Advanced Technologies in Biological Research:

Optical Spectroscopy and Biosensors

Jakub Dostalek

AIT - Austrian Institute of Technology GmbH
Biosensor Technologies Unit
Konrad-Lorenz-Strasse 24 | 3430 Tulln | Austria
T +43(0) 664 2351773

jakub.dostalek@ait.ac.at | http://www.ait.ac.at | http://www.jakubdostalek.cz

Outline

A. Introduction to light propagation and confinement: ray, wave, and electromagnetic optics. Total internal reflection, guided wave optics, surface plasmon resonance.

B. Optical micro- and nano-structures. Top down and bottom up fabrications, electron beam lithography, colloidal lithography, nano-imprint lithography.

C. Optical spectroscopy: absorption, fluorescence, FRET, Raman and IR spectroscopy.

D. Super-resolution microscopy: Diffraction limit and approaches to break it, STED, PALMS, STORM.

E. Optical tweezers: manipulation with cells and force measurements.

F. Biosensors. Transducers, key performance characteristics, application areas including medical diagnostics, and drug development.

G. Surface plasmon resonance (SPR) biosensors.

H. Reaction kinetics and evaluation of affinity binding constants for molecular interaction analysis (BIA).

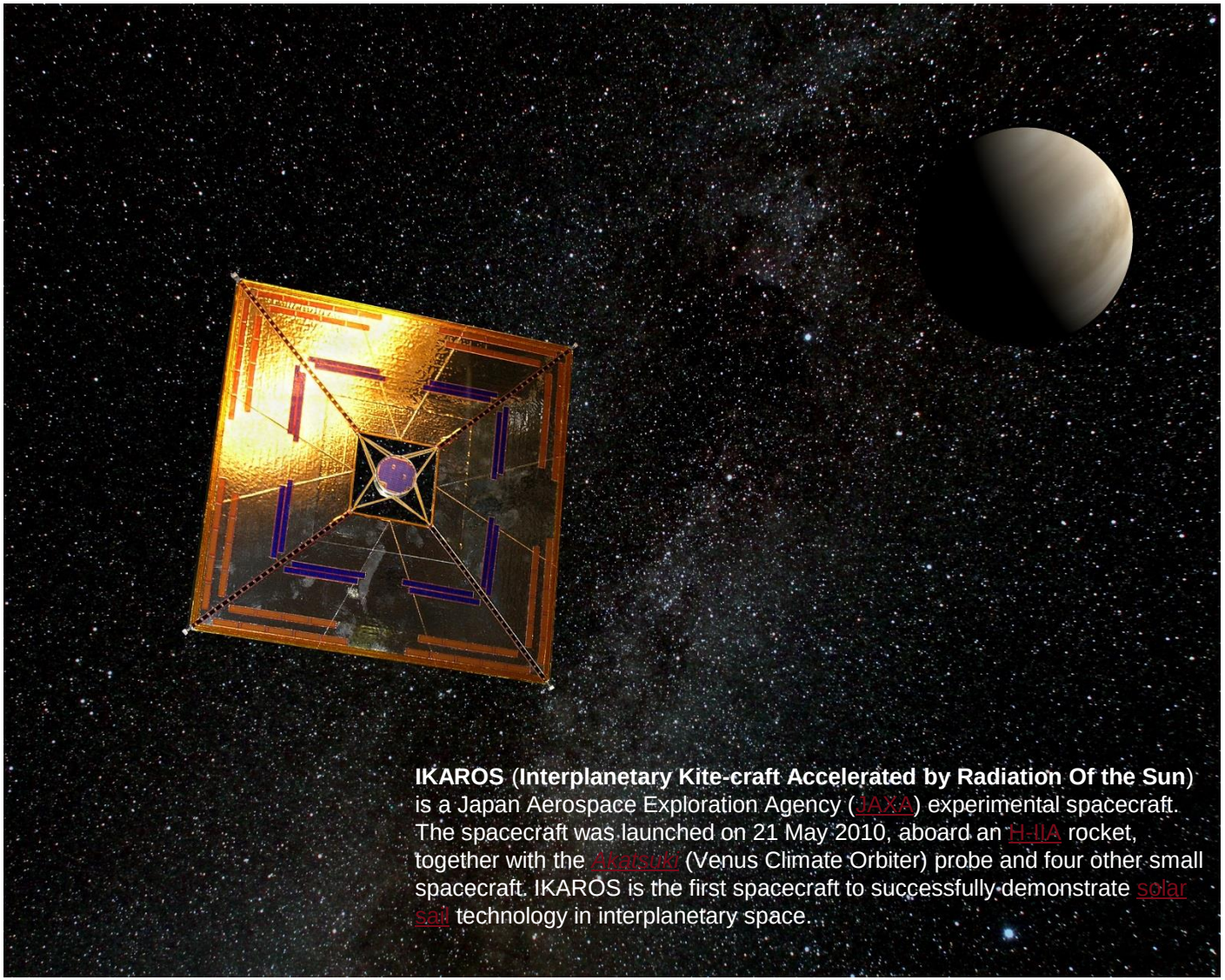
I. Single molecule interaction analysis by using fluorescence, FRET and LSPR monitoring.

J. Optical spectroscopy-based biosensors. Colorimetric, fluorescence, Raman.

E. “Optical Tweezers”

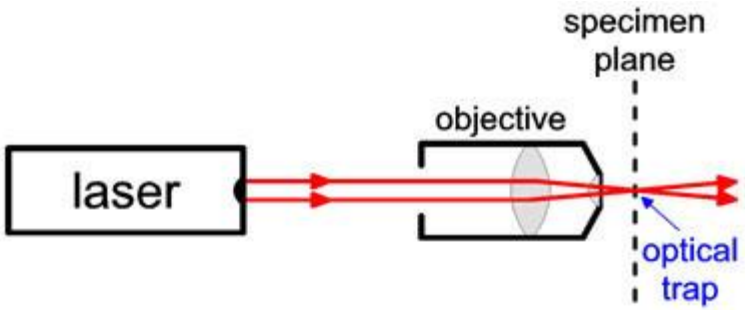
The Nobel Prize in Physics 2018 was awarded to Arthur Ashkin, Gérard Mourou and Donna Strickland. Their inventions have revolutionised laser physics. Extremely small objects and incredibly rapid processes are now being seen in a new light. Advanced precision instruments are opening up unexplored areas of research and a multitude of industrial and medical applications.

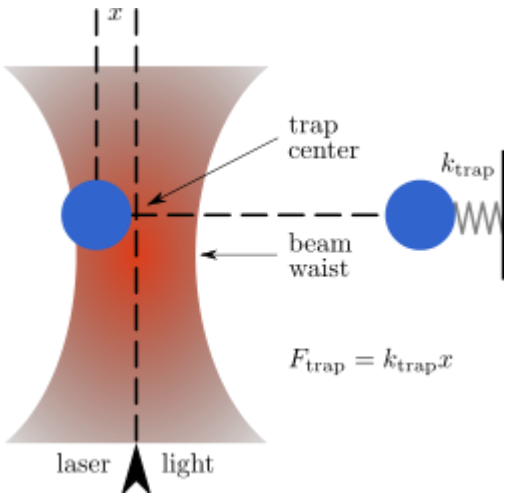
Solar Sail



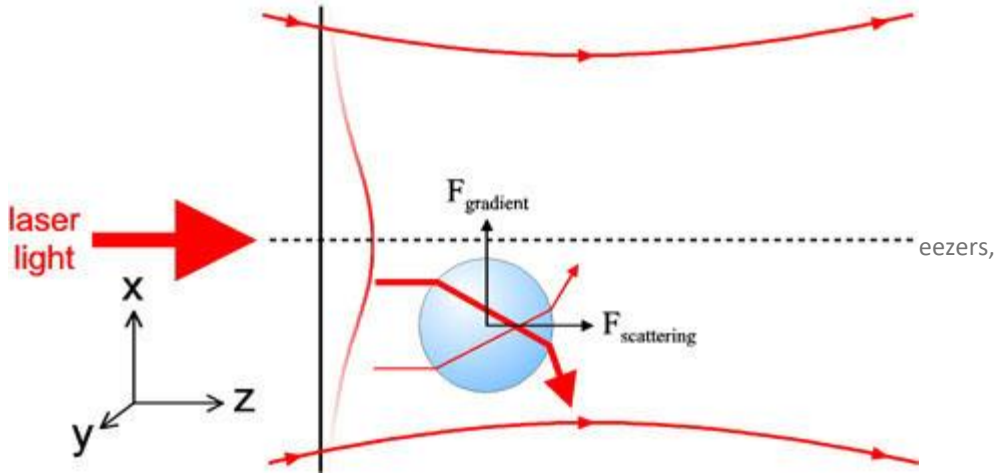
IKAROS (**Interplanetary Kite-craft Accelerated by Radiation Of the Sun**) is a Japan Aerospace Exploration Agency (JAXA) experimental spacecraft. The spacecraft was launched on 21 May 2010, aboard an H-IIA rocket, together with the *Akatsuki* (Venus Climate Orbiter) probe and four other small spacecraft. IKAROS is the first spacecraft to successfully demonstrate solar sail technology in interplanetary space.

Confinement of Light Creates Optical Trap





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

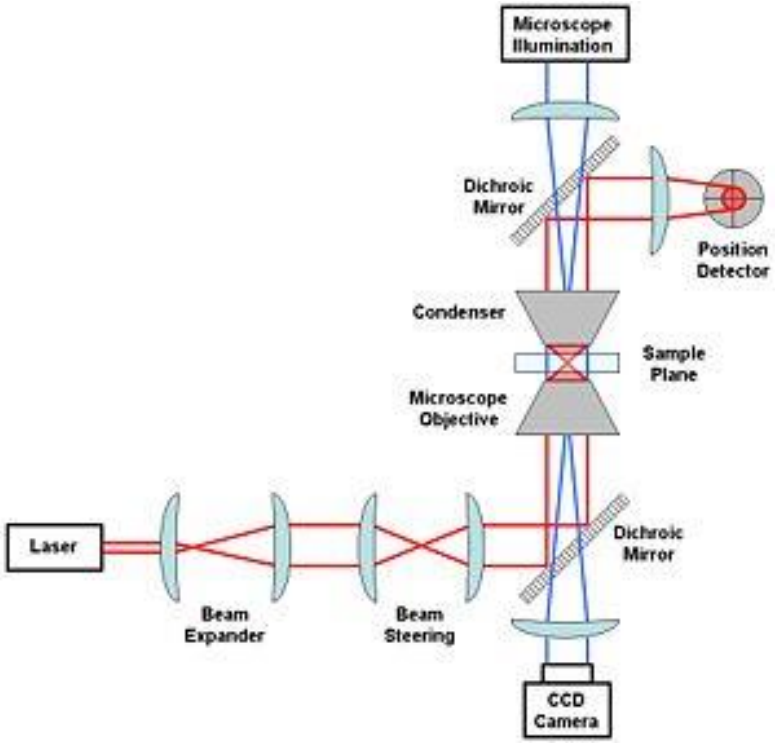


eezers,

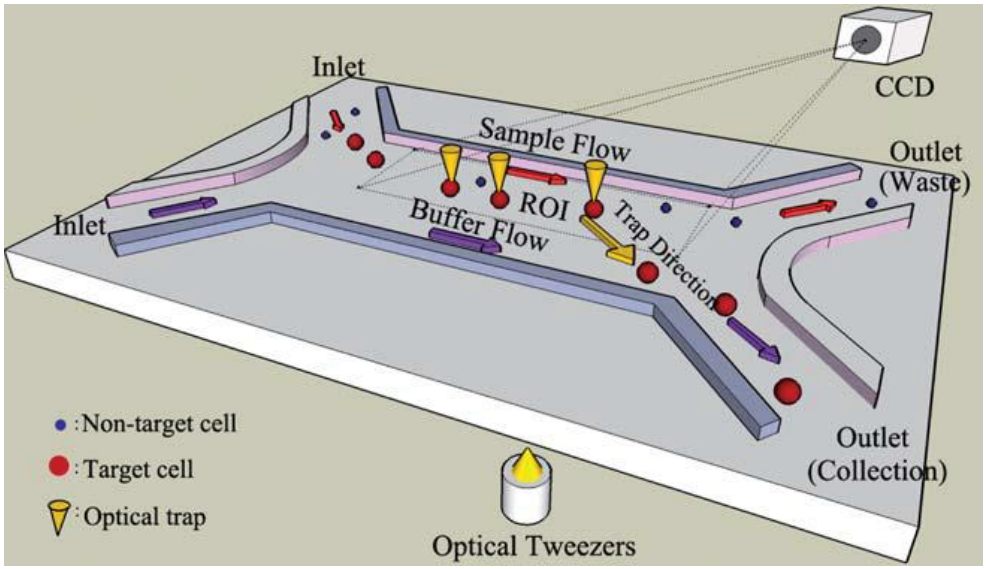
https://blocklab.stanford.edu/optical_tweezers.html

Ashkin, A. (1970). "Acceleration and Trapping of Particles by Radiation Pressure". *Phys. Rev. Lett*. **24** (4): 156–159.

Cell Sorting

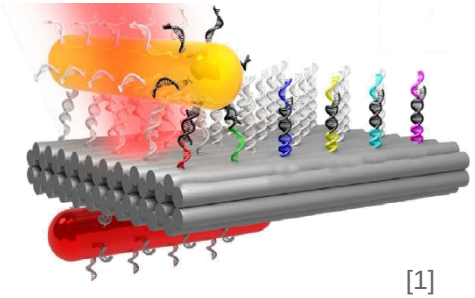


https://en.wikipedia.org/wiki/Optical_tweezers



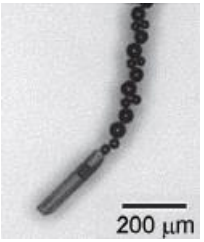
Lab Chip, 2011, 11, 3656–3662

Micromachines



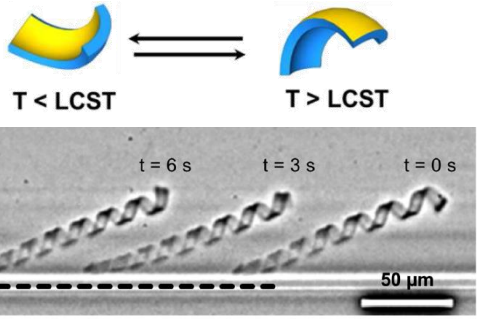
[1]

Affinity displacement reactions



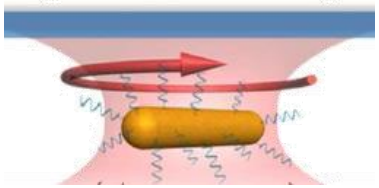
[2]

Catalytic reaction



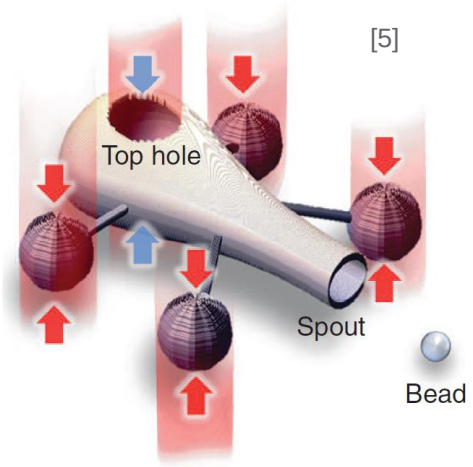
[3]

Non-reciprocal morphing



[4]

Optical torque

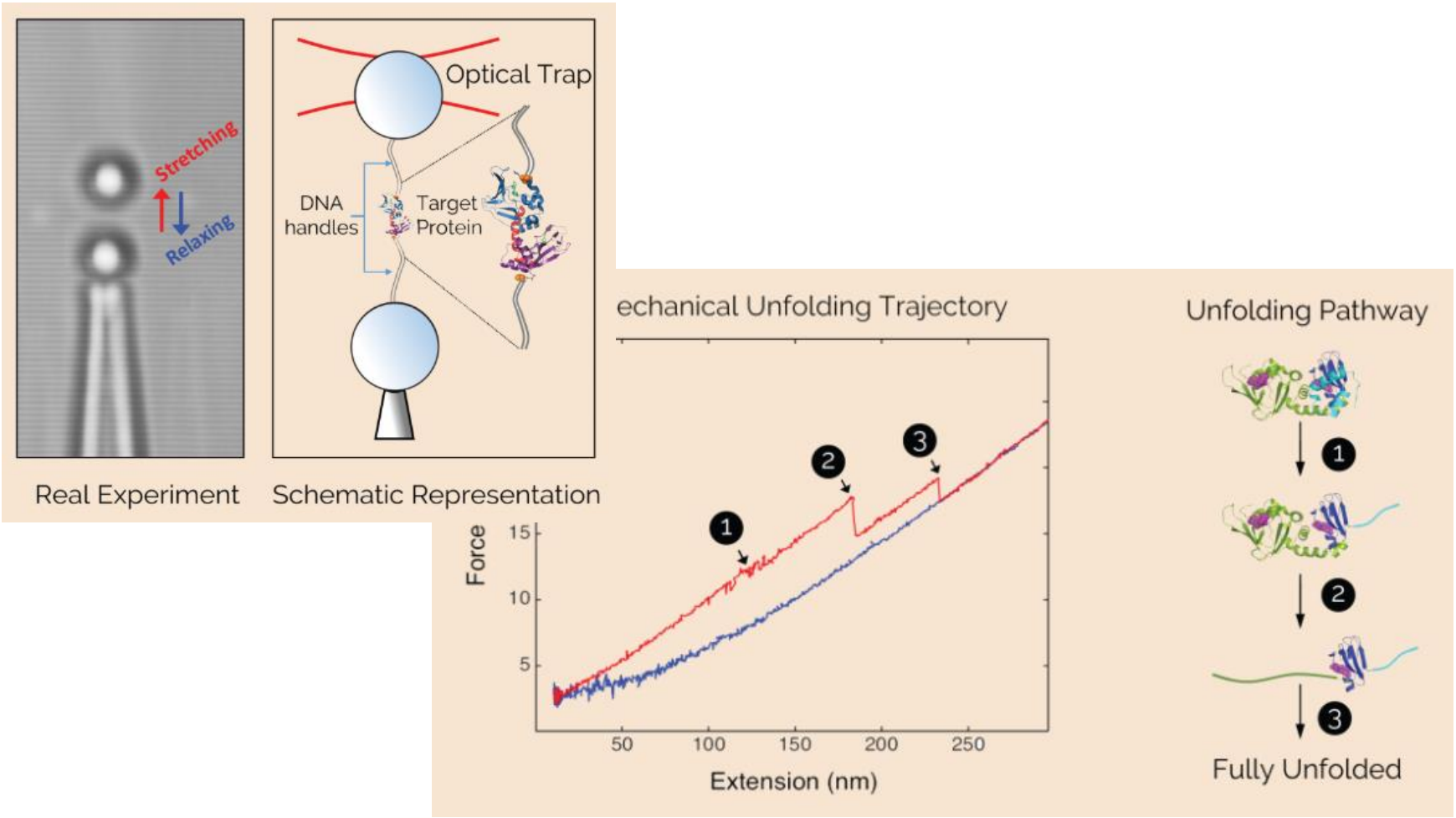


[5]

Optical tweezers and optical heating

[1] C. Zhou et al., Nature Commun. (2015), **6**, 8102. [2] Magdanz et al, Angew. Chem. 2014, 126, 2711 –2715. [3] A. Mourran et al., Adv. Mater. (2017) 29, 1604825 [4] Sipova et al, ACS Photonics, (2018), *5* (6), pp 2168–2175 [5] M. Jayson et al, Light: Sci & App (2016) 5, e16148

Force Measurements



http://maillardlab.org/optical-tweezers/

E - Summary

- Confined light can be employed for construction of traps for small dielectric / metallic objects.
- The technique of “optical tweezer” can be employed for *in situ* research in molecular interactions (force measurements) and e.g. cell sorting.
- Limiting factor is the need of using high power lasers and possible disturbance of cells through increased temperatures.

F. BIOSENSORS

Motivation

Common practice: Analysis of collected samples (e.g. blood, urine) in central laboratories which is time consuming, require trained personnel and is costly (ELISA, mass spectrometry, HPLC…)

Biosensors: Aimed at rapid and simplified analysis, portable device enables “on site” analysis, point-of-care applications, home diagnosis.

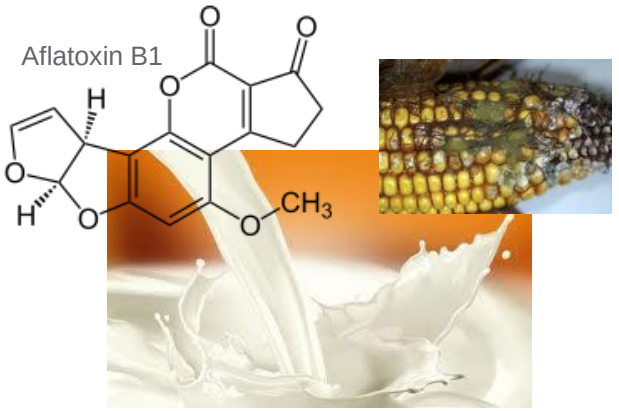
B 913 Apollo 11 ELISA Absorbance Reader

i-Stat: Portable Blood Gas Analyzer

Pregnancy test

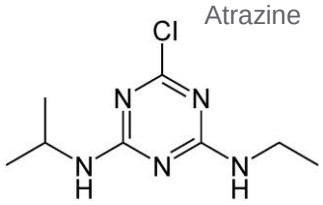
Aimed Application Areas

Food control (toxins, bacterial pathogens…)



Medical Diagnostics (biomarkers for cancer, cardiac, inflammation…)

Environmental Monitoring (pollutants in water and soil…)

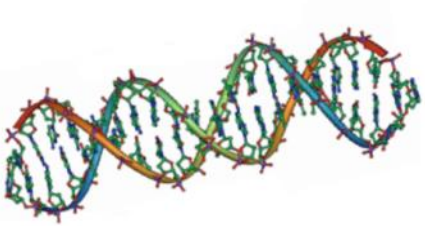


Homeland Security, **Forensics**….

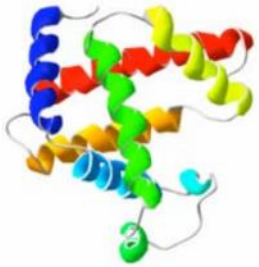
Biosensor

… is self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC 1996).

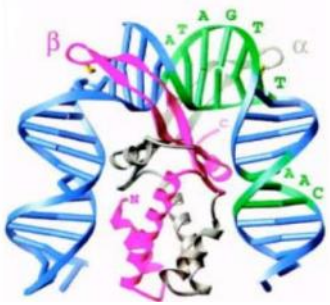
DNA/DNA



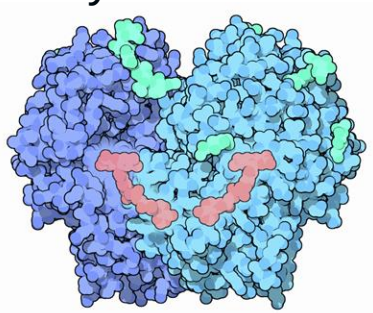
Protein/Protein

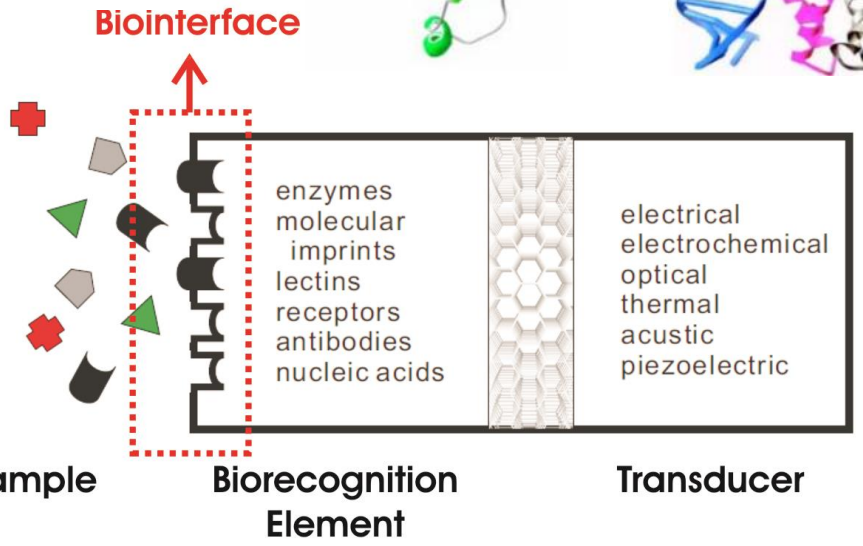


Protein/DNA



Enzyme





Physico-Chemical Transducers

Transducer converts molecular binding events to measurable (physical) signal. Those can be based on various physical quantities:

Mass (quartz crystal microbalance…)

Conductivity (amperometry, voltametry…)

Heat release or absorption (calorimetry)

Refractive index (surface plasmon resonance)

Absorption (colorimetric detection)

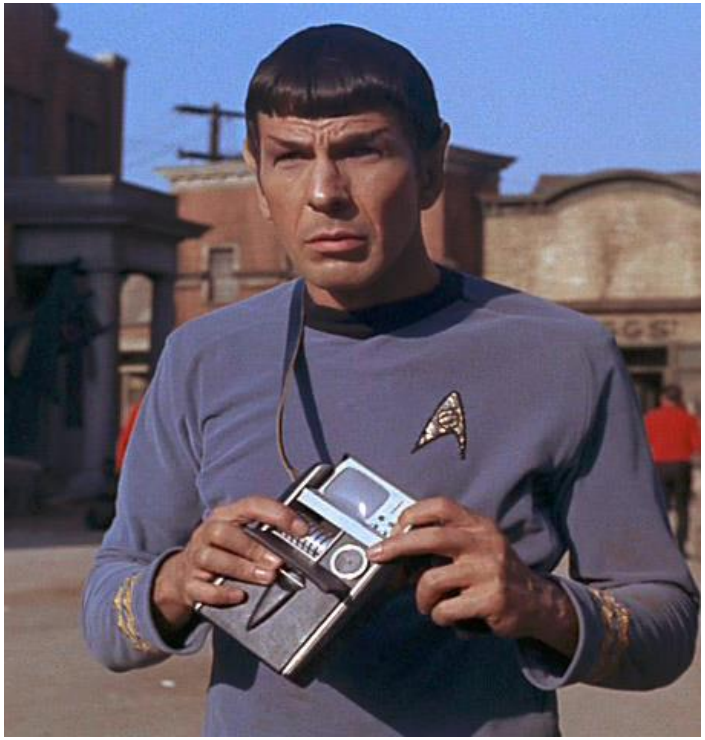
Non-linear optical interaction with matter (fluorescence, SERS)

…

Historical Examples

1962	Invention of a biosensor: an amperometric enzyme electrode for glucose (Clark).
1975	Commercial glucose biosensor (Yellow Springs Instruments)
1980	First fiber optic pH sensor for in vivo blood gases (Peterson)
1983	First surface plasmon resonance (SPR) immunosensor (Liedberg, Nylander, and Lundstrom)
1990	Commercial SPR based biosensor by Pharmacia BIACore
1992	Hand held blood biosensor by i-STAT
….	

Envisioned quite some time ago…

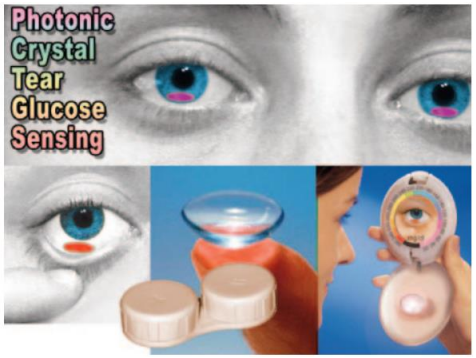


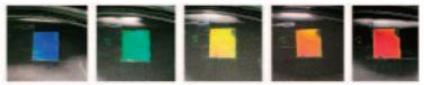
Vision of a device that can “analyze everything at once…”.

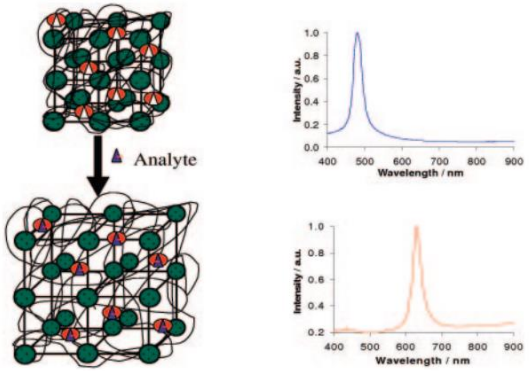
Tricorder were used for sensor scanning, data analysis, and recording data

http://www.rounds.com/blog/star-trek-predicted/

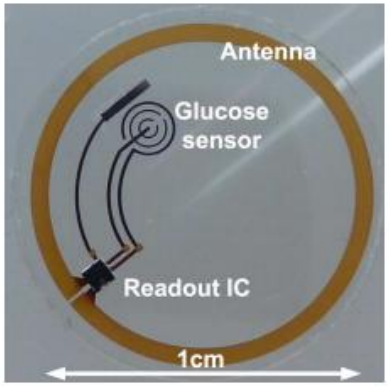
Contact Lens - Tear Fluid Analysis

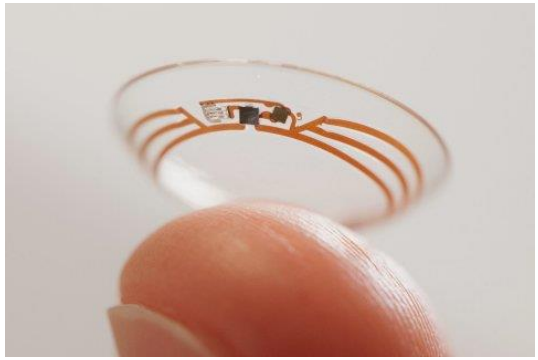






Photonic Crystal Glucose-Sensing Material for Noninvasive Monitoring of Glucose in Tear Fluid," V. Alexeev, S. Das, D.N. Finegold and S.A. Asher, Clinical Chemistry, 50, 2353 - 2360 (2004)

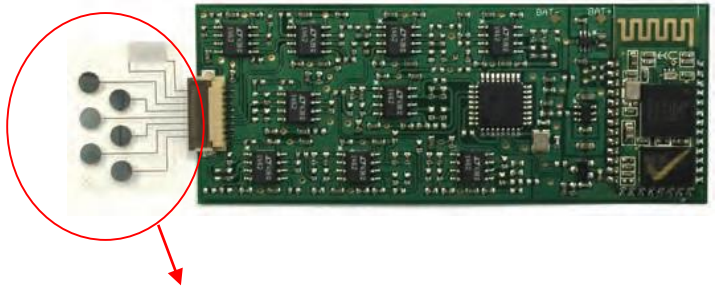




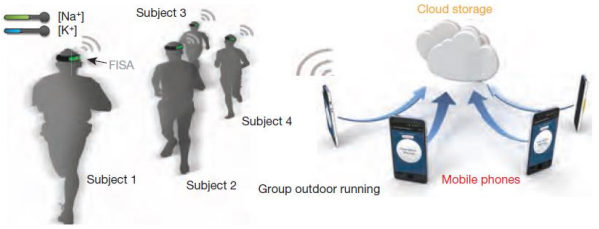
Liao Y-T, Yao H, Lingley A, Parviz B, Otis BP. A 3-uW CMOS glucose sensor for wireless contact-lens tear glucose monitoring. IEEE JSSC 2012;47:335Y44

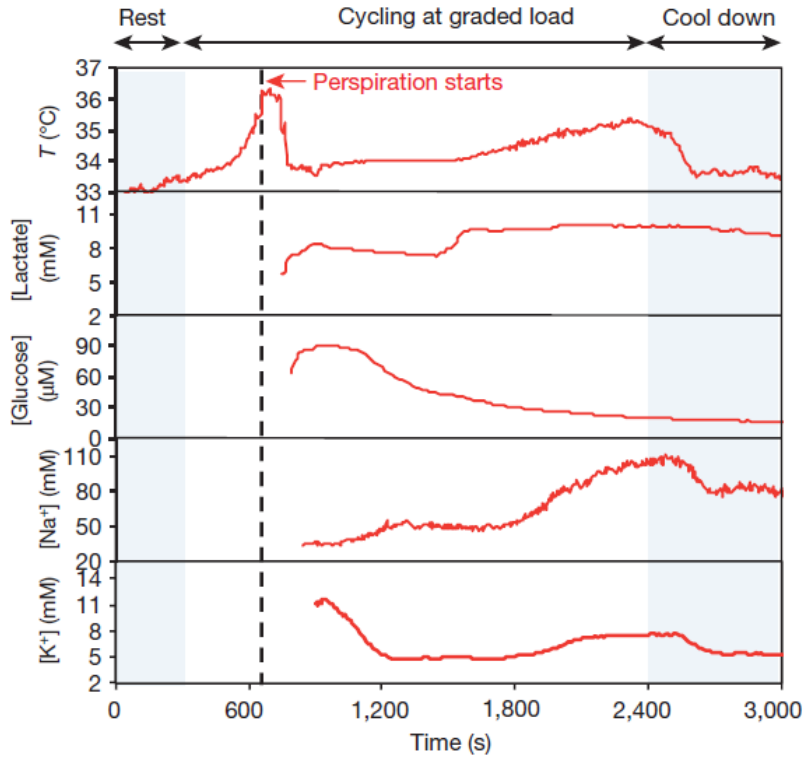
http://noviosense.com/

Wearable Sensors – Sweat Analysis



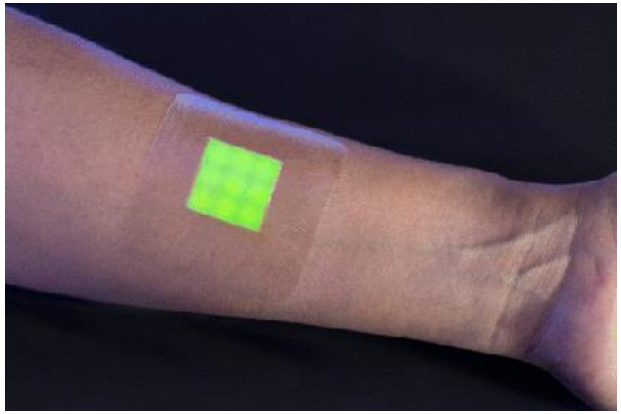
Electrochemical analysis of sweat at molecular level by arrays of sensors in close contact with skin.



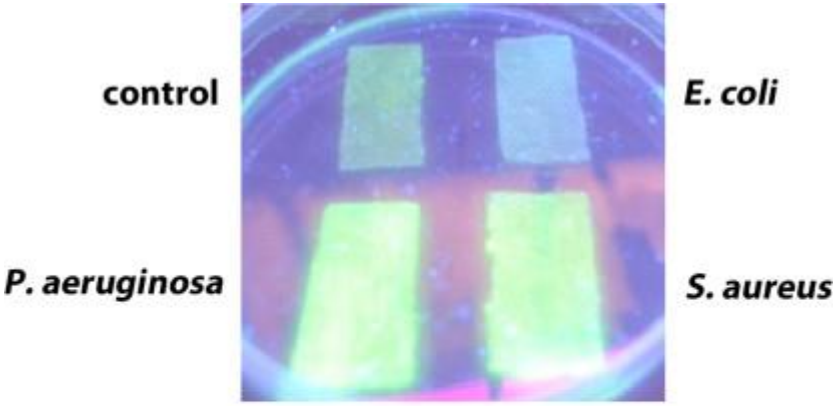


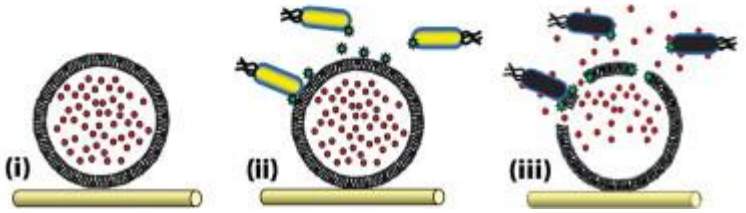
Nature 529(7587):509-514 · January 2016
DOI: 10.1038/nature16521

Smart Wound Dressing



Biosensors embedded in wound dressings to monitor bacterial infections. Possible incorporation of triggered release of a drug.





Dye self quenched

Dye bright

Fluorescent dye loaded to lipid vesicle, toxic bacteria destroy the lipid bilayer wall and leaches the dye reporter.

Toby Jenkins laboratory - 10.1021/acsami.5b07372

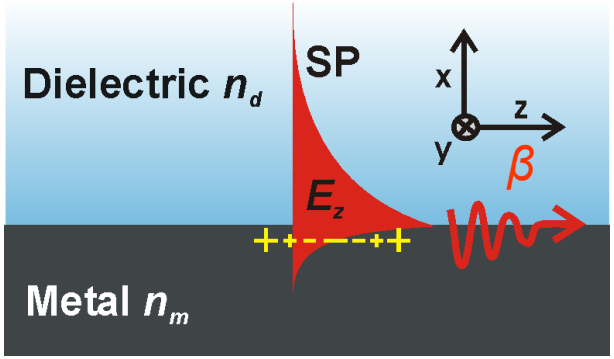
F - Summary

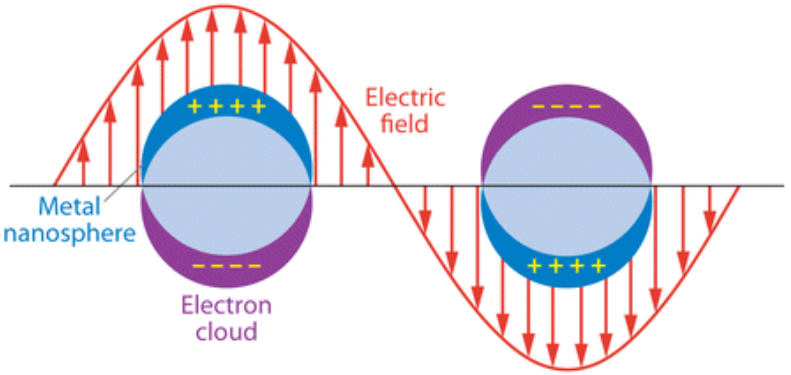
- Biosensor key elements – biomolecular recognition element and a transducer.
- Transducer converts the recognition of an analyte into a signal.
- Examples of biosensor transducers – QCM, amperometric biosensor
- Optical biosensors – based on detection of changes in properties of light resulting form the analyte binding to the sensor surface

G. SURFACE PLASMON RESONANCE – SPR – BIOSENSORS

Surface Plasmon (SP)

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.





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

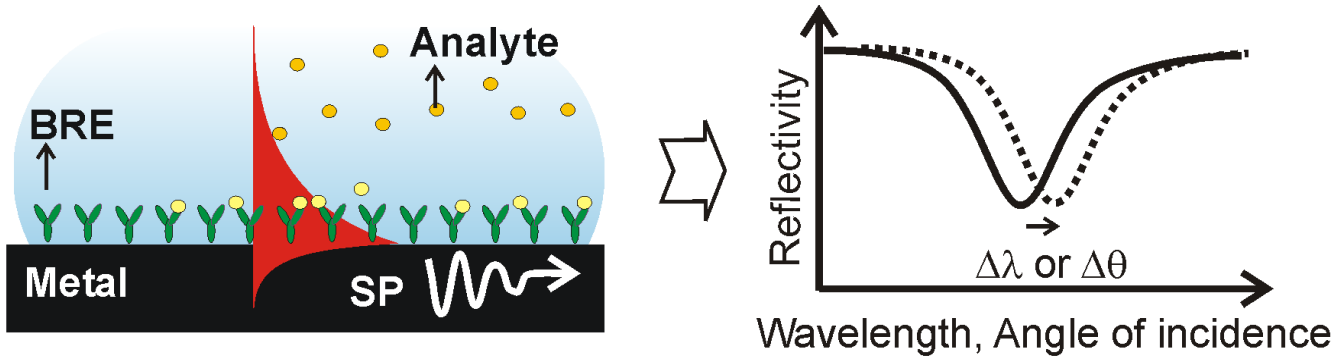
$$\beta = \frac{2\pi}{\lambda}\sqrt{\frac{n_m^2 n_d^2}{n_m^2 + n_d^2}}$$

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

- SPs allows for tight confinement of electromagnetic field at the interface.
- For visible near infrared wavelength typically gold and silver are used
- Majority of the field is probing the close vicinity to metal – 10^1-10^2 nm.

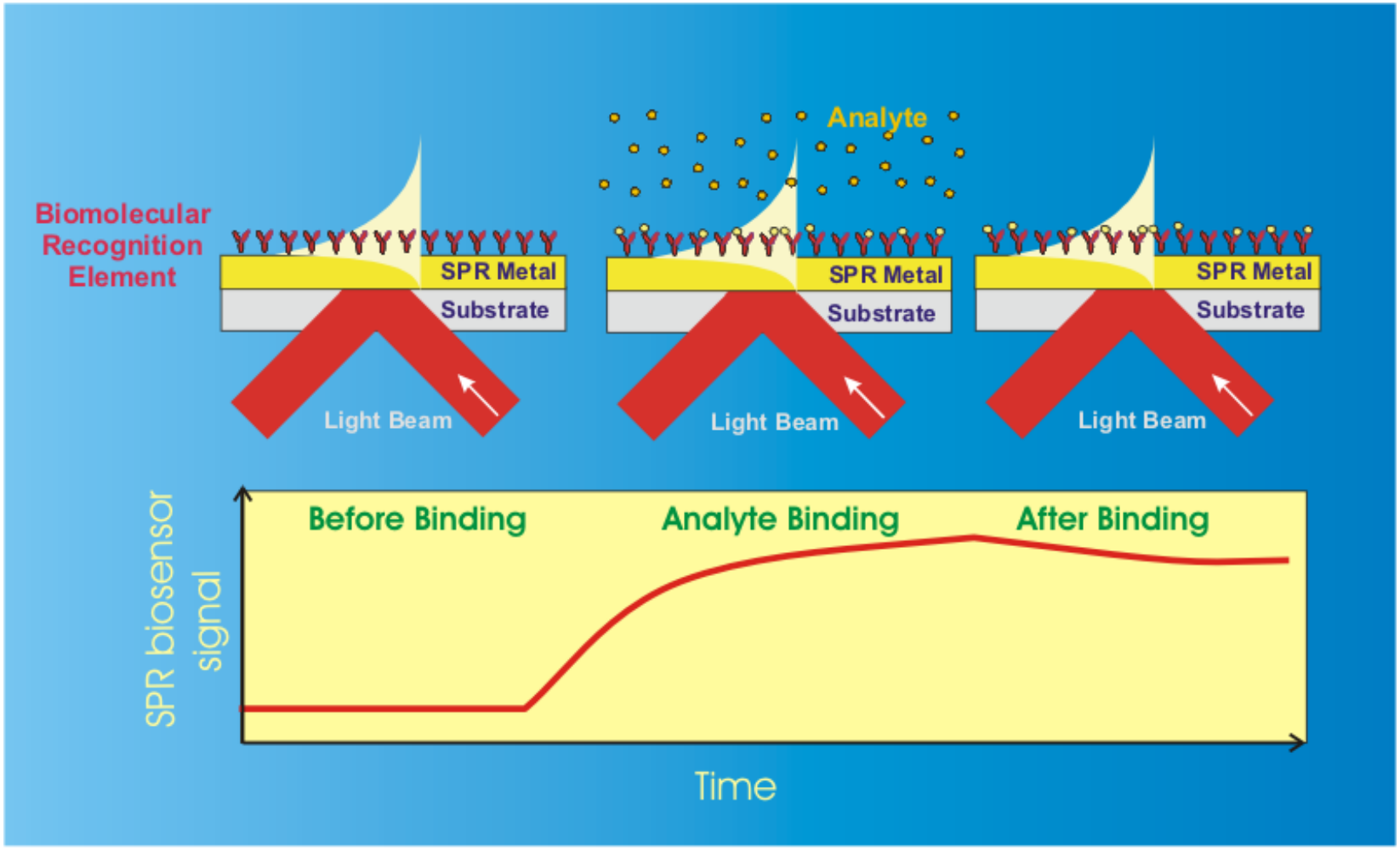
Surface Plasmon Resonance (SPR) Biosensor – Key Principle

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:



Analyte binding ⇒ Refractive index change δn_d ⇒ SP propagation constant change $\delta\beta$ ⇒ Coupling condition change $\delta\lambda$ or $\delta\theta$

SPR Binding Kinetics



SPR signal = LSPR wavelength λ_{LSPR}, SPR coupling angle, or wavelength

SPR Biosensors - Features

Observation of binding-induced refractive index changes

- **Direct label-free detection** principle
- Reatime observation of biomolecular binding kinetics
- Suitable for medium and large size molecules
- Sensitivity around nM range

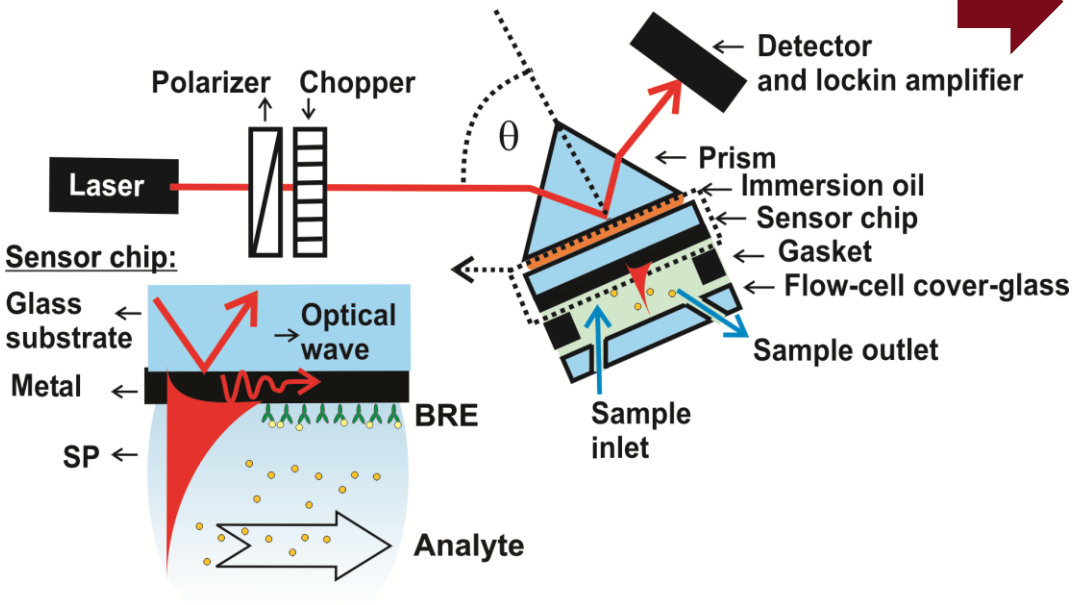
Rapid and sensitive analysis of molecular analytes

- Biomarkers, pathogens, toxins...

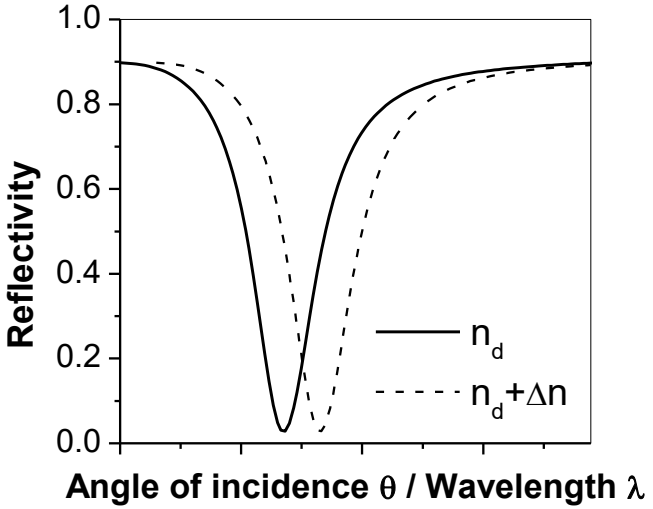
Biomolecular interaction analysis (BIA):

- Life sciences, drug development…
- Determining of kinetic constants of studied reaction (k_a, k_d, K_d).

Implementations of SPR Biosensor



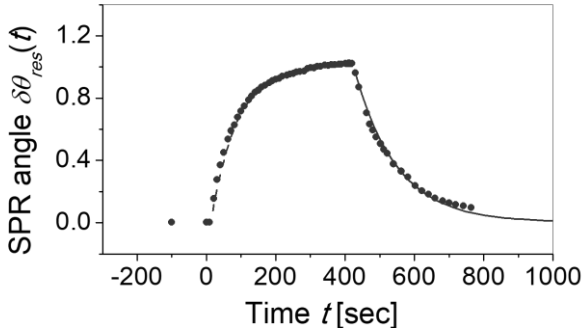
SPR reflectivity



Tracking of SPR angle

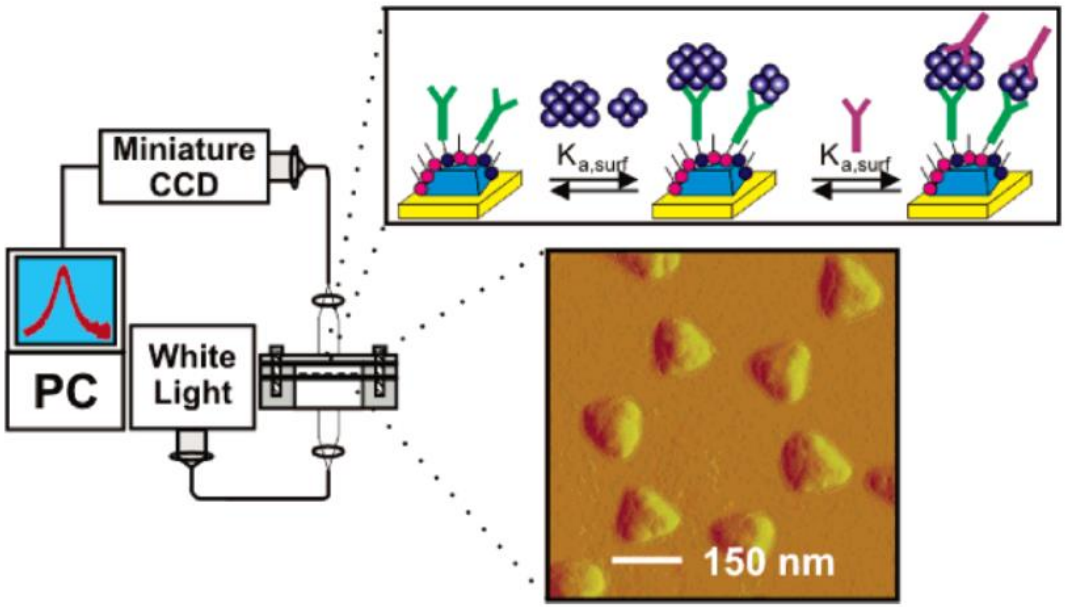
Example of an optical setup of angular modulation of SPR with a micro-fluidic unit for monitoring of affinity binding

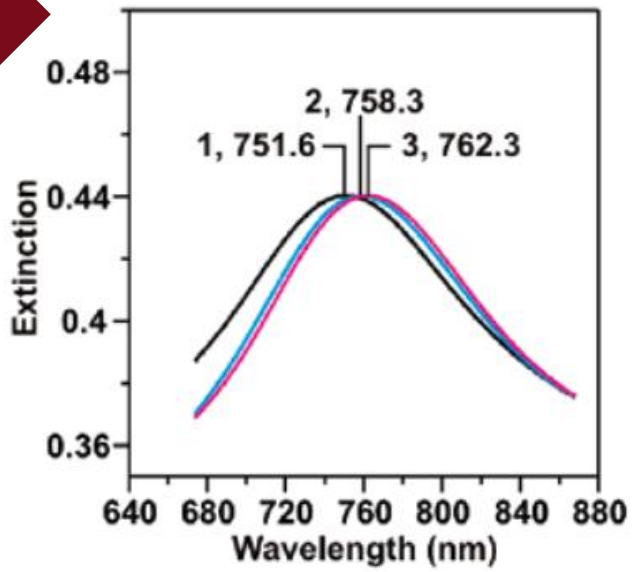
Analysis of reaction kinetics



Implementations of SPR Biosensor

LSPR transmission





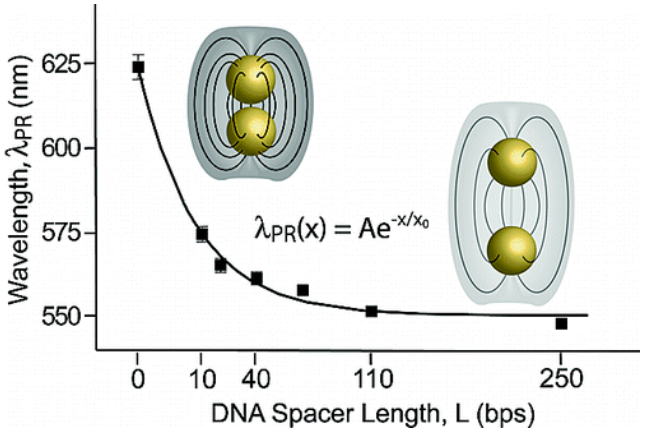
Example of first implementation of LSPR for analysis of biomarkers.

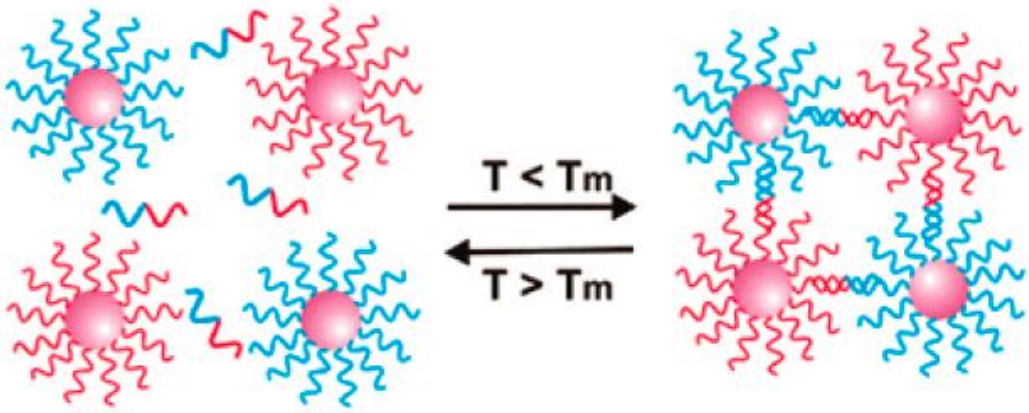
Tracking of LSPR wavelength λ_{LSPR}

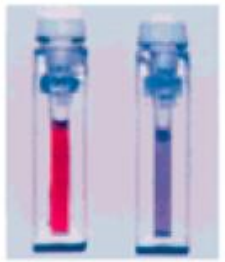
A.J. Haes et al,, *J. Am. Chem. Soc.*, **2005**, *127* (7), pp 2264–2271
DOI: 10.1021/ja044087q

LSPR Homogenous Assays

Concept of plasmonic ruler:







without DNA target

with DNA target

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).

Rosi, N. L.; Mirkin, C. A. *Chem. ReV.* ***2005, 105, 1547***

SPR and LSPR Comparison

Both SPR and LSPR biosensors were applied for the analysis of chemical and biological analytes and range of instruments become commercially available

	SPR	LSPR
Complexity of instrument	high	simple
Refractive index resolution	10^{-7}	an order of mag. lower
Probing depth	> 100 nm	>10 nm
Parallelized detection	propagation length > 1 µm	individual NP possible

Kvasnicka et al, BIOINTERPHASES **Volume:** 3 **Issue:** 3 **Pages:** FD4-FD11 **DOI:** 10.1116/1.2994687. Stewart et al, Chem. Rev. 2008, 108, 494−521. Homola, **Chemical Reviews**, 108, 462-493 (2008).

Availability of SPR Biosensors

Numerous commercially available SPR biosensor systems. From versatile to highly dedicated systems, price from 10-100kEuro.

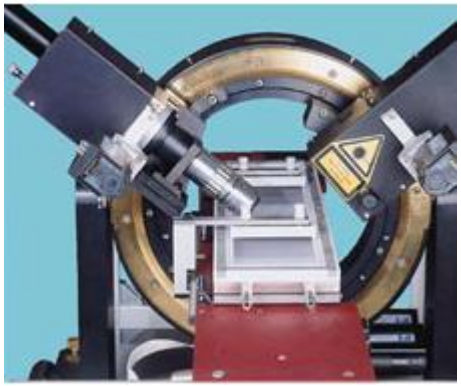
Biacore
label-free interaction analysis –
from research through drug discovery
and development to QC

www.biacore.com

HORIBA
Scientific

www.horiba.com/scientific/products/surface-plasmon-resonance-imaging-spri/

http://www.bionavis.com



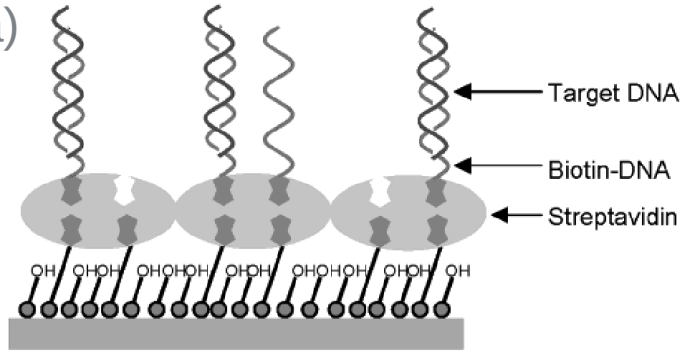
http://www.optrel.de/products_multiskop.html

Surface Architectures

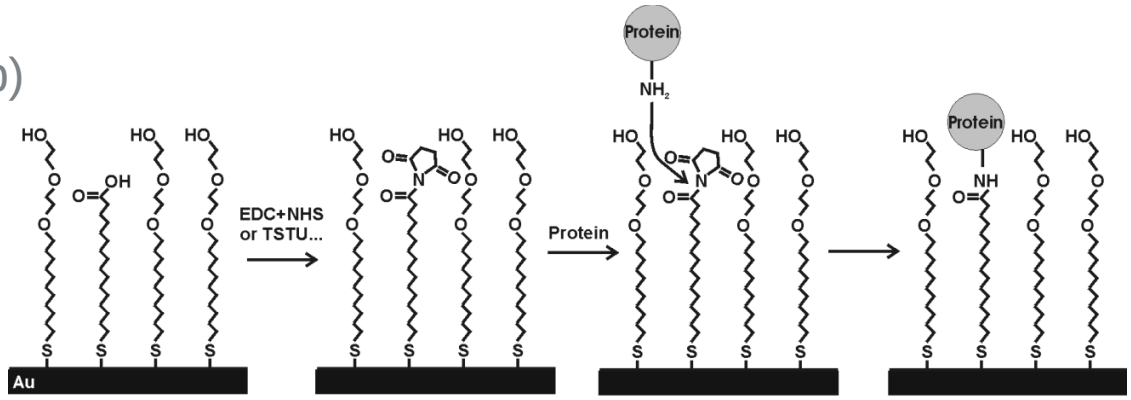
Examples of surface architectures for the immobilization of biomolecular recognition elements on the gold SPR biosensor surface:

a) Thiol self-assembled monolayer (SAM) for streptavidin – biotin coupling, b) thiol SAM for amine coupling, c) three-dimensional dextran brush matrix.

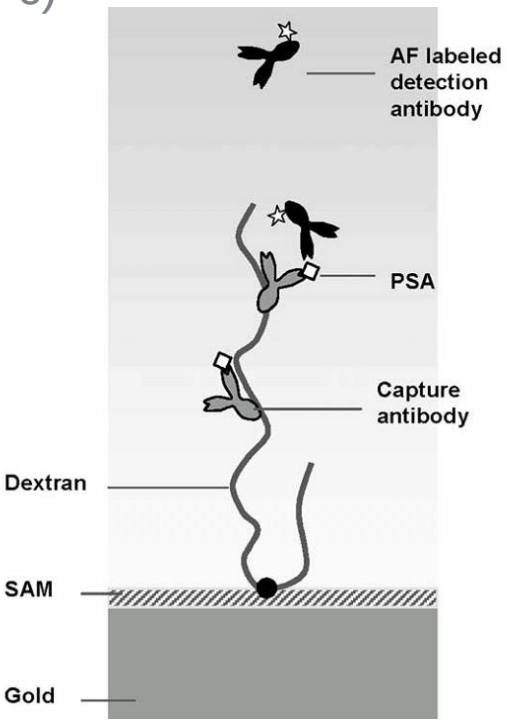
a)



b)

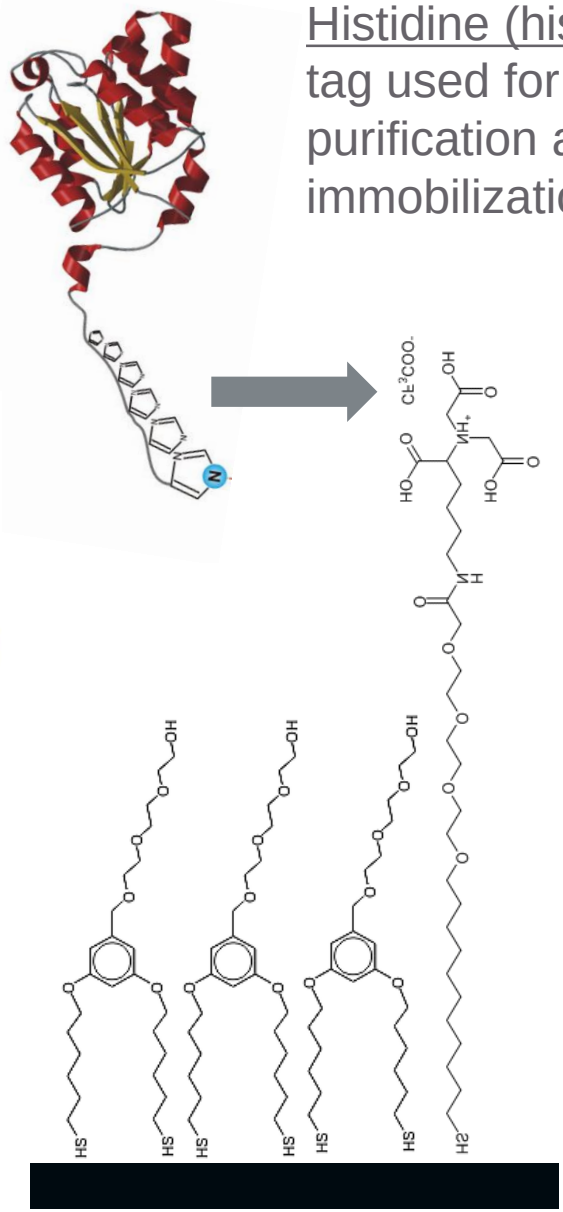


c)

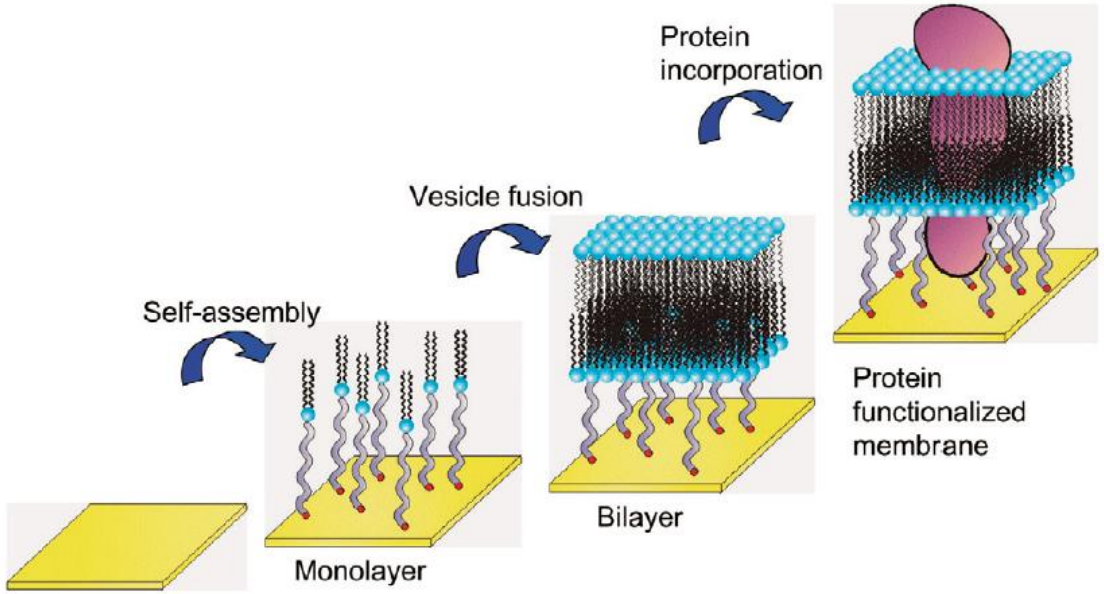


Surface Architectures

Histidine (his) tag used for purification and immobilization



Supported lipid bi-layer for membrane protein investigation



E-K. Sinner, S. Ritz, Y. Wang, J. Dostalek, U. Jonas, W. Knoll, Molecularly Controlled Functional Architectures at Biointerfaces, Materials Today (2010), 23, 4, 47-55

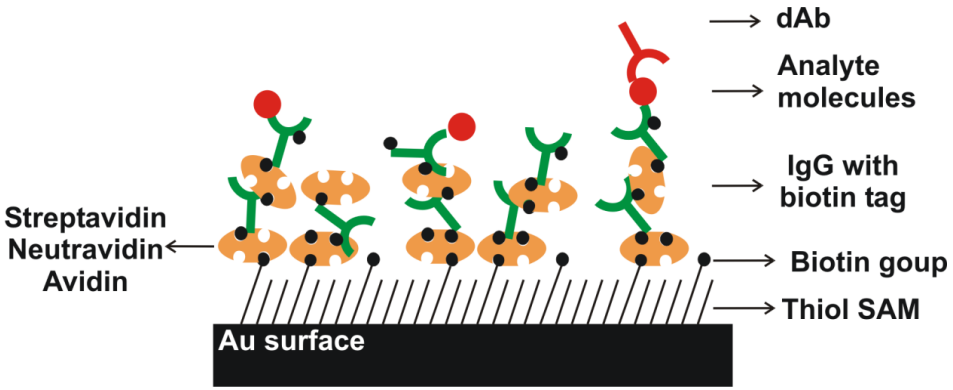
G - Summary

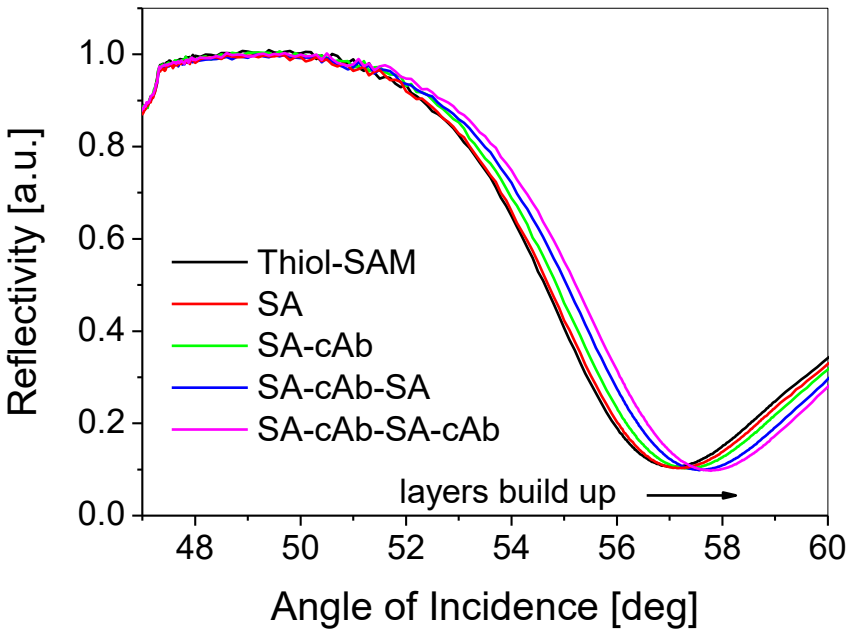
- SPR detection principle – measuring of binding induced refractive index changes.
- Implementations by using propagating surface plasmons (SPR) and localized surface plasmons (LSPR).
- Allows for direct detection of molecular binding events without the necessity of using labels.
- Surface architectures for immobilization of ligands.

H. BIA - BIOMOLECULAR INTERACTIONAL ANALYSIS ENSEMBLES OF BIOMOLECULES

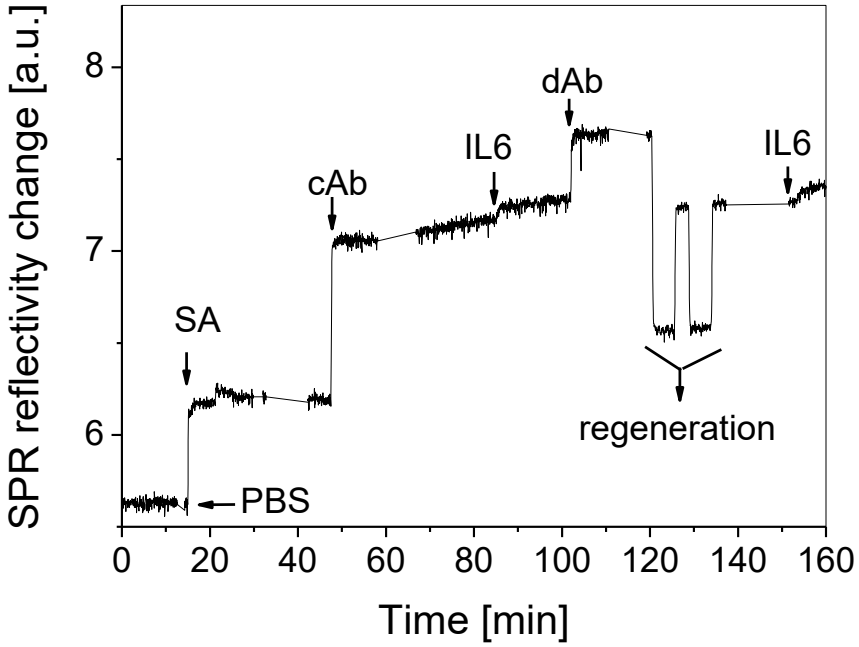
Observation of Thin Biomolecular Films

- SPR response ($\delta\theta_{res}$) proportional to mass adhered to the surface Γ.





Layer-by-layer building up of SA/cAb.



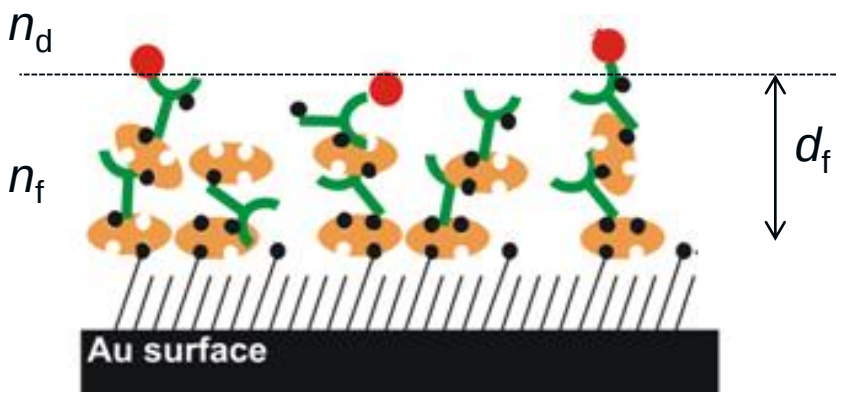
SPR IL6 sandwich assay.

Observation of Thin Biomolecular Films

SPR signal change can be converted to the surface mass density of bound biomolecules Γ. Useful tool for the investigation of surface coverage of proteins, binding capacity, developing assays….

Fitting of SPR spectra allows determining of refractive index n_f and / or thickness d_f of thin films (*e.g.,* implemented in a software Winspall).

$$\Gamma = (n_f - n_d) d_f \frac{\partial c}{\partial n_f}$$



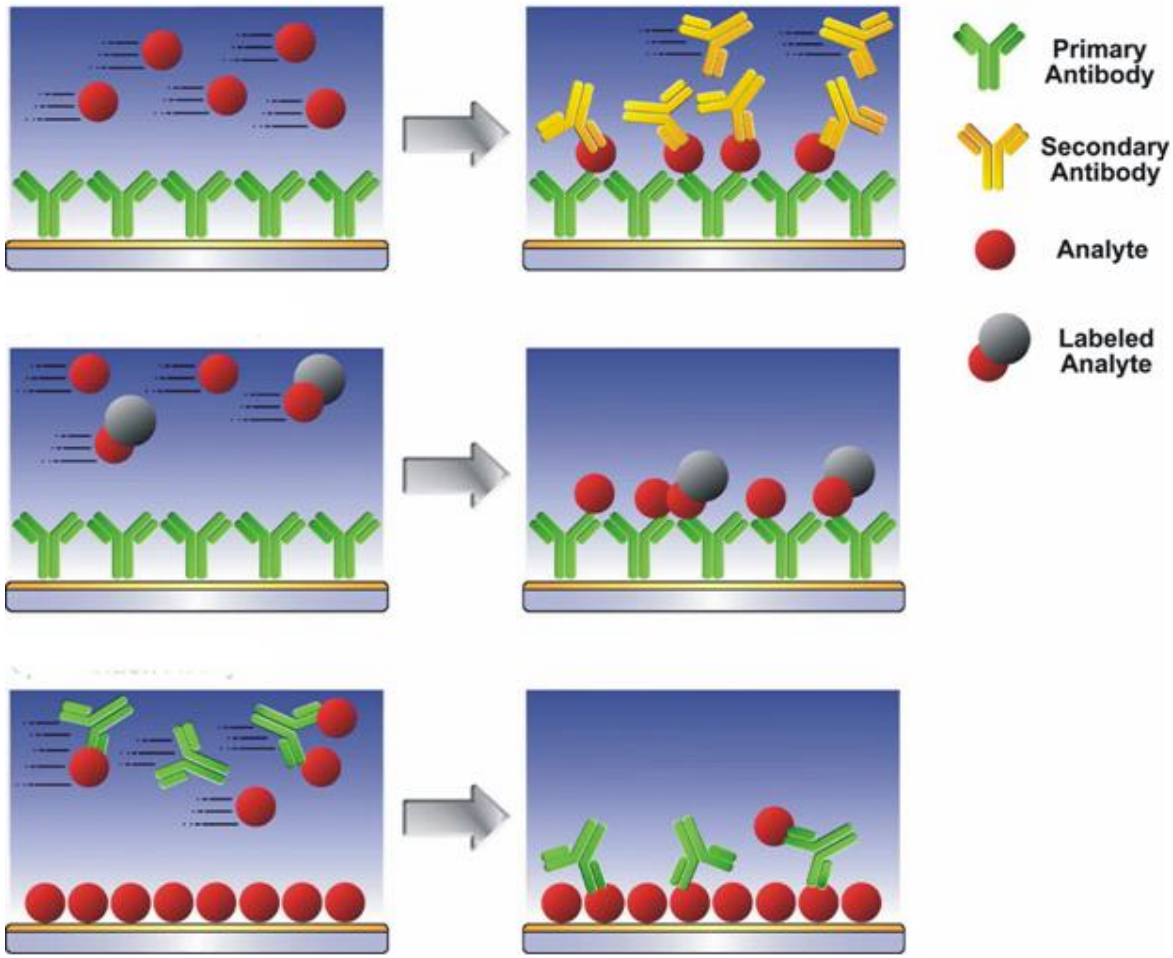
For most polymer materials holds $\partial n_f/\partial c \sim 0.2$ mm^3 mg^{-1} and in aqueous samples n_d=1.33. For instance a full packed IgG monolayer exhibits $\Gamma \sim 4$ ng/mm^2, IgG hydrodynamic radius $R_h \sim 5$ nm.

Heterogeneous Assays

Sandwich

Competitive

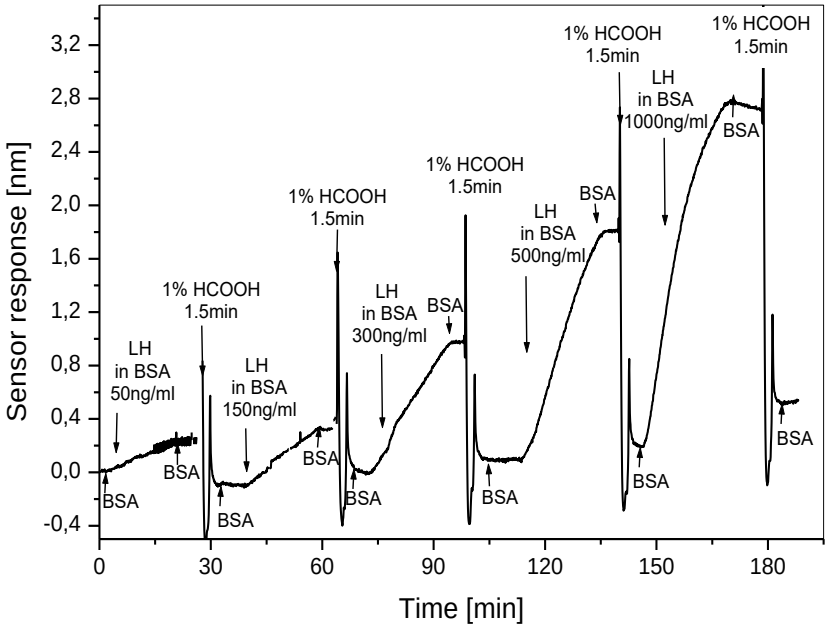
Inhibition

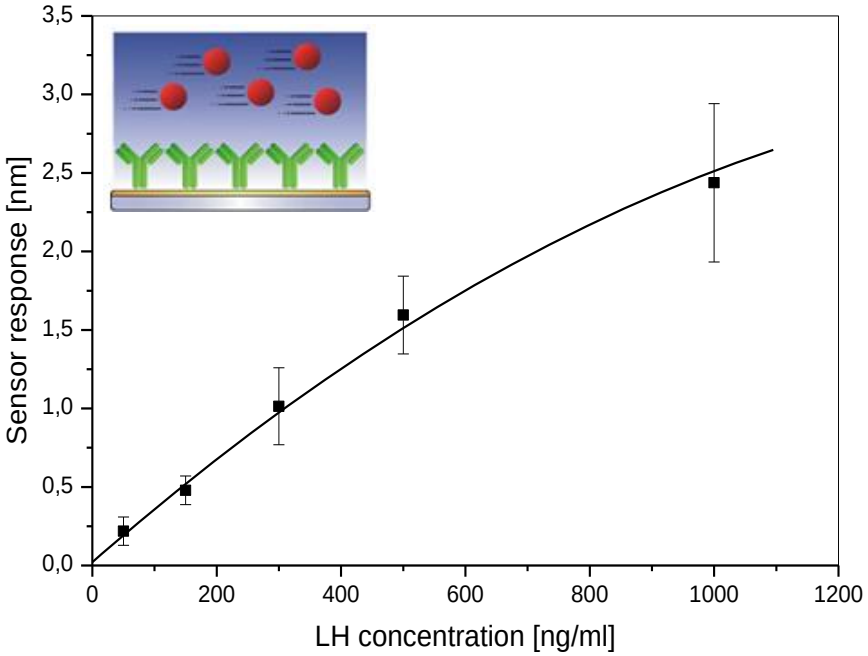


J. Homola (editor): Surface Plasmon Resonance Based Sensors, Springer, 2006.

Example of Direct Assay

Direct detection of luteinizing hormone (LH, triggers ovulation). Protein with molecular weight of 29 kDa.

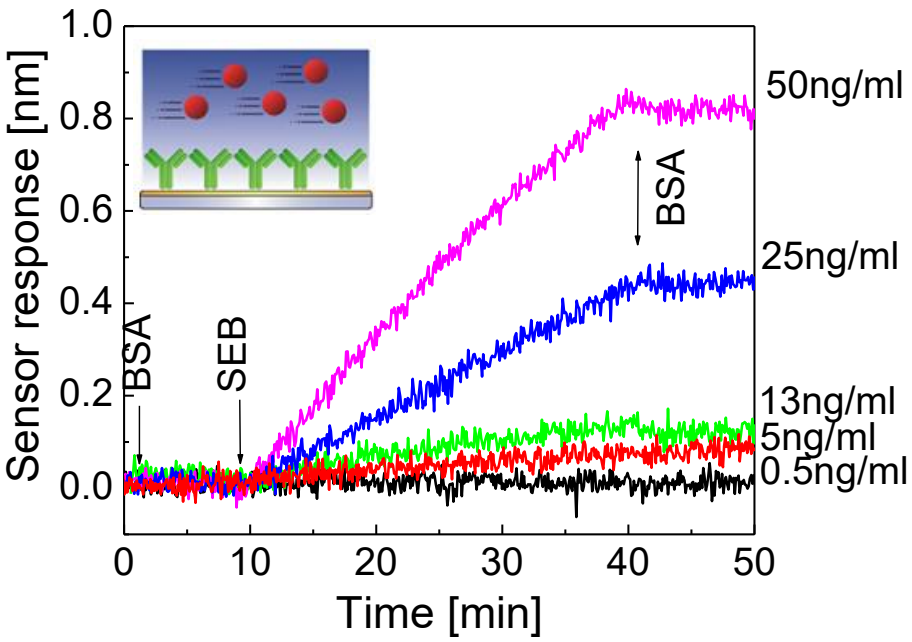


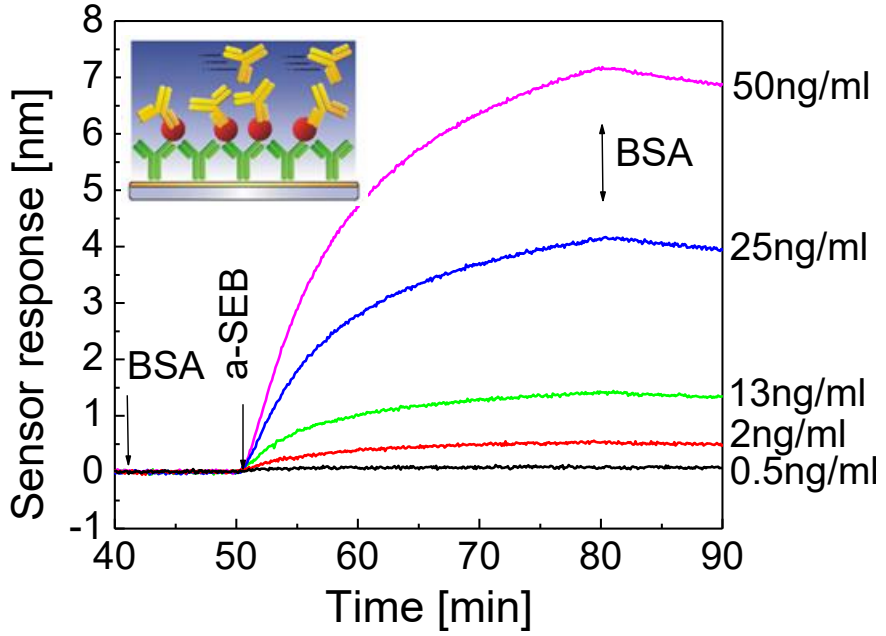


Binding kinetics for increasing concentrations of LH and regeneration between detection cycles (left) and the calibration curve (right).

Example of Sandwich Assay

Staphylococcal enterotoxin B (SEB) – toxin that commonly causes food poisoning, with severe diarrhea, nausea and intestinal cramping. Molecular weight 28 kDa. Response amplified by secondary polyclonal IgG with molecular weight of 160 kDa.

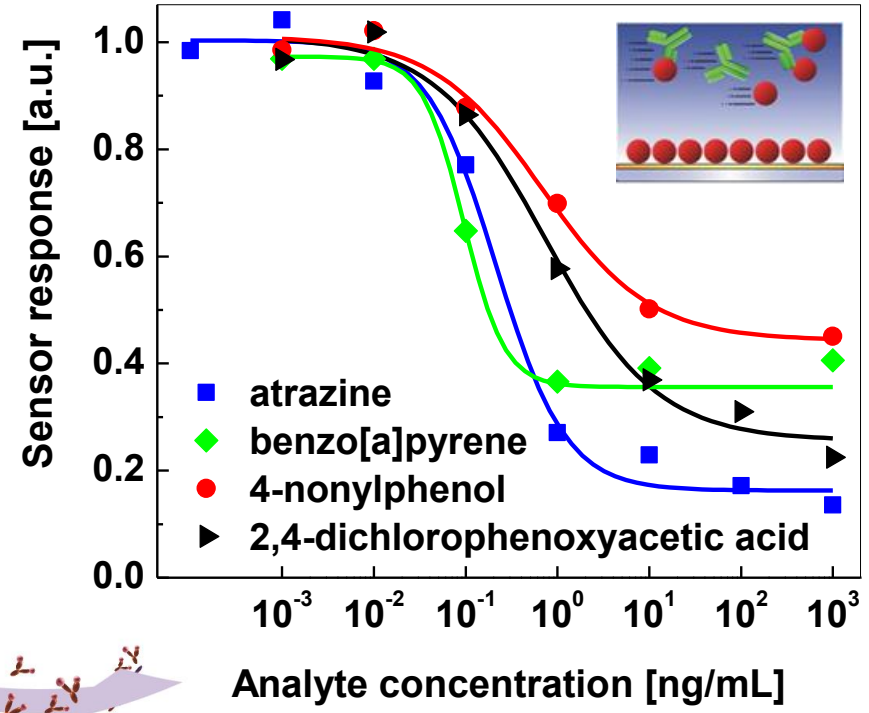


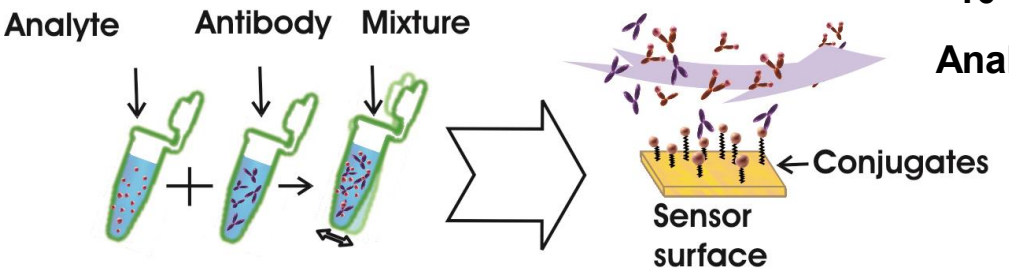


J. Homola, J. Dostalek, S. Chen, A. Rasooly, S. Jiang, S. S. Yee: Spectral Surface Plasmon Resonance Biosensor for Detection of Staphylococcal Enterotoxin B (SEB) in Milk , Journal of Microbiology, 75, (2002) 61-69.

Example of Inhibition Assay

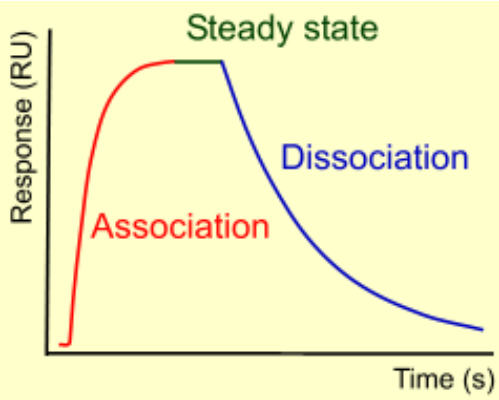
Atrazine – pesticide with molecular weight of 0.2 kDa. Too small to be detected directly and thus inhibition or competitive assays are used.



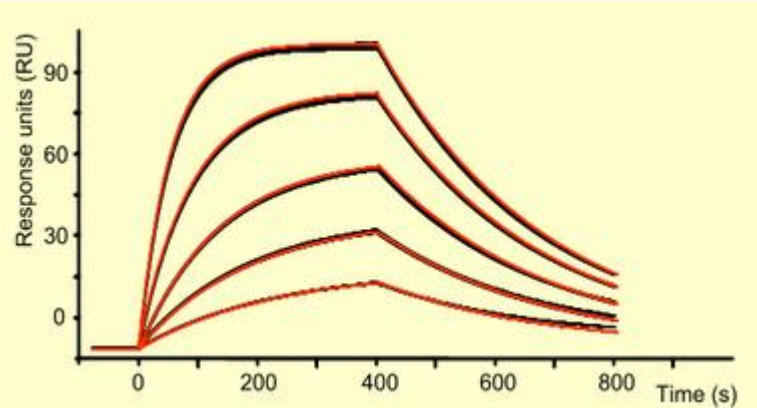


Dostalek, J. Pribyl, P. Skladal, J. Homola, Multichannel SPR biosensor for detection of endocrine disrupting compounds, Analytical and Bioanalytical Chemistry, (2007) 389:1841-1847

SPR Sensorgram



Fitting ⬇ kinetics



SPR kinetics - time dependent response (often plotted in relative units RU ~ change in n_d of 10^{-6} RIU)

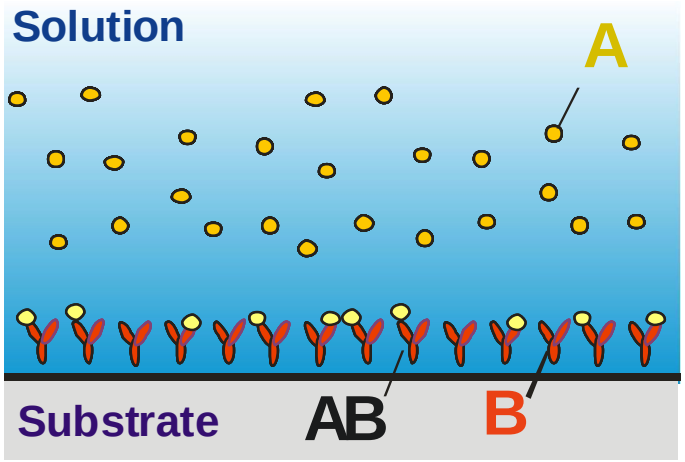
Association and dissociation binding rates (k_a and k_d, respectively) or more complex parameters can be determined by fitting with a model.

Analysis can be performed through specialized software (*e.g.* from BIAcore) or by another tools allowing fitting with non-linear functions (*e.g.* Origin).

Langmuir Adsorption Isotherm

Equilibrium of a reaction:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftarrows}} AB \qquad K = \frac{k_a}{k_d}$$



Kinetics of the reaction on a surface:

$$\frac{d\gamma}{dt} = k_a \alpha_0 (\beta - \gamma) - k_d \gamma$$

γ Concentration of [AB]

α_0 Concentration [A]

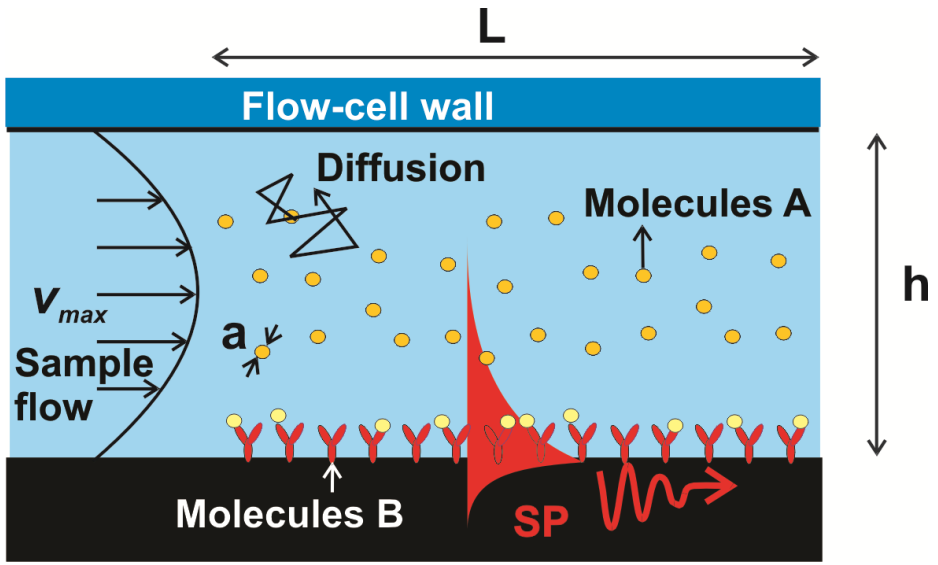
β Concentration [B]

Describes the interaction for:

a) Identical monovalent receptors B
b) Constant concentration of A in the solution ([A]>>[B])

(Possible to describe more complicated interactions e.g. multivalent receptors)

Two Compartment Model



D – diffusion coefficient

$$D \approx \frac{k_B T}{6\pi a \eta}$$

a – molecule A hydrodynamic radius

η - solution viscosity

In SPR biosensors, analyte molecules A in a liquid samples are flowed over the sensor surface.

Due to the friction, at the surface the flow velocity is v=0. Approximation that the analyte mass transfer rate occurs across an unstirred layer through diffusion:

$$k_m = \xi \left(\frac{v_{\max} D^2}{hL} \right)^{1/3}$$

Surface Reaction with Mass Transfer

Reaction kinetics become a function of mass transfer rate k_m.

$$\frac{d\gamma}{dt} = k_{on}\alpha(\beta - \gamma) - k_{off}\gamma$$

$$k_{on} = \frac{k_a}{1 + k_a\left[\beta - \gamma(t)\right]/k_m}$$

$$k_{off} = \frac{k_d}{1 + k_a\left[\beta - \gamma(t)\right]/k_m}$$

Fast diffusion

$k_m >> k_a\beta$

Reaction is affinity-controlled and $k_{on} \approx k_a$, $k_{off} \approx k_d$

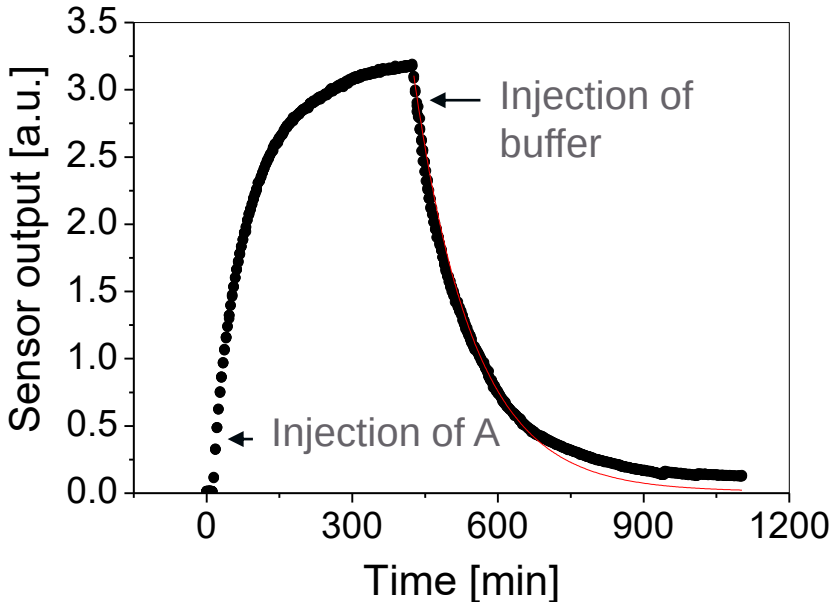
Slow diffusion

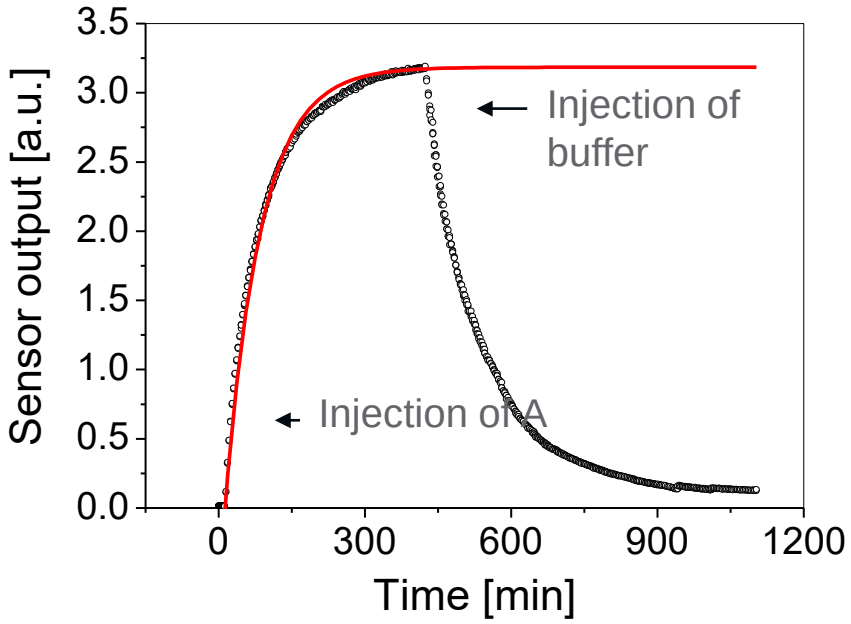
$k_m << k_a\beta$

Reaction is diffusion-controlled, $k_{on} \approx k_m\beta^{-1}$ and $k_{off} \approx k_m k_d (k_a\beta)^{-1}$

(low probe / ligand density, high flow rate)

Fitting of the SPR Sensor Kinetics





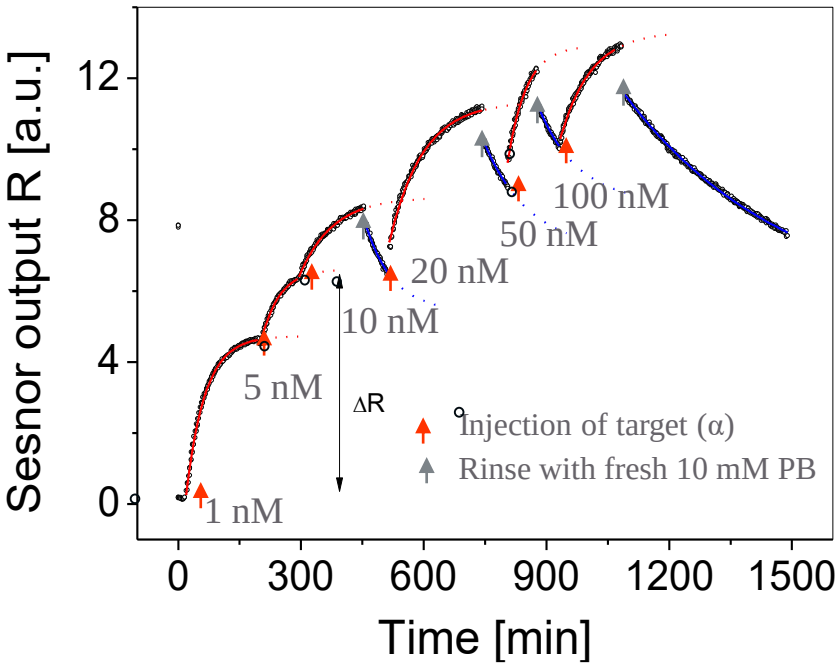
SPR biosensor output R(t) is proportional to $\gamma(t)$, one can fit k_a and k_d as:

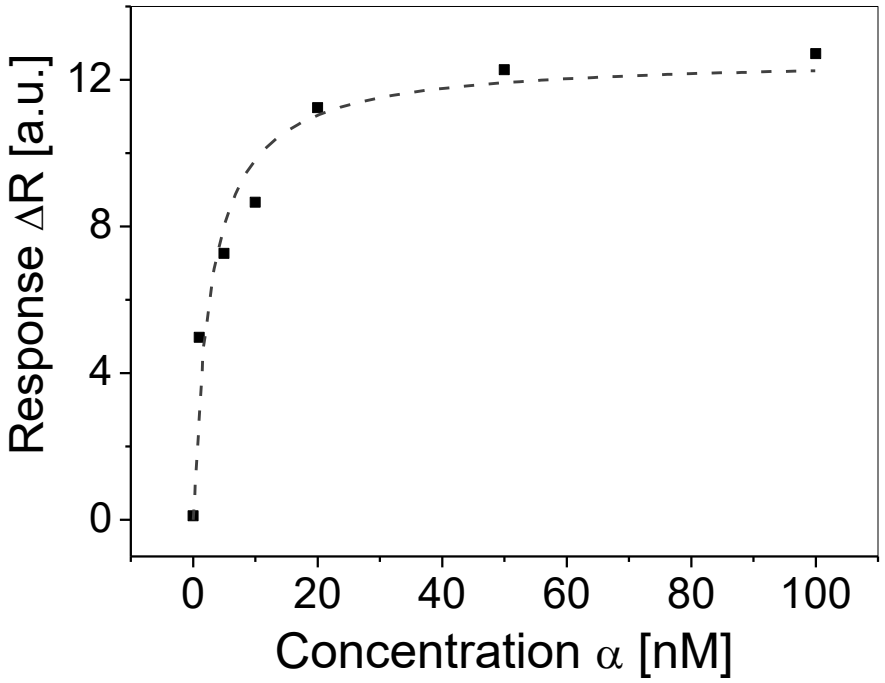
$$R_d(t) = (R_{max} - R_0)e^{-k_d(t-t_a)} + R_0 \qquad R_a(t) = (R_{max} - R_0)(1 - e^{-(k_a c_0 - k_d)(t-t_0)}) + R_0$$

k_d!

k_a!

Titration Experiment





By fitting the dependence of equilibrium sensor response *R* on the analyte concentration α the association affinity constant K_a can be fitted from a function:

$$\Delta R = const \frac{K\alpha}{1+K\alpha}$$

$$K_A = \frac{k_a}{k_d}$$

H - Summary

- Homogenous (in solution) and heterogeneous (on the surface) assays.
- Sandwich, competitive, and inhibition immunoassay.
- Evaluation of binding kinetics – determining of affinity binding constants k_a, k_d, K_d…., cascades of reaction studies.

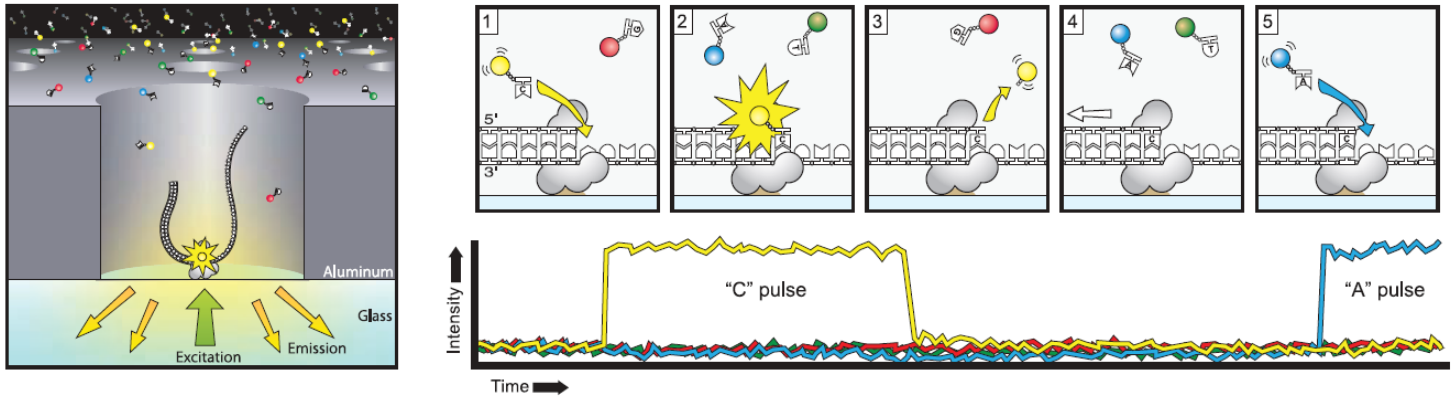
- Useful links: https://www.biacore.com
  http://www.sprpages.nl
  http://www.res-tec.de

I. BIA - BIOMOLECULAR INTERACTIONAL ANALYSIS – SINGLE BIOMOLECULES

Single Molecule Interaction Analysis / Detection

Optical technologies utilizing strong light confinement are a key for investigating of interactions at single biomolecule level:

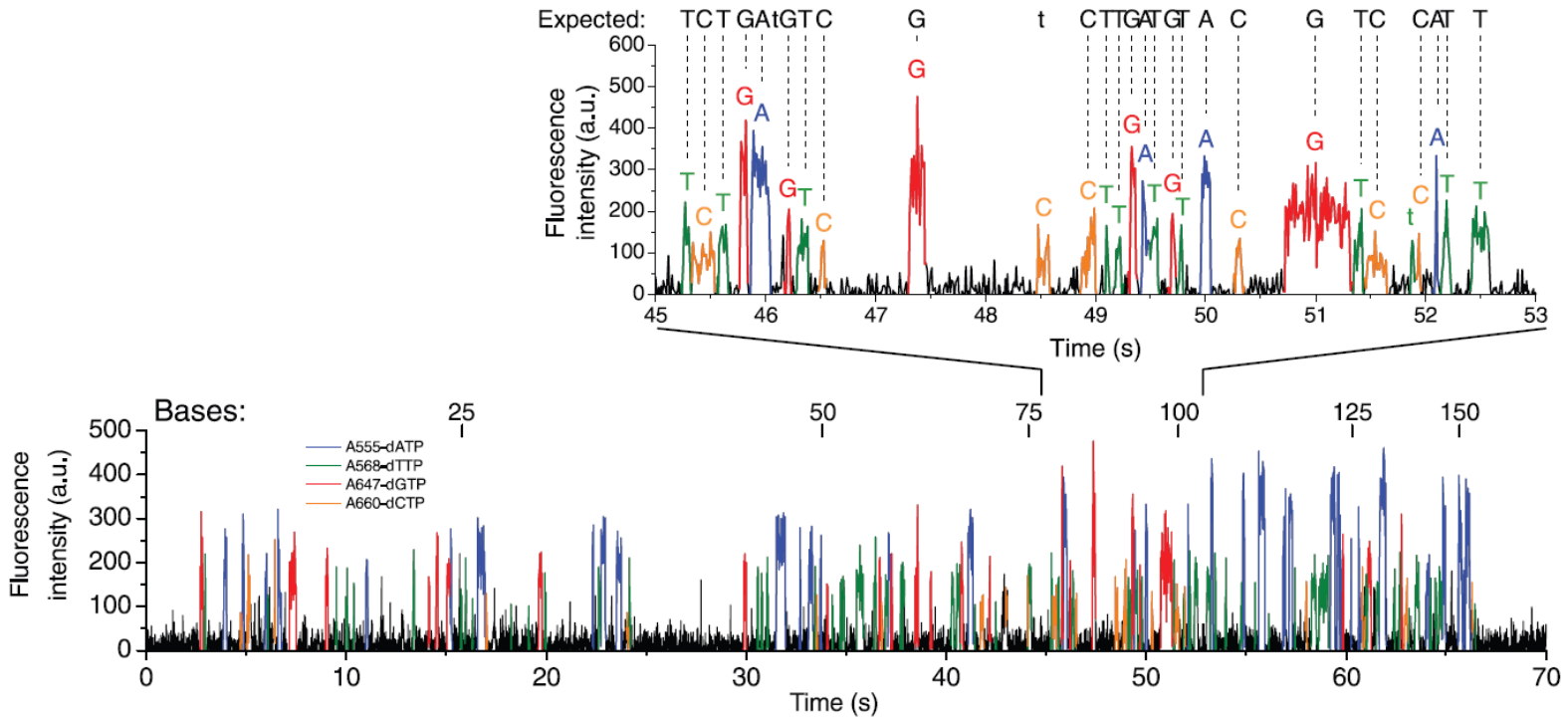
- Possibilities of observing new effects that are smeared by averaging.
- Peeking on subpopulations of biomolecules (e.g. with different folding).
- New functionalities not reachable with ensembles.



S. Real-Time DNA Sequencing from Single Polymerase Molecules. Science 2009, 323 (5910), 133–138.

Single Molecule Interaction Analysis

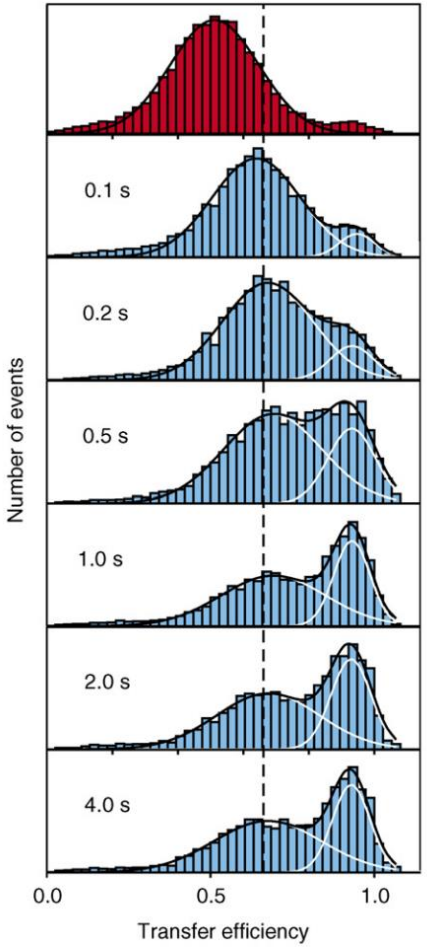
Labelling of bases with different dye molecules allows monitoring in real time assembly and prolongation of the duplex base by base.

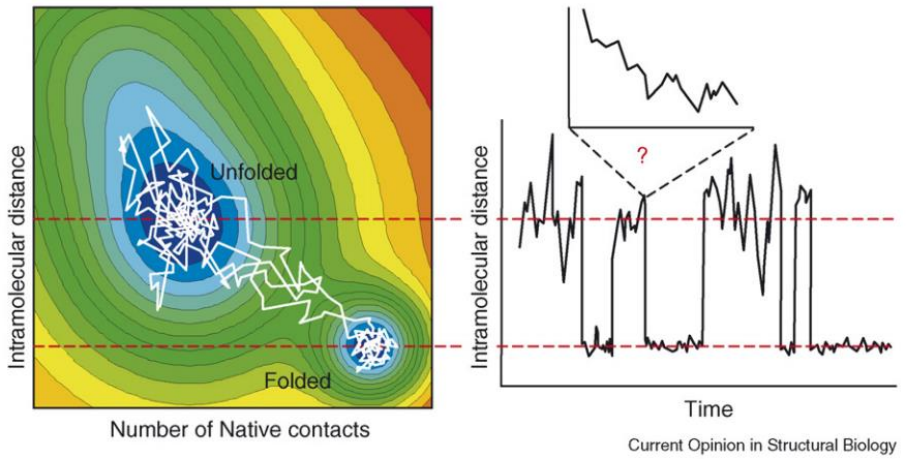


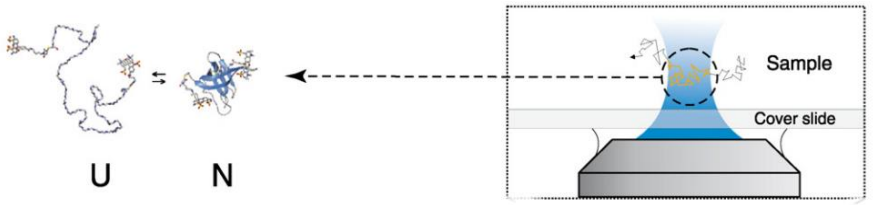
Pacific Biosciences: http://www.pacb.com/

S. Real-Time DNA Sequencing from Single Polymerase Molecules. Science 2009, 323 (5910), 133–138.

Protein Folding Observation by FRET

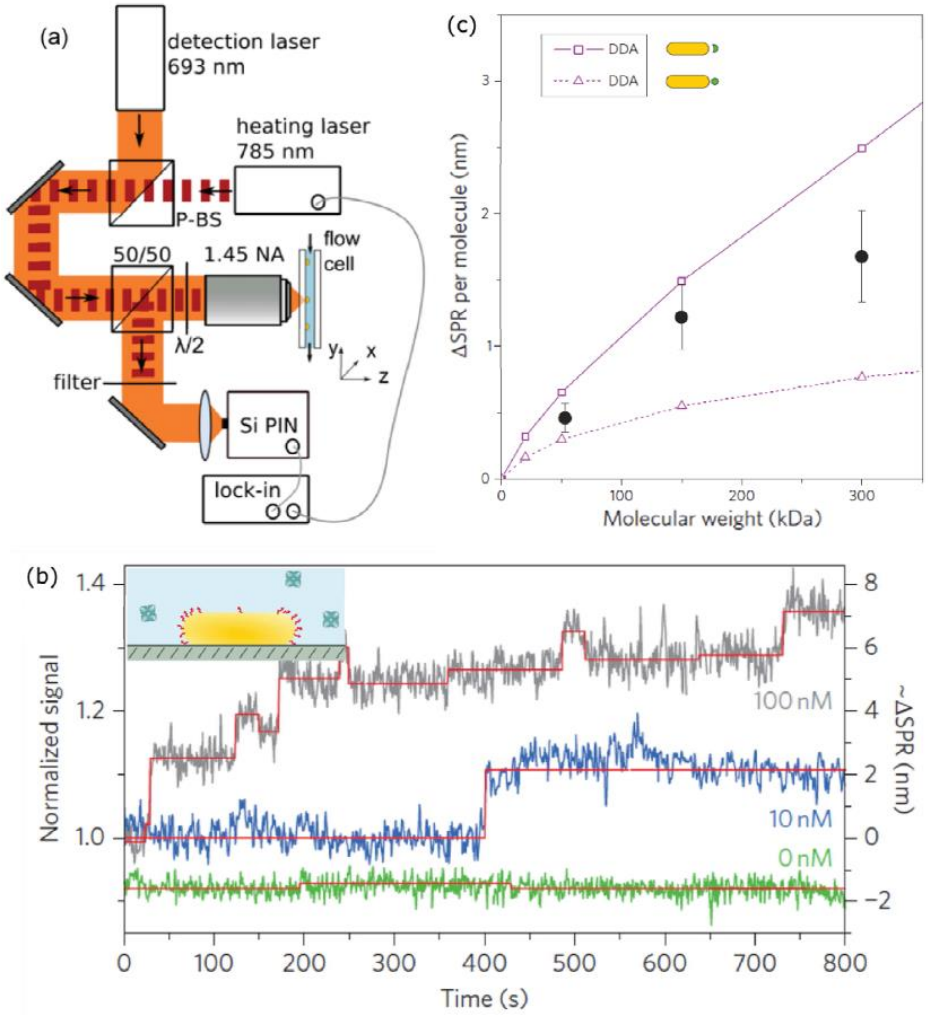






Schuler, B.; Eaton, W. A. Protein Folding Studied by Single-Molecule FRET. Curr. Opin. Struct. Biol. 2008, 18 (1), 16–26.

LSPR Observation of Single Molecule Binding Kinetics



Tracking LSPR wavelength allows for monitoring of individual on and off binding events.

Through statistics, access to additional information smeared for ensemble measurements.

Label – free without the need of using fluorophores.

Zijlstra, P.; Paulo, P. M. R.; Orrit, M. Optical Detection of Single Non-Absorbing Molecules Using the Surface Plasmon Resonance of a Gold Nanorod. Nat. Nanotechnol. 2012, 7 (6), 379–382.

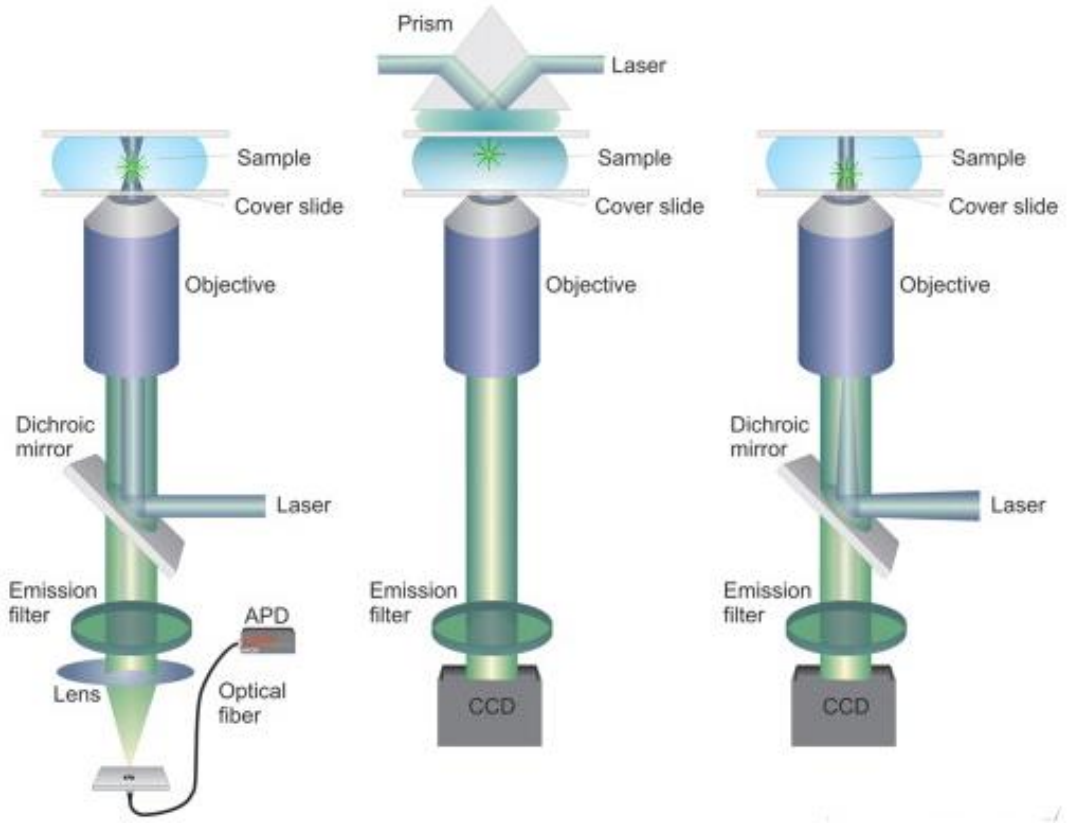
I - Summary

- Single molecule interactions can be optically observed by FRET, fluorescence from nanocavities, or LSPR on individual nanoparticles.
- Single molecule interaction analysis pursued for instance for protein folding and 3rd generation DNA sequencing.
- LSPR on individual gold nanoparticles offer the means to directly observe the biding events / eventually biomolecules conformation changes without the need of labels.

J. OPTICAL SPECTROSCOPY-BASED BIOSENSORS

Fluorescence Detection

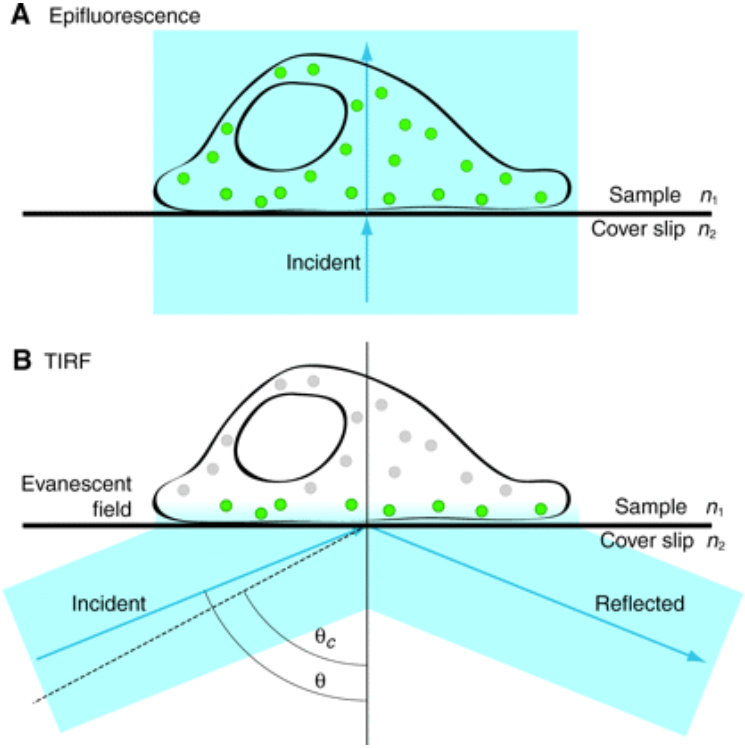
Most common configuration are: confocal fluorescence (left), total internal reflection (middle) and epi-fluorescence (right).

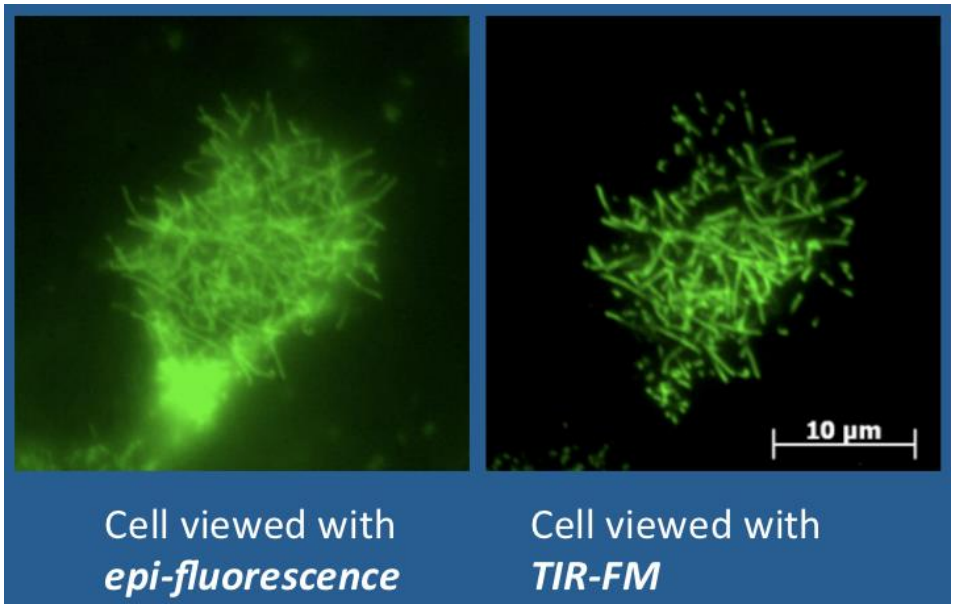


Current Opinion in Structural Biology Volume 14, Issue 5, October 2004, Pages 531–540

Total Internal Reflection Fluorescence (TIRF)

The excitation via evanescent field of totally internally reflected wave allows for the observation of fluorescence signal from close proximity (<1 µm) to the surface and decreasing background.

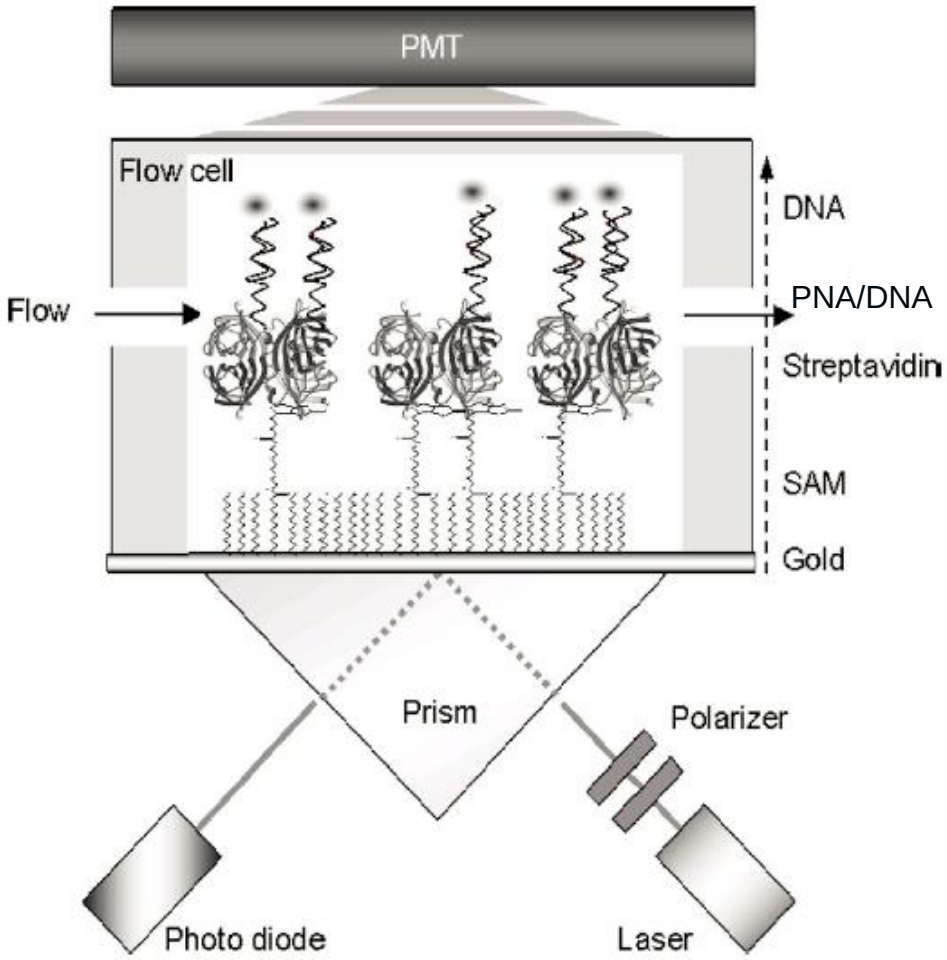




http://lightmicroscopy.ucdenver.edu

Mattheyses A L et al. J Cell Sci 2010;123:3621-3628

Example – DNA Hybridization



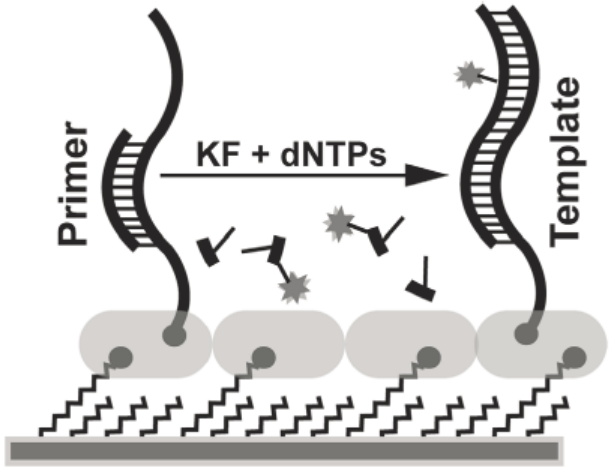
Example: investigation of peptide nucleic acid (PNA) probes for the detection of single nucleotide polymorphism (SNP).

Relevant to e.g. diagnosis of genetic diseases such as thalassemia.

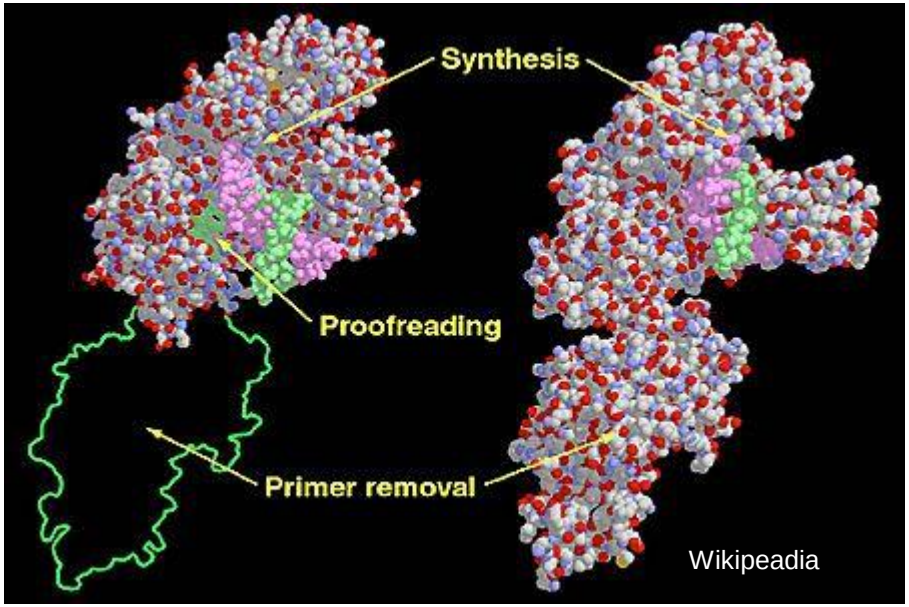
Instrumentation: combined SPR and surface plasmon-enhanced fluorescence spectroscopy (SPFS).

H. Park, A. Germini, S. Sforza, R. Corradini, R. Marchelli, and Knoll W. ,BioInterphases **1, 113 2006.**

DNA Hybridization: Primer Extension



dNTP – deoxynucleotide
KF – Klenow fragment

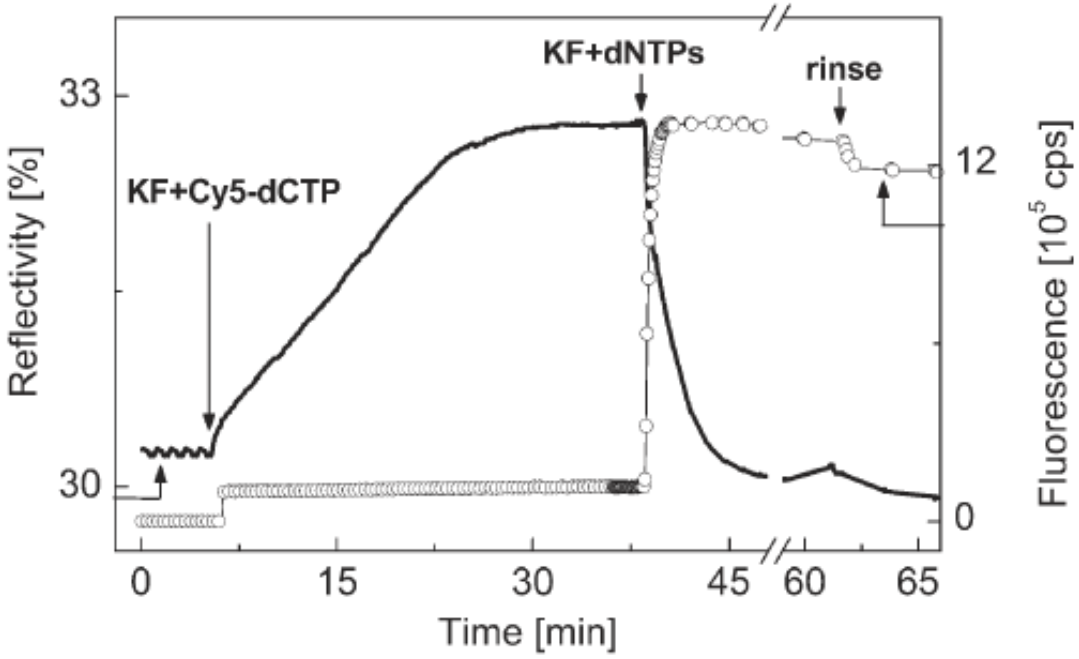


DNA polymerase

Combined SPR and SPFS applied for primer extension studies.

G. Stengel and W. Knoll, Nucleic Acids Res. **33, 69 2005**

DNA Hybridization: Primer Extension



SPR - Mostly sensitive to binding of large KF to DNA probes.
Fluorescence – "Zipping up" the duplex DNA strand.

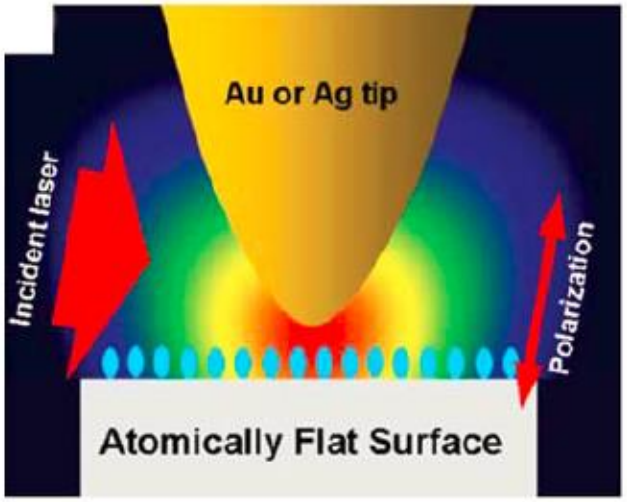
G. Stengel and W. Knoll, Nucleic Acids Res. **33, 69 2005**

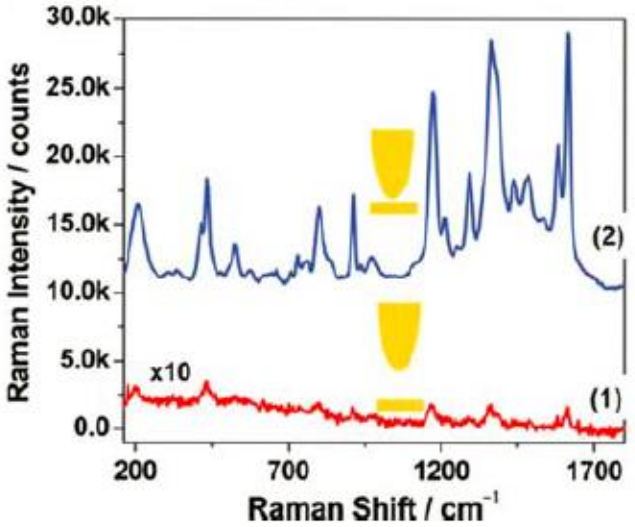
Surface Enhanced Raman Spectroscopy (SERS)

Raman scattering is extremely weak phenomenon, however since introduction of surface-enhanced Raman spectroscopy (SERS) it become important tool in analysis of biomolecules and for detection of chemical and biological species.

SERS originates from a) electromagnetic and b) chemical enhancement on (typically) rough metallic surfaces.

Huge increase in Raman signal >10^{11} can be achieved.





Tian Z-Q, Ren B, Li J-F, Yang Z-L (2007) Expanding generality of surface-enhanced Raman spectroscopy with borrowing SERS activity strategy. Chem Commn (34): 3514–3534

Blackie, Evan J.; Le Ru, Eric C.; Etchegoin, Pablo G. (2009). "Single-Molecule Surface-Enhanced Raman Spectroscopy of Nonresonant Molecules". *J. Am. Chem. Soc.* **131** (40): 14466–14472. doi:10.1021/ja905319w.PMID 19807188.

Surface Enhanced Raman Spectroscopy (SERS)

For small molecules (e.g. organic dyes), specific fingerprint spectrum can be identified and thus analyte directly detected (e.g. pesticides). However, this is not possible for larger biomolecules (protein, DNA) with much more complex spectra.

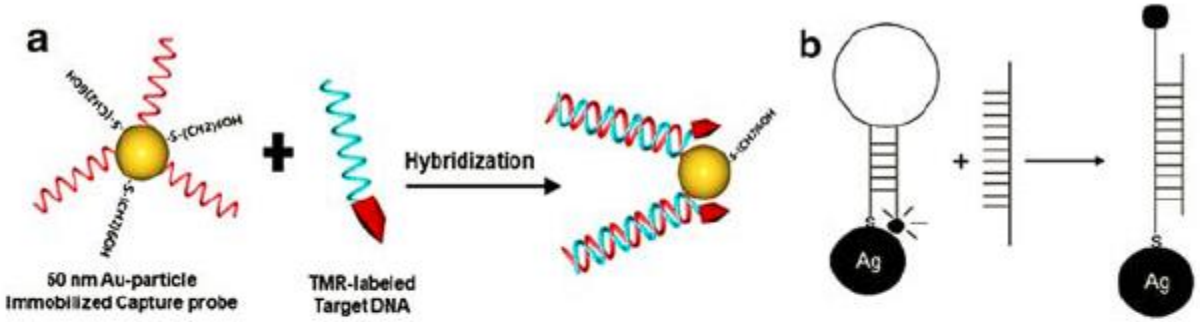
Assays employing labels (e.g. dyes) with SERS readout were pursued for the detection of protein and DNA biomolecules. The advantage over traditional fluorescence is narrow Raman spectra which are better suited for multiplexing and thus enabling detection of multiple reactions in parallel.

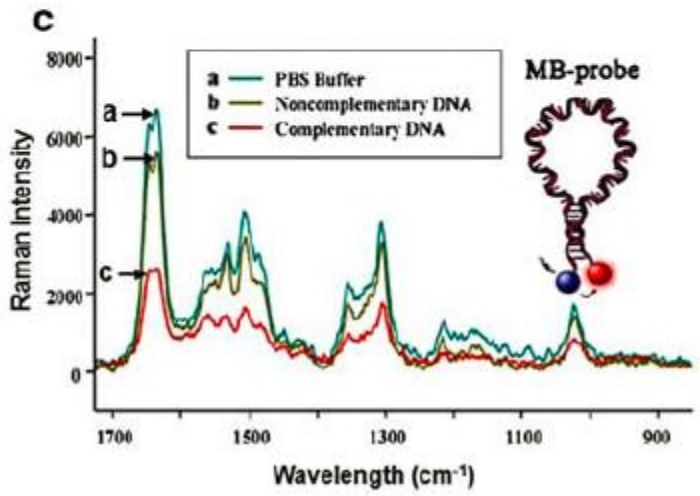
- Fluorescence emission band width for dyes >50 nm
- Fluorescence emission band width for quantum dots ~30 nm
- Raman peaks ~ 1 nm

Rapidly developing field, instrumentation advances resulted in portable devices available.

Ahura Scientific

SERS Assay - Example





a) Hybridization accompanied with a close contact of a dye and metallic NP – increase of SERS signal

b+c) Hybridization opens the hairpin and leads to a decrease in SERS signal

Microfluid Nanofluid (2009) 6:285–297, 123

J - Summary

- Fluorescence and its implementation for the readout of assays.
- Total internal reflection fluorescence – TIRF.
- Surface plasmons allow for the amplification of signal in fluorescence assays.
- Surface-enhanced Raman spectroscopy – SERS. Origin of the increasing of scattered intensity
- Examples of assays with SERS readout.

Final Remarks

Quick summary and questions in the exam:

- Confinement of light and its employment in probing biomolecules (evanescent field, surface plasmons, what they are).
- Basics of important spectroscopies (fluorescence spectroscopy, quantum yield, Stokes shift…)
- Surface plasmon resonance (SPR) for label-free analysis of biomolecules and their affinity interaction (principle of operation, example of implementation).
- Fluorescence – based analytical techniques, microscopy, TIRF, FRET.
- What is a biosensor and what are the key components.

Exam:

Two questions that will be based on the overview shown after each section.

Those will aim at principles and you will be asked to provide some examples / illustration of them.