

A Modified Resonance-Theoretic Framework for Structure–Property Relationships in a Halochromic Oxonol Dye

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Received January 1, 2010

Abstract: I demonstrate that a modification of the resonance color theory (in its form advocated by Brooker (*Rev. Mod. Phys.* **1942**, *14*, 275) and by Platt (*J. Chem. Phys.* **1956**, *25*, 80)) provides an accurate framework for rationalizing the *ab initio* excitation energies of the protonation states of the green fluorescent protein (GFP) chromophore (an asymmetric oxonol dye). I suggest that the original model space used in the resonance theory (specifically, a pair of Lewis structures) is formally inconsistent with a core aspect of the theory (specifically, a relationship between excitation energies and group-specific basicities (Brooker basicities) of the terminal rings). I argue that a more appropriate model space would consist of a complete active space ansatz based on group-localized orbitals. I then show that there is a solution to the state-averaged complete active space self consistent field (SA-CASSCF) problem with exactly this form. This family of SA-CASSCF solutions provides an objectively rigorous foundation for the resonance color theory. The solutions can be expressed in a localized set of active space orbitals, which display the same transferability pattern implied by the Brooker basicity scale. Using Platt's model Hamiltonian formulation of the resonance theory, I show that the accuracy of the set of excitation energies calculated with these solutions can be accurately reproduced using only two parameters per dye in the set. One of these parameters is the isoenergetic energy of the dye—the harmonic mean of the excitation energies of its symmetric parent dyes. The other parameter is a local basicity index (Brooker basicity), which is specific to each terminal ring and independent of the ring to which it is conjugated in a given dye. I proceed to show that the Brooker basicities, defined by differences between many-electron states, are also basicities in the usual (one-electron) sense and, finally, that Platt's construction of the color theory is an approximation to a *ab initio* effective Hamiltonian obtained by a minimum-norm block diagonalization procedure. What emerges is a powerful, simple, and *accurate* conceptual framework for thinking generally about color in monomethine dyes, and specifically about color tuning in the chromophore of green fluorescent proteins.

Introduction

Understanding relationships between the optical properties of molecules and their chemical constitution is a long-standing goal of theoretical chemistry.¹ *Methine dyes*, such as those in Figure 1, have a prominent place in the history

of these endeavors, and in chemistry itself. It is sometimes said that the modern organic chemical industry *began* with Perkin's discovery of the methine dye *Mauveine* in 1856, although the synthesis of the original *Cyanine* by Williams may predate this event.²

Methine is a limit of sp^2 carbon where the Lewis octet rule is satisfied by participation in multiple alternate struc-

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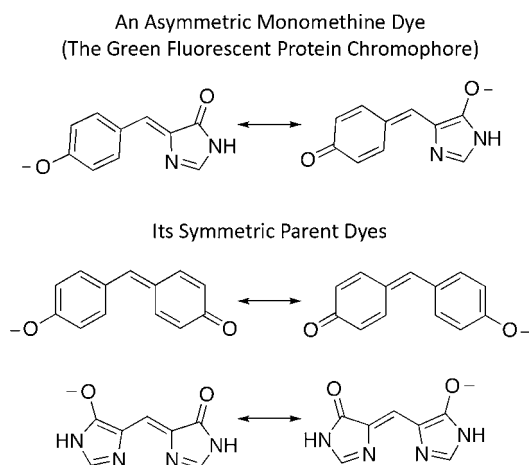


Figure 1. The classical resonance theory of methine dye color relates the color of an asymmetric methine dye (such as the Green Fluorescent Protein chromophore, top) to the colors of its symmetric parents (bottom), and to the energy difference between the resonance forms for the asymmetric dye. The latter is dominated by the difference in electronic basicities of the terminal heterocycles, which bear the charge in different structures.

tures, which differ by bond alternation and formal charge relocation. An archetypical *methine dye* is composed of two groups, usually heterocyclic rings, each of which can access two redox states, separated by a bridge containing one or more methine units. A *monomethine dye* has a single methine unit in the bridge, while a *polymethine dye* has more than one.

When the terminal groups are different, one of the resonating structures will dominate over the other. Within this context, the resonant methinic structure is a particular limit in a continuous set, the extreme elements of which are ionic polyenic structures with definite and opposing bond alternation. Though the dyes in Figure 1 are ions, the methine electronic structure is also relevant to neutral donor–acceptor chromophores. Within these systems, which resonate between neutral and zwitterionic closed shell states (as opposed to diametrically opposed charge structures as in Figure 1), it has been shown that the optical properties of the chromophores are strongly dependent on their proximity to the methine limit.³ For this reason, the methine electronic structure is a subject of continuing interest in the study of organic photonic and electronic materials.^{4–7}

The molecule that I use as an example at the top of Figure 1 is the chromophore of the green fluorescent protein, a system which has leapt to prominence⁸ for its utility in biooptical technologies.⁹ The chromophore is a *p*-hydroxybenzylidene-imidazolinone motif—an oxonol dye system. The chromophores of almost all fluorescent proteins are derivatives of this dye structure.¹⁰ Many of the technologies for which fluorescent proteins are used depend in some way on the color of the protein,⁹ which in turn depends on the color of the chromophore. The color of proteins with derived chromophores cluster together according to the nature of the derivation.¹⁰ Spectral variation within each group derives from variations in the interaction of the chromophore with its protein.¹¹ Each fluorescent protein chromophore is

synthesized within a particular protein and bound inside that protein for the duration of its functional existence.¹⁰ Fluorescent proteins are therefore very interesting natural laboratories for studying structure–property and environment–property relationships in methine dyes.

The issue of the color of fluorescent protein chromophores is a good example of how the general problem of color and constitution in methine dyes has maintained its relevance, despite its already long history.¹²

The Resonance Color Theory and the Brooker Basicity Scale

The problem of color and constitution in methine dyes received considerable attention during the early part of the twentieth century.^{1,13–19} Considerable motivation for development derived from the herculean experimental program of LGS Brooker and his group at Kodak, where a great many methine dyes were synthesized and studied (methine dyes are useful photographic sensitizers).¹⁸ Methine dyes had been known for almost a century when Brooker's work emerged, so his group was far from the first to study these systems. However, the scale and scope of the contribution have raised Brooker's name to prominence. For a thorough review of early work, I recommend a crucial (and very well-referenced) paper by Platt.¹⁴ I also recommend the books by Griffiths² and by Fabian and Hartmann,²⁰ as well as an article by Berneth in *Ullmann's Encyclopedia of Industrial Chemistry*.²¹ An early work of particular interest, which Platt's work builds upon, is Kuhn's description of the methine limit as a free-electron gas.¹³ Few theories can rival it for simple, effective insight—save possibly Platt's perimeter model of cata-condensed hydrocarbons, published in the same year.²²

The spectroscopy of methine dyes up to and including Brooker's studies had established several empirical rules,¹⁴ one of which will be our particular focus. The rule in question relates the color of an asymmetric methine dye (such as the GFP chromophore in Figure 1) to the color of the symmetric “parent” dyes produced by conjugating each terminus with a copy of itself (for the GFP chromophore, such dyes are shown at the bottom of Figure 1). This rule—*The Deviation Rule*¹⁸—is summarized as follows:

The absorbance wavelength of an asymmetric dye is no redder than the mean wavelength of its symmetric parents and deviates from this by a blue shift which increases as the difference in basicity of the terminal groups.

It is clear from Brooker's language^{17,18} that, when he used the word “basicity”, he was using it in the sense that it is normally meant—an energy associated with a *one-electron* process. This interpretation is implicit in theoretical works that followed Brooker's papers—specifically Herzfeld and Sklar's tight-binding Hamiltonian treatment²³ and Kuhn's free-electron model,¹³ both of which describe the chemical asymmetry in terms of a one-electron potential.

Brooker measured the absorption of many dyes and catalogued the deviations (“Brooker deviations”) of these from the mean of each dye's symmetric parents' parents.¹⁸ He used this data for formulation of an affine basicity scale for the terminal groups in the dyes (“the Brooker basicity

scale”).¹⁸ The Brooker basicity scale is correlated with other basicity scales such as the Hammett σ_R scale.² Platt's contribution was to verify that the deviations in Brooker's data set could indeed, to within the experimental error, be expressed by two numerical parameters: one specifying the mean parental wavelength and the other a basicity index specific to each terminal group, *and independent of the group to which it was conjugated a given dye*.¹⁴

In rationalizing his results, Brooker invoked a theory of the color of dyes, which was framed in a heuristic model space of resonating Lewis structures.^{17,19} Within this theory, the optical excitation of the dye emerges from the effective coupling between two isoenergetic *extreme* structures, which are analogues of those in Figure 1.¹⁶ Overlap arguments suggest that the direct coupling will be too small to explain the commonly measured wavelengths, so the theory hypothesizes *intermediate* structures, which place the formal charge on the bridge.^{16,19} The latter postulate is particularly important in long polymethine chains, where the exponential decay will decimate the bare coupling.²⁴ The intermediate structures are higher in energy than the extreme structures. They play an indirect role in the optical excitation, for which purpose they can be expressed as an effective potential.^{16,24} This potential creates a gap between the extreme structures. The excitation should be optically intense by very simple dipole length arguments.^{25,26}

There is only one bridge, and since the electronic structure on the bridge differs only by bond alternation in the extreme structures, the residual splitting between the extreme structures will be dominated by the difference in basicity of the terminal groups.¹⁸ This residual splitting is present in the absence of interaction and leads to a blue shift of the dye relative to its symmetric parents.^{14,16} This implies a two-state picture where the interaction matrix element is equal to the harmonic mean (in energy units, the arithmetic mean in wavelength) of the excitation energies of the symmetric parent dyes.¹⁴

Although the resonance theory in its classical form generated very effective heuristic explanations, attempts to translate it into quantitative models were problematic.^{14,16} It appears the resonance color theory faded into obscurity, a casualty of the early competition between molecular orbital (MO) theories of electronic structure and the valence-bond (VB) theories that were descendents of the early resonance theories.^{27–29} MO representations offer efficient techniques for storing and manipulating many-body states in the Born–Oppenheimer (clamped classical nuclei) electronic structure problem, because the one-electron density operator matrix elements span a Lie algebra.^{30,31} Valence bond theories have other strengths, particularly for constructing diabatic states whose character is maintained over an open neighborhood of nuclear geometries, and which can more easily accommodate nuclear motion and bond rearrangements.^{32,33} Their representation is less economical, so a computational threshold had to be crossed before they were incorporated into regular computational use.^{34,35} It is now well-known that MO and VB are only different ways of generating bases for the quantum mechanical state space.^{36,37} States are vectors in quantum mechanics, so the representa-

tion does not matter if both representations can be spanned within the same *complete* space.³⁸ Observables in both representations can be represented in the same algebra.^{39–41}

Platt's Construction

Platt constructed a quantitative empirical framework for the resonance color theory.¹⁴ Platt's construction is a recipe for generating 2×2 model Hamiltonians for a set of dyes built from a common set of terminal groups. The construction describes a given dye with two parameters, to be extracted from empirical data. The first of these is the “isoenergetic excitation energy”, which is the harmonic mean of the excitation energies of the parent symmetric dyes (proportional to the mean wavelength).

$$\frac{1}{E_I(A, B)} = \frac{1}{2} \left(\frac{1}{E_I(A, A)} + \frac{1}{E_I(B, B)} \right) \quad (1)$$

Here, $E_I(A, B)$ is the isoenergetic excitation of the dye (A,B) generated with terminal groups A and B. The isoenergetic excitation of a symmetric dye (such as (A,A) and (B,B)) is equal to its excitation energy. The second quantity in the Platt construction is the Brooker basicity difference $b(A, B)$.

$$b(A, B) = \sqrt{(\Delta E(A, B))^2 - (E_I(A, B))^2} \quad (2)$$

where $\Delta E(A, B)$ gives the excitation energy of the dye (A,B) generated with terminal groups A and B. Platt's construction yields a traceless model Hamiltonian $H^P(A, B)$.

$$H^P(A, B) = \frac{1}{2} \begin{pmatrix} b(A, B) & E_I(A, B) \\ E_I(A, B) & -b(A, B) \end{pmatrix} \quad (3)$$

The definition of $b(A, B)$ ensures that the splitting between the eigenvalues of $H^P(A, B)$ is equal to the dye excitation energy $\Delta E(A, B)$.

Platt's primary contribution was to show that the quantity $b(A, B)$ could be expressed as a difference between basicities that were *constant for each terminal group in the set*. He showed that Brooker's data could, within the experimental error, be described by a $b(A, B)$ formula with the simple parametric form (eq 4).

$$b(A, B) = b_A - b_B \quad (4)$$

where b_A and b_B are *constants* characteristic of groups A and B *and are independent of the conjugate groups with which they paired in any given dye*. Platt showed this by demonstrating that the $b(A, B)$'s extracted from Brooker's data obey the following “consistency rules”.

$$b(A, C) - b(B, C) = b(A, D) - b(B, D) \quad (5)$$

$$b(A, C) = b(A, B) + b(B, C) \quad (6)$$

The consistency rules above are not actually independent, since the second can be derived from the first, provided that the $b(A, B)$ actually can be written as a difference (so that $b(A, B) = -b(B, A)$).

Platt went on to demonstrate that a large data set published by Brooker could be compactly summarized and reproduced by his construction, using a suitable set of b_A parameters.¹⁴

In what follows, I will demonstrate that the excitation energies obtained for an example set of dyes within an *ab initio* representation that mimics the structure of the model space in color theory *can also be expressed this way*. That is, I will extract a set of b_A 's from a set of calculated excitations, and show that the excitation reconstructed with these parameters does not meaningfully deviate from the input set. I will then demonstrate that the b_A 's actually do measure a one-electron energy difference and that the Platt construction can be considered as a synthetic approximation to a quasi-diabatic *ab initio* effective Hamiltonian.

Revising the Model Space in the Resonance Color Theory

I want to point out that the information content implied by the Lewis structural representation of resonance theory, outlined in Figure 1, is *not formally consistent* with the definition of the Brooker basicity scale. There are different ways to highlight the problem. The problem is that a “basicity” usually means an energy associated with a *one-electron* process, while the Lewis structures in Figure 1 are rich in *pair* information (i.e., the bonding). Operators on a one-electron Hilbert space will generally not commute with pair operators defined on the tensor product of the space with itself. Although the set of one-electron operators spans a Lie algebra closed under commutation, the set composed of one- and two-electron operators generally does not.³⁰ This means that not only do the one- and two-electron operators not commute but expressing the commutator requires expanding the set of operators.⁴² *These statements imply that uncertainty relations prevent the precise, simultaneous specification of the basicity of a group and the bonding within the group.* A second argument simply notes that the underlying one-electron basis implied in the Lewis structure representation is one of the (perhaps orthogonalized) atomic orbitals. If the basicities of the different states in the group are not equal, then there will be *multiple detachment/attachment states, and a distribution of possible basicity values, associated with the ring*. This will be true even for a single dye molecule in a given Lewis structure. These problems are already apparent in discussions put forward by Brooker, who, *as expected*, had to invoke additional resonance structures beyond the canonical pair (e.g., Figure 1) for the dyes that he studied in order to rationalize his results.¹⁸

I propose that a more appropriate model space for the formulation of the resonance color theory could be obtained from a “methine-adapted” complete active space valence bond (CASVB) ansatz³⁸ built from group-localized orbitals, such as that outlined in Figure 2. This ansatz is consistent with the precise definition of group basicities, if the basicities are defined so that the group-localized orbitals are the relevant attachment/detachment states. Furthermore, the ansatz in Figure 2 still contains enough *pairing* degrees of freedom to index states in the model space spanned by the canonical resonating structures (Figure 1). This means that the information content of the theory can be preserved.

In molecular orbital theories, monomethine dyes are related to *odd alternate* systems.^{15,43} This means that, when the π

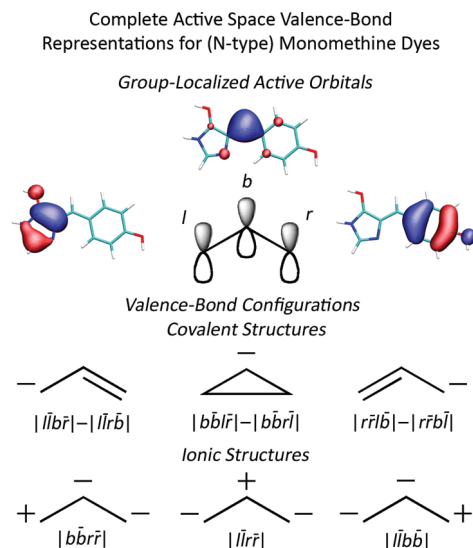


Figure 2. A model space for the resonance theory that is consistent with the precise basicity value for the rings would be a “methine-adapted” complete active space valence bond (CASVB) representation with one orbital state for each of the terminal rings (plus one for the bridge). The Lewis structure-based model space is not consistent with a precisely defined basicity, because there is too much information needed to specify the rings’ internal structure. As a result, given a dye in a precisely defined Lewis structure, there would be a spread of possible basicities.

molecular orbitals are paired according to their bonding or antibonding character, there will be a nonbonding orbital (NBO) left over. In diarylmethine dyes (for example Michler’s hydrol blue), the NBO is doubly occupied. In monomethine cyanines (for example Williams’ cyanine), this orbital is empty. In MO theories, the optical excitation of the former class is a HOMO–LUMO excitation from the occupied NBO to the lowest antibonding orbital. In the latter class, the excitation is a HOMO excitation from the highest bonding orbital into the NBO. The symmetry of odd alternate hydrocarbons in simple MO theories is such that these excitations would be the same for the anion and the cation formed from the same molecular frame. This implies that the four-electron/three-orbital CASVB representation outlined in Figure 2 unifies the state spaces of the resonance color theory and odd alternate MO theories for diarylmethines, and the corresponding two-electron/three-orbital CASVB space does the same for the monomethine cyanines. The GFP chromophore system is not strictly alternate, because it contains rings with an odd number of sites. Even so, it is *isoelectronic* with an odd-alternate hydrocarbon, and its orbitals can be identified with such a system. *Therefore, a model space over three frontier orbitals is also indicated by simple MO theories.*

A Self-Consistent Representation of the Resonance Color Theory

There is a methine-adapted solution to the two-state-averaged^{44,45} complete active space self-consistent field^{46,47} (SA2-CASSCF) problem with the form of Figure 2 for a

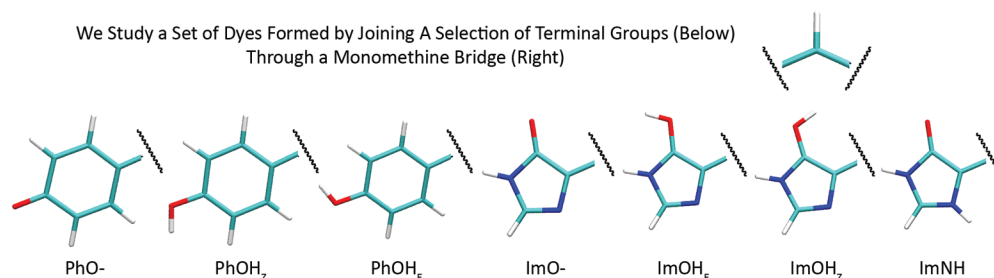


Figure 3. The data set used here consists of excitation energy calculations on a set of monomethine dyes generated by conjugating phenoxy and imidazolinoxy groups in different titration states (shown here). The generated dye set includes several conceivable protonation states of the green fluorescent protein chromophore, as well as several *bis*-phenoxy and *bis*-imidazolinoxy dyes.

large range of monomethine dyes. This includes the systems I use here as examples but appears to be much more general.⁴⁸ I conjecture that the existence and stability of such a solution can be safely used as an *operational definition* of the term “monomethine dye”. In a complete active space representation, the dyes I examine here have an active space with four electrons in three orbitals (SA2-CAS(4,3)). The solutions obtainable for monomethine cyanines have two electrons in three orbitals (SA2-CAS(2,3)).⁴⁹ In either case, the orbitals, after localization of the active orbitals with the Foster–Boys technique,⁵⁰ have the group-localized structure shown in Figure 2. The Boys localization procedure is unitary, so that the CASSCF state is invariant as long as the transformation respects the boundaries between the occupied, active and virtual spaces.^{45,51}

I argue that the methine-adapted SA-CASSCF solutions described above are *self-consistent representations* of the resonance color theory. This is important, because it highlights a strategy for making quantitative and objective predictions where the old resonance theory yielded only heuristic ones. It also means that the resonance color theory rests on stronger foundations than had previously been apparent.² The domain of application of the theory can be objectively assessed as the domain of applicability of the corresponding self-consistent field solutions.

A Set of Example Dyes

In what follows, I will support my assertion of correspondence between the resonance color theory. To do this, I will first describe a data set of excitation energies obtained by multistate⁵² multireference second-order perturbation theory^{53,54} (MS-MRPT2) on the methine-adapted SA-CASSCF reference space. Then, I will apply Platt’s synthetic Hamiltonian construction¹⁴ to this data set. Finally, I will show that *the accuracy of the excitation energies recalculated using Platt’s construction is within the expected accuracy of the original calculations themselves*. I will use a data set composed of 28 independent dye structures, all of which are monomethine pairings of different protonation states of a phenoxy and imidazolinoxy group (e.g., Figure 3). This set includes several protonation states of the GFP chromophore motif (which have been previously studied by quantum chemistry^{55–83}).

In general, methine dyes can have multiple *cis–trans* isomeric states. In the context of the example set here, there

is no meaningful distinction between *cis* and *trans* isomeric states of the phenoxy–methine bond (due to symmetry about its axis). Such a distinction is meaningful only for the imidazolinoxy–methine bond, for which I examine only the *cis* forms here (the imine nitrogen at position 2 is *Z* with respect to the conjugate terminal group). Each dye was relaxed in its *cis* conformation by performing an MP2⁸⁴ optimization with a cc-pvdz basis set.⁸⁵ For a few dyes in the set, the ground state minimum is a different *cis–trans* isomer and is not contained in the set. As my goal is to investigate the resonance color theory as a theory of the *electrons*, restricting the set of structures in this way makes sense. This procedure generates structures that are minima with respect to bond alternation coordinates. This is important, because one would expect that the model states of the resonance theory (e.g., Figure 1) are coupled through bridge bond-stretching vibrations.

For each dye in its relaxed *cis* geometry, I obtained the “methine adapted” SA2-CAS(4,3)/cc-pvdz solution space using unrestricted Hartree–Fock charge-density natural orbitals^{86,87} for the oxidized doublet radical as an initial guess for the self-consistent field optimization. I then calculated the excitation energy of the dye by applying a multistate multireference perturbation theory (MS-MRPT2) correction to the SA2-CAS(4,3) reference space. The MS-MRPT2 correction is formally size-extensive.⁸⁸ This is consistent with the interpretation of the underlying CASSCF as a form of maximum entropy inference.^{31,89} Only the highest-lying 32 orbitals were correlated, though, and some extensivity error might arise from this.⁹⁰ The perturbation theory calculations on the methine adapted SA2-CAS(4,3) solutions converged quickly and easily without the use of level shifts. The above procedure yielded 28 excitation energies for symmetric and asymmetric dyes generated by the groups in Figure 3. Additional details, as necessary to reproduce the wave function (i.e., state-averaged natural orbitals and occupation numbers, and MS-MRPT2 mixing matrices) are available in the Supporting Information. The most concrete result of this paper is that the resulting set of excitation energies can be accurately represented by Platt’s construction,¹⁴ where each group is assigned its own basicity index independent of the terminal group to which it is conjugated in a given dye.

I used the Molpro software package for all electronic structure computations.⁹¹

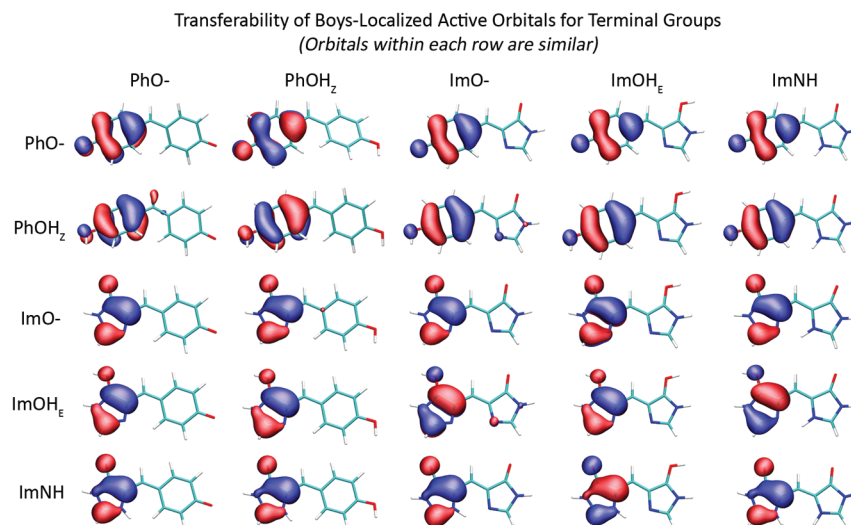


Figure 4. The Boys-localized active space orbitals obtained from the methine-adapted SA2-CAS(4,3) solution for the example dyes are transferrable in the manner suggested by the Brooker basicity scale. The shape of the orbital for each group is independent of the group to which it is paired in a given dye structure (i.e., orbitals within each row are similar). The Brooker scale associates a basicity (one-electron energy) to each group, independent of its context. Orbital isosurfaces are set at ± 0.04 . Overall, signs do not matter, by the SA-CASSCF convergence criteria.

My goal is to show that the methine-adapted solution to the SA-CASSCF problem *contains* the resonance color theory. We are now at a point where I can present the first pieces of suggestive evidence in defense of this notion. These are highlighted in Figures 4 and 5. Figure 4 shows that the Boys localized active space orbitals associated to each of the rings in the set of dyes maintain their shape when the conjugate dye is varied. This is exactly the pattern expected in order for a transferrable basicity scale—such as the Brooker scale—to apply.¹⁸ Figure 5 shows that the excitation energies obtained for the asymmetric dyes are consistent with Brooker's deviation rule, in that none of the asymmetric dyes shown is redder than the mean wavelength of its parents.⁹²

I want to highlight a particular structure to the excitation energies generated using the SA2-CAS(4,3) ansatz, not necessarily to argue for its absolute accuracy as an approximation to the exact Born–Oppenheimer electronic structure. It is convenient, however, that the excitations *do* compare well against observed excitations for chemically similar systems. In particular, the excitation energy of the anionic phenolate–imidazolinolate (PhO[−], ImO[−]) dye is in reasonable range of the measured excitation energies of green fluorescent protein (GFP) chromophore models in their anionic state,^{93–97} as well as the B band of GFPs,^{98–100} and is broadly consistent with computational models using larger active spaces.^{60,63,72,75,82} Moreover, the excitation energy of the bis-phenolate (PhO[−], PhO[−]) dye is quite close to the lowest excitation of benzaurin and phenolphthaleins in alkaline solution, and the corresponding diprotonated cations (PhOH/PhOH⁺) are close to the excitation of the benzaurin cation.² It does seem, interestingly, that there may be a systematic overprediction of the excitation energies of the neutral oxonol dyes in the set (e.g., (PhOH, ImO[−]), (PhO[−], ImOH), etc.). The calculated excitations of the (PhOH, ImO[−]) and (PhO[−], ImOH) dyes are both bluer than GFP chromophore models at neutral pH in several solvents,⁹⁶ models in an ion ring,⁵⁵ the A band of GFPs,^{98–100} and results from larger active

spaces.^{55,83} Similarly, the excitation of the (PhOH, PhO[−]) dye is bluer than the absorbance of benzaurin at neutral pH.² It may be that the estimates provided by the methine-adapted solution spaces overestimate the blue shift near the polyenic limits of the resonance scale. This question is not relevant to my purpose, which is to show that the excitations *within this model* follow a specific simple pattern, *and this pattern is predicted by the resonance color theory*. The difficulty of producing quantitatively accurate absolute excitation energies for dyes contained in the set has been highlighted in two recent benchmarking studies using similar techniques.^{60,61}

It is worth noting that the dimethyl derivative of the (PhO[−], ImO[−]) dye (HBDI), is autoionizing in its first excited state.⁶⁰ This is interesting, because one might expect this to artificially depress the excitation energy, and violate Brooker's deviation rule. Apparently, this is not happening (Figure 5). Possible explanations may be that (a) compensating artifacts occur in the parent dyes, so that the deviation rule is preserved, (b) the dye is nonresonant but appears so due to the artifact, (c) the calculation is (somehow¹⁰¹) managing to pick out the appropriate valence state from the embedding continuum, or (d) substituent effects induced by removing the methyl groups raise the ionization threshold above the first excited state.

Extraction and Validation of Basicity Indices for Terminal Groups

I extracted single basicity indices for each terminus in the following steps. First, I collected all basicity differences $b(A,C) - b(B,C)$ using Platt's construction,¹⁴ where A, B, and C ranged over the set of termini (Figure 3). I grouped these according to B and performed a linear regression fit within each of these groups. This step yielded the data in Figure 6. The lines for each B are parallel to a very good approximation. Therefore, the scales given by different B groups can be expressed relative to a common origin by

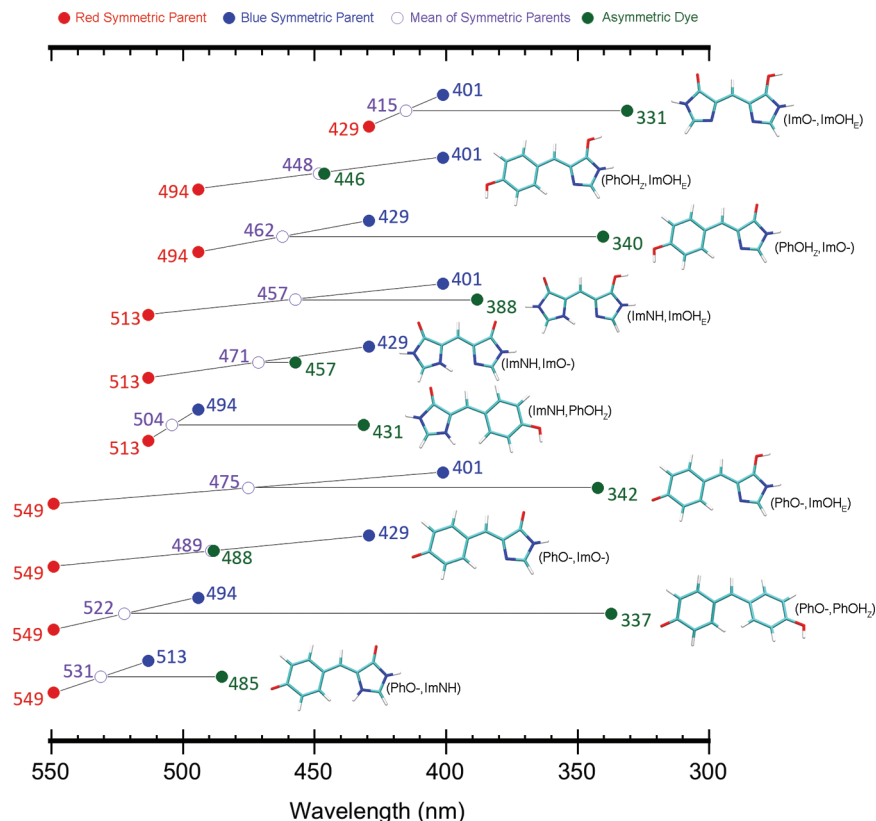


Figure 5. Excitation wavelengths calculated using the methine-adapted SA2-CAS(4,3) solutions are consistent with the Brooker deviation rule. Calculated excitation energies for a selection of dyes in the data set are shown. Excitations were calculated using MS-MRPT2 theory corrections to the methine-adapted SA2-CAS(4,3)/cc-pvdz reference model spaces. Excitations of asymmetric dyes are green dots and type, excitations of symmetric parents are red and blue dots and type, and mean wavelengths of the symmetric parents are purple circles and type. To the right of each row is a picture of the corresponding asymmetric dye, and a label. All of the calculated excitations are consistent with Brooker's deviation rule, because the excitation wavelengths of the asymmetric dyes are not redder than the mean wavelengths of their symmetric parents. Brooker deviations are greatest when the titration states of the aryloxy sites are different, indicating strong detuning from resonance. When they are the same, as in the anion (PhO⁻, ImO⁻) and dihydroxy cation (PhOH₂, ImOH₂), the deviation is small, indicating resonance.

shifting each group according to the y intercept of the respective regression line. This was also supported by the application of Friedman's test¹⁰² to the data, which indicated a high degree of agreement between scales corresponding to different choices of B.

After shifting, the basicities corresponding to each A are clustered together. Figure 7 shows a histogram of the shifted basicities colored according to the value of A.

I extracted a single basicity for each terminal group A by taking the median of the distribution of shifted basicities for each A. The median basicities and associated median absolute deviations are listed in Table 1.

If the resonance theory is a reasonable model for the excitations in the data set, then reconstructing the excitation energies for each of the asymmetric dyes in the set using Platt's construction¹⁴ should not significantly degrade the accuracy of the set. I tested this by back-calculating the excitation energies for each asymmetric dye in the set using the median shifted basicities and the isoenergetic excitations of the parent symmetric dyes. The set of reconstructed excitation energies fits the original set very well, as I show in Figure 8, by a linear fit between the two sets. The residuals of the fit (vertical distances to the regression line) were under 1000 cm⁻¹ (0.123 eV) for all of the dyes in the set. *This is*

as good a level of accuracy as would be expected a priori from quantum chemical estimates anyway,^{60,75,103–109} so the approximation of using group-specific basicities does not meaningfully degrade the accuracy of the set.¹¹⁰

Brooker Basicities are Correlated with One-Electron Basicities

An interesting feature of the resonance color theory is that the "Brooker basicity" is defined by a difference between observables on a *many-electron* Hilbert space. Yet, it is interpreted as a real chemical "basicity", which usually implies a *one-electron* energy. Even more interesting is that this relationship is experimentally verifiable.² I will now show that this relationship also emerges in the *ab initio* SA2-CAS(4,3) representation, by showing that the Brooker (many-electron) basicity scale extracted from the calculated excitations is correlated with a one-electron basicity scale extracted from the set of Boys-localized active space orbital energies (diagonal state-averaged Fock matrix elements).

I extracted group-specific one-electron basicities for the terminal groups in much the same way as for the Brooker basicities. Specifically, I collected differences $b'(A,C) - b'(B,C)$ where $b'(A,C)$ is the difference between the orbital

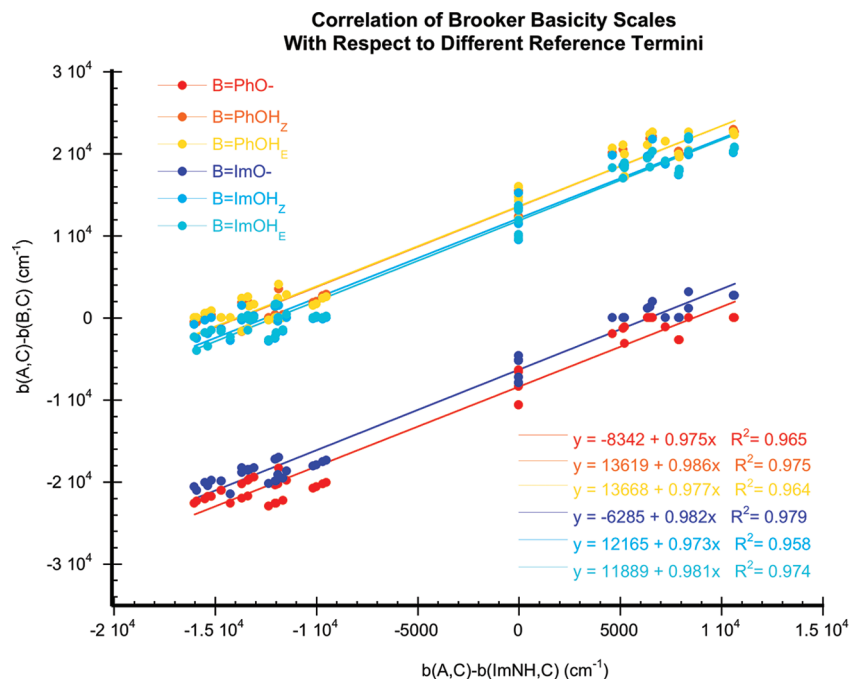


Figure 6. The Brooker basicities assigned to the different termini are consistent, if any single termini is chosen as a reference for the scale. The basicities with respect to the ImNH terminus are used as the abscissa, and basicities with respect to other termini are plotted against these. Relative Brooker basicity differences $b(A,C) - b(B,C)$ are plotted for all choices of groups B and C (see Figure 3). Groups corresponding to different B's were fit to a linear regression model. Coefficients of determination (R^2) are shown, indicating that each group $B \neq \text{ImNH}$ is well correlated with $B = \text{ImNH}$. The lines fit to different B's are parallel with a slope close to unity, indicating that they are also well correlated with each other. Groups corresponding to different choices of B can be brought into the same scale by shifting each according to the y intercept of its regression line.

energies (diagonal state-averaged Fock matrix elements) of Boys-localized active orbitals corresponding to the groups A and C in the dye (A,C). I then followed the same procedure that I used to extract the Brooker basicities b_A from the differences $b(A,C) - b(B,C)$ above. The data behaved in a very similar fashion to the Brooker deviation data at each step (Figures S1.1 and S2.2 in the Supporting Information are analogs to Figures 6 and 7 for the Brooker data). After extracting the group-specific one-electron basicities, I performed a linear regression fit between the Brooker and Boys orbital basicities to determine their correlation. I show the results of this comparison in Figure 9. The Brooker and Boys basicities are strongly correlated. This demonstrates that the *ab initio* Brooker basicity does measure a group “basicity” in the usual (one-electron) sense. *This is a core assertion of the resonance color theory.*^{14,18}

The relationship between the many-electron (Brooker) and one-electron basicity scales should reflect the importance of electron correlation in the electronic structures of the terminal groups. Specifically, the distance from the (*one-* vs *many-electron*) regression line should reflect the relative importance of electronic correlations to the basicities. With this in mind, Figure 9 suggests that electron correlations are most important for the oxygen-protonated termini PhOH_{E/Z} and ImOH_{E/Z}. The orderings of basicities of the PhOH_{E/Z} and ImOH_{E/Z} termini are apparently reversed between the one-electron and many-electron scales. The difference to the regression line is still small compared to the total range sampled, so it is probably reasonable to say that the basicities of the PhOH_{E/Z} and ImOH_{E/Z} termini are not operationally distinguishable. This viewpoint was supported by a statistical variance analysis

of the entire distribution of shifted basicities for all terminal groups in the set, wherein the distinguishability of these two specific groups depended sensitively on the parameters used in the test. These tests also indicated that the distributions corresponding to OH_E vs OH_Z conformations were indistinguishable, so that the basicity does not depend on the oxygen lone pair to which the proton is bound.

Platt's Construction Approximates an *Ab Initio* Effective Hamiltonian

Platt's construction¹⁴ is a recipe for *synthesizing* a 2×2 spectroscopic Hamiltonian from a collection of given absorption wavelengths. Though Platt's language¹⁴ suggests he had the Lewis structural representation in mind, he did not actually write down any constraints on the form of the representation to which his synthetic Hamiltonian corresponds. The representation is defined only through the matrix elements of the Hamiltonian he constructed. It is safe to conclude that *any* representation that obeys the right relationships between the absorbance wavelengths of a collection of asymmetric dyes and their symmetric parents, and for which a consistent set of basicities can be defined, is a candidate. One could, if one wished, consider an ensemble of representations consistent with the constraints (perhaps supplemented by other physically motivated constraints) and consider the state as a random variable.¹¹¹ In the context of the modified resonance-theoretic model space that I have proposed, it seems reasonable to insist that the constraints include a map between the energies defined in

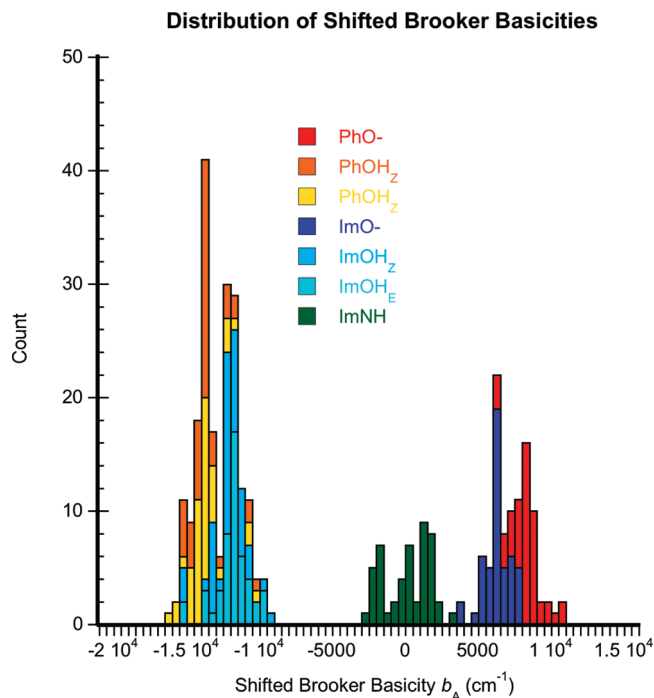


Figure 7. This figure shows the distribution of group-specific Brooker basicities generated when the relative basicity differences $b(A,C) - b(B,C)$ are shifted onto the same line (see Figure 6). This yields tightly clustered distributions for each group A, containing 25 data points each. The distribution for each A is sharply peaked and, for most cases, is concentrated within 5000 cm^{-1} of the peak. This suggests that the excitation data may be summarized without too much loss of accuracy using Platt's model Hamiltonian construction with one median Brooker basicity parameter assigned to each group.

Table 1. Median Brooker Basicities (cm^{-1}) for Different Terminal Groups in the Data Set and the Median Absolute Deviations of the Distributions for Each Terminal Group

group	median b_A (cm^{-1})	median absolute deviation (cm^{-1})
PhO-	8342	654
PhOH _Z	-13658	523
PhOH _E	-13668	576
ImO-	6288	614
ImOH _Z	-12165	664
ImOH _E	-11889	566
ImNH	0	1272

the one- and many-electron spaces, so that the Brooker basicities measure basicities calculated with the one-electron set.

I have shown above that the family of methine-adapted SA2-CAS(4,3) solutions for the example dye set obeys these requirements, to within the expected accuracy of the computations. We can use this to probe the relationship between the Platt Hamiltonian and other 2×2 Hamiltonians that can be extracted from the quantum chemical model space. For example, I could extract the angle between the Platt Hamiltonian and the Hamiltonian defined in the eigen representation of the SA-CASSCF solution (or its image under rotation by the MS-MRPT2 mixing matrix). Another interesting candidate for comparison would be the 2×2 Hamiltonian obtained by a *minimum-norm block diagonalization transformation*.¹¹² This is the transformation that does

Ab Initio vs. Reconstructed Excitation Energies

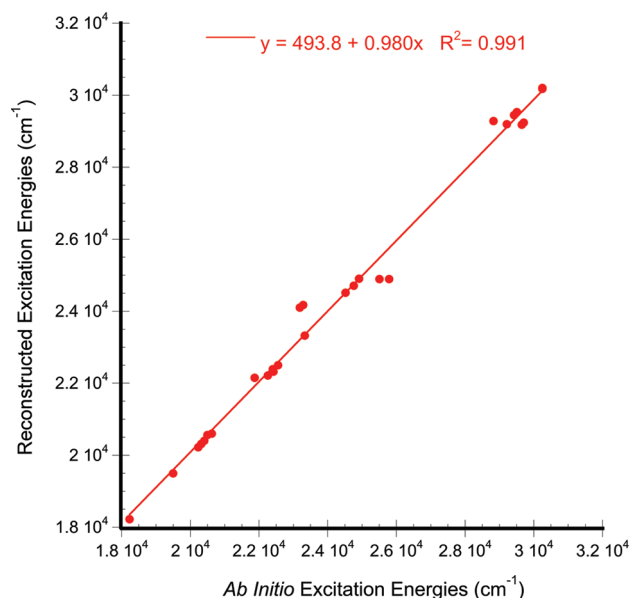


Figure 8. Reconstruction of the excitation energies in the data set using median Brooker basicity values (rather than the dye-specific values) does not meaningfully degrade the set. The calculated and reconstructed excitations (cm^{-1}) for dyes in the set are plotted against one another and fit to a linear regression model with parameters displayed. The coefficient of determination (R^2) is close to unity, showing that the data are strongly correlated. The slope of the regression line is also very close to unity, as expected. All of the residuals (vertical distances from the regression line) are less than 1000 cm^{-1} . This is much smaller than the range spanned by the set ($\sim 10\,000\text{ cm}^{-1}$) and comparable to the best accuracy expected for correlated quantum chemical excitation energy estimates. Therefore, the use of group-specific Brooker basicities summarizes the data set compactly and *with no significant loss of accuracy*.

as little as possible other than block-diagonalize the Hamiltonian, in the sense that it is closest to the identity on the space of configuration state functions (CSFs).^{112–114} The CASVB structure of the CSF basis in Figure 2 was a significant motivation for my assertion of correspondence between the family of methine adapted SA-CASSCF solutions and the resonance theory. One might therefore expect that the angles parametrizing the 2×2 unitary transformations that diagonalize the minimum-norm and Platt Hamiltonians would be close to each other (in some reasonable metric¹¹⁴). I show that this is true in Figure 10, where the distribution of angle differences is plotted in a histogram. The distribution appears to have two components: a large spike at 0° (which includes all of the symmetric dyes in the set, plus a few asymmetric ones) and a broader peak centered near -5° . Since I parametrized the Platt Hamiltonian using the MS-MRPT2 corrected energies, the minimum-norm Hamiltonians used in the comparison also used these energies. The eigenvector information required to build the minimum-norm Hamiltonian¹¹² was taken from the MS-MRPT2-mixed SA-CASSCF eigenstates (as has been done in previous work⁵⁸).

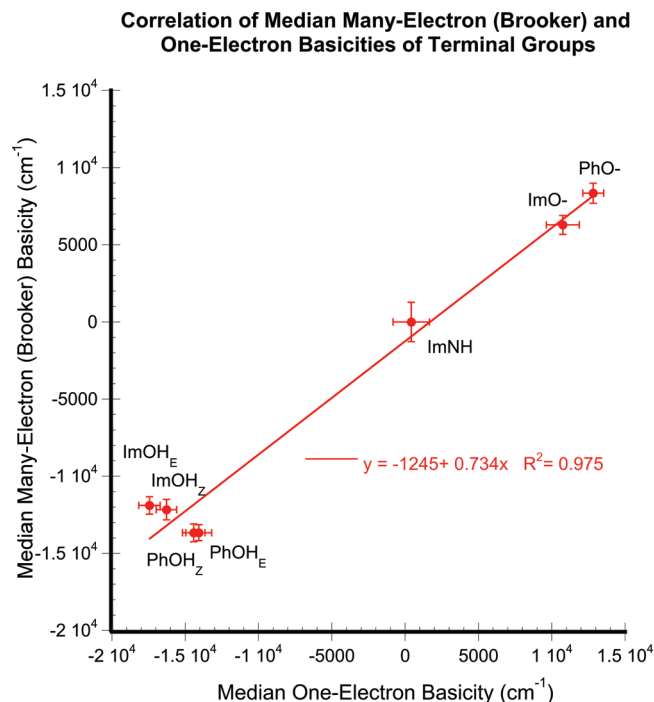


Figure 9. Many-electron (Brooker) and one-electron basicity scales are strongly correlated. This shows that the Brooker (many-electron) basicity *does* represent a “basicity” in the usual (one-electron) sense. Brooker basicities were calculated using the calculated many-electron excitation energies as input, while the one-electron basicities were derived from the diagonal Fock matrix elements of Boys-localized active orbitals. The basicities shown are the medians of the distribution corresponding to each terminal group (see Figure 3). The error bars show the median absolute deviations of each distribution. I include a similar figure showing the distributions themselves in the Supporting Information. Deviation from the regression line indicates the importance of many-electron correlations to the basicity values.

The small magnitude of the difference angles in Figure 10 broadly supports my argument that the family of methine-adapted SA-CASSCF solutions corresponds to the resonance color theory. Interestingly, the correlation between the diagonal elements of the minimum-norm Hamiltonian and the one-electron basicity differences was significantly worse than the correlation of the many-electron and one-electron basicity differences over the dye set. It seems, therefore, that Platt’s construction provides a higher fidelity mapping between one- and many-electron observables than does the minimum-norm block diagonalization.

Discussion

I have shown that a modified version of the resonance color theory^{14,16,18,19} can provide a systematic framework for understanding protonation-dependent color changes in an example set of monomethine oxonol dyes. I have done this in several steps. First, I have shown that there is a family of SA-CASSCF solutions which has the correct information structure for defining group basicities as was done in the theory, that this solution family has transferrability properties that mirror the Brooker basicity scale, and that the perturbed

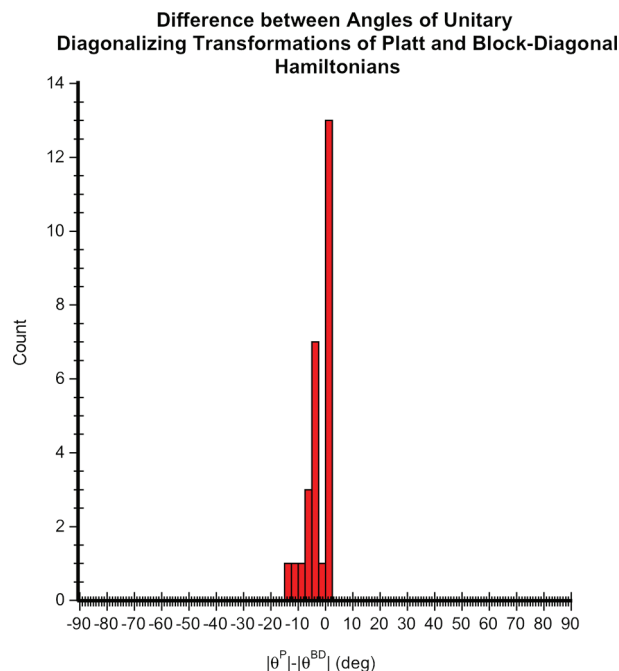


Figure 10. The differences between angles that parametrize the transformations diagonalizing the Platt (θ^P) and Block-Diagonalized (θ^{BD}) effective Hamiltonians are clustered near zero. The Block Diagonalized Hamiltonian was generated by a minimum-norm block diagonalization of the Hamiltonian defined on the SA-CASSCF states in the Boys localized representation. The Platt Hamiltonian was constructed from the excitation energies of asymmetric and symmetric parent dyes as discussed in the text. The difference in the angle between the Hamiltonians is small, falling between 0° and -10° for nearly all dyes in the set. This shows that Platt’s model Hamiltonian is a synthetic approximation to an *ab initio* effective Hamiltonian.

excitation energies calculated with this ansatz obey the Brooker deviation rule.^{14,18} Second, I have shown that group-specific basicity indices can be extracted from the excitation energies of the set. When these are used to construct model Hamiltonians via Platt’s construction,¹⁴ the reconstructed excitations reproduce the input set to within the accuracy expected of the calculations themselves.^{60,75,103–107} Third, I have demonstrated that these basicity indices are strongly correlated with an appropriately defined set of one-electron basicity indices, therefore showing that the representation is true to this core assertion of the resonance color theory. Finally, I have compared the Hamiltonians obtained via Platt’s construction to an *ab initio* minimum-norm Hamiltonian in order to show that it is, in fact, an approximation to such a Hamiltonian.

Although the results I report here were obtained for a relatively limited data set, my ongoing studies show that SA-CASSCF solutions of the form in Figure 2 can be readily obtained for a broad set of monomethine dyes. If this turns out to be generally true, then this provides a rigorous foundation for the resonance color theory in these systems. It immediately suggests a strategy for making quantitative, objective inferences using a conceptual framework that previously yielded only heuristic insights.

A formal validation of the resonance theory is important because the structure of the theory suggests many new strategies for modeling solvation and excited-state dynamics in complex environments. There has already been considerable work done in the field of nonequilibrium solvation, which, at least implicitly, invokes the resonance theory picture.^{115–117} Similar models for nonlinear optical chromophores have also been proposed.^{118–120} The “modular” nature of the relationship between color and group basicity in the theory is reminiscent of the modular nature of modern molecular dynamics force fields. This suggests that similar force fields for excited states may be achievable. This is a *very* interesting prospect, because there are a great many monomethine dyes which are already being used as probes of biomolecular structure and dynamics.^{121–123} A better understanding of the excited states of these systems would add value to many results *already in the literature*, in addition to stimulating new work.

The data set I employed here includes the canonical protonation states of the GFP chromophore—specifically, the anion (PhO[−], ImO[−]) and the phenolic neutral (PhOH, ImO[−])—that are almost universally assigned to the “A” and “B” absorbance bands (Boxer’s notation^{98–100}) of GFPs.^{10,124,125} It also includes the imidazol-3-ium “cationic” (PhOH, ImNH) and “zwitterionic” (PhO[−], ImNH) forms which were once considered as candidates for assignment to these bands.^{80,81} Our results suggest, as did previous ones, that this assignment would not be unreasonable if the absorption data alone were considered. Other data are contraindicative.^{96,126} The set also includes two protonation states which, as far as I am aware, have not been assigned to the observed spectra: a dihydroxy cation (PhOH, ImOH) and an imidazolinol neutral form (PhO[−], ImOH). The results I have obtained with these forms are interesting. First, I find that the (PhOH, ImOH) form, like the canonical (PhO[−], ImO[−]) form, is *resonant* by Brooker’s definition (small Brooker deviation) and is also quite red (446 nm). Though novel in this context, *this is not surprising* and could easily have been predicted on the basis of the *general* tendency of oxonol and hydroxyarylmethine dyes to develop deep color in acidic and alkaline solution.² The excitation energy of the (PhOH, PhOH) dye is bluer than the B bands of some GFP variants but is still comfortably in the range of wavelengths to which the label is applied.^{9,127} Possibly, this deserves more attention. I have also found that the absorbances of the two neutral dyes (PhOH, ImO[−]) and (PhO[−], ImOH) are very close (340 nm vs 342 nm) and would likely be *spectroscopically indistinguishable*. This suggests that the assignment of the A band in GFPs to a phenolic form should not be based on absorbance data alone. There is no immediate problem here: since their constituency is identical, the quantum chemical ground state energies of these forms can be directly compared. The (PhOH_Z, ImO[−]) form is lower in energy than the (PhO[−], ImOH_Z) form by 29 kcal/mol, so there is no *a priori* case for reassignment.

Every fluorescent protein chromophore is synthesized inside the protein to which it is bound—and with which it *interacts*—for the entirety of its functional existence.¹⁰ In the theory of open quantum systems, it is well-known that

bipartitioning of a pure state system (having an idempotent density matrix) will lead to mixed state (nonidempotent) reduced density matrices for the subsystems, if the subsystems are entangled across the boundary.^{128,129} If one naively assumes that the protein is described by a pure quantum state that is an eigenfunction of the proton number, then one is led, upon partitioning the chromophore from everything else, to consider that the most realistic assignment may include *ensembles* of protonation states. This possibility has already been suggested as an explanation for the spectra of positive-mode reversibly photoswitching GFPs.¹³⁰ The possibility that electronic and protonic states of the chromophore may be strongly coupled is consistent with the apparent ultrafast excited-state proton transfer speeds observed in some variants.¹³¹ This topic is fit for discussion in a later publication.

A recent experimental study by Dong et al. of the solvatochromism of GFP chromophore models has suggested a high polarizability for the anion.⁹⁶ This is broadly consistent with our result that the anion is a resonant system near the methine limit.³ In their study, Dong et al. fit the spectral solvatochromic data to a multivariate Kamlet–Taft equation¹³² (linear solvatochromic energy relationship) and obtained interesting results. At pH values consistent with neutral (most likely (PhOH, ImO[−])), the best-fit Kamlet–Taft coefficients were all negative (increasing solvent polarity, acidity, and (protic) basicity induced bathochromism). At pH values consistent with a cation (most likely (PhOH, ImNH)), all the coefficients were positive (hypsochromism). At pH values consistent with an anion, a bifurcation was observed, so that increased polarity induced bathochromism while increases in acidity or basicity induced hypsochromism. The model compound used was not strictly the same as ours, differing by two methyl substituents to the imidazolinone. Methyl groups are weak electron donors, and the relevant active orbital in our model has amplitude at the points of their substitution, so one might expect the substitution to raise the orbital energy and affect a small basicity increase in the imidazolinone.

The Platt construction can, in principle, describe the bifurcation of solvatochromic trends because it depends on two independent parameters.¹⁴ For a particular case, the relevant question is how to partition solvation effects into the E_i ’s and the b ’s. I would argue that nonspecific interactions whose influence on the rings is anticorrelated (such as a dipole field component parallel to the long molecular axis) should manifest in the b ’s alone, while ring-specific hydrogen bonding effects may be partly incorporated in the E_i parameters (as I have done here, taking protonation as a limit of hydrogen-bond donation). A specific effect that is localized on one terminal group and independent of the other can be incorporated into the definition of a new symmetric parent structure. There is no easy way to “mirror” interactions that affect the rings in an anticorrelated way. In the case of a dipole field, the directionality of the field implies that “mirroring” the field would destroy its dipolar character. It is possible that higher-order field components would be preserved (for example certain quadrupolar fields), but most solvation models consider dipolar fields to be dominant

contributors.¹³³ Interestingly, this partitioning into dipolar solvation effects and local (e.g., hydrogen-bonding) effects is exactly that suggested by the Kamlet–Taft analysis.⁹⁶ A bathochromic shift with increasing solvent polarity suggests a decrease in the absolute value of $b(A,B)$, which would only be detected if the magnitude of $b(A,B)$ was initially nonzero. It is possible, therefore, that the results of Dong et al. are indicative of electronic symmetry breaking in weakly polar solvents. It is interesting to note that the solvent with the highest dipolarity/polarizability parameter (π^*) studied by Dong et al. (water) reversed the trend toward increasing bathochromism. This may be indicative of crossover to a qualitatively distinct regime, but more data would be required to confirm or refute this idea. If protonation of the anion is considered as the extreme limit of hydrogen bond formation, then our results are consistent with hypsochromic shifts as the solvent hydrogen bond donation parameter is increased.

The resonance color theory predicts that the Brooker deviation should increase quadratically in the Brooker basicity difference. In this context, it is interesting to note the recent suggestion that a quadratic Stark effect is responsible for color tuning in red fluorescent proteins (RFPs).¹³⁴ RFPs are distinguished by an acylimine substitution to the imidazolinone ring. On the face of it, this suggests that the picture that emerges here for GFP chromophores may also be extended to chromophores from other subfamilies of the fluorescent proteins. A SA-CASSCF solution with the form of Figure 2 can also be obtained for an RFP chromophore model. In this case, there is one fragment orbital encompassing both the imidazolinone and acylimine moieties.

The resonance in the anionic dye (PhO^- , ImO^-) suggests high polarizability, as mentioned above. It is tempting to think that the prevalence of anionic chromophore forms in GFPs may have arisen *because* the resonant state allows greater flexibility for tuning the absorption. There are data supporting the idea that natural selection pressure drives emission color changes in these systems.^{135–138} Not all GFP homologues are fluorescent, so it is conceivable that similar pressure drives the tuning of absorbance.

While this paper was in review, Martínez, Lamothe and I demonstrated that Brønsted acid/base chemistry and double bond photoisomerization chemistry are linked in GFP chromophores through the methine chemistry.¹³⁹ This suggests the interesting possibility that the structure–property relations I have described may also be applied to the photoisomerization reaction, which is considered to be a major decay channel in these systems.¹²⁴ The protonation-dependent basicity differences that I report here are *easily* larger than the S_0 – S_1 energy gaps at favorable excited-state twisted configurations.^{58,65,74,75,82,139} In fact, they are of similar magnitude to the *excitation energies themselves*. It is possible that this may be important to understanding why HBDI is nonfluorescent even in its crystalline solid phase, where large-amplitude twisting motions are unlikely to occur.¹⁴⁰

Conclusion

I have made the case that the resonance theory of Brooker¹⁸ dyes provides a sound description for methine dye systems,

if it is expressed in a revised model space that better reflects its information content. I have demonstrated the effectiveness of this approach by pointing out that there is a self-consistent model space for such dyes with the same form and demonstrated that, when the results obtainable with this solution are used to parametrize Platt's model Hamiltonian construction,¹⁴ the set of excitations can be reconstructed without meaningful loss of accuracy. This provides a firmer theoretical foundation for the resonance theory and allows it to be used for quantitative, objective analyses where previously only heuristic insights could be obtained.

Acknowledgment. This work was supported by the Australian Research Council under Discovery Project DP0877875. Computations were done at the National Computational Infrastructure (NCI) Facility, Canberra, with time provided under Merit Allocation Scheme (MAS) project m03. I thank J. R. Reimers, N. S. Hush, and A. N. Tarnovsky for bringing Brooker's work to my attention; S. Boxer, S. Marder, S. Meech, T. Martínez, M. Prescott, M. Robb, M. Olivucci, G. Groenhof, L. Radom, T. Pullerits, T. Smith, M. Smith, S. Smith, and R. Jansen-Van Vuuren for helpful discussions; and R. McKenzie for research support, comments, and encouragement. Molecular graphics were generated using VMD.¹⁴¹

Supporting Information Available: Data pertaining to one-electron basicity extraction, Cartesian coordinates (Å), absolute SA-CASSCF and MS-MRPT2 energies (h), MS-MRPT2 mixing matrices, and SA-CASSCF natural and localized orbital graphics with state-averaged occupation numbers. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Lewis, G. N.; Calvin, M. *Chem. Rev.* **1939**, 25, 273.
- (2) Griffiths, J. *Colour and Constitution of Organic Molecules*; Academic Press Inc.: London, 1976; pp 2, 241; 248–250; 89–92; 258–259.
- (3) Marder, S. R.; Gorman, C. B.; Meyers, F.; Perry, J. W.; Bourhill, G.; Brédas, J.-L.; Pierce, B. M. *Science* **1994**, 265, 632.
- (4) Wu, W.; Hua, J.; Jin, Y.; Zhan, W.; Tian, H. *Photochem. Photobiol. Sci.* **2008**, 7, 63.
- (5) Jain, V.; Rajbongshi, B. K.; Mallajosyula, A. T.; Bhattacharjya, G.; Iyer, S. S. K.; Ramanathan, G. *Sol. Energy Mater. Sol. Cells* **2008**, 92, 1043.
- (6) Puyol, M.; Encinas, C.; Rivera, L.; Miltsov, S. *Sens. Actuators* **2006**, B115, 287.
- (7) Heilemann, M.; Margeat, E.; Kasper, R.; Sauer, M.; Tinnefeld, P. *J. Am. Chem. Soc.* **2005**, 127, 3801.
- (8) Weiss, P. S. *ACS Nano* **2008**, 2, 1977.
- (9) Tsien, R. Y. *Angew. Chem., Int. Ed.* **2009**, 48, 5612.
- (10) Remington, S. J. *Curr. Opin. Struct. Biol.* **2006**, 16, 714.
- (11) Shu, X.; Shaner, N. C.; Yarbrough, C. A.; Tsien, R. Y.; Remington, S. J. *Biochemistry* **2006**, 45, 9639.
- (12) Dahne, S. *Science* **1978**, 199, 1163.
- (13) Kuhn, H. *J. Chem. Phys.* **1949**, 17, 1198.

- (14) Platt, J. R. *J. Chem. Phys.* **1956**, 25, 80.
- (15) Dewar, M. J. *J. Chem. Soc.* **1950**, 2329.
- (16) Moffitt, W. E. *Proc. Phys. Soc.* **1950**, A63, 700.
- (17) Brooker, L. G. S.; Keyes, G. H.; Williams, W. W. *J. Am. Chem. Soc.* **1942**, 64, 199.
- (18) Brooker, L. G. S. *Rev. Mod. Phys.* **1942**, 14, 275.
- (19) Pauling, L. *Proc. Natl. Acad. Sci. U.S.A.* **1939**, 25, 577.
- (20) Fabian, J.; Hartmann, H. *Light Absorption of Organic Colorants*; Springer-Verlag: Heidelberg, Germany, 1980; pp 1–245.
- (21) Berneth, H. In *Ullmann's Encyclopedia of Industrial Chemistry*; 7th ed.; John Wiley & Sons Inc.: New York, 2009.
- (22) Platt, J. R. *J. Chem. Phys.* **1949**, 17, 484.
- (23) Herzfeld, K. F.; Sklar, A. L. *Rev. Mod. Phys.* **1942**, 14, 0299.
- (24) Reimers, J. R.; Hush, N. S. *Chem. Phys.* **1989**, 134, 323.
- (25) Feynman, R. P.; Leighton, R. B.; Sands, M. L. *Quantum Mechanics*; Addison-Wesley Publishing Company: Reading, MA, 1989; Vol. 3, pp 10–12.
- (26) Mulliken, R. S. *J. Chem. Phys.* **1939**, 7.
- (27) Dewar, M. J. S.; Longuet-Higgins, H. C. *Proc. R. Soc. London* **1952**, A214, 482.
- (28) Hoffmann, R.; Shaik, S.; Hiberty, P. C. *Acc. Chem. Res.* **2003**, 36, 750.
- (29) Shaik, S.; Hiberty, P. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Larter, R., Cundari, T. R., Eds.; John Wiley & Sons Inc.: New York, 2004; Vol. 20.
- (30) Lipkin, H. J. *Lie Groups for Pedestrians*; Dover Publications Inc.: Mineola, NY, 2002; pp 16–17.
- (31) Tishby, N. Z.; Levine, R. D. *Chem. Phys. Lett.* **1984**, 104, 4.
- (32) Shaik, S.; Shurki, A. *Angew. Chem., Int. Ed.* **1999**, 38, 586.
- (33) Truhlar, D. G. *J. Comput. Chem.* **2006**, 28, 73.
- (34) Hiberty, P. C. *THEOCHEM* **1998**, 451, 237.
- (35) Hiberty, P. C.; Shaik, S. *J. Comput. Chem.* **2007**, 28, 137.
- (36) Shaik, S. *New J. Chem.* **2007**, 31, 1981.
- (37) Hiberty, P. C.; Leforestier, C. *J. Am. Chem. Soc.* **1978**, 100, 2012.
- (38) Hirao, K.; Nakano, H.; Nakayama, K.; Dupuis, M. *J. Chem. Phys.* **1996**, 105, 9227.
- (39) Paldus, J.; Sarma, C. R. *J. Chem. Phys.* **1985**, 83, 5135.
- (40) Granucci, G.; Cassam-Chenaï, P.; Ellinger, Y. *J. Chem. Phys.* **1998**, 108, 2538.
- (41) Cassam-Chenaï, P.; Ellinger, Y.; Berthier, G. *Phys. Rev.* **1993**, A48, 2746.
- (42) An exception worth noting is in the case where there are exactly two electrons in a closed active space. Then, the complete set of number-conserving operators spans a Lie algebra and is composed of one- and two- electron operators.
- (43) Salem, L. *The Molecular Orbital Theory of Conjugated Systems*; W.A. Benjamin Inc.: New York, 1966; pp 36–43.
- (44) Docken, K. K.; Hinze, J. *J. Chem. Phys.* **1972**, 57, 4928.
- (45) Stålring, J.; Bernhardsson, A.; Lindh, R. *Mol. Phys.* **2001**, 99, 103.
- (46) Roos, B. In *Adv. Chem. Phys.*; Lawley, K. P., Ed.; John Wiley & Sons Ltd.: New York, 1987; Vol. 69, p 399.
- (47) Werner, H.-J.; Meyer, W. *J. Chem. Phys.* **1981**, 74, 5794.
- (48) Olsen, S.; McKenzie, R. H. *J. Chem. Phys.* **2009**, 131, 234306.
- (49) I have been able to obtain an analogous SA2-CAS(2,3) solution for the dication formed by removing two electrons from the GFP chromophore anion. The orbitals at convergence are visually indistinguishable from the four-electron case.
- (50) Foster, J. M.; Boys, S. F. *Rev. Mod. Phys.* **1960**, 32, 300.
- (51) Levy, B.; Berthier, G. *Int. J. Quantum Chem.* **1968**, 2, 307.
- (52) Finley, J.; Malmqvist, P.; Roos, B.; Serrano-Andrés, L. *Chem. Phys. Lett.* **1998**, 288, 299.
- (53) Celani, P.; Werner, H.-J. *J. Chem. Phys.* **2003**, 119, 5044.
- (54) Celani, P.; Werner, H.-J. *J. Chem. Phys.* **2000**, 112, 5546.
- (55) Rajput, J.; Rahbek, D. B.; Andersen, L. H.; Rocha-Rinza, T.; Christiansen, O.; Bravaya, K. B.; Nemukhin, A. V.; Bochenkova, A. V.; Solntsev, K. M.; Dong, J.; Kowalik, J.; Tolbert, L. M.; Petersen, M. Å.; Nielsen, M. B. *Phys. Chem. Chem. Phys.* **2009**, 11, 9996.
- (56) Li, X.; Chung, L.; Mizuno, H.; Miyawaki, A.; Morokuma, K. *J. Phys. Chem. B* **2010**, 114, 1114.
- (57) Ma, Y.; Rohlfing, M.; Molteni, C. *J. Chem. Theory Comput.* **2010**, 5, 257.
- (58) Olsen, S.; McKenzie, R. H. *J. Chem. Phys.* **2009**, 130, 184302.
- (59) Polyakov, I.; Epifanovsky, E.; Grigorenko, B.; Krylov, A. I.; Nemukhin, A. *J. Chem. Theory Comput.* **2009**, 5, 1907.
- (60) Epifanovsky, E.; Polyakov, I.; Grigorenko, B.; Nemukhin, A.; Krylov, A. *J. Chem. Theory Comput.* **2009**, 5, 1895.
- (61) Filippi, C.; Zaccheddu, M.; Buda, F. *J. Chem. Theory Comput.* **2009**, 5, 2074.
- (62) Luin, S.; Voliani, V.; Lanza, G.; Bizzarri, R.; Amat, P.; Tozzini, V.; Serresi, M.; Beltram, F. *J. Am. Chem. Soc.* **2009**, 131, 96.
- (63) Bravaya, K. B.; Bochenkova, A. V.; Granovskii, A. A.; Nemukhin, A. V. *Russ. J. Phys. Chem.* **2008**, B2, 671.
- (64) Vendrell, O.; Gelabert, R.; Moreno, M.; Lluch, J. M. *J. Chem. Theory Comput.* **2008**, 4, 1138.
- (65) Olsen, S.; Smith, S. *J. Am. Chem. Soc.* **2008**, 130, 8677.
- (66) Wang, S.; Smith, S. C. *Phys. Chem. Chem. Phys.* **2007**, 9, 452.
- (67) Camilloni, C.; Provati, D.; Tiana, G.; Broglia, R. *J. Phys. Chem.* **2007**, B111, 10807.
- (68) Zhang, L.; Xie, D.; Zeng, J. *J. Theory Comput. Chem.* **2006**, 5, 375.
- (69) Wang, S.; Smith, S. *J. Phys. Chem.* **2006**, B110, 5084.
- (70) Zhang, R.; Nguyen, M. T.; Ceulemans, A. *Chem. Phys. Lett.* **2005**, 404, 250.
- (71) Xie, D.; Zeng, J. *J. Comput. Chem.* **2005**, 26, 1487.
- (72) Sinicropi, A.; Andruniow, T.; Ferre, N.; Basosi, R.; Olivucci, M. *J. Am. Chem. Soc.* **2005**, 127, 11534.

- (73) Vendrell, O.; Gelabert, R.; Moreno, M.; Lluch, J. M. *Chem. Phys. Lett.* **2004**, 396, 202.
- (74) Toniolo, A.; Olsen, S.; Manohar, L.; Martínez, T. J. *Faraday Disc.* **2004**, 127, 149.
- (75) Martin, M. E.; Negri, F.; Olivucci, M. *J. Am. Chem. Soc.* **2004**, 126, 5452.
- (76) Laino, T.; Nifosì, R.; Tozzini, V. *Chem. Phys.* **2004**, 298, 17.
- (77) Das, A.; Hasegawa, J.; Miyahara, T.; Ehara, M.; Nakatsuji, H. *J. Comput. Chem.* **2003**, 24, 1421.
- (78) Helms, V. *Curr. Opin. Struct. Biol.* **2002**, 12, 169.
- (79) Helms, V.; Winstead, C.; Langhoff, P. *THEOCHEM* **2000**, 506, 179.
- (80) Voityuk, A.; Michel-Beyerle, M.; Rösch, N. *Chem. Phys.* **1998**, 231, 13.
- (81) Voityuk, A.; Michel-Beyerle, M.; Rösch, N. *Chem. Phys. Lett.* **1997**, 272, 162.
- (82) Altoe, P.; Bernardi, F.; Garavelli, M.; Orlandi, G. *J. Am. Chem. Soc.* **2005**, 127, 3952.
- (83) Topol, I.; Collins, J.; Polyakov, I.; Grigorenko, B.; Nemukhin, A. *Biophys. Chem.* **2009**, 145, 1.
- (84) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, 46, 618.
- (85) Dunning, T. H. *J. Chem. Phys.* **1989**, 90, 1007.
- (86) Bofill, J. M.; Pulay, P. *J. Chem. Phys.* **1989**, 90, 3637.
- (87) Pulay, P.; Hamilton, T. P. *J. Chem. Phys.* **1988**, 88, 4926.
- (88) Kutzelnigg, W. *Int. J. Quantum Chem.* **2009**, 109, 3858.
- (89) Canosa, N.; Rossignoli, R.; Plastino, A.; Miller, H. *Phys. Rev.* **1992**, C45, 1162.
- (90) Malrieu, J.-P.; Heully, J.-L.; Zaitsevskii, A. *Theor. Chem. Acc.* **1995**, 90, 167.
- (91) MOLPRO, version 2009.1, a package of ab initio programs: Werner, H.-J.; Knowles, P. J.; Lindh, R.; Manby, F. R.; Schütz, M. and others, see <http://www.molpro.net>.
- (92) There is one dye in the set that did violate the rule: the dye (PhOHZ/PhOHE). The excitation for this dye is very slightly below the mean wavelength of its parents, leading to an imaginary b value of $b(\text{PhOHZ/PhOHE}) = 372i \text{ cm}^{-1}$. The magnitude of this number is very small, consistent with the (reasonable) expectation that the dye is near-resonant, and the orientation of the hydrogen atom has a small effect. In this case, I replaced the imaginary number with its magnitude, after verifying that this substitution led to a difference of less than 1 nm in the recalculated excitation wavelength.
- (93) Forbes, M. W.; Jockusch, R. A. *J. Am. Chem. Soc.* **2009**, 131, 17038.
- (94) Andersen, L. H.; Lapierre, A.; Nielsen, S. B.; Nielsen, I. B.; Pedersen, S. U.; Pedersen, U. V.; Tomita, S. *Eur. Phys. J.* **2002**, D20, 597.
- (95) Nielsen, S. B.; Lapierre, A.; Andersen, J. U.; Pedersen, U. V.; Tomita, S.; Andersen, L. H. *Phys. Rev. Lett.* **1997**, 87, 228102.
- (96) Dong, J.; Solntsev, K. M.; Tolbert, L. M. *J. Am. Chem. Soc.* **2006**, 128, 12038.
- (97) Webber, N. M.; Meech, S. R. *Photochem. Photobiol. Sci.* **2007**, 6, 976.
- (98) Shi, X.; Abbyad, P.; Shu, X.; Kallio, K.; Kanchanawong, P.; Childs, W.; Remington, S. J.; Boxer, S. G. *Biochemistry* **2007**, 46, 12014.
- (99) McAnaney, T.; Park, E.; Hanson, G.; Remington, S.; Boxer, S. *Biochemistry* **2002**, 41, 15489.
- (100) Chattoraj, M.; King, B.; Bublitz, G.; Boxer, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93, 8362.
- (101) It is possible that the state-averaging procedure is removing contaminating continuum states from the active space, but this does not explain why they are not being re-introduced by the subsequent perturbation theory.
- (102) Langley, R. *Practical Statistics Simply Explained*; Dover Publications Inc.: Mineola, NY, 1971; pp 222–230.
- (103) Azizi, Z.; Roos, B.; Veryazov, V. *Phys. Chem. Chem. Phys.* **2006**, 8, 2727.
- (104) Serrano-Andrés, L.; Merchán, M. *THEOCHEM* **2005**, 729, 99.
- (105) Andersen, L. H.; Bochenkova, A. V. *Eur. Phys. J.* **2009**, D51, 5.
- (106) van Faassen, M.; de Boeij, P. L. *J. Chem. Phys.* **2004**, 120, 11967.
- (107) Schreiber, M.; Silva-Junior, M. R.; Sauer, S. P. A.; Thiel, W. *J. Chem. Phys.* **2008**, 128, 134110.
- (108) Grimme, S.; Neese, F. *J. Chem. Phys.* **2007**, 127, 154116.
- (109) Jacquemin, D.; Wathelet, V.; Perpète, E. A.; Adamo, C. *J. Chem. Theory Comput.* **2009**, 5, 2420.
- (110) In particular cases, accuracies exceeding these *a priori* expectations are achievable. See, for example: Schreiber, M.; Buss, V.; Fulscher, M. P. *Phys. Chem. Chem. Phys.* **2001**, 3, 3906.
- (111) Breuer, H.-P.; Petruccione, F. *The Theory of Open Quantum Systems*; Oxford University Press: Oxford, U.K., 2002; pp 5–10.
- (112) Cederbaum, L. S.; Schirmer, J.; Meyer, H.-J. *J. Phys. (Paris)* **1989**, A22, 2427.
- (113) Goldstein, J. A.; Levy, M. *Am. Math. Monthly* **1991**, 98, 710.
- (114) Dupré, M.; Goldstein, J.; Levy, M. *J. Chem. Phys.* **1980**, 72, 780.
- (115) Bianco, R.; Timoneda, J.; Hynes, J. J. *Phys. Chem.* **1994**, 98, 12103.
- (116) Kim, H.; Hynes, J. T. *J. Chem. Phys.* **1990**, 93, 5194.
- (117) Kim, H.; Hynes, J. T. *J. Chem. Phys.* **1990**, 93, 5211.
- (118) Thompson, W.; Blanchard-Desce, M.; Alain, V.; Muller, J.; Fort, A.; Barzoukas, M.; Hynes, J. T. *J. Phys. Chem.* **1999**, A103, 3766.
- (119) Thompson, W.; Blanchard-Desce, M.; Hynes, J. T. *J. Phys. Chem.* **1998**, A102, 7712.
- (120) Lu, D.; Chen, G.; Perry, J.; Goddard, W. A., III. *J. Am. Chem. Soc.* **1994**, 116, 10679.
- (121) Babendure, J.; Adams, S.; Tsien, R. *J. Am. Chem. Soc.* **2003**, 125, 14716.
- (122) Gonçalves, M. *Chem. Rev.* **2009**, 109, 190.
- (123) Ozhatici-Unal, H.; Pow, C. L.; Marks, S. A.; Jesper, L. D.; Silva, G. L.; Shank, N. I.; Jones, E. W.; Burnette, J. M.; Berget, P. B.; Armitage, B. A. *J. Am. Chem. Soc.* **2008**, 130, 12620.
- (124) Meech, S. R. *Chem. Soc. Rev.* **2009**, 38, 2922.
- (125) Tsien, R. Y. *Annu. Rev. Biochem.* **1998**, 67, 509.

- (126) Bell, A. F.; He, X.; Wachter, R.; Tonge, P. J. *Biochemistry* **2000**, *39*, 4423.
- (127) Pakhomov, A. A.; Martynov, V. *Chem. Biol.* **2008**, *15*, 755.
- (128) Jaynes, E. T. *Phys. Rev.* **1957**, *108*, 171.
- (129) Bengtsson, I.; Życzkowski, K. *The Geometry of Quantum States*; Cambridge University Press: Cambridge, U. K., 2006; pp 333–369.
- (130) Andresen, M.; Stiel, A. C.; Fölling, J.; Wenzel, D.; Schönle, A.; Egner, A.; Eggeling, C.; Hell, S. W.; Jakobs, S. *Nat. Biotechnol.* **2008**, *26*, 1035.
- (131) Kondo, M.; Heisler, I. A.; Stoner-Ma, D.; Tonge, P. J.; Meech, S. R. *J. Am. Chem. Soc.* **2009**, *132*, 1452.
- (132) Kamlet, M. J.; Abboud, J. L.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877.
- (133) Kamlet, M. J.; Abboud, J. L.; Taft, R. W. *J. Am. Chem. Soc.* **1977**, *99*, 6027.
- (134) Drobizhev, M.; Tillo, S.; Makarov, N. S.; Hughes, T. E.; Rebane, A. J. *Phys. Chem.* **2009**, *B113*, 12860.
- (135) Gruber, D. F.; Desalle, R.; Lienau, E. K.; Tchernov, D.; Pieribone, V. A.; Kao, H.-T. *Mol. Bio. Evol.* **2009**, *26*, 2841.
- (136) Labas, Y. A.; Gurskaya, N. G.; Yanushevich, Y. G.; Fradkov, A. F.; Lukyanov, K. A.; Lukyanov, S. A.; Matz, M. V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4256.
- (137) Field, S. F.; Bulina, M. Y.; Kelmanson, I. V.; Bielawski, J. P.; Matz, M. V. *J. Mol. Evol.* **2006**, *62*, 332.
- (138) Ugalde, J.; Chang, B.; Matz, M. *Science* **2004**, *305*, 1433.
- (139) Olsen, S.; Lamothe, K.; Martínez, T. J. *J. Am. Chem. Soc.* **2010**, *132*, 1192.
- (140) Dong, J.; Solntsev, K. M.; Tolbert, L. M. *J. Am. Chem. Soc.* **2009**, *131*, 662.
- (141) Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graph.* **1996**, *14*, 33.

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