Supporting information

Table of content

1. Materials and methods

1.1 *Materials*

Rink Amide AM resin (0.68 mmol/g) was obtained from Novabiochem. L-amino acids were used throughout the synthesis. Fmoc-β-(2-furyl)-L-alanine was purchased from Chem-Impex International. Dimethylformamide (DMF) peptide synthesis grade and N-methylpyrrolidone (NMP) were purchased from Biosolve. Dichloromethane (DCM) and N,N-diisopropylethylamine (DIPEA) were obtained from Aldrich. Trifluoroacetic acid (TFA), Hexafluoroisopropanol (HFIP), Fmoc-protected amino acids, and coupling reagents were obtained from Iris Biotech GmbH.

1.2 *General*

Crosslinking experiments were performed using Eppendorf Thermomixer Comfort for temperature control and Euromex Illuminator E_K-1 lamps (100W halogen lamp LE.5210), equipped with Euromex LE.5214 dual arm light conductor. The power of the lamps was measured using a TES 1335 light meter, equipped with a custom fitting for the lamp bulbs. During the crosslink reaction the temperature was kept constant at 25 °C.

Varian Cary 3E UV/VIS spectrophotometer or Trinean DropSense96 UV/VIS droplet reader were used for UV/VIS spectroscopy.

RP-HPLC analyses were performed on an Agilent 1100 Series HPLC system equipped with XTerra Shield RP18 Column, 125 Å (2.1 mm x 250 mm, 5 µm). A two solvent system was used: 0.1% TFA in H_2O (A) and 0.1% TFA in CH_3CN (B). Samples were eluted using a gradient from 30 % to 60 % B over 10 minutes at a flow rate of 350 µL/min at 40°C.

RP-HPLC-MS analyses were performed on an Agilent 1100 Series instrument with diode array detector, equipped with a Phenomenex Kinetex C18 100 Å (150 x 4.6 mm, 5 µm, at 35 °C), hyphenated to an Agilent ESI-single quadrupole MS detector type VL. Mass detection operated in the positive mode. A two solvent system was used: 0.1 % HCOOH in H_2O (A) and CH_3CN (B). Samples were eluted using a gradient from 0 % to 100 % B over 15 minutes at a flow rate of 1.5 mL/min at 35 °C.

MALDI-TOF-MS data were acquired on a Sciex/Applied Biosystems 4800plus MaldiTOF/TOF analyser equipped with a Nd-YAG solid state laser (355nm) and a pulse frequency of 200 Hz. For 1 mL matrix, 5 mg of α-cyano-4-hydroxycinnamic acid (CHCA) was dissolved in a 1:1 (CH_3CN:MQ water) solution containing 0.01% TFA and 0.01 M ammonium citrate buffer. First, 0.5 µL of the CHCA matrix, was spotted on a plate. Directly afterwards, 0.5 µL of the sample was pipetted on top of the matrix and finally, 0.5 µL of the matrix was added on top of the sample.

1.3 *Peptide synthesis*

1.3.1 Automated peptide synthesis

To synthesize the K_{coil} and R_{coil} peptides, 100 mg of the Rink amide resin (0.69 mmol/g) was used. For the E_{coil} peptides, 100 mg of the ChemMatrix rink amide resin was used (0.54 mmol/g). Automated peptide synthesis was done with a SYRO Multiple Peptide Synthesizer (Multisyntech) or with a MultiPep RSi (Intavis), making use of the Fmoc/tBu strategy. Usually, synthesis with double couplings was performed as following: the resin is swollen in DMF for 20 minutes. A mixture of 5 equivalents (eq.) amino acid in DMF (0.5 M), 5 eq. N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) in DMF (0.5 M), and 10 eq. DIPEA in NMP (2 M) is added to the resin, with subsequent reaction for 40 minutes at room temperature. The reaction mixture is removed and the

resin is washed with DMF (4 x 30 sec). The Fmoc group is removed by reaction with 20% piperidine in DMF for 12 minutes. In case the Intavis equipment was used, a capping step was performed after each amino acid coupling. For this purpose, a solution containing 5% acetic anhydride and 5% N-methylmorpoline in DMF was used.

1.3.2 Manual coupling of 4-Aminobenzoic acid

4-Acetamidobenzoic acid (Aba, 4 eq.) and HBTU (4 eq.) were dissolved in DMF (20 mL/g resin). Subsequently, DIPEA (8 eq.) was added and after 5 min, this mixture was added to the resin. After 1.5 hour of shaking, the solution was filtered off, the resin washed with DMF (3 x 30 sec), DCM (3 x 30 sec), DMF (3 x 30 sec), and a TNBS test is performed.

1.3.3 Manual coupling of 3-(2-furyl)propionic acid

To introduce the 3-(2-Furyl)propionic acid in the native E_{coil} peptides, Dap (2,3-diaminopropionic acid, 1 C chain), Dab (2,4-diaminobutyric acid, 2 C chain), Orn (ornithine, 3 C chain), and Lys (lysine, 4 C chain) with the side chain protected with a 4-methyltrityl (Mtt) group were incorporated by automated peptide synthesis at position 8. Then, the Mtt group was removed by treating the resin with a solution of HFIP (Hexafluoro-2-propanol)/DCM 1:1 with 0.5% HOBt (1 mL of solution per 10 µmol of peptide).[1] The resin was treated six times for three minutes with the deprotection cocktail. Once the deprotection cocktail remains colourless after adding a few drops of TFA, the deprotection can be regarded as complete. After deprotecting the Mtt group, 3-(2-furyl)propionic acid (4 eq.) and HBTU (4 eq.) were dissolved in DMF (20 mL/g resin). Subsequently DIPEA (8 eq.) was added and after 5 min, this mixture was added to the resin. After 1.5 hour of shaking, the solution is filtered off, the resin washed with DMF (3 x 30 sec), DCM (3 x 30 sec), DMF (3 x 30 sec), and a TNBS test is performed.

1.3.4 TNBS test

TNBS test is an easy detection method of free amino group. Few beads of the resin were transferred to a small test tube, and 10 µL of 10% DIPEA in DMF and 10 µL of a 5% solution of trinitrobenzene sulfonic acid in methanol were added at room temperature. The mixture is vortexed. If the coupling has not reached full conversion, the free amines present in the beads will react with the TNBS through nucleophilic aromatic substitution, and the beads will turn bright red. If the beads remain yellow, the amide bond formation is complete. When red beads are observed, the coupling must be repeated until TNBS test is negative.

1.3.5 Acetylation

To acetylate primary amines, the peptide resin was treated twice with 600 µL of a 89:5:6 DMF/Ac_2O/DIPEA solution for 1 minute.

1.3.6 Dde Deprotection

First a deprotection solution containing 5 g hydroxylamine hydrochloride and 3.6 g imidazole in 20 mL NMP was prepared. Just before the reaction, 5 volumes (1 mL) of this solution were diluted with 1 volume (200 µL) of DMF. This was added to the peptide resin.[2] The resin is subsequently shaken overnight. Afterwards the resin was washed with 3 x DMF, 3 x DCM, and 3 x DMF.

1.3.7 Coupling of pyrene butyric acid

The coupling of 1-pyrenebutyric acid (py) to obtain the pyrene-modified peptides was done manually. The coupling step was performed on resin on a 5 µmole scale. Py (5 eq.) and HBTU (4.9 eq.) were dissolved in DMF (400 µL). Subsequently DIPEA (10 eq.) was added and the mixture was allowed to activate for 2 min before addition to the pre-swollen resin. After 1 hour of shaking, the solution was filtered off and the resin washed with DMF.

1.3.8 Synthesis of pyrene-containing probes

C-terminally modified pyrene-probes: First the N-terminal fluorenylmethoxycarbonyl (Fmoc) protecting group was removed, and then the N terminus was acetylated. Finally, 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) was deprotected and 1-pyrenebutyric acid was coupled.

N-terminally modified pyrene probes: First the Fmoc was removed, then the N terminus was coupled to 1-pyrenebutyric acid. Finally, Dde was deprotected and the free amino group was acetylated.

1.3.9 Cleavage of the K_{coil} and R_{coil} peptides

The resin was treated with a mixture of TFA, triisopropylsilane (TIS), H_2O (95:2.5:2.5; 20 mL/g resin) and shaken for 2 hours. The mixture was then filtered off and the resin beads washed 3 times with the cleavage cocktail solution. The filtrate was collected in a falcon tube, and excess of TFA evaporated using an Ar or N_2 stream. The peptide precipitated with cold MTBE and centrifuged for 10 minutes at 5000 rpm at 4°C. The supernatant was removed. Subsequently, the peptide was dispersed in fresh ether and centrifuged (repeated twice), after which the remaining MTBE was evaporated, and the peptide dried in vacuo. The peptide was dissolved in MilliQ water/ACN (50/50), frozen in liquid N_2 and freeze-dried to store them until purification.

1.3.10 Cleavage of the furan containing E_{coil}-peptides and pyrene probes

The resin is treated with a mixture of TFA and m-Cresol (9:1, 500 µL/10 µmol) and shaken for 40 minutes. Subsequently, the mixture is collected in a falcon tube and the resin is treated a second time with this cleavage cocktail (40 minutes). The filtrate was collected in another falcon tube and the peptide precipitated with 10 volumes Et_2O, allowed to precipitate for 2h at -20°C, and centrifuged for 10 minutes at 5000 rpm per minute at 4°C. The supernatant is removed. The solid is resuspended in fresh Et_2O and centrifuged (repeated 3 times), after which the remaining Et_2O is evaporated. The peptide was dissolved in a MilliQ water/ACN (50/50), frozen in liquid N_2, and freeze-dried overnight.

1.3.11 Purification

Preparative purification was performed on an Agilent 218 solvent delivery system with a UV-VIS dual wavelength detector using a Phenomenex column (AXIA packed Luna C18(2), 250 x 21.2 mm, 5 µm particle size, 35°C) with a flow rate of 17 mL/min. The desired product was eluted with a gradient, using following solvent systems: 0.1% TFA in H_2O (A) and 0.1% TFA in CH_3CN (B), starting from 100% A, going up to 100% B in 30 min.

1.4 Circular dichroism (CD) spectroscopy

For this experiment a JASCO J7100 instrument (Tokyo, Japan) was used, equipped with a HAAKE cryostat temperature-controlled cell holder. The CD sp ectra of the peptides were recorded at 25 °C. The data are the average of nine scans. The concentration of each peptide in solution was 5 µM in phosphate buffered saline (100 mM NaCl, 10 mM phosphate, pH 7.4).

CD spectra of each peptide were recorded as well as a mixture containing both an E_{coil} type peptide and a K_{coil} or R_{coil} type peptide. The spectra were recorded at 50 nm/min scan rate and a bandwidth of 1 nm in a wavelength range 200-260 nm using a cell with 1 cm path length.

CD spectra are reported as the mean residue molar ellipticity ([θ], deg x cm^2/dmol), calculated by the equation:

$[\theta] = (\theta_{obs} \times MRW) / (10*l*c)$

where θ_{obs} is the ellipticity in millidegrees, MRW is the mean residue molecular weight (molecular weight of the peptide divided by the number of amino acid residues), l is the path length of the cuvette in

centimetres, and c is the peptide concentration in milligrams per millilitre. The degree of helical structure is directly proportional to the negative molar ellipticity ([θ]) at 222 nm and the $[\theta]_{222}/[\theta]_{208}$ ratio is typically 0.66-0.72 for the single α-helices and >1.0 for coiled-coil helical dimers E3 and K3 coils as previously reported.[3]

The negative molar ellipticity ([θ]) at 222 nm and the $[\theta]_{222}/[\theta]_{208}$ ratio of the single α-helices E3 (Ac-EIAALEKEIAALEKEIAALEK-NH2) and K3 (Ac-KIAALKEKIAALKEKIAALKE-NH2) coils, as well as a mixture containing both, as previously reported by [3] are below:

Table SI 2: The ellipticity ([θ]) at 222 nm and the [θ]222/[θ]208 ratio of the single α-helices E3 and K3 coils, as well as a mixture containing both.

	K_{Coil}	E_{Coil}	Coiled-coil
$[\theta]_{222}$	-11320	-9810	-30290
$[\theta]_{208}$	-15722	-14864	-28848
$[\theta]_{222}/[\theta]_{208}$ ratio	**0.72**	**0,66**	**1,05**

The E3/K3 coiled-coil complex was designed as a remarkably stable heterodimer with a dissociation constant of 70 nM.[3] In this paper, we assumed that we have similar K_D values for all K_{Coil} and R_{Coil} with E_{Coil} and variation thereof. At a concentration of 5 µM for each coil peptide, the monomer↔dimer equilibrium is shifted toward the formation of coiled-coil dimer in an 88.8%. See calculations below:

In equilibrium:

1. $[E_{Coil}][K/R_{Coil}] \leftrightarrow [\text{Coiled-coil}]$

Then the dissociation constant (k_D) is defined as:

2. $k_D = ([E_{Coil}]\,[K/R_{Coil}])/[\text{Coiled-coil}]$

where $[E_{Coil}]$, $[K/R_{Coil}]$, and [Coiled-coil] are the concentrations at equilibrium. The initial concentrations of the reactants are:

3. $[E_{Coil}]_i = [E_{Coil}] + [\text{Coiled-coil}]$ which can be rearranged as $[E_{Coil}] = [E_{Coil}]_i - [\text{Coiled-coil}]$

4. $[K/R_{Coil}]_i = [K/R_{Coil}] + [\text{Coiled-coil}]$ which can be rearranged as $[K/R_{Coil}] = [K/R_{Coil}]_i - [\text{Coiled-coil}]$

Substitute equation 3 and 4 into equation 2:

5. $k_D = ([E_{Coil}]_i - [\text{Coiled-coil}])\,([K/R_{Coil}]_i - [\text{Coiled-coil}])/[\text{Coiled-coil}]$

Rearrange the equation:

6. $[\text{Coiled-coil}] = (([E_{Coil}]_i - [\text{Coiled-coil}])\,([K/R_{Coil}]_i - [\text{Coiled-coil}]))/\,k_D$

If [Coiled-coil] is x and the initial concentrations are introduced, the equation is:

7. $x = ((5-x)\,(5-x))/0.07$; $x = 4.44$ µM

Then the coiled-coil concentration is 4.44 µM, this means that the equilibrium is shifted toward 88.8% of coiled-coil dimer formation. If we work at 5-10 µM, the ellipticity of the coiled-coil will be very similar, almost independent of peptide concentration.

1.5 Crosslink experiments

In a typical experiment, a 100 µM work solution of Rhodamine B (RhoB) was freshly prepared from a 1 mM stock solution. The stock solution of each peptide equalled 100 µM. In a 1.5 mL Eppendorf vial, 300 µL of an air saturated buffered solution (phosphate buffered saline, pH 7.4) containing 5 µM of the K_{coil}

peptide, 10 µM of the E_{coil} peptide was prepared and allowed to equilibrate for 5 min at 25°C, before the addition of RhoB at 10 µM final concentration. The lamp is then placed on top of the Eppendorf vial for 1 hour. Subsequently, 40 µL of solution were sampled at different irradiation times. The reaction mixture was left to react overnight at 25°C. Samples were taken for HPLC-UV trace and HPLC-MS analysis.

1.5.1 Calculation of crosslink yield

First, the integration of the peak corresponding to the R_{coil} peptide at t = 0 (I_{SM_0}) was measured at 260 nm. Subsequently the integrations of the peak corresponding to the crosslinked product (I_{XL}) were taken at 260 nm. The crosslinking yield (XL %) could then be calculated by:

$$XL\,\% = \frac{I_{XL}}{2 \cdot I_{SM_0}} \cdot 100 \qquad (1)$$

2. K_{coil}, E_{coil}, and Furan-modified-E_{coil} peptide characterization

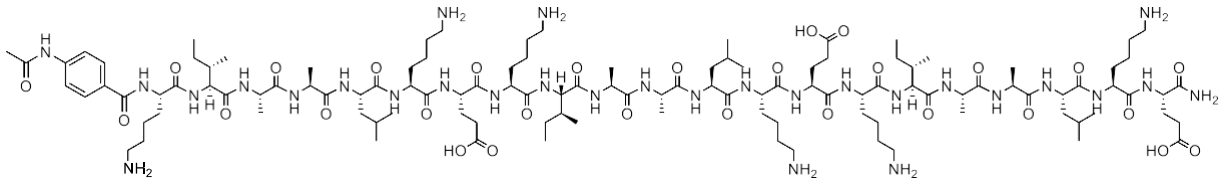
2.1 *Table of the K_{coil} and E_{coil} synthesized peptides*

Name	***Peptide Sequences***	***Calcd MW (Da)***
K_{coil}	Aba-K IAALKEK IAALKEK IAALKE-NH_2	2438.5
E_{coil}	Aba-E IAALEKE IAALEKE IAALEK-NH_2	2441.34
E_{coil}-FuA-8	Aba-E IAALEK-**FuA-** IAALEKE IAALEK-NH_2	2449.35
E_{coil}-Dap_{Fur}-8	Aba-E IAALEK-**$Dap_{(Fur)}$-**IAALEKE IAALEK-NH_2	2520.38
E_{coil}-Dab_{Fur}-8	Aba-E IAALEK-**$Dab_{(Fur)}$-** IAALEKE IAALEK-NH_2	2535.97
E_{coil}-Orn_{Fur}-8	Aba-E IAALEK-**$Orn_{(Fur)}$-**IAALEKE IAALEK-NH_2	2550.00
E_{coil}-Lys_{Fur}-8	Aba-E IAALEK-**$Lys_{(Fur)}$-**IAALEKE IAALEK-NH_2	2562.43

Table SI 1: Overview of the K_{coil}, E_{coil}, and Furan-modified E_{coil} peptide sequences, the calculated molecular weight (MW), and their corresponding codes: 4-Acetamidobenzoic acid (Aba), 2-furyalalnine (FuA), 2,3-diaminopropionic acid (Dap), 2,4-diaminobutyric acid (Dab), Ornithine (Orn), Lysine (Lys), and 3-(2-Furyl)propionic acid (Fur).

2.2 *K_{coil} peptide*

A)



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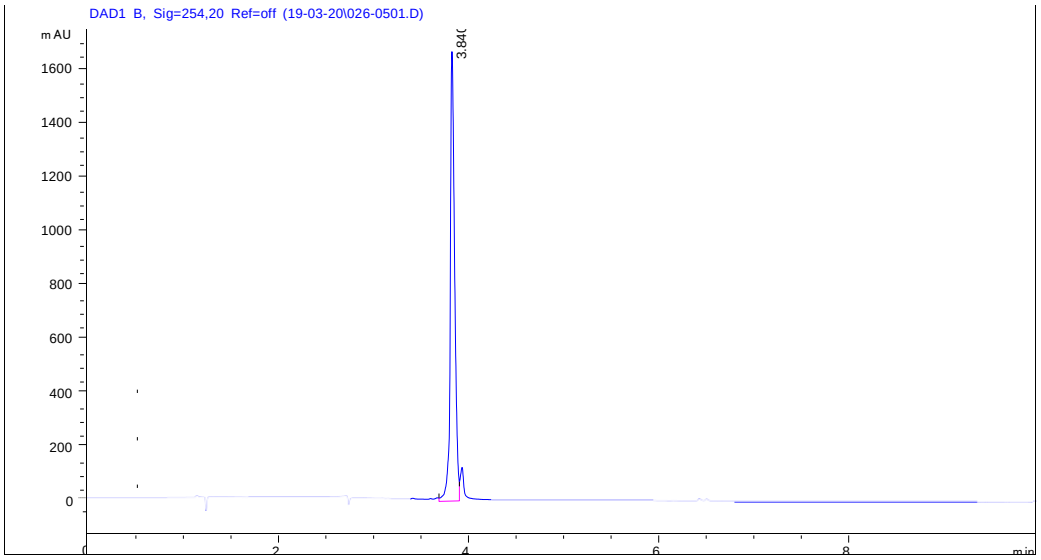


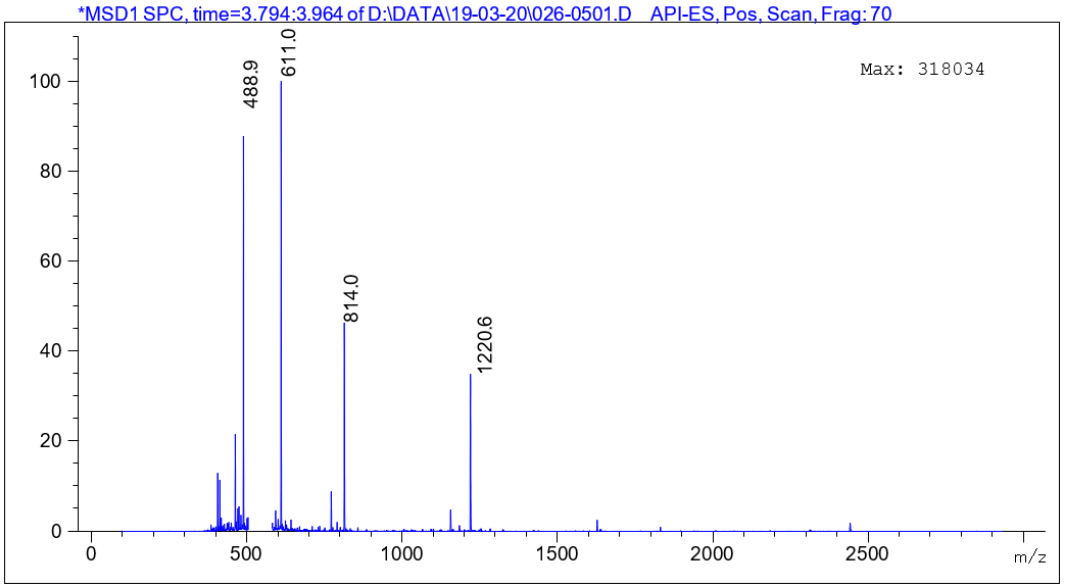
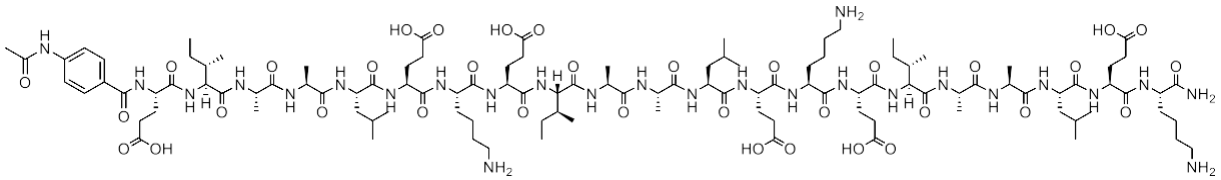


Figure SI 1: A) Structure of the k_{coil} peptide. B) HPLC-MS chromatogram of purified K_{coil}. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2438.5 Da. LC-ESI-MS m/z at t_r= 3.84: $[M+2H]^{2+}/2$= 1220.6; $[M+3H]^{3+}/3$= 814.0; $[M+4H]^{4+}/4$= 611.0; $[M+5H]^{5+}/5$= 488.9.

2.3 *E_{coil} peptide*

A)



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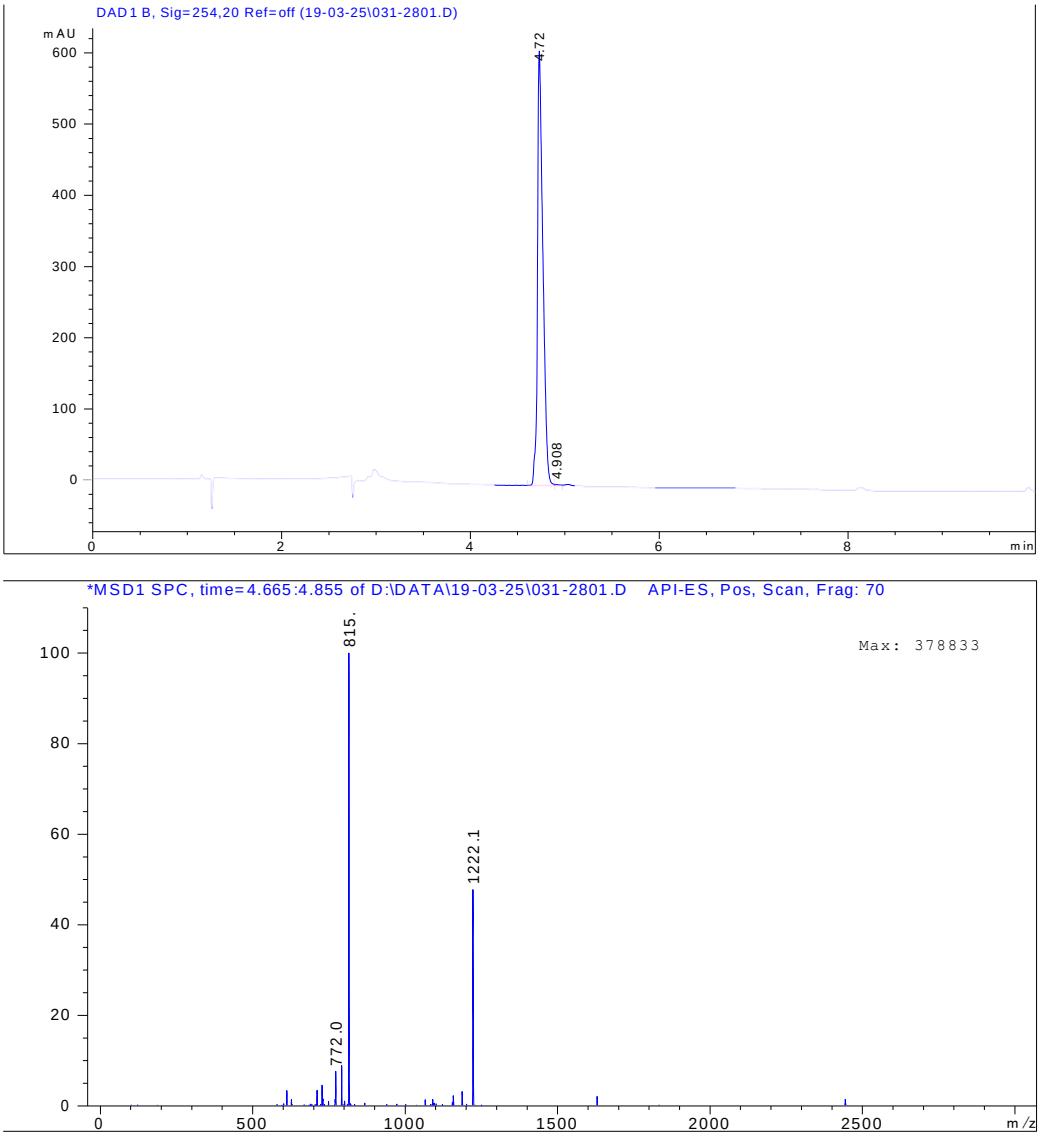
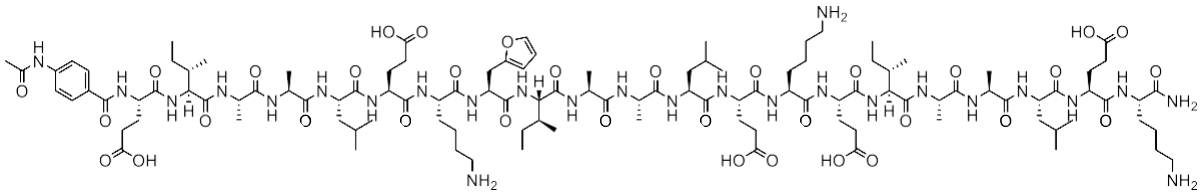


Figure SI 2: A) Structure of the E_{coil} peptide. B) HPLC-MS trace of purified E_{coil}. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2441.34 Da. LC-ESI-MS m/z at t_r= 4.73: $[M+2H]^{2+}/2$= 12221.1; $[M+3H]^{3+}/3$= 815.1.

2.4*E_{coil}-FuA-8 peptide*

A)



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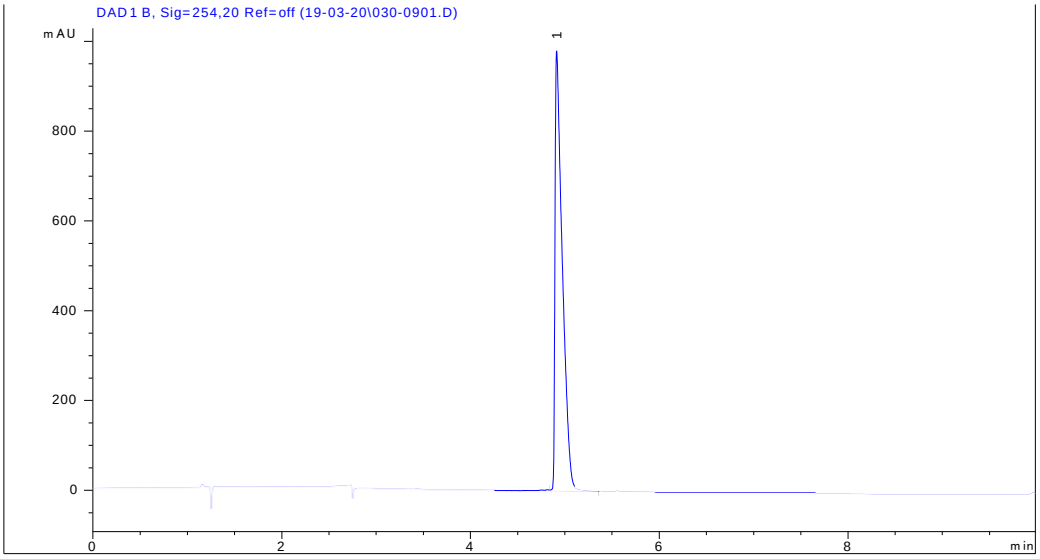


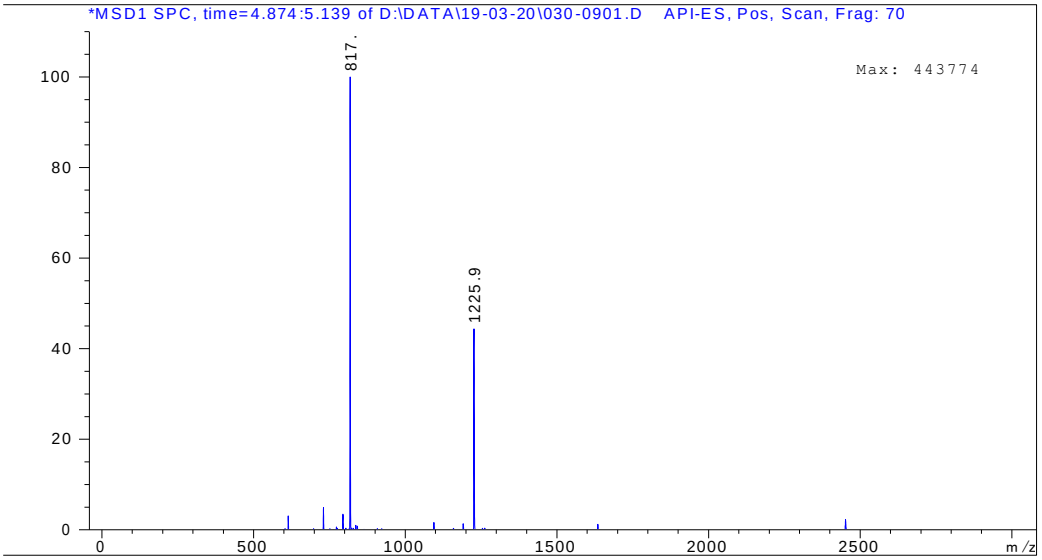
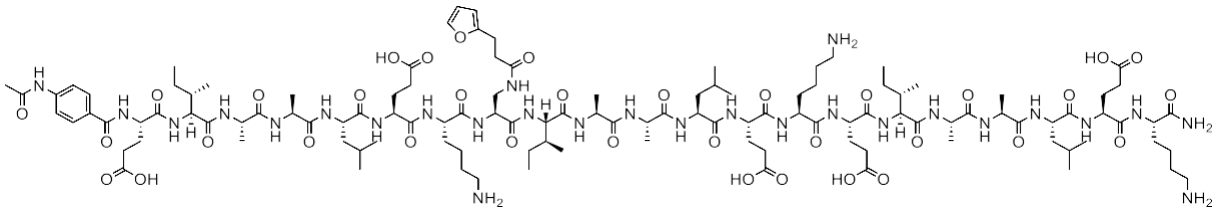


Figure SI 3: A) Structure of the E_{coil}-FuA-8 peptide. B) HPLC-MS trace of purified E_{coil}-FuA-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2449.35 Da. LC-ESI-MS m/z at t_r= 4.91: $[M+2H]^{2+}/2$= 1225.9; $[M+3H]^{3+}/3$= 817.7.

2.5 *E_{coil}-Dap_{Fur}-8 peptide*

A)



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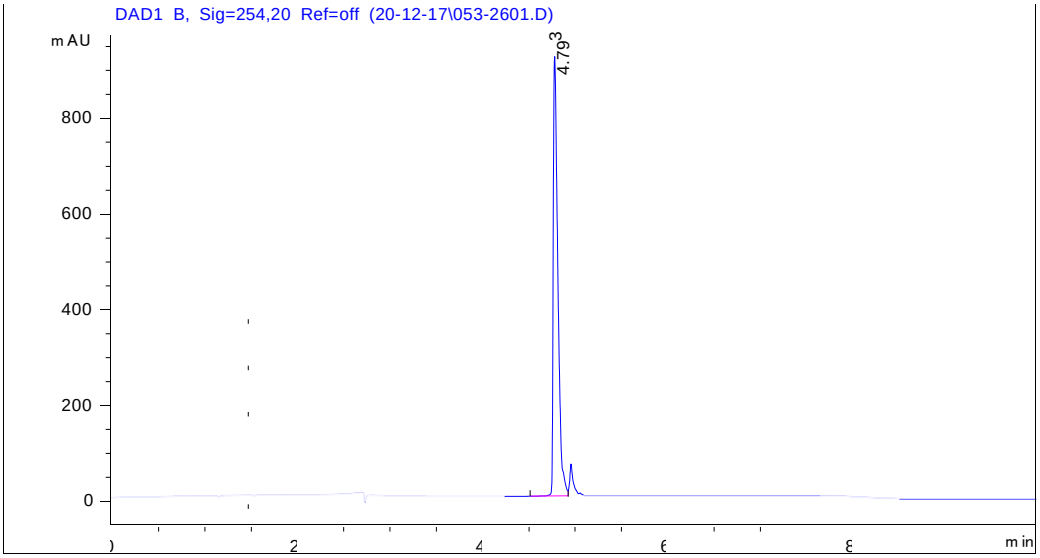


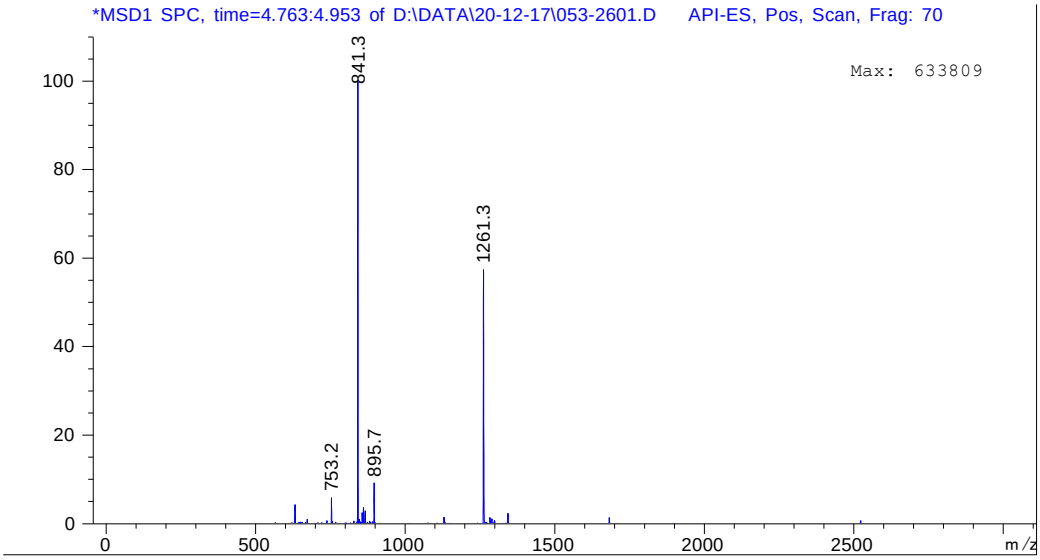
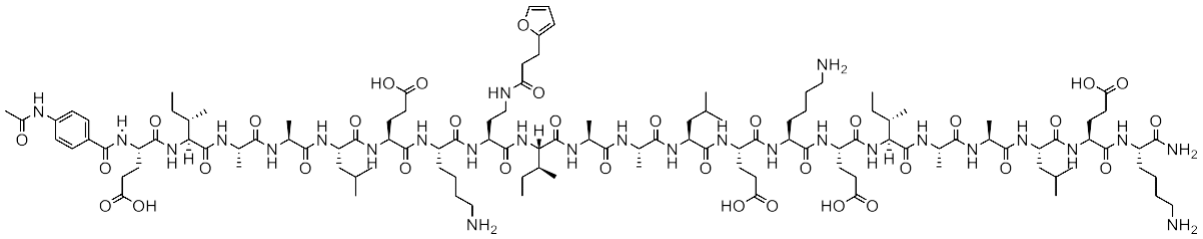


Figure SI 4: A) Structure of the E_{coil}-Dap_{Fur}-8 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.79: $[M+2H]^{2+}/2$= 1261.3; $[M+3H]^{3+}/3$= 841.3.

2.6 *E_{coil}-Dab_{Fur}-8 peptide*

A)



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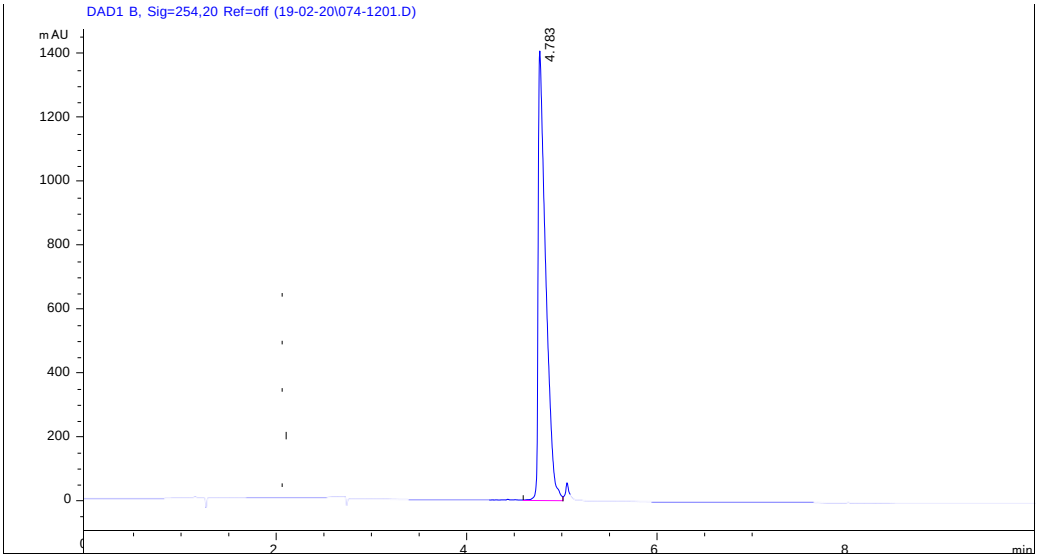
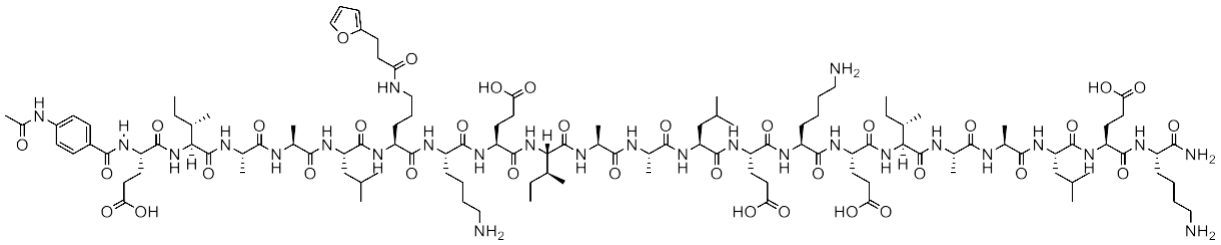


Figure SI 5: A) Structure of the E_{coil}-Dab_{Fur}-8 peptide. B) HPLC-MS trace of purified E_{coil}-Dab_{Fur}-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2535.97 Da. LC-ESI-MS m/z at t_r= 4.78: $[M+2H]^{2+}/2$= 1268.6; $[M+3H]^{3+}/3$= 846.2.

2.7 *E_{coil}-Orn_{Fur}-8 peptide*

A)



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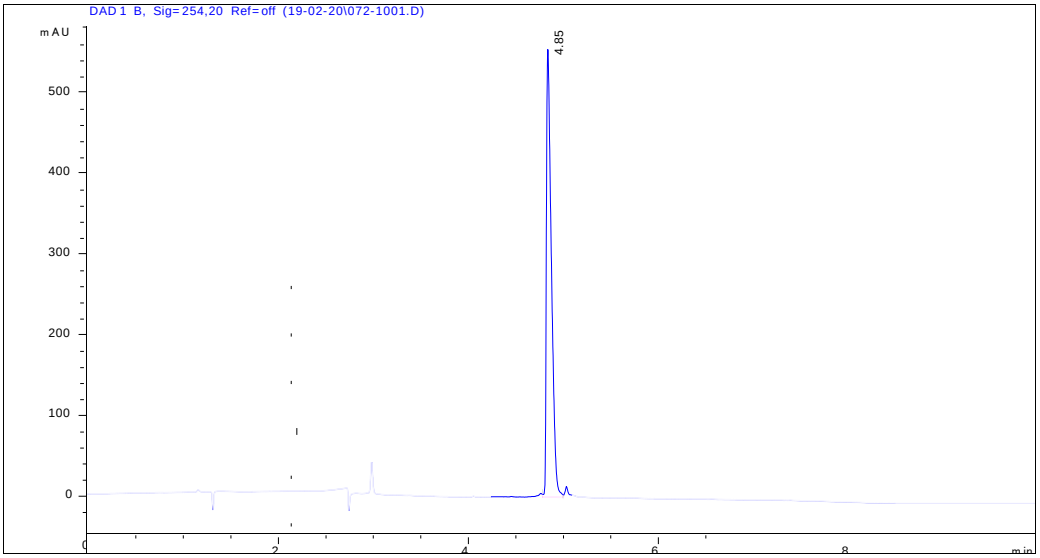


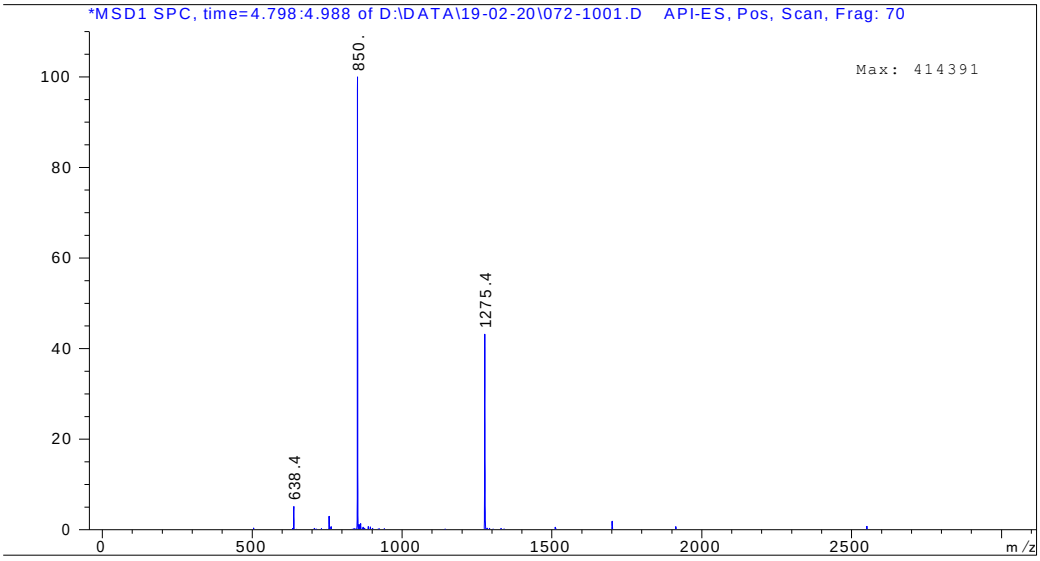
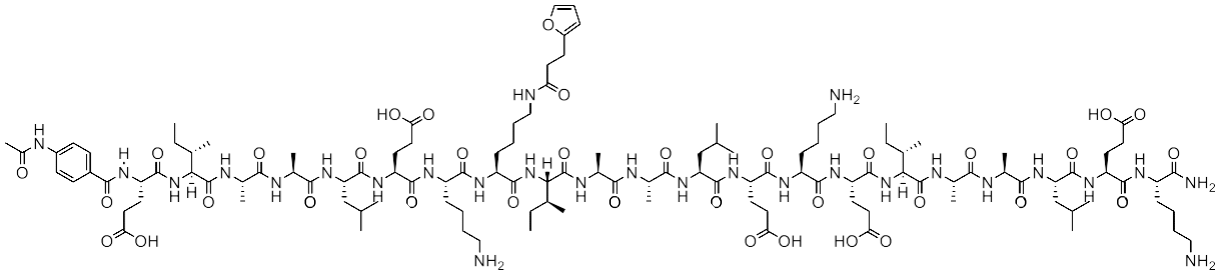


Figure SI 6: A) Structure of the E_{coil}-Orn_{Fur}-8 peptide. B) HPLC-MS trace of purified E_{coil}-Orn_{Fur}-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2550.00 Da. LC-ESI-MS m/z at t_r= 4.85: $[M+2H]^{2+}/2$= 1275.4; $[M+3H]^{3+}/3$= 850.8; $[M+4H]^{4+}/4$= 638.4.

2.8 *E_{coil}-Lys_{Fur}-8 peptide*

A)



B)

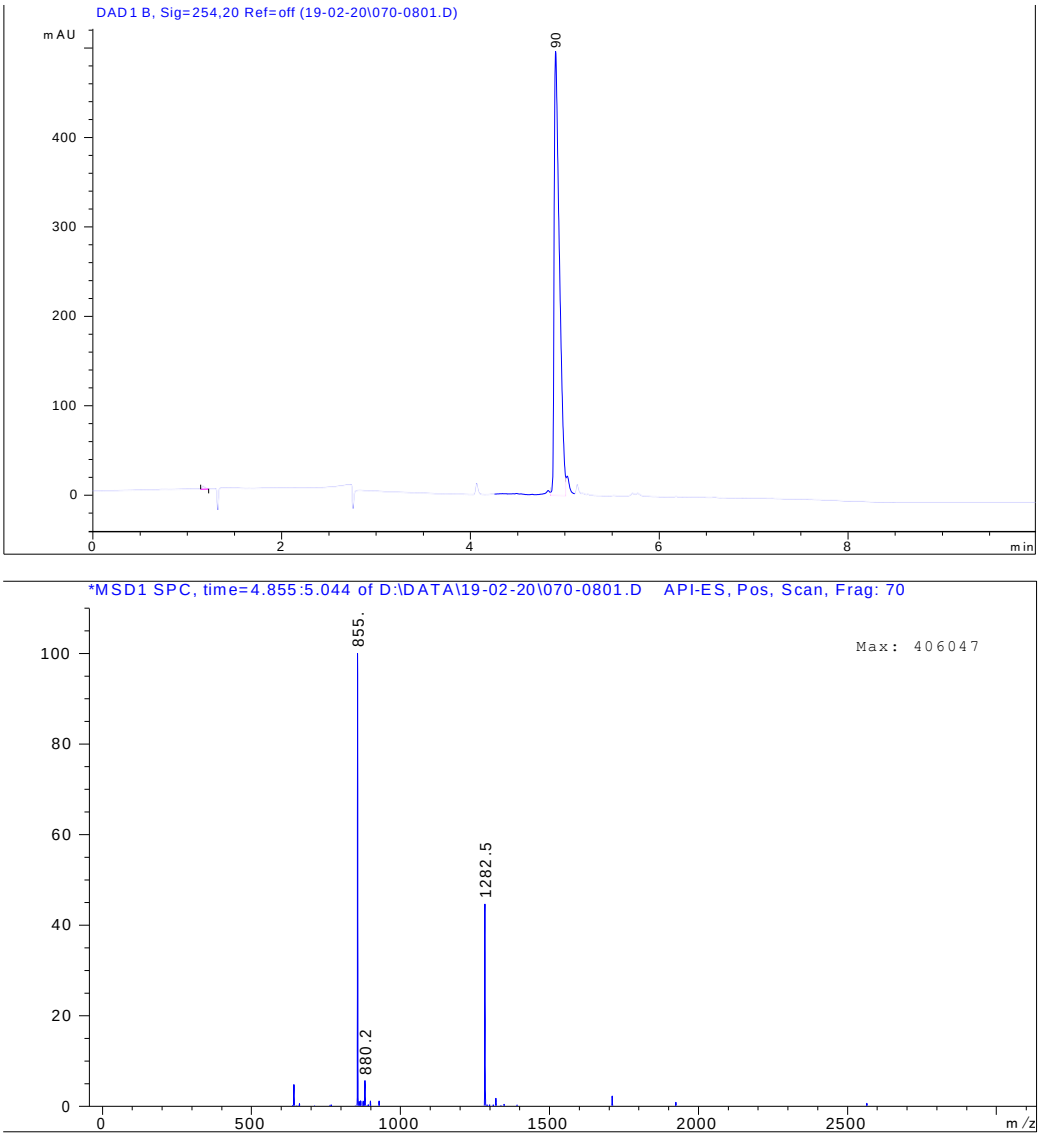


Figure SI 7: A) Structure of the E_{coil}-Lys_{Fur}-8 peptide. B) HPLC-MS trace of purified E_{coil}-Lys_{Fur}-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2562.43 Da. LC-ESI-MS m/z at t_r= 4.90: $[M+2H]^{2+}/2$= 1282.5; $[M+3H]^{3+}/3$= 855.5.

3. CD spectroscopy of K_{coil} with E_{coil}-FuA-8 and E_{coil}-X_{Fur}-8 peptides

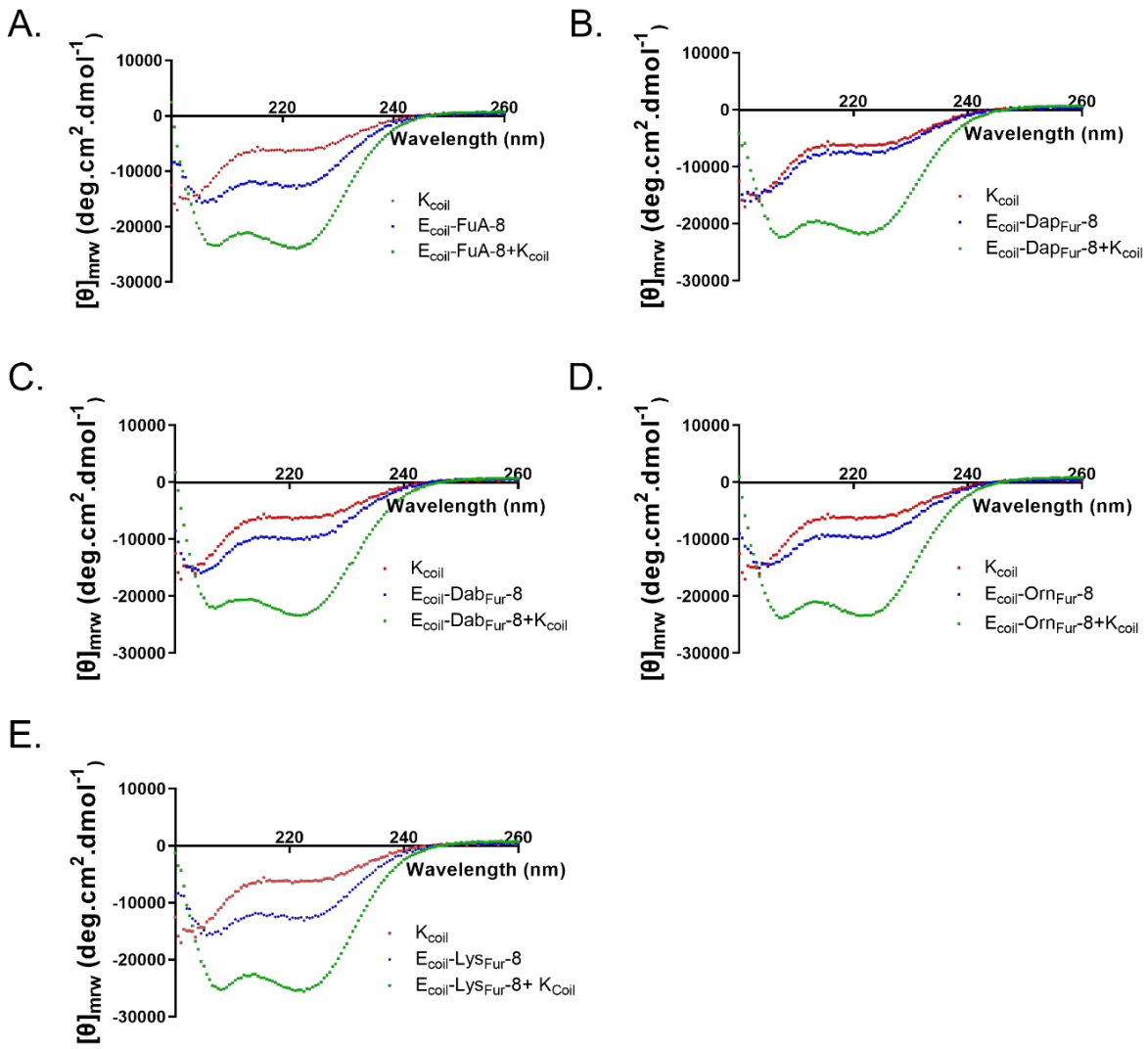


Figure SI 8: CD spectra obtained of the different furan-modified E_{coil} peptides (E_{coil}-FuA-8 and E_{coil}-X_{Fur}-8, blue trace): (A) E_{coil}-FuA-8, (B) E_{coil}-Dap_{Fur}-8, (C) E_{coil}-Dab_{Fur}-8, (D) E_{coil}-Orn_{Fur}-8, (E) E_{coil}-Lys-8, the K_{coil} peptide (red trace), and the equimolar mixture of both (green trace) in PBS solution (pH 7.4) at 5 μM concentration of each peptide.

4. K_{coil} crosslinks with E_{coil}-FuA-8 and E_{coil}-X_{Fur}-8

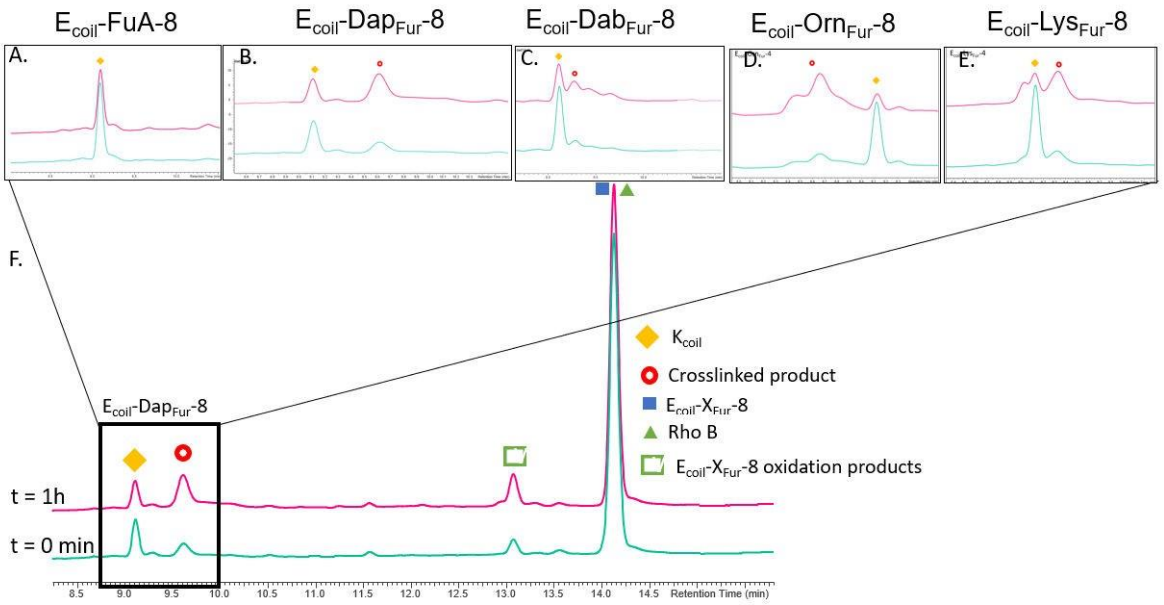


Figure SI 9: Zooms of HPLC chromatograms recorded at 260 nm of the reaction mixture (A) 5 µM K_{coil} + 10 µM **E_{coil}-FuA-8** + 10 µM Rho B (B) 5 µM K_{coil} + 10 µM **E_{coil}-Dap_{Fur}-8** + 10 µM Rho B (C) 5 µM K_{coil} + 10 µM **E_{coil}-Dab_{Fur}-8** + 10 µM Rho B (D) 5 µM K_{coil} + 10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B (E) 5 µM K_{coil} + 10 µM **E_{coil}-Lys_{Fur}-8** + 10 µM Rho B. (F) Complete HPLC chromatogram recorded at 260 nm of 5 µM K_{coil} + 10 µM **E_{coil}-Dap_{Fur}-8** + 10 µM Rho B. The green trace represents the mixture containing both **E_{coil}-X_{Fur}-8** and **K_{coil}** peptides as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation.

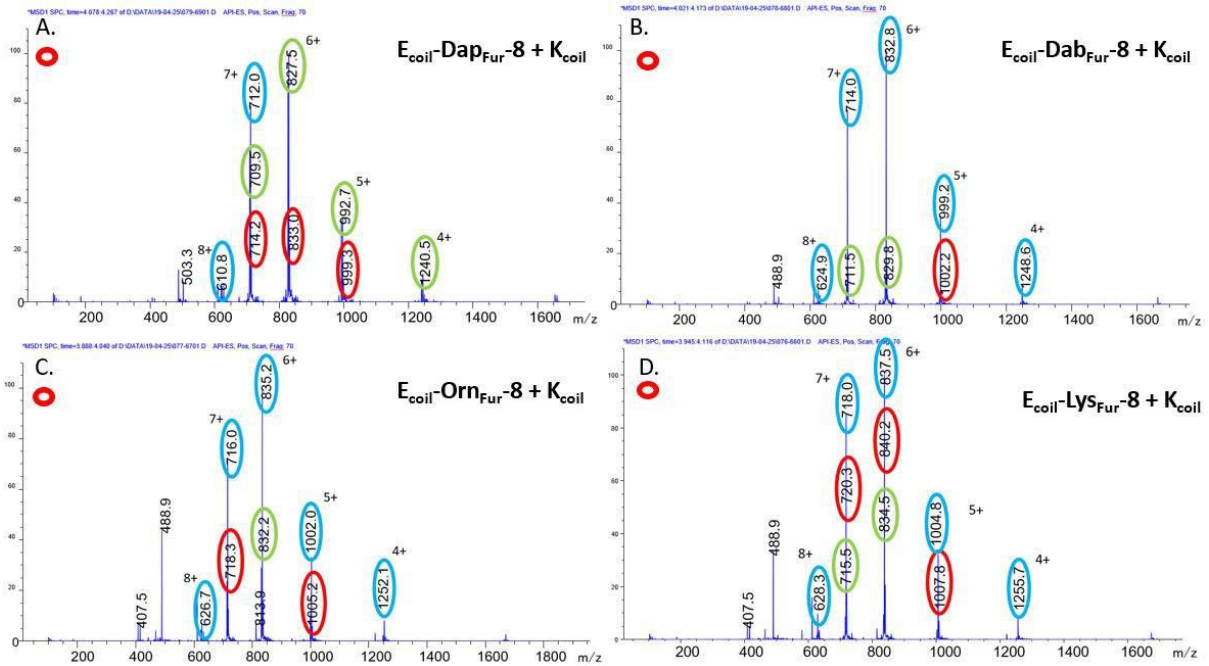


Figure SI 10: ESI-MS spectra of the peak corresponding to the crosslinked products obtained after 1h irradiation of (A) 5 µM K_{coil} + 10 µM **E_{coil}-Dap_{Fur}-8** + 10 µM Rho B (B) 5 µM K_{coil} + 10 µM **E_{coil}-Dab_{Fur}-8** + 10 µM Rho B (C) 5 µM K_{coil} + 10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B (D) 5 µM K_{coil} + 10 µM **E_{coil}-Lys_{Fur}-8** + 10 µM Rho B. The blue ovals correspond to the crosslinked product. Calcd MW (A) 4975.86 Da (B) 4989.88 Da (C) 5003.89 Da (D) 5017.91 Da and Found MW (A) 4976.94 Da (B) 4991.07 Da (C) 5005.18 Da D) 5019.15 Da. The green and red ovals represent the dehydrated (-18 Da) and the oxidised (+16 Da) form of the crosslinked product, respectively.

As can be seen in Figures SI 9-10, crosslinking to the K_{coil} can be obtained for E_{coil}-Dap_{Fur}-8, E_{coil}-Dab_{Fur}-8, E_{coil}-Orn_{Fur}-8, and E_{coil}-Lys_{Fur}-8. However, only in the case of E_{coil}-Dap_{Fur}-8 and E_{coil}-Orn_{Fur}-8, the peak corresponding to the crosslinked product appears base-line separated from the K_{coil} peak in the chromatogram (see Figure SI 9, panel B and D). Since we want to isolate and study the crosslinked product in more detail, base-line separated peaks are desired and therefore only E_{coil}-Dap_{Fur}-8 and E_{coil}-Orn_{Fur}-8 are considered as possible candidates for further experiments.

5. R_{coil}-Lys-X peptide characterization

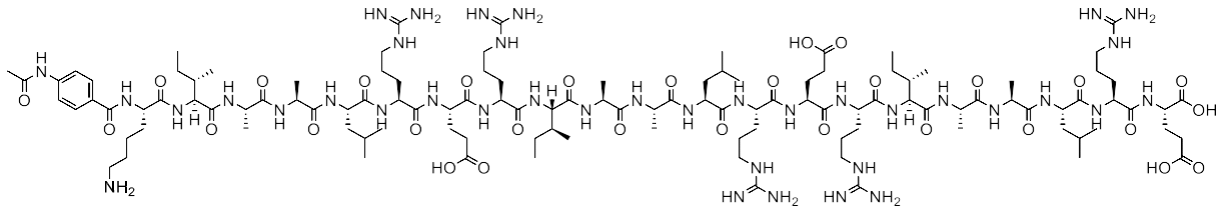
5.1 *Table of the R_{coil}-Lys-X synthesized peptides*

Name	***Sequence***	***Calcd MW (Da)***
R_{coil}-Lys-1	Aba-**K**IAALRER IAALRER IAALRE-NH_2	2578.53
R_{coil}-Lys-6	Aba-R IAAL**K**ER IAALRER IAALRE-NH_2	2578.53
R_{coil}-Lys-8	Aba-R IAALRE**K**IAALRER IAALRE-NH_2	2578.53
R_{coil}-Lys-13	Aba-R IAALRER IAAL**K**ER IAALRE-NH_2	2578.53
R_{coil}-Lys-15	Aba-R IAALRER IAALRE**K**IAALRE-NH_2	2578.53
R_{coil}-Lys-20	Aba-R IAALRER IAALRER IAAL**K**E-NH_2	2578.53

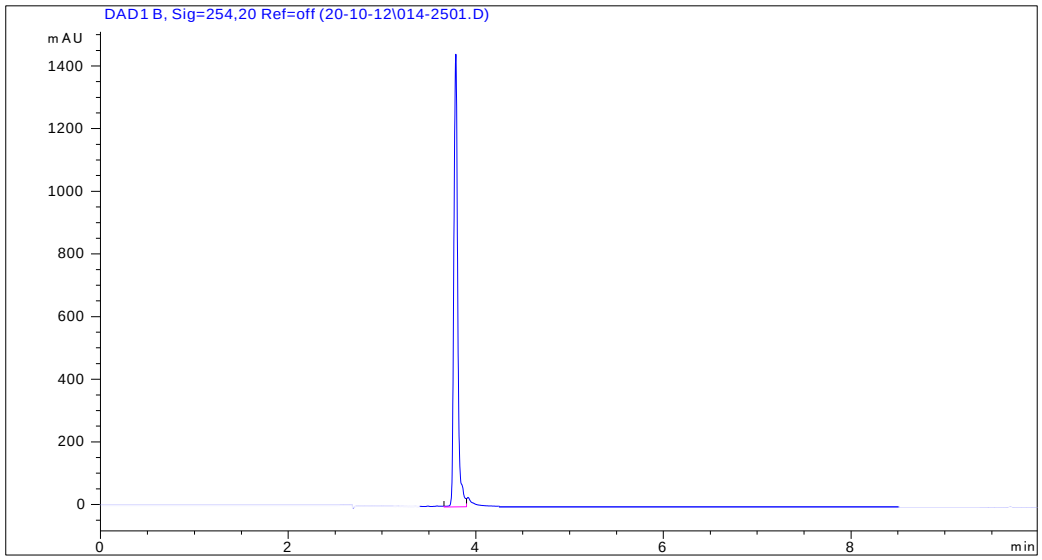
Table SI 2: Overview of the R_{coil}-Lys-X peptide sequences, the calculated molecular weight (MW), and their corresponding code: 4-Acetamidobenzoic acid (Aba)

5.2 *R_{coil}-Lys-1 peptide*

A)



B)



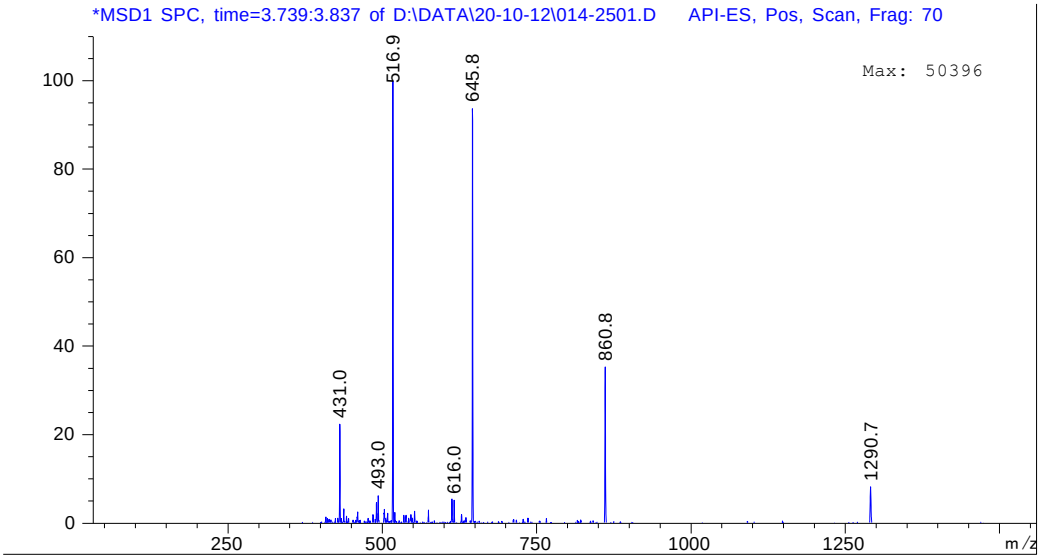
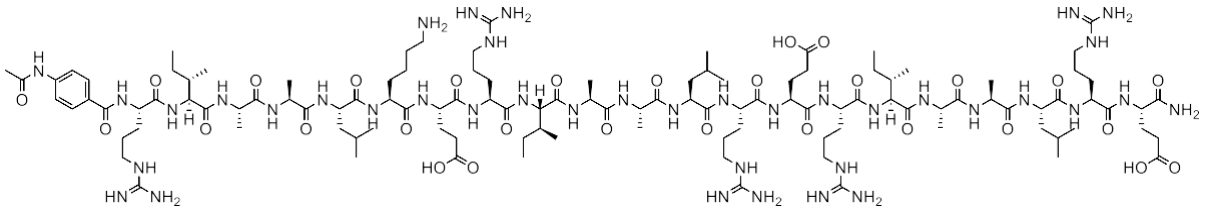


Figure SI 11: A) Structure of the R_{coil}-Lys-1 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-1. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.79: $[M+2H]^{2+}/2$= 1290.7; $[M+3H]^{3+}/3$= 860.8, $[M+4H]^{4+}/4$= 645.8, $[M+5H]^{5+}/5$= 516.9, $[M+6H]^{6+}/6$= 431.0.

5.3 *R_{coil}-Lys-6 peptide*

A)



B)

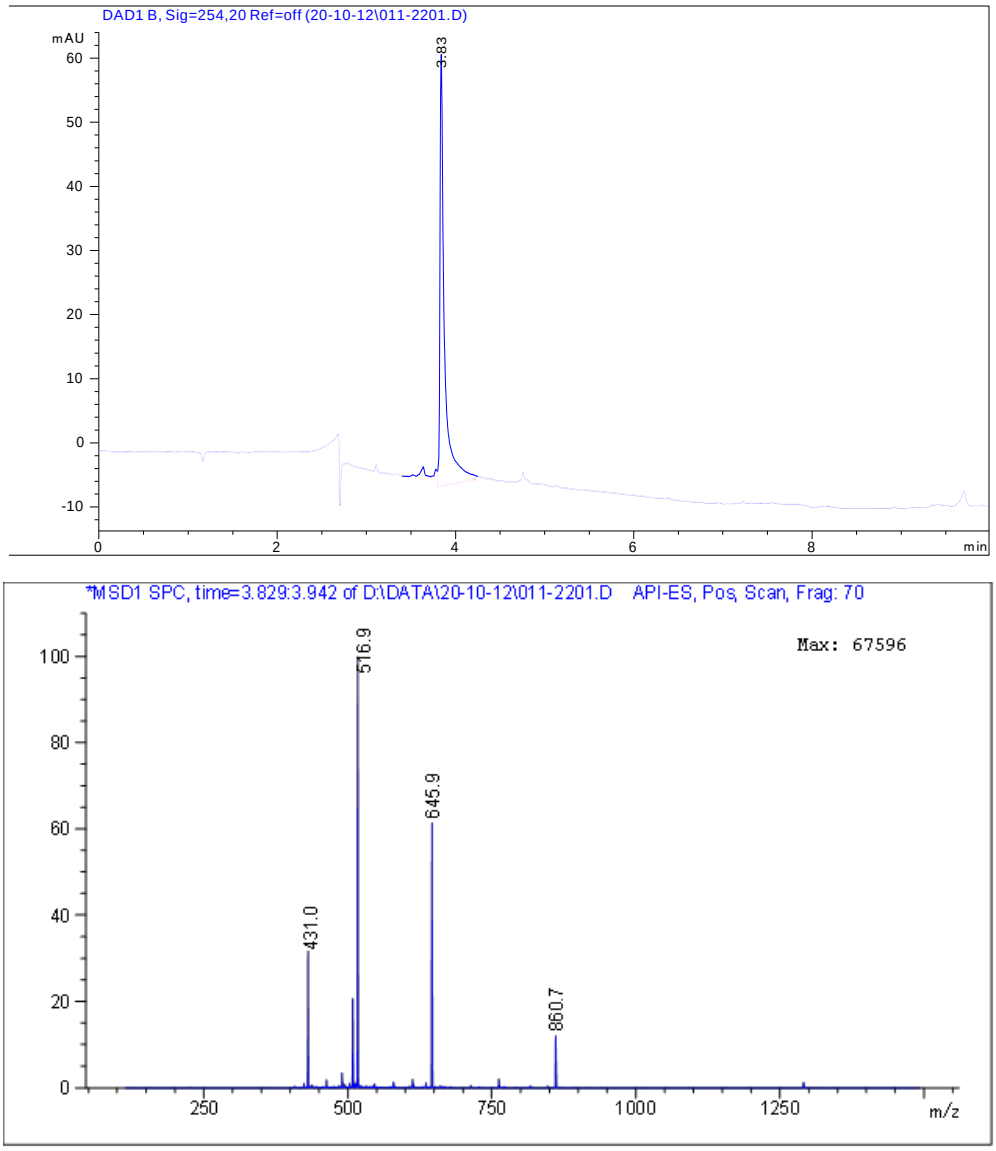
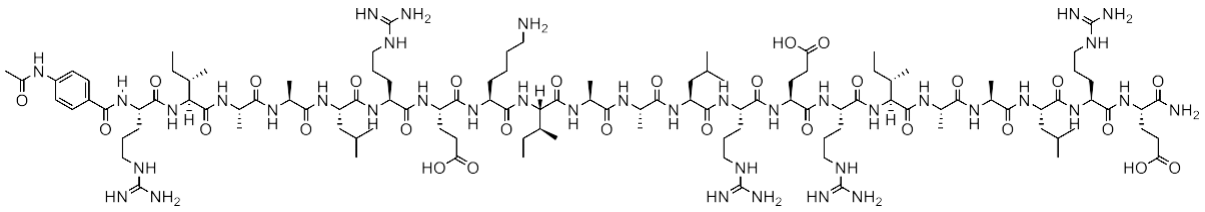


Figure SI 12: A) Structure of the R_{coil}-Lys-6 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-6. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.84: $[M+3H]^{3+}$/3= 860.7, $[M+4H]^{4+}$/4= 645.9, $[M+5H]^{5+}$/5= 516.9, $[M+6H]^{6+}$/6= 431.0.

5.4 *R_{coil}-Lys-8 peptide*

A)



B)

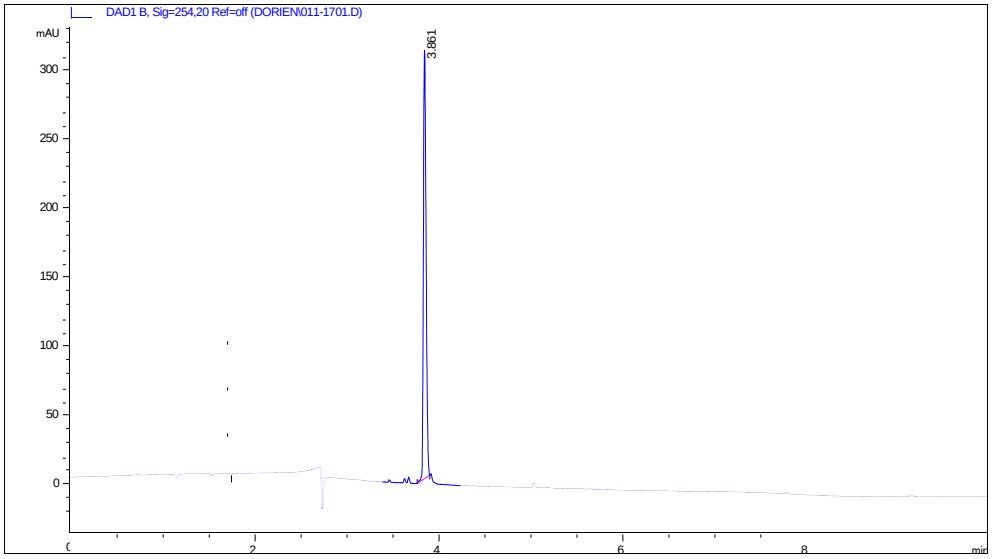
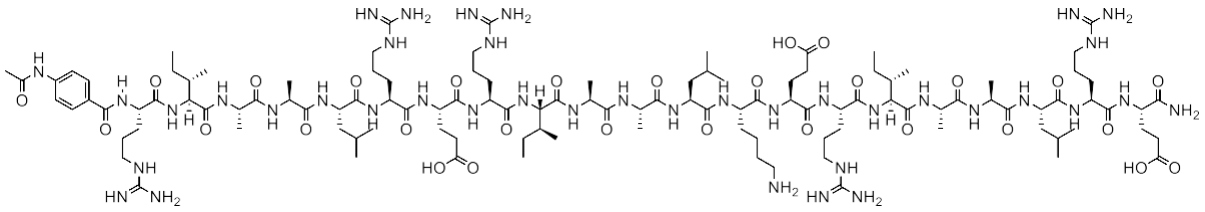


Figure SI 13: A) Structure of the R_{coil}-Lys-8 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-8. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.86: $[M+2H]^{2+}/2$= 1290.0, $[M+3H]^{3+}/3$= 860.7, $[M+4H]^{4+}/4$= 645.9, $[M+5H]^{5+}/5$= 516.9, $[M+6H]^{6+}/6$= 431.0.

5.5 *R_{coil}-Lys-13 peptide*

A)



B)

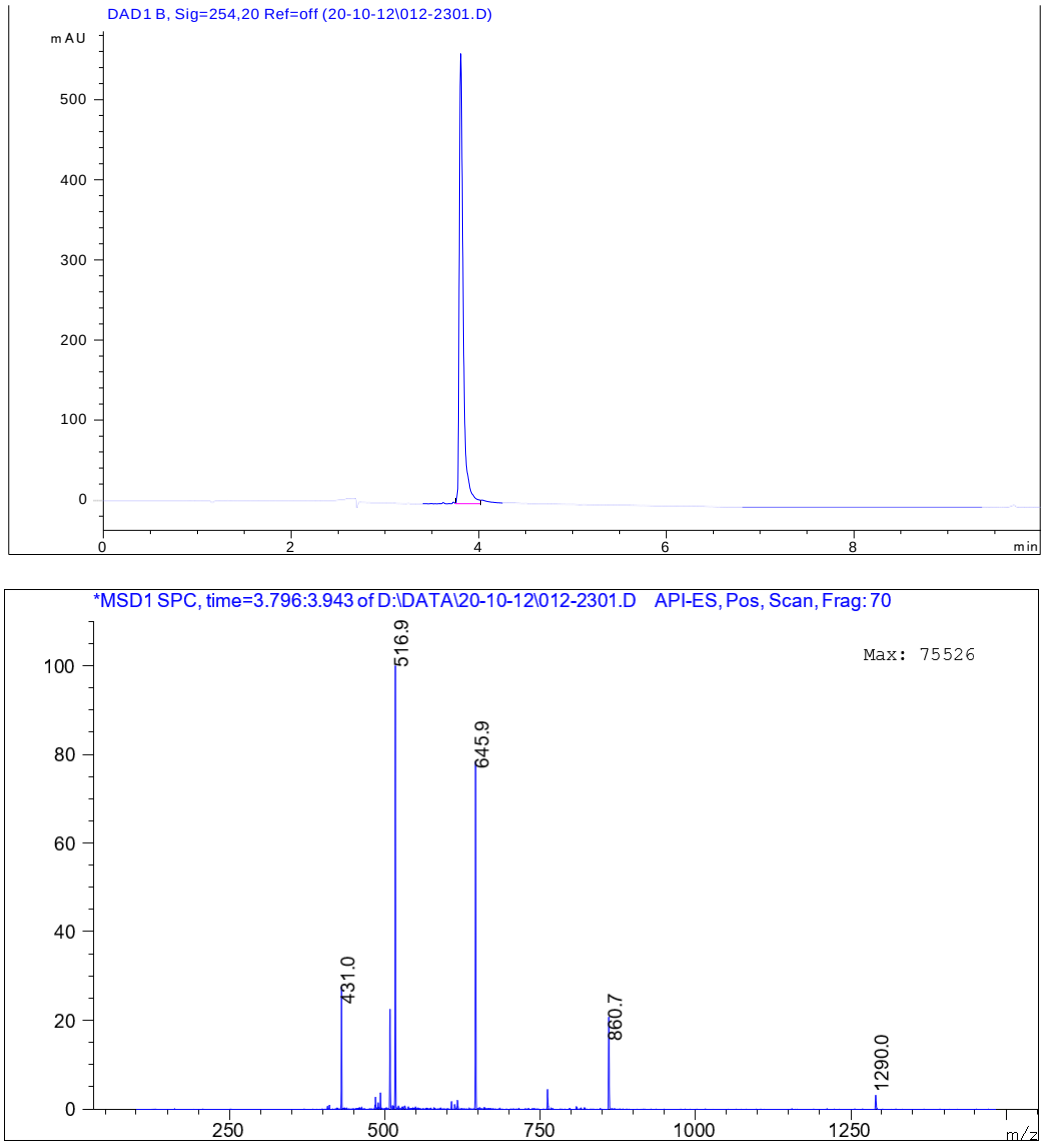
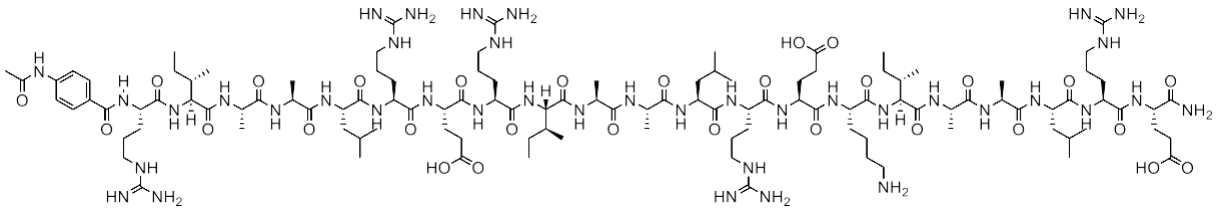


Figure SI 14: A) Structure of the R_{coil}-Lys-13 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-13. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.81: $[M+2H]^{2+}/2$= 1290.0, $[M+3H]^{3+}/3$= 860.7, $[M+4H]^{4+}/4$= 645.9, $[M+5H]^{5+}/5$= 516.9, $[M+6H]^{6+}/6$= 431.0.

5.6 *R_{coil}-Lys-15 peptide*

A)



B)

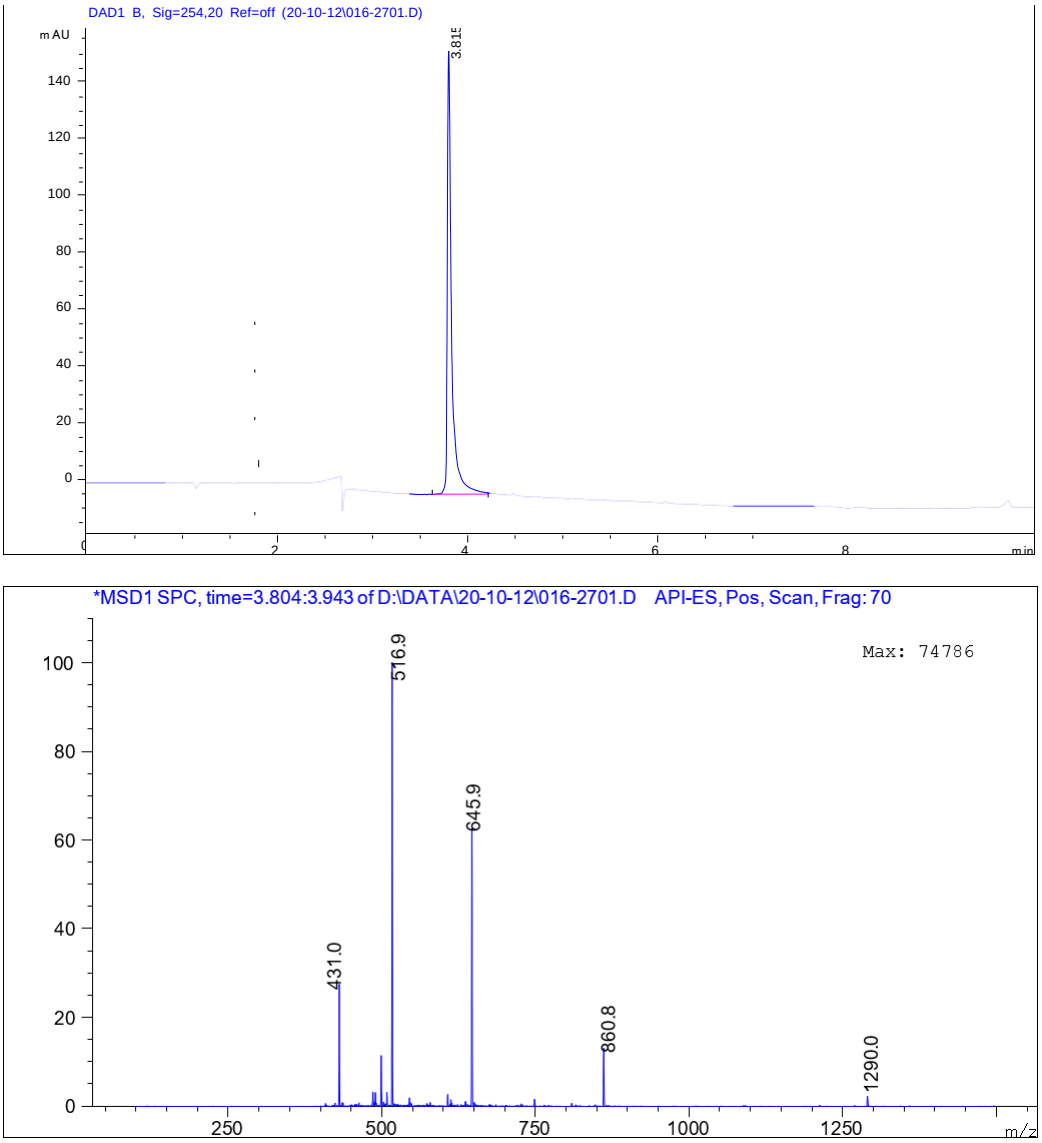
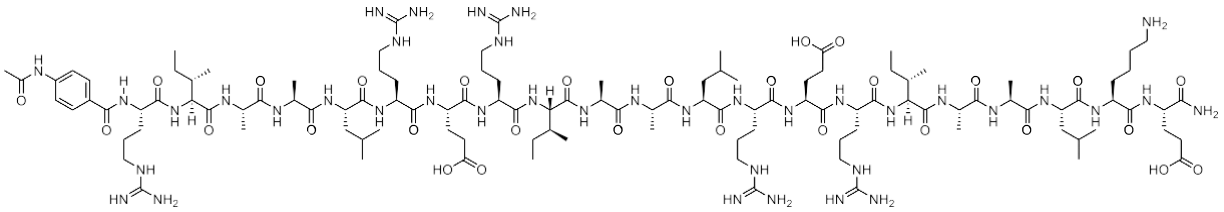


Figure SI 15: A) Structure of the R_{coil}-Lys-15 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-15. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.82: $[M+2H]^{2+}/2$= 1290.0, $[M+3H]^{3+}/3$= 860.8, $[M+4H]^{4+}/4$= 645.9, $[M+5H]^{5+}/5$= 516.9, $[M+6H]^{6+}/6$= 431.0.

5.7 *R_{coil}-Lys-20 peptide*

A)



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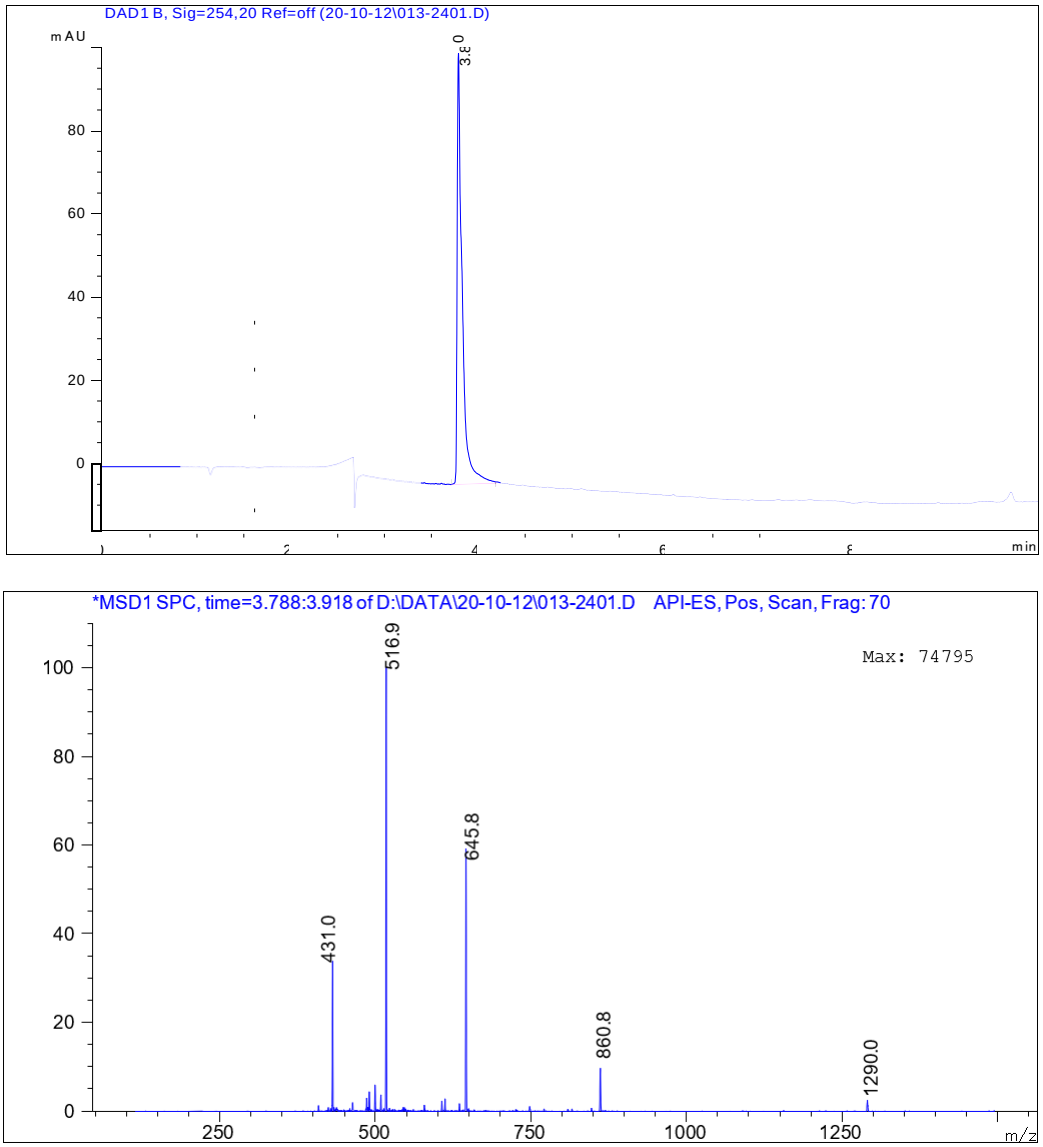


Figure SI 16: A) Structure of the R_{coil}-Lys-20 peptide. B) HPLC-MS trace of purified R_{coil}-Lys-20. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2578.53 Da. LC-ESI-MS m/z at t_r= 3.80: $[M+2H]^{2+}/2$= 1290.0, $[M+3H]^{3+}/3$= 860.8, $[M+4H]^{4+}/4$= 645.8, $[M+5H]^{5+}/5$= 516.9, $[M+6H]^{6+}/6$= 431.0.

6. Lysine scan performed with E_{coil}-Dap_{Fur}-8

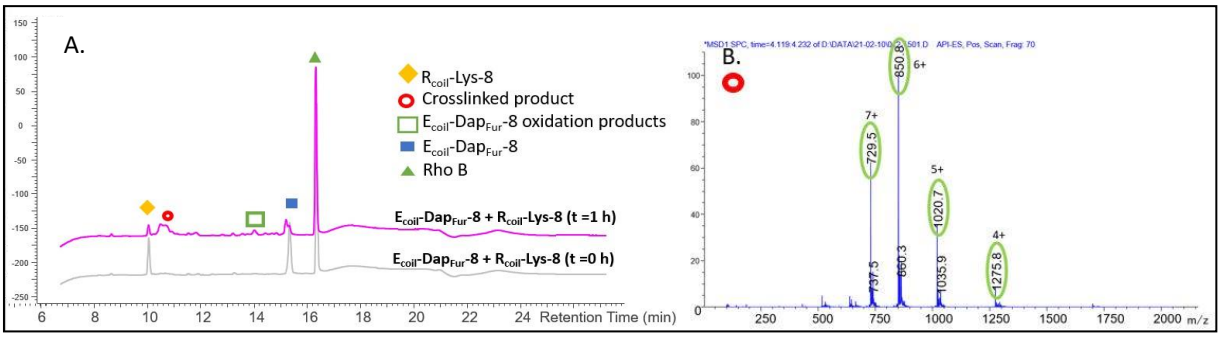


Figure SI 17: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-8** +10 µM **E_{coil}- Dap_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-8** and **R_{coil}-Lys-8** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The green ovals correspond to the dehydrated form of the crosslinked product of E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-8 with Rho B (Calcd MW 5099.90 Da): Found MW 5098.5: $[M+4H]^{4+}/4$= 1275.8, $[M+5H]^{5+}/5$= 1020.7, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5.

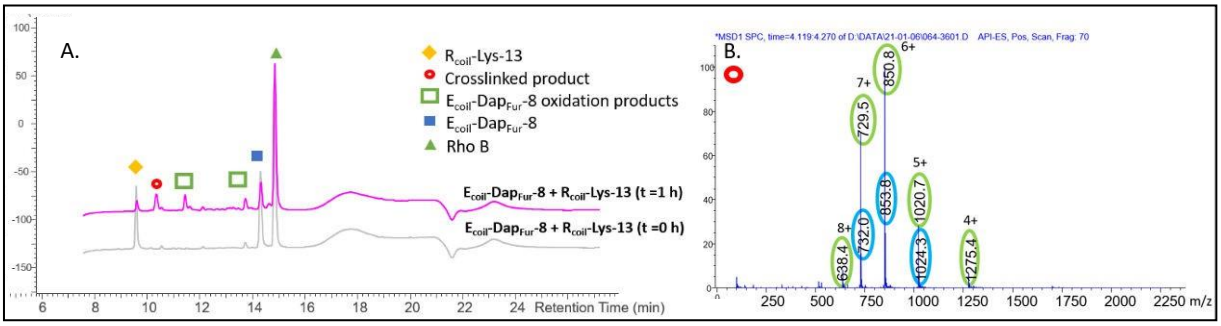


Figure SI 18: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-13** + 10 µM **E_{coil}-Dap_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-8** and **R_{coil}-Lys-13** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-13 with Rho B: Found MW 5116.5 $[M+5H]^{5+}/5$= 1024.3, $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0. The green ovals represent the dehydrated (Calcd MW 5099.90 Da) form of the obtained product: Found MW 5097.60: $[M+4H]^{4+}/4$= 1275.4, $[M+5H]^{5+}/5$= 1020.7, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

7. Lysine scan performed with E_{coil}-Orn_{Fur}-8

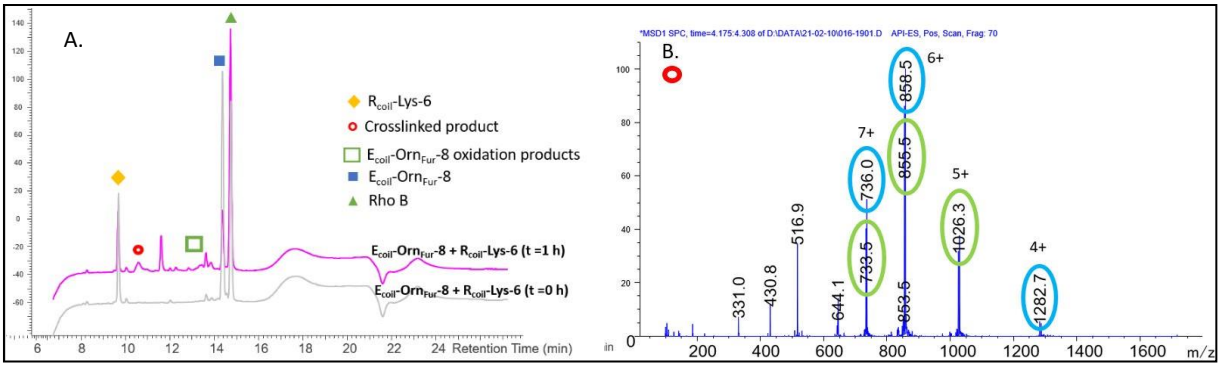


Figure SI 19: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-6** + 10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Orn_{Fur}-8** and **R_{coil}-Lys-6** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5143.92 Da) of E_{coil}-Orn_{Fur}-8 and R_{coil}-Lys-6 with Rho B: Found MW 5142.94: $[M+4H]^{4+}/4$= 1282.7, $[M+6H]^{6+}/6$= 858.5, $[M+7H]^{7+}/7$= 736.0. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5128.94 Da): Found MW 5126.5 $[M+5H]^{5+}/5$= 1026.3, $[M+6H]^{6+}/6$= 855.5, $[M+7H]^{7+}/7$= 733.5.

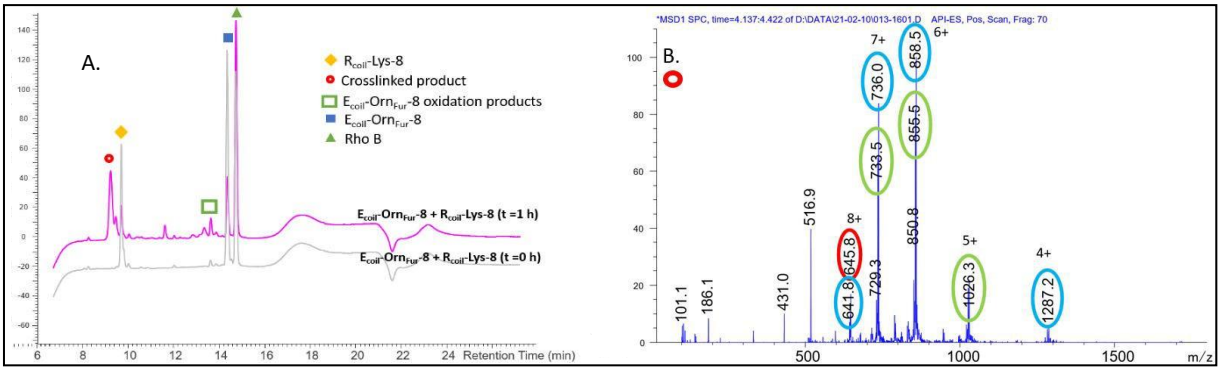


Figure SI 20: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-8** + 10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Orn_{Fur}-8** and **R_{coil}-Lys-8** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product. The blue ovals correspond to the crosslinked product (Calcd MW 5143.92 Da) of E_{coil}-Orn_{Fur}-8 and R_{coil}-Lys-8 with Rho B: Found MW 5142.94: $[M+4H]^{4+}/4$= 1287.2, $[M+6H]^{6+}/6$= 858.5, $[M+7H]^{7+}/7$= 736.0, $[M+8H]^{8+}/8$= 641.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5128.94 Da): Found MW 5126.5: $[M+5H]^{5+}/5$= 1026.3, $[M+6H]^{6+}/6$= 855.5, $[M+7H]^{7+}/7$= 733.5. The red oval represents the oxidised form of the crosslinked product (+16 Da): Found MW 5158.4: $[M+8H]^{8+}/8$= 645.8.

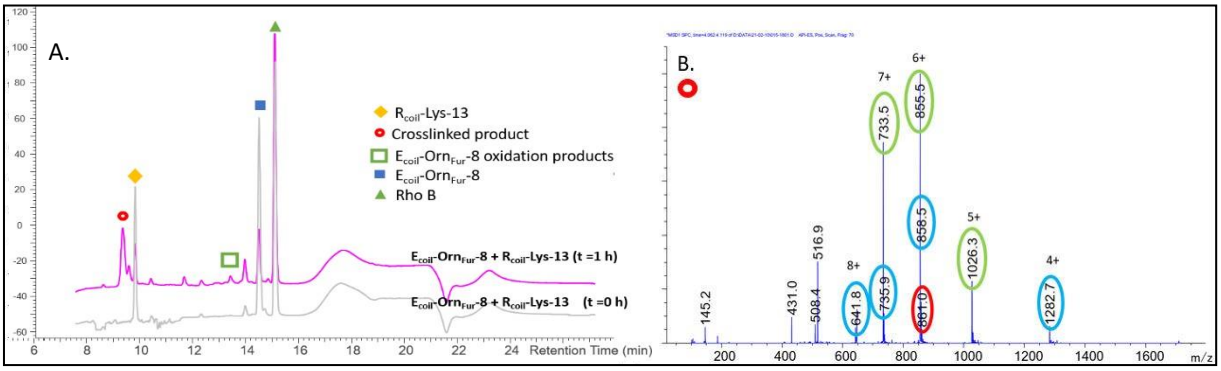


Figure SI 21: HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-13** +10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Orn_{Fur}-8** and **R_{coil}-Lys-13** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product. The blue ovals correspond to the crosslinked product (Calcd MW 5143.92 Da) of E_{coil}-Orn_{Fur}-8 and R_{coil}-Lys-13 with Rho B: Found MW 5142.94: $[M+4H]^{4+}/4$= 1282.7, $[M+6H]^{6+}/6$= 858.5, $[M+7H]^{7+}/7$= 735.9, $[M+8H]^{8+}/8$= 641.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5128.94 Da): Found MW 5126.5: $[M+5H]^{5+}/5$= 1026.3, $[M+6H]^{6+}/6$= 855.5, $[M+7H]^{7+}/7$= 733.5. The red oval represents the oxidised form of the crosslinked product (+16 Da): Found MW 5160: $[M+6H]^{6+}/6$= 861.0.

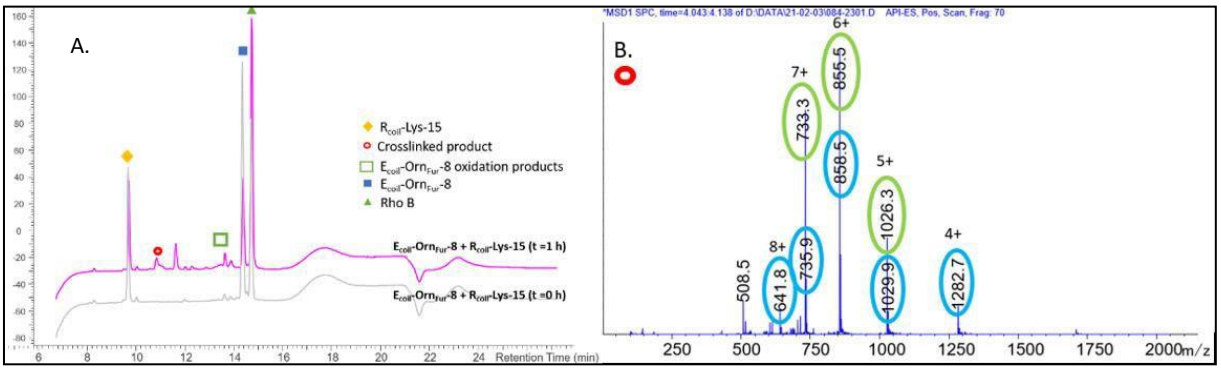


Figure SI 22: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-15**+ 10 µM **E_{coil}-Orn_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Orn_{Fur}-8** and **R_{coil}-Lys-15** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation (B) ESI-MS spectra of the peak corresponding to the crosslinked product. The blue ovals correspond to the crosslinked product (Calcd MW 5143.92 Da) of E_{coil}-Orn_{Fur}-8 and R_{coil}-Lys-15 with Rho B: Found MW 5142.94: $[M+4H]^{4+}/4$= 1282.7, $[M+5H]^{5+}/5$= 1029.9, $[M+6H]^{6+}/6$= 858.5, $[M+7H]^{7+}/7$= 735.9, $[M+8H]^{8+}/8$= 641.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5128.94 Da): Found MW 5126.5: $[M+5H]^{5+}/5$= 1026.3, $[M+6H]^{6+}/6$= 855.5, $[M+7H]^{7+}/7$= 733.3.

8. E_{coil}-Dap_{Fur}-X peptide characterization

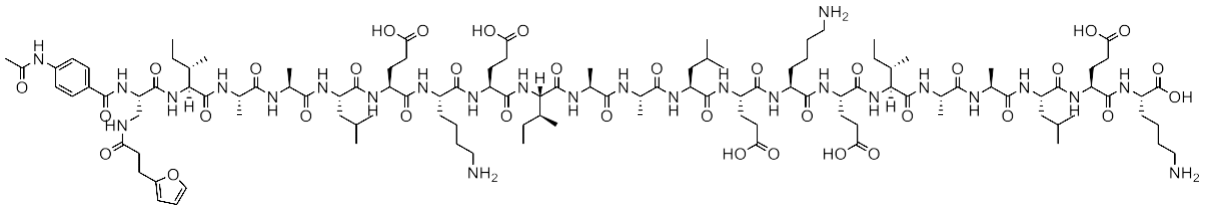
8.1 *Table of the E_{coil}-Dap_{Fur}-X synthesized peptides*

Name	***Sequence***	***Calcd MW (Da)***
E_{coil}-Dap_{Fur}-1	Aba-**Dap$_{(Fur)}$** IAALEKE IAALEKE IAALEK-NH_2	2520.38
E_{coil}-Dap_{Fur}-6	Aba-E IAAL**Dap$_{(Fur)}$**KE IAALEKE IAALEK-NH_2	2520.38
E_{coil}-Dap_{Fur}-13	Aba-E IAALEKE IAAL**Dap$_{(Fur)}$**KE IAALEK-NH_2	2520.38
E_{coil}-Dap_{Fur}-15	Aba-E IAALEKE IAALEK**Dap$_{(Fur)}$** IAALEK-NH_2	2520.38
E_{coil}-Dap_{Fur}-20	Aba-E IAALEKE IAALEKE IAAL**Dap$_{(Fur)}$**K-NH_2	2520.38

Table SI 3: Overview of all E_{coil}-Dap_{Fur}-X peptide sequences, the calculated molecular weight (MW), and their corresponding codes: 4-Acetamidobenzoic acid (Aba), 2,3-diaminopropionic acid (Dap), and 3-(2-furyl)propionic acid (Fur).

8.2 *E_{coil}-Dap_{Fur}-1 peptide*

A)



B)

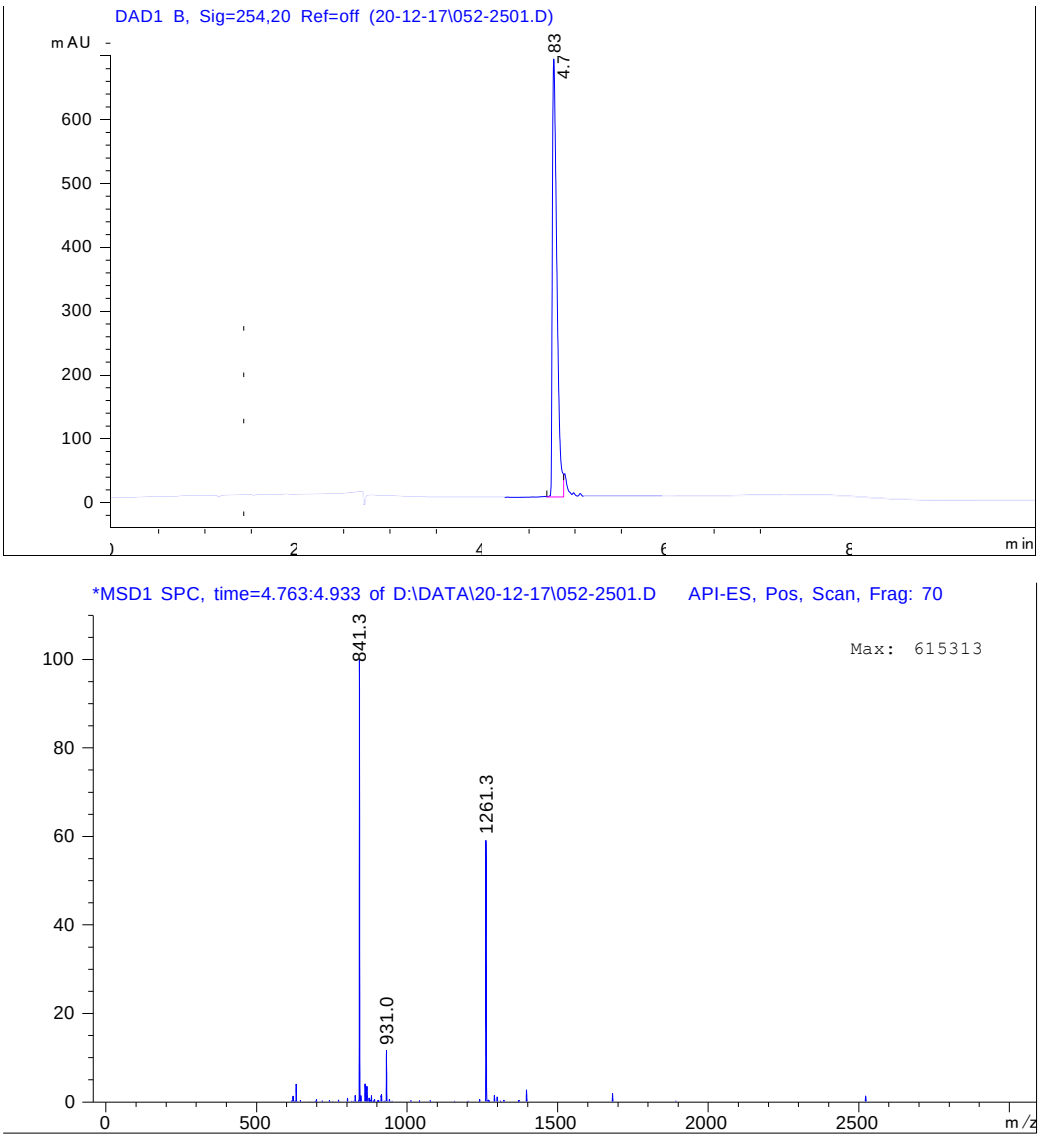
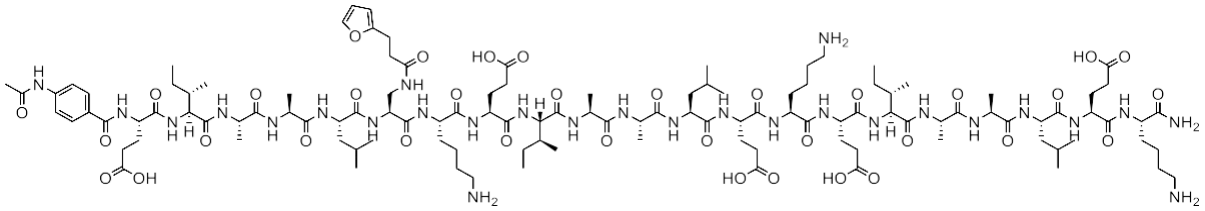


Figure SI 23: A) Structure of the E_{coil}-Dap_{Fur}-1 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-1. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.78: $[M+2H]^{2+}/2$= 1261.3, $[M+ 3H]^{3+}/3$= 841.3.

8.3 *E_{coil}-Dap_{Fur}-6 peptide*

A)



B)

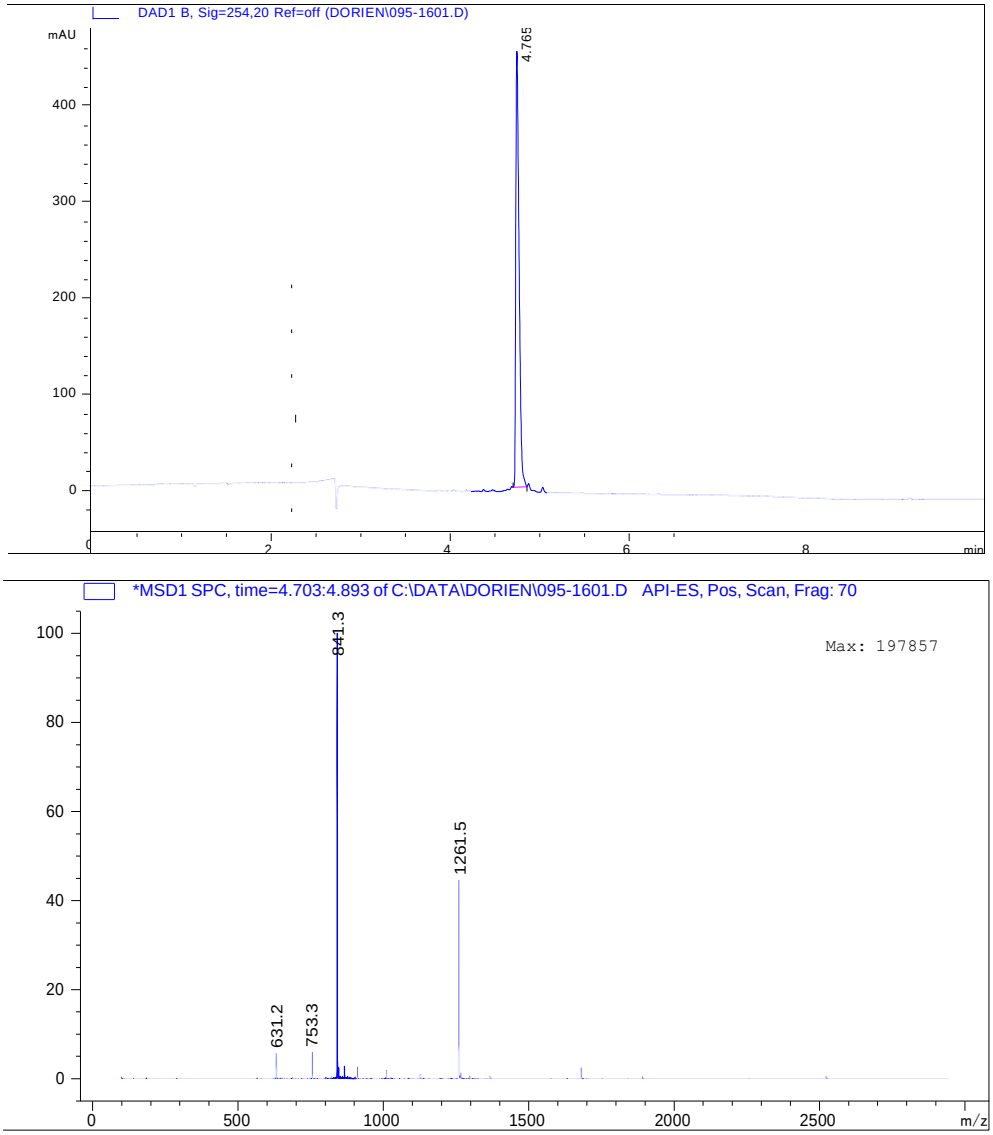
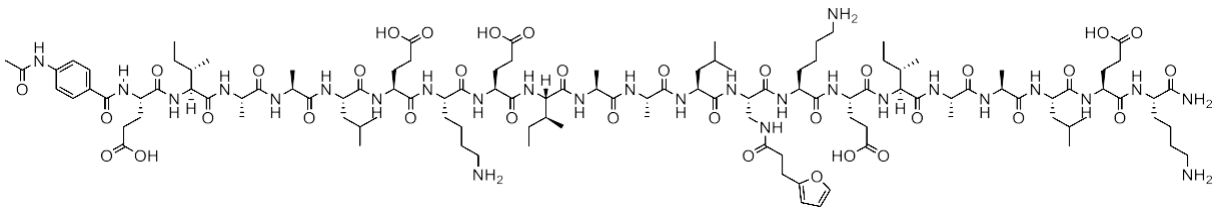


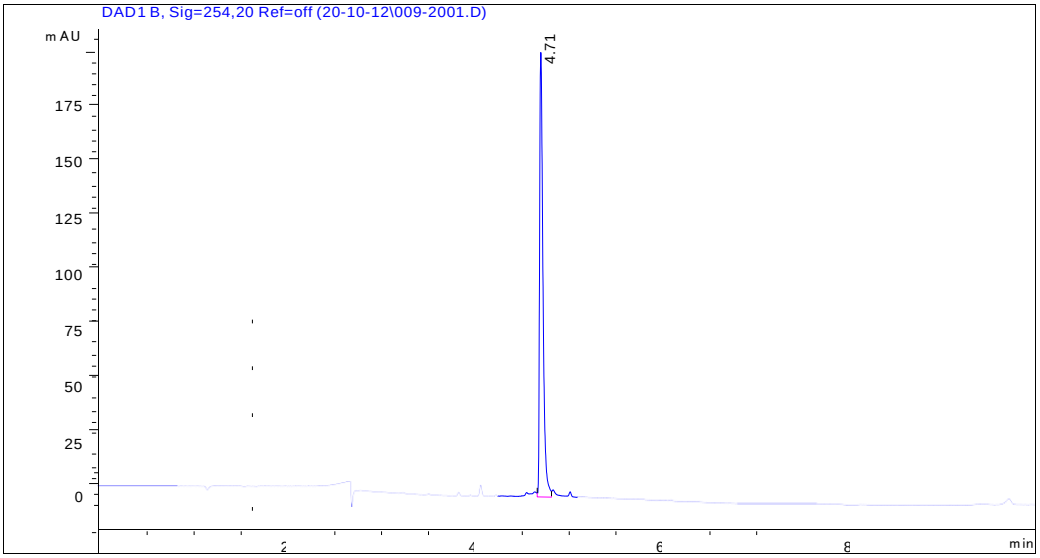
Figure SI 24: A) Structure of the E_{coil}-Dap_{Fur}-6 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-6. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.77: $[M+2H]^{2+}/2$= 1261.5, $[M+3H]^{3+}/3$= 841.3.

8.4 *E_{coil}-Dap_{Fur}-13 peptide*

A)



B)



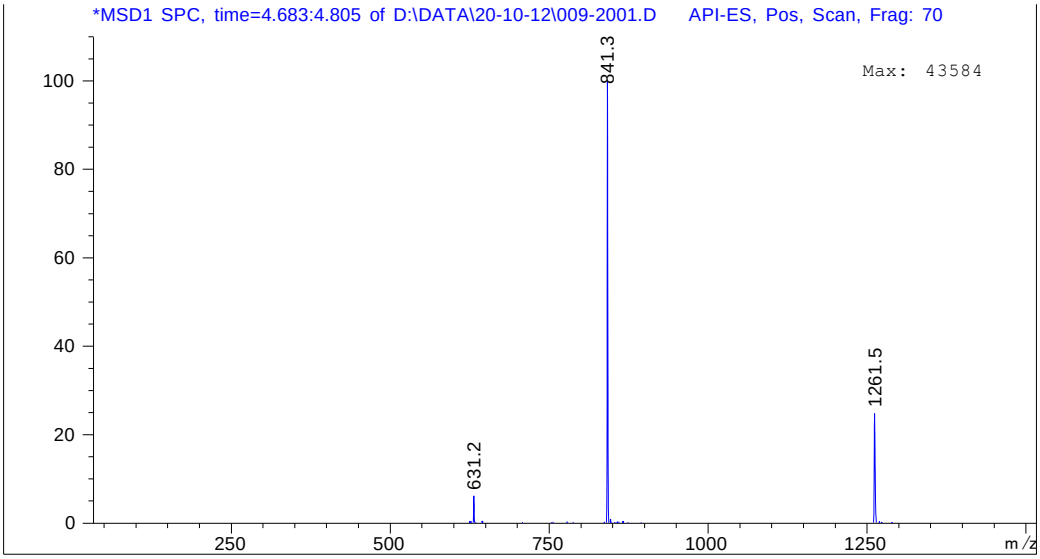
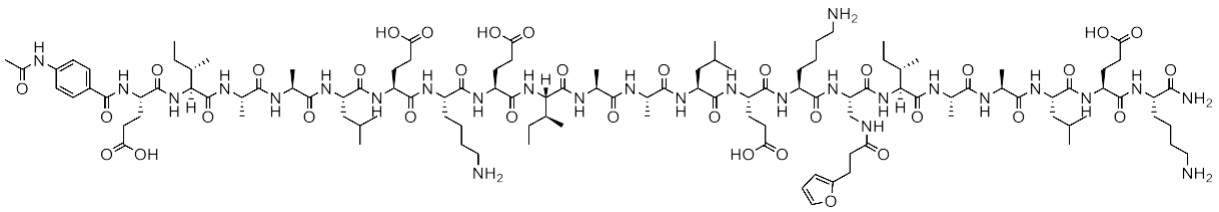


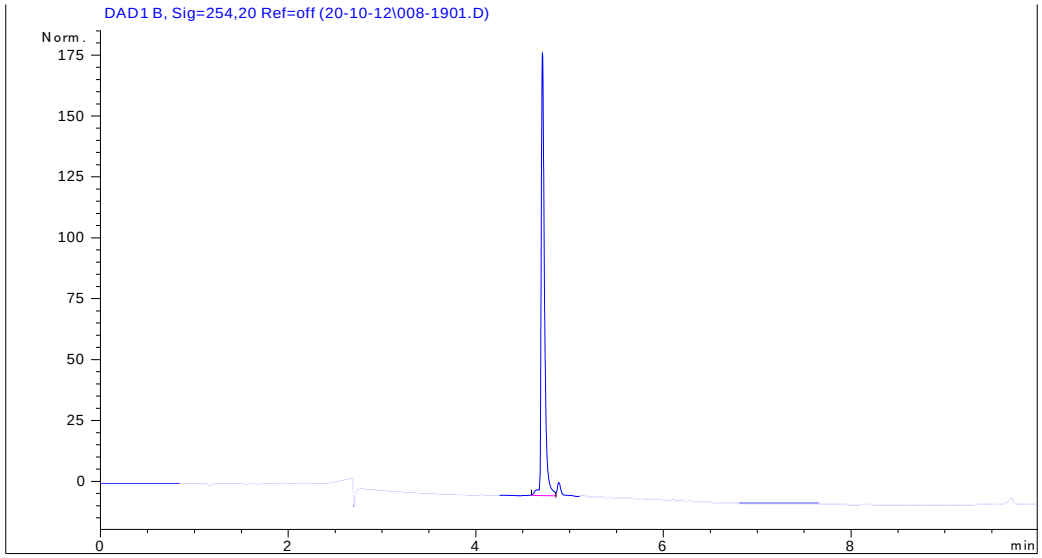
Figure SI 25: A) Structure of the E_{coil}-Dap_{Fur}-13 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-13. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.71: $[M+2H]^{2+}/2$= 1261.5, $[M+3H]^{3+}/3$= 841.3, $[M+4H]^{4+}/4$= 631.2.

8.5 *E_{coil}-Dap_{Fur}-15 peptide*

A)



B)



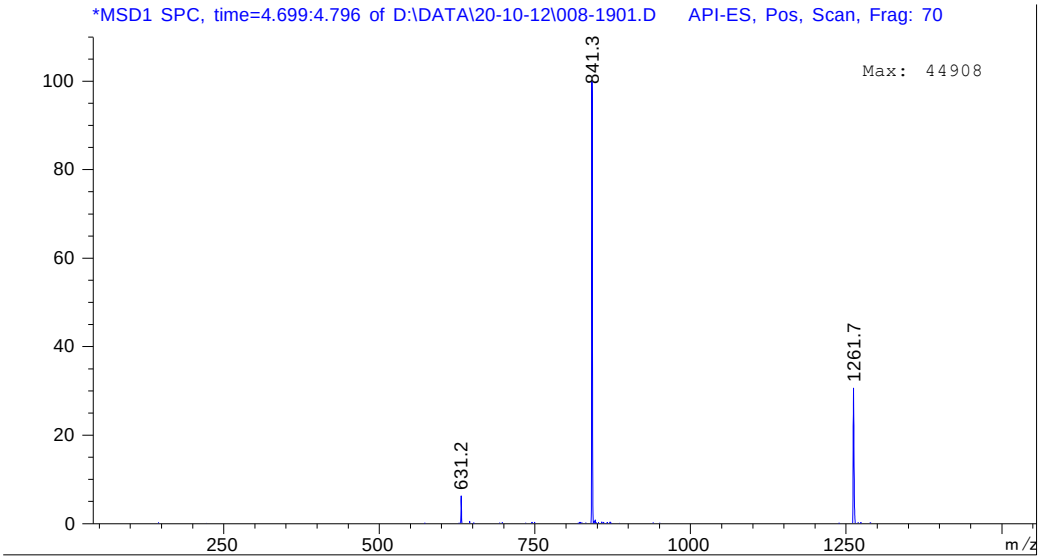
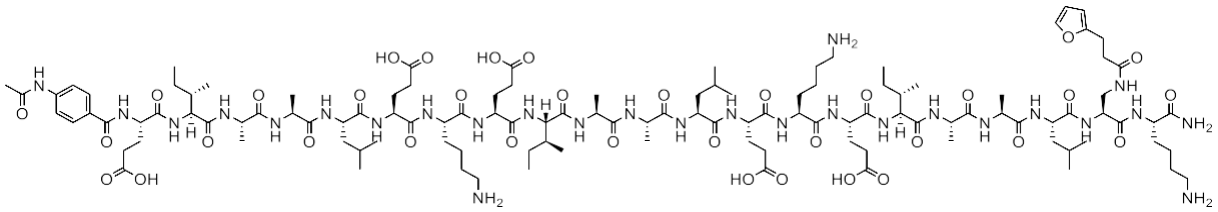


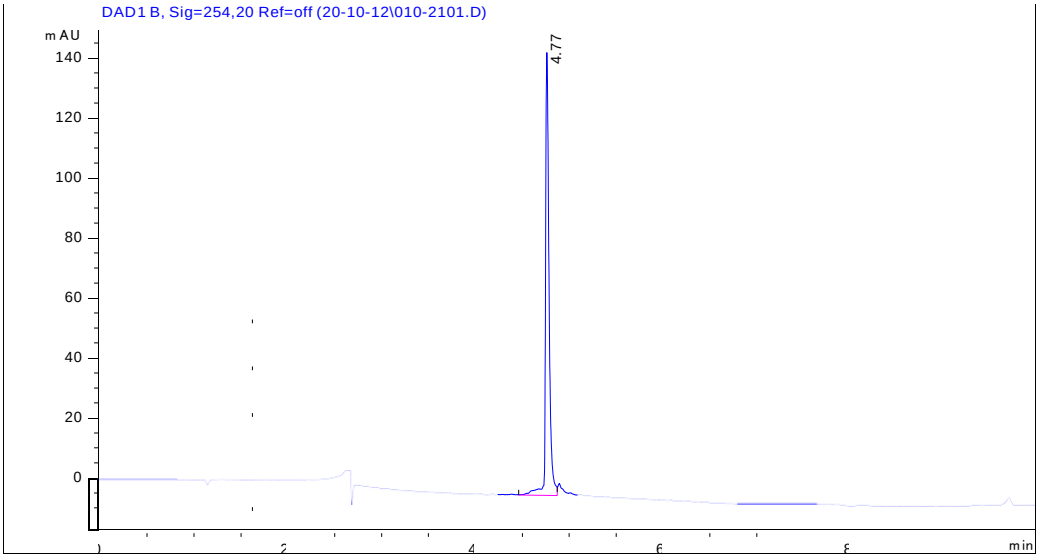
Figure SI 26: A) Structure of the E_{coil}-Dap_{Fur}-15 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-15. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.71: $[M+2H]^{2+}/2$= 1261.7, $[M+3H]^{3+}/3$= 841.3, $[M+4H]^{4+}/4$= 631.2.

8.6 *E_{coil}-Dap_{Fur}-20 peptide*

A)



B)



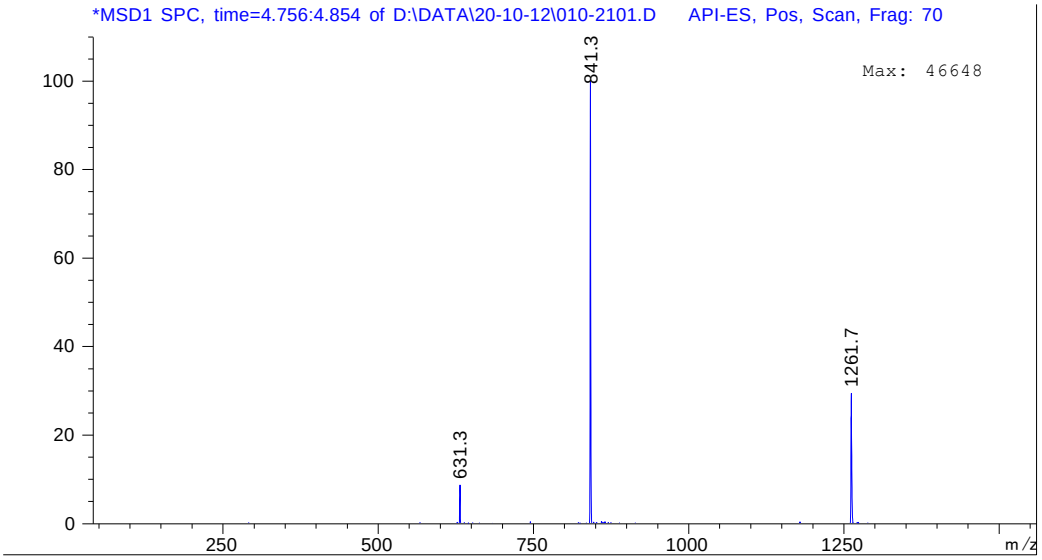


Figure SI 27: A) Structure of the E_{coil}-Dap_{Fur}-20 peptide. B) HPLC-MS trace of purified E_{coil}-Dap_{Fur}-20. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2520.38 Da. LC-ESI-MS m/z at t_r= 4.78: $[M+2H]^{2+}/2$= 1261.7, $[M+3H]^{3+}/3$= 841.3, $[M+4H]^{4+}/4$= 631.3.

9. Furan and Lysine scans performed with E_{coil}-Dap_{Fur}-X

In section 7, it was shown that E_{coil}-Orn_{Fur}-8 is able to crosslink to more than two R_{coil}-Lys-X peptides, which could result from the fact that an Orn (3 C chain) residue provides a longer spacer than a Dap (1 C chain) residue. To exclude the influence of linker flexibility and ensure formation of a single parallel or antiparallel crosslinked species, further experiments were performed with E_{coil}-Dap_{Fur}-X peptides. In this section, only the cases where crosslinked species was observed are reported.

15.1 Lysine scans with E_{coil}-Dap_{Fur}-1

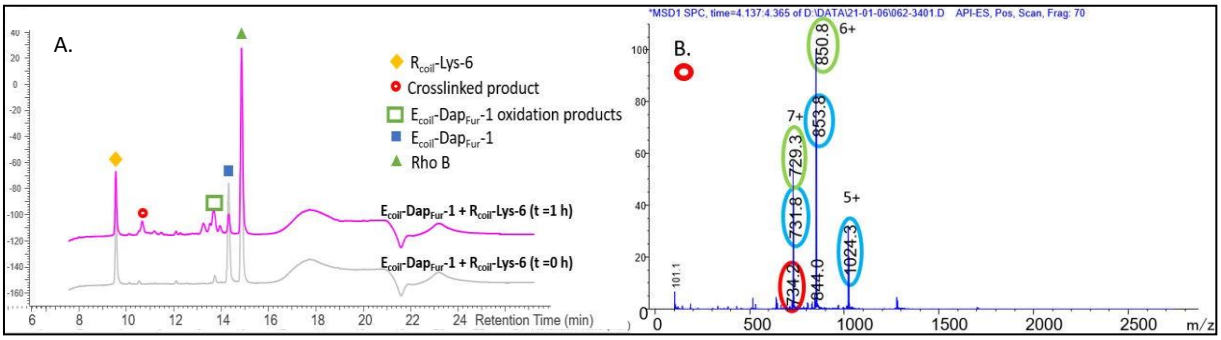


Figure SI 28: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-6** +10 µM **E_{coil}- Dap_{Fur}-1** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-1** and **R_{coil}-Lys-6** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-1 and R_{coil}-Lys-6 with Rho B: Found MW 5116.5: $[M+5H]^{5+}$/5= 1024.3, $[M+6H]^{6+}$/6= 853.8, $[M+7H]^{7+}$/7= 731.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+6H]^{6+}$/6= 850.8, $[M+7H]^{7+}$/7= 729.3. The red oval represents the oxidised form of the crosslinked product (+16 Da): Found MW 5132.4: $[M+7H]^{7+}$/7= 734.2.

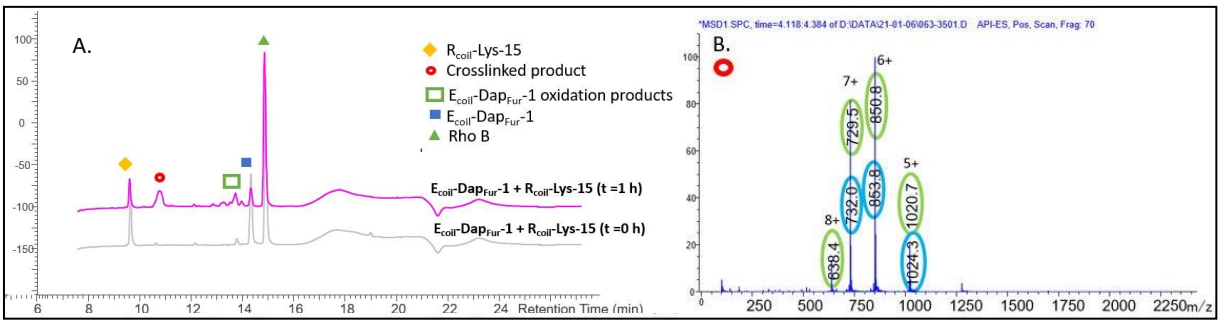


Figure SI 29: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-15** +10 µM **E_{coil}- Dap_{Fur}-1** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-1** and **R_{coil}-Lys-15** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the formed product. The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-1 and R_{coil}-Lys-15 with Rho B: Found MW 5116.5: $[M+5H]^{5+}$/5= 1024.3, $[M+6H]^{6+}$/6= 853.8, $[M+7H]^{7+}$/7= 732.0. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+5H]^{5+}$/5= 1020.7, $[M+6H]^{6+}$/6= 850.8, $[M+7H]^{7+}$/7= 729.5, $[M+8H]^{8+}$/8= 638.4.

15.2 Lysine scans with E_{coil}-Dap_{Fur}-6

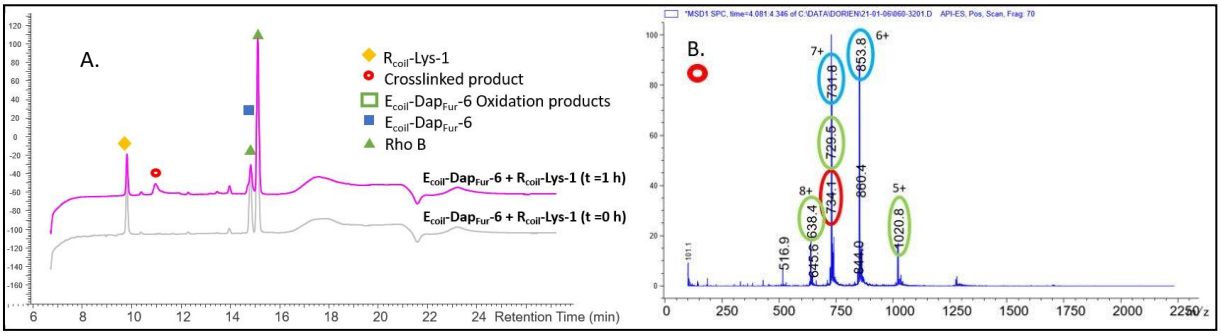


Figure SI 30: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-1** + 10 µM **E_{coil}- Dap_{Fur}-6** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-6** and **R_{coil}-Lys-1** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-6 and R_{coil}-Lys-1 with Rho B: Found MW 5116.5: $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 731.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+5H]^{5+}/5$= 1020.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4. The red ovals represents the oxidised form of the crosslinked product (+16 Da): Found MW 5131.7: $[M+7H]^{7+}/7$= 734.1.

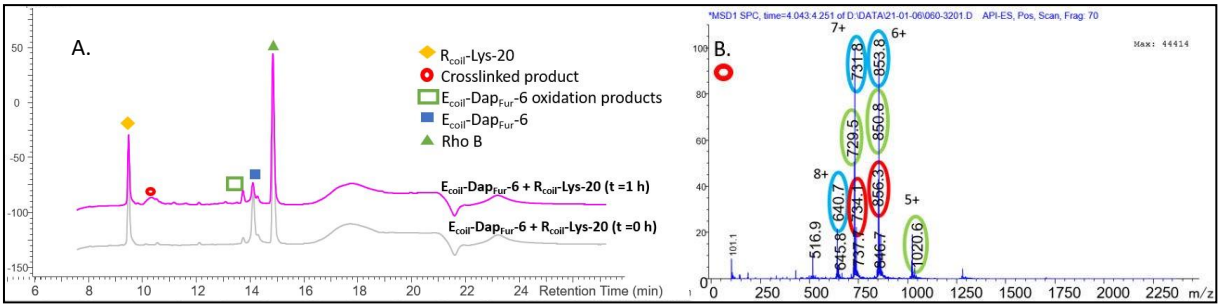


Figure SI 31: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-20** + 10 µM **E_{coil}- Dap_{Fur}-6** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing **both E_{coil}-Dap_{Fur}-6** and **R_{coil}-Lys-20** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-6 and R_{coil}-Lys-20 with Rho B: Found MW 5116.5: $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 731.8, $[M+8H]^{8+}/8$= 640.7. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+5H]^{5+}/5$= 1020.6, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5. The red ovals represents the oxidised form of the crosslinked product (+16 Da): Found MW 5131.7: $[M+6H]^{6+}/6$= 856.3, $[M+7H]^{7+}/7$= 734.1.

15.3 Lysine scans with E_{coil}-Dap_{Fur}-8

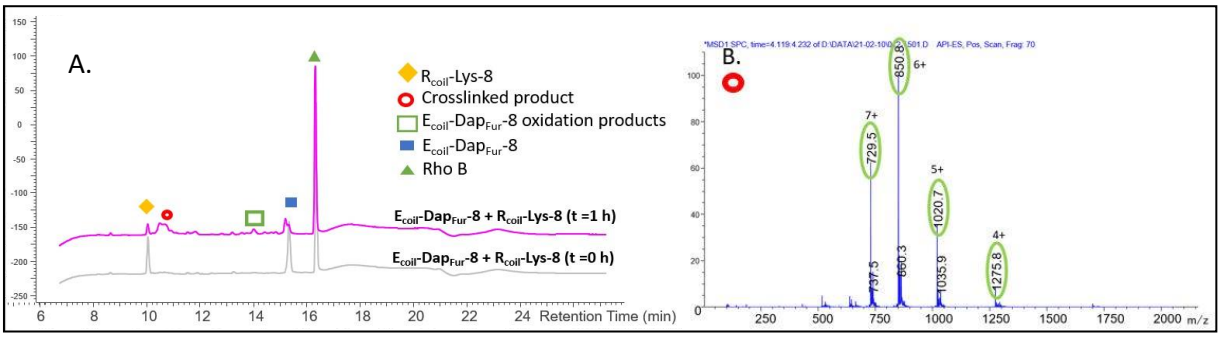


Figure SI 32: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-8** +10 µM **E_{coil}- Dap_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-8** and **R_{coil}-Lys-8** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The green ovals correspond to the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da) of E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-8 with Rho B: Found MW 5096.90: $[M+4H]^{4+}/4$= 1275.8, $[M+5H]^{5+}/5$= 1020.7, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5.

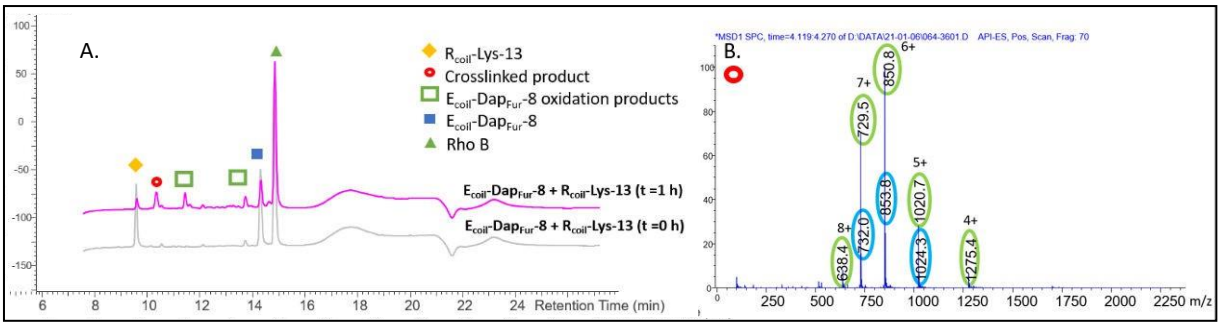


Figure SI 33: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-13** + 10 µM **E_{coil}- Dap_{Fur}-8** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-8** and **R_{coil}-Lys-13** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-13 with Rho B: Found MW 5116.5: $[M+5H]^{5+}/5$= 1024.3, $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$=1275.4, $[M+5H]^{5+}/5$= 1020.7, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

15.4 Lysine scans with E_{coil}-Dap_{Fur}-13

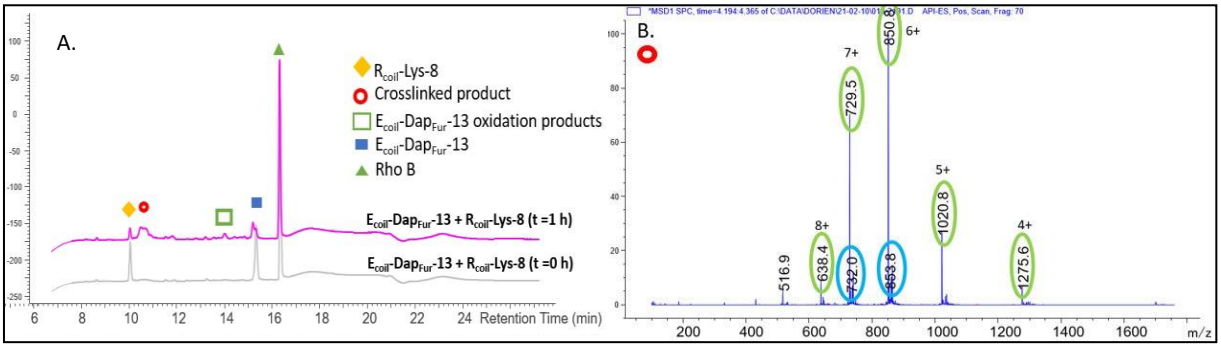


Figure SI 34: HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-8** + 10 µM **E_{coil}-Dap_{Fur}-13** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-13** and **R_{coil}-Lys-8** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-13 and R_{coil}-Lys-8 with Rho B: Found MW 5116.5: $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$=1275.6, $[M+5H]^{5+}/5$= 1020.8, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

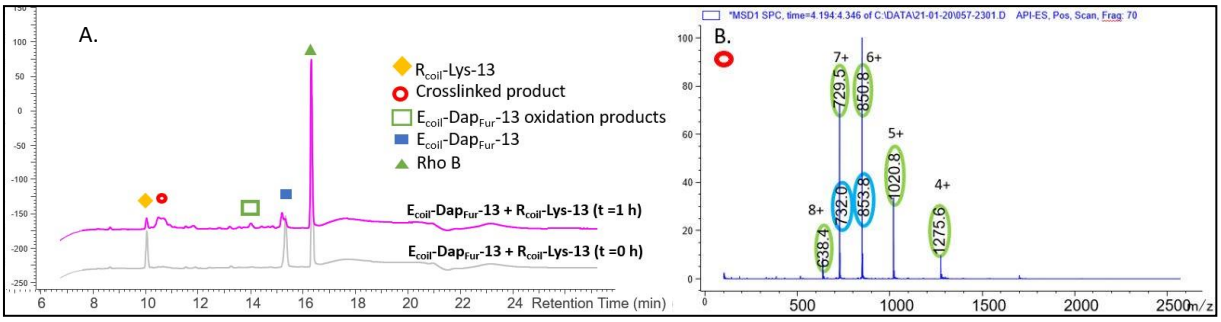


Figure SI 35: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-13** + 10 µM **E_{coil}-Dap_{Fur}-13** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-13** and **R_{coil}-Lys-13** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-13 and R_{coil}-Lys-13 with Rho B: Found MW 5116.5: $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$=1275.6, $[M+5H]^{5+}/5$= 1020.8, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

15.5 *Lysine scans with E_{coil}-Dap_{Fur}-15*

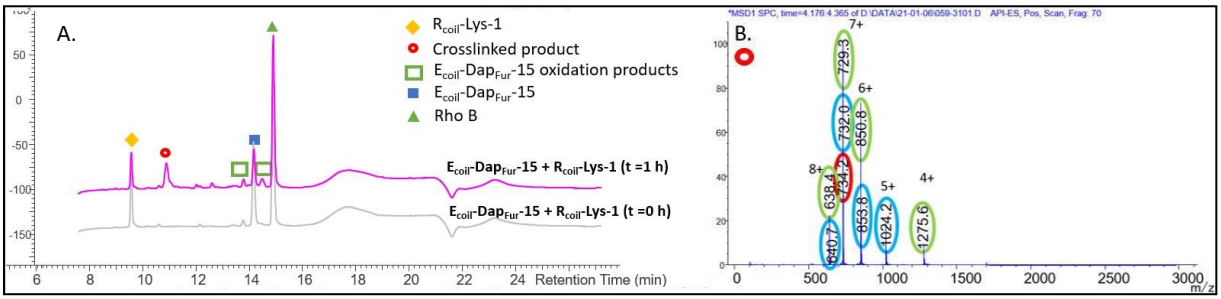


Figure SI 36: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-1** + 10 µM **E_{coil}-Dap_{Fur}-15** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing **both E_{coil}-Dap_{Fur}-15** and **R_{coil}-Lys-1** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-15 and R_{coil}-Lys-1 with Rho B: Found MW 5116.5: $[M+5H]^{5+}/5$= 1024.2, $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0, $[M+8H]^{8+}/8$= 640.7. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$= 1275.6, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.3, $[M+8H]^{8+}/8$= 638.4. The red oval represents the oxidised form of the crosslinked product (+16 Da): Found MW 5131.7: $[M+7H]^{7+}/7$= 734.2.

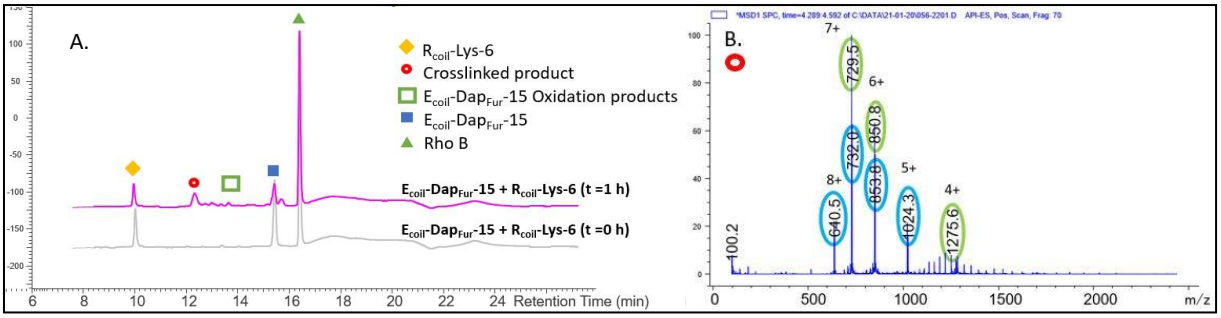


Figure SI 37: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-6** + 10 µM **E_{coil}-Dap_{Fur}-15** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-15** and **R_{coil}-Lys-6** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-15 and R_{coil}-Lys-6 with Rho B: Found MW 5116.5: $[M+5H]^{5+}/5$= 1024.3, $[M+6H]^{6+}/6$= 853.8, $[M+7H]^{7+}/7$= 732.0, $[M+8H]^{8+}/8$= 640.5. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$= 1275.6, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5.

15.6 Lysine scans with E_{coil}-Dap_{Fur}-20

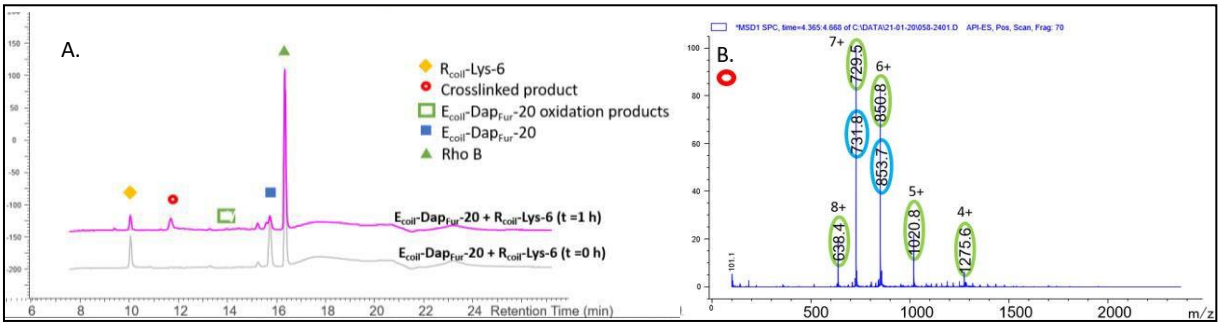


Figure SI 38: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-6** + 10 µM **E_{coil}-Dap_{Fur}-20** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-20** and **R_{coil}-Lys-6** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue ovals correspond to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-20 and R_{coil}-Lys-6 with Rho B: Found MW 5116.5: $[M+6H]^{6+}/6$= 853.7, $[M+7H]^{7+}/7$= 731.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$= 1275.6, $[M+5H]^{5+}/5$= 1020.8, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

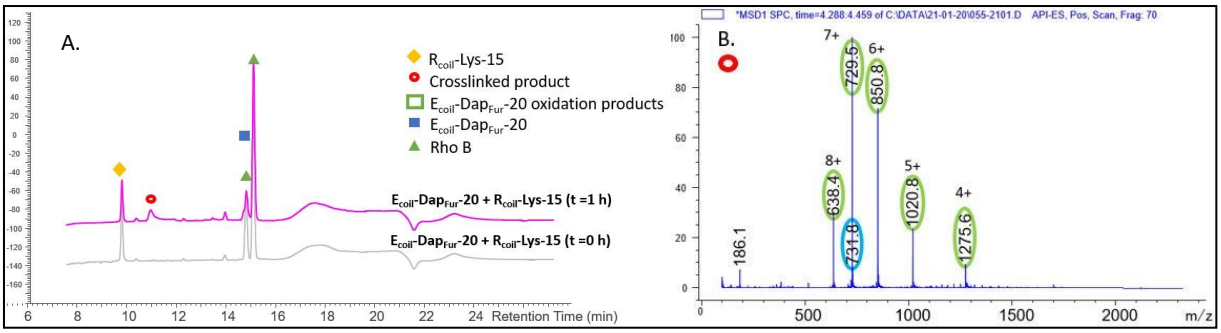


Figure SI 39: (A) HPLC chromatograms of the reaction mixture (5 µM **R_{coil}-Lys-15** + 10 µM **E_{coil}-Dap_{Fur}-20** + 10 µM Rho B) recorded at 260 nm. The grey trace represents the mixture containing both **E_{coil}-Dap_{Fur}-20** and **R_{coil}-Lys-15** as well as Rho B at t = 0 and the pink trace represents that same reaction mixture after 1 h of light irradiation. (B) ESI-MS spectra of the peak corresponding to the crosslinked product (red dot in panel A). The blue oval corresponds to the crosslinked product (Calcd MW 5115.89 Da) of E_{coil}-Dap_{Fur}-20 and R_{coil}-Lys-15 with Rho B: Found MW 5116.5: $[M+7H]^{7+}/7$= 731.8. The green ovals represent the dehydrated form of the crosslinked product (Calcd MW 5099.90 Da): Found MW 5098.8: $[M+4H]^{4+}/4$= 1275.6, $[M+5H]^{5+}/5$= 1020.8, $[M+6H]^{6+}/6$= 850.8, $[M+7H]^{7+}/7$= 729.5, $[M+8H]^{8+}/8$= 638.4.

10. CD spectroscopy of E_{coil}-Dap_{Fur}-13 with R_{coil}-Lys-X peptides

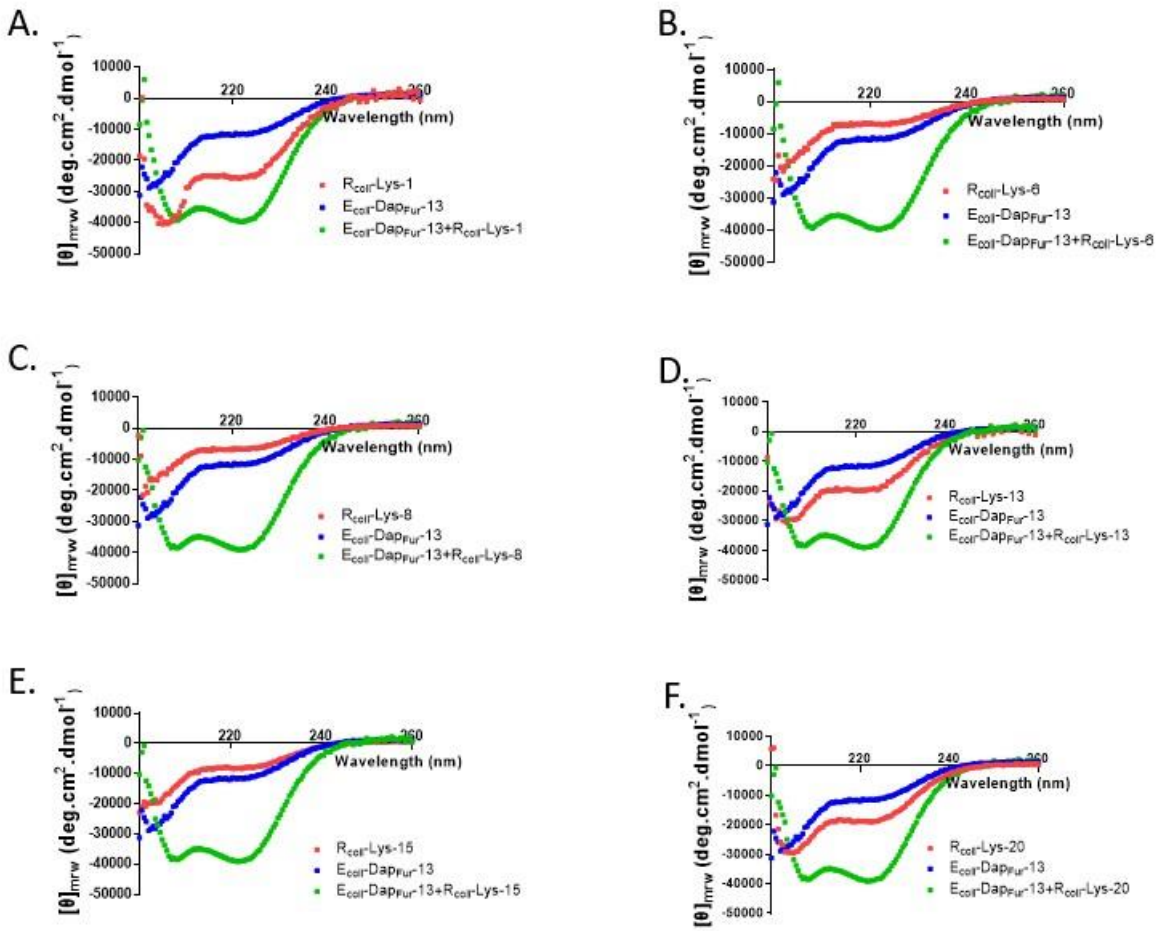


Figure SI 40: CD spectra obtained for the R_{coil} peptides (red trace) A) R_{coil} -Lys-1, B) R_{coil} -Lys-6, C) R_{coil} -Lys-8, D) R_{coil} -Lys-13, E) R_{coil} -Lys-15, F) R_{coil} -Lys-20, the E_{coil}-Dap_{Fur}-13 peptide (blue trace) and the equimolar mixture of both E_{coil}-Dap_{Fur}-13 and R_{coil} -Lys-X (green trace) in PBS solution (pH 7.4) at 5 µM concentration of each peptide.

11. Modelling studies

Parallel and antiparallel coiled-coil models were constructed using the webserver CCBuilder.0.1.[4] All the electrostatic charges in the e and g positions were set to zero to reduce complexity in the simulation analysis. Therefore, the coiled-coil models do not experience electrostatic frustration. Following computational predictions, six coiled-coil models were created: the E_{coil}-Dap_{Fur}-1 and R_{coil}-Lys-6, the E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-13, and E_{coil}-Dap_{Fur}-15 and R_{coil}-Lys-20, coiled-coil pairs that were considered to follow a parallel configuration, and the E_{coil}-Dap_{Fur}-1 and R_{coil}-Lys-15, the E_{coil}-Dap_{Fur}-8 and R_{coil}-Lys-8, and the E_{coil}-Dap_{Fur}-15 and R_{coil}-Lys-1, coiled-coil pairs that were considered to adopt an antiparallel conformation based on crosslinked experiments between the E_{coil}-Dap_{Fur}-X and the R_{coil}-Lys-X (see Figure 3C of the main manuscript). Additionally, a reciprocal version of every heterodimeric coil-coil pair was created, leading to a total of twelve coiled-coils. For example, for the heterodimeric E_{coil}-Dap_{Fur}-1 and R_{coil}-Lys-6 coiled-coil, the experimentally supported parallel version and an antiparallel version were created to check their preferred state computationally. For all models, the system was allowed to change the registry, the spontaneous sliding of one helix onto the other, however, the coiled-coil sliding was restricted by the maximum elongation that the Furan-Lysine linker can allow, which was set to be 11.9

Å. Validation of the coiled-coil models was performed by optimizing the knobs-into-holes (KIH) number using the SOCKET function of the CCBuilder, as shown in Figure SI 41.

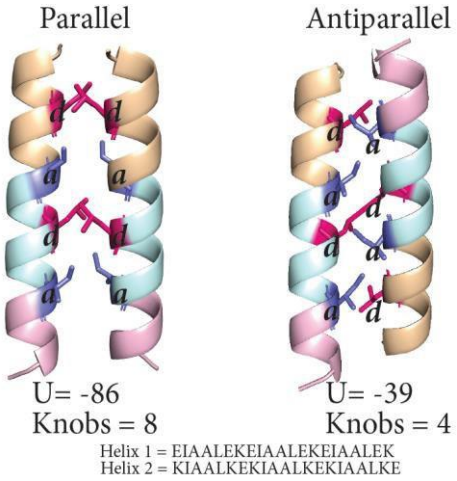


Figure SI 41: A model system of parallel (left) and antiparallel (right) configurations of the coiled-coils. Each coiled-coil consists of three heptad repeat units colored in light orange, cyan, and pink. Parallel coiled-coils are stabilized by hydrophobic interactions between the a_i-a_i' and the d_i-d_i' pairs, with i one of the three heptad repeats of the coiled-coil models. In antiparallel configurations, the stability is supported by the interactions between the a-d' and d-a' pairs. The a_i-a_i' become weaker due to the longer distances between these two residues in the antiparallel coiled-coil. The sequences of the two helixes are provided together with the KIH number of each representation.

Coarse-Grained Models

Coarse-grained models of the coiled-coil systems were created by representing each residue by two beads at the Cα and Cβ atoms. The peptide is simulated by a native topology-based model and uses the Lennard-Jones potential to represent native contact interactions.[5] All simulations were run for a total number of 3×10^5 steps. Every 1000 steps a snapshot of the coiled-coil configuration was taken.

The number of native contacts Q was used as the system order parameter, as described in our previous study.[5] In this representation, large Q values represent a folded coiled-coil with a high number of hydrophobic interactions and, therefore, a strong interface. Low Q values represent a coiled-coil with weak hydrophobic interactions, indicating poor stability of those configurations. Simulations were performed at a temperature of T=0.36, below the critical temperature of the system TF~0.4, defined as the temperature at which the CC is stable with a 50% probability. Using this temperature ensures that the coiled-coils remain in the bound states and enable us to capture the dynamics. For all simulations, the energetic strength of the Lennard-Jones potential was kept constant K=1. The crosslink between furan and lysine was defined as a covalent bond in the coiled-coil structure, with a bond strength of KCB=5.

The parallel and antiparallel coiled-coil conformations were represented based on their hydrophobic cores' geometry, as shown in Figure SI 41. In the parallel configuration, the hydrophobic interactions take place between the a-a' and d-d' positions in the coiled-coil. Here, the distances between the Cα atoms in the a_i-a_i' and d_i-d_i' positions, with i one of the three heptad repeats, are the same aa_i-aa_i= da_i-da_i= 6.3 Å. By contrast, the antiparallel configurations' hydrophobic core is supported by the interactions between a_1-d_3, d_1-a_3, a_2-d_2, d_2-a_2, a_3-d_1, and d_3-a_1, which have a distance of 5.9 Å.

The probability densities between the C_α atoms of the a_i-a_i' residues were plotted against the distance for the parallel and antiparallel coiled-coil models: E_{coil}-Dap_{Fur}-8/R_{coil}-Lys-13 and E_{coil}-Dap_{Fur}-15/R_{coil}-Lys-20 (see Figure SI 42), and the probability density of the number of native contacts (Q) was also calculated in the parallel and antiparallel coiled-coil system (see Figure SI 42).

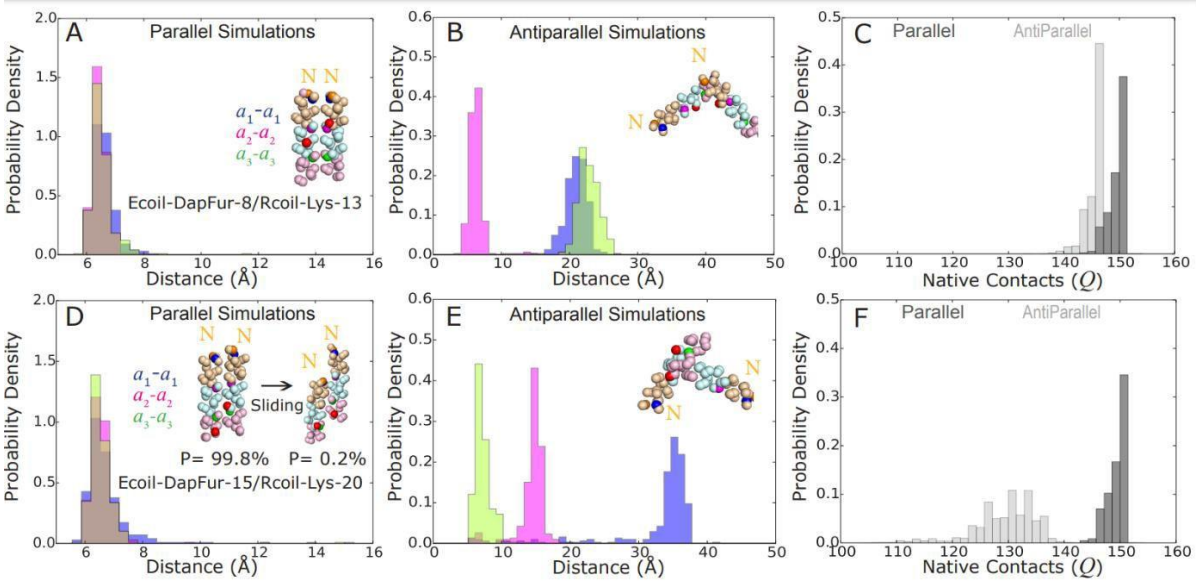


Figure SI 42: Probability densities of the distance between the Cα atoms of the a_i-a_i' pairs, with i one of the three heptad repeats of the coiled-coil models (1, 2 and 3). The considered parallel and antiparallel coiled-coils are: the E_{coil}-Dap_{Fur}-8+R_{coil}-Lys-13 (A, B) and the E_{coil}-Dap_{Fur}-15+R_{coil}-Lys-20 (D, E). Probabilities of staying in the initial or the shifted configuration, coiled-coil sliding, are denoted by P. Snapshots of the simulations are shown in every panel. Each of the heptad repeats of the coiled-coil model is represented with a different color: light orange (1st heptad), cyan (2nd heptad), and pink (3rd heptad). The a_i-a_i' pairs are represented in the snapshots as blue (a_1-a_1), magenta (a_2-a_2), and green (a_3-a_3). The Furan-Lysin linker is represented by two red beads, and the *N* terminus of each helix is represented by an orange bead. Probability densities of the number of native contacts Q in the parallel and antiparallel coiled-coils models are: the E_{coil}-Dap_{Fur}-8+R_{coil}-Lys-13 (C) and the E_{coil}-Dap_{Fur}-15+R_{coil}-Lys-20 (F).

The probability densities between the C_α atoms of the a_1-a_3, a_2-a_2, and a_3-a_1 residues were plotted against the distance for the antiparallel and parallel coiled-coil models: E_{coil}-Dap_{Fur}-8/R_{coil}-Lys-8 and E_{coil}-Dap_{Fur}-15/R_{coil}-Lys-1, and the probability density of the number of native contacts (Q) was also calculated in the antiparallel and parallel coiled-coil system (see Figure SI 43).

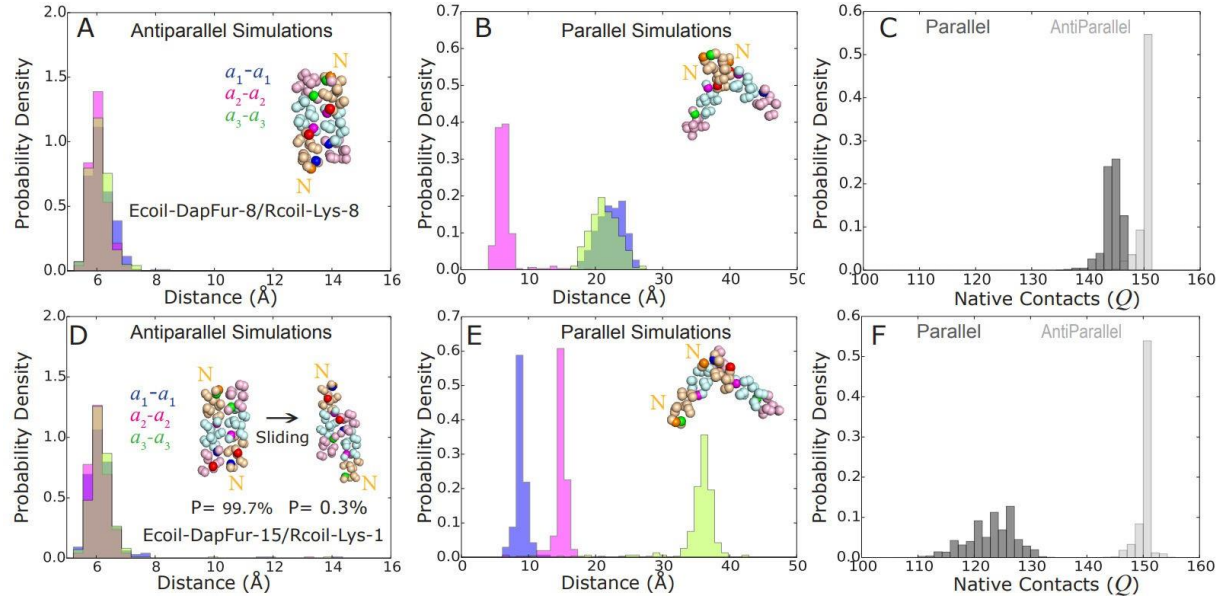


Figure SI 43: Probability densities of the distance between the Cα atoms of the a_1-a_3, a_2-a_2, a_3-a_1 pairs. The considered antiparallel and parallel coiled-coils are: the E_{coil}-Dap_{Fur}-8+R_{coil}-Lys-8 (A, B) and the E_{coil}-Dap_{Fur}-15+R_{coil}-Lys-1 (D, E). Probabilities of staying in the initial or the shifted configuration, coiled-coil sliding, are denoted by P. Snapshots of the simulations are shown in every panel. Each of the heptad repeats of the coiled-coil model is represented with a different color: light orange (1st heptad), cyan (2nd heptad), and pink (3rd heptad). The a_i-a_i pairs are represented in the snapshots as blue (a_1-a_3), magenta (a_2-a_2), and green (a_3-a_1). The Furan-Lysin linker is represented by two red beads, and the *N* terminus of each helix is represented by an orange bead. Probability

densities of the number of native contacts Q in the antiparallel and parallel coiled-coils models are: the E_{coil}-Dap_{Fur}-6+R_{coil}-Lys-20 (C) and the E_{coil}-Dap_{Fur}-8+R_{coil}-Lys-8 (F).

12 Pyrene E_{coil} and K_{coil} peptide characterization

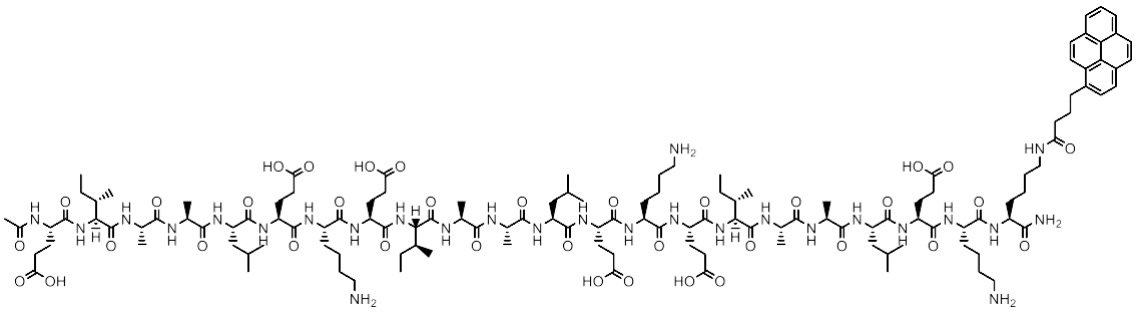
11.1 *Table of the pyrene E_{coil} and K_{coil} synthesized peptides*

Name	***Peptide sequences***	***Calcd MW (Da)***
E_{coil}-**C_{py}**	Ac-E IAALEKE IAALEKE IAALEK K(**Py**)-NH_2	2722.23
E_{coil}- **N_{py}**	**Py**-E IAALEKE IAALEKE IAALEK K(Ac)-NH_2	2722.23
K_{coil}-**C_{py}**	Ac- K IAALKEK IAALKEK IAALKEK K(**Py**)-NH_2	2719.39
K_{coil}- **N_{py}**	**Py**-K IAALKEK IAALKEK IAALKEK (Ac)-NH_2	2719.39

Table SI 4: Overview of pyrene-modified E_{coil}/K_{coil} peptide sequences, the calculated molecular weight (MW), and their corresponding codes: pyrene (Py) and acetylation (Ac).

12.2 E_{coil}-Cpy peptide

A)



B)

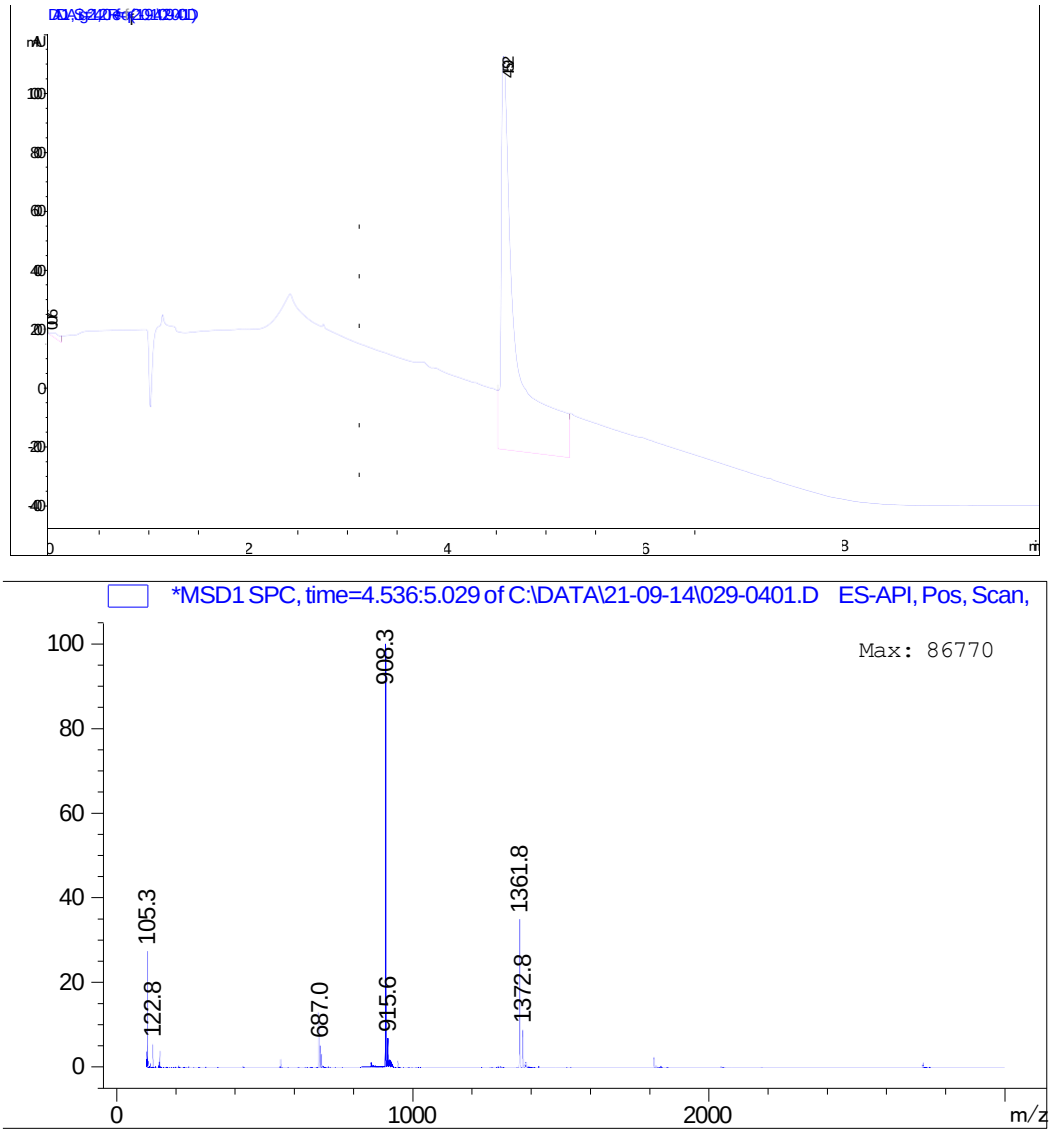
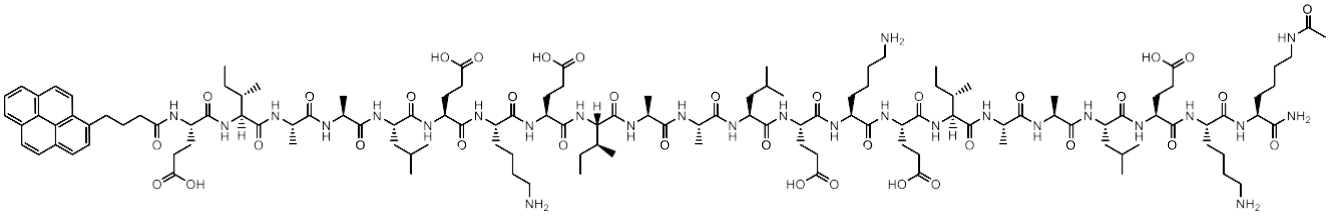


Figure SI 44: A) Structure of the E_{coil}-Cpy peptide. B) HPLC-MS trace of purified E_{coil}-Cpy. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2722.23 Da. LC-ESI-MS m/z at t_r= 4.59: $[M+2H]^{2+}/2$= 1361.8, $[M+3H]^{3+}/3$= 908.3.

12.3 E_{coil}-N_{py} peptide

A)



B)

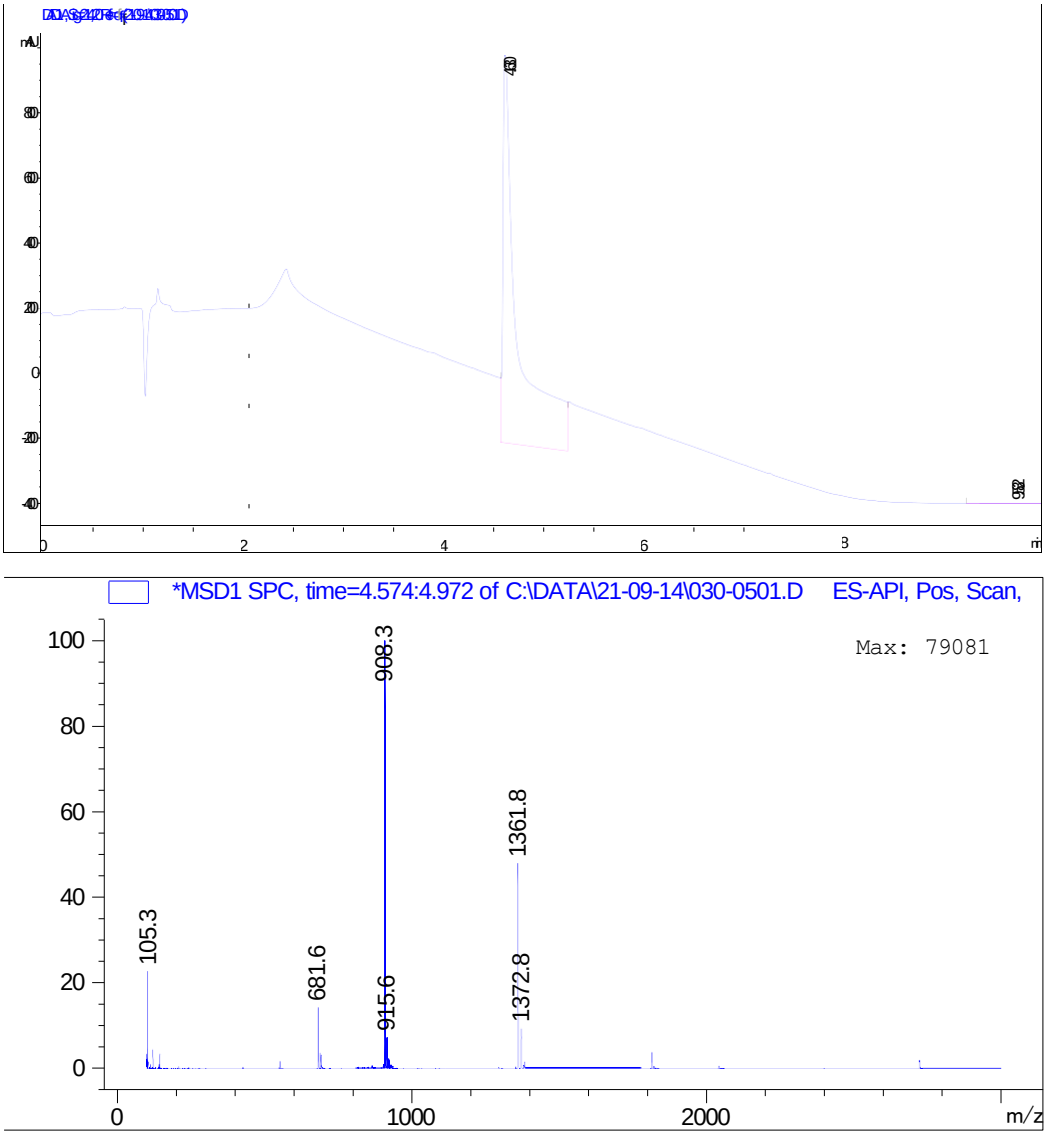
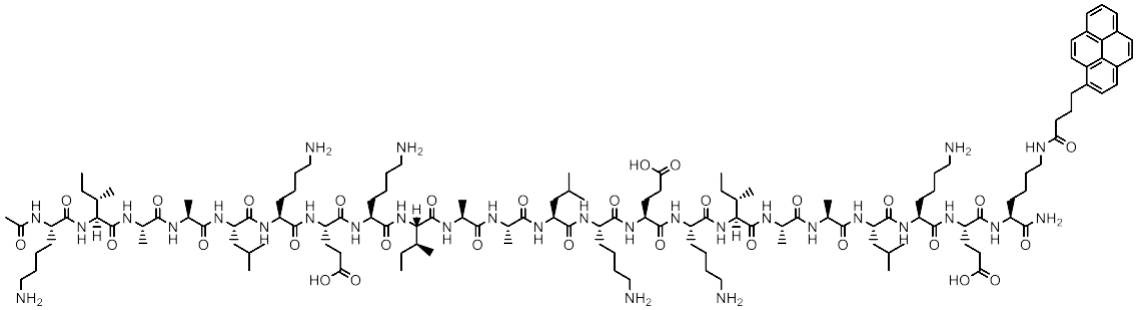


Figure SI 45: A) Structure of the Npy-E_{coil} peptide. B) HPLC-MS trace of purified Npy-E_{coil}. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2722.23 Da. LC-ESI-MS m/z at t_r= 4.63: $[M+2H]^{2+}/2$= 1361.8, $[M+3H]^{3+}/3$= 908.3.

12.4 K_{coil}-C_{py} peptide

A)



B)

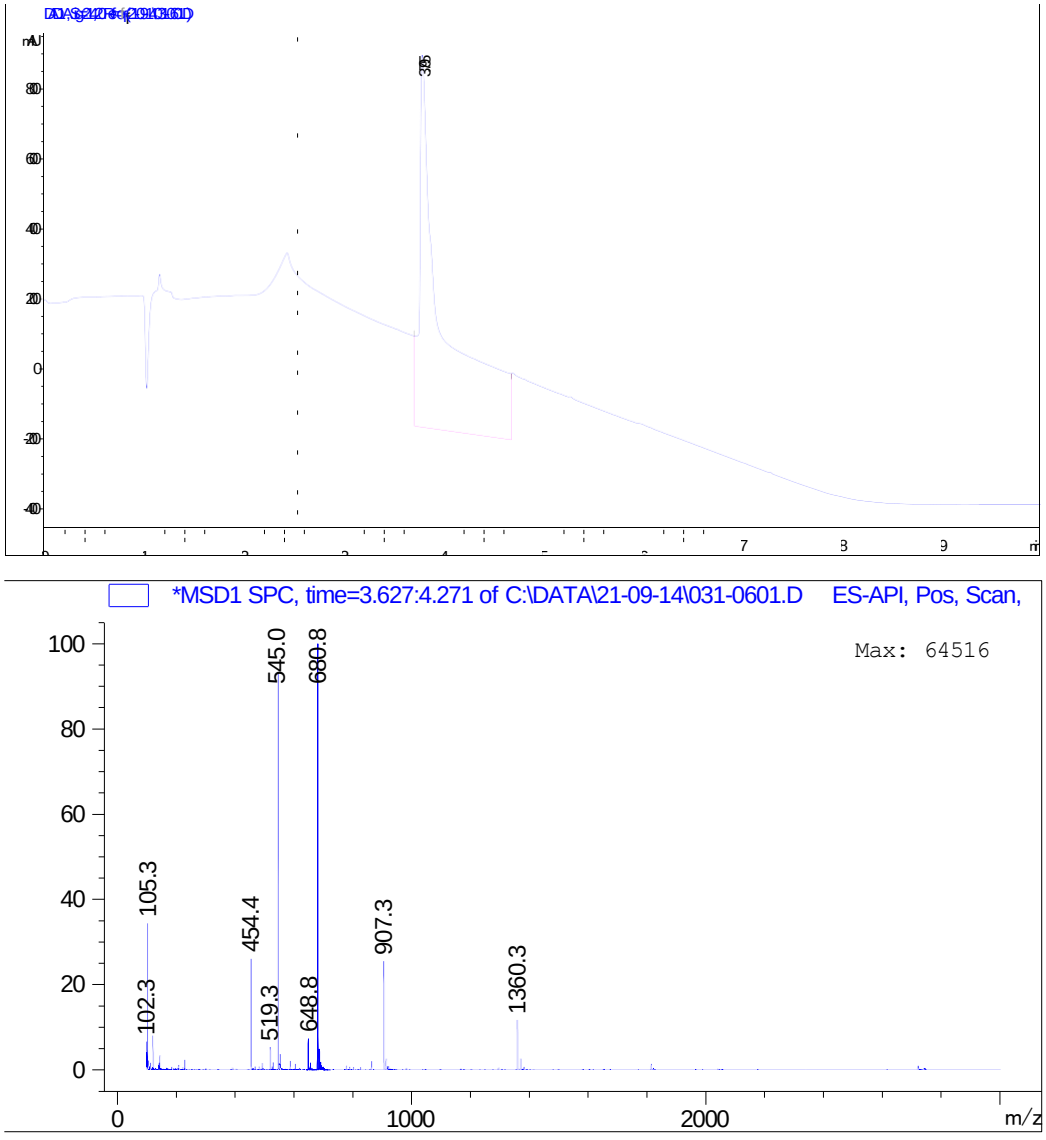
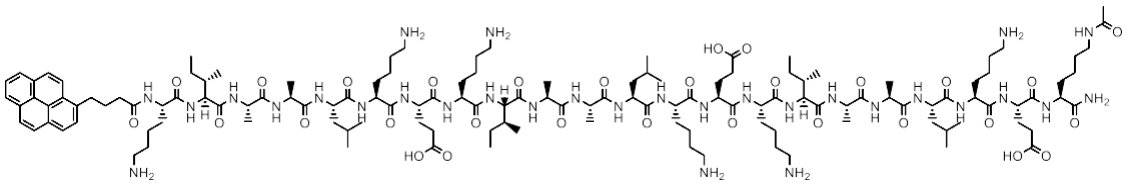


Figure SI 46: A) Structure of the K_{coil}-C_{py} peptide. B) HPLC-MS trace of purified K_{coil}-C_{py}. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2719.39 Da. LC-ESI-MS m/z at t_r= 3.80: $[M+2H]^{2+}/2$= 1360.7, $[M+3H]^{3+}/3$= 907.3, $[M+4H]^{4+}/4$= 680.8, $[M+5H]^{5+}/5$= 545.0, $[M+6H]^{6+}/6$= 454.4.

12.5K_{coil}-N_{py} peptide

A)



B)

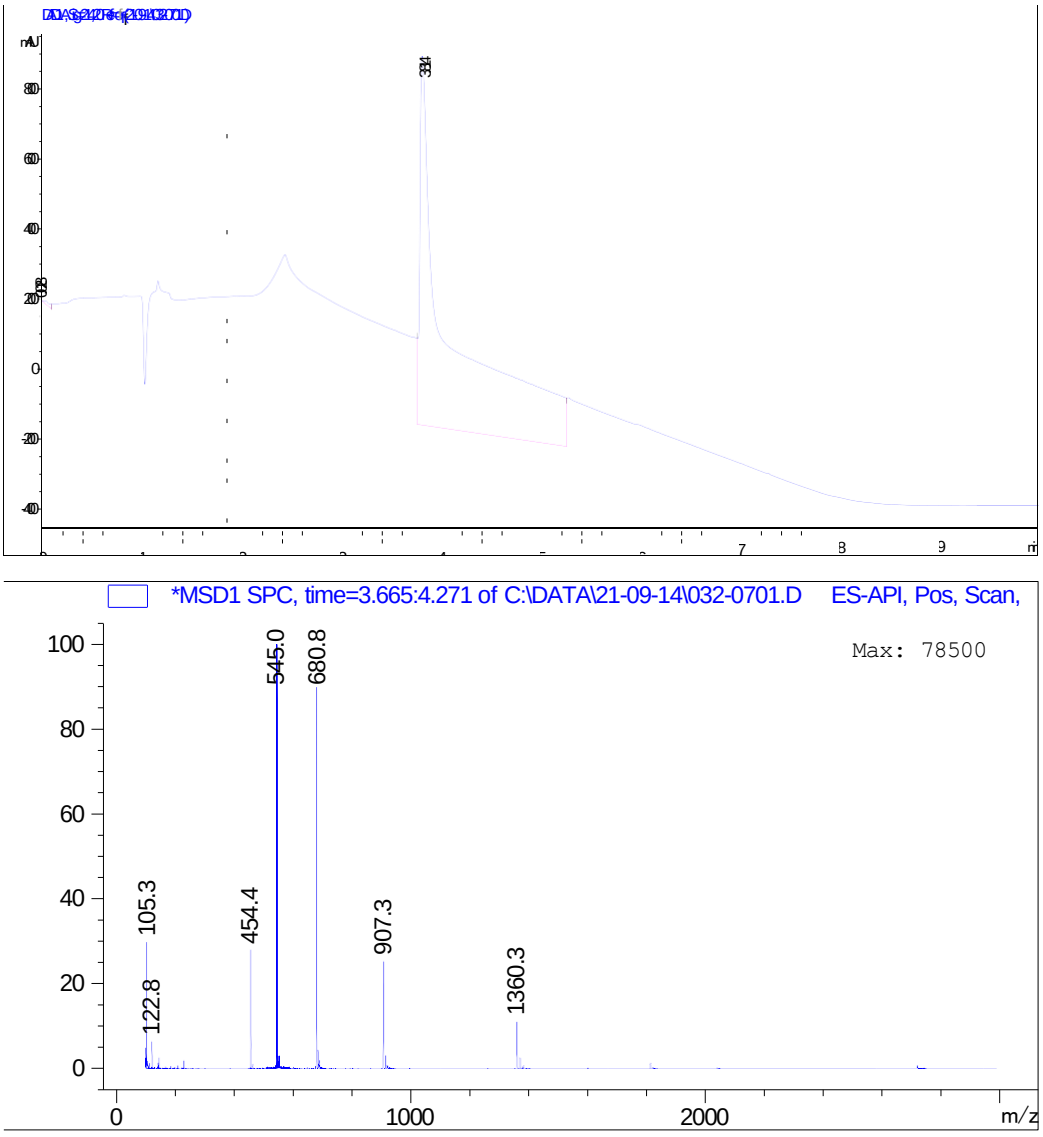


Figure SI 47: A) Structure of the K_{coil}-N_{py} peptide. B) HPLC-MS trace of purified K_{coil}-N_{py}. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2719.39 Da. LC-ESI-MS m/z at t_r= 3.81: $[M+2H]^{2+}/2$= 1360.3, $[M+3H]^{3+}/3$= 907.3, $[M+4H]^{4+}/4$= 680.8, $[M+5H]^{5+}/5$= 545.0, $[M+6H]^{6+}/6$= 454.4.

13 Fluorescence measurements

300 µL samples were prepared in triplicate in Nunc F96 MicroWell plates (Thermo Fisher) at 5 µM coils concentration in PBS (100 NaCl, 10 mM phosphate, pH 7.4) or 10 % DMF in PBS. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorometer equipped with a G9810A Agilent Microplate reader accessory, exciting at 343 nm (slit: 5.0 nm), scanning from 360 nm to 650 nm (slit: 5 nm). A scan speed of 200 nm/min was used.

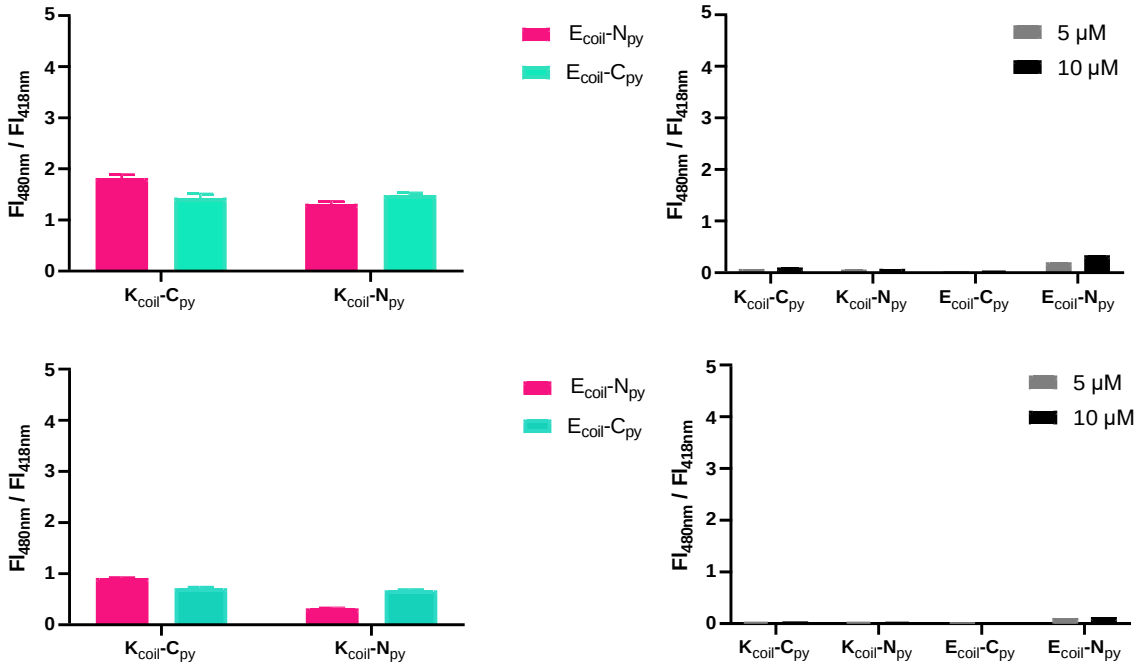


Figure SI 48: Excimer/monomer emission intensity ratio for the different coiled-coil systems (A, C) and coiled peptides (B,D) in PBS pH 7.4 (A,B) or 10% DMF in PBS pH 7.4 (C,D).

14 CD spectroscopy of pyrene E_{coil} and K_{coil} peptides

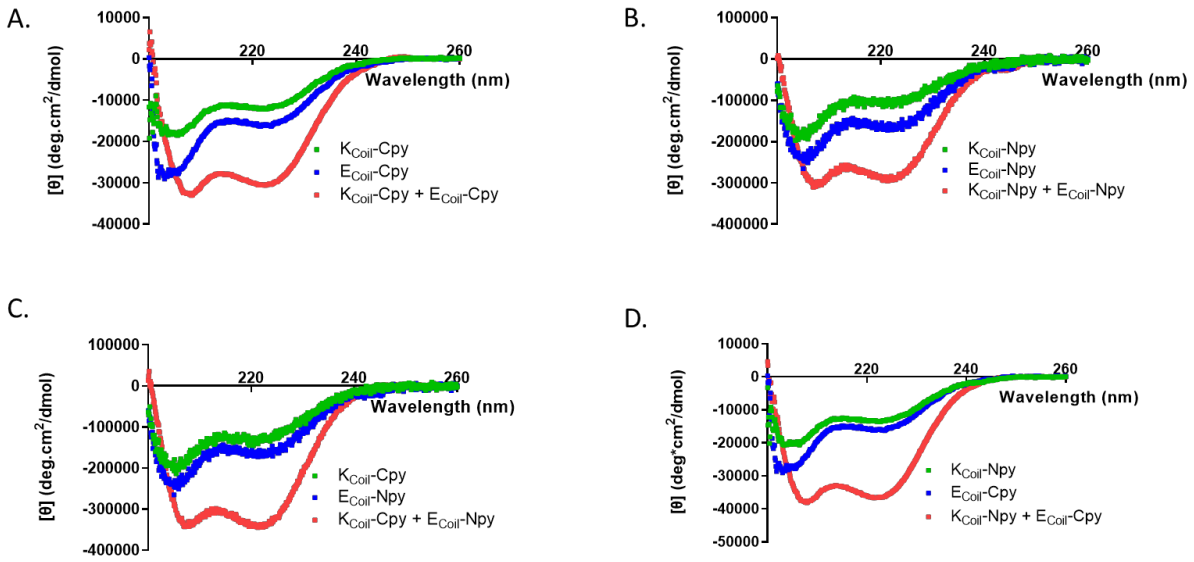


Figure SI 49: CD spectra obtained of the pyrene-modified E_{coil} peptides (blue trace) at 5 µM, the pyrene modified K_{coil} peptides (green trace) at 5 µM, and the equimolar mixture of both (red trace) in PBS solution (pH 7.4). A) E_{coil}-C_{py} + K_{coil}-C_{py}, B) E_{coil}-N_{py} + K_{coil}-N_{py}, C) E_{coil}-N_{py} + K_{coil}-C_{py}, and D) E_{coil}-C_{py} + K_{coil}-N_{py}.

15Thermal denaturation of pyrene E_{coil} and R_{coil}/K_{coil} peptides

CD Melting experiments were recorded on a Jasco J1500 equipped with Jasco CTU-100 circulating thermostat. Thermal denaturation profiles were measured by monitoring the CD signal at 222 nm from 20°C to 90°C with a heating rate of 0.5 °C/min and recording every 0.1 °C using a 3 mm optical path. Measurement conditions: strand concentration = 5 µM in pH 7.4 PBS buffer (100 mM NaCl, 10 mM phosphate). Melting temperatures were calculated from the first order derivative of a 10th order polynomial fitting function.

Sample	Tm (°C)	T'_{Trans} (°C)
E_{coil} + K_{coil}	50.8	*
E_{coil} + R_{coil}-Lys-1	55.3	*
E_{coil} + R_{coil}-Lys-20	56.6	*
K_{coil}-N_{py} + E_{coil}-N_{py}	73.3	47.7*
K_{coil}-N_{py} + E_{coil}-C_{py}	73.2	42.1*
K_{coil}-C_{py} + E_{coil}-C_{py}	77.7	*
K_{coil}-C_{py} + E_{coil}-N_{py}	78	41.4

Table SI 5: Overview of the melting temperatures determined for representative coiled-coil combinations. * indicates the presence of a possible transition that does not show a local maximum in the 1st order derivative.

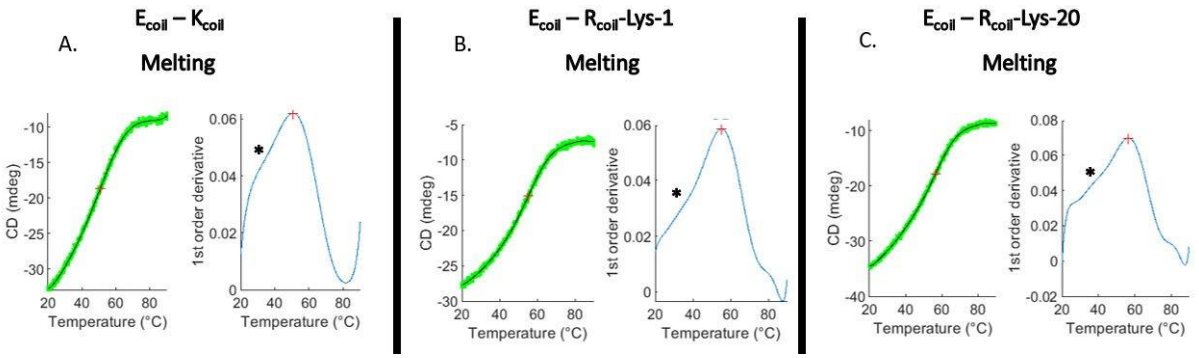


Figure SI 50: Thermal denaturation curves and their 1st order derivative obtained from the equimolar mixture of A) E_{coil} + K_{coil} B) E_{coil} + R_{coil}-Lys-1, C) E_{coil} + R_{coil}-Lys-20. * indicates the presence of a possible transition that does not show a local maximum in the 1st order derivative.

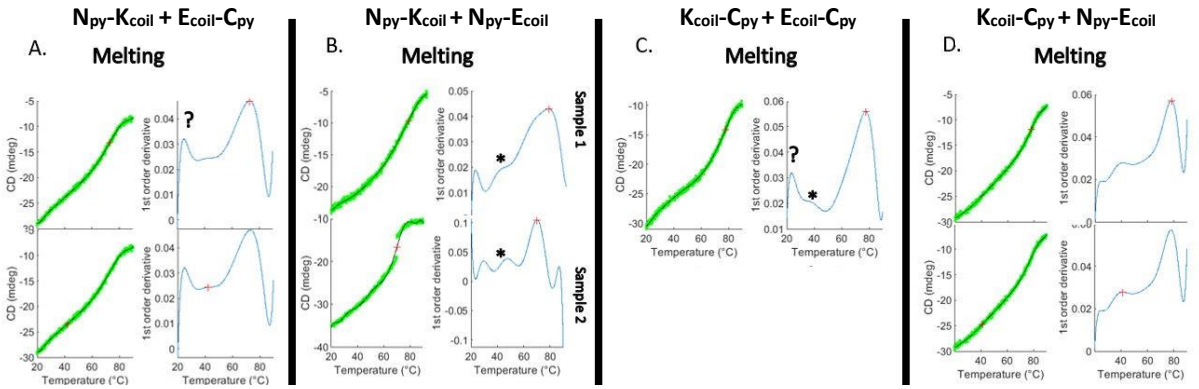


Figure SI 51: Thermal denaturation curves and their 1st order derivative obtained from the equimolar mixture of A) N_{py}-K_{coil} + E_{coil}-C_{py}, B) N_{py}-K_{coil} + N_{py}-E_{coil},C) K_{coil}-C_{py} + E_{coil}-C_{py}, and D) K_{coil}-C_{py} + N_{py}-E_{coil}. * indicates the presence of possible transition that did not show a local maximum in the 1st order derivative.

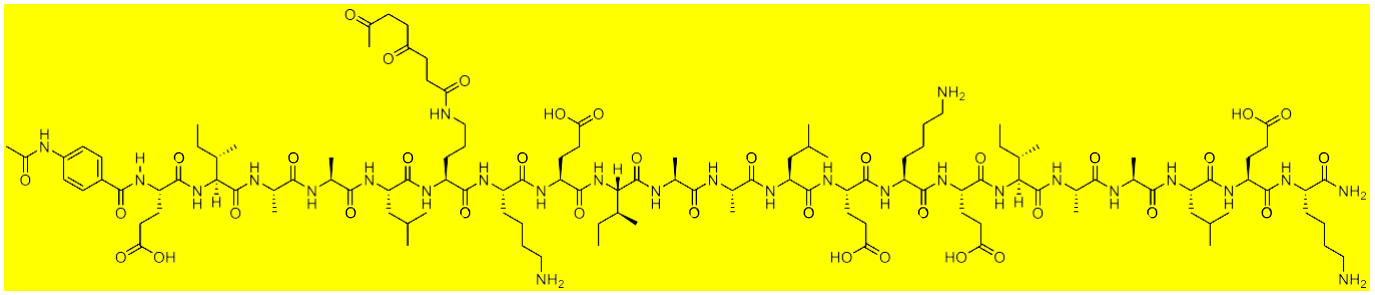
16E_{coil}-Orn_{DOP}-6 and R_{Coil}/K_{coil} peptide characterization

16.1 Table of the E_{coil}-Orn_{DOP}-6 and K_{Coil}/ R_{Coil} synthesized peptides

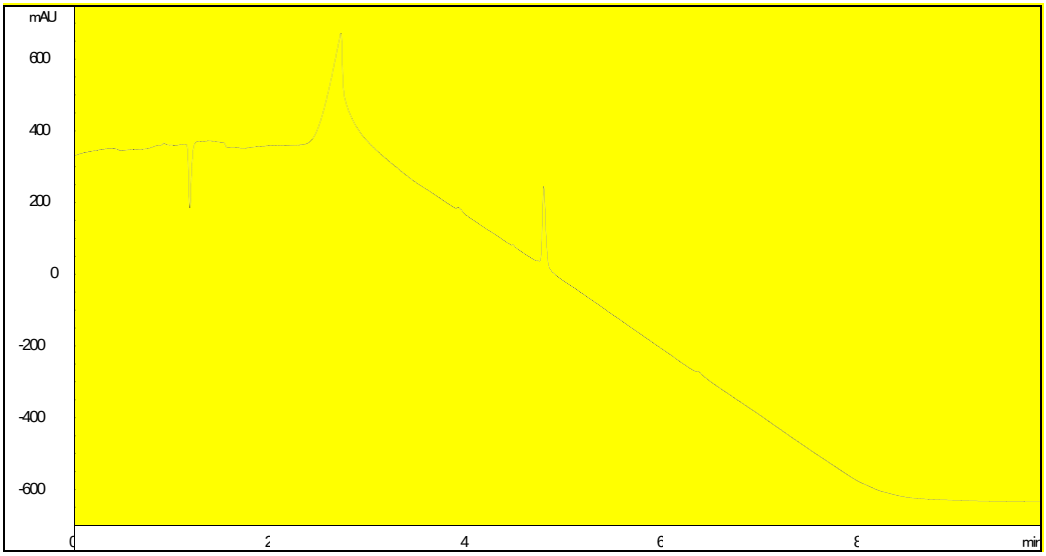
Name	***Peptide sequences***	***Calcd MW (Da)***
E_{coil}-Orn_{DOP}-6	ABA-EIAAL-$Orn_{(DOP)}$-KEIAALEKEIAALEK-NH_2	2562.43
K_{coil}-Orn_{Hy}-1	ABA-$Orn_{(Hy)}$-IAALKEKIAALKEKIAALKE-NH_2	2496.52
K_{coil}-Orn_{Hy}-20	ABA-KIAALKEKIAALKEKIAAL-$Orn_{(Hy)}$-E-NH_2	2496.52
R_{coil}-Orn_{Hy}-1	ABA-$Orn_{(Hy)}$-IAALRERIAALRERIAALRE-NH_2	2636.55
R_{coil}-Orn_{Hy}-20	ABA-RIAALRERIAALRERIAAL-$Orn_{(Hy)}$-E-NH_2	2636.55

16.2 E_{coil}-Orn_{DOP}-6

A)



B)



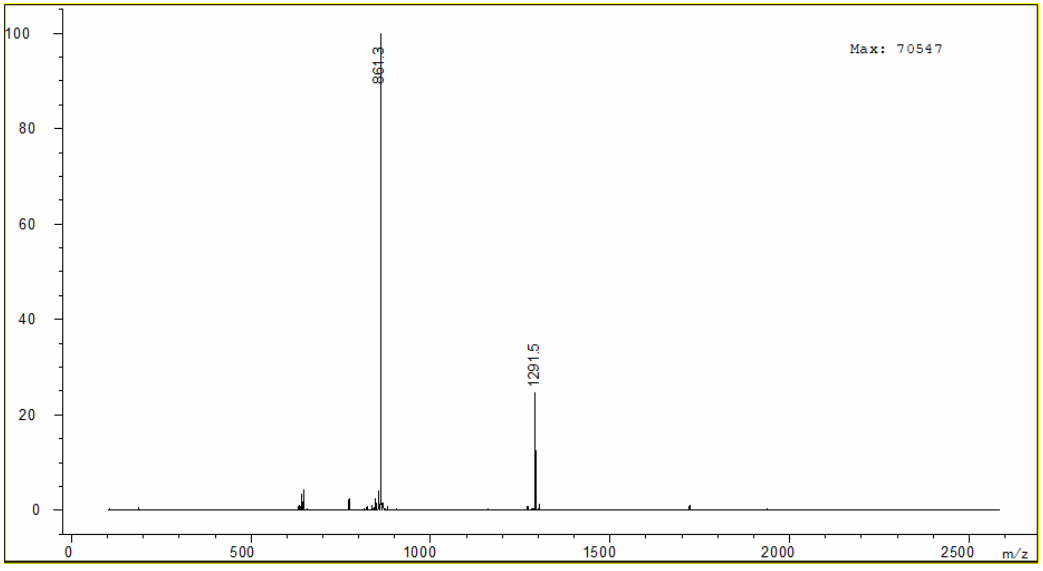
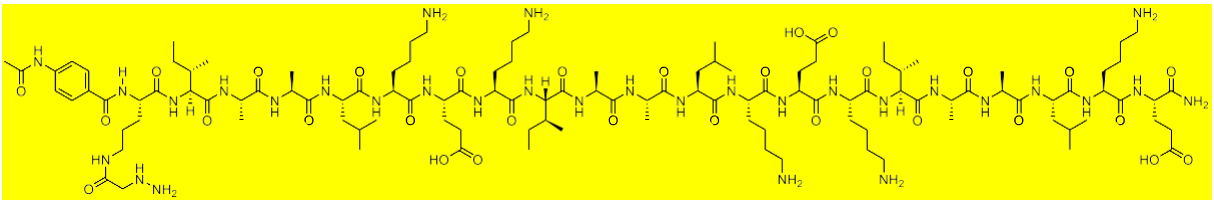


Figure SI 52: A) Structure of the E_{coil}-Orn_{DOP}-6 peptide. B) HPLC-MS trace of purified E_{coil}-Orn_{DOP}-6. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2562.43 Da. LC-ESI-MS m/z at t_r= 4.77: $[M+2H]^{2+}/2$= 1291.5, $[M+3H]^{3+}/3$= 861.3

16.3 K_{coil}-Orn_{Hy}-1

A)



B)

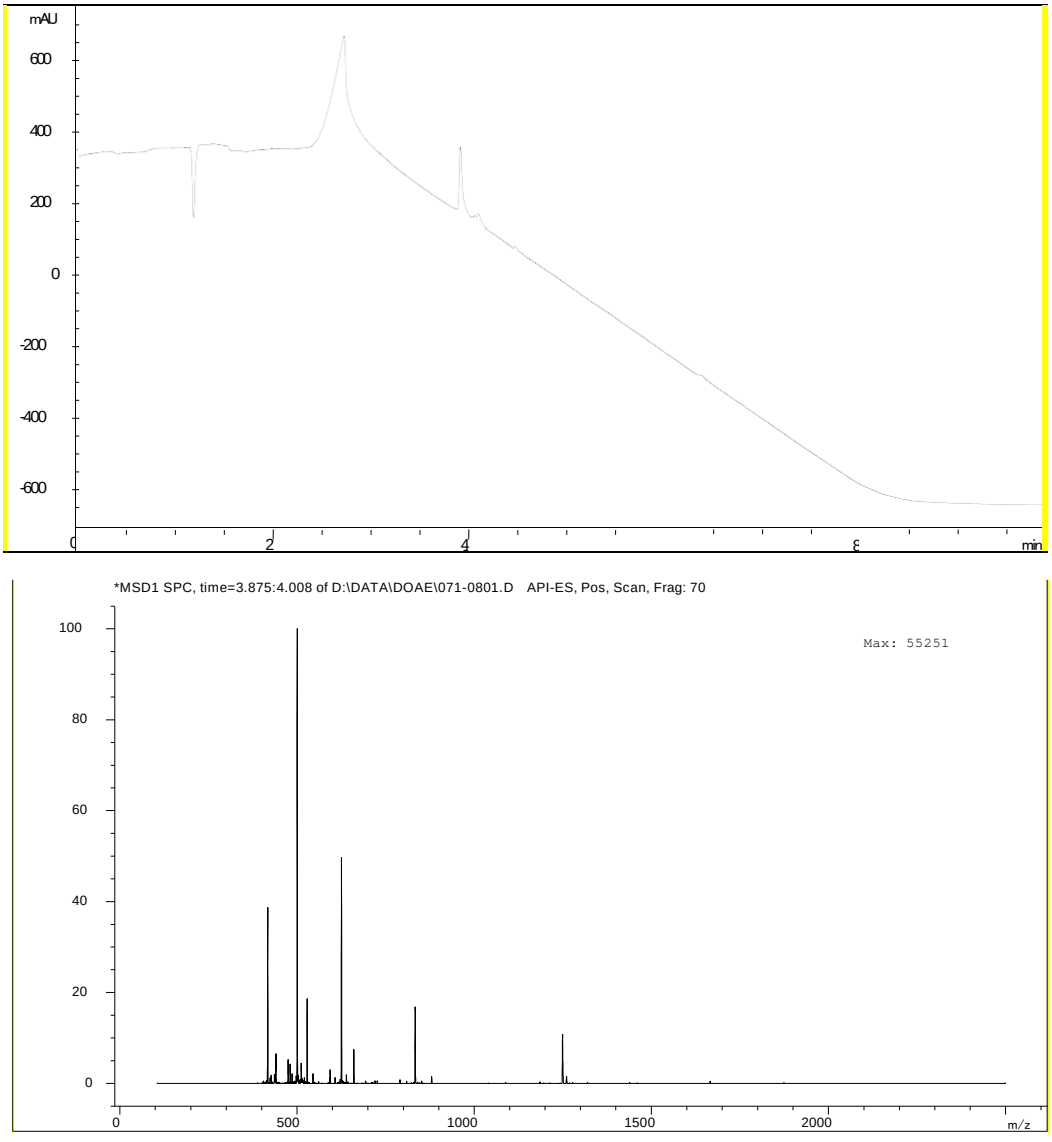
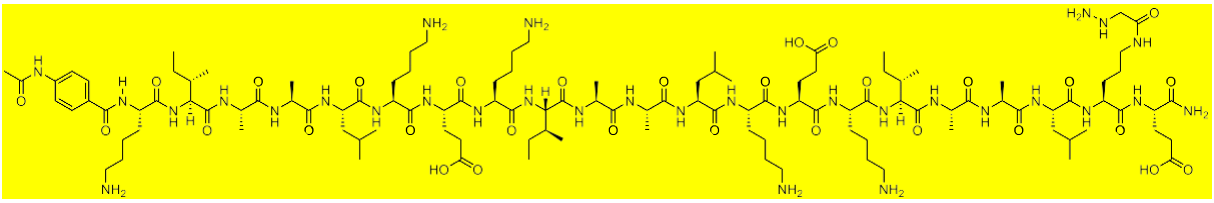


Figure SI 53: A) Structure of the K_{coil}-Orn_{Hy}-1 peptide. B) HPLC-MS trace of purified K_{coil}-Orn_{Hy}-1. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2496.52 Da. LC-ESI-MS m/z at t_r= 3.91: $[M+2H]^{2+}/2$= 1249.5, $[M+3H]^{3+}/3$= 833.4, $[M+4H]^{4+}/4$= 625.3, $[M+5H]^{5+}/5$= 500.5, $[M+6H]^{6+}/6$= 417.3.

16.4 K_{coil}-Orn_{Hy}-20

A)



B)

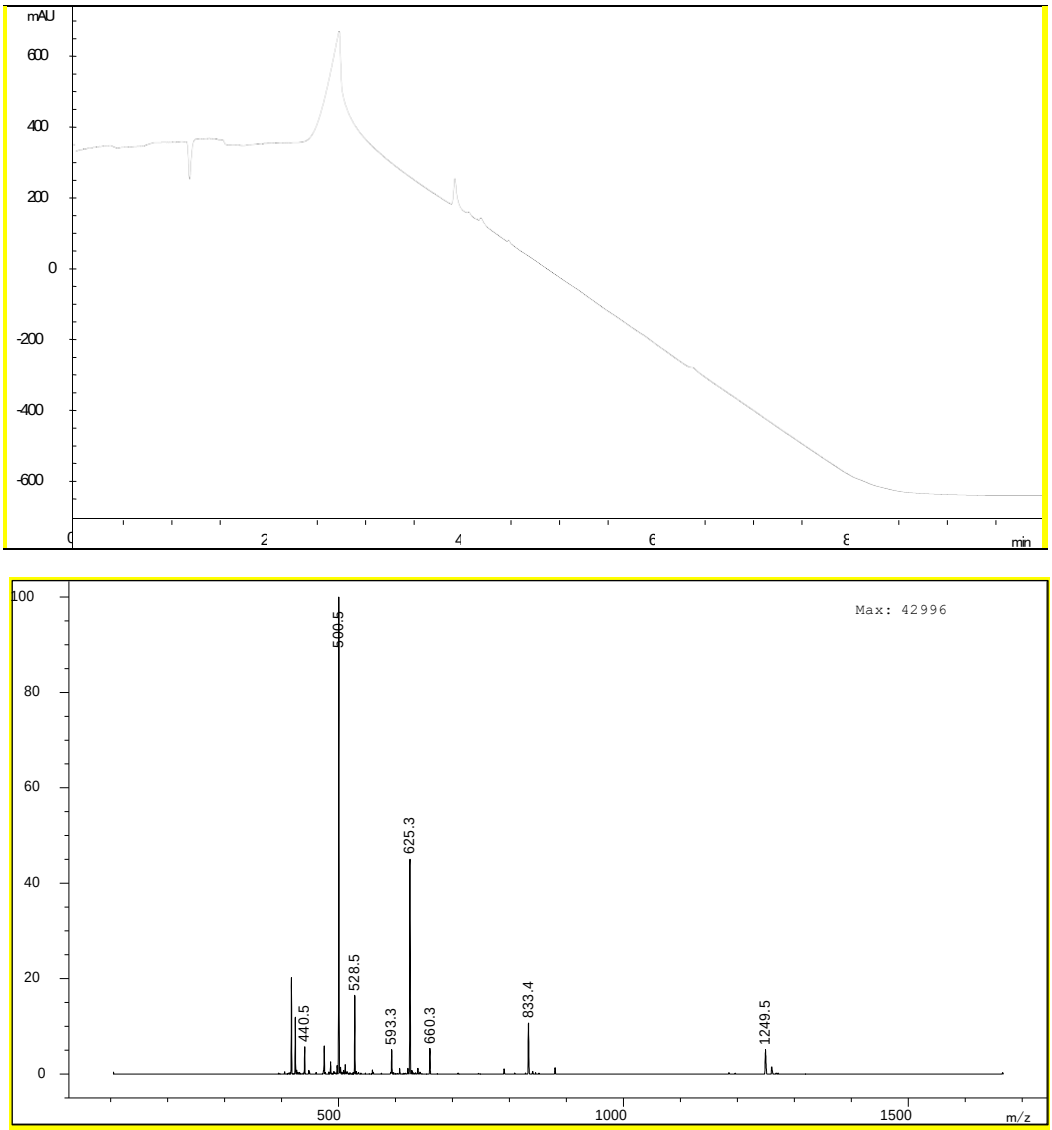
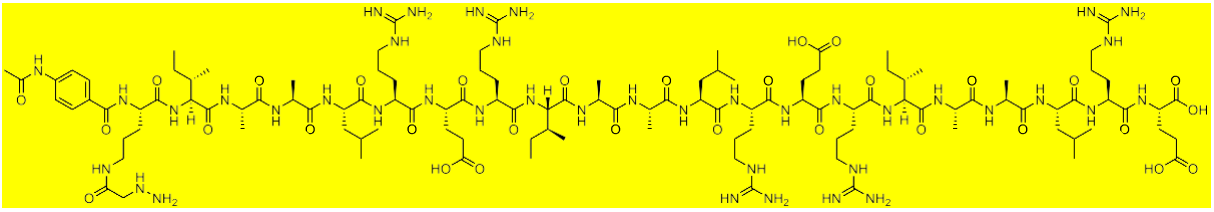


Figure SI 54: A) Structure of the K_{coil}-Orn_{Hy}-20 peptide. B) HPLC-MS trace of purified K_{coil}-Orn_{Hy}-20. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2496.52 Da. LC-ESI-MS m/z at tr= 3.91: $[M+2H]^{2+}/2$= 1249.5, $[M+3H]^{3+}/3$= 833.4, $[M+4H]^{4+}/4$= 625.3, $[M+5H]^{5+}/5$= 500.5

16.5 R_{coil}-Orn_{Hy}-1

A)



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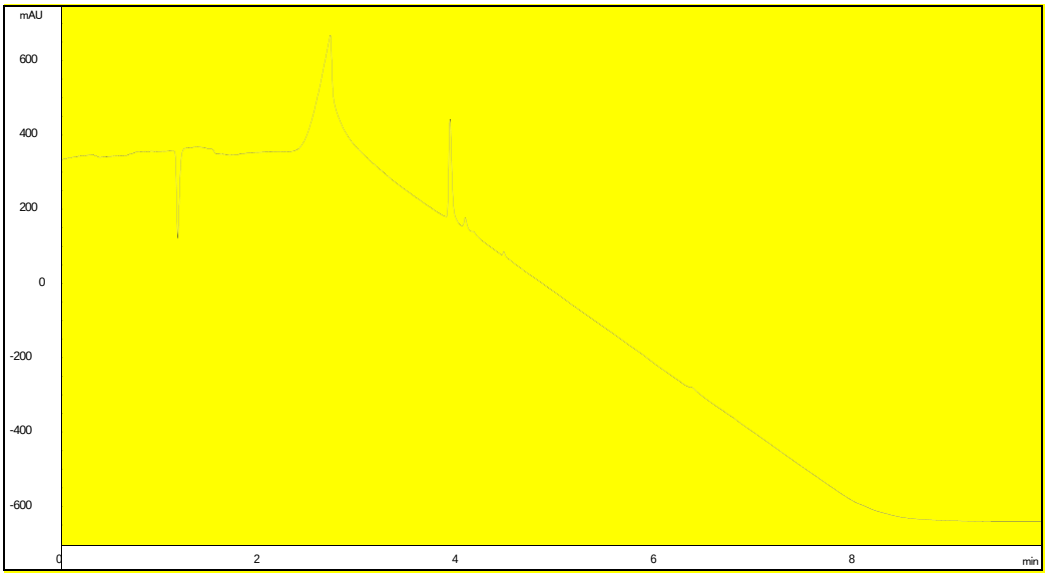


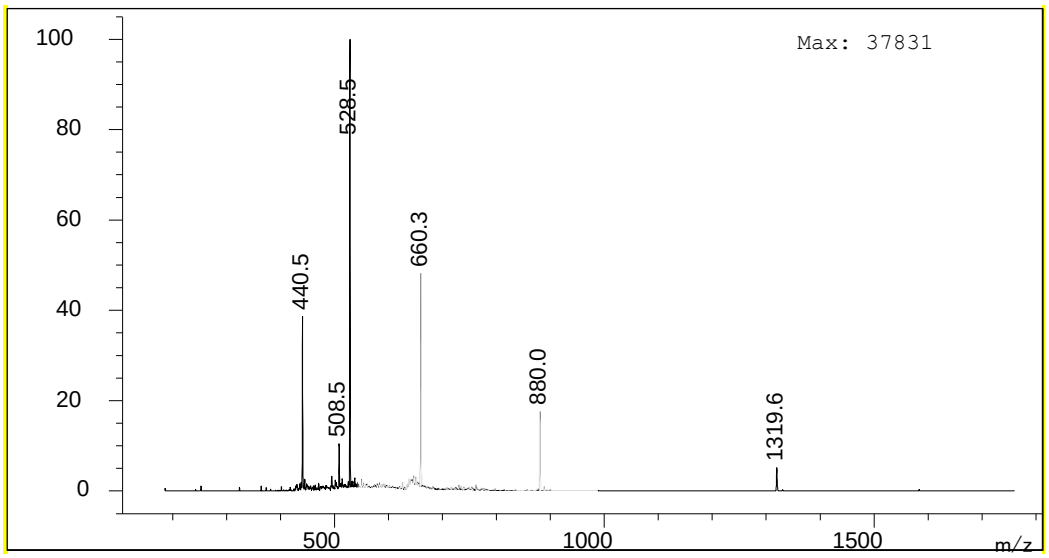
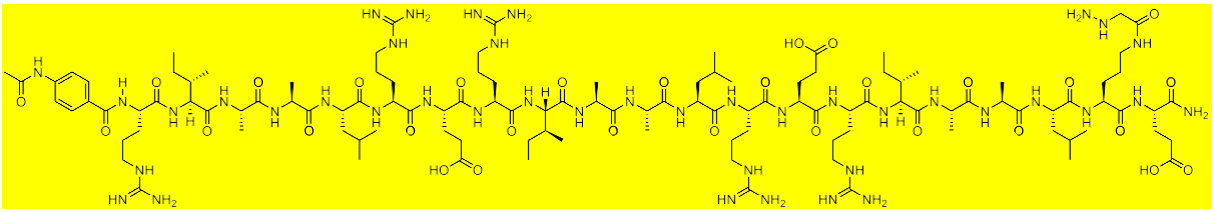


Figure SI 55: A) Structure of the R_{coil}-Orn_{Hy}-1 peptide. B) HPLC-MS trace of purified R_{coil}-Orn_{Hy}-1. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2636.55 Da. LC-ESI-MS m/z at tr= 3.90: $[M+2H]^{2+}/2$= 1319.6, $[M+3H]^{3+}/3$= 880.0, $[M+4H]^{4+}/4$= 660.3, $[M+5H]^{5+}/5$= 528.5, $[M+6H]^{6+}/6$= 440.5

16.6 R_{coil}-Orn_{Hy}-20

A)



B)

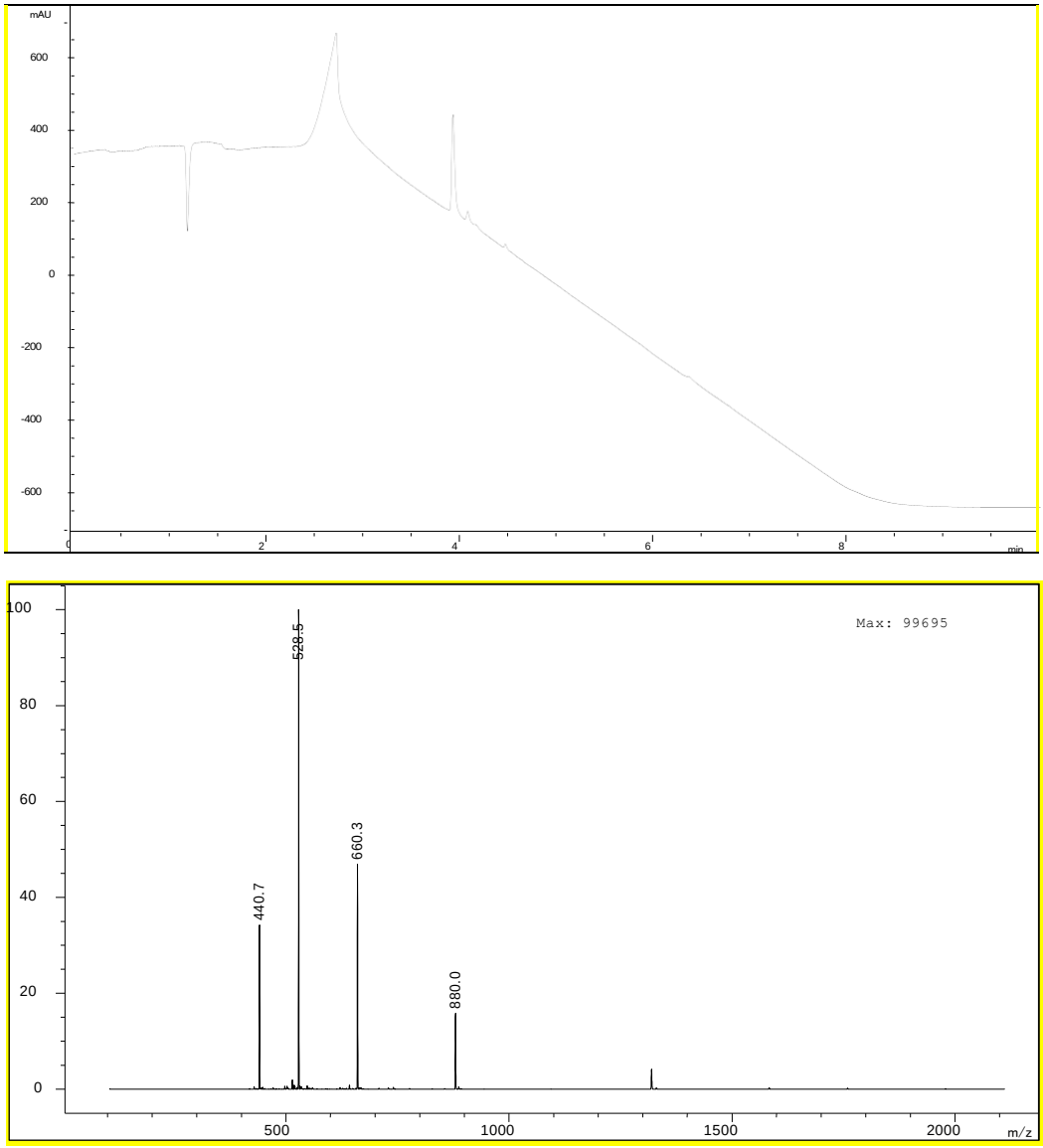


Figure SI 56: A) Structure of the R_{coil}-Orn_{Hy}-20 peptide. B) HPLC-MS trace of purified R_{coil}-Orn_{Hy}-20. HPLC-UV trace at 214 nm (top) and MS spectrum of the corresponding peak (bottom). Calcd MW: 2636.55 Da. LC-ESI-MS m/z at tr= 3.90: $[M+3H]^{3+}/3$= 880.0, $[M+4H]^{4+}/4$= 660.3, $[M+5H]^{5+}/5$= 528.5, $[M+6H]^{6+}/6$= 440.7

17E_{coil}-Orn_{DOP}-6 crosslink reactions with K_{coil}/R_{coil}-Orn_{Hy}-1/20

Before preparing the solutions, a small vial was filled with MQ water, which was then bubbled through with oxygen for 10 minutes. This solution was then used to prepare an air saturated buffer. Next, In a 1.5 mL Eppendorf vial, 200 µL of an air saturated buffered solution (phosphate buffered saline, 0.1 M NaCl, pH 7.4) containing 10 µM of the K_{coil}/R_{coil}-Orn_{Hy}-1/20 and 5 µM of E_{coil}-Orn_{DOP}-6 was prepared and allowed to react for 2h at 25°C, while stirring at 900 rpm. At different time points, 40 µL of sample were transferred to an Eppendorf tube and quenched with 8 µL of acetone.

17.1 K_{coil}-Orn_{Hy}-1 to E_{coil}-Orn_{DOP}-6 crosslinking

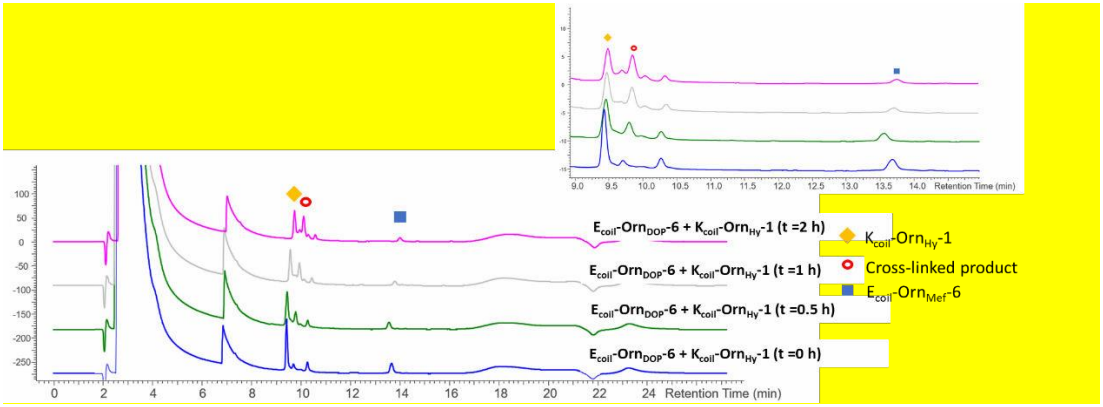


Figure SI 57: HPLC chromatograms of the reaction mixture (10 µM **K_{coil}-Orn_{Hy}-1** + 5 µM **E_{coil}-Orn_{DOP}-6**) recorded at 214 nm recorded at t = 0 (blue), t = 30 min (green), t = 1h (grey) and t = 2h (pink). The large signal around 3-4 min corresponds to the excess of acetone, which was added to quench the reaction.

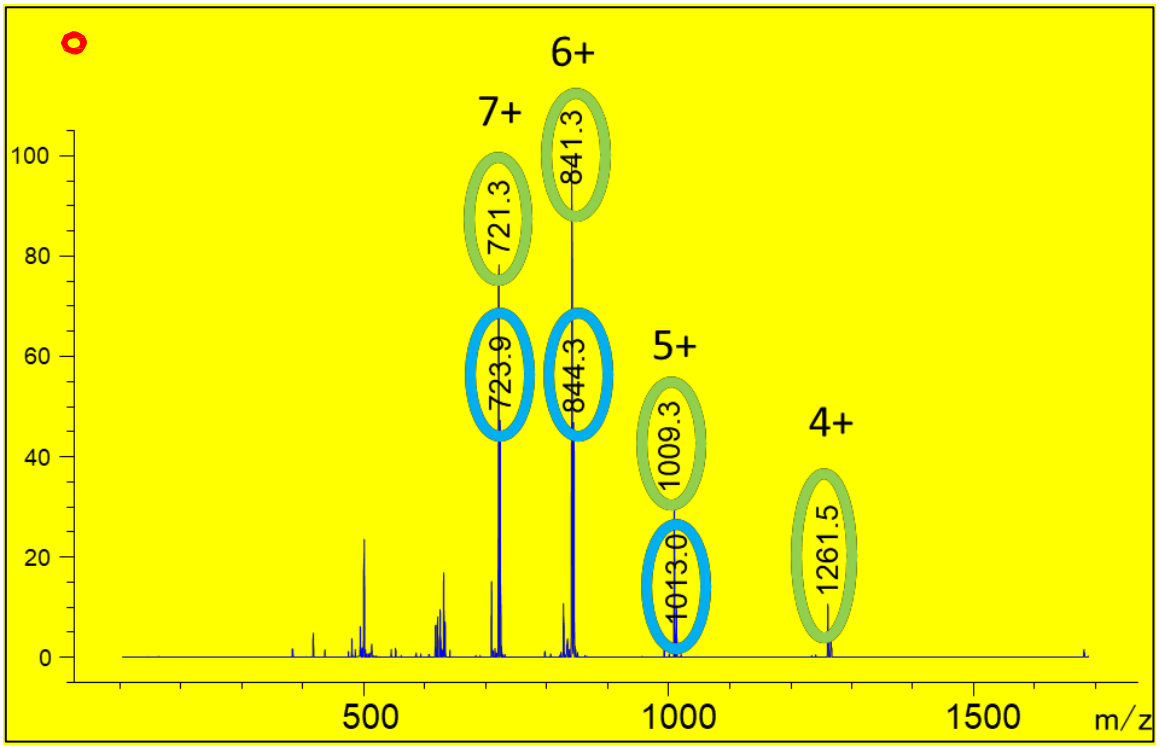


Figure SI 58: ESI-MS spectra of the peak corresponding to the crosslinked product (red dot). The green ovals indicate signals related to the crosslinked product (Calcd MW 5041.74 Da) of K_{coil}-Orn_{Hy}-1 + E_{coil}-Orn_{DOP}-6: Found MW (5042.0: $[M+4H]^{4+}$/4= 1261.5, $[M+5H]^{5+}$/5= 1009.3, $[M+6H]^{6+}$/6= 841.3, $[M+7H]^{7+}$/7= 721.3. The blue ovals indicate signals related to the hydrated form of the crosslinked product (Calcd MW 5059.8 Da): Found MW 5060.0: $[M+5H]^{5+}$/5= 1013.0, $[M+6H]^{6+}$/6= 844.3, $[M+7H]^{7+}$/7= 723.9.

17.2 K_{coil}-Orn_{Hy}-20 to E_{coil}-Orn_{DOP}-6 crosslinking

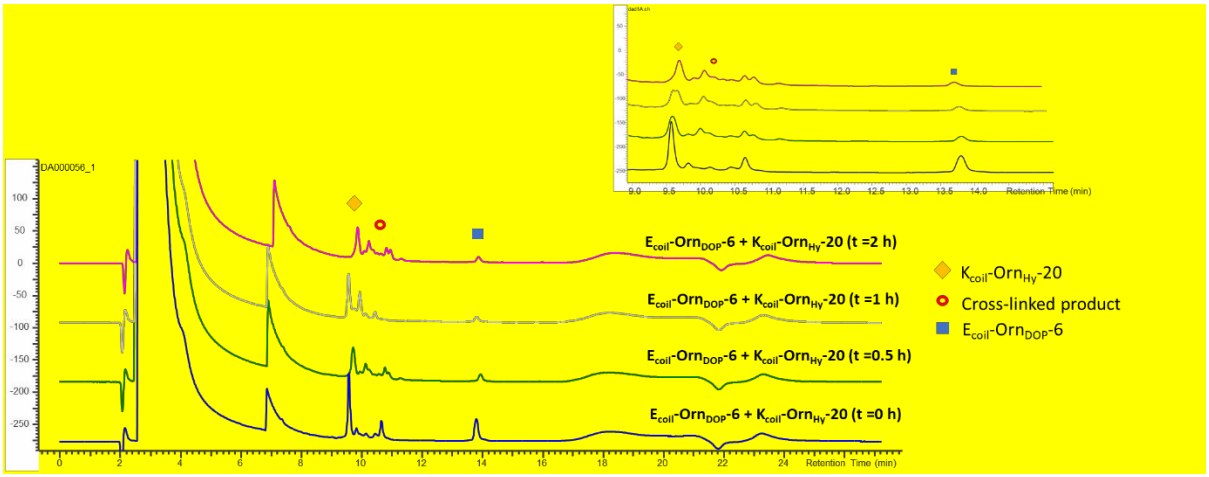


Figure SI 59: HPLC chromatograms of the reaction mixture (10 µM **K_{coil}-Orn_{Hy}-20**+ 5 µM **E_{coil}-Orn_{DOP}-6**) recorded at 214 nm recorded at t = 0 (blue), t = 30 min (green), t = 1h (grey) and t = 2h (pink).

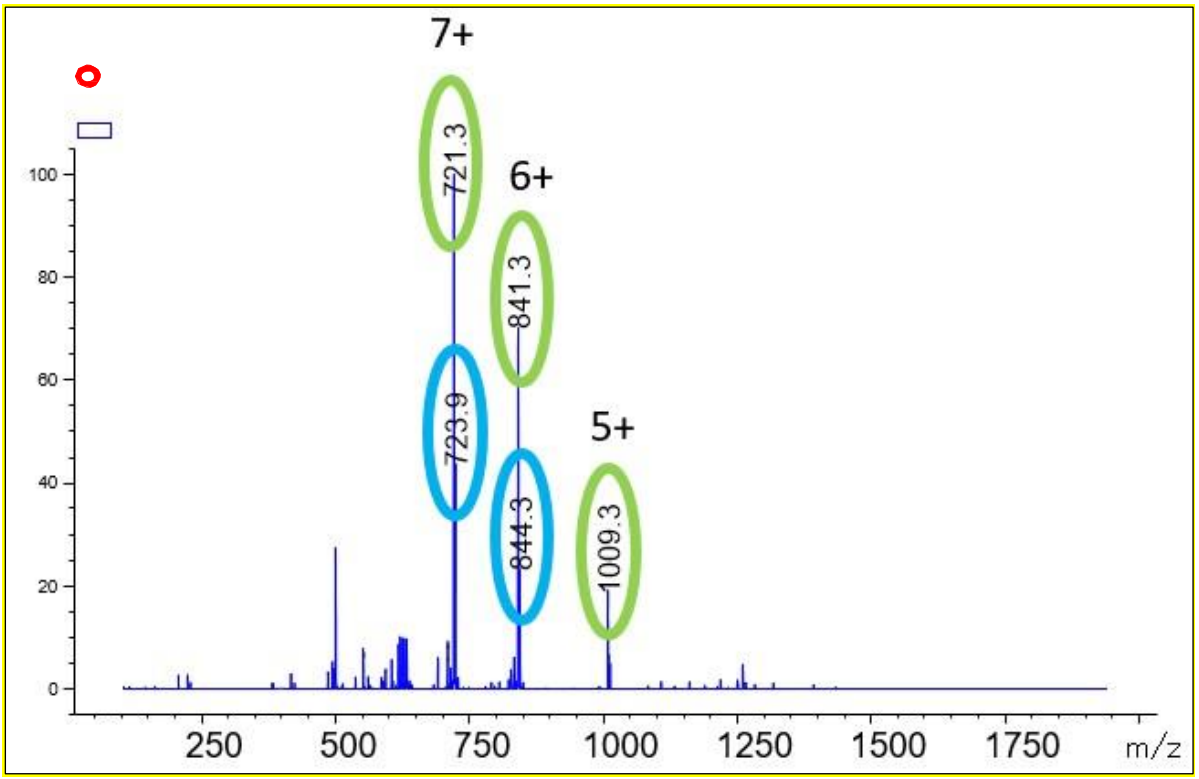


Figure SI 60: ESI-MS spectra of the peak corresponding to the crosslinked product (red dot). The green ovals indicate signals related to the crosslinked product (Calcd MW 5041.74 Da) of K_{coil}-Orn_{Hy}-20 + E_{coil}-Orn_{DOP}-6: Found MW (5042.0: $[M+5H]^{5+}/5$= 1009.3, $[M+6H]^{6+}/6$= 841.3, $[M+7H]^{7+}/7$= 721.3. The blue ovals indicate signals related to the hydrated form of the crosslinked product (Calcd MW 5059.8 Da): Found MW 5060.0: $[M+6H]^{6+}/6$= 844.3, $[M+7H]^{7+}/7$= 723.9.

17.3 R_{coil}-Orn_{Hy}-1 to E_{coil}-Orn_{DOP}-6 crosslinking

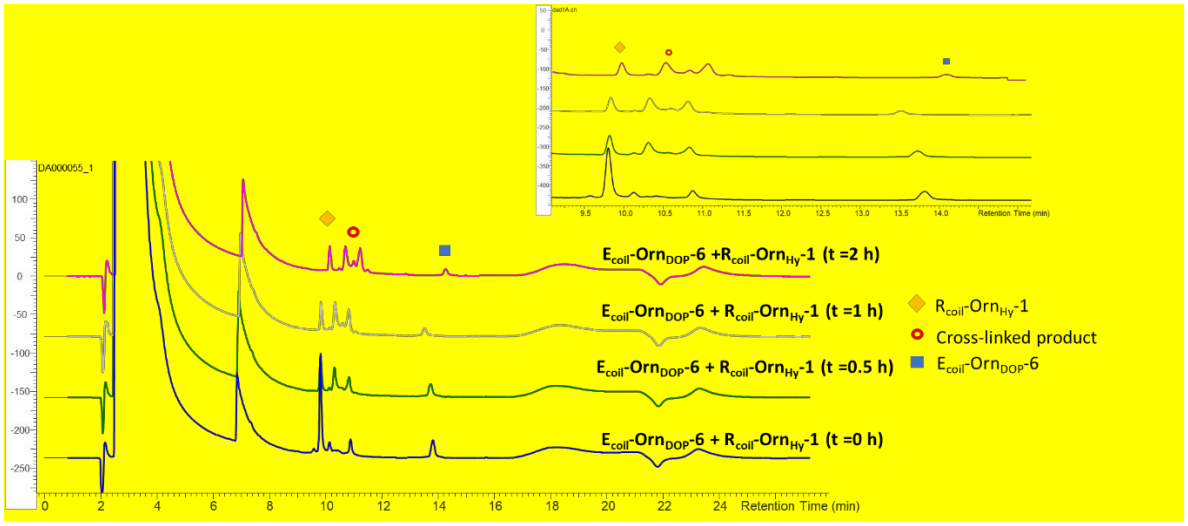


Figure SI 61: HPLC chromatograms of the reaction mixture (10 µM R_{coil}-Orn_{Hy}-**1** + 5 µM **E_{coil}-Orn_{DOP}-6**) recorded at 214 nm recorded at t = 0 (blue), t = 30 min (green), t = 1h (grey) and t = 2h (pink).

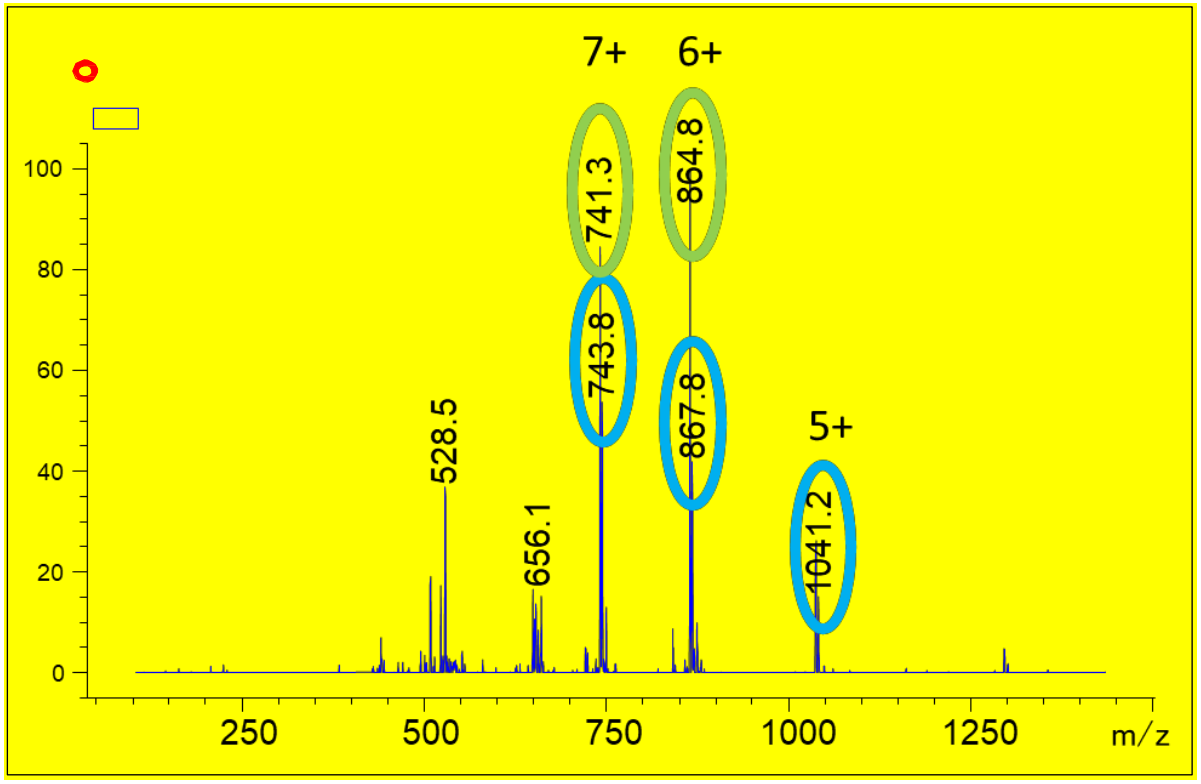


Figure SI 62: ESI-MS spectra of the peak corresponding to the crosslinked product (red dot). The green ovals indicate signals related to the crosslinked product (Calcd MW 5181.8 Da) of R_{coil}-Orn_{Hy}-1 + E_{coil}-Orn_{DOP}-6: Found MW (5182,8: $[M+6H]^{6+}/6$= 864.8, $[M+7H]^{7+}/7$= 741.3. The blue ovals indicate signals related to the hydrated form of the crosslinked product (Calcd MW 5199.9 Da): Found MW 5201.0: $[M+5H]^{5+}/5$= 1041.2, $[M+6H]^{6+}/6$= 867.8, $[M+7H]^{7+}/7$= 743.8.

17.4 R_{coil}-Orn_{Hy}-20 to E_{coil}-Orn_{DOP}-6 crosslinking

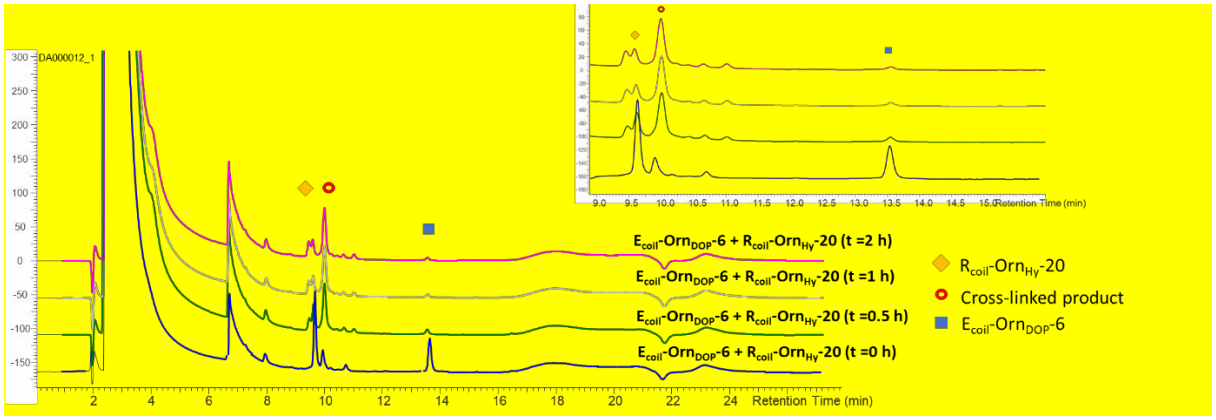


Figure SI 63: HPLC chromatograms of the reaction mixture (10 µM R_{coil}-Orn_{Hy}-**20** + 5 µM **E_{coil}-Orn_{DOP}-6**) recorded at 214 nm recorded at t = 0 (blue), t = 30 min (green), t = 1h (grey) and t = 2h (pink).

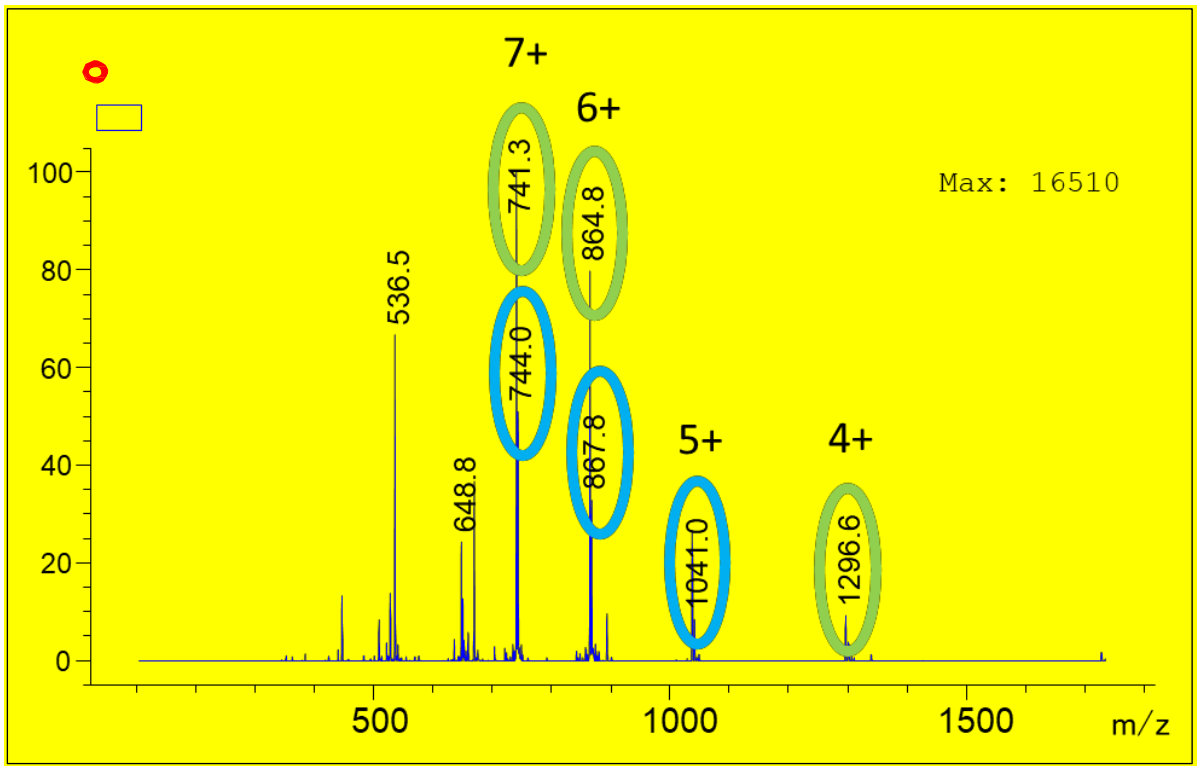


Figure SI 64: ESI-MS spectra of the peak corresponding to the crosslinked product (red dot). The green ovals indicate signals related to the crosslinked product (Calcd MW 5181.8 Da) of R_{coil}--20 + E_{coil}-Orn_{DOP}-6: Found MW (5182,8: $[M+4H]^{4+}/4$= 1296.6, $[M+6H]^{6+}/6$= 864.8, $[M+7H]^{7+}/7$= 741.3. The blue ovals indicate signals related to the hydrated form of the crosslinked product (Calcd MW 5199.9 Da): Found MW 5201.0: $[M+5H]^{5+}/5$= 1041.2, $[M+6H]^{6+}/6$= 867.8, $[M+7H]^{7+}/7$= 744.0.

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