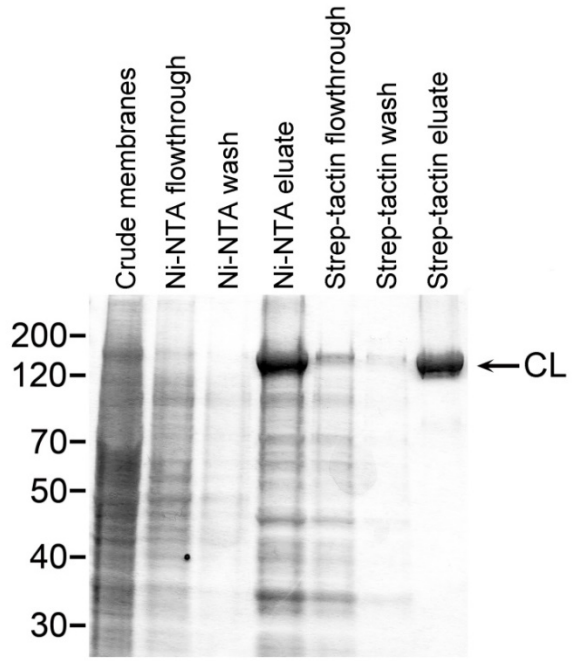
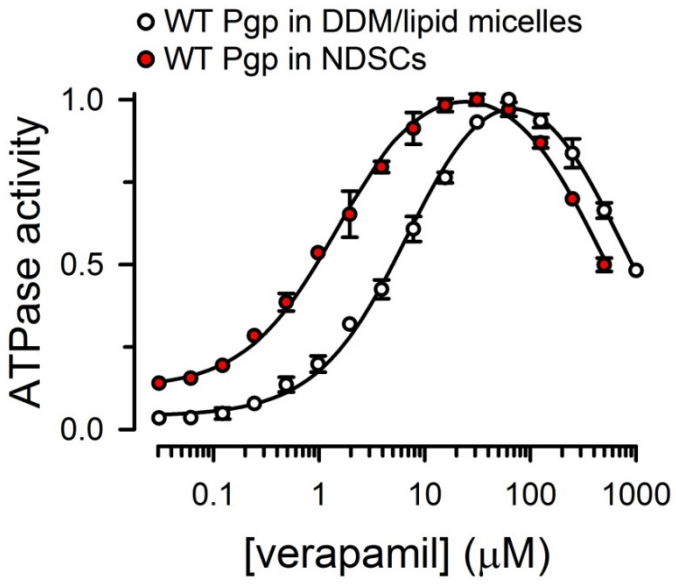
Supplementary Information
Substrate-induced conformational changes in the nucleotide-binding domains of lipid bilayer-associated P-glycoprotein during ATP hydrolysis

Maria E. Zoghbi[#1], Leo Mok[#2], Douglas J. Swartz[2] Anukriti Singh[2], Gregory A. Fendley[1], Ina L. Urbatsch[2,3] and Guillermo A. Altenberg[1,3]



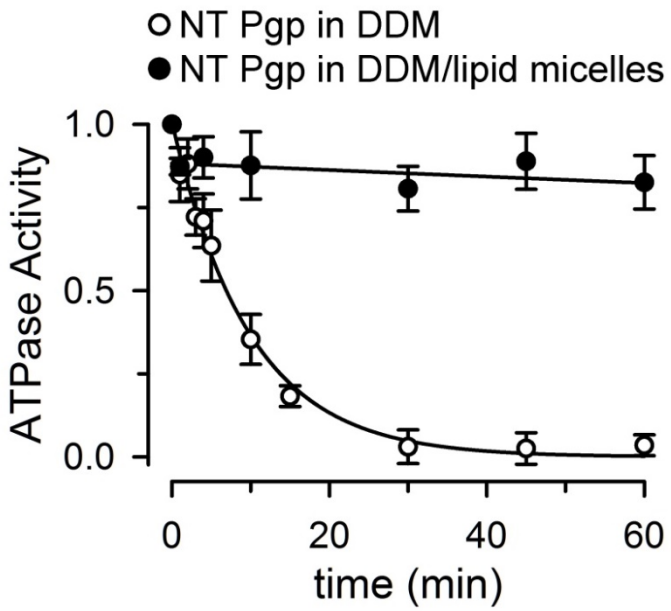
Supplementary Fig. 1. **Example of Pgp purification.** Samples were subjected to SDS-PAGE and the gel was stained with Coomassie blue. The example corresponds to the purification of Cys-less Pgp (CL), but the results were essentially identical for WT Pgp, NT Pgp and NT/AA Pgp. Molecular masses of selected markers, in kDa, are shown on the left. See main text for details.



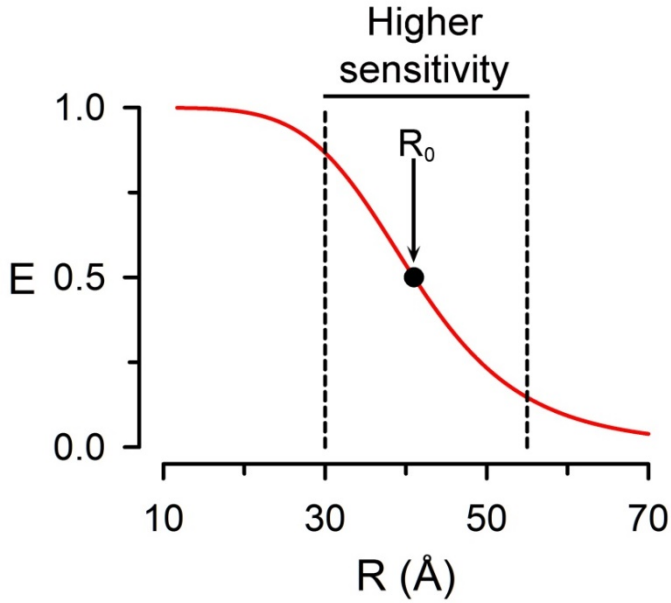
Supplementary Fig. 2. **Verapamil (Ver)-stimulated ATPase activity of WT Pgp.** WT Pgp activity was assayed at 37°C in the presence of 10 mM ATP and 12 mM $MgSO_4$ in 0.1% DDM supplemented with 0.6% (w/v) *E. coli* lipids (WT Pgp in DDM/lipid micelles) and in NDSCs. Data are means ± SD (n = 3 for each condition). SDs smaller than the symbols are not shown). Activities were normalized to maximal measured rates (5.0 ± 0.1 μmol/min/mg for Pgp for detergent/lipid micelles and 5.2 ± 0.1 μmol/min/ mg for Pgp in NDSCs). Lines represent fits to the equation:

$$V = V_b + ((V_{max} - V_b) \cdot Ver/(K_S + Ver)) \cdot (1 - (V_{max} - V_F)/(V_{max} - V_b) \cdot (Ver/K_I + Ver))$$

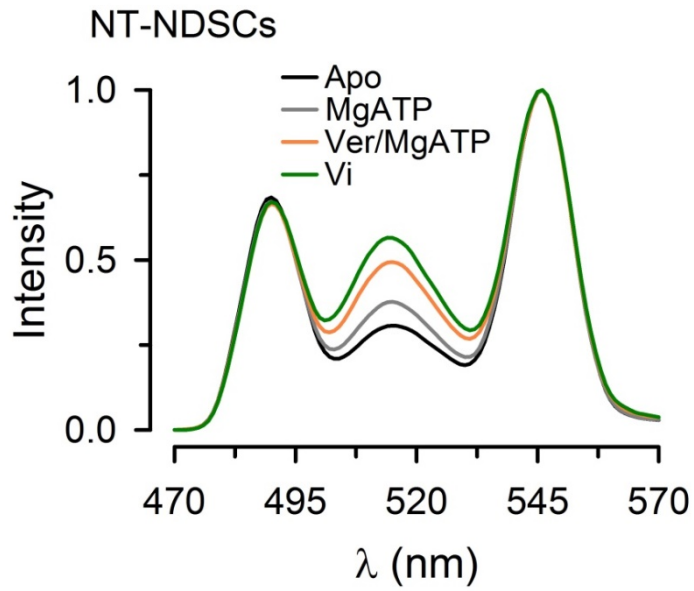
V: rate of ATP hydrolysis; V_b: basal ATPase activity; V_{max}: maximal ATPase activity; Ver: verapamil concentration; K_S: EC_{50} for the stimulatory effect; V_F: ATPase activity at ∞ Ver; K_I: IC_{50} for the inhibitory effect. The average K_S values for Pgp in NDSCs and micelles were 1.5 and 7.3 μM, respectively.



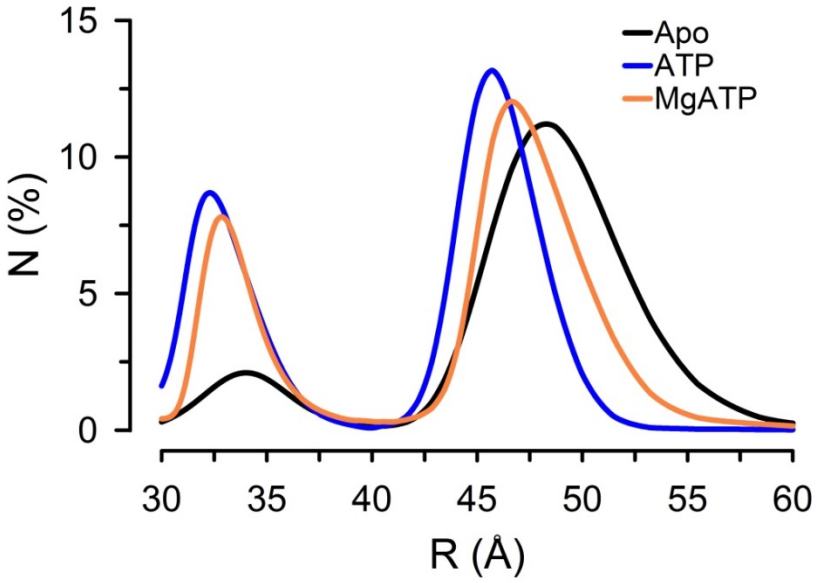
Supplementary Fig. 3. **Stability of the Pgp-NT ATPase.** Activities of Pgp NT in 0.1% n-dodecyl-β-D-maltopyranoside (DDM) in the absence of lipids (Pgp-NT in DDM) and after supplementation with 0.6% (w/v) *E. coli* lipids (Pgp-NT in DDM/lipid micelles). The ATPase activity was measured at 37°C and data are means ± SD (n = 3 for each condition).



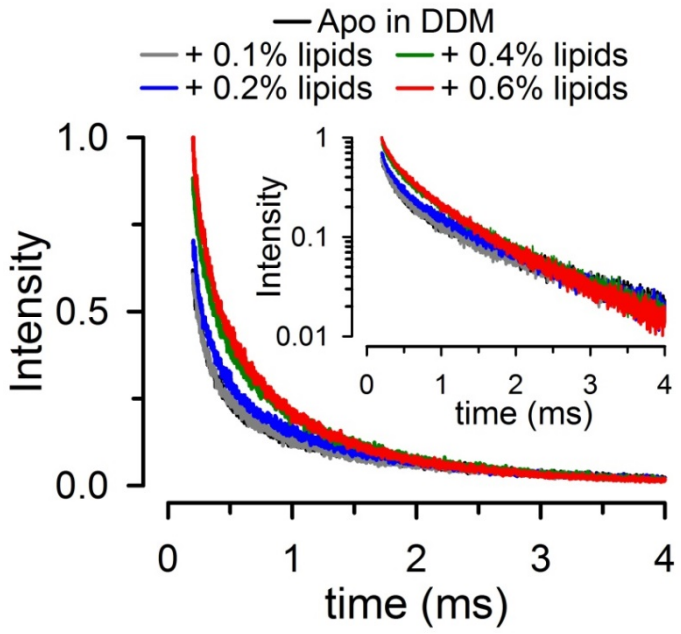
Supplementary Fig. 4. **Dependence of energy transfer (E) on Tb^{3+}-Bodipy FL distance.** The curve was generated from: $R = R_0 (E^{-1}-1)^{1/6}$, where R is the donor-acceptor distance and R_0 is the Förster distance (the distance at which E = 0.5). The R_0 determined for the Tb^{3+}/Bopidy Fl pair was 41 Å.



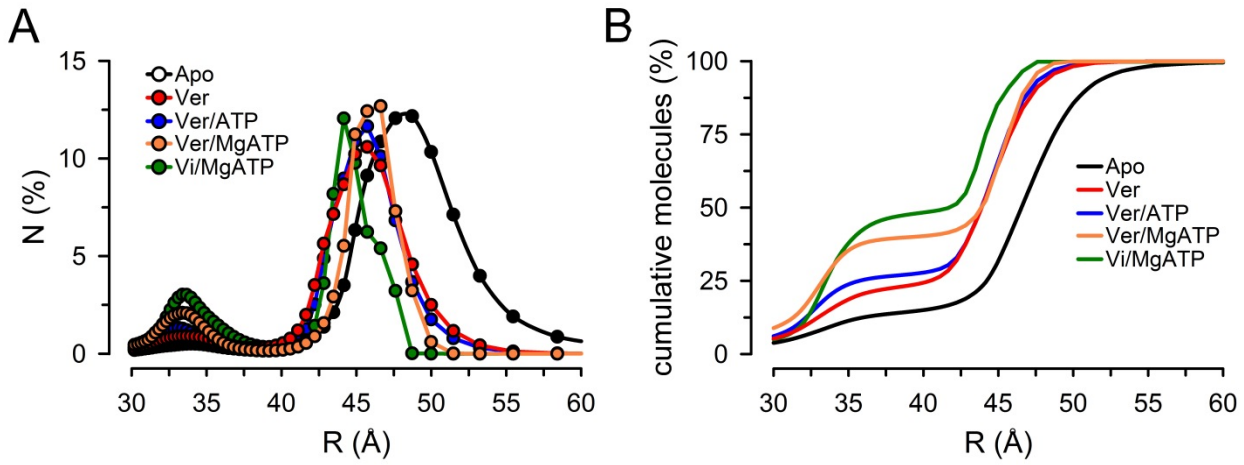
Supplementary Fig. 5. **Increase in sensitized Bodipy FL emission during hydrolysis by addition of verapamil.** Emission spectra from NDSCs containing Pgp NT labeled with donor (Tb^{3+} chelate) and acceptor (Bodipy FL). Traces obtained sequentially at 37°C were normalized to the Tb^{3+} emission at 546 nm and are representative of at least 3 independent experiments. Emission was recorded after a 200 μs delay from the 337-nm excitation pulse. Apo: nucleotide- and drug-free buffer with 1 mM EDTA; MgATP: + 5 mM MgATP; Ver/MgATP: + 30 μM verapamil; Ver/Vi: + 0.25 mM Vi.



Supplementary Fig. 6. **Distance distributions of Pgp NT in NDSCs in the absence of transport substrate.** Distance distributions calculated from LRET sensitized emission intensity decays analyzed by an exponential series method. N: % molecules. The percentage of molecules in each conformation was adjusted based on the cumulative number of molecules *vs*. donor-acceptor pair distance obtained from the exponential series method analysis. See Experimental Procedures and Supplementary Fig. 8 for details. Data correspond to the best fits obtained using Peak Fit (Systat Software Inc., San Jose, CA) using data from 7 independent experiments. Apo: nucleotide- and drug-free buffer with 1 mM EDTA; ATP: + 5 mM NaATP; MgATP: + 10 mM $MgSO_4$. Data from Pgp-NT in NDs at 37°C were used for all calculations.



Supplementary Fig. 7. **Conformational changes elicited by addition of lipids to Pgp NT in detergent in the apo state.** Bodipy FL sensitized emission decays, recorded at 520 nm, from Pgp NT in detergent (DDM; 0.1% n-dodecyl-β-D-maltopyranoside) before and after addition of *E. coli* lipids (in %; w/v) to produce mixed DDM/lipid micelles. Pgp NT was studied in the apo state (nucleotide- and drug-free buffer with 1 mM EDTA). The inset displays the same curves in a semi-log scale. Decays were normalized to the emission measured in the presence of 0.6% lipids at 200 μs. Traces are representative of at least 3 independent experiments and were obtained at 20°C. Emission was recorded after a 200 μs delay from the 337-nm excitation pulse.



Supplementary Fig. 8. **Distance distributions of Pgp NT in NDSCs under different states during the hydrolysis cycle.** *A*, Distance distributions calculated from LRET sensitized emission intensity decays analyzed by an exponential series method. N: % molecules. The symbols denote the calculated average values and are joined by spline lines. *B*, Cumulative number of molecules *vs*. donor-acceptor pair distance. Data were obtained by cumulative addition of N determined by the exponential series method analysis (panel *A*). The percentage of molecules in each conformation in Fig. 5 of the accompanying manuscript was calculated from the fractional intensity contribution of each exponential component divided by the rate of energy transfer ($k = 1/\tau_{DA} - 1/\tau_D$), and was adjusted based on the cumulative number of molecules *vs*. donor-acceptor pair distance obtained from the exponential series method analysis (panel *B*). Apo: nucleotide- and drug-free buffer with 1 mM EDTA; Ver: + 30 μM verapamil; Ver/ATP-bound: + 5 mM NaATP; Ver/MgATP: + 10 mM $MgSO_4$; Ver/Vi: + 0.25 mM Vi. Data from Pgp NT in NDs at 37°C were used for all calculations (n = 5). See Experimental Procedures for references and additional details.