Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 20 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

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New Mixed Vibrational-Electronic Methods in Fully Coherent Multidimensional

Spectroscopy: Progress Toward Metal Active Site Characterization

By

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Appendix A

For the public

A.1 Light-matter interaction in science

The great challenge of chemistry and many related fields of science is to determine what is happening right in front of you. Many of the important things we now study happen on a microscopic level – a drug binding to a receptor, a protein changing one molecule into another, chemical reactions depleting the ozone layer, or a solar cell turning light into electricity. In order to understand and control these things, we must first know what is happening, and be able to determine cause and effect. Observation is integral to the scientific method, but we need tools to match our senses to these microscopic processes.

It turns out the key tool in a vast number of cases is some kind of interaction of light with matter. Broadly, we refer to this as spectroscopy. The subdivisions of spectroscopy can seem infinite and I will only address a very small subset of them here. So why use light? The short answer might be that how a molecule or material interacts with light acts as a signature of sorts. The long answer – actually understanding why this is true – relies upon some basic understanding of quantum mechanics. Let's start there.

A.2 Quantum mechanics: it's not all Schrodinger cats

For most people, the term quantum mechanics (QM) conjures up an image of the popularized half-dead, half-alive cat, and all of its philosophical implications. But while those concepts are interesting, they actually constitute an extremely small part of what QM teaches us about the world – the rest is actually quite practical and well-defined! The field of QM seeks to describe how matter behaves, much like classical mechanics did before it. You may remember considering problems like billiard balls colliding or objects falling from the sky in an elementary physics course. Equations described their interaction in terms of mass, velocity, and so on. The key variable in these problems was energy (remember? kinetic? potential?), and the reason for this is the all-important thermodynamic Law of Conservation of Energy. If energy is conserved, it's basically the best choice for describing how something *is*.

Classical mechanics works great for large things. But around the turn of the 20th century, things got tricky when scientists discovered that not just light, but actually all matter, has a wave-like nature on a microscopic scale. Quantum mechanics acts as a patch on classical mechanics where this wave-like nature is accounted for, which is important whenever some part of the problem you're considering is small enough to have important wave properties (like light and electrons). Variables like momentum could then no longer be described as a product of mass and velocity, but needed a wave-like formalism.

When physics problems are transcribed into this new language, they need to be solved by a master equation called the Schrodinger Equation. In chemistry, the type of problem you need to solve is "how do I describe the interaction of this nucleus with the electrons around it?" and "how do I describe the interaction of this atom over here with those atoms over there?". The variables that come into play are whatever particles are involved – the nuclei, the electrons, and so on. You basically set up one of those billiard ball problems, but with a translation into quantum mechanical language (treating the wave-like nature).

This treatment results in a fairly beautiful result. It turns out that molecules can be very well described by a combination of three properties. The first is their "electronic state" – the nature of their electrons. These states will be based upon the s, p, d, etc. orbitals that describe where in space the electron is mostly likely to be, pictured in A.1(A).

The easiest way to understand why these orbitals form is to imagine an electron living on a circle in 2D space. Now give it a wavelength, and draw out its oscillations as you proceed around the circle. Only circles of certain diameters will result in exact overlap of the electron wave with itself when it gets back to where it started (Fig. A.1(B-D)). Therefore only those diameters, or states, are "allowed". In three dimensions instead of two, the answers look like the s, p, d, and f orbitals (in order of increasing energy) in Fig. A.1(A), rather than a simple circle. Only two electrons can sit in each orbital, so your molecular system will have a bunch of electrons in a bunch of different orbitals – filled from lowest energy, up.
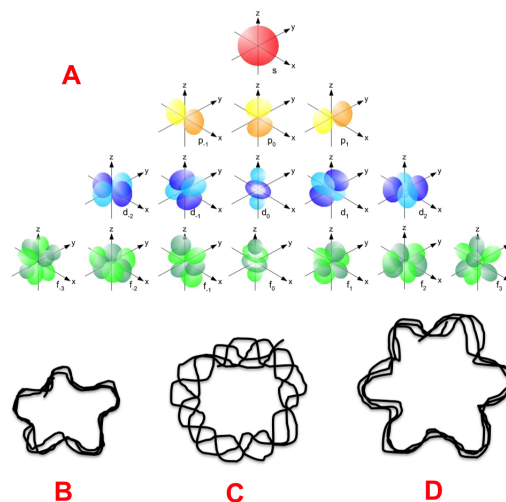

Figure A.1: (A) The shape the electron cloud takes in s, p, d, and f orbitals., from top to botttom.[121] (B-D) An electron of fixed frequency at varying radii around a nucleus, where (B) and (D) are allowed to exist, and (C) is not.

The second component found to be important in describing molecular systems is their "vibrational state". Application of Schrodinger's Equation to molecules reveals that the nuclei within them vibrate against each other as if connected by tiny springs. The frequency of the vibration depends on multiple factors, but the biggest is the mass of the nuclei involved. This is critical because it means a C-O bond vibrates at a different rate than an O-H, and so on, beginning to hint at how this characteristic can be a useful

signature. The frequency of a state is always proportional to its energy. The energies of vibrations are all much lower than the energies of electronic states.

The final component is "rotational state". In addition to vibrating against one another, subsets of the molecule can also rotate around one another. This final property is of significantly lower energy than vibrational energies and is usually drowned out in the liquid phase, where my research is performed. Therefore in my thesis work we can safely ignore it.

So, to recap, we can describe my molecular systems in terms of their electronic and vibrational states. Light is going to help us find out what those states are, which in turn theoretically describes how the molecule looks and behaves completely.

A.3   How to think about light-matter interaction?

We now know how to think about the energetic states of a molecule, and can picture how each has a wave-like component. This allows us to consider the impact of light. Light is an electromagnetic wave, whose wave-particle duality breaks it up into discrete photons, or *wavepackets* (Fig. A.2).

Picture two nuclei (say, in the molecule carbon monoxide, CO) vibrating back and forth. Then imagine a photon coming along with its oscillating electric field. When the field is positive, it will tug the nuclei one way, and when it is negative it will do the opposite. If it is synchronized correctly with the frequency of the vibration (if it is *resonant*), that vibration can actually take up the energy of the photon and convert it to an increased rate of nuclear vibration. A useful analogy is a child being pushed on a swing, where the child swinging is the molecule vibrating, and the adult
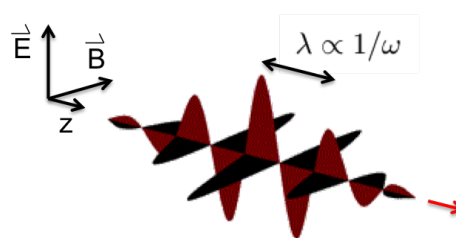

Figure A.2: Wavepacket of light containing an electric ($\vec{E}$) and magnetic ($\vec{B}$) field oscillating with frequency ω (wavelength λ) under an envelope defining its location as it propagates in space (z).

pushed on a swing, where the child swinging is the molecule vibrating, and the adult

pushing is the light oscillating. Quantum mechanically, this uptake of energy is equivalent to the vibrational state being promoted to a more energetic level (see Fig. A.3A) – one where it vibrates faster.
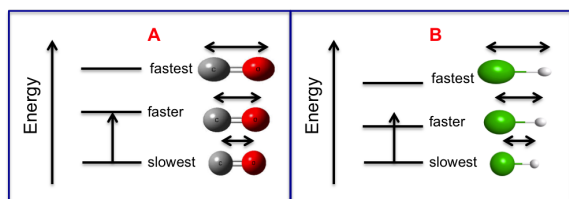
If the vibration is at the wrong frequency, like trying to push a child on a swing at completely random times, its effect will wash out, and the photon will not be absorbed. Therefore, if you send in light at a frequency corresponding to a CO vibration, CO molecules will absorb it, but HCl molecules will not (Fig. A.3B). Measuring what frequencies of light your sample is able to absorb (absorption spectroscopy) is a primary tool for determining what is in an unknown sample.

Figure A.3: A simplified *energy level diagram* of (A) the C-O stretch. The ground state has the slowest oscillation, but a resonant photon will excite the molecule to more rapid oscillation. There are an infinite number of states, analogous to the harmonics of a guitar string. In (B), the H-Cl vibration has different frequency, and so the same photon will fail to be absorbed.

Using light's infrared region, vibrational absorption tells you about the kinds of bonds present in your molecule. In the ultraviolet (UV) and visible regions, absorption instead tells you about the electronic states. Almost all electronic transitions occur in the UV, but a special subset occur in the visible. This subset is largely composed of so-called "d-to-d" transitions, where an electron is being moved from one type of d-orbital to another. These d-to-d transitions are characteristic of *transition metals* (Fig. A.4). Because transition metals are responsible for some incredibly important chemistry, this particular spectroscopic handle is very useful.

Figure A.4: Outlined are the d-block transition metals of the periodic table of elements.[122]

A.4   Some systems we're interested in

The need for additional techniques that would aid understanding of photosynthetic water splitting motivated my thesis work. Water splitting is the process of turning H_2O into O_2 gas plus either H_2 gas or H^+ ions and electrons, depending on the situation. It is the first step of photosynthesis in plants, algae, and bacteria, termed photo because it is driven by light.

Scientists would love to artificially reproduce this reaction. If we could use light to split water, we could collect the hydrogen gas product and use it as a fuel. Hydrogen combustion is clean – since it has no carbon atoms, its only product besides usable energy is water again. If the water splitting reaction was performed without consuming electricity, it would generate a clean, renewable fuel.

Unfortunately, water splitting is a famously difficult reaction to cause because oxygen is fiercely protective of its electrons. Between the pair of water molecules this reaction requires, four electrons have to be removed from the oxygen atoms (as well as four hydrogen ions) before they will be able to stabilize each other by forming O_2. To successfully perform the reaction, either all four electrons need to be removed at once, or

the unhappy intermediates need to be stabilized just right by a carefully designed environment.
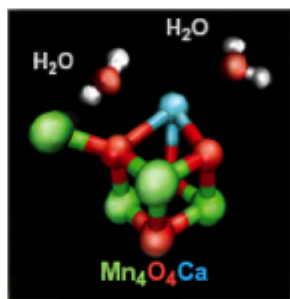

Figure A.5: A cartoon of the Oxygen Evolving Complex.[123]

The protein that achieves this reaction uses a little of both of these strategies. The water molecules are cradled by an Oxygen Evolving Complex (OEC) composed of a strange cube containing 4 manganese (Mn) atoms (Fig. A.5), as well as a number of carefully positioned amino acids. Each time light strikes the protein, its absorption by chlorophyll causes a cascade of events resulting in the removal of an electron from the OEC. The removal of the electron results in a charge the protein does not like, so an H^+ is also kicked out to neutralize the system. After this happens four times, only two oxygens remain from those water molecules, allowing O_2 gas to form and the process to start over.

Importantly, the OEC eases this process by initially removing electrons from the manganese atoms rather than the oxygens, allowing the electron deficiency to build up elsewhere until all four charges can be taken from the oxygens at once. Removal of electrons from the Mn atoms occurs more easily because transition metals have the critical property of being able to stably hold a few different charges. It is this property that makes them so incredibly important in chemistry. In fact, about half of all proteins have transition metals in them, and when they do, the metal often serves as an electron acceptor or donor that allows chemistry to be carried out just like this. It is the reason why many minerals are essential to our diet.

In addition to deferring the electron removal from oxygen to one swift event, the protein also stabilizes the hydrogen-depleted intermediates. Both the water molecules and the OEC are in an extremely fragile state throughout most of this process, but the protein carefully reorganizes itself to stabilize the intermediate structures until the next step occurs.

Though we know much about how this process works, there is still a lot that we

don't know. We aren't sure which Mn atoms accept the charge, or why. We don't know much about the evolution of the structure from one step to the next, or which parts of the chemical design are most critical. Until we know these things, it will be difficult to mimic this design artificially.

The OEC is enormously difficult to study because it is so small compared to the entire protein complex in which it is embedded. The vast machinery surrounding the core is necessary to coordinate light harvesting, electron and water motion, and overall structure, but it means that almost any experiment you do will have an incredible background from the protein itself. For example, if you took an absorption spectrum of this protein, the signal from one amino acid near the core would be perhaps $1/7000^{th}$ the contribution of the whole protein. For this reason, absorption spectroscopy is barely used when studying this process. Instead, a broad range of other techniques have taught us most of what we know today. These range from a spectroscopy based upon electrons' responses to magnetic fields, to non-spectroscopic techniques like the diffraction of light off a crystal made of this protein, and even advanced computational techniques. My research as part of the Wright group seeks a new tool to add to this toolbox.

There are a number of related problems that new techniques in this field would also address. As mentioned before, metal centers are extremely common in proteins. Proteins are in turn a widely popular field of study for their key role in all biological functions and for the insight they provide into successful chemical reaction mechanisms. You may be familiar with hemoglobin, the protein that carries oxygen through our blood (Fig. A.6). We understand how this works now, but we once had questions like, which amino acid does iron bind to, and why? And, how can I be sure O_2 binds to *this* protein? Or, does carbon monoxide bind to *this* protein when it gets into our blood? (The answer is yes.) And why does it *stay* there once it does?

One can also imagine using a technique like this to study an artificial catalyst (a molecule humans create in order to make a reaction happen repeatedly), and try to understand its function by following changes to its structure as it reacts.
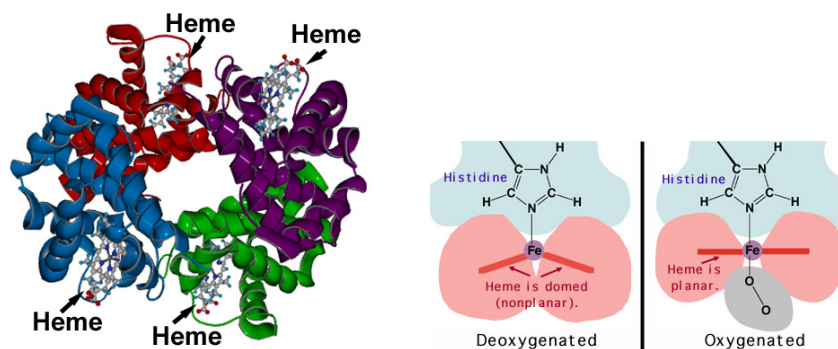
Figure A.6: (a) The protein hemoglobin, with its four attached heme molecules in grey. (b) Zooming in on the planar (flat) heme molecules, and viewing them from the side. You can see there is an iron (Fe) atom in the center of them, and that O_2 binds to that metal, which in turn binds to the amino acid histidine – part of the hemoglobin protein. The heme bends when binding occurs.[124]

A.5 What does multidimensional spectroscopy contribute?

The Wright group works within a field called coherent multidimensional spectroscopy (CMDS, sometimes also called *nonlinear* spectroscopy). To understand CMDS, imagine the following example: You have the idealized infrared (IR) absorption spectrum shown in Fig. A.7. It has five types of vibrations represented by five peaks, so you can assume that you have five types of vibrations in your sample.

Fig. A.8(B) is a hypothetical, simplified two-dimensional spectrum of the same sample. What we are doing in this two-dimensional spectrum is asking whether, at each (x,y) point in the grid, the vibrations occurring at the frequencies on the x- and y-axes are "coupled" to one another. You can think of coupling as broadly meaning *impacting one another*. The ability for two vibrations to impact one another is correlated to how spatially near to each other those sets of atoms are (among other things). If the two vibrations at x and y are coupled, there will be a peak at (x,y) in the plot. Along the diagonal of A.8(B), where $x=y$, you essentially reproduce the IR spectrum (A.7) because all vibrations are inherently coupled to themselves.
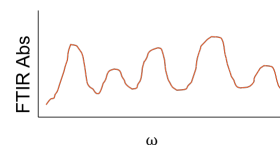

Figure A.7

In Fig. A.8(B), the three vibrations in orange
are coupled to one another, and the two in red to one another,
but coupling is not seen between them. This might be due
to a situation like that shown in Fig. A.8(A), where two types
of molecules are present in the sample, represented by the
two strings. One string contains the two red clusters of atoms
composing the two vibrations in red in Fig. A.8(B), and
the other string contains the three orange vibrations. Because
these sets of vibrations are localized on completely different
molecules, they cannot "talk to" one another. This is an
example of how the two-dimensional spectrum can teach us something about structure
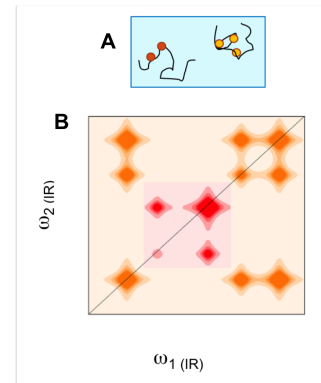that was not evident in the linear spectrum.

Figure A.8

This two-dimensional spectrum might be useful in this rather simple case where
only a few vibrations are present, but in reality most samples are *far* more complicated
than this. Most of my thesis research was related to developing a technique that was 3D
rather than 2D. Importantly, the third axis contains electronic transitions instead of
vibrations. The idea was this: imagine your new sample's IR absorption spectrum is now
the shapeless grey trace shown on the left in Fig. A.9 because there are a huge number of
overlapping peaks. You know those same vibrations from the first case (A.7) are present
(the orange peaks below the grey trace), but they are buried beneath signal from solvent
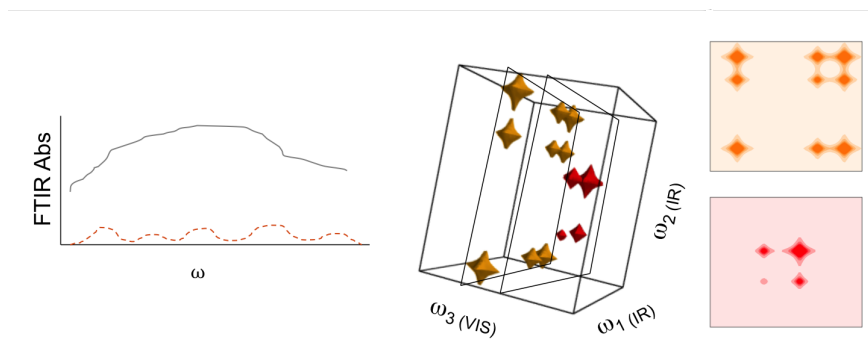and other solutes.

Figure A.9

Now imagine you collect the three-dimensional spectrum in the middle of Fig. A.9. Similar to before, we are now asking whether the transitions on the x-, y-, and z-axes are all coupled to one another. At each point in the three dimensional space, a peak only appears when the answer is yes. Imagine that these molecules in Fig. A.8(A) are colorful – they have visible electronic transitions – and the rest of the sample does not. (The solvent will have electronic transitions in the UV.) If those two molecules are different colors, their peaks will show up at different locations along the z-axis (or ω_3-axis), and everything else in the sample will appear outside the range probed.

Further, you can fix the visible frequency to one that interacts with just one of those molecule's electronic transitions and collect a 2D spectrum within that plane, as is drawn. This experiment would result in one of the spectra on the right of Fig. A.9, where much background has been removed relative to the standard infrared absorption spectrum due to the criterion of resonance with a unique electronic transition.

I pursued this type of experiment in my thesis work. Since transition metals often have unique visible electronic transitions, this type of experiment should help "see through" the sample background, such as the protein, and learn about vibrations – atoms – around the metal.

A.6   My specific results

My graduate work resulted in a new technique that works roughly as I've described. I was able to observe vibrations in my technique we couldn't see in the IR absorption spectrum. Figure A.10 shows the result. This 2D scan was taken with a visible frequency fixed to be resonant with an electronic transition of a molecule that is at very low concentration in my sample.[75] The blue line above is the IR absorption spectrum for the same range. The two peaks are due to solvent and a different molecule, not the molecule that appears in the 2D spectrum. Those two peaks are not observed in the 2D spectrum, because their electronic transitions are down in the UV. Therefore, I have successfully taken a slice through a 3D space as I described in Fig. A.9.

I have also collected preliminary evidence that we can "see through" proteins with this method in at least one case, shown in Fig. A.11. In this experiment, I used my 3d method on two forms of chlorophyll to find out what their 2d spectra looked like when the visible beam was electronically enhanced. I then looked at the photosynthetic protein complex I mentioned before using that visible frequency. The IR absorption spectrum of the protein, shown in the top figure, is a big smear because there is a lot going on. The diagonal slices

Figure A.10

of the 2d spectra of the chlorophylls and the protein are overlaid in the bottom plot. The protein clearly has four peaks it does not have in the IR absorption spectrum in the top plot, and those four peaks clearly correlate to chlorophyll. Therefore, we have preliminary evidence for using this method on proteins.
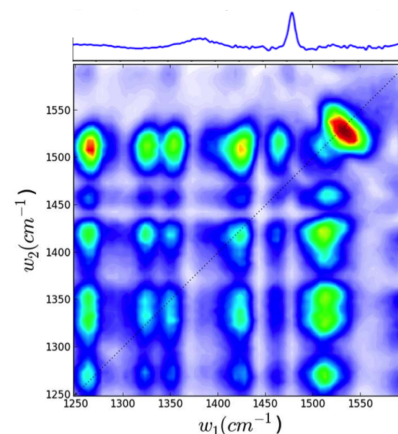
There are promising corollaries related to these discoveries, but one major step I haven't taken is to examine a sample containing an actual metal center. Both of these molecules had electronic transitions in light's visible region for a different reason. Future graduate students will be working on addressing that goal, as well as expanding on the capabilities of this technique now that the ground rules have been laid.

Though research of this kind can seem esoteric because of the minutiae that must be considered during its development, I believe this work represents a meaningful step forward within the field of CMDS. We hope this technique will be useful for understanding the
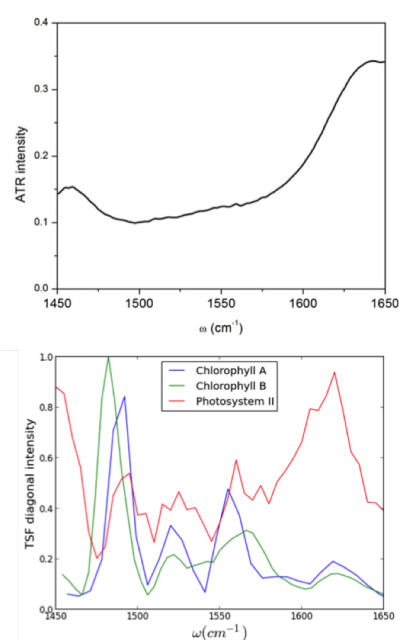

Figure A.11

behavior of biological and non-biological catalysts in the

near future. But it is always hard to predict where a

technique will go. A field quite similar to CMDS – NMR – began being seriously

developed back in the 1940s. It eventually expanded to being the most widely used

analytical technique in chemistry, as well as spawning other inventions such as the MRI,

which is ubiquitous in medical imaging. The work in my thesis lays a brick in the wall of

similar efforts, and we hope that it, too, will one day evolve into something so useful to

the general public.