The Investigation of Atomic-Level Interactions in the Cancer-causing BCR-ABL Tyrosine Kinase and Imatinib Through the Application of the ONIOM Method a work in progress

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Abstract

A computational chemistry approach was applied to a medically significant, biological system in the human body—the enzyme BCR-ABL tyrosine kinase, the origin of Chronic Myelogenous Leukemia (CML). By examining the complex formed by the inhibiter Imatinib (cancer drug known as Gleevax®) and the enzyme we hope to gain insight into the atomic-level interactions involved, accomplished through a classical approach (Molecular Mechanics). In addition, we aim to investigate the impact of quantum mechanics (QM) by use of the ONIOM approach, an approach that should allow treatment of a system that would otherwise be intractable through traditional QM approaches due to its large size (~10K atoms).

Background: Chronic Myelogenous Leukemia

Chronic Myelogenous Leukemia (CML)—a malignancy of a hematopoietic stem cell—results from a mutation involving chromosome 22 and 9. Sequences *abl* and *bcr* fuse and the resulting two Bcr-Abl fusion proteins form the Bcr-Abl tyrosine kinase, present in 95% of patients with CML.

The drug Gleevax® (the compound imatinib) is currently on the market and acts to inhibit the Bcr-Abl kinase (as seen in the figure to the left) by binding to its active site. It is this complex that I aim to study using a computational approach.

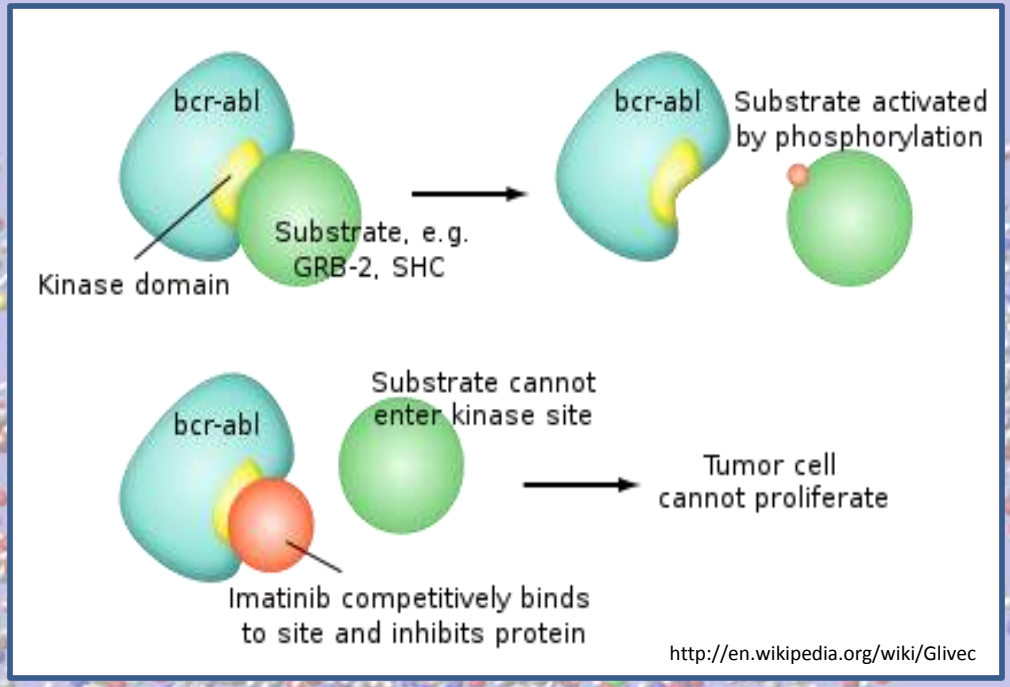


Figure 1. Depicts the biological mechanism by which the drug Gleevax® (imatinib) acts.

A Computational Approach

The foundation of this approach is characterized by the determination of the wave function (denoted with a psi, ψ) of the system which can be an atom, a molecule, or some combination. If we can determine ψ, we can calculate many different physical properties characteristic to the system such as dissociation energies, bond lengths and angles, etc.

How do we find ψ, the wave function? We apply Schrödinger's famous equation (figure 2) and the variation principle. Guess wave functions are chosen (a combination of hydrogenic wave functions, the most appropriate analytical wave functions we know) and the Hamiltonian operator ($\hat{H}$) is applied to determine the total energy (E). The guess wave function is then altered such that it will yield a lower total energy when the Hamiltonian operator is applied a second time. The variation principle says the total energy can never be less than the actual ground state energy. So, if we continue to change the guess wave function and obtain total energies that get lower and lower, we know we're approaching the actual wave function. Once the total energy stops changing considerably, we can say with some certainty that our final guess wave function is close enough to the actual wave function.

$$\hat{H}\psi = E\psi$$

$$-\frac{\hbar^2}{2m}\frac{\partial^2\psi}{\partial x^2} + V(x)\psi(x) = E\psi(x)$$

Figure 2. The Schrödinger equation: the general form (top) and the expanded form only using one dimension (bottom).

Different Computational Methods

Molecular Mechanics (MM)

- Newtonian mechanics are applied to molecular systems
- does not account for quantum effects
- potential energies for all systems are calculated using force fields

Hartree Fock (HF)

- A multi-electron ψ is written as product of one-electron ψ's
- The total ψ is represented as a Slater determinant
- The total Ψ is solved by the addition of the variation principle and solving the HF equations

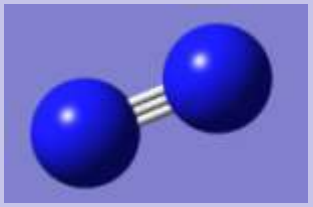
Density Functional Theory (DFT)

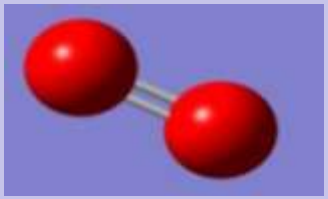
- Often shows better agreement with experimental values
- Energy is a function of electron density (ψ^2) instead of the wave function
- Solved iteratively similar to HF

Semi Empirical

- A step up from MM in that it begins to take quantum effects into account
- Solves the Hartree Fock overlap integrals and matrices using empirical spectroscopic data or ionization energies

Some Simple Example Applications





Tables 1 and 2 (right) show physical quantities calculated for O_2 and N_2 using different methods. Note that including more quantum effects by changing the method yields calculated values closer to the accepted values.

Note: the 3-21G basis set was used for the DFT calculation.

Table 1: Calculated Physical Data for Diatomic Nitrogen

Method	Bond Length	Vibrational Frequency
Semi Empirical	1.105 Å	2743.84 cm^{-1}
DFT	1.113 Å	2291.32 cm^{-1}
Accepted	1.12 Å	2,358.6 cm^{-1}

Table 2: Calculated Physical Data for Diatomic Oxygen

Method	Bond Length	Vibrational Frequency
Semi Empirical	1.085 Å	2093.79 cm^{-1}
DFT	1.3 Å	1386.99 cm^{-1}
Accepted	1.208 Å	1,580.2 cm^{-1}

Another Example Application

Figure 3 shows a small section of imatinib (Gleevax®) that was used as another example to demonstrate the differences that arise when utilizing different computation methods. A bond in the structure was chosen to see the differences in the corresponding dissociation energies.

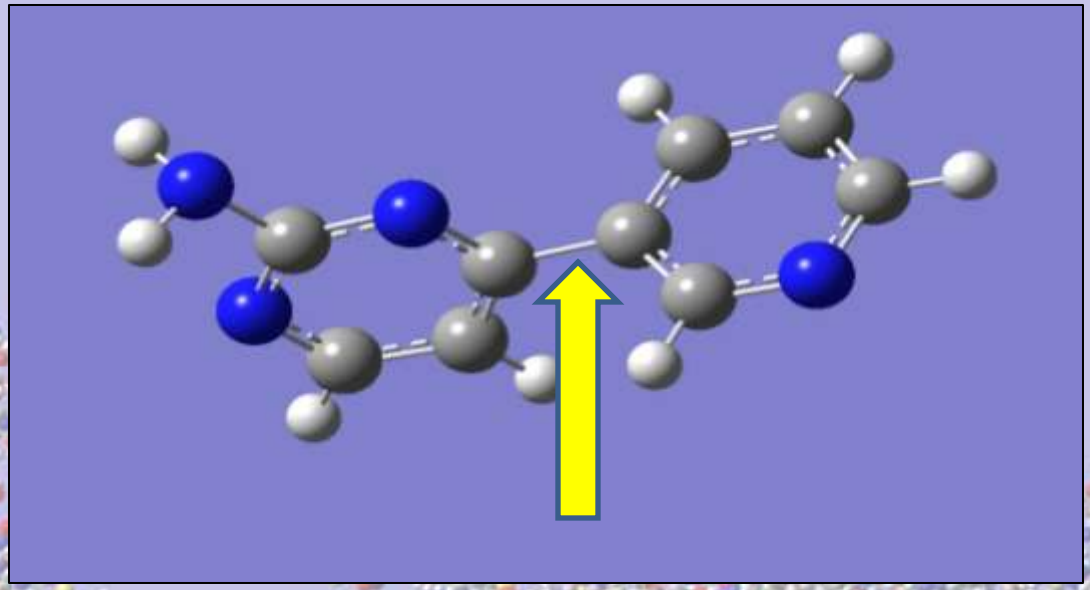


Figure 3. Shows the bond for which the dissociation energy was calculated using both semi empirical and DFT computational methods.

Table 3: Imatinib Segment Dissociation Energies

Calculation Method	Dissociation Energy
Semi Empirical	0.36233 a.u.
DFT	0.47831 a.u.

the ONIOM Method

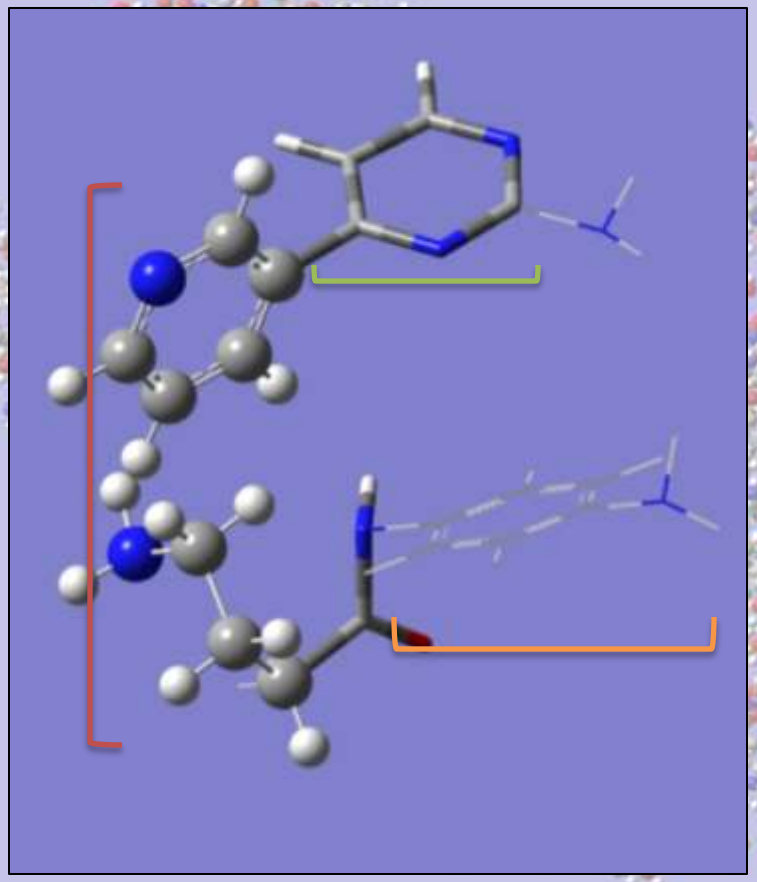


Figure 4. Shows an example of molecular “layers” for an ONIOM calculation. Each layer is represented by a different art style.

- The ONIOM method is characterized by the mixing of both Molecular Mechanics and Quantum Mechanics methods (MM/QM). It is frequently employed for larger structures where there is not enough computational power to do a complete QM calculation.

- QM is applied to the specific area of interest (the QM “layer”) and then MM or a semi empirical method, if the structure is small, is applied to the rest of the system (the MM or semi empirical layer).

The ONIOM Method and the Bcr-Abl Tyrosine Kinase Imatinib Complex

- the input file containing the Bcr-Abl tyrosine kinase complexed with imatinib was downloaded from an online protein database for use in the computational software *Gaussian 03W*

- the complex was optimized initially using MM

- because the complex is ~10,000 atoms, the ONIOM method was applied for the subsequent optimization
  - — 1 of the 2 binding sites (circled in yellow) was treated semi empirically at ~10 Å radially away from the bound imatinib molecule
  - — MM was applied to the remaining parts of the complex

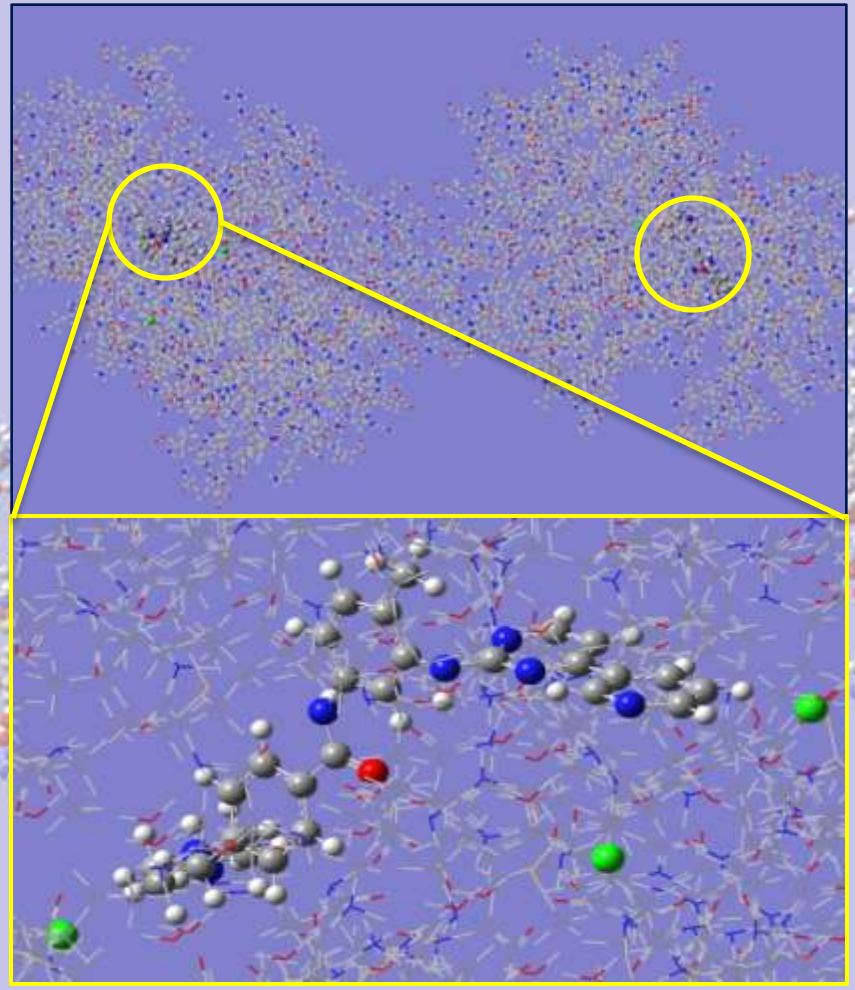


Figure 5. A close up view of the selected binding site

Some Initial Results

Table 4. Example bond length differences resulting from optimizing under different methods

Bond	Bond order	Location	MM	ONIOM (MM + SE)
1268(C)-1269(C)	single	Enzyme	1.50200 Å	1.50317 Å
507(C)-508(O)	double	Enzyme	1.25856 Å	1.25861 Å
534(C)-533(S)	single	Enzyme	1.82226 Å	1.82306 Å
532(C)-533(S)	single	Enzyme	1.82785 Å	1.82315 Å
1281(C)-1279(C)	aromatic	Enzyme	1.39992 Å	1.40047 Å
732(N)-733(C)	single	Enzyme	1.47004 Å	1.46972 Å
4485(C)-4500(O)	double	Imatinib	1.25988 Å	1.25918 Å
4484(N)-4482(C)	single	Imatinib	1.43908 Å	1.44151 Å
4474(N)-4475(C)	aromatic	Imatinib	1.36008 Å	1.36012 Å
4475(C)-4476(N)	aromatic	Imatinib	1.35944 Å	1.35986 Å
4467(C)-4471(C)	single	Imatinib	1.48274 Å	1.48375 Å
4470(C)-4469(N)	aromatic	Imatinib	1.35790 Å	1.35817 Å

Discussion

Considering the minute differences in the bond lengths between the MM and ONIOM optimizations, why bother even applying the ONIOM method?

Let's look at diatomic nitrogen again by varying the bond length and calculating the resulting total energy:

Bond length	Total energy
1.113 Å	-109.483892 a.u.
1.23 Å	-109.46619 a.u.

While the difference in total energy seems to be insignificant, 0.017702 atomic units, it can also be represented as 0.481696132 eV—a substantial deviation. Taking the sum of all these little energy differences around the binding site due to the bond lengths results in an overall drastically different energy.

Discussion and Future Work

Initial differences in bond lengths around the binding site and in imatinib itself were determined. Although some of these differences are relatively small, these values were taken prior to a complete optimization under the ONIOM method (still in progress). Once this is accomplished, another optimization will be performed adding a very small, DFT layer to specific areas around the binding site.

Using this optimized structure, I plan to exchange various functional groups on the imatinib molecule located in the binding site of the enzyme and investigate any changes energetically, hoping to find a better imatinib-like molecule that binds more effectively with Abl-Bcr tyrosine kinase.

Acknowledgements

I would like to thank Dr. Emily Jarvis for giving guidance and her time to assist with this project and my brother, Yuri Stippa, who suggested I study Gleevax®.

Works Cited

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PDB Files

- Downloaded from RCSB Protein Data Bank. April 28, 2009. http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=2HYY.