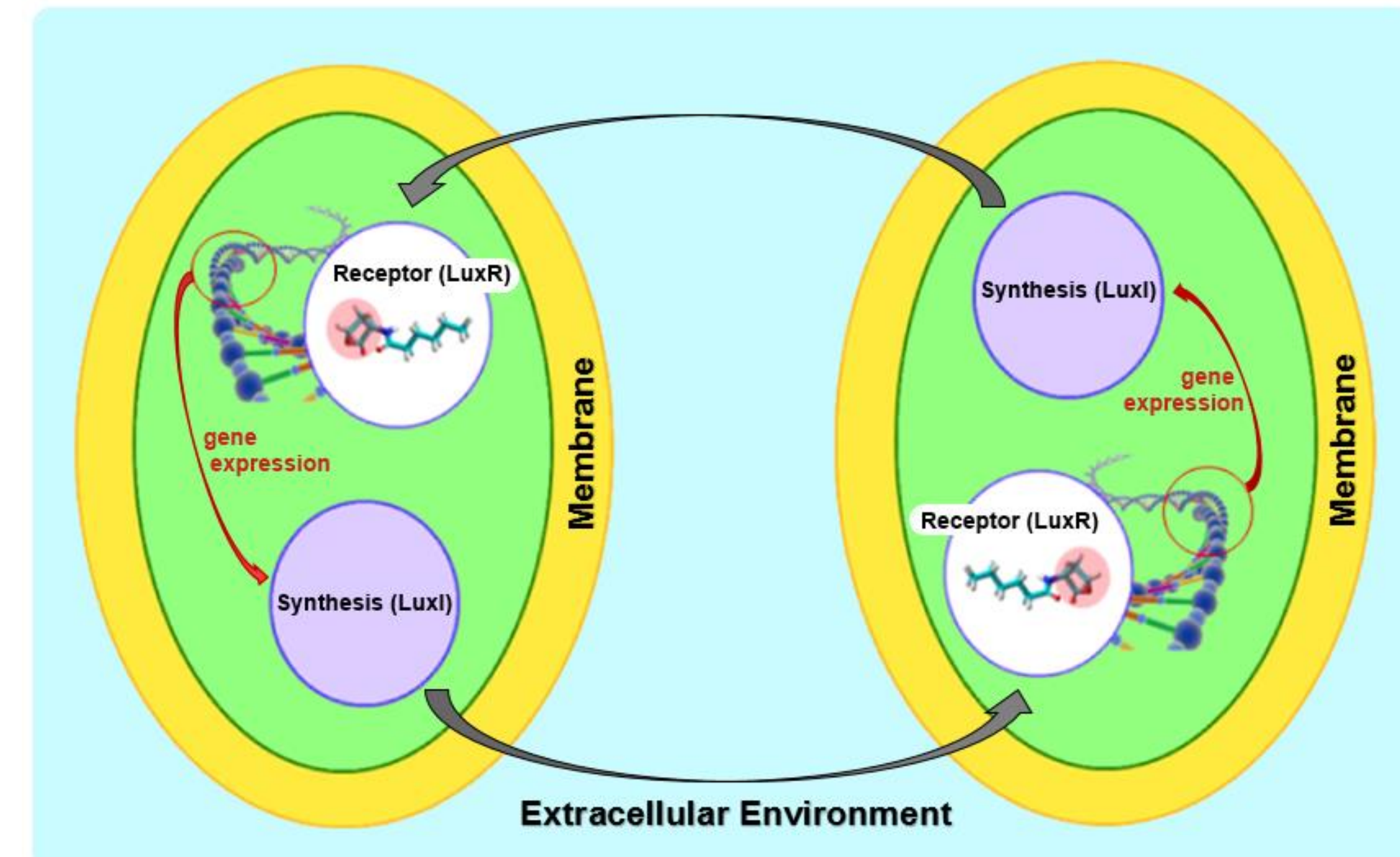


# Quorum Sensing Molecules for Unicellular Organisms: Spectroscopic and Computational Study of Conformational Behavior

## Background

Quorum sensing plays a vital role in unicellular communications. It has been recently found that unicellular bacteria communicate via small molecules that are created and released into the extracellular environment.<sup>[1-5]</sup> Detailed knowledge regarding the interactions of these quorum sensing molecules (QSM) with the molecular and cellular-scale environments can potentially lead to the manipulation of quorum sensing within a population. Studies have focused on the application of QS manipulation in phenol-degrading activated sludge in waste water treatment facilities, bio-char sequestering of QS molecules for agricultural purposes, and pharmaceutical applications.<sup>[2-4]</sup> The function of these molecules requires that they can readily diffuse through the polar environment of aqueous solution and the nonpolar environment of cell membranes.



**Figure 1.** Diagram showing the process of quorum sensing between two bacteria. When a signaling molecule is produced by a LuxI protein, it will diffuse through the cell membrane, where it will collect. Once a threshold concentration is reached in the extracellular environment, the signaling molecules will diffuse into another cell, bind to a LuxR receptor, and finally activate or repress gene expression of LuxI proteins. Adapted from reference 5, molecule images obtained from reference 7. When found in nature these molecules have varying acyl chain lengths, saturation levels, and oxidation states. These differences between QSMs can have a dramatic effect on the molecule's ability to migrate through the extracellular environment and ultimately signal another unicellular organism.

## Purpose

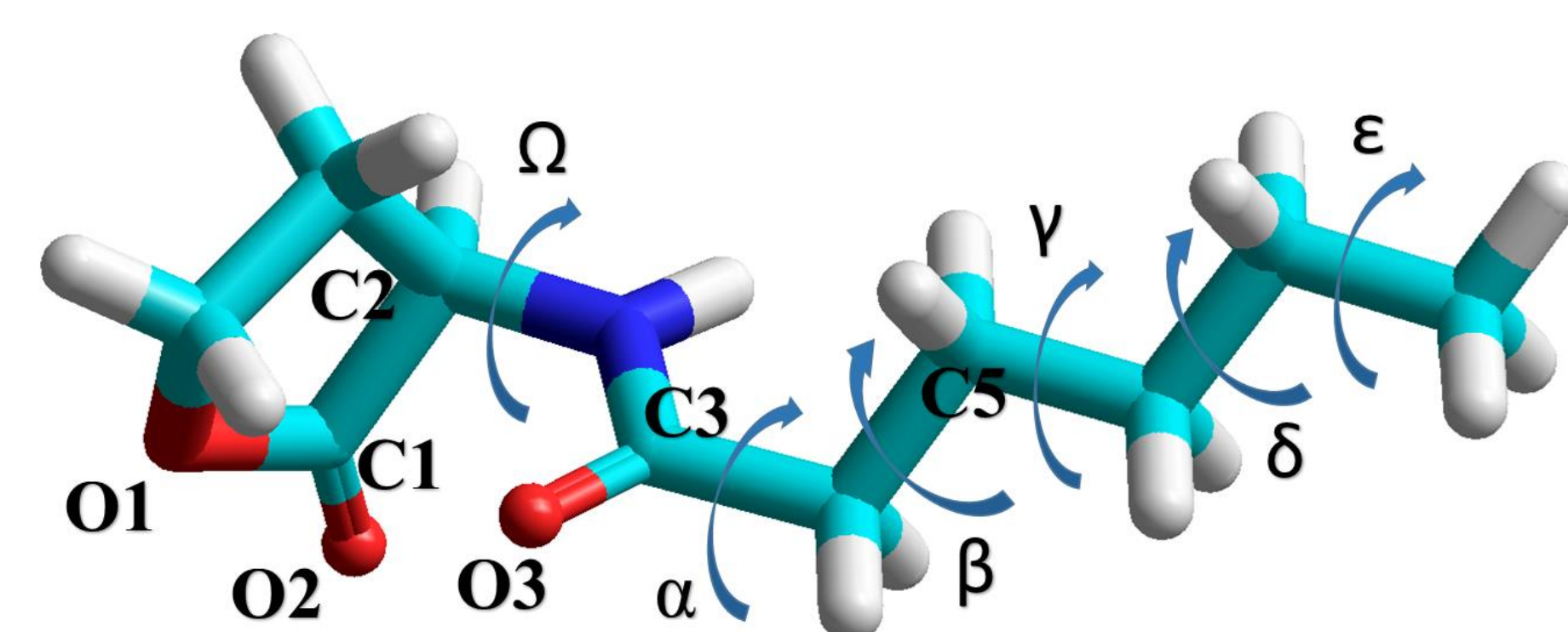
- To use computational chemistry in concert with spectroscopy to deduce the specific conformation these signaling molecules take in solution.
  - Can computational chemistry accurately predict the effects of solvation?

## Methodology

- 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.
- 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.

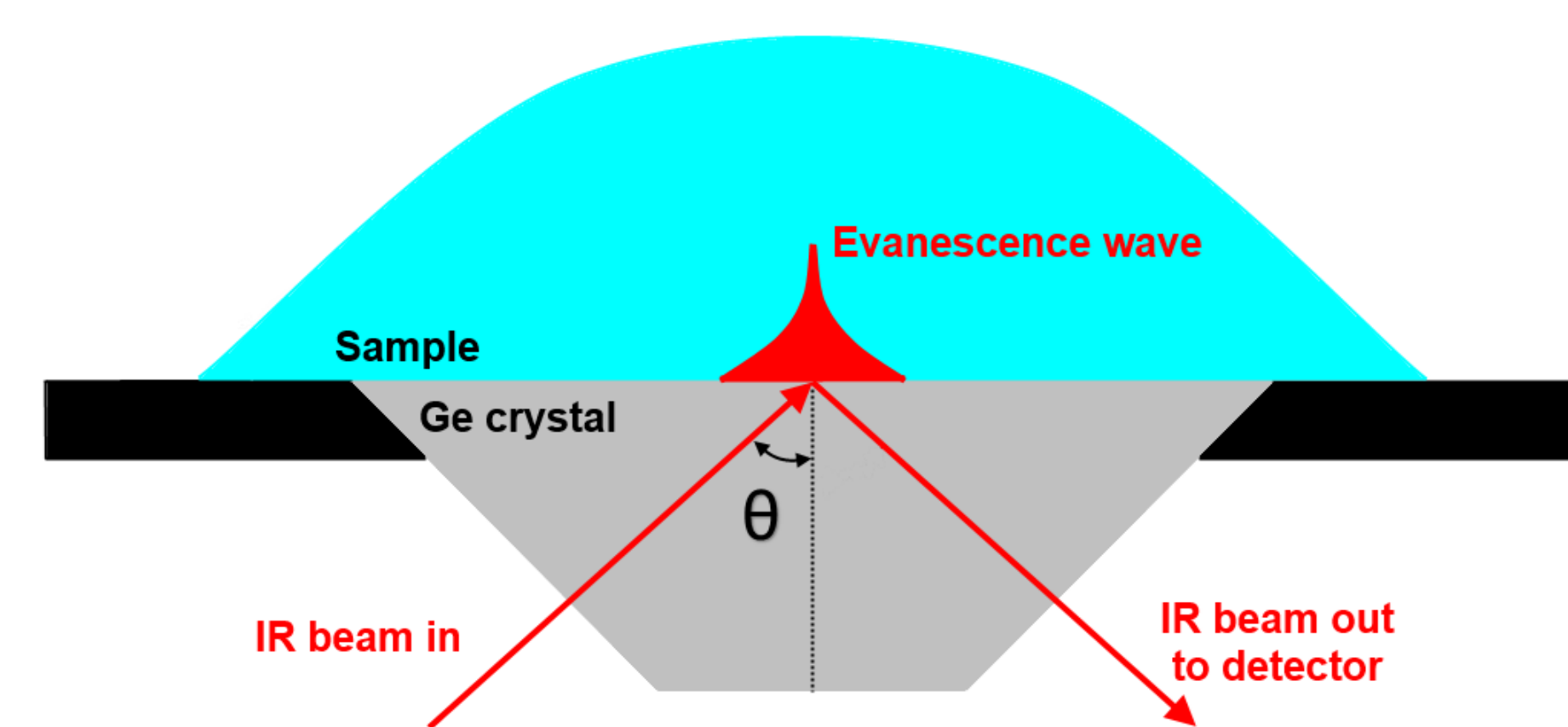
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## Experimental Spectra



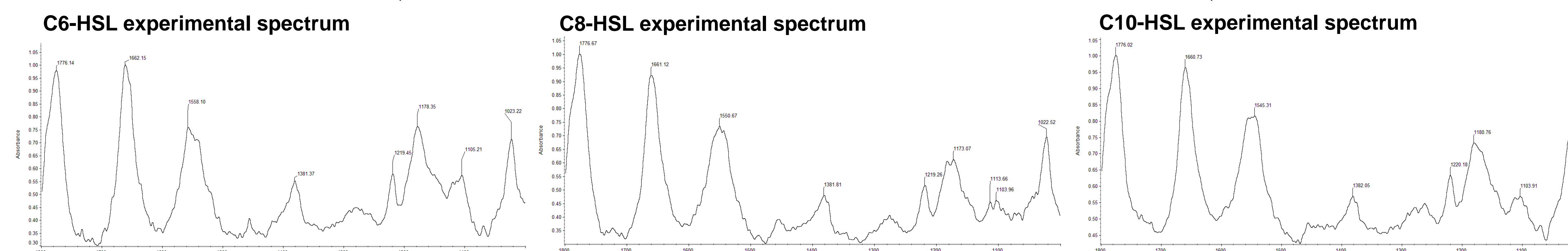
**Figure 2.** Structure of C6-HSL (N-hexanoyl-L-homoserine lactone) with adopted atom numbering and bond labels. Shown in the predicted endo low energy conformer which brings the amide carbonyl oxygen (O3) proximate to the lactone ring. The endo conformer is preferred when in solution. Image of molecule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

Solutions Prepared in D<sub>2</sub>O & Octanol to Replicate relevant molecular environments

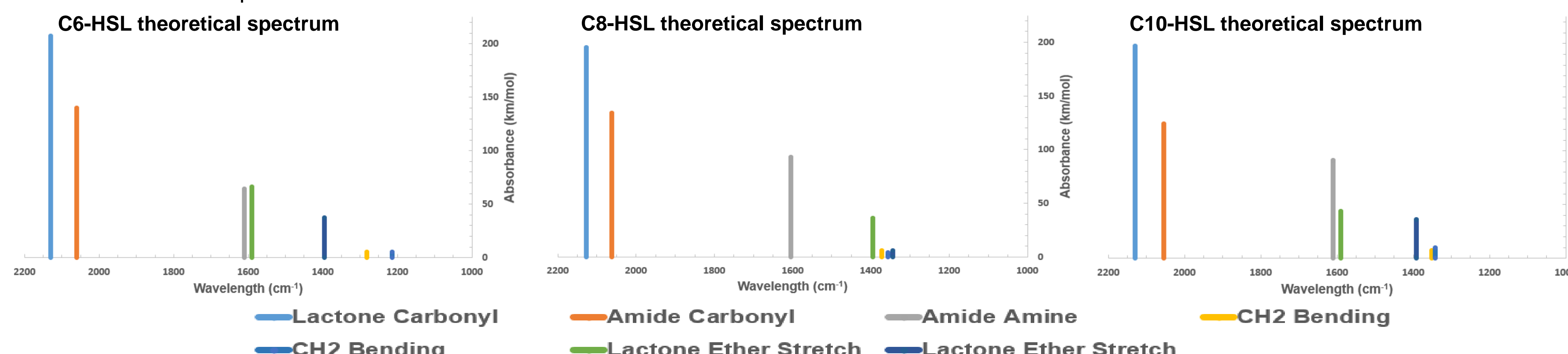


**Figure 3.** Representation of a Single Bounce Attenuated Total Reflection module. This instrument utilizes the property of total internal reflection resulting in an evanescent wave which penetrates 0.5-2 μm into the sample. Due to the extremely small penetration depth, sample volume can be very small.<sup>[6]</sup>

## Spectroscopic Measurements

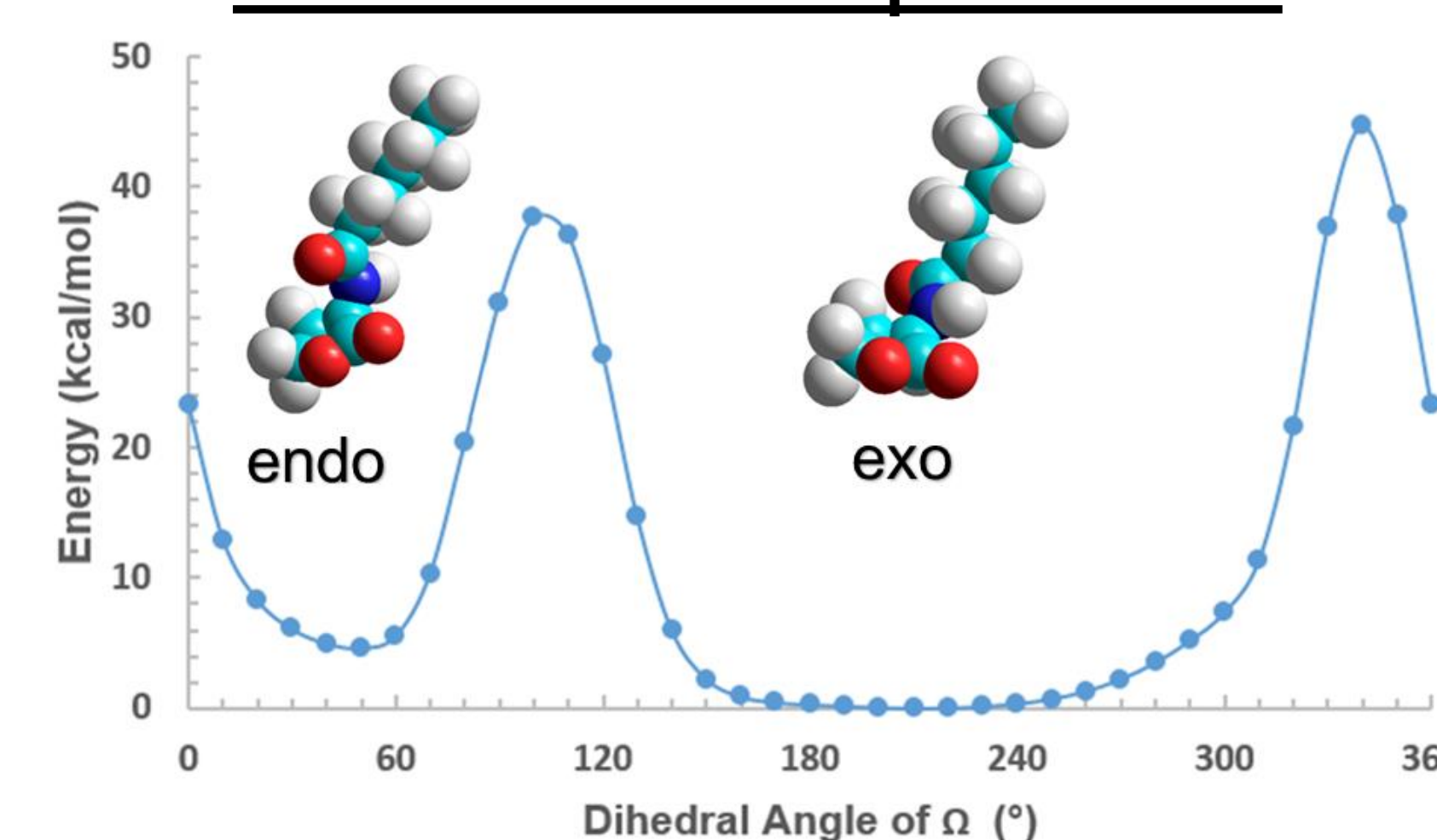


**Figure 6.** Acquired spectra of QS molecules in 1-octanol. Octanol was used as to replicate the molecular environment of a cell membrane. Each spectrum was collected with 128 scans with a resolution of 4 cm<sup>-1</sup>. The observable spectral features are located between 1000 and 1800 cm<sup>-1</sup>. Due to the insolubility of C8-HSL and C10-HSL in deuterium oxide, experimental spectra in deuterium oxide could not be obtained. Peak positions can be found in table 1.



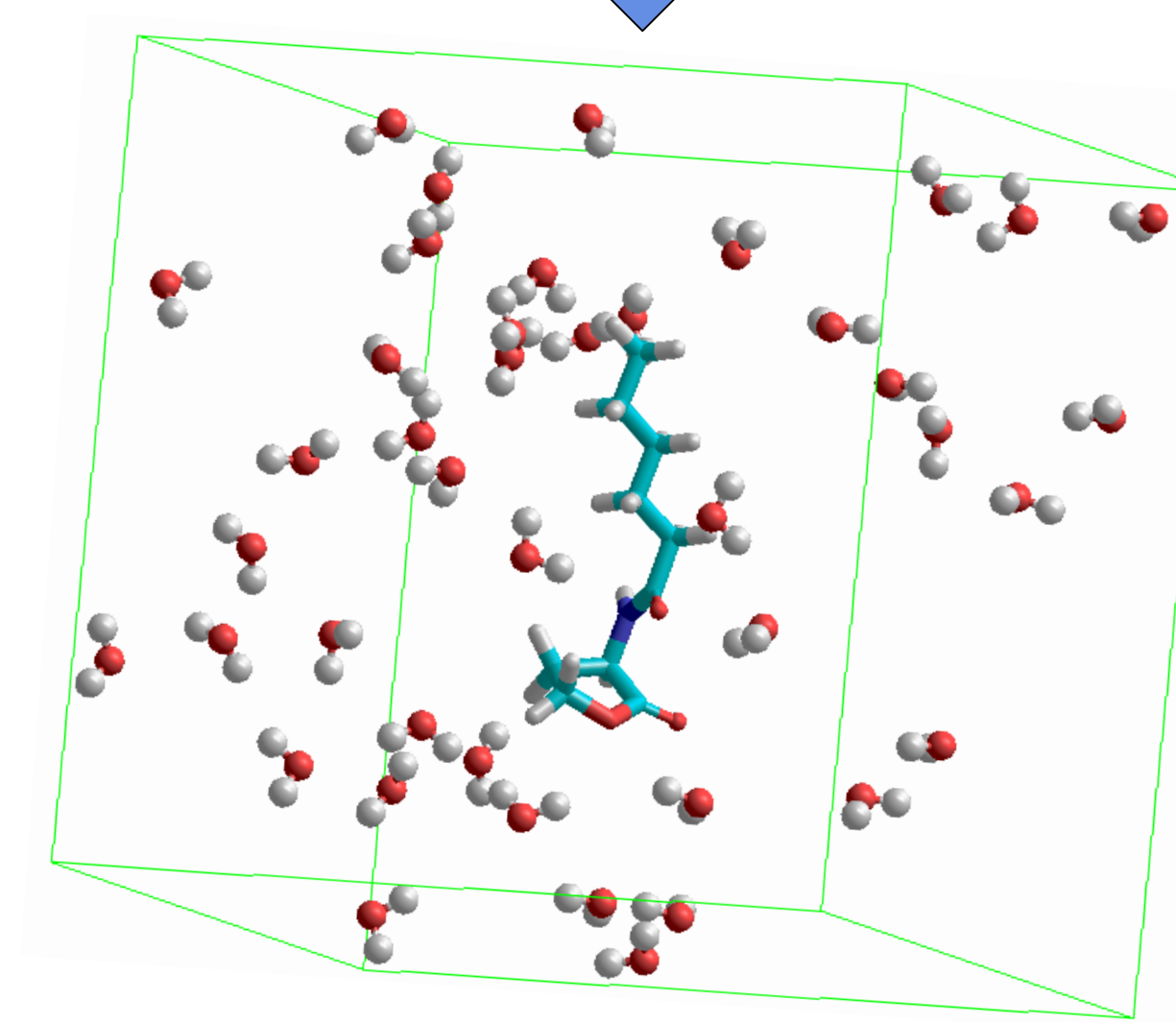
**Figure 7.** Theoretical spectrum predicted for each of the QS molecules. The vibrational frequencies were calculated using the semi-empirical AM1 method. Expected frequencies are seen, though far from observed locations in the experimental spectrum. More accurate vibrational frequencies can be calculated using more complex mathematical models, at the cost of much greater computational time.

## Theoretical Spectrum



**Figure 4.** Potential energy as a function of dihedral angle around the  $\Omega$  bond, 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.<sup>[7]</sup>

Search for low energy conformers and solvation



**Figure 5.** 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.<sup>[7]</sup>

## Theoretical Vibrational Analysis

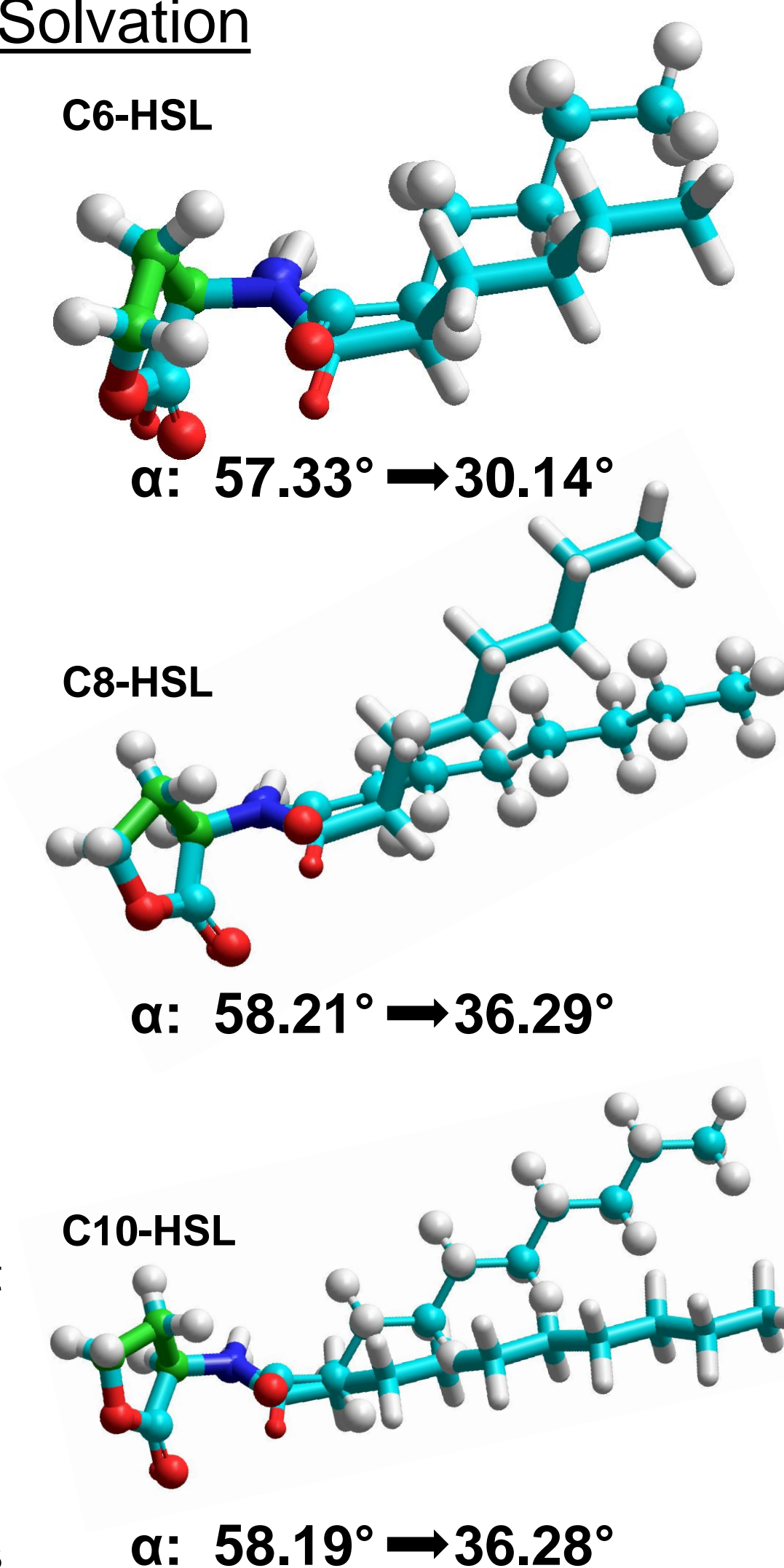
## Results

**Table 1.** Peak locations (cm<sup>-1</sup>) of the observable peaks of the QS molecules. Also listed are any spectral shifts.

Bond	Location of Peaks (cm <sup>-1</sup> )			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

## Effects of Solvation

**Figure 8.** The QS molecules in vacuum overlaid with the structure that was obtained from solvation. In all of the pictures, the molecule in vacuum is rendered in balls and sticks, while the solvated structure is shown in tube form (no balls). The C-C bond of the lactone ring was used as the coordination point, which is labeled in green. As expected, with all of the molecules, the solvent helps move the carbonyl oxygen (O3) closer to being over the carbonyl of the lactone ring. This is speculated to be due to the increase in molecular dipole moment as the bond dipole moments from the amide moiety and the lactone ring become co planar. The increased dipole moment will allow the QS molecule to be soluble in more liquid environments. The solvent can assist in twisting the amide linkage by hydrogen bonding to the amine hydrogen and the O3 oxygen. The red shift observed as the length of the acyl chain increases corresponds to the increasing effect of the solvent on the hydrogen bonding site. Images obtained via Hyperchem release 7.5.<sup>[7]</sup>



## 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.

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