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Electronic Supplementary Information

for

Synthetic Development of Cell-Permeable Polymer Colloids Decorated with Nanocrystal Imaging Probes Optimized for Cell Tracking

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MATERIALS AND METHODS

Materials. 2-(*N,N*-diethylamino)ethyl 2-methacrylate (DMAEMA) (Aldrich, inhibitor removed using a short path column of basic alumina), 2-aminoethyl 2-methacrylate hydrochloride (AEMA) (Aldrich), poly(ethylene glycol) dimethacrylate (PEGDMA) (MW ~ 330), ammonium persulfate (APS) (Aldrich) and 2-ethyl-2-thiopseudourea hydrobromide (Aldrich) were used as supplied unless described otherwise. Anhydrous solvents of the highest possible purity were used for all chemical transformations. Buffers and media were purchased from Sigma-Aldrich or prepared from biochemical grade salts and ultra-high purity water (Millipore) and sterile-filtered prior to use. QD 605 ITK Streptavidin conjugates (Life Tech) were used along with 4′,6-diamidino-2-phenylindole (DAPI) (Life Tech), and LysoTracker® Green (Life Tech) for confocal fluorescence microscopy experiments. Concentrations of nanocrystals were estimated using the procedure of Peng *et al*[1]. HeLa cells (ATCC# CCL-2) were obtained from ATCC (Manassas, VA) and grown according to guidelines.

Methods

Synthesis of amine-terminated polymer colloids. The chemical synthesis of the small library of guanidinylated colloids reported in this study builds greater molecular complexity from an approach we have detailed elsewhere[2] or have submitted[3] concurrently with this manuscript. Briefly, high-purity water (10 mL) containing DMAEMA (1.00 mL, 5 mmol) and PEGDMA (10 mg, 30 μmol) was degassed at 70 °C

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before introducing APS in degassed water (100 µL). After 3 h, a solution of AEMA (40 mg, 241 µmol) in degassed water (50 µL) was then added to grow the amine-containing shell. After an additional 1.5 h, the reaction mixture was cooled to 25 °C prior to purification by tangential flow filtration (0.05 µm cutoff, MicroKos TFF filters, Spectrum Laboratories) against Dulbecco's Phosphate Buffered Saline (PBS, pH 7.4). The materials were then stored at 4 °C at a concentration of ~50 mg mL^{-1}. The amount of APS used in the reaction scheme was varied according to the desired particle size: 10 mg (44 µmol) for large particles (d = 305 nm), 20 mg (88 µmol) for medium particles (d = 150 nm), and 40 mg (172 µmol) for small particles (d = 125 nm). Detailed characterization appears elsewhere[3].

Synthesis of guanidinylated polymer colloids. Guanidinylation of the amine terminated colloids (1 mL, 50 mg mL^{-1}) was carried out in a Shlenk tube at 70 ºC in degassed PBS containing 2-ethyl-2-thiopseudourea hydrobromide (93 mg, 0.50 mmol). During the course of the reaction, the evolution of ethyl mercaptan (*caution: toxic*) drives the reaction and thus should be conducted in a well-ventilated fume hood under a positive pressure of an inert carrier gas (e.g., N_2). After 3 h, the reaction mixture was cooled to 25 °C prior to purification by tangential flow filtration (0.05 µm cutoff, MicroKos TFF filters, Spectrum Laboratories) against Dulbecco's Phosphate Buffered Saline (PBS, pH 7.4). The materials were then stored at 4 °C at a concentration of ~5 mg mL^{-1}. FT-IR spectra indicating successful guanidinylation (Figure S1) were acquired for colloids deposited from stock dispersions of polymer vectors in PBS diluted 1:10 with MeOH (Perkin Elmer).

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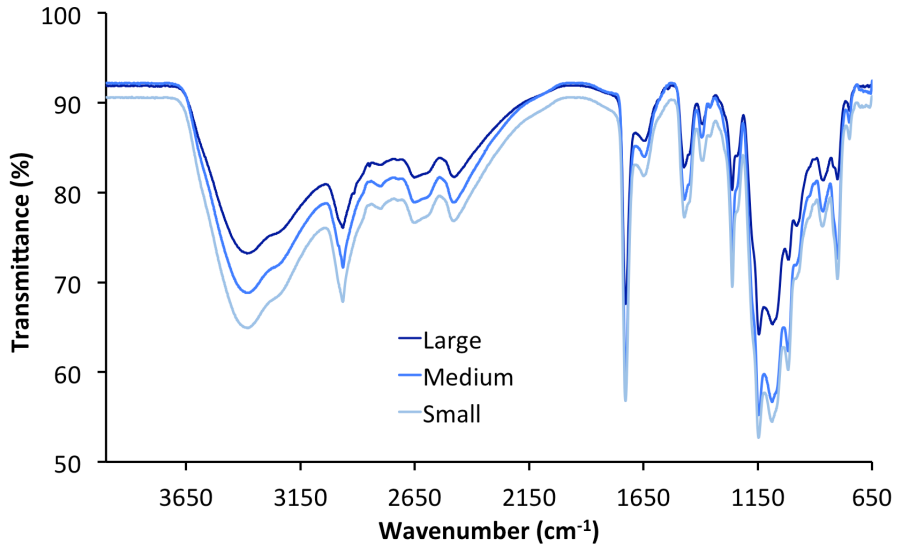

Fig. S1. FT-IR data for guanidinylated colloids.

Characterization of vector sizes by DLS. DLS measurements were made using a Malvern Zetasizer Nano ZS. For pH studies, core-shell polymer vectors were suspended in PBS at pH 7.4 or acetate buffer at pH 5.5 (1 mg mL^{-1}) and equilibrated at 37 °C for 10 minutes prior to analysis. These data are summarized in Table S1. For temperature studies, the vectors were again suspended in PBS (pH 7.4) and size measurements were taken as temperature varied. Specifically, a standard-operating procedure was created using Malvern Zetasizer software that increased or decreased the operating temperature in the cuvette by 1 °C every 2 minutes. Zeta potential measurements were performed by diluting one of the above mixtures 1:10 in MilliQ water. Data were collected until a count of 10^5 was reached.

TABLE S1. DLS measurements of guanidinylated colloidal polymer vectors. The diameter ratios ($d_{pH=5.5}$ / $d_{pH=7.4}$) and volume ratios ($V_{pH=5.5}$ / $V_{pH=7.4}$) are also given.

	Diameter (nm) at 37 °C			
Vector	**pH 7.4**	**pH 5.5**	**Diameter Ratio**	**Volume Ratio**
Small	150	461	3.1	29
Medium	175	350	2.0	8.0
Large	335	650	1.9	7.3

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Cell Viability Assays. The cytotoxicity of PDMAEMA-*co*-PEGDMA-*graft*-poly(guanidinyl ethyl methacrylate-*co*-PAEMA) cationic polymer vectors in the presence of SA-605-QDs (0 to 5 nM) was evaluated using a MTS colorimetric assay[2,3] (CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay, Promega). HeLa cells (5×10^4) were inoculated into a 96-well tissue culture plate (FALCON) with 100 µL of complete media (Dulbecco's Modified Eagle Medium supplemented with Fetal Bovine Serum, 10% *v/v*) and incubated for 24 h at 37 °C. The media was subsequently exchanged for complete media containing the polymer vector (*c* = 3.125 - 400 µg mL-1). The cells were incubated for up to 24 h at 37 °C and analyzed according to the manufacturer's instructions using a SpectraMax Plus 384 Spectrophotometer (Molecular Devices).

Flow Cytometry. HeLa cells were cultured in DMEM-FBS complete medium in 6-well plates (FALCON) and were labeled with SA-605-QDs as described above and similar to our previous protocols reported elsewhere[2,3]. The labeled cells were washed with D-PBS (3 x 1 mL) and harvested with 0.25% trypsin/EDTA (Sigma). DMEM-FBS complete medium (2 mL) was used to inactivate the trypsin and the labeled cells were pelleted by centrifugation at 1000 rpm for 8 minutes, discarding the supernatant. The resulting pellets were washed by resuspending he cells in D-PBS containing 1% *w/w* BSA (2 mL) and subsequent centrifugation. The pelleted cells were finally resuspended in PBS/1% BSA prior to analysis. Flow cytometry was performed on a BD Biosciences FACS Aria Flow Cytometer. Fluorescence signals from individual labeled cells (10,000 events in total) were obtained by exciting at λ_{ex} = 405 nm and monitoring the emission using a 605/40 band-pass filter. Data were acquired using FACSDiva *v.* 6.0 and processed using FloJo *v.* 7.5.

In Vitro Cell Imaging. 605-SA-QD delivery and subcellular localization over time was visualized using confocal fluorescence microscopy. For these experiments, HeLa cells were plated at a concentration of 500,000 cells/well (250,000 cells/mL) in a 6 well plate containing 22 x 22 mm glass substrates (OD 1.5, VWR). Cells were allowed to seed for 24 h. SA-605-QDs were allowed to bind to the guanidinylated vectors in PBS for up to 24 h at 4 ºC on a rotating carrousel prior to introduction to cells. Cells were incubated

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with SA-605-QDs (0.5 to 5 nM) bound to the guanidinylated polymer vectors (25 μg mL^{-1}) at 37 °C for 1 h in DMEM-FBS complete medium. The cells were then washed 2x with PBS and subsequently incubated at 37 °C in DMEM-FBS complete medium with the delivery process arrested at different times (0 h, 4 h, 8 h, etc) post-incubation. For endolysosomal co-localization studies, cells were incubated with LysoTracker® Green (75 nM) 1 h before the experimental endpoint. At the end of the designated time point, the media was replaced and cells were exposed to DAPI (400 nM) for 20 minutes. Cells were then further washed with PBS to remove the DAPI and imaged directly, or otherwise treated with freshly made cell fixing solution (2% *v/v* formaldehyde and 2.5% *v/v* glutaraldehyde in PBS) for 20 minutes. The fixing solution was then aspirated and the cells were washed 2x with PBS. The glass squares were removed from well plates and placed cell-side down onto glass slides (VWR) coated with Vectashield (Vectashield Corp, Burlingame, CA). Slides were sealed with clear nail polish and left to dry before imaging. Confocal fluorescence imaging of labeled and fixed cells were acquired using a Zeiss 710 Laser Scanning Confocal Microscope equipped with spectral detector, using a Plan-Apochromat 40x/1.40 oil objective. DAPI and SA-605-QDots were excited at λ = 405 nm using a HeNe laser, Lysotracker® Green and DiO was excited at λ = 488 nm using an Argon laser. Acquired images were processed identically with Zeiss ZEN software.

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