Supplementary Material for

The role of Tat peptide self-aggregation in membrane pore stabilization: insights from a computational study

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Content

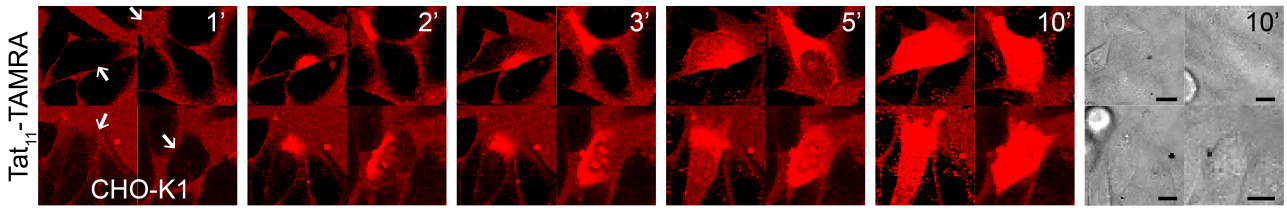

Figure S1. Transduction of Tat_{11} peptide, originating from spatially restricted zones (NZs) on the plasma membrane (white arrows). Time-lapse confocal microscopy at room temperature (with frames recorded every 30 seconds) for a free serum growth medium 15 μM solution of Tat_{11}-TAMRA administered to CHO-K1 cells. Five frames recorded at 1, 2, 3, 5 and 10 min are shown; transmitted-light channel recorded at 10 min is shown in the last frame. Scale bars: 10 μm.

Table S1. List of atoms of TAMRA model reporting atom type and atomic charge (i.e., standard CHARMM format is adopted).

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*>>>>>> CHARMM topology file generated by Molefacture <<<<<<
27 1

DEFA LAST NONE
AUTO ANGLES DIHE

RESI  TMR    -1.00
GROUP
ATOM   OO    O   -0.79810
ATOM   CC    C    0.74970
ATOM  OXT    O   -0.79810
ATOM   CA  CT1   -0.16350
ATOM    N  NH1   -0.38210
ATOM   HN    H    0.26810
ATOM   HA   HB    0.13960
ATOM   CB  CT2   -0.11960
ATOM  HB2   HA    0.14370
ATOM  HB3   HA    0.14370
ATOM    S    S   -0.30340
ATOM  CS4  CP1    0.04000
ATOM  CS3  CP2    0.04000
ATOM  CS2    C    0.45000
ATOM  OS2    O   -0.52700
ATOM  HS4   HA    0.01400
ATOM  H31   HA    0.01400
ATOM  H32   HA    0.01400
ATOM  CS1    C    0.45000
ATOM  OS3    O   -0.52700
ATOM   NS  NH1   -0.02900
ATOM   C1 CG2R61 -0.26300
ATOM   C6 CG2R61 -0.21100
ATOM   C5 CG2R61  0.27100
ATOM   C7    C    0.43600
ATOM   O2    O   -0.63400
ATOM   O3    O   -0.63400
ATOM   H6 HGR61   0.08400
ATOM   C2 CG2R61  0.00700
ATOM   H2 HGR61   0.11300
ATOM   C3 CG2R61 -0.23200
ATOM   H3 HGR61   0.10100
ATOM   C4 CG2R67 -0.00100
ATOM  C22 CG2R67  0.27000
ATOM   C8 CG2R61  0.03900
ATOM  C15 CG2DC1 -0.14100
ATOM   H8 HGA4    0.13300
ATOM  C14 CG2DC1 -0.18100
ATOM   H9 HGA4    0.14900
ATOM  C13 CG2DC2  0.10000
ATOM   N1  NH1    0.05100
ATOM  C20  CT3   -0.16600
ATOM  H16   HA    0.09300
ATOM  H17   HA    0.09300
ATOM  H18   HA    0.09300
ATOM  C23  CT3   -0.16600
ATOM  H13   HA    0.09300
ATOM  H14   HA    0.09300
ATOM  H15   HA    0.09300
ATOM  C12 CG2DC1 -0.23000
ATOM   H4 HGA4    0.14300
ATOM   C9 CG2R61  0.25700
ATOM   O1 OG3R60 -0.21800
ATOM  C10 CG2R61  0.25700
ATOM  C16 CG2R61 -0.23000
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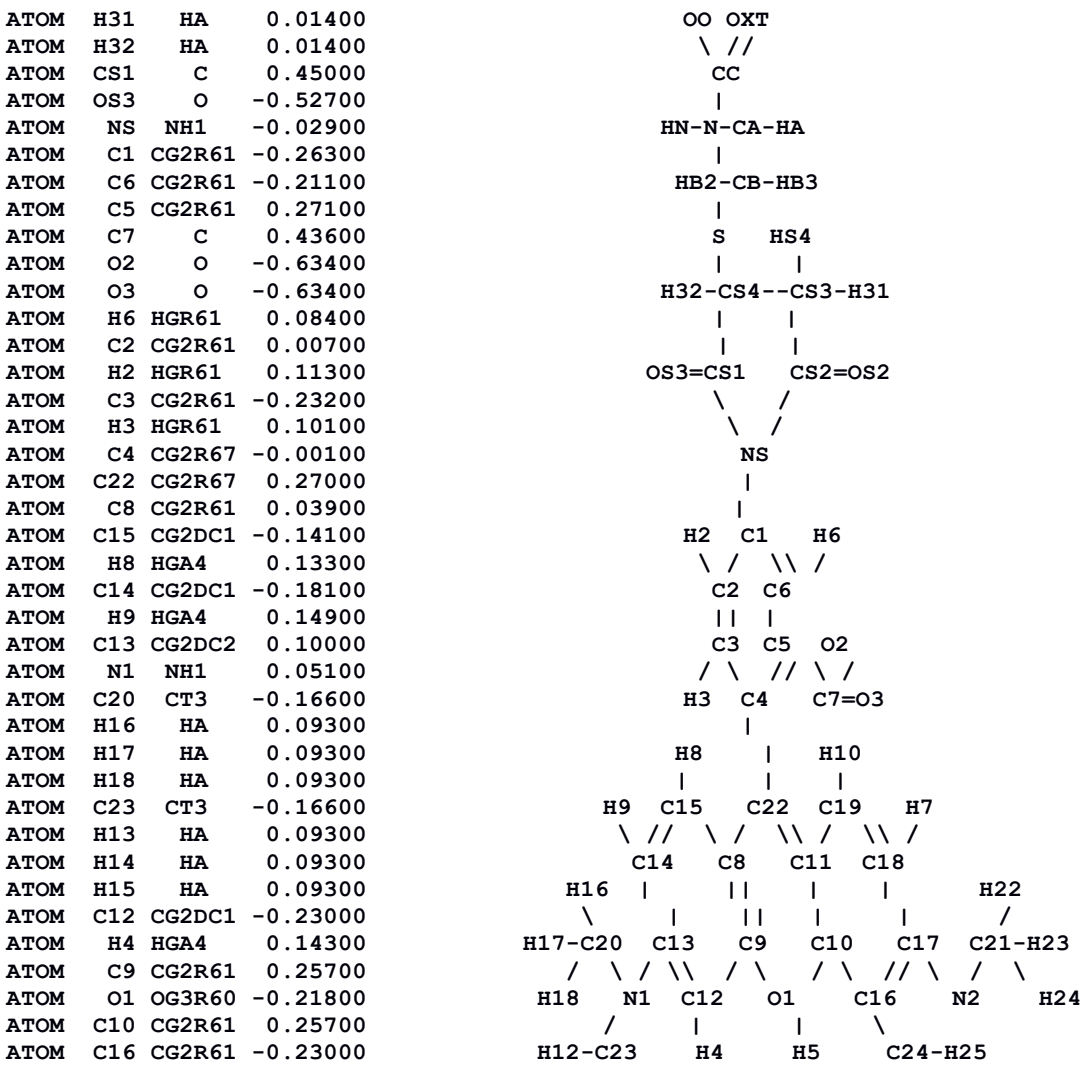

```
ATOM   H5 HGR61   0.14300                /  \                /  \
ATOM  C17 CG2R61  0.10000              H13  H14             H26  H27
ATOM   N2  NH1    0.05100
ATOM  C21  CT3   -0.16600
ATOM  H22   HA    0.09300
ATOM  H23   HA    0.09300
ATOM  H24   HA    0.09300
ATOM  C24  CT3   -0.16600
ATOM  H25   HA    0.09300
ATOM  H26   HA    0.09300
ATOM  H27   HA    0.09300
ATOM  C18 CG2R61 -0.18100
ATOM   H7 HGR61   0.14900
ATOM  C19 CG2R61 -0.14100
ATOM  H10 HGR61   0.13300
ATOM  C11 CG2R61  0.03900

BOND OO CC  CC OXT  CC CA
BOND CA HA  CA CB  CA N  N HN
BOND CB HB3  CB S  CB HB2  S CS4
BOND CS4 H32  CS4 CS1  CS4 CS3  CS3 HS4
BOND CS3 H31  CS3 CS2  CS2 NS  CS2 OS2
BOND CS1 OS3  CS1 NS  NS C1  C1 C6
BOND C1 C2  C6 H6  C6 C5  C5 C4
BOND C5 C7  C7 O2  C7 O3  C2 H2
BOND C2 C3  C3 H3  C3 C4  C4 C22
BOND C22 C11  C22 C8  C8 C15  C8 C9
BOND C15 H8  C15 C14  C14 H9  C14 C13
BOND C13 N1  C13 C12  N1 C23  N1 C20
BOND C20 H18  C20 H17  C20 H16  C23 H13
BOND C23 H15  C23 H14  C12 H4  C12 C9
BOND C9 O1  O1 C10  C10 C11  C10 C16
BOND C16 C17  C16 H5  C17 N2  C17 C18
BOND N2 C24  N2 C21  C21 H22  C21 H23
BOND C21 H24  C24 H26  C24 H25  C24 H27
BOND C18 H7  C18 C19  C19 H10  C19 C11

IMPR C7 C5 O3 O2
IMPR C17 C18 C16 N2
IMPR C13 C14 C12 N1
IMPR CC OXT OO CA
IMPR CS1 CS4 NS OS3
IMPR CS2 CS3 NS OS2

IMPR N  -C  CA  HN
DONOR HN N

END
```

Table S2. Molecular dynamics simulation details.

System	# lipids	# water mols	# ions	Replica / Length
DOPC lipid bilayer	400	33126	-	Unconstrained MD: 1; 100 ns
Tat_{11} monomer	320	19062	8 Cl^-	Unconstrained MD: 3; 400 ns
Tat_{11}-TAMRA monomer	320	19062	8 Cl^-	Unconstrained MD: 3; 600 ns Steered MD: 4; 40 ns
Tat_{11}-TAMRA dimer	320	19062	16 Cl^-	Unconstrained MD: 3; 0.6-1.0μs Steered MD: 4; 40 ns

Initial box size 105 x 95 x 95 $Å^3$.

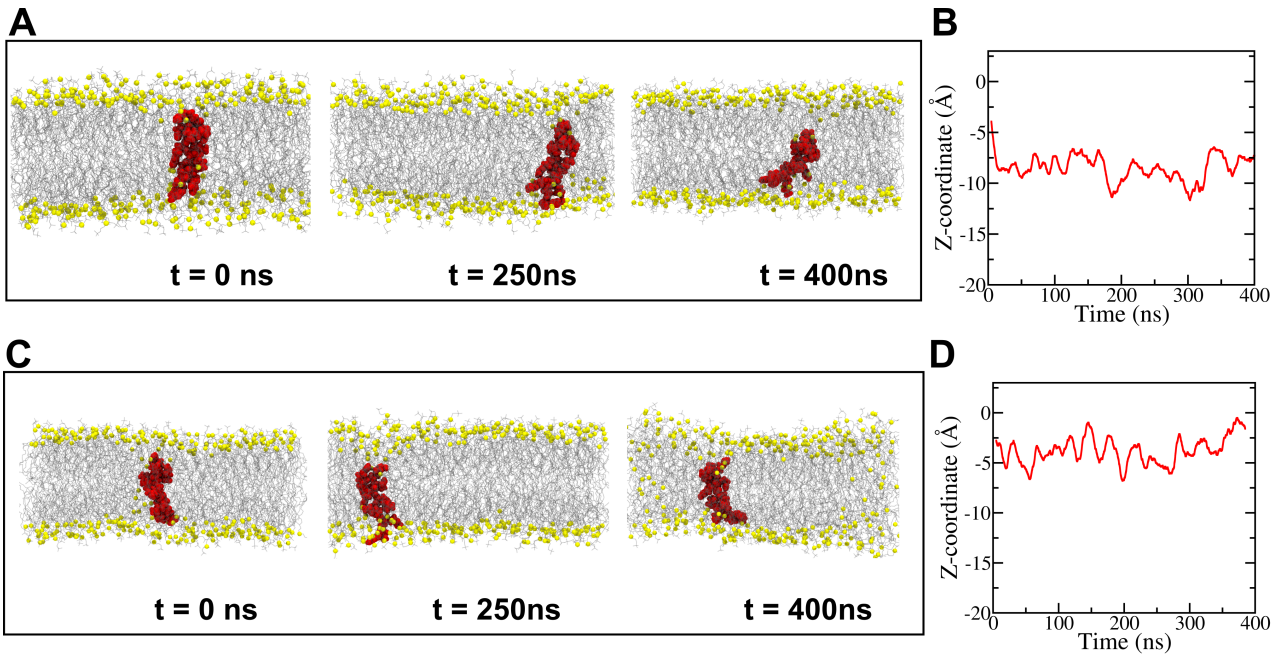

Figure S2. (A,C) Snapshot configurations issuing from Tat_{11} replica simulations taken at different time intervals. Peptides are depicted in red. (B,D) Corresponding translocation distance of monomer center of mass along the longitudinal pore axis (i.e., Z-coordinate).

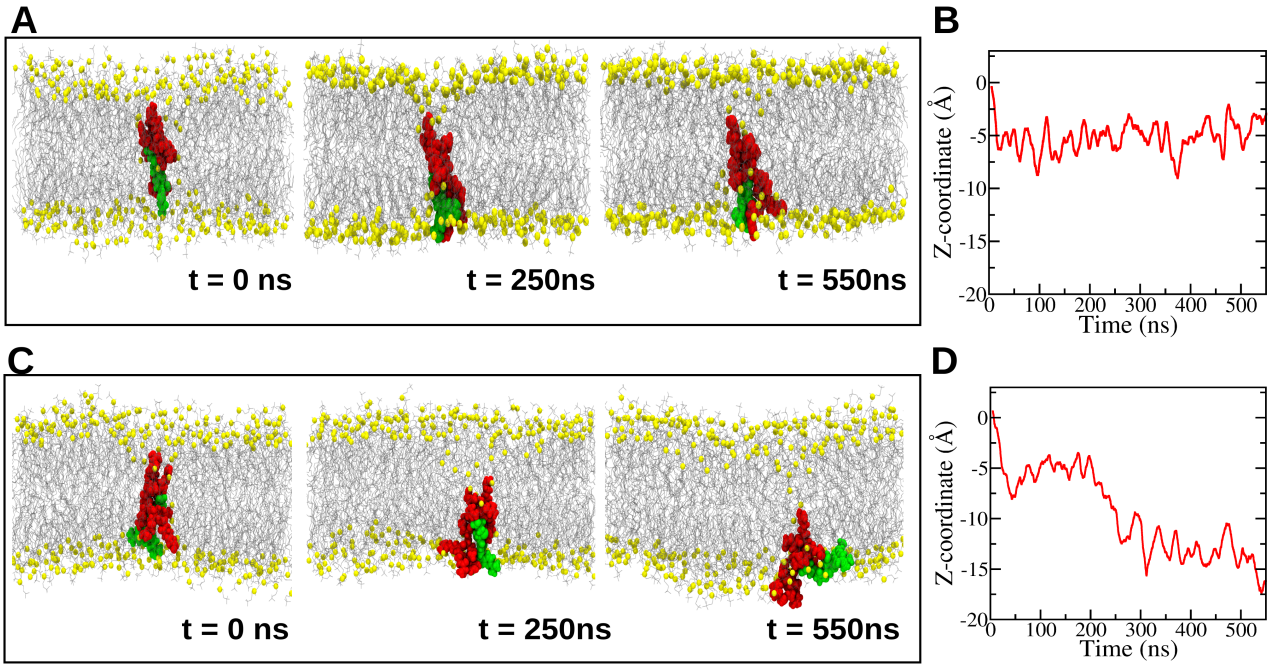

Figure S3. (A,C) Snapshot configurations issuing from Tat_{11}-TAMRA replica simulations taken at different time intervals. Peptides are depicted in red with TAMRA dye in green. (B,D) Corresponding translocation distance of monomer center of mass along the longitudinal pore axis (i.e., Z-coordinate).

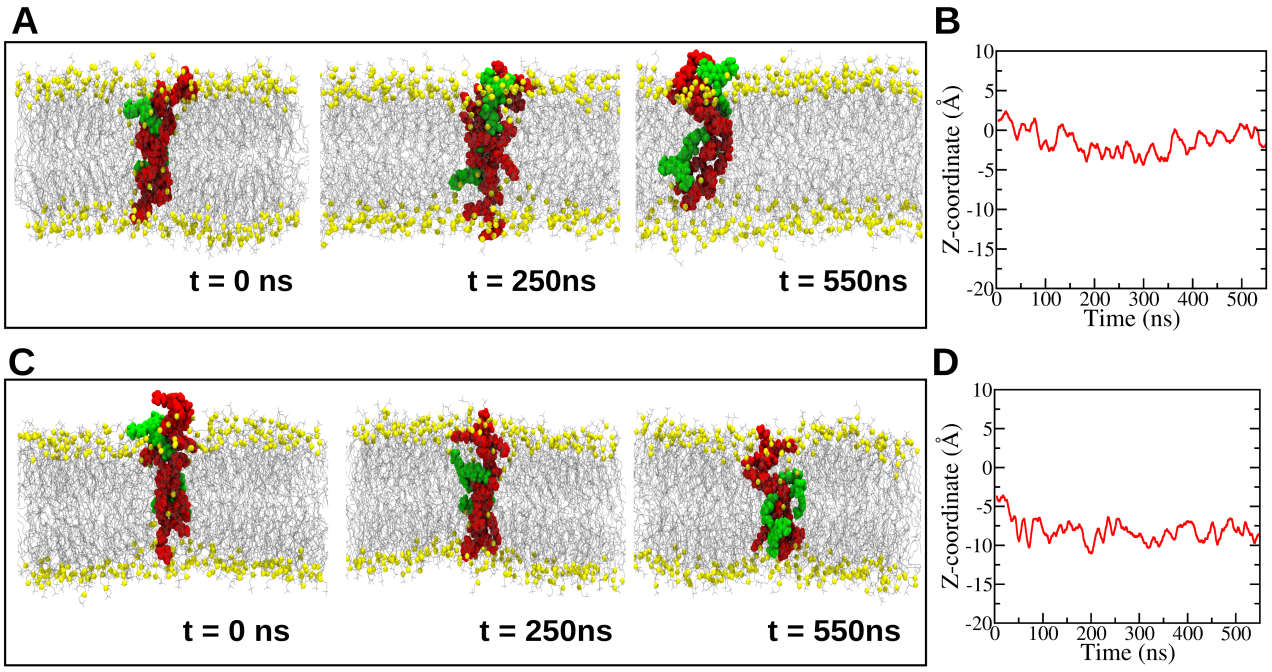

Figure S4. (A,C) Snapshot configurations issuing from Tat_{11}-TAMRA dimer replica simulations taken at different time intervals. Peptides are depicted in red with TAMRA dye in green. (B,D) Corresponding translocation distance of dimer center of mass along the longitudinal pore axis (i.e., Z-coordinate).

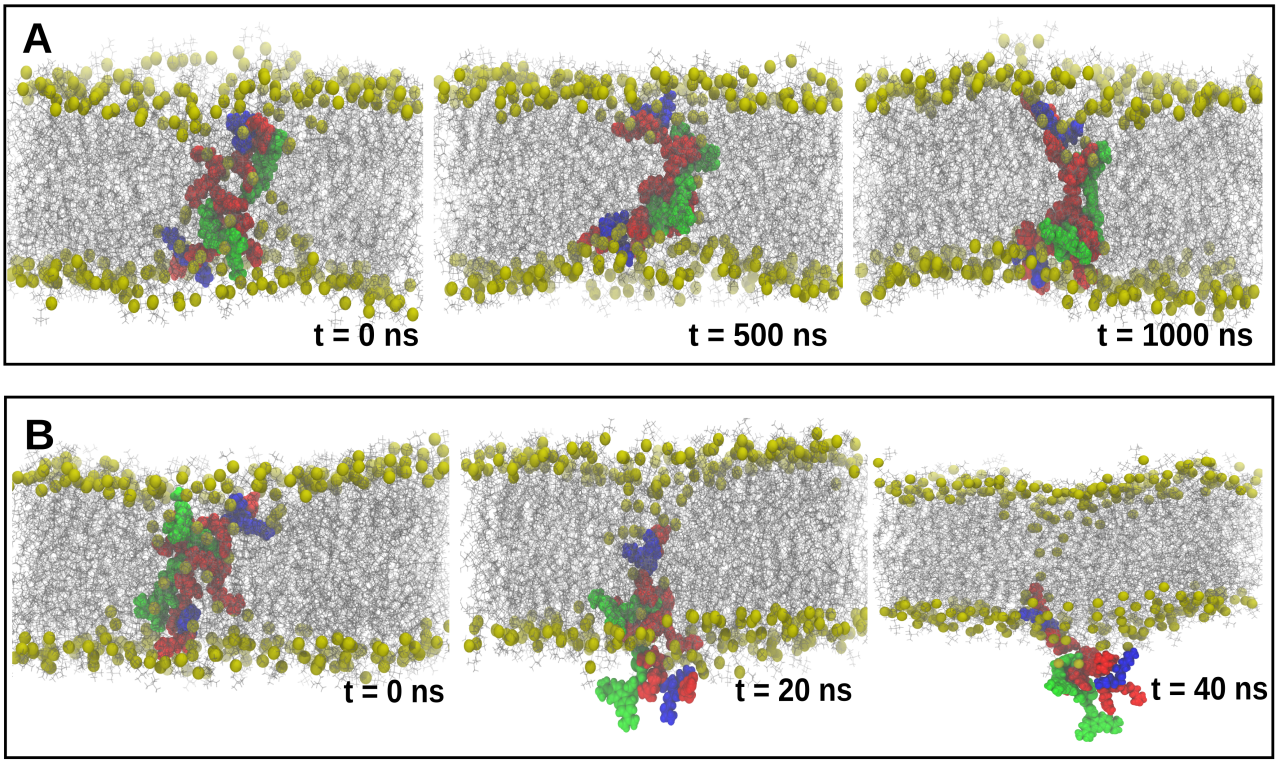

Figure S5. Snapshot configurations issuing from Tat11-TAMRA dimer (A) unconstrained simulations and (B) SMD simulations taken at different time intervals. Arginine residues are depicted in red and lysine residues are depicted in blue with TAMRA dye in green. Phosphate atoms are in yellow and lipids are in grey.