Supporting Information

**Calcium ions as bioinspired triggers to reversibly control the coil to helix transition in peptide-polymer conjugates**

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**Corresponding Author:** Hans G. Börner

**Other Authors:** Romina I. Kühnle, Denis Gebauer

*to whom correspondence is to be addressed

Prof. Dr. Hans G. Börner: Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany

E-Mail: h.boerner@hu-berlin.de

Phone: +49 (0)30-2093 7348

Fax: +49 (0)30 2093-7266
Materials: N-α-Fmoc protected amino acids and derivatives Fmoc-Glu(tBu) OH, Fmoc-Gly OH, Fmoc-Leu OH, Fmoc-\(\rho\)Phe OH (\(\nu\)Phe), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), N-Hydroxybenzo-triazole (HOBT), (Benztotriazol-1-ylloxy) tripyrroolidinophosphoniumhexa-fluorophosphate (PyBOP), tri-methylsilylbromide (TMSBr) and N-methyl-2-pyrroolidone (NMP, 99.9+ %, peptide synthesis grade) were used as received from IRIS Biotech GmbH (Marktredwitz, Germany). TentaGel PAP Resin (PEG attached peptide resin, loading: 0.27 mmol/g; \(M_w=3000\)) and TentaGel N NH2 resin (PEG attached peptide resin, loading 0.54 mmol/g; \(M_w=850\)) were obtained from Rapp Polymere GmbH (Tübingen, Germany). \(N, N\)-Diisopropylethylamin (DIPEA; Acros, peptide grade), piperidine (Acros, peptide grade), \(N, N\)-diisopropylcarbodiimide (DIC; Fluka, 99%) and methanol (Biosolve, HPLC grade) have been applied as received. Trifluoracetic acid (TFA; Acros, peptide grade) was distilled prior to use. Dichloromethane (DCM, IRIS Biotech GmbH, peptide grade) was distilled from CaH2 prior to use. Ethylene glycol tetraacetic acid (EGTA, Carl Roth GmbH, Karlsruhe, Germany) was applied as received. All other reagents were used as received from Aldrich without further purification.
Instrumentation

Mass spectrometry (MALDI-TOF MS) was performed on a Bruker Reflex III workstation with matrix-assisted laser desorption/ionization and time of flight detector. The samples were dissolved in 0.1 % TFA in acetonitrile-water (1:1, v/v) with a final concentration of 0.25 mg/mL. One µL of the sample solutions was mixed with one µL of the matrix solution consisting of 10 mg of α-cyano-4-hydroxy-cinnamic acid dissolved in 0.3 % TFA in acetonitrile-water (1:1, v/v) on the sample plate and air-dried at ambient temperature. Measurements were performed in reflectron positive mode for the peptide, in linear negative mode for conjugate I and in linear positive mode for conjugate II at an acceleration voltage of 20 kV with 100 shots per sample.

¹H nuclear magnetic resonance spectra (NMR) were recorded on a Bruker AV 500 spectrometer at 500 MHz in TFA-d₁ at room temperature.

Circular dichroism UV spectra (CD) were recorded on a Jasco J-710 CD spectrometer in solution, using a sample concentration of 0.17 mg in 1 mL TRIS/HCl buffer (0.1 M pH 8.9) in a sample cell with 1.0 mm width.

Fourier transformation infrared spectroscopy (FT-IR) was performed on a Jasco FTIR 4200 spectrometer. Samples were measured as solid on an ATR crystal.

Calcium binding studies: The utilized commercial Metrohm titration setup and experimental procedure are described in detail elsewhere (D. Gebauer, A. Völkel, H. Cölfen, Science 2008, 322, 1819). Dilute calcium chloride solution (10 mM, prepared by 1:10 dilution from 0.1 M standard solution, Metrohm No. 6.2301.070) was dosed into 20 mL Millipore water set to pH 8.5 at a rate of 10 µL/min under nitrogen flow. Upon addition of the calcium solution, the pH was kept constant by means of titration utilizing 10 mM NaOH solution as the calcium solution is slightly acidic (~pH 5). The calcium solution was added for ~700 s (final Ca²⁺ concentration ~60 µM), and the calcium potential was read-out every 10 s. This data was used for calibration of the electrode. Three samples of 20 mL Millipore water containing peptide (0.20 mg/mL), conjugate II (0.20 mg/mL) and conjugate I (0.18 mg/mL), respectively, were pre-set to pH 8.5 and measured analogously. In all cases, calcium solution was added for ~2500 s (final Ca²⁺ concentration ~190 µM). The calcium potential was read-out every 10 s and converted into calcium concentrations utilizing the calibration data. Activity corrections are not required owing to the minor ionic concentrations (cf. D. Gebauer, H. Cölfen, A. Verch, M. Antonietti, Adv. Mater. 2009, 21, 435).
Synthesis of the peptide

Ac-Leu-Leu-Glu-Glu-Leu-Leu-Glu-Glu-Leu-Leu-Glu-Leu-Glu-Glu-nPhe-Gly-COOH

The coupling of standard Fmoc-amino acid derivatives was performed on an Applied Biosystems ABI 433a peptide synthesizer, in NMP as solvent, following ABI-Fastmoc protocols (double coupling, no capping) and using a 2-Chlortritylchloride resin preloaded with Fmoc-Gly (loading 0.32 mmol/g, 0.1 mmol) as solid support. Fmoc amino acid coupling was facilitated by HBTU/DIPEA. After final Fmoc removal and acetylation, the resin was carefully washed with DCM and dried at 30 °C. Subsequent 1 h treatment of the resin with TFA-DCM (1:1, v/v) resulted in cleavage of the fully deprotected peptide-from the support. The peptide was isolated by diethyl ether precipitation and centrifugation, followed by lyophilization from water. A yield of 89 % was obtained without further purification.

Analysis of the peptide: 

\[ \delta = 1.03 \text{ (m, 42 H, -CH(CH}_3)^2, Leu), 1.72 \text{ (m, 14 H, -CH}_2-\text{CH(}} \text{CH}_3)^2, \text{Leu), 1.83 \text{ (m, 7 H, -CH(}} \text{CH}_3)^2, \text{Leu), 2.30 \text{ (m, 16 H, -CH}_2-\text{CH}_2-\text{COOH, Glu), 2.42 \text{ (s, 3 H, -N-CO-CH}_3), 2.71 \text{ (m, 16 H, -CH}_2-\text{CH}_2-\text{COOH, Glu), 3.40 \text{ (m, 2H, -CH}_2-\text{Ph, nPhe), 4.29 \text{ (d, 2H, -C}_a^\text{H}_2-, \text{Gly), 4.85 \text{ m, 15 H, -C}_a^\text{H}-, \text{Leu and Glu), 5.21 \text{ m, 1H, -C}_a^\text{H}-, nPhe), 7.58 \text{ (d, 2H, CH}_\text{ar} \text{ ortho, nPhe), 8.32 \text{ (d, 2H, CH}_\text{ar} \text{ meta, nPhe). FT-IR (cm}^{-1}: 1649 \text{ (s), 1539 \text{ (s), 1346 \text{ (s). Maldi-TOF-mass spectrometry: Peak signal: m/z 2157 corresponding to [M+Na}^+ (M_{th}=2134).} \]
Synthesis of the conjugates

Ac-Leu-Leu-Glu-Glu-Leu-Leu-Glu-Glu-Leu-Leu-Glu-Glu/Glu-Leu-Leu-Glu-Glu-nPhe-Gly-NH-PEO_{72}

The coupling of standard Fmoc-amino acid derivatives was performed on an Applied Biosystems ABI 433a peptide synthesizer, in NMP as solvent, following ABI-Fastmoc protocols (double coupling, no capping) and using a TentaGel® PAP resin (loading 0.27 mmol/g, 0.1 mmol) as solid support. Fmoc amino acid coupling was facilitated by HBTU/DIPEA. After final Fmoc removal and acetylation, the resin was carefully washed with DCM and dried at 30 °C. Subsequent 6 h treatment of the resin with TFA-TMSBr (99:1, v/v) resulted in cleavage of the fully deprotected peptide-PEG-conjugate from the support. The peptide-PEG-conjugate was isolated by diethyl ether precipitation and centrifugation, followed by lyophilization from water. A yield of 86 % was obtained without further purification.

Analysis of I: $^1$H-NMR (500 MHz; TFA-d$_1$) [ppm]: $\delta$ = 0.95 (m, 30 H, -CH(CH$_3$)$_2$, Leu), 1.65 (m, 14 H, -CH$_2$-CH(CH$_3$)$_2$, Leu), 1.75 (m, 7 H, -CH(CH$_3$)$_2$, Leu), 2.22 (m, 16 H, -CH$_2$-CH$_2$-COOH, Glu), 2.34 (s, 3 H, -N-CO-CH$_3$), 2.64 (m, 16 H, -CH$_2$-CH$_2$-COOH, Glu), 3.24 (m, 1H, -CH$_2$-Ph, nPhe), 3.41 (m, 1H, -CH$_2$-Ph, nPhe), 3.92 (s, 299 H, -O-CH$_2$-CH$_2$-O-, PEO), 3.65 (m, 2H, -CH$_2$-Ph, nPhe), 4.69 (m, 7 H, -CH$_2$-Ph, nPhe), 4.81 (m, 8 H, -CH$_2$-Ph, nPhe), 5.14 (t, 1H, -CH$_2$-Ph, nPhe), 7.50 (d, 2H, CH$_2$ ortho, nPhe), 8.23 (d, 2H, CH$_2$ meta, nPhe). FT-IR (cm$^{-1}$): 1653 (s), 1546 (m), 1342 (s), 1102 (s). Maldi-TOF-mass spectrometry: Peak signal: m/z 4979 corresponding to [M-O-H]$^-$ (M$_{th}$ = 4996) (cf. S.I. Figure S1).
Figure S1. MALDI-TOF mass spectrum of conjugate I confirms the successful synthesis of I by showing a mass distribution centered at m/z 4979 (M₀ = 4996). In source loss of oxygen of the nitrobenzyl moiety of p-nitro phenylalanine due to photodeoxygenation as earlier reported by Nielsen et al. (Letters in Peptide Science 1995, 2, 301-305). Characteristic spacing of 44 Da between adjacent signals corresponding to one ethylene oxide unit of the polyethylene oxide chain. n represents the number of ethylene oxide repeating units in the polymer chain.

Synthesis of II:
Ac-Leu-Leu-Glu-Glu-Leu-Glu-Glu-Leu-Glu-Glu-Leu-Glu-Glu-nPhe-Gly-NH-PEO₁₉
The coupling of standard Fmoc-amino acid derivatives was performed on an Applied Biosystems ABI 433a peptide synthesizer in NMP as solvent, following ABI-Fastmoc protocols (double coupling, no capping) and using a TentaGel® N NH₂ resin (loading 0.54 mmol/g, 0.1 mmol) as solid support. Fmoc amino acid coupling was facilitated by HBTU/DIPEA. After final Fmoc removal and acetylation, the resin was carefully washed with DCM and dried at 30 °C. Subsequent 6 h treatment of the resin with TFA-TMSBr (99:1, v/v) resulted in cleavage of the fully deprotected peptide-PEG-conjugate from the support. The peptide-PEG-conjugate was isolated by diethyl ether precipitation and centrifugation, followed by lyophilization from water. A yield of 85 % was obtained without further purification.

Analysis of II: ¹H-NMR (500 MHz; TFA-d₁) [ppm]: δ = 1.00 (m, 30 H, -CH(CH₃)₂, Leu), 1.75 (m, 21 H, -CH(CH₃)₂ and -CH₂-CH(CH₃)₂, Leu), 2.28 (m, 16 H, -CH₂-CH₂-COOH, Glu), 2.39 (s, 3 H, -N-CO-CH₃), 2.68 (m, 10 H, -CH₂-CH₂-COOH, Glu), 3.28 (m, 1H, -CH₂-Ph, nPhe), 3.46 (m, 1H, -CH₂-Ph, nPhe), 3.97 (s, 87 H, -O-CH₂-CH₂-O-, PEO), 4.10 (d, 2H, -CαH₂-, Gly), 4.73 (m, 7H, -CαH₂-, Leu), 4.86 (m, 8H, -CαH-, Glu), 5.19 (m, 1H, -CαH-, nPhe), 7.55 (d, 2H, CH₃ ortho, nPhe), 8.29 (d, 2H, CH₃ meta, nPhe). FT-IR (cm⁻¹): 1651 (s), 1541 (m), 1346 (s), 1092 (s). Maldi-TOF-mass spectrometry: Peak signal: m/z 2993 corresponding to [M+Na]⁺ (M₀=2970) (cf. S.I. Figure S2).
**Figure S2.** MALDI-TOF mass spectrum of conjugate II. Characteristic distribution with signal to signal distances of 44 Da corresponding to one ethylene oxide unit of the polyethylene oxide block. Signal corresponding to [M+Na]$^+$ can be found at m/z 2993 (M$_n$=2970). n represents the number of ethylene oxide repeating units in the polymer chain.

**Figure S3.** FT-IR-spectra of the carbonyl and finger print region of the peptide, conjugate I and conjugate II showing the typical amide I and II bands (conjugate I: 1653 (s) and 1546 (m); conjugate II: 1651 and 1541 cm$^{-1}$) as well as the C-O-stretch vibration of conjugated PEO (conjugate I: 1102 cm$^{-1}$; conjugate II: 1092 cm$^{-1}$).
Figure S4. CD spectra of the peptide (c=0.17 mg/mL in TRIS/HCl buffer pH 8.9) prior and after calcium ion addition.

Figure S5. Raw data of calcium detected in solutions containing the peptide, conjugate I and conjugate II. The reference line gives the expected development without binding of calcium. At first glance, the data indicate a minor initial amount of calcium present in the solutions. However, the presence of calcium in the peptide samples is excluded; thus, the offset and the flatter slope of the curves measured in the presence of the conjugates indicate interactions of the peptide conjugates with the membrane of the calcium ion-selective electrode that result in interference that changes calibrated electrode slope and intercept. In order to determine the calcium binding of the different peptides, the data of the samples containing peptide conjugates were offset corrected.
Figure S6. Offset-corrected amount of calcium detected in solutions containing the peptide (a), conjugate II (b) and conjugate I (c). The curves are linear when the calcium binding sites are saturated, and extrapolation of the linear part to the abscissa gives the amount of calcium bound to the respective peptide conjugates. With the molecular weights, we obtain a binding capacity of ~0.2-0.3 calcium ions bound per molecule in all cases. Since the slopes of the linear parts of the curves are flatter than the reference by ~10% (cf. Figure S5), owing to interactions of the conjugates with the electrode’s membrane, the binding capacities are systematically underestimated by ~10%. We estimate the relative error of the determined binding capacities to be of a similar magnitude.
Figure S7. CD spectra of I (c=0.17 mg/mL in TRIS/HCl buffer pH 8.9) after addition of 5 eq Ba\(^{2+}\), Mg\(^{2+}\) and respectively Ca\(^{2+}\) per COOH of the peptide segment. Neither barium nor magnesium has the power of inducing an effective coil-to-helix transition at this concentration.

Figure S8. CD spectra of II (c=0.17 mg in TRIS/HCl buffer pH 8.9) prior and after addition of increasing equivalents Ca\(^{2+}\) per carboxylic acid side chain of the peptide segment.
Figure S9. Cycling of II (random coil without Ca\textsuperscript{2+}, α-helix after Ca\textsuperscript{2+} addition and return to random coil structure after addition of EGTA\textsuperscript{4-}). Owing to the content of the additive EGTA the absorption under 200 nm reaches cut off for the respective spectra.