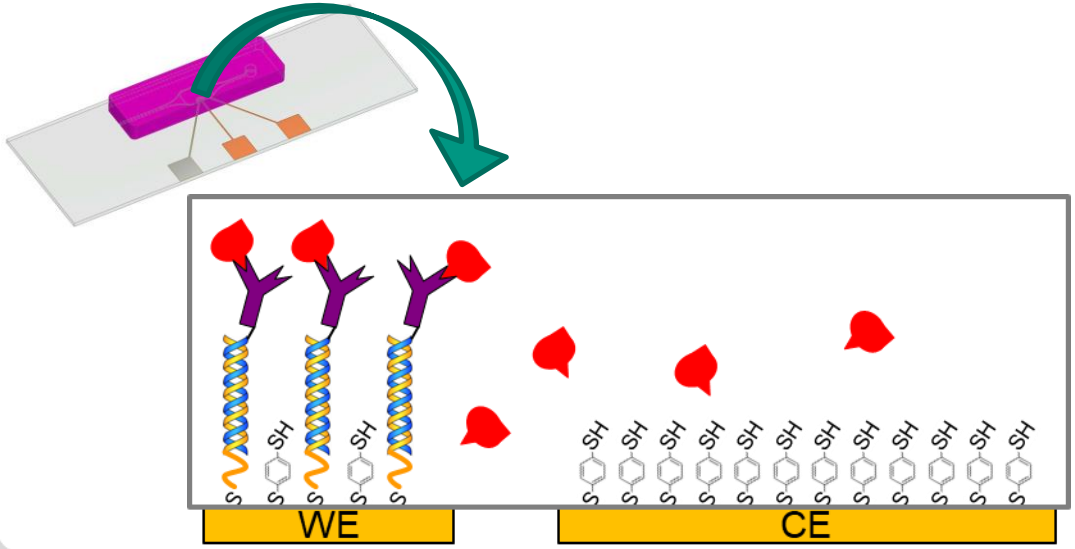
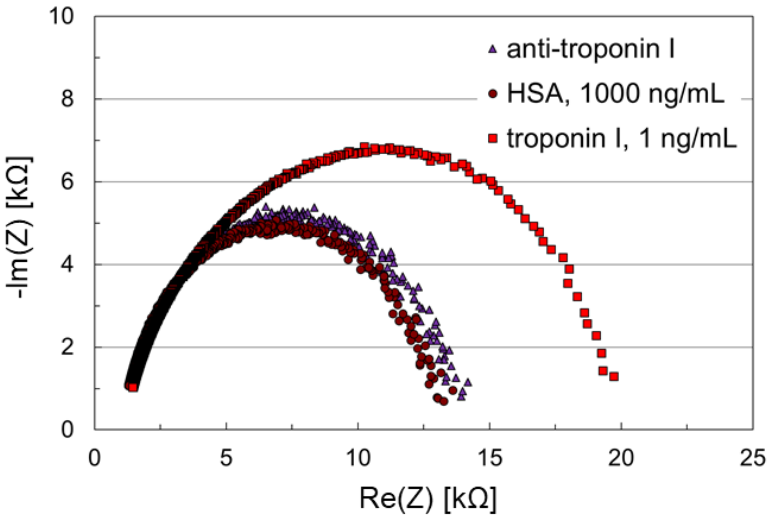
Microfluidic Impedance Biosensor Chip with DNA-Based Self-Assembled Monolayers for Label-Free Detection of Cardiac Biomarker Troponin I

Khaled Alsabbagh[1], Tim Hornung[1], Achim Voigt[1], Sahba Sadir[2], Taleieh Rajabi[1], Kerstin Länge[1]

[1] Institute of Microstructure Technology (IMT)
[2] Institute for Micro Process Engineering (IMVT)





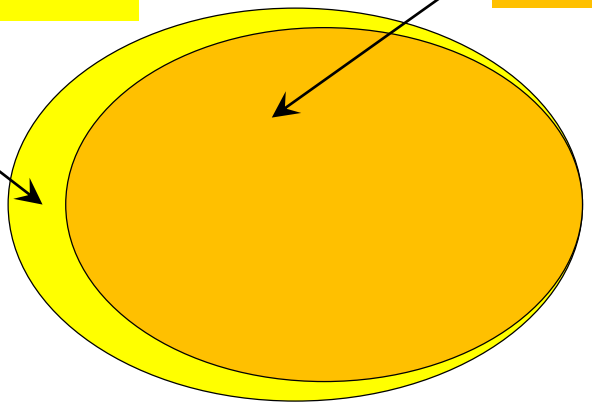
Outline

- Motivation for cardiac troponin I detection
- Microfluidic impedance biosensor chip
  - Background
  - Design and measurement setup
- Cardiac troponin I immunoassays with the microfluidic impedance biosensor chip using different SAMs
  - No SAM
  - Thiol-SAMs with hydrocarbon spacer
  - Thiol-SAM with DNA spacer
- Summary + Outlook

Cardiovascular Diseases (CVDs)

CVDs: global number 1 cause of death

85% of CVD deaths *(2016)*: heart attacks and strokes



WHO fact sheet 2017

37% of premature deaths (< 70 years of age) due to noncommunicable diseases *(2015)*: caused by CVDs

Myocardial Infarction, “Heart Attack”

- Diagnosis criteria include the **detection of cardiac markers in blood**
- **Cardiac-specific troponins I and T (cTnI, cTnT):** start of increase: 4–6 h

Troponin	**Normal levels** *(Dörner 2009)*	**Factor increase** *(Luppa & Junker 2018)*
cTnI	< 0.5–2.0 ng/mL (lab-specific)	up to 40-fold
cTnT	< 0.1 ng/mL	up to 40 to 60-fold (up to 300-fold)

Detection of Cardiac Markers
Cardiac Troponin I (cTnI) *et al.*

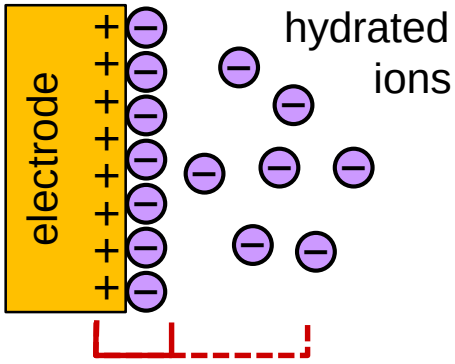
- **Early diagnosis increases survival rate**
  → Required: fast, sensitive and cost-effective detection method
- **Current methods**
  - Classical immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA): sensitive, but elaborate and time-consuming
  - Commercially available detection kits (with external readout device) e.g., Philips Minicare I-20, Quidel Triage Troponin I Test, Roche cobas h 232 System:
    - Detection time “less than 10 min” up to “ca. 20 min
    - Detection of only one cardiac marker or few cardiac markers
- **Advantages of impedance biosensor chip**
  - Label-free transduction principle: direct, rapid and easy detection method
  - Electrode design can easily be extended to an array for multi-analyte detection

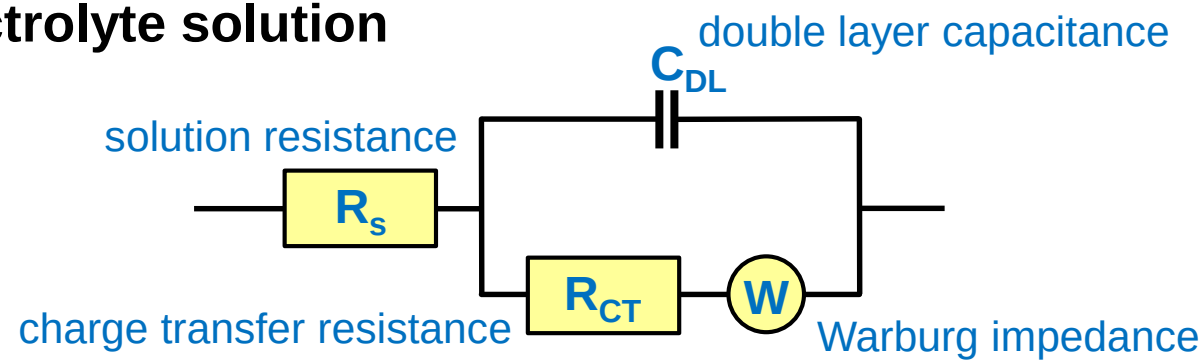
EIS: Electrochemical Impedance Spectroscopy

Impedance Z = the opposition a circuit presents to current when an alternating voltage is applied

$Z(j\omega) = \frac{U(j\omega)}{I(j\omega+\varphi)}$ $mit\ \omega = 2\pi f$ → Z is a complex number: Z = Re(Z) + j·Im(Z)

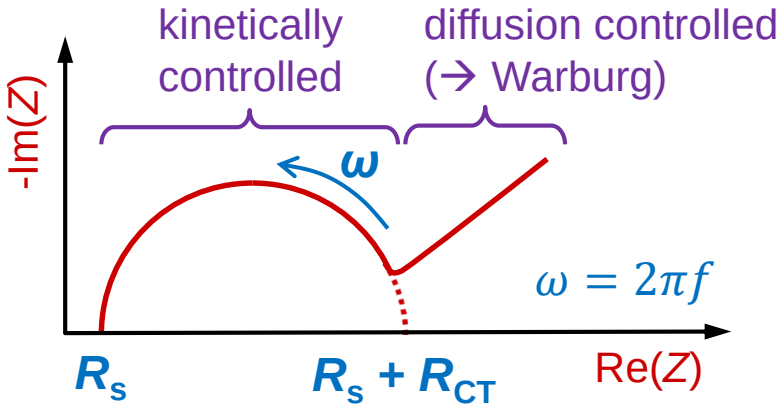
Electrode in an electrolyte solution





- Application of AC voltage: changes in double layer
- (Inner) Helmholtz double layer acts as plate condensator
- $Z = \sqrt{R^2 + |X_C|^2}$ with $X_C = \frac{1}{j\omega C} = \frac{-j}{\omega C}$

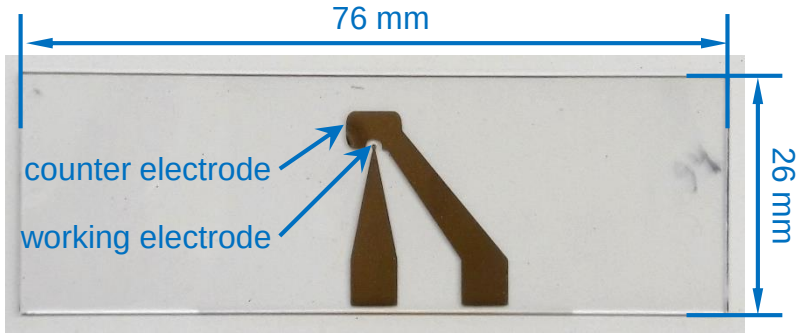
Nyquist plot



Microfluidic Impedance Biosensor Chip: Fabrication

Electrode Sputtering

1) Base plate: microscope glass slide
2) Cleaning: detergent, water, 2-propanol
3) Parylene C coating
4) Electrodes, conducting paths: gold sputtering

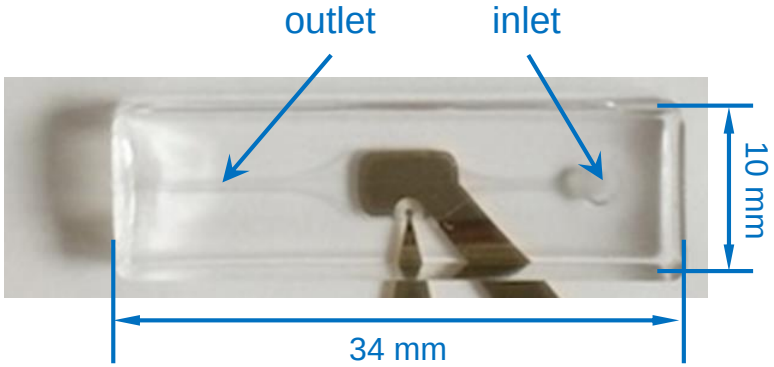


Introduction of Microfluidic Channel

Material: Polydimethylsiloxane (PDMS)

Fabrication: Casting of a mixture of PDMS base and curing agent into a milled form made of polymethyl methacrylate (PMMA)

Bonding: Plasma activation of chip and PDMS surface → connecting the parts



channel height: 0.2 mm

volume: < 10 µL

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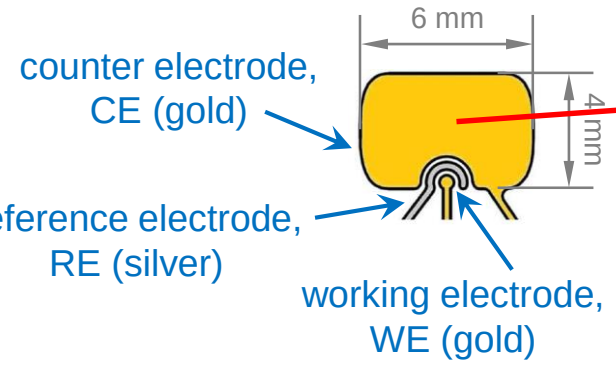
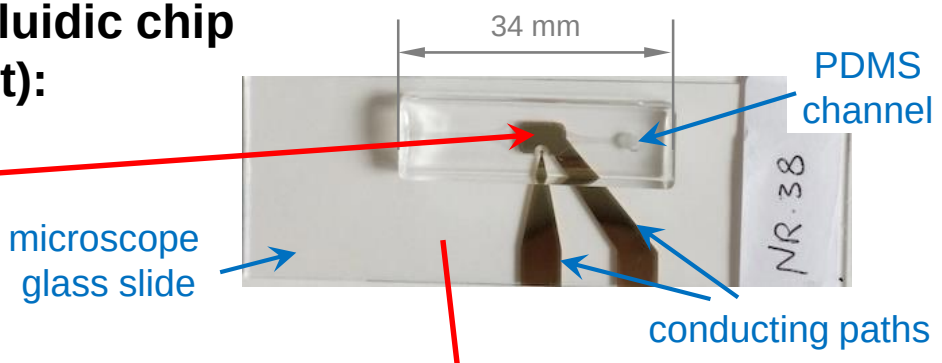
Microfluidic Impedance Biosensor Chip: Measurement Setup

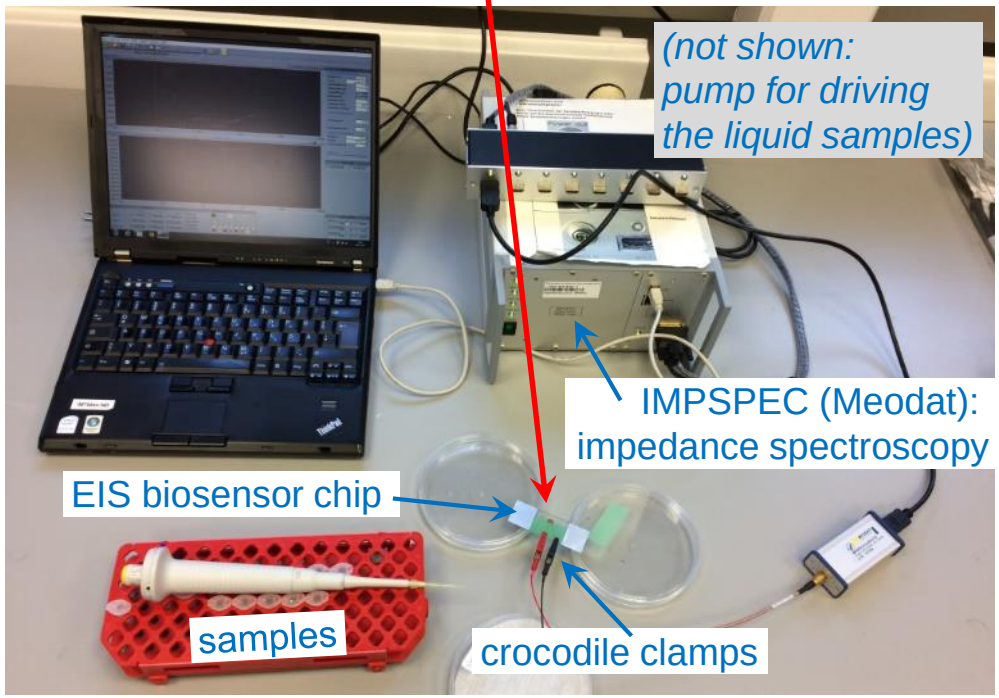
Electrodes:



Microfluidic chip (cutout):



Measurement setup:



To simplify chip fabrication and measurement setup, the following work was done without the reference electrode (RE)

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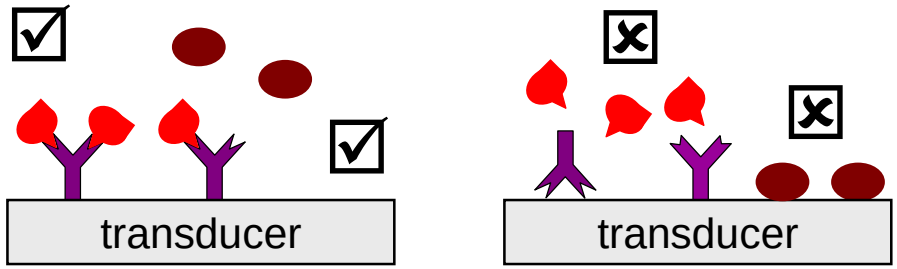
Impedance Biosensor Surface – Requirements

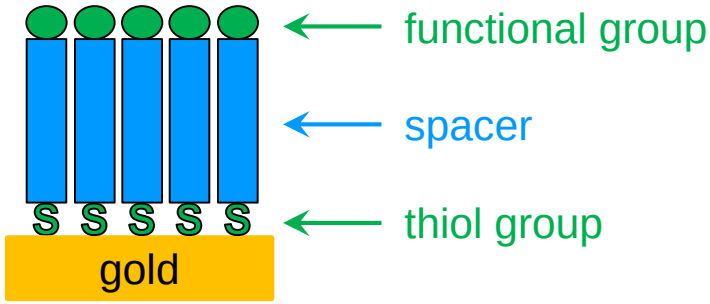
General biosensor surface requirements

- Analyte-specific binding – accessibility and integrity of binding sites
- Inhibition of non-specific protein adsorption



Introduction of functional groups on gold surfaces: via thiols

- Thiols: compounds with SH groups, form self-assembled monolayers (SAMs) on gold
- SAM: densely packed → inhibition of non-specific protein adsorption
- Biosensors: additional functional groups for further surface functionalization



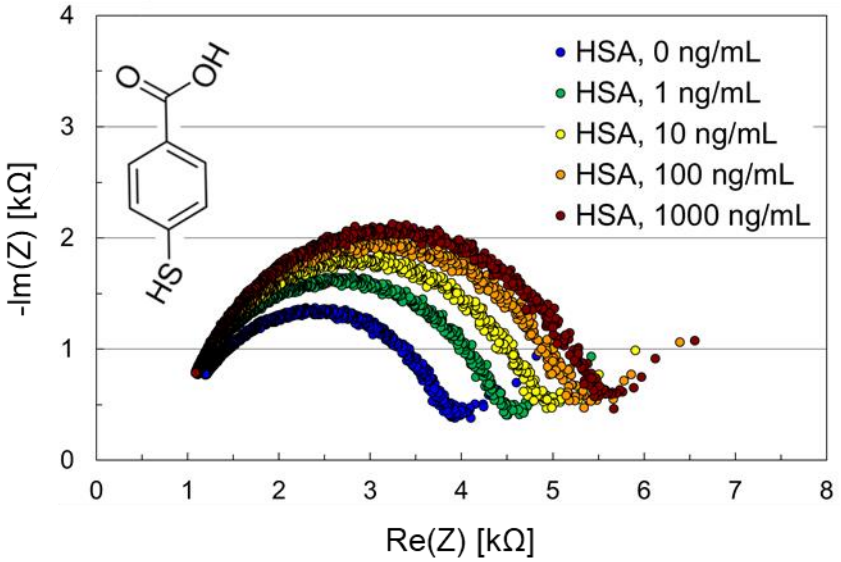
Additional requirements for impedance biosensors

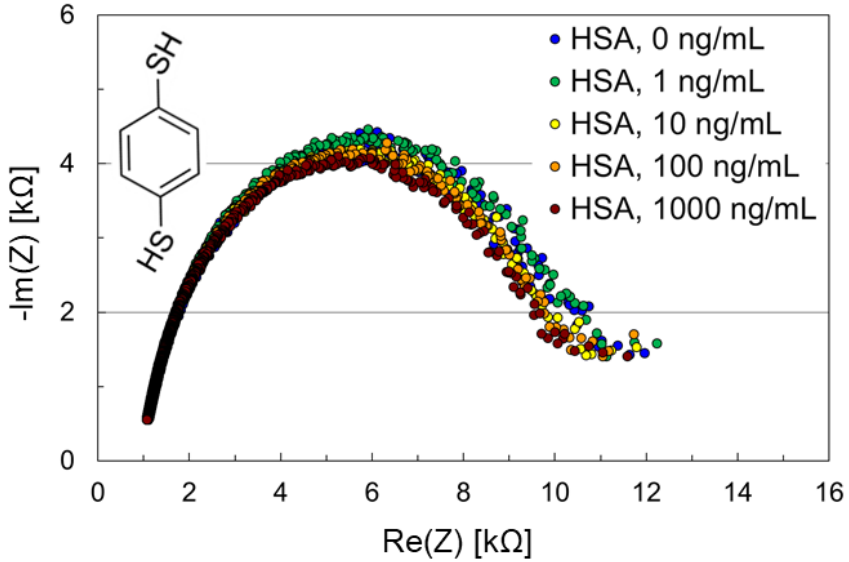
Transport of charge carriers to the electrode must be guaranteed, i.e., the sensing layer must allow permeability for charge carriers

→ Low initial impedance of the sensing layer

Thiol-SAM with Hydrocarbon Spacer: Testing the SAM Performance

- Both working and counter electrode were coated with the respective thiols
- Human serum albumin (HSA) samples were successively applied in increasing concentrations





4-Mercaptobenzoic acid:

Inhibition of non-specific protein adsorption insufficient, may be improved by subsequent antibody coupling

1,4-benezenedithiol:

- Excellent inhibition of non-specific protein adsorption
- Potential passivation layers for future counter electrodes

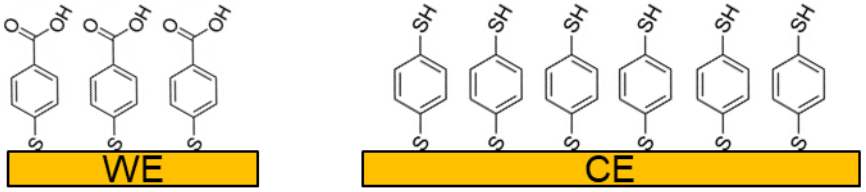
Thiol-SAM with Hydrocarbon Spacer: Antibody Immobilization and Troponin I Assay

1) **Plasma activation**

WE: working electrode
CE: counter electrode

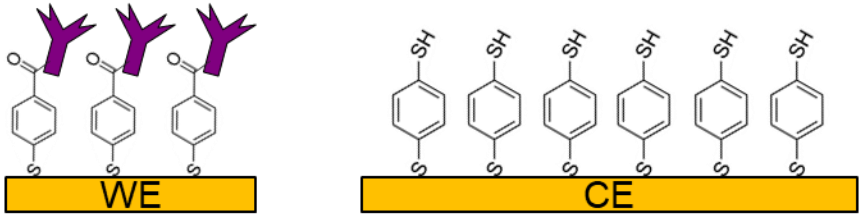
2) **4-Mercaptobenzoic acid: SAM on WE**

3) **1,4-Benzenedithiol: SAM on CE**

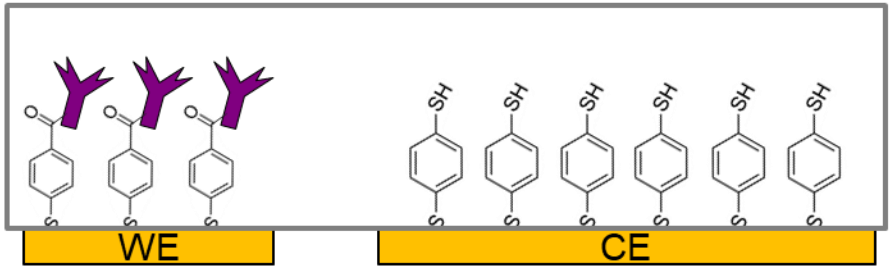


4) **Anti-troponin I: coupling via NHS ester**

NHS: N-hydroxysuccinimide

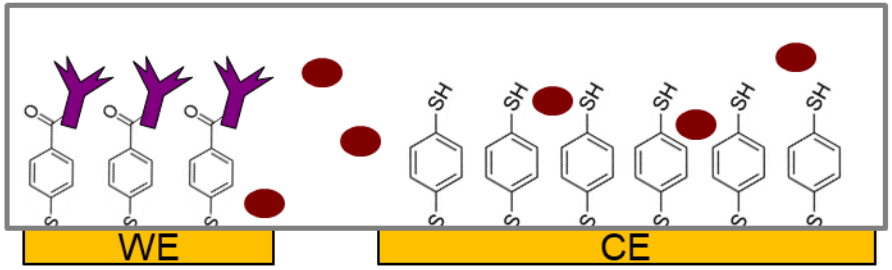


5) **Bonding of PDMS microfluidic channel**



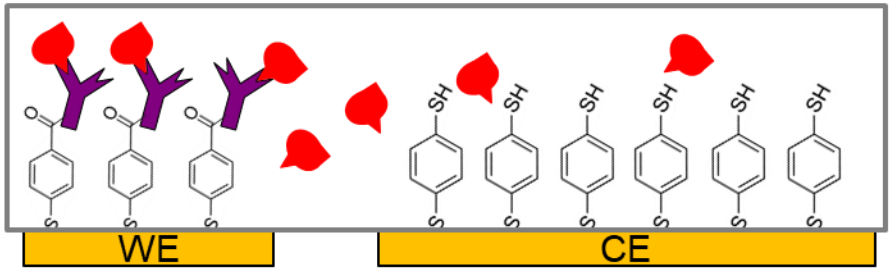
6) **Blocking with 1000 ng/mL HSA** in the flow

HSA: human serum albumin



7) **Sampling with 1 ng/mL cTnI** in the flow

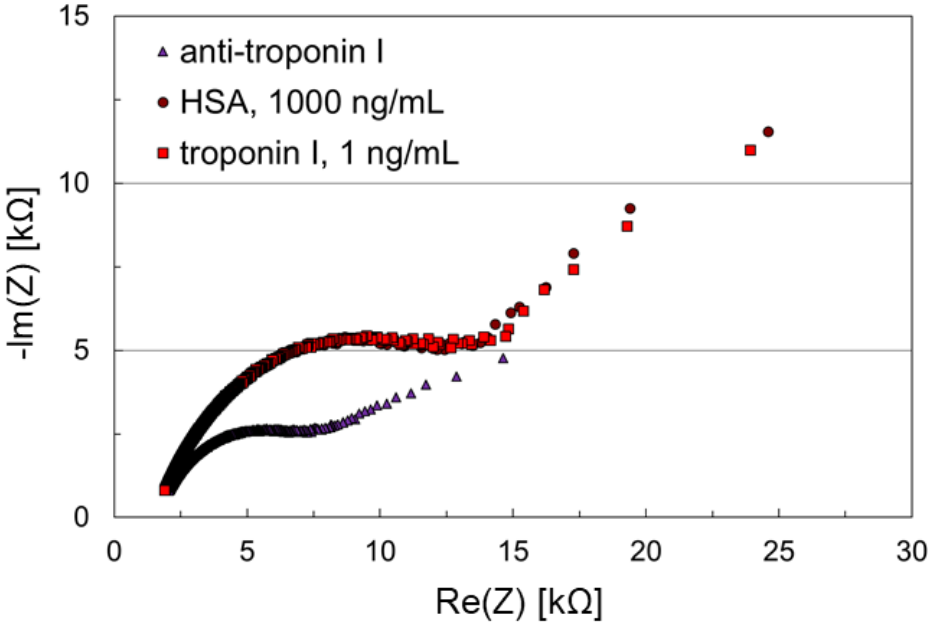
cTnI: cardiac troponin I



Thiol-SAM with Hydrocarbon Spacer: Antibody Immobilization and Troponin I Assay

Experimental:

- **Working electrode:**
  4 mercaptobenzoic acid SAM
  + **anti-troponin I** (via NHS ester)
- **Counter electrode:**
  1,4-benezenedithiol SAM
- **HSA** and **troponin I** samples were successively applied

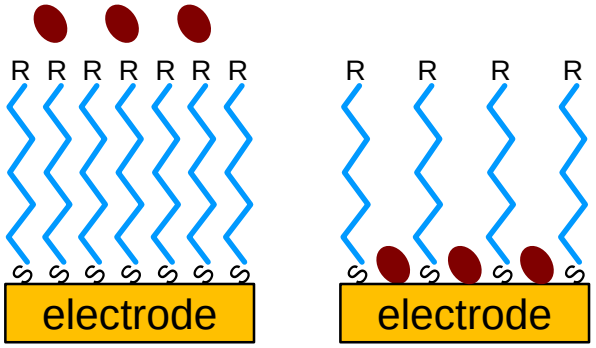


Results:

- Initial impedances increased from values < 10 kΩ (SAM) to values of almost 30 kΩ
- HSA and troponin I samples led to an increase of the Warburg impedance dominated region
- Inhibition of non-specific protein adsorption is (still) insufficient → SAM density not sufficient
- Troponin I cannot be detected

Other SAM layer required:
- Lower initial impedance
- Higher surface density

Thiol-SAM with DNA Spacer – Background



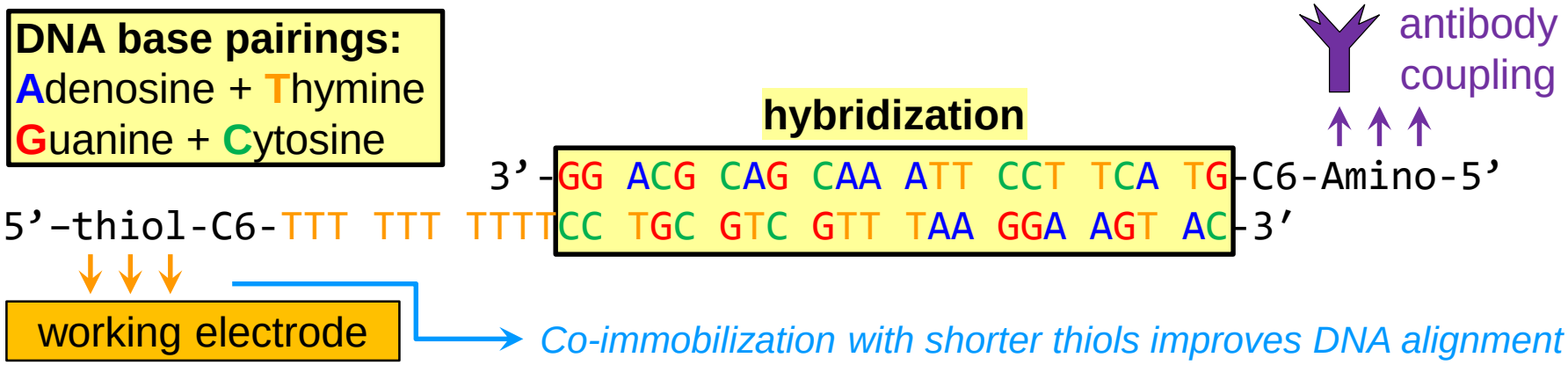
Inhibition of non-specific protein adsorption
by high surface density of thiol-SAMs and long-chain spacers

Spacer = hydrocarbon: high initial impedance

Spacer = DNA = 2 complementary single-strand DNAs (ssDNAs):

- Processable by wet chemistry, similar to thiols with hydrocarbon spacer
- Backbone made of alternating sugar (deoxyribose) and phosphate groups
  → negatively charged, **promising lower initial impedance**
- Sugar carries one of four bases forming bonds between the strands

DNA base pairings:
Adenosine + **T**hymine
Guanine + **C**ytosine



Co-immobilization with shorter thiols improves DNA alignment

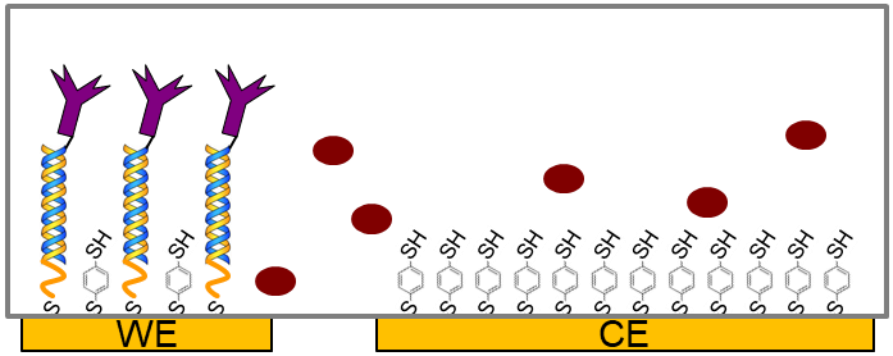
Thiol-SAM with DNA Spacer: Antibody Immobilization and Troponin I Assay

WE: working electrode
CE: counter electrode

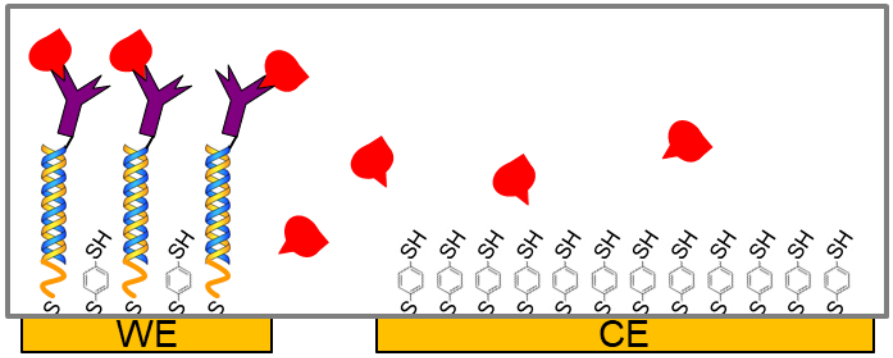
1) **Plasma activation**
2) **Mixture of thiol-ssDNA and 1,4-benzenedithiol: SAM on WE**
3) **1,4-Benzenedithiol: SAM on CE**

4) **Amino-ssDNA: hybridization**
5) **Glutaric anhydride: conversion of the amino groups to carboxyl groups**
6) **Anti-troponin I: coupling via NHS ester**
NHS: N-hydroxysuccinimide

7) **Bonding of PDMS microfluidic channel**
8) **Blocking with 1000 ng/mL HSA** in the flow
HSA: human serum albumin



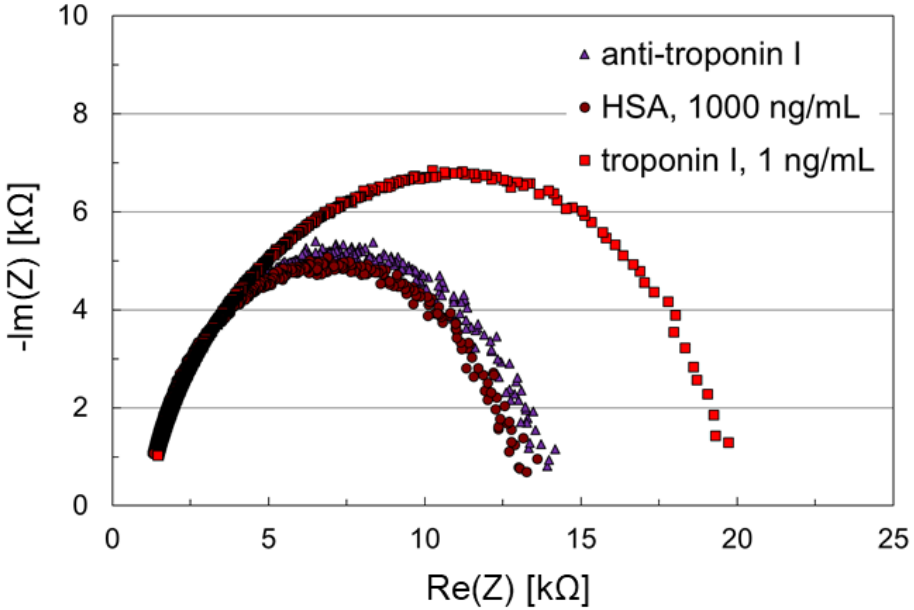
9) **Sampling with 1 ng/mL cTnI** in the flow
cTnI: cardiac troponin I



Thiol-SAM with DNA Spacer: Antibody Immobilization and Troponin I Assay

Experimental:

- **Working electrode:** DNA SAM: {thiol-ssDNA + 1,4-benezenedithiol} + amino-ssDNA + dicarboxylic acid + **anti-troponin I** (via NHS ester)
- **Counter electrode:** 1,4-benezenedithiol SAM
- **HSA** and **troponin I** samples were successively applied



Results:

- **Detection of 1 ng/nL troponin I**
- **Excellent inhibition of non-specific adsorption of 1000 ng/mL HSA**
- Initial impedances dropped back to values < 20 kΩ
- No Warburg impedance dominated region
- Sampling time: few minutes
- Measurement time (working range < 20 MHz): few seconds

Summary

- Microfluidic impedance biosensor chip:
  Own design; chip is fully functional and ready for biosensor assays
- Detection of cardiac troponin I:
  Best results obtained with thiol-SAM with DNA-spacer
  - Low initial impedance
  - Excellent inhibition of non-specific protein adsorption
  - Detection of 1 ng/mL troponin I
  - Only a few minutes required for sample application and measurement

Outlook

- Troponin I detection in real samples
- Detection of other cardiac markers
- Development of a microfluidic impedance biosensor array chip

Contact: kerstin.laenge@kit.edu