

# Software News and Updates

## A Toolkit to Assist ONIOM Calculations

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**Abstract:** A general procedure for quantum mechanics and molecular mechanics (QM/MM) studies on biochemical systems is outlined, and a collection of PERL scripts to facilitate ONIOM-type QM/MM calculations is described. This toolkit is designed to assist in the different stages of an ONIOM QM/MM study of biomolecules, including input file preparation and checking, job monitoring, production calculations, and results analysis. An iterative procedure for refitting the partial charges of QM region atoms is described and yields a more accurate treatment of the electrostatic interaction between QM and MM regions during QM/MM calculations. The toolkit fully supports this partial charge-refitting procedure. By using this toolkit for file conversions, structure manipulation, input sanity checks, parameter lookup, charge refitting, tracking optimizations, and analyzing results, QM/MM studies of large size biochemical systems can be much more convenient and practical.

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**Key words:** QM/MM; ONIOM; charge fitting; efficiency

### Introduction

Computational chemistry and biology are becoming routine methods to study many biological processes. In computational studies of biochemical reactions involving macromolecules, one of the most powerful tools available is combined quantum mechanics and molecular mechanics (QM/MM) methods.<sup>1–5</sup> In QM/MM methods, the large system of interest is divided into two (or more) parts. The larger part, which is not directly involved with the chemical reaction, is treated at a low cost level of theory, such as MM. The smaller part where the chemical reaction occurs is treated with a more accurate level of theory, such as QM, with a higher computational cost. Using this scheme, the entire system, for example, an enzyme with substrate and solvent molecules, can be treated computationally. The reaction of interest in the active site of the enzyme can be studied at an appropriate level of theory with an affordable computational cost.

Although different cases may vary, the general procedure for a typical QM/MM study of a biochemical system can be described as follows. First, reliable experimental structures, usually crystal structures, are identified and preprocessed. The preprocessing includes adding hydrogen atoms, determining the most probable protonation states of titratable amino acids, add counter ions and solvent molecules, geometry optimization with MM, and equilibration by molecular dynamics (MD) using MM. Second, representative geometries are selected from the MD simulations and are subject to optimization again before the

QM/MM study is initiated. Third, QM/MM geometry optimizations are set up from selected MD geometries and conducted for the initial structures, usually reactant complexes in the reaction sequence under study. Fourth, transition states (TS), intermediate structures and products of interest are generated from reactant structures and optimized. Constructing and optimizing representative structures along the reaction path is the most time consuming part of the study and needs to be carried out very carefully. Fifth, the structures identified along reaction path are subject to production calculations at a higher level of theory than used for the geometry optimizations. This general process is illustrated in Figure 1.

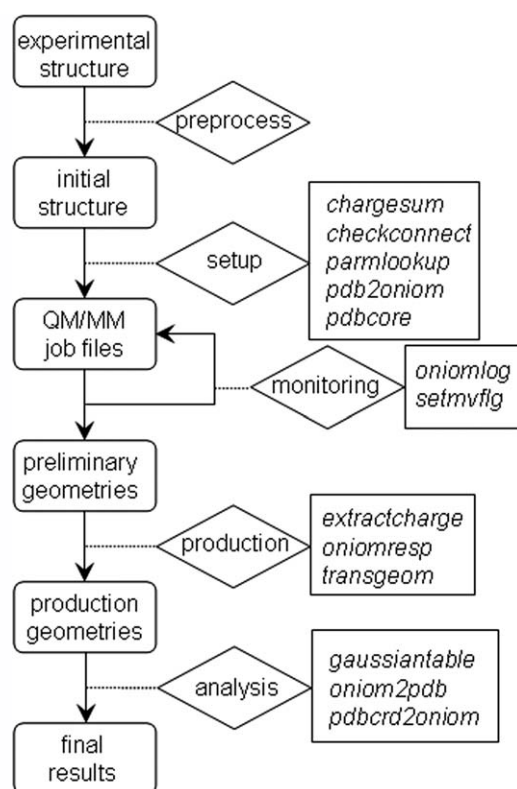
Based on the mechanism of coupling between the QM and MM region, two types of QM/MM methods are generally available, additive and subtractive.<sup>6</sup> ONIOM is one popular and robust subtractive QM/MM method.<sup>7,8</sup> The total ONIOM energy for a two-layer system is<sup>8</sup>

$$E^{\text{ONIOM}} = E^{\text{real,MM}} + E^{\text{model,QM}} - E^{\text{model,MM}}$$

Additional Supporting Information may be found in the online version of this article.

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**Figure 1.** Flowchart for a typical QM/MM study. Key steps are in rounded rectangles. Operations to connect steps are in diamond shapes. Programs developed to facilitate QM/MM studies are listed in regular rectangles.

In a two-layer ONIOM QM/MM calculation, the real system contains all the atoms (including both QM and MM regions) and is calculated at the MM level ( $E^{\text{real,MM}}$ ). The model system is the QM region treated at the QM level ( $E^{\text{model,QM}}$ ). To obtain the total ONIOM energy, the model system also needs to be treated at MM level ( $E^{\text{model,MM}}$ ) and be subtracted from real system MM energy.

Currently, the QM/MM methods implemented in the Gaussian package<sup>9</sup> are two- and three-layer ONIOM models for calculating the energy, gradient, and Hessian for geometry optimization and vibrational frequency analysis. ONIOM calculations using Gaussian have been applied in numerous studies in computational biology, chemistry, and material science.<sup>10–18</sup> In a subtractive QM/MM method, such as ONIOM, the MM force field is needed for entire system, including QM region. The MM force field includes parameters for bond and nonbond interactions and partial charges. Because of its subtractive nature, most bond and short-range nonbonded terms in the QM region cancel in the ONIOM QM/MM method.

Two schemes are generally applied to treat the electrostatic interaction between the QM and MM regions.<sup>19</sup> In the mechanical embedding (ME) scheme, this interaction is treated at the MM level. In the electrostatic embedding (EE) scheme, the partial charges of MM atoms are included in the Hamiltonian of the QM region. In the ME scheme, the electrostatic interaction

between the QM and MM regions does not cancel in the QM/MM energy. The partial charges of QM atoms play an important role in this scheme; therefore, they need to be treated carefully in QM/MM calculations.

As the size of biochemical systems studied by QM/MM can be rather large, some of the structure manipulation and processing of these systems can be cumbersome. During a QM/MM calculation, it is a good practice to monitor running jobs in terms of both energies and geometries. If problems in running jobs can be spotted at an early stage, much time and resources can be saved. However, as the sizes of structures and files in QM/MM studies can be rather large, it is not always convenient to check running QM/MM jobs on the fly, especially geometries. Although there are many graphical interfaces available to facilitate QM/MM calculations, the efficiency and convenience of QM/MM studies can be substantially increased when appropriate auxiliary tools are available for job preparation, monitoring, and analysis. In this article, we describe a toolkit to assist ONIOM calculations (TAO). The toolkit is written in PERL using an object-oriented design and was developed during our previous QM/MM studies.<sup>18</sup> With these tools, QM/MM studies using the ONIOM method in Gaussian become much easier and more efficient, especially for large enzymatic systems. To illustrate the use of the toolkit and to guide a user through an ONIOM QM/MM study of an enzymatic system, we also provide a tutorial in the Supporting Information.

In this article, the general procedure of a QM/MM study is discussed. The tools available for the various stages of this study are listed in Table 1.

## ONIOM Input Preparation

As many large-scale QM/MM studies are focused on biological systems, such as proteins, this article is directed mainly toward calculations of these systems. However, this toolkit can also be applied to any other system suitable for QM/MM studies. The Protein Data Bank (PDB) is a common resource for initial structures of biomolecules. The PDB file format is one of the most commonly used formats for biomolecule structure storage. The selected initial structures are usually subject to pretreatment by MM methods before they are used in QM/MM studies. Pretreatment typically includes geometry optimization, immersing target molecule in a box of explicit solvent molecules, equilibration by MD, etc. The resulting system after these pretreatment steps could be rather large (>10,000 atoms). Usually, it is not necessary to keep all the solvent molecules in QM/MM calculations. For potential energy surface (PES) studies, keeping several solvation shells may be sufficient. Then, the user needs to select a QM region for the QM/MM study. This step requires a compromise between completeness of reaction site and computational cost. Because of the large size of biomolecular systems, it is a common practice to select a region within certain distance of the QM atoms to be optimized during QM/MM study, while keeping other parts of the system frozen. This practice serves two purposes: it reduces the computational cost and avoids noise in the total energy from unrelated conformational changes in different parts of system other than reaction site.

**Table 1.** List of Programs in the Toolkit to Assist ONIOM Calculations

| Module            | Program              | Function  |
|-------------------|----------------------|---|
| Input preparation | <i>chargesum</i>     | Sum up the charges in each part of the ONIOM calculation                                      |
|                   | <i>checkconnect</i>  | Connectivity table sanity check   |
|                   | <i>parmlookup</i>    | Look up missing parameters in the AMBER force field   |
|                   | <i>pdb2oniom</i>     | Generate an ONIOM input file from a PDB file  |
|                   | <i>pdbcare</i>       | Generate a reduced size model system  |
| Job monitoring    | <i>oniomlog</i>      | Check the energy and geometry and generate a new input file                                   |
|                   | <i>setmvflg</i>      | Reset the optimization flag for each atom in an ONIOM input file                              |
| Production        | <i>extractcharge</i> | Extract partial charges from a Gaussian log file  |
|                   | <i>oniomresp</i>     | Facilitate the charge-refitting procedure   |
|                   | <i>transgeom</i>     | Generate an ONIOM input file for whole system containing geometry from a reduced model system |
| Analysis          | <i>gaussiantable</i> | Compare the energies from several ONIOM output files  |
|                   | <i>oniom2pdb</i>     | Generate a PDB file from an ONIOM file  |
|                   | <i>pdberd2oniom</i>  | Replace coordinates of an ONIOM input file by those from a PDB file                           |

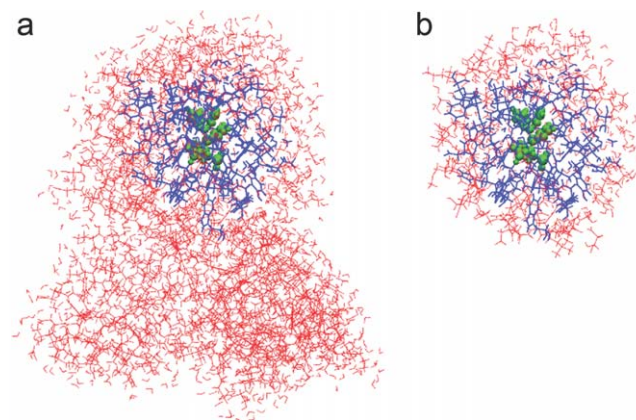
When studying a PES for a reaction, it is necessary to identify the reactant, product, TS, and intermediates along the reaction path. As general MM methods cannot be used to study bond forming and breaking, QM/MM methods are needed. Unlike for small molecules (<100 atoms), searching for reactants, products, and TSs in large biological systems (>5000 atoms) can be very cumbersome. Transition states can be particularly challenging. In our experience, using model QM/MM systems with reduced size can be very helpful when dealing with large systems. In early stages of a study, a large system can be reduced to a smaller model system which contains all of the QM region, the moving part of the MM region, and enough frozen part of the MM region that surrounds the first two parts (see Fig. 2). When compared with the whole system with solvent molecules, it is much easier to manipulate the reduced QM/MM model system to generate initial structures for the reactant, product, and TS, and optimizations are less costly to carry out. Of course, there will be numerous dangling bonds in the frozen MM region, but these missing bonds have little effect on the geometry optimizations in this preliminary study stage. The relative energies obtained from the reduced model system can be a guide for the production calculations on the entire system. Once a complete reaction path is identified using the reduced model, these geometries can be easily embedded back into the whole system, because the surrounding frozen part in the reduced model has coordinates identical to the whole system. In our

experience, once the initial geometries are optimized in the model system, the production geometry optimizations of the whole system are usually easy to complete.

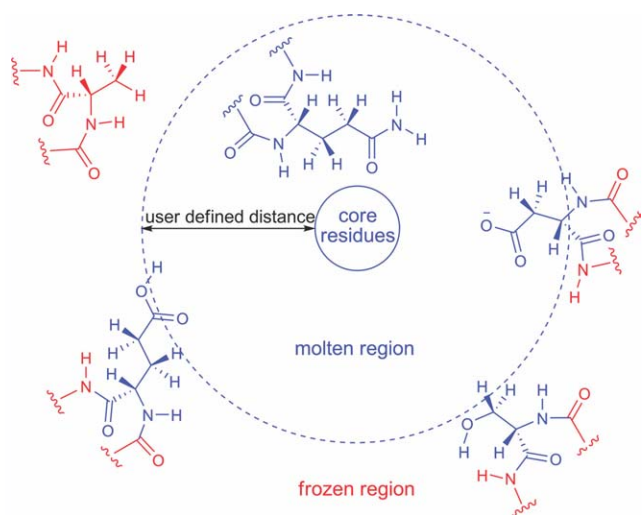
### Tools for ONIOM Input Preparation

Several tools are available in TAO to assist this stage of an ONIOM study. A program named *pdbcare* can be used to generate a reduced-size model for a large size system stored in a PDB file. By defining a core region with key residues, *pdbcare* truncates the full-size system to a reduced-size system by including residues within a certain distance (defined by user) of the core region.

Program *pdb2oniom* can be used to generate an ONIOM input file from a PDB file. There are other tools available to do similar tasks.<sup>20–25</sup> However, several features make *pdb2oniom* particularly useful for large biomolecular systems. A list of core residues and a distance can be set by the user. The core residues are those directly involved in the chemical reactions and any other important residues of system. In the input file generated by *pdb2oniom*, all residues which contain one or more atoms less than a given distance from any atom in the core residues are set to be freely optimized (molten region). All other residues further from core residues are frozen during optimization (frozen region). Therefore, each residue as a unit will be set to either free to move or frozen. Isolated free atoms are avoided in this setup (Scheme 1). This program uses amino acid and nucleic acid template files from AMBER<sup>26</sup> to assign the atom type and partial charge for each atom. A user can add similar template files to the library for any ligand or nonstandard residue for this purpose. The program can automatically read these template files and apply them to the corresponding residues. These template files are usually generated by a program or constructed by the users during the MM pretreatment of the system. It should be



**Figure 2.** ONIOM calculations. (a) Full size model, (b) reduced size model. The QM region is shown in green. Residues allowed to move during optimization are in blue. Frozen residues are in red. When key structures are identified in the reduced model, they can be used to generate the full size model. The frozen atoms in the reduced size model are used as anchors when generating the full size system model.



**Scheme 1.** Illustration of the optimization setup in an ONIOM calculation. Atoms shown in blue are allowed to move during geometry optimization. Atoms shown in red are frozen.

noted that this program does not generate a connectivity table for the biomolecular system. Such a table can be generated using other programs such as GaussView and must be copied to the end of the ONIOM input file. It is strongly recommended that the same connectivity be used for the QM region for all of the structures in the reaction sequence (reactant, TS, product, etc.) to ensure comparable ONIOM energies.<sup>8</sup> The *pdb2oniom* program will not set up the QM region, as it is not a common practice to add whole residues into the QM region. The user must explicitly choose what parts of which residues to include in the QM region for an ONIOM study using GaussView or other tools.

A correct connectivity table is important for the MM calculation, as the connectivity is used to assign partial charges and parameters for bond stretch, bend and dihedral angles, etc. The connectivity table in the ONIOM input file should be checked with the program *checkconnect*. This program will check for any isolated atoms and for atoms with too many bond connections. These atoms could easily disrupt an ONIOM calculation and are hard to diagnose. These atoms can also contaminate the total energy significantly.

Another sanity check is to sum up the total MM charges of the whole system and of each region of the system using the program *chargesum*. With this tool, the charge distribution within each region can be easily checked. It is particularly important for the QM region that the sum of MM partial charges should be close to the total QM charge.

When running a newly generated ONIOM job for a biomolecular system that includes a small ligand, the user will most likely encounter errors due to missing MM parameters. This is because the AMBER force field does not have parameters for the substrate and for non-natural amino acids or nucleosides in the systems. The program *parmlookup* can be used to find these missing parameters, first in the AMBER force field file (*parm99.dat*) and then in the general atom force field file

(*GAFF.dat*).<sup>27</sup> Users can easily replace these force field files used by *parmlookup* with updated force field files from newer versions of AMBER.

## ONIOM Job Monitoring

To maintain high efficiency in the computational study, it is a good practice to check that running jobs are producing meaningful results (both energies and geometries should be checked). When any suspicious behavior is detected for a running job, further attention may be needed before the job is finished. On the basis of the diagnosis, the job may need to be stopped and resubmitted with suitable modification. As ONIOM geometry optimization job files for biomolecular systems can be rather large, frequent checking of the energy and geometry along the optimization path with a text editor or visualization program may be tedious. A number of tools have been designed to make these tasks more convenient.

## Tools for ONIOM Job Monitoring

The *oniomlog* program was developed to facilitate job monitoring. It can extract the energy for each component in an ONIOM calculation along the optimization path and list either total energies in hartree or in relative values with respect to the last step of optimization in kcal/mol. With this information, users can easily follow the geometry optimization process. This check is particularly useful in detecting problems such as the energy alternating between two values rather than decreasing monotonically. To help the user check the geometry conveniently, this program can also extract a certain portion of the system either from the last step or from all steps in the optimization path. The extracted portion can be any layer or combination of layers in a two- or three-layer ONIOM system or all atoms not frozen in the optimization. The output file for the structure is in XYZ file format and can be visualized by many programs.<sup>22,24,28,29</sup> By visually inspecting the selected portion of the system, it may be possible to spot unusual behavior in the optimization before the job terminates with an error. Sometimes, it is desirable to restart an optimization job not from the last step in the current optimization attempt but from an earlier geometry along the optimization path. The *oniomlog* program can be used to generate a new ONIOM job file containing the geometry from a step specified by the user. With the various options provided by this program, users can monitor multiple ONIOM jobs and generate new jobs conveniently.

The *setmvflg* program helps a user to reset the optimization flag of an ONIOM job, for example, setting all the residues within 7 Å instead of 6 Å from core region allowed to move during optimization. The optimization flag of each atom can be easily reset by using this program during the study without going through the whole preparation process. Caution is needed when studying different structures from a reaction sequence. To obtain a meaningful energetic profile, the frozen region setup should be the same for all the structures in one reaction sequence.



## ONIOM Production Jobs

Once key structures in a reaction sequence (e.g., reactant, product, TS, etc.) are identified, final production calculations need to be carried out. If a reduced ONIOM model system was generated, the structures found for the model system must be reoptimized for the whole system. New input files for optimizing the whole system can be generated from the optimized geometries of the reduced model.

Another issue that needs to be addressed is the MM partial charges for atoms in the QM region. For ONIOM calculations, the MM force field is required for the entire system, including QM region. The MM force field includes parameters for bond and nonbond interactions and partial charges. Because of its subtractive nature, most bond and short-range nonbond terms of the QM region cancel in the ONIOM final energy, but the electrostatic interactions between the QM and MM regions remain. In the ME scheme for ONIOM calculations, the electrostatic interactions between the QM and MM regions are calculated at the MM level based on the partial charges. Therefore, it is critical that the partial charges accurately reflect the charge distribution of the QM region. In the EE scheme for ONIOM calculations, the partial charges of the MM atoms are included in the Hamiltonian of the QM region, and the MM partial charges of the QM atoms play a less important role in the total energy than in the ME scheme. However, ONIOM geometry optimization calculations using the EE scheme are generally more expensive than those with the ME scheme, especially for TS searching. Therefore, conducting ME ONIOM geometry optimization calculations accurately is still a common and practical approach at present. When studying different structures in a reaction sequence, the charge distribution of the whole system changes, and a convenient method is needed for dealing with the changes in the charge distribution in a reaction sequence.

The partial charges of natural amino acids and nucleosides are often taken from the AMBER force field. The partial charges for ligands are usually obtained from a charge-fitting process. One of the common procedures for charge fitting is the RESP method,<sup>30,31</sup> which was also used in the AMBER force field development. It would seem straightforward to obtain partial charges for the reactant and product and use them in QM/MM calculations. However, in many enzymatic systems, key amino acid residue side chains may form covalent bond with the substrate or may become protonated/deprotonated along the reaction path. In these cases, the charge distributions of substrate and residues at active site are strongly coupled. Not only the partial charges of the substrate but also those of the key residues in the active site of the protein need to be refitted in a reaction sequence to accurately reflect the structural changes.

## Tools for ONIOM Production Jobs

A program *transgeom* is available to generate an ONIOM input file for the full system containing geometry from a reduced model system. This program matches each atom from the model system to its counterpart in the full system to create a job file for the full system with the model system geometry. This pro-

gram also has the ability to retain the atom types, partial charges, optimization flags, and layer information from the model system, in case any of this setup information has been modified during preliminary study using the model system. With the optimized geometry from the model system, ONIOM optimization jobs for the full system generated by *transgeom* should converge rapidly. This program can also extract geometry from a full size model and build a partial model. In cases where a full frequency analysis is computationally prohibitive for the full size model but is affordable for a partial model, *transgeom* can be used to prepare the partial model input file with geometry from the full model.<sup>32</sup>

A program *oniomresp* is available to facilitate the fitting of a new set of partial charges for the QM region. This program is designed to apply the RESP charge-fitting protocol to be compatible with the AMBER force field, but other types of partial charges from quantum chemistry calculations can also be used. This program needs an ONIOM output file as input, because the coordinates of capping atoms (hydrogen in most cases) are needed in the charge-fitting calculation. This program works in three modes. Mode 1 generates a pure QM Gaussian input file for the QM region with capping atoms from the ONIOM output file. This input file can be used to run a QM single point calculation for charge fitting. In mode 2, an input file for the RESP program is generated based on the QM single point calculation output file for the charge fitting. If there are any capping atoms, it is recommended that they be constrained to have partial charges of zero. In this manner, the sum of the partial charges in the QM region will be constant and consistent for all the QM/MM calculations. This can be easily done by using a flag for the number of capping atoms. The zero charge constraint will be added automatically to all the capping atoms in the input file generated for the RESP calculation. In mode 3, a new ONIOM input file is generated with the partial charges of the QM region replaced by the RESP charges. Before using mode 3, an ONIOM input file should be generated from the ONIOM output file used for mode 1. The program *oniomlog* can be used for this purpose. This ONIOM input file contains the optimized geometry from the output file and the old partial charges. This ONIOM input file and the new partial charges fitted for the QM region by RESP are input files in mode 3 to generate an ONIOM input file with the new RESP partial charges. The newly generated ONIOM input file can be used for further QM/MM geometry optimization calculation.

In some cases, the user may want to refit the partial charges for different parts of the system other than the QM region. Mode 3 of *oniomresp* can fulfill this purpose by using a map file to match each charge to the corresponding atom in the ONIOM input file. Besides RESP, other types of atomic charges, such as APT, NPA, or Mulliken, could be used in ONIOM calculations. Program *extractcharge* is designed to extract partial charges from a QM calculation and to save to a file for use with mode 3 of *oniomresp*.

It is obvious that optimization of an input file generated with mode 3 of *oniomresp* will lead to a different geometry. It is intuitive to repeat the charge-fitting process after the new optimization calculation. This iterative procedure can be repeated until convergence. A well-defined convergence threshold is that the

optimization from the ONIOM job with refitted charges is finished at the very first step, that is, optimization is converged without any changes in the geometry. Thus, the partial charges for the QM region represent the charge distribution of the optimized geometry. However, this optimal convergence may not be achieved easily in some cases. A less stringent criterion used in our previous study is that the difference in the total ONIOM energies between the last two rounds of optimizations is less than 0.1 kcal/mol.<sup>18</sup> Obtaining this convergence typically requires four to six rounds of optimization and charge fitting.

### ONIOM Analysis Module

After finishing the ONIOM production calculations, the geometry and energy need to be analyzed to obtain insight into the systems under study. PDB is one popular file format for storing geometrical information of biomolecules. Many free and commercial graphical viewers are available to visualize and manipulate biomolecules in PDB file format. Therefore, it is convenient to convert the final geometry into PDB format for visualization and analysis.

The final ONIOM energy is calculated by subtracting the MM energy of the QM region from the MM energy for the whole system (the difference is denoted as the MM contribution) and plus the QM energy of the QM region. When studying a reaction sequence, comparisons of not only the ONIOM energy but also the QM energy and the MM contribution can be informative.

### Tools for ONIOM Analysis

The program *oniom2pdb* can translate the geometry from an ONIOM input or output file to a PDB file. It reads in a PDB file and an ONIOM file and uses the PDB file as a template to generate a new PDB file containing the geometry from the ONIOM file. It is recommended that the same template file be used for the various structures in a reaction sequence to ensure that all the PDB files generated in a reaction sequence have the same format. In this way, the setup for visualization can be conveniently shared among the different structures. Users may find this feature very convenient when comparing geometries and producing publication images for several structures. When using an ONIOM optimization output file to generate a PDB file, any geometry along the optimization path can be extracted with *oniom2pdb* and stored in a PDB file for further analysis.

Many users may find that it is easier to manipulate the geometry of biomolecules in PDB format than in other formats. Program *pdbrd2oniom* can generate a new ONIOM input file with coordinates from a given PDB file. *pdbrd2oniom* takes the coordinates from a given PDB file and replaces the coordinates in an existing Gaussian ONIOM input file. By contrast, *pdb2oniom* creates a new ONIOM input file using a PDB file. A user can easily create a PDB file with an optimized geometry from a Gaussian ONIOM file using *oniom2pdb*, make some changes to the structure of a PDB file using their favorite tools (to create TS, product, etc.) and then produce a new ONIOM input file with modified coordinates using *pdbrd2oniom*.

The program *gaussiantable* can read several ONIOM output files and compare the energies in these files. The ONIOM energy, QM energy, and MM contribution of each file are extracted and listed in hartree. Relative energies of these components with respect to the first output file (usually the reactant as reference) are also calculated and listed in kcal/mol. This program is a convenient tool for summarizing calculations to obtain reaction barrier heights and enthalpy changes.

### Conclusions

A set of tools has been developed to facilitate QM/MM calculation using the ONIOM method in Gaussian. Four modules with numerous programs were developed to assist in job preparation, monitoring, production runs, and analysis. Each program has a brief usage message and detailed documentation. This tool set has been used in several studies and proved to be efficient and effective. It is hoped that these tools will make the use of ONIOM calculations easier for QM/MM investigations of biochemical systems. This tool set is available free of charge from the website <http://www.chem.wayne.edu/schlegel/Software.html>. A tutorial on using the toolkit is available in the Supporting Information.

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