Structural characterization of the N-terminal domain of the *Dictyostelium discoideum* mitochondrial calcium uniporter

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Supporting Information (SI)

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Figure S1. The components of the mitochondrial calcium uniporter complexes in *Homo sapiens* and *Dictyostelium discoideum*. The uniporter components in *Homo sapiens* consist of mitochondrial calcium uniporter protein (MCU), MCU regulatory subunit b (MCUb) and essential MCU regulator (EMRE), together with the intermembrane space (IMS) proteins mitochondrial calcium uptake protein 1 (MICU1), MICU2 and probably MICU3. The *Dictyostelium discoideum* uniplex proteins only contain MCU homologue and a putative MICU1 homologue. The major difference for the pore-forming component MCU in *Homo sapiens* and *Dictyostelium discoideum* lies in the N-terminal domain (NTD).



Figure S2. A linear overview of the domain organization for the original sequence of DdMCU and the constructs used in this paper. For each protein construct, different colors represent different domain: the predicted mitochondrial targeting signal (MTS) (purple), 6×His tag (pink), N-terminal domain (NTD) (yellow), transmembrane domain (TM) (green), the conserved DXXE motif, and coiled-coil helix (CCH) (blue).



Figure S3. Structural alignment of MCU with different species homologues. Dali server was used to generate the structure alignment. (A) The sequence identity/similarity (top) and the z-score/RMSD values (bottom) of different fungal homologues comparing to HsMCU-NTD. (B) Superimposing protein structures of HsMCU-NTD (blue, PDB code: 4XTB) with MaMCU-NTD (PDB code: 6C5R) (yellow), CyMCU-NTD (PDB code: 6DNF) (violet), NcMCU-NTD (PDB code: 6DT0) (green), NfMCU-NTD (PDB code: 6D7W) (orange) (C) The aligned secondary structure between HsMCU-NTD and CyMCU-NTD (H/h: helix, E/e: strand, L/l: coil). Uppercase means structurally equivalent positions with CyMCU-NTD. Lowercase means insertions relative to CyMCU-NTD.

DdMCU MaMCU NcMCU CyMCU NfMCU HsMCU	MGHVLGGTLLAANRLAR MNCVRMRLQCRLMPSSNTLARWCLESP MRALVSRTPIAAALRSATLGSQCASIQ	PPAVVLGK. PR QKNLTLHR PR YNSLNILDRLPQP	VCCWRASPWPV ISNVALRQAST LPSRSFRIRYT MAAAAGRSLLL	IVSSALQFSSSS SVSPKTRETEAE GVSTSGRVTQTC LLSSRGGGGGGA	ARHINYISARYEARG Akakklogkrldehe RRSFQLSASSRDKRG GGCGALTAGCFPGLG
DdMCU MaMCU NcMCU CyMCU NfMCU HsMCU	RSTTORKVDDR.PWHRESSG EEVRAREQQVRRPWHREGAD PQSAEPDPLERLEVKKVQQQHENEKDD VSR.	SLPKSTSPDPTGGI KPPVEGNADP Sgrdtksggkvaki Hi	1 MNSF DATKGR IAKGK MTKG.K AMTKCDTIAGK RQQQHHRTVHQ	10 VIRNGFG <mark>L</mark> VRTF LLTTPTRLLKLI LLTTPSRLLKLV LLTTPSRLKLV LLTTPSRLFKLL RIASWQN <mark>L</mark> GAVY	20 NTRLFTTS LPIPFHPEQEYIN LPLPLRVEKDQKNNG LPLSTVDHN CSTVVPSDD
DdMCU MaMCU NcMCU CyMCU NfMCU HsMCU	AUCOLOGICA 30 40 	L SOURCE SO SO VSKLQEKLKIDP. LSYLERLIQAEIPI LSYLERLIQAELPI LSYLERLIQSEVPI RCOFTLKPISDSO	SUPERATING STREET	1 02 60 TFNDFKGIAKEV PEIIFRAEADYT PNVYFRAEDSEQ RSVTFRAMEAKD PAVSFIALQLEQ RGIDRVAIYSPD	QSND GIEESND GDQKPTSRAEARSKD DEIKPRK DAIRPKRGM.YEGTD GVRV
DdMCU MaMCU	β1 β2 α3 	β3 90 SGSIIYLPNSLI .YSGLGREGPS)	$\begin{array}{c} \alpha 1 \\ \alpha 4 \\ 1 0 0 \\ 1 0 \\ 1 0 0 \\ 1 0 \\$	β4 α5 <u>200</u> 0 110 PAHI. Y STEIGDFIRDAA	QQQQQQQ 129 QSLEHILD RGREFSVTIEGHAE.
NcMCU CyMCU NfMCU HsMCU	DGGEPSEYNTNLSHVAS .KADTEGGGGSDGSVQ AEIHRVEGGKDDATVAKRGEDFQEVDE AASTGIDLLLLD QQQQ a2	.YSGLGHRGPKRS: SYSGAGREGE.GI SYSGAGREGE.GI .GI TFSYLRRPGPGQGI DFKLVINDLTYHVI β5 β6	SODKRWVRWSS KDEGEFVRWSP DKEORFIRWSO RPPKRDLLSHE 2020 N1	STEMGDFIRDAA STEIGDFIRDAA STEIGDFIRDAA NAATLNDVKTLV 2	RGR <mark>E</mark> FAIE <mark>IE</mark> GYNI. Rakefeveiegspg. Rakefivtiegapag QQL <u>Y</u> TTLC <mark>IE</mark> QHQLN
DdMCU MaMCU NcMCU CyMCU NfMCU HsMCU	130 140 LNKLIESKKSEINSLRQ .ELRVAVPSFKDRTYYMRORLRKMS .ELRVAVPSFDDRTYYMRORLRKMS .VIKVAVPSFNDRTYYLRORLRKTSR LEQIHVAVPSFDERTYFLRMRLRKISR .KERELIERLEDLKE	150 KIQPLEEKKQVID EIDQMATVKRECD IDGLAKIKHECD KISKLAAIKEECD RIQGLAEIKHECD QLAPLEKVRIEIS	160 RKAHRRATAII LLAHKGAHALA LLAHKGAHALA LLAHKGAHRLA KAAHRGAQRIA ALAHRGAQRVA RKADKRTTLVL	170 18 WTGLGYCFAQAA KGGFAALAAWWG KGGFGLLAGWWG LACCGGLIGYWY LGGFGILAFWWY WGGLAYMATQFG	0 190 TLARLWW.DLSWDI IVYYTFHTDMGWDL VVYYTFHTEFGWDL IVYKLTFETDLGWDV IVYKLTFETDLGWDT TLARLWW.EYSWDI
DdMCU MaMCU NcMCU CyMCU NfMCU HsMCU	200 210 220 IDEVSWFLTFGSVLIGYTYTTWTKTEF VEPITYLAGLASIMGGYLWDLFISRDL VEPVTYLAGLTTIMGGYLWDLYINKDL MEPVTYLVGLSTLIGGYMWDLWHNREV MEPVTYVSLSTLMGGYLWDLYHNREV MEPVTYFITYGSAMAMYAYDVMTROEY	230 TYEALNHRLFSKR SYKAAMNVTVSR SYKAAMNVTVSR SYKAALNITVSAR SYRSALDFTIA YYPEARDRQYLLF	240 DKLFKRNNFP ONALYDERGFD DHALYDENGFD ONKLYDAKGFS OKKLYDNKCID FHKGAKKSRFD	250 KEDYENIVQAID PAKWDQLVHDAN IERWEQIVQDAN LQDWEGYLEEAN LQVWESIIDEAN LEKYNQLKDAIA	260 270 KKEKELKELELATKY GLRREIKFAATEYGV ALRREIKFAATEYGV AMRREIKAVASEYDV AIRREIKAVASEYDV QAEMDLKRLRDPLQV

Figure S4. Sequence alignment of different MCU homologues. The comparison of the sequence HsMCU with the fungal homologues and the comparison of the secondary structures of DdMCU-NTD and HsMCU-NTD using ClustalW and ESPript ¹ programs. The secondary structure elements on the top correspond to the DdMCU-NTD and the secondary structure elements below correspond to HsMCU-NTD.



Figure S5. The packing interfaces of DdMCU-NTD in the unit cell. Two interfaces are identified, including interface I (A) containing three pairs of inter-molecular hydrogen bonds formed by Q38-I88/Y111, K43-E115 and Q45-S85/S87 and interface II (B) containing three pairs of inter-molecular interactions formed by N59/D60-N93, K66-E74, E72-N76. Detailed views of the pairs of inter-molecular interactions are shown on the right side.



Figure S6. SEC-MALS analysis of different mutants. Chromatograms show the readings from the light scattering at 90° (green), refractive index (blue), and UV (red) detectors. The left and right axes represent the light scattering detector reading and molecular weight, respectively. The black curve represents the calculated molecular weight that calcium addition to WT shifts the average molecular weight to 20.2 kDa (A) and the average weight of the elution peaks of mutants Q38A (B), S85A/S87A (C), D60A (D), N93A (E), D60A/N93A (F), E71A (G), E72A (H), and E74A (I) are 58.7 kDa, 53.7 kDa, 33.8 kDa, 25.7 kDa, 19.0 kDa, 52.2 kDa, 42.5 kDa and 39.8 kDa, respectively.



Figure S7. Potassium effects on DdMCU-NTD oligomerization. (A) Gel-filtration analysis of the DdMCU-NTD in the absence (red) and presence (blue) of 50 mM K⁺ (Superdex 200 10/300 GL column (GE Healthcare)). (B) DLS intensity particle size distribution of DdMCU-NTD in the absence (red) or presence (blue) of 50 mM K⁺.



Figure S8. The oligomerization of DdMCU-NTD and HsMCU-NTD. (A) Gel-filtration results of DdMCU-NTD and HsMCU-NTD. (B) DLS results of DdMCU-NTD and HsMCU-NTD.



MaMCU K113-D242 CyMCU K15-D134 NcMCU K120-D264 NfMCU K139-D275



MaMCU R110-E235 CyMCU H40-E127 NcMCU R117-E257 NfMCU R136-E268

Figure S9. The conserved atomic interaction in the interface of two NTD subunits of the four fungal homologues. The salt bridge formed between an Asp and a Lys is identified in MaMCU-NTD (yellow), CyMCU-NTD (violet), NcMCU-NTD (green), NfMCU-NTD (orange).

References:

1. Robert, X., and Gouet, P. Deciphering key features in protein structures with the new ENDscript server, *Nucleic Acids Res.* **2014**, *42*, W320-324.