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INSTITUUT VOOR MATERIAALONDERZOEK

20 Years Materials for the Future

IMO - IMOMEC

Impedimetric biosensors: from point mutations in DNA to histamine in tuna brine

Technische Universität Dresden, May 19, 2011

Biosensors @ Hasselt University

IMO-MAF
BIOSensors, 2001
Patrick Wagner

BIOMED
Physiology, Genetics, Immunology
Luc Michiels
Marcel Ameloot

IMO-MAF
Wide Bandgap Materials
Ken Haenen
Milos Nesladek

IMO-Chem
Organic & Bio-Polymeric Chemistry, 2003
Thomas Cleij

Maastricht University & Academic Hospital
NUTRIM, CARIM
Freddy Troost
Mat Daemen

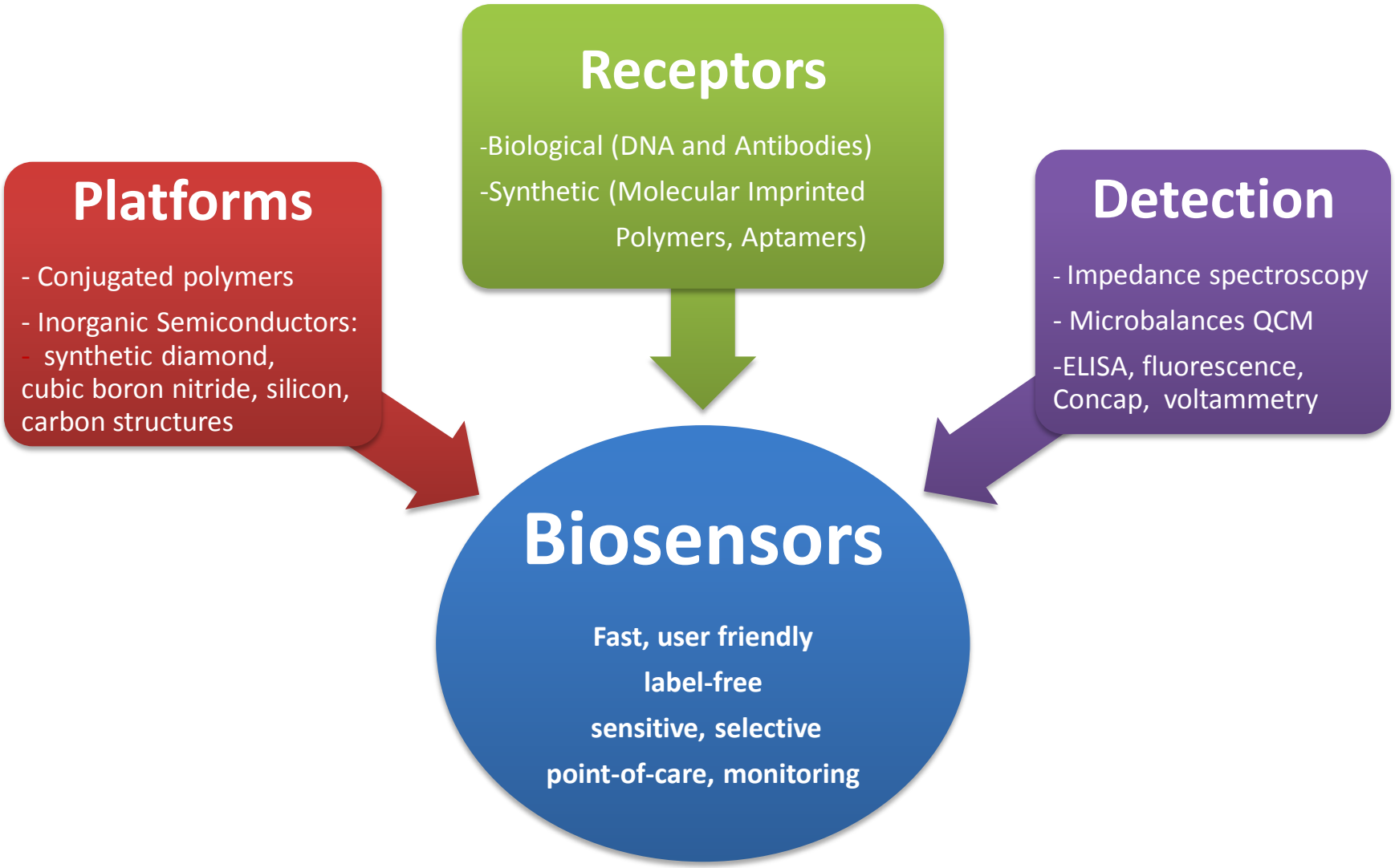
IMO-MAF
Electronic & Physical Characterization
Jan D'Haen
Ward De Ceuninck

IMO-Chem
Biochemistry, 2009
Wanda Guedens
Peter Adriaensens

XIOS
Electronical Engineering, 2009
Ronald Thoelen

IMO-MAF
Nanostructure Physics
Hans-Gerd Boyen

Sensor ingredients



Immuno-sensors for proteins

Small-molecule detection by MIP's

DNA-sensors for genetic tests

Enzymatic biosensors

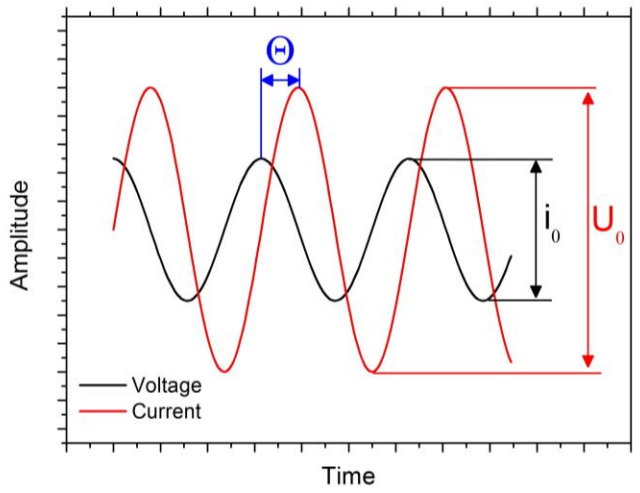
Immuno-sensors for proteins

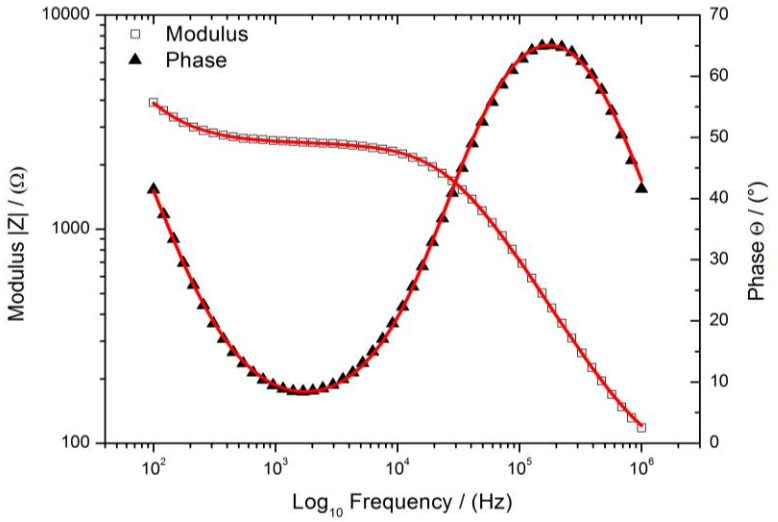
Small-molecule detection by MIP's

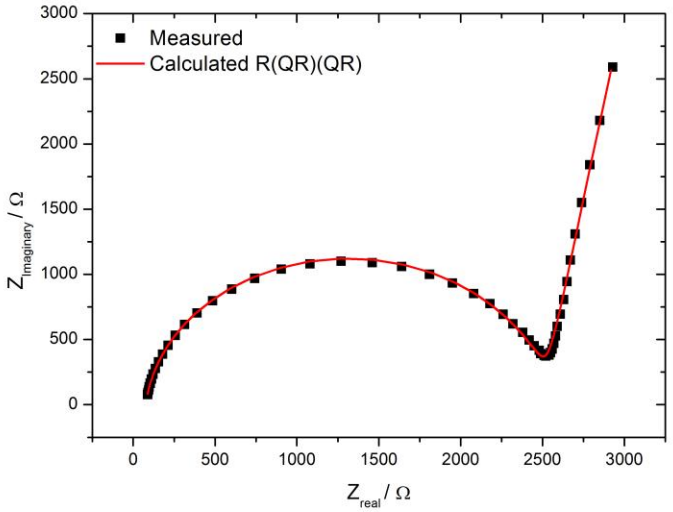
Enzymatic biosensors

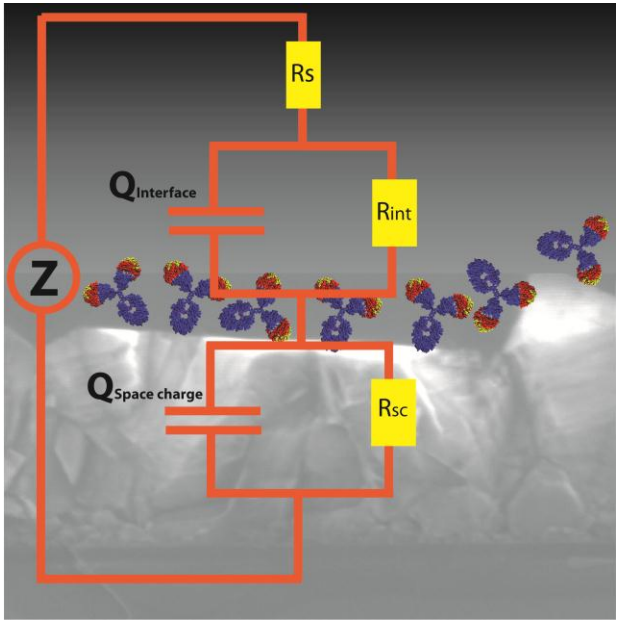
DNA-sensors for genetic tests

Impedance spectroscopy









Impedance analyzers

HP

100 Hz – 15 MHz
1 Channel

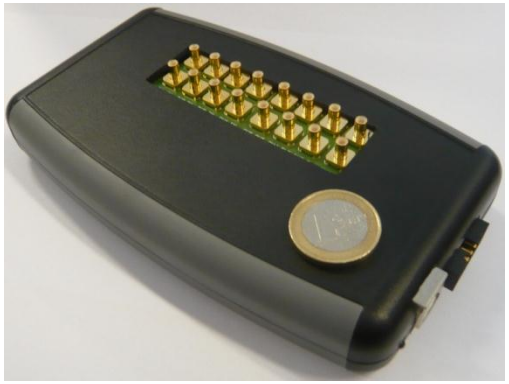
Iviumstat

10 µHz – 1 MHz
1 Channel

Bart

100 Hz – 100 kHz
8 Channels
5.7 sec / sweep

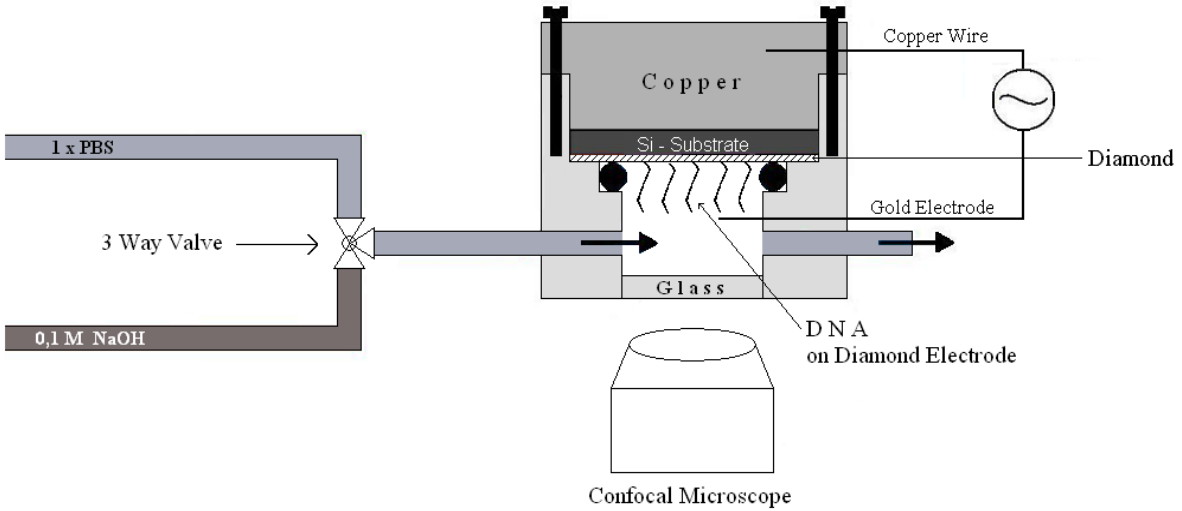
Jeroen



10 Hz – 100 kHz
8 Channels
9 sec / sweep

Part 1: Electronic sensors for DNA

DNA sensors



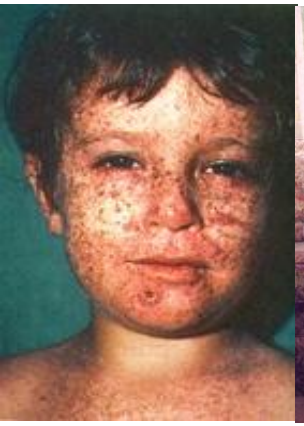
Single nucleotide polymorphisms (SNPs)

Hereditary diseases (phenylketonuria, breast cancers, mucoviscidosis,……397 others)

Key element in ´Theranostics`

Application examples

SNP mutations correlate with certain diseases…..

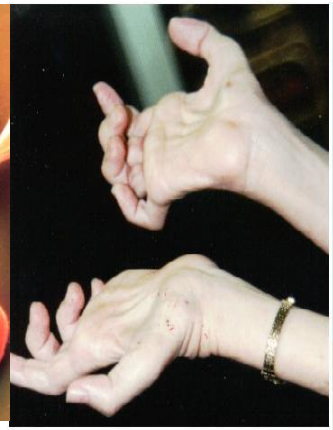


Xeroderma Pigmentosum

Cystic Fibrosis

Breast Cancer

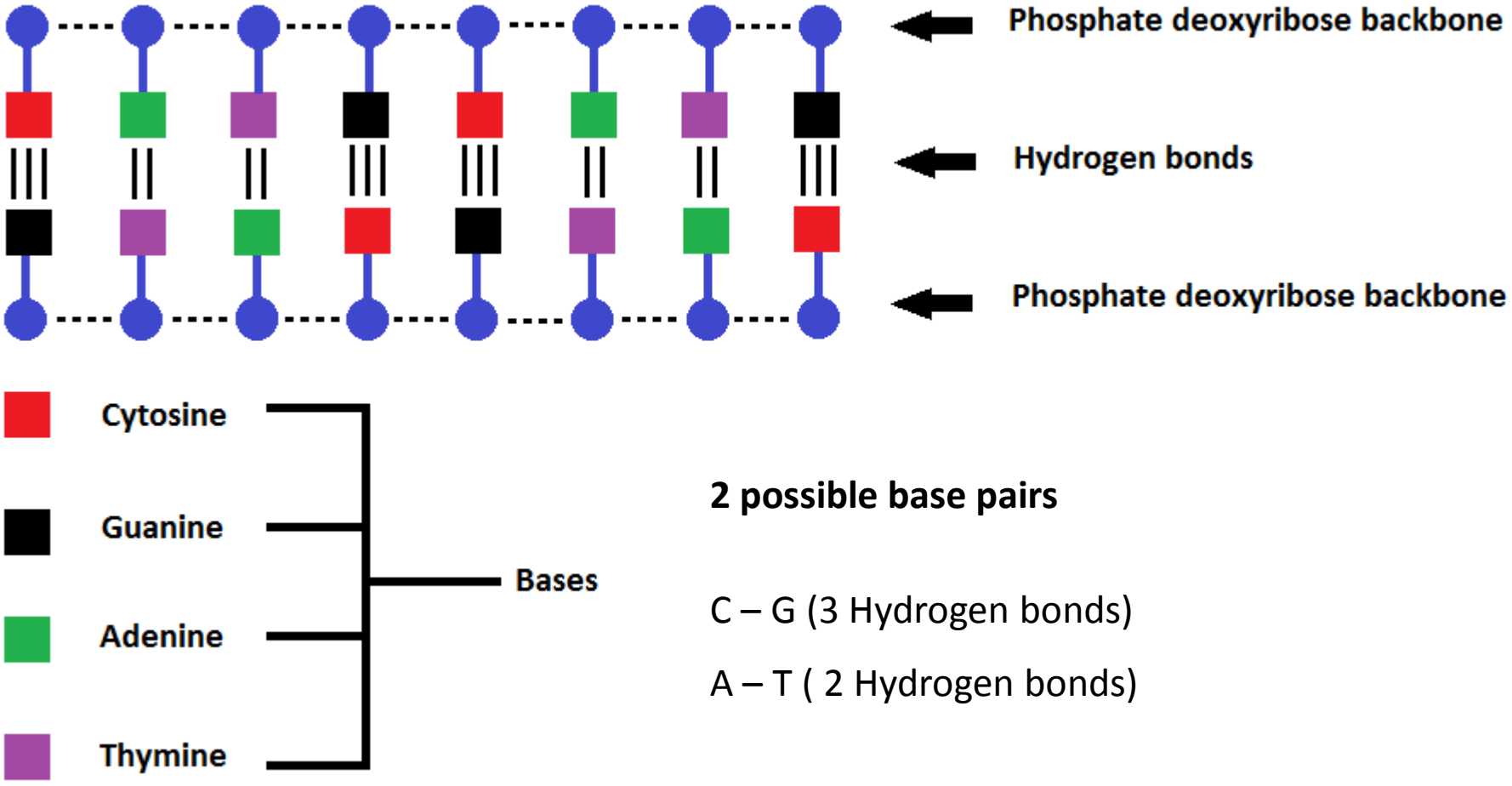
Sickle-cell Anemia



Charcot-Marie Tooth

Alzheimer

DNA – base pair coupling

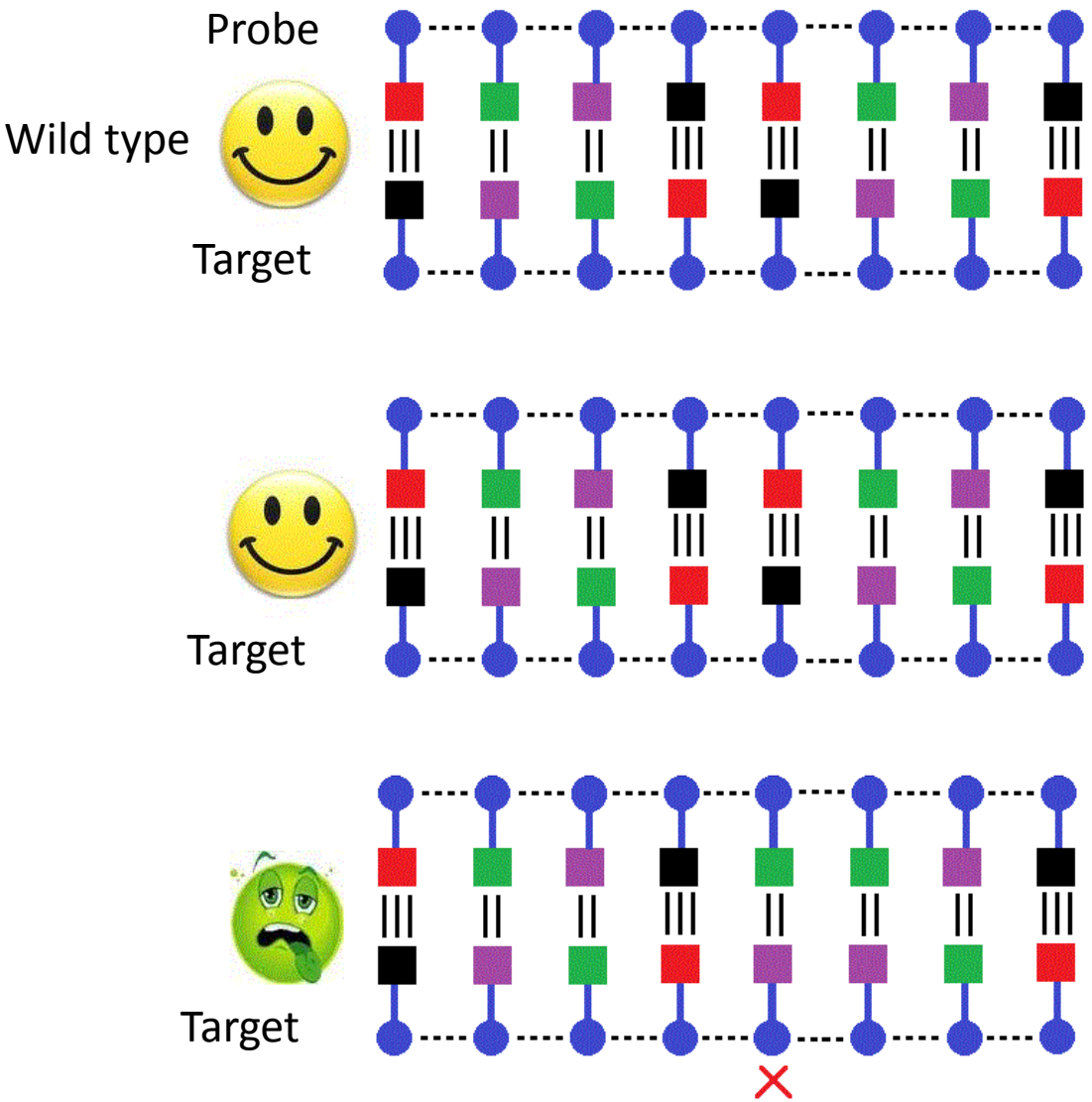


2 possible base pairs

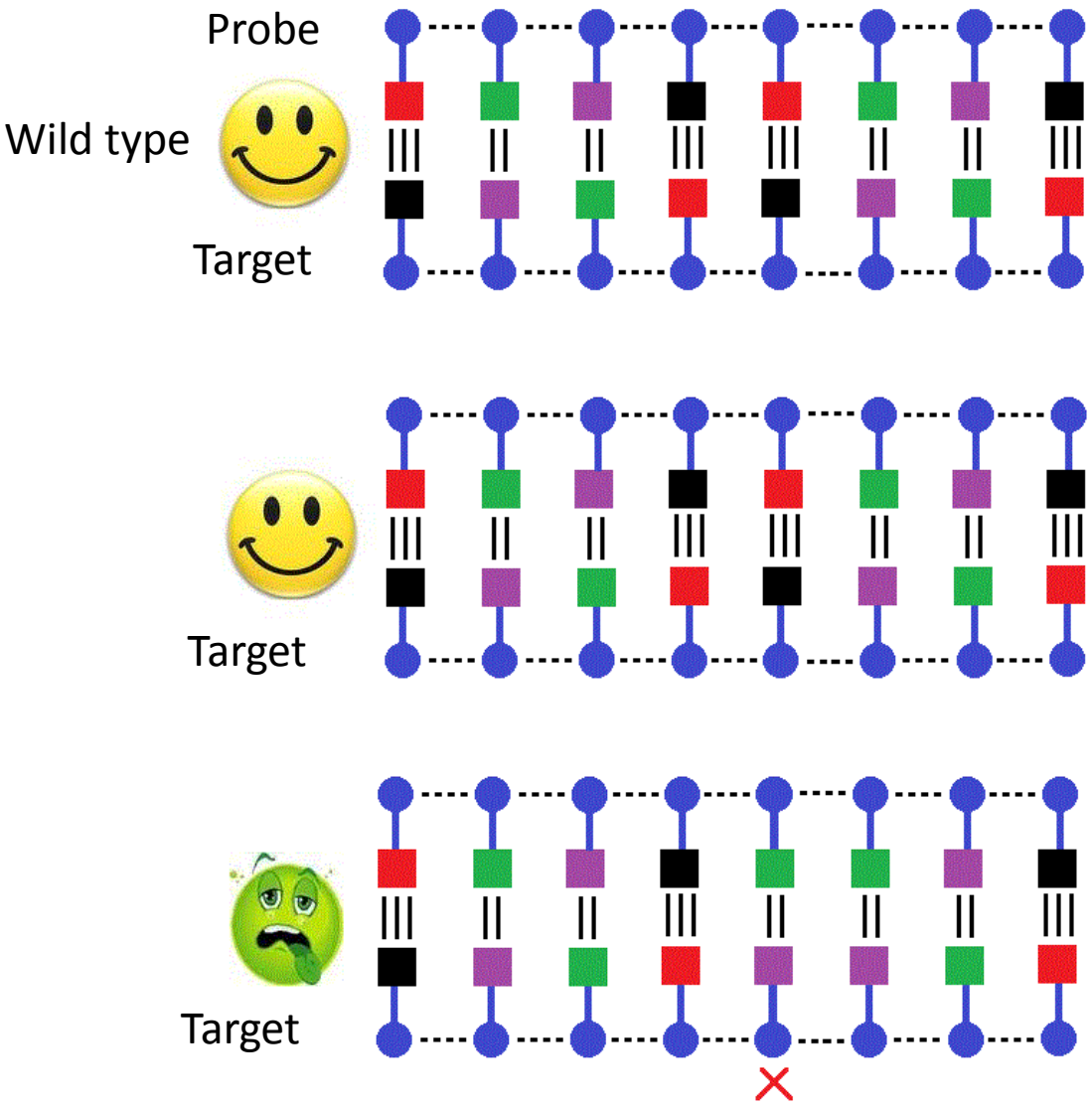
C – G (3 Hydrogen bonds)

A – T (2 Hydrogen bonds)

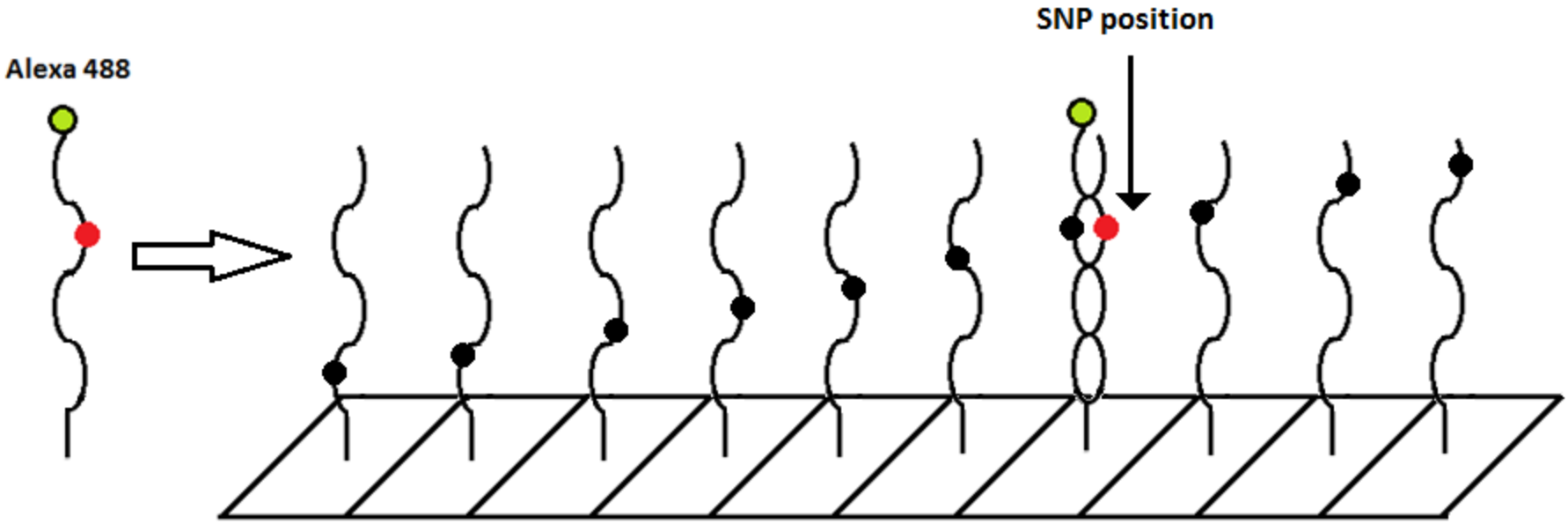
DNA Mutation



DNA Mutation



Finding SNPs with microarrays

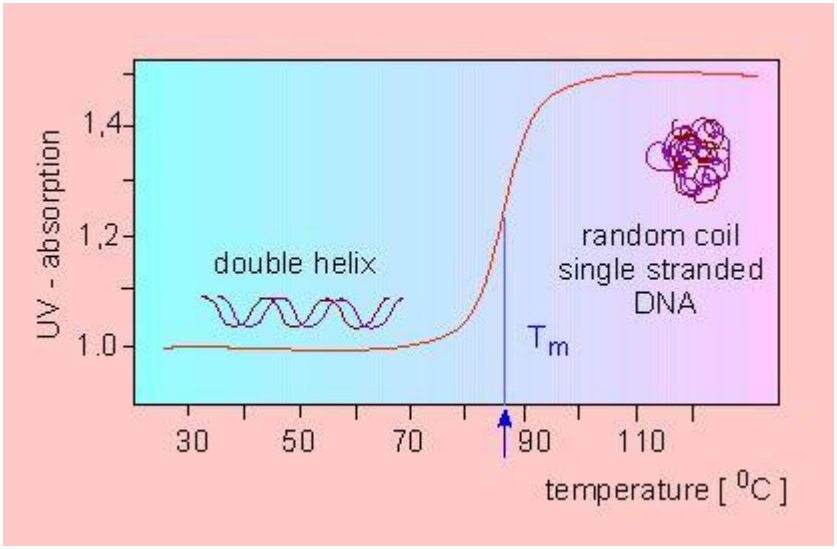


Schematic !

- Hybridisation at high temperature ≈ 80°C
- Long hybridisation time of 16 hours
- Only thermodynamically most-stable = complementary duplex forms

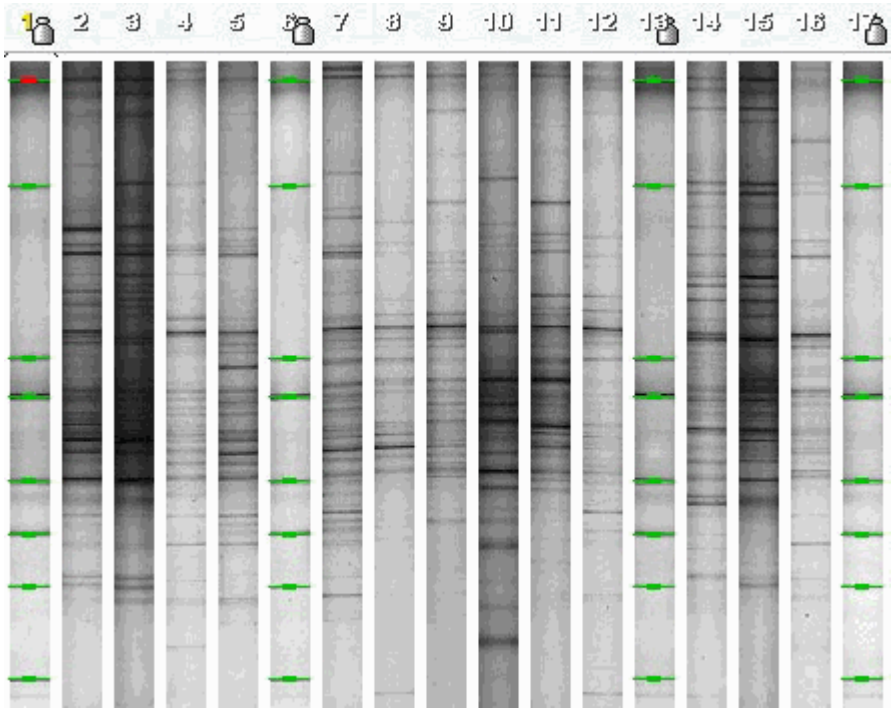
Denaturation–based SNP -detection

Denaturation by thermal melting



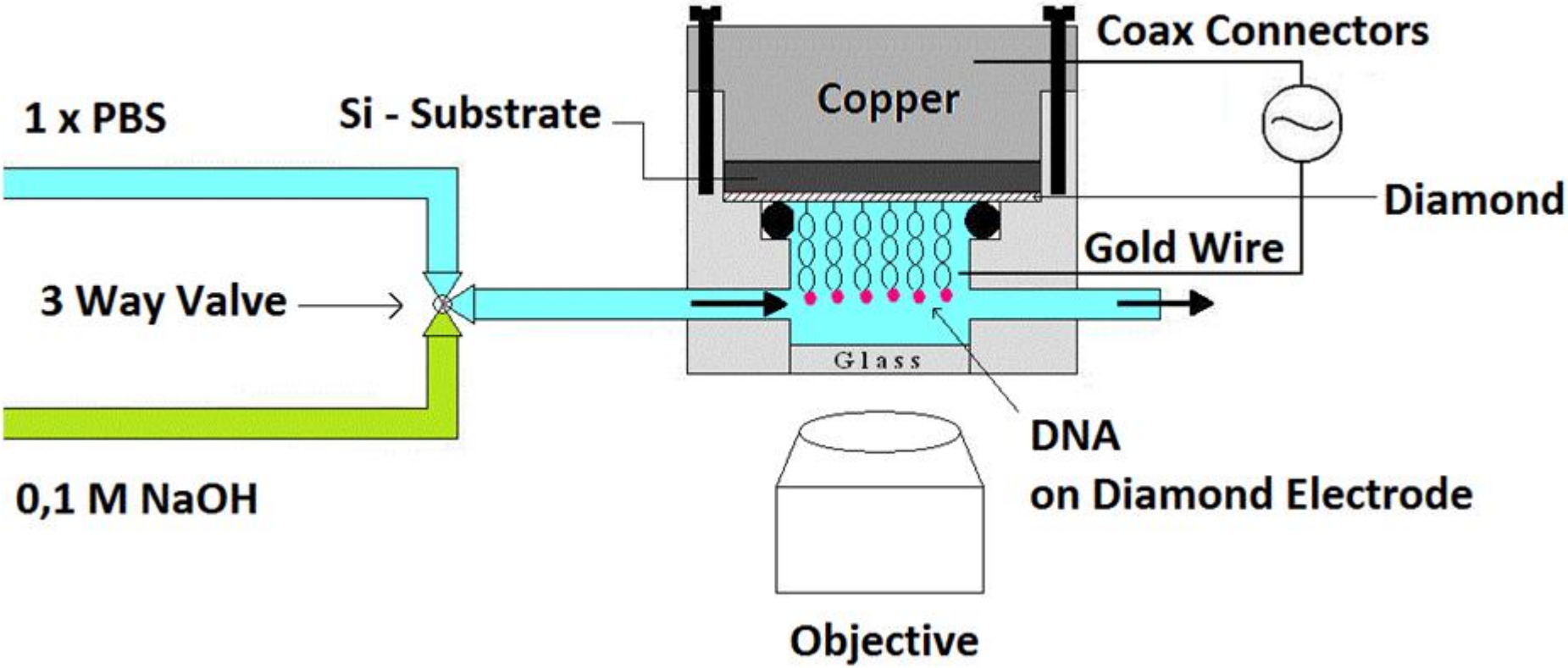
Stronger UV absorption in SS-DNA

Denaturing gradient gel electrophoresis (DGGE)

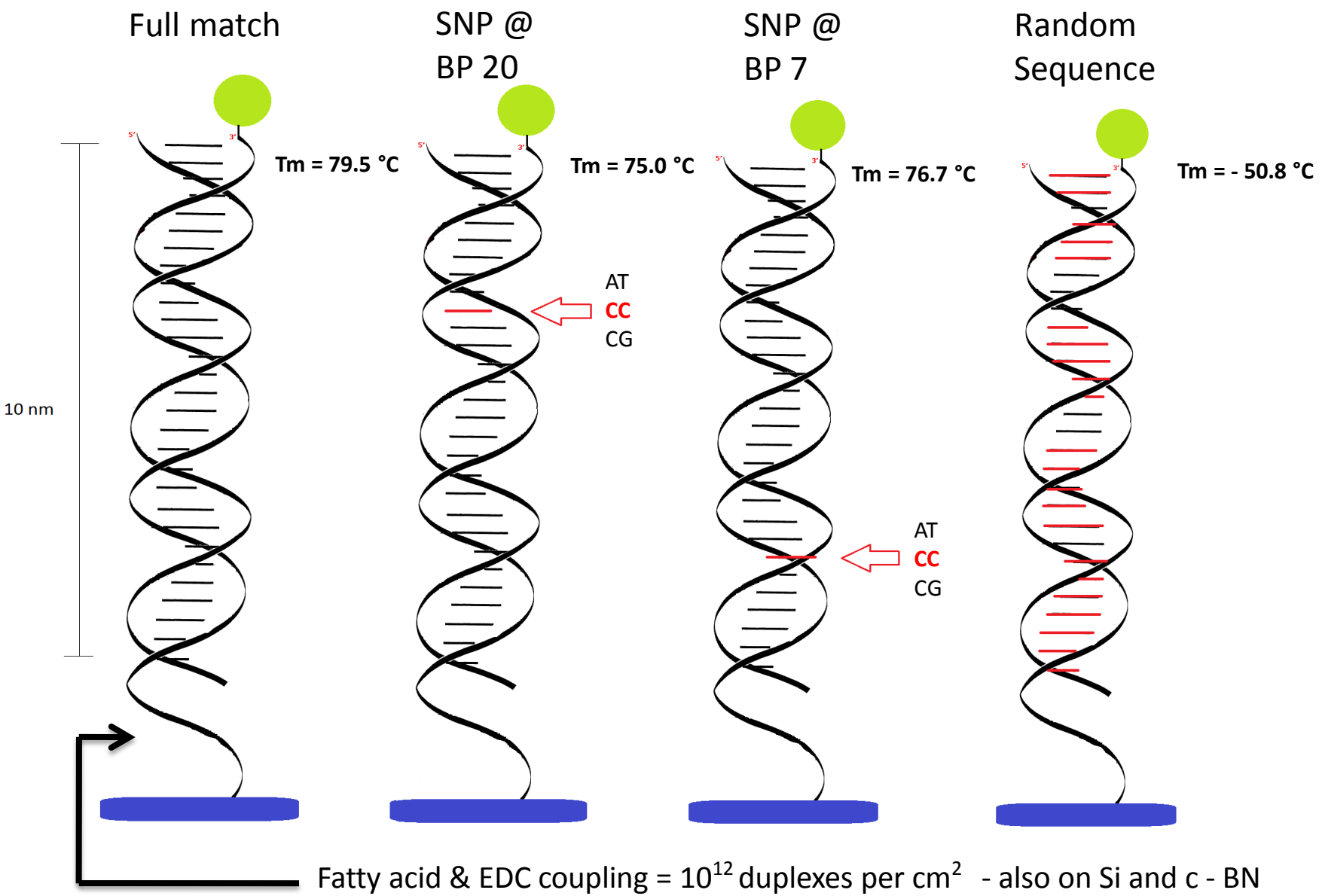


- Established, but time consuming: hours to 2 days
- Hard to integrate in high throughput analysis

Chemical denaturation at room temperature



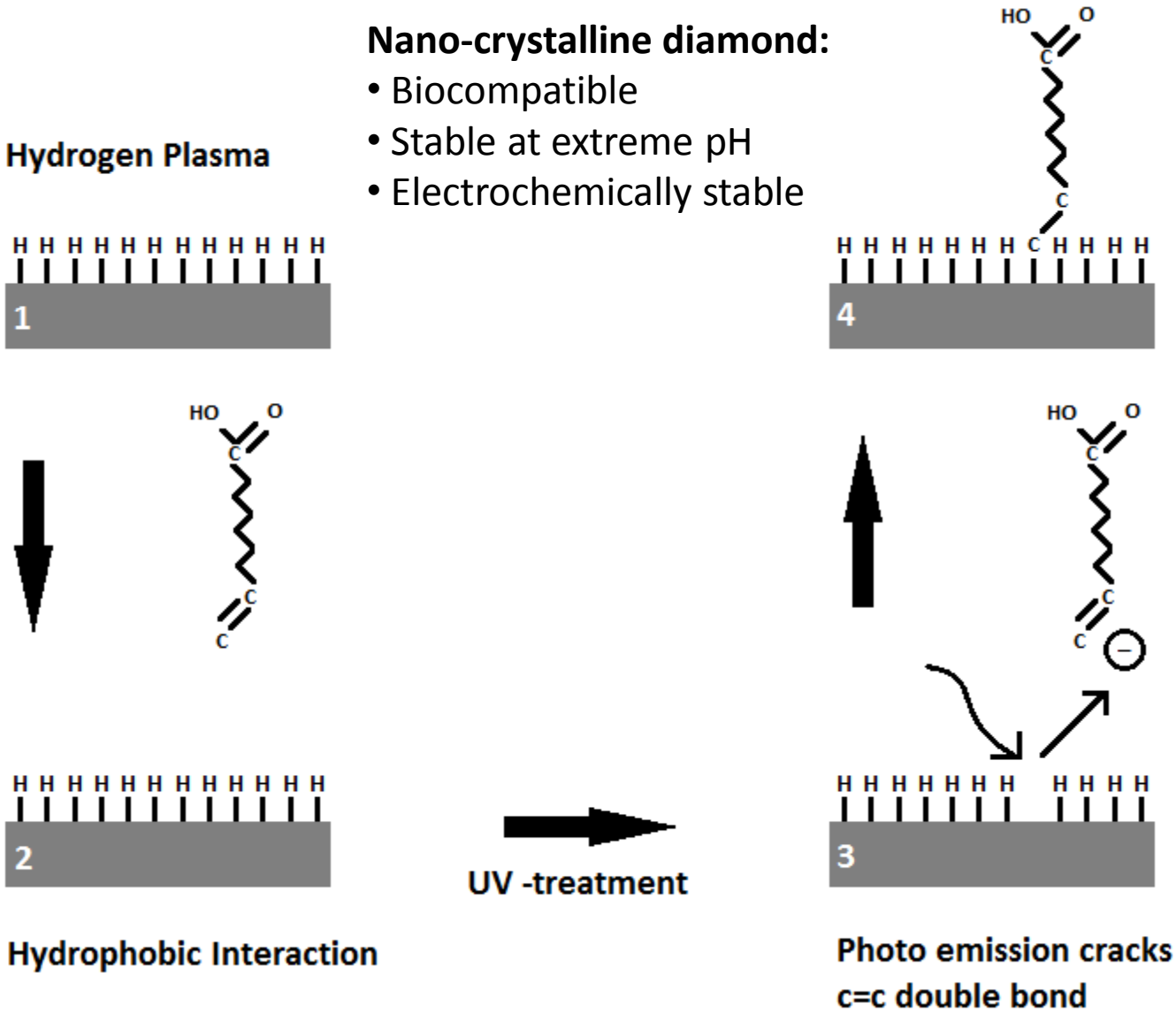
Test panel of DNA duplexes: 29-mers



Photo–coupling of fatty-acid crosslinker

Nano-crystalline diamond:

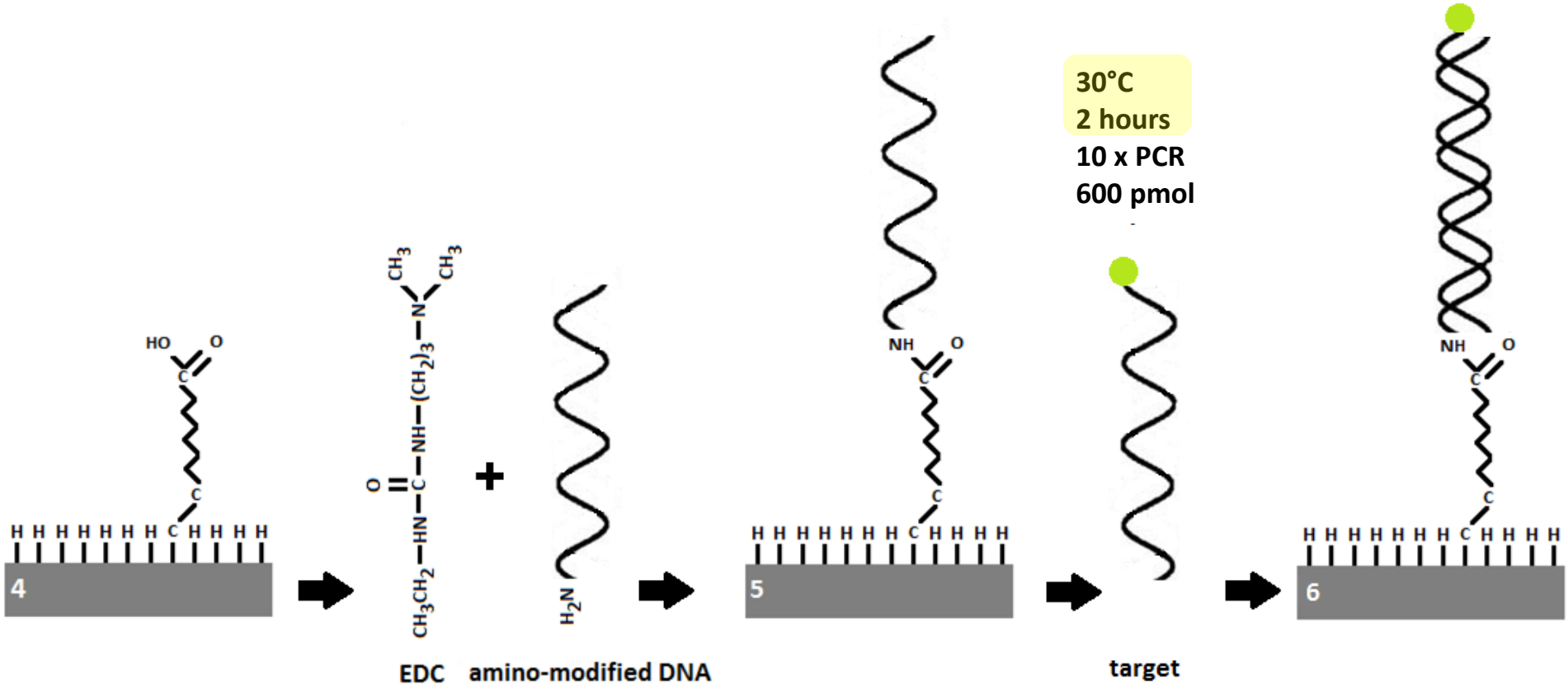
- Biocompatible
- Stable at extreme pH
- Electrochemically stable



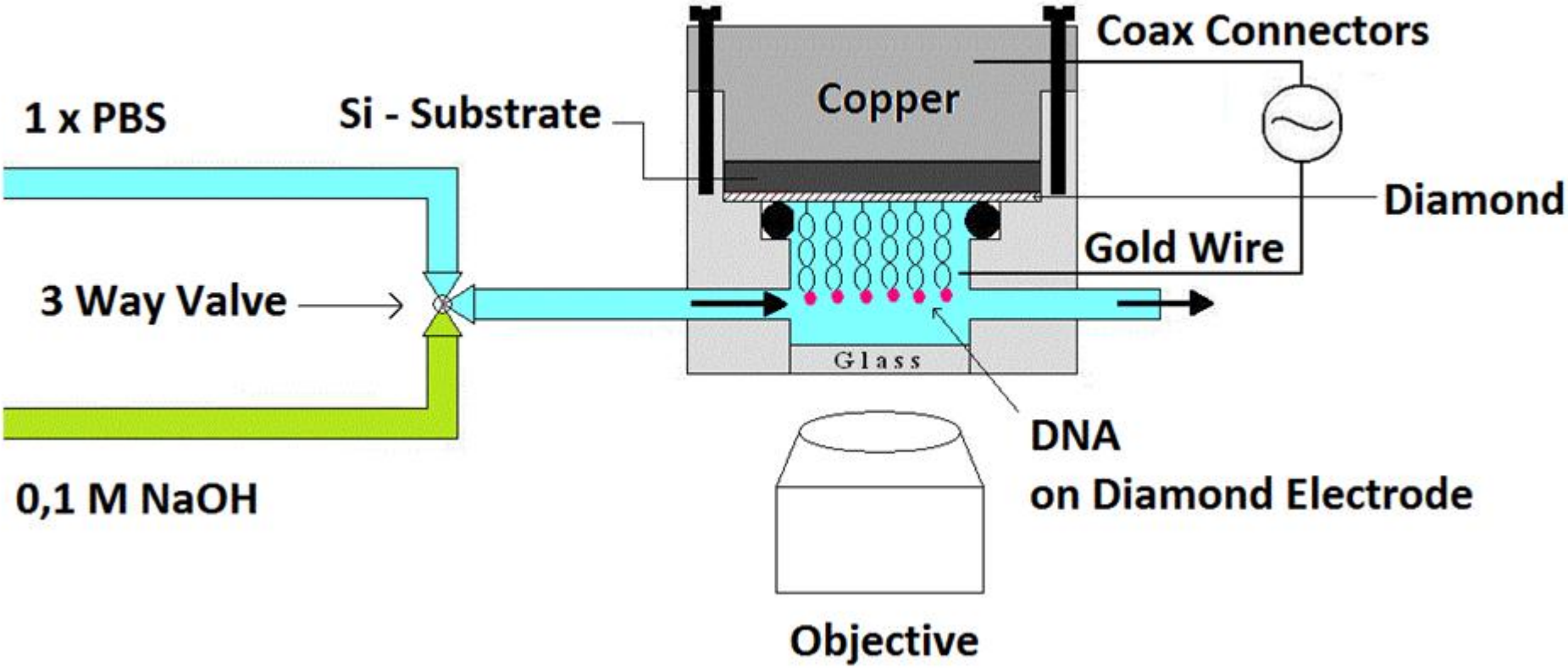
P. Christiaens et al., Biosens. Bioelectron., 2006, 22, 170–177.

X. Y. Wang et al., JACS 2010, 132, 4048–4049.

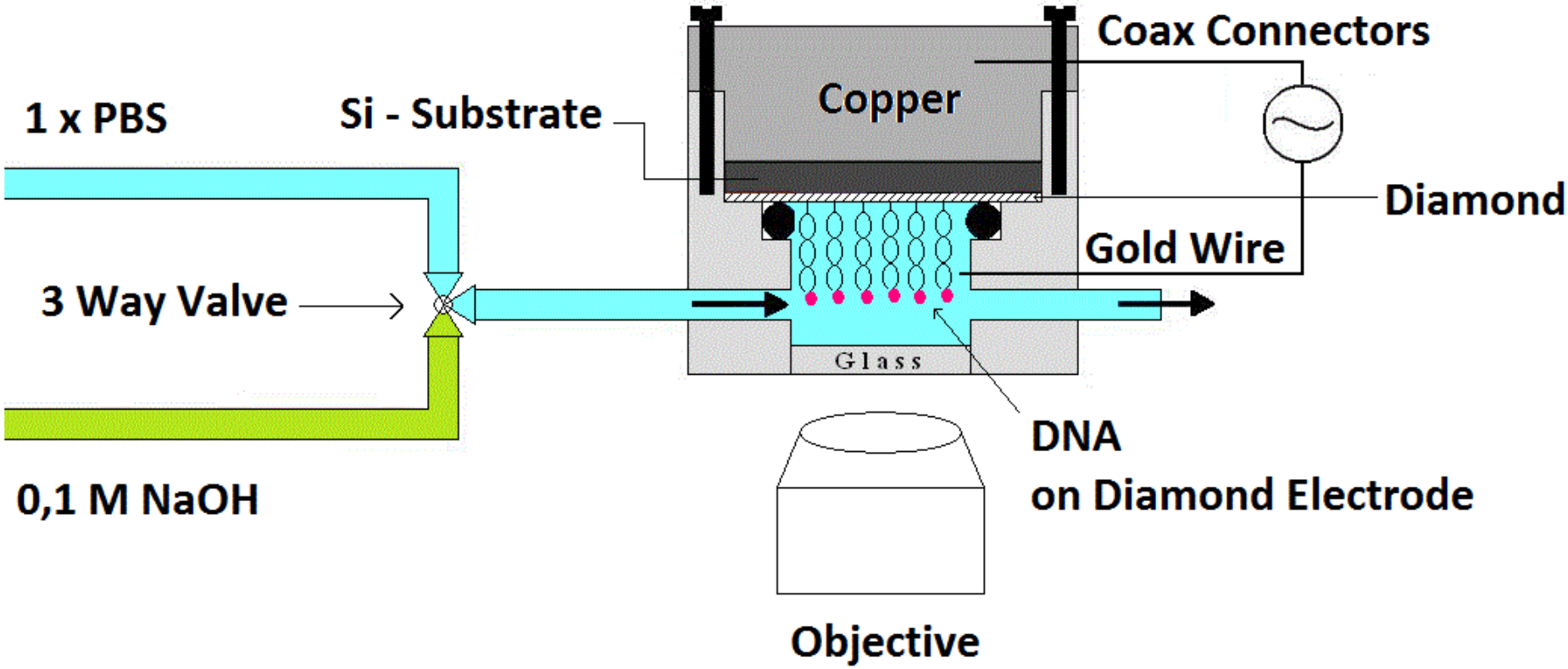
DNA attachment by EDC reaction



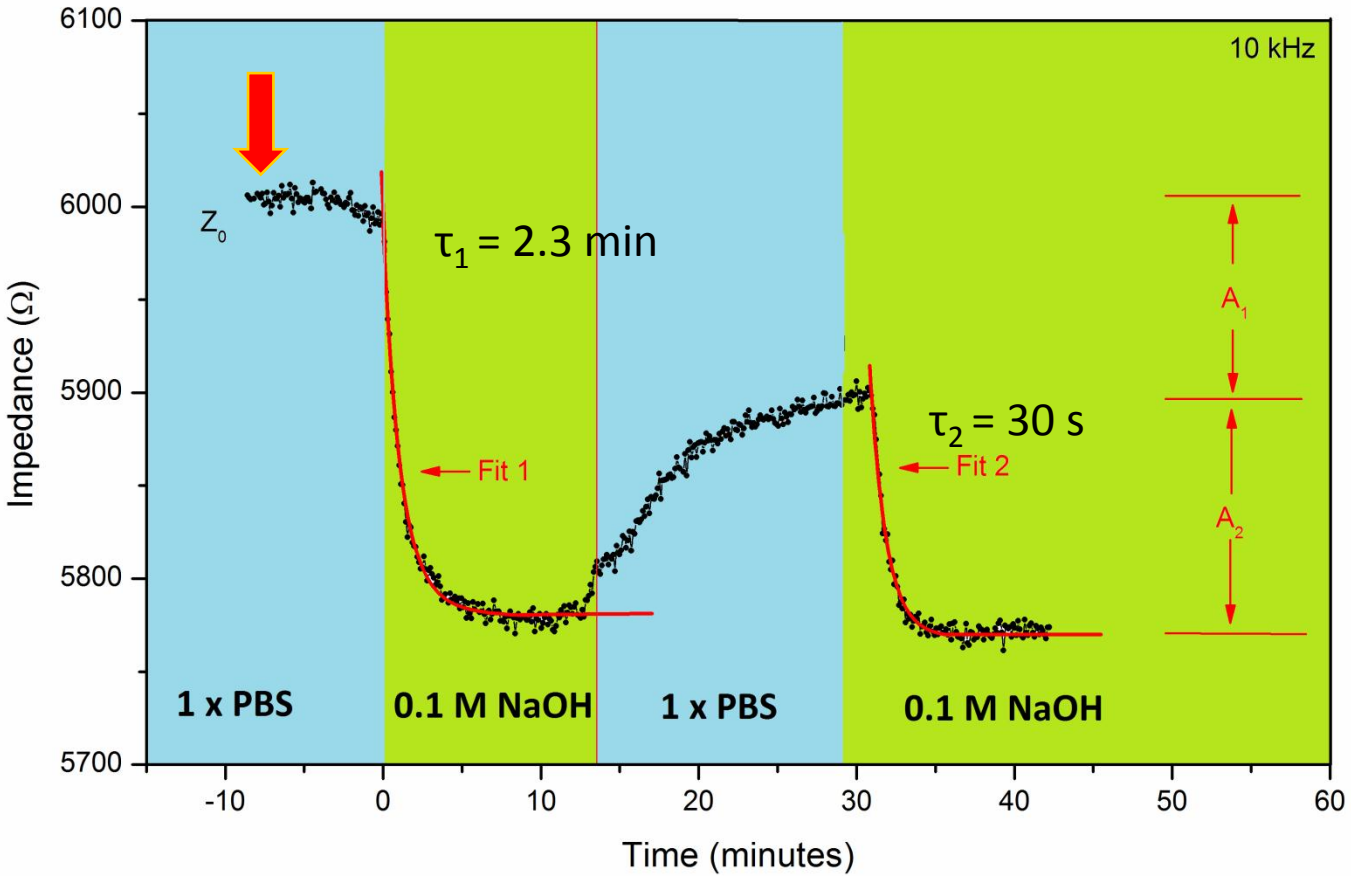
Impedance monitoring & double rinsing

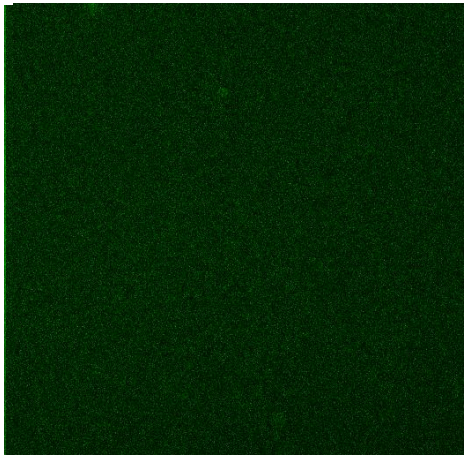


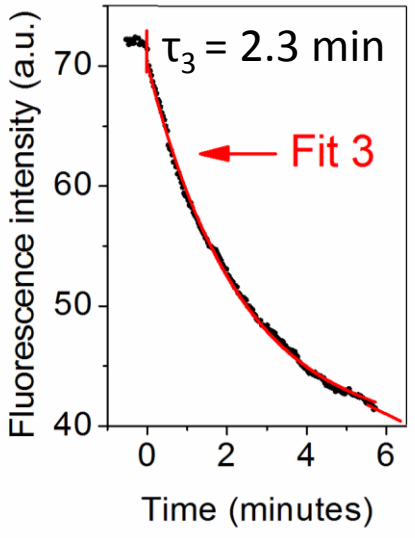
Impedance monitoring & double rinsing



Denaturation monitoring with double rinsing





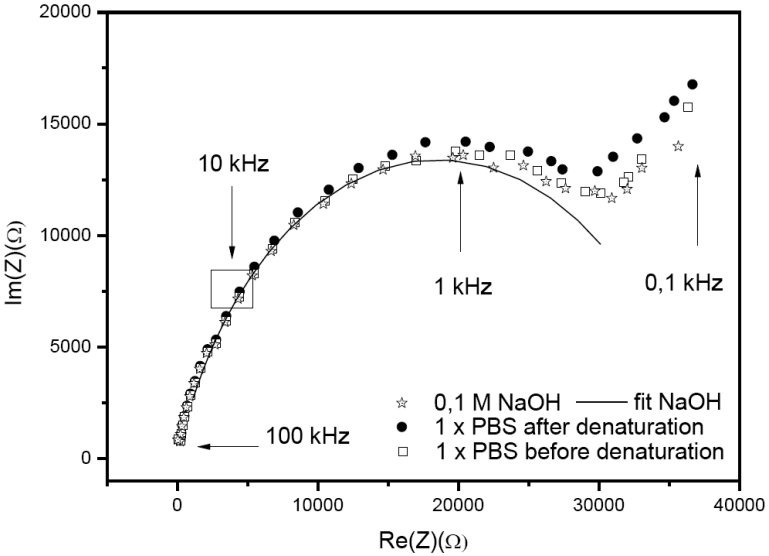


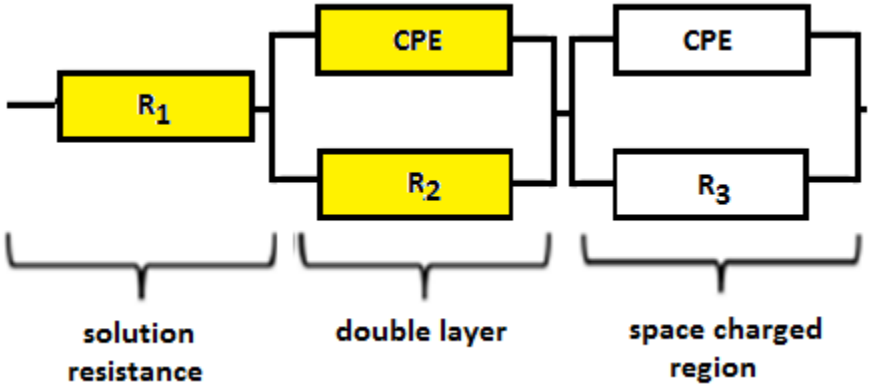
$$Z(t) = Z(t=\infty) + A_1 \exp\left\{-\frac{t}{\tau_1}\right\} + A_2 \exp\left\{-\frac{t}{\tau_2}\right\}$$

$$Z(t) = Z(t=\infty) + A_2 \exp\left\{-\frac{t}{\tau_2}\right\}$$

$$I(t) = I_0 + I_{DNA} \exp\left\{-\frac{t}{\tau_3}\right\}$$

Equivalent circuitry

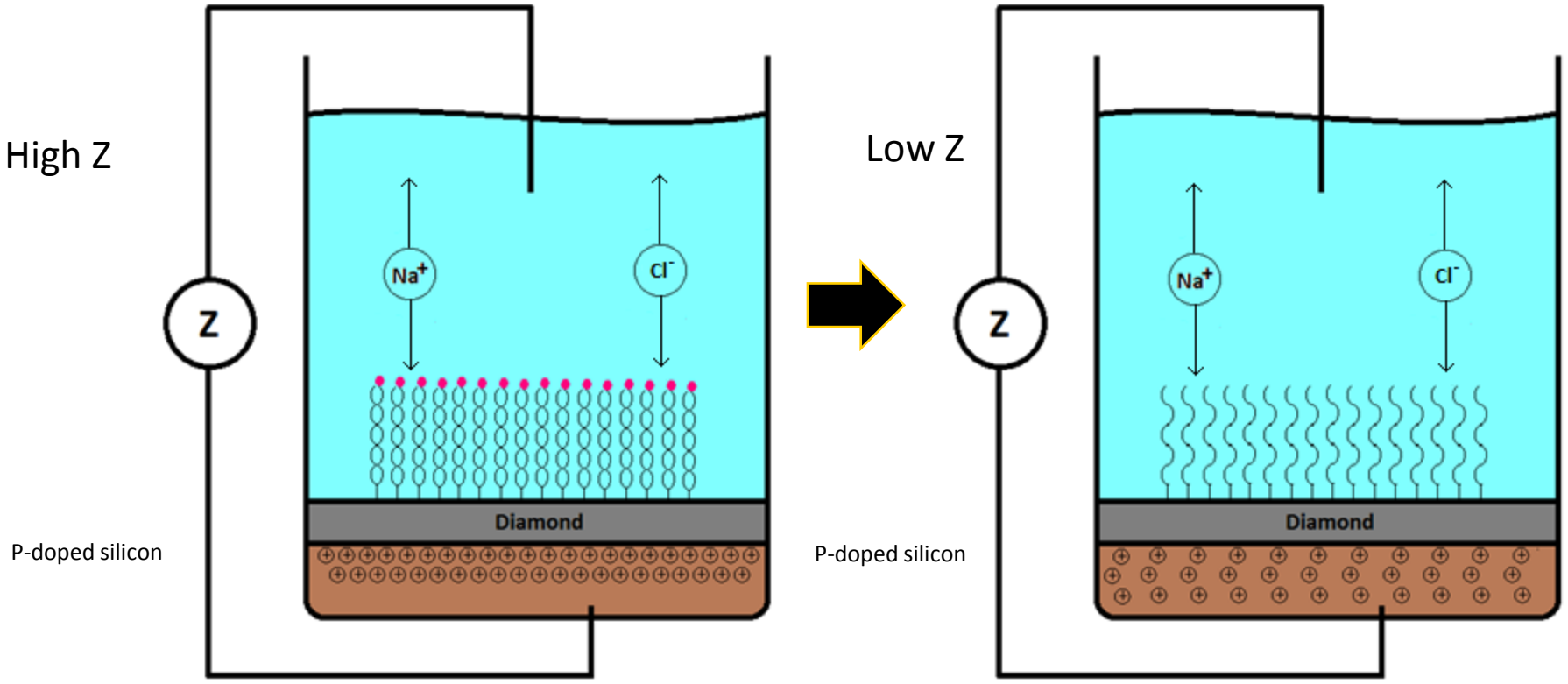




	1 x PBS before denaturation	1 x PBS after denaturation	0.1 M NaOH	Error (%)
R_1 (Ω)	142	142	140	2.5
CPE (nSs^n)	21.9	24.1	23.9	2.2
n	0.8	0.79	0.79	1.0
R_2 (kΩ)	39.3	38.0	37.1	1.2

Use data at 10 kHz for high signal/noise, capacitive sensing effect in CPE

Resistance effect of ds– and ss DNA brush

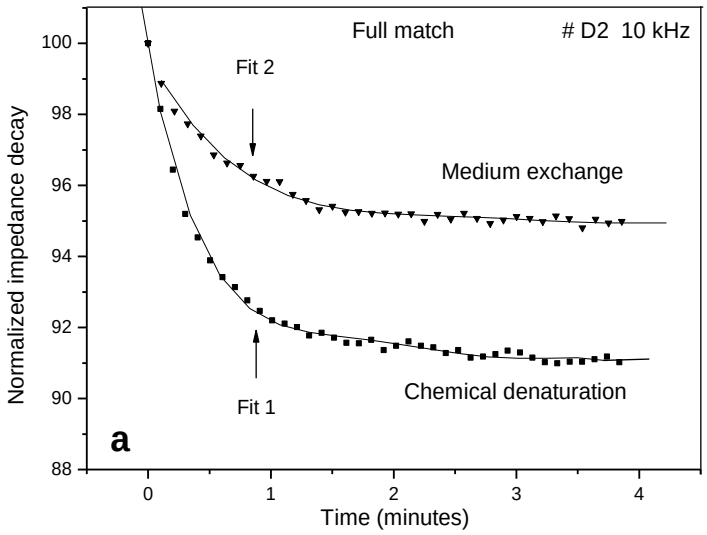


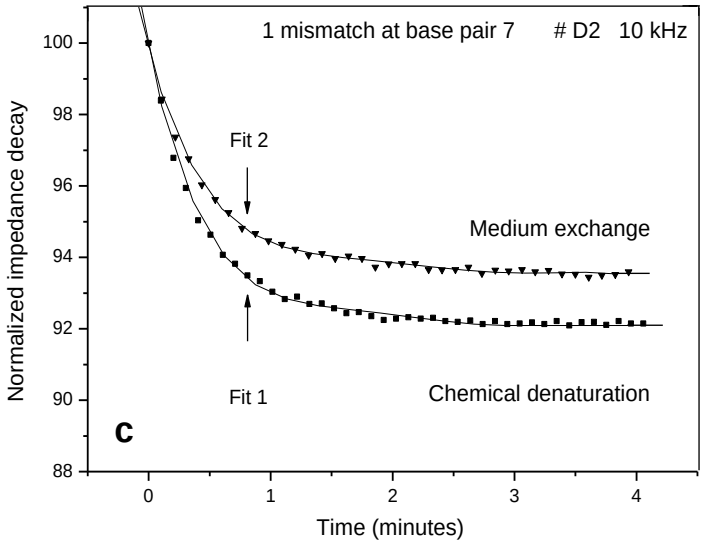
- Electric field effect in p-doped silicon ?
- Electric field effect in p-doped NCD ?

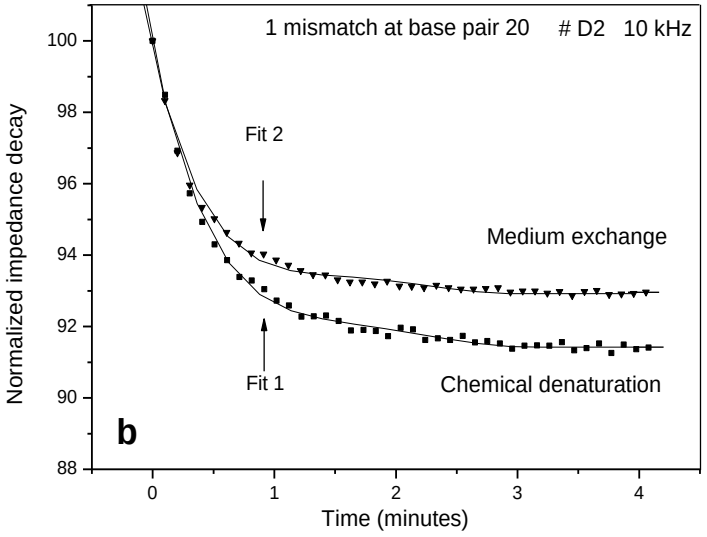
→ negative DNA charge

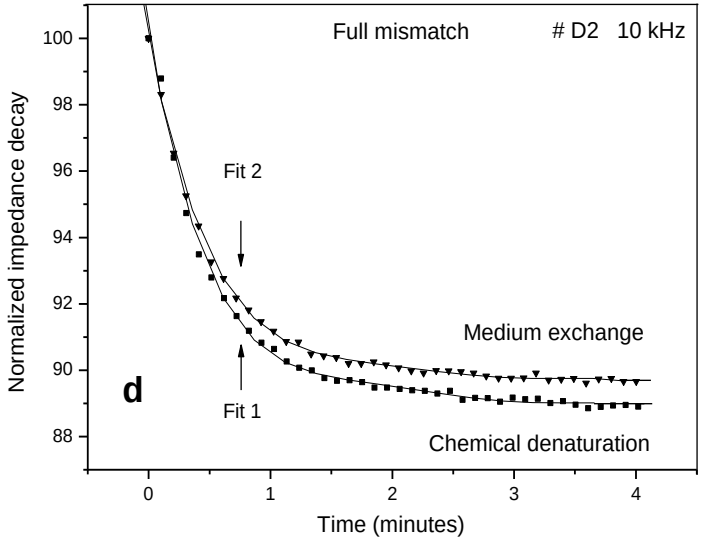
- ds-DNA brush impedes ion movement in buffer ?

Denaturation with 4 duplexes (x 5 electrodes)









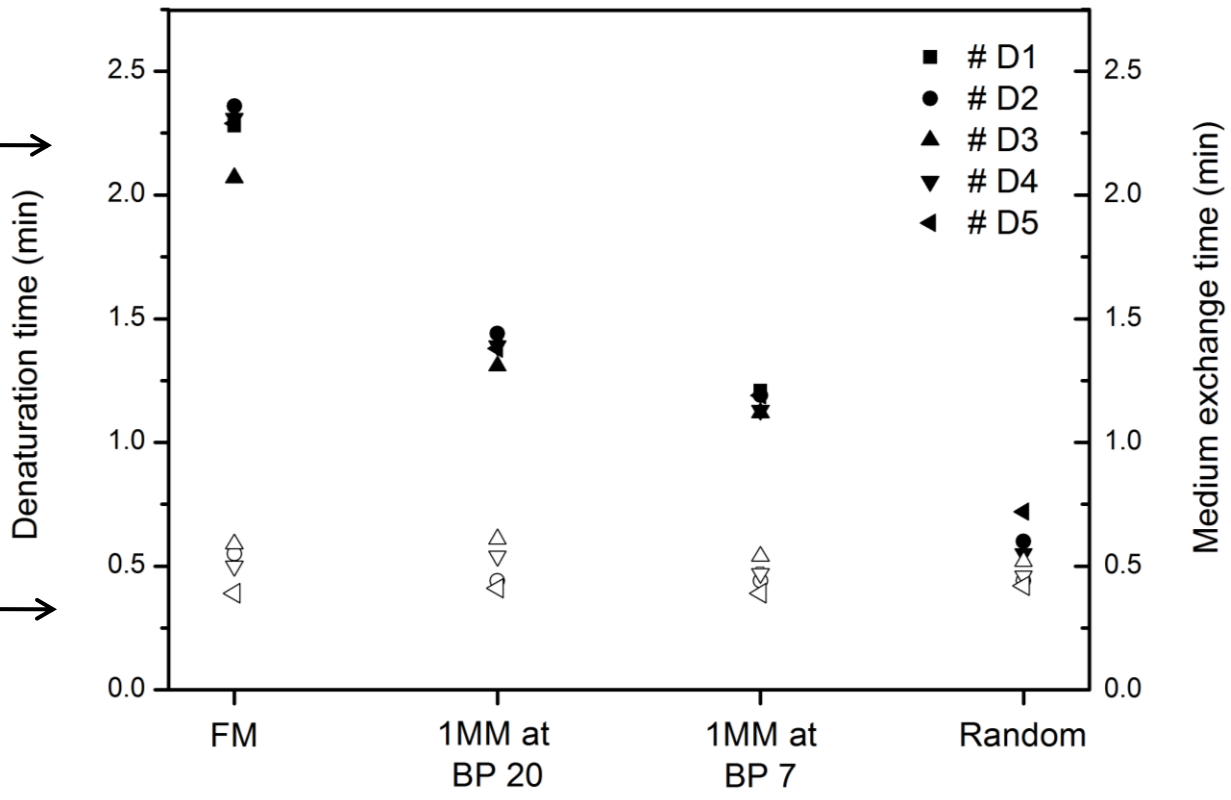
Compilation of denaturation data

Target DNA:	Complement	Mismatch BP 20	Mismatch BP 7	Random
T melting (° C) (FractTM)	91 (84)	85 (78)	88 (81)	- 33 (- 41)
T melting (° C) (HyTher)	79.5	75.0	76.7	- 50.8
$< \tau_1 >$ (min)	2.26	1.38	1.16	0.59
$\sigma < \tau_1 >$ (min)	0.11	0.05	0.04	0.08
$< \tau_2 >$ (min)	0.52	0.50	0.46	0.46
$\sigma < \tau_2 >$ (min)	0.08	0.09	0.06	0.04
$< A_1/Z(0) >$ (%)	3.4	2.0	2.0	0.4
$\sigma < A_1/Z(0) >$ (%)	1.1	0.3	0.7	0.2
$< A_2/Z(t_2) >$ (%)	4.9	6.9	5.6	9.8
$\sigma < A_2/Z(t_2) >$ (%)	1.3	0.5	1.2	1.1

Compilation of time-constant data

Denaturation time τ_1 →

Rinsing time τ_2 →



- **SNP resolution: same defect at different positions**
- **Reproducible data for several diamond electrodes**
- **No sensor regeneration between successive steps // stable under storage**

B. van Grinsven et al., *Lab Chip*, 2011, 11, 1656

Calculated melting temperatures vs. denaturation time

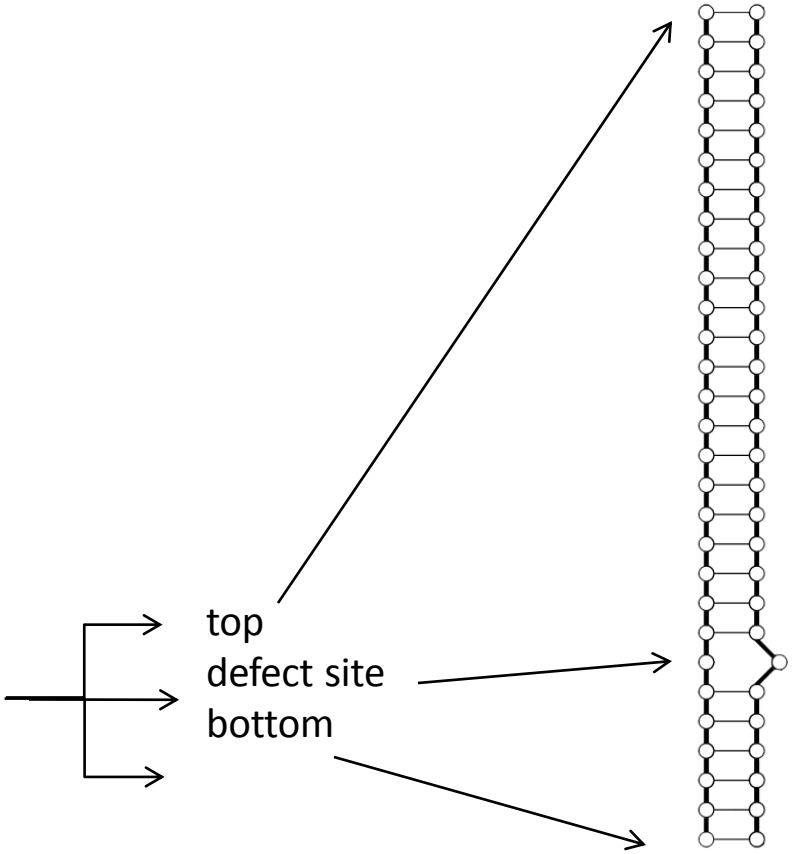
HyTher - online

http://ozone3.chem.wayne.edu

- base–pair interactions
- nearest neighbor effects
- salt concentration
- tethered terminus

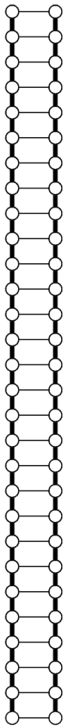
`Zipper – model rules`

- Cracking 1 base pair needs 1 time unit
- Chemical denaturation starts at …….



Denaturation in ‘zipper’ model

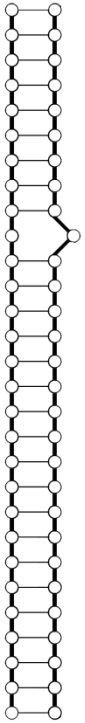
Tm = 79.5 °C



Zipper time: 14.5 units

Denaturation time: 2.26 min

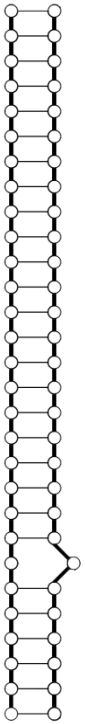
Tm = 75.0 °C



Zipper time: 9.5 units

Denaturation time: 1.38 min

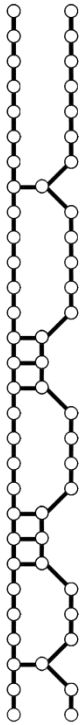
Tm = 76.7 °C



Zipper time: 11 units

Denaturation time: 1.16 min

Tm = - 50.8 °C

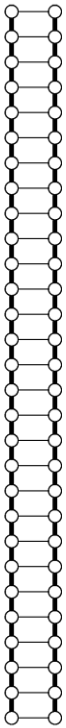


Zipper time: 1.5 units

Denaturation time: 0.59 min

Denaturation in ‘zipper’ model

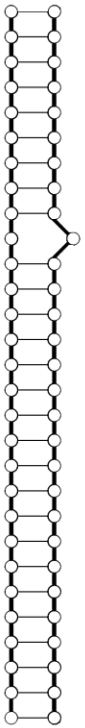
Tm = 79.5 °C



Zipper time: 14.5 units

Denaturation time: 2.26 min

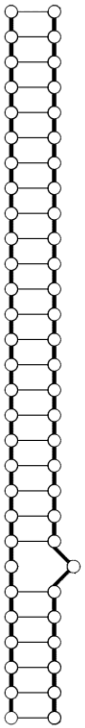
Tm = 75.0 °C



Zipper time: 9.5 units

Denaturation time: 1.38 min

Tm = 76.7 °C



Zipper time: 11 units

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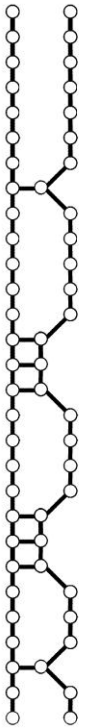
Tm = - 50.8 °C



Zipper time: 1.5 units

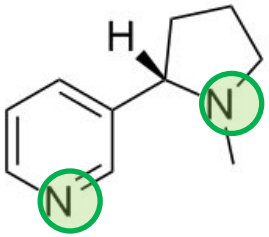
Denaturation time: 0.59 min

Summary of Part 1: DNA

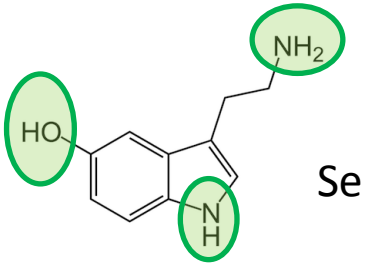
- Fehlerhafte DNA geht schneller kaputt
- Effect can be used to detect and localize SNP mutations
- Chemical equivalent of thermal denaturation
- Denaturation-time constants correlate with calculated melting temperatures
- To be studied: can we discriminate between different defects at identical positions ?

Part 2: Small molecule detection

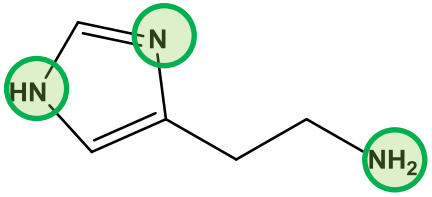
Molecularly Imprinted Polymers (MIP)



Nicotine



Serotonin



Histamine

Diagnostics

- Asthma
- Allergies
- Irritable bowel syndrome

Nicotine

Caffeine

Malachite green

Histamine &

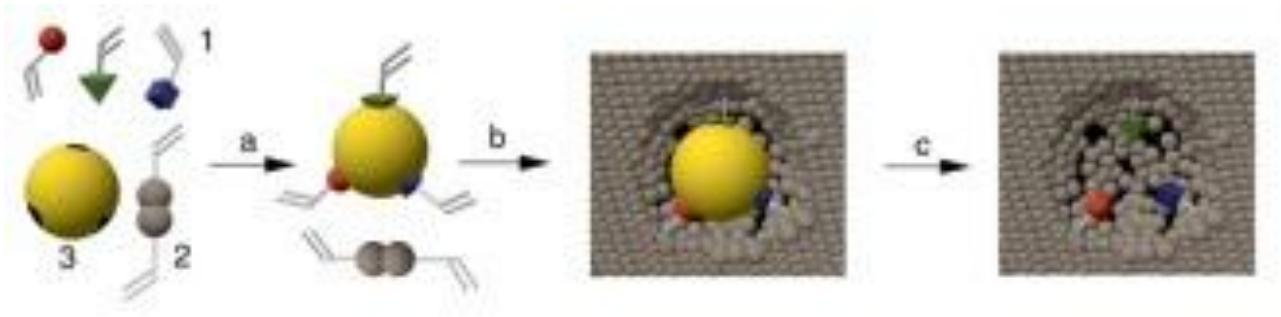
Biogenic amines

Serotonin

Food safety

- Aqua culture
- Fish, Wine, Cheese, "charcuterie"

MIP preparation principle



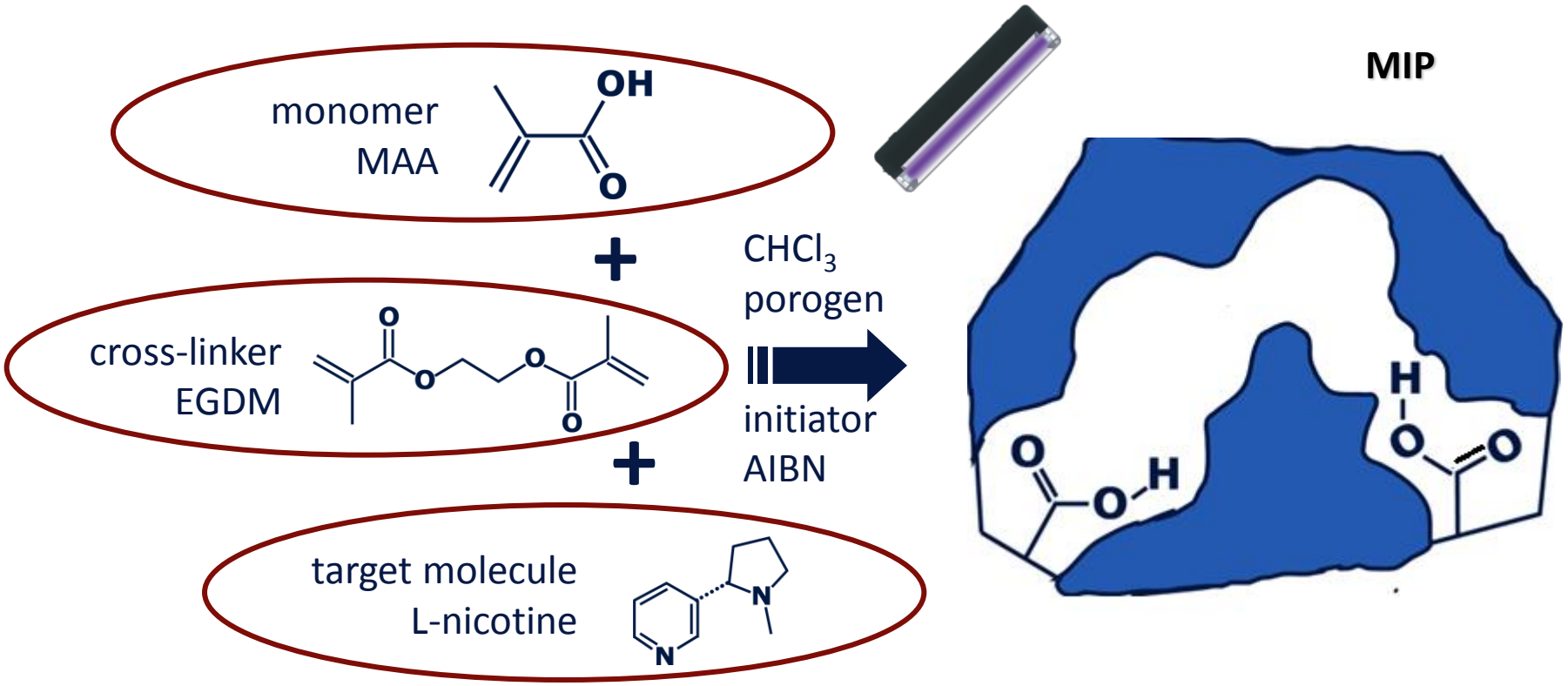
Solution | Pre-polymerization complex | Polymerization | Extraction

a: Template molecule forms a pre-polymerisation complex with functional monomers

b: Polymerisation in presence of cross-linker

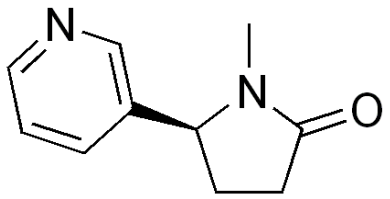
c: Removal of template leaves cavity with well-defined shape and complementary functional groups

Preparation of molecular imprints

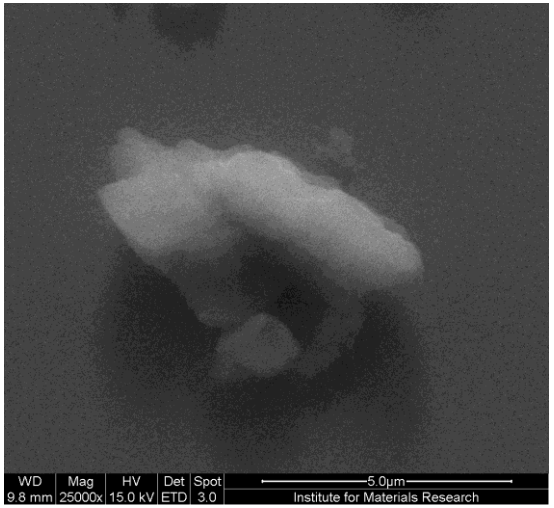


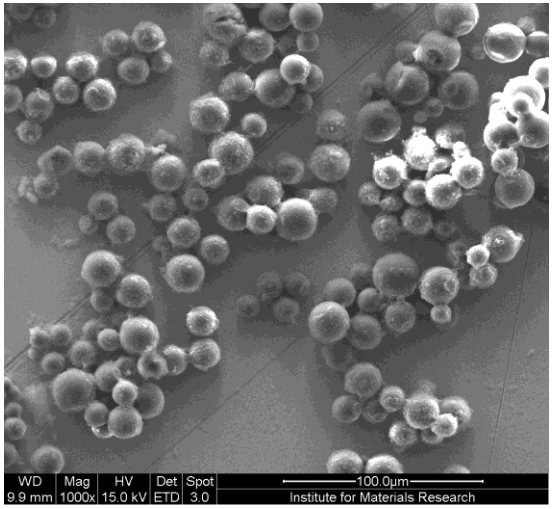
Reference:

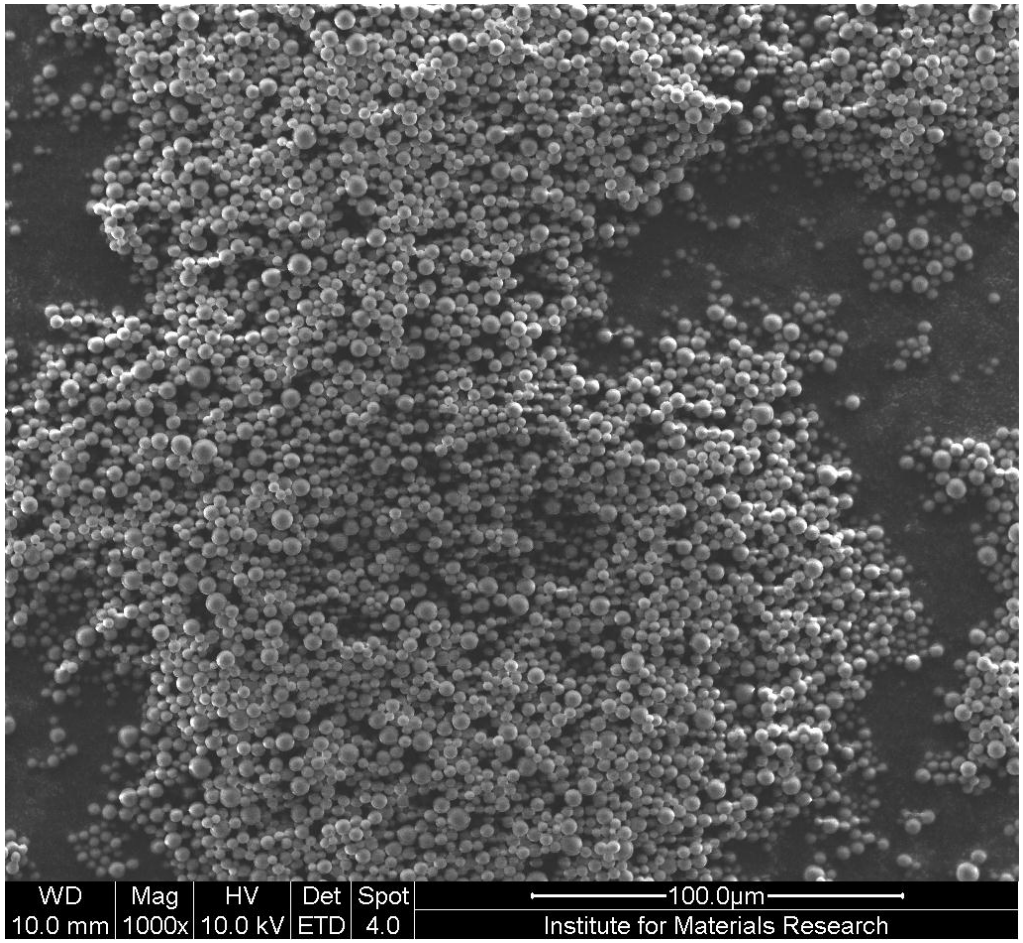
- **NIP:** same procedure <u>without</u> L-nicotine
- L-cotinine: similar molecule;
  two hydrogen atoms replaced by one oxygen atom



MIP-particle morphology



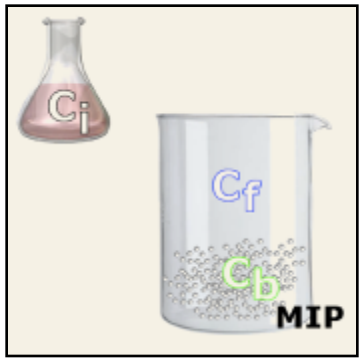




- Suspension polymerisation
- Diameter ± 5.0 µm

Optical batch rebinding experiments

UV-vis absorption
Wavelength ~ 276 nm



C_i = initial target concentration

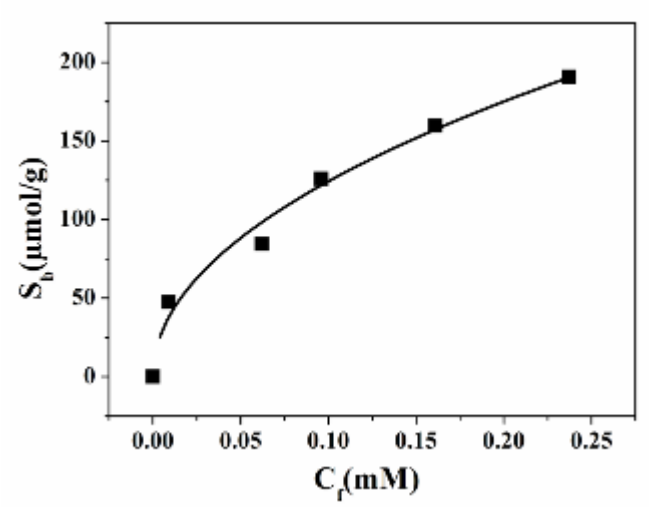
C_f = free target concentration

C_b = bound target concentration

S_b = bound target per gram MIP or NIP

➞ ***S_b plotted versus C_f***

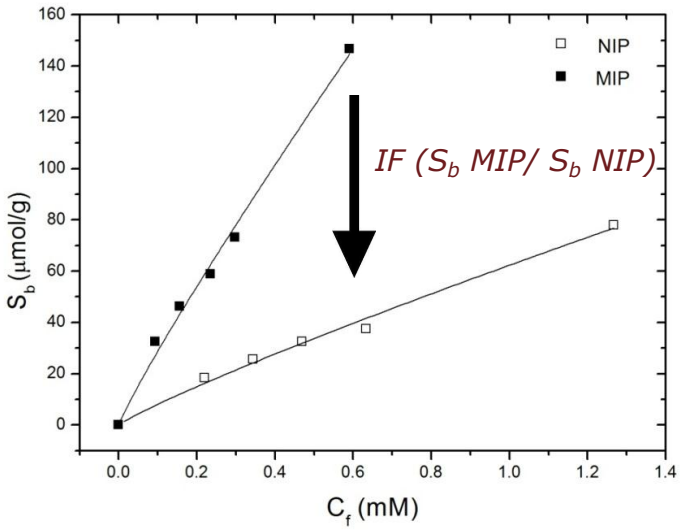
Target / MIP binding: specificity
Target / NIP binding: aspecificity
Analogue / MIP binding: selectivity



Binding isotherms predict MIP performance in sensor setup.

Test of aspecificity: MIP vs NIP

Serotonin



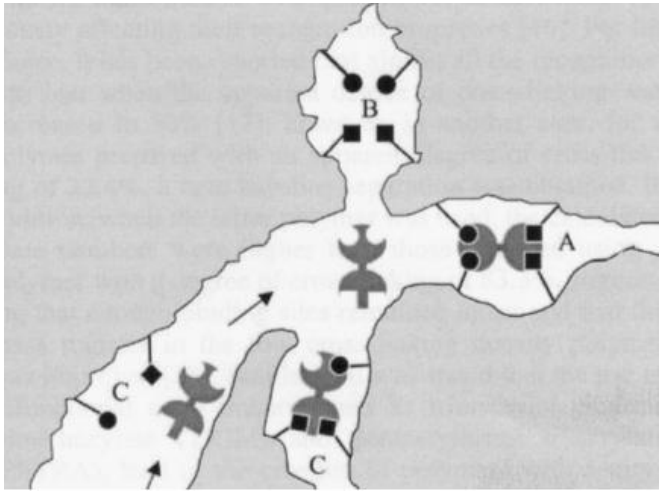
Imprint factor (IF) of 4.0 at 0.6 mM

Langmuir isotherm : $Sb = \frac{N * K * Cf}{1 + K * Cf}$

Freundlich isotherm : $Sb = a\, C_f^{\,b}$

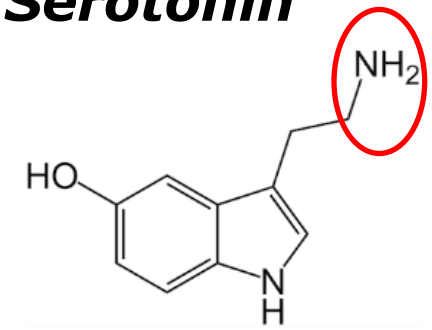
Classic allometric fit (R^2 = 0.99)

Fit parameters	NIP	MIP
A	62.3 ± 1.9	233 ± 12.9
b	0.88 ± 0.06	0.90 ± 0.05

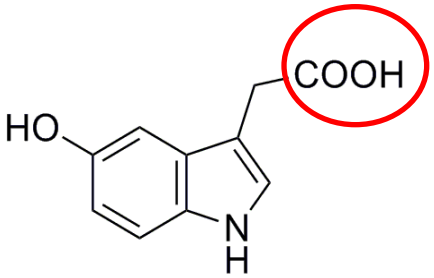


Selectivity test: serotonin vs. competitor

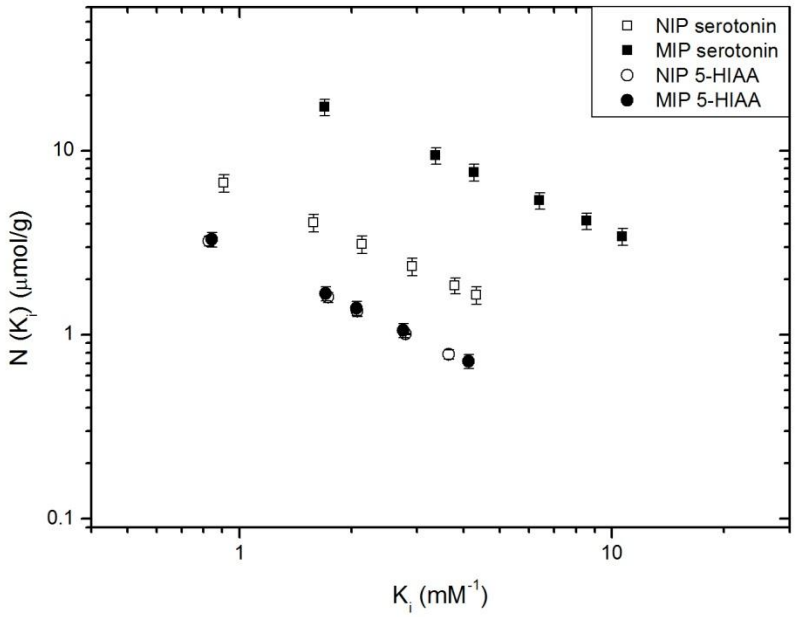
Serotonin



Competitor: 5-HIAA (metabolite)



Distribution of affinity constants:



Comparison of N_{total} :

MIP: 26.4 µmol/g

NIP: 5.63 µmol/g

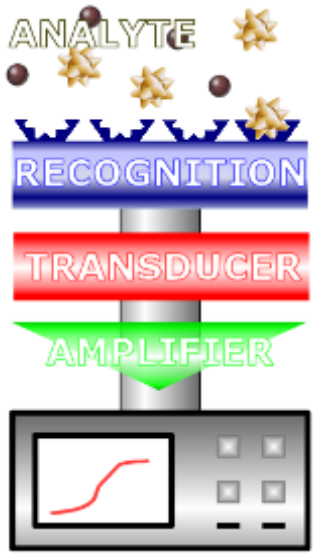
$$Sb = a\,C_f^{\,b}$$

Integration in sensing platforms

- Use the most specific and selective MIP
- MIP immobilization by **matrix entrapment** in a polymeric transducer layer
- Electronic detection of the MIP binding by two sensing principles:

1) Impedance Spectroscopy:

Target binding in the MIP → change of the complex resistance

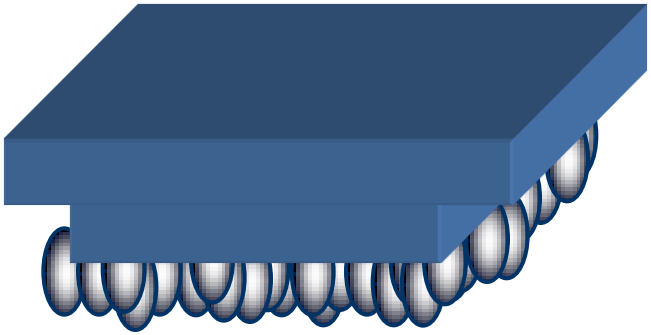


2) Quartz Crystal Microbalance (QCM):

Target binding in the MIP → change of resonance frequency due to mass increase

Matrix entrapment of MIP particles

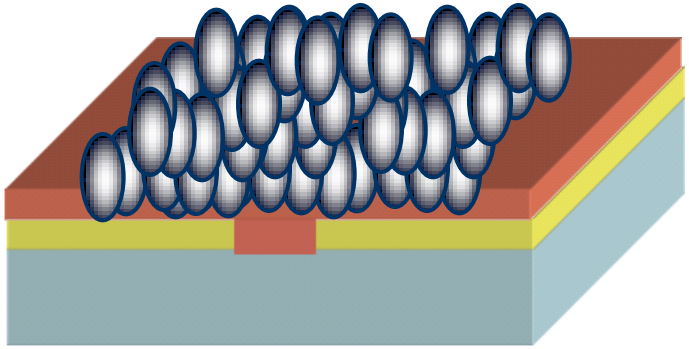
PDMS stamp



PPV/PVC adhesive

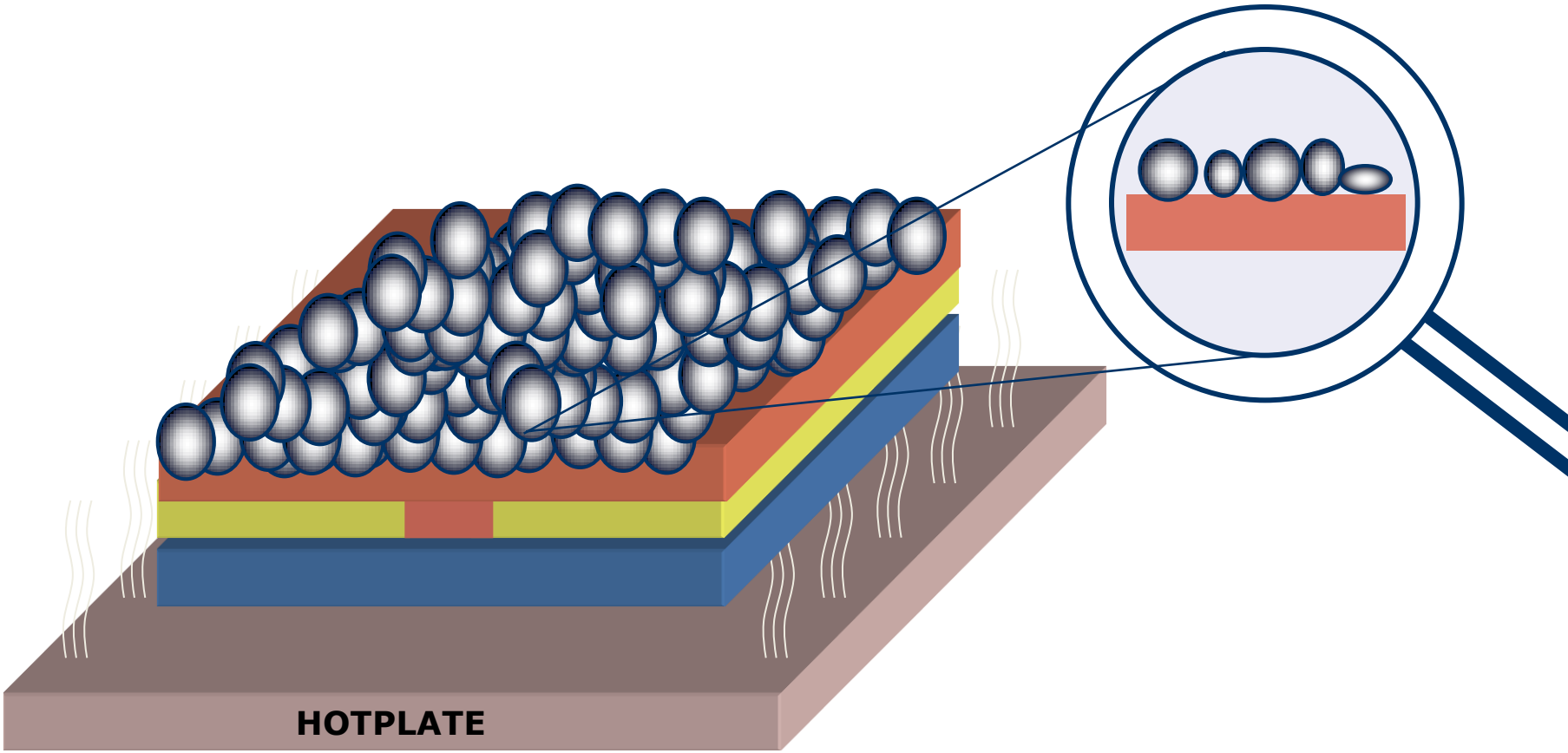
Aluminum

Glass



Straightforward, uniform coverage of the sensing surface : ~ 20%

Matrix entrapment of MIP particles

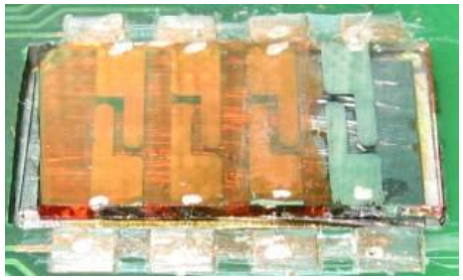


MIP immobilization by matrix entrapment

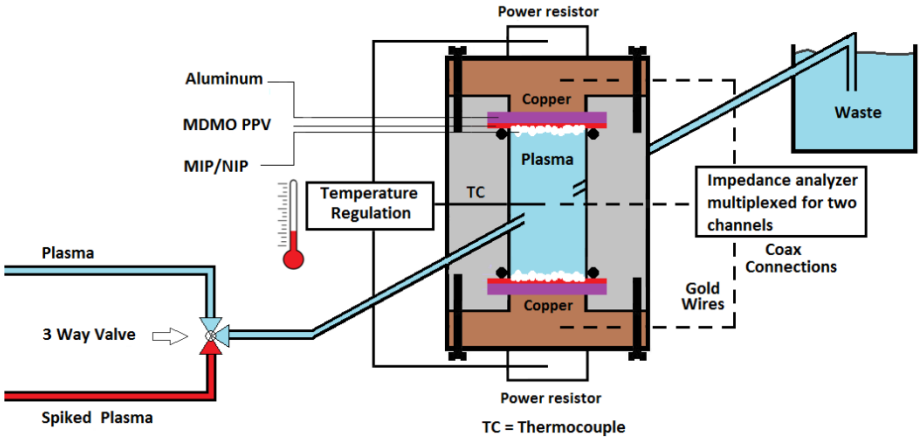
Layout of impedimetric cells

Impedance addition setup

Bongaers et al., 2010. Phys. Status Solidi A. 207, 837-843.



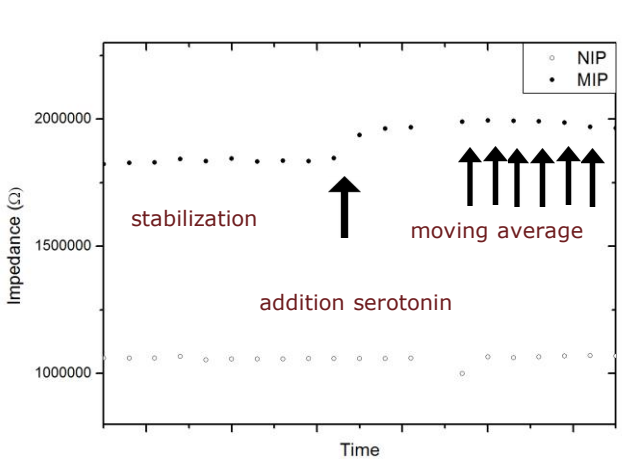
Impedimetric flow-through setup



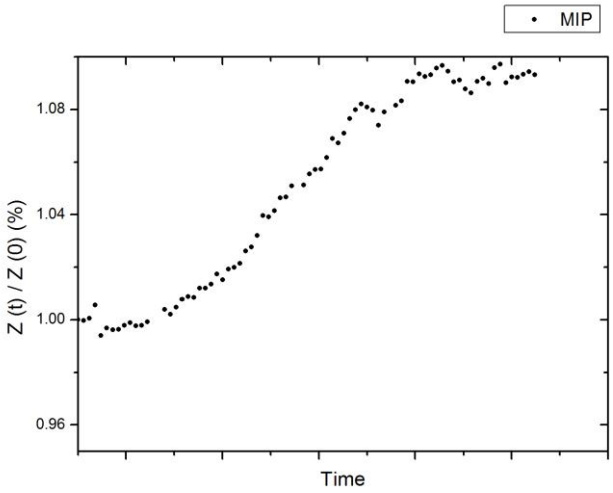
B. van Grinsven et al., 2010.

Phys. Status Solidi (a) 209, 2110-2113.

Impedance measurements in PBS buffer



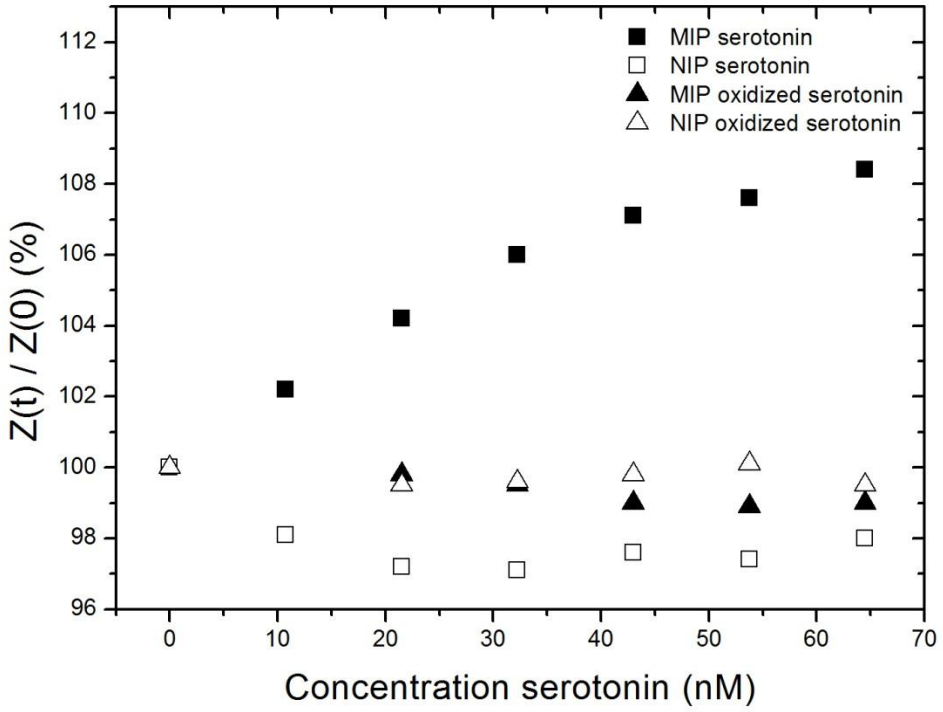
Normalized data



- First: stabilization
- Addition of serotonin of defined concentration
- Impedance normalized to stabilized value
- Increase calculated by moving average (6 points, 3 min in between)
- Per concentration : ± 30 min

Impedance measurement in buffer

Target: Serotonin in PBS buffer (addition setup)

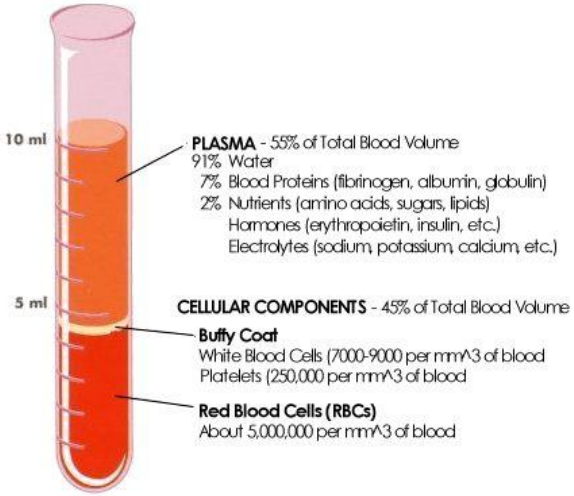


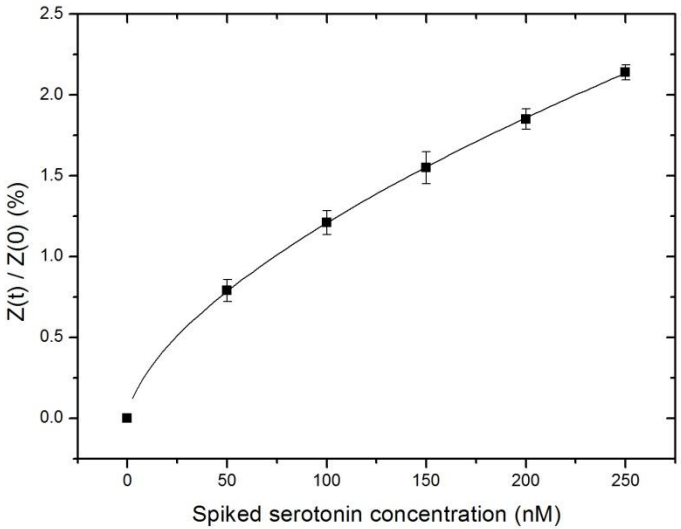
Nanomolar detection limit

Relevant concentrations: **5-20 nM** in plasma (Wymenga et al., Lancet, 1999)

Actual samples: blood plasma

- Detect serotonin in portal blood
- Addition of vitamin C to prevent oxidation





Start: non-spiked plasma

Spiking in impedance directly visible but…

Determine initial concentration ?

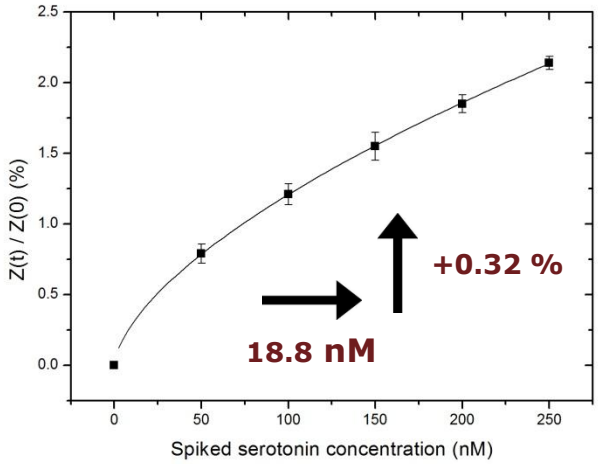
MIP

detection

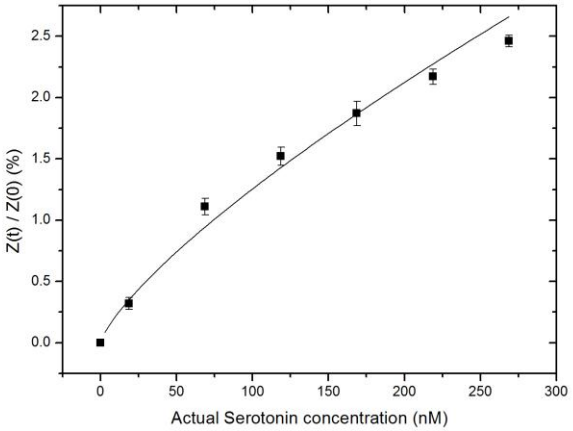
separation

Initial plasma concentration

- Healthy individuals : 5-20 nM (Wymenga et al., Lancet, 1999)
- Add 10 mg MIP to extract serotonin from plasma
- Upon addition of plasma with native serotonin: **+ 0.32% → 18.8 nM**



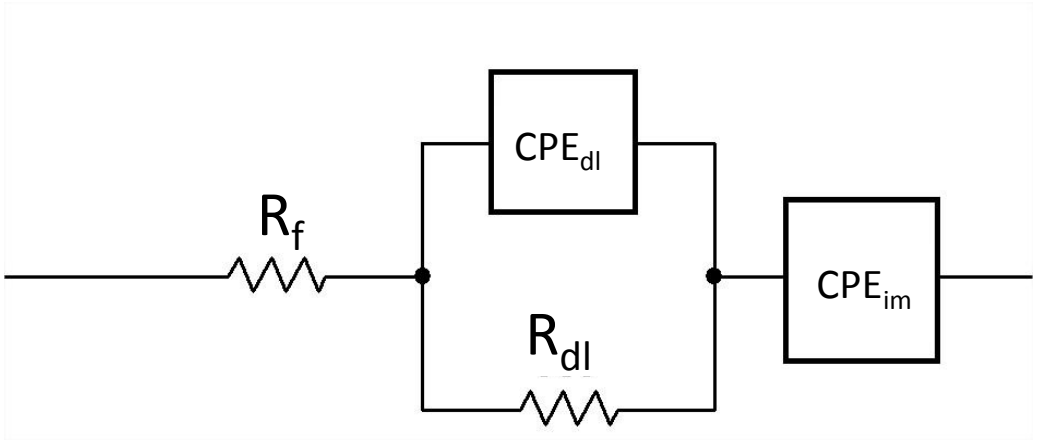
Correction



Parameters	Spiked serotonin
A	0.07 ± 0.002
b	0.62 ±0.005
R^2	0.99

Parameters	Offset corrected serotonin
A	0.06 ± 0.017
b	0.67 ±0.05
R^2	0.99

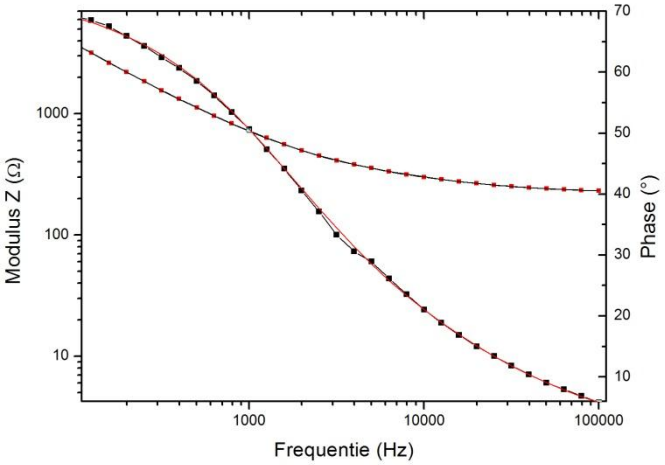
Impedimetric modeling

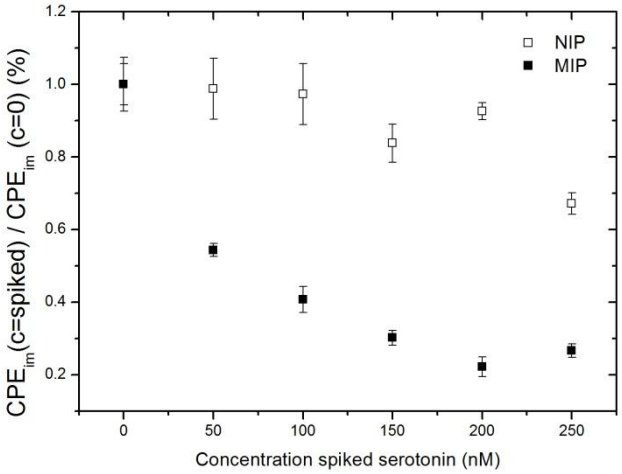


R_f	*fluid & counter electrode*
CPE_{dl}	*Al / PPV / fluid interfaces*
R_{dl}	*Al / PPV / fluid interfaces*
CPE_{im}	***imprinted polymers***

$\chi 2 = 1x\ 10^{-4}$

	MIP		NIP	
Spiked (nM)	CPE_{im} (µS*s^n)	n_{in}	CPE_{im} (µS*s^n)	n_{in}
0	13	0.62	54	0.46
50	7.0	0.69	53	0.46
100	5.3	0.72	52	0.46
150	3.9	0.75	45	0.48
200	2.8	0.79	50	0.47
250	3.4	0.77	36	0.51

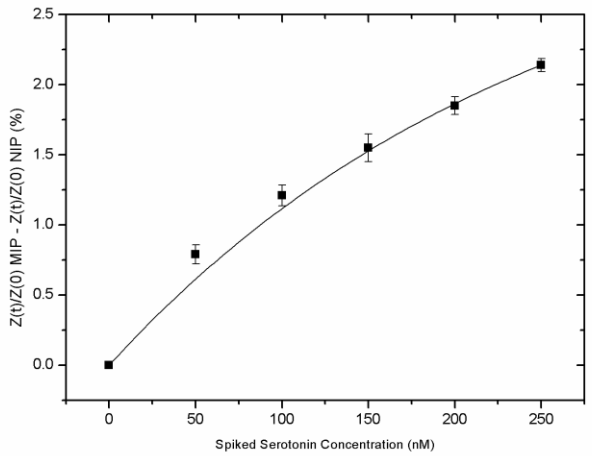




Impedance change from dielectric constant ?

- Change in CPE_{im}

$$Z = \frac{1}{j\,\omega\,C}$$

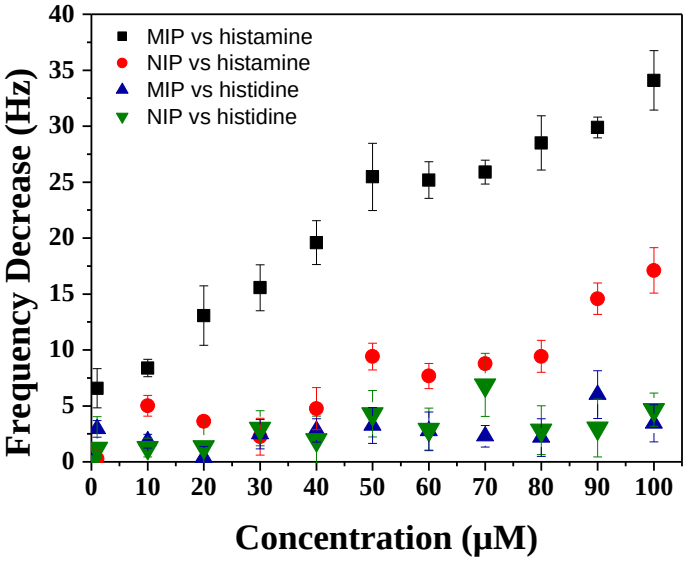


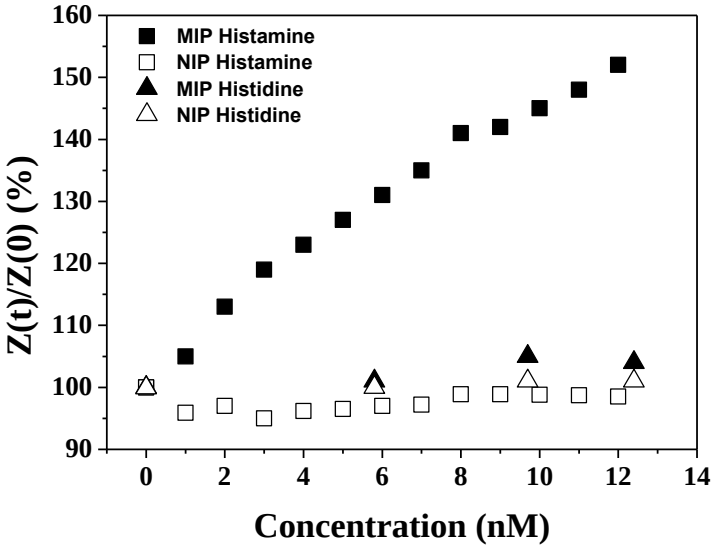
- Relative permittivity
  - ε_o and $\frac{A}{d}$ are constant
  - binding: changes local ε_r

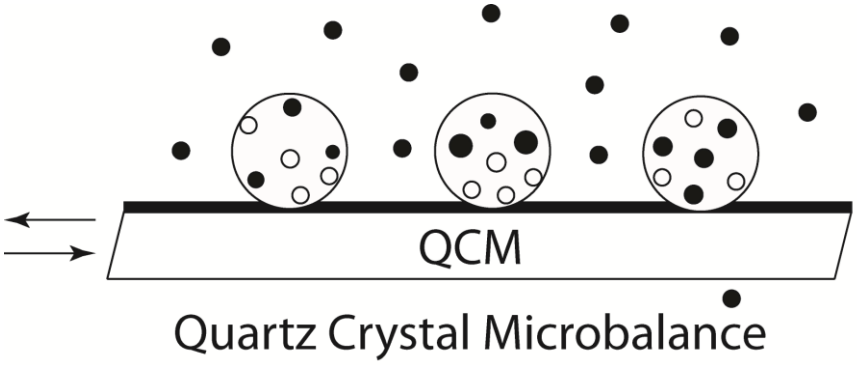
$$C = \varepsilon_r * \varepsilon_0 * \frac{A}{d}$$

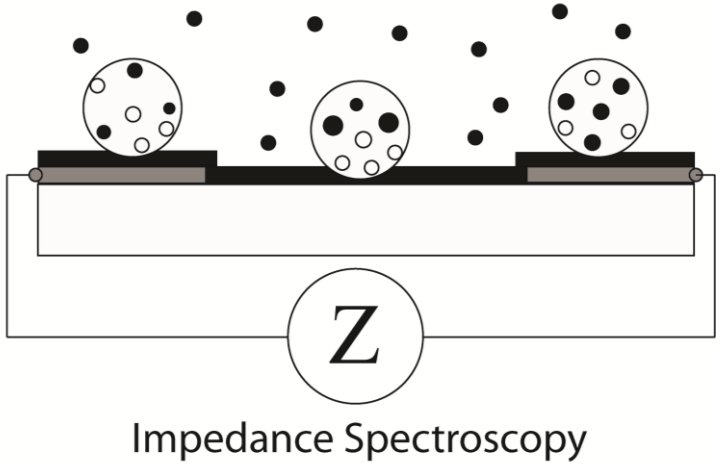
water ~ 81, organic molecules ~ 5-10

Similar: MIP-based histamine detection





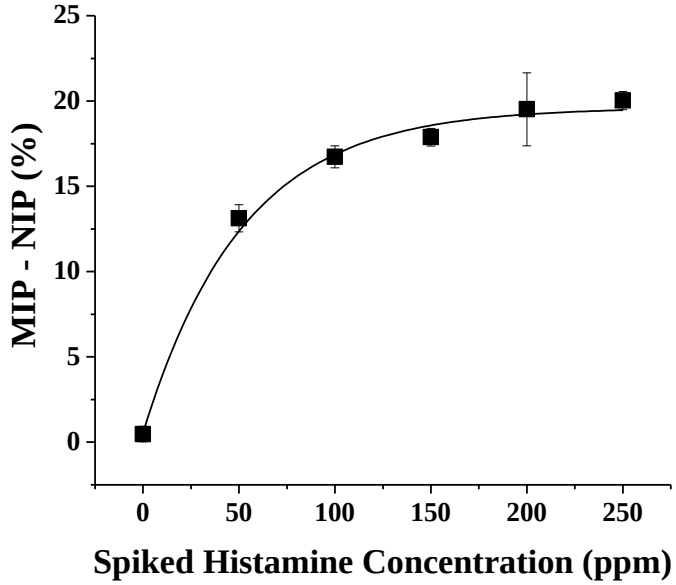




Histamine in food sample: fish

Canned tuna fluid extract

- **Spiked with Histamine**
- **Non - spiked**



Concentrations of spiked sample

1. **50 ppm**
2. **100 ppm**
3. **150 ppm**
4. **200 ppm**
5. **250 ppm**

Diluted 1000 x
=> nMolar range

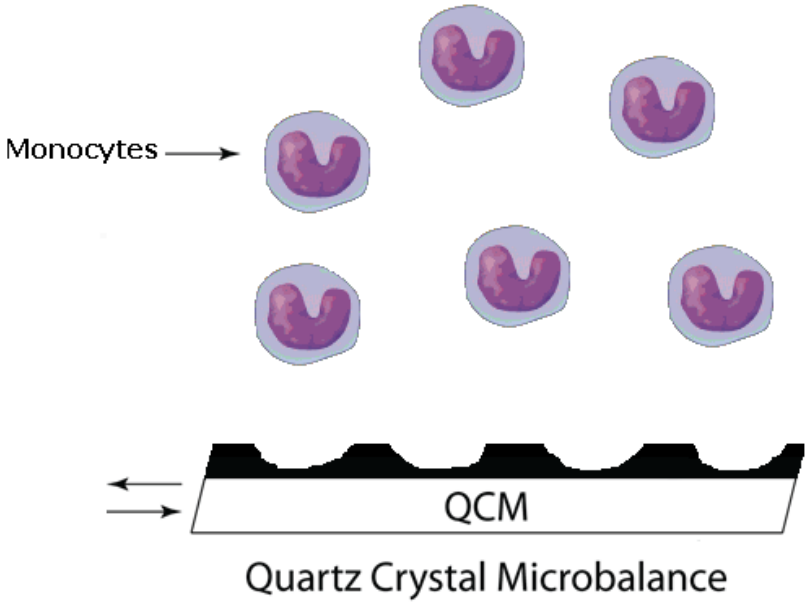
Offset concentration: 3 ppm

Summary of Part 2: MIPs

- Synthesis of selective and specific MIPs for serotonin, histamine, nicotine …
- Integration in a biomimetic sensor with impedance spectroscopy as read-out technique
- Nanomolar detection limit in plasma (biological matrix) and purified food samples
- Offset correction via target extraction
- Straightforward design, rapid and low-cost technique (as compared to chromatography)

Emerging activities 1

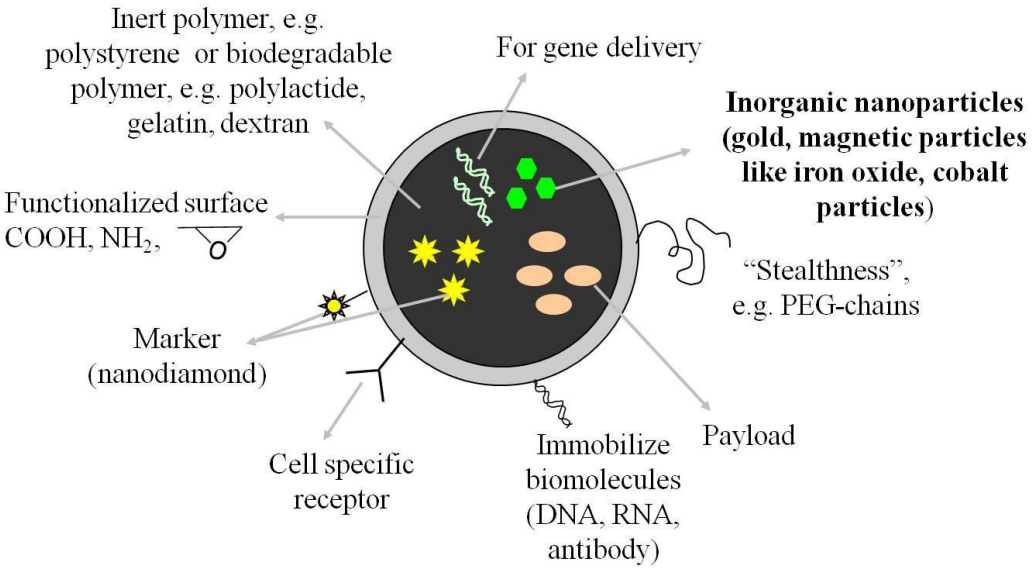
Structural Imprinting

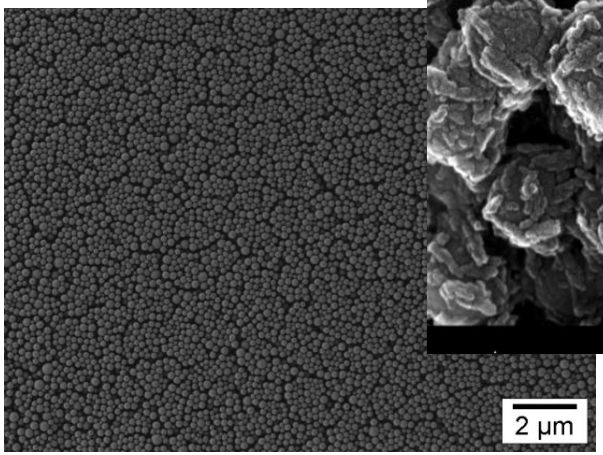


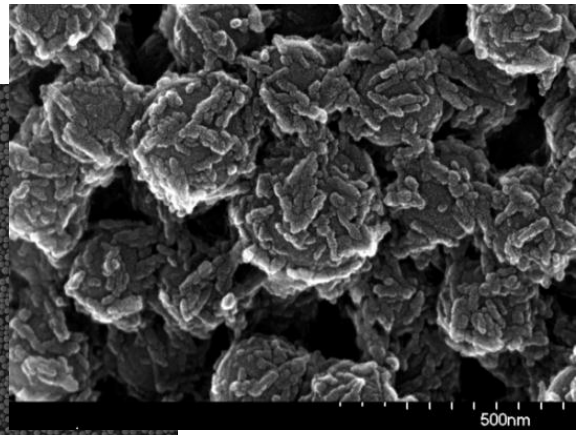
Detection of cells, bacteria or viruses (pathogens)

Here: monocytes → atherosclerosis (cardiovascular disease)

Multifunctional nanocarriers



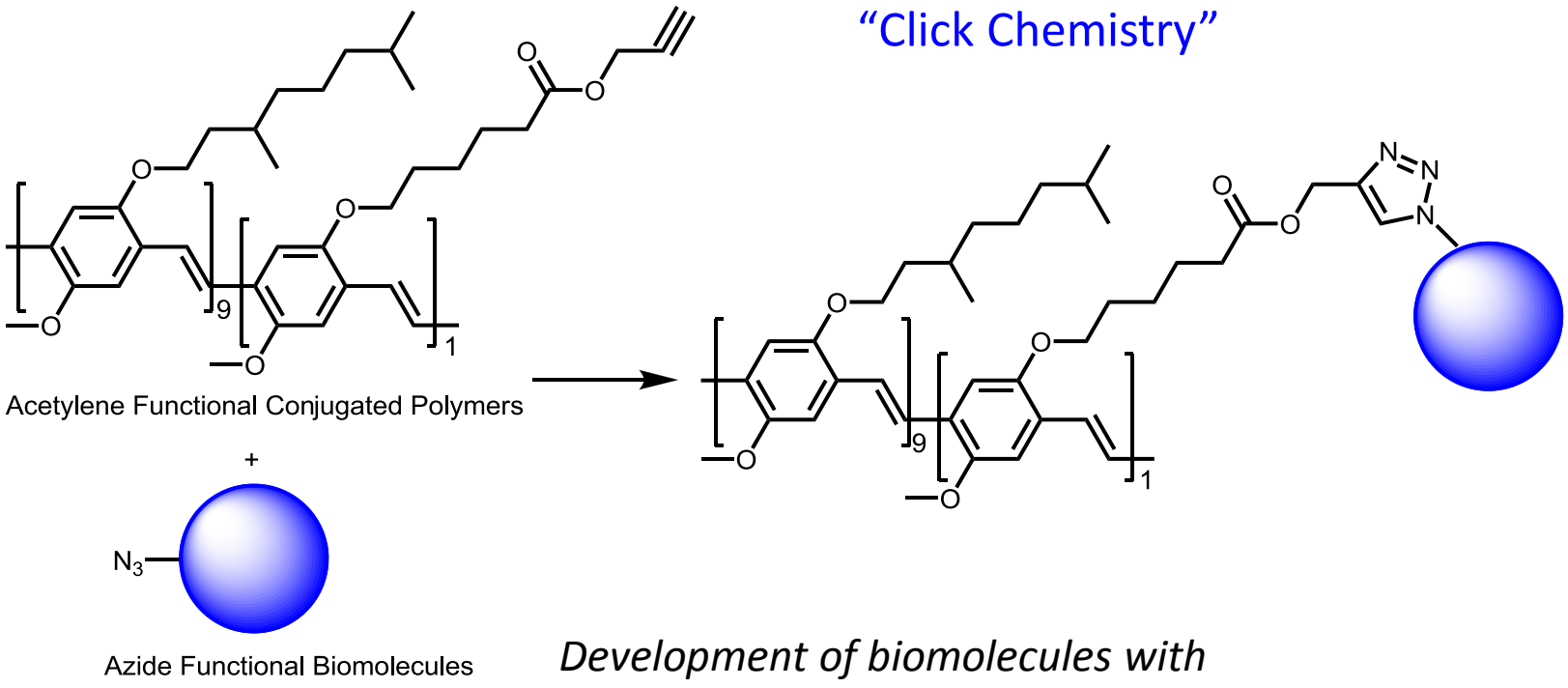




Emerging activities 2

Development of Plastic Bioelectronics

Development of novel functional materials, such as conjugated polymers

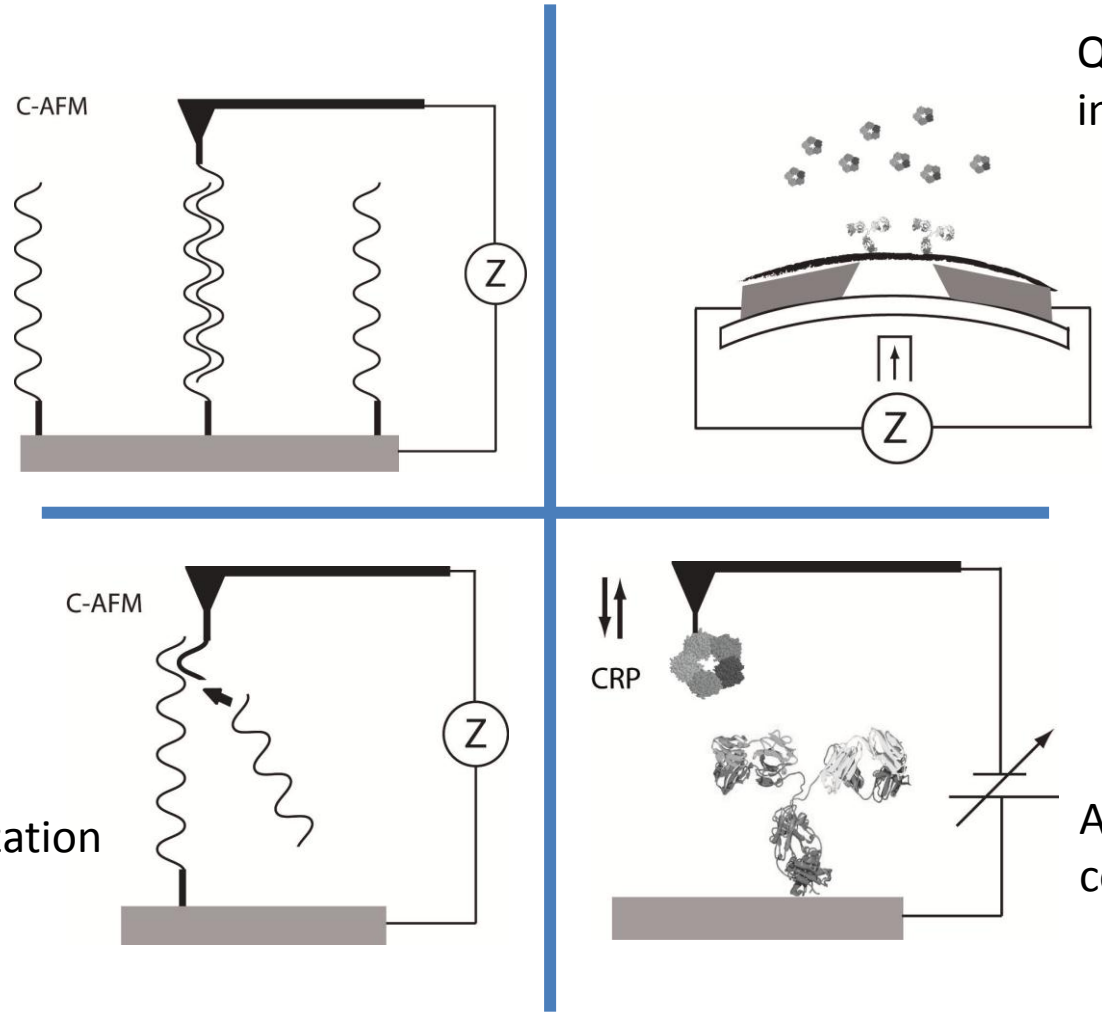


Development of biomolecules with well-defined functional groups

Future: single molecule interactions

σ ↔

- sequence
- temperature
- salt
- strain



Quantum-transport in polymer chains

Single hybridization events

Antibody-antigen coupling in E-fields

Partners and funding

- Aachen University of Applied Sciences
- Research Center Jülich
- ISAS & BESSY II Berlin
- K.U.Leuven: LVSM & MeBioS
- U Antwerpen: EMAT & CMT
- VITO Materials Technology
- IMEC - ESAT
- Siemens Corporate Technology
- University of Applied Sciences Kaiserslautern
- Cranfield Health

- UHasselt – Special Research Funds BOF
- Province of Limburg: Life-Sciences Impulse Programme
- Research Foundation Flanders FWO
- BELSPO Interuniversity Attraction Poles IUAP
- Methusalem NANO Antwerp-Hasselt
- IWT
- Interreg – NanoSensEU
- 7th Framework Programme - DINAMO