Computational Analysis of FRET type Fluorescence in Schiff Base Ligand Complexes

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Introduction

Metals are used in many biological functions and in other cases can lead to the detriment of both the cell and the organism. Therefore, it is important to track the presence or absence of some metals in the cell. A widely used way to track metals in a cell is through fluorescence spectroscopy; this is preferred to absorbance spectroscopy because of the low concentration of metal. Fluorescence occurs when an electron in an atom absorbs energy and raises to a higher energy level, when it relaxes back down to its ground state it gives off light. A subset of fluorescence spectroscopy is FRET. In FRET an electron is excited to a higher energy state, this is identical to normal fluorescence, however when the electron relaxes it transfers its energy to another electron, this electron can either be in the same molecule or in a separate molecule; it does this because it is faster for the electron to transfer the energy to another electron than to fluoresce. This new electron will fluoresce to relax to the ground state (see figure 1). FRET can be used to look at conformational changes because it only occurs when the two molecules or moieties are close together and have parallel pi systems that are facing each other.1 This means that a molecule could begin to fluoresce after a conformational change. This is especially useful when looking at ligands because if a metal is present and induces a conformational change that would cause the ligand to exhibit FRET then it could be used to track the presence or absence of that metal. This is useful for tracking metals that may serve an important biological role.

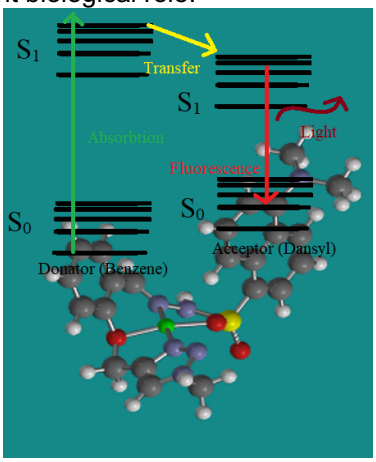

Figure 1: *An example of FRET based fluorescence*

Computational analysis can be used to show the structure of molecules that exhibit FRET as well as predict whether untested molecules will exhibit FRET or not. Two types of calculations that can be done to calculate the structure of these molecules are, molecular mechanics force field calculations and quantum mechanical calculations. The molecular mechanics force field calculations rely on classical mechanics between the bonds as well as interactions between atoms including van der waals interactions. These calculations are faster; however, these calculations are less accurate than the quantum mechanical calculations, so they are often used as a base for further testing. The quantum mechanical calculations look for where the electrons are likely to be on each atom and provides an estimate of what the structure would look like. This type of calculation is slower and is likely to better predict the structure of the molecule. Using these techniques computational analysis was carried out on ligands that exhibited FRET as well as ligands that were predicted to exhibit FRET.

Experimental

A ligand that was known to exhibit FRET (this occurred when the ligand was bound to Nickel) was placed into Spartan software which then calculated its lowest energy structure using molecular mechanics force field calculations and quantum mechanical calculations. This was tested when the ligand was bound to aluminum, calcium, cadmium, cobalt, copper, iron, mercury, magnesium, nickel, and zinc ligand (see figure 2). After this was complete other potential ligands were designed and tested using the using the same metals and methods. Once this was complete the ligands were analyzed and compared to the ligand that exhibited FRET. Interesting discoveries were made concerning distance, orientation, and pi overlap in regard to whether the ligand was thought to exhibit FRET. After this the data from the ligands were compiled and analyzed to determine the likelihood of FRET based fluorescence (see figure 3).

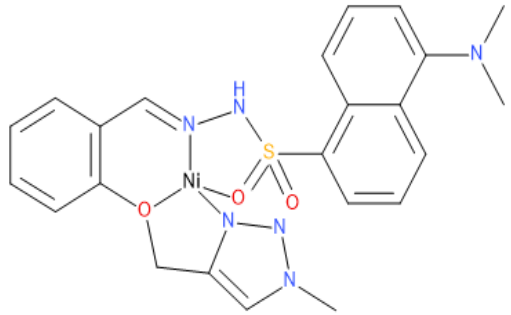

Figure 2: *An example of one of the metal complexes tested in this survey. This complex was tested and found to not exhibit FRET.*

	Al	Ca	Cd	Co	Cu	Fe	Hg	Mg	Ni	Ni
A		3, 2	3, 3	2, 2	2, 2		2, 2	2, 2	2, 2	2, 2
B		3, 3	2, 2	2, 2	2, 2		2, 2	2, 2	2, 2	2, 2
C		2, 3	2, 2	2, 3	2, 2		2, 2	2, 2	2, 2	2, 2
D		2, 2	2, 2	2, 2	2, 2		2, 2	2, 2	2, 2	2, 2
E	2, 2	3, 3	2, 2	2, 2	2, 3	2, 2	2, 2	2, 2	2, 2	2, 2
F	2, 2	2, 2	2, 2	2, 2	2, 4	2, 2	2, 3	2, 2	3, 1	2, 2
G	2, 2	2, 2	2, 2	2, 2	2, 2	2, 2	2, 2	2	2	2
H	2, 2	3	2, 3	2, 2	2, 2	4, 4	2, 2	2	2	2
Student 2			2, 2	2, 2	2, 2				1, 1	2, 2

Figure 3: *Evaluation of the calculation of the complexes using the code: 1, pi facial overlap , 2, no overlap , 3, ambiguous , 4, may need recalculation The results of the testing of metal complexes were compared between two students to analyze which complexes were likely to exhibit FRET.*

Results

The ligands that were predicted to exhibit FRET had three interesting similarities that are likely necessary for FRET to occur. First, distance between the two ring groups is an integral part of FRET. FRET is usually only seen in very short distances.2 This means that the ligand that exhibited FRET and those that are predicted to exhibit FRET all have two ring groups that are extremely close. The second factor that can be seen is the orientation of the two rings to each other. The rings must be parallel to each other in order to exhibit FRET. This seems to be necessary to transfer energy from one ring to another, because some of the tested ligands that did not exhibit FRET were close together but had rings that were not parallel. This factor is related to and affects the third factor. Pi overlap is the last factor that is necessary for FRET. While observing the HOMO and LUMO of the ligand that produced FRET it was noted that the likely place of the electrons went from one ring to another. This means that if an electron were excited into the LUMO energy level then it would be transferred into another ring. This seems to be a defining feature of FRET and has been tested by others.3 All three of these factors are necessary for FRET to occur. A theory for why this occurs is that when the ring transfers an electron from itself to another ring it also gives energy to the other ring which will then produce FRET. This is a reasonable theory because of the pi overlap as well as the orientation of the two rings. Due to the complexity of FRET it is hard to predict which molecules will produce FRET and which ones will not, however it seems likely that this pi overlap is essential to FRET and can be used as a way to predict if a ligand will exhibit FRET. This is a very useful gage that was used to predict if untested ligands would exhibit FRET. This allowed for the selection of metal complexes that might exhibit FRET when compared to the majority of metal complexes that could not exhibit FRET.

Conclusion

The information on the predicted parameters for FRET to occur as well as the data received from the models of the ligands was used to create a list of candidates that are likely to fluoresce using FRET. This list will be used to help synthesize ligands that will be useful in experimenting with FRET. These ligands will be cheaply manufactured and will be relatively small. Through this testing the lab will be able to see if the predictions made in this study were correct and to perhaps find ligands that only produce FRET under

one metal to allow for specificity. The end goal of this project is to create cheap ligands that exhibit FRET when certain metals are present in order to look at various biological functions. This study has concluded that there are certain parameters that a ligand must maintain to exhibit FRET and a list has been compiled of likely candidates. In the future this will be utilized to create ligands that will hopefully validate the predictions made in this study.

REFERENCES

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