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 50 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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Spectroscopic and Computational Investigations of the Cobalamin Containing Enzymes EutT, CblC, PceA, and QueG

By

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Katrina T. Forest, Professor, Bacteriology

CHAPTER EIGHT

Applications of B_{12} Chemistry to Trichloroethylene Respiring Bacteria

(Chapter Four for a Broader Audience)

This chapter was written in fulfillment of the requirements for Wisconsin Initiative for Science Literacy Graduate Thesis Award for Communicating Research to the Public

I have written this chapter to explain my research to a broad, non-scientific audience. While scientific research is performed with the goal, on some level, of being useful to a broader audience, often scientists' results and contributions are obscured by technical jargon and are only available behind paywalls. As the majority of my research was funded through taxpayer dollars (through the National Institute of Health and National Science Foundation), it is important to be able to share the content and impact of the research to a non-specialized audience. Indeed, science communication is a fundamental component of our responsibilities as scientists. Many thanks to the Wisconsin Initiative for Science Literacy at UW-Madison for providing this platform, and for sponsoring and supporting the creation of this chapter.

8.1 Introduction

Did you know that bacteria need some of the same vitamins and minerals as humans? My research has centered around the chemistry of derivatives of vitamin B_{12}. Vitamin B_{12} is a cobalt containing molecule also known as cyanocobalamin. The "cyano" prefix indicates that a cyanide molecule is directly bound to the cobalt. The molecule bound to the cobalt is known as a ligand. Just like a Gameboy plays different games depending on what cartridge you put in, you can swap cobalt's ligand to initiate different chemical reactions. Similar to how the Gameboy isn't changed depending on the game inserted but is necessary for the game to be played, the cobalamin derivatives serve as catalysts where they enable a chemical reaction but are unchanged in the end.

In our bodies, the molecule bound to the cobalt ion in vitamin B_{12}, the "cyano" ligand of cyanocobalamin, is exchanged with other molecules before the cobalamin can be used. Similarly,

bacteria scavenge B_{12} from their environment however they can get it and then recycle the cobalamin into whatever form is needed. Essentially, bacteria can take whichever Gameboy game came with the system and exchange it for the game they've been dying to play. One such B_{12} derivative has a water molecule bound to the cobalt creating aquocobalamin. The enzymes containing aquocobalamin were discovered relatively recently and it hasn't yet been determined how aquocobalamin catalyzes reactions, such as the conversion of trichloroethylene (TCE) a toxic dry-cleaning solvent, to a less toxic derivative (Figure 8.1). This process naturally aids bio-remediation by replacing TCE in the environment with a less toxic compound. I have spent a good part of my PhD studying how aquocobalamin can catalyze that reaction. In order to understand how we approached trying to understand how aquocobalamin does what it does, it is important to know a bit more about how other vitamin B_{12} derivatives work.

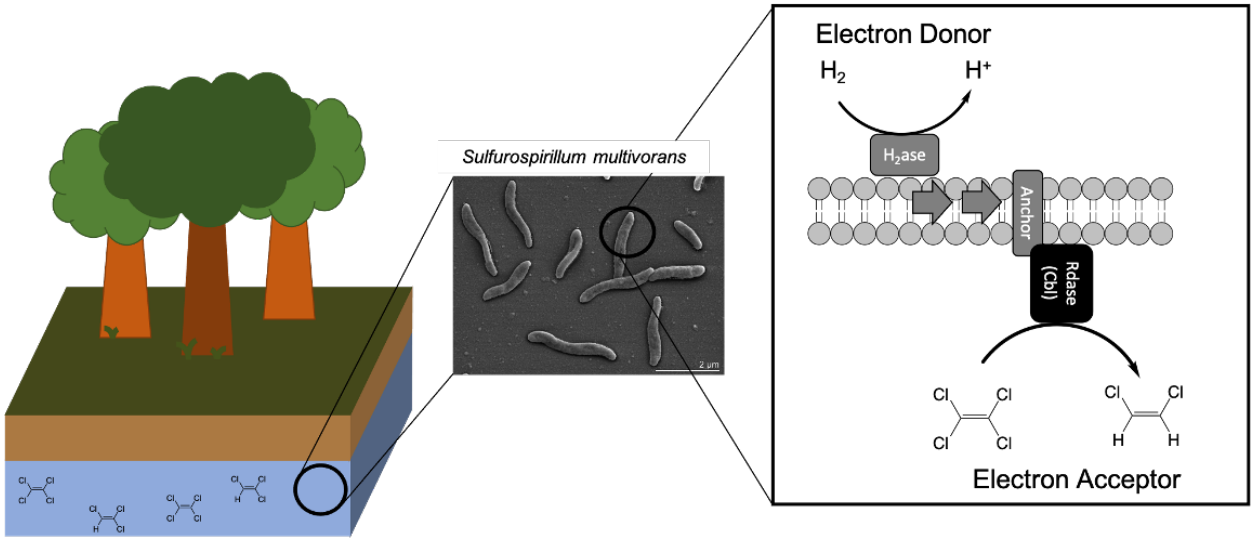

Figure 8.1 Role of PceA in bioremediation. TCE has polluted the environment in various locations including ground water. Organisms in these locations, such as *S. multivorans* naturally aid bioremediation by reducing the number of chlorine atoms on a substance as part of the organism's growth cycle.

8.2 The Vitamin

Let's take a step back for a minute – why don't bacteria, or humans for that matter, use the vitamin form of cobalamin with the cyano ligand? It turns out that the molecule bound to the cobalt ion, in this case cyanide, largely determines what type of chemical reactions the cobalamin can catalyze, and cyanocobalamin doesn't catalyze any useful reactions. Why is cyanocobalamin known as vitamin B_{12} then? When scientists were trying to identify and isolate the essential nutrient found in lamb and other livers, they first found the cyanide bound form of cobalamin (and the discovery resulted in the 1934 Nobel Prize in Medicine). Cyanide binds tightly to the cobalt so the isolated compound was identified as cyano-cobalamin. The three-dimensional structure of cobalamin was determined by Dorothy Hodgkin in 1955 confirming that the cyano ligand was bound to the Co (which, along with other structure work, led to the 1964 Nobel Prize in Chemistry). It wasn't until the late 1950s and 1960s that the active forms that are so important for human health, were identified (Figure 8.2).

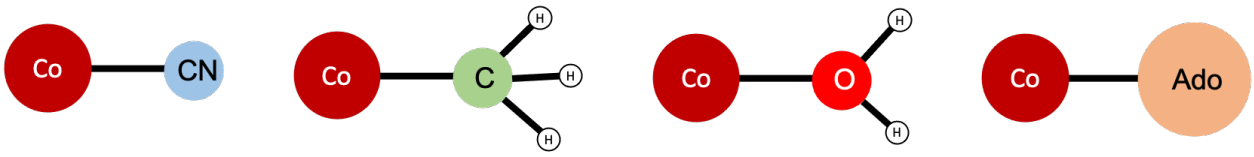

Figure 8.2 Common ligands for Cbl. Left to right the molecules represented are, cyanocobalamin, methylcobalamin, aquocobalamin, and adenosylcobalamin.

Whether in humans or bacteria, the active forms of cobalamin react in predictable ways. In general, the reactions catalyzed by adenosylcobalamin involve an unpaired electron, or radical. Radicals aren't overly stable, so the cobalamin and molecule being altered play a game of hot potato which rearranges the atoms of the starting molecule and ultimately regenerates the

adenosylcobalamin. These molecular rearrangements are necessary to form a compound which our bodies use to ultimately make heme to carry oxygen in our blood. On the other hand, methylcobalamin donates the methyl ligand to molecules in our bodies to form compounds including an important factor for the growth of new blood vessels. Since the methylcobalamin gave away the methyl ligand during the reaction, passing along its Gameboy game, another molecule in the cell has to provide a new methyl, a new version of the same game, to reform the methylcobalamin. Because aquocobalamin was discovered more recently, we don't yet know how it catalyzes molecular reactions.

8.3 The Enzyme

The enzyme that degrades TCE is known as PceA. The bacteria that I study that produces this enzyme was originally isolated from "toxic sludge" which is a fancy way of saying it was first found in sewage. Because the bacteria are found in environments where TCE has polluted the environment, such as in ground water, PceA is able to naturally aid bioremediation by replacing the toxic TCE compound with a less toxic derivative.

Let's talk more about PceA itself. PceA is a protein or, more specifically, an enzyme. Proteins are large molecules built up of building blocks called amino acids. Some amino acids just help the protein form the right shape, while other amino acids have special roles like assisting in chemical reactions. Proteins can have a wide range of functions from transport (hemoglobin in blood), to structure (keratin in skin, nails, etc) to storage like albumin (keeping fluid in your bloodstream). A protein that catalyzes a biochemical reaction is called an enzyme. PceA requires a molecule of aquocobalamin to function properly. If you were to take a molecule of cobalamin outside of an enzyme and try to have it do any of the chemical reactions we've talked about, you

might get some reaction, but it won't be terribly efficient. That's why organisms, such as bacteria and humans, bind cobalamin enzymes. Enzymes that bind to cobalamin, like PceA, serve multiple purposes. Primarily the enzyme a) prevents the cobalamin from floating away inside the organism and b) tunes the function of the cobalamin. The enzyme acts similar to a trellis for a rose bush. The rosebush will grow on its own, but the trellis helps guide the growth in a desired way. Specifically, the amino acids in PceA interact with the cobalamin such that there is a water molecule bound to the Co ion. PceA also requires two iron-sulfur clusters. These clusters serve like basecamp for electrons. The aquocobalamin is the summit of the mountain where the reaction takes place, but electrons can't go directly there. Instead, the electrons can be briefly stored in the iron-sulfur clusters so that they're available to be used on short notice.

8.4 The Toolkit

How do you figure out the mechanism for a process you can't directly observe? We have a tool kit of various spectroscopic and computational techniques that we can use to try to piece together the molecular mechanism. Just like doctors can use X-rays to get information on bones, or MRI to get information on soft tissues in the body, I can use electronic absorption (Abs) and magnetic circular dichroism (MCD) spectroscopies to get complementary information.

Electronic States

In order to better explain how Abs and MCD work, let's review some general chemistry. Cobalt, the central atom in a cobalamin molecule, sits at atom 27 on the periodic table. This places Co on the first row of the transition metals between Fe (iron) and Ni (nickel), arguably the better-known neighbors. While uncharged Co has 27 electrons, not all of these electrons are available for

chemical reactions. Only the electrons furthest from the nucleus, the valence electrons, can be borrowed or shared in chemical reactions. Losing an electron from the valence shell results in a charged species (Figure 8.3). In the case of Co in a cobalamin molecule, the cobalt prefers to be in the +1, +2, or +3 oxidation state meaning it is most stable when it has lost 1, 2, or 3 electrons.

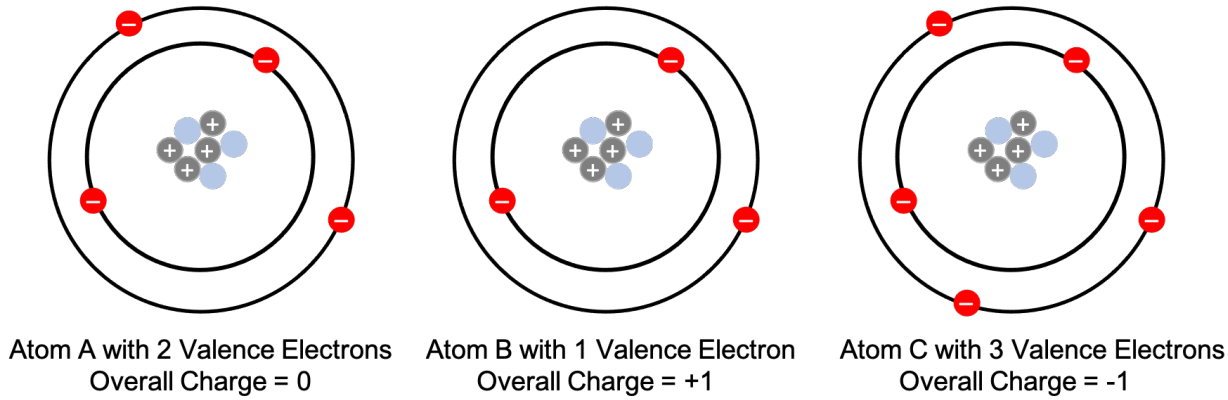

Figure 8.3 Valence electrons. The electrons can be lost or gained in the orbital furthest from the nucleus. Atom B and Atom C could share Atom C's extra electron to both have a neutral overall charge.

In a standard oxygen atmosphere, the +3 Co ion (which can also be written as Co^{3+} or Co(III)) is most stable. The Co(II) and Co(I) states can only be reached when there is little or no oxygen present. Why are these three Co ions more stable? Because of where the valence electrons are being stored. You might remember the octet rule from general chemistry where many atoms prefer to have 8 electrons in the valence shell and will borrow, steal, or share electrons to reach that magic number. For transition metals, such as Co, the rule is a little more flexible. In general, electrons are placed in the lowest energy compartments, or orbitals, first before filling the orbital next higher

in energy. For the relevant Co ions, the d_{z^2} orbital is empty for Co^{3+} and filled with one or two electrons for Co^{2+} and Co^{+} respectively. Adding or removing an electron from a different orbital has a much higher energetic cost (i.e. it can happen, but only in high energy conditions). The d in the d_{z^2} orbital indicates what type of orbital it is (compared to s, p, or f), and the subscript z^2 denotes the shape. The d_{z^2} orbital is shaped like an hourglass with a donut around its middle – the electron is most likely to be found somewhere in that shape with the Co atom at the very center (Figure 8.4). This orbital is oriented with the hourglass along the Co–ligand axis, the Co–water bond in aquocobalamin (Figure 8.4, right), which is useful for the spectroscopic techniques I use. Because the cobalamin is bound to PceA we can find out how the enzyme helps tune the cobalamin to reduce TCE by comparing the data collected with cobalamin in the enzyme and in water.

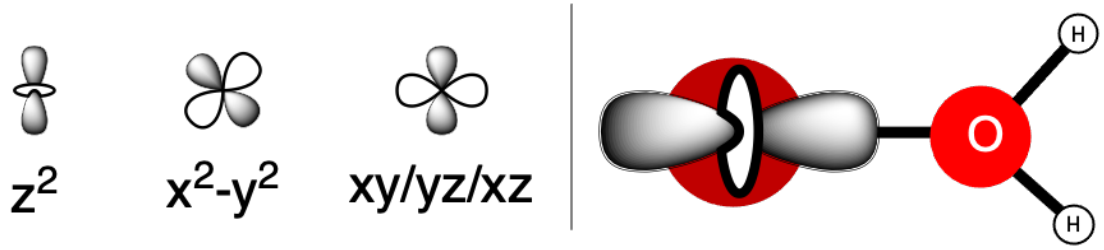

Figure 8.4 (Left) d-orbitals. Transition metals contain five d-orbitals that can be shaped in three ways. The d_{xy}, d_{yz}, and d_{xz} orbitals differ in which of the x,y,z axis they are aligned with. (Right) the orientation of the d_{z^2} orbital relative to the Co–water bond.

Earlier I said that both Abs and MCD are spectroscopies. But what exactly is a spectroscopy? At the most general, spectroscopy is the study of the interaction of light and matter. In this case, the matter will be our friend cobalamin and we'll be gathering information about how it interacts with light in different forms.

Absorption Spectroscopy

You probably remember from high school physics that light has amplitude, wavelength, and frequency and visible light is the rainbow or ROYGBIV. Bonus points to you if you remember that the energy of light is proportional to its frequency and inversely proportional to its wavelength. Abs spectroscopy arises from the fact that things absorb light. The energy in light excites an electron from the ground state (the most stable electron configuration) to an excited state (a previously empty or partially empty orbital gets an electron). In order for this excitation to happen, the energy of the light must have exactly the same energy as the gap. Think of this like playing a chord on a piano. All the keys are always there, but they aren't always being played (or occupied). In order to play a specific chord, your hand has to line up exactly with where the keys are. Spread your fingers too far or not far enough and you're not able to play that specific chord. Light that has energy that lines up with electronic transitions gets absorbed by the matter (exciting electrons to a different orbital) and any light that doesn't have a match bounces off. The light that isn't absorbed is what we see. Cobalamin is red because red light isn't absorbed. (Figure 8.5)

We can measure how much light is absorbed at any given wavelength using an instrument called a spectrophotometer. A spectrophotometer is a relatively simple instrument that has a source of light, diffraction grating (like a prism to pick the color of light), a slit to block out any unwanted light, the sample, and a detector. Interpreting the data is much harder than collecting it for Abs spectroscopy.

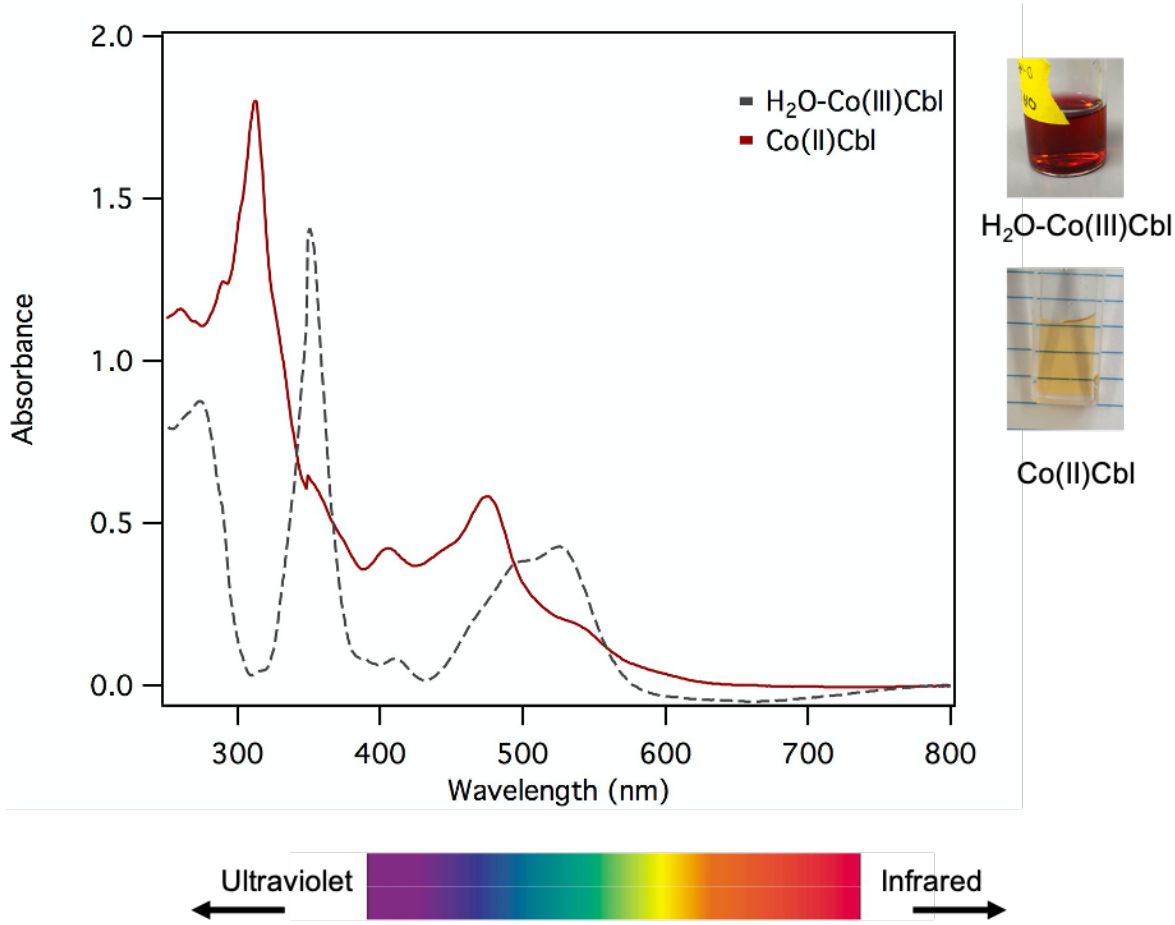

Figure 8.5 Abs spectra of H_2O-Co(III)Cbl and Co(II)Cbl. The color of H_2O-Co(III)Cbl and Co(II)Cbl correlate with the wavelengths that aren't absorbed.

In a completely perfect, theoretical world, the peak corresponding to light being absorbed would be infinitely narrow, aka a line. In reality it broadens to look more like a bell curve. We don't necessarily know how many transitions, or bell curves, are combining to form what we observe. If you look at Figure 8.5, you could probably draw many different sets of bell curves within the spectrum that would still average out to look like what is observed. Even if we were able to draw the perfect set of transitions, we don't necessarily know which states, or electron

configurations, the transition corresponds to. To help us figure it out, we can use complementary techniques like MCD spectroscopy.

Magnetic Circular Dichroism Spectroscopy

If you didn't guess by the name, magnetic circular dichroism requires a magnet. Unfortunately, a magnet off your fridge doesn't quite cut it. Similar to the inside of a MRI machine, the magnet for MCD has to be able to superconduct. This generates a strong magnetic current around the sample and the strength of the field decreases as you are further from the magnetic coils. The CD part of MCD arises from the use of circularly polarized light. Circularly polarized light looks like the threads of a screw and can either rotate to the left or the right (Figure 8.6). When you shoot circularly polarized light through a sample in a magnetic field, you collect MCD data (Figure 8.7).

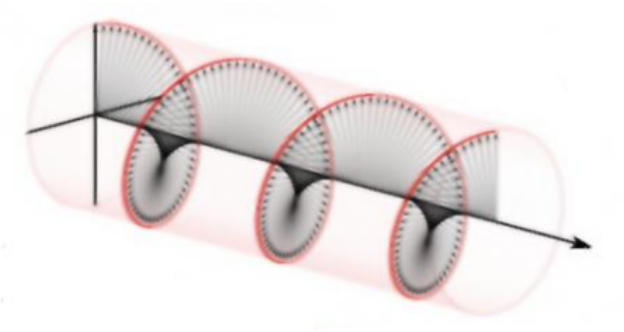

Figure 8.6 Circularly polarized light. The light travels in a circular path much like the threads of a screw.

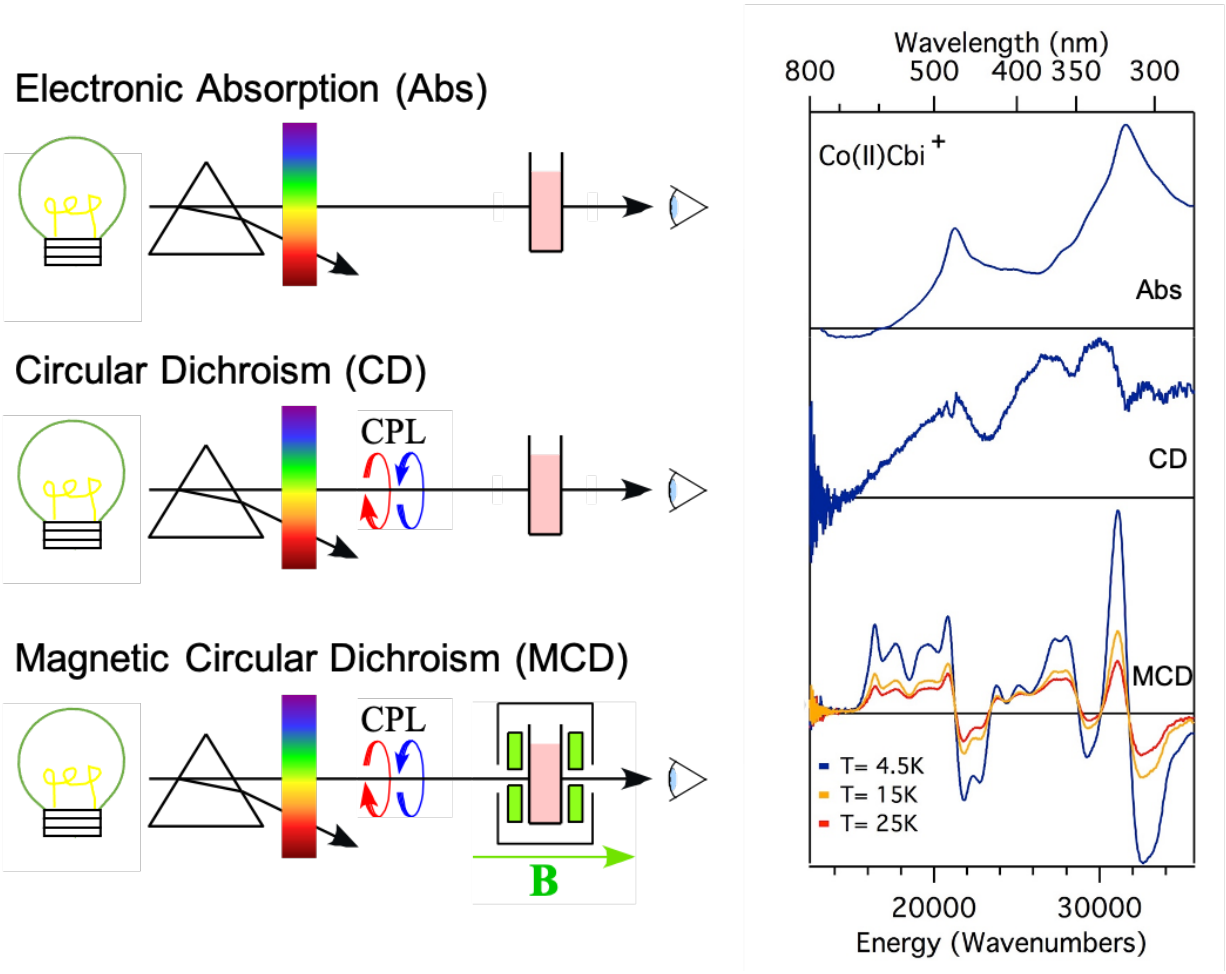

Figure 8.7 Comparison of Abs, CD, and MCD spectroscopies. In each case, as we go from left to right, there is a light source, a diffraction grating (which is like a prism to select the wavelength of light hitting the sample), the sample, and a detector. CD uses circularly polarized light (CPL) and MCD uses both circularly polarized light and a superconducting magnet at the sample. The far right shows data for each spectroscopy using a sample of $Co(II)Cbi^{+}$.

MCD spectroscopy is complementary to Abs spectroscopy because while they both arise from electronic transitions between the ground state and an excited state, MCD and Abs spectroscopies have different rules governing which electrons can be excited. MCD spectroscopy is particularly useful for PceA because atoms with an unpaired electron have an increased signal

making the signal for Co(II)cobalamin much more intense than the signal arising from the electron storing Fe-S clusters which have no unpaired electrons. Additionally, MCD transitions can result in positive or negative features where the intensity of the signal can be either positive or negative on the y-axis. By itself, the ability of MCD data to be either positive or negative can help us figure out what peaks in the Abs spectrum come from overlapping features since some of the MCD features point in the opposite direction. With a little bit of luck, MCD data can help identify most or all of the transitions in Abs spectroscopy, but we still don't know which electronic states those transitions correspond to. To figure that out, we turn to computational chemistry.

Quantum Mechanics

We can study steps in the mechanism that are too fast to capture experimentally with computations. In general, a series of equations can tell us where an electron could be. However, these equations can only be solved for systems with one electron. Unfortunately, this system has tens of thousands of electrons since it isn't a hydrogen atom or helium ion. Luckily, there are good approximations that make it so that we can get solutions for both the geometric structure (ie where the atoms are) and the electronic structure (ie where the electrons are and what their energies are).

The method I use to optimize the geometry of PceA is called quantum mechanics/molecular mechanics or QM/MM. The QM/MM method limits what atoms are explicitly included in the equations to just the atoms in the cobalamin and parts of the enzyme that we think are relevant to the reaction. These atoms form the QM region where the equations I mentioned in the last paragraph are being solved with approximations. The rest of the enzyme falls under the MM region meaning that the atoms are viewed in a much more simplistic way – essentially, they are treated as charged balls (atoms) on springs (bonds). In order to optimize the geometry, the computer

program iteratively moves atoms and then calculates energies and forces for both the QM and MM regions. It takes a computing cluster weeks to solve the lowest energy structure. Contrastingly, it would take months or more to find if I tried to just run the program on my laptop and it would be impossible to ever do by hand.

Once I have an optimized geometry from the QM/MM calculation, I can determine electronic structure information using another computation. This computation uses time dependent-density functional theory (TD-DFT, essentially electrons can move due to the time dependence) to determine where electrons can be. These states can then be used to predict electronic transitions, the peaks we observe with Abs spectroscopy, tying us back into what we can observe experimentally.

Now you may be asking if all the time that goes into the computational models is worth it: it is a lot of time, which means a lot of money. In the end, they are more than worth it because both the QM/MM and TD-DFT results give so much information! Beyond modelling the starting and ending states of a reaction, if we think a structure may be part of the chemical reaction, we can model it. The models then tell us how the structure changes the geometry and accessible electronic states (ie the Abs spectra). Being able to model intermediate steps of a reaction can both save us time experimentally by helping us know what reaction mechanisms are feasible, as well as give insight into structures that would be too short lived to be observed.

Due to the approximations necessary to solve any of the equations that describe the QM region, we have to validate the computations by comparing them to experimental data. If we use different approximations, you can end up with vastly different computational results. Experimental data serves as a checkpoint to make sure that the results we are getting are reasonable. Ideally,

computations lead to new questions to ask experimentally which lead to more models to probe computationally in a self-perpetuating cycle.

8.5 The Results

While absorption data is the easiest to collect, for PceA, it is not very informative. Because we know that the Co is bound to a water molecule in PceA, we would expect to see signals that are similar to a model molecule, Co(II)Cbi$^+$. As you can see in Figure 8.8, the signal of Co(II)Cbi$^+$ is essentially lost in the Abs of PceA. This is due to contributions to the signal from the iron-sulfur clusters. Think of it like a bush in the winter. When there is no snow, you can see an incredible amount of detail: the color, how many branches there are, if there are thorns on the stems, etc. As the snow begins to fall, you begin to lose detail and when enough snow has fallen, all you see is a white blob in the overall shape of the bush. To clear the snow off the bush, figuratively speaking, we can use MCD. Iron sulfur clusters have all their electrons paired (meaning they are diamagnetic), so they display minimal signal in MCD, allowing us to study just the cobalamin.

If you add one electron to Co(III) aquocobalamin in water, you end up with Co(II)Cbl which has a nitrogen atom as the cobalamin ligand. Contrast that to how PceA binds aquocobalamin which maintains the bond between the Co and the water, even with the number of electrons that gives the Co(II) oxidation state. Because we know that PceA binds aquocobalamin with a water molecule bound to the Co ion, we would expect to see MCD spectra that have the same shape as Co(II)Cbi$^+$, the model molecule that only binds water. By just looking at the shape of each spectrum in Figure 8.9, we can tell that PceA is in fact binding a water molecule to the Co ion. We can also use the position of the local maxima, the top of various peaks in the spectra, to glean information about the Co d-orbitals. Since the d_z^2 orbital is oriented along the Co-water

molecule bond (see Figure 8.4), shifts of the local maxima around 16,000 wavenumbers correspond to changes of the Co-water bond. A peak at lower energy means the Co-water bond is weaker and therefore longer and a peak at higher energy means the opposite, a stronger and shorter Co-water bond. Since PceA has that local maxima at a lower wavenumber (lower energy) than Co(II)Cbi$^+$, we know that the water molecule in PceA is bound more weakly in the enzyme than in Co(II)Cbi$^+$.

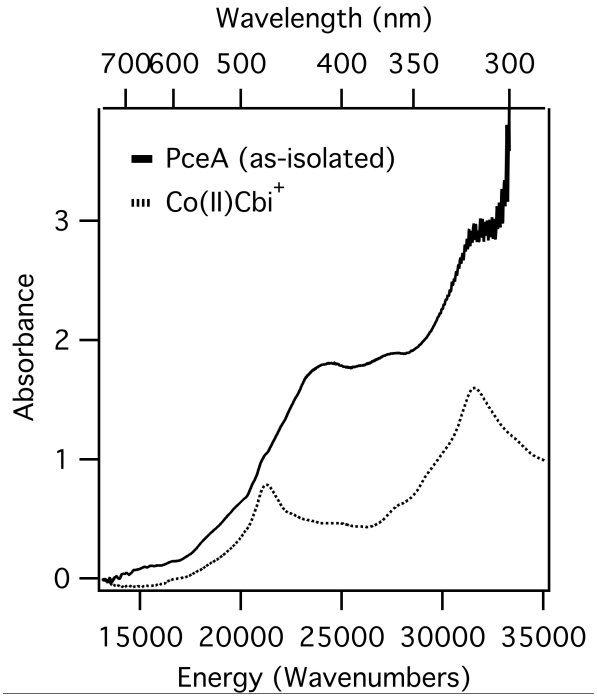

Figure 8.8 Abs spectra of PceA (solid) and Co(II)Cbi$^+$ (dashed). Co(II)Cbi$^+$ is a molecule that keeps the Co-water molecule bond at the Co(II) oxidation state.

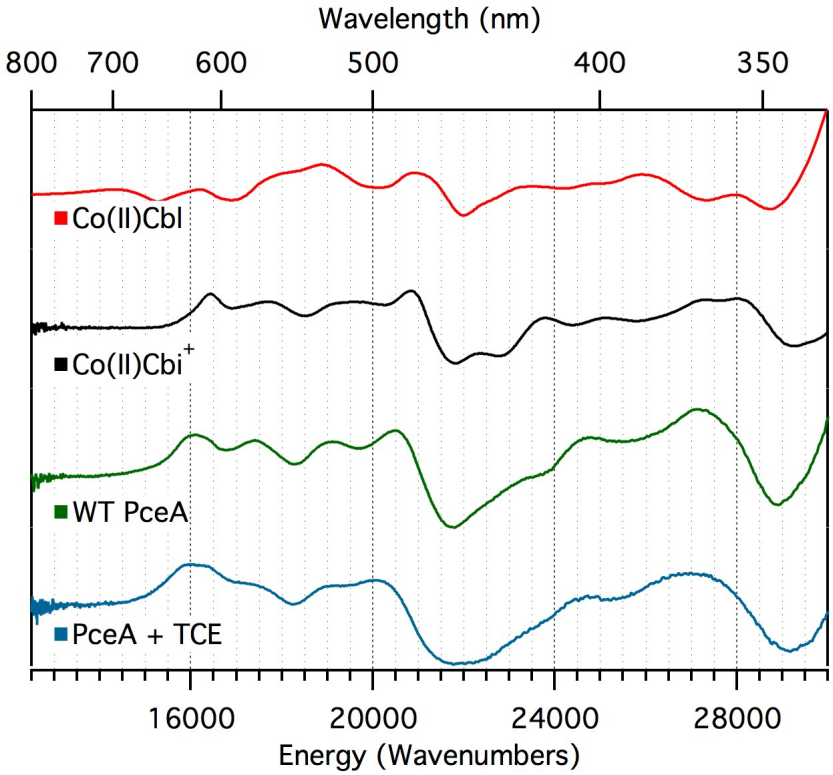

Figure 8.9 MCD spectra of Co(II)cobalamin (red), Co(II)Cbi$^+$ (black), PceA (green), and PceA+TCE (blue). The shape of the Co(II)cobalamin spectrum looks very different from PceA which indicates that there is not a nitrogen atom bound to the Co ion. The shifts of the peak around 16,000 wavenumbers for the other spectra correspond to changes in the strength of the Co–water bond.

Additionally, since the MCD data for Co(II)Cbi$^+$ and PceA with and without TCE are only moderately different, we can conclude that adding TCE to PceA does not appreciably change the environment around the Co ion. This was surprising! Other enzymes that use variations of Co(II)Cbl make the Co(II)Cbl really unstable by removing the axial ligand. By forcing the Co(II)Cbl to not have a ligand, it requires less energy to add an electron making Co(I)Cbl. Think of it like a domino, a thicker domino is easier to stand up but takes more of a push to knock over.

Make it thinner, the equivalent to removing the ligand, and it's not as stable standing up but knocks over much more easily. These results show that PceA does not use this method to reach Co(I)Cbl to initiate the chemical reaction.

Using these results as a guide, I could then generate and validate computational models. To validate the models, there were two things we had to reproduce: 1- the geometry of the atoms and 2- the electronic structure. Researchers had previously determined the atomic positions of the atoms in PceA with and without TCE bound. I generated models with the geometry optimized using the QM/MM method. The models were able to accurately reproduce these structures indicating that we had successfully reproduced the geometry (see Figure 8.10 for comparisons with TCE bound). What about the electronic structure?

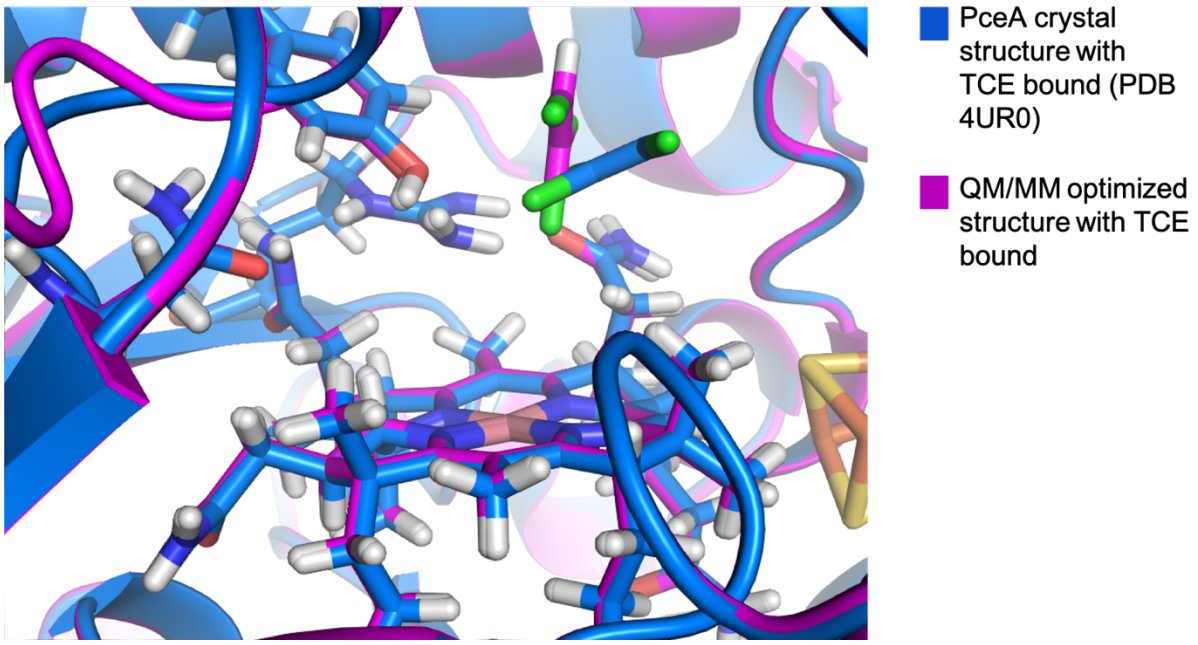

Figure 8.10 Comparison of the active site geometries for the crystal structure (blue) and optimized model (pink).

This is where we use the second type of calculation, TD-DFT. Since it is expensive to use this method on a large number of atoms, only the cobalamin and key residues from the enzyme were used. This means that the computed Abs spectra can't be directly compared to the experimental data since the Fe-S clusters, the largest contributor to the Abs data, aren't included. Instead, we can look at the identity of each transition, the sticks in Figure 8.11, and identify the transitions that involve the Co d orbitals. When we compare the transitions involving d orbitals to the trends observed using MCD, we see that there is a shift to lower energy for both PceA and PceA+TCE when compared to Co(II)Cbi$^+$, exactly what I observe experimentally! These shifts indicate a weakening of the Co-water bond which is important in order to form Co(I)Cbl and start the chemical reaction. Since I was able to reproduce the geometric and electronic structures, the same method can be used to probe intermediates in the reaction mechanism that have not been observed experimentally.

There have been a handful of mechanisms proposed for the reaction catalyzed by PceA. We can compare these mechanisms to painting. In the first, Co(I)Cbl passes an electron to TCE without touching it. If the Co(I)Cbl is the brush, and the electron the paint, this mechanism is like splattering the paint on the canvas, the TCE molecule, in the style of Jackson Pollock. The Co(I)Cbl could also use the two paired electrons in the d_z^2 orbital to attack either a carbon atom on the TCE molecule, or a chlorine atom on the TCE molecule. These two mechanisms are like comparing pointillism and impressionists: both apply the brush directly to the canvas but in two very different ways. We can model an intermediate structure from each mechanism and compare the energy and see which intermediate would be the most stable, and therefore the most likely to occur. The most probable reaction mechanism is like tossing a ball down a hill - the ball can't

choose where it goes, it just rolls to the lowest spot in its path. Likewise, the most likely reaction mechanism is whichever mechanism requires the least amount of energy.

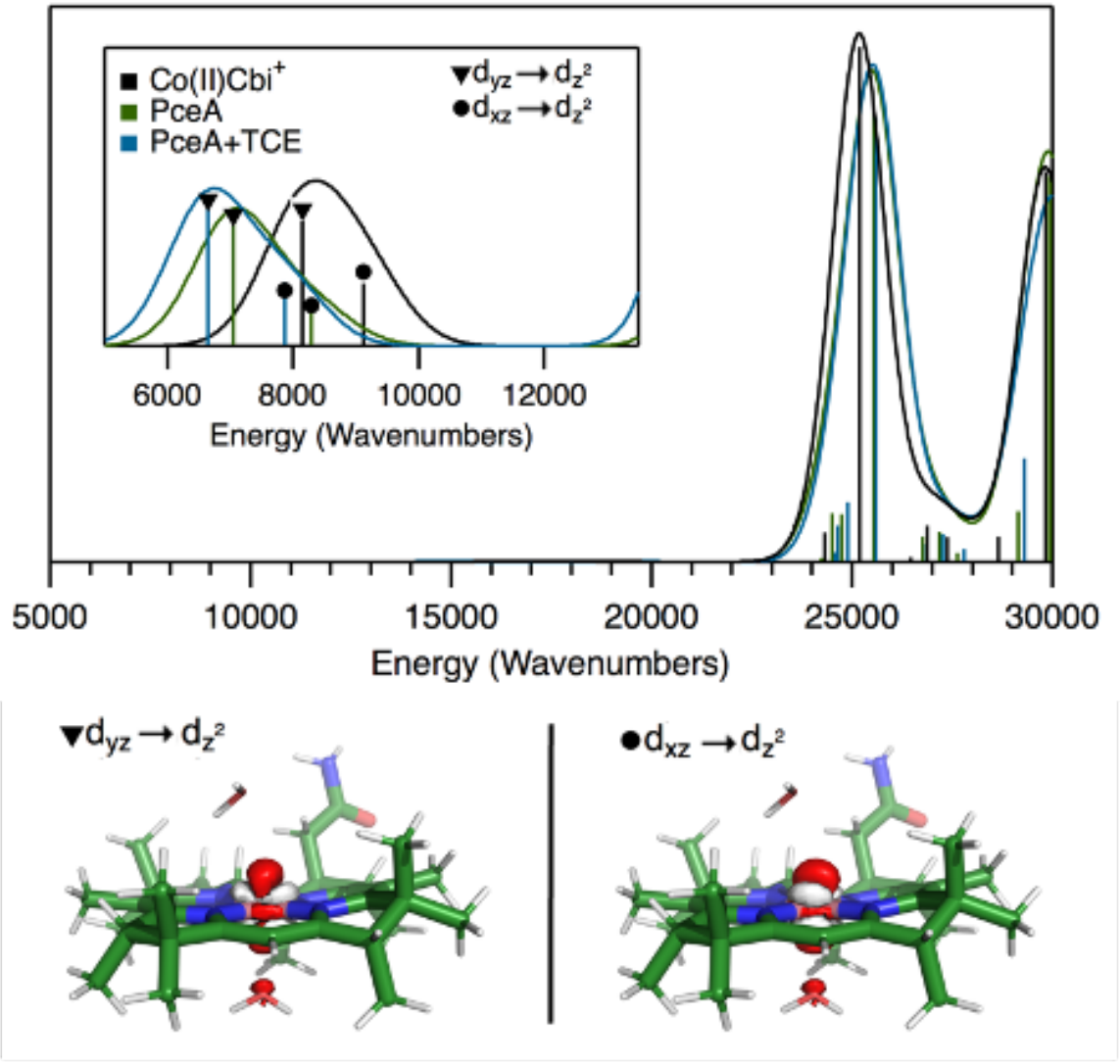

Figure 8.11 TD-DFT computed Abs spectrum (top) and electron density difference maps (bottom). The predicted transitions involving d orbitals match the trends observed in Figure 8.9 where the transitions for Co(II)Cbi$^+$ are highest in energy (inset). If we visualize these transitions (bottom), we see that the electron density is lost (white) from d_{yz} and d_{xz} and density is gained (red) in d_{z^2}.

Just like Figure 8.4, the subscript after the d describes the shape and for d_{yz} and d_{xz} the orientation relative to the x,y,z axes.

We can compare the energy of the intermediates using relative energy. Like you would measure the height of your kitchen table relative to the floor rather than relative to sea level, we compare the energy of the potential intermediates by comparing all of them to the lowest energy model (the equivalent of the kitchen floor). It turns out that the Co(I) tossing an electron to the TCE molecule without touching it is the most energetically accessible intermediate meaning it is the most likely route for the reaction to follow.

8.6 The Impact

If you made it this far, you probably have asked (at least once), so what? Why does this matter? Unlike some research, this project (and my PhD as a whole) doesn't yield a specific product, or improve a specific, commonly used reaction. This research isn't going to end up being sold on a shelf or even contributing to making something sold on the shelf. Instead, it falls under more fundamental research rather than applied. Fundamental research is important because it lays the foundation for future work. If you received an RNA based COVID-19 vaccine this year, you have first-hand appreciation for fundamental research into RNA that made the development of the vaccine possible.

The research you just read about contributes to our understanding of a fundamental vitamin. While humans don't natively use aquocobalamin, knowing what types of reactions the various forms of cobalamin can catalyze and how they do it is important for both potential drug

interactions in humans as well as understanding reactions in the bacteria that are all around us. Additionally, as PceA contributes to the global chlorine cycle through biodegradation of toxic chemicals such as TCE, this research can contribute to optimizing bioremediation strategies where these compounds have polluted the environment.