STRUCTURAL
BIOLOGY

Volume 76 (2020)

Supporting information for article:

An engineered disulfide bridge traps and validates an outward-facing conformation in a bile acid transporter

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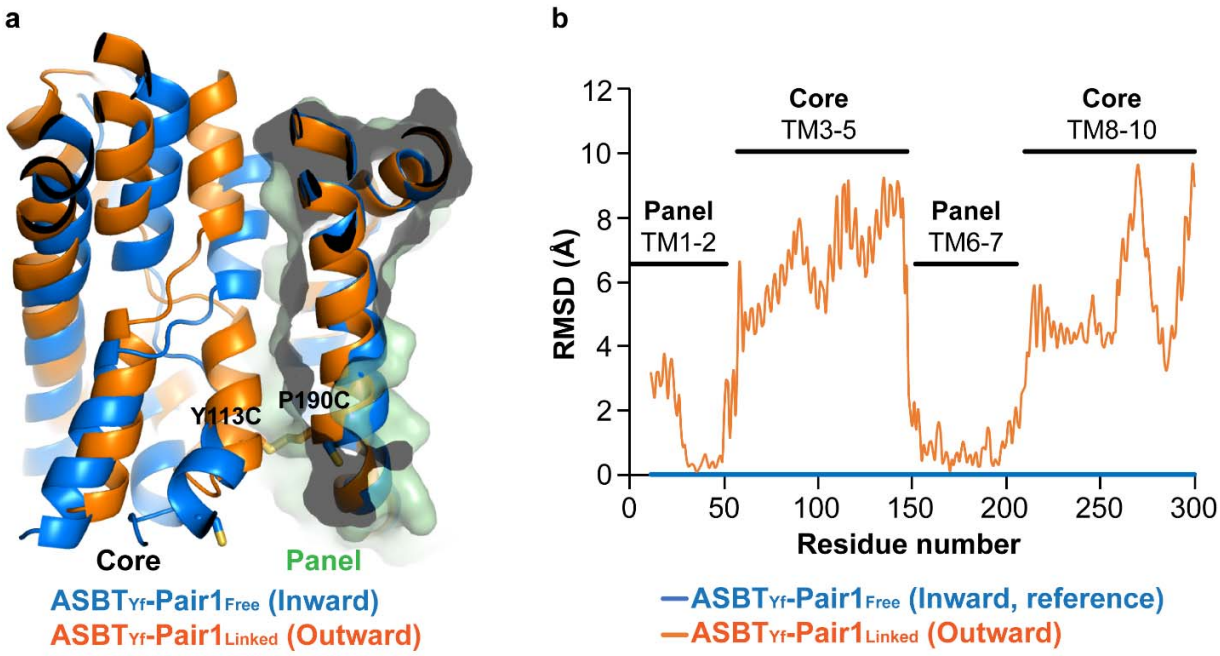

Figure S1 Domain movement during the conformational change of $ASBT_{Yf}$-Pair1 upon disulfide bridge formation. (a) Superposition of the outward-facing $ASBT_{Yf}$-$Pair1_{Linked}$ structure (orange) onto the inward-facing $ASBT_{Yf}$-$Pair1_{Free}$ structure (blue) by aligning their panel domains, which is also indicated by a green surface representation. Y113C and P190C are shown in stick mode. (b) RMSD vs. residue number plot for aligning $ASBT_{Yf}$-$Pair1_{Linked}$ structure (orange) to $ASBT_{Yf}$-$Pair1_{Free}$ structure (blue, reference). The two structures were aligned with their panel domains to reveal the movement of the core domain during the conformational transition upon disulfide bridge formation. The detailed RMSD per residue values are listed in Table S1.

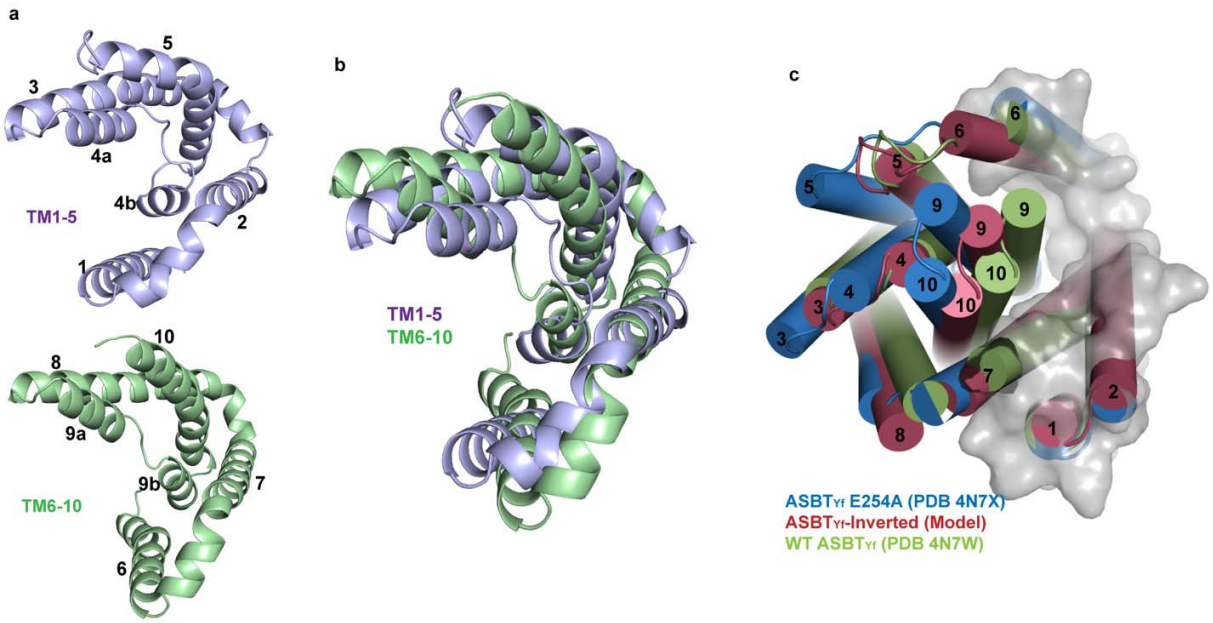

Figure S2 Modeling an outward-facing conformation from the inward-facing $ASBT_{Yf}$ structure (PDB: 4N7W). (a) The two inverted repeats in the 4N7W structure: TM1-5 (top) and TM6-10 (bottom). Transmembrane helices are numbered. (b) Superposition of TM6-10 onto TM1-5 of the 4N7W structure. (c) Superposition of the $ASBT_{Yf}$-Inverted model (by switching TM1-5 and TM6-10, red) with the outward-facing 4N7X structure (blue) and the inward-facing 4N7W structure (green) displayed in cylinder mode, viewed from the extracellular side. The grey surface represents the panel domain.

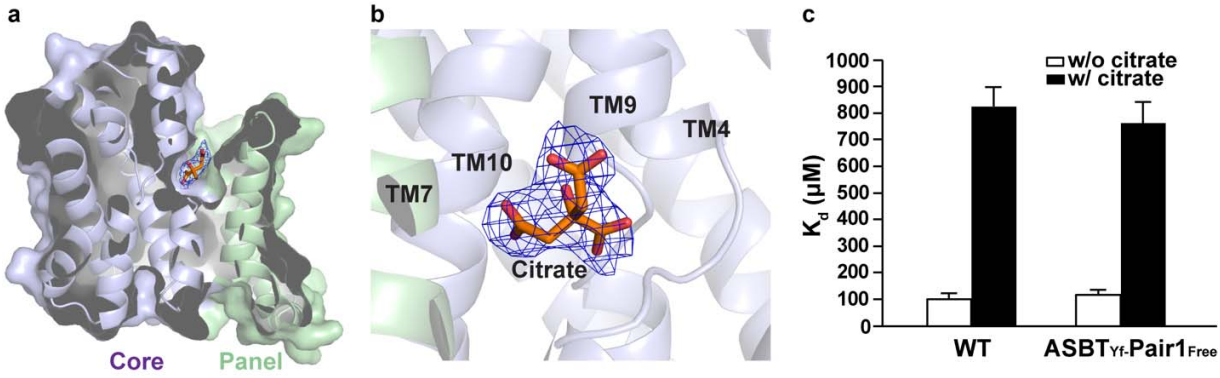

Figure S3 A citrate molecule captured in the $ASBT_{Yf}$-$Pair1_{Linked}$ structure within the central cavity. (a) Slab view of the $ASBT_{Yf}$-$Pair1_{Linked}$ structure showing a citrate molecule in the central cavity. (b) Close-up view showing the binding location of the citrate near the TM4/TM9 cross-over region. The blue mesh shows the simulated annealing 2Fo-Fc omit map contoured at 1.5 σ level with carve=2. (c) K_d determination of wild-type $ASBT_{Yf}$ and $ASBT_{Yf}$-$Pair1_{Free}$ for TCA in the absence or presence of 100 mM sodium citrate using MST. All measurements were performed in 4 independently repeated experiments (N = 4), and results were expressed as mean ± SD.