Gating in the LacY Symporter: Normal Mode Analysis
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Abstract
Lactose permease (LacY) is a typical facilitator, using energy stored in a transmembrane electrochemical proton gradient to drive cytosolic accumulation of galactosides against their concentration gradient. While the 3.5 Å crystal structure of LacY from E. coli [1] suggests a physical realization of the alternating access model [2] for the structural differences between its inward- and outward-facing conformations, the details of these gating transitions remain elusive. We probe large-scale conformational changes in this system by all-atom Normal Mode Analysis (NMA). We find that perturbing this system along the 3rd all-atom normal mode initiates opening and closing via global counter-rotations of the intracellular and extracellular domains around the TM helices, a response that is independent of whether the sugar binds or not.

Introduction
LacY encodes coiled-coil eukaryotic transporters that accumulate galactosides against a concentration gradient by utilizing the free energy released from downhill translocation of proton.

Computational Model
We use the IPK crystal structure of LacY at 3.5 Å resolution [1], a molecular system of 6625 protein atoms, 714 water molecules, 277 water molecules (515 atoms), totaling 7,716 degrees of freedom (bond lengths, bond angles, torsion and improper torsion angles) in the molecular system. The crystal structure of LacY was resolved for the C154 mutant, which was trapped in a state closed to the inside-in state.

Main observations:
• Global counter-rotations of the cytoplasmic and periplasmic helices (black arrow) are driven by the proton gradient.
• The protonated E325 residue is driven by the proton gradient.
• The E325 residue is driven by the proton gradient.

Tracking the low-frequency NMs using RTB-NC-MC
The low-frequency eigenvectors and/or their combinations were tracked using the RTB-NC-MC technique [8,7]. A number of RTB-NC-MC runs were executed for the G154C (native) mutant, always TDG-loaded, with protonated E325 always open. The lowest 3 frequency modes in the dataset for the G154C mutant show the same structural changes as those observed in the NMA calculations. The lowest mode shows large-scale conformational changes that are identical to those observed in the NMA analysis.

Conclusions
• NMA reveals that, for the first time, describing overall translocation and rotational motions of the entire system, the 7th and higher low-frequency NMs describe global conformational changes.
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References
5. http://people.brandeis.edu/~gennady/LacY.html

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