

P028 Theoretical Reduction Potentials for Engineered Flavoproteins

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The function of approximately one third of all known proteins is electron transfer (eT). The isoalloxazine nucleus of flavin redox cofactors comprises the electron sink in life-sustaining biological eT reactions. Much experimental evidence supports that natural control of flavoprotein mediated eT is fine-tuned by the protein environment. The change in Gibbs free energy for the first reduction of FMN, relative to the wild-type (WT) protein, imparted by 16 active-site neutral single-point mutations of *Anabaena* and *Clostridium beijerinckii* flavodoxins were computed using molecular dynamics free energy simulations. Concomitant changes in reduction potential ($\Delta E_{\text{ox/sq}}^{\ominus}$) were calculated consistent with experimental values for 9 *in silico* mutants ($R^2=0.94$ for linear regression). The sign of $\Delta E_{\text{ox/sq}}^{\ominus}$ was computed correctly for a further 4 mutations. 3 aliphatic and branched substitutions were not calculated in agreement with experimental observations. The method proved robust for aromatic \rightarrow aromatic mutations and those in which side-chains were reduced in size. Future *in silico* substitutions will be facilitated by the finding that rotamers yielding calculated $\Delta E_{\text{ox/sq}}^{\ominus}$ values which deviated least from experimental data corresponded to the most energetically favorable conformation. The method could be used to screen mutations imparting desired thermodynamic properties in eT flavoproteins of biotechnological interest where experimental reduction potentials and structural data are available for the WT protein.