TABLE S1. Dissociation constants for the myr(+)MA-CaM complex as calculated by fluorescence spectroscopy at different salt concentrations.

NaCl (mM)	K_d (μM)	χ^2	R^2
0	4.0 ± 1.0	0.000948	0.970
100	5.3 ± 0.5	0.00032	0.985
200	9.1 ± 0.8	0.0000618	0.995
300	11.4 ± 0.7	0.0000696	0.995
500	26.0 ± 2.0	0.0000943	0.993

χ^2 and R^2 are parameters that indicate the quality of fitting in ORIGIN.

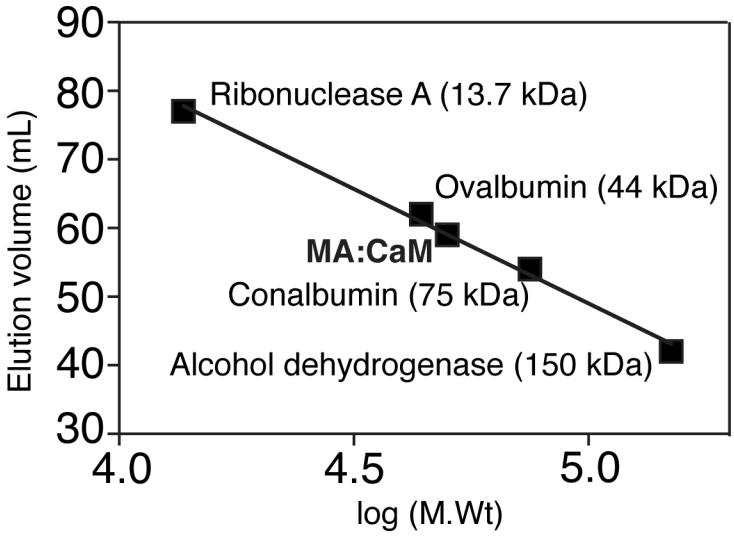

Figure S1. Gel filtration calibration assay showing mobility of the CaM-MA complex on a Sephacryl S-200 HR column (GE Healthcare).

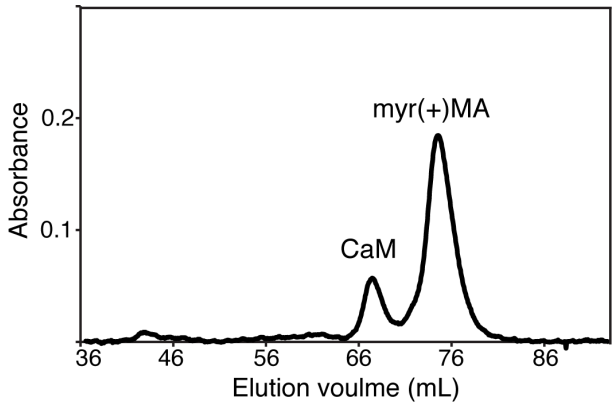

Figure S2. A gel filtration chromatogram showing no complex formation (~ 57 mL) in the absence of calcium. Sample was run on a HiLoad 16/60 Superdex 75 pg column (GE Healthcare).

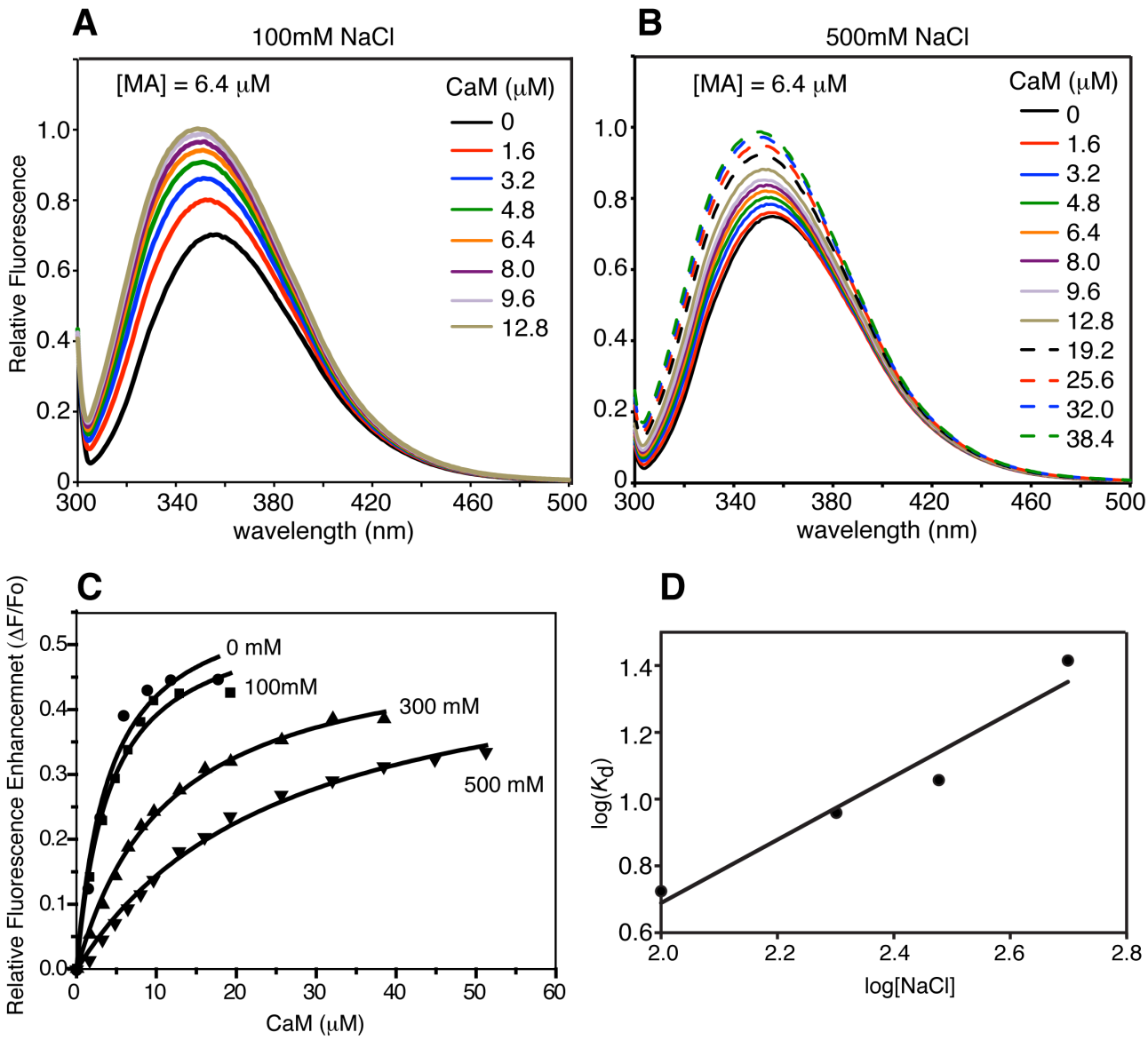

Figure S3. Salt effect on CaM-myr(+)MA interactions. Fluorescence emission spectra for myr(+)MA as titrated with CaM at (A) 100 mM and (B) 500 mM NaCl. (C) Change in fluorescence intensity for myr(+)MA as a function of salt ($\Delta F = F_n - F_o$, where F_n and F_o are fluorescence intensities for MA in the CaM-bound and free states, respectively). (D) A plot showing the correlation between dissociation constant (K_d) and salt concentration.

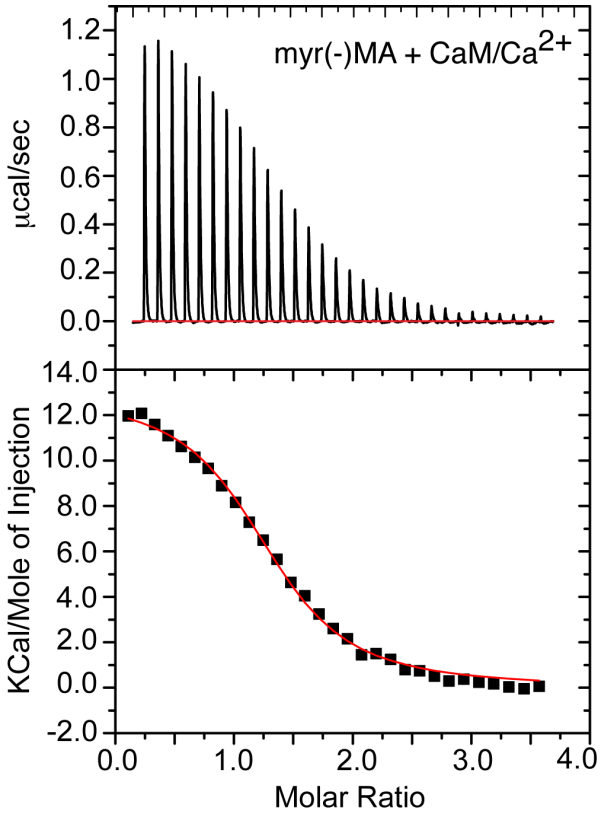

Figure S4. ITC data obtained upon titration of CaM (396 μM) into myr(-)MA (20.5 μM). Data best fit one-site binding mode and afforded a K_d of 2.1 μM (lower panel).

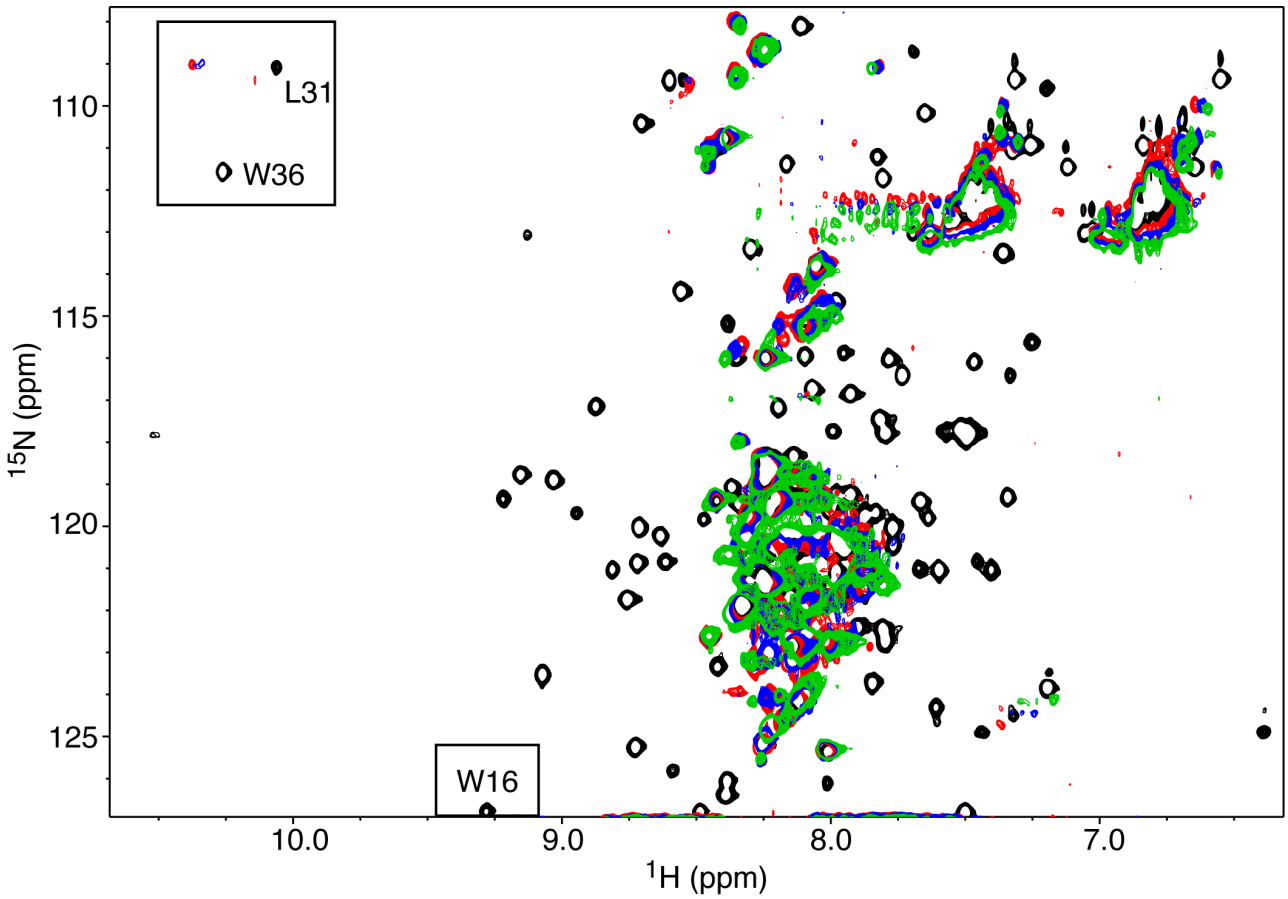

Figure S5. Overlay of 2D ^{1}H-^{15}N HSQC spectra obtained for ^{15}N-labeled myr(+)MA complexed with CaM as a function of temperature [unbound myr(+)MA at 35 ºC (black); myr(+)MA:CaM at 35 ºC (red), 25 ºC (blue) and 15 ºC(green)]. Boxed regions indicate significant chemical changes observed for Trp16 and Trp36 side chain signals.

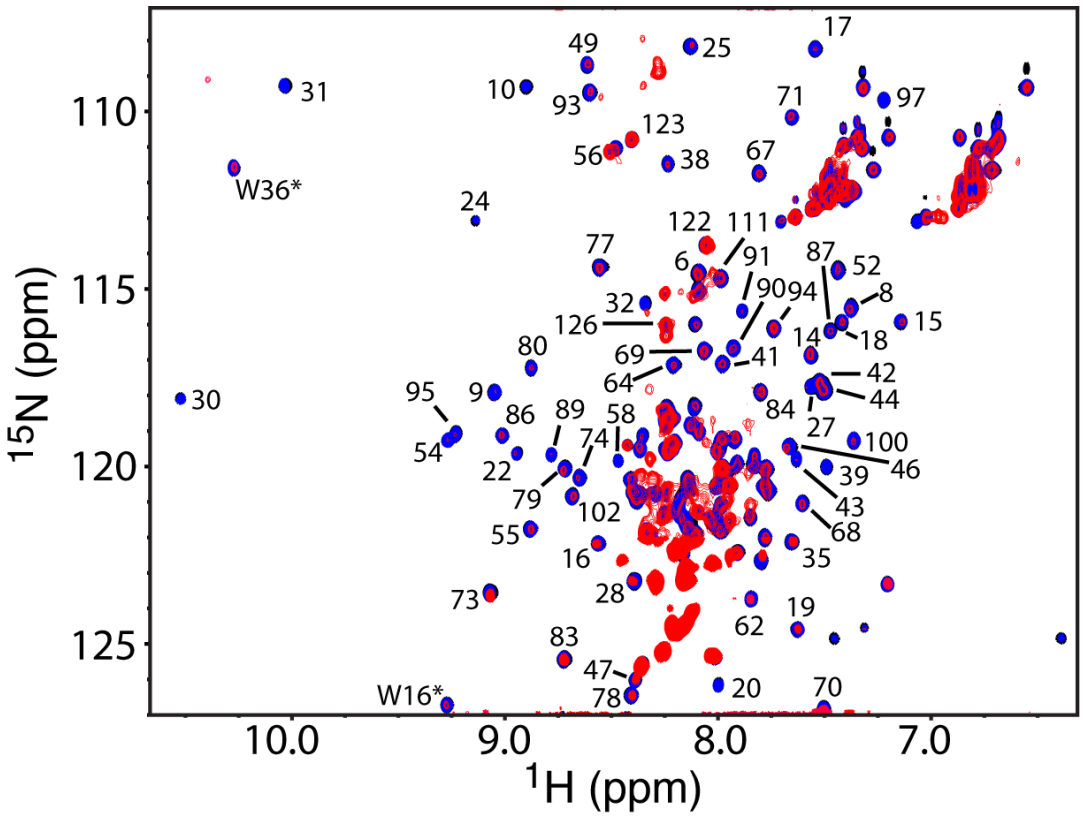

Figure S6. Overlay of 2D ^{1}H-^{15}N HSQC spectra obtained for ^{15}N-labeled myr(-)MA upon titration with unlabeled CaM [CaM:myr(-)MA = 0:1 (black), 0.25:1 (blue), 1:1 (red)].

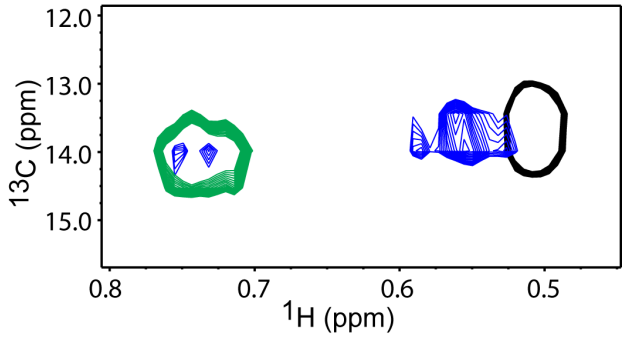

Figure S7. Overlay of 2D ^{1}H-^{13}C HMQC spectra obtained for myr(+)MA containing ^{13}C-labeled myr group upon titration with unlabeled CaM [CaM:myr(+)MA = 0:1 (black), 0.25:1 (blue), 1:1 (green)]. Only the ^{1}H-^{13}C signal for the myr terminal methyl group is shown. The two peaks observed at 0.25:1 indicate free and bound states in slow exchange.

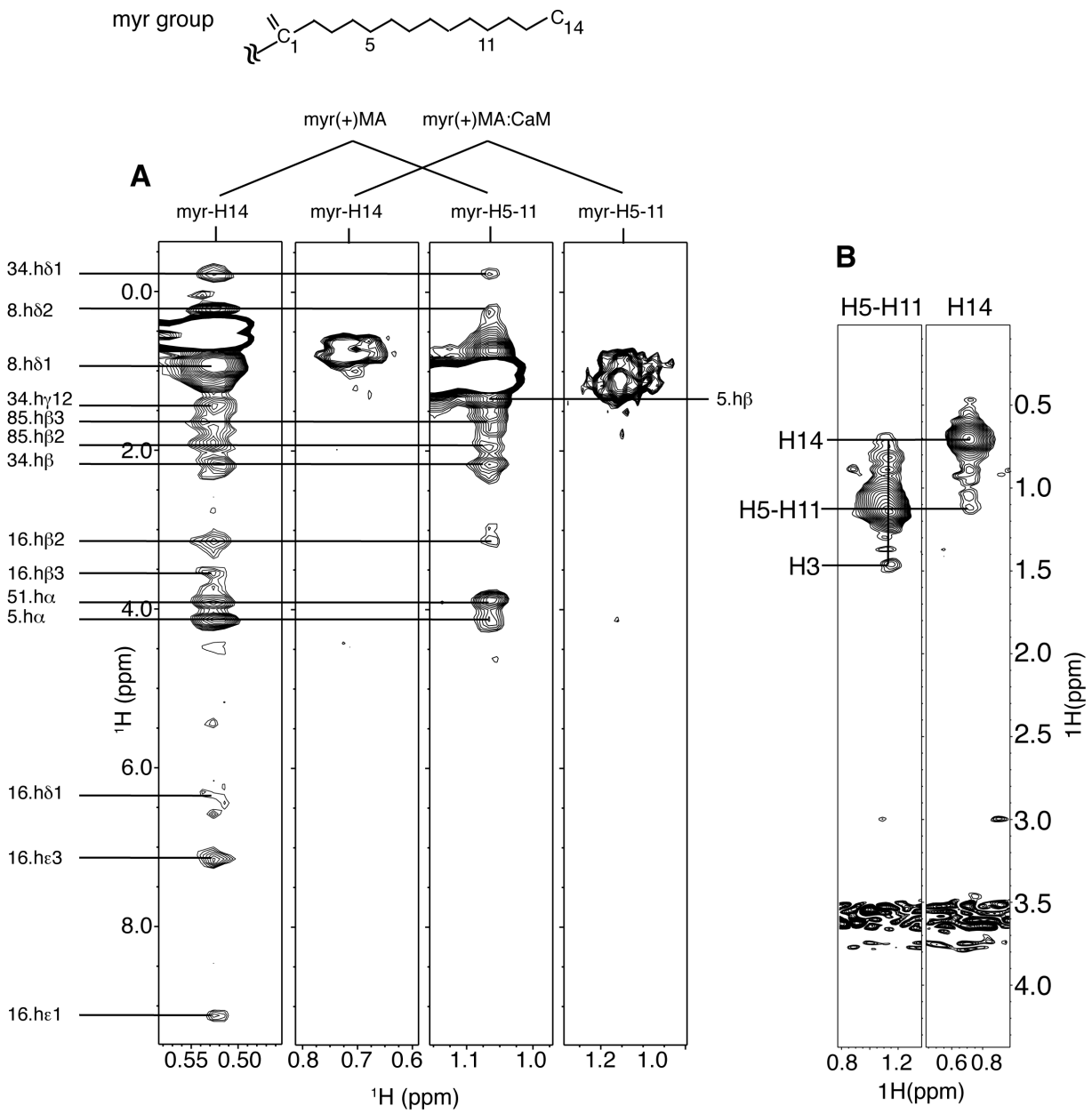

Figure S8. (**A**) Selected slices of the 3D ^{13}C-edited/^{12}C-double-half-filtered NOE data obtained for free and CaM-bound myr(+)MA showing assigned NOE cross-peaks between the myr group and key MA residues. These cross-peaks are absent in the myr(+)MA-CaM complex, indicating exposure of the myr group. Only the myr group is selectively ^{13}C-labeled. (**B**) For comparison, 3D ^{13}C-edieted HMQC-NOESY data obtained for the myr(+)MA-CaM complex show NOEs only between the methyl and methylene groups of the ^{13}C-myr group.

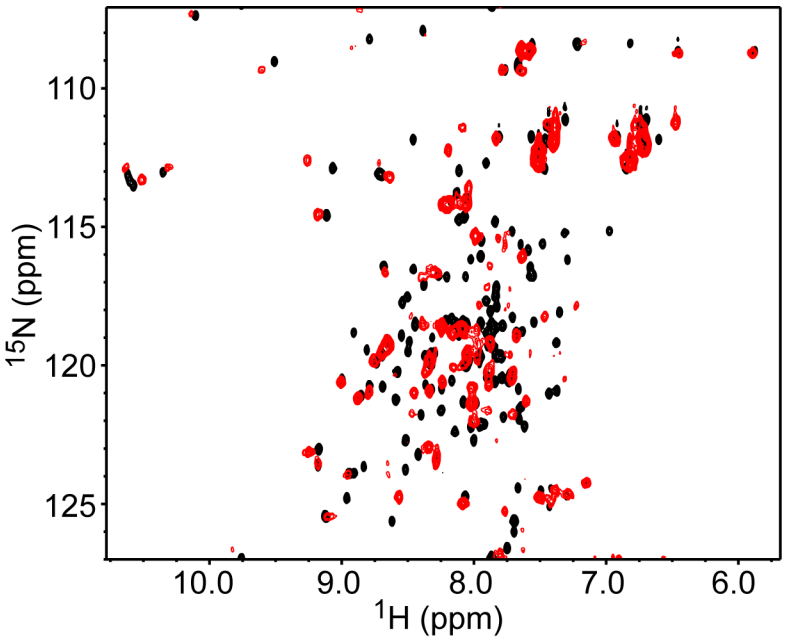

Figure S9. Overlay of 2D ^{1}H-^{15}N HSQC spectra obtained for ^{15}N-labeled CaM in the unbound state (black) and in complex with unlabeled myr(-)MA at 1.4:1 (MA:CaM).