Coupling the role of lipids to the conformational dynamics of the ABC transporter Pgp

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Abstract

The ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp) energizes the efflux of compounds through the plasma membrane and it is implicated in multidrug resistance in cancer. Its portal helices exhibit two conformations—straight and kinked—in high-resolution structures solved in detergent and nanodiscs, respectively. However, at the atomic level, the link between these two alternative inward-open conformations and Pgp function is unclear. We employ MD simulations to explore the dynamics of the two conformers in hepatocyte-like membranes and found that the access to the substrate cavity of the kinked conformer is restricted. Moreover, the cavity's volume and dynamics, influenced by cholesterol and ATP, differ between the conformers. Our findings suggest that the straight conformation precedes the kinked in the series of conformational transitions that underlie the functional mechanism, with the kinked conformer potentially playing a role in preventing substrate reuptake. Remarkably, in our unbiased simulations a spontaneous straight to kinked transition of one TM helix was observed. Our study highlights the lipid environment's impact on Pgp structural ensemble and elucidates the functional significance of its two inward-open states, shedding light on Pgp's overall mechanism.

In brief

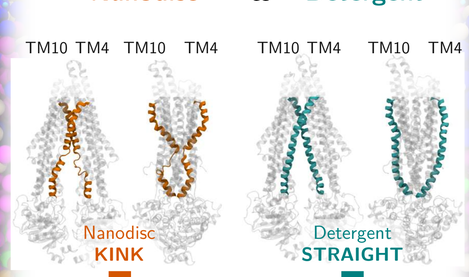
- ABC transporters are associated with multidrug resistance and cancer.
- The human ABC exporter P-gp is expressed in a highly specialized membrane bilayer enriched with cholesterol and sphingolipids.
- We use molecular modeling and MD simulations to study the effect of highly specialized membrane environment on the structural dynamics of P-gp.
- We investigate the functional connections between a complex lipid composition and the mechanism of substrate translocation.

Questions

- What is the role of specific lipids in the structure, dynamics and function of P-gp?
- What is the lipid substrate entrance path in P-gp?
- What is the dynamic role of the ATP in stabilization of the catalytic ABC dimer?
- What is the functional role of the two distinct inward-open conformers?

Methods

P-gp entrance gate helices TM4 and TM10 have a different conformation in **Nanodisc** & **Detergent**



INWARD OPEN CONFORMATIONS

Molecular Dynamics Simulations

± ATP ± Cholesterol

Coarse-Grained MD simulation 5μs × 3 reps (independent assemblies) - constrained - Martini2.2-ff 310 K 150mM NaCl

backmapping

All-atom MD simulation 1μs × 3 reps Charmm36m-ff 310 K 150mM NaCl

Membrane lipid composition (hepatocyte)

Chol:POPC:SM:POPE:POPS:POPI

inner leaflet: 40:27:23:10:0:0

outer leaflet: 40:8:10:17:15:10

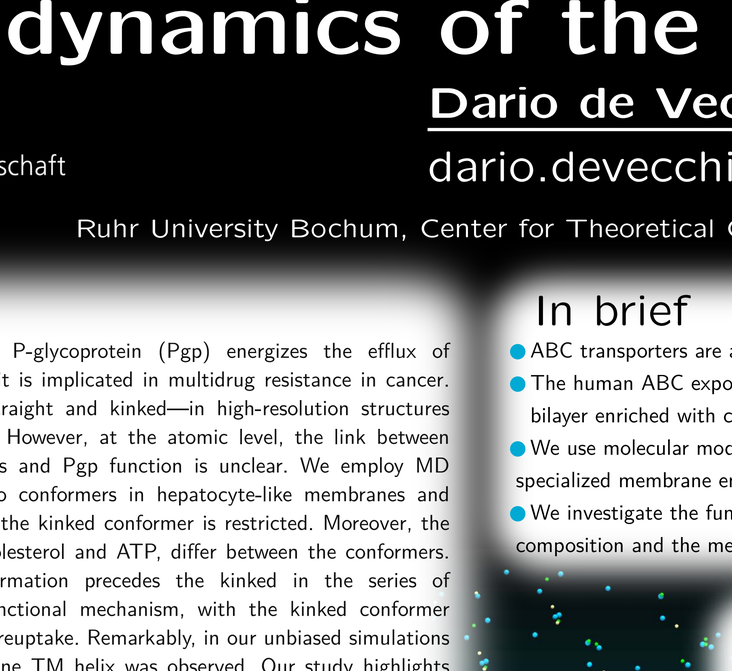


Figure 1 Human P-gp embedded in a native-like membrane mimicking the hepatocyte lipid composition. Shown is a fiction representation of the two resolutions adopted in this study and displayed next to each other. In this study, coarse-grained MD simulations were backmapped to all-atom resolution and both run independently.

P-gp dynamics in a lipid context

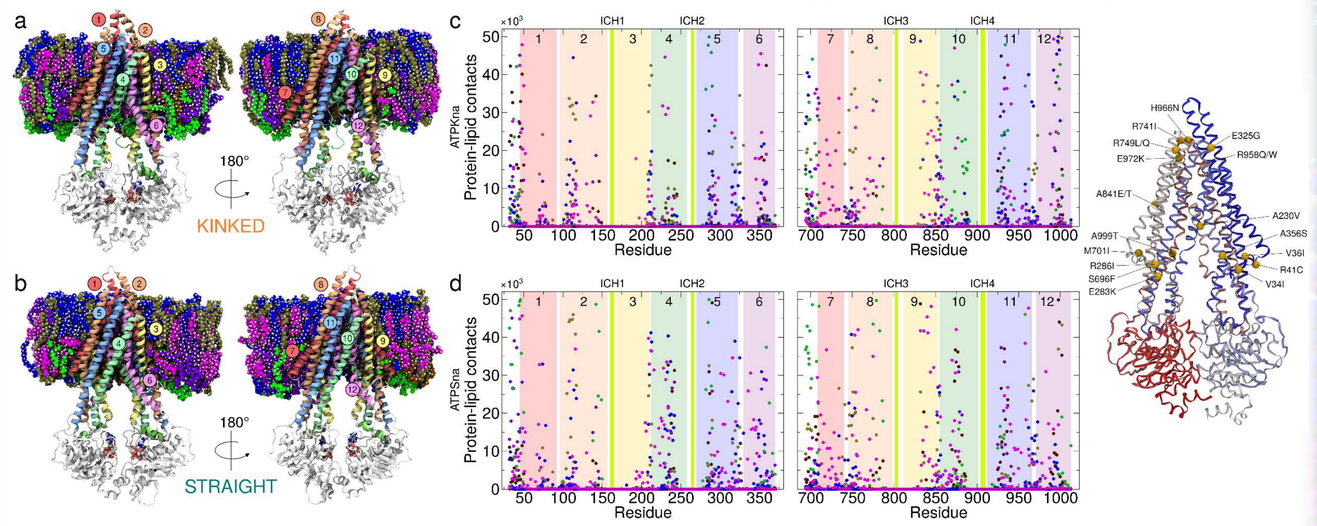


Figure 2: Membrane-embedded P-gp in the two different inward-open conformations. (a) Simulation snapshot of the P-gp kinked conformer embedded in the hepathocyte plasma membrane model. The snapshot is from the ATPKna system. The protein is shown in ribbon with the transmembrane helices numbered and colored in rainbow. The ATP and the membrane components are shown in van der Waals representation, cholesterol in magenta, sphingomyelin in blue, phosphatidylcholine in tan, phosphatidylethanolamine in violet, phosphatidylserine in brown, phosphatidylinositol in green. Solvent and ions are not shown for clarity. (b) As in a but for the P-gp straight conformer. The snapshot is from the ATPSna system. (c) Protein-lipid contact analysis of the P-gp kinked conformer. Phospholipid-P-gp and cholesterol-P-gp contacts during the accumulated 3 μs of all-atom MD simulation time of the ATPKna system for TM1 to TM6 (residues 22–381) and TM7 to TM12 (residues 684–1023). The P-gp transmembrane helices are numbered and colored as in a and b. The color legend for the lipids and cholesterol is the same as in a and b. Intracellular coupling helices ICH1 to ICH4 are indicated in green. (d) As in c but for the P-gp straight conformer (ATPSna system). The structure on the right indicates P-gp residues mutated in cancer and found in contact with membrane lipids.

Membrane milieu modulates P-gp cavity access

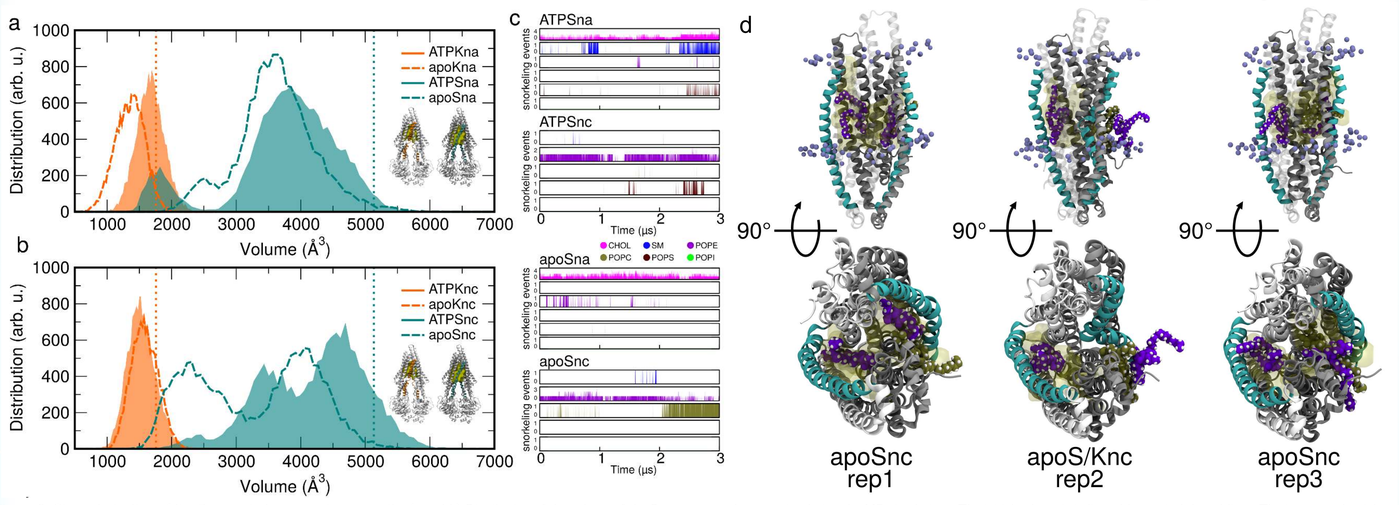


Figure 3: Characterization of the P-gp main transmembrane cavity. (a and b) Distribution of the volume of the P-gp transmembrane cavity during the MD simulations. The initial volume is indicated by vertical dotted lines. The insets on the right are the initial P-gp conformer models, the kinked conformer on the left (orange) and the straight conformer on the right cyan). The volume of their respective cavity is shown in yellow and both TM4 and TM10 are indicated. (c) Cholesterol and phospholipid snorkeling events from the membrane into the P-gp cavity during the MD simulations. A snorkeling event was recorded if the phosphorous or the oxygen atoms from phospholipids or cholesterol, respectively, were positioned inside the cavity volume shown in a, b. The three repeat simulations (of 1 μs each) were concatenated. The color legend is the same as in Fig. 1. (d) Snapshots from simulations showing the transmembrane cavity of the straight conformer with the membrane components (in van der Waals representation), snorkeling within the cavity (indicated in yellow). The phosphorous atoms from the lipid headgroups are indicated as spheres. Solvent, ions and NBD domains have been removed for clarity.

P-gp structural descriptors definealternative conformational states

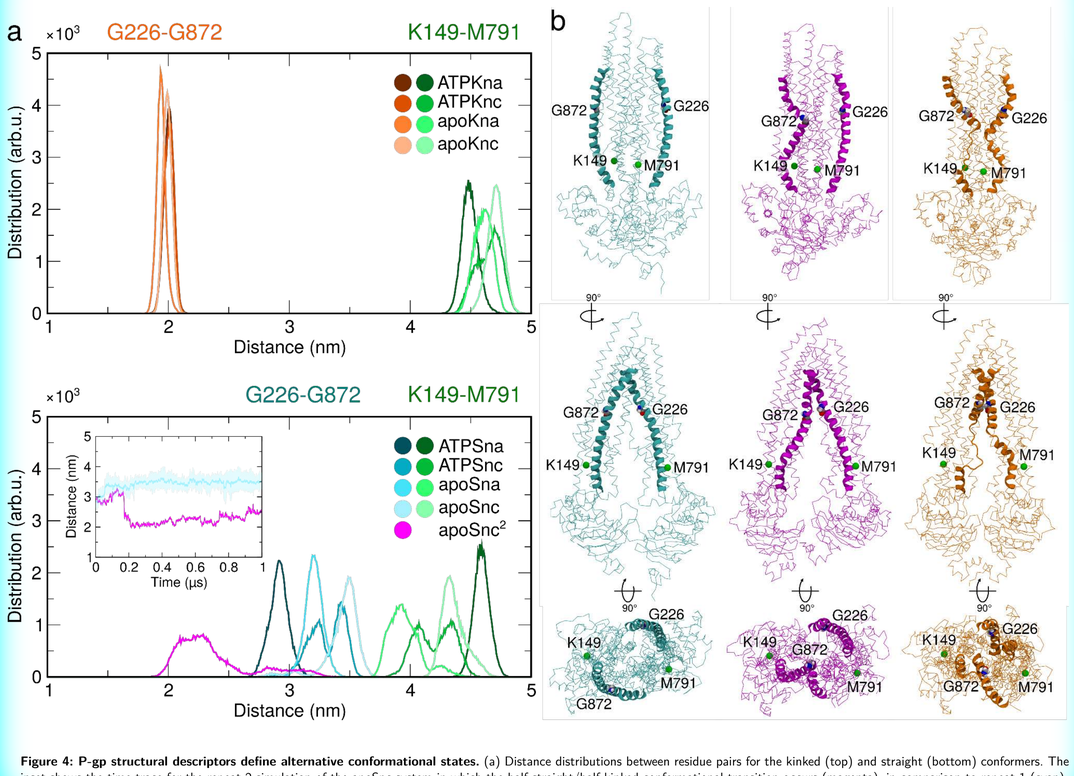


Figure 4: P-gp structural descriptors define alternative conformational states. (a) Distance distributions between residue pairs for the kinked (top) and straight (bottom) conformers. The inset shows the time trace for the repeat-2 simulation of the apoSnc system in which the half-straight/half-kinked conformational transition occurs (magenta), in comparison to repeat-1 (cyan). (b) Snapshots from simulations of the straight (cyan), kinked (orange) and half-straight/half-kinked (magenta) conformers. The residues used for the distance calculations are indicated.

Correlation between opening of the transmemrane bundles and substrate accessibility

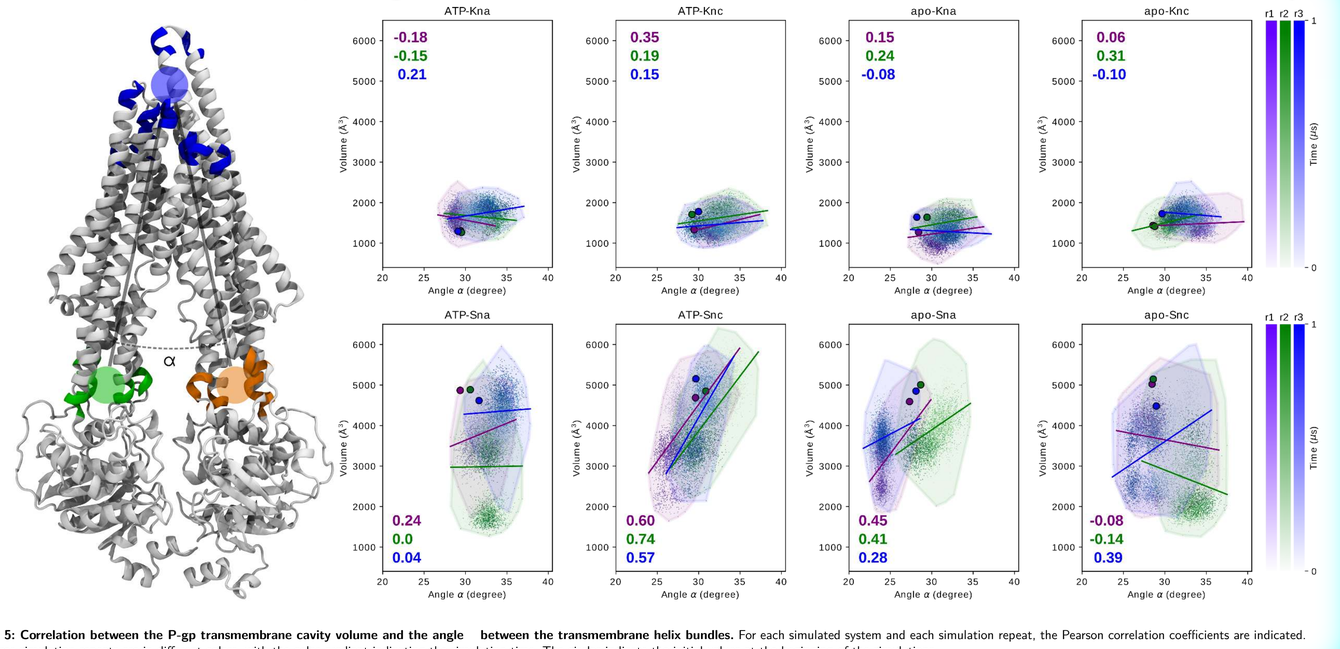


Figure 5: Correlation between the P-gp transmembrane cavity volume and the angle between the transmembrane helix bundles. For each simulated system and each simulation repeat, the Pearson correlation coefficients are indicated. The three simulation repeats are in different colors, with the color gradient indicating the simulation time. The circles indicate the initial values at the beginning of the simulations.

P-gp straight and kink conformers in a mechanistic context

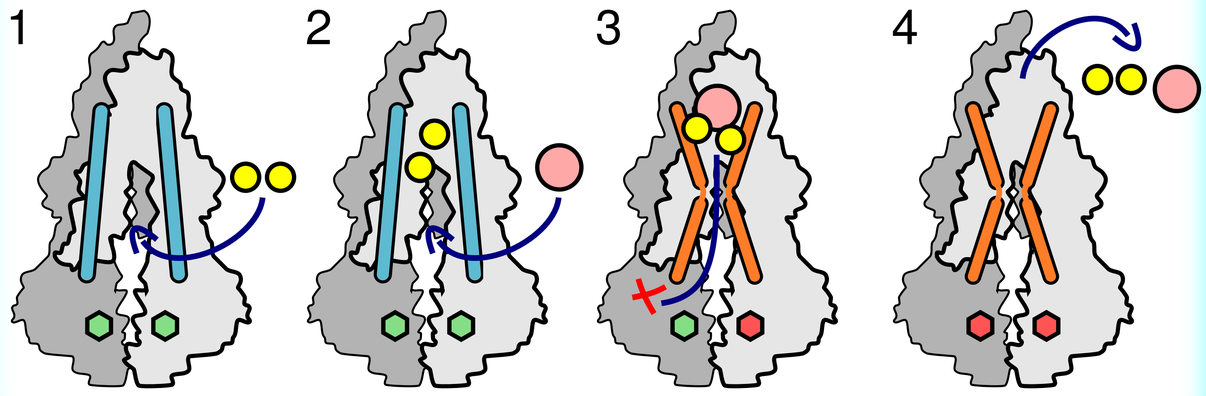


Figure 6: Proposed timeline mechanism for the straight and kinked inward-open P-gp conformers. P-gp straight (cyan) and kinked (orange) conformers are represented in cartoon. The ATP/ADP are shown as green and red hexagons, respectively. Phospholipids and substrate are depicted as yellow and pink spheres, respectively.

References

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Detection of structural tipping points on TM4 and TM10

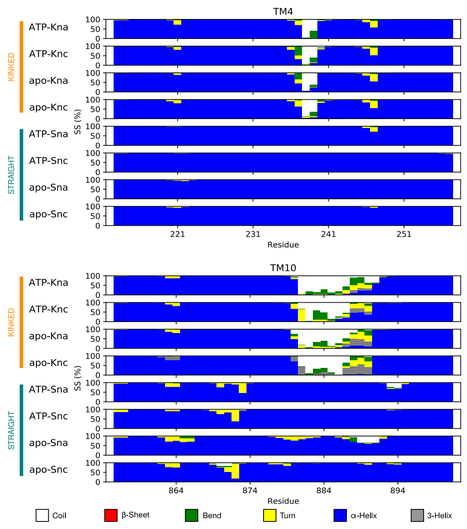

Figure 7: Evolution of the secondary structure of TM4 and TM10 in the simulated systems. The histograms show the persistence of each secondary structure element (color code at the bottom) calculated as a percentage during all concatenated repeat simulations.

Conclusions

- The **KINK** and **STRAIGHT** conformations have distinct dynamics and could play defined roles in the overall extrude mechanism
- The higly specialized lipid environment modulates protein flexibility and might play a role in substrate accessibility.
- The **KINK** conformation could be critical to avoid substrate withdrawal, and therefore it might play a role after the **STRAIGHT** conformation
- The combined sequential coarse-grained and all-atom MD simulations approach, proves effective to investigate possible Pgp lipid entrance paths.
- The presence of ATP-Mg stabilizes the NBD interface. However, in absence of ATP-Mg, dissociation events are more frequent for the **STRAIGHT** conformation.