Cryo-EM analysis of the conformational landscape of human P-glycoprotein (ABCB1) during its catalytic cycle

Gabriel A. Frank*, Suneet Shukla*, Prashant Rao, Mario J. Borgnia, Alberto Bartesaghi, Alan Merk, Aerfa Mobin, Lothar Esser, Lesley A. Earl, Michael M. Gottesman, Di Xia, Suresh V. Ambudkar[$], and Sriram Subramaniam[$]

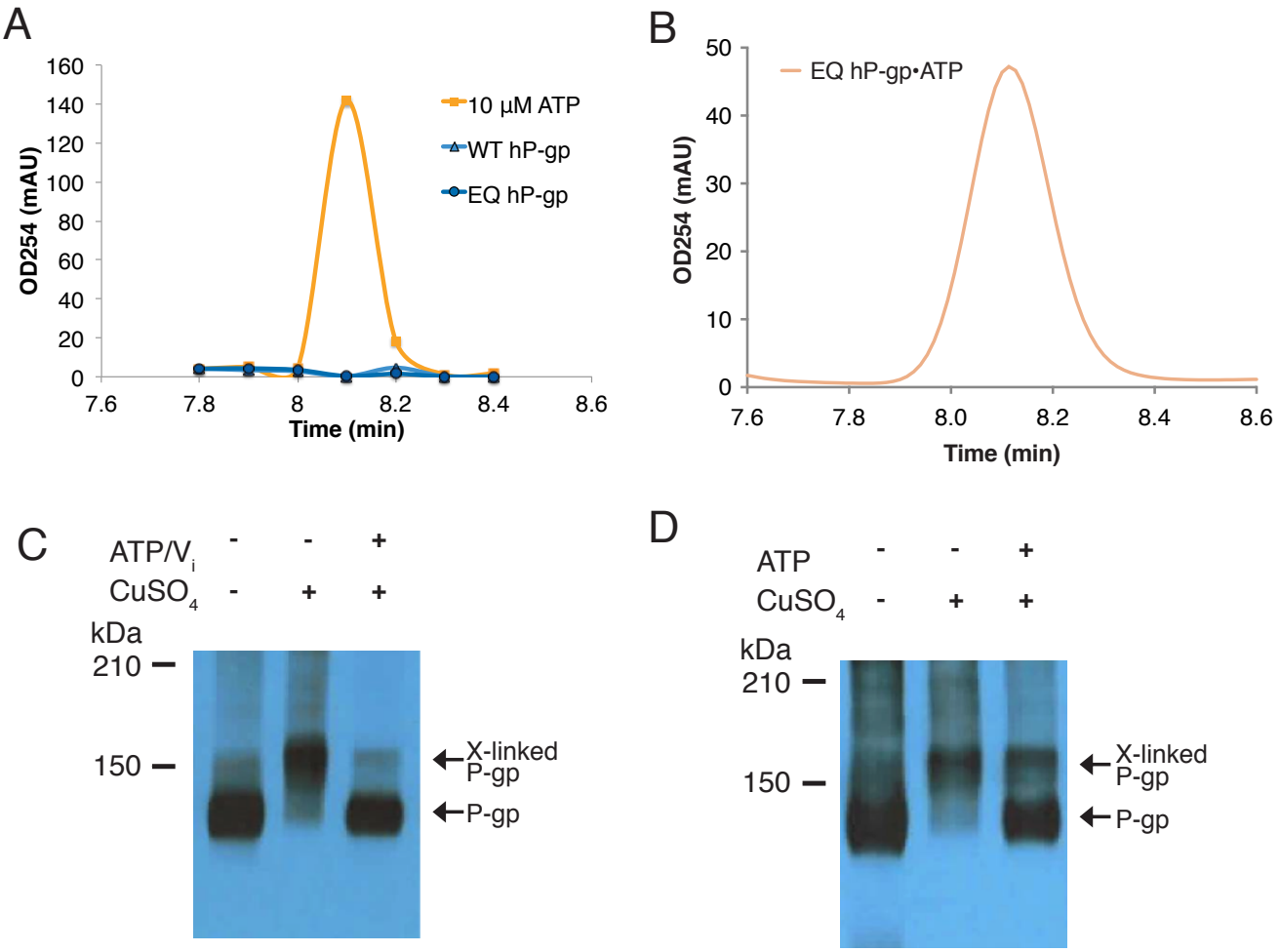
Supplementary Table 1

	Closed	**Open**		
WT P-gp 'apo'	5315	4340		
EQ P-gp 'apo'	34173	21815		
EQ P-gp•ATP	14158	11442		
WT P-gp•ATP•V_i	10085	---		
WT P-gp•ADP	---	5750	6443	5074

-----, Not detected.

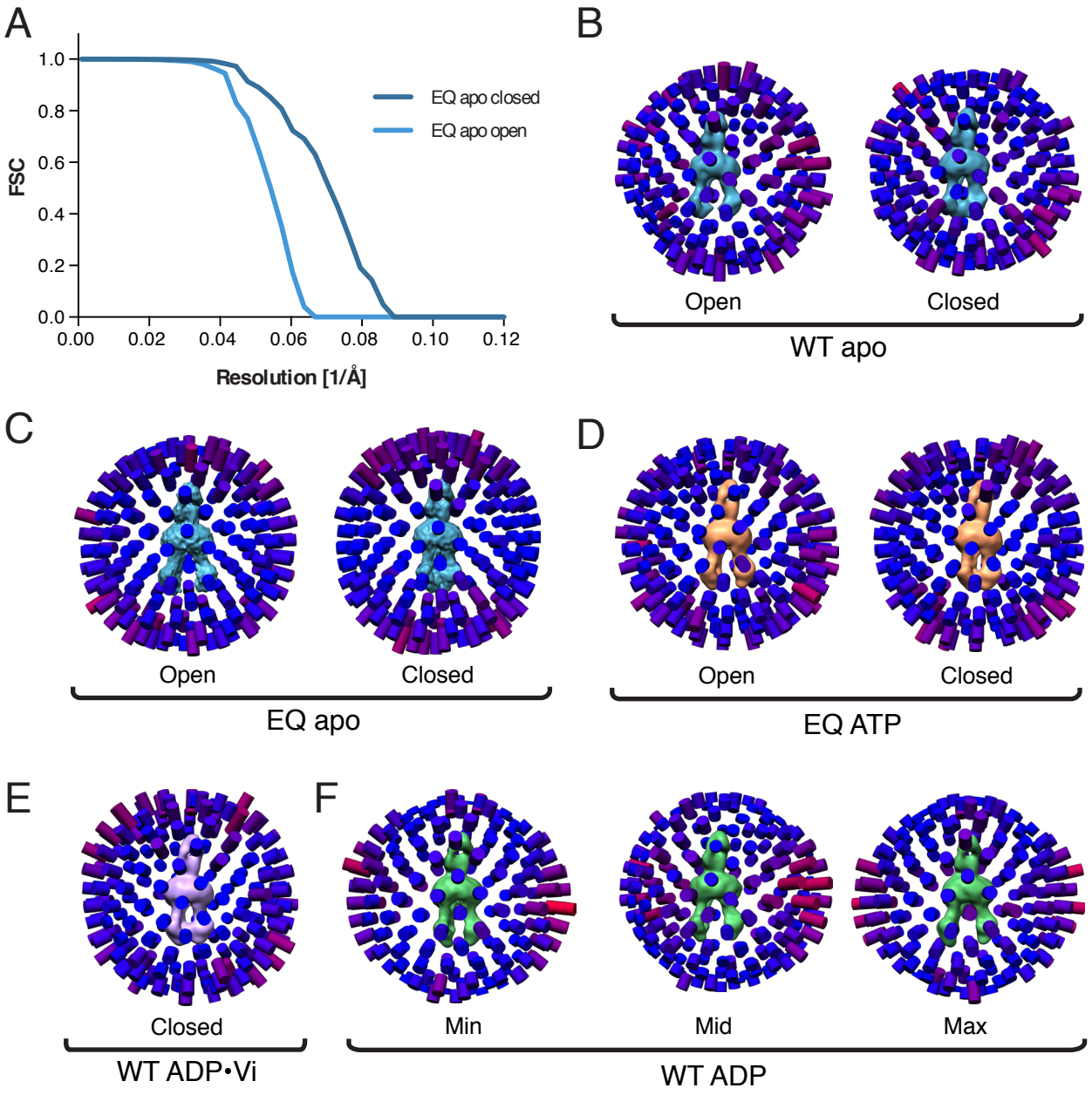
Supplementary Table 1: Number of particles in the 3D classes found in each of the states along the ATPase cycle of hP-gp. The numbers of particles in the last row are ordered as the maps in Figure 2. Dashes denote particles not detectable in indicated conformation.

Supplementary Figure 1



Supplementary Figure 1: Biochemical analysis and crosslinking of hP-gp. Extraction of ATP associated with purified hP-gp and CuSO4-induced disulfide crosslinking of Cys residues in the Walker A domain of NBDs. (A) ATP was extracted from either 10 µM of ATP in 200 µl of elution buffer (square, orange), WT (triangle, light blue) and EQ-hP-gp (circle, dark blue) purified samples (15 µM); and the samples were separated on a C18 reverse phase HPLC column, as described in materials and methods. Histogram of the elution of ATP is representative of three independent experiments. (B) Purified EQ hP-gp was incubated with 5 mM ATP at 30 ºC for 15 min. Free ATP was separated by passing the samples three times through desalting columns (Thermo Fisher Scientific, Waltham, MA), per the manufacturer's protocol. The occluded ATP was extracted and separated on a C18 reverse phase HPLC column. The histogram of the elution of ATP is representative of three independent experiments. (C, D) The crude membranes expressing WT hP-gp (C) or EQ h-Pgp (D) were incubated in the absence and presence of 5 mM ATP (plus 0.3 mM sodium orthovanadate for WT) and 10 mM MgCl2 at 37°C for 10 min as indicated, and crosslinked with CuSO4 for 15 min and analyzed by Western blot analysis probed with C219 antibody. The arrows indicate bands corresponding to non-crosslinked and crosslinked hP-gp. Western blot is representative of four independent experiments.

Supplementary Figure 2



Supplementary Figure 2: Purification and cryo-EM analysis of hP-gp. (A) FSC plots of hEQ-P-gp apo open (light blue) and closed (dark blue) reconstructions shown in this manuscript. Estimated resolutions of all maps are given in Table S1. (b-f) Analysis of orientation assignment of individual particles for WT P-gp apo (B), EQ P-gp apo (C), EQ P-gp + ATP (D), WT ADP•Vi (E), and WT P-gp +ADP (F) reconstructions.