Consulting the Experts on Trace Metal Speciation
EDventures grant final report
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Introduction:

Seated at the northern edge the Pyrenees in the South West of France, the ancient city of Pau is the birthplace of King Henry IV, location of the annual Formula racing Pau Grand Prix, and home of the world class Laboratory of Bioinorganic Analytical and Environmental Chemistry (LCABIE). For over a decade, the scientists at LCABIE have advanced the field of mass spectrometry based methods for analyzing organic-metal complexes in biological and environmental systems. My EDventures project to visit the University of Pau, France afforded me a valuable opportunity to discuss how these methods can be used to determine the chemical identity of trace metal ligands in marine environments with the chemical speciation experts at LCABIE. By visiting Pau, I have been exposed to cutting edge research in trace element speciation through (1) connecting with scientists at the university, (2) attending an international spectrometry conference on metal speciation, and (3) testing new methods for analyzing marine ligand samples. This experience has provided me with a better understanding of the techniques that I am using in my PhD research, as well as new ideas for future research projects that will continue beyond graduate school.

Figure 1: Sights from Pau: (left) The Pau Chateau, birth place of King Henry IV (middle) the Pau Grand Prix historic Formula car racing (right) the Laboratory of Bioinorganic Analytical and Environmental Chemistry (LCABIE).

Discussions with the experts:

The analysis of metal ligands in natural seawater involves three major challenges. The first is the difficulty to extract and analyze the organic iron ligands without degradation. Secondly, the samples must be detected with great sensitivity due to their low abundances, even in a concentrated organic extract sample. Finally, the compounds must be separated from an extremely complex mixture of other more abundant organic molecules. At Pau, I was able to gain insight into each of these challenges by working with a range of chemists and biologists who specialize in different aspects of the molecular level characterization of trace metals.

As an introduction to the community of Pau, I gave a lunchtime department seminar that described my current work on siderophores and iron binding ligands in marine cyanobacteria and seawater. Afterwards and throughout my stay, I had a chance to talk informally to many scientists with related interests. I discussed my project at length with Laurent Ouerdane, an assistant professor at the University of Pau, and his recently graduated student Paulina Flis. Their research focuses on the chemical speciation of transition metals, especially iron, in plants and plant-associated microbes, and faces similar sample preservation challenges. Laurent had many insights into best practices for preventing the degradation of iron organic complexes in samples during sample preparation and chromatographic analysis. I also had a chance to discuss molecular imaging techniques for the sensitive detection of trace metal concentrations and isotopes with Christophe Pecheyran and Dirk Schaumloffel. To learn about the separation of compounds in complex matrices, I talked with Sandra Mounicou and Brice Bouyssiere who specialize on the characterization of metal containing proteins and porphyrins from biological and petroleum samples. With this information, I am better prepared to tackle the characterization of marine trace metal ligands, and I have gained valuable contacts for future questions and collaborations.

Spectr'atom conference:

The first week of my stay coincided with Spectr'atom – a four-day analytical chemistry conference in Pau for scientists in francophone countries that focus on elemental determination and speciation. This conference was organized by my host, Olivier Donard, and gave me a unique opportunity to meet scientists from Europe and Canada and learn about some new and exciting methods that may be relevant to microbial oceanography. All of the presentations were in French, and it was a great way to expand my French vocabulary to include some technical/scientific terms (a useful transferrable skill). The talks and posters covered a wide range of topics, from the elemental composition of the surface of mars to the investigation of the fate of silver nanoparticles that enter the environment. At the core of every presentation were novel mass spectrometry and spectroscopy methods of sensitive elemental detection. Participating in this conference gave me a chance to connect with an international community of scientists with similar research interests.

Sample analysis:

During my visit, I also had the opportunity to analyze several marine samples with the help of Laurent Ouerdane and Paulina Flis. The first sample that I brought with me to Pau was an extract from the culture media of *Prochlorococcus*, the most abundant photosynthetic microbes on earth. Our goal was to discover the organic compounds that *Prochlorococcus* produces to bind and regulate trace metals. The second sample that we analyzed was an organic extract from the surface water of Station Aloha in the subtropical North Pacific. This sample was collected during the HOE PHOR research cruise in September 2013, and contains a series of iron binding ligands of currently unknown composition.

To analyze these samples, we used a combination of liquid chromatography (LC) coupled to both inductively coupled plasma mass spectrometry (ICP-MS) and Orbitrap mass spectrometry (Figure 2). These combined techniques enable the separation and chemical characterization/quantification of metal-organic compounds that are present in complex mixtures. From these analyses, we were able to detect a single iron complex as well as a single zinc complex from the *Prochlorococcus* extract (Figure 3, left). These compounds may play an important role in the biogeochemical cycling of iron and zinc in regions of the ocean that are dominated by *Prochlorococcus*, and future work will elucidate their chemical structure. Furthermore, we were able to detect several metal-organic complexes at very low concentrations in the seawater sample from Station Aloha. In addition to several iron and nickel compounds that we were targeting, we were also able to detect some vanadium and iodine complexes that had not been observed before (Figure 3, right). These compounds are likely linked to the currently unknown biological roles that these elements play in the ocean.

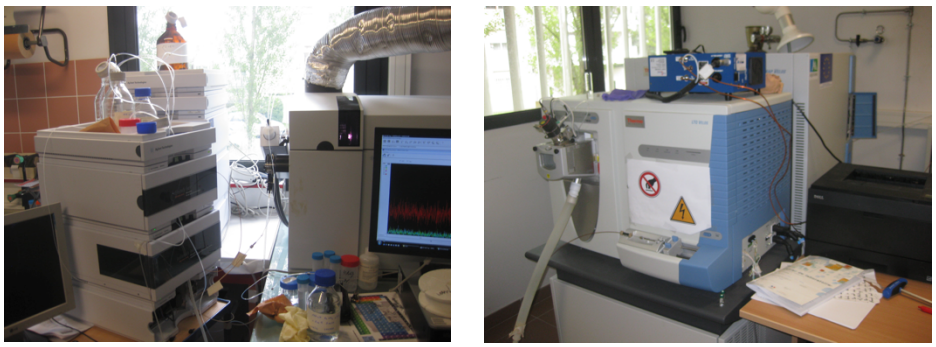

Figure 2: LC-ICPMS (left) and Orbitrap mass spectrometer (right) used to analyze seawater samples.

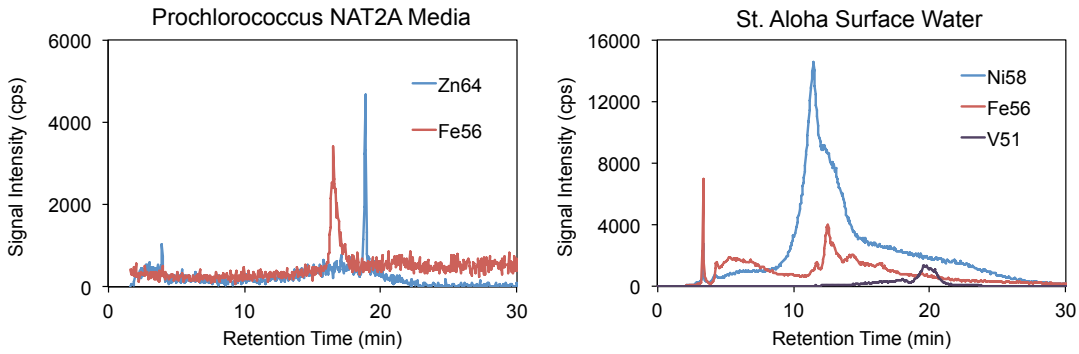

Figure 3: Liquid chromatography inductively coupled plasma mass spectrometry chromatograms of seawater samples. (left) The chromatograms of iron and zinc from the media of Prochlorococcus NATL2A reveal the presence of distinct compounds. (right) A number of peaks in the chromatogram of seawater extract from Station Aloha reveals the presence of naturally occurring nickel, iron, and vanadium complexes. Further work will be aimed at characterizing these compounds.

Beyond EDventures:

My short visit to Pau provided me with a strong basis for future collaborations. The success of our initial measurements suggests that mass spectrometry approaches are sensitive enough to characterize metal ligands in seawater samples. Furthermore, there

are several scientists at Pau, including Anne-Laure Bulteau and Laurent Ouerdane who were recently funded to study the uptake and regulation of iron by globally abundant phytoplankton including diatoms and coccolithophores – there is a critical link between this investigation and our research on the chemical forms of iron in the ocean. I am eager to continuing this work with LCABIE through an MIT International Science and Technology Initiative (MISTI) research grant or through a post-doctoral fellowship. Looking forward, Olivier Donard, has recently been funded to direct a new research facility that will operate an expanded arsenal of tools for developing next generation methods for determining metal species and their isotopes. There may be an opportunity for a productive partnership between this new research center in Pau and CMORE to study the biogeochemical cycling of trace nutrients in marine ecosystems.