Published in final edited form as:

Proteins. 2015 February ; 83(2): 318–330. doi:10.1002/prot.24717.

Methyltransferases do not work by compression, cratic, or desolvation effects, but by electrostatic preorganization

Jeronimo Lameira^{a,b}, B Ram Prasad^a, Zhen T. Chu^a, and Arie Warshel^{a,*}

^aUniversity of Southern California, Department of Chemistry, SGM 418, 3620 McClintock Avenue, Los Angeles, California 90089, United States.

^bFaculdade de Biotecnologia e Laboratório de Planejamento e Desenvolvimento de Fármacos; Universidade Federal do Pará, 66075-110, Belém, PA, Brazil.

Abstract

The enzyme catechol *O*-methyltransferase (COMT) catalyzes the transfer of a methyl group from *S*-adenosylmethionine to dopamine and related catechols. The search for the origin of COMT catalysis has led to different proposals and hypothesis, including the entropic, the NAC and the compression proposals as well as the more reasonable electrostatic idea. Thus it is important to understand the catalytic power of this enzyme and to examine the validity of different proposals and in particular the repeated recent implication of the compression idea. The corresponding analysis should be done by well-defined physically based analysis that involves computations rather than circular interpretations of experimental results. Thus we explore here the origin of the catalytic efficiency of COMT by using the empirical valence bond and the linear response approximation approaches. The results demonstrate that the catalytic effect of COMT is mainly due to electrostatic preorganization effects. It is also shown that the compression, NAC and entropic proposals do not account for the catalytic effect.

Keywords

methyltransferase; compression; NAC; electrostatic; preorganization

1. Introduction

Methyltransferases are an important group of enzymes that play key roles in normal physiology and human diseases.¹ This class of enzymes probably use a common bimolecular nucleophilic substitution (S_N2) methyl transfer mechanism, where *S*-adenosyl-L-methionine (AdoMet or SAM) usually donates a methyl to a nitrogen, oxygen or carbon atoms.² Recent studies on methyltransferases have revealed interesting links to human disease including cancer.³⁻⁴ For example, catechol *O*-methyltransferase (COMT) (a methyltransferase that catalyzes the transfer of a methyl group from *S*-adenosylmethionine to dopamine and related catechol) plays an important role in the metabolism of catecholamine neurotransmitters and catechol estrogens. In addition, COMT plays a key role

* Corresponding author: warshel@usc.edu.

in the extracellular metabolism of dopamine in the central nervous system and has attracted strong interest in regards to its role dopamine-related pathologies particularly Parkinson's disease.^{5,6} The reaction catalyzed by COMT removes dopamine from its signaling pathway and requires the magnesium cation (Mg^{2+}) in the active site of this enzyme.⁷ This reaction is speeded up by a remarkably large factor of about 10^{16} .⁸ In view of the importance of COMT and other methyltransferases it is crucial to understand the molecular origin of their catalytic power.

Our previous simulation studies have clearly indicated that the catalytic effect of enzymes is mainly due to electrostatic preorganization.⁹⁻¹⁰ However, several alternative proposals have also been advanced, including the near attack conformation (NAC),¹¹ the compression hypothesis¹²⁻¹³ and the “cratic” free energy.¹⁴ More specifically, Bruice and coworkers¹⁵ have invoked the NAC concept which implies, when formulated correctly (see Ref. 16), that the enzymes force the reactant state to a reactant state (RS) structure which is closer to the transition state structure than the corresponding RS structure in the reference reaction in water. This effect has been presumed to be the origin of the catalytic effect. The NAC proposal involved major problems in terms of its formulation (see section IV.2) and turned out in all the cases examined to either have minimal effect or to simply reflect the effect of the transition state stabilization (see Ref. 10 and 16 and section IV.2). Kollman and coworkers¹⁴ attributed a major part of the catalytic effect to the “cratic” free energy, which they assumed to be the free energy required to align the reacting groups into a favorable orientation for reaction. Unfortunately the estimate of the cratic entropy and the corresponding catalytic effect involved significant problems, that will be discussed in section IV. 4. Overall as will be shown in this work, the cratic free energy contribution of Ref. 14 appears reflect an inconsistent entropic consideration.

Schowen and coworkers have proposed that enzymes should compress S_N2 transition states, since the secondary kinetic isotopic effect (2° KIE) $k(CH_3)/k(CD_3)$ is larger and inverse for methyl transfer catalyzed by COMT than for reaction uncatalyzed in solution.^{17,18}

Subsequently, Zhang and Klinman proposed that an active site compaction in COMT can explain different values in the catalytic efficiency of the native and mutants,¹³ which is in agreement with Schowen's compression hypothesis and enzyme-supported hydrogen tunneling. Apparently although the compression proposal (which is related to the NAC idea) has major problems, it has been invoked repeatedly.^{17,18,13} We note, however, that computational works^{19,20} have indicated that there is no justification for this hypothesis. At any rate, this work will examine the validity of the compression idea in the case of COMT.

It seems to us that the main requirement, for moving from circular arguments about catalytic effects to having a verifiable proposal, is the ability of reproducing the observed effect by a consistent computational model (that can be of course subjected to careful scrutiny). In this respect, it is encouraging to note the QM/MM simulations of Roca et al²¹ who have proposed that electrostatic preorganization has an important contributions to the rate enhancement in the reaction catalyzed by COMT (see also recent work in Ref. 22). Although this analysis was not done at a sufficiently quantitative level it has provided a reasonable analysis. The corresponding results appear to be in agreement with our general

electrostatic preorganization idea that has been demonstrated in many cases, including the related S_N2 reaction of dehalogenase.¹⁰

The present work evaluates the activation free energy for COMT and its Y68A mutant to establish the origin of the catalytic effect of methyltransferases. It is found that the observed mutation experiments, that were put forward as a support for the NAC, cratic and compression hypothesis, are reproduced by the electrostatic preorganization effect. Considering the fact that any other proposed factors never reproduced such effects consistently, we find the present analysis to serve as an instructive support of the preorganization idea. Furthermore, our analysis provides a critical evolution of the problems associated with the alternative proposal.

II. Methods and Computational Approaches

Since the present study is focused on general mechanism for methyltransferases dependent of SAM as co-substrate, we have selected the COMT to understand the origin of catalytic power of these enzymes. The reaction catalyzed by COMT that will be studied in this work is described in Figure 1 which involves the attack of catecholate O^- on a methyl group bonded to sulfur atom of the SAM (Figure 1) in a direct bimolecular S_N2 process. The TS is almost certainly much less polar than the RS of the ternary enzyme-substrate complex, in which the S atom of SAM bears a positive charge and the catechol substrate should be in the form of the catecholate anion. The active site of COMT in complex with SAM and Mg^{2+} is shown in Figure 2. It should be noted that although the pKa of catechol in solution is 9.9 (and thus one may assume that the catechol binds in the neutral form to COMT), it is more likely that the catechol is deprotonated before the S_N2 reaction. That is, the deprotonated catechol is likely to form a stable salt bridge with Lys144 (Figure 2). This option was in fact confirmed by our PDL/D/S-LRA pKa calculations where it was found that ionizing the catechol in the presence of the positively charged Lys144 is -3 kcal/mol at pH=7. Similarly the energy of proton transfer from the catechol to the Lys144 is around -7 kcal/mol.

The initial coordinates for the simulation were taken from the crystal structure of the human, soluble form of COMT (s-COMT)⁶. In order to evaluate the activation barriers of the methyl transfer reaction for COMT and its Y68A mutant, we have used empirical valence bond (EVB) method.²³ This method, which is described in details elsewhere^{22,24} and in the SI, provides a powerful way of obtaining reliable activation free energies, while taking into account the full protein flexibility and configuration space. All the simulations were carried out by MOLARIS using ENZYMIX force field. The specific details about the setting of the simulation and EVB parameters are given in the Supporting Information. We also used the linear response approximation (LRA) in determining the different free energy contributions and analyzing the origin of the catalytic effect. The LRA approach²⁵ is described in details elsewhere (e.g. Ref. 26) and was applied here by keeping the LRA parameters and changing the residual charges to zero.

III. Results

To explore the origin of the catalytic power of methyltransferases, it is important to have a clear idea about the activation barrier of the reaction in both environments enzymatic and aqueous solution. Swain and Taylor²⁷ have reported experimental values of 30.1 kcal/mol for the activation free energy of the reaction of trimethylsulfonium ion $(\text{CH}_3)_3\text{S}^+$ and a phenolate ion (catO^-) in aqueous solution at 80 °C. Using their reported activation entropy we estimated the effect of temperature change and obtained an estimate of

$(\Delta g_w^\ddagger)_{\text{obs}} = 30.8 \text{ kcal/mol}$ at 25 °C (Table 1). Next we have to consider the free energy costs of bringing the reacting fragment to the same solvent cage. In the case of neutral molecules this about 2.5 kcal/mol.^{10,28} In the present case we have to consider the fact that we have an ion pair and we have to follow the prescription of the SI of Ref. 29 and estimate the ration of the partition function of being in a molar volume and of being in the cage. Since we have a high dielectric in water and since the volume element increases with the square of the distance we find that the cage effect is still significant and around 2.3 kcal/mol. Thus we obtain $\Delta g_{\text{cage}}^\ddagger = 28.5 \text{ kcal/mol}$ for the reaction involving $(\text{CH}_3)_3\text{S}^+$ and catO^- in water. Calibrating EVB model on the observed barrier of $(\text{CH}_3)_3\text{S}^+$ and catO^- we obtained the parameter given in Table S1). Interestingly, using the same EVB parameters (Table S1) for SAM and catO^- in water we obtain $\Delta g_{\text{cage}}^\ddagger = 27.0 \text{ kcal/mol}$ (Table 1). Therefore, one can assume that $\Delta g_w^\ddagger$ for the reference reaction in water is similar using SAM or $(\text{CH}_3)_3\text{S}^+$. The free energy profile for both reference water reactions ($(\text{CH}_3)_3\text{S}^+ \text{catO}^-$ and SAM + catO^-) are provided in the SI (Fig S3).

Recently, Zhang and Klinman have reported kinetic parameters for 3-*O*-Methylation of Dopamine catalyzed by Human s-COMT and some of its mutants.¹³ Taking their reported values of $k_{\text{cat}} = 12.3$ and 0.81 min^{-1} (at 37 °C), for the reaction catalyzed by the native COMT and its Y68A mutant, we obtained (using transition state theory and temperature of 25 °C) $\Delta g_{\text{cat}}^\ddagger = 18.4 \text{ kcal/mol}$ and $\Delta g_{\text{cat}}^\ddagger = 20.0 \text{ kcal/mol}$, respectively. The rate constant for the reaction involving COMT from rat liver and 3,4-dihydroxybenzoic acid (catechol derivative) ($k_{\text{cat}} = 24.0 \text{ min}^{-1}$)³⁰ is similar to that of the native COMT. Therefore, one can assume that the substitution in the catecholate ring does not change drastically the values of k_{cat} and can use the catecholate in place of dopamine to study the effects of mutations reported by Zhang and Klinman.¹³ Clearly, this comparison with experiment is only valid if the yet unmeasured rate constant of the reaction involving COMT and catecholate is similar to the reaction involving COMT and dopamine.

Having a reliable EVB surface, calibrated by using experimental information for the reaction in aqueous solution, allowed us to explore the free energy surface in the COMT without the challenge needs to obtain fully converging QM(ai)/MM. The calculated activation energy for reactions in water and in the native and mutant protein is compared to the corresponding observed values in the Table 1. The free energy profiles for COMT native and its Y68A mutant are presented in the SI (Fig. S3). Encouragingly, the calculated activation free energies for reactions in protein and water are in a reasonable agreement with the corresponding experimental values. Although, the effect of the mutation is overestimated. It

is likely that much more extensive sampling would improve the results for the mutational effect, but this is not the purpose of the present work where we focus on the origin of the main contributions to the mutational effect. However, the reproduction of the overall catalytic effect, more specifically reproducing the trend in the mutational effect cannot tell us if this effect is due to electrostatic preorganization or to other factors. To explore this issue we have calculated the electrostatic energies of the RS and TS in both the protein and water environments. Before we describe the corresponding analysis, we would like to point out that the methyl transfer reaction in COMT bears a neutral charge where we have +1, -1 for RS and +1/2, -1/2 at TS, (Figure 3). In this case the charge distributions change from localized charge at the RS to delocalized charge at the TS. Therefore it is hard for enzyme dipole to solvate the TS more than RS. However, it is even more difficult to obtain sufficient solvation by the solvent dipoles in the reference reaction.

The calculations of the solvation energies of the RS and TS in the protein and in water were performed by the LRA approach and are summarized in Tables 2, S2 and S3 as well as Figure 4. As can be seen from the tables and Figure 4, the protein solvate the RS more than water does, which is inconsistent with the desolvation proposal,³¹ where it is presumed that methyltransferases solvate RS less than water does.

Interestingly, the Y68A mutant solvates the RS more than the native does (Figure 4). This may be assumed to be inconsistent with the effects of the Y68A mutation reported by Zhang and Klinman,¹³ where K_m was found to increase by 4- and 22-fold for AdoMet and dopamine, respectively, upon the Y68A mutation. However, the reported experiments correspond to binding of one of the fragment when the other is already bound. Thus we evaluated the charging energy of each fragment when the other is already bound. The corresponding results (Table S4) seem to follow the trend of the observed K_m .

One key point, that is directly related to the problems with the NAC proposal, has emerged from the LRA calculations described in Table S3 and Fig 4 B. That is, the corresponding results show that in the RS the electrostatic energy decrease strongly upon shortening of the donor acceptor distance in the protein. The implication of this trend will be discussed below.

IV. Discussion

IV.1 Examining and eliminating the NAC and compression proposal

MD simulations and *ab-initio* quantum calculations by Bruice and coworkers suggested that electrostatic interactions of the enzyme with the substrate are stronger in the RS than in the TS.^{15,32} The authors interpreted these findings as evidence that the enzyme does not preferentially stabilize the TS relative to the RS. However, as explained above the issue is the comparison of the relative stabilization in the enzyme and water reactions, where as shown in Figure 4 the stabilization of the TS is larger in the enzyme. Regardless of the electrostatic stabilization problem it was assumed by Bruice and coworkers that enzyme catalysis is associated with the ability of the enzyme to bring the reacting fragments to the so-called near attack conformation (NAC) (e.g. Ref. 11). However, the definition of this proposal was based on selecting the critical distance and angle, where the NAC is supposed to occur, rather than on free energy surfaces that could be related directly to the difference

between $\Delta g_w^\ddagger$ and $\Delta g_{cat}^\ddagger$. That is, the activation free energy can only be defined by the difference between the free energy at the TS and the lowest point at the RS minima, or by the difference between the free energy in the TS and the overall free energy of the RS (see Ref. 32). Thus, selecting an arbitrary point along the reaction coordinate as a reference for the evaluation of the activation energy cannot give unique results. In fact, early forms of the NAC proposal constitute the reactant state destabilization (RSD) idea (Figure 6a) with all of its problems (see Ref. 9). Furthermore, the NAC proposal suffers from being ill defined in several other crucial points (see Ref. 16). Nevertheless, despite these problems a reasonable definition of what *must* be meant by the NAC proposal has eventually emerged and it is summarized in Figure 5b and Figure 6. These figures basically represent the current picture offered by the NAC proponents,^{16,34} which is now focused on $\langle R \rangle_{RS}^{enzyme}$ and $\langle R \rangle_{RS}^{water}$, where R is the solute contribution to the reaction coordinate. That is, if we evaluate $\langle R \rangle_{RS}^{enzyme}$ we may ask how much it would cost to reach $\langle R \rangle_{RS}^{enzyme}$ in water. We may approximate the NAC free energy by:

$$\Delta G_{NAC} \approx \Delta G\left(R=\langle R \rangle_{RS}^{enzyme}\right)_{water} - \Delta G\left(R=\langle R \rangle_{RS}^{water}\right)_{water} \quad (1)$$

where $G(R)_{water}$ is the value of the free energy profile in water at the indicated R . In the above approximation, $\langle R \rangle_{RS}^{enzyme}$ provides a proper definition for the NAC distance.

There are clear cases where it is easy to show that the NAC effect does not contribute significantly to catalysis.³⁵ However, in order to clarify our perspective we will intentionally take the COMT case where the NAC effect might seem to be very significant. A superficial examination of the results of different studies may suggest that we have here a case of RSD. For example, the MD studies of Hur and Bruice,^{34,36} indicated that the enzyme helps in bringing the reacting atoms of the substrate to typical short distances that are rarely attained in water. Thus they considered this NAC effect as the major reason for the catalytic power of COMT. Before examining whether the NAC represents a genuine reason for catalysis or if it merely reflects the result of electrostatic transition state stabilization. We performed similar analysis which is summarized in Figure S4. The figure shows the distribution of the values of the CO distance and SCO angle at the RS and TS in COMT and water. Apparently, the distribution of the CO distance and SCO angle show larger amplitude of movement in the RS in solution than in enzyme, while the distribution of CO distance for TS in solution and COMT is largely overlapping. In addition, the SCO angle is closer to linearity in both native and mutant than in solution. Although, this change in equilibrium distance and angle can be considered by some as a NAC effect, it is important to see if this structural change is simply the *result* rather than the *reason* of the catalytic effect. In order to analyze the above issue we turn back to the LRA calculation of Table 2 and Figure 4 that evaluate the binding free energy for RS and TS of the reaction in COMT and its Y68A mutant. As seen from Figure 4B and Table S3, while the electrostatic energy in water increases to a small extent upon decrease of the distance from around 6 to 4.5 Å, the electrostatic energy in the enzyme decreases very strongly. This means that moving to the ES structure that maximizes the polar preorganization of the TS also leads to an electrostatic stabilization of the RS ($\langle R \rangle_{RS}^{enzyme}$), which is smaller, however, than the TS stabilization. Although the decrease in

equilibrium distance can be called a NAC effect, it is simply the *result* rather than the *reason* of the catalytic effect as described above. That is, the distance is reduced since the electrostatic preorganization that is aimed at stabilizing the TS also leads to a larger stabilization of the RS in a distance that is shorter than the corresponding distance in water. This is, however, not an effect of some steric compression force.

In view of the above discussion of the NAC effect for moving from water to the enzyme we can turn to the presumed role of the NAC (compression) effect in determining the differences between the native COMT and its Y68A mutant. Here we note that the difference in the catalytic effect is almost entirely due to the difference in the electrostatic contribution to the activation barrier (see Table 2 and Figure 4). Here the electrostatic stabilization of the RS in the mutant is larger than in the native enzyme and this leads to a larger activation barrier in the mutant. Of course, this effect has very little to do with any NAC effect.

IV.2 Examining the compression idea

As stated in the introduction, mutational effects in COMT have been used to promote the compression idea.¹³ Originally it was proposed by Schowen and co-workers that have proposed a distance reduction of 0.03-0.29 Å between the donor and acceptor atoms in the native enzyme relative to uncatalyzed reaction.¹⁷ More recently it has been argued¹³ that the reduction in catalysis due to the Y68A mutation “proves” that the enzyme acts by using some residues to compress the reacting fragment and thus to presumably destabilize the ground state and leads to catalysis. The compression argument has been based on the observed isotope effect that presumably corresponds to an increase in the donor acceptor distance upon mutation. Here we do not wish to enter into arguments about the validity of the distance change deduced from the isotope effect, although this deduction has been carefully challenged.^{19,37} However, we have problems with almost any catalytic argument that has been based on isotope effects and, of course, other promotions of the compression idea (e.g. see our analysis in Ref. 37). For example, we would like to prevent the reader from being confused by arguments that actually have no clear relationship to catalysis. In particular, regardless if the $S^+ \cdots O^-$ distance (Figure 1) increases or not upon mutation, we do not have here any logical connection to the compression concept, since as shown in the NAC section the distance changes as a result of the electrostatic transition state stabilization and not of any steric compression (see above).

Trying to explore the magnitude of the compression effect by the simulations, we find that the average $S^+ \cdots O^-$ distance (Figure 1) for each frame of the mapping process are reduced, relative to the corresponding distance in water, in both the native COMT the Y68A mutant (Figure S4). This is apparently a result of the electrostatic stabilization discussed above. Now, in the enzymatic environment the compression idea implies¹³ that active site residues, such as Y68 in the case of COMT compress the reacting fragments and thus improves the catalytic efficiency. However, there are no significant differences between $S^+ \cdots O^-$ distance in the native COMT and the Y68A mutant (Fig S4 and Table 3). This indicates that there is no significant active site compression in the native COMT relative to the mutant Y68A that could explain the difference in enzymatic catalysis between the native and mutant enzymes.

One may still suggest that the small change in the angle bending energy is the reason for the change in catalysis and the most effective way to examine this proposal is the restraint release approach.³⁹ However, the structural changes are sufficiently small that we can use a much more direct free energy estimate by evaluating the probability distribution of being at different points in the conformational space. This is done in Figure S5, where we see that the free energy changing the SCO angle from its value in the mutant to the corresponding value in the wt is only 0.5 kcal/mol. The free energy of changing the bond length is even smaller. This means that there are no significant compression effects. In this respect we note that the compression effects must appear in the forces, namely the change in energy of wt upon moving to the mutant structure and not in the energy difference between the minima of the wt and the mutant.

IV.3 Analyzing and eliminating the cratic proposal and Entropic Effects in COMT

Although we addressed above the key questions that were the subjects of this work, it is useful to comments on the entropic proposal. Here we recognize that most of the discussion below is a repetition of our previous analysis (e.g. see Ref. 32) it is important for providing a complete picture. At any rate, the work of Ref. 14 that apparently could not reproduce the catalytic effect of COMT by its QM/MM calculations and thus was forced to use inconsistent considerations (see below) and entropic calculations that included major overestimates based on gas-phase vibrational analysis (see discussion in Ref. 32). This point should be clarified to the reader in order to prevent the reader from assuming that alternative explanations are valid.

Apparently the main conceptual problem in the work of Ref. 14 has been the misunderstanding of the nature of activation entropy and try related cratic entropy. That is, in evaluations enzyme catalysis (e.g. by the EVB method described in the previous section) one compares the activation barrier for a reaction step in an enzyme ($\Delta g_{cat}^\ddagger$) with the barrier for a corresponding process in a solvent cage ($\Delta g_{cage}^\ddagger$) and then considers the activation barrier in water in standard conditions ($\Delta g_w^\ddagger$). In the case of bimolecular enzymatic reaction in which the rate constant k_{cat} for conversion of the enzyme-substrate complex to an enzyme-product complex is rate-limiting $\Delta g_{cat}^\ddagger$ can be written as:

$$\Delta g_{cat}^\ddagger = \Delta g_{enz}^\ddagger - \Delta G_{bind} \quad (2)$$

Where $\Delta g_{enz}^\ddagger$ is the overall activation free energy and G_{bind} is the free energy of binding the reactants to the active site. G_{bind} Includes any free energy associated with the enzymesubstrate interactions and desolvation of reactants or enzyme, along with entropic cost of restricting the translational and rotational freedom of the reactants, therefore the difference between $\Delta g_{cat}^\ddagger$ and $\Delta g_{cage}^\ddagger$ should reveal the most important factors in the enzyme catalysis.

At any rate, for a bimolecular reaction of two small molecules in water, $\Delta g_{cage}^\ddagger$ is related to the overall activation free energy ($\Delta g_w^\ddagger$) by

$$\Delta g_{cage}^\ddagger = \Delta g_w^\ddagger - \Delta G_{V_0 \rightarrow V_c} \quad (3)$$

Where $\delta G_{V_0 \rightarrow V_c}$ is the free energy change for bringing one of the reactants into the solvent cage occupied by the other. (in case of charged fragments we have to use the treatment described in the SI of Ref 29 but the cage correction is still similar to that obtained above and its magnitude will not change our the consideration given below). The volume term reflects the loss of translational entropy when the second reactant is brought from a standard 1 M solution, where the volume of molecule is $V_0 \approx 1600 \text{ \AA}^3$, to an effective concentration of $1/V_c$, where V_c is the volume of the solvent cage:

$$\Delta G_{V_0 \rightarrow V_c} \approx -T \Delta S_{V_0 \rightarrow V_c} \approx k_B T \ln \{V_c/V_0\}. \quad (4)$$

Although V_c can be evaluated for any particular system by MD calculation,⁴⁰ $\delta G_{V_0 \rightarrow V_c}$ for most small molecules is given, to a good approximation, by the “cratic” or “mixing” free for energy,^{41,42}

$$\Delta G_{cratic} = -\beta^{-1} \ln \chi \quad (5)$$

where χ is the mol fraction of solute in the standard state.^{28,40} For a 1 M aqueous solution $\chi \approx 1/55.5$, which gives $\delta G_{cratic} \approx 2.4 \text{ kcal mol}^{-1}$ at 295 K and

$$\Delta g_{cage}^\ddagger = \Delta g_w^\ddagger - 2.4 \left(\text{kcal mol}^{-1} \right) \quad (6)$$

As defined originally by Gurney⁴¹ and as used here, the cratic free energy reflects only the change in the effective concentration of the reactant. It does not convey any information about the particular system. Some authors have used inconsistently the term “cratic free energy” in a different sense to mean the entropic contribution to the total change in free energy associated with binding and orienting a reactant in an enzyme's active site, in some cases including the entropy of solvation or desolvation. In particular Ref. 42 have estimated the cratic free energy δG_{cratic} using the equation:

$$\Delta G_{cratic} = \Delta G_{complex}^* - T \Delta S_{solute} \quad (7)$$

Where $\Delta G_{complex}^*$ is the free energy of complex formation and $-T \Delta S_{solute}$ is the sum of ideal gas, rigid rotor, harmonic-oscillator entropy.

Here it is important to clarify that (a) our ($\Delta g_{cage}^\ddagger$) can be evaluated rigorously by our restraint release approach⁴⁴ and (b) Kollman and coworkers unfortunately completely

misunderstood the fact that our ($\Delta g_{cage}^\ddagger$) does not include any contribution from frizzing the orientation of the reacting fragments or any of our preorganization effect. misunderstanding has resulted from the attempt to justify the idea that entropic effects play a major role in

enzyme catalysis.^{43,45,46} The problem with this idea stem from the little known fact that Page and Jencks⁴⁵ assumed implicitly that forming the TS for a bimolecular reaction, either in solution or in an enzyme, requires the complete loss of three rotational and three translational degrees of freedom. They assumed further that, in an enzymatic reaction, these degrees of freedom are lost during the formation of the enzyme-substrate complex, and that little additional change in entropy occurs as this complex evolves into the TS. In this picture (See Fig. 7) $\Delta S_{cat}^\ddagger \approx 0$ and $\Delta S_w^\ddagger \approx \Delta S_{enz}^\ddagger \approx \Delta S_{bind}$, which is very different that the correct entropic contribution.⁴⁰

To further clarify the above problem it is useful to consider the activation entropy contribution in a solvent cage in water, which can be approximated by:

$$-T\Delta S_{cage}^\ddagger \approx \Delta g_{cage}^\ddagger - \Delta g_{cage}^{\prime\ddagger} \approx \Delta G_{res}^{RS,w} - \Delta G_{res}^{TS,w} \quad (8)$$

Where the free energy difference $\Delta g_{cage}^{\prime\ddagger}$ represents the activation barrier that will be obtained while restraining the solute internal rotations and translations where $\Delta G_{res}^{RS,w}$ and $-\Delta G_{res}^{TS,w}$ are the free-energy costs of imposing artificial restraints in the reactant and TS, respectively (see Figure 8). The free-energy change for imposing the restraints can be evaluated by FEP calculations. Similarly, for the enzymatic reaction (Figure 8b), we have

$$-T\Delta S_{cat}^\ddagger \approx \Delta g_{cat}^\ddagger - \Delta g_{cat}^{\prime\ddagger} \approx \Delta G_{res}^{RS,enz} - \Delta G_{res}^{TS,enz} \quad (9)$$

Using the above equation and the cycle of Figure 7, one finds that the catalytic contribution from the solute entropy is

$$\begin{aligned} -T(\Delta S_w^\ddagger - \Delta S_{cat}^\ddagger) &= \left(\Delta G_{V_0 \rightarrow V_c} - T\Delta S_{cage}^\ddagger \right) - T\Delta S_{cat}^\ddagger \\ &= \left\{ \Delta G_{V_0 \rightarrow V_c} + \left(\Delta G_{res}^{RS,w} - \Delta G_{res}^{TS,w} \right) \right\} - \left(\Delta G_{res}^{RS,enz} - \Delta G_{res}^{TS,enz} \right) \\ &= \left\{ \Delta G_{V_0 \rightarrow V_c} + \left(\Delta G_{res}^{RS,w} - \Delta G_{res}^{RS,enz} \right) \right\} - \left(\Delta G_{res}^{TS,w} - \Delta G_{res}^{TS,enz} \right) \\ &= -T\Delta S_{bind} - \left(\Delta G_{res}^{TS,w} - \Delta G_{res}^{TS,enz} \right) \end{aligned} \quad (10)$$

On the other hand, some works^{14,43,46,47} have adopted part of this treatment to estimate entropic contributions to $\Delta g_{enz}^\ddagger$ for several reactions. For example, it was assumed,⁴⁶ as in the original proposal by Page & Jencks,⁴⁵ that the entropic cost of restraining the substrate in the TS is the same in solution and in the enzyme but that the enzyme pays this cost fully during the substrate binding, assuming that $\Delta S_w^\ddagger = \Delta S_{bind}$ and $\Delta S_{cat}^\ddagger = 0$ (see Figure 8). This work identified $-T\Delta S_{bind}$ with $\Delta G_{V_0 \rightarrow V_c} + \Delta G_{res}^{TS,w}$ and concluded incorrectly that, for the enzymes studied, entropic effects make the dominant contribution to the difference between $\Delta g_{enz}^\ddagger$ and $\Delta g_w^\ddagger$. Unfortunately it was assumed in Refs. 14, 42 and 45 that the contribution from the solute entropy is

$$-T(\Delta S_w^\ddagger - \Delta S_{cat}^\ddagger) \approx -T\Delta S_{bind} \quad (11)$$

On the other hand, the mathematically correct treatment (Eq. 10) used by Strajbl *et al.*⁴⁸ and Villà *et al.*⁴⁰ leads to the additional term $(\Delta G_{res}^{TS,w} - \Delta G_{res}^{TS,enz})$, which is missing from Eq. 11). If the interactions with the enzyme restrict the motions of the reacting species in the TS, as often appears to be the case, then $\Delta G_{res}^{TS,enz}$ (the free-energy cost of applying additional artificial restraints on these motions) will be small. $\Delta G_{res}^{TS,w}$ (the cost of applying the same restraints in solution) will be considerably larger, since water imposes only weak restrictions on the solute motions. The term $(\Delta G_{res}^{TS,w} - \Delta G_{res}^{TS,enz})$ will be thus comparable to $-T(\Delta s_w^\ddagger - \Delta s_{cat}^\ddagger)$. $T S_{bind}$ is small.

However, by far the most problematic result of the incorrect assumption is the fact that in Fig 5 (Fig 3 of Ref. 48, that corresponded to Fig. 5 of Ref. 14 the plateau in water is attributed to entropic effect that destabilize the water cage but actually the entropy in the water cage is much more positive (stabilizing free energy) than in the enzyme RS.

V. Concluding Remarks

This work analyzed and reproduced the catalytic activity of COMT and its Y68A mutant. Table 2 and Figure 4 establish the reduction of the observed activation barriers appears to result mainly from electrostatic effects. In looking at our results we see that while the enzyme “solvates” the RS of the reactive substrate more effectively than the corresponding RS solution, it does so even more effectively for the TS. The electrostatic stabilization of the TS may seem paradoxical because the electrostatic interaction energy of the substrate atoms with their surroundings in an enzyme is usually comparable to the corresponding interaction energy in solution. In fact, one might even have expected to find stronger electrostatic interactions in solution, where the solvent molecules are free to adopt configurations that optimize these interactions. However, one has to keep in mind that the folded enzyme provides a preorganized dipolar environment that is already partially oriented so as to stabilize the charge distribution in the TS. In reactions in water, orienting the polar environment towards the TS charges requires significant reorganization energy. The enzyme pays much of its reorganization energy in advance when the protein folds to its native configuration. The decrease in the activation free energy in the enzyme relative to water is accompanied by a decrease in the solvent reorganization energy.^{9,50} The fact that we account for both the catalytic effect of the native enzyme and the mutational effect by the electrostatic preorganization makes it hard to argue that these effects are due to entropy, NAC or compression effects. This issue was considered above but we will discuss below some of the main points.

The problem with the entropic proposal^{14,43} was considered (and reviewed) in section III.4. It was clarified that this proposal reflects simply an incorrect thermodynamic cycle. For skeptical readers we recommend to see the analysis in Ref. 50. The problems with the NAC proposal¹⁵ were analyzed in section III.2. Furthermore, it was found that the differences between the COMT native and its Y68A in catalytic effect are almost entirely due to the difference in the electrostatic contribution.

Our points on the entropic and NAC proposals were made before.^{16,33} Thus it might be most constructive to focus on the compression idea. The general problems with this compression idea were clarified in Ref. 37) and instead of repeating our previous arguments we can focus on this proposal in the case of COMT.¹³ The basic compression idea in the case of COMT presumes¹³ that active site residues such as Y68 compress the reacting fragments and increases catalysis. Unfortunately, this proposal is based on a non unique analysis of the change in isotope effects upon mutation (see section IV.3) which clearly cannot tell us if the change has anything to do with catalysis as it is not related to the activation barrier in any clear logical way. On the other hand, a well defined computer simulations that reproduce the observed change in catalysis show that the mutation just change the electrostatic free energy. Here it is useful to discuss the arguments presented by Zhang and Klinman¹³ in considering the work of Williams and coworkers.^{19,20} This argument includes the implication that the QM region in the QM/MM calculation might be too small. However, the size of the QM region has nothing to do with the examination of the compressing idea (as long as the atoms involved in the bond deformation are included. The reason is simply the compression effect is completely captured by the surrounding classical force field since it involves many small atomic displacements that are accurately represented by molecular mechanics. The second argument talks on the protein conformational sampling, which seem to be a recurring argument by some workers (see Ref. 51), as if this effect is missing in current consistent simulations. In fact, at least in the EVB simulations we include very extensive sampling and reproducing even temperature dependence of isotope effects⁵³ and we are also able to explore millisecond time scale.⁵² In particular, it is important to mention that while computer simulations actually correlate the structure with energetics and catalysis, the use of isotope effects to explore the compression idea does reflect any direct observation of compression and of course not of the energy of the presumed compression effect. In this, case finding a correlation between the change in catalysis and the isotope effect does not provide any direct proof on the validity of the compression idea.

Another point that can be consider in view of the present work is the desolvation proposal (see Ref. 9). This proposal presumes that enzyme work by moving polar or charged reacting fragments to non-polar environment and thus destabilizing the ground state. This type of general proposal has been shown to be extremely problematic in all the many cases examined computationally.⁹ However, a recent work of Wolfenden and coworkers that provided very useful information on the catalysis of alkyl transfer from SAM to halide ions³¹ suggested that SAM dependent halide alkylating enzymes catalyze their reaction by desolvation mechanism. However, as has been the case with other enzymatic reactions, we found here that in the related case of COMT (Table 2) that the RS is more stable in the enzyme than in water and that thus the desolvation idea does not applies to COMT. The problem with the analysis of Ref. 30 is exactly the same as that that led to the original desolvation idea. That is, observing the experiential fact that reactions in a test tube with polar RS and a test tube with less polar TS are accelerated upon moving from polar to non polar solvents lead in a superficial analysis to the conclusion that this is applicable to enzyme catalysis. However, not only that enzyme active sites are always polar, but as shown in our previous papers^{54,55} they are based on incomplete analysis (see Fig. 6 of Ref. 54) where the energy of changing the environment in moving the RS from a test tube with polar

solvent to non polar solvent should be taken into account. Considering this energy will show that moving to a non polar enzyme will lead to a positive binding energy. Of course, one can propose the use of the binding energy of distanced parts of the substrate, but this proposal has fail in any explicit test (see e.g. the analysis in section 5.6 of Ref. 9). However, the main point is that the polarity of enzyme active site can only be determined by computation (e.g. the LRA calculations of Table 2) and such computations have shown that COMT and other enzymes work by solvation rather than desolvation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants GM-24492 and GM-40283. J. L. thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Programa Ciência sem Fronteira for their financial support. We also would like to thanks the University of Southern California's High Performance Computing for computer time.

References

1. Copeland RA, Solomon ME, Richon VM. Protein methyltransferases as a target class for drug discovery. *Nature Reviews Drug Discovery*. 2009; 8(9):724–732.
2. O'Hagan D, Schmidberger JW. Enzymes that catalyse S(N)2 reaction mechanisms. *Natural Product Reports*. 2010; 27(6):900–918. [PubMed: 20372740]
3. Albert M, Helin K. Histone methyltransferases in cancer. *Seminars in Cell & Developmental Biology*. 2010; 21(2):209–220. [PubMed: 19892027]
4. Wagner T, Jung M. New lysine methyltransferase drug targets in cancer. *Nature Biotechnology*. 2012; 30(7):622–623.
5. Espinoza S, Manago F, Leo D, Sotnikova TD, Gainetdinov RR. Role of Catechol-O-Methyltransferase (COMT)-Dependent Processes in Parkinson's Disease and L-DOPA Treatment. *Cns & Neurological Disorders-Drug Targets*. 2012; 11(3):251–263. [PubMed: 22483291]
6. Rutherford K, Le Trong I, Stenkamp RE, Person VW. Crystal structures of human 108V and 108M catechol O-methyltransferase. *Journal of Molecular Biology*. 2008; 380(1):120–130. [PubMed: 18486144]
7. Axelrod J, Tomchick R. Enzymatic O-methylation of Epinephrine and other Catechols. *Journal of Biological Chemistry*. 1958; 233(3):702–705. [PubMed: 13575440]
8. Mihel IK, J. O, Coward JK, Schowen RL. Deuterium isotope effects and transition state structure in an intramolecular model system for methyl-transfer enzymes. *Journal of the American Chemical Society*. 1979:101.
9. Warshel A, Sharma PK, Kato M, Xiang Y, Liu H, Olsson MHM. Electrostatic basis for enzyme catalysis. *Chemical Reviews*. 2006; 106(8):3210–3235. [PubMed: 16895325]
10. Olsson MHM, Warshel A. Solute solvent dynamics and energetics in enzyme catalysis: The S(N)2 reaction of dehalogenase as a general benchmark. *Journal of the American Chemical Society*. 2004; 126(46):15167–15179. [PubMed: 15548014]
11. Bruice TC. A View at the Millennium: the Efficiency of Enzymatic Catalysis. *Acc Chem Res*. 2002; 35(3):139–148. [PubMed: 11900517]
12. Hegazi MF, Borchardt RT, Schowen RL. Alpha-Deuterium and C-13 isotope effects for methyl-transfer catalyzed by catechol O-methyltransferase -SN2-Like transition state. *Journal of the American Chemical Society*. 1979; 101(15):4359–4365.

13. Zhang J, Klinman JP. Enzymatic Methyl Transfer: Role of an Active Site Residue in Generating Active Site Compaction That Correlates with Catalytic Efficiency. *Journal of the American Chemical Society*. 2011; 133(43):17134–17137. [PubMed: 21958159]
14. Kollman PA, Kuhn B, Donini O, Perakyla M, Stanton R, Bakowies D. Elucidating the nature of enzyme catalysis utilizing a new twist on an old methodology: Quantum mechanical - Free energy calculations on chemical reactions in enzymes and in aqueous solution. *Accounts of Chemical Research*. 2001; 34(1):72–79. [PubMed: 11170358]
15. Lau EY, Bruice TC. Importance of correlated motions in forming highly reactive near attack conformations in catechol O-methyltransferase. *Journal of the American Chemical Society*. 1998; 120(48):12387–12394.
16. Strajbl M, Shurki A, Kato M, Warshel A. Apparent NAC effect in chorismate mutase reflects electrostatic transition state stabilization. *J Am Chem Soc*. 2003; 125(34):10228–10237. [PubMed: 12926945]
17. Rodgers J, Femec DA, Schowen RL. Isotopic mapping of transition-state structural features associated with enzymatic catalysis of methyl transfer. *Journal of the American Chemical Society*. 1982; 104(12):3263–3268.
18. Gray CH, Coward JK, Schowen KB, Schowen RL. alpha.-Deuterium and carbon-13 isotope effects for a simple, intermolecular sulfur-to-oxygen methyl-transfer reaction. Transition-state structures and isotope effects in transmethylation and transalkylation. *Journal of the American Chemical Society*. 1979; 101(15):4351–4358.
19. Kanaan N, Pernia JJR, Williams IH. QM/MM simulations for methyl transfer in solution and catalysed by COMT: ensemble-averaging of kinetic isotope effects. *Chemical Communications*. 2008; (46):6114–6116. [PubMed: 19082090]
20. Soriano A, Castillo R, Christov C, Andres J, Moliner V, Tunon I. Catalysis in glycine N-methyltransferase: Testing the electrostatic stabilization and compression hypothesis. *Biochemistry*. 2006; 45(50):14917–14925. [PubMed: 17154529]
21. Roca M, Marti S, Andres J, Moliner V, Tunon I, Bertran J, Williams IH. Theoretical modeling of enzyme catalytic power: Analysis of “cratic” and electrostatic factors in catechol O-methyltransferase. *Journal of the American Chemical Society*. 2003; 125(25):7726–7737. [PubMed: 12812514]
22. García-Meseguer R, Zinovjev K, Roca M, Ruiz-Pernía JJ, Tuñón I. Linking Electrostatic Effects and Protein Motions in Enzymatic Catalysis. A Theoretical Analysis of Catechol O-Methyltransferase. *The Journal of Physical Chemistry B*. 2014
23. Warshel A, Weiss RM. An empirical valence bond approach for comparing reactions in solutions and in enzymes. *Journal of the American Chemical Society*. 1980; 102(20):6218–6226.
24. Warshel, A. Computer modeling of chemical reactions in enzymes and solutions. Sons, JW., editor. New York: 1991.
25. Lee FS, Chu ZT, Bolger MB, Warshel A. Calculations of Antibody Antigen Interactions - Microscopic and Semimicroscopic Evaluation of the Free-Energies of Binding of Phosphorylcholine Analogs to Mcpc603. *Protein Eng*. 1992; 5(3):215–228. [PubMed: 1409541]
26. Florian J, Goodman MF, Warshel A. Theoretical investigation of the binding free energies and key substrate-recognition components of the replication fidelity of human DNA polymerase beta. *J Phys Chem B*. 2002; 106(22):5739–5753.
27. Swain CG, Taylor LJ. Catalysis by secondary valence forces. *Journal of the American Chemical Society*. 1962; 84(12):2456–&.
28. Warshel A, Parson WW. Dynamics of biochemical and biophysical reactions: insight from computer simulations. *Quarterly reviews of biophysics*. 2001; 34(4):563–679. [PubMed: 11852595]
29. Strajbl M, Shurki A, Warshel A. Converting conformational changes to electrostatic energy in molecular motors: The energetics of ATP synthase. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(25):14834–14839. [PubMed: 14657336]
30. Schultz E, Nissinen E. Inhibition of rat-liver and duodenum soluble catechol-ortho-methyltransferase by a tight-binding OR-462. *Biochemical Pharmacology*. 1989; 38(22):3953–3956. [PubMed: 2597177]

31. Lohman DC, Edwards DR, Wolfenden R. Catalysis by Desolvation: The Catalytic Prowess of SAM-Dependent Halide-Alkylating Enzymes. *Journal of the American Chemical Society*. 2013; 135(39):14473–14475. [PubMed: 24041082]
32. Kahn K, Bruice TC. Transition-State and Ground-State Structures and Their Interaction with the Active-Site Residues in Catechol O-Methyltransferase. *Journal of the American Chemical Society*. 1999; 122(1):46–51.
33. Warshel A, Parson WW. Dynamics of biochemical and biophysical reactions: insight from computer simulations. *Quart Rev Biophys*. 2001; 34:563–670.
34. Hur S, Bruice TC. The mechanism of catalysis of the chorismate to prephenate reaction by the *Escherichia coli* mutase enzyme. *Proc Natl Acad Sci*. 2002; 99:1176–1181. [PubMed: 11818529]
35. Shurki A, Štrajbl M, Villa J, Warshel A. How Much Do Enzymes Really Gain by Restraining Their Reacting Fragments? *J Am Chem Soc*. 2002; 124:4097–4107. [PubMed: 11942849]
36. Hur S, Bruice TC. Enzymes Do What Is Expected (Chalcone Isomerase versus Chorismate Mutase). *J Am Chem Soc*. 2003; 125:1472–1473. [PubMed: 12568595]
37. Ruggiero GD, Williams IH, Roca M, Moliner V, Tunon I. QM/MM determination of kinetic isotope effects for COMT-catalyzed methyl transfer does not support compression hypothesis. *Journal of the American Chemical Society*. 2004; 126(28):8634–8635. [PubMed: 15250699]
38. Kamerlin SCL, Mavri J, Warshel A. Examining the case for the effect of barrier compression on tunneling, vibrationally enhanced catalysis, catalytic entropy and related issues. *Febs Letters*. 2010; 584(13):2759–2766. [PubMed: 20433839]
39. Shurki A, Štrajbl M, Villa J, Warshel A. How much do enzymes really gain by restraining their reacting fragments? *Journal of the American Chemical Society*. 2002; 124(15):4097–4107. [PubMed: 11942849]
40. Villa J, Štrajbl M, Glennon TM, Sham YY, Chu ZT, Warshel A. How important are entropic contributions to enzyme catalysis? *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(22):11899–11904. [PubMed: 11050223]
41. Gurney, RW. *Ionic Processes in Solution*. 1935.
42. Kauzmann W. Some factors in the interpretation of protein denaturation. *AdvProtein Chem*. 1959; 14:1–63.
43. Kuhn B, Kollman PA. QM-FE and molecular dynamics calculations on catechol O-methyltransferase: Free energy of activation in the enzyme and in aqueous solution and regioselectivity of the enzyme-catalyzed reaction. *Journal of the American Chemical Society*. 2000; 122(11):2586–2596.
44. Singh N, Warshel A. Toward Accurate Microscopic Calculation of Solvation Entropies: Extending the Restraint Release Approach to Studies of Solvation Effects. *Journal of Physical Chemistry B*. 2009; 113(20):7372–7382.
45. Page MI, Jencks WP. Entropic Contributions to Rate Accelerations in Enzymic and Intramolecular Reactions and the Chelate Effect. *Proceedings of the National Academy of Sciences*. 1971; 68(8): 1678–1683.
46. Stanton RV, Perakyla M, Bakowies D, Kollman PA. Combined ab initio and free energy calculations to study reactions in enzymes and solution: Amide hydrolysis in trypsin and aqueous solution. *Journal of the American Chemical Society*. 1998; 120(14):3448–3457.
47. Donini O, Darden T, Kollman PA. QM-FE calculations of aliphatic hydrogen abstraction in citrate synthase and in solution: Reproduction of the effect of enzyme catalysis and demonstration that an enolate rather than an enol is formed. *Journal of the American Chemical Society*. 2000; 122(49): 12270–12280.
48. Štrajbl M, Sham YY, Villa J, Chu ZT, Warshel A. Calculations of activation entropies of chemical reactions in solution. *Journal of Physical Chemistry B*. 2000; 104(18):4578–4584.
49. Štrajbl M, Shurki A, Kato M, Warshel A. Apparent NAC effect in chorismate mutase reflects electrostatic transition state stabilization. *Journal of the American Chemical Society*. 2003; 125(34):10228–10237. [PubMed: 12926945]
50. Warshel A. Energetics of enzyme catalysis. *Proceedings of the National Academy of Sciences of the United States of America*. 1978; 75(11):5250–5254. [PubMed: 281676]

51. Singh N, Warshel A. A comprehensive examination of the contributions to the binding entropy of protein-ligand complexes. *Proteins-Structure Function and Bioinformatics*. 2010; 78(7):1724–1735.
52. Kamerlin SCL, Warshel A. At the dawn of the 21st century: Is dynamics the missing link for understanding enzyme catalysis? *Proteins-Structure Function and Bioinformatics*. 2010; 78(6): 1339–1375.
53. Liu H, Warshel A. Origin of the temperature dependence of isotope effects in enzymatic reactions: The case of dihydrofolate reductase. *Journal of Physical Chemistry B*. 2007; 111(27):7852–7861.
54. Warshel A, Aqvist J, Creighton S. Enzyme work by solvation rather than by desolvation. *Proceedings of the National Academy of Sciences of the United States of America*. 1989; 86(15): 5820–5824. [PubMed: 2762299]
55. Warshel A, Strajbl M, Villa J, Florian J. Remarkable rate enhancement of orotidine 5'-monophosphate decarboxylase is due to transition-state stabilization rather than to ground-state destabilization. *Biochemistry*. 2000; 39(48):14728–14738. [PubMed: 11101287]

Figure 1.

A schematic description of the transmethylation reaction catalyzed by COMT (B), where the methyl group is transferred from AdoMet to catecholate and a Mg²⁺ is required in the enzymatic reaction.

Figure 2.
The active site of human native COMT in complex with catechol, SAM and Mg^{2+} .

Figure 3.

A schematic description of the charge distribution during S_N2 reaction catalyzed by COMT.

Figure 4.

(A) The effects of the solvation free energies in the different systems studies. As seen from the figure the solvation energy of the TS is considerably larger in the enzyme than in water, and this is the origin of the catalytic effect of COMT. Note that the solvation energy of RS and TS in COMT Y68A is larger than in the native COMT, however, the difference between RS and TS leads to a larger activation barrier in the mutant. (B) The relative LRA estimates of the electrostatic free energy for a representative start point of RS and TS obtained along the reaction coordinate. Energies in kcal/mol are indicated over the corresponding bars.

Strong constraint of $100 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ was applied to R_1 and R_2 in the charging process, in order to keep the reaction coordinates similar to that found in evaluating the free energy surface profiles. Note that the solvation energy does not include the charge-charge electrostatic energy that is included in the total electrostatic energy.

Figure 5.

A schematic description of the free energy profiles in protein (bold and plain lines) and in water (dashed line) for the limiting cases of RSD and TSS, which are shown in the upper (a) and lower (b) panels, respectively. The figure focuses for simplicity on the profiles for the “chemical part” of the substrate (bold line) and describes in panel (a) the profile for the chemical plus nonreactive part of the substrate. For simplicity, we consider a case where the binding of the nonreactive part is along a coordinate orthogonal to the reaction coordinate (for discussion see Ref. 16). (Adapted from Ref 48).

Figure 6. Defining the NAC effect: (a) The energy diagram used to describe the NAC proposal. (b) Clarifies that the position and nature of the assumed plateau does not change any of the relevant activation barriers so that one simply have a regular TSS situation such in Figure 4B. (Adapted from Ref. 48).

Figure 7.

Analyzing the problematic entropic hypothesis. Apparently Page & Jencks⁴⁵ assumed that the overall entropy of activation in an enzymatic reaction ($\Delta S_{enz}^\ddagger$) is similar to that of the uncatalyzed reaction in solution ($\Delta S_w^\ddagger$). They proposed further that this entropy change occurs mainly when the substrate binds to the enzyme, so that $\Delta S_{bind} \approx \Delta S_w^\ddagger$ and $\Delta S_{cat}^\ddagger = 0$. (Adapted from Ref. 32).

Figure 8.

A procedure for a consistent evaluation of the entropic contributions to the activation barrier of a reaction in solution (*a*) and in an enzyme (*b*). In the states labeled “restrained” artificial constraints are applied to translational and rotational motions of the solute. The energy costs of applying these restraints are calculated by a FEP procedure for the reactant and transition states in solution and in the enzyme±substrate complex. For a system near the minimum of its potential of mean force, the difference between the costs of applying the restraints in a

reactant state and in the corresponding TS gives the entropic contribution to the activation free energy. (Adapted from Ref. 32).

Table 1

Activation free energies for the S_N2 step of the methyl transfer reaction in the Human s-COMT^(a).

	$g^{\ddagger}_{\text{calc}}$ (kcal/mol)	$g^{\ddagger}_{\text{exp}}$ (kcal/mol)
water ((CH ₃) ₃ S ⁺)	31.9	30.8
water cage ((CH ₃) ₃ S ⁺)	28.5	28.5
water cage (SAM)	27.0	-
Native	19.9	18.4
Y68A	24.4	20.0

^(a) The free energy perturbation mapping was performed with 41 frames of 20 ps each, for the movement along the reaction coordinate.

Table 2

The solvation free energies (kcal/mol) of the RS and the TS in the reference reaction in water and the catalytic reaction in human s-COMT^(a).

	<u>water reference</u>		<u>Native</u>		<u>Y68A</u>	
	RS	TS	RS	TS	RS	TS
$\langle U_Q - U_0 \rangle_Q$	-189.70	-64.18	-194.70	-100.2	-201.78	-105.86
$\langle U_Q - U_0 \rangle_0$	-19.31	-67.66	-68.90	-106.26	-72.39	-104.84
G_{solv}	-104.51	-65.92	-131.80	-103.23	-137.08	-105.35

^(a)The energies were obtained through the LRA approach using charged system as state I and uncharged system as State II. Note that the solvation energy reported in Fig 4 does not include the charge -charge interaction that is a part of the total electrostatic energy (see Tables S3 and S4).

Table 3Average distances computed along 20 ps of the EVB simulations.^(a)

	Water RS	Water TS	Native RS	Native TS	Y68A RS	Y68A TS
S-C	1.794	1.930	1.785	1.923	1.784	1.910
C-O	3.704	2.014	2.642	1.984	2.745	1.985
O-S	5.153	3.933	4.25	3.884	4.279	3.873

^(a) For the RS and TS we used the average distances of frame 1 and Frame 21, respectively.