Impedimetric analysis of abasic DNA sites

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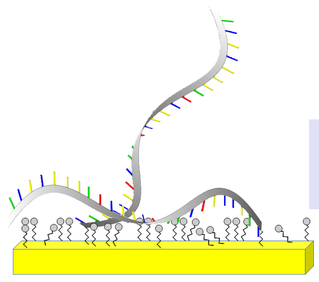
Introduction

- DNA is denoted as abasic when one base is missing at least. The formation can occur through many reasons, like radiation, genotoxic agents, intermediate base deletion due to DNA repair mechanism or spontaneous deletion through DNA breathing.
- Current detection methods need a kind of labeling, which is time and cost intensive.
- Therefore we have investigated three abasic DNAs with respect to their binding behavior to immobilized capture probes
- Detection possibilities have been evaluated with electrochemical impedance spectroscopy (EIS) and surface plasmon resonance (SPR)

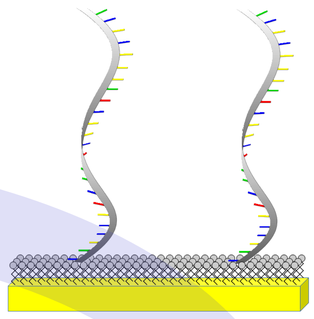
Electrode preparation / characterization

1) DNA immobilization



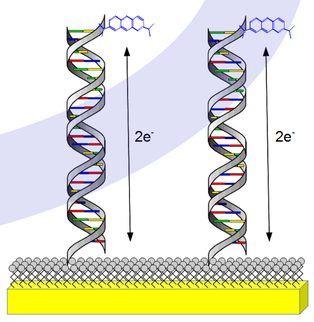
- Thiolized 25mer probe ssDNA (1 µM) + mercaptohexanol (0,5 µM)

2) Passivation



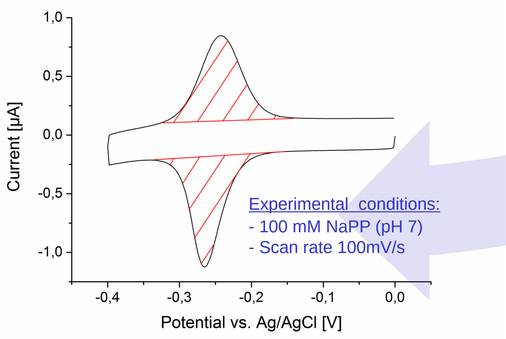
- Mercaptohexanol (1 mM)

3) Hybridization



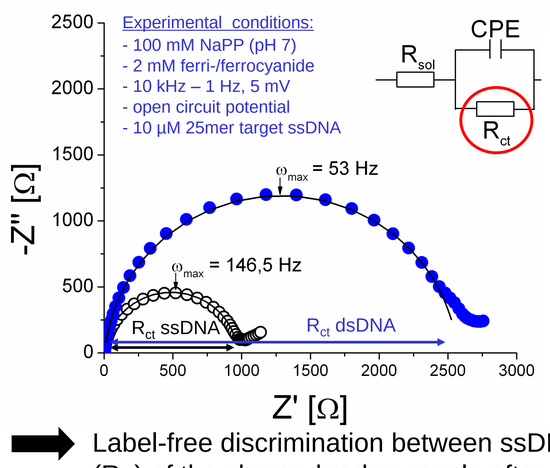
- Hybridization with methylene blue (MB) modified target DNA

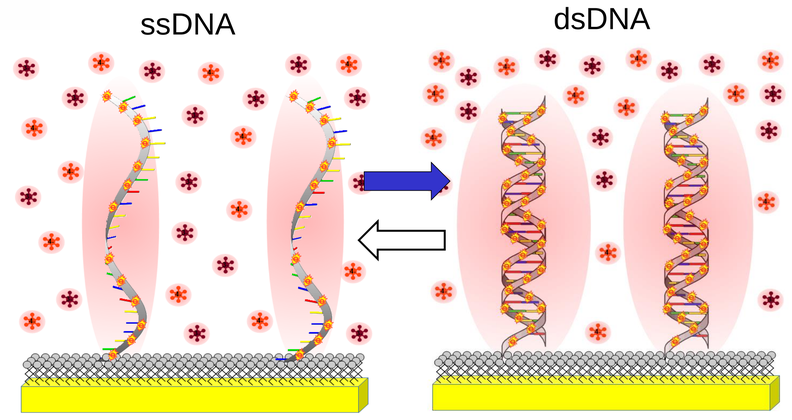
4) Evaluation of surface coverage



- Peak area corresponds to the transferred charge of MB and the surface coverage (10,1–12,6 pmol/cm^2)

Principle of impedimetric DNA detection



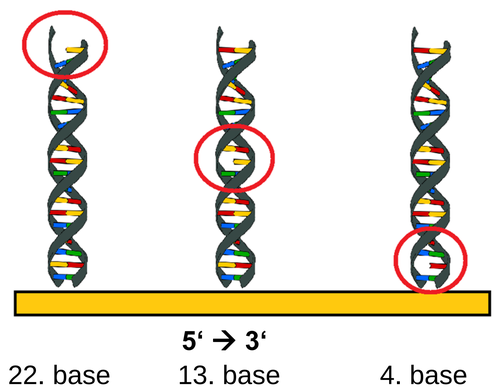


➡ Label-free discrimination between ssDNA and dsDNA due to the increased charge transfer resistance (R_{ct}) of the charged redox couple after hybridization

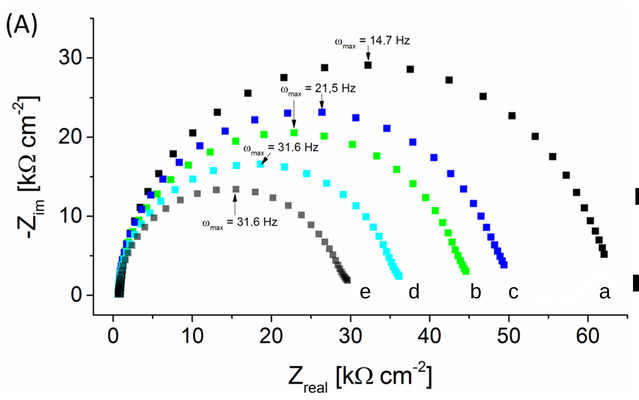
➡ Repeated hybridization-denaturation cycles are possible with deionized water as denaturation reagent

DNA sequences

type		sequence (5'→ 3')
target	fullmatch DNA (fmDNA)	CTT GAT GAG TCA GCC CGC TTG GAC G
target	abasic site near electrode (a-site$_{el}$ ssDNA)	CTT **X**AT GAG TCA GCC CGC TTG GAC G
target	abasic site in the middle (a-site$_{mid}$ ssDNA)	CTT GAT GAG TCA **X**CC CGC TTG GAC G
target	abasic site near solution (a-site$_{sol}$ ssDNA)	CTT GAT GAG TCA GCC CGC TTG **X**AC G



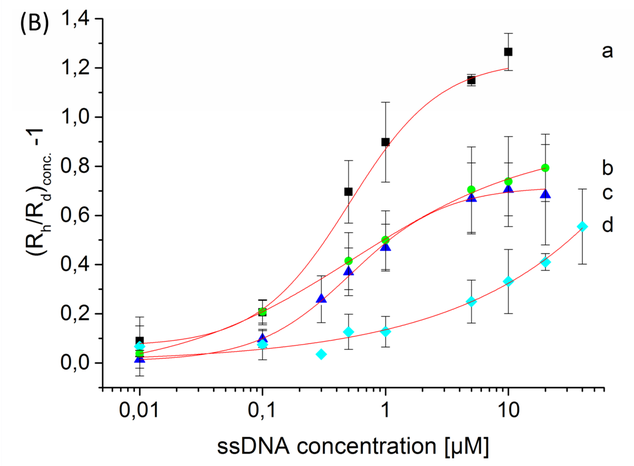
Results : a) EIS



- Similar shape of the Nyquist plots after hybridization of the different target strands

➡ EIS can be used to detect abasic DNA

➡ Also a dicrimination between the abasic sites seems to be feasible

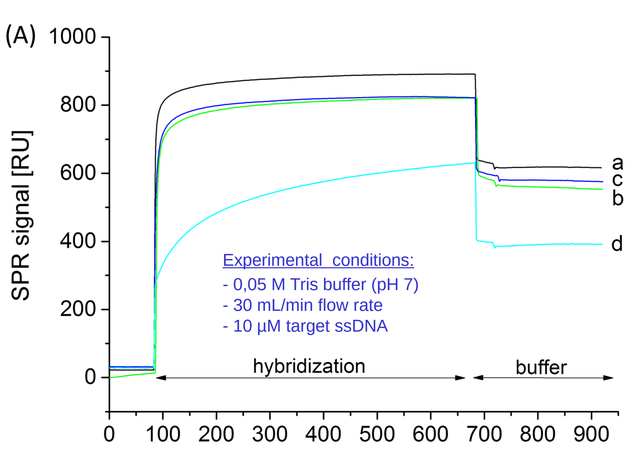


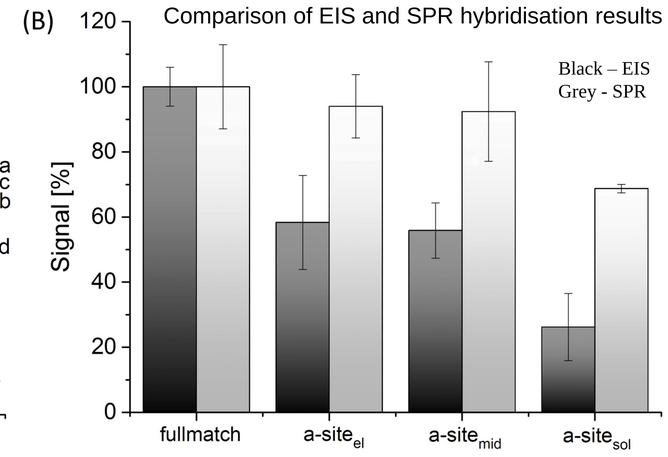
- No abasic DNA reaches the same maximum impedance signal as the fullmatch DNA after hybridization

? Different concentration bound on the surface or different binding structure

a – fmDNA dsDNA **b** – a-site$_{el}$ dsDNA **c** – a-site$_{mid}$ dsDNA
d – a-site$_{sol}$ dsDNA **e** – ssDNA

Results: b) SPR





a – fmDNA dsDNA **b** – a-site$_{el}$ dsDNA **c** – a-site$_{mid}$ dsDNA
d – a-site$_{sol}$ dsDNA

- Similar concentration and binding kinetics for fullmatch, a-site$_{mid}$ and a-site$_{el}$ ssDNA

➡ Slower binding kinetics and decreased concentration for a-site$_{sol}$ ssDNA (- 31 % compared to fullmatch DNA)

- The binding of abasic ssDNA results in a stronger signal change of the EIS-signal than for the SPR-signal compared to fullmatch DNA

➡ Indication of different dsDNA structures formed on the surface when abasic ssDNA binds.

Summary

- Concentration-dependent detection of 25mer abasic site containing ssDNA with EIS is feasible.
- Clearly distinction between abasic ssDNA and fullmatch ssDNA is possible - partly distinction between different abasic ssDNA.
- Rather similar binding kinetics of abasic ssDNA, with exception of solution exposed a-site
- Indication of different structure of abasic site containing dsDNA compared to fullmatch DNA after binding.

Literature

[1] Pänke, O; Kirbs, A.; Lisdat, F. *Biosens. Bioelectron.* **2007**, 22, 2656-2662.
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