Supporting Information

Dual-Fluorescence *L*-Amino Acid Reports Insertion and Orientation of Melittin Peptide in Cell Membranes

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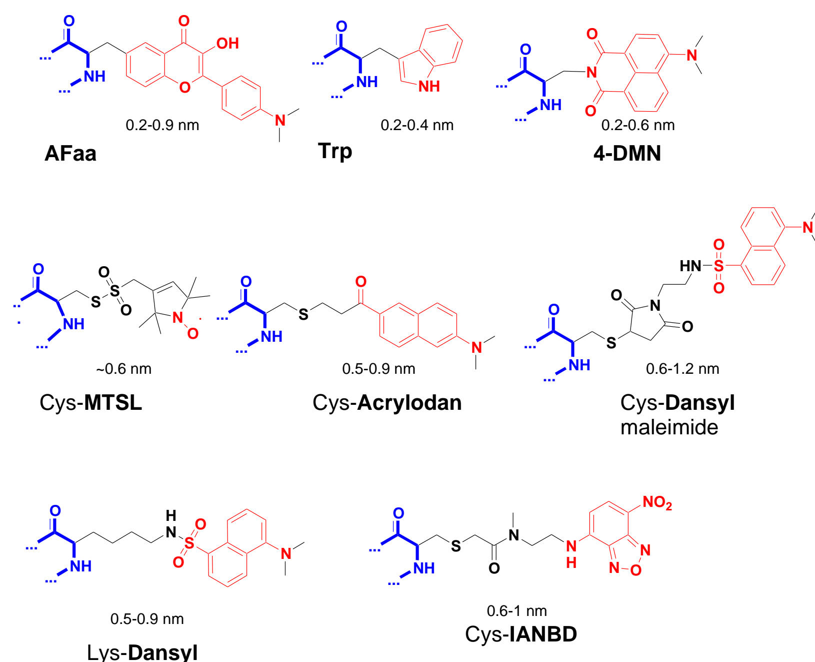

Figure S1. Distance from the peptide backbone (blue) to the environment-sensitive center (red) for AFaa, tryptophan, and some frequently used environment-sensitive peptide labels. Numbers are the approximate distances from Cα to the closet and furthest atom of fluorophores.

Proof of enantiomeric purity.

To check the enantiomeric purity of Boc-protected amino acid **4** it was coupled with R and **S** α-phenylethylamine and obtained diastereomeric amides (Fig. S2) were checked by ^{1}H-NMR (Fig. S3).

For the preparation of amides of protected amino acid **4** and enantiomeric R and **S** α-phenylethylamine was adopted common coupling procedure of peptide synthesis, in which HBTU/HOBt (1.25 eq.) solution and DIEA (2eq.) were used.

(R,S)-diastereomer. A mixture of 50 mg (0.1067mmol) of N-Boc-aminoacid **4**, 40 μL(3 eq.)of (R)-α-phenylethylaminein 100 μL of NMP, 55.76 μL (3eq.) of diisopropylethylamine and 0.9 mL (1.5 eq.) of solution of HBTU/HOBt (2/1) in NMP were stirred for 3h in the Ar-atmosphere. Then to the reaction mixture 3 ml of water was added. Orange precipitate was washed with water, dried and used for ^{1}H-NMR and mass spectroscopy. Yield of crude compound was 35.6 mg. Mass spectroscopy, ion polarity – positive, m/z: 572.2 $[M+H]^{+}$.

(S,S)-diastereomer was prepared by the same procedure as, utilizing (S)-α-phenyl-ethylamine. Yield of crude compound was 38.3 mg. Mass spectroscopy, ion polarity – positive, m/z: 572.2 $[M+H]^{+}$.

The racemization was estimated based on the signal intensities of amide protons (Fig. S3).

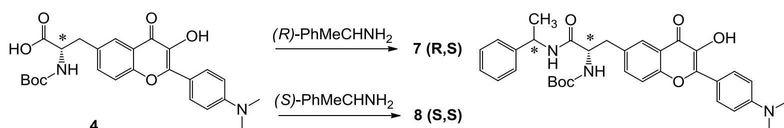

Figure S2. Synthesis of enantiomeric amides of 4.

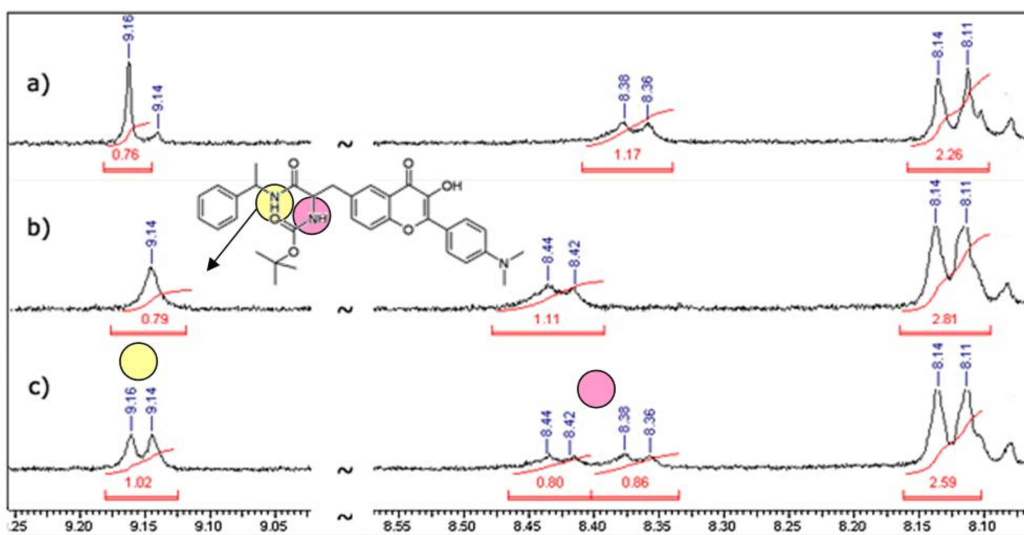

Figure S3. NMR spectra of enantiomeric amides 7(a), 8 (b) and the mixture (c).

Table S1. Spectral properties of AFaa and MFL labels[a]

Labeling	Media	QY, %	λABS, nm	λN*, nm	λT*, nm
N-MFL	Buffer	1	416	541	
	DOPC	37	407	492	560
	DOPC/Ch	45	405	473	560
L9	Buffer	6	401	550	
	DOPC	37	401	501	567
	DOPC/Ch	28	397	495	561
W19	Buffer	3	405	524	
	DOPC	34	405	514	568
	DOPC/Ch	24	400	507	565

[a]Spectra in buffer are after complete dissociation of melittin oligomers. DOPC/Ch is DOPC:cholesterol (6:4) LUVs. Conditions are the same as for spectra on Fig. 5.

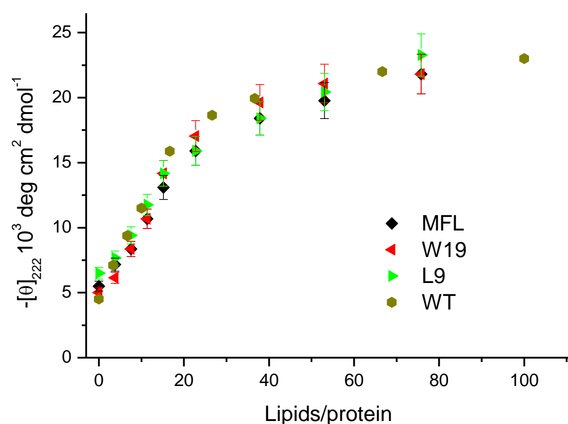

Figure S4. Conformation changes upon the titration of melittin variants by POPC vesicles.

Circular dichroism measurements. CD spectra were obtained with a Jasco J-720 spectropolarimeter. The melittin concentration was 10 µM. Spectra were recorded using a 2 mm pathlength cuvette, scanning from 250 to 200 nm with a step size of 0.2 nm and a scanning speed of 20 nm/min. For the determination of binding the 222±0.2 nm the signal was averaged and corrected for dilution (222nm is characteristic band for the α-helical conformation). Titration by POPC SUVs was performed in 10 mM Na-PO4 buff at 25±1°C.