Electrochemical Sensing of Cancer Biomarkers

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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
A. Direct immobilisation (binary SAM, thiol chemistry)

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

Prostate Specific Antigen (PSA)
PSA + glycosilated PSA

![Graph showing chemiluminescence vs PSA concentration for Aptamer-PSA-Antibody and Aptamer-PSA-Lectin]
Aptamer SAMs

B. Immobilisation on self assembled gold nanoparticles

1:50 ratio of Aptamer to MCH

ΔRct (%) vs [PSA] [ng/mL]

- 10 pg/mL
- 10 ng/mL

Gold nanoparticles

11 amino alkane thiol
• High non-specific binding with mercapto-hexanol (MCH) based SAM

\[ Z'' \text{ (kΩ)} \]

ca. 15%

• Alternative: Use of antifouling surface chemistry (Sulfo betaine moiety)
C. Use of Antifouling surface chemistry (Sulfo-betaine moiety)

1. SAM of co-immobilised thiol terminated Sulfo-betaine with 11-Mercaptoundecanoic acid
2. Activation of carboxylate groups of MUA via EDC/NHS chemistry followed by covalent attachment of amine terminated aptamers
3. PSA capture
Aptamer SAMs

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

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3. PSA capture

- HSA

Hydration shell by independent sulfo-betaine moieties
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
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
Aptamer MIPs: BioFET in serum

- Detection of low levels of PSA in serum
In mammalian cells, the enzyme is responsible for converting (2R)-methylacyl-CoA esters to their (2S)-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.
Aptamer on polypyrrole

DNA aptamer as bioreceptor

ANTA-Copper complex as redox marker

Polyethylene glycol for antifouling properties

Polypyrrole as sensor surface

Gold

Square Wave Voltammetry

CURRENT (µA)

VOLTAGE (V vs. Ag/AgCl)

Gold
Aptamer on polypyrrole

Capture of AMACR
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
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)
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 fingerprints 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
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 $\text{[Fe(CN)₆]}^{3-/-4-}$ 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

(a) Capture PNA probe (neutral)
(b) Spacer molecule
(c) Complementary strand (negatively charged) to capture PNA

Positively charged gold nanoparticles (self-assembled polyethylenimine)
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: $[\text{Fe(CN)}_6]^{3-/4-}$.
EIS: PNA with AuNPs

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

Charge transfer resistance \((R_{ct})\) significantly increased with target miRNA by increasing the electrostatic barrier.

Charge transfer resistance \((R_{ct})\) significantly decreased with AuNPs.
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

\[
C^* \equiv -\frac{1}{j\omega Z}
\]

\[
C' = \frac{-Z''}{\omega |Z|^2}
\]

\[
C'' = \frac{-Z'}{\omega |Z|^2}
\]

- \(Z'\): Real Part of impedance
- \(Z''\): Imaginary part of impedance
- \(|Z|^2\): \((Z')^2 + (Z'')^2\)
Monitoring non-Faradaic processes

Non-Faradaic EIS: PNA with AuNPs

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

Jolly et al., submitted
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
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

fPSA
PSA
AMACR
HER2
...

glycosilation
miRNAs

i-STAT®, Abbott
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