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Christopher Reynolds, Christopher J Illingworth, Kevin E Parkes, Chris R Snell, Vicent Moliner, Sergio Marti

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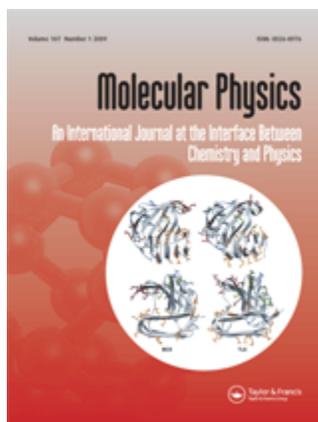
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The effect of MM polarization on the QM/MM transition state stabilization: application to chorismate mutase

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Complete List of Authors:	Reynolds, Christopher; University of Essex, Biological Sciences Illingworth, Christopher; UIniversity of Essex, Biological Sciences Parkes, Kevin; Medivir (UK) Snell, Chris; Medivir (UK) Moliner, vicent; Universitat Jaume I Marti, Sergio; Universitat Jaume I
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3 **The effect of MM polarization on the QM/MM**
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5 **transition state stabilization: application to**
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8 **chorismate mutase**
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17 C.J. R. ILLINGWORTH¹, K. E. PARKES², C. R. SNELL², S. MARTI³, V.
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19 MOLINER³, C. A. REYNOLDS^{1,*}
20

21
22
23 ¹Department of Biological Sciences, University of Essex, Wivenhoe Park,
24
25 Colchester, CO4 3SQ, United Kingdom

26
27 ²Medivir UK Ltd., Chesterford Research Park, Little Chesterford, Essex CB10 1XL,
28
29 United Kingdom

30
31 ³Departament de Química Física i Analítica, Universitat Jaume I, Box 224, Castellón,
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33 Spain.
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Abstract

Hybrid quantum mechanics / molecular mechanics (QM/MM) calculations provide a mechanism for studying enzyme catalysed reactions at the molecular level. Here, through applications on the chorismate to prephenate rearrangement within the enzyme chorismate mutase, we have demonstrated the feasibility of including MM polarization into these calculations using the method of induced charges. MM polarization is shown to be a short-range effect, such that 80% of the energy of MM polarization occurs within a 5 Å residue-based cut-off of the substrate. MM polarization was shown to have a greater magnitude within the enzyme catalysed reaction than in the aqueous reaction, indicating that MM polarization may in principle have a significant effect on enzyme rate enhancement and mechanism. In both the enzyme and the aqueous case, the percentage contribution of MM polarization to the total stabilization energy was towards the upper end of the expected value. For the specific structures studied here, MM polarization lowered the energy barrier for the aqueous reaction, but the calculated contribution of MM polarization to both the reactant and transition structure stability were similar.

Keywords: QM/MM, induced charge, polarization, chorismate mutase, transition state stabilization

1. Introduction

Transition state stabilization has been proposed as a major driving force behind enzyme rate enhancement [1] and since the transition state is believed to bind more tightly than the substrate, the use of transition state analogues has become a common strategy in rational drug design [2–7]. Here we propose to investigate the role of enzyme polarization on this transition state stabilization using hybrid quantum mechanical / molecular mechanics (QM/MM) methods with polarizable MM charges [8].

For particular reactions, the origin of enzyme rate enhancement may still be a matter of debate, and this is the case for the Claisen rearrangement of chorismate to prephanate. This reaction within the enzyme chorismate mutase has become the focus of a large number of hybrid quantum mechanical/molecular mechanics (QM/MM) computational studies, partly as an exemplar system for methodology development, because the reaction does not involve any covalent bonding between the enzyme and the substrate, thus avoiding the difficult issue of link atoms. Moreover, the mechanism is believed to occur with the same mechanism in solution, and as such the enzyme has played a central role in recent debates about theories of enzyme catalysis. Here we propose to investigate whether MM polarization, which is currently ignored in most QM/MM studies, can play a role in transition state stabilization.

Early studies of the system indicated, in line with the ideas of Pauling [1,9], that the catalysis proceeds by a mechanism of transition state stabilisation [10–12], in which the transition state is bound more strongly by the enzyme than the chorismate reactant. More recent controversy has focused around the argument that catalysis is instead due to the preferential binding by the enzyme of a reactive conformation of chorismate, known as a near-attack conformation.[13–15] Through improved computational techniques, more accurate simulations of the Claisen rearrangement have been carried out [16–18], leading to estimates

1
2 of the reaction energy barriers that are in close correspondence with experimentally derived
3 values [16,19], and seeming to confirm the importance of transition state stabilisation.
4
5 However, in these models electronic polarization is included in the QM model of the ligand
6 but it is not incorporated into the MM model of the enzyme. Noting the importance of
7 electrostatic effects in this system, [16,20] here we apply the method of induced charges
8 [8,21–23] (IC) to incorporate polarization into a QM/MM(IC) model of the chorismate
9 mutase reaction, with the aim of demonstrating the feasibility of studying the effect of
10 enzyme polarization on transition state stabilization.
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18 2. Methods

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23 Calculations were performed on reactant, product, and transition state structures of *Bacillus*
24 *subtilis* chorismate mutase (BsCM), and of the ligand in aqueous solution, taken from the
25 OH_{in} pathway calculated by Marti et al. [24]. In each case, the QM region was defined to be
26 the substrate. The enzymatic system was modelled using an MM region consisting of all
27 residues with at least one atom within 10 Å of the substrate, in at least one of the three
28 structures, making a total of 1438 MM points in each case. The aqueous solvent was
29 modelled using an MM region with a cut-off chosen to include the 364 water molecules
30 closest to the substrate, this being equivalent to a 10 Å cut-off in the reactant system. This
31 MM region is smaller than in other models of this system. In order to verify that this was
32 sufficient to capture the effects of polarization in the MM region, a series of calculations
33 were carried out measuring the MM polarization in the aqueous reactant system with cut-offs
34 varying from 3 to 11 Å, which were extrapolated to give an estimate of the total MM
35 polarization in an aqueous solvent with no cut-off.
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48 Initial charges for residues in the MM region were derived from the Amber03 [25] forcefield.
49 Gaussian03 [26] was used to carry out a QM optimisation on a water monomer at the
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2 B3LYP/6-31G(d) level of theory [27,28], and Stone's GDMA 1.3 program [29] was used to
3
4 convert the charge distribution into a multipole series, which was then converted into
5
6 potential derived point charges using the multfit methodology [21,22,30–32]. Both the
7
8 protein and the water charges are derived from correlated wavefunctions and therefore
9
10 suitable for polarization calculations [32]. These are potential derived charges in which the
11
12 potential is determined from a distributed multipole analysis up to hexadecapole in a
13
14 spherical shell around the multipole centre. The optimal charges that reproduce this
15
16 potential on the multipole centre and the atoms bonded to it are determined using an
17
18 analytical procedure that avoids the use of a numerical grid [30].

19
20 For each QM/MM system, the wavefunction was calculated using Gaussian03 [26] and
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22 expressed as a distributed multipole analysis using GDMA 1.3 [29]. The field was then
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24 calculated at the MM (enzyme and/or water) atoms from the multipole series using ORIENT
25
26 4.5 [33]. Induced dipoles, $\underline{\mu}$, were calculated for each atom in the MM region, according to
27
28 the equation

$$\underline{\mu} = \alpha \underline{E}$$

29
30 where \underline{E} is the field, and α is the isotropic atomic polarizability, taken from Miller and
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32 Savchik [34]. These dipoles were then expressed as induced charges using the multfit
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34 technology, described above [21,22,30–32]. These new induced charges were added to the
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36 permanent charges to generate a new QM/MM system, and in accordance with previous
37
38 work [8] this process was iterated four times, bringing the system very close to convergence.
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40 Examining the individual point charges, the largest change in the charge on an MM point as
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42 a result of the final iteration of the method was of magnitude 0.000011. In each case,
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44 calculations were performed at the B3LYP/6-31G(d) level of theory. Five energy values
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46 were calculated, labelled E_1 to E_5 (all of which are determined at the geometry of the
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48 complex). E_1 was defined as the energy of the monomer in vacuo. E_2 was defined to be the
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50 first SCF energy from the first SCF cycle of a QM/MM run, namely the energy of the
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2 monomer wavefunction in the presence of the point charges. Thus $E_2 - E_1$ gives the energy of
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4 the electrostatic interaction between the unperturbed wavefunction and the point charges. E_3
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6 was defined as the converged SCF energy for the initial QM/MM run, such that $E_3 - E_2$ is
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8 equal to the polarization energy of the QM system in response to the MM charges. E_4 was
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10 defined as the energy of the converged QM/MM run after one iteration of the classical
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12 polarization process, while E_5 was defined as the energy of the final QM/MM run, after four
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14 iterations of the classical polarization process. Hence the total MM polarization energy is
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16 derived from $E_5 - E_3$. The induced charge polarization energies included the self energy
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18 correction [8].

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20 Barrier heights for the reaction were calculated by comparing the energy values at different
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22 stages in the QM/MM calculation. For example, the height of the barrier to transition state,
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24 incorporating MM polarization, was given by subtracting the value of E_5 from the QM/MM
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26 run on the reactant from the value of E_5 from the QM/MM run on the transition state. The
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28 energy values E_1 to E_5 are tabulated in supporting information. However, the key focus of
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30 this article is not so much the magnitude of the energy barriers but the extent to which they
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32 are effected by MM polarization, which is essentially given by $E_4 - E_3$ (one iteration) and $E_5 -$
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34 E_3 (4 iterations).

36 37 **3. Results and Discussion**

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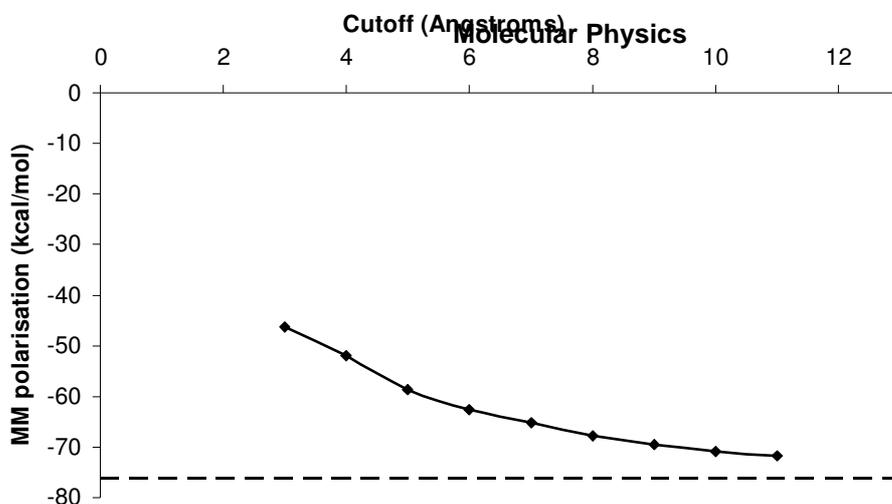


Figure 1 MM polarisation energy ($E_5 - E_3$) as a function of cut-off for the chorismate reactant in an aqueous solution, modelled using explicit waters. The calculated values are shown by the solid diamonds. The dotted line shows the asymptote of the extrapolated curve, that is, the estimated limit of the polarisation energy as the cutoff tends to infinity.

The MM polarisation energies for the reactant in aqueous solution as a function of the cutoff are shown in Figure 1. The MM energy of polarisation with a 10 Å cutoff was calculated to be -70.9 kcal mol⁻¹. The curve of the graph appears to increase asymptotically. Using the assumption that the polarisation energy in a shell of distance r from the ligand is proportional to r^{-3} (see supporting information), an estimate for the asymptote was calculated at -76.2 kcal mol⁻¹. The figure suggests that MM polarisation is primarily a short-range effect, with 77% of the MM polarisation energy occurring on molecules within 5 Å of the ligand, and 93% of the MM polarisation energy being captured by a simulation with a cutoff of 10 Å. This high percentage justifies the use of the 10 Å cutoff. Where different structures for the enzyme at different stages of the reaction are compared, the differences are likely to be close to the site of ligand binding. Thus, the effect on the energy of MM polarization of residues more than 10 Å from the binding site is likely not only to be small, but very similar between the structures. Because of this, the resultant error in the energy of transition state stabilisation caused by neglecting MM polarization of residues more than 10 Å from the ligand is likely to be negligible.

Table 1. Polarization energy values for the enzyme and aqueous systems, at different stages in the reaction pathway. The final column gives the MM polarization energy as a percentage of sum of the electrostatic and polarization energies.

	Energy (kcal mol ⁻¹)		% MM polarization
	QM polarization E ₃ -E ₂	MM polarization E ₅ -E ₃	
Enzyme system			
Reactant	-15.4	-94.9	14.9
Transition state	-14.4	-94.9	17.4
Product	-14.1	-91.2	16.2
Aqueous system			
Reactant	-10.2	-70.9	18.1
Transition state	-9.4	-72.3	19.1
Product	-9.4	-72.8	19.1

Table 1 shows QM and MM polarization energies for the reactants, transition structure and products in both the enzyme and water systems. The total MM polarization energy is relatively large, at about 17±2% of the total electrostatic and polarization interaction, which places this towards the upper end of the expected percentage value [32]. This high percentage is to be expected as the chorismate and prephanate are both highly charged and so will exert a large electric field. The Arg counterions will also exert a large field on their neighbours within the enzyme. Because the numerical MM polarization energy values are indeed large, MM polarization clearly has the potential to influence the rate and mechanism of enzyme catalysed reactions determined using QM/MM methods. Examination of the induced charges

1
2 in the MM system from the enzyme system showed that the largest changes occur on
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4 residues and water molecules close to the carboxylate groups in the ligand, e.g. Arg 90.
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8 Figure 2 shows a graphical representation of the changes in MM charges for the three
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10 systems. Comparison of the figures for reactant, transition state and product systems show
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12 that the changes in the MM charges are roughly constant across the three systems. Residues
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14 and water molecules containing atoms that have large induced charges (i.e. greater than 0.1)
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16 are listed in supporting information.
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18
19 Table 1 shows that the MM polarization of the enzyme in the reactant and transition structure
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21 configurations is also very similar (the MM polarization energies do not differ by more than
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23 4%). The effect of this in this case, is that inclusion of MM polarization has a small effect on
24
25 the energy barrier for the conversion of chorismate to prephenate since polarization increases
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27 the stability of the ligand in both the reactant and transition structure configurations by a
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29 similar amount of the energy. A similar conclusion was reached by Szefczyk et al. on the
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31 basis of quantum mechanical energy decomposition calculations on a limited number of
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33 active site residues [35]. Thus the increase in the barrier from reactant to transition state
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35 when MM polarization is incorporated into the model is only 0.1 kcal mol⁻¹ (once the full
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37 QM energies have been included, see supporting information).
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40 MM polarization in the product configuration is -91.2 kcal mol⁻¹ and this is smaller in
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42 magnitude than the -94.9 kcal mol⁻¹ for the transition structure. As a result of this, MM
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44 polarization lowers the
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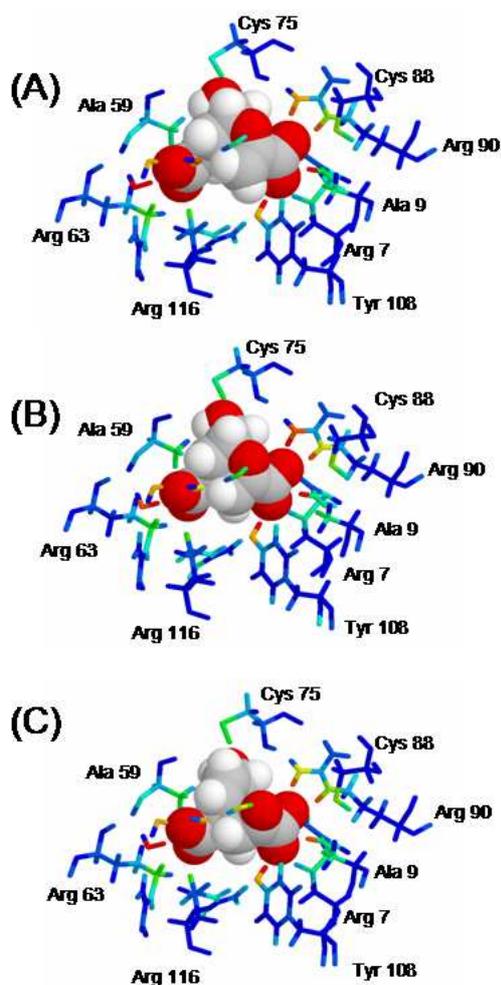


Figure 2. A graphical representation of the induced charges on the enzyme and key water molecules in (A) the reactant configuration, (B) the transition structure and (C) the product configuration. Atoms shown in red have the largest induced charges; atoms shown in blue have the smallest induced charges. The magnitude of the induced charge is equal to the change made to the original charge in the model. The chorismate ligand is shown in a space-filling representation in CPK colours. In each of the 3 cases, the induced charges are shown mapped onto the transition state configuration.

barrier for the reverse reaction by $7.5 \text{ kcal mol}^{-1}$ (once the full QM energies have been included)

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4 For the reaction in aqueous solution, the numerical values of the MM polarization of the
5 water are interesting for two reasons. Firstly, the electronic polarization effects are greater in
6 the enzyme environment than in the water and this suggests that MM polarization could in
7 general have more influence on reactions carried out within enzymes than on reactions
8 carried out in aqueous solution. Secondly, the effect of MM polarization on the environment
9 (i.e. the water) has a greater effect in the transition state ($-72.3 \text{ kcal mol}^{-1}$) than in the
10 reactant configuration ($-70.9 \text{ kcal mol}^{-1}$). These results mean that polarization lowers the
11 energy barrier for the forward reaction by $2.7 \text{ kcal mol}^{-1}$ (once the full QM energies have
12 been included). In this sense then, the calculations predict that MM polarization has an
13 unexpected indirect adverse effect on the enzyme rate enhancement.
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24 In contrast to the reaction in the enzyme, in aqueous reaction, MM polarization has
25 essentially no effect on the reverse reaction.
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29 Here we have demonstrated the feasibility of including classical polarization of MM charges
30 [8] in QM/MM studies of enzyme mechanisms. When considering the enzyme alone, our
31 calculations suggest that MM polarization does not affect the barrier height to the transition
32 state in the enzyme system. An implication of the lower predicted contribution of MM
33 polarization to transition state stabilization in the enzyme compared to water is that if MM
34 polarization plays a lower role than expected, then reactive conformation selection may play
35 a higher role than expected. However, a definitive answer on the impact of MM polarization
36 on the chorismate mutase reaction would require a more careful treatment of the enzyme
37 system, possibly by investigating multiple pathways [16].
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References

1. L. Pauling, Chem. Eng. News **24**, 1375 (1946).
2. L. Gan, M. R. Seyedsayamdost, S. Shuto, A. Matsuda, G. A. Petsko, and L. Hedstrom, Biochemistry **42**, 857 (2003).
3. M. J. Costanzo, S. C. Yabut, H. R. Almond, Jr., P. Andrade-Gordon, T. W. Corcoran, L. De Garavilla, J. A. Kauffman, W. M. Abraham, R. Recacha, D. Chattopadhyay, and B. E. Maryanoff, J Med. Chem. **46**, 3865 (2003).
4. S. P. Dharmasena, D. S. Wimalasena, and K. Wimalasena, Biochemistry **41**, 12414 (2002).
5. G. A. Kicska, P. C. Tyler, G. B. Evans, R. H. Furneaux, V. L. Schramm, and K. Kim, J Biol. Chem. **277**, 3226 (2002).
6. I. Basu, G. Cordovano, I. Das, T. J. Belbin, C. Guha, and V. L. Schramm, J Biol. Chem. **282**, 21477 (2007).
7. R. E. Rigsby, C. L. Rife, K. L. Fillgrove, M. E. Newcomer, and R. N. Armstrong, Biochemistry **43**, 13666 (2004).
8. C. J. Illingworth, S. R. Gooding, P. J. Winn, G. A. Jones, G. G. Ferenczy, and C. A. Reynolds, J. Phys. Chem. A **110**, 6487 (2006).
9. L. Pauling, Nature **161**, 707 (1948).
10. S. Marti, J. Andres, V. Moliner, E. Silla, I. Tunon, J. Bertran, and M. J. Field, J. Amer. Chem. Soc. **123**, 1709 (2001).
11. P. D. Lyne, A. J. Mulholland, and W. G. Richards, J. Amer. Chem. Soc. **117**, 11345 (1995).
12. K. E. Ranaghan, L. Ridder, B. Szefczyk, W. A. Sokalski, J. C. Hermann, and A. J. Mulholland, Mol. Phys. **101**, 2695 (2003).
13. S. Hur, T. C. Bruice, Proc. Natl. Acad. Sci. U. S. A **100**, 12015 (2003).
14. S. Hur, T. C. Bruice, J. Amer. Chem. Soc. **125**, 10540 (2003).
15. S. Hur, T. C. Bruice, J. Amer. Chem. Soc. **125**, 5964 (2003).
16. F. Claeysens, K. E. Ranaghan, F. R. Manby, J. N. Harvey, and A. J. Mulholland, Chem Commun. 5068 (2005).
17. K. E. Ranaghan, L. Ridder, B. Szefczyk, W. A. Sokalski, J. C. Hermann, and A. J. Mulholland, Org. Biomol. Chem **2**, 968 (2004).
18. F. Claeysens, J. N. Harvey, F. R. Manby, R. A. Mata, A. J. Mulholland, K. E. Ranaghan, M. Schutz, S. Thiel, W. Thiel, and H. J. Werner, Angew. Chem. Int. Ed. **45**, 6856 (2006).

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19. P.Kast, M. AsifUllah, and D. Hilvert, *Tetrahedron Letters* **37**, 2691 (1996).
20. M.Barbany, H. Gutierrez-de-Teran, F. Sanz, J. Villa-Freixa, and A. Warshel, *Chembiochem* **4**, 277 (2003).
21. S.R.Gooding, P. J. Winn, R. I. Maurer, G. G. Ferenczy, J. R. Miller, J. E. Harris, D. V. Griffiths, and C. A. Reynolds, *J. Comput. Chem.* **21**, 478 (2000).
22. P.J.Winn, G. G. Ferenczy, and C. A. Reynolds, *J. Comput. Chem.* **20**, 704 (1999).
23. J.H.Wu, P. J. Winn, G. G. Ferenczy, and C. A. Reynolds, *Int. J. Quant. Chem.* **73**, 229 (1999).
24. S.Marti, J. Andres, V. Moliner, E. Silla, I. Tunon, and J. Bertran, *J. Phy. Chem. B* **104**, 11308 (2000).
25. Y.Duan, C. Wu, S. Chowdhury, M. C. Lee, G. M. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. M. Wang, and P. Kollman, *J. Comput. Chem.* **24**, 1999 (2003).
26. Frisch MJ, Trucks GW, Shlegel HB, Scuseria GE, Robb MA et al. (2004) Gaussian 03, Revision C.02, version Wallingford CT:
27. A.D.Becke, *J. Chem. Phys.* **98**, 5648 (1993).
28. W.J.Hehre, R. Ditchfield, and J. A. Pople, *J. Chem. Phys.* **56**, 2257 (1972).
29. A.J.Stone, *J. Chem. Theory Comput.* **1**, 1128 (2005).
30. G.G.Ferenczy, *J. Comput. Chem.* **12**, 913 (1991).
31. G.G.Ferenczy, P. J. Winn, and C. A. Reynolds, *J. Phys. Chem. A* **101**, 5446 (1997).
32. P.J.Winn, G. G. Ferenczy, and C. A. Reynolds, *J. Phys. Chem. A* **101**, 5437 (1997).
33. Stone AJ, Dullweber A, Engkvist O, Fraschini E, Modges MP et al. (2002) Orient: a program for studying interactions between molecules, version 4.5, version University of Cambridge, Enquiries to AJ Stone, ajs1@cam.ac.uk. 2006.:
34. K.J.Miller, J. A. Savchik, *J. Amer. Chem. Soc.* **101**, 7206 (1979).
35. B.Szefczyk, F. Claeysens, A. J. Mulholland, and W. A. Sokalski, *Int. J. Quant. Chem.* **107**, 2274 (2007).

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Supporting information for CM paper

The effect of MM polarization on the QM/MM transition state stabilization: application to chorismate mutase

Christopher J. R. Illingworth¹, Kevin E. Parkes², Christopher R. Snell², S. Marti³, Vincent Moliner³, Christopher A. Reynolds^{1,*}

¹Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, United Kingdom, ²Medivir UK Ltd., Chesterford Research Park, Little Chesterford, Essex CB10 1XL, United Kingdom ³Departament de Ciències Experimentals, Universitat Jaume I, Box 224, Castellón, Spain,.

Table S1 Energy values (E1 to E5) for the different systems tested. The measured data gives the raw energy values, from the QM/MM run. The 'point charges' terms are the energy of the MM point charges, considered in isolation. These are subtracted from the measured energy values to give the corrected energy terms, which give the energy of the QM region. All values are in Hartrees.

Enzyme system					
Reactant	E1	E2	E3	E4	E5
Measured	-837.116367	-890.763672	-890.788264	-891.809700	-891.858188
Point charges		-52.561503	-52.561503	-53.288037	-53.328917
Corrected	-837.116367	-838.202169	-838.226761	-838.521662	-838.529271
Transition state					
Reactant	E1	E2	E3	E4	E5
Measured	-837.077742	-891.310861	-891.333852	-892.422144	-892.462504
Point charges		-53.291836	-53.291836	-54.085155	-54.118100
Corrected	-837.077742	-838.019025	-838.042016	-838.336988	-838.344403
Product					
Reactant	E1	E2	E3	E4	E5
Measured	-837.154240	-890.767666	-890.790086	-891.782741	-891.813676
Point charges		-52.538422	-52.538422	-53.246409	-53.274563
Corrected	-837.154240	-838.229243	-838.251663	-838.536332	-838.542113
Water system					
Reactant	E1	E2	E3	E4	E5
Measured	-837.118475	-934.850326	-934.866611	-937.768985	-937.836082
Point charges		-97.237494	-97.237494	-99.919034	-99.981136
Corrected	-837.118475	-837.612832	-837.629117	-837.849952	-837.854946

Transition state					
	E1	E2	E3	E4	E5
Measured	-837.067469	-934.813571	-934.828516	-937.601088	-937.667587
Point charges		-97.274160	-97.274160	-99.821504	-99.883029
Corrected	-837.067469	-837.539411	-837.554356	-837.779584	-837.784558

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Product					
	E1	E2	E3	E4	E5
Measured	-837.154179	-934.850215	-934.865217	-937.656686	-937.725032
Point charges		-97.218959	-97.218959	-99.783683	-99.846802
Corrected	-837.154179	-837.631257	-837.646258	-837.873003	-837.878230

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Table S2 Largest changes in point charges in MM region of reactant system

Residue	Atom	Initial charge	Final charge	Change
Tyr 108	HH	0.358	0.555	0.197
TIP3 86	H	0.372	0.567	0.195
Arg 7	2HH2	0.421	0.609	0.188
TIP3 86	O	-0.744	-0.931	-0.187
Arg 90	2HH1	0.421	0.599	0.179
Arg 90	HE	0.323	0.499	0.176
HOH 105	O	-0.744	-0.918	-0.174
Tyr 108	OU	-0.476	-0.642	-0.166
HOH 105	H	0.372	0.536	0.164
Arg 90	N2	-0.835	-0.998	-0.163
TIP3 70	H	0.372	0.531	0.159
TIP3 70	O	-0.744	-0.903	-0.158
HOH 41	H	0.372	0.524	0.152
HOH 41	O	-0.744	-0.885	-0.141
Arg 7	1HH1	0.421	0.557	0.137
Arg 90	NE	-0.511	-0.638	-0.127
Cys 88	SG	-0.243	-0.119	0.124
Arg 116	1HD	0.058	0.170	0.112
Arg 63	1HD	0.058	0.168	0.111
Arg 63	CD	0.101	-0.008	-0.109

Table S3 Largest changes in point charges in MM region of transition state system

Residue	Atom	Initial charge	Final charge	Change
Tyr 108	HH	0.358	0.552	0.193
TIP3 86	H	0.372	0.561	0.189
Arg 90	2HH1	0.421	0.607	0.187
Arg 7	2HH2	0.421	0.603	0.183
TIP3 86	O	-0.744	-0.922	-0.178
Arg 90	HE	0.323	0.499	0.176
Arg 90	N2	-0.835	-1.002	-0.167
Tyr 108	OU	-0.476	-0.638	-0.162

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3	HOH 105	H	0.372	0.531	0.159
4	HOH 41	H	0.372	0.530	0.158
5	TIP3 70	H	0.372	0.525	0.152
6	TIP3 70	O	-0.744	-0.894	-0.150
7	HOH 41	O	-0.744	-0.894	-0.150
8	HOH 105	O	-0.744	-0.888	-0.144
9	ARG7	1HH1	0.421	0.562	0.141
10	ARG90	NE	-0.511	-0.643	-0.131
11	CYS88	SG	-0.243	-0.121	0.122
12	ARG116	1HD	0.058	0.160	0.102
13	TIP3 51	O	-0.744	-0.845	-0.101
14	ARG63	1HD	0.058	0.158	0.101
15	TYR108	HH	0.358	0.552	0.193
16	TIP3 86	H	0.372	0.561	0.189

Table S4 Largest changes in point charges in MM region of product system

Residue	Atom	Initial charge	Final charge	Change
TIP3 86	H	0.372	0.558	0.185
TYR108	HH	0.358	0.539	0.180
TIP3 86	O	-0.744	-0.918	-0.173
ARG7	2HH2	0.421	0.592	0.171
ARG90	2HH1	0.421	0.588	0.168
ARG90	HE	0.323	0.484	0.160
HOH 105	H	0.372	0.529	0.157
TIP3 70	H	0.372	0.523	0.151
TYR108	OU	-0.476	-0.626	-0.150
HOH 41	H	0.372	0.518	0.146
TIP3 70	O	-0.744	-0.889	-0.144
HOH 105	O	-0.744	-0.885	-0.141
ARG90	N2	-0.835	-0.972	-0.138
ARG7	1HH1	0.421	0.556	0.136
HOH 41	O	-0.744	-0.880	-0.136
TIP3 51	O	-0.744	-0.870	-0.126
CYS88	SG	-0.243	-0.124	0.119
ARG90	NE	-0.511	-0.630	-0.119
ARG116	1HD	0.058	0.158	0.100
ARG63	1HD	0.058	0.157	0.100

Curve estimation

In the reactant water system, an estimate is given for the total MM polarisation energy, if the reactant was in an infinite amount of water. This is given by the following method:

Suppose that the total magnitude of the MM polarisation energy is equal to some limit, L . Dividing space according to its distance from the ligand into regions of thickness 1 \AA , and defining the polarisation energy of the waters in the shell of distance a to $a+1 \text{ \AA}$ from the ligand to be $e(a)$, we have:

$$L = \sum_{a=0}^{\infty} e(a)$$

If the polarization energy $e(a)$ of each shell is defined primarily in terms of charge-dipole interactions, the average strength of an individual interaction will be roughly proportional to a^{-4} , while the volume of the shell is roughly proportional to a^2 . Thus it roughly holds that

$$e(a) \propto a^{-4} * a^2 = a^{-2}$$

If we define

$$E(r) = \sum_{a=0}^{r-1} e(a)$$

we can then say that

$$L = E(r) + \sum_{a=r}^{\infty} e(a)$$

Given the proportionality relation defined for $e(a)$, it then holds roughly that

$$\sum_{a=r}^{\infty} e(a) \propto \sum_{a=r}^{\infty} \frac{1}{a^2}$$

Noting that

$$\frac{1}{a^2} - \frac{1}{a(a+1)} = \frac{1}{a^2(a+1)}$$

we then have the result

$$\sum_{a=r}^{\infty} e(a) \propto \sum_{a=r}^{\infty} \frac{1}{a^2} = \sum_{a=r}^{\infty} \left[\frac{1}{a(a+1)} + \frac{1}{a^2(a+1)} \right] = \sum_{a=r}^{\infty} \frac{1}{a(a+1)} + \sum_{a=r}^{\infty} \frac{1}{a^2(a+1)}$$

Looking at the first sum, we have

$$\sum_{a=r}^{\infty} \frac{1}{a(a+1)} = \sum_{a=r}^{\infty} \left[\frac{1}{a} - \frac{1}{a+1} \right] = \frac{1}{r}$$

since successive terms of the sum cancel out. For the second sum, we have

$$\sum_{a=r}^{\infty} \frac{1}{a^2(a+1)} = \sum_{a=r}^{\infty} \frac{1}{a} \left[\frac{1}{a} - \frac{1}{a+1} \right] < \sum_{a=r}^{\infty} \frac{1}{r} \left[\frac{1}{a} - \frac{1}{a+1} \right] = \frac{1}{r^2}$$

As r increases, this second sum becomes small, so we can say approximately that

$$\sum_{a=r}^{\infty} e(a) \propto \frac{1}{r}$$

Hence, for some constant c_1 , we have the model

$$L = E(r) + \frac{c_1}{r}$$

If, however, the energy $e(a)$ of each shell is defined primarily in terms of dipole-dipole interactions, the average strength of an individual interaction will be roughly proportional to a^{-6} . It would then follow, again roughly, that

$$e(a) \propto a^{-6} * a^2 = a^{-4}$$

$$\sum_{a=r}^{\infty} e(a) \propto \frac{1}{r^3} \text{ and}$$

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$$L = E(r) + \frac{c_2}{r^3} \text{ for some constant } c_2.$$

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Therefore, suppose that for some constant values k and c , the amount of polarisation energy in the volume up to r Ångstroms away from the ligand obeys the function

$$E(r) = L - \frac{c}{r^k} \quad \text{Eq.1}$$

This would imply that

$$E(r+1) - E(r) = \left(L - \frac{c}{(r+1)^k} \right) - \left(L - \frac{c}{r^k} \right) = c \left(\frac{1}{r^k} - \frac{1}{(r+1)^k} \right) \quad \text{Eq.2}$$

By looking at the calculated data, we then attempt to deduce the values of k and c , in order to create a model for the polarisation energy.

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Taking values of r from 8 to 11 Ångstroms gives the following observed differences:

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r	$E(r)$	$E(r)-E(r-1)$
8	-67.73682	-2.571943
9	-69.48578	-1.748963
10	-70.85499	-1.36921
11	-71.73236	-0.877372

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These differences can be compared to model difference values computed using different values of k , for example where k is equal to 1, as follows:

r	$E(r)$	$E(r)-E(r-1)$	Predicted difference	$c(r)$
8	-67.73682	-2.571943	0.017857143	-144.0288241
9	-69.48578	-1.748963	0.013888889	-125.9253718
10	-70.85499	-1.36921	0.011111111	-123.2288624
11	-71.73236	-0.877372	0.009090909	-96.51094997

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Here the predicted difference is equal to $E(r)-E(r-1)$ as given from Eq. 2 above, under the assumption that $c=1$, while $c(r)$ is the observed difference divided by the predicted difference at each r , that is, the value of c in Eq.2 needed to give a correct answer at that r .

By repeating this with different values of k , different sets of values of $c(r)$ can similarly be computed. The goodness of fit of the model is indicated by the consistency of the values $c(r)$ at each value of r – if $c(r)$ was constant that would indicate a perfect fit to the model, while a large variation in $c(r)$ would indicate a poor model fit.

The consistency of the value of $c(r)$ was measured at different values of k by calculating the standard deviation of the $c(r)$ values divided by the mean of the $c(r)$ values at that value of k . For simplicity, k was assumed to be an integer. This gave the following results:

k	stdev[c(r)] / mean[c(r)]
1	0.160008
2	0.059987
3	0.14485
4	0.273271

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As can be seen from the table, k=2 gives the best fit for this model. Given this result, the constant c was defined as the mean of the values c(8) to c(11) already calculated, giving the model:

$$E(r+1) - E(r) = -540.1 \left(\left(\frac{1}{r^2} \right) - \left(\frac{1}{(r+1)^2} \right) \right)$$

$$E(11) = -71.732$$

The limit of this sequence was calculated computationally up to r=10000, giving a limiting value for the energy of $-76.2 \text{ kcal mol}^{-1}$.

This model does not fit directly to either of the two scenarios presented above, but a number of factors need to be taken into account. Firstly, it is primarily a numerical procedure and there is not much data to extrapolate from. Secondly, it ignores higher order terms as they rapidly become small. For example if the energy varied according to $c_1/r + 0.3 c_2/r^3$ then at $r = 11 \text{ \AA}$, the first term is 50 times bigger than the second. It was argued above that the charge - (induced) dipole correction would be proportional to r^{-2} but this would only be true if the magnitude of the changes in the charges was constant – but this is unlikely to be true given the charged chorismate involved and this decrease in induced charge with distance from the chorismate may explain the preference for k=2 rather than k=1 in our model.