Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Tutorial 1: *In situ* Observation of Thin Polymer Films

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Content

- Types and functionalities of thin polymer films.
- Key characteristics of polymer hydrogel and brush films and respective optical properties.
- Ellipsometry, surface plasmon resonance (SPR)
- Optical waveguide spectroscopy (OWS) probing of films.
- Tutorial on design of the experiment, examples of fitting the acquired SPR / OWS spectra.

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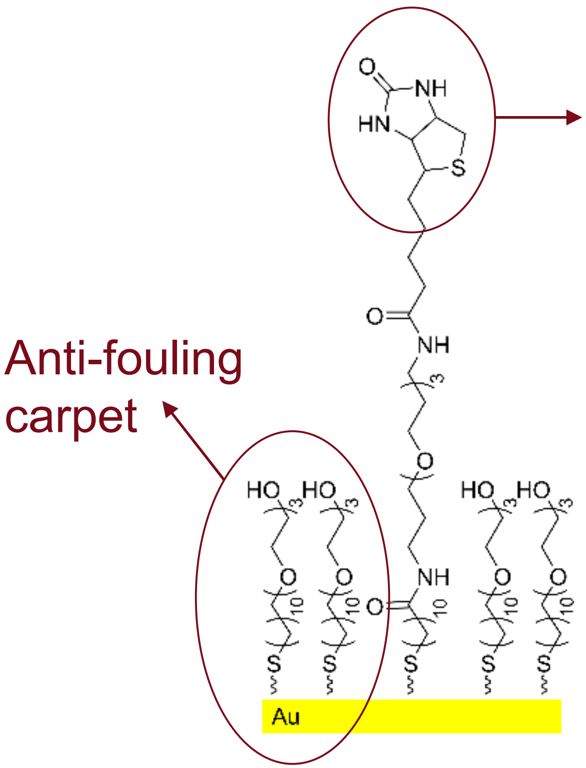
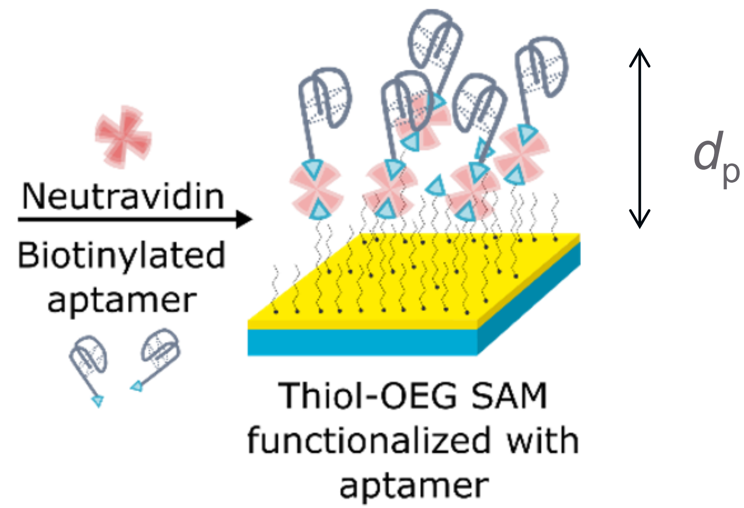
Mixed Thiol SAM





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

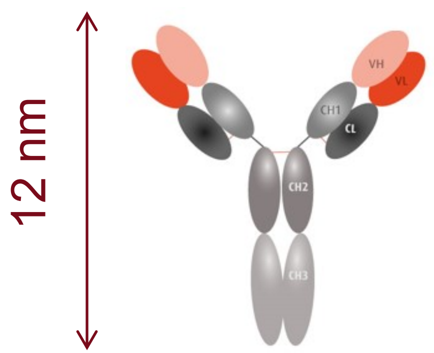
- Mixed thiol SAM allows combining different functionality.

Thiol SAM

Table 5. Wettability angle and ellipsometric measurements of surface after exposure to alkyl thiol solutions.

Polycrystalline gold	Wettability angle ±5°	Relative thickness (Å) (ellipsometry) ±10%	Thickness (Å) based on reflectivity ±10%	Theoretical all-*trans* length (Å)
Freshly deposited	48	0		
Hexane thiol	90	11	13	9.6
Dodecane thiol	105	16	23	17.1
Hexadecane thiol	105	23	30	22.1

- Alkyl thiol are stiff and form layers with thickness up to several nm, OEG more flexible and does not form ordered structures.



$$R_H = \frac{k_B T}{6\pi\eta D}$$

Hydrodynamic radius R_H defined from diffusion properties (for IgG ~6 nm).

- Size of biomolecules based on their structure, or hydrodynamic radius is used.

Surface Mass Density

$$\Gamma = (n_{p\text{-}water} - n_{water}) \cdot d_{p\text{-}water} / (\partial n / \partial c)$$

$$\Gamma = (n_{p\text{-}air} - 1) \cdot d_{p\text{-}air} \cdot (n_p - n_{water}) / (n_p - 1) / (\partial n / \partial c)$$

Γ – surface mass density of the polymer layer [ng/mm^2]

n_p – refractive index of the (compact dry) polymer layer

$n_{p\text{-}air}$ – refractive index of the dry polymer layer

$n_{p\text{-}water}$ – refractive index of the swollen polymer layer

n_{water} – bulk refractive index of the solvent

d_p – thickness of the layer

Γ/MW – surface density [mol/mm^2]

Surface Mass Density

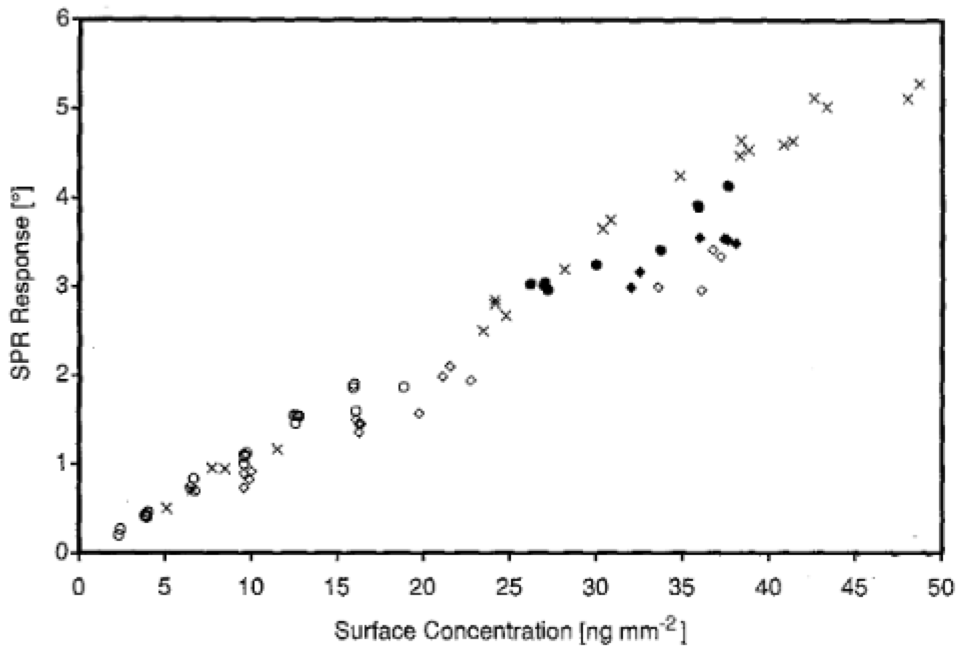


FIG. 7. Experimentally obtained SPR response as a function of surface concentration of the radiolabeled proteins studied. (○ and ● are ^{14}C-labeled chymotrypsinogen, ◇ and ♦ are ^{14}C-labeled transferrin, × are ^{35}S-antitransferrin monoclonal antibody. Open symbols are results from dish incubation.)

$\partial n / \partial c = 0.2$ mm^3/mg based on experiments on proteins:

10.1016/0021-9797(91)90284-F

Quantitative Determination of Surface Concentration of Protein with Surface Plasmon Resonance Using Radiolabeled Proteins

ESA STENBERG, BJÖRN PERSSON, HÅKAN ROOS, AND CSABA URBANICZKY

Pharmacia Biosensor AB, S-751 82 Uppsala, Sweden

Received August 21, 1990; accepted November 1, 1990

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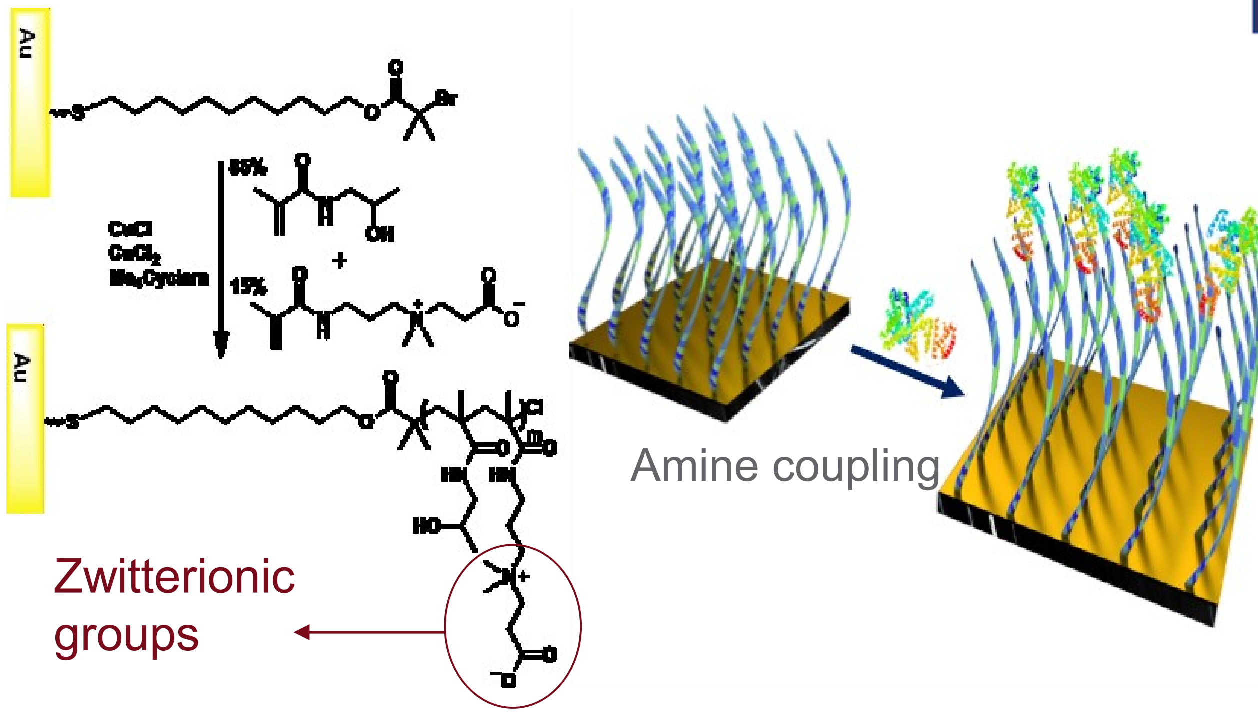
Antifouling Brushes

DWI Leibniz-Institut für Interaktive Materialien

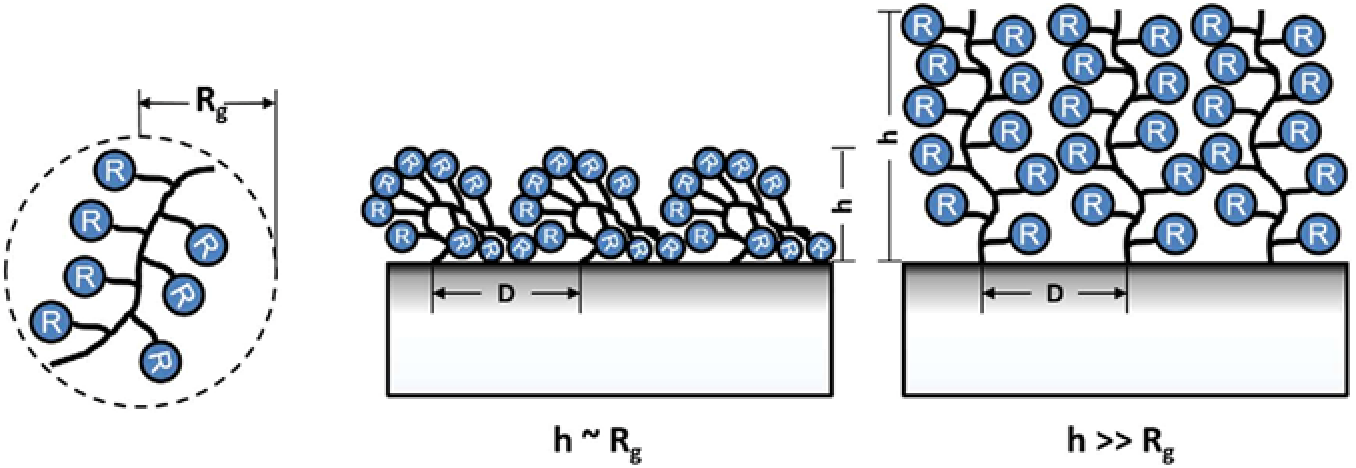


Dr. Riedel
Dr. Emmenegger
Dr. Lisalova

- 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.

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.

Polymer Brushes – Key Parameters



Gyration radius R_g

Mushroom conformation

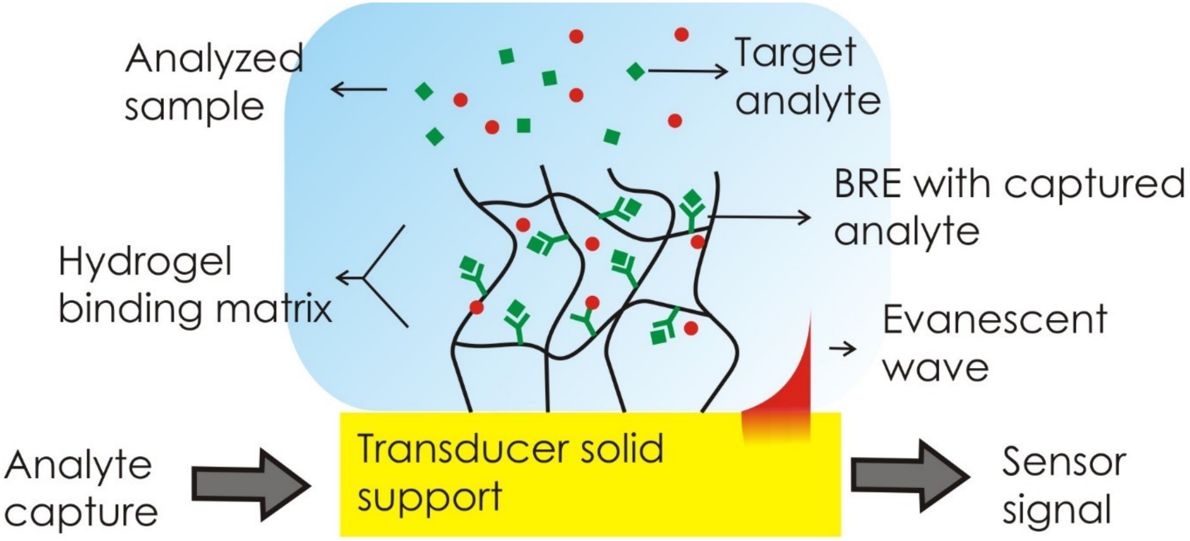
Brush conformation

10.1021/acsami.6b09068

$R_g = A_0 N^v$

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

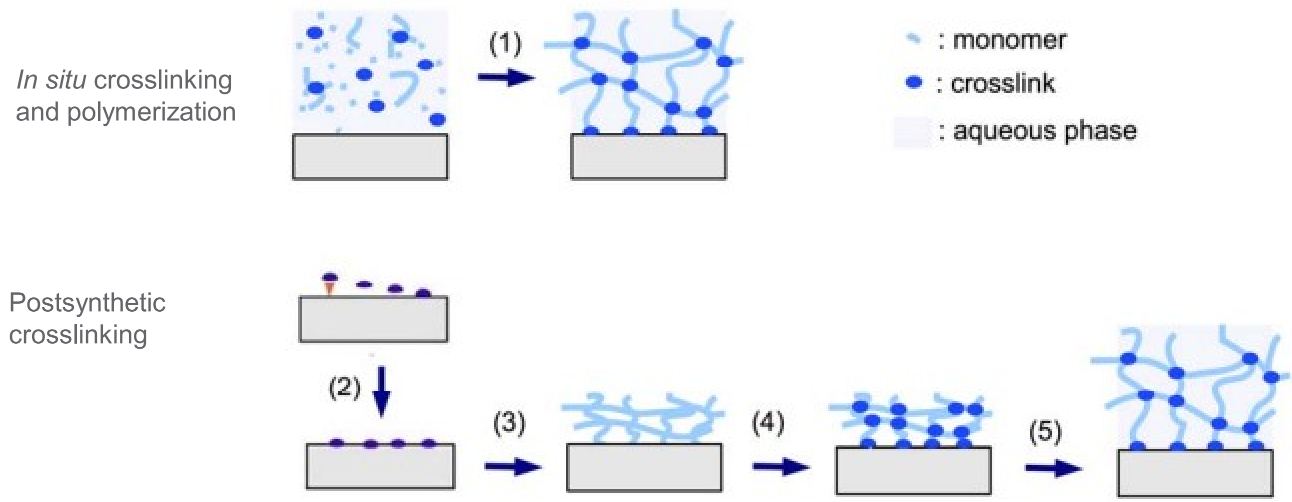
Hydrogels



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.

Polymer Networks – Key Parameters



Membranes 2012, 2, 40-69; doi:10.3390/membranes2010040

Swelling ratio SR = $d_h/d_{h\text{-dry}}$
Polymer volume content fraction *f=1/SR*
Crosslink density v_c
Pore size
Segment length
……

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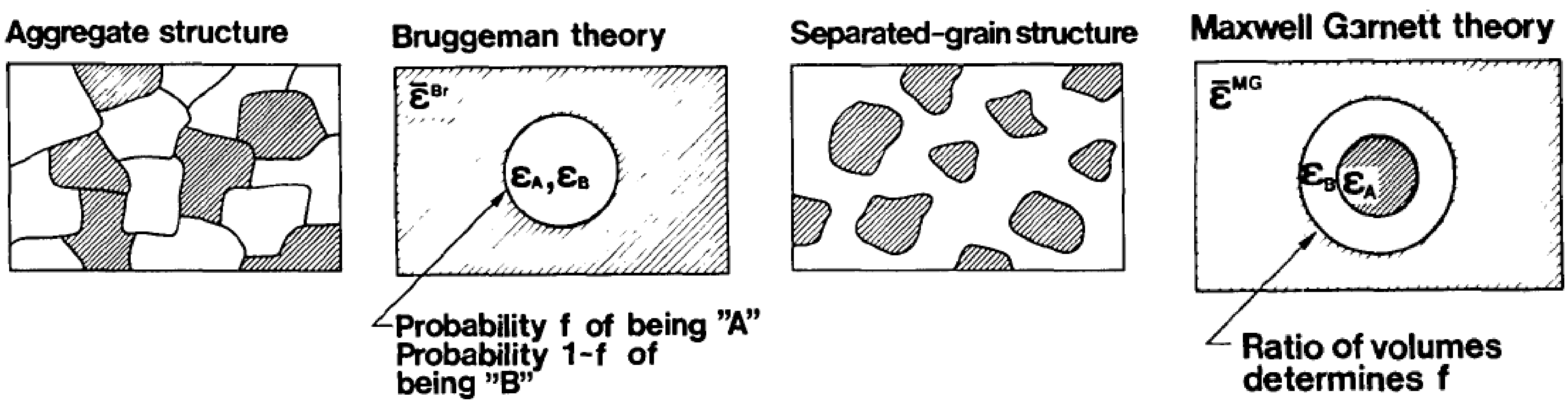
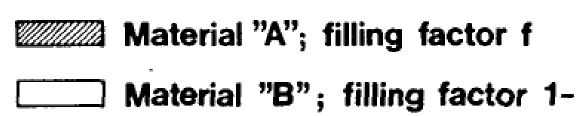
Effective Medium Theory

Refractive index of composite systems, when a material with permittivity ε_1 forms inclusions in another one with ε_2 (e.g. hydrogel layer formed by polymer networks swollen in water, biomolecules forming layers at interfaces…)

Bruggeman | Maxwell Garnett



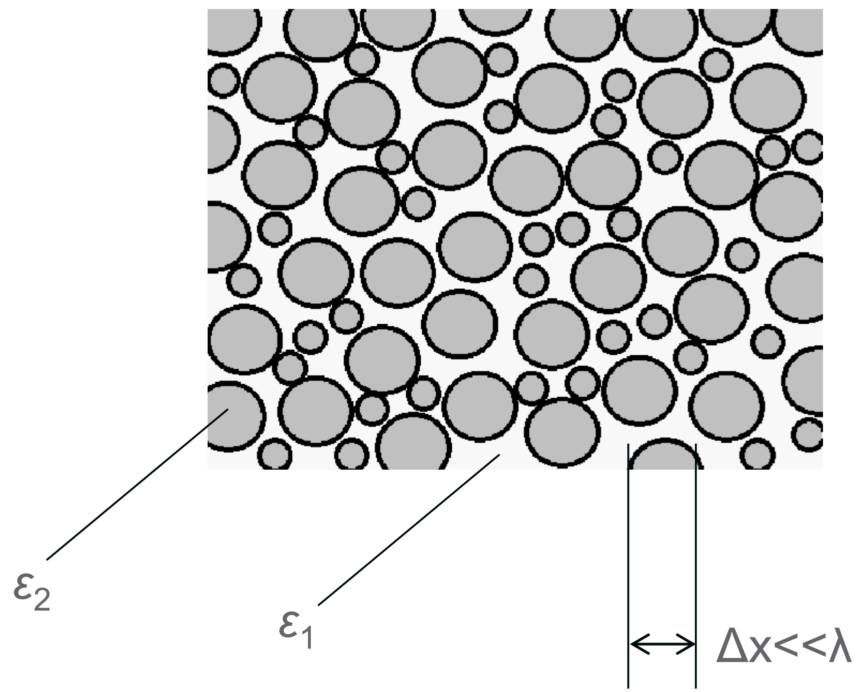
Can be treated by Mie scattering theory.



DOI: 10.1364/AO.20.000026

Maxwell Garnett Effective Medium Theory

$$\varepsilon_{eff} = \varepsilon_2 \frac{2f(\varepsilon_1 - \varepsilon_2) + \varepsilon_1 + 2\varepsilon_2}{\varepsilon_1 + 2\varepsilon_2 - f(\varepsilon_1 - \varepsilon_2)}$$



f – volume fraction of material 1 with percolations of material 2

Example – hydrogel polymer volume fraction *f*
n_h / $n_{h\text{-}dry}$ – refractive index of swollen / dry network
n_s – refractive index of solvent

$$f = \frac{(n_h^2 - n_s^2)}{(n_h^2 + 2n_s^2)} \frac{(n_{h-dry}^2 + 2n_s^2)}{(n_{h-dry}^2 - n_s^2)}$$

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Bruggeman Effective Medium Theory

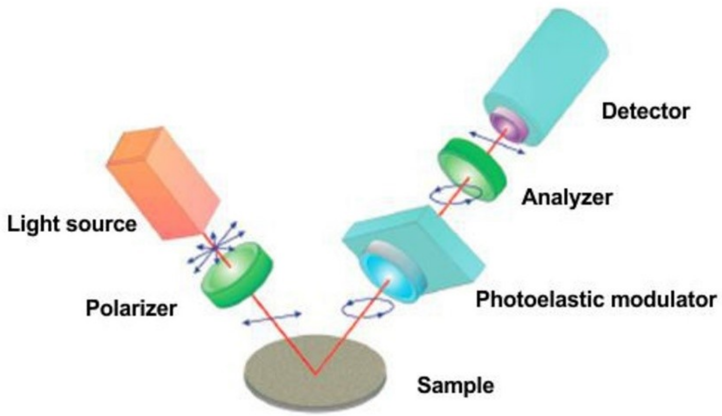
$$f\frac{\varepsilon_1-\varepsilon_{eff}}{\varepsilon_1+2\varepsilon_{eff}}+(1+f)\frac{\varepsilon_2-\varepsilon_{eff}}{\varepsilon_2+2\varepsilon_{eff}}$$

- Bruggeman and Maxwell Garnett theories give similar results, differs for large *f* and when, e.g. one of the material is metal (recall condition for localized surface plasmon resonance)

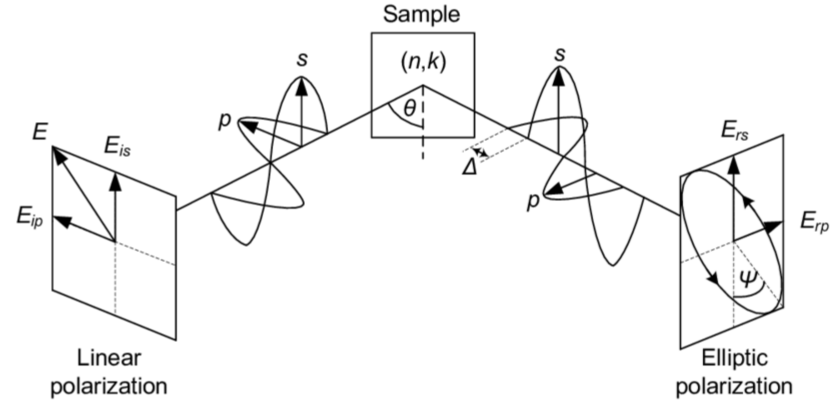
Methods to Measure Thickness and Density of Biopolymer layers

- Surface plasmon resonance / optical waveguide spectroscopy
- Ellipsometry
- Small angle neutron scattering

Ellipsometry

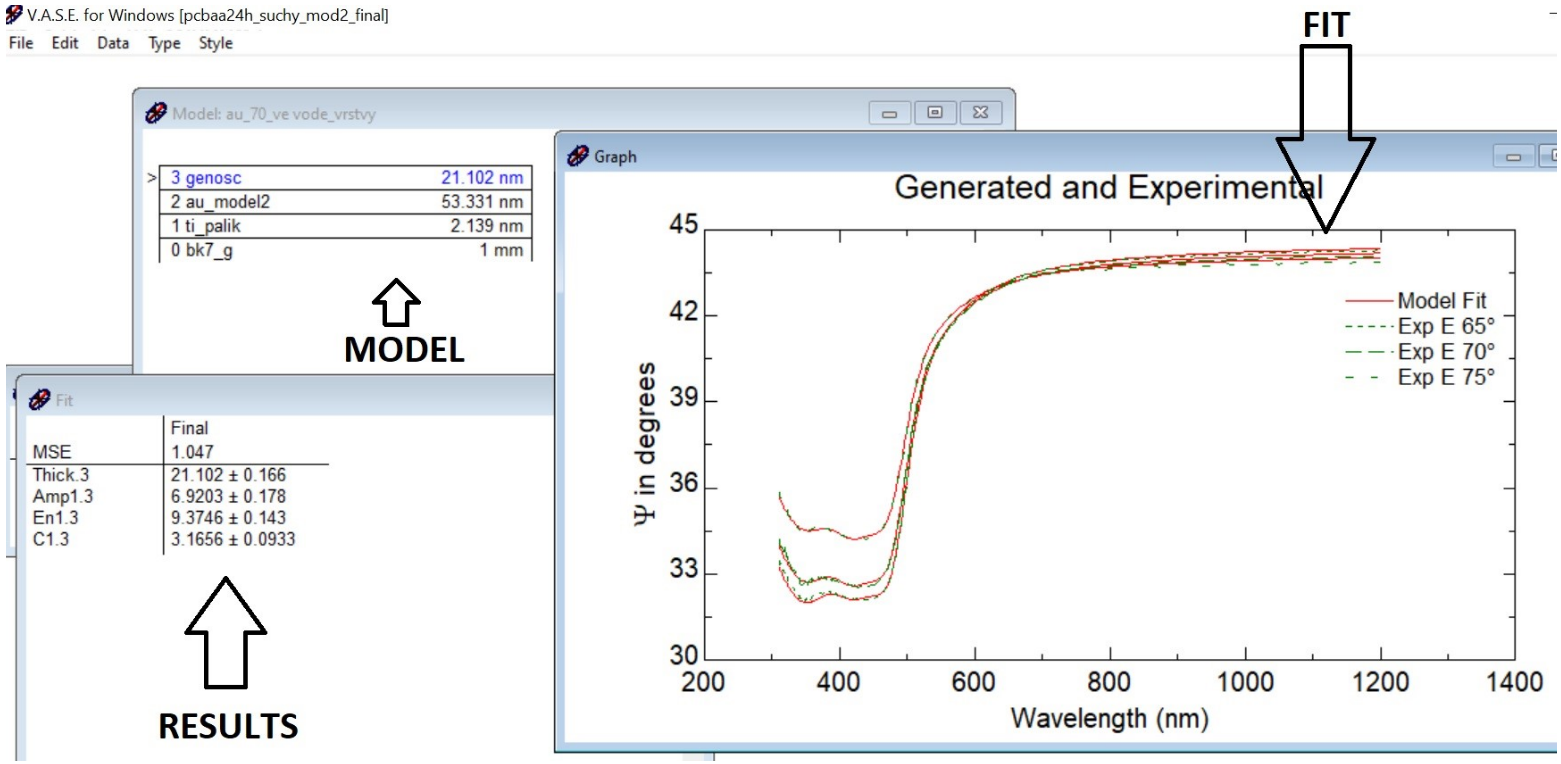


https://www.materize.com/device-categories/spectroscopy/



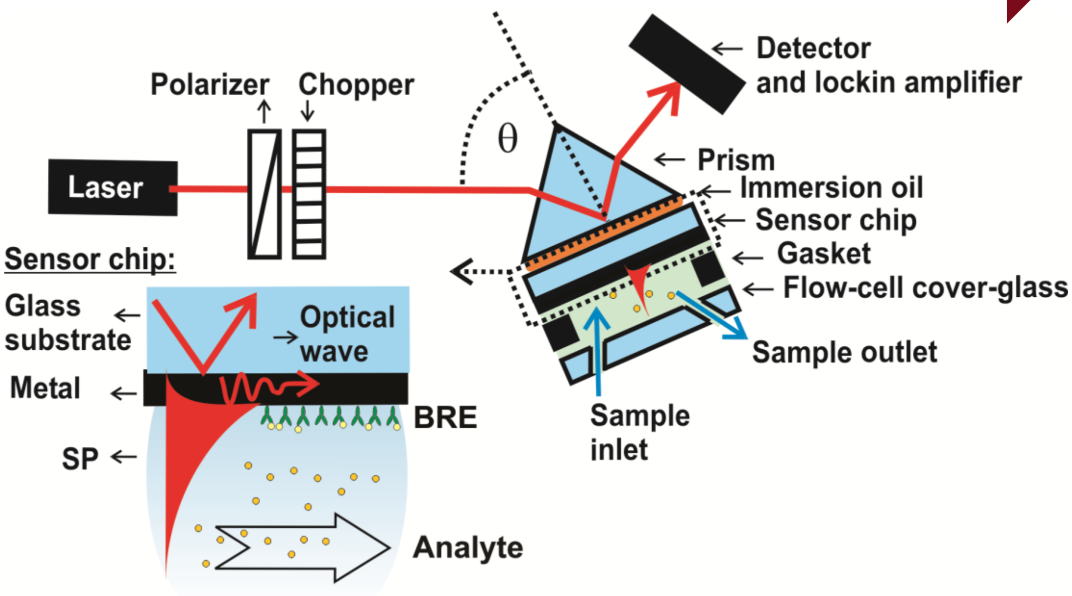
- Established technique for characterization of thin film stacks.
- Measurement and fitting of angular and wavelength spectra of phase shifts Δ, ψ. Determination of thickness and refractive index of each layer.
- Typically is performed in reflectivity mode for a stack in contact with air, possible using of a prism cuvette for measurement in contact with water.

Ellipsometry

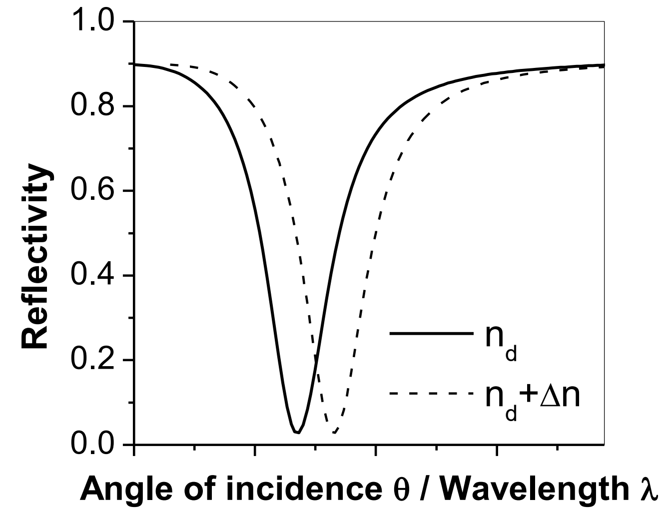


- Example of fitting a thin polymer brush film by V.A.S.E.

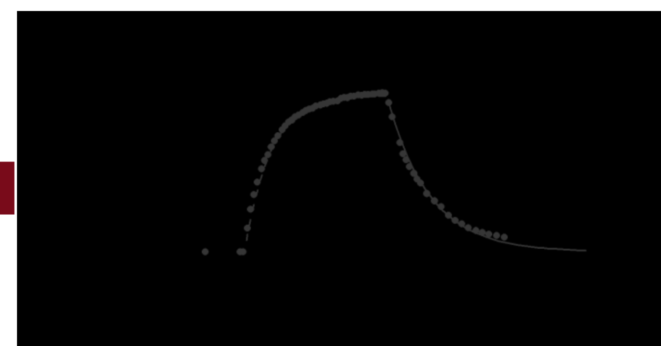
‘Mainz’ Design of SPR Biosensor



SPR reflectivity



Tracking of SPR angle

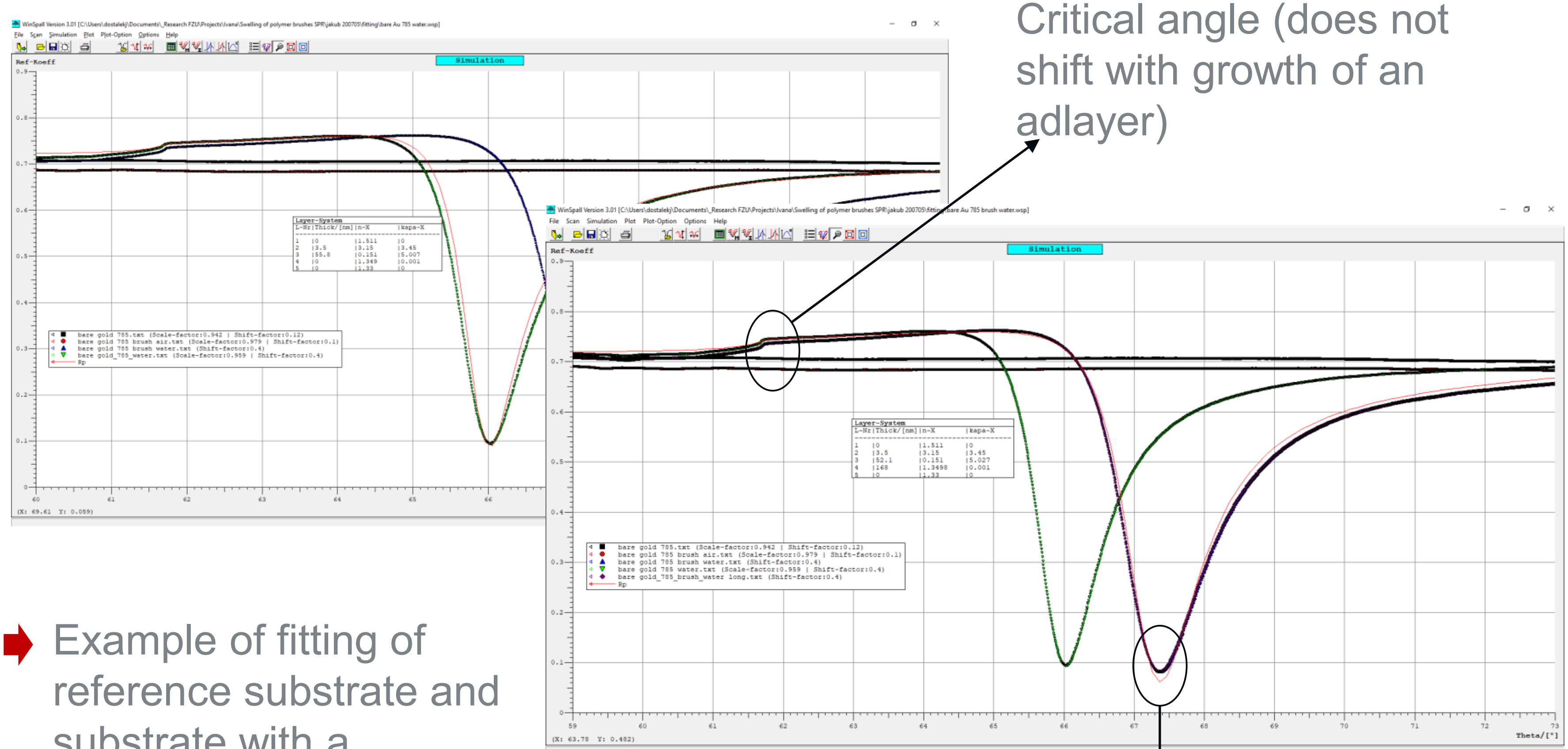


Analysis of reaction kinetics

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

SPR Biosensor

Critical angle (does not shift with growth of an adlayer)



SPR angle

- Example of fitting of reference substrate and substrate with a polymer layer by Fresnel reflectivity model.

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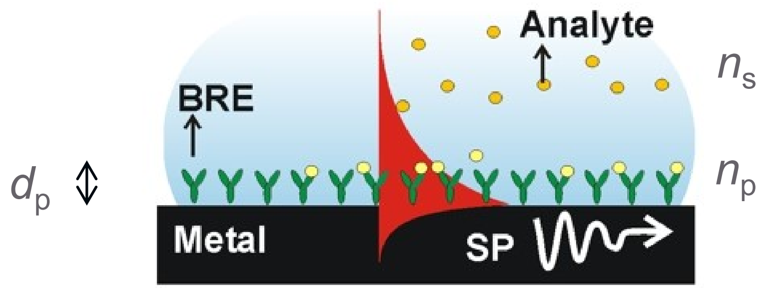
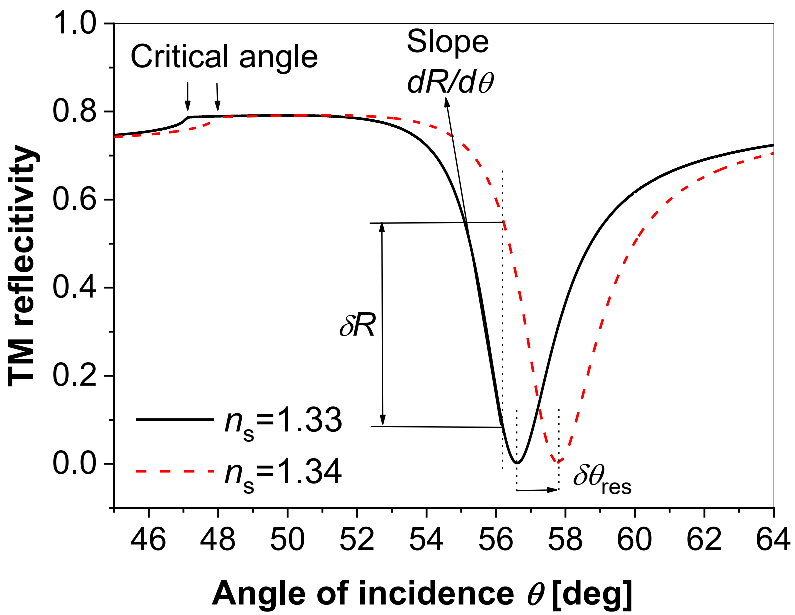
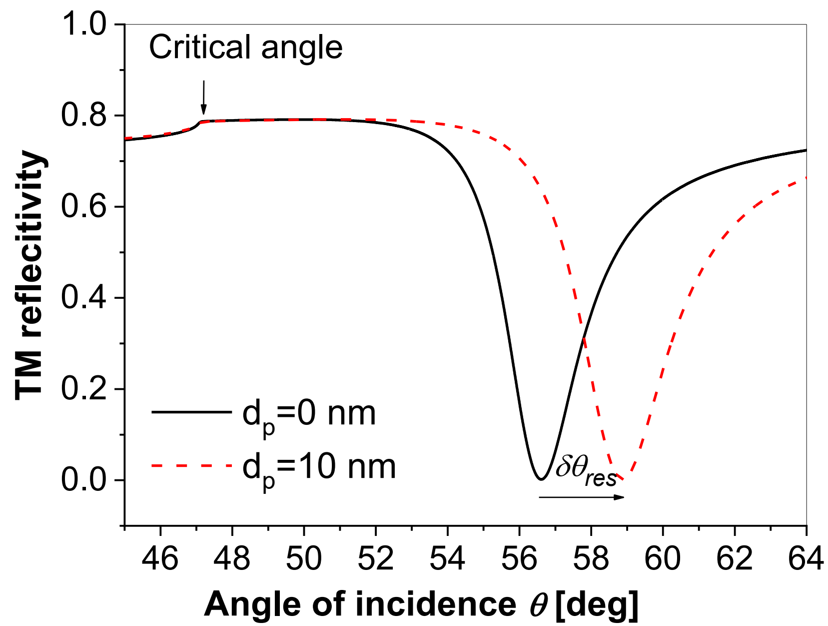
Refractive Index Sensitivity – Angular Interrogation

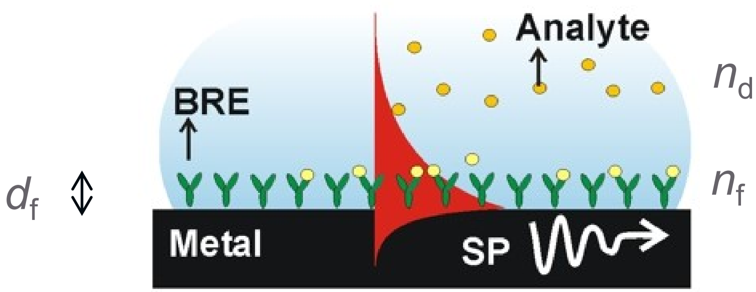


- SPR changes with both changes in the bulk n_p and surface refractive index changes.





‘Mainz’ Design of SPR – Typical Characteristics



$$\delta\theta_{res} = \delta R / \left(dR / d\theta \right)$$

Bulk sensitivity:

$$\delta\theta_{res} = S_b \delta n_d$$

Surface sensitivity:

$$\delta\theta_{res} = S_s \delta n_f d$$

Surface mass density change:

$$\gamma = \frac{\delta\theta_{res}}{S} \frac{\partial c}{\partial n_f}$$

SPR prism coupler		**Sensitivity of RI changes and molecular binding**	
Prism refractive index	n_p=1.845	Bulk RI sensitivity	S_b=118 deg
Gold film thickness	d_m=55 nm	Surface RI sensitivity	S_s=1.28 deg nm^{-1}
Gold film refractive index	n_m=0.1+3.5i	Protein induced RI	$\partial c/\partial n_f$=0.14-0.2 μL mg^{-1}
Sample refractive index	n_d=1.33	Surface conc. ($d \ll L^d_{pen}$)	γ=3-5 $\delta\theta_{res}$ ng $mm^{-2}deg^{-1}$

SPR – Observation of Thin Films

Difficult to distinguish independently thickness and refractive index for thin polymer films with a thickness d_h << surface plasmon probing depth L_p=133 nm (for gold in contact with water and red part of spectrum):

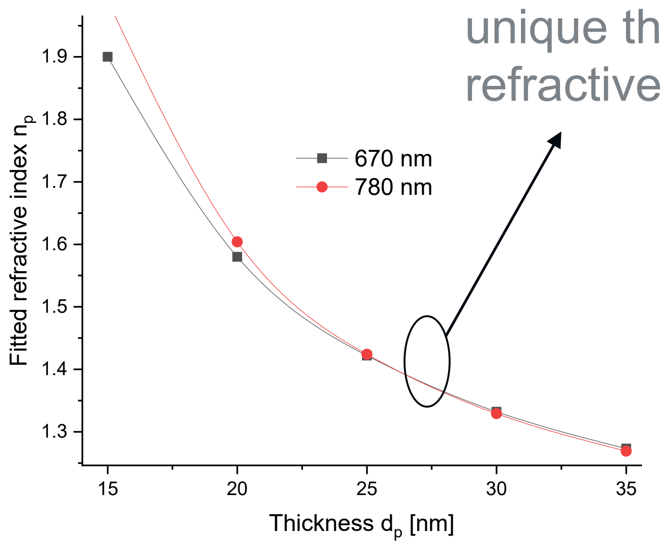
$$L_p = \frac{\lambda}{4\pi\sqrt{n_1^2 \sin^2(\theta) - n_2^2}}$$

n_1 – prim refractive index, n_2 – solvent (water) refractive index.

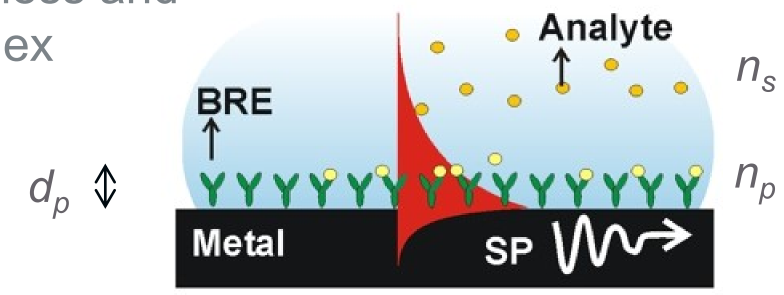
Approaches to measure the swelling of thin films with SPR that requires deconvoluting its refractive index n_h and thickness d_h.

(1) Multiple wavelength probing (10.1016/0030-4018(96)00238-6)
(2) Surface mass density matching (de Feijter approach, 10.1002/bip.1978.360170711)
(3) Exclusion heights (Gustav 10.1021/acs.jpcc.8b09171, 10.1016/0030-4018(91)90353-F)

SPR – Multiple Wavelength Probing



Cross-section corresponds to the unique thickness and refractive index



Dry polymer
d_p=26.4 nm
n_p=1.39 RIU (at 670 nm)
Dispersion omitted.

Dry polymer
d_p=28.5 nm
n_p=1.354 RIU (at 670 nm)
Dispersion corrected.

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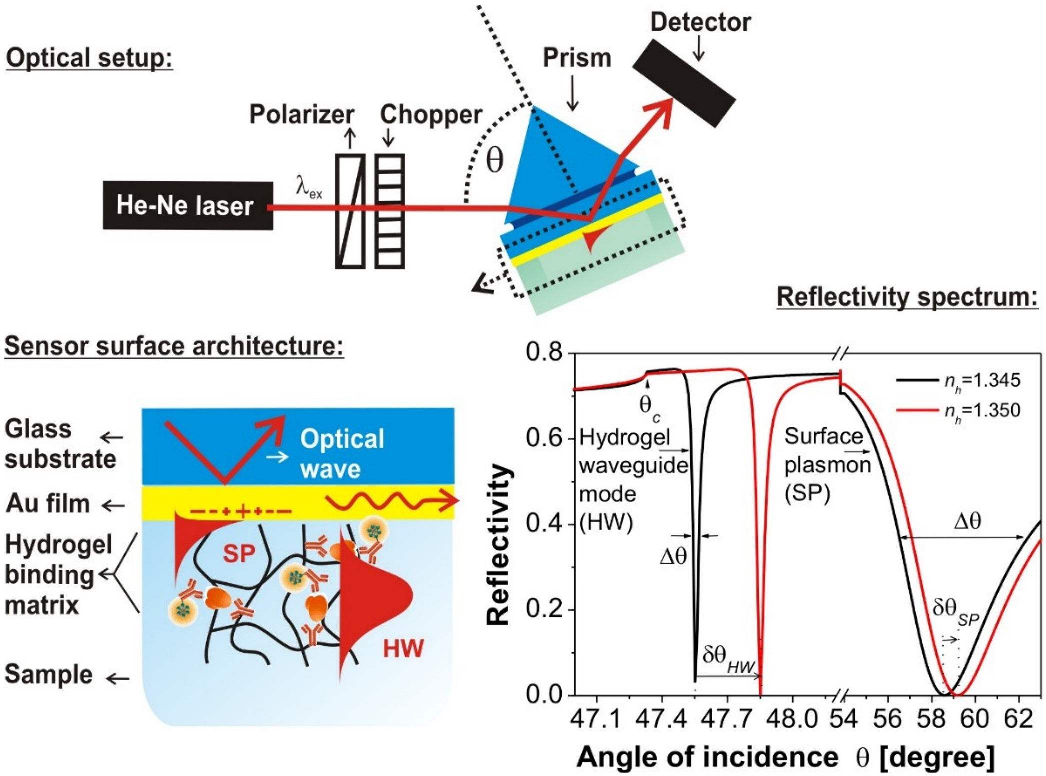
Observation of Thicker Polymer Films



Probing by surface plasmons (SP) and hydrogel optical waveguide (HOW) modes.

Analysis of the reflectivity spectrum

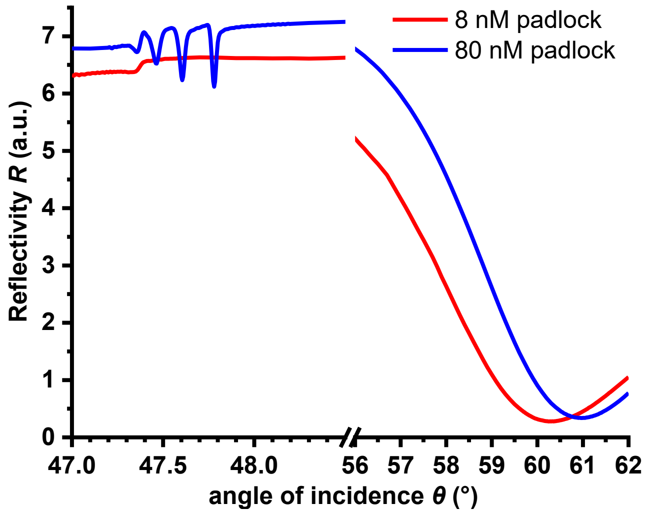
Monitoring of changes in the thickness d_h, refractive index n_h, surface mass of the gel Γ

- IgG surface mass density Γ up to 100 ng/mm^2
- Affinity binding studies
  Monitoring of responsive properties
- (1D) Swelling ratio $d_h/d_{h\text{-dry}} > 10$

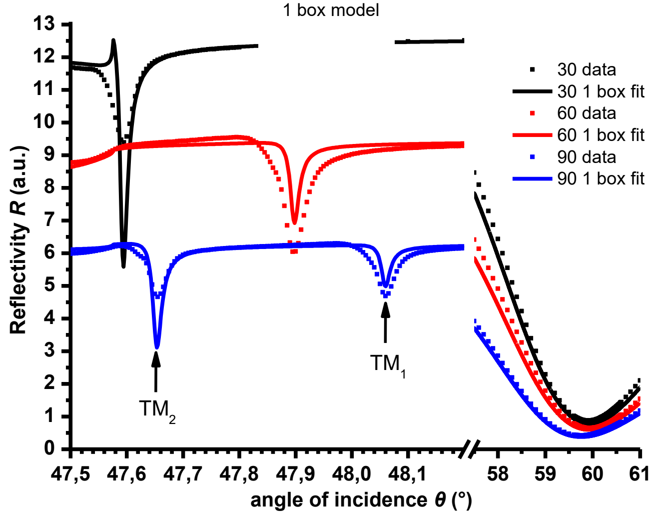
Aulasevich, R.F. Roskamp, U. Jonas, B. Menges, J. Dostalek, W. Knoll, Optical waveguide spectroscopy for the investigation of protein-functionalized hydrogel films, Macromolecular Rapid Communications, (2009), 30, 872-877.

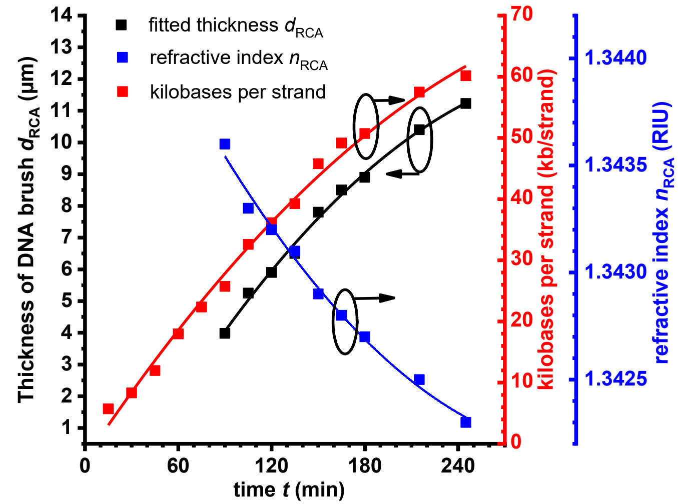
Dostálek J and Knoll W, Plasmonics in "Polymer Science: A Comprehensive Reference", Vol 2, pp. 647–659, Matyjaszewski K and Möller M (eds.), Amsterdam: Elsevier BV. (2012) ISBN: 978-0-08-087862-1.

Observation of RCA by OWS



1 box model

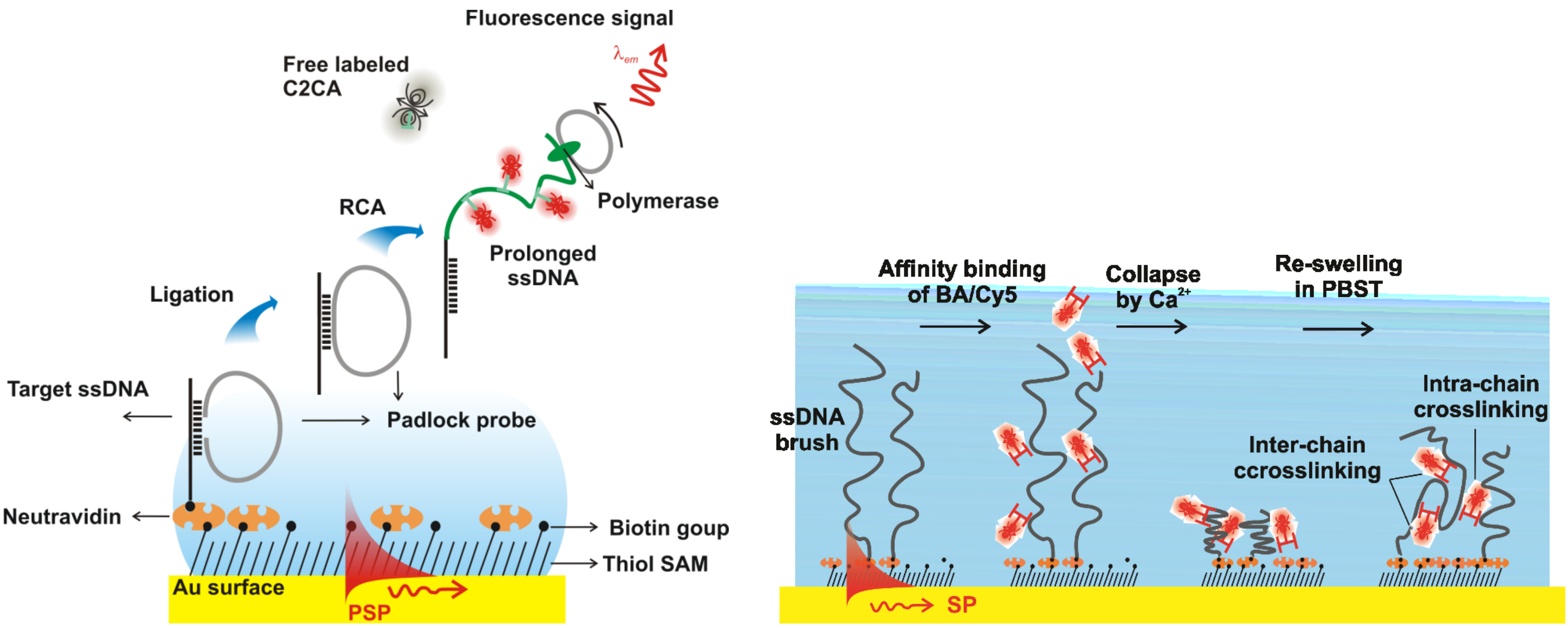




- The speed of ssDNA growths is about 5 nt/s at room temperature T=22℃ with polymerase Φ-19POL.
- Reaction can proceed without saturation to generate very long chains d_{RCA}>10 µm

Bernadette Lechner, Simone Hageneder, Marc P. Kreuzer, Rick, Cozemius, Ivan Barišić, Jakub Dostalek, *In situ* monitoring of rolling-circle amplification on a solid support by surface plasmon resonance and optical waveguide spectroscopy, in preparation.

PEF with Additional Enzymatic Amplification



- **Rolling circle amplification (RCA)** implemented to associate the presence of a single copy of analyte (bacterial ssDNA related to genes providing resistance to antibiotics) with **multiple fluorophore emitters**.

Bernadette Lechner, Simone Hageneder, Marc P. Kreuzer, Rick, Cozemius, Ivan Barišić, Jakub Dostalek, *In situ* monitoring of rolling-circle amplification on a solid support by surface plasmon resonance and optical waveguide spectroscopy, in preparation.