Supporting information

Role of the transmembrane domain in SNARE protein mediated membrane fusion: peptide nucleic acid/peptide model systems

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Table of contents

1) Materials 2

2) Solid phase synthesis of PNA/peptide hybrids 2

3) Analytic data for the synthesized PNA/peptide hybrids 4

4) Preparation of peptide associated liposomes 20

5) UV spectroscopy 20

6) Fluorescence spectroscopy 21

6a) Lipid mixing assays (time course measurements) 21

6b) Reduction of NBD/rhodamine-DOPE-labelled vesicles 21

6c) Reduction of NBD-labelled PNA/peptide hybrids 22

6d) Content mixing assay with SRB loaded vesicles 22

7) Supporting figure 23

8) Fusion lipid mixing experiments including error bars 24

9) References 29

1) Materials

Preloaded *Wang* resins and not preloaded *Nova PEG Rink amide* resin were purchased from *Nova Biochem*, Switzerland. *Rink amide MBHA* resin was obtained from *GL Biochem (Schanghai) Ltd*., China. Piperidine, DIEA, acetic acid anhydride, HATU and HBTU were ordered from *Carl Roth GmbH*, Germany. Lutidine came from *Acros Organics*, USA. Amino acid building blocks were purchased from either *GL Biochem (Schanghai) Ltd*., China or *Iris Biotech GmbH*, Germany. PNA building blocks were obtained from *ASM Research Chemicals*, Germany. Lipids were purchased from *Avanti Polar Lipids*, USA and extruder as well as extrusion membranes are from *Avestin*, Canada.

2) Solid phase synthesis of PNA/peptide hybrids

The peptide parts of the PNA/peptide hybrids were automatically synthesized *via* Fmoc-solid phase peptide synthesis on a pre-loaded *Wang* resin (0.1 mmol, 0.29-0.38 mmol/g, *Nova Biochem*, Switzerland). In case of amide modified compounds a manually pre-loaded *Rink amide MBHA* resin (0.36 mmol/g, *GL Biochem (Schanghai) Ltd*., China) or *Nova PEG Rink amide* resin (0.18 mmol/g, *Nova Biochem*, Switzerland) was used (0.1 mmol scale, respectively). Syntheses were performed by using the *Liberty* peptide synthesizer (*CEM*, Kamp-Lintfort, Germany) equipped with a *Discover* microwave reaction cavity (*CEM*). Standard reagents, protocols and procedures were used for deprotection (piperidine/NMP, 1:4, *v/v* with 0,1 M HOBt, 75 °C for 3,5 min), coupling (5 eq. HBTU/ 5 eq. HOBt/ 10 eq. DIEA in NMP, 75 °C for 5 min) and capping (Ac_2O/DIEA/NMP, 9:19:372, *v/v/v* with 15 mM HOBt, 65 °C for 1 min). The following amino acid building blocks were used in the automated synthesis: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Thr(^{t}Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(^{t}Bu)-OH and Fmoc-Val-OH (5 eq.). For all amino acids double couplings were performed. Special care was taken for the incorporation of Cys and Arg residues. For cysteine , the temperature of the microwave-assisted coupling, capping and deprotection steps was reduced to 50 °C to avoid racemization. Therefore, reaction times were elongated to 6 min. For arginine, the reaction time of the coupling step was elongated to 30 min (room temperature → 75 °C). Capping and deprotection steps were performed as described above. After synthesis, the resins were filtered off and washed successively with NMP and DCM (10 times each). Then the resins were dried *in vacuo*.

For subsequent manual elongation of the peptide chain with PNA-monomers 5 µmol of the resin with peptides were transferred in an acid/base resistant syringe (2 mL) equipped with a frit. After that, the resin was washed successively with NMP, DCM, MeOH and NMP (5 times each), swollen in NMP for 60min and washed again with NMP, DCM, NMP (5 times, respectively). PNA-monomers Fmoc-a(Bhoc)-aeg-OH [aeg = aminoethylglycine], Fmoc-c(Bhoc)-aeg-OH, Fmoc-g(Bhoc)-aeg-OH and Fmoc-t-aeg-OH were used for elongation. The coupling cycle was started with Fmoc-deprotection using piperidine/NMP (1/4, *v*/*v*, 2× 5 min), followed by washing with NMP, DCM and again NMP (NMP 3 times each, DCM 4 times). For each coupling, 5.45 equivalents of PNA-monomers were used; the solid building blocks were dissolved in stock solutions of the coupling reagents 5 min prior to addition of the bases. A mixture of HATU/HOAt (5.31 eq. / 5.45 eq.) in NMP was used as activator for coupling. DIEA and 2,6-lutidine (5,45 eq. and 8,17 eq.) were used as bases. The initial concentration of building blocks and HOAt in the reaction mixture were adjusted to 0.27 M, respectively. The concentration of HATU was adjusted to 0.26 M. The concentrations of bases were 0.27 M and 0.41 M for DIEA and 2,6-lutidine, respectively. 60 min coupling time were employed. For all PNA building blocks double couplings at RT were performed. After successive washing with NMP, DCM and again NMP (3 times each), capping with the mixture of Ac_2O/2,6-lutidine/NMP (1/2/7, vol%, 2×5 min) was performed. A final washing step with DIEA/NMP (5/95, *v*/*v*), DCM and NMP (3 times each) completed the coupling cycle.

In case of labelled PNA/peptide hybrids the fluorescence label NBD was attached to the N-terminal free amino group after synthesis. 4-Fluoro-7-nitrobenzofurazan (NBD-F) (25.0 µmol, 5 eq., 100 mM) and DIEA (125 µmol, 25 eq., 500 mM) in NMP were mixed and added to the resin to yield 250 µL reaction volume. The corresponding mixtures were shaken overnight under exclusion of light. The resins were filtered off, washed with DCM, NMP, MeOH and DCM (5 times each) and dried *in vacuo*.

Cleavage from the solid support for PNA/peptide hybrids was carried out within 2 h using TFA/TIS/EDT/*m*-cresol (87.5/2.5/5.0/5.0, vol%, 1 mL). After filtration, the filtrate was concentrated by using a nitrogen stream and the peptides were precipitated as white solids by addition of cold (-20 °C) diethylether. For compounds PNA1/SybK, PNA2/SxK and PNA1/SxK LC-MS runs using a *Thermo* (HPLC separation) and *Finnigan* (ESI-MS analysis) instrument were performed in addition to ESI analysis. A *Phenomenex MAX-RP Synergi* column (RP-C18, 150 mm x 2 mm, 4 µm) applying a linear gradient of eluent A (0.1 % TFA in H_2O) to B (0.1 % TFA in MeCN) was used. All compounds were used without purification.

3) Analytic data for the synthesized PNA/peptide hybrids

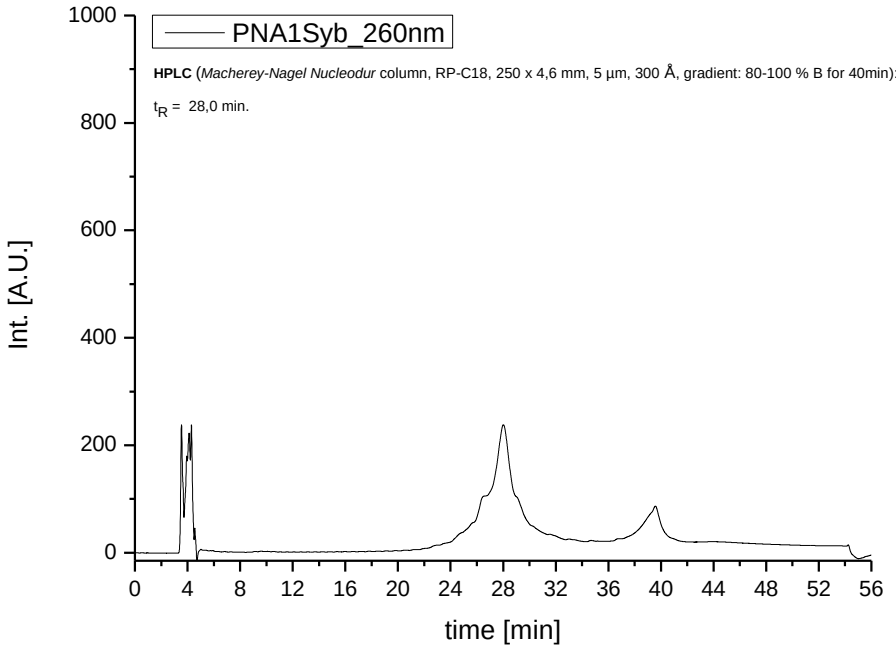
PNA1/Syb: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFST-OH

[$C_{298}H_{441}N_{99}O_{68}S_3$, 6594.52]

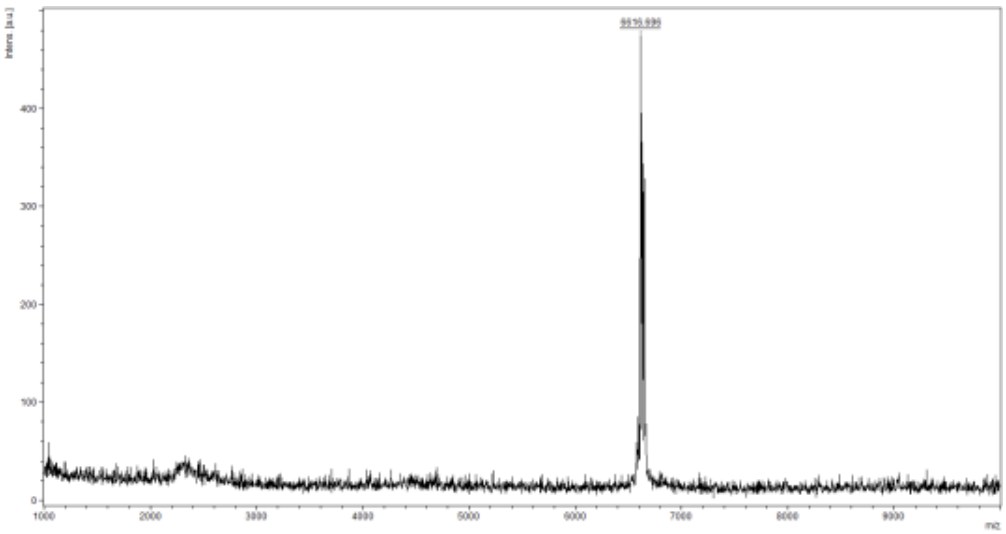
MS (ESI) *m/z*: 942.92 $[M+7H]^{7+}$, 1100.07 $[M+6H]^{6+}$, 1319.88 $[M+5H]^{5+}$, 6594.38 $[M]^{+}$

HR-MS (ESI) *m/z*: calcd. for $C_{298}H_{447}N_{99}O_{68}S_3$ $[M+6H]^{6+}$: 1100.0632, found: 1100.0639

calcd. for $C_{298}H_{446}N_{99}O_{68}S_3$ $[M+5H]^{5+}$: 1319.8744, found: 1319.8751.



MALDI-MS (Reflection positive mode)



PNA1/SybK: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFSK-OH

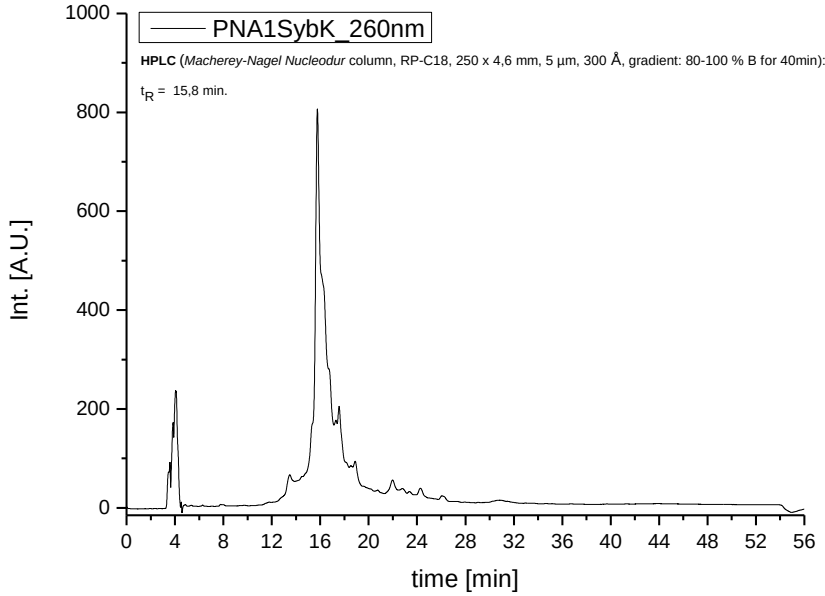
[$C_{300}H_{446}N_{100}O_{67}S_3$, 6621.60]

LC-MS (*Phenomenex MAX-RP Synergi* column, RP-C18, 150 mm x 2 mm, 4 µm, gradient: 5 to 70 % of solvent B for 30 min): t_R = 19.92 – 20.09 min.

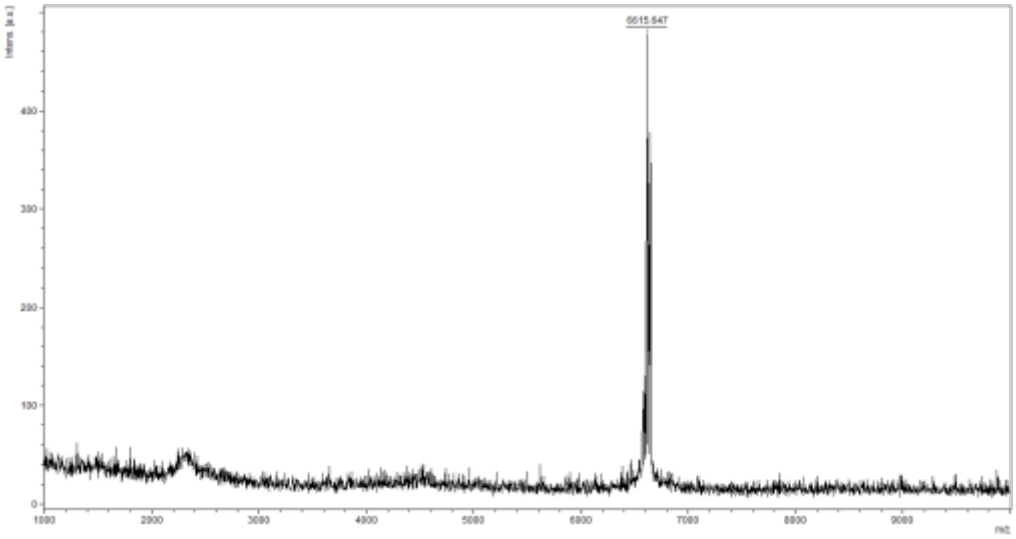
(ESI) *m/z*: 1104.66 $[M+6H]^{6+}$, 1325.12 $[M+5H]^{5+}$, 1655.94 $[M+4H]^{4+}$.

MS (ESI) *m/z*: 946.92 $[M+7H]^{7+}$, 1003.28 $[M+6H]^{6+}$, 1325.29 $[M+5H]^{5+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{300}H_{453}N_{100}O_{67}S_3$ $[M+7H]^{7+}$ 946.9191, found 946.9188.



MALDI-MS (Reflection positive mode)



PNA1/SybE: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFSE-OH

[$C_{299}H_{441}N_{99}O_{69}S_3$, 6622.54]

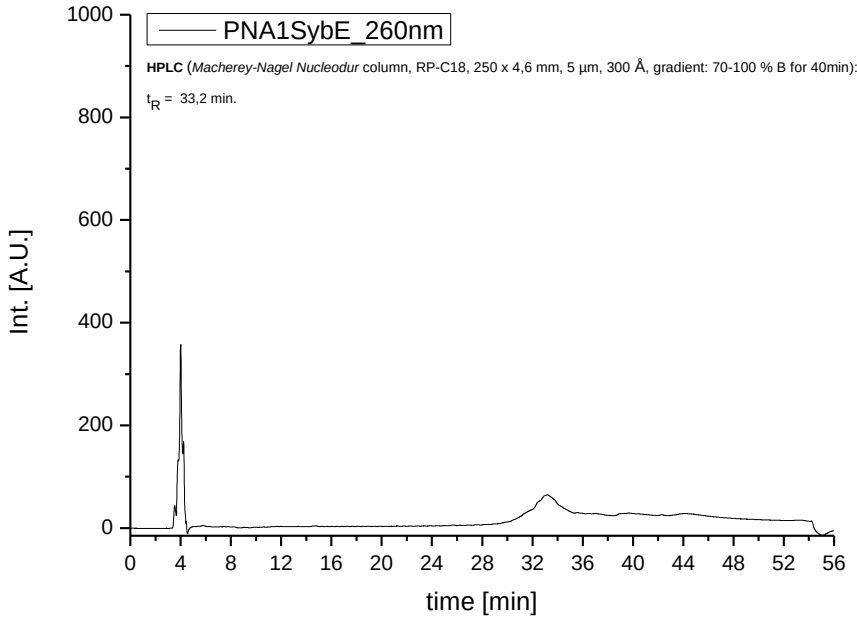
MS (ESI) *m/z*: 1104.7 $[M+6H]^{6+}$, 1325.5 $[M+5H]^{5+}$, 1656.6 $[M+4H]^{4+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{299}H_{448}N_{99}O_{69}S_3$ $[M+7H]^{7+}$ 947.0545, found 947.0532;

calcd. for $C_{299}H_{447}N_{99}O_{69}S_3$ $[M+6H]^{6+}$ 1104.7290, found 1104.7321;

calcd. for $C_{299}H_{446}N_{99}O_{69}S_3$ $[M+5H]^{5+}$ 1325.4734, found 1325.4758;

calcd. for $C_{299}H_{445}N_{99}O_{69}S_3$ $[M+4H]^{4+}$ 1656.5899, found 1656.5881.



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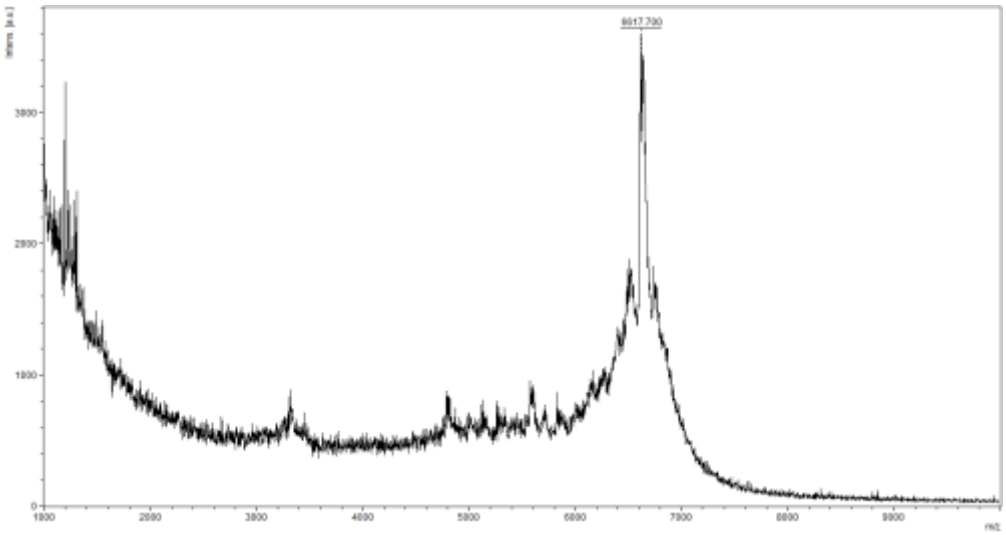


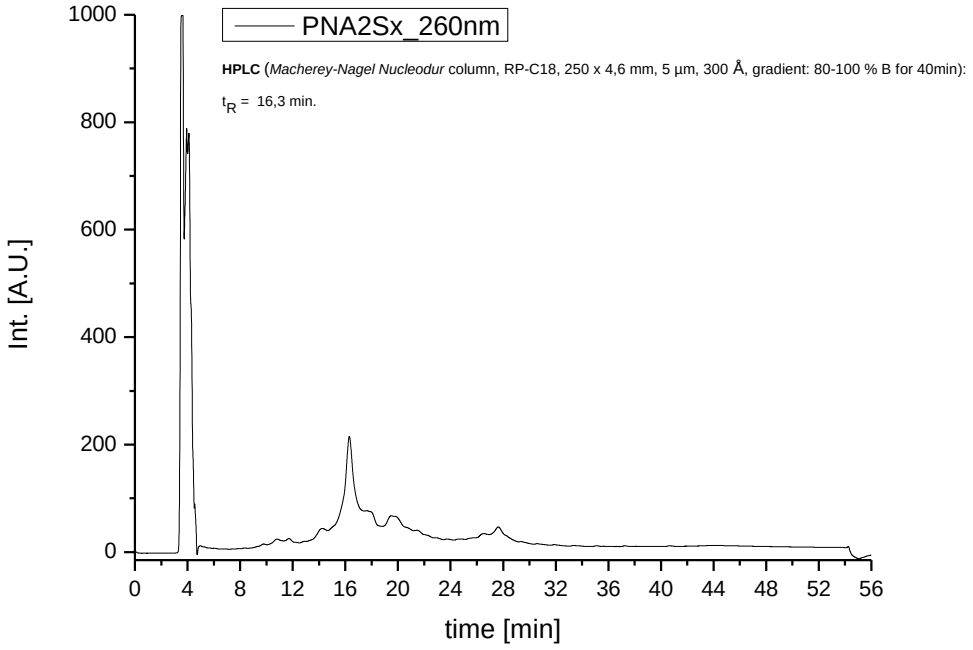
PNA2/Sx: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-OH

[$C_{273}H_{421}N_{101}O_{69}S_3$, 6318.11]

MS (ESI) *m/z*: 790.66 $[M+8H]^{8+}$, 903.46 $[M+7H]^{7+}$, 1053.87 $[M+6H]^{6+}$, 6317.18 $[M]^{+}$

HR-MS (ESI) *m/z*: calcd. for $C_{273}H_{428}N_{101}O_{69}S_3$ $[M+7H]^{7+}$: 903.4612, found: 903.4603;

calcd. for $C_{273}H_{427}N_{101}O_{69}S_3$ $[M+6H]^{6+}$: 1053.8702, found: 1053.8694.



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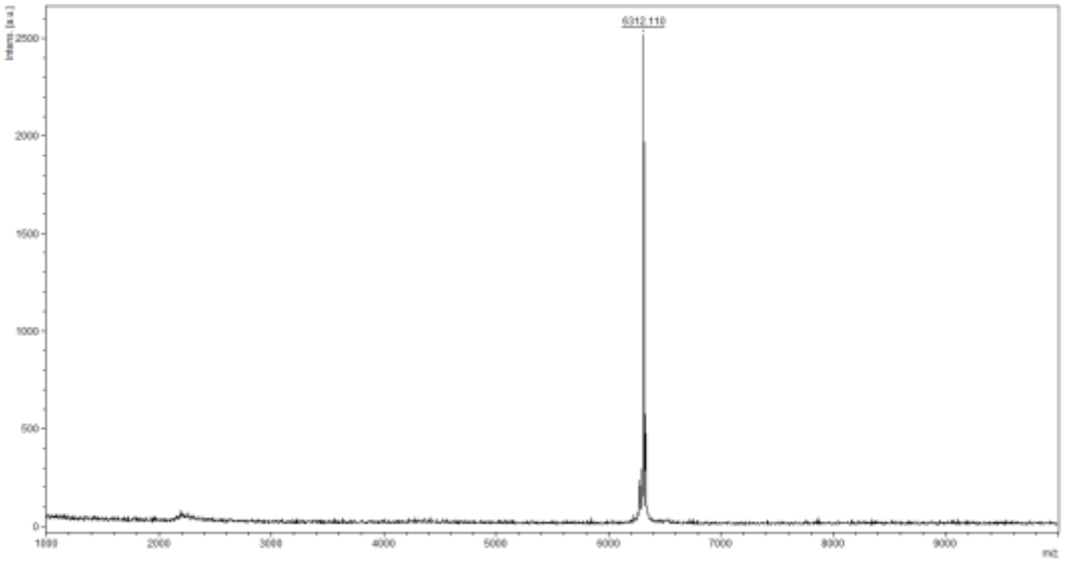


PNA2/SxK: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFK-OH

[$C_{277}H_{430}N_{102}O_{69}S_3$, 6389.23]

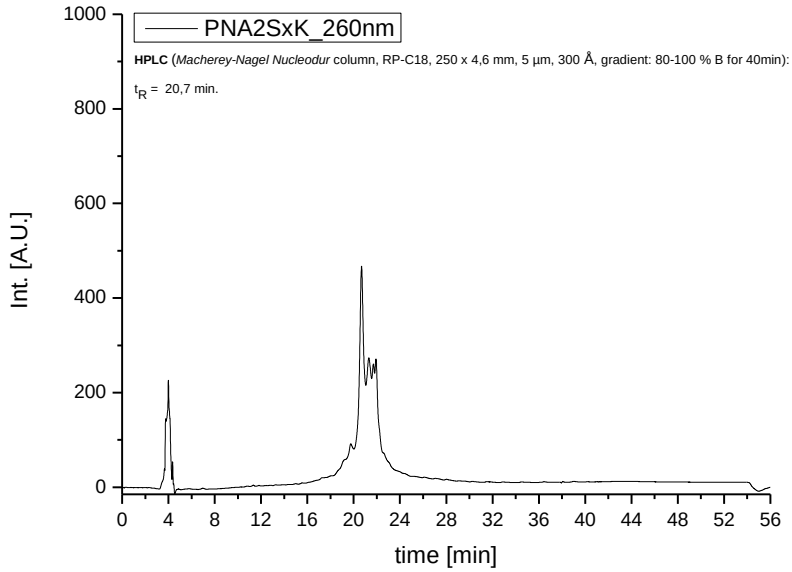
LC-MS (*Phenomenex MAX-RP Synergi* column, RP-C18, 150 mm x 2 mm, 4 µm, gradient: 5 to 70 % of solvent B for 30 min): t_R = 15.50 – 15.82 min.

(ESI) *m/z*: 799.57 $[M+8H]^{8+}$, 913.86 $[M+7H]^{7+}$, 1065.85 $[M+6H]^{6+}$, 1278.25 $[M+5H]^{5+}$, 1598.74 $[M+4H]^{4+}$.

MS (ESI) *m/z*: 799.5 $[M+8H]^{8+}$, 913.8 $[M+7H]^{7+}$, 1065.9 $[M+6H]^{6+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{277}H_{437}N_{102}O_{69}S_3$ $[M+7H]^{7+}$ 913.6146, found 913.6148;

calcd. for $C_{277}H_{436}N_{102}O_{69}S_3$ $[M+6H]^{6+}$ 1065.7158, found 1065.7145.



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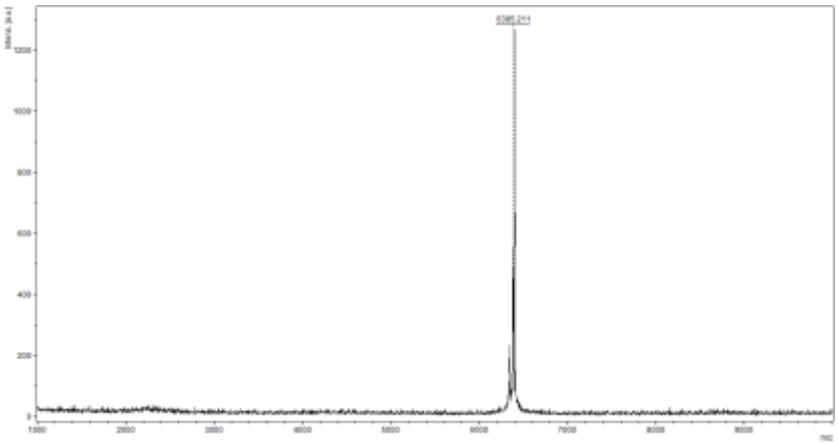
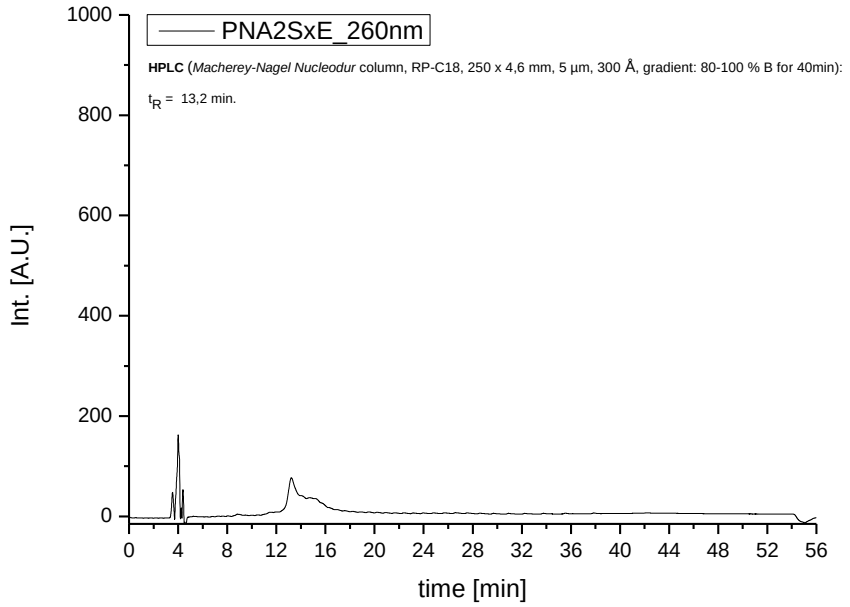


PNA2/SxE: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFE-OH

[$C_{276}H_{425}N_{101}O_{71}S_3$, 6390.18]

MS (ESI) *m/z*: 1065.9 $[M+6H]^{6+}$, 1279.0 $[M+5H]^{5+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{276}H_{431}N_{101}O_{71}S_3$ $[M+6H]^{6+}$ 1065.87, found 1065.87; calcd. for $C_{276}H_{430}N_{101}O_{71}S_3$ $[M+5H]^{5+}$ 1278.85, found 1278.85.



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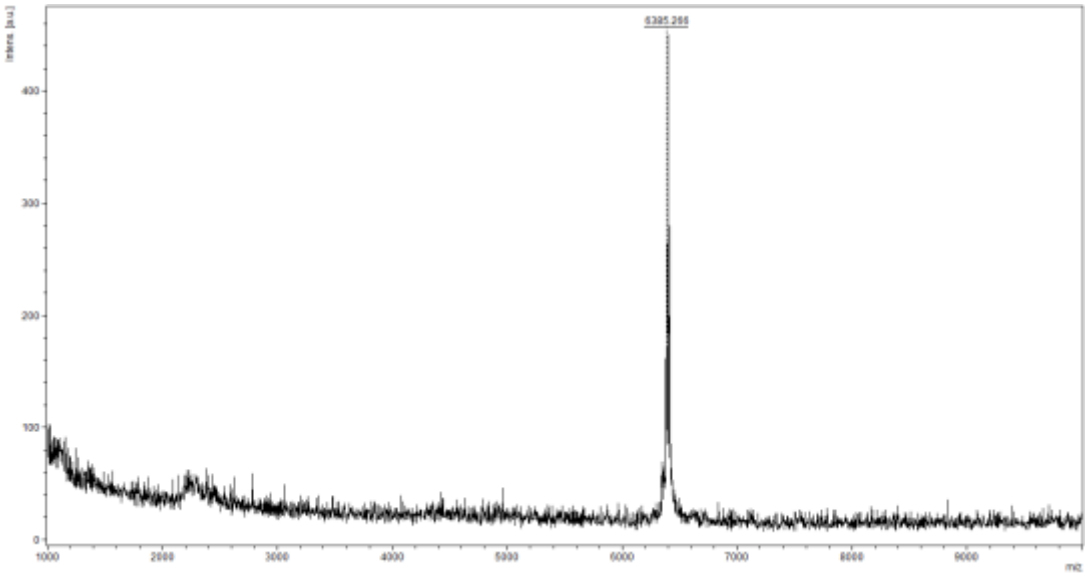


PNA1/SxK: H-gtagatcact-KYQSKARRKKIMIIICCVILGIIIASTIGGIFK-OH

[$C_{277}H_{430}N_{102}O_{69}S_3$, 6389.23]

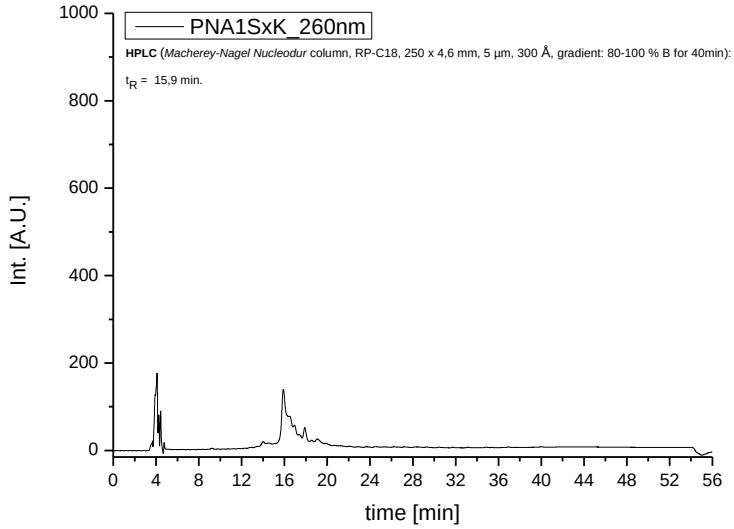
LC-MS (*Phenomenex MAX-RP Synergi* column, RP-C18, 150 mm x 2 mm, 4 µm, gradient: 5 to 70 % of solvent B for 30 min): t_R = 15.53 – 18.82 min.

(ESI) *m/z*: 912.11 $[M+7H]^{7+}$, 1066.28 $[M+6H]^{6+}$.

MS (ESI) *m/z*: 913.76 $[M+7H]^{7+}$, 1065.88 $[M+6H]^{6+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{277}H_{437}N_{102}O_{69}S_3$ $[M+7H]^{7+}$ 913.6146, found 913.6158;

calcd. for $C_{277}H_{436}N_{102}O_{69}S_3$ $[M+6H]^{6+}$ 1065.7158, found 1065.7149.



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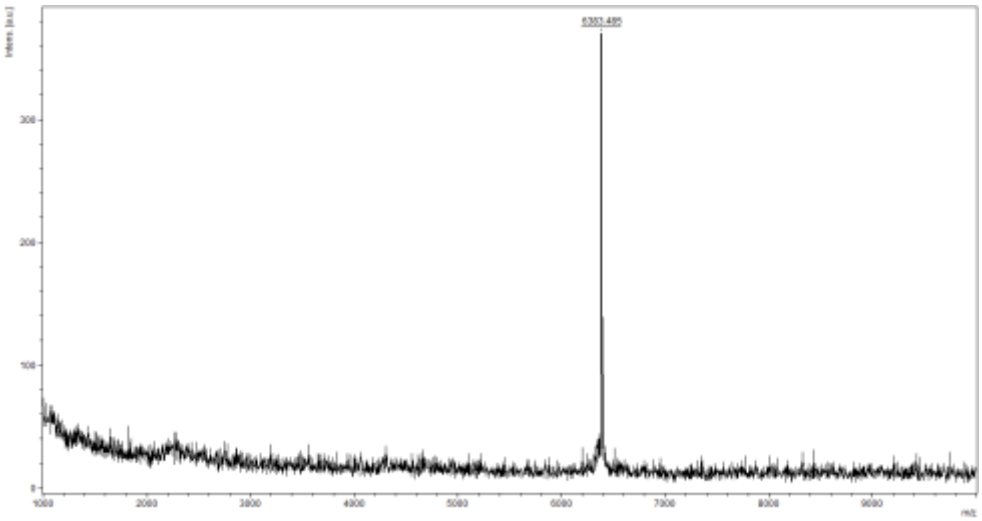


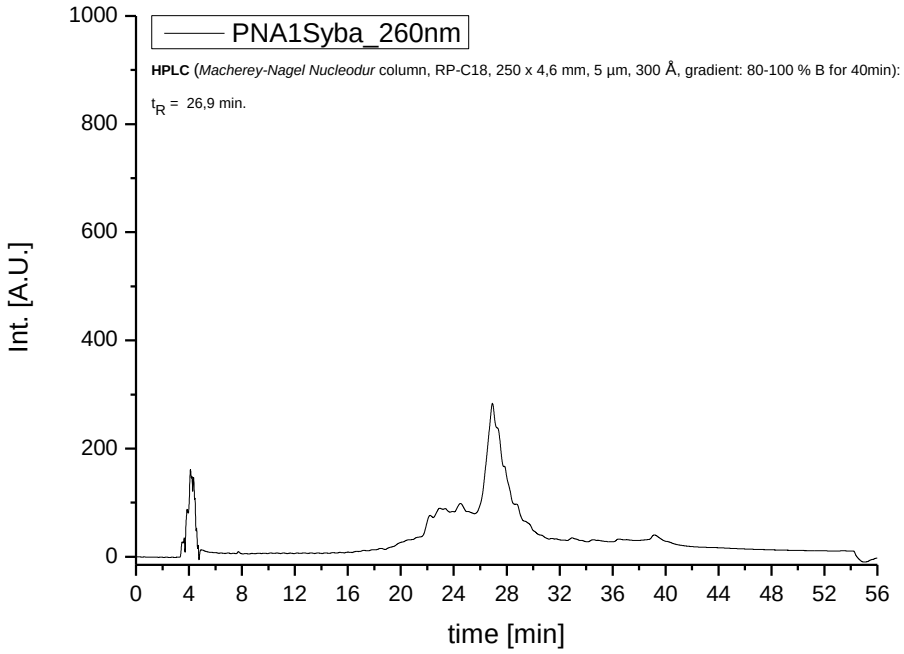
PNA1/Syba: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFST-NH_2

[$C_{298}H_{442}N_{100}O_{67}S_3$, 6593.54]

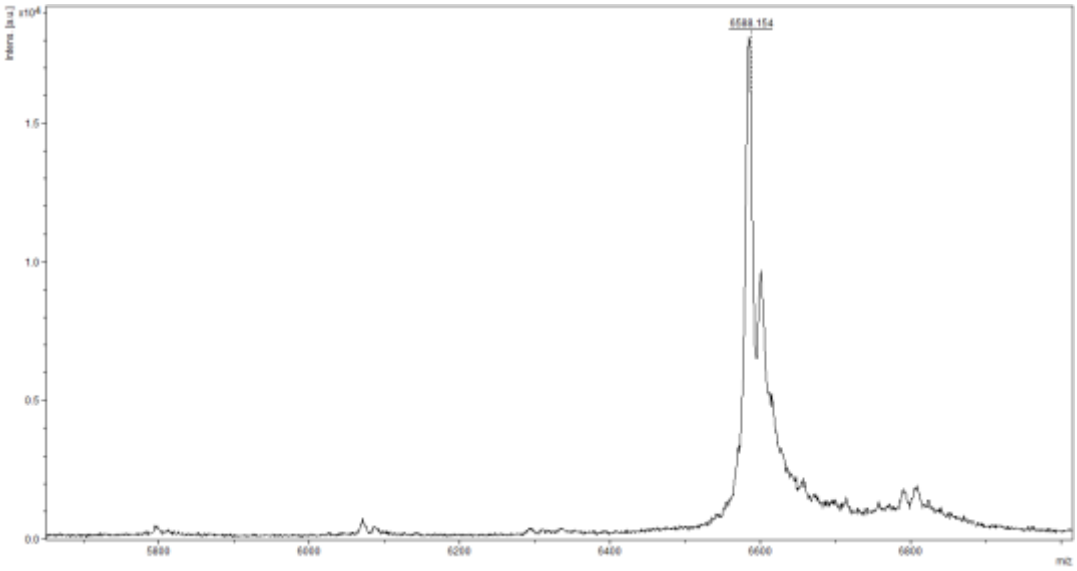
MS (ESI) *m/z*: 943.4 $[M+7H]^{7+}$, 1319.5 $[M+5H]^{5+}$, 1649.1 $[M+4H]^{4+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{298}H_{447}N_{100}O_{67}S_3$ $[M+5H]^{5+}$: 1319.4771, found: 1319.4735

calcd. for $C_{298}H_{446}N_{100}O_{67}S_3$ $[M+4H]^{4+}$: 1649.0946, found: 1649.0933.



MALDI-MS (linear positive mode)



PNA1/SybKa: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFSK-NH_2

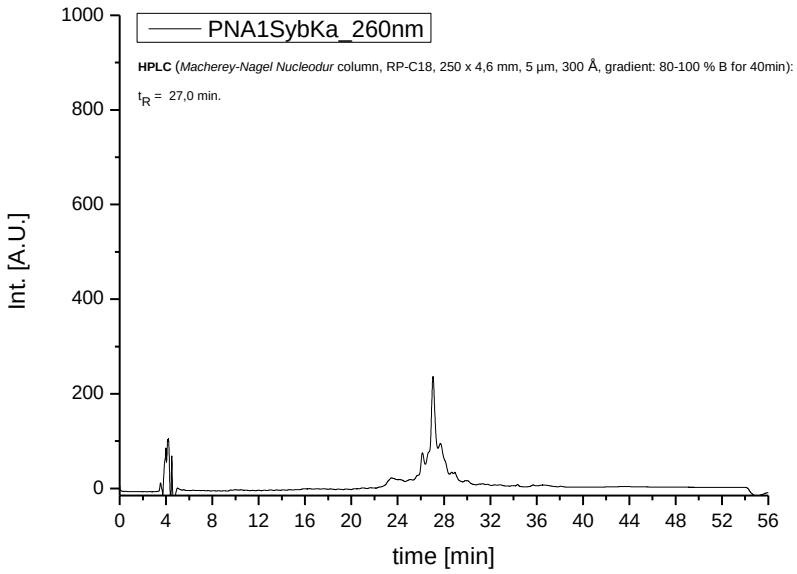
$[C_{300}H_{447}N_{101}O_{66}S_3, 6620.61]$

MS (ESI) *m/z*: 946.8 $[M+7H]^{7+}$, 1104.4 $[M+6H]^{6+}$, 1325.1 $[M+5H]^{5+}$.

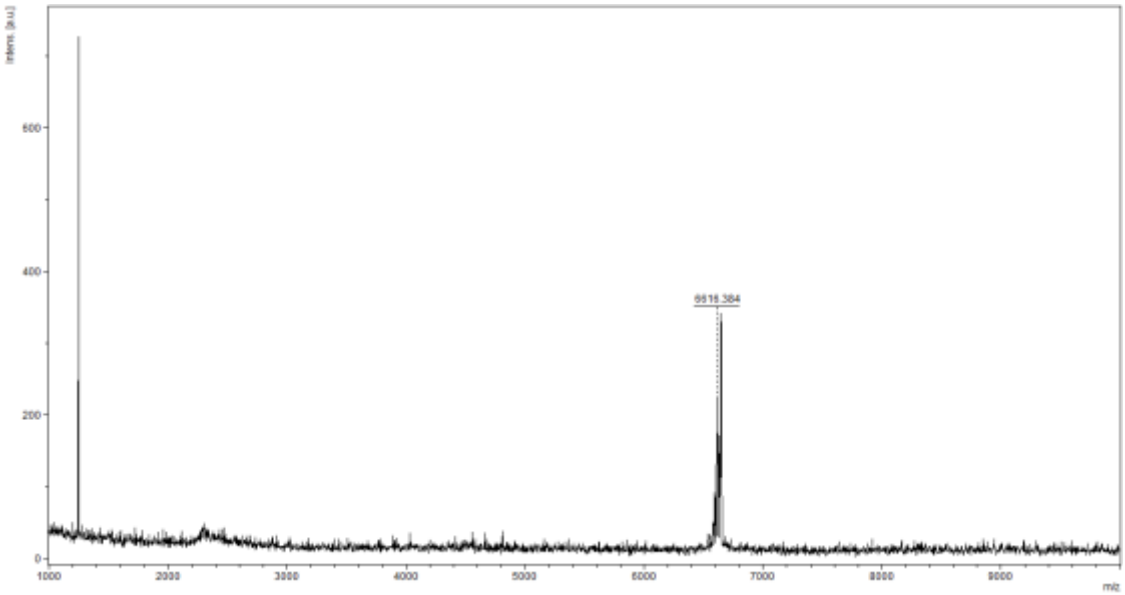
HR-MS (ESI) *m/z*: calcd. for $C_{300}H_{454}N_{101}O_{69}S_3$ $[M+7H]^{7+}$ 946.8, found 946.8;

calcd. for $C_{300}H_{453}N_{101}O_{69}S_3$ $[M+6H]^{6+}$ 1104.4071, found 1104.4079;

calcd. for $C_{300}H_{453}N_{101}O_{69}S_3$ $[M+5H]^{5+}$ 1325.0870, found 1325.0887.



MALDI-MS (Reflection positive mode)



PNA1/SybEa: H-gtagatcact-KRKYWWKNLKMMIILGVICAIILIIIIVYFSE-NH_2

$[C_{299}H_{442}N_{100}O_{68}S_3, 6621.55]$

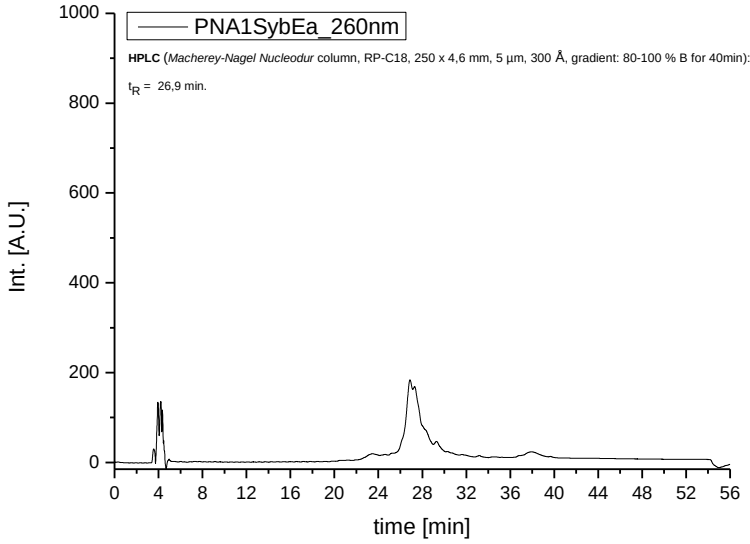
MS (ESI) *m/z*: 1104.6 $[M+6H]^{6+}$, 1325.3 $[M+5H]^{5+}$, 1656.1 $[M+4H]^{4+}$

HR-MS (ESI) *m/z*: calcd. for $C_{299}H_{449}N_{100}O_{68}S_3$ $[M+7H]^{7+}$ 946.9139, found 946.9052;

calcd. for $C_{299}H_{448}N_{100}O_{68}S_3$ $[M+6H]^{6+}$ 1104.5650, found 1104.5680;

calcd. for $C_{299}H_{447}N_{100}O_{68}S_3$ $[M+5H]^{5+}$ 1325.2766, found 1325.2783;

calcd. for $C_{299}H_{446}N_{100}O_{68}S_3$ $[M+4H]^{4+}$ 1656.3439, found 1656.3465.



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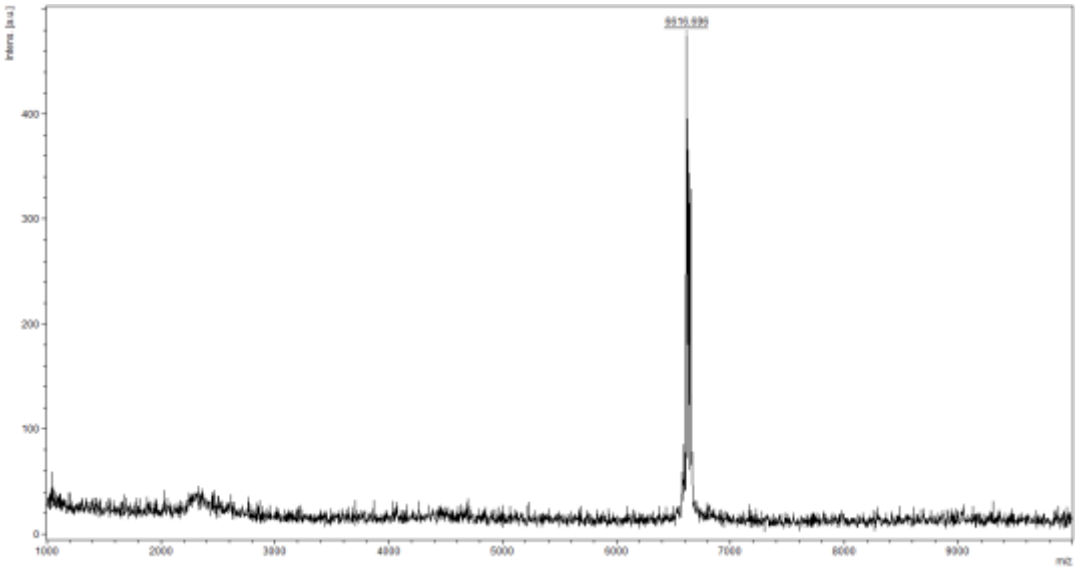


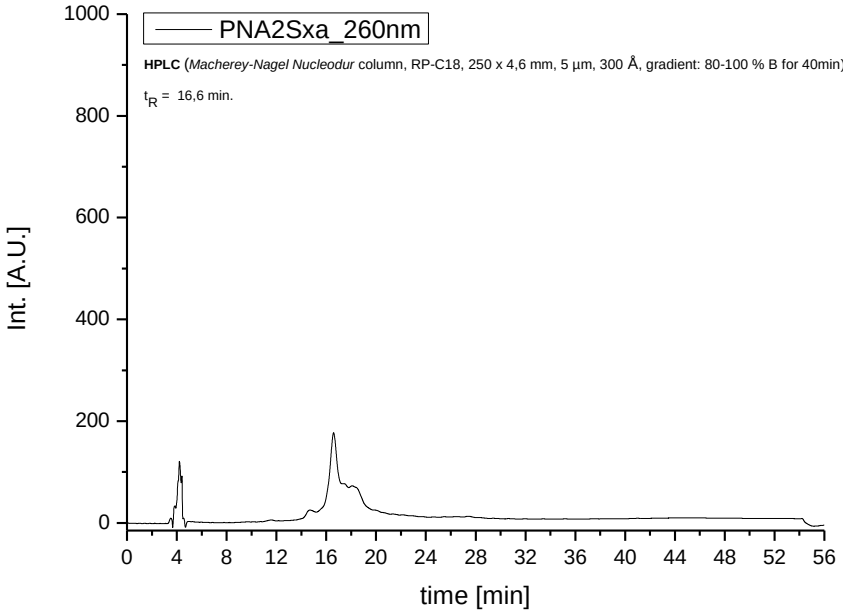
PNA2/Sxa: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFG-NH_2

[$C_{273}H_{422}N_{102}O_{68}S_3$, 6317.13]

MS (ESI) *m/z*: 1053.7 $[M+6H]^{6+}$, 1264.3 $[M+5H]^{5+}$, 1580.1 $[M+4H]^{4+}$, 6316.2 $[M]^{+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{273}H_{428}N_{102}O_{68}S_3$ $[M+6H]^{6+}$: 1053.7062, found: 1053.7044;

calcd. for $C_{273}H_{427}N_{102}O_{68}S_3$ $[M+5H]^{5+}$: 1264.2460, found: 1264.2429.



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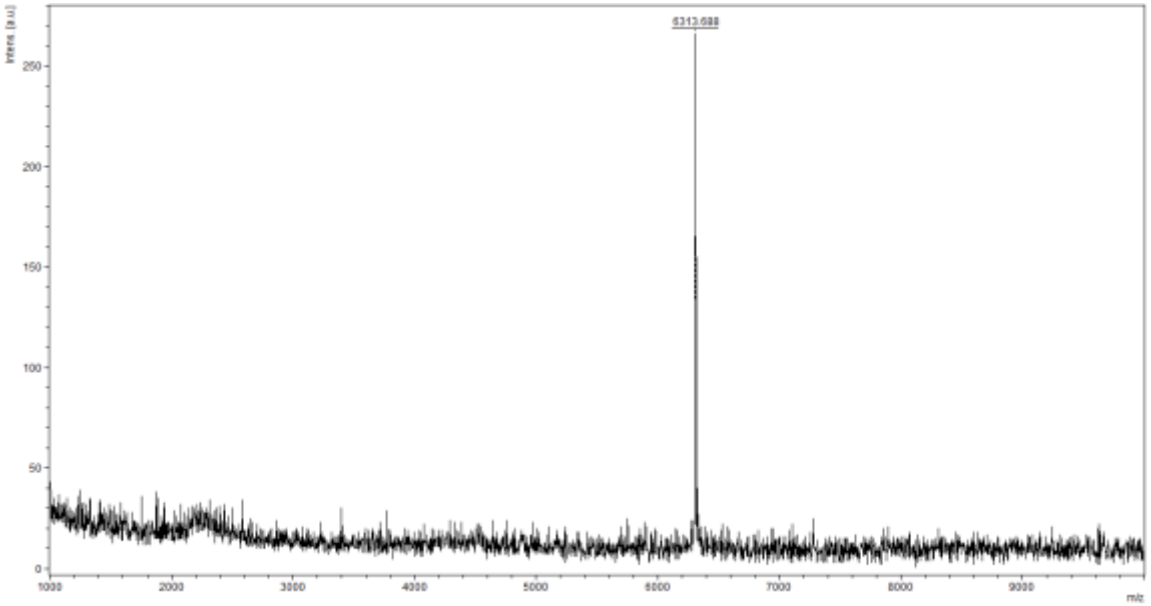
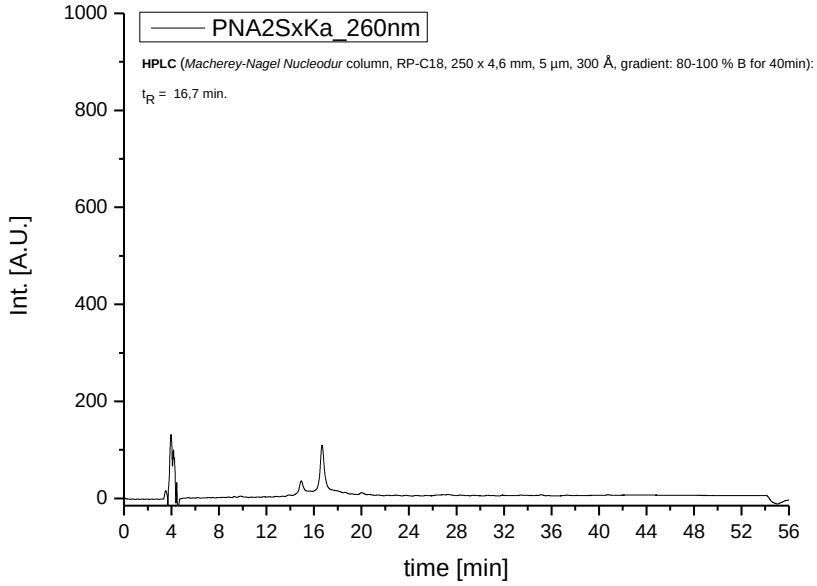


PNA2/SxKa: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFK-NH_2

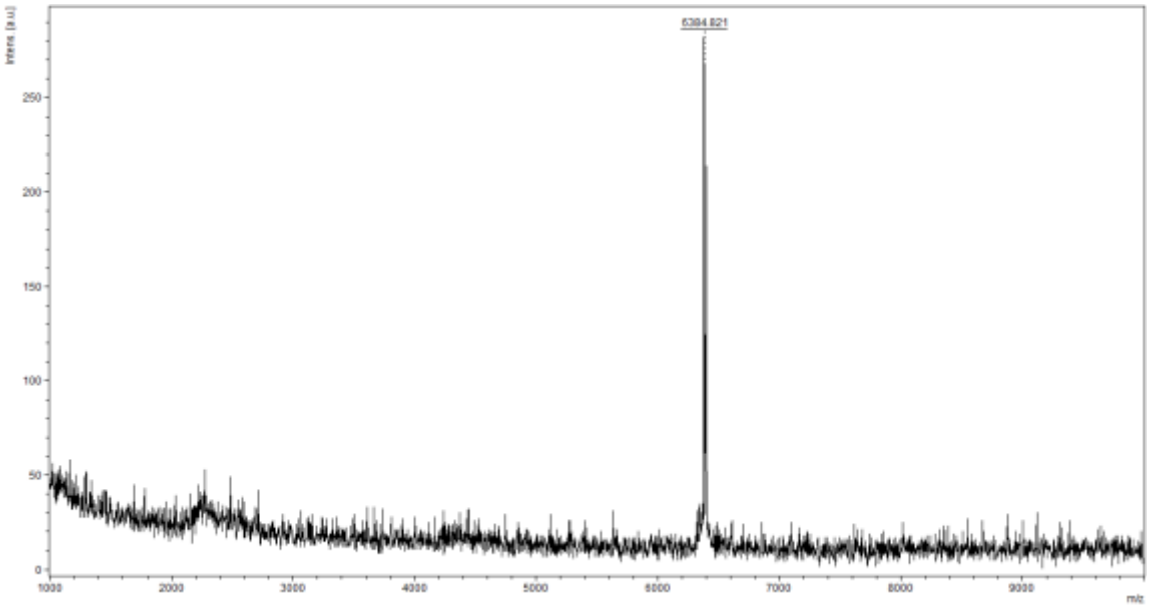
[$C_{277}H_{431}N_{103}O_{68}S_3$, 6388.25]

MS (ESI) *m/z*: 1065.6 $[M+6H]^{6+}$, 1278.5 $[M+5H]^{5+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{277}H_{437}N_{103}O_{68}S_3$ $[M+6H]^{6+}$ 1065.5518, found 1065.5703; calcd. for $C_{277}H_{436}N_{103}O_{68}S_3$ $[M+5H]^{5+}$ 1278.4607, found 1278.4623.



MALDI-MS (Reflection positive mode)



PNA2/SxEa: H-catctagtga-KYQSKARRKKIMIIICCVILGIIIASTIGGIFE-NH_2

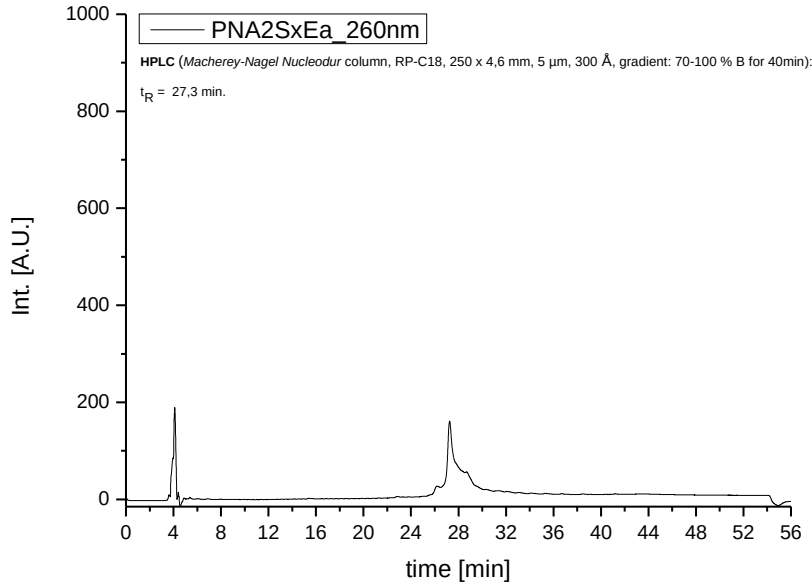
[$C_{276}H_{426}N_{102}O_{70}S_3$, 6389.19]

MS (ESI) *m/z*: 913.6 $[M+7H]^{7+}$, 1065.9 $[M+6H]^{6+}$, 1278.7 $[M+5H]^{5+}$.

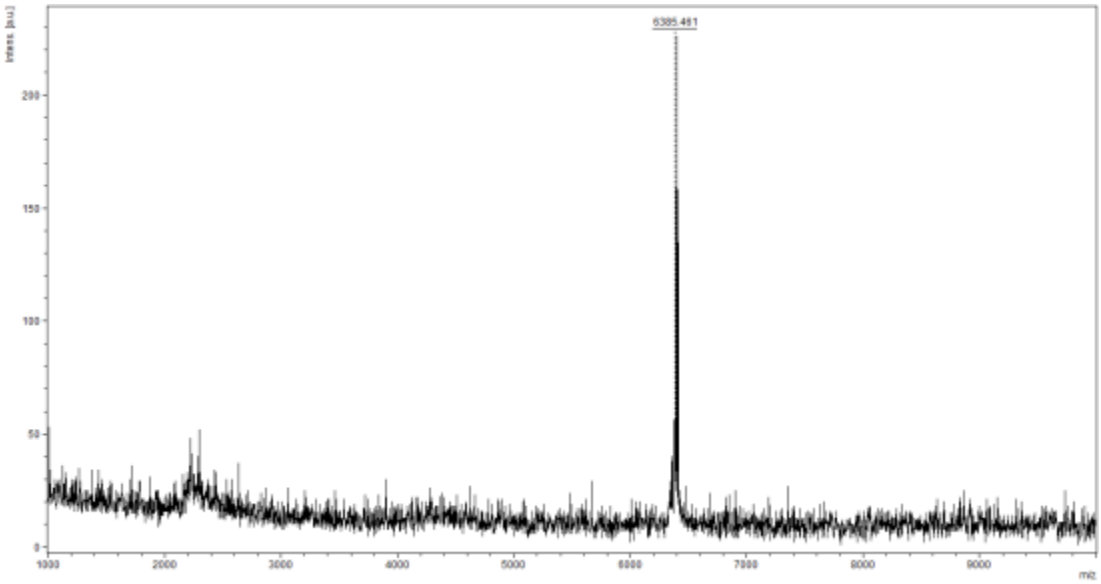
HR-MS (ESI) *m/z*: calcd. for $C_{276}H_{433}N_{102}O_{70}S_3$ $[M+7H]^{7+}$ 913.6094, found 913.6102;

calcd. for $C_{276}H_{432}N_{102}O_{70}S_3$ $[M+6H]^{6+}$ 1065.7097, found 1065.7104;

calcd. for $C_{276}H_{431}N_{102}O_{70}S_3$ $[M+5H]^{5+}$ 1278.6502, found 1278.6580.



MALDI-MS (Reflection positive mode)

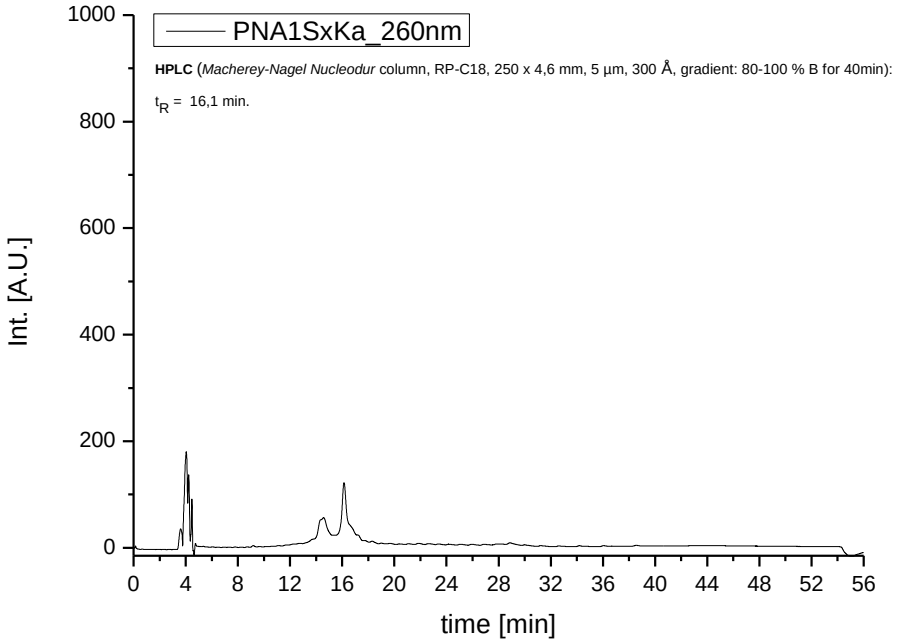


PNA1/SxKa: H-gtagatcact-KYQSKARRKKIMIIICCVILGIIIASTIGGIFK-NH_2

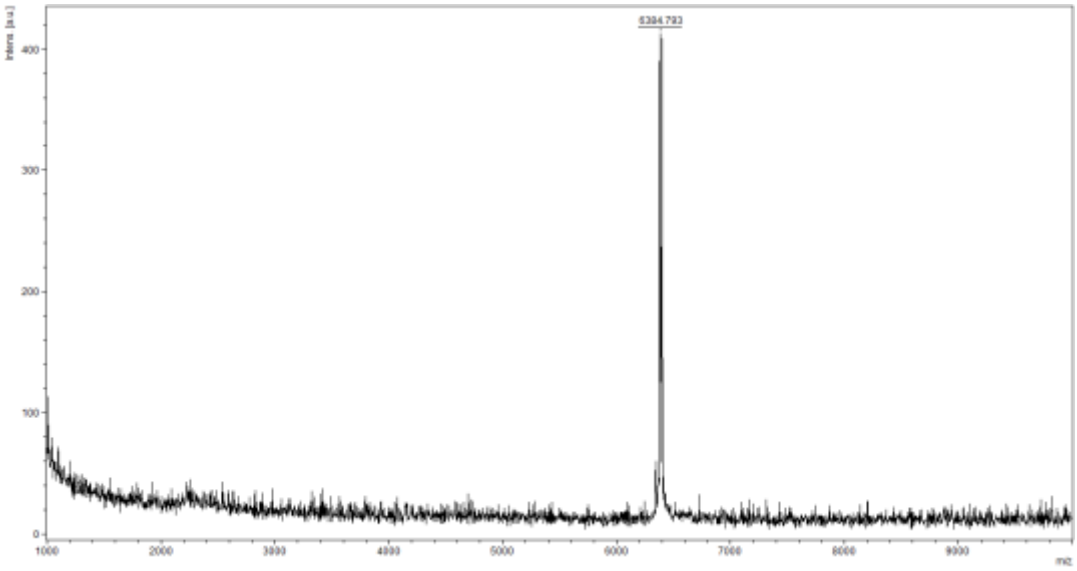
[$C_{277}H_{431}N_{103}O_{68}S_3$, 6388.25]

MS (ESI) *m/z*: 1065.6 $[M+6H]^{6+}$, 1278.5 $[M+5H]^{5+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{277}H_{437}N_{103}O_{68}S_3$ $[M+6H]^{6+}$ 1065.5518, found 1065.5635; calcd. for $C_{277}H_{436}N_{103}O_{68}S_3$ $[M+5H]^{5+}$ 1278.4607, found 1278.4608.



MALDI-MS (Reflection positive mode)

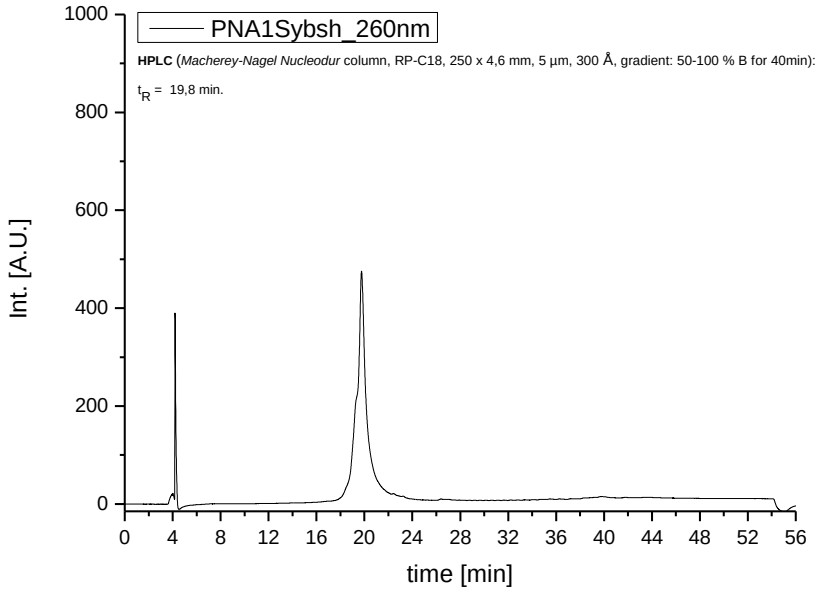


PNA1/Sybsh: H-gtagatcact-KRKYWWKNLKMMIILGVI-NH_2

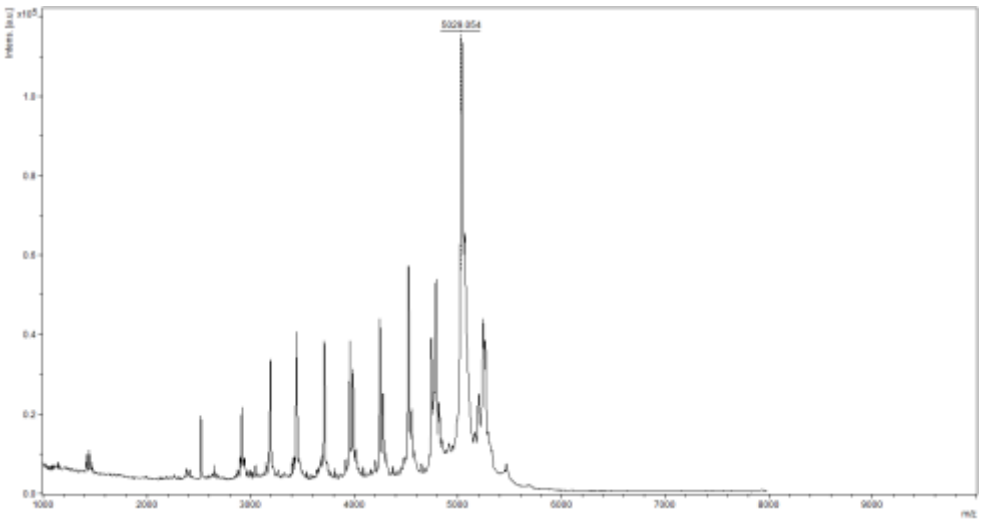
$[C_{220}H_{316}N_{86}O_{50}S_2, 5029.65]$

MS (ESI) *m/z*: 719.36 $[M+7H]^{7+}$, 838.92 $[M+6H]^{6+}$, 1006.70 $[M+5H]^{5+}$, 1257.87 $[M+4H]^{4+}$, 1676.82 $[M+3H]^{3+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{220}H_{322}N_{86}O_{50}S_2$ $[M+6H]^{6+}$ 838.7451, found 838.7458; calcd. for $C_{220}H_{321}N_{86}O_{50}S_2$ $[M+5H]^{5+}$ 1006.4932, found 1006.4927.



MALDI-MS (linear positive mode)

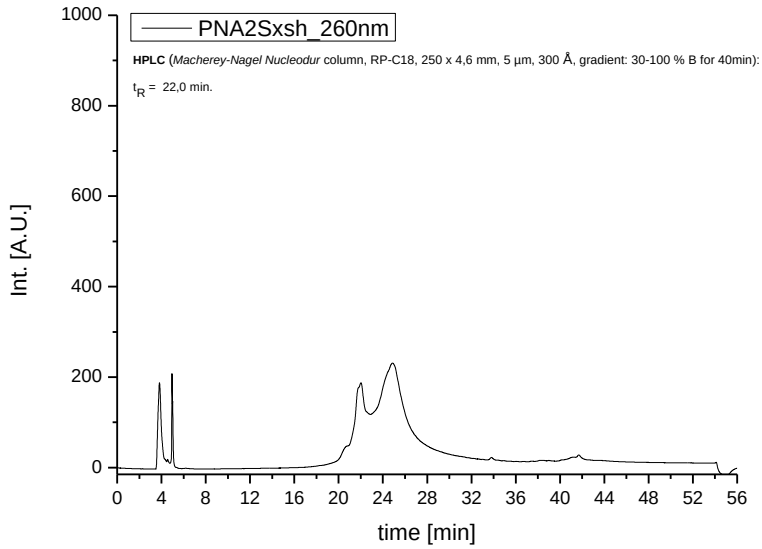


PNA2/Sxsh: H-catctagtga-KYQSKARRKKIMIIICC-NH_2

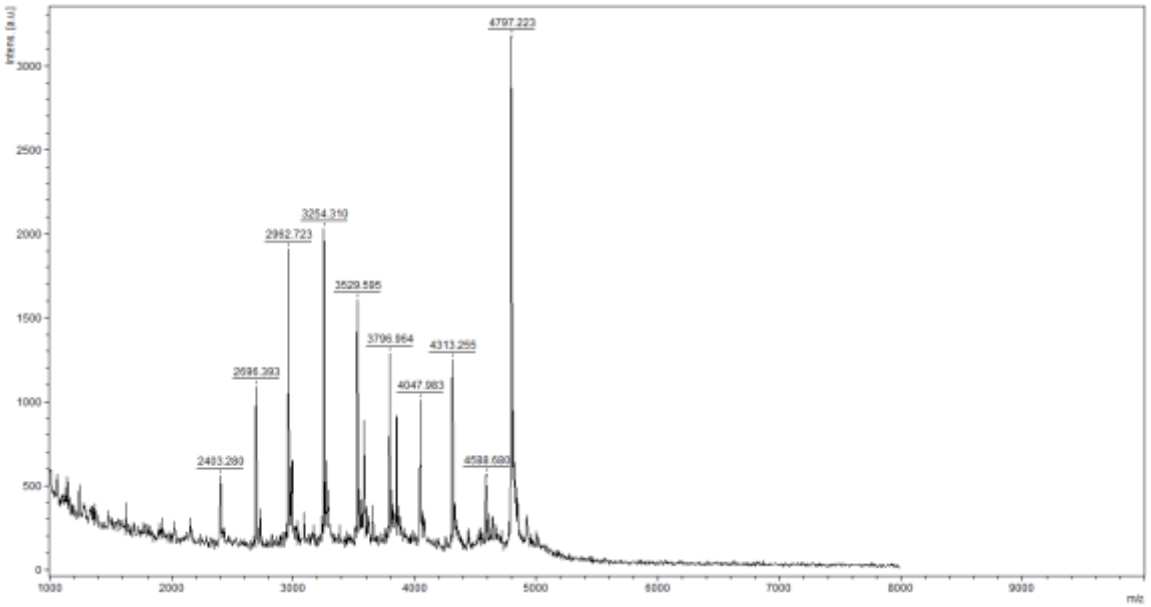
$[C_{199}H_{298}N_{86}O_{50}S_3, 4791.33]$.

MS (ESI) *m/z*: 799.22 $[M+6H]^{6+}$, 958.86 $[M+5H]^{5+}$, 1198.32 $[M+4H]^{4+}$.

HR-MS (ESI) *m/z*: calcd. for $C_{199}H_{304}N_{86}O_{50}S_3$ $[M+6H]^{6+}$ 799.0503, found 799.0494; calcd. for $C_{199}H_{303}N_{86}O_{50}S_3$ $[M+5H]^{5+}$ 958.6589, found 958.6596.



MALDI-MS (Reflection positive mode)



4) Preparation of peptide associated liposomes

Large unilamellar vesicles (LUVs) from the mixture of 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and cholesterol (50/25/25 mol%) were prepared in buffer solution (20 mM HEPES, 100 mM KCl, 1 mM EDTA, 1 mM DTT, pH = 7.4) following methods of MacDonald et al.[1] DOPC, DOPE and cholesterol dissolved in $CHCl_3$ (~20 mg/mL each) and the PNA/peptide constructs dissolved in trifluorethanol (TFE) were mixed yielding a solution in $CHCl_3$/TFE (1/1, *v*/*v*). Concentrations of PNA/peptide stock solutions were determined by UV absorption (see chapter 5). Removing the solvents in a nitrogen stream at 50 °C resulted in almost clear lipid/peptide films at the test tube walls. After removing of residual solvent under reduced pressure at 50 °C for 12 h, the lipid films were rehydrated with buffer solution. After 1 – 2 h of incubation at 50 °C, the hydrated lipid films were treated in a sonication bath to remove rests of lipid/peptide films from test tube walls. Afterwards, probes were vortexed. The milky suspensions were extruded 31 times through a polycarbonate membrane (100 nm nominal pore size) using a mini-extruder (*Liposofast*, *Avestin*, Ottawa, Canada) to produce an almost clear vesicle suspension. In the case of content mixing assays the lipid/peptide films were extruded with labelling buffer containing additionally 20 mM Sulforhodamine B (SRB). Because of the osmotically active SRB molecules, it was necessary to adjust the osmolality of the labelling buffer with respect to the reaction buffer.

5) UV spectroscopy

The concentrations of PNA/peptide stock solutions were estimated using a *Jasco V-550* UV spectrometer (Gross-Umstadt, Germany) controlled by a thermostat (*Jasco*, Model *ETC-505T*, Gross-Umstadt, Germany). All measuements were performed at 25 °C and the sample cell was floated with nitrogen. Molar absorption coefficients were calculated for each PNA/peptide hybrid by summation of the monomer molar extinction coefficients at a certain wavelength. Concentrations were calculated using the absorption at 260 nm under denatured conditions [$\varepsilon_{260\,nm}$(c) = 6600 L mol^{-1} cm^{-1}, $\varepsilon_{260\,nm}$(g) = 11700 L mol^{-1} cm^{-1}, $\varepsilon_{260\,nm}$(t) = 8600 L mol^{-1} cm^{-1}, $\varepsilon_{260\,nm}$(a) = 13700 L mol^{-1} cm^{-1}].[2] Concentrations were calculated from *Lambert-Beer's* law.

6) Fluorescence spectroscopy

Fluorescence spectra were obtained at the *Jasco FP 5600* spectrometer (Gross-Umstadt, Germany) with temperature control using a thermostat (Model 1162A, *VWR International*, Darmstadt, Germany). The excitation (460 nm) and emission bandwidths were set to 5 nm, the data pitch was 1 nm and the response time was adjusted to 1 s with the detector sensitivity "high". The emission measurement range was 500 – 610 nm. NBD-DOPE (emission at 535 nm) was used as a fluorescence donor, lissamine rhodamine-DOPE (emission at 585 nm) as an acceptor. The data were recorded at a P/L-ratio of 1/200 in DOPC/DOPE/cholesterol (50/25/25) LUVs.

6a) Lipid mixing assays (time course measurements)

Excitation was performed at 460 nm (bandwidth: 5 nm) with detection of fluorescence emission at 535 nm (bandwidth: 5 nm, detector sensitivity "high"). The measurement time was 1200 s (data pitch: 1 s). NBD/rhodamine-labelled vesicles (3 % of unlabeled DOPE was replaced by 1.5 % NBD-DOPE and 1.5 % lissamine rhodamine-DOPE), containing PNA/Syb-peptide constructs, were mixed with unlabeled vesicles (DOPC/DOPE/cholesterol, 50/25/25 mol%), containing PNA/Sx-peptide constructs at a defined ratio (1 to 4); the total lipid concentration in a cuvette was estimated to be between 150 and 160 µM assuming a lipid concentration of 185 µM before extrusion.[3] The reaction buffer composed of 20 mM HEPES, 100 mM KCl, 1 mM EDTA, 1 mM DTT (pH = 7.4) and the reaction temperature of 25 °C were used. The change in donor intensity is plotted as

$$F(\%) = 100\times(F_t\text{-}F_0)/(F_{total}\text{-}F_0)$$

with F_0 being the donor intensity at $t = 0$ before lipid mixing and F_{total} the donor intensity after disruption of the vesicles by addition of a Triton X-100 (10 vol% in buffer solution) to the reaction solution. The inner leaflet mixing assay was identically performed, except for the fact, that the NBD/rhodamine-labelled vesicles were treated with sodium dithionite as described below and with F_{total} being the donor intensity after treatment of labelled vesicles with Triton X-100/buffer solution without dithionite treatment.

6b) Reduction of NBD/rhodamine-DOPE-labelled vesicles

A 1:1 mixture of NBD/rhodamine-labelled vesicles (5 mM) and sodium dithionite (50 mM) was incubated on ice for 5 min. Free sodium dithionite was removed by gel filtration using a

Superdex G-50 Superfine columns (*GE Healthcare*). Reduction was carried out immediately before the use of the vesicles.

Alternatively, a 2:1 mixture of labelled vesicles (5 mM) and sodium dithionite (50 mM) was incubated at 4 °C for 12 h (oxidation of remaining dithionite by solvent/ air oxygen).

6c) Reduction of NBD-labelled PNA/peptide hybrids

Excitation was performed at 460 nm with a detection of the fluorescence emission at 535 nm. Data were recorded at a P/L-ratio of 1/200 in DOPC/DOPE/cholesterol (50/25/25) LUVs (5 mM), which contained NBD-labeled compounds PNA1/Syb and PNA1/Sx. The fluorescence emission from NBD-labeled vesicles (185 µM) was recorded for 60 sec at 535 nm, then 10 µL of 50 mM sodium dithionite was added followed by 10 µL of 10 vol % Triton X-100 after about 200 sec. The reaction buffer composed of 20 mM HEPES, 100 mM KCl, 1 mM EDTA, 1 mM DTT (pH = 7.4) and the reaction temperature of 25 ºC were used. The change in donor intensity was plotted as

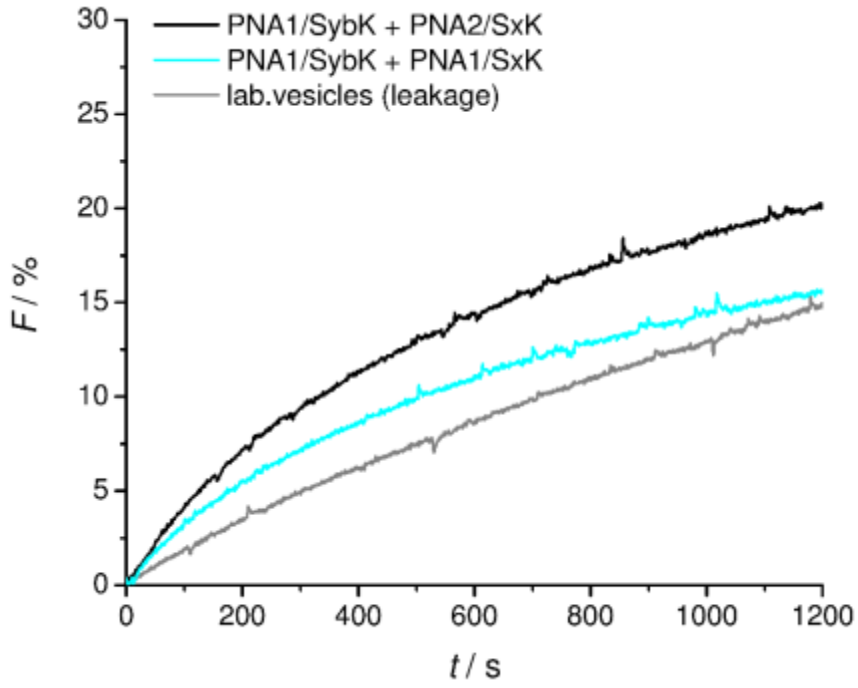
$$F(\%) = 100\times(F_t\text{-}F_{end})/(F_0\text{-}F_{end})$$

with F_0 being the donor intensity at $t = 0$ before addition of sodium dithionite and F_{end} being the donor intensity after disruption of the vesicles with Triton X-100.

6d) Content mixing assay with SRB loaded vesicles

Excitation was performed at 567 nm with detection of SRB emission at 583 nm. PNA1/SybK containing vesicles with self-quenching SRB as cargo were mixed with unlabelled vesicles containing PNA2/SxK or PNA1/SxK constructs (ratio 1 to 4). The total lipid concentration in the cuvette was 54 µM. Other parameters and standardization procedure were the same as described under 5a).

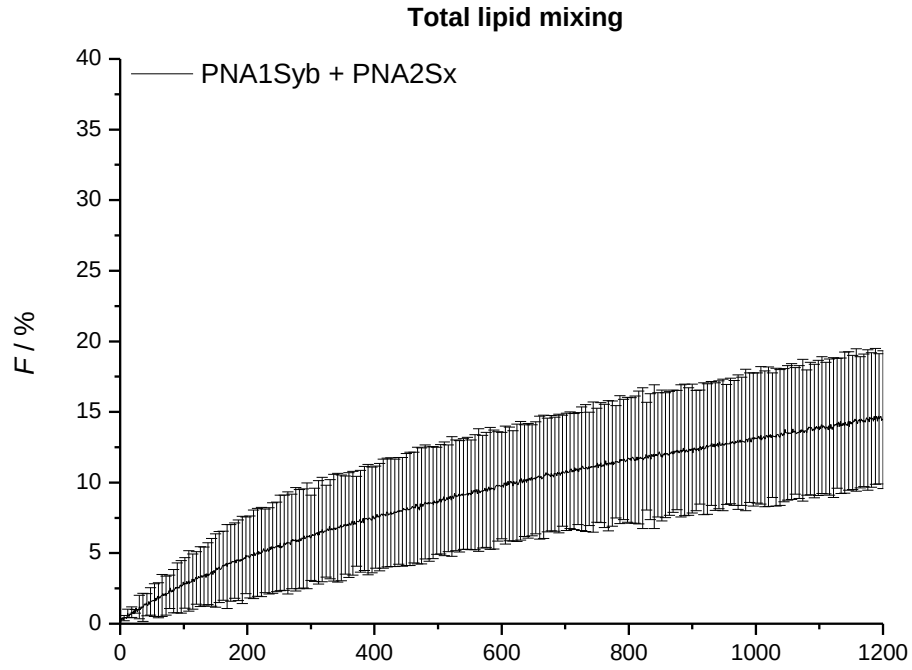
7) Supporting figure



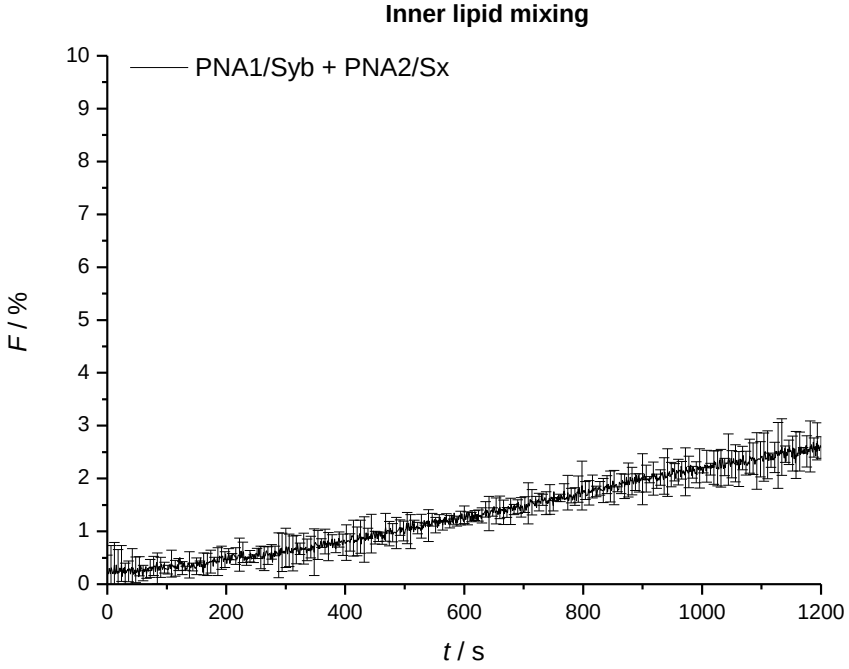
Supporting Figure S1. Content mixing assays with vesicles containing PNA/peptide hybrids with lysine at the negatively charged carboxylic acid C-terminus. Hybrids with parallel PNA strand orientation (PNA1/SybK + PNA2/SxK) show a significantly higher increase in fluorescence of SRB at 583 nm than the non-complementary hybrid combination (PNA1/SybK + PNA1/SxK) indicating mixing of the vesicles' contents. As a control labelled vesicles containing PNA1/SybK without unlabelled vesicles (lab. vesicles) were measured.

8) Fusion lipid mixing experiments including error bars

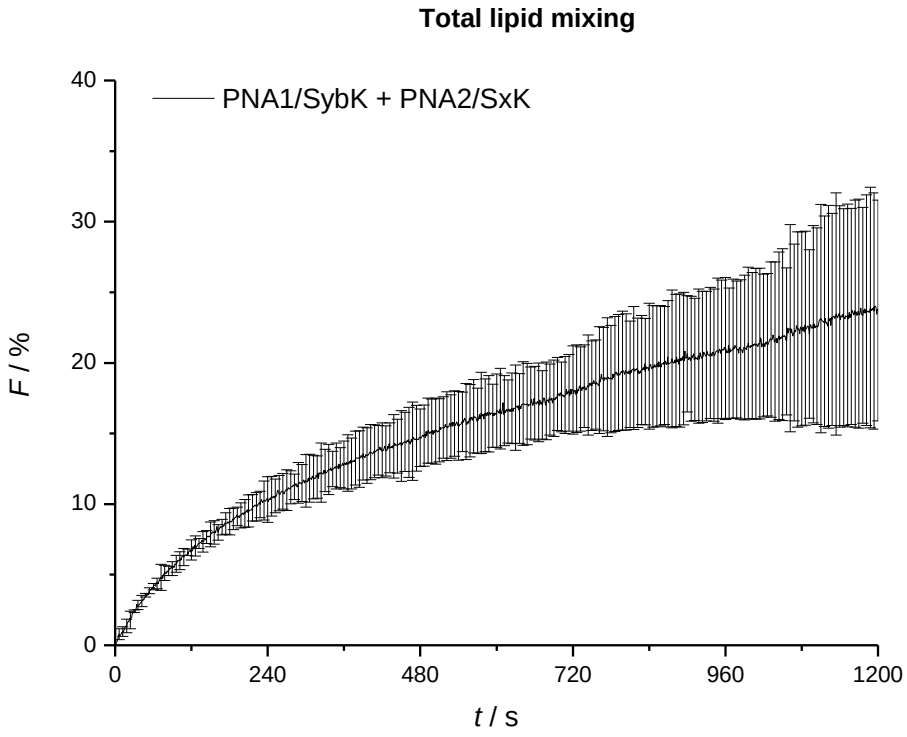
n = 3 (number of measurements), P = 0,95 (probability), t = 3,182 (t-factor)



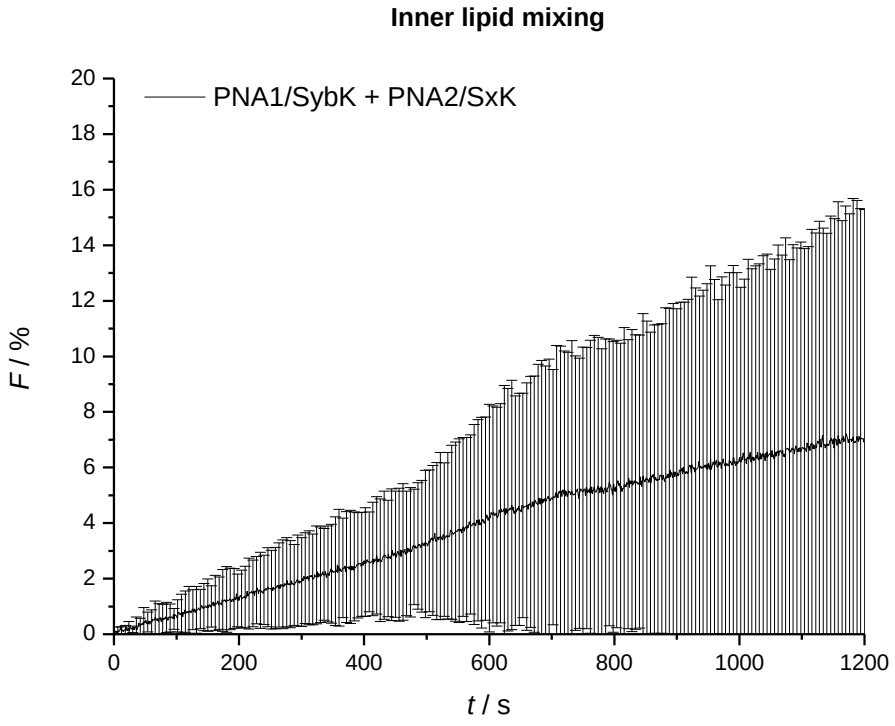
n = 3 (number of measurements), P = 0,95 (probability), t = 3,182 (t-factor)



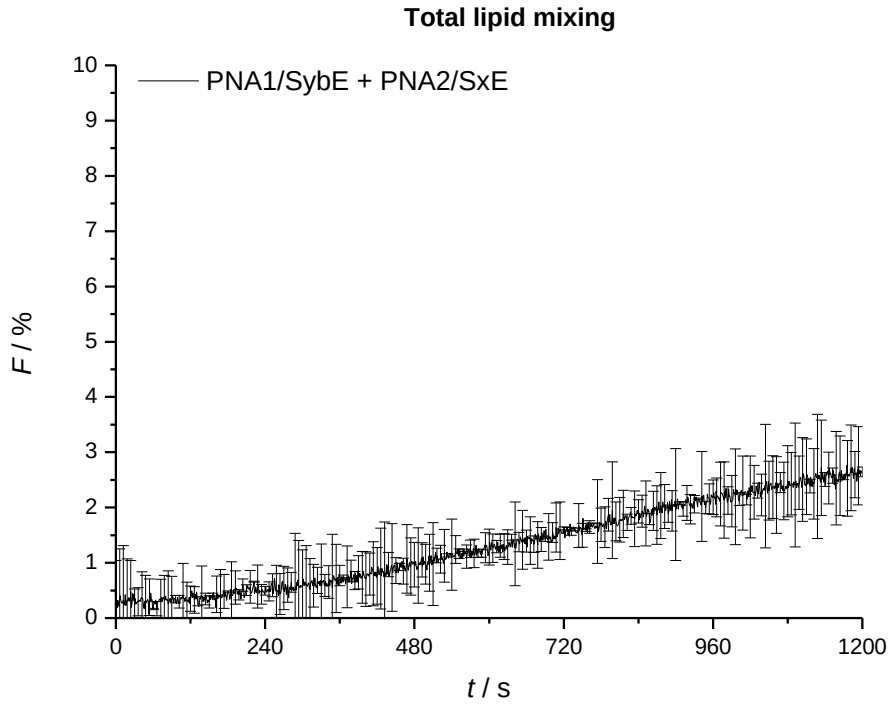
n = 3 (number of measurements), P = 0,95 (probability), t = 3,182 (t-factor)



n = 3 (number of measurements), P = 0,95 (probability), t = 3,182 (t-factor)



n = 2 (number of measurements), P = 0,95 (probability), t = 4,303 (t-factor)



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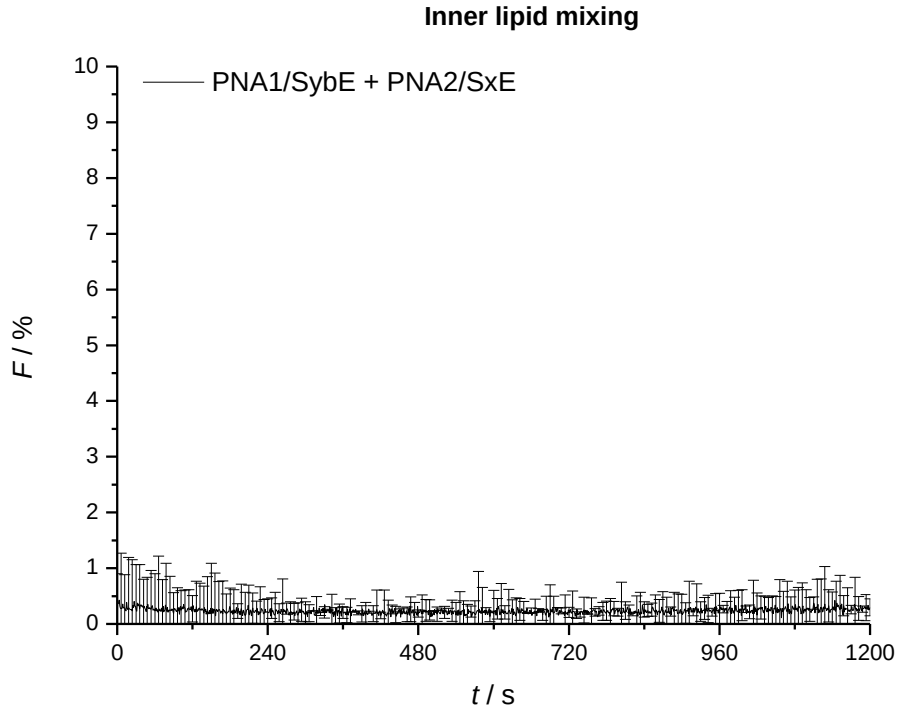
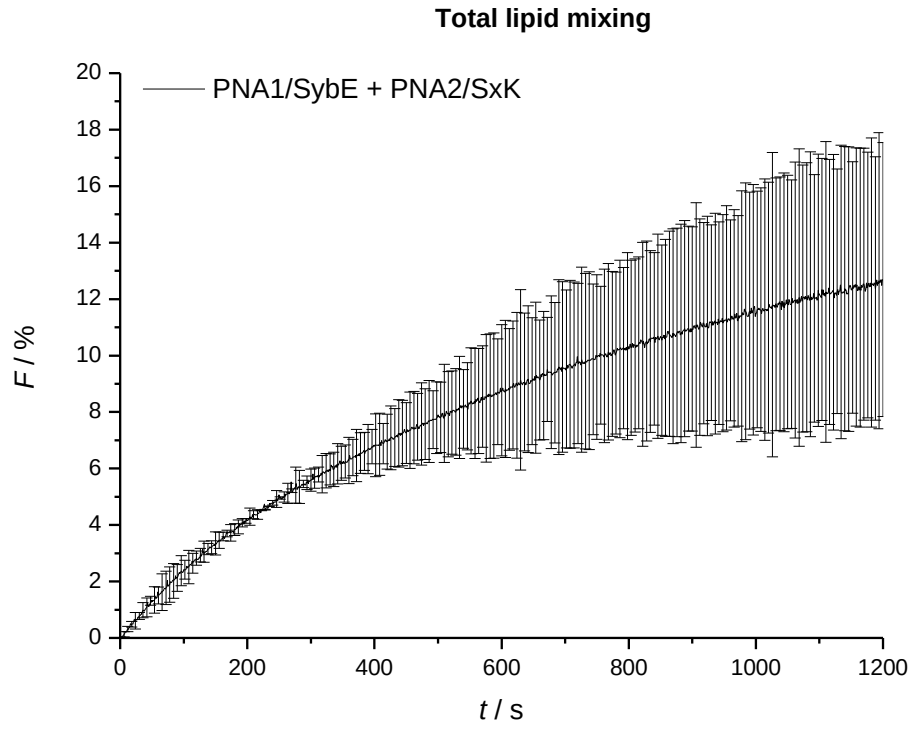


n = 2 (number of measurements), P = 0,95 (probability), t = 4,303 (t-factor)



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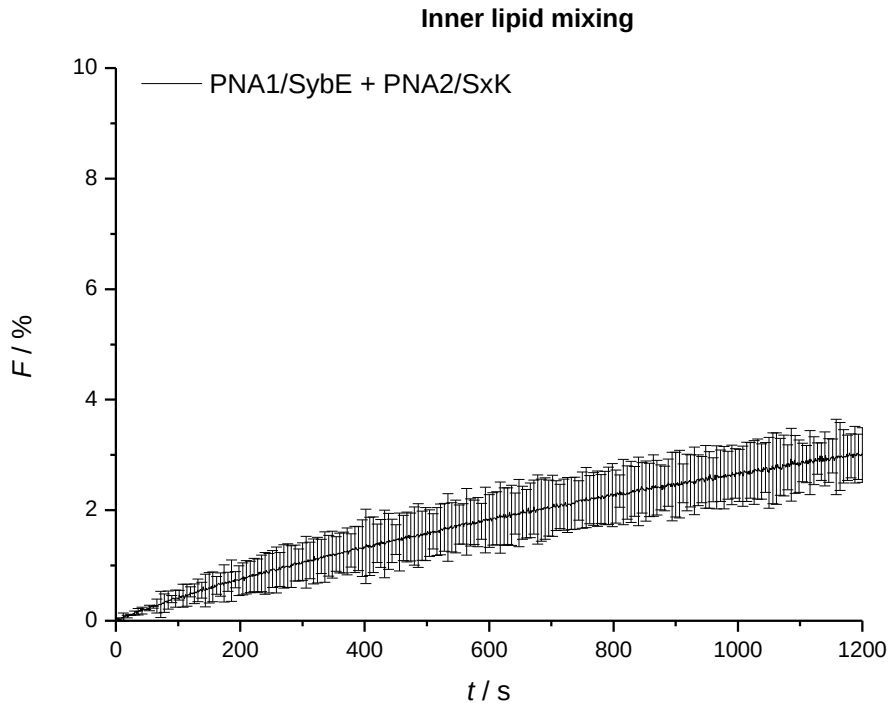

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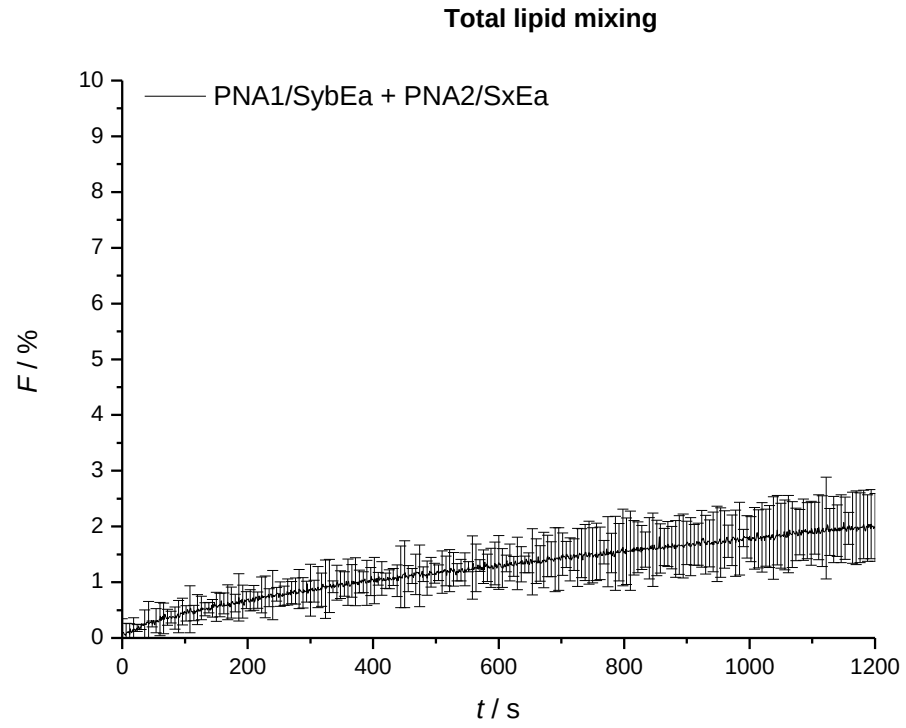

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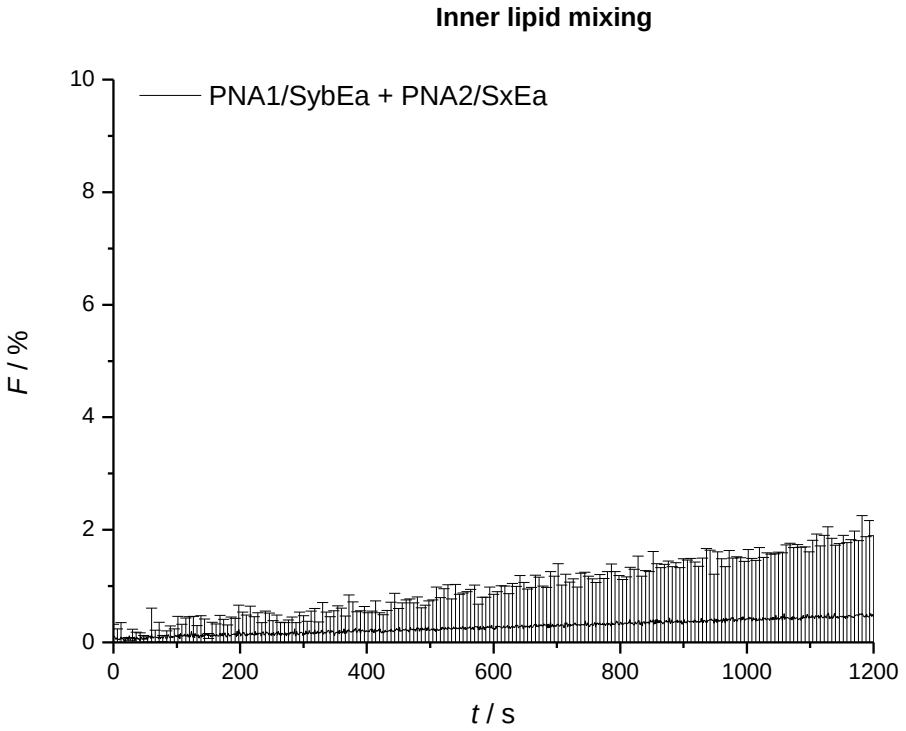

9) References

1 R. C. MacDonald, R. I. MacDonald, B. P. M. Menco, K. Takeshita, N. K. Subbarao and L. Hu, *Biochim. Biophys. Acta BBA-Biomembr.*, 1991, **1061**, 297 - 303.

2 P E. Nielsen, *PNA – Protocols and Applications*, Horizon Bioscience, Wymondham, 2004.

3 M. You, E. Li, W. C. Wimley,K. Hristova, *Anal. Biochem*. 2005, **340**, 154 - 164.