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Supporting information for article:

Structure and mechanism of copper–carbonic anhydrase II: a nitrite reductase

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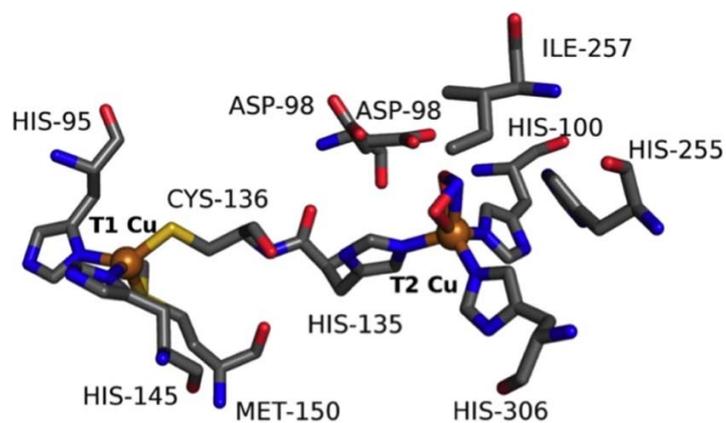


Figure S1 T1 and T2 Copper Binding Sites in *Achromobacter cycloclastes* Cu Nitrite Reductase. T1 and T2 copper sites with endogenously bound NO₂⁻ in T2 site. Adapted from Li *et al.* (2015) with permissions. Copyright 2015 American Chemical Society.

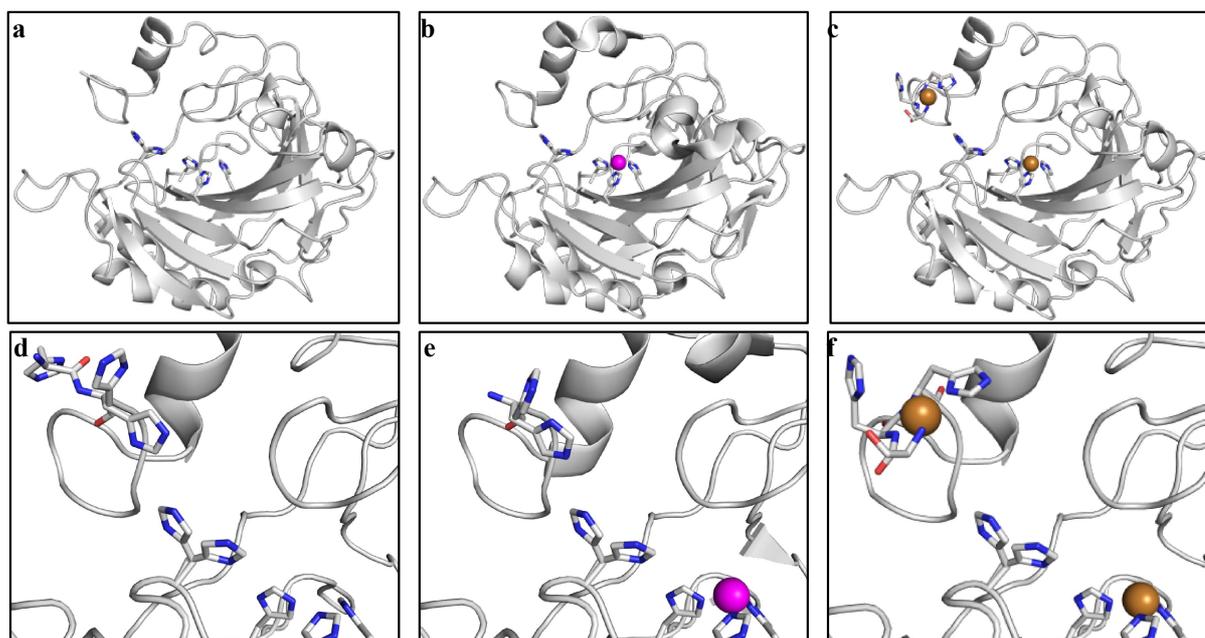


Figure S2 Metal binding in CA II. **A** and **D** Apo-CA II after treatment with DCA. The active site is empty and the N-terminus is disordered. **B** and **E** the Zn-CA II active site with zinc chelated by H94, H96, and H119. The N-terminus is disordered with density only for H4. **C** and **F** Cu-CA II with metal bound at both the T1 and T2 sites. The N-terminus is ordered around the copper atom, forming a ATCUN binding site.

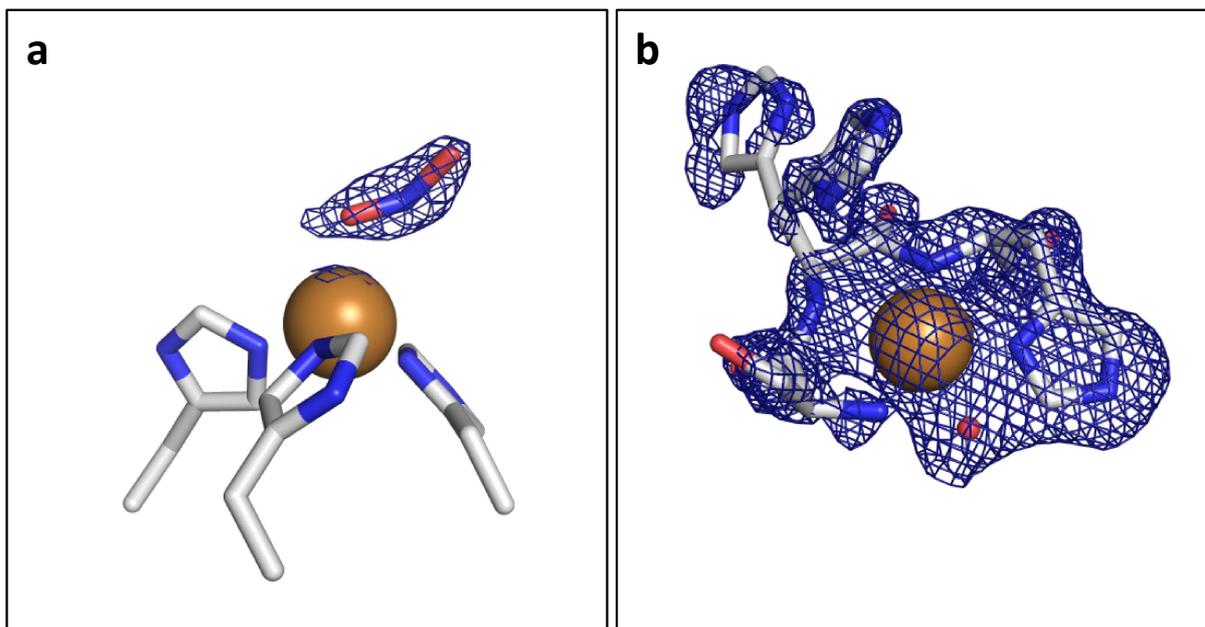


Figure S3 Electron density for Cu-CA II T1 and T2 sites. **A** Electron density for the NO₂⁻ bound at T2 site in copper substituted CA II, contoured to 1.5 sigma. **B** Electron density for the T2 site in Cu-CA II contoured to 0.8 sigma.

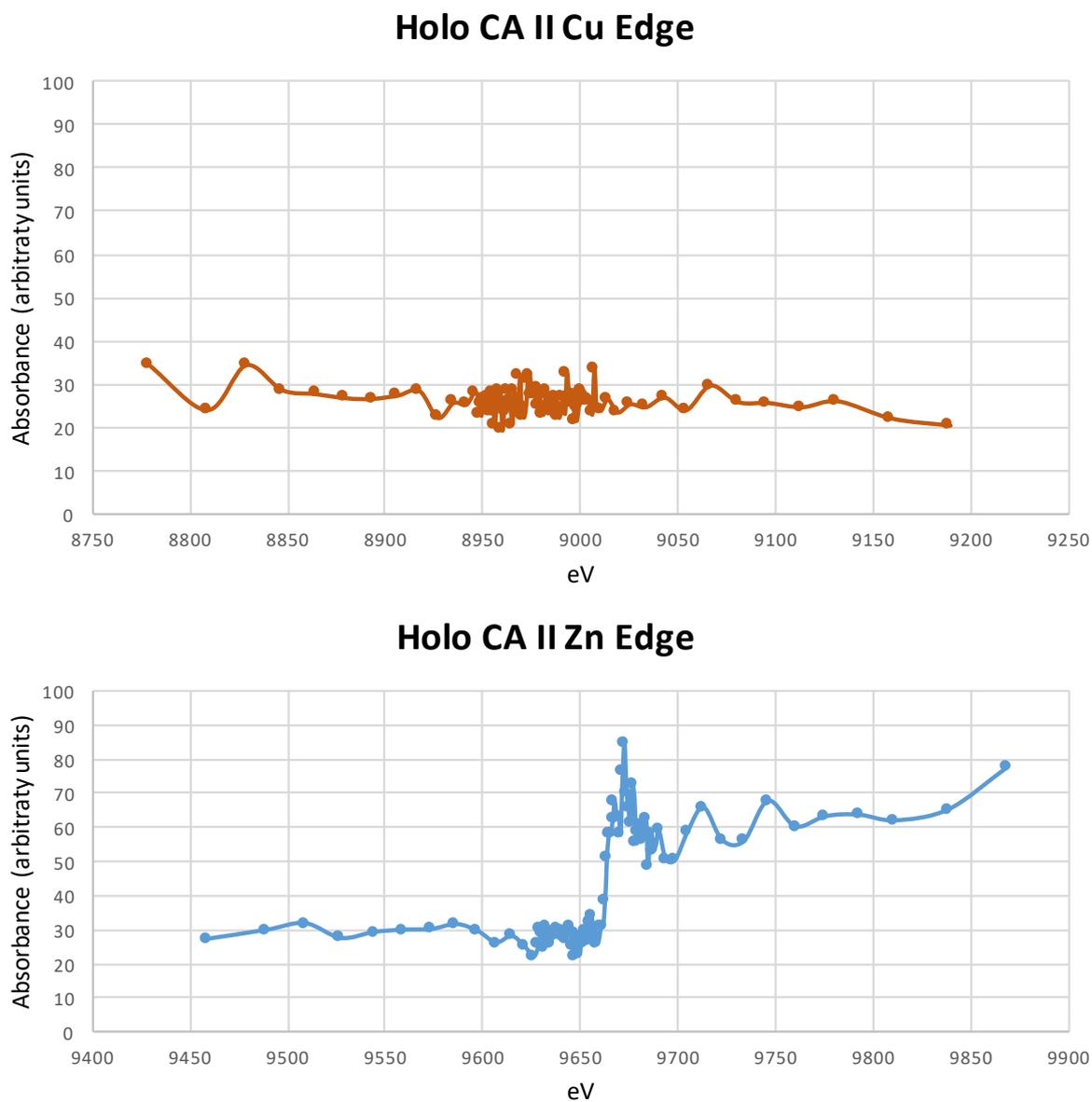


Figure S4 X-ray absorption edge spectra of Zn-CA II. Zn-CA II shows the expected absorption edge at ~9659 eV indicative of zinc bound.

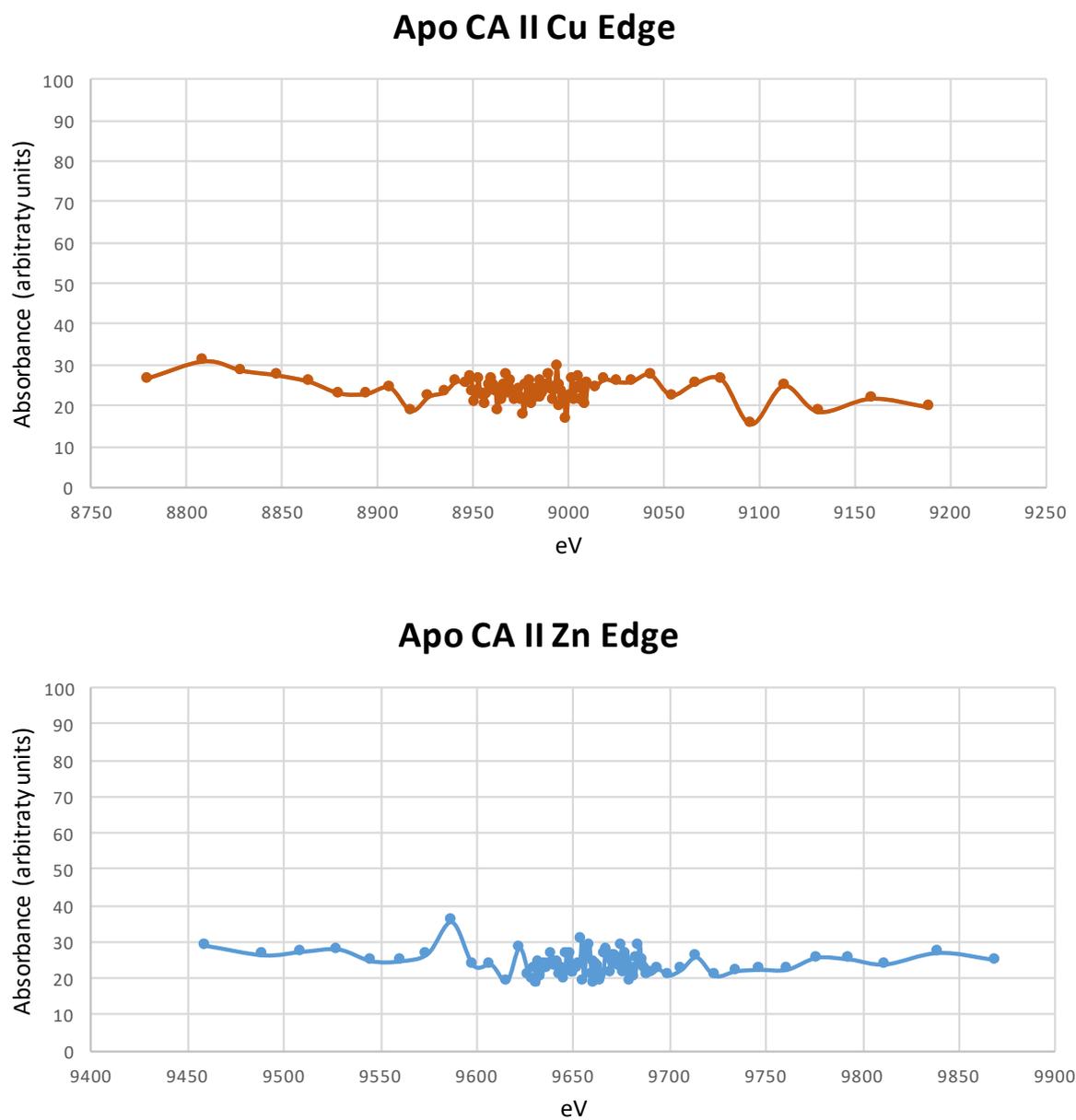


Figure S5 X-ray absorption edge spectra of Apo-CA II. Apo-CA II shows no absorption edge around 8979 eV nor 9659 eV indicating no metal present.

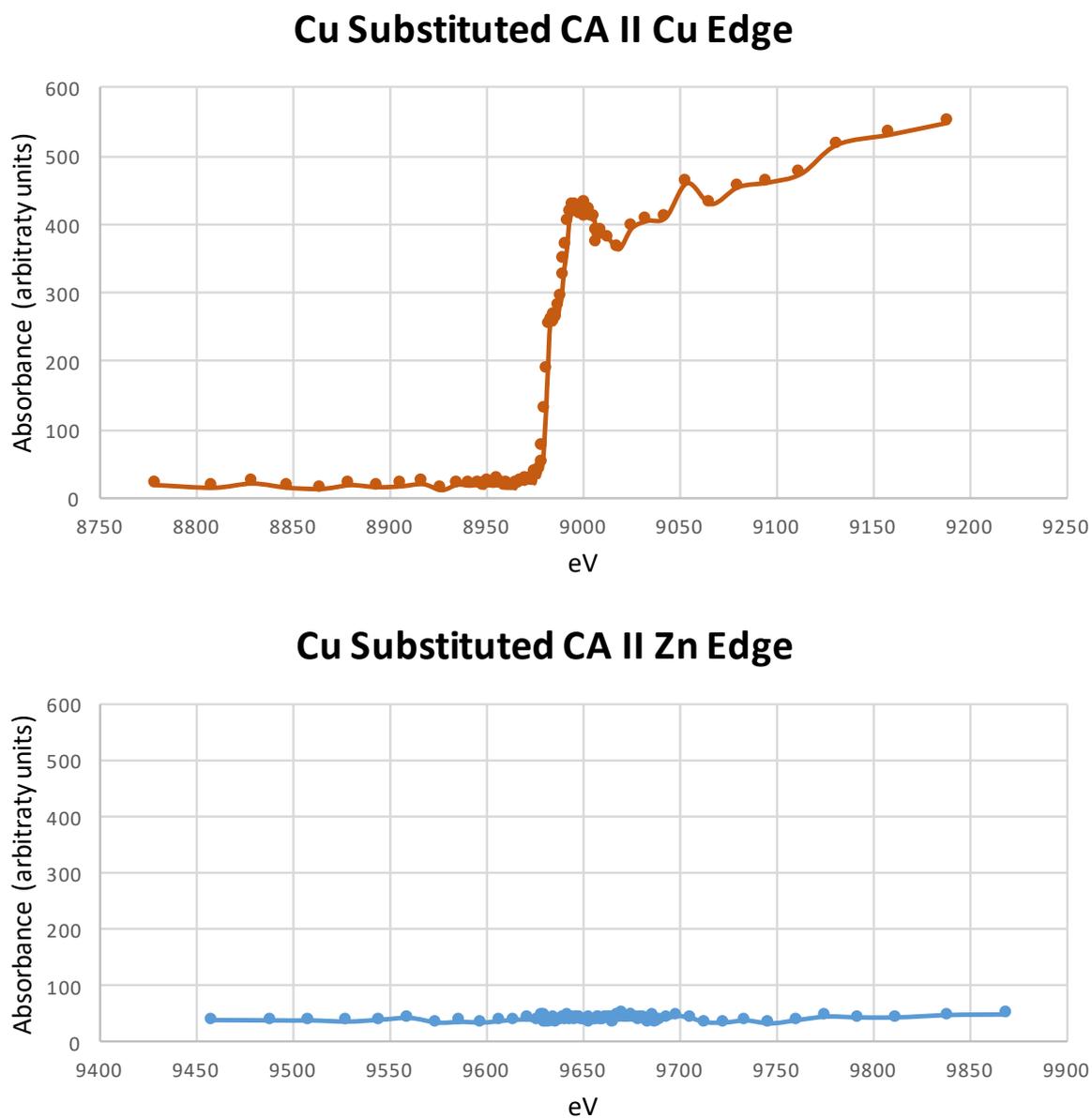


Figure S6 X-ray absorption edge spectra of Cu-CA II. Cu-CA II shows the copper absorption edge at ~8979 eV but not the zinc edge at 9659 eV indicating only copper bound.

Table S1 Data collection and refinement statistics.

Values in parentheses correspond to those of the highest-resolution shell.

| | Apo CAlI | Cu CAlI |
|--|----------------------------|----------------------------|
| Wavelength (Å) | 0.9795 | 0.9795 |
| Resolution range (Å) | 39.88 - 1.36 (1.41 - 1.36) | 34.90 - 1.23 (1.27 - 1.23) |
| Space group | P 1 21 1 | P 1 21 1 |
| Unit cell: a,b,c (Å) | 41.3, 42.3, 72.0 | 41.2, 42.4, 72.0 |
| α, β, γ (°) | 90, 104.2, 90 | 90, 104.3, 90 |
| Total reflections | 163082 (11244) | 379527 (20966) |
| Unique reflections | 49501 (4044) | 68889 (6488) |
| Multiplicity | 3.3 (2.8) | 5.5 (3.2) |
| Completeness (%) | 95.9 (76.5) | 98.1 (92.8) |
| I/I_σ | 30.6 (6.3) | 12.9 (1.6) |
| Wilson B-factor (Å²) | 11.9 | 13.1 |
| R_{merge}^a (%) | 2.17 (17.24) | 8.98 (63.41) |
| R_{work}^b (%) | 14.86 (18.83) | 15.68 (27.71) |
| R_{free}^c (%) | 16.41 (20.91) | 17.41 (28.60) |
| R_{pim}^d (%) | 1.40 (12.18) | 3.92 (41.76) |
| Reflections used in refinement | 50381 (4045) | 68841 (6471) |
| Reflections used for R-free | 2005 (156) | 1846 (180) |
| Number of non-hydrogen atoms | 2375 | 2406 |
| macromolecules | 2156 | 2188 |

| | | |
|-----------------------------------|-------|-------|
| ligands | 8 | 13 |
| solvent | 211 | 205 |
| Protein residues | 258 | 262 |
| RMS(bonds) (Å) | 0.007 | 0.014 |
| RMS(angles) (°) | 1.29 | 1.74 |
| Ramachandran favored (%) | 97.3 | 95.7 |
| Ramachandran allowed (%) | 2.7 | 4.3 |
| Ramachandran outliers (%) | 0 | 0 |
| Rotamer outliers (%) | 0.4 | 0 |
| Average B-factor(Å ²) | 17.2 | 19.2 |
| macromolecules | 16.2 | 18.4 |
| ligands | 35.8 | 29.1 |
| solvent | 26.7 | 27.6 |

$${}^a R_{\text{merge}} = (\sum |I - \langle I \rangle| / \sum \langle I \rangle) \times 100.$$

$${}^b R_{\text{work}} = (\sum |F_o - F_c| / \sum |F_o|) \times 100.$$

${}^c R_{\text{free}}$ is calculated in the same way as R_{cryst} except it is for data omitted from refinement (5% of reflections for all data sets).

$${}^d R_{\text{pim}} = [(\sum \sqrt{1/N} - 1) \sum |I - \langle I \rangle| / \sum \langle I \rangle] \times 100.$$