Electrochemical Sensing of Cancer Biomarkers

Pedro Estrela

Coordinator of the Marie Curie ITN PROSENSE

Department of Electronic & Electrical Engineering

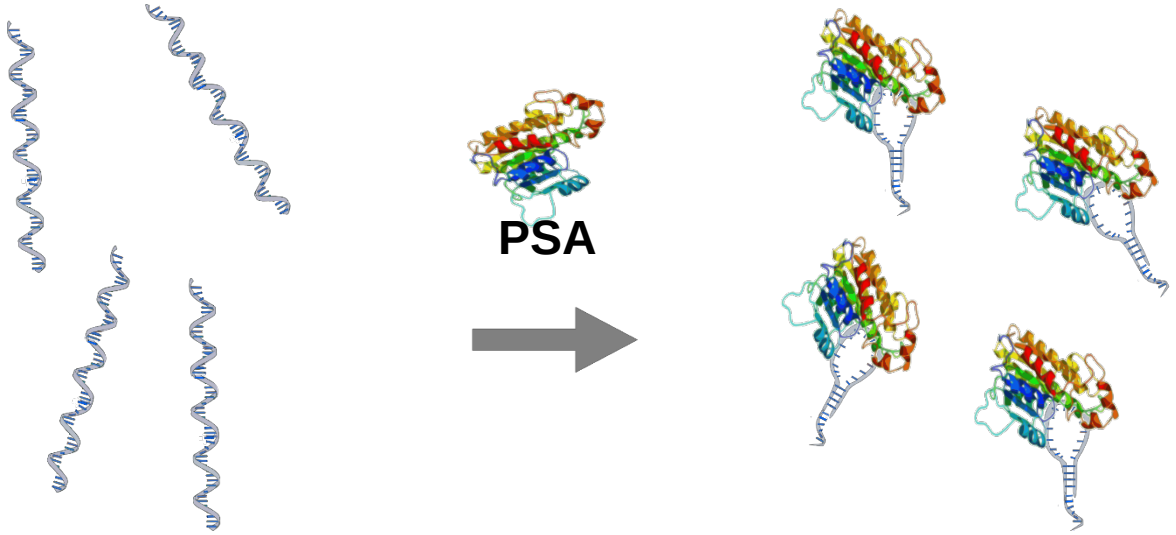
University of Bath, UK

Contents

- DNA Aptamers
- Aptamer Self-Assembled Monolayers (PSA)
  - Binary SAM
  - Sulfo-betaine moiety
- Aptamer within Molecular Imprinted Polymers (PSA)
- Aptamer on polypyrrole
  - PEG – ANTA/Cu^{2+} (AMACR)
  - Direct linking (PSA)
- microRNAs
  - PNA-AuNP

DNA Aptamers

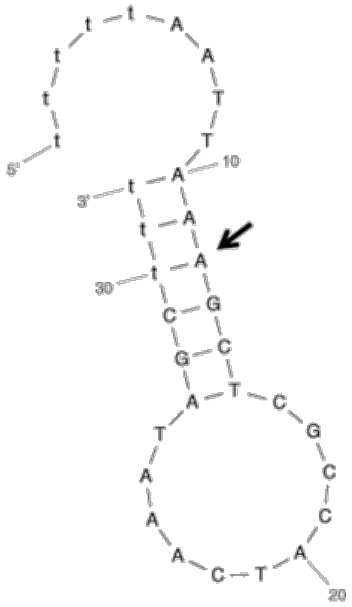
- DNA aptamers are single-stranded DNA that can bind to their targets with high affinity and specificity by undergoing conformational changes
- DNA aptamers have a number of advantages over antibodies, in particular with regards to their lower cost, easy manipulation and potential for controlled chemical attachment to electrodes



DNA Aptamers

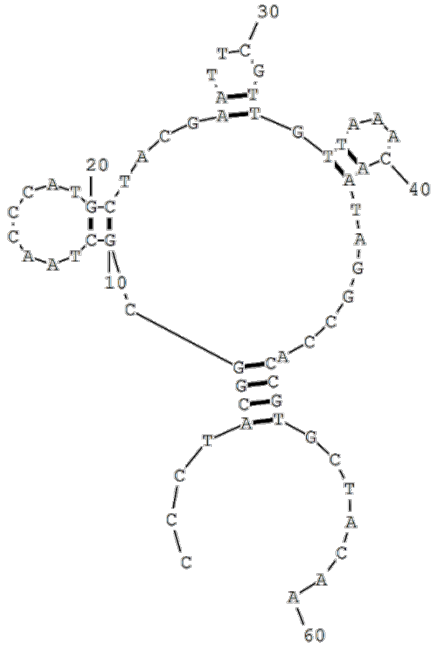
- Different DNA aptamers have different secondary structures

Single stem and loop



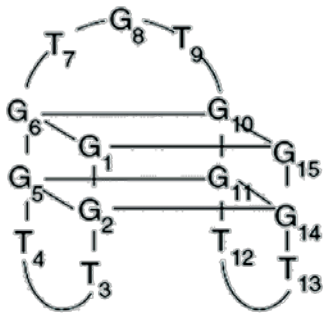
PSA aptamer

Multiple stem and loop



AMACR aptamer

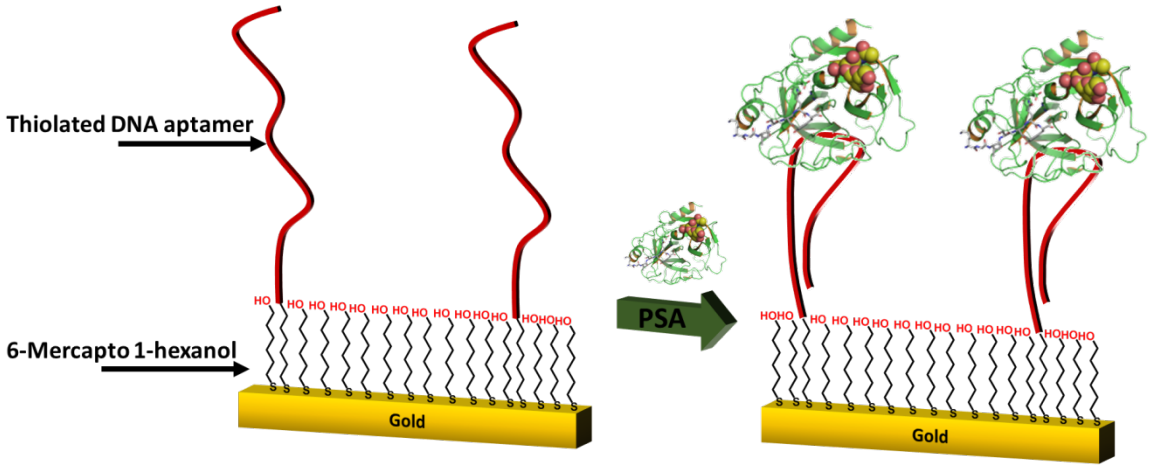
Quadruplex



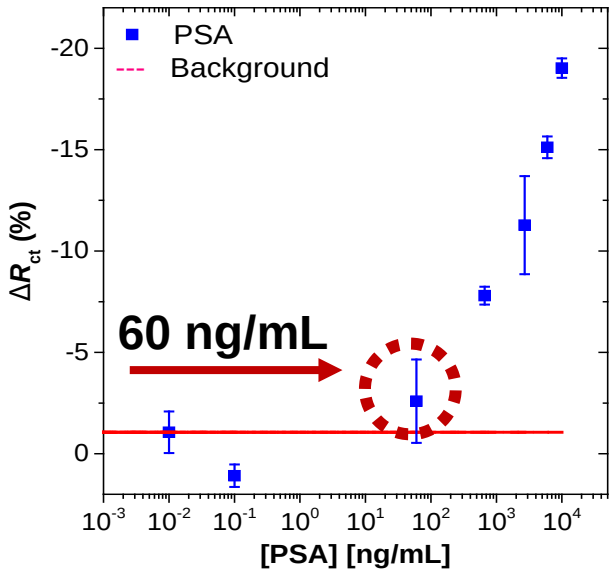
Thrombin aptamer

Aptamer SAMs

A. Direct immobilisation (binary SAM, thiol chemistry)



- **Detection via electrochemical impedance spectroscopy (EIS) the presence of redox markers**

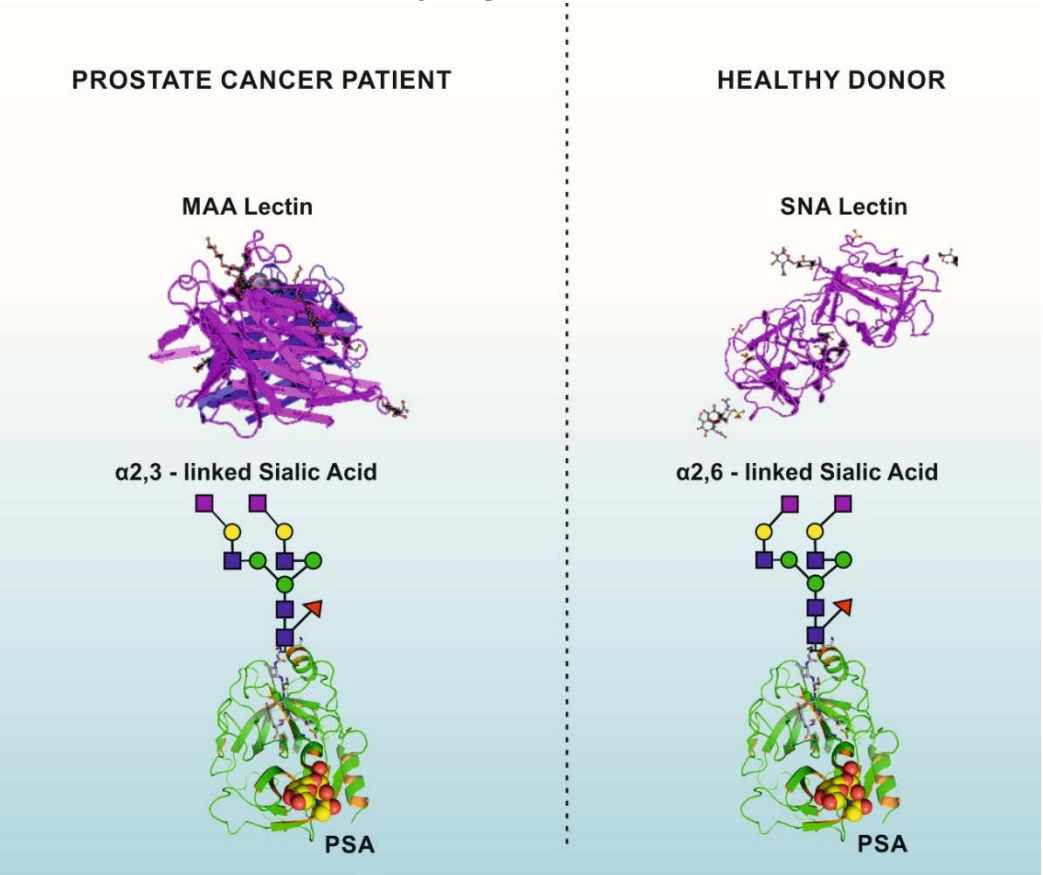


Electrochemical glycoprofiling of PCa biomarkers

Glycosylation – post-translational modification that attaches glycans (carbohydrate chains) to proteins, lipids, or other organic molecules

Glycoprofiling – determining the glycan composition of the protein, cell, tissue, etc

Aberrant glycosylation – characteristic for tumorigenesis, indication of cancer
→ studying structure of the oncomarker, rather than its level (PSA)



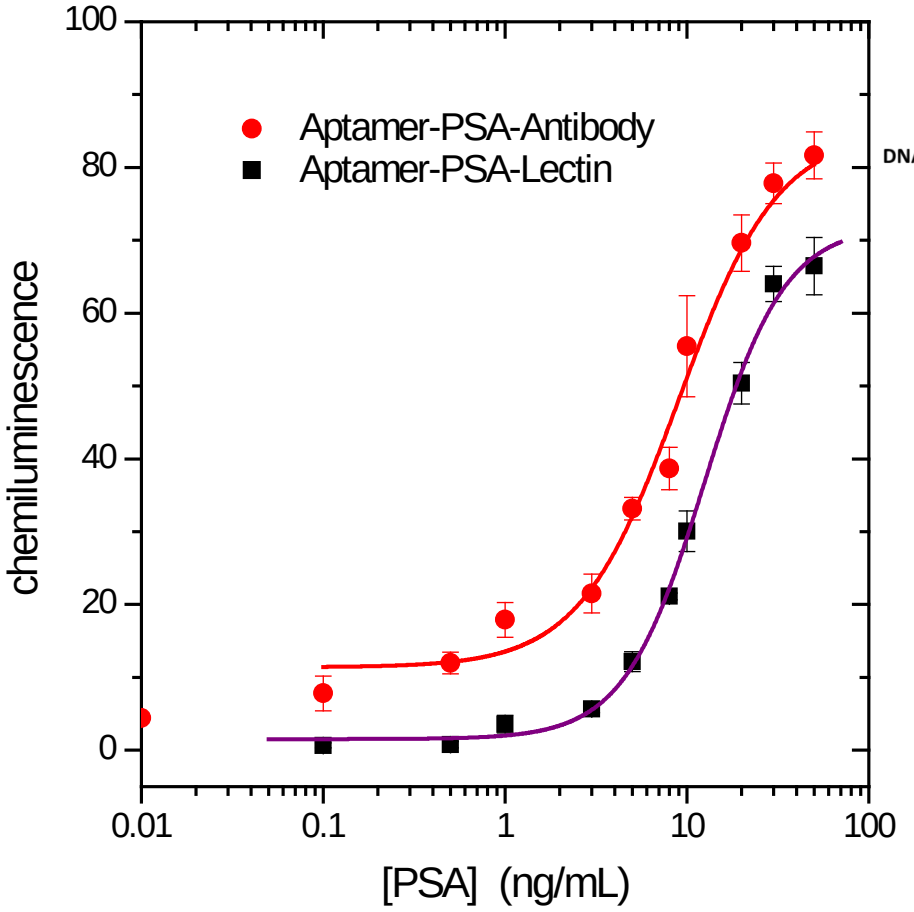
Lectins – proteins that react specifically with glycosidic residues of other molecules, act as a biorecognition element

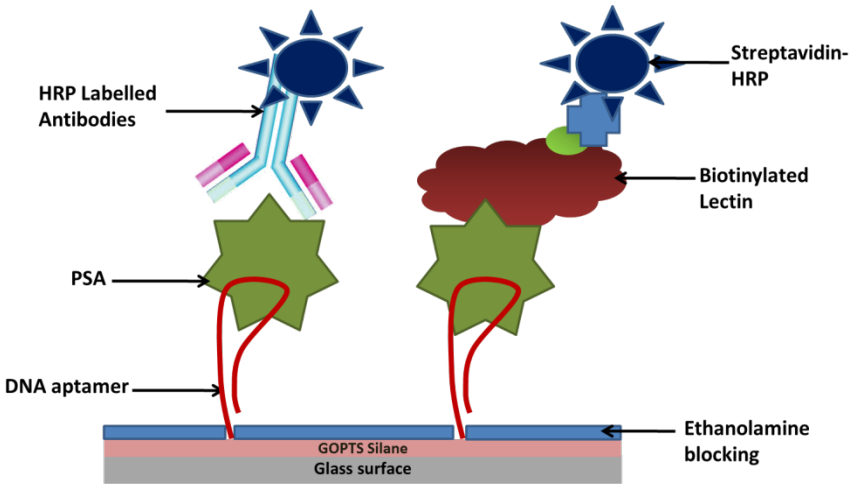
DNA aptamers / Lectins

UNIVERSITY OF BATH

Prostate Specific Antigen (PSA)

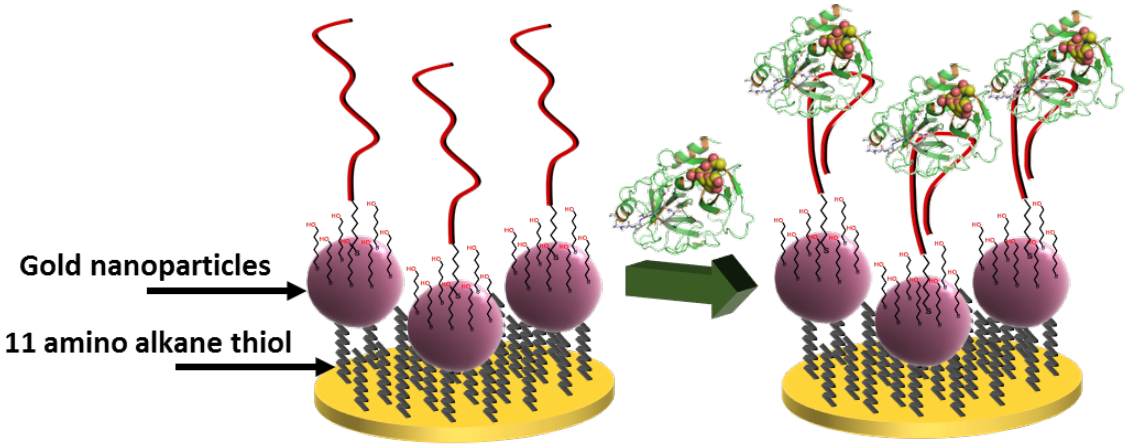
PSA + glycosilated PSA

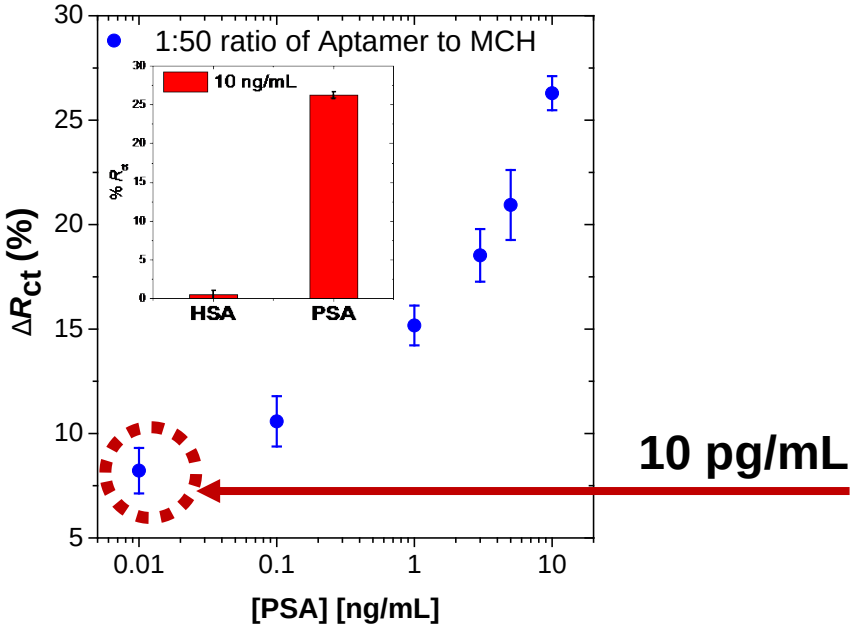




Aptamer SAMs

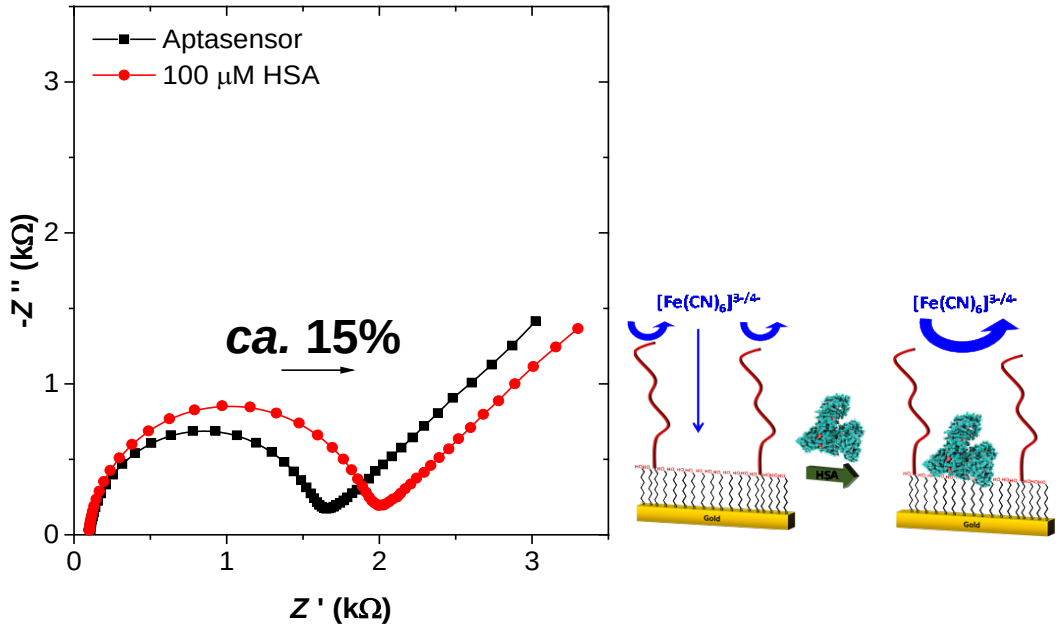
B. Immobilisation on self assembled gold nanoparticles



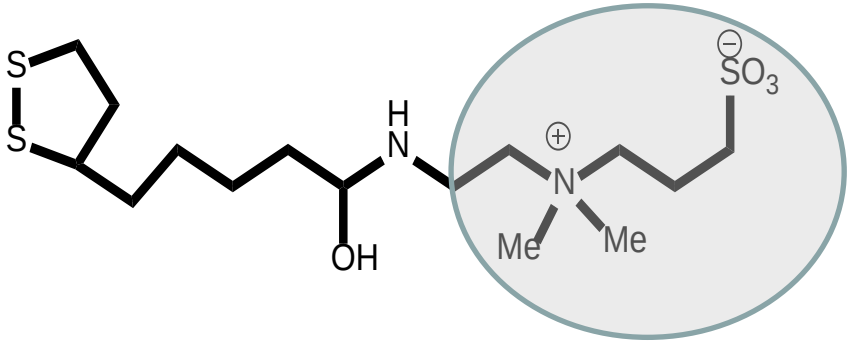


Aptamer SAMs

- **High non-specific binding with mercapto-hexanol (MCH) based SAM**



- **Alternative: Use of antifouling surface chemistry (Sulfo betaine moiety)**

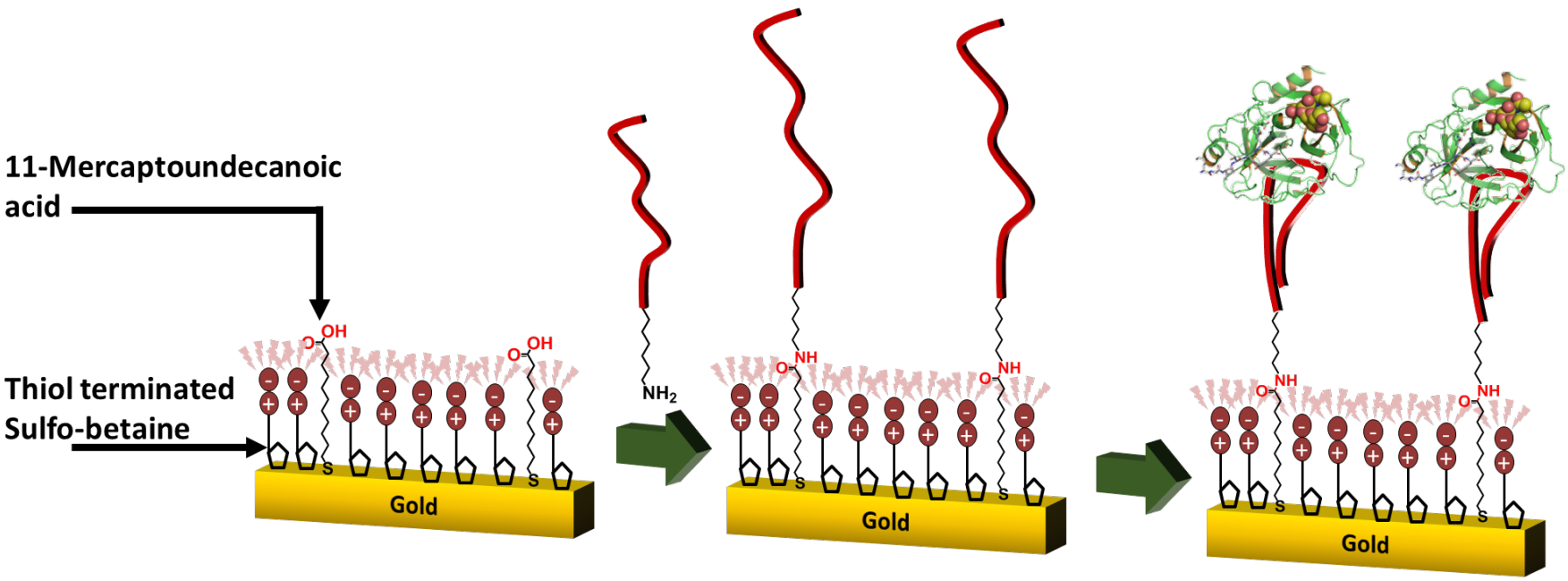


Jolly *et al.*, *Sens. Actuators B* 209 (2015) 306

Aptamer SAMs

UNIVERSITY OF BATH

C. Use of Antifouling surface chemistry (Sulfo-betaine moiety)

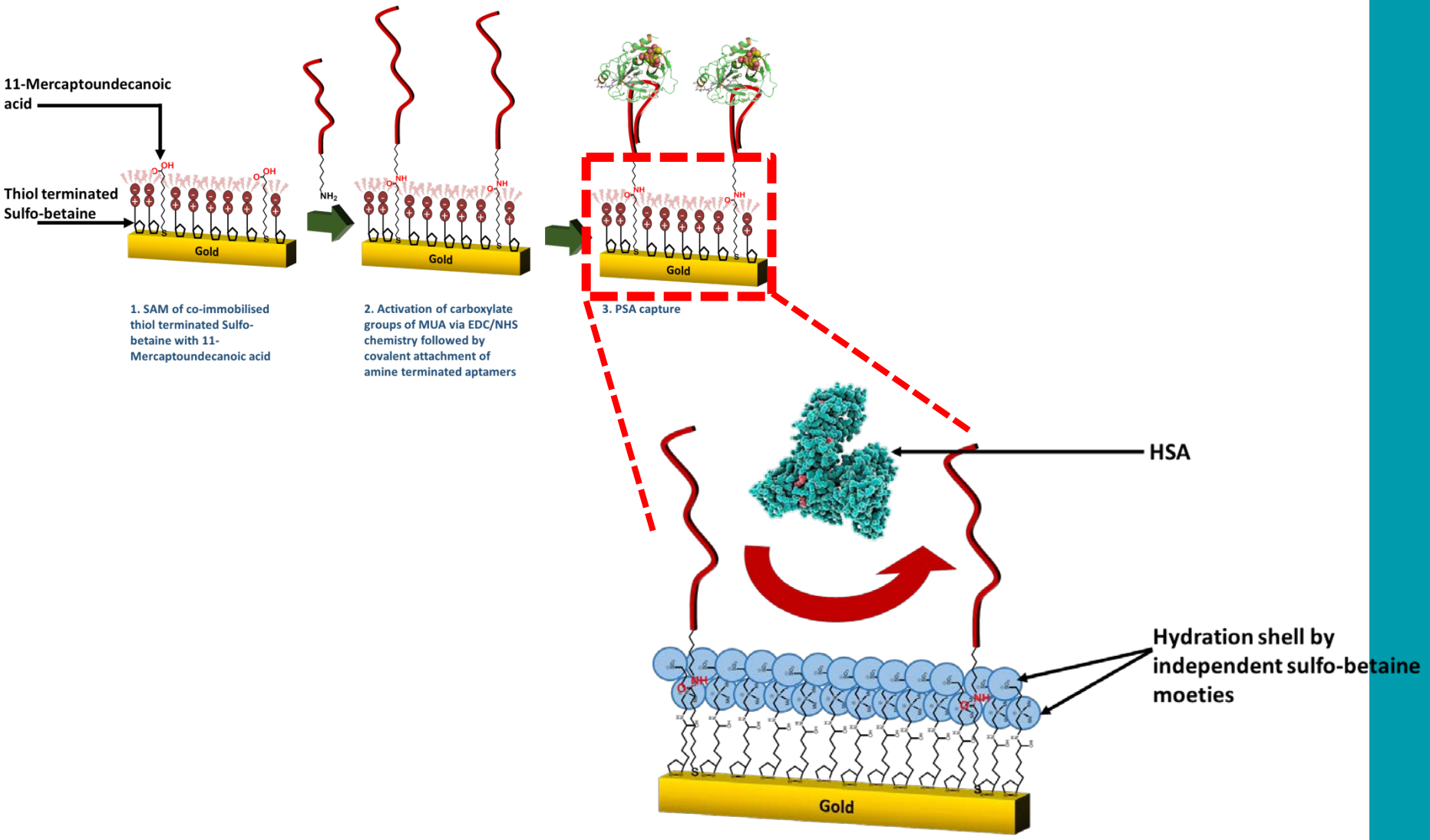


Jolly *et al.*, *Sens. Actuators B* 209 (2015) 306

Aptamer SAMs

UNIVERSITY OF BATH

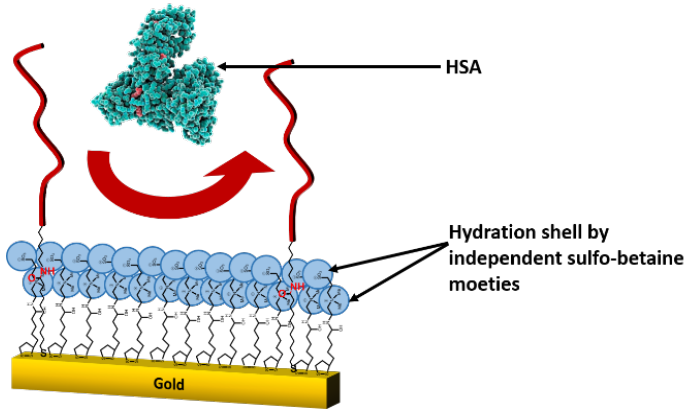
C. Use of Antifouling surface chemistry (Sulfo-betaine moiety)

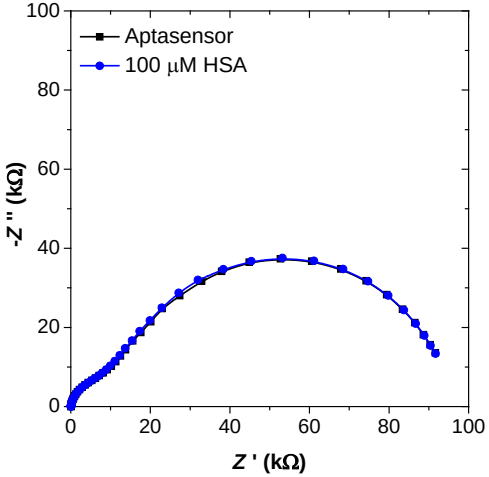


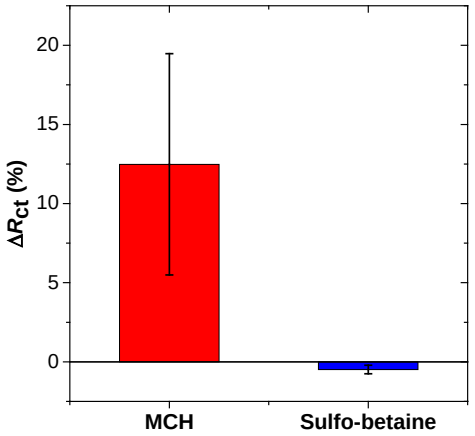
Jolly *et al.*, *Sens. Actuators B* 209 (2015) 306

Aptamer SAMs

- **Reduction of non-specific binding of HSA to less than 2%**

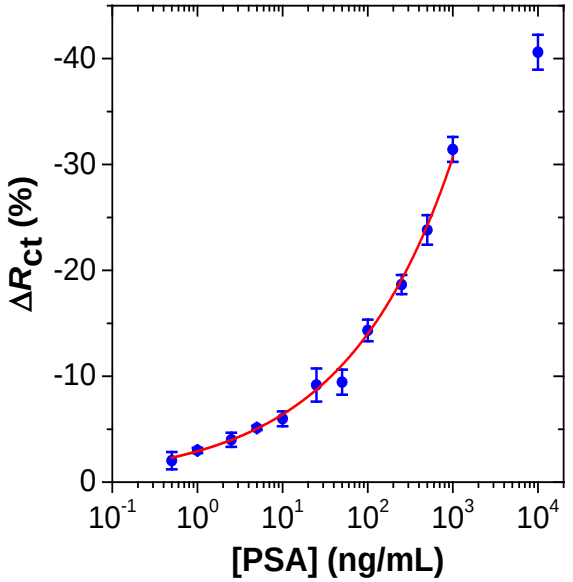






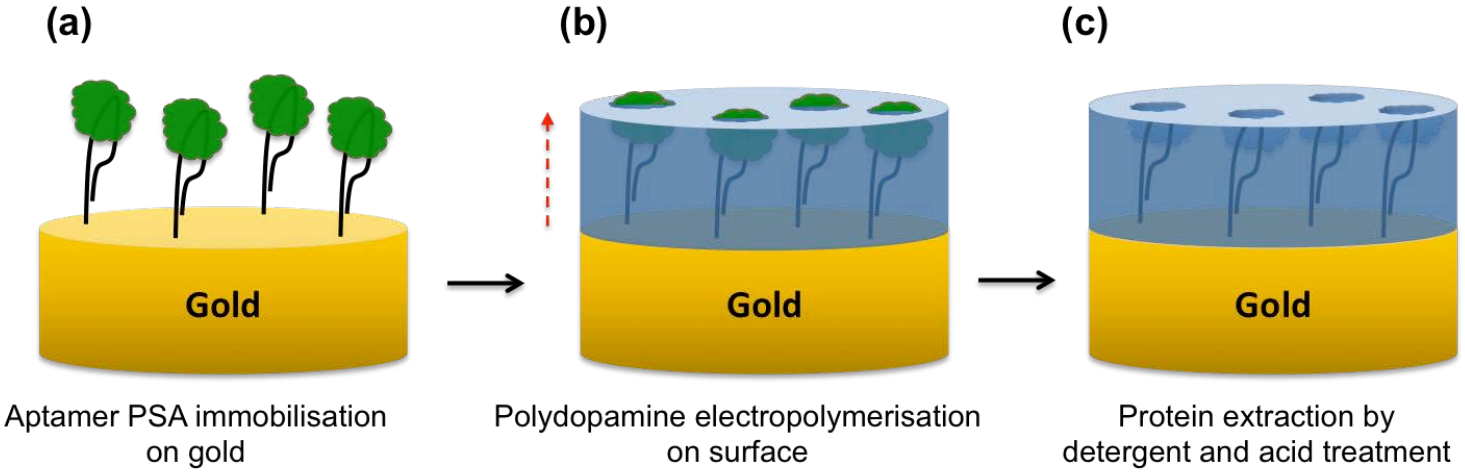
- **Increased sensitivity due to combined effect of linker length and sulfo-betaine**

Detection down to 1 ng/mL (60 times lower than MCH-based surface chemistry)



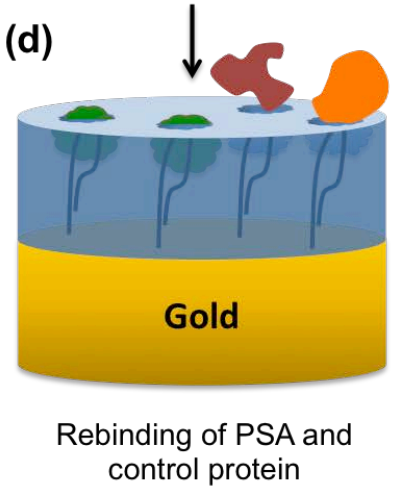
Aptamer MIPs

- **Hybrid DNA aptamer / molecular imprinted polymer**



Advantages:

- DNA aptamers used for controlled surface chemistry
- Resistant to stringent fabrication process: polymerisation, washing with 5% SDS and 5% acetic acid

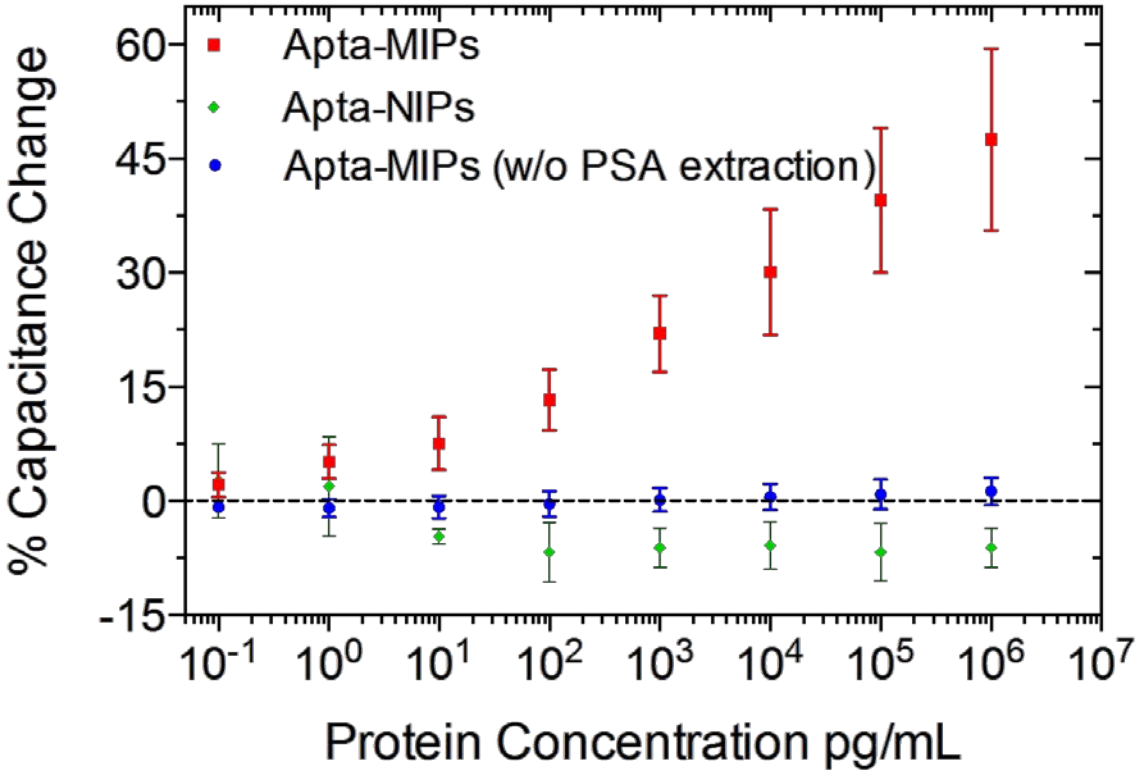


Jolly *et al.*, *Biosens. Bioelectron.* 75 (2016) 188

Aptamer MIPs

- **Increased sensitivity which can be attributed to imprinting effects**

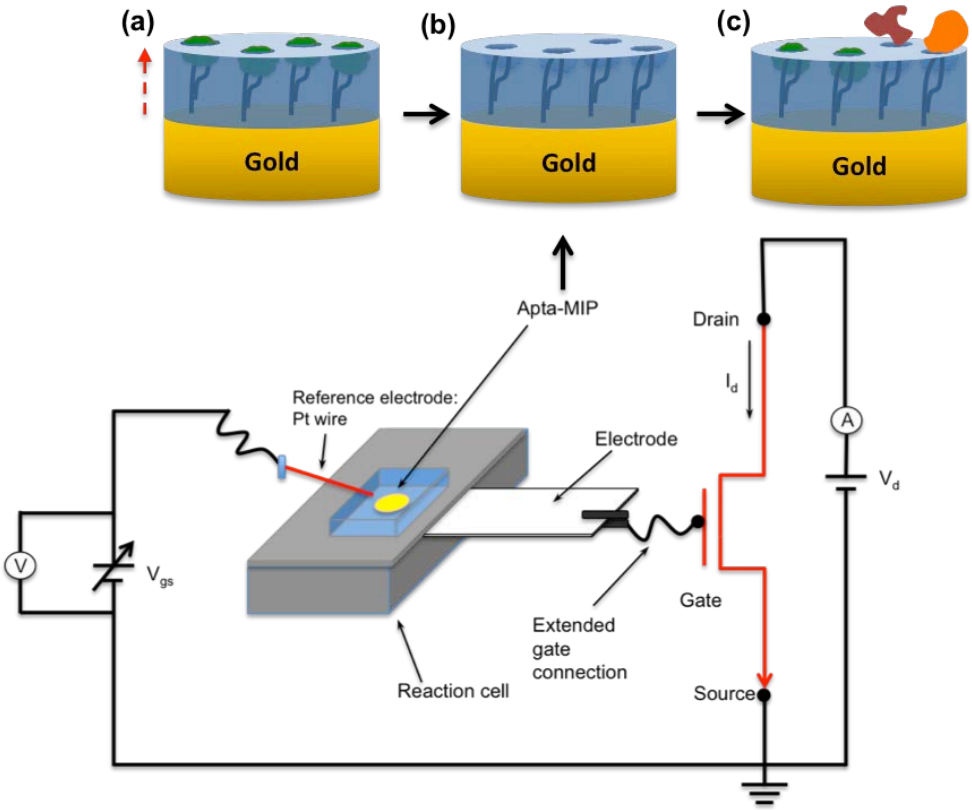
Detection down to 1 pg/mL (1000 times lower than betaine-based surface chemistry)

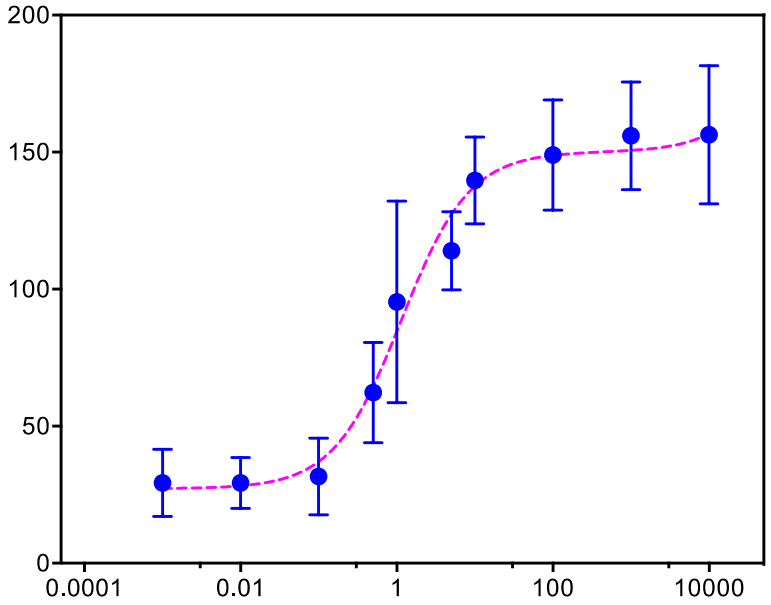


- **Potentially minimise nuclease degradation**

Tamboli *et al.*, submitted

Aptamer MIPs: BioFET in serum





- **Detection of low levels of PSA in serum**

AMACR (α-methylacyl-CoA racemase)

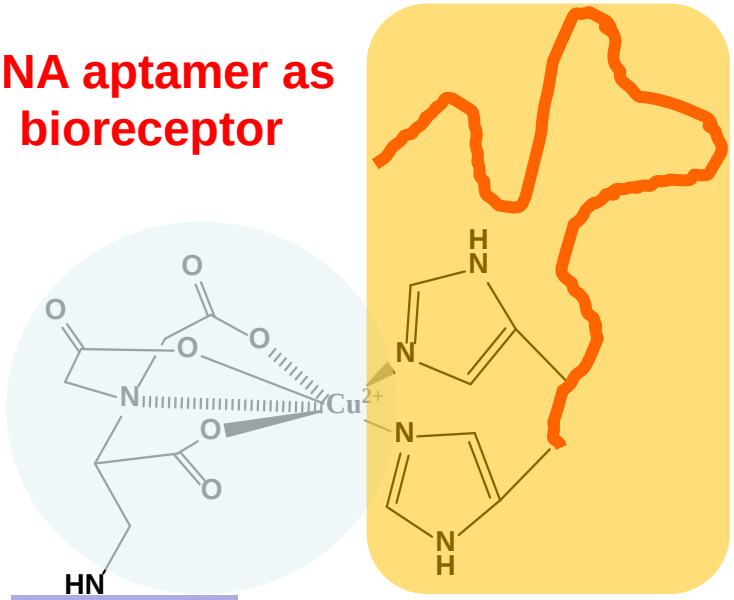
- In mammalian cells, the enzyme is responsible for converting (2*R*)-methylacyl-CoA esters to their (2*S*)-methylacyl-CoA epimers
- Biomarker for prostate cancer with high sensitivity of 77.8% and specificity of 80.6%
- It is still a tissue biomarker but studies have shown its presence in blood in the range of µg/mL and fg/mL in urine samples
- Have high potential to complement PSA screening in identifying patients with clinically significant prostate cancer, especially those with intermediate PSA levels

Jolly *et al.*, submitted

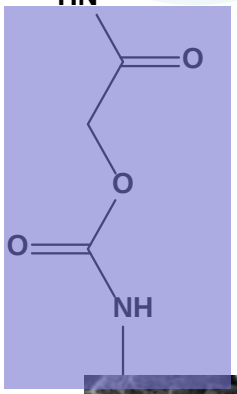
Aptamer on polypyrrole

DNA aptamer as bioreceptor

ANTA-Copper complex as redox marker

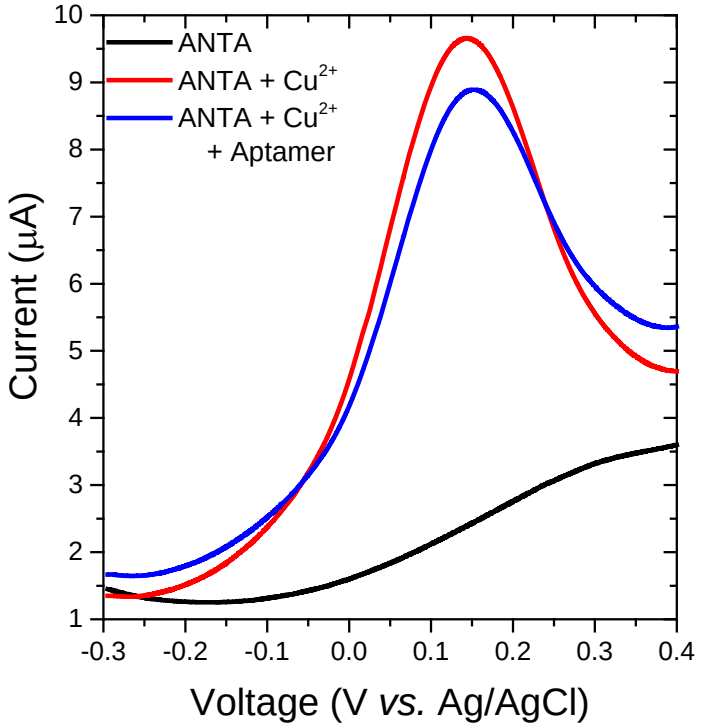


Polyethylene glycol for antifouling properties



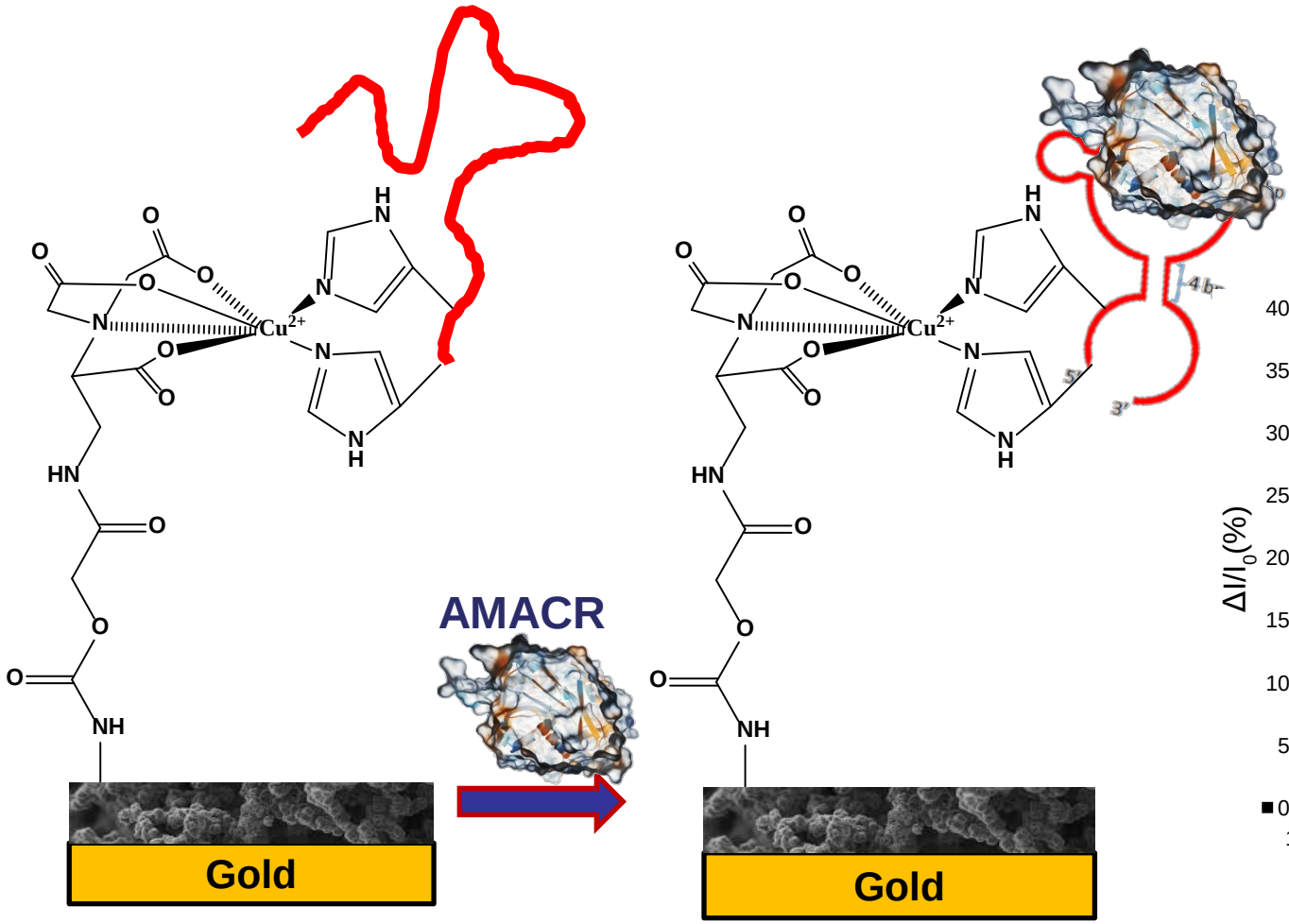
Polypyrrole as sensor surface

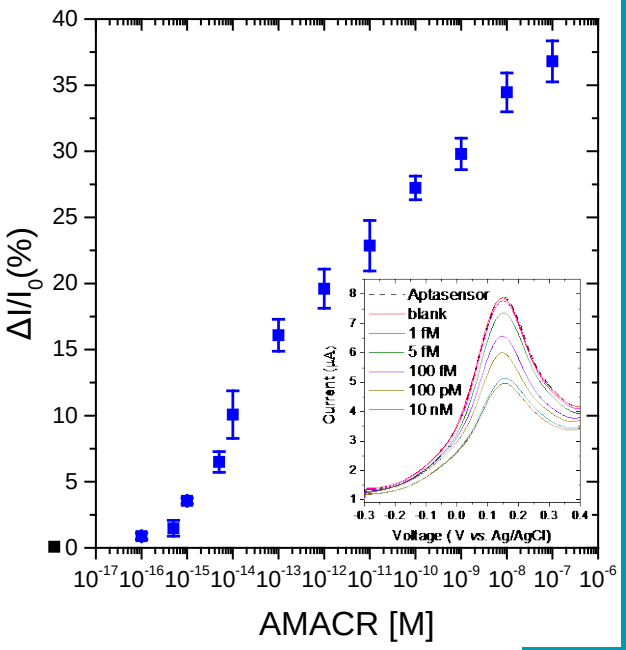
Square Wave Voltammetry



Aptamer on polypyrrole

Capture of AMACR

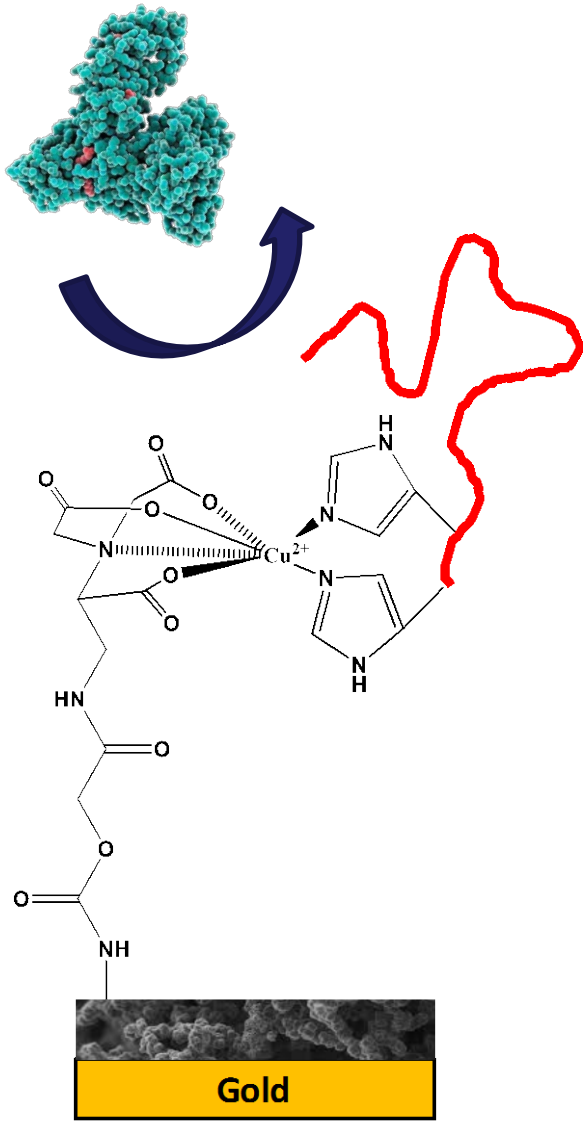




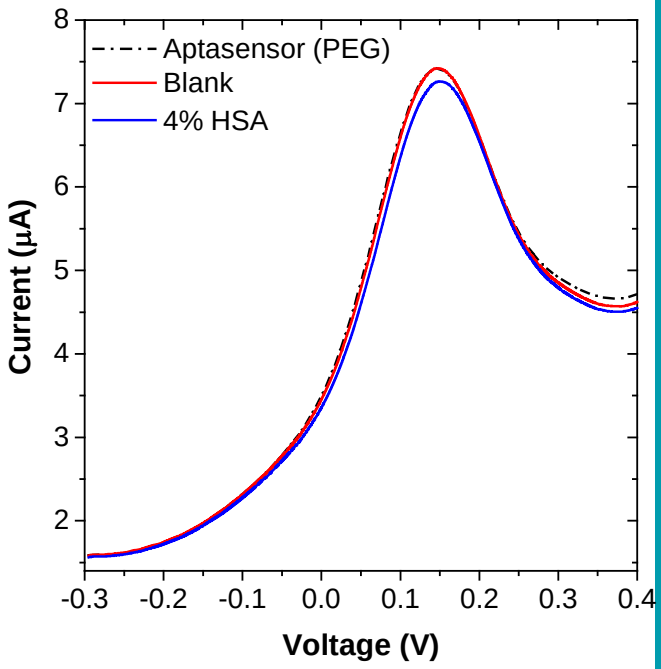
Jolly *et al.*, submitted

Aptamer on polypyrrole

Specificity study: 4% Human Serum Albumin (HSA)



Polyethylene glycol (PEG) based surface chemistry



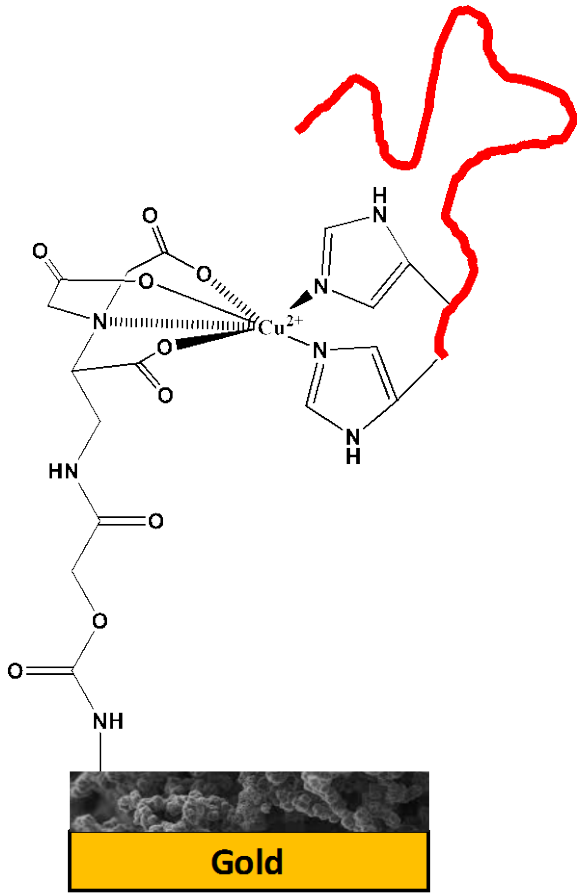
< 3% change in signal on incubation with 4 % HSA for 30 min

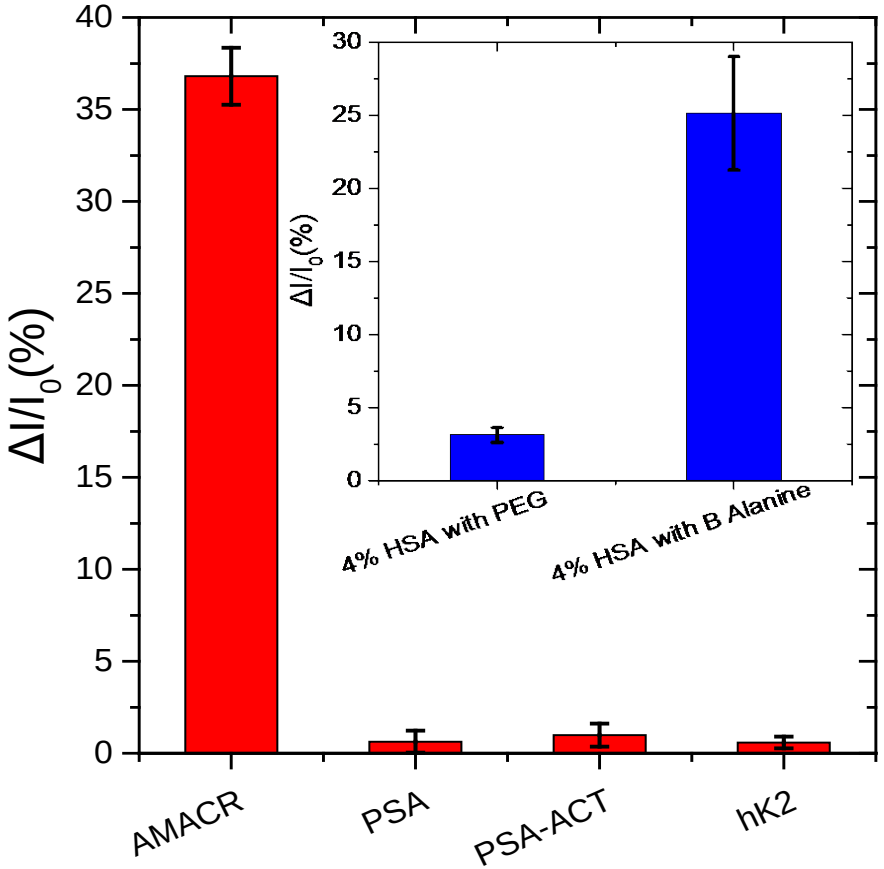
Jolly *et al.*, submitted

Aptamer on polypyrrole

Specificity study: other prostate cancer biomarkers

Negligible signal change with other prostate cancer biomarkers
(all proteins used were of same concentration 100 nM)



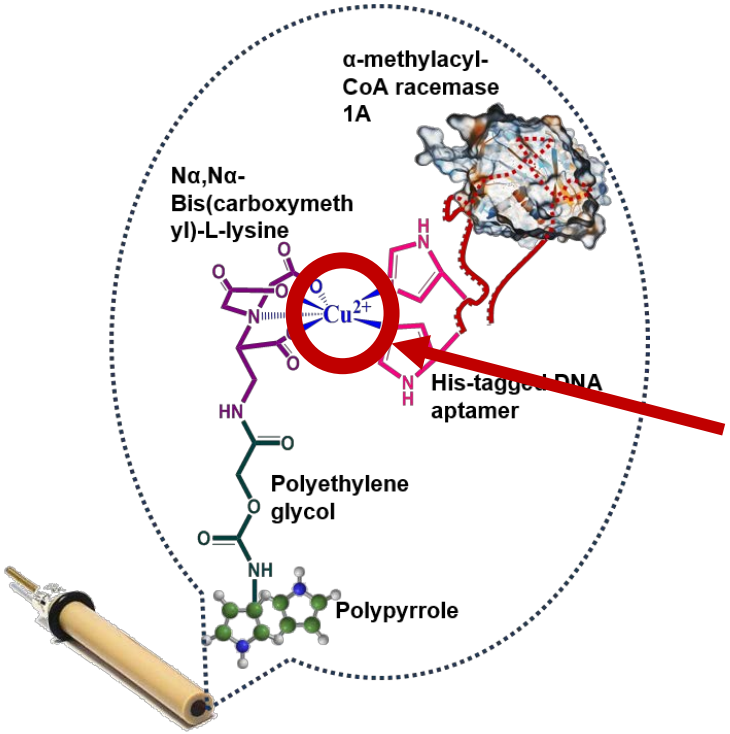


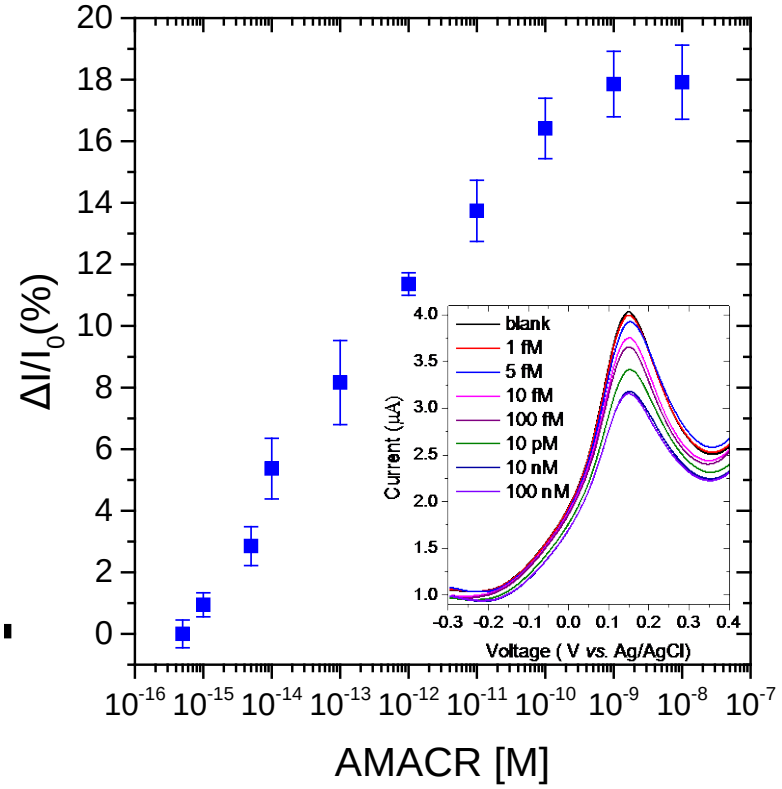
Jolly *et al.*, submitted

Aptamer on polypyrrole

Detection in human plasma samples

- Detection via square wave voltammetry
- Broad range from 0.1 fM to 10 nM
- Detection limit down to 1.4 fM



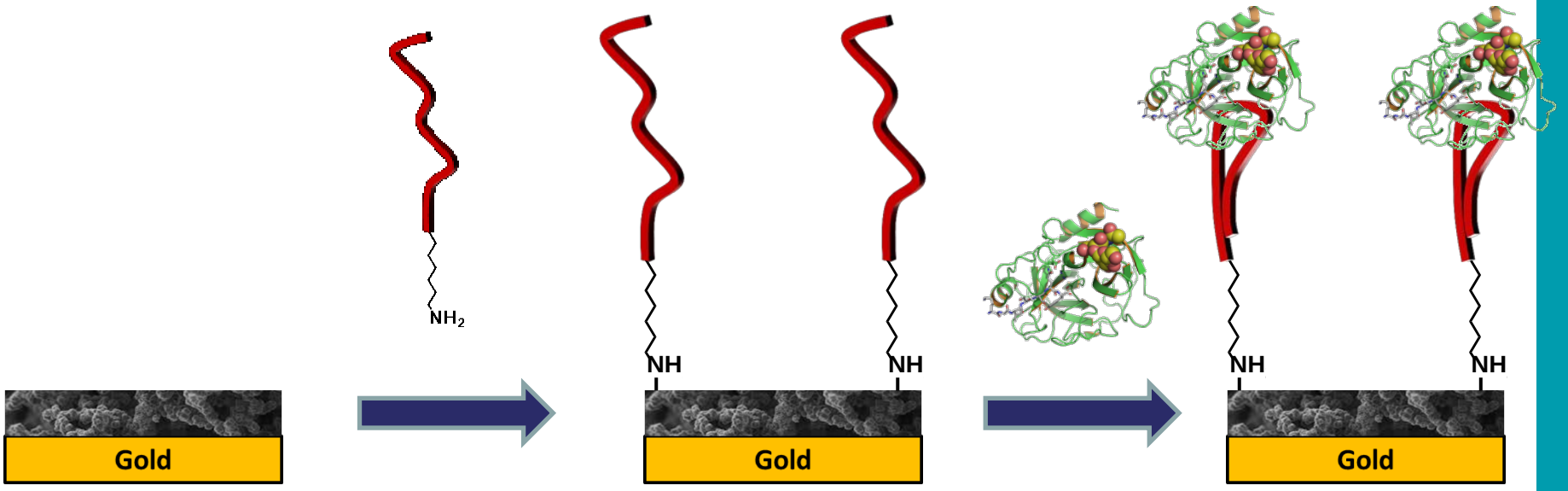


- Potential to develop multiplexed platform
- Copper can be replaced with other metal ions (nickel, zinc, etc.)

Aptamer on polypyrrole (direct)

Functionalisation of polypyrrole with carboxylate groups

- **One-step easy and fast deposition of probes bearing amines**
- **Detection method: EIS without any redox marker**

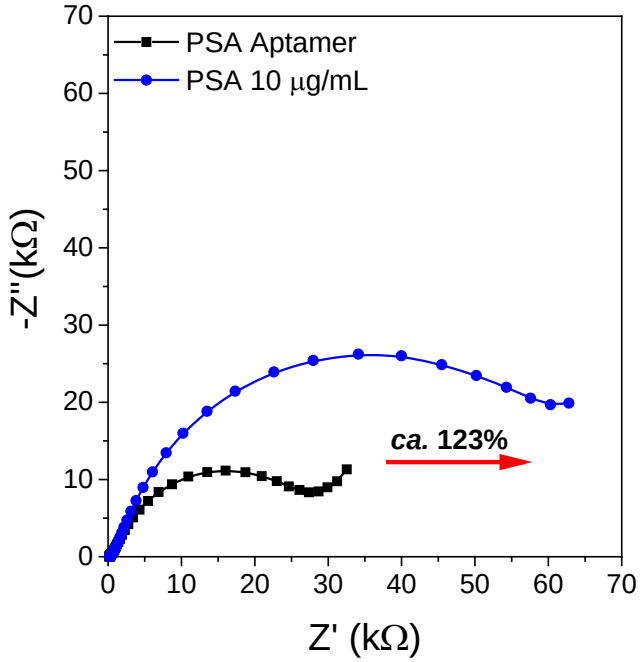


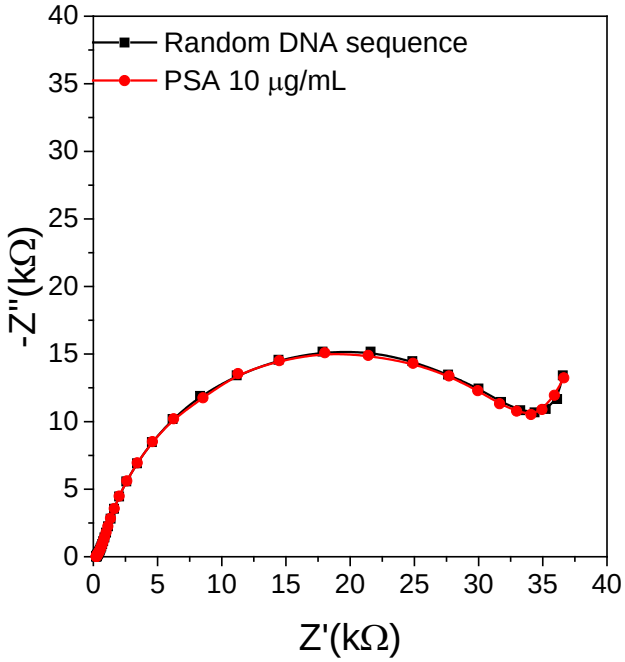
Electrochemical deposition of amine terminated aptamers

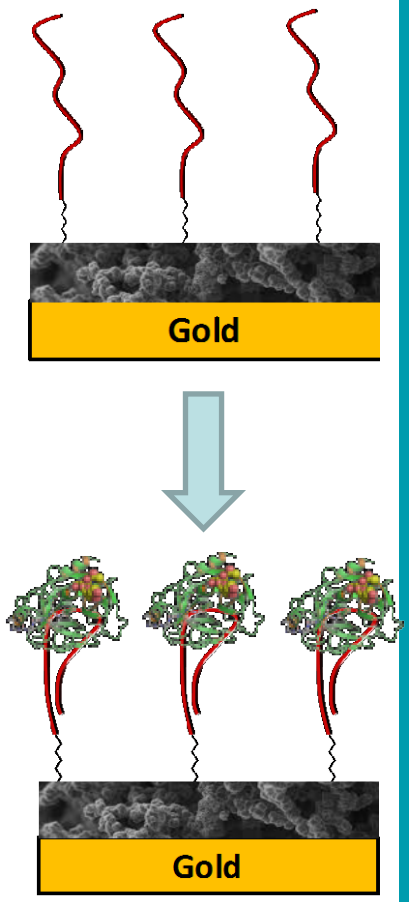
Capture of PSA

Aptamer on polypyrrole (direct)

- **Preliminary experiments with PSA aptamers**
- **Also being used for DNA/DNA hybridisation**

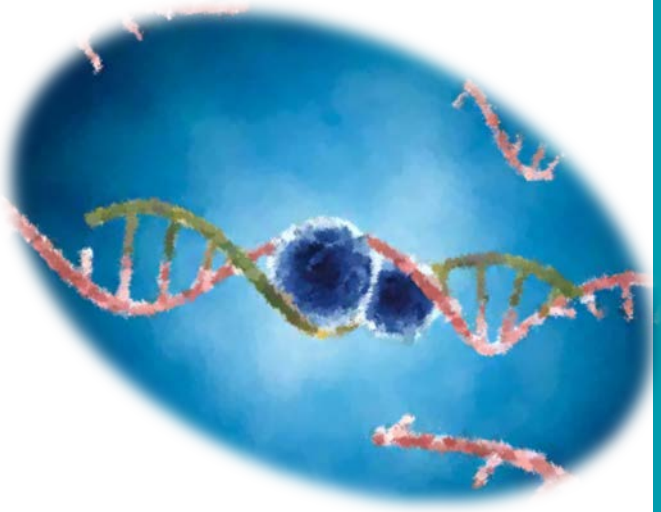






- **Negligible signal change with a random DNA sequence**

microRNAs



- Small (18-25 nt long) non-coding RNAs that are involved in regulation of gene expression (post transcriptional regulation)
- Increasing reports on role of miRNAs in **oncogenic processes** such as proliferation, apoptosis, differentiation and development of androgen independence
- Consequently, studies show that the altered levels of miRNA in blood can act like finger prints of cancer (diagnosis, prognosis and also the stage of the cancer)
- Different miRNAs are associated with different diseases and also published for essentially all cancer forms including prostate cancer

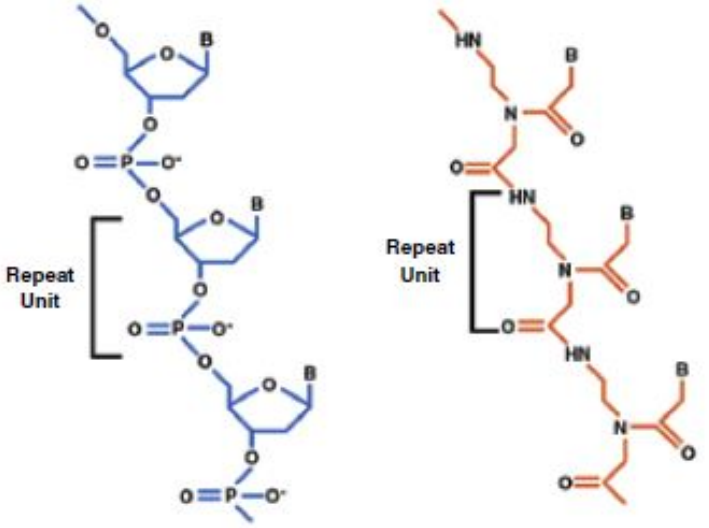
PNA

DNA-DNA interactions:

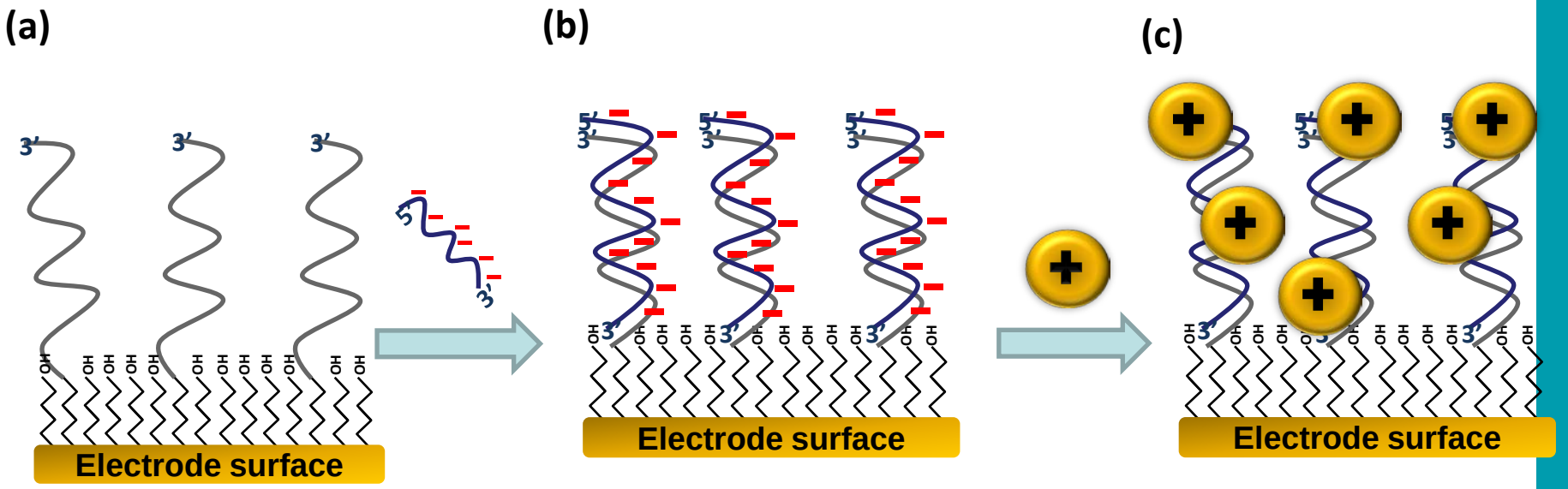
- due to charge screening / counterion condensation, change in net charge upon hybridisation is small
- formation of duplex “thickens” DNA layer, increasing electrostatic barrier to $[Fe(CN)_6]^{3-/4-}$ in-between DNA sites

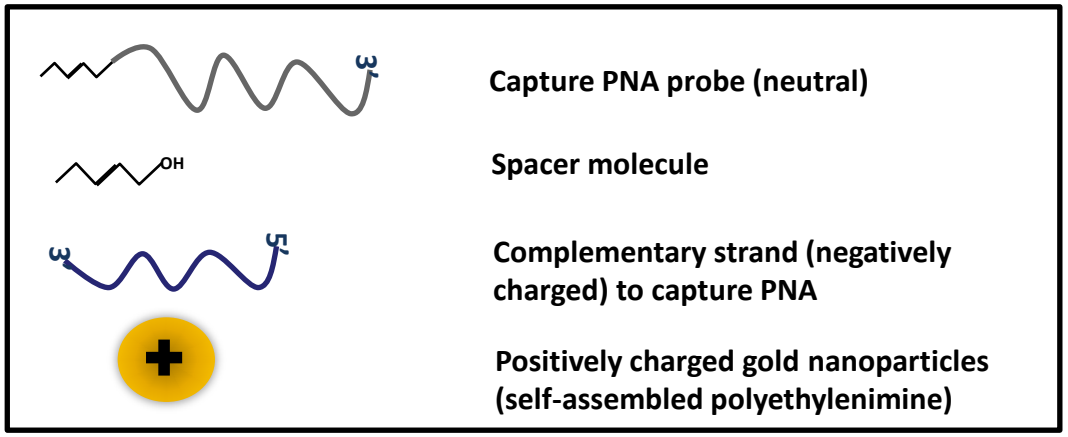
PNA-DNA interactions:

- initial probe layer has no electrostatic barrier
- hybridisation with DNA results in large increase in electrostatic barrier



EIS: PNA with AuNPs

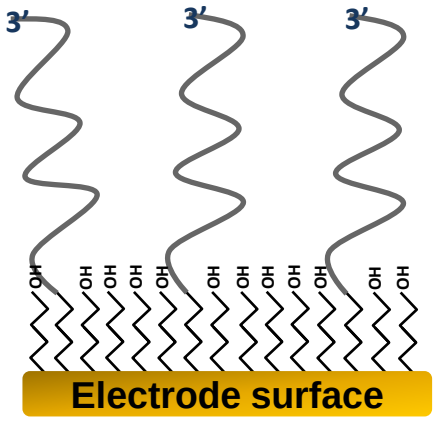


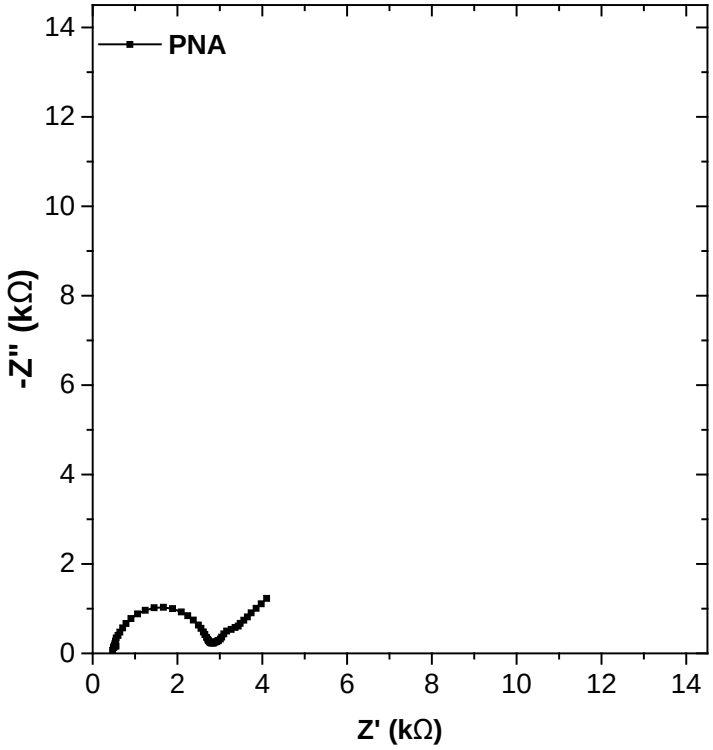


Jolly *et al.*, submitted

EIS: PNA with AuNPs

- Electrochemical Impedance Spectroscopy (EIS) was used in the presence of redox marker to confirm the concept
- PNA creates physical barrier to negatively charged redox couple in solution: $[Fe(CN)_6]^{3-/4-}$

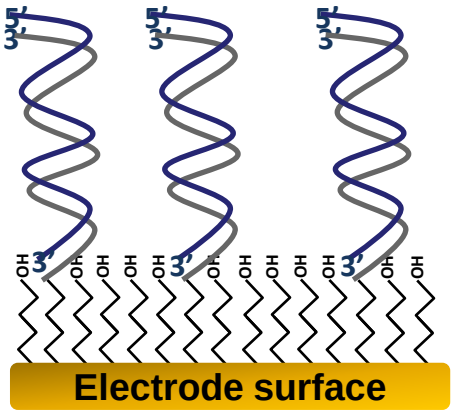


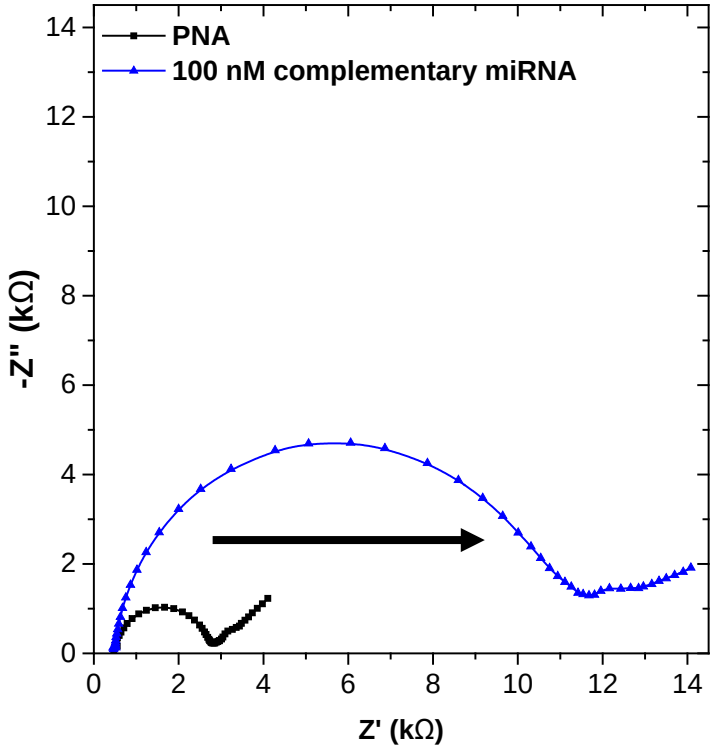


Jolly *et al.*, submitted

EIS: PNA with AuNPs

- Electrochemical Impedance Spectroscopy (EIS) was used in the presence of redox marker to confirm the concept
- PNA creates physical barrier to negatively charged redox couple in solution: $[Fe(CN)_6]^{3-/4-}$
- Charge transfer resistance (R_{ct}) significantly increased with target miRNA by increasing the electrostatic barrier

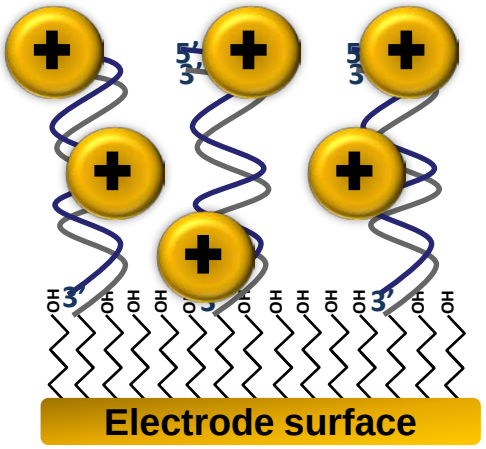


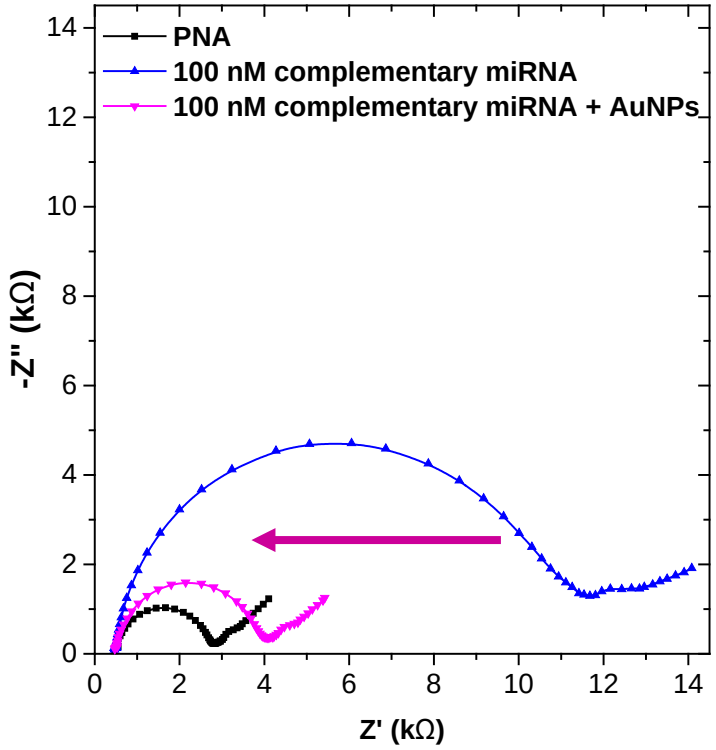


Jolly *et al.*, submitted

EIS: PNA with AuNPs

- Electrochemical Impedance Spectroscopy (EIS) was used in the presence of redox marker to confirm the concept
- PNA creates physical barrier to negatively charged redox couple in solution: $[Fe(CN)_6]^{3-/4-}$
- Charge transfer resistance (R_{ct}) significantly increased with target miRNA by increasing the electrostatic barrier
- Charge transfer resistance (R_{ct}) significantly decreased with AuNPs



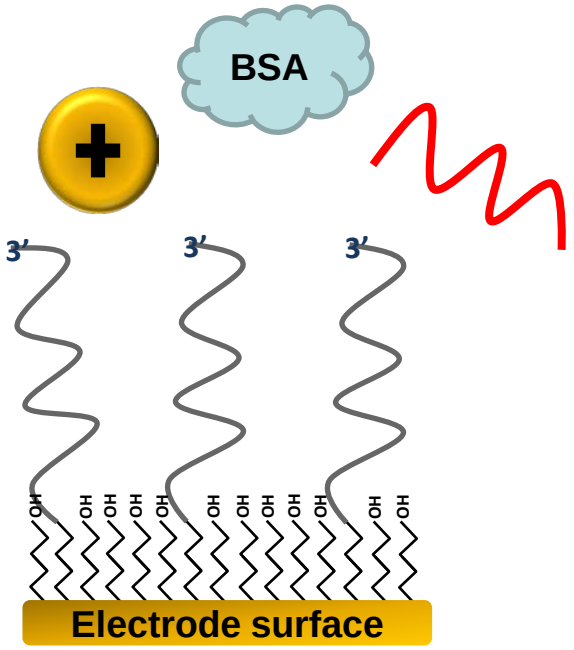


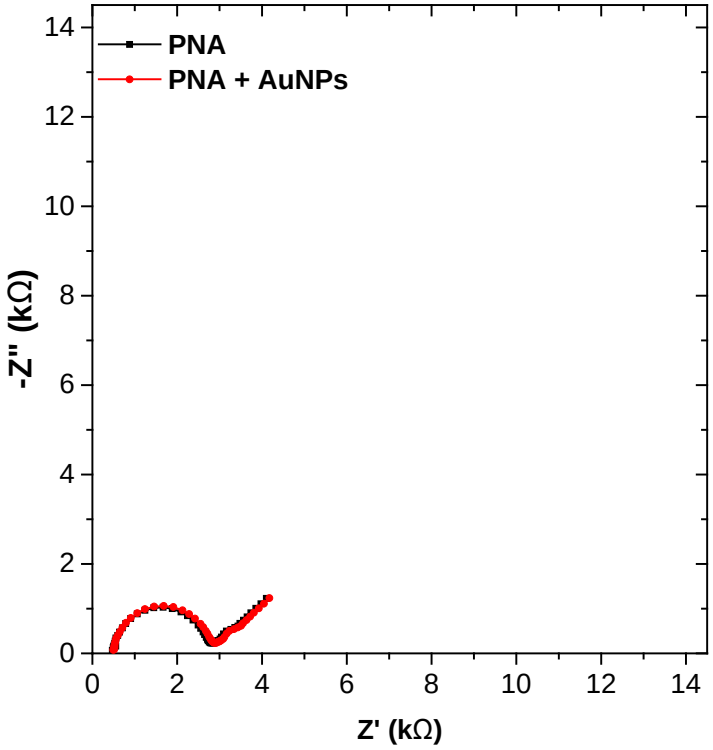
Jolly *et al.*, submitted

EIS: PNA with AuNPs

CONTROLS

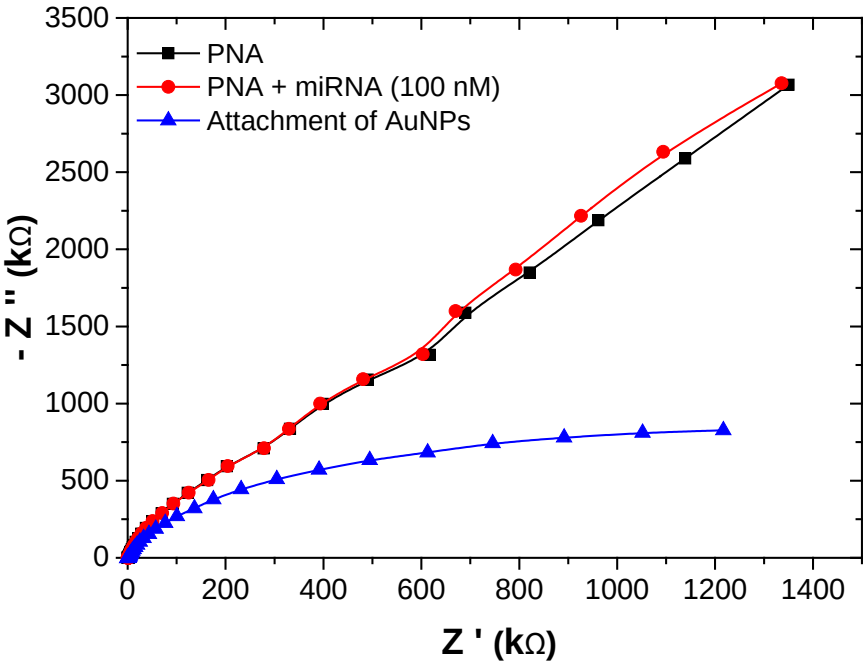
- AuNPs do not interact with SAM (red curve) (~2%)
- Negligible interactions with non-complementary DNA (1.08%) and also with BSA (Bovine Serum Albumin, 2.3%)





Jolly *et al.*, submitted

Non-Faradaic EIS: PNA with AuNPs



- Impedance measurements **without redox markers**
- Very high impedance is observed

$$C^* \equiv -1/j\omega Z$$

$$C' = \frac{-Z''}{\omega\,|Z|^2}$$

$$C'' = \frac{-Z'}{\omega\,|Z|^2}$$

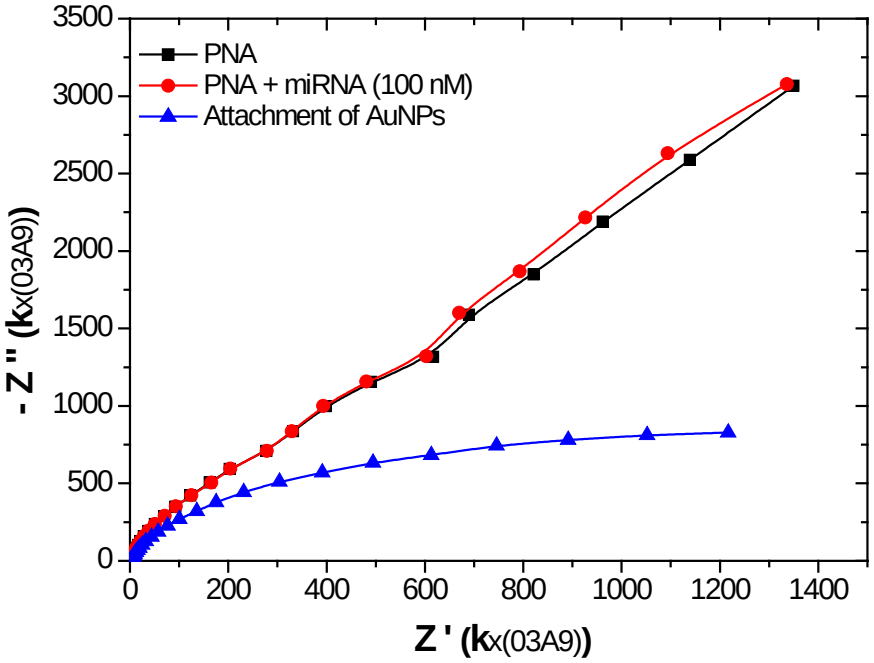
Z' : Real Part of impedance
Z'' : Imaginary part of impedance
$|Z|^2$: $((Z')^2 + (Z'')^2)$

Jolly *et al.*, submitted

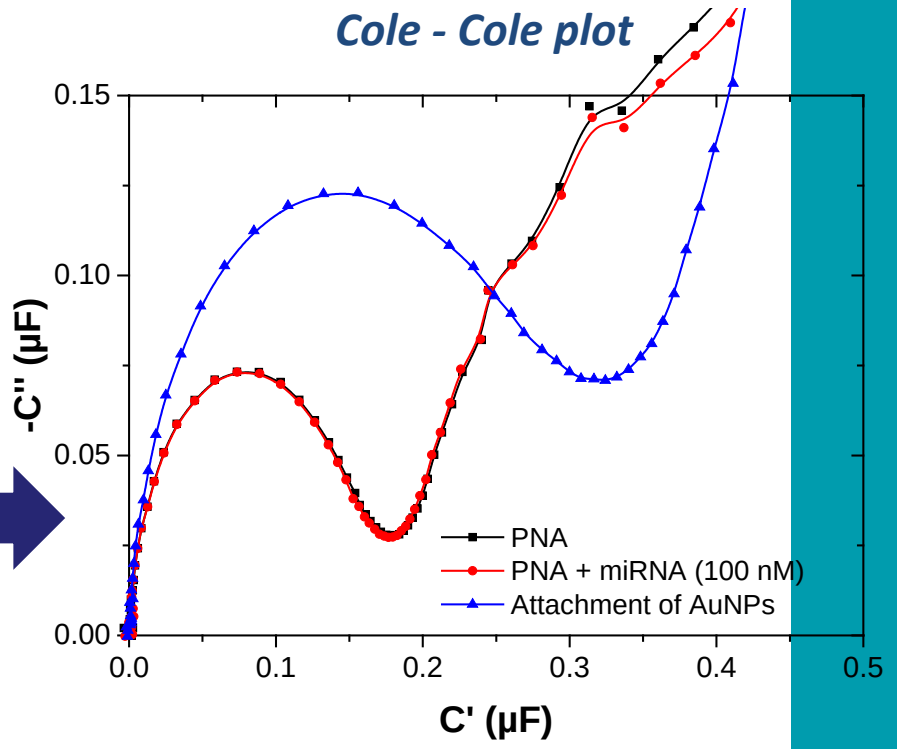
Non-Faradaic EIS: PNA with AuNPs

UNIVERSITY OF BATH

Nyquist plot



Cole - Cole plot

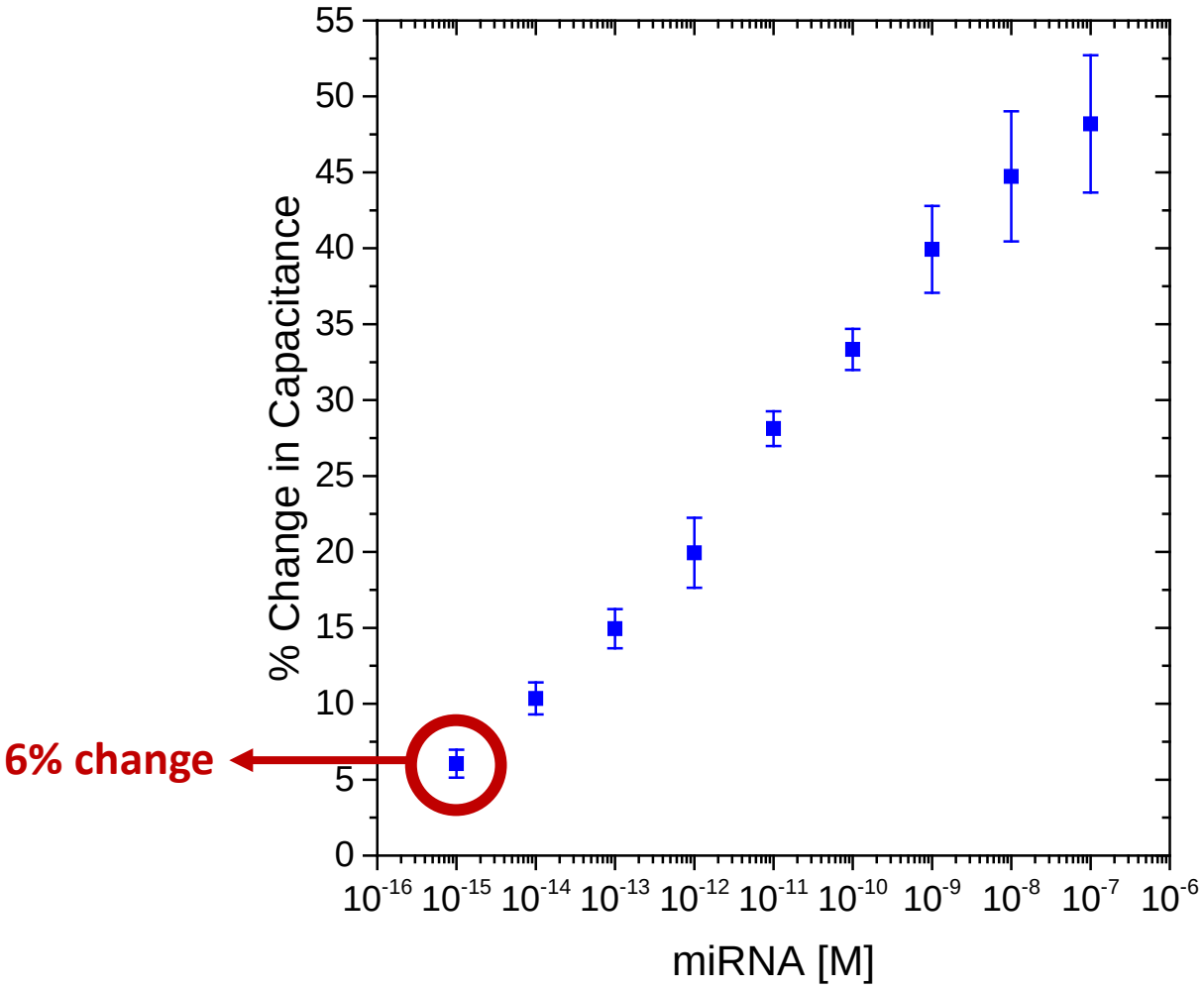


Monitoring non-Faradaic processes

Jolly *et al.*, submitted

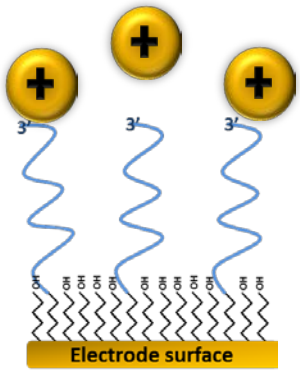
Non-Faradaic EIS: PNA with AuNPs

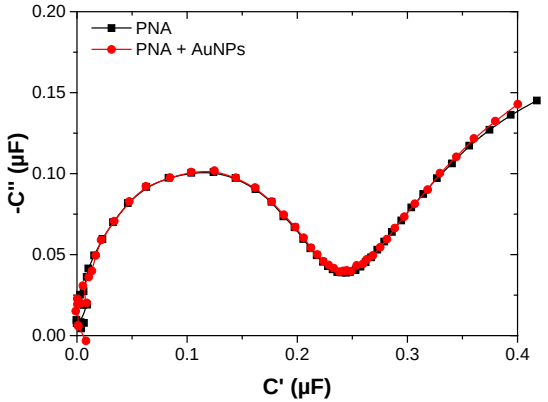
- Potential detection down to 1fM of complementary miRNA strand



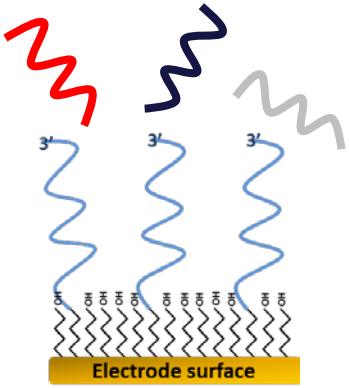
Non-Faradaic EIS: PNA with AuNPs

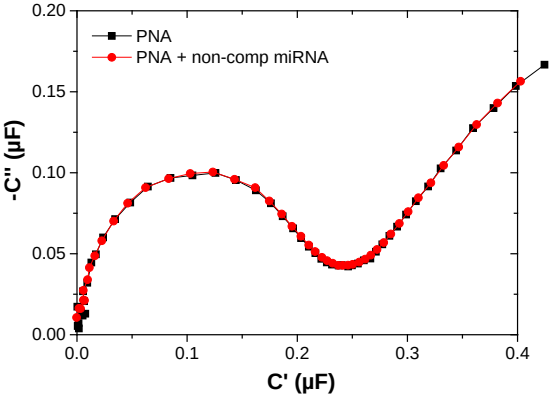
gold nanoparticles





100 nM non complementary miRNA target



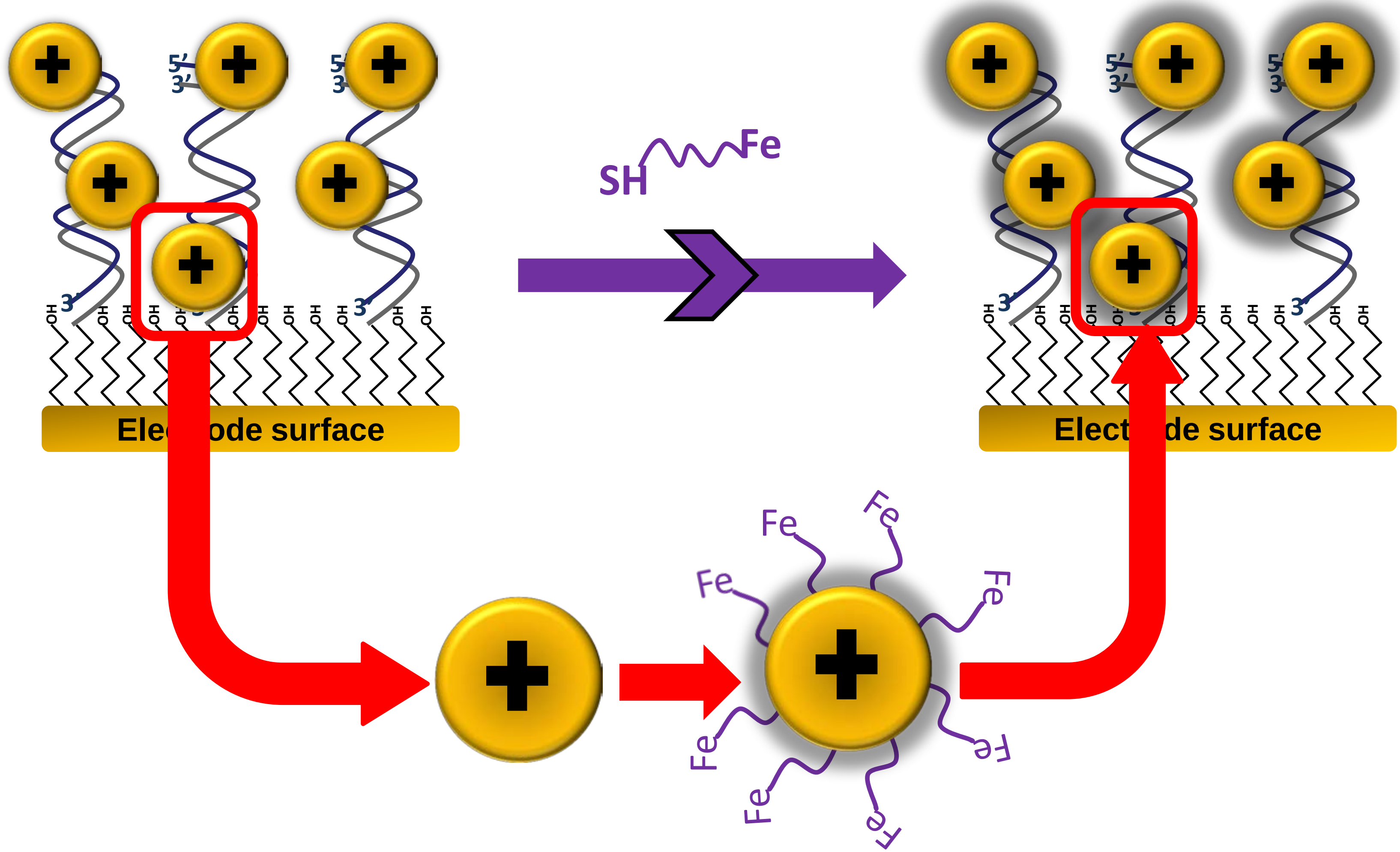


Control experiments output

- Around 1.5% capacitance change with just AuNPs was observed
- With non-complementary miRNA (100 nM), around 2% change was recorded
- With 100 nM of miRNA sequence with 2 mismatch, around 2.5% change was recorded
- With 1 mismatch sequence (100 nM), around 20% change was observed

Jolly *et al.*, submitted

Amperometric: PNA with AuNPs

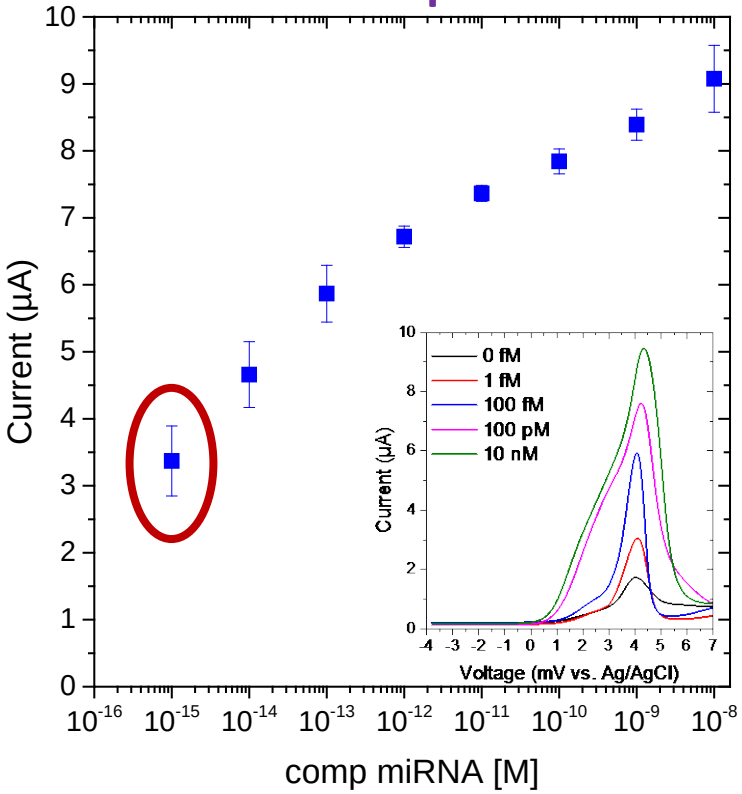


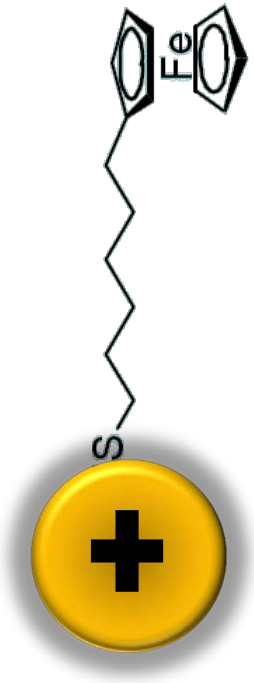
Jolly *et al.*, submitted

Amperometric: PNA with AuNPs

- Square wave voltammetry was used to monitor ferrocene peaks for different concentrations of miRNA
- Provision of dual detection technique

Dose Response





Control experiments output

- Around 1 µA peak current with just AuNPs
- With non-complementary miRNA (100 nM), around 1.2 µA
- With 100 nM of miRNA sequence with 2 mismatch, around 1.6 µA was recorded
- With 1 mismatch (100 nM) sequence, around 7 µA was observed

The near-future…

Potentiometric
Impedimetric
Amperometric

Antibodies
Antibody fragments
Peptides
Affimers
DNA aptamers
MIPs

Lectins

DNA, PNA, LNA

i-STAT®, Abbott

fPSA
PSA
AMACR
HER2
…

glycosilation

miRNAs

Acknowledgments

Biosensors Group:
Pawan Jolly, Anna Miodek

Dept Pharmacy & Pharmacology, Uni Bath:
Matthew Lloyd

Cardiff University:
Vibha Tamboli, Chris Allender, Jenna Bowen

University of São Paulo:
Marina Batistuti, Marcelo Mulato

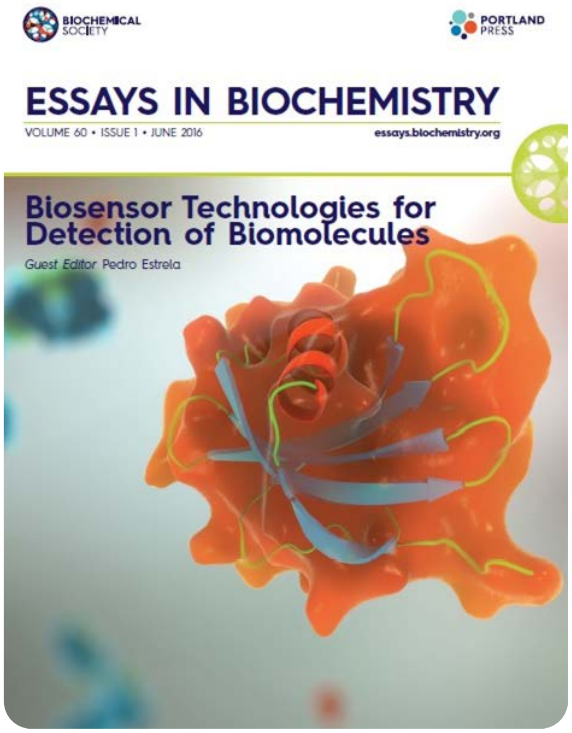
Slovak Academy of Sciences:
Peter Kasák, Jan Tkáč

National Taiwan University:
Deng-Kai Yang, Lin-Chi Chen

Marie Curie Initial Training Network
"Cancer Diagnosis: Parallel Sensing of Prostate Cancer Biomarkers"
(PROSENSE)
www.prosense-itn.eu

p.estrela@bath.ac.uk
go.bath.ac.uk/biosensors

Latest issue of *Essays in Biochemistry*



Covering an introduction to biosensors, discussion of analytical biosensors, and applications of biosensors, including in the biomedical field

Guest edited by Pedro Estrela, University of Bath, U.K.

Available at essays.biochemistry.org/content/60/1

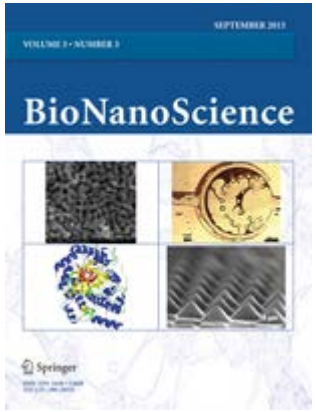
PROSENSE Conference on Prostate Cancer Diagnosis

Bath, 12-13 September 2016

Deadline for abstracts: **31 July 2016**

BioNanoScience: topic Issue on Prostate Cancer Diagnosis

Deadline for papers: **23 September 2016**



Follow links on http://go.bath.ac.uk/biosensors