STRUCTURAL BIOLOGY COMMUNICATIONS

Volume 72 (2016)

Supporting information for article:

Biochemical, spectroscopic and X-ray structural analysis of deuterated multicopper oxidase CueO prepared from a new expression construct for neutron crystallography

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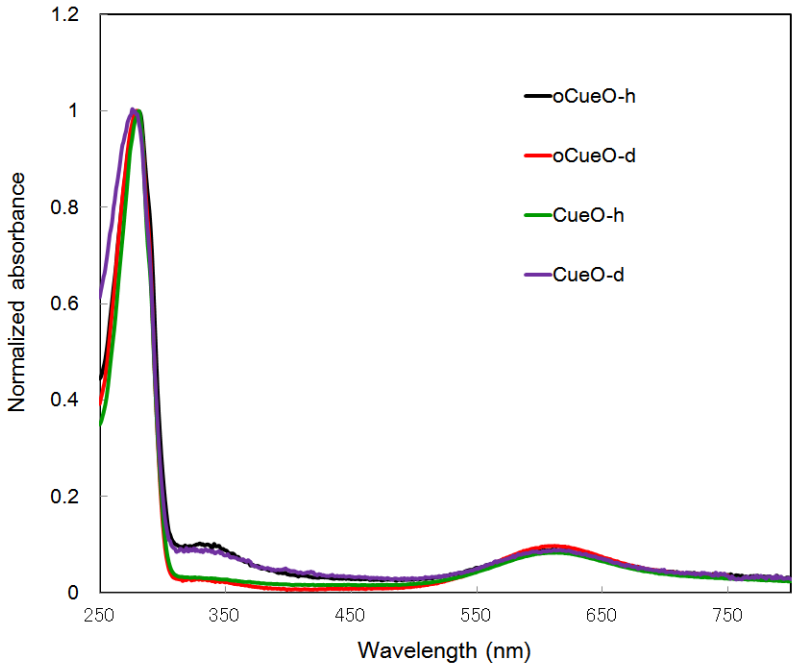

Figure S1 UV-Vis spectra of oCueO-h, oCueO-d, CueO-h, and CueO-d.

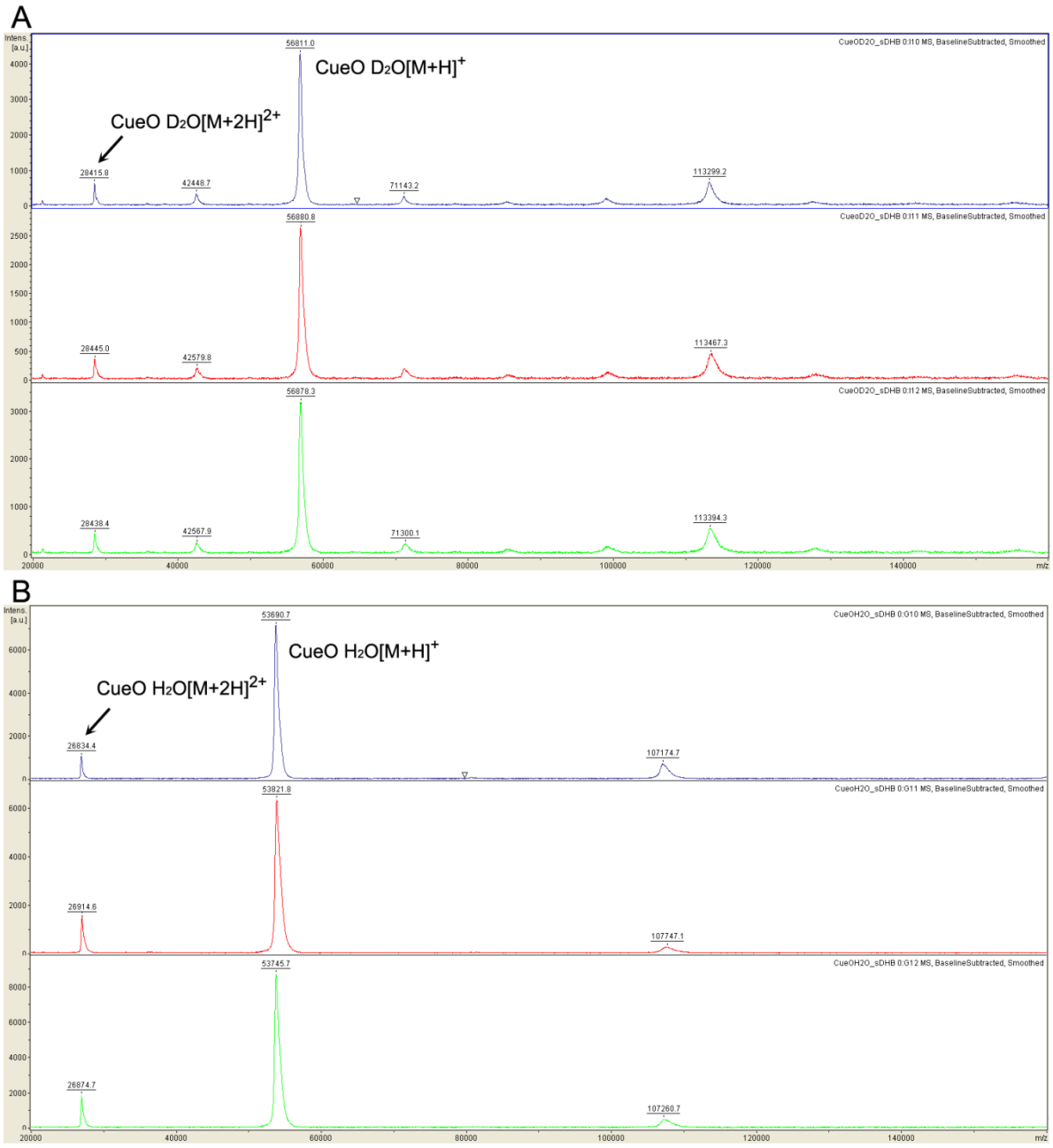

Figure S2 MALDI-TOF mass spectra. (*a*) CueO-d. (*b*) CueO-h.

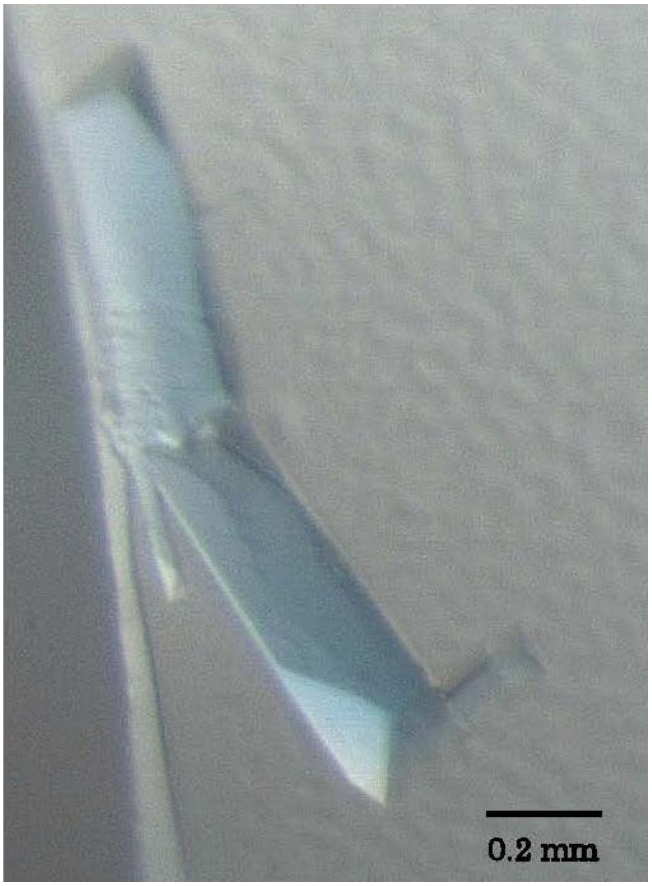

Figure S3 CueO-d crystal. Scale bar corresponds to 0.2 mm. The dimensions of the crystal are 0.5 x 0.2 x 0.2 mm^3.

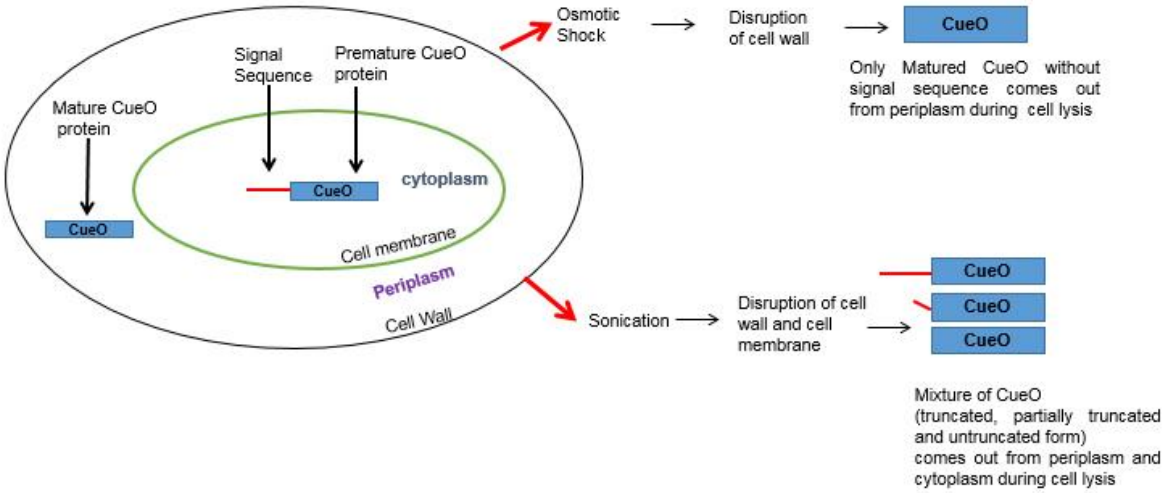

Figure S4 Effect of sonication to generate CueO protein with untruncated or partially truncated signal sequence during cell lysis.

Table S1 DNA and amino acid sequence of HRV3C-CueO

	Sequence
DNA of tag part	GCAAGTGGCGGTAGT**TGGAGCCACCCGCAGTTCGAGAAA**G GTGGAGGTTCCGGAGGTGGATCGGGAGGTGGATCG**TGGTCT CATCCGCAATTTGAGAAGG**GTTCAACTAGTGGTCTGGAAGT TCTGTTCCAGGGGCCCGGCGGT
Whole protein	MQRRDFLKYSVALGVASALPLWSRAVFAASGGS**WSHPQFEK**G GGSGGGSGGGS**WSHPQFEK**GSTSGLEVLFQGPGGAERPTLPIPD LLTTDARNRIQLTIGAGQSTFGGKTATTWGYNGNLLGPAVKLQR GKAVTVDIYNQLTEETTLHWHGLEVPGEVDGGPQGIIPPGGKRS VTLNVDQPAATCWFHPHQHGKTGRQVAMGLAGLVVIEDDEILK LMLPKQWGIDDVPVIVQDKKFSADGQIDYQLDVMTAAVGWFG DTLLTNGAIYPQHAAPRGWLRLRLLNGCNARSLNFATSDNRPLY VIASDGGLLPEPVKVSELPVLMGERFEVLVEVNDNKPFDLVTLP VSQMGMAIAPFDKPHPVMRIQPIAISASGALPDTLSSLPALPSLEG LTVRKLQLSMDPMLDMMGMQMLMEKYGDQAMAGMDHSQM MGHMGHGNMNHMNHGGKFDFHHANKINGQAFDMNKPMFAA AKGQYERWVISGVGDMMLHPFHIHGTQFRILSENGKPPAAHRA GWKDTVKVEGNVSEVLVKFNHDAPKEHAYMAHCHLLEHEDTG MMLGFTV

†Bold characters, single underlined and double underlined indicate Twin-Strep-tag, linker, and HRV3C protease recognition site, respectively.

Table S2 Copper-ligand distances (Å).

	CueO (PDB code 1KV7)	oCueO-d	CueO-h	CueO-d
T1 Cu – His 443	2.0	2.0	2.1	2.0
T1 Cu – Cys 500	2.2	2.2	2.2	2.2
T1 Cu – His 505	2.0	2.0	2.0	2.0
T1 Cu – Met 510	3.2	3.3	3.2	3.2
T2 Cu – His 101	1.9	2.0	1.7	1.9
T2 Cu – His 446	1.8	2.0	1.7	1.9
T3aCu – His 103	2.0	1.9	1.9	2.0
T3aCu – His 141	2.0	2.0	1.9	2.0
T3aCu – His 501	2.1	2.1	2.1	2.2
T3aCu – HOH	2.4	2.4	2.5	2.4
T3bCu – His 143	2.0	2.0	1.9	2.1
T3bCu – His 448	1.9	2.0	1.9	2.0
T3bCu – His 499	2.0	2.1	1.9	1.9
T3bCu – HOH	2.3	2.2	2.3	2.5
T2 Cu – T3aCu	4.0	4.1	4.0	3.9
T2 Cu – T3bCu	3.5	3.5	3.5	3.5
T3aCu – T3bCu	4.7	4.4	4.8	4.8