Supplementary Information

for

Single Particle Orientation and Rotational Tracking (SPORT) in Biophysical Studies

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Methods for Axonal Transport of Gold Nanorods in Primary Neuronal Cells

Preparation of transferrin surface-modified gold nanorods. **C**etyl trimethylammonium bromide (CTAB)-capped gold nanorod colloidal solution (25×73 nm, 1.3×10^{11} particles/mL) was purchased from Nanopartz (Loveland, CO). To facilitate their internalization into neuron cells, the gold nanorods were surface-modified with transferrin. A NHS-PEG disulfide linker (Sigma-Aldrich) was used by following a published protocol[1]. The NHS-PEG disulfide linker has both disulfide and succinimidyl functionalities for respective chemisorption onto gold and facile covalent coupling of TAT peptide. Briefly, excessive surfactant was first removed from 1.0 mL gold nanorod solution by centrifugation at 3000 g for 10 minutes and the particles were resuspended in 1.0 mL of 2 mM borate buffer. A proper amount of fresh NHS-PEG disulfide solution (in dimethyl sulfoxide) was added to reach a final thiol concentration of 0.2 mM and reacted with gold nanorods for 2 hours. The solution was then cleaned up by centrifugation and resuspended in 2 mM borate buffer. 40 μg of transferrin was added to the gold colloidal solution

[1] R. Narayanan, R. J. Lipert and M. D. Porter, *Anal. Chem.*, 2008, **80**, 2265-2271.

and reacted for 8 hours. The gold nanorods were then blocked by adding 100 mL 10% BSA solution (2 mM borate buffer) for over 8 hours. Before use, the colloidal gold nanorod probes were cleaned up by centrifugation and resuspended in 500 μL of 1% BSA (2 mM borate buffer). The concentrated colloidal solution was diluted in cell culture medium to a final concentration of 4.3×10^9 particles/mL for incubation with cells.

Cell culture. Hippocampal cultures were obtained from brains of embryonic Day 18 (E18) to Day 19 (E19) C57Bl/6 mice following standard protocols. The dissociated cells were plated on poly-L-lysine-coated coverslip and maintained in Neurobasal/B27 media (Invitrogen, Carlsbad, CA) supplemented with 0.5 mM glutamine. The cells were incubated with transferring-modified AuNRs in an incubator (37°C, 5% CO_2) for 4 hours. Then the coverslip was removed from the incubator and made into a chamber by using double sided tape and a clean glass slide.

Microscopy and live cell imaging. An upright Nikon Eclipse 80i microscope equipped with a heating stage was used in this study. The temperature of the heating stage was set at 37°C. The samples were illuminated through an oil immersion condenser (numerical aperture 1.40) and the optical signals were collected with a 100× Plan Apo/1.40 oil immersion objective. A bandpass filter (Semrock, Rochester, NY) with a central wavelength of 700 nm and a full width at half maximum (FWHM) of 13 nm was inserted into the light path. An Andor iXon^{EM}+ camera (512×512 imaging array, 16×16-μm pixel size) was used to record the events of axonal transport at 32 frames per second. MATLAB and NIH ImageJ were used to analyze the images and videos.