

Contents

1	Supplementary Figures.....	2
2	General Methods	6
2.1	Solvents and Reagents.....	6
2.2	High-Performance Liquid Chromatography (HPLC)	6
2.3	Melting Curve Analysis	7
2.4	Circular Dichroism Spectroscopy	7
3	Alanyl-PNA Monomer Synthesis.....	8
4	Peptide Synthesis.....	9
4.1	Synthetic Procedures	9
4.2	Peptide Sequences and Analytical Data	10
5	Fusion Assays.....	22
5.1	Preparation of Liposomes.....	22
5.2	TLM Assays	23
5.3	ILM Assays	24
5.4	Control Experiments	26
5.5	Statistical Calculations	26
6	FCCS Assays.....	27

1 Supplementary Figures

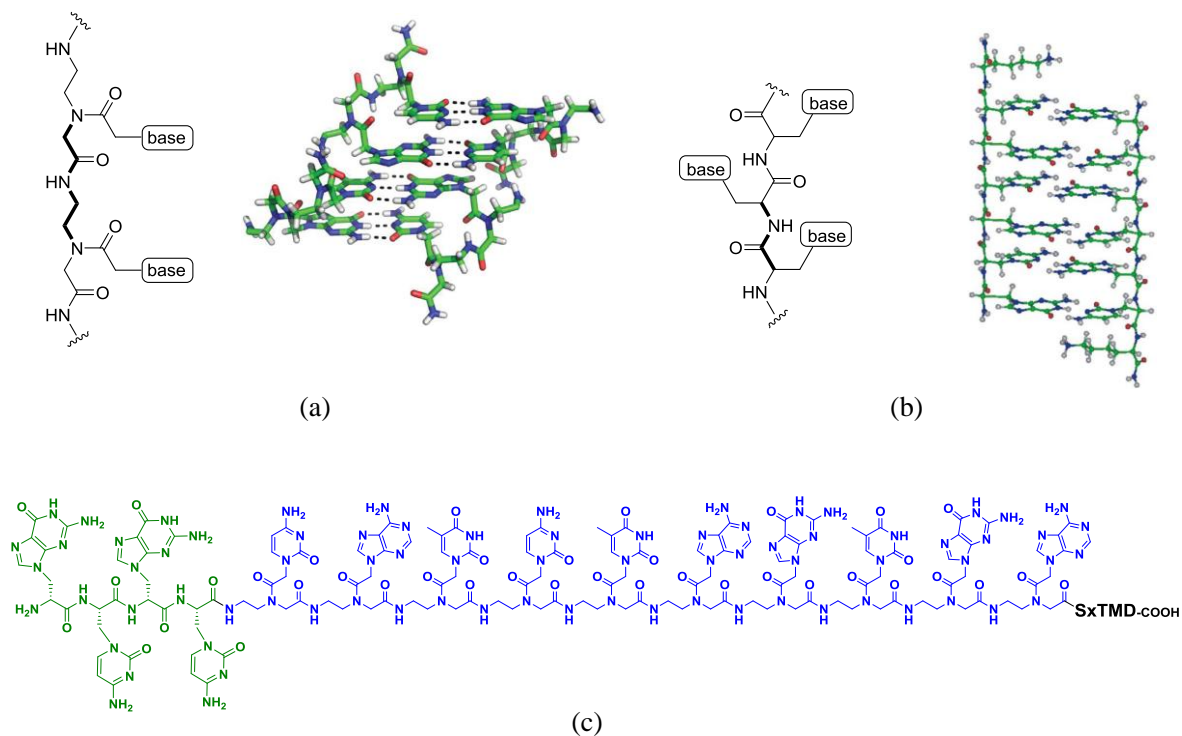


Figure S1. Backbone structure and topologies of aeg-PNA and ala-PNA duplexes. aeg-PNA and ala-PNA differ in the backbone structure which results in a different duplex topology. In aeg-PNA (a), each nucleobase, which is covalently attached via a methylene carbonyl linker to the poly-*N*-(2-aminoethyl)glycine backbone, is six backbone bonds apart from the next nucleobase (see bonds highlighted in black). The displacement of the nucleobases relative to the helical axis is 8.3 Å.^[1] The aeg-PNA duplex is helical because the strands are twisted to reach an optimal stacking distance.

The nucleobases in ala-PNA (b), which are attached to the C-atom of the alanyl side chain, are only three bonds apart from each other. If the monomers feature a D,L-alternating configuration, all nucleobases on a single strand have a distance of 3.6 Å and point towards the same direction.^[2] With this, ala-PNA single strands form a duplex with linear topology, because the nucleobase–nucleobase distance is close to the optimal stacking distance of 3.4 Å, obviating the need for strand helicalization.

The structure of the recognition unit of model peptide **4** is shown in (c) as an example to illustrate how the nucleobases are attached to the PNA backbones and how the two PNA types are connected in the PNA hybrid recognition unit. SxTMD = Syntaxin-1A (256-288).

The illustration of the aeg-PNA duplex in (a) was taken from Ref. [3] and the illustration of the ala-PNA duplex in (b) was taken from Ref. [4].

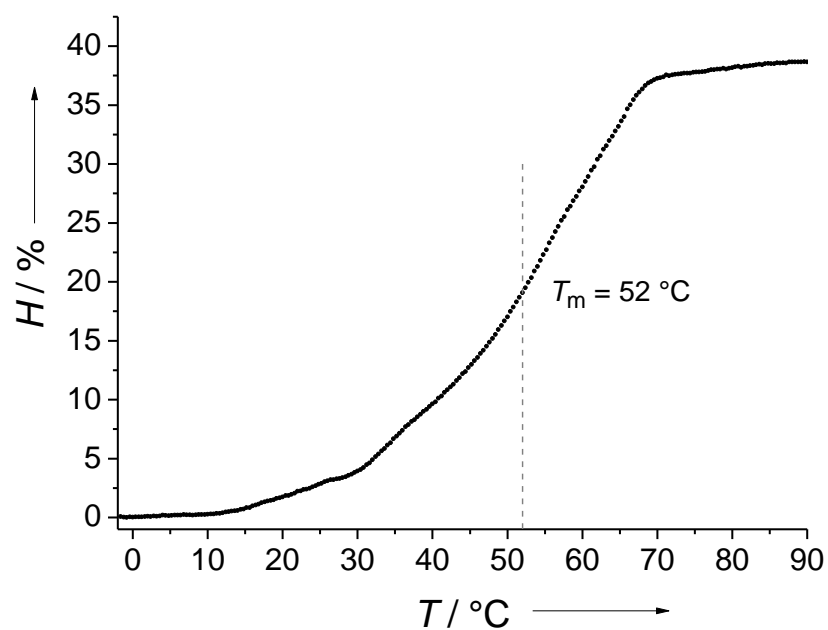


Figure S2. Melting curve analysis of ala-aeg-PNA hybrid oligomers **3+4** (without TMD sequences). A representative example melting curve is shown (recorded from the second heating phase). The average melting temperature was determined from 3 independent measurements to be (51 ± 3) °C. For technical details, see Section 2.3 in the Supporting Information.

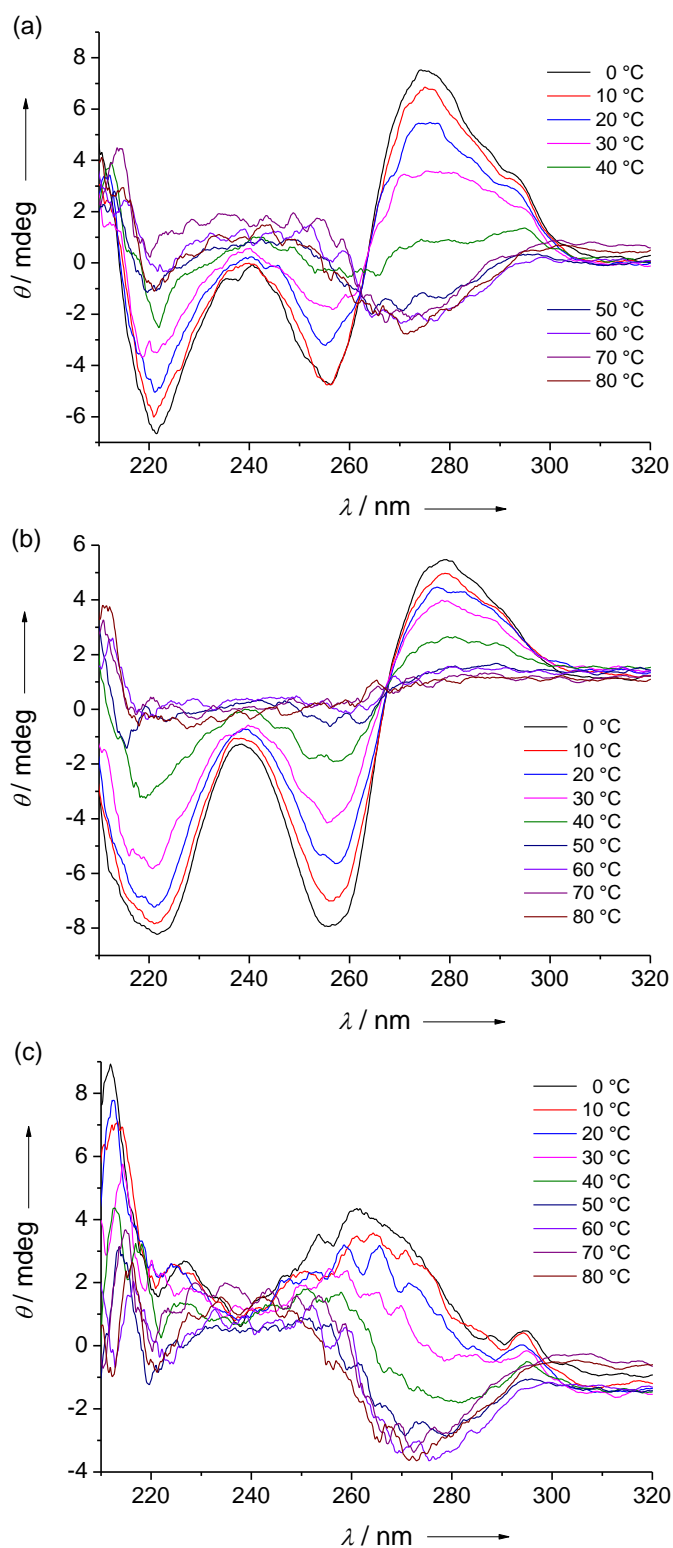


Figure S3. Circular dichroism (CD) spectra of ala-aeg-PNA oligomers **3+4** (a) and aeg-PNA oligomers **1+2** (b), at different temperatures (oligomers were used without the TMD sequences). The difference spectrum ((a) - (b)) is shown in (c). It is very similar to the reported spectra of pure ala-PNA oligomers.^[2] With this, it becomes clear that ala-PNA is contributing to PNA hybrid oligomer duplex formation. The concentration of the oligomers was 13.5 μ M (in phosphate buffer, 10 mM Na_2HPO_4 , 100 mM NaCl, H_3PO_4 , pH = 7). The presented spectra were calculated as the mean spectra from three measurements and were smoothed by using a Savitzky–Golay filter (100 points).

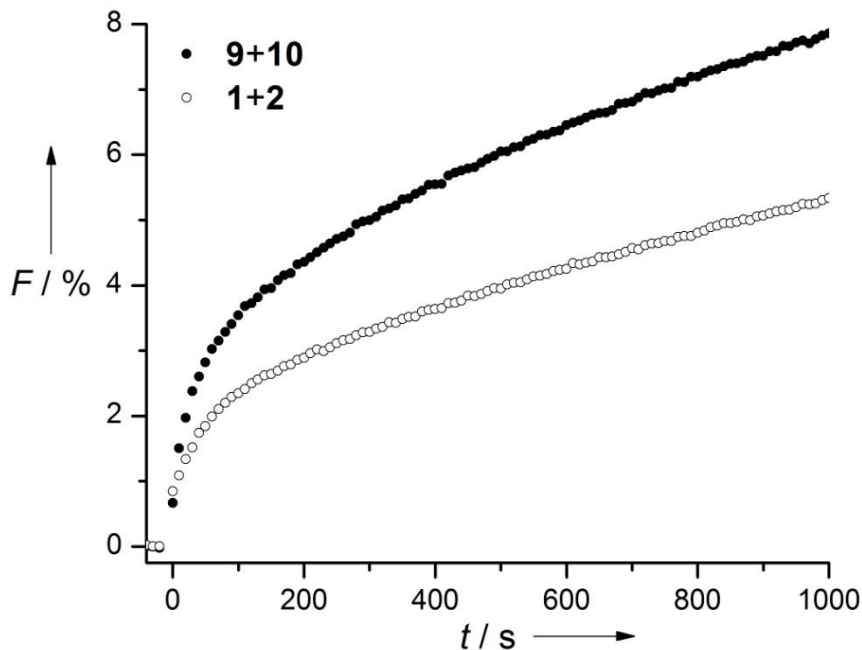


Figure S4. Comparison of the TLM efficiency of model peptides with a decameric aeg-PNA recognition sequence (**1+2**) to those with a pentameric aeg-PNA sequence (**9+10**). The graphs of two single measurements from the same day are shown. The peptide-to-lipid ratio was 1:1000.

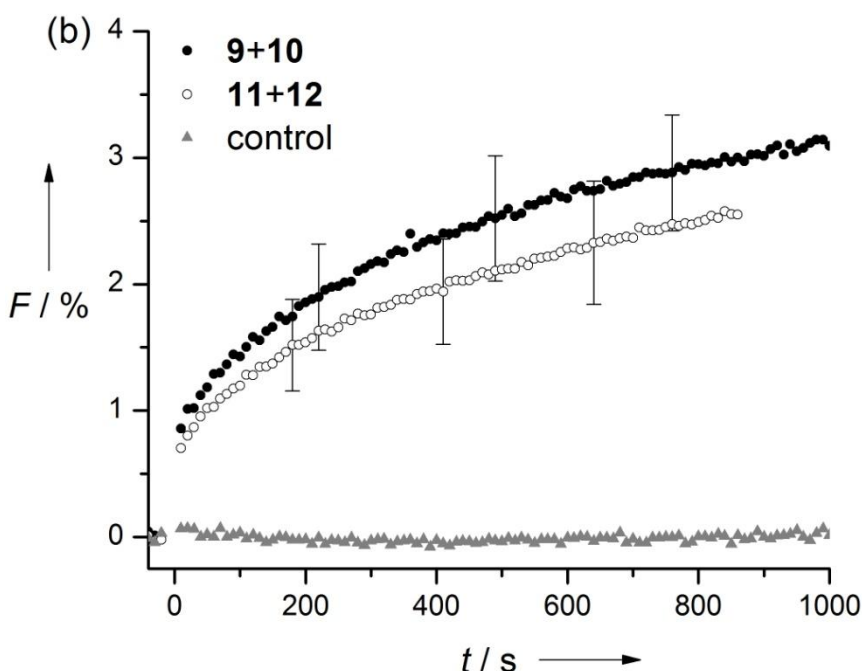


Figure S5. Inner leaflet mixing (ILM) assays performed with aeg-PNA analogues **9+10** and ala-aeg-PNA analogues **11+12**. The graphs represent the mean fusion curves obtained from at least three independently prepared assays. Controls were performed with labeled liposomes containing peptide **10** and unlabeled liposomes without peptides. The peptide-to-lipid ratio was 1:200 because at a ratio of 1:1000, which was used in the TLM assays, donor emission could not be detected reliably.

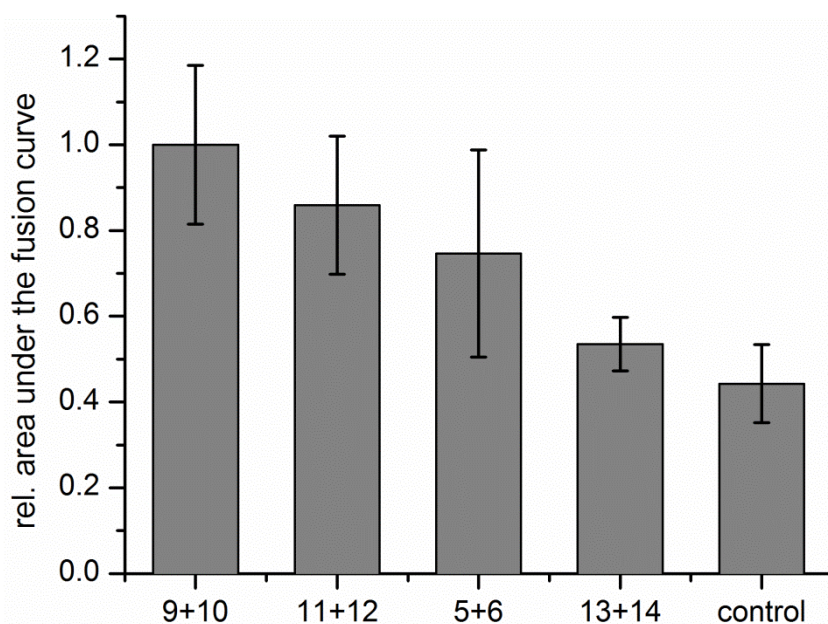


Figure S6. Comparison of the area under the fusion curves within the first 600 s. Relative values with reference to the system containing peptides **9+10** are shown. Values were obtained by calculating the integral of the mean fusion curves and the errors shown in Figures 3b and 3c.

2 General Methods

2.1 Solvents and Reagents

All applied solvents were of the grade pro analysi. Solvents for HPLC applications were of HPLC grade. Ultrapure water was obtained by purifying demineralized water with an arium mini (Sartorius, Göttingen, Germany) lab water system. Commercially obtained reagents were of the highest purity grade available. PNA monomers were obtained from ASM Research Chemicals (Hannover, Germany). Resins for solid-phase peptide synthesis were obtained from Novabiochem (Merck KGaA, Darmstadt, Germany). Amino acids were obtained from GL Shanghai Ltd. (Shanghai, China) or from IRIS Biotech GmbH (Marktredwitz, Germany). Lipids labeled with Oregon Green 488 (OG) and Texas Red (TR) were obtained from molecular probes (Eugene, USA); all other lipids were supplied by Avanti Polar Lipids, Inc. (Alabaster, USA).

2.2 High-Performance Liquid Chromatography (HPLC)

HPLC was performed using an HPLC system by JASCO (Tokyo, Japan), equipped with an *MD-2010Plus* detector and two *PU-2010Plus* pumps. Alternatively, an *Äkta basic 10* system by Amersham Pharmacia Biotech (Umeå, Sweden) equipped with a *UV-900* detector and two *P-900* pumps was used. Peptides were dissolved in small amounts of water and acetonitrile and injected after filtration. The flow was 1 mL/min for analytical runs, and 3 mL/min for

semipreparative runs. The following solvents were used: H₂O + 0.1% TFA (solvent A), acetonitrile + 0.1% TFA (solvent B1) and acetonitrile/H₂O = 8:2 + 0.1% TFA (solvent B2). A *Nucleodur* column by Macherey-Nagel (Düren, Germany) was used (RP-C18-ec, particle size: 5 µm, pore size: 100 Å, column length: 250 mm, column diameter: 4.6 mm (analytical), 10 mm (semipreparative)). HPLC was performed at room temperature.

2.3 Melting Curve Analysis

Experiments were carried out on a *V-550* spectrometer from JASCO (Tokyo, Japan) equipped with an *ETC-505T* temperature control unit. The cell was permanently purged with nitrogen. Quartz glass cuvettes by Hellma Analytics (Müllheim, Germany) with a path length of 10 mm were used. The sample volume was 500 µL. To avoid evaporation of the sample during heating, the sample surface was covered with a few drops of paraffin oil before capping the cuvette. The concentration of the oligomers was 4 µM (in phosphate buffer, 10 mM Na₂HPO₄, 100 mM NaCl, H₃PO₄, pH = 7). UV absorbance was measured at 260 nm. Each data point is the mean of 3 values measured in a row. The following temperature program was applied (based on a description in Ref. [5]): 1. sample conditioning: 24 °C → 80 °C (2.75 °C/min), 80 °C → 0 °C (4 °C/min), 0 °C (equilibration for 240 min); 2. data collection: 0 °C → 90 °C (0.5 °C/min), 90 °C → 0 °C (0.5 °C/min), 0 °C (equilibration for 240 min), 0 °C → 90 °C (0.5 °C/min), 90 °C → 0 °C (0.5 °C/min). The hyperchromicity *H* was calculated according to

$$H = \frac{A(T) - A_0}{A_0}$$

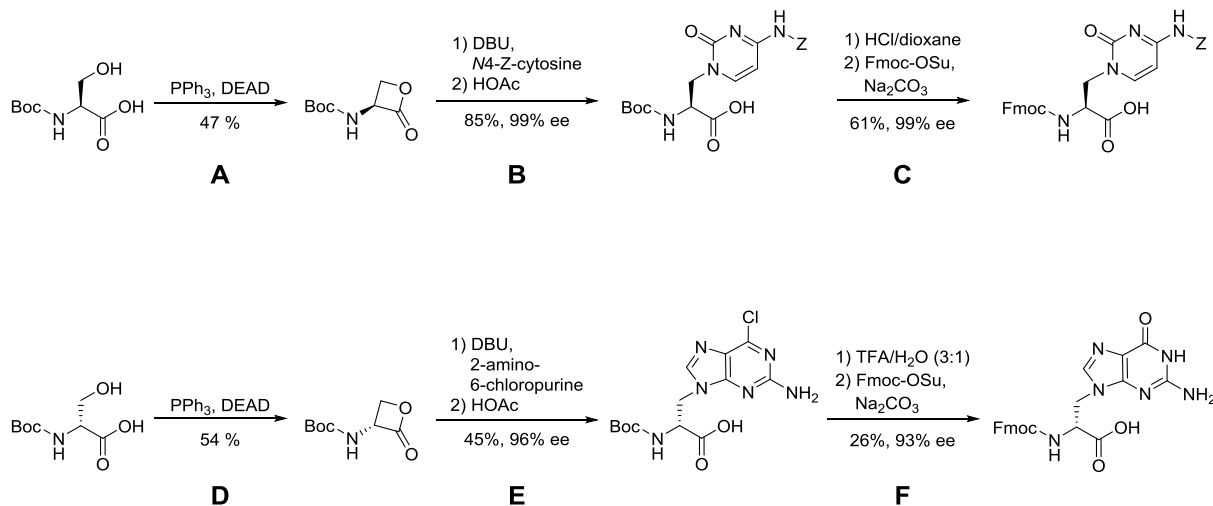
Here, *A(T)* is the absorbance at a temperature *T* and *A*₀ is the minimum absorbance measured during the experiment.

2.4 Circular Dichroism Spectroscopy

CD spectroscopy was performed by using a *J-810* spectrometer by JASCO (Tokyo, Japan) equipped with a *PTC-423S* temperature control unit. The cell was permanently purged with nitrogen. Quartz glass cuvettes by Hellma Analytics (Müllheim, Germany) with a path length of 10 mm were used. Samples were dissolved in a phosphate buffer (10 mM Na₂HPO₄, H₃PO₄, 100 mM NaCl, pH = 7) and cooled at -2 °C for 3 h prior to the measurement. After that, spectra were recorded at 0 °C and the temperature was raised in steps of 10 °C. After a new temperature had been set, an equilibration time of 10 min was applied before spectra were recorded. Spectra were recorded at a high sensitivity (5 mdeg), a scanning speed of 20 nm/min (response: 1.0 s), and a bandwidth of 1 nm. Each plotted spectrum is a mean spectrum of three single spectra. Raw spectra were corrected by a reference spectrum (buffer-only) and were smoothed by using a Savitzky-Golay filter (100 points).

3 Alanyl-PNA Monomer Synthesis

The Fmoc-protected ala-PNA monomers were obtained in a three-step synthesis starting from Boc-serine.



Scheme S1. Synthesis routes to the Fmoc-protected ala-PNA monomers, which were needed for the PNA hybrid recognition units. Details of steps A to F are explained in the text. Z = benzyl(oxycarbonyl) protecting group.

The enantioselective conversion of Boc-L-serine or Boc-D-serine into the respective Boc-serine β -lactone (step A and D, respectively) was performed as described in Ref. [6].

The subsequent nucleophilic ring opening with *N*4-[(benzyloxy)carbonyl]cytosine (*N*4-Z-cytosine, step B) or 2-amino-6-chloropurine (step E) was performed as described in Refs. [7,8]. *N*4-Z-Cytosine was synthesized from cytosine as described in Ref. [9].

The conversion of the Boc-protected alanyl nucleo amino acids into the Fmoc-protected building blocks was performed as described in Ref. [10].

The obtained yields are indicated in each step. The enantiomeric excess (ee) was determined by converting the respective building blocks into diastereomers with enantiomerically pure Boc-L-Ala-OSu on the basis of the description in Ref. [7]. Boc-protected building blocks (5 to 7 μmol , 1.0 eq) were dissolved in TFA (250 μL). The solution was stirred for 10 min and then TFA was removed in a nitrogen stream. Fmoc-protected building blocks (ca. 5 μmol , 1.0 eq) were dissolved in a solution of piperidine in DMF (1:4, *v/v*). The solution was stirred for 30 min and the solvent was removed *i. vac.*

The residue was suspended in H_2O (350 μL) and NaHCO_3 (1.2 M, 250 μL) to yield a pH of 9. A solution of Boc-L-Ala-OSu (6.0 eq) in THF (700 μL) was added and the mixture was stirred for 5 h. Afterwards, aqueous TFA (10 % in H_2O) was added to yield a pH of 2.5 and the diastereomers were chromatographically analyzed on a RP-HPLC column (C18-ec, solvents A + B1, 10 \rightarrow 50 % B1 in 30 min, see Section 2.2 for details of HPLC setup). Comparison of the integrals of the peaks at 260 nm yielded the indicated ee values.

4 Peptide Synthesis

4.1 Synthetic Procedures

Peptides were synthesized according to the following protocol adapted and modified from Ref. [11]. The sequences of the transmembrane domains (TMDs) of the model peptides were from Syntaxin-1A (256-288) and Synaptobrevin-2 (96-116) from *rattus norvegicus*. They were synthesized on a pre-loaded Wang resin (100 μmol , loading density < 0.4 mmol/g) by applying Fmoc solid-phase peptide synthesis (SPPS) using the *Liberty Blue* peptide synthesizer by CEM (Matthews, North Carolina, USA) which was connected to a *Discover* microwave unit by CEM. Melittin (1-26) from *apis mellifera* as well as the PNA sequences without a TMD attached were synthesized on a Rink amide MBHA resin (100 μmol , loading density 0.38 mmol/g). The Rink amide resin was manually preloaded with 5.0 eq of Fmoc-Gln(Trt)-OH (for melittin) or Fmoc-Lys(Boc)-OH (for PNA sequences without a TMD attached) by using HOBt (4.9 eq) and DIC (4.9 eq). The coupling step was performed twice using a *Discover* microwave unit by CEM (40 °C, 20 W, 10 min). Successful coupling was confirmed by the Kaiser test^[12] and a final capping with Ac₂O/2,6-lutidine/NMP (1:2:7, v/v/v) was performed. The resin was thoroughly washed with DCM and NMP between all steps.

For automatic peptide synthesis, the pre-loaded resins were swollen in DMF for at least 20 min. On the peptide synthesizer, deprotection was carried out with piperidine (20 % in DMF, 75 \rightarrow 90 °C, 65 s). The coupling step was performed after having added the Fmoc-amino acid (0.2 M in DMF, 5 eq), Oxyma (1 M in DMF, 5 eq) and DIC (0.5 M in DMF, 5 eq) to the deprotected resin (75 \rightarrow 90 °C, 125 s). The coupling step was performed twice for each amino acid. For the attachment of Fmoc-Arg(Pbf)-OH, the temperature did not exceed 75 °C and the reaction time was elongated to 1260 s in the first coupling step and to 300 s in the second coupling step. For the attachment of Fmoc-Cys(Trt)-OH, the temperature was reduced to 50 °C and the reaction time was 360 s in both coupling steps. During reaction steps, nitrogen was bubbled through the solution. The resin was washed four times with DMF after the deprotection step. A capping step was not performed. After the synthesis, the resin was transferred into a Discardit II syringe by Becton Dickinson (Heidelberg, Germany), washed with DCM (10x) and dried and stored in a desiccator.

The PNA monomers were attached to the TMDs (or to the Rink amide resin preloaded with Fmoc-Lys(Boc) in case of PNA sequences without a TMD attached) using manual SPPS at room temperature as proposed by Casale *et al.*^[13] The protocol described in Ref. [11] was followed with the following adaptations being made: The stock solutions of the reagents were prepared freshly each day. The resin which contained the transmembrane domain (1.0 eq) was swollen 2 h in NMP before the SPPS cycle started. Activator bases DIPEA and 2,6-lutidine were added to the aeg-PNA monomer immediately before transferring the mixture to the resin. The reaction time for coupling was set to 1 h and each coupling step was performed twice. The capping step with Ac₂O/2,6-lutidine/NMP (1:2:7, v/v/v) was performed twice, followed by a washing step with DIPEA (5% in NMP).

Attaching the ala-PNA monomers in principle followed the protocol above with the following specific adaptations: HATU (4.5 eq) and HOAt (5.0 eq) were added to the solid ala-PNA monomer (5.0 eq) and complete dissolving was achieved by treatment in an ultrasonic bath. The reaction mixture was shaken at room temperature for 2 h. After that, the coupling step was repeated. The capping step was omitted in all cases in which ala-PNA monomers were present on the growing peptide chain in order to avoid permanent acetylation of unprotected exocyclic amino groups.

If the synthesis was continued the next day, the resin was stored in NMP at $-20\text{ }^{\circ}\text{C}$ overnight. Otherwise, it was washed with DCM (10x) and dried and stored in a desiccator.

Cleavage from the resin was performed with a mixture of TFA/*m*-cresol/EDT/TIS (87.5:5:5:2.5, v/v/v/v) if the sequence contained aeg-PNA, with TFA/thioanisole/*m*-cresol/EDT/TIS (63:15:14.5:5:2.5, v/v/v/v/v) if ala-PNA was present and TFA/H₂O/TIS (95:2.5:2.5, v/v/v) for melittin.

The resin was shaken for 2 h (3 h if ala-PNA monomers were present) in the cleavage mixture. Afterwards, the resin beads were filtered off and volatile substances were removed in a nitrogen stream. Ice-cold diethyl ether was added to the concentrate and the peptide was obtained as a white solid. The precipitate was washed with ice-cold diethyl ether for 4 times and dried in a desiccator.

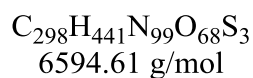
4.2 Peptide Sequences and Analytical Data

The peptides were analyzed via electrospray ionization mass spectrometry (ESI-MS) using a *maXis* spectrometer by Bruker (Bremen, Germany). Data was acquired and analyzed with the software provided by the manufacturer. Samples were prepared by dissolving the PNA/peptide hybrids in MeOH/TFE/formic acid = 1:1:0.1. Melittin was dissolved in H₂O/formic acid = 1:0.1. PNA sequences without a TMD attached were dissolved in methanol. For each compound the found *m/z* peaks and their relative intensities are indicated. High-resolution mass spectra (HR-MS) were measured as well; here the calculated (calc.) and the found peaks are indicated. For each compound, both the deconvoluted and the measured ESI-MS spectrum are shown with the peaks belonging to the compound being labeled. For PNA sequences without a TMD attached, purification via HPLC was possible and thus routinely performed; the applied conditions are indicated.

In the given sequences, aeg-PNA nucleobases are written in small letters (a=adenine, c=cytosine, g=guanine, t=thymine) and ala-PNA nucleobases are written in capitals with D-configured building blocks being underlined (G=D-alaG, C=L-AlaC); α -amino acids are given in capitals in the standard one-letter code. The nature of the C-terminus is given as -COOH for a carboxy C-terminus and -NH₂ for an amide C-terminus. Below the sequence, the chemical formula as well as the relative molecular mass is given for each compound. The relative molecular mass was calculated using ChemBioDraw 13.0 by PerkinElmer (Waltham, USA).

PNA1-SybTMD (1)

gtagatcact-KRKYWWKNLKMILGVICAILIIIVYFST-COOH



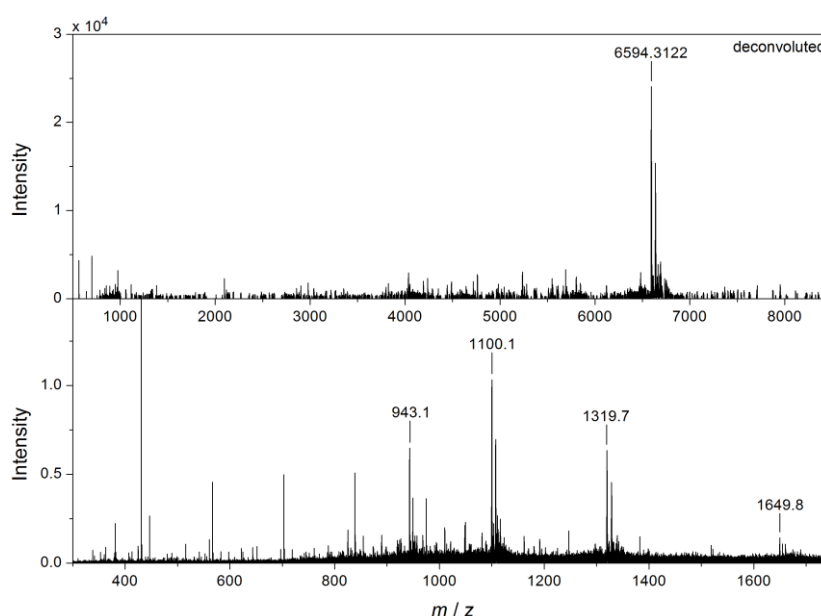
MS (ESI): m/z (%) = 943.1 (12) $[\text{M}+7\text{H}]^{7+}$, 1100.1 (43) $[\text{M}+6\text{H}]^{6+}$, 1319.7 (100) $[\text{M}+5\text{H}]^{5+}$, 1649.8 (18) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{298}\text{H}_{448}\text{N}_{99}\text{O}_{68}\text{S}_3$ ($[\text{M}+7\text{H}]^{7+}$): 943.0552, found: 943.0557;

calc. for $\text{C}_{298}\text{H}_{447}\text{N}_{99}\text{O}_{68}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 1100.0632, found: 1100.0645;

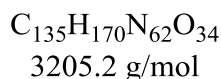
calc. for $\text{C}_{298}\text{H}_{446}\text{N}_{99}\text{O}_{68}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1319.8744, found: 1319.8763;

calc. for $\text{C}_{298}\text{H}_{445}\text{N}_{99}\text{O}_{68}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1649.5912, found: 1649.5849.



Fmoc-PNA1-KK (1 without TMD)

Fmoc-gtagatcact-KK-NH₂



MS (ESI): m/z (%) = 802.1 (37) $[\text{M}+4\text{H}]^{4+}$, 1069.1 (100) $[\text{M}+3\text{H}]^{3+}$, 1603.2 (58) $[\text{M}+2\text{H}]^{2+}$.

HR-MS (ESI): calc. for $\text{C}_{135}\text{H}_{175}\text{N}_{62}\text{O}_{34}$ ($[\text{M}+5\text{H}]^{5+}$): 641.8774, found: 641.8765;

calc. for $\text{C}_{135}\text{H}_{174}\text{N}_{62}\text{O}_{34}$ ($[\text{M}+4\text{H}]^{4+}$): 802.0949, found: 802.0944;

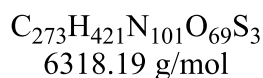
calc. for $\text{C}_{135}\text{H}_{173}\text{N}_{62}\text{O}_{34}$ ($[\text{M}+3\text{H}]^{3+}$): 1069.1241, found: 1069.1239;

calc. for $\text{C}_{135}\text{H}_{172}\text{N}_{62}\text{O}_{34}$ ($[\text{M}+2\text{H}]^{2+}$): 1603.1825, found: 1603.1851.

HPLC (0 → 50% B2 in 30 min): t_R = 25.9 min.

PNA2-SxTMD (2)

catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-COOH



MS (ESI): m/z (%) = 790.7 (18) [M+8H]⁸⁺, 903.5 (45) [M+7H]⁷⁺, 1053.9 (100) [M+6H]⁶⁺, 1264.4 (79) [M+5H]⁵⁺, 1580.3 (17) [M+4H]⁴⁺.

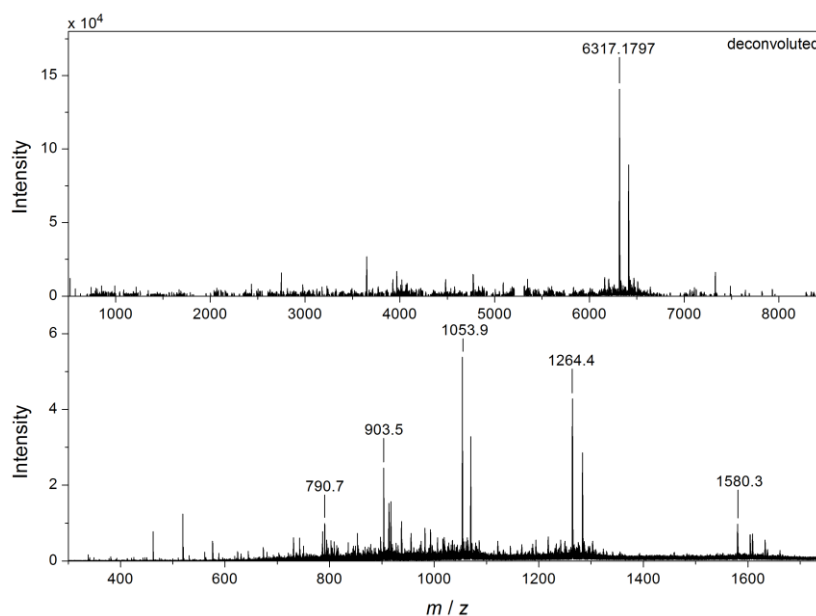
HR-MS (ESI): calc. for $\text{C}_{273}\text{H}_{429}\text{N}_{101}\text{O}_{69}\text{S}_3$ ([M+8H]⁸⁺): 790.6545, found: 790.6562;

calc. for $\text{C}_{273}\text{H}_{428}\text{N}_{101}\text{O}_{69}\text{S}_3$ ([M+7H]⁷⁺): 903.4612, found: 903.4629;

calc. for $\text{C}_{273}\text{H}_{427}\text{N}_{101}\text{O}_{69}\text{S}_3$ ([M+6H]⁶⁺): 1053.8702, found: 1053.8705;

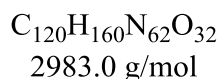
calc. for $\text{C}_{273}\text{H}_{426}\text{N}_{101}\text{O}_{69}\text{S}_3$ ([M+5H]⁵⁺): 1264.4428, found: 1264.4440;

calc. for $\text{C}_{273}\text{H}_{425}\text{N}_{101}\text{O}_{69}\text{S}_3$ ([M+4H]⁴⁺): 1580.3017, found: 1580.3014.



PNA2-KK (2 without TMD)

catctagtga-KK-NH₂



MS (ESI): m/z (%) = 597.5 (20) [M+5H]⁵⁺, 746.6 (83) [M+4H]⁴⁺, 995.1 (100) [M+3H]³⁺, 1492.1(26) [M+2H]²⁺.

HR-MS (ESI): calc. for $\text{C}_{120}\text{H}_{164}\text{N}_{62}\text{O}_{32}$ ([M+4H]⁴⁺): 746.5779, found: 746.5579;

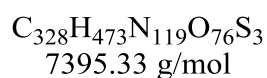
calc. for $\text{C}_{120}\text{H}_{163}\text{N}_{62}\text{O}_{32}$ ([M+3H]³⁺): 995.1014, found: 995.1010;

calc. for $\text{C}_{120}\text{H}_{162}\text{N}_{62}\text{O}_{32}$ ([M+2H]²⁺): 1492.1484, found: 1492.1492.

HPLC (0 → 50% B1 in 30 min): t_R = 14.4 min.

Ala1-PNA1-SybTMD (3)

CGCG-gtagatcact-KRKYWWKLNKMMILGVICAILIIIVYFST-COOH

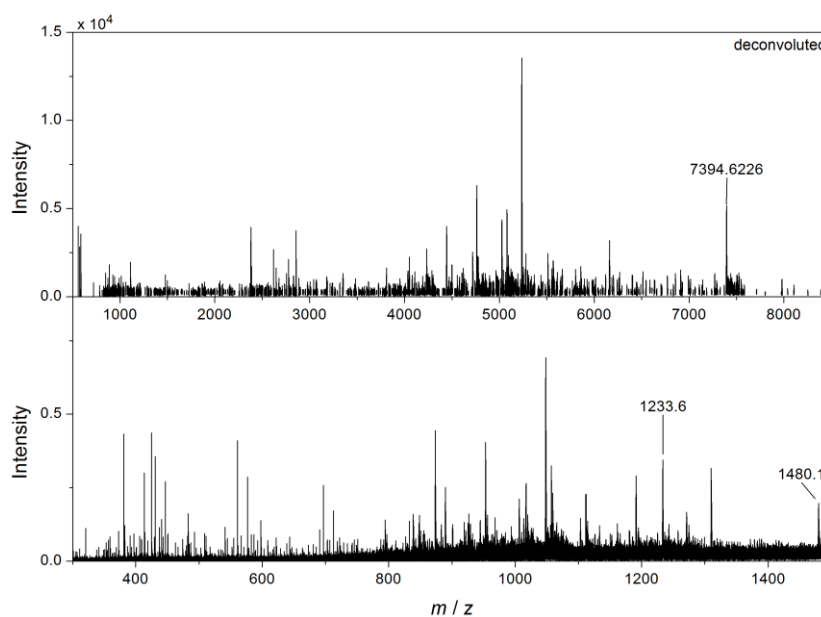


MS (ESI): m/z (%) = 1233.6 [M+6H]⁶⁺, 1480.1 [M+5H]⁵⁺.

HR-MS (ESI): calc. for $\text{C}_{328}\text{H}_{480}\text{N}_{119}\text{O}_{76}\text{S}_3$ ([M+7H]⁷⁺): 1057.3797, found: 1057.3761;

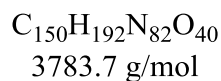
calc. for $\text{C}_{328}\text{H}_{479}\text{N}_{119}\text{O}_{76}\text{S}_3$ ([M+6H]⁶⁺): 1233.4417, found: 1233.4410;

calc. for $\text{C}_{328}\text{H}_{478}\text{N}_{119}\text{O}_{76}\text{S}_3$ ([M+5H]⁵⁺): 1479.9286, found: 1479.9283.



Ala1-PNA1-KK (3 without TMD)

CGCG-gtagatcact-KK-NH₂



MS (ESI): m/z (%) = 757.7 (13) [M+5H]⁵⁺, 946.9 (66) [M+4H]⁴⁺, 1262.2 (100) [M+3H]³⁺, 1892.8 (34) [M+2H]²⁺.

HR-MS (ESI): calc. for $\text{C}_{150}\text{H}_{196}\text{N}_{82}\text{O}_{40}$ ([M+4H]⁴⁺): 946.8966, found: 946.8968;

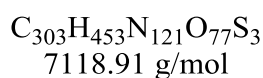
calc. for $\text{C}_{150}\text{H}_{195}\text{N}_{82}\text{O}_{40}$ ([M+3H]³⁺): 1262.1930, found: 1262.1917;

calc. for $\text{C}_{150}\text{H}_{194}\text{N}_{82}\text{O}_{40}$ ([M+2H]²⁺): 1892.7859, found: 1892.7851.

HPLC (5 → 18% B1 in 60 min): t_R = 43.7 min.

Ala2-PNA2-SxTMD (4)

GCGC-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-COOH

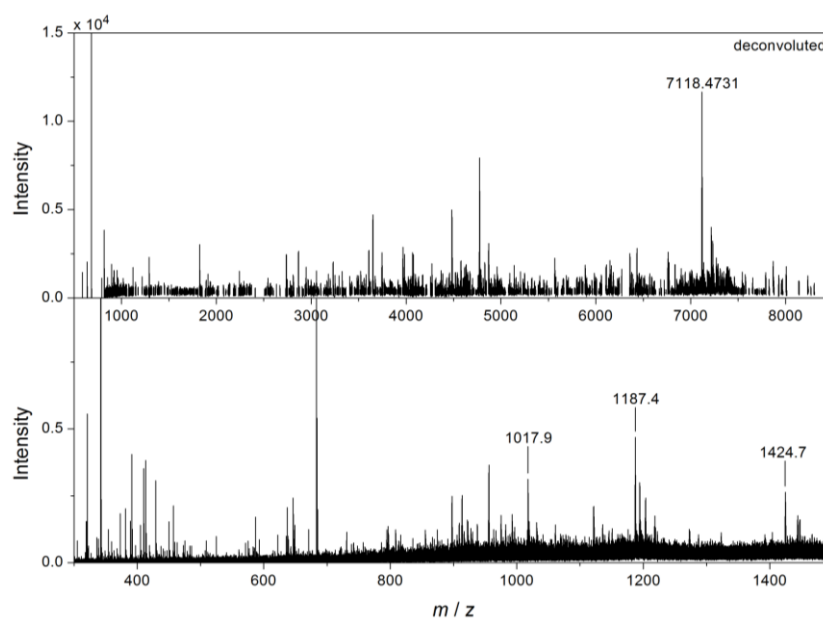


MS (ESI): m/z (%) = 1017.9 [M+7H]⁷⁺, 1187.4 [M+6H]⁶⁺, 1424.7 [M+5H]⁵⁺.

HR-MS (ESI): calc. for $\text{C}_{303}\text{H}_{460}\text{N}_{121}\text{O}_{77}\text{S}_3$ ([M+7H]⁷⁺): 1017.9288, found: 1017.9290;

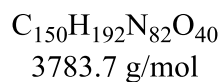
calc. for $\text{C}_{303}\text{H}_{459}\text{N}_{121}\text{O}_{77}\text{S}_3$ ([M+6H]⁶⁺): 1187.4158, found: 1187.4117;

calc. for $\text{C}_{303}\text{H}_{458}\text{N}_{121}\text{O}_{77}\text{S}_3$ ([M+5H]⁵⁺): 1424.6975, found: 1424.7014.



Ala2-PNA2-KK (4 without TMD)

GCGC-catctagtga-KK-NH₂



MS (ESI): m/z (%) = 631.6 (8) [M+6H]⁶⁺, 757.7 (20) [M+5H]⁵⁺, 946.9 (90) [M+4H]⁴⁺, 1262.2 (100) [M+3H]³⁺, 1892.8 (19) [M+2H]²⁺.

HR-MS (ESI): calc. for $\text{C}_{150}\text{H}_{196}\text{N}_{82}\text{O}_{40}$ ([M+4H]⁴⁺): 946.8966, found: 946.8952;

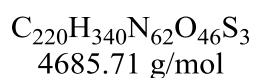
calc. for $\text{C}_{150}\text{H}_{195}\text{N}_{82}\text{O}_{40}$ ([M+3H]³⁺): 1262.1930, found: 1262.1910;

calc. for $\text{C}_{150}\text{H}_{194}\text{N}_{82}\text{O}_{40}$ ([M+2H]²⁺): 1892.7859, found: 1892.7862.

HPLC (5 → 20% B1 in 60 min): t_R = 40.6 min.

Ala1-SybTMD (5)

CGCG-KRKYWWKNLKMMLGVICAILLIIIIVYFST-COOH

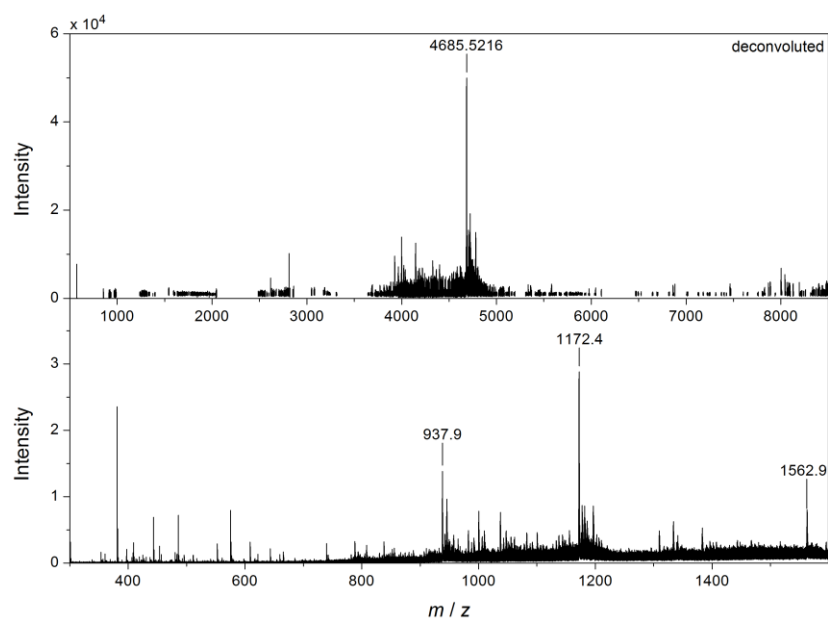


MS (ESI): m/z (%) = 937.9 (48) $[\text{M}+5\text{H}]^{5+}$, 1172.4 (100) $[\text{M}+4\text{H}]^{4+}$, 1562.9 (26) $[\text{M}+3\text{H}]^{3+}$.

HR-MS (ESI): calc. for $\text{C}_{220}\text{H}_{345}\text{N}_{62}\text{O}_{46}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 937.9150, found: 937.9137;

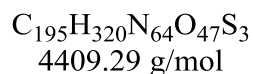
calc. for $\text{C}_{220}\text{H}_{344}\text{N}_{62}\text{O}_{46}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1172.3930, found: 1172.3911;

calc. for $\text{C}_{220}\text{H}_{343}\text{N}_{62}\text{O}_{46}\text{S}_3$ ($[\text{M}+3\text{H}]^{3+}$): 1562.8549, found: 1562.8517.



Ala2-SxTMD (6)

GCGC-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-COOH

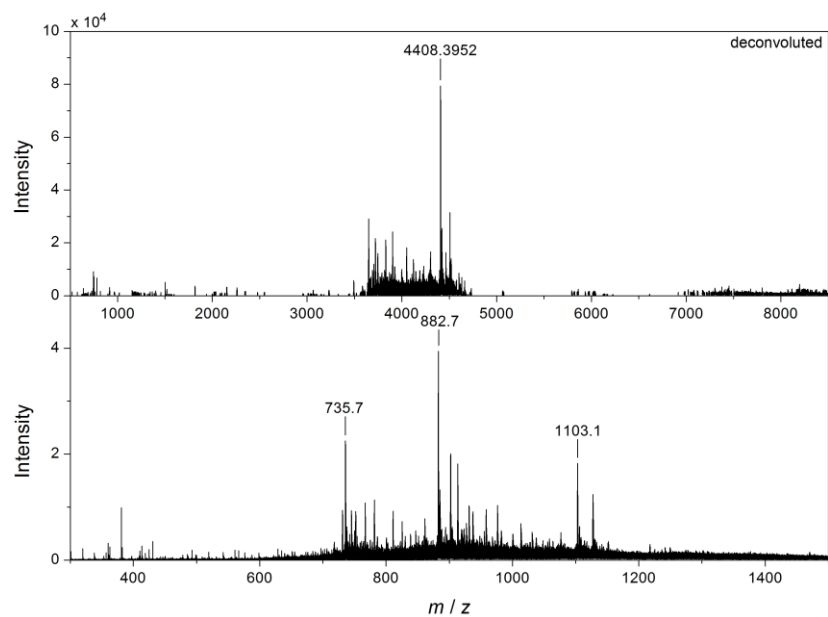


MS (ESI): m/z (%) = 735.7 (57) $[\text{M}+6\text{H}]^{6+}$, 882.7 (100) $[\text{M}+5\text{H}]^{5+}$, 1103.1 (46) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{195}\text{H}_{326}\text{N}_{64}\text{O}_{47}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 735.7378, found: 735.7379;

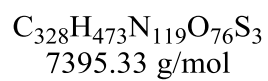
calc. for $\text{C}_{195}\text{H}_{325}\text{N}_{64}\text{O}_{47}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 882.6839, found: 882.6848;

calc. for $\text{C}_{195}\text{H}_{324}\text{N}_{64}\text{O}_{47}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1103.1030, found: 1103.1034.

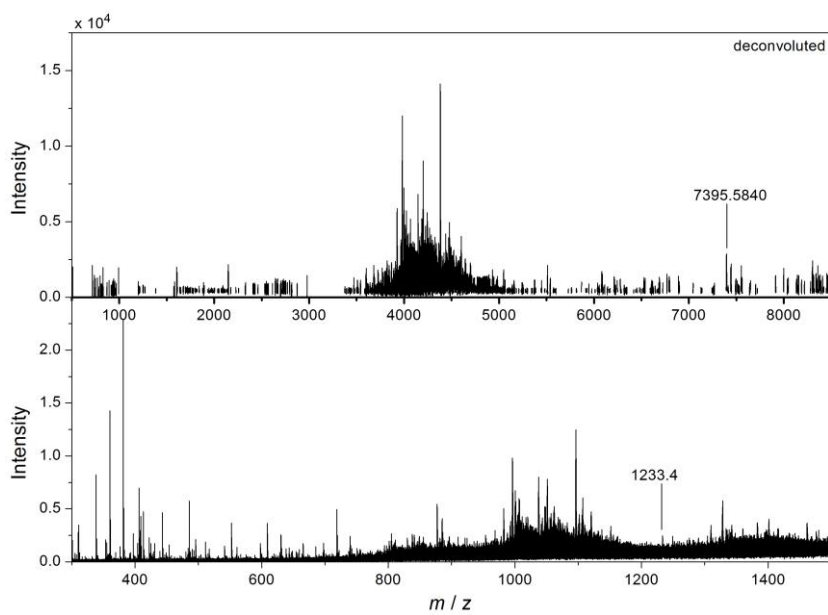


PNA1-Ala1-SybTMD (7)

gtagatcact-CGCG-KRKYWWKNLKMMLILGVICAILIIIVYFST-COOH

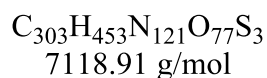


MS (ESI): m/z (%) = 1233.3 [M+6H]⁶⁺.



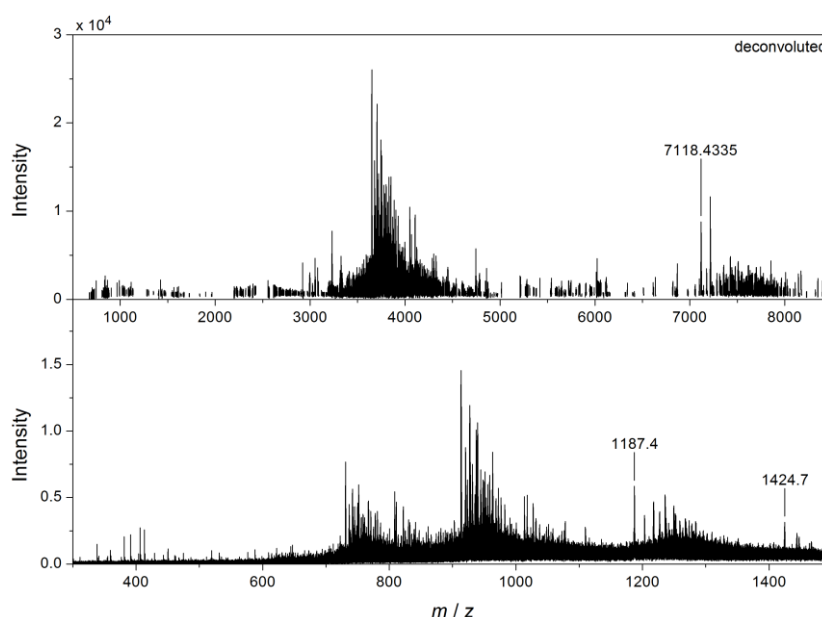
PNA2-Ala2-SxTMD (8)

catctagtga-GCGC-KYQSKARRKKIMIIICCVILGIIIASITGGIFG-COOH



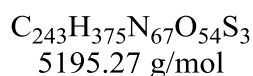
MS (ESI): m/z (%) = 1187.4 (100) $[\text{M}+6\text{H}]^{6+}$, 1424.7 (55) $[\text{M}+5\text{H}]^{5+}$.

HR-MS (ESI): calc. for $\text{C}_{303}\text{H}_{459}\text{N}_{121}\text{O}_{77}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 1187.4158, found: 1187.4094;
calc. for $\text{C}_{303}\text{H}_{458}\text{N}_{121}\text{O}_{77}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1424.6975, found: 1424.7050.



PNA1s-SybTMD (9)

tcact-KRKYWWKNLKMMLGVICAILIIIVYFST-COOH



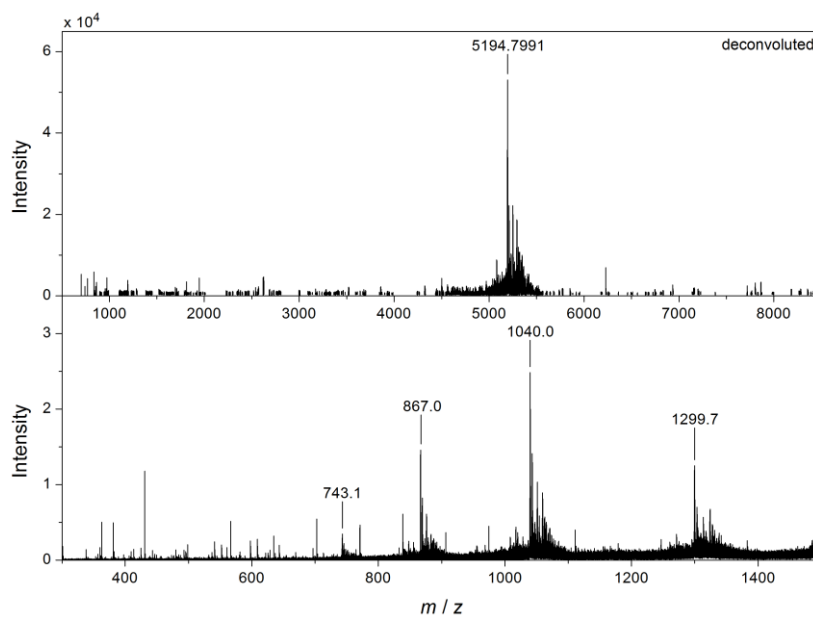
MS (ESI): m/z (%) = 743.1 (13) $[\text{M}+7\text{H}]^{7+}$, 867.0 (59) $[\text{M}+6\text{H}]^{6+}$, 1040.0 (100) $[\text{M}+5\text{H}]^{5+}$,
1299.7 (49) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{243}\text{H}_{382}\text{N}_{67}\text{O}_{54}\text{S}_3$ ($[\text{M}+7\text{H}]^{7+}$): 743.1204, found: 743.1190;

calc. for $\text{C}_{243}\text{H}_{381}\text{N}_{67}\text{O}_{54}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 866.9731, found: 866.9736;

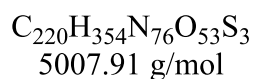
calc. for $\text{C}_{243}\text{H}_{380}\text{N}_{67}\text{O}_{54}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1039.9656, found: 1039.9660;

calc. for $\text{C}_{243}\text{H}_{379}\text{N}_{67}\text{O}_{54}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1299.7052, found: 1299.7067.



PNA2s-SxTMD (10)

agtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-COOH

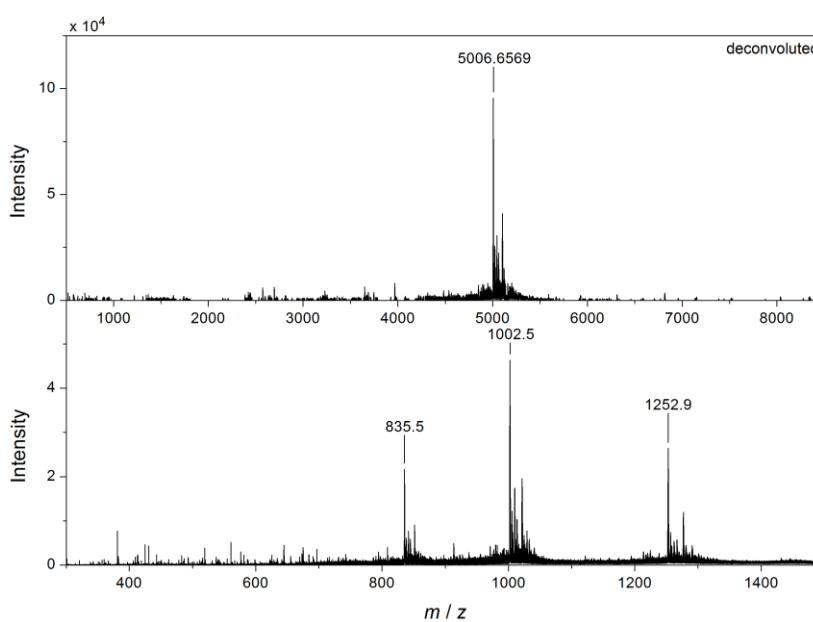


MS (ESI): m/z (%) = 835.5 (46) $[\text{M}+6\text{H}]^{6+}$, 1002.5 (100) $[\text{M}+5\text{H}]^{5+}$, 1252.9 (56) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{220}\text{H}_{360}\text{N}_{76}\text{O}_{53}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 835.4499, found: 835.4525;

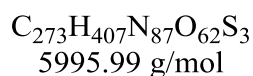
calc. for $\text{C}_{220}\text{H}_{359}\text{N}_{76}\text{O}_{53}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1002.5393, found: 1002.5409;

calc. for $\text{C}_{220}\text{H}_{358}\text{N}_{76}\text{O}_{53}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1252.9222, found: 1252.9232.



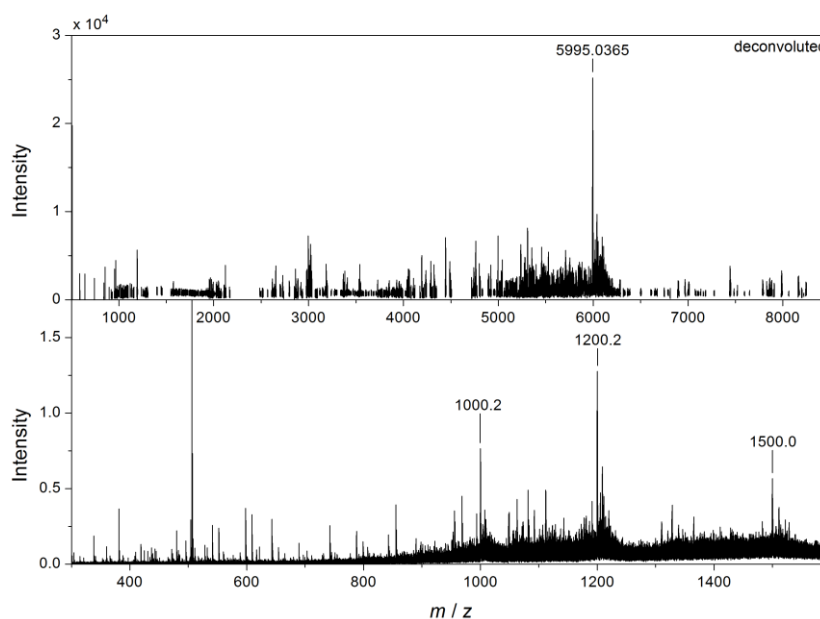
Ala1-PNA1s-SybTMD (11)

CGCG-tact-KRKYWWKNLKMMILGVICAILIIIVYFST-COOH



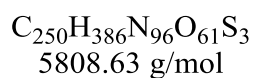
MS (ESI): m/z (%) = 1000.2 (60) $[\text{M}+6\text{H}]^{6+}$, 1200.2 (100) $[\text{M}+5\text{H}]^{5+}$, 1500.0 (42) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{273}\text{H}_{413}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 1000.1841, found: 1000.1828;
calc. for $\text{C}_{273}\text{H}_{412}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1200.2199, found: 1200.2181;
calc. for $\text{C}_{273}\text{H}_{411}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1500.0230, found: 1500.0225.



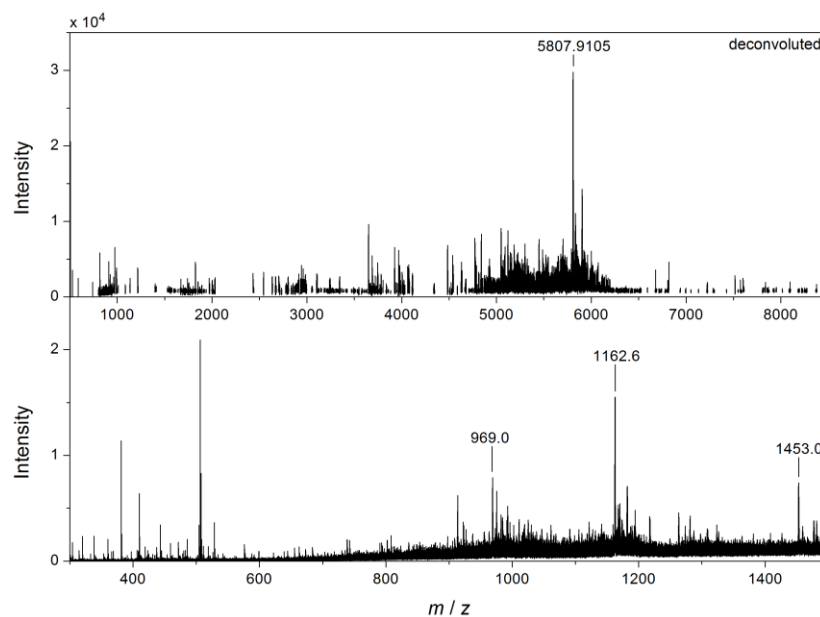
Ala2-PNA2s-SxTMD (12)

GCGC-agtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-COOH



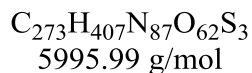
MS (ESI): m/z (%) = 969.0 (49) $[\text{M}+6\text{H}]^{6+}$, 1162.6 (100) $[\text{M}+5\text{H}]^{5+}$, 1453.0 (45) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{250}\text{H}_{392}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 968.9954, found: 968.9952;
calc. for $\text{C}_{250}\text{H}_{391}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1162.5930, found: 1162.5950;
calc. for $\text{C}_{250}\text{H}_{390}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1452.9895, found: 1452.9866.

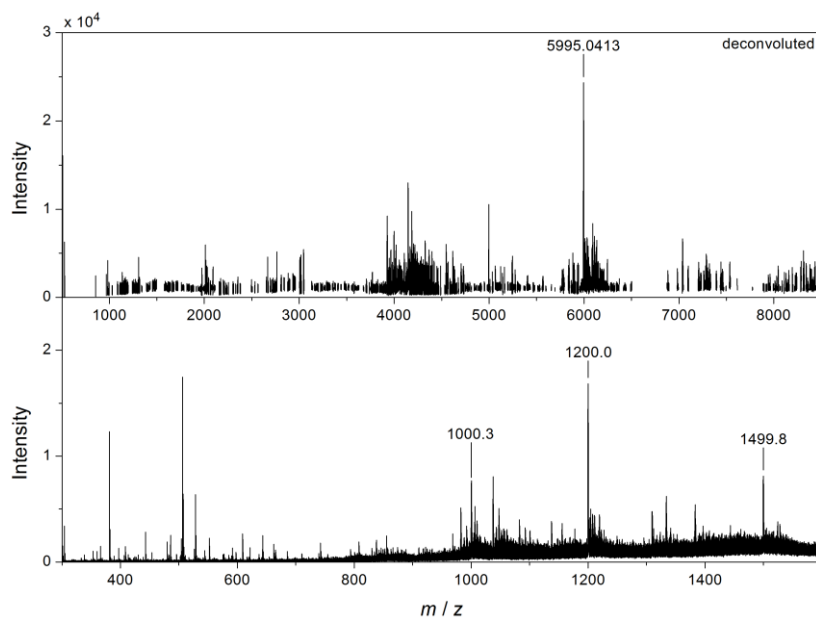


PNA1s-Ala1-SybTMD (13)

tcact-CGCG-KRKYWWKNLKMMLGVICAILIIIVYFST-COOH

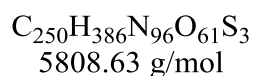


MS (ESI): m/z (%) = 1000.3 (45) $[\text{M}+6\text{H}]^{6+}$, 1200.0 (100) $[\text{M}+5\text{H}]^{5+}$, 1499.8 (45) $[\text{M}+4\text{H}]^{4+}$.
HR-MS (ESI): calc. for $\text{C}_{273}\text{H}_{413}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 1000.1841, found: 1000.1822;
 calc. for $\text{C}_{273}\text{H}_{412}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1200.0194, found: 1200.0186;
 calc. for $\text{C}_{273}\text{H}_{411}\text{N}_{87}\text{O}_{62}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1499.7725, found: 1499.7747.



PNA2s-Ala2-SxTMD (14)

agtga-GCGC-KYQSKARRKKIMIICCIVLGIHASTIGGIFG-COOH

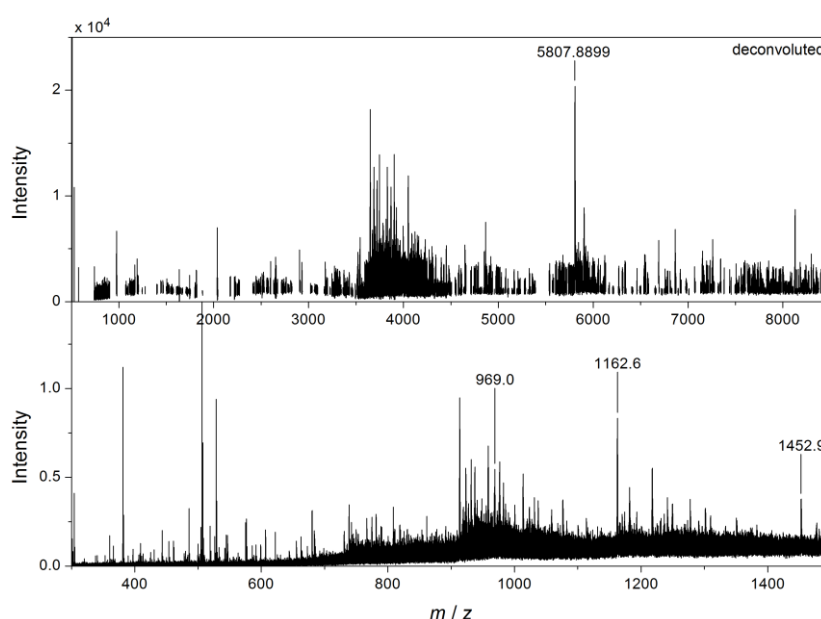


MS (ESI): m/z (%) = 969.0 (63) $[\text{M}+6\text{H}]^{6+}$, 1162.6 (100) $[\text{M}+5\text{H}]^{5+}$, 1452.9 (40) $[\text{M}+4\text{H}]^{4+}$.

HR-MS (ESI): calc. for $\text{C}_{250}\text{H}_{392}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+6\text{H}]^{6+}$): 968.9954, found: 969.0087;

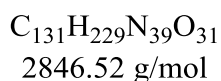
calc. for $\text{C}_{250}\text{H}_{391}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+5\text{H}]^{5+}$): 1162.5930, found: 1162.5956;

calc. for $\text{C}_{250}\text{H}_{390}\text{N}_{96}\text{O}_{61}\text{S}_3$ ($[\text{M}+4\text{H}]^{4+}$): 1452.9895, found: 1452.9881.



Melittin

GIGAVLKVLTTGLPALISWIKRKRQQ-NH₂



HPLC (column 3, 20 → 80% B1 in 30 min): t_R = 19.1 min.

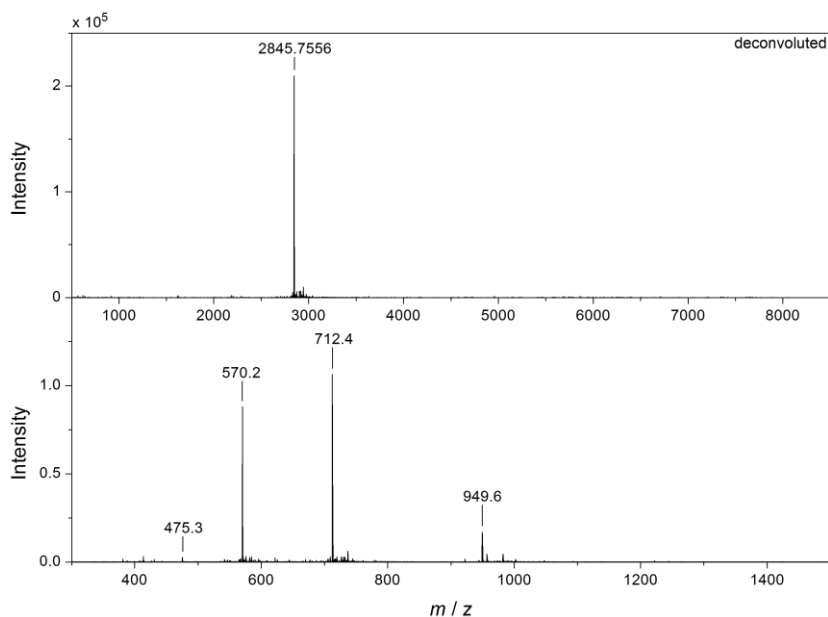
MS (ESI): m/z (%) = 475.3 (2) $[\text{M}+6\text{H}]^{6+}$, 570.2 (82) $[\text{M}+5\text{H}]^{5+}$, 712.4 (100) $[\text{M}+4\text{H}]^{4+}$, 949.6 (16) $[\text{M}+3\text{H}]^{3+}$.

HR-MS (ESI): calc. for $\text{C}_{131}\text{H}_{235}\text{N}_{39}\text{O}_{31}$ ($[\text{M}+6\text{H}]^{6+}$): 475.3001, found: 475.2995;

calc. for $\text{C}_{131}\text{H}_{234}\text{N}_{39}\text{O}_{31}$ ($[\text{M}+5\text{H}]^{5+}$): 570.1587, found: 570.1595;

calc. for $\text{C}_{131}\text{H}_{233}\text{N}_{39}\text{O}_{31}$ ($[\text{M}+4\text{H}]^{4+}$): 712.4465, found: 712.4474;

calc. for $\text{C}_{131}\text{H}_{232}\text{N}_{39}\text{O}_{31}$ ($[\text{M}+3\text{H}]^{3+}$): 949.5929, found: 949.5936.



5 Fusion Assays

5.1 Preparation of Liposomes

Liposomes were prepared according to the following protocol adapted and modified from Ref. [11].

Lipid stock solutions were prepared in CHCl_3 (20 mg/mL; lipids labeled with 7-nitro-2-1,3-benzoxadiazole (NBD) or Lissamine Rhodamine B (Rh): 2 mg/mL, lipids labeled with OG or TR: 1 mg/mL), and peptide stock solutions were prepared in TFE. All stock solutions were stored at -20°C . The concentration of the peptide stock solutions was estimated via UV absorption at 260 nm by making use of the Beer-Lambert law. A V-550 spectrometer by JASCO was used for absorption measurements. The molar extinction coefficient of the peptide was calculated as the sum of the extinction coefficients of all contributing units. The following units were considered:^[14] A ($\epsilon_{260\text{nm}} = 13700 \text{ L mol}^{-1} \text{ cm}^{-1}$), G ($11700 \text{ L mol}^{-1} \text{ cm}^{-1}$), C ($6600 \text{ L mol}^{-1} \text{ cm}^{-1}$), T ($8600 \text{ L mol}^{-1} \text{ cm}^{-1}$), Trp ($3300 \text{ L mol}^{-1} \text{ cm}^{-1}$),^[15] Tyr ($600 \text{ L mol}^{-1} \text{ cm}^{-1}$)^[15] and Phe ($145 \text{ L mol}^{-1} \text{ cm}^{-1}$)^[16]. Raw peptides were used without further purification for the peptide stock solutions.

Small glass tubes were used to prepare the lipid films which were prepared by mixing all components in a ratio dependent on the desired liposome composition. The following molar ratios were used: For unlabeled liposomes: 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) / 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) / cholesterol (Chol) = 50:25:25; for NBD- and Rh-labeled liposomes: DOPC/DOPE/Chol/NBD-DOPE/Rh-DOPE = 50:22:25:1.5:1.5; for OG-labeled liposomes: DOPC/DOPE/Chol/OG-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (OG-DHPE) = 50:23.5:25:1.5; and for TR-labeled liposomes: DOPC/DOPE/Chol/TR-DHPE = 50:24:25:1. The total amount of lipids per lipid film was $2.5 \mu\text{mol}$ for liposomes decorated with model peptides of the 1st generation and $0.625 \mu\text{mol}$ for liposomes decorated with model peptides of the 2nd generation.

The volume of the peptide stock solutions was adjusted to the desired peptide-to-lipid ratio, which was either 1:200 (for TLM assays with model peptides of the 1st generation, all ILM assays and the FCCS assays), 1:300 (for some assays with model peptides of the 1st generation) or 1:1000 (for TLM assays with model peptides of the 2nd generation and for the TLM assays in which **1+2** with **9+10** were compared (Figure S4)). When NBD and Rh were used as the FRET pair, peptides with a SybTMD were incorporated into labeled liposomes and peptides with a SxTMD were incorporated into unlabeled liposomes. When OG and TR were used, peptides with a SybTMD were incorporated into TR-labeled liposomes and SxTMD-based peptides were incorporated into OG-labeled liposomes.

The total volume in the glass tube was 500 μL (250 μL CHCl_3 and 250 μL TFE). Mixing of the components was carried out on ice to minimize loss of solvent. After that, the solution was warmed to room temperature, vortexed for 5 s, warmed to 50 $^\circ\text{C}$ and vortexed again for 5 s. The solvents were slowly removed in a nitrogen stream at 50 $^\circ\text{C}$ which resulted in a clear lipid film on the test tube wall. Test tubes with labeled lipid films were protected from light with aluminium foil. The lipid films were stored overnight in a vacuum oven at 50 $^\circ\text{C}$.

On the next day, the lipid films were rehydrated in 500 μL HEPES buffer (20 mM HEPES, 100 mM KCl, 1 mM EDTA, 1 mM DTT, pH = 7.4) for at least 2 h (170 rpm on a platform shaker, 40 $^\circ\text{C}$). A few glass beads were added to support lipid detachment from the walls. Immediately before extrusion, the lipid films were treated in an ultrasonic bath to homogenize the multilamellar vesicle mixture which was then extruded^[17] 31x through a polycarbonate membrane (100 nm pore diameter, by Avestin (Ottawa, Canada)) using a *LiposoFast-Basic* extruder by Avestin. The membrane was located in between two *Whatman* polyester drain discs by GE Healthcare (Little Chalfont, UK). The so prepared large unilamellar vesicles were stored in Eppendorf tubes. Labeled liposomes were protected from sunlight using aluminium foil. The size of the liposomes was spot-checked via dynamic light scattering (DLS) using a *Zetasizer Nano S* device by Malvern (Malvern, UK). DLS always confirmed a monodisperse distribution and the mean diameters were found to be around 155 nm (mean weighted average of the intensity size distribution).

5.2 TLM Assays

Total lipid mixing (TLM) assays were performed following a protocol adapted from Ref. [18]. The assays were conducted on an *FP-6200* spectrometer by JASCO (Tokyo, Japan) at 25 $^\circ\text{C}$. The temperature was controlled with an *ETC-272T* peltier thermostat by JASCO connected to a water recirculator. Quartz glass cuvettes equipped with a small magnetic stirrer by Hellma Analytics (Müllheim, Germany) were used. The light path was 10 mm for excitation and 4 mm for emission. Data was acquired with the software provided by the manufacturer.

The following parameters were set. Stirring speed: ca. 900 rpm, excitation band width: 5 nm, emission bandwidth: 5 nm, sensitivity: high, data pitch: 1 nm, scanning speed: 125 nm/min, response: fast. The wavelength for excitation of the NBD fluorophore was 460 nm and NBD emission was detected at 530 nm.

Before experiments were started, the buffer baseline was recorded and a spectrum of the labeled vesicles was measured to confirm that FRET was existent.

An experiment started with the addition of labeled vesicles (10 μL , final lipid concentration in the cuvette ca. 9 μM if the original lipid film contained 0.625 μmol lipids) to the buffer (1300 μL) in the cuvette. The NBD emission was recorded for at least 30 s and then the unlabeled liposomes (40 μL , final lipid concentration in the cuvette ca. 37 μM) were added. The emission was recorded for 20 minutes and then Triton X-100 (2.5 % in buffer, 25 μL ,

final concentration in cuvette: 0.68 μM) was added to destroy the liposomes and thus maximize the donor emission. In the end, a spectrum of the reaction mixture was recorded to check that no acceptor emission was present anymore.

The raw data was normalized according to the following equation:

$$F = \frac{F_t - F_0}{F_{\text{max}} - F_0}$$

F_t is the measured donor emission at the time t , F_0 is the mean value of the last 30 data points before the addition of the second liposome population and F_{max} is the donor emission after the addition of Triton X-100 which was calculated from the mean of the last 50 data points.

F is plotted in percent (%). The time $t = 0$ s is set to first data point after the addition of the second liposome population.

5.3 ILM Assays

Labeled liposomes were treated with dithionite to detect mixing of the inner leaflets, following a protocol adapted from Ref. [19]. For this, labeled liposomes (40 μL) were added to a cuvette containing buffer (1250 μL , final concentration of lipids in the cuvette: 37 μM). The donor emission was recorded as described for the TLM assays above for at least 30 s and then a solution of sodium dithionite (50 mM, 20 μL , final dithionite concentration in the cuvette: 760 μM) was added. The sodium dithionite solution was prepared directly before use. When the donor emission reached approx. 40 % of its initial value, O_2 was bubbled through the solution to oxidize remaining dithionite for 5 s. This was followed by a 5 s period of N_2 bubbling through the solution to remove the O_2 (see Figure S7 for a typical trace of the NBD emission during the reduction process). 323 μL of the so prepared reduced liposomes were diluted with buffer to reach a volume of 1310 μL and ILM mixing was performed as described above for the TLM assay.

To verify the ILM assay, the pore-forming peptide melittin (synthesized as described in Section 4) was added at distinct points of the reduction process (Figure S8). This was done following a description in Ref. [20]. The final concentration of melittin in the cuvette was 1.8 μM . Melittin stock solutions were prepared in ultrapure water and the concentration was determined via UV absorption at 280 nm ($\epsilon_{280\text{nm}} = 5690 \text{ L mol}^{-1} \text{ cm}^{-1}$ [15]).

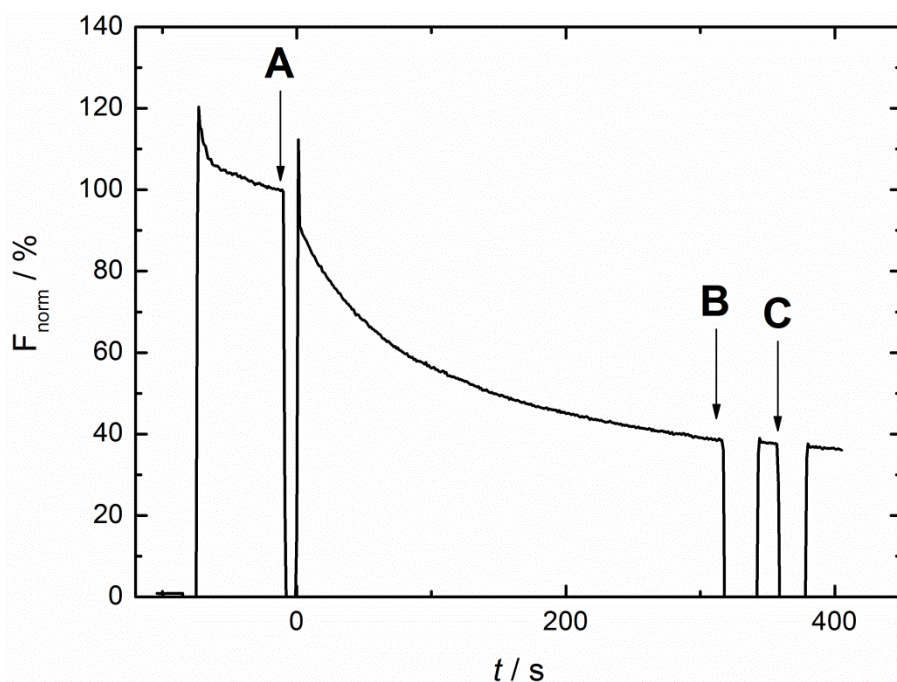


Figure S7. Typical trace of NBD emission during the reduction process of labeled liposomes with dithionite using the example of liposomes decorated with peptide **9**. After the addition of labeled liposomes and a waiting period of at least 30 s, dithionite was added (point **A**). When the NBD emission reached 40 % of its initial value, O₂ was applied for 5 s (point **B**), followed by N₂ for 5 s (point **C**). The labeled liposomes were then ready for submission to the ILM assay. In this plot, F_{norm} was calculated according to $F_{norm} = \frac{F_t}{F_{before}}$, with F_t being the measured fluorescence at time t and F_{before} being the mean value calculated from the last 5 data points before the addition of dithionite.

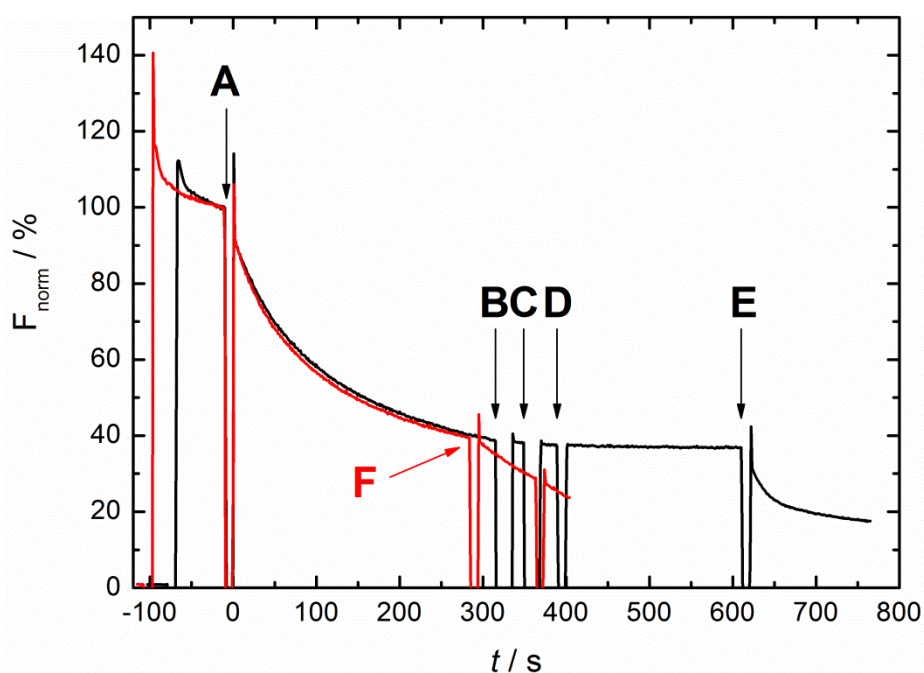


Figure S8. Melittin assay. Melittin was added at different points of the reduction process to verify that dithionite reduced NBD fluorophores located on the outer leaflet. At point **A** ($t = 0$ s, black line), dithionite was added. When the NBD emission reached 40 % of its initial

value, oxygen was bubbled through the cuvette for 5 s (point **B**), followed by nitrogen which was applied for 5 s as well (point **C**). Subsequent addition of melittin (point **D**) had no effect and confirmed that all remaining dithionite was oxidized. Further addition of dithionite (point **E**) led to a decrease in NBD emission and thus confirmed that melittin had formed pores. In a second test (red line), melittin was added directly as soon as the 40 % value was reached (point **F**). The observed following decrease in NBD emission showed that the amount of applied dithionite is sufficient to reduce all outer NBD fluorophores. On top, it showed that the liposomes are mainly impermeable against dithionite. In this plot, F_{norm} was calculated as indicated in the caption of Figure S7.

5.4 Control Experiments

Control experiments were performed either with both liposome populations containing no peptides (as shown in Figure 3) or with only one liposome population without peptides and the other one containing peptides. In both cases, identical control curves resulted as can be seen from Figure S9.

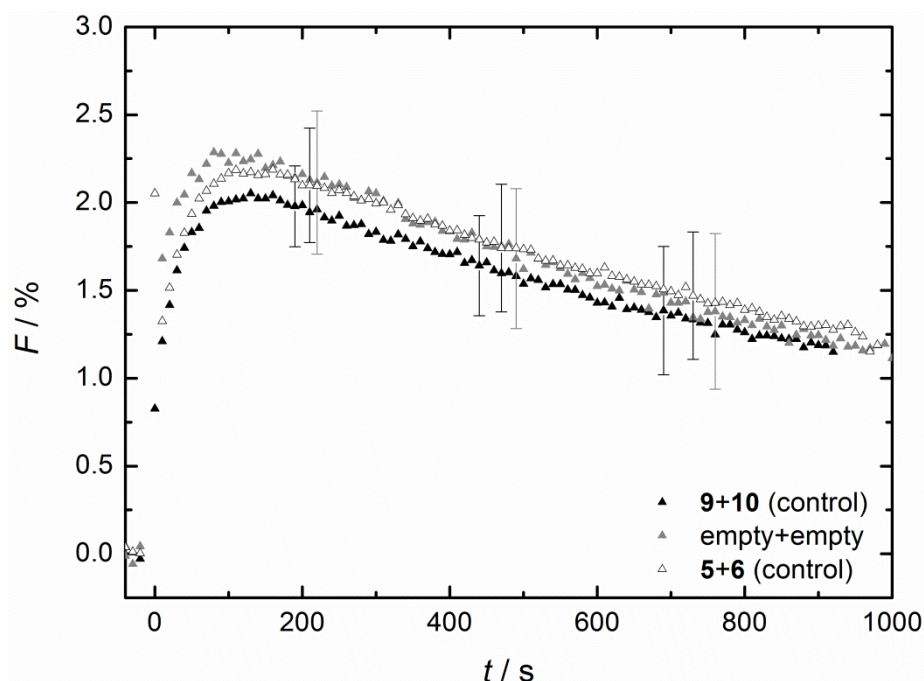


Figure S9. Control curves using the example of TLM assays of **5+6** and **9+10**. Here, the labeled liposome population contained the respective peptide and was mixed with an unlabeled liposome population that contained no peptides. Compared to control curves of assays in which none of the liposome populations contained peptides, no difference could be detected.

5.5 Statistical Calculations

Fusion curves presented with error bars are mean curves from at least three independently prepared samples. The error bars were calculated from the standard deviation of the mean including the respective t-value from Student's t-distribution. The t-values were based on a two-sided 95 % confidence interval.

6 FCCS Assays

For FCCS measurements, a setup as described in Ref. [21] was used. The differently labeled liposomes (either with OG or with TR) were excited simultaneously via two-photon-excitation using a *Chameleon* titanium-sapphire laser (800 nm, 90 MHz) by Coherent (Santa Clara, USA). The laser beam was expanded using a lens system and passed a variable neutral density filter to set the excitation energy to approximately 22 mW for all measurements. To purify the beam from shorter wavelengths, an *FEL 750* long pass filter by ThorLabs (Newton, USA) was used. The laser beam then was channeled by a first dichroic mirror (*715 DSCPXR* by AHF analysentechnik AG (Tübingen, Germany)) into a *UPlanSApo 60x/1.2-W* water immersion objective by Olympus (Tokyo, Japan). The emitted photons were collected with the same objective, passed the first dichroic mirror and were separated by a second dichroic mirror (*590 DCXR* by AHF). Each direction was filtered with an *HQ 645/75* or *HQ 535/50* band pass filter by AHF and focused on a separate *SPCM-AQR-13* avalanche photodiode (APD) by PerkinElmer (Waltham, USA). The APD signals were analyzed using the constant fraction discriminator inputs of a *DPC-230* 16-Channel Photon Correlator by Becker&Hickl (Berlin, Germany).

The measurement was started immediately after liposomes had been mixed in a 1:1 ratio. The ratio was based on the particle concentration which was determined beforehand individually for all liposome populations and which was set to ca. 0.5 particles at a time. The typical time for a measurement was 10 min. Each data point was the mean value of a cycle of 15 seconds. The number of measurements with independently prepared liposome samples was $n = 3$ for **9+10**, $n = 2$ for **11+12** and **5+6**, $n = 1$ for **13+14**, and $n = 6$ for the control. Data was acquired and processed with a homemade program. The fraction of docked particles was calculated via $\left(\frac{N_x}{N_G} + \frac{N_x}{N_R}\right)/2$, which N_x being the number of cross-correlated particles, N_G the particle number of the green channel and N_R the particle number of the red channel.

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