Precise functionalization of NPs towards chiral NP dimers

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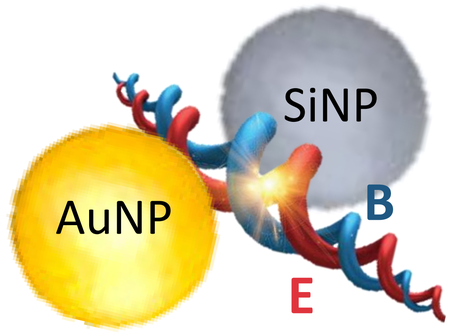
Abstract

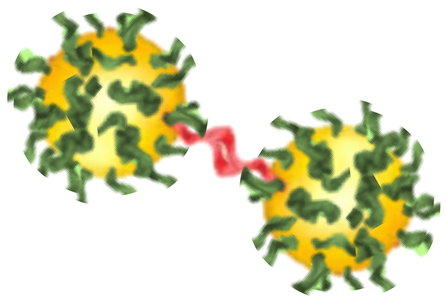
In the ChiroSense project we are developing a new chiroptical biosensor based on a dimer of gold and silicon nanoparticles (NPs), utilizing plasmonically enhanced circular dichroism (CD) measurements to probe molecule conformation in solution with increased sensitivity [1]. The development will strongly be guided by the use of consensus tetratricopeptide repeat (CTPR) proteins as a highly modular and well-defined structural probe, while simultaneously being employed as structural linker of the nano-particle dimer [2]. To deconvolute the structural and molecular components of the CD signal, the dimer will also be formed using single stranded DNA (ssDNA), which is also modular but lacks the distinct CD signal of CTPR [3]. Precisely controlling the loading ratio of molecules per NP is crucial for both approaches to achieve stable NPs that do not aggregate but are still able to form dimers.

Here we present our initial results on the development of these chiral NP dimers. To achieve functionalized NPs that enable dimer formation, it is important to achieve low loading ratios, to avoid aggregation, while maintaining the NP stability in solution. We developed a PEG based approach that allows us to precisely control the NP loading ratio, ranging from a few to several hundred molecules per NP. These particles were then used to synthesize chiral NP dimers with high yields.

Objectives

- Combine ssDNA and NPs for precise nano-engineering of **plasmonically amplified optical biosensors**, e.g. for CD or SERS
- Precisely **control molecular loading ratio** to optimize functionality and stability
- Connect NPs via **ssDNA and CTPR** to deconvolute structural and molecular CD





Conclusions

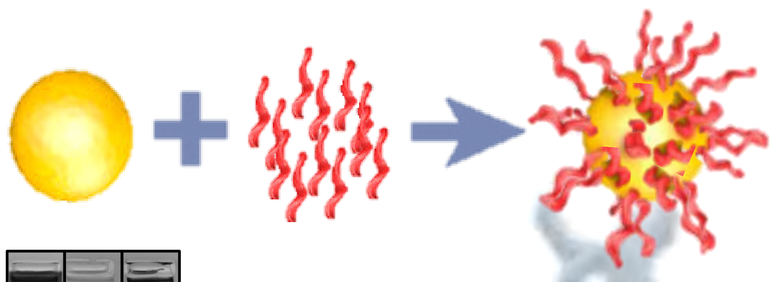
Freeze-thaw method for highly loaded, stable DNA-NPs ✓

Salt-ageing method for precise control of low DNA loading ratios ✓

PEGylation for stability of low loading ratio NPs ✓

DNA ligand exchange of PEGylated NPs for **high-yield dimer formation** ✓

High DNA loading ratio



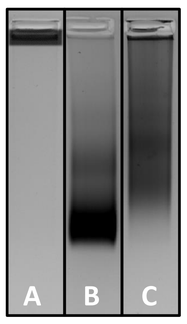


Figure 1: Agarose gel of citrate stabilized AuNPs (**A**), AuNPs with high DNA loading (**B**) and conjugated highly loaded AuNPs (**C**).

- Functionalization of AuNPs with high ssDNA loading ratios using rapid **freeze-thaw method** [4]
- **Good stability** in solution but slight aggregation, shown in agarose gel
- Strong **aggregation** upon conjugation

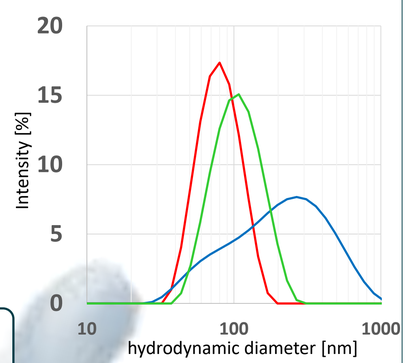
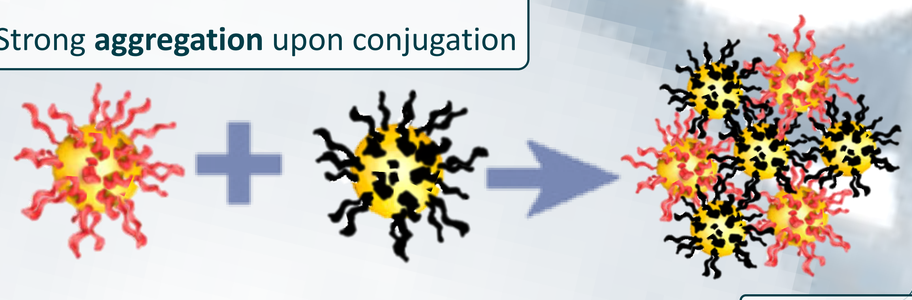


Figure 2: DLS measurements of the hydrodynamic radius of citrate stabilized AuNPs (**red**), AuNPs with high DNA loading (**green**) and conjugated highly loaded AuNPs (**blue**).



Low DNA loading ratio

Figure 3: Agarose gel of AuNPs with low DNA loading (**A**) and conjugated low loaded AuNPs (**B**).

- Controlled functionalization of AuNPs with **salt-ageing method** [5]
- Stable in solution but **prone to aggregation**, especially upon conjugation

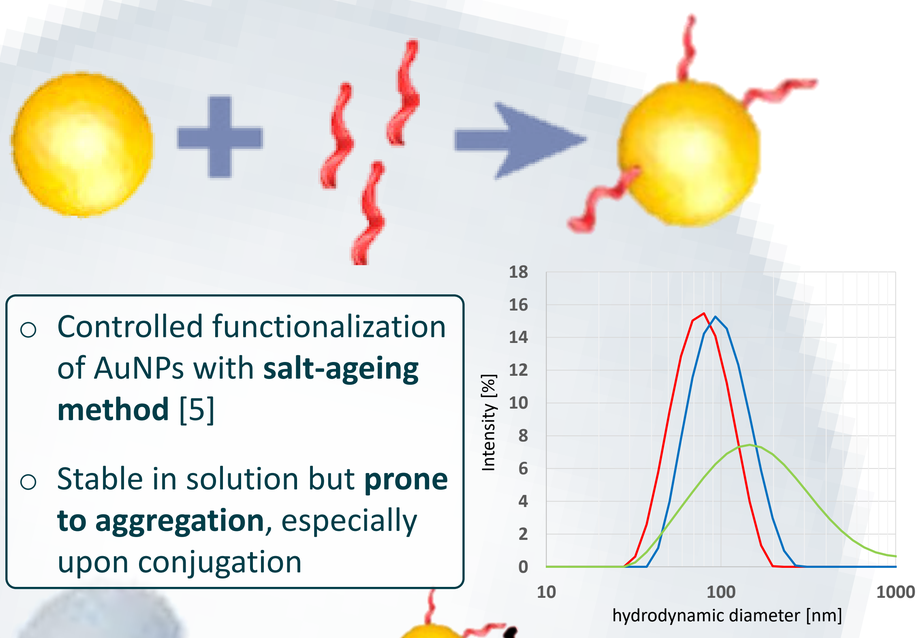
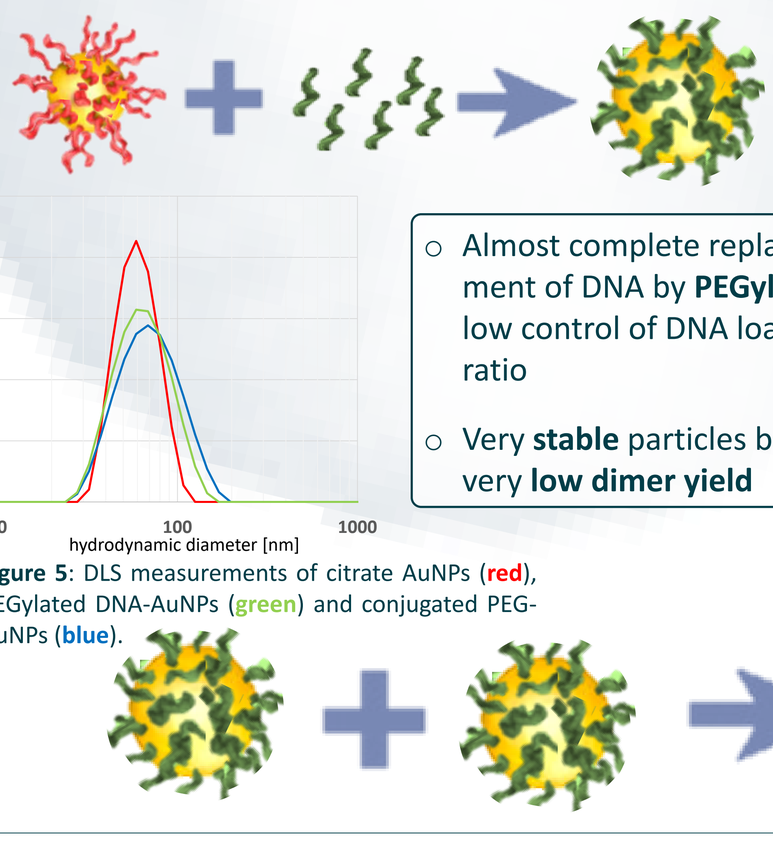


Figure 4: DLS measurements of citrate stabilized AuNPs (**red**), AuNPs with low DNA loading (**green**) and conjugated low loaded AuNPs (**blue**).

Complementary ssDNA strands

Polyethylene glycol

Adding PEG to DNA



- Almost complete replacement of DNA by **PEGylation**, low control of DNA loading ratio
- Very **stable** particles but very **low dimer yield**

Figure 5: DLS measurements of citrate AuNPs (**red**), PEGylated DNA-AuNPs (**green**) and conjugated PEG-AuNPs (**blue**).

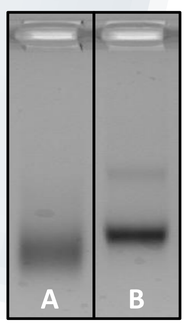
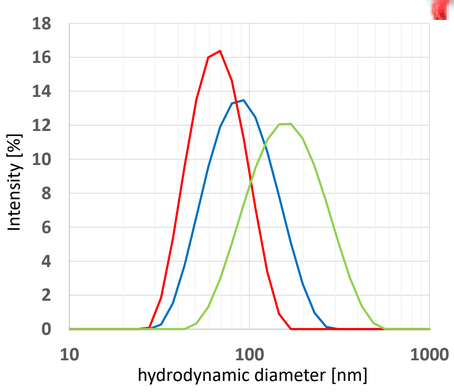


Figure 6: Agarose gel of PEGylated DNA-AuNPs (**A**) and conjugated PEG-AuNPs (**B**).

Adding DNA to PEG



- Slow PEG replacement by **DNA ligand exchange** enables high control over low loading-ratio
- Very stable particles with **high dimer yield** upon conjugation

Figure 7: DLS measurements of citrate AuNPs (**red**), DNA functionalized PEG-AuNPs (**green**) and conjugated DNA-PEG-AuNPs (**blue**).

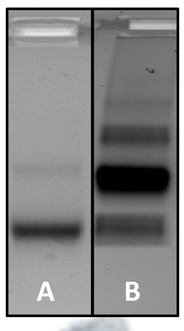
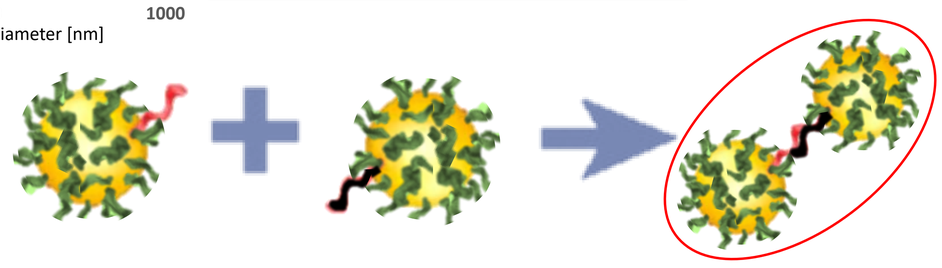


Figure 8: Agarose gel of DNA functionalized PEG-AuNPs (**A**) and conjugated DNA-PEG-AuNPs (**B**).



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Acknowledgements

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