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Collapse and Recovery of Green Fluorescent Protein Chromophore Emission through Topological Effects

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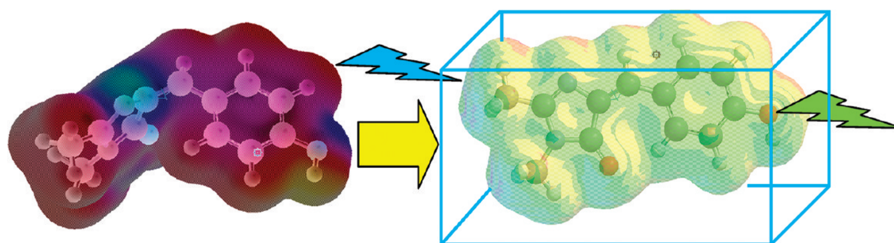
CONSPECTUS



Housed within the 11-stranded *β*-barrel of the green fluorescent protein (GFP) is the arylideneimidazolidinone (AMI) chromophore, the component responsible for fluorescence. This class of small-molecule chromophore has drawn significant attention for its remarkable photophysical and photochemical properties, both within the intact protein and after its denaturation. All of the proteins so far isolated that have visible light fluorescence have been found to contain an AMI chromophore. These proteins comprise an extensive rainbow, ranging from GFP, which contains the simplest chromophore, *p*-hydroxybenzylideneimidazolidinone (*p*-HOBDI), to proteins having molecules with longer conjugation lengths and a variety of intraprotein interactions. The fluorescence invariably almost vanishes upon removal of the protective β-barrel. The role of the barrel in hindering internal conversion has been the subject of numerous studies, especially in our laboratories and those of our collaborators.

A better understanding of these chromophores has been facilitated by the development of numerous synthetic protocols. These syntheses, which commonly use the Erlenmeyer azlactone method, have evolved in recent years with the development of a [2 + 3] cycloaddition exploited in our laboratory. The synthetic AMI chromophores have allowed delineation of the complex photophysics of GFP and its derivatives. Upon denaturation, AMI chromophores are marked by 4 orders of magnitude of diminution in emission quantum yield (EQY). This result is attributed to internal conversion resulting from conformational freedom in the released chromophore, which is not allowed within the restrictive *β*-barrel. To date, the photophysical properties of the AMI chromophore remain elusive and have been attributed to a variety of mechanisms, including cis–trans isomerization, triplet formation, hula twisting, and proton transfer. Advanced studies involving gas-phase behavior, solvent effects, and protonation states have significantly increased our understanding of the chromophore photophysics, but a comprehensive picture is only slowly emerging. Most importantly, mechanisms in structurally defined chromophores may provide clues as to the origin of the “blinking” behavior of the fluorescent proteins themselves.

One approach to examining the effect of conformational freedom on rapid internal conversion of the chromophores is to restrict the molecules, both through structural modifications and through adjustments of the supramolecular systems. We thus include here a discussion of studies involving the crystalline state, inclusion within natural protein-binding pockets, complexation with metal ions, and sequestration within synthetic cavities; all of this research affirms the role of restricting conformational freedom in partially restoring the EQY. Additionally, new photochemistry is observed within these restricted systems. Many of the studies carried out in our laboratories show promise for these molecules to be adapted as molecular probes, wherein inclusion turns on the fluorescence and provides a signaling mechanism.

In this Account, we present an overview of the AMI chromophores, including synthesis, overall photophysics, and supramolecular behavior. A significant amount of work remains for researchers to fully understand the properties of these chromophores, but important progress achieved thus far in photophysics and photochemistry is underscored here.

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Introduction

Formation of the chromophore *p*-hydroxybenzylideneimidazolidinone (*p*-HOBDI) in the green fluorescent protein (GFP) of the jellyfish *Aequorea victoria*[1] occurs via a post-translational autocatalytic cyclization followed by autoxidation within a tripeptide unit of the polypeptide sequence consisting of 238 amino acids (see Figure 1),[2] although the order of steps between dehydration and oxidation has been disputed.[3] X-ray diffraction reveals that the chromophore is protectively housed and covalently anchored along a coaxial helix threaded through the center of an 11-stranded β-barrel that secludes it from the aqueous medium surrounding the protein.[4,5] Additional noncovalent coupling of the chromophore to the protein backbone is facilitated via an extended hydrogen-bonded network.[6] The chromophore falls into a general class of hydroxyarenes in which the presence of electron-withdrawing groups, in this case, the methyleneimidazolidinone group, leads to a significantly increased acidity in the excited state.[7] Thus the "large Stokes shift"[8] associated with such proteins is not a true Stokes shift, since true Stokes emission arises *from the same electronic transition*,[9] but rather reflects an excited-state deprotonation to produce the bathochromically shifted anion emission. A better term would be "Förster shift".

The simplest chromophore (*p*-HOBDI) derived from the GFP has been investigated thoroughly by Meech[10] and others[11] and has become the standard representative chromophore for GFP (see Figure 2). Following removal from the protective β-barrel, the GFP chromophore exhibits several spectral changes including a 4 orders of magnitude decrease in emission quantum yield. The mechanism of radiationless decay has been established as internal conversion, promoted by motion along a volume conserving pathway, perhaps invoking a "hula twist",[12] nearly barrierless coordinate, which is disputed in favor of a phenyl rotation.[13] For recent reviews related to the synthesis and properties of GFP synthetic chromophores, see refs 14 and 15.

In this Account, we summarize our recent investigations in synthesis, photophysics, photochemistry, and sensor applications of the native GFP chromophore and its derivatives. We have established that their photophysics, for which excited state proton transfer (ESPT) is but one pathway, are characterized by a remarkable sensitivity to environment and have a wide variability in fluorescence efficiency, making them unique probes for a particular environment. When that environment is a protein, either the original β-barrel of the intact chromophore or a different protein host (as well as other biologically relevant analytes), the photophysics of the chromophore, including ESPT, emission wavelength, decay rate, and structure, provide detailed information about the chemical nature of the host. By working with synthetic chromophores, we can also vary electronic and steric effects on their photophysics and examine in detail subtleties in the decay pathways. In this context, we should emphasize that the dramatic difference between enclosed and free chromophore fluorescence quantum yields make such molecules

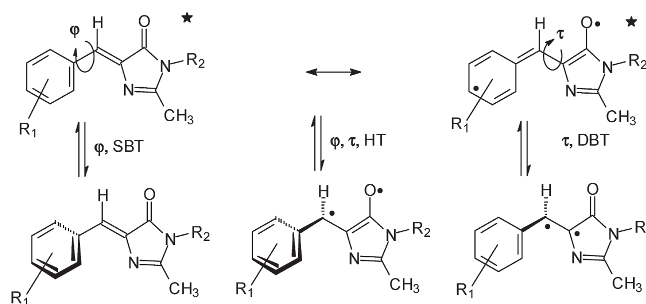

FIGURE 2. Single-bond twist (SBT), double-bond twist (DBT),and hula twist (HT) in internal conversion pathways for *p*-HOBDI.

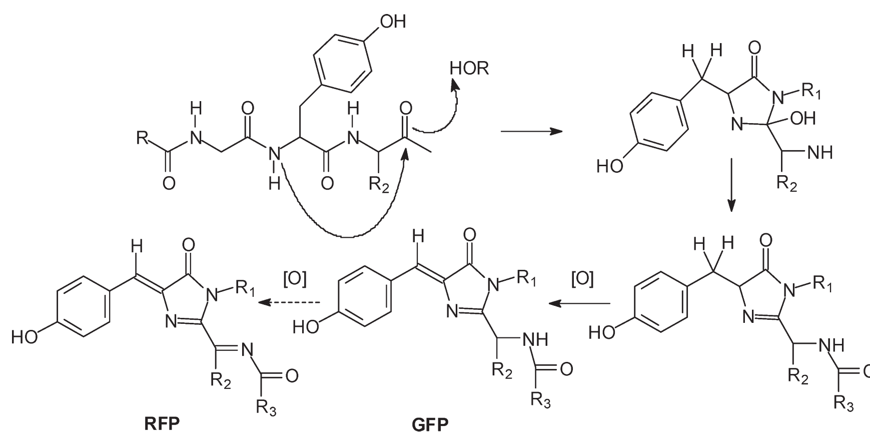

FIGURE 1. Cyclization/oxidation to yield GFP and RFP.

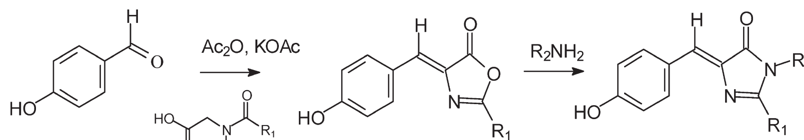

FIGURE 3. Standard method of BDI synthesis.

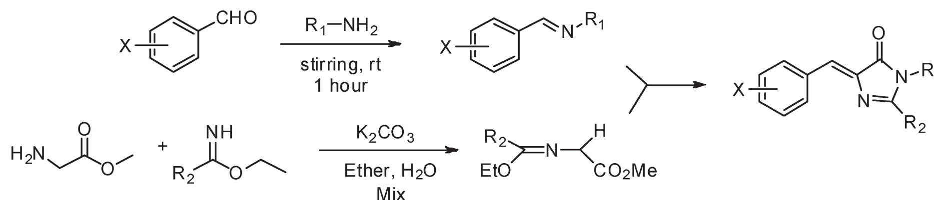

FIGURE 4. Synthesis of BDIs through a 2,3-cycloaddition.

ideal substrates for examining the influence of topological factors on internal conversion.

Synthetic Routes toward Arylideneimidazolidinone Derivatives

Synthesis of GFP chromophore analogs in the literature has commonly been carried out using Tonge's modification[16] of the Niwa synthesis,[17] often referred to as the Erlenmeyer azlactone synthesis, which involves the condensation of an arenecarboxaldehyde with an N-acylated amino acid derivative to produce an oxazolone intermediate, followed by reflux with a primary amine and potassium carbonate to yield the arylmethyleneimidazolinone (Figure 3). While the synthesis tolerates numerous aromatic aldehydes, efforts within our group using nitrogen-containing heterocycles (pyridine, pyrrole) yield no product under Erlenmeyer conditions. In contrast, a Knoevenagel condensation of the imidazolinone and various arenecarboxaldehydes, including pyrrole-2-carbaldehyde, provides chromophore analogs in good yields. Using an adaptation of condensation, reported by Wu and Burgess,[18] but with ionic liquids, our group is also able to synthesize chromophore analogs using pyridine-2-carboxaldehyde.

While efforts from multiple groups have contributed to the overall synthesis of chromophore analogs, both the Erlenmeyer azalactone method and the Knoevenagel condensation are nonconvergent and require multiple steps in generally modest yields. Recently, these concerns were addressed by our group using a $2 + 3$ cycloaddition originally proposed by Bazureau et al. (Figure 4).[19,20] This cycloaddition uses an aromatic imine, produced from the condensation of a primary amine with a given arenecarboxaldehyde, and an iminoglycine methyl ester produced from the condensation of an imidate with a glycine methyl ester. Overall yields of this synthesis range from fair to excellent and require minimal purification, providing an efficient overall synthetic process. The nature of the imine synthesis readily allows for a combinatorial approach for substitution on R_1 and R_2. Using robotic methods, we have now synthesized over 600 examples!

Finally, reported substitution on R_2 throughout all syntheses is limited and poses challenges. Recently, Lukyanov's group has developed an oxidation method using SeO_2 to convert the C-methyl of the imidazolinone ring to an aldehyde, allowing introduction of Wittig chemistry.[14] Using imine chemistry on this aldehyde as well provides for additional combinatorial substitution, currently in progress, that could provide model chromophores for the red fluorescent protein, which are outside the scope of this Account.

Spectroscopy in Solution and in the Gas Phase

The β-barrel provides a number of topological and nonbonding constraints in addition to the two covalent bonds, which are reflected on the fluorescence of the chromophore. In order to elucidate all of these effects on the photophysics, we have studied the properties of the unsolvated chromophore, that is, in the gas phase and in condensed media.

Gas-Phase Behavior. One intriguing aspect of the GFP absorption and emission wavelengths is the effect of a proximate arginine (Arg96), which can interact with the chromophore either through direct hydrogen bonding or through an electrostatic effect. Calculations by Tozzini[21] indicate that Arg96 might shift the absorption spectrum of the chromophore by as much as 40 nm, presumably by lowering the energy of the CT excited state.

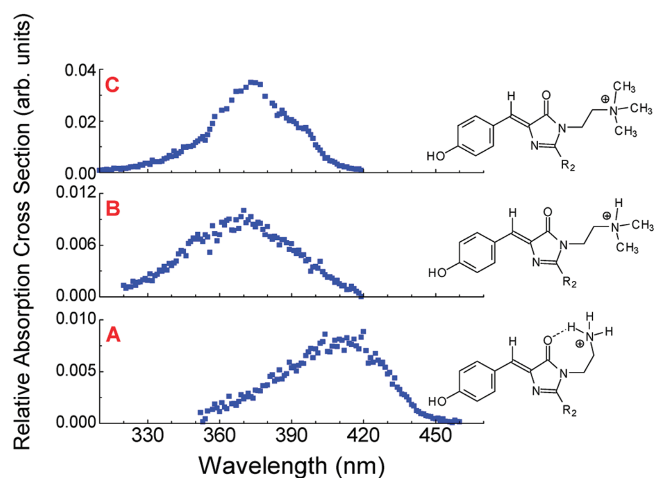

FIGURE 5. Absorption spectra of the GFP model chromophores in vacuum.

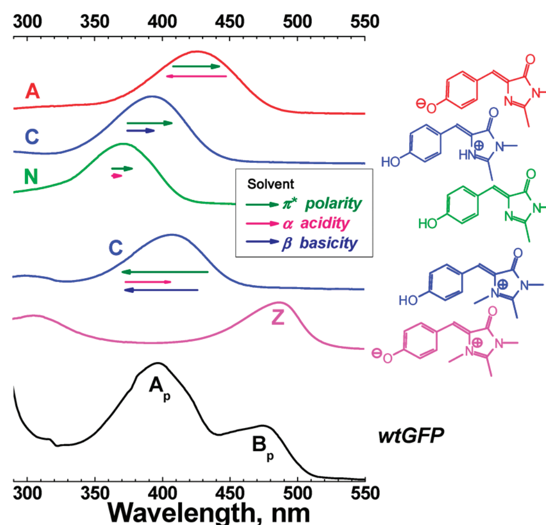

FIGURE 6. Absorption spectra of different protonation states of *p*-HOBDI and *p*-HOBDIMe+ in MeOH/H_2O. For comparison, we present the absorption spectra of wtGFP at pH 7. The results of the Kamlet–Taft solvatochromic analysis are presented as arrows. Their magnitude and directions show the relative contribution of solvent polarity, acidity, and basicity.

To mimic and separate these interactions, we have synthesized GFP chromophores carrying a spectator positive charge ($-NH_3^+$ and $-N(CH_3)_3^+$) attached to the molecule through an alkyl chain (Figure 5). In collaboration with Lars Andersen, we have performed gas-phase absorption experiments and theoretical studies on the neutral form of these chromophores using the electrostatic ion storage ring ELISA.[22,23] With this technique, photoabsorption spectra are recorded by measuring the count of neutral photo fragments generated after wavelength-dependent laser excitation of the ELISA ring, which can be related to the absorption cross-section. The structure of compound A shown in Figure 5, which allows intramolecular H-bonding, is found to give rise to a red shift in the photoabsorption spectrum compared with non-hydrogen-bonded compound C.[23] This observation supports the relevance of such interactions in GFP photophysics.

Solvent Effects and Protonation States. *p*-HOBDI has three distinct protonation states that exhibit significantly different spectroscopic properties: a protonated cationic form (C), pH < 2, a neutral form (N), and an anionic form (A), pH > 10 (Figure 6).[24] The absorbance spectrum of wild-type GFP (wtGFP) exhibits two forms of the chromophore with absorbance bands at 398 nm (A_p band) and 477 nm (B_p band), where the high-energy wt-GFP absorbance band corresponds to the absorbance of the *p*-HOBDI cation in water and the ratios very weakly depend on pH (Figure 6).

The solvatochromic behavior of various synthetic GFP chromophores displays a relative contribution of nonspecific (polarity) vs specific (hydrogen-bonding) solvation to the absorption spectra.[25] A Kamlet–Taft solvatochromic analysis[26] clearly demonstrates an increase of the dipole moment of the chromophores upon ionization and shows their amphoteric behavior. These experimental findings serve as a background for a number of theoretical efforts that predict the absorption spectra of GFP chromophores in solution and in proteinaceous environments.

We have also synthesized the ground-state zwitterion equivalent for HOBDI, namely, $^-$OBDIMe$^+$, which enforces a positive charge on nitrogen by methyl alkylation. This derivative exhibits a red-shifted absorption at 480 nm (Figure 6), which, curiously, coincides with that of the anion in wtGFP! We are tempted to conclude that the GFP absorption was misassigned, although a more practical explanation is the influence imposed by the obligatory Arg96 in GFP and all its fluorescent mutants, as discussed above.

The much higher efficiency of photoisomerization of free HOBDI in solution, compared with wtGFP, leads not only to significant fluorescence quenching but also to spectral broadening and band shifting.[27] We were able to reconstruct the wide structureless fluorescence spectra of HOBDI in glycerol–water solutions at various temperatures by convoluting the original wtGFP spectra with the function describing the distribution of the transition energies of the HOBDI chromophore (Figure 7).[27] The main coordinate of this distribution could be related to cis/trans isomerization. Another type of vibronic coupling in the wtGFP was recently studied by Mathies et al. who established an important correlation between several stretching modes of the

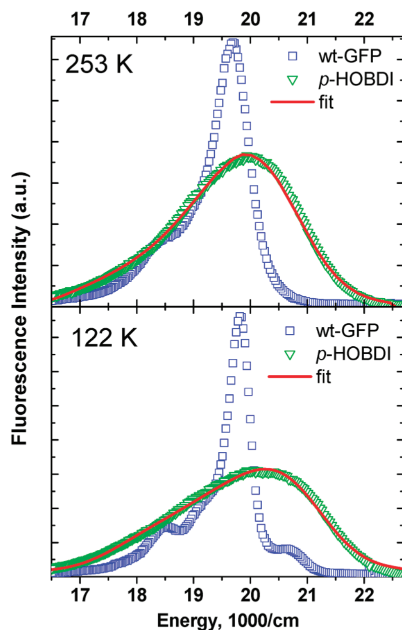

FIGURE 7. Reconstruction of *p*-HOBDI model spectra in glycerol/water (▽) at various temperatures by utilizing the protein spectra (□).

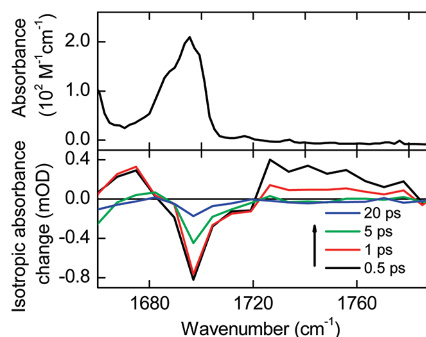

FIGURE 8. Steady-state (top) and transient (bottom) spectra showing the isotropic response of the C=O stretching bands in *p*-HOBDI in CD_3OD after electronic excitation.

chromophore and its ESPT efficiency.[28] These factors can be invoked in the "turn-on" phenomenon of particular ligand/host interactions.

Blinking and Isomerization. Fluorescent proteins are characterized by "blinking", the temporary conversion to a nonfluorescent "off" form, which is recovered either thermally or photochemically (often referred to as a "switchable" protein). A difficulty has been relating the off state to the photophysics. The off state has been variously attributed to triplet formation,[29] proton transfer,[30] or cis/trans isomerization (the trans form is almost invariably nonfluorescent). The identity of this off state remains a key unresolved issue in the photophysics of fluorescent proteins. Strong support for cis/trans isomerization as the origin of blinking is provided by the behavior of kindling fluorescent proteins,[31] which, upon irradiation into the long-wavelength band, are "kindled" to the fluorescent form. Recent single-crystal X-ray determinations of both forms of two kindling proteins, *dronpa* and *mTFP0.7*, differ in the stereochemistry about the benzylidene bond of the chromophores.[32] In the solid state, conformational freezing may or may not lead to fluorescence, indicating that excited-state decay is a complex function of structure.[33] Calculations[34] suggest that formal isomerization, that is, decay from the twisted intermediate onto the trans hypersurface, must be permitted. This phenomenon has been the subject of several studies[35] and relates well to other arylidene chromophores.

We have applied a variety of ultrafast optical techniques to study the photophysics and photochemistry of GFP chromophores. In collaboration with Erik Nibbering,[36] and independent of the work of Tonge and Meech,[37] we have measured the time-resolved infrared (TRIR) spectra of HOBDI (Figure 8). We observed the expected bleaching of the carbonyl band at 1696 cm^{-1} and a new band at 1746—1780 cm^{-1}, which Tonge and Meech do not report. The decay rate of this band is coincident with the fluorescence lifetime, which allows us to conclude that this new band is the excited-state carbonyl stretch, with some twisting about the formal double bond. We focused on the orientation of the transition moment of the IR-active carbonyl stretching vibration of HOBDI relative to that of the electronic transition dipole moment, which indicates that the C=O vibration acts in a "spectator mode" for the relative orientation of the phenolate and imidazolidinone groups, from which we were able to derive an effective near complete twisting around the ethylenic bridge upon electronic excitation of HOBDI.

The ultrafast deactivation of wtGFP chromophores is a subject of intensive experimental and theoretical work.[38] Together with Noam Agmon, we have developed a qualitative description of the unusual steady-state and time-resolved behavior of the HOBDI chromophore in the wide range of temperatures and pressures caused by isomerization-induced deactivation.[35b,39] We show that unlike small rigid aromatic dyes, which decay monoexponentially, the HOBDI anion in viscous solvents shows marked nonexponential behavior over a very wide temperature and pressure range. We find that a simple inhomogeneous model with a Gaussian sink term achieves an excellent quantitative fit with only one adjustable parameter, which is proportional to pressure and inversely proportional to temperature.

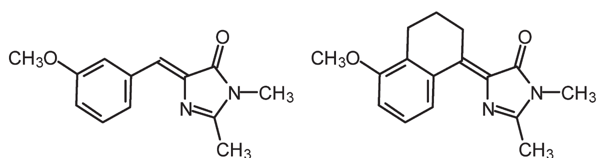

FIGURE 9. Locked (right) and unlocked (left) *Z m*-MeOBDI.

We have recently demonstrated a direct relation between the degree of HOBDI twisting and the rate of the excited-state deactivation by comparing the decays of HOBDI and 2,6-dimethyl-HOBDI.[40] In the latter, the highly twisted structure with 45° DFT-calculated dihedral angle between the phenyl and imidazolidinone planes results in an unprecedented short S_1 lifetime (110 fs for anion) compared with 530 fs for HOBDI anion (in ethanol). In an attempt to decouple ϕ and φ photoisomerization coordinates, we have synthesized the locked derivative of *m*-MeOBDI (Figure 9). Curiously, we have observed the counterintuitive result that the locked *m*-MeOBDI decays much faster than the unlocked one, with the lifetimes of 260 fs and 1.4 ps[41] (in methanol), respectfully. These results demonstrate that the rotation around the double bond is indeed a major coordinate leading to the conical intersection point. Single-crystal X-ray measurements demonstrate a dihedral angle increase from 3.90° for the unlocked[42] to 6.24° for the locked *m*-MeOBDI. Therefore, the twisted ground-state geometry appears to place the Franck–Condon state closer to conical intersection.[40] Such correlation between the degree of chromophore twisting and the fluorescence quantum yield is well documented for various fluorescent proteins.[43]

Femtosecond UV-pump UV-probe transient absorption data of *para*-hydroxy or *para*-methoxy derivatives demonstrates that each step of the one-photon pathway is characterized by a transition through a succession of states along a reaction coordinate that includes contributions from intramolecular twisting motion, solvation dynamics, and vibrational cooling.[41] Accordingly, the experimental time constants derived from this data can be considered as average values for a distribution of population in both the excited and ground states.[38]

Finally, with a view toward exploring the formation of "non-natural" fluorescent proteins, we have demonstrated higher emission quantum yields for *meta*- rather than *para*-HOBDI derivatives.[41,44] This allows studies of the ultrafast intermolecular ESPT that competes with isomerization. For *m*-HOBDI at neutral pH, a sequential ESPT is observed (Figure 10) including both diabatic and adiabatic ESPT.

While the mechanism of photoisomerization has been the subject of several studies, the mechanism of the thermal reverse isomerization is more problematic. The blinking phenomenon requires that isomerization, if involved, be thermally reversible. The results we have observed are consistent with the addition/elimination mechanism shown as path b in Figure 11.[45] Although a plausible mechanism, literature precedents are fairly rare. The isomerization of cinnamate anions has been shown to involve such processes. Such mechanisms have been proposed as possibilities in biological isomerizations, but no examples are reported.[46] A more immediate question is the relevance to the chemistry of fluorescent proteins, particularly the blinking phenomenon. As noted earlier, if blinking is associated with cis/trans isomerization, blinking "on" requires either reverse photoisomerization or a thermal process, and this work demonstrates that the unassisted process has too high a barrier to compete. Conversely, the addition/elimination mechanism, as a bimolecular diffusional process, is relatively slow. In a protein, however, such diffusion is irrelevant; rather, the question is whether there is a competent nucleophile to initiate such a mechanism. The crystal structure of rapidly isomerizing *trans-mTFP0.7* is characterized by the presence of a water molecule interposed between a glutamate and the benzylidene carbon of the chromophore such that the glutamate can promote addition of water to the double bond.[47] Addition/elimination emerges as a compelling mechanism for isomerization of fluorescent protein chromophores. The presence of an internal nucleophile thus becomes an additional point of mutation of such chromophores that may mediate their photophysical properties.

Supramolecular Behavior of Chromophores and Recovery of Fluorescence

The term "supramolecular" generally refers to nonbonding complexes of two or more species. Thus a fluorescent protein is not, in principle, supramolecular because it is held together primarily by bonding interactions. Nevertheless, its photophysical behavior is entirely due to nonbonding interactions. Thus one of our primary objectives has been to define such emission behavior in terms of supramolecular interactions, primarily topological ones, which inhibit twisting motions leading to facile decay.

Solid-State Fluorescence and Photoinduced Reactions. An efficient inhibition of photoisomerization is expected in the crystalline state. Recently, we noted that fluorescence "turn-on" occurs in the solid-state for alkyloxy derivatives, which are basically nonfluorescent in solution.[33] Steady-state

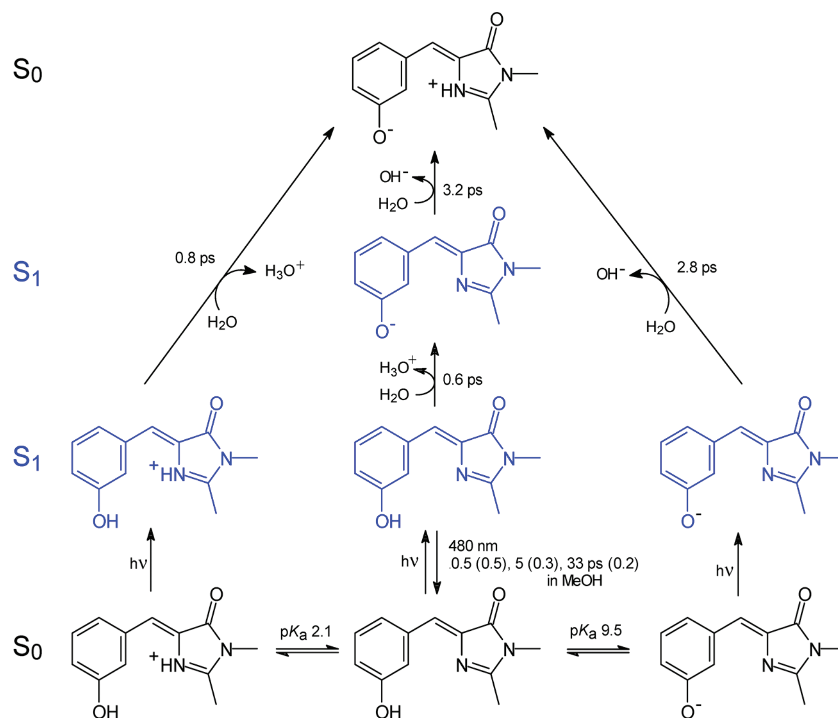

FIGURE 10. Photoprotolytic processes in *m*-HOBDI.

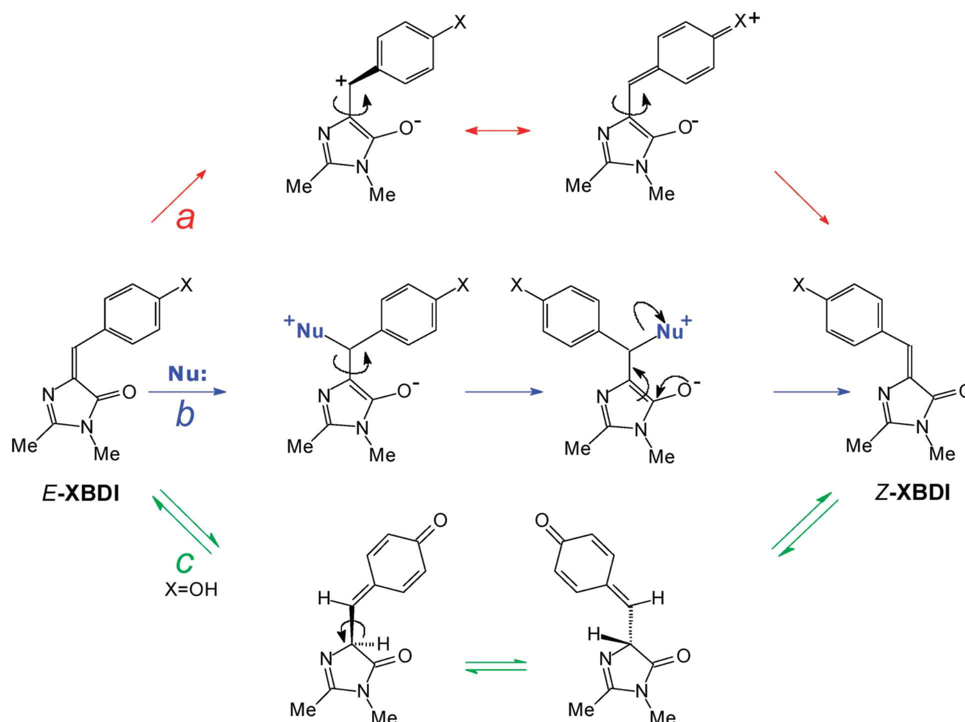

FIGURE 11. Pathways for the ground-state *E*-XBDI isomerization: (a) direct (traditional); (b) addition/elimination mechanism; (c) isomerization by tautomerization.

and time-resolved emission spectroscopy, as well as X-ray diffraction analysis, revealed the nature of complex emission in the crystals, including emission from monomers and aggregates. The size of the alkyloxy substituent plays a dramatic role in color tuning of the crystalline luminescence (Figure 12). Extending the alkyloxy group from methyl to hexyl to dodecyl weakens the interaction between the aromatic rings of the molecules in the lattice

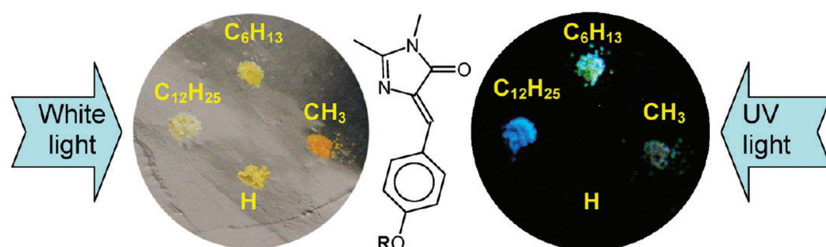

FIGURE 12. Real-color photograph of ROBDI crystals under daylight and UV-illumination. The structure of ROBDI molecules is presented in the middle.

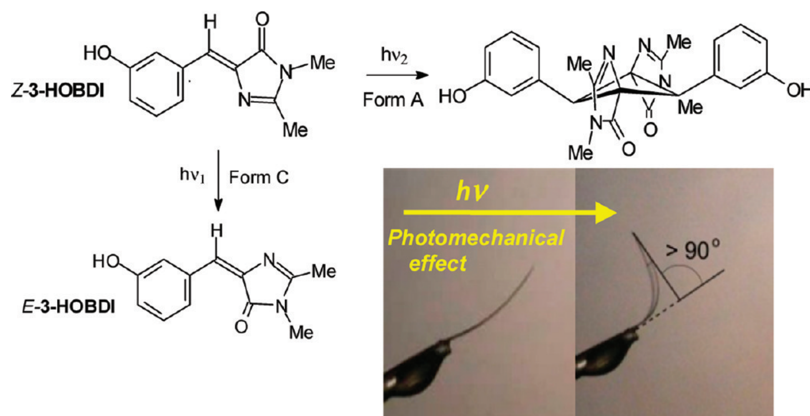

FIGURE 13. Solid-state photochemistry and photomechanical effect in *m*-HOBDI.

resulting in a hypsochromic shift of emission from the crystals. No emission was observed from the hydroxy derivative, which unlike the others exhibits hydrogen bonding between the phenolic OH and the imidazolinone carbonyl group of an adjacent molecule.[33] Additional solid-state studies, in collaboration with Pance Naumov, revealed the uniqueness of the meta hydroxy derivative compared with other chromophores. One exceptional property noted is a photomechanical effect resulting from a well-known[48] 2 + 2 cycloaddition (Figure 13).[42] This property was only observed in specific chromophore crystalline examples with stacked face-to-face geometry and an interplanar distance smaller than 4.5 Å.

Metal Complexation. Ultrafast photoisomerization in *p*-HOBDI chromophores can also be suppressed by metal ion complexation using an aza-derivative, for which the phenol moiety of *p*-HOBDI is replaced by a 2-pyridyl moiety. Dramatic enhancement of fluorescence for Zn^{2+} and Cd^{2+} ions is observed compared with other metals (Figure 14).[49] These zinc-selective derivatives of the GFP chromophore provide potential for optimization as sensors based upon inhibition of cis/trans isomerization.

Inhibition of Isomerization by Sequestration. The propensity for stilbene-like molecules to undergo twisting in the excited-state, leading to diminished fluorescence and, ultimately, conversion of the trans form to the cis form, has been one of the staples of organic photochemistry.[50] This is likely due to diminished bond order of the double bond in the excited state. As we have elaborated above, the photophysics of the GFP chromophore is a complex function of its protein environment, especially the restrictive β-barrel. The inhibition of isomerization has found practical applications with examples highlighted in the literature.[51,52] Inhibition of such twisting was the driving force for the development of stilbene antibodies, for which the complex with aminostilbene shows an emission enhancement of ca. 20-fold.

In collaboration with V. Ramamurthy, we discovered that sequestration of these chromophores within a deep cavity hydrophobic cavitand, the so-called "octaacid" (OA),[53] provides a 15-fold emission enhancement.[54] Curiously, the molecules "turning on" upon encapsulation are characterized by an ortho-substituent group, including methyl, trifluoromethyl, and various halogens, rather than by para or meta substituents, which NMR studies indicate are complexed at the bottom of the cavity.[55,56] From this, it is concluded that the ortho group inhibits phenyl bond rotation about the formal single bond and this contributes significantly to fluorescence recovery in addition to cis–trans

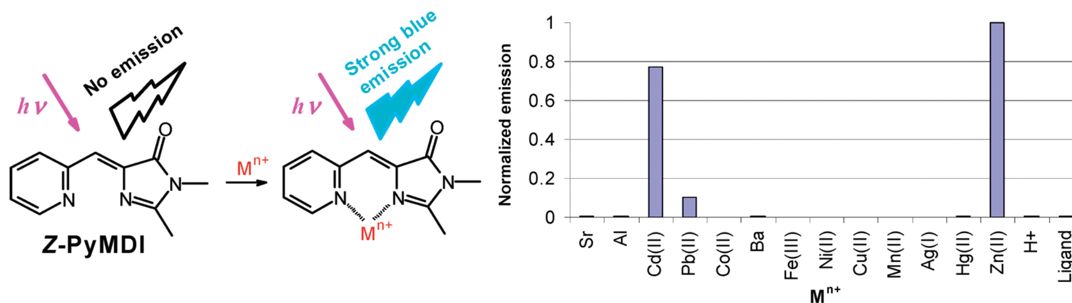

FIGURE 14. Effects of metal complexation on PyMDI fluorescence.

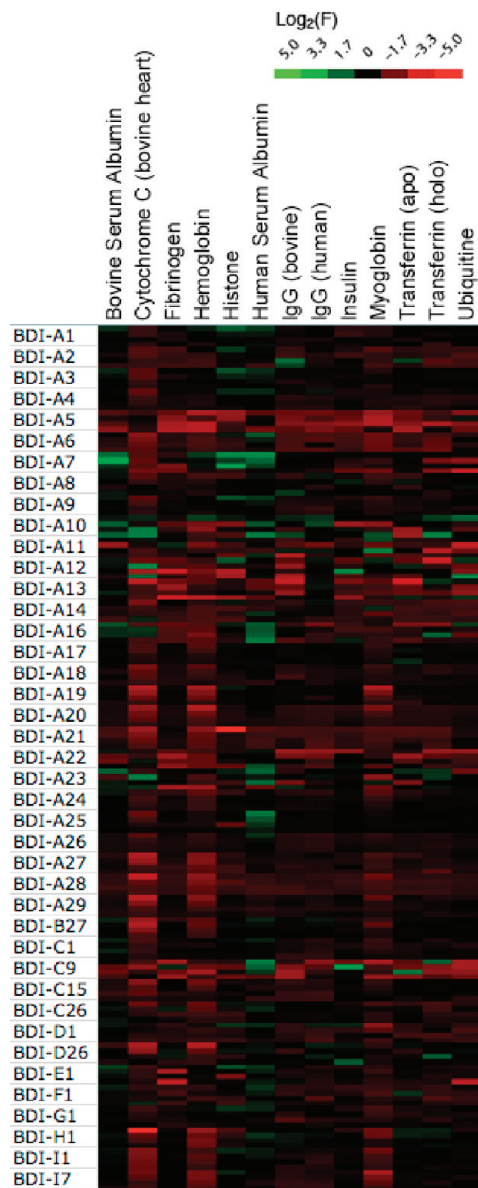

FIGURE 15. Heat map profile of protein class analytes (AMIs).

isomerization, an effect which supports the significance of phenyl rotation in fluorescence decay. This is corroborated by irradiation experiments that show quenching of fluorescence overtime attributed to the conversion of the fluorescent cis isomer to the nonfluorescent trans isomer.

From the spectral changes noted from sequestration, it follows that these chromophores can act as probes that fluoresce upon inclusion. Coupled with the combinatorial synthetic methods developed, a wide range of compounds can be synthesized to optimize the sensing capabilities of a given substitution pattern. In collaboration with Y.-T. Chang, 42 chromophores were screened against various analytes while monitoring the dose fluorescence response (Figure 15).[57] Our group has pursued several of these hits for GFP-based fluorescence probes for biologically important hosts. For instance, one derivative shows unique sensitivity for human but not bovine serum albumin, providing a wide sensing range as well as fluorescence enhancements of 100-fold.[58] Several other chromophores are shown to bind and activate a particular class of "lock and key" proteins, the nuclear receptors, such as human estrogen receptor α (ERα).[59] These findings show that such chromophores can provide a new class of probes based upon the internal structure of protein binding sites, resulting in a dramatic and unique fluorescence response. Such studies continue to engage our interest and excitement in this serendipitous field.

Perspective

The development of fluorescent proteins represents an extraordinary advancement in evolutionary biology. Harnessing of the genetic machinery to make proteins with new properties, especially color coding, represents a triumph for evolutionary biology, although limited as it is to a finite set of amino acids. At the heart of this advancement is the chromophore whose photochemistry is becoming defined but still somewhat unknown. The development of synthetic "turn-on" chromophores to the biological toolbox promises to add an additional sensitive probe for biomolecular structure and function. Such studies are now emerging.[60]

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BIOGRAPHICAL INFORMATION

Laren M. Tolbert is a Regents Professor, Fellow of the American Association for the Advancement of Science, and Fellow of the American Chemical Society. After receiving his Ph.D. at the University of Wisconsin, he did postdoctoral work at Harvard University and began his career at the University of Kentucky. He moved to Georgia Tech as a Professor in 1985 and was Chair of the School of Chemistry and Biochemistry from 1993 to 2002. His interests include ground- and excited-state structure of materials and synthesis of molecules to test theories about that structure. His work has been supported by the National Science Foundation, the National Institutes of Health, the Department of Energy, the Petroleum Research Fund, and Intel Corporation. As a true organic chemist, he has three beautiful and talented daughters.

Anthony Baldridge received his B.S. in Chemistry and Mathematics from Piedmont College in 2006 and his Ph.D. under the supervision of Dr. Laren Tolbert at Georgia Tech in 2011, where his thesis work focused on the synthesis, spectroscopy, and application of the green fluorescent protein chromophore.

Janusz Kowalik received both his M.Sc. and Ph.D. in chemistry from the Technical University of Wroclaw, Poland. After receiving his Ph.D. in 1985, he joined Laren Tolbert at the Georgia Institute of Technology, where he is currently a senior research scientist. He is pursuing various aspects of materials chemistry involving electroactive organic polymers and graphene, self-assembly of electroactive molecules, DNA sensors, and "molecular wires". A second interest involves developing synthetic procedures for the synthesis of a wide variety of GFP chromophore analogs.

Kyril M. Solntsev is a senior research scientist at Georgia Tech. He received his Ph.D. at Moscow State University in 1996 working with Michael Kuz'min on excited-state proton transfer in various microheterogeneous systems. After a joint postdoctoral stint in Israel with Noam Agmon and Dan Huppert in the field of photoinduced processes in novel photoacids, Kyril moved to Georgia Tech. He was first a postdoctoral fellow with Laren Tolbert and then a research scientist. He has coauthored more than 50 papers in the areas of photochemistry, spectroscopy, chemical kinetics, fluorescent probes, and function and design of fluorescent proteins.

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