

Supplementary Materials for
Structure of the Wilson disease copper transporter ATP7B

Ryan M. Bitter, SeCheol Oh, Zengqin Deng, Suhaila Rahman, Richard K. Hite*, Peng Yuan*

*Corresponding author. Email: hiter@mskcc.org (R.K.H.); yuanp@wustl.edu (P.Y.)

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Figs. S1 to S5
Table S1

xATP7B	1	-----MFPKRILDEEEGVQLLSTENEKCS TKRRSSPQFCVN---NTFSDSPVSVWKEAK
hATP7B	1	-----MPEQERQITAREGASRKILS---KLS--LPTRAWEPAM
xATP7A	1	METDSRAFLAFIPVEGMTCNCSVQTIEQKIGSINGVHSIKVSLEGKNAIIYDAKLQTPK
hATP7A	1	MDPSMGVNSVTISVEGMTCNCSVWTIEQQIGKVGNVHHIKVSLEEKNAIIYDPKLQTPK
LpCopA		-----
xATP7B	52	KPSCAFDNRGYEGSPDDLCSLPDDVGSVVVAIQGMTQCQSCVQSI EGRISKVSGVVGINVC
hATP7B	34	KKSFAFDNVGYEGGLDGLG-PSSQVATSTVRILGMTQCQSCVKSIEDRISNLKGIISMKVS
xATP7A	61	ALQEAVEDMGFESTLSYANPQPVPMDSTFLRLS-AEQSA--EQIRSGLSQFKGVLDVKTS
hATP7A	61	TLQEAIIDDMGFDAVIHNPDLPLVLTDTLFLTVT-ASLTLPWDHIQSTLLKTKGVTDIKIY
LpCopA		-----
xATP7B	112	LEQNNAIVNYLQTEITPHKICEEIEDMGFDASLSEQS-GMPSSVKSSYYGDNVIKIRVEG
hATP7B	93	LEQGSATVKYVPSVVCLQQVCHQIGDMGFEASIAEGK-AASWPSRSLPAQEAVVKLRVEG
xATP7A	118	LEGKVACVTFIPSVVNPGLLIQKIPGLSLDSMASKKTTSGSVESRSSVASDVLVKMKIEG
hATP7A	120	PQKRTVAVTIIPSI VNNANQIKELVPELSLDTGTLEKKSGACEDHSMQAAGEVVLKMKVEG
LpCopA		-----
xATP7B	171	MTCQSCVNTIEGKIGKIQGVQKIKVSLTGQEA VITYQSHIIQAEDLRKYIEDMGFEASIK
hATP7B	152	MTCQSCVSSIEGKVRKLGVRVVKVLSLNQEA VITYQPYLIQPEDLRDHVNDMGFEAAIK
xATP7A	178	MTCHSCTSTIEGKVGKLGVRQRIKVS LDSQEAQILYQPHLIKPEDIRTQIEEAGFIAQIK
hATP7A	180	MTCHSCTSTIEGKIGKLGQVQRIKVS LDNQEATIVYQPHLISVEEMKKQIEAMGFPAFVK
LpCopA		-----
xATP7B	231	NKPDPTKLG TIDIERLQN-----SIAENHS-----GHTNSNTVTLG--IDGMHCKSC
hATP7B	212	SKVAPLSLGPIDIERLQSTNPKRPLSSANQNFNNSETLGHQGSHVVTLQLRIDGMHCKSC
xATP7A	238	SKP-PQKLG AIDIERLTN-----TQTNSNGDLPQKIPKHQNDLIRAI FQIEGMHCKSC
hATP7A	240	KQPKYLKLG AIDVERLKN-----TPVKSSEGSQQRSPSYTNDST-ATFIIDGMHCKSC
LpCopA		-----
xATP7B	276	VHNIIEGYVSLGAGIQSIRVSLKNKNAV VCLSQGSTSLLSLKESIENLPPGKFKVTL PVGV
hATP7B	272	VLNIEENIGQLLG VQSIQVSL ENKTAQVKYDPSCTSPVALQRAIEALPPGNFKVSLPDGA
xATP7A	290	VVNIESNIGSQHGVS SIEVSL ENKLAVIH FYSNITTS DALIQAIEALS PGTFKVSLCNHP
hATP7A	292	VSNIESTLSALQYVSSIVVSL ENRS AIVKYNASSVTPESLRKAIEAVSPGLYRV SIT---
LpCopA		-----
xATP7B	336	EKG---QSLARNSTHSSHRDQSMG-----GNIAIISIGGMTQCQSCVSS IENMISQRKGV
hATP7B	332	EGSGTDHRSSSSHS PGSPPRNQVQGT----CSTTLIAIAGMTCASCVHSIEGMI SQLEGV
xATP7A	350	ELAETFMSQKNSEASC AKEIFSSPSNQDFCSKVTVINIEGMTCMSCVQSIEGLISKKPGV
hATP7A	349	--SEVESTSNSPSSSSLQKIP LNVVSQPLT-QETVINIDGMTCNCSVQSI EGVISKKPGV
LpCopA		-----
xATP7B	387	LHILVSLDEGN GNI FYNPCETNAEELRAAIEDMGFHSTLVSDNSPSISCS EYNSKEEENK
hATP7B	388	QQISVSLAEGTATVLYNPSVISPEELRAAIEDMGFEASV VSESCSTNPLGNHSAGNSMVQ
xATP7A	410	KSIQVSLVNNNGTVQYDPAVTS PEDIRAAIEDMGFDASLLANTGQADAF TSEAS-----
hATP7A	406	KSIRVSLANSNGTVEYDPLLTSPETLRGAIEDMGFDATLSDTNEPLV VVIAQPSS-----
LpCopA		-----

xATP7B	447	QTP--PKATRQISGSRDYILDVLPKKSHPDFANEKYDTAPEKCFLOITG MTCISC VSNI
hATP7B	448	TTDGTPTSVQEVAPHTGRLPANHAPDILAKSPQSTRAVAPQKCFLOIKG MTCASC VSNI
xATP7A	464	-----QKPFLLNSLLPKERTNKETPVHSHKPEKRTV-NKCFIQITG MTCASC VANIE
hATP7A	460	-----EMPLLTSTN-EFYTKGMPVQDKKEEGKNS-SKCYIQVTG MTCASC VANIE
LpCopA	1	-----MK

MBD5

xATP7B	505	RNLKKKDGIVSVLVALMSGKAEVKFYDPRIEPLIEIAQLVEDLGFASVMEDYTASDGNVE
hATP7B	508	RNLQKEAGVLSVLVALMAGKAEIKYDPEVIQPLEIAQFIQDLGFEEAVMEDYAGSDGNIE
xATP7A	513	RNLRREEGIYSVLVALMAGKAEVRYNPEVLQPSGIAELIQELGFEEAVLENYDDGTGILE
hATP7A	508	RNLRREEGIYSILVALMAGKAEVRYNPAVIQPPMIAEFIRELGFATVIENADEGDGVLE
LpCopA	3	HDHHQG-----HTHSGKGHACHH-----EHNSPKTQQAS

MBD6

xATP7B	565	LIITG MTCASC VHNIESRLMRTPGILQASVALATCKAQVKFDPEIVGPRDIRIEGIGF
hATP7B	568	LTITG MTCASC VHNIESKLTRTNGITYASVALATSKALVKFDPEIIGPRDIKIIIEEIGF
xATP7A	573	LLVRG MTCASC VHKIESRLMKTGVLYSVALATNKAHIKFDPEIVGPRDIMKIINDLGF
hATP7A	568	LVVVG MTCASC VHKIESLTKHRGILYCSVALATNKAHIKYDPEIIGPRDIHTIESLGF
LpCopA	32	SKMEGPVIVYTCPMHPEIRQSAPGHCPLCGMALEPETVTVS---EVSF-----

xATP7B	625	QASLAKRDPTAHKLDHKE E IKQWRNSFLFSLFSGIPVII LM IY ML AANK-----
hATP7B	628	HASLAQRNPNAHHLDHK E IKQWKKSFLCSLVFGIPVMAL MI Y ML IPSN-----
xATP7A	633	STSLVKKDRSASHLDHR V EIQRWKRSFLISLIFCIPVMGL MV H MM FMENHYLMPHH-HNM
hATP7A	628	EASLVKKDRSASHLDH K REIRQWRRSFLVSLFFCIPVMGL MI Y MM VMDHFFATLHHNQNM
LpCopA	77	----- E YLDMRRRFWIALMLTIPVVILE MG GHGLKH-----

xATP7B	674	-----DHHNTMVLDRNIVPGLSIIINLVFFILCTFVQTL GG RYFYVQAY K SLKHKATN MD
hATP7B	677	-----EPHQSMVLDHNIIPGLSILNLIFFILCTFVQL GG WYFYVQAY K SLRHRSAN MD
xATP7A	692	TMEDIASYHPTMVLEYQIIPGLSIMNLVSLLLCIPVQFL GG WYFYIQAY K ALKHRTAN MD
hATP7A	688	SKEEMINLHSSMFLERQILPGLSVMNLLSFLLCVPVQFF GG WYFYIQAY K ALKHRTAN MD
LpCopA	108	-----FISNGSS-WIQLLLATPVVLW GG WPFKRGWQSLKTGQLN MF

xATP7B	728	VLIVLATTIAYIYSVVILTVA-MVEKADKSP---ETFFD TP P ML FMFIAL GR W LE H IA K
hATP7B	731	VLIVLATSIAVYVSLVILVVA-VAEKAERSP---VTFFD TP P ML FVFIAL GR W LE H LA K
xATP7A	752	VLIVLATSVAFIYSLIVLIVA-IFEKSKVNP---ITFFD TP P ML FVFIAL GR W LE H IA K
hATP7A	748	VLIVLATTIAFAYSLIILLVA-MYERAKVNP---ITFFD TP P ML FVFIAL GR W LE H IA K
LpCopA	150	TLIAMGIGVAWIYSMAVAVLWPGVFPFAFRSQEGVVAVY F EAAAVITTLVLLGQVLE EL K AR

A domain

xATP7B	783	SKTSEALAKLISLQATEAAVVTFGANQIILREEQVAVELVQRGDIVKVVPGGKFPVDGKV
hATP7B	786	SKTSEALAKLSLQATEATVVTLGEDNLIIREEQVPMELVQRGDIVKVVPGGKFPVDGKV
xATP7A	807	SKTSEALSRLISLQATEATVILDPDNTVKSEEQVDVELVQRGDIVRVTGGKFPVDGRV
hATP7A	803	GKTSEALAKLISLQATEATIVTLSDNILLSEEQVDVELVQRGDIKVVPGGKFPVDGRV
LpCopA	210	EQTGSAIRALLKLVPESAHRIKEDG-----SEEEVSLDNVAVGDLRLVRPGEKIPVDGEV

A domain

xATP7B	843	IEGTSMADES LITGE PMPVRRKPGSMVIAGSINAHGTVLVEATHVGSSETTLAQIVKLVEE
hATP7B	846	LEGNTMADES LITGE AMPVTKKPGSTVIAGSINAHGSVLIKATHVGNDDTLAQIVKLVEE
xATP7A	867	IEGQSMVDES LITGE AMPVSKKPGSSVIAGSINQNGSLLIAATHVGSDDTLAQIVKLVED
hATP7A	863	IEGHSMVDES LITGE AMPVAKKPGSTVIAGSINQNGSLLICATHVGADTTLSQIVKLVEE
LpCopA	265	QEGRSFVDESMV TGE PIPVAKEASAKVIGATINQTSFVMKALHVGSDTMLARIVQMVS

			A domain	M3	
xATP7B	903	AQMSKAPIQQLA	DK ISGYFVPFIIISVVTLVTWIIIGFVNF	DI IKYFPSYSKNISKTE	
hATP7B	906	AQMSKAPIQQLA	DR FSGYFVPFIIIMSTLTLVWVIVIGFIDFGVVQRYFPNPNKHISQTE		
xATP7A	927	AQTSKAPIQQFA	DK LSGYFVPFIVAVSVLTLFVWIIIGYHNFEIVEKYFPGYNKSISKGE		
hATP7A	923	AQTSKAPIQQFA	DK LSGYFVPFIVFVSIATLLVWVIVIGFLNFEIVETYFPGYNRSISRTE		
LpCopA	325	AQRSRAPIQRLA	D TVSGWFPVAVILVAVLSFIVWALLG-----	PQ	
			M4A	M4B	P domain
xATP7B	963	VIIRVAFQTSITVLSIA	CPC ALGLATPTAVMVGTVGAAQNGILIKGGPELEMAHKIKAVM		
hATP7B	966	VIIRFAFQTSITVLCIA	CPC SLGLATPTAVMVGTVGAAQNGILIKGGKPLEMAHKIKTVM		
xATP7A	987	VIVRFAFQAAITVLCIA	CPC SLGLATPTAVMVGTVGVAQNGILIKGGPELEMAHKVKTVV		
hATP7A	983	TIIRFAFQASITVLCIA	CPC SLGLATPTAVMVGTVGVAQNGILIKGGPELEMAHKVQVVV		
LpCopA	365	PALSYGLIAAVSVLI	IACPC ALGLATPMSIMVGVGKGAQSGVLIKNAEALERMEKVNTLV		
			N domain		
xATP7B	1023	FDKTGT IITHGVPKVMRVLLLGDVVKMPLKRMLAVVGTAEASSEHPLGMAVTKYCKEELGT			
hATP7B	1026	FDKTGT IITHGVPRVMRVLLLGDVATLPLRKLAVVGTAEASSEHPLGVAVTKYCKEELGT			
xATP7A	1047	FDKTGT ITKGTTPVVMQVKVLVESNRMPSNKL LAIVGTAESNSEHPLGSAVIKYCKEVLNA			
hATP7A	1043	FDKTGT IITHGTPVNVQVKVLTESNRISHHKILAIIVGTAESNSEHPLGTAITKYCKQELDT			
LpCopA	425	VDKTG TLTEGHPKLTRIVT----DDFVEDNALALAAALEHQSEHPLANAIVHAAKEKG--			
			N domain		
xATP7B	1083	ELLYCYTDFQAVPGCGISCKVNNIESVLVQNEE---GLNEQNSYRNSLIGT-----TDSS			
hATP7B	1086	ETLGYCYTDFQAVPGCGIGCKVSNVEGILAHSER---PLSAPASHLN-----EAGS			
xATP7A	1107	ETLGTCTDFQAVPGCGISCKVNNIEPLLHAANG---PDNKRNAVVLVQIDGQHEDSILPSI			
hATP7A	1103	ETLGTCTDFQVVPGCGISCKVTNIEGLLHKNNWNIEDNNIKNASLVQIDASNEQSSTSSS			
LpCopA	479	LSLGSVEAFEAPTGKGVVQVD-----			
			N domain		
xATP7B	1135	LIITPELLGAQAPLAHTVLIGNREWMRRNGLHISTDVDEAMSSHEMKGQTAVLVAIDGEL			
hATP7B	1133	LPAEKDAV----PQTFSVLIGNREWLRRNGLTISSDVSDAMTDHEMKGQTAILVAIDGVL			
xATP7A	1164	TVSQNPSNGSSPQTYSVLIGNREWMNRNFLTTLTAETDSLMTHEHEKGRTAVLVAVDGVV			
hATP7A	1163	MIIDAQISNALNAQQYKVLIGNREWMIRNGLVINNDVNDFMTEHERKGRTAVLVAVDDEL			
LpCopA	501	-----GHHVAIGNARLMQEHGGDN-APLFEKADELGRGKASVMFMAVDGKT			
			P domain		
xATP7B	1195	CGMIAIADTVKQEAAALAVHTLKSMDIVVLTIGDNRKTAKAIATQVGIKKVF AEVLP SHK			
hATP7B	1189	CGMIAIADAVKQEAAALAVHTLQSMGVDVVLITIGDNRKTARAIATQVGINKVF AEVLP SHK			
xATP7A	1224	CGLIAIADTVKPEAALAIHSLRSMGLDIVLMTGDNSKTAKTIAAQVGITKVF AEVLP SHK			
hATP7A	1223	CGLIAIADTVKPEAELAIHILKSMGLEVLMTGDNSKTARSIASQVGITKVF AEVLP SHK			
LpCopA	546	VALLVVEDPIKSSTPETILELQQSGIEIVMLTGDSKRTAEAVAGTLGIKKVV AEIMPEDK			
			P domain		
xATP7B	1255	VAKVQALQSDNKRVMVGDGVNDSPALARADVGIAIGTGTDVAIEAADIVLIRNDLLDVV			
hATP7B	1249	VAKVQELQNKGGKVMVGDGVNDSPALAQADMGVVIAIGTGTDVAIEAADVVLIRNDLLDVV			
xATP7A	1284	VAKVKQLQEQGTRVAMVGDGINDSPALAMADVGITIGSGTDVAIEAADVVLIRNDLLDVV			
hATP7A	1283	VAKVKQLQEEGKRVAMVGDGINDSPALAMANVGIAIGTGTDVAIEAADVVLIRNDLLDVV			
LpCopA	606	SRIVSELKDKGLIVAMAGDGVNDAPALAKADIGIAMGTGTDVAIESAGVTL LHGDLRGIA			
			M5	M6	
xATP7B	1315	ASIHLSKRTVRRIRLNLFV FAL IYNLLG IPIAAGVFMPAG-LVLQPW	MGSAA	MAAS	SVSVV
hATP7B	1309	ASIHLSKRTVRRIRINLV LAL IYNLVG IPIAAGVFMPIG-IVLQPW	MGSAA	MAAS	SVSVV
xATP7A	1344	ASIDLSRKTVKRIRINLFV FAL IYNLVG IPIAAGVFMPIG-LILQPW	MGSAA	MAAS	SVSVV
hATP7A	1343	ASIDLSRKTVKRIRINLFV FAL IYNLVG IPIAAGVFMPIG-LVLQPW	MGSAA	MAAS	SVSVV
LpCopA	666	KARRLSESTMSNIRQNLFFAFIYNVLGVPLAAGVLYPLTGLLLSP	MAIAAA	MA	LS

		M6	C-terminus
xATP7B	1374	LSSLQLKCYRKPDSDRYEARAQGHMKPLTPSQISVHIGMDDRWRDLPKTKAWDQISYISQ	
hATP7B	1368	LSSLQLKCYKKPDLERYEAQAHGHMKPLTASQVSVHIGMDDRWRDSPRATPWDQVSYVSQ	
xATP7A	1403	FSSLLKLYRKPSREKLEQRVQGQMRQKSLSDISVHIGLTENRRTSTKLDLLDRIVNYSR	
hATP7A	1402	LSSLFLKLYRKPTYESYELPARSQIGQKSPSEISVHVGIDDTSRNSPKLGLLDRIVNYSR	
LpCopA	726	INALRLKRVTL-----	
<hr style="border: 1px solid red;"/>			
xATP7B	1434	VSR---ASQPKRHHGSLVEQQ-DKWSLLINETHEDQMI--	1467
hATP7B	1428	VSLSSLTSDKPSRHSAAADDDGDKWSLLNLRDEEQYI--	1465
xATP7A	1463	ASLNSFLSDKHSQHSLPLSEP-DKHSLLLGLKGEEDTFL	1501
hATP7A	1462	ASINSLSDKRSLSNVVTSEP-DKHSLLVGDFFREDDTAL	1500
LpCopA	736	-----	736

Fig. S1. Sequence alignment of Cu⁺-ATPases. The protein sequences of *Xenopus tropicalis* ATP7A (xATP7A, NCBI sequence: NP_001361526.1) and ATP7B (xATP7B, NCBI sequence: XP_002936778.2), *Homo sapiens* ATP7A (hATP7A, NCBI sequence: NP_000043.4) and ATP7B (hATP7B, NCBI sequence: NP_000044.2), and *Legionella pneumophila* Cu⁺-ATPase (LpCopA, NCBI sequence: WP_010946759.1) are aligned. Domain boundaries and transmembrane helices are indicated for xATP7B. Conserved amino acids critical for Cu⁺ transport are highlighted.

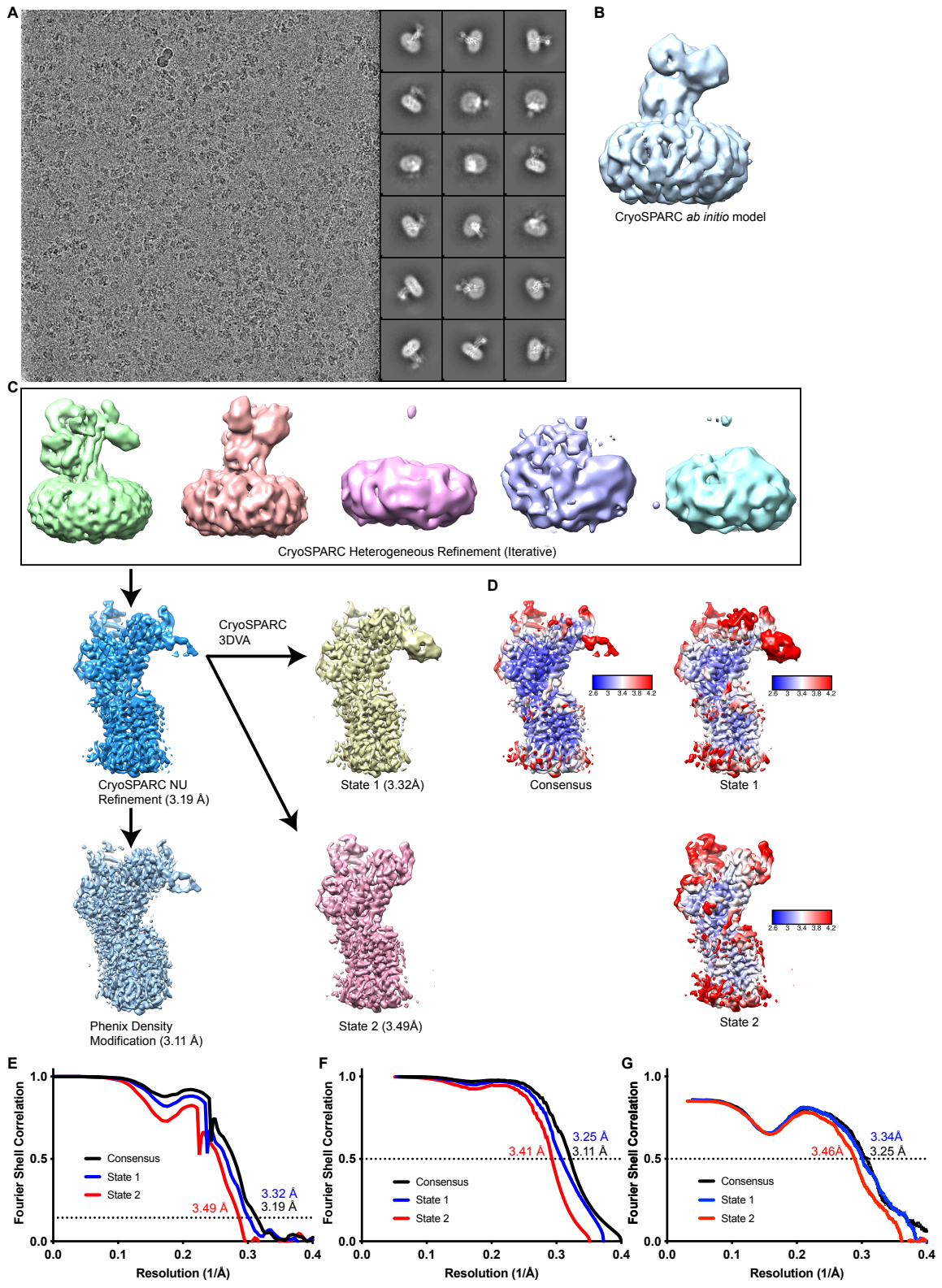


Fig. S2. Cryo-EM processing workflow. (A) Representative image and 2D class averages of xATP7B_{EM}. (B) CryoSPARC *ab initio* model. (C) Simplified Cryo-EM image processing workflow for xATP7B_{EM}. (D) Consensus, state 1 and state 2 density maps colored by local resolution using Blocres. (E-G) Fourier shell correlation (FSC) between two independent half-maps following non-uniform refinement (E), FSC between two independent half-maps following density modification (F) and FSC between density modified map and model following real space refinement (G). Black, blue and red curves correspond to consensus, state 1 and state 2 reconstructions, respectively.

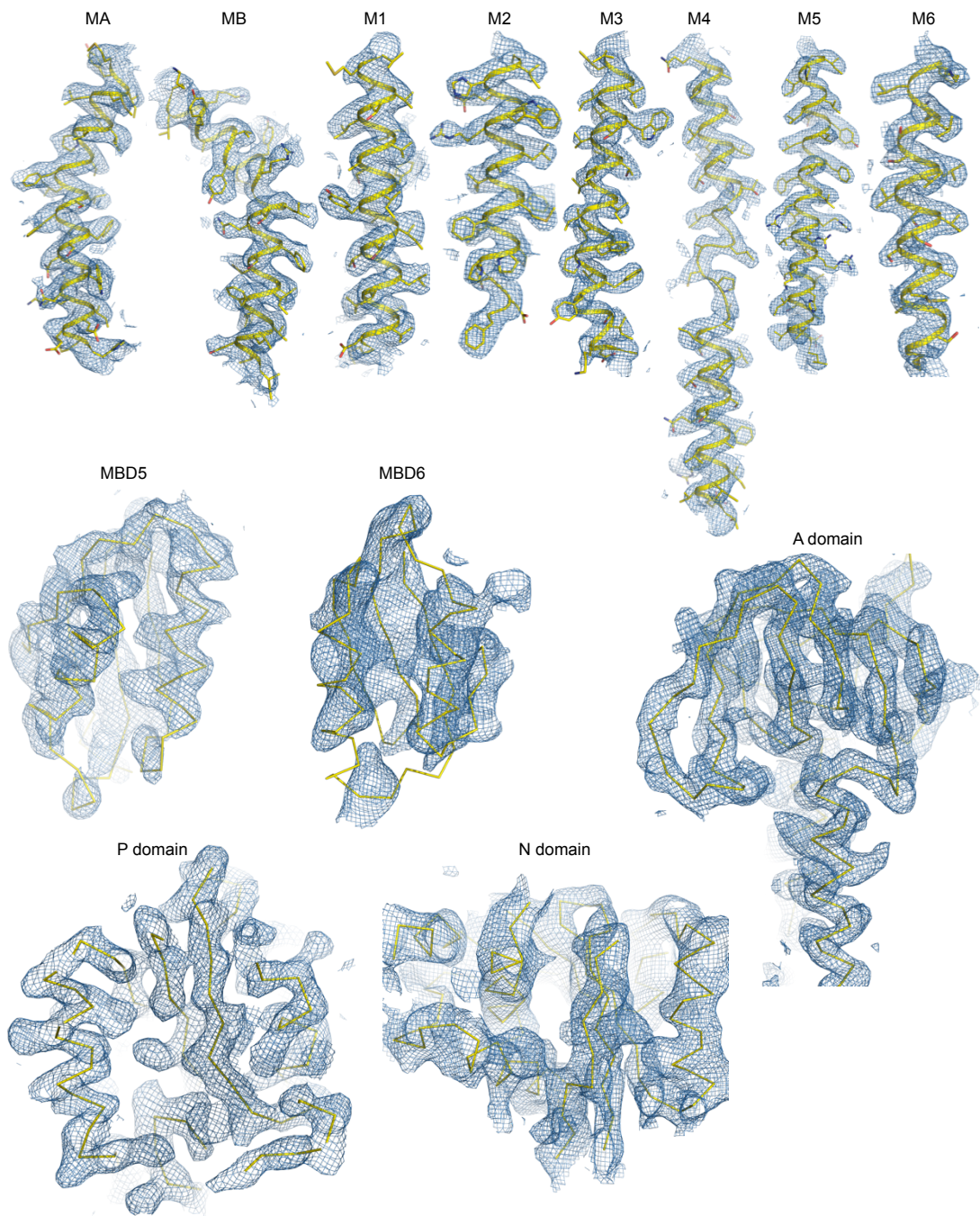


Fig. S3. Cryo-EM density. Representative densities of the TM helices and cytoplasmic domains for the state 1 reconstruction.

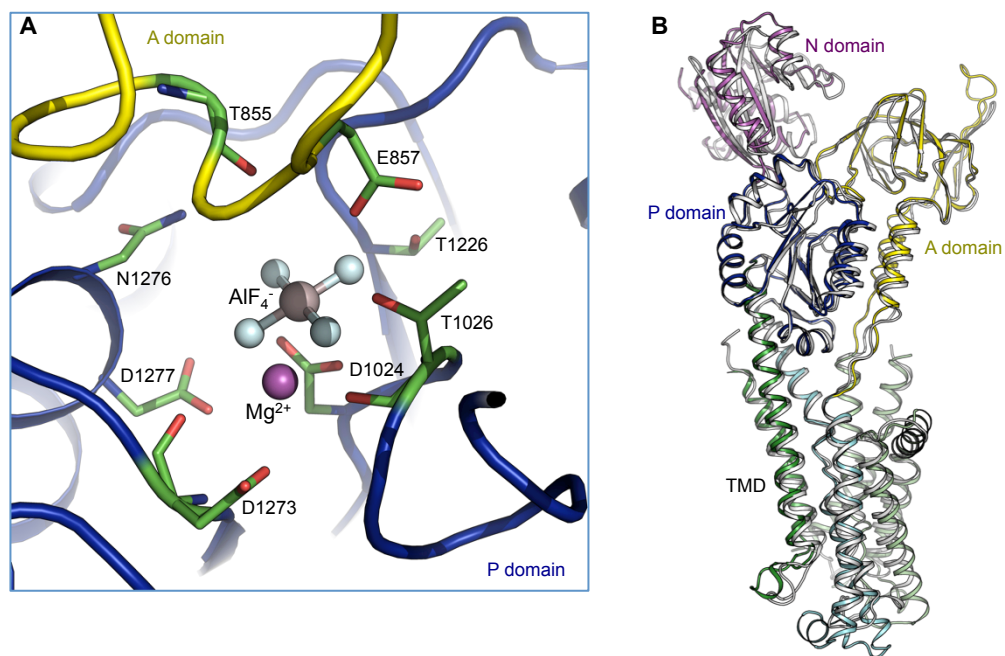


Fig. S4. Structure of xATP7B_{EM} in the E2-Pi state. (A) Active site in the P domain. **(B)** Superposition of structures of LpCopA (PDB: 3RFU) and the core of xATP7B_{EM}.

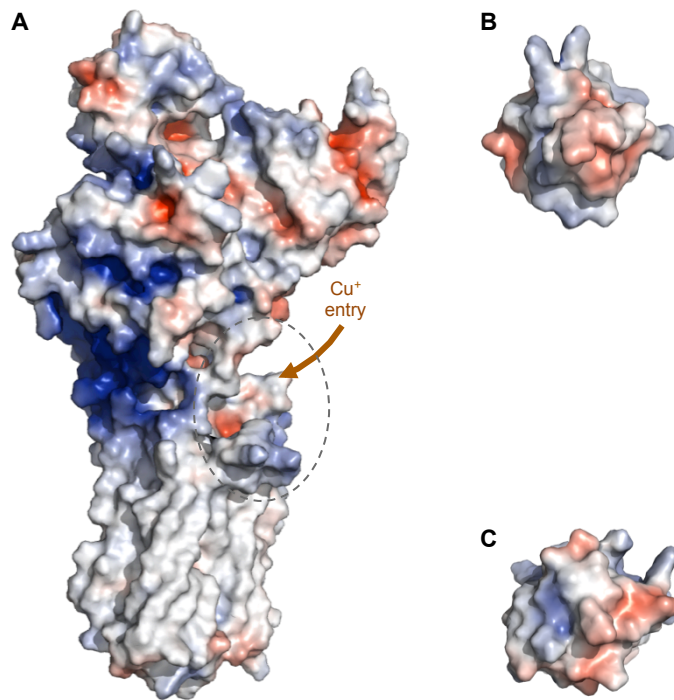


Fig. S5. Surface electrostatic potential of the xATP7B_{EM} core (A), MBD6 (B), and ATOX1 (C). Surface electrostatic potential is indicated by colors (red, -5 kT/e; white, neutral; blue, +5 kT/e). The frog ATOX1 model was generated on the basis of the crystal structure of human ATOX1 (PDB code: 1FEE).

Table S1. Cryo-EM data collection, refinement and validation statistics

	xATP7B _{EM} Consensus (EMD-25137) (PDB 7S13)	xATP7B _{EM} State 1 (EMD-25138) (PDB 7S16)	xATP7B _{EM} State 2 (EMD-25139) (PDB 7S17)
Data collection and processing			
Magnification	22,500x	22,500x	22,500x
Voltage (kV)	300	300	300
Electron exposure (e ⁻ /Å ²)	58.8	58.8	58.8
Defocus range (µm)	-1.2 to -2.5	-1.2 to -2.5	-1.2 to -2.5
Pixel size (Å)	0.85	0.85	0.85
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	1,889,296	1,889,296	1,889,296
Final particle images (no.)	257,208	138,790	118,418
Map resolution (Å)	3.19	3.32	3.49
0.143 FSC threshold			
Density modified resolution (Å)	3.11	3.25	3.41
Refinement			
Initial model used (PDB code)	3RFU	7S13	7S13
Model resolution (Å)	3.25/2.71	3.34/2.73	3.46/2.92
0.5 / 0.143 FSC threshold			
Model resolution range (Å)	300-3.1	300-3.2	300-3.4
Model composition			
Non-hydrogen atoms	6629	6629	5510
Protein residues	879	879	728
Ligands	2	2	2
<i>B</i> factors (Å ²)			
Protein	104	102	71
Ligand	41	51	48
R.m.s. deviations			
Bond lengths (Å)	0.002	0.002	0.002
Bond angles (°)	0.385	0.460	0.474
Validation			
MolProbity score	0.99	1.23	1.40
Clashscore	2.14	4.57	5.23
Poor rotamers (%)	0.00	0.00	0.00
Ramachandran plot			
Favored (%)	98.49	98.61	97.36
Allowed (%)	1.51	1.39	2.64
Disallowed (%)	0.00	0.00	0.00