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Membrane Tube Assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details about the Membrane Tube Assay.

Attachments



[416-897.pdf](#)

50KB

Materials

Materials:

- Biotinylated GUVs (0.001% mol fraction DSPE-PEG(2000) Biotin, Avanti Polar Lipids), formed by PVA swelling method.
- Small volume flow cells of the type commonly employed for in vitro single molecule imaging (melted parafilm sandwiched between no. 1.5 coverglass)
- Streptavidin functionalized silica beads, $\pm 1.56 \mu\text{m}$ diameter (Spherotech)
- Bovine serum albumin (BSA)
- Confocal fluorescence microscope modified with an optical trap.
- Fluorescently labeled protein

Imaging buffer





(iso- osmotic to GUV swelling solution)

	A	B
	Tris pH 8.0	20 mM
	NaCl	150 mM
	TCEP	5 mM
	MgCl ₂	2 mM

Troubleshooting



Membrane Tube Assay

- 1 Passivate flow cell with [M] 1 mg/mL BSA in imaging buffer.
- 2 Rinse flow cell with 2 flow cell volumes of imaging buffer. 
- 3 Mix GUVs with fluorescent protein and add to flow cell, allowing GUVs to settle on the bottom surface of the flow cell. 
- 4 Add  1 μ L of a 1:1000 dilution of silica beads to flow cell. 
- 5 Trap a bead in the optical trap, bring into contact with a GUV, and retract, forming a membrane tube.
- 6 Visualize protein recruitment to membrane tube with confocal microscopy. 