SURFACE PLASMON RESONANCE IMMUNOSENSORS FOR ENVIRONMENTAL WATER ANALYSIS

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Outline presentation:

- SPR
  - Introduction and Evanescent Field Sensors
  - Main features
  - Instruments
  - Functionalization on SPR sensors
- Pilot example of environmental analysis of organic pollutants
- Future and present work
- Conclusions

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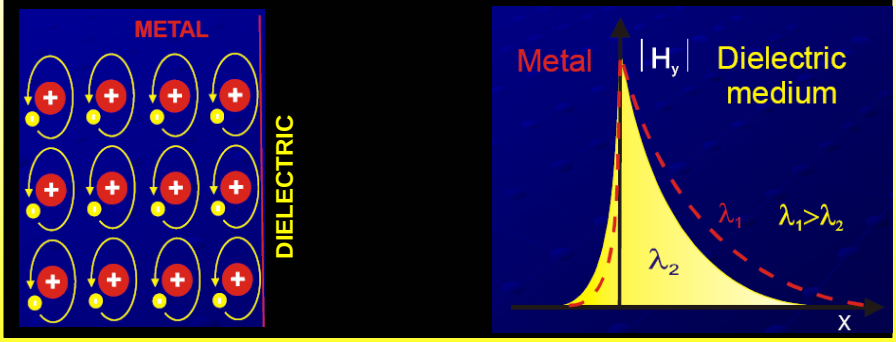
SPR theory

An incident monochromatic light at the interface between 2 substances with different refractive index can produce an evancescent wave



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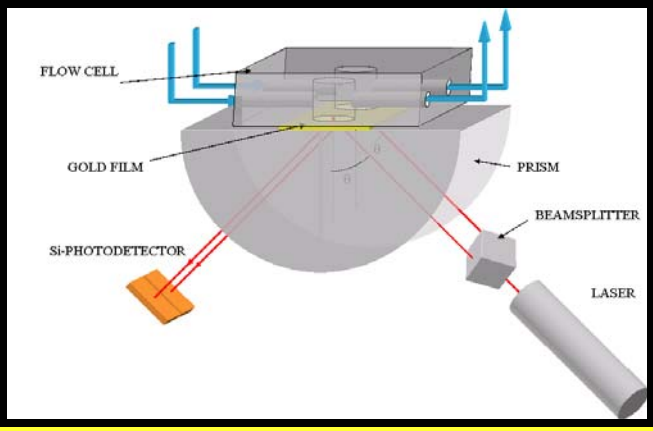
SPR theory and evanescent field sensors



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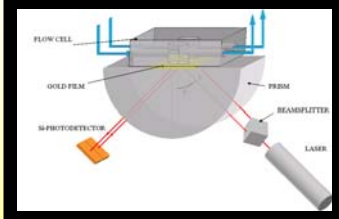

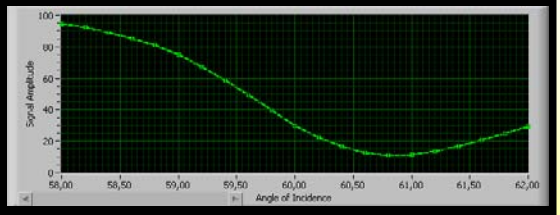

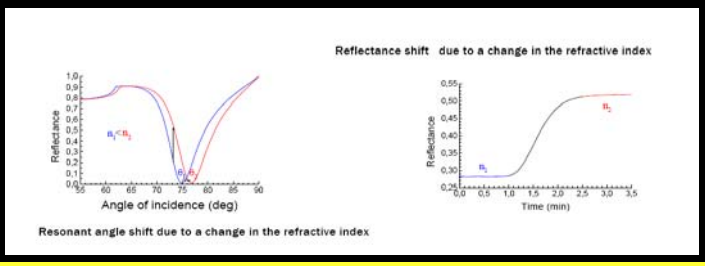

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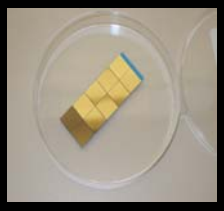
Biomolecular Recognition Elements in Evanescent Field Sensors:

SPR is a generic optical technology that can be combined with specific biological receptors against particular target analytes

- **Antibodies** → **Immunosensor Chips**
- **Proteins**
- **DNA**
- **RNA**
- **MIPs (plastibodies)**



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Affinity SPR Biosensing: Detection Strategies

DIRECT: Medium size and large analytes

INDIRECT COMPETITIVE: Small analytes at low concentration

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Affinity SPR Biosensing: Detection Strategies

DIRECT: Medium size and large analytes

INDIRECT COMPETITIVE: Small analytes at low concentration

→ Environmental analysis

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Surface Plasmon Resonance Biosensors:

Main Features

- **Direct** (can detect analyte in one-step).
- **Real-time** (analyte's binding to sensor surface can be continuously monitored).
- **Label-free** (no fluorescent or radioactive labels are required for analyte's detection).
- **Minimum interaction length/volume required** (small sample volumes can be analyzed).
- **Generic technology** (combines generic optical technology with receptors *specific* against particular target analytes).

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Laboratory SPR instruments based on angular Spectroscopy of surface plasmons:

- **BIAcore (S51, 3000)**
- **Spreeta SPR sensor**
- **SENSIA**

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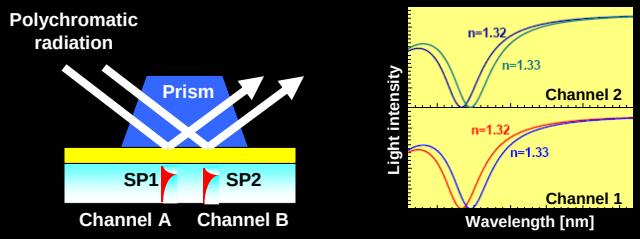
SPR Sensors with Referencing Channels:



Multichannel SPR sensor with parallel sensing channels and SPR spectra observed

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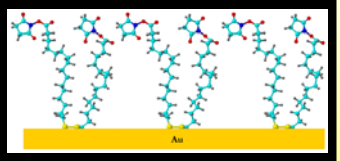
Functionalization of SPR Biosensors:

Alkanethiol Attachment Chemistry

SAM formation

Mercaptoundecanoic acid 0.05 mM in EtOH

Formation N-Hydroxysuccinimide ester

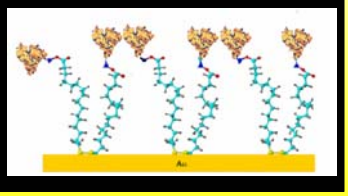


- 1-Ethyl-3(-3-dimethyl-amino-propyl)carbodiimide hydrochloride **(EDC) 0.2 M**
- N-Hydroxysuccinimide **(NHS) 0.05** M

Immunoreagent Immobilization

350+350 µL of 10 µg/L

Blocking agent: Ethanolamine 1 M, pH 8.5



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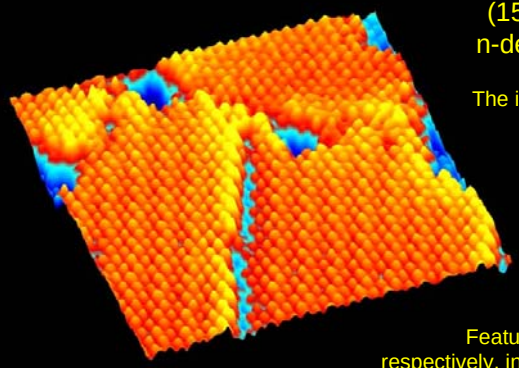

Scanning tunneling microscope (15 nm x 15 nm) of a SAM of n-decanethiolate on Au {111}.

The individual molecules closely pack into a hexagonal array.

Features of the assembly and surface, respectively, including domain boundaries (light blue stripe) and gold vacancy islands (circular blue depressions) are easily seen.

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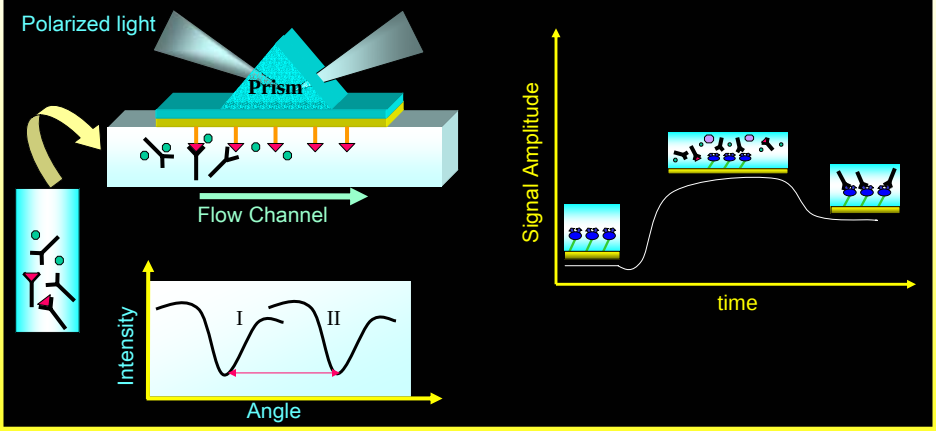
Indirect immunoassay



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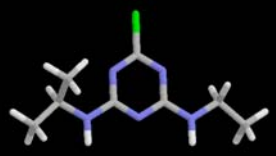
PILOT ANALYTE: ATRAZINE

Development of immunosensor chips

- Alkanethiol Attachment for SAM formation
- 2d-BSA atrazine conjugate



Immunoassay optimization

- Indirect format assay
- Purified polyclonal antibodies against Atrazine

Validation

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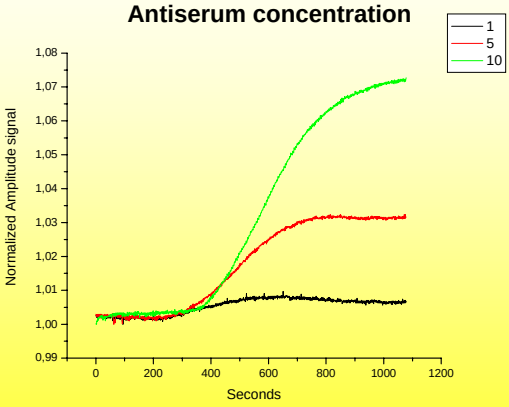
Immunoassay optimization:

- Antiserum concentration
- Incubation time
- Elution
- Buffers
- Regeneration

Antiserum concentration



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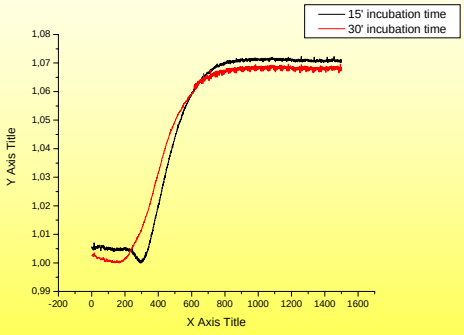
Incubation times



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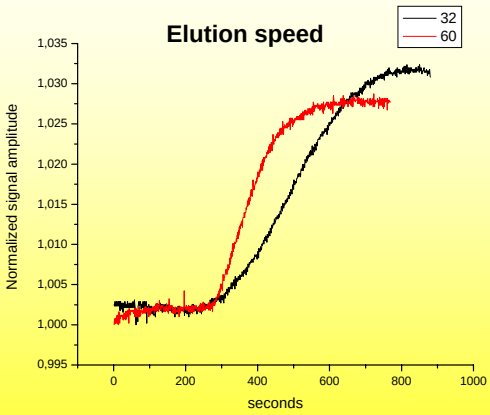

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Immunoassay optimization:

- Antiserum concentration
- Incubation time
- Elution
- Buffers
- Regeneration

Regeneration:

- HCl 0.05 M
- HCl 0.75 M
- HCl 0.1 M
- HCl 0.2 M
- NaOH 0.05 M
- NaOH 0.1 M

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Regeneration:

- HCl 0.05 M
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- NaOH 0.05 M
- NaOH 0.1 M

* More than 120 regeneration cycles can be performed

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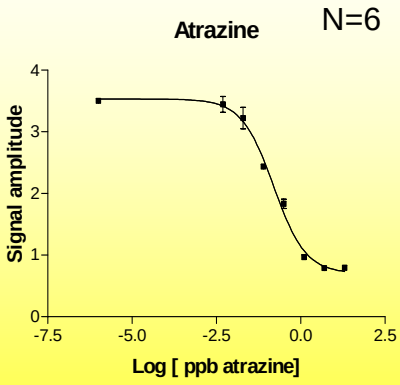
Optimized Assay

Acetic-acetate
Assay buffer: PBS
Coating: 10 μg/L 2d-BSA
Ab: 10 μg/L
Incubation time 15'
Elution speed 32 (23 μL/min)
Regeneration: NaOH 0.1 M



***IC50* = 0.17**
LOD = 0.02 ppb = 20 ng/L

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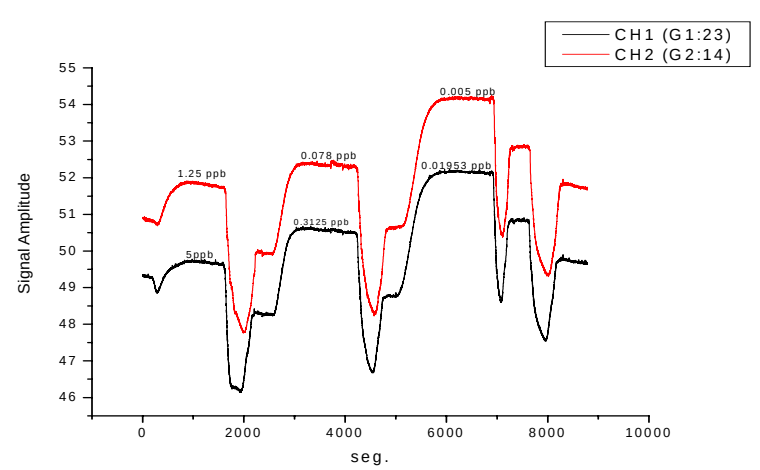

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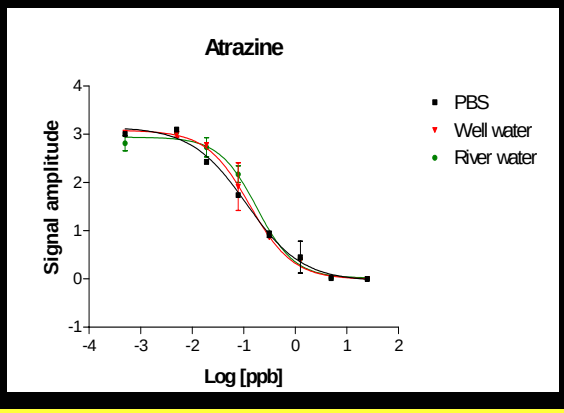
Matrix effects study



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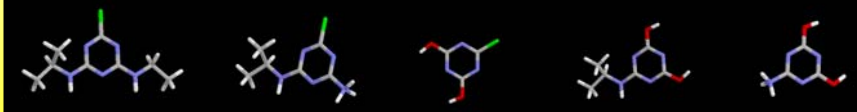
Cross Reactivity Studies

Compound	CR(%) = [*IC*50/*IC*50(atrazine)] 100
Atrazine	**100**
Simazine	25
Terbuthylazine	23
Desethyl-atrazine	5



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VERIFICATION:

Sample	SPR µg/L	SPE-HRGC/MS µg/L	Sum of Triazine µg/L	Sample	SPR µg/L	SPE-HRGC/MS µg/L	Sum of Triazine µg/L
1	0.05	0.05	0.25	8	0.10	0.07	0.29
2	0.10	0.08	0.13	9	0.12	0.07	0.27
3	1.00	0.82	1.07	10	0.06	0.05	0.1
4	0.26	0.24	0.46	11	0.10	0.07	0.15
5	0.22	0.19	0.51	12	0.07	0.06	0.09
6	0.20	0.20	0.59	13	0.06	0.06	0.09
7	0.11	0.07	0.27	14	0.10	0.07	0.11

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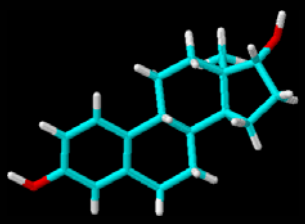
Future Work:

Development of immunosensing chips for analysis of emergent pollutants.

- Monoclonal antibodies
- Estrogens: E_2, E_1, EE_2
- Pharmaceutical compounds



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Conclusions:

- **Optical biosensors based on spectroscopy of surface plasmons provide label-free, fast, specific and sensitive measurements and it is a complementary approach to traditional laboratory analytical techniques.**
- **Real time analysis**
- **Inexpensive**
- **Portable systems**
- **Various immobilization procedures can be performed to meet needs of specific applications.**

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Conclusions:

- A rapid method for triazine screening have been developed
- This method is high sensitive LOD 20 ng/L
- No sample treatment is required just filtration and buffer adjustment
- Good agreement was observed validated vs. HRGC-MS

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SENSIA

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