Spying on Metal Complexes: Spectral Features of Ion-Specific Binding Motifs

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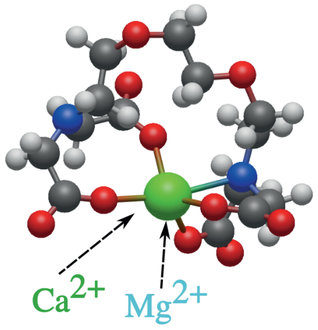
Background and Motivation

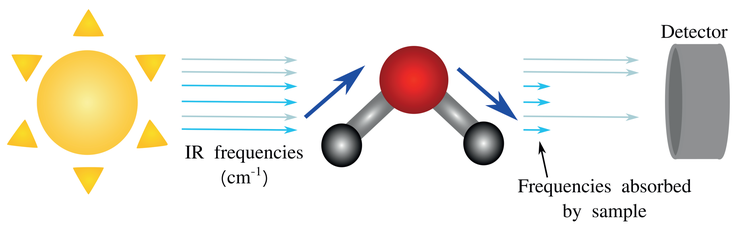
Ion binding behavior is not completely understood, even though it occurs in many biological processes. For example, the processes by which proteins discriminate between different ions remain, in many cases, unresolved.

Investigation of ion binding specificity in **EGTA** has been started in order to gain insight into the mechanisms by which biomolecules tell ions apart. **Fourier-transform infrared spectroscopy (FTIR)** is used as the main data source.



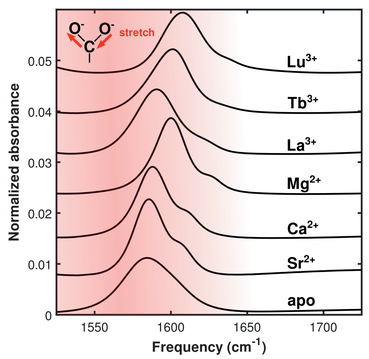
Above: EGTA was chosen as a model system due to its **metal-ion-dependent binding affinity**.

FTIR spectroscopy has the ability to detect **small changes in ion-specific behavior**. Results can be analyzed to aquire insights abount ion recognition mechanisms.



Above: In IR spectroscopy, molecules **absorb** infrared light at frequencies that correspond to the vibrational stretching modes of their chemical bonds. The IR light that is absorbed **excites** the vibrations within a molecule.

Since these vibrations are very sensitive to molecular structure, IR spectroscopy provides a powerful way to assess ion-binding-associated structural changes.



Left: Example IR spectra for different **EDTA**-ion complexes. Ionic radius changes of approximately 10 pm (0.1 Å) are readily detectable. [1] The magnitude of the shift depends on ionic radius and charge.

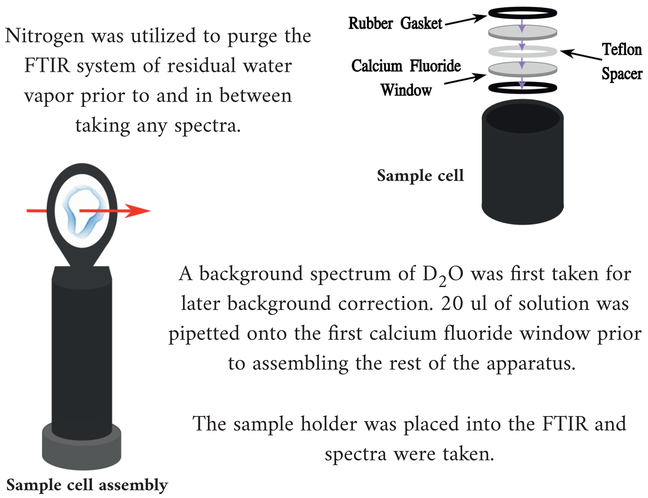
While both **EDTA** and **EGTA** bind ions, **EGTA** is more selective for Ca^{2+} ions.

Experimental Techniques and Setup

Solutions of EGTA in D_2O were prepared at 17 mM and 34 mM. The pH was adjusted to 8.5 with NaOD. 34 mM solutions of Ca^{2+} and Mg^{2+} were also made. All solutions utilized D_2O as solvent.

34 mM EGTA and metal ion solutions were combined in a 1:1 ratio to make final solutions with concentrations of 17 mM.

Nitrogen was utilized to purge the FTIR system of residual water vapor prior to and in between taking any spectra.



Sample cell assembly

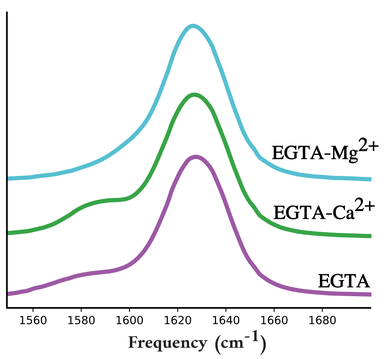
A background spectrum of D_2O was first taken for later background correction. 20 ul of solution was pipetted onto the first calcium fluoride window prior to assembling the rest of the apparatus.

The sample holder was placed into the FTIR and spectra were taken.

Results and Analysis

Utilizing Python, the resulting spectra were corrected using a D_2O background spectrum. Preliminary results exibit **one primary peak** around 1630 cm^{-1}.

Features can also be recognized near 1590 cm^{-1}. These features are specifically **ion-bound features** and can be seen very clearly in the EGTA-Ca^{2+} spectrum.



Left: It appears as if there is an absence of the 1590 cm^{-1} feature in the EGTA-Mg^{2+} spectrum.

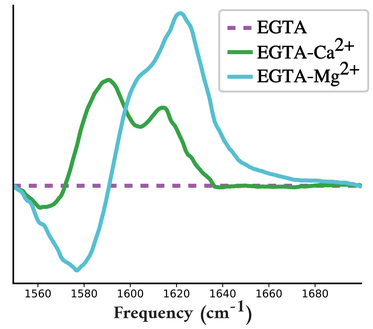
However, in comparison to unbound EGTA, the EGTA-Mg^{2+} spectrum **exhibits a shoulder** in the 1580 to 1620 cm^{-1} region. This is indicative of the presence of another binding configuration.

How can we make these ion-bound features more visible for analysis?

Right: In order to make the shifts more visible, an additional background correction of the spectra using the baseline-corrected EGTA was completed.

Here, **clearly visible features** are seen in the EGTA-Ca^{2+} spectrum around 1590 and 1620 cm^{-1}.

In the EGTA-Mg^{2+} spectrum, these features are **shifted** to 1600 and 1625 cm^{-1}, respectively.



A Ca^{2+} ion has a radius of 100 pm, whereas a Mg^{2+} ion has a radius of 72 pm. So, this **blue shift** occurs with a **decrease** in ionic radius. [2] This is consistent with prior results of EDTA ion complexes.

Conclusion and Outlook

- The binding of various metal ions to EGTA was studied with IR spectroscopy.
- We observed spectroscopic shifts in the carboxylate asymmetric stretching modes that were highly sensitive to binding geometry. We observed a blue shift with decrease in ionic radius.
- The current results for EGTA are consistent with prior results for EDTA.
- Future work will extend our EGTA dataset to include:
  - *Additional bound ions for comprehensive comparison to EDTA.*
  - *Temperature-dependent spectroscopy to measure stability of the complexes with different ions.*

These additonal experiments will help determine the structural features of EGTA that allow it to recognize certain ions.

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