Experiments and Simulations of Electrostatic Fields Inside Proteins

The changes in pK_a of titratable residues have long been used as reporters of local electrostatic fields in proteins. Though pK_a shifts are difficult to interpret because of the perturbative nature of the probe and sensitivity to non-Coulombic effects such as hydrogen bonding, significant effort has been dedicated to developing computational electrostatic models based on experimentally measured shifts in pK_a. Nitrile vibrational probes are potentially less disruptive and more direct reporters of local electrostatic field, but quantitative interpretation is similarly convoluted by the ability of the nitrile to accept a hydrogen bond. We address these challenges by comparing pK_a shifts and nitrile frequencies in the same positions in the same protein. To this end, we incorporated nitrile probes into 10 locations of staphylococcal nuclease (SNase) where pK_a shifts had already been reported. We find that nitrile frequencies and pK_a shifts do not correlate in this protein. We characterized the local environment of each nitrile probe experimentally, through temperature-dependent spectroscopy, and computationally, through molecular dynamics simulations, and show that hydrogen bonding interactions dominate the spectral line shapes. We demonstrate that the information provided by the line shape of the nitrile spectra, compared to scalar values of pK_a shift, describes local environments in proteins in a manner that will be useful for future computational efforts to predict electrostatics in complex biological systems.