Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information to

DNA-programmed spatial screening of heterobivalent binding to the endocytic AP-2 adaptor complex

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1 Materials and methods

Materials

Chemicals for DNA synthesis such as acetonitrile (water content < 30 ppm), DMT-removal reagent (3 % trichloroformic acid in dichloromethane), capping reagents I + II (1-methylimidazole/THF and acetic anhydride/pyridine/THF) and oxidizing reagent (iodine/water/pyridine/THF) were purchased from Roth (Karlsruhe, Germany). Dichloromethane (DNA-synthesis grade) was purchased from AppliChem (Darmstadt, Germany). DNA-monomers (DMT-dA(Bz)-β-cyanoethylphosphoramidite, DMT-dC(Bz)-β-cyanoethylphosphoramidite, DMT-dG(DMF)-β-cyanoethylphosphoramidite and DMT-dT-β-cyanoethylphosphoramidite) were obtained from Proligo (Sigma-Aldrich, Hamburg, Germany) and used as a 0.2 M solution in acetonitril for the coupling. Hyacinth-DMF (5-Benzylmercaptotetrazole) from emp-Biotech (Berlin, Germany) was used as 0.3 M solution in acetonitrile as coupling reagent. Fmoc-protected PNA-monomers were purchased from ASM Research Chemicals (Burgwedel, Germany). DMF for peptide synthesis as well as Fmoc-protected amino acids were purchased from Biosolve (Valkenswaard, Netherland), Fmoc-Lys(Mmt)-OH from Iris Biotech (Marktredwitz, Germany). Rink amide resin was supplied by Novabiochem (Schwalbach, Germany). HCTU was acquired from Chemcube (Bochum, Germany). Acrylamide-buffer for PAGE-analysis was supplied by Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Gene Ruler™ Ultra Low Range DNA-ladder (ready-to-use) was obtained from Fermentas GmbH (St. Leon-Rot, Germany) and 10000× SYBR-Gold for gel-staining was purchased from Invitrogen (Molecular Probes). All other chemicals were purchased from Acros, Sigma-Aldrich and Fluka and used without further purification. Water was used after purification with an Astacus Milli-Q Ultra Pure Water Purification System from membraPure GmbH (Bodenheim, Germany).

Instruments and Methods

Automated DNA-synthesis was carried out by using a 3400 DNA-Synthesizer (Applied Biosystems) in 1 μmol columns for DNA synthesis (Proligo, Sigma-Aldrich, Hamburg, Germany).

Manual solid phase synthesis was performed by using 2 mL polyethylene syringe reactors (from MultSynTech) equipped with a fritted disc. For automated linear solid phase synthesis of peptides and PNA-oligomers a MultipepRS Syntheziser and a ResPep-Parallel Synthesizer of Intavis Bioanalytical Instruments AG were used.

Analytical HPLC of peptide-DNA-conjugates was performed on a Waters-Acquity-UPLC system by using an UV/Vis-detector (260 nm detection wavelength) and a thermostated (55°C) analytical Waters-X-Bridge C18-column, BEH300, 1.7 μm, 100x2 mm. Eluent A (0.1 M triethylammonium acetate at pH 7.0) and eluent B (MeCN) were used in a linear gradient (3-50 % B in 2 min) at a flow rate of 0.6 mL/min. Preparative HPLC was performed by using a Gilson-HPLC system and an UV/Vis-detector (260 nm detection wavelength) and a thermostated (55°C) semi-preperative Waters-X-Bridge C18-column, BEH300, 5 μm, 250x10 mm. Eluent A (0.1 M triethylammonium acetate at pH 7.0) and eluent B (MeCN) were used in a linear gradient (3-50 % B in 40 min) at a flow rate of 4 mL/min.

For analytical and semi-preparative HPLC of peptides and PNA-conjugates an Agilent 1100 series instrument was used. Absorbance of analytical HPLC was measured by using a photo diode array detector at 210 and 260 nm and columns from *Macherey & Nagel* (semi-preparative: 5μ, 250x21 mm; analytical: 3μ, 125x4 mm). Samples were eluted using solvents A (98.9% water, 1% MeCN, 0.1% formic acid) and B (98.9% MeCN, 1% water, 0.1% formic acid) for analytical HPLC and A (98.9% water, 1% MeCN, 0.1% TFA) and B (98.9% MeCN, 1% water, 0.1% TFA) for semi-preparative

HPLC in linear gradients (see analytical details) at a flow rate of 16 mL/min (preparative) und 0.3 mL/min (analytical) at 55°C.

MALDI-TOF mass spectrometry of peptide-DNA-conjugates was performed on a Voyager-DE Pro Biospectrometry Workstation from PerSeptive Biosystems. A nitrogen laser at $\lambda = 337$ nm was used as excitation source. 0.15 M 2, 3, 4-Trihydroxyacetophenone in water/ethanol (1:1 [v/v]) with 0.5 M diammonium-citrate was used as matrix.

For the determination of the solid phase synthesis yields, the Fmoc-load was determined. The required UV-VIS-spectroscopical measurements were performed by using a Varian Carey 100 Bio (Agilent Technologies, Waldbronn, Germany). An aliquot of the filtrate obtained after Fmoc-cleavage was diluted and the absorption at 301 nm was determined. Loading was calculated applying Lambert law ($A = \varepsilon_{301} \cdot c \cdot d$). The concentration of PNA-/DNA-stock solutions was determined by measuring optical density at 260 nm using a quartz cuvette at 1 cm path length. The extinction coefficients were calculated using the OligoAnalyzer 3.1 from Integrated DNA Technologies (IDT) (http://eu.idtdna.com/analyzer/ applications/oligoanalyzer/).

Fluorescence anisotropy experiments were performed by using a SPEX Fluoromax 3 fluorescence spectrometer (HORIBA Jobin Yvon) equipped with a peltier thermostated single cell holder (set to 25 °C) and automated polarizers. The measurements were performed in a 0.25 mL fluorescence cuvette (105.250-QS, *Hellma*). Slits were set to yield an intensity of approximately $1.0 \bullet 10^6$ counts with both polarizers set to vertical orientation (8-13 nm). For each data point 10 values were measured and the last 5 were averaged. All protein containing solutions were kept on ice until measuring.

2 Synthesis

Synthesis of peptides

Manual solid-phase synthesis according to the Fmoc/tBu-strategy

Resin loading: TentaGel R RAM resin (100 µmol) was allowed to swell for 30 min in 2 mL DMF. A solution of 4 eq. (400 µmol) Fmoc-Lys(Mmt)-OH, 4 eq. (400 µmol) HCTU and 8 eq. (800 µmol) NMM in DMF (final concentration 0.2 M) was added to the resin. After 2 h the resin was washed (5x 1 mL DMF, 5x 1 mL CH_2Cl_2, 5x 1 mL DMF) and dried in vacuo. *Fmoc cleavage:* DMF/piperidine (4:1 [v/v], 1 mL) was added to the resin. After 5 min, the procedure was repeated once. The resin was washed (5x 1 mL DMF, 5x 1 mL CH_2Cl_2, 5x 1 mL DMF). *Coupling:* 4 eq. (corresponding to resin loading) amino acid was dissolved in DMF (final concentration 0.2 M) and 3.6 eq. of HCTU and 8 eq. of NMM were added. The solution was then added to the resin. After 30 min, the resin was washed (5x 1 mL DMF, 5x 1 mL CH_2Cl_2, 5x 1 mL DMF). *Capping:* Ac_2O/ 2,6-lutidine/DMF (5:6:89 [v/v/v]) was added to the resin. After 5 min the resin was washed (5x 1 mL DMF, 5x 1 mL CH_2Cl_2, 5x 1 mL DMF).

Automated solid-phase synthesis according to the Fmoc/tBu-strategy

TentaGel R RAM resin (25 µmol) was allowed to swell for 30 min in 1 mL DMF. During solid phase synthesis reactors were vortexed every 3 min for 30 s. *Fmoc cleavage:* DMF/piperidine (4:1 [v/v], 400 µL) was added to the resin. After 2 min, the procedure was repeated once. The resin was then washed with DMF (7x 800 µL). *Coupling*: A preactivation vessel was charged with a 0.6 M solution of HCTU in DMF (5 eq. corresponding to resin loading), a 4 M solution of NMM in DMF (10 eq.) and a solution containing 0.6 M amino acid and 0.6 M HOBt in DMF (5 eq). After 2 min the solution was added to the resin. After 30 min, the resin was washed with DMF (2x 800 µL). In synthesis of longer peptides coupling step was repeated as of 10th reaction cycle. *Capping:* Ac_2O/2,6-lutidine/DMF

(5:6:89 [v/v/v], 400 µL) was added. After 5 min the resin was washed with DMF (5x 800 µL). At the end of linear solid phase peptide assembly, resin was washed with DCM (4x 200 µL).

Mmt-cleavage: A solution of TFA in DCM (5% [v/v]) was added to the resin (5x 1 min). Afterwards the resin was washed (10x 1 mL DCM, 5x 1 mL DMF).

Coupling: (A) To yield fluorophore-labeled peptides (1-10) a solution of 10 eq. (corresponding to resin loading) of 5,6-carboxyfluorescein in DMF, 20 eq. NMM and 10 eq. PyBOP (final concentration 0.4 M) was added to the resin. After 6 h the resin was washed (at least 10x with DMF/Piperidin (4:1 [v:v]), 5x 1 mL DMF, 10x 1 mL DCM). (B) To obtain acetylated peptides (11-20) the ε-amino group of C-terminal lysine was capped with Ac_2O/ 2,6-lutidine/DMF (5:6:89 [v/v/v], 2x 5 min). At the end the resin was washed (5x 1 mL DMF, 10x 1 mL CH_2Cl_2). (C) To obtain maleimide peptides (21-30) a solution of 4 eq. 3-maleimide propionic acid in DMF, 8 eq. NMM and 3.6 eq. HCTU (final concentration 0.4 M) was added to the resin. After 2 h the coupling was repeated and the resin was washed (5x 1 mL DMF, 10x 1 mL CH_2Cl_2).

Cleavage from solid support: the resin was treated with a mixture of TFA/H_2O/triisopropylsilane (950 µL : 25 µL : 25 µL) for 90 min and subsequently washed with 500 µL TFA. The combined solutions were concentrated to 1/5 of the volume under reduced pressure following precipitation by addition of cold diethylether. The supernatant was discarded and the precipitate dissolved in water. The solution was lyophyllized. For HPLC-purification the crude product was dissolved in a water/MeCN mixture (max 900 µL, 0-50% MeCN). Product purity was verified by HPLC or UPLC analysis and MALDI-TOF/MS.

peptide sequence	**R = FAM**	**R = MIC**
Ac-Ser-Asp-Pro-Phe-Lys(**R**)-NH_2	**1**	-
Ac-Phe-Glu-Asp-Asn-Phe-Val-Pro-Lys(**R**)-NH_2	**2**	**18**
Ac-Ile-Asn-Phe-Phe-Glu-Asp-Asn-Phe-Val-Pro-Glu-Ile-Lys(**R**)-NH_2	**3**	-
Ac-Ile-Asn-Phe-Phe-Glu-Asp-Asp-Phe-Val-Pro-Glu-Ile-Lys(**R**)-NH_2	**4**	-
Ac-Leu-Asp-Gly-Phe-Glu-Asp-Asn-Phe-Asn-Leu-Gln-Ser-Lys(**R**)-NH_2	**5**	-
Ac-Pro-Asn-Asn-Trp-Ala-Asp-Phe-Ser-Lys(**R**)-NH_2	**6**	**19**
Ac-Pro-Asn-Asn-Trp-Ala-Asp-Phe-Ser-Ser-Thr-Trp-Pro-Lys(**R**)-NH_2	**7**	-
Ac-Lys-Gly-Trp-Val-Thr-Phe-Glu-Glu-Lys(**R**)-NH_2	**8**	**20**
Ac-Trp-Val-Thr-Phe-Asp-Asp-Asp-Lys(**R**)-NH_2	**9**	-
Ac-Asn-Pro-Lys-Gly-Trp-Val-Thr-Phe-Glu-Glu-Glu-Glu-Lys(**R**)-NH_2	**10**	-
Ac-Ile-Ser-Asn-Trp-Val-Gln-Phe-Glu-Asp-Asp-Thr-Pro-Lys(**R**)-NH_2	**11**	-

Table S1. Synthesized peptide sequences. The ε-amino group of C-terminal lysine was coupled to 5,6-carboxyfluorescein (R = FAM), fluoresceinisothiocyanate (R' = FITC) or 3-maleimido propionic acid (R, R' = MIC).

Characterization data for peptides

FAM-labeled peptides

Peptides were synthesized at 3 µmol scale. The yield was determined by measuring the absorption at 492 nm (ε_{492} (FAM) = 78000 M^{-1}•cm^{-1}) (R. P. Haugland, *Handbook of Fluorescent Probes and Research Products*, 9. Auflage, Molecular Probes Inc., Eugene, **2002**, S. 46-56) of peptides dissolved in 1% $NaCO_3$ in water.

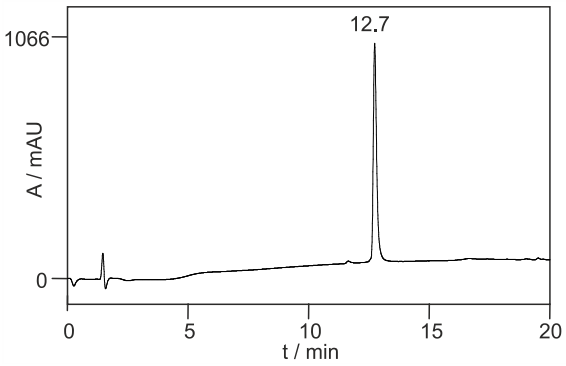
Ac-Phe-Glu-Asp-Asn-Phe-Val-Pro-Lys(FAM)-NH_2 **2**

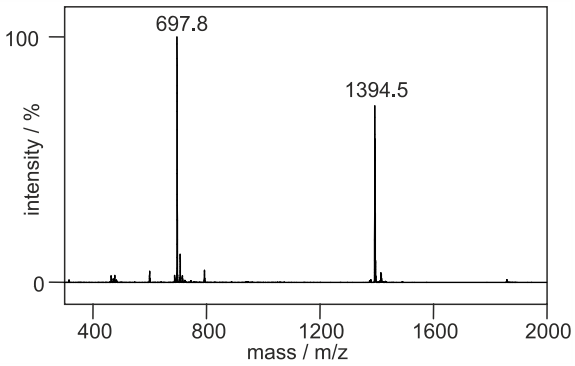
C_{70} H_{79} N_{11} O_{20} (1394.2 g/mol)

OD_{492} = 36.4 (467 nmol, 16 %), ε_{492} = 78000 M^{-1}•cm^{-1}

HPLC: t_R: 12.7 min (3-90% MeCN, 210 nm);

ESI/MS (m/z): calc.: 1394.2 $[M+H]^+$, 698.1 $[M+2H]^{2+}$; found: 1394.5 $[M+H]^+$, 697.8 $[M+2H]^{2+}$





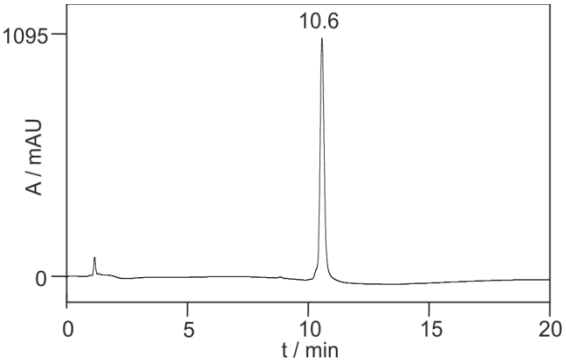
Ac-Ile-Asn-Phe-Phe-Glu-Asp-Asn-Phe-Val-Pro-Glu-Ile-Lys(FAM)-NH_2 **3**

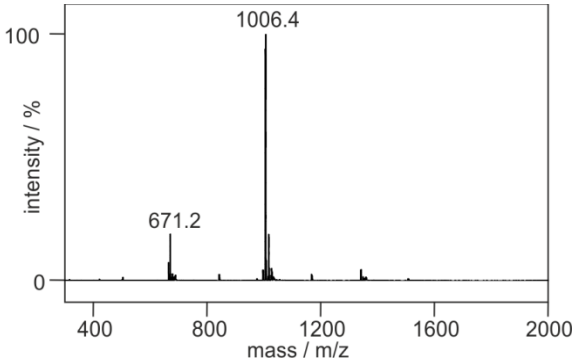
C_{100} H_{123} N_{17} O_{28} (2011.5 g/mol)

OD_{304} = 4.0 (6450 nmol, 43 %), ε_{304} = 616.8 M^{-1}•cm^{-1}

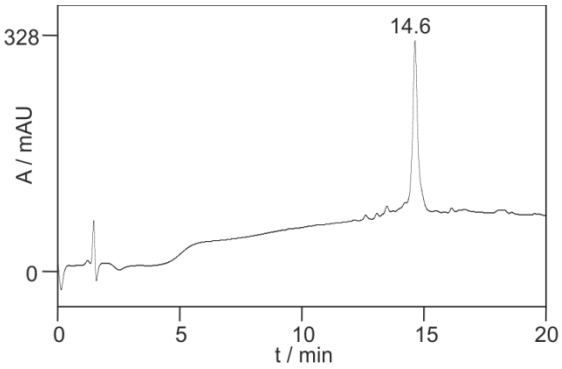
HPLC: t_R: 10.6 min (30-70% MeCN, 210 nm);

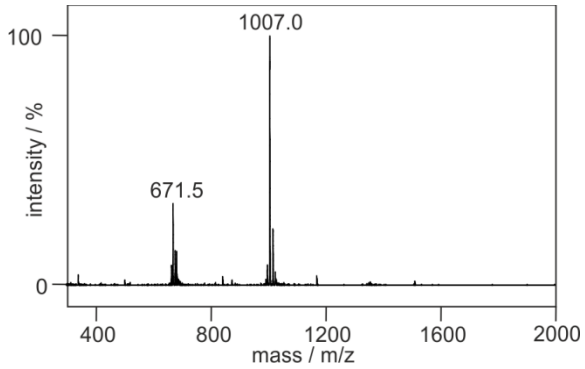
ESI/MS (m/z): calc.: 1006.8 $[M+2H]^{2+}$, 671.5 $[M+3H]^{3+}$; found: 1006.4 $[M+2H]^{2+}$, 671.2 $[M+3H]^{3+}$



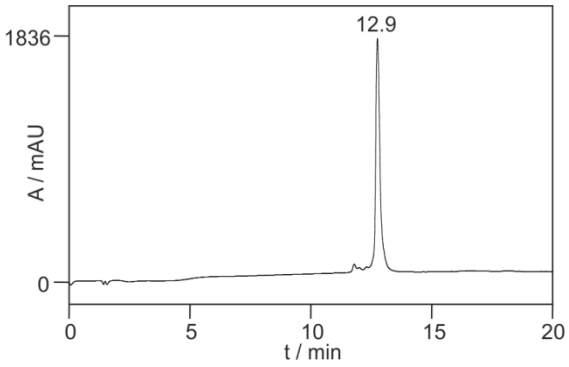


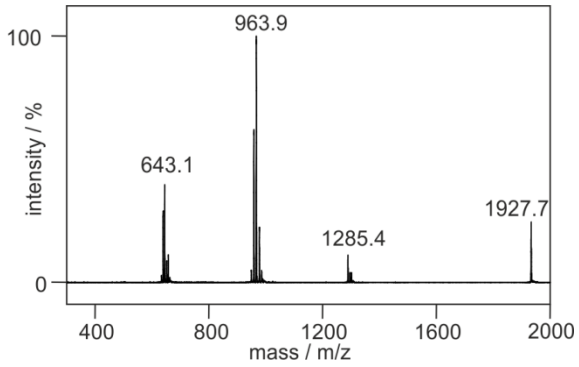
Ac-Ile-Asn-Phe-Phe-Glu-Asp-Asp-Phe-Val-Pro-Glu-Ile-Lys(FAM)-NH_2 **4**
C_{90} H_{122} N_{16} O_{29} (2011.9 g/mol)
OD_{492} = 1.2 (16 nmol, 1 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 14.6 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1007.0 $[M+2H]^{2+}$, 671.6 $[M+3H]^{3+}$; found: 1007.0 $[M+2H]^{2+}$, 671.5 $[M+3H]^{3+}$



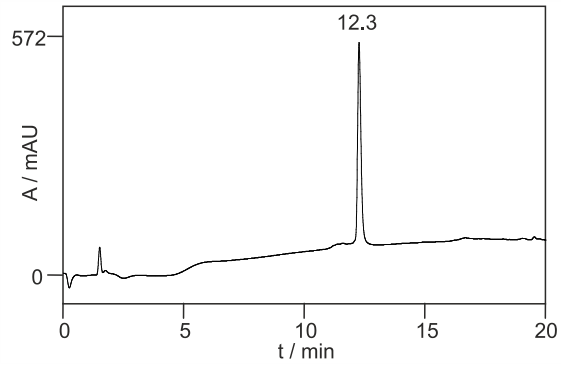


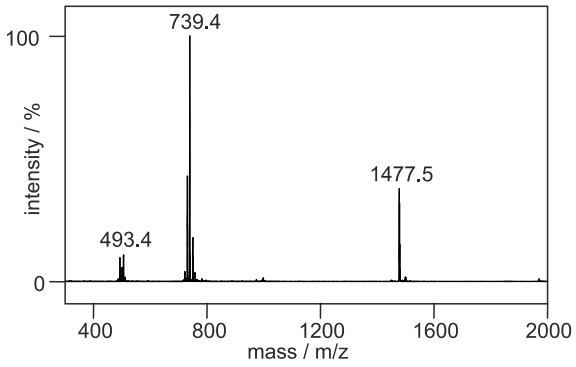
Ac-Leu-Asp-Gly-Phe-Glu-Asp-Asn-Phe-Asn-Leu-Gln-Ser-Lys(FAM)-NH_2 **5**
C_{90} H_{112} N_{18} O_{30} (1925.7 g/mol)
OD_{492} = 17.6 (225 nmol, 8 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 12.9 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1926.7 $[M+H]^{+}$, 963.9 $[M+2H]^{2+}$, 642.9 $[M+3H]^{3+}$;
found: 1927.7 $[M+H]^{+}$, 963.9 $[M+2H]^{2+}$, 643.1 $[M+3H]^{3+}$



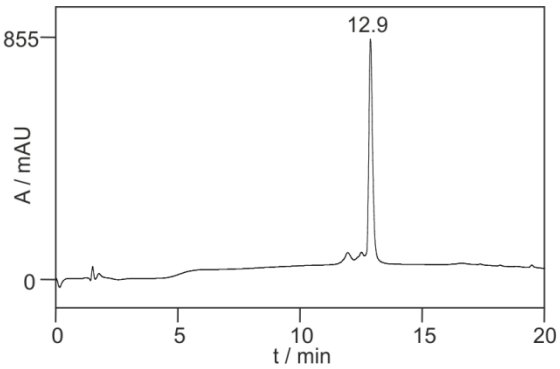


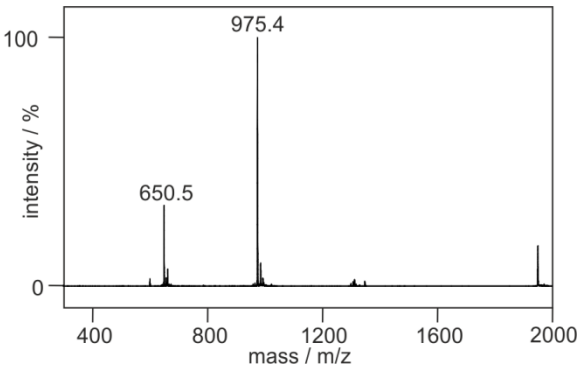
Ac-Pro-Asn-Asn-Trp-Ala-Asp-Phe-Ser-Lys(FAM)-NH_2 **6**
C_{72} H_{80} N_{14} O_{21} (1477.2 g/mol)
OD_{492} = 17.9 (229 nmol, 8 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 12.3 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1478.2 $[M+H]^+$, 739.6 $[M+2H]^{2+}$, 493.4 $[M+3H]^{3+}$;
found: 1477.5 $[M+H]^+$, 739.4 $[M+2H]^{2+}$, 493.4 $[M+3H]^{3+}$



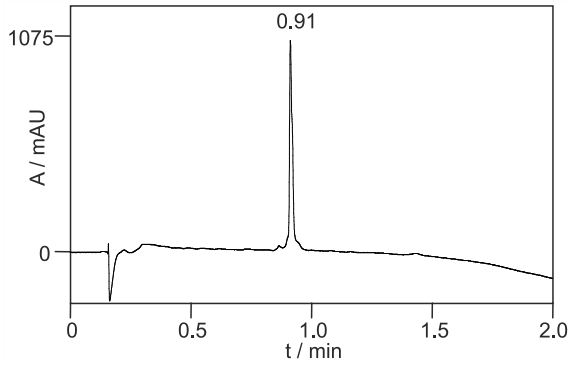


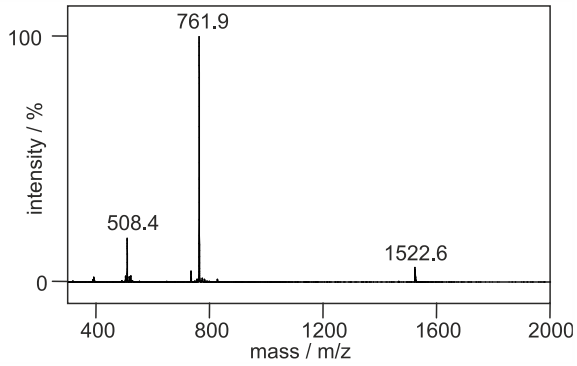
Ac-Pro-Asn-Asn-Trp-Ala-Asp-Phe-Ser-Ser-Thr-Trp-Pro-Lys(FAM)-NH_2 **7**
C_{95} H_{109} N_{19} O_{27} (1948.7 g/mol)
OD_{492} = 3.7 (47 nmol, 2 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 12.9 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 975.4 $[M+2H]^{2+}$, 650.6 $[M+3H]^{3+}$; found: 975.4 $[M+2H]^{2+}$, 650.5 $[M+3H]^{3+}$



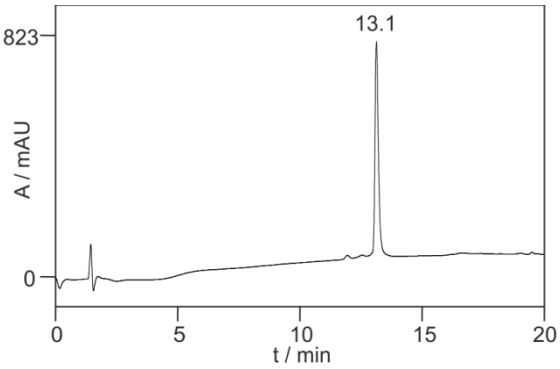


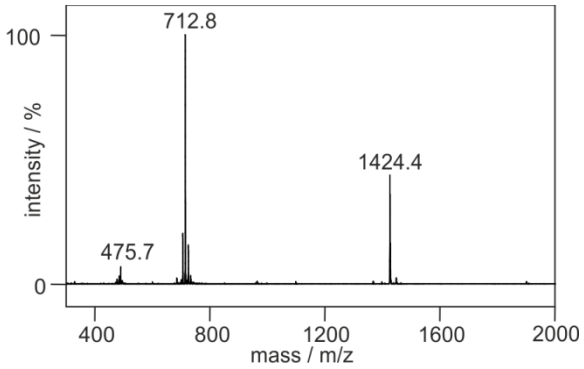
Ac-Lys-Gly-Trp-Val-Thr-Phe-Glu-Glu-Lys(FAM)-NH_2 **8**
C_{76} H_{91} N_{13} O_{21} (1521.6 g/mol)
OD_{492} = 23.9 (306 nmol, 10 %), ε_{492} = 78000 M^{-1}•cm^{-1}
UPLC: t_R: 0.91 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1522.6 $[M+H]^+$, 761.8 $[M+2H]^{2+}$, 508.2 $[M+3H]^{3+}$;
found: 1522.6 $[M+H]^+$, 761.9 $[M+2H]^{2+}$, 508.4 $[M+3H]^{3+}$



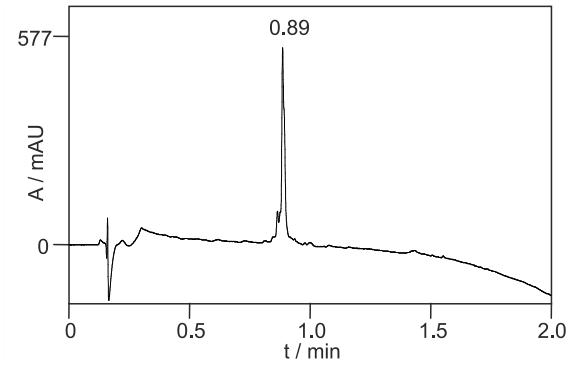


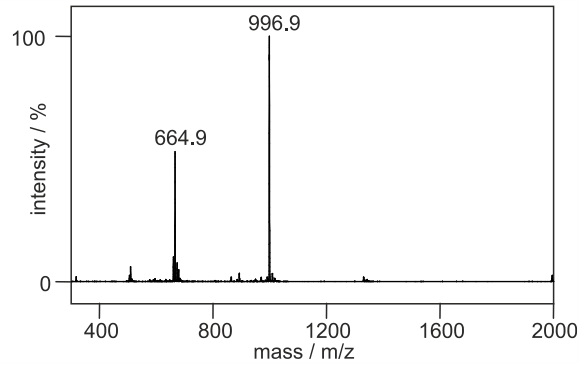
Ac-Trp-Val-Thr-Phe-Asp-Asp-Asp-Lys(FAM)-NH_2 **9**
C_{70} H_{77} N_{11} O_{22} (1423.5 g/mol)
OD_{492} = 22.7 (291 nmol, 10 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 13.1 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1424.5 $[M+H]^+$, 712.8 $[M+2H]^{2+}$, 475.5 $[M+3H]^{3+}$;
found: 1424.4 $[M+H]^+$, 712.8 $[M+2H]^{2+}$, 475.7 $[M+3H]^{3+}$



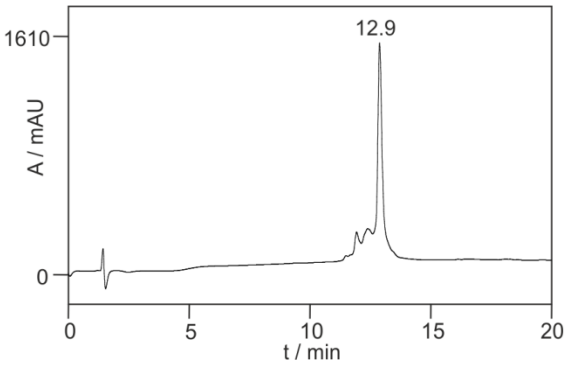


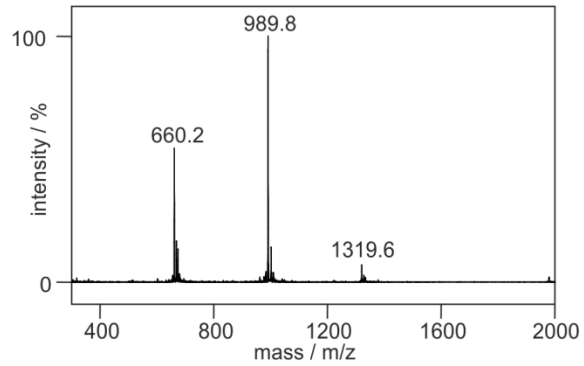
Ac-Asn-Pro-Lys-Gly-Trp-Val-Thr-Phe-Glu-Glu-Glu-Glu-Lys(FAM)-NH_2 **10**
C_{95} H_{118} N_{18} O_{30} (1991.8 g/mol)
OD_{492} = 17.0 (218 nmol, 7 %), ε_{492} = 78000 M^{-1}•cm^{-1}
UPLC: t_R: 0.89 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 996.9 $[M+2H]^{2+}$, 664.9 $[M+3H]^{3+}$; found: 996.9 $[M+2H]^{2+}$, 664.9 $[M+3H]^{3+}$





Ac-Ile-Ser-Asn-Trp-Val-Gln-Phe-Glu-Asp-Asp-Thr-Pro-Lys(FAM)-NH_2 **11**
C_{94} H_{116} N_{18} O_{30} (1977.8 g/mol)
OD_{492} = 2.1 (27 nmol, 1 %), ε_{492} = 78000 M^{-1}•cm^{-1}
HPLC: t_R: 12.9 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 989.9 $[M+2H]^{2+}$, 660.3 $[M+3H]^{3+}$; found: 989.9 $[M+2H]^{2+}$, 660.2 $[M+3H]^{3+}$

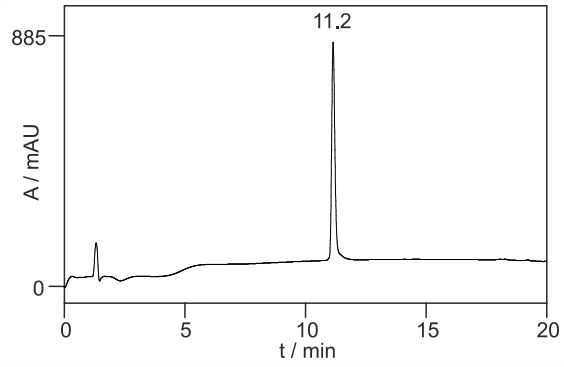


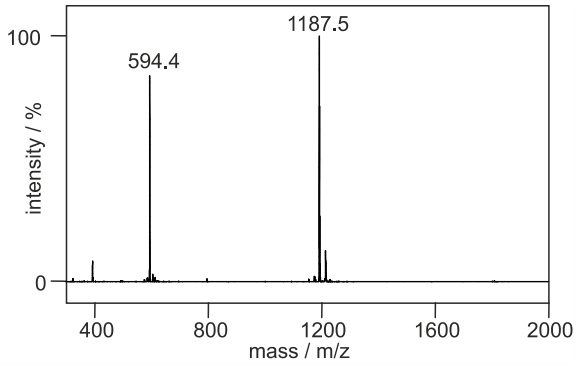


Maleimide peptides

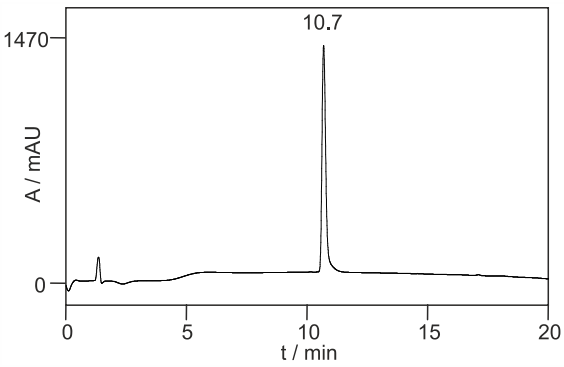
Peptides were synthesized at 15 µmol scale. The yield was determined by measuring the absorption at 304 nm (ε_{304} (MIC) = 616.8 M-1•cm-1.

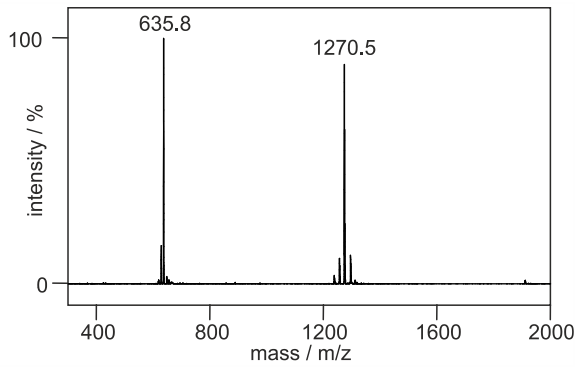
Ac-Phe-Glu-Asp-Asn-Phe-Val-Pro-Lys(MIC)-NH_2 **18**
C_{56} H_{74} N_{12} O_{17} (1186.5 g/mol)
OD_{304} = 3.8 (6200 nmol, 41 %), ε_{304} = 616.8 M^{-1}•cm^{-1}
HPLC: t_R: 11.2 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1187.5 $[M+H]^+$, 594.3 $[M+2H]^{2+}$; found: 1187.5 $[M+H]^+$, 594.4 $[M+2H]^{2+}$



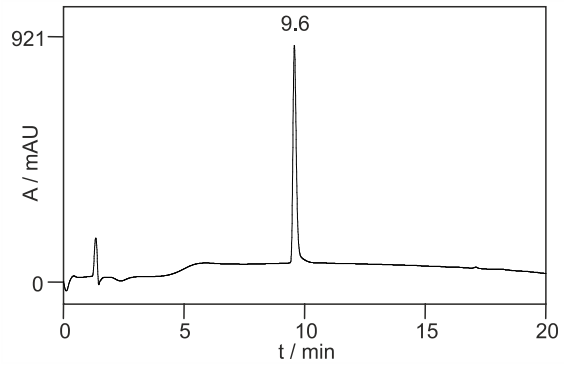


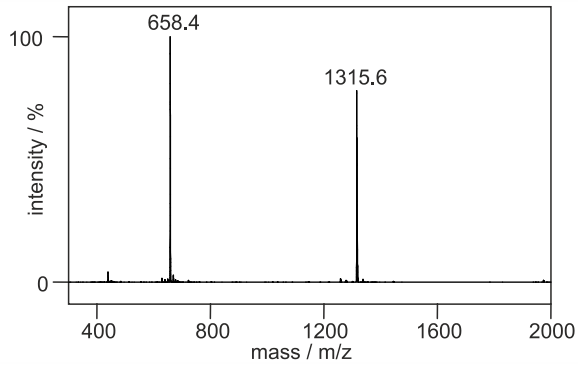
Ac-Pro-Asn-Asn-Trp-Ala-Asp-Phe-Ser-Lys(MIC)-NH_2 **19**
C_{58} H_{75} N_{15} O_{18} (1269.5 g/mol)
OD_{304} = 1.9 (3070 nmol, 28 %), ε_{304} = 616.8 M^{-1}•cm^{-1}
HPLC: t_R: 10.7 min (3-90% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1270.5 $[M+H]^+$, 635.8 $[M+2H]^{2+}$;found: 1270.5 $[M+H]^+$, 635.8 $[M+2H]^{2+}$





Ac-Lys-Gly-Trp-Val-Thr-Phe-Glu-Glu-Lys(MIC)-NH_2 **20**
C_{62} H_{86} N_{14} O_{18} (1314.6 g/mol)
OD_{304} = 2.6 (4240 nmol, 28 %), ε_{304} = 616.8 M^{-1}•cm^{-1}
HPLC: t_R: 9.6 min (3-60% MeCN, 210 nm);
ESI/MS (m/z): calc.: 1315.6 $[M+H]^+$, 658.3 $[M+2H]^{2+}$; found: 1315.6 $[M+H]^+$, 658.4 $[M+2H]^{2+}$





Synthesis of PNA-oligomers

Automated PNA synthesis was performed at 2 µmol scale by using Fmoc/Bhoc-protected PNA building blocks.

TentaGel R RAM resin (2 µmol) was allowed to swell for 30 min in 350 µL DMF. *Fmoc cleavage:* the resin was treated for 2 min with DMF/piperidine (4:1 [v/v], 2x 200 µL) and washed with DMF (7x 200 µL). *Coupling of PNA-monomers:* a preactivation vessel was charged with a 0.2 M solution of the PNA monomer in NMP (40 µL), a 0.6 M solution of HCTU in DMF (12 µL) and a 4 M solution of NMM in DMF (6 µL). After 1 min, the solution was transferred to the resin. After 30 min, the resin was washed (2x 200 µL DMF). *Capping:* a mixture of Ac_2O/2,6-lutidine/DMF (5:6:89 [v/v/v], 200 µL) was added to the resin. After 3 min the resin was washed (6x 200 µL). *Cleavage from the solid support:* the resin was treated with a mixture of TFA/H_2O/triisopropylsilane (950 µL : 25 µL : 25 µL) for 90 min and subsequently washed with 500 µL TFA. The combined solutions were concentrated to 1/5 of the volume under reduced pressure. Addition of cold diethylether yielded a precipitate. The supernatant was discarded and the precipitate dissolved in water. The solution was lyophyllized. For HPLC-purification the crude product was dissolved in water/MeCN mixture (max 900 µL, 0-50% MeCN). Product purity was verified by HPLC or UPLC analysis and MALDI-TOF/MS. The yield was determined by measuring the absorption at 260 nm and using the extinction coefficients calculated with the OligoAnalyzer 3.1 from Integrated DNA Technologies (IDT) (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/).

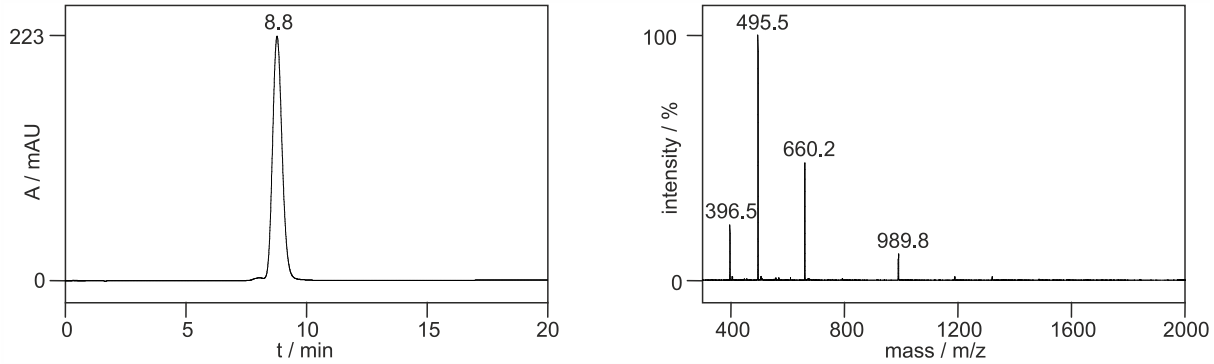
Ac-cgg cgg c-NH_2 **23**:

$C_{76}H_{96}N_{44}O_{22}$ (1977.9 g/mol)

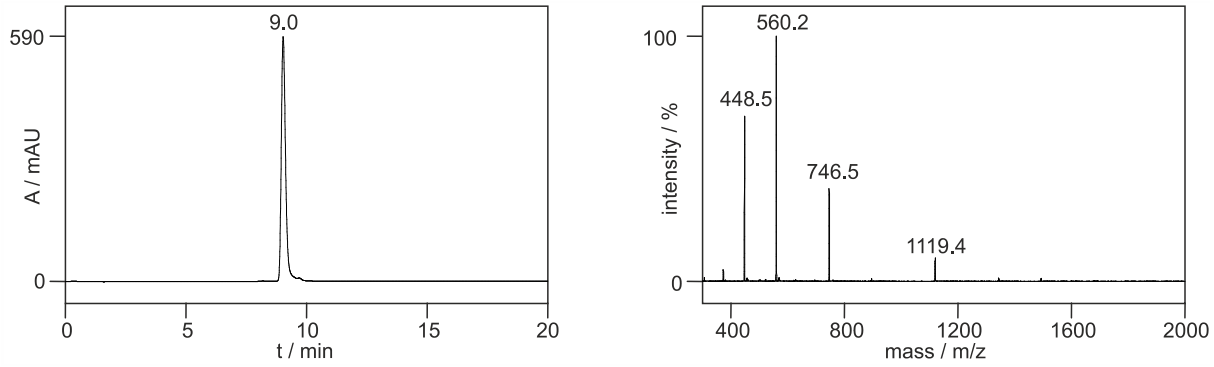
OD_{260} = 47.1 (857 nmol, 43 %), ε_{260} = 54900 M^{-1}•cm^{-1}

HPLC: t_R: 8.8 min (0-30% MeCN, 260 nm);

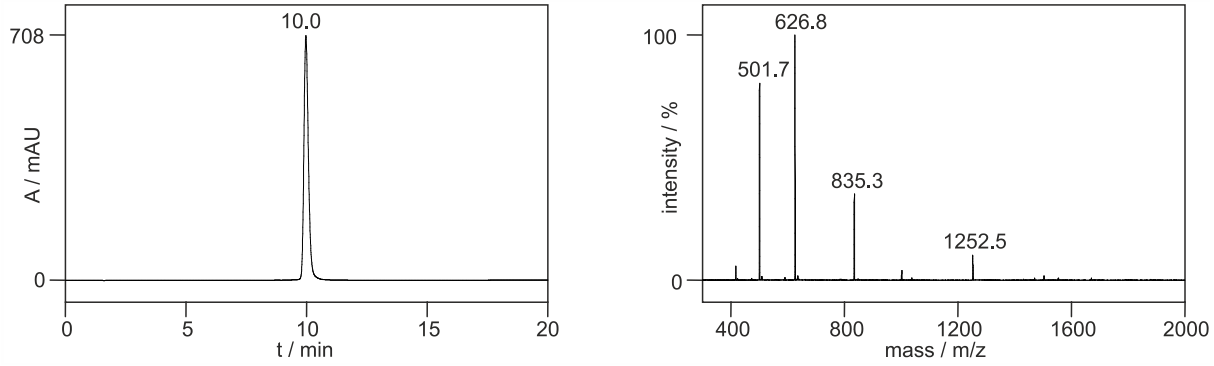
ESI/MS (m/z): calc.: 990.0 $[M+2H]^{2+}$, 660.3 $[M+3H]^{3+}$, 495.5 $[M+4H]^{4+}$, 396.6 $[M+5H]^{5+}$;
found: 989.8 $[M+2H]^{2+}$, 660.2 $[M+3H]^{3+}$, 495.5 $[M+4H]^{4+}$, 396.5 $[M+5H]^{5+}$



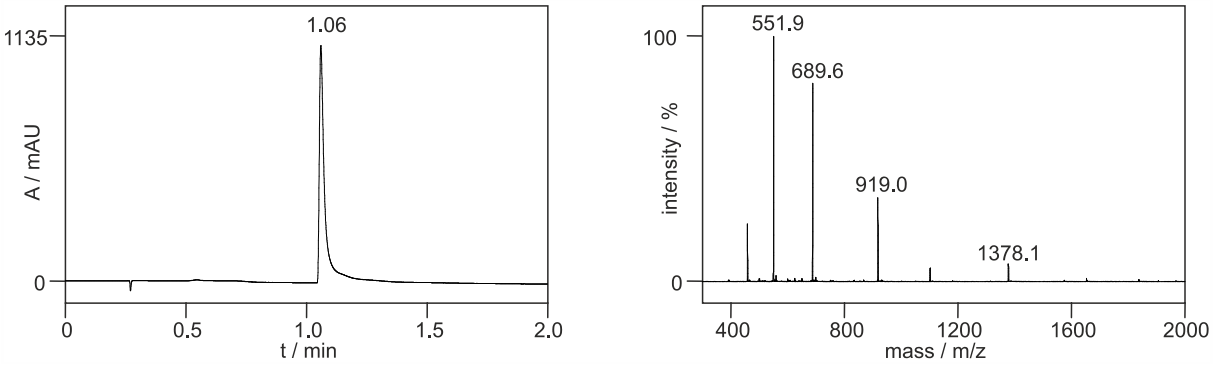
Ac-acg gca gc-NH_2 **24**:
$C_{87}H_{109}N_{51}O_{23}$ (2237.1 g/mol)
OD_{260} = 44.7 (576 nmol, 29 %), ε_{260} = 77500 M^{-1}•cm^{-1}
HPLC: t_R: 9.0 min (0-20% MeCN, 260 nm);
ESI/MS (m/z): calc.: 1119.6 $[M+2H]^{2+}$, 746.7 $[M+3H]^{3+}$, 560.3 $[M+4H]^{4+}$, 448.4 $[M+5H]^{5+}$;
found: 1119.4 $[M+2H]^{2+}$, 746.5 $[M+3H]^{3+}$, 560.2 $[M+4H]^{4+}$, 448.5 $[M+5H]^{5+}$



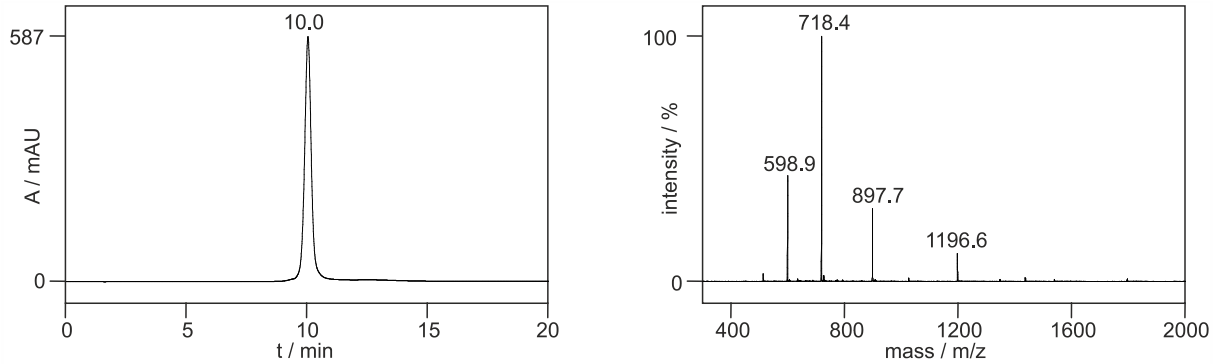
Ac-tac ggc agc-NH_2 **25**:
$C_{98}H_{123}N_{55}O_{27}$ (2503.4 g/mol)
OD_{260} = 43.1 (504 nmol, 25 %), ε_{260} = 85500 M^{-1}•cm^{-1}
HPLC: t_R: 10.0 min (0-20% MeCN, 260 nm);
ESI/MS (m/z): calc: 1252.7 $[M+2H]^{2+}$, 835.5 $[M+3H]^{3+}$, 626.9 $[M+4H]^{4+}$, 501.7 $[M+5H]^{5+}$
found: 1252.5 $[M+2H]^{2+}$, 835.3 $[M+3H]^{3+}$, 626.8 $[M+4H]^{4+}$, 501.7 $[M+5H]^{5+}$



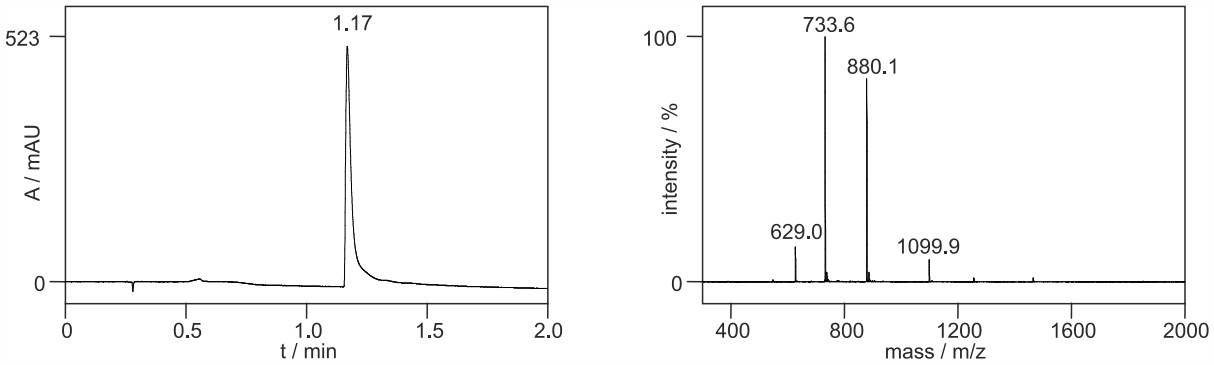
Ac-cta cgg cag c-NH_2 **26**:
$C_{108}H_{136}N_{60}O_{30}$ (2754.6 g/mol)
OD_{260} = 66.3 (772 nmol, 39 %), ε_{260} = 92000 M^{-1}•cm^{-1}
UPLC: t_R: 1.06 min (0-30% MeCN, 260 nm);
ESI/MS (m/z): calc: 1378.3 $[M+2H]^{2+}$, 919.0 $[M+3H]^{3+}$, 689.7 $[M+4H]^{4+}$, 551.9 $[M+5H]^{5+}$;
found: 1378.1 $[M+2H]^{2+}$, 919.0 $[M+3H]^{3+}$, 689.6 $[M+4H]^{4+}$, 551.9 $[M+5H]^{5+}$



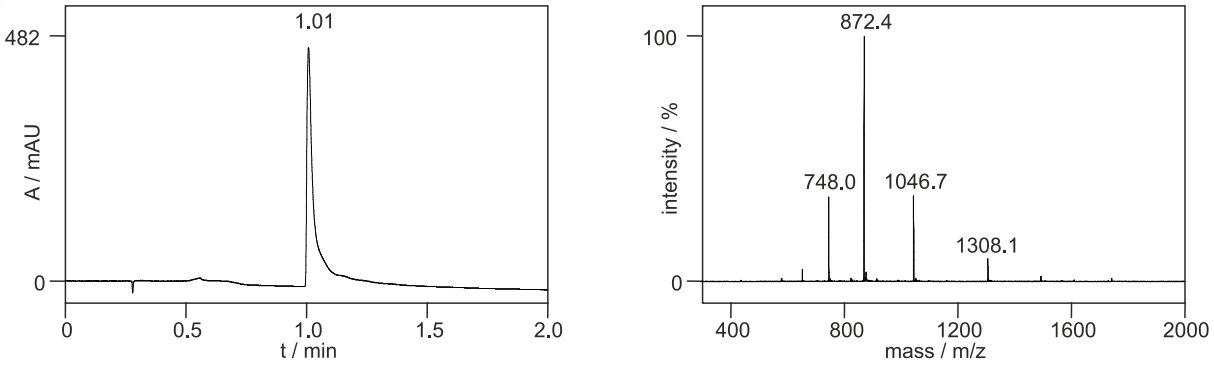
Ac-atg cta cgg cag c-NH_2 **27**:
$C_{141}H_{176}N_{78}O_{39}$ (3587.4 g/mol)
OD_{260} = 59.9 (484 nmol, 24 %), ε_{260} = 123800 M^{-1}•cm^{-1}
HPLC: t_R: 10.0 min (0-20% MeCN, 260 nm);
ESI/MS (m/z): calc.: 1196.8 $[M+3H]^{3+}$, 897.9 $[M+4H]^{4+}$, 718.5 $[M+5H]^{5+}$, 598.9 $[M+6H]^{6+}$;
found: 1096.6 $[M+3H]^{3+}$, 897.7 $[M+4H]^{4+}$, 718.4 $[M+5H]^{5+}$, 598.9 $[M+6H]^{6+}$



Ac-gtc atg cta cgg cag c-NH_2 **28**:
$C_{173}H_{216}N_{94}O_{49}$ (4396.2 g/mol)
OD_{260} = 61.7 (412 nmol, 21 %), ε_{260} = 149700 M^{-1}•cm^{-1}
UPLC: t_R: 1.17 min (0-30% MeCN, 260 nm);
ESI/MS (m/z): calc.: 1099.6 $[M+4H]^{4+}$, 880.2 $[M+5H]^{5+}$, 733.7 $[M+6H]^{6+}$, 629.0 $[M+7H]^{7+}$;
found: 1099.9 $[M+4H]^{4+}$, 880.1 $[M+5H]^{5+}$, 733.6 $[M+6H]^{6+}$, 629.0 $[M+7H]^{7+}$



Ac-atg gtc atg cta cgg cag c-NH_2 **29**:
$C_{206}H_{256}N_{112}O_{58}$ (5229.0 g/mol)
OD_{260} = 46.3 (255 nmol, 13 %), ε_{260} = 181400 M^{-1}•cm^{-1}
UPLC: t_R: 1.01 min (0-40% MeCN, 260 nm);
ESI/MS (m/z): calc.: 1308.3 $[M+4H]^{4+}$, 1046.8 $[M+5H]^{5+}$, 872.5 $[M+6H]^{6+}$;
found: 1308.1 $[M+4H]^{4+}$, 1046.7 $[M+5H]^{5+}$, 872.4 $[M+6H]^{6+}$



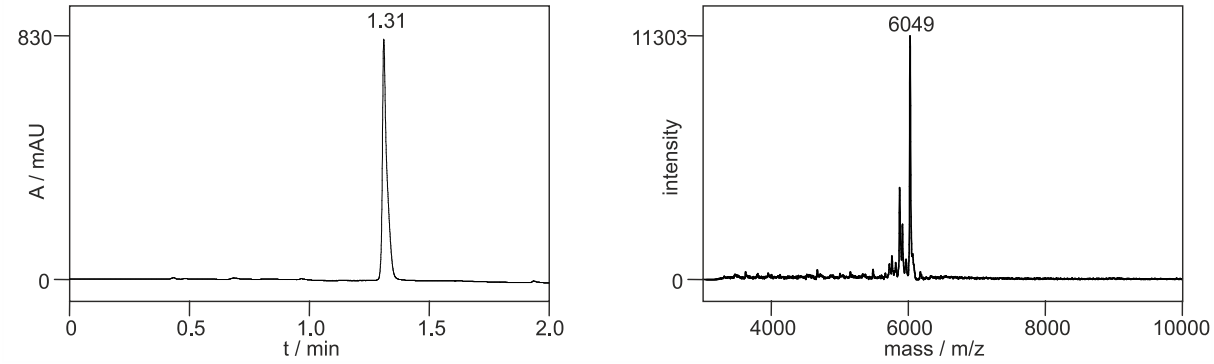
Synthesis of oligonucleotides

The DMT-5-S-*tert*.-Butylthio-cysteinyl-propragylamino-desoxyuridine-β-cyanoethyl-phosphor-amidite (CysT) was prepared as described elsewhere (F. Diezmann, H. Eberhard, O. Seitz, *Biopolymers* **2010**, *94*/4, 397-404) and directly used for DNA-synthesis. Automated DNA-synthesis was performed as described in *Biopolymers* **2010**, *94*/4, 397-404. Yields of the DNA-peptide-conjugates have been calculated by using the extinction coefficients calculated by using the OligoAnalyzer 3.1 from Integrated DNA Technologies (IDT) (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/). Unmodified and template oligonucleotides were purchased from Biotez GmbH, Berlin und used without further purifications. Cysteine-modified 50-mers were synthesized from NOXXON Pharma AG (Berlin, Germany) using our provided CysT. Concentration and purity was controlled by UV-Vis-spectroscopy and MALDI-TOF mass spectrometry.

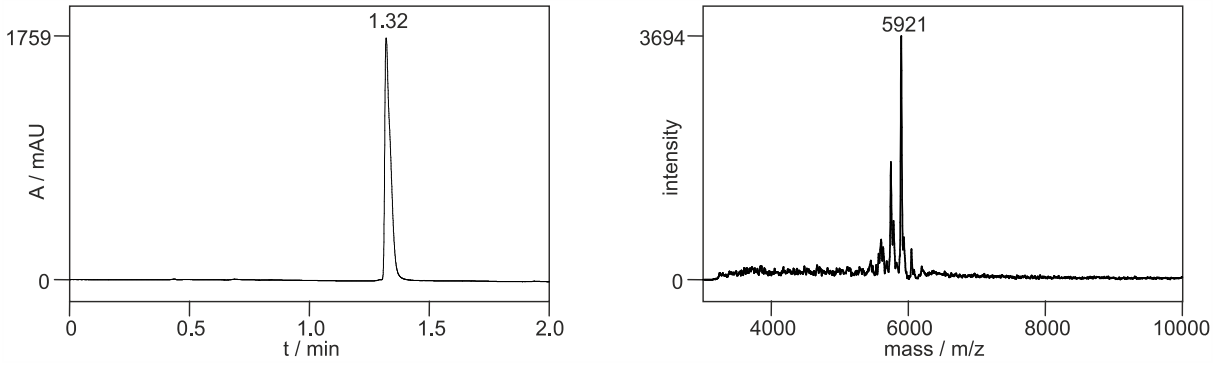
Synthesis of peptide-DNA-conjugates

The synthesis of peptide-DNA-conjugates was performed as previously published (H. Eberhard, F. Diezmann, O. Seitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 4146-4150). **Z** denotes the modified nucleoside cysteinyl-propargylamino-desoxyuridine and indicates the position of peptide conjugation.

5'-CGG CAT CGA GCG G(**Z**(Lys Gly Trp Val Thr Phe Glu Glu Lys))C-3' **30**
$C_{212}H_{259}N_{75}O_{110}P_{15}S$ (6048.9 g/mol)
OD_{260} = 23.9 (171 nmol, 34 %), ε_{260} = 139600 M^{-1}•cm^{-1}
UPLC: t_R: 1.31 min
MALDI-TOF (m/z): calc.: 6049.9 $[M+H]^+$; found: 6049 $[M+H]^+$



5'-C(**Z**(Phe-Glu-Asp-Asn-Phe-Val-Pro-Lys))G GCA CTG AGC GCG-3' **31**
$C_{206}H_{247}N_{73}O_{109}P_{15}S$ (5920.8 g/mol)
OD_{260} = 45.0 (331 nmol, 66 %), ε_{260} = 136100 M^{-1}•cm^{-1}
UPLC: t_R: 1.32 min
MALDI-TOF (m/z): calc.: 5921.8 $[M+H]^+$; found: 5921 $[M+H]^+$



3 DNA-complexes and thermal denaturation experiments

D1	**30** **T(D1)**	CGG CAT CGA GCG G**X**C GCC GTA GCT CGC CAG
D2	**31** **T(D2)**	C**X**G GCA CTG AGC GCG GAC CGT GAC TCG CGC
TC_3	**30 · 31** **T(TC_3)**	CGG CAT CGA GCG G**X**C-C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG GAC CGT GAC TCG CGC
$TC1_n$ (L1 L2); Y = T		
$TC1_6$	**30 · 31** **T($C1_6$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT-GAC CGT GAC TCG CGC
$TC1_8$	**30 · 31** **T($C1_8$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT TT-GAC CGT GAC TCG CGC
$TC1_{10}$	**30 · 31** **T($C1_{10}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT TTT T-GAC CGT GAC TCG CGC
$TC1_{13}$	**30 · 31** **T($C1_{13}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT TTT TTT T-GAC CGT GAC TCG CGC
$TC1_{18}$	**30 · 31** **T($C1_{18}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT TTT TTT TTT TTT-GAC CGT GAC TCG CGC
$TC1_{23}$	**30 · 31** **T($C1_{23}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TTT TTT TTT TTT TTT TTT TT-GAC CGT GAC TCG CGC
$TC2_n$ (L1 L2); Y = mixed		
$TC2_6$	**30 · 31** **T($C2_6$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TCG-GAC CGT GAC TCG CGC
$TC2_8$	**30 · 31** **T($C2_8$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-CGT CG-GAC CGT GAC TCG CGC
$TC2_{10}$	**30 · 31** **T($C2_{10}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GCC GCC G-GAC CGT GAC TCG CGC
$TC2_{11}$	**30 · 31** **T($C2_{11}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TGC CGT CG-GAC CGT GAC TCG CGC
$TC2_{12}$	**30 · 31** **T($C2_{12}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-ATG CCG TCG-GAC CGT GAC TCG CGC
$TC2_{13}$	**30 · 31** **T($C2_{13}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GAT GCC GTC G-GAC CGT GAC TCG CGC
$TC2_{16}$	**30 · 31** **T($C2_{16}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
$TC2_{19}$	**30 · 31** **T($C2_{19}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
$TC2_{22}$	**30 · 31** **T($C2_{22}$)**	CGG CAT CGA GCG G**X**C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_n (L1 L2); DNA-rigidified		
QC_{10}^{DNA}	**30 · R1 · 31** **T($C2_{10}$)**	CGG CAT CGA GCG G**X**C CGG CGG C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GCC GCC G-GAC CGT GAC TCG CGC
QC_{11}^{DNA}	**30 · R2 · 31** **T($C2_{11}$)**	CGG CAT CGA GCG G**X**C ACG GCA GC C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TGC CGT CG-GAC CGT GAC TCG CGC
QC_{12}^{DNA}	**30 · R3 · 31** **T($C2_{12}$)**	CGG CAT CGA GCG G**X**C TAC GGC AGC C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-ATG CCG TCG-GAC CGT GAC TCG CGC
QC_{13}^{DNA}	**30 · R4 · 31** **T($C2_{13}$)**	CGG CAT CGA GCG G**X**C CTA CGG CAG C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{16}^{DNA}	**30 · R5 · 31** **T($C2_{16}$)**	CGG CAT CGA GCG G**X**C ATG CTA CGG CAG C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{19}^{DNA}	**30 · R6 · 31** **T($C2_{19}$)**	CGG CAT CGA GCG G**X**C GTC ATG CTA CGG CAG C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{22}^{DNA}	**30 · R7 · 31** **T($C2_{22}$)**	CGG CAT CGA GCG G**X**C ATG GTC ATG CTA CGG CAG C C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC

QC_n (L1 L2); PNA-rigidified		
QC_{10}^{PNA}	**30 · 23 · 31** **T($C2_{10}$)**	CGG CAT CGA GCG G**X**C cgg cgg c C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GCC GCC G-GAC CGT GAC TCG CGC
QC_{11}^{PNA}	**30 · 24 · 31** **T($C2_{11}$)**	CGG CAT CGA GCG G**X**C acg gca gc C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TGC CGT CG-GAC CGT GAC TCG CGC
QC_{12}^{PNA}	**30 · 25 · 31** **T($C2_{12}$)**	CGG CAT CGA GCG G**X**C tac ggc agc C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-ATG CCG TCG-GAC CGT GAC TCG CGC
QC_{13}^{PNA}	**30 · 26 · 31** **T($C2_{13}$)**	CGG CAT CGA GCG G**X**C cta cgg cag c C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{16}^{PNA}	**30 · 27 · 31** **T($C2_{16}$)**	CGG CAT CGA GCG G**X**C atg cta cgg cag c C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{19}^{PNA}	**30 · 28 · 31** **T($C2_{19}$)**	CGG CAT CGA GCG G**X**C gtc atg cta cgg cag c C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC
QC_{22}^{PNA}	**30 · 29 · 31** **T($C2_{22}$)**	CGG CAT CGA GCG G**X**C atg gtc atg cta cgg cag c C**X**G GCA CTG AGC GCG GCC GTA GCT CGC CAG-TAC CAG TAC GAT GCC GTC G-GAC CGT GAC TCG CGC

Table S2. Composition of peptide-DNA-complexes. **X** = Z with conjugated peptide ligands L1 – L2.

Ternary and quaternary complexes were assembled by mixing equal amounts of required conjugates and templates, heating the mixture to 75°C and cool down slowly. For the preparation of pentameric complexes the solution was heated to 85°C and subsequently cooled to 20°C at 1°C/min.

Denaturation experiments were performed by using a Cary 100 Bio UV/Vis spectrophotometer (Varian, Agilent Technologies, Waldbronn, Germany) equipped with a peltier thermostated cell changer and a heating rate of 0.5 °C/min. The oligonucleotides were dissolved at 1.0 µM concentration in 10 mM NaH_2PO_4, 100 mM NaCl buffer at pH 7.0. The T_M-values were determined based on the mean of the maximum of the first derivative of at least three denaturation curves.
Figure **S1** shows denaturation experiments proving the stability of the modified peptide-DNA-conjugates. Similar stability studies have been published previously (F. Diezmann, H. Eberhard, O. Seitz, *Biopolymers* **2010**, *94*/4, 397-404; H. Eberhard, F. Diezmann, O. Seitz, Angew. Chem. Int. Ed. 2011, 50, 4146-4150).

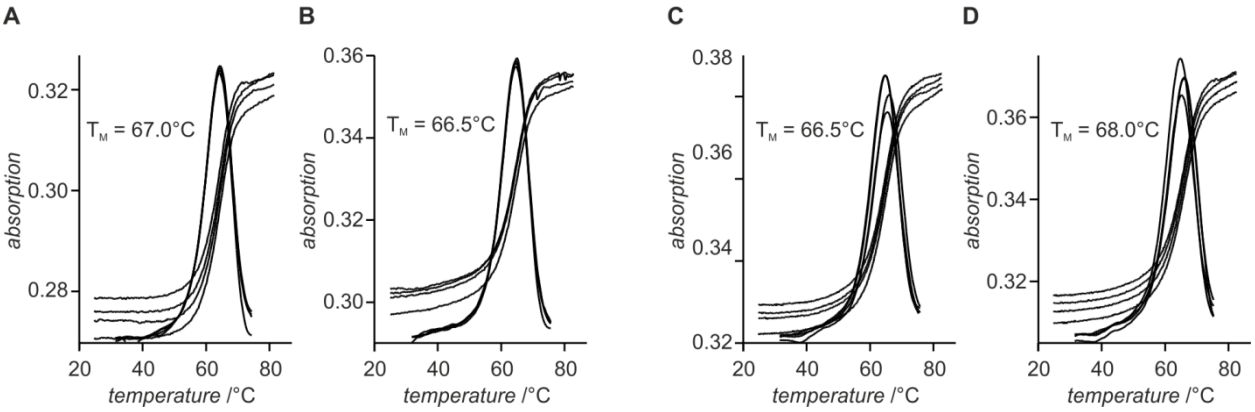


Figure S1. Thermal denaturation curves and their derivatives of peptide-modified duplexes D1 (A) and D2 (C) and their corresponding unmodified duplexes (B) and (D).

The complexes QC_n formed upon addition of rigidifiers were characterized by means of three independent thermal denaturation experiments which involved the template strand alone, template + DNA-rigidifier and template + PNA-rigidifier. Figure S2 shows denaturation curves measured for QC_{10} (A) and QC_{16} (B). The sigmoidal curve observed in the melt analyses of single stranded

templates point to intramolecular base pairing. The 13 nt long DNA- and PNA-rigidifiers successfully competed with intramolecular hybridization and provided duplexes that have higher T_M than the template strand alone (Fig S2B). In contrast to the 7 nt long PNA rigidifier, the 7 nt long DNA-rigidifier failed in competing with intramolecular base pairing (Fig. S2A).

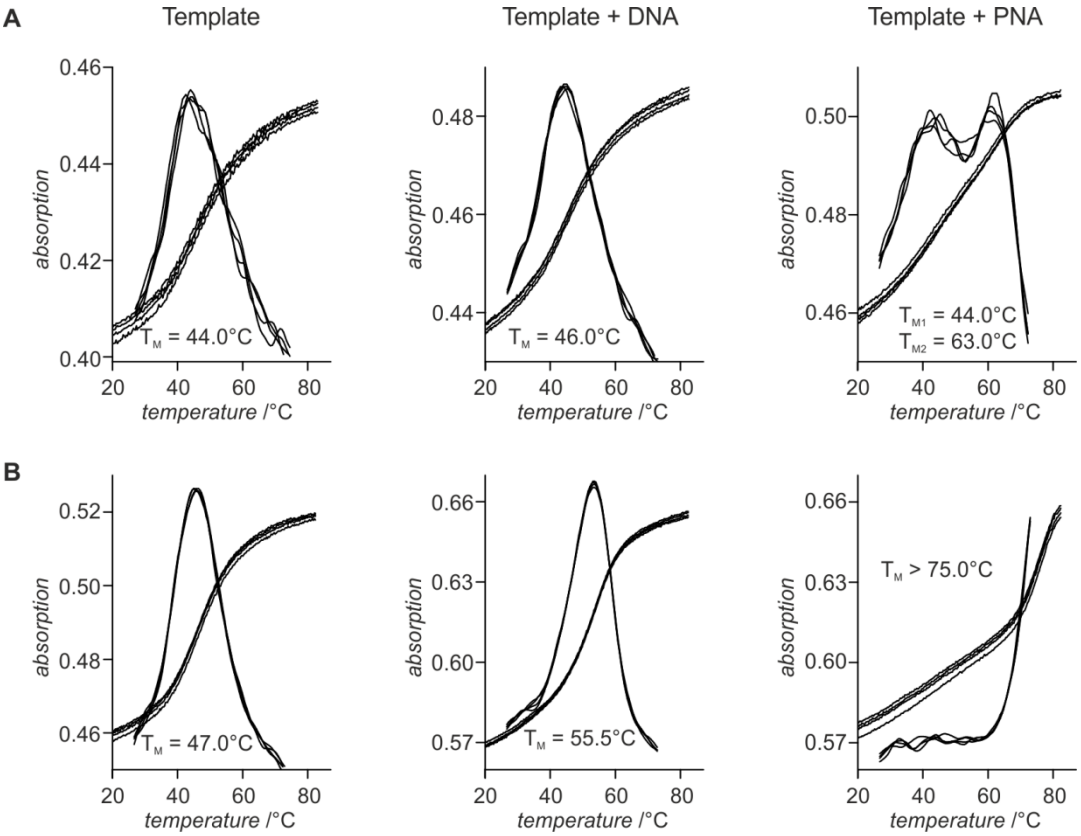


Figure S2. Thermal denaturation curves showing the stability of duplexes formed upon addition of rigidifiers in QC_{10} (A) and QC_{16} (B). A) Left: template **T(C2$_{10}$)**; middle: template **T(C2$_{10}$)** + DNA-7mer rigidifier **R1;** right: template **T(C2$_{10}$)** + PNA-7mer rigidifier **23**; B) Left: template **T(C2$_{16}$)** ; middle: template **T(C2$_{16}$)** + DNA-13mer rigidifier **R5**; right: template **T(C2$_{16}$)** + PNA-13mer rigidifier **27**.

4 Overexpression and purification of GST fusion proteins

E. coli bacteria were used as host organisms for overexpression of soluble recombinant proteins. As expression strains *E. coli* BL21 and ER2566 carrying the desired expression vector were used (Table **S3**).

Protein	vector	amino acids	kDa	extinction coefficient
GST- α-ear	pGEX 4T-1	702-938	53.568	51290 $l \cdot mol^{-1} \cdot cm^{-1}$
GST- Amphiphysin 1 B/C	pGEX 4T-1	250-578	38.507	52300 $l \cdot mol^{-1} \cdot cm^{-1}$

Table S3. Fusion proteins used in this study.

The LB/ampicillin-media (50 mL, 0.5 % [w/v] yeast extract, 1 % [w/v] trypton, 0.5 % [w/v] NaCl, pH 7.2 50 µg/mL ampicilin) were inoculated with a pipette tip of a bacteria glycerol stock and gently shaken at 37°C and 200 rpm overnight. The overnight culture was diluted (1:20) in selective 2xYT-medium (1 % [w/v] yeast extract, 1.6 % [w/v] trypton, 0.5 % [w/v] NaCl, pH 7.2) and incubated by shaking at 37°C until the OD_{600} reached 0.7 to 0.8. Protein expression was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM. Protein expression cultures were grown for 4 h at 30°C. After overexpression, the bacteria were harvested by centrifugation at 4000 x g for 15 min at 4°C. Cell pellets from 500 ml cultures were suspended in 35 mL ice-cold PBS and stored at -80°C. Bacterial pellets were supplemented with 1 mM PMSF, 1 mM DTT, 100 units of benzonase endonuclease (Sigma) and a "tip of a spatula" of lysozyme (Roth) and 1 % Triton X-100. The cells were sonicated by using a Microtip System Sonopuls from Bandelin (Berlin, Germany) for 90 s using 70 % power and 50 % duty cycle. The samples were centrifuged for 15 min at 17,000 x rpm at 4°C. The supernatant was added to pre-washed GST-binding resin (Novagen, 0.5 ml slurry per 500 ml expression culture) and incubated for 2 h at 4°C. The suspension was centrifuged at 2000 x g at 4°C for 2 min and beads were washed three times with PBS.
For protein elution, beads were incubated in 0.5 mL elution buffer (PBS containing 20 mM reduced glutathione) for 1 h at 4°C and centrifuged shortly. The protein solution was ultracentrifuged at 50.000x g at 4°C for 30 min and the protein buffer was exchanged. For this purpose, the protein solution was centrifuged in filter tubes (*Millipore,* Amicon Ultra-15, 30,000 MWCO) at 4300 rpm and 4°C until the total volume was reduced to ~1.0 mL. Then assay buffer (20 mM HEPES, 50 mM NaCl, 3 mM EDTA at pH 7.4) was added and the procedure was repeated four times. Finally, the solution was centrifuged until the total volume was reduced to ~1.0 mL. The concentration of protein in the resulting solution was determined via the absorption at 280 nm and by Bradford assay. Concentrations obtained were between 200 and 400 µM. The samples were aliquoted, snap-frozen in liquid nitrogen, and stored at -80 °C. Protein integrity was verified by SDS-PAGE (Figure S5).

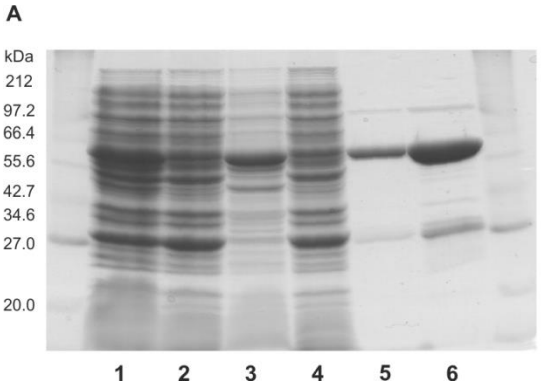


Figure S3. SDS-PAGE-gel of GST-α-ear. start (lane 1), pellet (lane 2), beads (lane 3), supernatant (lane 4), 1 µg (lane 5) and 5 µg (lane 6) of GST-α-ear. Conditions: 10% acrylamide gel loaded with protein marker (*Fermentas* prestained broad range 20-212 kDa, running time 60 min at 15-20 mA)

5 Estimation of distances

Distance between top site and side site binder of the AP-2 α-ear domain

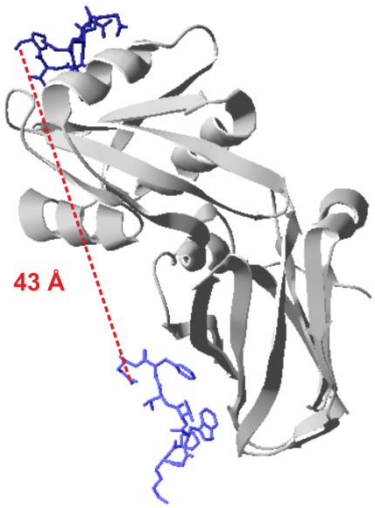


Figure S4. Crystal structure of AP-2 α-ear (pdb 2VJO) in complex with peptide ligands FEDNFVP (top site) and PKGWVTFE (side site). Distance between C-terminal prolin (anchor for attachment of DNA) of the top site binder and C-terminal glutamic acid of the side site binder was determined by using Swiss-PdbViewer 4.0.4.

6. Pull-down assay

The rat brain lysate (0.5 mL) was incubated with the immobilized GST-amphiphysin (25 µg) at 4°C for 1h as described in the methods section. After separation of the beads by centrifugation and washing the captured proteins were eluted by addition of SDS gel loading buffer (50 µL), as described in the methods section. Aliquots (25 µL) were analyzed by SDS gel electrophoresis. The gel was stained with Coomassie in order to prove that all samples contained the identical amount of GST-amphipysin or GST (Figure S5A). The gel also shows untreated rat brain extract. A second SDS gel (8%) was blotted after electrophoresis with a nitrocellulose membrane, which was stained by using PonceauS (Figure S5B). This stain confirmed that identical amounts of proteins were transferred onto

the membrane. After washing with 1% acetic acid and TBS the membrane was cut and the separate fragments incubated with different solutions of primary antibodies. An antibody against the AP-2 μ-domain was used for the detection of AP-2 (Figure S5C, lower segment). Because amphiphysin binds the α ear domain, positive staining confirmed the integrity of AP-2. Another antibody was used to detect clathrin (Figure S5C, upper segment). Clathrin also binds to amphiphysin, but at a different site. In the absence of competitors of the amphiphysin-AP2 interaction, the ratio between bands from AP-2 and clathrin should remain constant regardless of the concentration of rat brain lysate used for incubation with the bait. This proved true (Figure S5C).

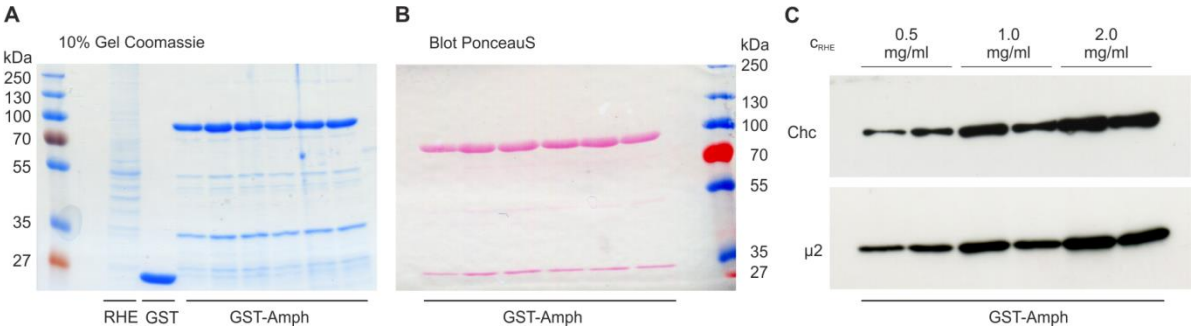


Figure S5. AP2 pull-down using diverse concentrations of rat brain extract. A) Coomassie-Gel (10%) of GST-proteins; B) Ponceau-staining of GST-Amphiphysin; C) Western Blot. Compared are different concentrations of rat brain extract (0.5, 1.0, and 2.0 mg/ml). Conditions: 25 μg GST or GST-Amph, 0.5 mL of 0.5, 1.0 or 2.0 mg/mL rat brain lysate, 1 h incubation, buffer: 20 mM HEPES, pH 7.4, 50 mM NaCl, 2 mM $MgCl_2$, 1% TritonX-100, 1 mM PMSF. Chc – Clathrin heavy chain.