Comparison of Cysteine and Penicillamine
Ligands in a Co(II) Maquette

<u>Supplimentary Material</u>

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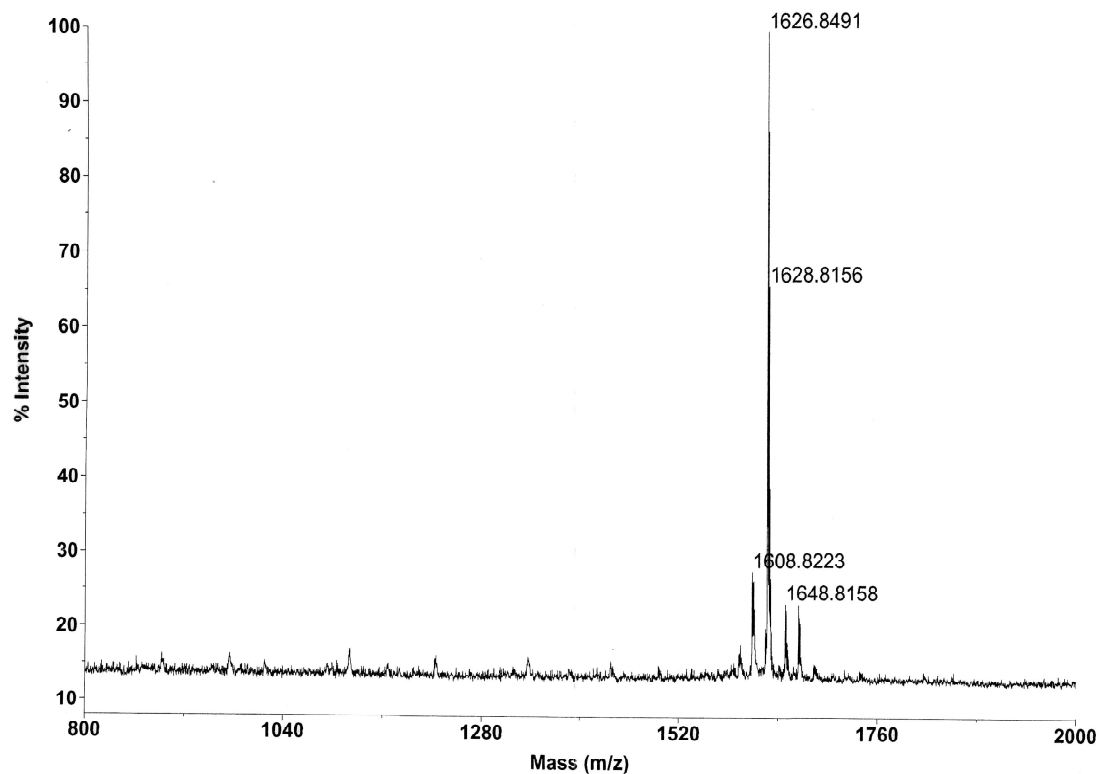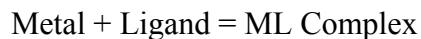[2]Department of Chemistry, University of New Mexico, Albuquerque, NM 87131

Figure S1. MALDI mass spectrum of the ligand **IGA-Pen**. Calculated mass: 1626.8 (M+2H)$^+$; expected mass 1626.9 amu.

Derivation of Fitting Equation.

For the general reaction:

$$\text{Metal} + \text{Ligand} = \text{ML Complex}$$

the dissociation constant, $K_d = ([M_{free}] * [\text{Ligand}]_{free})/[ML]$

Definitions

$[M]_{free}$ = concentration of free metal
$[M]_{bound}$ = concentration of metal bound to ligand
$[M]_{total}$ = total concentration of metal (variable) = $[M]_{free} + [M]_{bound}$

$[\text{Ligand}]_{free}$ = concentration of free ligand
$[\text{Ligand}]_{bound}$ = concentration of ligand bound to metal
$[\text{Ligand}]_{total}$ = total concentration of ligand (constant) = $[\text{Ligand}]_{free} + [\text{Ligand}]_{bound}$

$[ML]$ = concentration of Metal-Ligand Complex = $[\text{Ligand}_{bound}]$ = $[M_{bound}]$

ε_{bound} = Extinction coefficient of the metal-ligand complex
ε_{free} = Extinction coefficient of free metal
l = path length in cm

Absorbance = $\varepsilon_{bound}*l*[ML] + \varepsilon_{free}*l* [M]_{free} = \varepsilon_{bound}*l*[ML] + \varepsilon_{free}*l* ([M_{total}] - [ML])$

Take the K_d in terms of total [ligand]/[metal], and solve for [ML],

$K_d = ([M_{free}] * [\text{Ligand}]_{free})/[ML]$
$K_d = ([M]_{total} - [ML]) * ([\text{Ligand}]_{total} - [ML])/[ML]$

Multiply thorough by [ML] and expand terms

$K_d*[ML] = ([M]_{total} - [ML]) * ([\text{Ligand}]_{total} - [ML])$

$K_d*[ML] = ([M]_{total} *[\text{Ligand}]_{total} - [ML]([\text{Ligand}]_{total}) - [ML]* (M_{total}) + [ML]*[ML])$

Set equation to zero

$0 = -K_d*[ML] + [M]_{total} *[\text{Ligand}]_{total} - [ML]*[\text{Ligand}]_{total} - [ML]* [M]_{total} + [ML]*[ML]$

Solve the Quadratic,

$a=1$

$b= -K_d - [Ligand]_{total} - [M]_{total}$

$c= [M]_{total} * [Ligand]_{total}$

Thus, the concentration of the metal-ligand complex is solved for in terms of total ligand concentration (constant) and total metal concentration (variable).

Equation 1

$[ML]=$

$$\frac{(K_d+[Ligand]_{total}+[M]_{total}) + \sqrt{((K_d+[Ligand]_{total}+[M]_{total})^2-(4*1*[M]_{total}*[Ligand]_{total}))}}{2*1}$$

Substitution of *equation 1* into the absorbance equation below yields *equation 2* and allows for the calculation of the absorbance at each titration point and evaluation of the dissociation constant.

$$Abs= \varepsilon_{bound}*l*[ML] + \varepsilon_{free}*l*([M]_{total}- [ML])$$

Plot Abs (y-axis) vs. $[M]_{total}/[Ligand]_{total}$ (x-axis), thus $[M]_{total} = x*[Ligand]_{total}$

Equation 2

Abs (y)= $\varepsilon_{bound}*l*(0.5)(x*[Ligand]_{total} + K_d+[Ligand]_{total} -sqrt\{(x*[Ligand]_{total} + K_d+[Ligand]_{total})^2-4x*[Ligand]_{total}*[Ligand]_{total}\} + \varepsilon_{free}*l*(x*[Ligand]_{total}) - \varepsilon_{free}* 0.5(x*[Ligand]_{total} + K_d+[Ligand]_{total}) -sqrt\{(x*[Ligand]_{total} + K_d+[Ligand]_{total})^2-4x* [Ligand]_{total}*[Ligand]_{total}\}$

For Co(II) titrations into peptide, the value of ε_{free} is zero. In Kaleidagraph, the exact form of *equation 2* used to fit the **IGA** data in Figure 3 is, as follows:

m2*10*(0.5*(((m0*5e-6)+m1+5e-6)-(((m0*5e-6)+m1+5e-6)^2-4*5e-6*(m0*5e-6))^0.5));m1=1e-6; m2=1800; m3=0.0

where   m0 = x-axis value of each [Co(II)]/[Binding site]
                           m1 = K_d
                           m2 = ε_{bound} (comparison to known ε_{bound} provides an internal check of quality of
fit)

The value of 5e-06 is the concentration of Co(II) binding sites in molar and is equal to the peptide concentration because **IGA** has one Co(II) binding site per peptide.

The factor of 10 after m2 is the path length of the cuvette.

For the **IGA-Pen** data in Figure 3, the concentration of binding sites was 6.65e-06 molar, since each peptide (5µM peptide) contains 1.33 potential Co(II) binding sites (6.65µM binding sites).

Figure S2. Sedimentation equilibrium analytical ultracentrifugation of Co(II)-**IGA** (A) and

Co(II)-**IGA-Pen** (B). Both samples were equilibrated for 24 hrs at 50,000 RPM in a Beckman

XL-I ultracentrifuge at 20°C. The initial loading concentrations of the metallopeptides were 80-

95 µM in 20mM HEPES, 100 mM KCl, pH 7.5. Data reduction and analysis was performed

using WinNonlin, WinReedit, WinMatch and Sednterp. The radial distribution absorbance scan

data at 340 nm (CT band) were fit to a single exponential using WinNonlin The partial specific

volumes ($\overline{\upsilon}$) for the metallopeptides were calculated to be 0.55 using the method of Edelstein

and Schachman (Methods in Enzymol. **1973**, 27, 82-98). The bouyant molecular weight, M_b,

was converted to the average molecular weight of the molecular species in solution, M_r, with the

following relationship:

$$M_b = M_r (1 - \overline{\upsilon} \rho)$$

The Co(II)-**IGA** and Co(II)-**IGA-Pen** complexes sediment as single homogenous species with

molecular weights of 1900 and 2200 amu, respectively. These values are within experimental

error of the calculated molecular weights for the monomer complexes, 1587 amu and 1699 amu,

and demonstrate that these complexes are monomeric.

A

Absorbance @ 340nm vs (SQUARE OF RADIUS(CM))/2

B

Absorbance @ 340nm vs (SQUARE OF RADIUS(CM))/2
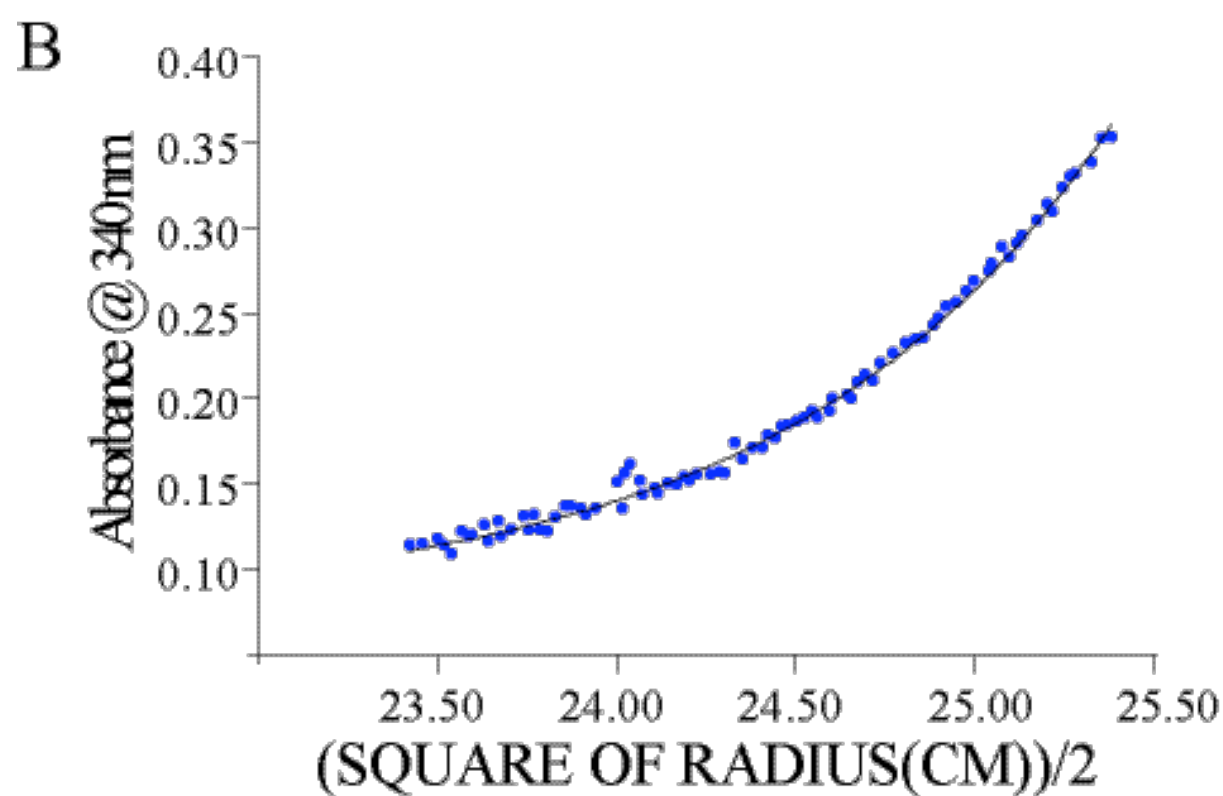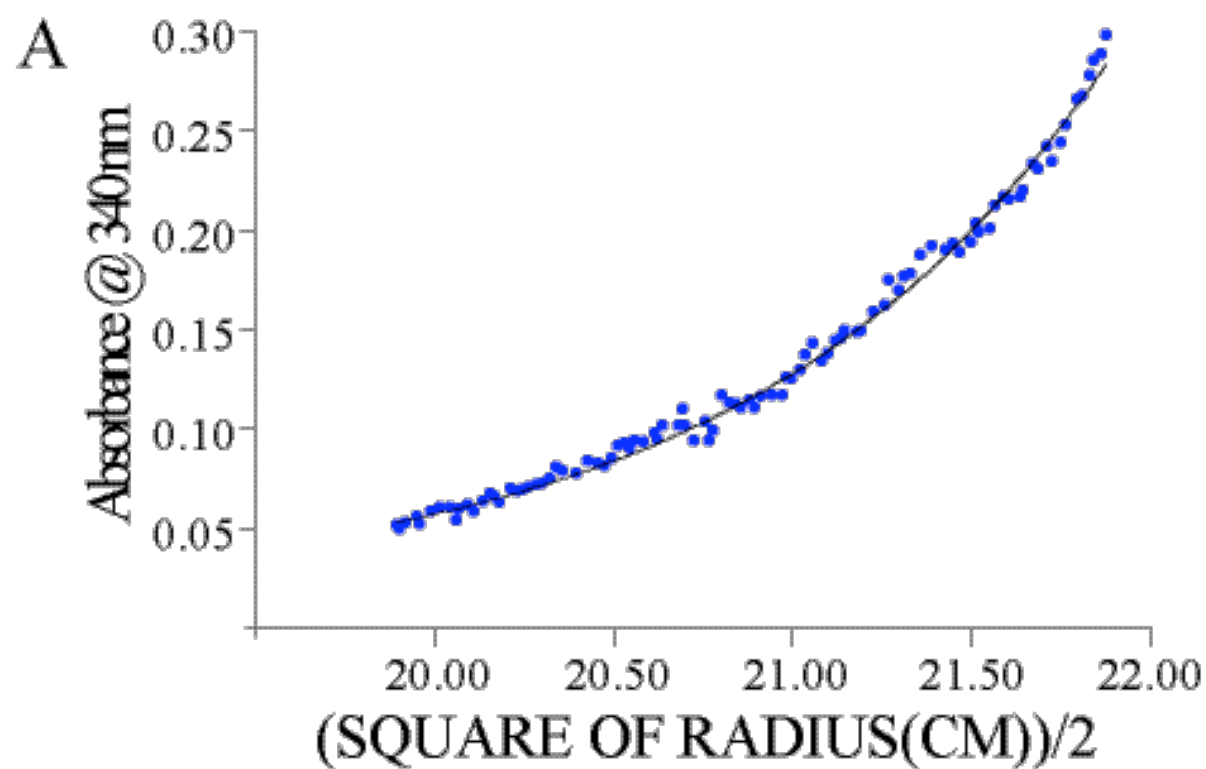
XAS Data Collection and Analysis

   X-ray absorption spectra were measured at the National Synchrotron Light Source, Beamline X9B. Samples were held at ca. 12 K using a Displex cryostat; samples were held in Lucite cuvettes with 6 μm polypropylene windows. EXAFS spectra were measured with 10 eV steps below the edge (7509 – 7689 eV for Co), 0.5 eV steps in the edge region (7689 – 7729 eV for Co), and 0.05 Å^{-1} steps in the EXAFS region. Integration times varied from ~ 1 s in the pre-edge region to 13 s at $k \approx 12$ Å^{-1} for a total integration time of approximately 45 minutes per scan. Total exposure time was approximately 4h. X-ray energies were calibrated by reference to the absorption spectrum of the appropriate metal foil, measured at the same time as the protein spectra. The first inflection point of the foil spectrum was assigned as 7709 eV (Co K).

   All fluorescence and ICR scans were examined prior to averaging to confirm the absence of artifacts. Final spectra are the result of averaging 5 scans per sample, ca. 10 channels per scan. Pre-edge subtraction was accomplished by fitting a Gaussian (centered near the K_ fluorescence energy) to the pre-edge region. EXAFS oscillations were isolated using a four-region spline of fourth order to the normalized data. Data were converted from energy (eV) to k-space (Å^{-1}) according to

$$k = \sqrt{2m_e(E - E_0)/\hbar^2}$$ with E_0 set at 7725 eV. Resultant EXAFS data were Fourier transformed over the range $k = 1 – 13.2$ Å^{-1}. Single-shell scattering was isolated by reverse Fourier transformation [$\Delta R = 0.9 – 2.4$, except fit #3 (below) for Co(II)-**IGA-Pen**, $\Delta R = 0.9 - 3.5$ Å] over the same k-range. The resulting Fourier filtered EXAFS data (ca. 9 degrees of freedom) were fit to equation (1) using a nonlinear least-squares algorithm contained in the program IFEFFIT (M. Newville, J. Synchrotron Rad., 2001, 8, 322-324) interfaced with "SixPack" (available from http://www-ssrl.slac.stanford.edu/~swebb/index.htm).

$$\chi(k) = \sum \frac{N_{as} A_s(k) S_c}{k R_{as}^2} \exp(-2k^2\sigma_{as}^2)\exp(-2R_{as}/\lambda)\sin[2kR_{as} + \phi_{as}(k)] \qquad (1)$$

   Scale factors, S_c's, and _E_0's were calibrated by fitting data for compounds of known structure. Models used for calibration were cobalt bis-trispyrazolylborate, Co(Tp)$_2$, for Co-N scattering and cobalt tetramesitylthiolate, Co(Smes)$_4$, for Co-S interactions. Optimum scale factors were: Co-S, $S_c = 0.83$ and Co-N, $S_c = 0.73$. Fits were carried out for all reasonable coordination numbers, holding S_c constant, while varying R_{as}, _$_{as}^2$ and _E_0.

Table S1: Single-scattering curve fitting results for Co(II)-**IGA** and Co(II)-**IGA-Pen**.[a]

		N [b]	R_{as} (Å)	_$_{as}^2$ [c]	_E_0 [d]	R [e]
Co(II)-**IGA**		4 S	2.31	4.3	-12	12.3
Co(II)-**IGA-Pen**	(1a)	4 S	2.27	6.3	-17	28.0
	(1b)	3 S	2.28	4.9	-14	15.9
		1 N/O	2.10	9.3		
	(2)	3 S	2.28	4.9	-14	7.3
		1 N/O	2.10	8.6		
		6 C	3.44	4.4		

[a]The fits shown are for Fourier filtered data; fits to unfiltered data gave similar results. [b]Integer coordination number giving the best fit. [c]Mean-square deviation in absorber-scatterer bond length in 10^{-3} Å^2. [d]When multiple atom types were used, the value of _E_0 was allowed to vary, with the restriction that _E_0 for S, O, and C were forced to be equal at all times. [e]Goodness of

fit (R) defined as $1000*\dfrac{\sum_{i=1}^{N}\left\{\left[\mathrm{Re}(\chi_{i_{calc}})\right] + \left[\mathrm{Im}(\chi_{i_{calc}})\right]\right\}}{\sum_{i=1}^{N}\left\{\left[\mathrm{Re}(\chi_{i_{obs}})\right] + \left[\mathrm{Im}(\chi_{i_{obs}})\right]\right\}}$, where N is the number of data points.
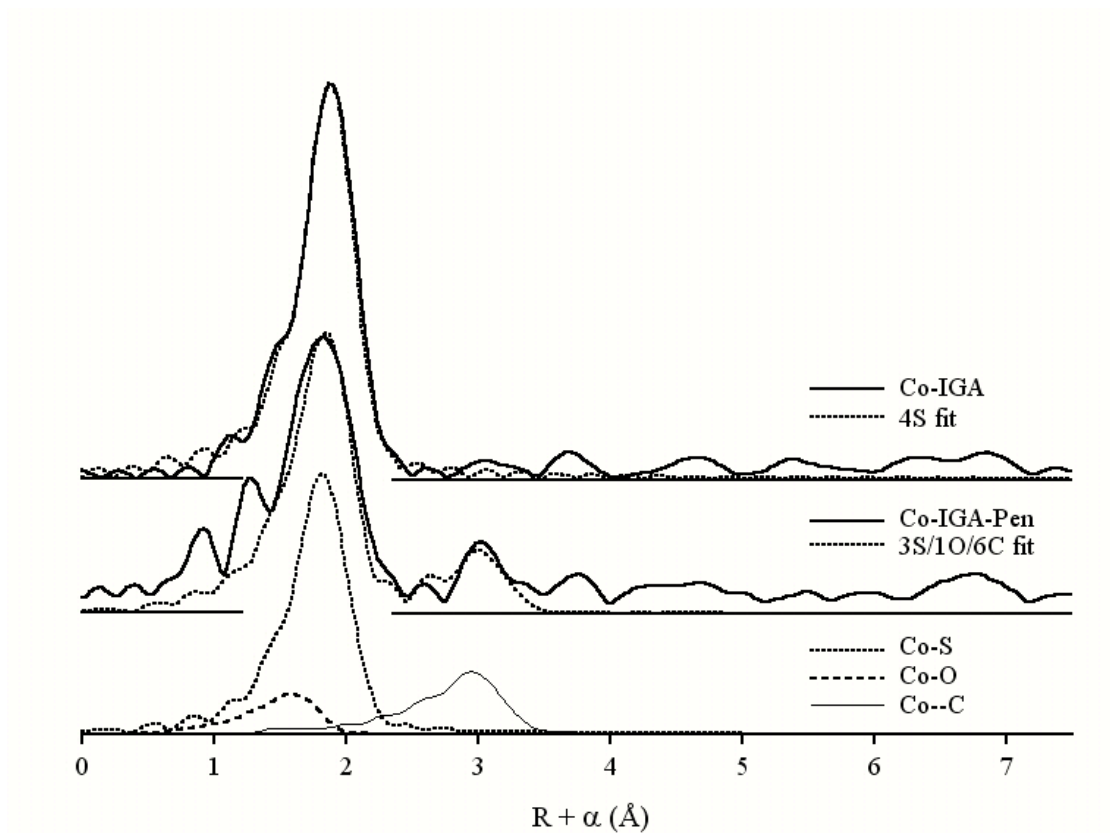
Figure S3. Best fits to Fourier filtered EXAFS data for Co(II)-**IGA** (top) and Co(II)-**IGA-Pen** (center), and individual contributions to the Co(II)-**IGA-Pen** fit (bottom), according to the parameters in Table S1.