Electronic Supplementary Material (ESI) for Metallomics.

Electronic supplementary information

Copper binding to naturally occurring, lactam form of angiogenin differs from that to recombinant protein, affecting their activity.

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Supporting Methods

ESI-MS measurement

Mass spectra were registered on a Q-Exactive mass spectrometer (Thermo Fisher) by direct infusion of the solutions at 3µl/min. Samples were prepared by mixing the proper amount of $Cu(NO_3)_2$ and Angiogenin solutions in order to obtain two different metal-to-ligand ratios (1:1 and 10:1). The Angiogenin concentration was 1.0×10^{-5} M. Spectra were carried out at pH 7.4. Deconvolution of the mass spectra was obtained by MagTran 1.03 (kindly provided by the software developer, Dr Zhongqi Zhang).

UV-visible measurements

UV-visible (UV-vis) spectra were recorded at 25 °C by using an Agilent 8453 or a Varian Cary 500 spectrophotometer. Measurements were performed using quartz cuvette with a 1 cm path length. Combined spectroscopic and potentiometric metal-peptide complex titrations were performed into a 3 ml quartz cuvette with a 1 cm path length to get the spectrum in the visible region at each pH value simultaneously with the potentiometric data These experiments were replicated at least three times for each copper(II)-peptide system. Spectroscopic data were processed by using the HYPERQUAD program (P. Gans, A. Sabatini, A. Vacca, *Talanta*, **1996**, *43*, 1739).

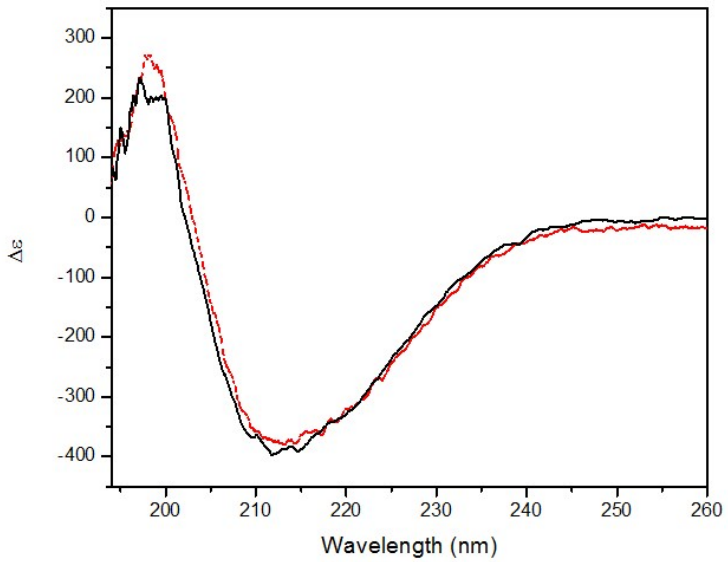
Peptide synthesis

The peptides were assembled using the solid phase peptide synthesis strategy on a Pioneer™ Peptide Synthesiser. All amino acid residues were added according to the TBTU/HOBT/DIEA activation method for Fmoc chemistry on Fmoc-PAL-PEG resin (loading 0.22 mmol/g, 0.33 mmol scale synthesis, 1.5 g of resin).

The peptides were purified by means of a preparative reversed-phase (rp)-HPLC. Purification was performed on a Varian PrepStar 200 model SD-1 chromatography system equipped with a Prostar photodiode array detector with detection at 222 nm. They were eluted with solvent A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) on a Vydac C_{18} 250 × 22 mm (300 Å pore size, 10-15 μm particle size) column, at a flow rate of 0.2 mL/min. Analytical rp-HPLC analyses were performed using an Agilent 1200 series instrument, equipped with a DAD detector. Samples were analyzed using gradient elution with solvent A and B on a Vydac C_{18} 250 × 4.6 mm (300 Å pore size, 5 μm particle size) column, at a flow rate of 1 mL/min. The peptides were eluted according to the following protocol: from 0 to 5 minutes isocratic gradient in 0% B, then linear gradient from 0 to 20% B over 20 min, finally isocratic gradient in 20% B from 20 to 35 minutes. Peptides were characterized by means of electrospray ionization mass spectrometry.

Supporting Figures

(a)



(b)

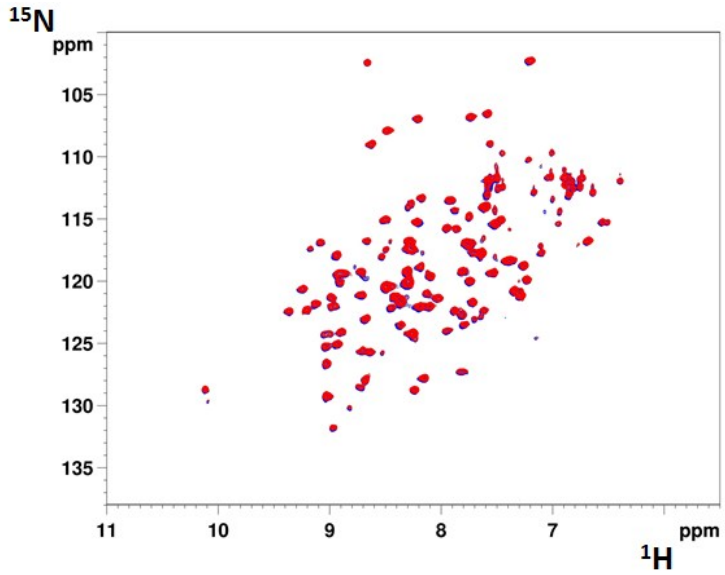


Figure S1. (a) Far-UV CD spectra of wt-Ang (black line) and r-Ang (red scattered line), in MOPS buffer at pH 7.4 ([Ang]= 50 μM). (b) Overlay of ^{1}H,^{15}N HSQC spectra of ^{15}N-enriched wt-Ang (blue contours) and r-Ang (red contours), in 50 mM phosphate buffer at pH 7.4 with 100 mM NaCl. The remarkable spectral similarity indicates no significant structural changes between wild-type and recombinant protein.

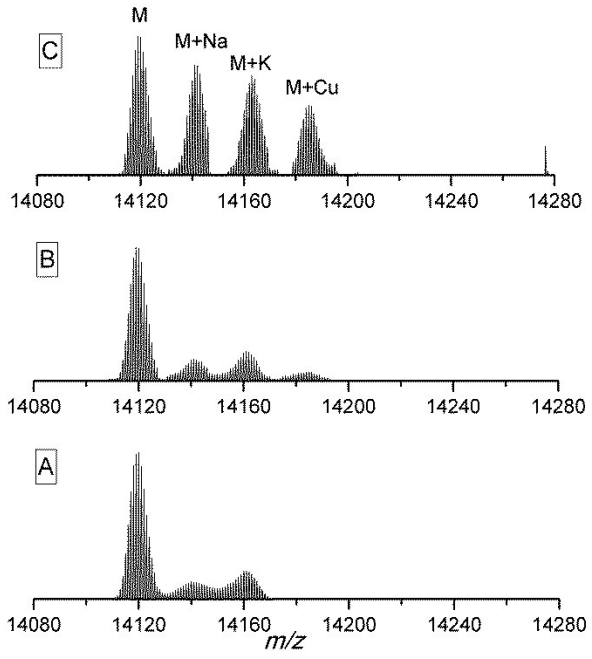


Figure S2. Deconvoluted ESI-MS spectra of wt-Ang alone (A) and mixed with copper(II) in a metal-to-protein ratio equal to 1:1 (B) and 10:1 (C), at pH = 7.4. The wt-Ang concentration was 1.0×10^{-5} M.

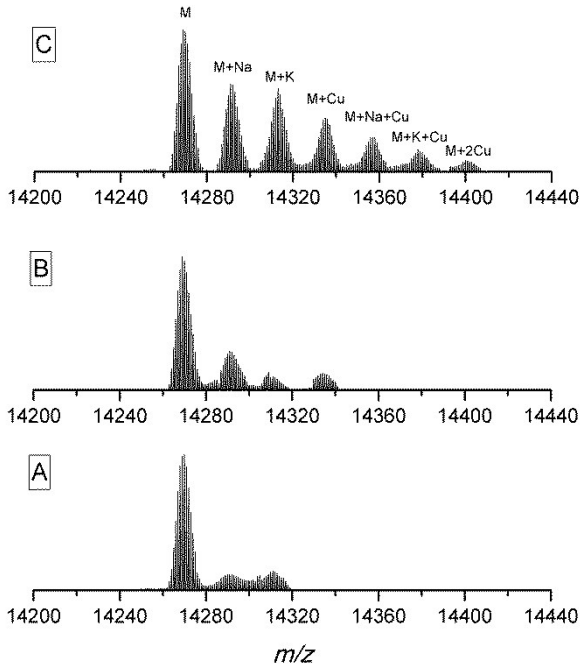


Figure S3. Deconvoluted spectra of r-Ang alone (A) and mixed with copper(II) in a metal-to-protein ratio equal to 1:1 (B) and 10:1 (C) at pH = 7.4. The r-Ang concentration was 1.0×10^{-5} M.

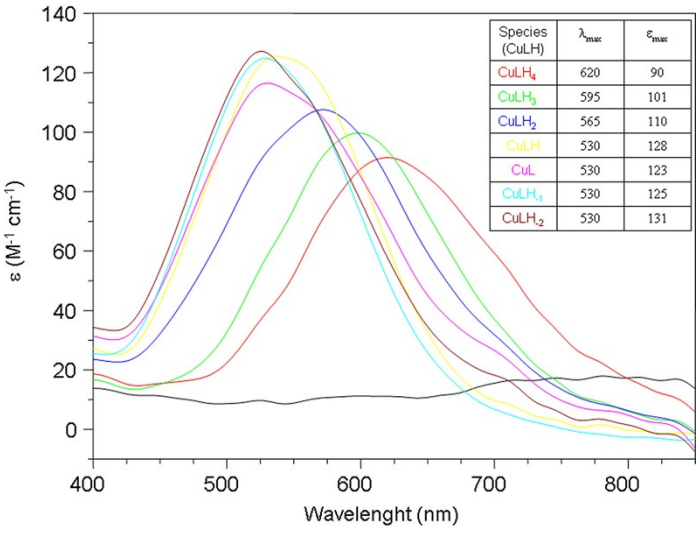


Figure S4. UV/Vis spectra of Cu-Ang(1–17) system obtained by titration experiments and processed by means of HYPERQUAD program. The peptide has eight protonation centres.

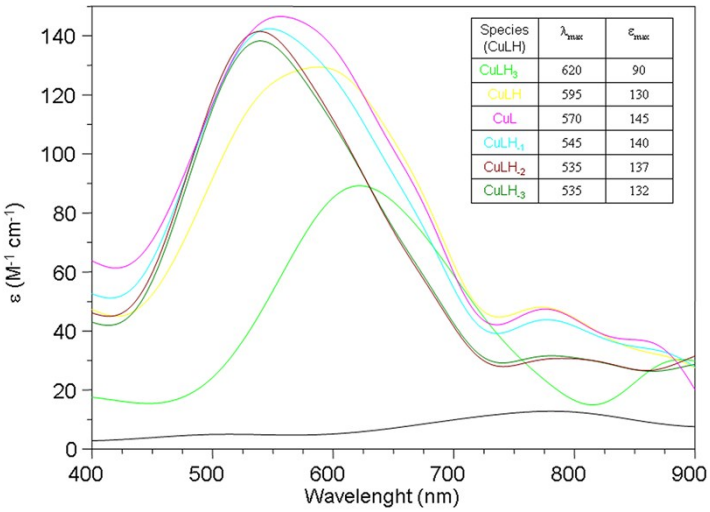


Figure S5. UV/Vis spectra of Cu-Ac-Ang(1-17) system obtained by titration experiments and processed by means of HYPERQUAD program. The peptide has seven protonation centres.

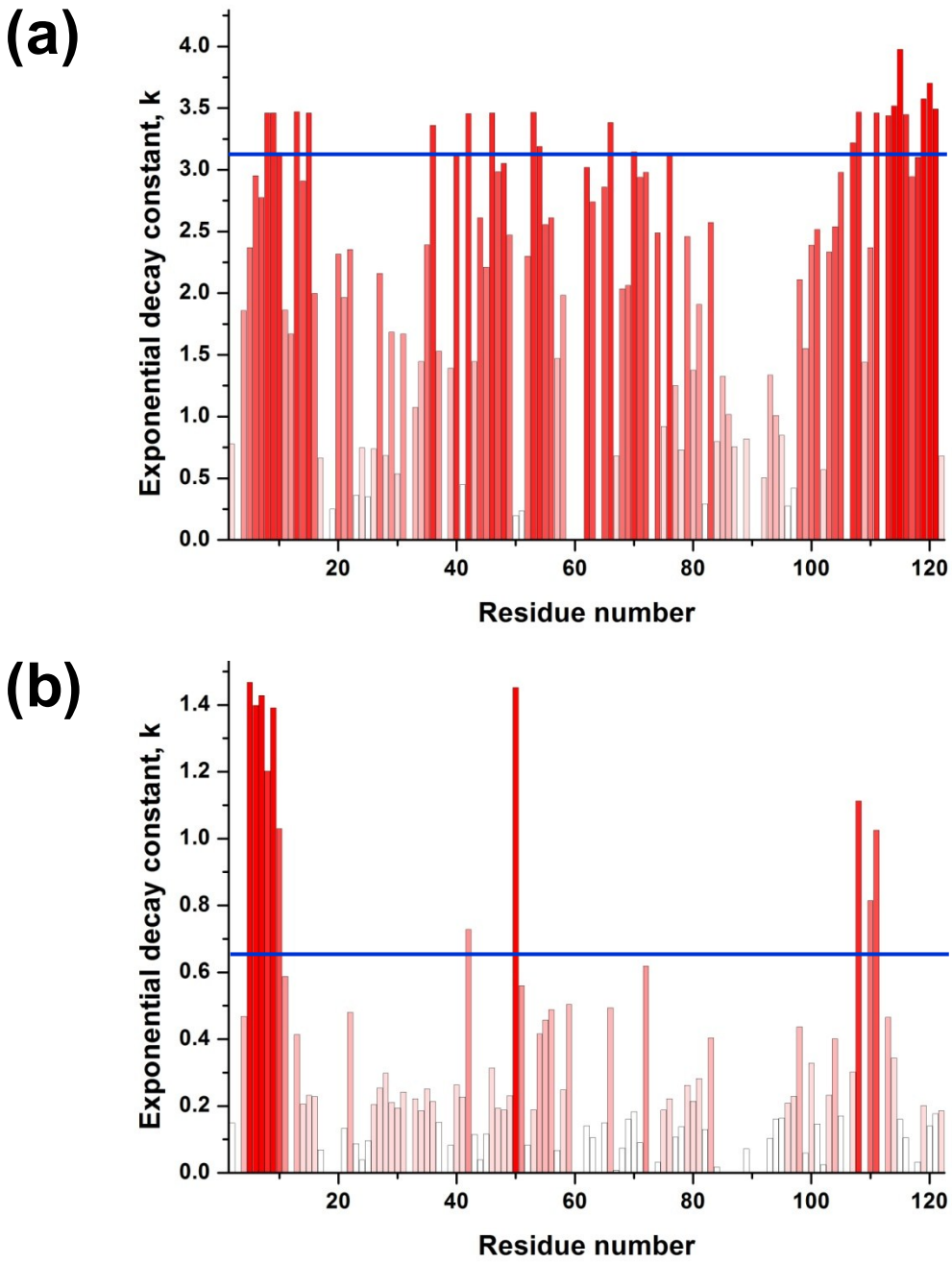


Figure S6. Bar plot of single-exponential decay constants of signal intensity (*k* values) of wt- (a) and r-Ang (b) residues affected by Cu(II) titration at pH 7.4. The color intensity of the bars is proportional to the *k* constant. The *k* value corresponding to the average plus one standard deviation, is indicated by a blue line.

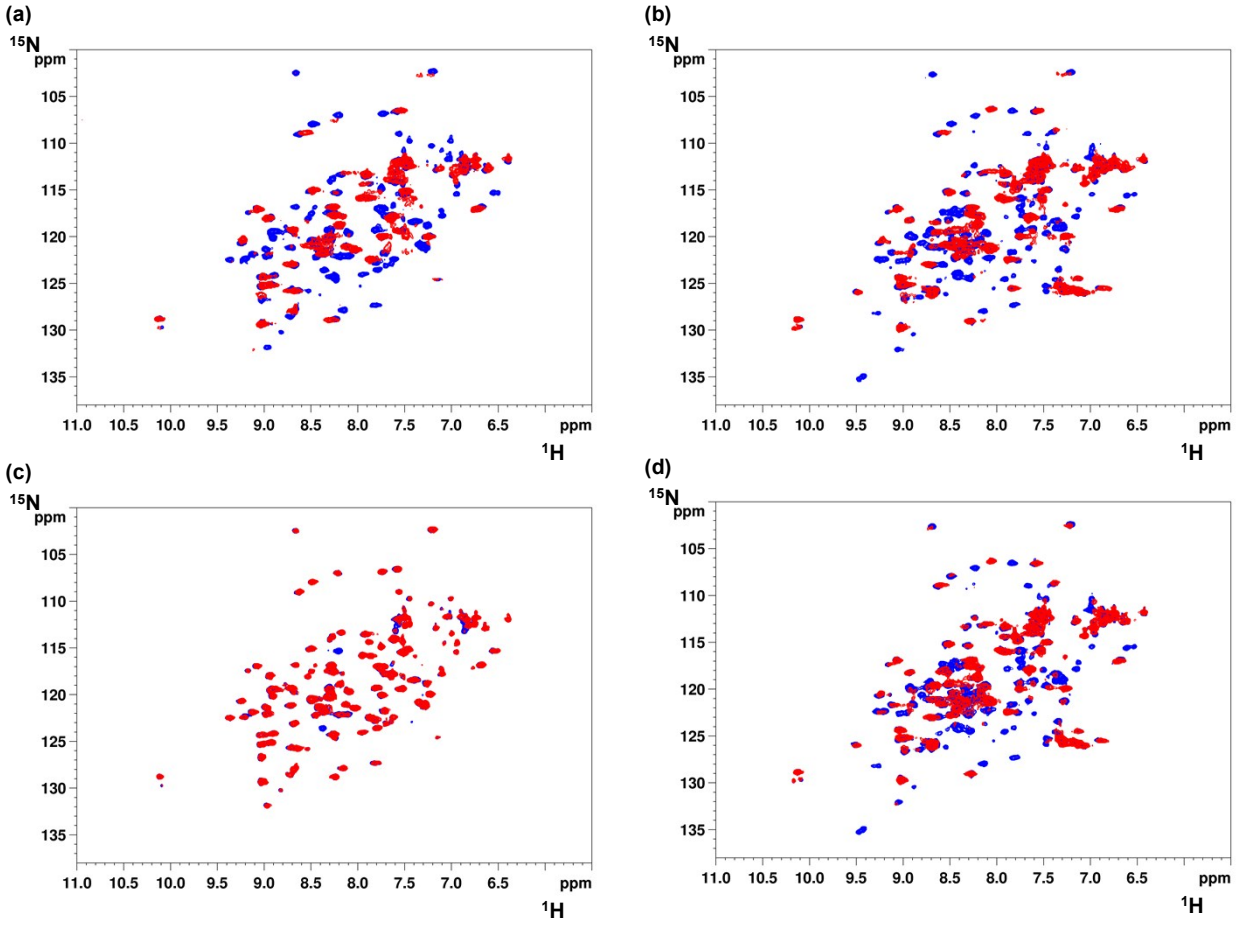


Figure S7. Overlay of ^{1}H,^{15}N HSQC spectra of wt- (a and b) and r-Ang (c and d) in the absence of Cu(II) (*blue* contours) and in the presence of 1 mol equiv of Cu(II) (*red* contours), recorded at pH 7.4 (a and c) and 5.5 (b and d). Note that, at physiological pH, only few cross-peaks in the N-terminal region of r-Ang are broadened beyond detection in the presence of paramagnetic Cu(II) (panel c).

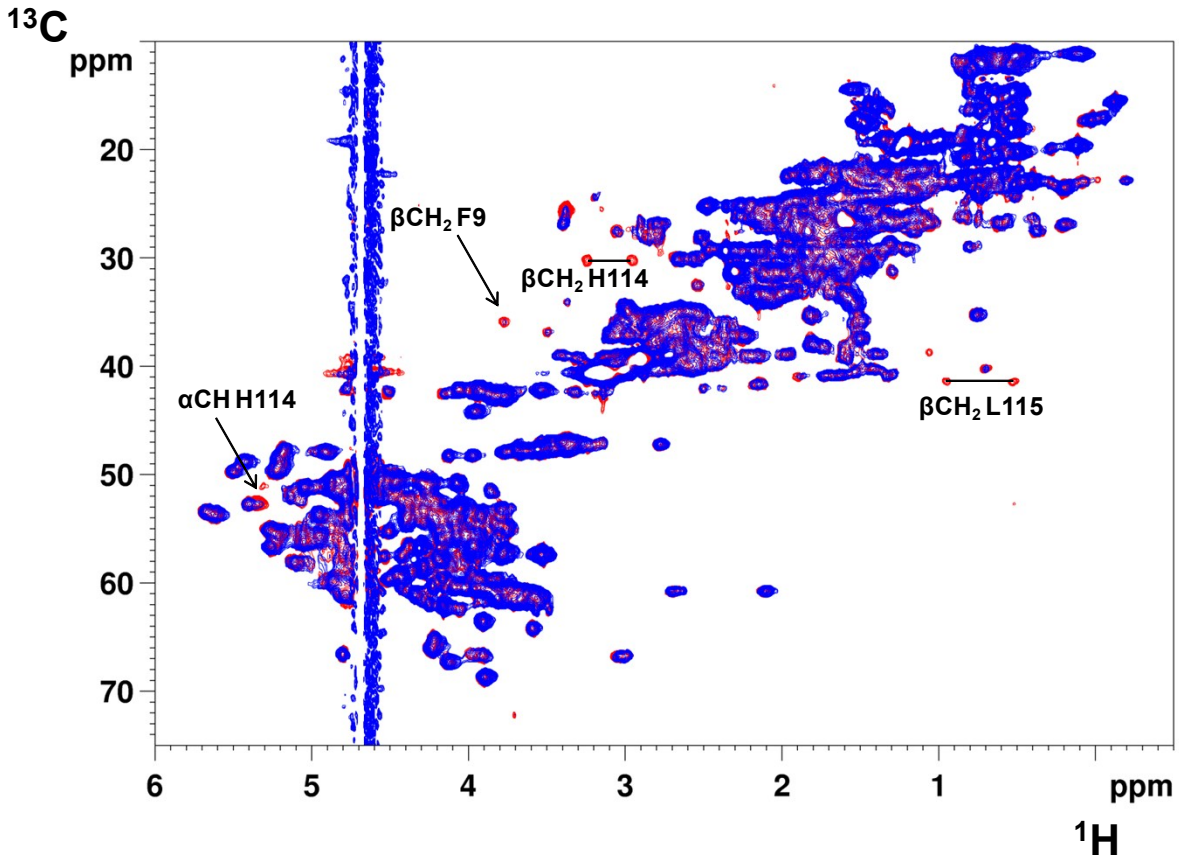


Figure S8. Overlay of $^{1}H,^{13}C$ HSQC spectra of wt-Ang in the absence of Cu(II) (*red* contours) and in the presence of 0.25 mol equiv of Cu(II) (*blue* contours) at pH 5.5. The cross-peaks experiencing large line-broadening effect are indicated.

Supporting Tables

Table S1. Backbone amide chemical shifts of wt-Ang (50 mM phosphate, 100 mM NaCl) at two different pH values.

wt-Ang				
	pH 5.5 Chemical shifts		pH 7.4 Chemical shifts	
Residue	^{15}N	$^{1}H_N$	^{15}N	$^{1}H_N$
Gln1				
Asp2	122,74	8,51	122,13	8,48
Asn3	121,66	8,58	121,48	8,55
Ser4	117,96	8,59	118,23	8,62
Arg5	122,99	8,58	122,74	8,57
Tyr6	123,91	8,42	123,59	8,36
Thr7	115,35	8,33	115,31	8,21
His8	120,96	8,24	122,11	8,19
Phe9	122,26	8,08	122,10	8,10
Leu10	119,89	8,33	119,27	8,31
Thr11	117,57	8,41	117,44	8,36
Gln12	115,90	7,74	115,88	7,74
His13	106,54	7,84	106,83	7,74
Tyr14	121,08	8,62	120,39	8,48
Asp15	127,95	8,13	127,86	8,15
Ala16	129,01	8,27	128,79	8,23
Lys17	116,88	8,28	116,81	8,28
Pro18				
Gln19	121,03	8,10	121,05	8,13
Gly20	108,74	7,39	108,73	7,39
Arg21	114,02	7,61	114,07	7,61
Asp22	117,51	7,73	117,69	7,72
Asp23	121,44	8,06	121,42	8,02
Arg24	117,82	7,65	117,79	7,64
Tyr25	122,42	7,88	122,42	7,88
Cys26	115,77	7,96	115,78	7,95
Glu27	117,44	8,28	117,44	8,27
Ser28	111,90	7,58	111,89	7,57
Ile29	123,06	8,69	123,11	8,68
Met30	119,99	7,75	120,03	7,74
Arg31	116,88	6,69	116,80	6,68
Arg32				
Arg33	113,44	7,92	113,54	7,92
Gly34	106,61	7,59	106,55	7,58
Leu35	119,68	8,10	119,66	8,09
Thr36	102,40	7,20	102,30	7,20
Ser37	114,98	7,45	115,10	7,46
Pro38				
Cys39	114,41	7,91	114,33	7,87
Lys40	127,34	7,82	127,32	7,82
Asp41	125,80	8,71	125,66	8,70
Ile42	116,84	7,76	116,98	7,78
Asn43	121,46	8,34	121,75	8,36
Thr44	124,74	8,61	124,72	8,23
Phe45	125,57	9,04	124,13	8,90
Ile46	121,60	8,90	121,36	8,99
His47	124,48	8,28	124,26	8,26
Gly48	107,91	8,49	107,93	8,48
Asn49	122,42	8,95	122,05	8,95
Lys50	126,48	8,82	126,56	8,90
Arg51	119,49	8,52	119,86	8,68
Ser52	114,66	7,76	114,87	7,75
Ile53	121,26	7,28	121,19	7,29
Lys54	119,39	7,76	119,26	7,81
Ala55	115,63	7,54	115,41	7,52
Ile56	121,18	7,65	121,75	7,72
Cys57	113,56	7,53	114,34	7,51
Glu58	119,65	7,46	119,38	7,53
Asn59	119,87	8,75	119,80	8,75
Lys60				
Asn61				
Gly62	108,95	7,67	109,00	7,56
Asn63	117,38	8,57	116,81	8,66
Pro64				
His65	122,61	8,29	124,10	8,26
Arg66	119,11	7,61	118,43	7,41
Glu67	119,29	8,70	119,28	8,71
Asn68	118,18	8,51	118,07	8,53
Leu69	120,25	8,17	120,00	8,29
Arg70	122,39	9,26	122,46	9,36
Ile71			120,11	8,91
Ser72	122,63	9,05	122,09	8,98
Lys73				
Ser74	113,13	8,13	113,34	8,17
Ser75	117,79	8,19	117,74	8,16
Phe76	120,63	8,43	120,46	8,49
Gln77	120,62	9,25	120,65	9,24
Val78	115,08	8,52	115,09	8,50
Thr79	121,69	9,12	121,86	9,13
Thr80	125,16	8,97	125,09	8,93
Cys81	126,61	8,98	126,70	9,03
Lys82	125,33	9,04	125,26	9,04
Leu83	132,09	9,06	131,85	8,97
His84	126,20	8,68	128,00	8,68
Gly85	112,39	8,23	112,36	8,23
Gly86	106,34	8,05	106,15	7,98
Ser87	116,00	7,90	115,83	7,86
Pro88				
Trp89	119,90	7,25	119,95	7,23
Pro90				
Pro91				
Cys92	124,38	9,04	124,31	9,00
Gln93	125,71	8,66	125,75	8,64
Tyr94	119,53	8,67	119,41	8,71
Arg95	118,20	8,92	117,92	8,94
Ala96	129,62	9,02	129,34	9,03
Thr97	116,82	9,07	116,90	9,09
Ala98	134,92	9,42	134,90	9,42
Gly99	108,98	8,62	109,02	8,62
Phe100	122,18	8,44	122,17	8,45
Arg101	121,07	8,69	121,15	8,70
Asn102	118,81	8,20	118,86	8,18
Val103	113,20	8,30	113,82	8,27
Val104	122,55	7,78	122,78	7,82
Val105	119,88	8,93	119,41	8,88
Cys107	120,06	8,89	119,46	8,92
Glu108	122,39	9,20	122,35	9,20
Asn109	125,90	9,50	125,92	9,51
Gly110	102,60	8,68	102,45	8,66
Leu111	118,29	7,38	118,38	7,39
Pro112				
Val113	107,06	8,23	106,97	8,21
His114	119,09	7,36	120,81	7,34
Leu115	128,19	9,26	128,60	8,72
Asp116	124,53	7,91	123,54	7,79
Gln117	126,13	8,54	126,22	8,40
Ser118	117,45	8,48	117,50	8,49
Ile119	115,58	6,61	115,28	6,55
Phe120	117,82	7,15	117,76	7,09
Arg121	119,06	7,29	118,78	7,26
Arg122	121,66	7,85	121,94	7,88
Pro123	117,93	7,50		

Table S2. Single-exponential decay constants of signal intensity (*k* values) of wt- and r-Ang residues affected by Cu(II) titration at pH 7.4.

	k constant	
Residue	wt-Ang	r-Ang
Met-1		
Gln1		
Asp2	0,774	0,149
Asn3		
Ser4	1,122	0,468
Arg5	2,423	1,468
Tyr6	2,960	1,399
Thr7	2,804	1,428
His8	3,460	1,202
Phe9	3,460	1,391
Leu10	3,103	1,030
Thr11	1,862	0,588
Gln12	1,445	0,000
His13	3,477	0,414
Tyr14	2,932	0,206
Asp15	3,460	0,232
Ala16	2,097	0,229
Lys17	0,687	0,070
Pro18		
Gln19	0,248	0,000
Gly20	2,359	
Arg21	2,061	0,134
Asp22	2,364	0,481
Asp23	0,362	0,087
Arg24	0,731	0,039
Tyr25	0,318	0,097
Cys26	0,731	0,205
Glu27	1,468	0,254
Ser28	0,684	0,298
Ile29	1,681	0,211
Met30	0,595	0,194
Arg31	1,686	0,242
Arg32		
Arg33	1,155	0,222
Gly34	1,446	0,186
Leu35	2,378	0,251
Thr36	3,340	0,213
Ser37	1,541	0,152
Pro38		
Cys39	1,390	0,083
Lys40	3,044	0,263
Asp41	0,452	0,227
Ile42	3,452	0,728
Asn43	1,445	0,115
Thr44	2,632	0,039
Phe45	2,356	0,116
Ile46	3,460	0,313
His47	1,619	0,194
Gly48	2,096	0,188
Asn49	2,517	0,231
Lys50	0,198	1,452
Arg51	0,247	0,560
Ser52	2,375	0,084
Ile53	3,468	0,188
Lys54	2,994	0,416
Ala55	2,631	0,457
Ile56	2,642	0,488
Cys57	1,444	0,067
Glu58	2,002	0,248
Asn59	0,000	0,504
Lys60		
Asn61		
Gly62	2,999	0,140
Asn63	2,987	0,106
Pro64		
His65	2,858	0,150
Arg66	3,340	0,494
Glu67	0,675	0,007
Asn68	2,091	0,074
Leu69	2,155	0,160
Arg70	3,107	0,183
Ile71	2,988	0,091
Ser72	2,977	0,619
Lys73		
Ser74	2,522	0,031
Ser75	0,734	0,188
Phe76	3,121	0,222
Gln77	1,231	0,107
Val78	0,740	0,138
Thr79	2,207	0,262
Thr80	1,367	0,214
Cys81	1,914	0,282
Lys82	0,292	0,130
Leu83	2,578	0,404
His84	0,783	0,018
Gly85	1,338	0,000
Gly86	0,281	0,000
Ser87	0,754	0,000
Pro88		
Trp89	0,822	0,072
Pro90		
Pro91		
Cys92	0,492	0,000
Gln93	1,433	0,104
Tyr94	1,006	0,161
Arg95	0,838	0,164
Ala96	0,275	0,209
Thr97	0,419	0,229
Ala98	2,125	0,437
Gly99	1,569	0,059
Phe100	2,387	0,329
Arg101	2,705	0,146
Asn102	0,618	0,024
Val103	2,345	0,233
Val104	2,574	0,401
Val105	2,952	0,171
Ala106		
Cys107	3,234	0,302
Glu108	3,471	1,112
Asn109	1,440	0,000
Gly110	2,429	0,815
Leu111	3,461	1,025
Pro112		
Val113	3,425	0,466
His114	3,548	0,344
Leu115	4,132	0,161
Asp116	3,440	0,105
Gln117	3,008	0,000
Ser118	3,142	0,032
Ile119	3,635	0,202
Phe120	3,815	0,140
Arg121	3,512	0,177
Arg122	0,687	0,186
Pro123		