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SUPPLEMENTARY INFORMATION

Detailed Material and Methods

a) Lipid vesicle composition

Experiments were performed with zwitterionic 1,2-dipalmitoylphosphatidylcholine (DPPC) cationic 1,2-dipalmitoyl-ethylphosphatidylcholine (EDPPC). EDPPC was used at 10%, 25% and 50% molar ratios.

As for viral and raft mimicking (VRM) vesicles, cholesterol to phospholipid ratio was kept at 0.83 and phospholipid composition was the following: 6.4% DPPC, 9.6% POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine), 33.1% egg-SM (sphingomyelin), 35.2% POPS (1-palmitoyl-2-oleoyl-phosphatidylserine) and 15.5% POPE (1-palmitoyl-2-oleoyl-phosphatidylethanolamine), in accordance with Brugger et al. [1]. Phospholipids were purchased from Avanti Polar Lipids (Albaster, AL, USA) and cholesterol was from Sigma (St. Louis, MO, USA).

100nm large unilamellar vesicles (LUV) were obtained by extrusion as described elsewhere [2].

b) Partition and Quenching Experiments

Partition experiments were performed as previously described [3]. Briefly, a solution of sifuvirtide was titrated with a concentrated lipid suspension to evaluate the affinity of this peptide towards vesicles by the changes on its fluorescence. Data was corrected for dilution and light scattering effects [4].

Quenching experiments were conducted according to Ref. [5, 6]. Briefly, acrylamide was used as an aqueous soluble quencher of the fluorescence of the Trp residues of sifuvirtide. Small aliquots of a 4 M solution of acrylamide were added to 15 µM peptide in the presence or absence of 3 mM lipid. To minimize the relative quencher/fluorophore light absorption ratios, the peptide was excited at 290 nm. Fluorescence emission was recorded at 360nm. Quenching data were corrected according to Coutinho and Prieto [7]. The quenching profiles were plotted using the Stern-Volmer representation.

In this experiment, a Stern-Volmer constant (K_{SV}) of 12.2±0.1 mM^{-1} was obtained for the quenching of sifuvirtide in aqueous solution by acrylamide. This value was retrieved using equation 1 [8].

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \qquad \text{Equation 1}$$

where I and I_0 are the fluorescence intensities of sifuvirtide in the presence and absence of acrylamide, while [Q] is the quencher concentration. To analyze the data in the presence of lipid vesicles, equation 2 was used, based on the Lehrer correction for the Stern-Volmer equation [9].

$$\frac{I}{I_0} = \frac{1 + K_{SV}[Q]}{1 + K_{SV}(1 - f_B)[Q]} \qquad \text{Equation 2}$$

Fixing the K_{SV} value, we were able to determine the fraction of peptide accessible to acrylamide in aqueous solution (f_B). From the f_B values we could then obtain apparent partition coefficient values (K_p) for the interaction of sifuvirtide with lipid vesicles, as described in equation 3 [6], where [L] is the lipid concentration and γ_L the molar volume of a lipid bilayer.

$$K_p = \frac{1 - f_B}{f_B} \times \frac{1}{\gamma_L[L]} \qquad \text{Equation 3}$$

c) Lipid mixing / Vesicle fusion

Lipid mixing between vesicles was evaluated using a Förster Resonance Energy Transfer (FRET) approach with NBD-PE (N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dipalmitoylphosphatidylethanolamine, from Invitrogen, Carlsbad, CA, USA) as donor and Rh-PE (rhodamine B 1,2-dipalmitoylphosphatidylethanolamine, also from Invitrogen) as acceptor molecule. Liposomes simultaneously labelled with 0.6% NBD-PE and 0.6% Rh-PE (molar ratio) and non-labelled liposomes were prepared by extrusion. Throughout this assay 100 µM lipid and 0-15 µM peptide were used. The samples were incubated for 10 min and fluorescence was recorded in the 490-650 nm range (NBD-PE and Rh-PE maximum emission peaks were 530 nm and 585 nm, respectively) with excitation at 470 nm (NBD-PE excitation maximum). Since sifuvirtide quenches NBD-PE, fluorescence intensities were corrected for this effect. Addition of Triton X-100 1% (v/v) was used as a positive control. Fusion efficiencies were calculated in accordance to Perez-Berna et al. [10], using equation 4. R, R_0 and $R_{100\%}$ correspond respectively to the NBD-PE/Rh-PE fluorescence intensity ratios in the presence of a given amount of peptide, absence of peptide and presence of Triton X-100.

$$\%\ \text{Fusion Efficiency} = \frac{R - R_0}{R_{100\%} - R} \qquad \text{Equation 4}$$

In the evaluation of the lipid mixing propensity of DPPC:EDPPC vesicles in the presence of sifuvirtide, non-labelled and labelled vesicles were kept at a 3:1 molar ratio.

For VRM vesicles, first we evaluated lipid mixing of 100 µM lipid vesicles (labelled and unlabelled vesicle ratio kept at 1:1) in the presence of sifuvirtide (0-15 µM). Then, DPPC:EDPPC (1:1) vesicles pre-incubated with 0-15 µM peptide were added to those samples. Total lipid concentration was kept at 100 µM and the ratio between VRM and DPPC:EDPPC membranes was 2:1.

d) Lipid mixing / Vesicle fusion

The location of sifuvirtide after presentation by DPPC:EDPPC (1:1) to VRM vesicles was performed using a FRET approach. We used the Trp residues of sifuvirtide as energy transfer donors and 1,6-diphenyl-1,3,5-hexatriene (DPH, from Invitrogen) in the VRM membranes as the acceptor molecule. The experiment was designed using the same lipid concentrations and DPPC:EDPPC (1:1) to VRM vesicle proportions used on the lipid mixing assays. Peptide concentration was 10 µM and viral-mimicking vesicles presented 0% or 2% DPH (molar ratio). Excitation was performed at 280 nm (Trp maximum excitation) and emission was recorded between 300-550 nm. Controls in the absence of peptide, of both DPPC:EDPPC (1:1) and viral-micking vesicles and with DPH-unlabelled vesicles were performed. DPH emission occurs on the 400-500nm range. Data was presented as normalized differential emission spectrum of sifuvirtide in the presence lipid vesicles subtracted to sifuvirtide spectrum in aqueous solution.

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