EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

FZU Institute of Physics of the Czech Academy of Sciences

Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

Jakub Dostalek

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

FZU – Institute of Physics of the Czech Academy of Sciences,
Na Slovance 1 | Prague 182 00 | Czech Republic
T+420 776767927

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

Biointerfaces for Optical Biosensors

Content

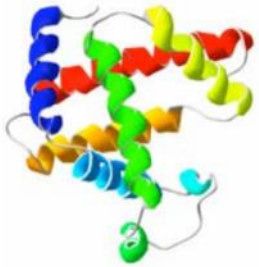
- Examples of optical biosensors
- Biosensor characteristics
- Biomolecular recognition elements
- Modification of oxide, metallic, and plastic surfaces
- Self-assembled monolayers
- Polymer brushes
- Polymer networks
- Immobilization strategies for coupling functional biomolecules

Biosensor

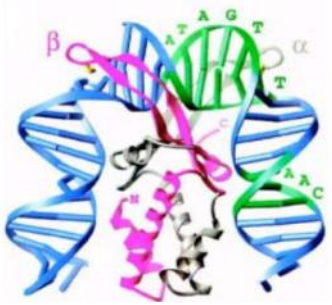
… is self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC 1996).

DNA/DNA

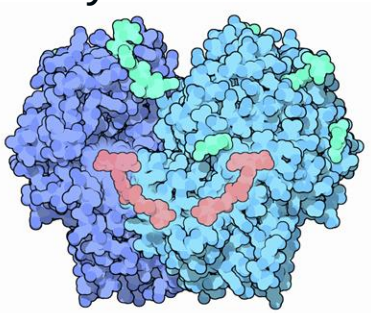
Protein/Protein

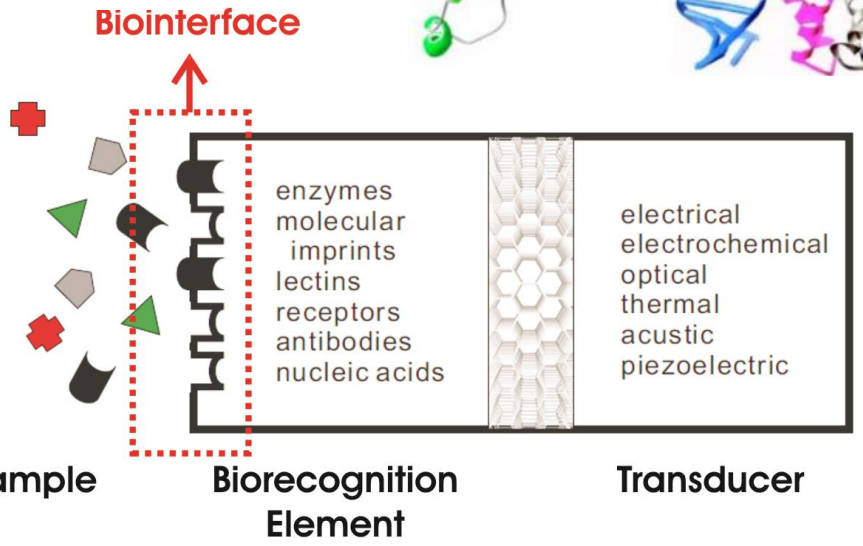


Protein/DNA



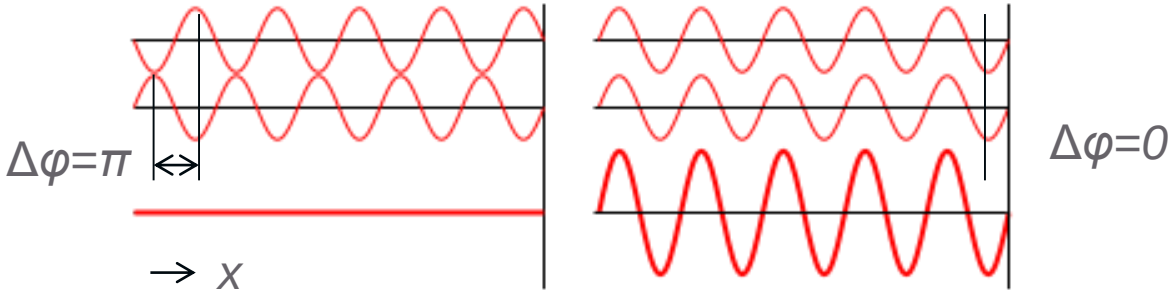
Enzyme





Interference

Optical phenomenon arising from (coherent) superimposing of amplitudes of two spatially overlapping waves. When changing a phase φ of one of the waves, intensity is varied.



Field amplitudes are subtracted – destructive interference

Field amplitudes are added – constructive interference

$\Delta\varphi = k_0 n d$ – a minute changes in refractive index shifts the phase and alters the intensity

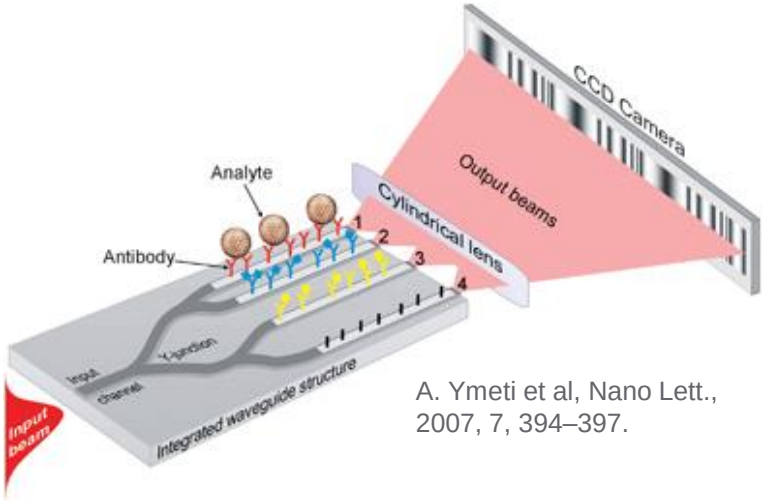
$$u(x,t) \propto \sin(k_0 n x - \omega t)$$

Exploited in (arguably) most sensitive optical measurements: Frequency stabilized lasers for metrology, microscopy with phase contrast, narrow optical filters,…

Dielectric Waveguides

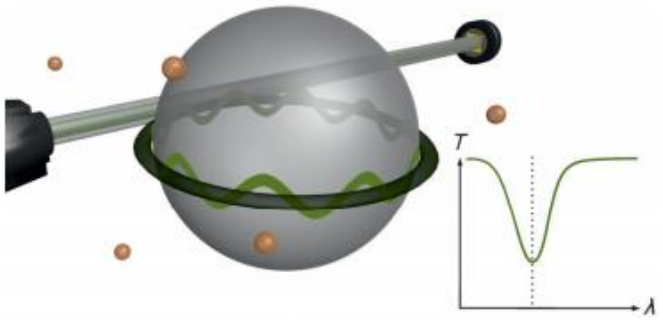
Various other optical biosensor platforms that are sensitive to binding-induced refractive changes have been developed. Further two examples that holds potential for highly compact devices based on integrated optics will be presented:

Waveguide interferometer



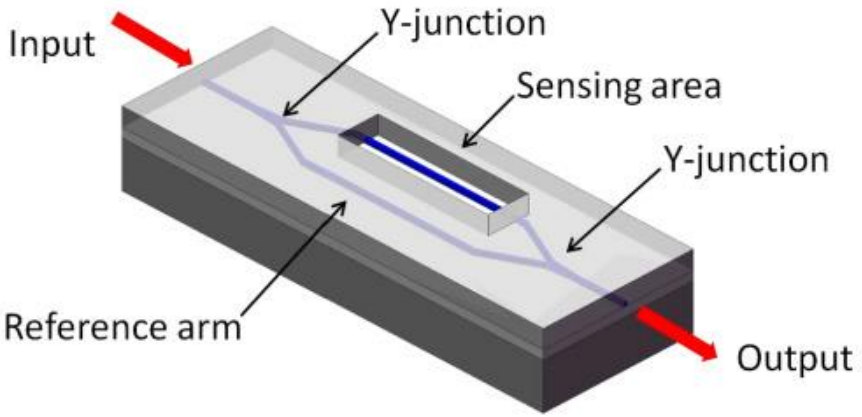
A. Ymeti et al, Nano Lett., 2007, 7, 394–397.

Ring resonators

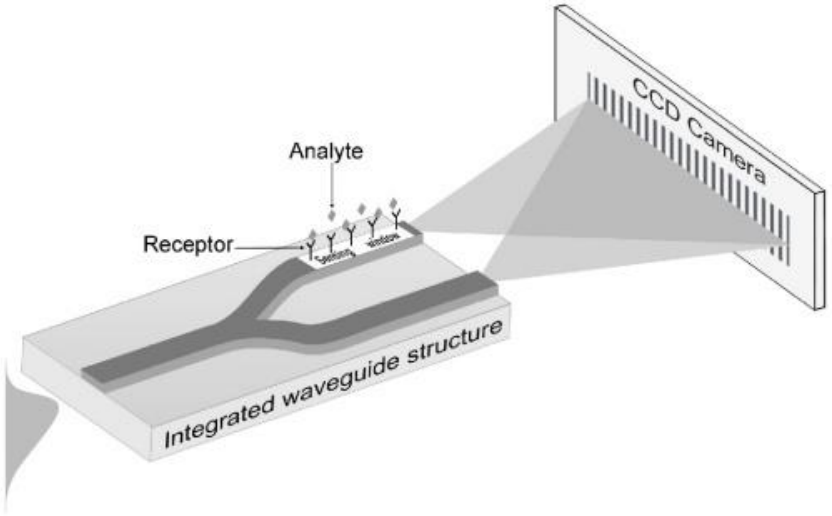


Martin Baaske and Frank Vollmer, ChemPhysChem 2012, 13, 427 – 436

Implementation of Interferometers



Optics Express, Vol. 20, Issue 7, pp. 7195-7205 (2012)



Optics Express, Vol. 20, Issue 19, pp. 20934-20950 (2012)

Mach-Zehnder interferometer:

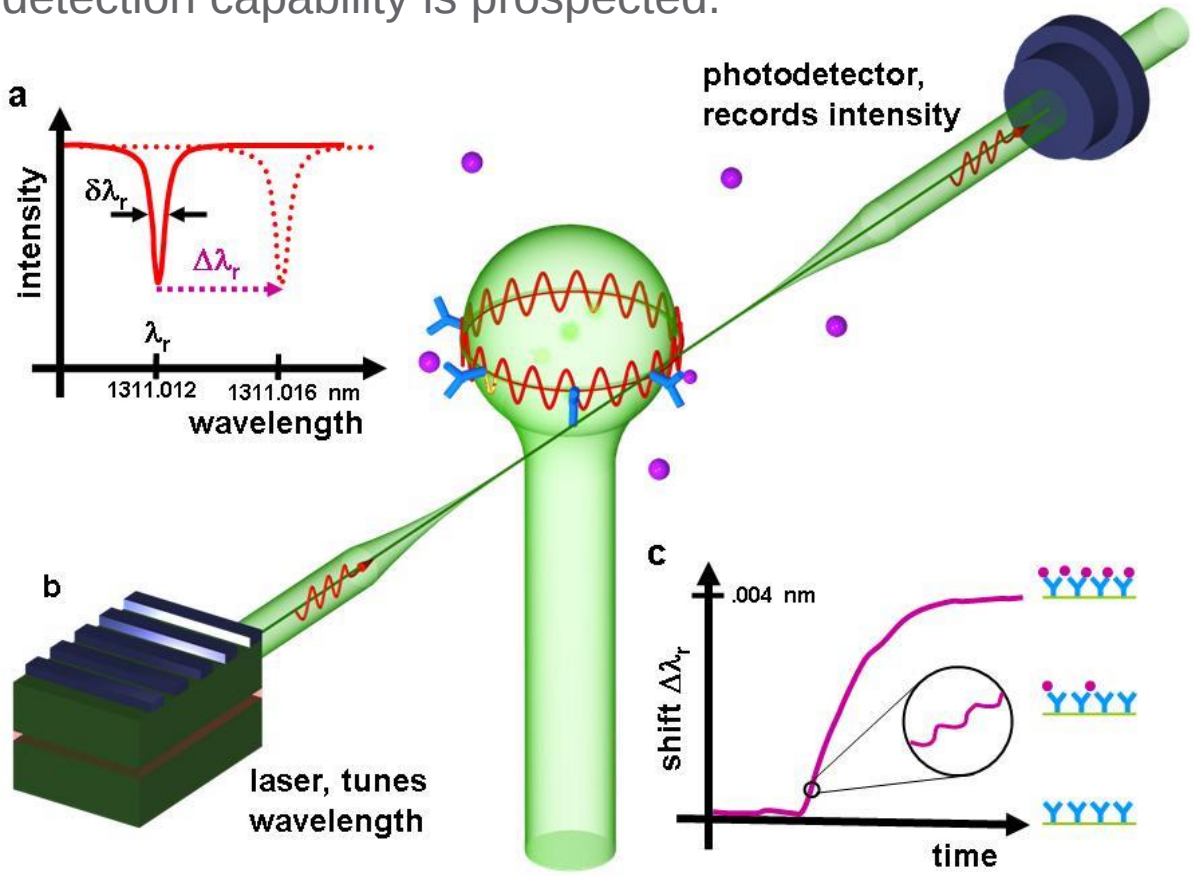
Detection of output intensity change induced by the capture of analyte on the sensing area

Young interferometer:

Detection of interference pattern shifts induced by the capture of analyte

Ring Resonators

Optical micro-resonators that exhibit a large Q-factor and small modal volume V (large Q/V) - highest sensitivity for label-free detection of molecules. Single-molecule detection capability is prospected.

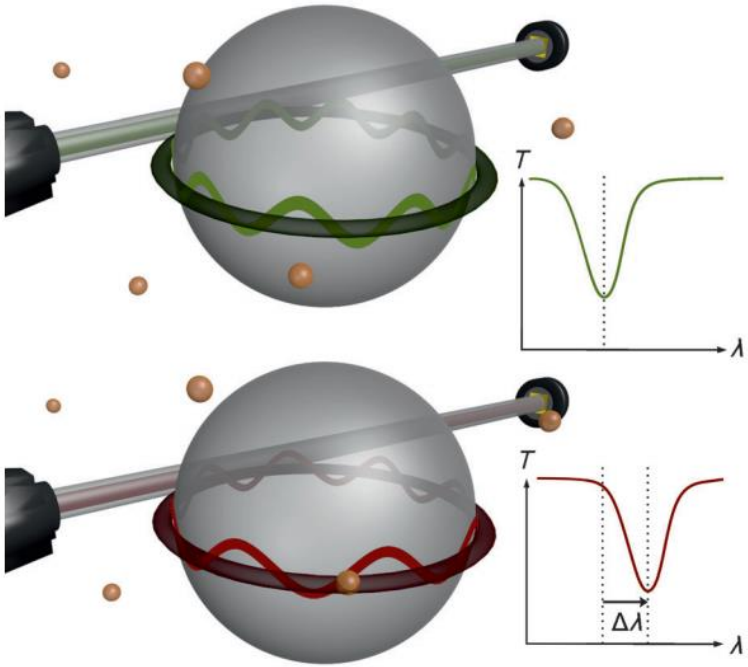


Vollmer group: http://mpl.mpg.de/mpf/php/bfp/research/wgm_biosensing.php

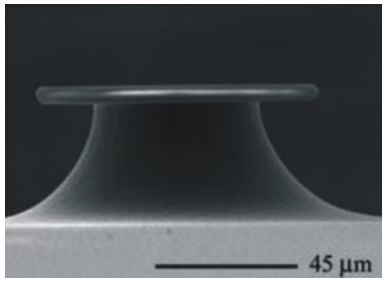
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education
MINISTRY OF EDUCATION, YOUTH AND SPORTS
FZU Institute of Physics of the Czech Academy of Sciences
AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

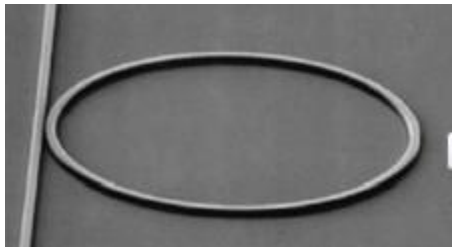
Implementations of Ring Resonators

Possible implementations include those based on silica microspheres coupled to tapered optical fibers (left) as well as integrated optical structures prepared by lithography (right).



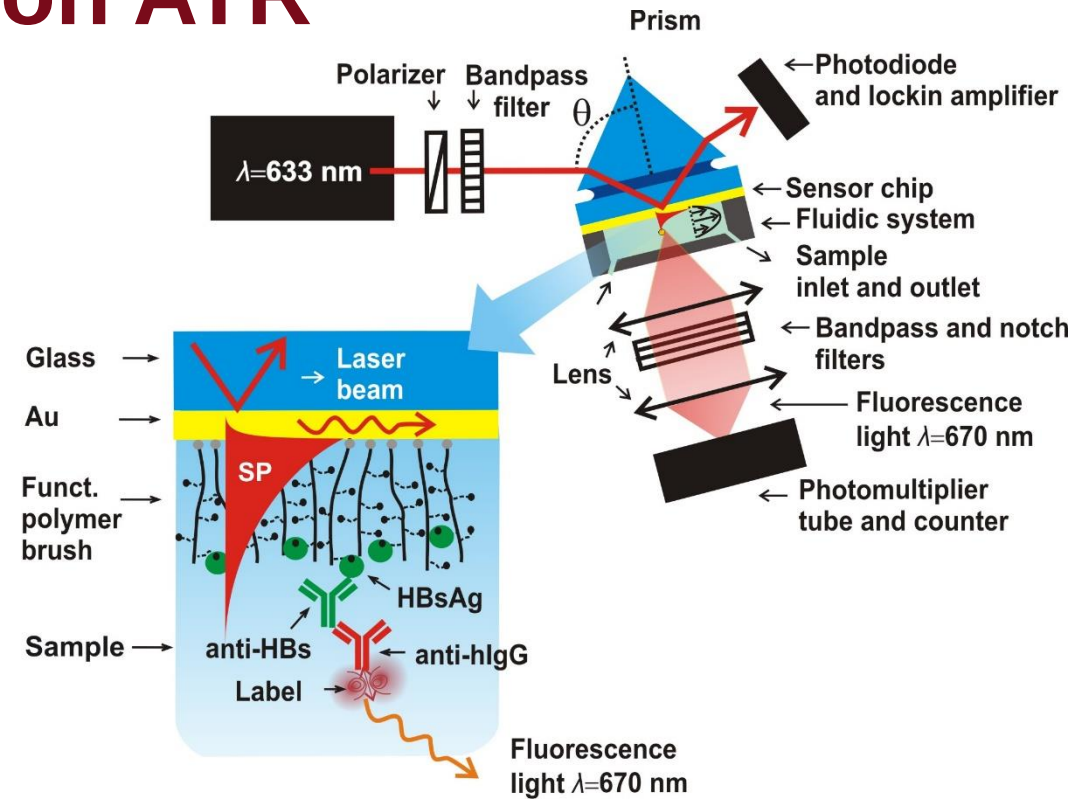
Martin Baaske and Frank Vollmer,
ChemPhysChem 2012, 13, 427 – 436





Analytica Chimica Acta
Volume 620, Issues 1–2, 14 July 2008, Pages 8–26

Implementation of SPR and PEF based on ATR

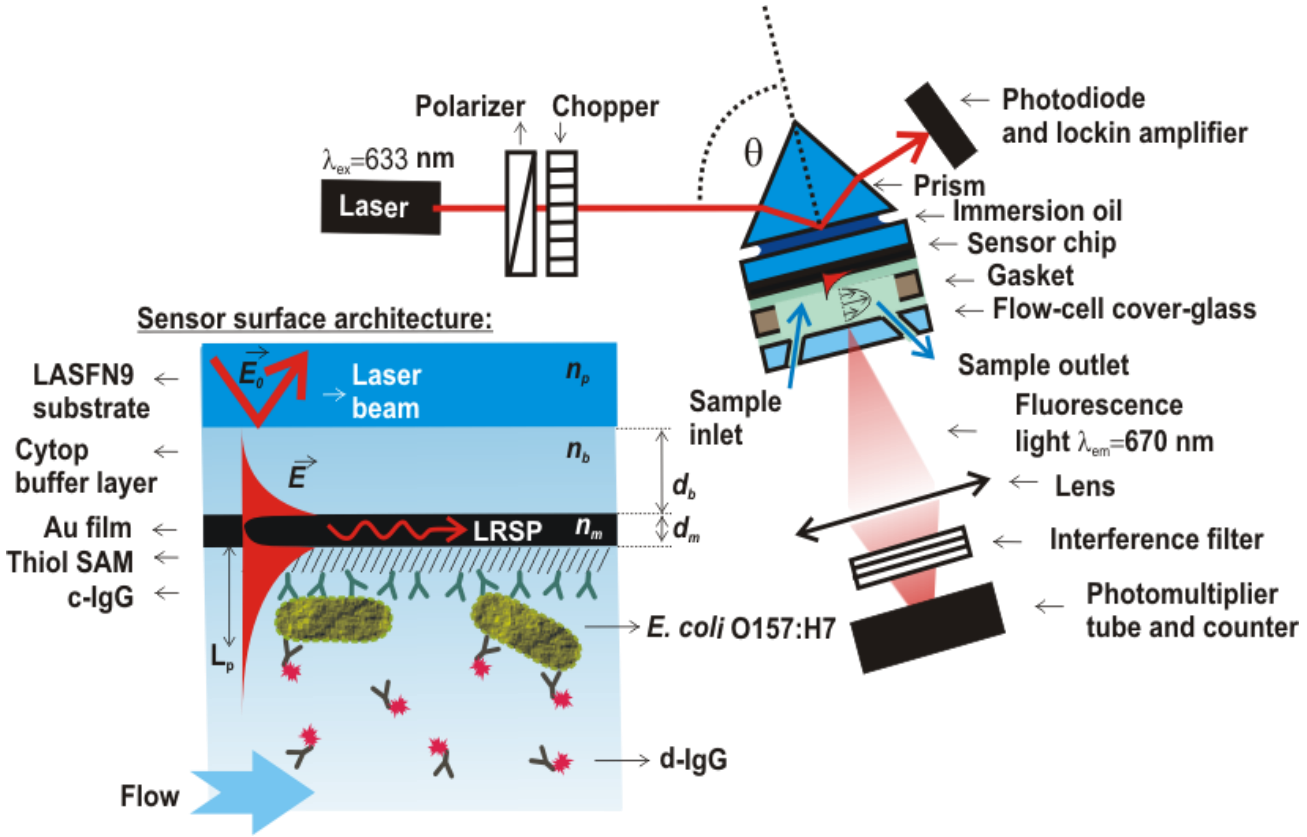


- Angular interrogation of SPR in **Kretschmann configuration** of attenuated total reflection method (ATR) that is combined with **plasmon-enhanced fluorescence spectroscopy** detection (SPFS).

T. Riedel, S. Hageneder, F. Surman, O.Pop-Georgievski, C. Noehammer, M. Hofner, E. Brynda, C. Rodriguez-Emmeneger, J. Dostalek, Plasmonic Hepatitis B biosensor for the analysis of clinical saliva, Analytical Chemistry, 2017, 89 (5), 2972.

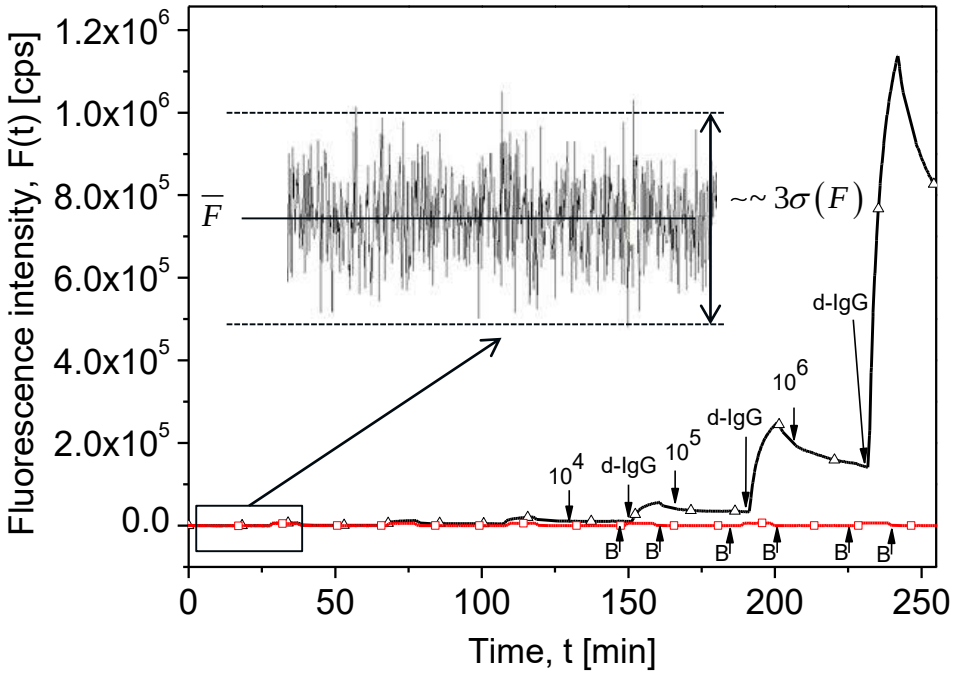
Biosensor Characteristics

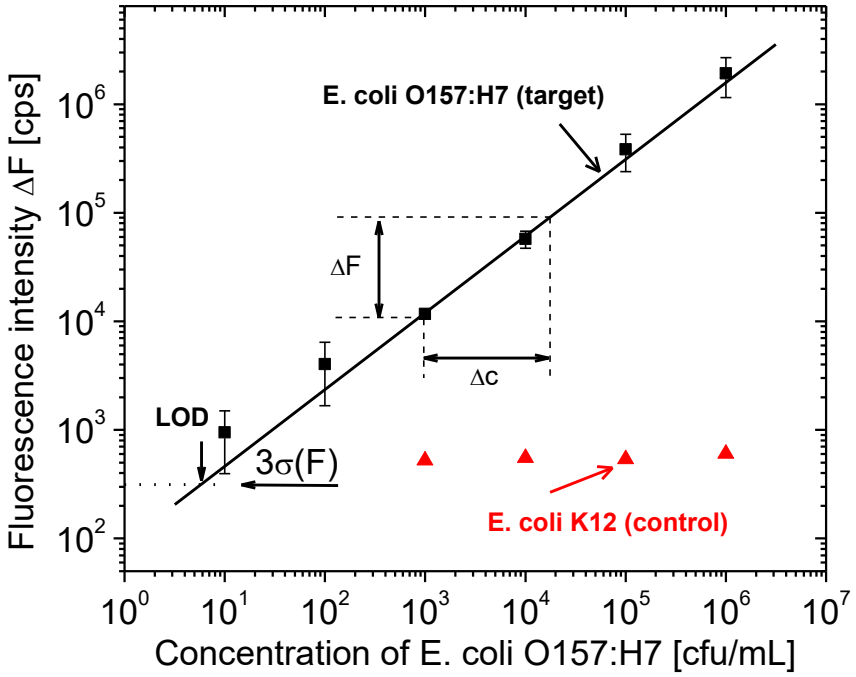
Long Range Surface Plasmon-Enhanced Fluorescence Spectroscopy (LRSP-FS)



J. Dostalek et al., Plasmonics (2007) 2, 97-106. K.Toma, et al, Optics Express (2011), Vol. 19, Iss. 12, pp. 11084–11089.
R. Mejard, C.J. Huang, J. Dostalek, H. Griesser, B. Thierry, Optics Materials, (2013), 35(12) pp 2507-2513.

Calibration Curve





C.J. Huang et al , Biosensors and Bioelectronics (2010), 26, 4, 1425-1431.

Sensitivity $S=\Delta F/\Delta c$

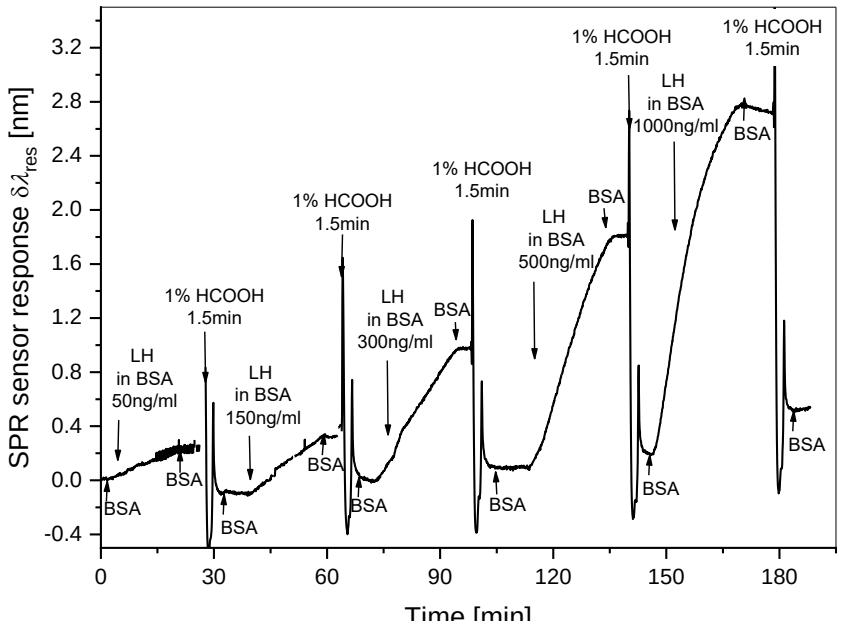
Sensor signal noise described by stand. deviation $\sigma(F)=\sqrt{\frac{1}{N-1}\sum_i\left(F_i-\overline{F}\right)^2}$

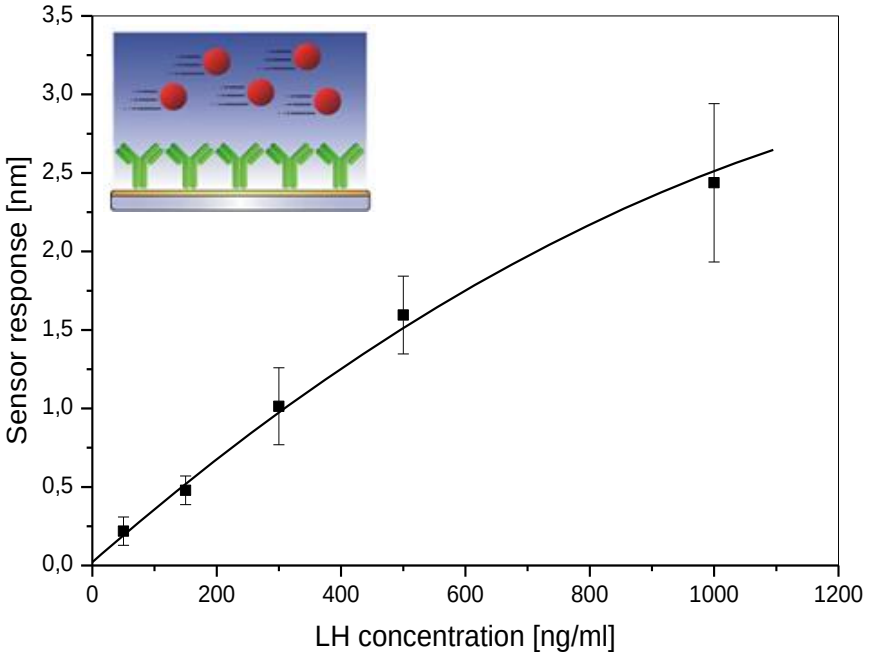
Limit of detection (LOD) determined from sensor noise as $LOD=3\sigma(F)/S$

Limit of quantification (LOQ) determined from sensor noise as $LOD=10\sigma(F)/S$

Regeneration of the Sensor

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).

Performance Characteristics

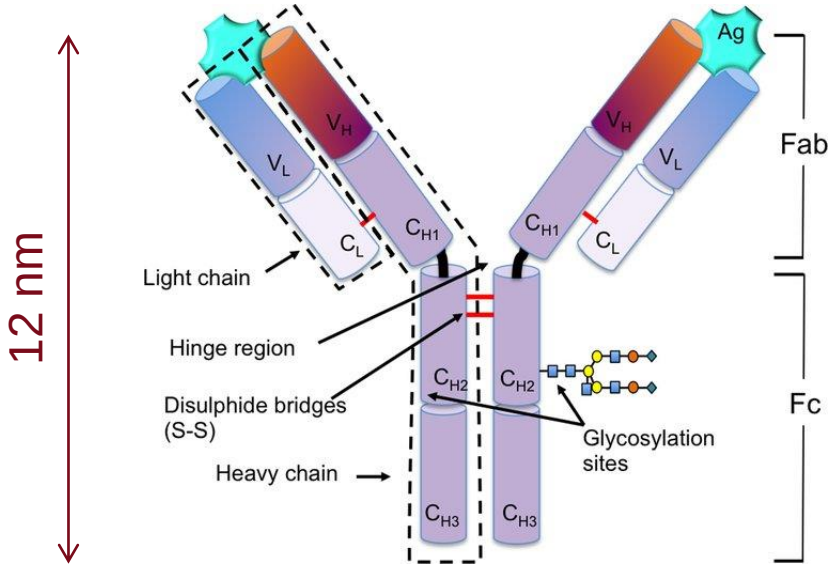
Detection range:	Concentrations of analyte that can be determined.
Sensitivity:	The value of the sensor response per analyte concentration.
Limit of detection	Minimum concentration of analyte that can be detected
Specificity / selectivity:	Interference of the presence of other compounds must be minimized for obtaining the correct result.
Matrix effect	Detection in real samples (e.g. blood serum) is rather more difficult than in model ones (e.g. buffer)
Analysis time:	The necessary time to carry out the analysis
Reusability:	Sensor chips are used only once or can be regenerated for multiple detections.

Biomolecular Recognition Elements

Polyclonal Antibodies

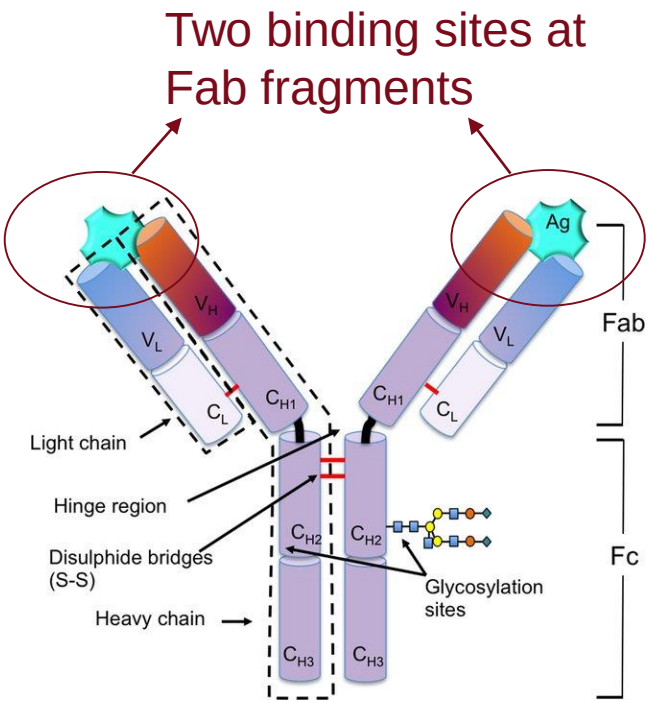
Immunoglobulin G antibody with MW of about 150 kDa.

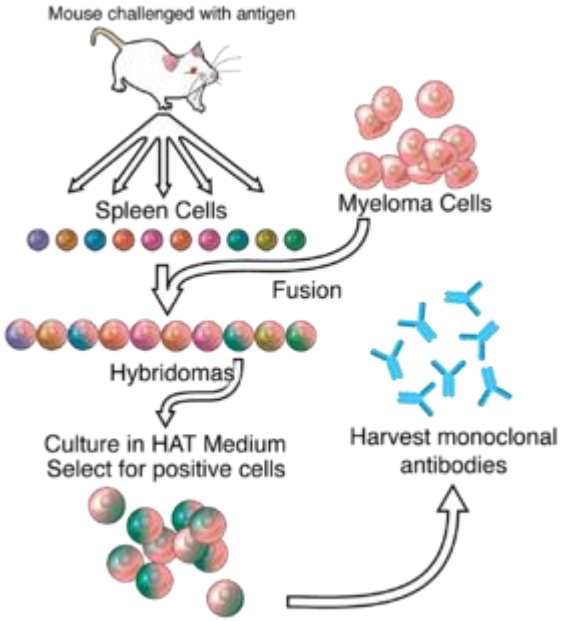
Hydrodynamic radius of about 7.5 nm



- 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

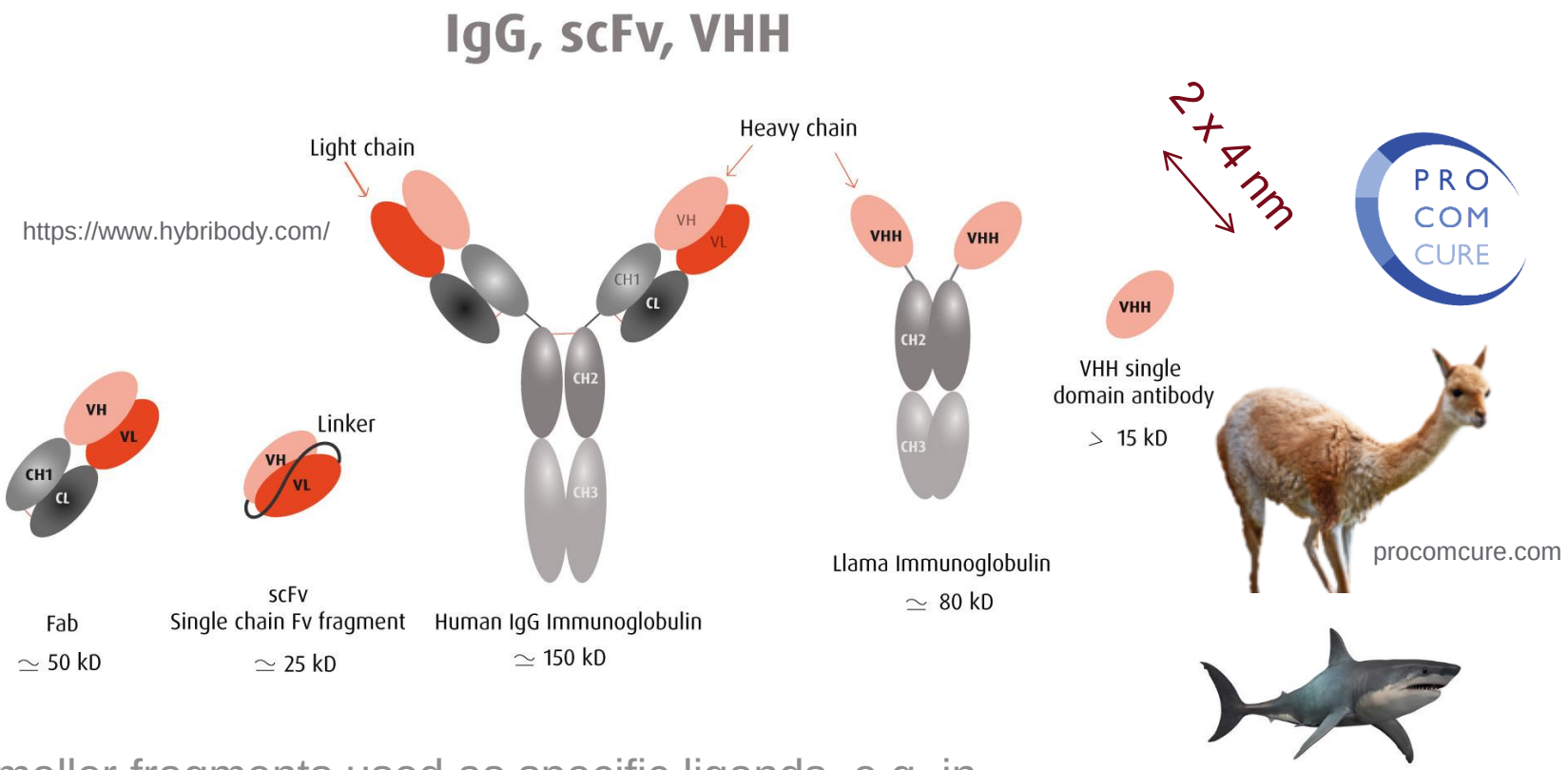




https://en.wikipedia.org/wiki/Monoclonal_antibody

- 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

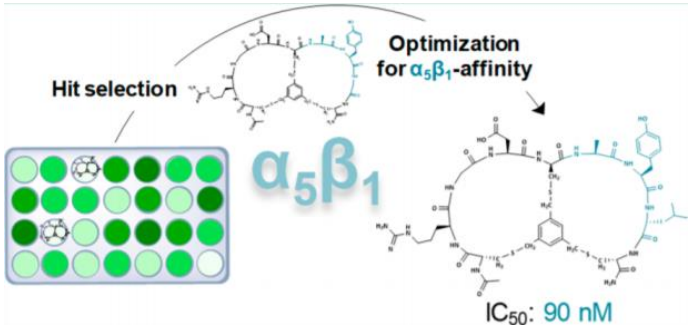
Antibody Fragments - Nanobodies



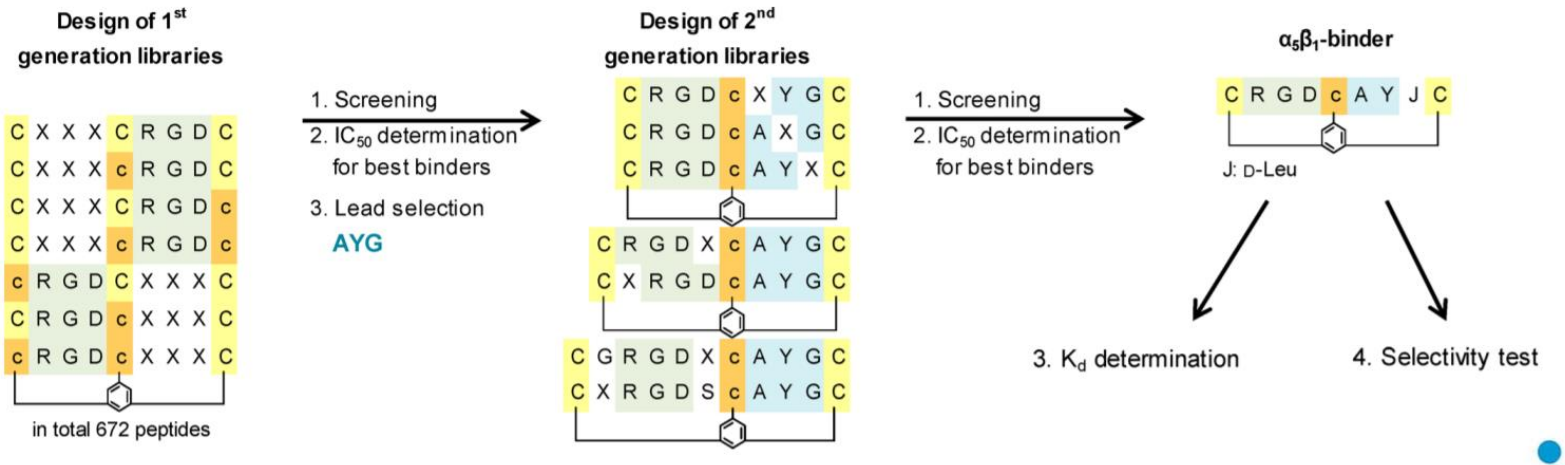
- Smaller fragments used as specific ligands, e.g. in therapeutic use. Accessibility of hidden epitopes and in biosensors more recognition sites can be attached to their surface.

Camelids and sharks

Peptides



Molecular weigh of > 1 kDa

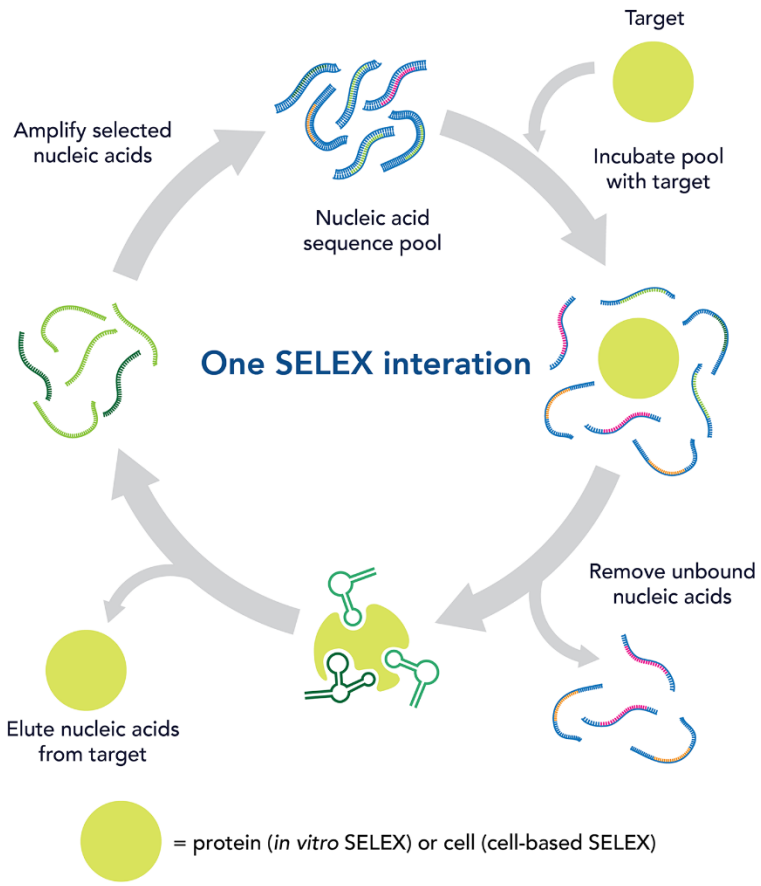


https://doi.org/10.1021/acscombsci.9b00081

PEPSCAN

- 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…)

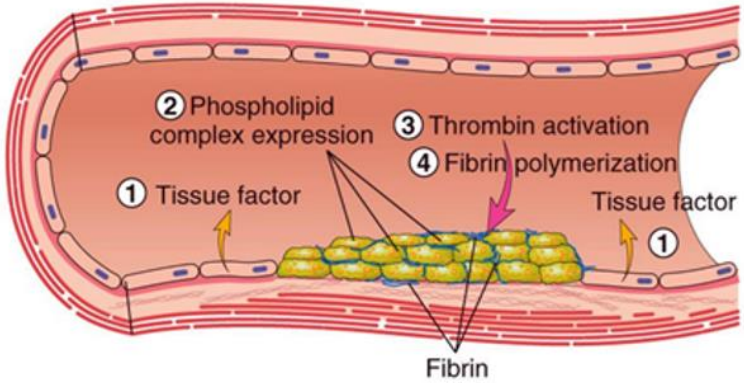
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

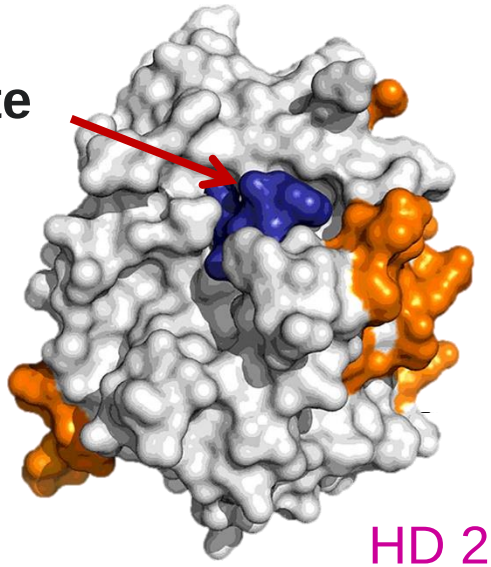
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

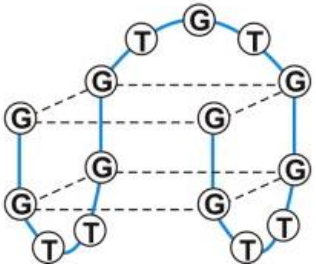
Thrombin Aptamer



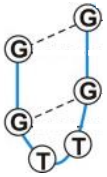
Periayah MH, Halim AS, Saad AZM. Mechanism action of platelets and crucial blood coagulation pathways in Hemostasis. Int J Hematol Stem Cell Res. 2017;11(4):319–27.



HD 1



HD 1 short



HD 22

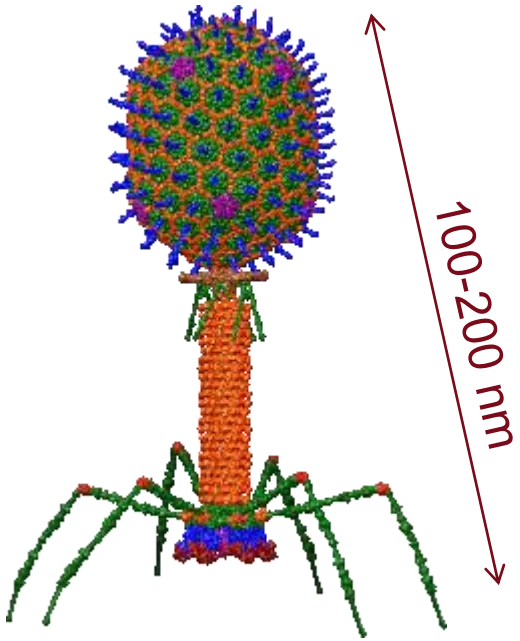
- A set of aptamers specifically recognizing thrombin with nM K_d constant discovered

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

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

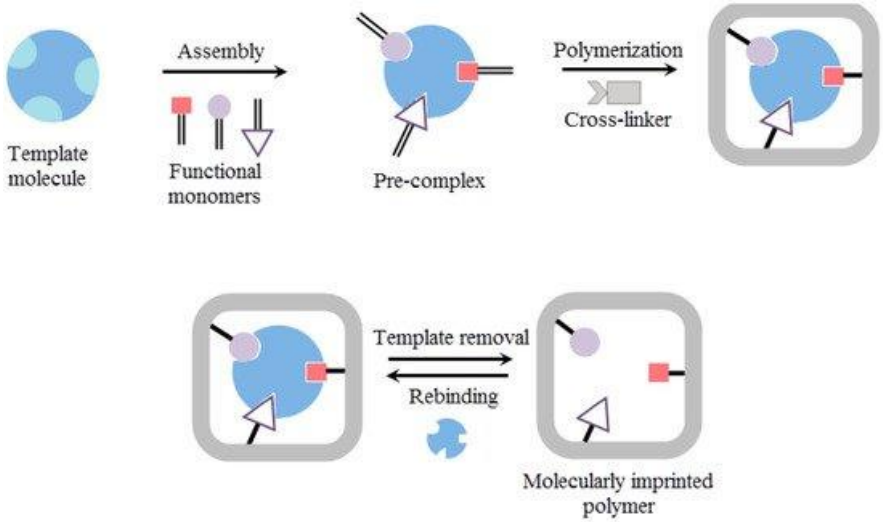
Bacteriophages

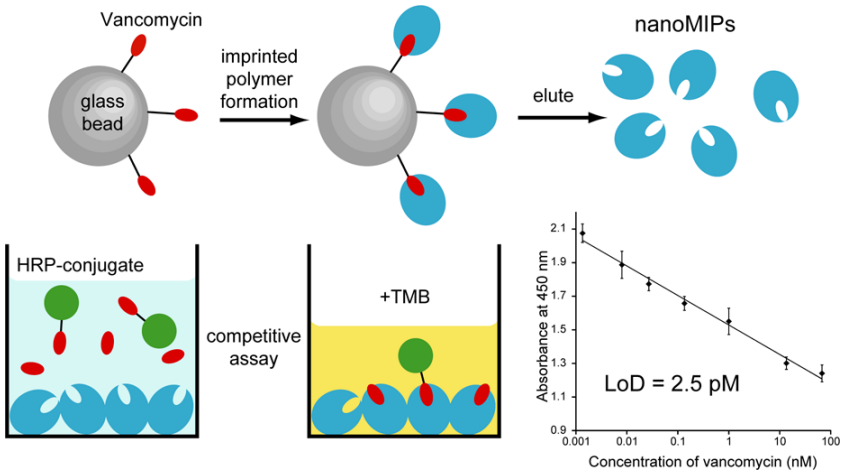
- Large viruses with the ability to recognize bacteria.
- Used since the late 20th century as an alternative to antibiotics in the former USSR. They are seen as a possible therapy against multidrug resistant strains of many bacteria.
- Explored as ligands in biosensing application areas.



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

Molecular Imprinted Polymers





https://doi.org/10.3390/s19061279

dx.doi.org/10.1021/la401891f

- Polymerized cavities in a presence of a template molecules. After eluting the template derived from a target molecule they serve as selective binding pockets.

Architectures of Biointerfaces

Surface Architectures

Transducer surface is typically from metal (e.g. gold), oxide (e.g. SiO2), or polymer (e.g. polystyrene). In order to couple functional biomolecules to the these surfaces, following approaches can be utilized:

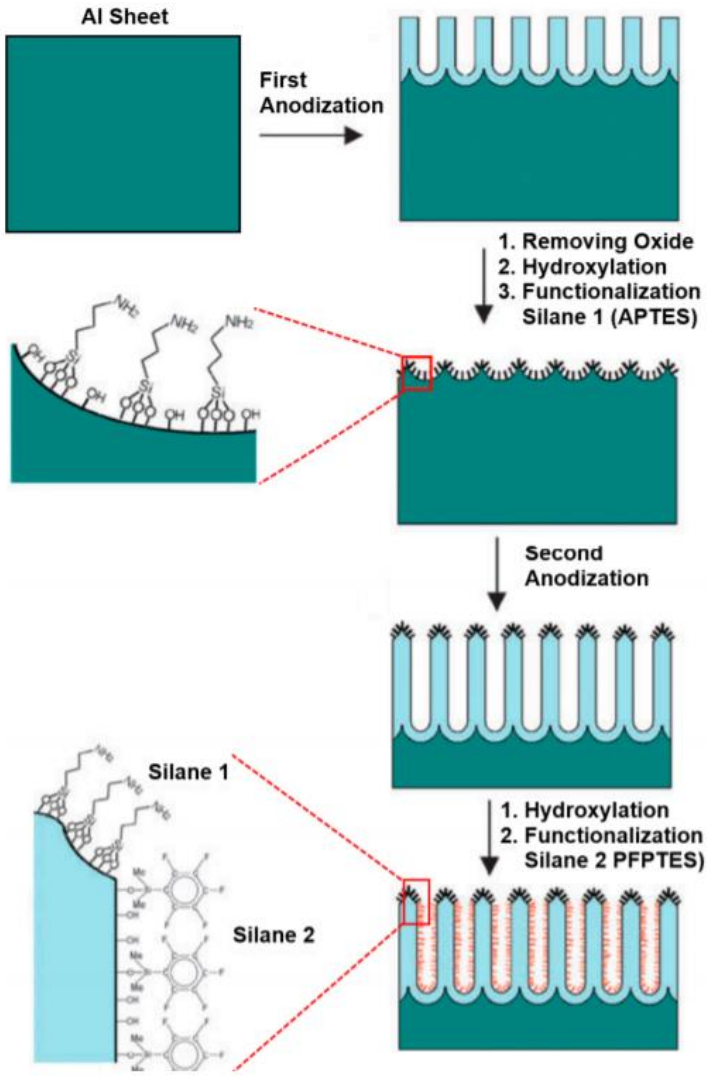
- Physisorption
- Covalent attachment (amine coupling, click reactions)
- Affinity coupling (streptavidin, protein G)
- Coulombic interaction (layer-by-layer)

Often biomolecules are be attached to the surface via linker molecules that can form 2D or 3D architectures:

- 2D monolayer systems (e.g. thiol SAM)
- 3D architecture (e.g. polymer brushes or hydrogels)

Silane Modification

- Oxide surfaces (SiO2, TiO2, Aluminum oxide,…) are often modified with silane-based molecules.
- Popular is APTES (3-Triethoxysilylpropylamine) providing groups for amine coupling or for layer-by-layer modification.
- Reacting with OH groups (Piranha cleaning), complications due to formation of multilayers.

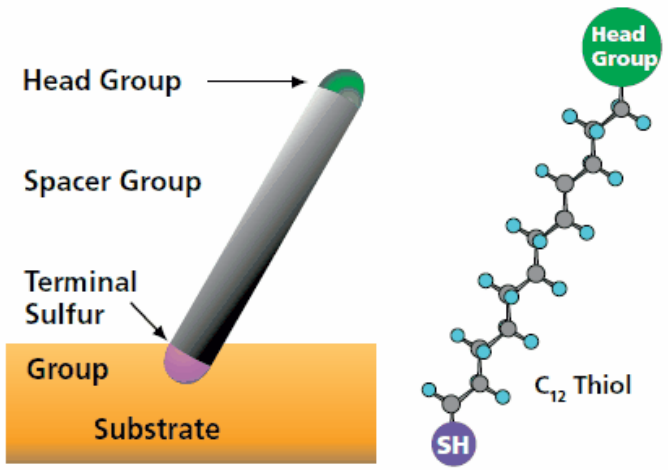


Sensors 2014, 14, 11878-11918; doi:10.3390/s140711878

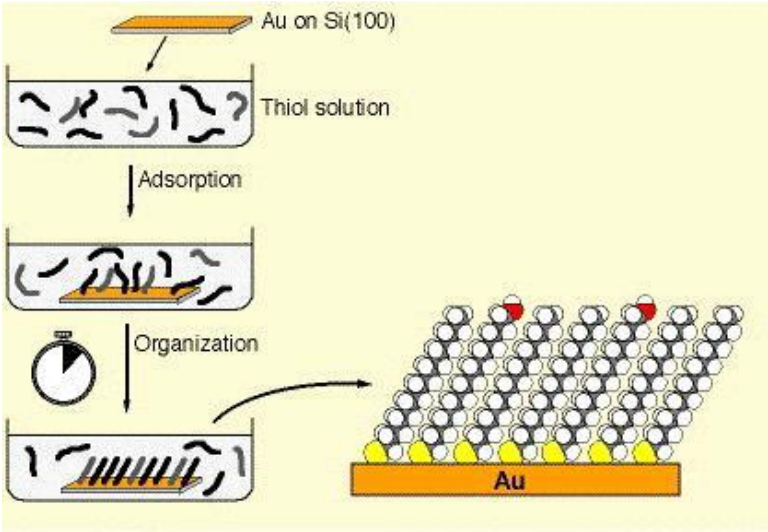
Mixed Thiol SAM

- Thiol monolayers studied heavily since 90ties of the last century. Forms well-defined monolayers on gold surface via self assembly (SAM).

- Wide range alkanethiols are available for functional modification of gold surface. Arguably reason for the success of SPR biosensors.



www.sigmaaldrich.com



https://www.ifm.liu.se/applphys/molphys/research/sam/sam-further/index.xml

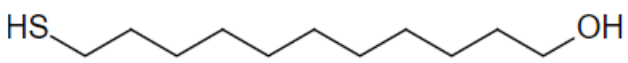
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

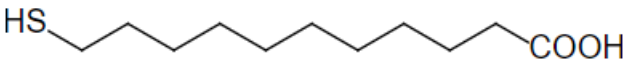
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

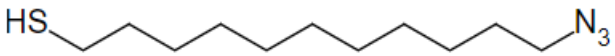
Thiol-based Modification



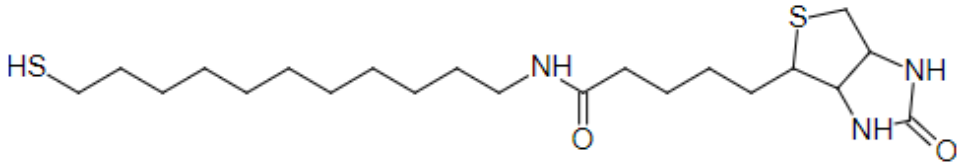
Hydrophilic surface



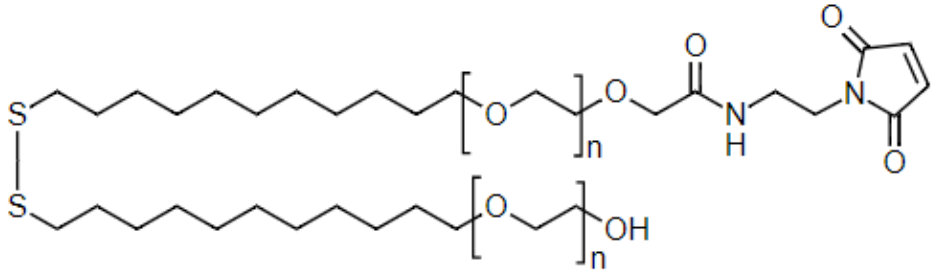
Amine coupling



Azide group for click coupling



Biotin



Oligo (ethylene glycole) / Maleimide

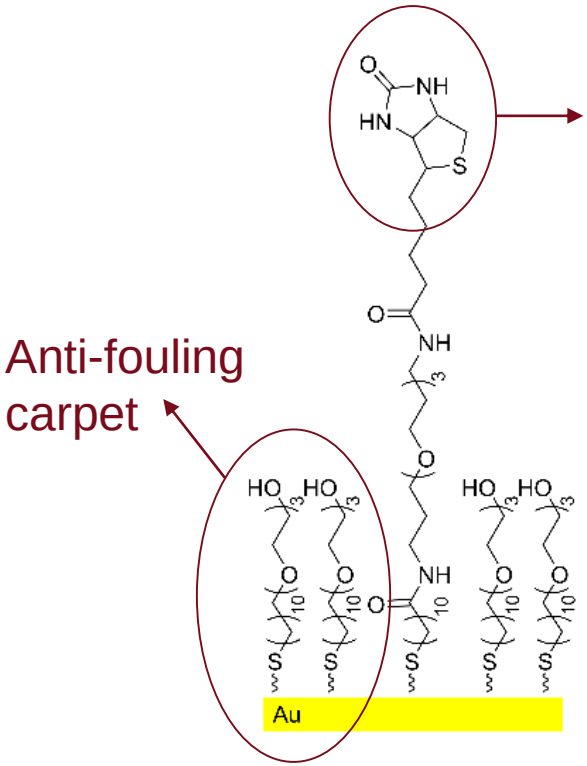
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

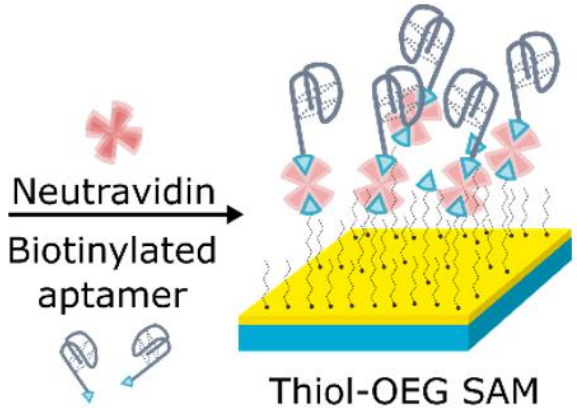
MINISTRY OF EDUCATION, YOUTH AND SPORTS

FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

Mixed Thiol SAM





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

- Mixed thiol SAM allows combining different functionality.

Coupling Chemistry – Streptavidin

Langmuir
The ACS Journal of Surfaces and Colloids

SEPTEMBER 1991
VOLUME 7, NUMBER 9

Letters

Biotin-Functionalized Self-Assembled Monolayers on Gold: Surface Plasmon Optical Studies of Specific Recognition Reactions

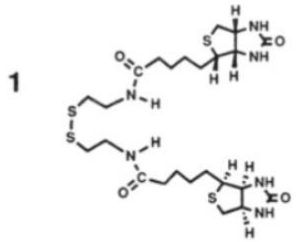
L. Häussling and H. Ringsdorf*

Institute of Organic Chemistry, Johannes Gutenberg University, J. J. Becherweg 22, D-6500 Mainz, Federal Republic of Germany

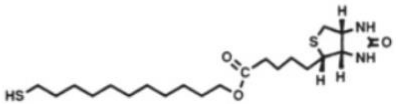
F.-J. Schmitt and W. Knoll*

Max Planck Institute for Polymer Research, Ackermannweg 10, D-6500 Mainz, Federal Republic of Germany

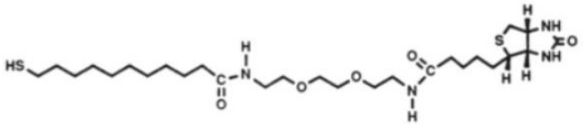
Received March 27, 1991. In Final Form: June 28, 1991



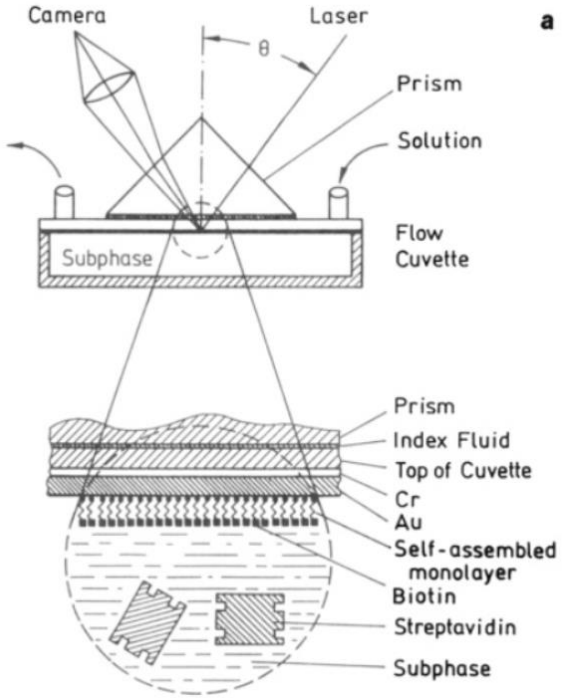
1



2



3



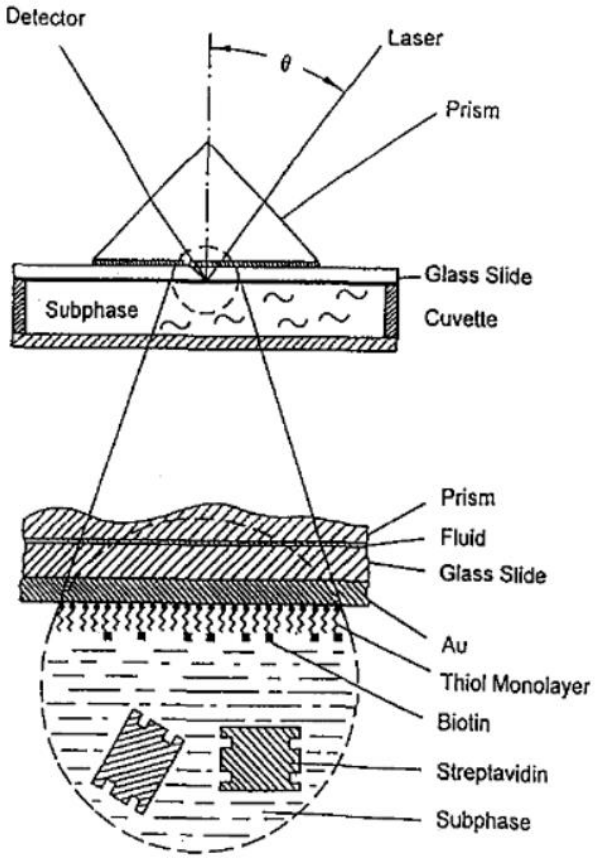
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

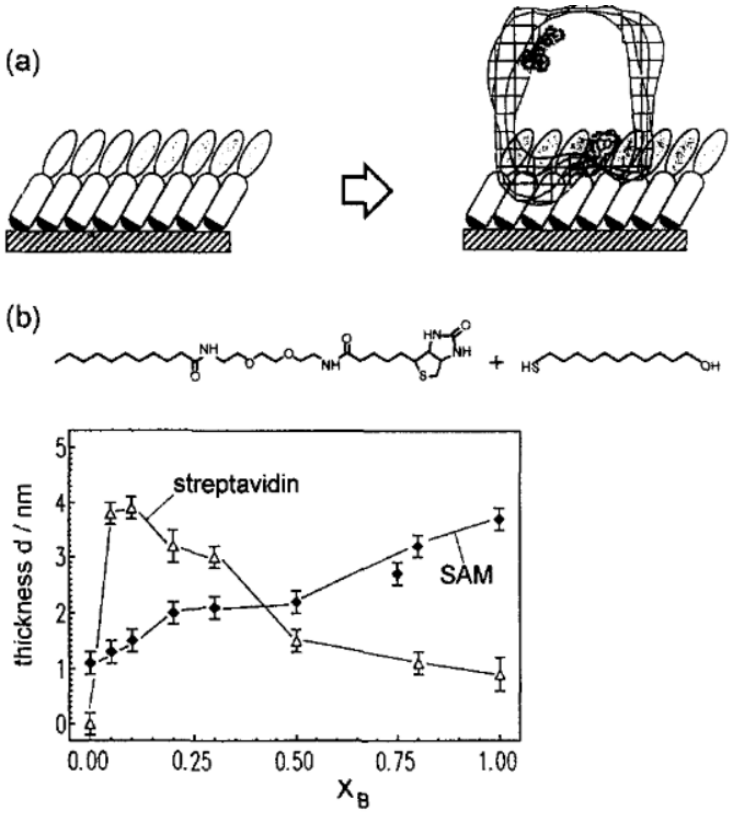
MINISTRY OF EDUCATION, YOUTH AND SPORTS

FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

Coupling Chemistry – Streptavidin on Mixed Thiol SAM





- Controlling of the density of biotin groups enables reaching optimum coverage with streptavidin

https://doi.org/10.1016/S0065-227X(97)89642-6

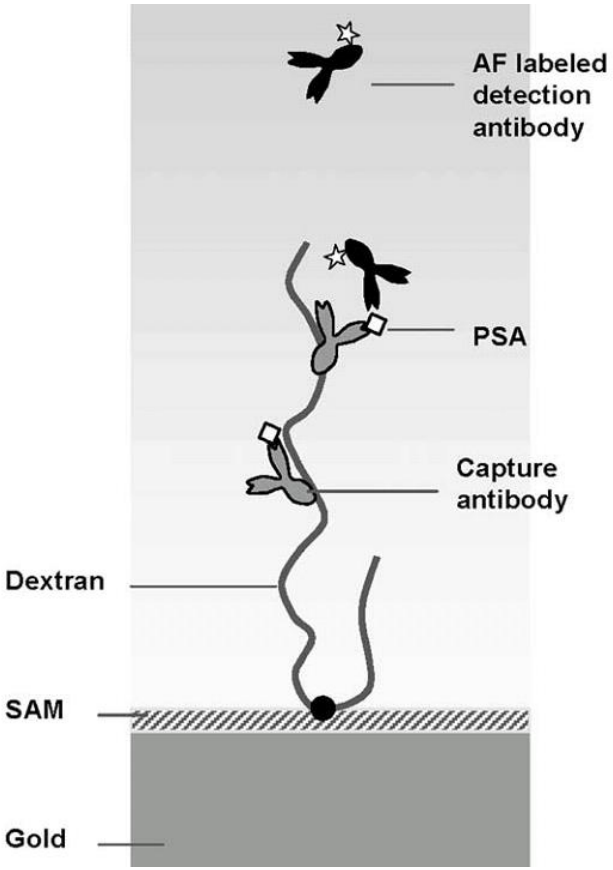
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

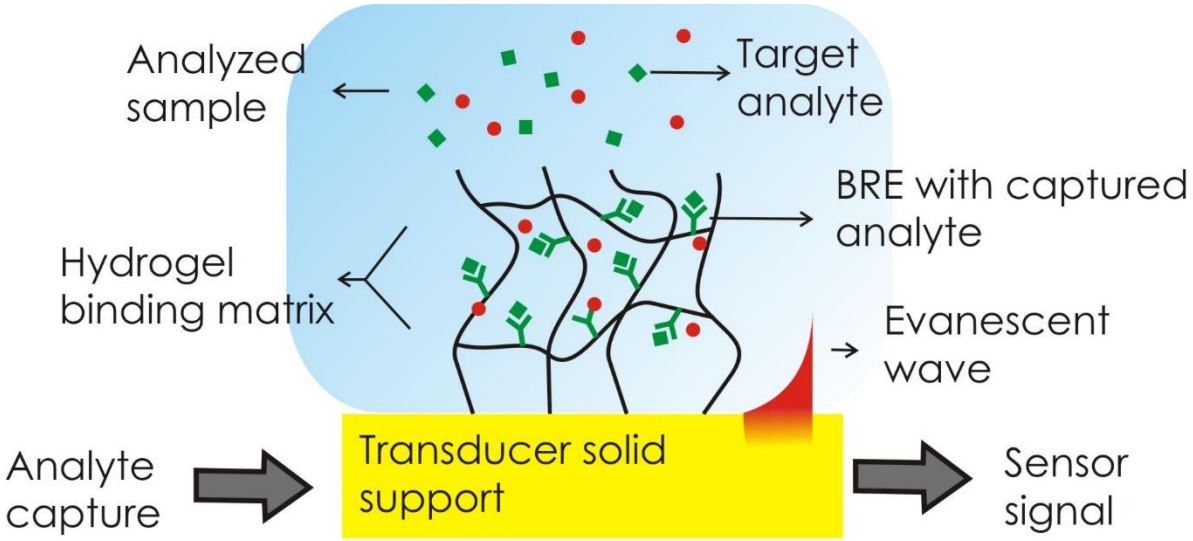
Polymer Brushes



https://doi.org/10.1016/0021-9673(92)80137-J

- ‘Grafted to’ carboxylated dextran polymer chains are used as a binding matrix on Biacore SPR chips (CM5).
- Attachment of ligands to flexible polymer chains allows suppressing the steric hindrance for affinity binding studies.
- Design allows diffusion of biomolecules through the brush layer and binding the attached ligands.

Hydrogels



Functionalized hydrogel to serve as a binding matrix in evanescent wave affinity biosensors for rapid detection of analytes

- **Crosslinked polymer chains** that forms a network, higher thickness > 1 μm
- **Large binding capacity** – accommodating large amounts of ligand.
- **Anti-fouling properties** – avoiding non-specific capture of non-target molecules.

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

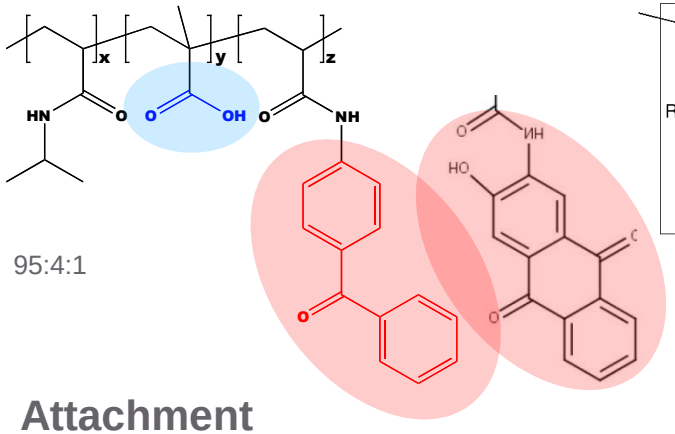
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

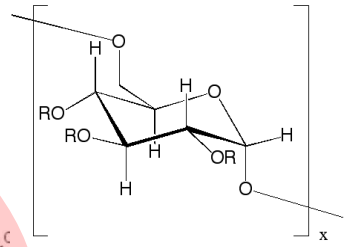
UNIVERSITÄT SIEGEN

Hydrogel Chemical Toolbox

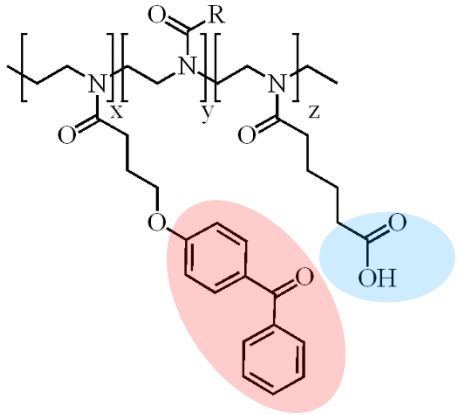
NIPAAm-based



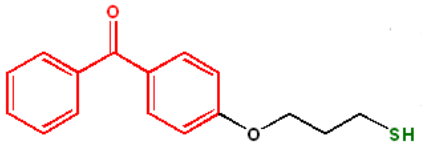
Dextran-based

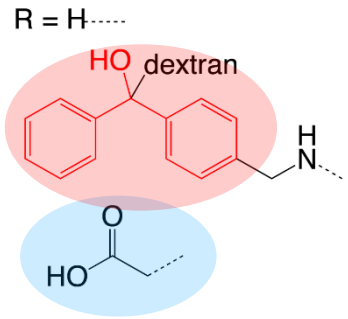


Oxazoline-based



Attachment





Various hydrogels were synthesized based on synthetic (e.g., NIPAAm), and natural (e.g. dextran) polymers

- Photocrosslinkable (●)
- Post-modification with functional moieties (●)

A. Mateescu, Y. Wang, J. Dostalek, U. Jonas, Thin Hydrogel Films for Optical Biosensor Applications, Membranes, (2012), 2(1), 40-69.
C. Petri, S. Hageneder, J. Dostalek, W. Knoll, U. Jonas, Novel Thermoresponsive and Photocrosslinkable Poly(2-alkyl-2-oxazolines) - Polymer Synthesis and Investigation of Responsive Hydrogel Coatings, Macromolecules, in preparation.

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

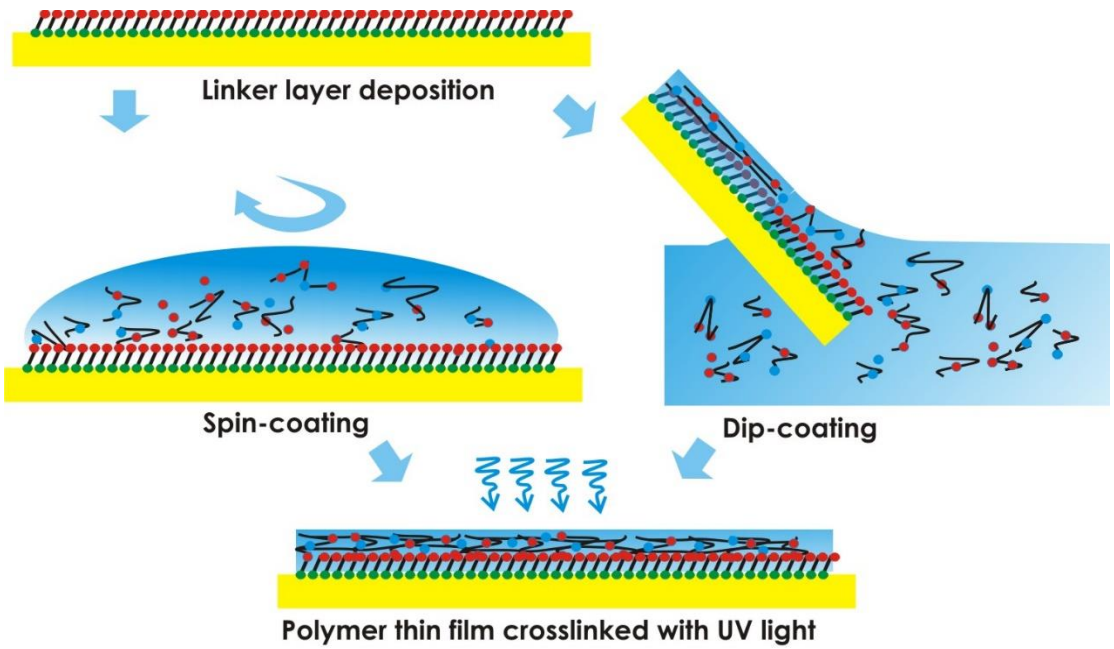
MINISTRY OF EDUCATION, YOUTH AND SPORTS

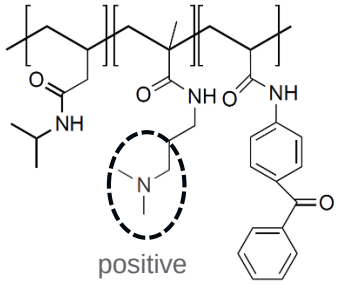
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

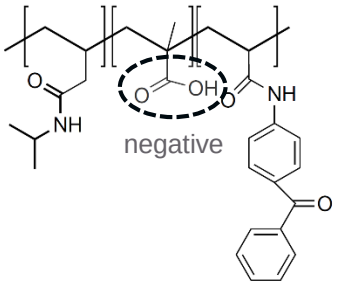
UNIVERSITÄT SIEGEN

Polymer Layer Deposition





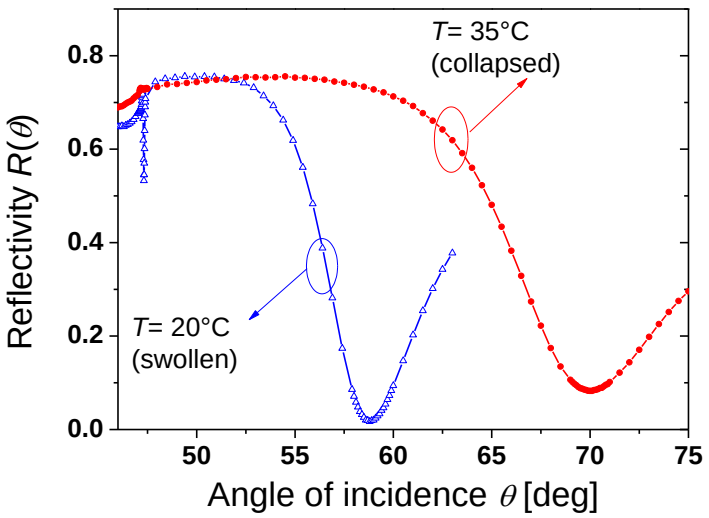
poly(NIPAAm-co-DMAPMAm-co-BPAAm)

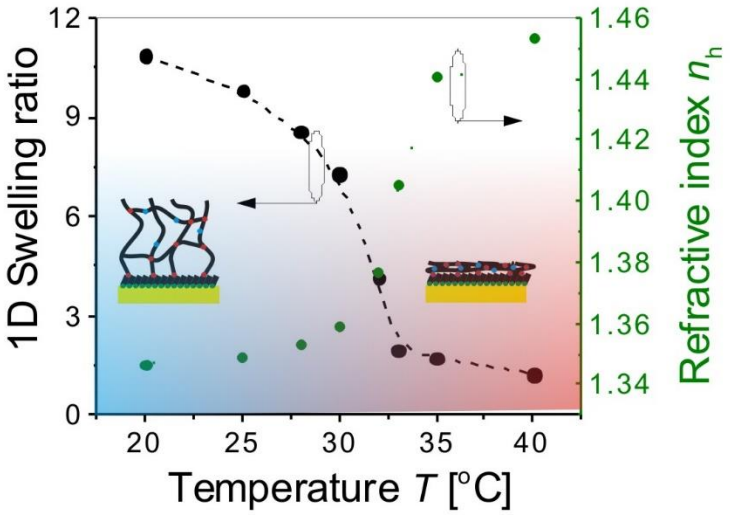


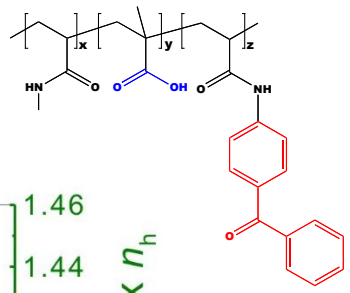
poly (NIPAAm-co-MAA-co-BPAAm)

- Spin-coating or dip-coating for films with thickness of nm - µm.
- Layer-by-layer films with thickness of nm.

Responsive NIPAAm-based Hydrogel Film





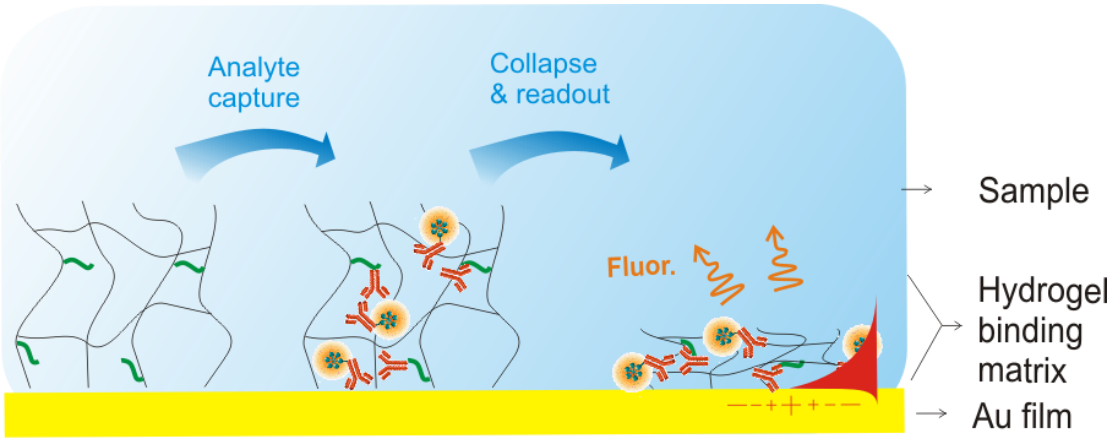


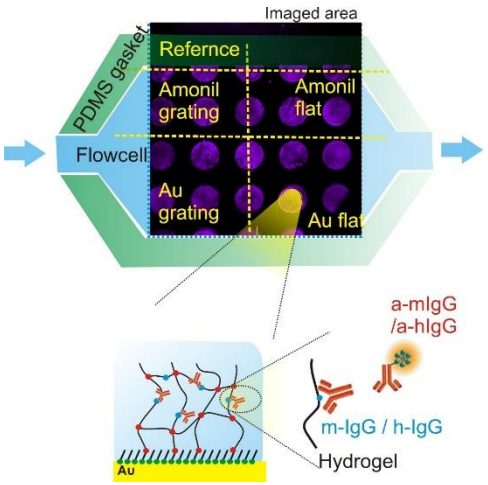
Equilibrium temperature-dependent swelling (left) and example of SPR angular scans upon probing the hydrogel (right).

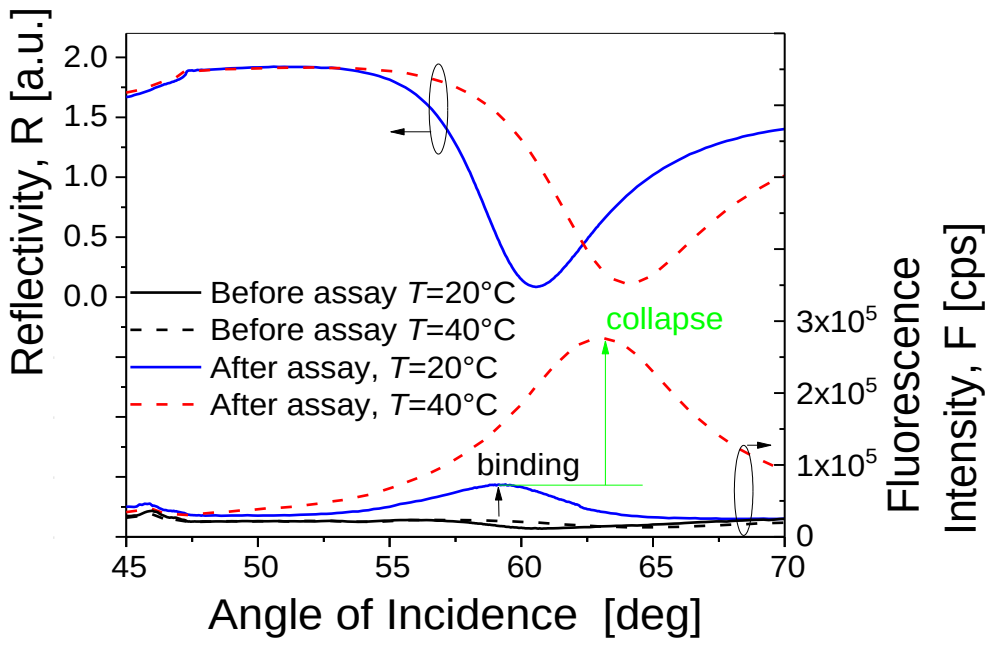
- Thermo-responsive NIPAAm – LSCT around 32 ^{0}C.
- Swelling ratio in 1D as d_h/d_{dry}> 10, refractive index change n_h up to 0.1

M. Toma, U. Jonas, A. Mateescu, W. Knoll, J. Dostalek, Active control of SPR by responsive hydrogels: towards active plasmonics for biosensor applications, Journal of Physical Chemistry C, (2013), 117(22), 11705.

Hydrogel Biointerface Collapse







- Increased binding capacity for peptide ligand attached to pNIPAAm-based hydrogel matrix (d_h~100 nm).
- Collapse of the matrix **enhances fluorescence signal 3×** by compacting the analyte in the evanescent SPP field.

S. Hageneder - performed in water with CA12 peptide receptor, a-CA12 mouse IgG that was reacted with anti mouse IgG conjugated with A670.

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

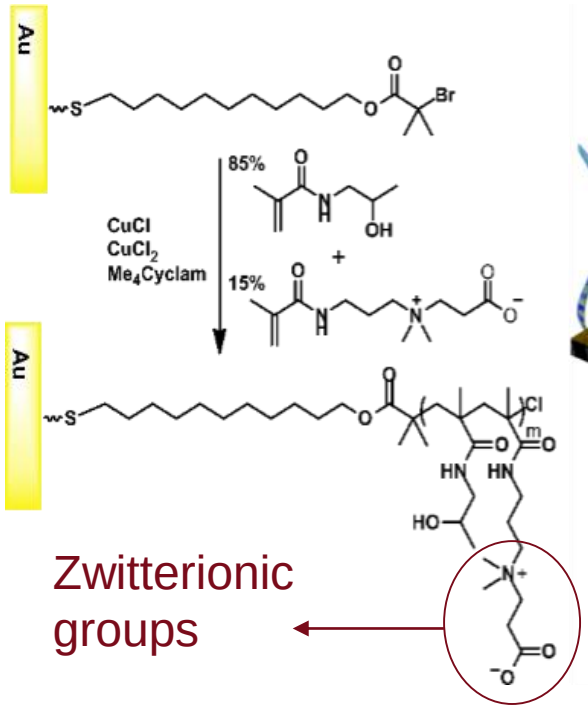
MINISTRY OF EDUCATION, YOUTH AND SPORTS

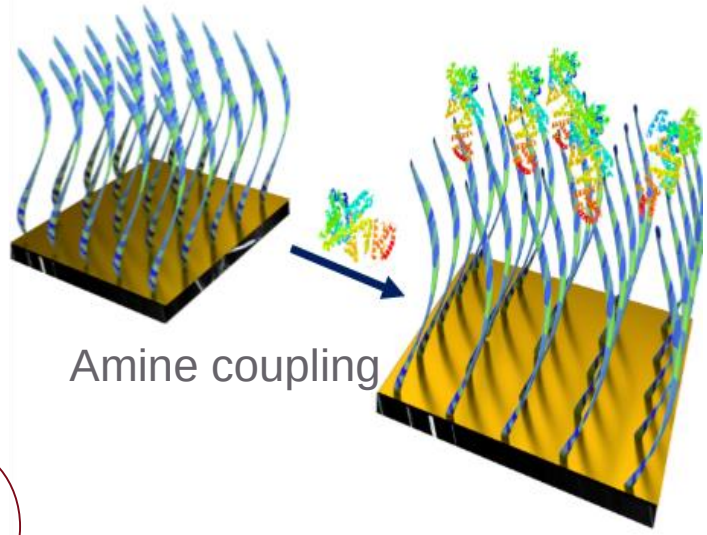
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

Antifouling Brushes

DWI Leibniz-Institut für Interaktive Materialien





macro PRAGUE

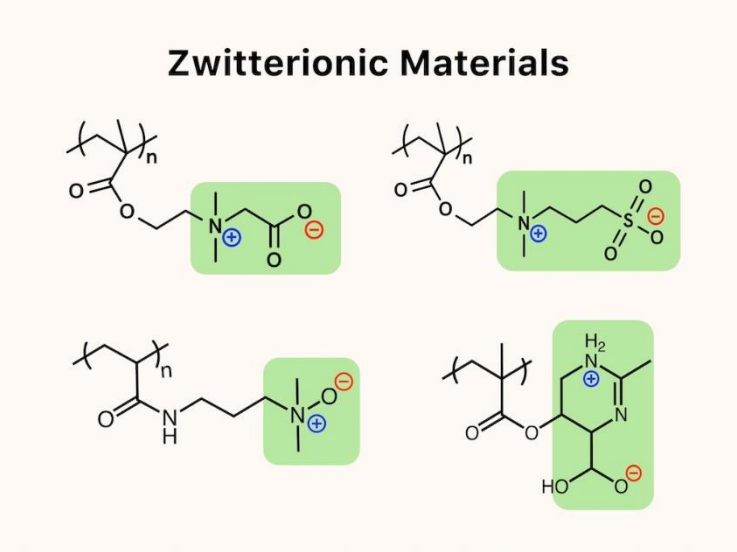
Dr. Riedel
Dr. Emmenegger
Dr. Lisalova

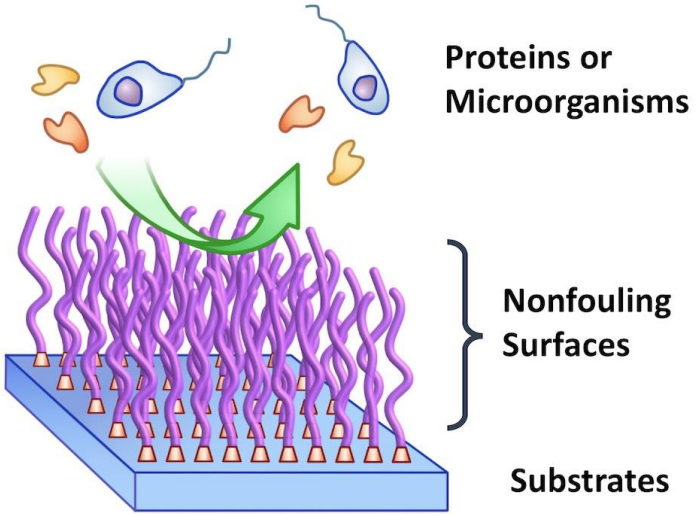
- Brushes based on poly[(N-(2-hydroxypropyl) methacrylamide)-co-(carboxybetaine methacrylamide)] - (poly (HPMA-co-CBMAA)
- Dense brushes that are ‘grafted from’ and can be designed to provide repelling of unspecific sorption from complex samples such as blood serum, plasma, and whole blood.

T. Riedel et al., J. Dostalek, Hepatitis B plasmonic biosensor for the analysis of clinical serum samples, Biosensors and Bioelectronics, 2016, 85, 272-279.

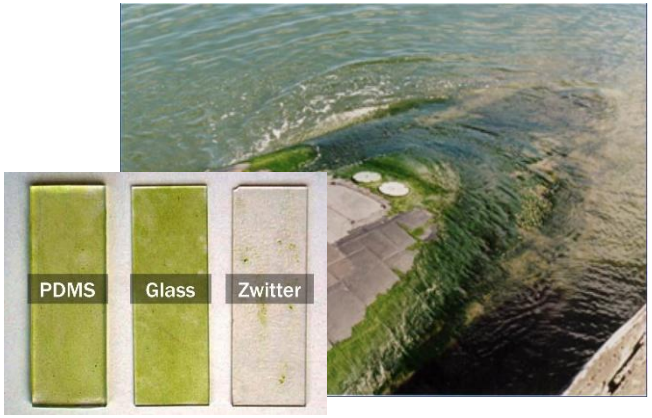
T. Riedel et al., Hepatitis B plasmonic biosensor for the analysis of clinical saliva, Analytical Chemistry, in preparation.

Zwitterionic Brushes





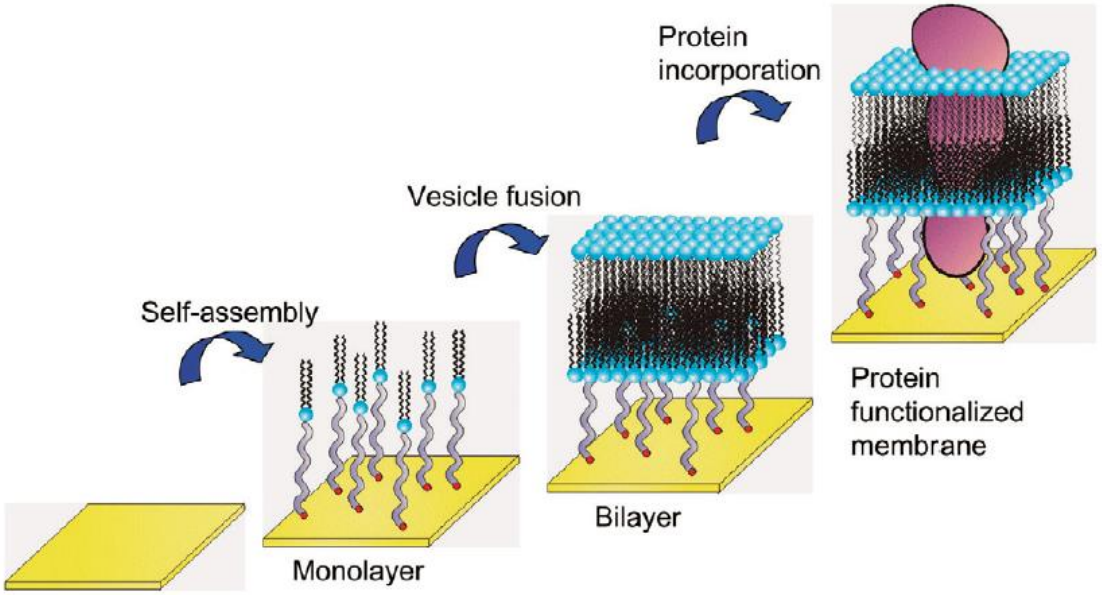
- Shaoyi Jiang (at Cornell since 2020) pionieer the materials based on zwitterionic polymer brushes.
- Biosensor, biomedical devices, marine fouling…



http://jgroup.bme.cornell.edu/research.html

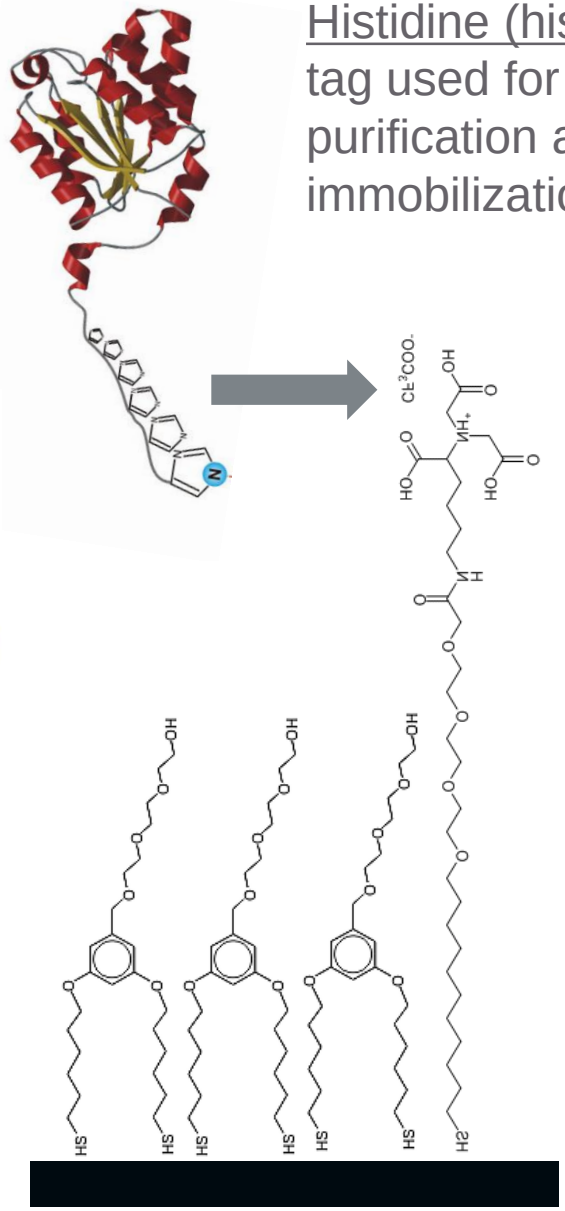
More Complex Architectures

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

Histidine (his) tag used for purification and immobilization



Coupling Chemistries

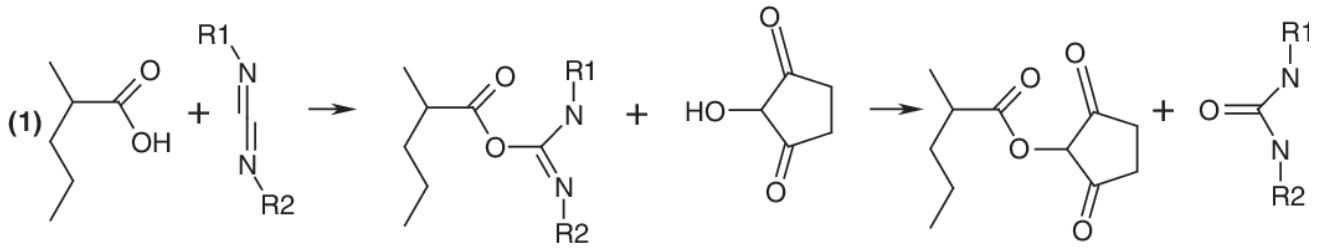
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

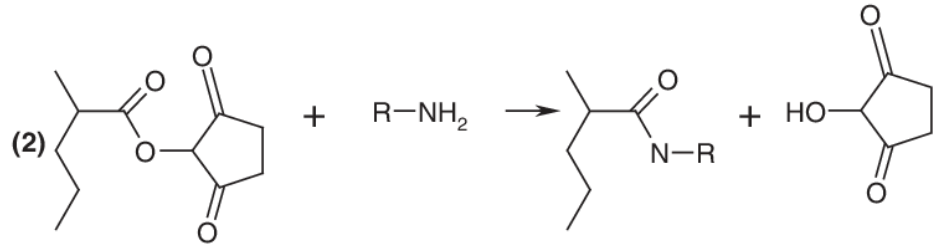
FZU Institute of Physics of the Czech Academy of Sciences

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

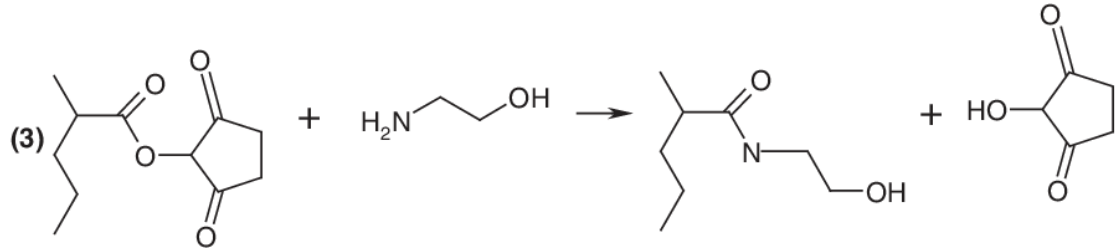
Amine Coupling



- Two-step activation

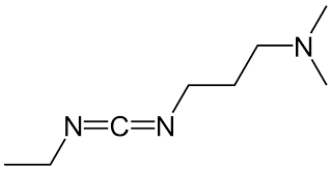


- Conjugation of biomolecules

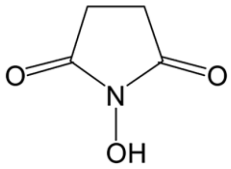


- Passivation unreacted groups

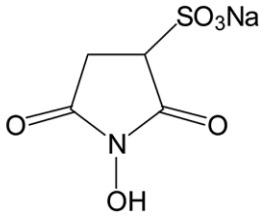
Amine Coupling



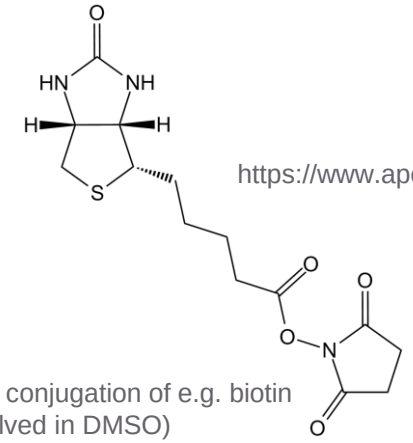
1-Ethyl-3-(3 -dimethylaminopropyl) Carbodiimide (EDC)



N-Hydroxysuccinimide (NHS)



N-hydroxysulfosuccinimide

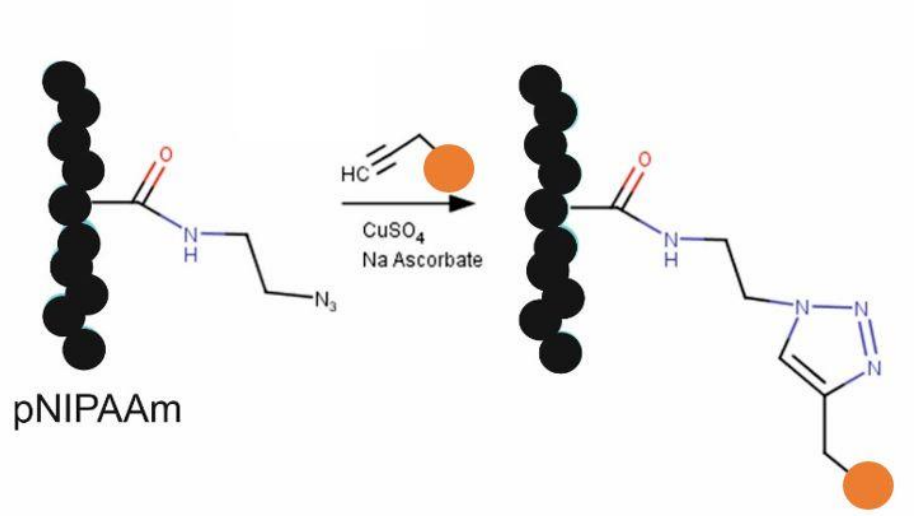


https://www.apexbt.com/

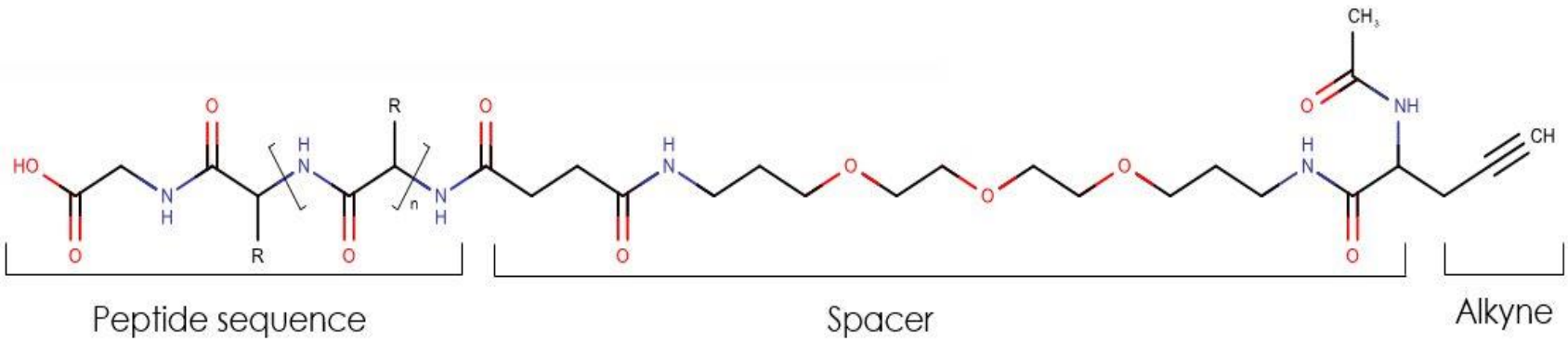
Facile conjugation of e.g. biotin (dissolved in DMSO)

- Two step reaction for gentle coupling - EDC, in conjunction with NHS allows, for 2-step coupling of without affecting the COOH groups of the protein.
- Activation is not long term stable in water environment and active esters hydrolyze back to carboxyl groups.
- Often the coupling reaction is affected by charge of the molecules, then using pH when the active ester and the biomolecules posses opposite charge.

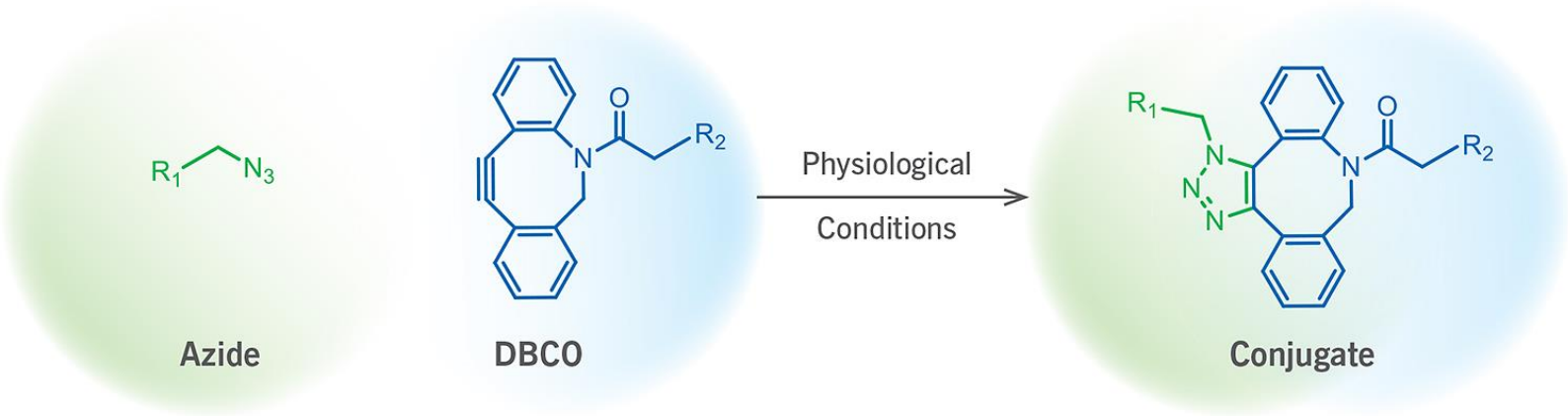
Click Chemistry

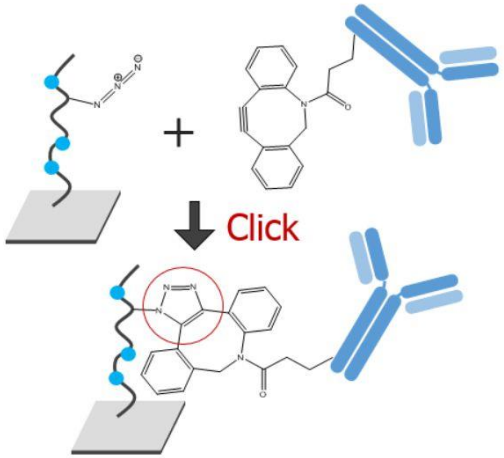


- Copper-mediated click reaction between azide (N_3) and alkyne (C≡C) groups.
- Oxygen quenches the reaction, solved by Na ascorbate.



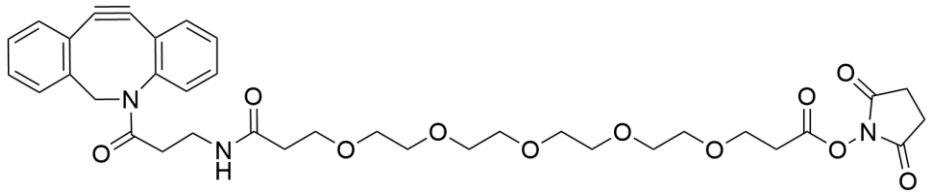
Copper-free Click Chemistry





https://www.xantec.com/news/whitepapers_newsletters_click_coupling.php

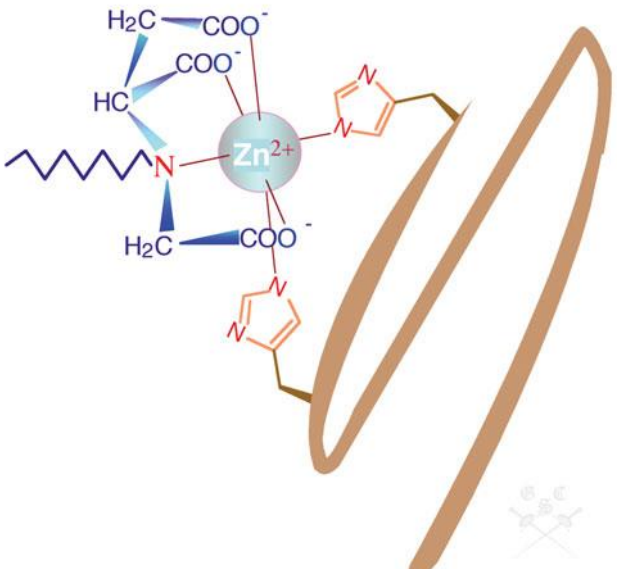
https://clickchemistrytools.com/



https://clickchemistrytools.com/product/dbco-peg5-nhs-ester/

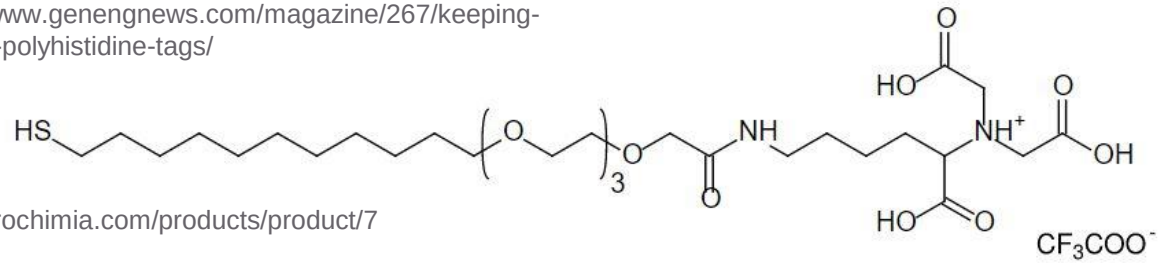
- Simple implementation, no need for pre-activation, increasingly popular.

Histidine Tag (His tag)



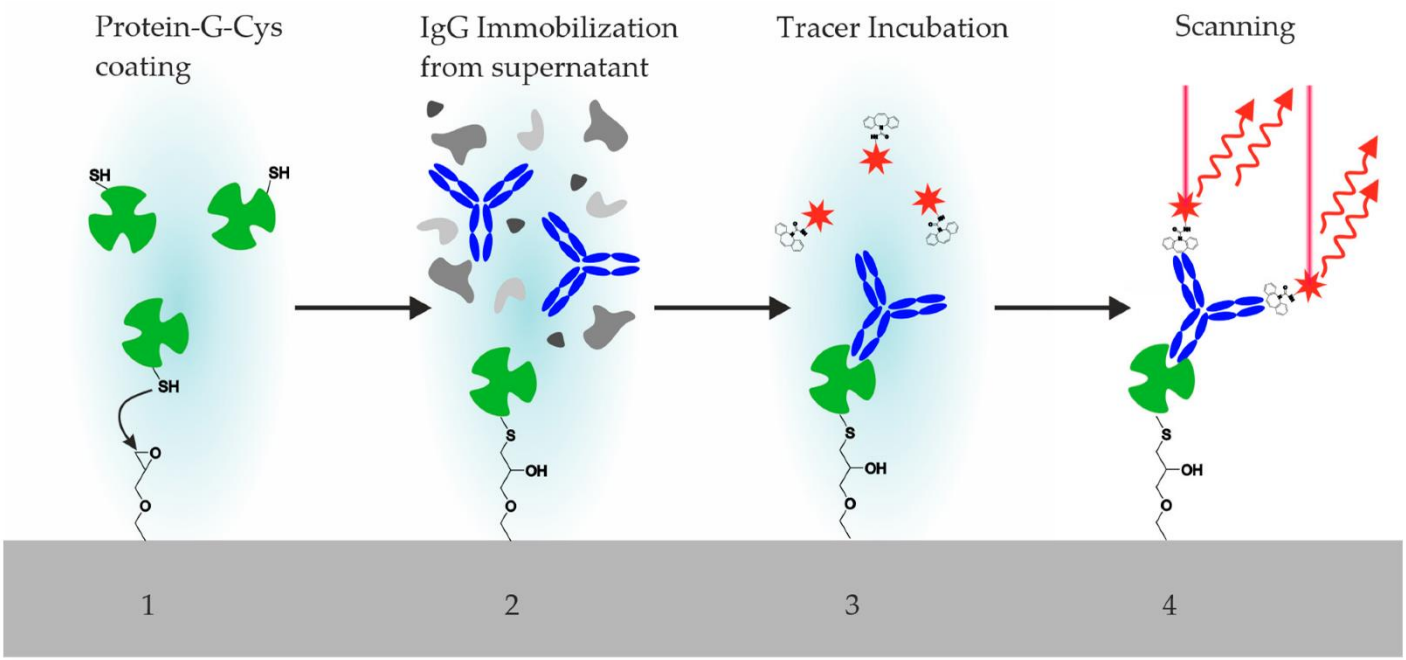
https://www.genengnews.com/magazine/267/keeping-tabs-on-polyhistidine-tags/

- Proteins often expressed with histidine tag (for purification), which can be exploited for the immobilization to a solid surface.
- SAMs terminated with a nitrilotriacetic acid (NTA-SAM) chelating agent can selectively bind Ni(II) and the His-Tag motif.
- In general weaker binding strength, but multiple histidine groups can be attached



https://prochimia.com/products/product/7

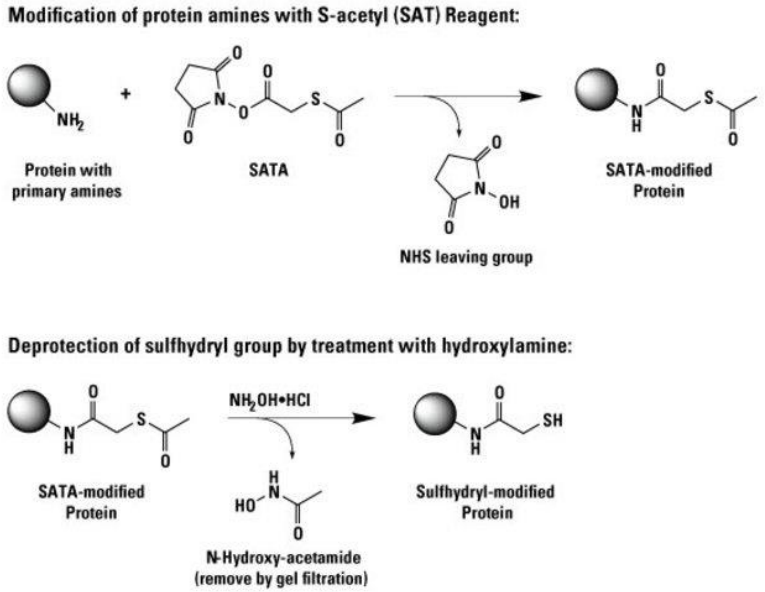
Protein G, A



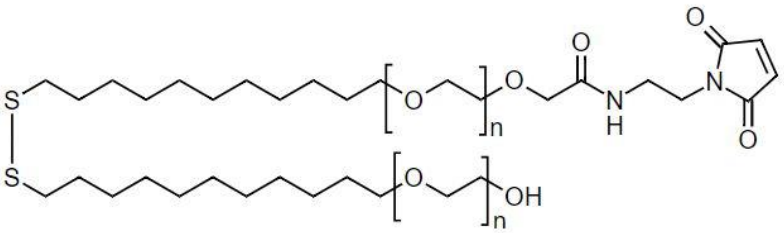
https://doi.org/10.3390/antib9010001

- Protein A and G affinity captures IgG antibodies via their Fc fragment, as exploited in purification columns.
- Allowing for controlled orientation of the immobilization without blocking the active Ab sides

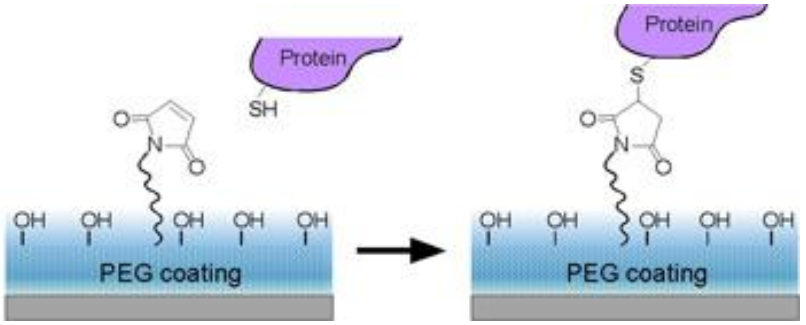
Maleimide - based Coupling



www.thermofisher.com



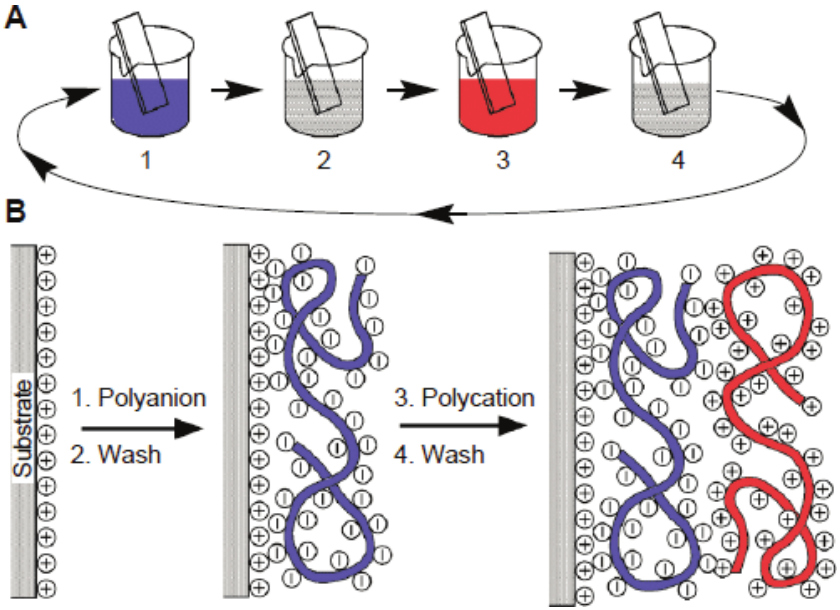
https://prochimia.com/products/product/46

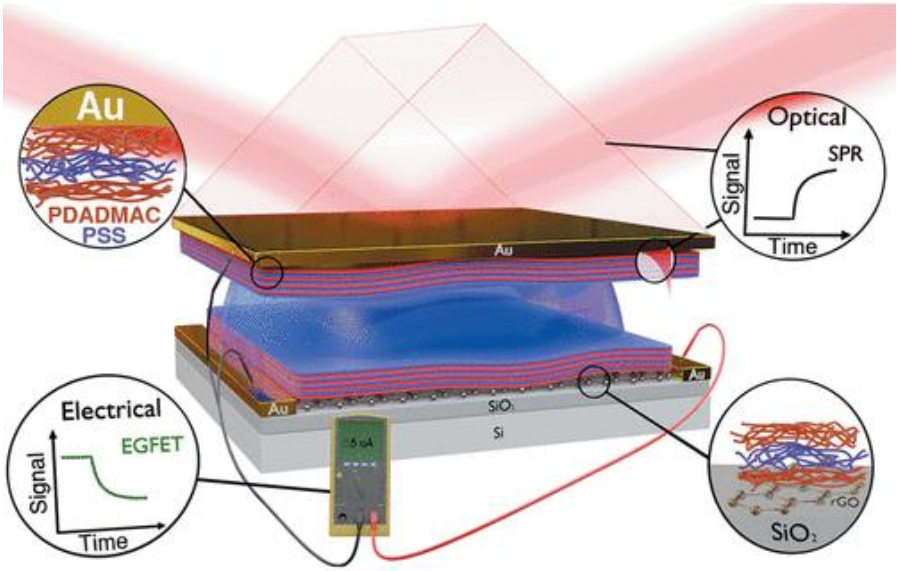


http://proteinslides.com/product-list

- Couples protein ligands via their SH group.
- In absence of the SH groups, they can be introduced based on e.g. amine coupling.

Layer-by-Layer

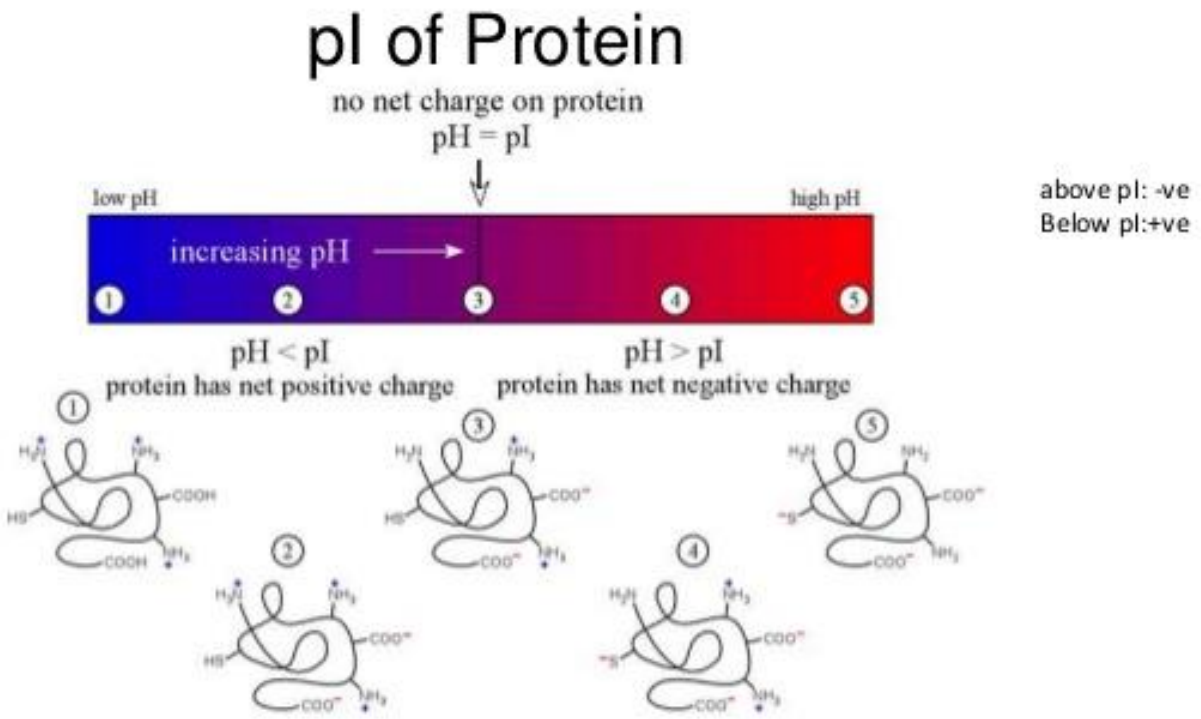




https://pubs.acs.org/action/showCitFormats?doi=10.1021/jacs.9b11835&ref=pdf

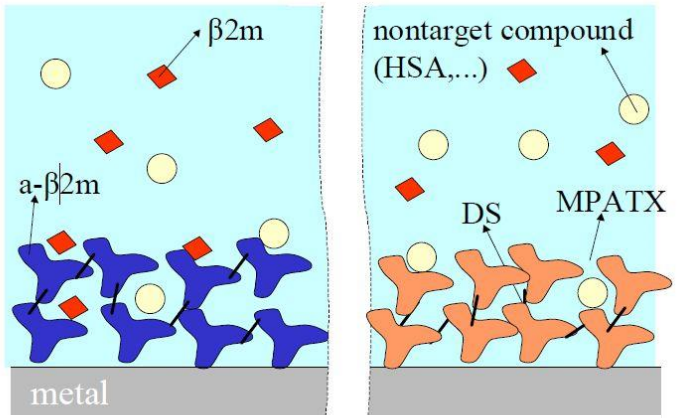
- Layer-by-layer (LbL) exploited with various (non-bio) polymers.
- The LbL layer is typically stable as polymer chains entangle. The schematics is idealized as the polymer layer interpenetrate.

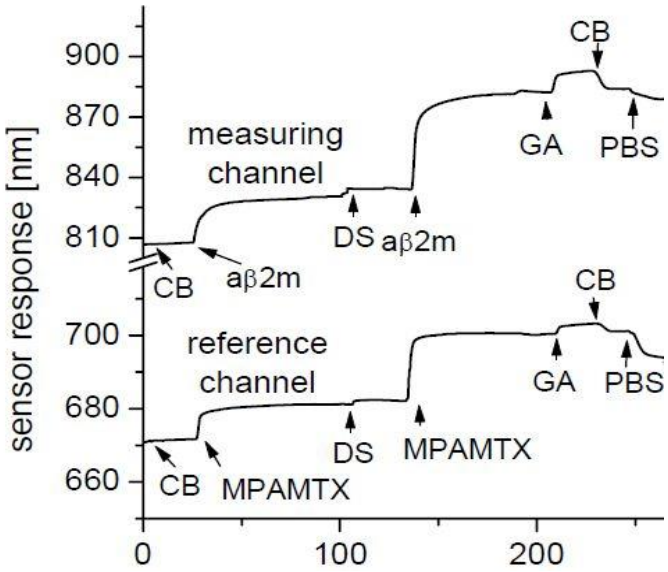
Isoelectric point



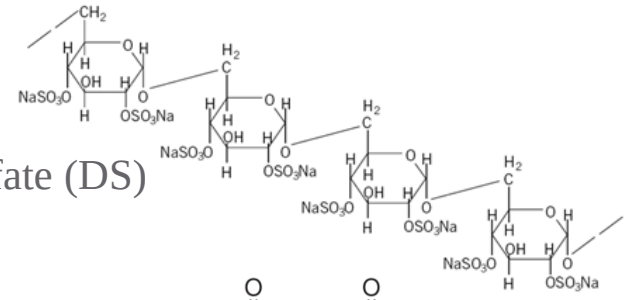
- pI defines pH, at which a protein has a neutral net charge.

Layer-by-Layer

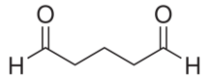




dextran sulfate (DS)



Glutaraldehyde (GA)



Citrate buffer (CA), pH=4 below pI

MPTAX, a-β2m – positively charged Ab in CB

- Layer-by-layer (LbL) – alternating deposition of oppositely charged molecules (Ab, DS)
- In order to keep the integrity o structure, crosslinking by GA before switching to physiological pH.

Jakub Dostalek – master thesis (2000)