Gold Nanoparticles (AuNP) linkage

Aim of the Experiment

In this experiment, two species of gold nanoparticles (AuNP) are cross-linked via a linker-RNA or DNA. The resulting aggregate can then be used for a nuclease cleavage assay. It is recommended to include a negative control with non-complementary oligonucleotide instead of the linker.

Materials

- DNA-labeled AuNP solution (see protocol "Gold Nanoparticles (AuNP)-DNA conjugation", diluted to 100 nM)
- Linker-oligonucleotide (100 μM) (Biomers, Germany)
- Linkage buffer (10x, 500 mM Tris [pH 8.3], 3 M NaCl)
- nuclease-free H_2O (Cart Roth, Germany)

Procedure

- Mix 28 μl of H_2O with 10 μl of buffer and add each 3 μl of the two AuNP-solutions.
- Incubate the mix at 37 °C for 30 min to remove potential secondary structures.
- Add 6 μl of linker oligonucleotide for a thousandfold molar excess of linker to combined oligonucleotides. Assuming 100 labels per AuNP, this would mean 10 linkers per label.
- The resulting mixture has the concentrations shown in table 1.

Table 1: Final linkage mix

Concentration	**Chemicals**
6 nM	AuNP 1
6 nM	AuNP 2
12 μM	linker oligonucleotide
2x	linkage buffer

- Heat the mixture to 70 °C for 2 min to remove potential secondary structures of the linker.
- Let cool down to room temperature for 15 min.
- Incubate at 4 °C for at least 6 h .
- Spin down at 2000 g for 10 min.
- After successful linkage, blue to purple aggregates should be visible as pellet in a clear supernatant. While these can hardly be brought back into solution, the pellet in the negative control can be easily solved by resuspension, leading to a red staining of the solution.