A new thiol-coated interface for the development of an aptasensor for lysozyme

<u>Iuliana Mihai</u>, Alis Vezeanu, Alina Vasilescu

[*]International Centre of Biodynamics, Bucharest,Romania

Greece, 21-25 October 2013

**COST Action TD1102-CGA-II Project,
Phototech project**

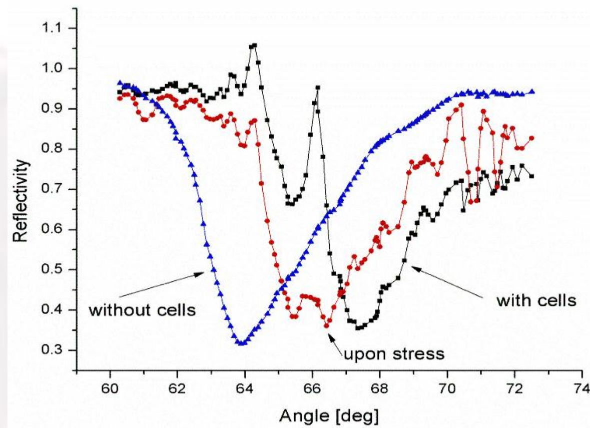
International Centre of
BIODYNAMICS
Bucharest-ROMANIA
www.biodyn.ro

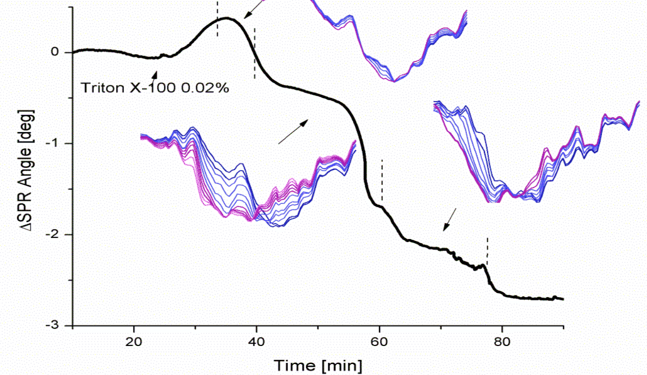
- ✓ *initiates and coordinates research programs;*
- ✓ *involved in a number of collaborative projects with other organizations;*

- ✓ *An International Postgraduate program "Master & Ph.D. in Biodynamics", is under development in collaboration with the University of Bucharest;*

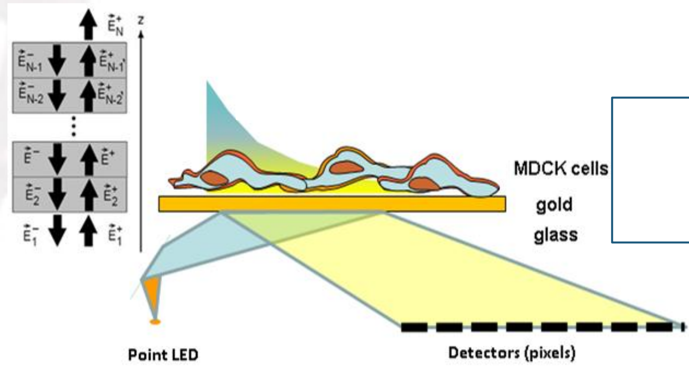
Assess cell dynamics: attachment, swelling, activation and cell-cell junctions using SPR

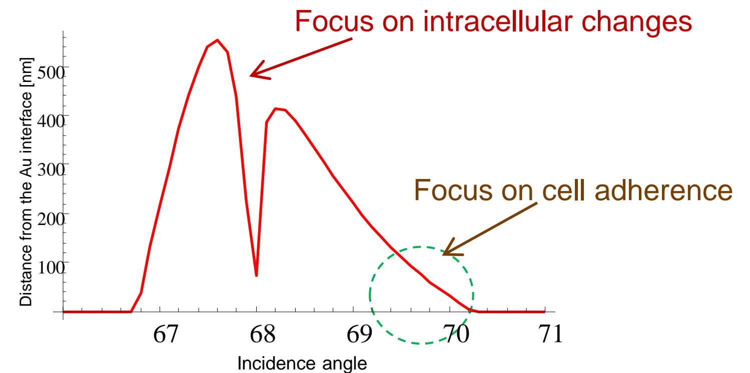
Gheorghiu *et al,* Biosensors and Bioelectronics (2014);
Gheorghiu et *al J. Alzheimer Disease* (2013)
Andreescu *et a*l *ACS Series* (2012)
Gheorghiu *et al*, Springer Series (2012);





Reveal the Analytic "zooming" capabilities at various heights provided by analysis of distinct angle domains !





Relevance?

Evaluate the effect of Aβ42 exposure on MDCK cells: a non monotonous, multiphasic process affecting both cell – surface interface and cell interior is revealed by combined Electro-Optical assays.

Novel sensing avenues based on Periodic Actuation (*) for:

(1) Fast Quantitation/Detection of low concentrations of **target** Target Cells / Microorganisms **in various media**.

Measurement principle:

- When applying a periodic magnetic field the induced oscillation of magnetically labelled target cells between a pair of electrodes is assessed by Electrochemical Impedance Spectroscopy, EIS measurements.
- Cell Concentration is derived based on the amplitude or/and phase of the oscillation exhibited by the electrical impedance at selected AC frequency.

Assessment of pathogenic bacteria using periodic actuation
Front Cover Lab Chip, 2013, 13,3192–98

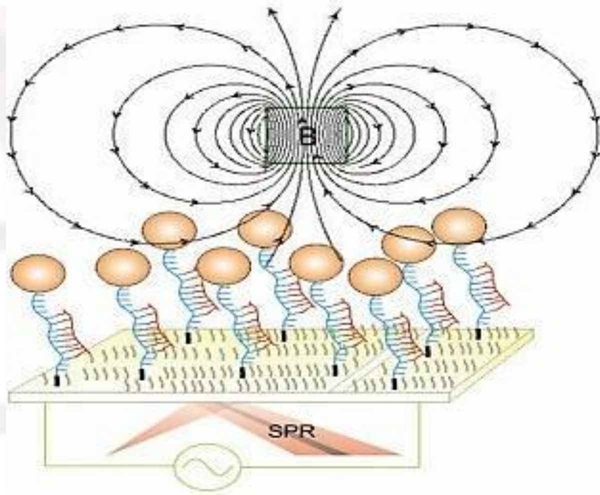
Distinctive Results:

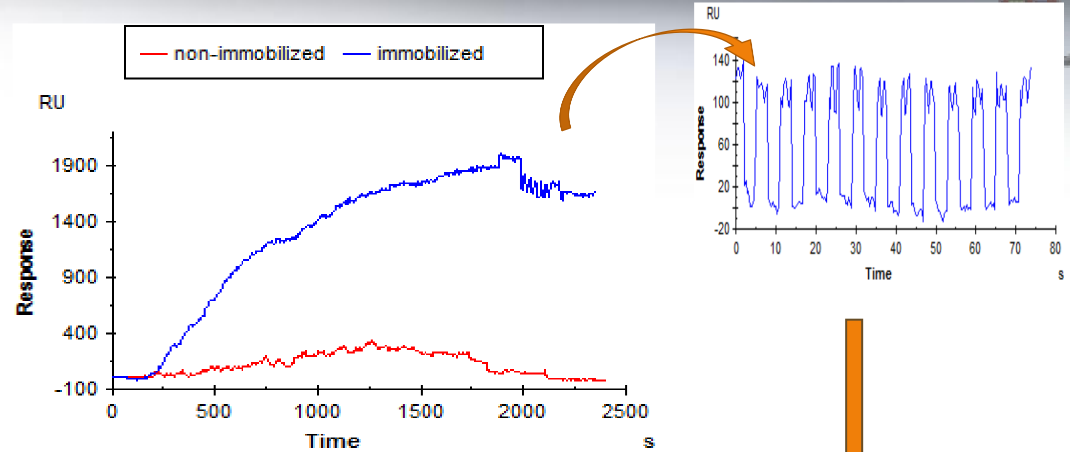
- Low limit of detection: $10^2 \pm 10$ cell/mL
- High specificity
- Fast analysis time < 1h (incubation period comprised)

(*) Gheorghiu et al, (2013). Systems and Methods for Detection and Quantitation of Analytes Using Periodic Actuation. *European Application No. 12733235.1, 2013. U.S. Patent Application No. 13/398,472/2012.*
Gheorghiu, E. (2011). Method to assess the amount of target analytes by controlled periodic actuation. *RO Patent Application A00136*

Novel sensing avenues based on Periodic Actuation (*) for:

(2) Effective assessment of DNA hybridization.

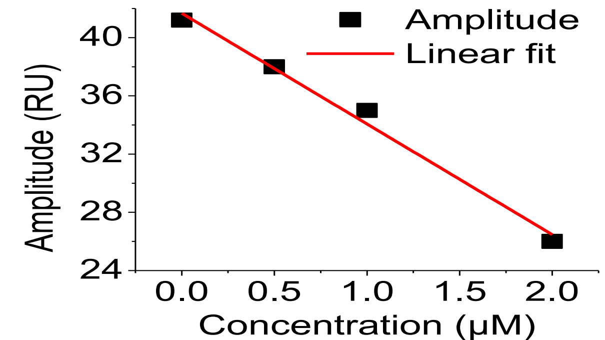




Measurement principle:

Oscillations of dsDNA-MB complexes

Periodic oscillations of ss and dsDNA-MB complexes are induced by controlled magnetic field gradient and monitored by electric measurements (EIS) and surface plasmon resonance (SPR); ssDNA oscillates with larger amplitude than dsDNA



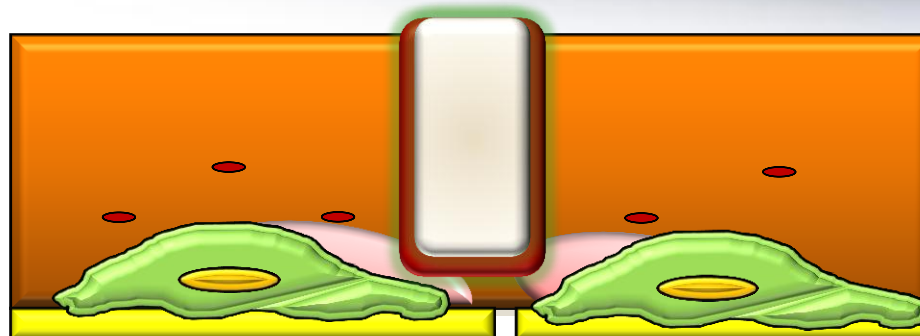
Calibration curve

Study carried out by Dr. C. Polonschii, grant winner of ICBS 2013 - Tsukuba, Japan & Finalist L'Oréal –UNESCO /2013

(*) Gheorghiu et al, (2013). Systems and Methods for Detection and Quantitation of Analytes Using Periodic Actuation. *European Application No. 12733235.1, 2013. U.S. Patent Application No. 13/398,472/2012.*
Gheorghiu, E. (2011). Method to assess the amount of target analytes by controlled periodic actuation. *RO Patent Application A00136*

Monitoring & Detection of ROS release at cellular level

Platforms for observing Reactive Oxygen Species, ROS at cellular level



← Enzymatic ECD

← Stressor

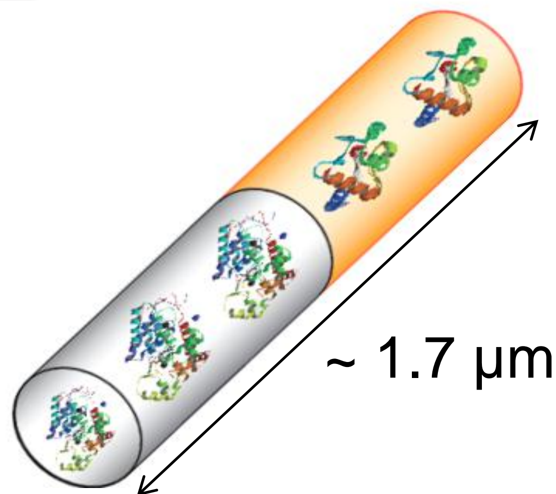
(calcium oxalate, nanoparticles)

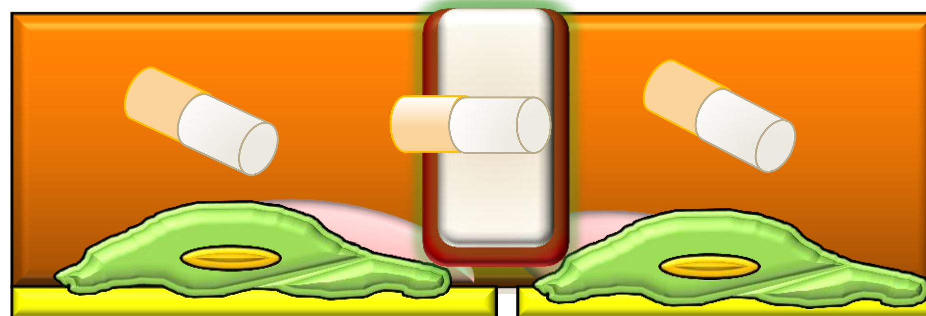
← ROS generation

← IS monitoring

1. Gáspár, et al Analytica Chimica Acta, (2012);
2. Gáspár et. al Biosensors and Bioelectronics, (2010);

Nanorods are modified with hemeproteins (e.g. HRP and Cyt c) to add selectivity and improved sensitivity.





Enzymatically enhanced motion of nanorods – towards autonomous motion of nanoparticles in biological systems

Bunea et al, Chemical Communications (2013), 49, 8803-8805.

Introduction

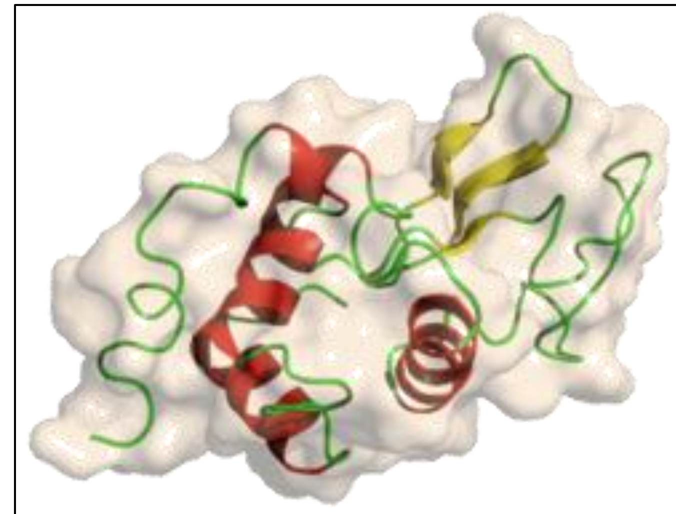
Aim: ***the development and the characterization of a new thiol coating allowing controlled and efficient immobilization of biorecognition elements and minimum non-specific adsorption with application <u>in lysozyme</u> detection.***

Lysozyme is a single chain polypeptide of 129 amino acids cross-linked with four disulfide bridges.

- Enzyme used for lysing bacterial cells by hydrolyzing the peptidoglycan present in the cell walls;
- MW: 14,307 kDa, pI: 11,35;
- It can form amyloid fibrils in vitro and it was extensively used as a model to study protein aggregation [1]

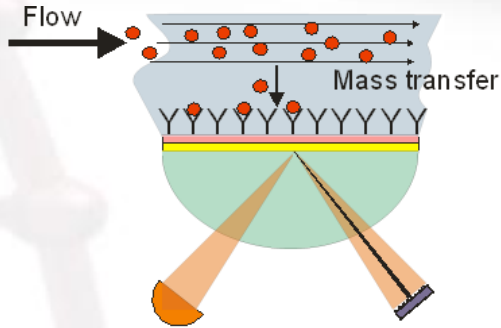
<u>Forced aggregation</u> - incubating the acidic solution of lysozyme at 60°C [2].

- ➢ 0 - 12 hours: monomer;
- ➢ 12-48 hours: dimers and higher oligomers;
- ➢ after 48 hours: protofibrils and fibrils.

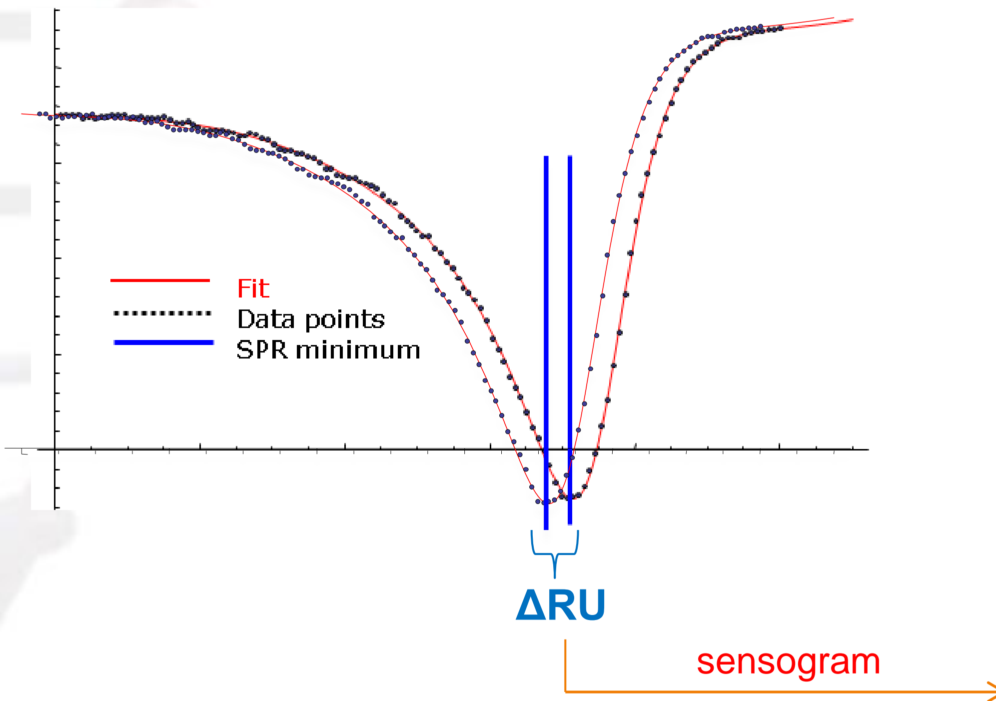


1. *Frare E. et al., 2006. Journal Of Molecular Biology 361, 551-561.*
2. *Vasilescu, A.,et al.,2013. Analyst 138, 3530–3537.*

Surface Plasmon Resonance

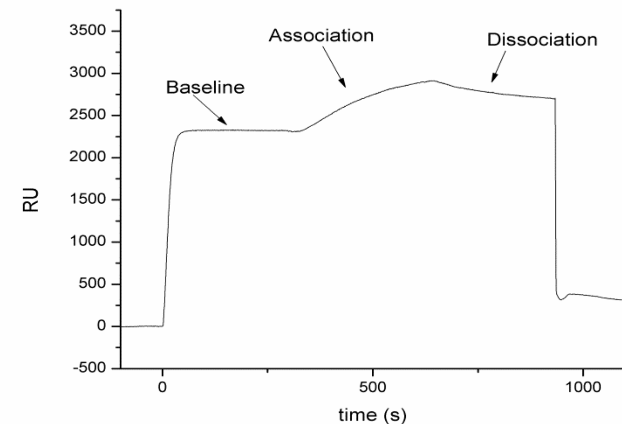


SPR is an optical technique that enables real-time monitoring of changes in the refractive index of a thin film close to a surface.



Data processing

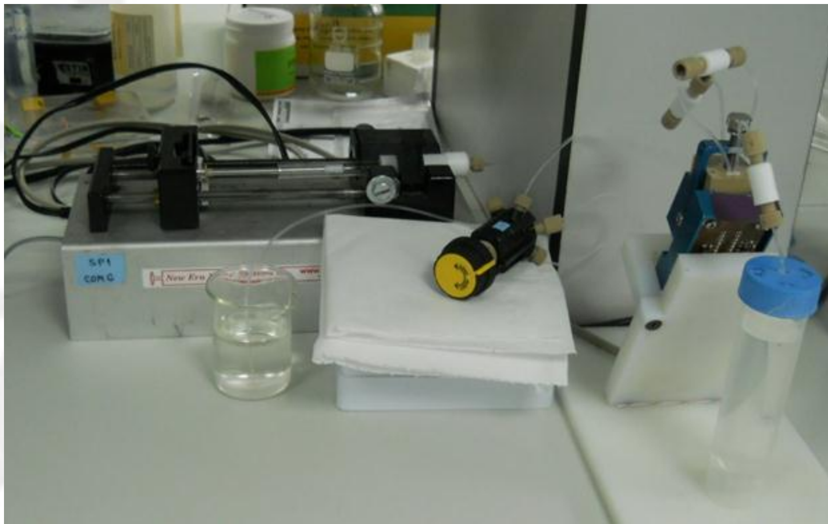
- Signal is acquired through a PC interface;
- Acquired signal is fitted with corresponding function and the SPR data is extracted;
- Refractive index values changes are monitored over time .

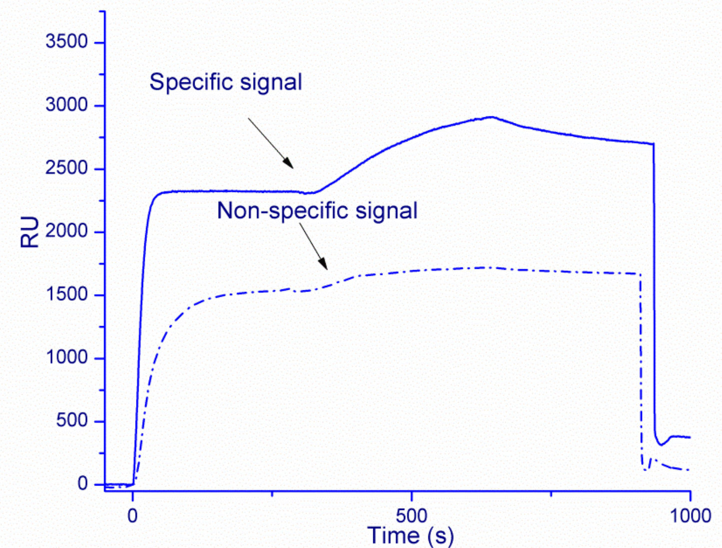


Surface Plasmon Resonance

Equipment

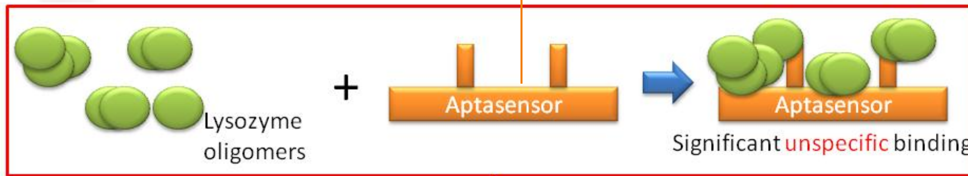
- 2 channels portable SPREETA SPR sensor (Texas Instruments) [3] .
- PC interface developed by ICB.

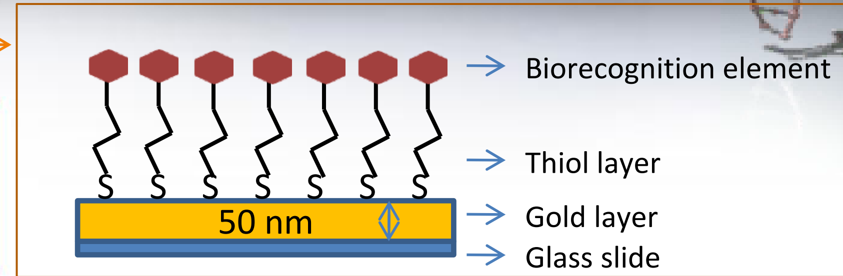


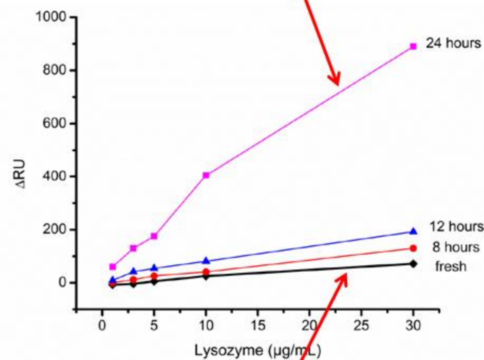


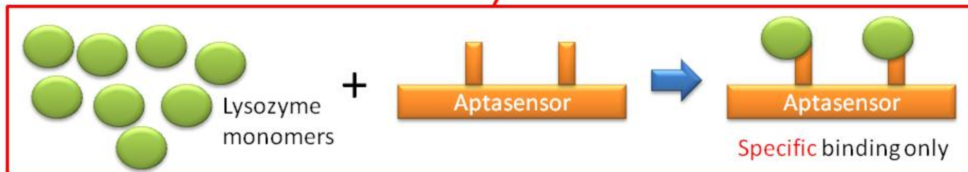
3. Polonschii et al., 2010. Talanta 80, 2157–2164.

Motivation









- ↗ Label-free detection of lysozyme;
- ↗ Linear range - 5-50 μg/mL;
- ↗ LOD -1 μg/mL;
- ↗ Monitoring the early stages of the aggregation of lysozyme;
- ↙ Large non-specific adsorption for aggregated lysozyme.

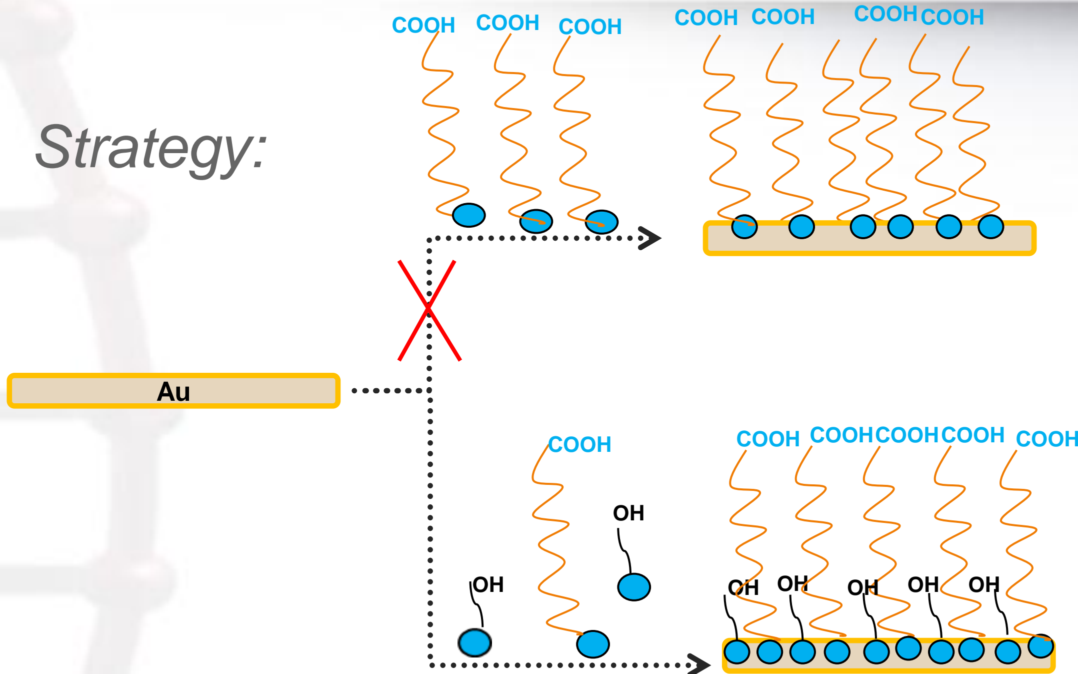
Aptamer sequence:

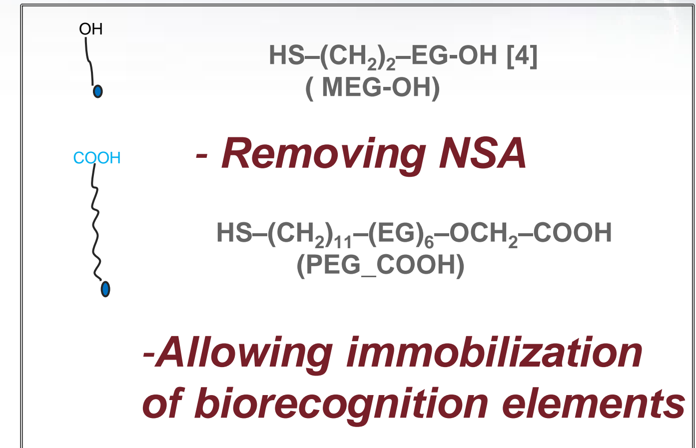
5'GGGAATGGATCCACATCTACGAATTCATCAGGGCTAAAGAG3'

2. Vasilescu, A.et al., 2013. Analyst 138, 3530–3537.

Manufacturing stages of an aptasensor

Strategy:





!! The conditions used for manufacturing a platform for biosensing applications need to be carefully chosen because they can affect the efficiency of the resulting sensor.

4. Avci, C. et al., 2013. Chemical Communications 49, 466–468.

Step 1: The cleaning procedure

The SPR gold chips were cleaned using two different procedures:

- oxidative treatment using a mixture of sodium hydroxide and hydrogen peroxide *Method 1* [5];
- reducing treatment using sodium borohydride ($NaBH_4$) -*Method 2* [6].

We studied the relevance of these cleaning procedure in the NSA evaluation of 1 mg/mL lysozyme solution:

Conditions: **A fresh solution of 1 mg/mL lysozyme in PBS was injected for 15 min at a rate of 30 µL/min. Next, the SPR cell was rinsed for 10 min with PBS buffer (100 µL/min).**

$$\Delta RU = RU_{after} - RU_{before}$$

5. Fischer, L.M.et al., 2009. Microelectronic Engineering 86, 1282–1285
6. Ansar, S.M. et al., 2013. Nanoletters 13, 1226–1229.

Step 2: SAM formation

a. The influence of time of SAM formation to the protein adsorption:

- Homogeneous MEG-OH

Lysozyme NSA was monitored on bare gold chips and compared with chips kept in 1mM HS–(CH2)2–EG-OH thiol solution for 30 minutes, 2 hours and 2 days respectively.

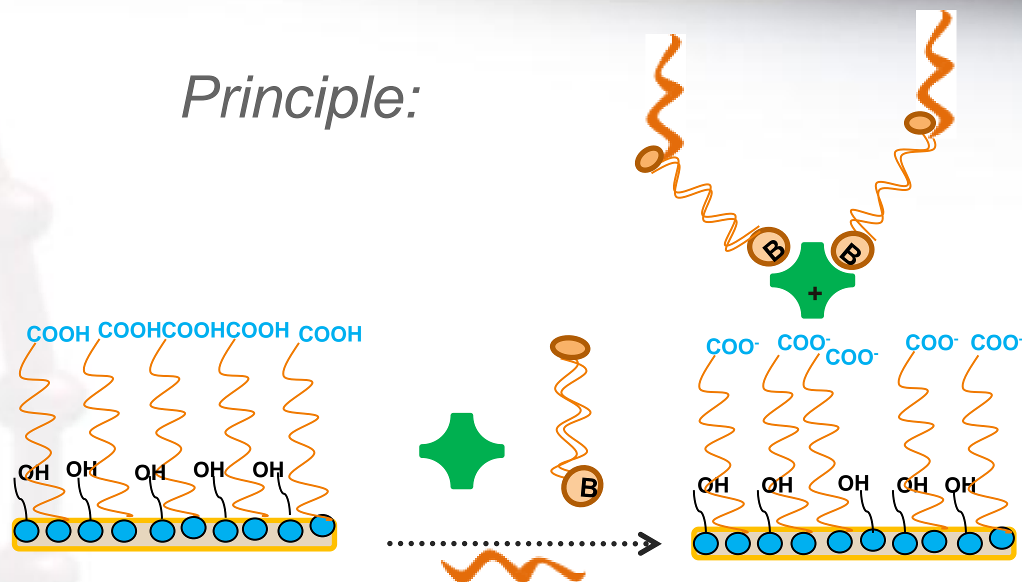
b. The composition of the thiols mixture

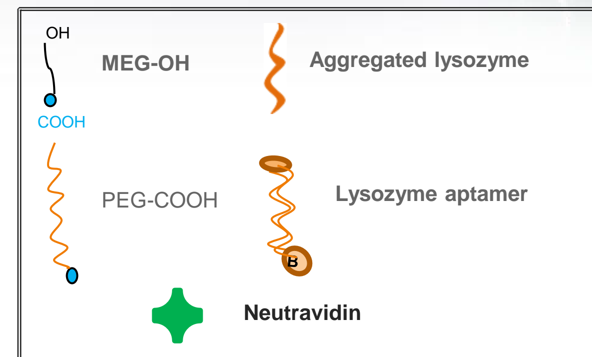
- **Homogeneous PEG-COOH – immobilization capacity - high NSA of Lysozyme oligomers;**
- **Homogeneous MEG-OH - does not allow the immobilization of ligands- good antifouling properties.**

=> PEG-COOH/MEG-OH mixed SAM

Step 3: Functionalization

Principle:





SAM type	Avg. RU $_{Neutravidin}$	RSD (%)	No. Det.
Homogenous PEG-COOH	1814	13.9	10
Mixed SAM	1474	8.5	5

✓ **The immobilization capacity of the sensor coated with the mixed SAM decreases only by 18% compared with the homogeneous carboxyl-ended SAM.**

Step 4: NSA measurements

Functionalization of sensor surfaces can influence drastically their properties with regards to protein resistance [7].

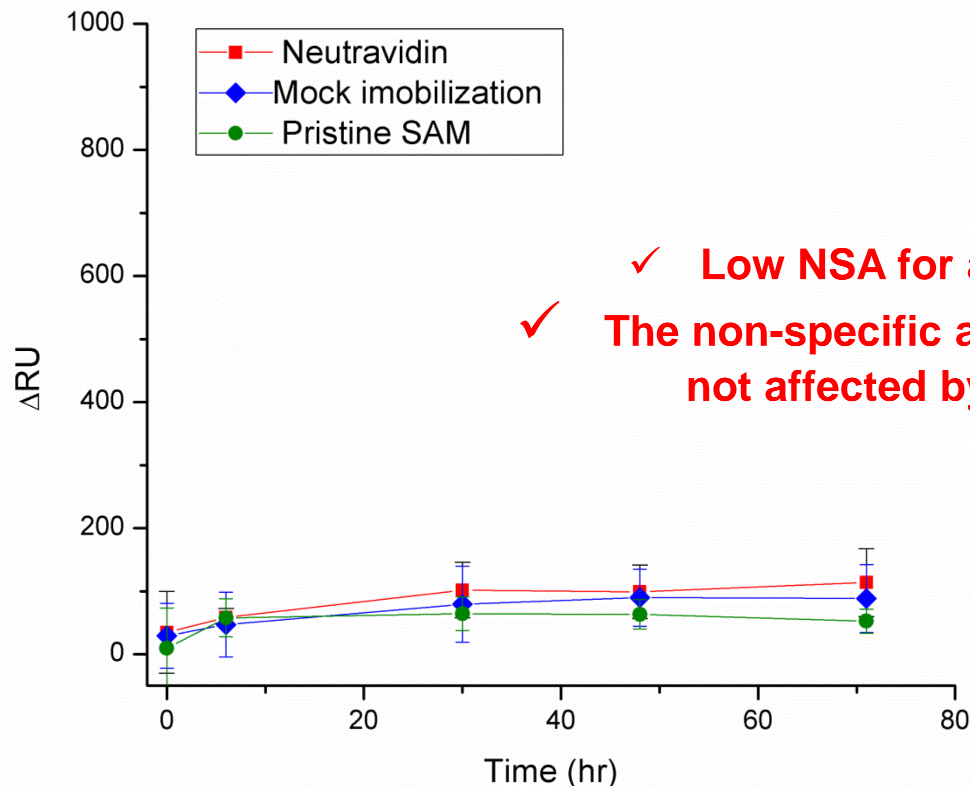
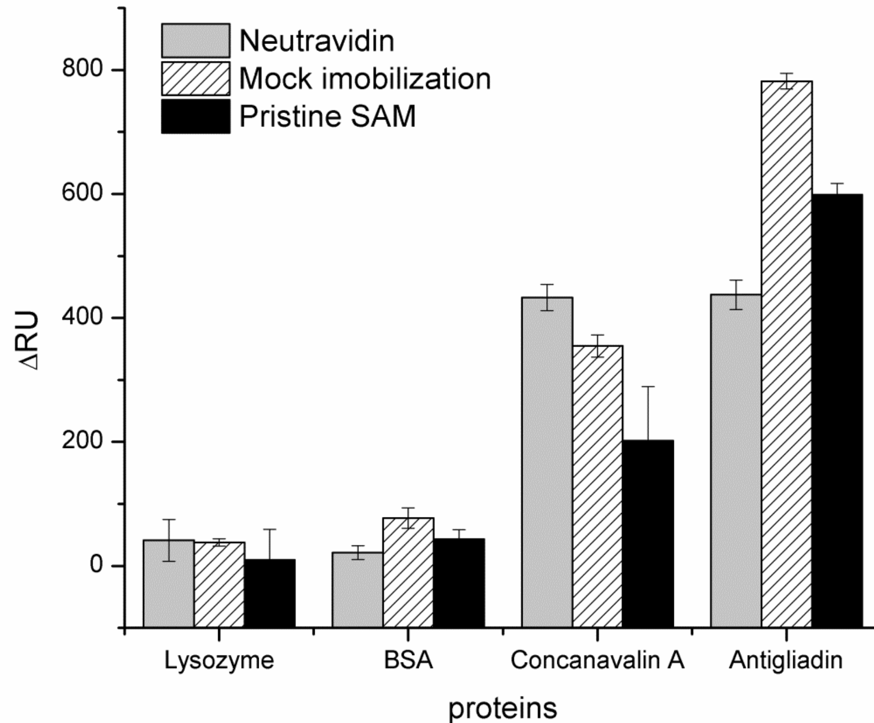


Figure 1. Variation of the SPR signal due to NSA of lysozyme and oligomers (t = 0 corresponds to monomeric lysozyme).

- ✓ **Low NSA for aggregated lysozyme;**
- ✓ **The non-specific adsorption of lysozyme was not affected by the functionalization.**

7. Vaisocherová H. et. al, 2014. Biosensors and Bioelectronics 52, 150-157].

Step 4: NSA measurements



Protein	MW (kDa)	pI
Lysozyme	14.3	11.35
BSA	66	4.7
Concanavalin A	104	5.5
Antigliadin antibody	150	-

Figure 2. Variation of the SPR signal due to NSA of several proteins having different molecular weight and isoelectric

✓ **The NSA is dependent on the physical properties of the protein.**

Step 5: Testing the operational stability of the sensor

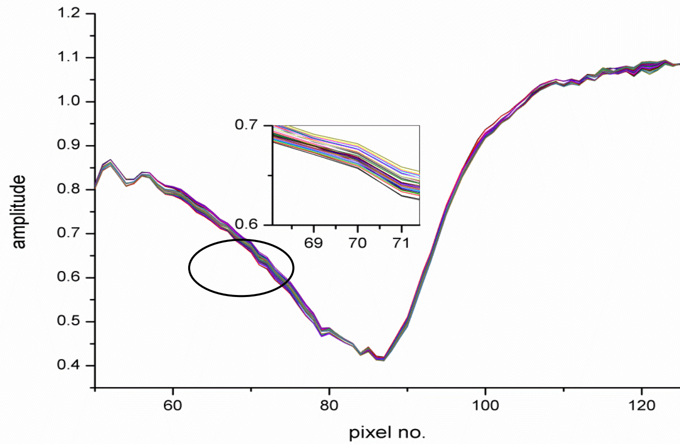
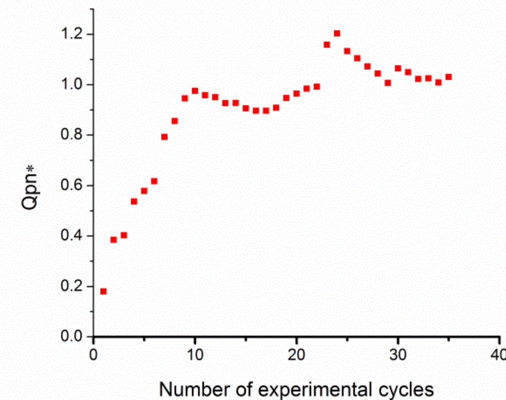


Figure 2. SPR curves for consecutive injections of lysozyme on Neutravidin functionalized surface- during 3 days.



- ✓ **The sensor is relatively stable after 3 days of repetitive regeneration steps.**

**Olaru A, Gheorghiu M, David S, Polonschii C, Gheorghiu E, Biosensors and Bioelectronics 45 (2013) 77–81*

Step 6: Proof-of-concept experiments

Lysozyme monomer

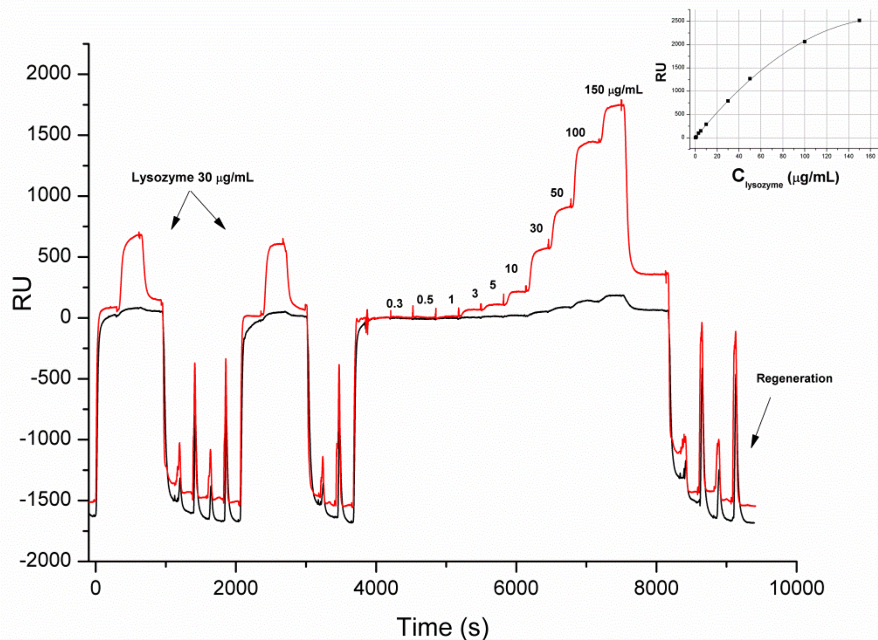


Figure 1. Determination of lysozyme with the SPR aptasensor by successive injections of increasing concentration: specific signal on the aptamer-modified channel, black: non-specific signal, inset: calibration plot.

Lysozyme oligomers

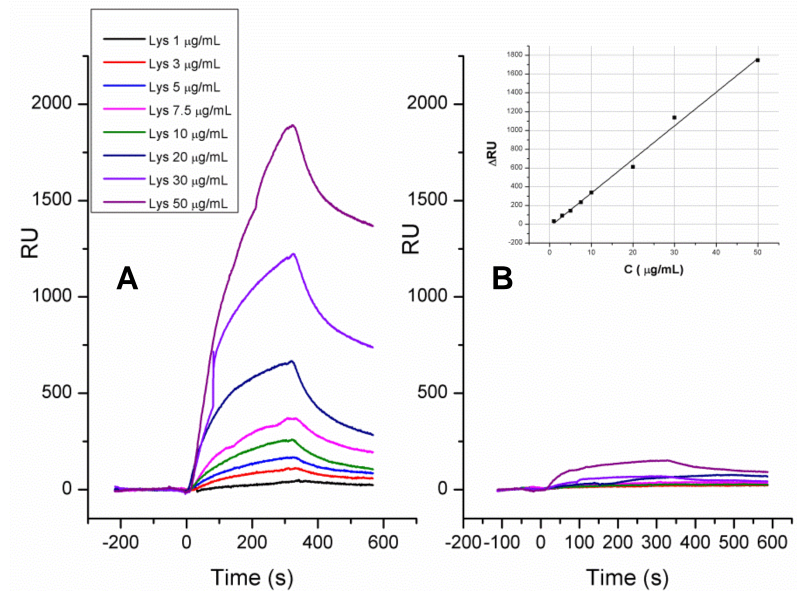


Figure 2. Determination of aggregated lysozyme (48 h) with the SPR aptasensor by injections of different concentration: specific signal (A),non-specific signal (B), inset: calibration plot.

Conclusions

- The PEG-COOH/MEG-OH mixed SAM is very effective in removing the non-specific binding of lysozyme (monomer and protofibrils) and provide stable interfaces with appropriate operational stability;

- The cleaning procedure applied to gold interfaces before SAM formation is critical for the formation of a SAM – The $NaBH_4$ treatment seems to be a good method for this application;

- On both, MEG-OH and mixed thiol, the non-specific signal was dependent on the nature of the protein while on the long PEG-COOH thiol the non-specific signal of different proteins was equally small ;

- The application of the new platform in SPR biosensors was demonstrated by developing an aptasensor for studying the aggregation of lysozyme.

Acknowledgments:

- *The authors acknowledge financial support from Romanian Research Project **PN-II-RU-TE-2011-3-0302** and **PN-II-PT-PCCA-2011-3** and from **COST Action TD1102-CGA-IIProject**, **Phototech project.***
- *We would like to express our acknowledgments to Professor Michael Thompson and Dr. Christophe Blaszykowski from University of Toronto, Canada for providing the "MEG-OH" thiol and to Dr. Cristina Polonschii and Dr. Sorin David from the International Centre of Biodynamics for support with the SPR experiments.*