

Gold nano-particle and crystal violet assay

This experiment integrates and adaption of the crystal violet assay¹ and an adaption of the golden nanoparticles experiment as described in².

- In a compartmented petri-dish with 4 compartments 4 ml of LB medium was added to each compartment with corresponding antibiotics.
- 300uL of the following overnight cultures were added:
 1. *E.coli* Δ CsgB untransformed
 2. *E.coli* Δ CsgB + BBa_K1316014 (CsgB + pRha, CsgA-His + pConst)
 3. *E.coli* Δ CsgB + BBa_K1316014 (CsgB + pRha, CsgA-His + pConst)
 4. *E.coli* Δ CsgB + BBa_K1316014 (CsgB + pRha, CsgA-His + pConst)
- To compartments 3 and 4 rhamnose was added to a final concentration of 0.5% (m/v)
- To compartments 3 and 4 a Dropsens gold on glass electrodes were added³
- To compartment 4 750 ul of 0.5 uM 5 nm Ni-NTA-Nanogold® (NiNTA-NG) is added⁴
- The cells are incubated with no shaking for 36h at 37°C

Conductance measurements

- The Dropsens electrodes were removed from compartments 3 and 4, and submerged in MQ 3 times to wash away loose cells and unbound NiNTA-NG.
- The electrodes from compartments 3 and 4 are laid to dry at 37°C for 24h.
- Alligator clamps are soldered to the electrode endings, and the resistance was measured with a conventional multimeter.

Crystal violet assay

- From dishes without electrodes liquid media was disposed of and the dish was submerged 3 times in clean MQ to wash away any loose cells and unbound NiNTA-NG.
- The dishes were now incubated with 1 ml Crystal Violet solution (8 g/L) shaking at 120 rpm for 20 min.
- The crystal violet was disposed of and the dish in submerged in MQ and shaken for 8 times, or until the water coming off was no longer visibly purple.
- The dishes were now incubated with 4 ml acetic acid in water solution (30% (w/w)) for 20 min at room temperature while shaking at 120 rpm to resuspend the biofilm.
- 1 ml of the acetic acid solution from the dish was now diluted in 10 ml acetic acid solution, and the absorbance was measured at 590 nm. The reference used was 30 % acetic acid in dH₂O (w/w).

¹ <http://amrita.vlab.co.in/?sub=3&brch=73&sim=208&cnt=2>

² Chen et al., Synthesis and patterning of tunable multiscale materials with engineered cells. *Nature Materials* 13, 515–523 (2014)

³ http://www.dropsens.com/en/interdigitated_electrodes.html

⁴ http://www.nanoprobes.com/products/Ni-NTA-Nanogold-His-tag-labeling-and-detection.html#Price_List