# Functional ssDNA

## for the development of a DNA origami biosensor

Gunnar Klös, Laura S. Peña, Valeri Pavlov, Aitziber L. Cortajarena

CICbiomaGUNE, Basque Research and Technology Alliance (BRTA)
Paseo de Miramón 194, Donostia-San Sebastián 20014, Spain

DEDNAED

EXCELENCIA MARÍA DE MAEZTU 07/2018 - 06/2022

Biomolecular Nanotechnology LAB

CICbiomaGUNE MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

## Abstract

The development of novel biosensors with increased performance strongly relies on the incorporation and combination of various nanotechnologies, such as functional nanoparticles (NPs) and DNA origami [1,2]. On the basis of these technologies, the DeDNAed project is creating an advanced bioanalytical sensor-platform with advanced sensitivity and versatility by utilizing SERS as an ultrafast optical analysis method. DNA origami will be used like a "nano-breadboard" to precisely control the nanoscale positioning of biorecognition elements (bioREs) with respect to the plasmonic hotspots of NPs, which are positioned on the DNA origami in a similar fashion, for enabling highly sensitive SERS measurements [3]. To combine these technologies while guaranteeing high spacial precision, we use short oligonucleotide sequences, an established method for the attachment of NP, and active elements to DNA origami [4]. Additionally, the concept includes the integration of metallic atomic cluster (AC) within the bioRE, providing enhanced fluorescence properties compared to other NP based systems while their synthesis is based on novel etching methods that avoid the denaturation of the bioRE [5]. Here we present first findings on the development of an aptamer based bioRE, with a strong focus on the synthesis of the AC and optimization of their fluorescent properties. The bioRE consists of three segments, one for its attachment to the DNA origami, one for the target specific binding and one for the AC coordination. The three segments have been analysed and optimised independently and in combination, verifying their combined functionality. Furthermore, we present the first development iteration of the plasmonic NPs for SERS and their functionalisation for the integration on the DNA origami.

## Objectives

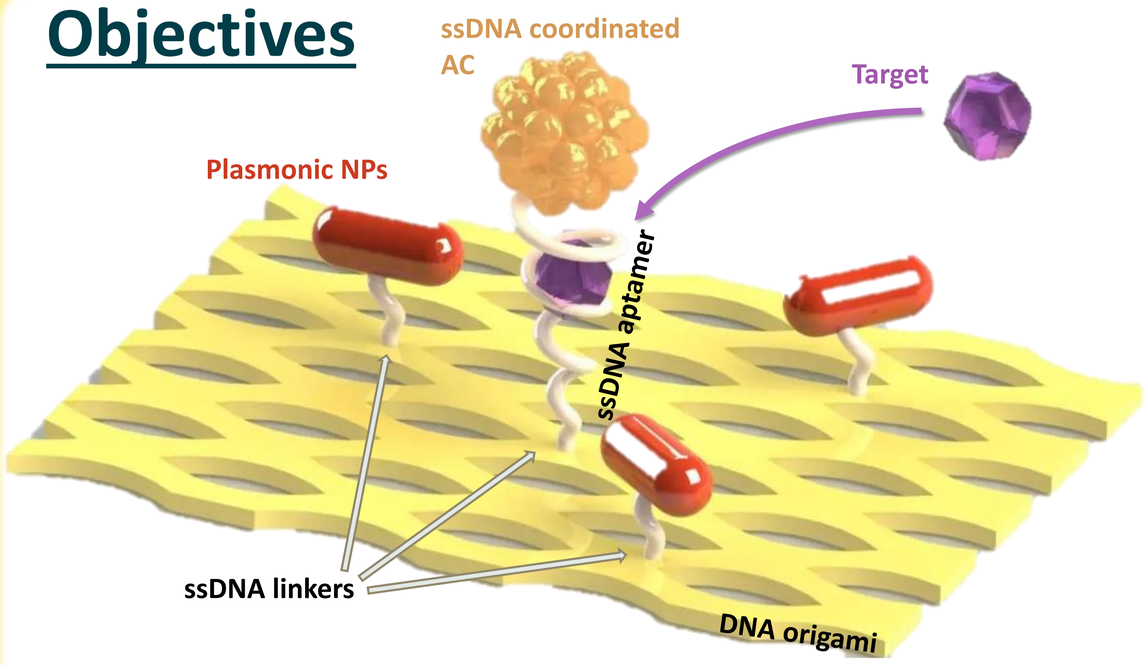



**Figure 1**: Schematic of a DNA origami scaffold for arrangement of plasmonic NP arrays for signal amplification of SERS measurements with an AC decorated ssDNA aptamer as bioRE.

- develop a **sensitive, versatile and ultrafast biosensing platform** by assembling and integrating sensing elements using DNA origami
- **single-stranded DNA (ssDNA)** as "**solder**" to attach elements to DNA origami
- ssDNA as **combined transducer and bioreceptor** with distinct segments functioning as aptamer and coordinator for synthesis of fluorescent ACs

## Conclusions

- ✓ synthesized multifunctional ssDNA sequence:
  - ✓ bioRE aptamer
  - ✓ attachable to DNA origami
  - ✓ coordinated synthesis of fluorescent AgACs
- ✓ For all three segments optimized individual and verified combined functionalities
- ✓ Functionalised spherical AuNP with ssDNA for DNA origami attachment
- ✓ Qualitative and quantitative analysis of ssDNA loading ratio per NP

## ssDNA functionalized plasmonic NPs

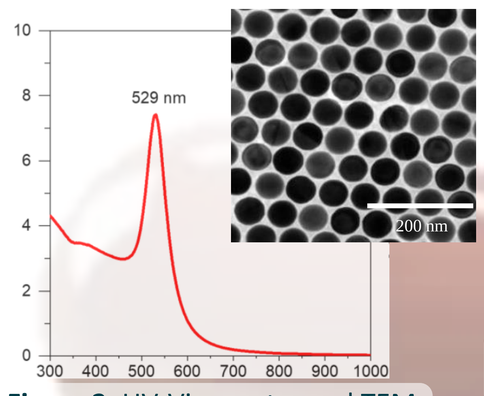



**Figure 2**: UV-Vis spectra and TEM image of 52 nm gold nanospheres.

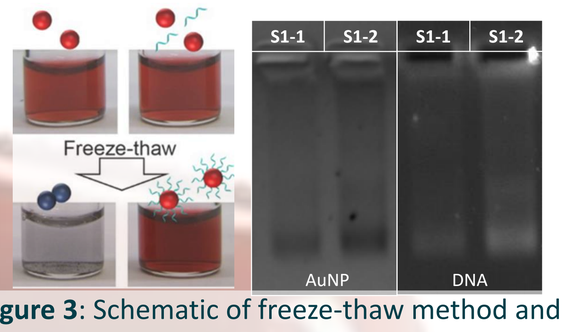
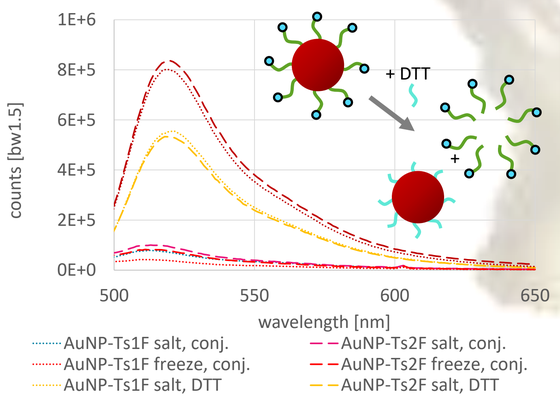



**Figure 3**: Schematic of freeze-thaw method and agarose gel showing overlap of NPs and ssDNA.

CTAB based synthesis of monodispers 52 nm diameter gold nanospheres. Comparing functionalization using freeze-thaw and salt-ageing method.

s1/s2 = ssDNA seq. | T= thiol mod . | F= fluorescein mod.




| | 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 |

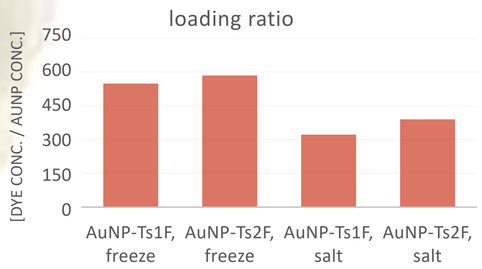



**Figure 4**: Determining ssDNA loading ratio per NP using fluorescein labeled ssDNA.

## Multifunctional ssDNA bioRE

ssDNA strand with three functional segments:

Ag = coordinating synthesis of fluorescent AgACs
A = bioRE aptamer for target specific binding
c = complementary to DNA origami linker

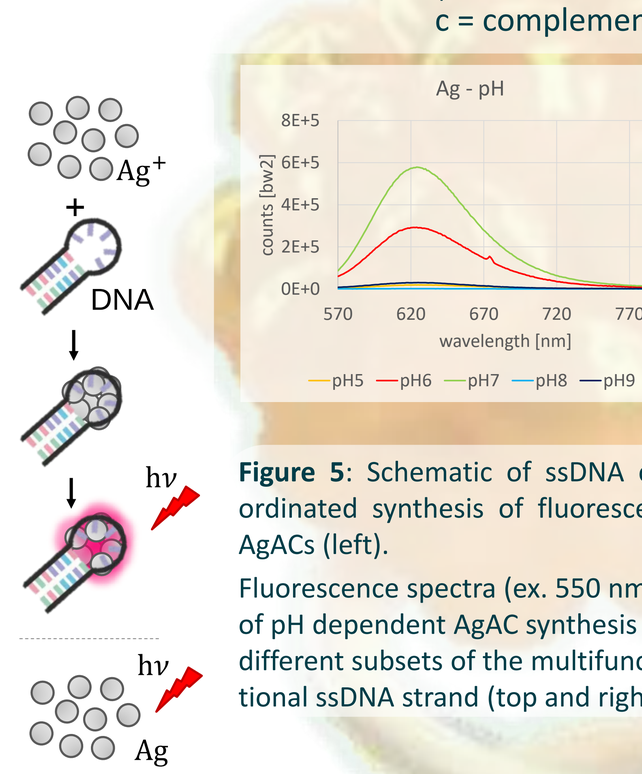



**Figure 5**: Schematic of ssDNA co-ordinated synthesis of fluorescent AgACs (left).

Fluorescence spectra (ex. 550 nm) of pH dependent AgAC synthesis in different subsets of the multifunctional ssDNA strand (top and right).

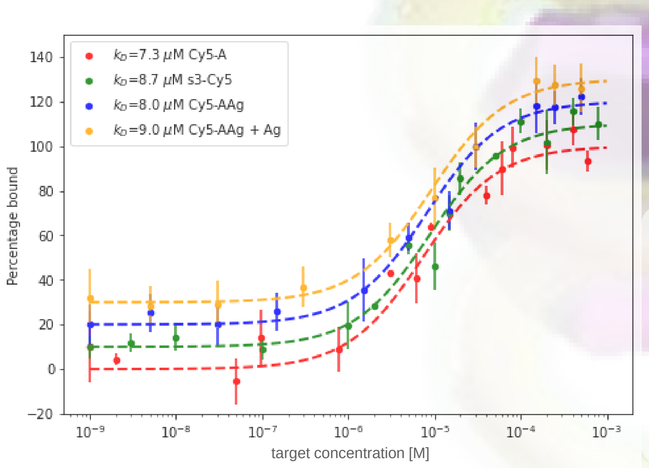



**Figure 6**: Schematic of Cy5 based determination of aptamer (A) target affinity (top).

Affinity ($k\_D$) measurements (Monolith) based on Cy5 fluorescence for different subsets of the multifunctional ssDNA strand. 10% curve shift to avoid overlap.

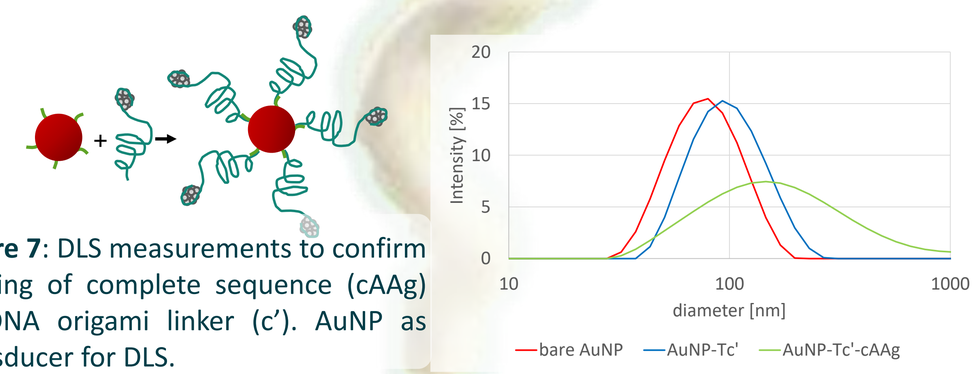



**Figure 7**: DLS measurements to confirm binding of complete sequence (cAAg) to DNA origami linker (c'). AuNP as transducer for DLS.

## References

[1] P. Wang, et al., Chem. 2 (2017) 359-382.
[2] J. Hahn, et al., Smart Systems Integration (SSI) (2021) 1-4.
[3] V. v. Thacker, et al. Nat. Comm. 5:1 (2014) 1–7.
[4] J. Zessin, et al., Nano Lett. 17:8 (2017) 5163-5170.
[5] L. Saa, et al. Nanoscale 6 (2014) 7405–7409.

## Acknowledgements

DeDNAed has received funding from the European Union's Horizon 2020 Research & Innovation Programme under grant agreement no 964248