“Supporting Information”

The role of peptide-peptide interactions in aggregation: Protonectins observed in equilibrium and replica exchange molecular dynamics simulations

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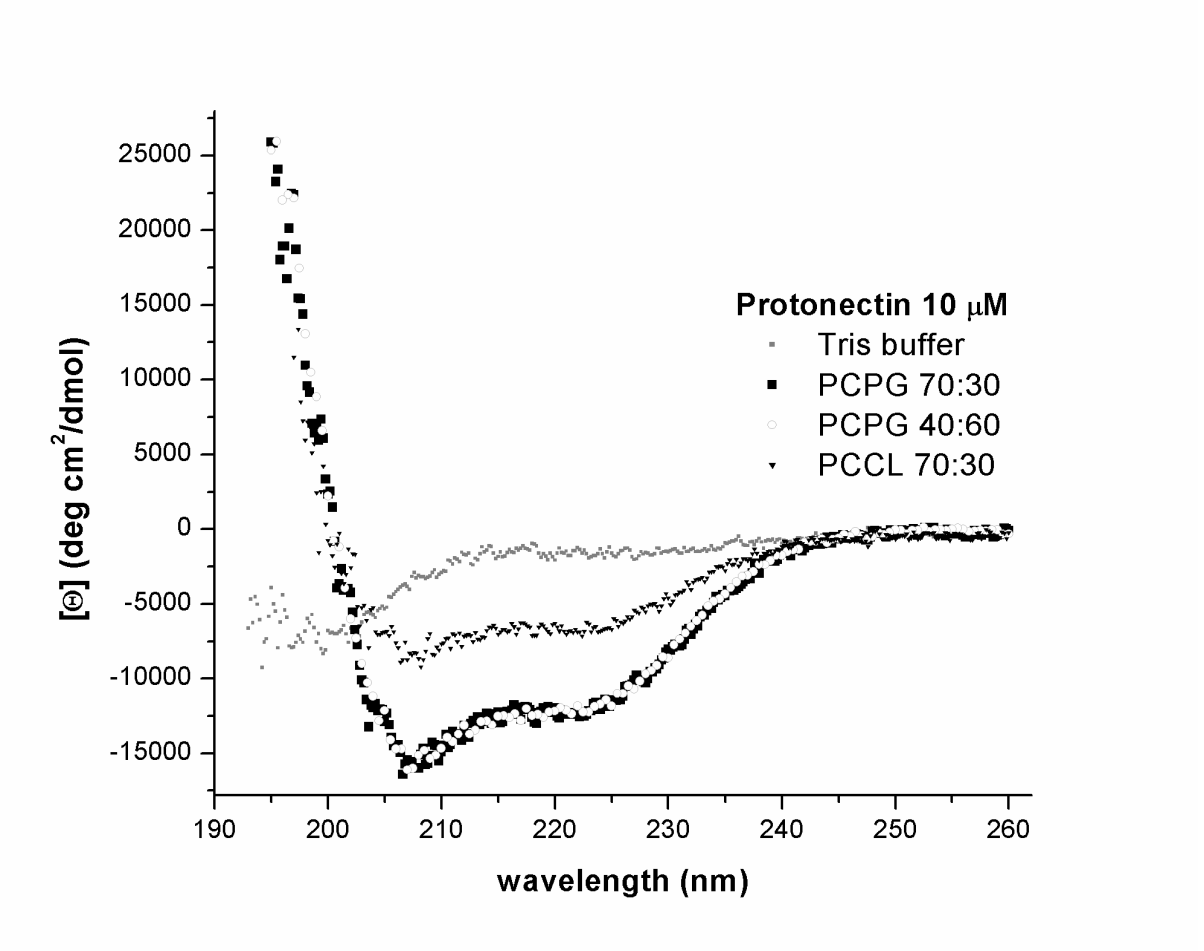
EXPERIMENTAL PROCEDURES

Circular Dichroism (CD) measurements. Peptides were synthesized by step-wise manual phase synthesis using N-9-fluorophenylmethoxy-carbonyl (Fmoc) chemistry with Novasyn TGS resin (NovaBiochem), according to the detailed procedure by Baptista-Saidemberg et al. (2010). Sigma-Aldrich Co. (S.Louis, MO) supplied phospholipids and other chemicals were of high quality analytical grade. Small unilamellar vesicles (SUV) were prepared by the evaporation (under N_2 flow and further under vacuum) of the solvents used to solubilize lipids, rendering homogeneous films on the walls of test tubes. These films were hydrated with Tris/H_3BO_3 5 mM, 150 mM NaF, 0.5 mM buffer to reach a final lipid concentration around 10 mM. Afterwards, the actual lipid concentration was confirmed by phosphorus analysis. After hydration, the suspensions were thoroughly vortex mixed at room temperature. SUVs were obtained after 50 minutes sonication (or until clear) with a tip sonicator in an ice/water bath, under N_2 flow; titanium debris was removed by centrifugation. SUVs were used within 24 hours of preparation as obtained after Ti debris removal. They were kept under refrigeration and protected from light. The following lipids were used: pure L-α-phosphatidylcholine (PC); PC and L-α-phosphatidyl-DL-glycerol (PG) at 70:30 (named PCPG 70:30) and at 40:60 (named PCPG 40:60), PC and cardiolipin (CL), at 70:30 (named PCCL 70:30).

CD spectra were obtained at 10 μM total peptide concentration in the presence of Tris buffer and of different SUVs. Buffer, peptide and lipid concentration have been chosen to minimize noise-to-signal ratio and light scattering. CD spectra were recorded from 260 to 190 nm with a Jasco-710 spectropolarimeter (JASCO International Co. Ltd., Tokyo, Japan), which was routinely calibrated at 290.5 nm using d-10-camphorsulfonic acid solution. Spectra have been acquired at 25°C using 0.5 cm path length cell, averaged over six scans, at

a scan speed of 20 nm/min, bandwidth of 1.0 nm, 0.5 s response and 0.1nm resolution. Following baseline correction, the observed ellipticity, θ (mdeg) was converted to mean residue ellipticity [Θ] (deg cm^2/dmol), using the relationship [Θ] = 100θ/(l c n), where "l" is the path length in centimeters, "c" is peptide milimolar concentration, and "n" the number of peptide residues.

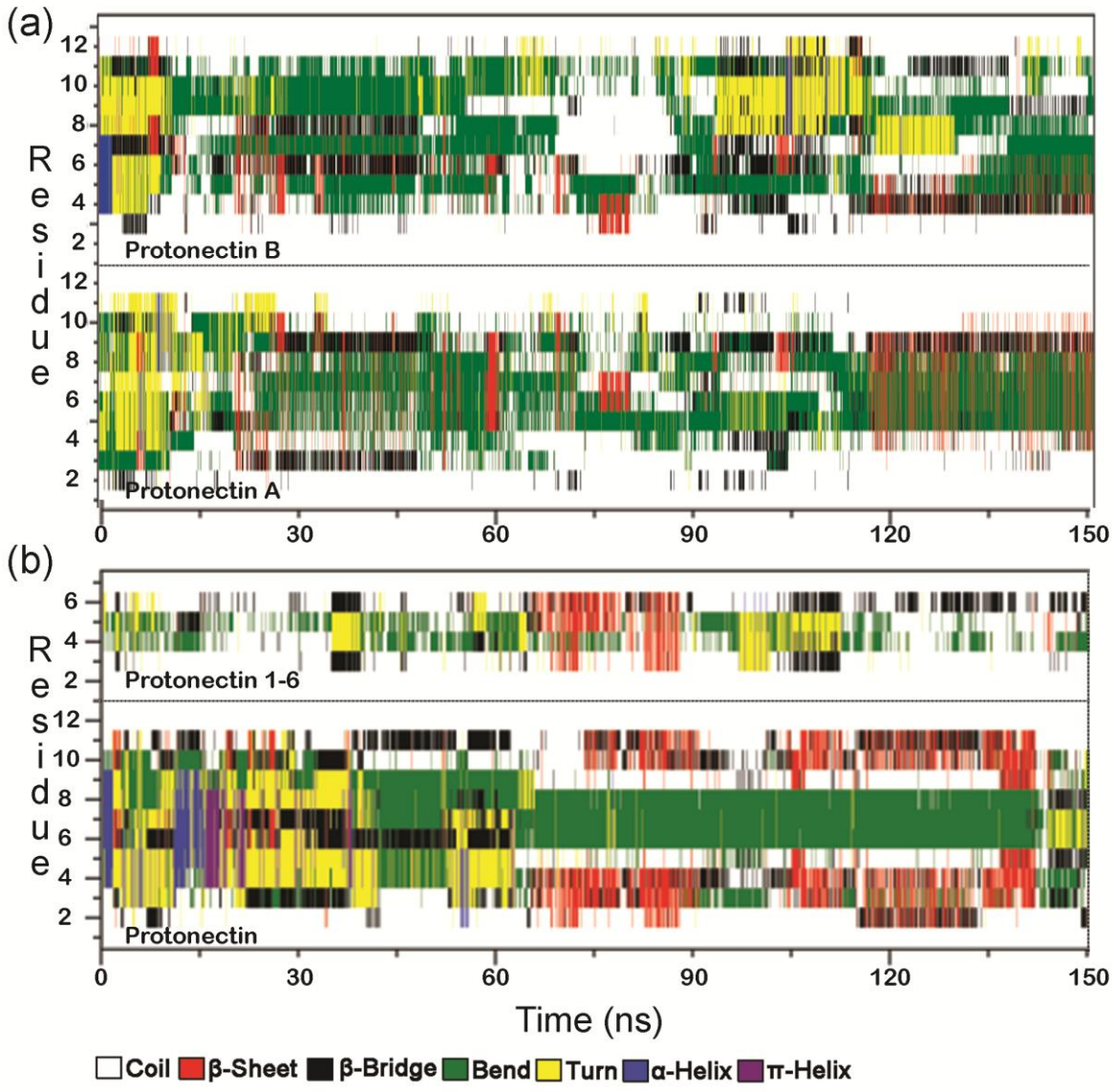
Supplementary Figure 1.



CD spectra of Protonectin obtained at 10 μM, at 25°C, in buffer and in the presence of small unilamellar anionic vesicles of different lipid composition, at 100 μM: PC/PG 70:30 (black squares); PC/PG 40:60 (empty gray circles); PC/CL 70:30 (black triangles). No smoothing has been applied.

The equivalent ellipticity of Protonectin in PC/PG 70:30 and PC/PG 40:60 and also the lower ellipticity in PC/CL 70:30 suggest the importance of the hydrophobic component to the interaction.

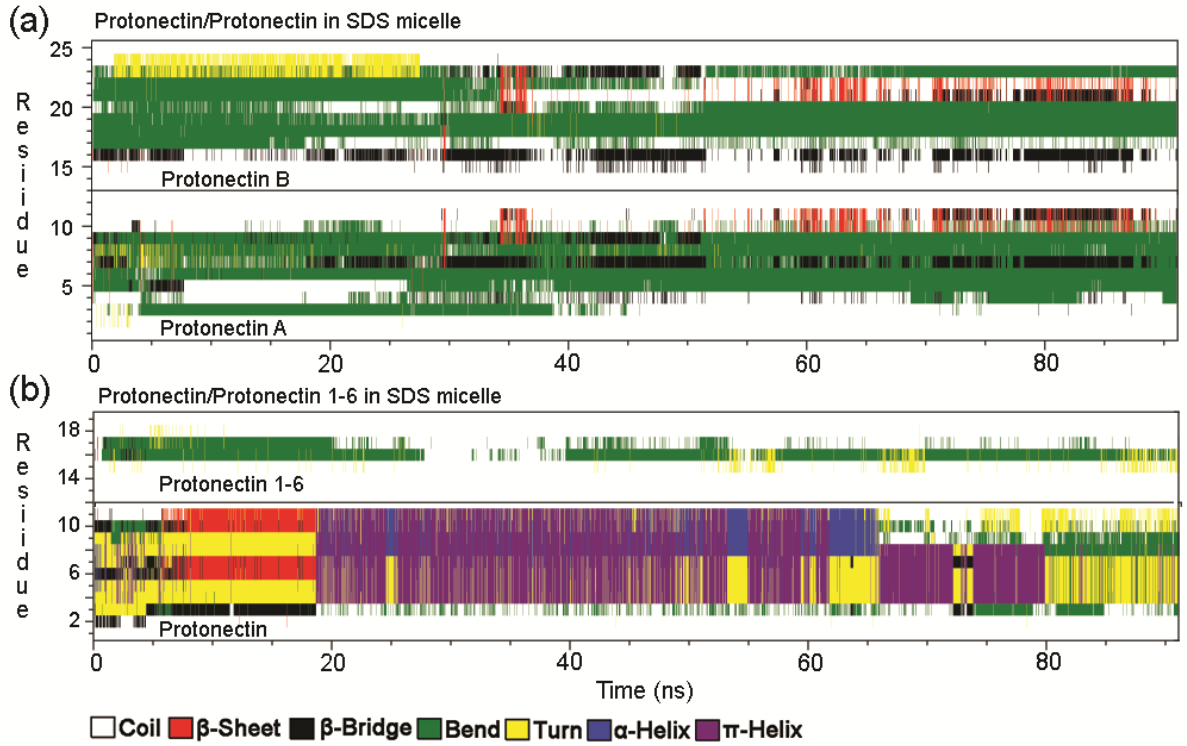
Supplementary Figure 2.



Secondary structure profile for the Protonectins and Protonectin/Protonectin (1-6) molecules obtained in simulations in water. (a) Protonectins, molecules A and B. (b) Protonectin and

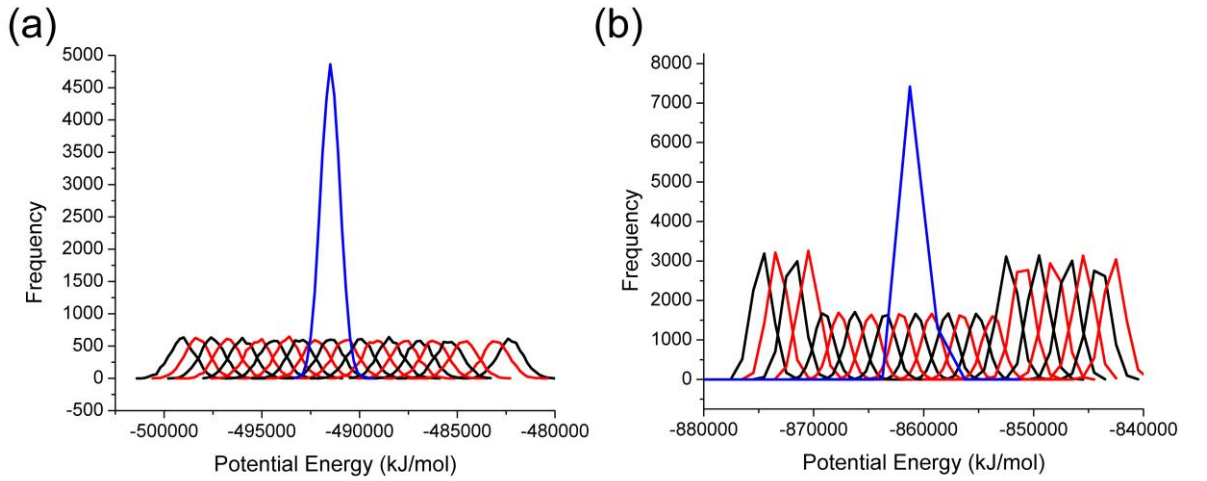
Protonectin (1-6). The bottom line of the figure shows the legend for the colors attributed to each type of secondary structure (for example: blue is for α-helix; red for β-sheets).

Supplementary Figure 3.



Secondary structure profile for the Protonectins and Protonectin/Protonectin (1-6) obtained in simulations in the presence of the SDS micelle. (a) Protonectins, molecules A and B. (b) Protonectin/Protonectin (1-6). The bottom line of the figure shows the legend for the colors attributed to each type of secondary structure (for example: blue is for α-helix; red for β-sheets).

Supplementary Figure 4.



Potential energy distribution of the equilibrium simulations (blue) and of the REMD (black and red) in the presence of the SDS micelle, showing that in the REMD simulation the conformational space is widely visited. (a) Protonectin and Protonectin 1-6 simulations. (b) Pure Protonectins.