SUPPORTING INFORMATION

Preparation of βNGF-QD complexes. Recombinant mouse βNGF (1156-NG/CF, R&D Systems) was biotinylated via carboxyl group substitution following procedures modified from Rosenberg et al.\textsuperscript{16} βNGF (100µM, 100ug in 74µl PBS) was diluted 1:10 with 10 mM pyridine-HCL at pH 4.8 (Aldrich). Biotin hydrazide (Sigma) (10µmol/ml in 1 DMSO:1 H\textsubscript{2}O) was added at a molar ratio of 2000 biotins per βNGF subunit. The coupling agent, 1-ethyl-3-(3-dimethylaminopropoyl)-carbodiimide (EDAC, Sigma) was added to this solution at a molar ratio of 2000 EDAC per βNGF and the resulting solution was incubated overnight at 23°C. This solution was supplemented with BSA and cytochrome C (Sigma) (1mg/ml each), ultrafiltered (Centricron MWCO 3kD, Millipore), and transferred to PBS. An excess molar ratio of 2000 EDAC: 1 βNGF and 2000 biotin: 1 βNGF is expected to biotinylate all βNGF molecules and result in 3 or less biotins per βNGF\textsuperscript{15}. βNGF-QD complexes were formed by gentle vortexing and 30 mins of incubation of biotinylated βNGF with red (655nm) QDs containing an outer shell of covalently bound streptavidin (1012-1,Quantum Dot Corp) at 1 βNGF: 1 QD. Based on Quantum Dot Corp’s estimates of 5-10 streptavidins/QD, it is expected that a biotinylated βNGF-QD of 1:1 would bind all available biotinylated βNGF.

Atomic force microscope visualization of βNGF-QD complexes. Freshly cleaved mica surfaces (SPI Supplies, West Chester, PA) were exposed to 23 mmHg vacuum and heated for 135 minutes at 180°C adjacent to a pool of APTES (1 mL/150 mm\textsuperscript{2} mica). After cooling to room temperature, 8µL of QD solution (5nM in PBS, pH 7.2) or βNGF-QD complexes (10nM in PBS, pH 7.2) was placed on the mica and allowed to adsorb for 15 minutes. Mica surfaces were then rinsed gently with a flow of 10mL DI water, dried under nitrogen stream, and immediately analyzed by AFM (Q-Scope 250, Quesant Instruments, Corp, Agoura Hills, CA). Quesant’s standard silicon tips with force constants ~40 N/m and resonant frequencies ~137 kHz were used to acquire the images under ambient laboratory conditions. Amplitude and phase images were simultaneously collected for samples at a scan rate of 2 Hz and scan area of 4 µm\textsuperscript{2}. Cross-section height profiles were extracted from the images with Quesant-supplied software program Q-Analysis.
Exposure of PC12 Cells to βNGF-QD complexes. PC12 cells (ATCC CRL-1721, ATCC) were grown in RPMI-1640 supplemented with 10% HS and 5% FBS at 37°C. For short-term receptor binding and uptake studies, cells (5×10^5 /well) were seeded in collagen coated poly-d-lysine glass bottom culture dishes (MatTek). Cells were incubated with βNGF-QD solution (10, 30, 60, and 100nM in DMEM). Controls were performed in parallel studies at the same concentrations using streptavidin-QDs. Cells were allowed to incubate in test and control solutions for 1 hour at 37°C, washed with DMEM(x2), fixed with 4-10% paraformaldehyde (15-20 mins), and mounted in glycerol for imaging. In longer-term neurite induction studies, cells were seeded (5x10^4 cells/well) in custom-constructed polystyrene-walled wells containing βNGF-QD (3nM and 30nM in RPMI). Controls were done in parallel studies using: 3nM and 30nM biotinylated βNGF; 3nM and 30 nM streptavidin-QD; 0.3nM βNGF in RPMI. Cells were exposed to test and control solutions for 3-5 days before fixation and image analysis.

Confocal microscopy. βNGF-QD-treated PC12 cells and controls were imaged with an Olympus BX-DSU spinning disk confocal microscope (Olympus, USA). All samples were examined using a 60x oil immersion objective lens (Plan Apochromat NA 1.4) or 60x water immersion (LUMPLANFL NA 0.9) objective lens. Quantum dots were imaged using a 75W xenon-arc lamp with an Olympus Filter cube (DSUMRFPHQ- ex: 535-555nm, em: 570-620nm) and a Hamamatsu ORCA high resolution, deep cooled monochrome CCD camera (Hamamatsu, Japan). Serial optical sections were taken using 0.5 µm optical slices and the DSU-Disk #3. Care was taken to collect test and control samples under equal exposure periods for suitable comparison. All image acquisition, processing and analysis were done using SlideBook software (v. 4.0, Intelligent Imaging Innovations, Denver, CO).

Image analysis. Quantification of neural induction was measured using ImagePro Plus version 4.1 (Media Cybernetics, Silver Spring, MD). Phase contrast images were randomly acquired and calibrated at 2.98 pixels/µm. Neurite growth was measured using the trace tool, with each extension defined from the tip of the axon to the end of the longest branch. Cells with no visible neurite outgrowth were given a value of 0. Histogram plots were created using Excel. n>700 cells were analyzed from images taken randomly in test experiments and n>450 cells for control experiments.