Localized surface plasmon resonance based gold nanoparticle-hairpin assay for optical detection of single DNA molecles

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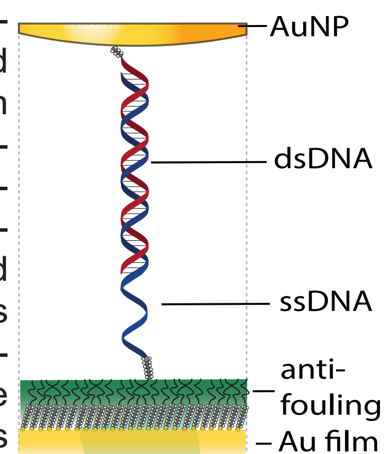
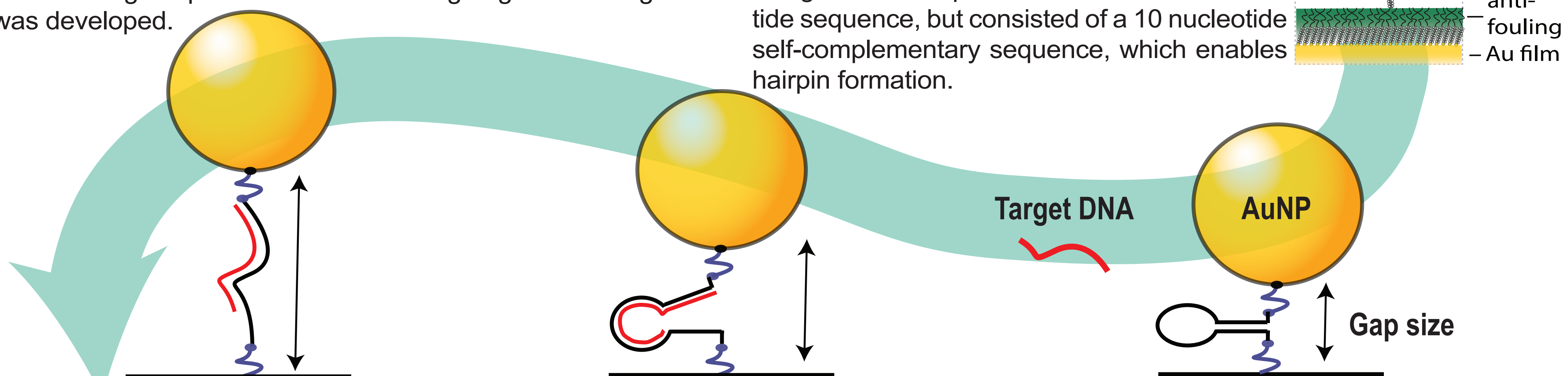
Introduction

Early diagnostics of several diseases requires sensing of ultra-low concentrations (< fM). Therefore, molecular diagnostics is moving towards single molecule detection assay. Localized surface plasmon resonance (LSPR) allows easy optical read-out, but lacks sensitivity towards single small molecules, like DNA. Available labeling methods experience coupling-distance limitations and/or consist of multiple assay steps. In this research an optical single sensing-step DNA sensor using hairpin-DNA without limiting target-DNA lengths was developed.

Assay details

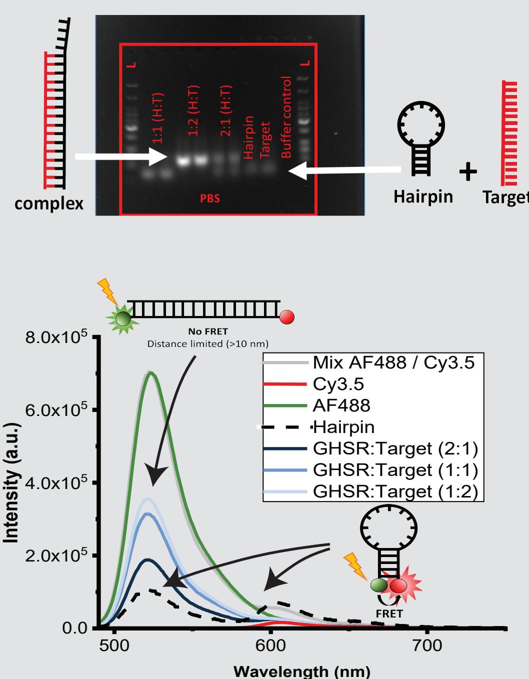
The sensor read-out is based on the displacement of gold nanoparticles (AuNPs) attached to hairpin-DNA relative to an Au-surface on target binding. The sensor consists of HS-hairpin-NH_2 DNA immobilized on thiol alkane ethylene-glycol self-assembled monolayers via EDC/NHS coupling. AuNPs are immobilized through thiol chemistry. The sensor was designed to be specific towards a 40 nucleotide sequence, but consisted of a 10 nucleotide self-complementary sequence, which enables hairpin formation.





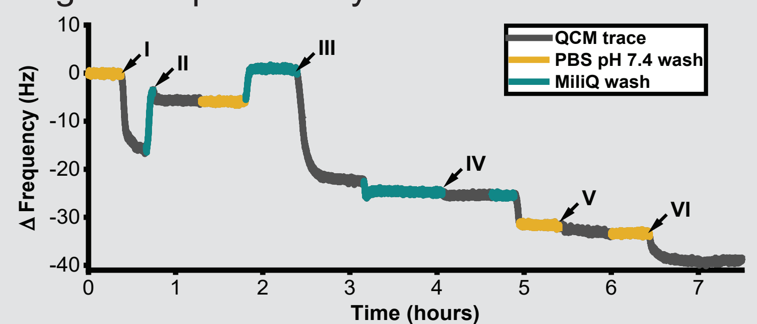
Hairpin testing

Hairpin-opening in the presence of target DNA was confirmed by gel electrophoresis, which showed different migration through the gel, and Förster Resonance Energy Transfer (FRET). Upon target sequence addition the energy transfer is reduced due to increased distance between the FRET pair. This confirms the opening of the hairpin free in solution



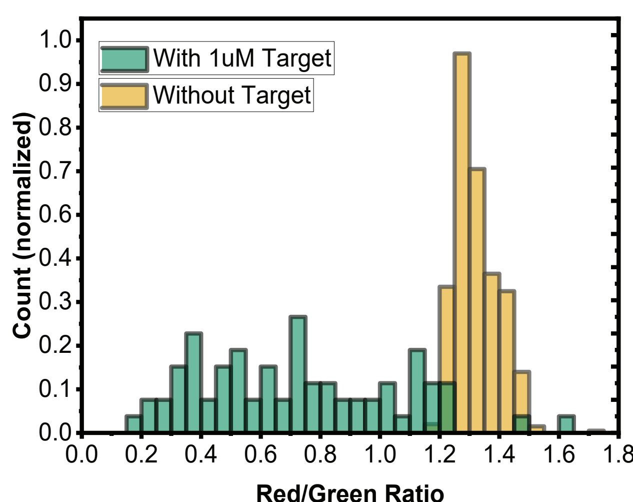
Assay assembly

Assembly steps and sequence specificity of the sensor are confirmed by Quartz Crystal Microbalance (QCM) measurements. (I) EDC/NHS activation of carboxyl-groups on self-assembled monolayers, (II) Hairpin immobilization via activated carboxyl–amine coupling, (III) AuNP immobilization via AuNP-thiol coupling, (IV) passivation of AuNPs with β-mercaptoethanol, (V) no binding of non-complementary DNA, (VI) binding of complementary DNA.



Optical detection

Binding of individual target DNA strands can be observed by darkfield microscopy as a color change from red to green due to distance-limited coupling of AuNPs to Au-surfaces



Difference	0.48
Standard error	0.02
95% CI	0.44 - 0.52
T test	26.87
DF	570
Significance	**P < 0.0001**

Significant difference in red/green ratio is observed after addition of target DNA.

Conclusion

We were able to confirm that our gold nanoparticle-hairpin assay can be used to optically detect the binding of target DNA as a shift in the red/green ratio. We confirmed the opening of the hairpin sequence in the presence of target DNA in solution and while assembled as a biosensor assay. With darkfield microscopy a significant color shift could be observed indicating the increased gap size between gold film and AuNP.

What is next?

- follow individual AuNPs over time upon target addition
- determine the limit of detection and linear range
- study the effect of non-coding sequences added
- measure patient samples in complex media

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