

Supporting Information for

**Membrane Mediated Regulation in Free Peptides of HIV-1 gp41:
Minimal Modulation of the Hemifusion Phase**

Cerasoli et al.

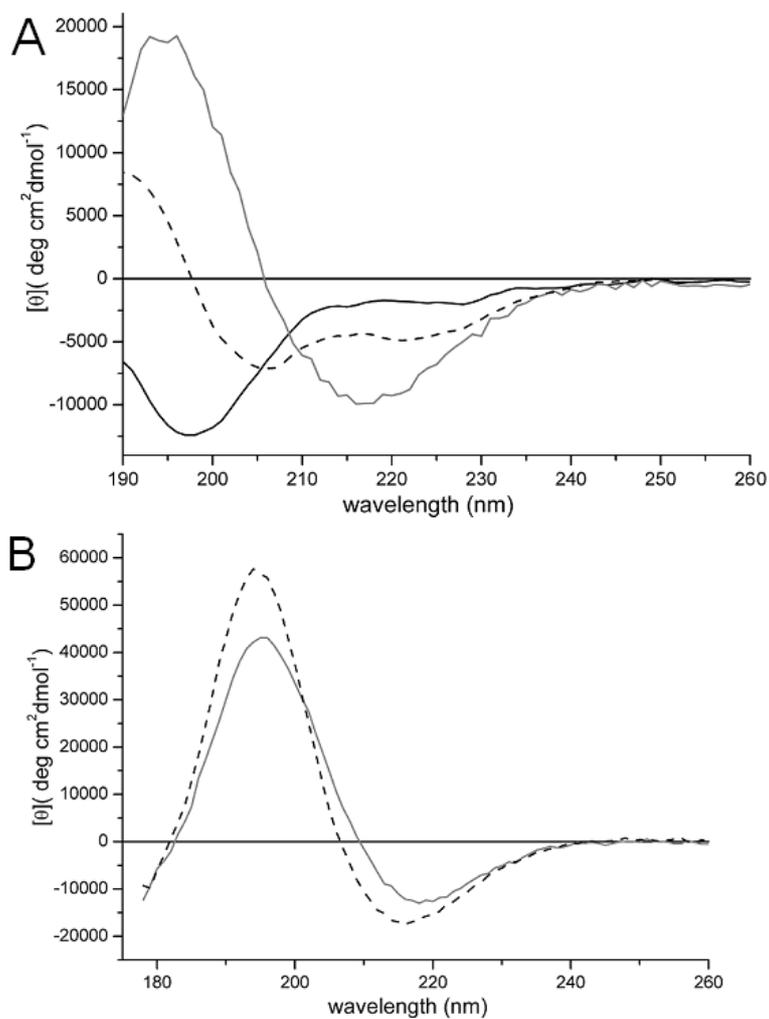


Figure S1: Folding of gp41₅₃₂₋₅₄₄ probed by CD spectroscopy. *A*, spectra for 40 μM peptide in 10 mM phosphate buffer (black solid line), 3 mM SDS (grey solid line) and 12 mM SDS (dashed line). *B*, spectra for 240 μM peptide in 3 mM SDS (black solid line) and 12 mM SDS (dashed line).

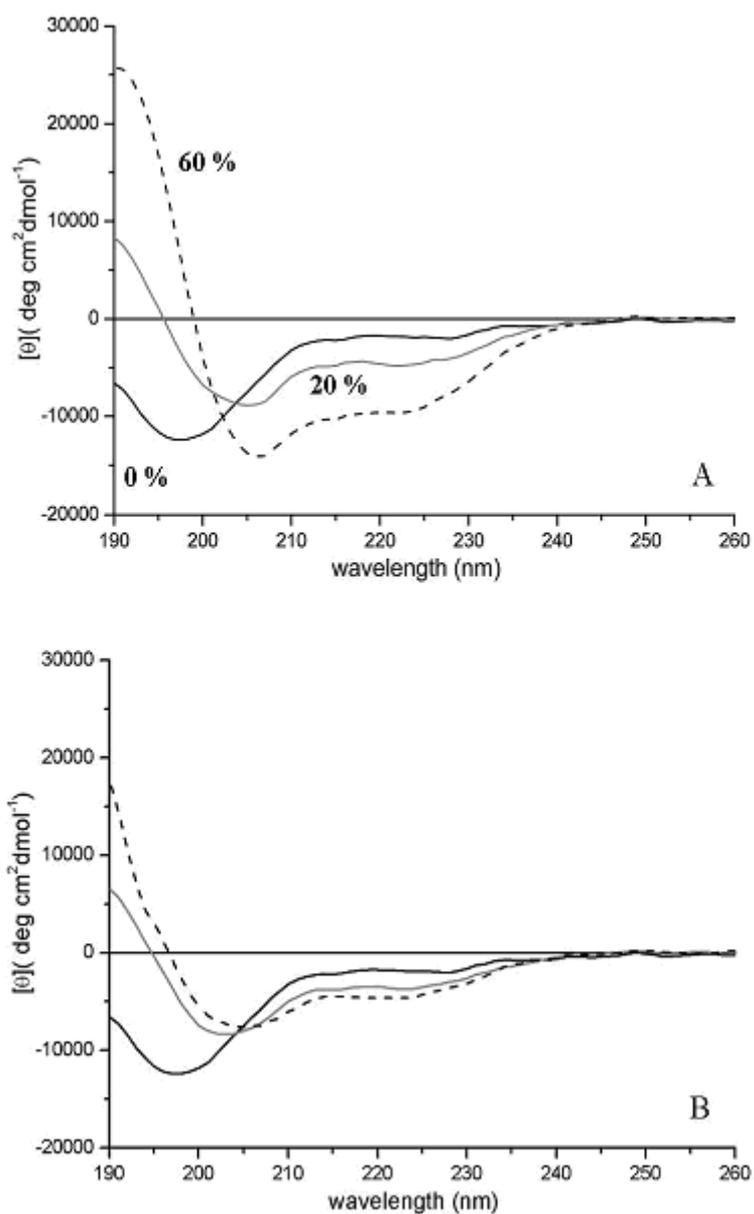


Figure S2: CD spectra for gp41₅₃₂₋₅₄₄ (40 μM). *A*, in 10mM phosphate buffer (black line) and in the same buffer with TFE, 20% (grey line) and 60% (black dashed line). *B*, in 10 mM PB (black line) and in 10 mM PB with 50% acetonitrile (grey line) and 50% methanol (black dash).

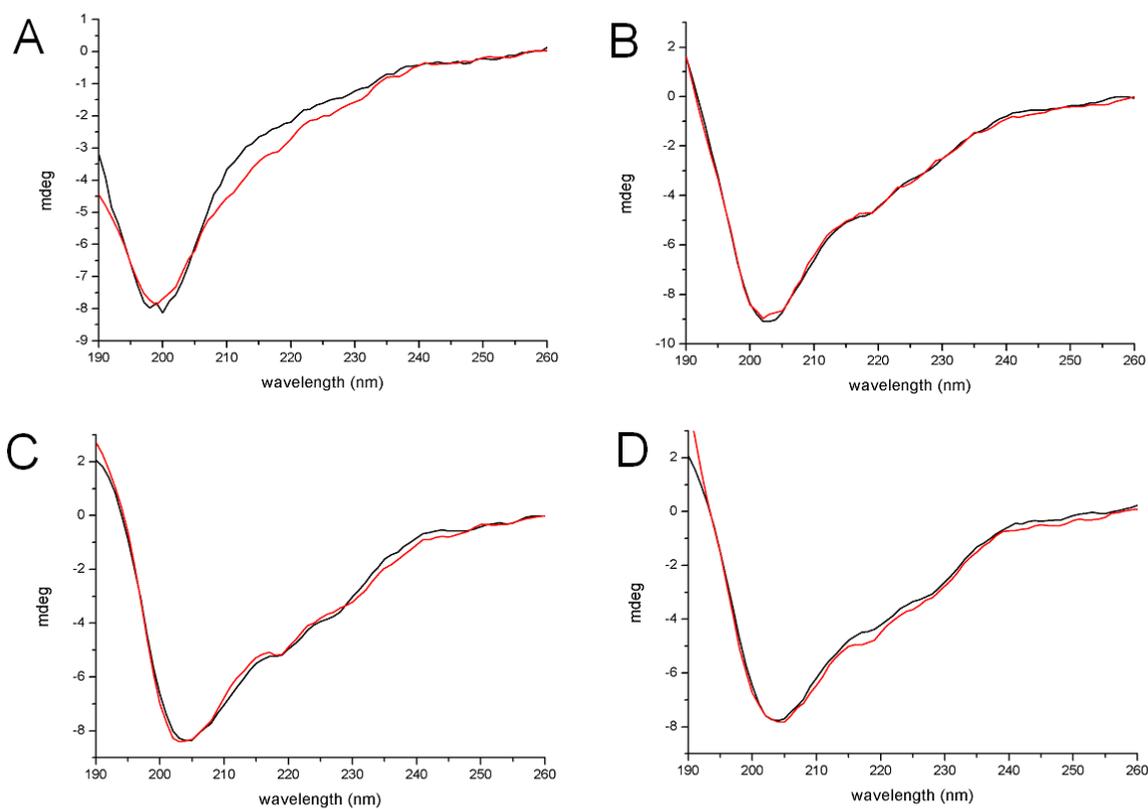


Figure S3: CD spectra for gp41_{532-544:659-671} in 10 mM phosphate buffer containing *A*, no additives, *B*, 50% acetonitrile, *C*, 12 mM SDS and *D*, 20% TFE. Linearly combined spectra of individual components and experimental spectra are shown in black and red, respectively. All spectra are for 40 μ M (total peptide).

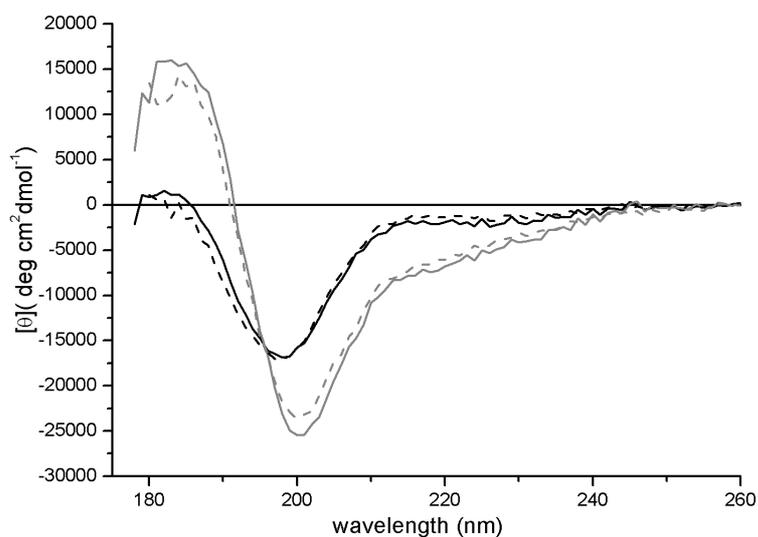


Figure S4: CD spectra for gp41₆₅₉₋₆₇₁ (black) and gp41₅₃₂₋₅₄₄ (grey) (200 μ M peptide) in the presence of zwitterionic membranes (1.6 mM total lipid) in 10mM phosphate buffer, pH 7-7.5, (solid line) and water at pH 3 (dashed line).

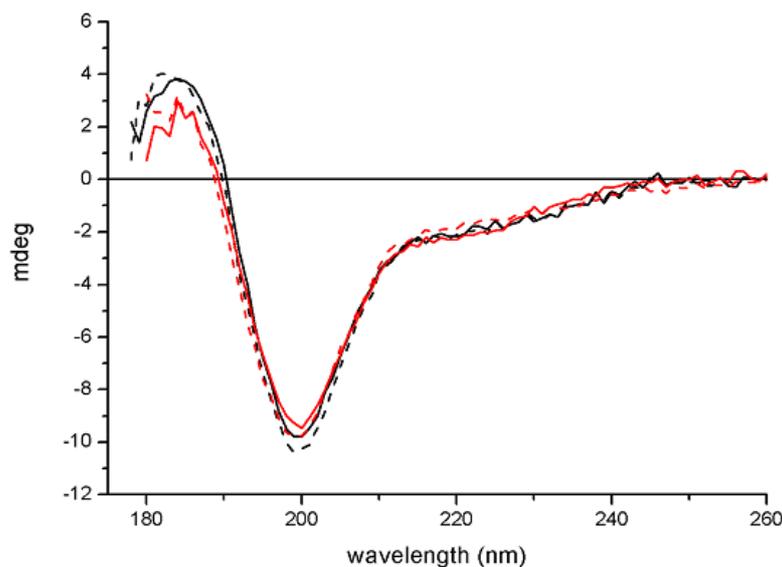


Figure S5: CD spectra for gp41_{532-544:659-671} (200 μ M in each peptide) in the presence of zwitterionic membranes (1.6 mM total lipid) in 10 mM phosphate buffer, pH 7-7.5 (solid black line) and in water at pH3 (solid red line). Linearly combined spectra of individual components and experimental spectra are shown in dashed and solid lines, respectively.

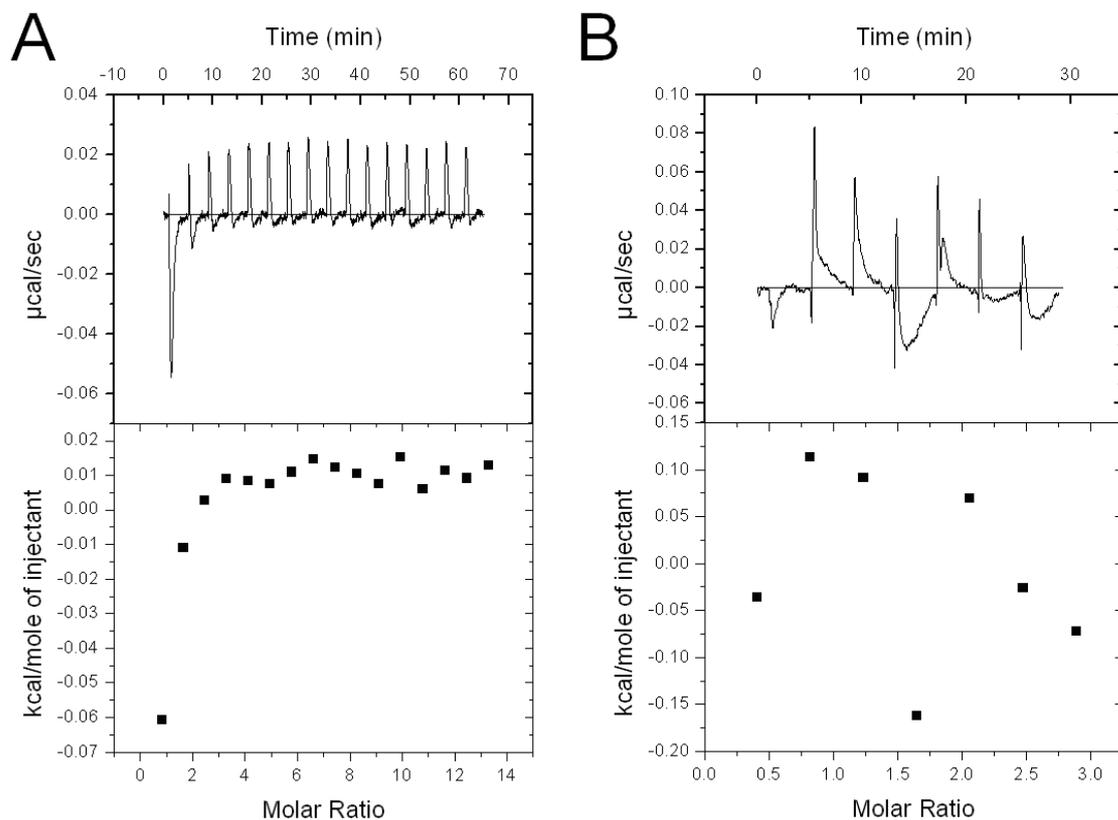


Figure S6: Peptide interactions with zwitterionic membranes probed by isothermal titration calorimetry. 40 μ M (total peptide) titrated with membrane solution (1.6 mM). A, gp41₅₃₂₋₅₄₄. B, gp41₆₅₉₋₆₇₁.

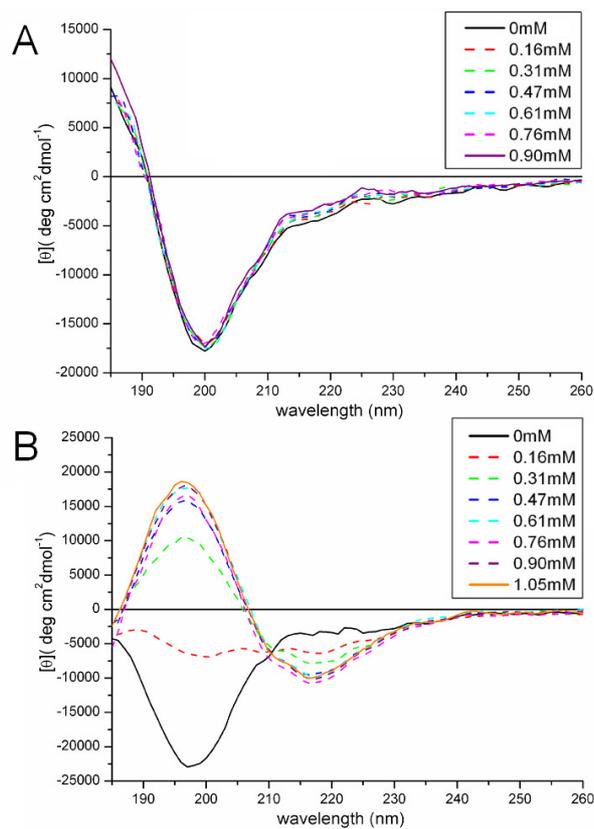


Figure S7: Folding of the individual peptides as a function of membrane concentrations. CD spectral changes for 40 μM peptide. *A*, gp41₆₅₉₋₆₇₁. *B*, gp41₅₃₂₋₅₄₄.

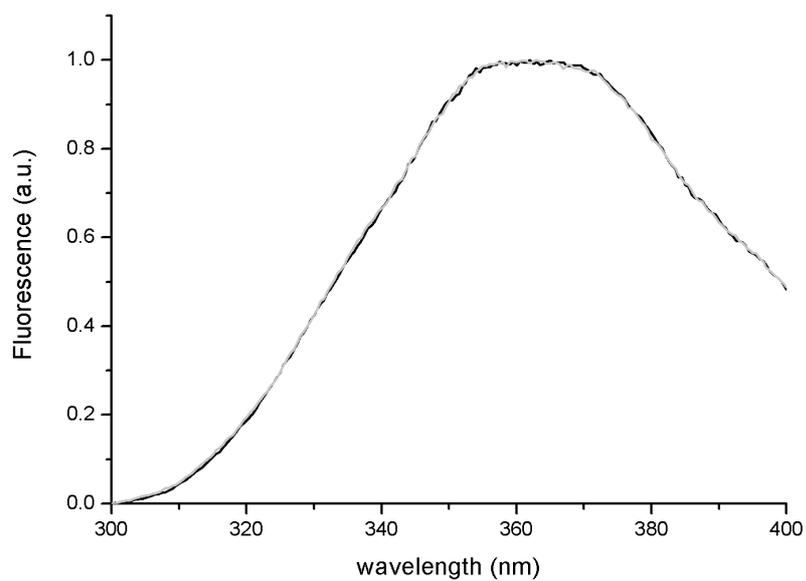


Figure S8: Normalised Trp fluorescence of gp41₆₅₉₋₆₇₁ (40 μM) with (grey line) and without (black line) anionic membranes.

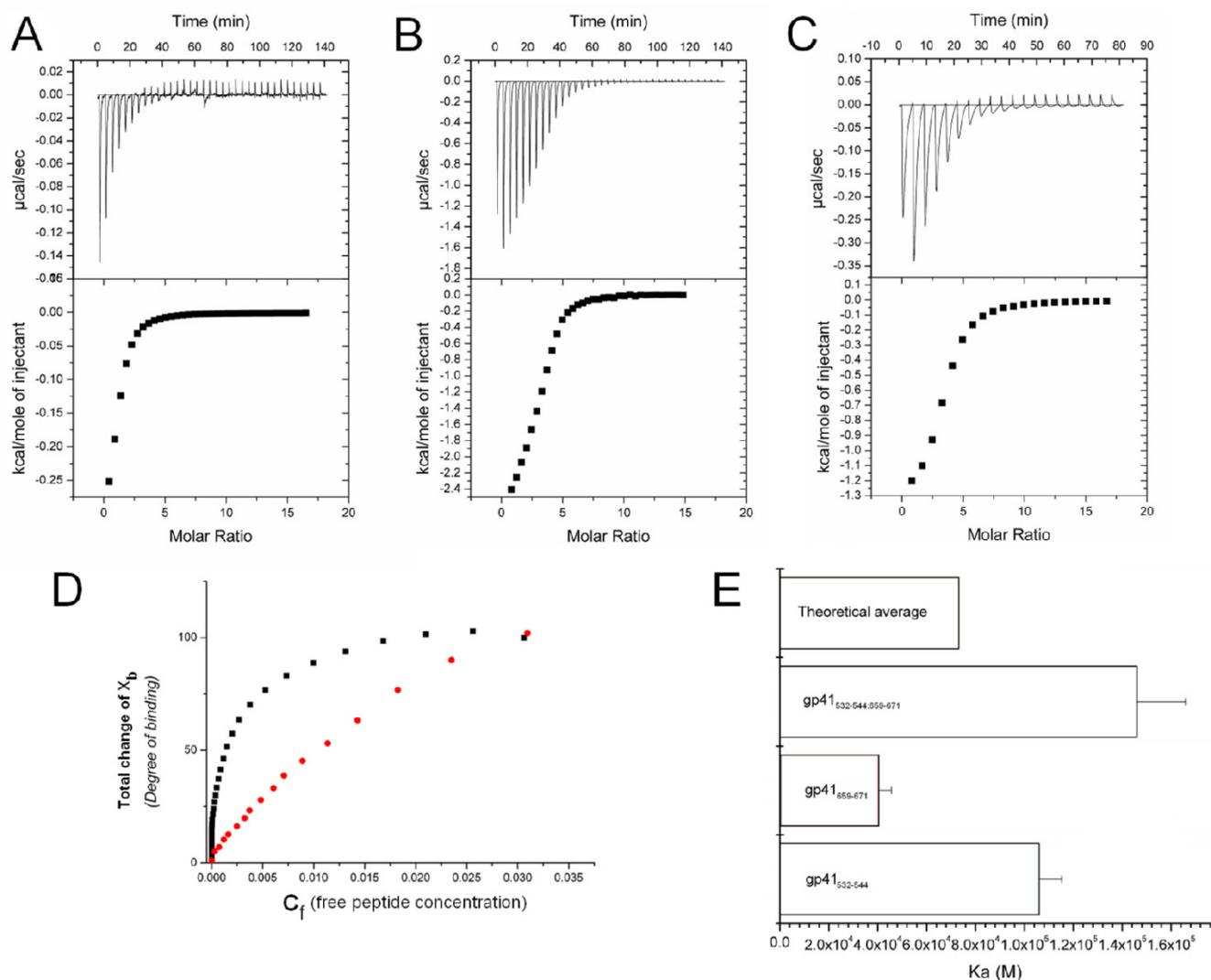


Figure S9: Peptide interactions with anionic membranes probed by isothermal titration calorimetry. A, gp41₆₅₉₋₆₇₁. B, gp41₅₃₂₋₅₄₄. C, gp41_{532-544:659-671}. 40 μM (total peptide) titrated with membrane solution (1.6 mM). D, degree of binding (X_b) as a function of free peptide concentration (C_f) for gp41₆₅₉₋₆₇₁ (red dots) and gp41₅₃₂₋₅₄₄ (black dots) E, K_a values derived from fitting the ITC data with a one-site binding model (22, 23).

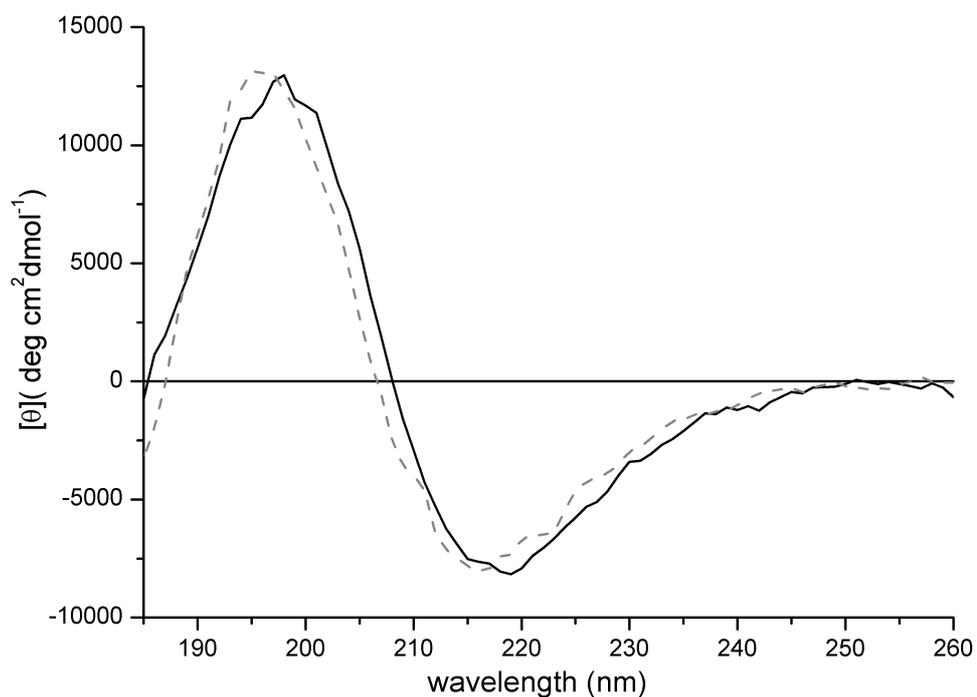


Figure S10: CD spectra for gp41₅₃₂₋₅₄₄ at 40 μM (black line) and 400 μM (grey dash) in the presence of anionic membranes (1.6 mM total lipid).

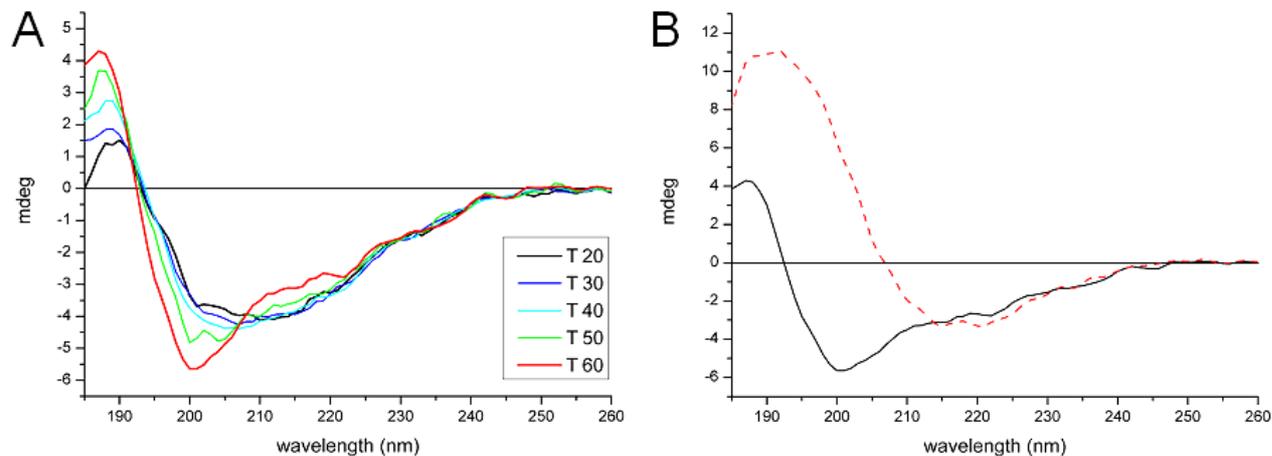


Figure S11: Folding of gp41_{532-544:659-671} in anionic membranes as a function of temperature (40 μM total peptide). *A*, CD spectra. *B*, CD spectra and linear spectral combinations for individual peptides at 60°C are shown in black and red, correspondingly while the lipid concentration is 0.45 mM.

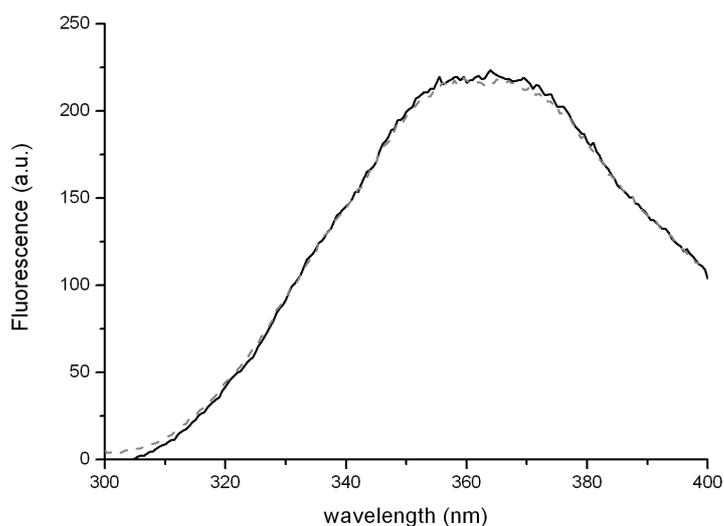


Figure S12: Intrinsic fluorescence spectra for gp41_{532-544:659-671} at 40 μM total peptide (grey dashed line) and gp41₆₅₉₋₆₇₁ at 20 μM (black solid line) in the presence of anionic membranes (1.6 mM total lipid).

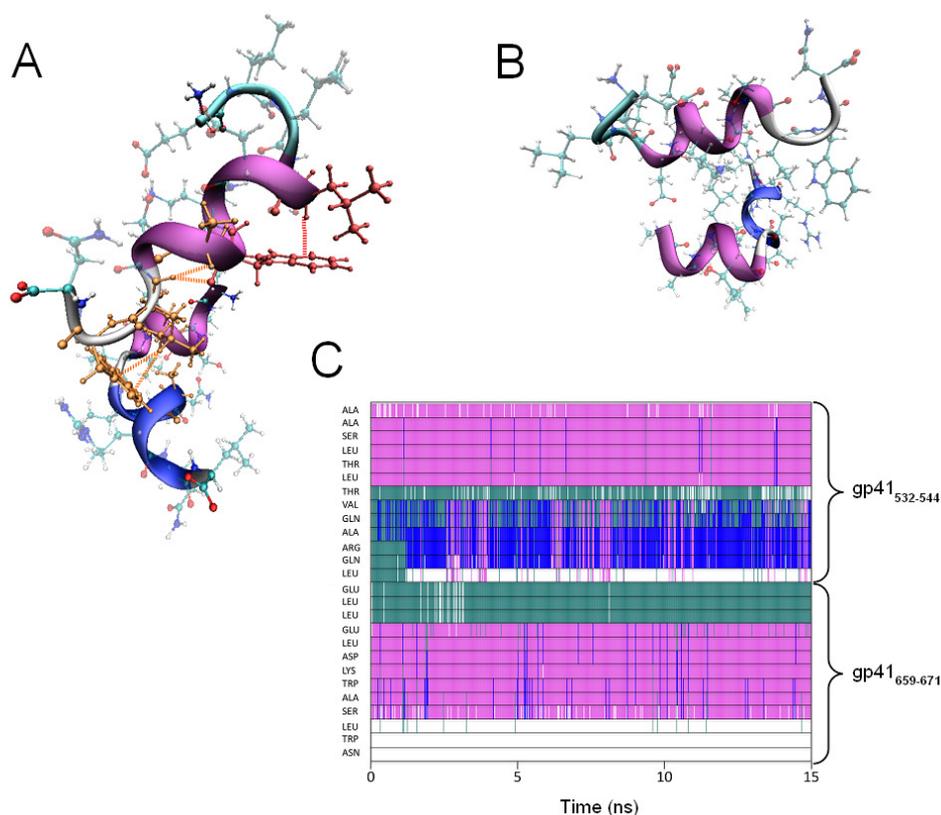


Figure S13: Snapshot of gp41_{532-544:659-671} simulation. *A*, Trp₆₆₆ residue leaving the hydrophobic interface (orange) to interact with Leu₆₆₃ (red). *B*, α-helical regions of gp41₅₃₂₋₅₄₄ and gp41₆₅₉₋₆₇₁ aligned in parallel. *C*, secondary structure of each residue of gp41_{532-544:659-671} as a function of time. Key: pink denotes α-helix, blue is for 3₁₀-helix, green is for turn and white is for unordered.

Table S1: Thermodynamic parameters calculated using a one-site binding model

	N	K_a (M)	ΔH (cal mol⁻¹)	ΔS (cal mol⁻¹ K⁻¹)
gp41 ₅₃₂₋₅₄₄	3.14 ± 0.04	1.06E05 ± 9.17E3	-2577 ± 46.91	14.5
gp41 ₆₅₉₋₆₇₁	1.32 ± 0.089	4.04E4 ± 5.27E3	-466.7 ± 38.20	19.5
gp41 _{532-544; 659-671}	3.23 ± 0.088	1.46E5 ± 1.99E4	-1367 ± 55.35	19.1