Electronic Supplementary Material (ESI) for Nanoscale
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Volume Labeling with Alexa Fluor Dyes and Surface Functionalization of Highly Sensitive Fluorescent Silica (SiO_2) Nanoparticles

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SUPPLEMENTAL MATERIAL

MitoHealth Staining (on live cells)

The MitoHealth stain accumulates in mitochondria in live cells proportional to the mitochondrial membrane potential. Scan the slide with filter appropriate for TRITC 595/620 (red).

1. Prepare MitoHealth stain solution by adding 3.25mL of media to 6 mLs of cell-growth media (RPMI 1640 media) without serum. Add 50mL per well followed by 150mL of growth media.
2. Incubate at 37°C for 30 minutes.
3. Rinse wells, 2x with PBS
4. Proceed with Nuclear Staining

Nuclear Staining with Hoescht 33342

1. Fix and stain cells with Hoescht by preparing adding 1.5mL of 16% Paraformaldehyde and 3ml of Hoescht stain in 4.5mLs of PBS.
2. Add 125mL/well
3. Incubate at room temperature (RT) for 15 minutes.
4. After incubation, rinse 2x with PBS
5. Add 125mL of 2.5% BSA to each well and incubate for 20 minutes at RT (blocking step).
6. Rinse wells 2x with PBS. You can visualize the cells at this point or proceed with actin staining. Make sure to add PBS to wells if you are stopping at this point.

Actin Staining (on fixed cells).

1. After rinsing with PBS after the blocking step 5 above, you must permeabilize the cells.
2. Add 125ml of 0.1% Triton X-100 per well and incubate at RT for 5 minutes.
3. Prepare AlexaFluor 633 Phalloidin by adding 2-3 units (10-15mL) of dye to 200mL of PBS. Scale up according to number of wells to cover.
4. Add 125mL of dye/well and incubate at RT for 20 minutes.
5. Wash 2x with PBS, add PBS to wells and store in a dark area (preferably 4°) until ready to visualize