

1

Supplementary Information

2

Catalytic and inhibitory architecture of comammox ammonia monooxygenase

3

4

5

6

7

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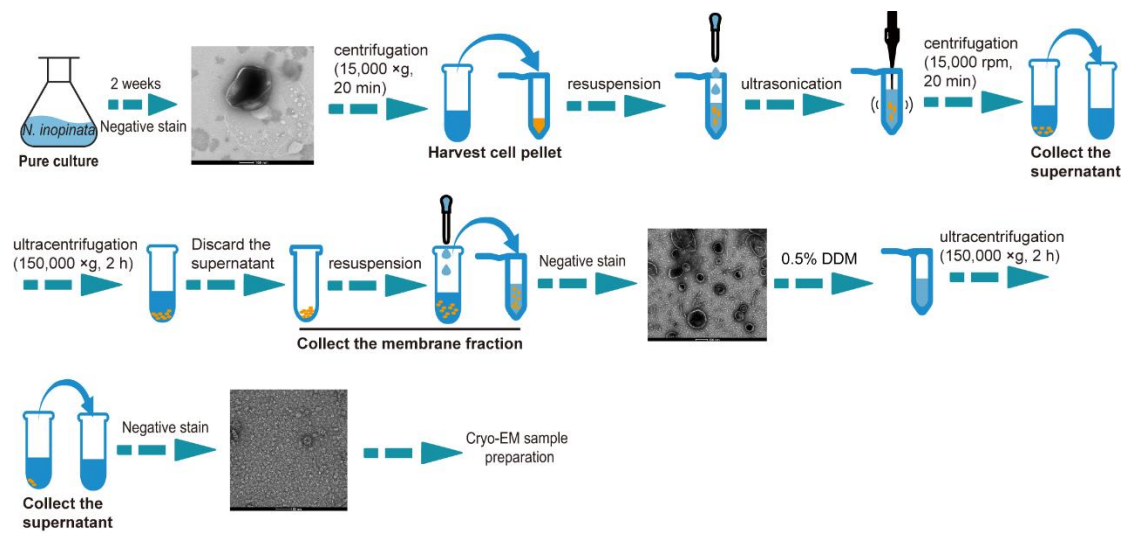
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Supplementary Figures 1-22

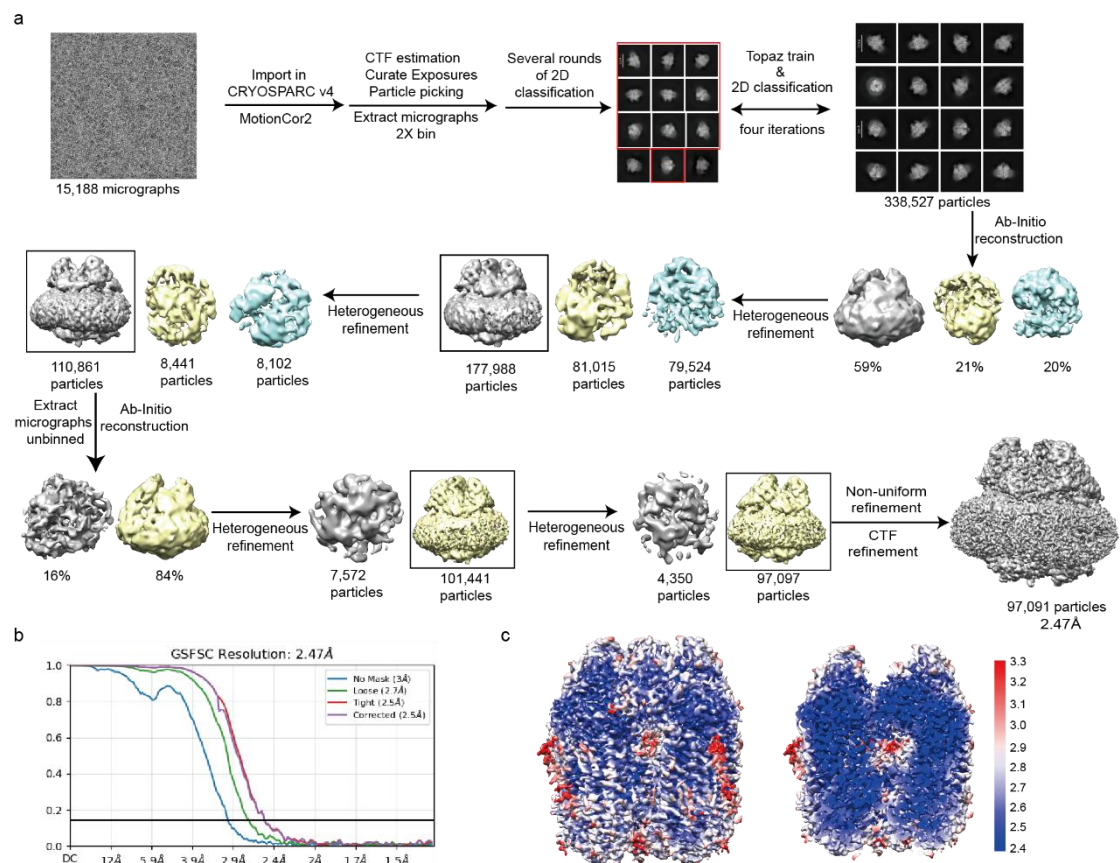
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Supplementary Tables 1-6

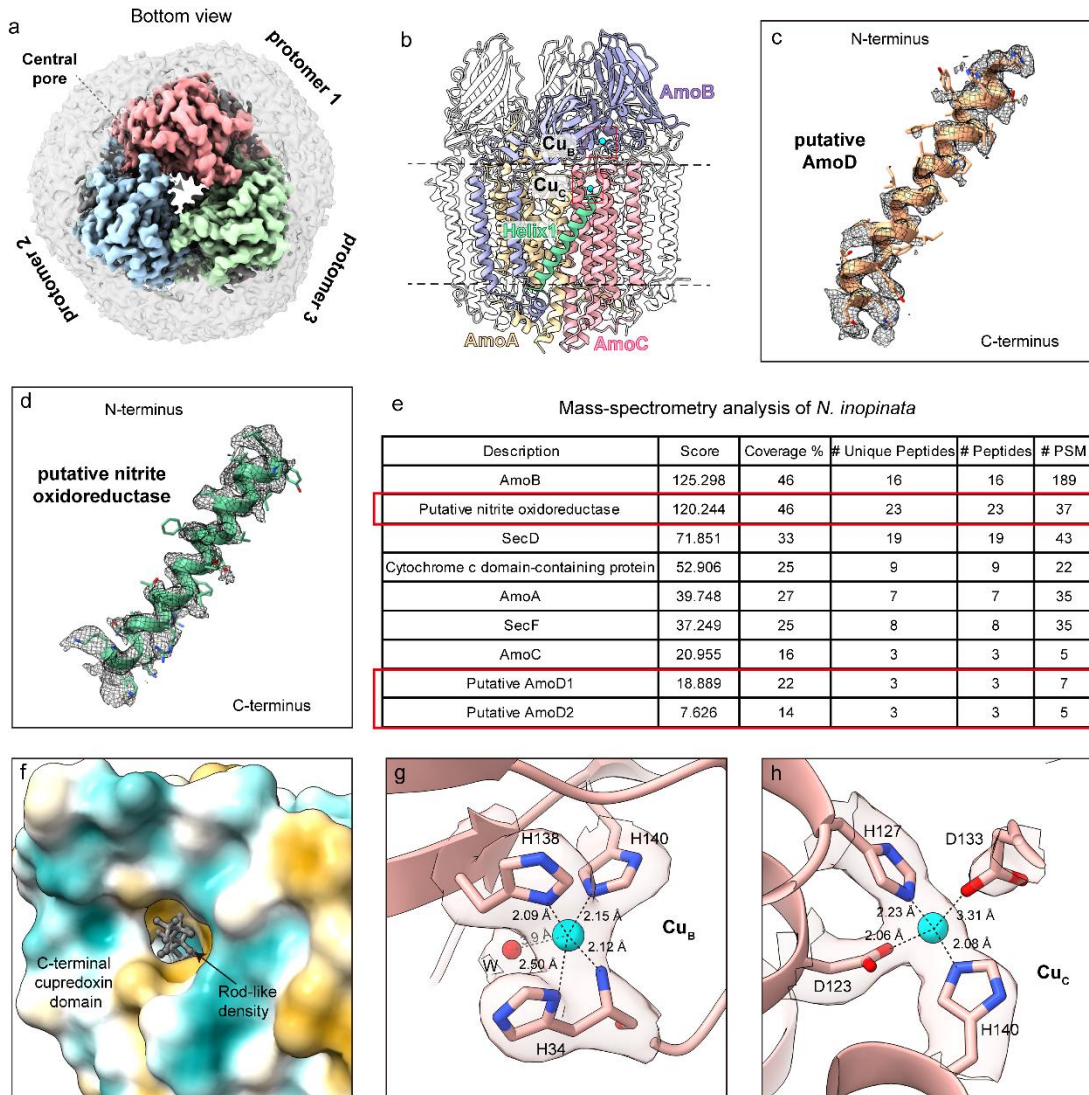
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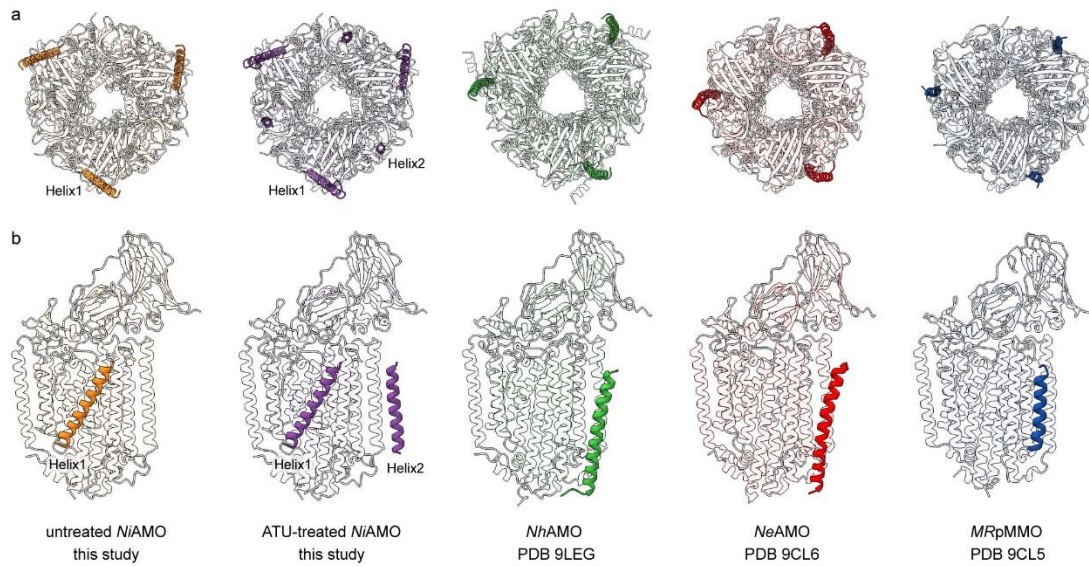
Supplementary Fig. 1 | Schematic diagram depicting the purification of AMO from *N. inopinata*. *Ni*AMO was purified in 0.5% DDM for cryo-EM sample preparation after checking by negative staining.



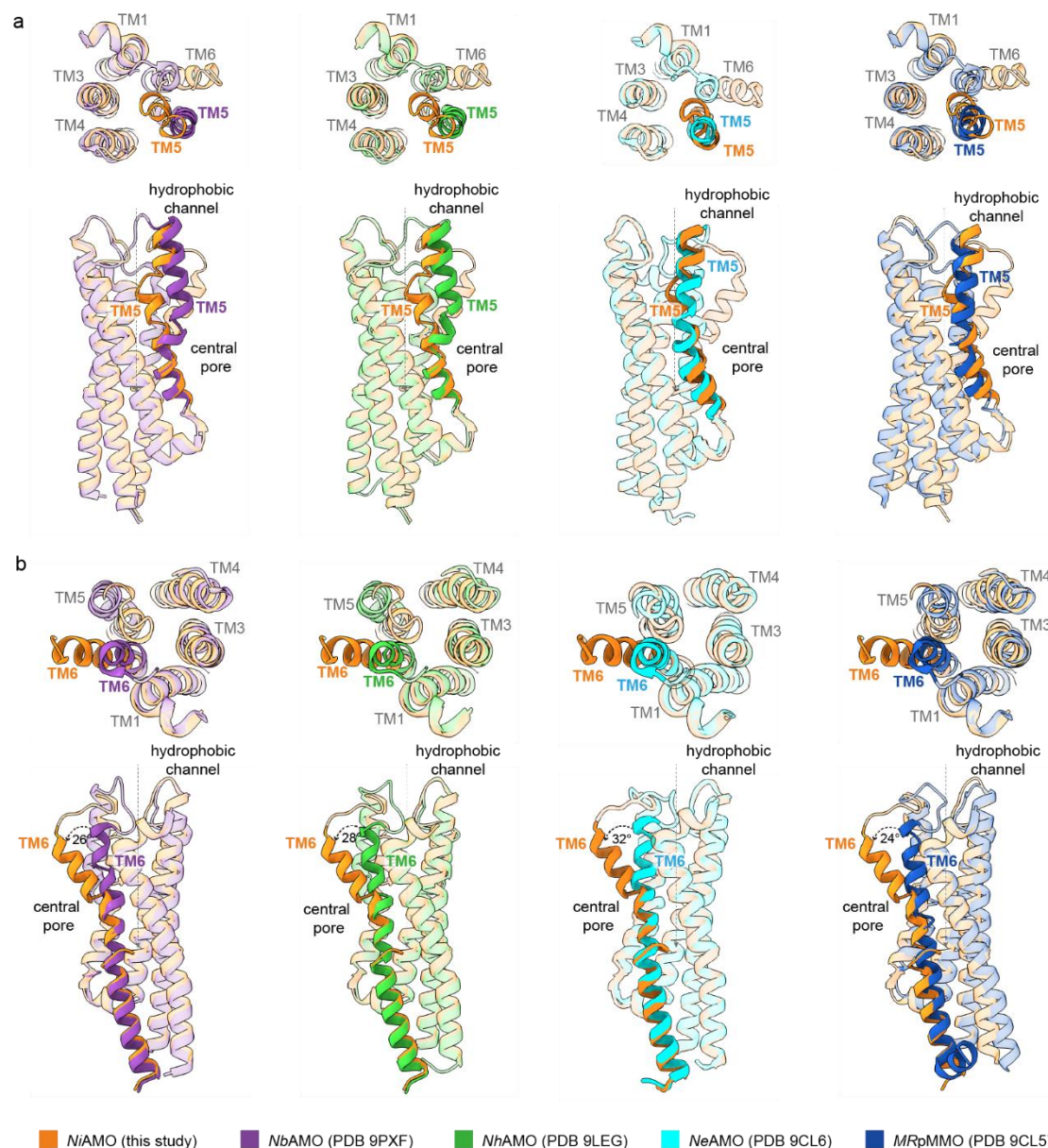
Supplementary Fig. 2 | Cryo-EM data analysis of *NiAMO*. **a**, The flowchart of cryoEM data processing of *NiAMO*. Details can be found in the “Method” section. **b**, Gold-standard Fourier shell correlation (GSFSC) curve for the *NiAMO* map generated using cryoSPARC 4.3.2. The threshold of 0.143 was used to determine the overall resolution of the map. **c**, Local resolution for the overall reconstruction (left) and a central slice (right) of *NiAMO*.



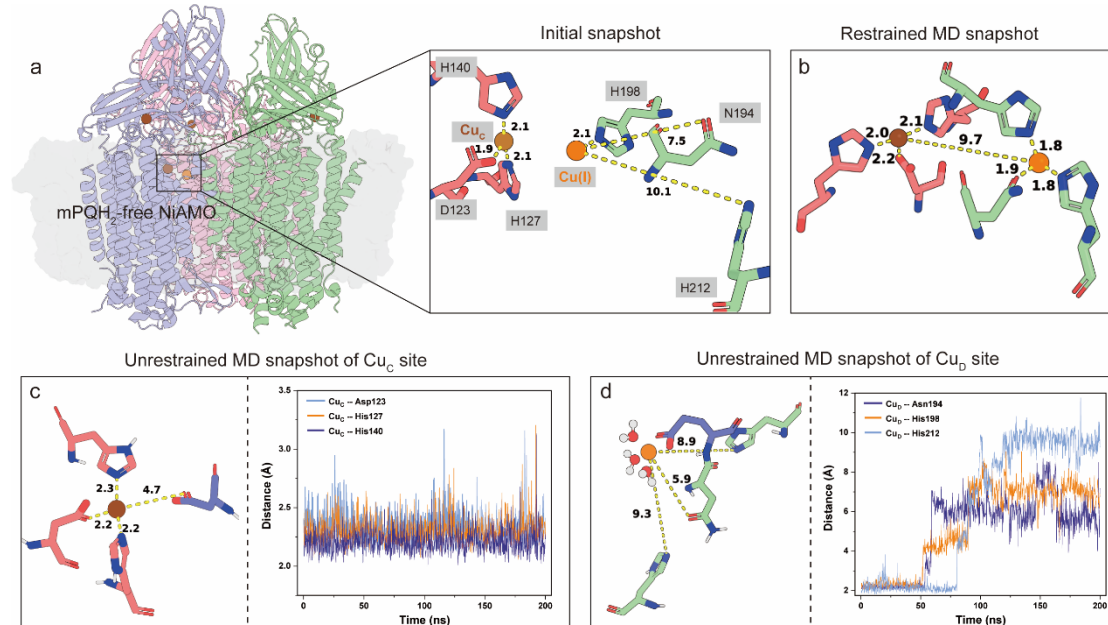
Supplementary Fig. 3 | Cryo-EM structure of NiAMO. **a**, Cryo-EM structure from bottom view showing three protomers (colored in pink, blue and green, respectively) of *NiAMO*. **b** Atomic model of *NiAMO* monomer. Four components of *NiAMO* are colored in yellow (*AmoA*), purple (*AmoB*), coral (*AmoC*) and green (*Helix1*). Cu_B site and Cu_C site are highlighted by red box, with their coppers colored in cyan. **c-d**, *Helix1* with transparent cryo-EM density superimposed. **e**, Mass-spectrometric results of *N. inopinata* identifying peptide candidates for *Helix1*. Two of these candidates, highlighted by red boxes, were considered as potential matches. **f**, Rod-like density (colored in gray) located in the C-terminal cupredoxin domain of *AmoB*. The hydrophobic and hydrophilic surface characteristics of the cupredoxin domain are depicted. **g-h**, Cu_B site (**e**) and Cu_C site (**f**) and their coordination environments with transparent cryo-EM density superimposed.



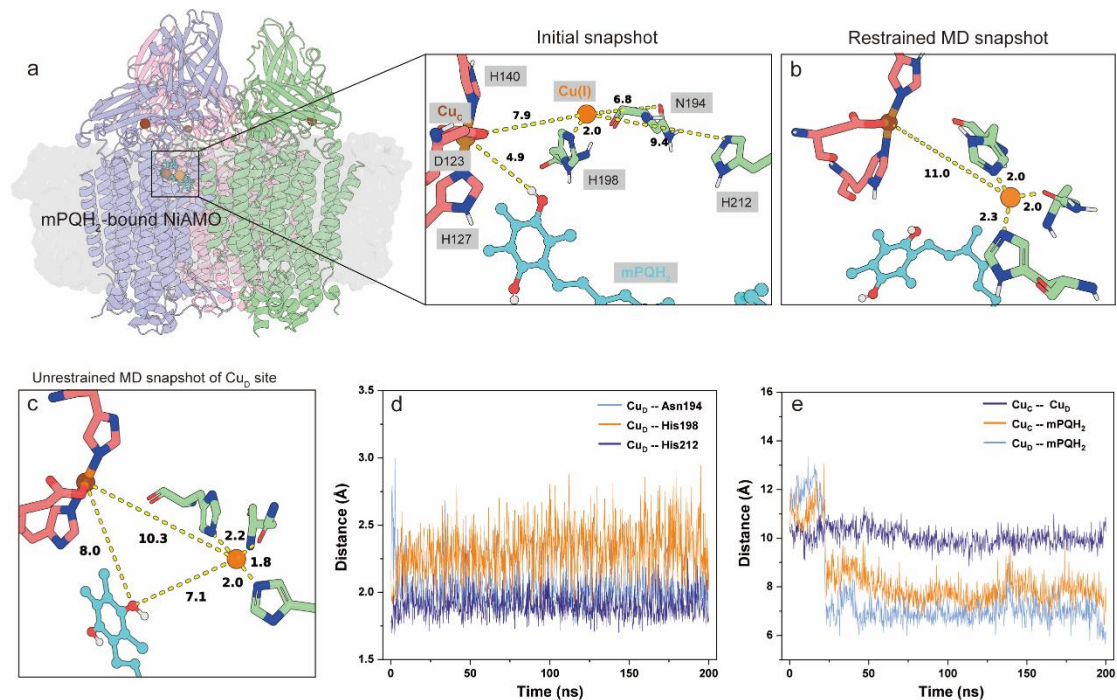
Supplementary Fig. 4 | Comparison of AMO and pMMO determined by cryo-EM SPA. The extra helices are highlighted in the corresponding atomic models of the monomer from top view (a) and the protomer from side view (b) of AMOs and pMMO. *Ni*AMO, ATU-treated *Ni*AMO in this study, *Nh*AMO (PDB: 9LEG), *Ne*AMO (PDB: 9CL6) and *MRp*MMO (PDB: 9CL5) were colored in orange, purple, green, red and blue.



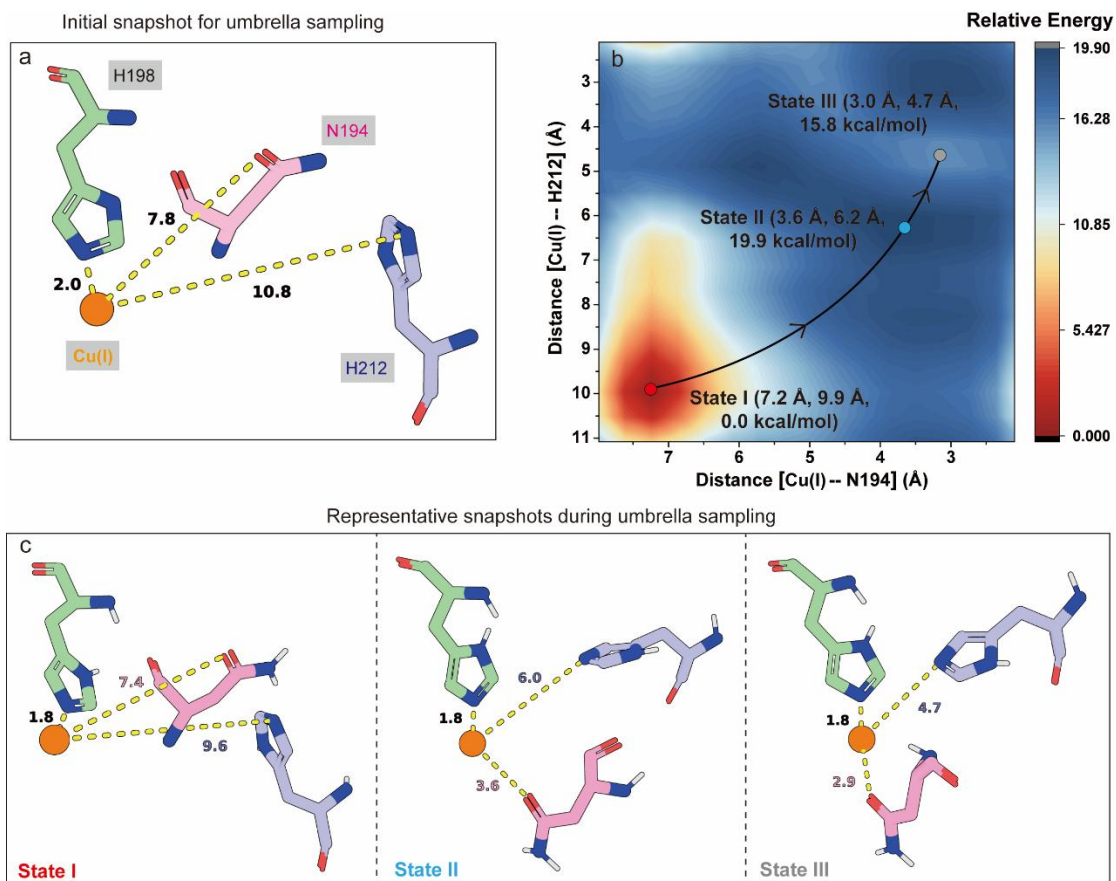
Supplementary Fig. 5 | Comparison of hydrophobic channel from AMOs. a, Alignment of conserved hydrophobic channel highlighting TM5 in Amoc from AMOs shown in top view (top) and side view (bottom). **b,** Alignment of conserved hydrophobic channel highlighting TM6 in Amoc from AMOs shown in top view (top) and side view (bottom). *Ni*AMO in this study, *Nb*AMO (PDB: 9PXF), *Nh*AMO (PDB: 9LEG), *Ne*AMO (PDB: 9CL6) and *MRp*MMO (PDB: 9CL5) were colored in orange, purple, green, cyan and blue.



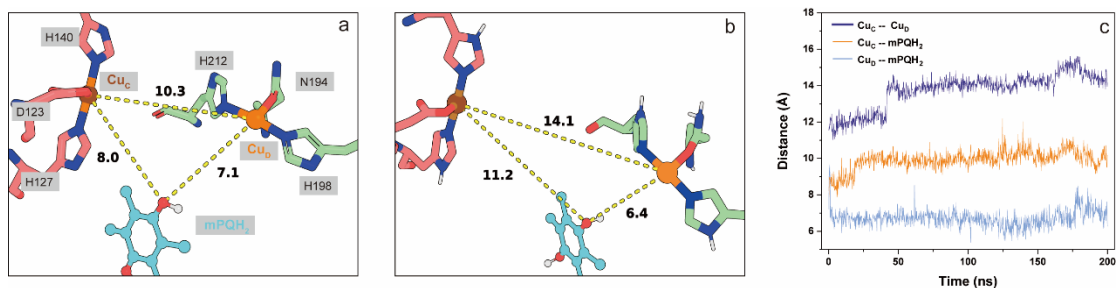
Supplementary Fig. 6 | Evaluation of the Cu ion capture capability of the Cu_D site in the mPQH_2 -free NiAMO . **a**, Overall structure of membrane-bound mPQH_2 -free NiAMO and the initial configuration for MD simulations, in which a Cu(I) ion was randomly positioned near the Cu_D site for conformational sampling. **b**, Stable Cu(I) coordination geometry at the Cu_D site obtained from restrained MD simulations by applying distance and angle restraints between the Cu(I) ion and the surrounding coordinating residues. **c-d**, Representative conformations of the Cu_C and Cu_D sites obtained from unrestrained MD simulations without predefined coordination bonds, together with the time-dependent distances between the Cu(I) ions and their surrounding coordinating residues. The membrane is shown as a gray surface. The Cu(I) ions resolved in the cryo-EM structure are represented as brown spheres, whereas the Cu(I) ions randomly placed near the Cu_D site are shown as orange spheres. The coordinating residues at the Cu_C and Cu_D sites are depicted as red and green sticks, respectively. Key distances are given in angstrom (Å).



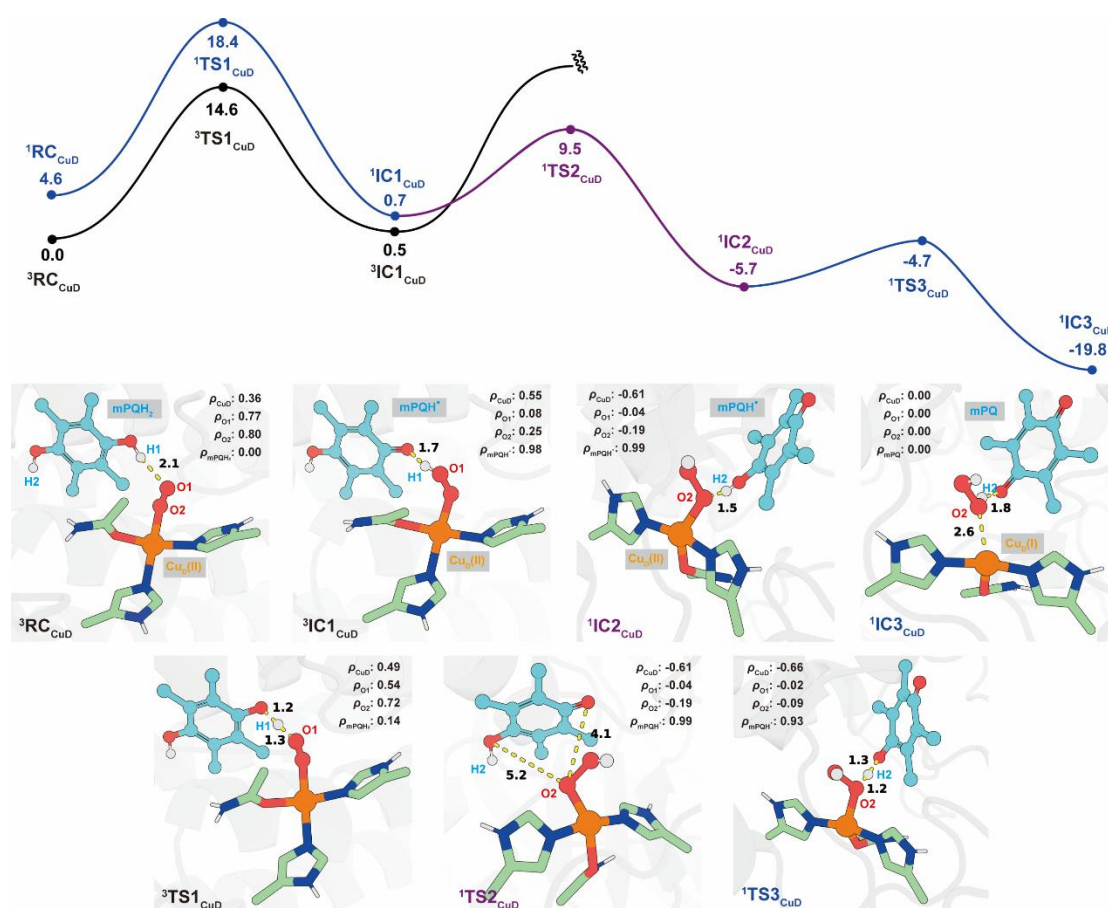
Supplementary Fig. 7 | Evaluation of the Cu ion capture capability of the Cu_D site in the mPQH₂-bound NiAMO. **a**, Overall structure of the membrane-bound NiAMO-mPQH₂ complex and the initial snapshot for MD simulations, in which a Cu(I) ion was randomly positioned near the Cu_D site for conformational sampling. **b**, Stable Cu(I) coordination geometry at the Cu_D site obtained from restrained MD simulations by applying distance and angle restraints between the Cu(I) ion and the surrounding coordinating residues. **c**, Representative conformations from unrestrained MD simulations with coordination bond parameters applied only to the Cu_C site. **d-e**, Time-dependent distances between the Cu(I) ion at the Cu_D site and its surrounding coordinating residues, as well as mPQH₂. The membrane is shown as a gray surface. The Cu(I) ions and mPQH₂ resolved in the cryo-EM structure are represented as brown and cyan spheres, respectively, whereas the Cu(I) ions of Cu_D site are shown as orange spheres. The coordinating residues at the Cu_C and Cu_D sites are depicted as red and green sticks, respectively. Key distances are given in angstrom (Å).



Supplementary Fig. 8 | Free energy profile for Cu ion capture by the CuD site in the mPQH₂-free structure. **a**, Initial snapshot for umbrella sampling simulations, in which Cu(I) was placed near H198. Asn194, His198, and His212 are depicted as pink, green, and blue stick models, respectively, while Cu(I) is represented as an orange sphere. **b**, Umbrella-sampling-calculated the free energy profile (in kcal/mol) for the transition of Cu(I) from the initial non-coordinating state (State I) to the Cu-coordinating state (State III). The reaction coordinates were defined as the distances between Cu(I) and N194 and between Cu(I) and H212, respectively. **c**, Representative snapshots sampled along the transition pathway of Cu(I) from the uncoordinated to the coordinated state. Key distances are given in angstrom (Å).

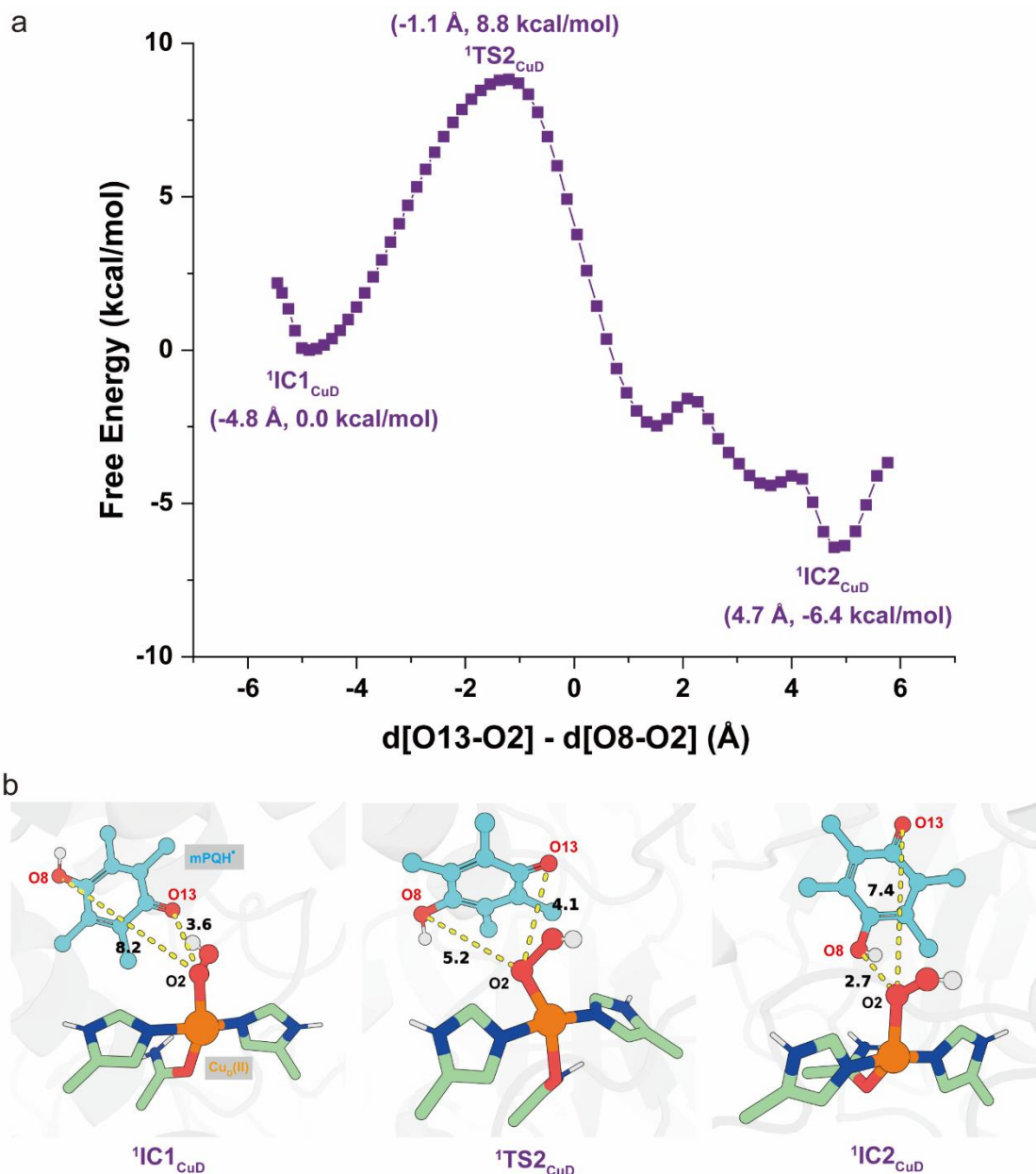


Supplementary Fig. 9 | Binding mode in the active site of mPQH₂-bound NiAMO. a-b, The initial and representative configurations of NiAMO containing mPQH₂ and simultaneously occupied Cu_C and Cu_D sites during MD simulations. c, Time-dependent distances between mPQH₂ and the Cu_C and Cu_D sites, as well as between the Cu_C and Cu_D copper ions. The Cu_C and Cu_D sites are shown as brown and orange spheres, respectively, whereas mPQH₂ is shown as cyan spheres. The coordinating residues at the Cu_C and Cu_D sites are depicted as red and green sticks, respectively. Key distances are given in angstrom (Å).

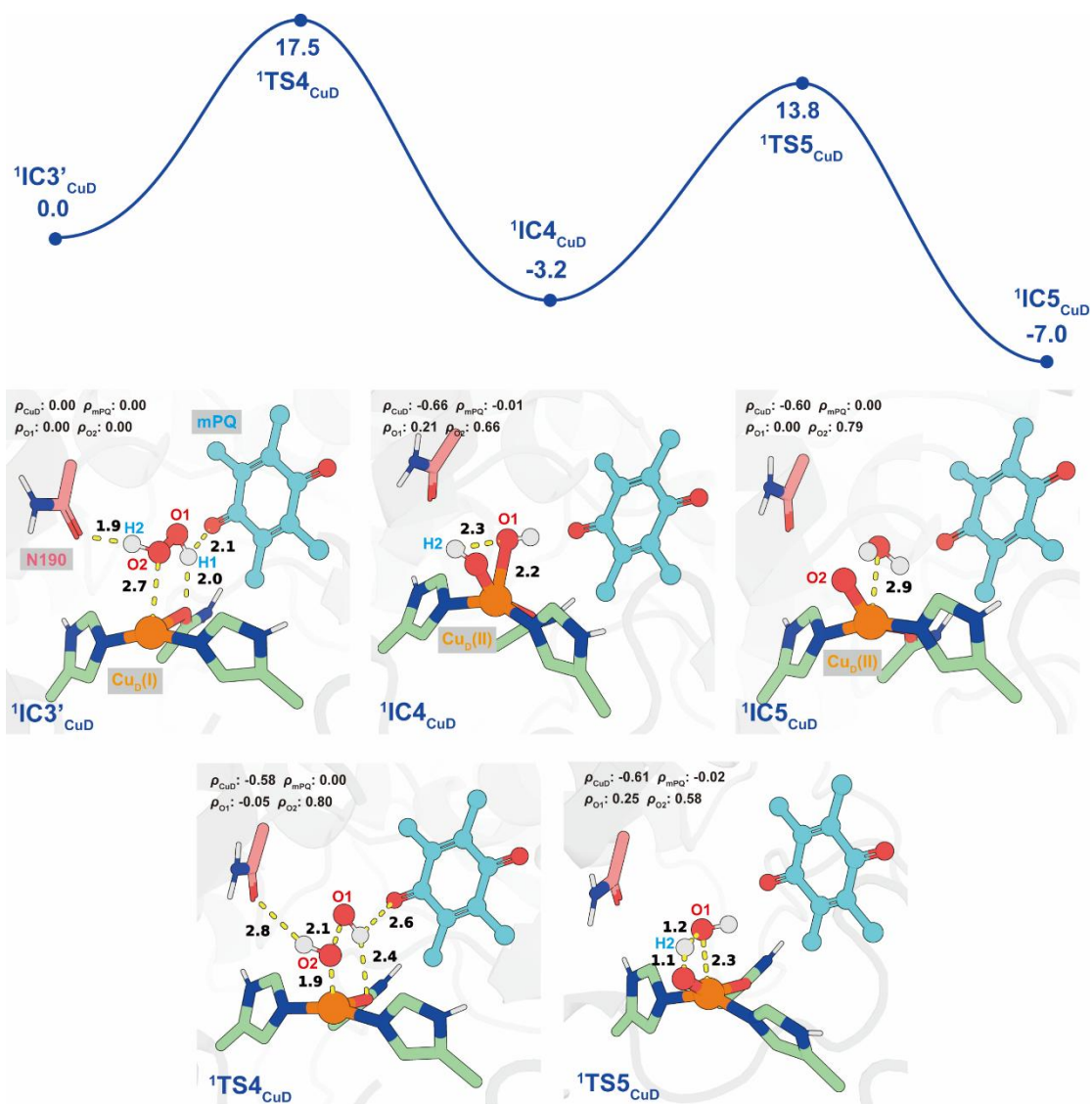


Supplementary Fig. 10 | Calculated the mechanism of $\text{Cu}_D(\text{I}) \cdots \text{H}_2\text{O}_2$ intermediate generation.

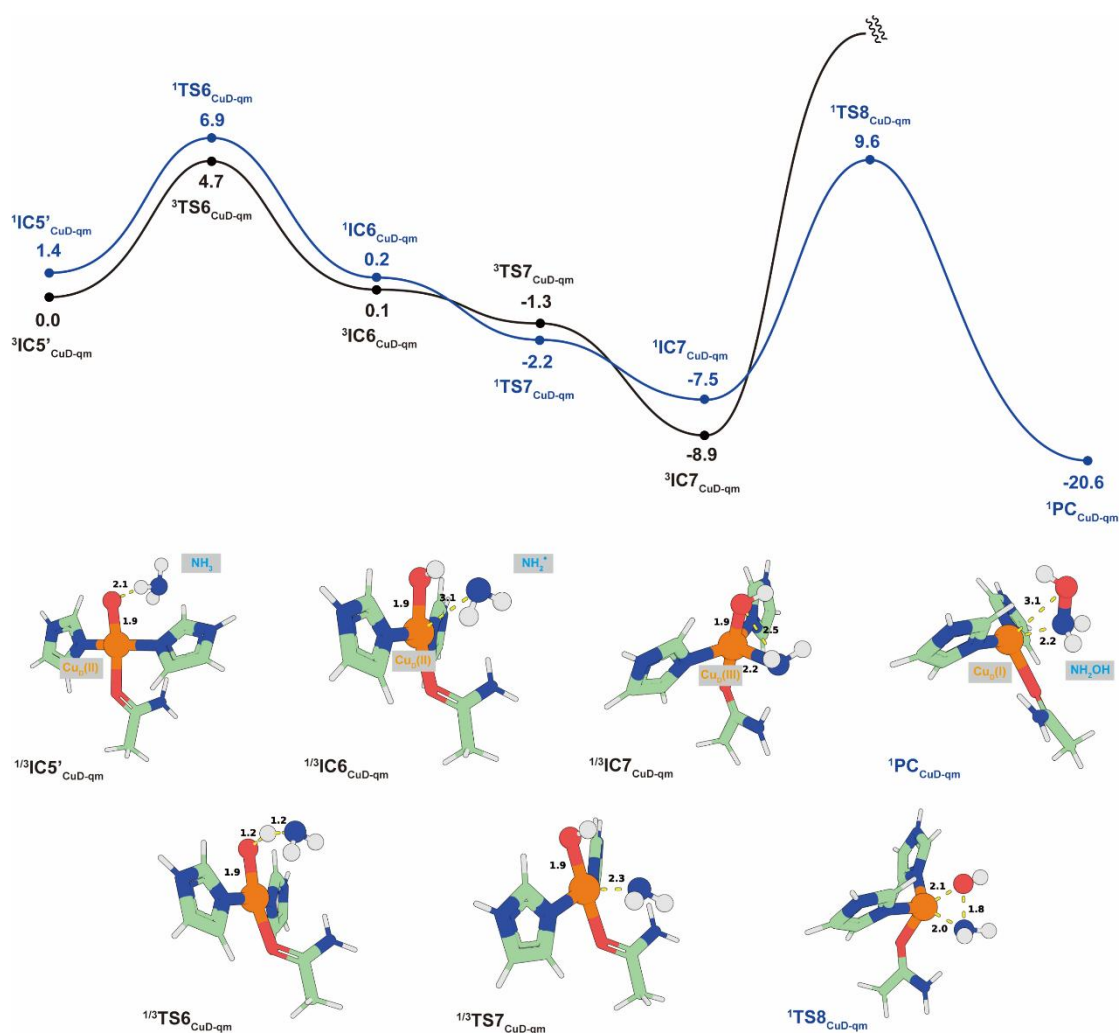
QM(UB3LYP-D3/def2-TZVP)/MM relative energies (in kcal/mol) for $\text{Cu}_D(\text{I}) \cdots \text{H}_2\text{O}_2$ (IC3_{CuD}) formation from $\text{Cu}_D(\text{II})-\text{O}_2^{\bullet-}$ and mPQH_2 complex (RC_{CuD}) via the two HAT processes in the open-shell broken-symmetry singlet and triplet states. QM(UB3LYP-D3/def2-SVP)/MM optimized geometries of key species involved in the reaction are presented. Key distances are given in angstrom (Å).



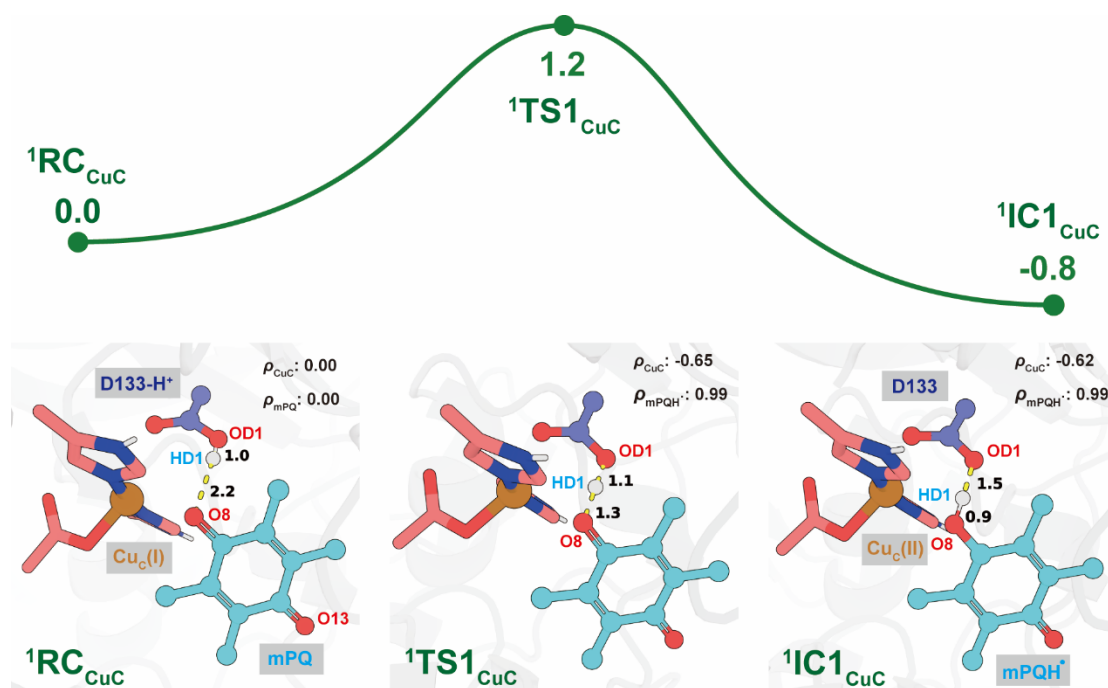
Supplementary Fig. 11 | Calculated the mechanism of mPQH[•] radical conformational reorganization near the Cu_D site. a, QM/MM metadynamics-calculated free energy profile (in kcal/mol) for the conformational reorganization of the mPQH[•] radical near the Cu_D site. The reaction coordinate is defined as the difference between the distance from O2 of the Cu_D(II)-OOH⁻ species to O13 of the mPQH[•] radical (d1) and the distance between O2 and O8 of the mPQH[•] radical (d2). **b**, The geometry of intermediates and transition states. Key distances are given in angstrom (Å).



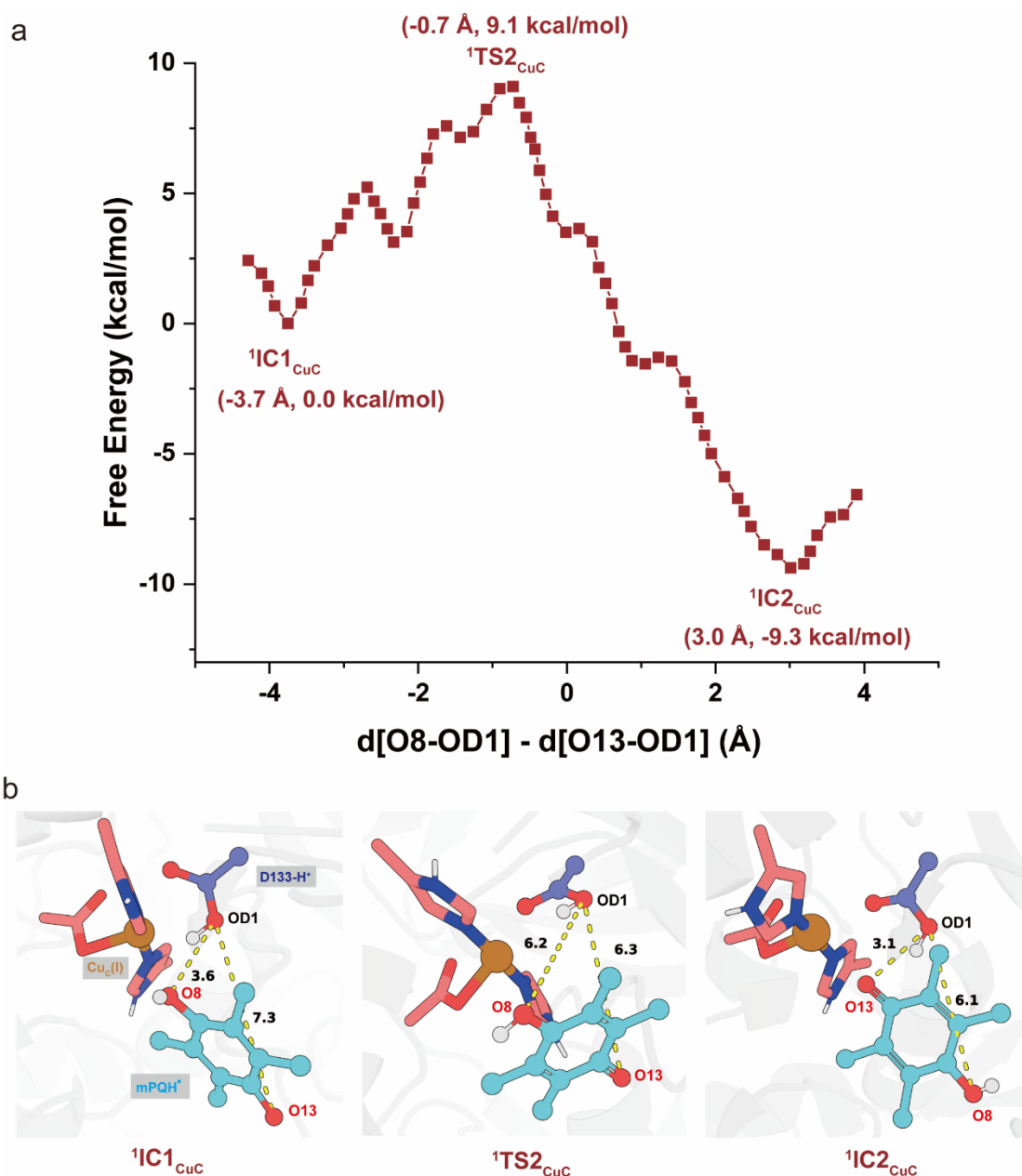
Supplementary Fig. 12 | Calculated the mechanism of reactive oxygen species generation. QM(UB3LYP-D3/def2-TZVP)/MM relative energies (in kcal/mol) for $\text{Cu}_D(\text{II})-\text{O}^{\bullet-}$ (${}^1\text{IC5}_{\text{CuD}}$) formation from $\text{Cu}_D(\text{I})\cdots\text{H}_2\text{O}_2$ (${}^1\text{IC3}'_{\text{CuD}}$) in the open-shell broken-symmetry singlet state. QM(UB3LYP-D3/def2-SVP)/MM optimized geometries of key species involved in the reaction are presented. Key distances are given in angstrom (Å).



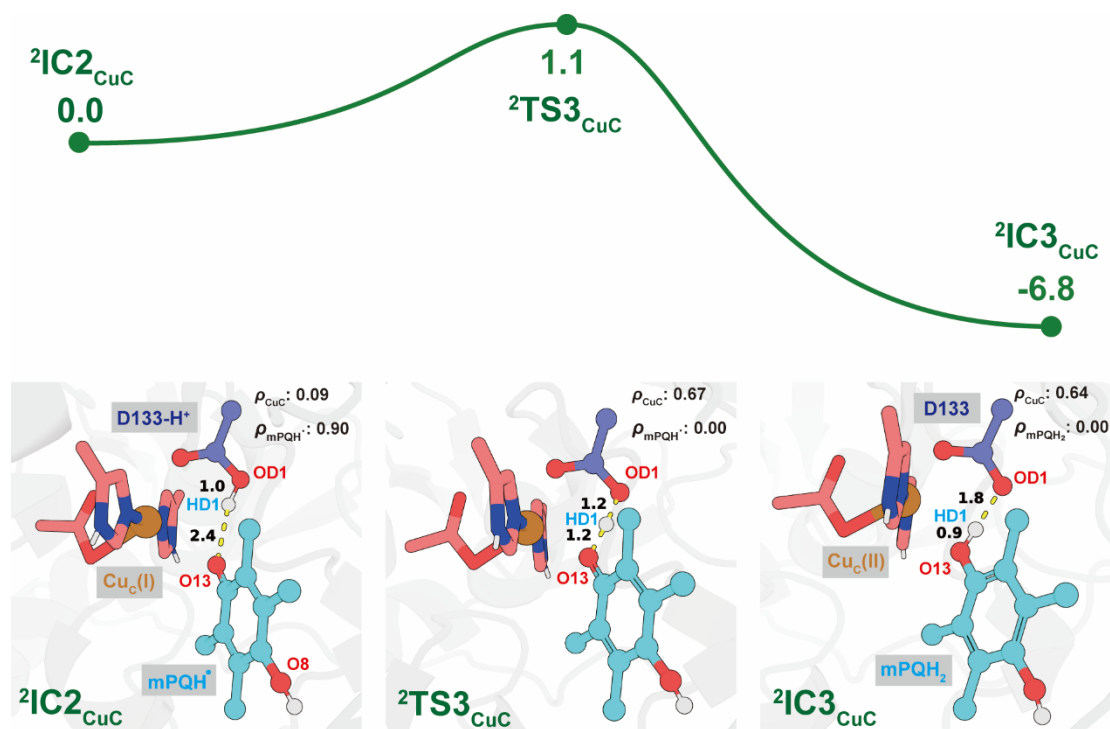
Supplementary Fig. 13 | Calculated mechanism of CuD-mediated ammonia oxidation. QM (UB3LYP/def2-TZVP//def2-SVP) calculated potential energy profile (in kcal/mol) for CuD(II)–O[•]-mediated ammonia hydroxylation to hydroxylamine via the HAT/oxygen rebound mechanism in both open-shell broken-symmetry singlet and triplet states. Key distances are given in angstrom (Å). The spin density population of key atoms for all species are summarized in Supplementary Table S3.



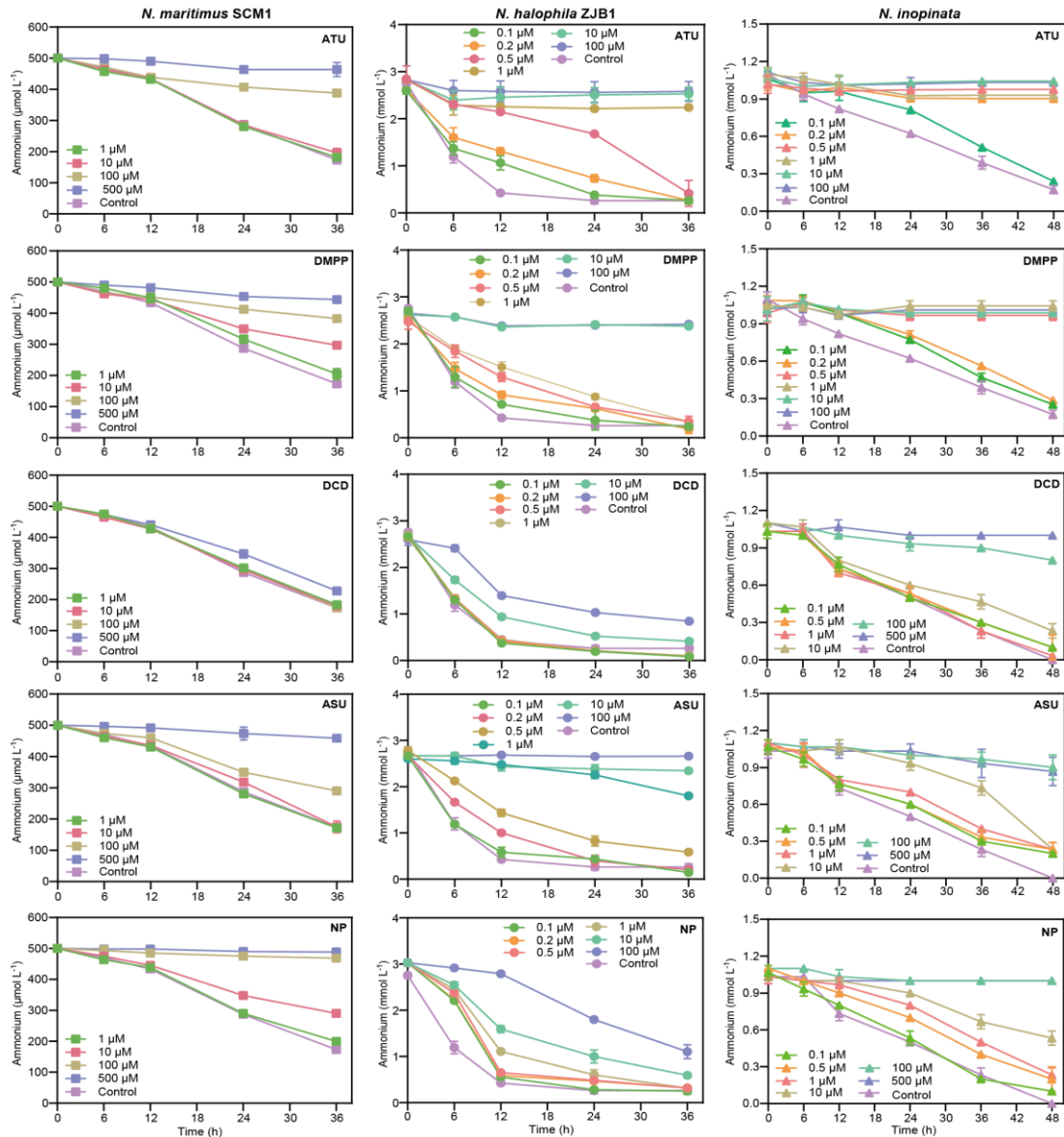
Supplementary Fig. 14 | Calculated mechanism of the first proton-coupled reduction of mPQ to the mPQH• radical at the Cu_C site. QM(UB3LYP-D3/def2-TZVP)/MM-calculated relative energies (in kcal/mol) for the first proton-coupled electron transfer (PCET) from oxidized mPQ ($^1\text{RC}_{\text{CuC}}$) to form the mPQH• radical ($^1\text{IC1}_{\text{CuC}}$) at the Cu_C site in the open-shell broken-symmetry singlet state. QM(UB3LYP-D3/def2-SVP)/MM optimized geometries of key species involved in the reaction are presented. The proton donor residue D133 is highlighted in a blue ball-and-stick model. Key distances are given in angstrom (Å).



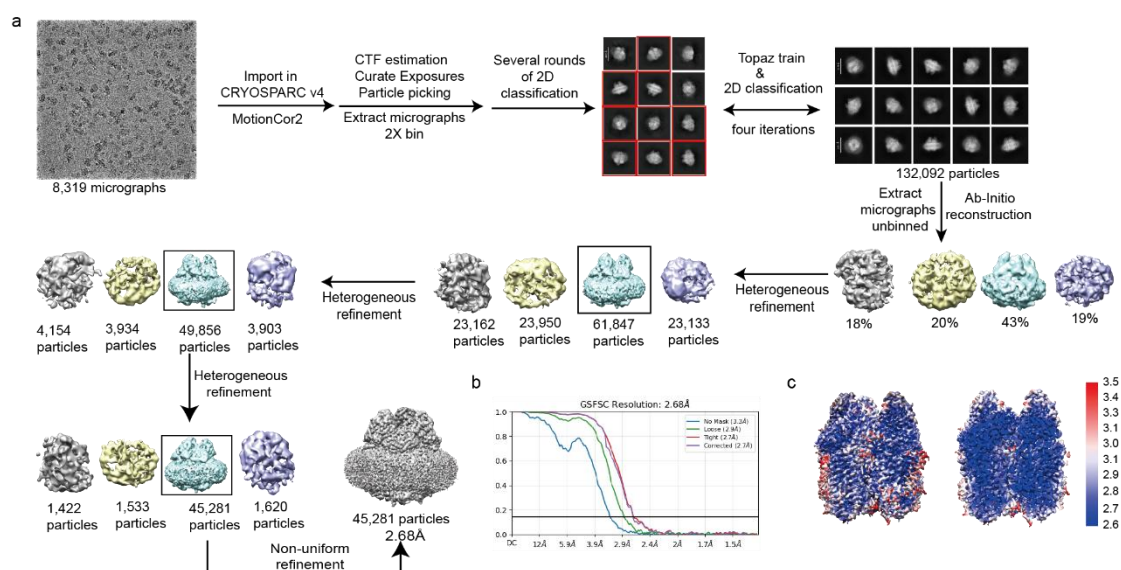
Supplementary Fig. 15 | Calculated the mechanism of mPQH[•] radical conformational reorganization near the Cu_C site. a, QM/MM metadynamics-calculated free energy profile (in kcal/mol) for the conformational reorganization of the mPQH[•] radical near the Cu_C site. The reaction coordinate is defined as the difference between the distance from OD1 of the residue D133 to O8 of the mPQH[•] radical (d1) and the distance between OD1 and O13 of the mPQH[•] radical (d2). **b**, The geometry of intermediates and transition states. Key distances are given in angstrom (Å).



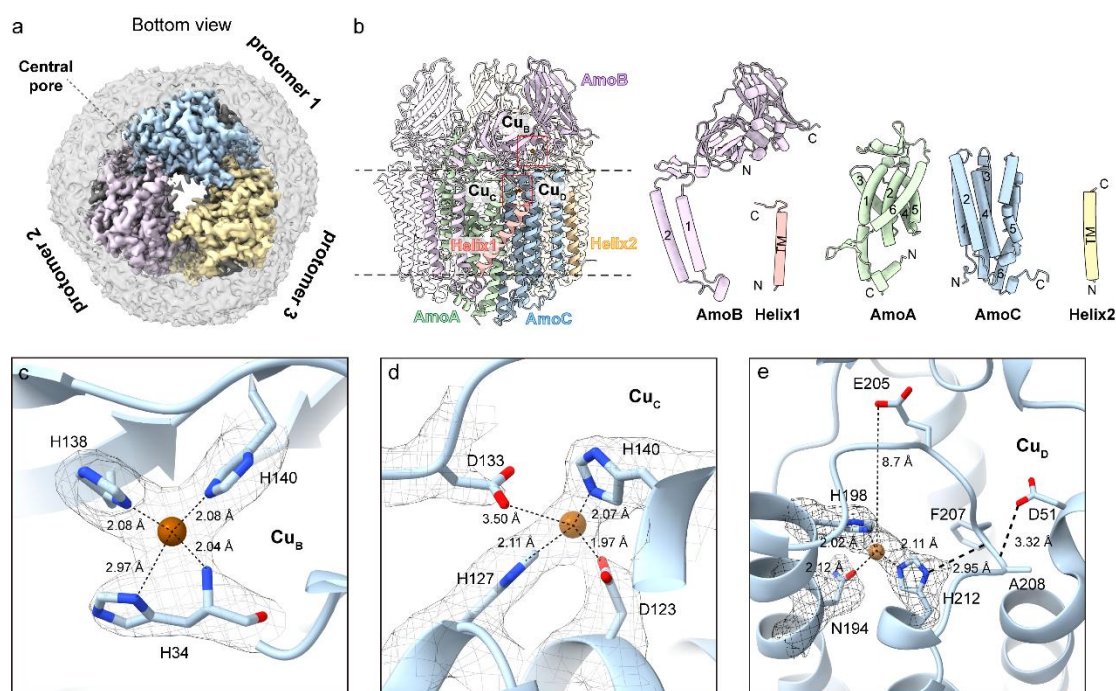
Supplementary Fig. 16 | Calculated mechanism of the second proton-coupled reduction of the mPQH• radical to mPQH₂ at the Cu_C site. QM(UB3LYP-D3/def2-TZVP)/MM-calculated relative energies (in kcal/mol) for the second proton-coupled electron transfer (PCET) from the mPQH• radical (${}^2\text{IC2}_{\text{CuC}}$) to form mPQH₂ (${}^2\text{IC3}_{\text{CuC}}$) at the Cu_C site in the open-shell double state. QM(UB3LYP-D3/def2-SVP)/MM-optimized geometries of key species involved in the reaction are presented. The proton donor residue D133 is highlighted in a blue ball-and-stick model. Key distances are given in angstroms (Å).



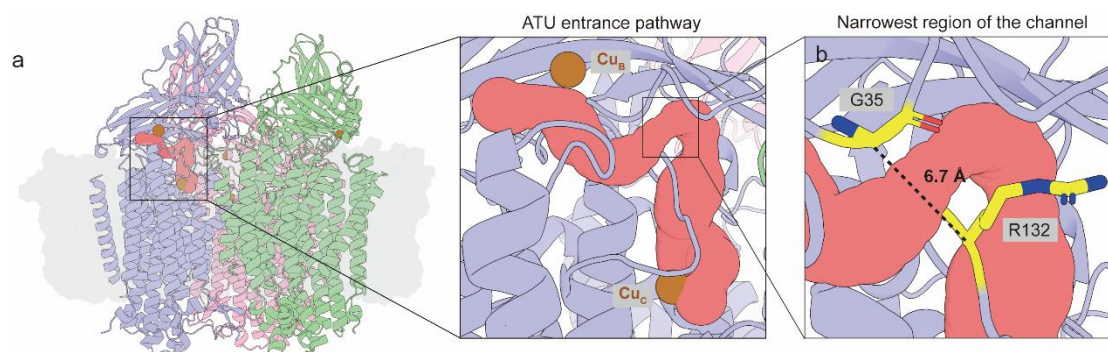
Supplementary Fig. 17 | Average inhibition of AO in AOA (left panels), AOB (middle panels) and comammox (right panels) under varying concentrations of NIs. Each subplot corresponds to a specific NI: ATU, DMPP, DCD, ASU and NP. Different colors and symbols represent different concentrations of the respective inhibitor. Control treatments lack NIs. Error bars indicate standard deviations derived from ≥ 3 biological replicates, each performed in triplicate. These data correspond to Fig. 3b.



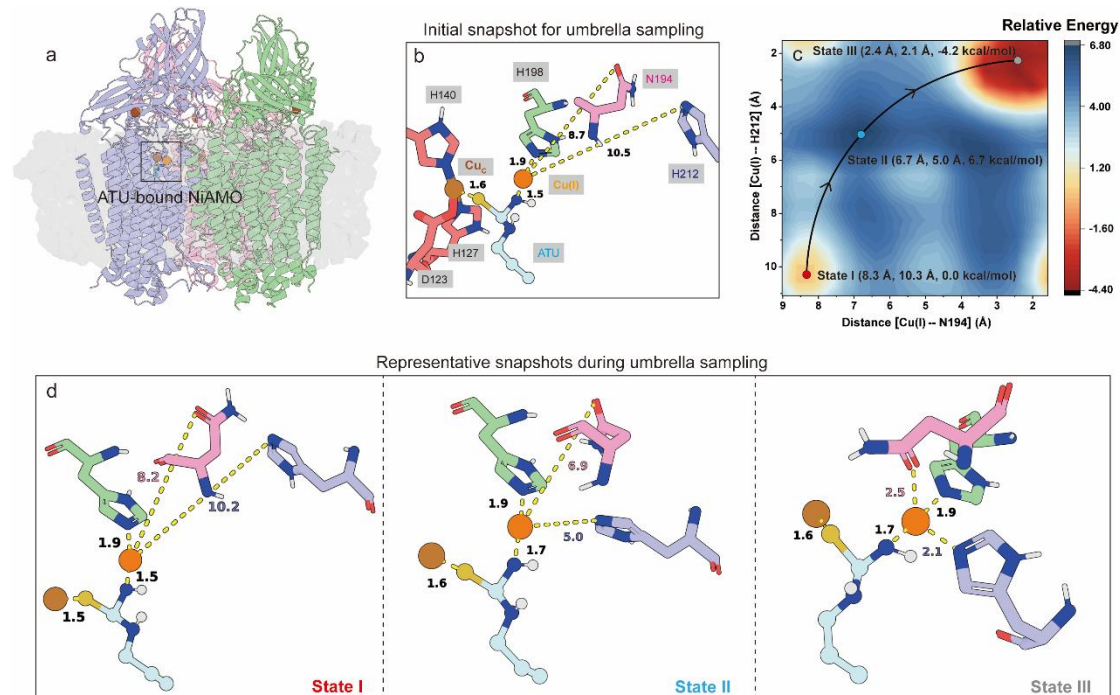
Supplementary Fig. 18 | Cryo-EM data analysis of ATU-treated *NiAMO*. **a**, The flowchart of cryoEM data processing of ATU-treated *NiAMO*. Details can be found in the “Method” section. **b**, Gold-standard Fourier shell correlation (GSFSC) curve for the ATU-treated *NiAMO* map generated using cryoSPARC 4.3.2. The threshold of 0.143 was used to determine the overall resolution of the map. **c**, Local resolution for the overall reconstruction (left) and a central slice (right) of ATU-treated *NiAMO*.



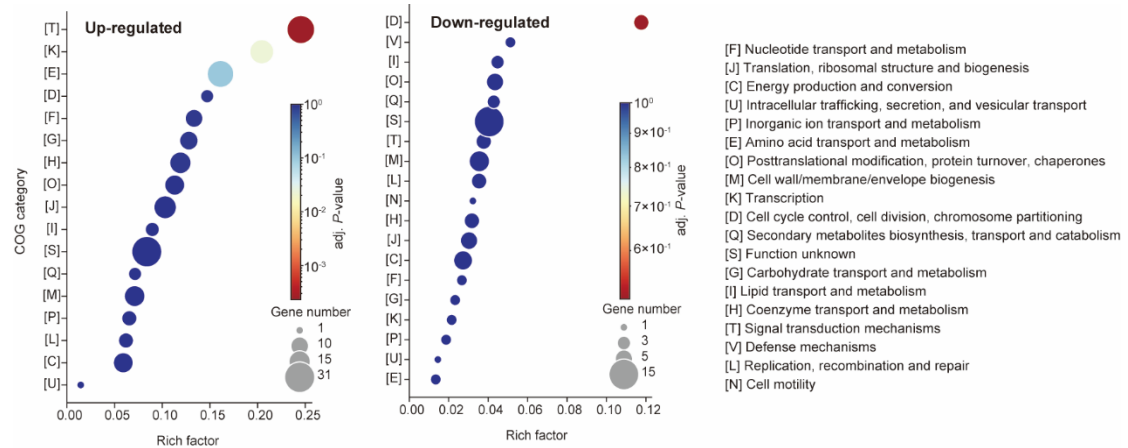
Supplementary Fig. 19 | Cryo-EM structure of ATU-treated NiAMO. **a**, Cryo-EM structure from bottom view showing three protomers (colored in blue, light purple and yellow, respectively) of ATU-treated NiAMO. **b**, Atomic model of ATU-treated NiAMO monomer. Five components of ATU-treated NiAMO are colored in green (AmoA), purple (AmoB), blue (AmoC), coral (Helix1) and yellow (Helix2). Cu_B site, Cu_C site and Cu_D site are highlighted by red box, with their coppers colored in brown. **c-e**, Cu_B site (**c**), Cu_C site (**d**) and Cu_D site (**e**) and their coordination environments with mesh cryo-EM density superimposed.



Supplementary Fig. 20 | Pathway of ATU migration into the *NiAMO* active site. a, Overview of the channel pathway mediating ATU transport from the external environment to the active site of *NiAMO*. **b**, The narrowest region of the channel for ATU entry. The amino acid residues at the narrowest regions are displayed as yellow stick models.



Supplementary Fig. 21 | Free-energy profile for Cu(I) ion capture by the Cu_D site in *NiAMO* following ATU entry. **a**, Overall structure of apo *NiAMO* with ATU and the Cu(I) ion at the Cu_D site superimposed based on the cryo-EM structure of ATU-treated *NiAMO*. The membrane is shown as a gray surface. Cu(I) ions at the Cu_C and Cu_D sites are shown as brown and orange spheres, respectively, and ATU is shown as a cyan ball-and-stick model. **b**, Initial snapshot for umbrella sampling simulations, with ATU and the Cu(I) ion at the Cu_D site positioned according to the ATU-treated *NiAMO* structure. Cu_C-site coordinating residues are shown as red sticks, while Cu_D-site residues N194, H198, and H212 are shown in pink, green, and blue, respectively. **c**, Umbrella-sampling-calculated free energy profile (in kcal/mol) for the transition of Cu(I) from the initial non-coordinating state (State I) to the Cu-coordinating state (State III). The reaction coordinates were defined as the distances between Cu(I) and N194 and between Cu(I) and H212, respectively. **d**, Representative snapshots sampled along the transition pathway of Cu(I) from the uncoordinated to the coordinated state. Key distances are given in angstrom (Å).



Supplementary Fig. 22 | Enrichment analysis of differentially proteins based on EggNOG database in every cluster from proteomic heatmap. Thresholds: $|\log_2FC| > 1.2$ and adjusted $P < 0.05$.

Supplementary Table 1. QM/MM energies (in Hartree, a.u.) of all species

UB3LYP/def2-TZVP//def2-SVP				
Species	QM	MM	QM/MM	ZPE
¹ RC _{CuD}	-3221.5200	-90.0915	-3311.6115	0.5520
³ RC _{CuD}	-3221.5261	-90.0928	-3311.6189	0.5057
¹ TS1 _{CuD}	-3221.5007	-90.0867	-3311.5874	0.5498
³ TS1 _{CuD}	-3221.5048	-90.0884	-3311.5932	0.5032
¹ IC1 _{CuD}	-3221.5289	-90.0887	-3311.6176	0.5518
³ IC1 _{CuD}	-3221.5282	-90.0895	-3311.6177	0.5053
¹ IC2 _{CuD}	-3221.4708	-56.9803	-3278.4511	0.5490
¹ TS3 _{CuD}	-3221.4696	-56.9782	-3278.4479	0.5473
¹ IC3 _{CuD}	-3221.4583	-57.0139	-3278.4722	0.5477
¹ IC3' _{CuD}	-3430.6902	-54.1615	-3484.8517	0.6270
¹ TS4 _{CuD}	-3430.6571	-54.1588	-3484.8159	0.6191
¹ IC4 _{CuD}	-3430.6918	-54.1612	-3484.8530	0.6233
¹ TS5 _{CuD}	-3430.6608	-54.1618	-3484.8226	0.6200
¹ IC5 _{CuD}	-3430.7034	-54.1558	-3484.8593	0.6234
¹ RC _{CuC}	-3167.8553	-58.9868	-3226.8421	/
¹ TS1 _{CuC}	-3167.8591	-58.9812	-3226.8403	/
¹ IC1 _{CuC}	-3167.8621	-58.9814	-3226.8435	/
² IC2 _{CuC}	-3168.5869	-59.0611	-3227.6480	/
² TS2 _{CuC}	-3168.5938	-59.0525	-3227.6462	/
² IC3 _{CuC}	-3168.6068	-59.0520	-3227.6588	/

212

Supplementary Table 2. QM energies (in Hartree, a.u.) of all species

UB3LYP/def2-TZVP//def2-SVP			
Species	EE	ZPE	EE+ZPE
¹ IC5' _{CuD}	-2434.244	0.262	-2433.981
¹ TS6 _{CuD}	-2434.229	0.256	-2433.973
¹ IC6 _{CuD}	-2434.242	0.259	-2433.983
¹ TS7 _{CuD}	-2434.243	0.258	-2433.985
¹ IC7 _{CuD}	-2434.256	0.261	-2433.996
¹ TS8 _{CuD}	-2434.228	0.260	-2433.968
¹ PC _{CuD}	-2434.281	0.264	-2434.016
³ IC5' _{CuD}	-2434.246	0.262	-2433.984
³ TS6 _{CuD}	-2434.232	0.256	-2433.976
³ IC6 _{CuD}	-2434.242	0.259	-2433.984
³ TS7 _{CuD}	-2434.244	0.258	-2433.986
³ IC7 _{CuD}	-2434.259	0.261	-2433.999

213

214

Supplementary Table 3. Spin density population of key atoms for all species

Species		Spin density		
QM/MM model	Cu _D	O1	O2	mPQH ₂ / mPQH [•] / mPQ
³ RC _{CuD}	0.36	0.77	0.8	0
³ TS1 _{CuD}	0.49	0.54	0.72	0.14
³ IC1 _{CuD}	0.55	0.08	0.25	0.98
¹ RC _{CuD}	-0.45	0.37	0.18	0
¹ TS1 _{CuD}	-0.57	0.37	0.26	0.09
¹ IC1 _{CuD}	-0.56	-0.07	-0.26	0.99
¹ TS2 _{CuD}	-0.61	-0.04	-0.19	0.99
¹ IC2 _{CuD}	-0.61	-0.04	-0.19	0.99
¹ TS3 _{CuD}	-0.66	-0.02	-0.09	0.93
¹ IC3 _{CuD}	0	0	0	0
¹ IC3' _{CuD}	0	0	0	0
¹ TS4 _{CuD}	-0.58	0.8	-0.05	0
¹ IC4 _{CuD}	-0.66	0.66	0.21	-0.01
¹ TS5 _{CuD}	-0.61	0.58	0.25	-0.02
¹ IC5 _{CuD}	-0.6	0.79	0	0
QM model	Cu _D	O	NH ₃ / NH ₂ [•] / NH ₂ OH	
¹ IC5' _{CuD}	-0.62	0.83	0.0	
¹ TS6 _{CuD}	-0.58	0.32	0.52	
¹ IC6 _{CuD}	-0.59	-0.06	0.90	
¹ TS7 _{CuD}	-0.57	-0.24	0.97	
¹ IC7 _{CuD}	-0.52	0.08	0.57	

¹ TS8 _{CuD}	-0.28	0.05	0.27
¹ PC _{CuD}	0.0	0.0	0.0
³ IC5' _{CuD}	0.50	1.24	0.0
³ TS6 _{CuD}	0.54	0.73	0.56
³ IC6 _{CuD}	0.59	0.31	0.91
³ TS7 _{CuD}	0.59	0.24	0.97
³ IC7 _{CuD}	0.59	0.30	0.85
QM/MM model	Cu _C	mPQH ₂ / mPQH [•] / mPQ	
¹ RC _{CuC}	0.0000	0.0000	
¹ TS1 _{CuC}	-0.6500	0.9900	
¹ IC1 _{CuC}	-0.6200	0.9900	
² IC2 _{CuC}	0.0900	0.9000	
² TS3 _{CuC}	0.6700	0.0000	
² IC3 _{CuC}	0.6400	0.0000	

216

217

Supplementary Table 4. Data collection, refinement and validation statistics

	PDB 45KL EMDB-83338	PDB 45LB EMDB-83349
Data collection and processing		
EMDB code	83338	83349
PDB code	45KL	45LB
Magnification	130,000	130,000
Voltage (kV)	300	300
Camera	Falcon4i	Falcon4i
Electron exposure (e ⁻ /Å ²)	50	50
Defocus range (μm)	-1.2 ~ -2.0	-1.2 ~ -2.0
Pixel size (Å)	0.6584	0.6584
Micrographs (no.)	15,188	8,319
Initial particle images (no.)	338,527	132,092
Final particle images (no.)	97,091	45,281
Symmetry imposed	C3	C3
Map resolution (Å)	2.47	2.68
Map sharpen B factor (Å ²)	85.4	84.4
FSC threshold	0.143	0.143
Map resolution range (Å)	2.4-3.3	2.6-3.5
Refinement		
Model resolution (Å)	2.47	2.68
FSC threshold	0.143	0.143
Model composition		
Non-hydrogen atoms	23168	23555
Protein residues	2820	2898
Ligands	CU: 6	CU: 9, ATU: 3
Waters	476	437
ADP (B-factors)		
Protein	8.54/125.85/52.45	3.46/141.00/40.10
Ligand	73.82/98.55/85.83	43.66/101.55/61.16
Water	30.87/75.25/47.89	13.96/71.87/38.02
R.m.s. deviations		
Bond lengths (Å)	0.004	0.003
Bond angles (°)	0.645	0.554
Validation		
MolProbity score	2.07	1.68
Clashscore	6.09	5.41
Rotamer outliers (%)	2.46	1.57
Ramachandran plot		
Favored (%)	93.24	96.41
Allowed (%)	6.15	3.49
Disallowed (%)	0.61	0.10

Supplementary Table 5. Copper content in active and inactivated *Ni*AMO samples measured by ICP-MS

Sample type	Sample name	Sample weight (g)	Final vol. (mL)	Element	Measured conc. (µg/L)	Cu (mg/kg) ^a
Active <i>Ni</i> AMO	AMO_WT-1	0.011	10	Cu	112.00	104.67 ^b
	AMO_WT-2	0.011	10	Cu	111.80	104.48
	AMO_WT-3	0.011	10	Cu	111.62	104.31
Inactivated <i>Ni</i> AMO	AMO_ATU-1	0.0051	10	Cu	52.67	103.27
	AMO_ATU-2	0.0051	10	Cu	52.67	103.28
	AMO_ATU-3	0.0051	10	Cu	52.65	103.23
Buffer	buffer-1	0.0049	10	Cu	2.21	4.50
	buffer-2	0.0049	10	Cu	2.17	4.44
	buffer-3	0.0049	10	Cu	2.25	4.59

^athe net copper mass in the sample was calculated as: $m(\text{Cu})_{\text{net}} = m(\text{Cu})_{\text{sample}} - m(\text{Cu})_{\text{buffer}}$. Analogous to pMMO, AMO accounted for approximately 80% of the total membrane-bound protein abundance.

^bthe molecular weight of *Nm*AMO trimer is 328.53 kDa.

Supplementary Table 6. QPCR-based quantification of *amoA* gene abundance in

AOA, AOB, and comammox

Target genes	Primer sequence (5'-3')	PCR conditions	Efficiency	R ²	References
AOA- <i>amoA</i>	1F: STAATGGTCTGGCTT AGACG 2R: GCGGCCATCCATCTG TATGT	95°C for 10min; 40× (95°C for 30s; 56°C for 45s; 72°C for 50s).	92%	0.99	Francis et al., 2005 ¹
β -AOB- <i>amoA</i>	1F: GGGGHTTYTACTGGT GGT 2R: CCCCTCKGSAAAGCC TTCTTC	95°C for 10min; 40× (95°C for 30s; 58°C for 40s; 72°C for 60s).	85%	0.99	Kowalchuk et al., 1999 ²
CMX clade A- <i>amoA</i>	Ntsp-162F: GGATTCTGGNTSGA TTGGA Ntsp-359R: WAGTTNGACCACCA STACCA	95°C for 10min; 40× (95°C for 15s; 48°C for 30s; 72°C for 45s).	85%	0.99	Fowler et al., 2018 ³

Reference

- 1 Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E. & Oakley, B. B. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14683–14688 (2005). <https://doi.org/10.1073/pnas.0506625102>
- 2 Kowalchuk, G. A. *et al.* Molecular analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in compost and composted materials. *Appl. Environ. Microbiol.* **65**, 396–403 (1999). <https://doi.org/10.1128/aem.65.2.396-403.1999>
- 3 Fowler, S. J., Palomo, A., Dechesne, A., Mines, P. D. & Smets, B. F. Comammox Nitrospira are abundant ammonia oxidizers in diverse groundwater-fed rapid sand filter communities. *Environ. Microbiol.* **20**, 1002–1015 (2018). <https://doi.org/10.1111/1462-2920.14033>