SFB 1078

Protonation Dynamics in Protein Function

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Webex

Link via e-mail

Colloquium

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Pre-organization & Evolution of Enzyme Active Sites using the Vibrational Stark Effec

Local electric fields in complex systems like proteins can be determined using the vibrational Stark effect (VSE). A direct proportionality has been established in earlier work between the activation free energy and electric field projected onto the bond undergoing charge reorganization at the active site an enzyme from which we obtain the electrostatic contribution to catalysis. In all cases, mutations made the field and catalytic rate smaller, begging the question whether larger fields and corresponding larger rates can be created either by design or by evolution? Using the hydride transfer enzyme liver alcohol dehydrogenase, we show that mutations and metal replacements at the active site can produce both larger fields and faster rates, extending and strengthening the concept of electrostatic catalysis. By using an aldehyde inhibitor, we can measure projections both on the carbonyl C=O bond and on the C-H (with H replaced by D) at the same carbon. This 2-directional probe can be studied both in simple solvents and at the active site of the enzyme. We find that while the fields depend strongly on solvent, the ratio of the fields projected on C=O and C-D is approximately constant over a wide range of solvent polarities. By contrast, the ratio of the field projections at the active site of LADH is substantially different, consistent with the idea that the protein creates a unique pre-organized electrostatic environment. Implications of both observations for enzyme design will be discussed.

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