

A quantum chemical benchmark study of the green fluorescent protein chromophore

Evgeny Epifanovsky, Anna I. Krylov

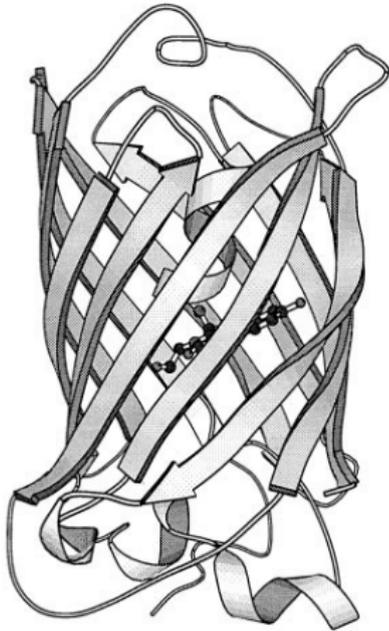
(University of Southern California, Los Angeles, CA)

Igor Polyakov, Bella Grigorenko,
Alexander Nemukhin

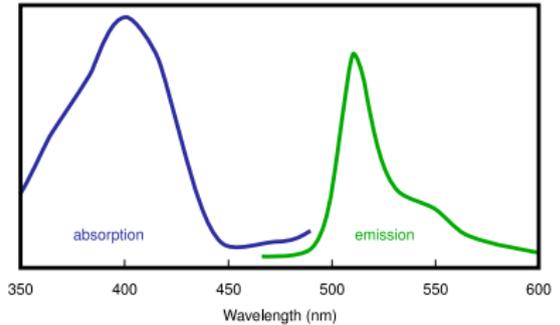
(Department of Chemistry, Moscow State University, Russia)

64th OSU Symposium on Molecular Spectroscopy
Columbus, OH
June 26, 2009

Green fluorescent protein (GFP)



The chromophore is synthesized within the protein that has a cylindrical shape.



Combined absorption/emission spectrum of wild-type GFP.



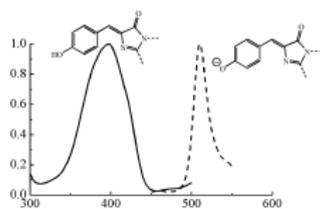
The relatively short DNA sequence of GFP enables its use as a genetic marker.

¹R. Heim *et al.*, PNAS 91 (1994), 12501.

²R.Y. Tsien, *Annu. Rev. Biochem.* 67 (1998), 509.

GFP chromophore

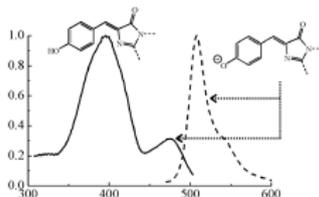
There are cationic, neutral, and anionic forms of the GFP chromophore. They exist in solution at different pH, which makes the absorption spectra pH-dependent.



pH=4

Absorption:
395 nm.

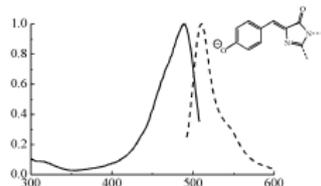
Fluorescence:
508 nm.



pH=7

Absorption:
395 nm, 475 nm.

Fluorescence:
508 nm.

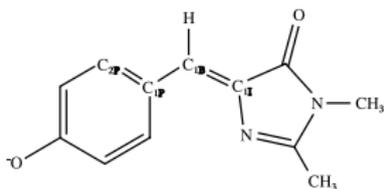


pH=9

Absorption:
475 nm.

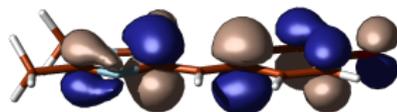
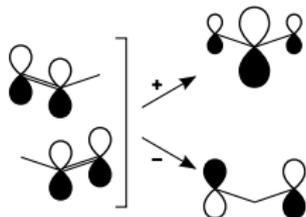
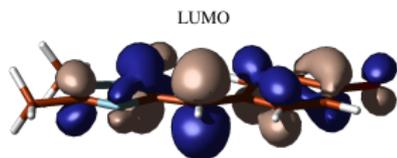
Fluorescence:
508 nm.

The anionic form exists inside the protein and is responsible for its green fluorescence.



Molecular orbitals of HBDI

4'-hydroxybenzylidene-2,3-dimethylimidazolinone (HBDI) anion



HOMO

Delocalization

Charge on oxygen atoms:

-0.65 (ph) v. -0.66 (im).

C-C bond length:

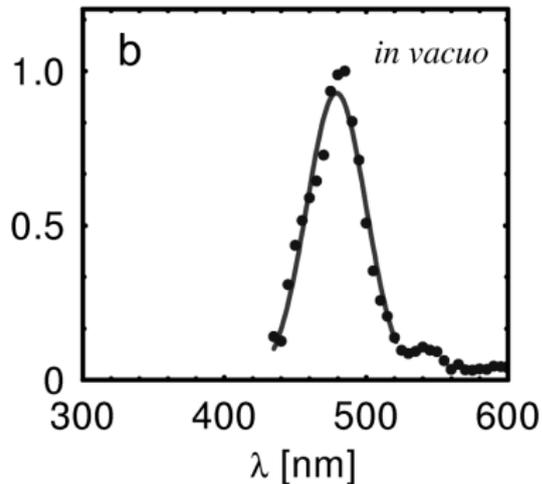
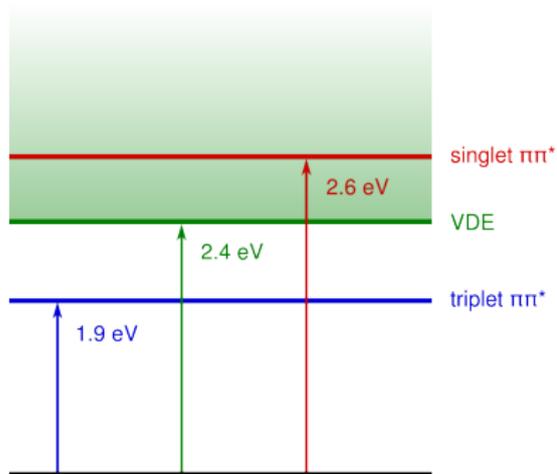
1.384 Å (ph) v. 1.378 Å (im).

Compare to 1.333 Å for C=C and 1.470 Å for C-C (sp^2 hybridized).

1.8 electrons assigned to the allylic bridge (NBO).

Electronic states of HBDI

- ▶ Vertical electron detachment continuum starts at 2.4 eV, below the bright $\pi\pi^*$ state at 2.6 eV.
- ▶ The $\pi\pi^*$ state is a resonance state coupled to the continuum.
- ▶ Small feature at 540 nm (2.3 eV) is due to electron detachment.
- ▶ Large feature at 479 nm (2.6 eV) is due to the $\pi\pi^*$ transition.

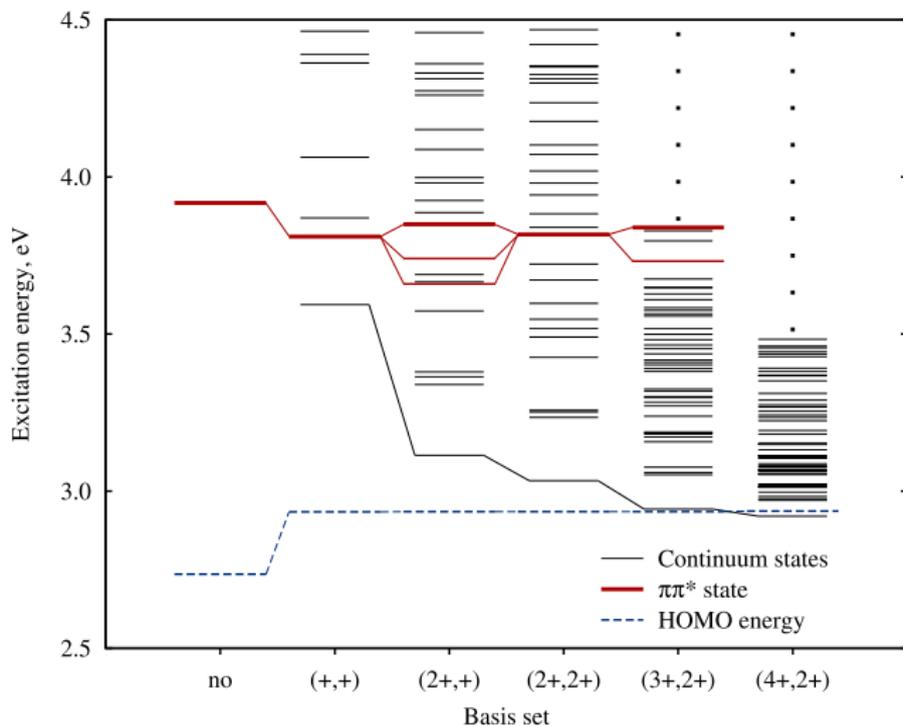


¹S.B. Nielsen *et al.*, Phys. Rev. Lett. 87 (2001), 228102.

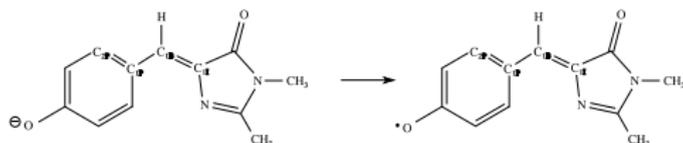
²L.H. Andersen *et al.*, PCCP 6 (2004), 2617.

Stabilization of the $\pi\pi^*$ excited state

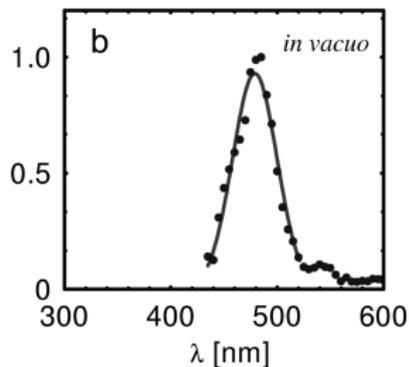
The $\pi\pi^*$ state lies higher than the onset of an electronic detachment continuum.
It is therefore not a bound state, but a resonance embedded in the continuum.



Vertical detachment energy

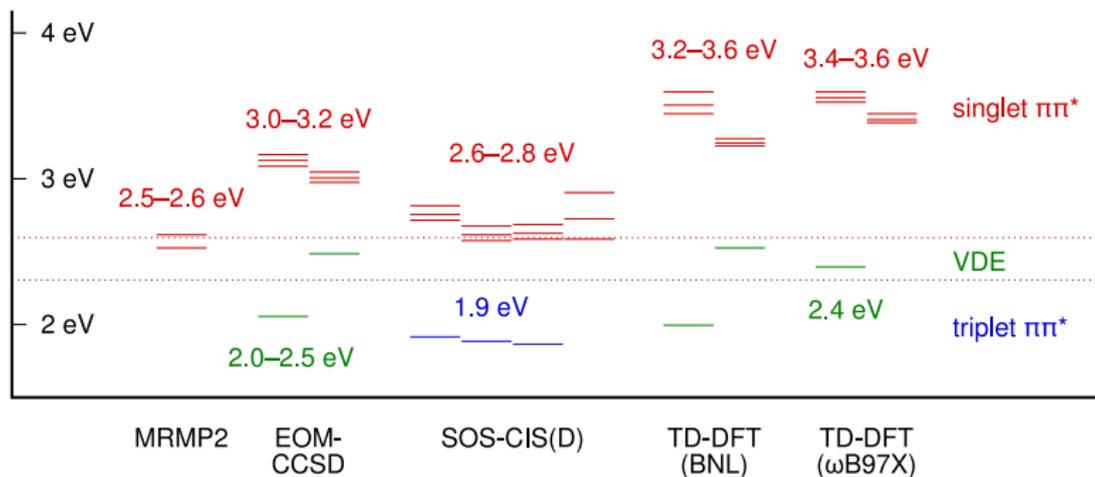


Method	Energy, eV
Koopmans' theorem, 6-311(2+,+)G(2df,2pd)	2.93
B3LYP/cc-pVDZ	2.46
ω B97X/cc-pVDZ	2.39
BNL/6-311G(3+,2+)G(2pd,2df)	2.53
EOM-IP-CCSD/6-31+G*	2.48
EOM-IP-CCSD/6-311(2+,+)G(2df,2pd) (<i>projected</i>)	2.4–2.5



$$\begin{aligned}
 & \text{EOM IE (large basis)} \\
 & = \\
 & \text{EOM IE (small basis)} \\
 & + \\
 & \text{Koopmans' IE (large basis)} \\
 & - \\
 & \text{Koopmans' IE (small basis)}
 \end{aligned}$$

Electronic states of HBDI



- ▶ Effect of geometry: 0.1 eV.
- ▶ Effect of basis set: 0.1–0.2 eV.
- ▶ Highly correlated MRMP2 method is accurate, but too complicated.
- ▶ Equation-of-motion method requires the inclusion of triples and basis set corrections.
- ▶ SOS-CIS(D) has one parameter and performs nicely.
- ▶ TD-DFT methods systematically overestimate the transition energy.

Conclusions

- ▶ The bright excited $\pi\pi^*$ state is a resonance state embedded in a continuum of electron-detached states.
- ▶ Wave function-based and DFT methods agree on the character of the states.
- ▶ SOS-CIS(D) proves to be an inexpensive and uncomplicated method to compute electronic transition energies for $\pi\pi^*$ excitations.

More on this topic:

- ▶ Cis-trans isomerization of the chromophore in the gas phase and solution.
- ▶ Oxidative redding of GFP.

Acknowledgments



- ▶ Prof. Anna Krylov
- ▶ Dr. Ksenia Bravaya

From Moscow State U:

- ▶ Prof. Alexander Nemukhin
- ▶ Dr. Bella Grigorenko
- ▶ Igor Polyakov

iOpenShell (<http://iopenshell.usc.edu/>), Q-Chem. Funding: CRDF, NSF.

E. Epifanovsky *et al.*, J. Chem. Theory Comput. (in press).

I. Polyakov *et al.*, J. Chem. Theory Comput. (in press).