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Supporting Material

Cell penetrating HIV1 TAT peptides can generate pores in model membranes

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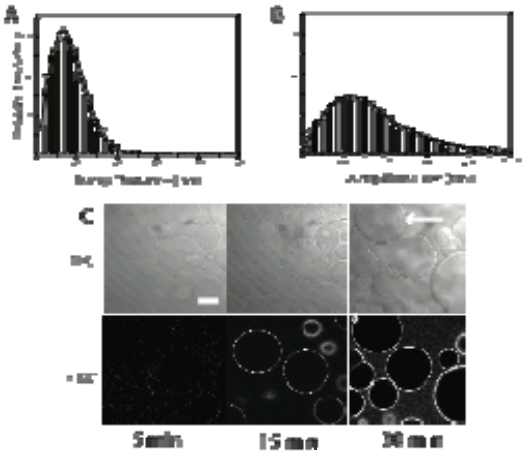
Online supplemental material:

Supplemental Figure S1:

Interaction of TAT peptides with neutral model membranes containing PC

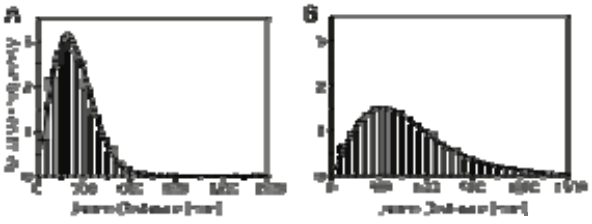
Jump distance analysis for TR-DHPE in neutral GUVs: DPPC (A) or DOPC (B). TR-DHPE moves more rapidly within GUVs known to have an unordered phase with a diffusion coefficient D of 2.20±0.01 $\mu m^2/s$ compared to GUVs known to comprise an ordered phase (D=0.56±0.01 $\mu m^2/s$). (C) Confocal imaging of TAT peptide-membrane interaction with neutral GUVs in glucose solution. The upper panel shows the time series of DIC images of the GUVs in a glucose solution, and the lower panel illustrates the confocal fluorescence images of the GUVs incubated with 2 µM R-TAT in the rhodamine fluorescence channel. R-TAT does not translocate, but just accumulates on the GUV membranes. Similar results were obtained for AF-TAT. The arrow marks a GUV containing several internal membranes. Scale bar, 20 µm.



Supplemental Figure S2:

Mobility of lipid tracers in anionic model membranes

Jump distance analysis for TR-DHPE in anionic GUVs: (A) DPPC/DPPS and (B) DOPC/DOPS. The anionic charge was 40 mol%. Similar to neutral membranes, TR-DHPE moved more rapidly within GUVs with a liquid-disordered phase with a diffusion coefficient D of 2.01±0.01 $\mu m^2/s$ compared to GUVs with liquid-ordered phase, D=0.54±0.01 $\mu m^2/s$.



Supplemental Figure S3:

Confocal images of GUVs with 40 mol% PS in presence of 2 µM R-TAT

Time series of GUVs incubated with 2 µM R-TAT. Images were taken (A) 10, (B), 20 and (C) 30 min after mixing GUVs and TAT. Scale bar, 50 µm. There was a special case where the TAT import could be well observed in a GUV containing two internal membranes (see arrow). In order to simplify the identification of GUVs over time, we labeled the dominant ones by numbers. After 30 min GUVs 4, 5, 6, 12 and 13 were disrupted. The diameters of the GUVs remained constant during the incubation as demonstrated in Table S1. Slight changes were probably due to a change of the axial position.

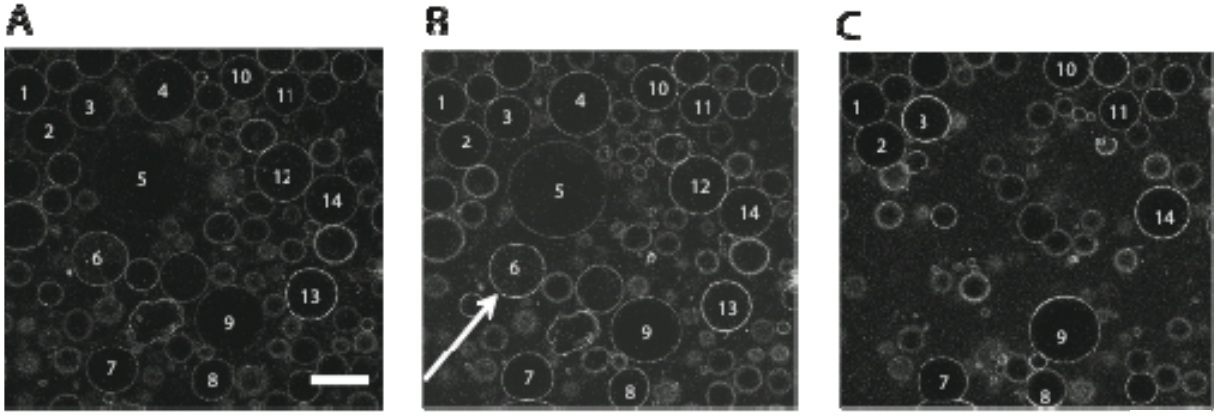


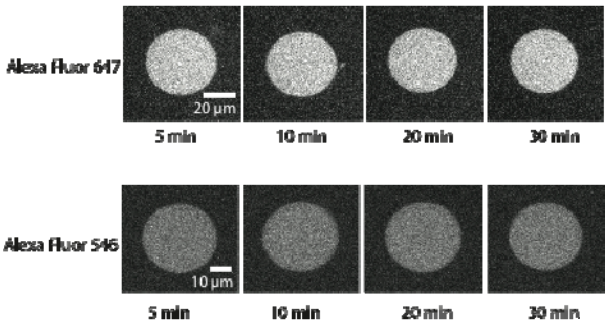
Table S1: Diameter of GUVs as a function of time.

GUV	Diameter of GUV in µm		
	10 min (A)	20 min (B)	30 min (C)
1	37.8	37.7	37.8
2	42.4	42.5	42.5
3	42.5	42.5	42.7
4	53.3	53.3	-
5	88.9	88.9	-
6	46.7	46.7	-
7	42.5	42.5	46.7
8	33.3	33.3	42.5
9	55.6	55.6	55.4
10	35.5	35.6	35.5
11	35.6	35.6	35.6
12	48.9	48.9	-
13	42.2	42.2	-
14	44.4	44.4	44.3

Supplemental Figure S4:

Control experiments for Alexa Fluor 647 and Alexa Fluor 546 photobleaching

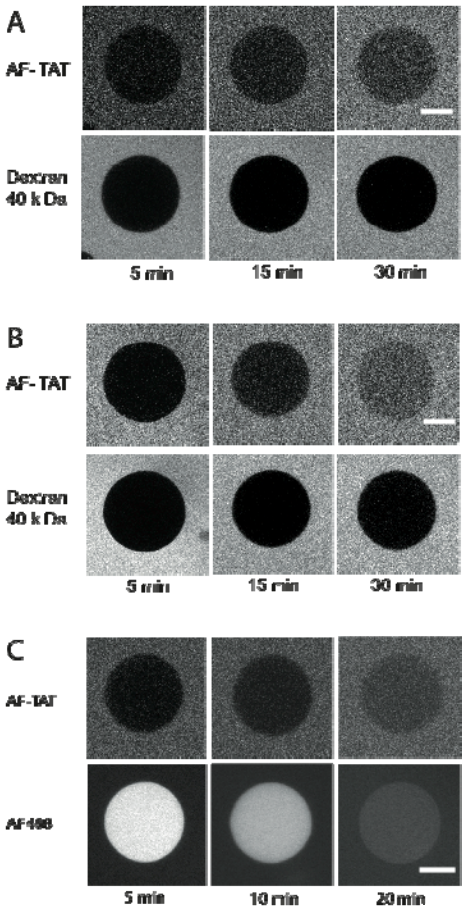
GUVs were loaded with fluorescent markers in the absence of the TAT peptide. The fluorophores were stable; there was no change in fluorescence intensity even after repetitive imaging scans.



Supplemental Figure S5:

Confocal images of GUVs in PBS in presence of 2 µM AF-TAT as a function of time

(A) Neutral GUVs were made from DOPC/DPPC/Chol at a ratio of 40/40/20. The time series clearly demonstrates that TAT peptides can translocate even into neutral GUVs in the presence of PBS (upper panel). The lower panel demonstrates that the GUV membrane did not allow the passage of large dextran molecules. (B, C) Anionic GUVs made were made from DOPC/DPPS/Chol at a ratio of 60/20/20. The time series clearly demonstrates that TAT peptides can translocate into anionic GUVs placed into PBS in these GUVs (upper panels), which was not the case in a solution without ions. 40 kDa dextran molecules remained excluded from the GUV interior (B), but a small tracer molecule – AF488 – could pass the membrane (C).



Supplemental Movie S1:

Single molecule imaging of TR-DHPE in GUVs prepared from DPPC/cholesterol

Single diffraction-limited fluorescence spots representing single TR-DHPE molecules could be observed diffusing within the GUV membrane in the high-speed video microscope operated at a frame rate of 30 Hz. The tracer was added to the lipids at a ratio of 10^{-7} mol %. The field of view was 12.2 x 12.2 μm^2. The movie was contrast-enhanced.

Supplemental Movie S2:

Single molecule imaging of AF-TAT peptides on DPPC/cholesterol GUVs

Single diffraction-limited fluorescence spots representing single AF-TAT peptide molecules could be observed diffusing on the GUV membrane in the high-speed video microscope operated at a frame rate of 100 Hz (display 30 Hz). The peptides were added at a concentration of 0.25 nM to GUVs. The field of view was 12.2 x 12.2 μm^2. The movie was contrast-enhanced.