**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% POP (1-palmitoyl-2-oleoyl-phosphatidylserine) and 15.5% POPE (1-palmitoyl-2-oleoyl-phosphatidylethanolamine), in accordance with Brugger et al. Phospholipids were purchased from Avanti Polar Lipids (Albaster, AL, USA) and cholesterol was from Sigma (St. Louis, MO, USA).

**b) Partition and Quenching Experiments**

Partition experiments were performed as previously described. Briefly, acrylamide was used as an aqueous soluble quencher 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 fluorophore light absorption ratios, the peptide was excited at 290 nm. Fluorescence emission was recorded in the 490-650 nm range (NBD-PE and Rh-PE maximum emission peaks) with excitation at 470 nm and presence of Triton X-100.

**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).

% Fusion Efficiency = \( \frac{R - R_0}{R_{100\%} - R} \) \hspace{1cm} (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-mimicking 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.

References