Passivated Tracer Particles with PLL-g-PEG

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This protocol passivates 200nm fluorescent sulfate beads and 3um sulfate beads. They are observed to be 100% non-sticky and freely diffusing when mixed in 2mM MgCl2 buffer (usually makes normal beads sticky), 0.8% PEG, and 1mg/ml microtubules.

PEG was from Laysan Bio: mPEG-SCM, MW 20kDa

PLL was Sigma: number P7890 MW, 15-30kDa

Prepare PLL-grafted-PEG Stock

Want molar ratio of approximately 3.5 Lysine : 1 PEG
Lysine: MW 137Da

1) Make 50mM sodium borate buffer, pH 8.5
    a. 1.906g into 100ml
2) Dissolve 1.7mg of p-lysine and 71.4mg PEG into 1.25ml of sodium borate buffer
    a. Final concentrations: 10mM Lysine and 2.86mM PEG
3) Fill up small dialysis membrane to capacity (so that volume does not change much).
    a. Use 2x 500uL capsules with pore size 5kDa.
    b. Dialyze against DI H2O overnight, then replace H20, let go another night.
4) Recover and aliquot.
    a. For long term storage, flash freeze and store in -20C.

Coating microspheres with PLL-PEG

The PLL is positively charged, so you must use beads with a negative surface charge.
Carboxylated beads supposedly work, according to this protocol:
(Title: **Ligand-Specific Targeting of Microspheres to Phagocytes by Surface Modification with Poly(L-Lysine)-Grafted Poly(Ethylene Glycol) Conjugate**).
Sulfate beads are supposed to be very negatively charged, and thus work very well.

Ratio of PLL-PEG:beads should be dependent on the surface area of the beads.

3um beads:

1) Mix 10uL PLL-PEG(~1.37mg/ml of PLL) + 100uL HEPES(100mM) + 12uL beads (10%) + 878uL H2O
2) Incubate an hour on a rotating platform.
    a. **Ultra-sonicating at 20% (low setting) inside the 1.5ml eppendorf tube was very helpful for the 3um beads and ESSENTIAL for the 200nm beads. This prevents aggregation into clusters of beads.**
    b. Sonicate once every 20 minutes for the 1 hour.
        i. Each time, sonicate 3X for 3 second bursts (too much can melt the beads).
    c. Put back on rotating platform.
3) Transfer to 500uL eppendorf tubes. Spin at 4k (5140g) for 5 minutes. Will get some beads glazing the walls, but should get a reasonable pellet, too.
4) Aspirate supernatant and consolidate pellets to another 500uL tube.
5) Exchange buffer (whatever buffer you like). Vortex and pellet should resuspend easily.
6) Spin again as in step 3.
7) Aspirate and rususpend in whatever volume you want. (approximately 50ul)
    a. Transfer to new tube for storage in 4C.

200nm beads:

1) Mix 15uL PLL-PEG + 20uL HEPES (100mM) + 2.4uL bead stock + 162.6 H20
2) Incubate an hour on a rotating platform.
    a. **Ultra-sonicating at 20% (low setting) inside the 1.5ml eppendorf tube was very helpful for the 3um beads and ESSENTIAL for the 200nm beads. This prevents aggregation into clusters of beads.**
    b. Sonicate once every 20 minutes for the 1 hour.
        i. Each time, sonicate 3X for 3 second bursts (too much can melt the beads).
    c. Put back on rotating platform.
3) Transfer to 500uL eppendorf tubes. Spin at 14k (18,000g) for 5 minutes. Will get some beads glazing the walls, but should get a reasonable pellet, too.
4) Aspirate supernatant and consolidate pellets to another 500uL tube.
5) Exchange buffer (whatever buffer you like). Vortex and pellet should resuspend easily.
6) Spin again as in step 3.
7) Aspirate and rususpend in whatever volume you want. (approximately 40ul)
    a. Transfer to new tube for storage in 4C.