Optimisation of an Electrochemical Impedance Spectroscopy aptasensor by exploiting Quartz Crystal Microbalance with Dissipation signals

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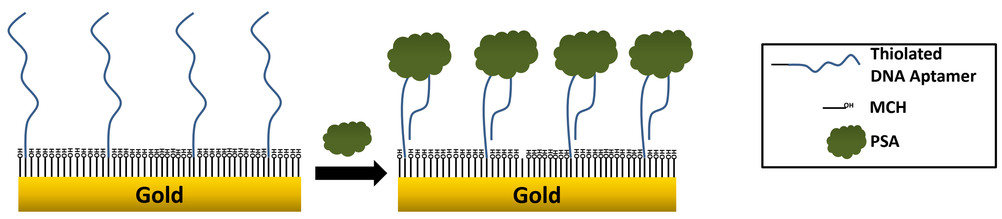
Introduction

- **Electrochemical Impedance Spectroscopy (EIS) is a fast, sensitive and relatively inexpensive label-free tool, which can be employed for a wide range of applications including for cancer diagnostics.**
- **EIS-based biosensors using DNA/RNA aptamers require a careful design in order to be most efficient. In this study we investigated the performance of an impedimetric aptasensor by comparing EIS to Quartz Crystal Microbalance with Dissipation (QCM-D) signals.**

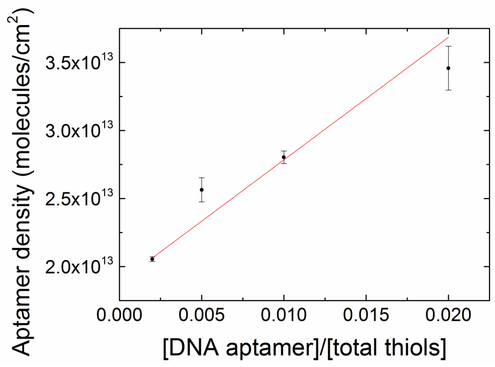
Experimental setup

(a)



- **This study focused on Prostate Specific Antigen (PSA) detection by using a target-specific DNA aptamer.**
- **The self assembled monolayer comprises a mixture of 6-mercaptohexanol (MCH) and thiolated-DNA aptamer.**

Experimental setup

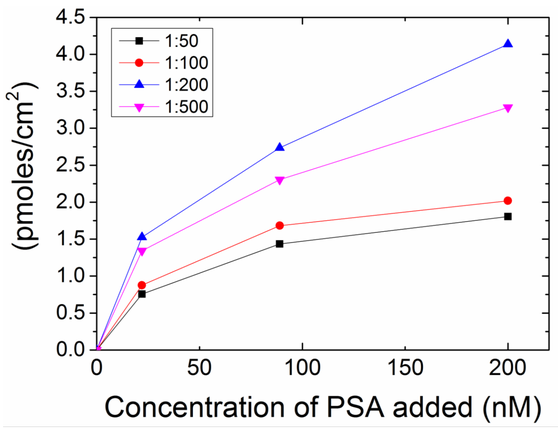


- **The biosensor behaviour was analysed varying three different buffers for the assay and four ratios of MCH/Aptamer for the sensor modification.**
- **QCM-D and EIS measurements were then recorded.**

Results

QCM-D measurements performed in:

- **EIS buffer (50 mM PB, 100 mM K_2SO_4, pH 7.0), HMCKN buffer (20 mM HEPES, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 2 mM KCl and 150 mM NaCl, pH 7.4), TBS buffer (10 mM Tris HCl, 150 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH 7.4).**
- **Only TBS buffer (with which the aptamer was raised) showed binding.**



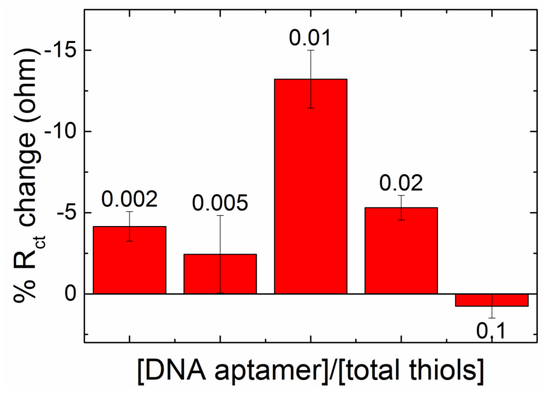
- **Using TBS buffer, three aptamer/MCH ratios have been tested for the surface coverage:**

  1:50, 1:100, 1:200, 1:500.

Results

EIS measurements:

- **The impedance of the biosensor is due to a combination of the obstruction effect towards $[Fe(CN)_6]^{3-/4-}$ redox couple and screening of the DNA aptamer charge.**
- **Negative shifts in R_{ct} were recorded as a result of the prevalence of screening of the DNA aptamer charge.**
- **Although 1:200 was the ratio that ensures maximum extent of PSA binding (from QCM-D results), this did not correspond to the ratio that maximize the shift in R_{ct} concentration fraction.**



R_{ct} change upon incubation with 300 nM PSA in different SAM over range of aptamer/MCH

Conclusions

- **Regardless of the great potential of aptamers versus antibodies in biosensing, aptasensors need a careful design in order to provide an acceptable binding efficiency. The conditions which allow a maximum analyte binding do not necessarily also provide the best settings in terms or impedance recordings.**
- **QCM-D results confirmed the importance of buffer conditions and surface optimization for efficient aptamer-protein binding. In particular a ratio 1:200 of aptamer:MCH provides the maximum PSA binding.**
- **However, EIS data demonstrated a different optimal surface coverage ratio as compared to QCM-D because of additional factors governing the EIS measurements. The results of this study can be applied to future label-free, reliable and cost effective aptamer-based sensors exploiting EIS.**

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