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Quantum chemical modelling in the research of molecular mechanisms of enzymatic catalysis

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Abstract. The advantages and disadvantages of various methods of theoretical modelling of mechanisms of enzymatic reactions based on quantum theory are discussed. Molecular mechanical, quantum mechanical and hybrid approaches are considered. Detailed analysis is performed in relation to a superfamily of enzymes, serine hydrolases, which include cholinesterases playing a crucial role in higher nervous activity. As another example, the results of modelling of enzymatic hydrolysis of nucleoside phosphates are considered. The bibliography includes 177 references.

I. Introduction

Catalysis by enzymes plays a key role in the chemical transformations in living systems.[1–3] It is not surprising that so much prominence is given to studies of the mechanisms of enzymatic catalysis. Numerous experimental works of enzymatic catalysis by methods of chemical kinetics using the whole range of spectral tools are available. Important information is provided by X-ray diffraction analysis of enzymes and their complexes with a variety of ligands contained in the Protein Data Bank (PDB).[4] Using the results of these works, the views on how chemical transformations of substrates in protein matrices occur were formulated for numerous classes of enzymes.

While admitting the undoubted priority of experimental studies in this interdisciplinary field of science on the border of chemistry, biology and physics, in this review, we would like to point out the achievements in the theoretical modelling of mechanisms of enzyme action, which adds computational mathematics to the above list of disciplines involved. Theoretical description of the cleavage and formation of chemical bonds related to changes in the electronic structures of reactants whether this be in the gas phase, in solution or in the protein matrix requires using the quantum theory formalism. Owing to advances in computer technology and long-year efforts of software specialists, numerical solutions of quantum mechanical (QM) equations for electron – nuclear systems and their interpretation in the chemical language have now become rather customary (this area is now commonly called quantum chemistry and that accounts for a large part of computational chemistry[5]). Nevertheless, it took considerable effort to approach complicated processes such as chemical reactions in proteins from the standpoint of quantum chemistry the achievements of which only two or three decades ago had been limited to the description of gas-phase reactions between an atom and a diatomic molecule. The most considerable progress was made upon combination of quantum chemistry methods with another approach to modelling of the properties of macromolecules that does not claim to describe reactions, namely, molecular mechanics (MM). The use of this hybrid approach, the quantum mechanics/molecular mechanics (QM/MM),[6–9] for theoretical investigation of chemical reactions in condensed media proved to be fairly popular.

This paper differs from reviews[10–27] devoted to the achievements of computational chemistry in modelling the enzyme-catalyzed reactions and published after 2000 both in the structure and in the selection of supporting material. In our paper, we first of all draw attention to the possibility of analyzing qualitative aspects of enzyme-catalyzed reac-

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tions by quantum calculations. After discussing the procedural aspects (Sections II, III and IV), we consider two examples of such modelling carried out mainly by QM/MM methods. First, we chose one but a very important and the most abundant class of enzymes, serine hydrolases (Section V). The mechanism of action of these enzymes is described in a number of study books but numerous questions still remain open. Second, we compare the mechanisms of hydrolysis of one class of substrates, namely, nucleotide phosphates, catalyzed by various enzymes; in this area, there are even more unsolved problems (Section VI). In both cases, the application of advanced molecular modelling methods considerably augments the knowledge obtained in experimental research and is useful for planning new experiments and also provides visualization of processes taking place in molecular systems.

II. Molecular mechanical approaches

This review is devoted to the use of quantum mechanical approaches to modelling enzymatic reactions; nevertheless, it appears necessary to consider the achievements and problems of modelling of the properties of biomolecular systems based on the principles of molecular mechanics.[5,28,29] All approaches of this type including, in particular classical molecular dynamics (MD) and molecular docking, are based on representing the potential energy or free energy of a model molecular system by analytical formulae taking into account the energy contributions from the deformation of chemical bonds and bond angles, from changes in the torsion angles, from electrostatic interactions of atoms with the partial charges ascribed to them, and from the dispersion interactions of the chemically non-bonded atoms. The parameters appearing in these relations are called force field parameters; over the decades of application of the molecular mechanical modelling, several force field libraries have formed (the most popular ones for the modelling of the properties of biomolecular systems are AMBER, CHARMM, OPLS and GROMOS); the force fields present there have been selected in different ways and actually do not always provide concordant results.

The most comprehensivle application of modelling by means of force fields is the calculation of equilibrium geometric parameters and classical molecular dynamics of macromolecules including analysis of conformational changes. The key point in the use of MM and MD for solving the problems of enzymatic catalysis is the evaluation of the structures of enzyme-substrate complexes (ES) or possible intermediates and reaction products. It is noteworthy that the structures placed in the PDB contain, as a rule, atom coordinates corrected after computer processing of the primary experimental information in terms of MM methods. Molecular docking techniques,[30–32] which are used to identify the possible ligand (substrate) binding sites of the protein macromolecule, fall in the same category of theoretical approaches. From the computational mathematics standpoint, the problem of structure determination of protein–substrate complexes is a problem of optimization of a large number of parameters (atom coordinates) with respect to the energy minimum. In view of the conformational mobility of proteins, the difficulty of this problem is obvious, and considerable effort was put into its solution.[5,33]

The degree to which the results of molecular mechanical modelling of, in particular, ES structures are considered satisfactory depends on the general objective of the particular study. For qualitative estimates, the MM methods provide a valuable means of visualization; therefore, it is not surprising that programme products of some companies based on molecular mechanics principles are in great demand. It is difficult to overestimate the significance of display visualization of the geometric configurations of complex molecular systems for both educational purposes and for gaining new knowledge. Skilled specialists formulate the views on the mechanisms of possible chemical transformations in the protein considering the database structure obtained after MM processing and other experimental observations.

However, the results of molecular mechanics-based computations that claim to be more accurate should be treated with caution. This statement will be illustrated by considering the modelling of guanosine triphosphate (GTP) hydrolysis with the growth factor protein EF-Tu.[34–39] The mechanism of hydrolysis of nucleotide phosphates, in particular, GTP is considered in more detail in Section VI. Here we will compare the structures of the enzyme–substrate complex (GTP molecule and water inside the protein) calculated by MM and QM/MM methods. The equilibrium geometric configuration of ES determined by quantum theory (QM/MM) calculations[34] is shown in the inset of Fig. 1.

It is noteworthy that the reactive water molecule designated by Wat and circled in the inset of Fig. 1 is oriented by the hydrogen bonds with side chains of residues His85 and Thr62 in the optimal fashion for nucleophilic attack on the P_γ atom of the terminal phosphate group of the GTP molecule. This type of orientation of the reactive water molecule and the distance from the water oxygen atom to P_γ (3.1 Å) is characteristic of the mechanism of enzymatic hydrolysis of nucleoside triphosphates according to which the configuration of the $P_\gamma O_3^-$ group in the transition state formed upon the $P_\gamma - O_{\beta\gamma}$ bond cleavage induced by the protein environment is planar. The bond cleavage is followed by the nucleophilic attack by the reactive water molecule on the P_γ atom with proton transfer up to the formation of inorganic phosphate molecule. In a number of studies discussed in Section IV, it was shown that this scenario is characterized by quite realistic activation barriers.

Figure 1 shows a superposition of the enzyme–substrate complex structures for GTP hydrolysis in the EF-Tu active site determined by the QM/MM method (shown in brown) and MM (shown in blue). The structures were aligned with respect to the γ-phosphate group of the GTP molecule. In the second case, the calculations were performed using the molecular dynamics programme package NAMD[40] with the CHARMM force field.[41] Starting from the ES geometry found by QM/MM, the coordinates of all atoms of the model system were optimized with respect to the energy minimum with the given force field parameters. Then the molecular dynamics trajectory of 4 ns duration was calculated from the atom positions obtained, and at the end of the trajectory, the coordinates of the whole system were again optimized with respect to the energy minimum. This gave a new ES structure consistent with the CHARMM force field interaction potentials.

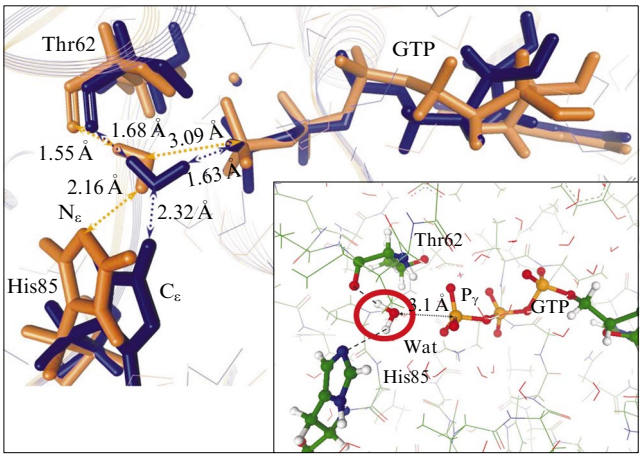


Figure 1. Comparison of the structures of enzyme–substrate complex for GTP hydrolysis in the active site of the protein EF-Tu found by QM/MM (shown in brown) and MM methods (shown in blue).[34, 35]
The inset shows in detail a QM/MM-optimized fragment of the ES (traditional designations are used, namely, green indicates carbon atoms, red stands for oxygen atoms and blue corresponds to nitrogen atoms).

Generally, the structures do not differ much, which is consistent with the common views on the adequacy of molecular dynamics analysis of the conformational transformations in proteins. However, the structure obtained using force fields does not give any idea of the possible scenario of hydrolysis if one analyzes the orientation of the reactive water molecule relative to the GTP γ-phosphate group and the His85 side group. The water oxygen atom does not point to the P_γ atom and the formation of its chemical bond with P_γ, which is necessary for moving towards the reaction product, inorganic phosphate, would require additional energy. Obviously, attempts to model the nucleophilic attack of the water molecule on the γ-phosphate group of GTP starting from this configuration would produce substantially overestimated activation barriers as compared with those obtained for the reaction pathway starting from the QM/MM structure (inset of Fig. 1).

Another example refers to the ES structure for the hydrolysis of penicillin catalyzed by penicillin acylase (PA). This enzyme belongs to the class of serine hydrolases (considered in more detail in Section V) and is important as a catalyst of amide bond hydrolysis in penicillin antibiotics. According to the accepted mechanism of catalysis, PA functions as a hydrolase with N-terminal nucleophile.[42–45] The attack by the O^γ atom of the N-terminal serine (Ser1) residue on the carbonyl carbon atom of the substrate is accompanied by the proton transfer from the hydroxyl group to the own α-amino group. This gives rise to oxyanion, which is stabilized by the interaction with the amide group of Asn241 and the backbone nitrogen atom of Ala69. The residues Asn241 and Ala69 form the oxyanion hole.

The fragment of the ES structure for the hydrolysis of a penicillin molecule (shown in the inset) by penicillin acylase is presented in Fig. 2. The structures were optimized in the QM/MM calculations, on the one hand, and by the MM method with the OPLS-AA force field,[46] on the other hand. We compare the most important distances, namely, those between the O^γ atom of Ser1 and the carbonyl carbon atom of the substrate and between the carbonyl oxygen of the substrate and the nitrogen atoms of the residues Asn241 and Ala69 of the oxyanion hole. The most important observation is that according to quantum theory-based calculations, penicillin is much more tightly incorporated in the protein matrix, in particular, the intermolecular contacts between the substrate and the enzyme are shorter when determined by QM/MM calculations than in the case of force field modelling. Experience in the modelling of the energy reaction profiles indicates that the calculated activation barriers are very sensitive to the initial distances between the reactants in the ES configuration. Consider the publication[47] estimating the activation barriers to reactions of serine hydrolases as functions of the critical distance between the Ser O^γ atom and the His nitrogen atom of the catalytic triad in the enzyme–substrate complex. An increase in this distance by only 0.1–0.2 Å may result in the calculated barrier to increase by 3 kcal mol^{-1} (this is matched by a ~3 orders of magnitude decrease in the predicted rate constant). Thus, rather loose arrangement of the substrate in the protein predicted by the molecular mechanics force field modelling may produce overestimated activation barriers on the reaction path when the modelling is started from the corresponding ES structure.

As a conclusion of this Section, note once again that traditional force field modelling cannot claim to describe the whole reaction path the movement along which is accompanied by cleavage of some chemical bonds and formation of other bonds. The parameters of the molecular mechanics potential functions (force fields) are most often calibrated for particular properties of molecular systems at geometric configurations near the equilibrium, and moving beyond the borders of these regions does not offer prospects of a reasonable reproduction of the energy profile. Perhaps, by additional parameterization of contributions to the potential function, it is possible to extend the scope of

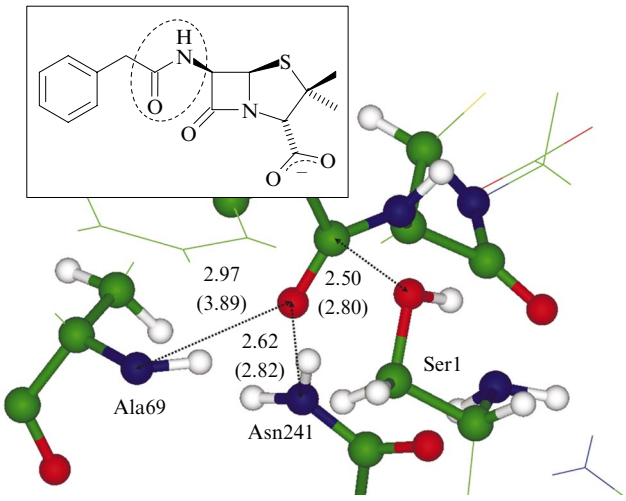


Figure 2. Fragment of the ES structure for the reaction of penicillin catalyzed by penicillin acylase.
Penicillin molecule is shown in the inset; the dashed line circles the area that is shown as the ES in the figure. The values are the distances between heavy atoms (Å) determined in the QM/MM optimization and molecular mechanics calculations with the OPLS-AA force field (in the parentheses) (authors' data).

molecular mechanical methods to describing reactions but there are only few systematic works along this line

III. Cluster models in quantum chemical calculations

In this review, by a molecular cluster, we mean a rather large group of atoms (up to two to three hundred) arranged according to the pattern of an appropriate structure from the PDB. The interatomic interaction energies and forces for the cluster are calculated by quantum chemistry methods using suitable computer software. The calculation is aimed at determining the energy surface cross-sections for the desired reaction in the protein. Obviously, this strategy brings about lots of practical questions of which the most difficult are the following: which model cluster would represent adequately the enzyme active site and what is to be done with the peripheral tails of the molecular groups that constitute the cluster, *i.e.*, should they be omitted or fixed during the geometry optimization for the movement along the presumptive reaction coordinate? One more, equally important group of questions is formed by the proper problems of quantum chemical approaches: whether consideration should be restricted to electron density functional theory (DFT) approximations for calculations of potential energy surfaces (and, correspondingly, what functionals should be chosen) or should more labour-consuming but more theoretically substantiated multiconfigurational approaches be used? which basis sets are better to be used? and other details. This technical aspect of modelling is not widely addressed in this review. Semiempirical quantum chemical calculations based on simplified Hartree–Fock equations (AM1 and PM3 type methods) are not mentioned in the review at all because the reliability of these approaches are difficult to estimate.

Considering the selection of a model cluster in a number of particular applications, there are actually not many options. For example, in the calculation of energy profiles for the reactions of serine hydrolases,[48,49] it appears evident to select a cluster that includes fragments of the side chains of the catalytic triad residues, Ser, His, Asp (Glu), the oxyanion hole and the substrate.† Figure 3 shows a small-size cluster for which the energy profile was calculated along the path from reactants to the first tetrahedral intermediate.[50]

All participants of the acylation step of serine protease reactions have formally been introduced: side-chain functional groups of Ser, His, Asp; a substrate-mimicking molecule with a C–N bond to be cleaved, and two water molecules mimicking the oxyanion hole. In the study cited,[50] no constrains on the model cluster coordinates were imposed in the calculation of the minimum energy path: first, the geometric parameters were fully optimized with respect to the energy minimum, then the reaction coordinate was chosen to be the distance R_{OC} between the Ser oxygen atom and the substrate carbon atom, and upon successive decrease of this distance, the other coordinates were optimized in each step. Surprisingly, this simple (as appears now) model gives a reasonable result — the calculated geometric configurations of the cluster and electron density distribution along the reaction path from the reactants that model the ES to a local minimum that models the tetrahedral intermediate and lies higher in energy predict qualitatively correctly the changes in the active site of serine proteases. Another parameter could be chosen instead of R_{OC} as the reaction coordinate, namely, the R_{HN} distance between the Ser proton and the His nitrogen atom or a linear combination of R_{OC} and R_{HN}, and this would have given a similar result. In a later study,[51] a somewhat larger cluster was constructed: extended models of the Ser, His and Asp side chains were considered, an Asn fragment was taken as the oxyanion hole and a methylacetamide molecule served as the substrate. The key conclusions of these calculations were the same as the earlier estimates for the small cluster.[50]

The problems of this strategy, *i.e.*, the construction of the energy profile of the reaction path by a series of geometry optimizations of the molecular cluster without constrains imposed, for example, on the coordinates of peripheral atoms, are as follows. First, the intermolecular interactions between relatively small molecules (see Fig. 3) that simulate the participants of the reaction in the proteins do not necessarily hold to the mutual orientations that occur in the protein macromolecule with an extended hydrogen bond network providing the optimal substrate arrangement for enzymatic catalysis. During the calculation, although using correct quantum theory equations, of the movement along the presumed reaction coordinate, the minimum energy path would sooner or later lead the system to a region of a deeper minimum that does not match the real structure existing in the protein. Second, the energy paths calculated for molecular clusters produce unrealistically overestimated potential barriers, such as the reported[50,51] values of $\sim$30 kcal mol^{-1}, and this problem cannot be attributed to the use of low-level approximations of quantum chemistry. Reasonable values that are to be two or three times lower still cannot be attained by using the Hartree–Fock method, multiconfigurational methods or DFT or by varying basis sets. There are some publications in which the activation barriers calculated for cluster models of enzymatic reactions (even in very good quantum chemical approximations) are 50–60 kcal mol^{-1}. Third, unlucky combination of circumstances may bring about even a qualitatively incorrect result. In particular, in Ref. 51, according to calculations in one of quantum chemical approximations, the Asp fragment was protonated in the tetrahedral intermediate configuration by means of the His residue, indicating double proton transfer mechanism in serine hydrolases. The higher-level calculations of the QM/MM approach did not support this mechanism.

The problems of quantum chemical cluster models of enzymatic reactions are partially eliminated by either considerable extension of the size of the molecular system or by using a rather simple expedient: it is necessary to fix the coordinates of peripheral atoms in the cluster (for example, the coordinates of C^{α} atoms of amino acid side chains) as in the suitable structure from the PDB and to provide free optimization of the coordinates of species in the local region corresponding to the enzyme active site. A recent review [27] summarizes the key results of the cluster quantum chemical modelling of the enzymatic reaction mechanisms based on the 15-year experience. Today the quantum chemical calculations of the energy profiles of reactions using density

† The mechanism of action of serine hydrolases can be viewed in the website http://www.sciencemedia.com/website/demos/biochem/xerProtease.html

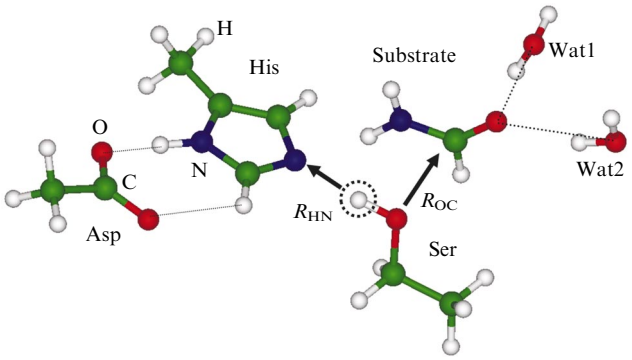


Figure 3. Small cluster for modelling of the acylation step in the catalysis by serine proteases.[50]

functional theory methods for clusters comprising about 150 atoms are considered to be routine.

The applications of the cluster approach for modelling transformations in the active sites of metal ion-containing enzymes are especially popular.[27, 52–57] In some cases, the results of DFT calculations led to quite reasonable conclusions. For example, the quantum chemical calculations of the energy profiles of the reaction of cyclic disulfides and azodicarbonamide with zinc-containing sites mimicking so-called zinc finger regions in the HIV-1 nucleocapsid protein (NCp7)[58–60] were carried out with the goal of theoretical interpretation of experimental data stating that these reagents, which are promising pharmaceutical agents, destroy the coordination sphere of zinc. The results of modelling confirmed the presumable reaction mechanism: on approaching the Zn site, the cyclic disulfide molecules detach one of the ligands of the inner coordination sphere of the metal and the site configuration switches from tetrahedral to planar. The same is characteristic of the azodicarbonamide molecule protonated at the central nitrogen atom, the reaction in this case occurring without an activation barrier.

However, modelling of a system containing transition metals that can change the oxidation state requires certain caution as not any quantum chemistry method can describe such systems without particular efforts.[61, 62] The processes related to dioxygen activation in iron-containing enzyme sites are fairly complicated for modelling (see, for example, Refs 63–74). In these applications, it is necessary to consider various spin states and to keep check on the populations of the metal ion orbitals upon the possible electron transfers between groups that constitute the cluster.

Figure 4 illustrates cluster modelling[72–74] of the mechanism of dioxygen activation by the FIH protein (HIF-inhibiting factor; HIF is hypoxia-inducible factor) containing non-haem iron. The reaction is accompanied by elimination of a CO_2 molecule from the cofactor, 2-oxoglutarate (2OG), which requires electron transfer from the iron ion to the O_2 molecule and the formation of a number of intermediates, in particular, with an active FeO group. By far not all details of this reaction are now clear; nevertheless, from the modelling results,[74] it was concluded that upon hypothetical replacement of an iron ion in the natural FIH enzyme by nickel or cobalt ions, the enzyme activity should decrease and the activation barriers are expected to increase.

The calculations within the framework of cluster models can be performed using well-known quantum chemistry programme packages such as Gaussian, GAMESS, NWChem, *etc*., by extending the experience of small molecules to large biomolecular systems. This trend became possible only in recent years owing to the access to modern tetra- and penta-flop supercomputers and the progress in the development of relevant software. While admitting that a skilled person can obtain interesting and important results on the mechanism of enzyme action along this line, we still advocate for addressing this problem by the combined quantum mechanics — molecular mechanics approach considered in the main part of the review.

IV. Quantum mechanics/molecular mechanics methods

According to the basic idea of the quantum mechanics/molecular mechanics hybrid approximation, a part of the protein system, where chemical bond redistribution is expected upon enzymatic reaction, is included in the quan-

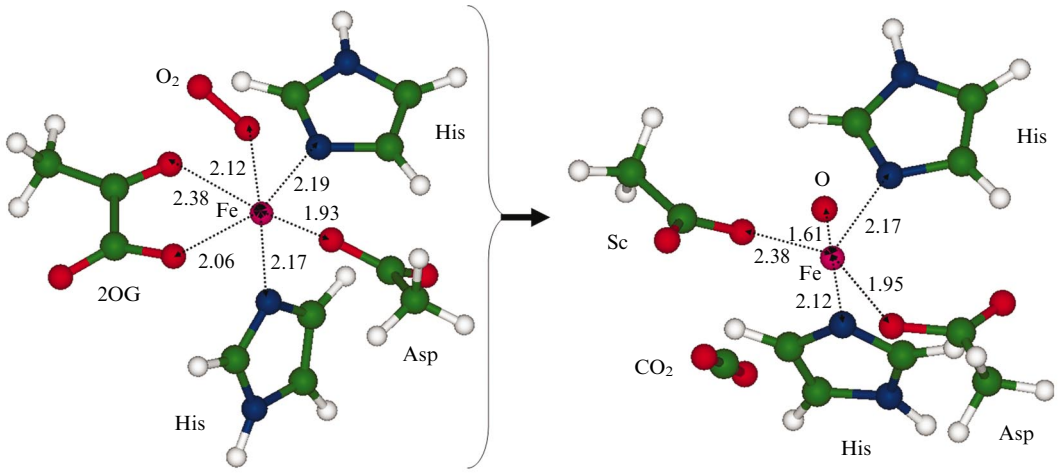


Figure 4. Cluster model for description of dioxygen activation by iron-containing enzymes with the cofactor 2-oxoglutarate.[74] One of the decarboxylation intermediates is the FeO-containing structure shown on the right. Sc is succinate.

tum subsystem (QM subsystem) and the energies and forces in this subsystem are calculated by quantum mechanical equations in various quantum chemistry approximations. Most of the protein molecular groups that surround this distinguished central part refer to the molecular mechanics subsystem (MM subsystem). It is assumed that QM/MM approximations should take into account the conformational changes in the protein matrix that accompany the chemical reaction and simultaneously take into account the effect of the conformational changes in the large system on the energy profile of the reaction.

Selection of the molecular groups for inclusion into the QM subsystem is practically equivalent to the molecular cluster selection for quantum chemical calculations (see Section III): it is not always possible to guess beforehand which part of the model system would adequately reflect the enzyme active site. In some cases, trial-and-error method is to be used. However, the problem of what is to be done with the ends of molecular groups that constitute the QM subsystem is solved more definitely than in the cluster approach. No forced fixing of the atom coordinates in the QM part is required, and upon proper modelling, the geometry of the whole model system (QM + MM) reproduces adequately the structure in the protein macromolecule.

Figure 5 illustrates the separation of the model system into the QM and MM parts. Consider once again the example (see Section II) of penicillin (substrate) hydrolysis by the enzyme penicillin acylase. It is clear that the substrate molecule (at least its part in which electron redistribution during the reaction occurs) and the side chain of the catalytic amino acid Ser1 should be assigned to the QM part. The computation experience indicates that if there is a technical possibility not to separate the substrate molecule into the QM and MM parts, it is better to do without the separation and to assign the whole molecule to the QM subsystem. In addition, the experience of modelling of reactions in serine hydrolases shows that the side chains of the oxyanion hole amino acids (here Ala69 and Asn241) should better be included in the quantum part.

Thus, for the example shown in Fig. 5, the QM subsystem would comprise the whole substrate molecule (penicillin) and parts of the side chains of Ser1, Asn241 and Ala69 residues. The border between the QM and MM subsystems thus runs along the $C^{\alpha}-C$ covalent bond in Ser1 and $C^{\alpha}-C^{\beta}$ bond in Asn241 and twice along the C–N bonds in Ala69. Other versions are also possible. For example, the border can be moved even farther from the functional groups of the chosen amino acid residues if this is allowed by the computer and software capability. Currently the size of the QM subsystem may be $\sim$100 atoms; further increase is desirable but it can still result in too long waiting for the calculation to complete. For saturation of the broken valences in the quantum subsystem, a simple procedure is used: so-called link hydrogen atom is placed along each chemical bond that is cut to separate the QM part (they are shown in Fig. 5 as grey circles and for clarity their positions are marked by arrows). Other ways for treating the border between the QM and MM parts have also been reported but the idea of link hydrogen atoms proved to be most popular in the software implementation of QM/MM.

The principles of the QM/MM techniques and some details of its computer implementation in various program packages are discussed in a number of recent reviews.[24–26] Without going into details, note that most implementations of the QM/MM approach are based on the so-called embedded cluster concept.[75–77] In this version, the QM subsystem (embedded cluster) is attached to the surrounding MM subsystem by intermolecular interactions between groups of the QM and MM parts. In practice, explicit expressions for the potential functions describing these intermolecular interactions are written down and, in the calculation of energies and forces in the whole model system, the contributions of QM and MM components and QM/MM cross components are summed up. According to a simpler version of mechanical embedding, the electron density polarization in the QM subsystem caused by the partial charges on the atoms of the MM subsystem is neglected, while in the electron embedding model, these effects are considered. Currently, attempts are made to further upgrade the QM/MM techniques as implemented in the electron embedding model and to envisage the effect of electron density variations in the QM part on the force field parameters in the MM subsystem, *i.e.*, the use of ideas of the polarizable force field approach is considered.

Yet another implementation[78–81] of the QM/MM method suitable for both fairly accurate calculation of the contribution of the MM subsystem to the quantum Hamiltonian and for estimation of the back influence of the quantum subsystem on the MM subsystem is based on the effective fragment concept.[82–85] By the effective fragment, one means a group of atoms with fixed internal coordinates. The most comprehensible example of effective fragment procedure is modelling of processes in solutions where each of the set of solvent molecules (MM subsystem) is regarded as an effective fragment, while the solute molecules refer to the QM subsystem. This modification of the QM/MM techniques has been used and is still actively used to model chemical transformations in solutions, most often in aqueous solutions (see recent reviews[84,85] and original papers[86–96]).

The initial version of the QM/MM method with the MM subsystem being represented by effective fragments[82,83] is not easily adjusted to modelling of enzymatic reactions; therefore, a modification providing a sufficient flexibility of the MM environment has been proposed and implemented.[78–81] A specific feature of our approach is splitting of the amino acid side chains into small effective fragments (*e.g.*, CO_2^-, CH, NH_3^+, CH_2, CONH) that interact with the QM part and modelling of the fragment–fragment interactions by means of known force fields. The effective fragment concept proved also to be fruitful for separating the model system into the QM and MM parts along covalent bonds. For this purpose, a buffer fragment common to both subsystems was introduced, the quantum part being closed by means of link hydrogen atoms, for example, as shown in Fig. 5. This procedure was described in several reviews[97–99] of 2004–2006. The latest software implementation[100] of the QM/MM method with conformationally labile fragments was performed in terms of the quantum chemical software GAMESS (US).[101,102]

V. Serine hydrolases. Cholinesterases

To pass from procedural issues to the results of modelling of the mechanisms of particular enzymatic reactions, we start this Section by citing a recent study[103] where the energy profile of the acylation step in the hydrolysis of acetylcholine (ACh) by the enzyme acetylcholinesterase (AChE) was

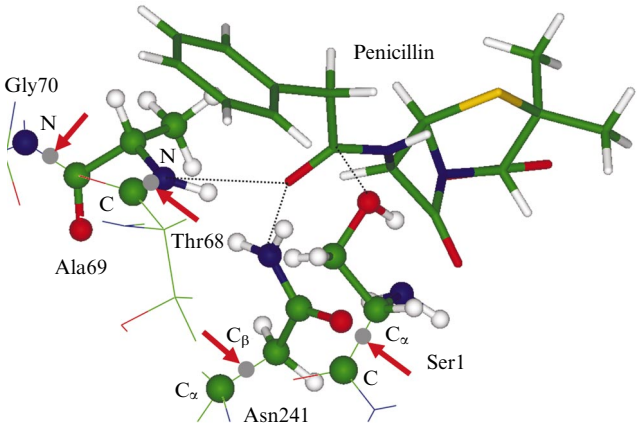
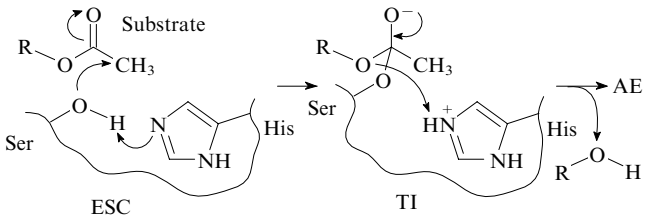


Figure 5. Example of the separation of the system into the QM and MM parts in the penicillin acylase active site.
The sticks (substrate) and balls and sticks (part of the enzyme) mark the atoms assigned to the QM subsystem. The arrows point to the positions of link hydrogen atoms, which are shown by grey circles.

calculated by the QM/MM method with systematic change of the protein region referred to the quantum subsystem.

At the acylation step of reactions of serine hydrolases (AChE, in particular),[104–106] the chemical transformations formally involve the substrate molecule and the functional groups of Ser and His side chains. As shown in Scheme 1, after proton transfer from Ser to His and the covalent bonding of the serine oxygen atom and the substrate carbon atom, the system passes to the tetrahedral intermediate (TI) configuration.[104] The first transition state (TS1) separates the ES and TI structures. The second transition state (TS2) separates the TI and the acyl-enzyme (AE) structure; in this section of the reaction path, the proton migrates from His to the leaving group.

Scheme 1



Thus, it is possible to analyze the energy profile of the reaction in the route ES → TS1 → TI → TS2 → AE by identifying each structure of this series as a stationary point on the potential energy surface in which the energy gradient is equal to zero. For the ES, TI and AE structures, these must be the minimum energy points (unconstrained minimum points), while for TS1 and TS2 these must be saddle points. Using QM/MM molecular modelling, it is possible to estimate how this profile depends on the composition of the model system. One can start from assigning a minimum set of groups to the QM part, in particular, the substrate and the Ser and His side chains and then relocate more and more participants from the MM to QM subsystem, including the whole catalytic triad, the oxyanion hole and other protein fragments.

The results of implementation of this algorithm are presented in Fig. 6 (below we use the numbering of amino acid residues consistent with the crystallographic structure of human AChE with the PDB code 1B41).[107] For constructing the model system of the protein with the substrate (more than 3500 atoms), we chose the peptide groups of the central fragment of ~20 Å radius around the catalytic triad (Ser203, His447, Glu334). The positions of atoms spaced by more than 8–10 Å from the active site were fixed during the geometry optimization in the stationary points on the potential surface.

The energies and forces in the QM subsystems were calculated by DFT with the hybrid functionals BB1K [108]

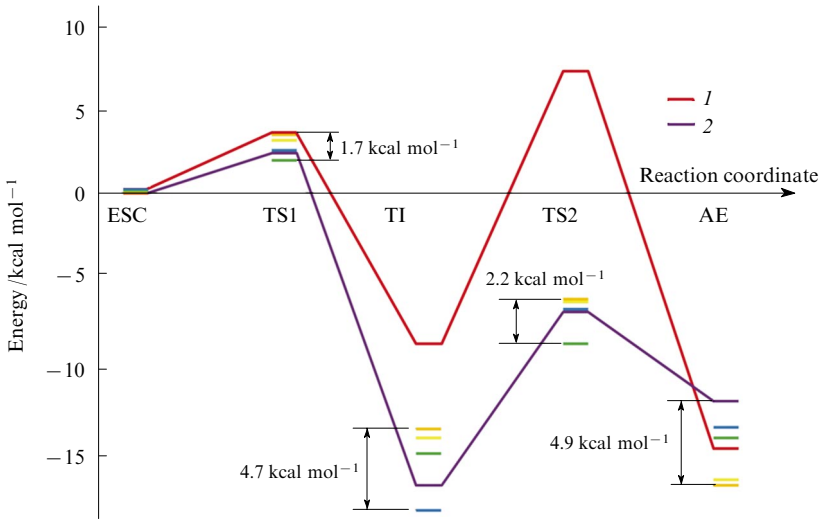


Figure 6. Energy diagrams for substrate acylation by acetylcholinesterase for different ways of separation of the model system into the QM and MM parts (see the text).[103]
(*1*) Minimum QM subsystem (substrate + Ser + His); (*2*) QM subsystem comprising the substrate, the catalytic triad (Ser, His, Glu), the oxyanion hole and the nearest amino acid residues.

and B3LYP [109, 110] and the basis sets 6-31G* (for geometry optimization) and 6-311G** (for the final estimates of energies). The MM part was described using the AMBER parameters.[111] The calculations were carried out using a NWChem program package [112] in the electron embedding approximation to take account of the interactions of QM and MM subsystems. Other technical details were reported.[103]

The calculated energy profile shown by red-coloured lines in Fig. 6 corresponds to the minimum quantum subsystem that comprises only the direct participants of the reaction, Ser203, His447 and the substrate molecule. The profile shown by violet-coloured lines corresponds to the model problem where the QM part comprises the substrate molecule, the catalytic triad amino acids (Ser203, His447, Glu334), the oxyanion hole amino acids (Gly121, Gly122, Ala204) and the groups of the nearest residues Gly120, Glu202, Gly206, Ala207 and Ser229. The other levels are intermediate between the maximum and minimum QM parts.

Several methodically important results are worth noting. First, the profile obtained while using the minimum quantum subsystem (red lines) differs substantially from the profiles obtained in other versions by both an overestimated TS2 energy barrier and too high TI energy with respect to the ES starting point. As soon as the third element of the catalytic triad, Glu334, is included in the QM subsystem, the relative energies of the TS2 and TI sharply go down. On further consecutive extension of the QM subsystem, no systematic changes in the calculated energies in the stationary points can be followed, the profiles obtained for all versions starting from the system comprising the substrate and the catalytic triad fit without any regularity into a rather narrow corridor near the results obtained for the largest QM subsystem (violet line in Fig. 6). One more unexpected finding (see Fig. 6) is that the scatter of the relative energies of the transition states (1.7 and 2.2 kcal mol^{-1}) caused by the selection of the quantum part was less pronounced than the scatter of the minimum energies (4.7 and 4.9 kcal mol^{-1}). Although it is estimation of activation barriers rather than of relative energies of transition states that is believed to be most problematic.

In an earlier study,[113] the mechanical embedding QM/MM approach was used to construct the full energy profile for the hydrolysis of acetylcholine in the AChE active site including the acylation and deacylation steps. Together with the substrate, all amino acid residues of the catalytic triad and the oxyanion hole were included in the quantum subsystem. It was shown that deacylation is the rate-limiting step of the process, the activation barriers do not exceed 10 kcal mol^{-1}, and the tetrahedral intermediates are fairly stable structures. These and more recent [103] results are in good agreement with experimental data.[114–117] Studies of the secondary isotope effect in the acetylcholine hydrolysis by AChE demonstrated [116, 117] that the stable tetrahedral intermediate is accumulated and that its hydrolysis is the rate-limiting step.

However, another opinion on the stability of the tetrahedral intermediates formed in the acetylcholine hydrolysis by AChE was stated based on the results of calculations with the QM(B3LYP/6-31*)/MM(AMBER) approximation energies.[118] For splitting of the model system, the researchers chose to include the side chains of the catalytic triad (Ser203, His447, Glu334) without the oxyanion hole or the substrate into the QM part. At the deacylation step, a water molecule was added to the QM subsystem. The energy profiles are characterized by high energy of tetrahedral intermediates with respect to the energies of the starting species (ES and AE): 11.3 and 16.3 kcal mol^{-1} for the two reaction steps. These energies of tetrahedral intermediates virtually do not differ from the energies of the corresponding saddle points (the differences are within 1 kcal mol^{-1}). This paper illustrates the problems caused by description of the results of QM/MM calculations by different authors: the computation details reported in the paper [118] are obviously do not suffice for the understanding how the values indicated in the energy profile drawings have been obtained. It cannot be ruled out that the addition of oxyanion hole amino acids to the quantum subsystem could correct the results. Other papers devoted to the computer modelling of cholinesterase [119–124] illustrate process details but do not change the mechanism in principle.

The cholinesterase family attracts steady attention of researchers in relation to the development of new pesticides and drugs and also due to the essential role of these enzymes in the study of human molecular polymorphism.[125] The molecular modelling of the mechanisms of action of cholinesterase contributes to this important area. In the works [126, 127] devoted to the specific features of AChE catalysis studied by the QM/MM techniques, the reactivities were predicted for a number of non-charged substrates, alkyl acetates, which were in good agreement with experimental data.[128] Within the framework of the studies of human molecular polymorphism, it was possible to explain, based on calculations, the role of Asp70Gly polymorphic modification of butyrylcholinesterase in the transition of succinylcholine from the class of muscle relaxant drugs to the class of poisons.[129]

Turning back to the QM/MM modelling of enzymatic reactions for a more extensive class of serine hydrolases, pay attention to the study [130] describing the full cycle of chemical transformations of the enzyme–substrate complex to the enzyme–product complex for trypsin type enzymes. The stationary points on the potential surface corresponding to all known intermediates of this cycle were located and characterized. The qualitative picture of chemical transformations described in textbooks [1] was fully reproduced in the calculations with the only exception: no indications supporting the mechanism of double proton transfer in serine hydrolases,[48] which is often discussed, were provided by the QM/MM calculations. The activation barrier of the rate-limiting step (acylation) determined to be up to 10 kcal mol^{-1} should be considered as an advantage of the cited study.[130] As was discussed in Section III, the cluster models, *i.e.*, calculations without adequate accounting for the protein matrix, typically gave activation barriers of 20–30 kcal mol^{-1} for reactions of serine proteases.

An unusual class of serine hydrolases is constituted by serine-carboxyl peptidases called sedolisins [131–137] with the catalytic triad Ser-Glu-Asp (instead of the traditional triad Ser-His-Asp). Before the QM/MM modelling,[138] detailed mechanism of sedolisin catalysis was unknown. For QM/MM calculation of the energy profile of the hydrolysis of the peptide substrate (a fragment of the oxidized insulin chain B) with sedolisin, a molecular model was constructed for a 2500-atomic protein system where 48 atoms of the active site were assigned to the quantum part. The energies and forces of the quantum subsystem were calculated using

the DFT method in the PBE0/6-31+G** level of theory. Considering the modelling results, it was concluded that the mechanism of action of serine-carboxyl peptidases has common features with those of serine proteases and aspartyl proteases.

Thus, in relation to the chemical transformations in the active sites of serine hydrolases, it was demonstrated for a whole series of particular enzymes that by using the QM/MM method with the minimum acceptable QM subsystem, it is possible to adequately calculate the energy profiles of the elementary steps of these significant reactions. In this case, the QM subsystem should comprise all of the amino acid residues of the catalytic triad, the substrate and (desirably) the oxyanion hole groups. Failure to meet this condition may result in qualitatively and quantitatively incorrect results.

A more objective approach to selection of the quantum subsystem can be based on the use of bioinformatics techniques suitable for comparison of various proteins and identification of the conservation of amino acids that form the enzyme catalytic site.[47, 139–143] This strategy is based on programmed alignment of the amino acid sequences of the proteins having a particular degree of homology and catalyzing the same reaction. Statistically valid results were obtained if the sample comprises more than 100 proteins (multiple amino acid sequence alignment taking account of insertions and deletions).

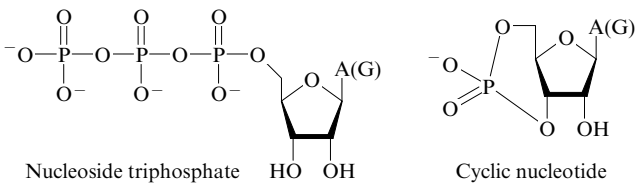
A remarkable phenomenon was observed: the amino acids that form the enzyme catalytic site occupy conserved positions in the sequences of a multitude of related proteins and have zero information entropy. As a rule, these are amino acids bearing acid–base or charged groups and present in the group of five amino acids with the highest conservation rates (Asp, His, Glu, Arg, Ser). Using this approach, it is possible to identify the groups that form the enzyme catalytic site.

VI. Enzymatic hydrolysis of nucleoside phosphates

Among phosphorylation processes, which affect lots of events in the living cells, of special note as the most important biochemical reaction is hydrolysis of phosphoric esters of nucleosides. The role of enzymatic hydrolysis of nucleoside triphosphates (NTP), first of all, adenosine triphosphate (ATP) and guanosine triphosphate (GTP), can hardly be overestimated due to the special role played by these macroenergetic compounds in the body. A great number of works, including works on molecular modelling, are devoted to the mechanism of transformation of the NTP molecule to the diphosphate (NDP) and so-called inorganic phosphate (Pi) upon the reaction with a water molecule

$$NTP + H_2O \longrightarrow NDP + Pi.$$

Yet another important group is formed by cyclic nucleotides, in particular, monophosphates (cNMP), which are hydrolyzed with ring opening at the P–O3′ bond. These reactions, which are catalyzed by phosphodiesterases, are responsible for maintenance of the concentrations of secondary mediators, cAMP and cGMP, which are used in the body for intracellular signal transduction of particular hormones. The chemical structures of the nucleoside phosphate molecules that are discussed in this Section are shown below.

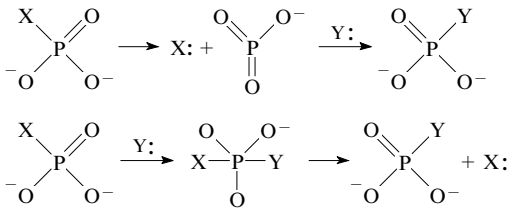


Nucleoside triphosphate Cyclic nucleotide

A is adenine, G is guanine

According to the accepted views,[144–148] the phosphorus–oxygen bond cleavage in esters may occur as either dissociative (the upper reaction in Scheme 2) or associative type of reaction (the lower reaction in Scheme 2), which differ in the geometric configuration of the transition state (or reaction intermediate) of the limiting step.[145] Discussions on the assignment of each particular case of nucleoside phosphate hydrolysis in solution or in the protein to one or the other mechanism still go on.

Scheme 2



In order to avoid repeating the previous review[149] published five years ago, which was concerned with QM/MM modelling of the mechanisms of enzymatic hydrolysis of nucleoside triphosphates, we will briefly summarize the results[34–36, 150–156] obtained earlier for GTP and ATP hydrolysis and describe new data concerning the hydrolysis of nucleoside phosphates.

In the QM/MM calculations of the energy profiles of the reaction path for GTP hydrolysis in various proteins (the proteins and protein complexes Ras, Ras-GAP and EF-Tu were considered), most important was to construct the model system corresponding to the enzyme–substrate complex proceeding from the heavy atom coordinates of an appropriate structure taken from the PDB. Figure 1, Section II shows the configuration of reactants (H_2O + GTP) in the ES structure obtained for one of the protein systems. Analogous structures are also typical of other proteins. In all cases, after thorough analysis of the hydrogen bond network predicted based on the energy minimum of the model QM/MM system, it could be seen that the reactive water molecule and the GTP molecule (with fully deprotonated phosphate groups) are arranged by the side chains of the nearest amino acids and by the magnesium cation in the optimal manner for nucleophhilic attack on the P_γ phosphorus atom of the GTP terminal γ-phosphate group. The reactants are tightly embedded in the protein macromolecule cavity without any voids, which finally ensures the relatively low activation barriers during chemical transformations. As the distance between the water oxygen and P_γ atoms is regularly decreased with appropriate adjustment of all other coordinates of the model system, *i.e.*, as the system moves along the QM/MM minimum energy path, it is possible to identify the first saddle point on the energy surface. It corresponds to the configuration in which the GTP $P_\gamma-O_{\beta\gamma}$ bond has already been cleaved but the new

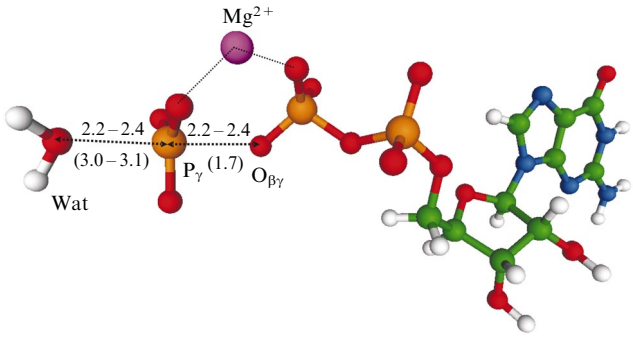


Figure 7. Geometric configuration of the first transition state in the enzymatic hydrolysis of GTP.[151, 155]
Distances (Å) for the TS and ES (in parentheses) are indicated.

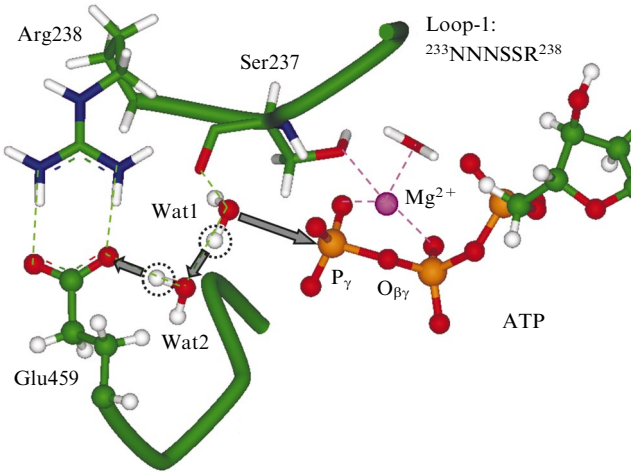


Figure 8. Fragment of the substrate-binding myosin region.[156, 157]
The large arrow indicates the direction of the nucleophhilic attack by the reaction water molecule Wat1 and the small arrows show the directions of proton migration.

bond of the γ-phosphate group has not yet been formed (Fig. 7).

The TS structures tend to have a nearly linear arrangement of the (Wat)O–P_γ–$O_{\beta\gamma}$ group of three atoms with similar distances in the range of 2.2–2.4 Å between the oxygen and phosphorus atoms and almost planar configuration of the PO_3^- group. According to the classification presented in Scheme 2, this reaction corresponds to the dissociative type. This is followed by the formation of a new bond between oxygen of the water molecule and P_γ, which is accompanied by proton transfer up to the formation of inorganic phosphate molecule, possibly, through the next transition state. In all cases, it was shown that activation barriers of 10–15 kcal mol^{-1} on the potential surface are obtained for this type of scenario.

The alignment of the reactants, *i.e.*, the reactive water molecule and the triphosphate fragment with the charge of −4, appropriate for the first transition state of this type (see Fig. 7) is also characteristic of both methyl triphosphate hydrolysis in water[92] and adenosine triphosphate hydrolysis with the protein myosine.[156, 157] The results for the reaction

$$H_2O + ATP \longrightarrow ADP + Pi,$$

catalyzed by the motor protein myosin should be discussed in somewhat more detail.

Figure 8 shows the fragment of the substrate-binding region of myosin in the configuration constructed by the QM/MM techniques following the 1VOM crystallographic motif from PDB.[158] Loops (^{233}NNNSSR^{238}) and (^{454}DISGPE^{459}) form a cavity, which is closed, in particular, by the Arg238–Glu459 salt bridge. The cavity accommodates two water molecules (Wat1 and Wat2 in Fig. 8), oriented according to the hydrogen bond network. The direction of the attack by the reactive water molecule Wat1 on the P_γ phosphorus atom of the terminal γ-phosphate group of ATP is shown by the large arrow. The second water molecule Wat2 and the side chain of Glu459 assist proton transfers, as shown by small arrows, finally resulting in the formation of inorganic phosphate HPO_4^{2-} inside the cavity and in the protonation of Glu459. A mechanism like this involving two water molecules was in general hypothesized[159, 160] based on consideration of the crystallographic structures of myosin (with the initial composition and with point mutations). After the QM/MM calculations,[156, 157] the pattern of chemical transformations involved in the ATP hydrolysis by myosin for the two-water model becomes better understood.

The structure of the transition state, which is the only one in this reaction up to the formation of inorganic phosphate, is fully consistent with the characteristic motif shown in Fig. 7; in other words, the ATP hydrolysis in myosin should correspond to the dissociative mechanism. It is noteworthy that other quantum theory calculations of the energy profiles of ATP hydrolysis in myosin were also published in the last decade.[161–167] In these works, it was assumed that the reaction mechanism should be consistent with an associative type transition state (see Scheme 2), *i.e.*, a species with pentacoordinate phosphorus atom was assumed to exist in the reaction path. The activation barriers in the minimum energy path calculated in terms of this approach were not lower than 20 kcal mol^{-1}, which poorly agrees with the expected results, if for no other reason than because the heat produced during ATP hydrolysis is about 9 kcal mol^{-1}. The experimental estimates of the activation barrier to ATP hydrolysis by myosin are based on two observations. First, the measured[168] rate constant for the step of hydrolysis of myosin-captured substrate (160 s^{-1}) can be used to calculate the free energy activation barrier (14.6 kcal mol^{-1}) if one relies on the simple expression of the transition state theory. Second, a recent publication[169] reports the results of kinetic measurements of ATP hydrolysis by myosin carried out by ^{31}P NMR spectroscopy. From the temperature dependence of the rate constant in the range of 20 to 50 °C, the activation energy was estimated at 10.3 kcal mol^{-1}.

The mechanism of ATP hydrolysis by myosin was modelled[156, 157] using two versions of the QM/MM method (in the conformationally mobile effective fragment approximation and in the embedded cluster approximation) and two software packages (GAMESS and NWChem). The two approaches lead to consentient results: the reaction channel with the assumption of associative mechanism has a much higher activation barrier (as in other works[161–167]) than the dissociative path in terms of the two-water model. According to DFT calculations with the functionals B3LYP or BB1K and the basis sets 6-311++G(d,p) (for geometry

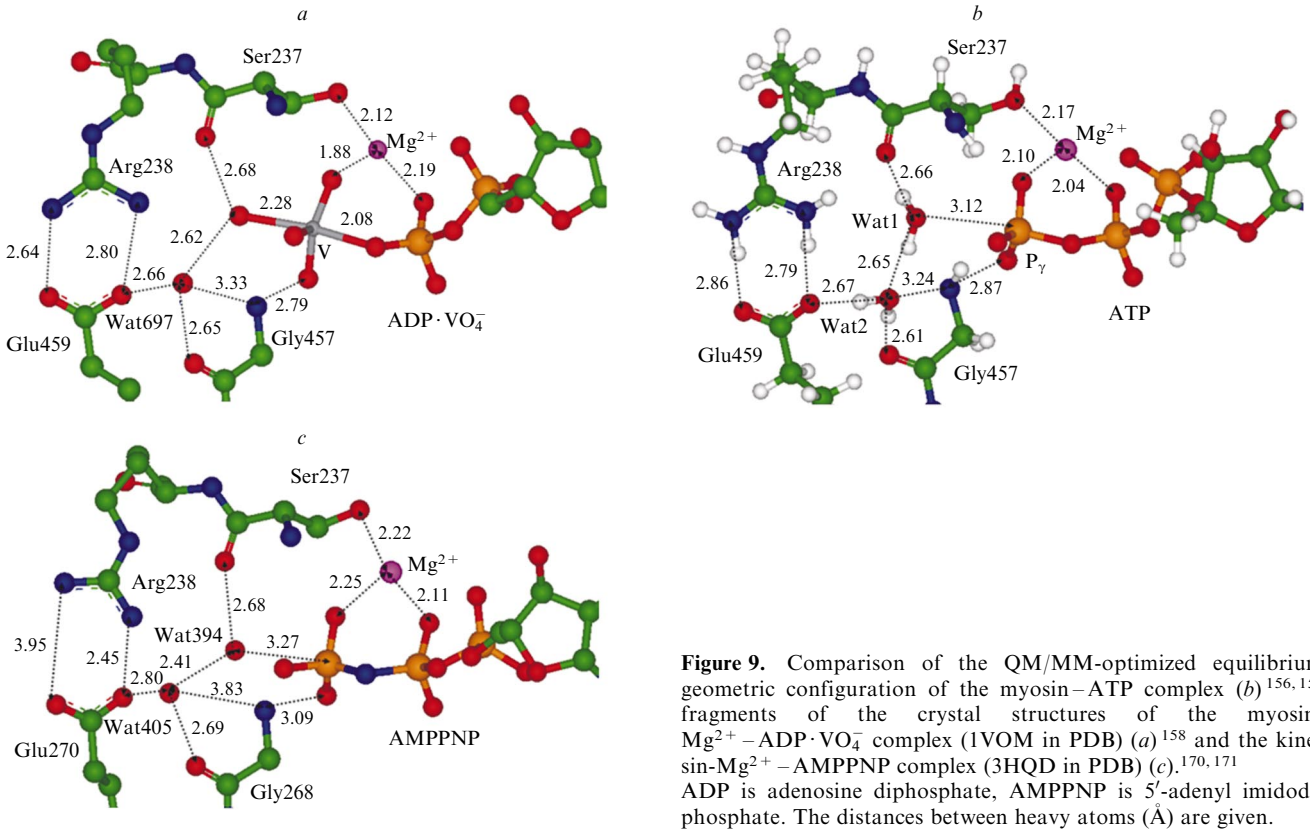


Figure 9. Comparison of the QM/MM-optimized equilibrium geometric configuration of the myosin – ATP complex (*b*)[156, 157] fragments of the crystal structures of the myosin-Mg^{2+} – $ADP \cdot VO_4^-$ complex (1VOM in PDB) (*a*)[158] and the kinesin-Mg^{2+} – AMPPNP complex (3HQD in PDB) (*c*).[170, 171]
ADP is adenosine diphosphate, AMPPNP is 5′-adenyl imidodiphosphate. The distances between heavy atoms (Å) are given.

optimization) and 6-311 + + G(2d,2p) (for energy estimates) in the QM subsystem comprising the ATP phosphate groups, two water molecules, the magnesium cation with its coordination sphere and the side chains of Glu459, Arg238, Ser236, Ser237, Ser181 and Thr186 and with the AMBER force field in the MM subsystem,[157] the activation barrier on the potential surface was estimated at 8.1 – 10.4 kcal mol^{-1}.

There are grounds to believe that this two-water model of ATP hydrolysis would also be valid for another motor protein, kinesin. Recent studies[170, 171] describe the crystallographic structure of kinesin with ATP analogue (PDB code is 3HQD). In the substrate-containing cavity of the protein, the positions of key molecular groups including two water molecules, similar to those in the myosin structures, are clearly distinguished. Figure 9 shows for comparison the structure fragments for the following systems: myosin with ATP analogue (PDB code 1VOM),[93] kinesin with another ATP analogue (PDB code 3HQD)[170, 171] and the model ES structure optimized by the QM/MM method for the myosin – ATP complex.[156, 157] The similarity of the structures is obvious and the assumption of a similar mechanism of ATP hydrolysis in both cases is quite justified.

The chemical transformations involved in ATP hydrolysis in a closed protein cavity end in the formation of products, adenosine diphosphate and inorganic phosphate (HPO_4^{2-}) (Fig. 10). The protonation of Glu459 side chain should be temporary: after opening of the salt bridge and exit of the products from the cavity, the state of Glu459 should be restored. The QM/MM methods, like any other quantum theory approach, are not meant, strictly speaking, for the modelling of so pronounced conformational changes of protein macromolecules. Molecular dynamics calculations[172] showed that opening of the Arg238 – Glu459 salt bridge in the ES before the chemical reaction (non-protonated Glu459) requires approximately twice more energy than that after the product formation in the cavity (protonated Glu459). Thus, the temporary protonation of Glu459 should promote the exit of the reaction products from the myosin cavity.

Unlike the hydrolysis of nucleoside triphosphates, hydrolysis of cyclic monophosphates follows the associative mechanism (see Scheme 2). Figure 11 illustrates the cGMP ring opening at the P – O3′ bond in water studied by QM/MM.[96] Water molecules align along the minimum energy path by means of hydrogen bonding in such a way as to facilitate the proton transfer from the reactants (on the left in Fig. 11 *a*) to the products (on the right in Fig. 11 *a*). The structure of the reaction intermediate with pentacoordinate phosphorus is shown in Fig. 11 *b*.

Qualitatively, the reasons for different type mechanisms of the hydrolysis of nucleoside triphosphates (closer to the dissociative mechanism) and cyclic monophosphates (closer to the associative mechanism) are clear. The high negative charge (−4) of ATP and GTP does not favour the formation of a compact structure with pentacoordinate phosphorus at the γ-end; it is preferable to detach the PO_3^- group. In the case of the cNMP monoanion, the intermediates and transition states resembling more closely the configuration with pentacoordinate phosphorus are preferred. However, none of the classifications of reaction mechanisms can be perfect and the assignment of the phosphorylation mechanism to either of the two types is not always unambiguous.[173, 174]

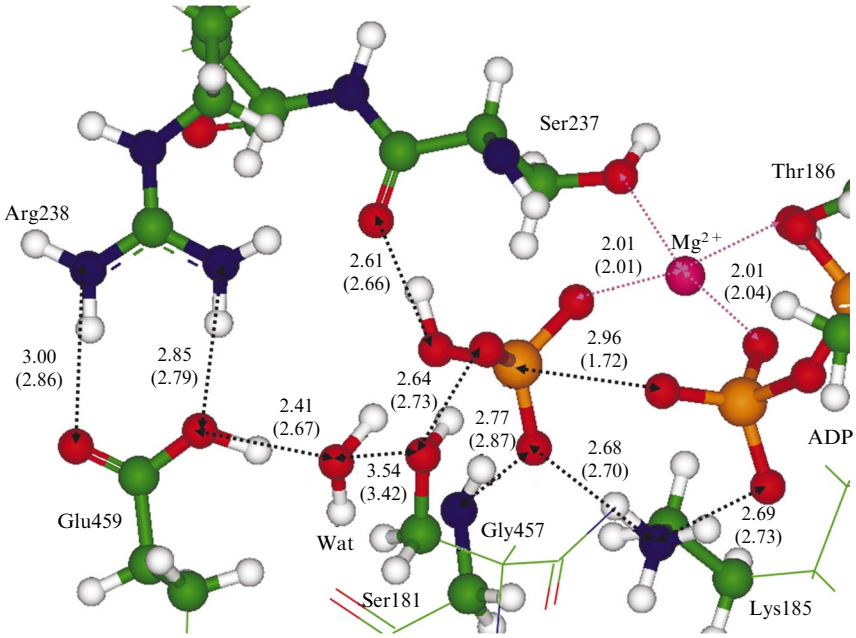


Figure 10. Fragment of the structure of the enzyme complex with products (ADP + Pi) for ATP hydrolysis by myosin.[157]
The distances between heavy atoms (Å) are given for the enzyme complex with products and for ES (in parentheses).

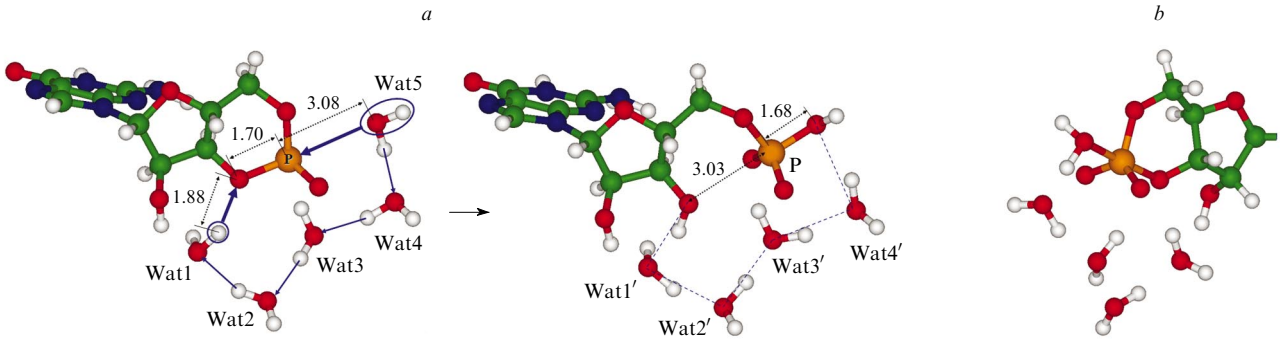


Figure 11. Mechanism of cGMP hydrolysis in aqueous solution illustrating changes in the QM subsystem (*a*) and structure of the reaction intermediate (*b*).[96]
The water shell molecules forming the MM subsystem are not shown. The distances are given in Ångströms.

VII. Conclusion

Molecular modelling becomes a more and more popular tool for the investigation of biochemical and biophysical events. The application of computational technologies markedly supports the experimental works in elucidating the mechanisms of enzymatic catalysis by sharply reducing the research time and cost. As noted in this review, the techniques of molecular mechanics actively used to model the three-dimensional structures of biomolecules or for molecular docking fail to describe the cleavage and formation of chemical bonds as the force field parameters are based on the properties of reference systems near the equilibrium configurations. The use of quantum mechanical approaches based on cluster models that can describe changes in the electronic structures of participants of chemical reactions is restricted by the size of molecular system.

Reliable results of a fully quantum analysis for systems incorporating >300 atoms can hardly be expected in the near future, and even in this case, it is necessary to take into account the effect of rather extended molecular environment on the isolated small fragment of the system that is analyzed in quantum approximations. Most promising is the use of the hybrid QM/MM method. The subject of investigation of a reaction mechanism is analysis of the stationary points on the hybrid (QM + MM + QM-MM) potential energy surface, *i.e.*, analysis of the geometric configurations of the local minima corresponding to the reactants, products and possible intermediates and the saddle points through which the system travels from one minimum to another. Particular attention is devoted to calculation of the energy barriers encountered in the route

from the reactants to the products. Examples of successful modelling of reactions in the proteins using QM/MM methods are presented in this review.

The future of this area of research will be related, in all probability, to calculations of free energy surfaces and potentials of the QM/MM scheme and, in the long view, also with fully quantum potentials. Despite the great expenditure of computational resources, the first steps along this line have already been made.[175–177]

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