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Supplemental Information

Membrane Remodeling by the Lytic Fragment of SticholysinII: Implications for the Toroidal Pore Model

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Supplemental information

Tables

Table S1: Parameters obtained at 500 ns of simulations of pre-associated $StII_{1-30}$ in different lipid membrane systems

Parameter	Membranes	
	POPC:SM	POPC:SM:POPE
Pore diameter (nm) [a]	1.9 ± 0.4	2.0 ± 0.5
Tilt angle (degree) [b]	89.0 ± 12.0	98.0 ± 15.0
Average distance of the N-terminus to the pore center (nm) [c]	1.1 ± 0.4	1.0 ± 0.3
Lipid count [d]	7.0 ± 3.0	18.0 ± 2.0*
Membrane thickness fluctuation (nm) [e]	0.30 ± 0.03	0.50 ± 0.02*

[a]Pore diameter was determined as the maximum distance between two water molecules located at the *xy* plane within the pore, during the last 50 ns of the simulation time. [b]Tilt angle was calculated considering the angle formed between each peptide and the *z* axis perpendicular to membrane plane. [c]Distance between the center of mass of the peptide and the center of the pore, calculated as the *z*-coordinate of the peptide respect to the center of the bilayer (z'=0) averaged during the last 100 ns of simulation. [d]Lipid count at 500 ns, defined as lipids that are in contact with the peptides, within a distance of 0.5 nm. [e]Membrane thickness fluctuation was calculated at 500 ns as the deviation of the distance phosphate-phosphate (between each monolayer) within a cut-off of 1.5 nm from the pore center. Values correspond to the mean and standard deviation from the three replicates of the system. *indicates that the values are statically different, with p values calculated from a two-tailed distribution, reported with a confidence level of 0.05.

Table S2: RMSD values of the structures obtained at 0 and 500 ns of the simulations of pre-associated $StII_{1-30}$ in different lipid membrane systems

Parameter	RMSD (Å)			
Composition	**POPC:SM**		**POPC:SM:POPE**	
Peptide region	**1-10**	**11-30**	**1-10**	**11-30**
Peptide 1	3.7 ± 0.2	1.8 ± 0.2	4.4 ± 0.4	2.2 ± 0.3
Peptide 2	3.4 ± 0.3	2.1 ± 0.3	3.5 ± 0.3	1.3 ± 0.2
Peptide 3	3.5 ± 0.3	2.3 ± 0.2	2.9 ± 0.4	2.0 ± 0.4
Peptide 4	2.8 ± 0.4	1.9 ± 0.3	3.1 ± 0.2	2.1 ± 0.3

The RMSD values were calculated for the hydrophobic (1-10) and amphipathic (11-30) regions of each peptide molecules forming pre-associated $StII_{1-30}$, in the membrane systems. Values were calculated with respect to the initial structure (0 ns), using the gmx_rms tool included in GROMACS. The values are reported as the mean and the standard deviation.

Table S3: Parameters that characterize the activity of $StII_{1-30}$ in lipid model membranes

Parameters	Membrane			
	POPC:SM		**POPC:SM:POPE**	
	$StII_{1-30}$ WT	**$StII_{1-30}$ L2W**	**$StII_{1-30}$ WT**	**$StII_{1-30}$ L2W**
π_c [a] (mN.m^{-1})	32.4 ± 0.6	ND	35.7 ± 0.4*	ND
F/F_0 max [b]	1.20 ± 0.02	2.52 ± 0.02	1.78 ± 0.01*	2.88 ± 0.02 *
Lip_{50} [c] (mM)	22.2 ± 0.2*	4.5 ± 0.1	14.5 ± 0.3	5.0 ± 0.2*
C_{50} x 10^{-4} [d] (mol:mol)	3.2 ± 0.1*	ND	0.9 ± 0.1	ND

[a]Critical pressure (π_c), which corresponds to the pressure that must be applied to avoid incorporation of the peptide to the monolayer and it is directly correlated with its affinity for the lipids. [b]Maximum fluorescence intensity ratio (F/F_0) obtained upon saturation conditions. F_0 and F are the fluorescence in the presence and absence of vesicles, respectively. [c]Amount of lipid necessary to bind half of the peptide molecules (Lip_{50}), estimated by fitting to a Boltzmann function. [d]Peptide:lipid ratio (C_{50}) necessary to achieve 50% of vesicles permeabilization. Values correspond to the mean and standard deviation from at least three independent experiments. *indicates that the values are statically different, with p values calculated from a two-tailed distribution reported with a confidence level of 0.05.

Figures

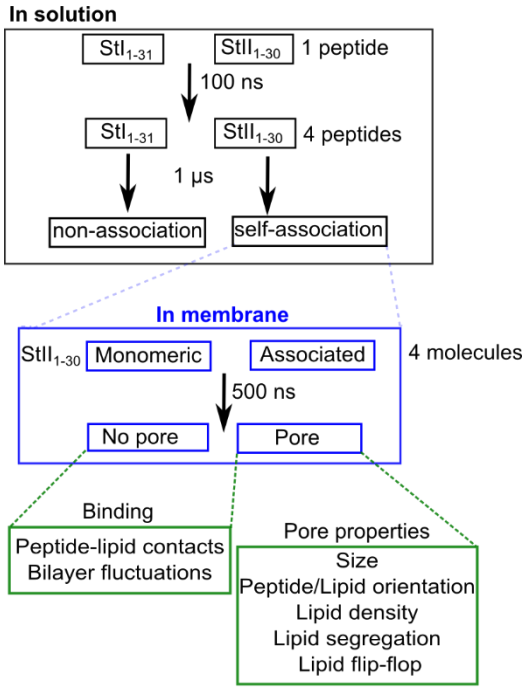

Figure S1. Workflow of the simulations and analysis.

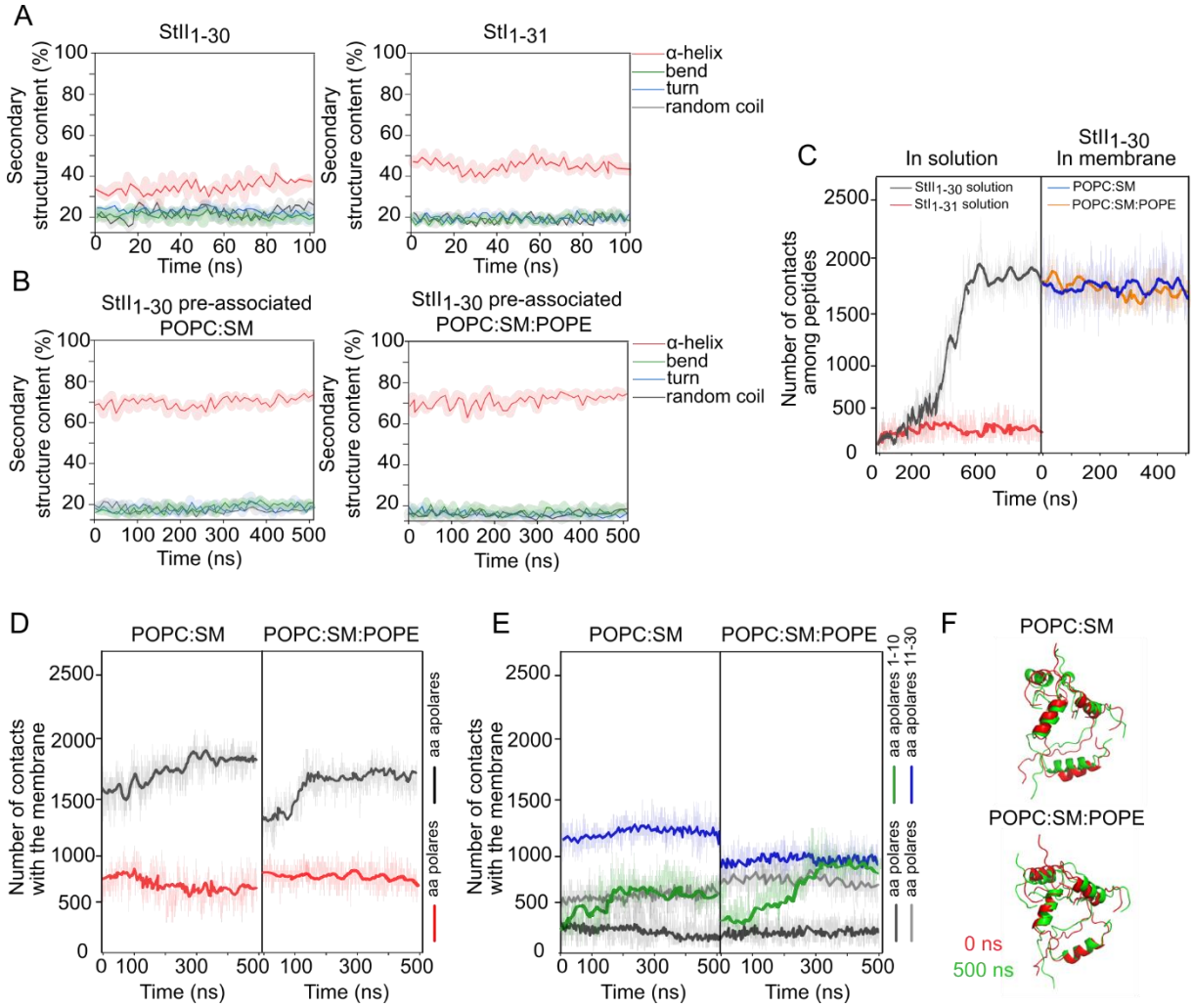

Figure S2. Analysis of the secondary structure of the peptides in solution and membranes. **(A)** Stability of structure of $StII_{1-30}$ **(left)** and StI_{1-31} **(right)** obtained during equilibration time. The initial structure of these segments corresponds to the one obtained in the parental sticholysins. **(B)** Stability of the structure of $StII_{1-30}$ in membranes of POPC:SM **(left)** or POPC:SM:POPE **(right)**. The initial structure corresponds to pre-associated $StII_{1-30}$ in solution. **(C)** Interaction of individual molecules of $StII_{1-30}$ in solution and in membranes of POPC:SM or POPC:SM:POPE. **(D-E)** Kinetics of the interaction of polar and apolar amino acid residues with the membrane, calculated for the full sequence of $StII_{1-30}$ **(D)** or the segments 1-10 (hydrophobic) and 11-30 (amphipathic) **(E)**. Amino acid residues were separated in two groups depending on their polar/apolar properties and their interaction with the membrane was calculated within a distance of 0.5 nm as the number of contacts with the lipids. **(F)** Superposition of the structures of pre-associated $StII_{1-30}$ obtained at the beginning and the end of the simulations in POPC:SM **(upper)** or POPC:SM:POPE membranes **(bottom)**. In **C-E**, dark lines in the plots represent the main values and shadow lines the standard deviation among three replicates.

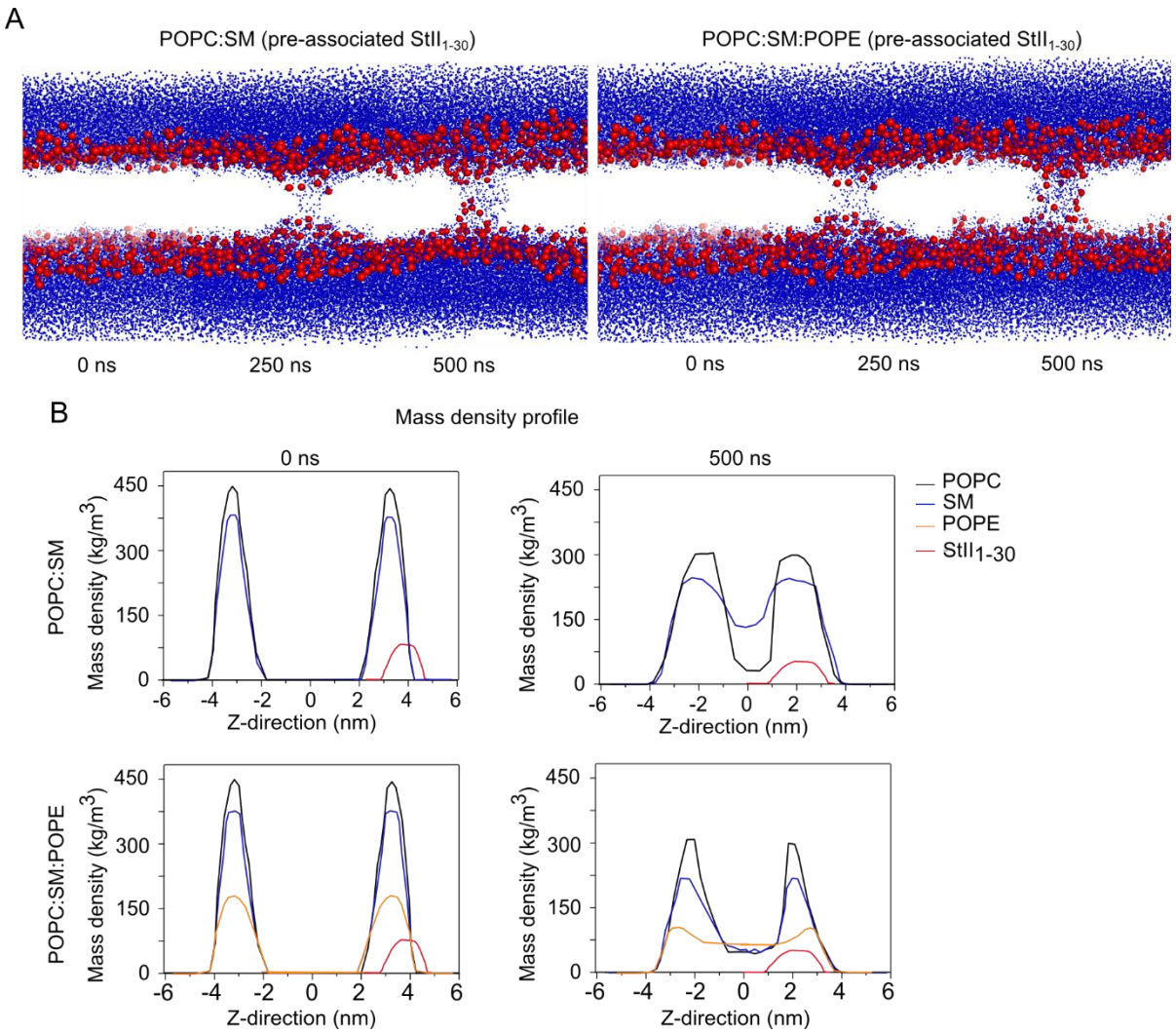

Figure S3. Membrane alterations as a consequence of pore-formation by $StII_{1-30}$. **(A)** Representative snapshots of the sequence of events in the simulations in POPC:SM **(left)** and POPC:SM:POPE **(right)** membranes. Phospholipid head groups are represented in in red and water molecules in blue. **(B)** Mass density profile of $StII_{1-30}$ and the head groups of the lipids. Values were calculated at time 0 and 500 ns in POPC:SM **(top)** or POPC:SM:POPE **(bottom)** membranes. Plots correspond to a representative profile from each simulation.

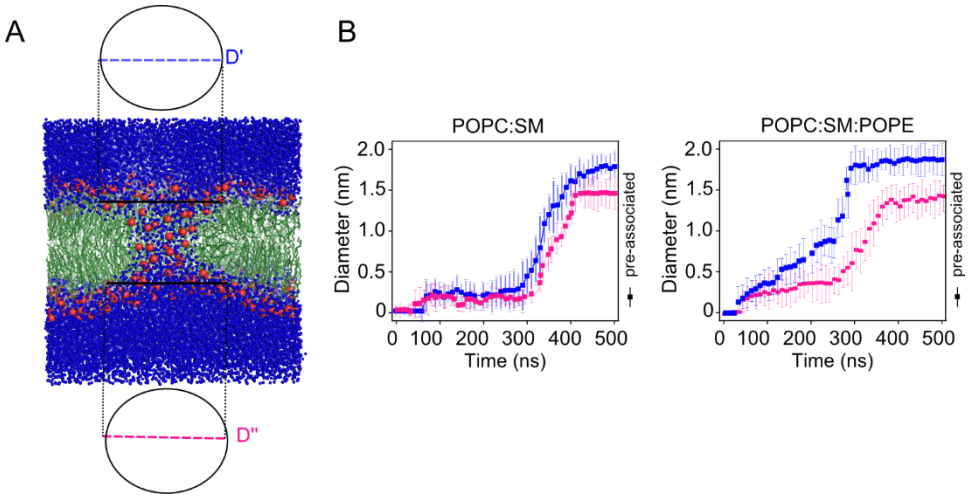

Figure S4. Determination of the pore size. **(A)** Representation of the diameter sections. D' and D'' are the diameter of the outer and inner leaflets, respectively. **(B)** Increase in size kinetics of the pore formed by pre-associated $StII_{1-30}$ in POPC:SM **(left)** or POPC:SM:POPE **(right)** membranes. The diameter of the pore was calculated as the diameter of the area fitting the phosphate groups at the lipid-water interface, in both the inner and outer monolayers. Values in **B** are shown as the mean values and the standard deviation among three replicates.

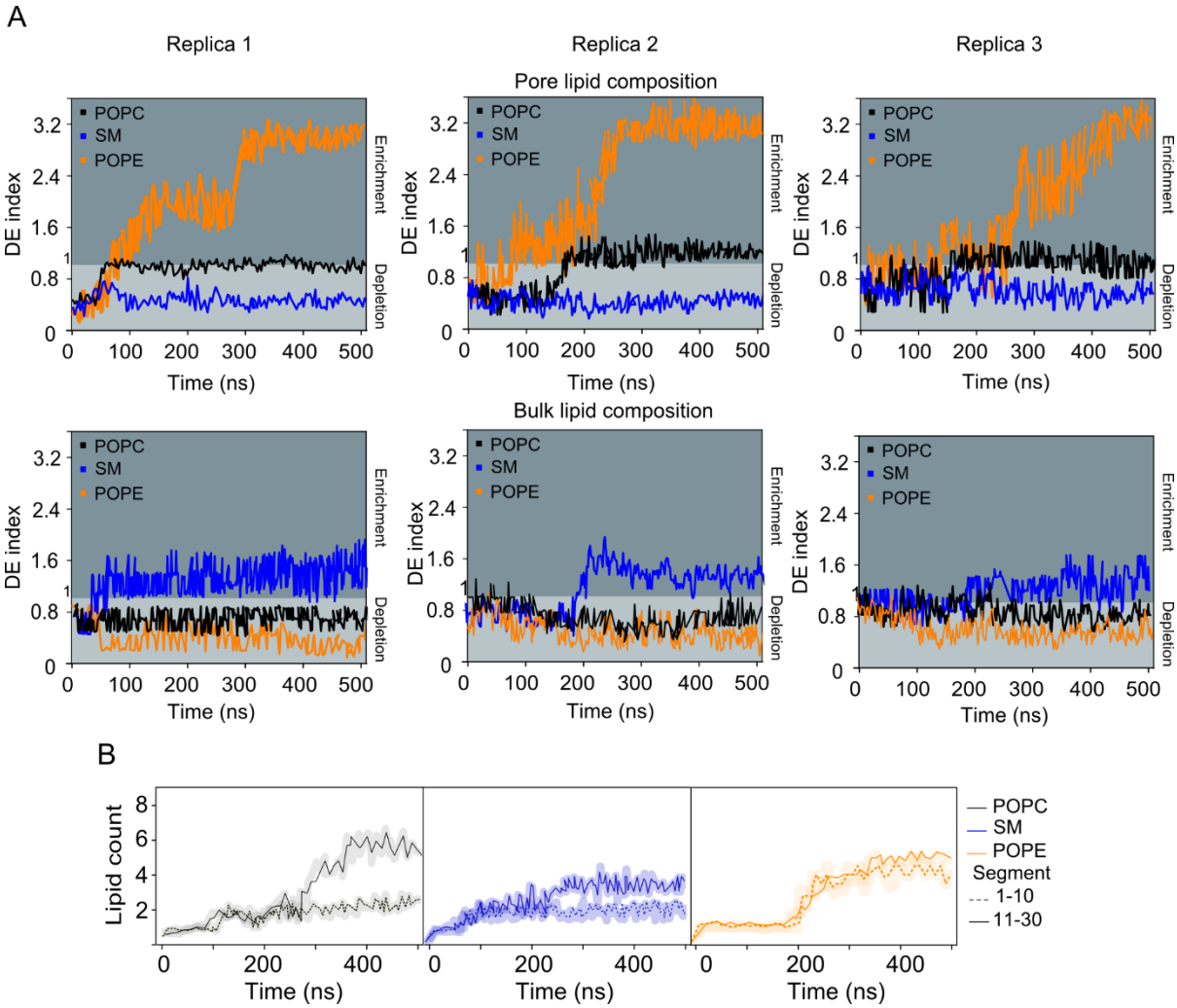

Figure S5. Lipid segregation during $StII_{1-30}$ interaction with membranes of POPC:SM:POPE. **(A)** DE index of the lipids was calculated for single replicates of the systems over time. DE was calculated at the pore region (**top**) or the bulk membrane (**bottom**). **(B)** Number of lipids that interact with unfolded (1-10) or folded (11-30) regions of $StII_{1-30}$. Contacts with the polar head of individual phospholipids were considered within a 0.5 nm distance. Dark lines in the plots represent the main values and shadow lines the standard deviation among three replicates.

Methods

Systems for the MD simulations

In solution

Peptide systems were created in an 8 x 8 x 8 nm water box in solution or 12 x 12 x 12 nm in membranes. First, the structures of $StII_{1-30}$ and StI_{1-31} were obtained from the structure of StII (PDB 1O72) (1) and StI (PDB 2KS4) (2) respectively, and equilibrated in solution for 100 ns. Then, four peptides in solution were simulated during 1 µs to allow peptide self-association. All systems in solution were created with a distance of 2 nm in the *x*-dimension and 4 nm in the *y*- and *z*- dimensions between each peptide in an 8 x 8 x 8 nm water box.

In membranes

An overview of our simulations in membranes is shown in table S4. Membrane systems were created with four peptides of $StII_{1-30}$ starting from two different initial configurations: i) monomeric or non-associated or ii) pre-associated in solution. Each peptide system was simulated in the presence of lipid membranes of different compositions: POPC:SM (50:50) and POPC:SM:POPE (50:40:10) using a box dimension of 12 x 12 x 12 nm. Lipid systems contained 240 lipid molecules, which corresponded to a peptide/lipid (P/L) ratio of approximately 1/60. Peptide molecules were added to one side of the membrane, mimicking in vitro experiments in which peptide molecules are initially added to the external monolayer of liposomes.

Table S4: Overview of the $StII_{1-30}$-membrane simulations

Composition	No. of peptides	Associated peptides	Simulation Length (ns)	Replicates/Number of simulations
POPC:SM (50:50)	4	No	500	3
POPC:SM (50:50)	4	Yes	500	3
POPC:SM:POPE (50:40:10)	4	No	500	3
POPC:SM:POPE (50:40:10)	4	Yes	500	3

Preparation of lipid vesicles

Films of POPC:SM (50:50) or POPC:SM:POPE (50:40:10) were prepared by evaporation of stock chloroform solutions using a stream of wet nitrogen and submitted to vacuum for not less than 2 h. For permeabilizing assays, MLVs were obtained by subsequent hydration in the presence of 80 mM CF, pH 7.4 (adjusted by adding NaOH) in water and subjected to six cycles of freezing and thawing. LUVs were prepared by

extruding a solution of MLVs through a two-syringe LiposoFast Basic Unit extruder (Avestin Inc., Ontario, Canada) equipped with two stacked polycarbonate filters with 100 nm pore size (Nuclepore, Maidstone, UK). To remove untrapped fluorophore, vesicles were filtered through a mini-column (Pierce, Rockford, USA) loaded with Sephadex G-50 pre-equilibrated with TBS. To study binding to liposomes, SUVs were prepared by sonication of a suspension of MLVs, prepared as described above, using an ultrasonicator (Branson 450, Danbury, USA) equipped with a titanium tip and subjected to 15 cycles of 2 min sonication with intervals of 1 min rest. Titanium particles released from the probe were removed by further centrifugation at 10 000 g for 10 min at 22 °C. Lipid concentration used are given in the text.

Supporting citations.

1. Mancheno, J. M., J. Martin-Benito, M. Martinez-Ripoll, J. G. Gavilanes, and J. A. Hermoso. 2003. Crystal and electron microscopy structures of sticholysin II actinoporin reveal insights into the mechanism of membrane pore formation. Structure 11(11):1319-1328.
2. Castrillo, I., J. Alegre-Cebollada, A. M. del Pozo, J. G. Gavilanes, J. Santoro, and M. Bruix. 2009. 1H, 13C, and 15N NMR assignments of the actinoporin Sticholysin I. Biomol NMR Assign 3(1):5-7.