Investigating Crystal Violet Reactivity and Color with Quantum Theory and Interactive Webpages

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Response to Reviewer Comments March 18, 2025

Editor	
Comment	Response
manuscript falls under a Laboratory Experiment	Changed on ACS submission page
the corresponding requirements apply. The manuscript satisfies most of them except for reporting assessment outcomes, as noted by Reviewer 3. Are students' completed worksheets assessed? How well do they do? Please comment on the extent to which the intended learning outcomes are achieved. Reporting aggregated grades or commenting on student outcomes	Worksheets from 125 students in 7 lab sections in the Spring 2025 class were analyzed. Aggregate results of 7 sections are plotted in a figure and table in SI. Summary paragraphs address the strengths and weaknesses of student responses.
I advise against using GC as an acronym for general chemistry.	Done
Reviewer 1	
it is a rather unusual and complex choice from the point of view of examining reaction coordinates, transitions states, and chemical reactions. That is because in this case the barrier is largely to due desolvation of OH-. In contrast, we tend to focus on gas-phase reactions in gen. chem., and talk about barriers due to simultaneous bond breaking and bond making. That is still true here — but one is forced to consider the hydrogen bonds!	Considering changes in hydrogen bonds during this reaction allows students to follow the fate of these bonds throughout the reaction progress. Although it is not emphasized in the worksheet, the magnitude of E_{act} is consistent with the breaking or weakening of several hydrogen bonds. The treatment of transition states in general chem texts varies tremendously. Chang and Overby (13th ed) show no examples at all. Burdge and Overby (4th ed) show a transition state of the Cl..ClNO gas phase reaction. Gilbert, Kirss, et al (2nd ed.) give a nice discussion with diagrams of the O_3...NO gas phase reaction. Talk about exotic! Students must think: Who ever heard of these reactions and why do I care? On the other hand, with this crystal violet exercise students have just seen these substances reacting on the lab bench with their own eyes.
The choice of the 4-water OH- cluster is unusual. Why choose "one of the most stable" clusters, rather than the most stable? Otherwise, this seems arbitrary. Admittedly this does not likely influence the pedagogical values, but still seems odd.	"Four" waters were chosen because this strikes a balance between obtaining realistic energy change values and somewhat simplified cluster structures. As pointed out in the student handout, in the actual water solution probably many different clusters exist and are reacting with CV. The 4-water cluster is a model that is treatable by QM methods. Figure-S1-OH-4waters.pdf, which details the structures and energies of several hydroxide-water clusters, has been added to the SI. The bridged cluster used in the CV exercise is the most stable at this level of theory.
Similarly, is 4c a true transition state? Has this been verified (e.g. by identifying a single imaginary mode)? This should be done.	Already answered in the original ms. See p.3, line 54 and p.4 line 3.

* On a similar note, there is good pedagogical value of examining the nature of this imaginary mode (which essentially corresponds to the reaction coordinate, locally!). The note of a reaction coordinate would also tie into several of the author's worksheet questions.	The reviewer is correct that the imaginary vibrational mode of the TS is important and interesting. In fact, an earlier version of our JSmol website included an animation of the imaginary mode, as well as a reaction trajectory taken from the intrinsic reaction coordinate (IRC) calculation. ---However, reference to this phenomenon was removed from the handout and worksheet to reduce the number of concepts necessary for understanding the exercise, which is already large. This would be ideal for a version of the exercise aimed at P. chem or organic students.
* Does the authors have his students examine the bond lengths in the CV+ or CV species? One might see examples of the resonance hybrid in the bond length changes for those bonds involved in the hybridization.	This is a good suggestion. A sub-section that addresses C-N bond lengths has been added to Part A of the worksheet for the Spring 2025 lab, which results are included in the ms.
Some of the fine details in the "WebMO and Compute Servers" section appears to be implementation and location specific. Perhaps this is better places in SI?	Some location-specific information has been removed or moved to the SI. However, the materials unavoidably mention the course number for this class, such as the custom WebMO template "Molecular Energy – 106". We feel that this is an important detail that helps students identify with, and feel ownership of, this laboratory exercise. Adopters can name their custom template any way they wish.
The arrowing in Eq. 1is strangely slanted.	Arrow angle corrected.
Reviewer 2	
To educate readers who may not be knowledgeable or fluent in the concepts and terms of computational chemistry, I suggest that the authors add an annotated glossary	This is a good suggestion. A glossary PDF will be made available to students. A copy is included in the SI.
Please justify or explain why the B3LYP/6-31G(d,p) level of theory was chosen for the calculations in this exercise.	The indicated theory was chosen for several reasons based on the information in the following Table. - Density functional theory (DFT) was chosen over semi-empirical theory. xTB is a fast and fairly accurate semi-empirical method, but the xTB energies are off (line 1). - A very large basis set (line 5) gives the most accurate ΔE_{rxn}. However, it is about 4x slower than if a smaller basis set is used. - The basis set with p-functions on H atoms (6.31G(d,p)) is better on ΔE_{rxn} and but is slightly slower than if p-functions are not included (6-31G(d)). - Why B3LYP and not one of the other 100 or so DFTs? Because this is the most common DFT by far and it gives good geometries. Trivially, the acronym is easy to explain in terms of the developers' initials. - The calculations include solvation by a polarizable continuum model because the reaction takes place within a highly polar solvent. If solvation is not taken into account, the

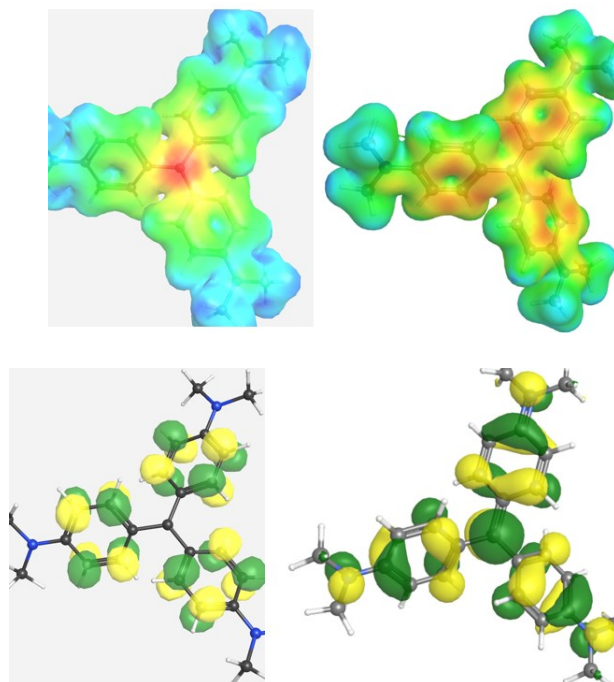|		reaction energy for combining a cation and anion is far too negative (line 4) This is now addressed in the ms.

Table. Energies of the CV + OH(H$_2$O)$_4$ → CVOH(H$_2$O)$_4$ reaction, and single-point calculation times for the CVOH(H$_2$O)$_4$ complex, using different model chemistries

Model chemistry	Polarizable continuum model	# Basis functions[‡]	ΔE_{rxn} kcal mol^{-1}	ΔE_{act} kcal mol^{-1}	Calculation elapsed time[‡§] sec
GFN2-xTB	ALPB	171	-22.499	2.367	0.2
B3LYP/6-31G(d)	SMD	540	-11.281	6.482	28
B3LYP/6-31G(d,p)	SMD	657	-10.603	6.885	32
B3LYP/6-31G(d,p)	(none)	657	-78.444	n.d.	n.d.
B3LYP-D3BJ/def2-TZVP	SMD	1257	-8.618	6.350	111
Experiment	-	-	-6.3*	15.3*	-

[‡]CVOH-4-water complex. [§]Core i9-13900K cpu (8 cores) *ΔH ; Ritchie, C. D. et al. *J. Am. Chem. Soc.* **1975**, 97(5), 1163-1170.

A very serviceable version of WEBMO is available as a free iPhone or Android app and offers many of the computational features used in this exercise. As access to the WEBMO smartphone app is easy and immediate and does not require the complex IT steps required to install it on desktops and local servers, please consider and describe what elements of the lab exercise could be accomplished using the smartphone apps.	This is an interesting suggestion. However, the Android app uses the Hückel molecular orbital method, which leads to the erroneous conclusion that the crystal violet's central carbon is electron-rich (the red spot on the upper left image (Hückel-surface). Whereas this carbon actually carries a very small charge (yellow on the upper right image). Also, the Hückel method shows a LUMO with a node (zero amplitude) on the central carbon (lower left), whereas all DFT and ab initio methods indicate there is a large LUMO amplitude on that carbon (lower right). This is why OH$^-$ bonds at that position. Therefore, the Android app will not work for this exercise.
My test drive of the Android WEBMO app let me import the structure of CV; calculate energy content, bond distances, and orbital energies; display electron density, electrostatic potential, and molecular orbitals;	There is no need to install WebMO on any student device. The student's browser (Chrome or Firefox or others) connects to the WebMO server and receives data and structures from it. The true nature of WebMO can be appreciated by logging on as guest, password guest, at https://www.webmo.net/demoserver/cgi-bin/webmo/login.cgi.

and calculate excited states and visualize a UV-Vis spectrum. (screenshots of example outputs are attached)	The exercise described in this ms requires that a chemistry department have a working WebMO server and one or more remote server computers. This would require obtaining a WebMO license, installing one or two Linux computers with at least the ORCA computational program, and making the appropriate network and Internet connections.
Page 4. "Learning Objectives": Please enumerate the specific learning objective here, so readers do not have to search for them in the supplementary materials.	A Learning Objectives summary is added to the ms. ahead of the existing sections that describe the different sections of the worksheet.
Page 6, lines 9-16: Please expound upon the reasons why the calculated lambda(max) values for crystal violet and CVOH (and HOMO-LUMO gaps) are at higher energy levels than the experimental values. Possible reasons for these discrepancies could be queried in the student worksheets.	The student worksheet already notes that the $\Delta E = E_{LUMO} - E_{HOMO}$ calculation is a crude approximation because electronic excitations actually involve promotions from multiple orbitals. The more basic issue regarding the nature of the excited state, as explained below, has been added to the ms and the student worksheet. Calculating λ_{max} using $\Delta E = h\nu$, where $\Delta E = E_{LUMO} - E_{HOMO}$ as in this CV exercise, makes the crude assumption that the CV orbitals are unchanged when an electron is promoted from HOMO to LUMO. The molecular geometry remains the same (this is a "vertical excitation" as defined by the Born-Oppenheimer approximation), however in reality, the orbitals instantly adjust to stabilize the excited state, which results in an excitation energy 12 kcal/mol less than expected from the crude approximation. Taking this adjustment into account requires solving, or approximating a solution to, the time-dependent Schrödinger Equation. (Note that one cannot simply put an electron into the LUMO and calculate new orbitals: the self-consistent field process will instead revert to the ground state.) A TDDFT calculation at the B3LYP/6-31G(d,p)/SMD level of theory corrects about half the excitation energy discrepancy, giving a λ_{max} of 510 nm. Asking students to explain this energy discrepancy - beyond parroting the explanation already given - is beyond the scope of a general chemistry worksheet. The point of this calculation is to explain why one molecule is purple and the other one colorless, and this has been done.
Define TDDFT and M06-2X/def2-TZVP in the glossary.	These terms have been removed.
Reviewer 3	
...there should also be an assessment of how the experiment improved the learning process of students and whether the pedagogical goals were achieved.	Analysis of student reports is now included.
Further, the instruction to authors states that "Limitations of the experiment...should be noted as an indication of whether it can be used in certain settings." I comment on the limitations requirement because I would not be able to replicate the instructions for setting up WebMO and ORCA and running the specific	Limitations section added.

experiments without the assistance of an IT-proficient colleague.	
Pre-Lab Answer Key: Question 5 has "-18.3" as an answer. I end up with -19.3 kcal/mol.	corrected
On the worksheet, Part A, question 1c, the instructions below the structures state to add four "C=C's and one +" symbol. This is not quite correct as one of the bonds is a N=C bond.	corrected
In the worksheet, Part B, question 1: A bit of context for what these numbers mean might be useful to a student. For example, this is a reasonably slow reaction at room temperature. What activation energy might correspond to a fast reaction at rt? The same sort of analogy might be useful for the reaction energy. This section also has me wondering if the general chemistry textbook uses kJ or kcal. If mostly kJ, it might be worth staying consistent.	These are useful suggestions. The Burdge Atoms First text does use kJ rather than kcal, and this can be changed in future editions of the worksheet. We have considered adding questions related to the expected energy changes in the CV + OH reaction. However, we cannot make big changes in the materials at this point in the semester. This article is based on the existing student materials contained in the SI. WebMO and many ACS journal articles use kcal/mol. The assignment here is to do the calculations and then relate these numbers to the energy plot already given. This is in fact what scientists do: First get the data, THEN attempt to rationalize what the data means. The worksheet now includes a hint to check Figure 2 in the student handout, which contains the correct labels.
In the worksheet, Part B, question 2: I think I would find it useful if the figures used the same color scheme as in the discussion handout, with hydroxide-colored fuchsia.	The fuchsia-colored O of OH is now included in the handout, worksheet, worksheet key, and JSmol webpages.
In part C, students are asked to compare their lambda max answers to the spectrum in the handout. Here, I would suggest acknowledging the discrepancy between experimental and calculated values. It would be appropriate to discuss the limitations of calculations, the trade-off between time and lower levels of theory, etc.	Added statement: "Since this is a rather crude approximation, your calculated λ_{max} values will be much less, i.e. at higher energy, than the experimental values. Nevertheless, you should still be able to clearly distinguish between the spectral properties of CV and CVOH." We choose not to delve into the time/accuracy trade-off in this exercise which is already stuffed with new and unfamiliar material. Several aspects of the calculations such as timing, solvation, theory and basis set, etc., are either not mentioned, or are mentioned only in the Appendix of the student handout.