Gold Nanoparticles (AuNP) cleavage assay for Cas13a

Aim of the Experiment

In this experiment, gold nanoparticles (AuNP) are used to detect target-induced activity of Cas13a on paper. For this particular enzyme it is required to have the AuNP linked via RNA containing an unbound, single-stranded and Uracil-containing segment susceptible to Cas13a-dependent cleavage. Ultimately, this setup serves to use Cas13a and AuNP to detect specific target-RNAs.

Materials

- Heat plate (magnetic stirrer, RH basic, IKA, Germany)
- Nuclease-free filter paper (glass microfiber paper 934-AH RTU, Whatman, GE healthcare, Germany)
- Nuclease-free BSA (bovine serum albumine, 5%) (VWR life sciences, Germany)
- Aggregated AuNP (see protocol "Gold Nanoparticles (AuNP) linkage")
- Cas13a Lbu protein (from the species *Leptotrichia Buccalis*)
- Lbu-specific and target-specific crRNA s(extracted from cell lysate or *in vitro* transcribed)
- Target-RNA (DNA template for *in vitro* transcription: Biomers)
- Murine RNase inhibitor (NEB, Germany)
- RNase A (IDT, Germany)
- RNase processing buffer (10x, 200 mM HEPES, 500 mM KCl, 50 mM $MgCl_2$, 50% glycerol, pH 6.8)
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, USA)

Table 1: Cas13a mix final concentrations

Concentration	**Chemicals**
300 nM	Cas13a
50 nM	crRNA
10 nM	target RNA
1,5 U	RNase inhibitor
1x	RNase processing buffer

Procedure

- Block filter paper ($\sim$1 cm^2 per reaction) with BSA over night followed by thorough rinsing with nuclease-free water and drying on a heat plate at 80 °C for 20 min.
- Remove most of the supernatant from aggregated AuNP and wash twice with 1x RNase processing buffer and remove most of supernatant.
- Agitate pellet and spot $\sim$1 µlon blocked paper.
- Dry paper at room temperature for $\sim$10 min.
- Mix H_2O , buffer and crRNA and heat to 65 °C for 5 min.
- Add Cas13a and incubate at 37 °C for 10 min.
- Add RNase inhibitor (for positive control: add RNase A instead).
- Add target RNA, resuspend and spot 1 µl onto the AuNP placed on the paper.
- Observe or document the reaction on paper: For a positive result, cleaved AuNP are seen as spreading of a red circle around the aggregate while not observable in negative control.