# Gold Nanoparticles (AuNP) - DNA conjugation

## Aim of the Experiment

This experiment is used to label Gold Nanoparticles (AuNP) with thiolated DNA strands. These DNA labels then enable crosslinking of AuNP upon addition of complementary oligonucleotides.

## Materials

- Centrifugal filter (Amicon Ultra,100 kDa Millipore, USA)
- Concentrated AuNP (see protocol "Preparation of Gold Nanoparticles")
- Thiolated DNA-strands (100 μM, Biomers)
- Tris (carboxyethyl) phosphine hydrochlorid (100 mM)(TCEP, Sigma Aldrich, Germany)
- Citrate buffer (100 mM, pH 3) (Simga Aldrich, Germany)
- HEPES (100mM, pH 7) (Carl Roth, Germany)
- 0,5x TBE buffer (Carl Roth, Germany)
- 1x Control buffer (1x TAE, 12,5 mM $MgCl_2$)

## Procedure

- Incubate thiolated DNA strands with 2 μl of TCEP for at least 30 min.
- Mix the thiolated DNA strands and AuNP with a molar ratio of 100:1.
- Add 0.25 volumes of citrated buffer for a final concentration of 20 mM and pH 3.
- Incubate for 3 min at room temperature. For challenging strands like G-rich sequences, extend incubation time.
- Add 0.25 volumes of HEPES for a final concentration of 20 mM and pH 7.

- To test the success of the conjugation, spot $\sim$2 µl of conjugate on a clean surface and add 2 equal volume of 1x control buffer. If the solution remains red, the conjugation was successful.
- The next step, removal of unbound DNA, is recommended to conduct shortly before linkage assays.
- Wet centrifugal filters by adding of 400 µl 0,5x TBE buffer and centrifugation in a table-top centrifuge at 2000 rcf for 5 min.
- Remove unbound DNA by addition of the labelled AuNP to the columns and centrifugation at 10000 rcf for 10 min and discard flowthrough.
- Repeat this step four times while adding 400 µl before each step.
- Retrieve AuNPs from column by placing the filter upside down into the column and centrifuging at 10000 rcf for 10 min.
- Concentration of labelled AuNP is determined at wavelength $\lambda = 518$ nm and extinction coefficient $\varepsilon = 8.5 \cdot 10^{7}$ $mol^{-1}$ $cm^{-1}$.