Supporting Information

for

Spin Hamiltonian Parameters for Cu(II)-Prion Peptide Complexes from L-band EPR Spectroscopy

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Fourier transform analysis. The use of Fourier transform analysis for the determination of nitrogen coupling to Cu(II) by EPR spectroscopy appears to have first been suggested by Hyde and colleagues (Pasenkiewicz-Gierula, M., Froncisz, W., Basosi, R., Antholine, W. E. and Hyde, J. S. [1987] Multifrequency ESR of Cu(II)-$(His)_n$. 1. Immobile Phase. *Inorg. Chem. 25*, 3006-3010), who applied the method to mobile phase spectra of Cu(II) complexes. Their approach was essentially a sophisticated Fourier filtering approach that aimed to simplify the spectrum by extracting the nitrogen superhyperfine pattern from the underlying copper hyperfine pattern. It was subsequently reported by one of those investigators that more general information could be extracted from the Fourier transform (Della Lunga, G., Pogni, R. and Basosi, R. [1995] Discrimination of Copper-Nitrogen Ligand Coordination by Fourier Analysis of EPR Spectra in Mobile Phase. *J. Magn. Reson. A. 114*, 174-178). Specifically, Basosi showed that the parity of the nitrogen coordination number, i.e. odd or even, was coded into a region of the Fourier transform of the experimental spectrum. Therefore, by comparison of the Fourier transforms of experimental spectra with either (i) those of simulated spectra assuming different nitrogen coordination number or else with (ii) experimental spectra of models with known nitrogen coordination number, the parity of the nitrogen coordination number can be determined in a very straightforward manner. As in the original Hyde study, the method was applied to motionally averaged spectra. As the nitrogen couplings of interest are dominated by Fermi contact, individual nitrogen atom couplings are very similar and each is essentially isotropic, whether in a mobile or immobile system. In a mobile system, the copper hyperfine interaction is motionally averaged and the average value is of the order of five times greater than the nitrogen coupling. Hence the nitrogen-sensitive region

of the Fourier transform is insensitive, for the purposes of parity determination, to the precise values of the anisotropic copper hyperfine values.

In the present study, and as originally suggested by Hyde and colleagues, the Fourier transform method was applied to immobile Cu(II) spectra of the prion peptide complexes. In immobile square-planar Cu(II) systems, the copper perpendicular hyperfine interaction, $A^{Cu}\perp$, is of the same order (generally 1 to 2 times as much) as the nitrogen hyperfine interaction. This pattern is expected to give rise to an additional Fourier transform component that could potentially have a contribution in the nitrogen-sensitive region. An analysis of the effects of spin Hamiltonian parameters upon the Fourier transforms was, therefore, carried out and is presented here.

Figure S1 shows the effects of the Zeeman parameters on the Fourier transforms of X-band (9.6 GHz) spectra with parameters around those for the simulations of PrP Component 3; in each case, the dashed box surrounds the nitrogen-sensitive region of the Fourier transform. The traces S1A show that any chemically reasonable change in $g||$ will have no effect on the nitrogen coordination number parity determined from the Fourier transform. Trace S2B shows that, assuming an axial Zeeman matrix, a difference in $g\perp$ of 0.015 (corresponding to about 25 G [2.5 mT] at X-band), has very little effect indeed on the nitrogen sensitive region. A difference $\delta g\perp$ = 0.05 (80 G [8 mT]) has the effect of changing the "wavelength" of the pattern, preserving the coherence in the center of the pattern but not at the outer edges. The change in $g\perp$ needed to see this effect is far larger than any error expected in simulation. One parameter that does have a significant effect on the nitrogen-sensitive region of the Fourier transform is the degree of rhombicity of **g**, i.e. (g_x - g_y). As shown in traces S1C, the intense low-wavenumber part of the Fourier transform is insensitive to g_x - g_y when (g_x + g_y) is invariant. However, as shown in traces S1D, the nitrogen-sensitive region is sensitive to rhombicity in **g**. As the rhombicity increases, the intensity of the Fourier transform decreases to a minimum, at g_x - g_y = 0.0175 (blue trace), corresponding to a difference in resonance positions of 24 G or about 2 × A^N. It is worth pointing out that a much larger rhombicity of about 0.09 would be required to fulfill this

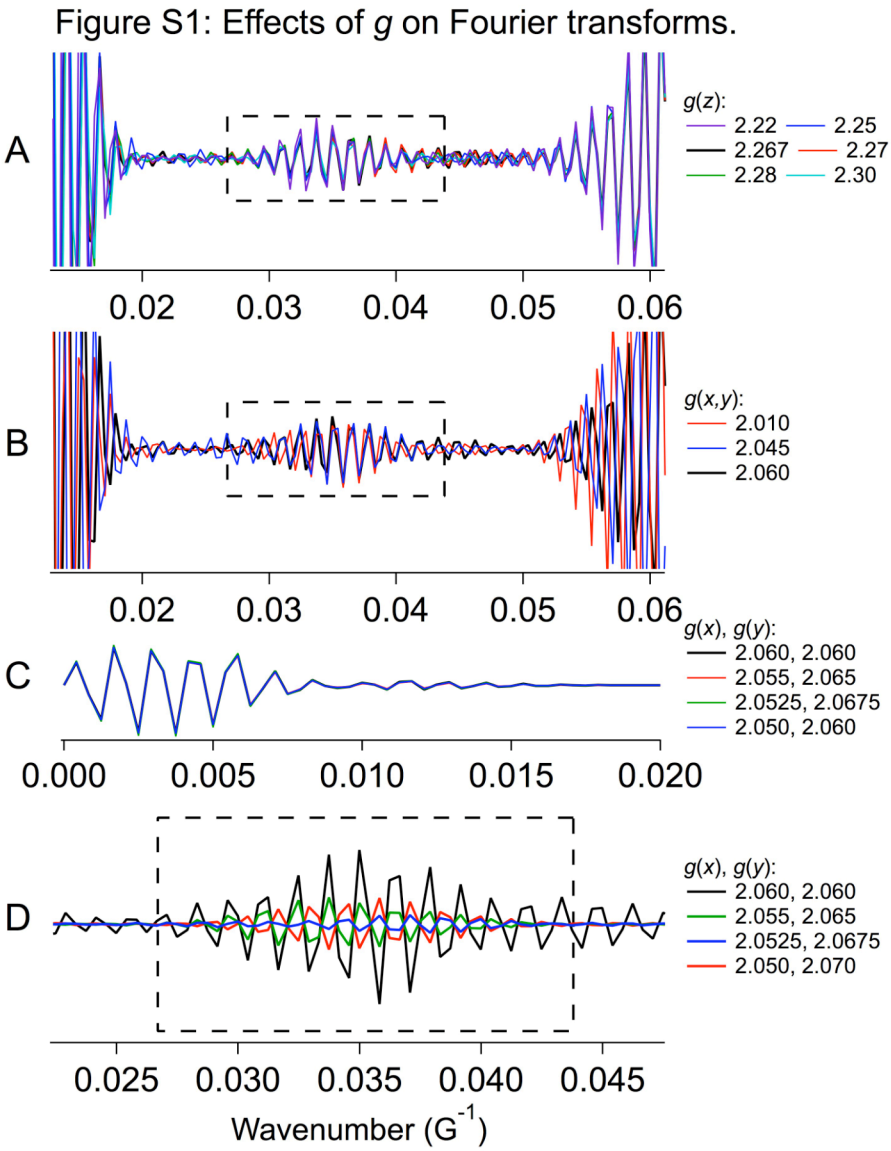

Figure S1. Effects of g on Fourier transforms of calculated EPR spectra. Parameters were varied as shown in the panel and the Fourier transforms calculated. Unspecified parameters were taken from the set $g(x,y,z)$ = 2.060, 2.060, 2.269; $A^{Cu}(x,y,z)$ = 11, 11, 182 × 10^{-4} cm^{-1}; $A^{N}(x,y,z)$ = 13.6, 13.9, 12.6 × 10^{-4} cm^{-1}. The dashed box surrounds the region sensitive to the nitrogen coordination number.

condition at L-band. As the rhombicity is further increased, at X-band, the Fourier transform intensity increases again but with opposite phase (red trace). In the present work, the simulations indicated rhombicities generally of 0.01, corresponding to the green trace of S1D. These values are comfortably within the range of rhombicities for which the Fourier transform pattern in the

nitrogen-sensitive region differs only in intensity, and larger rhombicities would be clear from the X-band spectra and simulations. Nevertheless the point should be made that high quality simulations using a multiple frequency approach are desirable. Fourier transform analysis at L-band would require the rhombicity needed to affect the determination of nitrogen coordination number parity to be beyond that chemically reasonable, and improvement in signal-to-noise at L-band to facilitate this approach is highly desirable.

Figure S2 shows the effects of hyperfine parameters on the nitrogen-sensitive region of the Fourier transform. As is evident from traces S2A and S2B, the transforms are perhaps surprisingly insensitive to either the magnitude or the rhombicity of the copper hyperfine parameters (some small intensity differences, up to a factor of 2, were observed and the traces shown in S2A and B were normalized in the nitrogen-sensitive region). This is particularly reassuring as the values for $A^{Cu}{}_x$ and $A^{Cu}{}_y$ rely heavily on the L-band spectra, whereas high precision values for g_x and g_y are better determined to high precision from higher frequency (X- and Q-band) spectra. Similarly, the nitrogen-sensitive region of the Fourier transforms were relatively insensitive to the magnitudes and rhombicities of the nitrogen superhyperfine couplings, within the somewhat restricted range of chemically reasonable values (traces S2C) and, again perhaps somewhat surprisingly, to quite large angles of noncoincidence of **g** and $\mathbf{A}^{Cu}$, including those indicated in the present work from the frequency dependence of $A^{Cu}||_{app}$.

Some conclusions arise from the above analysis of the use of Fourier transforms for determination of nitrogen coordination number parity. It should be appreciated that if (super)hyperfine patterns are further split, by e.g. anisotropy in **g**, by a field that is of the order of twice the original splitting or more, then that split pattern will be indistinguishable from two genuinely independent patterns. As it is the effect of such an additional splitting (i.e. a separation in field) that affects the Fourier transform, rather than the cause of the splitting (e.g. rhombicity in **g**), then carrying out the Fourier analysis at a frequency that minimizes the effects of parameters in the field dimension will decrease the sensitivity of the analysis to those parameters. In the case of immobilized square planar Cu(II), most of the parameters have very little effect when varied within even a generous range corresponding to that which is chemically reasonable. Even rudimentary simulations should provide parameters sufficiently close to the

real ones as to be used for Fourier analysis, except for one important exception. There is some sensitivity to the magnitude of '$g\perp$' [more precisely, $(g_x + g_y)/2$], but this is readily determined to

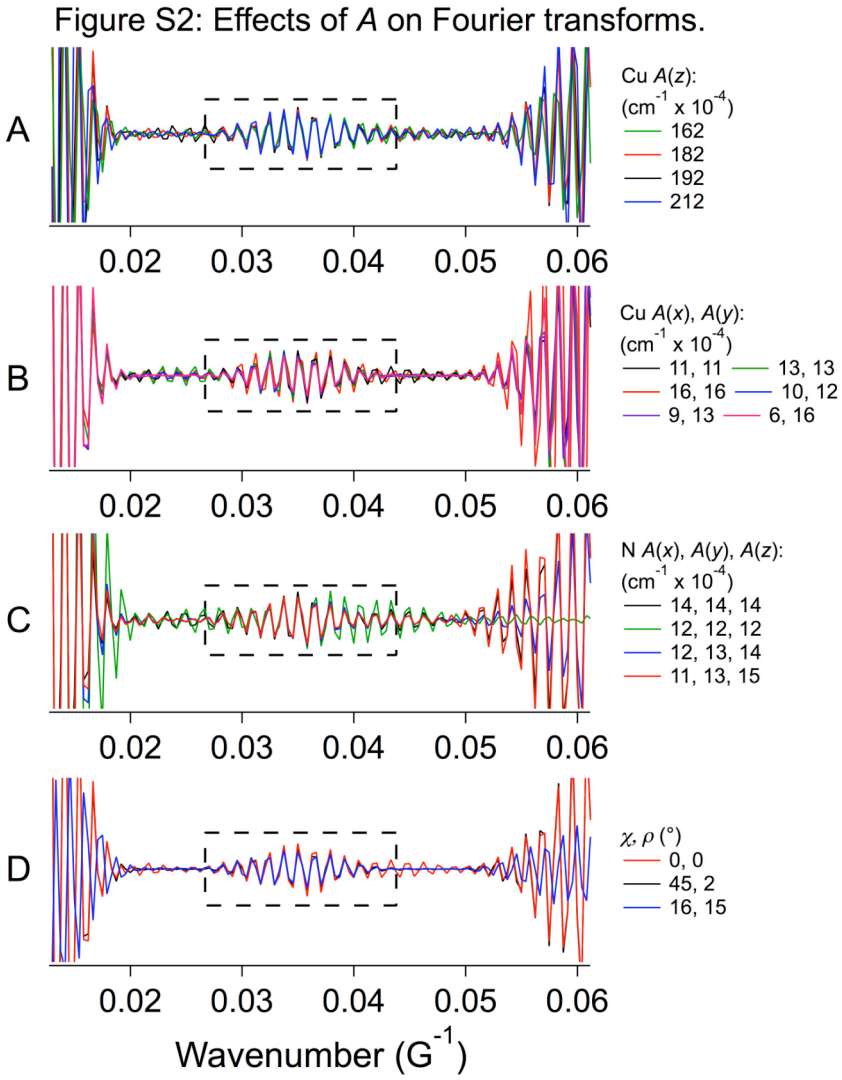

Figure S2. Effects of A on Fourier transforms of calculated EPR spectra. Parameters were varied as shown in the panel and the Fourier transforms calculated. Unspecified parameters were taken from the set $g(x,y,z)$ = 2.060, 2.060, 2.269; $A^{Cu}(x,y,z)$ = 11, 11, 182 × 10^{-4} cm^{-1}; $A^{N}(x,y,z)$ = 13.6, 13.9, 12.6 × 10^{-4} cm^{-1}. The dashed box surrounds the region sensitive to the nitrogen coordination number.

high precision by high quality simulations that include at least one high frequency (X-band or higher), or by examination of Q- or W-band spectra. The only area where significant uncertainty

may still arise is that of the rhombicity, g_x - g_y. Rhombicities corresponding to around 25 G (2.5 mT) or more will provide an incorrect value for the parity unless they are accounted for in simulations. High quality simulations at multiple frequencies and sensitivity analyses, as carried out in the present work, provide one method for determining whether this case may apply (in the present work, it doesn't). Alternatively, one can carry out the analysis at a frequency where the field corresponding to the rhombicity in **g** is reduced to well below 25 G, e.g. at S- or L-band. Nascent advances in low frequency spectroscopy, as discussed below, will provide the signal-to-noise adequate to carry this out.

Potential for improvements in L-band EPR spectroscopy. The major drawback with EPR spectroscopy at L-band is the poor signal-to-noise (S/N). There are numerous deficiencies with the current instrumentation; fortunately, there are solutions that either exist or are in development. The three fundamental aspects of an EPR spectrometer are (i) the RF source, (ii) the coupling of the RF to the sample, and (iii) detection of the RF absorption as a function of field. The current source is an octave-band 1 - 2 GHz bridge with a mechanically-tuned oscillator. The bridge was designed for frequency flexibility, at the costs of high phase noise and a very non-linear dependence of power output on frequency. The relatively recent appearance of low phase-noise synthesizers with very high frequency stability, such as the Agilent E4438C, will provide significant improvements in the RF quality and, thus, in signal quality. In addition, synthesizer sources allow for improvements in detection. Improved coupling of the RF to the sample essentially means improved resonators, and this is an ongoing program at the National Biomedical EPR Center at the Medical College of Wisconsin. Resonators are designed *in silico* using Agilent's High Frequency Structure Simulation software which solves Maxwell's equations for an RF structure. Resonators can be designed with samples, sample tubes, couplers etc. *in situ*, and optimized for available sample volume, desired frequency, temperature, quality factor Q_0 and RF field $\mathbf{B}_1$. The high frequency and phase stability of synthesizer sources will allow for resonators with a higher Q_0 and, hence, higher signal intensity. The greatest problem with S/N appears to come from the detection system. As with almost all EPR systems, phase-sensitive detection with magnetic field modulation is currently employed. The field modulation causes signal-degrading mechanical and microphonic effects known collectively as 'potato' (Sealy, R. C., Hyde, J. S. and Antholine, W. E. [1985] Electron spin resonance. In Modern Physical Methods in

Biochemistry, Part A (Neuberger, A. and Van Deenen, L. L. M., eds.). pp. 69-148, Elsevier B.V., Amsterdam). In addition, field modulation introduces artifactual changes to the lineshapes in high-resolution spectra. Rapid field scanning in the absence of field modulation provides a method of avoiding modulation-related artifacts and provides a pure absorption spectrum that can later be derivatized or pseudomodulated under conditions optimized for the experiment (Kittell, A.W., Camenisch, T.G., Hyde, J.S. [2010] Nitroxide Lineshape Analysis With X-Band Pure Absorption Rapid Scan (PARS) Electron Paramagnetic Resonance. Abstracts of the 52nd Rocky Mountain Conference on Analytical Chemistry, A177, p.52; Hyde, J.S., Pasienkewicz-Gierula, M., Jesmanowicz, J., and Antholine, W.E. [1990] Pseudo field modulation in EPR spectroscopy. *Appl. Magn. Reson. 1*, 483-496). Direct detection has been developed that is time-locked to the synthesizer source (Hyde, J. S., Camenish, T. G., Ratke, J. J., Strangeway, R. A. and Froncisz, W. [2005] Digital detection by time-locked sampling in EPR. *Biol. Magn. Reson. 24*, 199-222). A commercially available digital signal channel is now available from Bruker. Time-locked digital data can be decomposed into absorption, dispersion, and higher harmonic displays by automated computer processing, and the consequent removal of the hardware associated with traditional phase sensitive detection from the signal chain further improves the quality of the data. Each of these advances promises significant improvements in S/N and in data quality in the future.