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Experimental Methods

Materials

All solvent and reagents used during peptide chain assembly were peptide synthesis grade and purchased from commercial suppliers unless otherwise stated.

Peptide Synthesis

General method for automated SPPS

Where peptides were synthesized by automated SPPS the following procedure was followed using the Liberty CEM microwave peptide synthesiser.

Deprotection: The *N*α-Fmoc group was deprotected before addition of *N*α-Fmoc protected amino acids by consecutive additions of 20% v/v piperidine in DMF for 0.5 min and 3 min. The maximum microwave power was set to 25 W and the maximum temperature was set to 75 °C. Resin was then washed with DMF (4x7ml). *Amino acid couplings:* The *N*α-Fmoc protected amino acid in DMF (5 equiv., 0.2 M), HCTU in DMF (4.5 equiv., 0.45 M) and DIPEA in NMP (10 equiv., 2 M) were added to the resin and subjected to 25 W microwave irradiation for 6 min. Each coupling was performed twice with a maximum temperature of 50 °C for both couplings. The exception was Fmoc-Arg(Pbf)-OH, which was coupled for 25 min at rt, followed by coupling for 5 min at 25 W microwave irradiation and the maximum temperature of 75 °C.

General method for manual SPPS

Deprotection: The *N*α-Fmoc group was removed by treatment of the resin with a solution of 20% v/v piperidine in DMF containing HOBt (0.1 M) for 10 min. *Amino acid coupling:* The *N*α-Fmoc protected amino acid (5 equiv.) was pre-activated with a solution of HATU in DMF (5 equiv., 0.45 M) and DIPEA (neat) (10 equiv.) for 2-10 min at rt. The activated solution was added to the resin and the mixture was left for 60 min at rt. *Unusual amino acid couplings: N*α-Fmoc protected *Fmoc-Lys(Mmt)-OH, Fmoc-Asp(Opip)-OH,* were coupled as detailed below. *N*α-Fmoc protected amino acid (2.5 equiv.) was pre-activated with a solution of HATU in DMF (2.5 equiv.) and DIPEA (neat) (5 equiv.) for 2 min at rt. The activated solution was added to the resin and the mixture was left for 16 h at rt. To confirm reaction had reached completion 10 mg of resin was cleaved (general method III), and the peptide analysed by HPLC and ESI-MS. If the reaction was found to be incomplete a second coupling was performed with fresh reagents. Coupling and deprotection solutions were drained between each step and the resin was washed with DMF (3 x 5 mL), DCM (3 x 5 mL) and DMF (3 x 5 mL). Reactions were monitored *via* the TNBSA test[1] for primary amines.

General SPPS of β3LAC A-D

Peptides β3LAC A-C were assembled using a combination of manual and automated Fmoc-solid-phase synthesis using HCTU as coupling reagent and Rink Amide resin. Initially manual deprotection of Fmoc from the resin, and addition of Trp was achieved following methods detailed in the General method for manual SPPS (see above). Residue were then assembled on a peptide synthesiser (Liberty, CEM) from K(738) to K(729), F(730), E(733), R(734) for β3LAC A, B, C and D respectively as detailed in the General method for automated SPPS. The peptides were then assembled manually to D(723) as detailed in the General method for manual SPPS.

Fully protected resin bound peptides (0.1 mmol) were successively washed with DMF (5 x 5 mL) and DCM (5 x 5 mL). A solution of DCM, TFA, TIPS (94:1:5, 5 mL) was added to the resin to selective remove the side chain protecting groups, monomethoxytrityl (Mmt) and 2-phenylisopropyloxy (Opip) of lysine (Lys) and aspartic acid (Asp) respectively. The solution was stirred for 2 min, and then washed with DCM (5 x 5 mL). This procedure was repeated 10 times. The resin was then washed with DCM (5 x 5 mL) and DMF (5 x 5 mL). The resin bound peptide was treated with PyBOP (52 mg, 0.1 mmol,) and DIPEA (86 μL, 0.5 mmol,) in DMF. After 18 h the reagents were removed by filtration, and a sample of the resin (10 mg) was cleaved (see Cleavage from solid support and peptide purification

below) and analysed by HPLC and ESI-MS. If the cyclisation had not reached completion, the coupling was repeated with fresh reagents. The overall cyclisation reaction varied from 1 to 3 days as shown in each example below. Once cyclisation was completed addition of the final His residue was achieved following the General method manual SPPS.

Cleavage from solid support and peptide purification

Cleavage of the peptide from the resin was performed using 2.5% triisopropylsilane (TIPS), 2.5% 3,6-dioxa-1,8-octanedithiol (DODT) and 2.5% water in TFA (1 ml per 50mg of resin) for 2 hours. Half the TFA was removed under nitrogen, and peptide was precipitated by addition of diethyl ether. The suspended peptide was isolated by centrifugation and residue dissolved in 30% acetonitrile in water and lyophilised. Peptides were purified on a Gilson semi prep RP-HPLC system (GX-271 liquid handler, UV/VIS-155, and 322-pump) fitted with a Supleco (C18, 300 Å, 5 μm, 10 mm x 250 mm) column. Peptides were generally purified with a gradient of 20-45% ACN/H_2O with 0.1% TFA over 25min. Combined pure fractions were lyophilised to yield the desired peptide as a white powder.

Analytical methods

Peptide purity was determined on an Agilent 1260 analytical RP-HPLC system fitted with an analytical (C18, 300 Å, 5 μm, 4.6 mm x 150 mm) column. The eluents were 0.1% aqueous TFA and 0.1% TFA in acetonitrile. High-resolution mass spectra (HMRS) were acquired with an Agilent 1260 infinity TOF LC/MS 6230, running Agilent Mass Hunter software. The process of elongation was monitored during peptide synthesis using an electrospray ionization ion trap mass spectrometer (Bruker, HCT mass spectrometer). 1H NMR, 13C NMR and 2D spectra were recorded using the Varian Oxford NMR 600 MHz, with Agilent DD2 console running on Agilent VnmrJ 4.2 software. Chemical shifts are reported in parts per million (ppm) on the δ scale.

<u>β3MP(722-739) (NH2-HDRKEFAKFEEDRARAKW-CONH2)</u>

β3MP(722-739): The resin bound peptide was synthesised on a 0.1 mmol scale on rink amide resin. The initial deprotection and coupling of *C*-terminal Fmoc-Trp(OtBu)-OH to the resin was performed according to methods described in General procedure for manual SPPS. Elongation of the sequence from K(738) to R(724) was achieved following General procedure for automated SPPS. Addition of final residues D(723) and H(722) was then completed following General procedure for manual SPPS. The desired peptide, with free N_α-terminal, was cleaved from the resin. The crude peptide was purified and lyophilised to afford β3MP(722-739) as a white powder. HRMS (ESI+) $[M+3H]^{+3}$ calculated for $[C_{104}H_{158}N_{34}O_{28}]$: 778.0741; observed: m/z 778.0712.

β3LAC-A (NH2-HDK*KEFD*KFEEERARAKW-CONH2)

β3LAC-A was synthesised according to the procedure General SPPS of β3LAC A-D with Fmoc-Asp(Opip)-OH and Fmoc-Lys(Mmt)-OH coupled at positions 728 and 724. HRMS (ESI+) $[M+4H]^{+4}$ calculated for $[C_{105}H_{156}N_{32}O_{29}]$: 583.3008; observed: m/z 583.3020.

β3LAC-B (NH2-HDRK*EFAD*FEEERARAKW-CONH2)

β3LAC-B was synthesised according to the procedure General SPPS of β3LAC A-D with Fmoc-Asp(Opip)-OH and Fmoc-Lys(Mmt)-OH coupled at positions 729 and 725. HRMS (ESI+) $[M+4H]^{+4}$ calculated for $[C_{102}H_{149}N_{33}O_{29}]$: 576.0379; observed: m/z 576.0371.

β3LAC-C (NH2-HDRKEFKK*FED*ERARAKW-CONH2)

B3LAC-C was synthesised according to the procedure General SPPS of β3LAC A-D with Fmoc-Asp(Opip)-OH and Fmoc-Lys(Mmt)-OH at the positions 734 and 729. HRMS (ESI+) $[M+4H]^{+4}$ calculated for $[C_{106}H_{161}N_{35}O_{27}]$: 590.0655; observed: m/z 590.0660.

β3LAC-D (NH2-HDRKEFAK*FEED*RARAKW-CONH2)

β3LAC-D was synthesised according to the procedure General SPPS β3LAC A-D, with Fmoc-Asp(Opip)-OH and Fmoc-Lys(Mmt)-OH coupled at the positions 733 and 729. HRMS (ESI+) $[M+3H]^{+3}$ calculated for $[C_{103}H_{154}N_{34}O_{27}]$: 767.3987; observed: m/z 767.3983.

Circular dichroism spectroscopy

Circular dichroism experiments were carried out at the Biophysical Characterisation Facility, Sansom Institute for Health Research, UniSA and AIB Lab. Samples were prepared in 10 mM phosphate buffer (pH 6.1), with a final peptide concentration of 25 μM. Peptide concentrations were determined by UV absorption of tyrosine and tryptophan residues at 280 nm using Nano drop 2000 (Thermoscientific).

CD measurements for all peptides were performed using a Jasco J-815 circular dichroism spectropolarimeter (Jasco Corp., Japan), calibrated with *d*-10-camphorsulfonic acid. The quartz cell temperature of 298K was stabilised using a Peltier temperature controller. Secondary spectra for all peptides were obtained for wavelengths between 190-260 nm under the same parameters, where scan speed was 10 nm/min, bandwidth was 1.0 nm and the resolution was 1 nm with a 1 second response. 0.1 mm quartz cuvettes were used, and three repeat scans were compiled to generate the average spectrum. The CD signal resulting from solvent alone was subtracted from the spectrum of each peptide solution. The results were evaluated using the Jasco Spectra Manager and smoothed using Savitzky-Golay convolution (width: 9).

The α-helical content of each peptide was determined from the mean residue molar ellipticity at 222 nm.

$$[\theta] = \theta / (10\, l\, c\, n) \qquad \textit{eq. 1}$$

Where [θ] is the mean residue molar ellipticity (deg.cm^2.dmol^{-1}), θ is ellipticity (mdeg), l is the path length (cm), c is the concentration (mol L^{-1}) and n is the number of residues.

Percent helicity was calculated from the ratio $[\theta]_{222}/[\theta]_{max}$, where

$$[\theta]_{max} = (-44\,000 + 250T)(1 - k/n) \qquad \textit{eq. 2}$$

and is the maximum mean residue molar ellipticity (deg.cm^2.dmol^{-1}), T is the temperature (°C), k is the number of non-hydrogen-bonded peptide carbonyls, n is number of residues[2].

For our system we used $[\theta]_{max} = -23\,400$ for k = 4.0 and n = 18 (number of amino acid residues in the peptide).

Protein Preparation

U-^{15}N-labeled talin PTB domain (F3) was expressed and purified as described previously[3]. Note that, for reasons of protein stability, this construct incorporates a serine residue at position 336 (cysteine in the wild-type protein).

NMR Spectroscopy

All NMR experiments were performed at 298K on an Agilent Inova 600MHz spectrometer equipped with a cryo-probe. Samples were prepared in 50 mM phosphate buffer (pH 6.1), containing 100 mM NaCl, 100μM 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) and 10% D_2O. ^{1}H signals were referenced to DSS at 0 ppm, and ^{15}N signals were referenced indirectly using a ^{15}N-^{1}H frequency ratio of 0.101329118[4]. Spectra were processed using VnmrJ 4.2 software (Agilent Technologies) and analysed using CCPNMR Analysis software[4].

HSQC titrations

^{15}N-^{1}H HSQC titrations of U-^{15}N-labeled talin F3 domain (0.1 mM), with peptide ligands, were performed as described previously (de Pereda et al). HSQC spectra were obtained with 256 increments in F1, 2K data points in F2 and 32 scans per increment. Ligand concentrations were as follows: for βMP 1, 2, 3.5 5.5, 7.9, 10.5, 13.1 mM; βLAC-A 0.5, 1, 2, 5, 8, 11.8 mM; βLAC-B 0.15, 0.36, 0.66, 1.1, 2, 3, 5, 8, 10 mM; βLAC-C 1,2,4,10 mM; βLAC-D 1, 2, 3.5, 5.5, 8, 11.5, 15 mM. Titration samples also contained Complete protease inhibitors (Roche Applied Science).

Combined ^{1}H and ^{15}N amide chemical shift perturbations (Δδ(H,N)) were calculated as the weighted sum of the absolute shift changes i.e. $\Delta\delta(H,N) = |\Delta\delta(H)| + 0.15|\Delta\delta(N)|$ where $\Delta\delta = \delta_{bound} - \delta_{free}$. Dissociation constants (*K*d) were determined by fitting chemical shift perturbations to the following

equation, $\Delta\delta(H,N) = \Delta\delta(H,N)_{max}\frac{[P] + [L] + Kd - \sqrt{([P] + [L] + Kd)^2 - 4[P][L]}}{2[P]}$

where *K*d is the dissociation constant, $\Delta\delta(H,N)$ is the weighted shift change, $\Delta\delta(H,N)_{max}$ is the perturbation at saturation, and [P] and [L] are the protein and ligand concentrations, respectively. For each titration, data from the ten most perturbed peaks, unobscured by overlap, were fit simultaneously to this equation using Graphpad Prism 6, extracting a single shared *K*d and multiple $\Delta\delta(H,N)_{max}$ values.

Peptide structures

For structure studies, peptides were prepared to a final concentration of 5.7mM β3MP, 4.7mM β3LAC-A, 5.5mM β3LAC-B, 6.6mM β3LAC-C, 5.5mM β3LAC-D, in the standard buffer (see above). ^{1}H resonances were assigned from zTOCSY (80ms mixing time), DQF-COSY and NOESY spectra (200ms mixing time), recorded at 298K using ChemPack (Agilent) pulse sequences. All spectra were acquired over 9615.4Hz and 2048 complex data points in F2, and 256 (zTOCSY, DQF-COSY) or 512 (NOESY) increments in F1. zTOCSY and NOESY experiments used 16 scans per increment, while DQF-COSY experiments were acquired with 8. Water suppression was achieved using presaturation. Spectra were processed using VnmrJ 4.2. NH temperature coefficients were determined from a series of 1D ^{1}H spectra, recorded every 5K from 288-318K. $^3J_{HNH\alpha}$ scalar coupling constants were obtained from high-resolution ^{1}H 1D spectra, by fitting.

Proton resonance assignments were obtained using the standard assignment strategy of Wüthrich [5]. Peptide structures were calculated using a version of ARIA 2.3, modified to deal with lactam constrained peptides (Benjamin Bardiaux, Unite de Bioinformatique Structurale, Institut Pasteur[6]). Lactams were identified as being in the trans conformation, by the presence of large NOEs between the lactam 'aspartate' Hβ protons to the lactam amide proton, as well as the absence of NOEs from these same Hβs to the Hε protons of the lactam 'lysine' residue, so lactam φ-angles were set to -180°. Initial structures were calculated from NOE distance restraints and scalar coupling restraints. Hydrogen bond restraints were then introduced, provided the likely acceptor/s could be identified from the preliminary round of calculations (>50% of structures). If multiple acceptors were identified, then the hydrogen bond restraints were made ambiguous. Twenty structures were calculated for iterations 1-7, and 200 structures for iteration 8. From these structures, twenty structures were further refined in water. Default parameters were used, apart from the number of steps, which were increased for all parts of the calculation to 40000 high-temperature steps, 80000 cool1 and cool2 steps, and 20000 refine steps. Final structures were validated using the Protein Structure Validation Suite (http://psvs-1_5-dev.nesg.org). Final structures had no violations > 3Å. Structure figures were prepared using Chimera[7].

Chymotrypsin degradation assay

Purified peptide and bovine α-chymotrypsin (Sigma catalog C4129) stock solutions (50mM Tris, pH 7.5) were mixed to a ratio of 2200:1 (100μM final peptide concentration), and incubated at room temperature. Aliquots were taken at the time points indicated in Figure S6, before quenching with TFA (1% final concentration). Samples were snap-frozen and stored at -20°C, before subsequent analysis by LC/MS using an eclipse XDB-C18 column (4.6 x 150 mm, 5 μm, Agilent System 1260 Infinity) coupled to a mass spectrometer (Bruker HCT, Germany). The samples were eluted with a gradient of 1-100% ACN/H_2O with 0.1% formic acid over 20 minutes. Spectra were analysed using Hystar software (3.1). The relative protein concentration of the remaining peptide was determined from the area under the curve using extracted ion mass chromatogram[2, 8]. Three independent experiments were performed for each peptide. Chymotrypsin activity was assessed at different time points using the protocol reported in [9].

Table S1. Predicted and reported effects of alanine mutations on β3-integrin binding to the talin F3 domain.

	Changes in binding energy (ΔΔG) from alanine substitution	
	Robetta prediction[a,b,c] Kcalmol^{-1} (kJmol^{-1})	Reported[10] KJmol^{-1}
F727	1.62 (6.78)	4.97 (combined[d])
F730	2.46 (10.29)	
E733	1.15 (4.81)	N/D
W739	4.39 (18.37)	8

[a] – Robetta online alanine-scanning server.
[b] – Residues with ΔΔG>1.0 are considered to be 'hot-spot' residues.
[c] – Using structure 2H7E from the PDB, an NMR structure of talin F3 in complex with β3-integrin peptide.
[d] – FF727/730AA double mutation.

Table S2 NMR restraints and structural statistics for the top twenty calculated structures.

	β3MP	β3LAC-A	β3LAC-B	β3LAC-C	β3LAC-D
No. of distance restraints					
Unambiguous NOEs	84	161	154	156	144
Ambiguous NOEs	78	67	117	67	70
Total NOEs	162	228	271	223	214
Hydrogen bond restraints	5	8	10	9	6
No. scalar coupling restraints	10	12	5	11	8
RMS distances from ideal values					
Bonds (Å)	0.004	0.004	0.004	0.004	0.004
Angles (degree)	0.5	0.6	0.5	0.6	0.5
Nonbonded energies					
Electronic ($kcalmol^{-1}$)	-591±65	-627±64	-592±52	-659±86	-554±66
van der Waals ($kcalmol^{-1}$)	-127±4	-144±4	-136±3	-136±3	-132±3
Ramachandran (%)[a]					
Most favoured	71.2	94.1	92.2	93.1	72.5
Additionally allowed	26.2	5.9	7.5	6.9	25.9
Generously allowed	2.5	0	0.3	0	1.6
Disallowed	0	0	0	0	0
Global RMS distance (Å)[b]					
All residues					
backbone (N,CA,C,O)	2.1	0.8	0.8	0.8	1.8
all atoms	3.9	1.8	1.6	1.8	3
Well-defined residues[c]					
backbone (N,CA,C,O)	0.2 (730-736)	0.3 (724-737)	0.2 (724-738)	0.2 (725-738)	0.2 (728-737)
all atoms	1.3 (730-736)	0.9 (724-737)	1.2 (724-738)	1.2 (725-738)	1.1 (728-737)

[a] Determined using Procheck[11]
[b] From PSVS[12]

Table S3 NMR data collected for the parent βMP peptide, and the lactam-constrained series.

β3MP						
Residue	NH Temp Coeffs (ppb/K)	3JHNHA (Hz)	$I_{\alpha N(i,i+1)}/I_{NN(i,i+1)}$	$I_{\alpha N(i,i+1)}/I_{\alpha N(i,i)}$	αH CSI[a]	NH CSI[a]
His722	-	-	-	-	-	-
Asp723	-	-	-	-	0.1	-
Arg724	-10.6	6.3	-	4.6	-0.031	0.405
Lys725	-7.1	6.3	1.8	2.2	-0.002	0.128
Glu726	-5.3	6.2	5.4	2.1	-0.023	-0.041
Phe727	-6.9	OV[b]	D[c]/AMB[d]	AMB	-0.074	-0.054
Ala728	-6.3	OV	3.3	1.9	0.024	0.114
Lys729	-6.2	6.5	D	1.9	0.003	-0.107
Phe730	-4.4	6.5	AMB	AMB	-0.137	-0.009
Glu731	-3.9	5.6	D/AMB	AMB	-0.138	0.011
Glu732	-3.7	5.76	D	AMB	-0.089	-0.132
Glu733	-3.3	5.7	1.7	1.2	-0.175	-0.177
Arg734	-4.6	OV	AMB	AMB	-0.19	-0.237
Ala735	-3.5	5.2	1.7	1.2	-0.116	-0.338
Arg736	-3.6	OV	D	AMB	-0.201	-0.393
Ala737	-3.6	OV	AMB	AMB	-0.058	-0.307
Lys738	-3.6	OV	1.8	1.3	-0.072	-0.366
Trp739	-4.7	7.4	-	-	-0.001	-0.088

β3LAC-A						
Residue	NH Temp Coeffs (ppb/K)	$^3J_{HNHA}$ (Hz)	$I_{\alpha N(i,i+1)}/I_{NN(i,i+1)}$	$I_{\alpha N(i,i)}/I_{\alpha N(i,i+1)}$	αH CSI[a]	NH CSI[a]
His722	-	-	-	-	-	-
Asp723	-	-	-	-	0.133	-
X(K)724	-7	3.3	1.4	1.1	-0.127	0.247
Lys725	-2	4.2	AMB[d]	AMB	-0.15	0.003
Glu726	-7	6.2	0.4	AMB	-0.15	-0.469
Phe727	-9.4	4.8	0.4	0.4	-0.405	0.128
X(N)728	-15	4.5	0.1	0.9	0.029	0.647
Lys729	-1.4	5.6	1.0	1.1	-0.038	-0.576
Phe730	-2	5.3	0.5	0.8	-0.233	-0.149
Glu731	-4.6	5.2	0.5	0.7	-0.271	-0.044
Glu732	-1.4	5.3	D[c]/AMB	AMB	-0.129	-0.271
Glu733	0	5.6	D/AMB	AMB	-0.251	-0.372

Arg734	-3	5.4	0.7	AMB	-0.303	-0.318
Ala735	-1	5.4	0.8	1.3	-0.168	-0.464
Arg736	-1.2	OV[b]	D/AMB	AMB	-0.266	-0.528
Ala737	-1.6	OV	D/AMB	AMB	-0.071	-0.456
Lys738	-2	OV	D/AMB	AMB	-0.095	-0.506
Trp739	-4	OV	-	-	-0.003	-0.145

β3LAC-B						
Residue	NH Temp Coeffs (ppb/K)	$^3J_{HNHA}$ (Hz)	$I_{\alpha N(i,i+1)}/I_{NN(i,i+1)}$	$I_{\alpha N(i,i+1)}/I_{\alpha N(i,i)}$	αH CSI[a]	NH CSI[a]
His722	-	-	-	-	-	-
Asp723	-	-	-	-	0.1	-
Arg724	-9.2	5.5	7.1	3.6	-0.02	0.39
X(K)725	-3.7	4.7	1.5	AMB	-0.02	-0.046
Glu726	-2.6	OV[b]	0.4	0.4	-0.207	0.165
Phe727	-8.3	OV	0.6	1.0	-0.194	-0.545
Ala728	-6.3	OV	0.7	0.6	-0.103	0.14
X(N)729	-12.4	OV	0.2	0.9	0.151	0.333
Phe730	-2.8	OV	0.9	1	-0.171	-0.277
Glu731	-2.8	OV	D[c]	1.0	-0.251	0.076
Glu732	-2.1	5.3	0.7	0.8	-0.101	-0.19
Glu733	0.9	OV	D	1.1	-0.235	-0.376
Arg734	-2.6	4.6	0.9	0.7	-0.296	-0.308
Ala735	-1.3	OV	AMB	AMB	-0.156	-0.433
Arg736	-2.4	OV	D/AMB	AMB	-0.256	-0.473
Ala737	-2.4	OV	D/AMB	AMB	-0.074	-0.405
Lys738	-1.7	OV	D	0.9	-0.09	-0.467
Trp739	-3.6	7.4	-	-	-0.001	-0.13

β3LAC-C						
Residue	NH Temp Coeffs (ppb/K)	$^3J_{HNHA}$ (Hz)	$I_{\alpha N(i,i+1)}/I_{NN(i,i+1)}$	$I_{\alpha N(i,i+1)}/I_{\alpha N(i,i)}$	αH CSI	NH CSI[a]
His722	-	-	-	-	-	-
Asp723	-	-	-	-	0.124	-
Arg724	-9.7	6.6	-	3.1	-0.051	0.483
Lys725	-5.2	OV[b]	AMB[d]/D[c]	AMB	-0.044	0.152
Glu726	-4.5	OV	1.9	1.2	-0.065	0.034

Phe727	-6.8	7.0	0.8	0.8	-0.109	-0.130
X(K)728	-2.3	OV	0.6	AMB	-0.019	-0.208
Lys729	-2.4	OV	0.4	0.3	-0.189	-0.249
Phe730	-6.4	6.2	0.4	0.0	-0.294	-0.578
Glu731	-5.2	5.4	0.4	0.1	-0.345	0.089
X(N)732	-9.5	5.2	0.3	0.1	0.051	0.469
Glu733	-2.7	OV	0.5	0.5	-0.13	-0.545
Arg734	-1.8	5.2	0.7	0.8	-0.248	-0.512
Ala735	-2.1	5.5	0.9	AMB	-0.141	-0.455
Arg736	-1.4	6.2	1.0	0.7	-0.232	-0.583
Ala737	-2.1	5.3	AMB/D	AMB	-0.062	-0.407
Lys738	-2.2	6.2	D	0.9	-0.075	-0.439
Trp739	-3.8	6.2	-	-	0.001	-0.127

β3LAC-D						
Residue	NH Temp Coeffs (ppb/K)	$^3J_{HNHA}$ (Hz)	$I_{\alpha N(i,i+1)}/I_{NN(i,i+1)}$	$I_{\alpha N(i,i+1)}/I_{\alpha N(i,i)}$	αH CSI[a]	NH CSI[a]
His722	-	-	-	-	-	-
Asp723	-	-	-	-	0.117	-
Arg724	-10.3	5.8	17.1	2.8	-0.031	0.427
Lys725	-6	6.1	D[c]	1.7	-0.016	0.148
Glu726	-5.4	5.9	3.0	1.5	-0.04	0.07
Phe727	-6.8	5.6	AMB[d]/D	AMB	-0.12	-0.058
Ala728	-4	OV[b]	1.3	0.4	0.036	0.105
X(K)729	-3.4	4.3	0.6	0.4	0.015	-0.285
Phe730	-4.2	OV	0.4	0.4	-0.405	-0.025
Glu731	-6.4	5.8	0.3	0.4	-0.347	-0.304
Glu732	-3.5	5.1	0.3	0.3	-0.181	-0.216
X(N)733	-6.9	5.8	AMB	AMB	-0.088	0.219
Arg734	-1.7	OV	AMB	AMB	-0.289	-0.646
Ala735	-1.7	OV	AMB	AMB	-0.147	-0.557
Arg736	-3.9	OV	AMB	AMB	-0.258	-0.498
Ala737	-1.8	OV	AMB	AMB	-0.072	-0.515
Lys738	-2	OV	AMB	AMB	-0.085	-0.493
Trp739	-4	OV	-	-	-0.002	-0.128

[a] Difference in chemical shift from random coil values, as determined from Kjaergaard and Poulsen[13].
[b] Overlapped
[c] Too close to diagonal
[d] Ambiguous

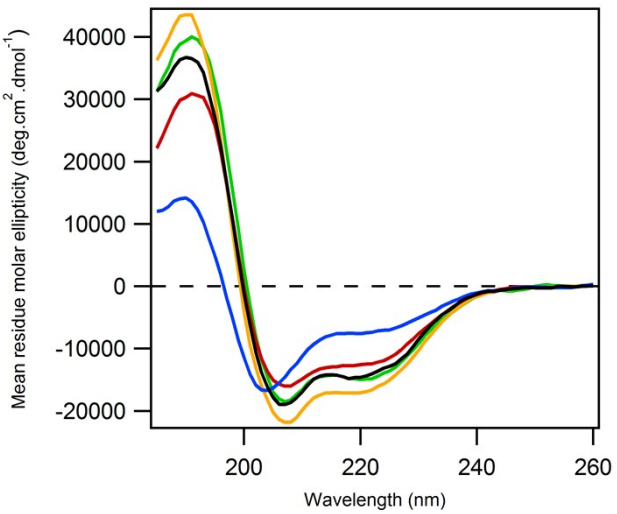
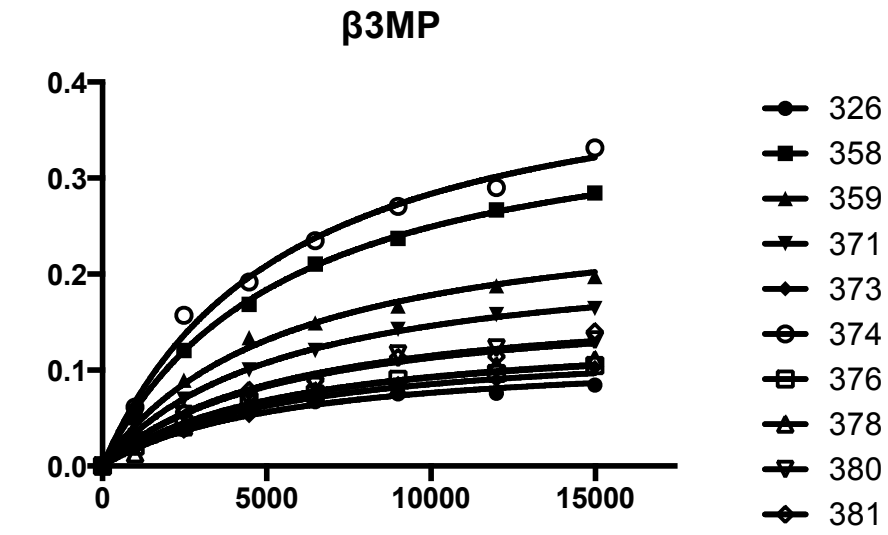
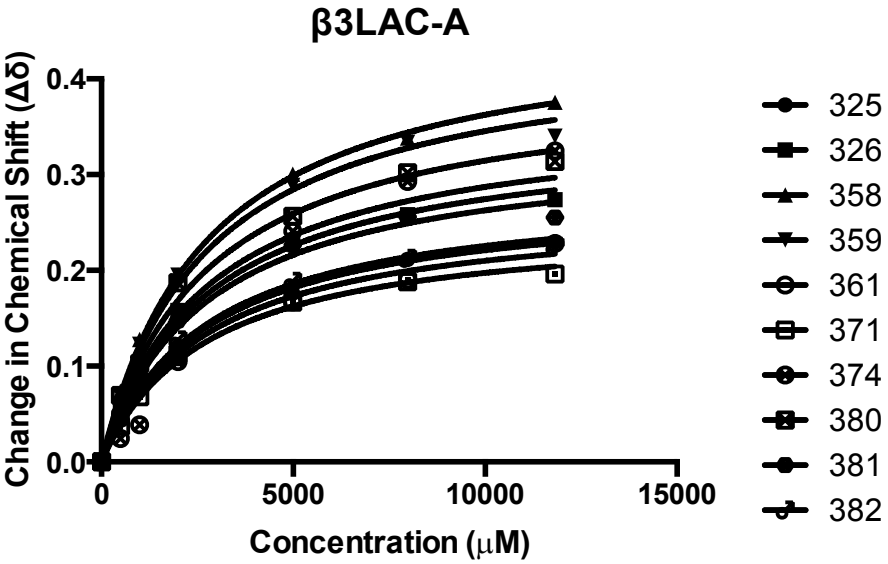
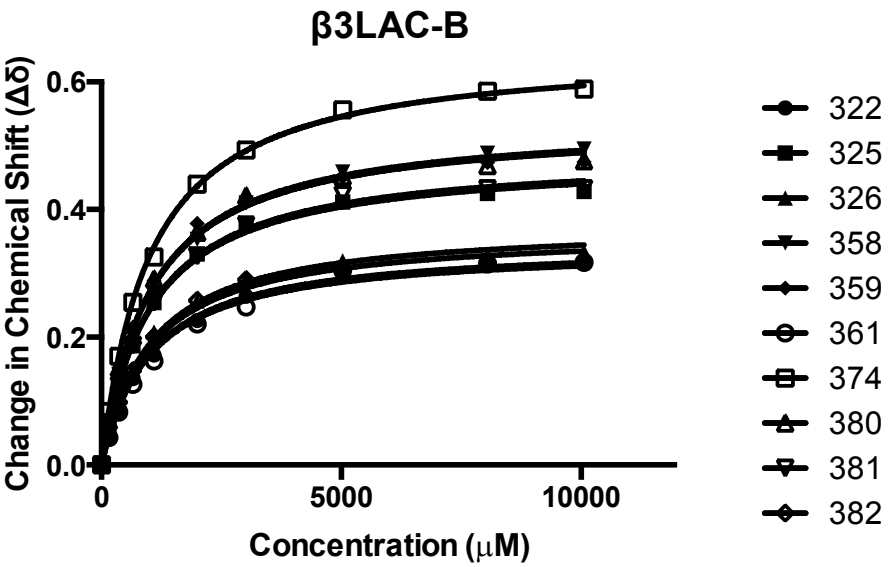


Figure S1. Circular dichroism spectra for βMP (blue) and four lactam bridged peptides, βLAC-A (black), βLAC-B (yellow), βLAC-C (red) and βLAC-D (green) in 10mM phosphate buffer, pH 6.5, 298K.


β3MP
0.4
0.3
0.2
0.1
0.0
0
5000
10000
15000
326
358
359
371
373
374
376
378
380
381


β3LAC-A
Change in Chemical Shift (Δδ)
0.4
0.3
0.2
0.1
0.0
0
5000
10000
15000
Concentration (μM)
325
326
358
359
361
371
374
380
381
382


β3LAC-B
Change in Chemical Shift (Δδ)
0.6
0.4
0.2
0.0
0
5000
10000
Concentration (μM)
322
325
326
358
359
361
374
380
381
382

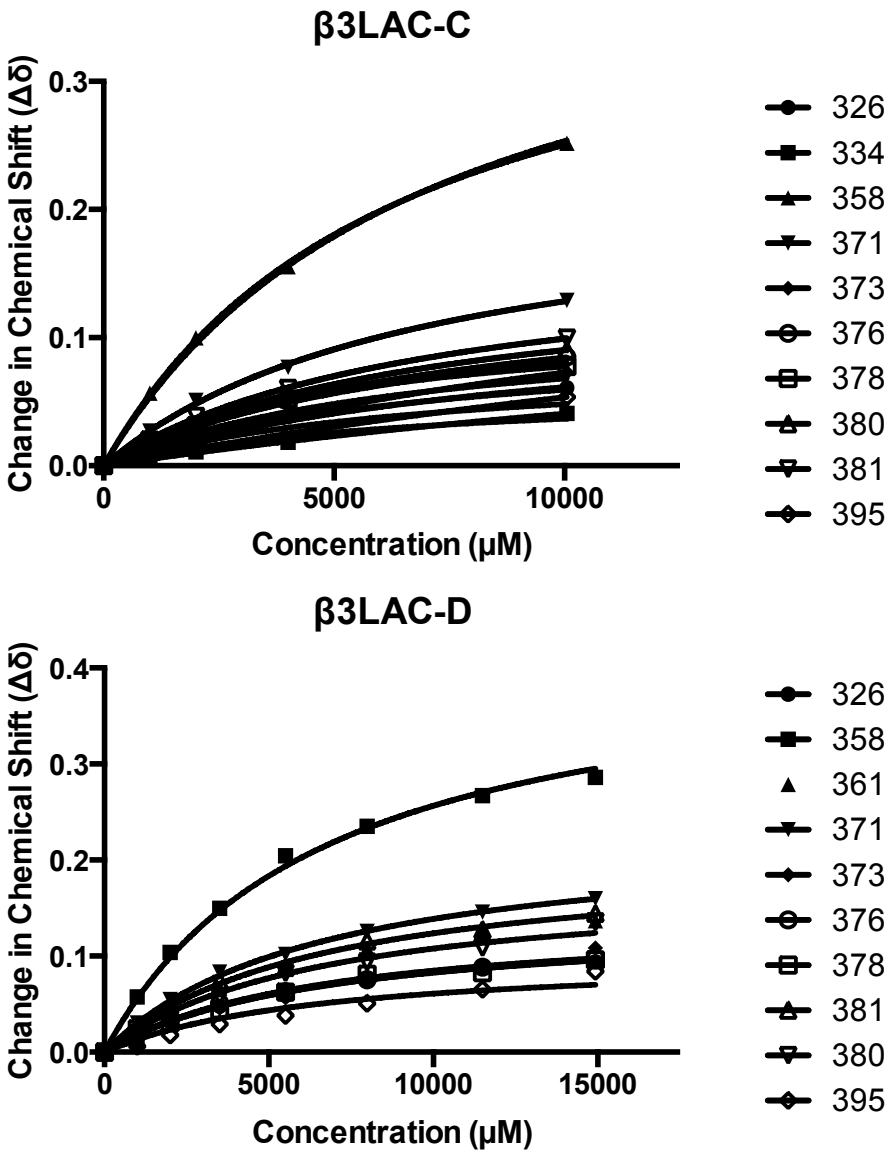


Figure S2. Plots of change in chemical shift (Δδ) versus peptide ligand concentration (μM), for the ten most shifted peaks in each HSQC spectrum. Residue identities are indicated to the right of each chart.

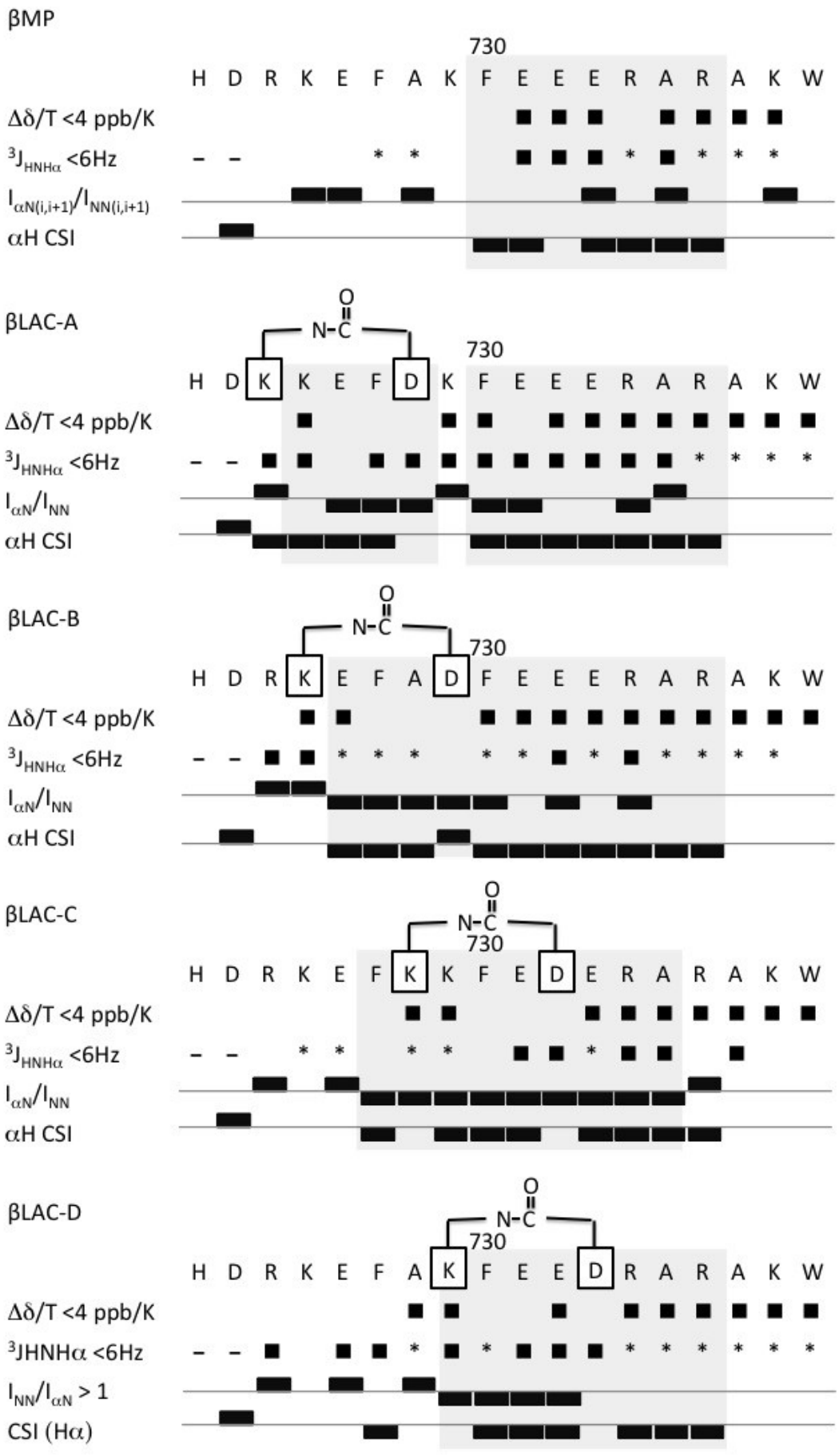
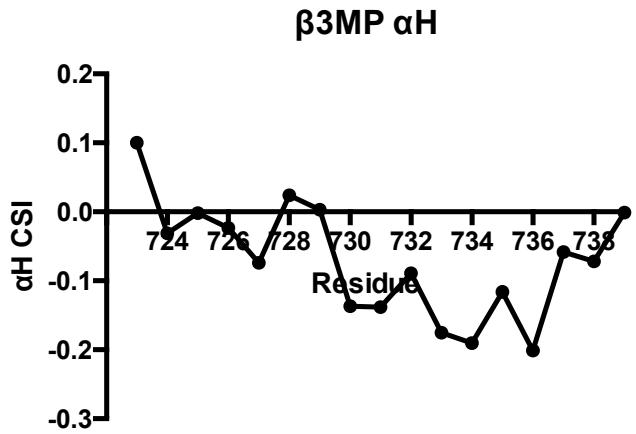
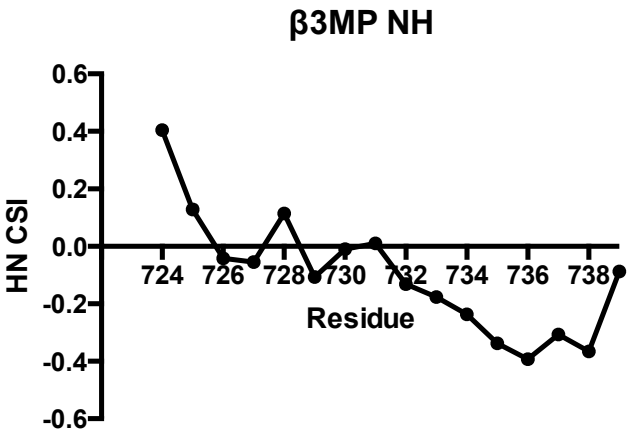
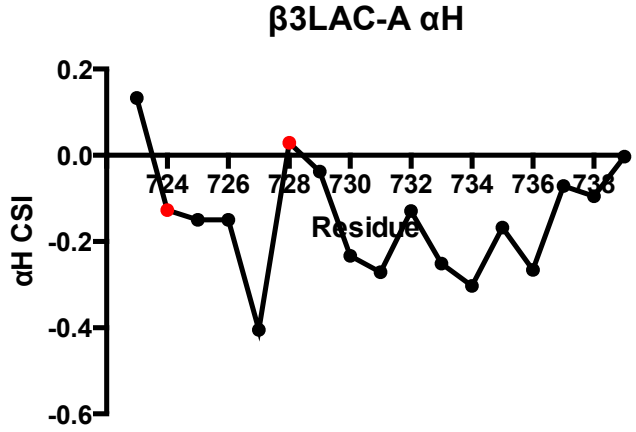


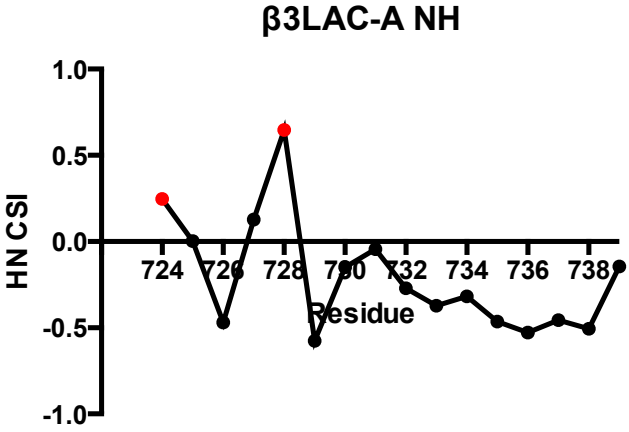
Figure S3. NMR data summary. NH temperature coefficients (Δδ/T) < 4ppb/K, and $^3J_{HNH\alpha}$ < 6Hz are indicated by black squares. (*) Coupling constants obscured by overlap, (-) NHs absent due to exchange.

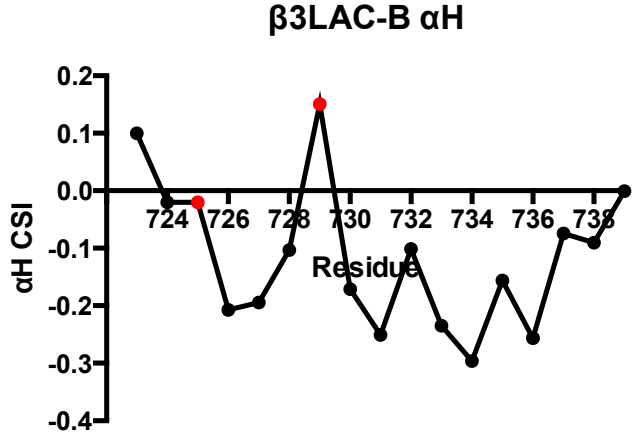
Ratios of sequential NN(i,i+1) to αN(i,i+1) NOE intensities of less than one ($I_{NN}/I_{\alpha N} < 1$) are indicated. Here, the horizontal line indicates a value of one, with boxes above the line for values >1, and boxes below for values <1. Chemical shift indices (CSI) for αH resonances are also indicated, where boxes above and below the line signify differences from random coil values of > 0.1 or < 0.1, respectively. Grey shaded boxes indicate consensus helical regions suggested by the data.

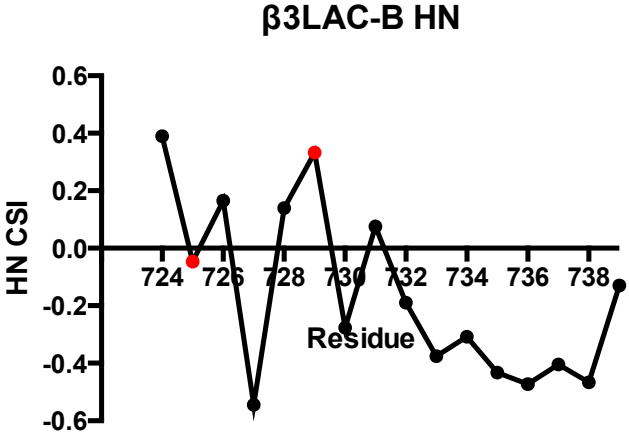

β3MP αH
αH CSI
0.2
0.1
0.0
-0.1
-0.2
-0.3
724 726 728 730 732 734 736 738
Residue


β3MP NH
HN CSI
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
724 726 728 730 732 734 736 738
Residue


β3LAC-A αH
αH CSI
0.2
0.0
-0.2
-0.4
-0.6
724 726 728 730 732 734 736 738
Residue


β3LAC-A NH
HN CSI
1.0
0.5
0.0
-0.5
-1.0
724 726 728 730 732 734 736 738
Residue


β3LAC-B αH
αH CSI
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
724 726 728 730 732 734 736 738
Residue


β3LAC-B HN
HN CSI
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
724 726 728 730 732 734 736 738
Residue

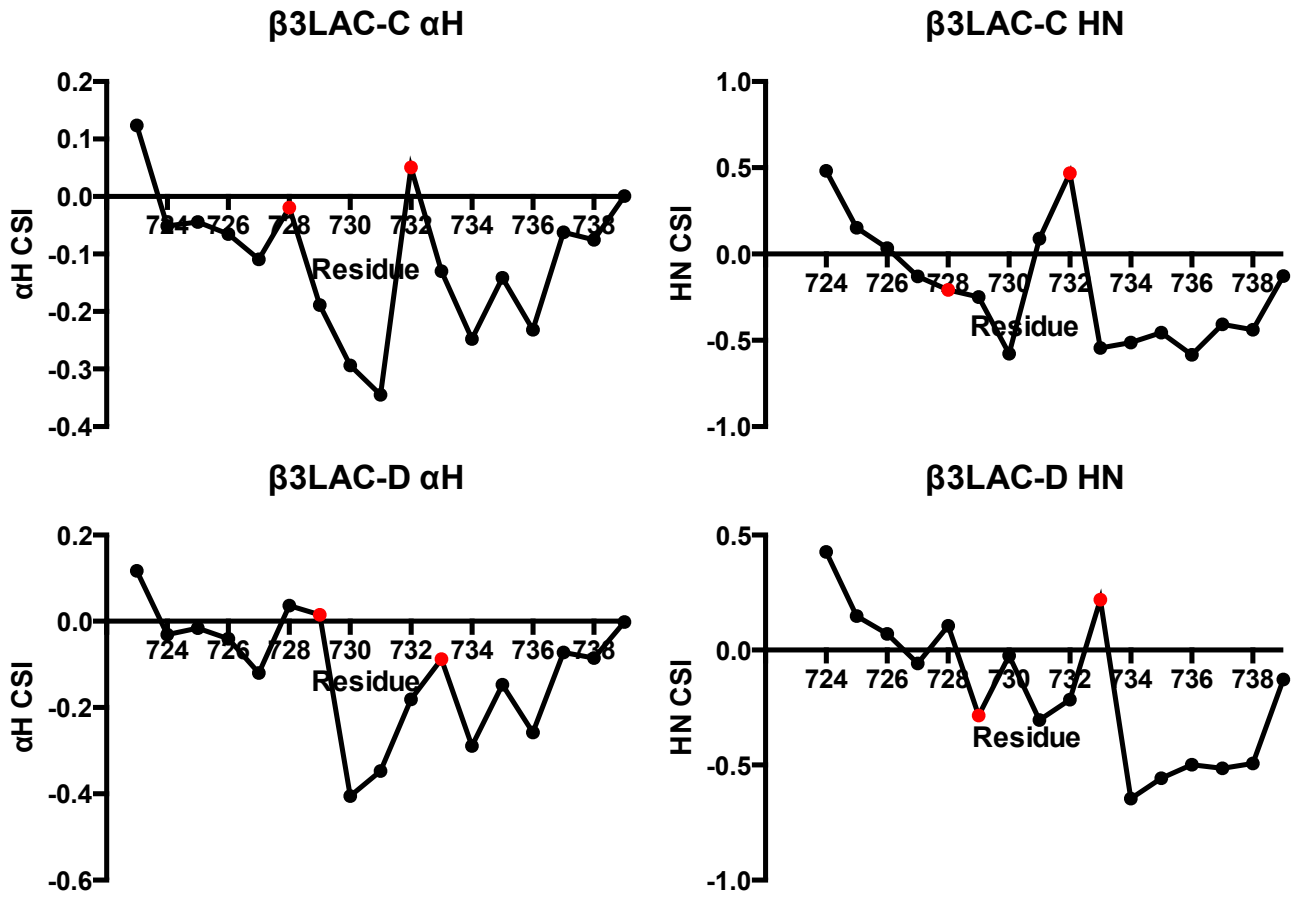
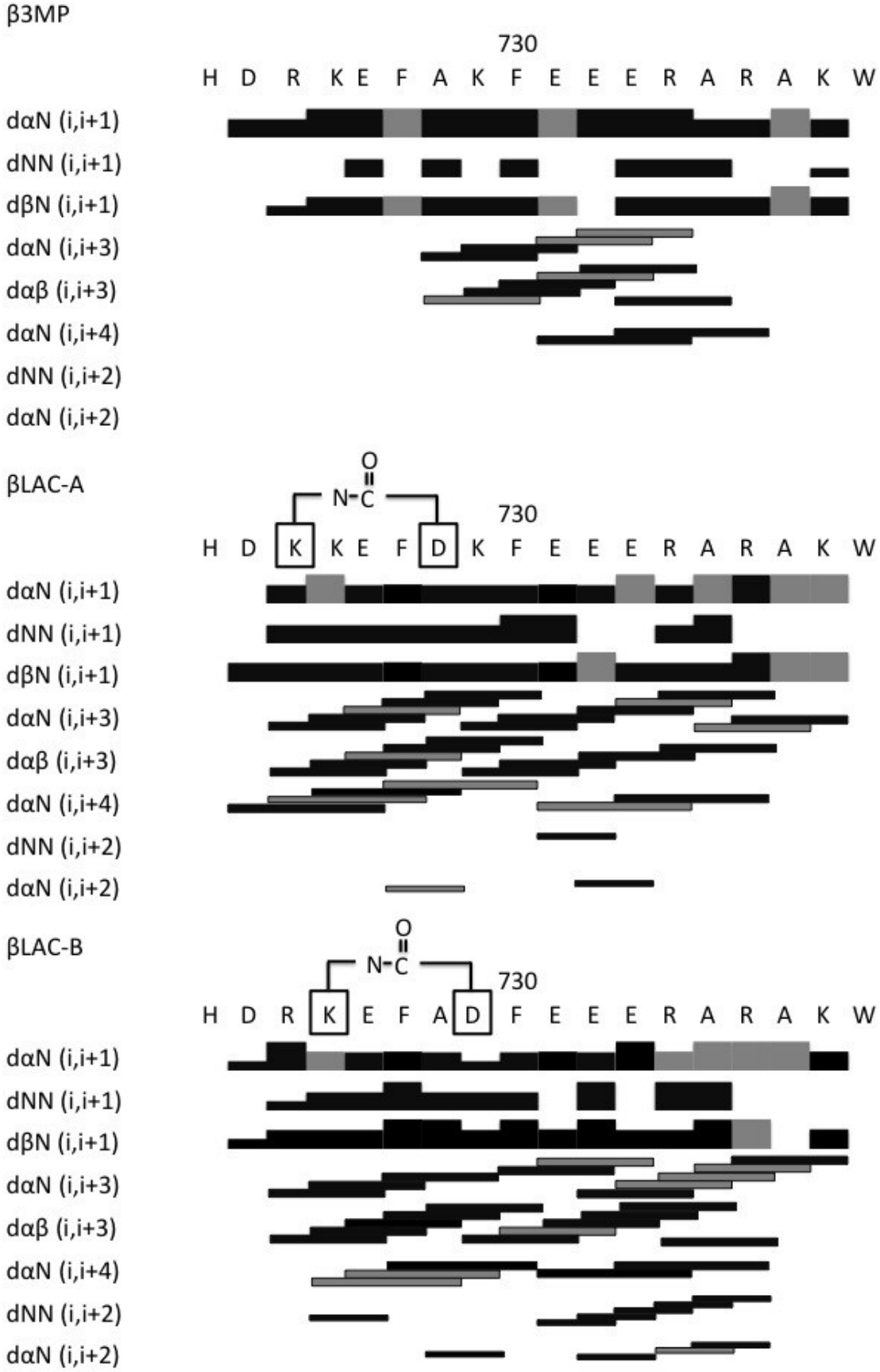


Figure S4. Plots of the difference in αH and NH shifts from random coil values, for each peptide. Linker residues are indicated in red.


β3MP
730
H D R K E F A K F E E E R A R A K W
dαN (i,i+1)
dNN (i,i+1)
dβN (i,i+1)
dαN (i,i+3)
dαβ (i,i+3)
dαN (i,i+4)
dNN (i,i+2)
dαN (i,i+2)
βLAC-A
O
N-C
730
H D K K E F D K F E E E R A R A K W
dαN (i,i+1)
dNN (i,i+1)
dβN (i,i+1)
dαN (i,i+3)
dαβ (i,i+3)
dαN (i,i+4)
dNN (i,i+2)
dαN (i,i+2)
βLAC-B
O
N-C
730
H D R K E F A D F E E E R A R A K W
dαN (i,i+1)
dNN (i,i+1)
dβN (i,i+1)
dαN (i,i+3)
dαβ (i,i+3)
dαN (i,i+4)
dNN (i,i+2)
dαN (i,i+2)

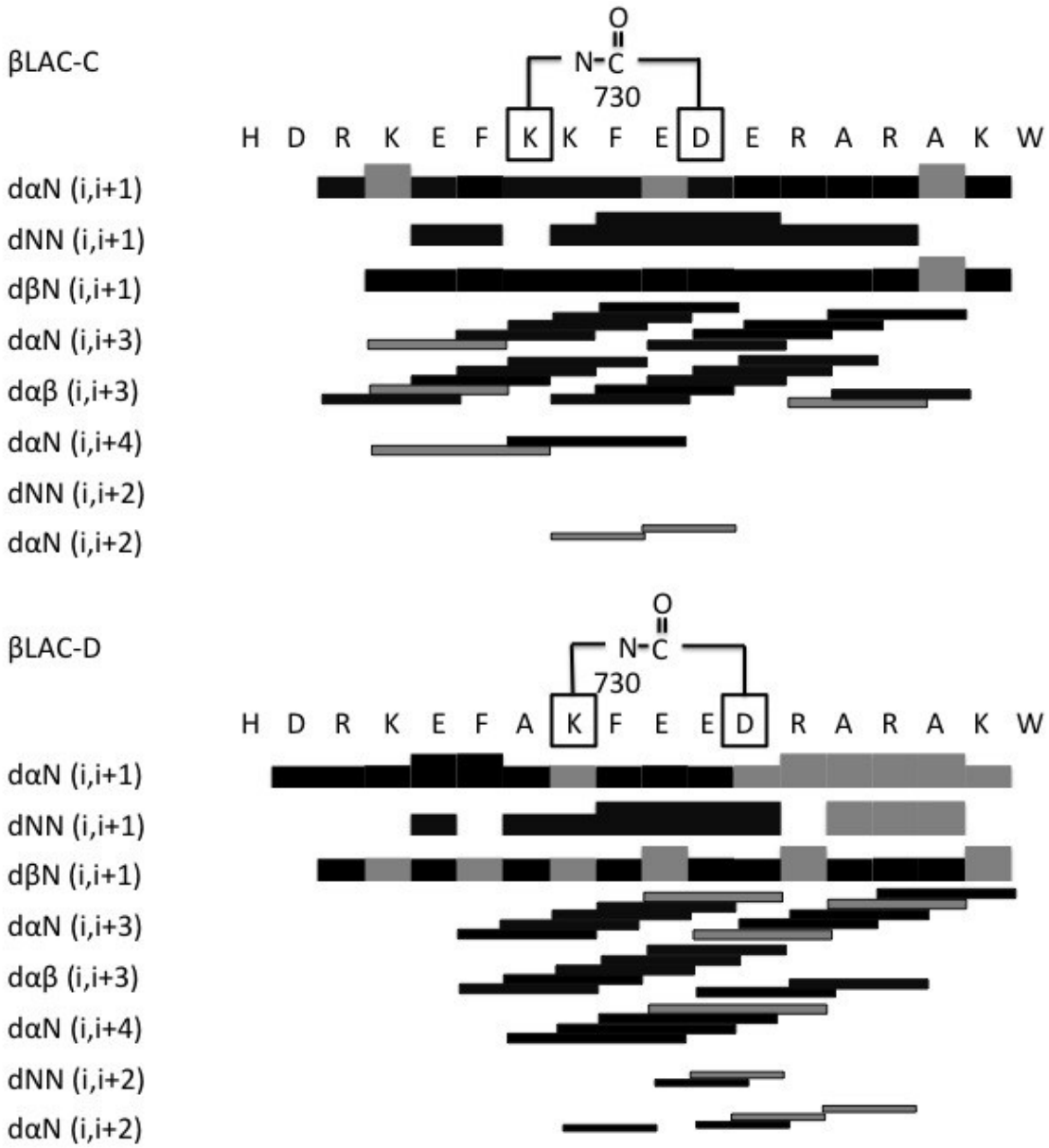


Figure S5. NOE connectivity maps. Ambiguous NOEs are indicated in grey.

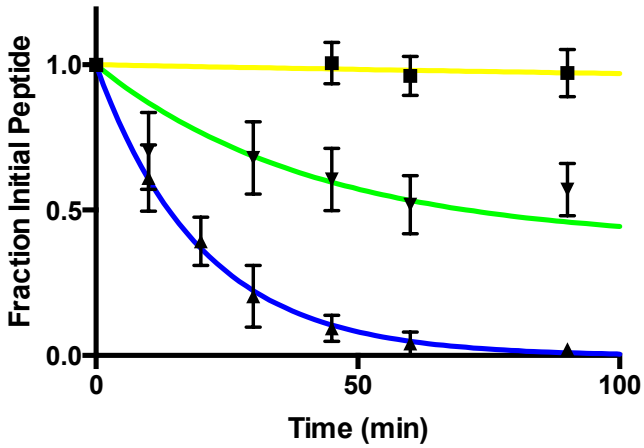


Figure S6. Time course of β3 peptide cleavage by chymotrypsin. βMP (▲), βLAC-B (■), βLAC-D (▼). Curves indicate the fit to a one-phase exponential decay equation (Graphpad Prism).

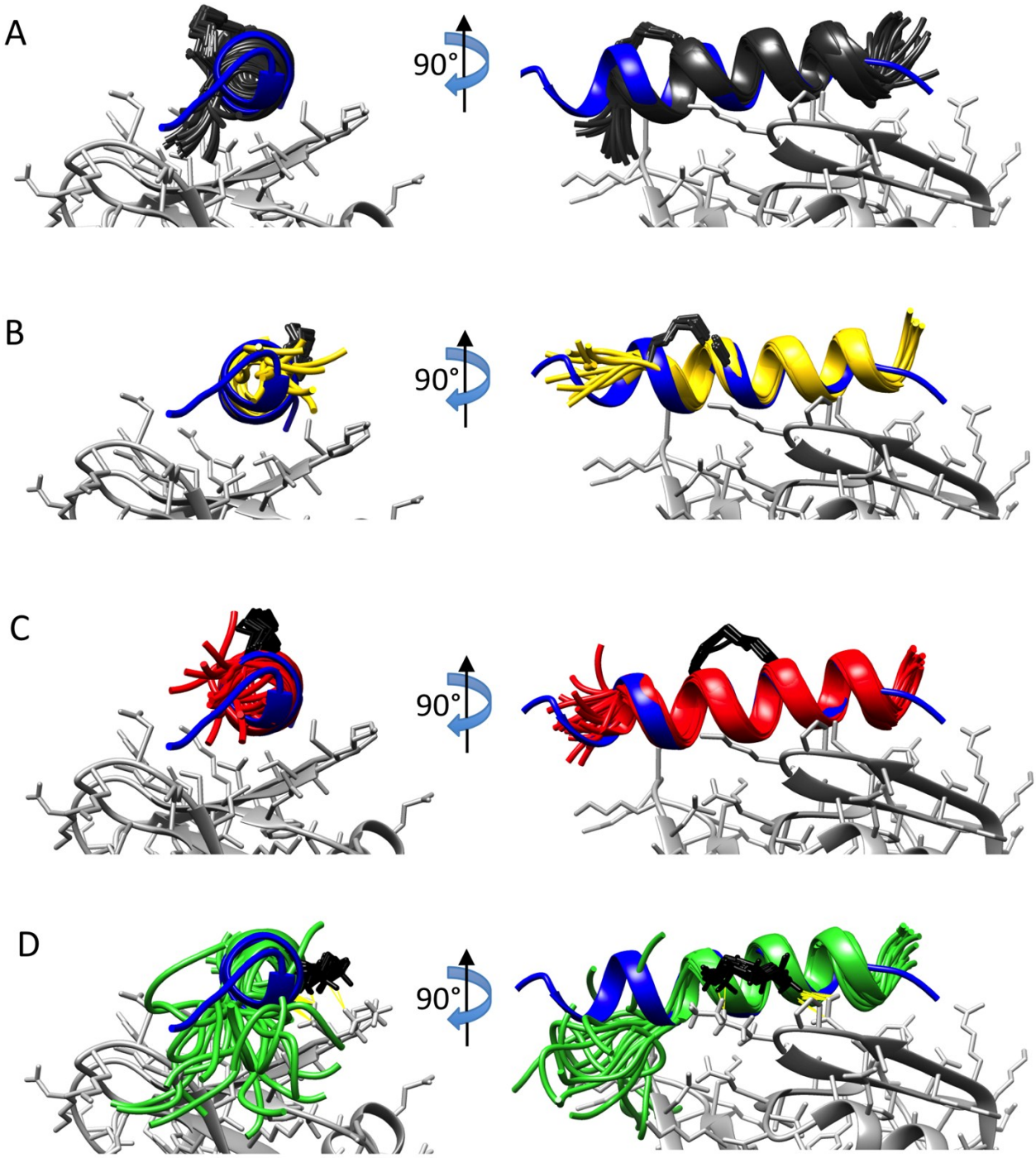


Figure S7. Calculated structures of the βLAC peptides superimposed onto the integrin β3 peptide (blue) from the talin/integrin NMR complex structure (PDB 2H7E). The talin F3 domain is shown in grey. A) βLAC-A (black), B) βLAC-B (yellow), C) βLAC-C (red), and D) βLAC-D (green). Lactam bridges are indicated in black. Protons from βLAC-D lactam bridge are also indicated in order to indicate steric clashes with the talin protein (yellow).

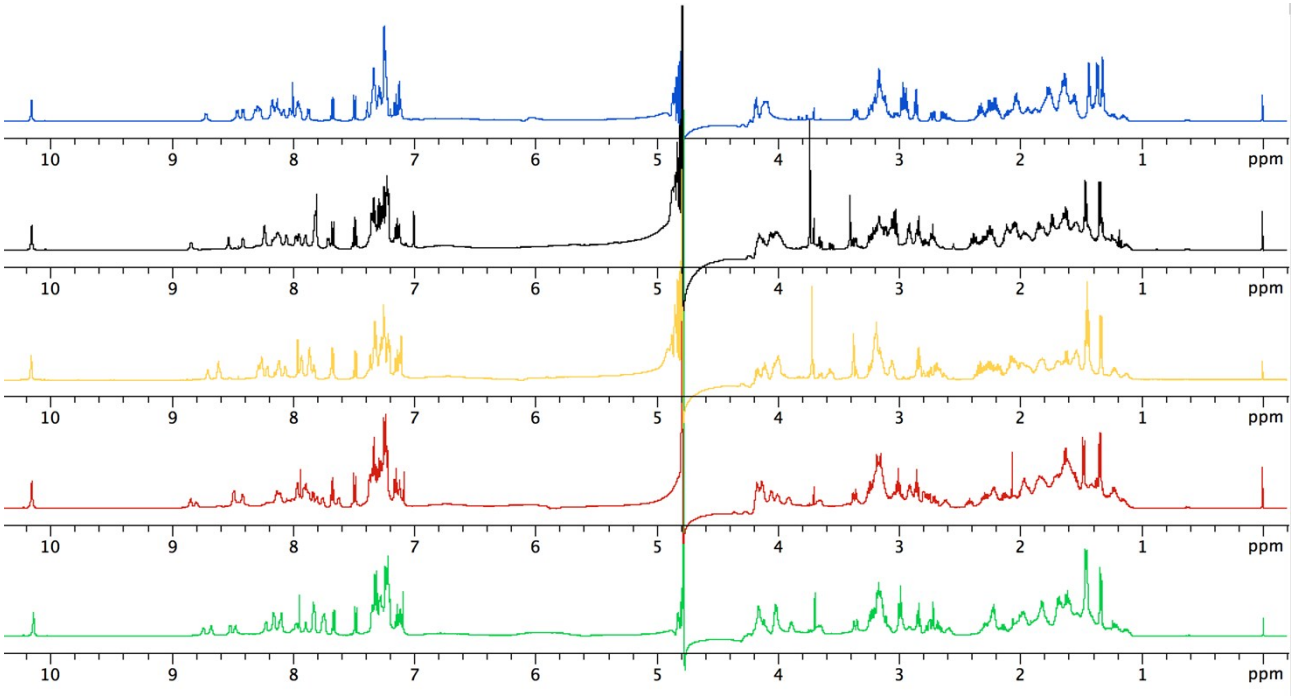


Figure S8. One-dimensional ^{1}H NMR spectra for βMP (blue), βLAC-A (black), βLAC-B (yellow), βLAC-C (red), and βLAC-D (green) in 50mM phosphate buffer (pH 6.1) with 100mM NaCl, recorded at 298K.

Supplementary References

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