Electronic Supplementary Material (ESI) for Soft Matter

Enhanced activity of cyclic transporter sequences driven by phase behavior of peptide-lipid complexes

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Supplementary Materials

Materials

Lipids are DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), and DOPS {1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt)}, from Avanti Inc.

SAXS sample preparation and data analysis

Detailed procedures have been published elsewhere.[23] In summary, mixture of lipids at different ratios are prepared in chloroform, which are then vacuum dried overnight, resuspended in Millipore water with salt added (NaCl, 100-150 mM), incubated overnight at 37 ^{o}C, and then sonicated and filtered (0.2 um pore size) to get Small Unilamellar Vesicles (SUVs). To make SAXS samples, those lipid solutions then are mixed with peptide solutions, which also contain the same salt concentrations, at desired peptide/lipid ratios. Then the mixture is transferred and hermetically sealed in 1.5 mm quartz-glass capillaries. SAXS measurements are performed at the Stanford Synchrotron Radiation Laboratory (BL4- 2) and at the Advanced Photon Source (BESSRC-CAT BL-12IDC). SAXS data are analyzed using the Nika 1.2 package (usaxs.xor.aps.anl.gov/staff/ilavsky/nika.html) data reduction package for Igor Pro and FIT2D (www.esrf.eu/computing/scientific/FIT2D/). The entire sample preparation process and SAXS measurements are done at room temperature.

Cell culture

The MCF7 cell line is grown in Dulbecco's Modified Essential Medium with high glucose supplemented with 44.1 mM sodium bicarbonate, 10% FBS, 1% L-glutamine, 1% nonessential amino acids, and 1% penicillin/streptomycin, at a pH of 7.4. The cell line is maintained in a 37°C humidified atmosphere with 5% CO_2 and handled with standard sterile tissue culture protocols.

Peptide uptake

MCF7 cells are seeded at a density of $1x10^6$ cells/cm^2 and incubated overnight prior to the experiment. The cells are seeded onto 8-well chambered coverglasses for confocal microscopy experiments and 35 mm tissue culture plates for Fluorescence-Activated Cell Sorting (FACS). On the day of the experiment, fluorescently-labeled peptides are separately diluted in serum-free media and incubated with MCF7 cells for 5 hrs to allow the peptides to be internalized into the cells. Subsequently, the medium containing the peptides is aspirated, and the cells are washed three times with PBS to remove nonspecifically attached peptides on the cell surface. Afterwards, the cells are subjected to either confocal microscopy or FACS to determine the extent of peptide uptake.

Laser scanning confocal microscopy (LSCM)

The LSCM images of the cells are taken on a Leica Inverted TCS-SP MP Spectral Confocal and Multiphoton Microscope (Heidelberg, Germany) equipped with an argon laser (488 nm blue excitation: JDS Uniphase), a diode laser (DPSS; 561 nm yellow-green excitation: Melles Griot), a helium-neon laser (633 nm red excitation), and a two photon laser setup consisting of a Spectra-Physics Millenia X 532 nm green diode pump laser and a Tsunami Ti-Sapphire picosecond pulsed infrared laser tuned at 768 nm for UV excitation.

Fluorescence-activated cell sorting (FACS)

FACS analyses of MCF7 cells incubated with the peptides are performed on a BD FACScan™ (BD Bioscience, San Jose, CA) system equipped with an argon laser (488 nm blue excitation) and two filters: a green filter (530 ± 30 nm) and an orange filter (585 ± 42 nm). A total of 10,000 cells per sample are used for the analysis. The mean fluorescence intensity is used as a metric to represent the degree of peptide internalization into the cells.

Measurement of cytotoxicity using the MTS cell proliferation assay

The MTS cell proliferation assay (CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay) is performed to assess the cytotoxicity level of the peptides [28]. The uptake experiments are performed with MCF7 cells seeded on 96-well plates with triplicates of each condition. After the 5-hr incubation period, the medium is aspirated and fresh medium containing 20% MTS reagent is added to the cells. The cells are incubated again at 37°C for 1 hr, and the absorbances at 490 nm and 700 nm are measured using the Infinite F200 plate reader (Tecan Systems Incorporated, San Jose, CA). The relative survival of cells compared to control cells (*i.e.*, cells incubated in growth medium without the peptides) is calculated by determining the ratio of the ($A_{490} - A_{700}$) values. The toxicity of TAT is also measured as a standard sample for comparison. The result (Figure S1) shows that all the peptides, including TAT, are non-cytotoxic, indicated by the relative survival being nearly equal to the survival of the control group (cells only). This result indicates a low cellular cytotoxicity of each peptide, which suggests that cytotoxicity does not affect our confocal and FACS results.

SAXS measurements of target-specific CPPs

We investigate the membrane curvature generation of target-specific CPPs PepI(cyclo(D-Arg-D-Arg-D-Thr-Pip-Nal-Arg-Gln)) and PepJ (cyclo(D-Arg-D-Arg-D-Thr-Pip-Nal-Arg-D-

Arg-D-Arg-D-Arg-Gln)) (Figure S2), which are specific for target protein Pin1 (Note: Pin1 is the target protein and PepI/PepJ are the CPPs that have the specific motif to bind to Pin1). Pin1 regulates the function and/or stability of phosphoproteins by altering the conformation of specific pSer/pThr-Pro peptide bonds. After the phosphorylation of Thr in both PepI and PepJ, D-pThr-Pip-Nal can serve as a binding motif to Pin1, leading to inhibition of Pin1 activity upon binding. It has been suggested that cancer cells have a high expression level of Pin1 and are more sensitive to Pin1 inhibitors, so these Pin1-targeting CPPs can have potential applications in cancer treatment. Our SAXS measurement results show that both peptides generate strong negative Gaussian curvature, consistent with data from cell uptake experiments.[7]

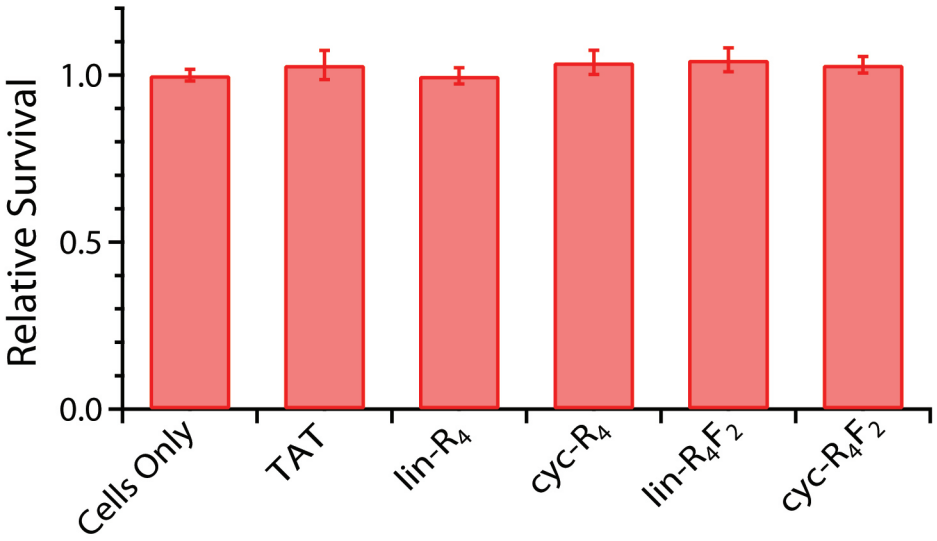

Figure S1. Cytotoxicity of different peptides on MCF7 cells. Cells are incubated with 10 μM of each peptide for 5 hrs under serum-free conditions. Error bars represent standard errors of the mean.

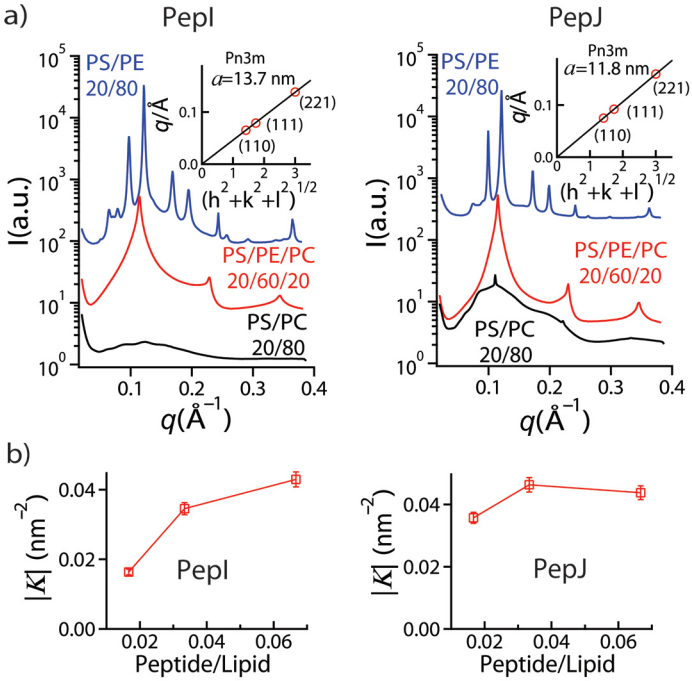

Figure S2. Effect of lipid composition and peptide/lipid molar ratio on the membrane activities of target-specific CPPs PepI and PepJ. a) SAXS data show the effect of lipid composition on the membrane activities of PepI and PepJ for P/L = 1/30. The SAXS intensity curves are shifted for clarity. b) Calculated average magnitude of Gaussian curvature $|K|$ at different peptide/lipid molar ratios for DOPS/DOPE/DOPC = 20/80/00. Insets in a) show indexation of the Pn3m cubic phase by agreement between the measured peak q positions and the Miller indices h, k, l, with relationship $q = 2\pi(h^2+k^2+l^2)^{1/2}/a$, for a cubic phase with lattice constant, a. For PepI and PepJ, the Pn3m lattice constants are a_{PepI} = 13.7 nm and a_{PepJ} = 11.8 nm, respectively (DOPS/DOPE/DOPC = 20/80/00, and P/L = 1/30).