Computational and theoretical approaches to understand the functional properties of enzymes at multiple scales: from molecular interactions to systems biology

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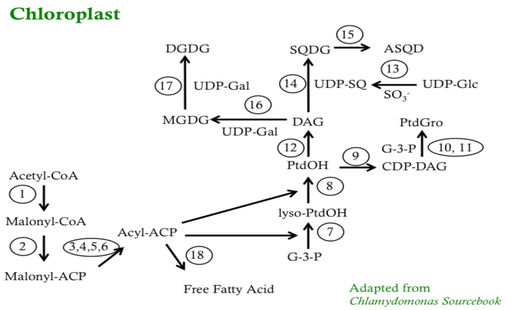
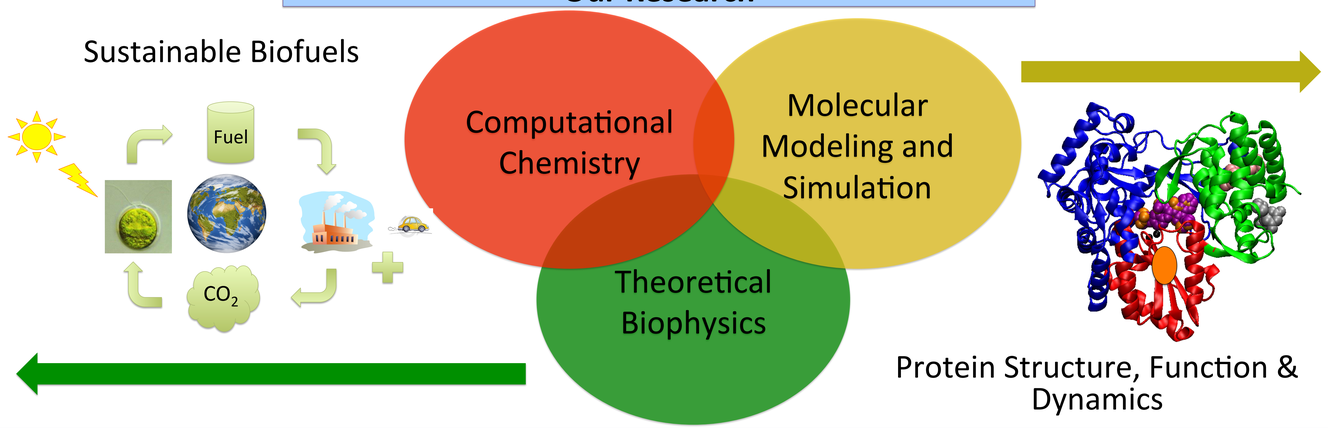
Biofuels from Microalgae

A worldwide effort to find renewable alternatives to fossil fuels is underway. Potential sources of renewable fuel include microalgae. Under certain conditions, these organisms produce large amounts of triacylglycerides (TAGs), lipids that can be converted to biodiesel. However, the lipid biosynthetic pathway of microalgae is not fully understood. To better understand the conditions that govern lipid production in microalgae, we employ theoretical and computational methods to understand the topology, flux and regulatory properties of the metabolic pathway involved in TAG biosynthesis in microalgae. In particular, we seek to understand the differences that lead to altered TAG production in different microalgal species. Thus far, we have studied the lipid pathway in the well-studied microalgal organism *Chlamydomonas reinhardtii*. We have created a structural kinetic model of the pathway following the example of Steuer et al. (PNAS (2006) 103, 11868-11873). Such models pinpoint areas of instability in the pathway and may highlight instances where overexpression of an enzyme may lead to increased TAG. Predictions from the model will ultimately be validated by comparison with *in vitro* and *in vivo* experiments. By understanding more about lipid production in microalgae we hope to guide rational genetic engineering approaches to increase oil production in these organisms. We anticipate that these studies will ultimately provide insights into lipid biosynthesis for a wide range of other organisms.

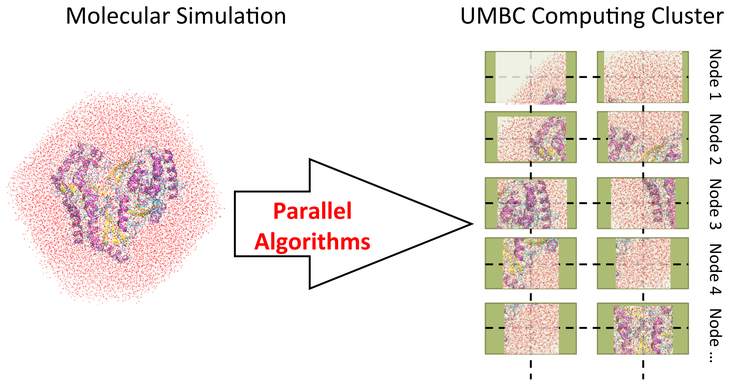
Lipid Synthesis Pathway in *C. reinhardtii*



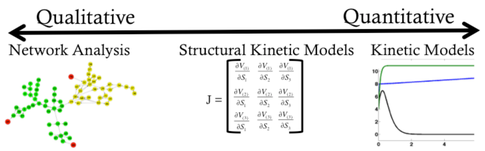
Our Research



High Performance Computing



Structural Kinetic Modeling



Structural Kinetic Modeling

Jacobian = $\begin{bmatrix} \frac{\partial V_{(1)}}{\partial S_1} & \frac{\partial V_{(1)}}{\partial S_2} & \frac{\partial V_{(1)}}{\partial S_3} \\ \frac{\partial V_{(2)}}{\partial S_1} & \frac{\partial V_{(2)}}{\partial S_2} & \frac{\partial V_{(2)}}{\partial S_3} \\ \frac{\partial V_{(3)}}{\partial S_1} & \frac{\partial V_{(3)}}{\partial S_2} & \frac{\partial V_{(3)}}{\partial S_3} \end{bmatrix}$

V = Flux
S = Substrate Concentration

$J_x = \Lambda \bullet \theta_x^{\mu}$

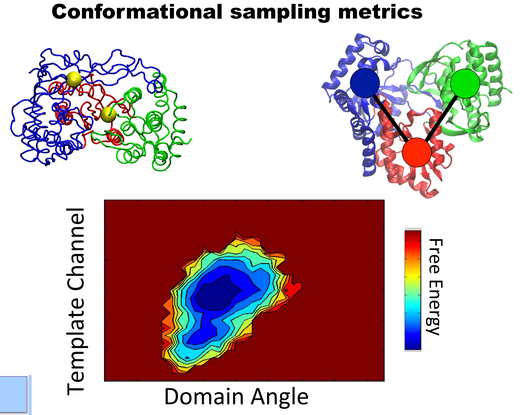
Λ = reflects the metabolic and stoichiometric state
θ_x^{μ} = effective kinetic order of the normalized saturation

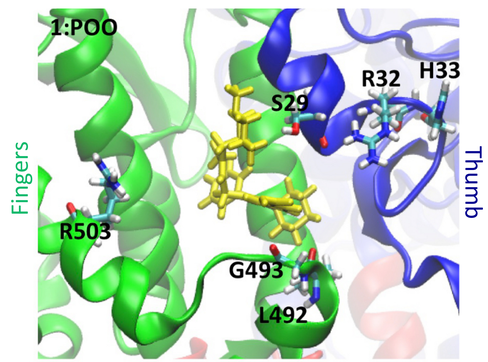
$$\Lambda_{ij} = N_{ij} \frac{V_j(S^0)}{S_i^0}$$

N = stoichiometric matrix

Molecular Modeling

Conformational sampling metrics

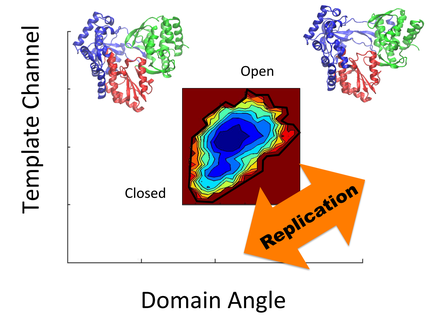




Allostery in the Hepatitis C Virus Polymerase

The Hepatitis C Virus (HCV) infects approximately 200 million people throughout the world. Currently, there is no cure and current treatments have limited efficacy and sever side effects. Therefore, new HCV treatments are in high demand. The RNA polymerase (gene product NS5B) from HCV is a validated drug target because of its importance for viral replication. NS5B functions through *de novo* initiation in a closed conformation, while elongation occurs in the open conformation. Currently, there are four known allosteric binding sites to which diverse inhibitors can bind. However, the molecular mechanisms that underlie allosteric inhibition are unclear from the structural data alone. We employ molecular dynamics simulations and various computational analyses in order to understand how the presence of allosteric nonnucleoside inhibitors (NNIs) impacts the structure and dynamics of NS5B. Our results suggest that ligand binding prevents the enzyme from achieving functional conformations. Moreover, we find that non-overlapping NNI sites are compatible with simultaneous binding of dual inhibitors. We observe that both inhibitors act in concert to induce novel enzyme conformations and motional behavior. This knowledge will be useful in optimizing combinations of inhibitors to target NS5B, helping to prevent the acquisition of viral resistance that remains a significant barrier to the development of HCV therapeutics.

Conformational states in free enzyme



Conformations sampled when two inhibitors are present