Design, synthesis, and characterization of metallic nanoparticles as amplifiers for the optical readout of a novel SERS-based sensor system.

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Introduction

In the realm of biosensing, the quest for innovative platforms with unprecedented sensitivity and versatility has spurred the integration of diverse nanotechnologies, including functional nanoparticles (NPs) and DNA origami structures (Figure 1). This fusion of cutting-edge methodologies holds the promise of revolutionizing bioanalytical sensing capabilities, offering new avenues for rapid and precise detection across various applications. [1, 2]

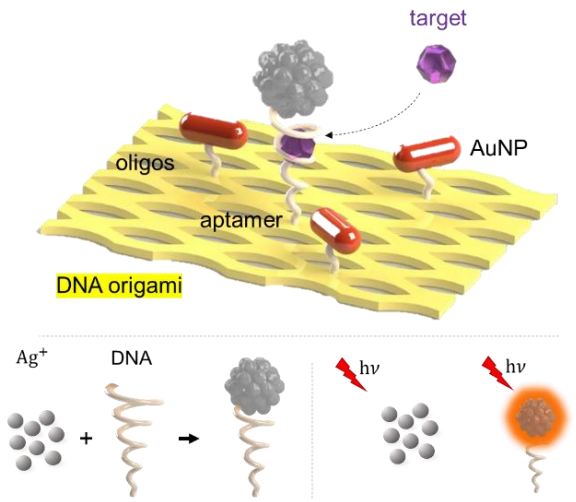


Figure 1: Schematic of DNA origami scaffolds for the arrangement of plasmonic ssDNA attached NP arrays for SERS signal amplification, with an AC-decorated DNA aptamer as bioRE. Bottom: Multifunctional ssDNA oligonucleotide including a sequence for the coordination of fluorescent AgACs.

At the vanguard of this transformative pursuit lies the DeDNAed project, a pioneering initiative poised to reshape the landscape of biosensing technology. Central to its mission is the strategic incorporation of surface enhanced raman spectroscopy (SERS) as a state-of-the-art optical analysis method, facilitating the rapid and precise detection of analytes with unparalleled sensitivity. [3]

Integral to the innovative architecture of the DeDNAed project is the strategic utilization of DNA origami as a versatile "nano-breadboard," providing a robust scaffold for the precise positioning of biorecognition elements (bioREs) in proximity to the plasmonic hotspots of functionalized nanoparticles (NPs).

This orchestrated arrangement promises to elevate the sensor platform to new heights of performance and efficacy. To achieve seamless integration and ensure utmost spatial precision, the project employs short oligonucleotide sequences as a well-established method for anchoring NPs and active bioREs onto the DNA origami scaffold. [4] Furthermore, the incorporation of metallic atomic clusters (ACs) within the bioREs enhances the sensor platform's fluorescence properties and signal amplification capabilities, setting it apart from conventional NP-based systems.[5]

As such, the DeDNAed project represents a pioneering endeavor poised to catalyze significant advancements in biosensing technology. By pushing the boundaries of innovation and harnessing the synergistic potential of nanotechnologies, it heralds a new era of bioanalytical sensing, offering transformative solutions to address pressing challenges in healthcare, environmental monitoring, and beyond.

Results and discussion

To integrate gold nanoparticles (AuNPs) with the DNA origami into a hybrid structure, the DNA origami was designed to have protruding single-stranded DNA (ssDNA) as attachment points (see Figure 1). The AuNPs were functionalized with complementary ssDNA oligonucleotides. This was accomplished by synthesizing oligonucleotides with a thiol group at the 5'-end. For assessing the efficiency of functionalization, the oligonucleotides were also tagged with a fluorescein dye at the 3'-end. These oligonucleotides were synthesized in-house using the Applied Biosystems 3400 DNA synthesizer to facilitate this attachment process. The three components of the bioRE (attachment to DNA origami, target-specific binding, and AC coordination) were individually analyzed and optimized.

For the synthesis of the AuNPs, previously reported CTAC/$NaBH_4$ seeded growth approach was employed. [6] To obtain the gold seeds 50 µL of a 0.05 M $HAuCl_4$ solution was added to 5 mL of a 0.1 M CTAC solution. 200 µL of a freshly prepared 0.02 M NaBH4 solution was then injected under vigorous stirring and after 3 min the mixture was diluted 10 times in CTAC 100 mM. With the aim of obtaining 50 nm particles, two growing steps were then carried out. In the first growth step, 10 nm nanoparticles (see Figure 2) were obtained by mixing 900 µL of the seed solution and 40 µL of 0.1 M ascorbic acid in 10 mL of 25 mM CTAC solution. Then, 50 µL of a 0.05 M HAuCl4 solution was injected under vigorous stirring 10 min. After that, in other to obtain 50 nm gold nanoparticles, the last growth step was then repeated using 30 µL of 10 nm gold nanoparticles as nucleating particles and adding 10 µL of a dilute sodium hypochlorite solution after one hour under vigorous stirring.

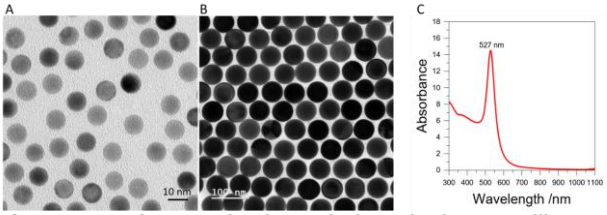
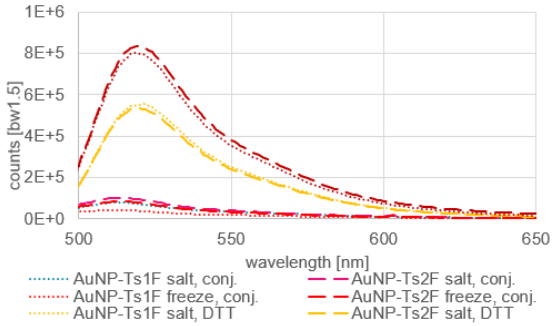


Figure 2. Characterization of the single-crystalline Au nanospheres. A: TEM image of intermediate growth step, showing NPs with an average diameter of 10 nm. B: TEM image of final growth step, showing 55 nm diameter AuNPs (after ligand exchange from CTAB). C: UV-Vis-NIR spectrum of the AuNPs in water displaying a sharp plasmonic absorption peak at 527 nm.

To attach the thiolated oligos to the AuNPs, two different approaches were employed, the salt-aging and the freeze-thaw method [7], [8]. The first step for both methods is the activation of the thiol by the addition of tris(2-carboxyethyl)phosphine (TCEP) to the oligo solution which was then mixed with the AuNPs in a concentration ratio depending on the size of the AuNPs (50-60 nm diameter NPs require a ration of 1:2000 [AuNP]:[oligo]).
To further distinguish between the loading ratios (oligos per NP) achieved with the two different functionalization methods, we developed a method to measure the oligo concentration after functionalization by utilizing the attached fluorescein dye and fluorescence spectroscopy (Figure 3).

a)



b)

	dye conc. [nM]	NP conc. [nM]	loading ratio
AuNP-Ts1F, freeze	164.7	0.30	549
AuNP-Ts2F, freeze	169.4	0.29	584
AuNP-Ts1F, salt	96.6	0.30	322
AuNP-Ts2F, salt	101.4	0.26	390

c)

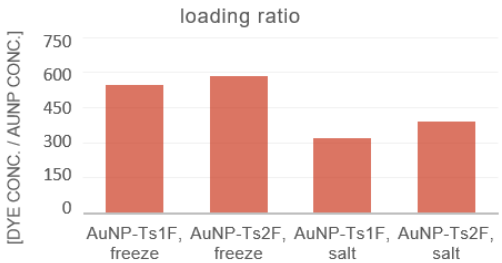


Figure 3: Quantitative loading analysis comparing the loading efficiencies of the salt-aging (*salt*) and freeze-thaw (*freeze*) functionalization methods. **A**: Fluorescence spectra of the fluorescein labelled oligos, before (*conj.*) and after (*DTT*) detaching them from the surface of the AuNPs. **B**: Table with the values for the dye and AuNPs concentration and the resulting loading ration of dye-labelled oligos per NP. **C**: Column chart visualising the resulting loading ratios for the two different oligo species and functionalization methods, taken from the table in **B**.

Figure 3 shows the results of this approach for the two functionalization methods. Using the previously calibrated peak values of the fluorescence spectra of the resuspended oligos together with UV-Vis-NIR spectra to determine the NP concentration, measured before dissociation, we determined the concentrations of the labeled-oligos and the AuNPs. Based on these values, we then calculated the loading ratio of oligos per NP. The measured values are given in Figure 3, B and the loading ratio is displayed as a column chart in Figure 83, C. The resulting ratios appeared to be slightly higher for the freeze-thaw method, making this the method of choice for the preparation of oligo-functionalized nanoparticles as amplifiers for the optical readout of a novel SERS-based sensor system

Conclusions

In summary, we successfully synthesized a DNA oligonucleotide sequence designed for a bioRE compatible with DNA origami bearing AC. This bioRE comprises three functional segments: a complementary DNA strand for attachment to the DNA origami, an aptamer for specific target binding, and a sequence for the coordinated synthesis of fluorescent AgACs. Additionally, we have successfully prepared gold nanoparticles functionalized with these sequences and quantitatively and qualitatively characterized the oligonucleotide-to-nanoparticle ratio.

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Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964248. We like to thank Andreas Herwig and Michael Mertig of the Kurt-Schwabe-Institut Meinsberg for the DNA origami synthesis.