Biophysical Journal, Volume 99

Supporting Material

Cell Penetrating Peptide Induces Leaky Fusion of Liposomes Containing Late Endosome Specific Anionic Lipid

Sung-Tae Yang, Elena Zaitseva, Leonid V Chernomordik, and Kamran Melikov

SUPPLEMENTAL FIGURES

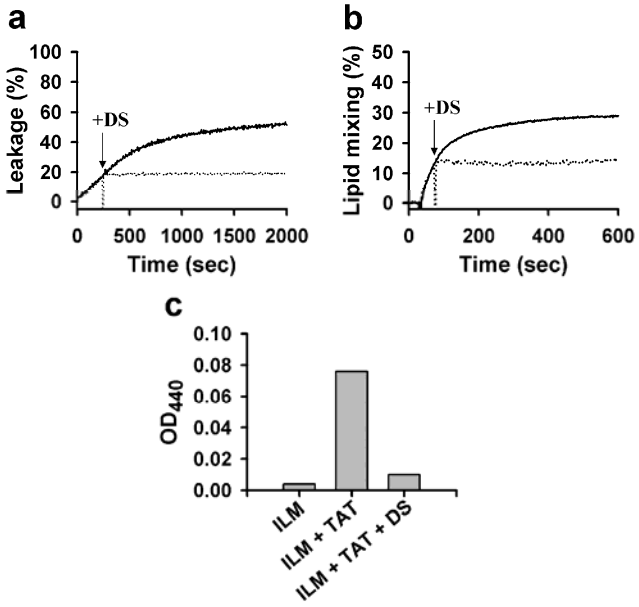

Figure S1. Dextran sulfate inhibits leakage of encapsulated water soluble dye, lipid mixing and reverses liposome aggregation. a) Addition of 20 μM DS (+DS) inhibits TAT-induced (2 μM peptide concentration) fluorescence dequenching due to release of ANTS/DPX from ILM liposomes (dotted line). Control experiment without addition of DS is shown as solid line. b) Addition of 20 μM DS (+DS) inhibits TAT-induced (2 μM peptide concentration) Rh-PE dequenching due to lipid mixing between ILM liposomes (25 μM final total lipid concentration) induced by 1 μM TAT at pH 5.5 (dotted line). Control experiment without addition of DS is shown as solid line. c) OD_{440} measured after 30 min incubation of ILM liposomes without TAT or DS (ILM), with 2 μM of TAT alone (ILM+TAT) or after 30 min incubation with 2 μM of TAT followed by 30 minute incubation with 20 μM DS (ILM+TAT+DS).

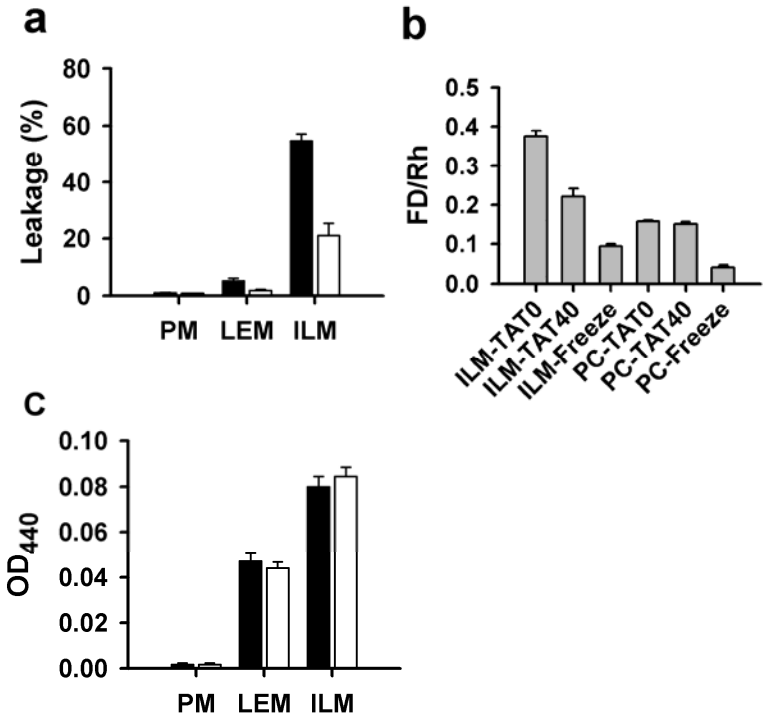

Figure S2. a) TAT-induced release of HPTS from LUVs of different lipid composition. 2 μM of TAT was added to 25 μM of ILM-, LEM- or PM-liposomes at pH 5.5 (black bars) or at pH 7.4 (white bars). Percent of leakage was measured at 30 min after addition of TAT. Mean and standard deviation of 3 independent experiments are shown. b) TAT mediates release of 70kDa fluorescein tagged dextran from Rhodamine PE labeled ILM liposomes. The ratio of fluorescein- (λex=495 nm, λem=520 nm) and rhodamine- (λex=560 nm, λem=585 nm) fluorescence was measured after separation of released dextran on density gradient following 30 min incubation of ILM or PC liposomes (500 μM final concentration) without TAT (ILM-TAT0 and PC-TAT0) or with 40 μM TAT (ILM-TAT40 and PC-TAT40) or after 3 cycles of freezing-thawing (ILM-freeze and PC-freeze). Decrease in ratio reflects release of encapsulated water soluble dextran. c) TAT-induced vesicle aggregation. The turbidity of liposomes was measured at λ=440nm 30 min after addition of 2 μM TAT to 25 μM ILM-, LEM- or PM-liposomes at pH 5.5 (black bars) or at pH 7.4 (white bars). Mean and standard deviation of 3 independent experiments are shown.

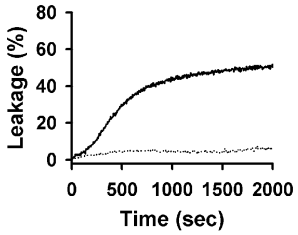

Figure S3. Replacement of BMP with PG in ILM liposomes inhibits aqueous dye leakage. Kinetics of dye dequenching due to release of ANTS/DPX from 25 μM of ILM- (solid line) and PG: PC: PE (77:19:4) (dotted line) liposomes upon addition of 2 μM TAT peptide at pH 5.5.

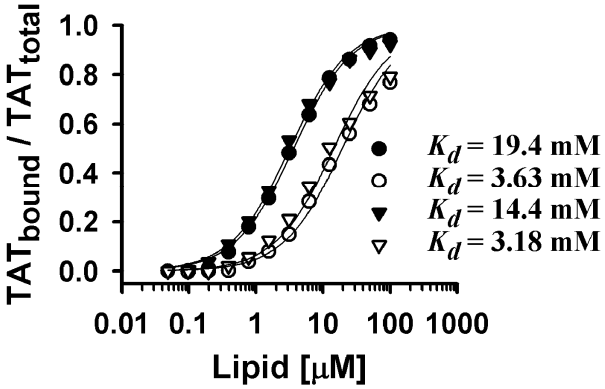

Figure S4. TAT peptide binds with similar affinity to BMP:PC (1:1) and PG:PC (1:1) liposomes at both acidic pH 5.5 and neutral pH 7.4. Dependence of fraction of bound peptide TAT_{bound}/TAT_{total} on lipid concentration for BMP/PC (1:1) (circles) and PG/PC (1:1) (triangles) liposomes in pH 5.5 (filled symbols) or pH 7.4 (open symbols) is shown. To determined the apparent dissociation constant K_d data was fitted by equation $[P_b]/[P_{tot}]=[L]/(K_d+[L])$, where $[P]_b$ is the amount of bound peptide, $[P]_{tot}$ is the total peptide concentration by measuring the fraction of bound peptide, and [L] is lipid concentration. Fit is shown as solid curves and K_d for different data sets are listed on the figure.

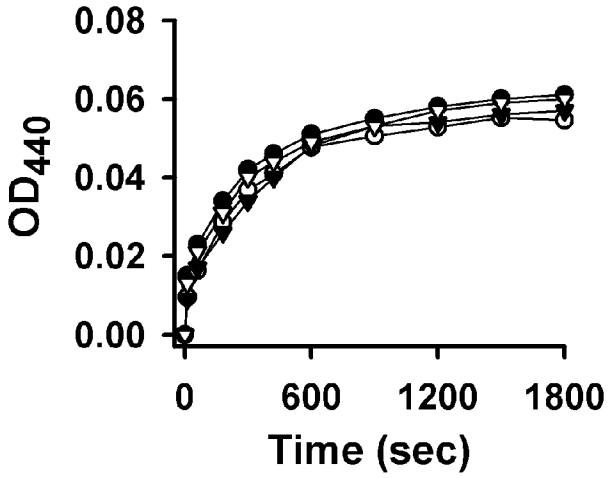

Figure S5. TAT-induced vesicle aggregation. TAT-induced change in the turbidity (440 nm) was measured for BMP:PC (1:1) (circles) and PG:PC (1:1) (triangles) liposomes in either pH 5.5 (filled symbols) or pH 7.4 (open symbols)was measured at different times after peptide addition. TAT concentration was 1 μM and total lipid concentration was 25 μM.

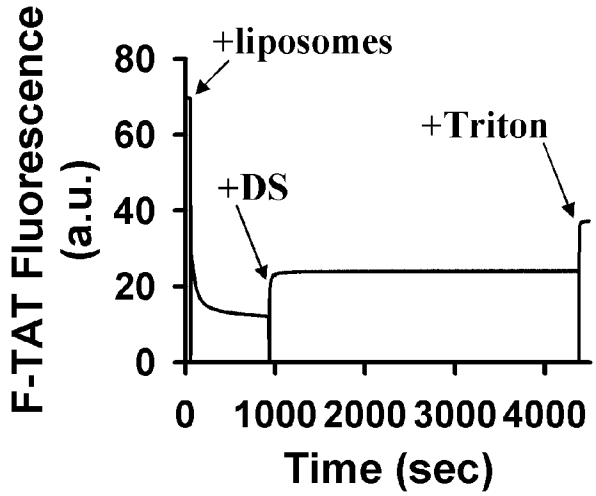

Figure S6. TAT peptide that entered liposomes remains entrapped after extended incubation. Change in fluorescence of fluorescein tagged TAT (2 μM) at pH 5.5 after addition of 25 μM of ILM liposomes (+liposomes), 20 μM dextran sulfate (+DS), and finally 0.1% Triton X-100 (+Triton).

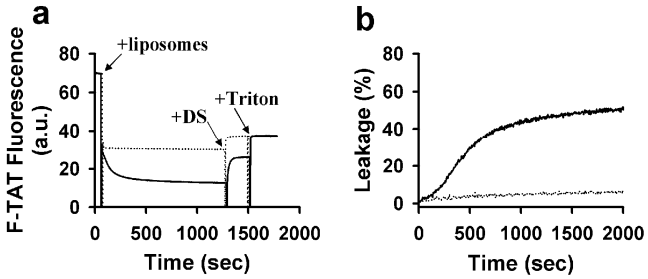

Figure S7. Inclusion of 2 mol% of PEG-PE lipid in ILM liposomes inhibits TAT peptide translocation and aqueous dye leakage. (a) Change in fluorescence of fluorescein tagged TAT (2 μM) at pH 5.5 after addition of 25 μM of ILM-(solid line) and BMP: PC: PE:PEG-PE (77:17:4:2) (dotted line) liposomes (+liposomes), 20 μM dextran sulfate (+DS), and finally 0.1% Triton X-100 (+Triton). (b) Kinetics of dye dequenching due to release of ANTS/DPX from 25 μM of ILM-(solid line) and BMP: PC: PE:PEG-PE (77:17:4:2) (dotted line) liposomes upon addition of 2 μM TAT peptide at pH 5.5.

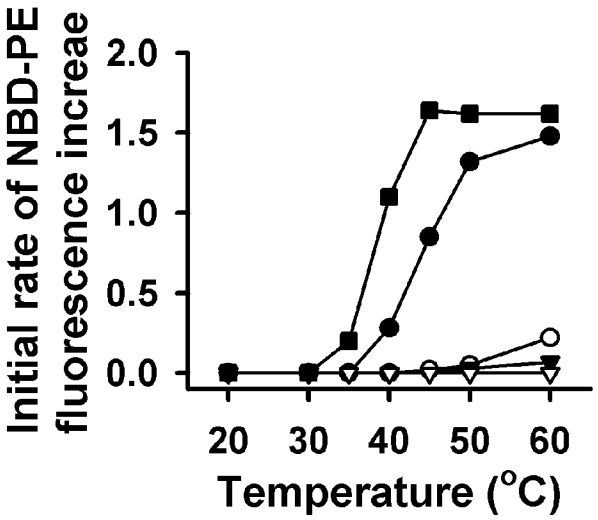

Figure S8. Effect of BMP and PG on the Lα → H_{II} phase transition temperature (T_H) of PE. Dependencies of initial rate of NBD fluorescence increase upon injection into pH 10 buffer on buffer temperature is shown for PE- (filled squares), PE:BMP (95:5) (filled circles), PE:BMP (9:1) (open circles), PE:PG (95:5) (filled triangles) and PE:PG (9:1) liposomes are shown. All liposomes contain 0.1 mol% of NBD-PE fluorescent lipid.