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Letter

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Vibrationally Resolved Photoelectron Spectroscopy of the Model GFP Chromophore Anion Revealing the Photoexcited S_1 State Being Both Vertically and Adiabatically Bound against the Photodetached D_0 Continuum

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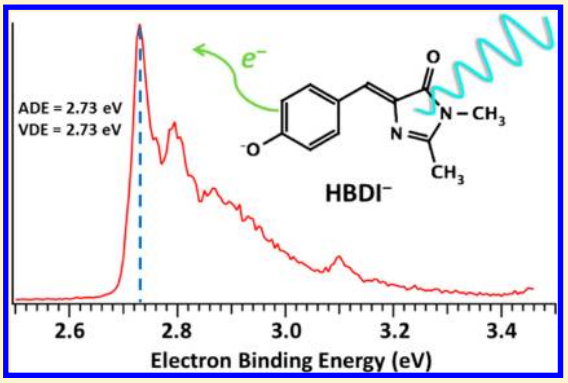
Supporting Information

ABSTRACT: The first excited state of the model green fluorescence protein (GFP) chromophore anion (S_1) and its energy level against the electron-detached neutral radical D_0 state are crucial in determining the photophysics and the photoinduced dynamics of GFP. Extensive experimental and theoretical studies, particularly several very recent gas-phase investigations, concluded that S_1 is a bound state in the Franck−Condon vertical region with respect to D_0. However, what remains unknown and challenging is if S_1 is bound adiabatically, primarily due to lack of accurate experimental measurements as well as due to the close proximity in energy for these two states that even sophisticated high-level ab initio calculations cannot reliably predict. Here, we report a negative ion photoelectron spectroscopy study on the model GFP chromophore anion, the deprotonated *p*-hydroxybenzylidene-2,3-dimethylimidazolinone anion ($HBDI^-$) taken under low-temperature conditions with improved energy resolution. Despite the considerable size and low symmetry of the molecule, resolved vibrational structures were obtained with the 0−0 transition being the most intense peak. The adiabatic (ADE) and vertical detachment (VDE) energies therefore are determined both to be 2.73 ± 0.01 eV, indicating that the detached D_0 state is 0.16 eV higher in energy than the photon excited S_1 state. The accurate ADE and VDE values and the well-resolved photoelectron spectra reported here provide much needed robust benchmarks for future theoretical investigations.



SECTION: Spectroscopy, Photochemistry, and Excited States

The green fluorescent protein (GFP) from jellyfish *Aequorea victoria* has attracted great interest as a biological fluorescence marker as it provides an ideal tool for in vivo observation of the biological processes.[1−3] The wild-type GFP is a single-chain protein with 238 amino acids, which folds into a *β*-barrel structure and places the GFP chromophore, formed by autocatalytic cyclization of the -Ser65-Tyr66-Gly67- tripeptide, in the center.[4,5] It displays two absorption bands peaked at 395 and 480 nm, ascribed to the neutral and deprotonated anionic forms of the chromophore, respectively.[6] Excitation of either form results in green fluorescence at 508 nm, originating from the anionic chromophore as the neutral form deprotonates upon excitation.[6,7]

The most common model compound of the GFP chromophore is *para*-hydroxybenzylidene-2,3-dimethylimidazolinone (HBDI, Figure1a). The remarkably similar absorption spectrum of the HBDI "neutral"[8] and its deprotonated anion ($HBDI^-$)[9] obtained in vacuo compared to the GFP absorption bands demonstrates that the electronic structure of the GFP chromophore can be properly represented by HBDI. The sum of the perturbations in the wild-type GFP environment has very little effect on the absorption wavelength. However, it is known that GFP is rather weakly fluorescent in solutions at room

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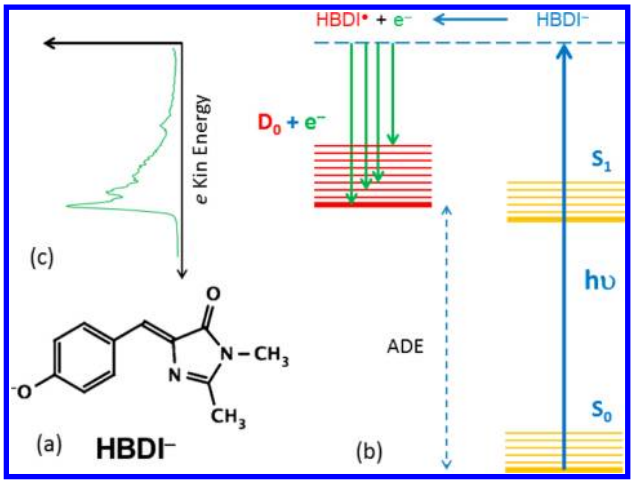

Figure 1. (a) Model GFP chromophore HBDI. The deprotonated (anionic) form is shown. (b) Energy diagram of the electronic ground state, S_0, and the first electronic excited state, S_1, of the anion and the ground state, D_0, of the electron detached neutral. (c) Kinetic energy photoelectron spectrum as a result of direct electron detachment.

temperature (but strong fluorescence restores when cooled to 77 K)[4] and is nonfluorescent in the gas phase.[10] This indicates that although the initial photoexcitation of the anionic GFP chromophore can be properly described using $HBDI^-$ as a model compound, the deactivation pathways following excitation from the S_0 ground state to the S_1 excited state are critically dependent upon the chromophore environments. The outer β-barrel in GFP provides the chromophore a hydrophobic "vacuum-like" binding pocket and keeps the chromophore in a preferred configuration through its hydrogen-bond network,[1] which helps prevent undesired intramolecular rotations and internal conversions that are detrimental to the fluorescence quantum yield.

Motivated by obtaining a molecular level understanding of the GFP fluorescence mechanism and its dependence upon the chromophore local environments, extensive gas-phase studies on the isolated $HBDI^-$ have been performed.[8−10] The gas-phase absorption spectra of both "HBDI"[8] and $HBDI^-$ show strikingly similar bands in both position and intensity with the respective GFP absorption bands ascribed to the neutral and anionic forms of the chromophore, proving that the actual environment of the GFP chromophore is much closer to vacuum. The primary deactivation pathways following excitation from the S_0 ground state to the S_1 excited state are found to be electron detachment (simultaneously also generating the $HBDI^•$ neutral radical), fragmentation, and internal conversion to S_0, but not fluorescence.[10] The dynamics of the S_1 state has been investigated using time-resolved photoelectron spectroscopy,[11] ultrafast fluorescence spectroscopy,[12] and theoretical calculations,[13,14] revealing that the S_1 state evolves from the Franck−Condon (FC) region to the fluorescence state (FS) geometry on an ultrafast time scale, followed by a rotation around the bridging C−C−C bonds between phenol and imidazolinone rings that leads to formation of a twisted intermediate geometry, and then subsequently undergoes internal conversion to the anionic ground state S_0. The fate of the S_1 state is also inherently connected to the electron-detachment continuum, D_0 (Figure 1b). In 2012, three prominent groups[15−17] conducted independently negative ion photoelectron spectroscopy (NIPES) of isolated $HBDI^-$ and reported the vertical detachment energy (VDE), measured from the maximum of the first band of the respective NIPE spectra, to be 2.8 ± 0.1,[15] 2.85 ± 0.1,[16] and 2.68 ± 0.1 eV.[17] Comparing these VDE values to the measured gas-phase $S_1 \leftarrow S_0$ absorption maximum of 482.5 nm (2.57 eV),[10] which is also the origin of the transition, indicates that the S_1 state is bound with respect to the vertical electron detachment in the FC region.

Regarding the adiabatic detachment energy (ADE) of $HBDI^-$, that is, the adiabatic $D_0 \leftarrow S_0$ transition compared to the $S_1 \leftarrow S_0$ transition, a recent action spectroscopy work (published in 2009) indicated that multiple photons were needed to observe the electron detachment channel at 482.5 nm, which implied that the ADE should be larger than 2.57 eV (482.5 nm).[10] However, no ADE value was given in that study, and furthermore, the observed photodetached channel was not from direct measurements but instead was inferred from the difference between the parent ion intensity before laser irradiation and the sum of the parent and fragment ion intensity following irradiation.[10] Therefore, considerable discrepancies still exist regarding ADE values. The only one reported ADE of 2.6 ± 0.2 eV estimated from the threshold of the $HBDI^-$ NIPES by Horke and Verlet in 2012[15] seems consistent with the theoretically calculated values spanning from 2.38 to 2.69 eV[18,19] considering the 0.2 eV experimental error bar. This would render ADE considerably smaller than VDE and put the D_0 state in close proximity if not nearly degenerate with the S_1 state. As a consequence, the S_1 state could also be unbound with respect to the D_0 state. Ironically, the theoretical simulated NIPE spectra based on the Franck−Condon factors (FCFs) calculated from the optimized S_0 and D_0 states indicate that the most intense vibrational transition in $D_0 \leftarrow S_0$ is the 0−0 transition,[15−19] and hence, the ADE and VDE should be the same or nearly the same. If that were true, then the S_1 state would be a truly bound state, not only with respect to the vertical but also to the adiabatic electron detachment processes. Unfortunately, no vibrational structure has been resolved in the NIPE spectra reported so far, leaving accurate determination of ADE, VDE, and the energy level of D_0 relative to S_1 unsettled.

Considering that the electron detached D_0 state plays an important role in the dynamics of the S_1 state, it is desirable to accurately measure its energy level. Here, we report a NIPES study of $HBDI^-$ carried out employing a low-temperature NIPES coupled with an electrospray ionization (ESI) source and a cryogenic ion trap. Well-resolved vibrational structures are observed in the spectra, exhibiting that the most intense peak corresponds to the 0−0 vibrational transition in the $D_0 \leftarrow S_0$ photodetachment process, which confirms the theoretical simulation based on the FCFs' calculations. The well-resolved spectrum also allows accurate ADE and VDE to be determined, both as 2.73 ± 0.01 eV. Compared to the $S_1 \leftarrow S_0$ absorption maximum of 482.5 nm (2.57 eV) reported recently,[10] our study indicates a true bound state for S_1 against both vertical as well as adiabatic electron detachment.

The NIPES experiments were conducted on a home-built apparatus consisting of an ESI source, a low-temperature ion trap, a time-of-flight (TOF) mass spectrometer, and a magnetic-bottle TOF photoelectron spectrometer.[20] HBDI was synthesized according to published methods.[21] Briefly, $HBDI^-$ anions were produced via spraying ~0.1 mM HBDI in a mixture solvent of methanol and water (3:1), titrated with a small amount of NaOH aqueous solution. Abundant $HBDI^-$ anions were readily generated by ESI via deprotonation of the

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most acidic proton on the phenol moiety. The produced anions were transported by a RF-only quadrupole ion guide into a quadrupole mass filter and then guided by a 90° ion bender into a cryogenically controlled 3D Paul trap, where the anions were accumulated and cooled via collisions with ~1 mTorr cold buffer gas (20% H_2 balanced with He) for a period of 20−100 ms with the ion trap temperature set to 20 K (the actual "ion temperature" should be higher than the setting of 20 K). The cooled ions were ejected out of the Paul trap at a 10 Hz repetition rate to the extraction zone of a TOF mass spectrometer for mass analysis. The $HBDI^-$ anions were mass-selected and maximally decelerated before being intercepted by a probe laser beam in the photodetachment zone. In the current study, photon energies of 355 nm (3.496 eV), 266 nm (4.661 eV) both from a Nd:YAG laser, and 193 nm (6.424 eV) from an ArF excimer laser were used. The laser was operated at a 20 Hz repetition rate with the ion beam off at alternating laser shots, thus allowing for shot-by-shot background subtraction. The photoelectrons were collected at nearly 100% efficiency by a magnet bottle and analyzed in a 5.2 m long flight tube. The TOF photoelectron spectra were collected and converted to kinetic energy spectra, as shown in Figure 1c, calibrated with the known spectra of I^-, ClO_2^-, and $Cu(CN)_2^-$. The electron binding energy (EBE) spectra presented in this Letter were obtained by subtracting the kinetic energy spectra from the photon energies. The energy resolution (ΔE/kinetic energy) was 15 meV (full width at half-maximum, fwhm), as shown in the 355 nm spectrum of I^-.

Well-resolved NIPE spectra of $HBDI^-$, obtained with 355 and 266 nm lasers, are shown in Figure 2a and b, respectively. The 355 nm spectrum features a large peak, marked X, at an EBE of 2.73 ± 0.01 eV. It is followed by several partially resolved smaller peaks at EBEs between 2.75 and 3.1 eV. The 266 nm spectrum has features similar to those of the 355 nm spectrum at EBEs between 2.7 and 3.5 eV, but at higher EBEs, the 266 nm spectrum shows a new set of well-resolved peaks, starting with A at EBE = 3.885 eV. Besides the X and A bands, the 193 nm spectrum (Figure 2c) exhibits two more sets of peaks, that is, the partially resolved B series at EBEs between 4.3 and 4.8 eV and a very strong but congested C band centered at EBE = 5.1−5.2 eV. Our 193 nm spectrum is slightly better resolved but qualitatively similar to that reported by Horke and Verlet at 201.5 nm.[15] However, our spectra at both 266 and 355 nm reveal well-resolved vibrational structures for the X and A bands, in striking contrast to all previously reported spectra that were obtained at similar photon energies (Horke and Verlet at 268.1 and 355 nm;[15] Toker et al. at 355 nm;[17] and Mooney et al. at 269 and 330 nm[16]) but displayed two broad X and A bands with no vibrational structures being resolved (see the comparison in Figure S1 for the X band in the Supporting Information (SI)).

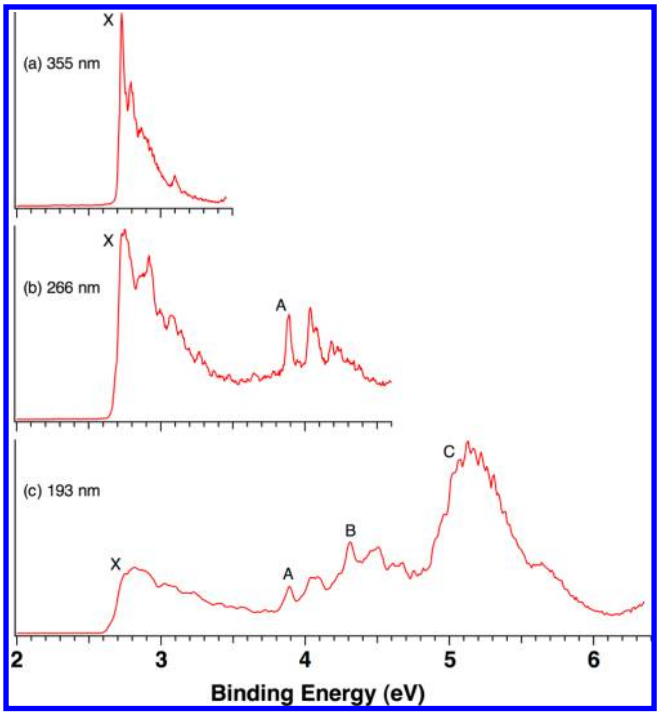

Figure 2. Low-temperature (with the ion trap temperature set for 20 K) photoelectron spectra of $HBDI^-$ at 355 (3.496 eV) (a), 266 (4.661 eV) (b), and 193 nm (6.424 eV) (c).

The NIPE spectral features (X, A−C) in Figure 2 represent transitions from the ground state of the anion $HBDI^-$ (closed-shell) to the ground and excited states of the corresponding neutral radical $HBDI^•$. In Koopmans's approximation, these features can be viewed alternatively as removing electrons from each occupied molecular orbital (MO) in the anion. Thus, the ground state of the neutral has an electronic configuration with a single electron residing in the highest occupied MO (HOMO), and the excited states of the neutral can be obtained from the ground-state electronic configuration by single excitation to promote one electron from the deeper-occupied MOs to the utmost singly occupied MO (HOMO). Horke and Verlet[15] carried out time-dependent density functional calculations, and they assigned the dominant contributions to band A as HOMO−2 → HOMO, band B as HOMO−1 → HOMO, and band C as HOMO−3 and HOMO−4 → HOMO.

We compare our low-temperature 355 nm spectrum with the FCF simulated stick spectrum calculated by Bravaya and Krylov at T = 300 K[19] by setting the simulated peak position and intensity of the 0−0 band to match that in the experimental spectrum in Figure 3. In this figure, we also show a convoluted spectrum of the stick spectrum using 15 meV fwhm of the instrument resolution for each calculated vibrational peak.

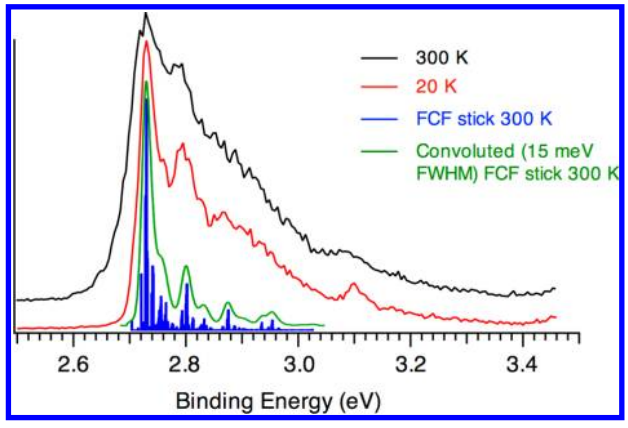

Figure 3. Comparison of the experimental 355 nm photoelectron spectra (20K, red; 300 K, black, which is upshifted for clarity) and the simulated FCF stick spectrum (blue stick) calculated by Bravaya and Krylov at T = 300 K[19] and its convoluted spectrum (green) using a 15 meV fwhm Gaussian profile for each stick. The simulated peak position and intensity of the 0−0 transition have been set to match those in the experimental spectrum.

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Excellent agreement in peak positions is found, allowing us to assign the peaks at EBEs of 2.76, 2.80, and 2.87 eV in the X band as $\upsilon_{48}/\upsilon_{49}$, υ_{43}, and υ_{19} vibrational excitation bands of the detached neutral, respectively. These vibrational modes correspond to the bending vibrations in the HBDI$^{\bullet}$ skeleton, mostly in the imidazolinone and bridge regions. However, the partially resolved peak at EBE = 2.76 eV, which is also reproduced in the simulated spectrum with 15 meV fwhm resolution, is no longer observable in the simulated spectrum using 30 meV fwhm (Figure S2, SI), consistent with the 15 meV instrument resolution.

While our low-temperature spectrum shows all major spectral features that the simulated spectrum (with 15 meV convolution width) predicts, it is less resolved compared to the simulated one. However, our spectrum agrees better with the FCF simulated spectrum by Toker et al.,[17] also calculated at a 300 K ion temperature (see Figure S3 in the SI). The fact that there is good agreement between our low-temperature spectrum and the simulations at 300 K by Toker et al. (Figure S3, SI) indicates that the real ion temperature is significantly higher than the trap setting of 20 K. The observable differences between two sets of simulations (both at 300 K) are likely due to the different theoretical methods and approximations that were used in the calculations.[17,19]

The peak at EBE = 3.1 eV, which is not shown in the simulated spectrum, corresponds either to C−H stretching of ∼3000 cm^{-1} or to resonant autodetachment at 355 nm, resulting in non-Franck-Condon features.[22] It is likely that this peak is due to resonant detachment because the C−H stretching mode is absent in all simulations.

It is worth noting that the most intense peak in both the 355 and 266 nm spectra corresponds to the 0−0 band. Therefore, experimentally, both ADE and VDE are the same, as 2.73 eV. However, at 193 nm, where the instrumental resolution deteriorates (the fwhm of the first I$^-$ peak is 85 meV, compared to 15 meV at 355 nm and 30 meV at 266 nm), the maximum of the X band is 2.82 eV, 0.1 eV higher than the true ADE and VDE of 2.73 eV, but this number is in excellent agreement with the reported VDEs of 2.8 eV by Horke and Verlet[15] and 2.85 eV by Fielding and co-workers.[16] Consequently, we suspect the low instrumental resolution to be the reason that leads to the observed maximum of the band shifting to a higher EBE as a result of convolution of many individual vibrational peaks.[17,19]

The 355 nm spectrum shown in Figure 2a was taken with a 15 meV fwhm instrumental resolution for I$^-$ and also under low-temperature (20 K trap temperature) conditions. In order to untangle the temperature effects from the resolution effects, we conducted 355 nm NIPE measurements by keeping the same resolution but at room temperature. Appreciable thermal broadening and additional hot band transitions at EBEs between 2.6 and 2.7 eV were observed by comparing the room-temperature spectrum with the 20K one (Figure 3), and the threshold of the room-temperature spectrum started at 2.6 eV, although the maximum of the spectrum still corresponds to the 0−0 peak. This fact indicates that the ions at the 20 K setting are indeed much colder than those with the room-temperature setting. The better agreement between the cold spectrum (rather than the room-temperature spectrum) and the simulated ones calculated at 300 K suggests that the actual ion temperature is significantly higher than the setting temperature of 20 K. Therefore, we believe both high instrumental resolution and cold temperature play important roles in determining accurate ADEs and VDEs from the spectra. Our newly determined ADE (of 2.73 eV) also indicates that the low-energy photoemission at a wavelength of 480 nm (2.58 eV, the peak of $S_1 \leftarrow S_0$ absorption) observed previously[10,15,17] was not from direct photodetachment. Instead, it resulted from either absorption of multiple photons[10] or thermionic emission via internal conversion[15] or resonant electron emission from vibrational hot anions[17] following $S_1 \leftarrow S_0$ excitation.

In summary, we report in this Letter a low-temperature photoelectron spectroscopy study of the model GFP chromophore anion, HBDI$^-$. Well-resolved vibrational structures were obtained, with the 0−0 transition being the most intense peak. Thus, ADE and VDE are accurately determined to be 2.73 ± 0.01 eV. This value leads to the conclusion that the bright S_1 state of the anion, which is responsible for the 508 nm green fluorescence, is a true bound state against both the vertical and adiabatic electron detached D_0 state. In addition, vibrationally resolved features are observed for the excited state of HBDI$^{\bullet}$. Our ADE and VDE values provide key benchmarks to test high-level theoretical calculations, and we hope that this study can stimulate more theoretical studies toward understanding excited states of the neutral radical as well.

■ ASSOCIATED CONTENT

Supporting Information

The low-temperature 355 nm spectrum reported in this work compared with four previously reported spectra[10,15−17] (Figure S1); the simulated FCF stick spectrum calculated by Bravaya and Krylov at T = 300 K,[19] and its convoluted spectra with different resolutions, that is, full width at half-maximum (fwhm) of 15, 30, 50, and 100 meV (Figure S2); and comparison of the low- and room-temperature 355 nm spectra reported in this work with the FCF simulated spectrum by Toker et al. at 300 K[17] and the FCF simulated spectrum by Bravaya and Krylov,[19] also calculated at 300 K convoluted with 15 meV fwhm for each of the calculated vibrational peaks (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Tsien, R. Y. The Green Fluorescence Protein. *Annu. Rev. Biochem.* **1998**, *67*, 509−544.

(2) Day, R. N.; Davidson, M. W. The Fluorescent Protein Palette: Tools for Cellular Imaging. *Chem, Soc. Rev.* **2009**, *38*, 2887−2921.

(3) Meech, S. R. Excited State Reactions in Fluorescent Proteins. *Chem, Soc. Rev.* **2009**, *38*, 2922−2934.

(4) Niwa, H.; Inouye, S.; Hirano, T.; Matsuno, T.; Kojima, S.; Kubota, M.; Ohashi, M.; Tsuji, F. I. Chemical Nature of the Light Emitter of the *Aequorea* Green Fluorescent Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13617−13622.
(5) Ormö, M.; Cubitt, A. B.; Kallio, K.; Gross, L. A.; Tsien, R. Y.; Remington, S. J. Crystal Structure of the *Aequorea victoria* Green Fluorescent Protein. *Science* **1996**, *273*, 1392−1395.
(6) Chattoraj, M.; King, B. A.; Bublitz, G. U.; Boxer, S. G. Ultra-fast Excited State Dynamics in Green Fluorescent Protein: Multiple States and Proton Transfer. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8362−8367.
(7) Lossau, H.; Kummer, A.; Heinecke, R.; Pöllinger-Dammer, F.; Kompa, C.; Bieser, G.; Jonsson, T.; Silva, C. M.; Yang, M. M.; Youvan, D. C.; Michel-Beyerle, M. E. Time-Resolved Spectroscopy of Wild-Type and Mutant Fluorescent Proteins Reveals Excited State Deprotonation Consistent with Fluorophore−Protein Interactions. *Chem. Phys.* **1996**, *213*, 1−16.
(8) Rajput, J.; Rahbek, D. B.; Andersen, L. H.; Rocha-Rinza, T.; Christiansen, O.; Bravaya, K. B.; Erokhin, A. V.; Bochenkova, A. V.; Solntsev, K. M.; Dong, J.; et al. Photoabsorption Studies of Neutral Green Fluorescent Protein Model Chromophores In Vacuo. *Phys. Chem. Chem. Phys.* **2009**, *11*, 9996−10002 ; the reported "neutral" absorption spectra were derived from a substituted HBDI chromophore with charges introduced on the substituents, corrected by the charge-induced spectral shift from calculations..
(9) Nielsen, S. B.; Lapierre, A.; Andersen, J. U.; Pedersen, U. V.; Tomita, S.; Andersen, L. H. Absorption Spectrum of the Green Fluorescent Protein Chromophore Anion In Vacuo. *Phys. Rev. Lett.* **2001**, *87*, 228102/1−228102/4.
(10) Forbes, M. W.; Jockusch, R. A. Deactivation Pathways of an Isolated Green Fluorescent Protein Model Chromophore Studied by Electronic Action Spectroscopy. *J. Am. Chem. Soc.* **2009**, *131*, 17038−17039.
(11) Mooney, C. R. S.; Horke, D. A.; Chatterley, A. S.; Simperler, A.; Fielding, H. H.; Verlet, J. R. R. Taking the Green Fluorescence out of the Protein: Dynamics of the Isolated GFP Chromophore Anion. *Chem. Sci.* **2013**, *4*, 921−927.
(12) Mandal, D.; Tahara, T.; Meech, S. R. Excited-State Dynamics in the Green Fluorescent Protein Chromophore. *J. Phys. Chem. B* **2004**, *108*, 1102−1108.
(13) Polyakov, I. V.; Grigorenko, B. L.; Epifanovsky, E. M.; Krylov, A. I.; Nemukhin, A. V. Potential Energy Landscape of the Electronic States of the GFP Chromophore in Different Protonation Forms: Electronic Transition Energies and Conical Intersections. *J. Chem. Theory Comput.* **2010**, *6*, 2377−2387.
(14) Bochenkova, A. V.; Andersen, L. H. Ultrafast Dual Photoresponse of Isolated Biological Chromophores: Link to the Photo-induced Mode-Specific Non-Adiabatic Dynamics in Proteins. *Faraday Discuss.* **2013**, *163*, 297−319.
(15) Horke, D. A.; Verlet, J. R. R. Photoelectron Spectroscopy of the Model GFP Chromophore Anion. *Phys. Chem. Chem. Phys.* **2012**, *14*, 8511−8515.
(16) Mooney, C. R. S.; Eugenia Sanz, M.; McKay, A. R.; Fitzmaurice, R. J.; Aliev, A. E.; Caddick, S.; Fielding, H. H. Photodetachment Spectra of Deprotonated Fluorescent Protein Chromophore Anions. *J. Phys. Chem. A* **2012**, *116*, 7943−7949.
(17) Toker, Y.; Rahbek, D. B.; Klærke, B.; Bochenkova, A. V.; Andersen, L. H. Direct and Indirect Electron Emission from the Green Fluorescent Protein Chromophore. *Phys. Rev. Lett.* **2012**, *109*, 128101/1−128101/4.
(18) Epifanovsky, E.; Polyakov, I.; Grigorenko, B.; Nemukhin, A.; Krylov, A. I. Quantum Chemical Benchmark Studies of the Electronic Properties of the Green Fluorescent Protein Chromophore. 1. Electronically Excited and Ionized States of the Anionic Chromophore in the Gas Phase. *J. Chem. Theory Comput.* **2009**, *5*, 1895−1906.
(19) Bravaya, K. B.; Krylov, A. I. On the Photodetachment from the Green Fluorescent Protein Chromophore. *J. Phys. Chem. A* **2013**, *117*, 11815−11822.
(20) Wang, X. B.; Wang, L. S. Development of a Low-Temperature Photoelectron Spectroscopy Instrument Using an Electrospray Ion Source and a Cryogenically Controlled Ion Trap. *Rev. Sci. Instrum.* **2008**, *79*, 073108−1−8.
(21) Voliani, V.; Bizzarri, R.; Nifosì, R.; Abbruzzetti, S.; Grandi, E.; Viappiani, C.; Beltram, F. *Cis*−*Trans* Photoisomerization of Fluorescent-Protein Chromophores. *J. Phys. Chem. B* **2008**, *112*, 10714−10722.
(22) Schiedt, J.; Weinkauf, R. Resonant Photodetachment via Shape and Feshbach Resonances: *p*-Benzoquinone Anions as a Model System. *J. Chem. Phys.* **1999**, *110*, 304−314.