

## Visualization of the Differential Transition State Stabilization within the Active Site Environment

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**Abstract:** Increasing interest in the enzymatic reaction mechanisms and in the nature of catalytic effects in enzymes causes the need of appropriate visualization methods. A new interactive method to investigate catalytic effects using differential transition state stabilization approach (DTSS) [1, 2] is presented. The catalytic properties of the active site of cytidine deaminase (E.C. 3.5.4.5) is visualized in the form of differential electrostatic properties. The visualization was implemented using scripting interface of VMD [3]. Cumulative Atomic Multipole Moments (Camm) [4,5,6] were utilized for efficient yet accurate evaluation of the electrostatic properties. The implementation is efficient enough for interactive presentation of catalytic effects in the active site of the enzyme due to transition state or substrate movement. This system of visualization of DTSS approach can be potentially used to validate hypotheses regarding the catalytic mechanism or to study binding properties of transition state analogues.

**Keywords:** Differential Transition State Stabilization; Catalytic properties; Visualization; Enzymatic catalysis; Molecular electrostatic potential; Molecular Electrostatic field; Multipole moments.

This work is dedicated to prof. Henryk Chojnacki on his 70th anniversary.

## 1 Introduction

The modeling of catalytic properties of enzymes is a subject of active research. Due to the size and complexity of the protein environment and the accuracy required to study the kinetic properties of enzyme catalysts, this type of research requires specialized tools and tends to be rather demanding computationally. In fact, the rational engineering of active site properties falls behind the contemporary experimental techniques, which allow to produce novel catalyst by means of combinatorial synthesis and then screening.

On the other hand, even the „combinatorial design” boils down to the proper selection of mutation sites, which requires the detailed knowledge of the roles of active site residues. Only few ones can be the subject of combinatorial mutation experiment due to the vast number of possible mutants.

It is currently possible to study the details of the nature of interactions within the active site environment by means of interaction energy decomposition analysis [7]. Results from such calculations in most cases support the earlier observations [8,9] that the most important contribution to the catalytic interactions is electrostatic in nature. This observation was confirmed for interatomic distances of  $\sim 2.7$  Å or larger [10], which are optimal for non-bonding interactions. The electrostatic interactions for such distances can be inexpensively and accurately approximated by atomic multipole expansion.

The hypothesis of electrostatic complementarity of the catalytic site, first suggested by Pauling [14], shall be supplemented by chemical kinetics theory by Marcus [11, 12] according to which the driving force of chemical reactions is the dynamic fluctuation of the environment. A proper description of the catalytic effects should take into account at least the electrostatic and dynamic contributions.

Another problem with rational design of novel catalysts is the lack of appropriate visualization tools. There is a number of software packages targeted on analysis and visualization of either *ab initio* or forcefield based results but the former are limited to *a posteriori* analysis of expensive calculations, and the latter are not applicable for studies of chemical reactions. There are some attempts to fill the gap with semiempirical tools e.g. [13] but to our knowledge, lacking are tools that would allow to interactively visualize the catalytic properties of the active site and not only the binding interactions.

In this contribution, we present a method of visualization of catalytic properties of the active site and discuss the application of real-time modeling of the catalytic interactions. The approximate yet efficient evaluation of interaction energies, potential and fields was implemented based on Cumulative Atomic Multipole Moments.

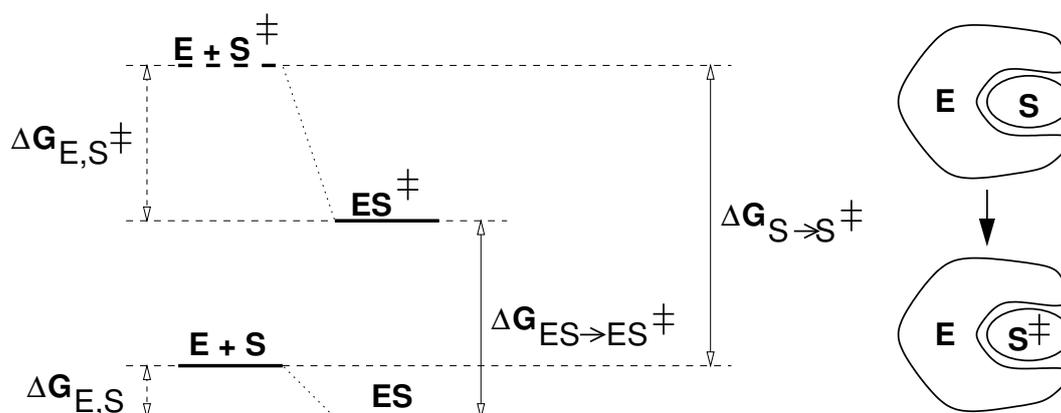


Figure 1: Illustration of the transition state stabilization by the enzyme environment (Eq. 1). Note that  $S^\ddagger$  (transition state) and  $S$  (substrate) are equivalent to  $TS$  and  $SC$ , respectively, used elsewhere (e.g. in [1]).

## 2 Methods

### 2.1 The Differential Transition State Stabilization

Assuming that the presence of the enzyme active site environment lowers the reaction barrier, the barrier lowering  $\Delta^{DTSS}$  can be calculated as the difference between interaction free energies of the enzyme with the transition state ( $\Delta G_{E,S^\ddagger}$ ) and with substrate(s) ( $\Delta G_{E,S}$ , Eq. 1). The vibrational enthalpic contributions mostly cancel in the interaction energies, and the entropic terms are believed to be small [8]. Therefore, an useful approximation of  $\Delta^{DTSS}$  can be obtained using the difference in electronic energies  $\Delta E$ . As discussed in [8, 15] and presented in [10], one can use the electrostatic interaction term in place of  $\Delta E$ , too.

$$\Delta^{DTSS} = \Delta G_{E,S^\ddagger} - \Delta G_{E,S} \approx \Delta E_{E,S^\ddagger} - \Delta E_{E,S} \sim -RT \ln k_{cat} \quad (1)$$

Molecular electrostatic potential (MEP) distribution is best stabilized by a complementary charge distribution. Using the electrostatic approximation, ideal catalytic environment can be devised as the difference of electrostatic potential distributions complementary to  $S^\ddagger$  and  $S$  molecular electrostatic potentials. Same way, the difference between complementary electrostatic gradients (molecular electrostatic fields, MEF) can illustrate the preferable forces stabilizing the transition state  $S^\ddagger$  over  $S$ .

## 2.2 The Cumulative Atomic Multipole Moments

An arbitrary charge distribution could be described as a multipole series expansion on selected points. The expansion of molecular charge distribution can be calculated directly from the density matrix  $P$ :

$$m_a^{klm} = Z_a u_a^k v_a^l w_a^m - \sum_{r \in a} \sum_s^{\text{AO}} P_{rs} \langle r | u^k v^l w^m | s \rangle - \sum_{k'=1}^{k-1} \sum_{l'=1}^{l-1} \sum_{m'=1}^{m-1} \binom{k}{k'} \binom{l}{l'} \binom{m}{m'} u_a^{k-k'} v_a^{l-l'} w_a^{m-m'} m_a^{k'l'm'} \quad (2)$$

where  $u, v, w$  are coordinate components;  $m_a^{klm}$  is the  $(k+l+m)$ -th order multipole moment on atom  $a$  (0-th order – charge, 1-st order –  $x, y, z$  dipole components etc.);  $Z_a$  is nuclear charge;  $\langle r | u^k v^l w^m | s \rangle$  is so-called multipole integral over atomic orbitals  $r, s$ ; and the last term subtracts from  $m_a^{klm}$  the contribution described by lower-order multipole moments [5].

For large but periodic systems the necessary density matrix evaluation can be accomplished using a finite set of properly blocked molecular fragments. The molecular electrostatic charge distribution of polypeptides can be constructed from CAMM multipole moments calculated for separate aminoacids with sufficient accuracy [6].

The molecular electrostatic potential is the simplest measure of the binding or stabilizing properties of a ligand or the catalytic site. It can be derived from the atom-centered CAMM distribution using the formula:

$$V_{\text{CAMM}} = \sum_a [q_a R_a^{-1} + (\vec{\mu}_a \vec{R}_a) R_a^{-3} + (\vec{R}_a \vec{\Theta}_a \vec{R}_a) R_a^{-5} + \vec{R}_a (\vec{R}_a \vec{\Omega}_a \vec{R}_a) R_a^{-7} + \dots]$$

where  $R$  is the distance from the CAMM expansion center  $a$ , and  $q_a, \vec{\mu}_a, \vec{\Theta}_a, \vec{\Omega}_a$  are moments of subsequent orders (charge, dipole, quadrupole etc). For the purposes of visualization of the electrostatic potential, it was calculated on a fixed grid on the border between the active site and the reagents.

The electrostatic field was calculated as the analytical gradient of  $V_{\text{CAMM}}$ .

## 2.3 Visualization

The Visual Molecular Dynamics (VMD) software package provided the capabilities of molecular display and manipulation. VMD is developed at the National Institute of Health by Schulten *et al.* [3] The scripting engine of VMD using the Python language [16] was used to implement the visualization of the electrostatic potential and the fields. The potential is drawn using a color scale and the field vectors are presented as cones which allows for simultaneous presentation of both properties. The differential properties describing the catalytic instead of binding properties are calculated as the respective differences

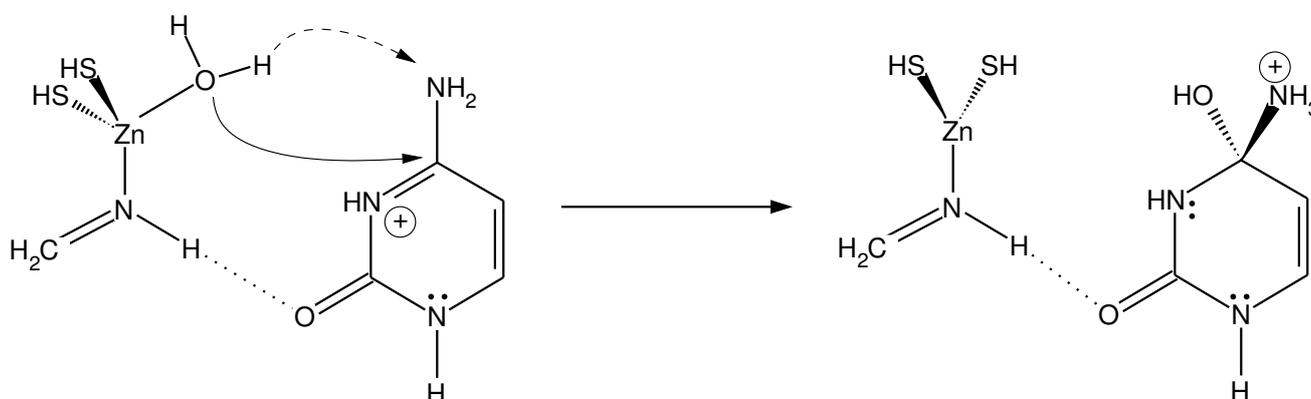


Figure 2: Model of the slowest step of the deamination reaction performed by cytidine deaminase – nucleophilic attack of water molecule on the C4 atom of the N3-protonated cytidine.

on the grid points. The grid was generated on the inner surface of the active site model using a method by Connolly [17] and the point density of  $4/\text{\AA}^2$ .

#### 2.4 The reaction model

A putative reaction pathway and the structures of stationary points was recently published for cytidine deaminase [18]. Cytidine deaminase is an important enzyme of the nucleotide metabolism. It catalyzes the conversion of cytidine to uridine and ammonia. The active site contains a  $\text{Zn}^{+2}$  cation, coordinated by the imidazole group of histidine 102 and by two cysteine sulfides (129 and 132). The substrate water occupies the fourth coordination position [19].

The model structures of the bottleneck step of the deamination pathway are presented in Fig 2. These structures were optimized using Perdew-Wang density functional and LanL2DZ basis set, and verified by means of harmonic vibration analysis. For the purposes of this work, only the cytosine base and the water molecule were included as the model of substrates and transition state. The coordinated zinc cation was treated as part of the catalytic environment. Cumulative Atomic Multipole Moment distributions for the reactants were calculated using B3LYP/6-31G\*\* basis set, for compatibility with the library of CAMM [6] used to assemble the CAMM distribution of the active site model. The B3LYP density matrices  $P$  (Eq. 2) for all molecules were obtained using Gaussian 94 package [20], and the CAMM electrostatic moments were calculated with our software. The active site was represented using all aminoacids within  $8\text{\AA}$  from the cytidine substrate. The coordinates of the protein were taken from Protein Data Bank structure 1CTT and optimized with the transition state and substrate structures using InsightII molecular modeling suite [21].

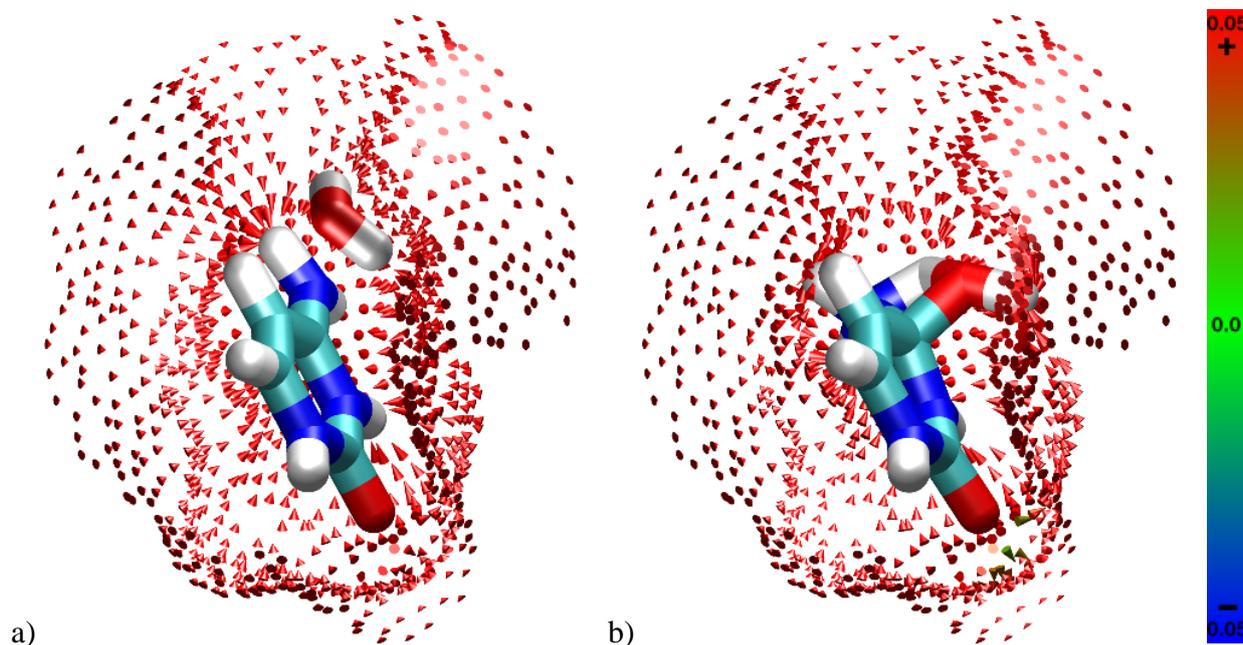


Figure 3: a) Substrate complex (cytosine + H<sub>2</sub>O) within the grid representing the active site cavity. The substrate is protonated (+1) causing highly positive molecular electrostatic potential in the proximity. The molecular electrostatic potential is color-coded and the electrostatic field vectors are represented as cones.

b) Transition state and its electrostatic potential and field on the active site grid.

### 3 Results

#### 3.1 Static properties

The DTSS method was used to compare the real and idealized catalytic properties of the cytidine deaminase active site. The results are presented in Figures 3–4. Figure 3 present the electrostatic potentials and fields around the substrate complex and the active complex, respectively. The fields are relatively weak but the potential is highly positive due to +1 protonation state of the system.

Differential DTSS properties of an idealized catalyst are presented in Fig. 4a. Here one can observe that the efficient catalyst shall present a localized island of positive charge near the C4 carbon of cytosine, where the  $\text{-NH}_2$  group is replaced by a hydroxyl  $\text{-OH}$ . The other regions seem relatively unimportant for the reaction. Fig. 4b depicts the DTSS potential and field generated by the protein environment. It can be observed that the catalytic positive potential near C4 is provided by the zinc cation (gray ball). On the other hand, significant differential field is observed within the active site. It can be interpreted as the force driving the enzyme towards the substrate. This is due to our sign convention; in reality, the enzyme

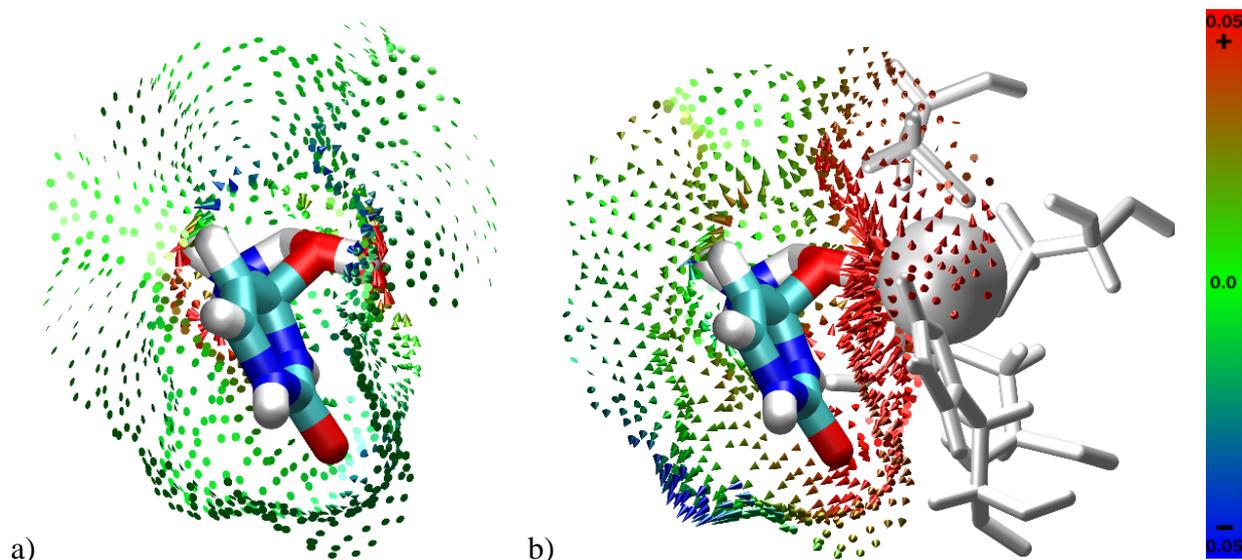


Figure 4: Differential transition state stabilization of the transition state by the cytidine deaminase active site. Only the four catalytic residues are shown but the DTSS properties were calculated using the active site model of 8Å radius. Significant electrostatic field is observed within the proximity of the zinc cation which seem to be responsible for drawing the ligand into the active site. a) the „idealized” catalytic environment – difference in MEP and MEF between  $S^\ddagger$  and S. b) the catalytic environment provided by the enzyme.

is expected to pull the ligands into the active site cavity.

### 3.2 Dynamic visualization

The code calculating differential MEP and MEF distribution is efficient enough for interactive presentation of catalytic effects in the active site of the enzyme due to transition state or substrate movement. The visualization presenting real-time response of the differential potential and fields to the movement of the reactants was prepared as a movie in MPEG-1 format. The movie can be downloaded from [http://www.ccmsi.us/leszczynski/deamination\\_dtss.mpeg](http://www.ccmsi.us/leszczynski/deamination_dtss.mpeg).

The animation shows the insertion of the transition state into the active site of the cytidine deaminase, along with the response of the differential electrostatic potential and field on the active site surface (presented like in Fig. 4). Formation of an island of high differential electrostatic field can be observed near the catalytic water and the C4 atom of cytosine. The sideways movement of the ligand cause rapid growth of the generated potential and field vectors, which is expected for suboptimal placement of the reagents. It can be observed that the DTSS description of the interactions depicts the optimal binding,

according to the least action principle, while retaining the properties of necessary catalytic field.

#### 4 Conclusions

DTSS visualization was implemented using the scripting interface of VMD [3]. The implementation is efficient enough for interactive exploration of active site properties and for design of optimal catalytic environment. The DTSS approach was applied to study the catalytic properties of cytidine deaminase. The distribution of stabilizing potential within the active site environment was found qualitatively equivalent to one predicted from transition state and substrate properties alone.

The DTSS visualization system presented here can be used to validate various possible hypothetical enzyme reaction mechanisms. In addition it could be applied to inspect possible binding modes of inhibitors – transition state analogues. The implementation is efficient enough for interactive presentation of catalytic effects in the active site of the enzyme due to transition state or substrate movement. Therefore, it seems well suited to use for relatively large systems of biological interest.

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