

# ***Lanosterol Biosynthesis in the Membrane Environment***

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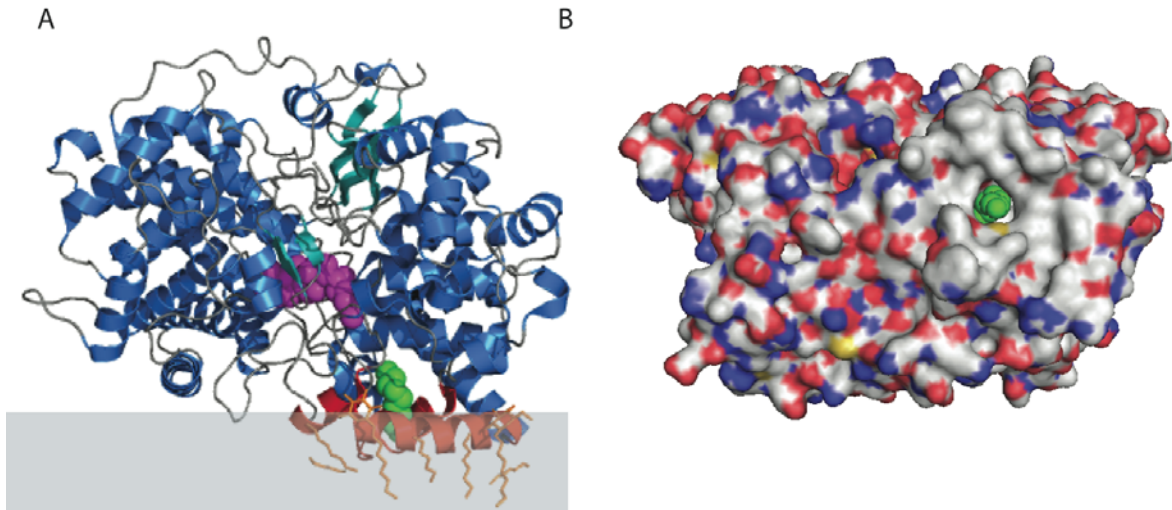
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## **Background**

Membrane enzymes have evolved to catalyze reactions whose hydrophobic substrates are part of the membrane. These enzymes must actively influence the structure of the lipid bilayer in order to access, steer, and release their reactants. Among the enzymes specialized in lipidic substrates, is the family of monotopic enzymes. Members of this family permanently reside in the bilayer, employing large hydrophobic surfaces to submerge into the non-polar part of only one leaflet of the bilayer. This unique integration into the bilayer allows them to reach into the membrane's hydrophobic-hydrophilic interface and the hydrophobic core to access their target substrates.<sup>1</sup> Monotopic enzymes participate in central cellular pathways, such as steroid synthesis, and lipid mediated signaling, e.g. the endocannabinoid in the nervous system and eicosanoids in homeostasis. The unique adaptation of monotopic proteins to their lipidic surrounding brings forth important questions: How do monotopic proteins interface with the membrane and deliver their substrate and products to and from the lipid bilayer via hydrophobic channels? What unique features arise when protein conformational changes are linked to the membrane bulk environment? Does the unique interface with the lipid bilayer assist in substrate channeling in biosynthetic pathways?

These fundamental questions regarding the behavior of monotopic membrane enzymes will be studied using 2,3-oxidosqualene cyclase (OSC; lanosterol synthase). OSC catalyzes the one-step cyclization of lanosterol, the precursor for cholesterol and all other steroids.<sup>2</sup> OSC is a prime therapeutic target for treatment of hypercholesterolemia and atherosclerosis.<sup>3</sup> In animals studies OSC inhibitors were shown to be highly effective in reducing serum and low-density lipoprotein cholesterol without effecting levels of non-sterol isoprenoids (unlike the action of statins). As an added benefit OSC inhibition also increases the production of 24(S),25-epoxycholesterol, an activator of the nuclear hormone receptor liver X receptor, which regulates liver functions. *In vivo* OSC is active in two radically different membranous structures: the lipid bilayer of the endoplasmic reticulum and the lipid monolayer of lipid droplets.<sup>4</sup> (Lipid droplets - also known as lipid bodies and lipid particles - are independent organelles, consisting of core neutral lipids coated by a lipid and proteins monolayer, that are used for storage and steroid synthesis.<sup>5</sup>) Understanding the differences in OSC activity and substrate accessibility between these two membrane systems promises to help the design of potent inhibitors and optimization of cellular delivery from the membrane to the OSC active site.



Structure of lanosterol (magenta) bound human OSC.<sup>6</sup> A) The active site is linked to the membrane interior via a 15 Å hydrophobic channel, which mediates substrate uptake and product release. The disordered lipid in the channel's entrance (green) illustrates the substrate path from the membrane to the active site. OSC position in the membrane (grey) was inferred from the position of crystallographically observed detergent molecules (orange). B) The surface representation of hOSC showing the hydrophobic patch that resides in the membrane. A disordered lipid is blocked from entering the channel by the gating contraction (green).

### Project goals

- 1) Expression, purification and reconstitution of the human OSC into membranes of well defined composition and organization.
- 2) Investigate the membrane conditions required for efficient substrate presentation and the effect of the membrane's physicochemical properties on catalysis.
- 3) Prepare <sup>13</sup>C enriched transition state analogues will be utilized which will be enzymatically prepared using OSC mutants that prematurely abort the cyclization reaction.
- 4) Identify several of the membrane embedded and gate residues by solid-state NMR spectroscopy using <sup>13</sup>C isotopically enriched ligands.

### Perspective

Our long term goal is to understand the correlation – as mediated by the membrane - between the conformational changes of the protein and the transfer of the substrate and product to and from the lipid bilayer.

### References

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