# SITE-SPECIFIC CHARACTERIZATION OF P450CAM SUBSTRATE RECOGNITION *VIA* 2D IR SPECTROSCOPY

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Determining the mechanism by which Cytochrome P450s (P450s) can catalyze oxidation reactions of substrates with differing specificity and regioselectivity is crucial for aiding drug development and understanding drug metabolism. Cytochrome P450cam (P450cam), a model P450, catalyzes the hydroxylation of *d*-camphor to 5-exo-hydroxycamphor with high specificity and regioselectivity, it can also act upon camphor-like analogs at the expense of regioselectivity. Previous studies have suggested conformational dynamics may play a role in the recognition and hydroxylation of substrates with varying degrees of regioselectivity. To investigate the role of dynamics in regioselectivity, we characterized P450cam when bound to a camphor and norcamphor, substrates acted upon with 100% and 45% regioselectivity respectively. 2D IR spectroscopy was paired site-specific labeling and used to measure protein side-chain dynamics with high spatial and temporal resolution. Cyanophenylalanine was used as a vibrational probe and incorporated in five distinct locations of P450cam, three sites in the active site and two progressively distal from the active site. The results suggest different parts of the protein active site are preferentially involved in substrate binding and contributions from inhomogeneous broadening are more significant for substrates acted upon with high regioselectivity.