The Role of High-Level Calculations in the Assignment of the Q-Band Spectra of Chlorophyll

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Abstract.We recently established a novel assignment of the visible absorption spectrum of chlorophyll-a that sees the two components Q_x and Q_y of the low-energy Q band as being intrinsically mixed by non-adiabatic coupling. This ended 50 years debate as to the nature of the Q bands, with prior discussion poised only in the language of the Born-Oppenheimer and Condon approximations. The new assignment presents significant ramifications for exciton transport and quantum coherence effects in photosystems. Results from state of the art electronic structure calculations have always been used to justify assignments, but quantitative inaccuracies and systematic failures have historically limited usefulness. We examine the role of CAM-B3LYP time-dependent density-functional theory (TD-DFT) and Symmetry Adapted Cluster-Configuration Interaction (SAC-CI) calculations in first showing that all previous assignments were untenable, in justifying the new assignment, in making some extraordinary predictions that were vindicated by the new assignment, and in then identifying small but significant anomalies in the extensive experimental data record.

INTRODUCTION

Recently, we demonstrated a new assignment of the Q-band spectrum of chlorophyll-a (Chl-a) and many other chlorophyllides.[1-3] Its key features, plus those of the previously competitive "traditional" 1960's [4-7] and "modern" 1980's [8-12] proposals are depicted in FIG. 1. Shown for Chl-a in ether are the observed absorption band contour ($\Delta A/\nu(\nu)$ where A is the absorption coefficient [12] and ν is frequency), reflected emission band contour ($E(\nu)/\nu^3(\nu)$ where $E(\nu)=E(\lambda)/\nu^2$ is the emission strength [13]), and magnetic circular dichroism (MCD) band contour.[12] The Q-band is made up of two independent electronic transitions Q_y and Q_x, each with a dominant origin band and associated vibrational sideband tail tohigher energy; the lowest-energy band is clearly the intense Q_y origin, the debated issue concerns the location of the weak Q_x origin somewhere amidst the Q_y sideband. As the emission is much weaker at Q_y+~2000 cm^{-1} than is the absorption, the Q_x origin is likely to be located here, [7] leading to the "traditional" assignment. MCD spectra are like absorption spectra except that $Q_y$appears with positive signal whilst the Q_x appears negative, and the relative intensity ratios (known as the "*B/D* ratio") are very different for Q_x and Q_y.

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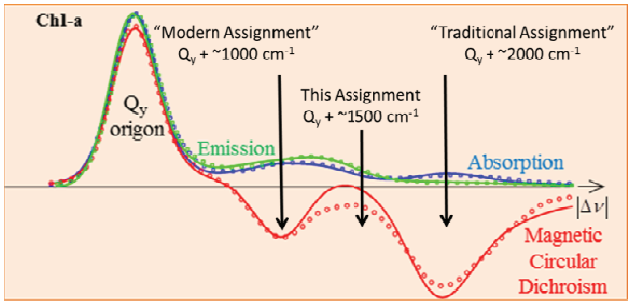

FIGURE 1. The observed absorption band contour in Q-band (670-550 nm) region for Chl-a in ether is shown as a function of frequency difference Δν from the intense Q_y origin (blue) where it is compared to the observed emission band contour reflected about this origin (green) and the MCD band contour (red).

FIG. 1 locates the most intense negative MCD peak at a similar location, supporting the "traditional" assignment. However, a second negative MCD peak at Q_y+~1000 cm^{-1} is also apparent, and the "modern" assignment attributes this to Q_x instead. It was proposed based on polarized fluorescence-excitation spectra in diethylether at low temperatures, [8-12] and strongly supported by both linear dichroism [11] and careful MCD analyses.[12] Neither assignment could explain the existence of two negative bands in the Q_x region, however. Research proceeded based on the assumption that one of the two options was fundamentally correct and that some unknown auxiliary process was responsible for the observed anomalies, and for 50 years the quest was to measure some *truly* indicative property to resolve the fundamental assignment question. In this vein, we developed high-resolution spectroscopic techniques in 2009 and 2010 whose results appeared consistent with, in one case,only the "traditional" assignment, [14] and in the other case,only the "modern" assignment (showing here significantly that the emission spectrum is also depressed at Q_y+~1000 cm^{-1}). [13] All analyses of photosystem function and coherence have used the "traditional" assignment, both for its simplicity and because key information such as the Q_x/Q_y dipole-strength ratios, required to utilize the "modern" assignment, had never been properly determined.

Both the "traditional" and "modern" assignments are based on the Born-Oppenheimer approximation and the Franck-Condon principle that portray electronic spectral bands as being dominated by a single structure centered about the vertical excitation energy,which for chlorophylls manifests as an intense origin plusweak vibrational sideband(s).Instead, our new assignment, which is consistent with *all* known experimental data, allows the Q_y and Q_x states to become strongly mixed by vibronic-coupling effects that make the electronic wavefunctions dependent on nuclear momentum, in violation of the Born-Oppenheimer approximation. As a result, for Chl-a the usual single observed origin+sidebandstructure becomes split into two equal-intensity origin+sideband systems, with very little absorption or emission occurring at the band's actual vertical excitation energy; this assignment places the Q_x state at Q_y+~1500 cm^{-1}, as depicted in FIG. 1. The consideration of such a scenario was first contemplated by Gouterman [5, 15] and others in the 1960's: while it couldaccount for the observed spectra of Chl-a, analogous spectra for other chlorophyllides such asbacteriochlorophyll-a (BChl-a) and pheophytin-a (Pheo-a) showed only one peak whereas two would be expected. Our recent work has identified the missing peaks for all chlorophyllides. [1, 2] These peaks can be quite weakand identification required the development of a range of new data-analysis methods. [1, 2]

Our primary interest herein concerns the role of high-level electronic-structure calculations in the establishment of our new assignment. There are three ways in which calculations can readily contribute. First, from a qualitative perspective, calculations can potentially provide alternate explanations to the observation of two *x*-polarized bands in the MCD results, for example by showing that a third electronic stateis located near Q_x. Second, calculations can readily predict the bandgap ΔE, a quantity central to the assignment options. Finally, calculations can predict the Q_x/Q_y dipole-strength ratio but this aspect has not been of great historical significance as the "modern" assignment was never applied to yieldthis quantity for comparison.

Theoretical understanding of the spectra of porphyrins and chlorophylls dates back to Gouterman's "4-orbital" model. [16] This considers only the two highest occupied orbitals and two lowest unoccupied ones, depicting four independent electronic transitions Q_x, Q_y, and the analogous Soret-band components B_x and B_y. Higher-energy transitions are known for porphyrins starting at the N band, but the Soret bandshape is complex for chlorophylls and the N bands have never been reliably identified. Early empirical,semi-empirical, and ab initio molecular-orbital calculations confirmed Gouterman's analysis, placing the N bands etc. at much higher energy than the Q and Soret bands. Historically, it was firmly believed that the Q bands were isolated and that no other electronic state could contribute significantly to the two observed *x*-polarized bands. These calculations almost invariablygave a large

energy gap ΔE that would seem to be consistent only with the "traditional" assignment, though likely shortcomings in the calculations were known to exceed the differences in ΔEas perceived by the various assignments.

During the 1990's Time-Dependent Density-Functional Theory (TD-DFT) provided an alternate path to excited-state analysis, and calculations [17, 18] depicted a significantly different excited-state manifold in which unidentified electronic states sat very close to Q_x, explaining the observation of two *x*-polarized bands to vindicate the "modern" assignment.As TD-DFT includes a much better description of electron correlation than did the older Hartree-Fock-based methods, the new results were considered as more reliable. However, Multi-Reference Configuration Interaction(MRCI)-DFT results became available soon afterwards [19] which predicted a state to be between the Q and Soret bands but close to the Soret band, more supporting "traditional" analyses. As MRCI-DFTembodies an empirical enhancement to normal DFT, that at the time was not supported by a wide range of successful applications, it was thennatural to think of these results as inferior. Also at that time the accurate molecular-orbital Symmetric Adapted Cluster-Configuration Interaction (SAC-CI)method was applied to chlorophylls, again vindicating the "traditional" analysis. [20] Searching for answers as to the quality of the TD-DFT results, we measured and analyzed oligoporphyrin and polyacetylene structure and spectra, [21, 22] pointing out systematic failings of TD-DFT when applied using generalized-gradient approximation (GGA) and hybrid functionals, of the type used previously for Chl-a. We then showed that the novel low-energyband that such functionals predict [17, 18] is in fact the N band, a band of charge-transfer type that GGA-type functionals seriously underestimate in energy, and that these methods incorrectly predict this state to be the lowest-energy state in PhotosystemI. [23] The newly suggested CAM-B3LYP density functional [24] containing corrections for this effect was then programmed [25] and demonstrated [26] to provide realistic answers for porphyrins and chlorophylls whilst other newly created functionals designed to address the same issues still gave the old failures. All current high-level computational methods thus indicate that only Q_x and Q_y contribute significantly to the spectrum of chlorophyll in the Q-band region.

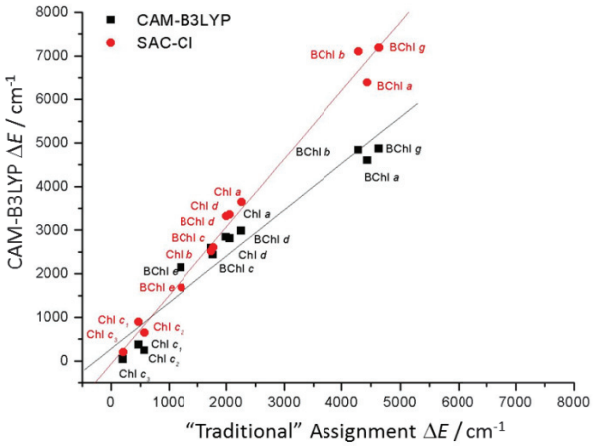

FIGURE 2. Original SAC-CI and SAC-B3LYP calculations using continuum solvent models appear to support the "traditional" assignment but cannot interpret observed large explicit solvation effects.

Between 2005 and 2007 we appliedCAM-B3LYP/6-31G* and SAC-CI/6-31G* to calculate the band gap ΔE and thus discriminate between the "traditional" and "modern" assignments. Calculations were performed for 34 chlorophyllide-solvent combinations, initially using continuum dielectric solvation models. Apparently good results were obtained, as illusrtated in FIG. 2, but these were misleading as the coordinaton of the magnesium atom can be either five-coordinate (5CO) or six-coordinate (6CO) [27, 28] and the corresponding large change inΔEcannot be accounted for. This led us to consider chlorophyllide-solvent complexes. Unfortunately, the extreme truncation of integrals required to make SAC-CI calculations feasible introduced errors that were of the order of the effects being investigated, leaving only CAM-B3LYP as a viable computational method. Results for explicitly solvated molecules in addition to a surrounding dielectric continuum model are shown in FIG. 3, though we found that dielectric solvation had an insignificant effect on explicitly solvated chlorophyllides and so for simplicity published previously only results for gas-phase complexes. [1] Again the "traditional"assignment shows a general correlation with CAM-B3LYP but the data can be blocked into groups that behave quite differently. The insert in the figure highlights this, considering the change in ΔEon lowering the temperature,converting 5CO species into 6CO ones. This is a property that the calculations would be expected to describe well, yet there is no correlation with the

"traditional" assignment. These aspects again reoccur for the "modern" assignment. However, good agreement is found with our new vibronic-coupling assignment, although free-basechlorophyllides (e.g., pheophytins) appear displaced by 1000 cm^{-1} from metal-containing analogues, and the slopes of the lines of best fit are near 0.75 instead of unity. These differences are attributed to unknown shortcomings of CAM-B3LYP.

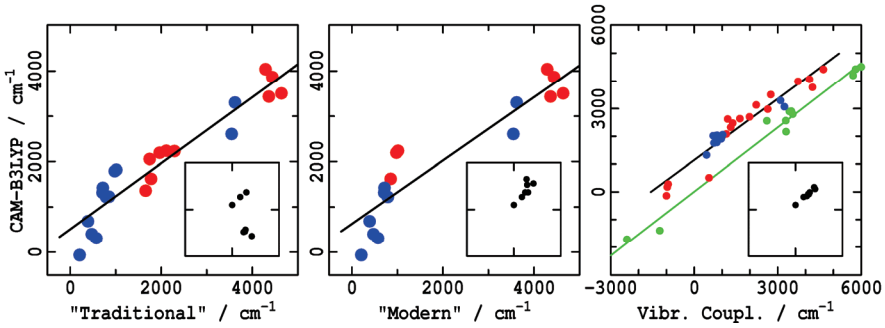

FIGURE 3. CAM-B3LYP calculated Q_x-Q_y band gaps ΔE are compared to observed values based on the "traditional", "modern", and our new vibronic-coupling assignment of chlorophyllide spectra. Red- 6CO species, blue- 5CO species, green- free-base species. The inserts show the changes in ΔE (scale -2000 to 0 cm^{-1}) from 6CO to 5CO species in the same solvent.

When the CAM-B3LYP calculations were completed in 2007,results were difficult to interpret as it was unknown as to whether or not the method had delivered the required quantitative accuracy. In the following years we demonstrated that CAM-B3LYP could predict the exciton couplings and energy dispersion amongst chlorophylls in Photosystem-I using coordinates from a PW91/6-31(+)G* optimization of the 150000-atom photosystem trimer, [29] suggesting that the Q_y band is described well. [30] Later we also showed that it could predict and interpret the unexpected large asymmetry between absorption and emission observed for BChl-a, [31] indicating that CAM-B3LYP gives a good representation$Q_y$as a function of Condon-allowed nuclear coordinates. These results suggested that the CAM-B3LYP results for ΔE were reliable, highlightingthe need for a new assignment.

While the results shown in FIG. 3 then provided strong theoretical endorsement for the new assignment, even more important results turned out to be the prediction of a 7-fold variability in the relative Q_x intensity, a completely unforeseen property that emerged during the spectral fitting to the vibronic-coupling model that was initially interpreted as arising from a flawed assignment. [1] Also, spectroscopic data in ether was initially highly inconsistent with the CAM-B3LYP predictions, leading to the discovery that 30 years of critical data had been compromised (to a small but significant extent) through trace water contamination.[3]

In conclusion we see that the availability of high-quality calculated data contributed significantly to the understanding that a new assignment was required, and thenwas essential to the establishment of its legitimacy. The assignment not only explains all available experimental data but is also consistent with basic theory. It was only because a wide range of experimental and theoretical data was available that the assignment could be made.

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