Electronic Supplementary Material (ESI)

Functional stapled fragments of human preptin of the minimized length

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peptide **1**

peptide **2**

peptide **3**

Scheme S1. Continued on the next page

peptide **4**

peptide **5**

peptide **6**

Scheme S1. Studied preptin fragments (peptides **1** and **2**) and their stapled derivatives (peptides **3-6**).

Triazole ring staples

C$_4$-azide based

4-1

C$_3$-azide based

3-1

1-4

1-3
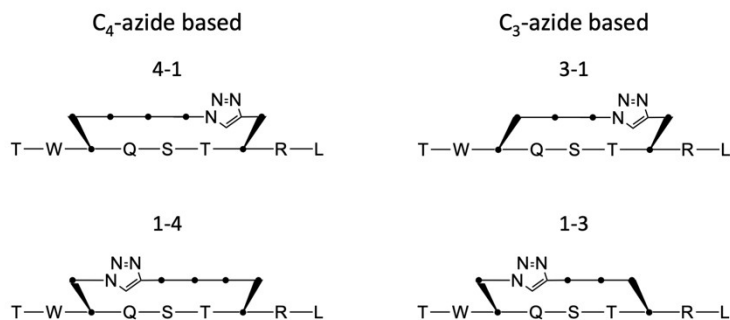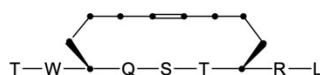
Olefine staples

Figure S1. Tested links within the computational design of stapled peptides. We considered only the (*S*, *S*) configurations on both amino acids connected by the triazole or the olefin staple (3 and 7).

Table S1. The helical content (in %) in all considered stapled peptides from Figure S1 during 100 ns long MD simulations. All simulations started from ideal α-helical conformations (ϕ=-52°, ψ=-53)

C$_3$ azide based		C$_4$ azide based	
1-3	3 %	1-4	22 %
3-1	15 %	4-1	22 %

Scheme S2. Synthetic scheme for the preparation of stapled peptides **4-6** by a ring-closing olefin metathesis reaction (RCM). Peptide 6 does not contain N-terminal threonine. SPPS means solid phase peptide synthesis. Amino acids are shown in single letter codes (T, W, Q, S, R and L). Black dot is Rink Amide resin. Amide means C-terminal carboxamide. Configuration (S) of Cα atoms of non-standard amino acids is also shown.
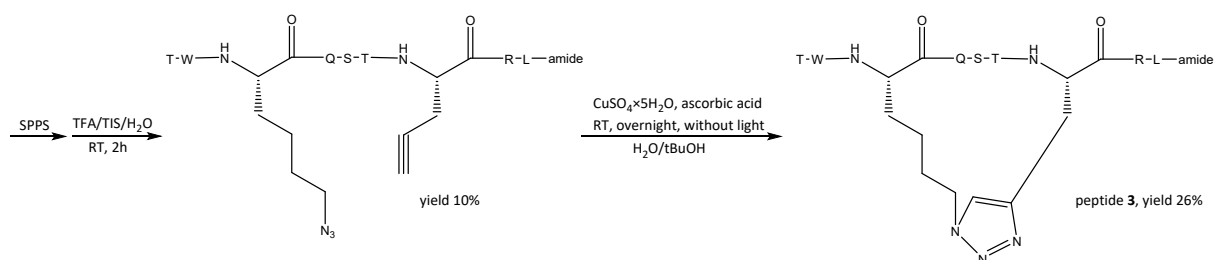
Scheme S3. Synthetic scheme for the preparation of stapled peptide **3** by a Cu[(I)]catalysed cycloadditon of alkyne and azide. SPPS means solid phase peptide synthesis. Amino acids are shown in single letter codes (T, W, Q, S, R and L). Amide means C-terminal carboxamide. Configuration (S) of Cα atoms of non-standard amino acids is also shown.

Table S2. The helical content (in %) in studied peptides as provided by MD simulations or CD spectroscopy.

% helix	10 ns MD	100 ns MD	from CD
peptide **1**	8	7	0
peptide **2**	6	0	0
peptide **3**	45	22	9
peptide **4**	18	7	0
peptide **5**	44	11	8
peptide **6**	17	8	0
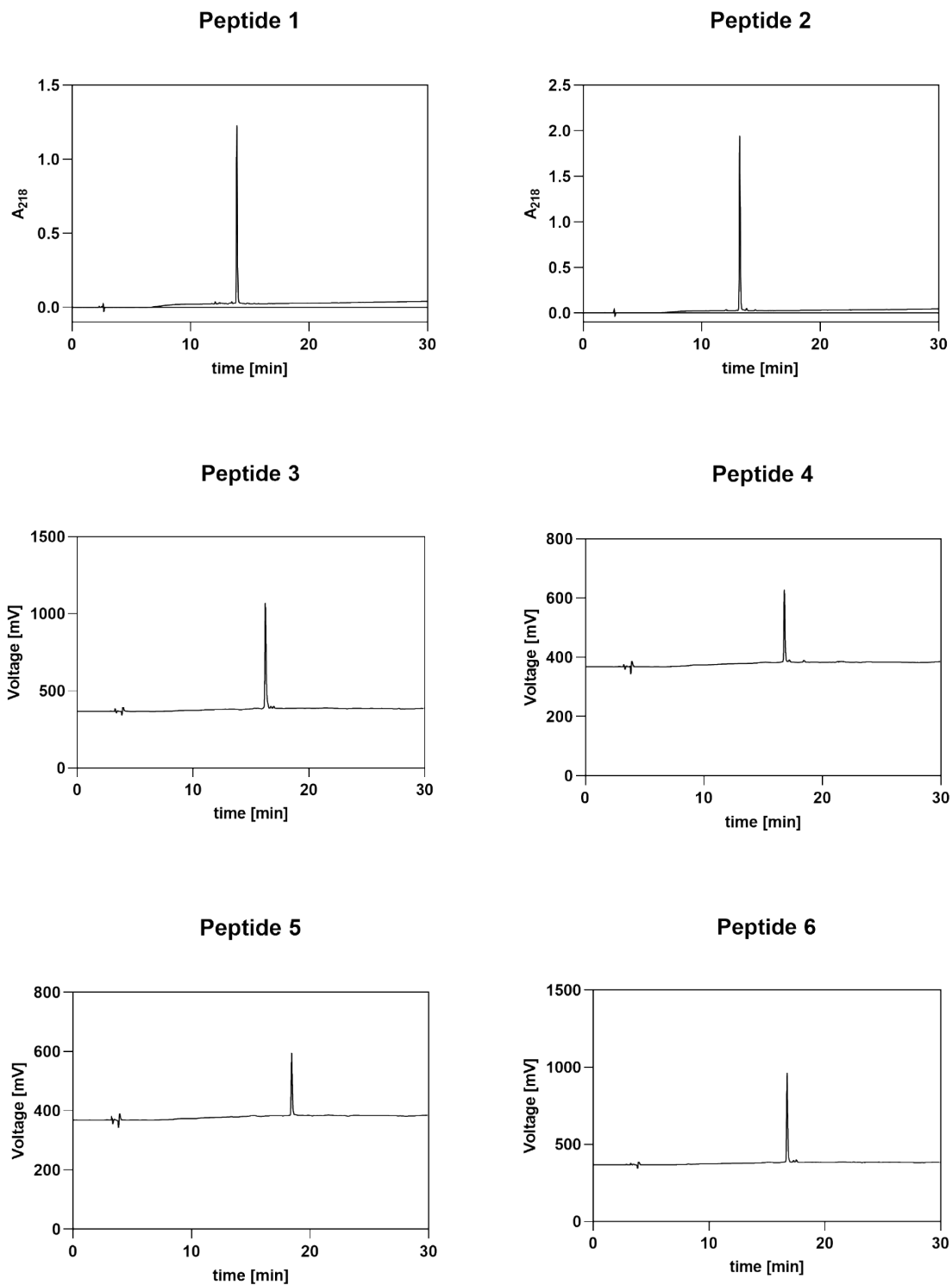
Figure S2. Analytical RP-HPLC profile of purified peptides **1**-**6**.

Figure S3. HR-MS of human preptin.

061221_servisHR_40_XT_00001_M_ #1 RT: 1.00 AV: 1 NL: 2.91E6
T: FTMS + p ESI Full ms [200.00-2000.00]

1145.63258
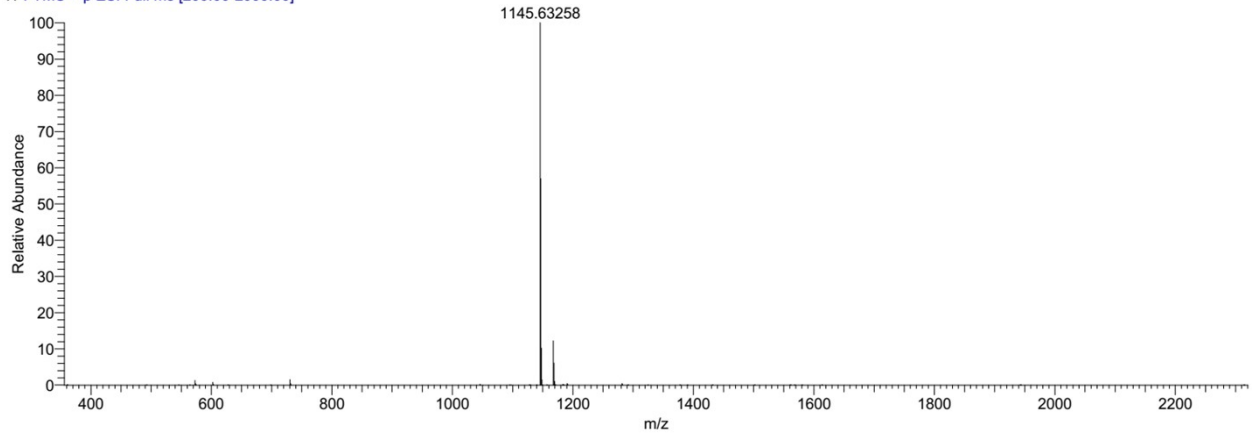
Figure S4. HR-MS of peptide **1**.

061221_servisHR_27_211206124736_XT_00001_M_ #1 RT: 1.00 AV: 1 NL: 3.91E5
T: FTMS + p ESI Full ms [200.00-2000.00]

1044.58327

Figure S5. HR-MS of peptide **2**.

141220_servisHR_+14_XT_00001_M_ #2 RT: 2.00 AV: 1 SB: 2 1.00 , 1.00 NL: 7.99E7
T: FTMS + p ESI Full ms [200.00-2000.00]

1138.59680

441.21302   806.43964   1103.55567   1258.62326   1588.71407   1949.95178   2278.20130
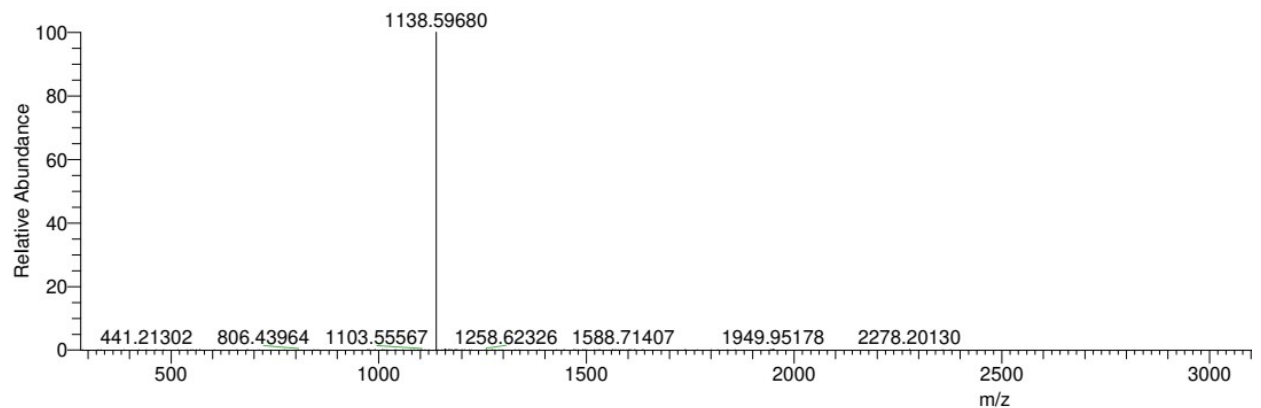
Figure S6. HR-MS of peptide **3**.

141220_servisHR_+12_XT_00001_M_ #2 RT: 2.00 AV: 1 SB: 2 1.00 , 1.00 NL: 1.71E8
T: FTMS + p ESI Full ms [200.00-2000.00]

Figure S7. HR-MS of peptide **4**.

141220_servisHR_+13_XT_00001_M_ #2 RT: 2.00 AV: 1 SB: 2 1.00 , 1.00 NL: 6.29E7
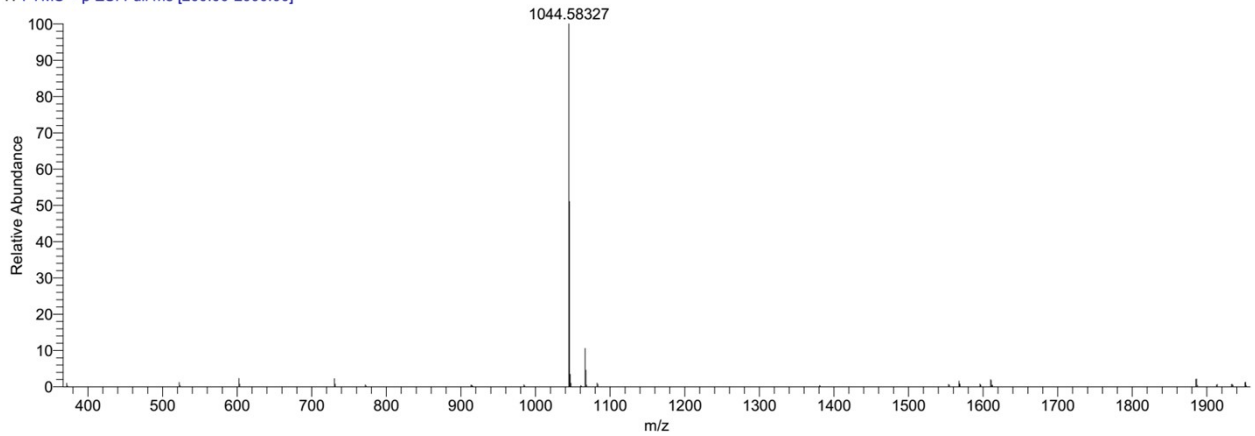T: FTMS + p ESI Full ms [200.00-2000.00]
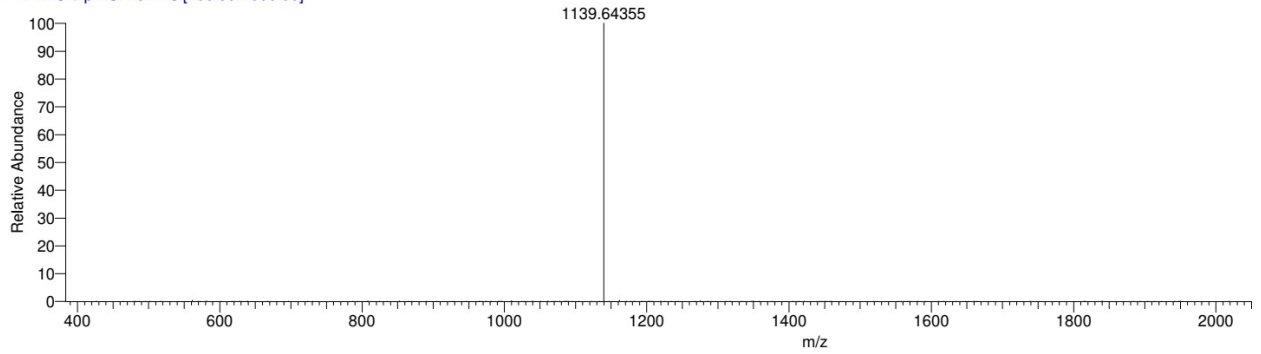
Figure S8. HR-MS of peptide **5**.
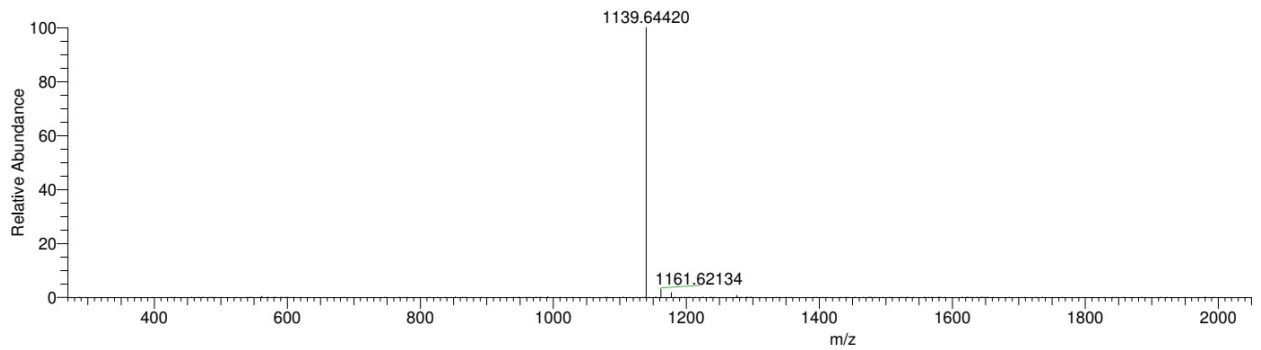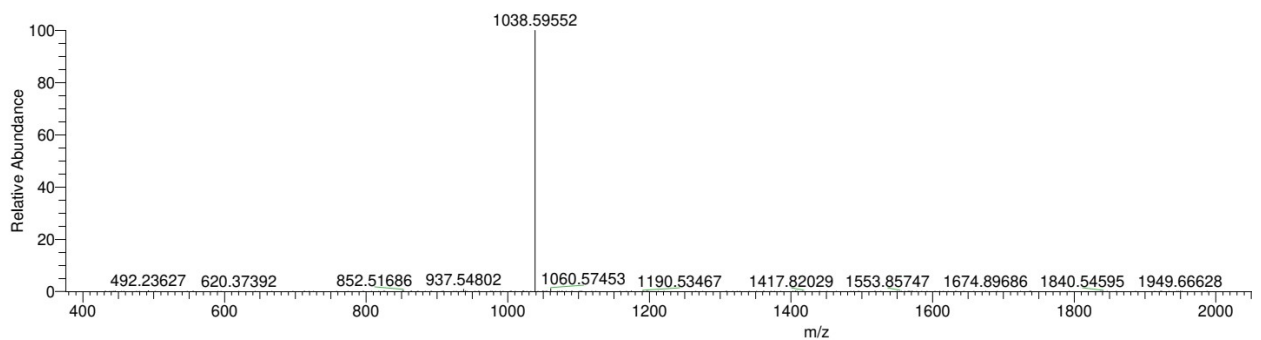

141220_servisHR_+11_XT_00001_M_ #2 RT: 2.00 AV: 1 NL: 3.14E7
T: FTMS + p ESI Full ms [200.00-2000.00]

Figure S9. HR-MS of peptide **6**.

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Table S3. Proton chemical shifts of peptide **1** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

Residue	NH	Hα	Hβ	Hγ	Others
Thr-1	*a*	3.89	4.16	1.29	
Trp-2	8.76	4.70	3.28; 3.25		10.11 (N1H), 7.27 (H2), 7.60 (H4), 7.23 (H5), 7.14 (H6), 7.48 (H7)
Lys-3	8.03	4.13	1.67	1.22	1.56 (Hδ), 2.91 (Hε)
Gln-4	8.14	4.15	2.02; 1.64	2.32	7.50; 6.87 (NεH$_2$)
Ser-5	8.39	4.47	3.92; 3.86		
Thr-6	8.17	4.33	4.25	1.19	
Gln-7	8.30	4.31	2.07; 1.97	2.34	7.50; 6.84 (NεH$_2$)
Arg-8	8.37	4.32	1.83; 1.75	1.61	3.18 (Hδ), 7.17 (NεH)
Leu-9	8.25	4.33	1.67	1.61	0.92 (Hδ1), 0.86 (Hδ2), 7.59; 7.04 (CONH$_2$)

a not detected (fast exchange with water).

Table S4. Proton chemical shifts of peptide **2** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

Residue	NH	Hα	Hβ	Hγ	Others
Trp-1	*a*	4.31	3.40; 3.35		10.21 (N1H), 7.29 (H2), 7.57 (H4), 7.25 (H5), 7.14 (H6), 7.50 (H7)
Lys-2	8.32	4.24	1.72	1.31	1.64 (Hδ), 2.95 (Hε)
Gln-3	8.37	4.20	2.04; 1.97	2.36	7.51; 6.88 (NεH$_2$)
Ser-4	8.48	4.50	3.93; 3.86		
Thr-5	8.21	4.34	4.25	1.20	
Gln-6	8.33	4.32	2.06; 1.97	2.33	7.51; 6.85 (NεH$_2$)
Arg-7	8.39	4.32	1.82; 1.75	1.61	3.18 (Hδ), 7.17 (NεH)
Leu-8	8.26	4.33	1.66	1.59	0.92 (Hδ1), 0.86 (Hδ2), 7.60; 7.04 (CONH$_2$)

a not detected (fast exchange with water).

Table S5. Proton chemical shifts of peptide **3** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

Residue	NH	Hα	Hβ	Hγ	Others
Thr-1	*a*	3.98	4.31	1.31	
Trp-2	8.90	4.63	3.30		10.11 (N1H), 7.26 (H2), 7.60 (H4), 7.23 (H5), 7.14 (H6), 7.48 (H7)
Xxx-3	8.24	3.93	1.77	0.96	1.36 (Hδ), 1.65 (Hε)
Gln-4	7.96	3.82	2.00; 1.93	2.32	7.51; 6.86 (NεH$_2$)
Ser-5	7.70	4.36	3.90; 3.85		
Thr-6	7.91	4.18	4.14	1.08	
Xxx-7	8.05	4.72	3.28; 3.20		7.74 (Hδ)
Arg-8	8.20	4.34	1.89; 1.79	1.64	3.20 (Hδ), 7.20 (NεH)
Leu-9	8.18	4.31	1.72; 1.66	1.60	0.90 (Hδ1), 0.87 (Hδ2), 7.50; 7.04 (CONH$_2$)

a not detected (fast exchange with water).

Table S6. Proton chemical shifts of peptide **4** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

Residue	NH	Hα	Hβ	Hγ	Others
Thr-1	a	3.86	4.13	1.28	
Trp-2	8.84	4.81	3.30; 3.24		10.18 (N1H), 7.31 (H2), 7.72 (H4), 7.18 (H5), 7.24 (H6), 7.48 (H7)
Xxx-3	7.81	--	1.40; 1.65	2.01	1.84 (Hδ), 5.42 (Hε)
Gln-4	7.75	3.93	1.93	2.30; 2.08	7.47; 6.83 (NεH₂)
Ser-5	7.22	4.48	3.95; 3.74		
Thr-6	8.04	4.27	4.27	1.24	
Xxx-7	7.77	--	1.30; 1.65	2.01	1.84 (Hδ), 5.32 (Hε)
Arg-8	7.77	4.31	1.72; 1.89	1.59	3.18 (Hδ), 7.15 (NεH)
Leu-9	a	4.02	1.73	1.73	0.96 (Hδ1), 0.95 (Hδ2), 7.48; 6.83 (CONH₂)

a not detected.

Table S7. Proton chemical shifts of peptide **5** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

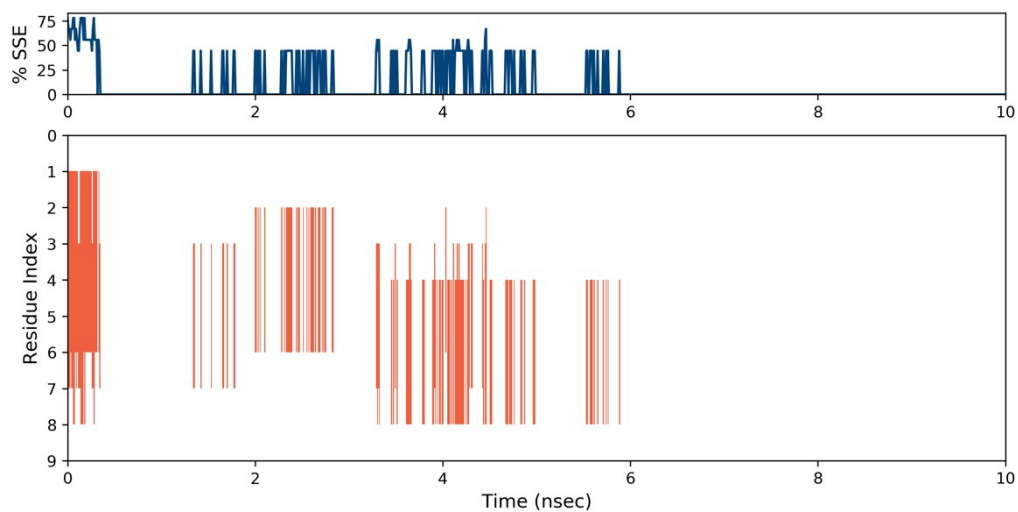| Residue	NH	Hα	Hβ	Hγ	Others
Thr-1	a	3.89	4.20	1.30	
Trp-2	8.96	4.85	3.42; 3.25		10.21 (N1H), 7.36 (H2), 7.74 (H4), 7.25 (H5), 7.18 (H6), 7.50 (H7)
Xxx-3	8.60	--	1.82; 1.37	1.96	1.76 (Hδ); 5.43 (Hε)
Gln-4	7.86	3.87	1.86; 1.70	2.37	7.14; 7.21 (NεH₂)
Ser-5	7.94	4.20	4.03; 3.96		
Thr-6	7.44	4.05	4.22	1.16	
Xxx-7	8.03	--	1.82; 1.41	1.96	1.76 (Hδ); 5.36 (Hε)
Arg-8	7.65	4.16	1.90	1.69	3.18 (Hδ), 7.22 (NεH)
Leu-9	7.62	4.22	1.83; 1.76	1.63	0.96 (Hδ1), 0.88 (Hδ2), 7.45; 6.85 (CONH₂)

a not detected (fast exchange with water).

Table S8. Proton chemical shifts of peptide **6** (at 600 MHz; in H_2O + D_2O 95:5 + CD_3COOD; pH=3.0; T = 25 deg).

Residue	NH	Hα	Hβ	Hγ	Others
Trp-1	a	4.36	3.45; 3.35	--	10.30 (N1H), 7.37 (H2), 7.72 (H4), 7.20 (H5), 7.28 (H6), 7.51 (H7)
Xxx-2	7.88	--	1.76; 1.41	1.90	1.61 (Hδ); 5.41 (Hε)
Gln-3	7.88	4.12	1.95; 2.11	2.37	7.50; 6.87 (NεH₂)
Ser-4	7.99	4.44	3.99; 3.82		
Thr-5	7.89	a	4.22	1.23	
Xxx-6	7.86	--	1.76; 1.40	1.90	1.61 (Hδ); 5.34 (Hε)
Arg-7	7.89	4.30	1.89; 1.75	1.60	3.18 (Hδ), 7.20 (NεH)
Leu-8	7.99	4.27	1.72	1.62	0.93 (Hδ1), 0.86 (Hδ2), 7.44; 7.10 (CONH₂)

a not detected.

Figure S10. SSE (α-helices) composition for peptide **1** over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).
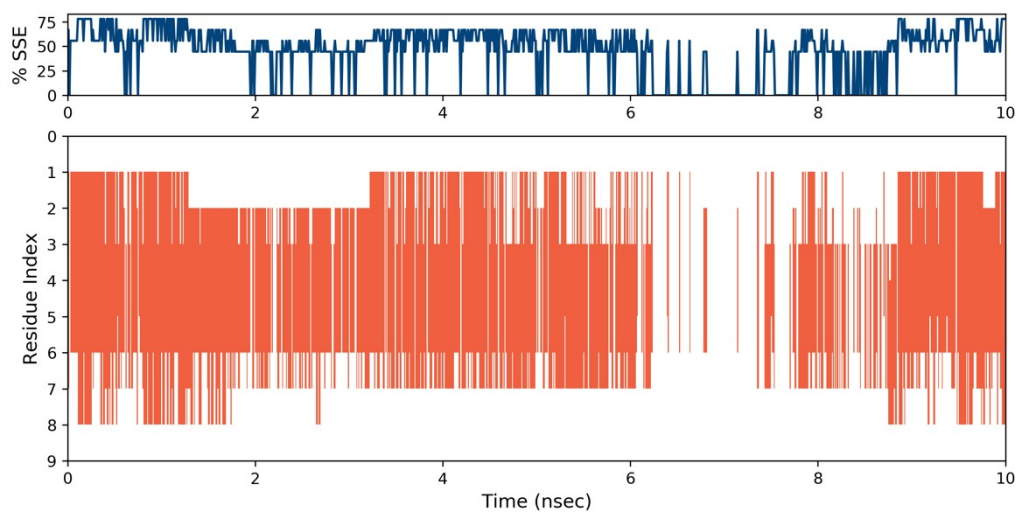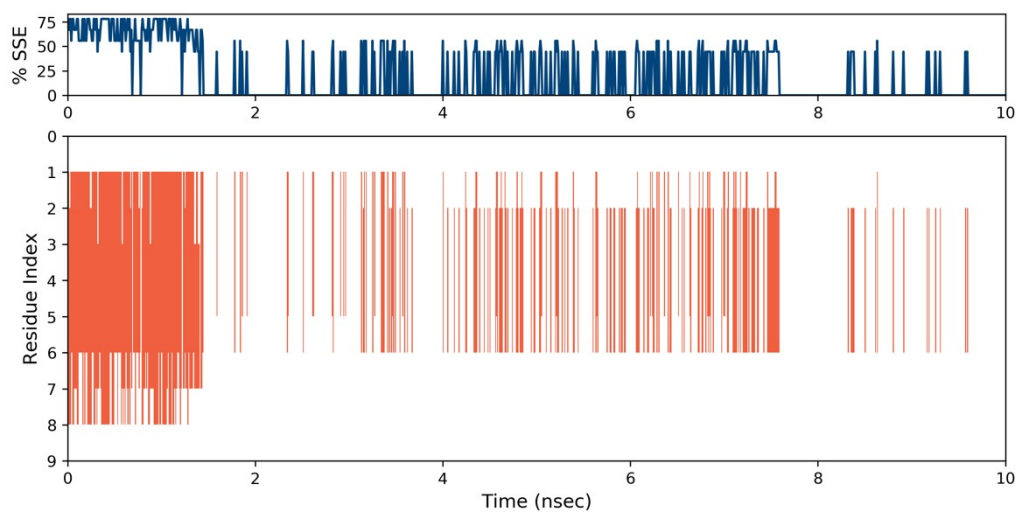
Figure S11. SSE (α-helices) composition for peptide **2** over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).

Figure S12. SSE (α-helices) composition for peptide **3** over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).
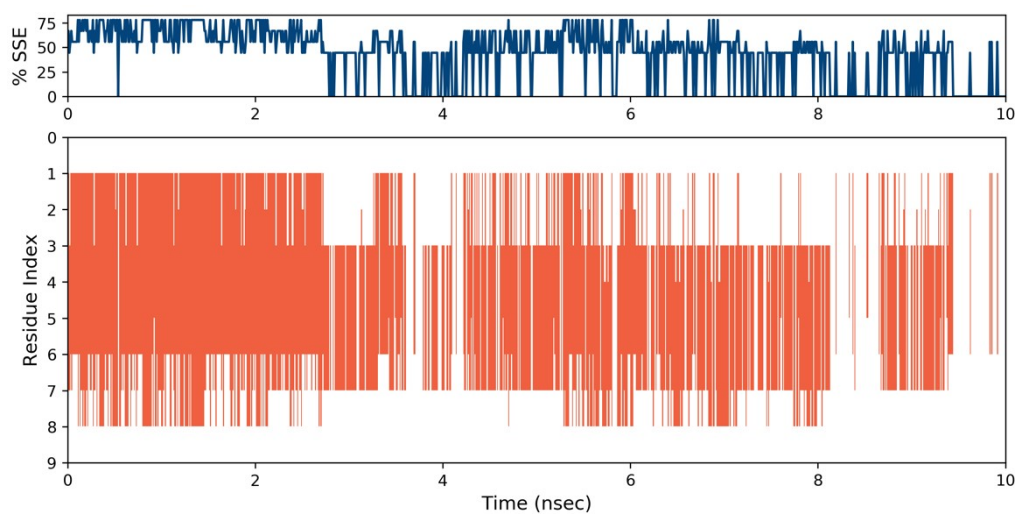
Figure S13. SSE (α-helices) composition for peptides **4** (*trans*-isomer) over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).

Figure S14. SSE (α-helices) composition for peptides **5** (*cis*-isomer) over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).

Figure S15. SSE (α-helices) composition for peptide **6** over the time (blue), and the α-helical secondary structure monitored for individual amino acid residues over the time (red).
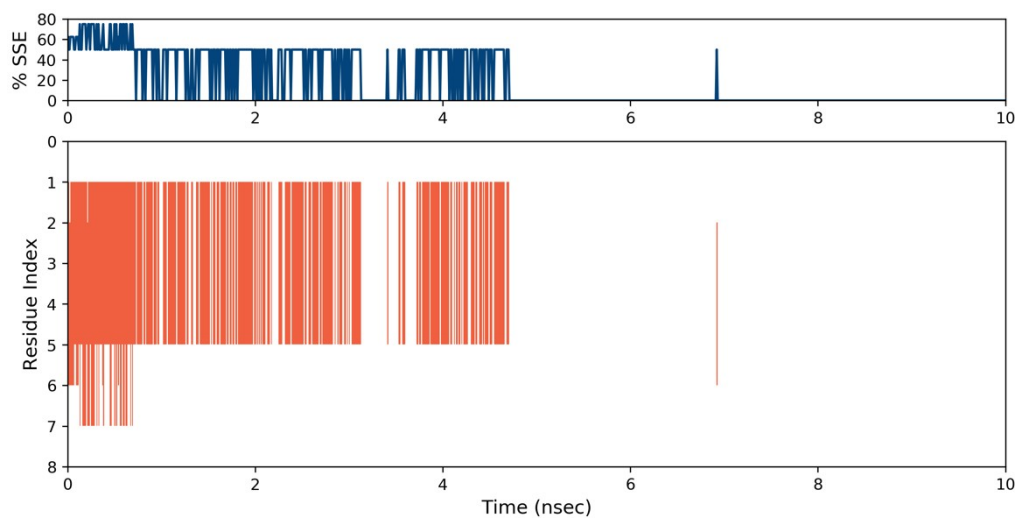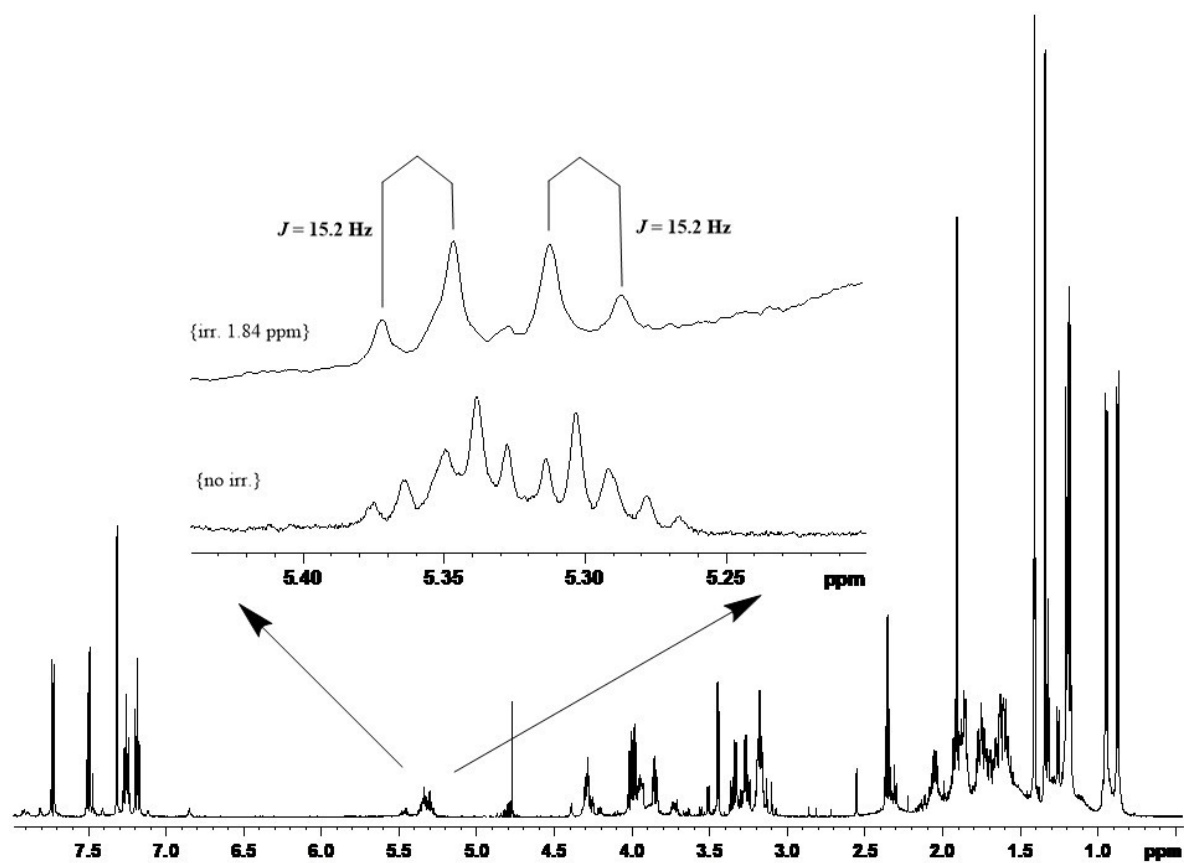
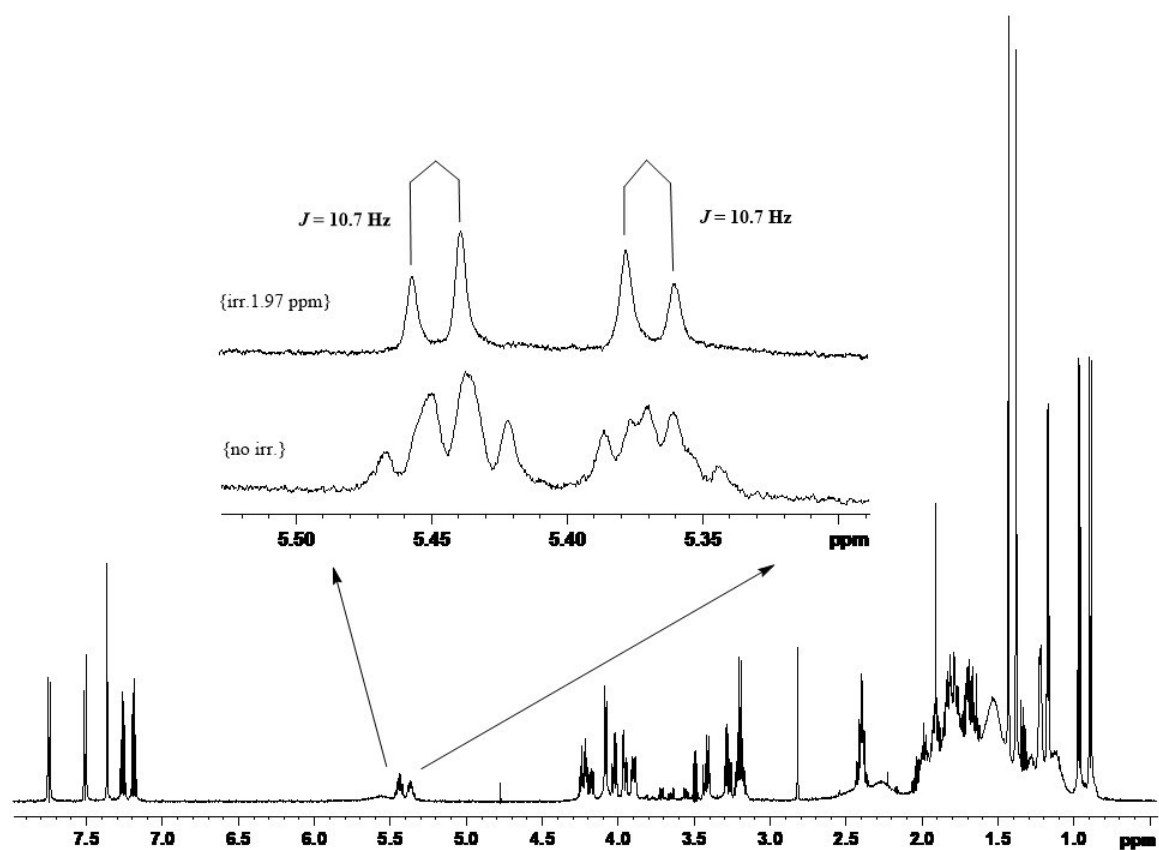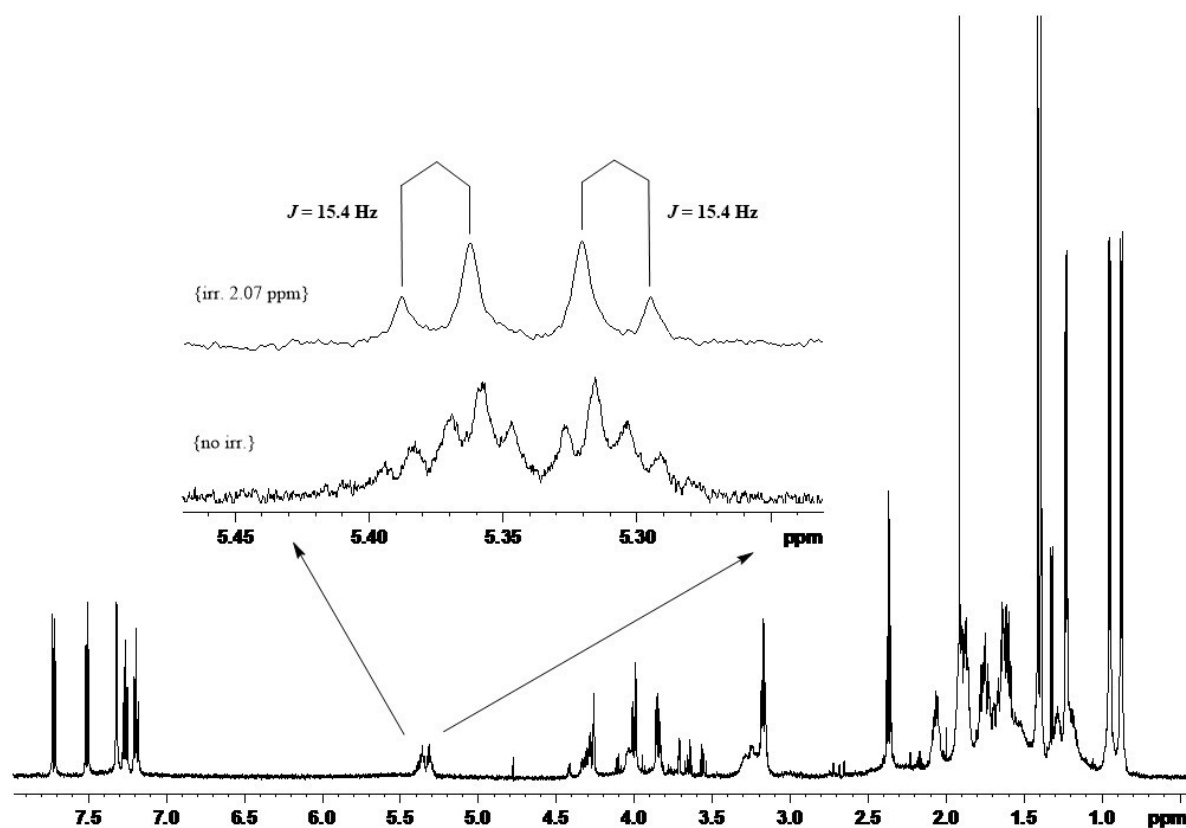**Figure S16.** Proton NMR spectrum of peptide **4** in D$_2$O. The signals of olefinic protons are expanded and shown also at irradiation of neighbouring CH$_2$ groups.
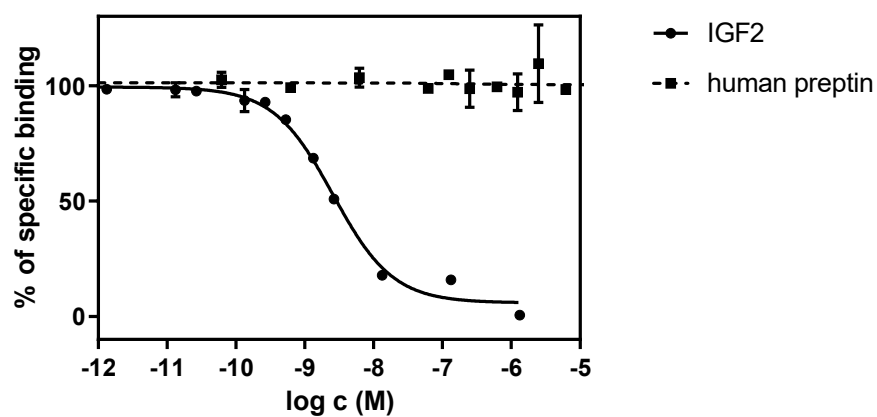
Figure S17. Proton NMR spectrum of peptide **5** in D_2O. The signals of olefinic protons are expanded and shown also at irradiation of neighbouring CH_2 groups.

Figure S18. Proton NMR spectrum of peptide **6** in D_2O. The signals of olefinic protons are expanded and shown also at irradiation of neighbouring CH_2 groups.
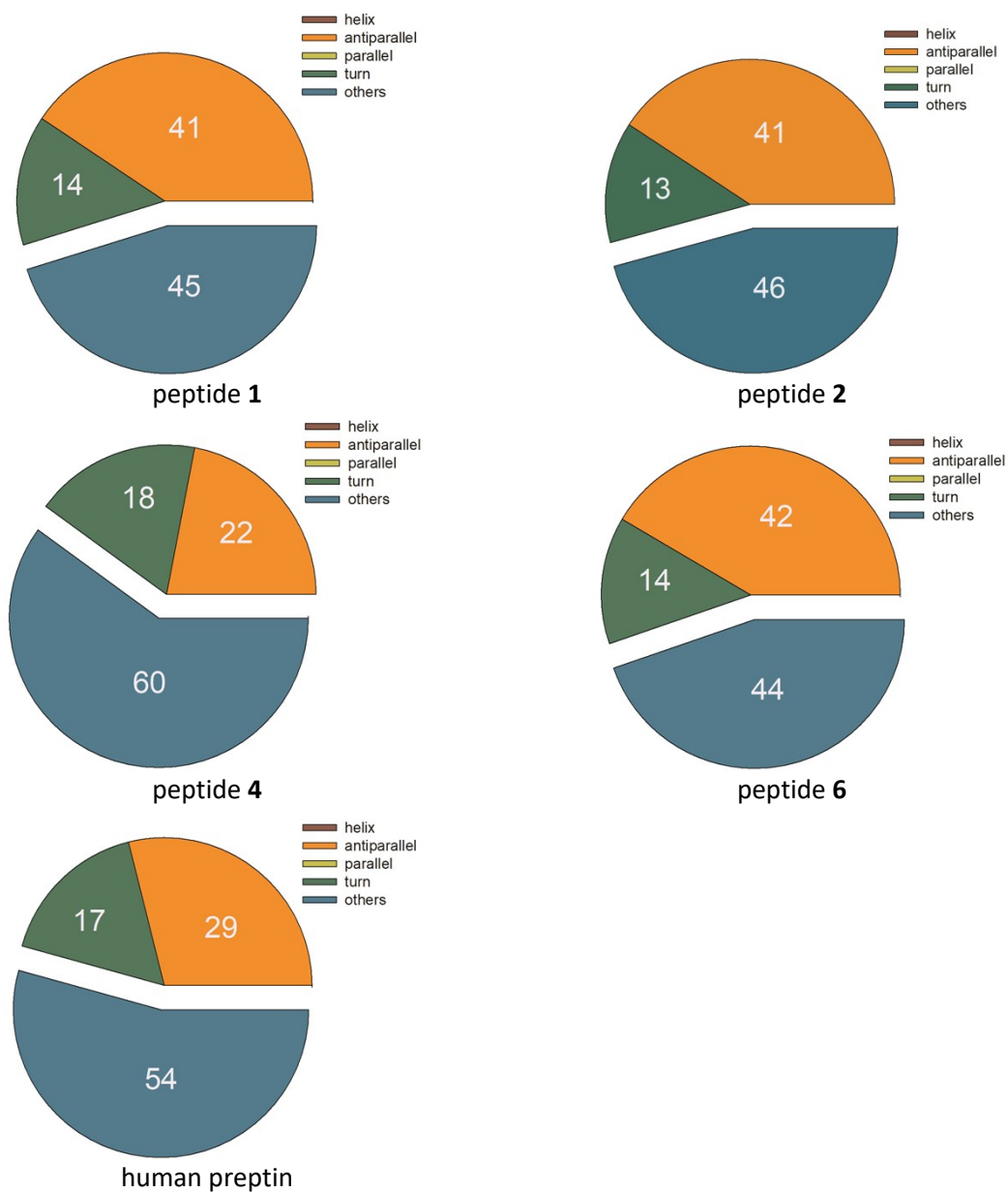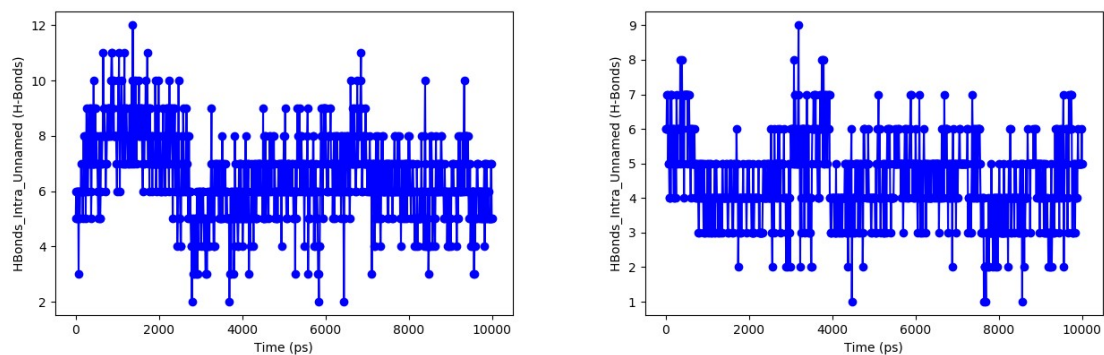
Figure S19. Representative binding curves of IGF2 and preptin on immobilized D11. Inhibition of binding of human [^{125}I]-monoiodotyrosyl-Tyr2-IGF2 to D11 by human IGF2 (solid line) and by human preptin (dashed line).

Figure S20. Estimated secondary structure content (%) for peptides **1**, **2**, **4**, **6** and human preptin provided by CD spectroscopy.

Figure S21. Number of intramolecular H-bonds occurring in peptide **5** (left) and peptide **6** (right) during the 10 ns MD simulation. The MD simulations performed for peptide 5, and peptide 6, revealed an average number of seven intramolecular H-bonds occurring in the former, while only five for the latter
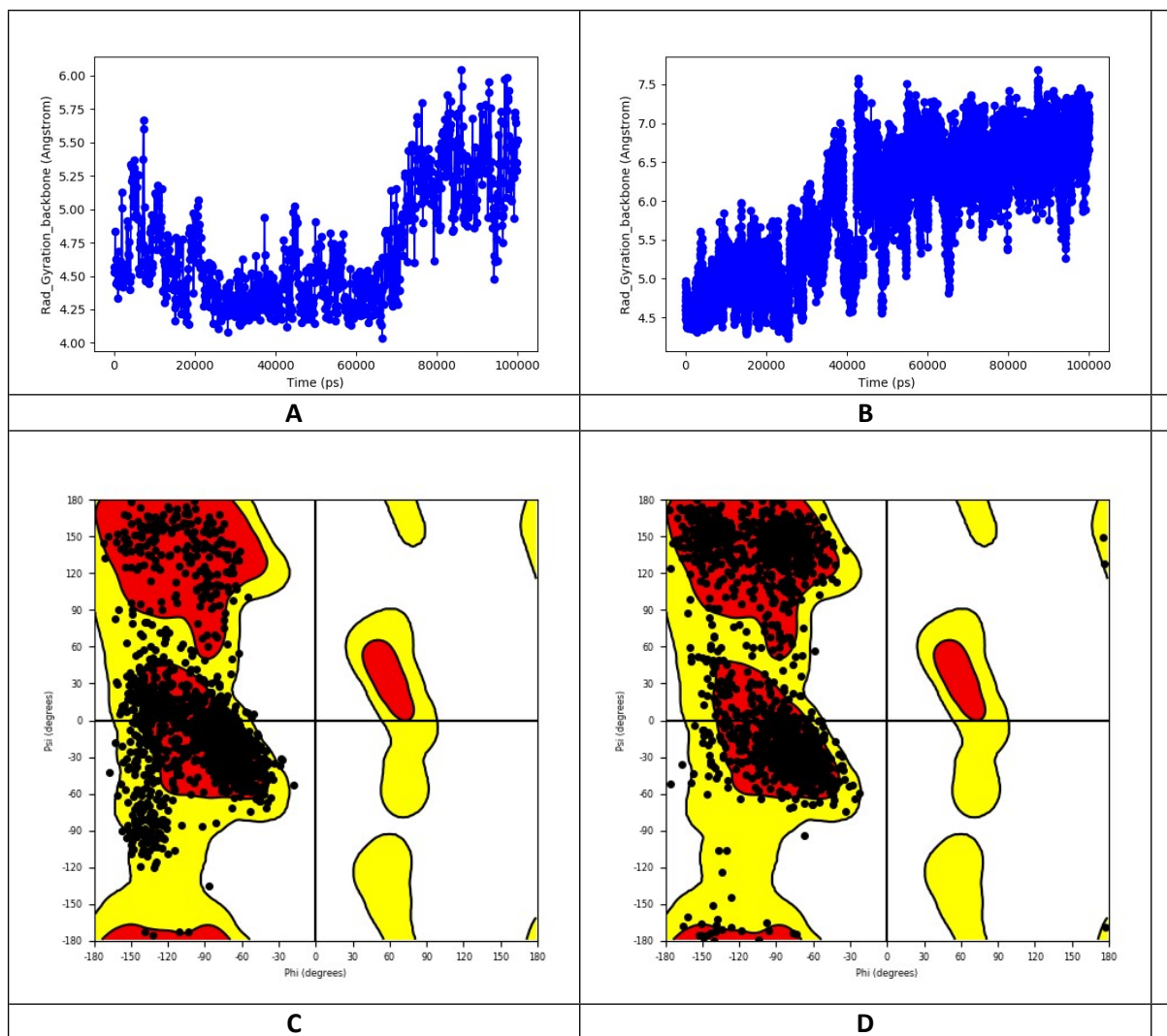
Figure S22. Average deviations (in Å) of backbone atoms (top row) of peptide **4** (A) and peptide **5** (B) from the reference geometry as monitored during the 100 ns MD simulation. The bottom row shows the Ramachandran plots for 200 geometries of peptide **4** (C) and peptide **5** (D) sampled during the 100 ns MD.

The figure explains slightly higher flexibility of backbone atoms of peptide **5** over peptide **4**. Although the MD run was affected by the initial helical structure, after 80 ns we can expect that the system is equilibrated. After that time, we still see higher average deviations of backbone atoms for peptide **5** (6.5 Å) than for peptide **4** (5.5 Å). Analysis of the (ϕ, ψ) backbone torsion angles of both peptides also revealed a slight tendency of peptide **4** to adopt δ_D conformation (ca. -150 °, -90 °; notation taken from Ref. {Kaminský, 2016 #4266}) that we did not observe for peptide **5** (Figure S22, ESI).

α-helix   **3$_{10}$**   **disordered**
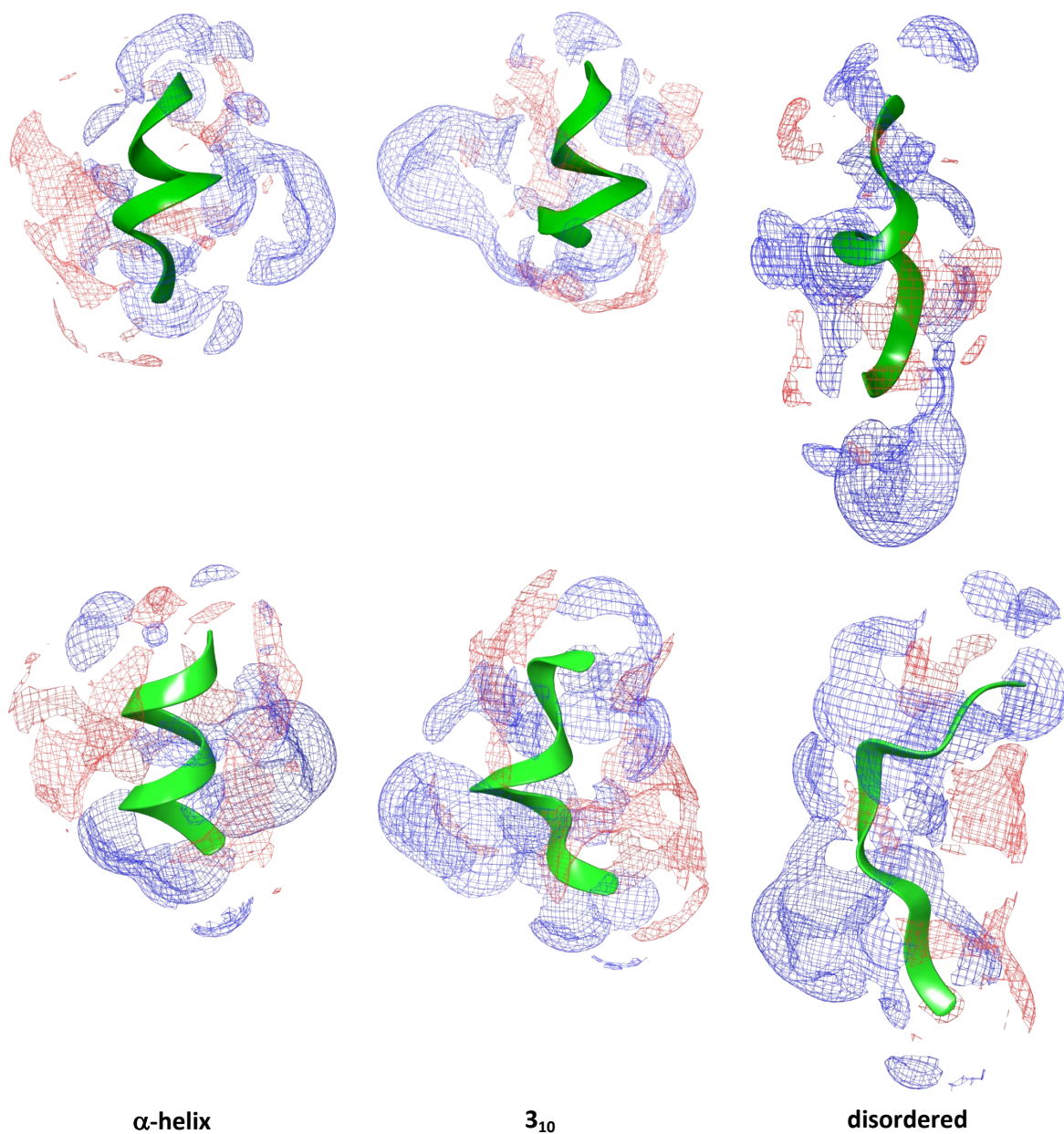
Figure S23. Hydrophobic (red) and hydrophilic (blue) surfaces for selected representative geometries of α-helix, 3$_{10}$-helix, and a disordered structure of peptide **4** (top) and peptide **5** (bottom).

Table S9. Experimental absorption and CD band wavelengths λ (nm) and intensities (Δε).

	λ (nm)	Δε (M^{-1}cm^{-1})
Human preptin	200	-3.8
	226	-0.3
peptide **1**	198	-2.6
	225	0.1
peptide **2**	198	-4.1
	228	0.1
peptide **3**	189	3.1
	207	-2.0
	225	-1.1
peptide **4**	201	-4.4
	226	-0.3
	233	-0.5
peptide **5**	190	1.9
	208	-1.4
	227	-0.8
peptide **6**	200	-1.8
	226	0.1