Suporting Information

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SI Text

1. Cell-Penetrating Peptide (CPP) Cellular Uptake. Intracellular CPP trafficking experiments were performed to investigate the cellular uptake of TAT peptide and R_9 in the presence of specific inhibitors. HeLa cells, stained with DAPI, were pretreated with inhibitors cytochalasin D and nystatin, chemicals that inhibit macropinocytosis and caveolae-dependent endocytosis, respectively. Representative confocal images are shown in Fig. S1. TAT and R_9 readily crossed the membrane in the control cells (cells not treated with transport inhibitors) (Fig. S1 *A* and *B*). Although treating the cells with nystatin had a small effect (Fig. S1 *C* and *D*), cytochalasin D, which inhibits actin polymerization, significantly reduced the uptake of the peptides (Fig. S1 *E* and *F*). This result indicates an important role for actin polymerization in the uptake mechanism, consistent with a parallel endocytotic pathway.

2. MTS (3-(4,5-Dimethylthiazol-2-yl)-5-(3-Carboxymethoxyphenyl)-2-(4-Sulfophenyl)-2H-Tetrazolium) Cell Survival Assay. The toxicity of TAT, R_9, and endocytosis inhibitors was assessed with the MTS cell proliferation assay (CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay, Promega). The HeLa cells used in the uptake experiments were seeded at a density of 4×10^4 cells/cm^2 in a 48-well plate (Lab-Tek) at least 12 h before the start of experiment. The cells were washed with PBS and pretreated with either no inhibitor drug, nystatin at 54 μM, or cytochalasin D at 1.9 μM in complete cell culture medium for 30 min. The medium containing inhibitors was aspirated, and the cells were washed with PBS to remove any residual amount of inhibitors. The cells were then incubated in complete cell culture medium containing 12.5 μM of FITC-labeled TAT or R_9 peptide for 15 min at room temperature. The medium was aspirated and complete cell culture medium containing 20% MTS reagent was added to the cells. The cells were placed back into the 37 °C humidified atmosphere with 5% CO_2 for 2 h, and the absorbance at 490 nm was read with an Infinite F200 plate reader (Tecan Systems Inc.). The survival of cells relative to the control (cells incubated with growth medium containing no TAT, R_9, or drug) was calculated by taking the ratio of the A490 values (Fig. S1 *G* and *H*).

3. Actin Encapsulated Giant Unilamellar Vesicles (GUVs). GUVs labeled with DiO/DiI (Invitrogen) were prepared using electroformation. Lipid mixtures in chloroform, with calcium ionophore A23187 (Sigma-Aldrich) (1–5%) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)] (Avanti Polar Lipids) (0.5%), were deposited and dessicated on indium tin oxide-coated glass slides, before swelling with 4–8 μM rhodamine phalloidin (Invitrogen) (542 Ex/565 Em), 7–15 μM globular actin (Cytoskeleton, Inc.), and 100 mM sucrose in g buffer (2 mM TRIS/HCl pH 8.0, 0.2 mM CaCl2, 0.2 mM Na2ATP, 0.2 mM DTT, 0.01% NaN3) under 10 Hz AC electric field. After GUV detachment via 4 Hz square AC, the suspension was diluted with glucose (160 mM) in g buffer.

4. TAT-Nanoparticle (NP) Synthesis. ***(a) Synthesis of Cy5-PLA conjugate.*** In a glove box, Cy5 (2.5 mg, 0.005 mmol) was dissolved in anhydrous THF (1 mL). (BDI)ZnN(TMS)$_2$ (7.0 mg, 0.01 mmol) in THF (100 μL) was added to the Cy5 solution. The mixture was stirred for 15–20 min at room temperature. Lactide (LA; 144 mg, 1.0 mmol) in anhydrous THF (2 mL) was added dropwise to the vigorously stirred mixture of Cy5 and (BDI)ZnN(TMS)$_2$. The polymerization was monitored by following the lactone band at 1,772 cm^{-1} using FTIR or by checking the methine (-CH-) peak of LA using ^{1}H NMR. After the polymerization was complete, an aliquot of the polymerization solution was measured by HPLC to quantify the unreacted Cy5 in order to determine the incorporation efficiency of Cy5 to the Cy5-PLA conjugate.

(b) Synthesis of PLA-PEG3.4k-MAL. In a glove box, HO-PEG3.4k-MAL (hydroxyl-poly(ethylene glycol)-maleimide, molecular weight approximately 3,400, Laysan Bio, AL; 34 mg, 0.01 mmol) was charged into thick-wall tube. Tin(II) 2-ethylhexanoate (4.1 mg, 0.01 mmol) and LA (144 mg 1.0 mmol) were mixed in toluene solution (3 mL) and added to the tube. The tube was tightly sealed, moved out from the box, and heated at 120 °C over 24 h. The resulted solution was dried and PLA-PEG3.4k-MAL polymer was precipitated by hexane. The precipitate was further washed by cold ether and methanol twice, and dried in vacuum.

(c) Synthesis of NP-TAT conjugates. The Cy5-PLA/ PLA-PEG3.4k-MAL NPs conjugated NPs were readily prepared through the nanoprecipitation of Cy5-PLA conjugates in the presence of PLA-PEG3.4k-MAL. Briefly, Cy5-PLA conjugate (100 μL dimethylformamide (DMF), 10 mg/mL) and PLA-PEG3.4k-MAL (100 μL DMF, 10 mg/mL) were mixed and then added dropwise to nanopure water (4 mL). The resulting NP suspension was purified by ultrafiltration (15 min, 9,700 × *g*, Ultracel membrane with 10,000 nominal molecular weight limit, Millipore) and then characterized by dynamic light scattering. The obtained Cy5-PLA/PLA-PEG3.4k-MAL NPs (wt/wt = 1/1, 1 mL, 1 mg/mL in DNase RNase-free water) were dispersed in PBS solution at room temperature. The NPs were allowed to react with Cys-TAT peptide (0.5 mg/mL in DNase RNase-free water, 50 μL) over 12 h. The resulting NP-TAT bioconjugates were washed with ultrapure water (20 mL) by ultrafiltration (5 min, 1,000 × *g*, Ultracel membrane with 10,000 nominal molecular weight limit, Millipore). The NP-TAT conjugates were resuspended (1 mg/mL in DNase RNase-free water) for further study.

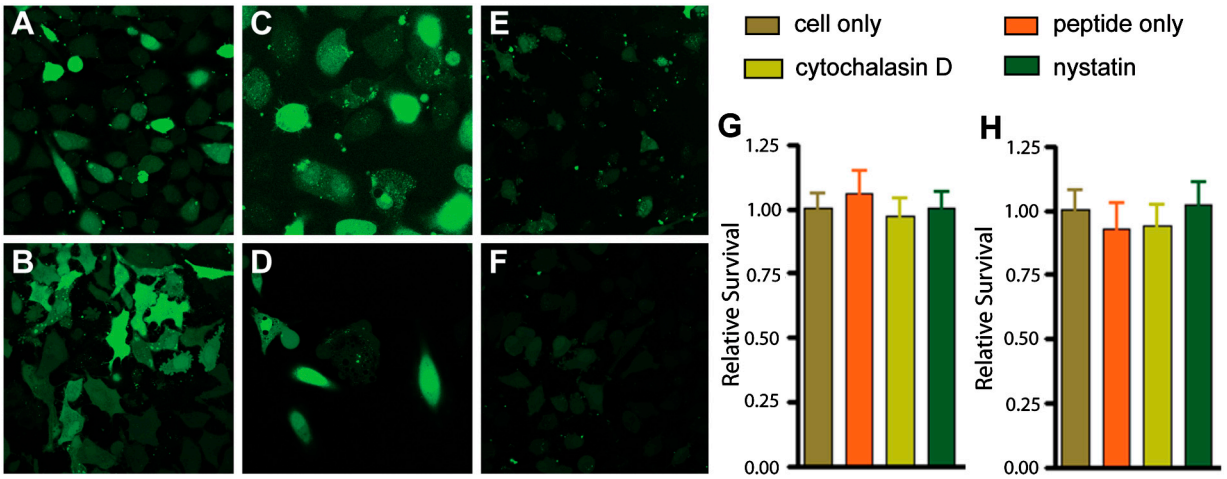

Fig. S1. Cellular uptake suppressed by inhibitor of actin polymerization. Confocal images of the cellular uptake studies performed with HeLa cells and FITC-TAT and FITC-R_9 using (*A* and *B*) no inhibitor drug, (*C* and *D*) nystatin, and (*E* and *F*) cytochalasin D, respectively. The peptides and inhibitor drugs caused little to no cytotoxicity at the conditions used (*G* and *H*).

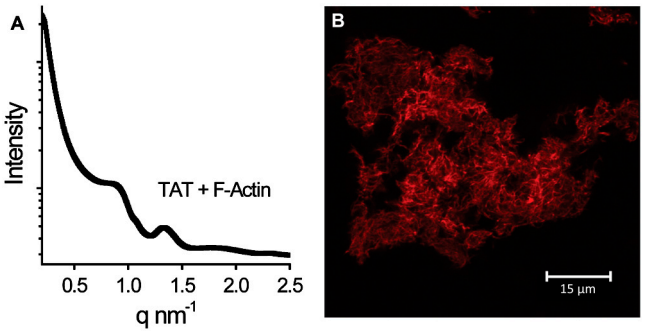

Fig. S2. Interaction of TAT peptide with actin. (*A*) SAXS data show that the TAT peptide condenses F-actin filaments into close-packed bundles. Two correlation peaks are observed at $q = 0.83$ nm^{-1} and 1.34 nm^{-1} consistent with a distorted hexagonal lattice. (*B*) TAT peptide polymerizes monomeric G-actin into filament. Rhodamine phalloidin binds specifically to actin filaments.