close to the determined LOD. The horizontal dotted lines correspond to the variability of the sensor response, var_{SR} . Each concentration of miR-125b was measured at least in triplicate.

Surface Plasmon Imaging - SPRI



- Surface plasmon imaging (SPRI) is technique used for probing of arrays of spots, imaging of cells....
- Spatial resolution typically of several μm , utilizing intensity modulation of SPR with refractive index resolution of typically 10^{-5} .

Invention in 1988

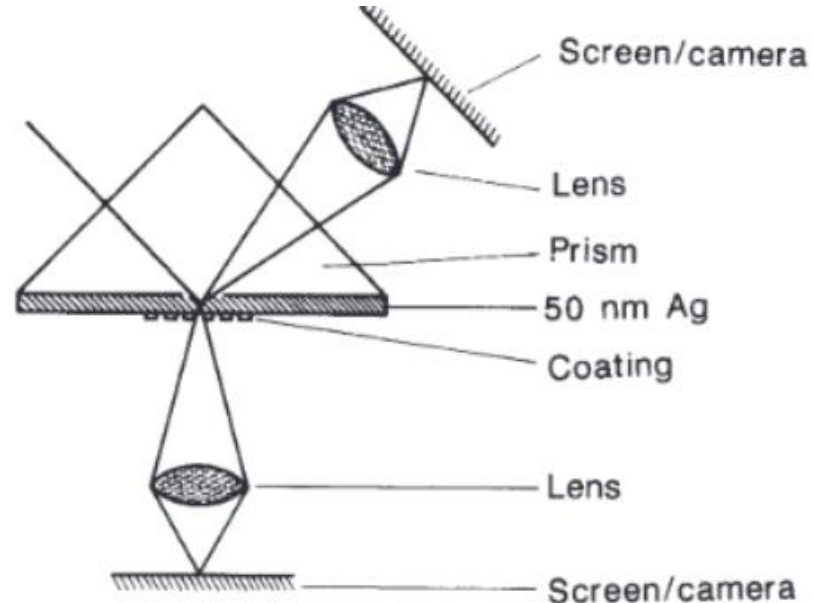
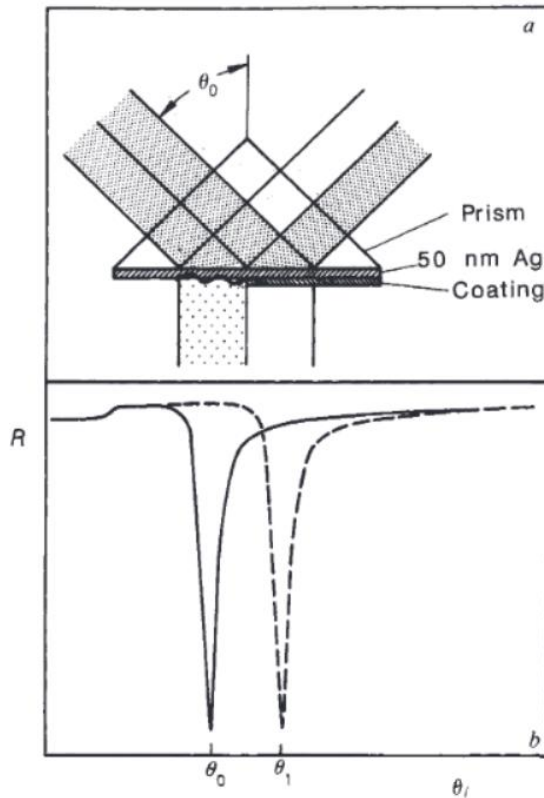
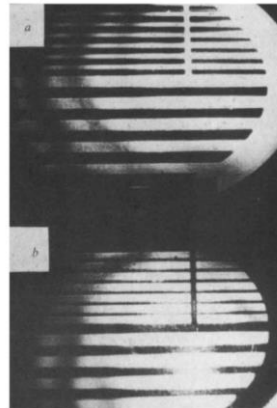
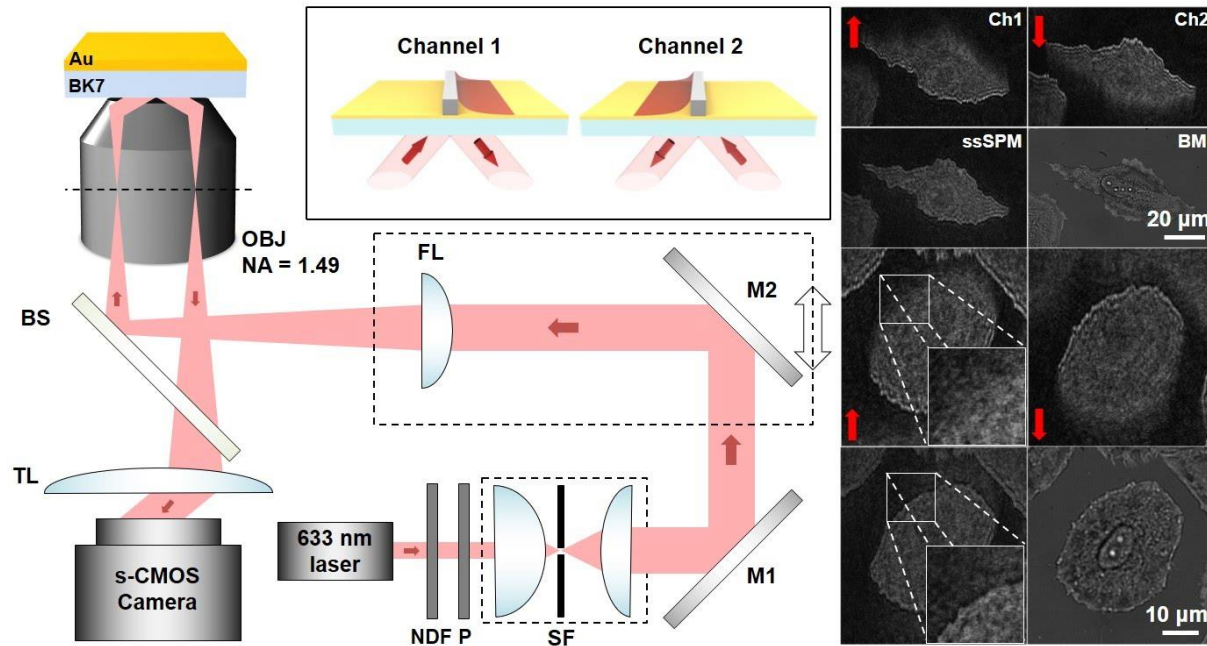


Fig. 1 Schematic of the optical set-up of SPM. Not to scale.



- ➔ Rothenhaesler and Knoll invented the method coined the term Surface Plasmon Microscopy – SPM.

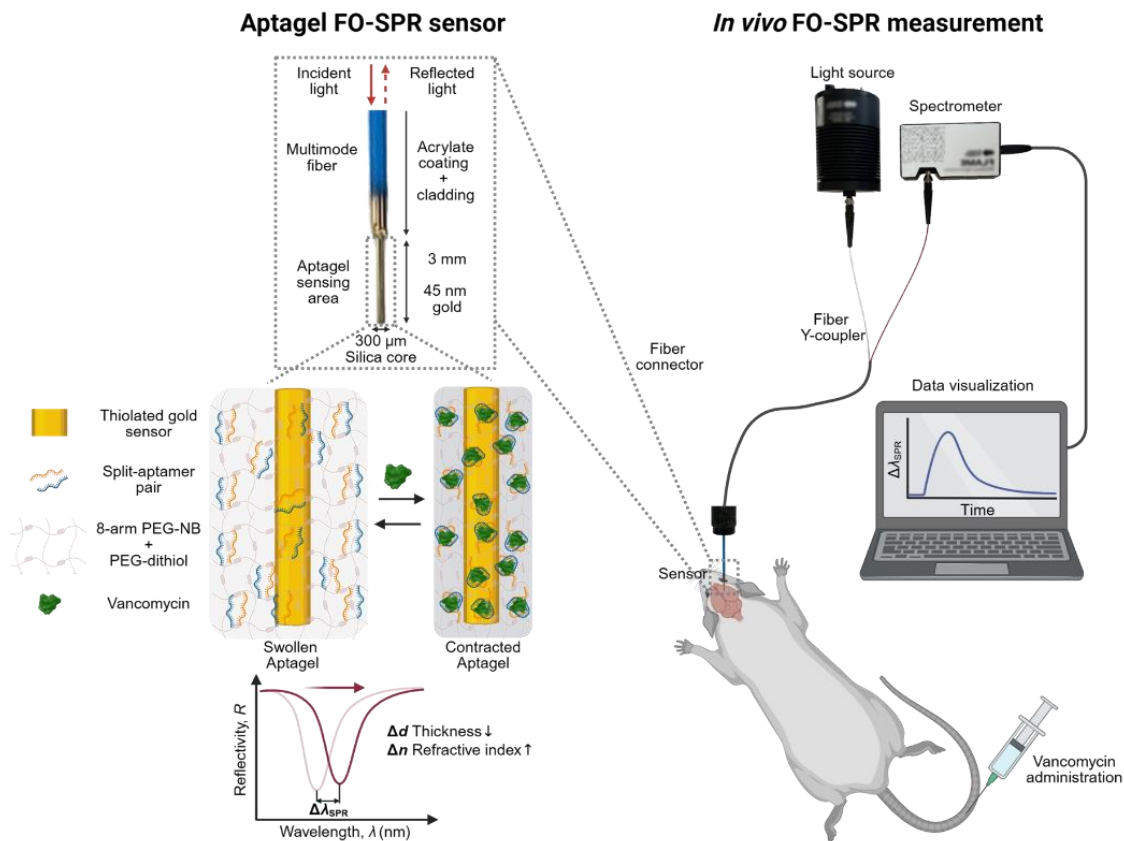
Surface Plasmon Microscopy - SPM



<https://sites.google.com/site/taehwangson89/>

- ➔ Implemented with using a microscope with scanned objective lens to form the image.
- ➔ Spatial resolution typically of better than SPRI.

Fiber optic SPR



➔ Implementations for *in vivo* pharmacokinetics measurements.

BIACORE

Parameter

Biacore T200

Affinity range

fM to mM

Analysis temperature

4°C to 45°C (maximum 20°C below ambient temperature)

Analysis time per cycle

Typically 2 to 15 min

Application¹

Kinetics/affinity characterization, kinetics/affinity screening, single-cycle kinetic analysis, fragment screening, epitope mapping¹, immunogenicity, concentration free concentration analysis, thermodynamics, comparability, sample recovery MS

Association rate constant (k_a)

Proteins 10^3 to 3×10^9 $M^{-1}s^{-1}$
LMW molecules 10^3 to 5×10^7 $M^{-1}s^{-1}$

Automation

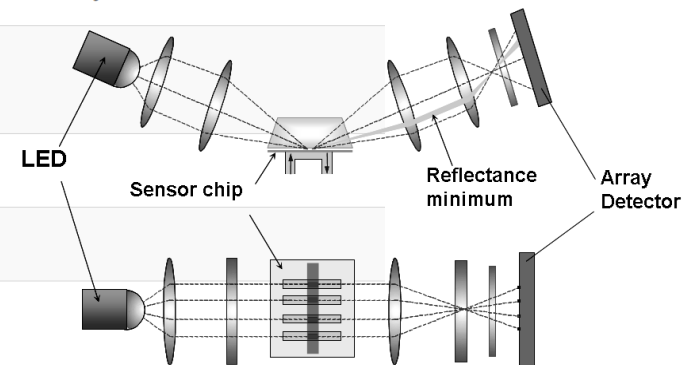
48 h unattended operation

Baseline drift

< 0.3 RU/min

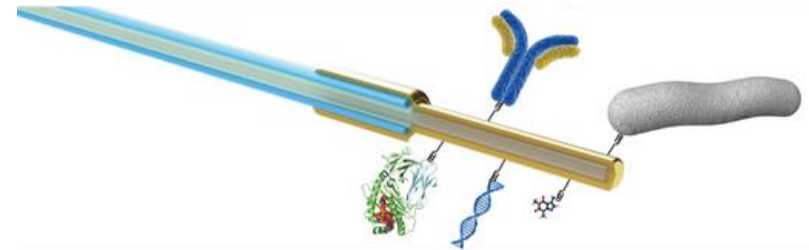
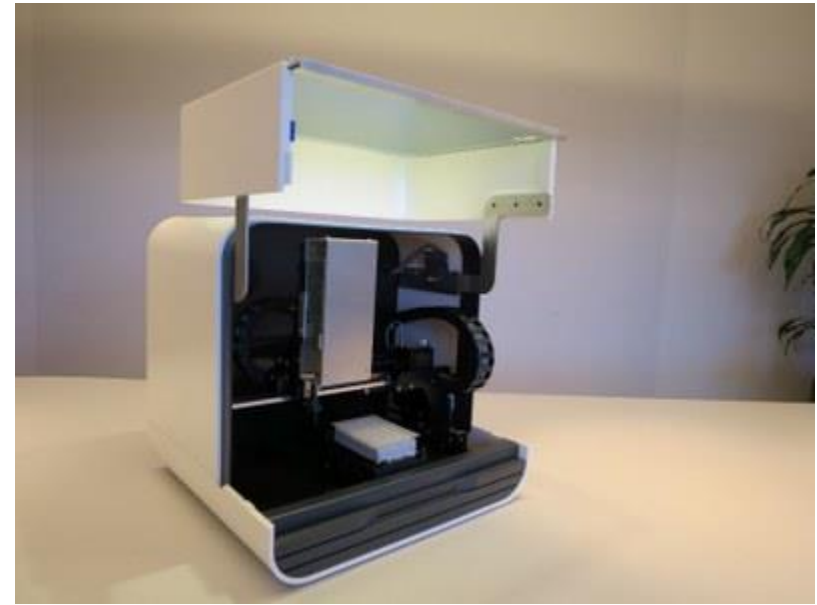
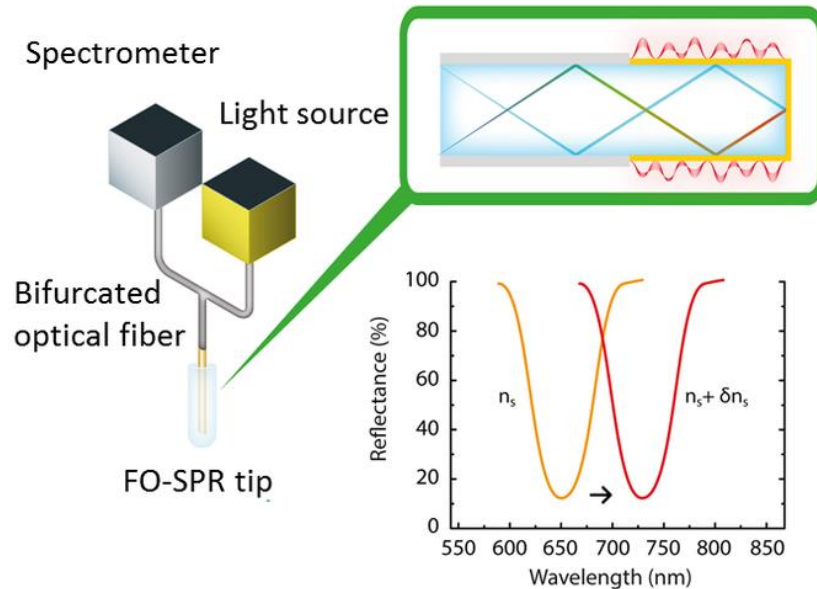
Baseline noise

< 0.03 RU (RMS)



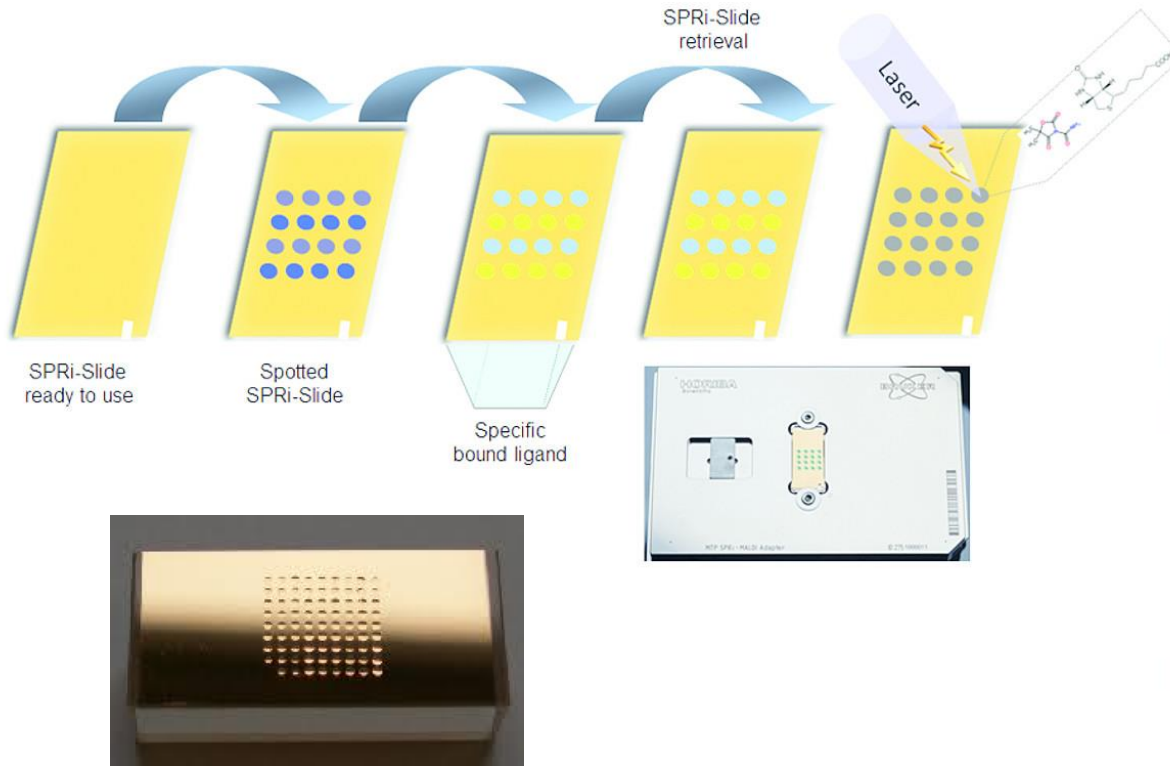
➔ Originally Pharmacia, GE Healthcare, now Cytiva. Covering the interaction analysis market, drug development.

FOX DIAGNOSTICS



- ➔ Coupled with microtiter plates for sample delivery replacing the fluidic system (similar to Octet technology)

HORIBA



- ➔ Implemented SPR imaging (SPRI) for array detection format. Prims coupler-based with recyclable prims-shape chips.

BIONAVIS



- ➡ Conceptually similar to 'Mainz' design of SPR with versatile applications in life sciences and materials research.
- ➡ Up to four flow chambers, angular interrogation of SPR at multiple wavelength for air and aqueous samples.