Supplementary Information

Computational evolution of threonine rich β-hairpin peptides mimicking specificity and affinity of antibodies.

Hongxia Hu, Christian Kofoed, Ming Li, Juliana Gonçalves, Jonas Hansen, Martin Wolfram, Axel Kornerup Hansen, Camilla Hartmann Friis Hansen, Frederik Diness, Sanne Schoffelen,* and Morten Meldal*

Center for Evolutionary Chemical Biology, Department of Chemistry, University of Copenhagen, Denmark, Corresponding author email : meldal@nano.ku.dk

TABLE OF CONTENTS

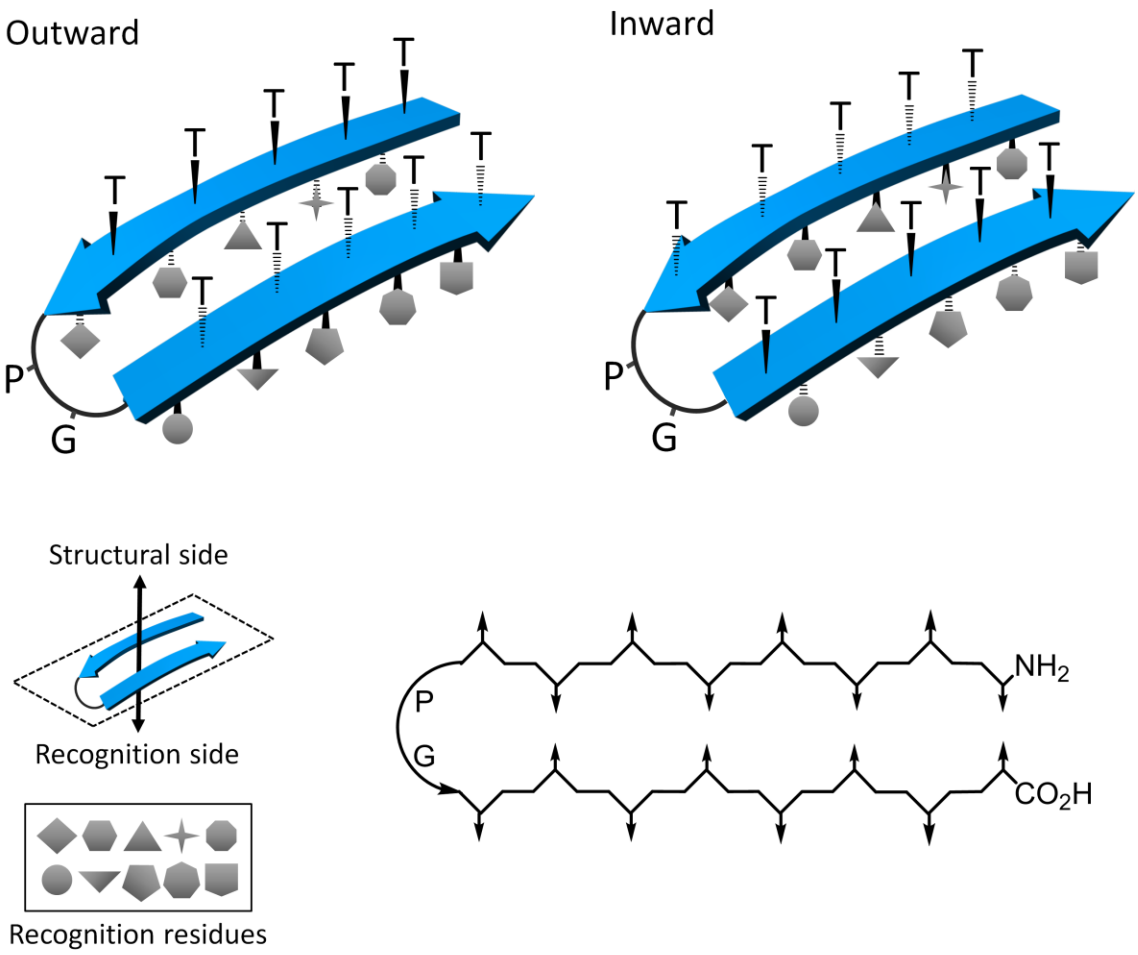


Figure SI-1. Schematic representation of the two types of β-bodies. Depending on the register with respect to the two turn residues, side-chains of the recognition residues are pointing either away from each other (outward orientation) or towards each orther (inward orientation), as illustrated by arrows in the drawing below the two hairpin cartoons. Inwards orientation may be selected for binding to cavities involved in ligand or substrate binding while outward orientation is favoured for more planar protein surfaces.

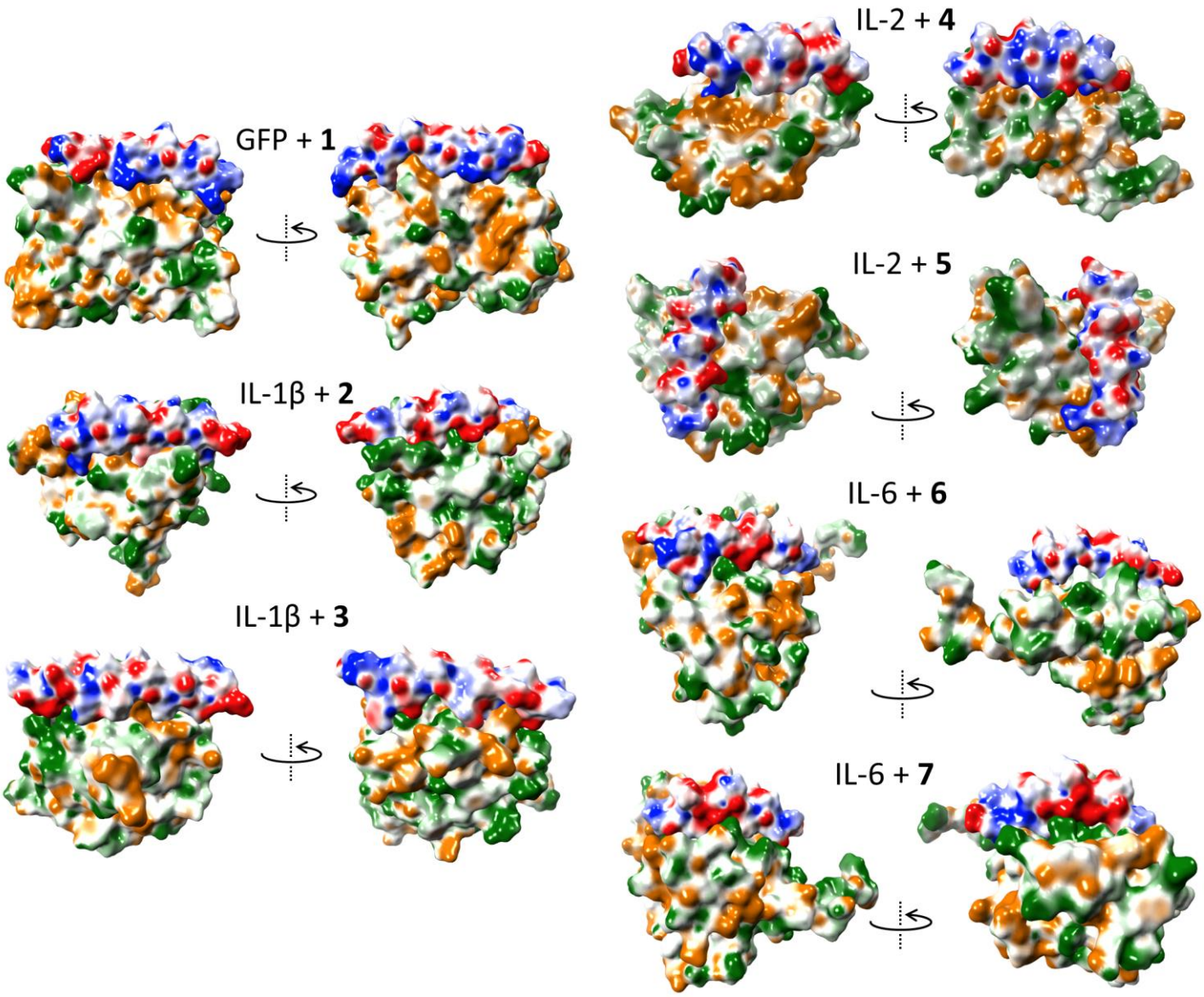


Figure SI-2. 3D-representation of the seven designed β-bodies in complex with their target proteins. β-bodies are shown in red/blue and target proteins in orange/green. The surfaces are color-coded according to electrostatic potential (positive: blue/green, negative: red/orange). Note the spatial and electrostatic complementarity between the two surfaces. Each β-body is presented from two sides to illustrate the surface and charge complementarity derived computationally through the process illustrated in Figure 2. Details of the interaction between IL-1 β and **2** are shown in Figure 3.

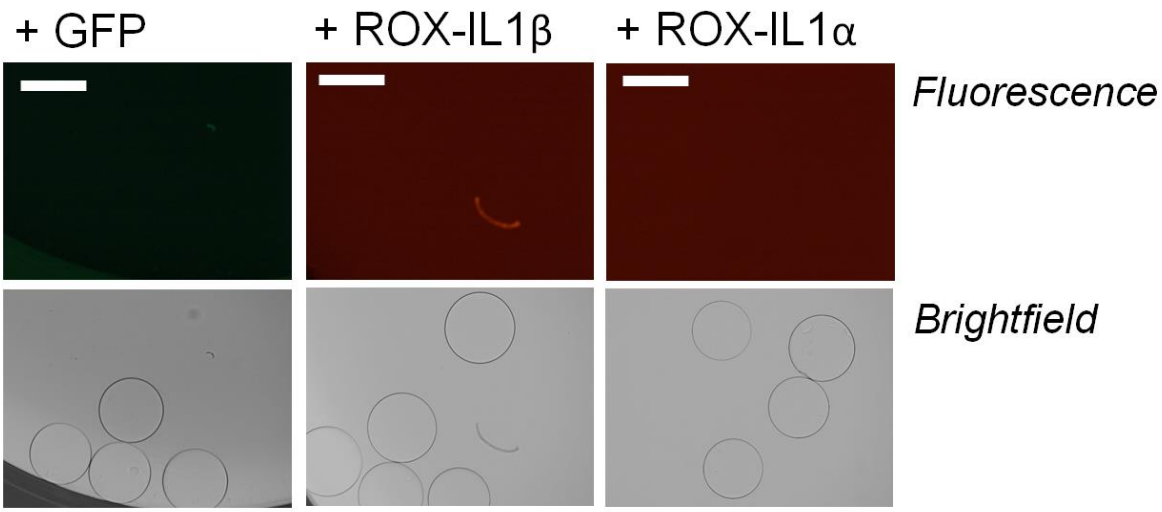


Figure SI-3. Microscope images of *N*-acetylated beads without β-bodies incubated with GFP, ROX-labeled IL-1 β and ROX-labeled IL-1α, respectively; negative control for Figure 4. Scale bars are 500 µm.

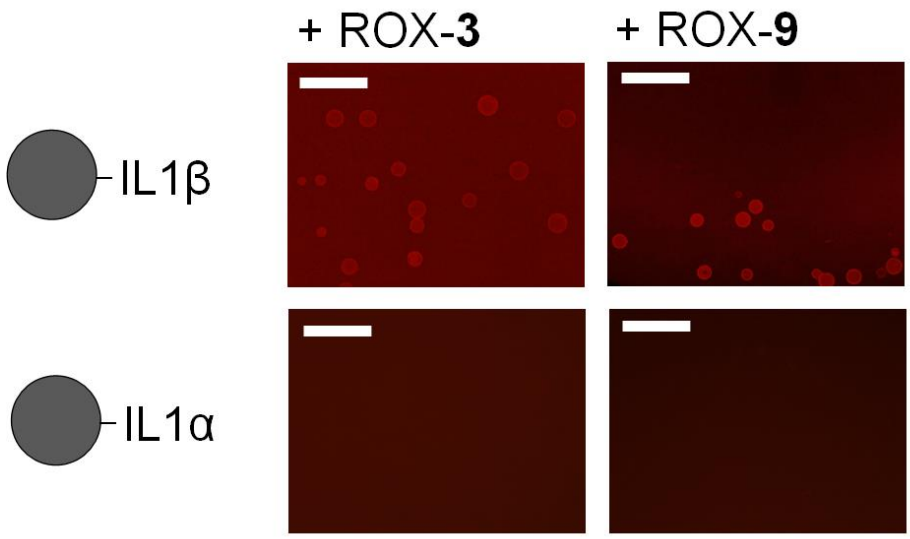


Figure SI-4. Binding of ROX-labeled **3** and **9** (a triazole-bridged version of **2**) to IL-1β immobilized on Ni-NTA agarose beads. As negative control, IL-1α was used. Scale bars are 500 µm.

LCMS and NMR of compound 2 (ETDTYTETYPGRTITWTITDG) and 9 (*c*-E-Pra-DTYTETYPGRTITWTI-Abu-DG).

An aliqoute of the NMR - solutions described below were diluted 20 times and injected in an UPLC-MS instrument, (Waters, Xevo G2-S QTOF).

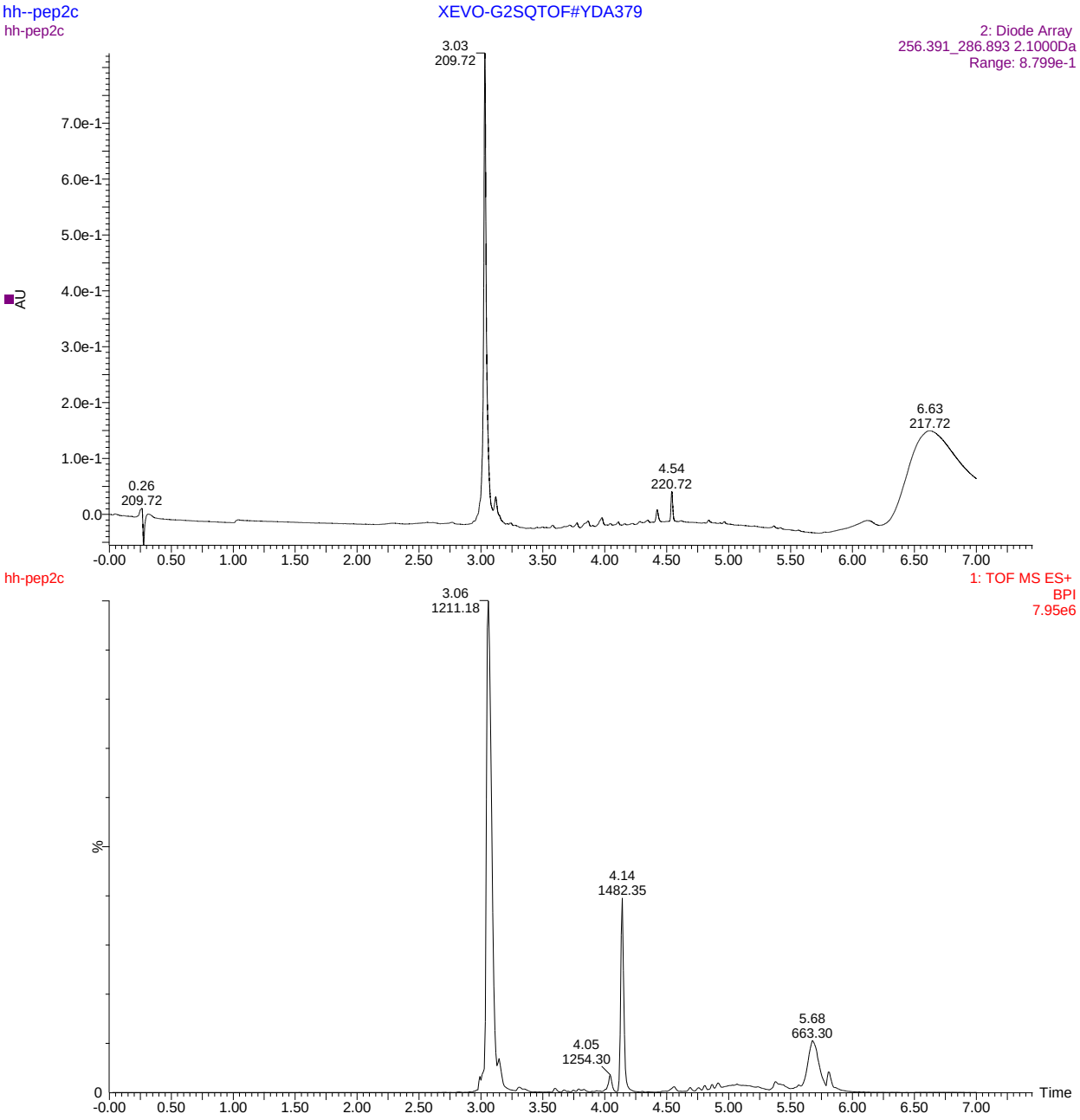


Figure SI-5. LCMS of compound **2**, upper trace UV-trace, lower trace TIC.

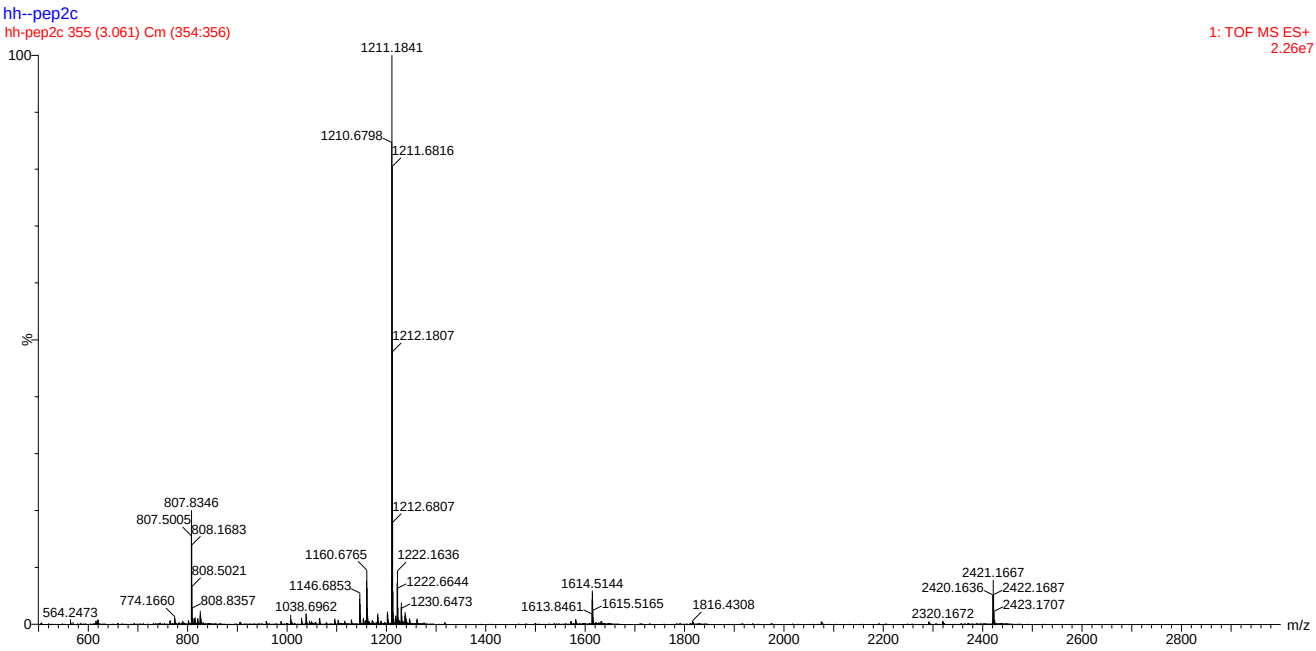


Figure SI-6. MS spectrum showing [M+H$^+$], [M+2H$^+$], [M+3H$^+$] for the compound **2** - NMR sample.

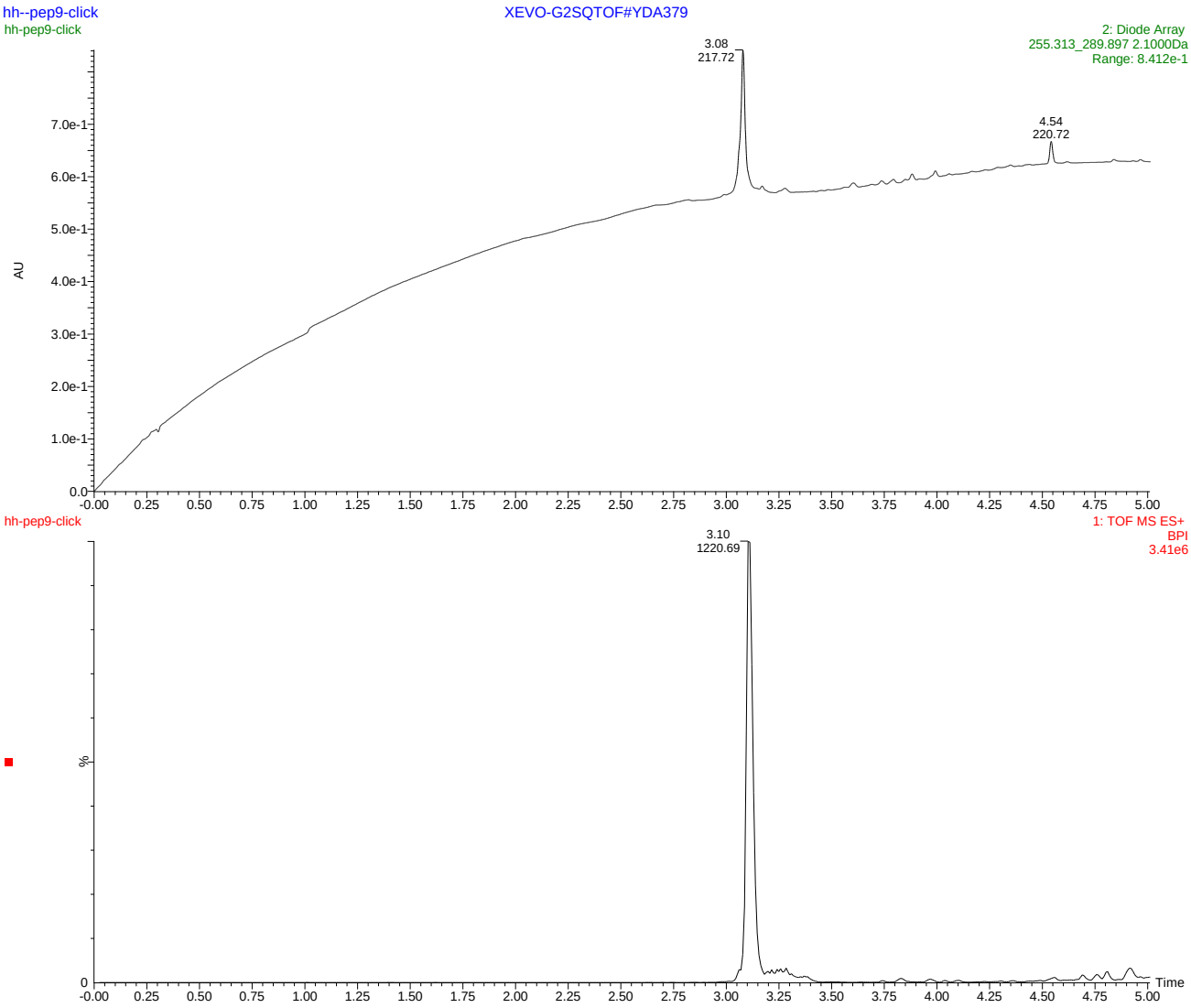


Figure SI-7. LCMS of compound **9**: upper trace UV-trace, lower trace TIC.

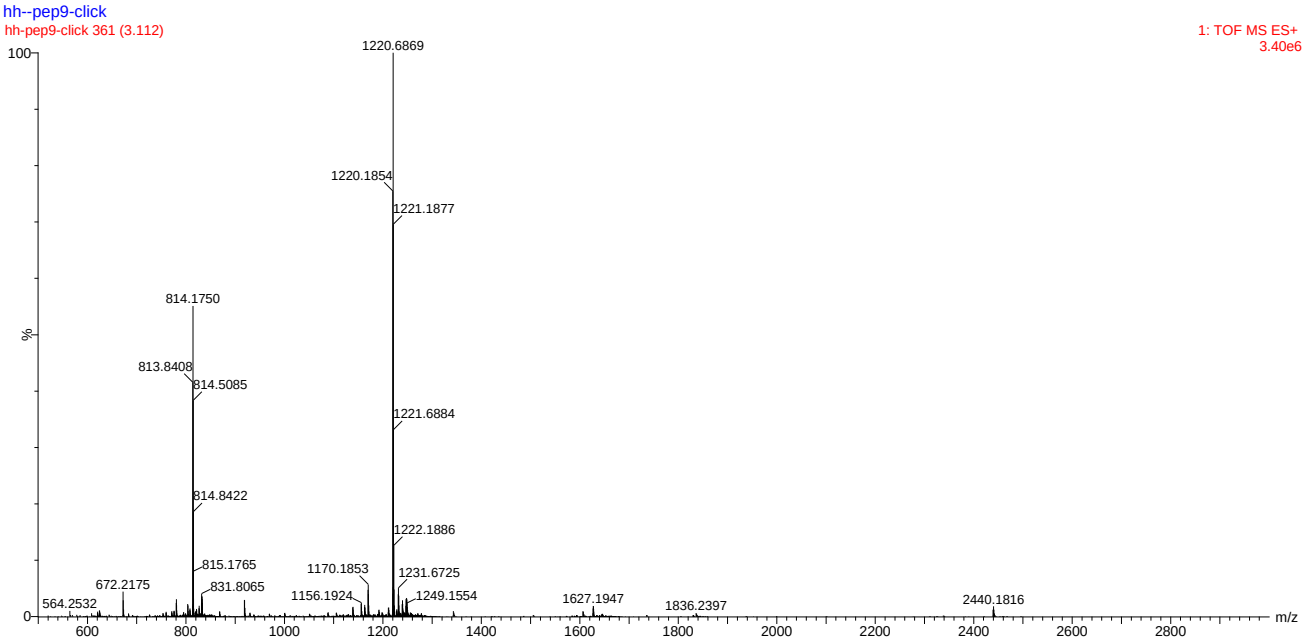
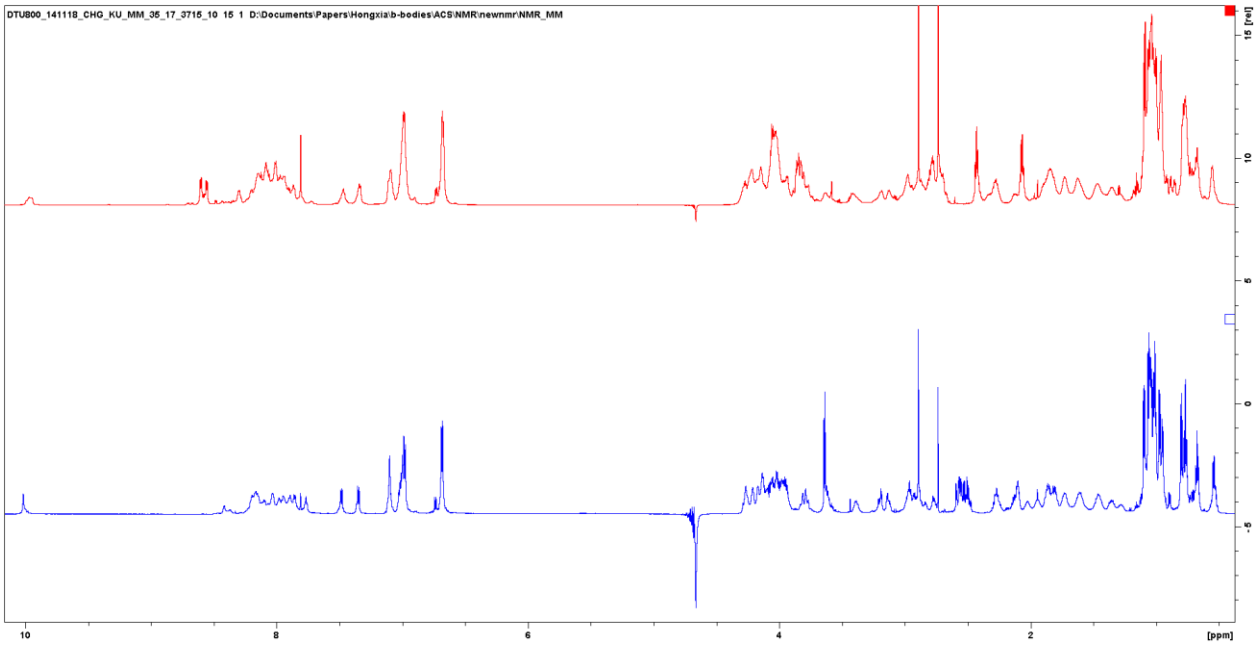


Figure SI-8. MS spectrum showing [M+H$^+$], [M+2H$^+$], [M+3H$^+$] for the compound **9** - NMR sample.

A)



B)

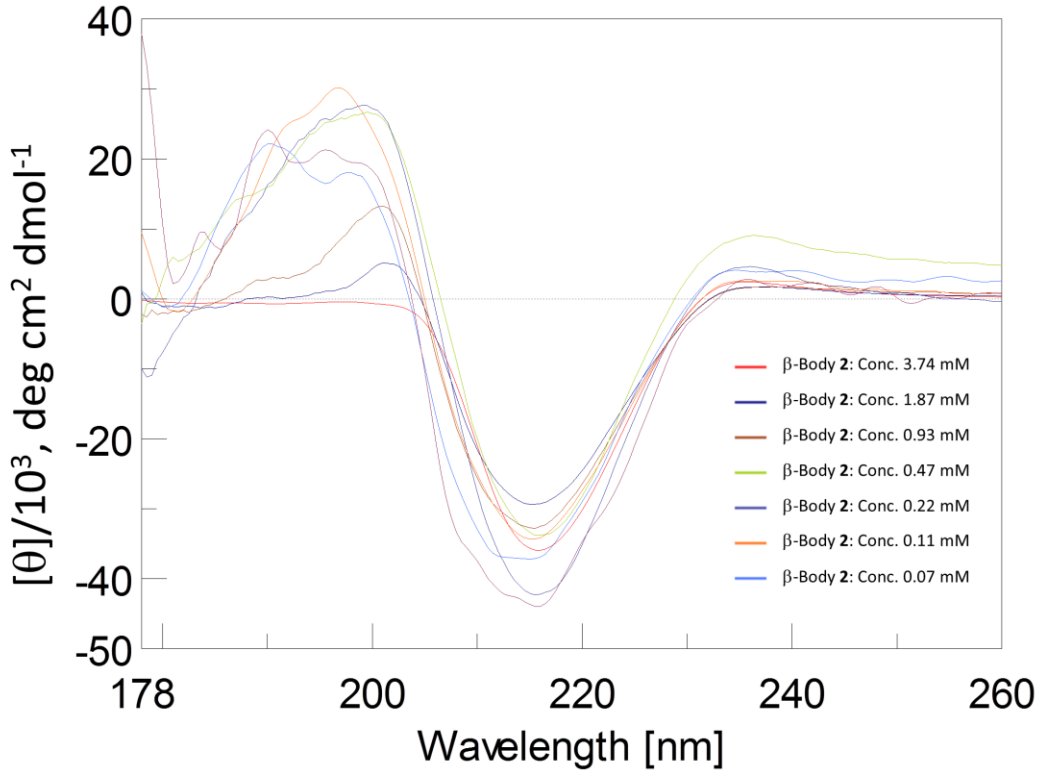
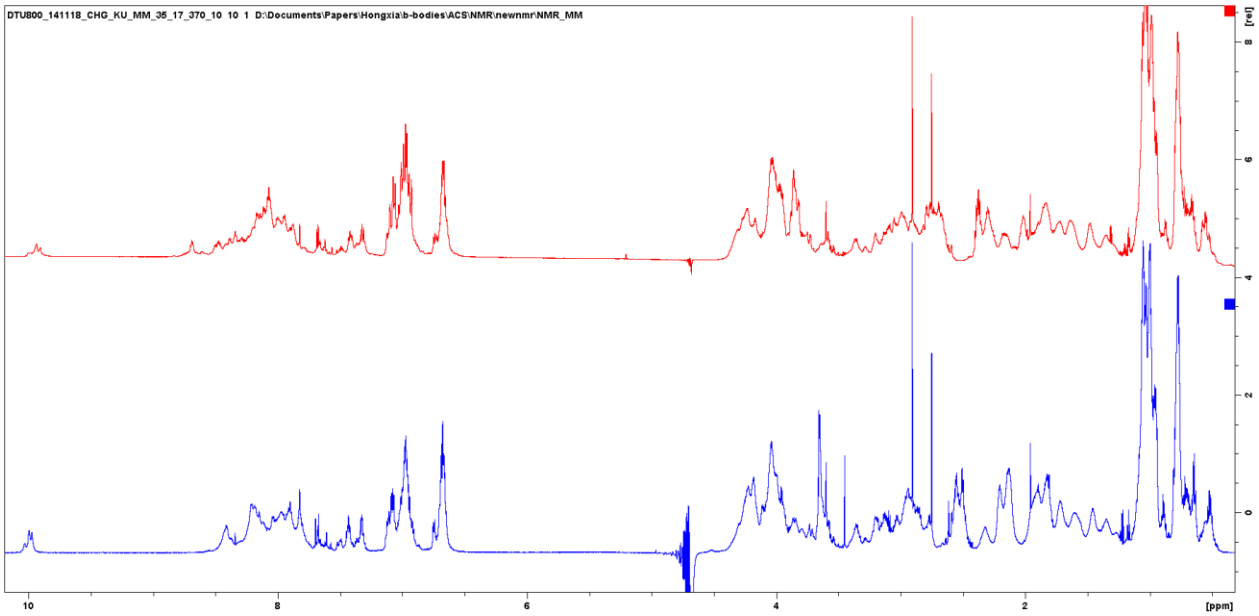


Figure SI-9. A) Transition to a second conformer by dilution of the NMR sample of compound **2**. B) Circular dichroism of compound **2** depends on concentration in the mM to μM range. At 3.74 mM compound **2** has a tendency to aggregate. Through a dilution series showing a transition through several conformational states a CD spectrum corresponding to a β-hairpin [max 198 nm, min 216 nm] [1] eventually appeared below 0.11 mM.

A)



B)

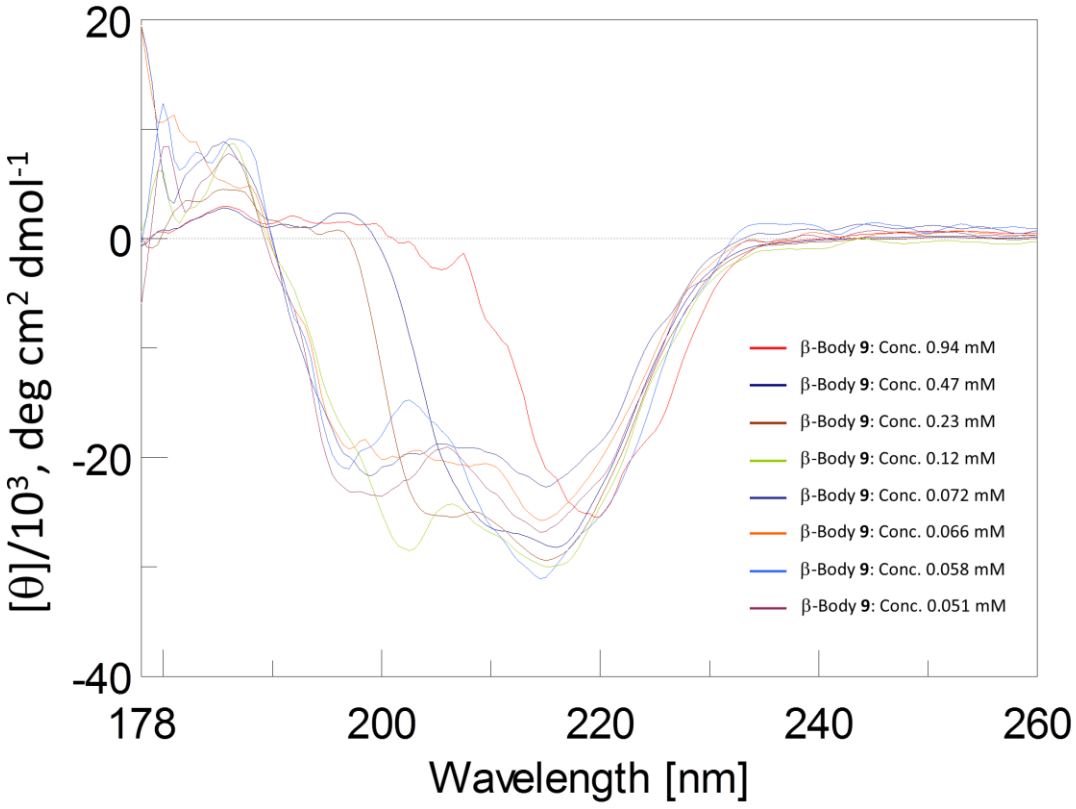
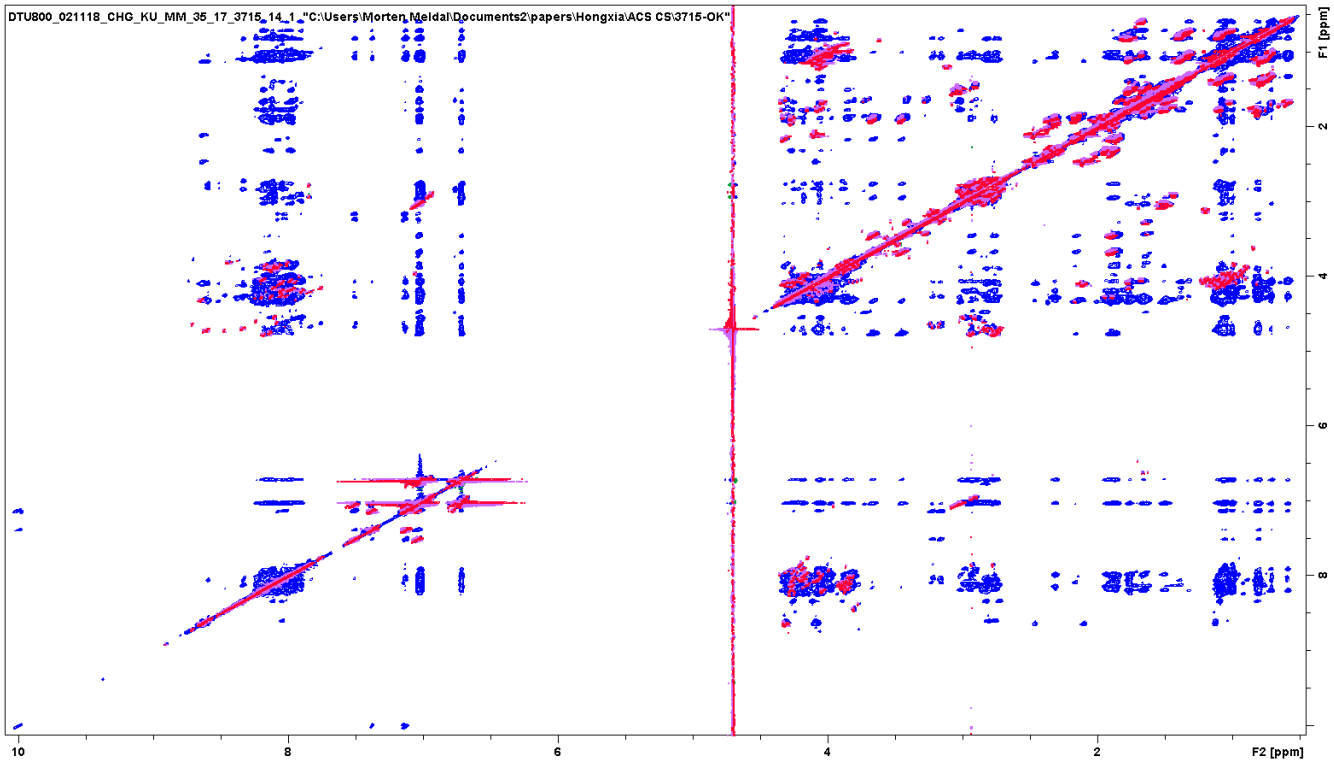
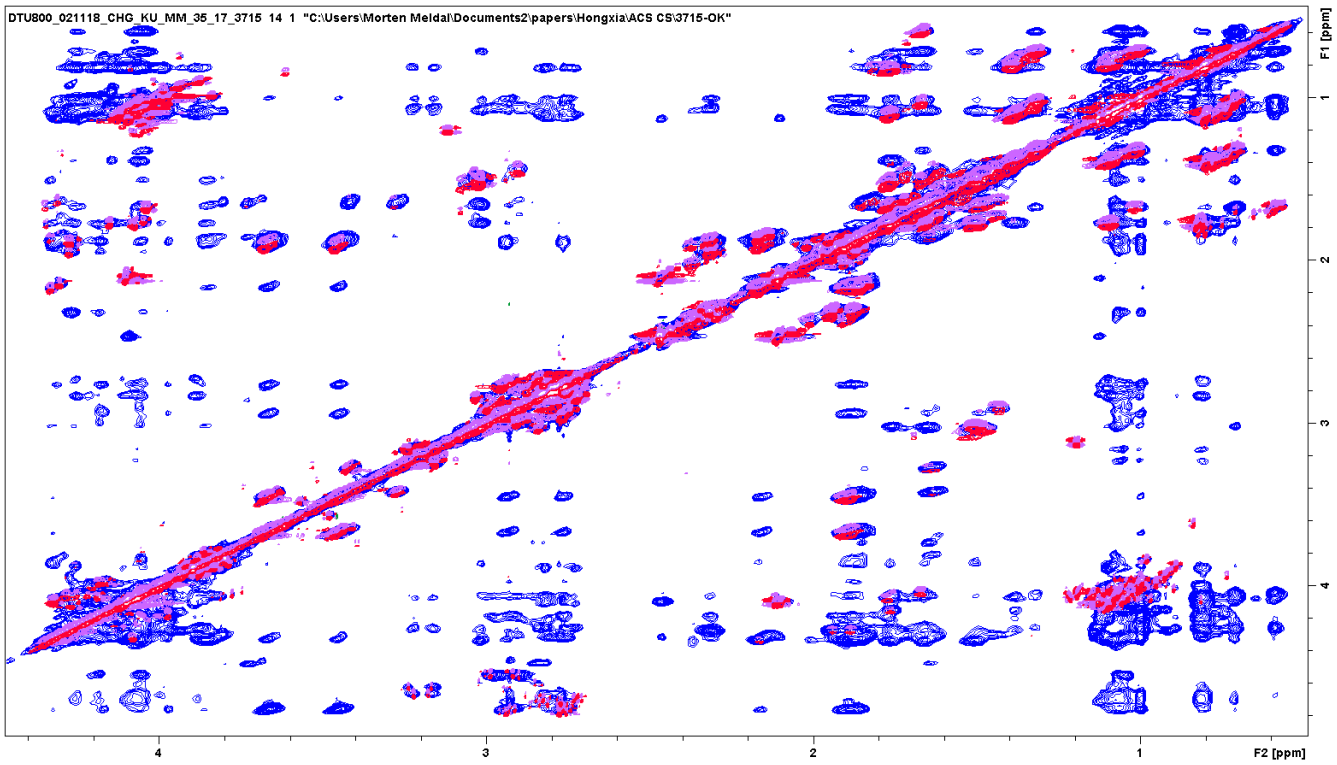
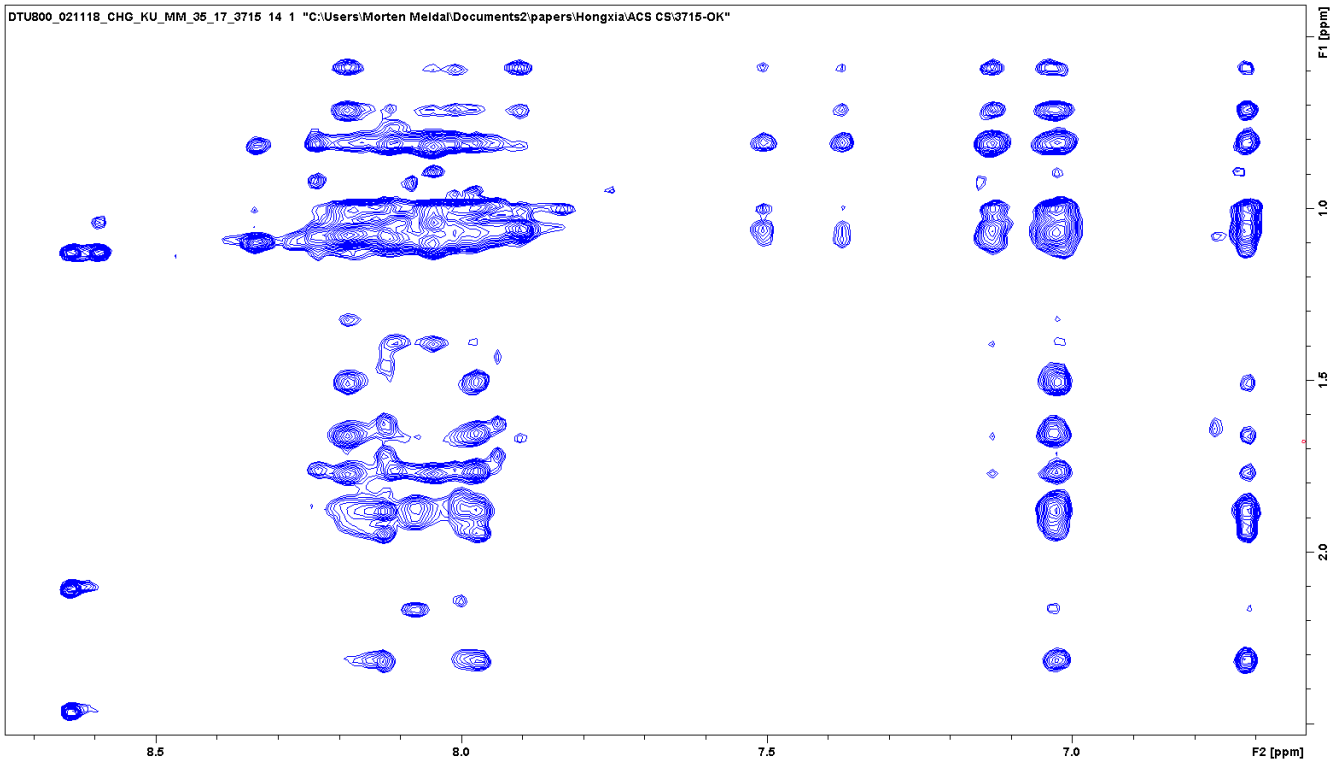


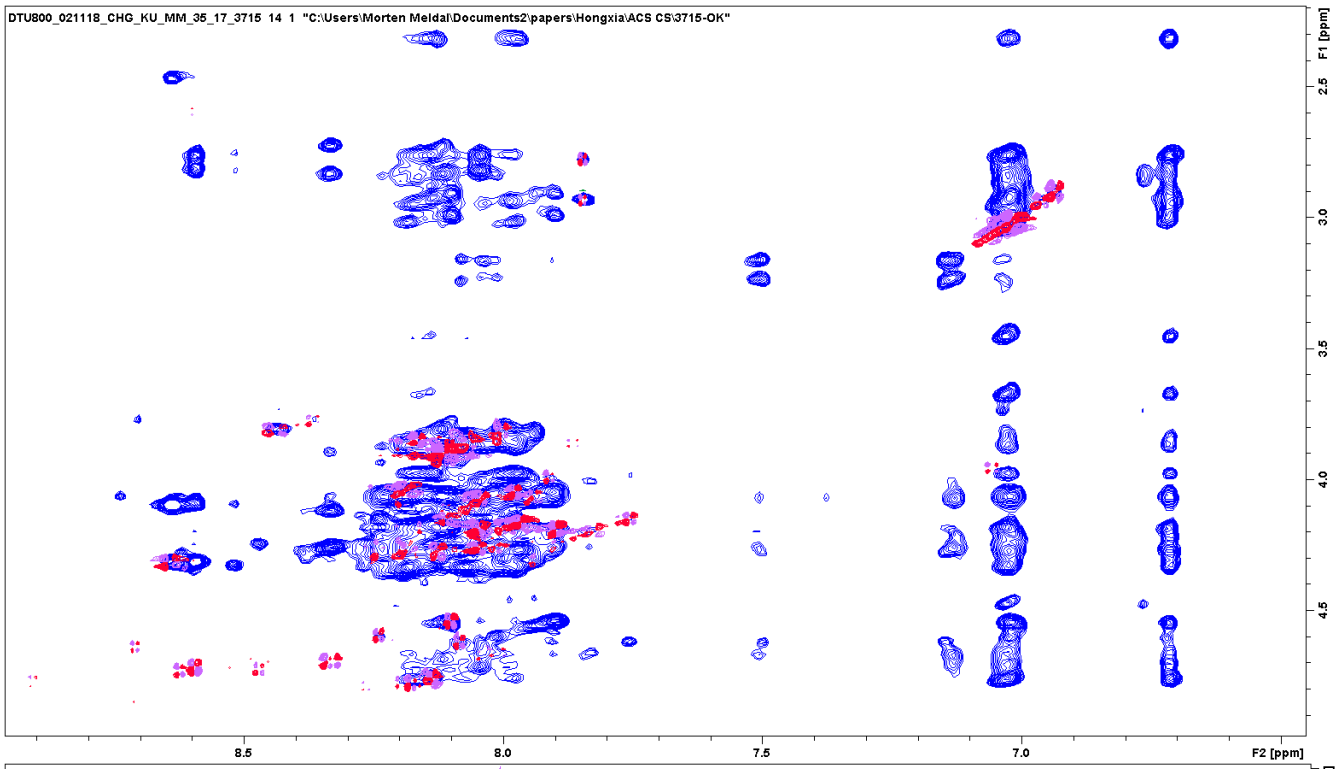
Figure SI-10. A) Transition of conformers by dilution of the NMR sample of **2**. B) Circular dichroism of compound **9** depends on concentration in the mM to μM range. Through a dillution series of the solution of compound **9** used for NMR it presented a more complex transition than compound **2** in agreement with the NMR results.

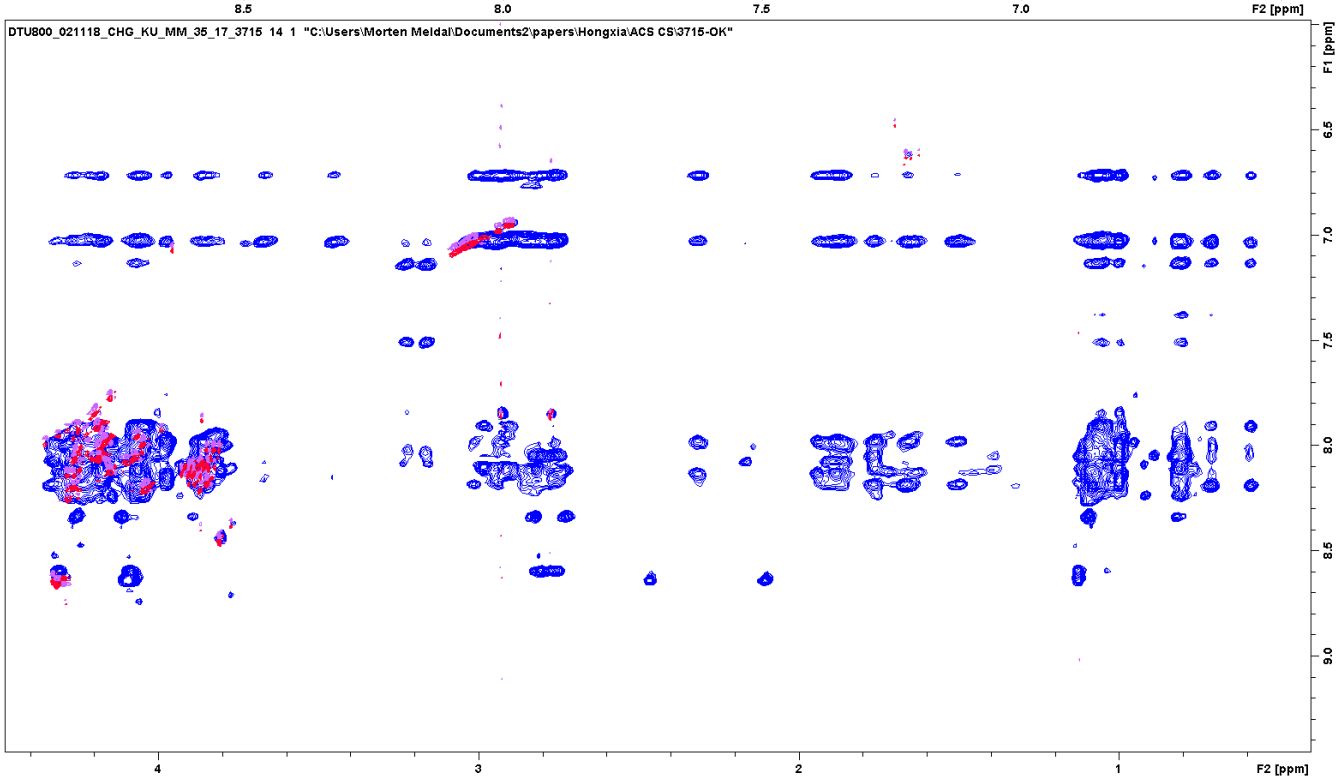
Assignments of the spectra of compound **2** (ETDTYTETYPGRTITWTITDG) and **9** (*c*-E-Pra-DTYTETYPGRTITWTI-Abu-DG) were complicated by the fact that they contained up to 8 threonines and that they showed some formation of two – three well-defined oligomers in slow exchange at the high NMR concentrations. As shown by NMR and CD above the complexe formation is reduced upon dilution. The spectra were aligned using first the tyrosine signals at 6.68 and subsequent fine adjustment using the isolated COSY and NOESY crosspeaks at 7.8468, 2.9300. Below overlays of the DQF-COSY and the NOSEY of **2** and of **9**, respectively, are presented. The NOESY was recorded under conditions that allow some spin diffusion in order to facilitate assignment. The aromatic signals of the two tyrosines and of Trp were first assigned and the NOESY crosspeaks were used to connect these to their H^{β}, H^{α} and NH protons. Then the isoleucine side-chains were assigned and their NOESY connectivities to the H^{α} and H^{β}-regions were analyzed. The arginine proline, glutamic and aspartic acid spinsystems were located based on their unique chemical shifts and coupling patterns. The connections between Thr H^{γ}, H^{β} and H^{α} were established by combined use of the overlayed NOESY and DQF COSY spectra. The relative positions of the identified threonines in the sequence was established via analysis of the NOESY connectivities from neighbouring residues to the threonine H^{γ}, H^{β}. Finally the NH - H^{α} connectivities were carefully aligned with the other assignments by use of COSY crosspeaks and their NOESY alignment with all assigned resonances. The entire process of assignment was performed three times with corrections to eliminate any unambiguous assingments. The NOESY crosspeaks that did not coincide with COSY crosspeaks and according to the assignments belong to different residues in peptide **2** are listed in Table SI-2.




DTU800_021118_CHG_KU_MM_35_17_3715 14 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\3715-OK"
F1 [ppm]
1
2
3
4
4
3
2
1
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3715 14 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\3715-OK"
F1 [ppm]
1.0
1.5
2.0
8.5
8.0
7.5
7.0
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3715 14 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\3715-OK"
F1 [ppm]
2.5
3.0
3.5
4.0
4.5
8.5
8.0
7.5
7.0
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3715 14 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\3715-OK"
F1 [ppm]
6.5
7.0
7.5
8.0
8.5
9.0
4
3
2
1
F2 [ppm]

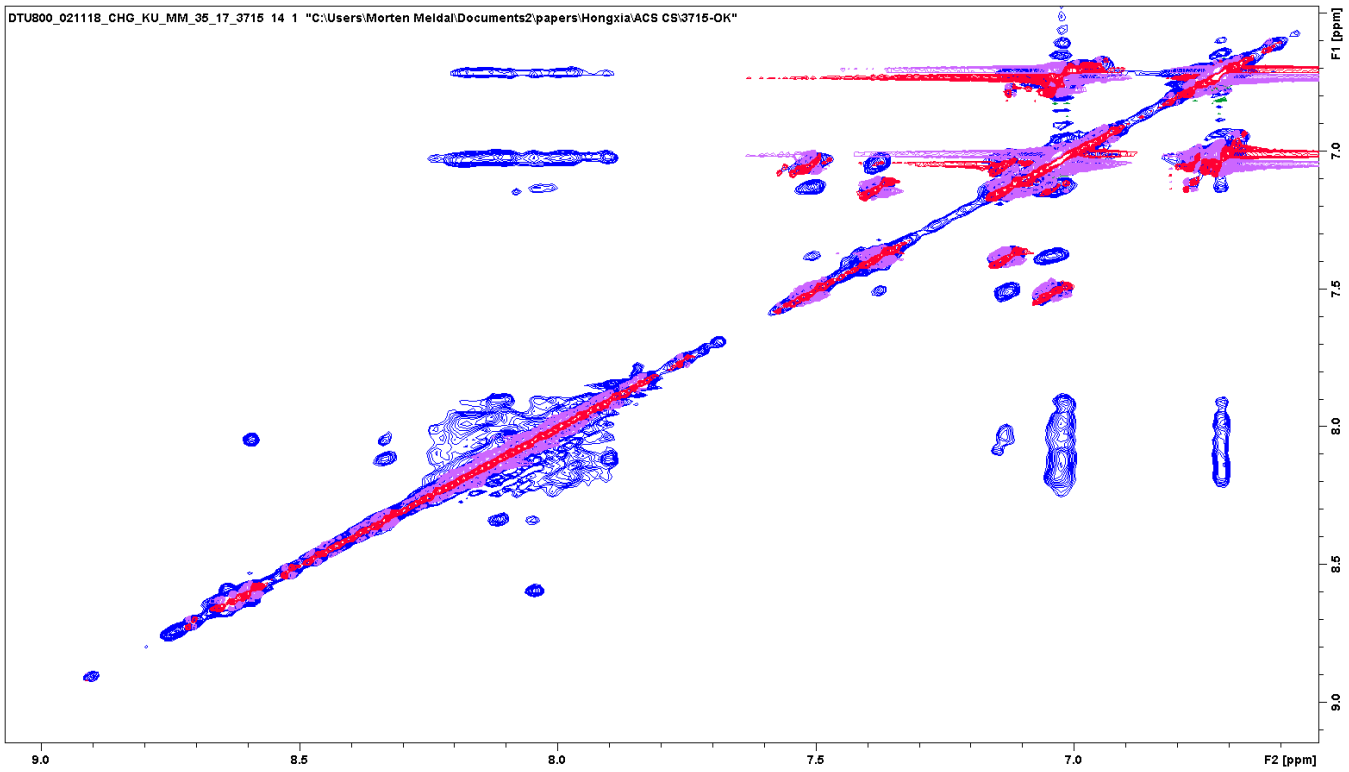


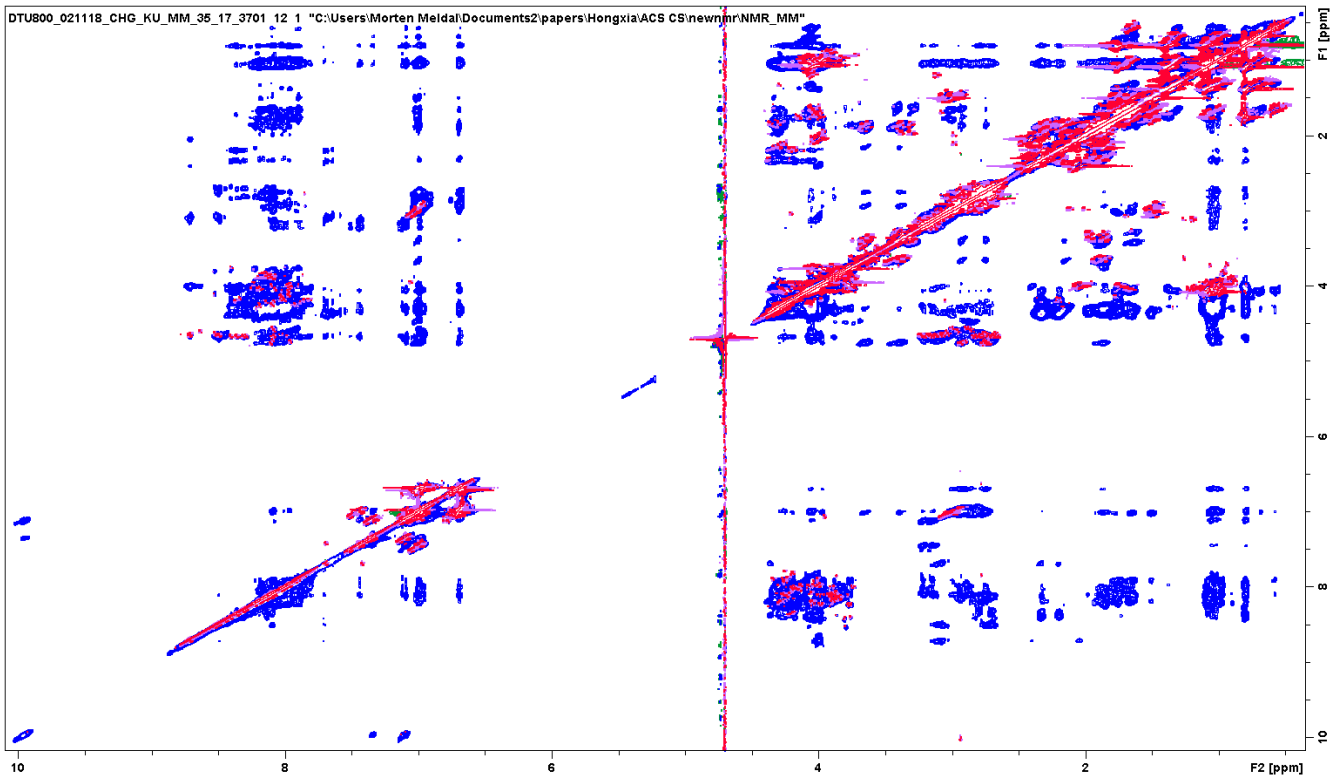
Figure SI-11. NOESY with overlay of DQF_COSY ^{1}H-NMR spectrum for compound **2** with expansion of important regions. The **s**pectra were analyzed by TOPSPIN (Bruker) and assigned (Table SI-1). Analysis of the NOE crosspeaks provided intra-residue NOE's (Table SI-2).

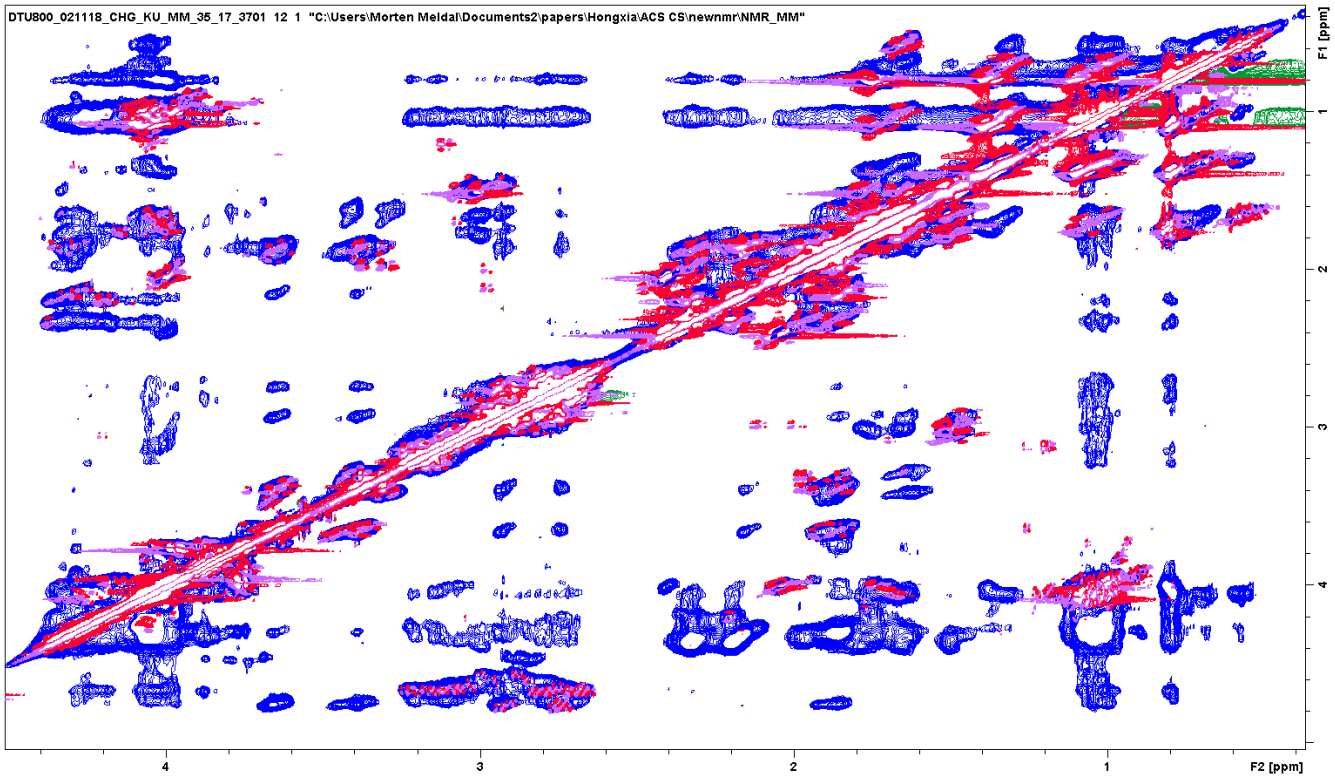
Table SI-1. Assignment of ^{1}H-NMR signals for compound **2**.

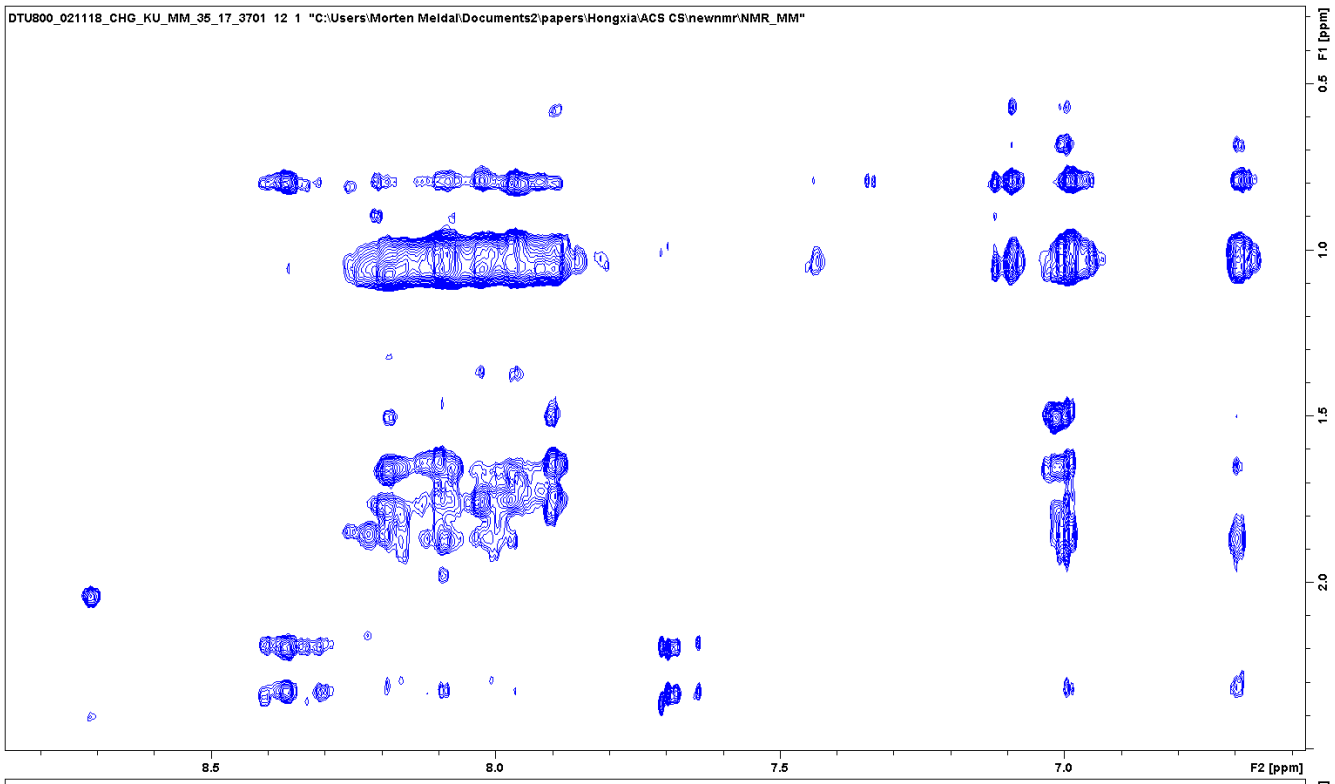
Peptide **2**		ETDTYTETYPGRTITWTITDG								
	NH	Ha	Hb1	Hb2	Hg1/H2	Hg2/H3	Hd1/H4	Hd2/H5	H6	H7
E		4.286	2.083		2.441					
T	8.626	4.295	4.068		1.106					
D	8.580	4.695	2.795	2.743						
T	7.966	4.160	3.954		0.984					
Y	8.086	4.522	2.983	2.901	7.011	6.690				
T	8.026	4.167	4.047		1.021					
E	8.105	4.249	1.926		2.299					
T	7.877	4.166	4.038		1.044					
Y	8.116	4.738	2.903	2.737	7.051	6.700				
P		4.317	2.139		1.863		3.648	3.426		
G	8.149	3.845	3.823							
R	8.029	4.058	1.755	1.643	1.487		2.993			
T	8.110	4.237	4.036	0.040	1.026					
I	8.169	4.019	1.645	1.486	0.995	0.572	0.693			
T	7.959	4.249	3.957		0.975					
W	8.063	4.599	3.215	3.148	7.123	9.966	7.359	7.112	7.019	7.488
T	7.734	4.123	4.055		1.055					
I	8.029	4.053	1.748	1.487	1.078	0.795	0.787			
T	8.029	4.236	4.096		1.080					
D	8.307	4.671	2.711	2.805						
G	8.144	3.830	3.850							

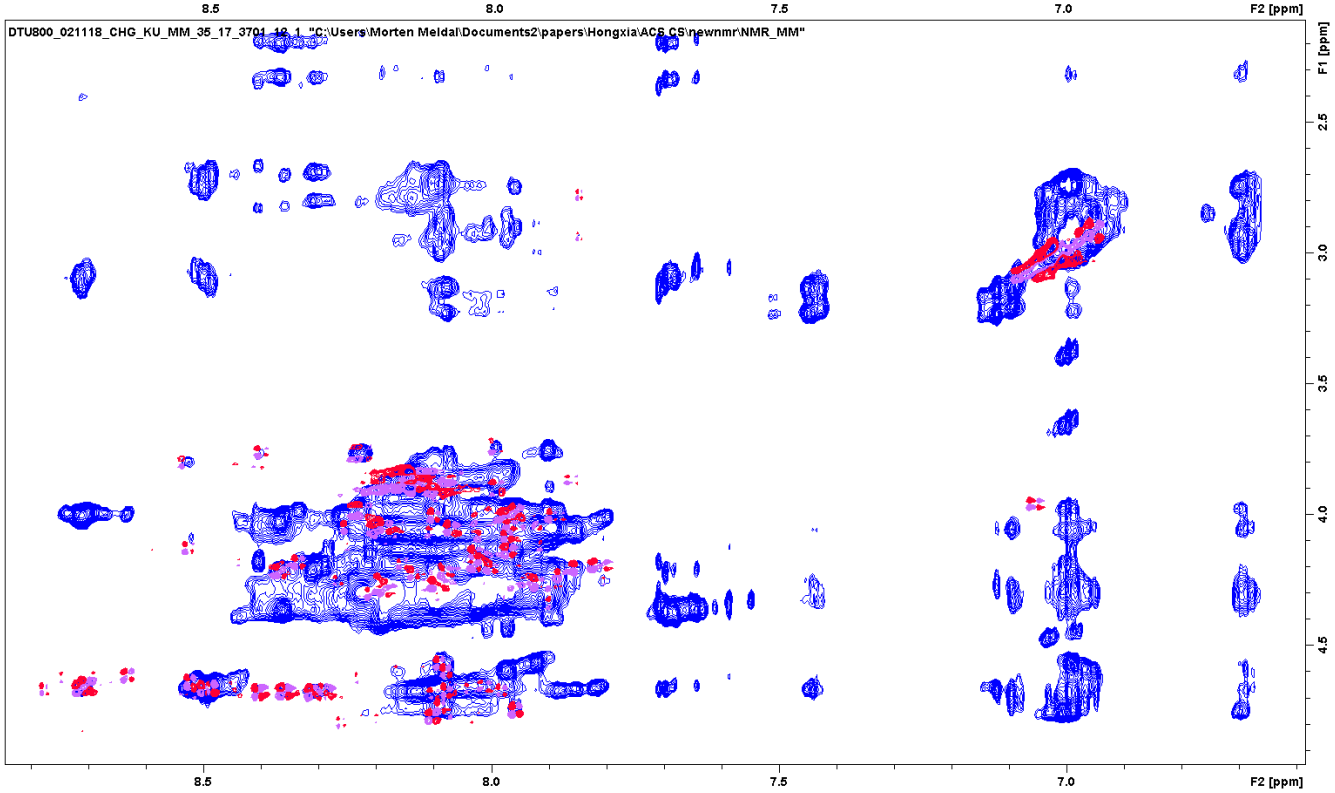
Table SI-2. Intra-residue NOE's identified for compound **2**. Only unambigously assigned NOE crosspeaks were included.

Peptide **2**	Intra-residue NOE's							
	NH	Ha	Hb1	Hb2	Hg1/H2	Hg2/H3	Hd1/H4	Hd2/H5
E					4.058			
T			2.441					
D			0,795					
T	7.011				8.026			
Y		7.877	7.123	7.123		0.787		
T	7.011							
E								
T	0.572							
Y	3.648/3.959		1.863	1.863		0.693		
P			2.737				8.116/7.959	7.959/8.116
G								
R			7.011	7.011			0.693	
T		1.755						
I						2.993		
T	3.959/3.648							
W			6.700		2.973	6.690	0.787	
T								
I		9.966	2.805			7.959	9.966	
T			8.307					
D	1.080/0.795			4.096				
G								


DTU800_021118_CHG_KU_MM_35_17_3701 12 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\newnmr\NMR_MM"
F1 [ppm]
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3701 12 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\newnmr\NMR_MM"
F1 [ppm]
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3701 12 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\newnmr\NMR_MM"
F1 [ppm]
0.5
1.0
1.5
2.0
8.5
8.0
7.5
7.0
F2 [ppm]


DTU800_021118_CHG_KU_MM_35_17_3701 12 1 "C:\Users\Morten Meldal\Documents2\papers\Hongxia\ACS CS\newnmr\NMR_MM"
F1 [ppm]
2.5
3.0
3.5
4.0
4.5
8.5
8.0
7.5
7.0
F2 [ppm]

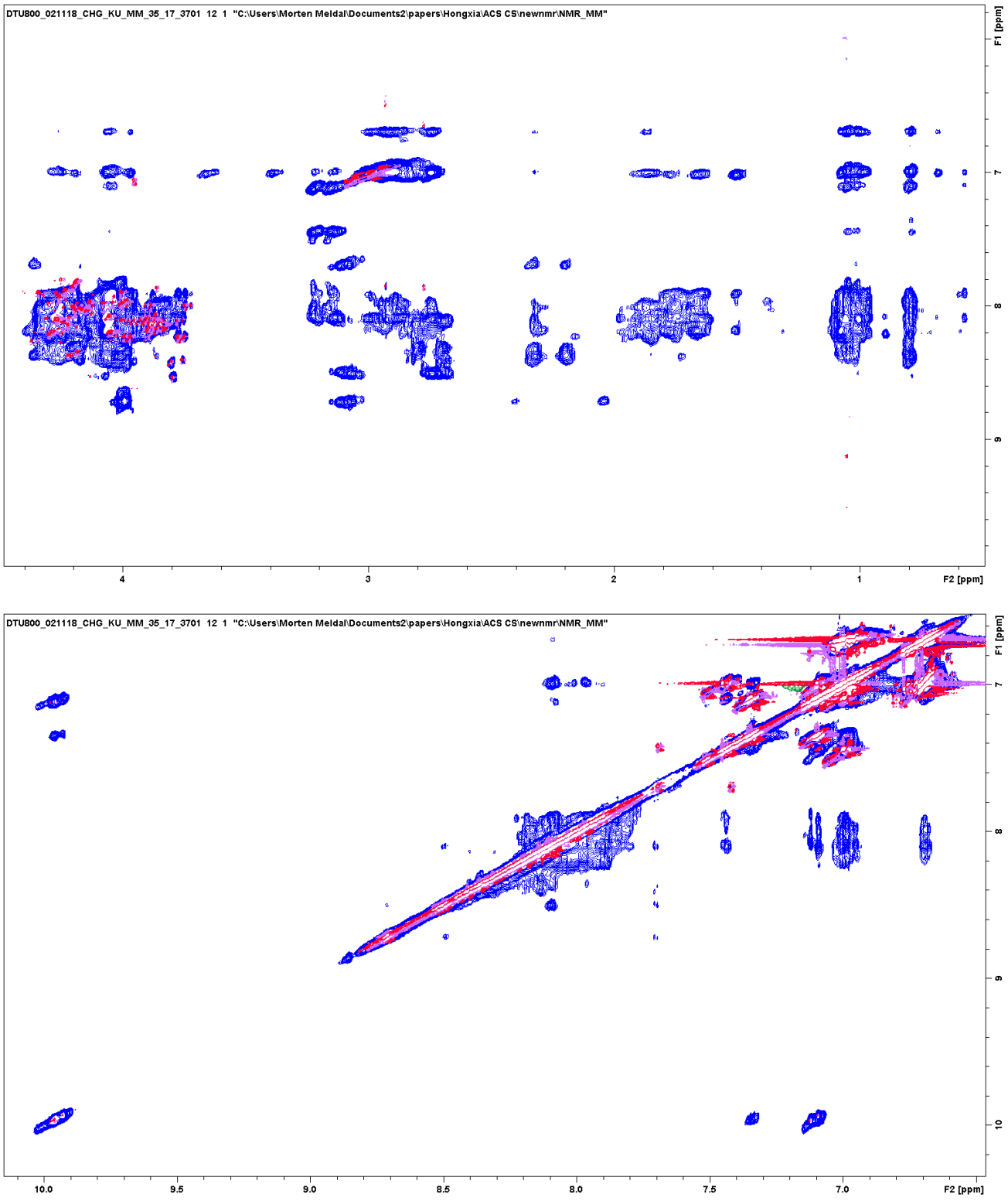


Figure SI-12. NOESY with overlay of DFQ-COSY ^{1}H-NMR spectrum for compound **9** with expansion of important regions. Spectra were analyzed by TOPSPIN (Bruker) and assigned (Table SI-3). Overlay of the NOESY spectra of **2** and **9** (not shown) indicated that the structure of the two compounds were very similar except for signals caused by the two residues linked by triazole in compound **9**.

Table SI-3. Assignment of ^{1}H-NMR signals for compound **9**.

Compound **9**	*c*-E-Abu-DTYTETYPGRTITWTI-Pra-DG								
	NH	Ha	Hb1	Hb2	Hg1/H2	Hg2/H3	Hd1/H4	Hd2/H5	H6	H7
E		3.986	2.026		2.398					
Abu	8.704	4.645	2.392	2.029	3.988					
D	8.488	4.659	2.807	2711						
T	7.995	4.190	3.960		1.000					
Y	8.086	4.553	2.983	2.876	6.990	6.690				
T	8.088	4.255	4.054		1.072					
E	8.024	4.183	2.185		2.315					
T	7.914	4.254	4.012		1.051					
Y	8.116	4.758	2.918	2.739	6.990	6.700				
P		4 .223	2.147		1.955	1.851	3.648	3.426		
G	8.215	3.746	3.968							
R	7.960	3.985	1.755	1.653	1.495		2.993			
T	8.174	4.250	4.007		1.000					
I	8.064	4.047	1.654	1.308	1.001	0.568	0.693			
T	7.889	4.310	4.068		1.044					
W	8.063	4.653	3.216	3.122	7.115	9.951	7.331	7.083	6.984	7.433
T	8.157	4.307	4.008		1.035					
I	7.966	4.018	1.745	1.362	1.076	0.795	0.785			
Pra	8.502	4.650	2.920	2.744		7.881				
D	8.304	4.656	2.681	2.777						
G	7.985	3.720	3.884							

Sequences and mass spectrometry data of β-bodies 1–10

Purity based on the HPLC UV trace at 214 nm or cumulative from PDA

ROX-labeled peptides contain both fluorophore isomers. In some cases the β-bodies showed two or three peaks with identical masses in LCMS. Methionine containing peptides oxidized over time in dilute solutions.

GFP-β-body **1**

TETKTVTITRPKMTWTFTHTVTG

Chemical formula: $C_{117}H_{189}N_{31}O_{36}S$

Calculated mass: 2636.36; found: $[M+2H]^{2+}$ 1319.1788; $[M+3H]^{3+}$ 879.7903; $[M+4H]^{4+}$ 660.0964; $[M+5H]^{5+}$ 528.2790

IL1β-β-body **2**

ETDTYTETYPGRTITWTITDG

Chemical formula: $C_{106}H_{157}N_{25}O_{40}$

Purity: 94%

Calculated mass: 2420.10; found: $[M+2H]^{2+}$ 1211.0486; $[M+3H]^{3+}$ 807.7087; found as well: $[M+2H]^{2+}$ 1202.0496; $[M+3H]^{3+}$ 801.7070 (N-term. Glu>pyro-Glu), and $[2M+H]^{3+}$ 1614.4115

IL1β-β-body **3**

TWTETYTWTEPGDTQTLTITNTG

Chemical formula: $C_{115}H_{169}N_{27}O_{43}$

Purity: 93%

Calculated mass: 2616.19; found: $[M+2H]^{2+}$ 1309.0953; $[M+3H]^{3+}$ 873.0563; $[M+4H]^{4+}$ 660.1065

IL2-β-body **4**

NTVTNTMTRPGVTETVTQTDG

Chemical formula: $C_{89}H_{151}N_{27}O_{37}S$

Calculated mass: 2222.05; found: $[M+H]^{1+}$ 2223.3430; $[M+2H]^{2+}$ 1112.1760; $[M+3H]^{3+}$ 741.7905

IL2-β-body **5**

TRTLTYTEPGITQTKTEAG

Chemical formula: $C_{88}H_{146}N_{24}O_{33}$

Purity: 90%

Calculated mass 2067.05; $[M+2H]^{2+}$ 1034.4596; $[M+3H]^{3+}$ 689.9866; $[2M+3H]^{3+}$ 1378.9807

IL6-β-body **6**

HTWTDTLTRPGYTVTHTLTLG

Chemical formula: $C_{106}H_{163}N_{29}O_{33}$

Calculated mass 2370.20; found: $[M+2H]^{2+}$ 1186.0591; $[M+3H]^{3+}$ 791.0399; $[M+4H]^{4+}$ 593.5319

IL6-β-body **7**

TMTDTDTYPGFTDTLTHAG

Chemical formula: $C_{87}H_{129}N_{21}O_{34}S$

Calculated mass 2043.87; found: $[M+2H]^{2+}$ 1022.9030; $[M+3H]^{3+}$ 682.2690

Bridged GFP-β-body **8**

c-Pra-ETKTVTITRPKMTWTFTHTV-Dab(N_3)-G, clicked

Chemical formula: $C_{118}H_{186}N_{34}O_{34}S$

Calculated mass: 2655.36; found: $[M+2H]^{2+}$ 1328.6852; $[M+3H]^{3+}$ 886.1251; $[M+4H]^{4+}$ 664.8465; $[M+5H]^{5+}$ 532.0791

Bridged IL1β-β-body **9**

c-E-Pra-DTYTETYPGRTITWTI-Dab(N_3)-DG, clicked

Chemical formula: $C_{107}H_{154}N_{28}O_{38}$

Calculated mass: 2439.10; found: $[M+2H]^{2+}$ 1220.5137; $[M+3H]^{3+}$ 814.0156; $[2M+3H]^{3+}$ 1627.0590

IL1β-β-body **10**

TWTDTATEPGYTMTATGTG

Chemical formula: $C_{83}H_{124}N_{20}O_{33}S$

Calculated mass: 1960.84; found: $[M+H]^{1+}$ 1961.7426; $[M+2H]^{2+}$ 981.3540; $[2M+3H]^{3+}$ 1308.1576; $[M+3H]^{3+}$ 654.5898

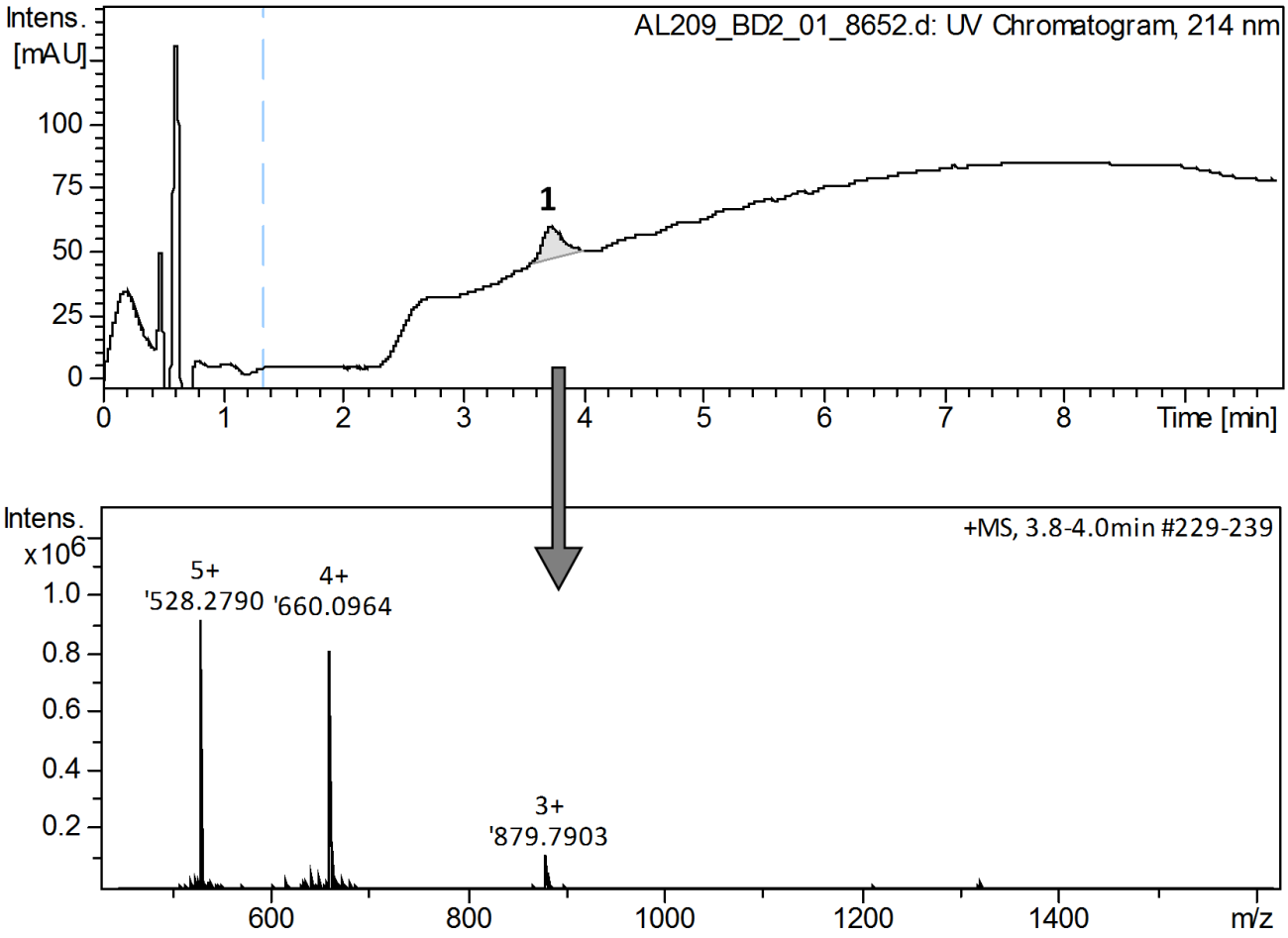


Figure SI-13. HPLC chromatogram and MS spectrum of purified GFP β-body **1**.

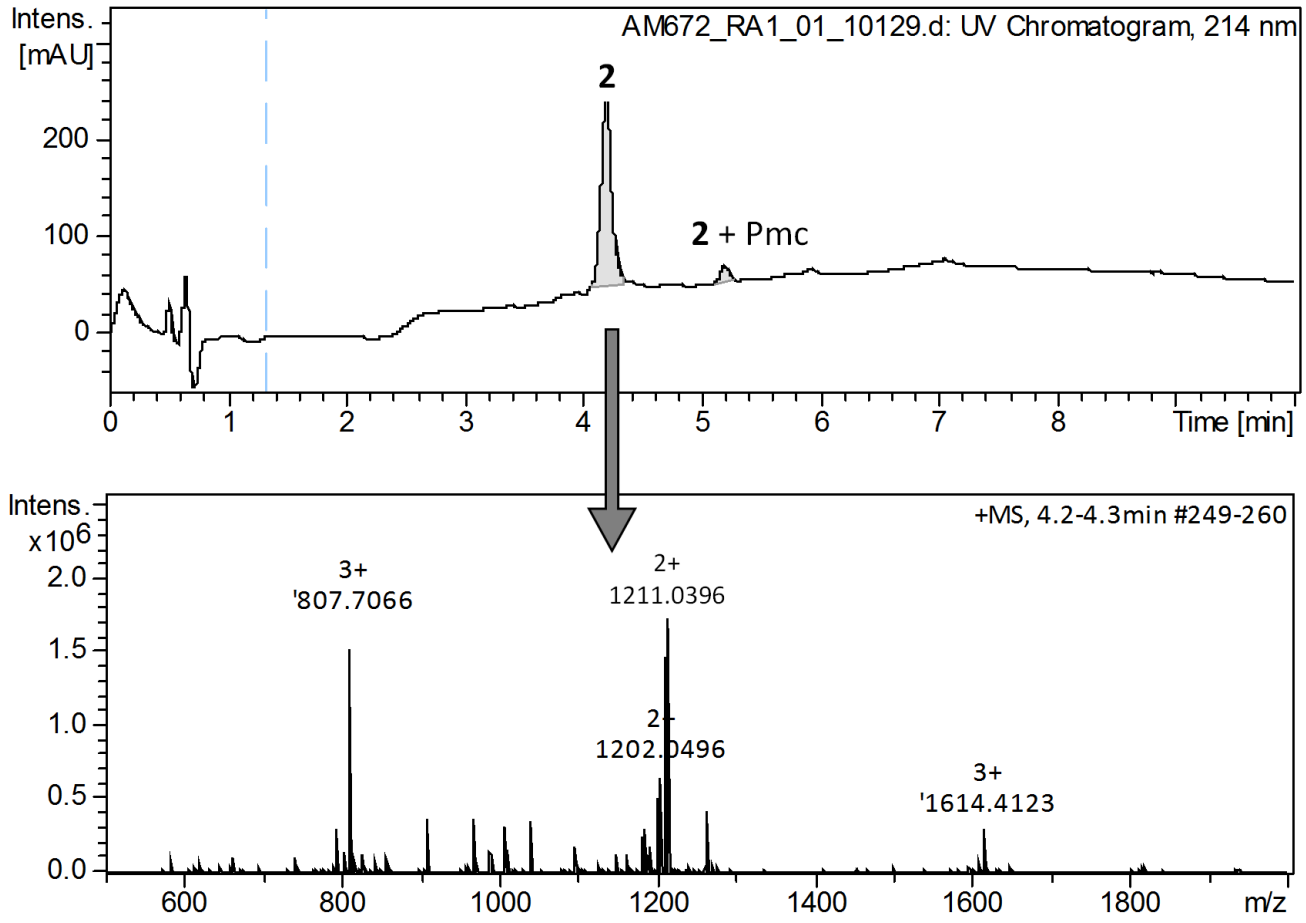


Figure SI-14. HPLC chromatogram and MS spectrum of purified IL-1β β-body **2** (calculated mass: 2420.10). The derivative in which the N-terminal Glu was converted in pyro-Glu was present as well ($[M+2H]^{2+}$ 1202.0496).

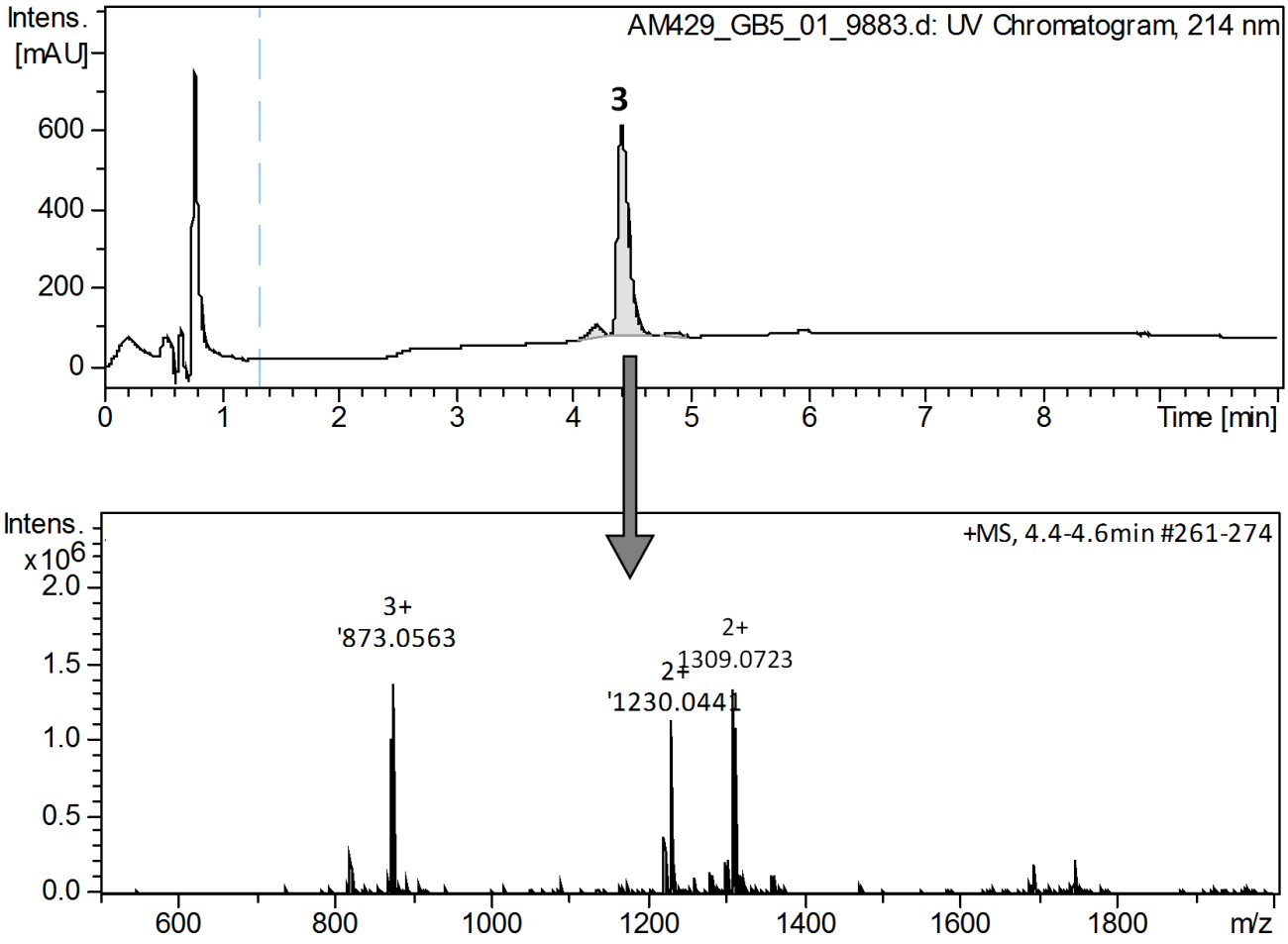


Figure SI-15. HPLC chromatogram and MS spectrum of purified IL-1β β-body **3** (calculated mass: 2616.19). A truncated version missing the two C-terminal residues Tyr-Gly (calculated mass: 2458.12) co-eluted with the full-length peptide ($[M+2H]^{2+}$ 1230.0441).

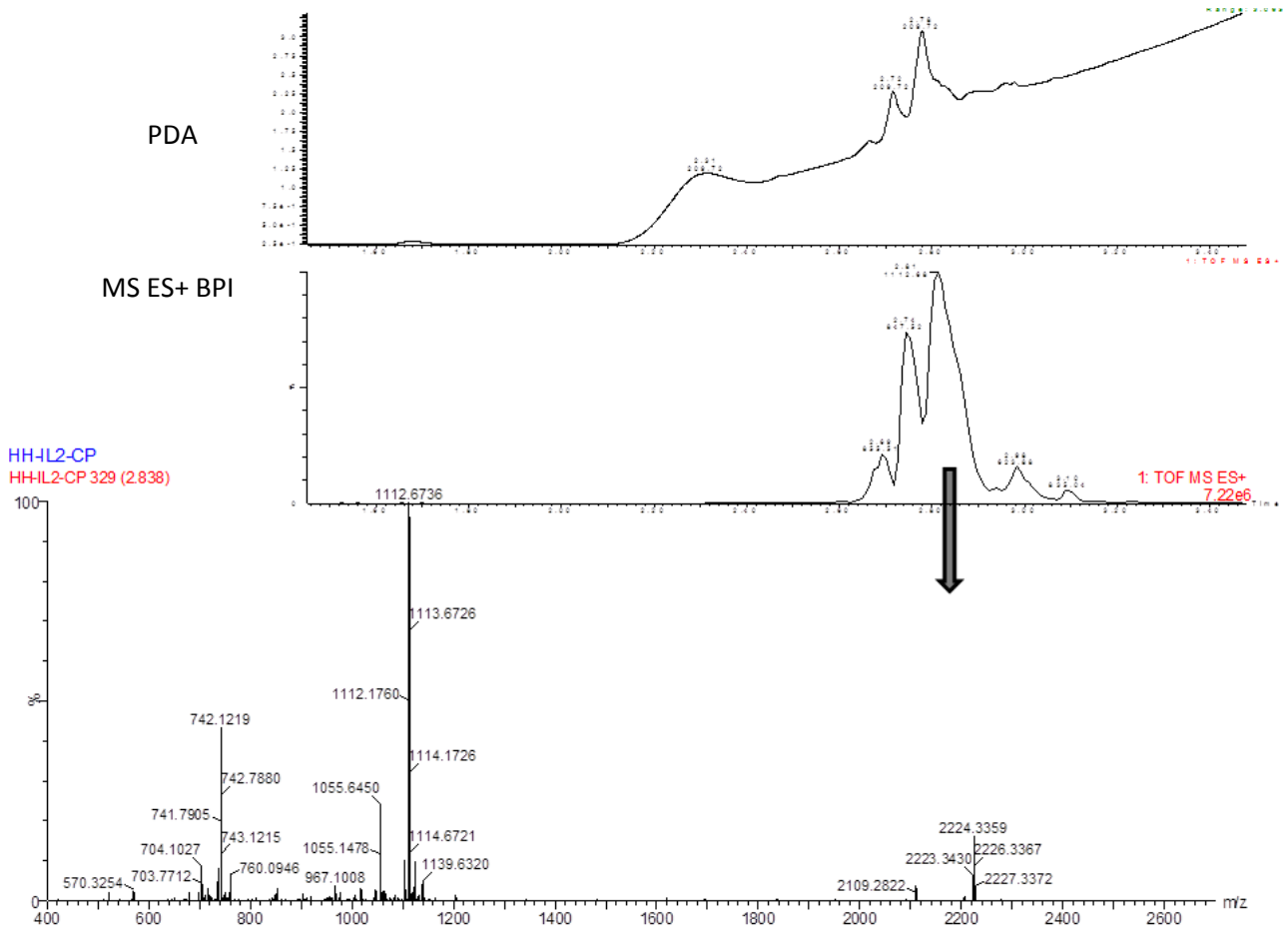


Figure SI-16. HPLC chromatogram and MS spectrum of IL-2 β-body **4**, The peaks all have the same mass and converge into one peak upon high dilution of the injection sample.

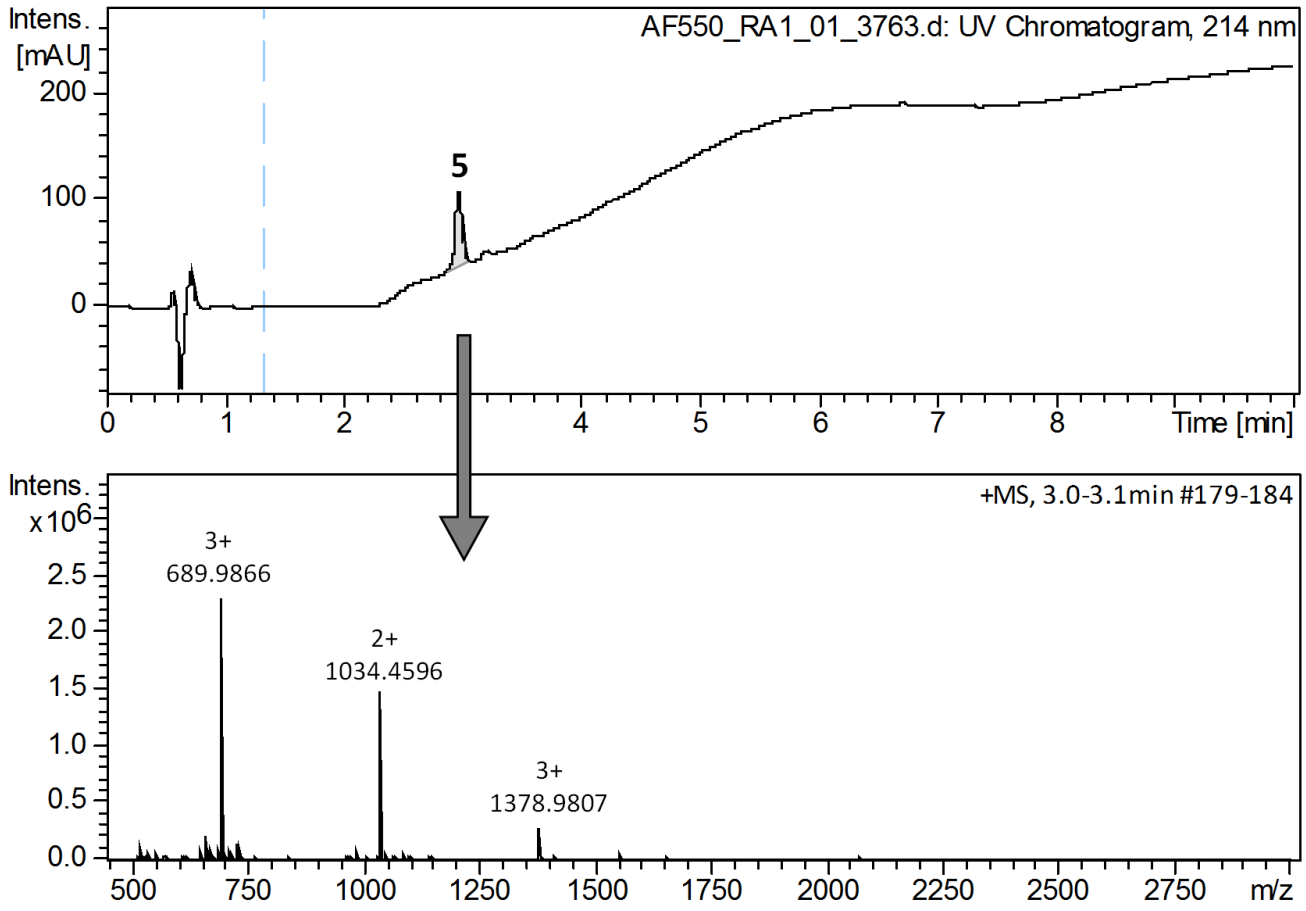


Figure SI-17. HPLC chromatogram and MS spectrum of IL-2 β-body **5**.

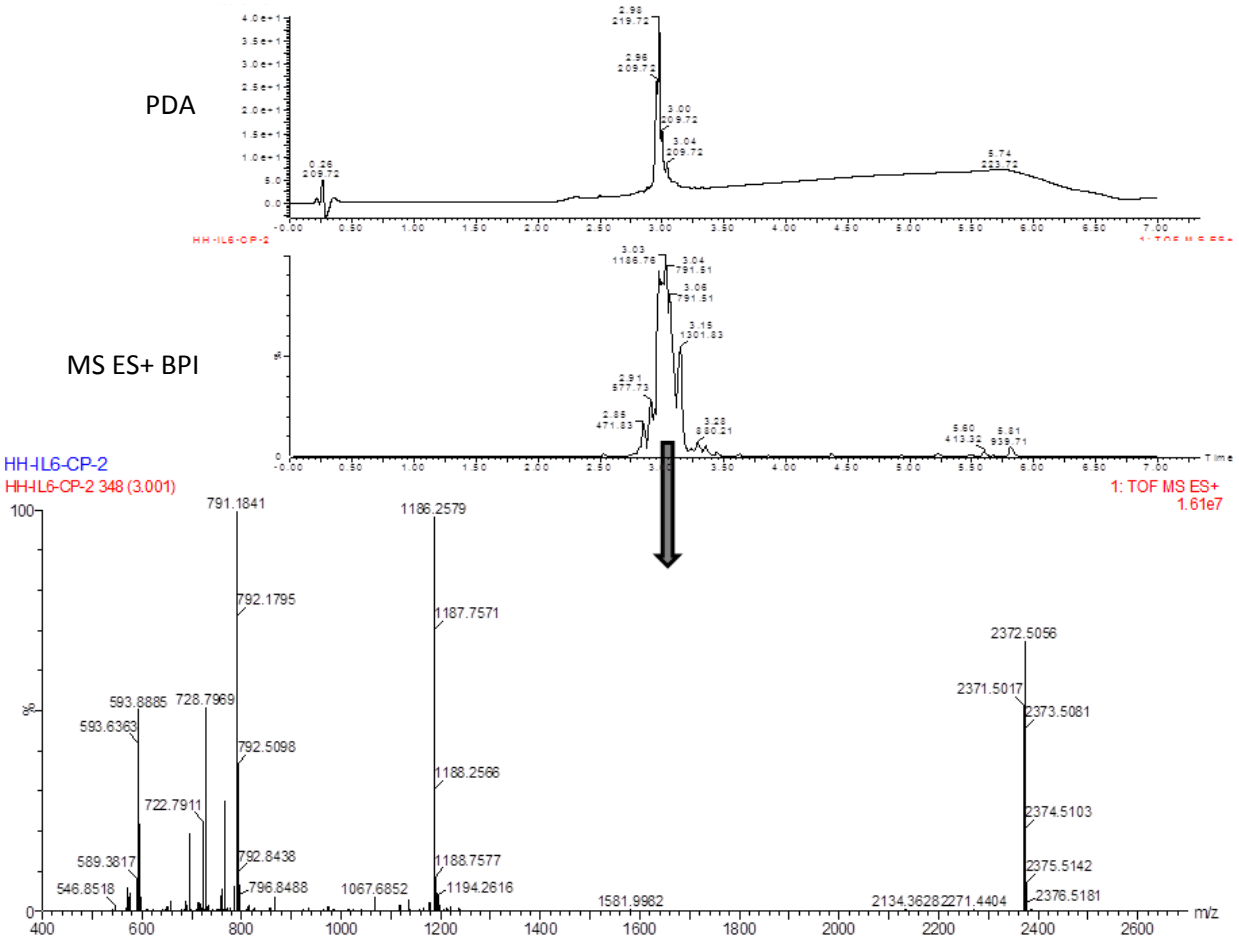


Figure SI-18. HPLC chromatogram and MS spectrum of IL-6 β-body **6**.

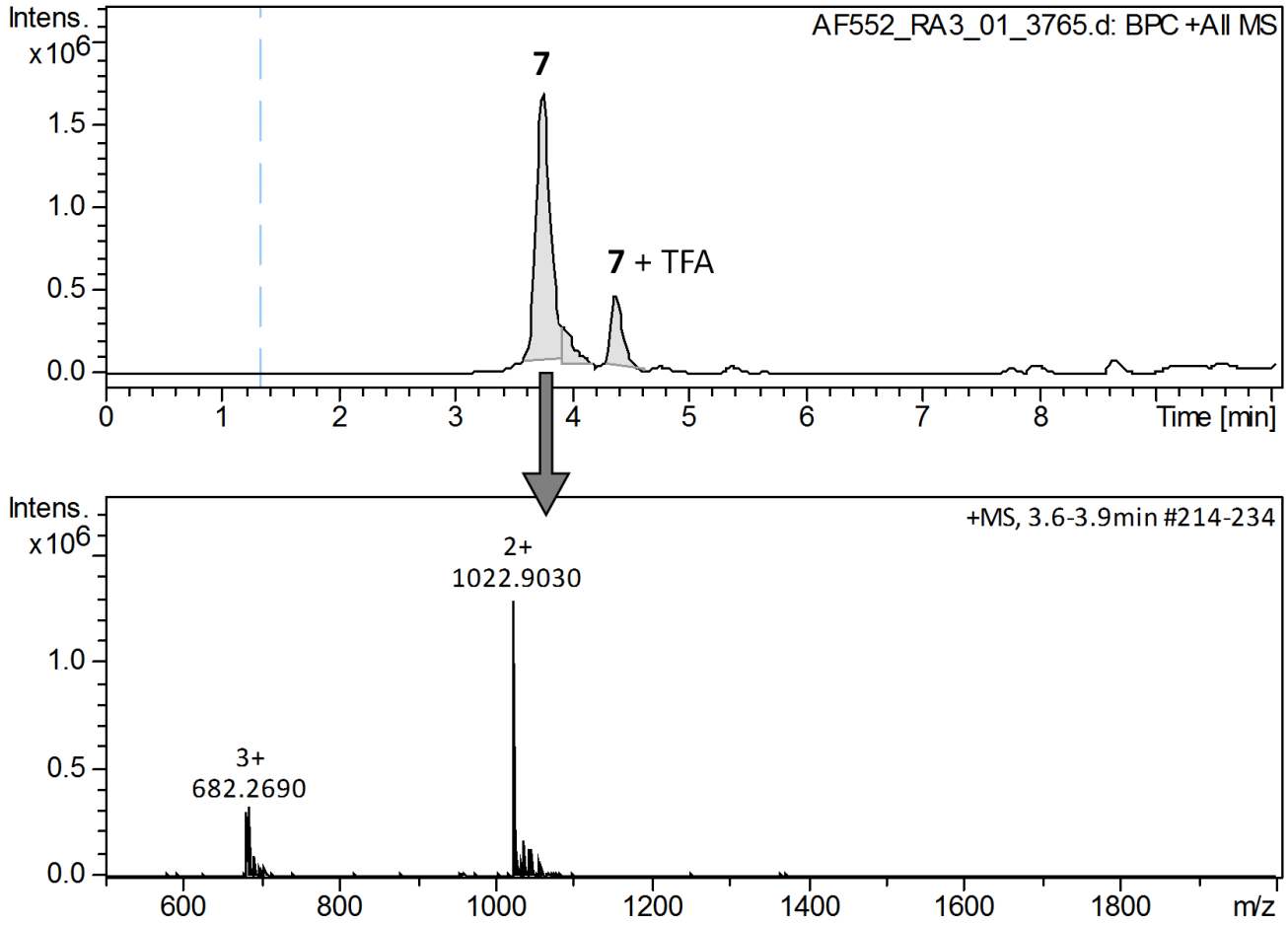


Figure SI-19. HPLC chromatogram and MS spectrum of IL-6 β-body **7**.

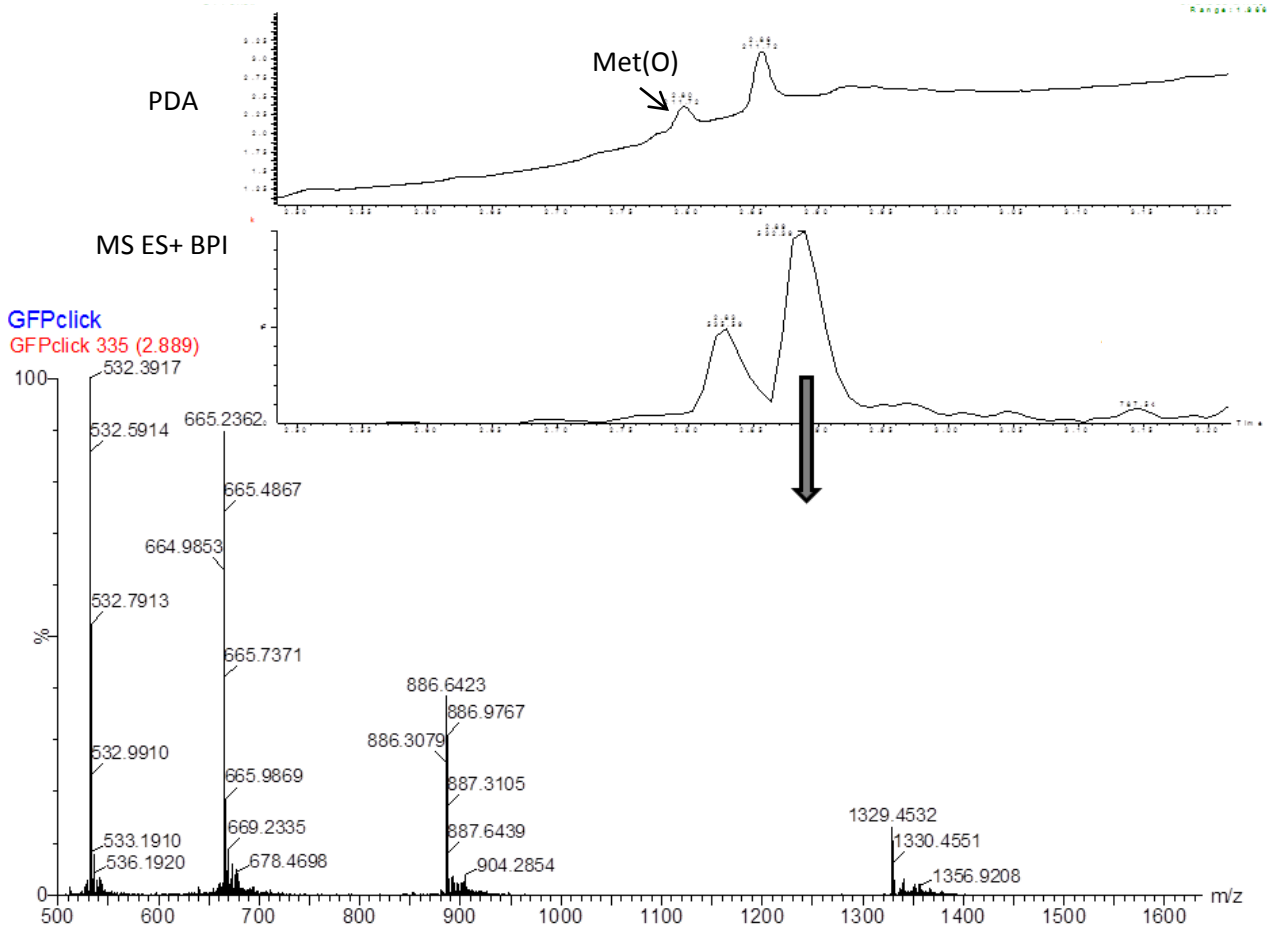


Figure SI-20. HPLC chromatogram and MS spectrum of triazole-bridged GFP β-body **8**.

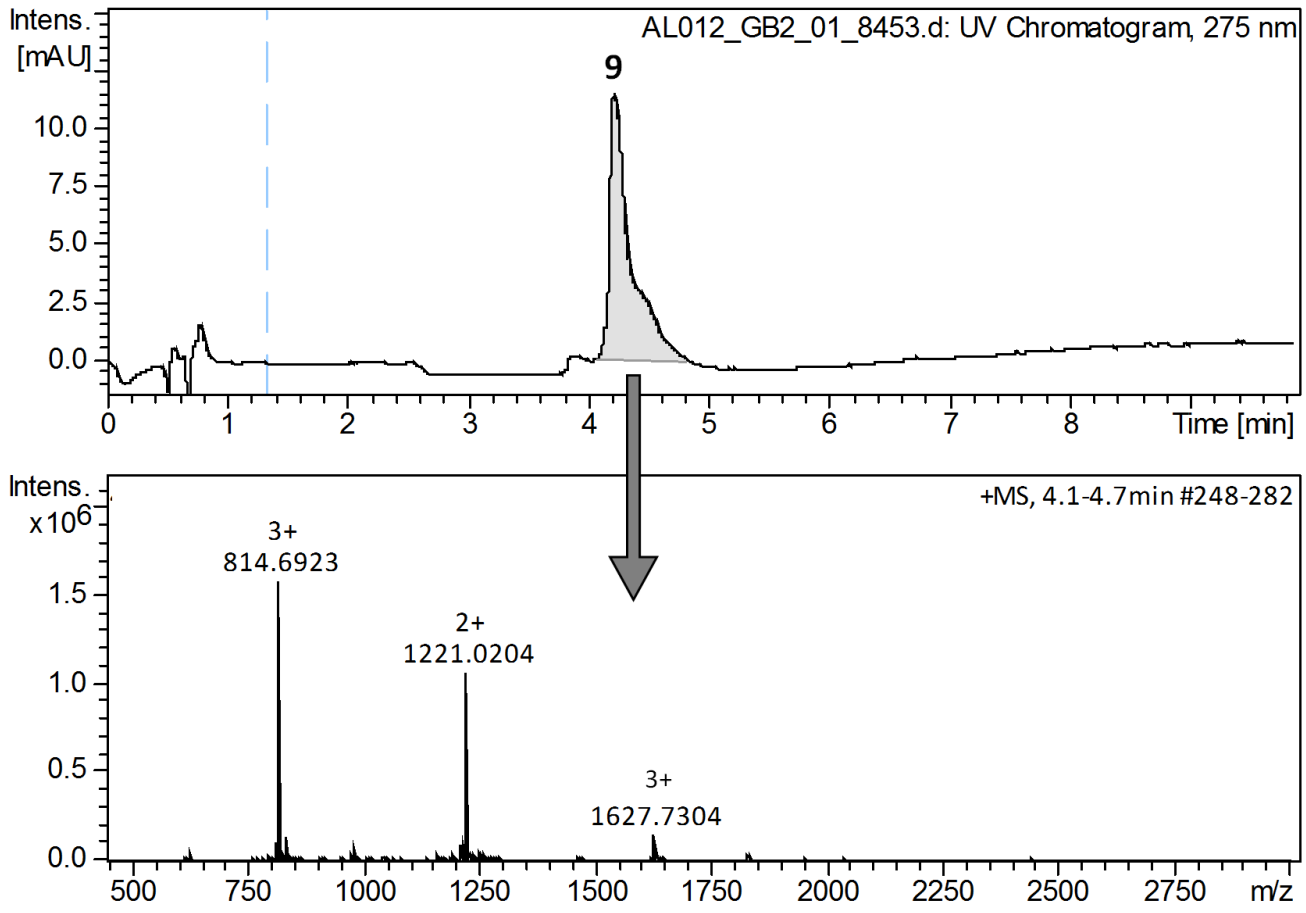


Figure SI-21. HPLC chromatogram and MS spectrum of triazole-bridged IL-1β β-body **9**.

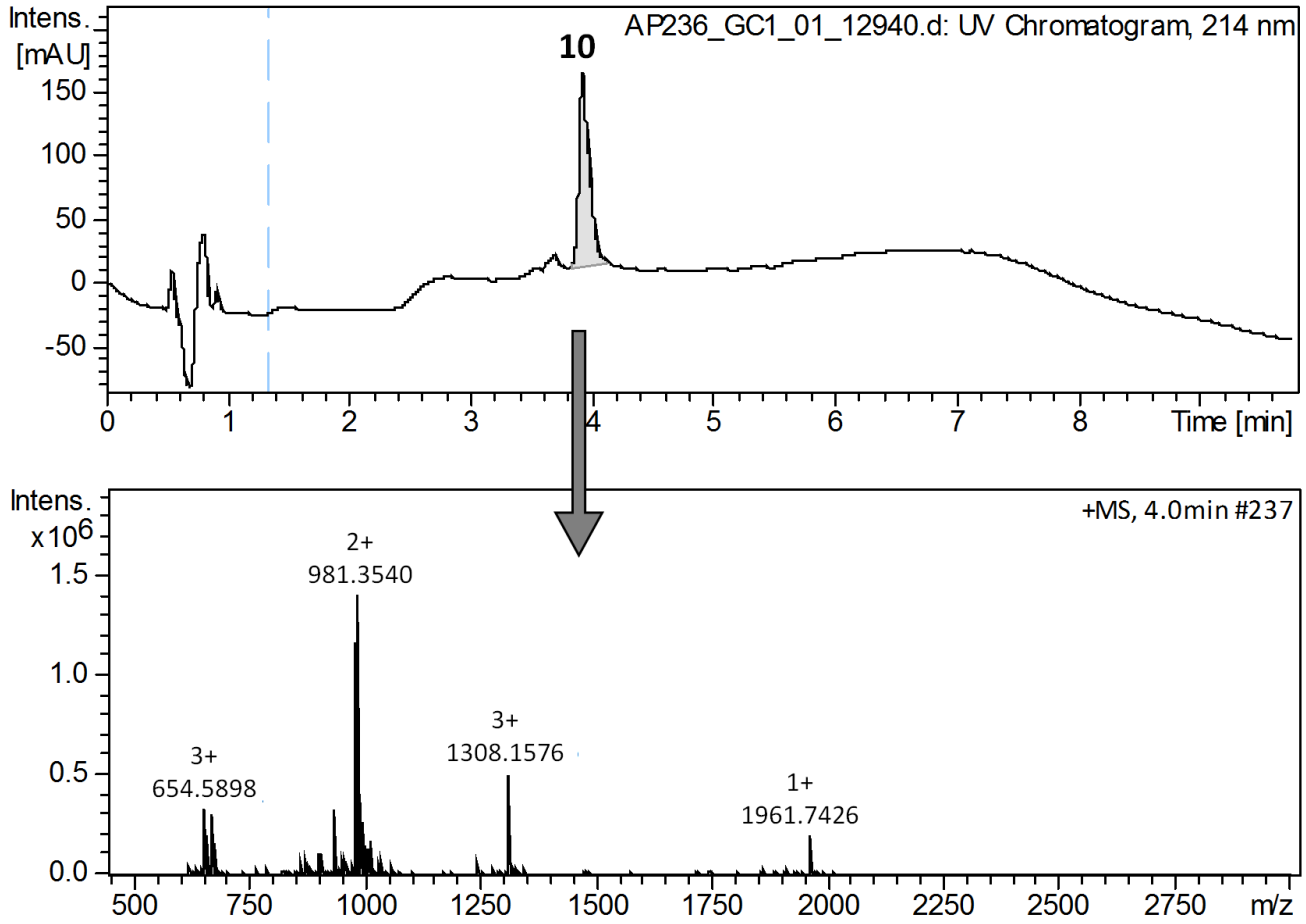


Figure SI-22. HPLC chromatogram and MS spectrum of IL-1β β-body **10**.

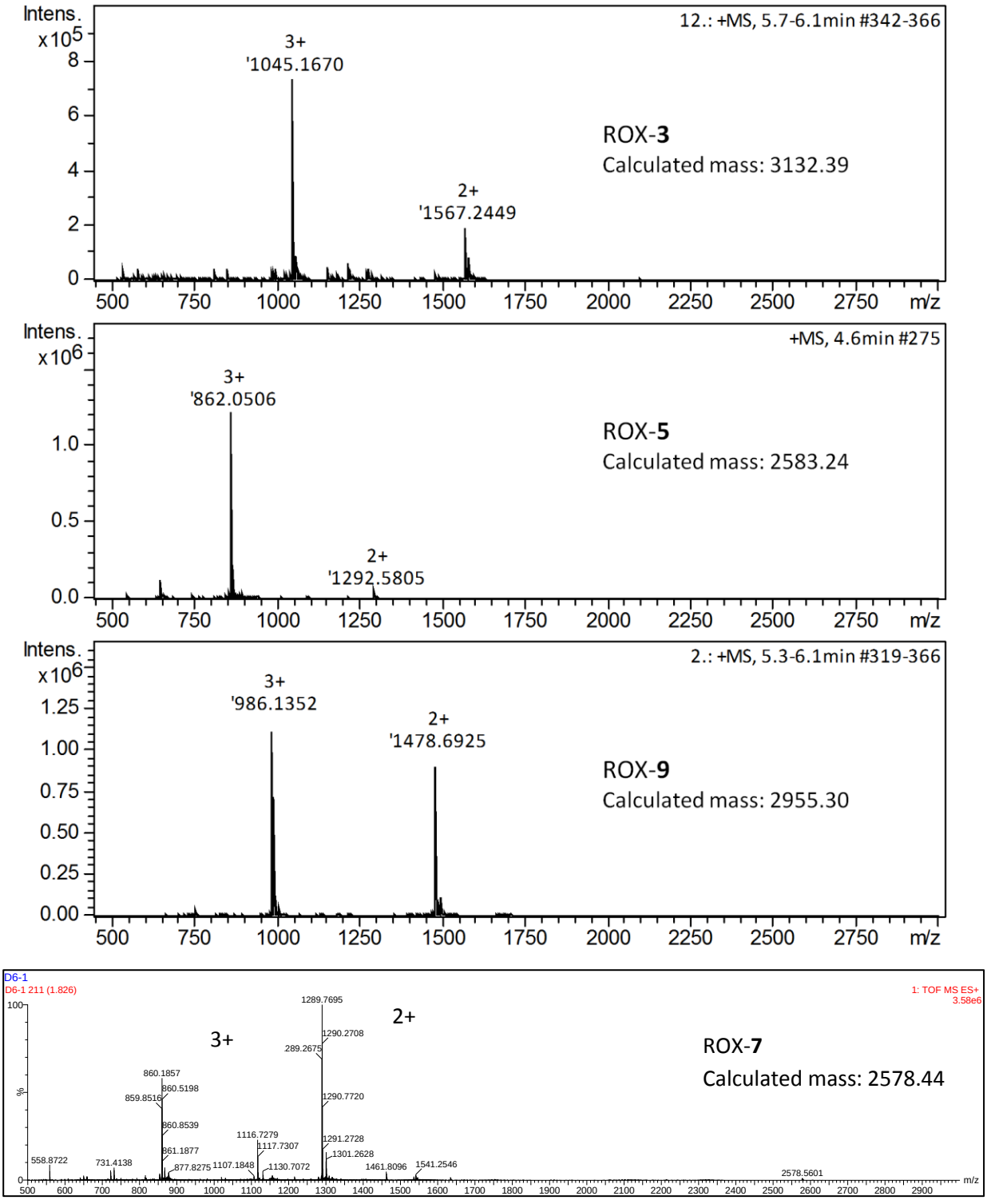


Figure SI-23. MS spectra of ROX-labeled β-body **3**, **5**, **7** and **9**.

Sequences and mass spectrometry data of GFP, IL-1β and IL-1α

GFP

GSSHHHHHHSSGLVPRGSHMLEKREAEAGRLGAGGPVATMVSKGEELFTGVVPILVELDGDVNGHKF
SVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQE
RTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKV
NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLG
MDELYK

Molecular formula (after formation of the fluorophore): $C_{1377}H_{2126}N_{380}O_{416}S_9$
Calculated average molecular weight: 30948.55 Da
Detected M_r: 30947.44, and 31125.34 (gluconoylated)

IL1β

GSSHHHHHHSSGLVPRGSAPVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQG
EESNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNKLEFESAQFP
NWYISTSQAENMPVFLGGTKGGQDITDFTMQFVSS

Molecular formula: $C_{852}H_{1334}N_{234}O_{260}S_8$
Calculated average molecular weight: 19271.67 Da
Found M_r: 19270.84, 19400.73 (retention of N-terminal Met) and 19449.13 (gluconoylated)

IL1α

SAPFSFLSNVKYNFMRIIKYEFILNDALNQSIIRANDQYLTAAALHNLDEAVKFDMGAYKSSKDDAKIT
VILRISKTQLYVTAQDEDQPVLLKEMPEIPKTITGSETNLLFFWETHGTKNYFTSVAHPNLFIATKQDYW
VCLAGGPPSITDFQILENQALEHHHHHH

Molecular formula: $C_{870}H_{1325}N_{225}O_{253}S_4$
Calculated average molecular weight: 19112.48 Da
Found M_r: 19111.22, and 19511.18 (unknown modification)

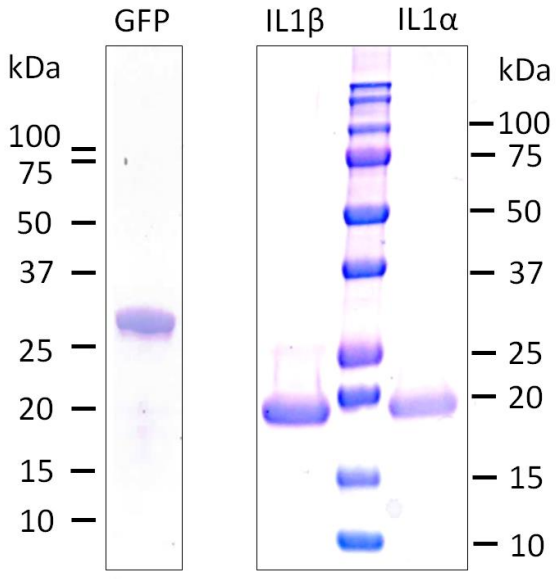


Figure SI-24. SDS-PAGE gels depicting the purity of GFP (30.9 kDa), IL-1β (19.3 kDa) and IL-1α (19.1 kDa). The Precision Plus Protein™ Dual Color standard (BioRad) was used as molecular weight marker.

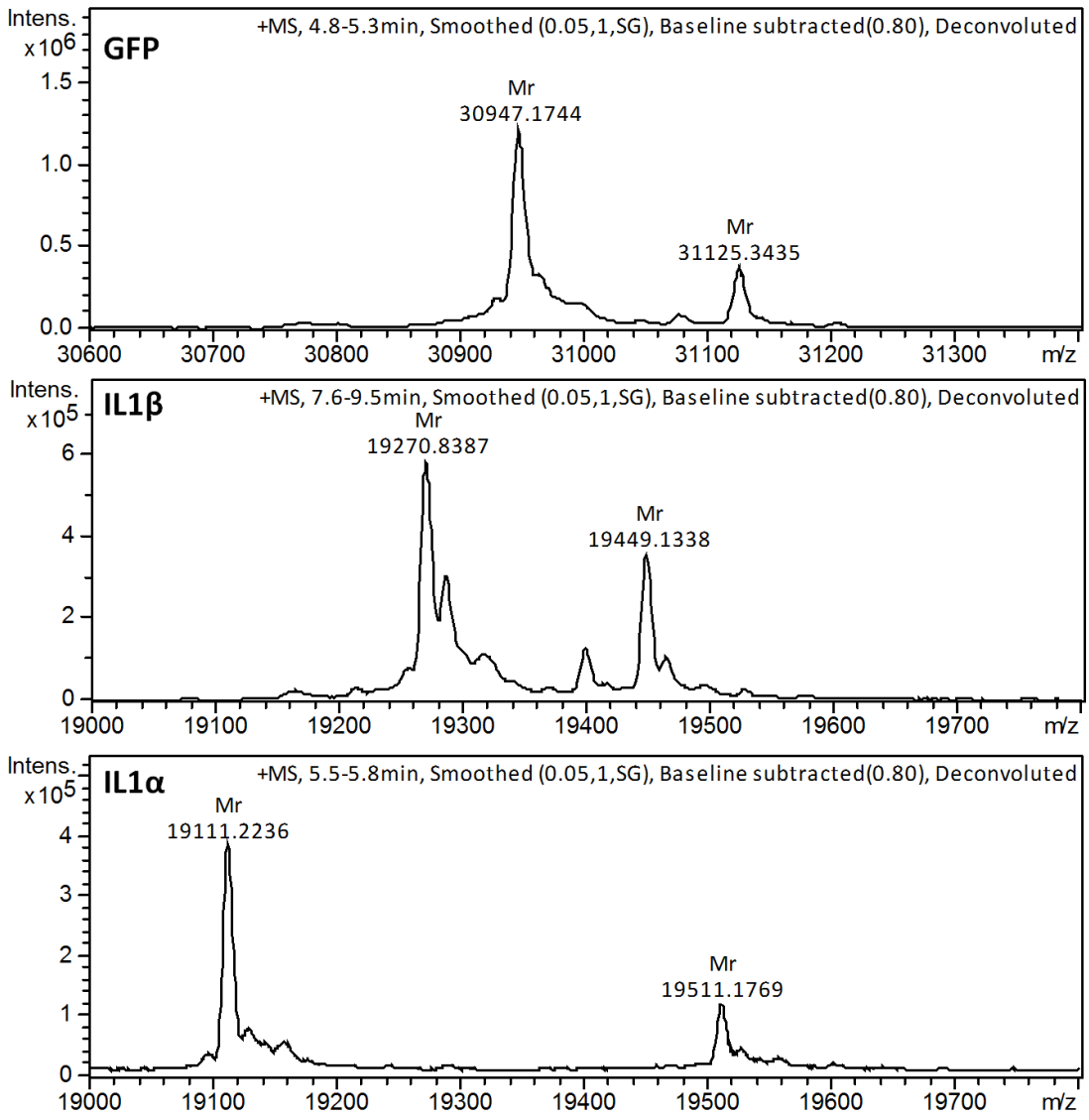


Figure SI-25. Deconvoluted MS spectra of purified GFP, IL-1β and IL-1α. In addition to the unmodified proteins, adducts were detected corresponding to the gluconoylated protein (GFP and IL-1β, +178 Da) and an unknown derivative (IL-1α, +400 Da). Spontaneous non-enzymatic gluconoylation is known to occur in proteins that are recombinantly expressed in *E. coli* BL21(DE3), particularly when they contain an N-terminal His-tag.[1]

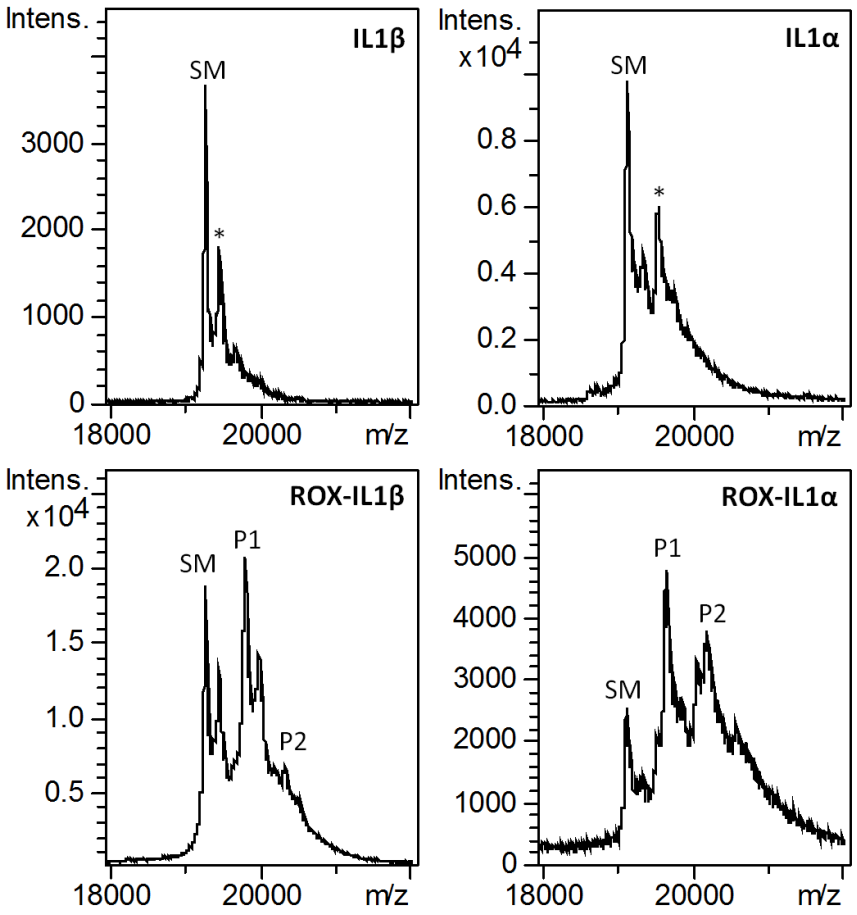


Figure SI-26. MALDI-TOF spectra of (top) unlabeled and (bottom) ROX-labeled IL-1β and IL-1α. Unlabeled and posttranslationally modified proteins are indicated with SM and with a '*', respectively. Product peaks are assigned with P, where the number indicates the number of fluorophores attached (calculated mass shift per fluorophore moiety: 516.2 Da).

In vivo test of immunogenicity

A selection of 15 female 8 weeks old BALB/cJTac mice (Taconic Europe, Lille Skensved, Denmark) were housed at the barrier protected animal facility, Faculty of Health and Medical Sciences, University of Copenhagen under standard conditions in open cages without filter lids. Housing and health monitoring was conducted according to FELASA guidelines.[2] All mice had free access to standard quality tap water and fed ad libitum Altromin 1324 diet (Brogaarden, Lynge, Denmark). The experiment was conducted in line with the Danish Act on Animal experimentation (LBK 474 of 15/05/2014), which implements the Directive 2010/63/EU on the protection of animals in scientific research. The study was approved by the Animal Experimentation Inspectorate, Ministry of Environment and Food, Denmark (License No 2017-15-0201-01262). Eight mice were weighed and injected IP with 1 mg/kg β-body **2** dissolved in PBS and seven mice were weighed and injected IP with similar amounts of PBS. 16 hours later, mice were weighed again and blood was sampled from the oral cavity of anaesthetized mice which were subsequently euthanized. The blood was centrifuged at 8000g for 8 min and plasma was used to measure cytokines with a multiplex V-PLEX plus proinflammatory panel mouse kit (Mesoscale Discovery, Rockville, MD) according to manufacturer's instructions. IFN-γ, TNF-α, IL-1β, IL-2, IL-5, IL-6, IL-10, CXCL1, IL-12p70, IL-4 were included in the kit. IL-4 was below detection range. Results were read and analyzed on a MESO QuickPlex SQ120.

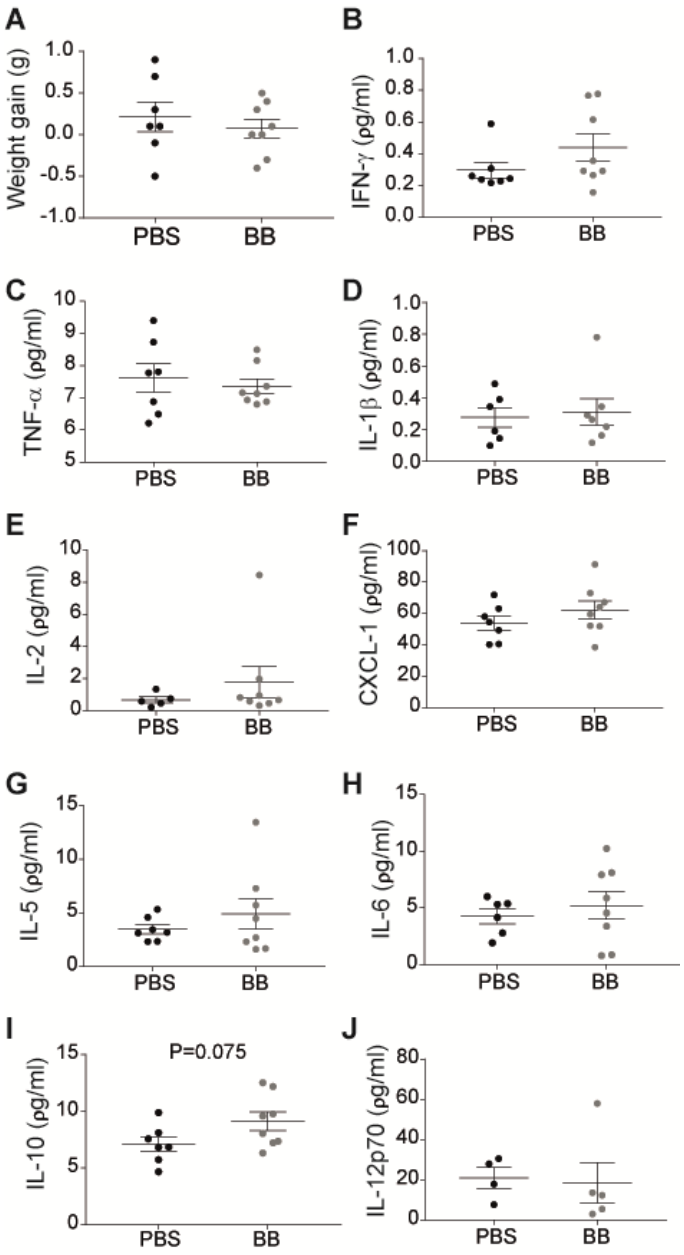


Figure SI-27. Measurement of weightloss and cytokine levels as a measure of immunogenicity in Balb-C mice upon IP-administration of β-body **2** as described above. Over a 16 h period neither weightloss nor cytokine levels indicated any effects of β-body **2** on the immune system. There seemed to be no toxic effects of the β-body. A) Loss of body mass; B) INF-γ; C) TNF-α; D) IL-1β) IL-2; F) CXCL-1; G) IL-5; H) IL-6; I) IL-10; J) IL-12. For IL-4 no signal was observed in the control assay and the IL-4 data were therefore not included.

References

1. C.E. Stotz R.T. Borchardt C.R. Middaugh T.J. Siahaan D. Vander Velde E.M. Topp. Secondary structure of a dynamic type I'β-hairpin peptide. J. Peptide Research, **2004**, *63*, 371-382. **DOI:** 10.1111/j.1399-3011.2004.00129.x
2. Geoghegan, K. F.; Dixon, H. B. F.; Rosner, P. J.; Hoth, L. R.; Lanzetti, A. J.; Borzilleri, K. A.; Marr, E. S.; Pezzullo, L. H.; Martin, L. B.; LeMotte, P. K.; McColl, A. S.; Kamath, A. V.; Stroh, J. G., Spontaneous α-N-6-phosphogluconoylation of a His tag in Escherichia coli: The cause of extra mass of 258 or 178 Da in fusion proteins, *Analyt. Biochem.* **1999**, *267*, 169–184. **DOI:** 10.1006/abio.1998.2990
3. Mähler, M.; Berard, M.; Feinstein, R.; Gallagher, A.; Illgen-Wilcke, B.; Pritchett-Corning, K.; Raspa, M., FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units, *Lab. Anim.*, **2014**, *48*, 178-192. **DOI:** 10.1177/0023677213516312