Electric Fields and Enzyme Catalysis

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We have developed the *vibrational Stark effect* to probe electrostatics and dynamics in organized systems, in particular in proteins where vibrational probes can report on functionally important electric fields. The strategy involves deploying site-specific vibrational probes whose sensitivity to an electric field is measured in a calibrated external electric field. Once calibrated, these probes, typically nitriles or carbonyls, can be used to probe *changes* in electric field due to mutations, ligand binding, pH effects, light-induced structural changes, etc. We can also obtain information on *absolute* fields by combining vibrational solvatochromism and MD simulations, checked by the vibrational Stark effect calibration. This frequency-field calibration can be applied to quantify *functionally relevant* electric fields at the active site of enzymes. Using ketosteroid isomerase as a model system, we correlate the field sensed at the bond involved in enzymatic catalysis with the rate of the reaction it catalyzes, including variations in this rate in a series of mutants and variants using non-canonical amino acids. This provides the first direct connection between electric fields and function: for this system electrostatic interactions are a dominant contribution to catalytic proficiency. Using the vibrational Stark effect, we can now consistently re-interpret results already in the literature and provide a framework for parsing the electrostatic contribution to catalysis in both biological and non-biological systems. Extensions of this approach to other classes of enzymes, to effects of electrostatics on pathways of photoisomerization in proteins, and to the evolutionary trajectories of enzymes responsible for antibiotic resistance will be described if time permits.