**M2 Internship 2023-2024**

Subject : Study of the regulatory mechanisms of the Drs2p lipid flippase embedded in a lipidic bilayer using all-atom molecular dynamics simulation

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Project :

Flippases are membrane proteins that help maintain transbilayer lipid asymmetry, by actively transporting lipids from the exoplasmic to the cytosolic layer of cell membranes. In humans, mutations of the flippase ATP8B1 cause intrahepatic cholestasis, a hereditary disease (1). Several high-resolution structures of the yeast flippase Drs2p have been determined in the laboratory, providing detailed insights into the regulatory mechanism of these molecular machines (2). Drs2p transports phosphatidylserine lipids and is autoinhibited by its N- and C-termini. Moreover, the activity of Drs2p is regulated by the presence of a phosphoinositide, PI4P (3). Indeed, we have shown that Drs2p is fully active when truncated in N- and C-termini and in the presence of PI4P (4).

We propose to the student to study the mechanisms by which PI4P activates Drs2p by all-atom molecular dynamics (MD) simulations in the presence of a lipid bilayer. Indeed, MD makes it possible to obtain information at the atomic scale on the dynamics of systems and on the interactions between proteins and lipids.

Several systems comprising the flippase Drs2p embedded in a lipid bilayer have been already constructed. Two different membrane types were chosen: a pure POPC bilayer and a mixed bilayer comprising a mixture of lipids mimicking a biological membrane. In each of these two lipid environments, PI4P is either absent or present. The trajectories have already been computed in two replica of one microsecond each. The objective is to analyze and compare the simulations in the presence and absence of PI4P to highlight the role of PI4P in Drs2p-catalyzed lipid transport.

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cholestasis type 1. Semin. Liver Dis. 30, 117–124 (2010).

(2) Timcenko M, Lyons JA, Januliene D, Ulstrup JJ, Dieudonné T, Montigny C, Ash MR, Karlsen JL, Boesen T, Kühlbrandt W, Lenoir G, Moeller A, Nissen P. Structure and autoregulation of a P4-ATPase lipid flippase. Nature:366-370 (2019).

(3) Zhou, X., Sebastian, T. T. & Graham, T. R. Auto-inhibition of Drs2p, a yeast phospholipid

flippase, by its carboxyl-terminal tail. J. Biol. Chem. 288, 31807–31815 (2013).

(4) Azouaoui H, Montigny C, Dieudonné T, Champeil P, Jacquot A, Vázquez-Ibar JL, Le Maréchal P, Ulstrup J, Ash MR, Lyons JA, Nissen P, Lenoir G. High phosphatidylinositol 4-phosphate (PI4P)-dependent ATPase activity for the Drs2p-Cdc50p flippase after removal of its N- and C-terminal extensions. J Biol Chem. 292(19):7954-7970 (2017).

Figure presented on the web site: Simulation box comprising Drs2-Cdc50 membrane protein complex (in colored ribbon representation) embedded in a hydrated lipidic bilayer (red spots for water molecules and light blue sticks for lipids). PI4P molecule is in van der Waals representation.