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 µM 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 µl SH-PEG-biotin for 30 min in room temperature for attachment to particles
2. Rinse with MilliQ
3. Incubate in 300 µl Covering substance for 1.5 h in room temperature for attachment between particles
4. Rinse with PBS
5. Incubate the surfaces in 300 µl linker (Neutravidin) for 20 min in room temperature
6. Rinse with PBS
7. Incubate the surfaces in 300 µl 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 µl SH-PEG-biotin for 30 min in room temperature for attachment to particles
2. Rinse with MilliQ
3. Incubate the surfaces in 300 µl linker (Neutravidin) for 20 min in room temperature
4. Rinse with PBS
5. Incubate the surfaces in 300 µl 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 µl SH-PEG for 30 min in room temperature to shield the particles
10. Rinse with MilliQ
11. Incubate in 300 µl 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