[Cu(aq)]²⁺ is structurally plastic and the axially elongated octahedron goes missing

Patrick Frank,^{1,2,a)} Maurizio Benfatto,^{3,a)} and Munzarin Qayyum^{1,b)}

¹*Department of Chemistry, Stanford University, Stanford, California 94305, USA*

²*Stanford Synchrotron Radiation Lightsource, SLAC, Stanford University, Menlo Park, California 94025, USA*

³*Laboratori Nazionali di Frascati-INFN, P.O. Box 13, 00044 Frascati, Italy*

(Received 2 February 2018; accepted 2 May 2018; published online 23 May 2018)

High resolution ($k = 18 \text{ \AA}^{-1}$ or $k = 17 \text{ \AA}^{-1}$) copper K-edge EXAFS and MXAN (Minuit X-ray Absorption Near Edge) analyses have been used to investigate the structure of dissolved [Cu(aq)]²⁺ in 1,3-propanediol (1,3-P) or 1,5-pentanediol (1,5-P) aqueous frozen glasses. EXAFS analysis invariably found a single axially asymmetric 6-coordinate (CN6) site, with $4 \times O_{eq} = 1.97 \text{ \AA}$, $O_{ax1} = 2.22 \text{ \AA}$, and $O_{ax2} = 2.34 \text{ \AA}$, plus a second-shell of $4 \times O_{water} = 3.6 \text{ \AA}$. However, MXAN analysis revealed that [Cu(aq)]²⁺ occupies both square pyramidal (CN5) and axially asymmetric CN6 structures. The square pyramid included $4 \times H_2O = 1.95 \text{ \AA}$ and $1 \times H_2O = 2.23 \text{ \AA}$. The CN6 sites included either a capped, near perfect, square pyramid with $5 \times H_2O = 1.94 \pm 0.04 \text{ \AA}$ and $H_2O_{ax} = 2.22 \text{ \AA}$ (in 1,3-P) or a split axial configuration with $4 \times H_2O = 1.94$, $H_2O_{ax1} = 2.14 \text{ \AA}$, and $H_2O_{ax2} = 2.28 \text{ \AA}$ (in 1,5-P). The CN6 sites also included an 8- H_2O second-shell near $3.7 \text{ \AA}$, which was undetectable about the strictly pyramidal sites. Equatorial angles averaging $94^\circ \pm 5^\circ$ indicated significant departures from tetragonal planarity. MXAN assessment of the solution structure of [Cu(aq)]²⁺ in 1,5-P prior to freezing revealed the same structures as previously found in aqueous 1M HClO₄, which have become axially compressed in the frozen glasses. [Cu(aq)]²⁺ in liquid and frozen solutions is dominated by a 5-coordinate square pyramid, but with split axial CN6 appearing in the frozen glasses. Among these phases, the Cu–O axial distances vary across $1 \text{ \AA}$, and the equatorial angles depart significantly from the square plane. Although all these structures remove the $d_{x^2-y^2}$, d_{z^2} degeneracy, no structure can be described as a Jahn-Teller (JT) axially elongated octahedron. The JT-octahedral description for dissolved [Cu(aq)]²⁺ should thus be abandoned in favor of square pyramidal [Cu(H₂O)₅]²⁺. The revised ligand environments have bearing on questions of the Cu(I)/Cu(II) self-exchange rate and on the mechanism for ligand exchange with bulk water. The plasticity of dissolved Cu(II) complex ions falsifies the foundational assumption of the rack-induced bonding theory of blue copper proteins and obviates any need for a thermodynamically implausible protein constraint. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5024693>

INTRODUCTION

At least since the work of Latimer,¹ the structure of dissolved aqueous cupric ion, [Cu(aq)]²⁺, has been under continuous investigation.^{2–43} The earliest studies found in favor of the Jahn-Teller (JT) axially elongated octahedron [Cu(H₂O)₆]²⁺, based upon the newly developed crystal field theory and experiments indicating spectroscopic similarity between crystalline hexaaqua complexes and dissolved [Cu(aq)]²⁺. This structural deduction from theory and experiment dominated thinking about dissolved Cu(II) during the succeeding 50 years. Although Peisach and Mims reported linear electric field effect EPR experiments in 1976 revealing that many dissolved Cu(II) complex ions were D_{2d} distorted,¹⁰ these results were generally overlooked during subsequent years.^{44–49}

Renewed interest in the structure of dissolved [Cu(aq)]²⁺ followed the neutron diffraction experiments and molecular dynamics (MD) simulations of Pasquarello *et al.*, who proposed the predominance of square pyramidal [Cu(H₂O)₅]²⁺ in solution.²⁵ This idea placed the structures of the dissolved cupric aqua and ammine complex ions into apparent experimental conformance.⁵⁰ The inference was quickly corroborated when an examination of copper K-edge x-ray absorption spectroscopy (XAS) spectra using the extended continuum multiple scattering (ECMS) theory employed in Minuit XANES (MXAN) analysis derived a square pyramidal solution structure for [Cu(aq)]²⁺.^{27,51–53}

The MXAN approach extracts structural information from the first 200 eV of an XAS spectrum, including the rising edge (XANES) energy region where the symmetry constraints of bound-state transitions apply. Inclusion of the XANES energy region allows MXAN simulations to return both distances ($\pm 0.01 \text{ \AA}$) and angles ($\pm 2^\circ$) of the immediate ligand environment about the central absorber, ranging out to about $5 \text{ \AA}$. MXAN analysis is thus able to produce structural models

^{a)}Authors to whom correspondence should be addressed: pfrank@slac.stanford.edu and Maurizio.Benfatto@lnf.infn.it

^{b)}Present address: Intel Corporation, Hillsboro, OR 97124, USA.

of solution-phase complexes of near x-ray crystal diffraction resolution.

We have combined EXAFS and MXAN analyses to investigate the structure of several dissolved Cu(II) complex ions using K-edge XAS spectra.^{42,54–58} EXAFS fits are first combined with external physico-chemical information (such as using the cupric equatorial square as a constraint) to derive a reasonable “first guess” structure. This structure is then subjected to MXAN analysis. During MXAN fits, the computed and measured XAS are compared and the difference is minimized by iterative adjustments of the structure. Assessments of intermediate fits allow rational alterations of the structural model. Ideally, the final result is a fully three-dimensional structural model that reproduces the measured XAS spectrum in a calculation from ECMS theory.

In previous work, we employed the combined EXAFS/MXAN approach to derive three-dimensional structural models for aqueous dissolved Cu(II) in its imidazole, ammine, and aqua complex ions, all of which included a proximate second-shell of solvent molecules.^{30,42,59,60} In every case, the K-edge liquid solution XAS spectrum was best fit with an axially elongated square pyramidal $[\text{CuL}_5]^{2+}$ core structure. However, this core structure invariably included a non-bonded, but associated, axial water molecule at a Cu–O distance of approximately 3 Å. All three complex ions also featured equatorial departures from D_{4h} symmetry. Likewise, others have found axially elongated square pyramidal complex ions for Cu(II) dissolved in methanol, DMSO, and acetonitrile.^{61,62}

In water solution, $[\text{Cu}(\text{aq})]^{2+}$ is evidently distributed between two such square pyramidal $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ sites, one of which includes both an ~ 3 Å axially associated water and a discernable second-shell.^{42,43} Departure of the 3 Å axially associated water apparently allows the solvation shell about $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ to become disorganized. The same structural motifs dominate $[\text{Cu}(\text{aq})]^{2+}$ in frozen aqueous 1M HClO_4 solution. However, during freezing a perchlorate ion migrated into the axial position of the CN5 $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ site, coming to rest at Cu–O = 3 Å. In both phases, the structures show equatorial departures from D_{4h} symmetry.

Here we have extended these studies to include $[\text{Cu}(\text{aq})]^{2+}$ dissolved in frozen aqueous glasses. Freezing into a glass can capture water in a configuration approximating the initial amorphous liquid state.^{63,64} Glass formation avoids exclusion of Cu(II) from growing water crystallites, with regions of increased Cu(II) concentration and their induced re-equilibrations. The glassing agents chosen were 1,3-propanediol (1,3-P) and 1,5-pentanediol. These molecules do not have α -hydroxyl groups, thus vitiating the possibility of complex formation. We present here the combined EXAFS/MXAN structural analysis of the K-edge XAS of $[\text{Cu}(\text{aq})]^{2+}$ in two alternative frozen glasses. The set of two $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ structures already observed in liquid and frozen water are here shown to expand to a second set of two structures in these rigid glassy matrixes. Dissolved $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ is revealed to be structurally plastic, apparently achieving an energetic ground state within an extended variety of ligand environments.

MATERIALS AND METHODS

Sample preparation

Stock 0.10M $\text{Cu}(\text{ClO}_4)_2$ in 1M HClO_4 was prepared using 99.995% CuO (Aldrich Chemicals, lot # 08909TD) containing 0.26 ppm Zn, as described previously.⁴² Solutions for frozen glass experiments were prepared by diluting appropriate volumes of the stock dissolved $\text{Cu}(\text{ClO}_4)_2$ to 60% v/v with the remaining 40% made up of either 1,3-propanediol or 1,5-pentanediol (Sigma/Aldrich Chemical Co.). The homogeneous blue solutions were thus 60 mM $\text{Cu}(\text{ClO}_4)_2$ dissolved in aqueous 0.60M HClO_4 with 40% glassing agent by volume.

Samples for frozen glass XAS measurements were held in a $2 \times 2 \times 20$ mm Delrin pinhole cell, faced with a Kapton window of 35 μm thickness. Filled cells were dropped into a freezing iso-pentane slush (-159.9 C) to yield clear frozen glasses. These were stored in liquid nitrogen prior to measurement.

XAS measurements

Frozen copper K_α fluorescence excitation XAS spectra were measured on Stanford Synchrotron Radiation Light-source (SSRL) beam line 7-3 using a Si(220) monochromator, fully tuned at 9684 eV and optimized at 9200 eV, and with ring conditions of 3 GeV and about 350 or 450 mA current. Baseline I_0 was measured using an in-line nitrogen-filled ionization chamber situated in front of the sample.

The glassy frozen solution samples were held near 10 K using an Oxford Instruments CF1208 continuous flow liquid helium cryostat. Each sample was positioned 45° to the beam and K_α fluorescence excitation XAS spectra were measured using a Canberra 30-element liquid-nitrogen-cooled germanium array detector. Beam size was set to 1×5 mm using in-hutch slits. Four beam-spots were chosen per frozen sample, and two XAS scans were measured per spot. The second scans showing traces of photo-reduction were not used for subsequent analysis. A liquid solution sample of dissolved 0.060M $\text{Cu}(\text{ClO}_4)_2$ with 0.60M HClO_4 in 40% aqueous 1,5-pentanediol was also measured, but as a transmission XAS spectrum using a Teflon spacer cell of 2 mm path length. The cell was windowed with a 5 μm polypropylene film. All measured XAS spectra were calibrated using the XAS of a copper foil positioned after the I_1 chamber, which was simultaneously measured using a third in-line nitrogen-filled ionization chamber (I_2).

The measured XAS spectra were processed as described previously.⁴² PySpline was used to normalize the measured XAS and extract the EXAFS spectra. PySpline was written by Dr. Adam Tenderholt and is available as open source software at <http://pyspline.sourceforge.net>.⁶⁵ The choice of spline included the criterion that it passes through the XANES features at unit intensity. Normalized XAS spectra were obtained by dividing the baseline-subtracted XAS spectrum by the fitted polynomial spline.

XAS spectra were analyzed using the program EXAFS-PAK which was written by Professor Graham George, University of Saskatchewan. EXAFSPAK is available free of charge

on the SSRL website: <http://ssrl.slac.stanford.edu/~george/exafspak/exafs.htm>. For calibration, the first inflection of the first derivative of the rising K-edge XAS of the copper calibration foil was set to 8980.30 eV.

EXAFS fits

FEFF8 was used to fit the experimental EXAFS spectra, using the program OPT within the EXAFSPAK suite as has been described previously.^{59,60} Muffin tin (MT) radii were assigned within FEFF based on the Norman criterion.⁶⁶ Phase shifts were calculated within FEFF. Hydrogen atoms were not included in the fits. EXAFS spectra were fit over the range $k = 2\text{--}18 \text{ \AA}^{-1}$ (1,3-propanediol glass) or $k = 2\text{--}17 \text{ \AA}^{-1}$ (1,5-pentanediol glass). The expected resolution between shells was 0.1 Å. Equation (1) is minimized during the fit,

$$F = \left[\sum k^6 (\chi_{\text{exp}} - \chi_{\text{calc}})^2 / \sum k^6 \chi_{\text{exp}}^2 \right]^{1/2}. \quad (1)$$

The number of statistically independent points (N_{ind}) in an EXAFS fit was calculated using the following Stern equation:⁶⁷

$$N_{\text{ind}} = (2 \times \Delta R \Delta k / \pi) + 2. \quad (2)$$

N_{ind} was used in calculations of the Michalowicz F_M value, used to evaluate EXAFS fits using alternative structural models that include unequal degrees of freedom.⁶⁸ FEFF8 was also used to calculate EXAFS phases and amplitudes and to simulate the Cu K-edge EXAFS of $[\text{Cu}(1,3\text{-p})(\text{H}_2\text{O})_4]^{2+}$ (see the text: 1,3-p is 1,3-propanediol). Structural models for the EXAFS fits and for the MXAN fits described in the following were prepared using Chem3D (Perkin-Elmer Informatics).

MXAN fits

The normalized Cu K-edge XAS spectra were fitted over the relative energy range, $E - E_0 = -7.5 < \Delta E < 200$, where $E_0 = 8990.00 \text{ eV}$. The extended continuum multiple scattering (ECMS) theory of the MXAN method has been described in detail.^{52–54,69} MXAN uses the muffin tin (MT) approximation for the atomic potentials. Although the effects of the non-MT corrections on XANES calculations are still not well understood, evidence exists that their influence, if present, is confined to the very low energy part of the spectrum with a very weak influence on the structural determination and can be approximated by judicious optimization of the radii and the potential.^{30,42,70} These considerations form the basis of the potential optimization procedure normally applied to MXAN analysis.^{37,53}

MT potentials were optimized during MXAN fits to all tested models. Trans-equatorial water ligand distances and trans $L_{\text{eq}}\text{--Cu--}L_{\text{ax}}$ θ -angles were linked and varied in concert. Equation (3) shows the function, R_{sq} , minimized during the MXAN fit,

$$R_{sq} = n \frac{\sum_{i=1}^m w_i [(y_i^{\text{th}} - y_i^{\text{exp}}) \epsilon_i^{-1}]^2}{\sum_{i=1}^m w_i}. \quad (3)$$

Here “ n ” is the number of independent parameters, “ m ” is the number of data points, “ y_i^{th} ” and “ y_i^{exp} ” are the theoretical and

experimental values of the absorption, respectively, “ ϵ_i ” is the error in each point of the experimental data set, and “ w_i ” is a statistical weight. When $w_i = 1$, the square residual function R_{sq} becomes the statistical χ^2 function.⁷¹ In this work, $w_i = 1$ was assumed, and the experimental error (ϵ) was set to a constant 1.0% of the main experimental edge jump over the whole data set. Hydrogen atoms were included in the MXAN fits. Statistical errors were calculated by the MIGRAD routine. MXAN also introduces a systematic error of 1%–2% into the bond lengths that must be added to the MIGRAD statistical error. Atomic coordinates for the MXAN input files were derived from structural models constructed within the program Chem3D. Final systematic error in the bond lengths for the two-site fits was calculated as the root-sum-square (r.s.s.) of 1.5% systematic error in each of the step-wise iterations (see the text).

RESULTS

When employing samples of dissolved transition metal ions in solutions with high concentrations of glassing agents in order to prepare frozen aqueous glasses, it is necessary to know that the glassing agent does not form a complex with the metal ion. For example, $\text{Cu}(\text{CF}_3\text{SO}_3)_2$ dissolves in pure ethylene glycol (eg) to produce the complex ion $[\text{Cu}(\text{eg})_3]^{2+}$. The structure of $[\text{Cu}(\text{eg}\text{--}_{2\text{H}})_2](\text{Ba}(\text{eg})_3)$ is known, and in alkaline solution, both ethylene glycol and glycerol will form complexes with dissolved $\text{Cu}(\text{II})$.^{72–75}

Although the experimental solutions employed here were acidic, which condition suppresses complex formation with α -diol ligands,^{73,74,76} the glassing agents were highly concentrated and the samples were frozen glasses rather than room temperature liquids. Previous XAS studies of $\text{Cu}(\text{ClO}_4)_2$ dissolved in 1M HClO_4 showed that freezing causes the unbound perchlorate ion to associate with $\text{Cu}(\text{II})$.⁴² If freezing likewise induced 1,3-propanediol to bind to $[\text{Cu}(\text{aq})]^{2+}$, an artifactual complex ion with a 6-membered chelate ring could result.

To test this idea, a chemically plausible molecular chelate of $[\text{Cu}(1,3\text{-p})(\text{H}_2\text{O})_4]^{2+}$ (1,3-p = 1,3-propanediol) was constructed in Chem3D (Fig. S1 of the [supplementary material](#)). The model was made to have the same Cu–O bond lengths as the EXAFS fitted model of $\text{Cu}(\text{II})$ in the 1,3-propanediol frozen glass (see below). FEFF8 was then used to simulate the Cu K-edge EXAFS and Fourier transform EXAFS spectra of this chelate complex ion (Fig. S2 of the [supplementary material](#)). Comparison with the corresponding experimental spectra of dissolved 0.060M $[\text{Cu}(\text{ClO}_4)_2]$ with 0.6M HClO_4 in 40% 1,3-propanediol frozen glass provided no evidence of the nearby carbon shells expected in a 1,3-propanediol chelate. The XAS spectrum of $\text{Cu}(\text{ClO}_4)_2$ in the 1,5-pentanediol frozen glass was nearly identical to that of the 1,3-propanediol, providing no evidence of the homologous chelate carbon shell (see below). 1,5-Pentanediol is also the less likely to form a chelate, in part because of the greater number of internal >CH--HC< gauche interactions.^{77–79} Simulation of the Cu K-edge EXAFS of a $[\text{Cu}(1,5\text{-p})(\text{H}_2\text{O})_4]^{2+}$ chelate complex ion was thus deemed not necessary.

Figure 1 compares the copper K-edge XANES and EXAFS spectra of $[\text{Cu}(\text{ClO}_4)_2]$ dissolved in dilute aqueous

FIG. 1. Left panel: Copper K-edge XANES spectra of $\text{Cu}(\text{ClO}_4)_2$ dissolved in dilute aqueous HClO_4 , under conditions of liquid aqueous solution (black line), frozen aqueous solution (blue line), 40% 1,3-propanediol frozen glass (red line), and 40% 1,5-pentanediol frozen glass (violet line). The spectra have been offset 0.25 normalized intensity units for clarity. Right panel: The EXAFS spectra of the same samples. Inset: 5-point smoothed expansion of the $k = 14\text{--}18\text{ \AA}^{-1}$ EXAFS region, showing emergence of phase differences.

HClO_4 in liquid or frozen solution, or in 40% 1,3-propanediol or 1,5-pentanediol frozen aqueous glass. Direct inspection shows a strong similarity of the liquid aqueous solution and the frozen glass XANES spectra, implying the common presence of $[\text{Cu}(\text{aq})]^{2+}$.⁴² The K-edge XANES spectrum of frozen aqueous $[\text{Cu}(\text{aq})]^{2+}$ in 1M HClO_4 solution exhibits a pronounced rising edge shoulder at 8990.4 eV (Fig. 1), indicating trans-axial scatterers.⁴² Consistent with this, the $[\text{Cu}(\text{aq})]^{2+}$ was found to include either a distant unbound axial perchlorate ion ($\text{Cu}\text{--}\text{OClO}_3 = 3.2 \pm 0.2\text{ \AA}$) or unbound axial water molecule ($\text{Cu}\text{--}\text{O}_w = 3.0 \pm 0.1\text{ \AA}$).⁴²

Although the liquid aqueous solution and the two frozen glass samples produced very similar K-edge XANES spectra, intensity differences were found in the rising K-edge energy region (Fig. 2). These differences are unlikely to reflect errors in splining or baseline subtraction (Fig. S3 of the supplementary material). Axial scatterers contribute to the intensity in this energy region.⁴² Thus, the XANES difference intensities most

FIG. 2. Double-y plot. Right ordinate: (green line) Cu K-edge XANES of 0.10M $\text{Cu}(\text{ClO}_4)_2$ dissolved in liquid aqueous 1M HClO_4 . Left ordinate: Difference Cu K-edge XAS spectra (liquid aqueous minus frozen glass) for 40% 1,3-propanediol (red line) or 40% 1,5-pentanediol (blue line). The dip at 8984.3 eV in the 1,3-propanediol difference spectrum (red arrow) indicates a trace of $\text{Cu}(\text{I})$ from photo-reduction.

likely indicate subtle axial disparities around $\text{Cu}(\text{II})$ within the frozen glass environments.

EXAFS fitting results

The immediate ligation structure about $\text{Cu}(\text{II})$ was first explored. Fourier-filtered first-shell ($k = 2\text{--}18\text{ \AA}^{-1}$) EXAFS spectra were obtained by windowing and back-transformation. Gaussian windows were of the range 0.8–2.4 $\text{\AA}$ for the 1,3-propanediol glass and 0.7–2.5 $\text{\AA}$ for the 1,5-pentanediol frozen glass, respectively (see Fig. S4 of the supplementary material).

The back-transformed first-shell EXAFS spectra were then fit with three alternative first-shell Cu–O models. These were axially elongated square pyramid (CuO_5), axially elongated Jahn-Teller (JT) octahedron (CuO_6), and split axial ($\text{CuO}_{(5+1)}$). The split axial fit allowed the two Cu–O axial distances to relax independently. Previous work had shown that these three models fit the EXAFS of $[\text{Cu}(\text{aq})]^{2+}$ in liquid HClO_4 solution equally well to about $k = 14\text{ \AA}^{-1}$ but diverged thereafter.⁴² Figure 3 shows that the same proved true for $[\text{Cu}(\text{aq})]^{2+}$ in the frozen aqueous glasses. The three alternative model first-shell EXAFS fits show similarly intense unfit residuals through about $k = 13\text{ \AA}^{-1}$, for both frozen glasses [Figs. 3(a) and 3(c), bottom]. After $k = 13\text{ \AA}^{-1}$, the split axial model proved the best fit, with a much less intense unfit residual. This model also produced the lowest intensity residual across nearly the entire R-range of the Fourier transform spectrum.

The EXAFS fit using the split axial model has two more degrees of freedom than do either of the others. The Michalowicz F_M goodness of fit metric allows comparative evaluation of fits differing in degrees-of-freedom.⁶⁸ $F_M > 1$ reveals that an improved fit is statistically valid. The goodness-of-fit F_M and $\Delta\chi^2$ for the alternative first-shell models, presented in Table I, indicated a choice for the split axial structure.

All the first-shell frozen glass structural models produced Cu–O distances very similar to the K-edge EXAFS of $[\text{Cu}(\text{aq})]^{2+}$ in liquid aqueous 1M HClO_4 solution.⁴² The two split-axial Cu– O_{ax} distances differed by 0.14 $\text{\AA}$ which is greater than the estimated 0.10 $\text{\AA}$ inter-shell resolution of the $k = 2\text{--}18\text{ \AA}^{-1}$ EXAFS fitting range.

Fits over the full EXAFS [k -range: $2\text{--}18\text{ \AA}^{-1}$ (1,3-propanediol) or $2\text{--}17\text{ \AA}^{-1}$ (1,5-pentanediol)] were then carried out. The axially elongated square pyramidal, JT-octahedral, and split axial models were again all tested. However, the full-fit models now also included four additional oxygen scatterers representing a more distant solvation shell. The fit weighted F-values for the three models (square pyramidal, JT-octahedral, split axial) were 0.2207, 0.1817, 0.1567 (1,3-propanediol) and 0.1796, 0.1449, 0.1141 (1,5-pentanediol), respectively. The Michalowicz F_M was again evaluated to account for differing degrees of freedom in the fits. Relative to the split axial model, the fit F_M values were 9.22, 3.19 (square pyramidal, JT-octahedral) for 1,3-propanediol, respectively, and 10.96, 5.57 (square pyramidal, JT-octahedral) for 1,5-pentanediol, respectively. These results again definitively favor the split axial structure for $[\text{Cu}(\text{aq})]^{2+}$. The square pyramidal and JT-octahedral fits are shown in Fig. S5 and Table S1

FIG. 3. Back transformed Fourier-filtered first-shell EXAFS spectra [(a) and (c)] and the Fourier transform of this filtered EXAFS [(b) and (d)] for 0.06M $\text{Cu}(\text{ClO}_4)_2$ dissolved in 0.6M HClO_4 in 40% aqueous (top) 1,3-propanediol or (bottom) 1,5-pentanediol frozen glass. The fitted models are as follows: (blue line) square pyramid, (red line) JT-octahedron, or (green line) split axial. The dotted lines of the same color indicate the unfit residual.

of the [supplementary material](#). The split axial model fits for $[\text{Cu}(\text{aq})]^{2+}$ in the two frozen glass solutions are shown in Figs. 4(a)–4(d).

Visual comparison of the fit residuals in Fig. 4 and Fig. S5 of the [supplementary material](#) clearly shows the superiority of split axial models in the $k = 12\text{--}18 \text{ \AA}^{-1}$ EXAFS region as

TABLE I. Fits of Fourier filtered first-shell K-edge EXAFS for $[\text{Cu}(\text{aq})]^{2+}$ in 40% aqueous frozen glass.

1,3-Propanediol						
Scatterer	JT-octahedral		Square pyramid		Split axial	
	[CN] R (Å) ^a	σ ² × 10 ³ (Å ²)	[CN] R (Å) ^a	σ ² × 10 ³ (Å ²)	[CN] R (Å) ^a	σ ² × 10 ³ (Å ²)
O _{eq}	[4] 1.97 ± 0.002	4.19	[4] 1.96 ± 0.002	4.27	[4] 1.96 ± 0.001	4.32
O _{ax1}	[2] 2.29 ± 0.004	9.10	[1] 2.29 ± 0.007	5.04	[1] 2.24 ± 0.003	1.90
O _{ax2}	...	...	...	...	[1] 2.38 ± 0.004	2.58
ΔE (eV)	−6.925 0		−7.375		−7.597	
Fit Δχ ^{2,b}	0.131 2		0.2100		0.084 3	
F _M ^c	3.89		10.4		...	
1,5-Pentanediol						
O _{eq}	[4] 1.97 ± 0.001	4.15	[4] 1.97 ± 0.002	4.25	[4] 1.97 ± 0.001	4.30
O _{ax1}	[2] 2.29 ± 0.004	10.66	[1] 2.28 ± 0.007	5.96	[1] 2.23 ± 0.002	2.44
O _{ax2}	...	...	...	...	[1] 2.37 ± 0.003	3.03
ΔE (eV)	−6.433 3		−7.0545		−6.690 3	
Fit Δχ ^{2,b}	0.077 55		0.1269		0.039 72	
F _M ^c	3.14		7.26		...	

^a[CN] is the coordination number; uncertainty in Cu–O distance is the e.s.d. calculated by the fit; systematic error in Cu–O is about $\pm 0.01 \text{ \AA}$.

^b $\Delta\chi^2 = [\sum (\text{data} - \text{fit})^2] / (\text{No. of points} - \text{No. of variables})$.

^c $F_M = [(\Delta\chi_1^2 - \Delta\chi_2^2) / (\nu_1 - \nu_2)] / (\Delta\chi_2^2 / \nu_2)$, where $\nu = N_{\text{ind}} - N_{\text{par}}$, and F_M was calculated relative to the split axial fit.^{67,68}

FIG. 4. (Points) Cu K-edge EXAFS [(a) and (c)] and Fourier transformed EXAFS [(b) and (d)] of 0.06M $\text{Cu}(\text{ClO}_4)_2$ with 0.6M HClO_4 in 40% aqueous frozen glass. Glasses are (top) 1,3-propanediol and (bottom) 1,5-pentanediol. Full blue line represents the fits using the split axial model, and dashed red line represents the unsplit residual.

well as in the FT spectra over $1.5 \text{ \AA} < R < 2.5 \text{ \AA}$. The metrics of the final fits are given in Table II. The EXAFS models of $[\text{Cu}(\text{aq})]^{2+}$ in the two frozen glasses are effectively identical. Also of interest is that the frozen glass EXAFS models are almost identical to the model of $[\text{Cu}(\text{aq})]^{2+}$ in liquid 1M HClO_4 solution, including the second shell.⁴² Thus, it appears that the frozen glasses did indeed trap the liquid state structure.

Figure 5 shows the consensus EXAFS model for $[\text{Cu}(\text{aq})]^{2+}$ in the frozen aqueous glasses, including a distributed array of four second-shell scatterers.

TABLE II. EXAFS structural models for $[\text{Cu}(\text{aq})]^{2+}$ in 40% frozen aqueous glass.

Scatterer	1,3-propanediol			1,5-pentanediol		
	[CN] ^a	R (Å) ^b	$\sigma^2 \times 10^3$ ^c	[CN] ^a	R (Å) ^b	$\sigma^2 \times 10^3$ ^c
O _{eq}	[4]	1.97 ± 0.001	4.31	[4]	1.97 ± 0.001	4.29
O _{ax1}	[1]	2.24 ± 0.002	2.11	[1]	2.22 ± 0.002	2.29
O _{ax2}	[1]	2.37 ± 0.003	2.75	[1]	2.37 ± 0.002	2.74
O _{w1}	[1]	3.20 ± 0.03	15.10	[1]	3.20 ± 0.02	14.79
O _{w2}	[1]	3.37 ± 0.02	8.14	[1]	3.37 ± 0.02	10.38
O _{w3}	[2]	3.81 ± 0.01	8.70	[1]	3.78 ± 0.01	2.94
O _{w4}	...	...	...	[1]	3.89 ± 0.01	2.87
Avg. 2nd shell (Å)		3.6 ± 0.3			3.6 ± 0.3	
ΔE (eV)		-6.378			-5.654	
F-value		0.1567			0.1141	

^a[CN] is coordination number.

^bUncertainties in Cu–O are the average statistical e.s.d.'s calculated by the fit. Systematic error is of order $\pm 0.01 \text{ \AA}$.

^cMean square displacement ($\text{\AA}^2$).

MXAN fitting results

The MXAN approach uses extended continuum multiple scattering (ECMS) theory to derive structural models from the first 200 eV of an XAS spectrum, including the XANES energy region.^{53,69,80–82} The XANES region is not affected by thermal disorder, which benefits MXAN structural precision. The 5–10 $\text{\AA}$ photoelectron mean free path and $1/kR$ intensity dependence of low energy XAS photoelectrons mean that

FIG. 5. The consensus split axial EXAFS model for $[\text{Cu}(\text{aq})]^{2+}$ in the 40% aqueous diol frozen glasses; see Table II for Cu–O distances. The four second-shell waters about Cu(II) have been distributed to alternate above and below the equatorial plane at their Cu–O EXAFS distances. Hydrogens were added to the illustration but were not included in the fits.

scattering features from distant atoms can appear in the XANES energy region, with cautionary implications to the assignment of rising K-edge features to two-electron processes.^{16,20,30,42,80,83,84}

In prior work, MXAN examination of the Cu K-edge XAS of 0.1M $\text{Cu}(\text{ClO}_4)_2$ with 1M HClO_4 in both liquid and frozen aqueous solutions invariably found evidence of two core axially elongated square pyramidal structures, in a near 1:1 ratio. These are $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ ($\text{Cu}-\text{O}_{\text{ax}} = 2.23 \pm 0.11 \text{ \AA}$) with no discernable solvation shell, coexisting with $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ having both a shorter axial distance ($\text{Cu}-\text{O}_{\text{ax}} = 2.06 \pm 0.07 \text{ \AA}$) and a distant axial but non-bonded water molecule ($\text{Cu}-\text{O}_{\text{w}} = 3.0 \pm 0.2 \text{ \AA}$) as well as eight second-shell water molecules ($\text{Cu}-\text{O}_{\text{w}} = 3.8 \pm 0.1 \text{ \AA}$). These two sites must be in dynamical equilibrium.^{43,85,86} The same MXAN analyses had definitively excluded single-site axially elongated square pyramidal or JT-octahedron models. Extended range ($k = 2\text{--}18 \text{ \AA}^{-1}$) EXAFS analyses also excluded these latter two models.^{27,30,42}

Thus, it no longer seemed necessary to again here consider either of the single-site $[\text{Cu}(\text{aq})]^{2+}$ ligation environments that were excluded by previous work. The present MXAN approach to $[\text{Cu}(\text{aq})]^{2+}$, therefore, was to separately consider the two square pyramidal sites, i.e., the square pyramidal $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ alone, and $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ with a distant axial water molecule and a second shell of eight water molecules. These were tested first as individual single sites and then conjointly within a two-site model. This approach was described in detail previously.⁴²

Single-site MXAN fits

Figure 6 shows the XAS spectrum of $[\text{Cu}(\text{aq})]^{2+}$ in the 1,3-propanediol glass with a test of each of the two single-site pyramidal models (see Table III for the fit results). The difference in R_{sq} values is significant, indicating that in the frozen aqueous glass the split axial plus second-shell model is a better representation of $[\text{Cu}(\text{aq})]^{2+}$ than the bare square pyramidal model. Within liquid HClO_4 solution, by contrast, these two models produced fits of nearly equivalent quality ($R_{\text{sq}} = 1.96$ and 2.12 , respectively).

The final split axial model also included a surprisingly short Cu–O axial distance of $1.91 \pm 0.06 \text{ \AA}$ (Table III) which is indistinguishable from the four equatorial Cu–O distances. This makes the inner coordination environment an almost perfect square pyramid. The longer $2.17 \text{ \AA}$ axial distance in the split axial model is also notable in that it is much shorter than the second axial distance in any of the other split axial structures previously reported for $[\text{Cu}(\text{aq})]^{2+}$, including in frozen aqueous solution. Each of the three axial distances in Table III is associated with a relatively large uncertainty, implying the positional variation due to the contribution of the alternative structures to the total XAS spectrum. For example, it is certain that the axial waters dynamically exchange distances within the Cu(II) rotational correlation time.^{87–90}

The same two individual structures were also tested in MXAN fits to the K-edge XAS of $[\text{Cu}(\text{aq})]^{2+}$ in the 1,5-pentanediol frozen glass (see Fig. S5 and Table S1 of the supplementary material). A similar spread of split axial and

FIG. 6. (Points) The copper K-edge XAS spectrum of 0.06M $\text{Cu}(\text{ClO}_4)_2$ in 0.6M HClO_4 in 40% 1,3-propanediol frozen glass. MXAN fits: (top, blue line) the split axial model plus second-shell model and (bottom, red line) the bare elongated square pyramidal model. See Table III for the fit metrics.

square pyramidal R_{sq} values was obtained (1.94 and 3.75, respectively). The axial Cu–O distances in the former model ($2.12 \pm 0.04 \text{ \AA}$ and $2.29 \pm 0.05 \text{ \AA}$) are longer than those within the 1,3-propanediol frozen glass and are much closer to the axial Cu–O bond lengths typical of the JT-octahedral crystalline aquaions.⁹¹

Thus in both frozen glasses the split axial structures are fully and uniquely 6-coordinate despite the internally disparate

TABLE III. MXAN single-site metrics for $[\text{Cu}(\text{aq})]^{2+}$ in 1,3-propanediol frozen glass.

	Split axial		Square pyramidal	
	CN ^a	R (Å)	CN ^a	R (Å)
Water				
(H ₂ O) _{eq}	4	1.95 ± 0.02	4	1.94 ± 0.01
(H ₂ O) _{ax1}	1	1.91 ± 0.06	1	2.18 ± 0.08
(H ₂ O) _{ax2}	1	2.17 ± 0.05	...	...
Second shell H ₂ O ^b	8	3.5 ± 0.3^c	...	...
$\angle \text{O}_{\text{ax}}\text{--Cu--O}_{\text{eq}}$ (deg) ^d		92 ± 5		94 ± 0.5
R_{sq}		1.90		4.07

^aCoordination number.

^bSystematic uncertainty in the second-shell water distances is about $\pm 0.1 \text{ \AA}$.

^cThe second-shell distance variation of eight water molecules.

^dBond angle systematic uncertainty is $\pm 2^\circ$.

axial bond lengths. In addition, the eight second-shell water molecules in the 1,5-pentanediol frozen glass averaged a somewhat more distant 3.8 ± 0.4 Å from copper (cf. Table III) relative to the 1,3-propanediol glass. By contrast, the square pyramidal models in the two frozen glasses are very comparable. Nevertheless, none of the structures can be described as a Jahn-Teller axially elongated octahedron.

Two-site MXAN fits

Following these initial single-site fitting experiments, the structure of $[\text{Cu}(\text{aq})]^{2+}$ in the frozen glassy solutions was explored by conjoint fitting over the prior two individual sites. Briefly, in conjoint MXAN fits, two structurally distinct sites are included in an iterative fitting routine. Each of the two sites in the fit has half-unit weight, implying a 1:1 site structural ratio. In a first step, one site structure is iteratively relaxed to achieve a local minimum in R_{sq} , while the second site structure is held constant. In the second step of the first round, the first site is now held fixed in its fitted relaxed state, while the second site is iteratively relaxed to a new local minimum in R_{sq} . Each full round yields two R_{sq} values, and each R_{sq} reflects the overall improvement in the fit for that half-step. In a successful fit, the individual R_{sq} values will decrease and converge toward a single value. Typically, this MXAN two-site fitting approach

TABLE IV. MXAN two-site fit metrics for $[\text{Cu}(\text{aq})]^{2+}$ in 40% diol frozen glasses.

40% 1,3-Propanediol frozen glass				
Ligand	Split axial		Square pyramidal	
	CN ^a	R (Å)	CN ^a	R (Å)
(H ₂ O) _{eq}	4	1.95 ± 0.01	4	1.95 ± 0.01
(H ₂ O) _{ax1}	1	1.93 ± 0.03	1	2.23 ± 0.08
(H ₂ O) _{ax2}	1	2.22 ± 0.04	...	...
Second shell H ₂ O ^b	8	3.5 ± 0.3 ^c	...	...
∠O _{ax} –Cu–O _{eq} (deg) ^d	93 ± 3.5		96 ± 0.4	
R _{sq}	1.75		1.74	
40% 1,5-Pentanediol frozen glass				
(H ₂ O) _{eq}	4	1.94 ± 0.01	4	1.95 ± 0.01
(H ₂ O) _{ax1}	1	2.14 ± 0.11	1	2.24 ± 0.06
(H ₂ O) _{ax2}	1	2.28 ± 0.10	...	...
Second shell H ₂ O ^b	8	3.8 ± 0.4 ^c	...	...
∠O _{ax} –Cu–O _{eq} (deg) ^d	89 ± 8.8		97 ± 1.8	
R _{sq}	1.82		1.81	

^aCoordination number.

^bSystematic uncertainty in the second-shell water distances is about ± 0.1 Å.

^cThe second-shell distance variation of eight water molecules.

^dThe split axial angles are relative to the shorter axial ligand. Bond angle systematic uncertainty is $\pm 2^\circ$.

FIG. 7. (Points) The copper K-edge XAS of 0.060M $\text{Cu}(\text{ClO}_4)_2$ with 0.6M HClO_4 as a frozen glass in (top) 40% 1,3-propanediol or (bottom) 40% 1,5-pentanediol. The lines are the two-site MXAN fits. See the text for details. Insets show the XANES energy region of the XAS spectra and the fits. The fit metrics appear in Table IV.

converged after about five rounds. In prior work, two structural sites were found for dissolved $[\text{Cu}(\text{aq})]^{2+}$ in both liquid and frozen aqueous solutions.⁴²

For the two-site fitting experiments exploring Cu(II) in the frozen glasses, the two “first-guess” candidate structures were the axially elongated square pyramid and the split axial model with eight water molecules in a second solvation shell, as were found present in liquid water solution.⁴² This choice was deemed reasonable on the grounds of the similarity with the XANES and EXAFS spectra of Cu(II) in the 40% frozen glasses (cf. Fig. 1 and the section titled Discussion). Additionally, this choice was made to avoid biasing the two-site fits with the single-site frozen glass structures.

As noted previously, the inability to discern a solvation shell in the fit using the square pyramidal model implies

FIG. 8. Left, the axially elongated square pyramidal model, and, right, the six-coordinate structural model that together represent the dominant forms of dissolved Cu(II) with 0.06M HClO_4 in the 40% aqueous 1,3-propanediol frozen glass. The depressed equatorial $\text{O}_{\text{ax}}\text{--Cu--O}_{\text{eq}}$ angles are visually apparent.

FIG. 9. Left, the axially elongated square pyramidal model and, right, the six-coordinate structural model that together represent the dominant forms of dissolved Cu(II) with 0.06M HClO₄ in the 40% aqueous 1,5-pentanediol frozen glass. The non-planarity of the equatorial water ligands is again visually apparent.

only that solvating waters were undetectable, rather than not present. In water solution, solvation shells are necessarily present. Lack of detection in the MXAN fits implies that the second shell is disorganized. In this case, the photo-electron back-scattering waves emerging from this shell are out of phase and self-damping. It was previously found that the second shell improved the MXAN fits only when the [Cu(aq)]²⁺ model included an associated but non-bonded water molecule in the trans-axial position. As before, the first guess models were alternately relaxed during the two-step iterative fits. Inclusion of the two structural models improved the fits and produced lower R_{sq} values, Fig. 7. The coordination numbers and Cu–O bond lengths obtained from these fits are given in Table IV.

The previously developed⁵⁹ error metric $fe(E) = \sqrt{(y_i^{th}(E) - y_i^{exp}(E))^2}$ was calculated for each fit to further evaluate the relative improvement of the two-site fits over single-site fits. The results for the 1,3-propanediol and the 1,5-pentanediol frozen glasses are presented in Figs. S8 and S9 of the [supplementary material](#), respectively. Improvement over the single-site fits is obvious in the XANES energy region ($E - E_0 = -7.5$ –30 eV). However, improvement over the single site CN6 model is uneven throughout the lowest rising edge energy region and at $E - E_0 > 100$ eV. This mixed result is reflected in the relatively small comparative improvement in R_{sq} of the two-site MXAN fit over that of the single-site split axial fit.

The two structural sites derived for [Cu(aq)]²⁺ in 40% 1,3-propanediol frozen glass are shown in Fig. 8. Figure 9 presents the two fitted structures best representing [Cu(aq)]²⁺ in the 40% 1,5-pentanediol frozen glass.

DISCUSSION

The results reported here establish a revision of the 60-year standing default view of the coordination environment of Cu(aq)²⁺. Namely, they complete a demonstration that the Jahn-Teller octahedron does not describe the coordination environment of Cu(aq)²⁺ under any set of aqueous conditions.

Apart from other considerations, this work also introduces 1,3-propanediol and 1,5-pentanediol as new glassing agents that neither coordinate metal ions under ordinary conditions nor apparently change the metal ion immediate solvation environment (see below and Fig. S10 of the [supplementary material](#)). Such glassing agents may have general utility in studies of solution structure, especially in acquiring low-temperature XAS spectra of metalloproteins.

A coherent interpretation of structural differences between dissolved [Cu(aq)]²⁺ in liquid and frozen glass phases requires that, initially, the initial liquid state solution structure of [Cu(aq)]²⁺ in, e.g., 40% 1,5-pentanediol was identical with or extremely similar to that in liquid 1M HClO₄. In this regard, Fig. S10 of the [supplementary material](#) compares the room temperature liquid solution K-edge XAS spectra of 1M Cu(ClO₄)₂ in 1M HClO₄ with that of liquid-phase 0.06M Cu(ClO₄)₂ with 0.6M HClO₄ and 40% 1,5-pentanediol. The 1,5-pentanediol solution was chosen for this comparison because the additional methylene groups of that additive are more disruptive of the native structure of liquid water, relative to the 1,3-propanediol additive.^{92,93} Figure S10 of the [supplementary material](#) compares the XAS spectra of [Cu(aq)]²⁺ in 1M HClO₄ solution and in the 1,5-pentanediol solution with 0.6M HClO₄. The XANES spectra are nearly superimposable [Fig. S10 of the [supplementary material](#), inset (a)], implying very similar electronic bound states and thus ligand environments. In the continuum energy region, the difference XAS displays negligible residual oscillations, again indicating very small structural differences in the extended radial environment of [Cu(aq)]²⁺ in these two media.

To test this idea further, two-site MXAN fits to the XAS of [Cu(aq)]²⁺ in liquid aqueous 40% 1,5-pentanediol were carried out (see Fig. S11 of the [supplementary material](#)). The fits yielded structures identical to those found in liquid aqueous solution, showing that prior to freezing [Cu(aq)]²⁺ dissolved in 40% 1,5-pentanediol occupied ligation and solvation states virtually identical to those within liquid 1M HClO₄ alone (Fig. S12 and Table S3 of the [supplementary material](#)). This conclusion can be extended by default to [Cu(aq)]²⁺ in 1,3-propanediol on XAS spectroscopic grounds (cf. Fig. 1). The near identity of the initial ligand and solvation environments allows a coherent structural comparison of [Cu(aq)]²⁺ in liquid solution and frozen glass.

In each of the two frozen glass solutions, [Cu(aq)]²⁺ is distributed between axially elongated square pyramidal and six-coordinate split-axial ligand environments. A second shell of water was detectable only around the six-coordinate site. However, neither of the CN6 frozen glass structures included the 3 Å axially associated water of [Cu(aq)]²⁺ in liquid aqueous 1 M HClO₄. The 3 Å axial perchlorate of [Cu(aq)]²⁺ in frozen 1 M HClO₄ solution was also missing. The absence of perchlorate in the glassy solution structures, despite that $[HClO_4]_{total} = 0.72M$, indicates the non-migration of solutes during freezing of the viscous diol solutions. Nevertheless, the discerned environments are clearly derived from the homologous structures of [Cu(aq)]²⁺ in liquid 1M HClO₄ solution.⁴²

The square pyramidal structures within the two frozen glass environments are identical within the uncertainty bounds

of the fits. Their equatorial and axial distances fall within known limits,^{91,94} and in both cases, the equatorial water ligands are bent about 4° below the formal $d_{x^2-y^2}$ plane of Cu(II). Within error, the two frozen glass square pyramidal structures are also identical with the analogous site in liquid aqueous solution ($4 \times \text{Cu}-\text{O}_{\text{eq}} = 1.95 \pm 0.02$ Å, $1 \times \text{Cu}-\text{O}_{\text{ax}} = 2.23 \pm 0.11$ Å).⁴² All four frozen glass structures include one axial Cu–O distance near 2.24 Å.

However, the two frozen glass CN6 ligand environments are strikingly different, both from one another and from the split axial structure found in liquid solution. In the 1,3-propanediol glass, the shorter axial distance is identical within the fit uncertainty to the equatorial Cu–O distances. This structure may be described as a mono-capped regular square pyramid. The longer Cu–O = 2.22 Å axial bond provides the JT-asymmetry, breaking the $d_{x^2-y^2}$, d_{z^2} degeneracy, and is identical within error with the square pyramidal axial bond length.

By contrast, in the 1,5-pentanediol glass, the shorter axial Cu–O distance is 2.14 Å and, combined with the longer 2.28 Å axial water ligand, yields an axially asymmetric CN6 $[\text{Cu}(\text{aq})]^{2+}$. Both frozen glass CN6 structures are split axial; neither can be described as an axially elongated octahedron. Likewise, both CN6 structures exhibit significant excursions in the $\text{O}_{\text{ax}}-\text{Cu}-\text{O}_{\text{eq}}$ angles, especially that in the 1,5-pentanediol glass.

Both inner shell Cu–O distances and the second-shell water molecules are more distant from copper in the 1,5-pentanediol glass, relative to those in the 1,3-propanediol glass. In addition, the systematic uncertainties in the axial bond lengths for $[\text{Cu}(\text{aq})]^{2+}$ within the 1,5-pentanediol glass are about three times the larger. This difference gains significance upon noting that the equatorial bond lengths in both frozen glass phases exhibit very similar, and small, systematic uncertainties. Thus, these larger uncertainties in the axial bond lengths are not reflective of a lower quality fit. The axial distinction may indicate a significantly greater spread of axial bond lengths in the 1,5-pentanediol medium.

There is no obvious reason why $[\text{Cu}(\text{aq})]^{2+}$ should inhabit different structural sets in the two alternative frozen glass phases. The MXAN fit of liquid-state 40% 1,5-pentanediol noted above strongly implies that both glassy complex ions began from the $[\text{Cu}(\text{aq})]^{2+}$ ligand environment of water itself. The frozen glass complexes have the identical ligand array. Their inequivalent ligand configurations must therefore reflect discrepant forces within the frozen glasses. Such discrepancies may arise from microscopic or fluctuational variances between the freezing mechanisms. Nevertheless, it seems clear that relatively small environmental differences can have a large effect on the ligation environment of Cu(II).

Unusual crystalline $[\text{Cu}(\text{aq})]^{2+}$ structures include $(\text{H}_3\text{O})[\text{Cu}(\text{H}_2\text{O})_5](\text{ClO}_4)_3$, which exhibits an axially elongated square pyramid of $\text{Cu}-\text{O}_{\text{eq}} = 2 \times 1.97$ Å, 2×2.00 Å, with $\text{Cu}-\text{O}_{\text{ax}} = 2.31$ Å and $\text{O}_{\text{ax}}-\text{Cu}-\text{O}_{\text{eq}} = 90.1^\circ \pm 1.1^\circ$. In this complex, a 3.3 Å bidentate $\text{Cu}-\text{O}_2\text{ClO}_2$ axial interaction completes the nearest neighbor array.⁹⁵ $[\text{Cu}(\text{H}_2\text{O})_6](\text{BrO}_3)_2$ crystallizes as a completely regular octahedron with 6×2.079 Å Cu–O distances. The hexaaquadinitrate complex exhibits an unusually distorted octahedron with a short average

$\text{Cu}-\text{O} = 2.052 \pm 0.027$ Å (range 2.014–2.084 Å). However no known crystalline aqua complex of Cu(II) resembles the low symmetry structures found to typify $[\text{Cu}(\text{aq})]^{2+}$ in the frozen glass phases.

The structure of solution-phase $[\text{Cu}(\text{aq})]^{2+}$ has now been determined in four distinct environments. These are liquid and frozen 1M HClO_4 and the two frozen glass samples reported here. The structure of $[\text{Cu}(\text{aq})]^{2+}$ is at least bimodal in every phase investigated. Figure 10 provides a visual summary of these structures. PDB-format files of all these structures plus those reported here are included with the [supplementary material](#).

Among these structures, the three square pyramidal structures distributed among liquid solution and the two frozen glasses are all nearly identical, including the axial Cu–O distances and the equatorial bond angles. The solution-phase pyramidal sites did not acquire a distant axial perchlorate in the frozen glass.

The split axial structure in liquid solution displayed what was, at the time, an unexpectedly short axial bond length of 2.06 ± 0.07 Å. The other axial position included only a distant 3 Å interaction with a non-bonded water molecule. It is now seen that the 1,3-propanediol freezing process evidently reduced the 2.06 Å distance to 1.93 Å and brought the 3 Å water to within a 2.22 Å axially bonding distance. In some contrast, freezing the 40% 1,5-pentanediol solution lengthened the liquid solution short axial distance slightly, but again moved the initially 3 Å axial water into bonding distance. As noted above, these differences between the two frozen glass structures apparently imply that microscopic variations in the energetic environment provided by the frozen solvent can induce alternative structural ground states for $[\text{Cu}(\text{aq})]^{2+}$. That is, the 0.35 Å spread in axial bond distances implies a potential range of structures in which the ground state of the complex ion depends on the configurational details of the frozen medium. Furthermore, variation in axial distances dominates the structural divergence but can be accompanied by some variation in equatorial distances and angles.

All these variations demonstrate the structural plasticity of dissolved Cu(II), a trait that has already been extensively discussed with respect to crystalline solids.^{47,91,96} It is become clear that Cu(II), unconstrained by chelating ligands, can entertain a variety of axial bond lengths and several degrees of equatorial non-planarity. $[\text{Cu}(\text{aq})]^{2+}$ can also migrate between CN5 and axially asymmetric CN6 ground states in response to environmentally determined energetic gradients.

The observed structural shifts corroborate elements of recent molecular dynamics (MD) simulations, carried out at the TIP5P level of theory. These *in silico* simulations predicted dissolved $[\text{Cu}(\text{aq})]^{2+}$ to be about 55% CN6 and 43% CN5, in dynamical exchange. This equilibrium implies an energetic difference of only about 0.15 kcal mol^{−1} separating the two structures. The CN6 form in the MD simulation was structurally split axial, with average Cu–O_{ax} distances of 2.24 Å and 2.61 Å.⁴³ The 2.61 Å axial water was loosely bound, with the Cu–O_{ax} distance ranging across 2.3–3.6 Å (median 2.47 Å). The 2.61 Å average is identical to the Shannon Cu(II)–O ionic limit,⁹⁷ implying a generally electrostatic interaction. The extensive Cu–O_{ax} range of the 2.61 Å water

FIG. 10. The bimodal structures of dissolved $[\text{Cu}(\text{aq})]^{2+}$. Top: panels [(a1) and (a2)] in liquid 1M HClO_4 and panels [(b1) and (b2)] in frozen 1M HClO_4 . Bottom: panels [(c1) and (c2)] with 0.6M HClO_4 in 40% 1,3-propanediol frozen glass and panels [(d1) and (d2)] with 0.6M HClO_4 in 40% 1,5-pentanediol frozen glass. In the liquid and frozen 1M HClO_4 solutions [panels (a2), (b1), and (b2)], the more distant axial oxygen is non-bonded and about 3 Å from $\text{Cu}(\text{II})$. The [supplementary material](#) includes pdb files of these structures.

molecule indicates that even this CN6 MD structure was, at times, a square pyramid with a non-bonded but axially associated water molecule. Other MD studies also have indicated a bimodal CN5, CN6 solution structural distribution.⁸⁵ However, still other MD simulations have predicted preference for CN5 or CN6 alone, with the difference in result apparently depending upon the theory used.^{29,39,98–101}

These considerations lead to an explanation for the consistently lower magnitudes of the EXAFS σ^2 values of the axial water ligands, relative to those of the far more strongly bonded equatorial waters (cf. Tables I and II). Recent molecular dynamics simulations have indicated that the radial distributions (RD) of the axial water distances are not likely to be Gaussian (see Fig. 4 in Ref. 43).⁴³ From the perspective of theory, if the RD of the $\text{Cu}(\text{II})\text{--OH}_2$ angular distance is not Gaussian, the simple EXAFS treatment in terms of $\exp(-k^2\sigma^2)$ is no longer accurate.^{102,103} This being true, the EXAFS fitted axial σ^2 values lose their physical significance. Corrections to account for an asymmetric RD of bond distances can significantly increase the magnitude of a fitted σ^2 .

Furthermore, the $[\text{Cu}(\text{aq})]^{2+}$ complex ion is invariably structurally heterogeneous. MXAN analysis has shown that the $[\text{Cu}(\text{aq})]^{2+}$ complex ion occupies at least two co-existent structures in every tested aqueous medium.^{37,42} The fractional distribution of these two structures is always nearly 1:1, and the radial inequity is always concentrated in the axial distances. In such cases, EXAFS fits yield parameters adjusted to reflect a non-existent average structure. This circumstance must

necessarily produce composite fitted distances and unreliable σ^2 values.

In fits to the copper K-edge EXAFS of $[\text{Cu}(\text{L}_4)(\text{H}_2\text{O})]^{2+}$ complex ions in liquid aqueous solution, where $\text{L} = \text{NH}_3$ or imidazole, the axial σ^2 values were well-behaved,^{59,60} almost certainly because these two complex ions are structurally homogeneous, unlike $[\text{Cu}(\text{aq})]^{2+}$. In Tables I–IV, all the equatorial EXAFS and MXAN $\text{Cu}\text{--OH}_2$ distances are the same within error. On the other hand, not one of the axial $\text{Cu}\text{--OH}_2$ EXAFS distances is the same as its MXAN analog. However, for both nitrogenous complex ions, the EXAFS and MXAN fitted axial $\text{Cu}\text{--NH}_3$ and $\text{Cu}\text{--ImH}$ distances were nearly the same within 1 σ error. This comparison is shown in Tables S3–S7 of the [supplementary material](#).

The extended continuum multiple scattering theory employed within MXAN automatically includes all multiple scattering pathways. For this reason, MXAN distances are more accurate (if not more precise) than those derived from EXAFS fits. Thus, by the criteria of the more complete theory and the axial inhomogeneity of the ligands, the disparate EXAFS $\text{Cu}\text{--OH}_2$ axial distances are judged unreliable. The corresponding $[\text{Cu}(\text{aq})]^{2+}$ axial σ^2 values are thus also unreliable. See the [supplementary material](#) for more details.

Application to metallo-biochemistry

We turn here to appraise the immediate biochemical implications of the very significant excursions of solution phase

Cu(II) complex ions away from the canonical JT-octahedral symmetry. The rack-bonding theory of blue copper metalloproteins has as its foundational assumption that Cu(II) has a strict preference for four rigidly planar equatorial ligands.^{47,104,105} The results reported in this work and previous work indicate that unconstrained Cu(II) instead has a clear preference for square pyramidal ligand arrays, with slightly depressed equatorial bond angles.^{10,27,30,42,59,60,106} The notably irregular ligation of unconstrained Cu(II) thus directly gainsays the basis of rack-bonding theory.^{49,104,105} In addition, the low thermodynamic stability of the protein fold makes the notion of protein-imposed racks a very uncertain explanation for the low-symmetry blue copper site.^{30,37} Rather, the idea that the unusual ligand array of blue copper protein metal sites is an unstrained ground state for both copper oxidation states, first advanced by Dorfman *et al.*,¹⁰⁷ is sufficient to explain the biophysical system.^{30,37,108–112} This notion of an unstrained ground state in both copper redox states also includes the idea that the high covalence of the Cu(II)–S_{Cys} bond^{37,107,108,113,114} restricts the metal redox valence change to about 0.6 e[−].¹¹³ In this light, it is suggested that the structural plasticity of Cu(II) described here along with the relatively small biological redox change (Cu¹⁺ to Cu^{1.6+}) of blue copper proteins permits a unitary and unstrained ground-state structure that is identical for the two oxidation states.^{30,37}

The above notion of an unstrained metal site directly obviates the need to invoke protein imposition in order to explain the retention of weakly bound active site ligands. When the protein active site geometry is also the thermodynamic ground state, weakly bound ligands are not energetically disposed to dissociate from the active site metal ion. Given a shallow but negative energetic gradient, the retention of weakly bound ligands is readily understood when it is noted that protein amino acid side chain groups are unable to diffuse away. This check on ligand departure is produced by the secondary and tertiary structure of the protein without any energetic cost and produces a very large effective ligand concentration at any metalloprotein active site.

Non-diffusion of ligands requires a statistical adjustment of standard equilibrium thermodynamics. The tertiary structure of the blue copper proteins azurin or plastocyanin (Pc) places an active site methionine thioether permanently within about 5 Å from the open bonding face of Cu(II), with no intervening solvent molecules. From a solution equilibrium perspective, the local persistence of the ligand equates to a very high solution concentration.

To model ligand persistence in terms of solution equilibria, a construct can be envisioned that includes a cube of 10 Å per diagonal around a tetrahedral site, with Cu(II) at the center. All eight points of the 10 Å diagonal cube must be occupied by thioether to assure the 100% ligand availability, as provided by the protein, to any arbitrary open bonding site on a coordinatively unsaturated tetrahedral Cu(II). The statistically effective ratio of thioether to copper is thus 8:1.

The strength of the Cu(II)–Met₉₂ thioether bond in plastocyanin (Pc) has been experimentally determined to be ΔH = −4.6 kcal mol^{−1}.^{115,116} Assuming the entropy of transition from free to bound methionine sulfur can be approximated by the per-atom maximum entropy of an alloy order/disorder

conversion, $k_B \ln 2/\text{atom} = -0.41 \text{ kcal mol}^{-1}$ at 298 K,¹¹⁷ a binding ΔG = −4.2 kcal mol^{−1} for Cu(II)–SMet₉₂ is found. This produces K_{eq} = 1.15 × 10³ mol^{−1} at 298 K. Equilibrium consideration yields

$$\text{Cu}^{\text{II}} + \text{Met}_{92}\text{SCH}_3 \rightleftharpoons [\text{Cu}^{\text{II}}\text{S}(\text{CH}_3)\text{Met}_{92}]$$

and

$$K_{eq} = \frac{[\text{Cu}^{\text{II}}\text{S}(\text{CH}_3)\text{Met}_{92}]}{[\text{Cu}^{\text{II}}][\text{Met}_{92}\text{SCH}_3]} = 1.15 \times 10^3 \text{ mol}^{-1}. \quad (4)$$

Let “x” be the fraction of bound Cu(II), “1-x” be the fraction of unbound Cu(II), and “8-x” be the fraction of unbound thioether at the statistically effective metal-site. Then,

$$1.15 \times 10^3 = \frac{x}{(1-x)(8-x)}. \quad (5)$$

Solving the quadratic yields a physically meaningful root at x = 1.00. Thus ΔG = −4.2 kcal mol^{−1} yields a Cu(II)–Met₉₂ binding site occupancy of 100% in Pc at physiological temperature (298 K) without any need for a protein rack. The similar calculation for Cu(I)–SMet₉₂, for which ΔH = −1 kcal mol^{−1},¹¹⁶ yielded an occupancy of 95% at 298 K even for this very weak bond. Thus, ligands that cannot diffuse away do not require enforced bonding even when the thermodynamic syncline is shallow. High effective ligand concentration combined with the observed structural plasticity of Cu(II) explains most of the biophysics of the blue copper site, with high Cu(II)–S_{Cys} covalence accounting for the rest.^{30,37,107,108,110,113}

Consideration of ligand non-diffusion can be extended to other redox protein metal sites, such as the iron site of cytochrome c (cyt c). In this metalloprotein, the Fe(II)–S_{Met} and Fe(III)–S_{Met} bond enthalpies have been estimated as ΔH = −2.6 and −5.5 kcal mol^{−1}, respectively.¹¹⁸ The same maximum entropy approach yields estimated equilibrium binding −ΔG values of 2.19 kcal mol^{−1} and 5.09 kcal mol^{−1}, respectively. The equivalent full occupancy 5 Å statistical model will assign one thioether sulfur to each of the four corners of a square with sides of 4.085 Å, centered over the single open face of heme iron. This produces a statistical thioether/iron ratio of four. The equivalent equilibrium calculations yield unforced Fe–S_{Met} occupancies of 0.99 and 1.00 for the Fe(II) and Fe(III) site, respectively.

High bond occupancy is thus always achieved spontaneously when ligands cannot diffuse away from a shallow thermodynamic syncline into a weak bond. This condition is attained without the need of any protein-enforced constraint or any imposed rack and at no energetic cost to the protein. Similar considerations should apply to the amino-acid ligand of any protein metal site that exhibits even a relatively small negative thermodynamic gradient favoring bond formation. Unforced high ligand occupancy is the only metal active site bonding model that is completely consistent with the very low thermodynamic stability of the protein fold, a fold that is incapable of applying or sustaining the large thermodynamically unfavorable +ΔG required by the rack model.^{30,37} The evolutionary choice of a specific transition metal, copper, which is uniquely able to sustain both biological redox states within an unstrained and unitary ground state structure, is the source of the of blue copper protein “*entasis*” that facilitates rapid electron transfer. No energetic poise is needed.

In conclusion, this XAS study has extended to frozen glasses the previous finding that unconstrained dissolved $[\text{Cu}(\text{aq})]^{2+}$ is structurally diverse. MXAN analysis, in particular, has revealed that $[\text{Cu}(\text{aq})]^{2+}$ alternates among apparently nearly iso-energetic structural states. Migrations between states exchange coordination number and modify the distances and angles of the immediate ligation sphere both within and between aqueous phases.⁴² The same apparently holds true for $[\text{Cu}(\text{solv})]^{2+}$ in non-aqueous media.^{61,62} These new findings have fundamental bearing on the associative/dissociative mechanism of Cu(II) solvent exchange,^{90,119} the thermodynamics of the Cu(I)/Cu(II) self-exchange rate and solvent reorganization,^{120,121} and the prior question of the low symmetry sites in copper redox proteins.^{30,37,42}

Across this series of liquid and frozen solutions and the two frozen aqueous glasses, the immediate ligand environment of $[\text{Cu}(\text{aq})]^{2+}$ favors the CN5 pyramid, the Cu–O axial distances vary across 1 Å, and the equatorial angles depart significantly from the square plane. Although all the structures remove the $d_{x^2-y^2}$, d_{z^2} degeneracy, no structure can be described as a Jahn-Teller axially elongated octahedron. The JT-octahedral consensus for dissolved liquid-phase $[\text{Cu}(\text{aq})]^{2+}$ should thus be abandoned in favor of a core CN5 axially elongated $[\text{Cu}(\text{H}_2\text{O})_5]^{2+}$ square pyramid. In liquid solution and about half the time, the CN5 structure associates with a distant (~ 3 Å) axial water molecule, with concomitant organization of the immediate solvation second shell. After more than 60 years of study, $[\text{Cu}(\text{aq})]^{2+}$ still yields new insights that ramify across the disciplines of modern chemistry.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the following: Fig. S1: Equatorial 1,3-propanediol chelate of aqueous Cu(II); Cu(pd)(H₂O)₄ Model.xyz. Fig. S2: Cu(II) plus 1,3-propanediol: simulated and measured EXAFS spectra. Fig. S3: Cu K-edge XAS in 1,3-propanediol and 1,5-pentanediol. Fig. S4: Fourier windows for back-transformation. Fig. S5: First-shell EXAFS fits. Table S1: Alternative EXAFS models; $[\text{Cu}(\text{aq})]^{2+}$ in 1,3-propanediol frozen glass. Fig. S7: MXAN fits; Cu K-edge XAS in 40% 1,5-pentanediol frozen glass. Table S2: MXAN single-site metrics; $[\text{Cu}(\text{aq})]^{2+}$ in 1,5-pentanediol frozen glass. Fig. S8: The $fe(E)$ metric; MXAN fit of $[\text{Cu}(\text{aq})]^{2+}$ in 1,3-propanediol frozen glass. Fig. S9: The $fe(E)$ metric; MXAN fit of $[\text{Cu}(\text{aq})]^{2+}$ in 1,5-pentanediol frozen glass. Fig. S10: Comparison of XAS $[\text{Cu}(\text{aq})]^{2+}$ in liquid water solution or 40% 1,5-pentanediol. Fig. S11: MXAN fit: $[\text{Cu}(\text{aq})]^{2+}$ in liquid 40% 1,5-pentanediol solution. Table S3: MXAN fit metrics for $[\text{Cu}(\text{aq})]^{2+}$ in liquid 1,5-pentanediol solution. Fig. S12: Structures of $[\text{Cu}(\text{aq})]^{2+}$ in liquid 40% 1,5-pentanediol solution. Cu_Liq_CN5.pdb; Cu_Liq_CN5+1.pdb; Cu_Froz_CN5+1.pdb; Cu_Froz_CN5+ClO4.pdb; Cu_1,3-P_CN5.pdb; Cu_1,3-P_CN6.pdb; Cu_1,5-P_CN5.pdb; Cu_1,5-P_CN6.pdb; PDB file description.docx.

ACKNOWLEDGMENTS

The authors thank Dr. Adam Tenderholt for his critical review prior to submission. This work was supported by Grant

No. P41GM103393 (KOH). Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research and by the National Institutes of Health, National Institute of General Medical Sciences (including Grant No. P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

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