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Optical Biosensors: Implementation of Surface Plasmon Resonance Biosensors

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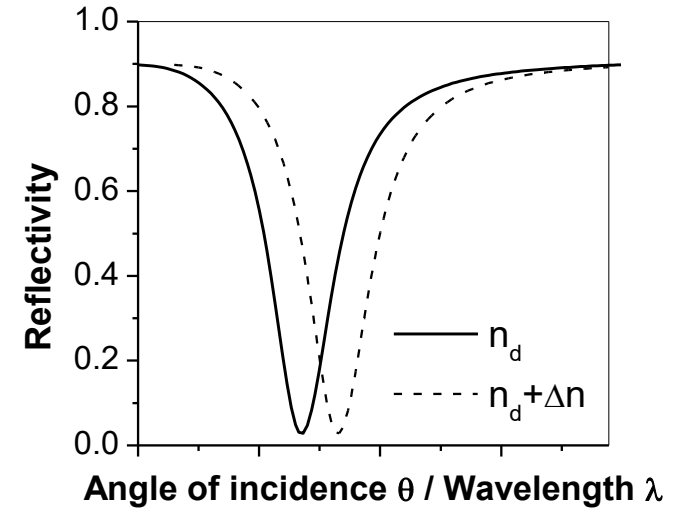
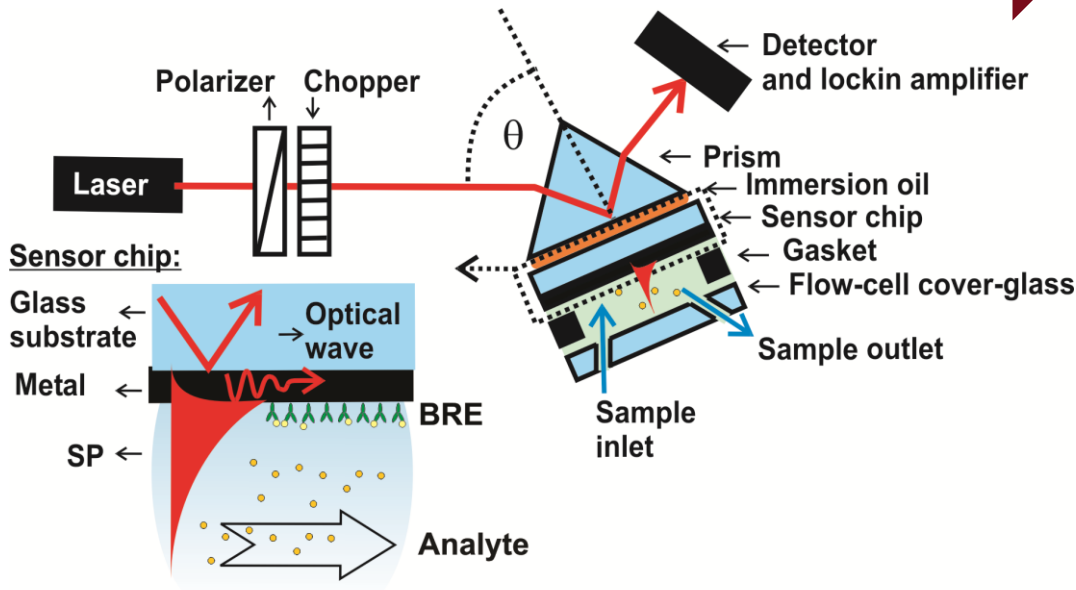
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Content

- **Kretschmann configuration of SPR**
- **Key characteristics of thin polymer films.**
- **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.**

'Mainz' Design of SPR Biosensor

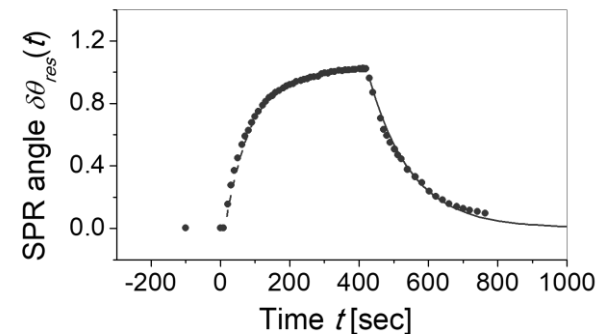
SPR reflectivity



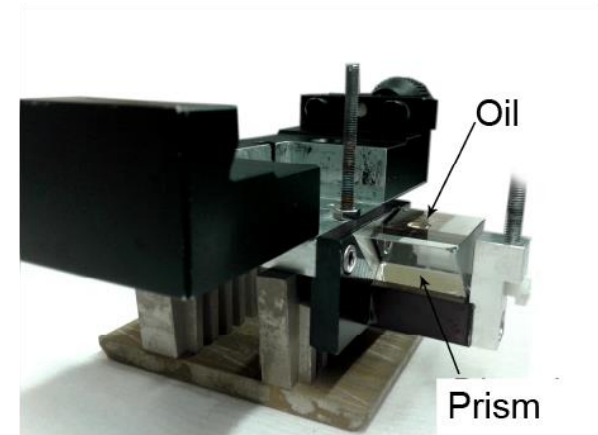
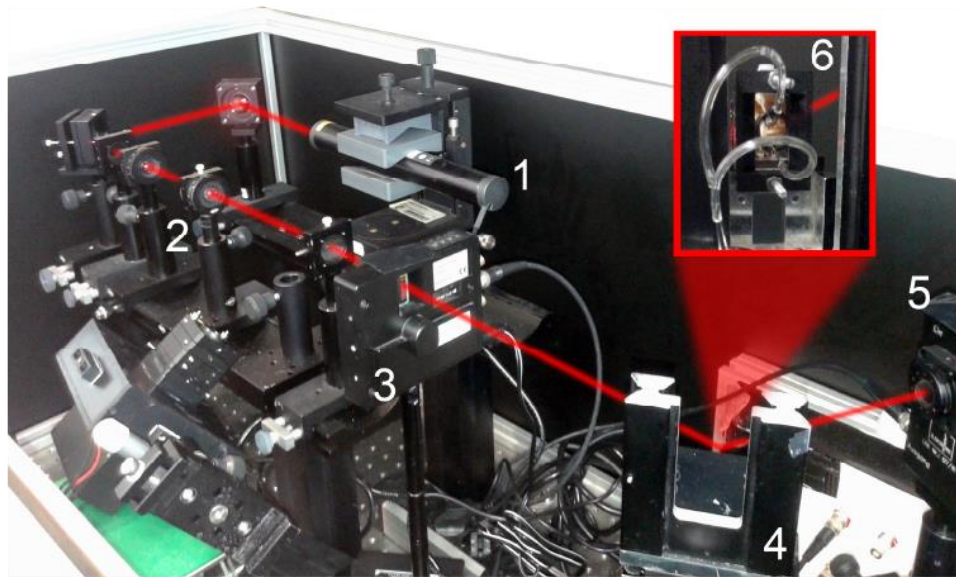
Tracking of
SPR angle

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

Analysis of
reaction
kinetics

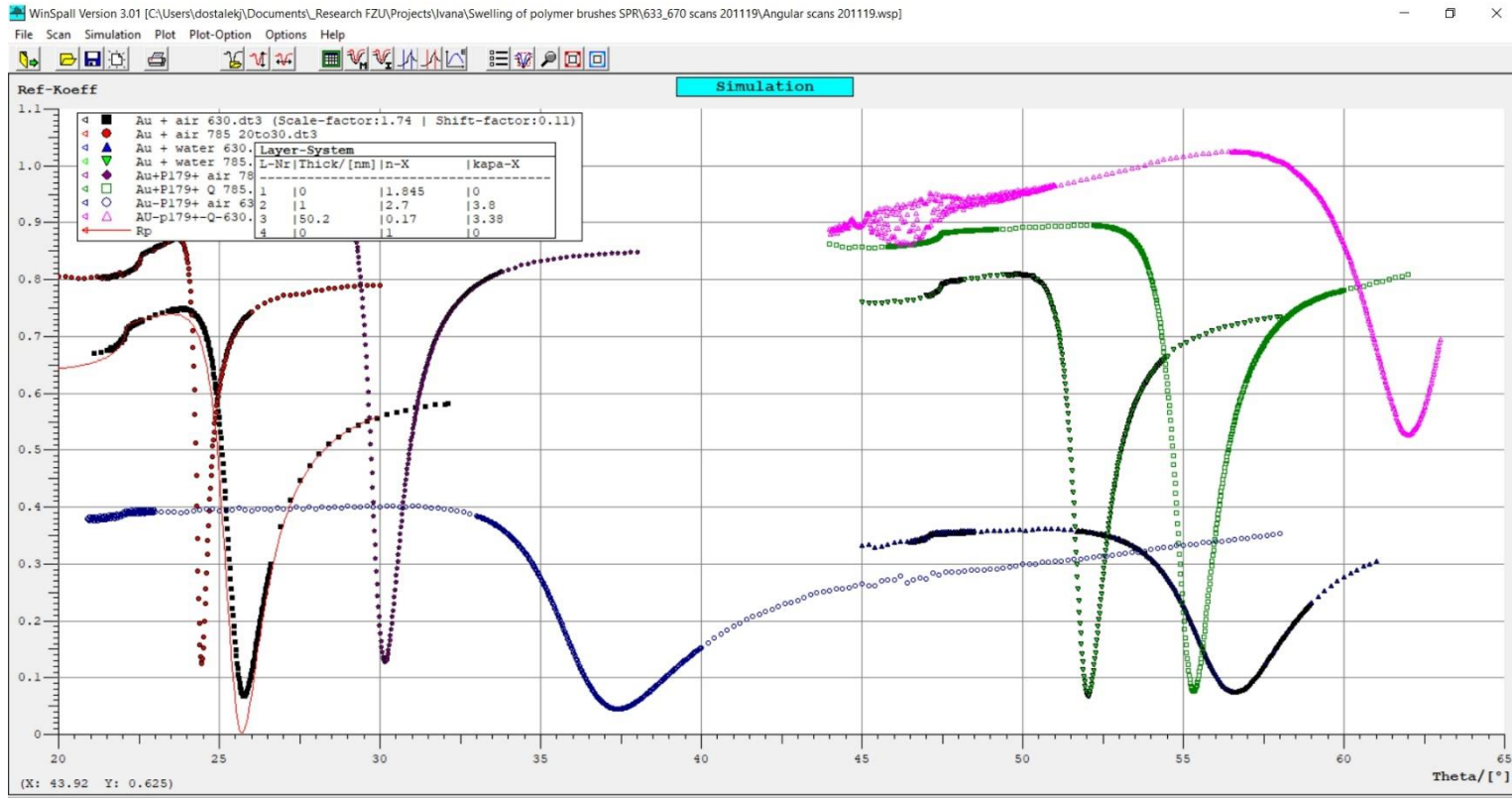


Implementation at DPU



Left: SPR setup, with (1) He-Ne laser, (2) polarizer, (3) chopper, (4) goniometer, and (5) detector. (6) front view of sample with flow cell. Right: Sample holder with the prism and immersion oil.

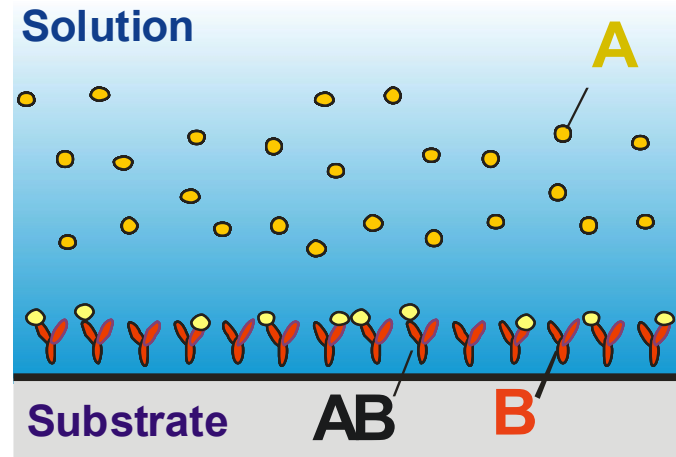
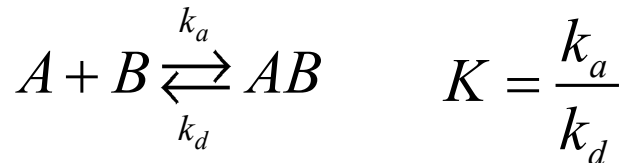
'Mainz' SPR Biosensors



➔ Fitting of reflectivity curves allows to determine the thickness d_f and refractive index n_f in order to determine the surface mass density Γ .

Langmuir Adsorption Isotherm

Equilibrium of a reaction:



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:

- Identical monovalent receptors B
 - Constant concentration of A in the solution ($[A] \gg [B]$)
- (Possible to describe more complicated interactions e.g. multivalent receptors)

Surface Mass Density

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

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

Γ – surface mass density of the polymer layer [ng/mm²]

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/mm²]

Surface Mass Density

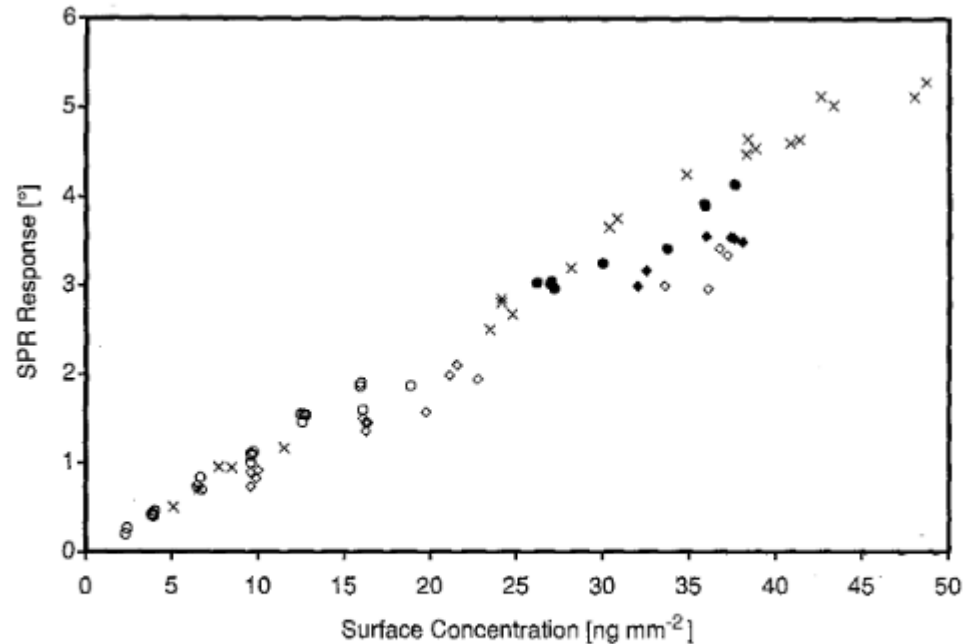


FIG. 7. Experimentally obtained SPR response as a function of surface concentration of the radiolabeled proteins studied. (○ and ● are ¹⁴C-labeled chymotrypsinogen, ◇ and ◆ are ¹⁴C-labeled transferrin, × are ³⁵S-antitransferrin monoclonal antibody. Open symbols are results from dish incubation.)

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

[10.1016/0021-9797\(91\)90284-F](https://doi.org/10.1016/0021-9797(91)90284-F)

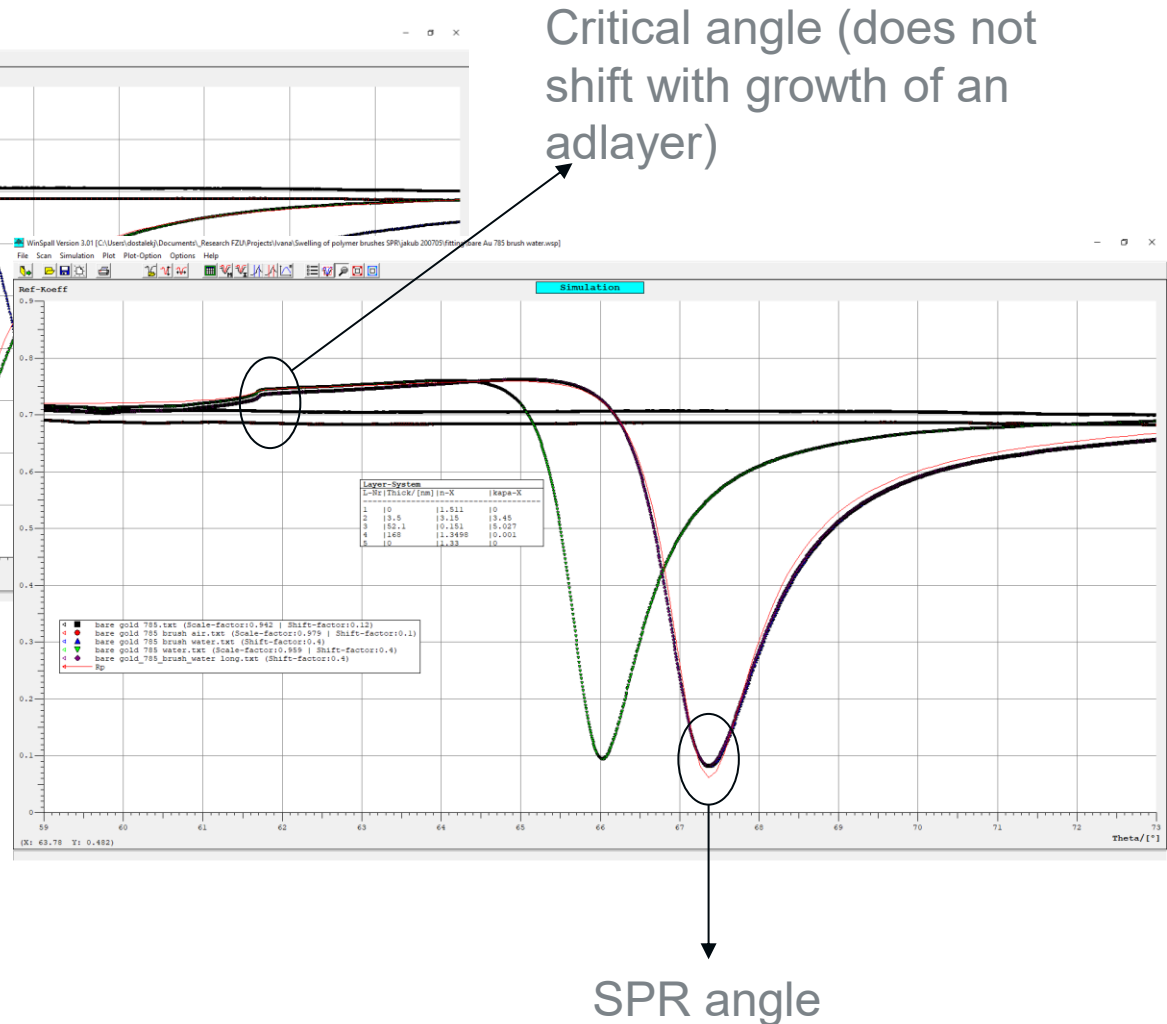
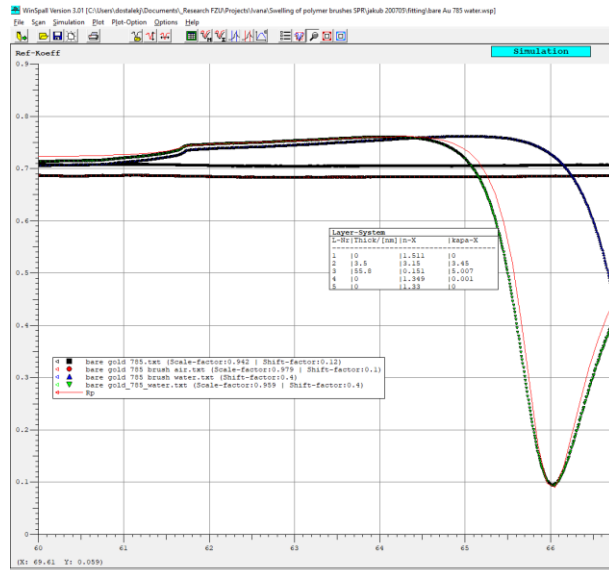
Quantitative Determination of Surface Concentration of Protein with Surface Plasmon Resonance Using Radiolabeled Proteins

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

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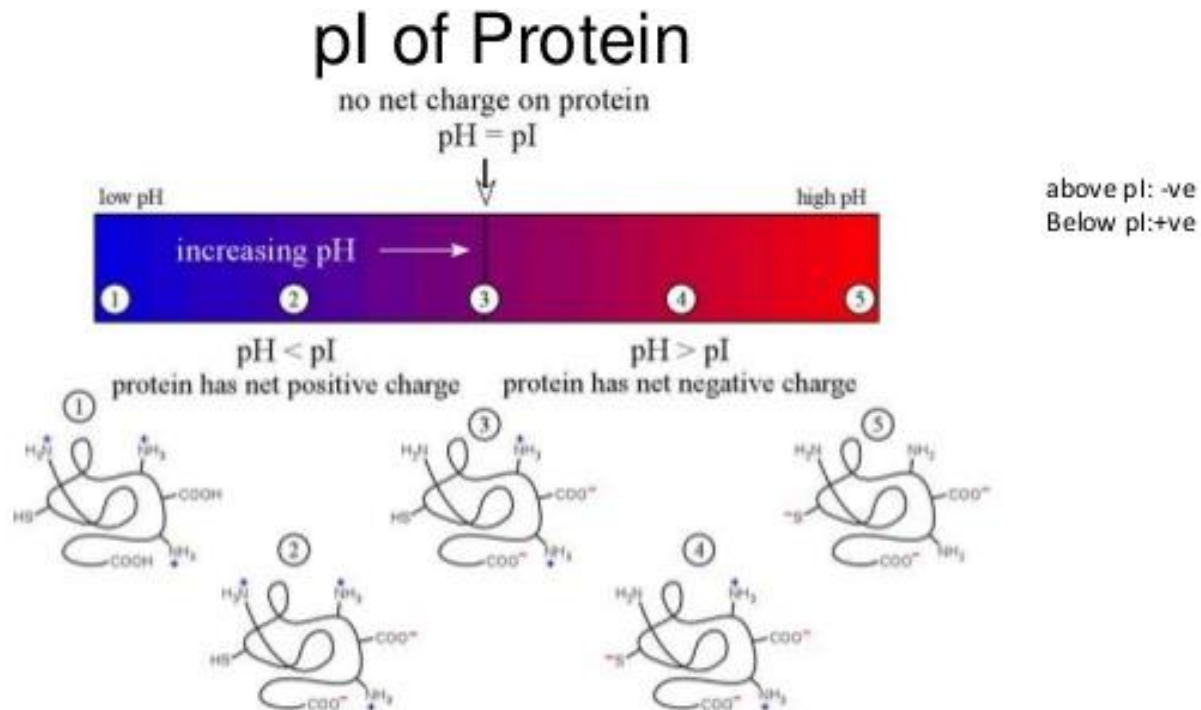
Received August 21, 1990; accepted November 1, 1990

SPR Biosensor



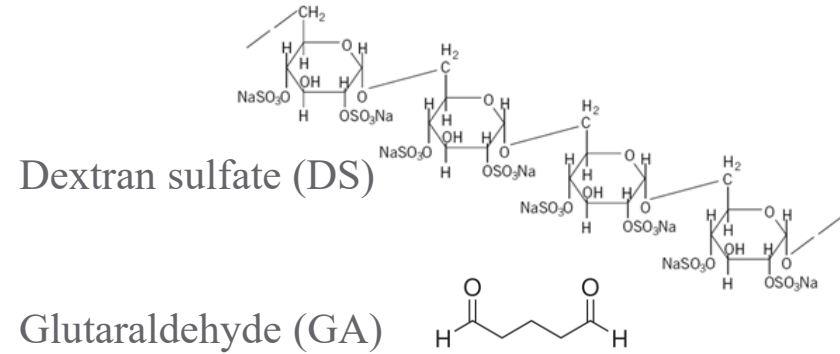
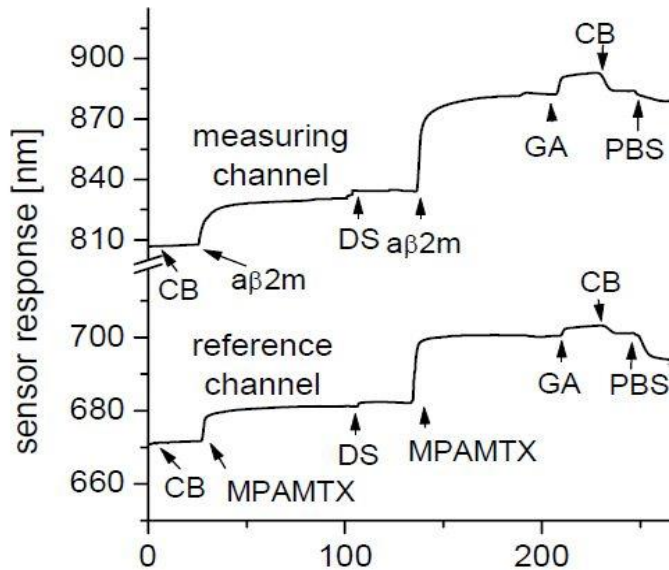
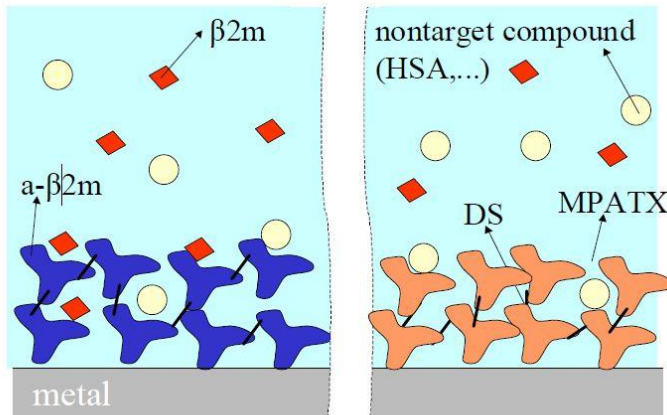
➔ Example of fitting of reference substrate and substrate with a polymer layer by Fresnel reflectivity model.

Isoelectric point



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

Layer-by-Layer

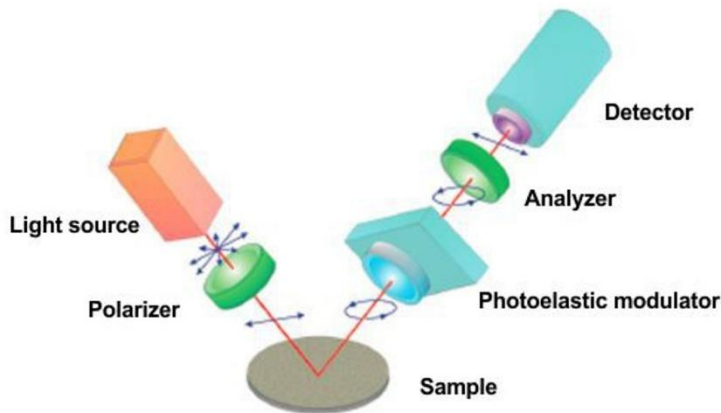


Citrate buffer (CA), pH=4 below pI

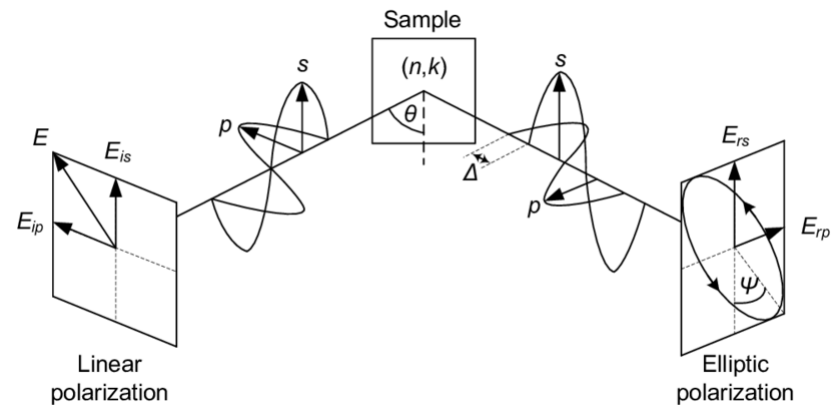
MPTAX, α - β 2m – positively charged Ab in CB

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

Ellipsometry



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

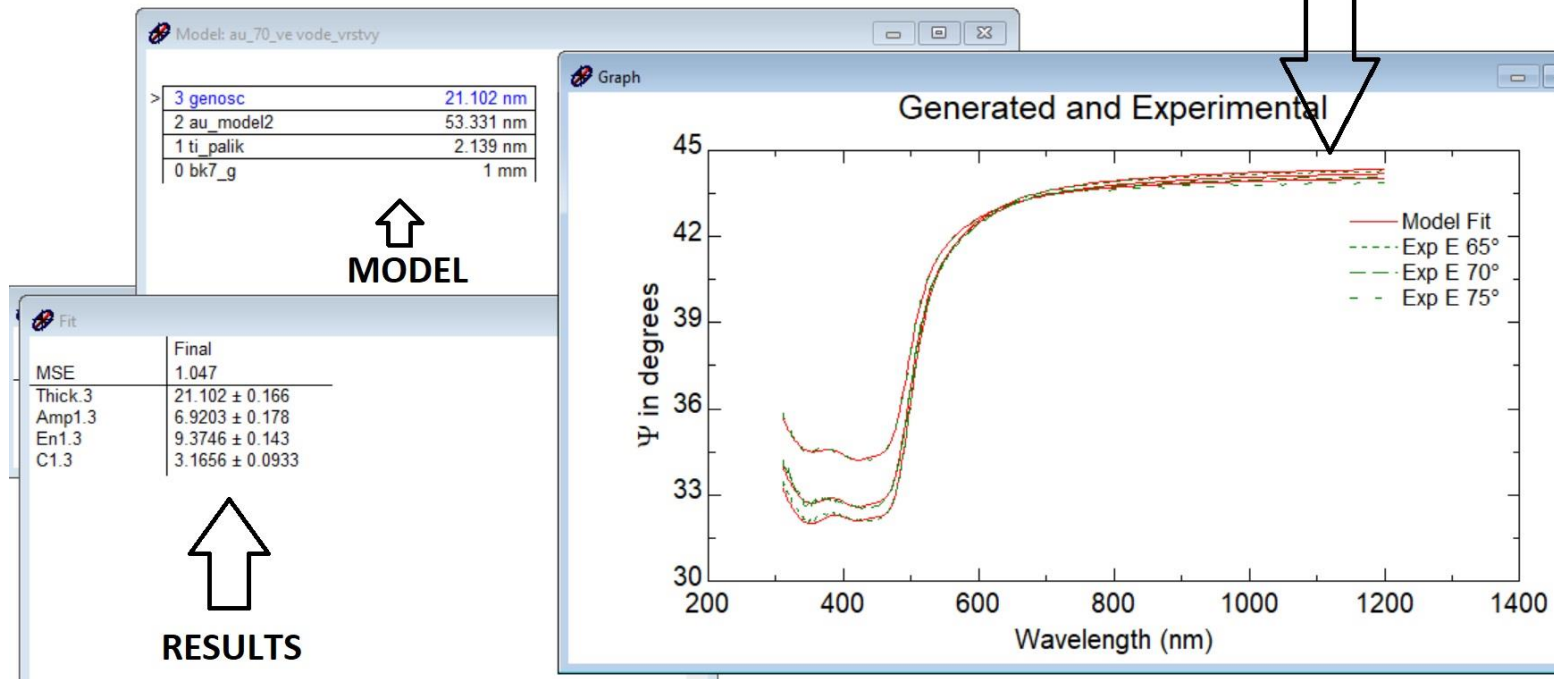


- ➡ Established technique for characterization of thin film stacks.
- ➡ Measurement and fitting of angular and wavelength spectra of phase shifts Δ , ψ . Determination of thickness and refractive index of each layer.
- ➡ Typically is performed in reflectivity mode for a stack in contact with air, possible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

Ellipsometry

V.A.S.E. for Windows [pcb24h_suchy_mod2_final]

File Edit Data Type Style



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

SPR – Observation of Thin Films

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

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

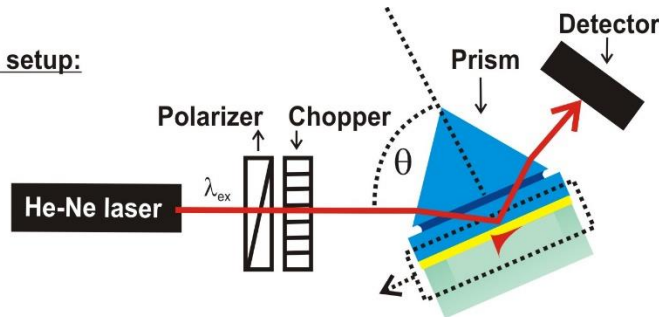
n_1 – prim refractive index, n_2 – solvent (water) refractive index.

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

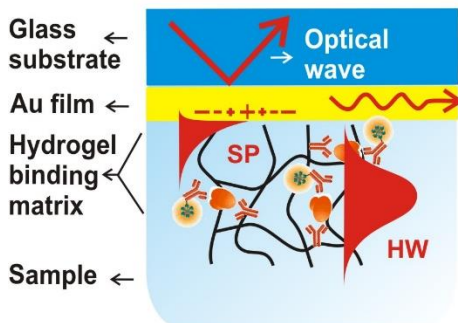
- (1) Multiple wavelength probing ([10.1016/0030-4018\(96\)00238-6](https://doi.org/10.1016/0030-4018(96)00238-6))
- (2) Surface mass density matching (de Feijter approach, [10.1002/bip.1978.360170711](https://doi.org/10.1002/bip.1978.360170711))
- (3) Exclusion heights (Gustav [10.1021/acs.jpcc.8b09171](https://doi.org/10.1021/acs.jpcc.8b09171),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

Observation of Thicker Polymer Films

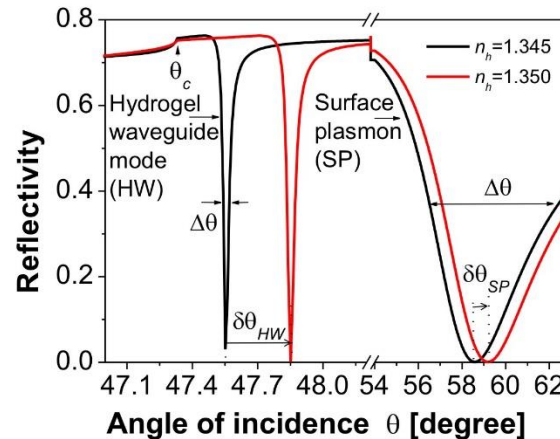
Optical setup:



Sensor surface architecture:



Reflectivity spectrum:



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

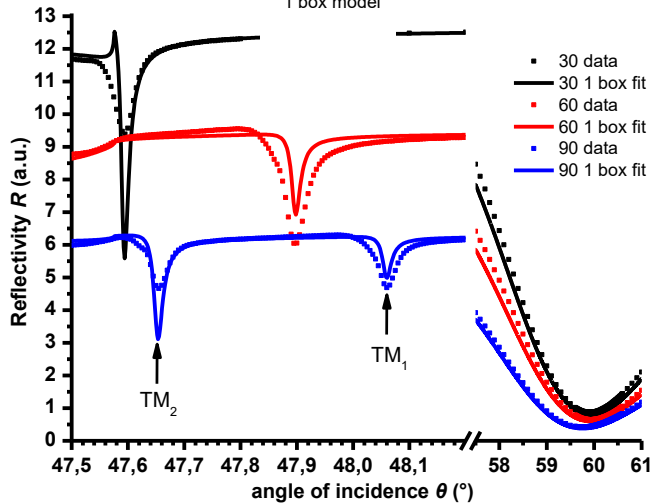
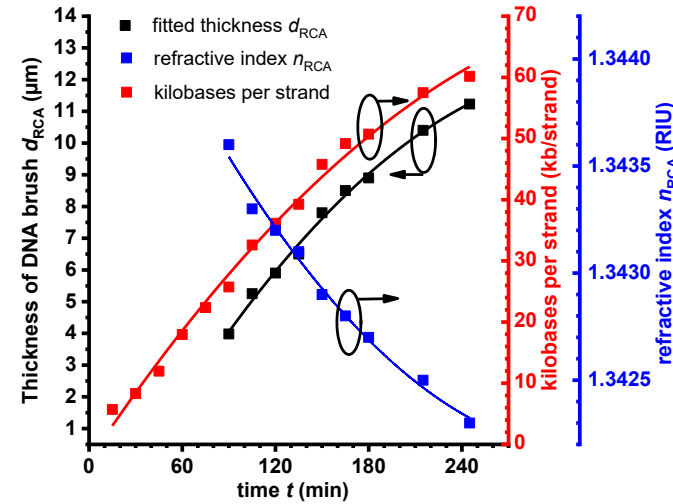
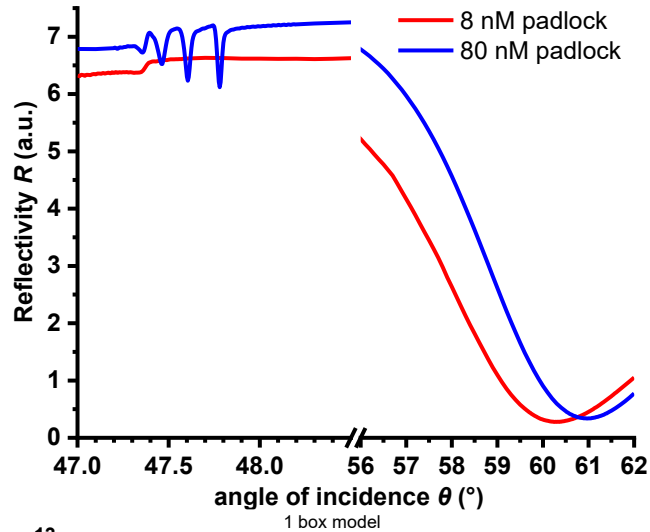
Analysis of the reflectivity spectrum



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 $d_h/d_{h\text{-dry}} > 10$

Observation of RCA by OWS



- ➔ The speed of ssDNA growths is about 5 nt/s at room temperature $T=22\text{ }^\circ\text{C}$ with polymerase Φ -19POL.
- ➔ Reaction can proceed without saturation to generate very long chains $d_{RCA} > 10\text{ }\mu\text{m}$