May 09, 2024

Preparation and imaging of lipid bilayer-coated silica microspheres

DOI
dx.doi.org/10.17504/protocols.io.rm7vzjy2rlx1/v1

Ezra Bruggeman[1]

[1]University of Cambridge

ASAP Collaborative Rese…

Ezra Bruggeman
University of Cambridge

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.rm7vzjy2rlx1/v1

External link: https://doi.org/10.1101/2023.02.07.527479

Protocol Citation: Ezra Bruggeman 2024. Preparation and imaging of lipid bilayer-coated silica microspheres. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzjy2rlx1/v1

Manuscript citation:
Bruggeman et al., POLCAM: Instant molecular orientation microscopy for the life sciences. bioRxiv 2023.02.07.527479 (Feb 2023), doi: https://doi.org/10.1101/2023.02.07.527479

Protocol status: Working
We use this protocol and it's working

Created: May 08, 2024

Last Modified: May 09, 2024

Protocol Integer ID: 99426

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000509

Abstract

This protocol describes the preparation and imaging of lipid bilayer-coated micrometer-scale glass beads. This protocol was used to generate the data presented in **Figure 2d-g** in the following publication:

- Bruggeman et al., POLCAM: Instant molecular orientation microscopy for the life sciences. bioRxiv 2023.02.07.527479 (Feb 2023), doi: https://doi.org/10.1101/2023.02.07.527479

The described protocol is based on a previously published protocol that uses lipid extrusion instead of a tip probe sonicator to generate lipid vesicles:

- Tingting Wu, Jin Lu and Matthew D. Lew. *pixOL: pixel-wise dipole-spread function engineering for simultaneously measuring the 3D orientation and 3D localization of dipole-like emitters.* bioRxiv (2022). https://doi.org/10.1101/2021.12.30.474544

Guidelines

Chloroform is toxic (**SDS**).

Materials

Reagents:

- DPPC in chloroform (**850355C**, Avanti Polar Lipids, sold by Merck). *Note: solid at -20 °C.*
- Cholesterol (**C8667-5G**, Sigma-Aldrich). *Note: will need to be diluted in chloroform, after which it is liquid at -20 °C.*
- Chloroform (**366927**, Sigma-Aldrich). *Note: chloroform is toxic (**SDS**).*
- Tris buffer: 100 mM NaCl, 10 mM **Tris base**, pH 7.4
- Tris-Ca2+ buffer: 100 mM NaCl, 3 mM CaCl2, 10 mM **Tris base**, pH 7.4
- 5 µm diameter silicon dioxide (SiO2, i.e. silica) microspheres **(44054-5ml-F, Sigma-Aldrich)**
- 0.01% poly-L-lysine solution (P4707, Sigma-Aldrich).

Equipment:

- Tip sonicator.
- Plasma cleaner.
- Small centrifuge for Eppendorfs **(Cat. No. 5453000015, MiniSpin plus, Eppendorf)**.
- Heated water bath that can reach 65 °C

Other:

- 0.02 µm syringe filters (6809-1102, Whatman) with compatible syringes.
- Glass viles with PTFE (Teflon) cap liners (**14-955-327**, Thermo Fisher Scientific, or **600460**, Avanti Polar Lipids, sold by Merck). If you don't have vials with PTFE caps, you can use PTFE sealing tape (**Z104388-1PAK**, Merck) before screwing a regular cap on a vial.
- Parafilm (**P7543-1EA**, Sigma-Aldrich)
- Cover glass.
- Frame-seal slide chamber (9x9 mm, SLF0201, Bio-rad).

Preparation of reagents

1 Prepare Tris buffer: 100 mM NaCl, 10 mM **Tris base**, pH 7.4

2 Prepare Tris-Ca2+ buffer: 100 mM NaCl, 3 mM CaCl2, 10 mM **Tris base**, pH 7.4

3 Filter 50 ml of Tris buffer and Tris-Ca2+ buffer using a 0.02 µm syringe filter (6809-1102, Whatman).

Preparation of DPPC + 40% cholesterol lipid vesicles

1h 22m

4 Dissolve DPPC (850355C, Avanti Polar Lipids) in chloroform (366927, Sigma-Aldrich) to a concentration of [M] 25 mg/mL in a glass vial with a PTFE (Teflon) lined cap (14-955-327, Fisher Scientific). You will need 23 µL per sample.

5 Dissolve cholesterol (C8667-5G, Sigma-Aldrich) in chloroform (366927, Sigma-Aldrich) to a concentration of [M] 10 mg/mL in a glass vial with a PTFE (Teflon) lined cap (14-955-327, Fisher Scientific). You will need 20 µL per sample.

6 Prepare a DPPC + 40% cholesterol mixture by combining 23 µL [M] 25 mg/mL DPPC with 20 µL [M] 10 mg/mL cholesterol in a new glass vial with a PTFE (Teflon) lined cap (14-955-327, Fisher Scientific).

7 Evaporate the solvent Overnight under vacuum.

8 Re-hydrate the lipids/cholesterol mixture using 1 mL of Tris-Ca2+ buffer ([M] 100 millimolar (mM) NaCl, [M] 3 millimolar (mM) CaCl2, [M] 10 millimolar (mM) Tris base, pH 7.4).

9 00:40:00 Vortex for 00:00:30 .

40m 30s

10 Sonicate the solution using a tip sonicator for 00:40:00 (cycles of 45 seconds on and 15 seconds off, 60% amplitude) until the solution runs clear. 40m

11 Centrifuge the solution for 00:01:30 at 14000 rcf to remove residu from the sonicator probe. Keep the supernatant. 1m 30s

Coating silica microspheres with the DPPC/40% cholesterol mixture 35m

12 Dilute the glass beads (5 µm diameter, 44054-5ML-F, Sigma-Aldrich) to approximately [M] 2.8 mg/mL .

13 Clean the beads by centrifuging and replacing the supernatant with Tris-Ca2+ buffer.

14 Heat the glass beads and the lipid vesicle solutions to 65 °C using a heated water bath.

15 Once heated to 65 °C , mix the glass bead and lipid vesicle solution together in a 1:1 ratio. Leave the mixture at 65 °C and wait for 00:30:00 . 30m

16 Turn the heating element of the water bath off, and let the glass bead/lipid vesicle mixture slowly cool down to room temperature inside the water bath. The lipid vesicles will attach to the glass beads, open and form a lipid-bilayer on the glass surface of the beads.

17 Gradually replace the buffer by Tris (100 mM NaCl, 10 mM Tris base, pH 7.4):

17.1 Centrifuge for 00:05:00 at 0.3 rcf . 5m

17.2 Gently replace two thirds of the supernatant with Tris.

17.3 Repeat the last two steps (14.1 and 14.2) a total of 6 times.

18 Store the lipid-coated glass beads at 4 °C and use as soon as possible, or within a week of preparation.

Imaging 50m

19 Argon plasma clean cover glass (VWR collection, 631-0124) for 00:30:00 in a plasma cleaner (Expanded Plasma Cleaner, PDC-002, Harrick Plasma). 30m

20 Create a sample well on the cleaned cover glass by sticking a frame-seal slide chamber (9x9 mm, SLF0201, Bio-rad) on the cover glass.

21 Pipet 70 µL of 0.01% PLL (0.01% poly-L-lysine solution, P4707, Sigma-Aldrich) into the well and wait for 00:15:00. The PLL will coat the surface of the cover glass. 15m

22 Use a pipet to remove the excess PLL from the well and immediately replace it with 70 µL of filtered PBS.

23 Use a pipet to remove the excess filtered PBS from the well and immediately replace with 70 µL filtered PBS. Gently pipet up and down in the corners of the well. Repeat this step 2 more times.

24 Use a pipet to remove the excess PBS from the well and immediately replace with 50 µL of the lipid bilayer-coated beads. Wait for 00:05:00. 5m

25 Use a pipet to gently remove the excess PBS from the well and immediately replace with 50 µL of a dye of choice, *e.g.* [M] 1 nanomolar (nM) Nile red for PAINT of the lipid membrane.

26 Image the sample the same day.