Supporting Information

SNARE derived peptide mimic inducing membrane fusion

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Content

1. Materials and Methods

Solvents were of the highest grade available. Acetonitrile (HPLC-grade) was obtained from *FisherScientific GmbH* (Nidderau, Germany). Ultra pure water was derived using a water purification device *Simplicity* (*Millipore*, Bedford, UK). All reagents were of analytical grade and used as supplied. All amino acid derivatives as well as coupling reagents and the resins for solid phase synthesis were obtained from *NovaBiochem* (Darmstadt, Germany), *IRIS Biotech* (Marktredwitz, Germany), *GL Biochem* (Shanghai, China), *Bachem* (Bubendorf, Switzerland), *Merck* (Darmstadt, Germany), *ABCR* (Karlsruhe, Germany). All other chemicals were purchased from *Sigma-Aldrich* (Taufkirchen, Germany), *Fluka* (Taufkirchen, Germany), *Acros Organics* (Geel, Belgium), *Merck*, *Lancaster* (Karlsruhe, Germany), *Alfa Aesar* (Karlsruhe, Germany) and *ABCR* in analytical grade. ESI-MS data were obtained with a *Finnigan* instrument (type *LGC* or *TSQ 7000*) or *Bruker* spectrometers (types *Apex-Q IV 7T* and *micrOTOF API*). High resolution spectra were obtained with the *Bruker Apex-Q IV 7T* or the *Bruker micrOTOF*, respectively.

Concentrations of peptide solutions were determined via UV absoprtion using a *NanoDrop ND-2000c* spectrophotometer (*Thermo Scientific*, Bremen, Gemany).

2. Peptide synthesis and purification

The peptides were synthesized via Fmoc solid phase peptide synthesis on preloaded *Wang*-resins (0.1 – 0.3 mmol g^{-1}). Chain elongation was performed on the microwave assisted automatic peptide synthesizer LibertyTM (CEM Corporation, Matthews, NC) with an additional module of *DiscoverTM* (CEM Corporation, Matthews, NC) that combines microwave energy at 2.450 MHz. Fmoc deprotection was performed with 20% piperidine in NMP. Fmoc amino acids were prepared as 0.2 M solutions in NMP. Coupling reactions were performed with 0.5 M HBTU/HOBt (5 equiv) in DMF, 0.2 M amino acids in NMP (5 equiv) and of 2 M DIPEA (10 equiv) in NMP. To enhance the coupling efficiency, couplings were performed twice. Capping was done for every cycle using a solution of acetic anhydride (10%), DIPEA (5%) and HOBt (0.2%) in NMP. Deprotection, coupling and capping reactions were performed with microwave energy and N_2 mixing. The microwave cycle was characterized by two deprotection steps (30 seconds, 180 seconds). All microwave coupling reactions except for arginine and cysteine were of 300 seconds at 75°C. Arginine was

coupled under N_2 mixing (1500 s) at room temperature followed by microwave assisted coupling time (300 s) at 75°C. The conditions for cysteine were equal to the standard conditions, but the maximum temperature was 50°C for every microwave assisted step. For the peptide syntheses the following amino acid building blocks have been used: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Val-OH. After coupling of the last amino acid the resin was washed thoroughly with DCM, MeOH and diethylether and dried over night. Cleavage from the solid support was carried out using TFA/water/EDT/TES (v/v/v/ v = 94/2.5/2.5/1, 10 mL/g resin) within 2 h. The resin was filtered off and the solution was concentrated by using a flow of N_2. The peptides were precipitated from cold MTBE and centrifuged. The supernatant was discarded and the peptide pellet was washed with MTBE several times, centrifuged and lyophilized.

HPLC analysis was performed using a *Pharmacia Äkta Basic* (*GE Healthcare*, Uppsala, Sweden) with a pump type P900, variable wavelength detector UV-900 using a linear gradient of A (0.1% TFA in H_2O) to B (0.1% TFA in MeCN/H_2O 8/2). Peptides were analyzed using a *YMC* column ODSA, RP-C18, 250 × 4.6 mm, 4 µm, 80 Å, with a flow rate of 1 mL·min^{-1}. Preparative purification was performed using a *YMC* column ODS-A, RP-C18, 250 × 20 mm, 5 µm, 120 Å, with a flow rate of 10 mL·min^{-1}.

Fluorescence labeling was performed on the solid support using Texas Red®-X succinimidyl ester (TxR; *Invitrogen*, Paisley, UK). A solution of the dye (5 eq) and *N,N*-diisopropylethylamine (5 equiv) was dissolved in dimethylformamide and added to the resin. After 24 h of shaking at room temperature under exclusion of light, the resin was thoroughly washed and the peptide was cleaved like mentioned above.

Transmembrane peptides were difficult to purify. First experiments with unpurified peptides showed only specific fusion so that all further experiments were performed without purification after cleavage from the resin. Such an approach has been reported elsewhere.[1]

3. Protein constructs and purification

The SNARE proteins from rat (*Rattus norvegicus*) were heterogenously expressed in *Escherichia coli*. Protein expression, purification and the reconstitution in vesicles was performed as reported.[2,3]

4. Synthesized peptides (analytical data)

K3-syntaxin [$C_{279}H_{479}N_{73}O_{66}S_3$, 6008.4]

H-WWG(KIAALKE)$_3$QSKARRKKIMIIICCVILGIIIASTIGGIFG-OH

ESI-MS: m/z = 668.52 $[M+8H]^{8+}$, 859.24 $[M+7H]^{7+}$, 1202.53 $[M+5H]^{5+}$

HR-MS: calcd. $[M+7H]^{7+}$ 858.8006, found 858.7999

E3-VAMP2 [$C_{288}H_{473}N_{65}O_{71}S_3$, 6078.4]

H-G(EIAALEK)$_3$RKYWWKNLKMMIILVICAIILIIIIVYFST-OH

ESI-MS: m/z = 1013.93 $[M+5H]^{5+}$

HR-MS: calcd. $[M+6H]^{6+}$ 1013.5838, found 1013.5851

peptide E3' [$C_{106}H_{182}N_{26}O_{36}$, 2396.79]

H-G(EIAALEK)$_3$G-OH

HPLC (C18, gradient = 30-80% B in 30 min): t_R = 22.82 min.

ESI-MS: m/z (%) = 2397.33 $[M+H]^+$ (100), 799.78 (82) $[M+3H]^{3+}$, 1199.17 (20) $[M+2H]^{2+}$

HR-MS: calcd. 799.4476, found 799.4473

peptide Texas Red-E3 [$C_{143}H_{221}N_{29}O_{43}S_2$, 3098.55]

TxR-G(EIAALEK)$_3$G-OH

HPLC (C18, gradient = 40-80% B in 30 min): t_R = 17.76 min.

ESI-MS: m/z = 3098.57 $[M+H]^+$

K3-VAMP [$C_{106}H_{182}N_{26}O_{36,}$ 6390.13**]**

H-(KIAALKE)$_3$GWKRKYWWKNLKMMIILGVICAIILIIIIVYFST-OH

ESI-MS: m/z = 931.83 $[M+7H]^{7+}$, 1065.98 $[M+8H]^{8+}$

HR-MS: calcd. 1065.4712, found 1065.4722

E3-Syntaxin [$C_{263}H_{453}N_{67}O_{72}S_3$, 5802.14]

H-G(EIAALEK)$_3$YQSKARRKKIMIIICCVILGIIIASTIGGIFG-OH

ESI-MS: m/z = 967.89 $[M+6H]^{6+}$, 1161.27 $[M+5H]^{5+}$

5. Vesicle preparation

Vesicles were prepared from chloroform stock solutions of a 5:2:2:1 molar ratio of phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl serine (all from pig brain) and cholesterol (*Avanti Polar Lipids*, Alabaster, USA). Removal of solvents was carried out in a N_2 stream followed by 12 h under reduced pressure. The lipid film was rehydrated in buffer (20 mM HEPES, 150 mM KCl, pH = 7.4, used in all experiments) and sodiumcholate as detergent. Peptides were dissolved in buffer containing 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS, 5%) as detergent. The concentration of the peptide solution was determined via UV-280 absorption and added to the micellar lipid solution. Removal of the detergent was performed using *Sephadex* G-50 Superfine (*GE Healthcare*, Buckinghamshire, UK) resulting in < 100 nm-sized small unilamellar vesicles (SUVs).

Vesicles for content mixing experiments were prepared via extrusion.[4] Lipids and cholesterol were dissolved in $CHCl_3$ (about 20 $mg \cdot mL^{-1}$), mixed with peptide stock solutions in trifluoroethanol with a defined protein to lipid-ratio. Removal of the organic solvents was carried out in a N_2 stream followed by 12 h under reduced pressure. The lipid film was rehydrated and the milky multilamellar vesicle suspension was extruded 31 times through a polycarbonate membrane (100 nm nominal pore size) using a *Liposofast* mini-extruder (*Avestin*, Ottawa, Canada) to produce an almost clear vesicle suspension containing vesicles of 100 nm diameter. For preparation vesicles with encapsulated sulforhodamine B (*Sigma Aldrich*, St. Luis, USA), the lipid film was rehydrated with buffer containing 20 mM of the dye, extruded like mentioned before and followed by removal of excess dye by size exclusion chromatography. (*Sephadex* G-50 Superfine).

6. Fluorescence experiments

Time-dependent fluorescence spectra were recorded on a *Fluorolog* (Horiba) or a *Fluoromax* (Horiba) at 20 °C with 1 nm bin width at a final volume of 1.2 mL in a quartz cuvette (Quartz Suprasil, *Hellma*, Mühlheim, Germany) under magnetic stirring. Lipid mixing experiments were performed with the FRET pair 1,1'dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD, 1.5 mol% of total lipids, acceptor) and oregon green 488 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (OG-DHPE, 1.5 mol%, donor) (*Invitrogen*, Paisley, UK). The excitation wavelength was 496 nm (1 nm slit) and the emission wavelength was 670 nm (5 nm slit). The total lipid concentration of each population was 80 µM and the molar peptide/lipid ratio was 1:2000 (Figure 2) and 1:1000 (Figure 3). The change of fluorescence signal (acceptor) was monitored over time.

Content mixing experiments were performed with sulforhodamine B encapsulated in one vesicle population. A solution of peptides in trifluoroethanol was mixed with lipids dissolved in methanol providing a molar peptide/lipid ratio of 1:200. After removal of the solvent, the lipid/peptide film was rehydrated in buffer solution containing 20 mM sulforhodamine B (SRB). The excitation wavelength was 495 nm (1 nm slit) and the emission wavelength was 515 nm (5 nm slit). SRB vesicles in a total lipid concentration of 40 µM were mixed with 120 µmol of unlabeled vesicles. The change of the fluorescence signal was recorded for 30 min followed by an addition of Triton-X100 (0.1%, w/v). The normalized SRB fluorescence over time $F_{\%}(t)$ is plotted as:

$$F_{\%}(t) = 100 \times (F(t) - \mathrm{F}(0)) / (F_{\mathrm{total}} - F(0))$$

with $F(0)$ the SRB intensity at t = 0 prior to content mixing and F_{total} the total SRB fluorescence after full membrane lysis of the vesicles with Triton X100.

For the fluorescence anisotropy measurements, TxR-labeled peptide was excited at 590 nm and the emission measured at 610 nm. First the G factor was determined according to $G = I_{HV}/I_{HH}$. Subsequently, the anisotropy (r) was determined according to $r = (I_{VV} - G \times I_{VH}) / (I_{VV} + 2 \times G \times I_{VH})$, where I is the fluorescence intensity, and the first and second subscript letter indicate the polarizations of the excitation light and emission mirrors, respectively. Fluorescence anisotropy reports local conformational flexibility of the labeled residue and is a measure of mobility. The anisotropy changes upon complex formation or dissociation.[2]

7. Fluorescence anisotropy

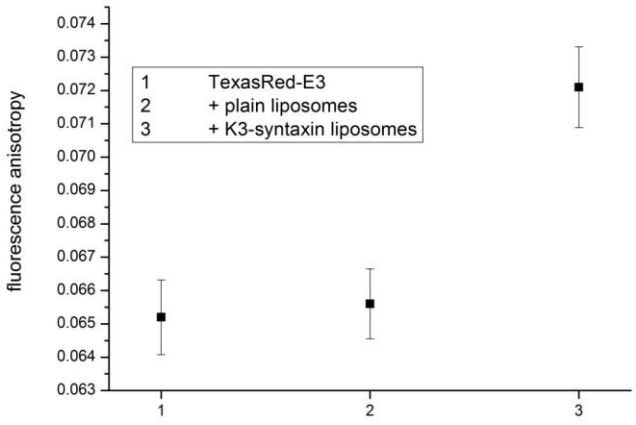

Fig S1: Fluorescence anisotropy of fluorescently labeled peptide E3 (8 µMl) was measured over a time period of 100 seconds with 2 s binning. Upon addition of plain vesicles (40 µM lipids) no significant change in anisotropy was observed. In contrast, vesicles containing K3-syntaxin (40 µM lipids) resulted in an increase of the anisotropy. This indicated specific binding of the E3'-peptide to the K3-syntaxin in the vesicles. Error bars represent the standard deviation of the fluorescence anisotropy over 100 seconds.

8. Comparison E3-VAMP2/K3-syntaxin and K3-syntaxin/E3-VAMP2

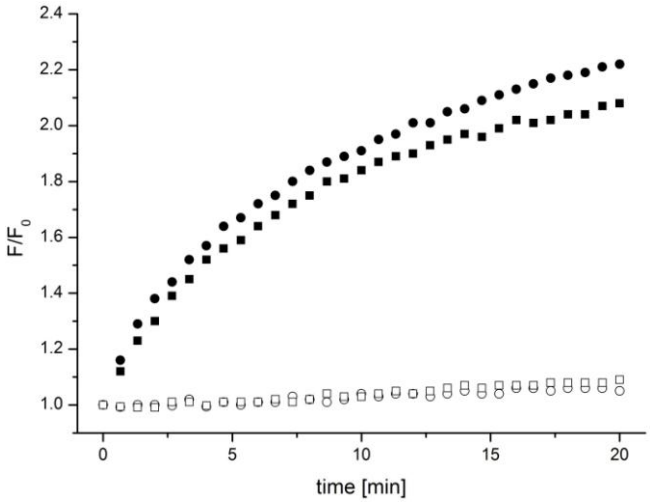

Fig. S2: FRET experiment with vesicles containing E3-VAMP2/K3-syntaxin (■) and K3-VAMP2/E3-syntaxin (●). An increase of fluorescence indicates lipid mixing. Addition of the non membrane-anchored peptide E3' to vesicle incorporated K3-syntaxin (□) or K3-VAMP2 (○) results in only little increase of fluorescence. Fluctuations in the increase of fluorescence among various systems may result from small differences in the concentrations.

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2. A. V. Pobbati, A. Stein, D. Fasshauer, *Science*, 2006, **313**, 673.
3. G. van den Bogaart, M. G. Holt, G. Bunt, D. Riedel, F. S. Wouters, R. Jahn, *Nat. Struct. Mol. Biol.* 2010, **17**, 358.
4. P. E. Schneggenburger, S. Müllar, B. Worbs and U. Diederichsen, *J. Am. Chem. Soc.*, 2010, **132**, 8020.