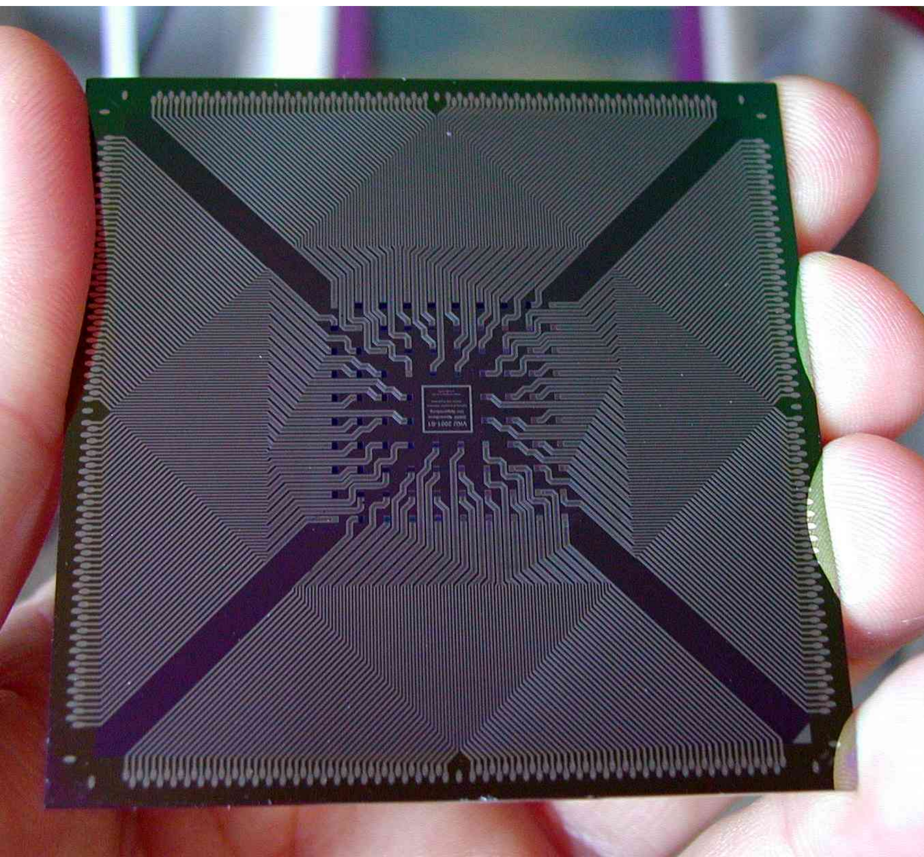
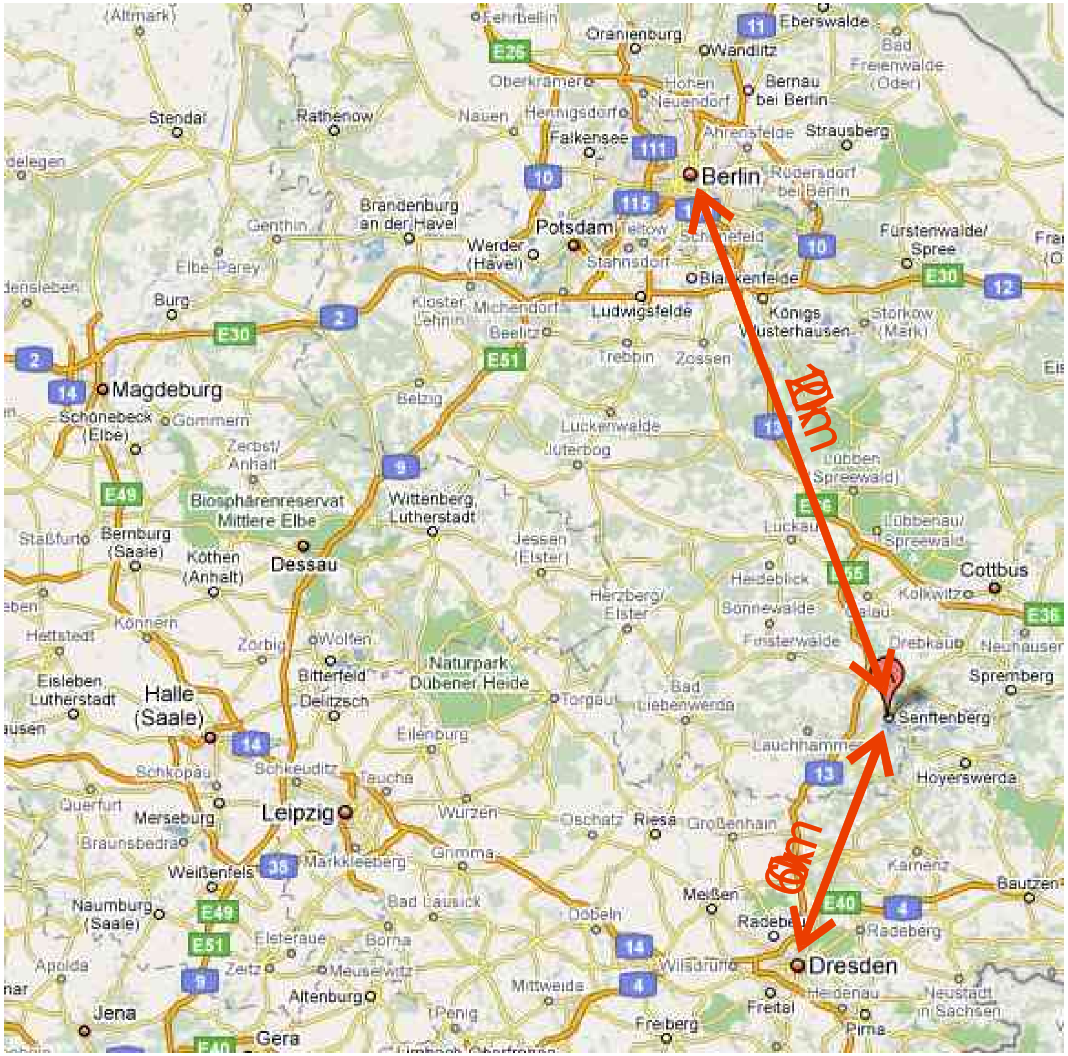
Affinity chemo- and biosensors: development trends and applications in food industry



Vladimir Mirsky
Lab. of Nanobiotechnology
Department of Biotechnology
Faculty of Natural Sciences
Lausitz University of Applied Sciences,
Senftenberg, Germany

Senftenberg



before 1990:
- production of brown coal

now (minings=>lakes):
- recreation area
- chemical and bio-technological industry
- Lausitz University of Applied Sciences

Main directions of our scientific work:

1. Development of transducing principles: optical (SPR, SPR-imaging), impedometric and conductometric (capacitive, electrochemical transistors)

2. Surface science, self assembly and immobilization of biomolecules and nanoparticles (LbL in flow mode, molecular tacking, µ-contact chemoprinting ...)

3. Numerous applications

Irreversible freezing indicator (for Timestrip Ltd., UK)

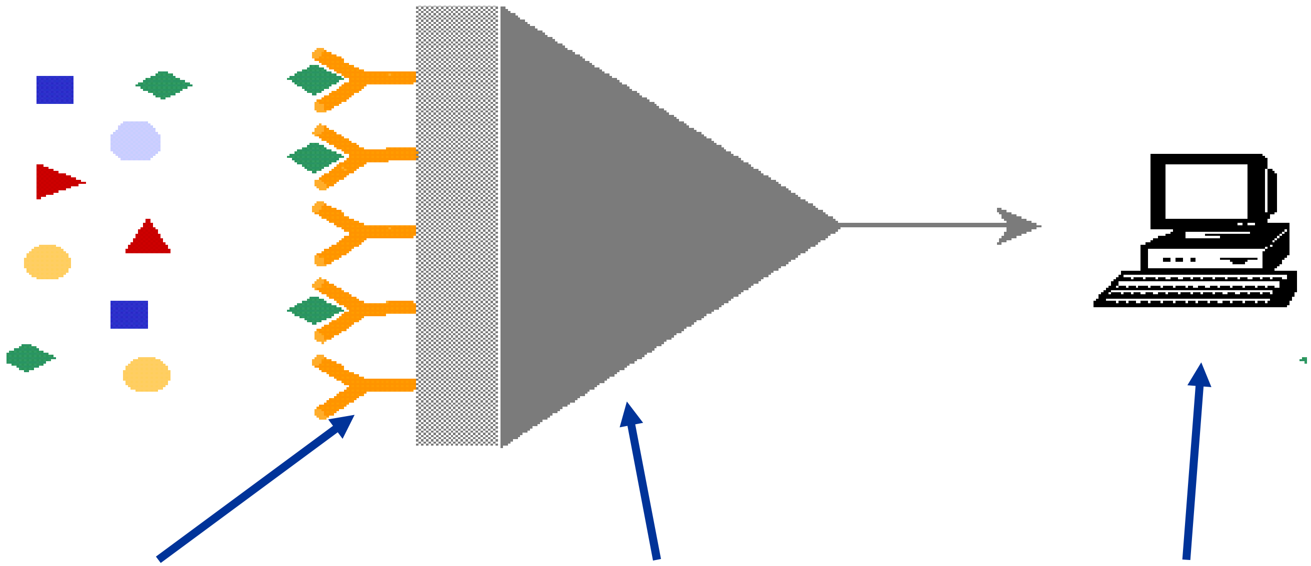
Problem: about 30 % vaccines are damaged during storage (freezing!)

Solution: Freezing indicator
(irreversible color changes after freezing)

Principle: aggregation of gold nanoparticles

Technical solution:
Au-nanoparticles,
nucleator (initiating of ice cristals grow),
D_2O (adjustment of freezing temperature)...

General structure of chemo- and biosensors



Receptor → Transducer → Signal processing

Affinity (bio) sensors

General structure of a biosensor:

Receptor → Transducer→ Data analysis system

Definition: (Bio)sensors based on the measurements of surface coverage of the receptor layer by analyte.

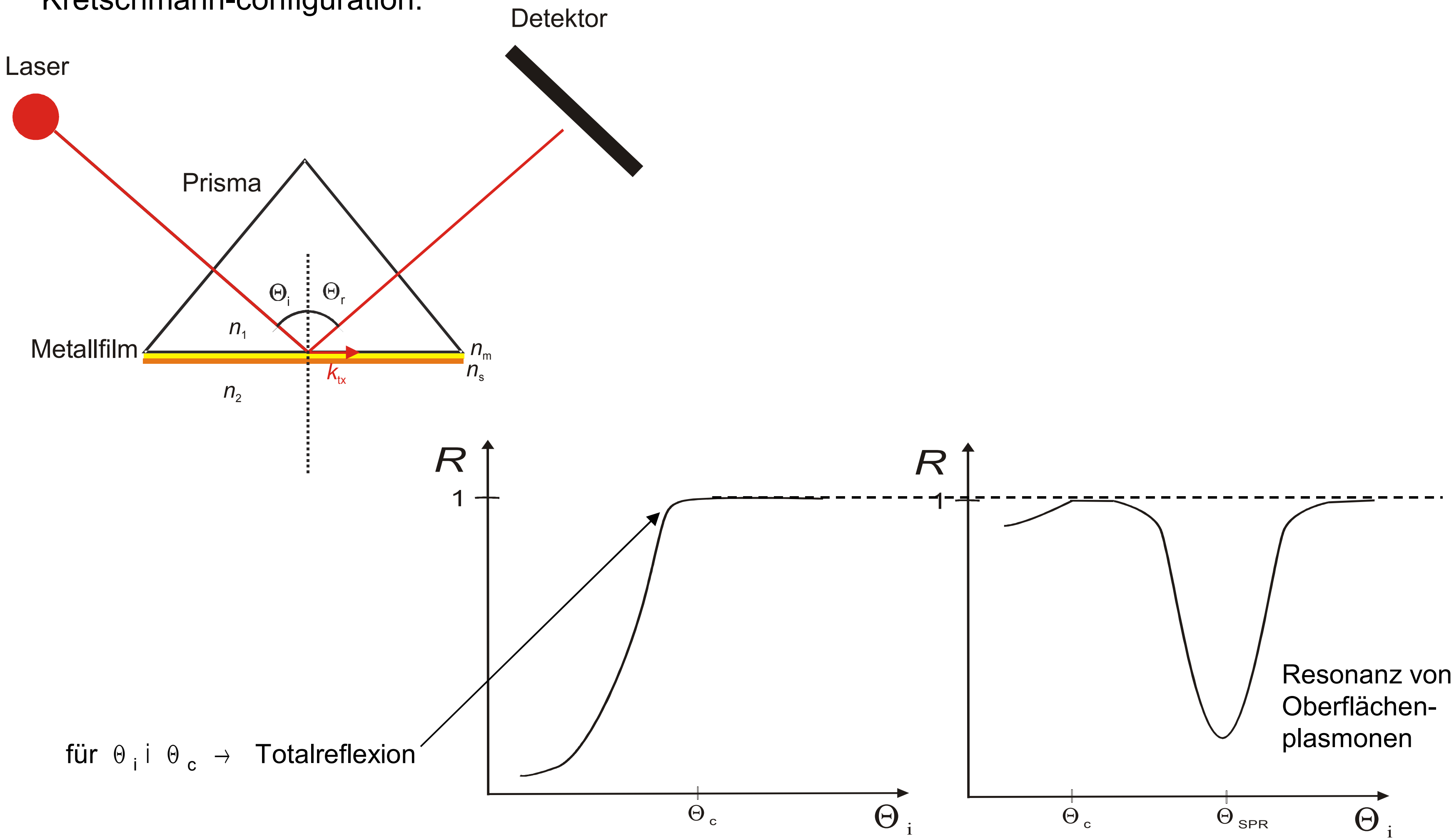
Main types of transducers for affinity sensors:

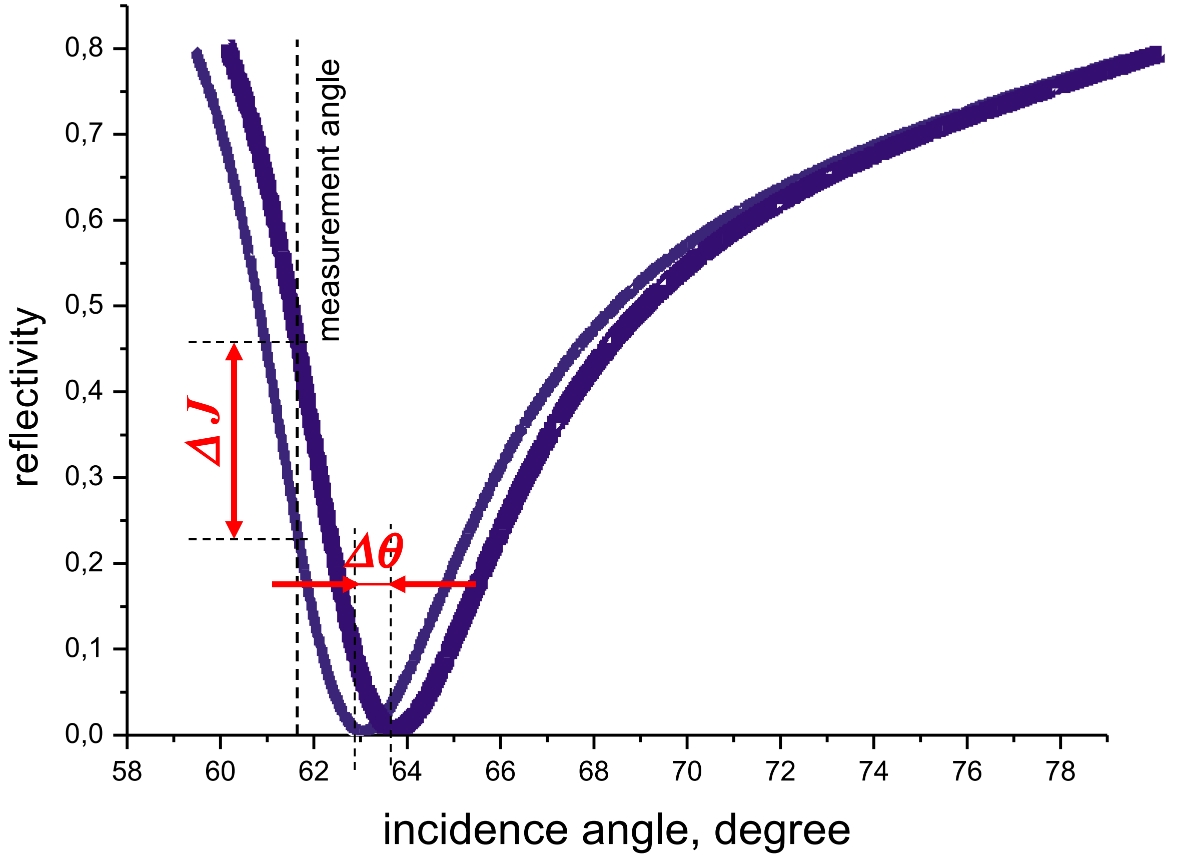
- Optical
  - Surface plasmon resonance
  - Interferometry
  - Ellipsometry
- Mechano-acoustical
  - Quartz microbalance (QMB)
  - µ-Cantilevers
  - Devices based on surface acoustic waves (SAW)
- Electrical
  - Capacitive or impedometric transducers

SPR

Surface plasmon resonance (SPR): principle

Kretschmann-configuration:



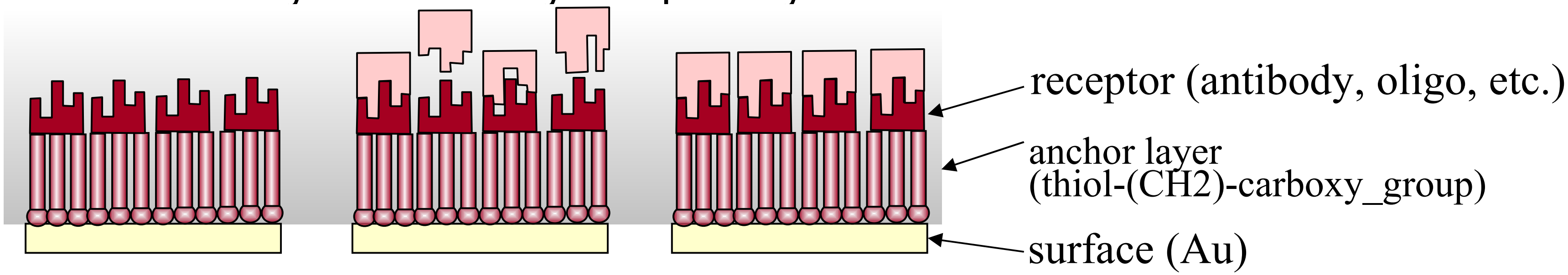


reflectivity
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
58
60
62
64
66
68
70
72
74
76
78
incidence angle, degree
measurement angle
ΔJ
Δθ

Main idea of refractometric transducing

$\Delta n = f$ *(total concentration near surface)*

⇒ changes of the refractive index are not selective
⇒ the selectivity is defined by receptor layer



calibration curve

concentration on the sensing surface ⇢ *volume concentration*

The sensor provides information on total concentration of species near the surface (≈ surface coverage).

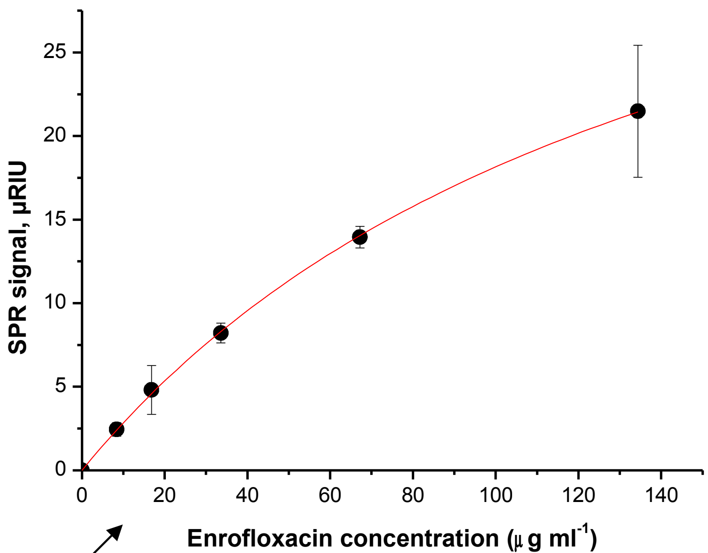
A determination of volume concentration is performed through calibration curve (adsorption isotherm).

A sensor which delivers a signal proportional to surface coverage belongs to ***affinity*** sensors.

Other types of affinity sensors: quartz microbalance (QMB), impedometric (capacitive), interferometric, some of fluorescent sensors.

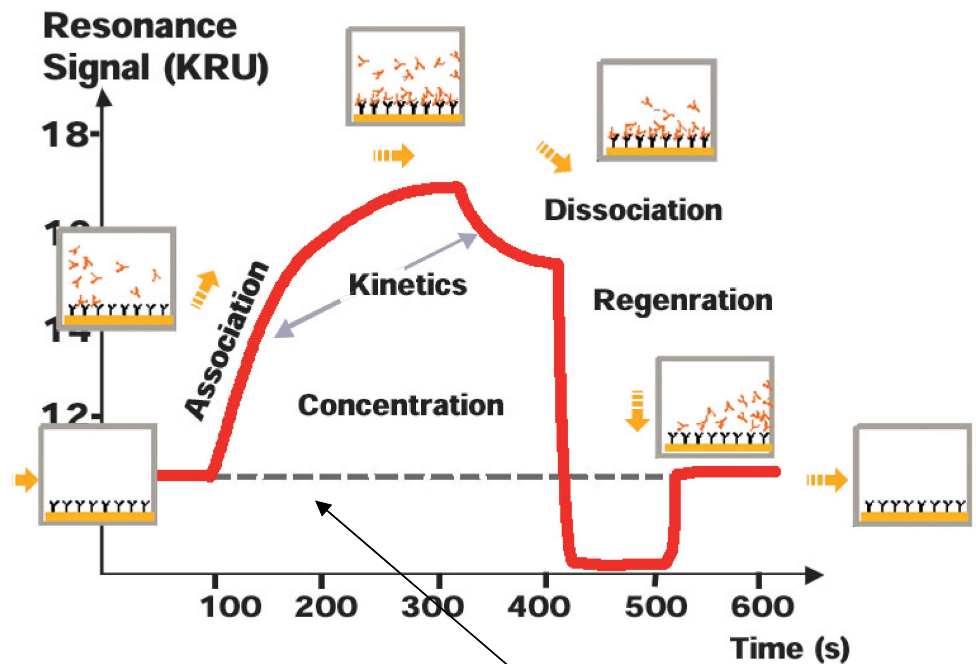
Applications of SPR in biosensors

Stationary measurements



calibration curve
(~ dependence between surface and volume concentration = adsorption isotherm)

Kinetical measurements



sensorgram (a plot of the signal of affinity sensor vs. time)

Commercial SPR-devices

Biacore A100

about 15 SPR-companies:

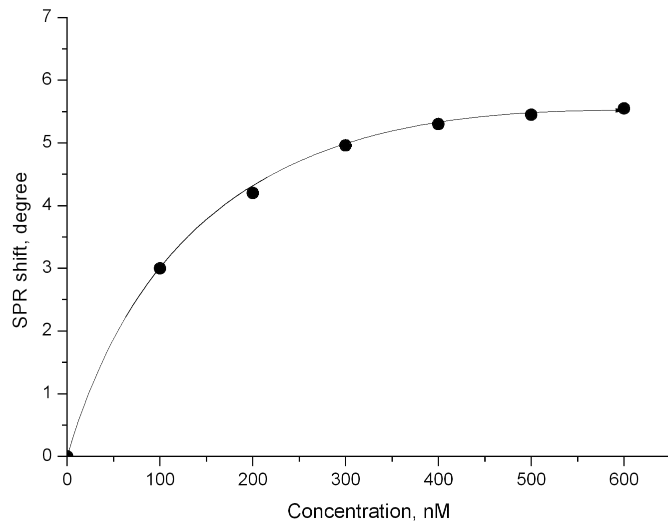
- Biacore – GE
- ESPRIT-EcoChemie
- KSV
- SPRimager / GWCtechnol
- Miltiscop / Optrel
- SPR670 / Nippon Laser Electr.
- IBIS
- Plasmonics
- Biosuplar / Mivitec
- SPRETA / Texas Instruments
- …

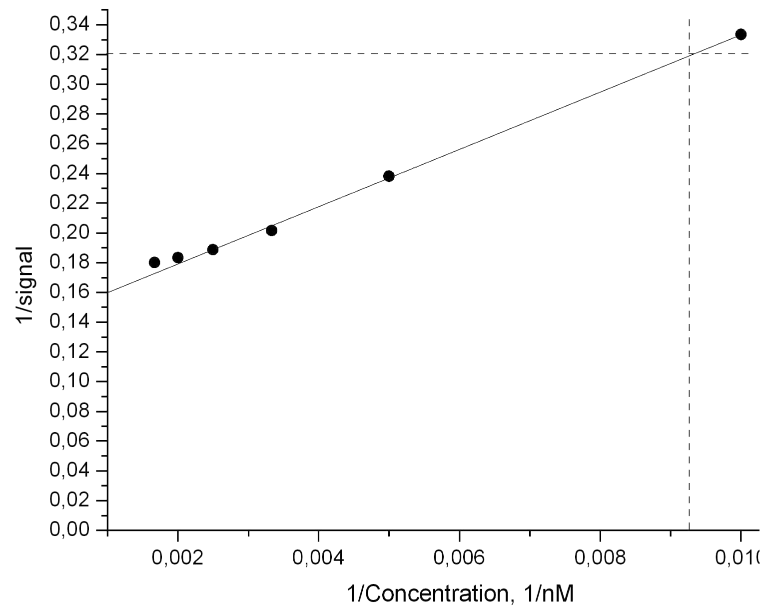
Physical resolution:
Biacore A100: 0.3 µRIU
other devices: 5 – 100 µRIU

Oberflächenplasmonenresonanz als Transduktor in Biosensoren

Beispiel 1. Biosensor für anti-HSA.

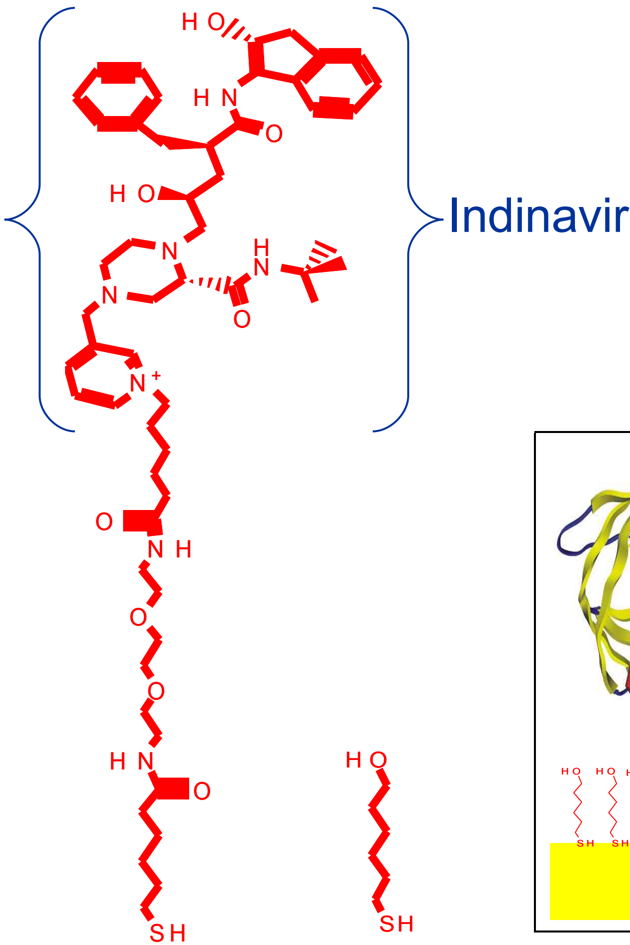
Rezeptor: HSA
Beschichtung: chemische Immobilisierung (EDC-Technik, auf HS-$(CH_2)_{15}$-COOH - SAM) unten SPR-Kontrolle
Messung: SPR

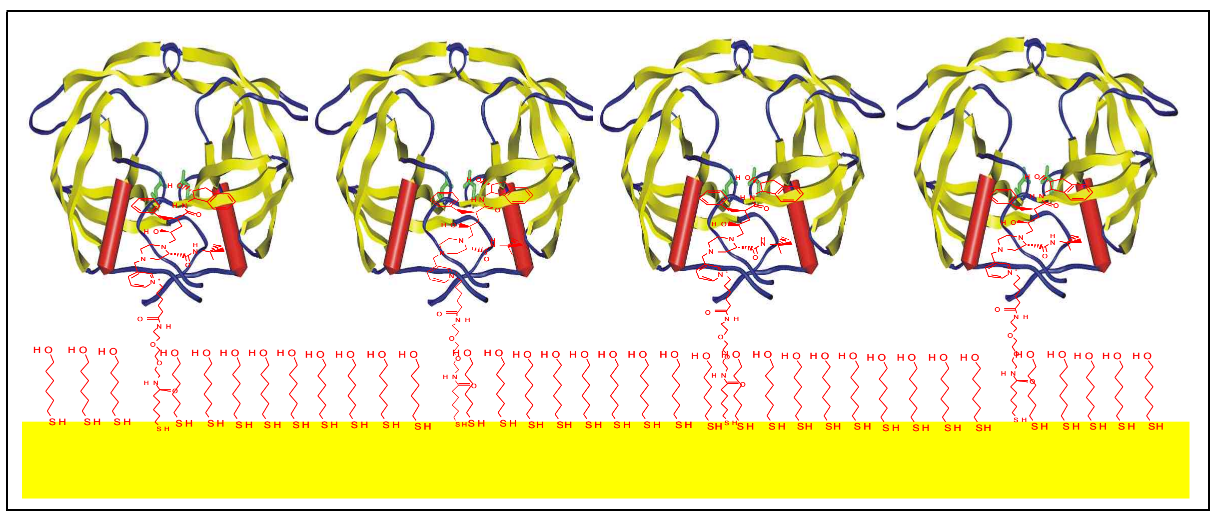




Oberflächenplasmonenresonanz als Transduktor in Biosensoren

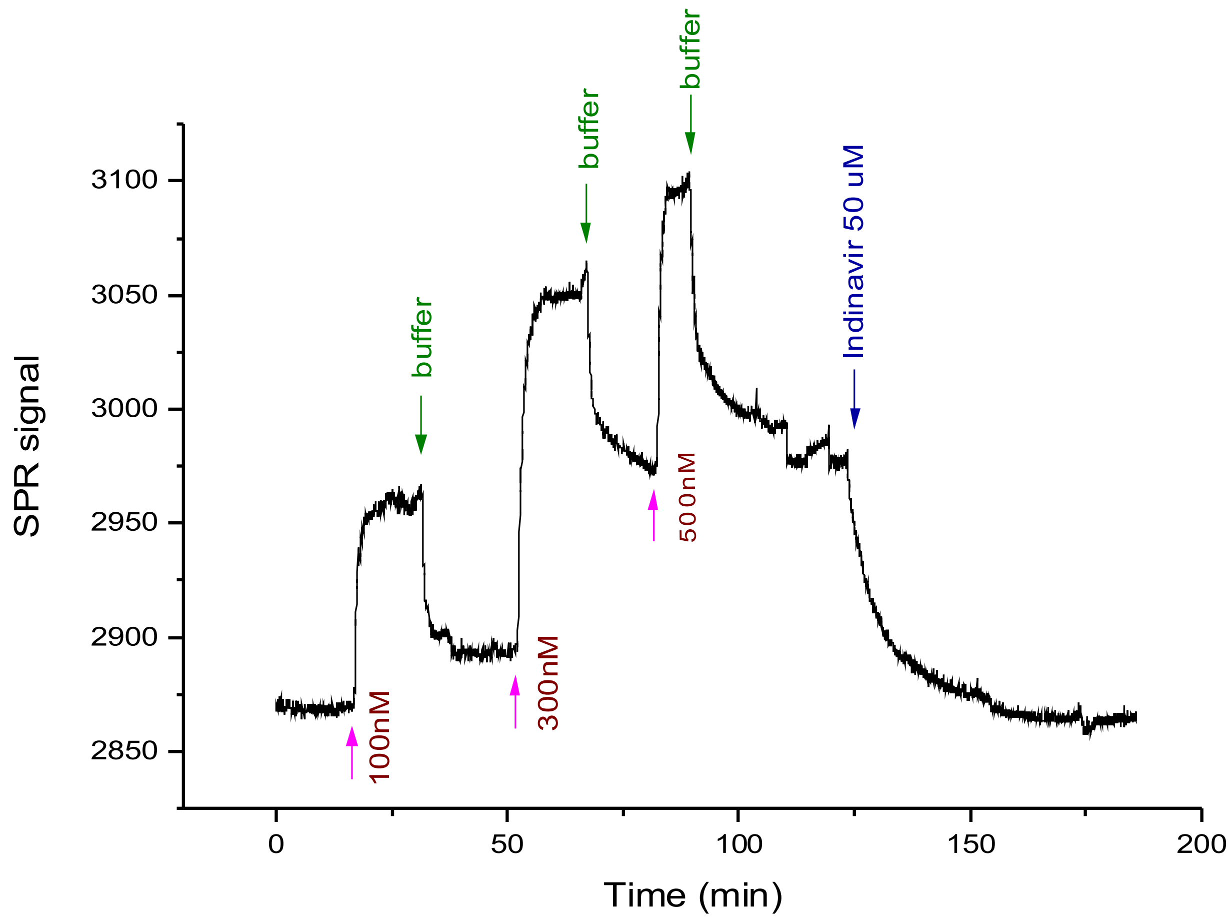
Beispiel 2. Biosensor für die Bestimmung von Drug-Resistänz von HIV-Protease (Kooperation mit P. Cigler, V. Kral, J. Konvalinka).





Oberflächenplasmonenresonanz als Transduktor in Biosensoren

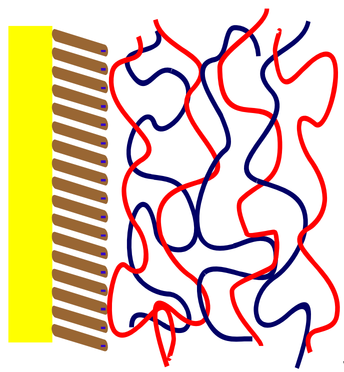
Beispiel 2. Biosensor für die Bestimmung von Drug-Resistänz von HIV-Protease

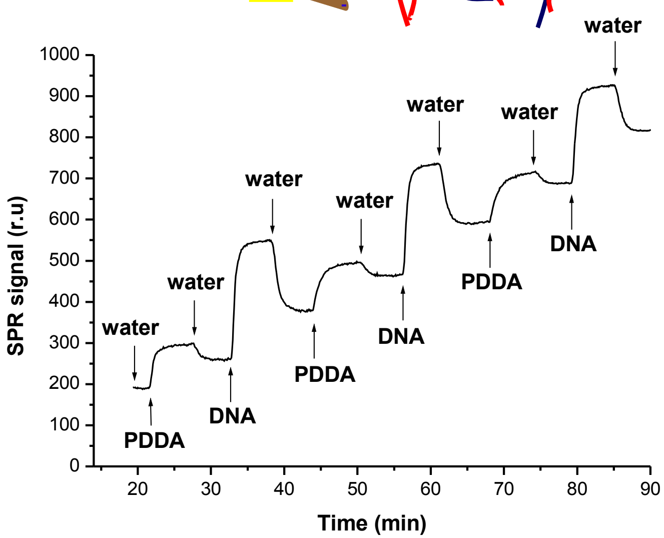


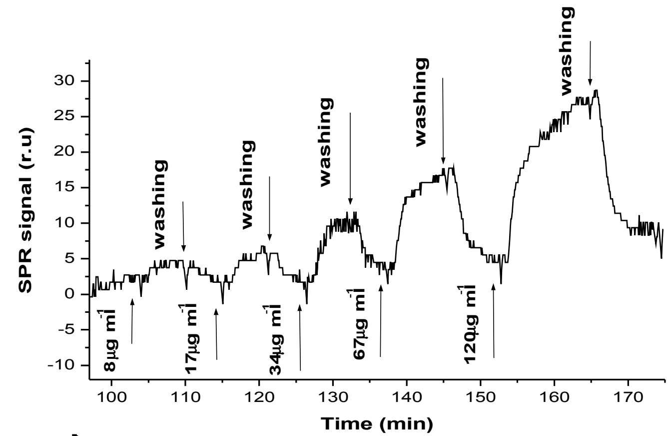
Oberflächenplasmonenresonanz als Transduktor in Biosensoren

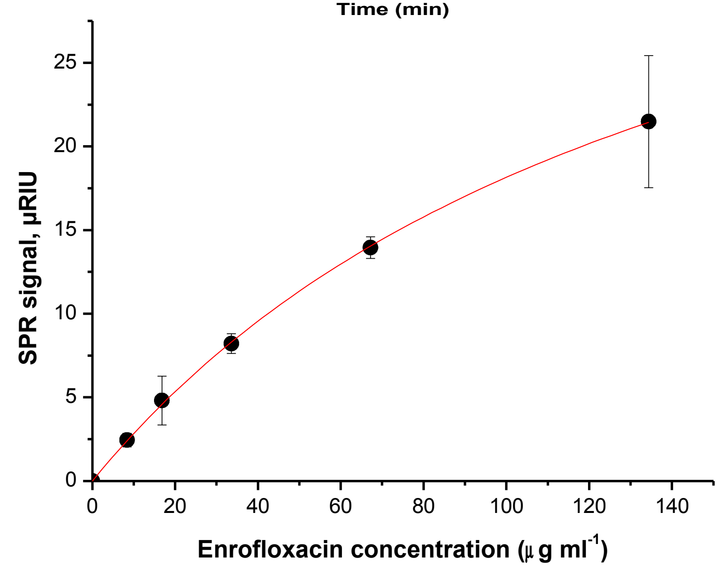
Beispiel 3. Biosensor für die Bestimmung von Antibiotika (Enrofloxacin) in Milch

Rezeptor: DNA (Enrofloxacin hat Affinität zur DNA)
Beschichtung: LbL-Technik unten SPR-Kontrolle
Detektion: SPR



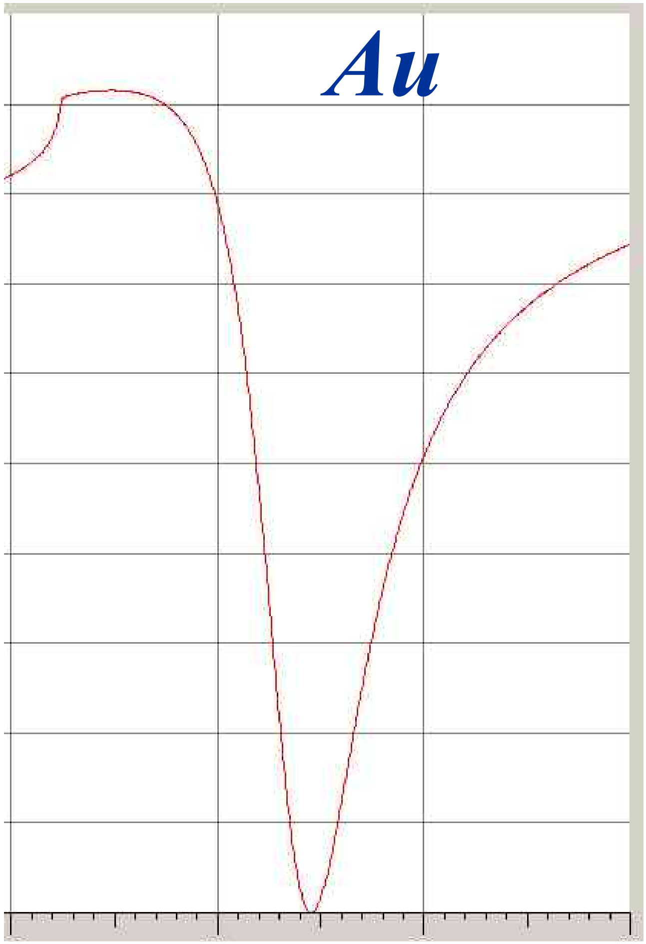


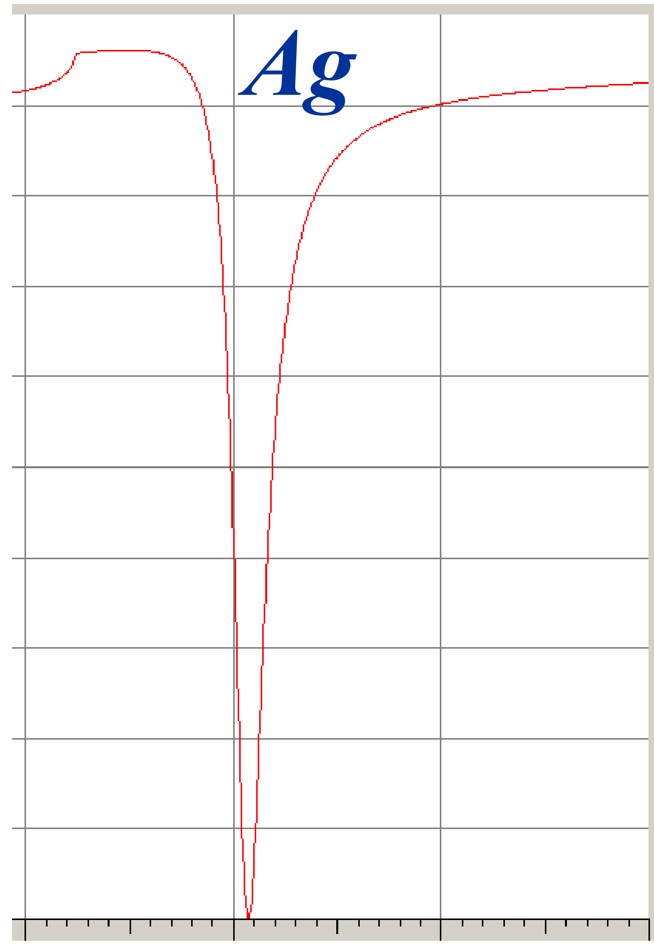




L. Cao, H. Lin, V. M. Mirsky. Anal. Bioanal. Chem., 2007; Anal. Chim. Acta, 2007

SPR: improvement of the resonant layer



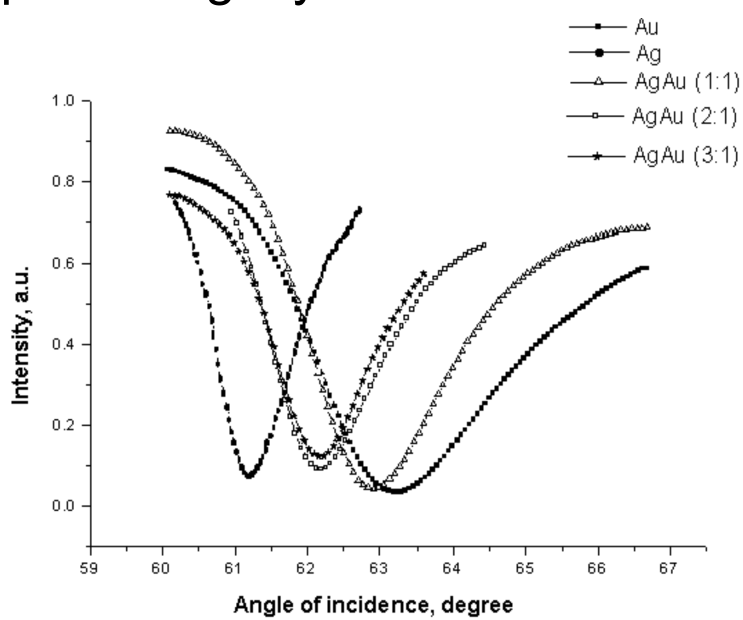


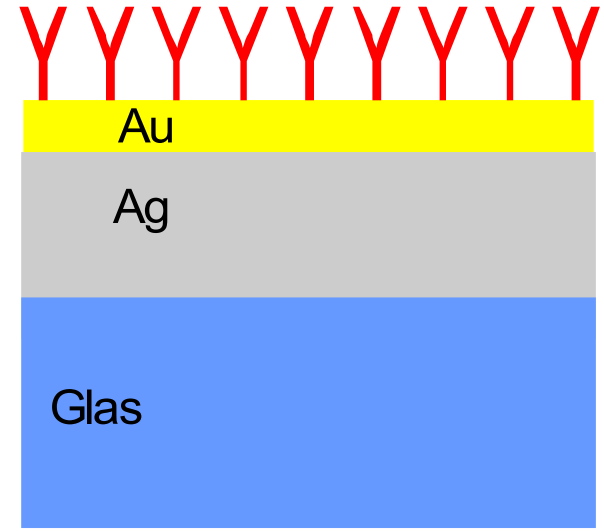
Gold is chemically inert

Silver is not chemically inert but provides better sensitivity of SPR transducing

Bimetalic coatings in SPR-chips

Solution:
Ag as the main resonator layer,
Au as a thin protecting layer



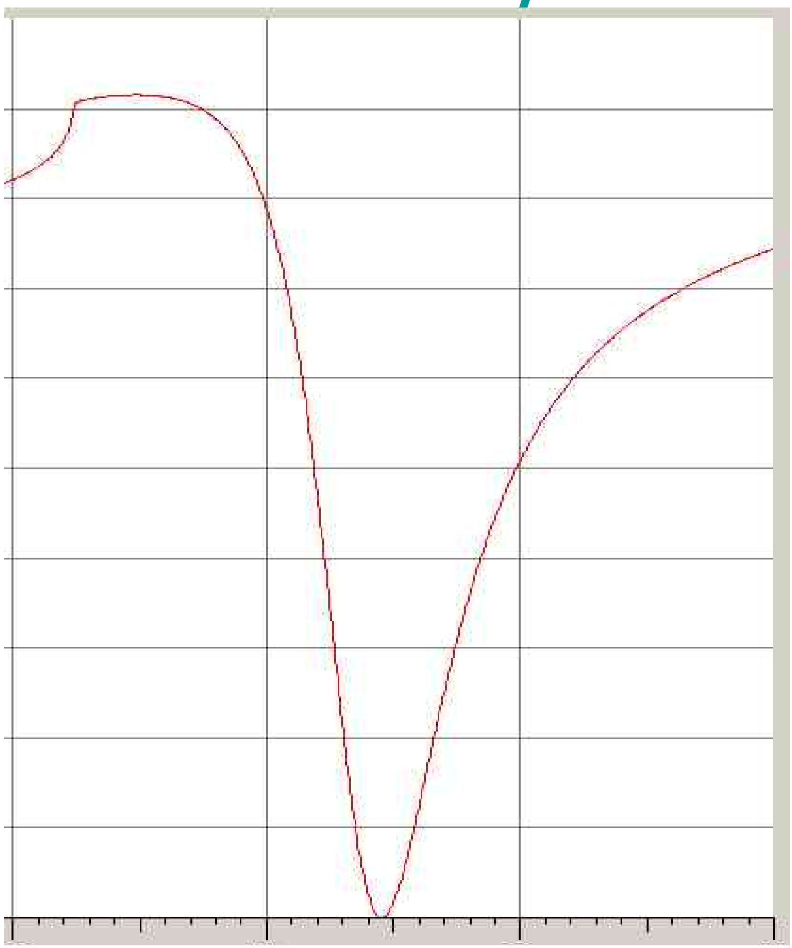


S/N improvement:
our results: 1.4
later literature (Zhai et al., *Anal. Chem.*, 2007): **2.7**

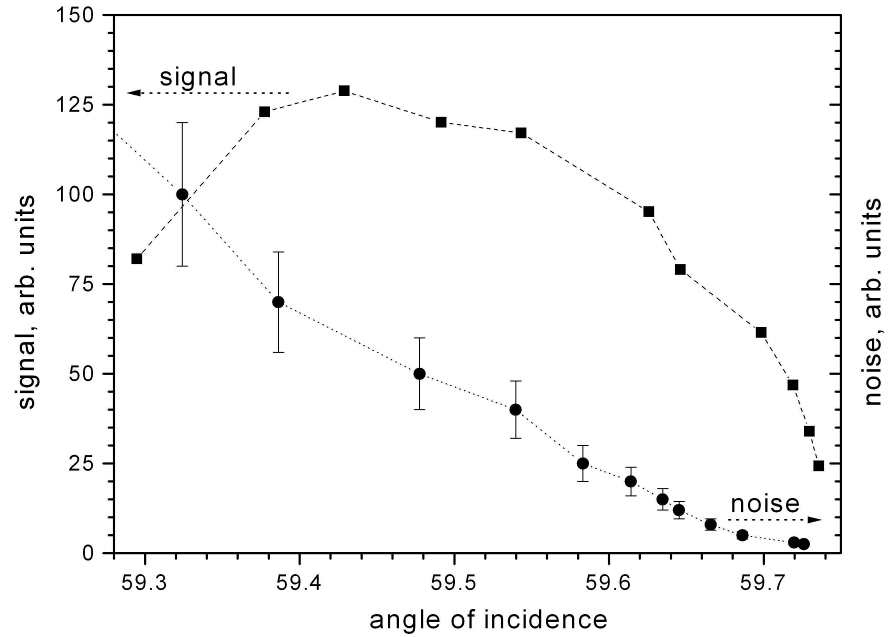
S. A. Zynio, A. V. Samoylov, E. R. Surovtseva, Y. M. Shirshov, V. M. Mirsky. *Sensors, 2002,* ***2****, 62-70*

Optimization of the detection angle

max ($dR/d\theta$) ?



but noise ≠ const !



Advanced SPR-techniques:

- compensation of microfluctuations of temperature (also of analyte pressure, concentration, velocities...)

- separation of surface and volume effects…

A postulate to discuss:

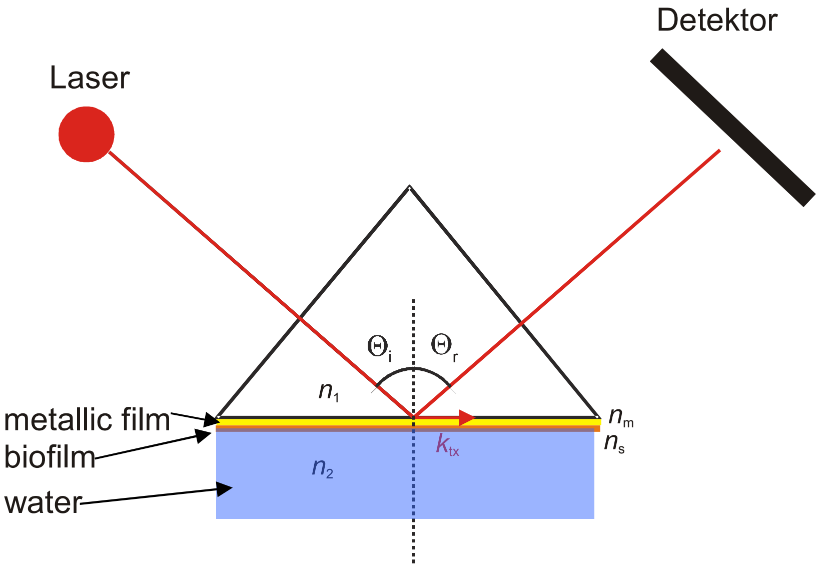
Resolution of each well-developed analytical technique is limited by temperature fluctuations.

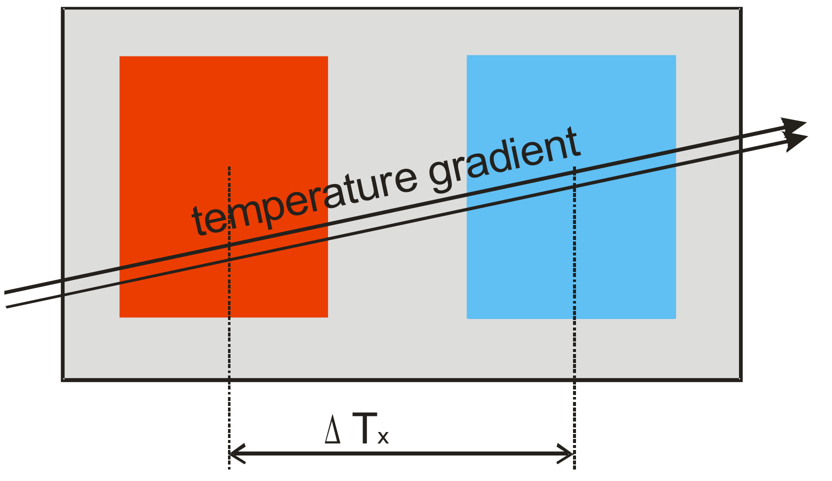
☺ The consequence:

If a resolution of an analytical technique is not limited by temperature fluctuations, the technique is not well developed.

Surface plasmon resonance is a well developed analytical technique…

The problem of temperature stabilization:

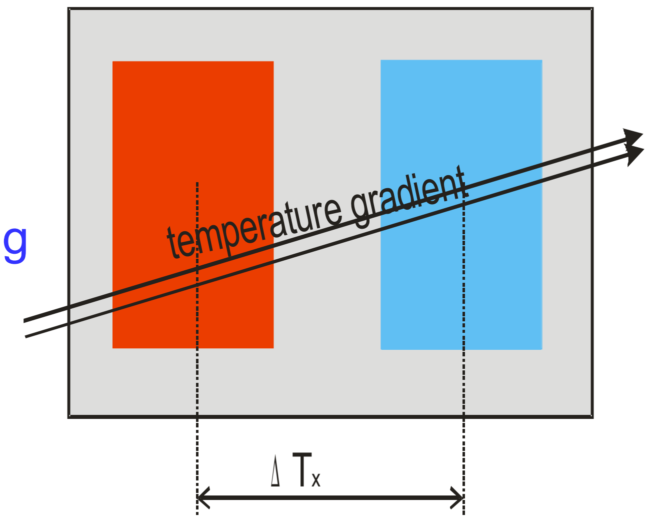




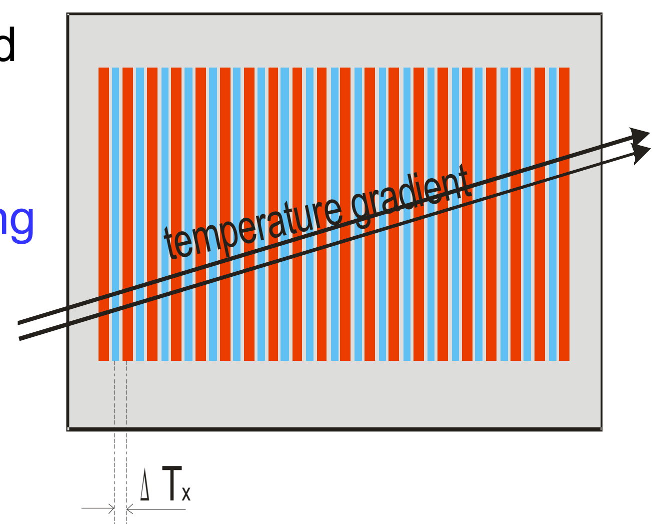
Role of temperature microfluctuations:
Temperature coefficient of water:
100 μRIU / K,
therefore, a stabilization with precision of 0.0003 K°
is required for resolution 0.1 μRIU!

Distributed sensing and referencing spots

single
sensing
and
referencing
spots

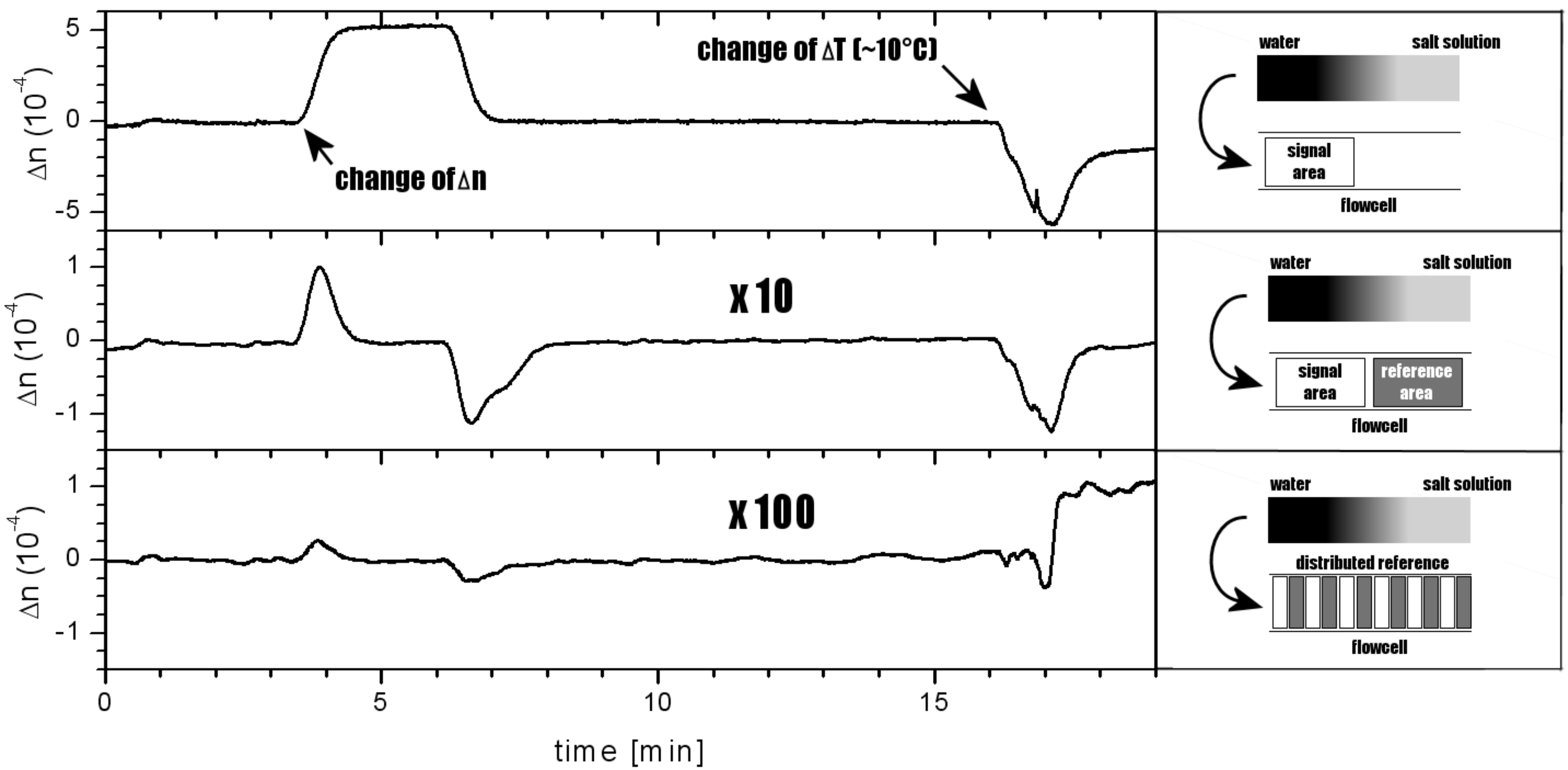


distributed
sensing
and
referencing
spots



Distributed sensing and referencing spots

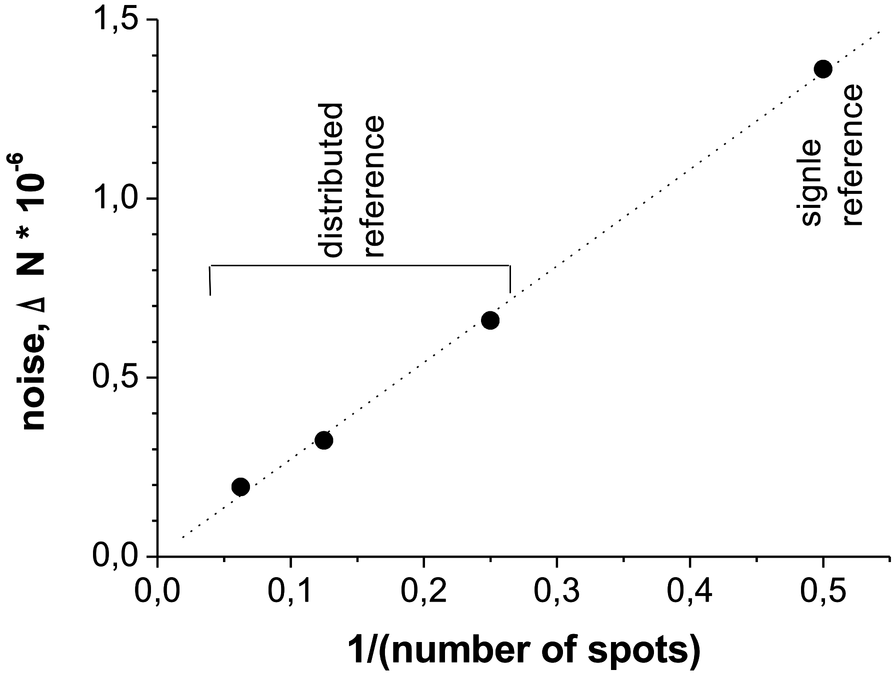
the break through sensitivity limitations caused by physical and chemical surface inhomogenities (fluctuations of temperature, of reagents concentrations, etc.)



Zybin, A.; Boecker D., Grunwald, C.; Niemax K., Mirsky, V. M., *Rev. Sci. Instr.*, **2008**, 79

Distributed sensing and referencing spots

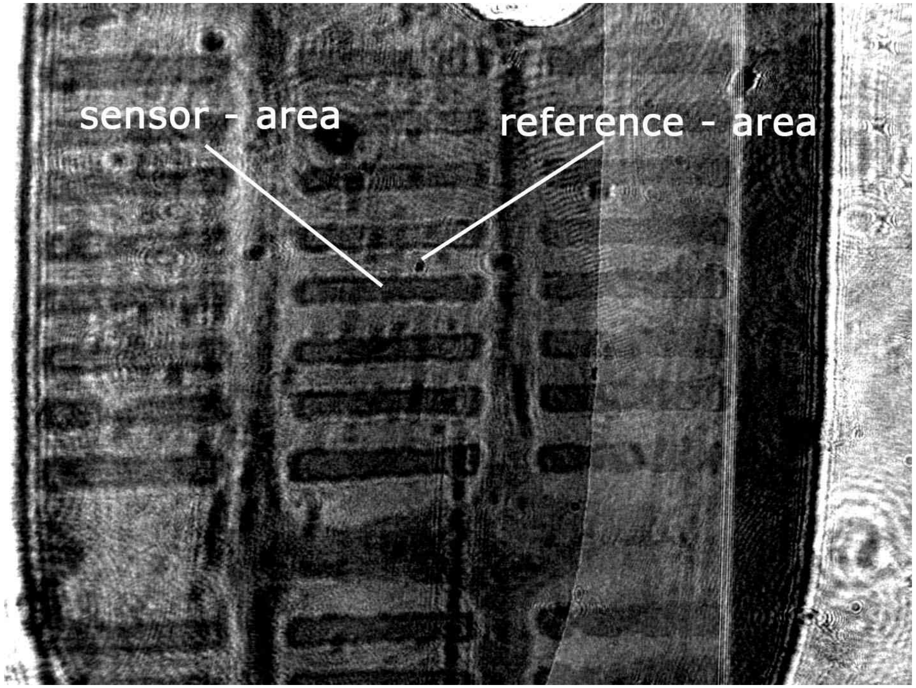
the break through sensitivity limitations caused by physical and chemical surface inhomogenities (fluctuations of temperature, of reagents concentrations, etc.)



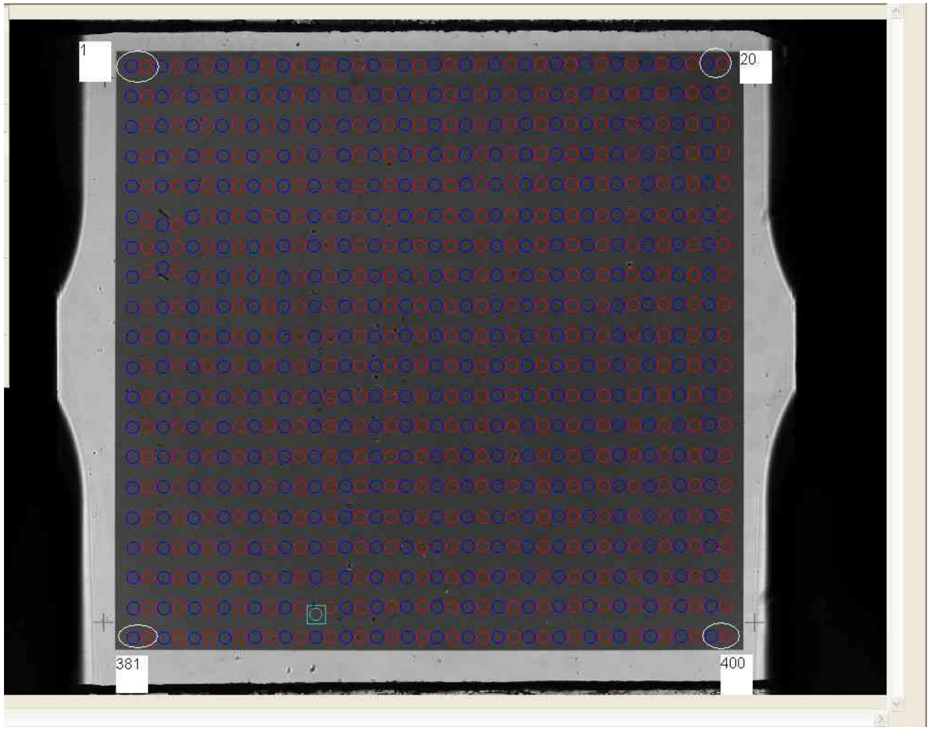
Result: >10-fold (!) increase of the signal/noise ratio.

Zybin, A.; Boecker D., Grunwald, C.; Niemax K., Mirsky, V. M., *Rev. Sci. Instr.*, **2008**, 79

Structures for distributed referencing

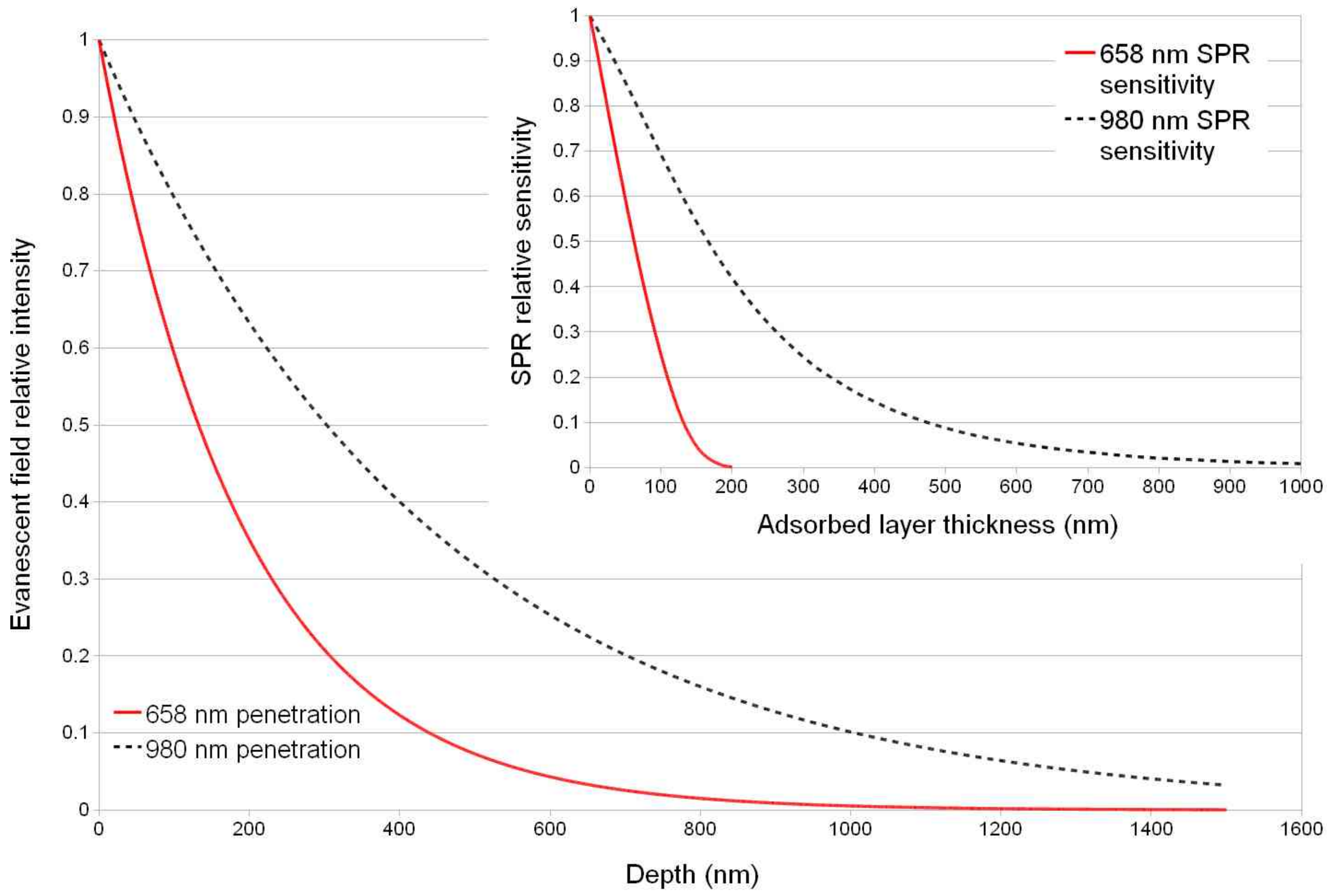


μ-printing technology



flexchip (GE)

Again on the double wavelengths technique…



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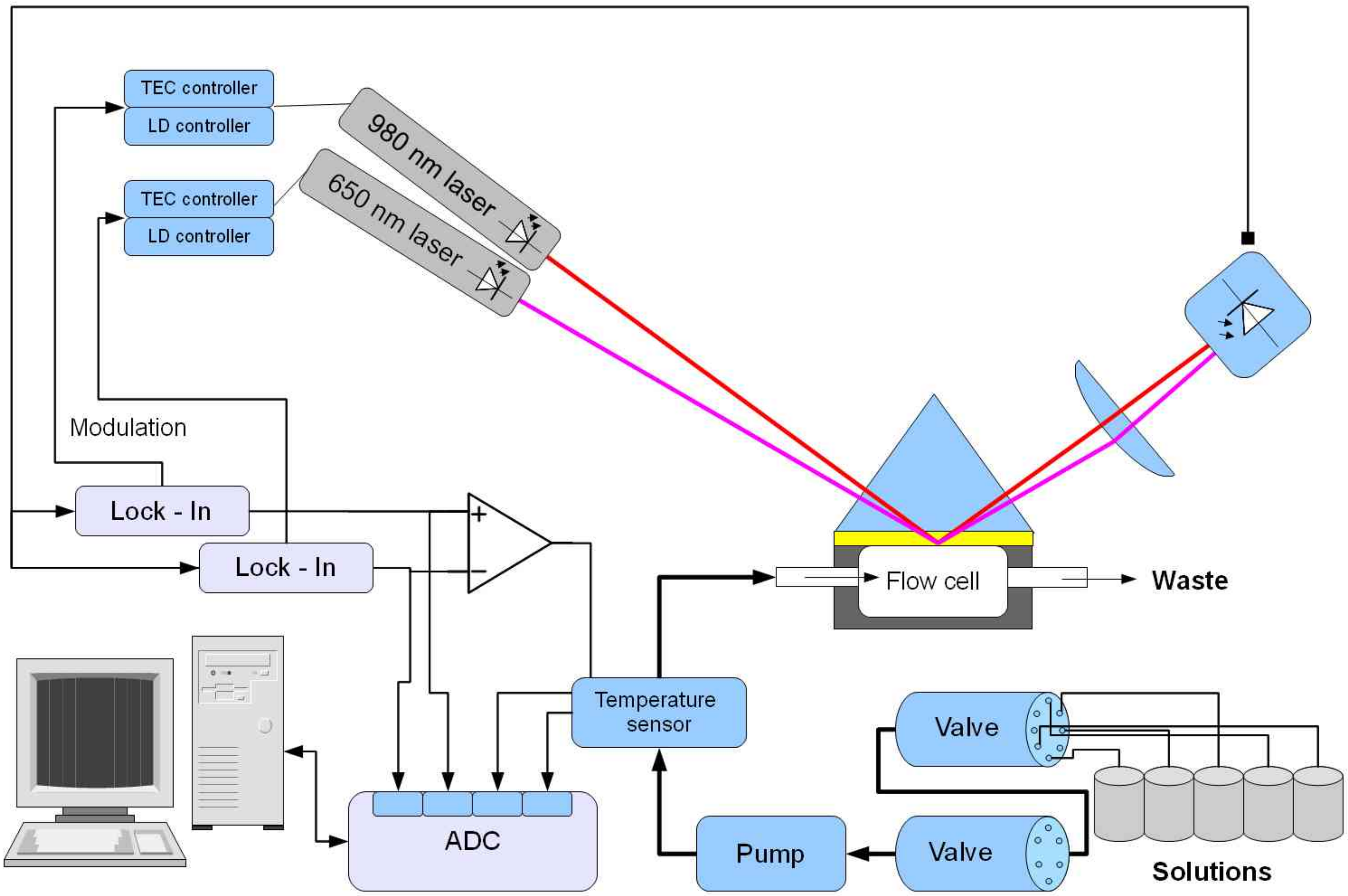
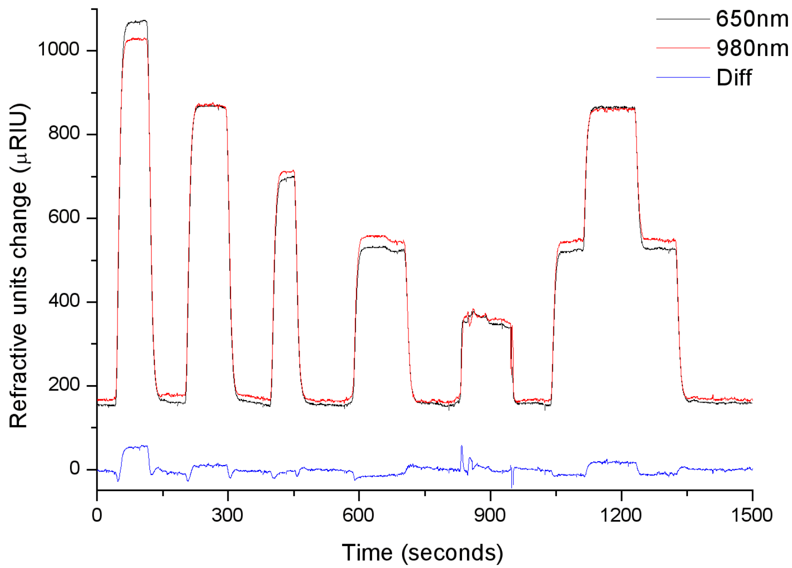
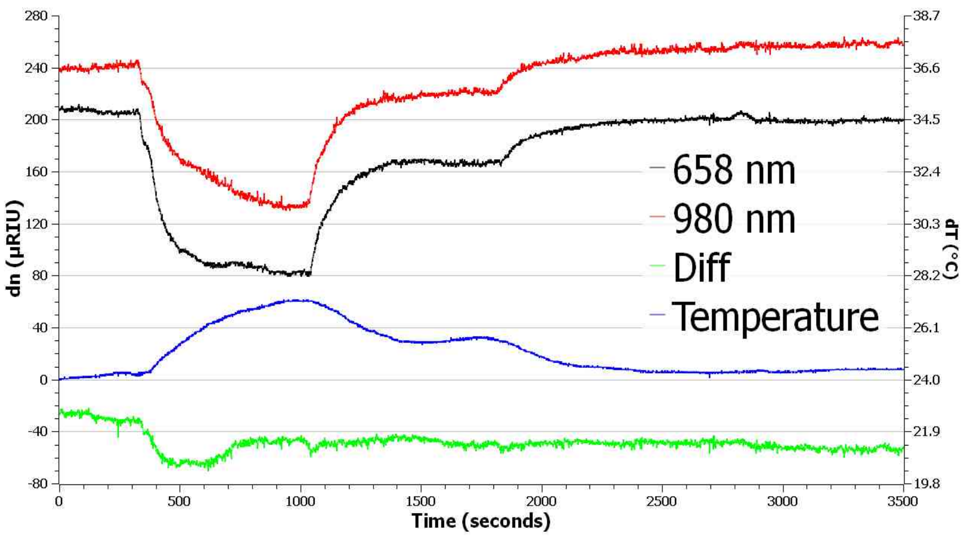


Again on the double wavelengths technique…

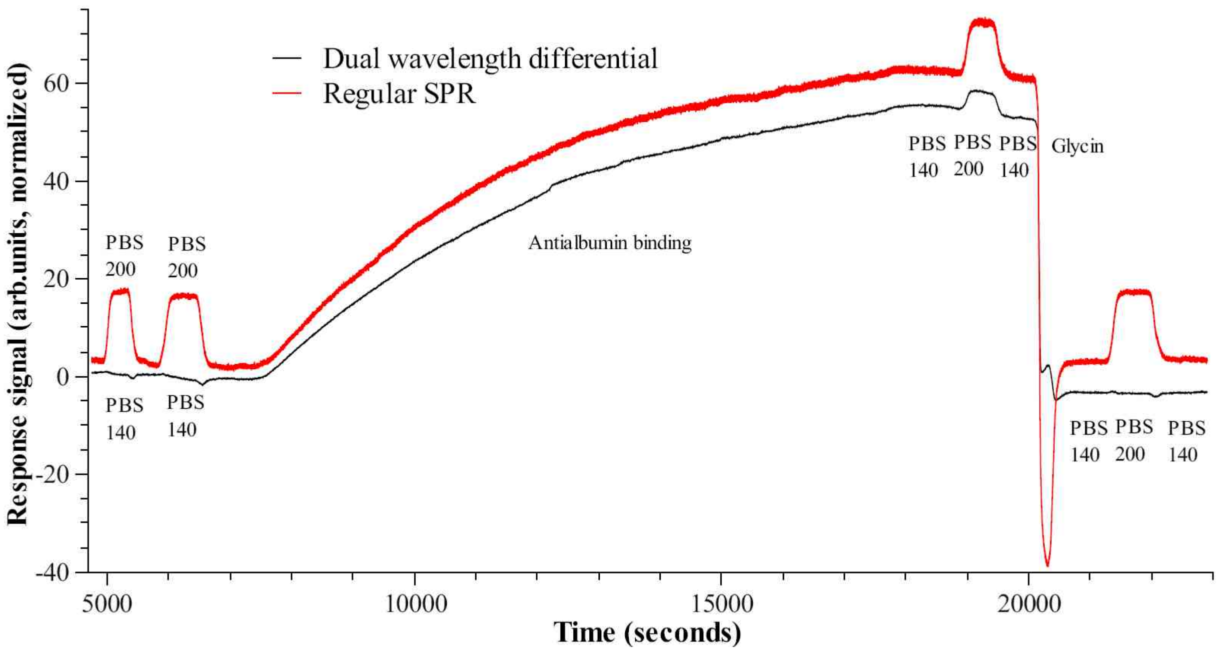


Adjusted compensation of influence of bulk refractive index



Consequence: Compensation of influence of temperature

Again on the double wavelengths technique...



Detection of antigen – antibody binding

Conductometric chemosensors:

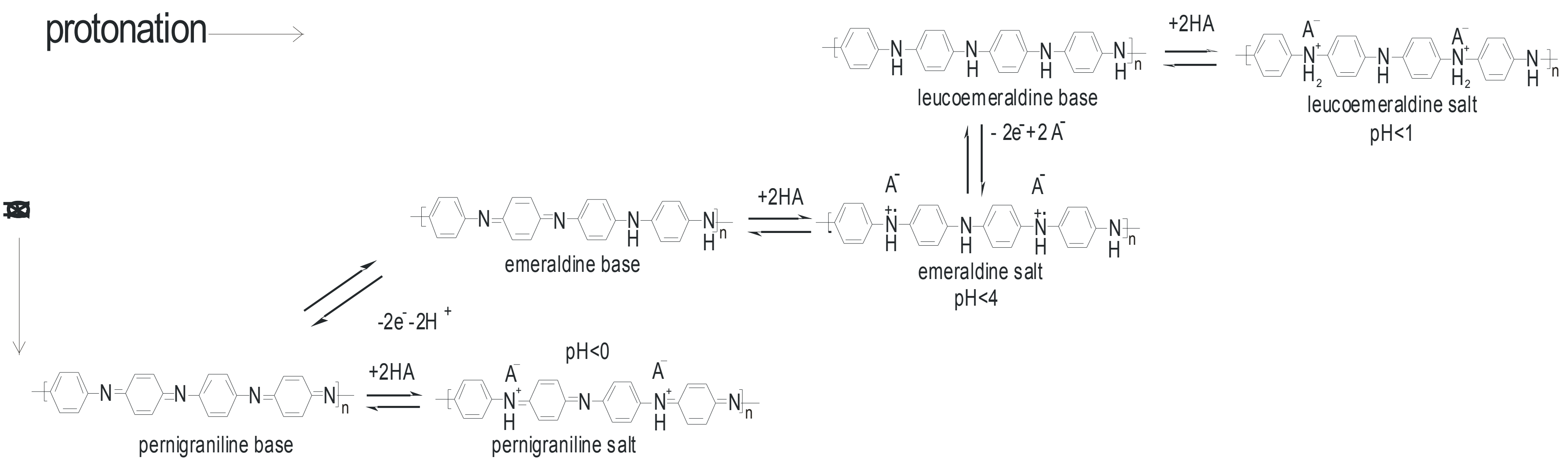
- chemosensors for Hg-vapor
- chemosensors based on conducting polymers

Conducting polymers and integrated electrochemical chemotransistors

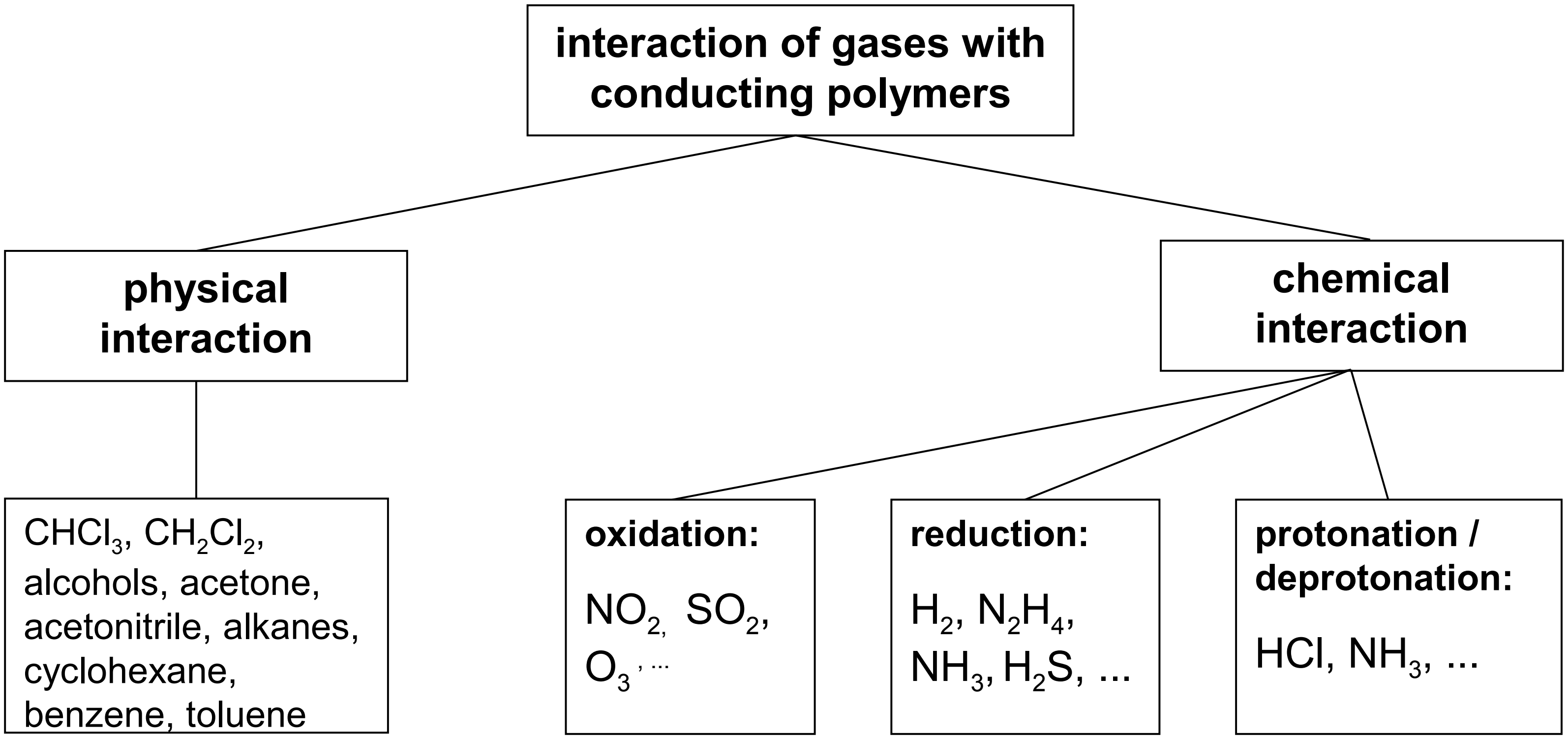
Conducting polymers as receptors

Conductometric [or optical] transducing:

- **pH-sensitivity of CP** (pH sensors, **acidic/basic gases,** pH-transducer in biosensors)
- **CP as red-ox probe** (sensor for oxidizing or reducing gases)



Gas sensors

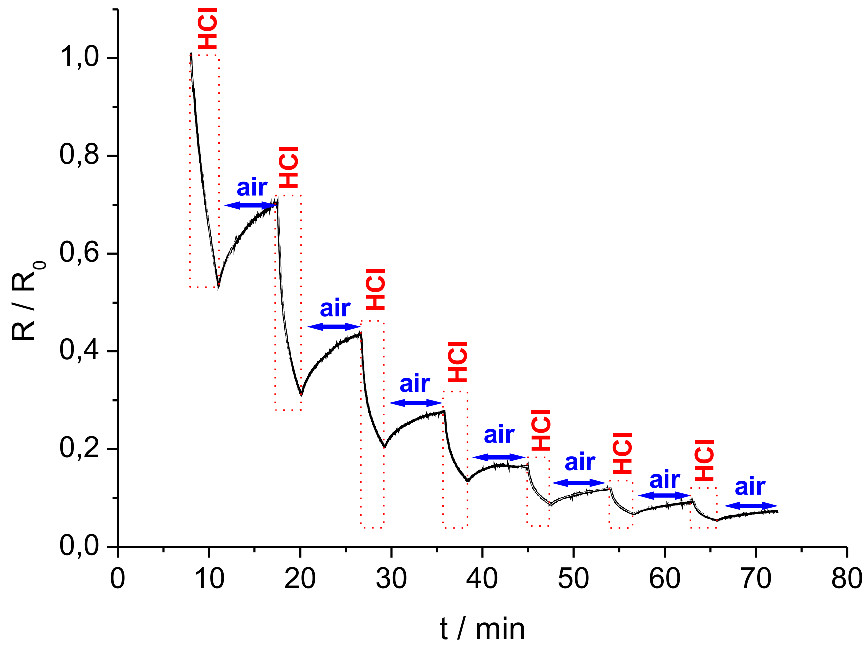


Problems:

- pH-effects are coupled with redox-effects =>...
- high affinity => irreversible behavior (desorption takes tens of minutes)
- poor selectivity

Gas sensors: poor reversibility

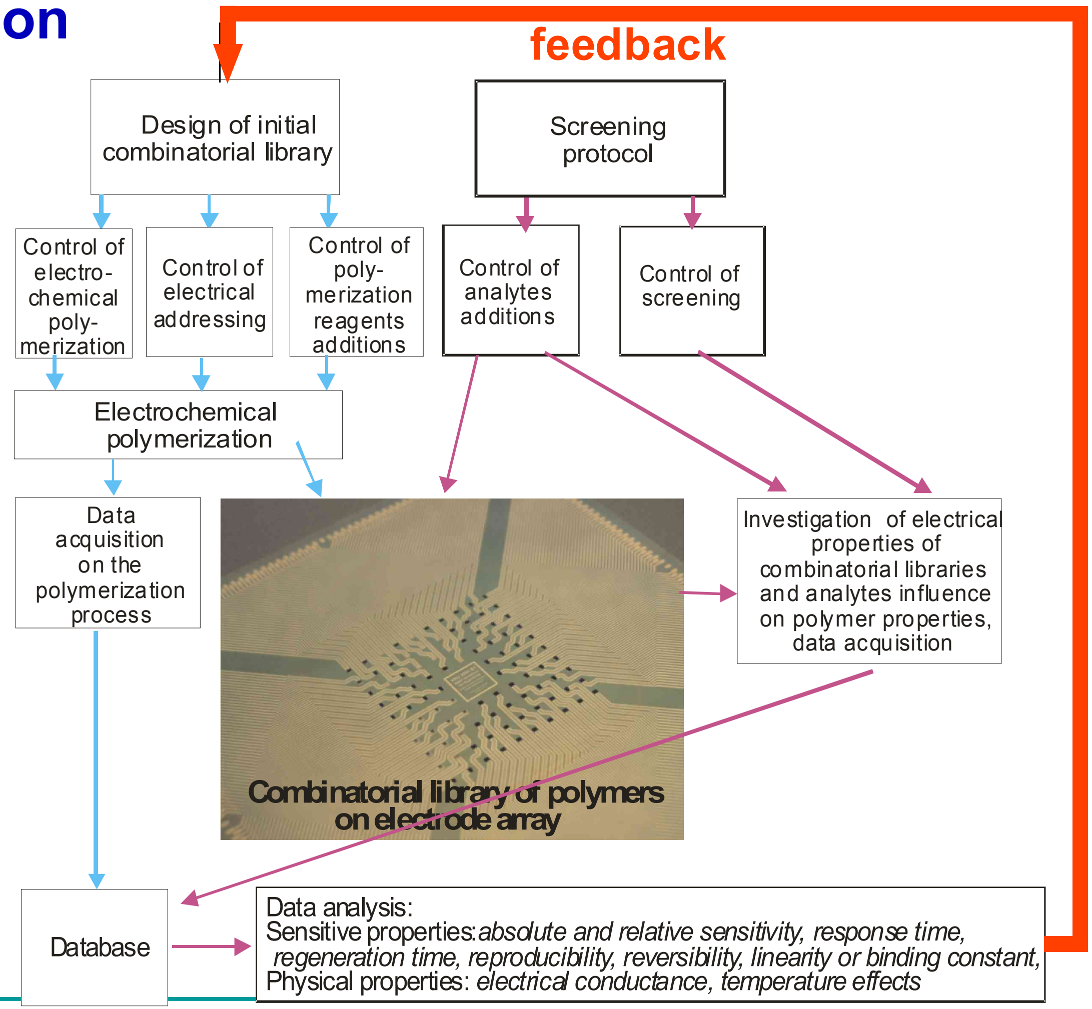
HCl:

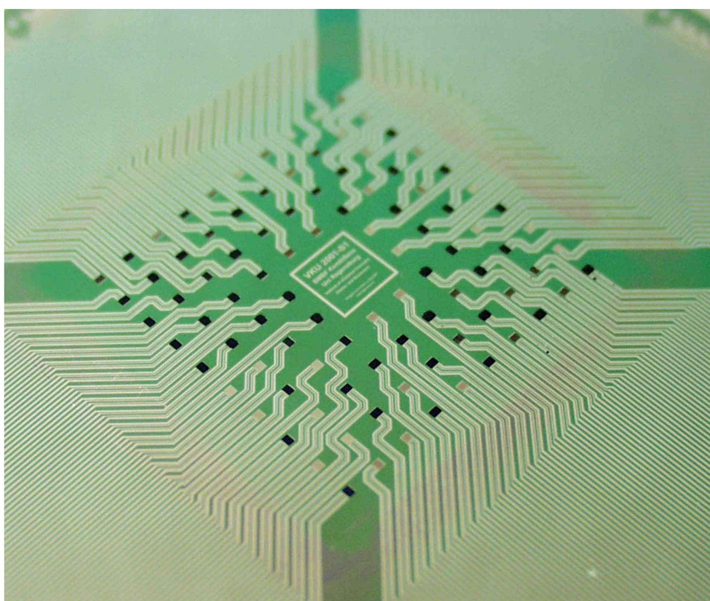


Other applications of PANI-Sensor: amino-containing compounds => fish products!

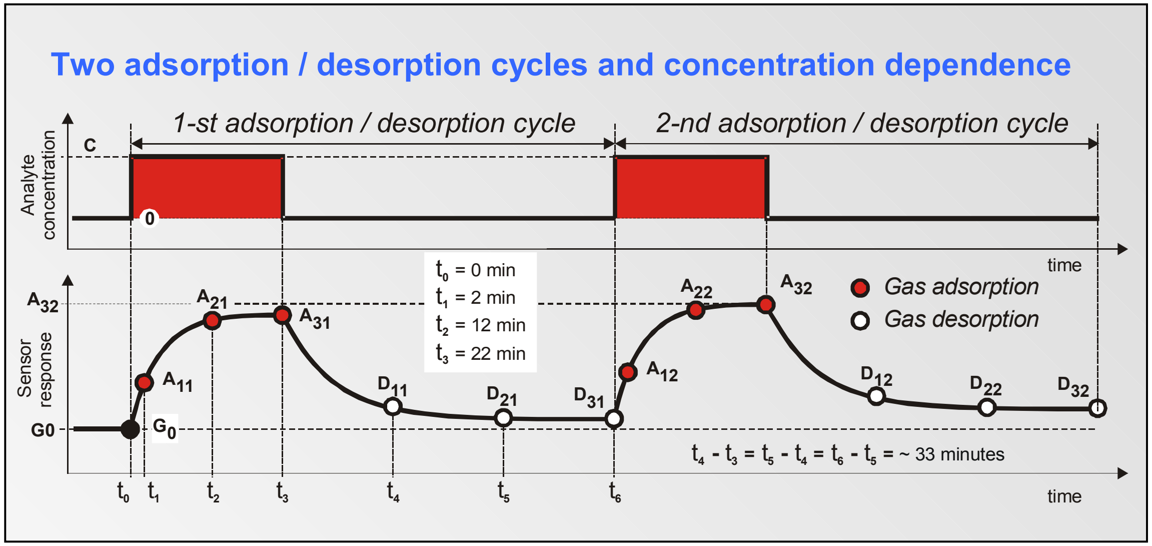
Combinatorial electropolymerization and High-Throughput screeining of polymer properties

Information flow:





Formalisation of the measurement protocol for high-throughput quantitative characterization of chemosensitive properties

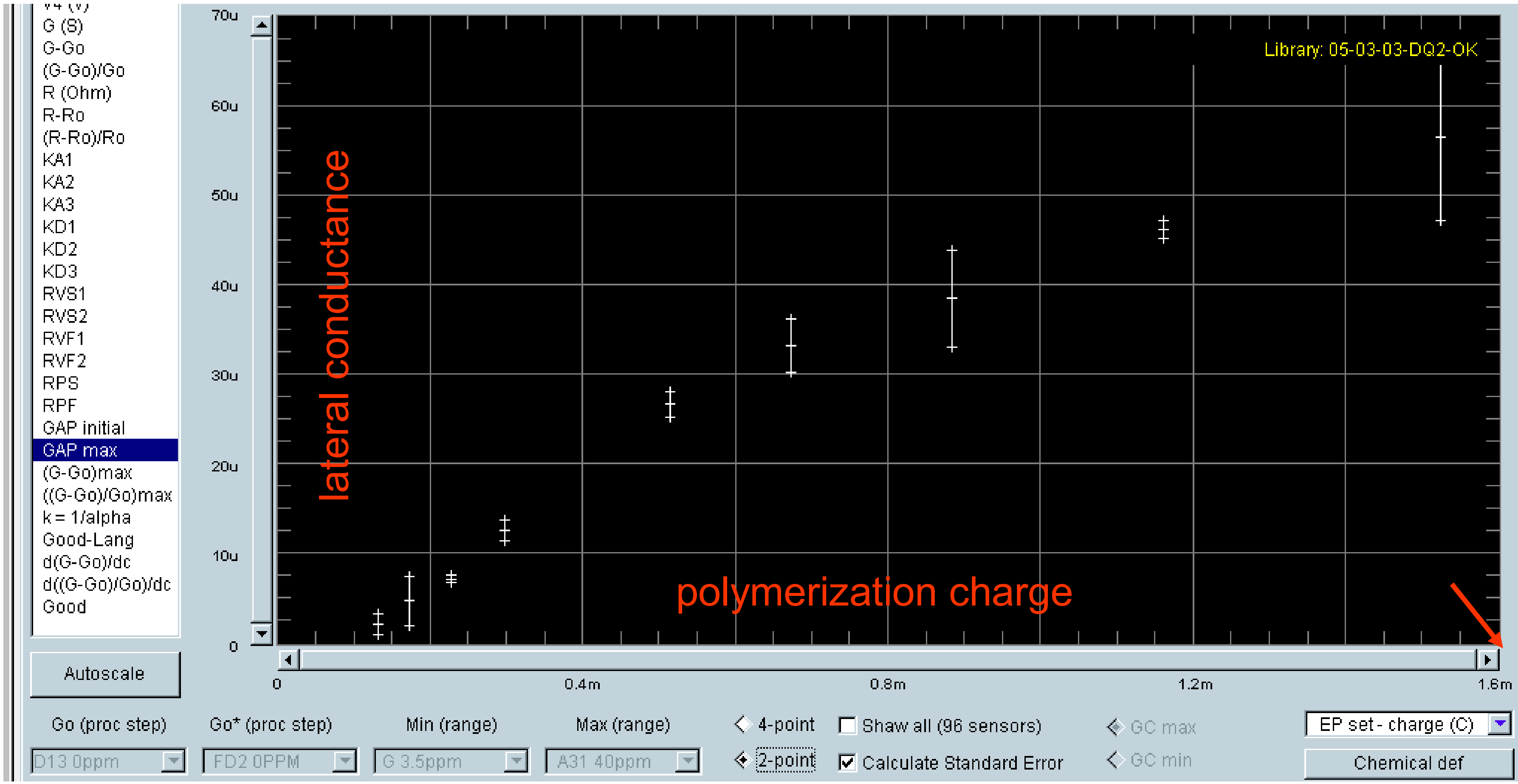


Extracted parameters:

•*analytical sensitivity*
•*relative sensitivity*
•*response time*
•*desorption efficiency*
•*reproducibility*
•*reversibility*
•*fitting by Langmuir isotherm (binding constant)*
or
•*fitting by Henry isotherm (linearity)*

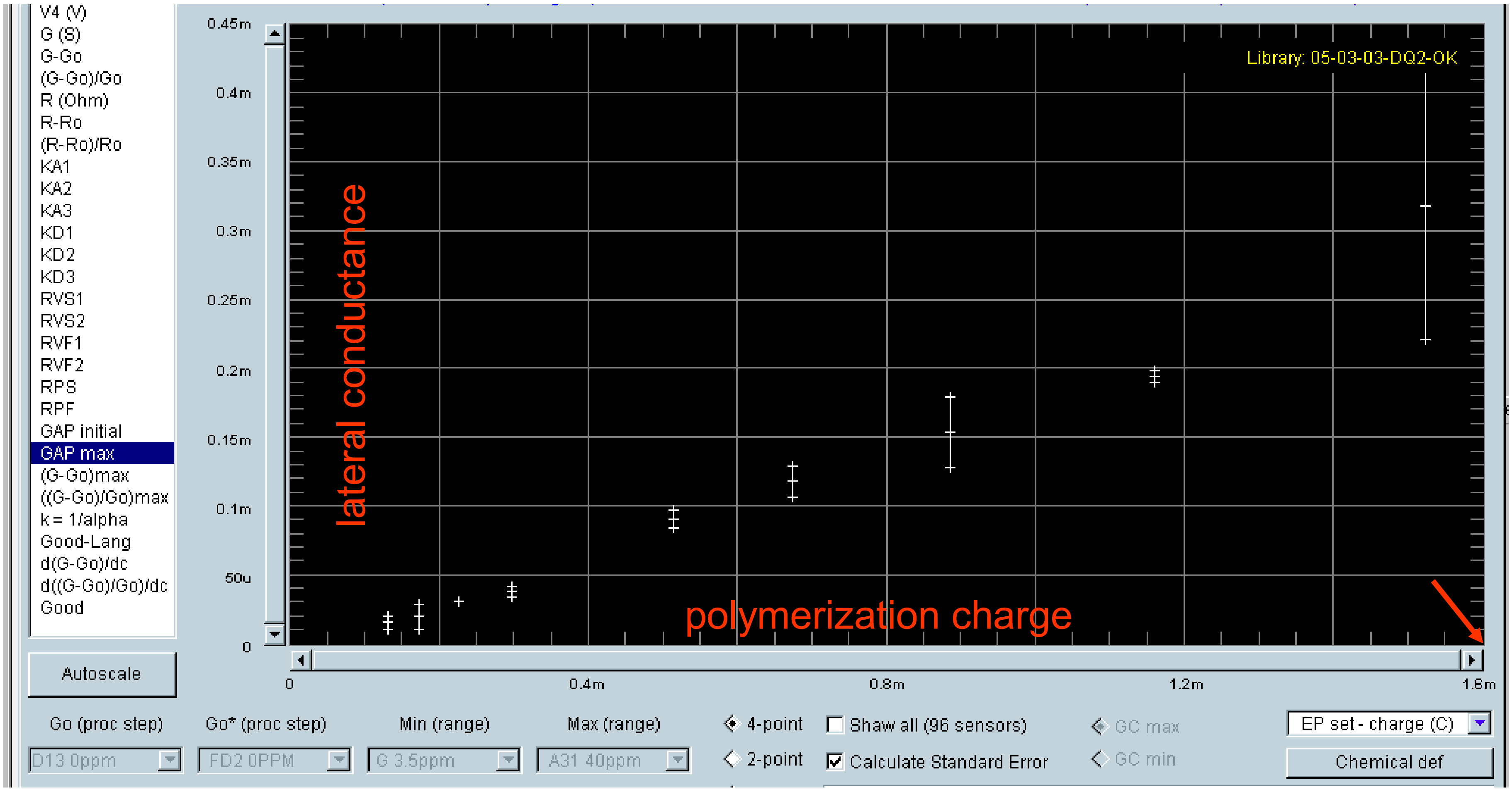
HCl-sensor

Influence of polymer thickness (polymerization charge) on G_2



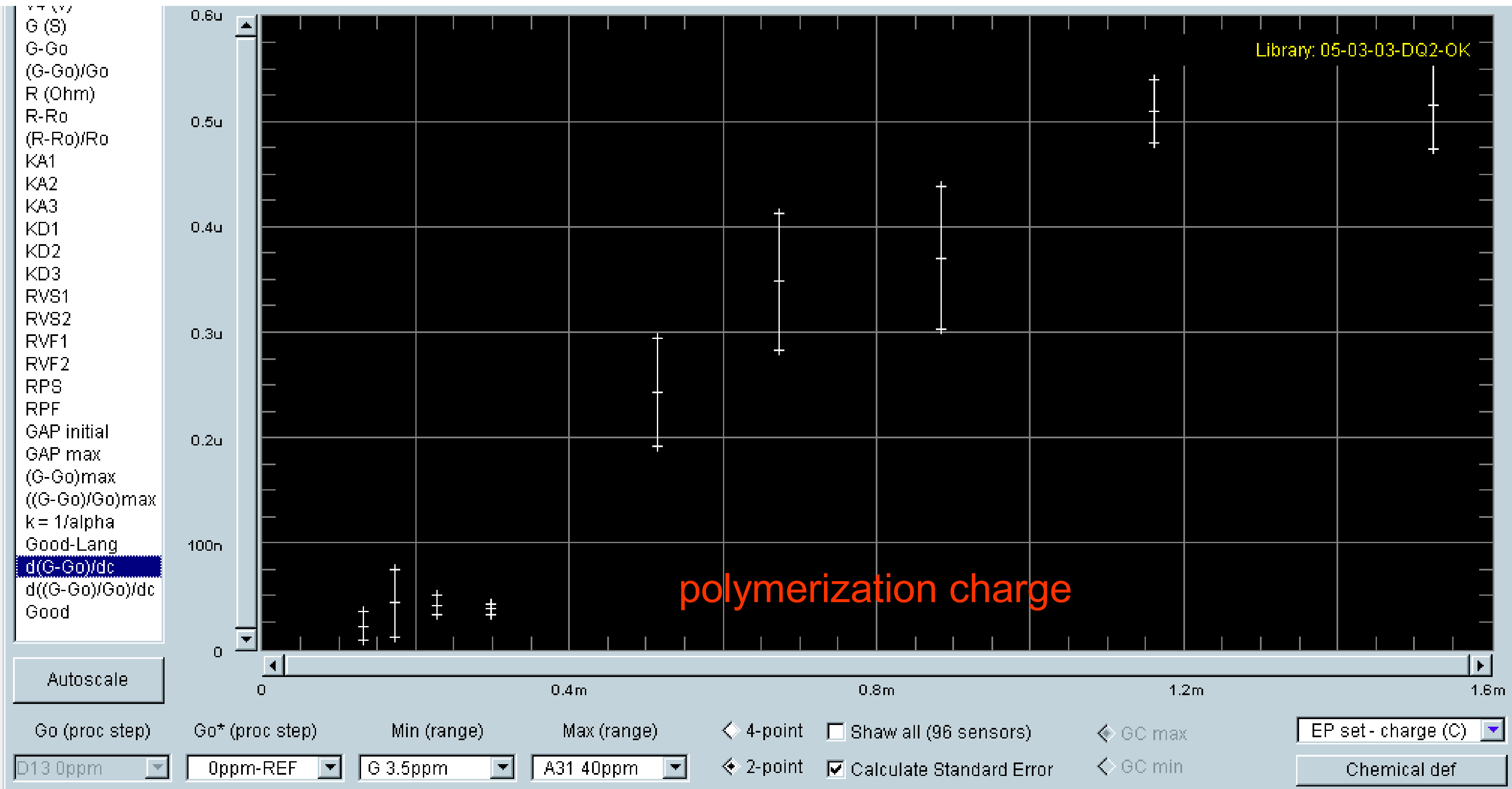
HCl-sensor

Influence of polymer thickness (polymerization charge) on G_4



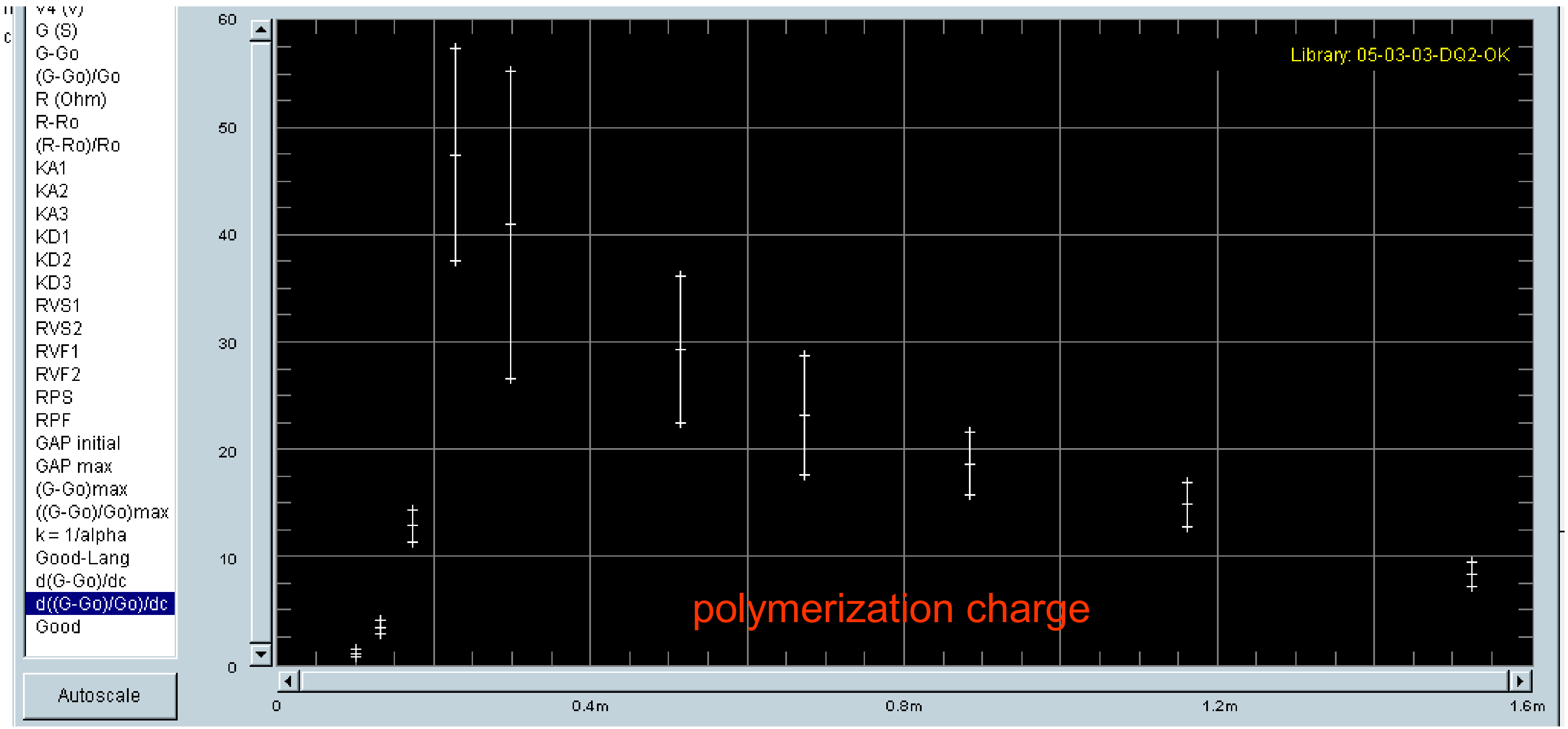
HCl-sensor

Influence of polymer thickness (polymerization charge) on absolute sensitivity



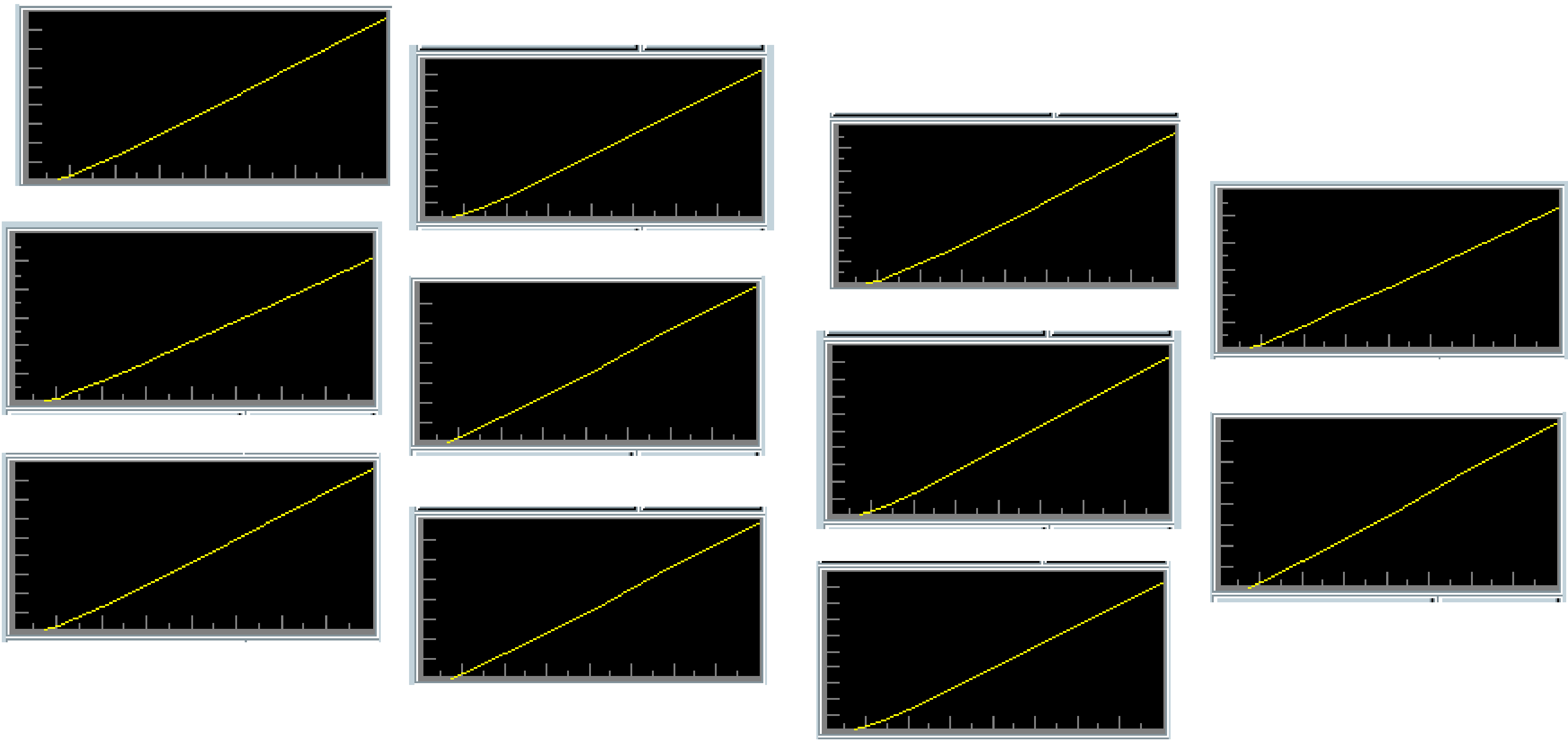
HCl-sensor

Influence of polymer thickness (polymerization charge) on relative sensitivity



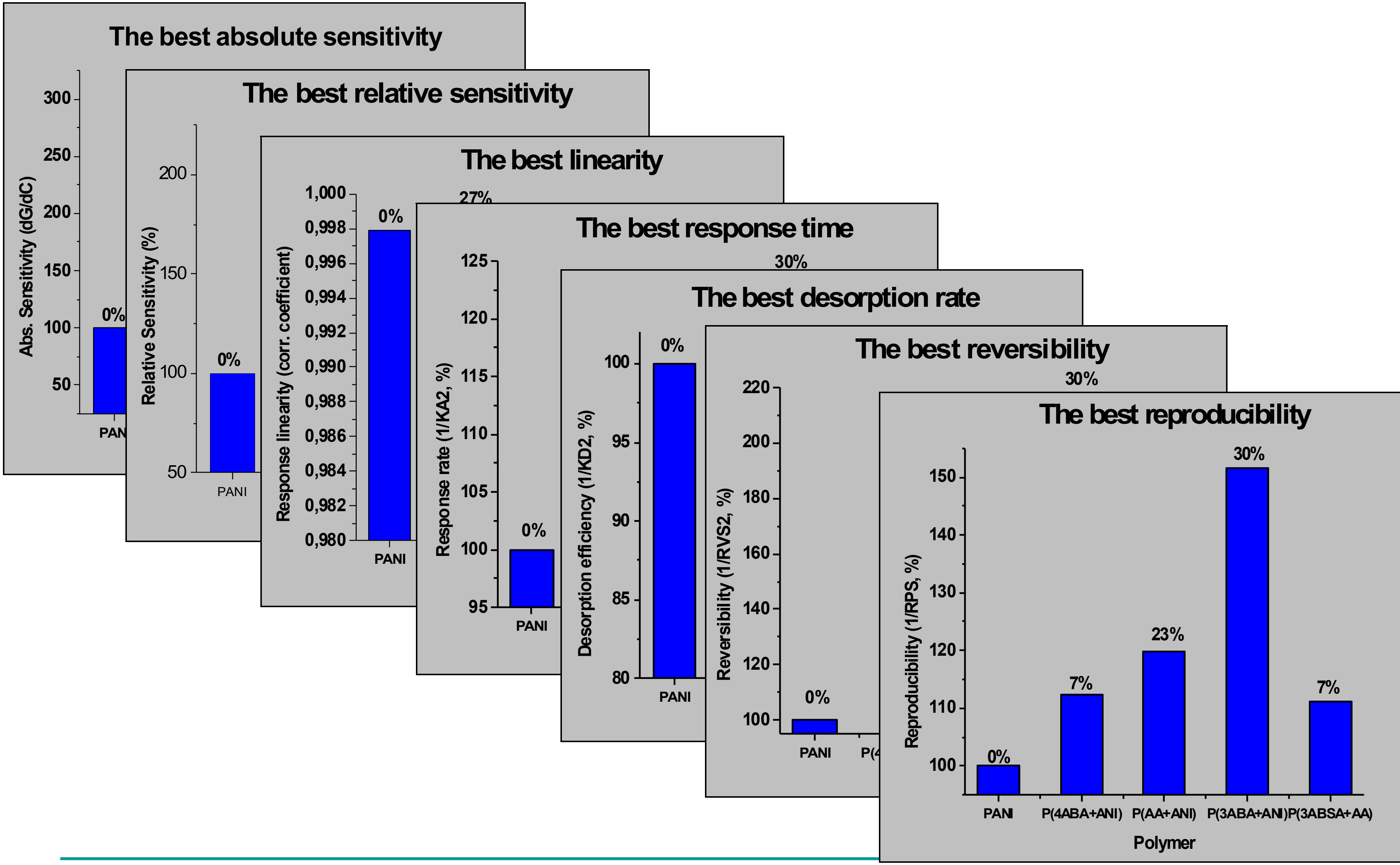
Sensor properties

conductance vs. concentration



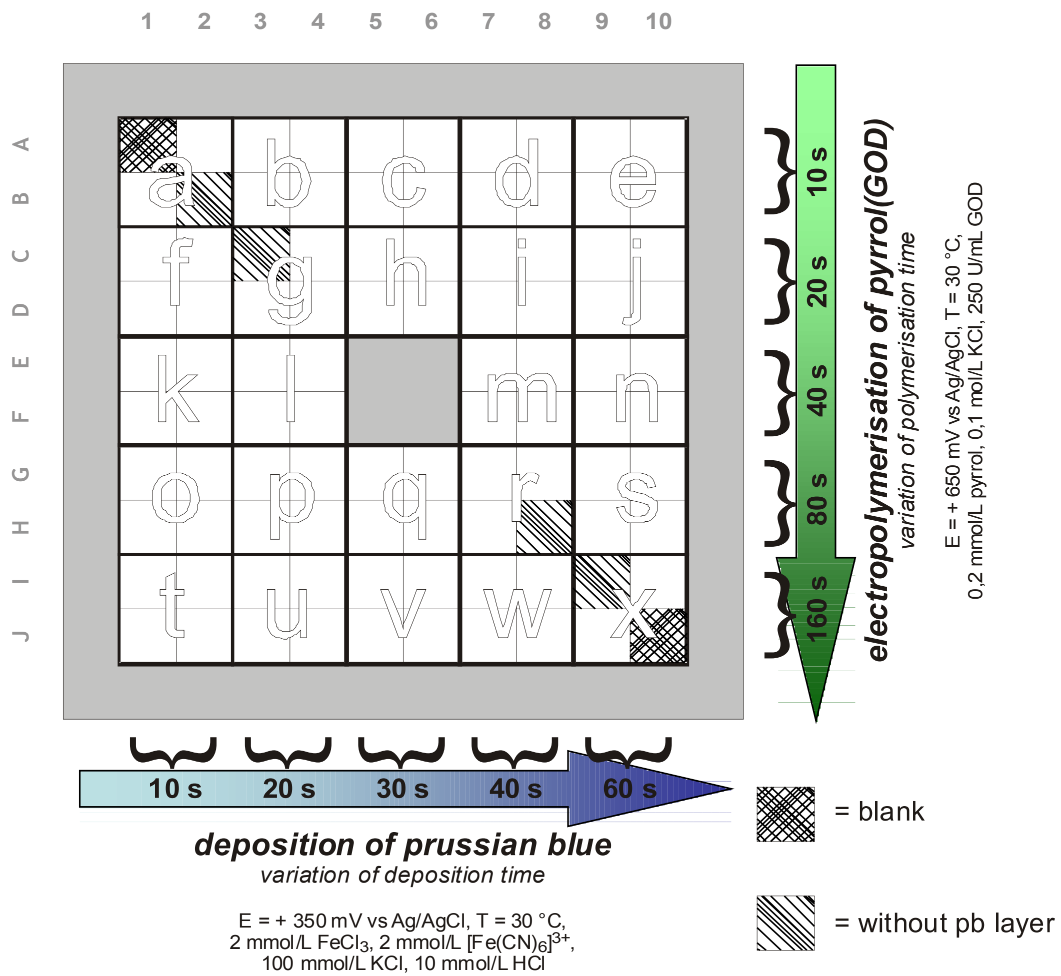
conclusion: linear dependence

HCl-sensor: combinatorial screening of copolymers



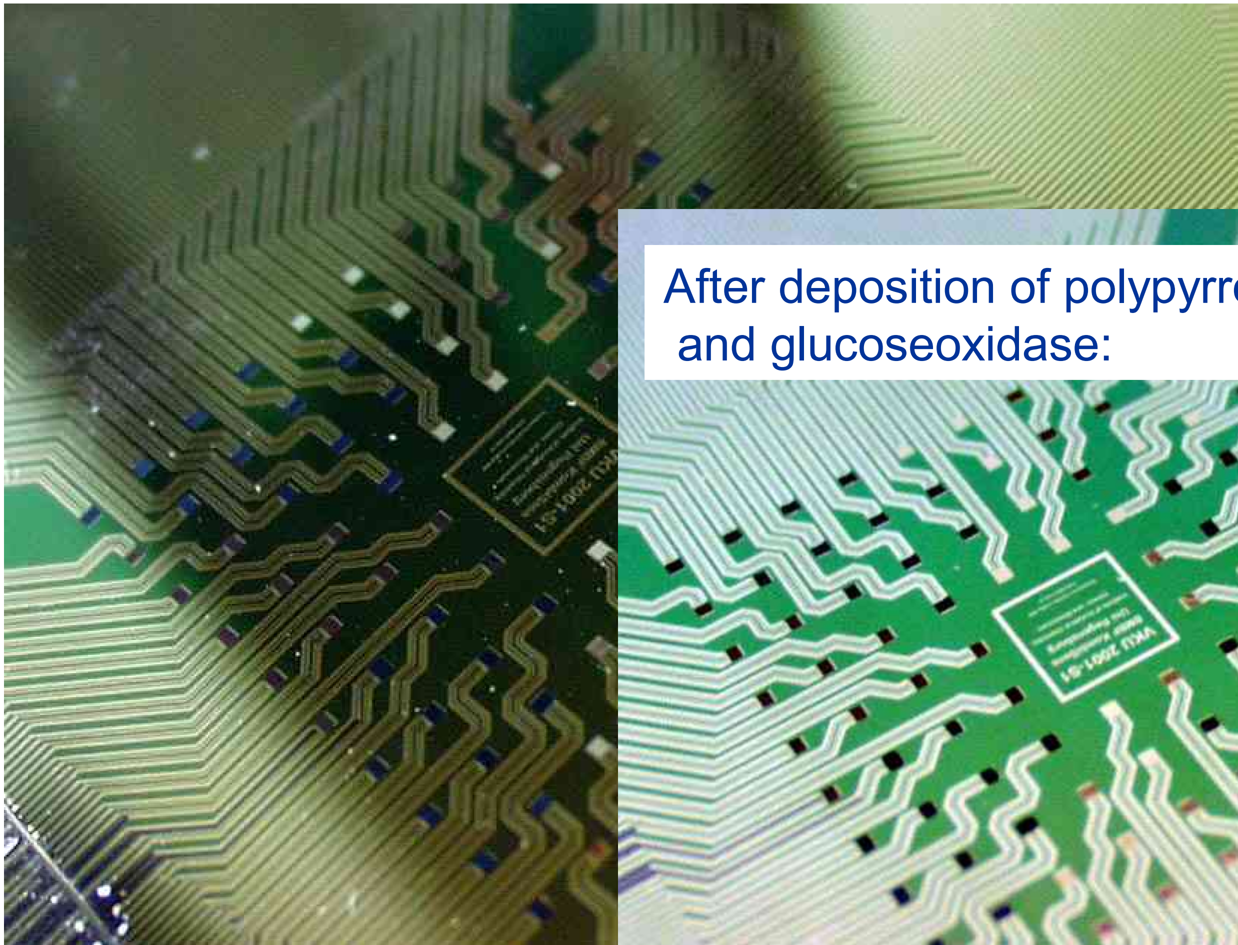
Optimization of amperometric biosensors

Screening:

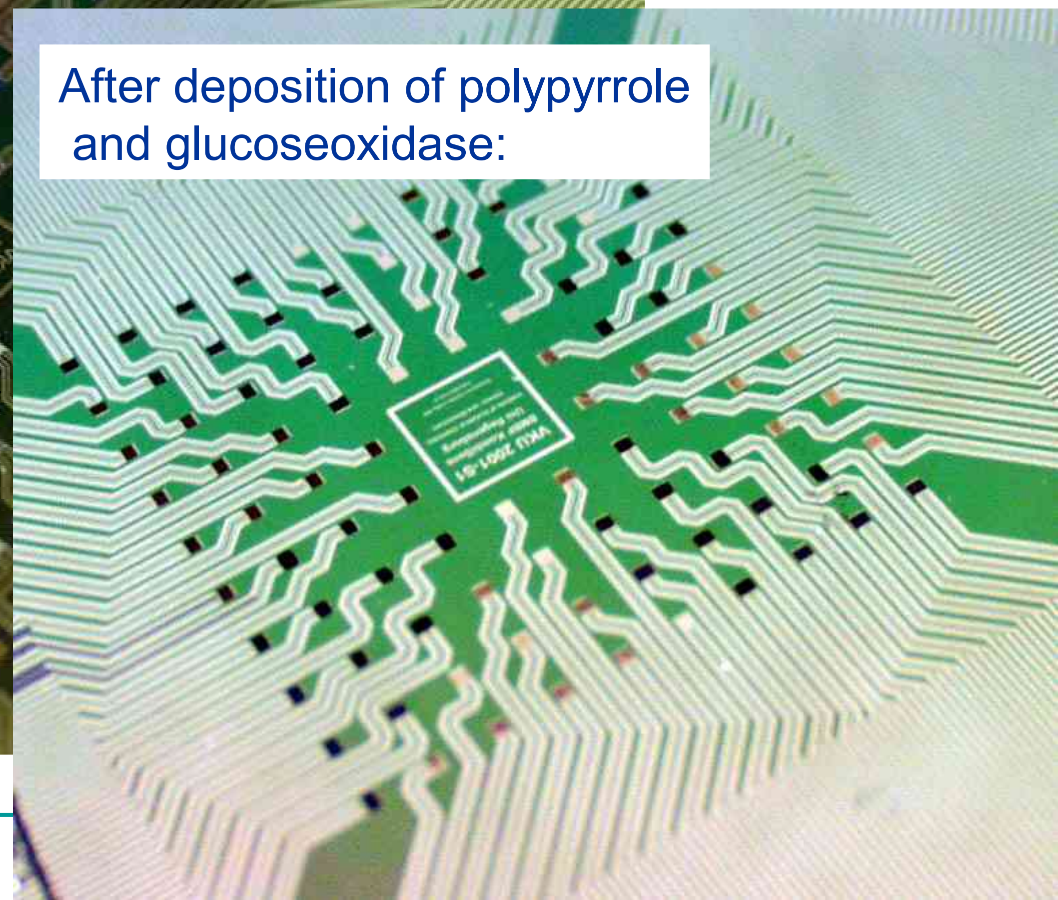


Optimization of amperometric biosensors

After deposition of Prussian blue:



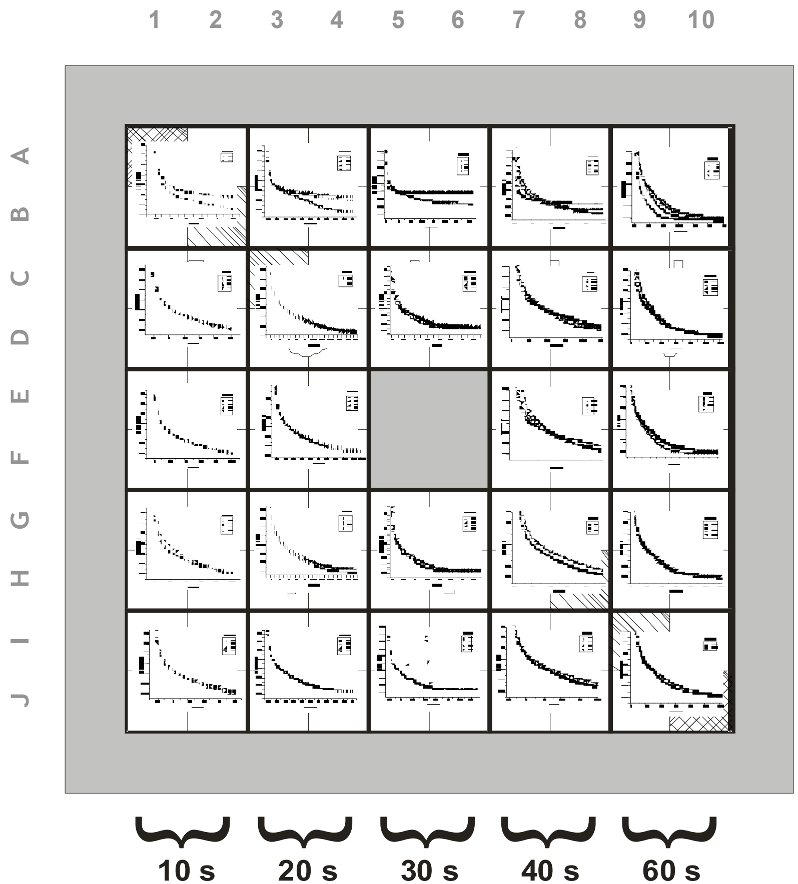
After deposition of polypyrrole
and glucoseoxidase:



Optimization of amperometric biosensors

deposition of prussian blue

variation of deposition time



E = + 350 mV vs Ag/AgCl, T = 30 °C,
2 mmol/L $FeCl_3$ 2 mmol/L $[Fe(CN)]_6{}^{3+}$
100 mmol/L KCl, 10 mmol/L HCl

A general problem in the development of highly-sensitive affinity sensors:

a usual goal: a possibly high binding constant K_b

but $K_b = k_{ads} / k_{des}$

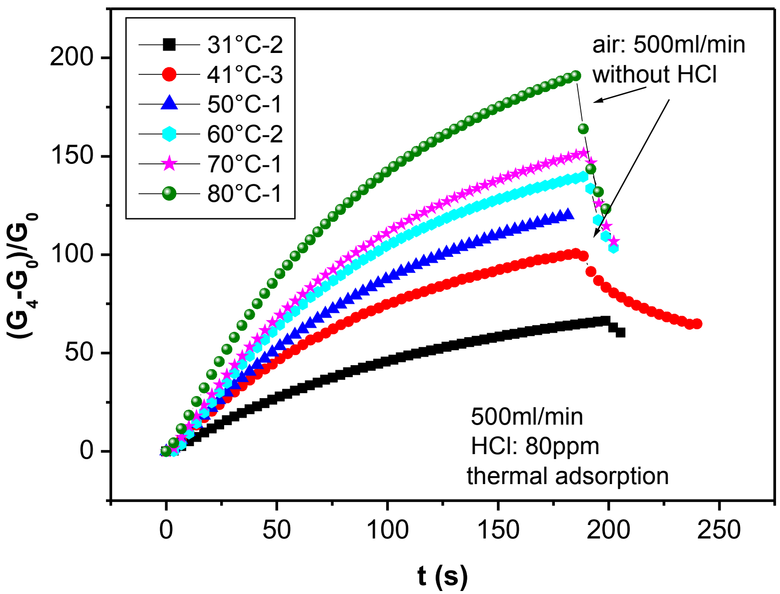
in many cases increasing of k_{ads} / k_{des} is based on decreasing of k_{des}

=> extremely slow desorption, not reversible sensor behaviour

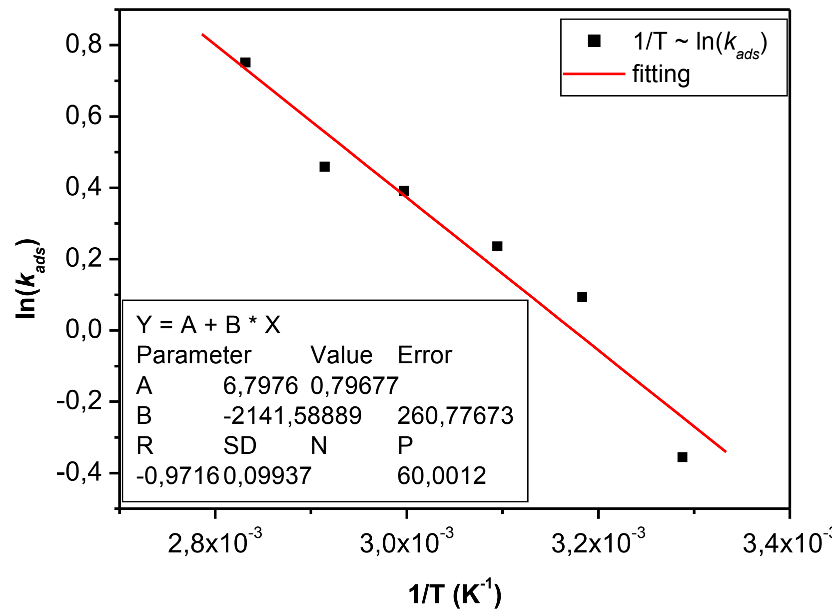
Solution: electrical (or optical) control of affinity

Temperature influence on HCl binding

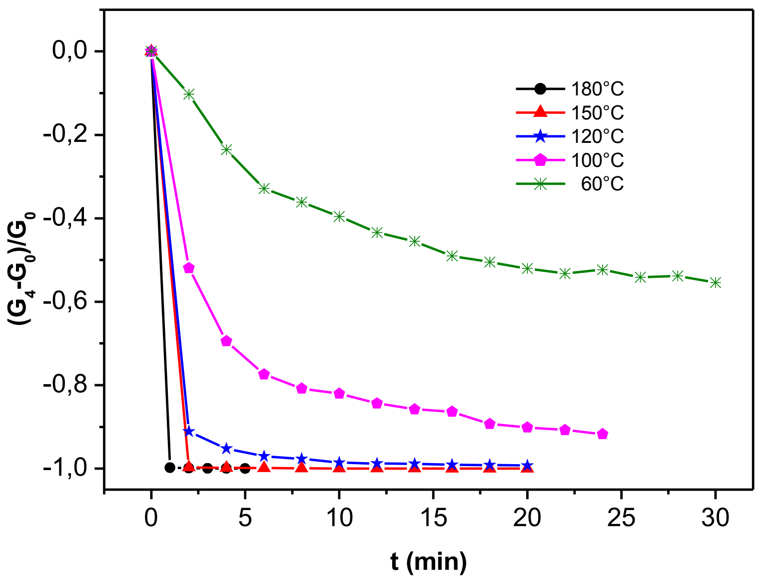
kinetics

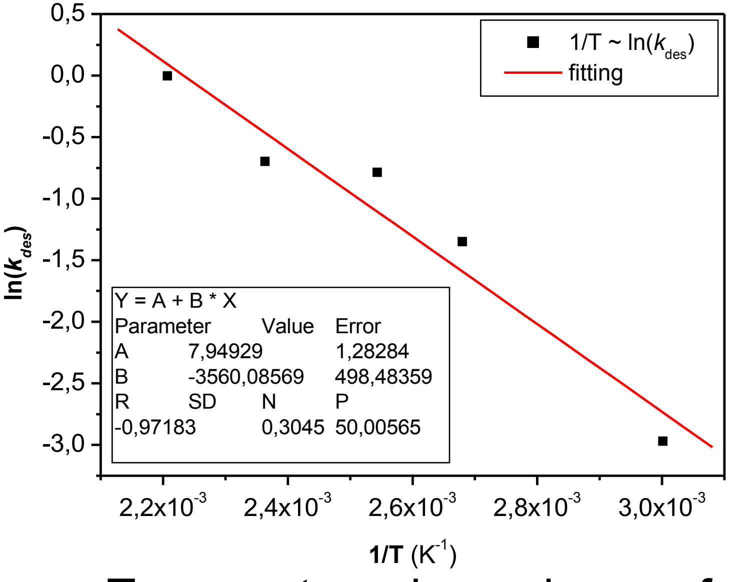


Arrhenius plot



Temperature influence on HCl desorption





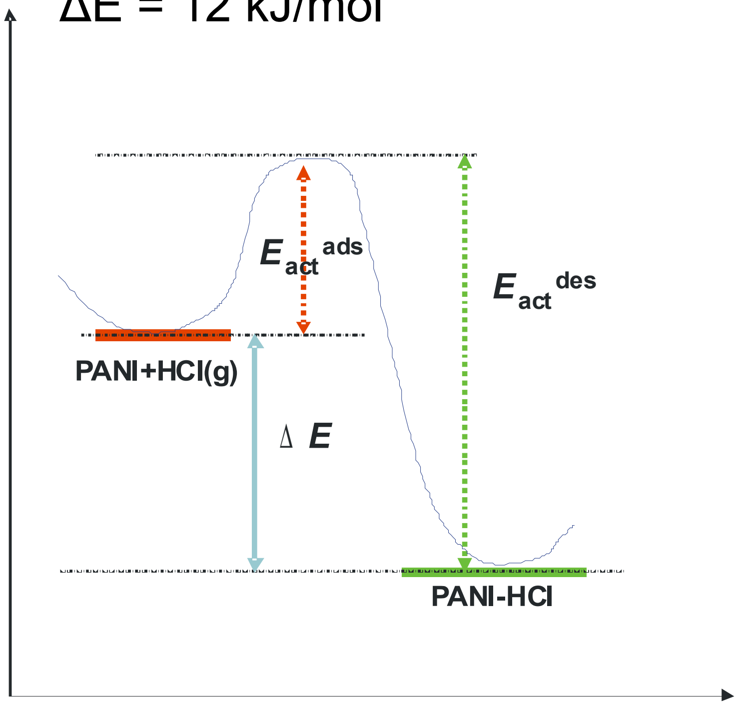
Temperature dependence of the desorption rate

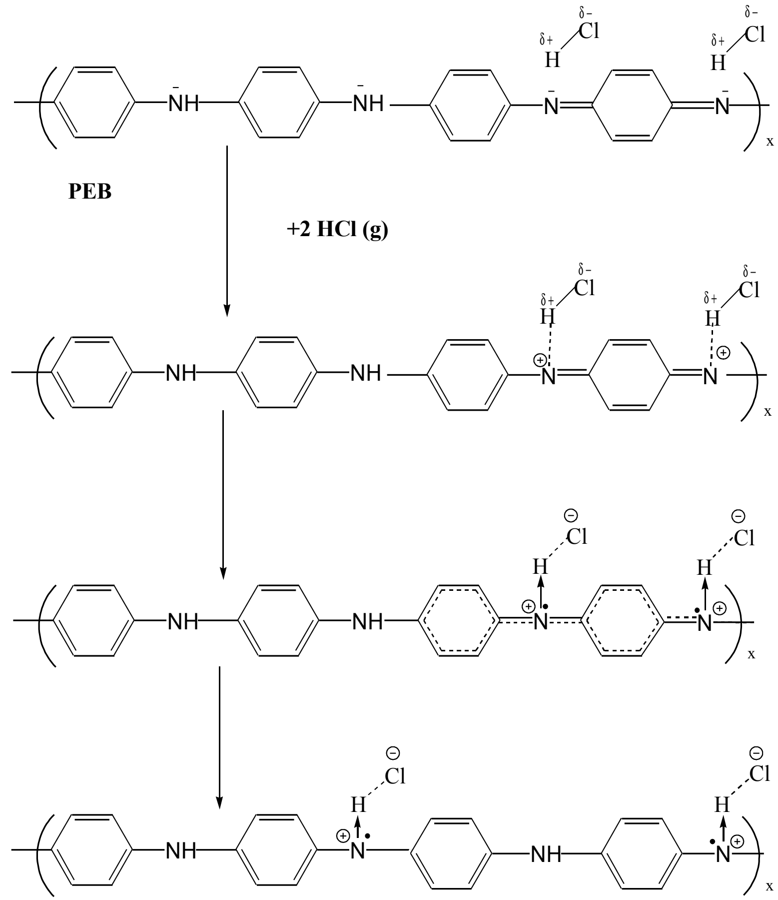
Adsorption and binding energies

$E_{act(ads)} \approx 18$ kJ/mol

$E_{act(des)} \approx 30$ kJ/mol

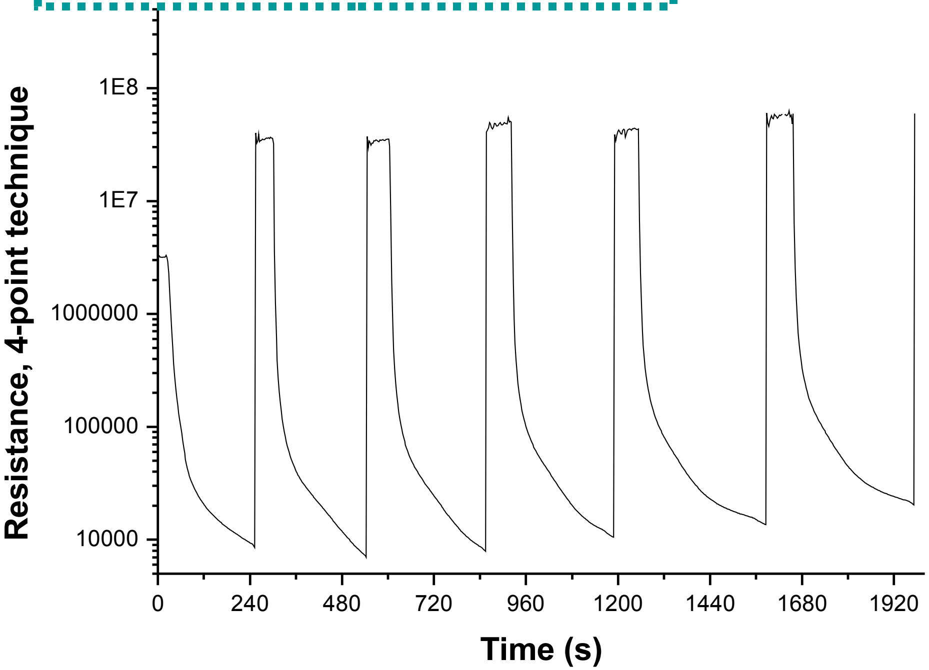
$\Delta E = 12$ kJ/mol





HCl-sensor

additions of 10 ppm HCl and subsequent regeneration 10 min at 150°



extremely high response (>1000 times)

=> very high sensitivity (detection limit < 0.1 ppm)

binding constant (calculated from kinetic curves):

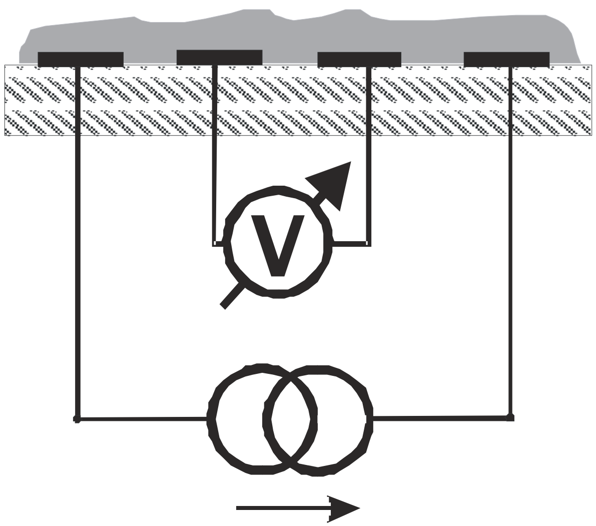
~ 1 / [60 ppm] (60°C)

=> linearity in the range 0 – 50 ppm

good reversibility
good reproducibility
response time < 30 s
life time > 1 year

Measurements of lateral resistance: 4-electrode configurations

“classical” configuration



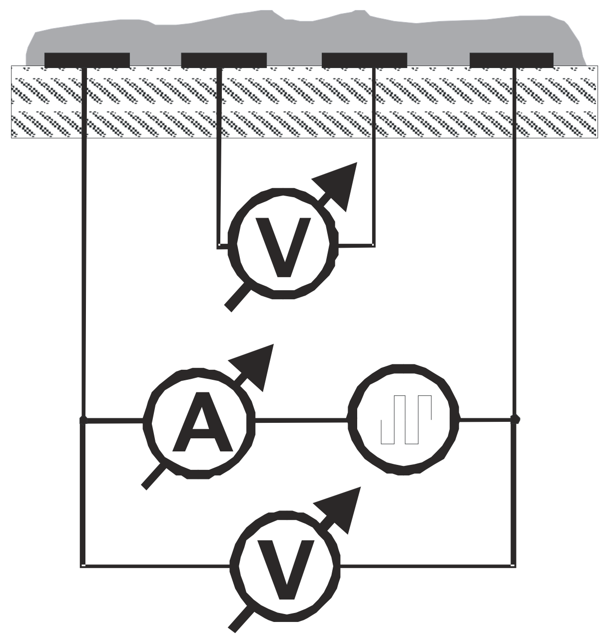
• characterization of bulk polymer resistance

• *but:*

• not information on contact resistance

• applied voltage should be minimized, better – AC voltage

simultaneous 2- and 4-electrode measurements

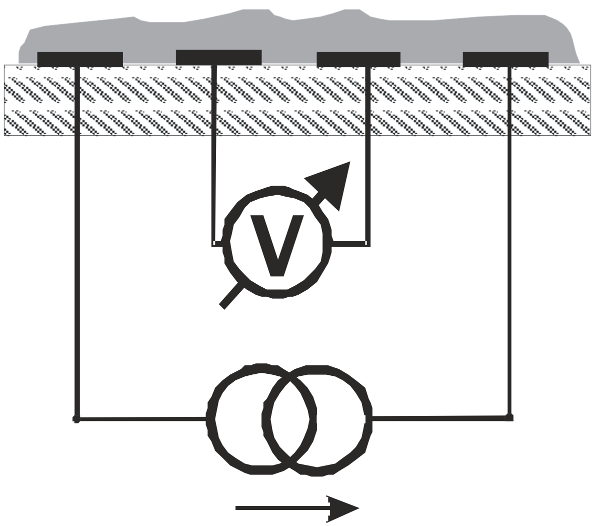


• characterization of bulk polymer resistance

• R2/R4=>characterization of the “contact quality”

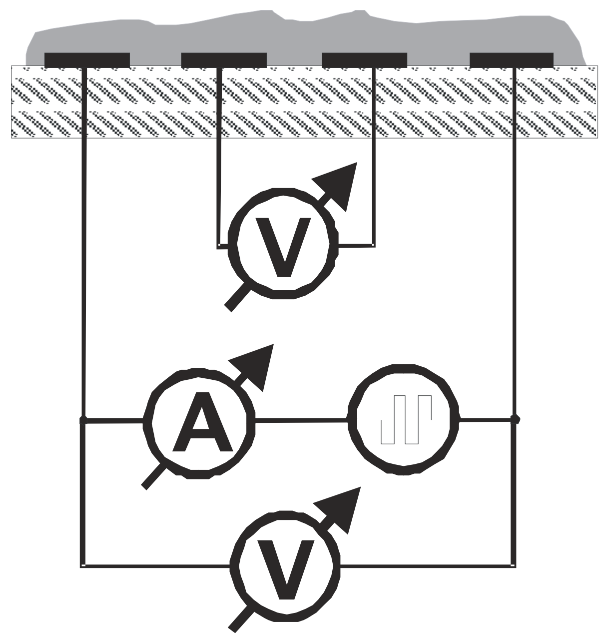
Problem: red/ox state of the polymer is not fixed!

“classical” configuration



- characterization of bulk polymer resistance
- *but:*
- not information on contact resistance
- applied voltage should be minimized, better – AC voltage

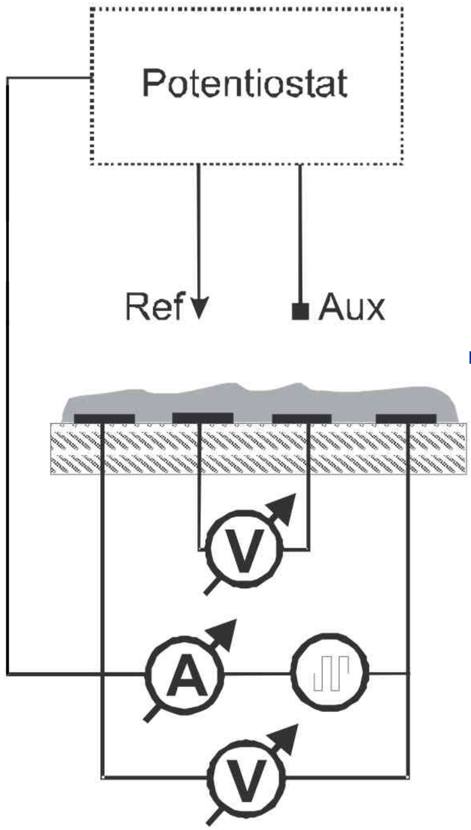
simultaneous 2- and 4-electrode measurements



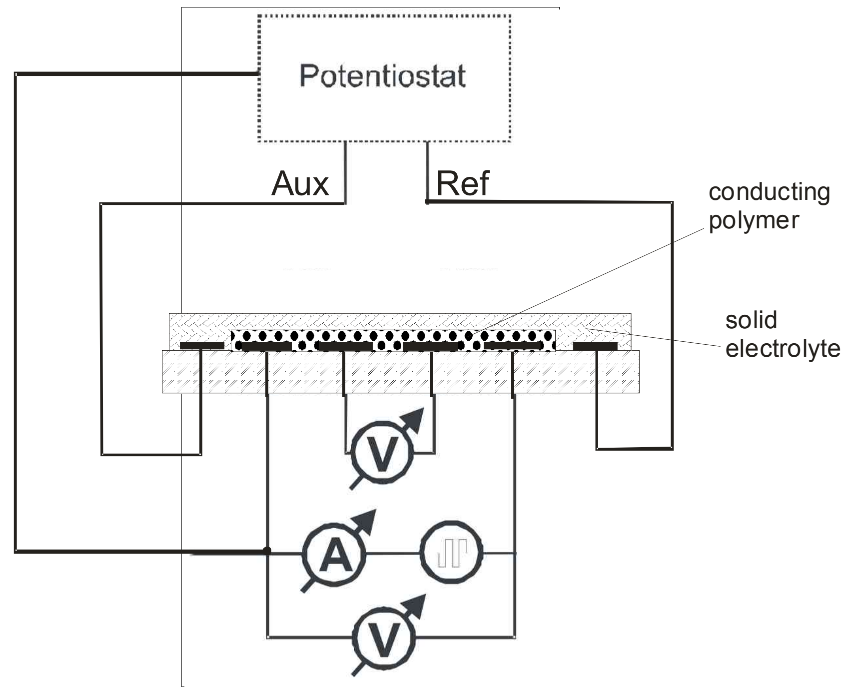
- characterization of bulk polymer resistance
- R2/R4=>characterization of the “contact quality”

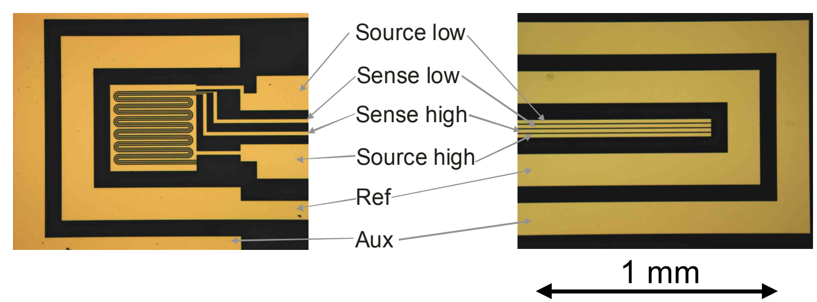
6-electrode configurations

in liquid electrolyte:

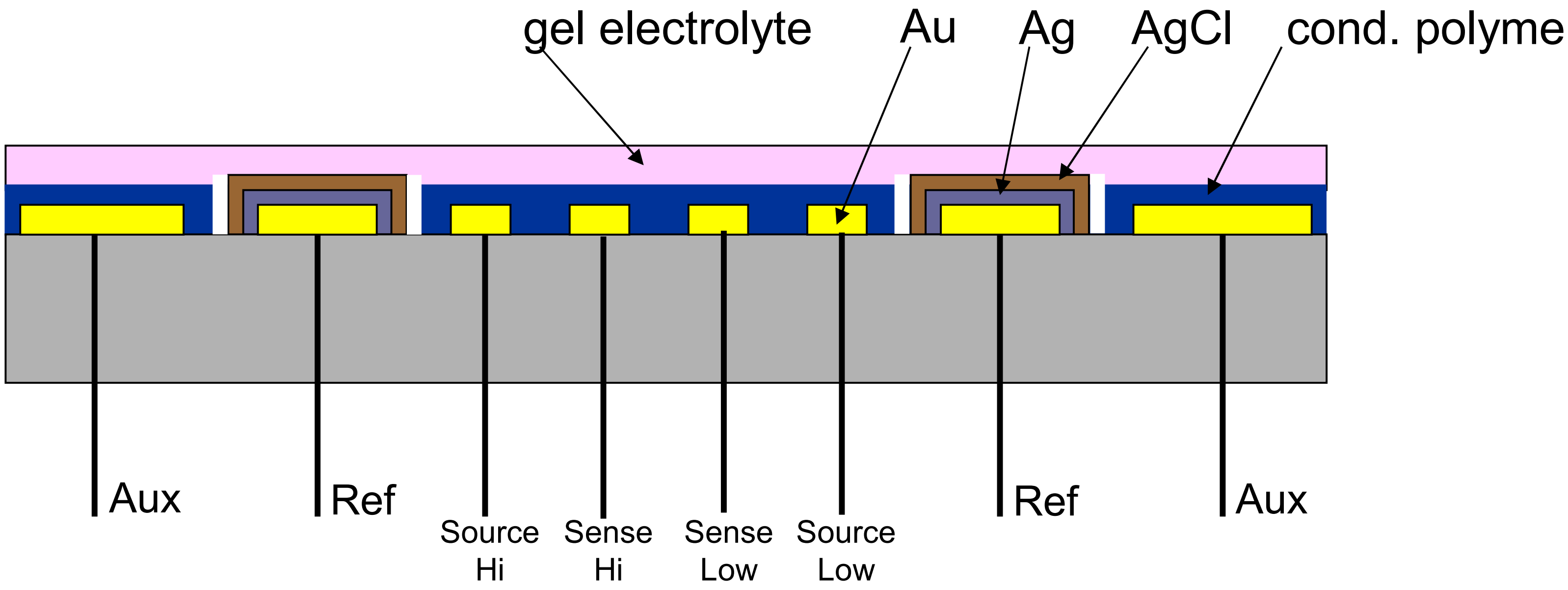


as solid-state chemosensor:

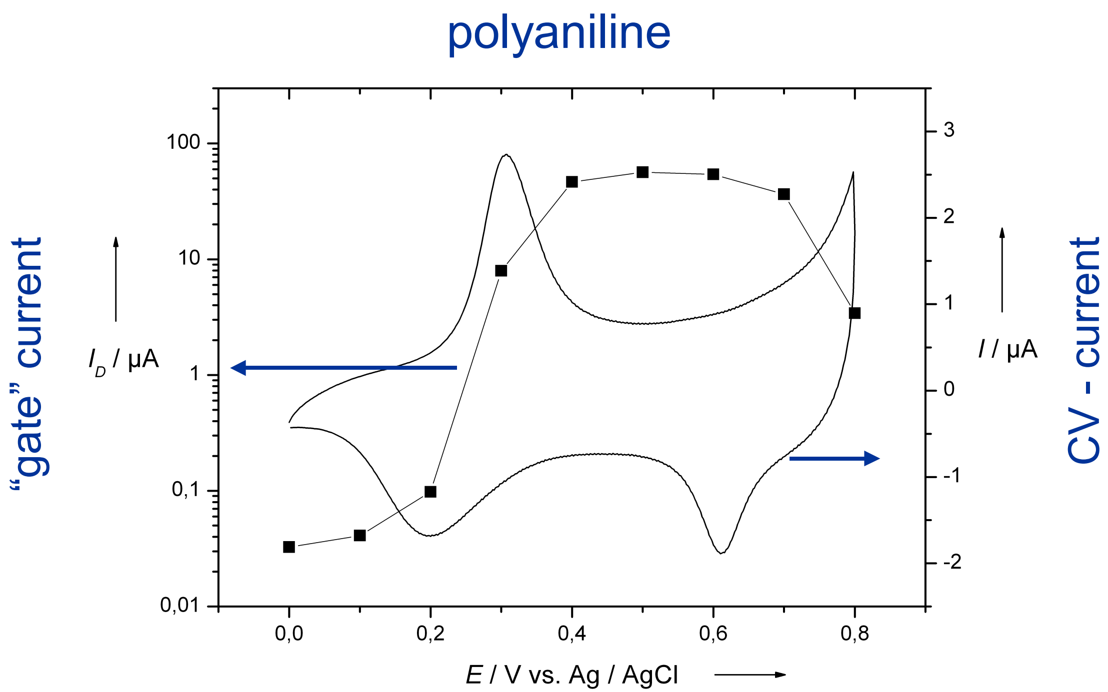




Solid state sensor for 6-point measurements (integrated electrochemical chemotransistor)

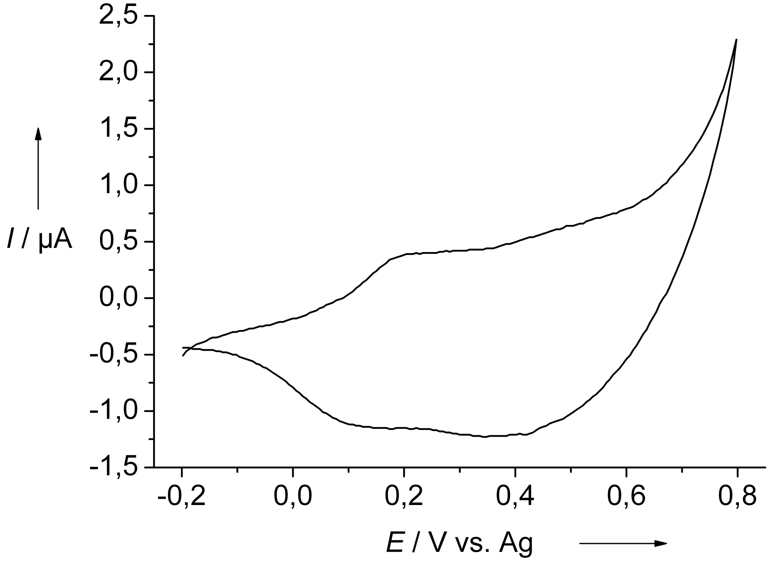


Test of 6-point solid state sensor: electrical control of the redox state of the conducting polymer



Note: no liquid phase!
The measurement was performed in air using 6-point configuration

Test of 6-point solid state sensor: electrical control of the redox state of the conducting polymer

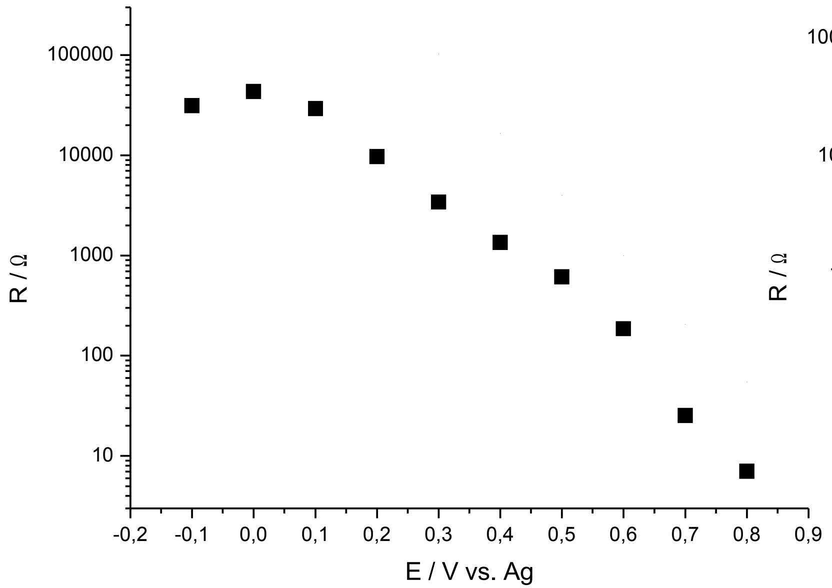


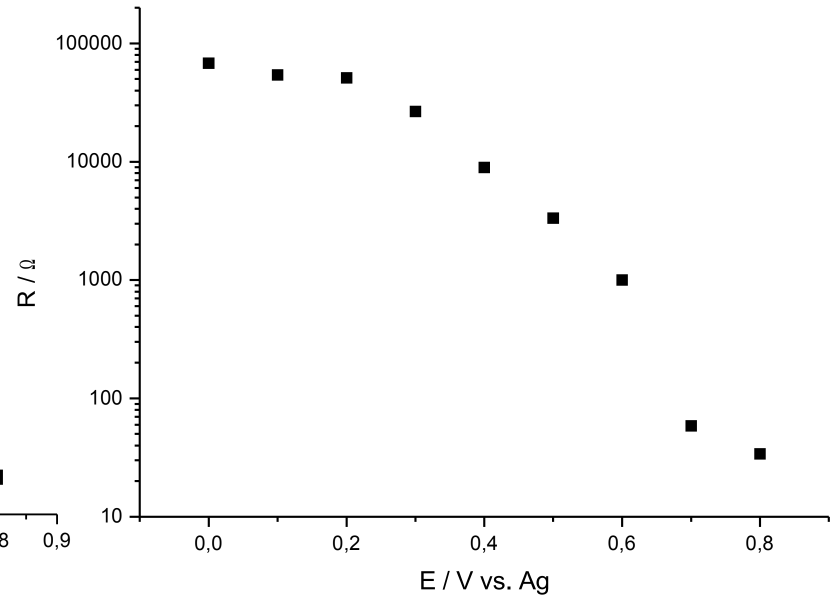
polythiophene

Note: no liquid phase!
The measurement was performed in air using 6-point configuration

Test of 6-point solid state sensor: electrical control of the redox state of the conducting polymer

polythiophene



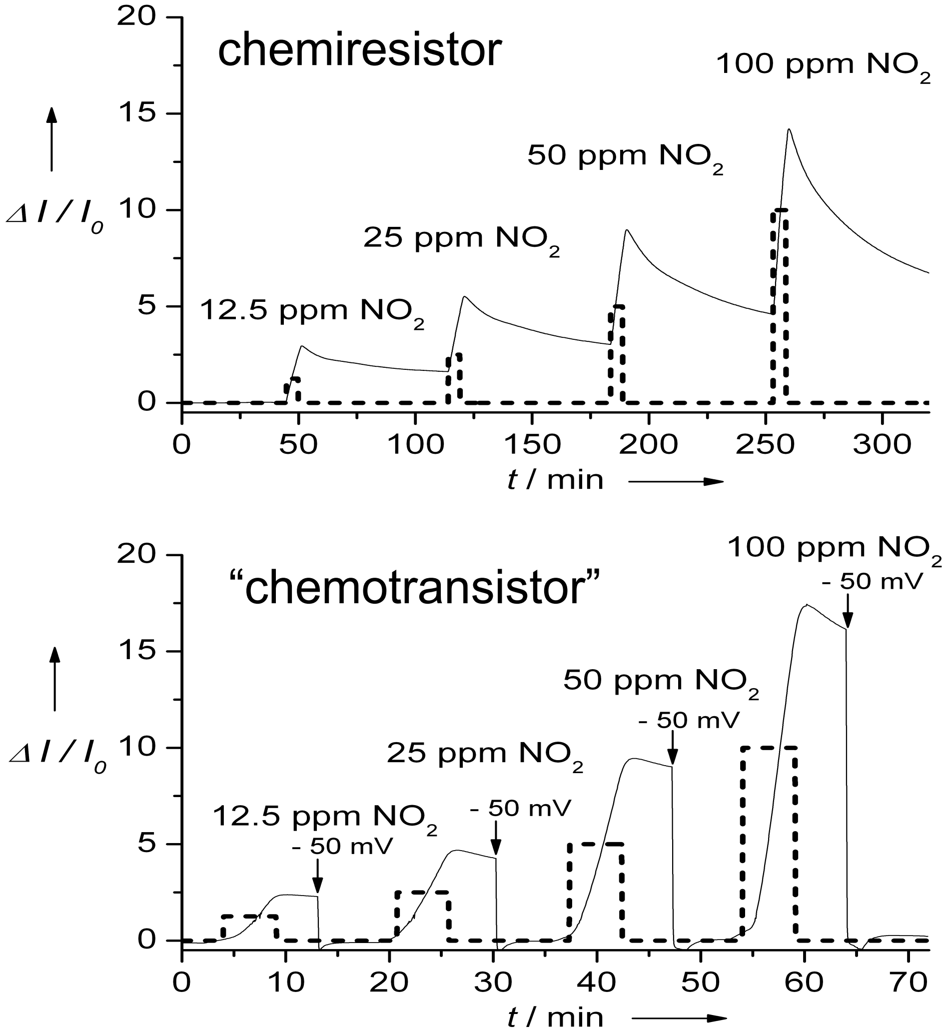


The measurement was performed:

in air using 6-point configuration

in aqueous solution

Solid state sensor for 6-point measurements



- NO_2 oxidizes the polymer
- Reduction process on air quite slow
- Regeneration of the sensor by applying a short reduction pulse

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- German ministry for research and education
- European commission (project RASP)
- Industry

Thank you
for attention!

Equipment:

SPR (surface plasmon resonance) and SPR-imaging

Electrochemistry (incl. impedance spectroscopy and 8-channel capacitance monitoring)

FTIR (also IRRAS for monolayers)

Dynamic Light Scattering (size, ζ-potential)

Contact angle / Oil-drop tensiometer

Set-up for conducting polymers

Set-up for combinatorial electropolymerization and high-throughput screening of conducting polymers

Set-up for planar lipid bilayers

Laser ablation system

Microspotter (“Nanoplotter”)