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

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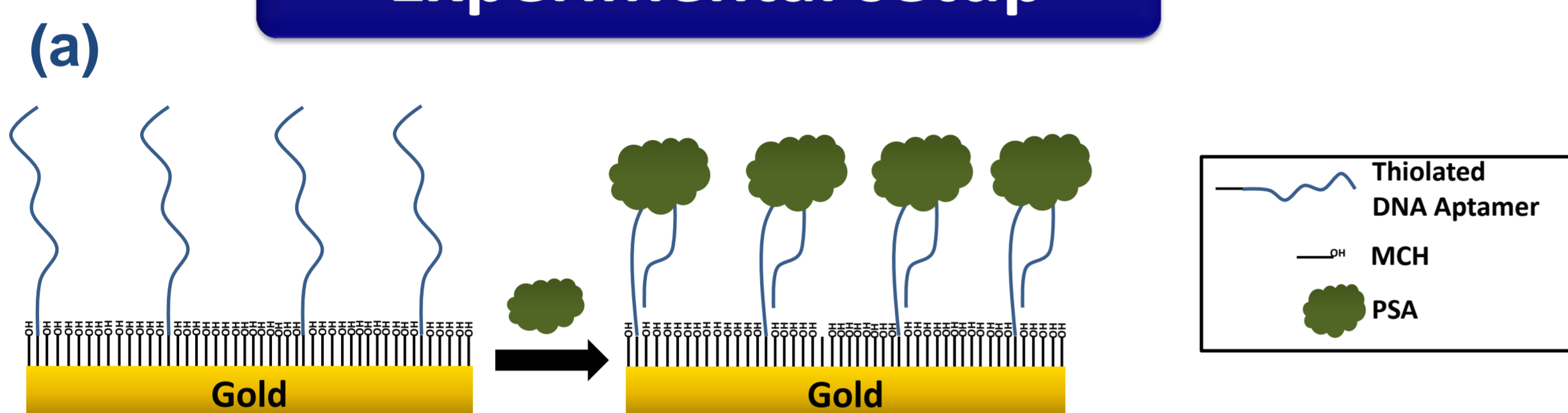
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Introduction

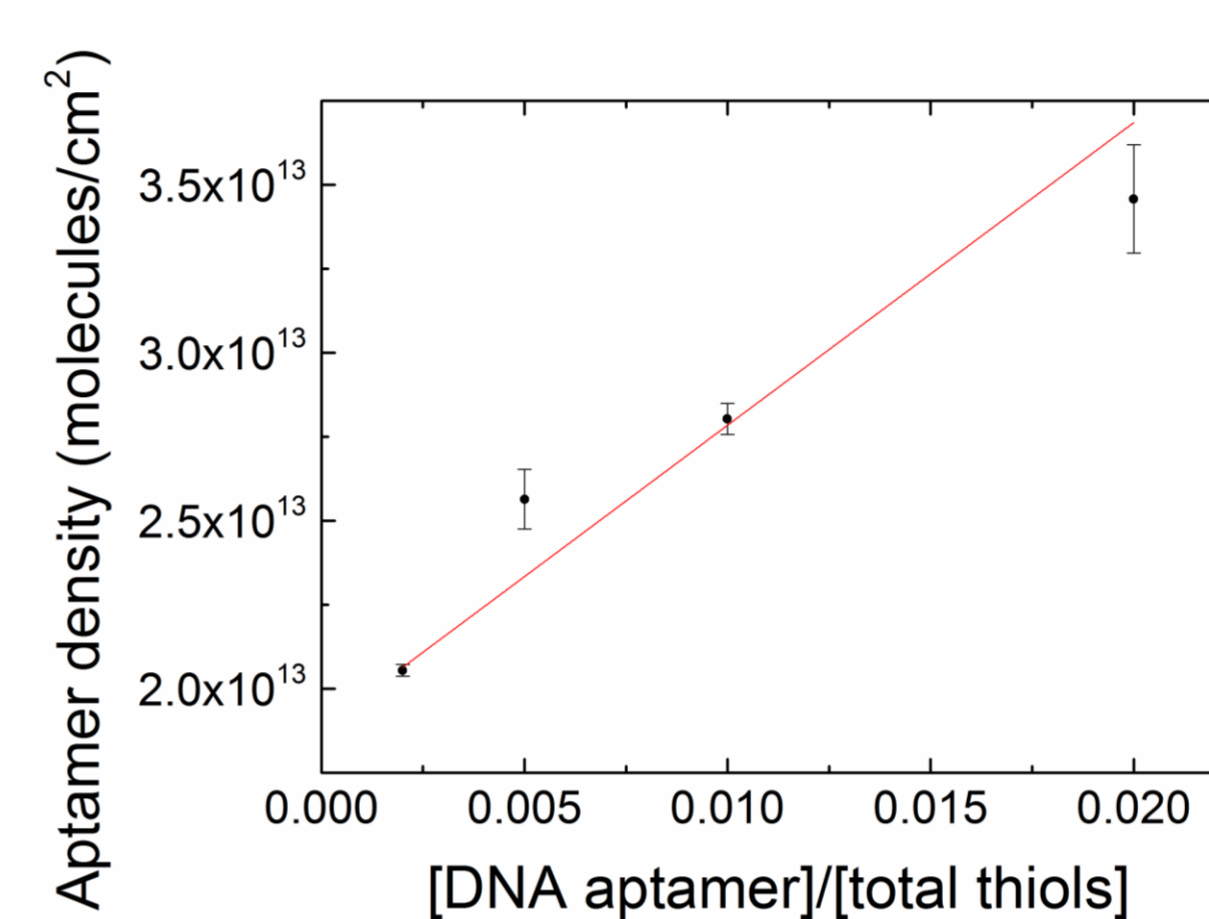
- Electrochemical Impedance Spectroscopy (EIS) is a fast, sensitive and relatively inexpensive label-free tool, which can be employed for a wide range of applications including for cancer diagnostics.
- EIS-based biosensors using DNA/RNA aptamers require a careful design in order to be most efficient. 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



- 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

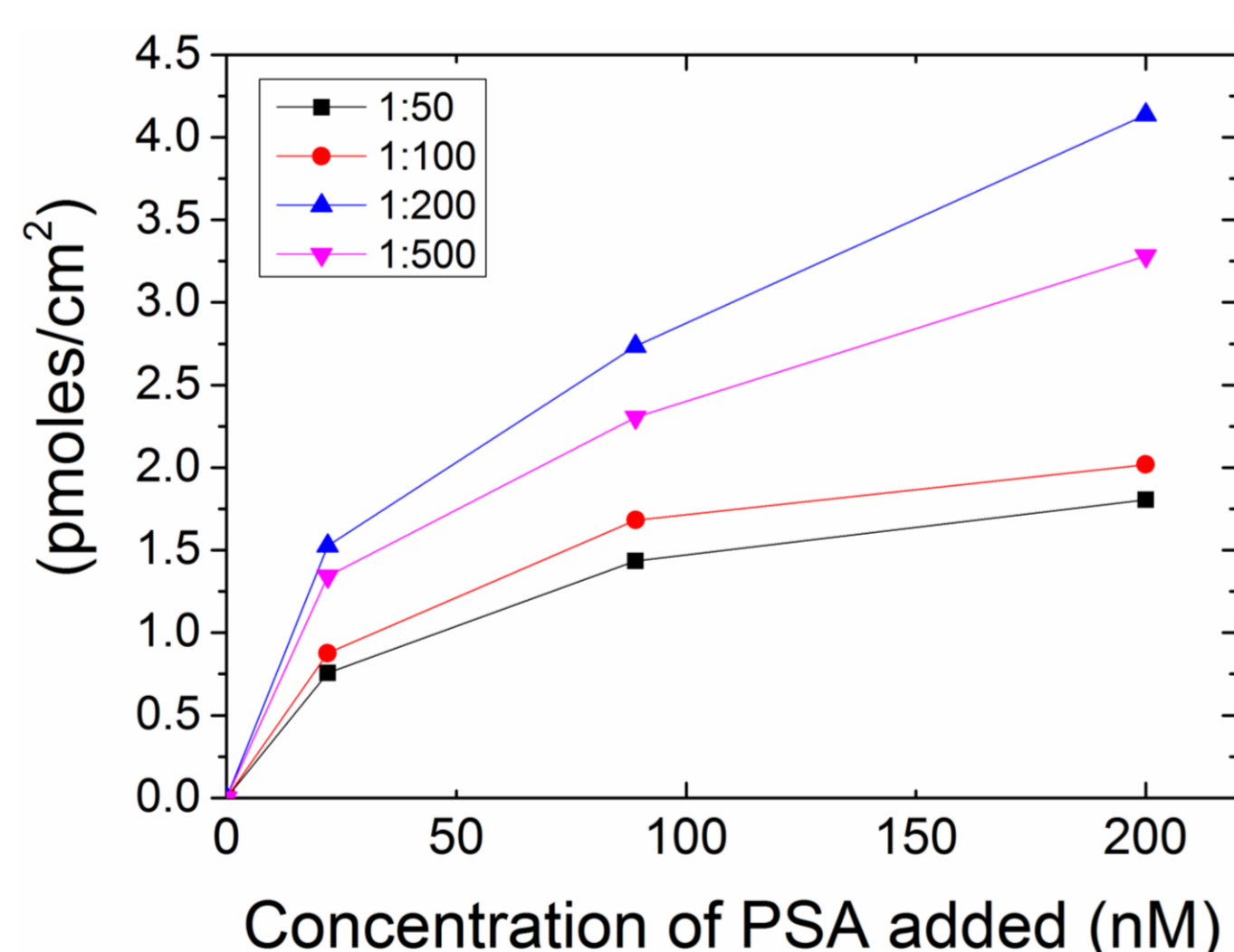


- The biosensor behaviour was analysed varying three different buffers for the assay and four ratios of MCH/Aptamer for the sensor modification.
- QCM-D and EIS measurements were then recorded.

Results

QCM-D measurements performed in:

- EIS buffer (50 mM PB, 100 mM K₂SO₄, pH 7.0), HMCKN buffer (20 mM HEPES, 2 mM MgCl₂, 2 mM CaCl₂, 2 mM KCl and 150 mM NaCl, pH 7.4), TBS buffer (10 mM Tris HCl, 150 mM NaCl, 5 mM KCl, 5 mM MgCl₂, pH 7.4).
- Only TBS buffer (with which the aptamer was raised) showed binding.

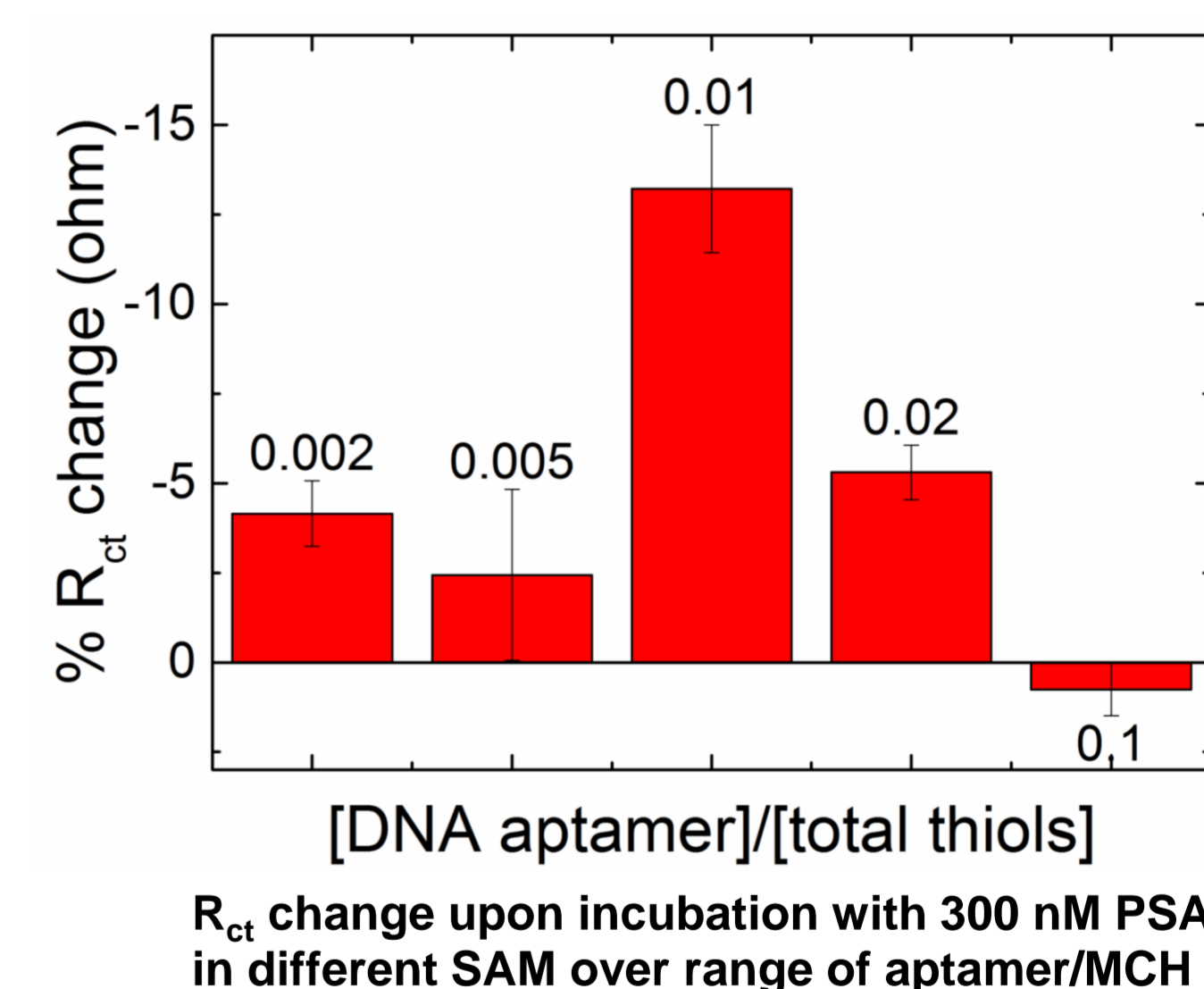


- Using TBS buffer, three aptamer/MCH ratios have been tested for the surface coverage: 1:50, 1:100, 1:200, 1:500.

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.



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.

