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Using Fluorescence Spectroscopy to Evaluate Hill Parameters and Heterogeneity of Ligand Binding to Cytochromes P450[1] GLENN A. MARSCH, BENJAMIN CARLSON, JENNIFER HANSEN, ELAINE MIHELC, Grove City College Physics Department, MARTHA V. MARTIN, F. PETER GUENGERICH, Vanderbilt University School of Medicine — The cytochromes P450 (CYPs) are hemoproteins that oxidize many drugs and carcinogens. Binding interactions of two CYPs with Nile Red, pyrene, and alpha-naphthoflavone were studied using fluorescence quenching. Upon interaction with CYPs, fluorescence from pyrene excited-state dimers was quenched more efficiently than fluorescence from pyrene monomers. Quenching data was fit to the Hill equation to determine binding affinities and the Hill parameter n for the interaction of substrates with CYPs. All ligands showed strong binding to the CYPs, especially alpha-naphthoflavone, but exhibited little or no cooperativity in the interaction. Modified Stern-Volmer plots were used to confirm binding affinities, and suggested heterogeneous populations of amino acid fluorophores. Fluorescence anisotropy experiments suggest that CYP molecules tumble more rapidly when alpha-naphthoflavone is added.

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