Cholesterol transport

Cholesterol binding and transport by the hedgehog receptor PTCH1

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Project Partners

V. M. Korkhov, ETH Zürich and Paul Scherrer Institute

In Short

- Motions of PTCH1 in hydrated lipid membranes
- Dynamics of interactions between protein and cholesterol
- Hydrogen-bond networks in the transmembrane region of the receptor
- Ligand binding alters conformational dynamics of the receptor
- Combining experiments and simulations to understand the role of specific protein groups

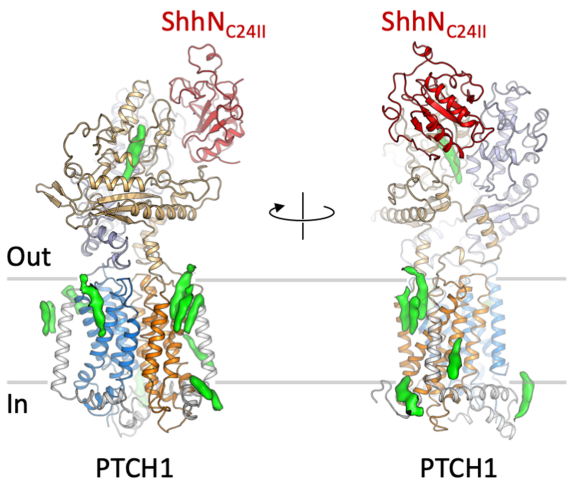

***Figure 1:** Cryo-EM structure of PTCH1 bound to ligand. The 3.4Ångstrom resolution structure of PTCH1 devoid of its C-terminus and carrying a stabilizing mutation bound to a modified sonic hedgehog reveals 10 bound sterol molecules (green density) at the protein-lipid interface; an additional sterol is bound in the PTCH1 ectodomain.*

Patched (PTCH1) is the receptor for sonic hedgehog (Shh), a secreted morphogen protein that controls embryonic development and adult tissue homeostasis. This protein belongs to a large family of proteins, the Resistance-Nodulation-Division (RND) transporter family. Mutations in PTCH1 that lead to aberrant hedgehog signaling have been linked to basal cell carcinoma, the most commonly occurring form of skin cancer. Other types of cancer, such as medulloblastoma, have also been linked to the hedgehog pathway. Furthermore, mutations disrupting the PTCH1 activity are linked to holoprosencephaly and related genetic diseases. Although the medical importance of PTCH1 for human development and pathophysiology is immense, its mechanism of action is unclear. PTCH1 has been proposed to act as a transporter for cholesterol inhibited by Shh binding, although how the protein performs its transporter function is unclear. In this proposal we outline a strategy to probe the molecular mechanisms of PTCH1 activity.

The research plan will build on the recent success in the Korkhov group at Paul Scherrer Institute in determining the high resolution cryo-EM structure of PTCH1 bound to a modified Shh ligand. The cryo-EM structure, solved at 3.4Ångstrom resolution, revealed ten sterol molecules bound to the protein-lipid interface of PTCH1 bound to a hedgehog ligand, suggesting a possible pathway for substrate translocation by PTCH1 (Fig. 1) [1]. This 3D reconstruction can now provide the basis for understanding how cholesterol transport is facilitated by PTCH1, and how this activity is controlled by the hedgehog protein. However, the structure alone merely represents a snapshot of the protein in one conformation. A deep understanding of the molecular events that occur during the activity of the protein requires a description of the motions of the protein in a hydrated lipid membrane environment at room temperature.

To characterize the motions of PTCH1 we will perform atomistic molecular dynamics simulations of PTCH1 in a membrane patch (Fig. 2). The simulation system will consist of PTCH1 with and without substrate, cholesterol, lipid membrane, water molecules and ions. The simulation system will consist of about 320.000 atoms, which highlights the importance of efficient parallel computing.

We will explore the motions of PTCH1 with and without ligand bound, and probe the response of the protein to mutants that alter amino acid residues thought important for function. For these data analyses we will focus on dissecting the dynamics of hydrogen bonds, and utilize specialized tools developed for analyses of hydrogen-bond networks in protein environments and at lipid membrane interfaces[2–4]. The computer simulation work will be performed in close collaboration with experiments

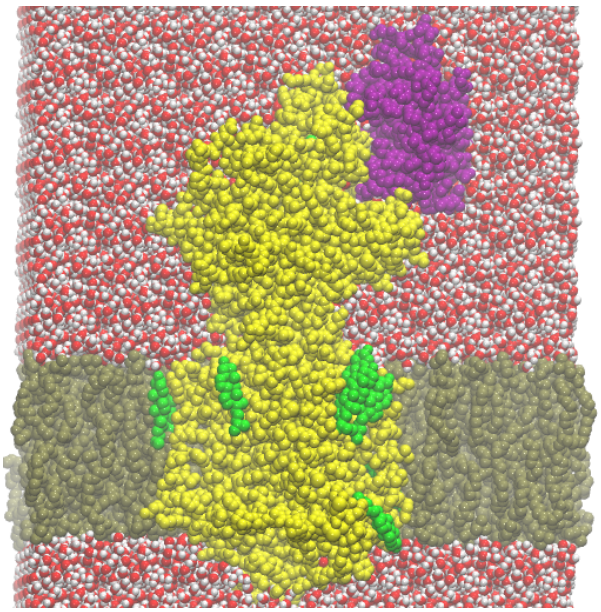

Figure 2: *Preliminary simulation setup for the reeptor-ligand complex in a hydrated lipid membrane environment. The receptor and the ligand are shown as yellow and magenta van der Waals spheres, respectively. Cholesterol molecules are colored green, and POPC lipids are brown. Lipids and water molecules are shown as a cut-away view. For clarity, we depict only hydrogen atoms of waters, and only part of the water layers on the two sides of the membrane.*

pursued in the laboratory of our cooperation partner, Dr. V.M. Korkhov.

We anticipate that results of the research planned here will allow us to generate and test hypotheses of the mechanism of substrate translocation by PTCH1. These results will help us understand how this protein, together with the hedgehog morphogen, controls this important signaling pathway. Moreover, because PTCH1 likely operates similarly to other disease-related transporters (such as Nieman Pick disease protein, NPC1), our findings may extend far beyond the hedgehog signaling field, pointing to the conserved features of ion-coupled regulation and substrate transport in the RND transporters.

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WWW

http://www.physik.fu-berlin.de/en/einrichtungen/ag/ag-bondar/

More Information

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