

# Spectral properties of environmentally sensitive probes associated with horseradish peroxidase

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The environmentally sensitive fluorescent probes 6-propionyl-2-(N,N-dimethylamino)naphthalene (PRODAN) and 2'-(N,N-dimethylamino)-6-naphthoyl-4-trans-cyclohexanecarboxylic acid (DANCA) form complexes with the heme binding site of apohorseradish peroxidase. The dissociation constants of the PRODAN and DANCA complexes were determined from anisotropy titration data to be approximately  $8.7 \times 10^{-5}$  and  $3.3 \times 10^{-4}$  M, respectively. From comparison of the steady state fluorescence spectra of PRODAN and DANCA in solvents of varying dielectric constants, and in the apohorseradish peroxidase complex, we conclude that the heme binding site of horseradish peroxidase is relatively polar. The lifetimes of PRODAN and DANCA in organic solvents of varying polarities can be fit to single exponential decays. However, the lifetimes of PRODAN and DANCA associated with apohorseradish peroxidase, determined using a background subtraction method to correct for the non-negligible fluorescence of unbound probe, fit best