QM/MM Study Tutorial
using GaussView, Gaussian, and TAO package

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Introduction

This tutorial is the supporting information for the article "A Toolkit to Assist ONIOM Calculations" (TAO) by Peng Tao and H. Bernhard Schlegel. This tutorial has been developed to demonstrate the general procedure for a quantum mechanics / molecular mechanics (QM/MM) study of a biochemical system using Gaussian, GaussView and the TAO package. The example used in this tutorial is the inhibition mechanism of matrix metalloproteinase 2 (MMP2) by a selective inhibitor (4-phenoxyphenylsulfonyl)methylthiirane (SB-3CT). This QM/MM study was described in the article "Matrix Metalloproteinase 2 Inhibition: Combined Quantum Mechanics and Molecular Mechanics Studies of the Inhibition Mechanism of (4-Phenoxyphenylsulfonyl)methylthiirane and Its Oxirane Analogue." *Biochemistry* **2009**, *48*, 9839-9847.

This tutorial is designed for users who are familiar with general use of Gaussian, GaussView and Unix/Linux, and who are planning to conduct QM/MM studies of biological systems using the ONIOM method available in the Gaussian package. For more details, please refer to the user manuals and information about general usage of Gaussian, GaussView and Unix/Linux system.

In this tutorial, we use the MMP2·SB-3CT complex to demonstrate ONIOM input job preparation, job monitoring, production calculations, and analysis carried out in a typical QM/MM study. In particular, the structure of the reactant complex is optimized in this tutorial. The initial structure for the complex was generated from docking and molecular dynamics (MD) studies of SB-3CT with the crystal structure of MMP2. The preprocess stage described in the article is not part of this tutorial. The details of the preprocess using the molecular dynamics package AMBER can be found in the computational method session of the *Biochemistry* article. For more information about molecular mechanics simulations of biomolecules, please refer to the AMBER website (http://ambermd.org/) or related books (e.g. Schlick, T. (2002). *Molecular Modeling and Simulation, An Interdisciplinary Guide*, Springer).

The structure of the MMP2·SB-3CT complex with necessary water solvent molecules from the preprocess treatment is stored in PDB file, *mmp2_full_r.pdb*. This file served as the starting structure for **3-R** in Figure 5 in the *Biochemistry* paper.

Currently the TAO package is available and tested for any Unix/Linux platform with PERL installed. It can also be run on either Windows or Mac with PERL installed. Users are advised to work through this tutorial under the Unix/Linux environment and use a text editor to view and modify the Gaussian ONIOM job files. The example used in this tutorial came from an actual research project. The sizes of the protein model and job files are not small. This could be cumbersome for beginners of Gaussian and

ONIOM. However, the authors believe that using an example from a real study could help users to find the best way to conduct their own research, and shows the usefulness and effectiveness of the toolkit. Please note that all the calculations shown in this tutorial are for demonstration purposes only, and a low level of theory is chosen so that calculations are relatively fast. For publication quality calculations, users should consult related references for the appropriate level of theory for their own studies.

When using text files to run Gaussian calculation on Unix/Linux, please make sure these files are in Unix/Linux text format (with line break or end-of-line recognized by Unix/Linux systems). Otherwise, these files cannot be run properly on Unix/Linux platforms.

For any of the tools available from the TAO package, typing the command by itself will display brief information about that command. Typing the command with the flag **-h** or **--help** will display a detailed UNIX style manual page for that command.

The user must have access to Gaussian and GaussView. TAO is compatible with Gaussian (versions 03 and 09), and GaussView (versions 3 to 5). Gaussian 09 is used to carry out calculations in this tutorial. To start this tutorial, the user needs to obtain a copy of TAO (available from http://www.chem.wayne.edu/schlegel/Software.html), and install it on the system they are using. Please refer to the installation guide of the TAO package.

In this tutorial, all the file names are in italic, and command names and flags are in bold. Example command lines are in blue. The example command output and file contents are in smaller font than regular text.

ONIOM input preparation

I. Initial Gaussian job preparation

The user should copy the file *mmp2_full_r.pdb* and *corelist.txt* into their working directory. These two files and other files which will be generated by the user following this tutorial are available from http://www.chem.wayne.edu/schlegel/Software.html.

The program **pdb2oniom** was used to generate a preliminary ONIOM input file from the PDB file *mmp2_full_r.pdb*. To run this program, a file with a core residue list is needed. In this case, the core residue list, *corelist.txt*, reads:

```
[INH] "339"
[ZN] "335"
[HID] "288"
[HID] "292"
[HID] "298"
```

[GLU] "289"

Core residues in this example include the inhibitor, the active site zinc, three histidine residues and one glutamic acid residue. Both the residue name (in square bracket) and index number (in double quotation marks) are needed to identify each core residue. The following command uses **pdb2oniom** to generate a preliminary ONIOM input file with all residues containing any atom within 6 Å from any atom in the core residues allowed to move during optimization:

pdb2oniom **-o** *mmp2_full_r.gjf* **-resid** *corelist.txt* **-near** *6* **–i** *mmp2_full_r.pdb*

For the *mmp2_full_r.pdb* input file, this command produces the following output.

```
Core residues list file corelist.txt provided.
All residues within 6 angstroms from core region are free to move (0) during geometry optimization.
Atom type cannot be assigned to atom H1 in residue LYS 1.
Partial charge cannot be assigned to atom H1 in residue LYS 1.
Element type cannot be decided for atom H1 in residue LYS 1.
Atom type cannot be assigned to atom H2 in residue LYS 1.
Partial charge cannot be assigned to atom H2 in residue LYS 1.
Element type cannot be decided for atom H2 in residue LYS 1.
Atom type cannot be assigned to atom H3 in residue LYS 1.
Partial charge cannot be assigned to atom H3 in residue LYS 1.
Element type cannot be decided for atom H3 in residue LYS 1.
Atom type cannot be assigned to atom OXT in residue PRO 334.
Partial charge cannot be assigned to atom OXT in residue PRO 334.
Element type cannot be decided for atom OXT in residue PRO 334.
Residue     ZN does not exist in database. Atom with name    ZN may not be defined.
Residue     ZN does not exist in database. Atom with name    ZN may not be defined.
Residue     KA does not exist in database. Atom with name    KA may not be defined.
Residue     KA does not exist in database. Atom with name    KA may not be defined.
There are 1599 residues in the PDB file.
Write ONIOM input file mmp2_full_r.gjf from PDB file.
Opening file mmp2_full_r.gjf for output ...
Successfully wrote mmp2_full_r.gjf file.
```

Two files are generated: *mmp2_full_r.gjf* and *mmp2_ full_r.gjf.onb*. *mmp2_full_r.gjf* is a Gaussian input file. The other file, *mmp2_full_r.gjf.onb*, has both atom and residue information, and will be needed for later use in the production stage. Some atoms in residues Lys1 and Pro334 cannot be processed correctly, because these two residues are the N- and C-terminal residues. The names and atom types for the three hydrogens in the protonated N-terminal amine group in Lys1 and the oxygen in the unprotonated C-terminal carboxylate group in Pro334 cannot be assigned, and need to be fixed manually. Residue ZN and KA are zinc and calcium, respectively. The missing parameters need to be added for them as well. The correct parameters for these atoms are obtained from the related AMBER force field and are given in *mmp2_full_r_02.gjf*. Please note that the partial charges for the other atoms in the terminal residues also need to be changed since charge distributions are different for internal and terminal residues.

The inhibitor SB-3CT (residue SB3 in the PDB file) is recognized by **pdb2oniom**, because its PREP file, *SB3_resp_int.prep*, is available in the ESPT/prepfiles folder of the TAO package. This file was generated during the preprocess stage using the AMBER program suite. For more information about this

file format, please refer to the AMBER manual which can be obtained from http://ambermd.org. Users can put PREP files for any substrates in their systems in the same folder. **pdb2oniom** automatically reads these files and processes corresponding residues in the PDB input file.

The Gaussian job file generated by **pdb2oniom** does not contain a connectivity table. This table can be generated by GaussView by reading *mmp2_full_r_02.gjf*, and saving to another Gaussian input file *mmp2_full_r_03.gjf*.

Since the full system of MMP2 with water molecules is rather large (~8800 atoms), it is convenient for structure manipulation to build a reduced size (partial) model for preliminary calculations. The partial system can be built using any software that users are comfortable with, such as VMD, PyMol, etc. The program **pdbcore** can also be used to generate a partial model system.

pdbcore -o *mmp2_partial_r.pdb* **-resid** *corelist.txt* **-near** *12* **-i** *mmp2_full_r.pdb*

This command generates partial model, *mmp2_partial_r.pdb*, containing all the residues within 12 Å of the core residues. Both the full model, *mmp2_full_r.pdb*, and the partial model, *mmp2_partial_r.pdb*, are illustrated in Figure S1.

A Gaussian ONIOM job file needs to be generated for the partial model similarly to the full model.

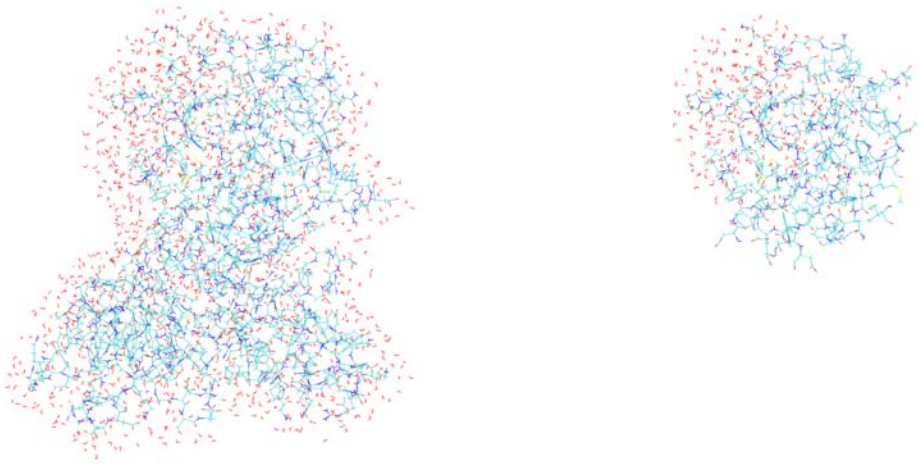
pdb2oniom -o *mmp2_partial_r.gjf* **-resid** *corelist.txt* **-near** *6* **-i** *mmp2_partial_r.pdb*

mmp2_partial_r.gjf then needs to be modified for correct atom types and charges (*mmp2_partial_r_02.gjf*), and the connectivity table must be added using GaussView, (*mmp2_partial_r_03.gjf*).

If the core residue file is not provided, no atoms in the Gaussian input file will be marked as frozen for the optimization.

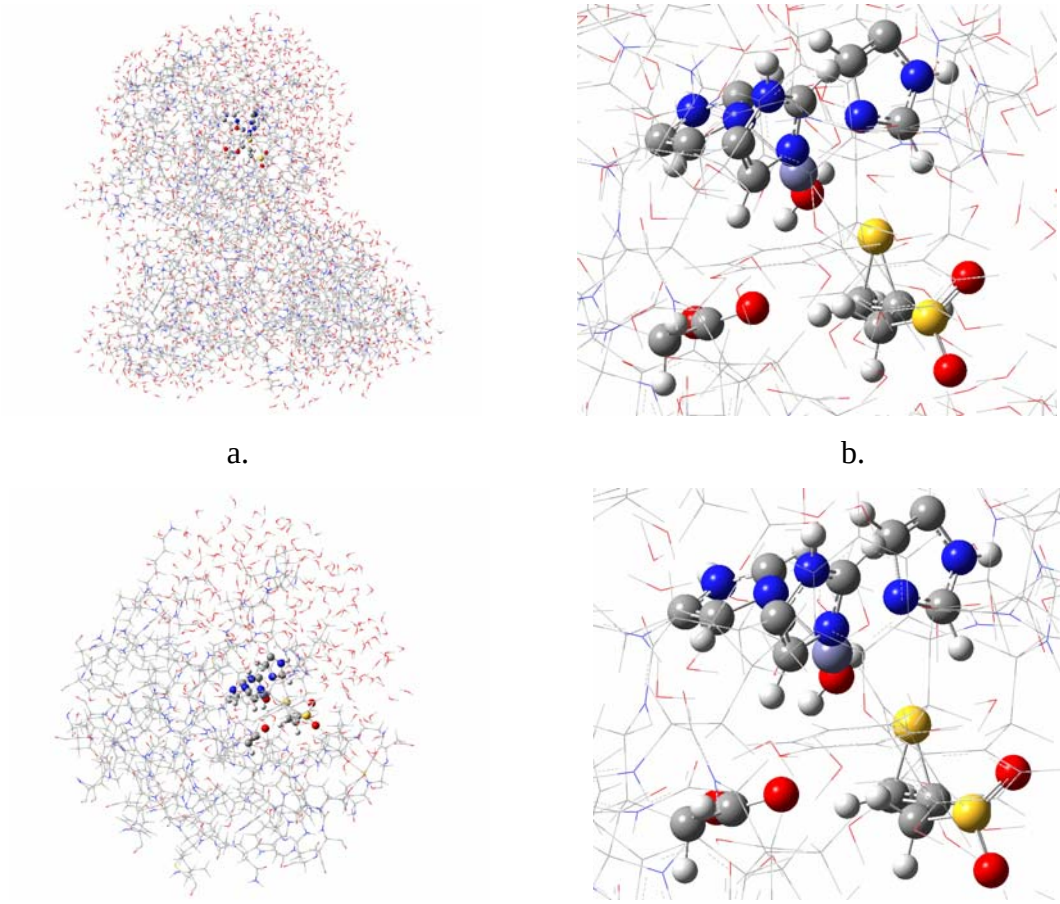
II. QM region setup

The next step is to use GaussView to set up the QM region in both the partial model (*mmp2_partial_r_04.gjf*) and the full size model (*mmp2_full_r_04.gjf*) (Figure S2). Users can use Layer Selection Tool from GaussView (choose Edit -> Select Layer…) to set up desired QM region. Please refer to GaussView manual for more information.



a. b.

Figure S1. Model systems for the QM/MM tutorial. (a) full size model; (b) partial model.



a. b.

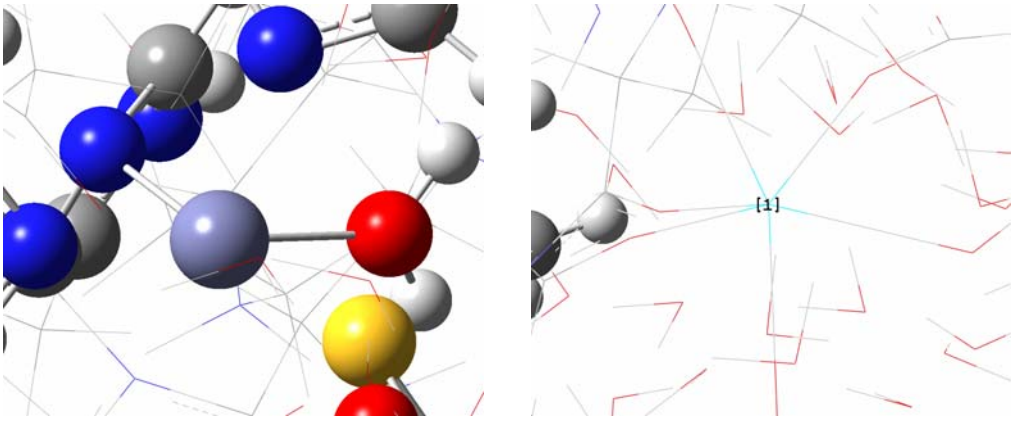
c. d.

Figure S2. QM region for the full and partial model systems. (a) full model; (b) close up view of the QM region of the full model; (c) partial model; (d) close up view of the QM region of the partial model.

III. ONIOM job files clean up

Before running these Gaussian jobs, the connectivity of some atoms need to be fixed (e.g. the bonds shown in GaussView, see Figure S3). These connections can be detected using **checkconnect**. This

program can help users to find atoms based on their numbers of connections in the connectivity table of a Gaussian input file. In most cases, metal ions are assigned multiple connections by GaussView. These connections need to be removed for proper behavior in the MM part of the ONIOM calculations. This program also detects all the isolated atoms as a sanity check.



a. b.

Figure S3. Connections which need to be removed for ONIOM calculations. (a) zinc ion in QM region (purple) with two connections; (b) a calcium ion (highlighted and labeled as [1]) with six connections.

Using **checkconnect** program to check the Gaussian input file of the partial model:

checkconnect -g *mmp2_partial_r_04.gjf* **–c** *5*

Part of the output reads:

```
Opening mmp2_partial_r_04.gjf for processing...
Treat the file as a Gaussian input file.
This is an ONIOM input file.
There are 2493 atoms.
Atom 1605 (Zn) has 5 connections.
Atom 1606 (Ca) has 6 connections.
```

This shows that atoms 1605 (zinc) and 1606 (calcium) have at least five connections (defined by flag **–c** *5*) and need to be fixed. The connectivity of atom 1607 (calcium) also needs to be cleaned. The connectivity of calcium (1607) is 4, and can be displayed using flag **-c** *4*. Since many carbons have four connections, extra caution is needed to identify this. The corrected input file is saved to *mmp2_partial_r_05.gjf*. A similar process is needed for the full model input file, *mmp2_full_r_04.gjf*, with new input in *mmp2_full_r_05.gjf*.

To set up a Gaussian ONIOM job, the user needs to assign the net electric charge and spin multiplicity for each layer. The user can use **chargesum** to quickly add up the MM partial charges in each layer.

chargesum -g *mmp2_full_r_05.gjf*

The output reads:

```
Opening mmp2_full_r_05.gjf for process
Given file mmp2_full_r_05.gjf is not Gaussian log file.
Treat it as Gaussian input file.
Total charge of real system is -11.721594.
Total charge of high layer is   0.355254.
Total charge of medium layer is   0.000000.
Total charge of low layer is -12.076848.
Total charge of high plus medium layer is   0.355254.
Dipole moment (Debye) (X, Y, Z) is
(    -2484.0827,      -3100.5310,      -2279.4550).
Total Dipole moment (Debye) is       4580.3793.
```

Since we did not add counter ions to this protein, the total charge of the whole protein is not zero. Due to the setup of the QM region (with covalent bonds across the QM/MM boundary), the total charge of the QM region is not an integer. The QM region should carry a net charge of +1, because the zinc ion has a +2 charge, and the glutamate side chain has a –1 charge. The charge and spin multiplicity for the full protein model ONIOM job is

```
-11 1 1 1 1 1
```

The first pair of numbers, –11 and 1, are the charge and spin multiplicity for the low level of theory (MM in this case) calculation of the real system (including both QM and MM regions in this case). The second pair of numbers, 1 and 1, are the charge and spin multiplicity for high level of theory (QM) calculation of the model system (QM region in this case). The third pair of numbers, 1 and 1, are the charge and spin multiplicity for the low level of theory (MM) calculation of the model system (QM region). Please refer to the Gaussian manual for more detailed information.

For the partial model system, we have

```
-3 1 1 1 1 1
```

For the next step, the command line for each ONIOM job needs to be changed to the following:

```
#p oniom(pm3:amber=hardfirst) nosymm geom=connectivity iop(2/15=3) test opt=quadmac
```

The PM3 semi-empirical level of theory is used for the QM region to reduce the computational time in this tutorial. Users should use a more suitable level of theory for their research. Memory and number of CPUs also need to be changed to values appropriate for the level of theory. Please refer the Gaussian manual for details of the job setup. The checkpoint file name should be modified as well. *mmp2_partial_r_06.gjf* was used for the Gaussian run.

From this point forward, we will use the partial model system to carry out the calculations. Once we have identified the key structures, (e.g. reactant, transition state (TS), product etc.) we can construct inputs for the full systems using the partial models.

IV. Missing parameters lookup

The Gaussian job *mmp2_partial_r_06.gjf* fails with a complaint about missing parameters in the log file *mmp2_partial_r_06.log*:

```
Read MM parameter file:
Define ZN        1
Define C0        2
Include all MM classes
Bondstretch undefined between atoms   1617   1618 S-O [H,H] *
Bondstretch undefined between atoms   1617   1619 S-O [H,H] *
Bondstretch undefined between atoms   1617   1620 S-CA [H,L]
…
…
Angle bend  undefined between atoms   1630   1631   1632 OS-CA-CA [L,L,L]
Angle bend  undefined between atoms   1630   1631   1640 OS-CA-CA [L,L,L]
* These undefined terms cancel in the ONIOM expression.
MM function not complete
Error termination via Lnk1e in XXXXXXXXXXXXXX/g09/l101.exe at XXXXXXXXXXXXXX 2009.
Job cpu time:  0 days  0 hours  0 minutes  1.8 seconds.
File lengths (MBytes):  RWF=      5 Int=      0 D2E=      0 Chk=      1 Scr=      1
```

This error message tells us that the MM parameters for zinc and calcium and numerous other MM parameters are missing from the input file. These parameters can be found in the AMBER force field file and need to be added at the end of the Gaussian input file. The parameters for zinc and calcium are

```
VDW    Zn  1.10    0.0125
VDW    C0  1.7131  0.459789
```

The **parmlookup** program can be used to look up these missing parameters from AMBER force field files.

parmlookup -g *mmp2_partial_r_06.log* **-o** *mmp2_partial_r_06_parm.txt*

The missing parameters are listed in the output file, *mmp2_partial_r_06_parm.txt*, in the format used by Gaussian.

```
Hrmstr1    S    O   194.8000     1.8020
Hrmstr1    S   CA   277.9000     1.7390
Hrmstr1    S   HS   286.4000     1.3530
Hrmstr1   CA   OS   372.4000     1.3730
HrmBnd1    N   CT   HC     0.0000     0.0000
HrmBnd1   CT    S    O     0.0000     0.0000
HrmBnd1   CT    S   CA     0.0000     0.0000
HrmBnd1   CT    S   HS     0.0000     0.0000
HrmBnd1    S   CA   CA    62.2000   122.5500
HrmBnd1    O    S    O     0.0000     0.0000
HrmBnd1    O    S   CA     0.0000     0.0000
HrmBnd1    O    S   HS     0.0000     0.0000
HrmBnd1   CA   CA   OS    69.8000   119.2000
HrmBnd1   CA   OS   CA    63.6000   118.9600
```

Some of the parameters cannot be found by **parmlookup** in the AMBER force field and are set to zero in the output. Values for these missing parameters can be estimated by looking up the corresponding parameters for similar atom types. This process may need to be repeated several times until all the parameters are provided.

The user may also encounter the following error message (shown in *mmp2_partial_r_06_02.log*)

```
The combination of multiplicity 1 and  3505 electrons is impossible.
Error termination via Lnk1e in XXXXXXXXXXXXXX/g09/l101.exe at XXXXXXXXXXXXXX 2009.
 Job cpu time:  0 days  0 hours  0 minutes  2.1 seconds.
 File lengths (MBytes):  RWF=     52 Int=      0 D2E=      0 Chk=      1 Scr=      1
```

This is because a total charge of –3 is assigned to the whole system, but this does not correspond to a closed shell configuration. The user needs to adjust them to appropriate values. After using **chargesum** to check total charges, a charge of –2 and a multiplicity of 1 are used. Following this correction, still one more round of parameter look up is needed. The final working Gaussian job file is *mmp2_partial_r_07.gjf*.

ONIOM Job Monitoring

Actively monitoring running ONIOM jobs can save a tremendous amount of time and computational resources. Program **oniomlog** was developed for this purpose.

I. Check energies

When an ONIOM geometry optimization job is running, this program can be used to check its progress. *mmp2_partial_r_07_part.log* is an unfinished geometry optimization log file. The following command produces a summary of the energies in the log file.

oniomlog -o -i *mmp2_partial_r_07_part.log*

The output of this command reads:

```
Gaussian out file is mmp2_partial_r_07_part.log

Optimization -o was used
Final ONIOM energy report for a two-layer ONIOM calculation (hartree):
  This corresponds to the last complete step of an unfinished geometry optimization job.

High Level Model:            0.117316
Low  Level Model:           -0.099033
Low  Level Real :           -7.941107
Low  Level Real-Model :      -7.842074

ONIOM Energy :               -7.724758


Dipole moment (Debye) (X, Y, Z) is
(     -272.4224,      -431.8687,      -519.2448).
Total Dipole moment (Debye) is       728.2443.

Attention: this Gaussian calculation did not terminate normally!

OPT flag -o was turned on. Energy of each step will be printed.
Energies along the optimization path (kcal/mol):
Step Number        ONIOM          High Model       Low (Real-Model)
  1           139.704978         95.029033         44.675945
  2            61.415425         70.264397         -8.848971
  3            44.086133         50.729131         -6.642998
  4            29.244689         33.199647         -3.954959
  5            19.479058         21.517017         -2.037959
```

```
6          10.567483          11.196136          -0.628653
7          4.583601           4.318795           0.264807
8          0.000000           0.000000           0.000000
There are 8 steps of optimization in this job.
```

The ONIOM energy of the final step is displayed in hartree. The geometry optimization path is displayed at the end, because the flag **-o** is used. It shows that seven steps of the optimization were finished so far, because the step 1 is the initial structure. The ONIOM energy, QM region energy (high model) and contribution from the MM calculation, MM_{real}-MM_{model} (Low(real–model)) are displayed in kcal/mol with the last step as reference. If the flag **-ha** is specified, all the energies are printed as total energies in hartree rather than relative energies in kcal/mol. From the energies, it is clear that the calculation is stepping toward a minimum.

II. Check geometries

This program can also extract certain parts of the structure along the optimization path for a quick check.

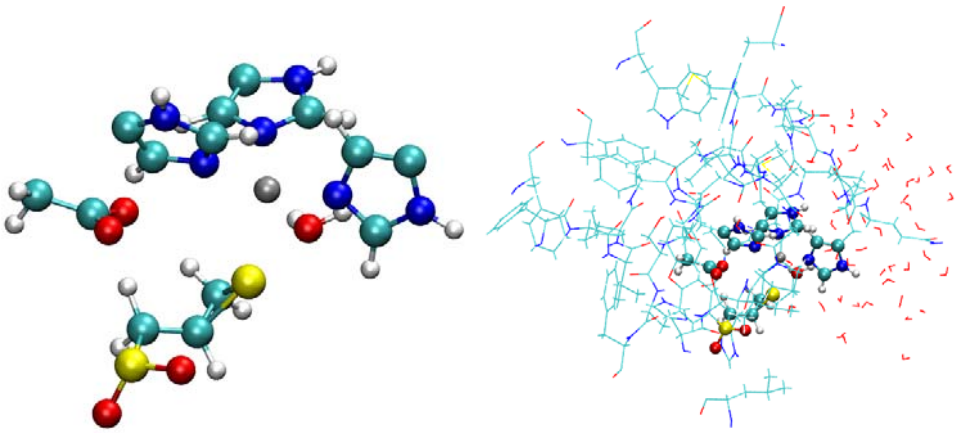
oniomlog -s *mmp2_partial_r_07_partqm.xyz* **-o -i** *mmp2_partial_r_07_part.log*

The QM region is extracted along the optimization path with flag **-o**, and saved to the file *mmp2_partial_r_07_partqm.xyz* in XYZ format. If flag **-o** is not given, only the last geometry will be saved.

oniomlog -s *mmp2_partial_r_07_partqmmov.xyz* **-g -l** *1* **-o -i** *mmp2_partial_r_07_part.log*

The QM region and all of the moving part of system are extracted along the optimization path with flag **-g -l** *1* **-o**, and saved to *mmp2_partial_r_07_partqmmov.xyz***.**

mmp2_partial_r_07_partqm.xyz and *mmp2_partial_r_07_partqmmov.xyz* are relatively small, and can be loaded into visualization software, such as VMD for quick viewing (Figure S4). Flags **-l**, **-g** and their combinations can be used to extract structures from ONIOM calculations in many different ways. Please refer to the manual page of **oniomlog** for more details. With these files, the user can quickly check the progress of a Gaussian ONIOM optimization job.



a. b.

Figure S4. Structures extracted by **oniomlog**. a) QM region only; b) QM region and all the atoms that move during the optimization.

III. Generate new input files

If the user needs to create a new Gaussian ONIOM job with the optimized geometry from a previous ONIOM job, this can also be done using **oniomlog**.

oniomlog -oi -t *mmp2_partial_r_07.gjf* **-fo** *mmp2_partial_r_08.gjf* **-i** *mmp2_partial_r_07_part.log*

The flag **-oi** tells the program that a new Gaussian ONIOM input file needs to be generated. The program uses *mmp2_partial_r_07.gjf* as a template (after flag **-t**), and extracts the last geometry from *mmp2_partial_r_07_part.log* (after flag **-i**) and generates a new Gaussian ONIOM input file *mmp2_partial_r_08.gjf* (after flag **-fo**). The flag **-fn** *number* can be used to generate a new input file from a specific geometry along the optimization path instead of the last geometry (*number* is the step number of the desired geometry along the optimization path). Finding an appropriate level of theory and getting the geometry optimization converged to the appropriate state may involve many rounds of calculations, and is beyond the scope of this tutorial. With the help of this toolkit, these calculations can be conducted conveniently and efficiently.

Another important task in studying a reaction is finding the transition state (TS). After obtaining an optimized reactant structure, the user can modify this structure toward a targeted TS, by conducting a series of geometry optimizations with key geometric parameters fixed (*mmp2_partial_TS_01.gjf*). These fixed geometric parameters are usually related to the breaking and forming of chemical bonds. For example, in *mmp2_partial_TS_01.gjf*, two bond lengths and one bond angle are fixed. The user will find that it is much easier to manipulate the partial model system than the full size model system. Once the partially optimized geometry is close enough to the desired TS, it can be used in a TS optimization. There are many ways to search for a TS (please refer to Hratchian, H. P.; Schlegel, H. B.; “Finding

Minima, Transition States, and Following Reaction Pathways on Ab Initio Potential Energy Surfaces", in *Theory and Applications of Computational Chemistry: The First 40 Years*, Elsevier, **2005**, pg 195-259; Jensen, F, (1999), *Introduction to Computational Chemistry*, Wiley; Wales, D. (2004), *Energy Landscapes: Applications to Clusters, Biomolecules and Glasses*, Cambridge University Press). This topic is beyond the scope of this tutorial.

Sometimes, it will take a rather long time to finish an ONIOM geometry optimization job. In some cases, a geometry optimization job does not converge. For example an optimization may cycle between two very close geometries after many optimization steps, and total energy oscillates within a very narrow range before the number of allowed optimization steps is exceeded. **oniomlog** is particularly useful in this case. Long before the Gaussian ONIOM job exits with an error message, the user can see if the optimization is stuck. When this happens, **oniomlog** can be used to check the geometries along the optimization and to generate a new Gaussian ONIOM input file with the necessary changes for another optimization job (for example, using a different level of theory, changing the maximum step size allowed in geometry optimization, etc.). All of these can be done using **oniomlog** while the current job is still running. To save time and computational cost, users are encouraged to actively monitor ONIOM optimization jobs.

In the present case, the geometry optimization job needed to be restarted several times before convergence was achieved. The final geometry optimization log file is *mmp2_partial_r_08_final.log*. An ONIOM input file *mmp2_partial_r_08_final_geom.gjf* is generated containing the optimized geometry from *mmp2_partial_r_08_final.log* using **oniomlog**.

oniomlog -oi -t *mmp2_partial_r_07.gjf* **-fo** *mmp2_partial_r_08_final_geom.gjf*
-i *mmp2_partial_r_08_final.log*

To save time and computational cost, it is recommended that users do not wait until the jobs in this tutorial complete before proceeding. Using a Gaussian log file with several iterations of geometry optimization or the Gaussian log file provided in this tutorial is sufficient for the rest of the tutorial.

IV. Reset optimization flag

If a user wants to reset the optimization flags of an ONIOM job, for example, to set all the residues within 7 Å instead of 6 Å from core region allowed to move during optimization, **setmvflg** can do this easily. An example of this program is

setmvflg -b -i *mmp2_partial_r_07.gjf* **-onb** *mmp2_partial_r.gjf.onb* **-resid** *corelist.txt* **-near** *7*
-o *mmp2_partial_r_07_newflg.gjf*

setmvflg needs an ONIOM input file, *mmp2_partial_r_07.gjf*, the ONB file, *mmp2_partial_r.gjf.onb*, produced by **pdb2oniom** when the ONIOM input file was generated from the PDB file, and a core

residue list file, *corelist.txt*. This command resets the optimization flags in *mmp2_partial_r_07.gjf*. All the residues within 7 Å of the core region will be allowed to move during the geometry optimization in *mmp2_partial_r_07_newflg.gjf*.

The command line listed above generates the following output.

```
Use coordinates from file mmp2_partial_r.gjf.onb to reset optimization flag.
Reading ONIOM input file mmp2_partial_r_07.gjf...
There are 2493 atoms in file mmp2_partial_r_07.gjf.
Done!
Reading ONIOM ONB file mmp2_partial_r.gjf.onb...
There are 2493 atoms in file mmp2_partial_r.gjf.onb.
There are 393 residues in file mmp2_partial_r.gjf.onb.
Done!
Output file name is mmp2_partial_r_07_newflg.gjf
In file mmp2_partial_r_07.gjf, there are 784 atoms, 89 residues subject to geometry optimization.
After reset, there are 1124 atoms, 132 residues subject to geometry optimization.
Open mmp2_partial_r_07_newflg.gjf to write new ONIOM input file...
Successfully wrote mmp2_partial_r_07_newflg.gjf file.
```

The output shows that 132 residues (1124 atoms) are allowed to move in *mmp2_partial_r_07_newflg.gjf*, compared to 89 residues (784 atoms) in *mmp2_partial_r_07.gjf*. Flag **-b** tells **setmvflg** to use the coordinates from the ONB file to reset the optimization flags. Without this flag, **setmvflg** will use coordinates from the Gaussian input file to reset the optimization flags. In either case, the newly generated input file always has the same coordinates as the given input file.

ONIOM Production Calculations

I. Generating input files for the full protein model

After obtaining appropriate structures in a reaction sequence using a partial model system, these structures (reactant, TS, product, etc.) need to be re-optimized using the full protein model. **transgeom** was developed for this purpose. This program needs an input file:

transgeom *mmp2transgeom.in*

Note that no flag is required before the input file, which must be the last item in the command line. The input file *mmp2transgeom.in* reads

```
Modelgjf         mmp2_partial_r_08_final_geom.gjf
Modelonb         mmp2_partial_r.gjf.onb
Productiongjf    mmp2_full_r_05.gjf
Productiononb    mmp2_full_r.gjf.onb
Productioninput  mmp2_full_r_06.gjf
```

Four input files are needed and one file will be generated. *mmp2_partial_r_08_final_geom.gjf* (“Modelgjf”) is the Gaussian ONIOM input file for the partial model system. *mmp2_partial_r.gjf.onb* (“Modelonb”) was produced by **pdb2oniom** when the ONIOM input file for the partial model system was generated from the PDB file. *mmp2_full_r_05.gjf* (“Productiongjf”) is the Gaussian ONIOM input file for the full protein model with solvent molecules prepared earlier in this tutorial. *mmp2_full_r.gjf.onb* (“Productiononb”) was generated by **pdb2oniom** when generating the ONIOM input file of the full model system from the PDB file. *mmp2_full_r_06.gjf* (“Productioninput”) is the Gaussian ONIOM input for the full system with the optimized geometry from *mmp2_partial_r_08_final_geom.gjf.*

After generating the Gaussian ONIOM input file for the full system, the user needs to copy the added MM parameters in the partial model input file to the full model input file, and make necessary adjustment to the full model input file similar to those for the partial model input file (e.g. charge and multiplicity, etc.). Users are also encouraged to check the completeness and validity of the input file for the full model production run. File *mmp2_full_r_06.gjf* is a valid Gaussian ONIOM job for the full system. The converged geometry optimization log file of the full size model is *mmp2_full_r_06_final.log*.

By default, program **transgeom** takes the atom, atom type, partial charge, moving flag (0 or -1), layer setup (H, M or L), and coordinate information for those atoms included in the partial model system if they are different from the given full model input file. In this way, any changes made in the partial model will automatically be carried through to the new full model input file. If for any reason a user chooses to keep any of the information from the full model input file, several flags (**-atmp**, **-chgp**, **-movep**, **-layerp**, **-coordp**) can be used to keep any of these values from the full model input file. Please refer to the **transgeom** manual page for more details.

In some cases, users may want to take the geometry from a full size model and build a partial model. For example, when full frequency analysis is computationally prohibitive for the full size model, but such an analysis can be afforded for a partial model. By simply setting the full size model as “Model” and the partial model as “Production” in the input file, **transgeom** will extract the corresponding portion of the geometry from the full size model and build a partial model. All other options of **transgeom** apply as well.

II. Fit partial charges for new structures

For different structures in a reaction sequence, it is obvious that the MM partial charges of the atoms in the QM region change and need to be refitted. **oniomresp** was developed for this purpose.

The partial charge refitting procedure used in this tutorial can be described as follows. After finishing the geometry optimization using the full model, the QM region with capping atoms (usually hydrogen) is extracted from the log file. The extracted structure is subject to a single point QM calculation using the Merz-Singh-Kollman scheme for electrostatic potential derived charges. The QM single point calculation results are used in the Restrained Electrostatic Potential (RESP) charge fitting program. The fitted partial charges replace the previous set of partial charges for QM atoms in a new Gaussian ONIOM input file. The geometry optimization is repeated with newly fitted partial charges for QM atoms. This whole procedure can be repeated until convergence. Please refer to *Biochemistry* **2009**, *48*, 9839-9847 for details of this procedure. The refitting of charges is particularly important for the mechanical embedding scheme in ONIOM calculations since the electrostatic interactions between the QM region and the rest of the system are handled at the MM level. **oniomresp** has three modes for charge fitting.

In mode 1, the QM region with capping atoms is extracted from a Gaussian ONIOM log file and saved to a Gaussian input file.

oniomresp -m1 -g *mmp2_full_r_06_final.log* **-o** *mmp2_full_r_06_final_4ESP.gjf*

File *mmp2_full_r_06_final.log* is the converged log file for the full size model, and *mmp2_full_r_06_final_4ESP.gjf* contains the extracted structure. Users are recommended to visually check the extracted structure using GaussView. With an appropriate level of theory and a suitable population analysis scheme, a single point calculation is carried out using *mmp2_full_r_06_final_4ESP.gjf*. Note that geometry optimization should not be conducted for this extracted structure, since it is not a minimum as a stand-alone structure. By default, **oniomresp** only extracts the QM region with capping atoms. When an atom list file is given with the flag **-list**, **oniomresp** can extract the corresponding atoms from the ONIOM log file and write them to a Gaussian input file. Please refer to the **oniomresp** manual page for more details of this option.

The log file, *mmp2_full_r_06_final_4ESP.log*, generated from a QM single point calculation using *mmp2_full_r_07_sp_4ESP.gjf* is used for mode 2 of program **oniomresp**.

oniomresp -m2 -# *5* **-g** *mmp2_full_r_06_final_4ESP.log* **-o** *mmp2_full_r_06_final_4ESP_RESP.in*

Flag **-#** *5* tells **oniomresp** there are 5 capping atoms. Zero charge constraints are added to these capping atoms in the RESP input file, *mmp2_full_r_06_final_4ESP_RESP.in*. Before using **resp**, from the AMBER program package, to do the charge fitting, the electrostatic potential needs to be extracted from *mmp2_full_r_06_final_4ESP.log*. **espgen**, another program from the AMBER program package, can be used for this purpose. The extracted electrostatic potential is saved to *mmp2_full_r_06_final_4ESP.esp*.

espgen -i *mmp2_full_r_06_final_4ESP.log* **-o** *mmp2_full_r_06_final_4ESP.esp*

resp can perform the charge fitting using files generated above.

resp -i *mmp2_full_r_06_final_4ESP_RESP.in* **-o** *mmp2_full_r_06_final_4ESP_RESP.out*
-p *mmp2_full_r_06_final_4ESP_RESP.pch* **-t** *mmp2_full_r_06_final_4ESP_RESP.qout*
-e *mmp2_full_r_06_final_4ESP.esp*

mmp2_full_r_06_final_4ESP_RESP.qout contains the fitted partial charges for the QM atoms. The last five (capping) atoms have zero partial charges because of the constraints added in *mmp2_full_r_06_final_4ESP_RESP.in*. Please refer to the AMBER user manual for more details of **resp** usage. The *qout* file with the fitted charges reads:

```
 0.536331 -0.581191  0.447401  0.011234  0.246270  0.020309 -0.742044  0.488682
-0.004958  0.014470  0.028681  0.763294 -0.902114 -0.792329  0.594623 -0.724348
 0.506225  0.129609  0.174016  0.001224 -0.604498  0.328291  0.627675 -0.754995
 0.492897  0.358836  0.132775 -0.151578 -0.646392  0.291544  0.429922 -0.023872
 0.268593  0.096902 -0.283048  0.096459  0.164431 -0.522630  0.325696  0.190850
 1.325617 -0.668203 -0.647399 -0.724312  0.314251  0.366800  0.000000  0.000000
 0.000000  0.000000  0.000000
```

Both **espgen** and **resp** are available free of charge from the AMBER website (http://ambermd.org/).

Since *mmp2_full_r_06_final.log* is a geometry optimization log file, a new ONIOM input file needs to be generated containing the new geometry. This can be done using **oniomlog**.

oniomlog -oi -t *mmp2_full_r_06.gjf* **-fo** *mmp2_full_r_06_final_geom_oldchg.gjf*
-i *mmp2_full_r_06_final.log*

The new partial charges can be added to the ONIOM input file, *mmp2_full_r_06_final_geom_oldchg.gjf*, used in mode 3 of **oniomresp**, where the resp charge file, *mmp2_full_r_06_final_4ESP_RESP.qout*, is needed.

oniomresp -m3 -g *mmp2_full_r_06_final_geom_oldchg.gjf*
-qin *mmp2_full_r_06_final_4ESP_RESP.qout* **-o** *mmp2_full_r_07.gjf*
-c *mmp2_full_r_06_07_chgcomp.txt*

mmp2_full_r_07.gjf is an ONIOM input file with refitted partial charges. *mmp2_full_r_06_07_chgcomp.txt* lists the old and new sets of partial charges and atom information for the user's reference.

Users are not limited to RESP charges in their studies. Partial charges from any type of population analysis can be used when appropriate. **extractcharge** can extract designated partial charges and save them to a file which can be used by **oniomresp** in mode 3. Other type of partial charges can be used conveniently when there is no covalent bond across the QM/MM interface since no capping atoms are needed.

extractcharge -c1 -g *mmp2_full_r_06_final_4ESP.log* **-o** *mulcharge.txt*

In this example, Mulliken charges are extracted from file *mmp2_full_r_06_final_4ESP.log* and saved to *mulcharge.txt*.

By default, **oniomresp** assigns partial charges from a given charge file to QM atoms in sequence. When a map file is given by flag **-p**, **oniomresp** assigns partial charges to those atoms listed in the map file. Please refer to the **oniomresp** manual page for more details of this option. Flags **-list** and **-p** are useful when partial charges of a particular part of the ONIOM system other than QM region need to be refitted.

ONIOM Results Analysis

I. Generating a PDB file from an ONIOM file

The results of production calculations usually require further analysis. For biological system, it is convenient to analyze the structures in PDB format using visualization software like VMD, UCSF Chimera, PyMol, etc.. **oniom2pdb** can generate a PDB file from Gaussian ONIOM file using a template PDB file. Either a log file or an input file can be used to generate the PDB file. Two examples of using **oniom2pdb** are

oniom2pdb -g *mmp2_full_r_06_final.log* **-pdb** *mmp2_full_r.pdb* **-o** *mmp2_full_r_06_final_log.pdb*

oniom2pdb -g *mmp2_full_r_06.gjf* **-pdb** *mmp2_full_r.pdb* **-o** *mmp2_full_r_06_gjf.pdb*

In these two examples, the PDB file, *mmp2_full_r.pdb*, used when generating ONIOM input file in the preparation stage is the template file. When an optimization job log file is specified, the flag **-n** *number* can be used to choose a particular geometry along the optimization path to generate the PDB file.

II. Assigning coordinates from a PDB file to an ONIOM file

Many users may find that it is easier to manipulate the geometry of biomolecules in PDB format than in other formats. **pdbcrd2oniom** can generate a new ONIOM input file with coordinates from a given PDB file.

pdbcrd2oniom **-g** *mmp2_full_r_06.gjf* **-pdb** *mmp2_full_r.pdb* **-o** *mmp2_full_r_06_pdb.gjf*

pdbcrd2oniom needs a Gaussian ONIOM input file, e.g. *mmp2_full_r_06.gjf*, and a PDB file, e.g. *mmp2_full_r.pdb*. These two files should have exactly the same number and types of atoms and in the exactly same order. **pdbcrd2oniom** then takes coordinates from the PDB file and replaces the coordinates in the Gaussian ONIOM input file and saves to *mmp2_full_r_06_pdb.gjf*. **pdbcrd2oniom** is very different from **pdb2oniom**, which reads a PDB file, assigns an AMBER force field atom type and partial charge to each atom, and creates a new Gaussian ONIOM input file. **pdbcrd2oniom**, on the other hand, reads a PDB file and a Gaussian ONIOM input file, takes coordinates ONLY from the PDB file and replaces coordinates ONLY in the Gaussian ONIOM input file.

Using both **pdbcrd2oniom** and **oniom2pdb**, users can easily create a PDB file with the optimized geometry from a Gaussian ONIOM file using **oniom2pdb**, make some changes to the structure in a PDB file using their favorite tools (to create TS or product, etc.), and then create a new ONIOM input file with modified coordinates using **pdbcrd2oniom**.

Please keep in mind that PDB files only keeps three decimal places per coordinate value. This is less than those in ONIOM job files (6 decimal places).

III. Extract Energetic Information

When comparing energies of different structures in a reaction sequence, **gaussiantable** can automatically extract the ONIOM energy and its components from a series of files and list them as both absolute and relative values. One example of running this program reads

gaussiantable **-i** *gaussiantable.in* **-o** *gaussiantable.out*

Two Gaussian ONIOM log files, *01_3_R_Th.log* and *02_3_TS_Th.log*, are given in *gaussiantable.in*:

```
# Example input file for gaussiantable
#

<Label>Example</Label>
<ONIOM>
01_3_R_Th.log
02_3_TS_Th.log
```

</ONIOM>

All blank lines and comment lines starting with # are ignored. The list of log files is organized in the block defined by the *<ONIOM>* and *</ONIOM>* lines. The keyword *ONIOM* tells the program the following are ONIOM log files. The keyword *GAUSSIAN* will be used for regular Gaussian log files in future development. Two files are listed in this example. An arbitrary number of log files can be listed in the block. The first file (usually the reactant structure in a reaction sequence) will be used as the reference values for the relative energies. Each input file can have an arbitrary number of blocks.

The output file *gaussiantable.out* contains the ONIOM energy information.

```
================================================
Output for block 1: Example
Absolute values in Hartee:
        ONIOM Total          Model High          Model Low           Real Low        Real-Model Low

01_3_R_Th.log
        -3861.352671        -3827.772029           -0.250204          -33.830845          -33.580642

02_3_TS_Th.log
        -3861.312040        -3827.731645           -0.091761          -33.672155          -33.580395

------------------------------------------------
Relative values in kcal/mol:

        ONIOM Total          Model High          Model Low           Real Low        Real-Model Low

01_3_R_Th.log
           0.000000            0.000000            0.000000            0.000000            0.000000

02_3_TS_Th.log
          25.496088           25.340972           99.424437           99.579553            0.155116

================================================
```

The first half has ONIOM energies, and each component is listed in its absolute value in hatree. The second half has these energies listed in relative values in kcal/mol with respect to the first log file listed in this block. If multiple blocks are given in the input file, the output blocks are listed in the same format and same order as the blocks listed in the input file.

With this program, the final energies from QM/MM study can be easily extracted, analyzed and compared.

Summary

This tutorial describes a general procedure for a QM/MM study of a biochemical system using Gaussian and GaussView with the help of the PERL toolkit TAO. This tutorial is designed for users with some basic experience with Gaussian, GaussView and Unix/Linux systems. The TAO toolkit is distributed free of charge from http://www.chem.wayne.edu/schlegel/Software.html.