

Abstract Submitted
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A Fluorescence Spectroscopic Study of Cytochromes P450 1A2 and 3A4. GLENN MARSCH, Grove City College, F.P. GUENGERICH, Vanderbilt University, JOSHUA INKS, Grove City College — Fluorescence spectroscopy was used to study cytochromes P450 1A2 and 3A4. Spectra of P450s were acquired in the presence and absence of acrylamide quencher. In both P450s, quenching revealed three distinguishable species of amino acid fluorescence, with maxima at 297, 323, and 345 nm. The 345 nm tryptophan fluorescence was quenched by low levels of acrylamide; the 297 nm tyrosine fluorescence was resistant to quenching. The 323 nm fluorescence was observed at intermediate concentrations of quencher. Stern-Volmer plots of P450 quenching were non-linear, but were well-fitted to a superposition of linear plots for each fluorophore species. The effect of P450 1A2 binding on pyrene fluorescence was also examined. Upon binding to P450 1A2, the intensity of the 383 nm pyrene vibronic band was decreased relative to the intensities of the 372 and 393 nm bands. Fluorescence quenching of pyrene and other ligands upon binding to P450s will be used to evaluate distances between ligands and the P450 heme moiety by fluorescence resonance energy transfer. Fluorescence quantum yields of ligands, overlap integrals, and Förster distances of many ligand-heme donor-acceptor pairs were calculated. Steady-state spectra and time-resolved data of bound ligand will be used to calculate substrate-heme distances in the P450 enzymes.

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