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**A Fluorescence Spectroscopic Analysis of the Binding of Pyrene to Cytochromes P450 1A2 and 3A4.** JUDAH HENRY, Grove City College, F. PETER GUENGERICH, Vanderbilt University, GLENN MARSCH, 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 the P450's 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. Both P450's showed binding of the pyrene, but 1A2 demonstrated significantly more excimer emission than did the 3A4, which suggests that more than one pyrene molecule binds to 1A2's active site. The results of these analyses will be used in further characterization of these enzymes.

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