Supporting Information

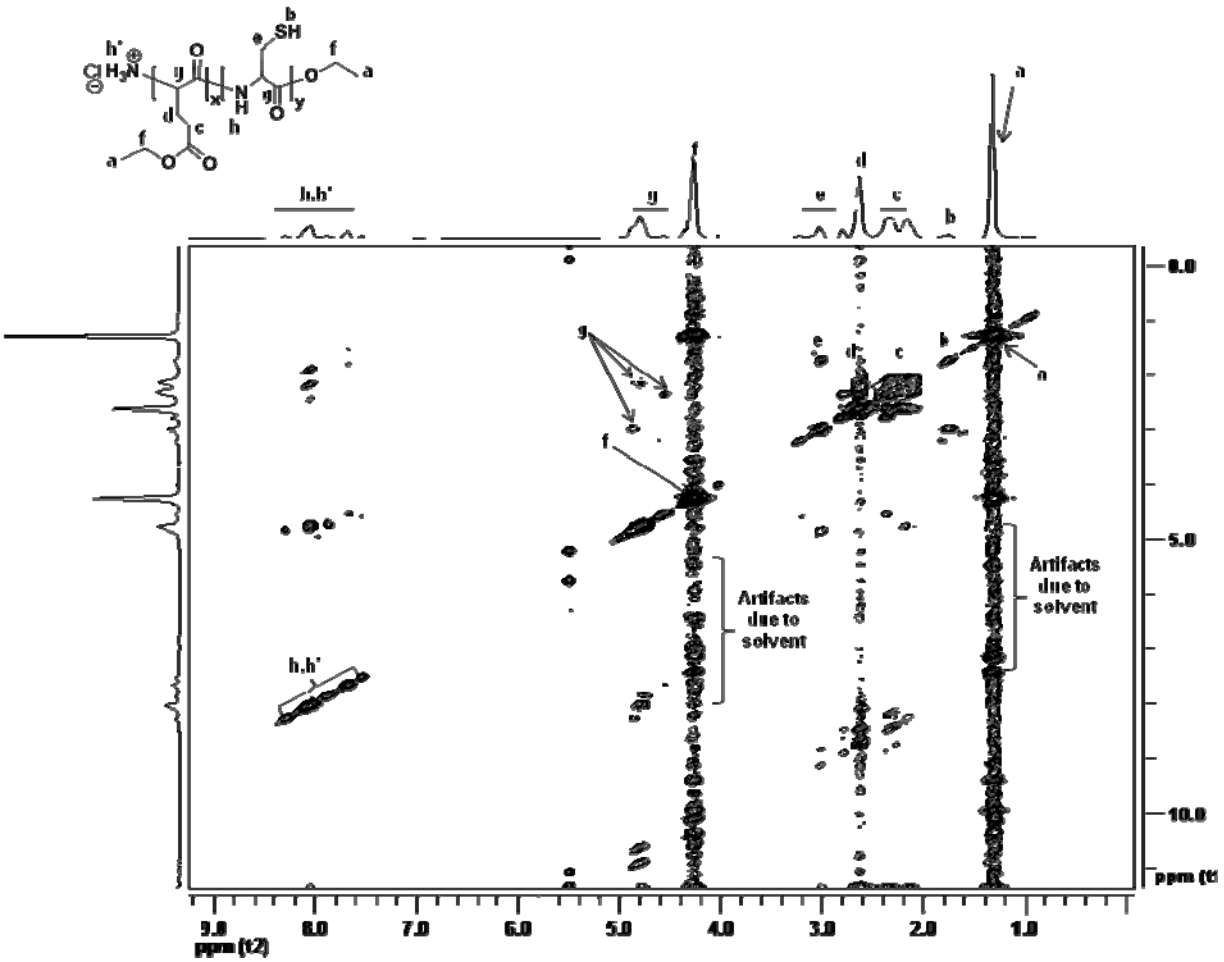


Figure S1. 2D (^{1}H-^{1}H) COSY90 NMR (300 MHz, 3:1 TFA:TFA-*d*) spectrum of oligo(L-Glu-co-25%L-Cys) synthesized using from 7:3 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h.

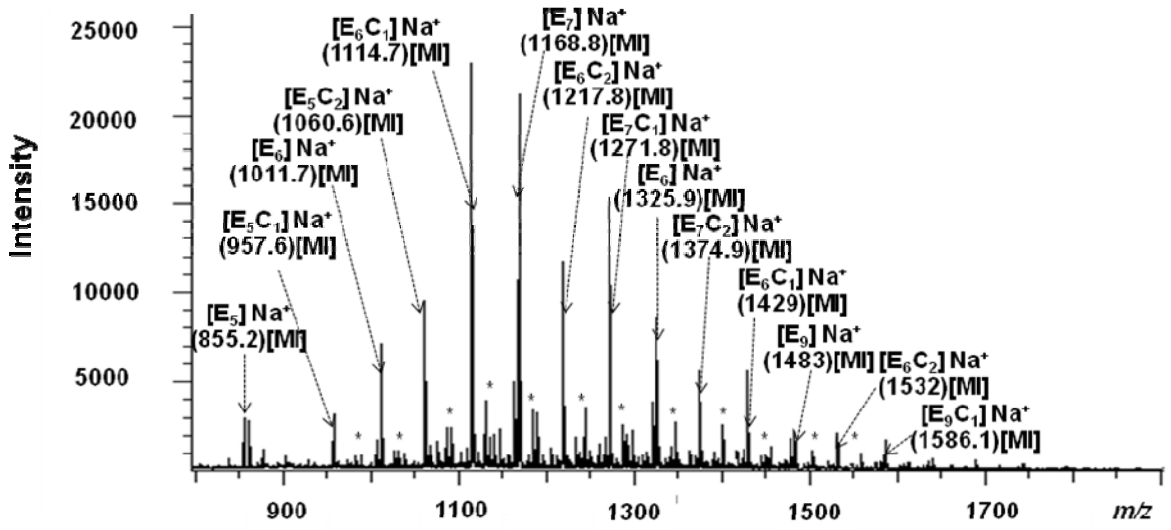


Figure S2. MALDI-TOF spectra of products consisting of oligo(γ-L-Et-Glu-*co*-L-Cys)[E_xC_y] where 'x' and 'y' represents the number of repeat units, synthesized from 7:3 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h. Peaks corresponding to oligopeptides with one de-esterified L-Glu unit are designated by an asterisk [*]. The m/z values observed are ±1 Da of those expected for the molecular ion peaks.

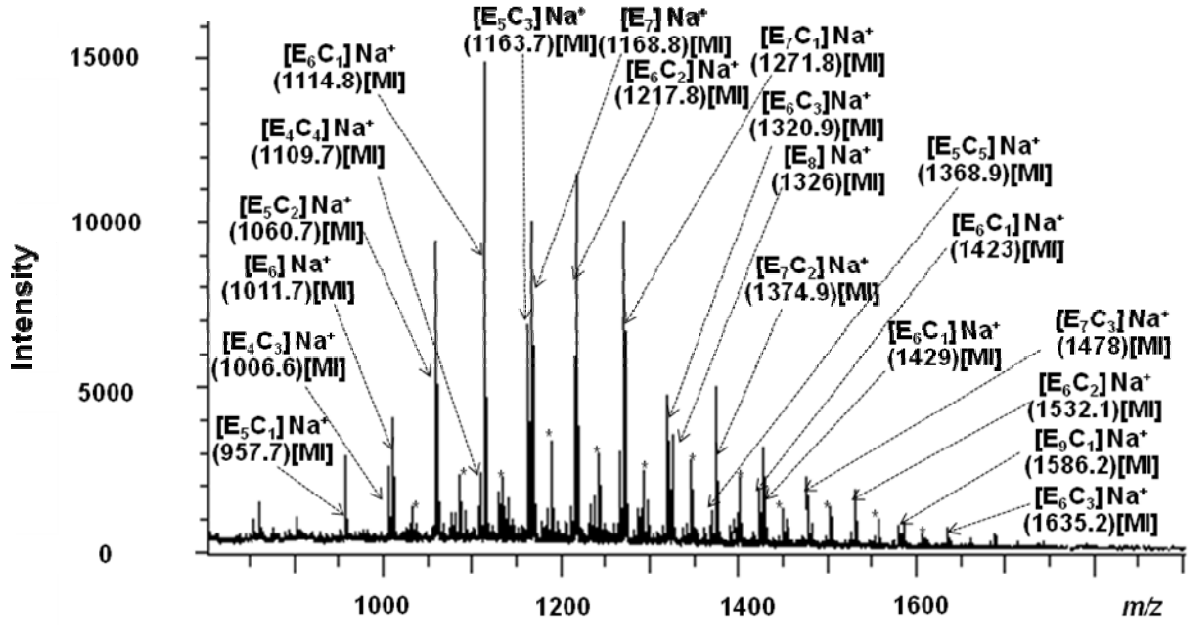


Figure S3. MALDI-TOF spectra of products consisting of oligo(γ-L-Et-Glu-*co*-L-Cys)[E_xC_y] where 'x' and 'y' represents the number of repeat units, synthesized from 6:4 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h. Peaks corresponding to oligopeptides with one de-esterified L-Glu unit are designated by an asterisk [*]. The m/z values observed are ±1 Da of those expected for the molecular ion peaks.

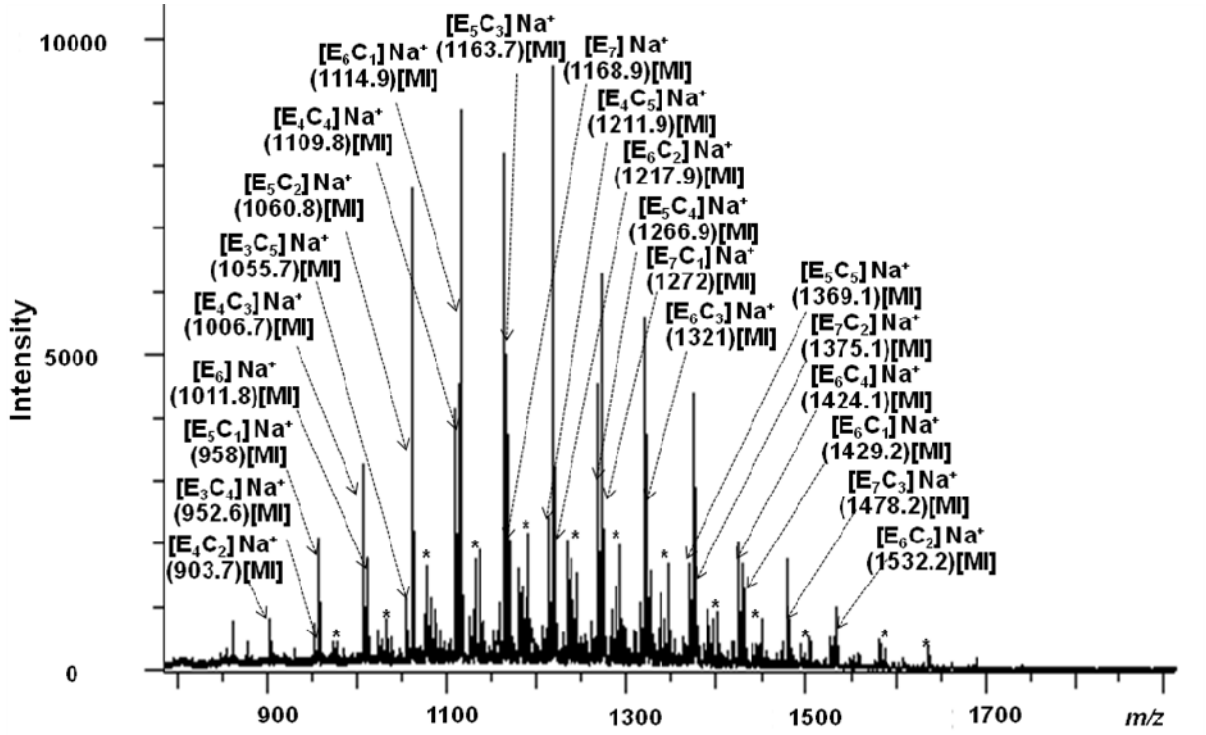


Figure S4. MALDI-TOF spectra of products consisting of oligo(γ-L-Et-Glu-*co*-L-Cys)[E_xC_y] where 'x' and 'y' represents the number of repeat units, synthesized from 5:5 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h. Peaks corresponding to oligopeptides with one de-esterified L-Glu unit are designated by an asterisk [*]. The m/z values observed are ±1 Da of those expected for the molecular ion peaks.

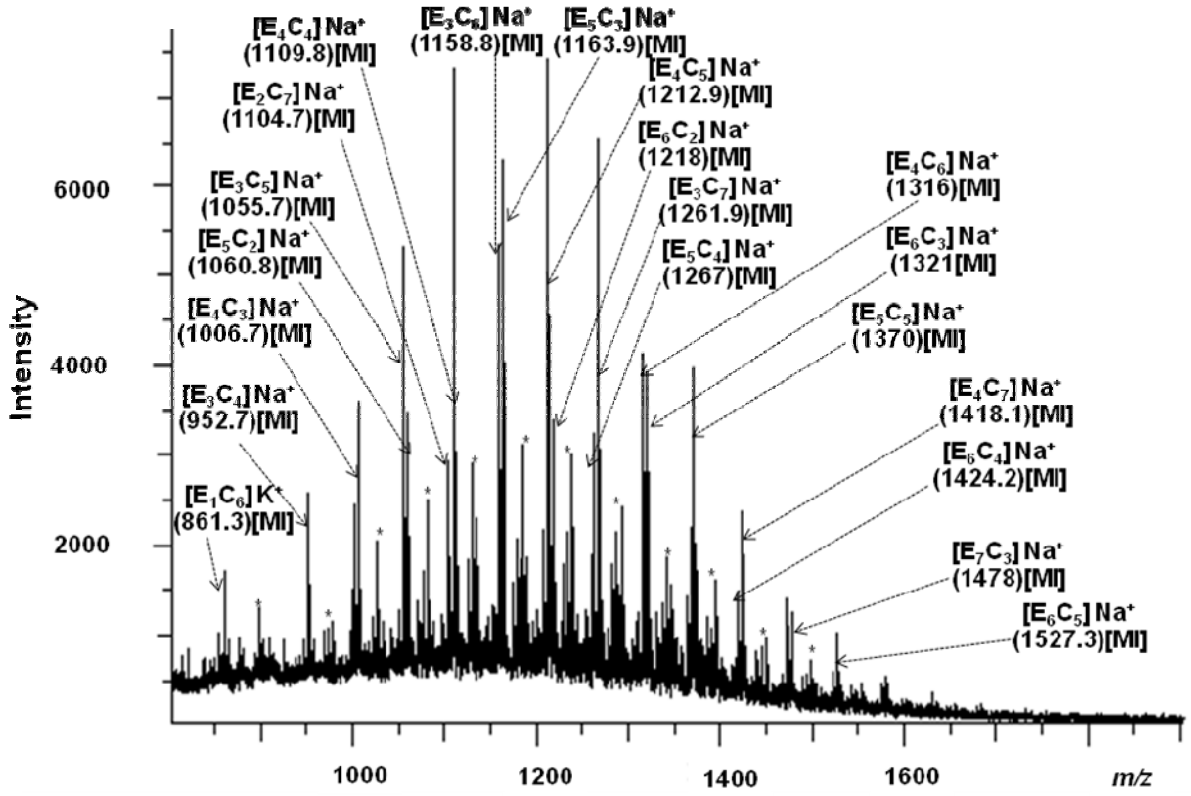


Figure S5. MALDI-TOF spectra of products consisting of oligo(γ-L-Et-Glu-*co*-L-Cys)[E_xC_y] where 'x' and 'y' represents the number of repeat units, synthesized from 3:7 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h. Peaks corresponding to oligopeptides with one de-esterified L-Glu unit are designated by an asterisk [*]. The m/z values observed are ±1 Da of those expected for the molecular ion peaks.

Note: We were unsuccessful in attempts to record a MALDI-TOF spectrum of oligo(L-Cys).

MALDI-TOF spectrum peak validations

The following procedure was followed to authenticate peak assignments for MALDI-TOF spectra using the isotope pattern calculator (IPC) program available from the website **OMNICS.PNL.GOV**.

All peaks were assigned based on the theoretical masses calculated for co-oligomers of $[Glu_x\text{-}Cys_y]_n$ (where n = x+y; x = 0 to 12, y = 0 to 12) using MS-EXCEL 2007. Monoisotopic masses were calculated for the molecular ion (MI) and de-esterified (DE) peaks with adducts of H^+, Na^+ and K^+. The masses of corresponding intra disulfide linked peptides and *m/z* corresponding to doubly charged species were also calculated. Some peaks have more than one candidate of peptide (overlap sequence). To find which peptide was observed, the isotopic abundance was

calculated using X-massOMNIFLEX6.0.0 and compared with the experimentally observed abundance.

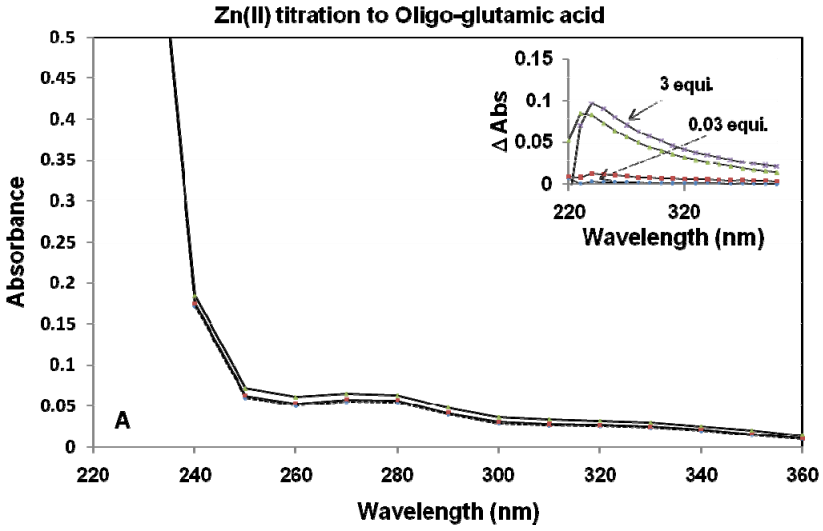
Two examples are shown in Table S1 for oligo(γ-L-Et-Glu-*co*-L-Cys)[E_xC_y], prepared using papain as catalyst from a 7:3 L-$(Et)_2$-Glu:L-Et-Cys monomer feed ratio. Comparison of the abundance for the peak at 1325.95 correlates best to the abundance calculated for [MI] corresponding to $E_8 + Na^+$. Likewise, the abundance for the peak at 1168.84 correlates well with the abundance calculated for [MI] corresponding to $E_7 + Na^+$. Experimental peaks obtained for each of the feed ratios studied in this paper were further authenticated by comparing the isotopic abundance of the experimental peaks with the abundance generated by the IPC program.

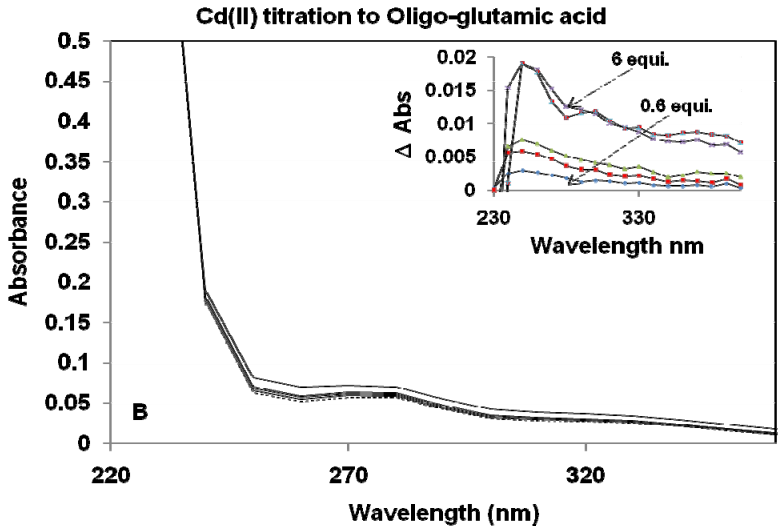
Table S1. Best fit obtained for a given set of isotopically resolved peaks which have possible contributions from two different Glu_x-Cys_y oligopeptide sequences. The oligopeptide was synthesized from 7:3 L-Glu-$(Et)_2$:L-Cys-Et, 0.5 M total substrate concentration, 16 units/mL papain, 0.9 M phosphate buffer (pH 8.0) at 40 °C for 3h. Values of isotopic abundance for a given molecular ion totals 100%

integral mono-isotopic mass	exp. mass	overlap sequences	isotopic abundance (IPC program)		isotopic abundance (Experimental)
			(a)	(b)	
1326(a) 1325(b)	1325.95	(a)E_2C_9[MI]+ K^+ (b)E_8[MI] + Na^+	[34,20,23,11,7]	[48,33,13,4,1]	46,33,13,5,2
1169(a) 1168(b)	1168.84	(a)C_9E_1[MI] + K^+ (b)E_7[MI] + Na^+	[37,19,23,10,7]	[53,31,12,3]	51,33,12,4

Determination of thiol group content for metal binding assay: A previously reported protocol was adapted.[1] In summary, 100 mM Tris•HCl (pH 7.4), 100 mM sodium phosphate buffer (pH 7) and 100 mM sodium phosphate buffer (pH 8) were prepared. Nitrogen was bubbled through these solutions for 60 min to maintain anaerobic conditions for the final assay. To prepare *solution A*, 39.6 mg of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was dissolved into 10 mL of sodium phosphate buffer (100 mM, pH 7). *Solution B* consists of 0.025 mg/mL oligo(L-Glu-co-47%L-Cys) in Tris•HCl buffer (100 mM, pH 7.41). *Solution C* was then prepared by transferring 3 mL *solution B*, 2 mL sodium phosphate buffer (100 mM, pH 8) and 5 mL DI water to a screw cap glass vial fitted with a septum and vortexing for 2 min. Finally, 3 mL of *solution C* was transferred to a quartz cuvette followed by addition of 20 µL of *solution A*. Absorbance was measured (412 nm) against a blank (3 mL of 100 mM Tris•HCl buffer, pH 7.4) after 3 hours. Similarly a literature protocol was adopted that uses the trinitrobenzene sulfonic acid (TNBS)[2] assay to determine the concentration of fully water soluble oligoglutamic acid.

UV-Vis spectroscopy: Analogous experiments were conducted with oligo(L-Glu) synthesized by papain catalysis. The peptide concentration was measured using the TNBS assay. A 40 µM solution of oligo(L-Glu) was titrated with increasing quantities of the metal ions Co(II), Ni(II), Cd(II) and Zn(II). Inspection of Figure S6(a-d) shows there is little change in absorption due to metal binding to oligo(L-Glu) in for $[M]_{total}/[P]_{total}$ from 0.6 to 4 equivalents. However, careful inspection of titration curves shows small changes in difference spectra. Most apparent are plots of ΔA as a function of wavelength for titrations of oligo(L-Glu) with Zn(II) and Cd(II). Specifically, ΔA values up to 0.1 at $[M]_{total}/[P]_{total} = 3$ and 0.02 at $[M]_{total}/[P]_{total} = 6$ were measured for Zn(II) and Cd(II), respectively. At $[M]_{total}/[P]_{total}$ ratios up to 1, there is no significant change in the UV-visible spectra for Zn(II), Cd(II), and Ni(II). The change for Co(II) is due to the absorption by the metal ion (Figure S7(c)). Thus, in the metal concentration regions of interest explored for oligo(L-Glu-co-47%L-Cys), absorption due to binding of metals by oligo(L-Glu) can be taken as spectroscopically silent.





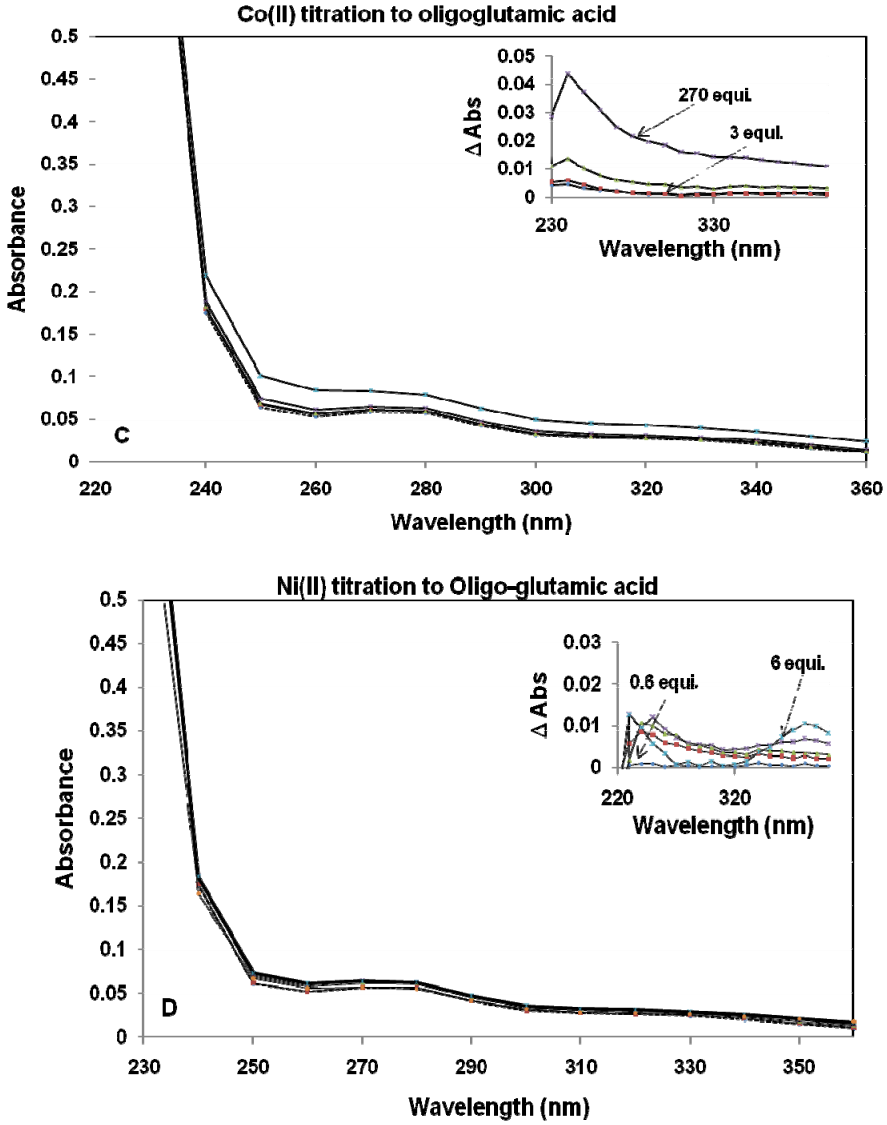


Figure S6. Absorption spectra of oligo(L-Glu) (synthesized by papain catalysis) titrations with A) Zn(II), B) Cd(II), C) Co(II) and D) Ni(II). Titrations were conducted at room temperature, in 100 mM Tris-HCl buffer at pH 7.4, with peptide concentration of 40 μM (peptide concentration determined by **TNBS** assay) and monitored by UV-visible spectroscopy. Spectra were recorded 20 min. after metal ion addition. The inset shows the difference absorption spectra obtained by subtracting the absorbance contribution of oligo(L-Glu).

To estimate the contribution of metal ions, the absorption spectrum was obtained by titrating a Tris buffer solution with a solution of a metal ion at varying metal ion concentrations. The results, displayed in Figure S7(A-D), show a small but regularly increasing absorbance at 250 nm for titrations with Co(II), from about 0.02 at 3 equivalents to ~0.12 at 300 equivalents. However, the other metals did not show any significant absorption.

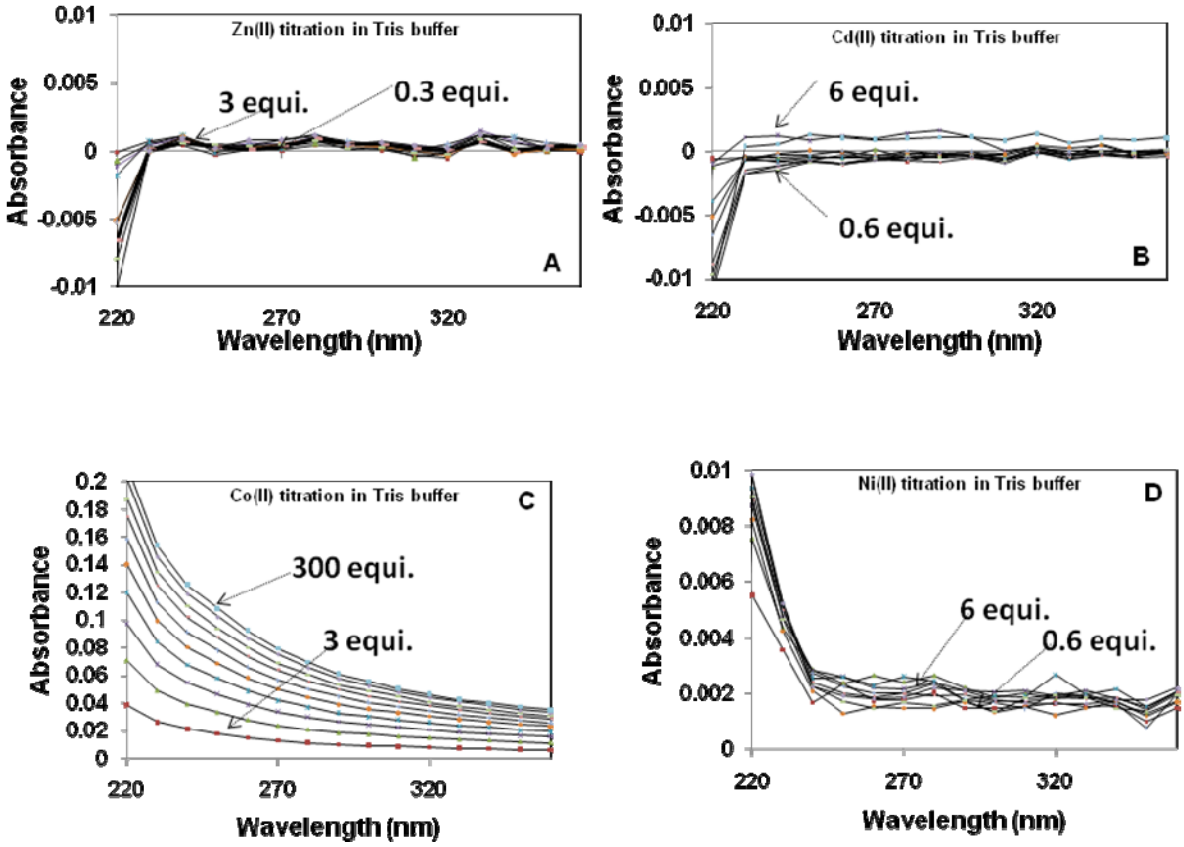


Figure S7. Absorption spectra of oligo(L-Glu) (synthesized by papain catalysis) titrations with A) Zn(II), B) Cd(II), C) Co(II) and D) Ni(II). Titrations were conducted at room temperature, in 100 mM Tris-HCl buffer at pH 7.4, with peptide concentration of 40 μM (peptide concentration determined by **TNBS** assay) and monitored by UV-visible spectroscopy. Spectra were recorded 20 min. after metal ion addition.

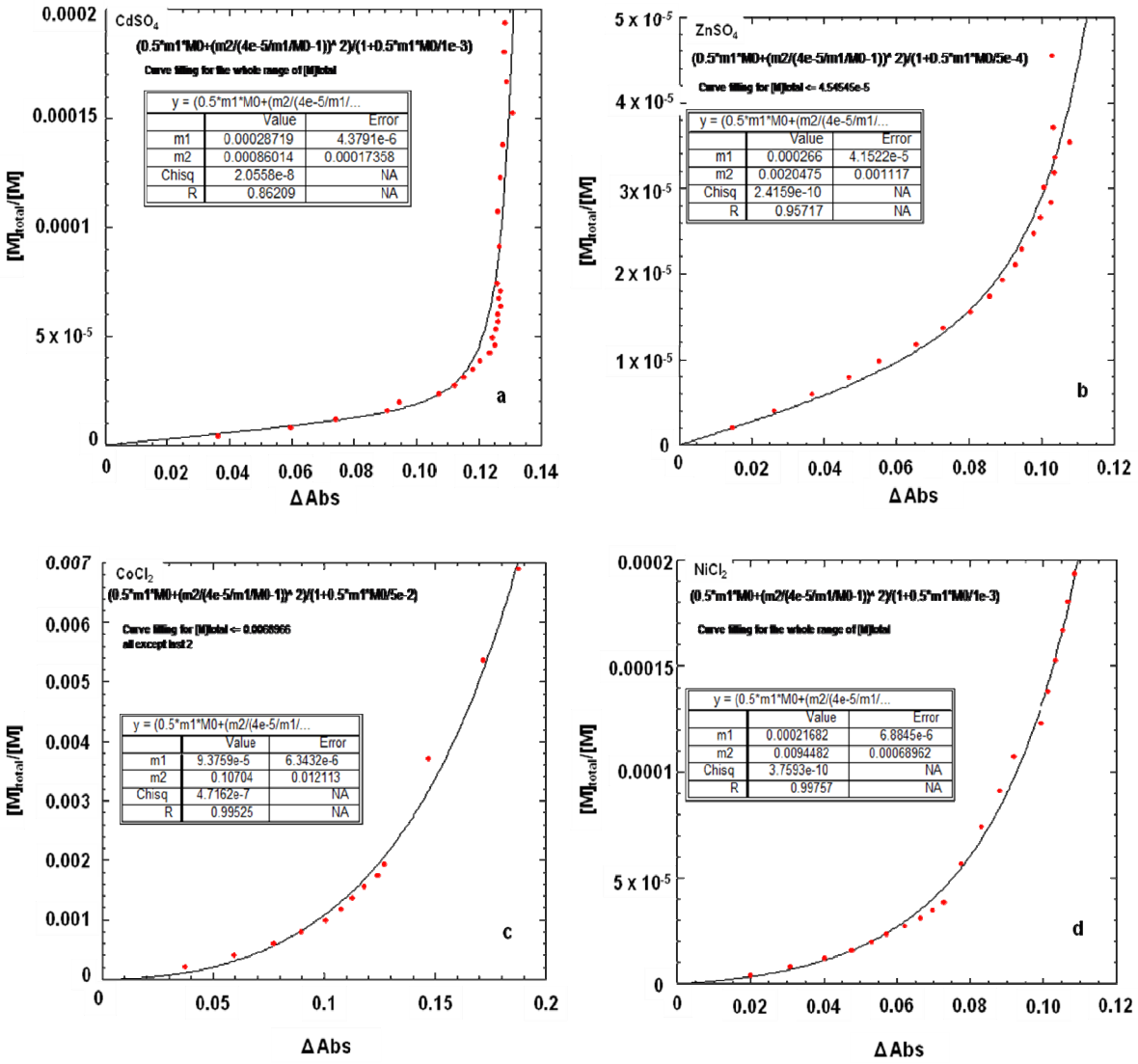


Figure S8. Curve fits (KaleidaGraph) of experimental data from metal binding experiments. Equation 13 with eq 7 was used for the fitting. The inset gives fitting parameters m1 = K_d, m2 = $b(\varepsilon_{PM}-\varepsilon_P)$ or $b(\varepsilon_{PM}-\varepsilon_P-n\varepsilon_M)$.

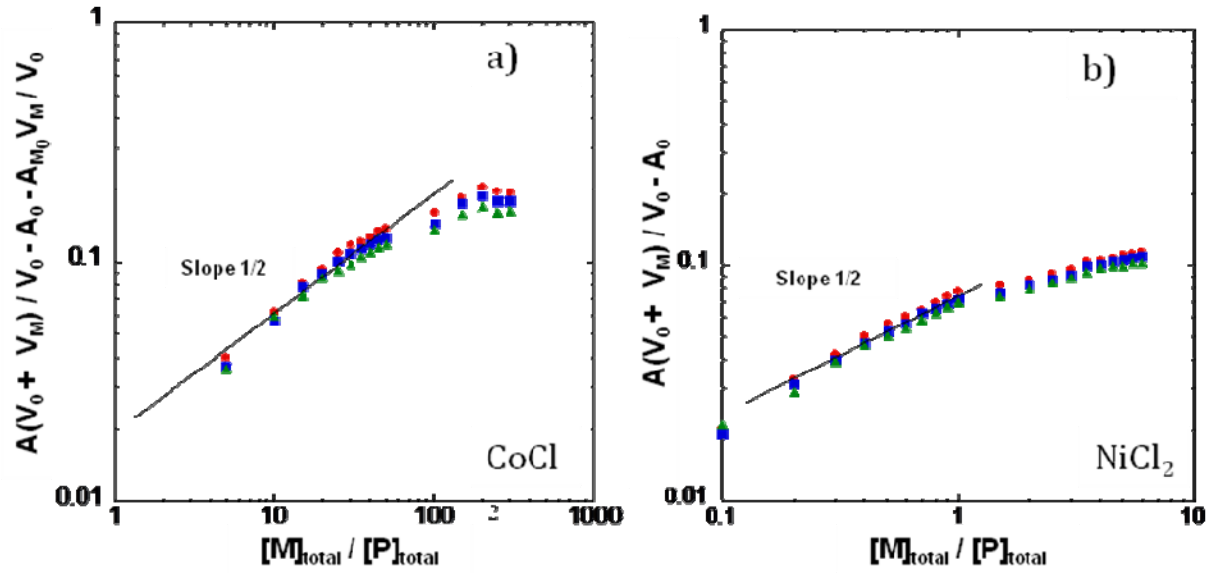


Figure S9. Double log plots for metal ion titrations of oligo(L-Glu-*co*-47%L-Cys) as monitored by the change in the absorbance at 260 nm for (a) $CoCl_2$,, and (b) $NiCl_2$. Titrations were conducted at room temperature and pH 7.41 in 100 mM Tris-HCl. The *x* coordinate is the ratio of the metal ion concentration to the total peptide concentration. The *y* coordinate is the difference absorption value after subtracting the contribution of free peptide and free Metal ion solution. The straight line has a slope of 1/2.

CD spectropolarimetry Experiments analogous to those conducted on EC peptide were performed with oligo(L-Glu). Figure S10 (--) depicts CD spectra of oligo(L-Glu) (100 μM) titrated with saturating concentrations of different metal ions similar to the concentrations used for EC peptide. The spectra in Figure S10 (a-d) do not show any appreciable change due to metal binding.

The mean residue ellipticity ($deg \cdot cm^2 \cdot dmol^{-1}$) on the y axis was calculated for oligo(L-Glu), using values for average molecular weight, average chain length, and mean residue weight (MRW) of 1050.9 g/mol, 8 and 150.12 g/mol, respectively. For the EC peptide, values used for average molecular weight, average chain length, and mean residue weight (MRW) are 1076.2 g/mol, 9 and 134.52 g/mol, respectively. The following equation was used to calculate the mean residue ellipticity.

$$[\theta] = \frac{\theta_{deg} \cdot \mathrm{MRW}}{l \cdot C_{MR}}$$

where C_{MR} = concentration in mg/mL, θ_{deg} = CD machine units in degrees and l = path length of the cell in mm. The mean residue ellipticity (an average of three experiments) was plotted to determine if conformational change are observed on titrating oligo(L-Glu) with different metal ion solutions.[3-5]

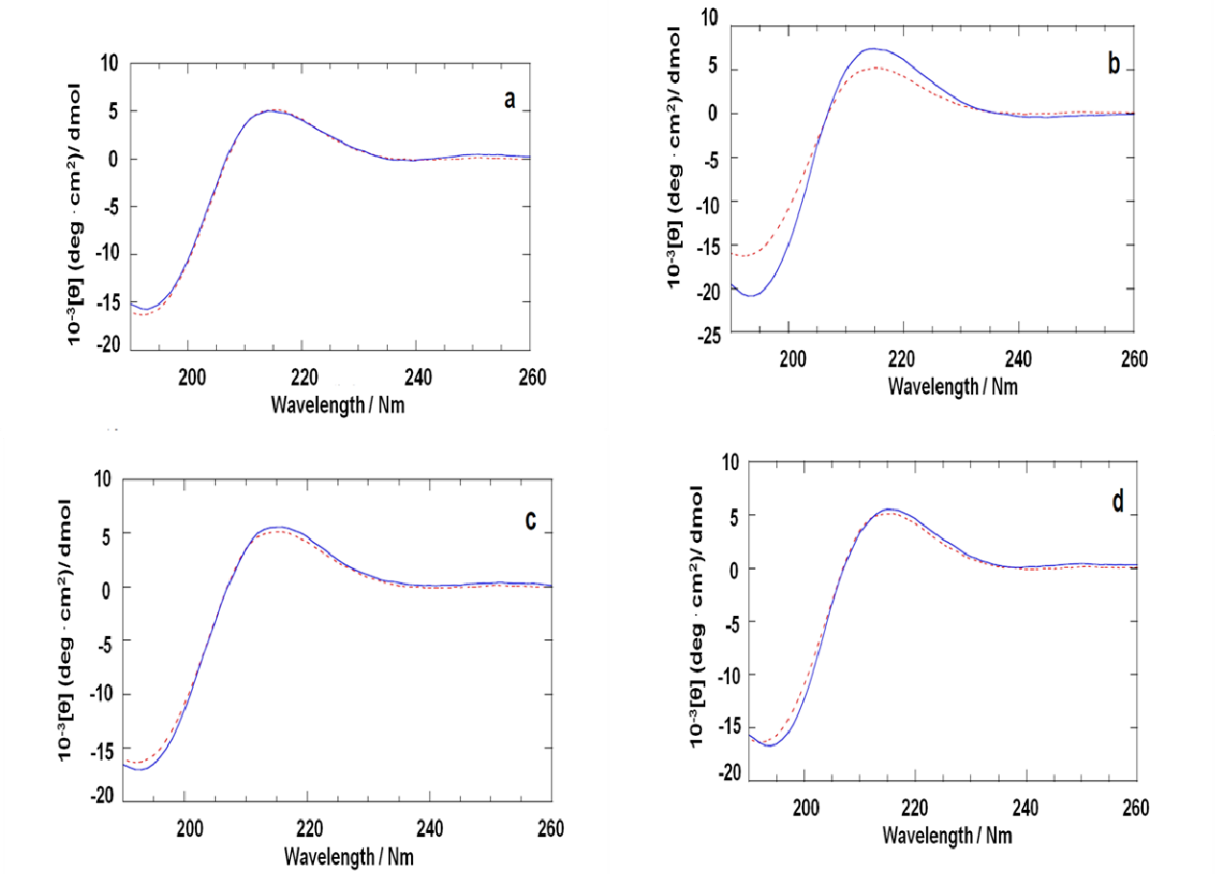


Figure S10. The far UV-CD spectra of oligo-glutamic acid, Cd(II), Zn(II), Co(II) and Ni(II) complexes shown in a-d, respectively, were obtained in 10 mM Tris-HCl buffer (pH 7.41) at room temperature. The total peptide concentration is 100 µM and the concentration of Cd(II), Zn(II), Co(II) and Ni(II) are 400, 400, 1600 and 800 µM respectively. The CD spectra of oligo-glutamic acid is designated as dashed (-----) lines. The CD spectra of metal complexes are designated as solid (——) lines.

References

1. Riddles, P. W.; Blakeley, R. L.; Zerner, B. *Analytical Biochemistry* **1979,** 94, 75-81.
2. Requena, J. R. F., M. X.; Ahmed, M. U.; Jenkins, A. J.; Lyons, T. J.; Baynes, J.W. *Biochemistry Journal* **1997,** 322, 317-325.
3. Greenfield, N. J.*Nat. Protocols* **2007,** 1, 2876-2890.
4. Greenfield, N. J.; Fasman, G. D. *Biochemistry* **1969,** 8, 4108-4116.
5. Savitzky, A.; Golay, M. J. E. *Anal. Chem.* **1964,** 36, 1627-1639.