Supporting Information for

Exploring the Specificity of Rationally Designed Peptides Reconstituted from the Cell-Free Extract of *Deinococcus radiodurans* toward Mn(II) and Cu(II)

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Figures and Tables

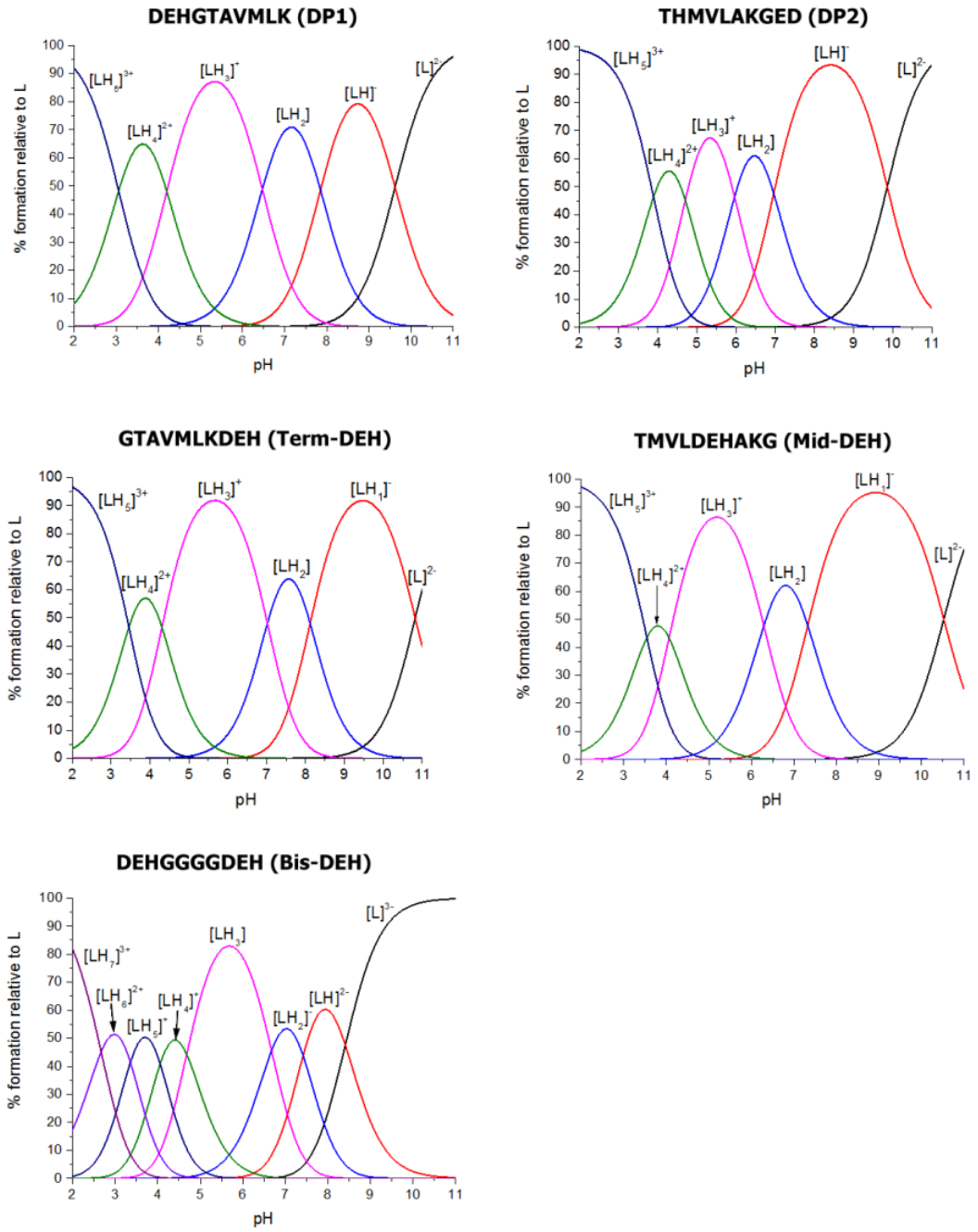

Figure S1. Representative distribution diagram for the protonation equilibria of DEHGTAVMLK (DP1), THMVLAKGED (DP2), GTAVMLKDEH (Term-DEH), TMVLDEHAKG (Mid-DEH) and DEHGGGGDEH (Bis-DEH), at T = 298 K and I = 0.1 M $NaClO_4$.

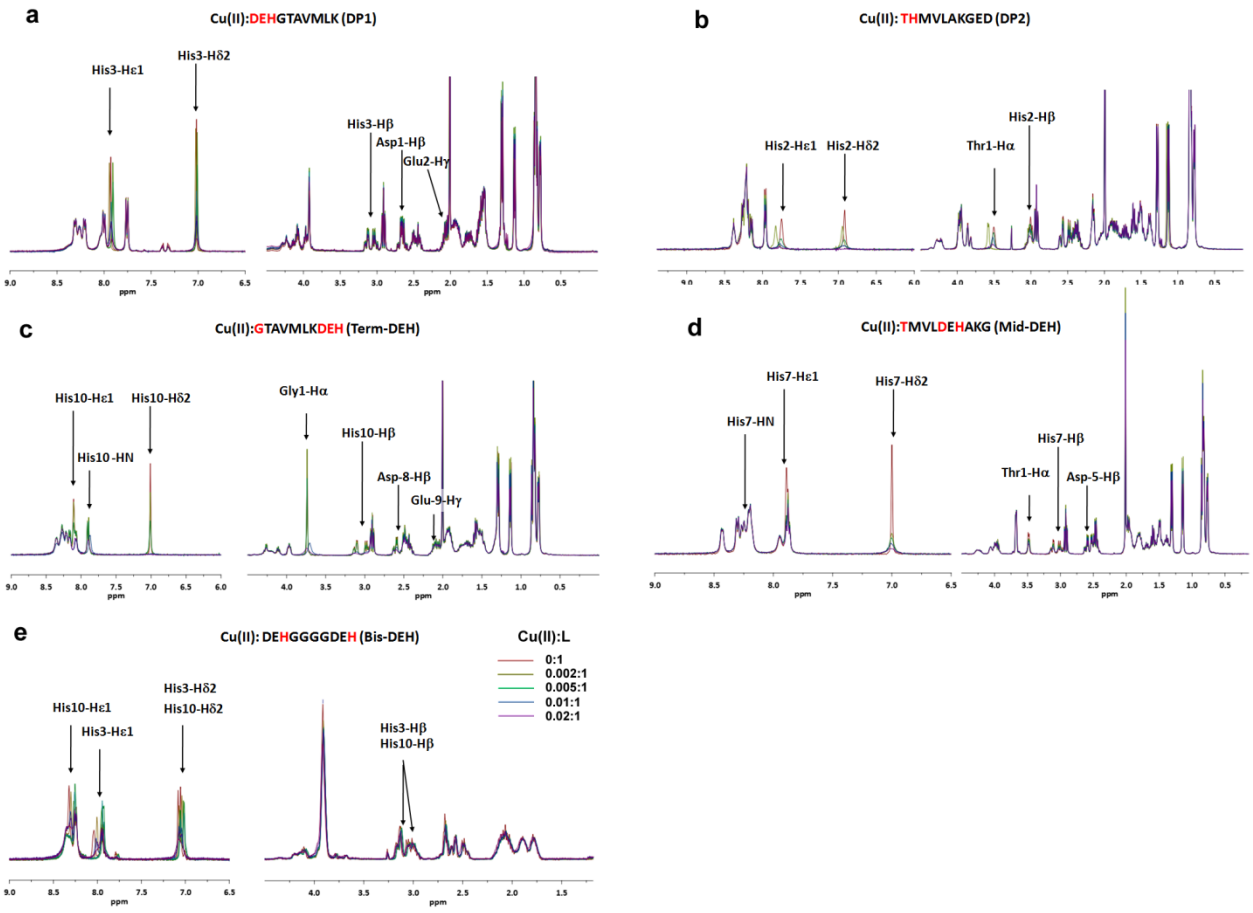

Figure S2. Superposition of 1D ^{1}H NMR spectra for a) DEHGTAVMLK (DP1), b) THMVLAKGED (DP2), c) GTAVMLKDEH (Term-DEH), d) TMVLDEHAKG (Mid-DEH) and e) DEHGGGGDEH (Bis-DEH) peptides by increasing amounts of Cu(II) from 0:1 to 0.02:1 metal to ligand molar ratio, at pH ~7**.** The residues experiencing the largest relaxation effects are labeled in the spectra and highlighted along the sequence in red color.

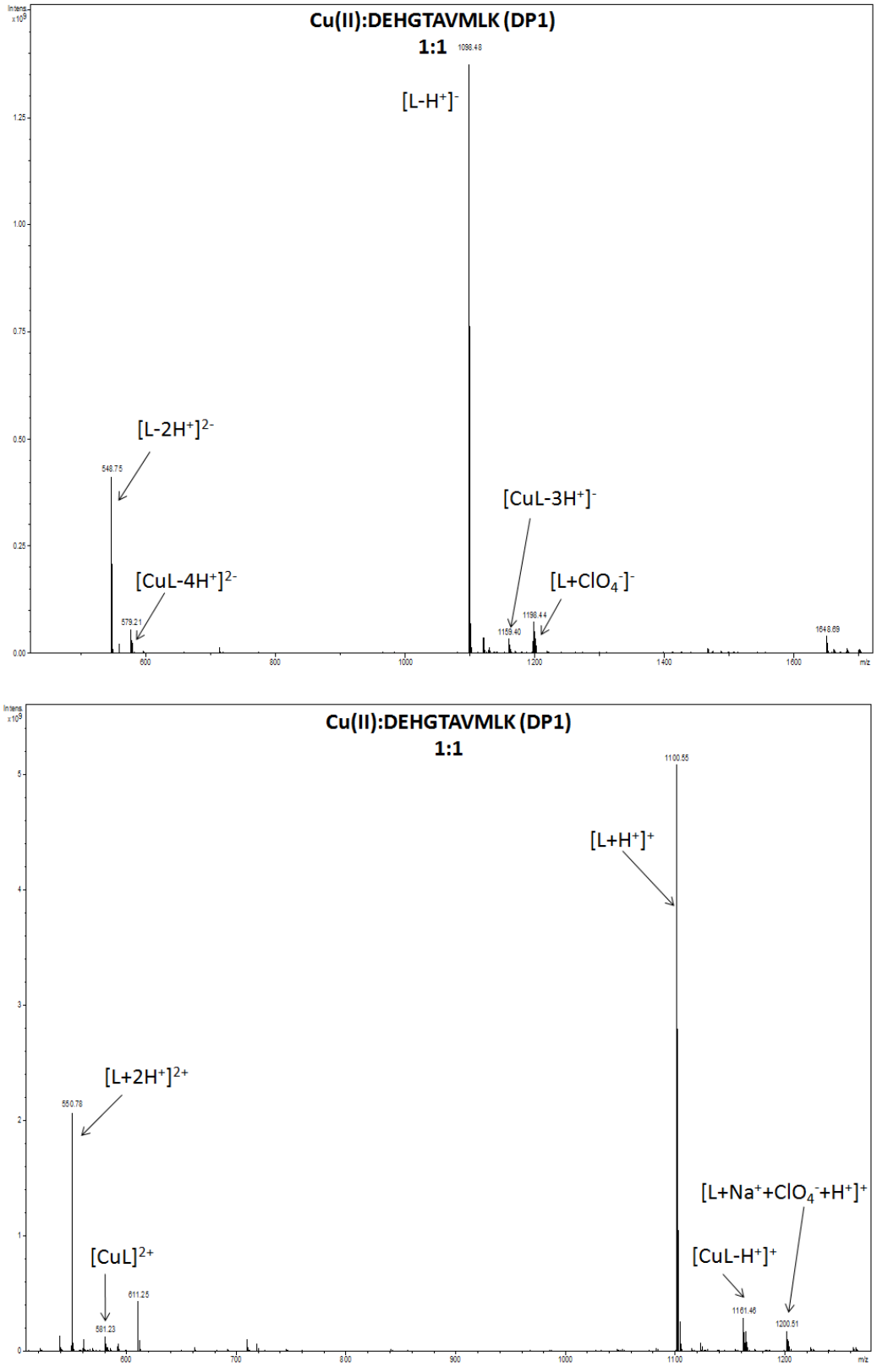

Figure S3. ESI-MS of Cu(II):DEHGTAVMLK (DP1), at 1:1 molar ratio and pH 7.

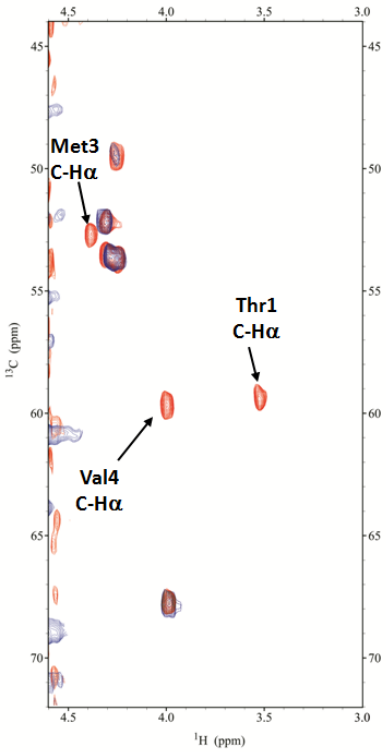

Figure S4. Aliphatic region of ^{1}H-^{13}C HSQC spectrum of DP2 free (red) and Cu(II):DP2 system at 0.02:1 molar ratio (blue), and pH 10. Disappearing peaks are labeled.

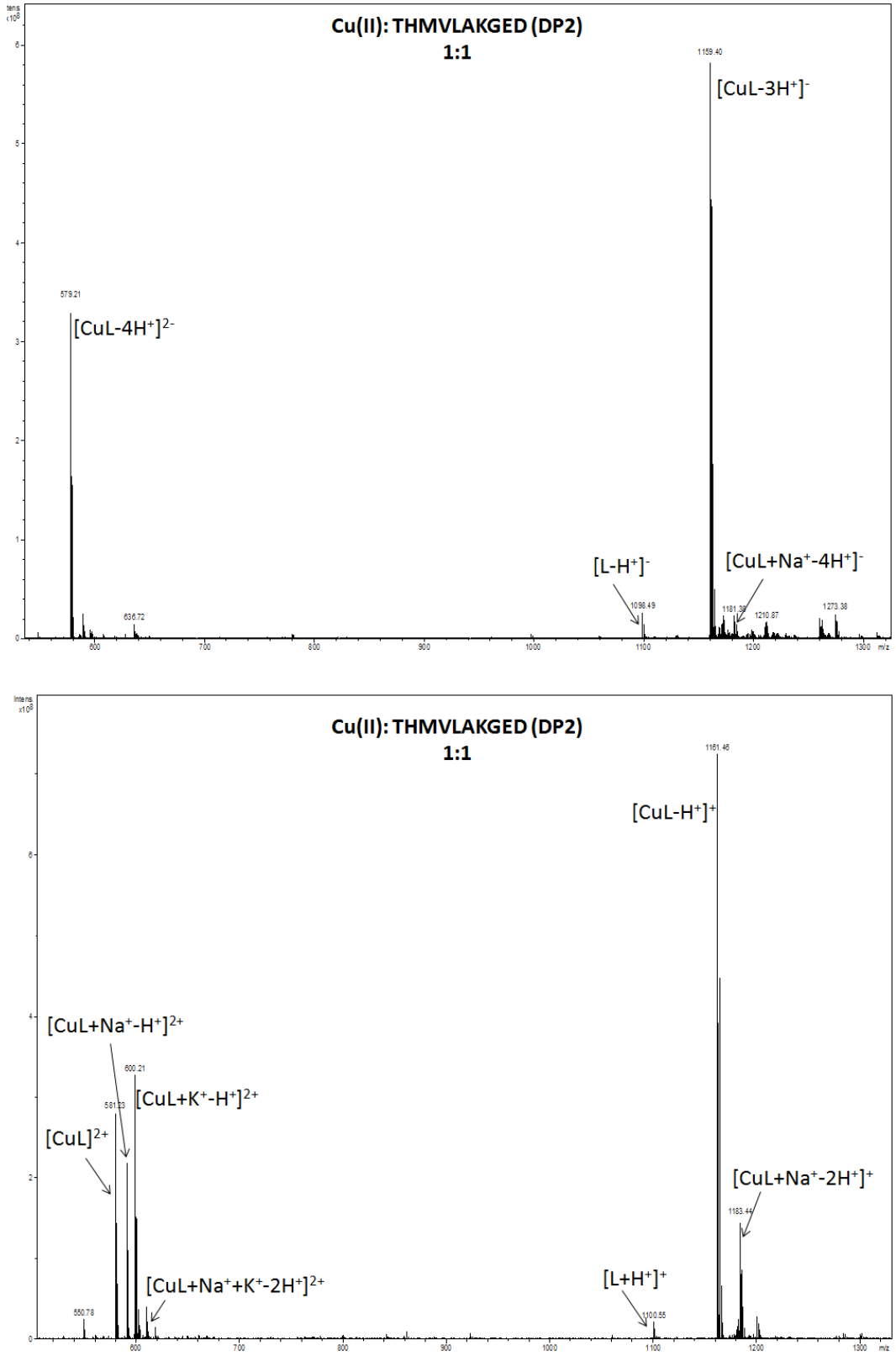

Figure S5. ESI-MS of Cu(II):THMVLAKGED (DP2), at 1:1 molar ratio, and pH 7.

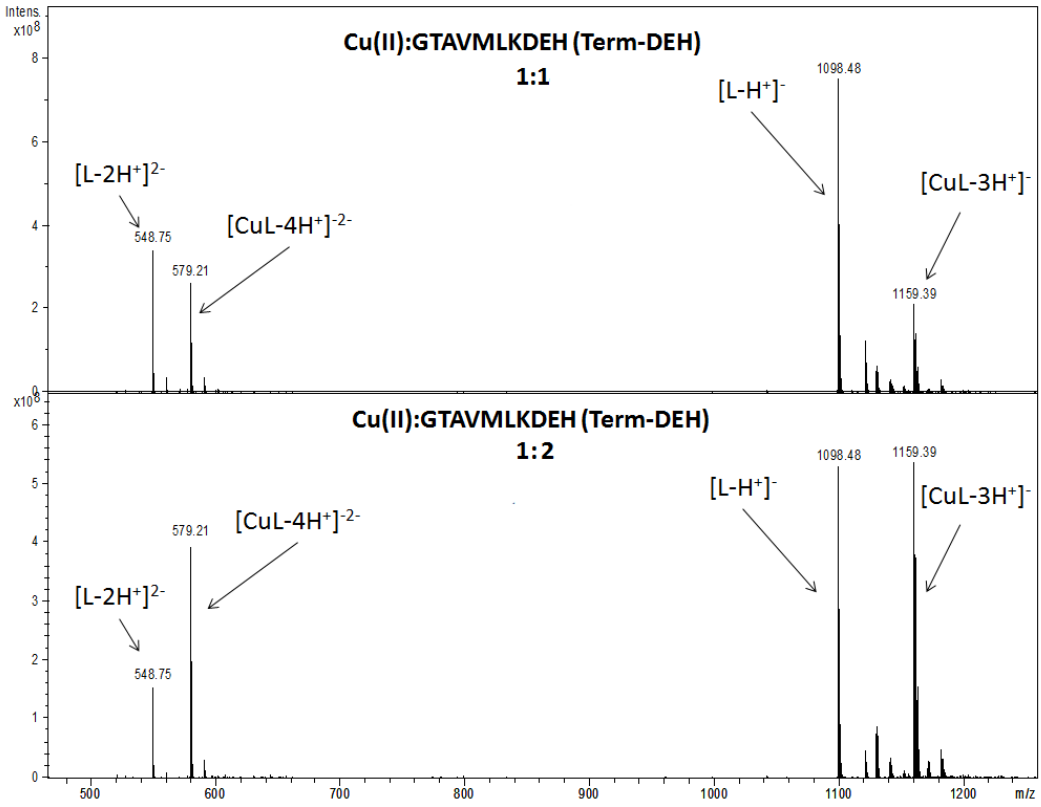

Figure S6. ESI-MS of Cu(II):GTAVMLKDEH (Term-DEH), at 1:1 and 1:2 molar ratio, and pH 7.

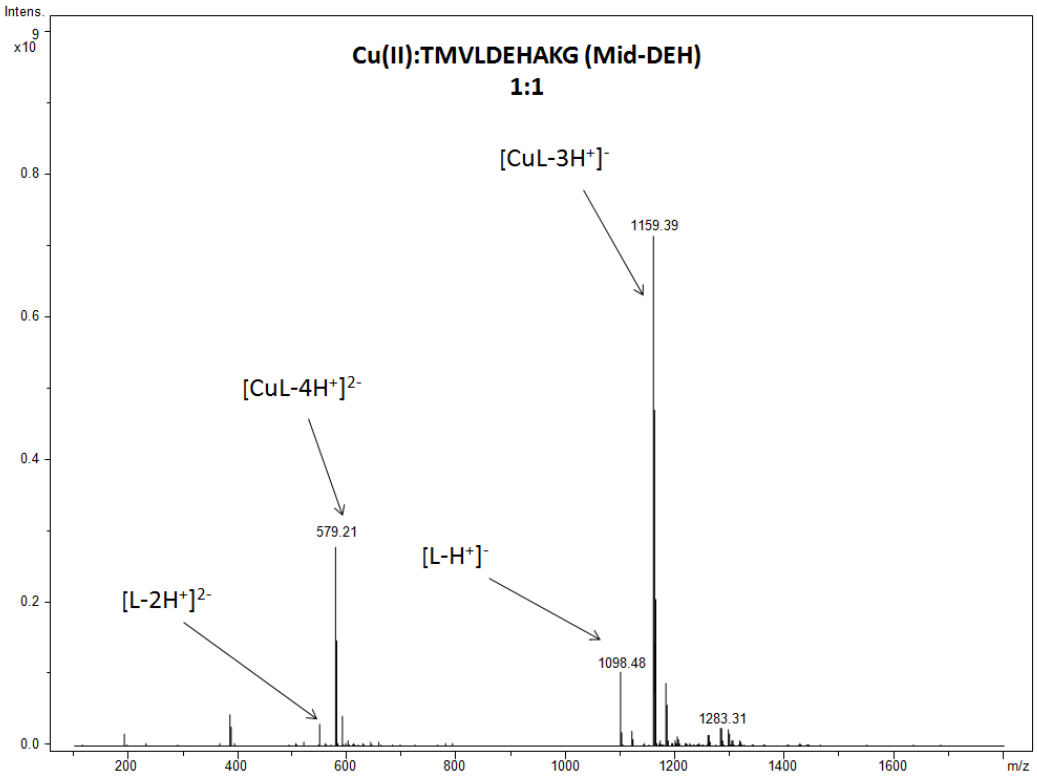

Figure S7. ESI-MS of Cu(II):TMVLDEHAKG (Mid-DEH), at 1:1 molar ratio, and pH 7.

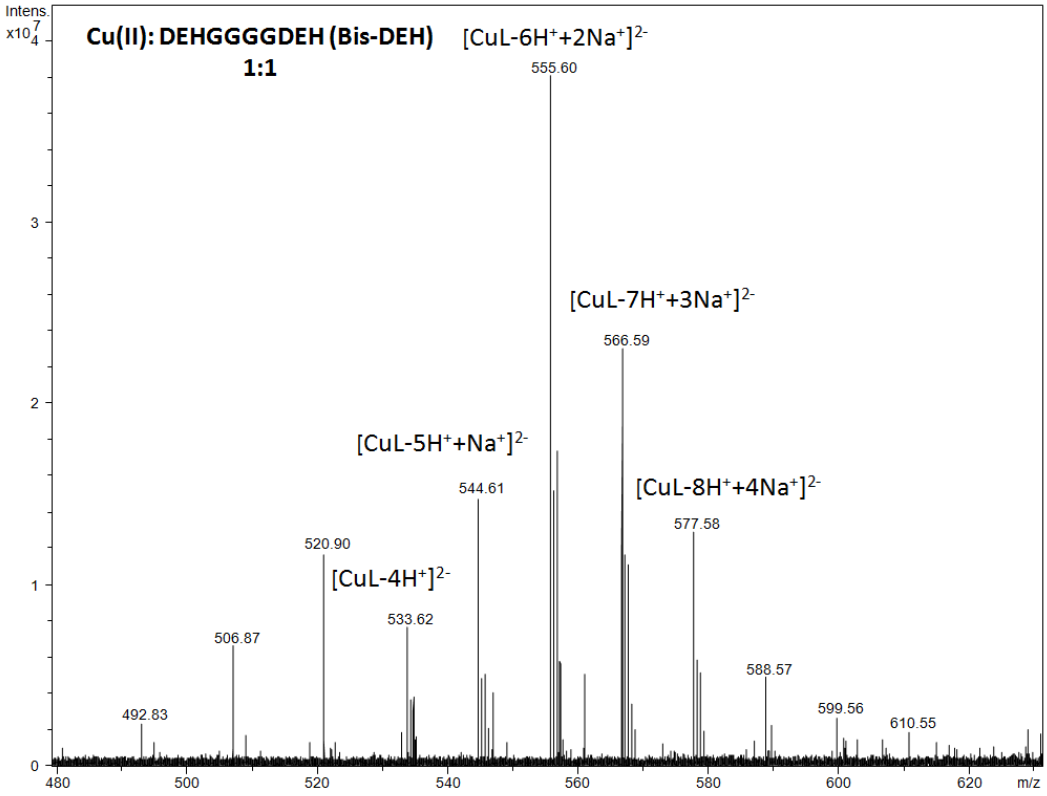

Figure S8. ESI-MS of Cu(II):DEHGGGGDEH (Bis-DEH), at 1:1 molar ratio, and pH 7.

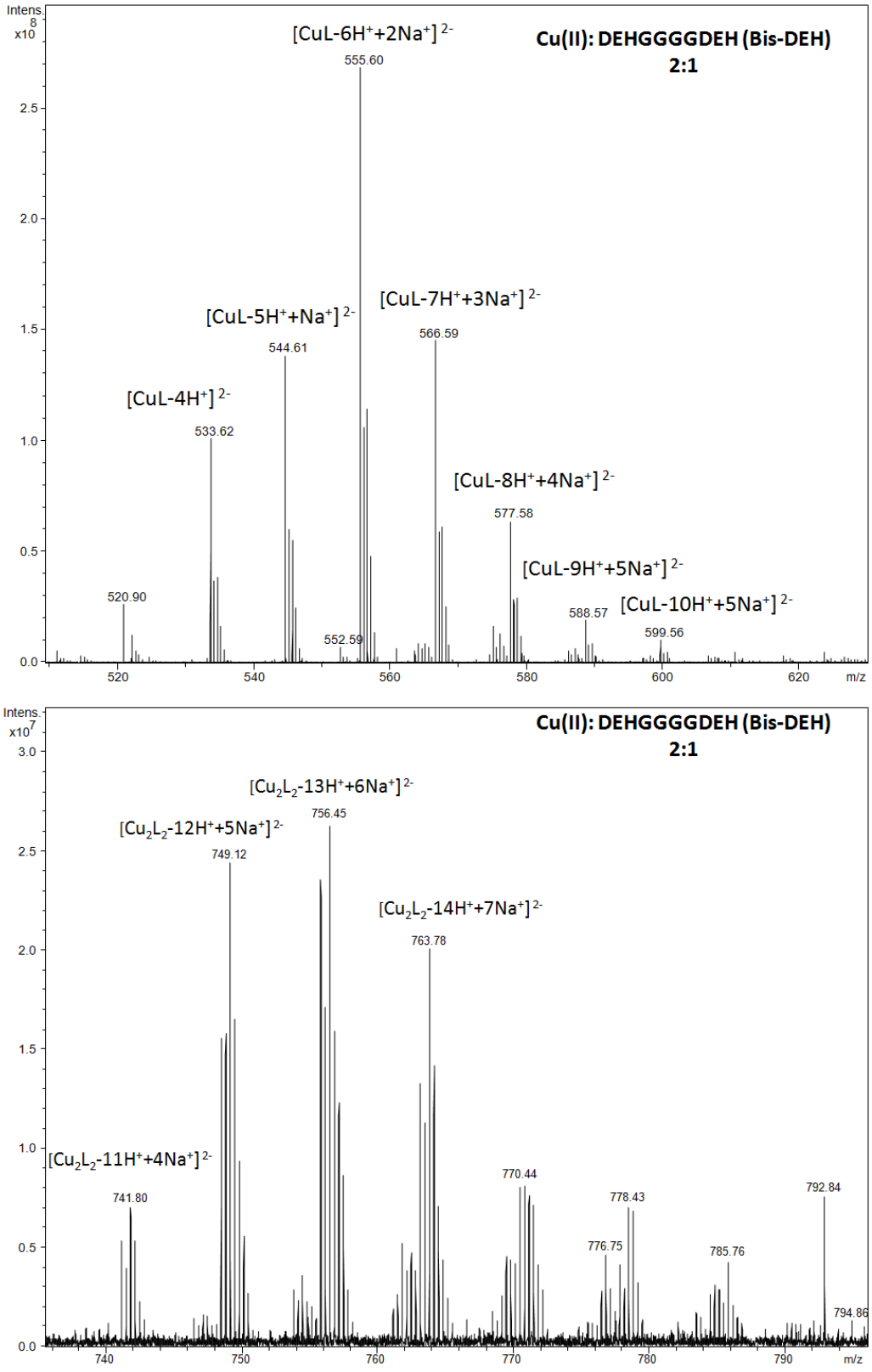

Figure S9. ESI-MS of Cu(II):DEHGGGGDEH (Bis-DEH) at 2:1 molar ratio, and pH 7.

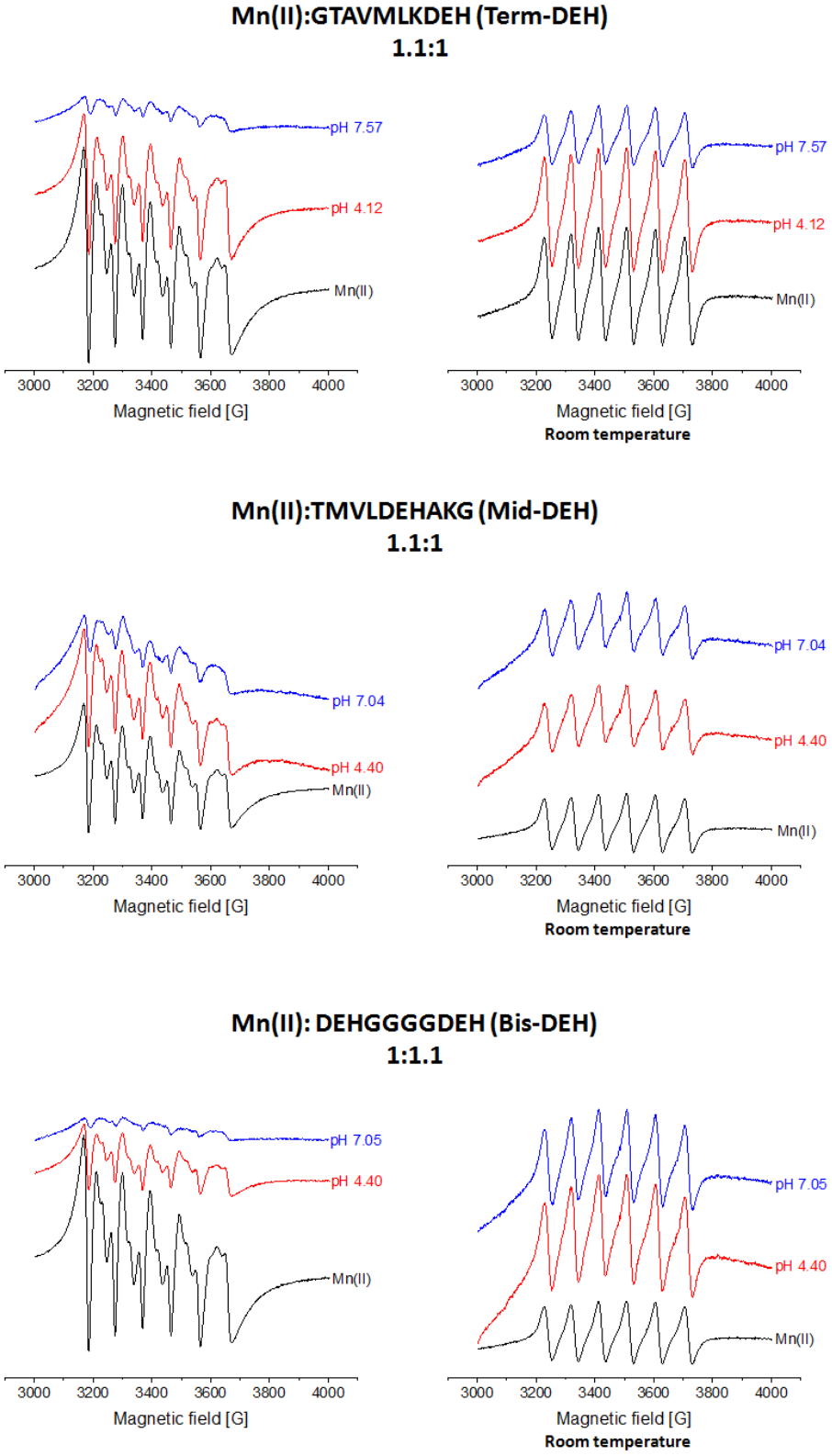

Figure S10. EPR spectra of the Mn(II)-peptides system as a function of pH.

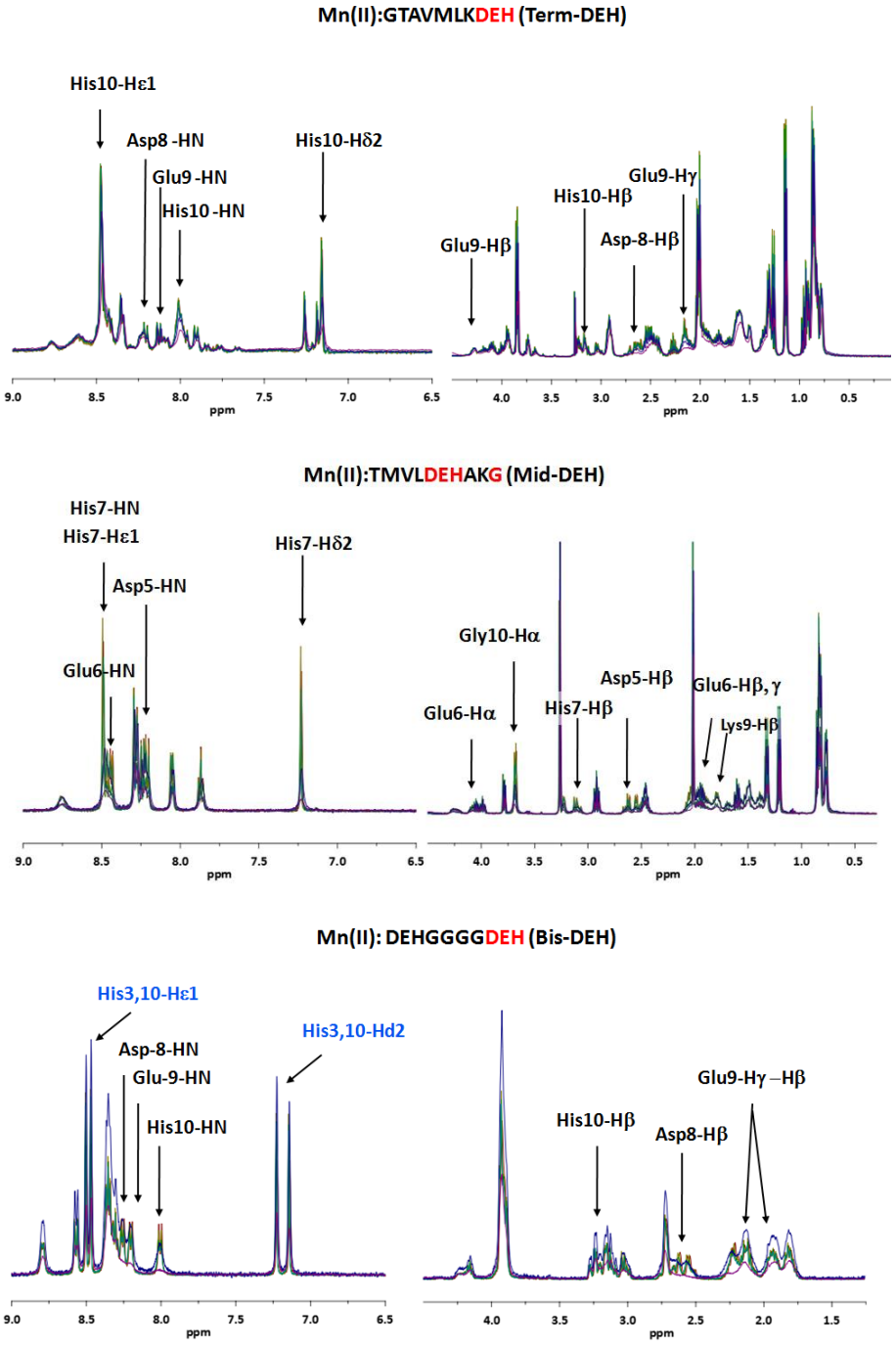

Figure S11. Superposition of 1D ^{1}H NMR spectra for GTAVMLKDEH (Term-DEH), TMVLDEHAKG (Mid-DEH) and DEHGGGGDEH (Bis-DEH) peptides by increasing substochiometric Mn(II) to ligand molar ratio, at pH 5**.**

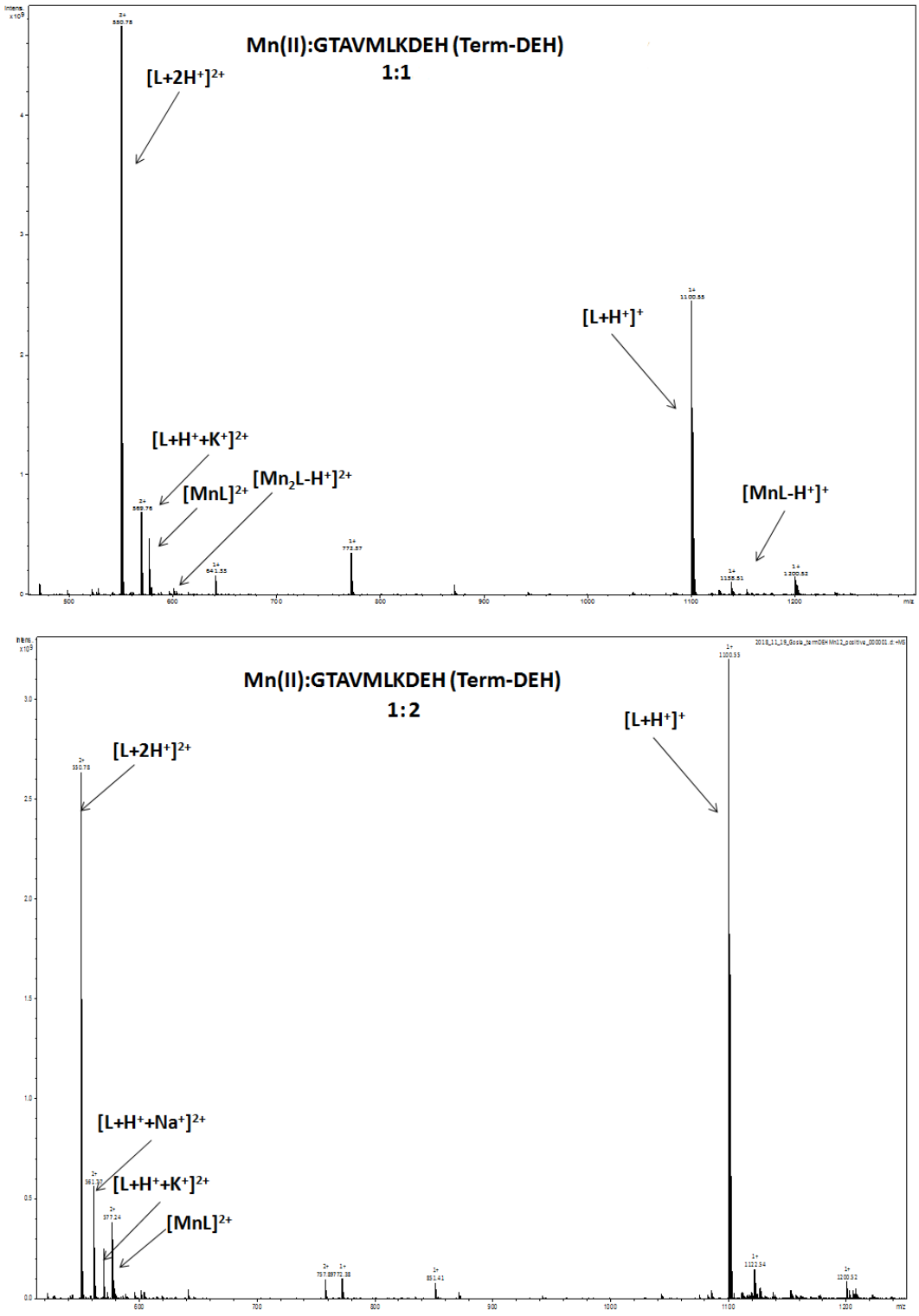

Figure S12. ESI-MS of Mn(II):GTAVMLKDEH (Term-DEH), at 1:1 and 1:2 molar ratio, and pH 7.5.

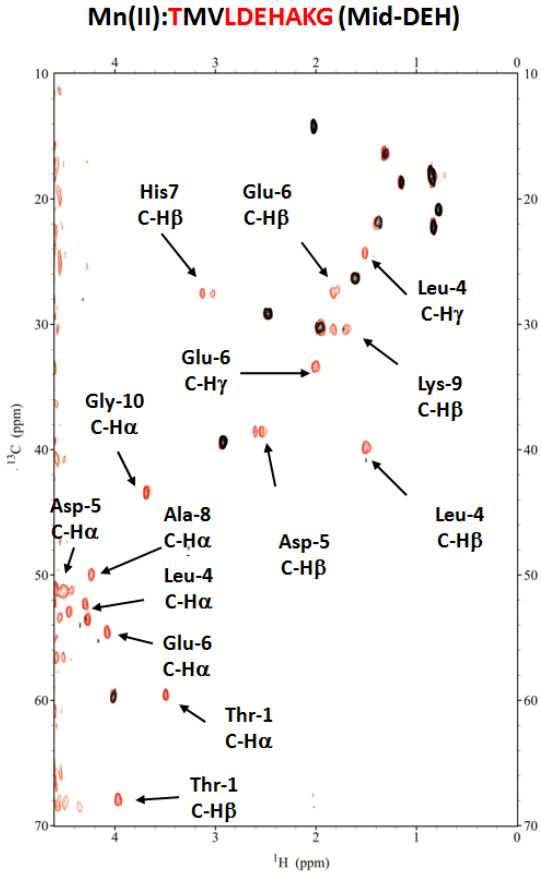

Figure S13. Aromatic and aliphatic region of ^{1}H-^{13}C HSQC spectrum of free Mid-DEH (red) and Mn(II): Mid-DEH at 0.1:1 molar ratio (blue) and pH 7.2. Disappearing peaks are labeled.

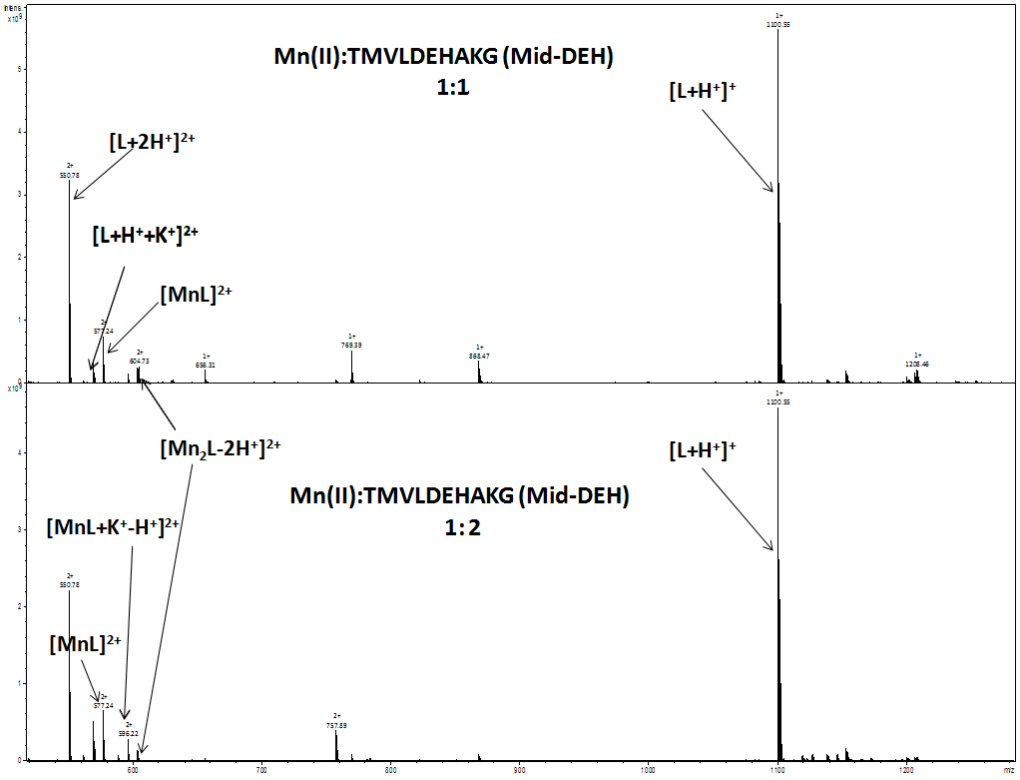

Figure S14. ESI-MS of Mn(II):TMVLDEHAKG (Mid-DEH), at 1:1 and 1:2 molar ratio, and pH 7.5.

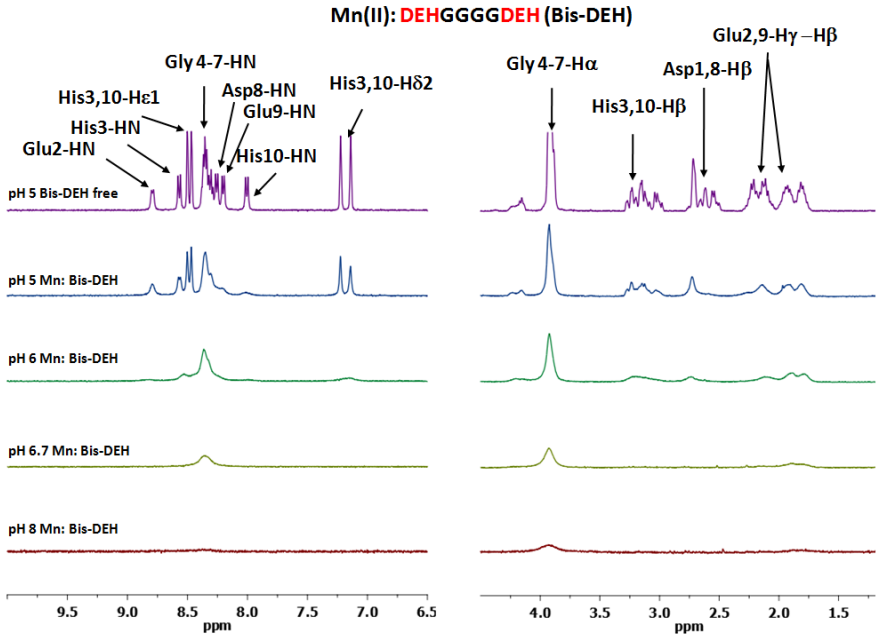

Figure S15. Comparison of 1D ^{1}H NMR spectra for free DEHGGGGDEH (Bis-DEH) at pH 5, and Mn(II):Bis-DEH system (0.05:1 molar ratio) at pH 5, 6, 6.7 and 8, respectively.

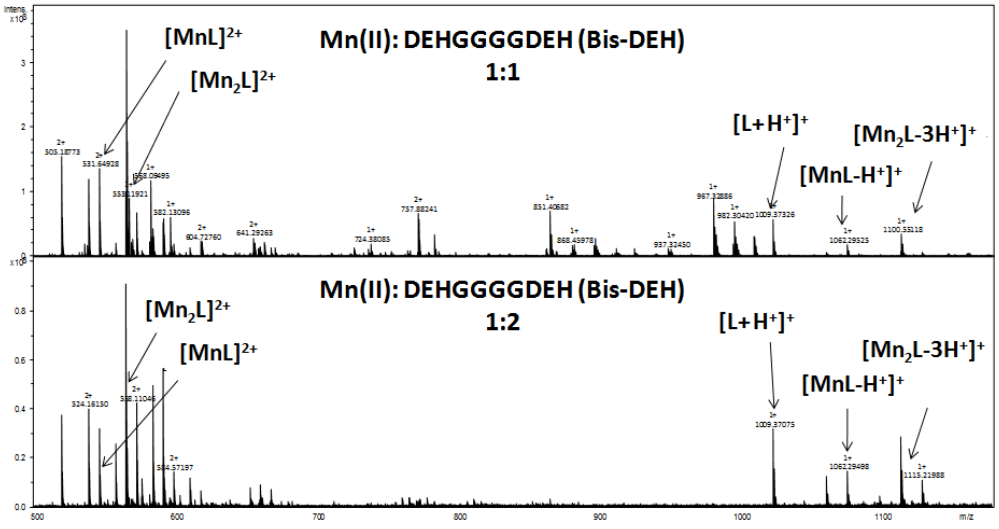

Figure S16. ESI-MS of Mn(II):DEHGGGGDEH (Bis-DEH), at 1:1 and 1:2 molar ratio, and pH 7.5.

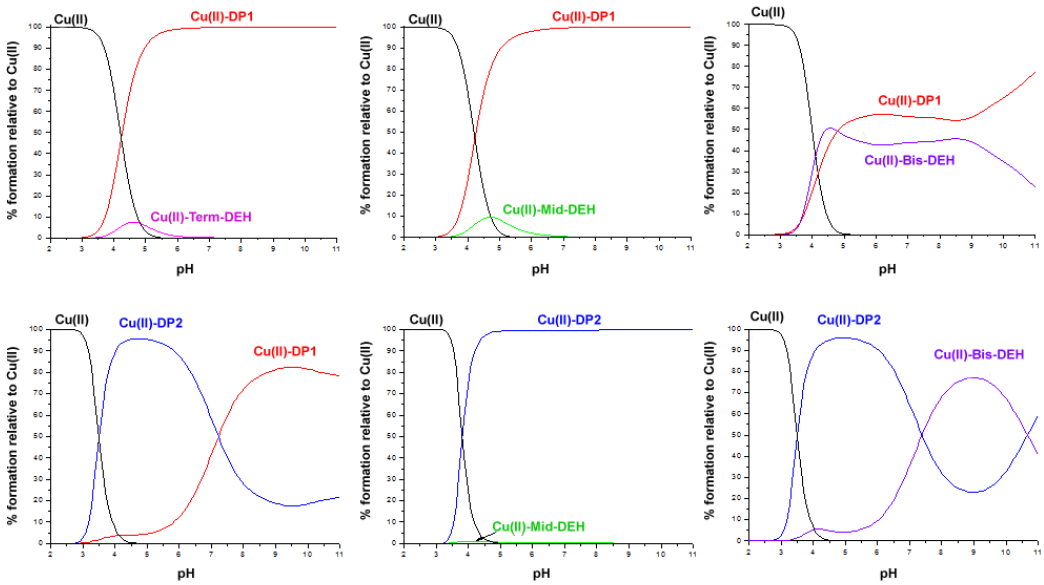

Figure S17. Competition plots for a pair of peptides between DEHGTAVMLK (DP1), THMVLAKGED (DP2), GTAVMLKDEH (Term-DEH); TMVLDEHAKG (Mid-DEH) and DEHGGGGDEH (Bis-DEH).
The calculated stability constants are applied to a theoretical situation, in which equimolar amounts of pair of peptides are competing for the binding with Cu(II) ion in 1:1:1 metal-to-ligand molar ratio.

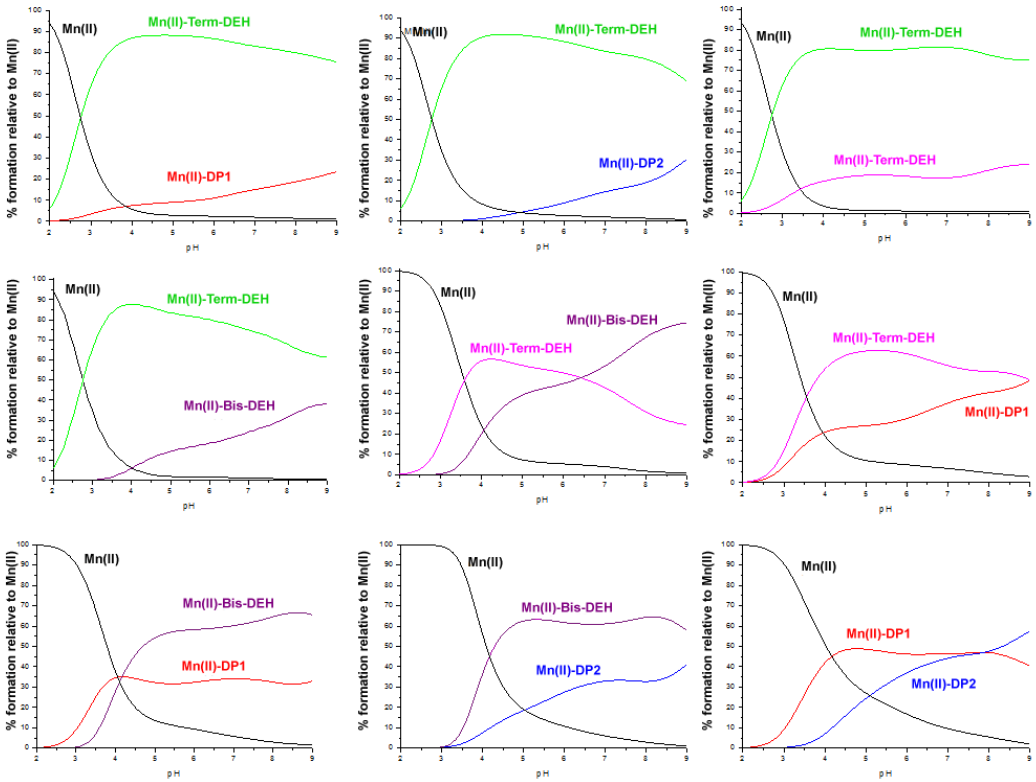

Figure S18. Competition plots for a pair of peptides between DEHGTAVMLK (DP1), THMVLAKGED (DP2), GTAVMLKDEH (Term-DEH); TMVLDEHAKG (Mid-DEH) and DEHGGGGDEH (Bis-DEH).
The calculated stability constants are applied to a theoretical situation, in which equimolar amounts of pair of peptides are competing for the binding with Mn(II) ion in 1:1:1 metal-to-ligand molar ratio.

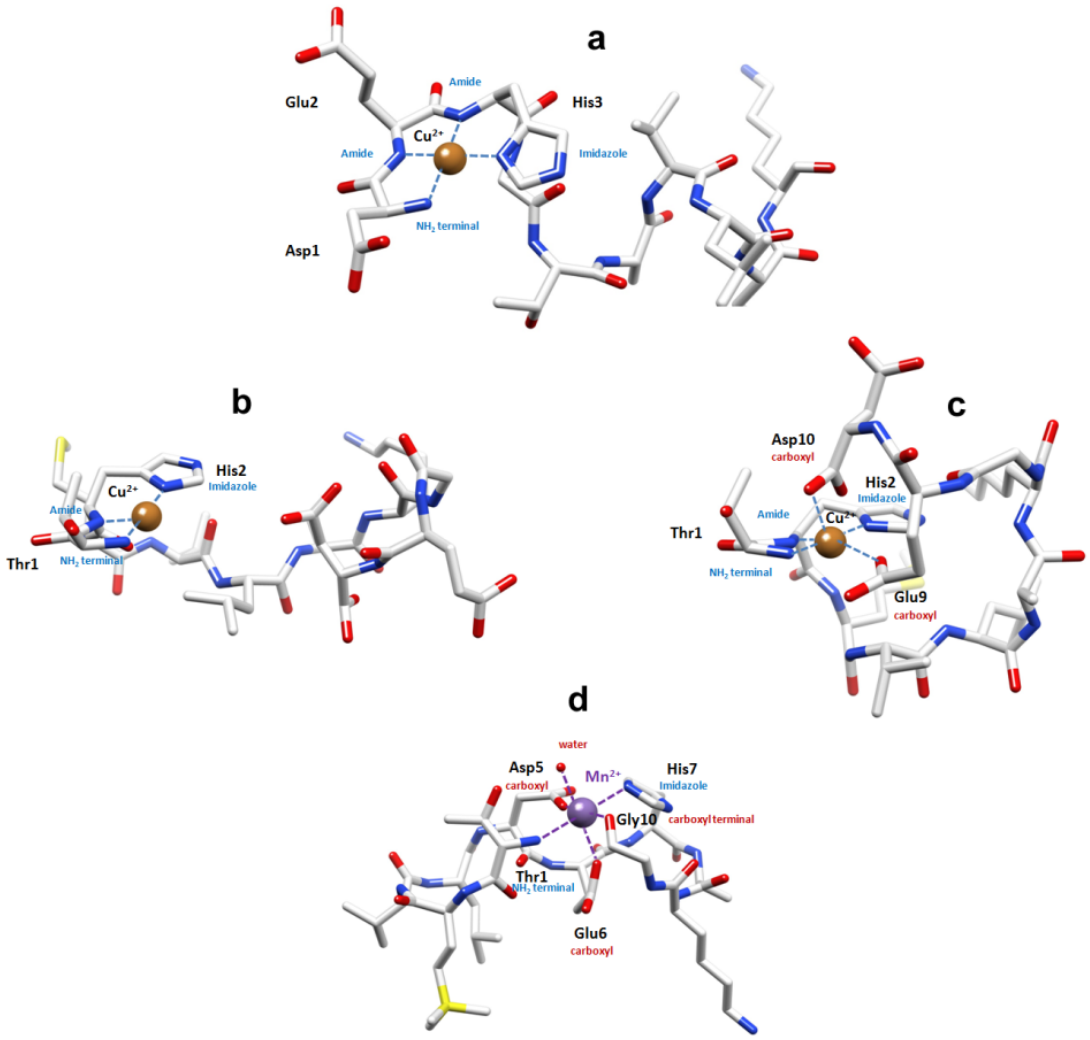

Figure S19. Structural models of a) DP1-Cu in 4N{N_{term}, N_{im}, $2N^-_{amide}$} *albumin-like*, b) DP2-Cu in 3N {N_{term}, $N_{im,}$ N^-_{amide}}, c) DP2-Cu in 3N2O { N_{term}, $N_{im,}$ N^-_{amide}, 2O}, and d) Mid-DEH-Mn in 2N4O {N_{term}, N_{im}, 4O} coordination modes, respectively. The proposed models should be taken with appropriate caution, since they were constructed by selective imposition of a number of constraints; however, they can still represent an interesting structural hypothesis for the complexes formed in solution, in agreement with experimental evidences..

For Cu(II)-DP1 model a), the Cu(II) ion was constrained in a 4N square planar coordination mode using the metal-donor distances and backbone dihedral angles derived from X-ray crystal structures of aqua-((aspartic acid)-alanyl-histidinyl-lysinyl)-copper(II) chloride hexahydrate (CSD Entry: OZEBOL). Moreover, meaningful ROEs involving a through-space correlation of the side-chain aliphatic protons of Glu-3 with the aromatic and aliphatic β-protons of His-3 were also used and introduced as constraints for model generation. These through-space correlations were evaluated using diamagnetic Ni(II) (at pH 10) to probe paramagnetic Cu(II) in the same square planar coordination mode. The geometry of the rest of the peptide was optimized without any constraints allowing all the atoms, bonds and dihedral angles to change simultaneously to reach the lowest overall energy within the HyperChem™ 8.0.3 program (Hypercube, Inc., Gainesville, FL, 2007). For Cu(II)-DP2 (b model), the Cu(II) ion was constrained in a 3N planar coordination involving N_{term} from Thr-1 and $N_{im\ and}$ N^-_{amide} from His-2. The N-Cu(II) distances were derived from the same template (CSD Entry: OZEBOL). Moreover, in c model, additional constraints were imposed to the carboxylic oxygen donors of Asp-9 and Glu-10 in order to be connected to the metal ion. The other residues and backbone dihedral angles have been left free to move. As for the d model, Mn(II) bound in an octahedral {N_{term}, N_{im}, 4O} coordination mode with Thr-1 (N_{term}), Asp-5 (O), Glu-6 (O), His-7 (N_{im}), Gly-10 (O) and H_2O, the metal-donor distances were chosen using those found in the structure of MNTR mutant E11K complexed with Mn^{2+} in octahedral geometry (PDB 4HX4) while the position of all the other atoms and backbone dihedral angles were optimized without any constraints.

Tables

Table S1. ^{1}H and ^{13}C resonance assignment (δ ppm) for DEHGTAVMLK (DP1), THMVLAKGED (DP2), GTAVMLKDEH (Term-DEH), TMVLDEHAKG (Mid-DEH) and DEHGGGGDEH (Bis-DEH) peptides.

DEHGTAVMLK (DP1), pH 7.2										
Nucleus\residue	**D1**	**E2**	**H3**	**G4**	**T5**	**A6**	**V7**	**M8**	**L9**	**K10**
CA	51.12	54.02	53.76	42.82	59.25	49.91	59.63	52.44	52.49	54.87
CB	38.04	27.43	27.95		67.06	16.47	30.16	29.27	39.54	31.16
CD										26.43
CD1									22.27	
CD2			117.40						20.67	
CE								14.25		39.50
CE1			135.60							
CG		33.24			18.89			30.15	24.16	21.98
CG1							18.31			
CG2							17.77			
HA	4.047	4.206	4.570		4.237	4.279	3.972	4.425	4.290	4.082
HB					4.142		1.954			
HB2	2.682	1.886	3.119					2.513		1.729
HB3	2.592	1.777	3.013					2.438		1.617
HD2			6.976							
HE1			7.815							
HG									1.542	
HN				8.272	8.026	8.258	8.001	8.310	8.203	7.760
QA				3.921						
QB						1.302			1.542	
QD										1.586
QD1									0.843	
QD2									0.785	
QE								2.013		2.913
QG		2.053			1.131			1.947		1.301
QG1							0.840			

THMVLAKGED (DP2), pH 7.3										
Nucleus\residue	**T1**	**H2**	**M3**	**V4**	**L5**	**A6**	**K7**	**G8**	**E9**	**D10**
CA	59.51	53.63	52.68	59.63	52.05	49.48	53.66	42.46	53.45	52.11
CB	68.00	28.46	29.09	30.12	39.77	16.56	30.48		27.85	39.50
CD							26.37			
CD1					22.14					
CD2		116.90			20.78					
CE			14.16				39.36			
CE1		136.00								
CG	18.44		30.32		24.20		21.68		33.61	
CG1				18.46						
CG2				17.87						
HA	3.435	4.564	4.367	3.973	4.292	4.240	4.232		4.285	4.287
HA1								3.853		
HA2								3.970		
HB	3.952			1.931						
HB2		3.000	2.395				1.739		2.047	2.586
HB3		2.996	2.365				1.695		1.816	2.477
HD2		6.903								
HE1		7.700								
HG					1.517					
HN				8.134	8.263	8.213	8.234	8.384	8.208	7.964
QB					1.514	1.282				
QD							1.605			
QD1					0.836					
QD2					0.783					
QE			2.002				2.931			
QG	1.111		1.893				1.382		2.159	

GTAVMLKDEH (Term-DEH), pH 7.4										
Nucleus\residue	**G1**	**T2**	**A3**	**V4**	**M5**	**L6**	**K7**	**D8**	**E9**	**H10**
CA	41.27	59.21	49.87	59.66	52.54	52.40	53.56	51.75	53.81	54.62
CB		67.19	16.58	30.24	29.27	39.61	30.50	38.46	27.94	28.02
CD							26.40			
CD1						22.21				
CD2						20.74				117.50
CE					14.28		39.40			
CE1										134.30
CG		18.91			30.27	24.25	21.76		33.58	
CG1				18.23						
CG2				17.93						
HA		4.277	4.293	3.980	4.428	4.285	4.234	4.528	4.198	4.353
HB		4.120		1.951						
HB2					2.502	1.527	1.714	2.617	1.939	3.129
HB3					2.439	1.493	1.674	2.491	1.777	2.980
HD2										7.022
HE1										8.120
HG						1.546				
HN			8.265	8.086	8.361	8.262	8.215	8.292	8.169	7.909
QA	3.758									
QB			1.305							
QD							1.599			
QD1						0.841				
QD2						0.786				
QE					2.021		2.916			
QG		1.148			1.939		1.322		2.094	
QG1				0.844						

TMVLDEHAKG (Mid-DEH), pH 7.2										
Nucleus\residue	**T1**	**M2**	**V3**	**L4**	**D5**	**E6**	**H7**	**A8**	**K9**	**G10**
CA	59.54	52.94	59.67	52.32	51.27	54.51	53.39	49.93	53.60	43.45
CB	67.86	29.08	30.32	39.87	38.56	27.33	27.54	16.42	30.38	
CD									26.34	
CD1				22.13						
CD2				20.83			117.40			
CE		14.22							39.48	
CE1							135.40			
CG	18.66	30.32		24.36		33.41			21.97	
CG1			18.43							
CG2			17.99							
HA	3.489	4.463	4.015	4.290	4.505	4.069	4.536	4.229	4.269	
HB	3.962		1.954							
HB2					2.601	1.837	3.125		1.816	
HB3					2.533	1.791	3.016		1.701	
HD2							7.010			
HE1							7.899			
HG				1.506						
HN			8.263	8.308	8.204	8.438	8.235	7.953	8.190	7.880
QA										3.690
QB		2.481		1.499				1.318		
QD									1.618	
QD1				0.836						
QD2				0.787						
QE		2.027							2.930	
QG	1.151	1.952				2.002			1.395	
QG1			0.837							

DEHGGGGDEH (Bis-DEH), pH 7.0							
Nucleus\residue	**D1**	**E2**	**H3**	**G4-G7**	**D8**	**E9**	**H10**
CA	50.96	54.18	53.42	42.60	51.54	54.09	54.34
CB	37.57	27.43	27.47		38.57	27.44	27.48
CD2			117.50				117.30
CE1			135.00				133.70
CG		33.30				33.43	
HA	4.118	4.221	4.608		4.543	4.156	4.376
HB2		1.911	3.160		2.602	1.924	3.159
HB3		1.792	3.062		2.488	1.798	3.002
HD2			7.069				7.090
HE1			8.044				8.332
HN			8.419	8.347	8.255	8.257	7.955
QA				3.925			
QB	2.695						
QG		2.077				2.119	

Table S2. Spectroscopic parameters and coordination modes for the Cu(II):peptide systems.

DP1	**UV-Vis**[a]			**CD**[a]			**EPR**					**NMR**	
DEHGTAVMLK	**pH**	**λ**	**ε**	**pH**	**λ**	**Δε**	**pH**	**A_{II}**	**g_{II}**	**Complex**	**Donors**	**pH**	**Residue donors**
DP1 species $[CuH_2L]^{2+}$-$[CuHL]^{+}$							3.85	155.0	2.33	Not determined			
[CuL]							4.95	208.7	2.18	4N	$\{N_{term}, N_{im}, 2N^-_{amide}\}$	4.3	{D1 N_{term}, E2 N^-_{amide}, H3 N_{im}, N^-_{amide}}
$[CuLH_{-1}]^-$	7.04	525	297	7.04	560	-0.65	7.20	208.7	2.18	4N	$\{N_{term}, N_{im}, 2N^-_{amide}\}$	7.0-7.6	{D1 N_{term}, E2 N^-_{amide}, H3 N_{im}, N^-_{amide}}
					485	0.43							
					310	1.20							
					272	-2.80							
$[CuLH_{-2}]^{2-}$	10.02	525	325	10.05	560	-0.77	9.85	208.7	2.18	4N	$\{N_{term}, N_{im}, 2N^-_{amide}\}$	9	{D1 N_{term}, E2 N^-_{amide}, H3 N_{im}, N^-_{amide}}
					485	0.43							
					310	1.20							
					272	-2.80							
$[CuLH_{-3}]^{3-}$	10.95	525	339	11.07	560	-0.84				4N	$\{N_{term}, N_{im}, 2N^-_{amide}\}$	10.0	{D1 N_{term}, E2 N^-_{amide}, H3 N_{im}, N^-_{amide}}
					485	0.43							
					310	1.20							
					272	-2.80							

[a]The reported errors on λ_{max}= ±2 nm, ε = ± 5%.

DP2	UV-Vis			CD			EPR					NMR	
THMVLAKGED	**pH**	**λ**	**ε**	**pH**	**λ**	**Δε**	**pH**	**A_{II}**	**g_{II}**	**Complex**	**Donors**	**pH**	**Residue donors**
DP2 species													DP2:Cu 1:0.02
							3.13	119.27	2.414	$Cu_{(aq)}$			
$[CuHL]^{+}$	4.58	597	169	4.34	585	0.22	4.55	190.0	2.225	3N	$\{N_{term}, N_{im}, N^{-}_{amide}\}$	4.25	{ H2 $N_{im,}$ (E9 O^{-}_{carb}, D10 O^{-}_{carb},)}
					300	-0.74							
					265	0.76							
[CuL]	7.55-8.17	595	210	6.2-8.2	585	0.22	6.95	190.0	2.225	3N	$\{N_{term}, N_{im}, N^{-}_{amide}\}$	7.0	{T1 N_{term}, H2 $N_{im,}$ N^{-}_{amide}, (E9 O^{-}_{carb}, D10 O^{-}_{carb},)}
					300	-0.74							
					265	1.06							
$[CuLH_{-1}]^{-}$	7.55-8.17	595	210	Not determined			9.08	190.0	2.225	3N	$\{N_{term}, 2N^{-}_{amide}\}$		
$[CuLH_{-2}]^{2-}$	10.0	540	251	10.1	620	-0.24				4N	$\{N_{term}, 3N^{-}_{amide}\}$	10.1	{T1 N_{term}, H2 N^{-}_{amide}, M3 N^{-}_{amide}, V4 N^{-}_{amide}, (E9 O^{-}_{carb}, D10 O^{-}_{carb},)}
					537	0.55							
					290.0	-1.62							
$[CuLH_{-3}]^{2-}$	10.8	530	262	11.06	620	-0.24	Not determined			4N	$\{N_{term}, 3N^{-}_{amide}\}$		
					537	0.36							
					290	-1.62							

Term-DEH	**UV-Vis**			**CD**			**EPR**					**NMR**	
GTAVMLKDEH	**pH**	**λ**	**ε**	**pH**	**λ**	**Δε**	**pH**	**A_{II}**	**g_{II}**	**Complex**	**donors**	**pH**	**Residue donors**
Term-DEH species													Term-DEH:Cu 1:0.02
							2.80-3.09	119.30	2.416	$Cu_{(aq)}$			
$[CuH_2L]^{2+}$	4.96	715	25	Not determined			5.13	140	2.320	1N	$\{N_{im}\}$	5.12	{ D8 O^-_{carb}, H10 $N_{im,}$}
$[CuHL]^{+}$	6.05	660	60	6.12	695	-0.07	5.97	160	2.290	2N	$\{N_{term}, N_{im}\}$	6.30	{G1 N_{term}, D8 O^-_{carb}, H10 $N_{im,}$ N^-_{amide}}
					320	0.25							
					270	-0.25							
[CuL]	7.44	625	96	8.00	680	-0.21	7.84-8.78	169	2.230	3N	$\{N_{term}, N_{im}, N^-_{amide}\}$	7.4	{G1 N_{term}, D8 O^-_{carb}, H10 $N_{im,}$ N^-_{amide}}
					320	0.62							
					270	-0.69							
$[CuLH_{-1}]^{-}$	Not determined			Not determined			Not determined			Not determined		9.0	{G1 N_{term}, D8 O^-_{carb}, E9 N^-_{amide}, H10 $N_{im,}$ N^-_{amide}}
$[CuLH_{-2}]^{2-}$	10.31	520	162	10.06	540	-1.06	10.07	204	2.185	4N	$\{N_{term} / N_{im}, 3N^-_{amide}\}$	10.1	{ G1 N_{term}, D8 $N^-_{amide,}$ E9 N^-_{amide}, H10 N^-_{amide}}
					310	1.1							
					275	-2.61							
					250	1.45							
$[CuLH_{-3}]^{3-}$	11.01	520	170	11.03	537	-1.11	10.97	204	2.185	4N	$\{N_{term} / N_{im}, 3N^-_{amide}\}$		
					310	1.14							
					275	-2.75							

Mid-DEH TMVLDEHAKG	UV-Vis pH	λ	ε	CD pH	λ	Δε	EPR pH	A_{II}	g_{II}	Complex	Donors	NMR pH	Residue donors
Mid-DEH species													Mid-DEH:Cu 1:0.02
							3.17-4.19	119.3	2.416	$Cu_{(aq)}$			
$[CuH_2L]^{2+}$	4.85	710	37	5.22	630	-0.13	5.14	151.9	2.323	1N	$\{N_{im}\}$	4.9	$\{D5O^-_{carb}, H7\ N_{im,}, G10\ O^-_{term\text{-}carb}\}$
					340	0.10							
					235	-2.34							
$[CuHL]^{+}$	5.34	655	62	5.22	630	-0.13				2N	$\{N_{term}, N_{im}\}$	5.5	$\{D5O^-_{carb}, H7\ N_{im,}, G10\ O^-_{term\text{-}carb}\}$
					340	0.10							
					235	-2.34							
				5.98	630	-0.37							
					335	0.24							
					240	-1.0							
[CuL]	7.56	617	150	6.47	610	-0.55	6.24	170	2.218	3N	$\{N_{term}, N_{im}, N^-_{amide}\}$	7.2	$\{T1\ N_{term},\ D5\ O^-_{carb}, H7\ N_{im,}\ N^-_{amide}\}$
					335	0.32							
					285	-0.17							
					245	-0.66							
				7.01	610	-0.56							
					335	0.32							
					285	-0.19							
				8.29	610	-0.56							
					335	0.33							
					290	-0.21							
					260	0.60							
$[CuLH_{-1}]^{-}$	9.00	590	139	9.1	615	-0.38	9.37	170	2.218	3N	$\{N_{im}, 2N^-_{amide}\}$	9.0	$\{D5\ N^-_{amide}, H7\ N_{im,}\ N^-_{amide}\}$
					325	0.12							
					293	-0.18							
					258	1.98							
$[CuLH_{-2}]^{2-}$	10.48	520	170	10.1	532	-0.64	10.54	204	2.172	4N	$\{N_{im}, 3N^-_{amide}\}$		
					350	-0.23							
					313	0.35							
					285	-0.60							
					260	2.05							
$[CuLH_{-3}]^{3-}$	11.00	520	178	11.04	530	-0.91	11.34	204	2.172	4N	$\{N_{im}, 3N^-_{amide}\}$		
					350	-0.19							
					312	0.57							
					280	-0.90							
					258	1.80							

Bis-DEH 1:1	**UV-Vis**			**CD**			**EPR**					**NMR**	
DEHGGGGDEH	**pH**	**λ**	**ε**	**pH**	**λ**	**Δε**	**pH**	**A_{II}**	**g_{II}**	**Complex**	**Donors**	**pH**	**Residue donors**
Bis-DEH species							3.18	119.3	2.416	$Cu_{(aq)}$			
$[CuH_2L]^{2+}$	4.1	728	21				4.11	161	2.315	1N	$\{N_{im}\}$	3.86-4.48	NA (severe broadening)
[CuL]	Not determined			Not determined			Not determined			Not determined			NA (severe broadening)
$[CuLH_{-1}]^{-}$	6.4-9.8	521	172	6.4-9.8	560	0.66	6.1-9.2	207	2.170	4N	$\{N_{term}, N_{im}, 2N^{-}_{amide}\}$	7.2	{H3 $N_{im,}$ N^{-}_{amide}, H10 N_{im}}
					490	-0.39						8	{D1 N_{term}, E2 N^{-}_{amide}, H3 $N_{im,}$ N^{-}_{amide}}
					310	-1.05						8.9	{D1 N_{term}, E2 N^{-}_{amide}, H3 $N_{im,}$ N^{-}_{amide}}
					270	2.34							
$[CuLH_{-2}]^{2-}$	10.3-11.0	521	172	10.3-11.0	560	0.66	11.30	207	2.170	4N	$\{N_{term}, N_{im}, 2N^{-}_{amide}\}$	10.5	{D1 N_{term}, E2 N^{-}_{amide}, H3 $N_{im,}$ N^{-}_{amide}}
					490	-0.39							
					310	-1.05							
					270	2.34							

Bis-DEH 2:1 DEHGGGGDEH	UV-Vis			CD			EPR					NMR	
	pH	λ	ε	pH	λ	Δε	pH	A_{II}	g_{II}	Complex	Donors	pH	Residuedonors
Bis-DEH species													
$[CuH_2L]^{2+}$	Not determined			Not determined			Not determined			Not determined		Not determined	
$[CuL]$	Not determined			Not determined			Not determined			Not determined		Not determined	
$[CuLH_{-1}]^{-}$	Not determined			Not determined			Not determined			Not determined		Not determined	
$[CuL_2H_{-2}]^{2-}$	7.06	690	64	7.06	575	-0.29	7.06	207	2.170	4N	$\{N_{term}, N_{im}, 2N^{-}_{amide}\}$	Not determined	
		525	140		494	0.26							
					310	0.39							
					273	-0.97							
$[CuL_2H_{-3}]^{3-}$	8.00	540	180	8.00	592	-0.44	7.06	207	2.170	4N	$\{N_{term}, N_{im}, 2N^{-}_{amide}\}$	Not determined	
					502	0.41							
					308	0.37							
					271	-0.19							
					247	0.76							
$[CuL_2H_{-4}]^{4-}$	10.21	553	230	10.21	608	-0.62	Not determined			Not determined		Not determined	
					509	0.65							
					308	0.42							
					268	1.20							
$[CuL_2H_{-5}]^{5-}$	Not determined			Not determined			Not determined			Not determined		Not determined	

Table S3. Coordination mode for the Mn(II):peptide species.

Peptide	Species	Residue donor
DP1	$[MnH_3L]^{3+}$	{D1 O^-_{carb}, E2 O^-_{carb}, K10 $O^-_{term\text{-}carb}$}
DEHGTAVMLK	$[MnH_2L]^{2+}$	{D1 O^-_{carb}, E2 O^-_{carb}, H3 N_{im}}
	$[MnHL]^{+}$	{D1 N_{term}, D1 O^-_{carb}, E2 O^-_{carb}, H3 N_{im}}
	[MnL]	{D1 N_{term}, D1 O^-_{carb}, E2 O^-_{carb}, H3 N_{im}}
DP2	$[MnH_3L]^{3+}$	{E9 O^-_{carb}, D10 O^-_{carb} $O^-_{term\text{-}carb}$}
THMVLAKGED	$[MnH_2L]^{2+}$	{H2 N_{im},E9 O^-_{carb}, D10 O^-_{carb}}
	$[MnHL]^{+}$	{T1 N_{term}, H2 N_{im},E9 O^-_{carb}, D10 O^-_{carb} $O^-_{term\text{-}carb}$ }
	[MnL]	{T1 N_{term}, H2 N_{im},E9 O^-_{carb}, D10 O^-_{carb} $O^-_{term\text{-}carb}$ }
Term-DEH	$[MnH_3L]^{3+}$	{D8 O^-_{carb}, E9 O^-_{carb}, H10 $O^-_{term\text{-}carb}$}
GTAVMLKDEH	$[MnH_2L]^{2+}$	{D8 O^-_{carb}, E9 O^-_{carb}, H10 N_{im} $O^-_{term\text{-}carb}$}
	$[MnHL]^{+}$	{G1 N_{term}, D8 O^-_{carb}, E9 O^-_{carb}, H10 N_{im} $O^-_{term\text{-}carb}$}
Mid-DEH	$[MnH_3L]^{3+}$	{D5 O^-_{carb}, E6 O^-_{carb}, H7 N_{im}, G10 $O^-_{term\text{-}carb}$}
TMVLDEHAKG	$[MnH_2L]^{2+}$	{D5 O^-_{carb}, E6 O^-_{carb}, H7 N_{im}, G10 $O^-_{term\text{-}carb}$}
	$[MnHL]^{+}$	{T1 N_{term}, D5 O^-_{carb}, E6 O^-_{carb}, H7 N_{im}, G10 $O^-_{term\text{-}carb}$}
	[MnL]	{T1 N_{term}, D5 O^-_{carb}, E6 O^-_{carb}, H7 N_{im}, G10 $O^-_{term\text{-}carb}$}
Bis-DEH	$[MnH_3L]^{+}$	{D8 O^-_{carb}, E9 O^-_{carb}, H10 $O^-_{term\text{-}carb}$}
DEHGGGGDEH	$[MnH_2L]$	{D8 O^-_{carb}, E9 O^-_{carb}, H10 N_{im} $O^-_{term\text{-}carb}$}
	$[MnHL]^{-}$	{D1/8 O^-_{carb}, E2/9 O^-_{carb}, H3/10 N_{im}}
	$[MnL]^{2-}$	{ D1 N_{term}, D1/8 O^-_{carb}, E2/9 O^-_{carb}, H3/10 N_{im}}