

# **Quorum sensing molecules for unicellular organisms: Spectroscopic and computational study of conformational behavior**

Dan Tollefson

Dr. John Thoemke

*Department of Chemistry and Geology*

*Minnesota State University, Mankato*

# Background

- Quorum sensing plays a vital role in unicellular communications.<sup>[1-5]</sup>
- Unicellular bacteria communicate via small molecules that are created and released into the extracellular environment.

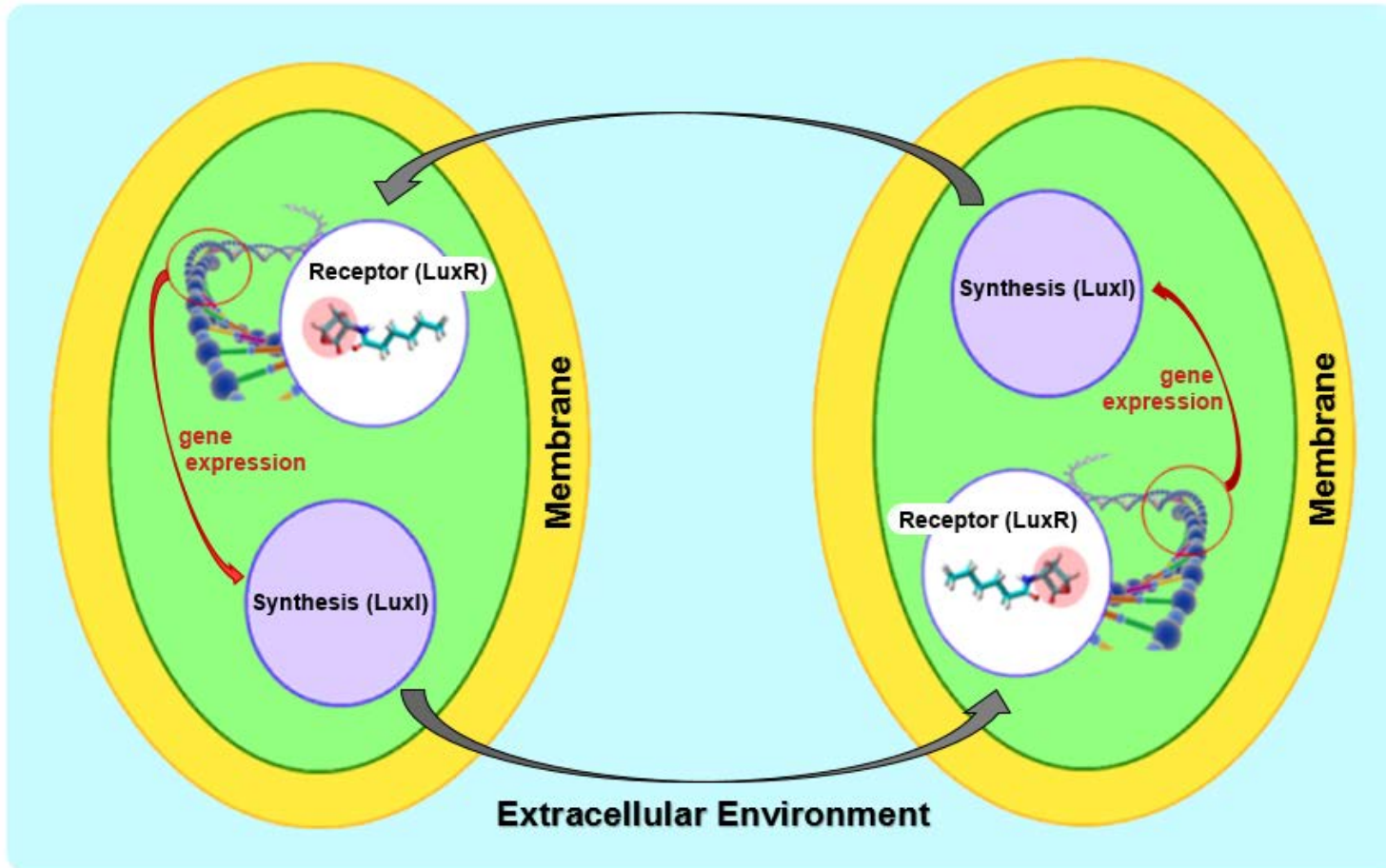


Figure 1. Diagram showing the process of quorum sensing between two bacteria. Adapted from reference 5, molecule images obtained from reference 7.

# Applications

- Studies have focused on...
  - QS manipulation in phenol-degrading activated sludge in waste water treatment facilities<sup>[2]</sup>
  - Bio-char sequestering of QS molecules for agricultural purposes<sup>[3]</sup>
  - Pharmaceutical applications<sup>[4]</sup>

# Purpose

- To use computational chemistry in concert with spectroscopy to deduce the specific conformation these signaling molecules take in solution.

## Questions to be answered...

- Can computational chemistry be used to predict stable conformations of these signaling molecules?
- Can computational chemistry accurately predict the effects of solvation?

# Structure

When found in nature, these molecules have varying...

- Acyl chain lengths
- Saturation levels
- Oxidation states

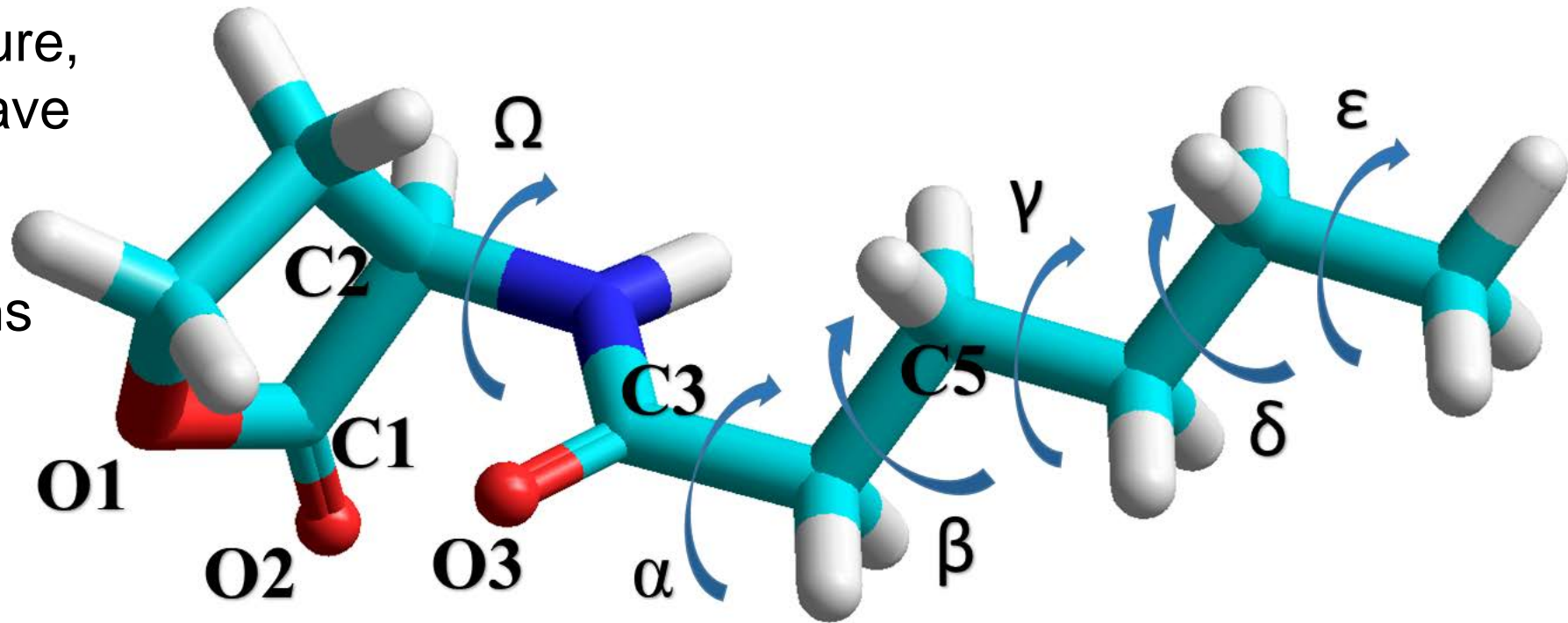


Figure 2. Structure of C6-HSL (N-hexanoyl-L-homoserine lactone) with adopted atom numbering and bond labels. Shown in the predicted endo low energy conformer which brings the amide carbonyl oxygen (O3) proximate to the lactone ring. The endo conformer is preferred when in solution. Image of molecule obtained via Hyperchem release 7.5.<sup>[7]</sup>

# Structure Continued

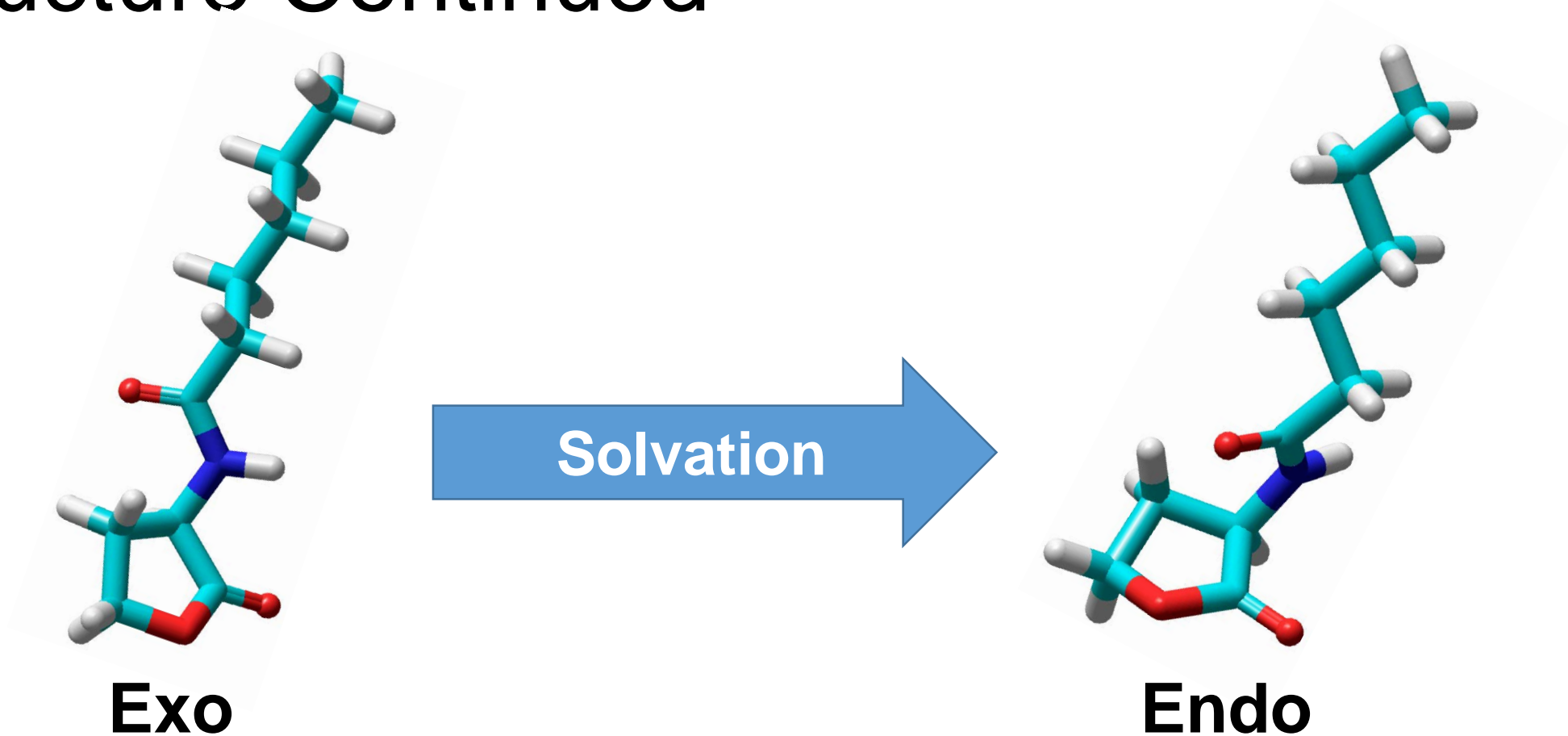


Figure 3. Conformational differences of C6-HSL when found in a vacuum (exo) compared to that which is found in a solution (endo).

# FT-IR Analysis

- For FT-IR analysis, solutions of QSMs were prepared in deuterated water and octanol.
  - Nicolet iS10 spectrometer with a single bounce ATR system (Smart iTR module).
  - 20 mg/mL solutions were prepared in octanol and deuterium oxide.
- Due to the insolubility of C8-HSL and C10-HSL in deuterium oxide, experimental spectra in deuterium oxide could not be obtained.

# Single Bounce ATR Module

- Utilizing a single bounce ATR module allowed the direct analysis of liquid samples.
- As the evanescence wave enters the sample, some of the energy of the evanescence wave is absorbed by the sample, and the affected IR beam is sent to the detector.
- Penetration depth of evanescence wave depends on...
  - 1) Wavelength of light
  - 2) Angle of incidence
  - 3) Indices of refraction of the ATR crystal and sample

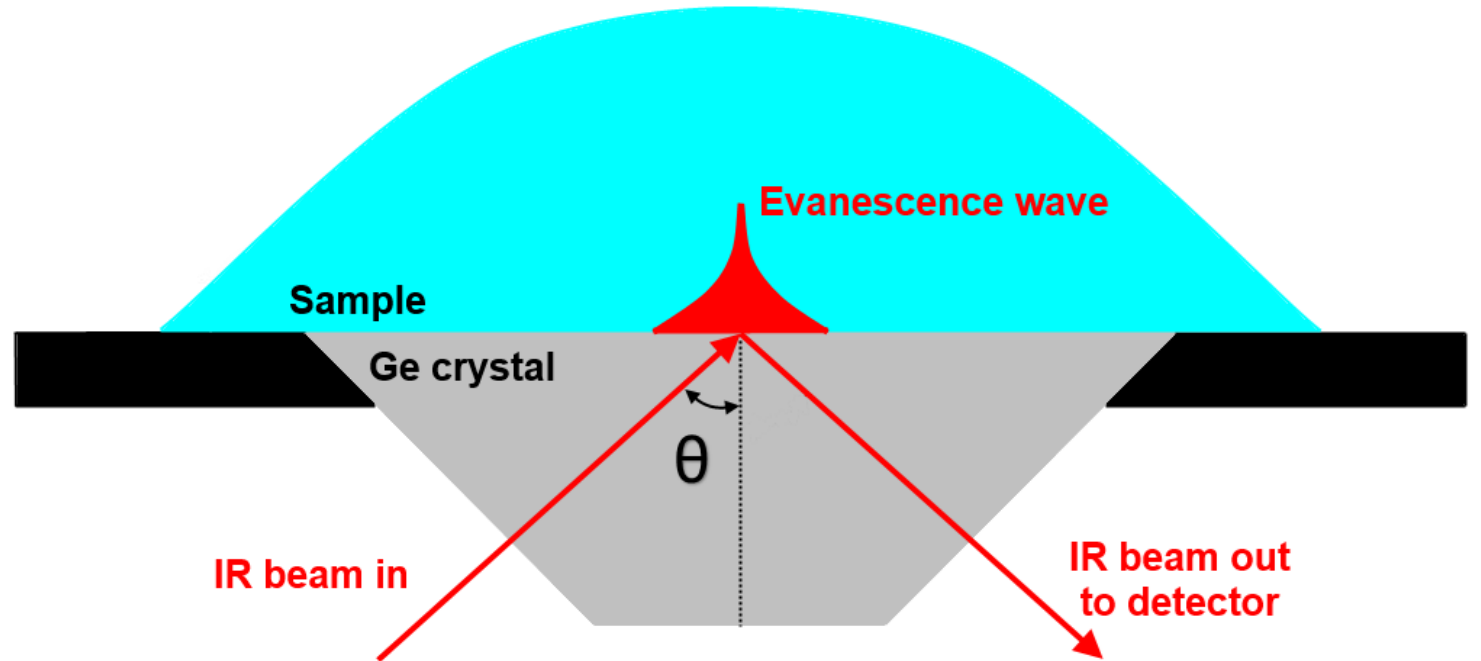


Figure 4. Representation of a Single Bounce Attenuated Total Reflection module. This instrument utilizes the property of total internal reflection resulting in an evanescence wave which penetrates 0.5-2  $\mu\text{m}$  into the sample. Due to the extremely small penetration depth, sample volume can be very small.<sup>[6]</sup>



# Spectroscopic Measurements

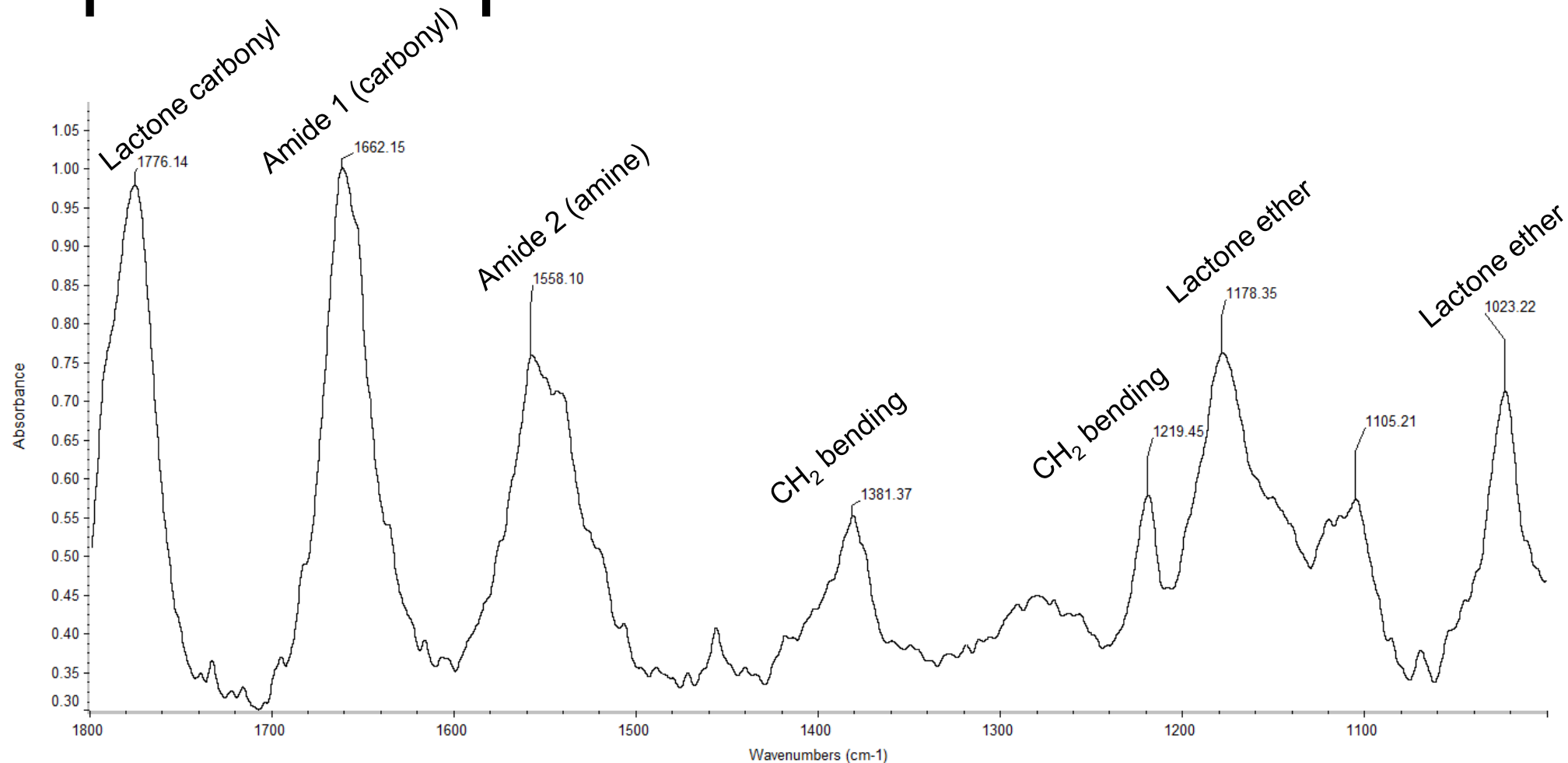


Figure 5. Acquired spectrum of C6-HSL in 1-octanol. Octanol was used as to replicate the molecular environment of a cell membrane. Each spectrum was collected with 128 scans with a resolution of 4 cm<sup>-1</sup>. The observable spectral features are located between 1000 and 1800 cm<sup>-1</sup>.

# Spectroscopic Measurements

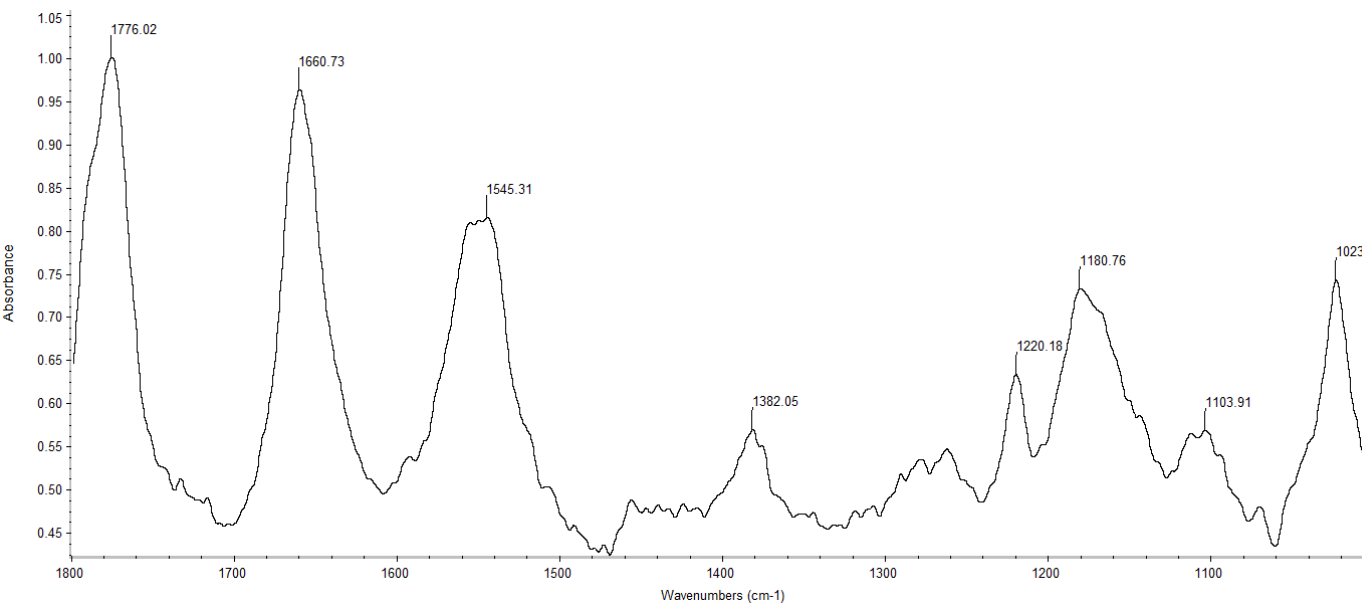
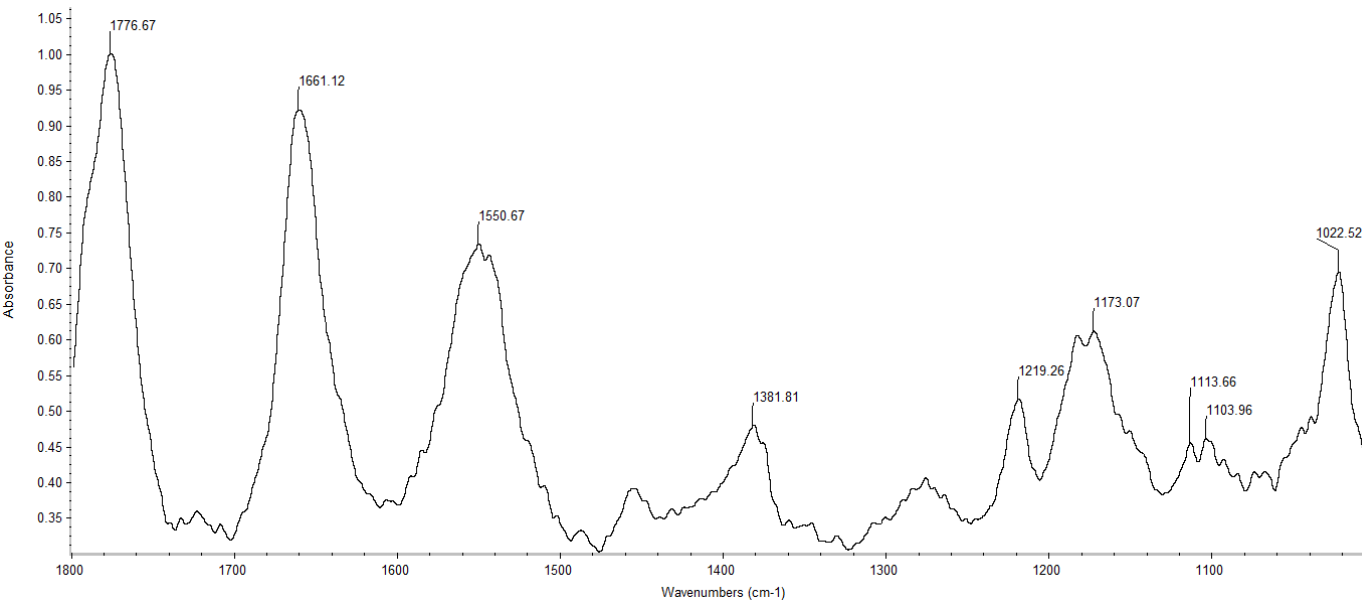


Table 1. Peak location of C6, C8, and C10-HSLs, respectfully.

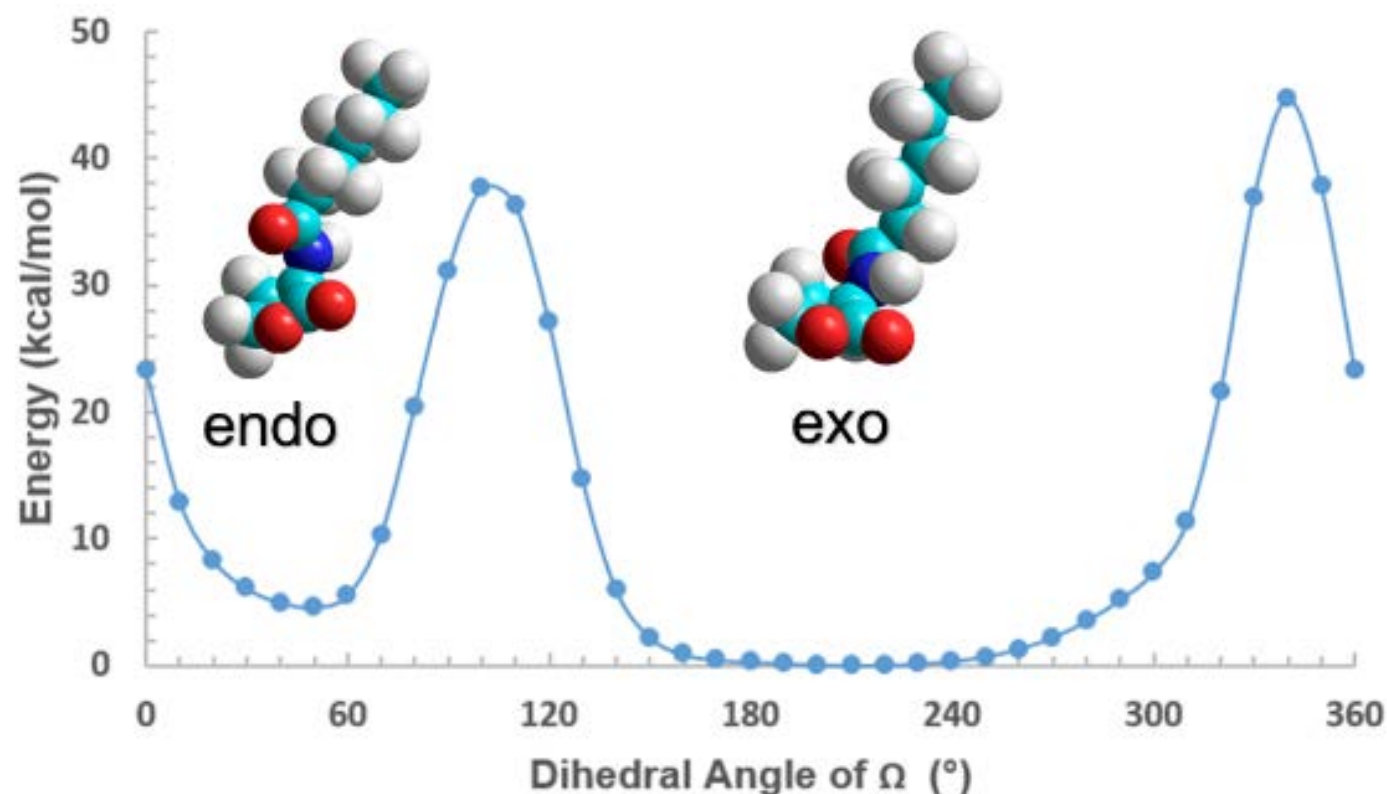
Bond	<u>Location of Peaks (cm<sup>-1</sup>)</u>			shift
	C6-HSL	C8-HSL	C10-HSL	
Lactone carbonyl	1776.14	1776.67	1776.02	none
Amide carbonyl	1662.15	1661.12	1660.73	none
Amide amine	1558.10	1550.67	1545.31	red
CH <sub>2</sub> bending	1381.37	1381.81	1382.05	none
CH <sub>2</sub> bending (2nd)	1219.45	1219.26	1220.18	none
Lactone ether	1178.35	1173.07	1180.76	none
Lactone ether (2nd)	1023.22	1022.52	1022.5	none

Figure 6. Experimental spectra of C8-HSL (upper) and C10-HSL (lower) in octanol. Spectra were collected 128 scans with a resolution of 4 cm<sup>-1</sup>.

# Theoretical Spectra

- Experimental spectra were compared to theoretical spectra produced via computational methods.
  - Initial systematic search for possible low energy conformations was conducted using the AMBER force-field and the semi-empirical AM1 methods.
  - The low energy structures were optimized using ab initio methods at the 3-21G\* level.
    - DFT methods incorporating correlation exchange were to computationally costly
  - Optimized structures were solvated in a periodic box of equilibrated (at 300 k) TIP3P model water molecules.
    - The number of water molecules in the periodic box was reduced to more closely resemble the corresponding number of octanol molecules that would be present.
    - Scaled to the dielectric constant of octanol.
  - Theoretical IR spectra obtained via vibrational analysis.

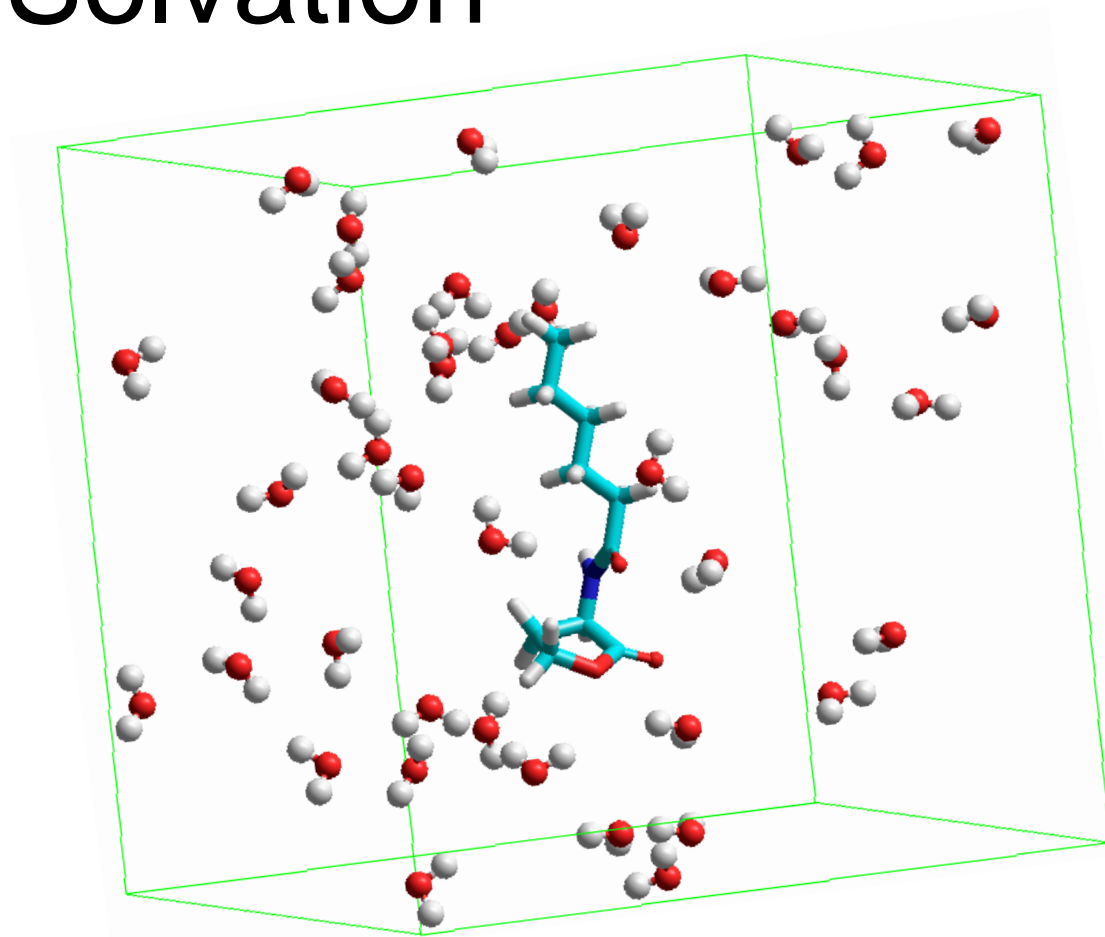
# Search For Low Energy Conformations



- $\Omega$  depends on environment
- $\Psi$  is an amide bond  $\rightarrow$  trans
- $\alpha$  can vary widely
- The remainder of the acyl chain is very close to values found in usual trans or gauche conformers of n-butane

Figure 4. Potential energy as a function of dihedral angle around the  $\Omega$  bond of C6-HSL, which links the lactone ring with the side chain. There are two predicted conformers, endo and exo, which correspond to the carbonyl oxygen either positioned over the lactone ring, or out and away from the ring, respectively. The endo conformer is preferred in solutions. Images of molecules obtained via Hyperchem release 7.5.[7]

# Solvation



- This process of inducing the effects of a solvent is essentially flawed.
  - Hyperchem v7.5 can only solvate a molecule using a periodic box, which it can only perform calculations at a molecular mechanics level.
- The polarized continuum model is most commonly used now.
  - Instead of individual solvent molecules, a polarized continuum is used
    - Dielectric PCM
    - Conductor-like PCM

Figure 8. C6-HSL in a periodic box of mock octanol molecules. In order to replicate the effects of the solvent, TIP3P models of water (equilibrated at 300 K, one atm) were used with a scaled dielectric constant to mimic the effects that would be felt in the presence of octanol. Image obtained via Hyperchem release 7.5.[7]

# Calculated Vibrational Frequencies

C6-HSL Theoretical Spectrum

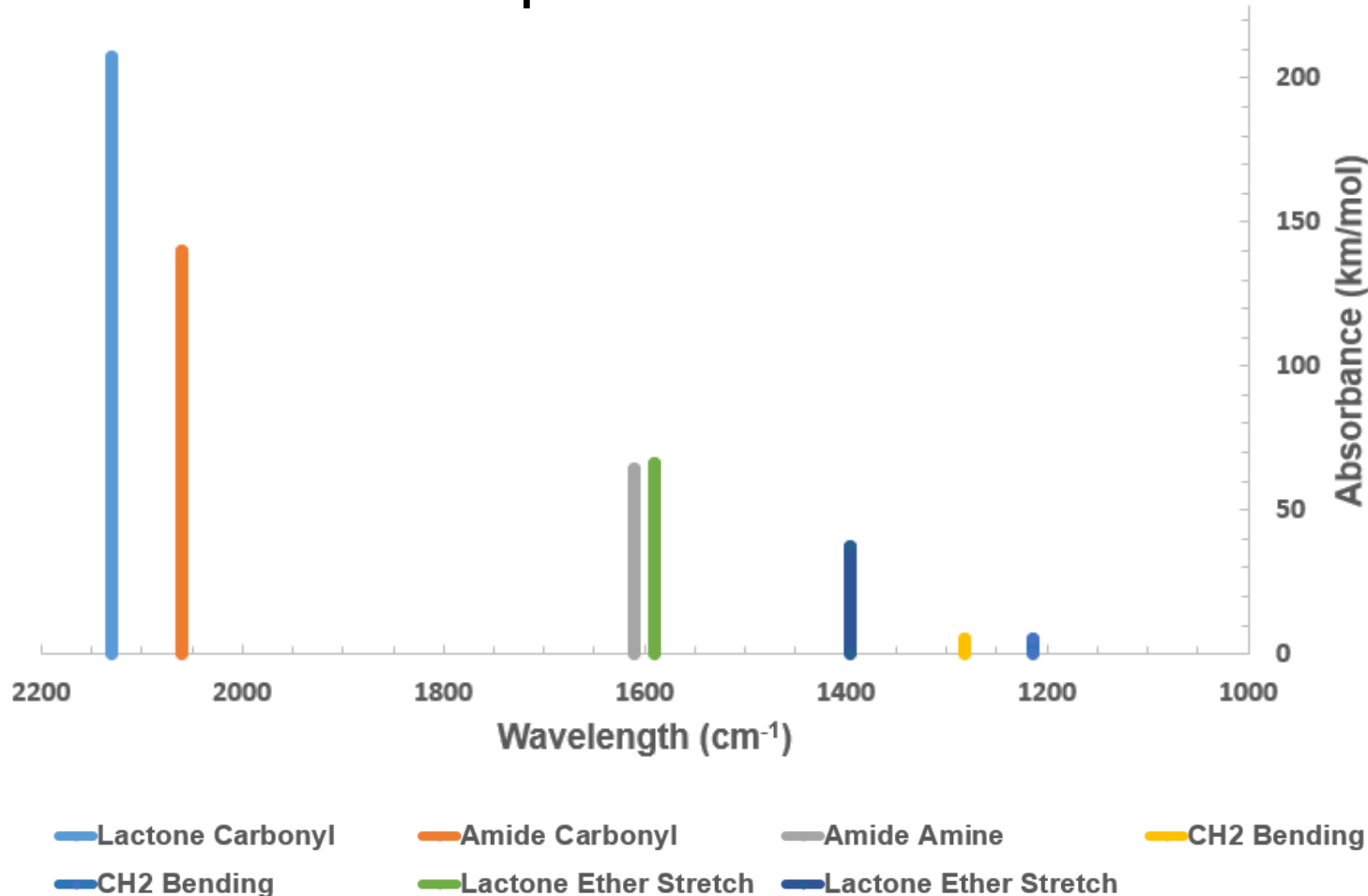


Figure 9. Theoretical spectrum predicted for each of the QS molecules. The vibrational frequencies were calculated using the semi-empirical AM1 method. Expected frequencies are seen, though far from observed locations in the experimental spectrum. More accurate vibrational frequencies can be calculated using more complex mathematical models, at the cost of much greater computational time.

# Conclusion

- Unfortunately, due to the level of calculations used, accurate theoretical spectra were not obtained. It is promising that the expected peaks are there and they are in the proper order.
  - The levels of calculations had to be lowered in order to complete time efficient calculations (<24 hours)
- Further work would be needed to obtain accurate spectra.

# Future Work

The shortcomings of this research can largely be overcome by using larger and more complex mathematical models along with newer and more advanced software. All of this requires a higher computational cost. More accurate results can be obtained using DFT methods at the B3LYP/6-31+G\* level. These calculations would not only use larger basis sets, it would also take into the account of electron correlation and excited states. This would also lead to much more accurate calculated vibrational frequencies.



# References

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# Any Questions?

## Acknowledgments:

We acknowledge the URC and the MSU, Mankato Foundation for funding for this research. We also acknowledge Dr. Brian Groh for the use of specialized glassware and instruction on spectrographic techniques, as well as the MSU Department of Chemistry and Geology.