Supporting Information

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Pyrenebutyrate-mediated Delivery of Quantum Dots Across the Plasma Membrane of Living Cells

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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).

Nuclear and Membrane Staining: The cell nucleus was stained with 55 μM 4',6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen) at 37°C for one hour. The plasma membrane was stained with 1,1'-dioctadecyl-3,3,3'3',-tetramethyl-indocarbocyanine perchlorate (DiI, Invitrogen) following quantum dot incubation to minimize staining of intracellular membranes. DiI was diluted to 3.2 μM in PBS and centrifuged briefly to remove dye aggregates. The dilute solution of DiI was added to the cells and incubated at 37°C for ten minutes.

Trypan Blue Assay: BS-C-1 cells were incubated with 4 μM pyrenebutyrate for 60 minutes, washed, incubated with a 0.004 % solution of trypan blue, and assessed for viability. No difference was observed between pyrenebutyrate-treated and control cells. As further tests of membrane integrity, cells were incubated with trypan blue following the combined pyrenebutyrate-PA-QD treatment. In both cases, no indication of membrane permeabilization was observed. It should be noted that pyrenebutyrate is removed from the cells upon replacement of the cell culture medium. (Takeuchi, T.; Kosuge, M.; Tadokoro, A.; Sugiura, Y.; Nishi, M.; Kawata, M.; Sakai, N.; Matile, S.; Futaki, S. *ACS Chem. Biol.*

2006, *1*, 299-303.) Pyrenebutyrate exposure is limited to six minutes in imaging experiments suggesting that any decrease in long term viability will be minimal.

Zeta Potential Measurements: The zeta potentials of PA-QDs were measured with a Malvern Zetasizer (Nano-ZS, Malvern Instruments, Worcestershire, UK) using a universal dip cell in disposable cuvettes. Unless otherwise noted, zeta potentials were measured in ultrapure water. The Smoluchowski approximation was used to convert the electrophoretic mobility to a zeta potential. Experiments consisted of 30 runs per measurement and all experiments were carried out in triplicate.

Membrane Potential. To reduce the membrane potential of the cells, PBS was supplemented 136.9 mM KCl, 1.5 mM KH_2PO_4, and 8.3 mM K_2HPO_4 (Mallinckrodt Chemicals, Phillipsburg, NJ). This solution is referred to as PBS(K) and was used at a concentration of 50% providing 75 mM extracellular potassium. As with PBS, the addition of pyrenebutyrate resulted in a decrease in zeta potential indicating that potassium does not affect the ability of pyrenebutyrate to interact with polyarginine. The reduction of the membrane potential due to the extracellular potassium was verified with FluoroGrade™ bis-(1,3-dibutylbarbituric acid) trimethine oxonol ($DiBAC_4(3)$, Invitrogen) and a BD LSR Flow Cytometer (Franklin Lakes, NJ). A $DiBAC_4$ stock solution of 2mg/mL was made and stored at -20°C for no more than a week. Cells were incubated at 37°C with 50% PBS(K) and 2µM $DiBAC_4$ for 30 minutes then washed twice with Dulbecco's phosphate buffered saline (DPBS, Invitrogen) and scraped with 500µL of DPBS. Data (20,000 events) were collected with the FITC filter of the BD LSR Flow Cytometer. A 36% increase in fluorescence was observed confirming a reduction in membrane potential with the addition of potassium.

Microscopy and Image Analysis: Confocal microscopy was carried out with a LSM 510 confocal microscope (Carl Zeiss Inc., Germany) using a 1.30 N.A., 40x, oil immersion objective. Quantum dots (655 nm, Invitrogen, Cat. Q25021MP) were excited with the 457 nm line of an argon ion laser. The excitation laser was separated from fluorescent emission with a 585 nm long pass filter. DiI was excited with the 543 nm line of HeNe laser. Emission was filtered through a 560-615 nm band pass filter. DAPI was excited with the 364 nm line of an UV laser. The excitation laser was separated from fluorescent

emission with a 385-470 nm band pass filter. The pinhole was set to 50 μm. For single particle tracking to determine the trajectories of PA-QDs and to measure colocalization with FITC-dextran, an inverted microscope (Olympus IX70, Center Valley, PA) in an epi-fluorescent configuration with a 1.20 N.A, 60x, water immersion objective (Olympus) was used. Quantum dots (705 nm, Invitrogen, Cat. Q25061 MP) were excited with the 457 nm line of an argon ion laser (Melles Griot, Carlsbad, CA). The laser light was directed onto the cells using a 457 nm dichroic mirror (Chroma, Rockingham, VT). Quantum dot emission was separated from the excitation laser with a 620 nm long-pass mirror (Chroma) and imaged onto a CCD camera (DU-888, Andor, South Windsor, CT). FITC-dextran (10,000 MW, 0.1 mg/mL, Invitrogen, Cat. D1820) and 1 nM PA-QDs were incubated for two hours at 37°C. For the pyrenebutyrate (Sigma-Aldrich, Cat. 257354) treatment, cells were incubated for two minutes with 1 μM pyrenebutyrate then incubated for four minutes with 0.75 mg/mL FITC-dextran, 1 nM PAQDs, and 1 μM pyrenebutyrate. The higher concentration of FITC-dextran was necessary to observe any FITC-dextran uptake during such a short period. After incubation, cells were washed with cell culture medium and Leibovitz medium. Images were recorded at a rate of 2 frames/second with a 500 ms exposure. Image J (http://rsb.info.nih.gov/ij/) was used for tracking and quantifying cellular fluorescence. Particle tracking was performed with the Image J plugin, "Manual Tracking" (http://rsb.info.nih.gov/ij/plugins/track/track.html). The "Region of Interest Manager" in Image J was used for fluorescence measurements. The cell outline, excluding the nucleus, was defined in transmitted light images and transferred to fluorescence images for analysis. In an analysis of 26 cells, no difference was observed between the use of transmitted light images and DAPI fluorescence to define the nucleus. The intensity of each cell was normalized by the area of the cell. The brightness and contrast of the images were increased for publication purposes with all images increased to the same level. Fluorescence intensities listed in Table S1 and S2 were generated from raw data and normalized against the maximum signal. Using this method, the background intensity, with no QDs present, has a normalized fluorescence of 8%.

Table S1. Relative fluorescence intensity as a function of PA-QD concentration. Fluorescence was measured from confocal images of cells following a four minute incubation at 37°C, n is the number of cells analyzed. The ratio of pyrenebutyrate to quantum dots is held constant.

PA-QD (nM)	PBS (% fluorescence)	PBS-pyrenebutyrate (% fluorescence)
4	19 ± 4, n=18	100 ± 14, n=16
1	26 ± 4, n=42	50 ± 4, n=37

Table S2. Relative fluorescence intensity as a function of temperature. Fluorescence was measured from confocal images of cells following a four minute incubation with 1 nM PA-QDs and 1μM pyrenebutyrate at either 4°C or 37°C, n is the number of cell analyzed.

Temperature (°C)	PBS (% fluorescence)	PBS-pyrenebutyrate (% fluorescence)
4	15 ± 1, n=13	58 ± 5, n=16
37	26 ± 4, n=42	50 ± 4, n=37

Figure S1. Colocalization (yellow) of PA-QDs (red) with FITC-dextran (green), a fluid phase marker of endocytosis. (a) Two hour incubation at 37°C in full cell culture medium (MEM, 10% FBS) shows colocalization following endocytic uptake. (b) In the presence of pyrenebutyrate, a four minute incubation allows very little time for FITC-dextran uptake and shows no colocalization with PA-QDs. Scale bar; 10 μm.

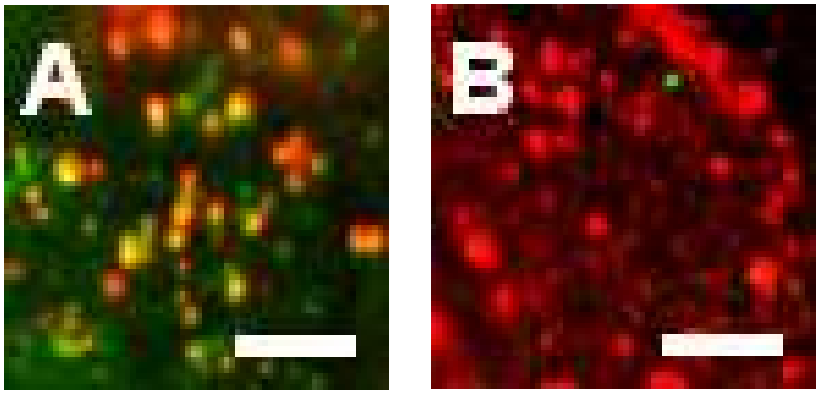

Figure S2. Representative trajectories for PA-QDs incubated for four minutes with PBS (black) or PBS-pyrenebutyrate (gray) followed by a one hour incubation at 37°C in full cell culture medium (MEM, 10% FBS). For the PBS-treated cells, the additional hour incubation allows time for endocytosis. For the PBS-pyrenebutyrate-treated cells, the additional hour incubation does not result in any change in motion of the PA-QDs and is used only for comparison with the PBS-treated cells. Each trajectory is 50 s in duration. Black circles indicate the beginning and end of each trajectory.