ATP2, the essential P4-ATPase of malaria parasites, catalyzes lipid-stimulated ATP hydrolysis in complex with a Cdc50 β-subunit

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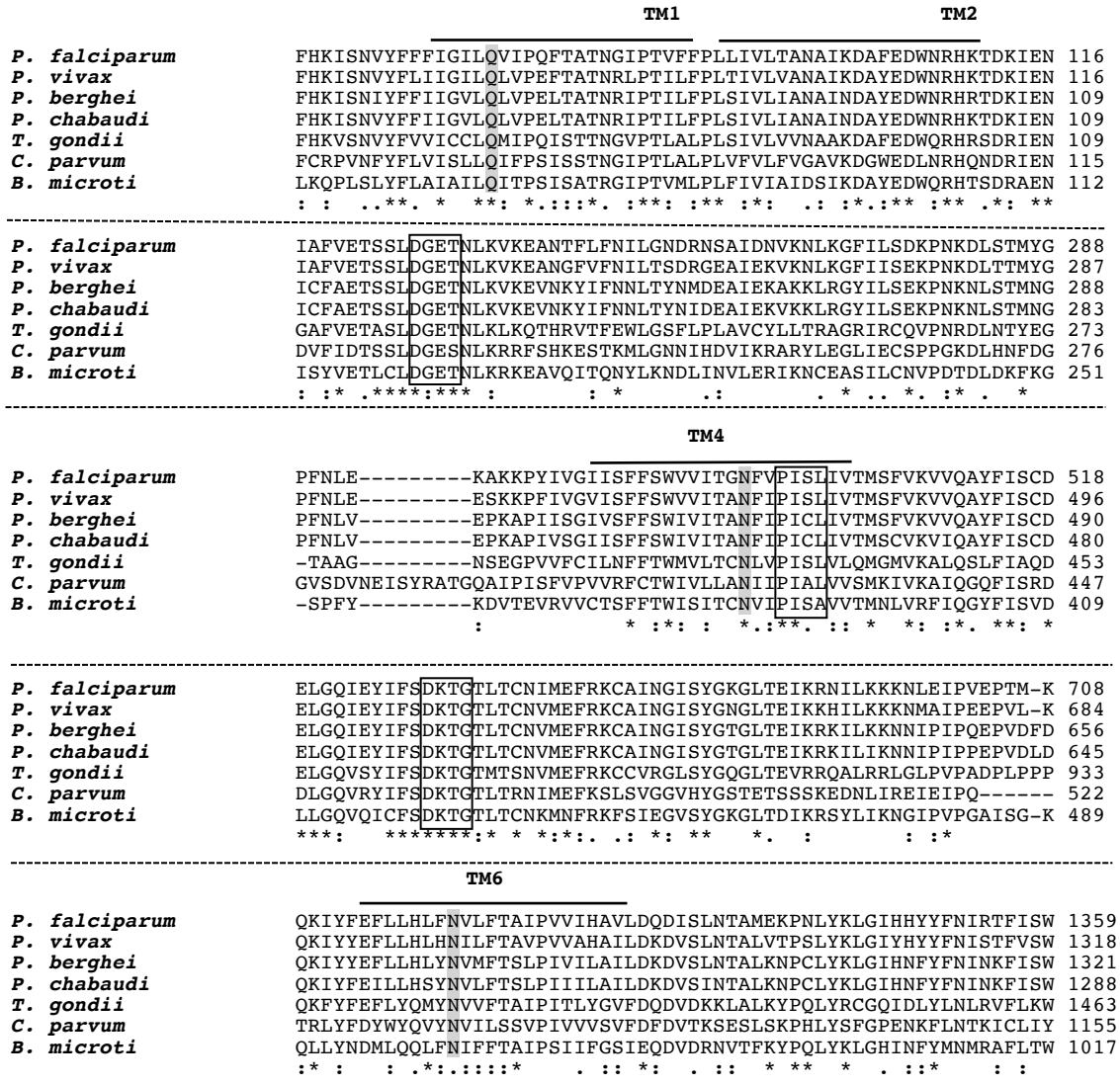
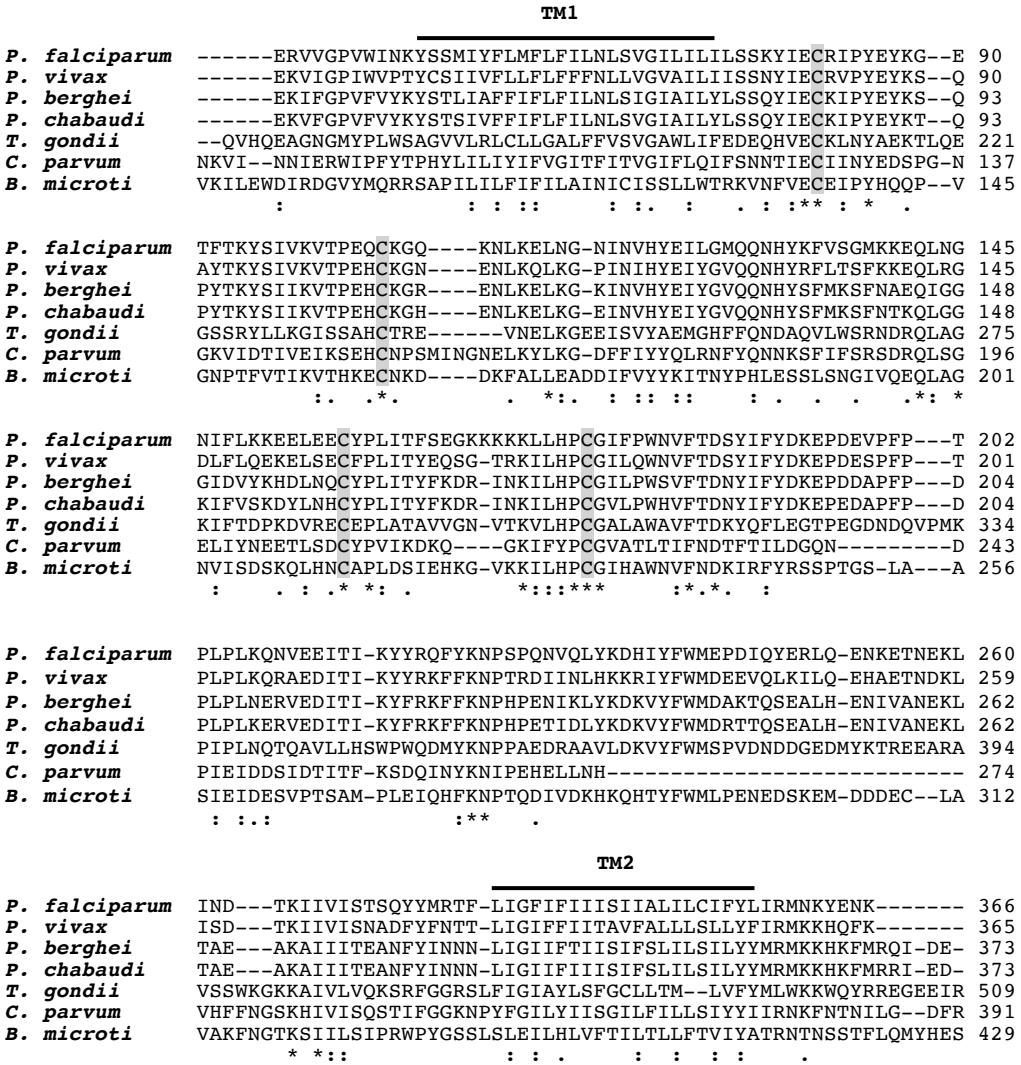
SUPPLEMENTARY FIGURES

Figure supplement 1



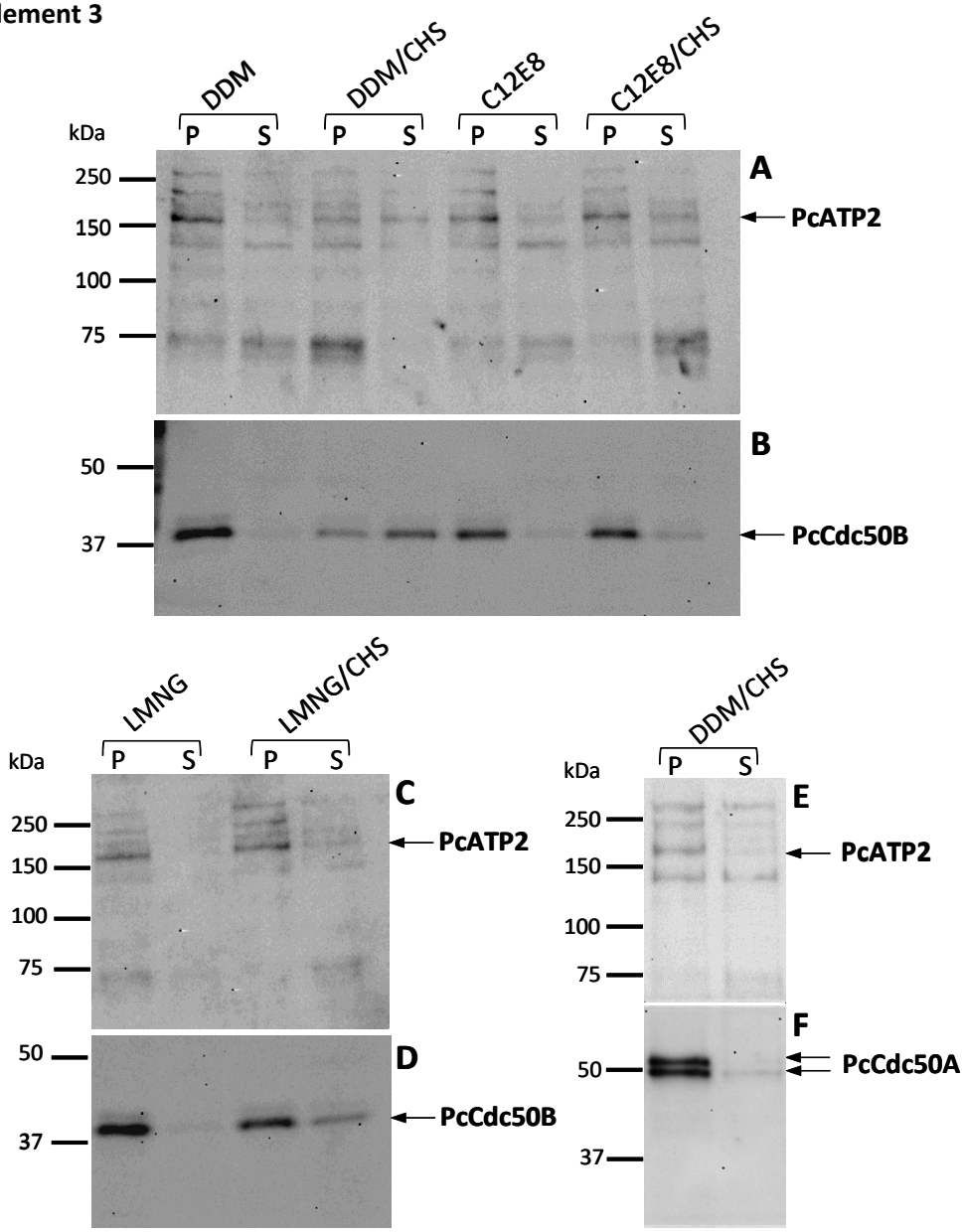
Multiple sequence alignments of ATP2 homologs encoded by Apicomplexan parasites. The figure only shows the regions where conserved residues or motifs mentioned in the main text are located. Lines above define the predicted transmembrane segments (TM) of the *P. falciparum* ATP2 (PfATP2) calculated using TOPCONS [1]. Conserved regions of these sequences specific to P-type ATPases or P4-ATPases are framed. Residues involved in the coordination of the PS head group in the human ATP8A1 [2] are highlighted with a grey background. Eukaryotic Pathogen, Vector & Host Database Resources (VEuPathDB) ID codes: **PF3D7_1219600**: *P. falciparum* ATP2 (PfATP2); **PVX_123625**: *P. vivax* ATP2; **FBANKA_1434800**: *P. berghei* ATP2; **PCHAS_1436800**: *P. chabaudi* ATP2 (named PcATP2 in this work); **TGME49_247690**: *Toxoplasma gondii* ATP2 homolog; **cgd7_1760**: *Cryptosporidium parvum* ATP2 homolog, and **BMR1_01G01915:** *Babesia microti* ATP2 homolog. (*) indicates positions which have a single, fully conserved residue, (:) indicates conserved residues of strongly similar properties, and (.) indicates conserved residues of weakly similar properties.

Figure supplement 2



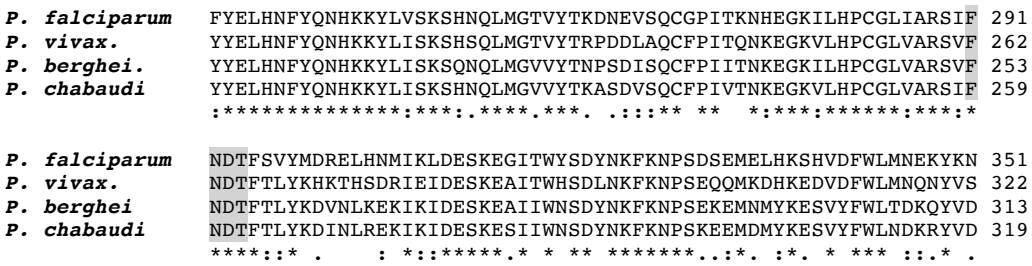
Multiple sequence alignments of *Plasmodium* and Apicomplexan Cdc50B homologs. The figure only shows the regions where conserved residues or motifs mentioned in the main text are located. Lines above define the predicted transmembrane segments (TM) of the *P. falciparum* Cdc50B (PfCdc50B) calculated using TOPCONS [1]. The residues highlighted with a grey background correspond to the four conserved cysteines involved in the formation of the two disulfide bridges of the Cdc50 ectodomain. Eukaryotic Pathogen, Vector & Host Database Resources (VEuPathDB) ID codes: **PF3D7_1133300**: *P. falciparum* Cdc50B (PfCdc50B); **PVX_092270**: *P. vivax* Cdc50B; **PBANKA_091510:** *P. berghei* Cdc50B; **PCHAS_093090**: *P. chabaudi* Cdc50B (named PcCdc50B in this work); **TGME49_230820:** *Toxoplasma gondii* Cdc50B homolog; **cgd5_360**: *Cryptosporidium parvum* Cdc50B homolog, and **BMR1_03g01157**: *Babesia microti* Cdc50B homolog. (*) indicates positions which have a single, fully conserved residue, (:) indicates conserved residues of strongly similar properties, and (.) indicates conserved residues of weakly similar properties.

Figure supplement 3



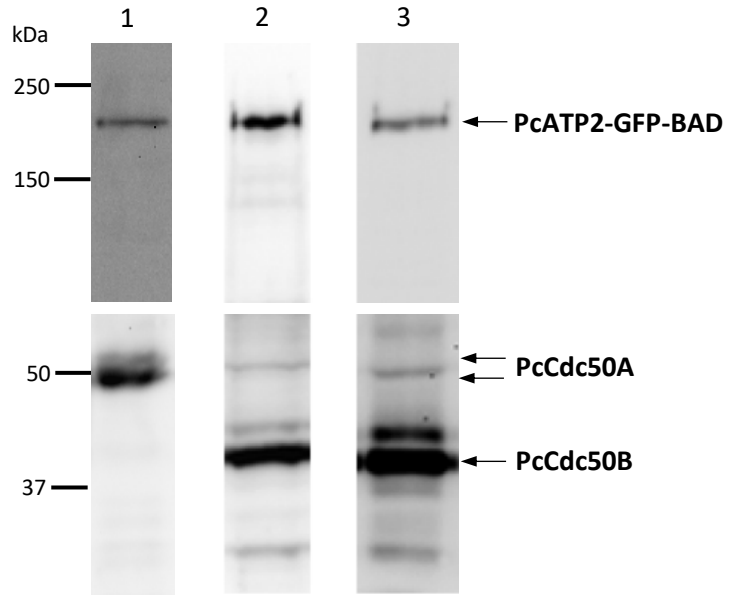
Detergent solubilization of P3 membranes co-expressing PcATP2 with either PcCdc50A or PcCdc50B. Membranes co-expressing BAD-PcATP2/PcCdc50B-His (panels *A* to *D*) or BAD-PcATP2/PcCdc50A-His (panels *E* and *F*) at 2 mg/ml of total protein concentration were solubilized in 1 % (w/v) of the indicated detergent for 1 h at 20°C, in the presence or absence of 0.2 % (w/v) of cholesteryl hemisuccinate (CHS). After ultracentrifugation, 1 µg of total protein of the pellet (*P*, non-soluble material) and the supernatant (*S*, soluble material) were loaded on each lane. Panels *A*, *C* and *E*, western blots revealed with the probe against the BAD. Panels *B*, *D* and *F*, western blots revealed with the HisProbe™ to detect the 10xHis tag. *DDM*, N-dodecyl-β-D-maltopyranoside, *LMNG*: Lauryl maltose neopentyl-glycol, C12E8, Octaethylene glycol monodecyl ether.

Figure supplement 4



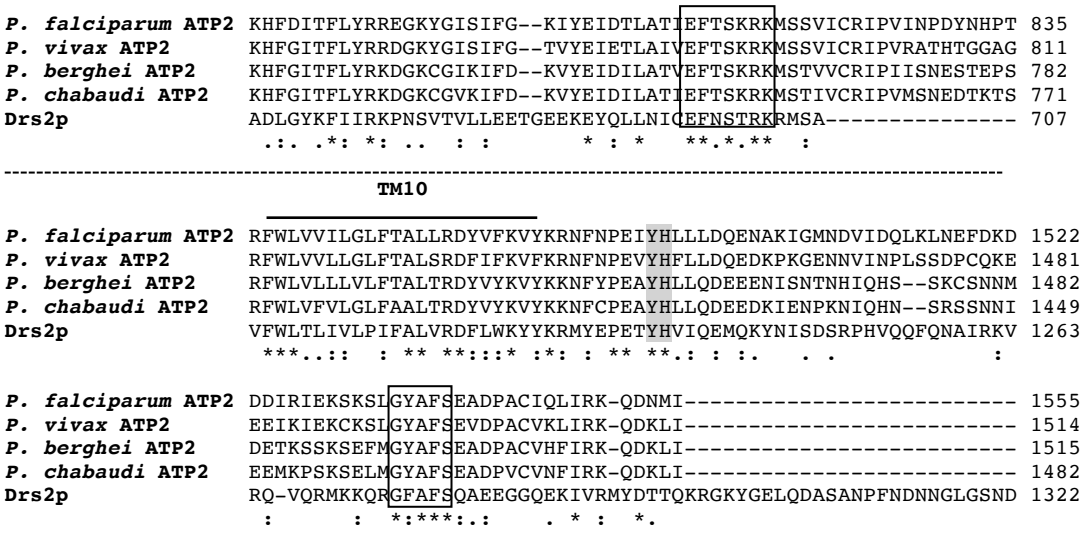
Conservation of a predicted N-glycosylation site in *Plasmodium* Cdc50A homologs. The figure only shows the aligned regions where conserved residues or motifs mentioned in the main text are located. The residues highlighted with a grey background correspond to a conserved N-glycosylated sequence [3,4], situated in the extracellular or luminal domain of the protein between TMs 1 and 2. Eukaryotic Pathogen, Vector & Host Database Resources (VEuPathDB) ID codes: **PF3D7_0719500**: *P. falciparum* Cdc50A (PfCdc50A); **PVP01_0315200**: *P. vivax* Cdc50A; **PBANKA_061700:** *P. berghei* Cdc50A; **PCHAS_061870**: *P. chabaudi* Cdc50A (named PcCdc50A in this work). (*) indicates positions which have a single, fully conserved residue, (:) indicates conserved residues of strongly similar properties, and (.) indicates conserved residues of weakly similar properties.

Figure supplement 5



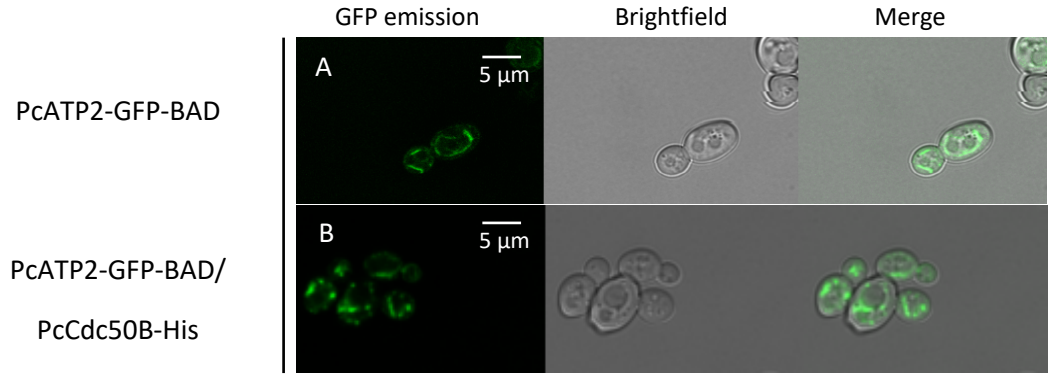
Western blot analysis of P3 membranes co-expressing PcATP2-GFP-BAD (wild-type and D596N mutant) with PcCdc50B-His, and PcATP2-GFP-BAD with PcCdc50A-His. Lane 1, co-expression of PcATP2-GFP-BAD with PcCdc50A-His; Lane 2, co-expression of PcATP2-GFP-BAD with PcCdc50B-His; Lane 3, co-expression of D596N-PcATP2-GFP-BAD with PcCdc50B-His. Top panels, western blots revealed with an antibody against the GFP to detect PcATP2-GFP-BAD. Bottom panels, western blots revealed with the HisProbe™ to detect the 10xHis tag of PcCdc50 proteins.

Figure supplement 6



Conservation in *Plasmodium* ATP2 orthologs of the PI4P binding-site and the autoinhibitory domains of Drs2p. The figure shows the partial sequences alignments of *Plasmodium* ATP2 sequences and the *S. cerevisiae* P4-ATPase, Drs2p. Lines above define the predicted transmembrane segments (TM) of the *P. falciparum* ATP2 (PfATP2, PF3D7_1219600) calculated using TOPCONS [1]. Residues at the C-terminal end involved in the binding of PI4P in Drs2p [5], and also conserved in the *Plasmodium* sequences are highlighted with a grey background. Protein motifs in Drs2p involved in protein autoinhibition, EFNSTRK at the nucleotide domain and GFAFS at the C-terminal, are framed [5]. Eukaryotic Pathogen, Vector & Host Database Resources (VEuPathDB) ID codes: **PF3D7_1219600**, *P. falciparum* ATP2 (PfATP2); **PVX_123625**, *P. vivax* ATP2; **PBANKA_1434800**, *P. berghei* ATP2; **PCHAS_1436800**, *P. chabaudi* ATP2 (named PcATP2 in this work). Drs2p is a P4-ATPase of *S. cerevisiae*. (*) indicates positions which have a single, fully conserved residue, (:) indicates conserved residues of strongly similar properties, and (.) indicates conserved residues of weakly similar properties.

Figure supplement 7



Localization in *S. cerevisiae* of GFP-tagged PcATP2 or PcCdc50B alone or co-expressed with their respective non-tagged partner.

Confocal GFP fluorescence microscopy images of *S. cerevisiae* cells expressing PcATP2-GFP-BAD (Panel A), and co-expressing PcATP2-GFP-BAD and PcCdc50B-His (Panel B). Pictures were taken with a Leica SP8 confocal microscope, using the 63x oil objective and an excitation wavelength of 488 nm.

Table supplement 1.

Detergents used in the membrane solubilization experiments.

	Mw (g/mol)[a]	CMC (mM)[b]
n-decyl-β-D-maltopyranoside (DM)	482.56	1.6
n-undecyl-β-D-maltopyranoside (UDM)	496.59	0.59
n-dodecyl-β-D-maltopyranoside (DDM)	510.62	0.17
5-cyclohexyl-1-pentyl-β-D-maltoside (CYMAL-5)	494.57	2.4
lauryl maltose neopentyl glycol (LMNG)	1000.19	0.01
n-Octyl-β-D-glucopyranoside (OG)	292.37	18
n-Octyl-β-D-thioglucopyranoside (OTG)	308.43	9
Laurydimethylaminoxide (LDAO)	229.4	1
n-dodecyl phosphocholine 12 (FosC12)	351.5	1.5
octaethylene glycol monododecyl Ether (C12E8)	538.75	0.09
3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS)	614.88	8

[a, b] data extracted from the commercial suppliers listed in the methods section.

REFERENCES FROM SUPPLEMENTARY INFORMATION

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5 Timcenko M, Lyons JA, Januliene D *et al.* Structure and autoregulation of a P4-ATPase lipid flippase. *Nature* 2019; **571**: 366–370.