Exploring the molecular mechanism of PIP2-regulated sterol transport by StarD4

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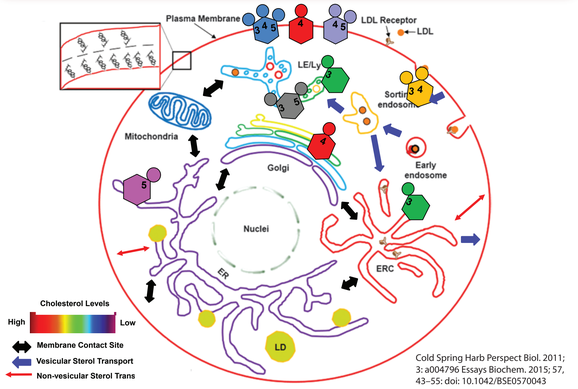
1. Abstract

Complex mechanisms regulate the cellular distribution of cholesterol (CHL), a critical component of eukaryote membranes involved in regulation of membrane protein functions directly and through the physiochemical properties of membranes. StarD4, a member of the steroidogenic acute regulator-related lipid-transfer (StART) domain (StARD)-containing protein family, is a highly-efficient sterol-specific transfer protein involved in cholesterol homeostasis. Experimental data show that sterol transfer by StarD4 is modulated by the organelle-dependent composition of phosphatidylinositol phosphate (PIP) subtypes present in the sterol donating or accepting membrane. The kinetics of StarD4-mediated sterol transport between vesicles containing PIP2 are higher compared to vesicles containing Phosphatidylserine (PS), and PI(4,5)P_2 cause a stronger acceleration effect than PI(3,5)P_2.

To understand the molecular mechanisms underlying the modulation of StarD4 efficiency through PIP-subtype recognition, we study the effect of membrane composition on the StarD4 cholesterol trafficking process using molecular dynamics (MD) simulations. We find that StarD4 embeds differently into membranes containing PI(4,5)P_2, PI(3,5)P_2 or PS, and have identified different modes of lipid binding to StarD4 in each system. The interaction modes of StarD4 with the different membrane lipid compositions depend on the specific anionic lipid molecules, suggesting a potential mechanism of PIP2-subtype recognition of StarD4. Membrane embedded StarD4 exhibits significant differences in the preference of allosteric gate opening conformations and lipid interaction, resulting in different kinetics of sterol transport as evaluated by the free energy barrier along the CHL release pathways. These findings suggest a detailed model of the molecular mechanism of regulation of sterol transport and organelle preference by StarD4 recognition of different PIP-subtypes in the target membranes.

2. Background: Cholesterol and PIP distribution in mamalian cells

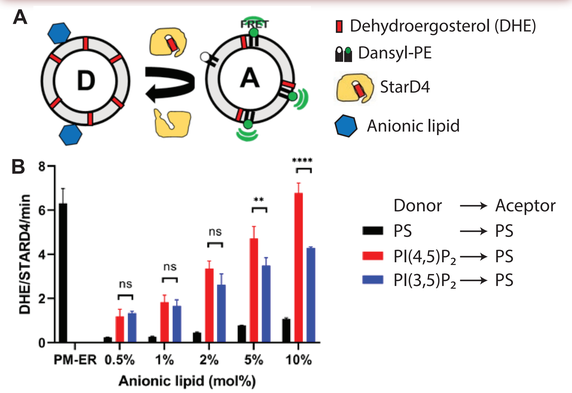


Cold Spring Harb Perspect Biol. 2011; 3: a004796 Essays Biochem. 2015; 57, 43–55: doi: 10.1042/BSE0570043

Significant differences in cholesterol distribution are maintained among cellular organelles. Membrane cholesterol levels in mamalian cells are displayed adove as a heat map, with membranes enriched in cholesterol labelled red and membranes lacking in cholesterol labelled blue. In plasma membrane (PM), cholesterol constitutes 30~40 mol% of total PM lipid, whereas in the endoplasmic reticulum (ER) where cholesterol is synthesized, it amounts to 5 mol% of the lipid molecules.

The predominant localization of particular PIP species in subcellular compartments is labeled in the hexagons. PIPs concentrate in the cytosolic leaflet of membranes, serving as discrete determinants of membrane identity. The activity of several sterol transport proteins have been shown to modulated by membrane specific PIPs.

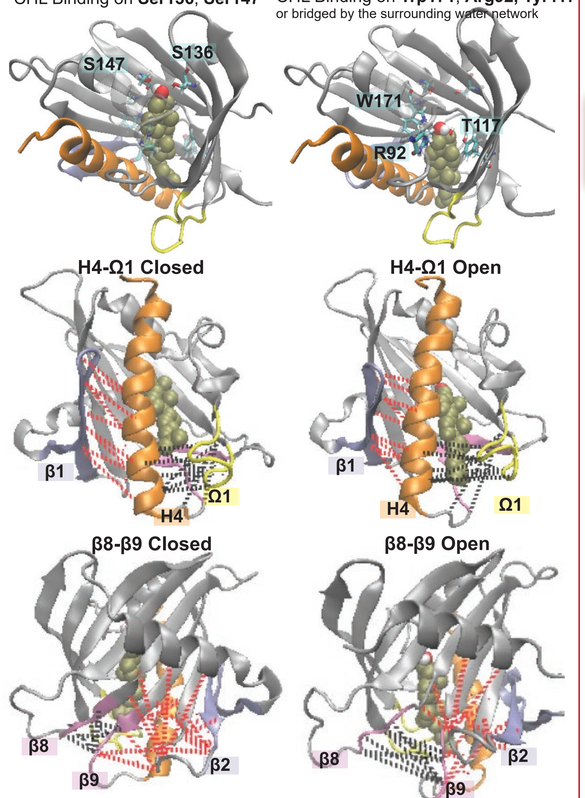
3. Experiments of sterol transfer between liposomes show that StarD4 preferentially extracts sterol from membranes containing PI(4,5)P_2



(A) The kinetics of sterol transfer between liposomes was quantified by the DHE transfer assay, which measures the FRET signal resulting from mixing the two fluorescent lipids.

(B) When PIPs replaced the PS in either donor or acceptor membranes, the sterol transfer activity of StarD4 was modulated.

4. Spontaneous CHL translocation in the hydrophobic pocket is found to occur concurrently with motif movements that open the gate



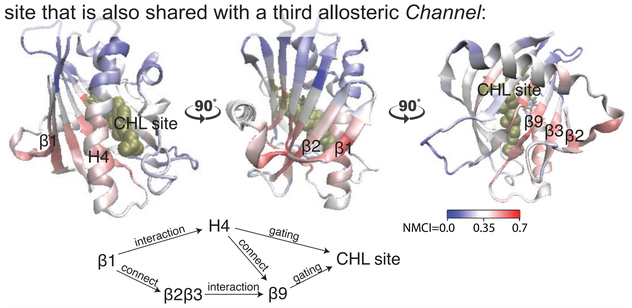
In 403μs simulation of CHL-StarD4 complex in water, the Rare Event Detection (RED) algorithms revealed a major event in StarD4 that opens the gates between H4-Ω1 and between β8-β9. This event is consistently concurrent with the translocation of CHL. Remarkably, the RED algorithms is based only on the protein conformation, without taking the information of CHL as the input, which suggests a mechanism of coupled dynamics in the CHL-StarD4 complex between the structural rearrangements of the protein frame, and the transition of CHL in the binding pocket.

5. Allosteric network that connects the structural motifs and the CHL binding site in StarD4

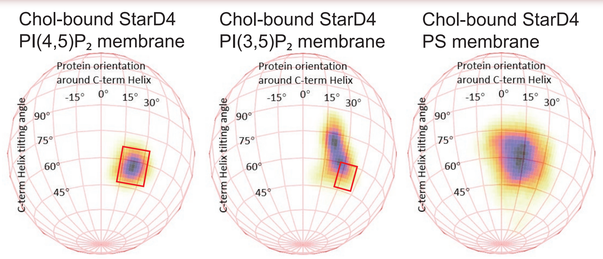
Normalized Coordination Information: the portion of information in *Receiver* motif that is shared with a *Transmitter* motif:

Receiver: \ Transmitter:	β1	β2β3	B23 loop	H4head	Ω1loop	β9	B78loop-nearβ9	B78loop-mid	β78loop-nearβ6	CHLsite
β1	19.48	22.1%	8.2%	16.8%	7.7%	14.0%	6.2%	5.7%	6.8%	14.5%
β2β3	30.6%	14.68	31.1%	24.3%	13.5%	20.8%	7.7%	6.5%	7.3%	24.6%
β23loop	10.0%	35.4%	10.11	9.4%	7.0%	11.0%	4.9%	3.9%	3.8%	11.6%
H4head	26.5%	27.9%	9.9%	16.50	10.6%	25.1%	7.2%	6.1%	5.9%	22.8%
Ω1loop	8.8%	12.5%	6.6%	12.7%	7.49	11.8%	6.0%	6.6%	6.4%	15.5%
β9	16.0%	20.6%	12.6%	22.0%	8.8%	6.45	7.1%	6.8%	6.3%	20.2%
B78loop-nearβ9	4.9%	4.8%	2.7%	3.3%	3.4%	6.1%	8.90	32.9%	18.8%	9.3%
B78loop-mid	3.8%	4.6%	2.8%	3.1%	1.9%	5.3%	27.4%	13.62	38.1%	6.6%
β78loop-nearβ6	4.5%	4.2%	3.4%	3.5%	1.5%	5.2%	18.4%	38.0%	9.57	8.3%
CHLsite	33.8%	38.4%	24.6%	30.5%	21.4%	29.7%	23.2%	18.2%	23.1%	1.70

Normalized Mutual Coordination Information: measures the portion of information flow between *Transmitter* β1 and *Receiver* CHL-site that is also shared with a third allosteric *Channel*:



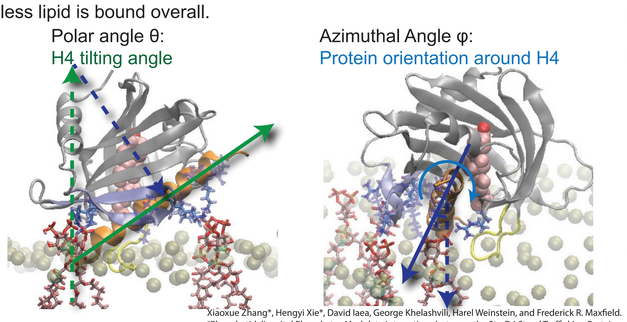
6. StarD4 embeds in the membrane with β1&2, and H4+Ω1. Different binding modes of StarD4 were identified on membranes containing PI(4,5)P2; PI(3,5)P2; or PS



The StarD4 orientation distribution calculated from the last 2/3 segment of the 4 μs simulation trajectories of membrane-embedded StarD4 is displayed in a spherical coordinate system.

The squares in the density map highlight the orientations of StarD4 where gate-opening and cholesterol-exposure were observed (θ≈55°, φ≈25°) (as shown in the example below).

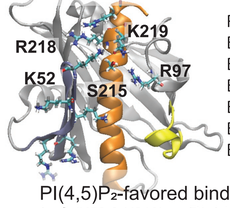
In the preferred orientation of chol-bound StarD4 in PI(3,5)P_2 membranes the C-term Helix leans down more (larger tilting angles) compared to the orientation in PI(4,5)P_2 membranes. The anchoring of StarD4 in PS membranes is weaker, as indicated by the wider sampling of orientations, and less lipid is bound overall.



Xiaoxue Zhang*, Hengyi Xie*, David Iaea, George Khelashvili, Harel Weinstein, and Frederick R. Maxfield. "Phosphatidylinositol Phosphates Modulate Interactions between the StarD4 Sterol Trafficking Protein and Lipid Membranes." J Biol Chem. 2022 Jul;298(7):102058.

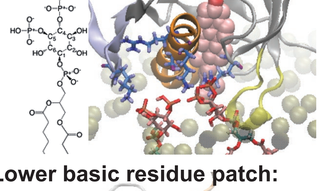
7. PI(4,5)P2 and PI(3,5)P2 show distinct binding mode prefernces, suggesting a mechanism of PIP2-subtype specific recognition

Upper basic residue patch:

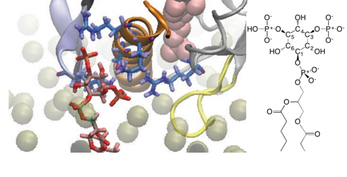


Representative binding mode	Population of the mode: PI(4,5)P2	PI(3,5)P2
Binding K219	4%	9%
Binding K52 & K219, close to R218 (<6Å)	4%	19%
Binding K52 & K219, far from R218 (>6Å)	22%	13%
Binding K52 & K219, far from R97 (>8Å)	14%	10%
Binding K52 & K219 & S215 & R97	12%	23%
Binding R97 & K219	41%	23%

PI(4,5)P_2-favored binding modes:
Left (K52 & K219) or Right (K219 & R97)

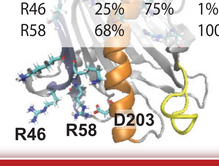


PI(3,5)P_2-favored binding modes:
Traverse from left to right (K52, R218, K219, R97)

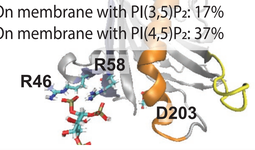


Lower basic residue patch:

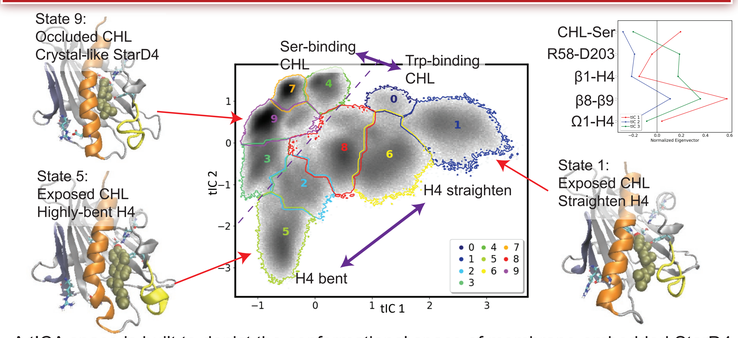
Probability of binding #lipid simutaneously	PI(4,5)P2 1	PI(4,5)P2 2	PI(3,5)P2 1	PI(3,5)P2 2
R46	25%	75%	1%	99%
R58	68%		100%	



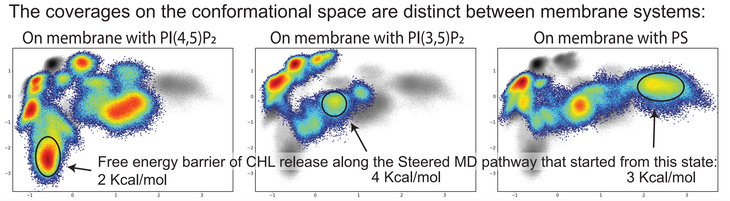
Probability of R58-D203 salt bridge bearking:
In water: 2.7%
On membrane with PI(3,5)P2: 17%
On membrane with PI(4,5)P2: 37%



8. On different membrane systems, CHL-bound StarD4 favors distinct metastable states in the tICA conformational space



A tICA space is built to depict the conformational space of membrane-embedded StarD4 (mid), with Collective Variables capturing the dynamics of aforementioned interactions as tICA parameters (upper right). On the tICA space, 10 macrostates are clustered based on kinetics similarities. Representative conformations are shown for some macrostates.
The coverages on the conformational space are distinct between membrane systems:



Conclusion

Together, our findings of (1)-an allosteric network in StarD4 that coordinates the dynamics of the CHL at the binding site with peripheral motifs β1,H4 as *transmitters*, of (2)-a differential mode of PI(4,5)P_2 and PI(3,5)P_2 binding to the basic residues around the *transmitter* motifs, and of (3)-different cholesterol-exposure conformation observed on PI(4,5)P_2- and PI(3,5)P_2-containing membrane with distinct energy barriers, suggest that the StarD4-membrane interaction mode is a factor in the PIP$_2$-mediated regulator mechanism of StarD4 kinetics.

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