

Supplementary Information for

Structural basis for amino acid exchange by a human heteromeric amino acid transporter

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Other supplementary materials for this manuscript include the following:

Movies S1 to S2

Fig. S1. Sample preparation and validation. **(A)** Fluorescence micrographs of uninduced and induced Flp-In™ T-REx™293-b(0,+)AT1-eGFP-rBAT cells. The results confirmed the successful production and trafficking to the plasma membrane. **(B)** SDS PAGE analyses of fractions from the tandem affinity purification procedure. **(C)** SEC profile of purified b(0,+)AT1-rBAT complex. **(D)** Native PAGE and Western immunoblotting results from the peak fraction of the SEC run. Samples subjected to electrophoresis were additionally treated with10 mM DTT to confirm the inter-subunit disulfide bond. **(E)** DSF profile and T_m value of purified $b(0,+)AT1$ -rBAT complex indicate for a

stable complex preparation. The measured T_m is 65.5 ± 0.1 °C. Data is mean \pm SD, n = 5 replicates. **(F)** Cell based uptake assay of 50 µM L-[3H]Arginine (54.5 Ci/mmol, 0.5 Ci/well) using different cell lines **(G)** Cell based competition assay of 50 µM L-[3H]Arginine (54.5 Ci/mmol, 0.5 Ci/well) with varying concentrations of cystine using different cell lines. Data in **(F)** and **(G)** are mean ± SEM. n = 3 replicates. The results confirm the activity of the heterologously produced $b^{(0,+)}AT1$ -rBAT complex.

Fig. S2. Cryo-EM data processing workflow. (A) Exemplary micrograph and 2D class averages from an initial negative staining EM experiment. **(B)** Exemplary micrograph and CTF estimation (Gctf) of the cryo-EM dataset. **(C)** 2D class averages from the cryo-EM dataset. **(D)** Schematic workflow of pre-processing, classification and refinement of cryo-EM data (see Supplementary Methods for details). Different processing approaches are indicated by color. Reported resolution values correspond to FSC = 0.143.

Fig. S3. Map quality analysis of the b^(0,+)AT1-rBAT structure. (A) Local resolution map of the heterotetrameric b^(0,+)AT1-rBAT complex with corresponding **(B)** 3DFSC plot, and **(C)** Euler angle distributions. **(D)** Local resolution map of the heterodimeric $b^{(0,+)}AT1$ -rBAT complex with corresponding **(E)** 3DFSC plot, and **(F)** Euler angle distributions. **(G)** Map-to-model correlation curve of maps from three different focused refinement jobs.

Fig. S4. Comparison of overall conformation and substrate-binding sites between $b^{(0,+)}$ AT1, LAT1, GkApcT and AdiC. The overall structure of b^(0,+)AT1 was captured in an inward-facing conformation. Its overall fold resembles that of LAT1, GkApcT and AdiC**.** The putative binding pocket of $b^{(0,+)}$ AT1 is characterized by a local environment which is common among SLC7 family amino acid transporters. LAT1 (PDB 6IRT), GkApcT (PDB 5OQT) and AdiC (PDB 3L1L) are colored green, red and yellow, respectively.

Fig. S5. The inward-facing conformation of b^(0,+)AT1. Schematic illustration of extracellular and cytoplasmic barriers of $b^{(0, +)}$ AT1 in the inward-facing conformation.

Fig. S6.

LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6
y+LAT1_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A8
ASC1_H.sapiens_SLC7A11
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A11

LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6 y+LATT_H.sapiens_SLC7A6
LATT_H.sapiens_SLC7A7
LATZ_H.sapiens_SLC7A8
D(0,+)ATT_H.sapiens_SLC7A8
ASC1_H.sapiens_SLC7A1
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A13

LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6
y+LAT1_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A8
b(0.+)AT1_H.sapiens_SLC7A8
ASC1_H.sapiens_SLC7A11
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A11

LAT1 H.sapiens SLC7A5 LAIT_In:Sapiens_SLC/AS

y+LAT1_H.sapiens_SLC7A6

y+LAT1_H.sapiens_SLC7A7

LAT2_H.sapiens_SLC7A8

b(0,+)AT1_H.sapiens_SLC7A8

ASC1_H.sapiens_SLC7A10

xCT_H.sapiens_SLC7A11

xCT_H.sapiens_SLC7A11

xCT_H.sapiens_SLC7A11 AGT1_H.sapiens_SLC7A13

LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6
y+LAT1_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A8 LAT_T.Saplents_SLC7A9
ASC1_H.sapiens_SLC7A9
ASC1_H.sapiens_SLC7A10
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A13

LAT1_H.sapiens_SLC7A5

y+LAT2_H.sapiens_SLC7A6

y+LAT1_H.sapiens_SLC7A7

LAT2_H.sapiens_SLC7A7

bnothers_SLC7A9

ASC1_H.sapiens_SLC7A10

xCT_H.sapiens_SLC7A11

xCT_H.sapiens_SLC7A11

xCT_H.sapiens_SLC7A11 AGT1_H.sapiens_SLC7A13

LAT1_H.sapiens_SLC7A5 LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6
y+LAT1_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A7
b(0,+)AT1_H.sapiens_SLC7A8
ASC1_H.sapiens_SLC7A10
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A13

LAT1_H.sapiens_SLC7A5
y+LAT2_H.sapiens_SLC7A6
y+LAT1_H.sapiens_SLC7A7
LAT2_H.sapiens_SLC7A8
b(0.+)AT1_H.sapiens_SLC7A9
ASC1_H.sapiens_SLC7A11
xCT_H.sapiens_SLC7A11
AGT1_H.sapiens_SLC7A11

Fig. S6. Sequence alignment of human SLC7 family members. Red stars indicate the

conserved G(S/A)G motif and residues involved in formation of substrate binding sites.

 \star

Fig. S7.

rBAT(H.sapiens)
Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus) Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

rta-t (rt. sapiens)
Trehalose_Synthase(D.radiodurans)
Oligo-1, 6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462)
Neopullulanase(G.stearothermophilus)
4F2hc(H.sapiens)

rBAT(H.sapiens)

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Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2nc(H.sapiens)

rBAT(H.sapiens)

Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus) Origo-1, o-Giucosidase (B.cereus)
Alpha-Glucosidase (Geobacillus_sp.HTA-462)
Neopullulanase (G.stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus) Alpha-Glucosidase(Geobacillus_sp.HTA-462)
Neopullulanase(G.stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

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Trehalose_Synthase(D.radiodurans)
Oligo-1, 6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

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Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus) Alpha-Glucosidase(Geobacillus_sp.HTA-462)
Neopullulanase(G.stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

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Trehalose_Synthase(D.radiodurans)
Oligo-1, 6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462)
Neopullulanase(G.stearothermophilus)
4F2hc(H.sapiens)

rBAT(H.sapiens)
Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens)

Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus) Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase(G.stearothermophilus)
4F2hc(H.sapiens)

rBAT(H.sapiens)

Trehalose_Synthase(D.radiodurans)
Oligo-1,6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462) Neopullulanase (G. stearothermophilus) 4F2hc(H.sapiens)

rBAT(H.sapiens) rBAT (H. sapiens)
Trehalose_Synthase(D.radiodurans)
Oligo-1, 6-Glucosidase(B.cereus)
Alpha-Glucosidase(Geobacillus_sp.HTA-462)
Neopullulanase(G.stearothermophilus)
4F2hc(H.sapiens)

Fig. S7. Full length sequence alignment of human rBAT, 4F2hc and GH13 α-amylase family

members. Red stars indicate conserved residues participating in Ca²⁺ binding and coordination.

Fig. S8.

GCQTNNGFVHNEDILEQTPDPGSSTDNLKHS<mark>TRGILGSQEPDFKGVQP\</mark>
GCQTNNGFVQNEDIQEQDPD---SRDTPQSNAVSIPAPEEPQLKVVRP\
GCQTNNGFVQNEDIPELDLD------PGSQEHILGPEEPNAKNIQP\
GCRTNNGFVQNEDIPEQDPDPG-SRDTPQPNAVSIPAPEEPHLKAVRP\
EGQTNNGFVQNEDIRETDLDPS MAED<mark>KSK</mark>
MNEDKDK
MAEEGSK
MDEDKGK
MAED<mark>KS</mark>K <mark>RDSIEMSMK</mark>
RDSIQMSM<mark>K</mark>
RDSIKMNM<mark>K</mark>
RD<mark>PIQ</mark>MSL<mark>K</mark> rBAT(H.sapiens) 70 PYAGMP
PYAGMP
PYAGMP rBAT(R.norvegicus)
rBAT(O.cuniculus) 67
62 69
 70 rBAT(M.musculus) $rBAT(B. taurus)$ **AGMF** ns $|G|N$ rBAT(O.aries) GOT **DLDPSSPAAGPQHNTVDILGPG** 70 rBAT(H.sapiens) 71 140 NE ILE WIT Y YSVELLIGATIA I I I I SP
REILE WIT Y YSVELLIGATIA I I AISP
REILE WIT Y YSVELLIGATIA I I VISP
REVLE WIT Y ASVLVLIAATIA I I AISP EVLFQFSGQA
EVLFQFSGQA
EVLFQFSGQA<mark>I</mark> SE
SE
SE
SE **CDS**
CDS
CDS rBAT(R.norvegicus) 68 CLDWWQAGPMYQIY<mark>P</mark>
CLDWWQAGPMYQIYP DGNG
DGNG 137 $\frac{80}{63}$ VP
VP 132 rBAT(O.cuniculus) .
CLDWWQA<mark>GP</mark>IYQ
CLDWWQAGPMYQ **DGNG**
DGDG rBAT(M.musculus) 70
71 139 140 rBAT(B.taurus) AC $rBAT(O.aries)$ 71 EVL $DGDG$ 140 rBAT(H.sapiens) 141 $\begin{array}{c}\n1 \\
1 \\
0 \\
1\n\end{array}$ 210 DIE
DIE PERSON RYAVEDFI
RYGVEDFI
RYAVEDFI
RYAVEDFI KEIDPIFGTM<mark>K</mark>DFENLVAAVHDKGL
REIDPIFGTMEDFENLLAAIHDKGL
KEIDPIFGTMKDFENLLAAIHDKGL
REIDPIFGTMKDFENLVAAIHDKGL D
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D
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L :GIQEH
:GIQDH
:GIQEH 138 207 rBAT(R.norvegicus) LDYI IWI EDTI
LDYI
LDYI
LDYI rBAT(O.cuniculus) 133 $\frac{1}{2}$
 $\frac{1}{2}$ ESSE
ESSEF
ESSEF 202 rBAT(M.musculus) $\frac{140}{141}$ 209 rBAT(B.taurus) GIQD 210 **VW** rBAT(O.aries) 141 оū T_D 210 GIOD **DFENLVAAIHD** ★ 211
208 rBAT(H.sapiens) 280 IWHDCTHENGKTI
IWHNCTHANGVTT
IWHDCAHENGITT
IWHDCNRENGTTI NNWLSVYGNSSWOFDEE
NNWLSVYGNSSWHFDEVF
NNWLSVYGNSSWHFDEVF
NNWLSVYGNSSWHFDEVF KHPWFQSSRTRSG
KHAWFQLSRTRTG
KHPWFQSSRTRSG
KHAWFQWS<mark>RNQTG</mark> KROCYFHOFLK
KROCYFHOFLK
KROCYFHOFLK
KROCYFHOFMK rBAT(R.norvegicus) 277 **NHT** S_D 200 F LENITS
203 F LPNHTSD
210 F LPNHTSD
211 F LPNHTSD TDYY
TDYY rBAT(O.cuniculus) 272 rBAT(M.musculus) 279 rBAT(B.taurus) 280 $rBAT(O.aries)$ 211 HAWEOW: **I WHOCNYENG EHOFM** 280 **FIPNHTSP NMI** PDLNF RNPDVQEE |
RNPAVQEE |
RNPDVQEE |
RNPAVQEE | 281
278 rBAT(H.sapiens) 350 EQPDLNF
EQPDLNF
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EQPDLNF **KEILRFWLTF**
KEIIKFWLSF
KEIIMQFWLTF
KEIIQFWLSF
KEIIQFWLSF
KEIIQFWLSF KFLLEAKHLF
KFLLEAKDLF
KFLLEAMHLF
KFLLEAKDLF
PFLLEAKHLF
PVLLEAKHLF SLDAVKF
SFDAVKF
SFNAVKF
SFDAV<mark>KF</mark>
SFDAL<mark>PF</mark> :Q|PDTV
:Q|PDTV
:Q|PD<u>T</u>V **GVDGF** 347 rBAT(R.norvegicus) ERN SELY **HDF** RNE I QVI
RNE I QVI 'SELYHDF
'SELYHDF
'SQLHHDF $\frac{273}{280}$ GVDGF
GVDGF rBAT(O.cuniculus) 342 rBAT(M.musculus) 349 $rBAT(B.taurus)$ 281 **DVOI** ĀF 350 rBAT(O.aries) 281 **FNA** 350 $rBATH$ saniens) 351 **VGMHDI** 10^c V M^{λ} 420 **ISMLD** QVGMHDIVRSFI
QVGMHDLVRSFI
QEGMHDIVRSFI
QVGMHDIVRSFI
QVGMHDIVRSFI {FMG|EAYAESTLR|VMYYGLPF|QEADFPFNKY
|FMGTEVSAESTERTMYYYGLSF|QEADFPFNKY
|FLGTEAYAES|DRTMRYYGLSF|QEADFPFNKY
|FMGTEAHGES|TKTMVYYGLPF|QEADFPFNSY
|FMGTEAHGES|TETMVYYGLPF|QEADFPFNSY
|FMGTEAHGES|TETMVYYGLPF|QEADFPFNSY MDQTSTEP
M<mark>NQFSREP</mark>
MDKYSREP
MNQYSREP Y LSMLDT VS 420
YLATLDT LS 417
YFTT LDT LS 412
YFTT IGT LS 419
YLSKLDKPS 420 rBAT(R.norvegicus) $rac{1}{348}$ **THE** rBAT(O.cuniculus) 343
350 ıо MDK lõ rBAT(M.musculus) $rBAT(B.taurus)$ 351 MNIC KLDKP: 420 351 rBAT(O.aries) 420 rBAT(H.sapiens) GEE I GMGN I V 490 421 GN SVIERITSWWENWPEG
FVYEVITAWMENMPEG
FVYEVITSWMENMPEG
SVSEIITSWMENMPEG WPNWMIGGPDSSRLISK
WPNWMIGGPETSRLTSR
WPNWMTGGPDITRLTSR
WPNWMTGGPDSVRLTSR
WPNWMTGGPDSVRLTSR RIGGEYVNAMMILLFTLPGTPITYYGEEIGMGDIS487
RIGNOYVNAMMILLFTLPGTPITYYGEEIGMGDIS487
RIGNOYVNAMMILLFTLPGTPITYYGEEIGMGDIS489
RIGEKYVNAMMILVFTLPGTPITYYGEEIGMGDIS489
RIGEKYVNAMMILVFTLPGTPITYYGEEIGMRNIL490 rBAT(R.norvegicus) 418 GH1 413 GNT
420 GHT rBAT(M.musculus) 421
 421 LGEKY
LGEKY rBAT(B.taurus) **GEETGMRNTL** $rBAT(O.aries)$ 490 491 AANLI
488 ITNL
483 ATNL
491 AANLI
491 AANLI NLNESYD INTLRSKSPMQWDN:
NLNERYDTNALLSKSPMQWDN:
NLNESYDVNTLLSKSPMQWDN:
NFNESYDSTTLVSKSPMQWDN:
NLNETYDAGTLFSKSPMQWDN:
NLNENYDTGTLFSKSPMQWDN: rBAT(H.sapiens) ASN¹ 560 SSNAGFSEASNIWLP
SSNAGFSEGNHTWLP
SSNAGFSEGNHTWLP
SSNAGFSEGNHTWLP
SSNAGFSEGNHTWLP
SSNAGFSEGNHTWLP *Y*QDLSLLHA
*Y*QDLSLLHA
*Y*QALSLLHA
*Y*QELSLLHA
*Y*QELSLLHA
*Y*QELSLLHA NRK
KLL
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KLL TNSDYHTVNVDVQKTQPS
TSSDYHTVNVDVQKTQPT
TSSDYHTVNVDVQKTQPT
TSSDYHTVNVDVQKTQPR SALF
SALF
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SALF 557 rBAT(R.norvegicus) rBAT(O.cuniculus) 552 rBAT(M.musculus) 559 rBAT(B.taurus) 560 $rBAT(O.aries)$ 560 **561 NELLLNRGWFCHLRNDSHYVVV**
558 RELLLSRGWFCLLRDDNHSVVV
553 NELLLSRGWFCLLRNDSRVLVV
560 TELVLSRGWFCLLRDDSHSVV
561 NELLLGRGWFCFLGNYNHSIM
561 NELLLSRGWFCYLRNDNHSIM rBAT(H.sapiens) **ADK 629** RELDGIDRVFLVVLNFGESSTVLNLQETISOVPTKLRIRLSTNPASK 627
RELDGIDRVFIVVLNFGESSTVLNLQEMISGLPVRLSIKLSTNPASK 627
RELDGIDNVFLVVLNFGESSTVLNLQGIISOLPPELRIRLSTNSASK 629
RELDGINRIFLMVLNFGES-SVLNLKEMISNIPTRVRIRLSTNSAYG 629
RELDGINRIFLMVL rBAT(R.norvegicus) rBAT(O.cuniculus) rBAT(M.musculus) rBAT(B.taurus) rBAT(O.aries) \overline{s} 629 rBAT(H.sapiens) 630 685 630 GSKVDTSG I FLDKGEGL I FEHNTKNLLHRQTAFRDRCFVSNRACYSSVLN I LYTSC
628 GSDVDTHAVSLEKGEGL I LEHSMKTLLHHQKAFRDKCF I SNRACYSSVLDLLYSSC
622 GSQVDTRG I FLERGEGLVLEHSMKNLLHRQTAFRDRCF I SSRACYSSALD I LYSSC
630 GSAVDTRA I SLEKGEGL SGIFLD rBAT(R.norvegicus) 683 rBAT(O.cuniculus) 677 rBAT(M.musculus) 685 rBAT(B.taurus) 685 $rBAT(O.aries)$ 685

Fig. S8. Full length sequence alignment of mammalian rBAT homologs. Red stars indicate conserved residues participating in $Ca²⁺$ binding and coordination.

 $b^{(0,+)}AT1$ Domain A of rBAT Domain B of rBAT Domain C of rBAT Fig. S9. Cystinuria causative mutations of the b^(0,+)AT1-rBAT complex. (A) Overall structure of the heterodimeric $b^{(0,+)}$ AT1-rBAT complex. Locations of cystinuria-related mutations are indicated in red. (**B**) Closeup view of domain C mutations. The M467 mutation is found at the interface between domains B and C. Residues C666 and C673 are involved in formation of disulfide bonds within the C-loop of rBAT. (**C**) Closeup view of domain A and B mutations. T216 is in immediate vicinity of the Ca²⁺-bind site. (D) Closeup view of b^(0,+)AT1 mutations. W230 completes the substrate binding site of $b^{(0,+)}$ AT1and acts as a gating residue. G105 locates in the IL1 at a close position to

TM3. A detailed summary of the respective mutations and their physiological effects is given in the Table. S2.

Fig. S10. Interaction interfaces of rBAT and $b^{(0, +)}$ AT1. (A) Atomic model and density map of the C-terminal loop of rBAT and EL2 of b^(0,+)AT1. **(B)** Atomic model and density map of the β₄/α₅ loop of rBAT and EL2 and EL4b of $b^{(0,+)}$ AT1. EL = extracellular loop.

Fig. S11. Conformational heterogeneity of the b^(0,+)AT1-rBAT complex revealed by principle **component analysis.** (**A**) The most dominant component of the conformational heterogeneity is shown. We observe a relative rotational movement between the two HAT units along the membrane axis. The rotation center is found to be at the interaction interface between of the two extracellular rBAT domains. For a more comprehensive illustration of the movement see movies S1 and S2. The initial conformational is colored in blue. The highest rotational sampling angle is colored in red.

Fig. S12. Structural comparison of the β4/α4 extension between human rBAT and the bacterial trehalose synthase. Ribbon representation of the extracellular domain of human rBAT and the trehalose synthase from *D. radiodurans* (PDB 4TVU). The β4/α4 extensions of the two structures highlighted in red indicate that the rBAT domain possesses a larger loop segment than the rehalose synthase. Despite the structural resemblance between rBAT and the trehalose synthase, the bacterial member of the glycoside hydrolase (GH13) family is lacking critical residue required for dimer formation.

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

Mutation	Location	Structural comments	Mutation defect summary
$b^{(0,+)}AT1$			
Gly105Arg Gly105Glu	IL ₁	In the IL1 region and is close to TM3	Mutated charge and/or larger side chains may interfere with the conformation switches
Thr123Met	TM ₃	In the middle of TM3 and near the binding pocket zone	Mutated hydrophobic side chain may form van der Waals contacts with residues from neighbour helix and affect its behaviour even the overall conformation
Trp230Arg	TM6 (binding pocket)	Act as occlusion gate of the binding pocket on the extracellular side	Loss of hydrophobic interaction and steric hindrance would prevent the successful transport of substrates
Tyr232Cys	TM ₆	Locates right in the binding pocket zone	Mutated smaller side chain may result in loose packing and disrupt the substrate interaction
Asp233Glu	TM ₆ (binding pocket)	Play a crucial dual role as part of the binding pocket	A slightly larger residue may affect the tight packing pattern of the binding pocket
Ser379Arg	TM10	In the middle of TM10 and near the binding pocket	Mutated charge and/or larger side chain may change the size of the binding pocket and disrupt substrate interaction
Ala382Thr	TM10	In the middle of TM10 and near the binding pocket	Mutated polar and/or larger side chain may change the size of the binding pocket and disrupt the substrate interaction
rBAT			
Thr216Met	Domain B	Adjacency of Asn214 and forms hydrogen bond with Tyr237	Loss of hydrogen bond and mutated longer residue may disrupt the stabilization of the ion binding site
Arg270X Arg270Leu	Domain B	Forms hydrogen bond network along with Thr234, Gln272 and Asp241	Arg270X: Truncated ectodomain Arg270Leu: Loss of hydrogen bond network

Table S2. Representatives of cystinuria-associated mutations in b(0,+)AT1 and rBAT

Movie S1 (separate file). Principle components of multibody analyses (part I)

Repositioning of the reconstructed body densities along three major motion for the heterotetrameric complex reveals the flexibility of the TMH domain over the extracellular domain. Soft masks for extracellular domain and TMH domain were applied for conducting this multibody refinement.

Movie S2 (separate file). Principle components of multibody analyses (part II)

Repositioning of the reconstructed body densities along three major motion for the heterotetrameric complex reveals the flexibility between two heterodimeric subunits. Soft masks for heterodimeric bodies were applied for conducting this multibody refinement.