Solvent Entropy Contributions to Catalytic Activity in Designed and Optimized Kemp Eliminases

Supplementary Material

Saurabh Belsare[1†], Viren Pattni[7†], Matthias Heyden[7*], Teresa Head-Gordon[1-6*]
[1]The UC Berkeley-UCSF Graduate Program in Bioengineering
[2]Kenneth S. Pitzer Center for Theoretical Chemistry, [3]Department of Chemistry, [4]Department of Bioengineering, [5]Department of Chemical and Biomolecular Engineering, University of California, [6]*Chemical Sciences Division, Lawrence Berkeley National Labs*
Berkeley, CA, 94720

[7]Max-Planck-Institut fur Kohlenforschung Mülheim an der Ruhr, Germany

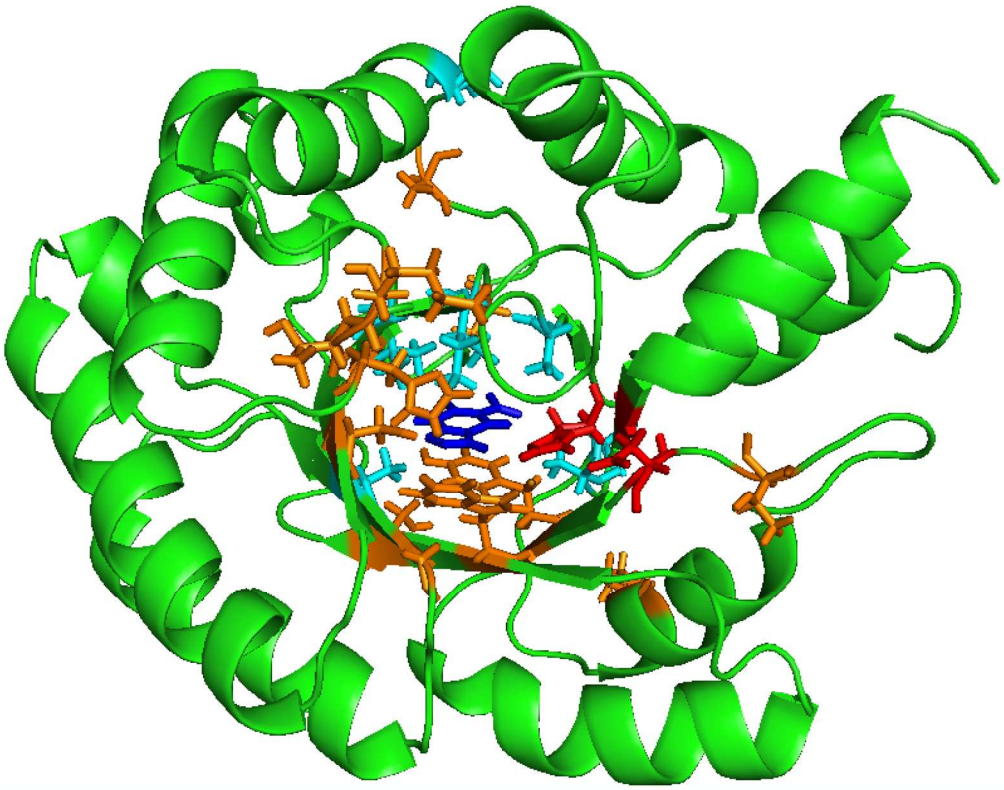

Figure S1. *Kemp Eliminase KE70 enzyme*. The catalytic residues (red) and additional stabilizing residues (cyan) to create the designed KE07 enzyme, and the corresponding mutations introduced by laboratory directed evolution (orange) for the best evolved variant R6. The ligand is shown in dark blue.

[†]authors contributed equally
[*]corresponding authors: heyden@kofo.mpg.de, thg@berkeley.edu

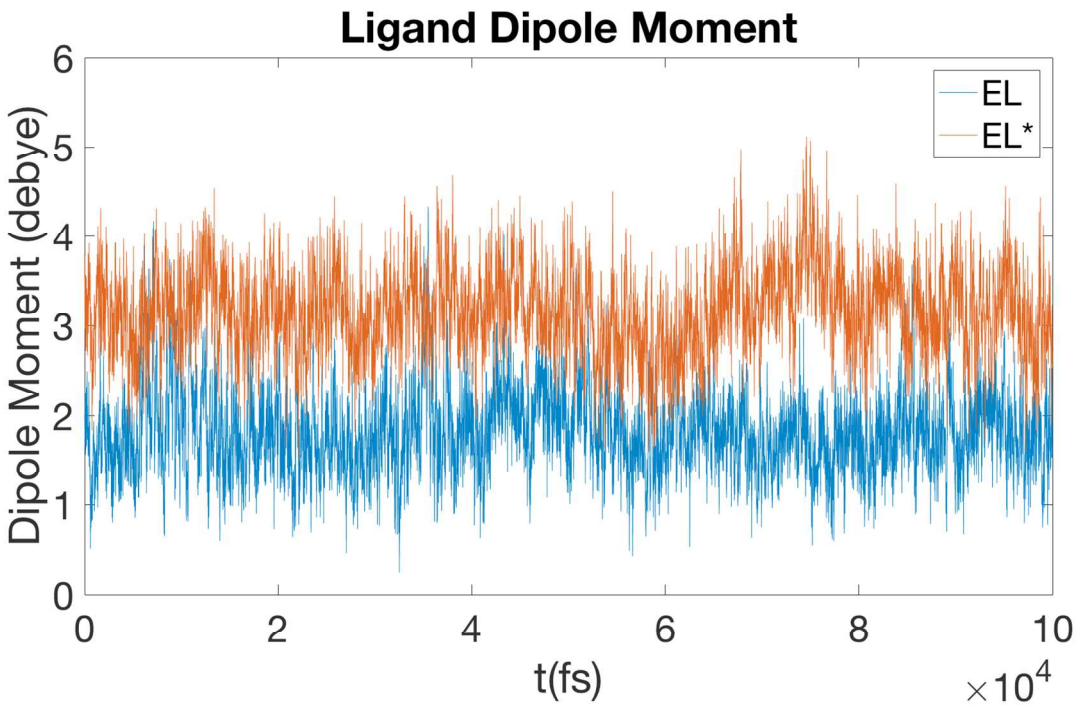

Figure S2. *Simulated molecular dipole moment of the nitrobenzisoxazole substrate in the EL and EL* states*. The average molecular dipole moment in the EL state is 1.81(0.47) D and for the EL* state 3.16(0.48) D.

Table S1. *Laboratory directed evolution mutations for KE07.* Amino acid and chemical changes are examined for the 8 positions between the designed and best evolved (R7) enzyme variant.

Residue Number	**Original residue**	**Mutated residue**	**Chemical change**
7	Ile	Asp	Hydrophobic →Polar Acidic
12	Val	Met	No change
77	Phe	Ile	No change
102	Ile	Phe	No change
146	Lys	Thr	Polar Basic → Polar Uncharged
202	Gly	Arg	Hydrophobic →Polar Basic
224	Asn	Asp	Polar uncharged →Polar Acidic
229	Phe	Ser	Hydrophobic →Polar Uncharged

Table S2. *Laboratory directed evolution mutations for KE70.* Amino acid and chemical changes are examined for the 12 positions between the designed and best evolved (R6) enzyme variant.

Residue Number	Original Residue	Mutated Residue	Chemical Change
29	Lys	Asn	Polar Basic→Polar Uncharged
43	Thr	Asn	No change
48	Tyr	Phe	Polar Uncharged→Hydrophobic
72	Trp	Cys	Hydrophobic →Polar Uncharged
74	Ser	Gly	Polar Uncharged → Hydrophobic
101	Gly	Ser	Hydrophobic → Polar Uncharged
138	Ser	Ala	Polar Uncharged → Hydrophobic
166	His	Asn	Polar Basic → Polar Uncharged
178	Ala	Ser	Hydrophobic → Polar Uncharged
197	Lys	Asn	Polar Basic → Polar Uncharged
198	Thr	Ile	Polar Uncharged → Hydrophobic
204	Ala	Val	No change