SUPPORTING MATERIAL

STRUCTURED FUNCTIONAL DOMAINS OF MYELIN BASIC PROTEIN: CROSS-TALK BETWEEN ACTIN POLYMERIZATION AND Ca^{2+}-DEPENDENT CALMODULIN INTERACTION

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SUPPORTING MATERIAL

MATERIALS AND METHODS

Materials: Electrophoresis grade acrylamide, ultrapure TRIS base, and ultrapure Na_2EDTA were purchased from ICN Biomedicals (Costa Mesa, CA). Other chemicals were reagent grade and acquired from either Fisher Scientific (Unionville, ON) or Sigma-Aldrich (Oakville, ON). The Ni^{2+}-NTA (nitrilotriacetic acid) agarose beads were purchased from Qiagen (Mississauga, ON). Dodecylphosphocholine (DPC) was obtained from Avanti Polar Lipids (Alabaster, AL). For uniform labeling of protein or peptide for NMR spectroscopy, the stable isotopic compounds ^2H_2O (D_2O), $^{15}NH_4Cl$, and $^{13}C_6$-glucose were obtained from Cambridge Isotope Laboratories (C.I.L., Cambridge, MA).

Construction of MBP-α-peptides: Three different regions of the gene for murine 18.5 kDa myelin basic protein (MBP) coding for amino acids (A22-K56), (S72-S107), and (S133-S159) (murine MBP sequence numbering, *cf.*, (12;17), and **Figure S1**) were cloned into the Champion™ pET *SUMO (small ubiquitin modifier)* Expression System (Invitrogen Life Technologies, Burlington, ON) and were named α1, α2, and α3, respectively. The pET22b rmMBP plasmid containing the complete open reading frame for 18.5 kDa MBP rmC1 (4) was used as template DNA for all polymerase chain reaction (PCR) amplifications. Synthetic oligonucleotides were purchased from Sigma-Aldrich (Oakville, ON). The PCR products coding for peptide regions (A22-K56), (S72-S107), and (S133-S159) of 18.5 kDa murine MBP were amplified using three (3) forward and reverse primer sets: (*i*) 5'-GCC AGG CAT GGC TTC CTC CC-3', 5'-TCA CTT GCC AGA GCC CCG CT-3' (for the α1-peptide); (*ii*) 5'-TCG CAG CAC GGC CGG ACC CA-3', 5'-TCA GGA CAG GCC TCT CCC CT-3' (for the α2-peptide); and (*iii*) 5'-TCG GCT CAC AAG GGA TTC AAG G-3', 5'-TCA GCT GTC TCT TCC TCC CAG C-3' (for the α3-peptide). All PCR reactions were performed using *Taq* polymerase (Invitrogen Life Technologies) with the following cycle parameters: initial denaturation temperature of 92°C for 10 min, 25 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min, followed by a final extension of 72°C for 7 min. The PCR products were ligated and TA-cloned directly into the pET *SUMO* vector, and positive plasmids were confirmed by sequencing (Laboratory Services Division, University of Guelph). Subsequent plasmids coding for SUMO-MBP-α-peptide fusion proteins were transformed into *E. coli* BL21-CodonPlus (DE3)-RP cells (Stratagene, La Jolla, CA) and were expressed and purified as described below.

Expression and purification of full-length 18.5 kDa recombinant murine MBP (rmC1): The unmodified 18.5 kDa recombinant murine MBP isoform (rmC1) was expressed in *E. coli* BL21-CodonPlus(DE3)-RP cells (Stratagene, La Jolla, CA) and purified by nickel-affinity chromatography, followed by ion exchange chromatography to remove minor contaminating material as previously described (4;17). Protein eluate from the column was dialyzed and lyophilized as previously published (2). Protein concentration was determined by measuring the absorbance at 280 nm, using the extinction coefficient ε=0.676 $Lg^{-1}cm^{-1}$ (as calculated by SwissProt for protein in 6.0 M guanidium hydrochloride, 0.02 M phosphate buffer, pH 6.5). Purity of the protein preparation was assayed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining.

Expression and purification of MBP-α-peptides: Three MBP-α-peptides (α1, α2, and α3) fused to His-tagged SUMO at their N-terminus were expressed in *E. coli* BL21-CodonPlus(DE3)-RP cells (Stratagene, La Jolla, CA). Cells were grown at 37°C in 130 mL LB kanamycin $(kan)^+$-chloramphenicol $(cam)^+$ (25 μg/mL and 33 μg/mL, respectively) until an A_{600nm}~1.0 was reached. Cells were harvested by centrifugation, and the pellet was suspended in 1 L M9 minimal media $(kan)^+$-chloramphenicol $(cam)^+$ (25 μg/mL and 33 μg/mL, respectively), and further grown at 37°C until A_{600nm}~0.7–0.8. The culture was then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and grown for a further 4-5 h. Cells were harvested by centrifugation, and the pellet was frozen at -20°C until used.

The following procedure was applied for each MBP-α-peptide construct. Frozen pellets from the 1 L culture were suspended in 30 mL lysis buffer (phosphate-buffered saline (PBS), pH 8.0, 150 mM NaCl, 1% v/v Tween-20, 10 mM imidazole, 1 mM phenylmethylsulfonylfluoride (PMSF)), homogenized and sonicated on ice six times for 10 s, with 10 s cooling between bursts. Lysates were centrifuged at 25,000 g for 30 min at 4°C to pellet cell debris, and the supernatants were applied to a column containing a 4 mL bed volume of Ni^{2+}-NTA pre-equilibrated with lysis buffer. The column was washed with 40 mL of lysis buffer, followed by another 40 mL of wash buffer (PBS, pH 8.0, 15 mM imidazole), and finally, SUMO-fused MBP-α-peptides were eluted with 25 mL of elution buffer (PBS, pH 8.0, 0.5 M imidazole). Samples from 1.3 mL fractions eluted from these columns were evaluated by discontinuous SDS-PAGE (5% stacking, 14% separating), and pure fractions of each SUMO-fused peptide, separately, were combined and dialyzed against protease buffer (10% glycerol in PBS, pH 8.0), two changes of 2 L each.

Following dialysis, SUMO was removed by Ulp1 protease at a 1:1000 protease to SUMO-peptide w/w ratio at 30°C for 3 h in the presence of 2 mM dithiothreitol (DTT). Recombinant SUMO Ulp1 protease was expressed from a pET28b plasmid (Novagen, Gibbstown, NJ) containing His-tagged Ulp(403-621), which was a kind gift from Dr. Christopher Lima (Sloan-Kettering Institute, NY) (23). Digestion mixtures were applied onto three Ni^{2+}-NTA (4 mL bed volume) columns pre-equilibrated with protease buffer. The columns were washed with 20 mL of 10 mM imidazole in protease buffer (wash 1), followed by another 20 mL of 15 mM imidazole in the same buffer (wash 2), and bound material was eluted in 20 mL of 0.5 M imidazole in protease buffer. Using Tricine-PAGE (28), MBP-α-peptides were detected in the flow-through and two wash fractions.

The MBP-α-peptides were further purified by reversed-phase high-performance liquid chromatography (HPLC) using a Waters (Mississauga, ON) apparatus with a Symmetry 300 C18, 5 mm, 4.6 x 250 mm column. Acetonitrile constituted the mobile phase, and 0.1% TFA (trifluoroacetic acid) was the ion-pairing agent. Detection was at 214 nm, the flow rate was 1 mL/min, and the column was maintained at 22°C. The elution gradient was begun at 80% Solvent A (ddH_2O with 0.1% TFA) and 20% Solvent B (acetonitrile with 0.1% TFA), and run at a rate of 1% Solvent B/min for 30 min, followed by 5% Solvent B/min for 10 min. Peptides were collected and their purity was assessed using the same method. To confirm the molecular mass, a sample of each MBP-α-peptide was analyzed by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry (Protein Analysis Facility, Hospital for Sick Children, Toronto, ON).

The results obtained from these purification procedures are presented in **Figure S2**.

Purification of calmodulin: The expression vector for recombinant *Xenopus laevis* calmodulin (CaM) was obtained from Dr. M. Ikura (Ontario Cancer Institute, Toronto, ON), and this protein was expressed in *E. coli* and purified as described (27).

Purification of actin from chicken muscle: Chicken muscle acetone powder was prepared, and actin was extracted from 8 g of it at a time as described (26). The purity of actin was checked using MALDI-TOF mass spectrometry, supported by SDS-PAGE, and it was clear that no further purification steps were required. Protein concentration was determined by measuring the absorbance at 280 nm, using the extinction coefficient ε=0.62 $Lg^{-1}cm^{-1}$. The protein suspended in G-buffer (2 mM TRIS-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM $CaCl_2$, and 0.2 mM 2-mercaptoethanol) was aliquoted into 1.5 mL microfuge tubes, flash frozen in liquid nitrogen, and stored at -80°C. The pyrene labeling of actin was performed as previously published (16).

Circular dichroism (CD) spectroscopy: The experiments were performed using a Jasco J-815 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) using an initial peptide concentration of 0.5 mg/mL in a sample volume of 150 µL in a quartz demountable cuvette with a path length of 0.05 cm. The far-UV CD spectra (180–250 nm) were scanned at a rate of 50 nm/min, with data collection at 1 nm intervals at 30°C. The blank was subtracted after each measurement, and the spectra from six successive scans were averaged for each sample. The molar ellipticities were calculated using the MBP-α-peptide concentrations and molar mass. The CD measurements were carried out for each MBP-α-peptide in the following solutions: (*i*) ddH_2O; (*ii*) CD buffer (20 mM Tris-H_2SO_4, pH 7.4, 100 mM Na_2SO_4); (*iii*) 10% TFE in CD buffer; (*iv*) 20% TFE in CD buffer; (*v*) 30% TFE in CD buffer; (*vi*) 40% TFE in CD buffer; (*vii*) 50% TFE in CD buffer; (*viii*) 0.5 mM DPC in CD buffer; (*ix*) 1 mM DPC in CD Buffer; (*x*) 10 mM DPC in CD buffer; and (*xi*) 30 mM DPC in CD buffer.

The results obtained from these CD experiments are presented in **Figure S3**.

To study the α1- and α3-peptide interactions with Ca^{2+}-CaM, CD spectra were collected using a demountable cuvette with a path-length of 0.01 cm in the buffer comprising 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$ [*cf.*, (20)]. For this experiment, we used 0.5 mg/mL peptides (131 µM and 176 µM, for α1 and α3, respectively) with equimolar amounts of Ca^{2+}-CaM.

Isothermal titration calorimetry (ITC): Experiments were carried out using a VP-ITC instrument (Microcal, Inc., Northampton, MA). Lyophilized apo-calmodulin (apoCaM) was dissolved in 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$, and was extensively dialyzed against the same solution (at least 4 changes). Following the dialysis, the protein was filtered (0.22 µm pore size) and the concentration was estimated from the absorbance at 280 nm = 1.48 (1%, 1 cm). The MBP-α-peptides were dissolved in the same solution prior to experiment at the concentration range of 0.9-1 mM. Samples were degassed in a Thermovac (Northhampton, MA) at 30°C for 10 min. The MBP-α-peptide (either α1, α2, or α3) solution (0.9-1.0 mM) was injected into the sample cell, containing Ca^{2+}-CaM (0.07-0.10 mM) in the above solution. Typically, the titrations were carried out with a preliminary injection of 2 µL followed by 28 injections of 10 µL of

peptide solution with 300 s spacing between each. All experiments were carried out at 30°C. Before analysis, data from the preliminary 2 μL injection were discarded, and heats of dilution of the peptide into solution of 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$ (in the absence of CaM) were subtracted from the "peptide into CaM" titration experiments. The corrected data were integrated and plotted as a function of the molar ratio, and the binding isotherms obtained were fitted to the Origin "one set of sites" model (Origin 5.0, Microcal). To study the effect of Ca^{2+}, the same experiments were performed in the Ca^{2+}-depleted buffer (20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 8 mM EDTA, 2 mM EGTA).

The thermodynamic parameters derived from these ITC experiments are presented in **Table S1**.

Actin polymerization assay: Actin polymerization was followed by measuring an increase in fluorescence intensity of pyrene-labeled actin via an automated microplate fluorescence reader (Polarstar Omega, BMG Laboratory Technologies) using a 367-10 nm filter for excitation and a 407-10 nm filter for emission channels. The stock of 15 μM G-actin was prepared in G-buffer by mixing pyrene-labeled actin with unlabeled actin to a final proportion of 5% label, and 33 μL were added to each well followed by an additional volume of G-buffer calculated to give a final volume of 100 μL after addition of MBP-α-peptides. Stocks of MBP-α-peptides were also prepared in G-buffer at a concentration of 33.75 μM and different volumes were automatically injected across the half area 96-well plate to reach the final volume of 100 μL in each well. Thus, the concentration of MBP-α-peptide ranged from 0.5 μM to 22.5 μM or from 1:0.1 to 1:4.5 [actin]:[α1/α2/α3] molar ratio. In the case of the N-terminal MBP-α-peptide (α1), to reach a higher molar ratio, the stock was prepared at a concentration of 60 μM, and different volumes were injected to reach a 1:8 molar ratio of actin to α1-peptide. The plate was mixed for 10 s, and the emission intensity was measured for 30 min at 30°C.

In the experiments where the effect of dodecylphosphocholine (DPC) on α1-induced actin polymerization was studied, 33.75 μM stock of the peptide was prepared in G-buffer in the presence of 27 mM DPC. To determine the effect of Ca^{2+}-CaM on MBP-α-peptide-induced actin polymerization, we used a previously described method (5). Briefly, to 1.1 nmol of G-actin in 450 μL G-buffer, we added 13.3 nmol of MBP-α-peptide in 20 μL G-buffer, and followed the increase in pyrene fluorescence intensity (excitation at 365 nm and emission at 407 nm) for 30 min. After a plateau was achieved, 13.3 nmol Ca^{2+}-CaM in 53.2 μL G-buffer was added and the emission was measured for another 30 min. The experiment was performed at 30°C and the final molar ratio of [actin]:[MBP-α-peptide]:[Ca^{2+}-CaM] was 1:12:12, respectively.

Transmission electron microscopy (TEM): Transmission electron microscopy was used to examine the morphology of the MBP-α-peptide-induced F-actin assemblies. Samples were prepared and examined at a 4.5 (or 6.5) to 1 [MBP-α-peptide]:[actin] molar ratio as previously described (1;2).

The results of these analyses are presented in **Figure S4**.

Analysis of actin bundling: To test the ability of α1 to bundle actin in the presence or absence of dodecylphosphocholine (DPC), samples were prepared at a 1:1, 1:4.5, 1:6, and 1:8 actin to peptide molar ratios in a final volume of 600 μL. The samples were incubated at 30ºC for 30 min

followed by centrifugation at 18,000*g* for 2 h. Pellets and supernatants were analyzed by SDS-PAGE for the presence of actin and α1-peptide as previously described (2). The amount of DPC bound to the peptide upon interaction with actin was determined by the Micro-Bartlett phosphorus assay (3). The amount of α1 bound to actin was determined by analysis of the HPLC profile of the resuspended pellets and comparing the integrated areas of peak related to the MBP-α-peptide to a standard curve obtained from running samples with known amount of MBP-α-peptide under the same chromatographic conditions.

A demonstration of this actin assembly assay is presented in **Figure S5**.

Solution NMR spectroscopy: For solution NMR spectroscopy of the interaction of the α1- or α3-peptides of 18.5 kDa mMBP with calcium-activated calmodulin (Ca^{2+}-CaM), uniformly ^{13}C,^{15}N-labeled α1- or α3-peptide was prepared as described before, but this time the M9 minimal media was supplemented with $^{15}NH_4Cl$, and $^{13}C_6$-glucose (C.I.L., Cambridge, MA). The freeze-dried peptide was dissolved in the NMR sample buffer composed of 20 mM HEPES, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$, and 90% H_2O/10% D_2O. For titration experiments, the initial peptide concentration was 1.5 mM. Then, unlabeled CaM was titrated by adding CaM stocks dissolved in NMR sample buffer stepwise to achieve [α3]:[CaM] molar ratios of 0.1, 0.2, 0.3, 0.5, 0.8, 0.9, 1.0, 1.1, and 1.2 (ditto for the α1-peptide). In other titration experiments, the α3-Ca^{2+}-CaM complex was titrated with EDTA-EGTA to prove the Ca^{2+}-dependence of the interaction.

A series of two-dimensional ^{1}H-^{15}N heteronuclear single-quantum coherence (HSQC) (6;13;25;30) spectra was collected for the α1- or α3-Ca^{2+}-CaM sample at each of the molar ratios. For sequence-specific resonance assignments of the α3-peptide alone in solution, another sample was prepared at molar concentration of 1.6 mM in the same NMR buffer. All spectra were collected on a Bruker Avance III spectrometer operating at a proton Larmor frequency of 600.13 MHz and equipped with a triple resonance cryogenic-operated probe. All resonance assignments were done at 22°C. Sequential connectivities of backbone resonances were established using the standard Bruker suite of 3D heteronuclear experiments: HNCO (9;29), HN(CA)CO (7;14), HNCACB (24;31), and CBCA(CO)NH (10;24). The acquisition parameters for all spectra are summarized in **Table S2**. All spectra were processed using NMRPipe (8) as follows: a cosine-squared window function was applied in all dimensions; then the time domains were zero-filled up to 512 x 512 x 2048 complex points in the F_1 x F_2 x F_3 dimensions, respectively, prior to Fourier transformation. Details of the backbone resonance assignments are given next.

Backbone resonance assignments: All sequence-specific assignments were done within the environment of CARA (Computer-Aided Resonance Assignment) (15) and using a set of in-house scripts written in LUA (www.lua.org), and executed using the built-in LUA interpreter. The first script used the HNCO spectrum to pick peaks and create spin-systems automatically, based on ^{13}C, ^{15}N, and ^{1}H ppm values of $^{13}C'[i-1]$, $^{15}N[i]$, and $^{1}H^{N}[i]$, respectively. Then, another script picked and assigned C'[i] for each spin-system from HN(CA)CO followed by picking and assigning $C^{\alpha}[i]$, $C^{\alpha}[i-1]$, $C^{\beta}[i]$, and $C^{\beta}[i-1]$ of each spin-system from CBCA(CO)NH and HNCACB and two other LUA scripts. The created spin-systems were then linked based on the predecessor/successor matching scores calculated by CARA, based on the matching of C'[i]-C'[i-1], $C^{\alpha}[i]$-$C^{\alpha}[i-1]$, and $C^{\beta}[i]$-$C^{\beta}[i-1]$ pairs of spins. Linking the spin-systems and matching the

fragments of spin-systems to fragments of the amino acid sequence of the peptide was done manually due to the simplicity of the sequence. **Figure S6** shows an example of a fragment of spin-systems after assignment of the α3-peptide.

The ^{1}H-^{15}N HSQC spectra of both the α3-peptide (**Figure S7**), and the α1-peptide (**Figure S8**), demonstrated peak shifts upon interaction with Ca^{2+}-CaM. Since the C-terminal α3-peptide is the primary CaM-target originally predicted (17-19;21), we analyzed it in detail first. (Solid-state and solution NMR structural studies of the α1-peptide in association with actin and with Ca^{2+}-CaM, respectively, are currently underway and will be part of a future publication.) Here, current backbone assignments are provided in **Tables S3** and **S4** for the α3-peptide alone and complexed with Ca^{2+}-CaM, respectively.

SUPPORTING TABLES and FIGURES

Table S1: Summary of thermodynamic parameters obtained from ITC for the interaction of the α1- and α3-peptides with Ca^{2+}-CaM.

Peptide	*n*	K_a, M^{-1}	ΔH, kcal mol^{-1}	$-T\Delta S$, kcal mol^{-1}
α1	1.02±0.01	(8.13±0.90) 10^5	-6.51±0.07	-1.69±0.18
α3	1.07±0.01	(2.08±0.27) 10^5	-5.41±0.07	-2.14±0.32

All measurements were carried out in triplicate at 30ºC. The values shown represent the fitting parameters obtained using the "one set of sites" model. For each variant, "K_a" is the association constant, "ΔH" is the change in enthalpy, "ΔS" is the change in entropy, and "*n*" is the number of ligands bound. Errors correspond to 95% confidence levels in the determination of the parameters. The "$-T\Delta S$" is presented as the mean ± standard deviation of at least 3 independent experiments.

Table S2: Summary of NMR acquisition parameters[a,b].

Acquisition Parameter / Spectrum	Transients	Carrier Frequency (ppm)			Sweep Width (ppm)			Number of Points			**Quadrature Detection[a]**	
		F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2
HNCACB	8	40	118	4.7	76	35	14	144	44	2048	S-TPPI	**S-TPPI**
CBCA(CO)NH	8	40	118	4.7	76	35	14	144	44	2048	S-TPPI	**S-TPPI**
HNCO	16	175	118	4.7	25	35	14	72	44	2048	S-TPPI	**S-TPPI**
HN(CA)CO	16	175	118	4.7	25	35	14	72	44	2048	S-TPPI	**S-TPPI**
HSQC	**10**	**115**	**4.7**	**-**	**30**	**16**	**-**	**128**	**2048**	**-**	**E-A-E**	**-**

[a] S-TPPI = States-TPPI (22), E-A-E = Echo-AntiEcho (13).

Table S3. The ^{1}H and ^{13}C chemical shifts of the 18.5 kDa murine MBP α3-peptide alone in 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$, and 90% H_2O/10% D_2O, referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid).

#	AA	H_N	N	C`	C^{α}	C^{β}
133'	Ser	-	-	-	-	-
134'	Ala	-	-	-	-	-
135'	His	-	-	-	-	-
136'	Lys	-	-	-	-	-
137'	Gly	7.899	109.787	170.759	42.318	-
138'	Phe	8.125	120.315	173.054	55.029	36.994
139'	Lys	8.406	124.211	173.737	53.619	30.092
140'	Gly	8.434	110.266	170.905	42.393	-
141'	Ala	8.118	123.528	174.714	49.760	16.510
142'	Tyr	8.208	119.289	172.761	55.104	35.881
143'	Asp	8.173	122.502	173.347	50.947	38.404
144'	Ala	8.208	124.690	175.398	50.428	16.139
145'	Asn	8.331	117.991	174.079	53.619	26.307
146'	Gly	8.297	109.241	171.882	42.635	-
147'	Thr	8.105	114.368	172.093	59.705	66.904
148'	Leu	8.317	124.348	174.812	52.803	39.443
149'	Ser	8.221	116.418	171.882	55.920	60.967
150'	Lys	8.242	123.049	173.542	53.842	30.092
151'	Ile	7.927	120.588	173.249	58.443	35.807
152'	Phe	8.255	124.553	172.663	55.104	36.920
153'	Lys	8.160	123.528	173.298	53.248	30.463
154'	Leu	8.294	123.953	175.153	52.803	39.443
155'	Gly	8.481	110.266	171.931	42.486	-
156'	Gly	8.290	108.694	171.345	42.465	-
157'	Arg	8.242	120.315	173.347	53.396	28.162
158'	Asp	8.475	121.409	172.517	51.764	38.479
159'	Ser	7.824	120.725	175.788	57.182	62.154

Table S4. Resonance assignments of the 18.5 kDa murine MBP α3-peptide complexed with Ca^{2+}-CaM in 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5 mM $CaCl_2$, and 90% H_2O/10% D_2O, referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid).

#	AA	H_N	N	C`	C^{α}	C^{β}
133'	Ser	-	-	-	-	-
134'	Ala	8.765	124.382	177.351	52.612	19.014
135'	His	8.614	117.956	174.482	55.412	28.813
136'	Lys	8.559	122.468	177.159	56.381	32.905
137'	Gly	8.573	109.480	177.124	45.612	-
138'	Phe	8.231	118.913	-	-	-
139'	Lys	-	-	-	-	-
140'	Gly	-	-	-	-	-
141'	Ala	-	-	-	-	-
142'	Tyr	-	-	-	-	-
143'	Asp	-	-	-	-	-
144'	Ala	-	-	-	-	-
145'	Asn	-	-	-	-	-
146'	Gly	-	-	-	-	-
147'	Thr	-	-	-	-	-
148'	Leu	8.094	111.667	-	54.119	-
149'	Ser	7.840	120.691	178.548	59.827	64.780
150'	Lys	-	-	-	-	-
151'	Ile	-	-	-	-	-
152'	Phe	-	-	-	-	-
153'	Lys	-	-	-	-	-
154'	Leu	-	-	-	-	-
155'	Gly	-	-	-	-	-
156'	Gly	8.176	108.249	174.297	45.289	-
157'	Arg	8.217	120.281	176.139	55.950	30.859
158'	Asp	8.505	121.374	175.190	54.119	40.766
159'	Ser	7.840	120.691	178.548	59.827	64.780

Figure S1: Potential α-helical molecular recognition fragments (A) in the intrinsically disordered 18.5-kDa "classic" murine MBP isoform (B). The amino acid sequence of murine recombinant MBP (rmMBP, or rmC1 for the unmodified form) consists of 168 residues with an additional LEH_6 purification tag at the C-terminus of the protein (panel B); the N-terminal methionine is cleaved post-translationally and is not included in numbering. Three rectangles represent the sequences of polypeptides generated for the purpose of the present study (A22-K56, S72-S107, S133-S159, for α1, α2, and α3, respectively). The insets in panel (A) show strongly amphipathic α-helical regions in the rmC1 sequence suggested also by solution NMR studies (11;17), *inter alia*. Arrows show the vertical alignment of hydrophobic moment vector. The helical wheel representations were redrawn from the results generated using the HeliQuest program (http://heliquest.ipmc.cnrs.fr/), and are adapted from reference (11).

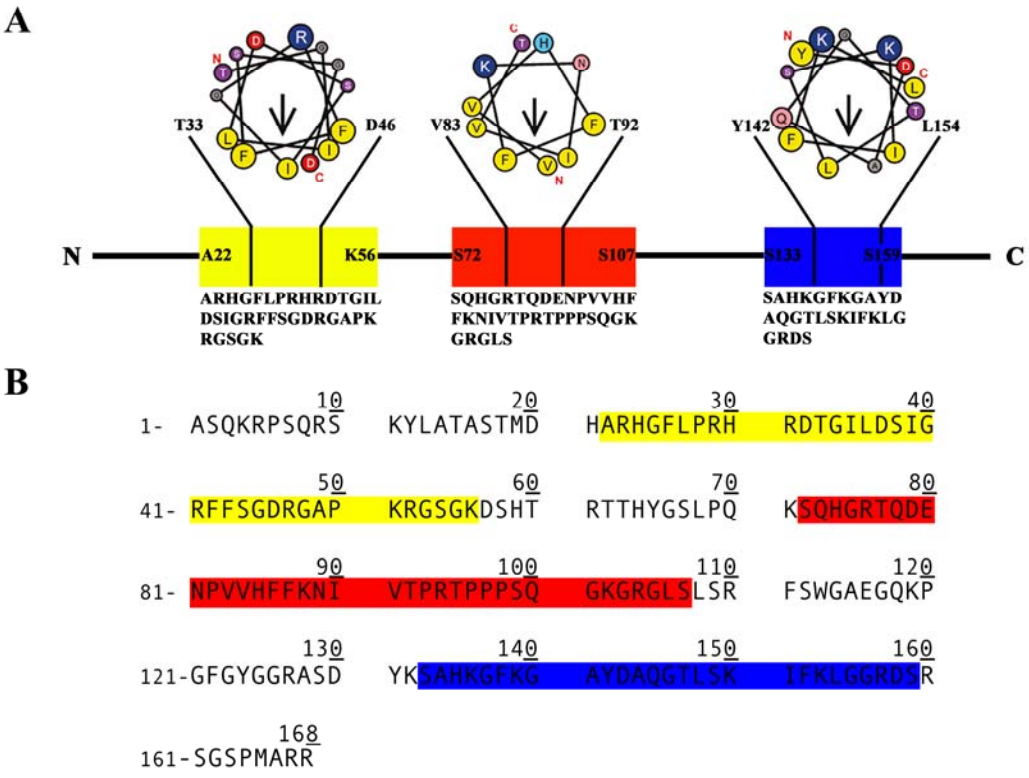


Figure S2: Expression and purification of recombinant murine 18.5 kDa MBP (unmodified rmC1 variant) α-peptides. Three MBP-α-peptides (α1, α2, and α3) were expressed using the Champion™ pET SUMO Expression System. Peptides fused with SUMO at their N-terminus were over-expressed and purified using immobilized Ni^{2+}-affinity chromatography. The SDS-PAGE analyses presented on the left-hand side of panels (A,C,E) compare the crude lysates "C" and the eluates "E" of three MBP-derived SUMO-peptides (α1, α2, and α3, respectively). The right-hand sides of panels (A,C,E) confirm the enzymatic cleavage of SUMO (lane D) from the peptides by Tricine-PAGE analysis. Peptides were further purified by the second round of immobilized Ni^{2+}-affinity chromatography followed by an HPLC polishing step. The final purity of the MBP-α-peptides was assessed by analytical HPLC, as shown in panels (B,D,F) for α1, α2, and α3, respectively.

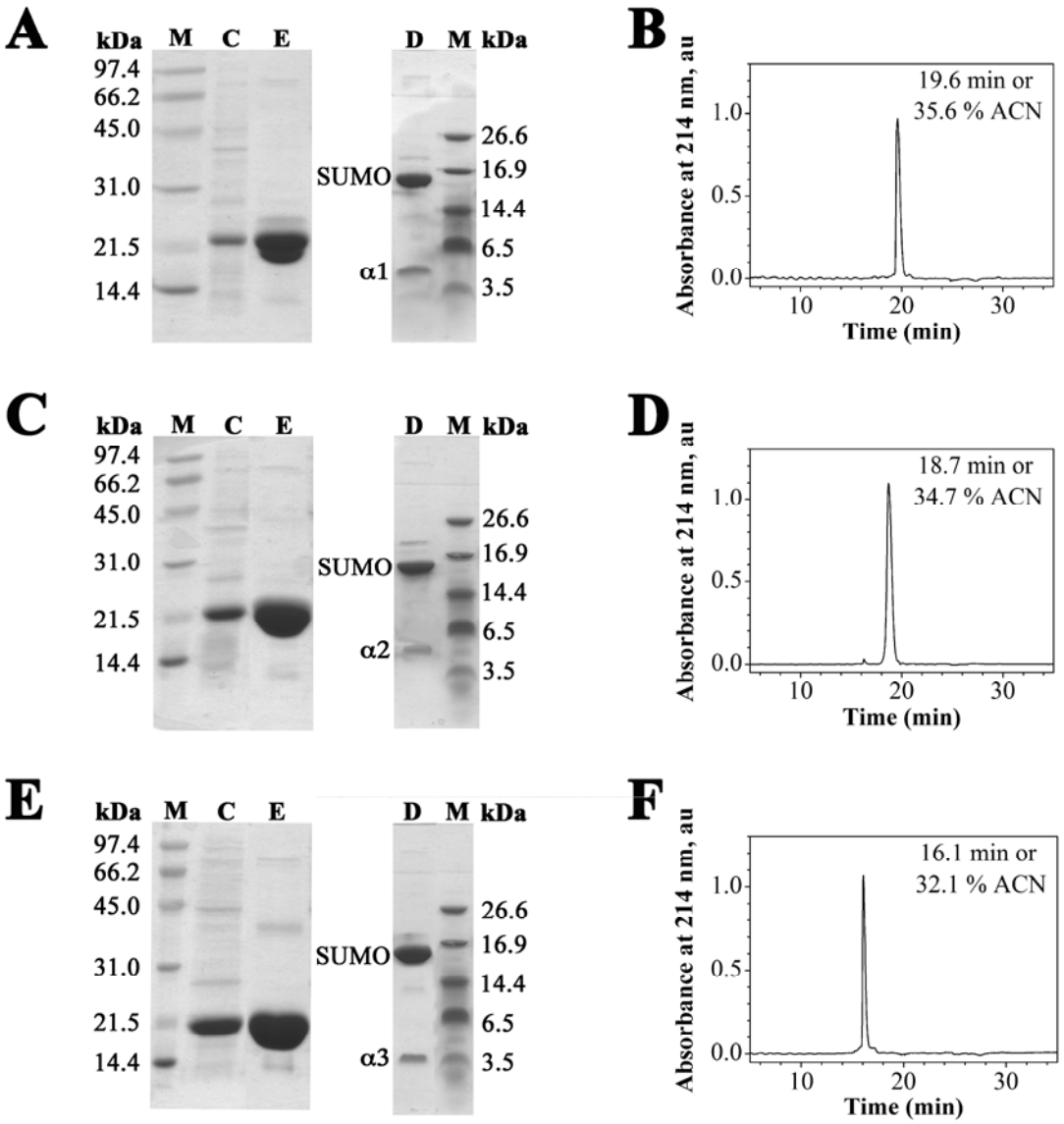


Figure S3: Circular dichroism (CD) spectroscopy of MBP-derived peptides in different chemical environments. The CD spectra under 8 different conditions of 0.5 mg/mL of α1-, α2-, and α3-peptides are shown in panels (A and D), (B and E), and (C and F), respectively. Panels (A,B,C) and (D,E,F) compare the effects of TFE and DPC, respectively. Solid line: 20 mM Tris-H_2SO_4, pH 7.4, 100 mM Na_2SO_4; dashed line: 10% TFE or 0.5 mM DPC; dotted line: 30% TFE or 1 mM DPC; dashed-dotted line: 40% TFE or 10 mM DPC; and dash-dot-dotted line: 30 mM DPC.

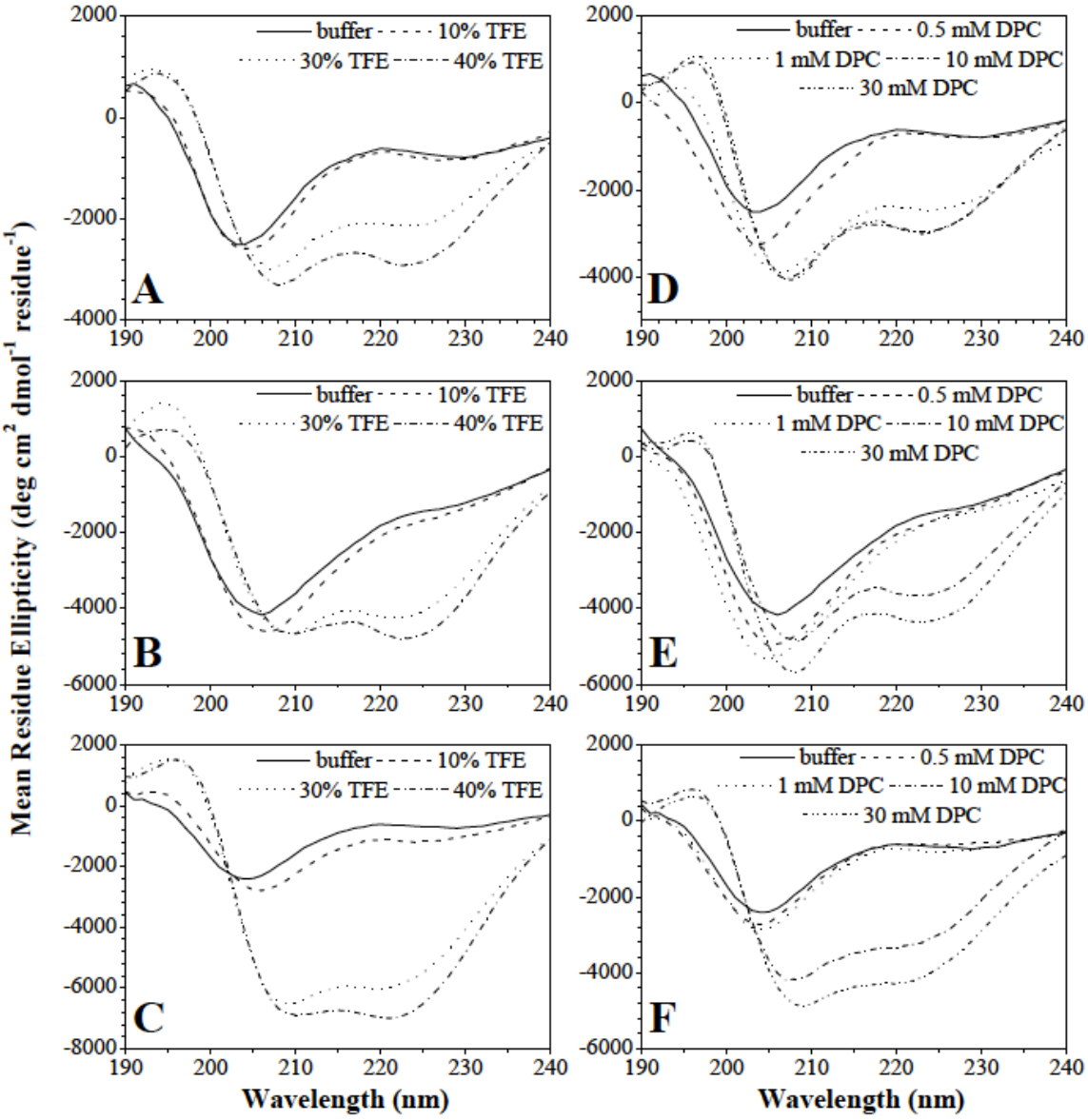


Figure S4: Transmission electron microscopy (TEM) of the actin assembly induced by MBP-derived peptides. The MBP-α-peptides: α1 (A), α2 (B), and α3 (C), were added to 5 μM of G-actin (D) at a 4.5 to 1 peptide:actin molar ratio. Following a 1 h incubation at room temperature, samples were stained with uranyl acetate, and the TEM image was captured on a Philips CM10 transmission electron microscope. All scale bars represent 200 nm at the object level.

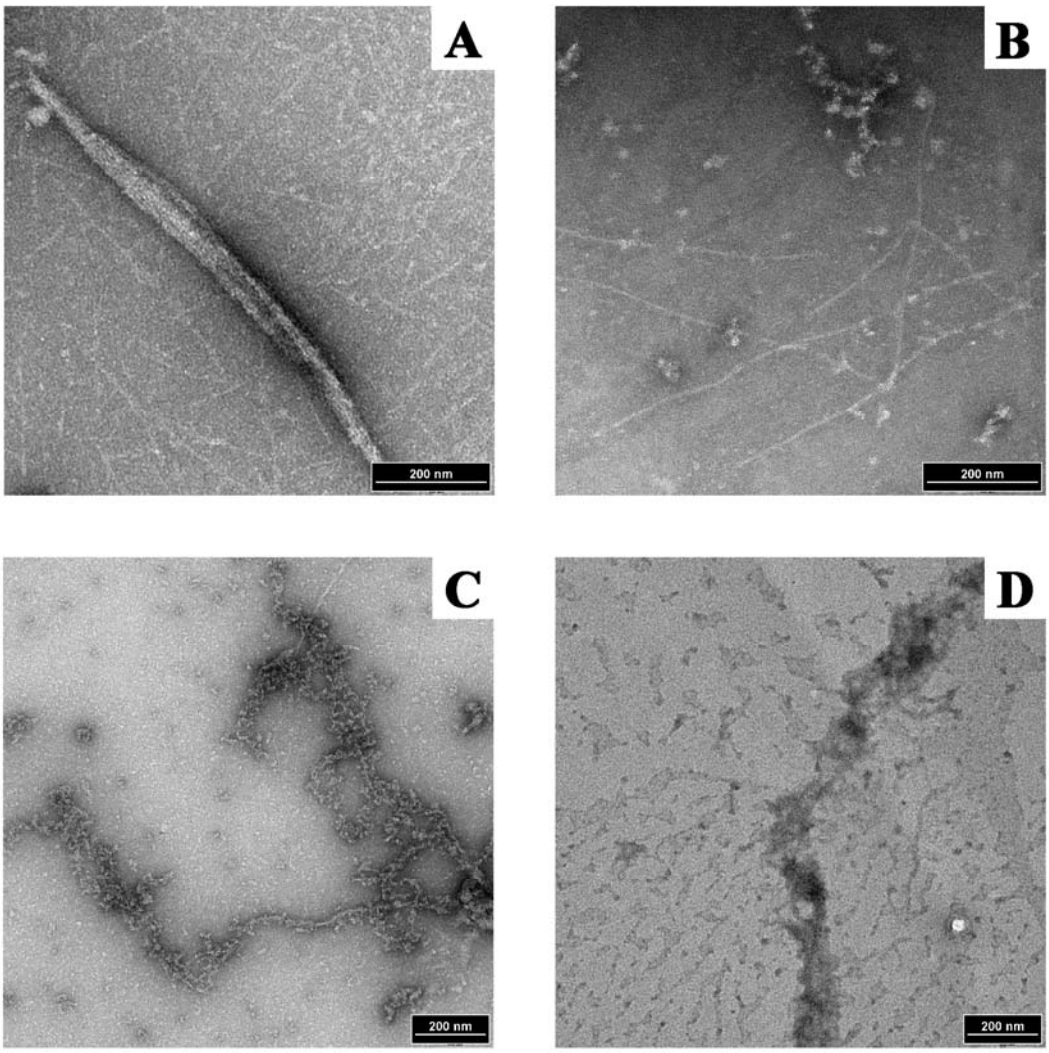


Figure S5: Demonstration of Tricine-PAGE-based assessment of actin bundling and binding by the N-terminal α1-peptide in the presence and absence of DPC. Shown is a representative binding experiment, where 5 μM of G-actin was mixed with different concentrations of α1-peptide in G-buffer to reach the molar ratios indicated above the gels. Reaction mixtures were analyzed as indicated in the Materials and Methods section for the presence of bound and free peptide. (**A**) Proteins in the pellet following sedimentation assay; (**B**) supernatants of the corresponding reaction mixture; (**C**) binding plot of fractional saturation *vs*. free peptide.

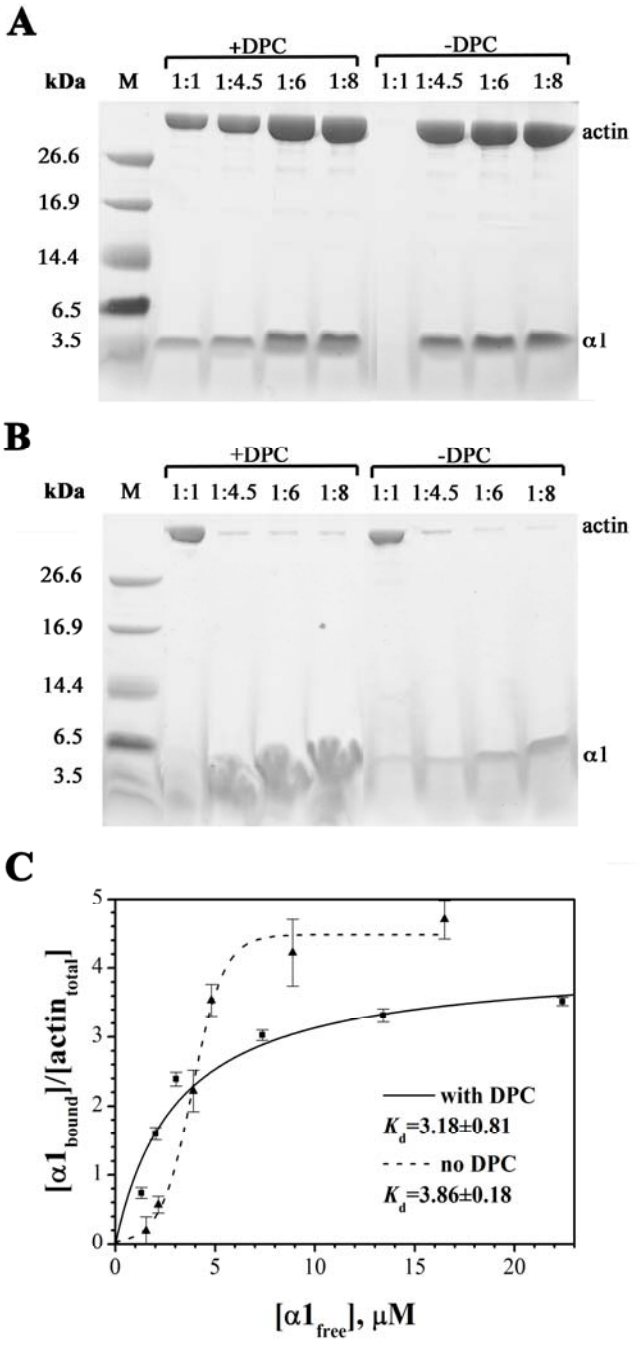


Figure S6: Sample strip-plot of HN(CA)CO (top), and HNCACB (bottom), for the fragment of the C-terminal α3-peptide, corresponding to residues (G155-S159) of the full-length 18.5 kDa murine MBP. Red contours are due to positive C' and C^{α} peaks, whereas blue contours represent negative C^{β} peaks.

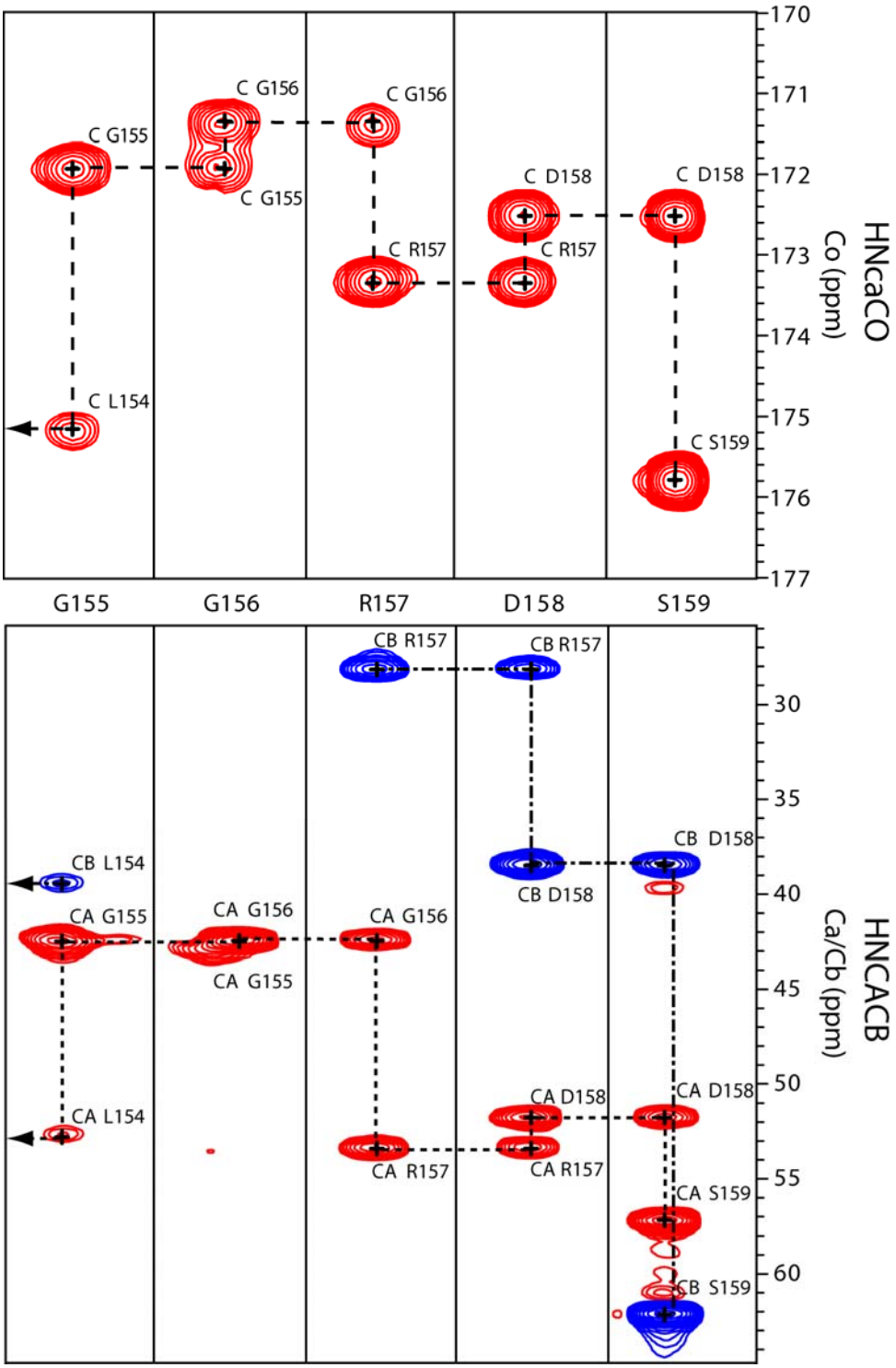


Figure S7: The ^{1}H-^{15}N HSQC spectra for the C-terminal α3-peptide alone, for the α3-Ca^{2+}-CaM complexes at a 1:1 molar ratio, and for the α3-Ca^{2+}-CaM complexes at a 1:1 molar ratio titrated with EDTA-EGTA to a maximum concentration of 5 mM (4 mM EDTA and 1 mM EGTA). All spectra were acquired using identical acquisition parameters and at the same temperature as the HSQC spectrum for the α3-peptide alone as given in **Table S1**.

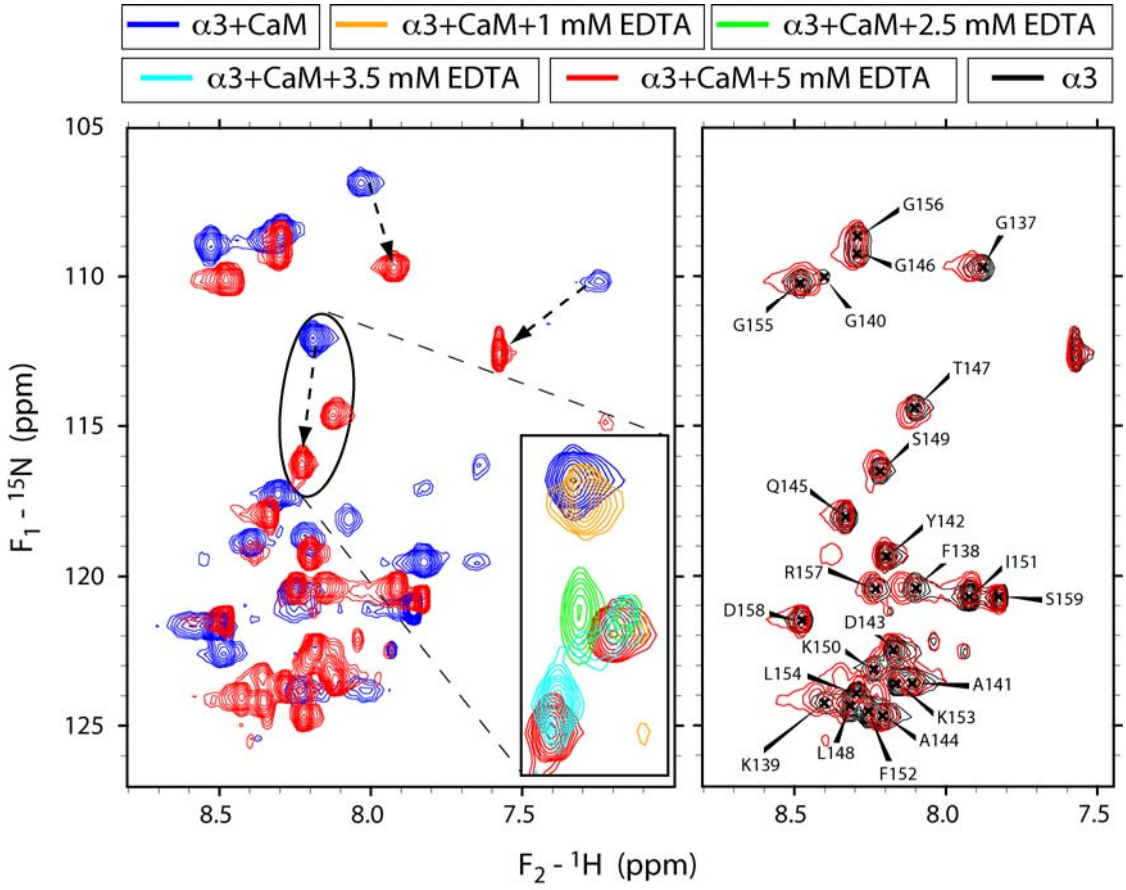
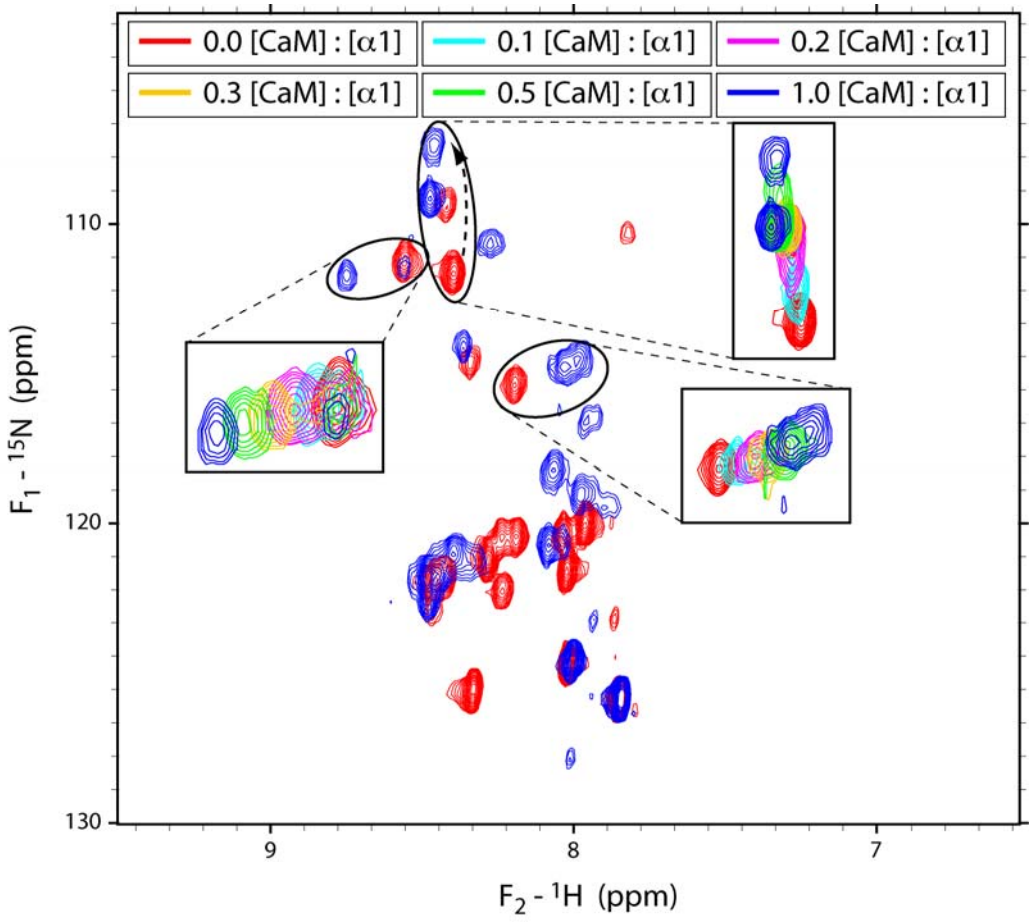


Figure S8: The ^{1}H-^{15}N HSQC spectra for the N-terminal α1-peptide, and for the α1-Ca^{2+}-CaM complexes at 1:1 and lesser molar ratios (both zoom-in regions in the insets).



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