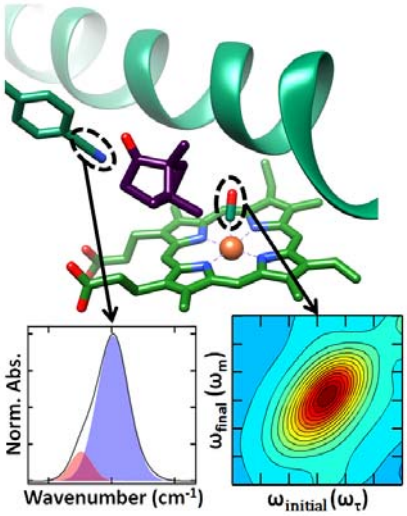
Quantitative & Chemical Biology Graduate Training Program

QCB Evenings

Wednesday, March 22 • 5:30pm • Chemistry C122

Dynamics and Conformational Heterogeneity in Cytochrome P450s via Infrared Spectroscopy

EDWARD BASOM, THIELGES LAB



Cytochrome P450s are a superfamily of enzymes which catalyze hydroxylation of unactivated hydrocarbons, however the means by which P450s control regioselectivity of this reaction remain largely elusive. Toward investigation of regioselectivity in the archetypal cytochrome P450cam, we combined infrared spectroscopy and the site-specific incorporation of vibrational probes which permit characterization of fluctuations on fast timescales with high spatial resolution. Our results from a heme-bound carbon monoxide probe suggest that the binding of different substrates to P450cam variably stabilizes the active site into two distinct states associated with different levels of regioselectivity. Incorporation of nitrile probes at different P450cam microenvironments enabled us to discern changes experienced at each of those environments when a substrate binds in the active site. Finally, the putative P450cam-substrate hydrogen bonding interaction was more rigorously investigated with both CN and CO probes.

Mutations in the primary sigma factor bypass the critical ComW requirement for natural transformation in *Streptococcus pneumoniae*

YANINA TOVPEKO, WINKLER/MORRISON LAB

Streptococcus pneumoniae is an opportunistic pathogen that resides in the human nasopharynx and is naturally transformable, or able to take up and integrate exogenous DNA into its genome. Competence for genetic transformation is tightly regulated, transient, and occurs in two phases, early and late. The early genes encode a quorum-sensing system and peptide pheromone that signal an entire population to become competent in synchrony. One early gene encodes σ^X, the only known streptococcal alternative σ factor, which is responsible for coordinated synthesis of the late genes, those which are necessary for DNA uptake and recombination. In *S. pneumoniae*, elevated σ^X is insufficient for development of full competence without co-expression of a second competence-specific protein, ComW, which is regulated by the same pheromone circuit that controls σ^X. *comW* mutants display several phenotypes, a 10^4-fold reduction in the amount of transformants, a 10-fold reduction in σ^X activity, and a 10-fold reduction in the amount of σ^X protein. To identify proteins that may be interacting with ComW during competence, a suppressor screen was performed seeking mutants that were partially restored for transformation in the Δ*comW* mutant background. Whole genome sequencing of suppressor strains revealed ten different single-base substitutions in *rpoD*, the gene encoding the primary σ factor, σ^A, that each bypass the ComW requirement for transformation. Eight of the ten single-base substitutions mapped to residues previously implicated in σ^A binding affinity to core RNA polymerase. 3D structure analysis suggests ComW increases σ^X access to core RNA polymerase, pointing to a role for ComW in σ factor exchange during genetic transformation in *S. pneumoniae*, and a novel mechanism of σ factor activation in Gram positive bacteria.

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

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