Some bacteria, including *Rhodococcus jostii* RHA1 and *Mycobacterium tuberculosis* (Mtb) can degrade steroids such as cholesterol and use them as their sole sources of carbon for growth. Besides playing roles in carbon cycling, steroid degrading bacteria are important for remediation of harmful steroid wastes in the environment and have been used for the biotransformation of plant sterols to steroid drugs, such as the anti-inflammatory compound hydrocortisol. More recently, cholesterol utilization by *Mtb* was implicated for its persistence in host alveolar macrophages. With the increased prevalence of drug resistant strains of *Mtb*, the cholesterol degradation pathway has become a promising target for the development of new antibiotics against this pathogen.

Catabolism of steroid side chains is analogous to fatty acid β-oxidation, where repeating enzymatic reactions shorten the chain by 2-3 carbons, yielding central metabolites for energy generation and biosynthesis. One crucial enzyme for this process is an enoyl-CoA hydratase (EcHd). The steroid catabolic gene clusters of *R. jostii* RHA1 and *Mtb* encode putative EcHds with atypical structures, including modified non-catalytic domains which may facilitate binding of bulky steroid substrates. Using in-vitro activity assays and in-vivo gene knockout studies I will determine if distinct EcHds with different chain length specificities are involved in the steroid degradation pathways of *Mtb* and *R jostii* RHA1. Site specific mutagenesis and 3D structural determination will be used to investigate the structural features that facilitate substrate binding and determine side chain length specificity.