Probing Substrate Sequestration in Carrier Proteins

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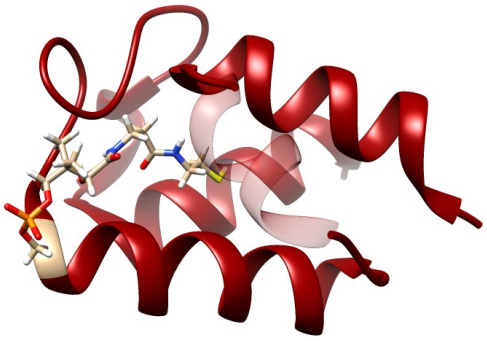
Carrier proteins are considered “lynchpin” enzymes of biosynthetic pathways. The *E. coli* acyl carrier protein (ACP) is composed of multiple alpha helices that form a hydrophobic, solvent-protected pocket that provides a hiding spot to protect substrates bound to the ACP’s phosphopantetheine (Ppant) arm. The action of “chain sequestration” is thought to be important for driving the acyl chain biosynthetic process. Site specific vibrational spectroscopy techniques are being used to investigate Ppant arm dynamics via nitrile probe groups incorporated into the peptide sequence and onto the end of substrate loaded by the Ppant arm, as well as via unnatural amino acids incorporated into the sequestration cavity. Computational simulations in GROMACS aim to characterize Ppant arm dynamics and the arm's shifting conformational distribution by analyzing geometric quantities and solvent exposed surface area (SASA) of specific pieces of the arm. Ultimately, incorporating a nitrile probe group into the simulation will provide an interpretive aid for spectroscopic lineshapes. A thorough understanding of Ppant arm dynamics will also inform further characterization of experimental data from carrier proteins and efforts to manipulate ACP's interactions with partner enzymes.



***E. coli* Acyl Carrier Protein** (PDB: 2FAC) with phosphopantetheine arm carrying growing substrate tucked into internal hydrophobic cavity.