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The role of reorganization energy in rational enzyme design☆

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Computational design is becoming an integral component in developing novel enzymatic activities. Catalytic efficiencies of man-made enzymes however are far behind their natural counterparts. The discrepancy between laboratory and naturally evolved enzymes suggests that a major catalytic factor is still missing in the computational process. Reorganization energy, which is the origin of catalytic power of natural enzymes, has not been exploited yet for design. As exemplified in case of KE07 Kemp eliminase, this quantity is optimized by directed evolution. Mutations beneficial for evolution, but without direct impact on catalysis can be identified based on contributions to reorganization energy. We propose to incorporate the reorganization energy in scaffold selection to provide highly evolvable initial designs.

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Dedicated to Arieh Warshel to congratulate his Nobel prize in Chemistry, 2013. He provided a seminal contribution to use reorganization energy for interpretation of enzymatic catalysis.

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Tailoring activities of biomolecules is a dream for both computational and experimental biochemists. Enzymes that catalyze nonbiological reactions are awaited and utilized in biomedicine and biotechnology. De novo enzyme design comprises two main steps. First a computational process [1,2] provides a model with the desired function, albeit with moderate activity. This is followed by experimental optimization of the initial model by repeated rounds of random mutagenesis and natural selection [3,4]. In general, directed evolution increases k_{cat} by 10^2 to 10^3 fold. Currently, owing to the synergistic effort of computational design and laboratory optimization, artificial enzymes with efficiencies close to that of catalytic antibodies could be engineered, but reaction rates are still far from what has been optimized by Nature [5]. Although the success of a recently evolved Kemp eliminase is promising [6••], enzyme designs still seem to lack major catalytic factors.

Computer-assisted model generation requires an in-depth understanding of structure–function relationships of enzymes. Albeit it has been debated for long, the stabilization of the transition state (TS) is the origin of enzymatic catalysis [7]. The modes and mechanisms of how this is actually achieved however, remain to be clarified [8,9]. Various factors, such as proximity effects [10], acid–base catalysis, near attack conformation [11], strain [12], dynamics [13], desolvation [14] etc. contribute to lowering the activation barrier as compared to solution reactions. The individual effect of these factors is moderate and results in a rate acceleration $< 10^4$ fold. The only factor with major impact on catalysis is the electrostatic preorganization [15••], which can provide 10^7 to 10^{10} fold rate acceleration [16]. On the basis of the Marcus theory electrostatic preorganization can be quantified by the reorganization energy (λ) [17]. This expresses the work of the protein while it responds to changing charge distribution of the reactant along the reaction pathway (Figure 1). Although reorganization energy is the concerted effect of all enzyme dipoles, group contributions could be approximated (see Box 1).

Current design approaches aim to maximize the binding energy of the TS, but do not evaluate the free energy profile of the catalyzed reaction [18]. Thus response of the enzymatic environment to changes in charge distribution from ground state to TS is not correctly represented. Furthermore, steric strain is ignored, if significant deformations between the ground and TS geometries occur. All these effects are critical for the energetics of the reaction and are influenced by the interplay between the active site groups and the enzymatic environment. Hence considering only key interactions in the TS can result in different mechanism in the design and the real enzyme. Catalytic antibodies might provide a misleading impression that a few residues, which contact or located in the proximity of the reactants are sufficient for catalytic activity [19]. Indeed, the efficiencies of enzyme designs with complex scaffolds are comparable that of simple models [20•] or even re-engineered cavities [21]. This suggests that design strategies mostly optimize *proximity* or *medium* effects, which can be exerted by simply

Figure 1

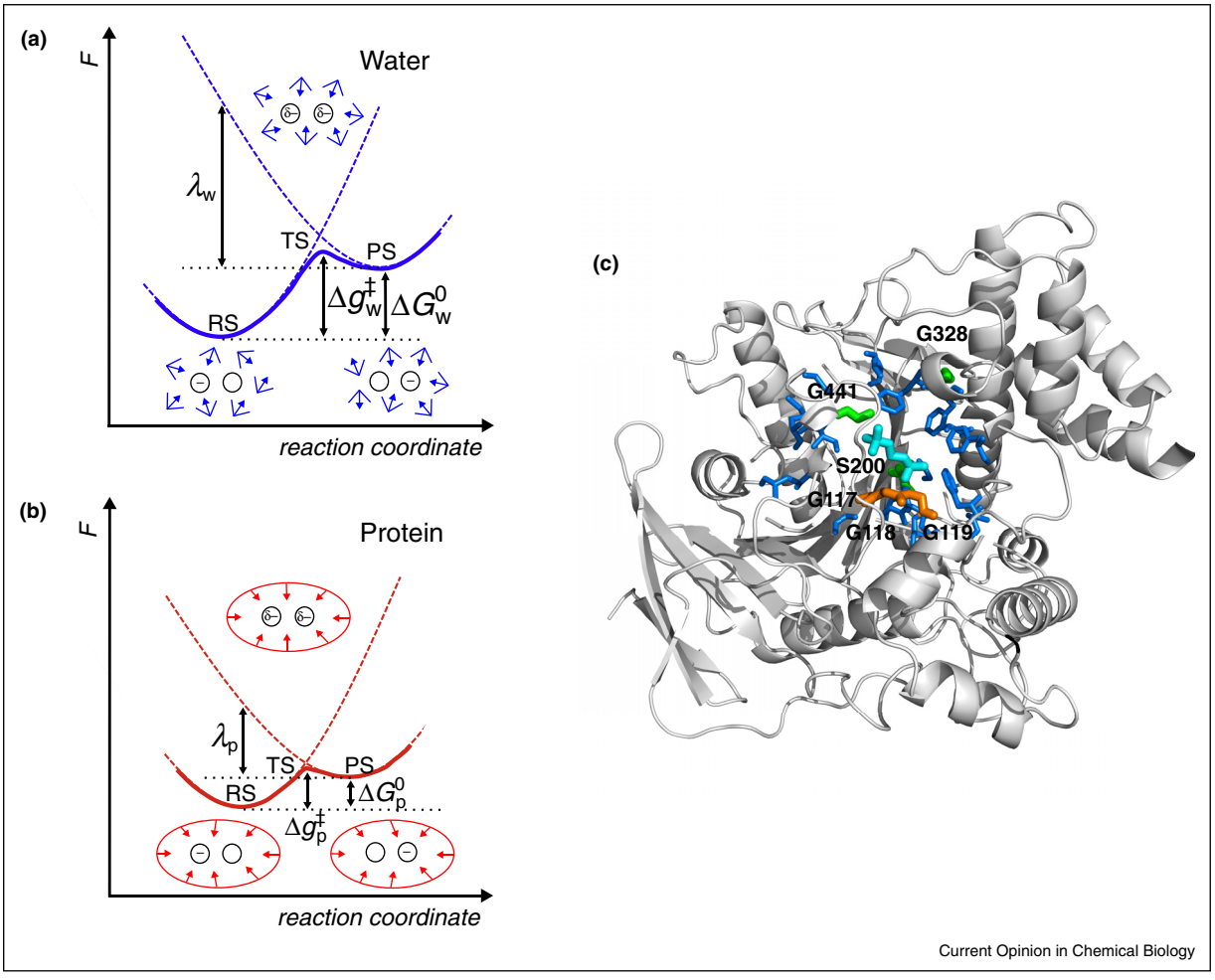

Illustration of the reorganization effect. General scheme of the adiabatic (solid lines) and the diabatic (dashed lines) free energy profiles as functions of a hypothetical reaction coordinate in **(a)** water solution and **(b)** enzymatic environment. The relationship between the activation barrier ($\Delta g^{\ddagger}$), reaction free energy (ΔG°) and reorganization energy (λ) is shown. Schematic pictures at reactant (RS), transition (TS) and product (PS) states represent that reorganization of dipoles is considerably smaller in protein than in solution. **(c)** In acetylcholinesterase (PDB: 1ace) reduction in reorganization energy accounts for 10 kcal/mol out of 15 kcal/mol decrease in the activation barrier [16]. This is mostly due to main chain dipoles of Gly117, Gly118 and Gly119 (orange), which establish hydrogen bonds with the substrate (cyan). Other residues with favorable contributions to catalysis are displayed by green. Although the active site mostly consists of hydrophobic residues (blue), desolvation is destabilizing for the TS.

changing the macroscopic dielectric properties of the system. Activities of enzyme designs are also lowered by structural instabilities (floppiness) [22,23]. Inclusion of flexibility [24] or molecular dynamics (MD) thus significantly improves the efficiency of computed variants [25,26,27•] (see below).

Here we overview the basic concepts, which are implemented in computer-aided enzyme design and assess their performance in directed evolution. We find that electrostatic preorganization is significantly optimized in laboratory as it was quantified in case of KE07 Kemp eliminase [28•]. We exemplify how contributions to reorganization energy could be exploited for screening. We propose that reorganization energy is a missing key catalytic factor in computational design, incorporation of which can be a promising approach to yield highly evolvable enzyme variants.

Design strategies

Computer-aided enzyme design is comprised of three main steps [29]: (i) determination of the TS geometry and optimal arrangement of the key functional groups (theozyme) [30]; (ii) scaffold selection and optimization of the active site environment; (iii) ranking the candidates. De novo design normally utilizes three to four functional groups for catalysis [18] as more complex theozymes can be prohibitory in scaffold selection.