Energy differences behind green fluorescent protein's glow in jellyfish skirts and biological studies

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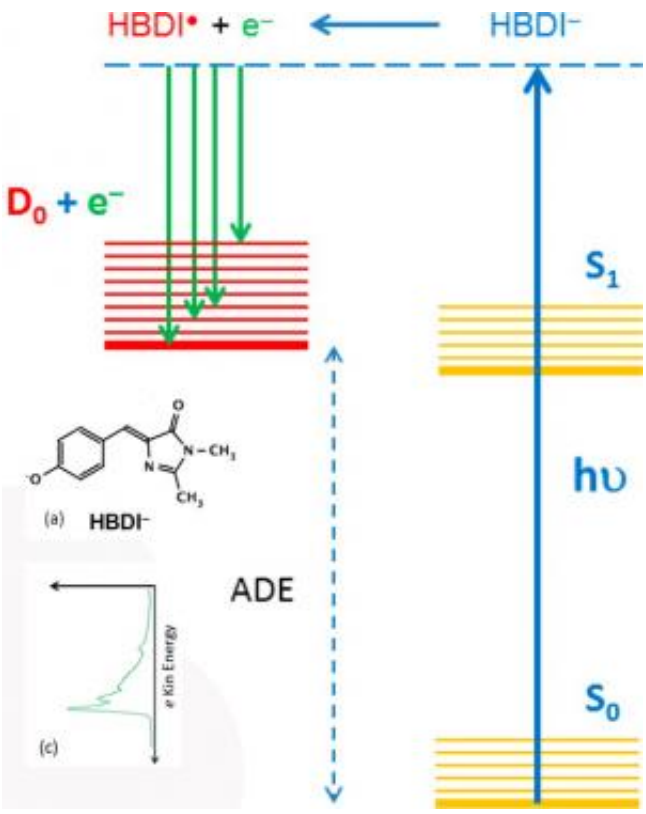


The research beings with (a) the model GFP chromophore HBDI-. The deprotonated form is shown. The researchers determined the (b) energy diagram of the electronic ground state, S_0, and the first electronic excited state, S_1, of the anion and the ground state, D_0, of the electron detached neutral. To achieve this result, they produced well-resolved (c) kinetic energy photoelectron spectrum showing direct electron detachment in HBDI-. Copyright 2014: American Chemical Society

Credited with revolutionizing scientific studies, green fluorescent proteins or GFPs let scientists track molecules in complex reactions inside cells. Found in jellyfish and other marine animals, GFP glows green when hit with light. For all the interest in GFP, the exact changes in its energy and structure as it winks on and off were not clearly understood, so scientists at Pacific Northwest National Laboratory (PNNL) and their collaborators conducted an exacting series of measurements. For the first time, they determined—unequivocally—that the energy level of the excited state of the molecule or chromophore anion solely responsible for the fluorescence after light is absorbed lies below the energy level of the neutral form. Their results unravel key molecular-level energetic conditions that explain the extremely high fluorescence efficiency of GFPs.

"Our study also provides much needed benchmarks for this important molecule," said Dr. Xue-Bin Wang, a chemical physicist at PNNL who worked on the study.

Understanding the photophysics and the photo-induced changes in the chromophore anion is critical to scientists using and altering GFP for its encores in biological and energy sciences, including the production of bio-based fuels. The large size and low symmetry of the chromophore anion have made such understanding difficult. In this study, the scientists overcame complications caused by the size and symmetry issues to determine the anion's structure and energy levels before and after it absorbed light.

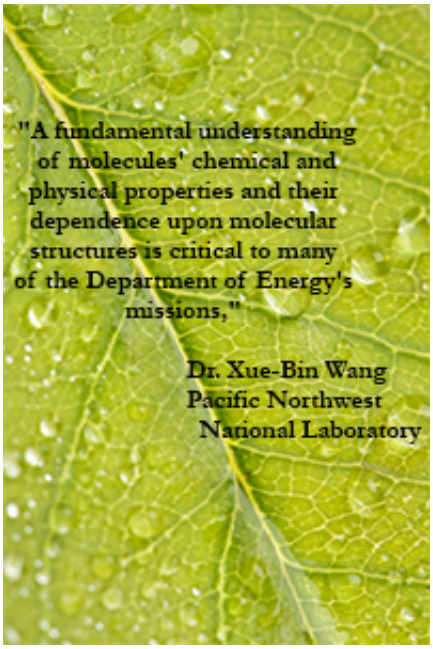
"A fundamental understanding of molecules' chemical and physical properties and their dependence upon molecular structures is critical to many of the Department of Energy's missions," said Wang.

The scientists began with a model chromophore anion. It absorbs packets of light or photons, and its energy goes from a ground state to an excited

or S_1 state. The molecule then either fluoresces and immediately falls back to its ground energy state, or emits electrons to form the electron-detached neutral radical D_0 state. The relative energy-level between S_1 and D_0 is crucial in determining the fluorescence yield. Scientists wanted to know the precise energy difference between the S_1 and D_0 states.

Previous studies, both experimental and theoretical, could not accurately identify the energy levels of these two states.

Scientists at PNNL and their collaborators in China used negative ion photoelectron spectroscopy to study HBDI-, which serves as a model for the actual GFP chromophore. The full name of HBDI- is the deprotonated p-hydroxybenzylidene-2,3-dimethylimidazolinone anion.



The team coupled the spectroscopy with a cryogenic trap for running the

experiments at low temperatures. The temperature is a critical issue as HBDI- is initially generated thermally hot. The heat causes the molecules to contain a broad distribution of energy content and prevents the energy levels from being accurately pinned down.

The team gathered spectra that are much better resolved than any of those obtained in previous studies. The spectra clearly show that the dominant spectral peak is the transition from the vibrational ground state of the anion to the vibrational ground state of the corresponding neutral. The absolute energy level of the neutral D_0 state lies at 2.73 ± 0.01 eV above the anion's level, and 0.16 eV higher in energy than the bright S_1 state of the anion. In other words, the bright state of the anion, S_1, which is responsible for the green fluorescence, is a true bound state and, generally, can only release its energy via emitting green lights instead of emission of electrons.

"Our findings provide a molecular basis towards understanding the amazing trait of GFP's fluorescence in many creatures and have great implications for those people trying to model and understand the mechanics of GFP and the model compounds used to understand it," said Wang.

Wang and his colleagues at PNNL are continuing to answer basic questions about the energetics of GFP and other molecules of importance and to explore potential local binding environments' effects.

More information: Deng SHM, XY Kong, GX Zhang, Y Yang, WJ Zheng, ZR Sun, DQ Zhang, and XB Wang. 2014. "Vibrationally Resolved Photoelectron Spectroscopy of the Model GFP Chromophore Anion Revealing the Photoexcited S1 State Being Both Vertically and Adiabatically Bound against the Photodetached D0 Continuum." *Journal of Physical Chemistry Letters* 5:2155-2159. DOI: 10.1021/jz500869b

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