

# FUNCTIONALIZATION OF GRADIENT

## Materials

- Gradient Surfaces
- Pipette

## Chemicals

- SH-PEG-Biotin or SH-PEG
- Biotinylated protein (Table 1)
- Covering substance between AuNP (e.g. Laminin, Fibronectin, Collagen, PEG-5000) (Table 1)
- Linker (Neutravidin)
- PBS
- MilliQ-water

Table 1

Location	Name	Necessary combination	Concentrations (Ca) in solvent
On particles	Protein	SH-PEG-Biotin + Linker + Biotin-protein	100 $\mu$ M in MilliQ + 50 mg/ml in PBS + 40 nM in PBS
Between particles	Covering substance	Covering substance	20 nM in PBS (for PEG-5000: 1 mM in MilliQ)

## Functionalize surfaces

### Protein on particles and covering substance in-between (double gradient):

1. Incubate the surfaces in 300  $\mu$ l SH-PEG-biotin for 30 min in room temperature for attachment to particles
2. Rinse with MilliQ
3. Incubate in 300  $\mu$ l Covering substance for 1.5 h in room temperature for attachment between particles
4. Rinse with PBS
5. Incubate the surfaces in 300  $\mu$ l linker (Neutravidin) for 20 min in room temperature
6. Rinse with PBS
7. Incubate the surfaces in 300  $\mu$ l Biotinylated protein in the fridge over night
8. Store the glass slides with the functionalized surfaces in PBS

### Protein on particles, nothing in-between (single gradient)

1. Incubate the surfaces in 300  $\mu$ l SH-PEG-biotin for 30 min in room temperature for attachment to particles
2. Rinse with MilliQ
3. Incubate the surfaces in 300  $\mu$ l linker (Neutravidin) for 20 min in room temperature
4. Rinse with PBS
5. Incubate the surfaces in 300  $\mu$ l Biotinylated protein in the fridge over night
6. Store the glass slides with the functionalized surfaces in PBS

### Covering substance in-between AuNP without protein on the particles (single gradient):

9. Incubate the surfaces in 300  $\mu$ l SH-PEG for 30 min in room temperature to shield the particles
10. Rinse with MilliQ
11. Incubate in 300  $\mu$ l Covering substance for 1.5 h in room temperature for attachment between particles
12. Rinse with PBS
13. Store the glass slides with the functionalized surfaces in PBS