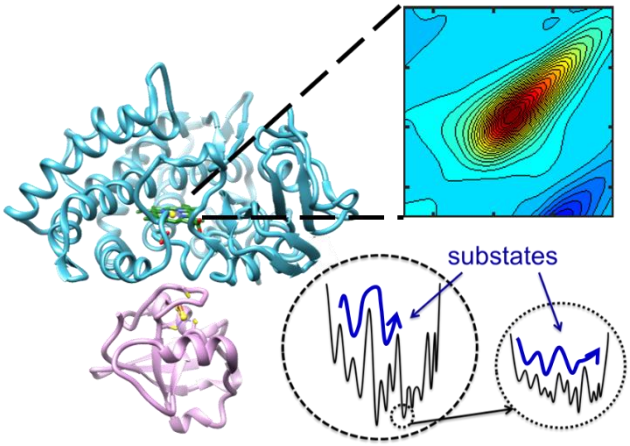
QCB Evenings

Wednesday, April 18th • 5:30pm • Chemistry C033

QCB
Quantitative and Chemical Biology
Indiana University

Conformational Dynamics of the P450cam-Putidaredoxin Complex Probed via 2D IR Spectroscopy

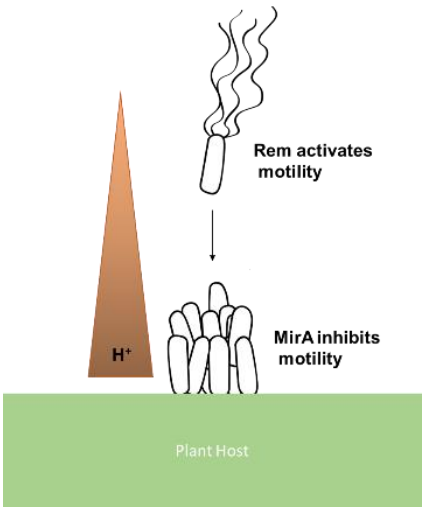
❖ **Sashary Ramos, Thielges Lab**



Cytochrome P450s are a family of heme oygenases found in all kingdoms of life; the well-studied P450cam from *Pseudomonas putida* is considered an archetypical P450. P450cam catalyzes the hydroxylation of *d*-camphor with the aid of its electron transfer partner putidaredoxin (Pdx). Pdx has been known to play an effector role in its reduction of the O_2 complex of cytochrome P450cam. However, the conformational dynamics contributing to this role are not fully understood. To investigate how Pdx binding impacts ferrous, CO-ligated, P450cam, we applied 2D IR spectroscopy to measure the conformations and dynamics of wild-type enzyme in the absence and presence of Pdx, as well as a mutant, L358P P450cam, which has served as a putative model for the Pdx complex. The CO vibrations of the Pdx complex and L358P report population of two conformational states in which the CO experiences distinct environments. This talk will discuss the distinct conformational dynamics reported by the 2D IR experiments and provide insight toward the effector role of Pdx on P450cam reduction.

Acid-Responsive Motility Inhibition by MirA in *Agrobacterium tumefaciens*

❖ **Melene Thompson, Fuqua lab**



Agrobacterium tumefaciens is a plant pathogenic bacterium that swims via flagellar propulsion. When *A. tumefaciens* cells encounter an acidic plant wound environment, they undergo a transition to a sessile state. This transition is mediated in part by the acid-responsive periplasmic regulator ExoR, which controls activity of the two-component system ChvG-ChvI. Under acidic conditions, ChvG-ChvI blocks the activity of the primary motility regulator Rem, a novel transcription factor, leading to a loss of motility gene expression. We have discovered that the motility inhibition exerted through an active ExoR-ChvG-ChvI pathway requires the function of a previously unrecognized gene which we have named the motility inhibitor of Rem, or *mirA.* Initial characterization of *mirA* indicates that it encodes a small protein (76 aa) which lacks any identifiable amino acid motifs indicative of its structure or function. The upstream region of MirA contains a small RNA defined in previous genome-wide RNA screens. Expression of *mirA* is activated by the ChvG-ChvI two component system, thus linking this pathway and Rem. Plasmid-borne expression of *mirA* broadly inhibits motility through modulation of motility gene expression. Current work is aimed to understand how MirA can impose such a potent inhibitory activity on Rem-dependent transcriptional control.

All QCB Trainer Lab Personnel are invited to attend.
Refreshments will be provided.