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Dear Dr. Wu,

I am pleased to submit the attached revisions to our manuscript entitled “Characterizing Lewis Pairs Using Titration Coupled with ReactIR Spectroscopy” under consideration for publication as an article in the *Journal of Visualized Experiments*. Thank you very much for the opportunity to address the comments from the expert reviewers. We feel that we have been able to adequately address their concerns. Further, the resulting changes have improved the discussion in the manuscript. I have attached to this letter the reviewer comments as well as our response to them.

Submitted materials include the revised manuscript (in MS Word format), the revised manuscript with changes tracked (in MS Word format), this cover letter, along with eight Figures (in PSD format), the Table of Materials (in xls format), and the Author License Agreement. All authors have reviewed the contents of this revision and approved of its submission.

If you require any further information, I would be happy to provide it.

Best wishes,

James J. Devery, III, Ph.D.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We believe we have caught all errors at this time with the revised submission.

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Response: We have cited the corresponding figures accordingly and have attached the appropriate explicit copyright permission.

3. Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please check the iThenticateReport attached to this email.

Response: We have updated the corresponding sections that contained significant overlap with our previously published work and have made the following changes:

INTRODUCTION:

The utilization of Lewis acids to activate substrates containing carbonyls is ubiquitous in organic synthetic methods¹⁻⁴. The study of these interactions has relied on solid state X-ray crystallography, as well as *in situ* NMR spectroscopy². Limitations of these techniques manifest from artifacts that arise from crystallization, or the inability to probe paramagnetic Lewis acids via NMR analysis. To overcome these issues, chemists have employed infrared (IR) spectroscopy to determine the exact structure of Lewis pairs. Further, IR has been utilized to determine Lewis acidity⁴⁻⁹. The Susz lab studied the solid-state interactions of Lewis acids and carbonyls in the stoichiometric regime. Utilizing IR in conjunction with elemental analysis, the Susz group was able to elucidate the structures of neat, 1:1 mixtures of Lewis pairs. This analysis provided a great deal of insight into structural ramifications of the interactions of simple carbonyl compounds with commonly utilized Lewis acids in the solid state, and of particular interest to our lab: FeCl₃^{10,11}. We posited that we could add to the existing understanding of the interactions of these important Lewis pairs via an *in situ* method that examines synthetically relevant conditions.

ReactIR spectroscopy enables chemists to measure functional group specific reaction trends and profiles in real-time *in situ*, providing highly specific information about kinetics, mechanism, pathways, and the influence of reaction variables on performance. Using ReactIR spectroscopy, chemists can directly track reactants, reagents, intermediates, products and byproducts as their structures change over the course of the reaction. This tool provides critical information to chemists that facilitates the development of new compounds and the optimization of synthetic routes and new chemical processes.

Employing ReactIR spectroscopy as a detection method, we probed the substrates and intermediates that participate in the catalytic cycle of metal-mediated carbonyl-olefin metathesis¹². The Fe(III)-catalyzed carbonyl-olefin metathesis process, developed by the Schindler lab, exemplifies a powerful method for the production of C=C bonds from functional groups utilized ubiquitously in the construction of complex molecules¹³⁻¹⁵. Since the original report, this

process has inspired a plethora of synthetic developments beyond the utilization of Fe(III)^{16–25}. Importantly, this reaction requires that the Lewis acid catalyst differentiate between a substrate carbonyl and a product carbonyl for successful reactivity. To observe this competitive interaction under synthetically relevant conditions, we combined titration with the continuous observation provided by ReactIR.

We believe this method is of general importance to chemists studying carbonyl-centered reactions catalyzed by Lewis acids. This detailed video aims to help chemists apply this technique to their system of study.

4. For in-text referencing, please insert the superscripted text number before a comma or a period.

Response: We have corrected this error.

5. Please combine some short steps so that each step contains 2-3 actions.

Response: We have corrected this error.

6. The highlighted protocol steps are over the 2.75 page limit (including headings and spacing). Please highlight fewer steps for filming.

Response: We have corrected this error.

7. Please do not abbreviate journal titles for references.

Response: We have corrected this error.

8. Please sort the items in alphabetical order according to the name of material/equipment.

Response: We have corrected this error.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This manuscript must be revised and added more information before publication.

Major Concerns:

1. Reviewer can not find the useful of this manuscript. Author needs to add the discussion about the real application, for instance author should try other kinds of Lewis acids more than these.

Response: We have studied two Lewis acids that have been employed to catalyze carbonyl-olefin metathesis reactions: both ring-closing, and ring-opening metathesis. The GaCl₃ system offers an example of simple complexation (i.e. 1:1 complexation); whereas, the FeCl₃ system offers an example of more complicated complexation (i.e. highly-ligated complexes like complex 4). These examples provide a foundation for the types of interactions that could be observed for other Lewis acid/carbonyl systems. The extension of this analysis method to other Lewis acids would merely provide additional examples of the same displayed phenomena. Additionally, the findings from this method have been utilized to propose the mechanism of other metathesis reactions (*Org. Lett.* **2019, *21*, 8132). This citation was published while our manuscript was under review. We believe it is appropriate to include it in the conclusion, so we have added the following sentence to the last paragraph: “Findings from this method have been utilized to propose the mechanism of other metathesis reactions.”**

2. Why author select only both Lewis acid. I think author needs to try strong Lewis acid as well if possible.

Response: We focused on GaCl_3 and FeCl_3 due to their ability to catalyze the carbonyl-olefin metathesis reaction that is of interest to our group, as stated in the introduction. Furthermore, FeCl_3 is one of the strongest Lewis acids routinely used, with only AlCl_3 being stronger (*JACS*, 2017, 139, 10832; *Chem. Rev.* 1969, 69, 251; *Chem. Rev.* 1978, 78, 1). We have also previously reported that when a stronger Lewis acid, AlCl_3 , is employed, it does not facilitate the carbonyl-olefin metathesis reaction.

3. Did author consider about the reduction of FeCl_3 to FeCl_2 ? Please explain on this reason

Response: We did consider this electron transfer a possibility. We previously reported electron paramagnetic resonance spectroscopy studies that show no change in the oxidation state of Fe(III) either during the metathesis reaction, or when simply complexed with acetone. (*JACS*, 2017, 139, 10832).

4. Ketone or Carbonyl groups have several types. Here, because wavenumber of carbonyl groups have several positions, author should consider more than these 2 types.

Response: We have shown interactions of a simple aliphatic ketone, a simple aromatic aldehyde, and a complex aromatic ketone with two different Lewis acids. We have also previously studied the interactions of a simple aromatic ketone with GaCl_3 and FeCl_3 . (*JACS*, 2019, 141, 11870). These precedents showcase a simple interaction between the carbonyl of the titrant and GaCl_3 as well as a complicated interaction between the simple titrants and FeCl_3 . We have also included a competition study between a simple aliphatic ketone and a complex aromatic ketone to illustrate the competitive interactions between Lewis bases. Additional ketones, aldehydes, and esters would provide analogous data, and not provide additional insight. We believe that these examples showcase the capability of this analysis to identify the solution interactions between simple carbonyls and Lewis acids, and as these examples are relevant to carbonyl-olefin metathesis, we have shown its extension to aid in mechanistic investigations.

5. If the real analysis has many functional groups (in the case of Complicated), how your research can apply it? Please discuss on this.

Response: We have included competition studies between two different carbonyl-containing compounds to elucidate their respective binding affinities with these Lewis acids. This analysis involves fairly complicated IR spectra (Figure 7), where we observed at least 4-5 different carbonyl-containing species in solution, including native carbonyl compounds, Lewis acid/carbonyl pairs, and highly ligated compounds with both same ligand, and mixed ligand structures. We believe that additional complexity would obfuscate the fundamental observations that are possible via this method and make the work less accessible to the general audience of *JoVE*.

6. Author needs to explain on chemical mechanism such as electron density on carbonyl group and moving of electron.

Response: It is not clear what the reviewer is asking, as the electronic interactions of carbonyls and Lewis acids have been well-characterized in the literature. Further, as we have previously reported, the oxidation state of FeCl_3 is not changing. As a result, there is no electron movement in these systems. The Susz lab dedicated more than a decade of work measuring and calculating the interactions of a significant number of Lewis acids and bases. We have cited the relevant literature that corresponds to the examples presented.

However, this manuscript provided new technique and many interesting idea but it needs to be provided more details based on above comments. I also suggested author give more information about application, why have to use your research to solve problems.

Response: The application is to provide insight into the ground state structures that are possible in solution during catalytic mechanisms facilitated by the interactions of Lewis acids and bases, which

we state in the introduction. We show the direct application of these data in determining the operating mechanism of an important new reaction (*JACS*, 2019, 141, 11870). Further, this work has been used as the basis of mechanistic proposals by other labs (*Org. Lett.* 2019, 21, 8132). Because of the wide range of Lewis acid-catalyzed reactions that exist in the literature (References 1-4), we believe that this method can be widely applied to a broad range of mechanisms to support proposals.

Reviewer #2:

Manuscript Summary:

The activation of chemical functionalities with Lewis acids is a crucial process in organic chemistry and the quantitative measurements of the association of carbonyl compounds with Lewis acids is very useful tool for understanding the nature (structure, reactivity...) of the reactive intermediates and complexes involved in a catalytic cycle.

Major Concerns:

- I would be surprised that there are no UV-Vis studies on the association of carbonyl compounds with Lewis acids? The second line of the introduction should be certainly revised.

Response: We agree with the Reviewer that it is surprising that there are no UV/Vis studies on the interactions between carbonyls and Lewis acids. We have performed exhaustive searches through the literature and have been unable to find UV/Vis analyses on these types of interactions. The interactions between carbonyls and Lewis acids have been primarily observed through IR and/or NMR spectroscopy, as we have cited.

- In figure 3, the starting absorbance is -0.01 whereas the maximum is 0.04. Does this mean that the background was not performed correctly?

Response: We agree with the reviewer. Our original intent for Figure 3 was to show a live feed of raw data collection and only show the first 5 additions of acetone. An entire titration typically includes 39 or more additions. We apply a baseline correction after data collection across the entire spectra as is apparent in Figure 4, 5, and 7. However, we agree with the reviewer that this presentation could be confusing to a general reader and have updated Figure 3.

- The Job plot method is often used for determining the stoichiometry of donor-acceptor complexes, would that possible to use this method to confirm the stoichiometry in complex 4?

Response: Unfortunately, the Job plot method cannot be applied to this system because FeCl_3 is moisture sensitive and therefore cannot be added incrementally to a reaction flask with our described setup. Additionally, FeCl_3 is insoluble in DCE as we have previously mentioned, which does not allow for a solution of FeCl_3 to be added incrementally. Lastly, the Job method requires all components to be homogeneous at all times.

This question is linked to the fact that the authors mentioned on page 6/6 that the FeCl_3 is largely insoluble in DCE, and I do not see in the protocol if this solution become homogeneous when acetone is added.

Response: We agree with the reviewer and apologize that we did not address this observation in the protocol initially. FeCl_3 becomes homogenous in DCE once 1 equiv acetone has been added. We have included these observations in the protocol as well as in the discussion and have highlighted below where these changes were made.

2.3 Under inert atmosphere, add Lewis acid to a flame-dried 25 mL 2-neck round bottom flask charged with a stir bar (Figure 2B). Seal the flask with rubber septa and attach an Ar-filled balloon to the flask. Add desired volume of anhydrous solvent via syringe (minimum 3 mL) (Figure 2C).

Note: FeCl_3 is not soluble in DCE. GaCl_3 is soluble in DCE.

4.4 Repeat steps 4.1-4.3 until desired amount of analyte is added.

Note: FeCl_3 mixture becomes homogeneous once 1 equiv **1** is added and GaCl_3 mixture remains homogeneous regardless of amount of **1** added.

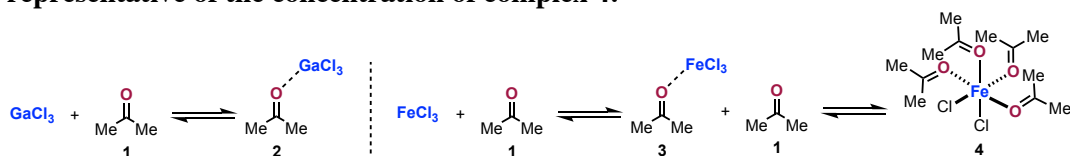
The updated Discussion is below:

DISCUSSION:

Under anhydrous conditions, Lewis acids can have a range of solubilities. The two examples we have presented are GaCl_3 and FeCl_3 in DCE. GaCl_3 is homogeneous at the onset of the titration, while FeCl_3 is largely insoluble. Beginning with the homogeneous solution of GaCl_3 , we completed a titration from 0-4 equiv **1** in 10 μL increments and extracted the IR spectra (Figure 4A). Examination of the transitions that occur over the course of the titrations shows a formation of a single species in the carbonyl region at 1630 cm^{-1} , which grows from 0-1 equiv **1** (Figure 4B) ^{26,27}. When greater than 1 equiv **1** is added to the solution, no change in the peak at 1630 cm^{-1} occurs and unbound **1** is observed at 1714 cm^{-1} (Figure 4C). These results are consistent with the formation of **2**. When the same titration is performed with FeCl_3 (Figure 4D), a peak at 1636 cm^{-1} forms from 0-1 equiv **1**, which is consistent with **3** (Figure 4E). **Importantly, the mixture becomes homogenous once 1 equiv **1** is achieved.** When the titration proceeds beyond 1 equiv **1**, unbound **1** is observed at 1714 cm^{-1} , **3** decreases in intensity, an isosbestic point resolves at 1648 cm^{-1} , and a new peak at 1663 cm^{-1} forms.

- I do not understand why the association constants K of the equilibria shown in Figure 1 have not been determined. The manuscript and video will gain in interest if these equilibrium constants are determined. As the titration experiments are already done, and that the data are available, it is expected that the values of the equilibrium constant between **1** and GaCl_3 and between **1** and FeCl_3 should be calculated and given in the discussion section.

Response: We agree with the Reviewer that the determination of the equilibrium constants between acetone and GaCl_3 and between acetone and FeCl_3 would be beneficial. However, in the case of the equilibrium between **1** and GaCl_3 , we observe no **1** in solution, so the equilibrium concentration of acetone is a small number and effectively 0 between 0-1 equiv acetone added. Once we get beyond 1 equiv acetone added, the concentration of the GaCl_3 is also a small number and effectively 0; therefore, the most we can say about the equilibrium constant is that it is a large value. In the case of the FeCl_3 system, we observe a similar issue with respect to **1**, which is compounded by the insolubility of FeCl_3 , having a concentration near 0. Alternatively, once we get beyond 1 equiv acetone added, we have proposed complex **4** as one possible structure that is found in solution (*JACS*, 2019, 141, 11870); however, it is likely that there is a mixture of different types of highly-ligated structures that are formed in solution as is indicated by our non-whole number slope in our consumption analysis in Figure 6. Therefore, we cannot assume that the signal we observe around 1650 cm^{-1} is solely representative of the concentration of complex **4**.



To address this comment, we have added the following language to the Discussion: “However, it is likely that there is a mixture of different types of highly-ligated structures that are formed in solution

as is indicated by our non-whole number slopes in our consumption analysis in Figure 6, and the crystal structure we observe may simply be the one complex that precipitates.”

The manuscript is overall of very good technical quality and shows that IR methods and ReactIR spectroscopy can be used for performing quantitative evaluations of Lewis base interactions in the context of organic catalysis.

Interesting aspect such as competition experiments, investigations of catalytic cycles and determination of the structures of reactive Lewis acid-base adducts are investigated. This will attract the attention of organic chemistry, but also chemists interested in physical organic chemistry, catalysis, mechanistic and structural investigations and I would recommend to accept this manuscript and to proceed with the experimental part.