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Supporting Information

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HIV TAT Perforates Membranes by Inducing Saddle-Splay Curvature: Potential Role of Bidentate Hydrogen Bonding

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Supplementary Information:

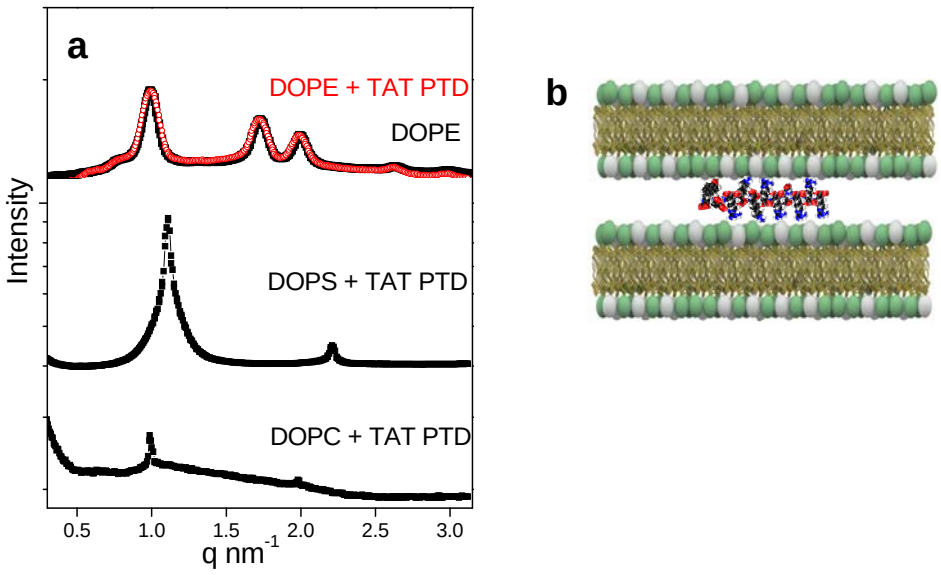
1. Crystallography of Cubic Pn3m phase

Before making the assignment of a cubic phase to the observed diffraction pattern, the possibility of phase coexistence was considered. This is especially important for cubic phases, from which different subsets of the diffraction peaks can be indexed to simpler phases. In the present case, one can generate a model diffraction pattern using a coexistence of a lamellar phase (q_1 = 1.13 nm^{-1}) and an inverted hexagonal phase (q_{10} =1.01 nm^{-1}), except that it misses the peak at q = 0.84 nm^{-1}. This would imply a three-phase coexistence for the present three-component system. Since we see essentially the same diffraction pattern for different peptide/lipid molar ratios, different PS:PE weight ratios, and different temperatures (three degrees of freedom), this coexistence scheme would violate the Gibbs phase rule and hence, is not possible for a system at equilibrium.

2. Electron density reconstruction of H_{II} phase

The phases used for the amplitudes at q_{10}, q_{11}, q_{20} are (+,-,-), in agreement with those from previous reconstructions on pure lipid H_{II} phases.[1] Regions of the lowest electron density (green-blue, ~0.169 e/Å^3) correspond to lipid hydrocarbon tails, which surround high-density, hexagonally-coordinated circular rims (red, ~0.410 e/Å^3) corresponding to phospholipid head groups. In turn, these surround intermediate-density channels of the H_{II} phase. For the pure PE H_{II} phase, an electron density of 0.337 e/A^3 is found in these holes, which corresponds well to the electron density of water. For the polylysine-induced PS:PE=20:80 structure, the electron density in the corresponding region is ~0.286 e/A^3, which is consistent with a mixture of water and polypeptide.

3. TAT PTD interactions with different lipid species



(a) SAXS data show that the TAT PTD 'glues' together DOPS (middle) and DOPC (bottom) membranes into a lamellar phase with the PTD intercalated between the bilayers. The native inverted hexagonal diffraction pattern of DOPE (top, black) remains unchanged upon addition of TAT PTD (top, red). The two diffraction patterns can be superimposed. (b) Schematic showing the composite TAT PTD intercalated lamellar phase.

4. Experimental Section

(a) Peptide synthesis

The TAT PTD (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg), Rh-PTD and $(Lys)_8$ are synthesized using automated solid-state synthesis.

(b) SUV preparation

The lipids 1,2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (sodium salt) (DOPS), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine (DOPE), and 1,2-Dioleoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)] (Sodium Salt) (DOPG) are purchased from Avanti Polar Lipids and used without further preparation.. Stock solutions of lipids in chloroform are mixed at the desired ratios, dried under N_2 and desiccated under vacuum overnight. The dried lipids are rehydrated with Millipore water to a final concentration of 30 mg/ml at 37 °C overnight. This solution is sonicated and extruded through a 0.2 μm Nucleopore filter to make liposomes. TAT PTD and $(Lys)_8$ are dissolved in millipore water at 10 mg/ml. Peptides and liposomes are mixed at different peptide-to-lipid molar ratios and salt conditions and subsequently sealed in quartz capillaries.

(c) Synchrotron x-ray scattering

Small angle x-ray scattering (SAXS) data is collected at Stanford Synchrotron Radiation Laboratory (Palo Alto, CA) (BL4–2) and Advanced Photon Source (Argonne, IL, BESSRCAT BL-12ID) using 9-keV and 12-keV x-rays respectively. The scattered intensity is collected using a MAR-Research (Hamburg) charge-coupled device detector (pixel size 79 μm). All experiments are conducted at room temperature. We observe no evidence of radiation damage to the samples. Representative samples are re-measured after several months to ensure that they are fully equilibrated. Absolute electron densities in the unit cell reconstructions are calibrated by using standard measured values for typical head groups and terminal methyl groups.

(d) GUV preparation

The lipids DOPS, DOPE, DOPC, 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (POPC), and 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphoethanolamine (POPE) are purchased from Avanti Polar Lipids and used without further purification. Lipids in chloroform solution are mixed in the following weight ratios: POPC:DOPS 80:20, DOPE:DOPC:DOPS 20:60:20 and 40:40:20, with the fluorescent membrane dye 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (BODIPY, purchased from Invitrogen) incorporated at 0.5 mol%. These solutions are desiccated in a glass vial under vacuum overnight. The resulting film is hydrated with 100 mM NaCl solution containing 1%v/v glycerol to achieve a lipid concentration of 1 mg/mL, refrigerated overnight, and sonicated for 5-10 minutes. The resulting suspension is deposited in 5 μL drops on glass coverslips, dried for 1-2 hours, and rehydrated with 50 μL of 100 mM NaCl solution. Vesicles tens of microns in diameter form and adhere to the glass substrate.[2,3]

(e) Confocal microscopy

Leica inverted laser scanning confocal microscopes are used for all imaging. Laser lines of 488 nm and 514 nm, respectively, are used sequentially to excite the BODIPY membrane dye and the Rhodamine fluorophore of Rh-PTD, which is added to the sample in successive 1-10 μL drops of 1.4 mM concentration. All experiments are conducted at room temperature.

5. Movie showing TAT PTD crossing membranes in GUVs

filename: TAT membrane crossing.avi

(top) Confocal micrographs of Rhodamine-tagged TAT PTD (false-color red) being added to the exterior of BODIPY-tagged PS:PC:PE 20:40:40 membranes (false-color green) in the form of GUVs. The initial frame shows a 5 μm scale bar. Movie is approximately real-time. (bottom) For the lower vesicle, the circularly-integrated Rhodamine intensity is plotted as a function of radius from the center of the GUV to the exterior circle drawn in the initial frame.

Initially, the system is at a ratio of lipid:PTD 1.15:1 and the Rhodamine intensity is equilibrated on both sides of the membrane, where it is the highest. In the fifth frame, more Rh-TAT-PTD is added to the region under view, so that the system-wide ratio of lipid:PTD is 1:1.30; the local concentration of Rh-TAT-PTD is much higher. The Rh-TAT-PTD crosses the membrane so that the interior and exterior intensities equilibrate.