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TOMORROW TODAY

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# Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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# Combinations of Optical Spectroscopy with other Techniques



# Content

**Combinations of SPR biosensors with other techniques for more complex investigations of biointerfaces, interaction analysis, and materials research:**

- **Fluorescence spectroscopy (SPFS)**
- **Quartz crystal microbalance (QCM)**
- **Electrochemistry (EC-SPR)**
- **Field effect transistor (FET)**
- ~~**Mass spectrometry**~~
- ~~**SERS, TERS...**~~

**Date: May 31<sup>st</sup>**



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# Fluorescence



# Surface Plasmon-Enhanced Fluorescence



ELSEVIER

Colloids and Surfaces

A: Physicochemical and Engineering Aspects 171 (2000) 115–130

COLLOIDS  
AND  
SURFACES

A

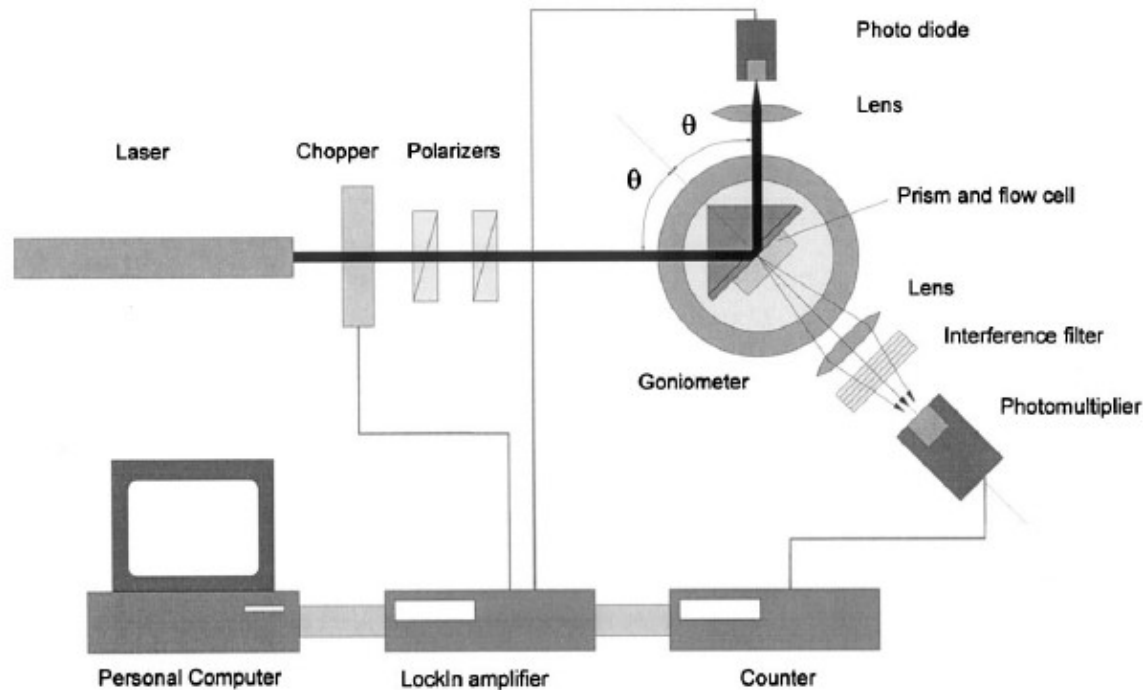
[www.elsevier.nl/locate/colsurfa](http://www.elsevier.nl/locate/colsurfa)

## Surface-plasmon field-enhanced fluorescence spectroscopy

Thorsten Liebermann \*, Wolfgang Knoll

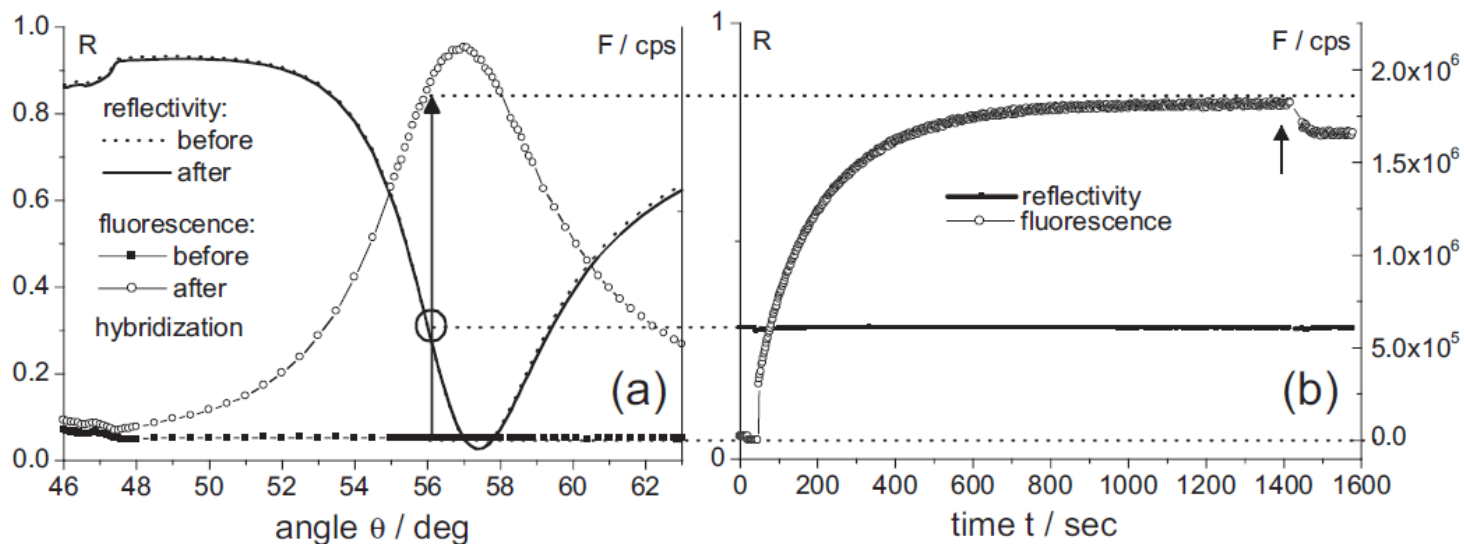
*Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz, Germany*

# Surface Plasmon-Enhanced Fluorescence ( $\lambda_{\text{ex}}$ )



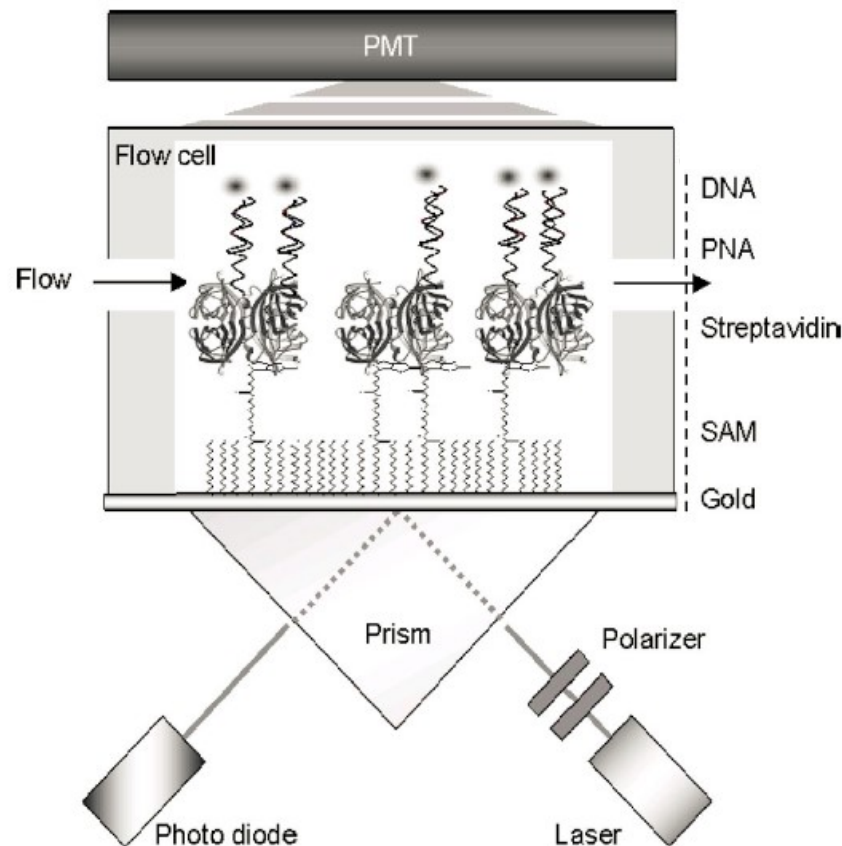
- ➡ Combination of surface plasmon resonance (SPR) and surface plasmon field enhanced fluorescence excitation (SPFS).

# Surface Plasmon-Enhanced Fluorescence ( $\lambda_{ex}$ )



- ➡ Comparison of SPR and SPFS for investigation of short oligonucleotide hybridization. Sensitivity motivation for low molecular weight analytes.

# Surface Plasmon Resonance (SPR) and Surface Plasmon-Enhanced Fluorescence (SPFS)

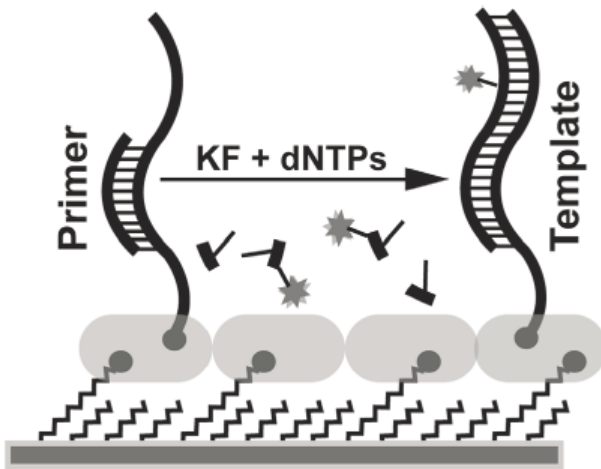


Example: investigation of peptide nucleic acid (PNA) probes for the detection of single nucleotide polymorphism (SNP).

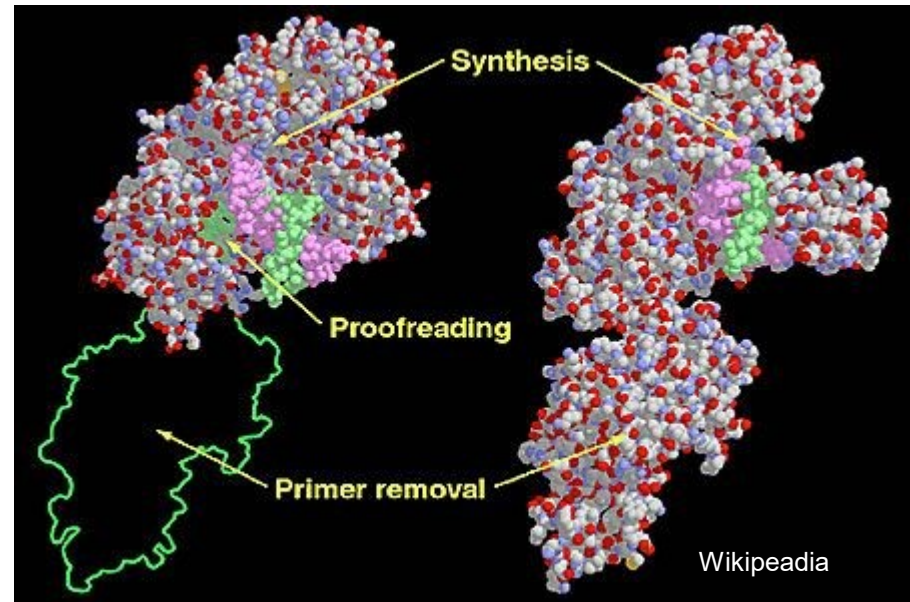
- ➔ Relevant to e.g. diagnosis of genetic diseases such as thalassemia.
- ➔ Instrumentation: combined SPR and surface plasmon-enhanced fluorescence spectroscopy (SPFS).



# DNA Hybridization: Primer Extension



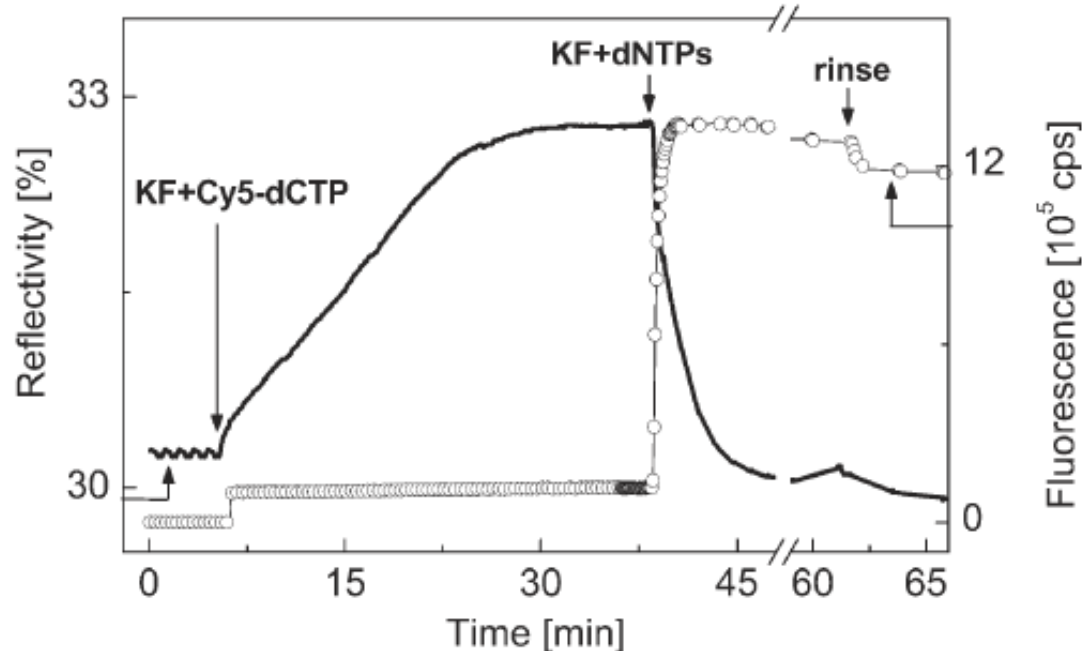
**dNTP** – deoxynucleotide  
**KF** – Klenow fragment



DNA polymerase

- ➡ Besides sensitivity, combined SPR and SPFS offers means to probe more parameters at the same time: applied for primer extension studies.

# DNA Hybridization: Primer Extension



## Complementary information obtained:

- ➡ **SPR:** Mostly sensitive to binding of large KF to DNA probes.
- ➡ **Fluorescence:** “Zipping up” the duplex DNA strand (low molecular weight).



# Ultrasensitive Detection – Relating Fluorescence Signal with Number of Molecules

J|A|C|S  
COMMUNICATIONS

Published on Web 07/01/2004

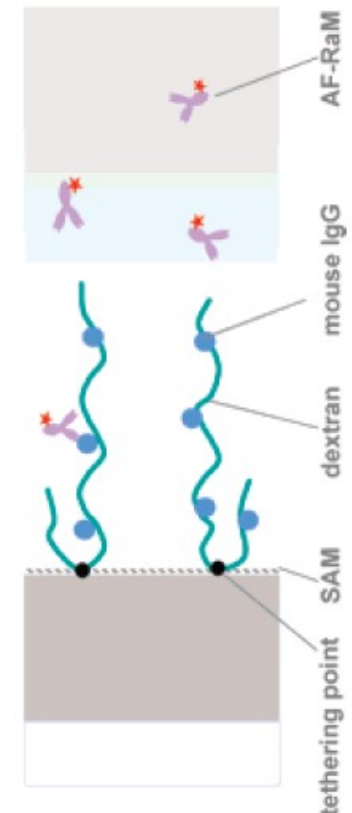
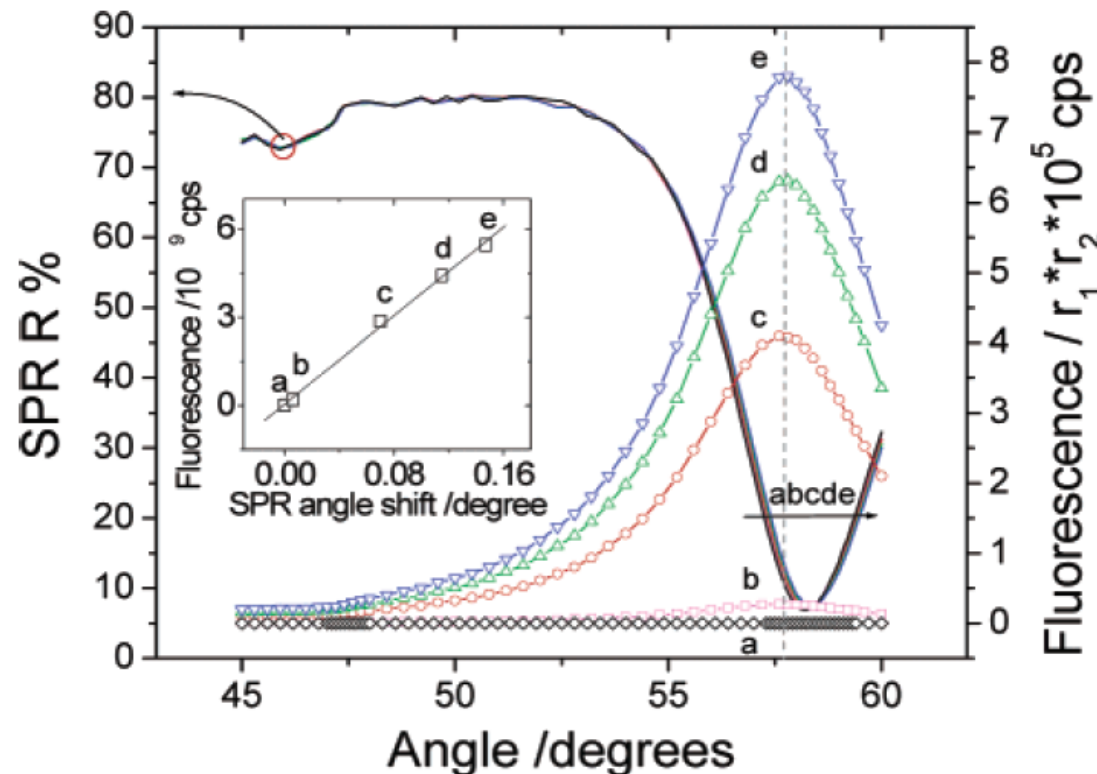
## Attomolar Sensitivity in Bioassays Based on Surface Plasmon Fluorescence Spectroscopy

Fang Yu,<sup>†</sup> Björn Persson,<sup>‡</sup> Stefan Löfås,<sup>‡</sup> and Wolfgang Knoll<sup>\*,†</sup>

*Max-Planck-Institute for Polymer Research, Ackermannweg 10, D-55128 Mainz, Germany, and Biacore AB, Rapskatan 7, SE-754 50 Uppsala, Sweden*

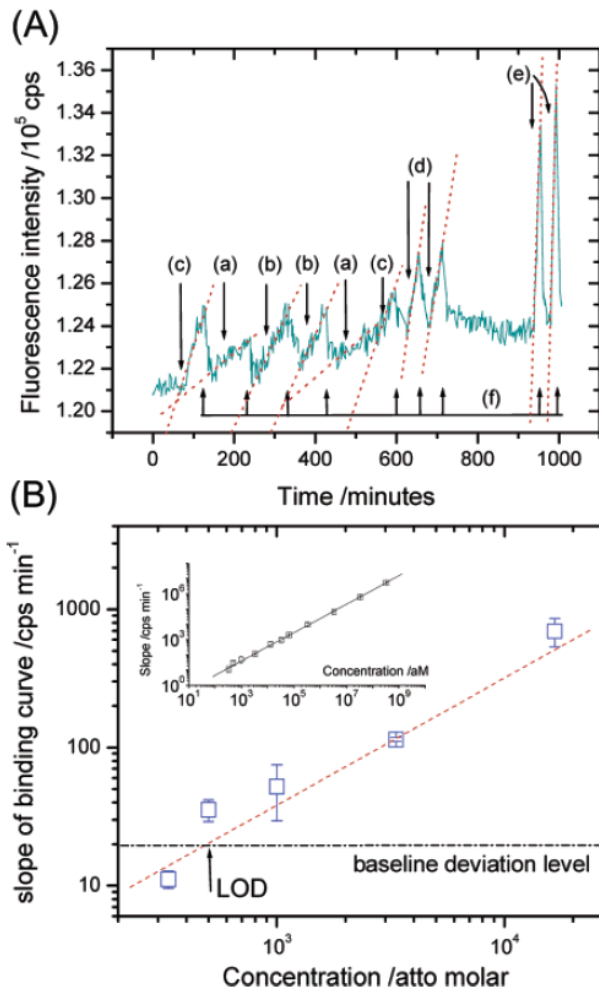
Received March 11, 2004; E-mail: knoll@mpip-mainz.mpg.de

# Calibration of Fluorescence Signal by SPR / SPFS



- ➡ Calibration: SPR allows for determining the captured molecules surface density (for high analyte concentrations), relate that to the fluorescence signal, and extrapolate (also for concentration much lower where SPR is blind)

# Calibration of Fluorescence Signal by SPR / SPFS



- ➡ Achieved 0.5 fM limit of detection (SPFS with very high excitation power and long incubation time of 60 min)
- ➡ By SPFS related to SPR, there was determined that one can detect 600 molecules / mm<sup>2</sup>.
- ➡ Taking advantage of time kinetics and processing all data measured over long time (corresponding to averaging).



# Surface Plasmon-Enhanced Fluorescence (Study in Early 90ties)

## **Sensitivity enhancement of optical immunosensors by the use of a surface plasmon resonance fluoroimmunoassay**

**J. W. Attridge, P. B. Daniels, J. K. Deacon, G. A. Robinson & G. P. Davidson**

Serono Diagnostics Ltd., Unit 21, Woking Business Park, Albert Drive, Woking, Surrey GU21 5JY, UK

(Received 4 May 1990; revised version received 7 August 1990; accepted 9 August 1990)

*Biosensors & Bioelectronics* **6** (1991) 201-214

# Surface Plasmon-Enhanced Fluorescence ( $\lambda_{\text{ex}}$ , $\lambda_{\text{em}}$ )

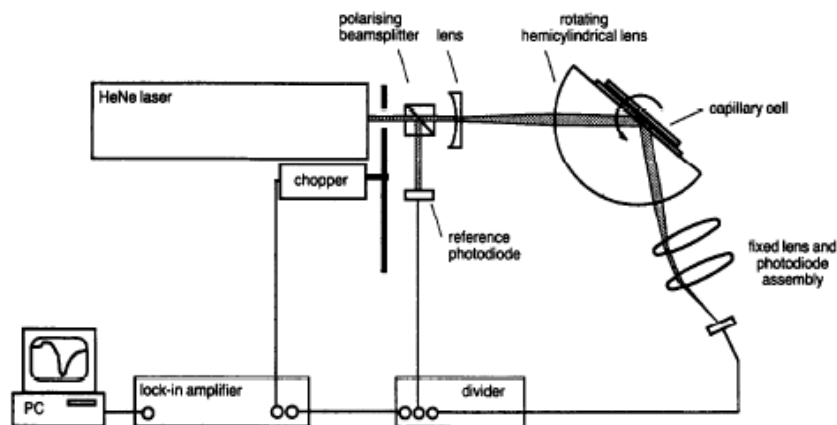
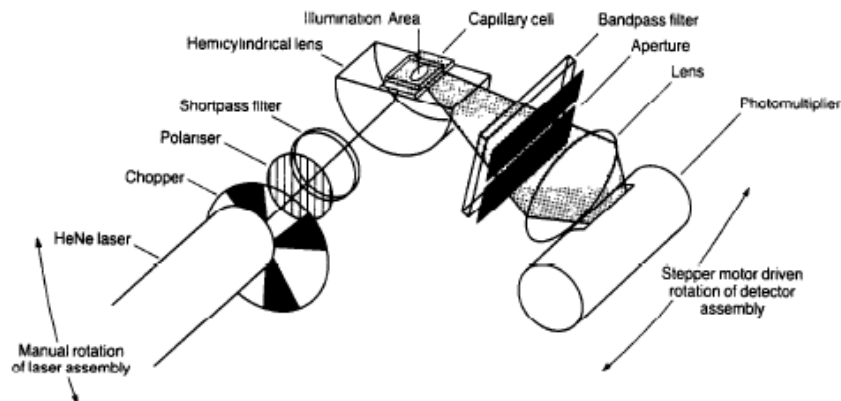


Fig. 4. Experimental apparatus used for SPR reflectance measurements.





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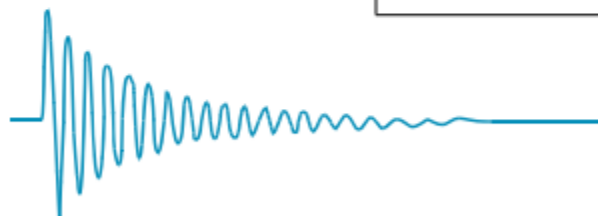
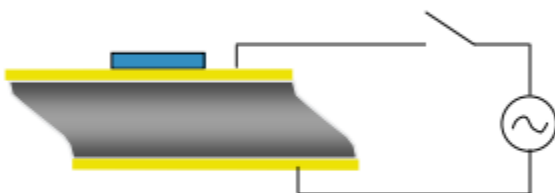
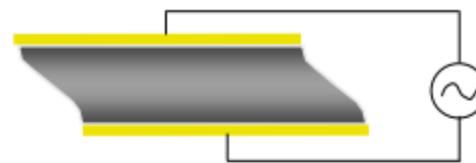
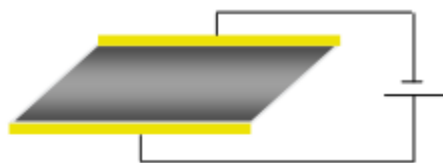


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# Quartz Crystal Microbalance



# Quartz Crystal Microbalance (QCM)

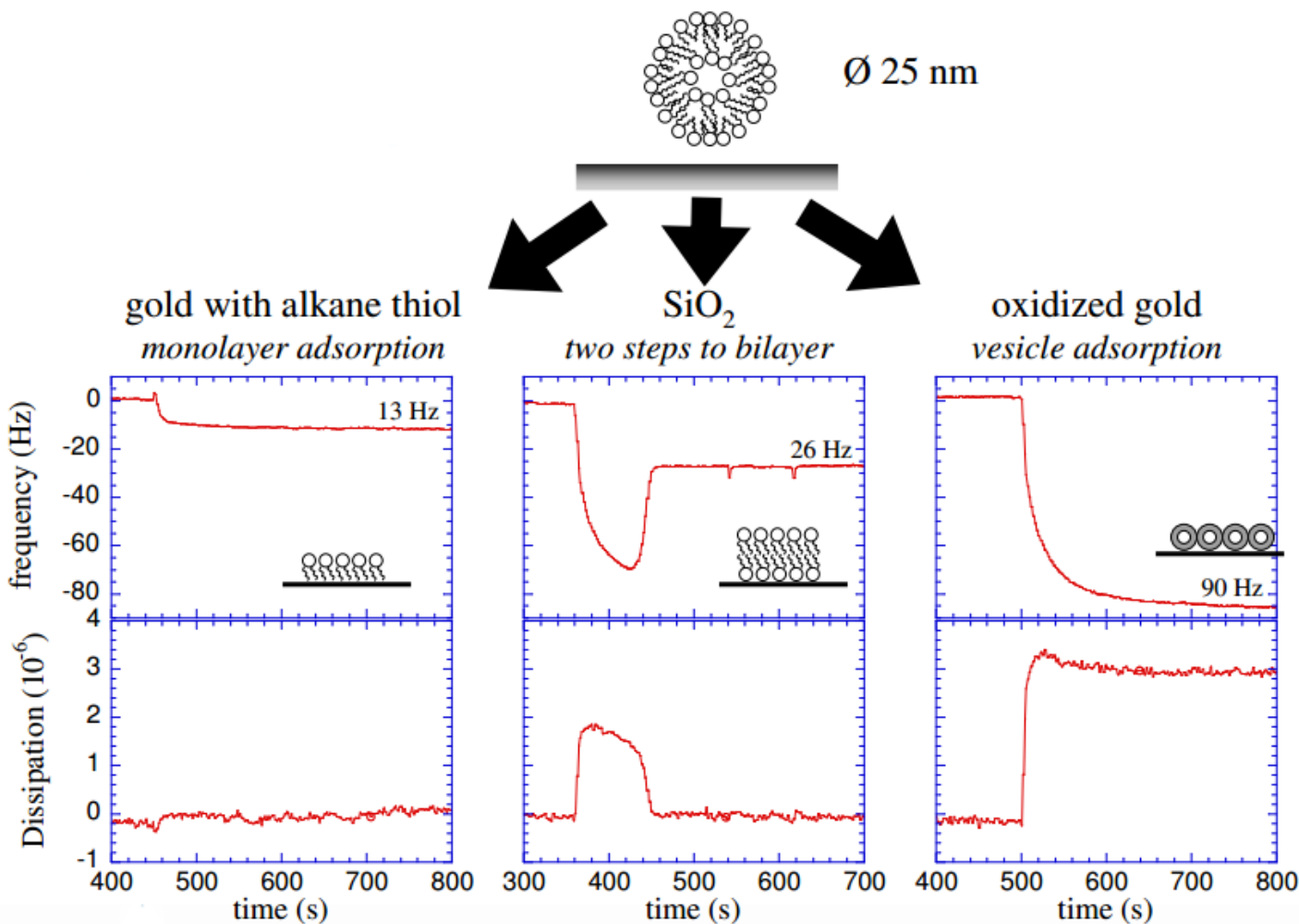


$\Delta f$  is related to the mass  
of the attached film  
(Sauerbrey relation)



$\Delta D$  is related to the  
viscoelasticity

# Quartz Crystal Microbalance (QCM)



C.A. Keller and B. Kasemo, Biophysical J. 75 (1998) 1397.



*Langmuir* **2002**, *18*, 479–489

## Using Surface Plasmon Resonance and the Quartz Crystal Microbalance to Monitor in Situ the Interfacial Behavior of Thin Organic Films

Larry E. Bailey,<sup>†</sup> Dev Kambhampati,<sup>‡</sup> Kay K. Kanazawa,<sup>†</sup> Wolfgang Knoll,<sup>†,§</sup> and Curtis W. Frank<sup>\*,†</sup>

*Department of Chemical Engineering, Stanford University, Stanford, California 94305-5025,  
Thermo Hybaid Interactiva, Sedanstrasse 10, D-89077 Ulm, Germany, and Max Planck  
Institute for Polymer Research, Ackermannweg 10, 55128, Mainz, Germany*

*Received August 10, 2001*

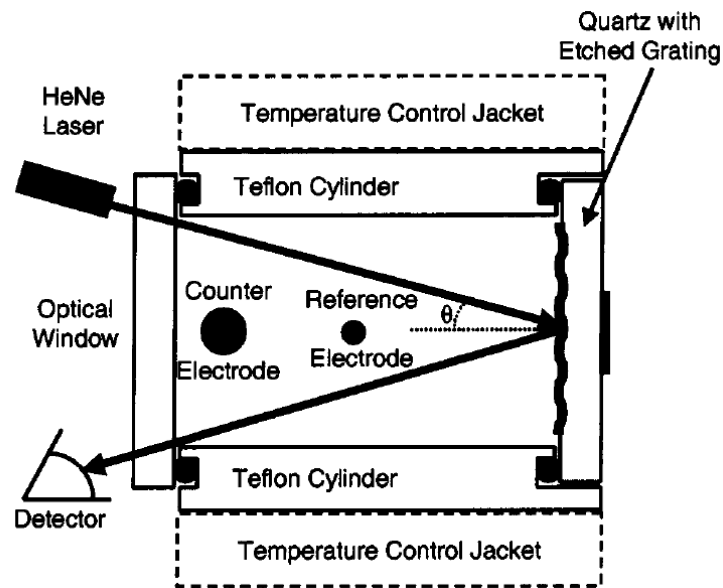
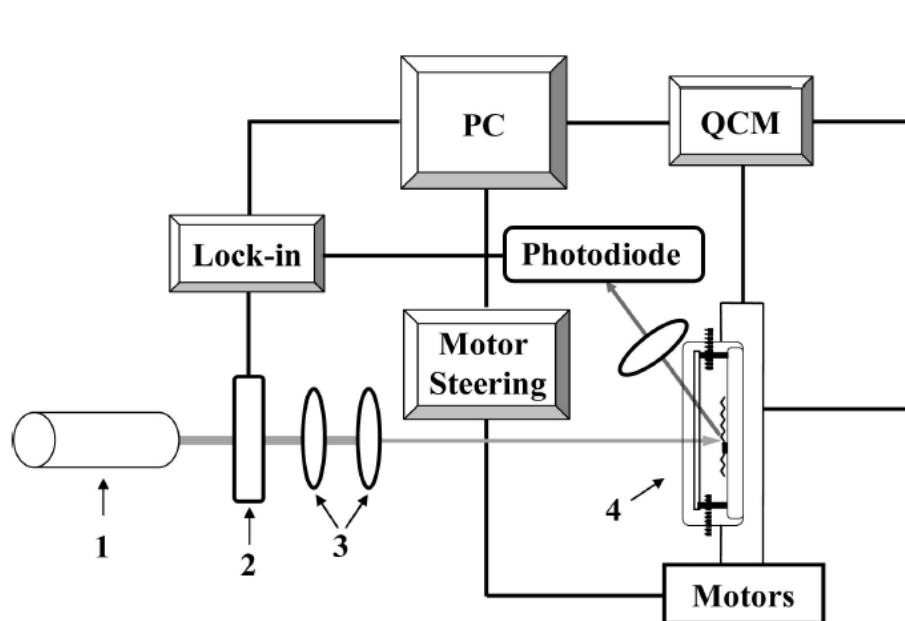
*Anal. Chem.* **2008**, *80*, 5246–5250

## Quartz Crystal Microbalance with Integrated Surface Plasmon Grating Coupler

Yun Zong,<sup>†</sup> Fei Xu,<sup>†</sup> Xiaodi Su,<sup>†</sup> and Wolfgang Knoll<sup>\*,†,‡</sup>

*Institute of Materials Research and Engineering, A\*STAR (Agency for Science, Technology and Research), 3  
Research Link, Singapore 117602, and Max-Planck Institute for Polymer Research, Ackermannweg 10,  
55128 Mainz, Germany*

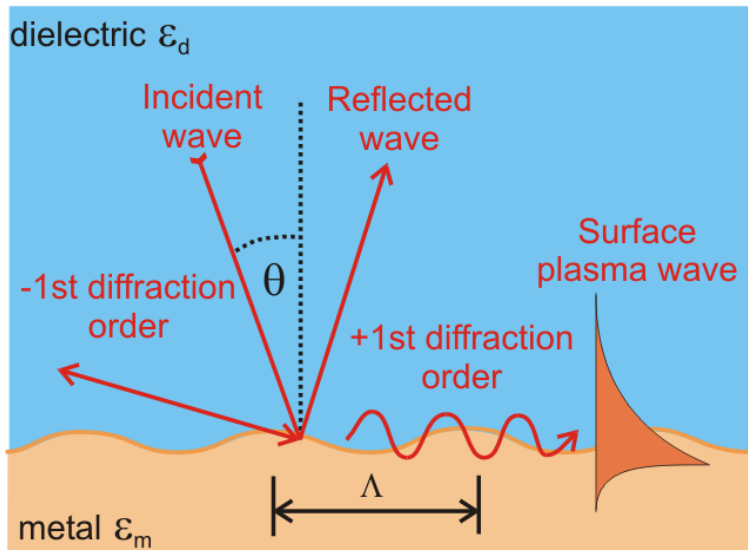
# QCM with Grating-Coupled SPR



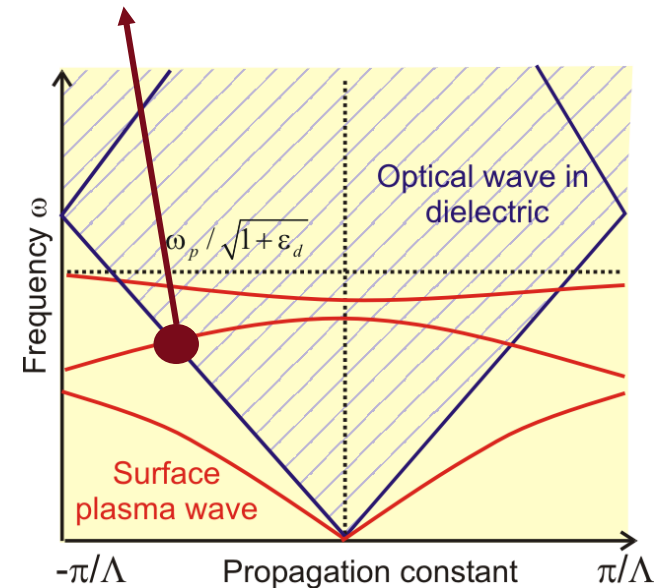
*Langmuir* **2002**, 18, 479–489

*Analytical Chemistry*, Vol. 80, No. 13, July 1, 2008

# Grating Coupling to PSPs

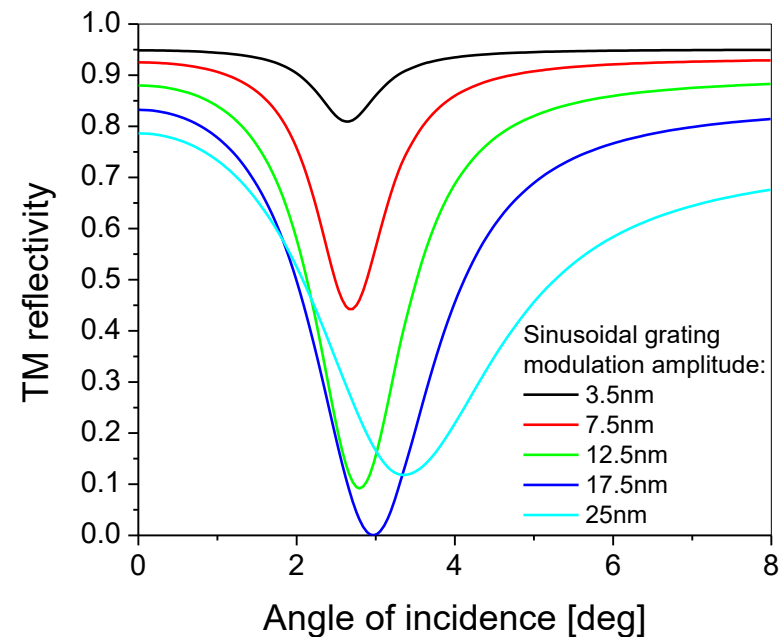
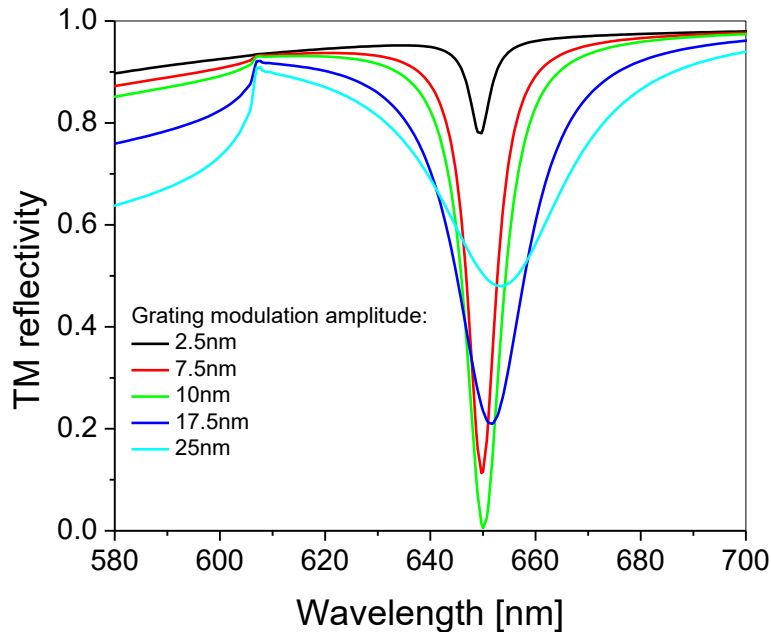


$$\text{Re}\{\beta\} = \frac{2\pi}{\lambda} n_d \sin(\theta) \pm p \frac{2\pi}{\Lambda}$$



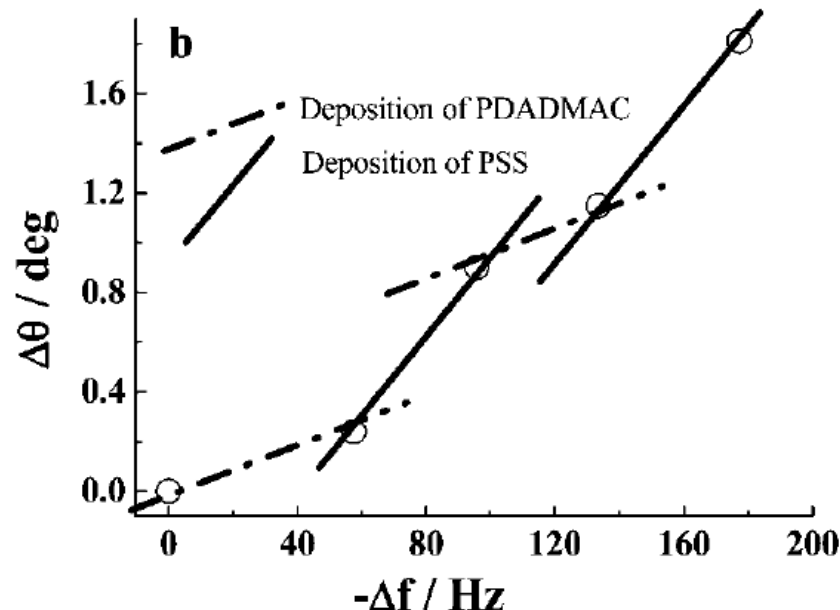
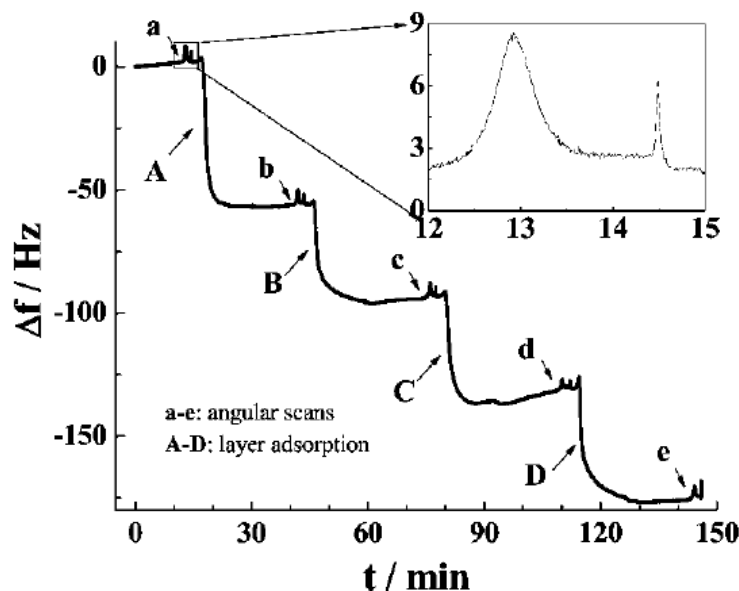
- Phase-matching can be investigated by using dispersion of the modes, manifested as a cross-section for certain diffraction order  $p$ .

# Grating Coupling to PSPs



- ➡ On metallic diffraction gratings, the coupling strength to PSPs is controlled by the modulation depth.

# QCM with Grating-Coupled SPR



- ➡ Demonstrated that upon LbL deposition the 'optical' and 'acoustic' thickness differs. Information on the viscoelastic properties, bound water molecules, ions....



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# Electrochemistry





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*Analytica Chimica Acta* 558 (2006) 150–157

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[www.elsevier.com/locate/aca](http://www.elsevier.com/locate/aca)

# In situ electrochemical and surface plasmon resonance (SPR) studies of aniline-carboxylated aniline copolymers

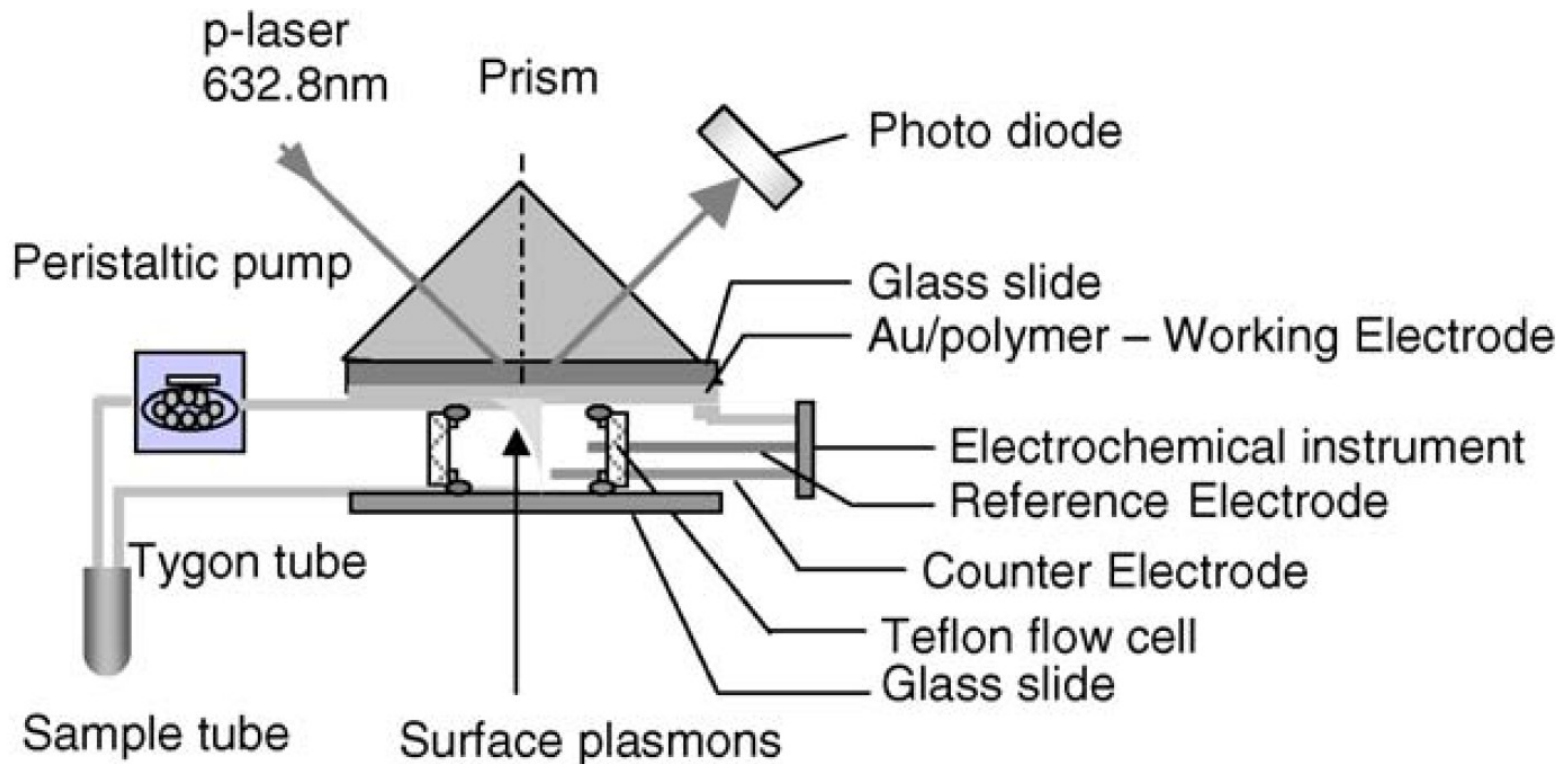
Yanju Wang, Wolfgang Knoll\*

*Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz, Germany*

Received 23 August 2005; received in revised form 30 October 2005; accepted 1 November 2005

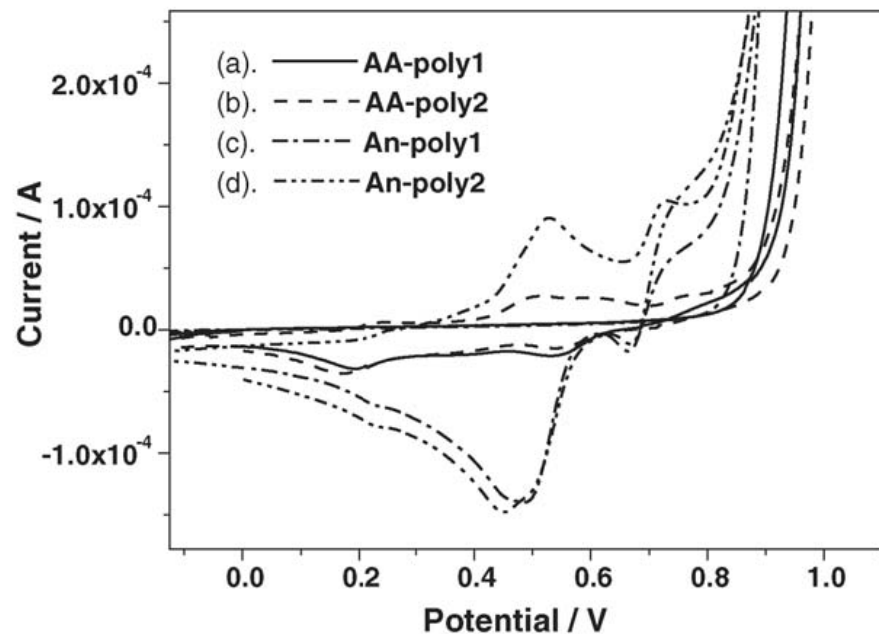
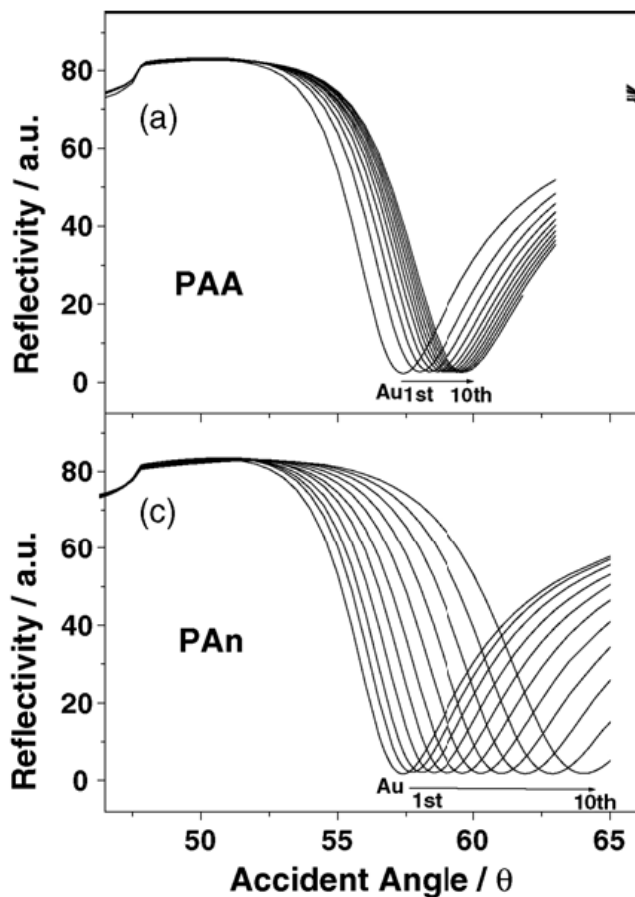
Available online 20 December 2005

# EC-SPR Setup



➡ SPR-active gold surface used as a working electrode.

# EC-SPR Setup



➔ Cyclic voltammetry combined with SPR was used for the monitoring of polymerization and conductive polymer film stability.

# SPR Sensitivity to Ions on the Metallic Surface

Biosensors and Bioelectronics 126 (2019) 365–372



Contents lists available at [ScienceDirect](#)

Biosensors and Bioelectronics

journal homepage: [www.elsevier.com/locate/bios](http://www.elsevier.com/locate/bios)



Biomolecular charges influence the response of surface plasmon resonance biosensors through electronic and ionic mechanisms

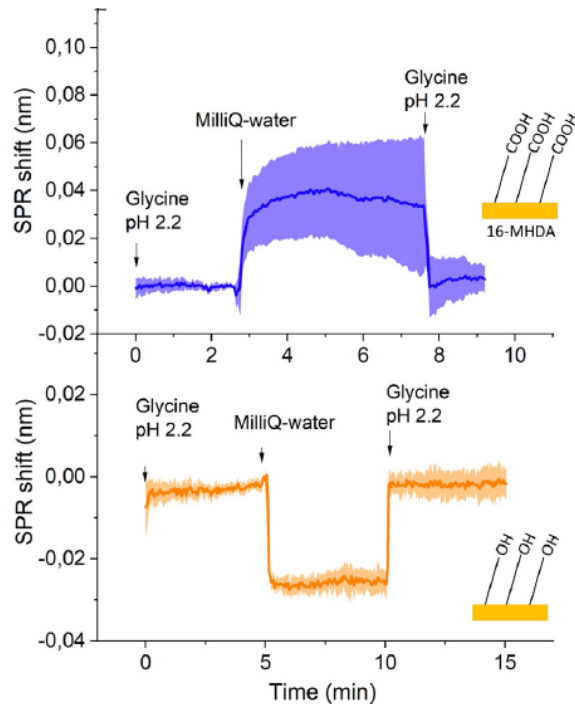
Hana Šípová-Jungová<sup>\*,1</sup>, Ludmila Jurgová, Kateřina Mrkvová, Nicholas Scott Lynn, Barbora Špačková<sup>1</sup>, Jiří Homola<sup>\*</sup>

*Institute of Photonics and Electronics, Czech Academy of Sciences, Chaberská 57, 182 51 Prague, Czech Republic*



- ➡ 1) the formation of an electrical double layer (**ionic mechanism**), and 2) changes in the electron density at the surface of a metal (**electronic mechanism**).

# SPR Crosstalk With Ions on the Metallic Surface

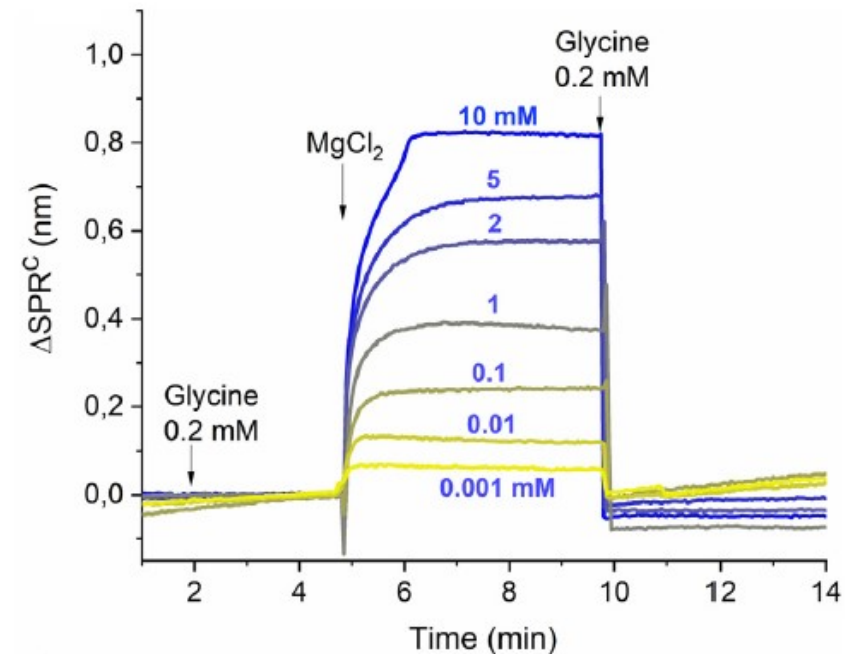


Metal RI  
change

$$\omega = \frac{1}{m_e c}$$



Dielectric RI  
change



- ➡ Under low ionic strength conditions, the electronic mechanism is dominant and the SPR wavelength shift is linearly proportional to the surface concentration of biomolecular charges. At high ionic strength conditions, both electric and ionic mechanisms contribute to the SPR shift.



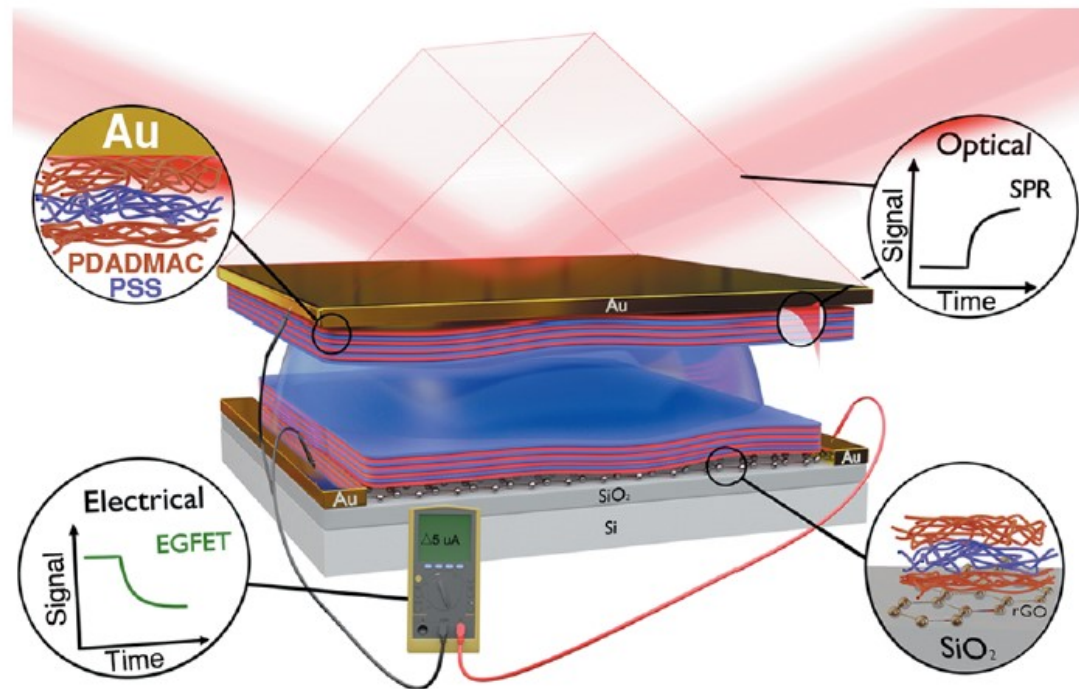
# Field Effect Transistor (FET)



# Dual Monitoring of Surface Reactions in Real Time by Combined Surface-Plasmon Resonance and Field-Effect Transistor Interrogation

Patrik Aspermaier,<sup>⊥</sup> Ulrich Ramach,<sup>⊥</sup> Ciril Reiner-Rozman, Stefan Fossati, Bernadette Lechner, Sergio E. Moya, Omar Azzaroni, Jakub Dostalek, Sabine Szunerits, Wolfgang Knoll, and Johannes Bintinger\*

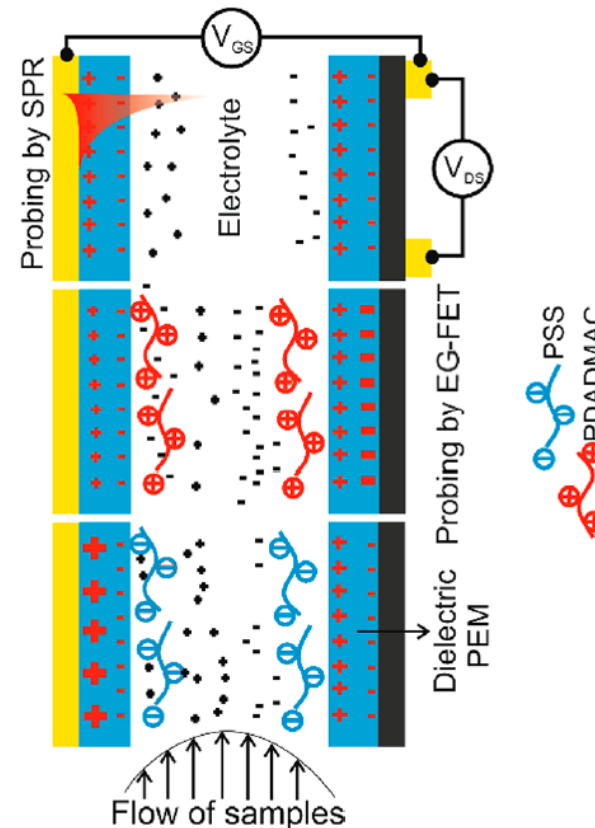
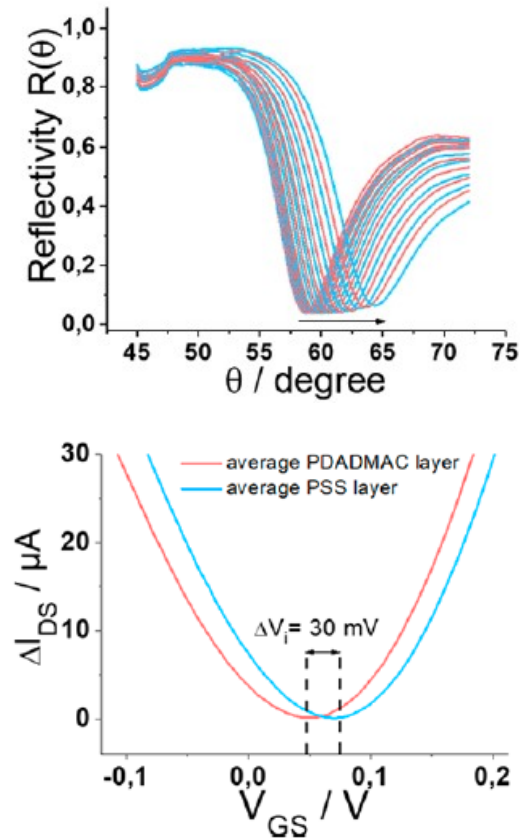
# SPR Crosstalk With Ions on the Metallic Surface



- ➡ SPR-active gold film used as a gate electrode in electrolyte-gated field-effect transistor.

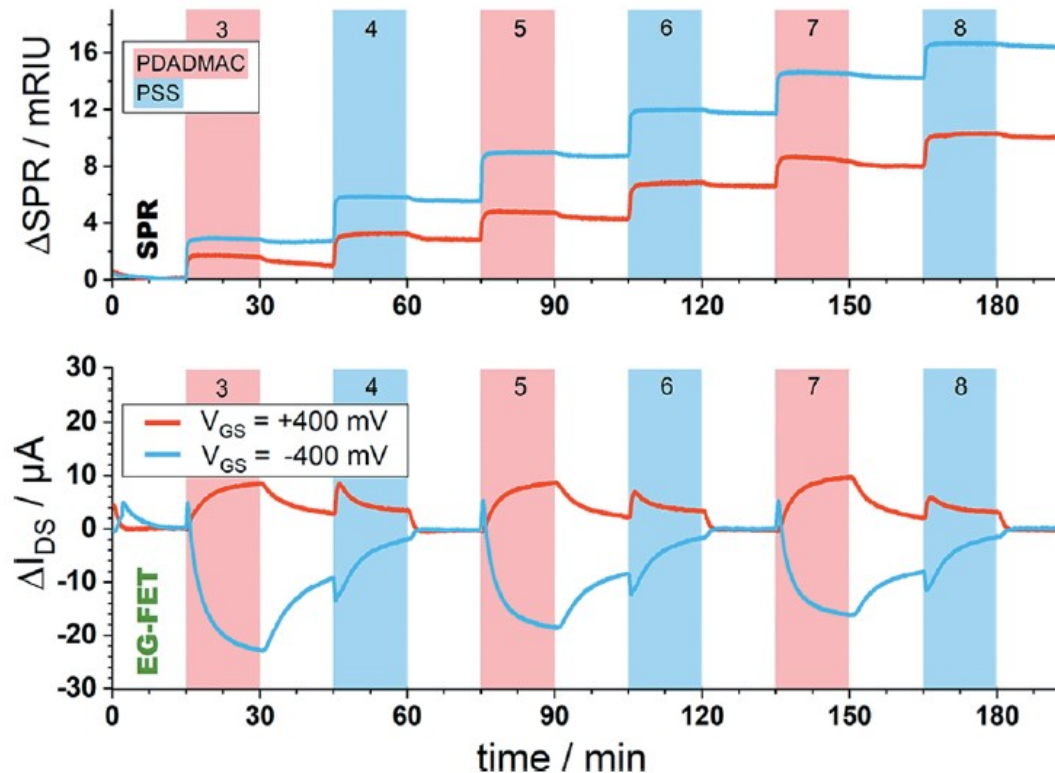


# SPR Crosstalk With Ions on the Metallic Surface



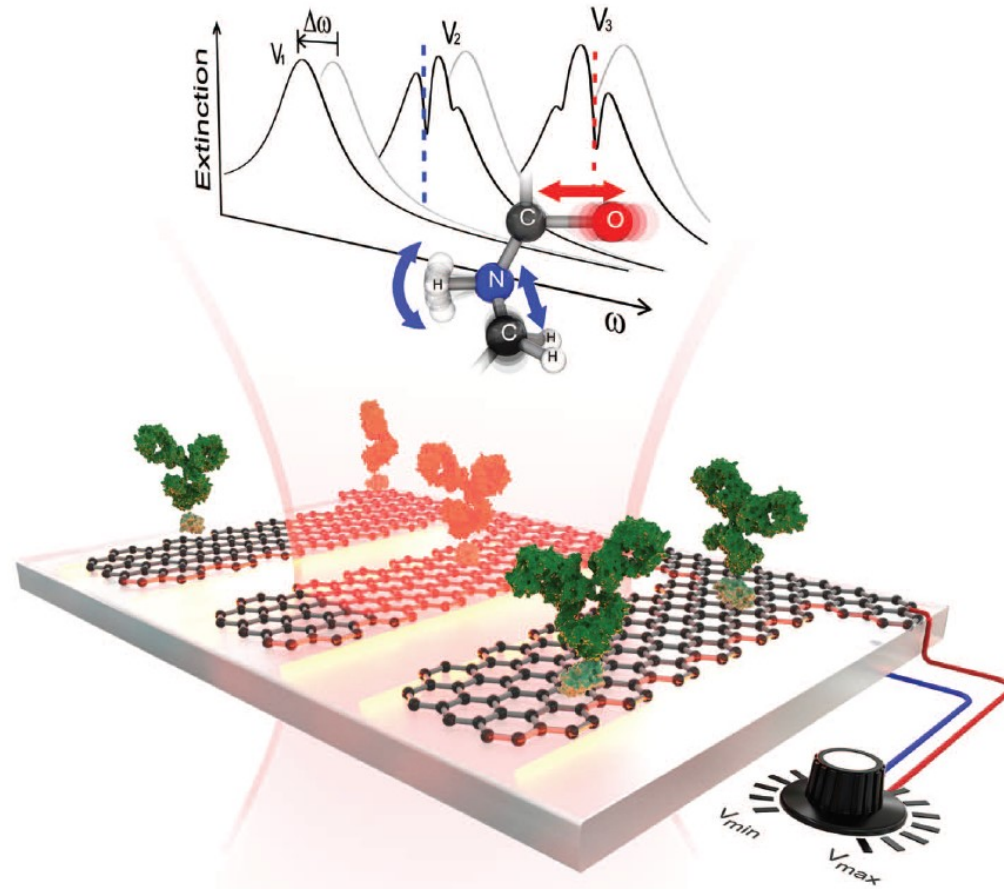
- ➡ Dual readout of the interfacial processes manifested by changes in the refractive index (SPR) and charge density (FET).

# SPR Crosstalk With Ions on the Metallic Surface



- ➡ SPR and FET response kinetics are different and peaks on different aspects of the process of LbL formation.

# Graphene Plasmonics



- ➔ Graphene is semiconductive material and exhibits surface plasmons in the NIR spectral range (lower plasma frequency)
- ➔ Its properties can be electronically tuned and thus enable for active tuning of its plasmonic properties.