

Supporting Information for

Ca(II) and Zn(II) cooperate to modulate the structure and self-assembly of S100A12

Qian Wang[‡], Aleksey Aleshintsev^{‡,⊥}, David Bolton[§], Jianqin Zhuang[‡]
Michael Brenowitz[‡] and Rupal Gupta^{‡,⊥,§,*}

[‡]Department of Chemistry, College of Staten Island, City University of New York, 2800 Victory Blvd. Staten Island, New York, 10314, United States

[§]Laboratory of Molecular Structure and Function/Mass Spectrometry Facility, Department of Molecular Biology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York, United States

[⊥]Departments of Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461

[⊥]Ph.D. Programs in Biochemistry and Chemistry, The Graduate Center of the City University of New York, United States

***Corresponding author:** Rupal Gupta, Department of Chemistry, College of Staten Island, The City University of New York, USA, Tel: (718) 982-3936; Email: rupal.gupta@csi.cuny.edu

Mass Spectrometry Method and Analysis

Mass spectrometry sample preparation. 10 μL of the cloned S100-A12 protein (1 mM in 50 mM Tris, 150 mM NaCl buffer, pH 8.3) or bovine ribonuclease (Sigma R-5125; 1 mM in 4.5 mM NaHPO_4 , 23 mM NaCl, pH7.2) were denatured in 8 M urea in 1 M ammonium bicarbonate (ABC), pH 8 and analyzed (1) untreated, (2) after disulfide reduction with DTT, (3) alkylation with iodoacetamide and (4) digestion with trypsin. Samples from steps 1 – 3 were analyzed by LC-MS and those from step 4 were analyzed by LC-MS/MS as described below. Reduction of disulfide bonds was done in 28 mM DTT at 50C for 30 min followed by addition of iodoacetamide (final concentration of 55.6 mM) and incubation in the dark at room temperature for 20 min. An equal volume of 1 M ABC was added, followed by 1.5 μg of trypsin (Sigma T-6567) and the protein samples were digested overnight at 37C. Samples removed after (1) urea denaturation, (2) disulfide reduction and (3) alkylation with iodoacetamide were diluted to the same final volume (36 μL ; protein concentration = 278 pmole/ μL) with LC-MS water and stored at -20C until processed for LC-MS.

LC-MS analyses. Protein samples from steps 1 – 3 above were diluted in aqueous 0.1% formic acid (buffer A; Pierce/Thermo Scientific) to concentrations of 10 and 1 pmole/ μL and a range of sample amounts from 250 fmole to 20 pmole were analyzed. The results reported herein are taken from analyses of 1 and 2 pmole injections of S100A12 and 0.5 and 1 pmole injections of RNase A. LC-MS analyses were performed using a Waters nanoAcquity UPLC system with dual pump trapping to a C4 column (Waters BEH C4, 300 \AA , 1.7 μm , 300 μm x 50 mm) at 10

$\mu\text{L}/\text{min}$ followed by analysis on a C4 analytical column (Waters BEH C4 300 Å, 1.7 μm , 150 μm x 100 mm). Protein separation was achieved at 1 $\mu\text{L}/\text{min}$ using a linear gradient of 95% buffer A and 5% buffer B (0.1% formic acid in acetonitrile; Pierce/Thermo Scientific) to 90% buffer B over 14 min beginning at 1 min post injection. The total column flow was directly infused into a Qtof Micro tandem mass spectrometer using a nanoflow electrosprayer with the capillary voltage set to 3200 V and the cone voltage set to 30 V. MS data were collected over the range of m/z 400 to 1990 in 1 sec scans with 0.1 sec data transfer interleaves from 0 to 44 min with reference scans of leucine enkephalin taken at 60 sec intervals from the reference sprayer. The MS data were analyzed in MassLynx 4.1 using lock mass-corrected data and centered MaxEnt 1 analyses to determine the average protein masses. LC peaks for S100A12 and RNase A were integrated to determine the starting and ending scans for MaxEnt 1 analyses and the entire peaks were analyzed, then the spectra from the top 80% of each peak were analyzed. In the case of S100A12, a later-eluting shoulder was detected so the individual peak segments (main peak and shoulder) were also analyzed individually. LC data sets were selected from the different protein loads for analysis with MaxEnt 1 based on optimal ion signal intensities: 1 pmole samples for unmodified and alkylated S100A12, 2 pmole for reduced S100A12, 0.5 pmole for unmodified and alkylated RNase A and 1 pmole for reduced RNase A. For MaxEnt 1 analyses, the output mass range was set from 5,000 to 40,000 with 24 ppm channel resolution (0.25 Da for S100A12; 0.33 Da for unmodified and reduced RNase A and 0.34 Da for alkylated RNase A). The simulated isotope pattern spectrometer blur width was set to the full width at half maximum for the lowest mass peak in each spectrum m/z range used. The m/z ranges for

S100A12 and RNase A were set to include all detected peaks, which included the 6+ ions through 15+ ions for S100A12, and the 8+ to 13+ ions for unmodified RNase A, 8+ to 17+ ions for reduced RNase A, and 8+ to 18+ ions for alkylated RNase A. The MaxEnt 1 target intensities were set to 10% of the maximum for left and right segments for S100A12, and 10%/30% (L/R) for unmodified RNase A, 30% left and right for reduced RNase A and 20% left and right for alkylated RNase A. Each analysis was run to convergence, which took between 34 and 61 iterations depending on the data set analyzed. MaxEnt 1 results were centered using MassLynx using the top 80% of the peak with the number of channels set to the full peak width at half maximum as determined for each result.

LC-MS/MS analyses. Peptides from the tryptic digests were diluted with buffer A to a nominal concentration of 1.7 pmole/ μ L and 1, 3 or 5 μ L (1.7, 5.1 and 8.5 pmoles) were injected and trapped on a C18 trapping column (Waters nanoAcquity UPLC 2G-VM Symmetry C18 180 μ m x 20 mm) at 15 μ L/min for 4 min. Peptides were separated on a Waters BEH130 C18 column (1.7 μ m, 75 μ m x 250 mm) using a complex gradient of 5% to 90% acetonitrile in aqueous 0.1% formic acid (solvent B) at a flow rate of 250 nL/min over 104 min with a total run time of 151 min. The outflow of the column was directly infused into a Qtof Micro tandem mass spectrometer using a nanoflow electrosprayer with the capillary voltage set to 3200 V and the cone voltage set to 30 V. Data dependent acquisition (DDA) MS/MS spectra were collected from 15 min to 120 min using 1 sec MS survey scans from m/z 350 to 1800 with 0.1 sec data transfer interleaves and 1 sec MS/MS scans from m/z 50 to 1990 for up to eight 1+ to 5+ ions for a total of up to 12 sec per parent ion. Parent ions were selected above a threshold of 15 ions per sec (ips) using

charge state peak selection with deisotoping and were then excluded for 30 sec. MS/MS collision energies were set according to profiles developed for each charge state from 1+ to 5+. Lock mass scans of leucine enkephalin (556.2771 Da) were collected at 60 sec intervals from a secondary nanoflow electrosprayer positioned orthogonally to the primary sprayer. Continuum MS and MS/MS spectra were collected, stored and analyzed offline using the ProteinLynx Global Server software package (PLGS version 2.3; Waters Corp.). The raw data was converted to deisotoped centroided spectra after applying adaptive noise reduction and smoothing (Savitsky-Golay method; three channels and two iterations) using the Fast mode with these parameters: 3% threshold, centroid 80% top; 4 channel minimum peak width, 5000 TOF resolution and 0.7 Np multiplier. The processed data was used to search two different databases for proteins having masses of 0 to 200,000 Da and pI values of 0 to 14 using the following parameters: 50 ppm peptide tolerance, 0.1 Da fragment tolerance, 5 mDa calibration error, tryptic peptides allowing 1 missed cleavage and a range of variable modifications, including carbamidomethyl C, deamidation N/Q, dehydration S/T, oxidized M, and phosphorylated S/T/Y. Proteins identified in the database search were subsequently analyzed using the Automod search function using the same tolerances but allowing for nonspecific cleavage and one substitution per peptide with the substitution likelihood value set to 11 (only the most likely substitutions allowed). All identified sequences contained a series of at least three consecutive yⁿ ions in the MS/MS spectrum, as specified in the validation rules set by the program. The searched databases included a modified Swissprot database containing 105,293 entries and the human-specific RefSeq database containing 37,971 entries. For S100A12, peptides identified in the initial survey analyses were

excluded by m/z and retention time and additional LC-MS/MS data sets were collected and analyzed to identify additional peptides present in lower concentrations. Several additional sequences and modifications were identified from those analyses.

Results

LC-MS Results. S100A12 was identified as the major component of the sample, with the peak ion intensity eluting at 13.4 minutes with a later eluting shoulder at 13.6 min (Figure S1A). The main peak was found to contain primarily unmodified S100A12 by MaxEnt 1 analysis (74%; Table 1; Figure S1B - C), but the shoulder contained approximately equal amounts of the unmodified S100A12 and a form having a mass 28 Da larger. That component was confirmed to be *N*-formylated Met₁ by LC-MS/MS analysis (22%; Figure S1C, D; Tables S2 & S3). Several additional forms were observed in very small amounts (<2%; Figure S1D; Table S2) and two of those were examined in more detail. One, having a mass 18 Da smaller than the unmodified protein, was provisionally identified as S100A12 having dehydration at Thr₂ (dehydroamino-2-butyric acid-Thr₂). The second one, having a mass 17 Da larger than S100A12, was provisionally identified as S100A12 having oxidized Met₁ (Met₁ sulfoxide) and deamidation at Asn₁₃ or Gln₆₈ (Figure S1D; Tables S2 and S3). The average masses measured for all forms of S100A12 were well within the expected error for this instrument (<50 ppm) and were not altered by reduction or alkylation, confirming the absence of cysteine residues within the protein (Table S1).

Better measurements were obtained for S100A12 and the *N*-formylated form when spectra were taken from the top 80% of the mass chromatographic peaks based on the 8+ ions for each form (m/z 1322.8 S100A12; m/z 1326.4 *N*-formyl-S100A12), probably due to the elimination of lower quality signals outside this range. The mass measurements of the other modified forms were of lower quality due to the relatively weak signal intensities within the spectra analyzed; no effort was made to analyze the higher concentration samples to obtain more precise values.

LC-MS/MS Results. Sequence determination by LC-MS/MS confirmed 90.2% (amino acids 1 – 83 of 92) of the S100A12 protein sequence (RefSeq accession number NP_005612.1). The sequence from amino acid 84 to 92 (AAHYHTHKE) was not observed in any MS/MS analyses. Probable protein modifications were identified at positions 1, 2, 13, 64 and 68 (Table S3). The modifications were not identified uniformly, i.e., each peptide was seen in both the modified and unmodified form. This is expected considering about 74% of the protein appears to be in the unmodified form with about 23% having only the *N*-formyl-Met₁ modification. Thus, approximately 97% of the S100A12 protein appears in those two forms. All other forms were present in amounts below 2% of the unmodified S100A12 protein. Several peptides were found to have carbamyl or carbamidomethyl (monoCAM) modifications at the amino terminus. These are most likely artifacts of the alkylation procedure due to the known reaction of iodoacetamide with primary N-terminal nitrogen in the tryptic peptides that would not be present in the intact protein. Modifications identified by the ProteinLynx Global Server program were assigned to the most probable positions after manual review of the MS/MS fragmentation spectra (Table S4). The fragmentation spectra only allowed definitive assignment of one modification (deamidation

at Asn₁₃) but restricted the positions of the other modifications to only a few residues. The manual assignments agree with the PLGS assignments except in the case of acetylation in peptide 32. The PLGS assignment is to Gly₃₁ as an N-terminal acetylation, which would not be likely in the intact protein. Assignment to Lys₃₄ is more likely if this modification exists in the whole protein and was not introduced during the tryptic digestion.

Table S1: Mass measurements for S100A12 and RNase A control

Protein	Treatment	Average Mass		Error		Mass Delta Vs Unmodified (Da)	
		Expected	Measured	Da	ppm	Measured	Expected
S100A12	Denatured (8M urea)	10575.05	10575.08	0.03	2.8	0.03	0
S100A12	Reduction with DTT	10575.05	10575.04	-0.01	-0.9	0.01	0
S100A12	Iodoacetamide alkylation	10575.05	10574.88	-0.17	-16.1	-0.17	0
RNase A	Denatured (8M urea)	13682.31	13682.21	-0.10	-7.3	-0.10	0
RNase A	Reduction with DTT	13690.37	13689.97	-0.40	-29.2	7.66	8.06
RNase A	Iodoacetamide alkylation	14146.79	14147.10	0.31	21.9	464.79	464.48

Average masses measured from spectra included in the top 80% of the LC peak. S100A12 peak was defined from the mass chromatogram based on the 8+ ion of the unmodified S100A12 to differentiate it from the *N*-formylated form.

Table S2: Mass measurements for S100A12 forms and RNase A control

Protein	Modification	Average Mass*		Error		Average Distribution in Sample	
		Calculated	Measured	Da	ppm	%	S.D. (%)
S100A12	None	10575.05	10574.95	-0.10	-9.1	73.8	5.6
S100A12	<i>N</i> -formyl-Met ₁	10603.06	10602.72	-0.34	-32.1	22.3	4.6
S100A12	Dehydroamino-2-butyric acid-Thr ₂	10557.04	10557.12	0.08	7.32	1.9	0.3
S100A12	Met ₁ sulfoxide, deamidation N ₁₃ /Q ₆₈	10592.04	10592.15	0.11	10.4	1.2	0.4

S100A12	<i>N</i> -acetyl-Met ₁ / <i>N</i> -acetyl-X	10617.09	10617.23	0.14	12.9	0.8	0.4
---------	--	----------	----------	------	------	-----	-----

Average masses measured from spectra included the entire S100A12 LC peak and all observed ions (6+ to 15+). S100A12 peak was defined from the mass chromatogram based on the total ion chromatogram for the 1 pmole injection of the urea denatured (unmodified) S100A12 sample.

Table S3: Peptides identified by LC-MS/MS analysis

#	Peptide Ion		Measure d Mass	Peptide Mass	Delta (Da)	Ladder Score	Position		Sequence	Modification(*)
	m/z	Charge					Start	End		
1	632.063	4	2524.221	2524.290	- 0.068	34.1	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Dehydration ST (2)
2	848.430	3	2542.267	2542.300	- 0.033	80.5	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	
3	636.817	4	2543.238	2543.284	- 0.046	17.9	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Deamidation N (13)
4	640.568	4	2558.241	2558.295	- 0.054	54.5	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Oxidation M (1)
5	857.758	3	2570.251	2570.295	- 0.044	76.4	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Formyl N-TERM
6	647.071	4	2584.251	2584.311	- 0.060	38.2	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Acetyl N-TERM
7	647.318	4	2585.240	2585.306	- 0.066	44.7	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	Carbamyl N- TERM
8	867.432	3	2599.271	2599.322	- 0.051	60.2	1	21	(-)MTKLEEHLEGIVNIFHQYSVR(K)	MonoCAM N-Term
9	668.590	4	2670.330	2670.395	- 0.065	22.5	1	22	(-)MTKLEEHLEGIVNIFHQYSVRK(G)	

#	Peptide Ion		Measure d Mass	Peptide Mass	Delta (Da)	Ladder Score	Position		Sequence	Modification(*)
	m/z	Charge					Start	End		
10	603.803	4	2411.182	2411.260	-0.077	63.2	2	21	(M)TKLEEHLEGIVNIFHQYSVR(K)	
11	771.064	3	2310.168	2310.212	-0.044	12.6	3	21	(T)KLEEHLEGIVNIFHQYSVR(K)	
12	614.302	3	1839.882	1839.916	-0.033	41.4	4	18	(K)LEEHLEGIVNIFHQY(S)	
13	728.372	3	2182.094	2182.117	-0.023	84.8	4	21	(K)LEEHLEGIVNIFHQYSVR(K)	
14	546.772	4	2183.058	2183.101	-0.043	53.3	4	21	(K)LEEHLEGIVNIFHQYSVR(K)	Deamidation N (10)
15	742.704	3	2225.089	2225.123	-0.034	57.1	4	21	(K)LEEHLEGIVNIFHQYSVR(K)	Carbamyl N-TERM
16	747.374	3	2239.097	2239.139	-0.041	61.0	4	21	(K)LEEHLEGIVNIFHQYSVR(K)	MonoCAM N-Term
17	578.549	4	2310.165	2310.212	-0.047	45.9	4	22	(K)LEEHLEGIVNIFHQYSVRK(G)	
18	690.678	3	2069.011	2069.033	-0.022	53.5	5	21	(L)EEHLEGIVNIFHQYSVR(K)	
19	647.660	3	1939.958	1939.990	-0.032	78.5	6	21	(E)EHLEGIVNIFHQYSVR(K)	
20	716.884	2	1431.752	1431.762	-0.010	60.9	10	21	(E)GIVNIFHQYSVR(K)	

#	Peptide Ion		Measured Mass	Peptide Mass	Delta (Da)	Ladder Score	Position		Sequence	Modification(*)
	m/z	Charge					Start	End		
21	631.830	2	1261.645	1261.657	-0.012	86.0	12	21	(I)VNIFHQYSVR(K)	
22	582.296	2	1162.576	1162.588	-0.013	88.2	13	21	(V)NIFHQYSVR(K)	
23	525.277	2	1048.537	1048.545	-0.008	66.7	14	21	(N)IFHQYSVR(K)	
24	468.731	2	935.447	935.461	-0.015	74.4	15	21	(I)FHQYSVR(K)	
25	395.199	2	788.382	788.393	-0.011	57.6	16	21	(F)HQYSVR(K)	
26	487.259	3	1458.754	1458.783	-0.030	50.7	22	34	(R)KGHFDTLSKGELK(Q)	
27	474.226	2	946.436	946.451	-0.014	64.4	23	30	(K)GHFDTLSK(G)	Carbamyl N-TERM
28	481.234	2	960.453	960.467	-0.013	53.3	23	30	(K)GHFDTLSK(G)	MonoCAM N-Term
29	666.346	2	1330.677	1330.688	-0.011	84.1	23	34	(K)GHFDTLSKGELK(Q)	
30	569.305	2	1136.594	1136.608	-0.014	57.9	25	34	(H)FDTLSKGELK(Q)	
31	515.310	2	1028.605	1028.623	-0.018	70.6	31	39	(K)GELKQLLTK(E)	

#	Peptide Ion		Measure d Mass	Peptide Mass	Delta (Da)	Ladder Score	Position		Sequence	Modification(*)
	m/z	Charge					Start	End		
3 2	536.315	2	1070.615	1070.634	- 0.019	35.3	31	39	(K)GELKQLLTK(E)	Acetyl N-TERM
3 3	536.814	2	1071.611	1071.629	- 0.017	62.7	31	39	(K)GELKQLLTK(E)	Carbamyl N- TERM
3 4	543.826	2	1085.635	1085.644	- 0.009	52.9	31	39	(K)GELKQLLTK(E)	MonoCAM N-Term
3 5	645.401	1	644.393	644.386	0.008	77.8	35	39	(K)QLLTK(E)	Carbamyl N- TERM
3 6	707.919	2	1413.823	1413.819	0.004	60.9	35	46	(K)QLLTKELANTIK(N)	Carbamyl N- TERM
3 7	831.470	1	830.462	830.450	0.012	79.5	40	46	(K)ELANTIK(N)	Carbamyl N- TERM
3 8	593.828	2	1185.639	1185.672	- 0.032	29.8	40	49	(K)ELANTIKNIK(D)	Carbamyl N- TERM
3 9	546.323	1	545.315	545.317	- 0.002	70.4	42	46	(L)ANTIK(N)	
4 0	1274.62 8	3	3820.861	3820.940	- 0.079	43.3	50	83	(K)DKAVIDEIFQGLDANQDEQV DFQEFISLVAIALK(A)	
4 1	1193.57 7	3	3577.707	3577.819	- 0.111	50.8	52	83	(K)AVIDEIFQGLDANQDEQVDF QEFISLVAIALK(A)	
4 2	1193.89 3	3	3578.656	3578.803	- 0.147	36.0	52	83	(K)AVIDEIFQGLDANQDEQVDF QEFISLVAIALK(A)	Deamidation Q (17)

#	Peptide Ion		Measure d Mass	Peptide Mass	Delta (Da)	Ladder Score	Position		Sequence	Modification(*)
	m/z	Charge					Start	End		
4 3	888.447	3	2662.318	2662.349	- 0.031	34.0	60	83	(Q)GLDANQDEQVDFQEFISLV AIALK(A)	
4 4	1097.07 2	2	2192.127	2192.137	- 0.009	60.4	65	83	(N)QDEQVDFQEFISLV AIALK(A)	
4 5	464.308	2	926.600	926.617	- 0.016	72.5	75	83	(F)ISLV AIALK(A)	
4 6	727.514	1	726.506	726.500	0.005	69.2	77	83	(S)LV AIALK(A)	
4 7	515.358	1	514.350	514.348	0.002	70.4	79	83	(V)AIALK(A)	

*Modifications to S100A12 (RefSeq accession number NP_005612.1) as assigned by ProteinLynx Global Server (PLGS ver 2.3).

The number in parentheses indicates position of the modified amino acid within the peptide from the amino terminus. "MonoCAM" denotes a single carbamidomethyl modification. Carbamyl and monoCAM modifications are known to be caused by the cysteine alkylation procedure.

Table S4: Position assignments of probably S100A12 modifications

Peptide Number	Modification	Assigned Position	Sequence Range Limits
1	Dehydration S/T	Thr ₂	Met ₁ , Thr ₂ , Lys ₃
3, 14	Deamidation	Asn ₁₃	Asn ₁₃
4	Oxidation	Met ₁	Met ₁ , Thr ₂
5	Formylation	Met ₁	Met ₁ , Thr ₂
32	Acetylation	Lys ₃₄	Gly ₃₁ , Glu ₃₂ , Leu ₃₃ , Lys ₃₄
42	Deamidation	Gln ₆₈	Asp ₆₆ , Glu ₆₇ , Gln ₆₈

Peptide numbers refer to Table S3. Modifications likely to have been introduced during the alkylation procedure are not considered here.

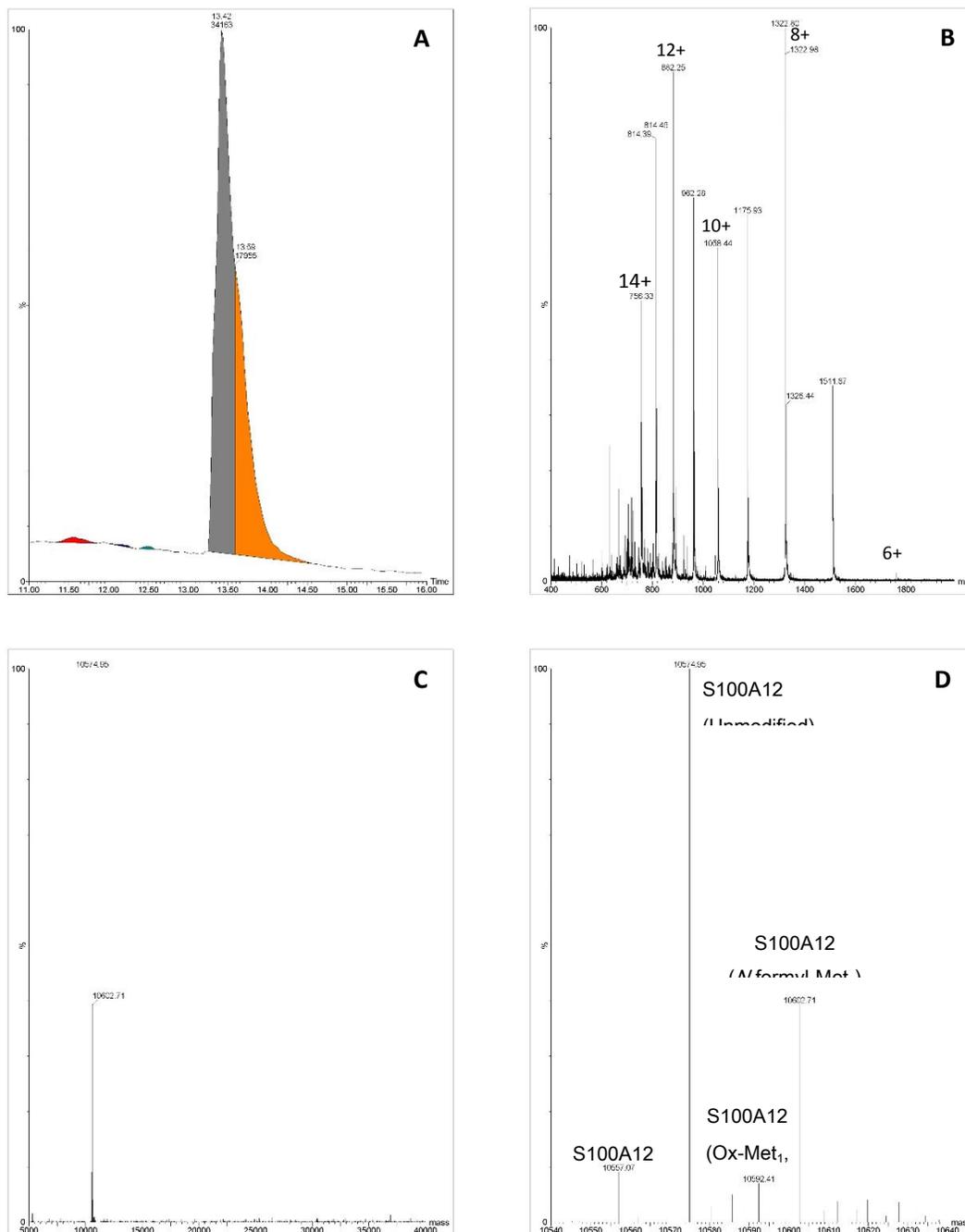


Figure S1. *LC-MS analyses of S100A12.* (A) Total ion current (TIC) LC-MS chromatogram of urea-denatured unmodified S100A12 (1 pmole) showing the main S100A12 peak eluting at 13.42 min (dark grey) with the *N*-formyl-S100A12 shoulder at 13.59 min (orange). (B) Combined spectra from the peak in (A). Even numbered protein ions are labeled from 6+ to 14+. (C) Example MaxEnt 1 analysis of (B). (D) Narrowed view of (C) emphasizing the mass range of unmodified S100A12 and the modified forms,

with the *N*-formylated-Met₁, dehydroamino-2-butyric acid Thr₂, Met₁ sulfoxide and deamidated forms labeled.

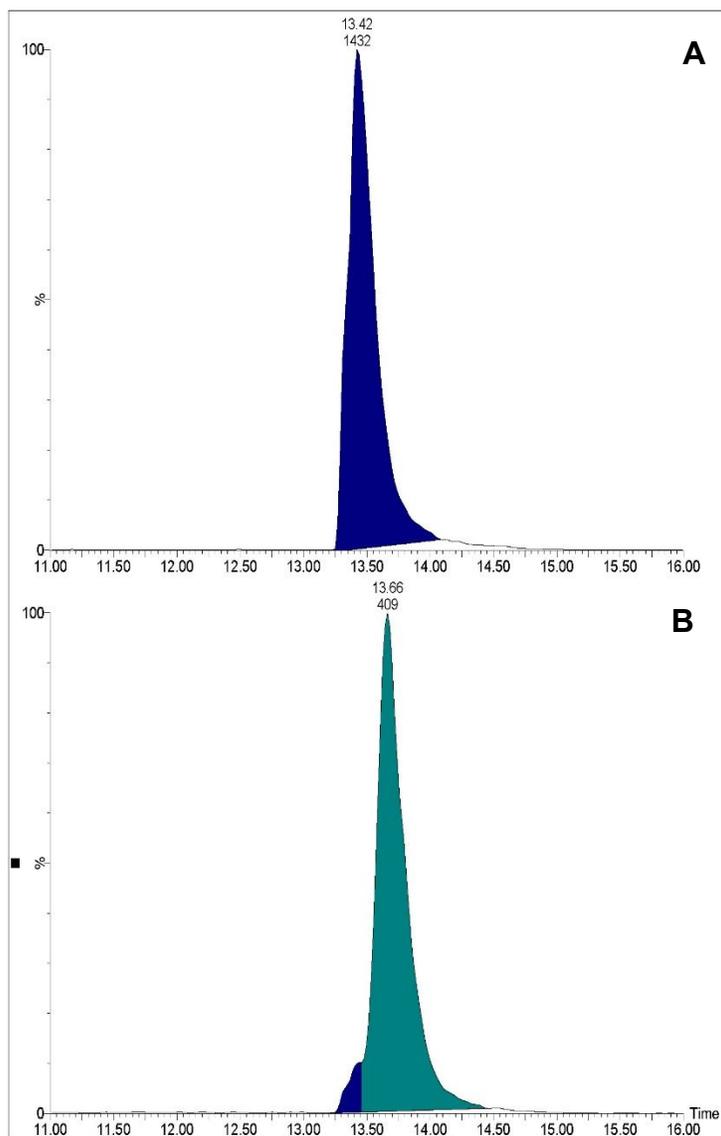


Figure S2. Mass ion chromatograms of (A) unmodified S100A12 8+ ion (m/z 1322.8; peak at 13.42 min) and the *N*-formyl-Met₁ S100A12 (m/z 1326.4; peak at 13.66 min).

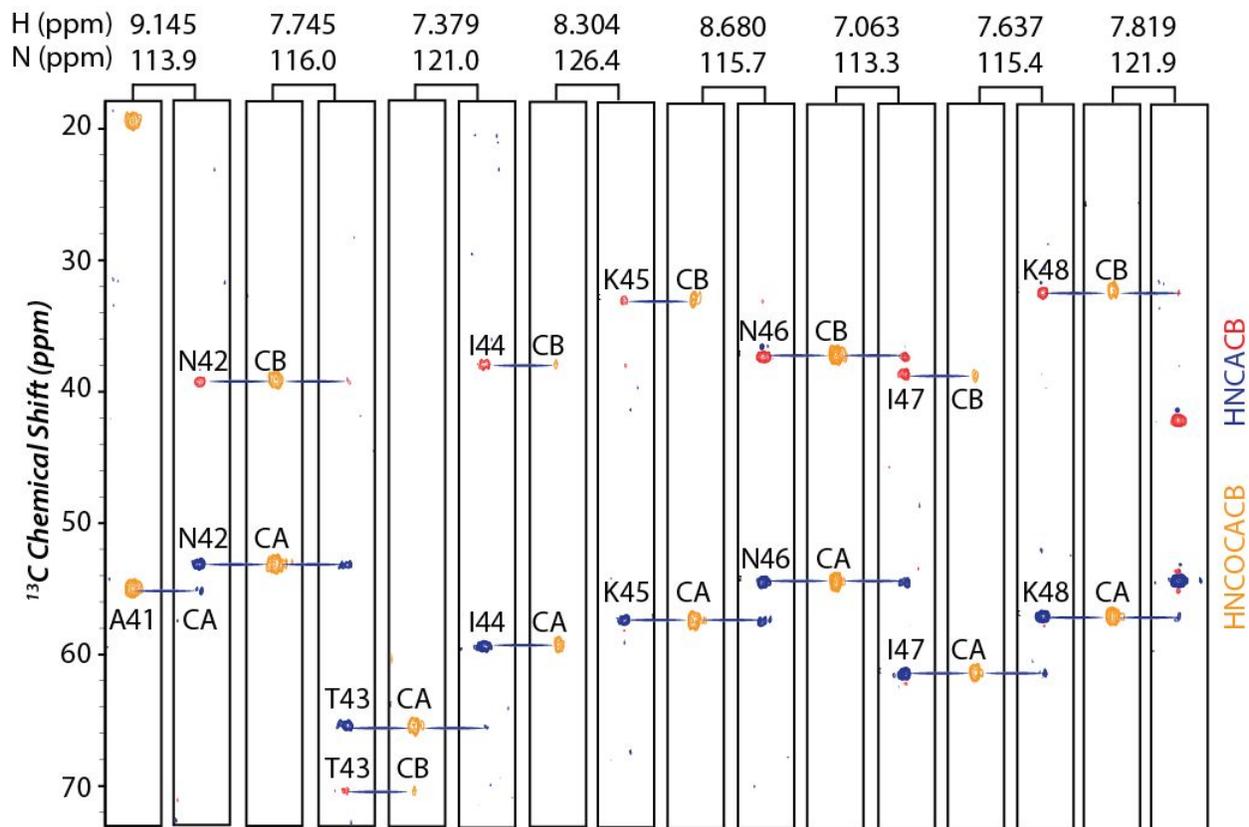


Figure S3. Sequential assignments showing strip plots from ¹H-¹³C-¹⁵N CBCACONH (orange) and HNCACB (blue) NMR spectra of Ca²⁺-S100A12 acquired at 14.1 T for residues A41-K48.

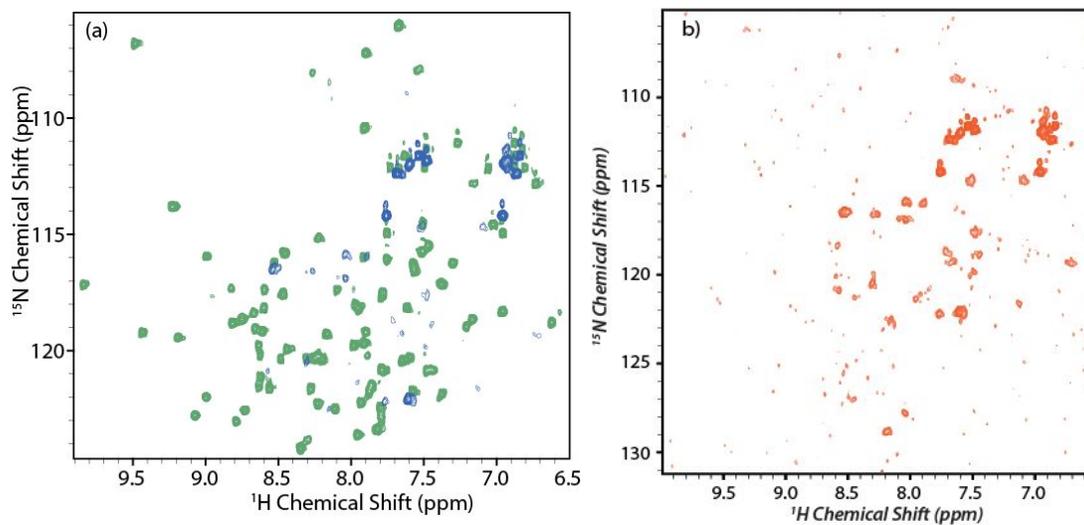


Figure S4. a) Overlay of 18.8 T ^1H - ^{15}N HSQC spectra of apo-S100A12 homodimer (green) and Zn^{2+} bound S100A12 (blue) and; b) ^1H - ^{15}N HSQC spectrum of Zn^{2+} bound S100A12 with contour level adjusted so that the spectral noise is visible.

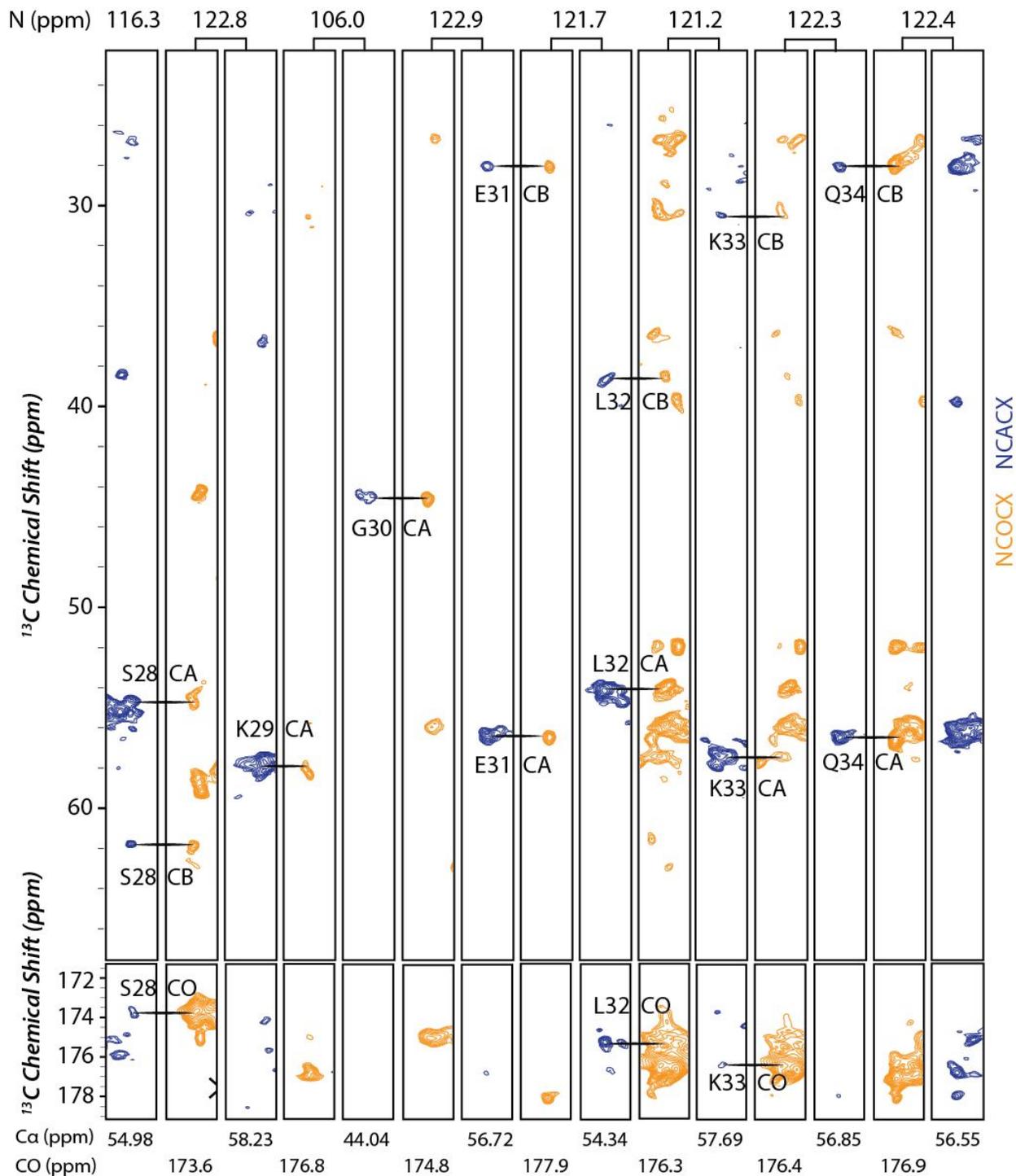


Figure S5. Sequential NCACX (blue) and NCOCX (orange) backbone-walk used to assign ^{13}C - ^{13}C DARR correlation spectrum of Zn bound S100A12 protein showing strip plots for residues S28-Q34.

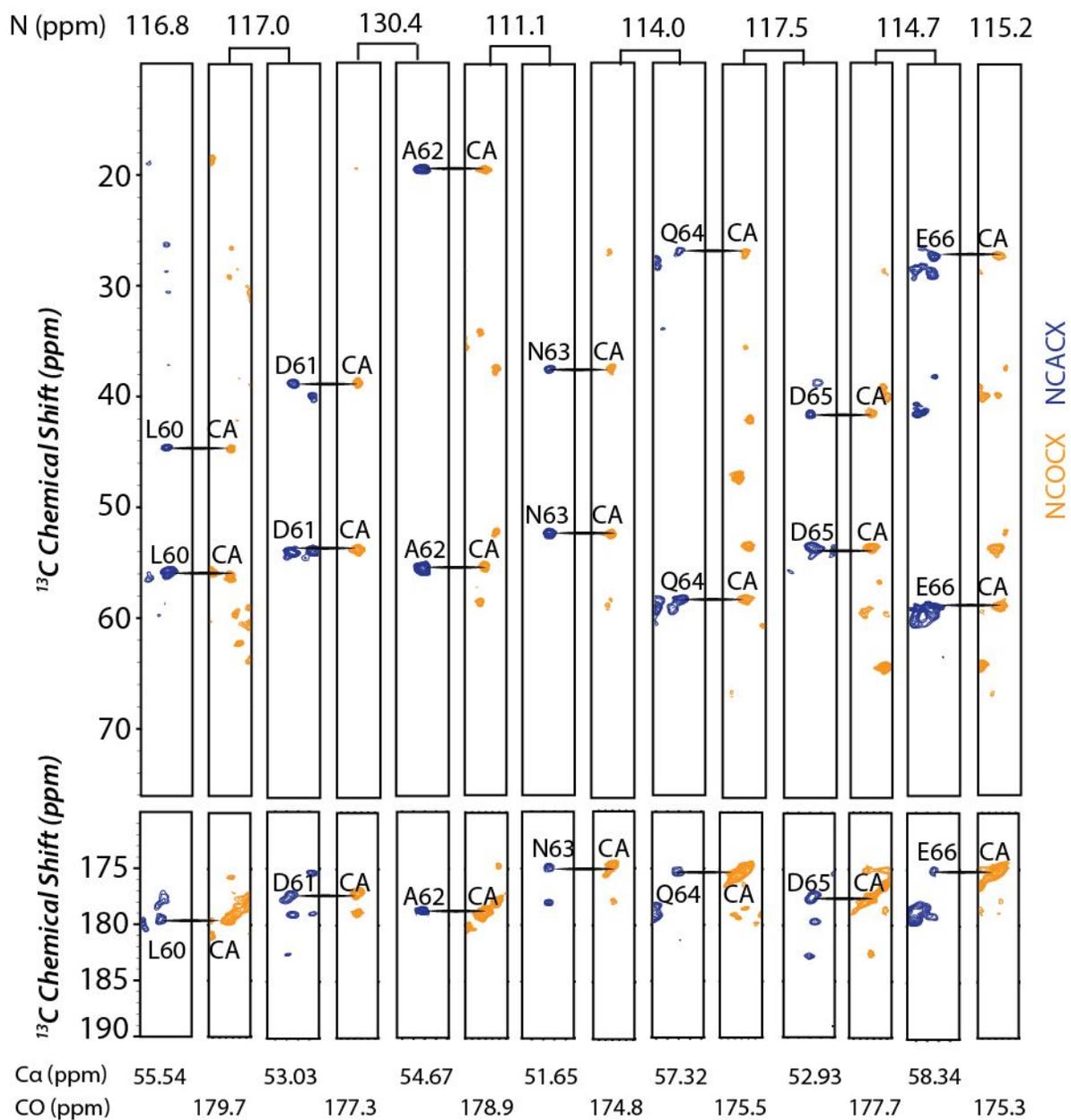


Figure S6. Sequential NCACX (blue) and NCOCX (orange) backbone-walk used to assign ^{13}C - ^{13}C DARR correlation spectrum of Ca^{2+} , Zn^{2+} - S100A12 protein showing strip plots for residues L60-E66.

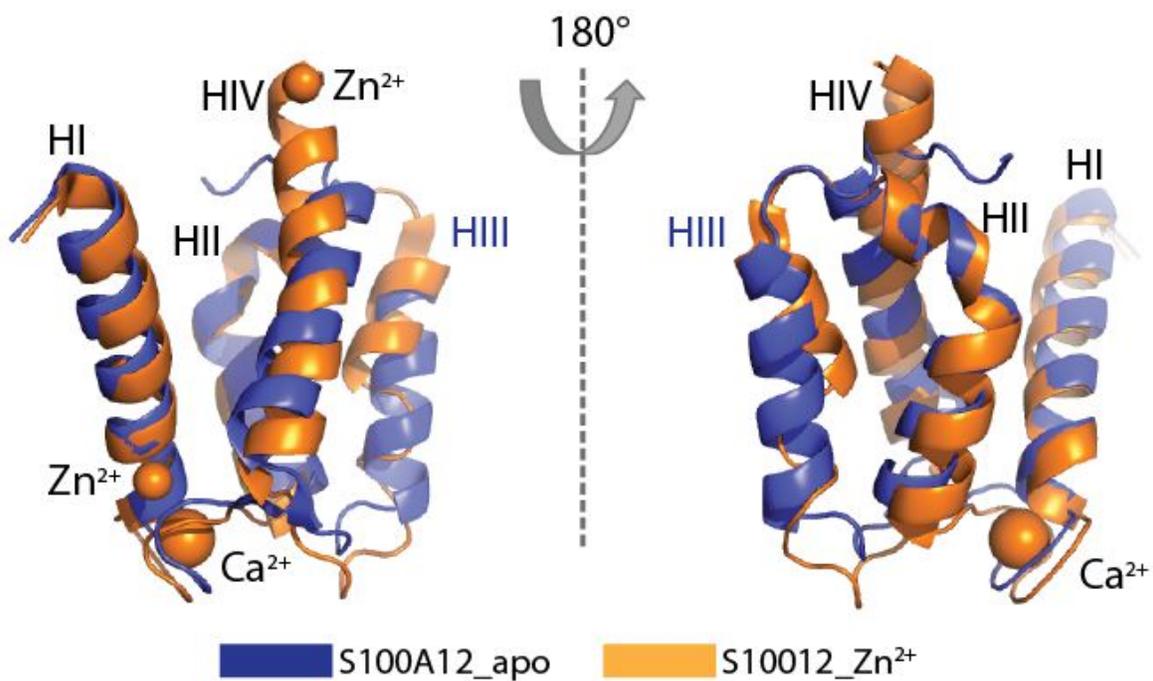


Figure S7. An overlay of the crystal structures of apo (PDB: 2WCF) and zinc bound (PDB: 2WCB) S100A12. The figure shows only the S100A12 monomers for clarity.

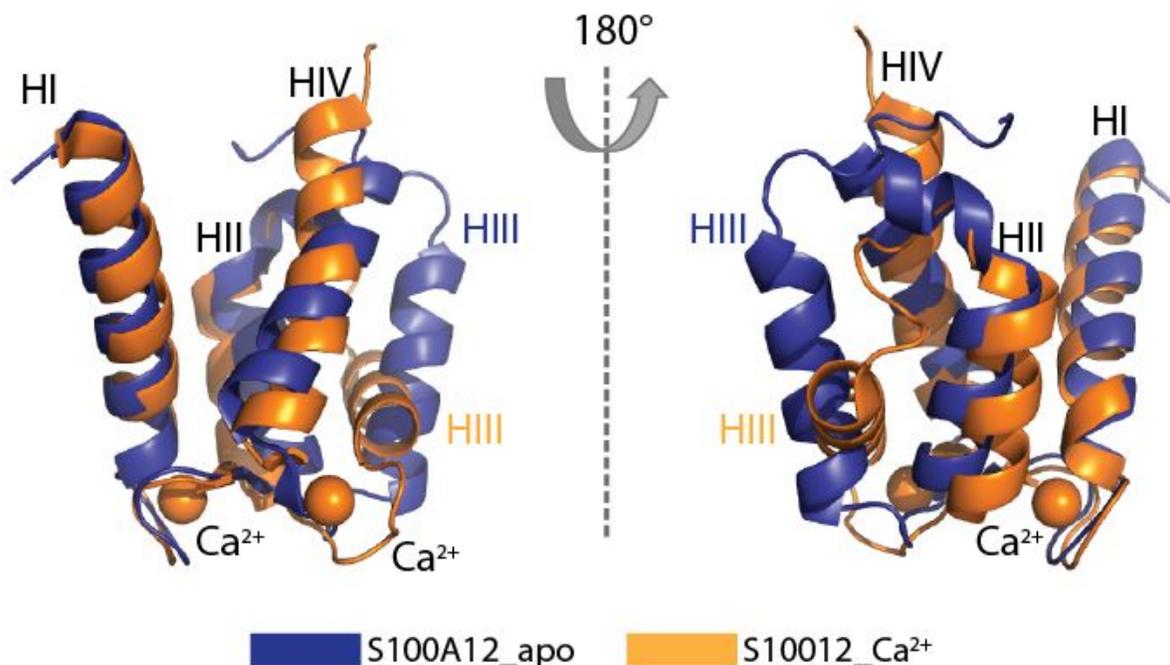


Figure S8: An overlay of the crystal structures of apo (PDB: 2WCF) and calcium bound (PDB: 1E8A) S100A12. The figure shows only the S100A12 monomers for clarity.

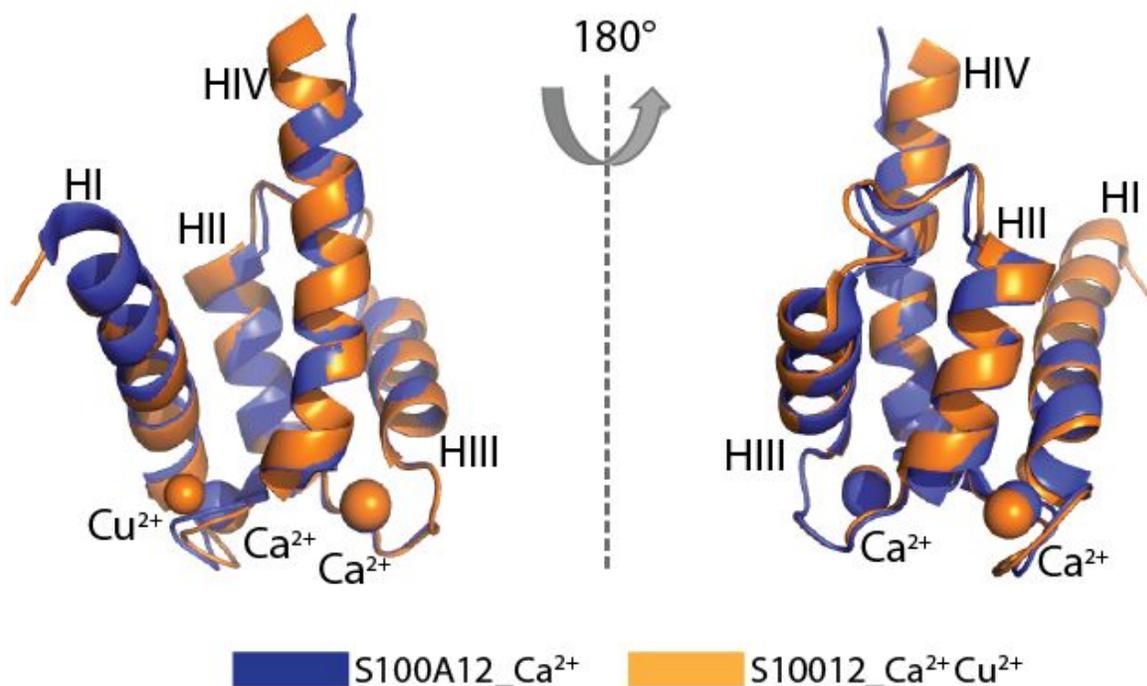


Figure S9: An overlay of the crystal structures of calcium (PDB: 1E8A) and copper, calcium bound (PDB: 1ODB) S100A12. The figure shows only the S100A12 monomers for clarity.

