Pyrenebutyrate Leads to Cellular Binding, Not Intracellular Delivery, of Polyarginine-Quantum Dots

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Cell Culture: BS-C-1 cells (ATCC, Manassas, VA) were maintained in a 37°C, 5% carbon dioxide environment in modified Eagle's medium (MEM, Invitrogen, Carlsbad, CA) with 10% (v/v) fetal bovine serum (FBS, Invitrogen). Cells were passaged every 3 days. For fluorescence imaging, cells were cultured in 35 mm glass-bottom cell culture dishes (MatTek, Ashland, MA) and imaged in Leibovitz medium (Invitrogen) or PBS (Invitrogen).

Nuclear Staining: The cell nucleus was stained with 55 µM 4',6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen) at 37°C for one hour. Cells were washed twice with PBS before additional experiments.

Microinjection: A 2 nM streptavidin-QD (655 nm emission, Q10121MP, Invitrogen) solution supplemented with 3% bovine serum albumin (BSA) was used for microinjection (Injectman/FemtoJet, Eppendorf, Hamburg, Germany). After microinjection, cells were washed twice with cell culture medium (modified Eagle's medium supplemented with 10% fetal bovine serum) and incubated at 37°C for 10 min before imaging. PA was not included during microinjection as it led to some endocytosis in the time between microinjection and imaging.

Microscopy and Image Analysis: Epi-fluorescence microscopy was carried out with an inverted microscope (Olympus IX71, Center Valley, PA). The magnification and N.A. of the objective used in each experiment is noted in the text. A xenon lamp was used for excitation and a CCD camera (DU-897, Andor, South Windsor, CT) was used for detection. A "DAPI" filter cube (DAPI-1160A, Semrock) was used to image DAPI and a "QD" filter cube which includes a 500 nm long-pass filter was used to image QDs (QDLP-A, Semrock). Confocal microscopy was carried out with a LSM 510 confocal microscope (Carl Zeiss Inc., Germany) using a 40x, 1.30 N.A., oil immersion objective. Quantum dots (655 nm, Invitrogen) were excited with the 488 nm line of an argon ion laser. The excitation laser was separated from fluorescent emission with a 585 nm long pass filter. DAPI was excited with the 364 nm line of an UV laser. The excitation laser was separated from fluorescent emission with a 385 nm – 470 nm band pass filter. The pinhole was set to collect a 0.8 μm optical slice. TEM images were recorded on a Hitachi H-7500 TEM following standard fixation and embedding protocols. Silver enhancement was used to better resolve the QDs. Image J (http://rsb.info.nih.gov/ij/) was used for fluorescence image analysis and processing. The brightness and contrast of the fluorescent images were increased for publication purposes with all comparable images increased to the same level.

Figure S1. Confocal z-stack of PA-QDs (20 nM, red) bound to the plasma membrane of BS-C-1 cells collected at 2 μm intervals. Cells were incubated at 4°C to prevent internalization. This high concentration of PA-QDs bound to the plasma membrane demonstrates the contribution of out-of-plane fluorescence from the QDs in the thin BS-C-1 cells. DAPI (blue) was used as a nuclear stain.