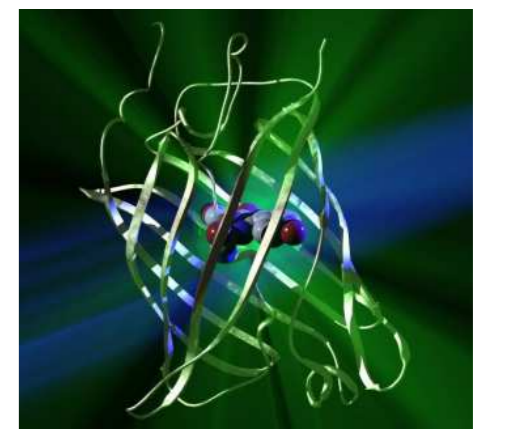
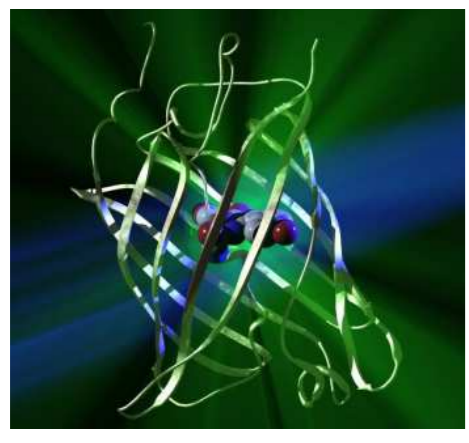
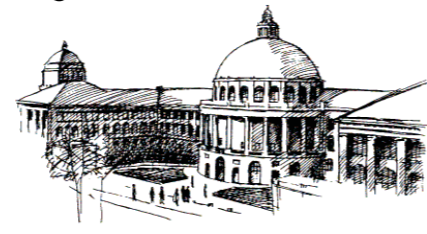


COMPETITION BETWEEN PROTON AND H-ATOM TRANSFER IN GREEN FLUORESCENT PROTEINS



Carine Manca

Laboratorium für Physikalische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland



ETH Eidgenössische Technische Hochschule Zürich

Ecole polytechnique fédérale de Zurich Politecnico federale di Zurigo Swiss Federal Institute of Technology Zurich

Introduction

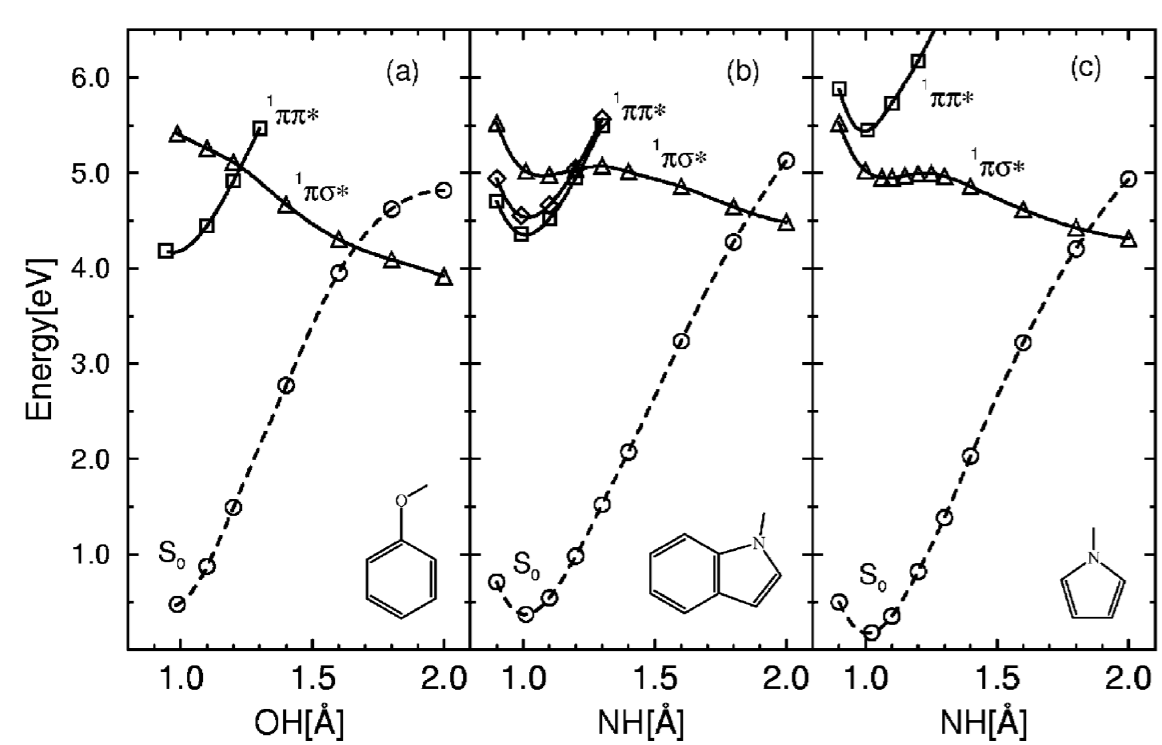
The **Green Fluorescent Proteins (GFP)** have been found in numerous bioluminescent organisms, like the jellyfish *Aequorea victoria* and the sea pansy *Renilla reniformis* [1, 2]. They can be used as fluorescent marker in cell biology and therefore, many studies are devoted to understand their **complex photochemistry**. The **chromophore** responsible for the green fluorescence (8p-hydroxybenzylidene-imidazolinone) is involved in a **hydrogen bonded wire** composed of a water molecule hydrogen-bonded to a serine residue (**Ser205**) that finally connects to a glutamate residue (**Glu222**).

The GFP exhibits two absorption maxima at ~395 and 475 nm which correspond to the neutral and anionic forms of the chromophore, respectively [3]. The second absorption is the experimental proof of the tautomerization of the chromophore via **proton transfer** along the wire. Nevertheless, recently, analog to the **7-hydroxyquinoline-(NH₃)₃** system [5, 6], Vendrell *et. al.* [7] have shown that **H-atom transfer** occurs between the GFP chromophore and the water molecule in the *C_s* symmetry arising from a $\pi\pi^*-\pi\sigma^*$ state crossing (CASSCF calculations).

Questions arising

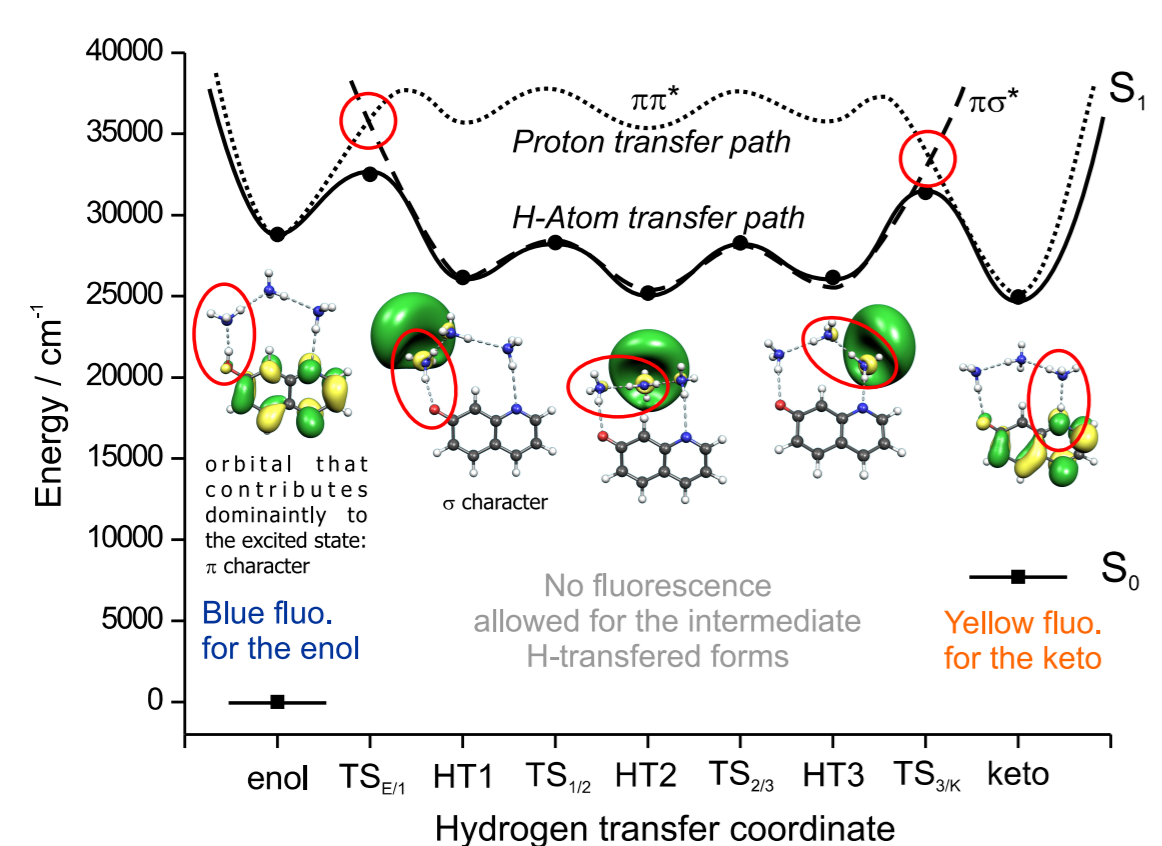
- Does the GFP chromophore exhibit the same behavior as 7-hydroxyquinoline (i. e. $\pi\pi^*-\pi\sigma^*$ state crossing) when involved in a H-bonded wire (see below) ?
- Could a $\pi\pi^*-\pi\sigma^*$ state crossing be predicted with a computational 'low cost' method and an appropriate basis set ?
- Does the environment of the chromophore play a role in the stabilization of the $\pi\sigma^*$ state ?

The $\pi\pi^*-\pi\sigma^*$ state crossing



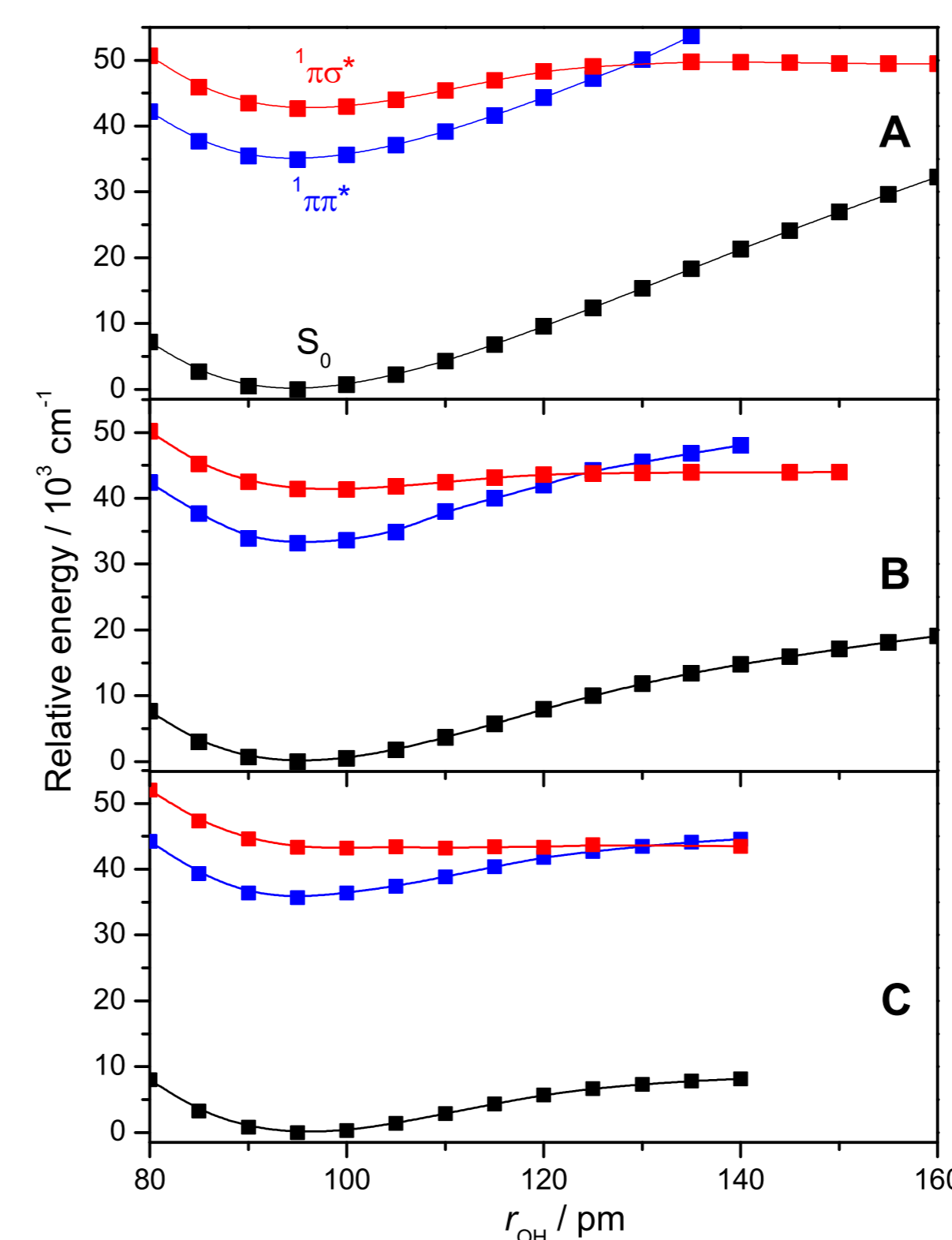
Potential energy profiles of S_0 , $\pi\pi^*$ and $\pi\sigma^*$ states of (a) phenol, (b) indole, and (c) pyrrole.

The **optically dark $\pi\sigma^*$ state** [4]: the $\pi\sigma^*$ state crosses the $\pi\pi^*$ excited state along the O-H / N-H stretching coordinate \Rightarrow new type of photochemical reaction pathway.

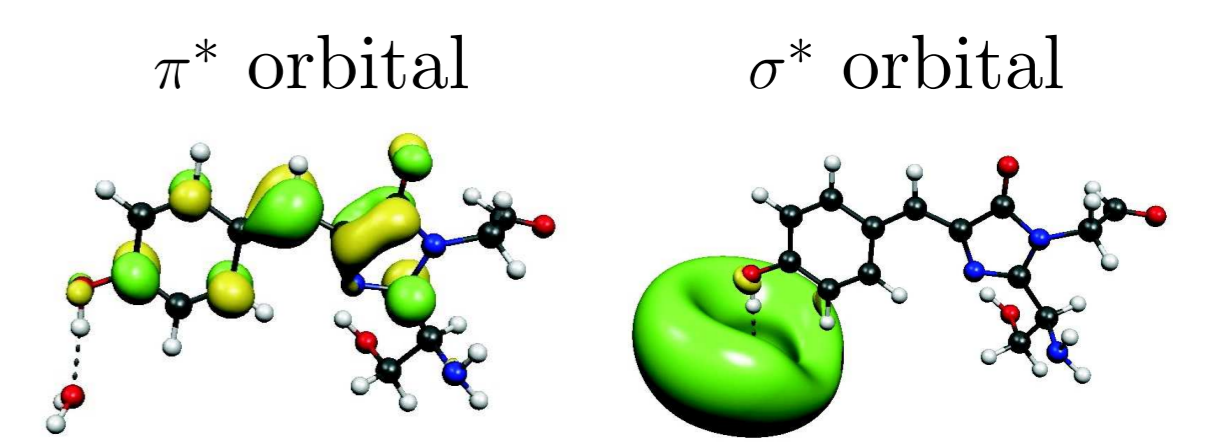


Multistep H Atom transfer in 7-Hydroxyquinoline(NH₃)₃ [5, 6]: The hydrogen bonded wire connecting back to the 7-hydroxyquinoline prevents the cluster dissociation or relaxation to the ground state and leads to successive H atom transfer \Rightarrow no excited proton transfer.

Newton paths

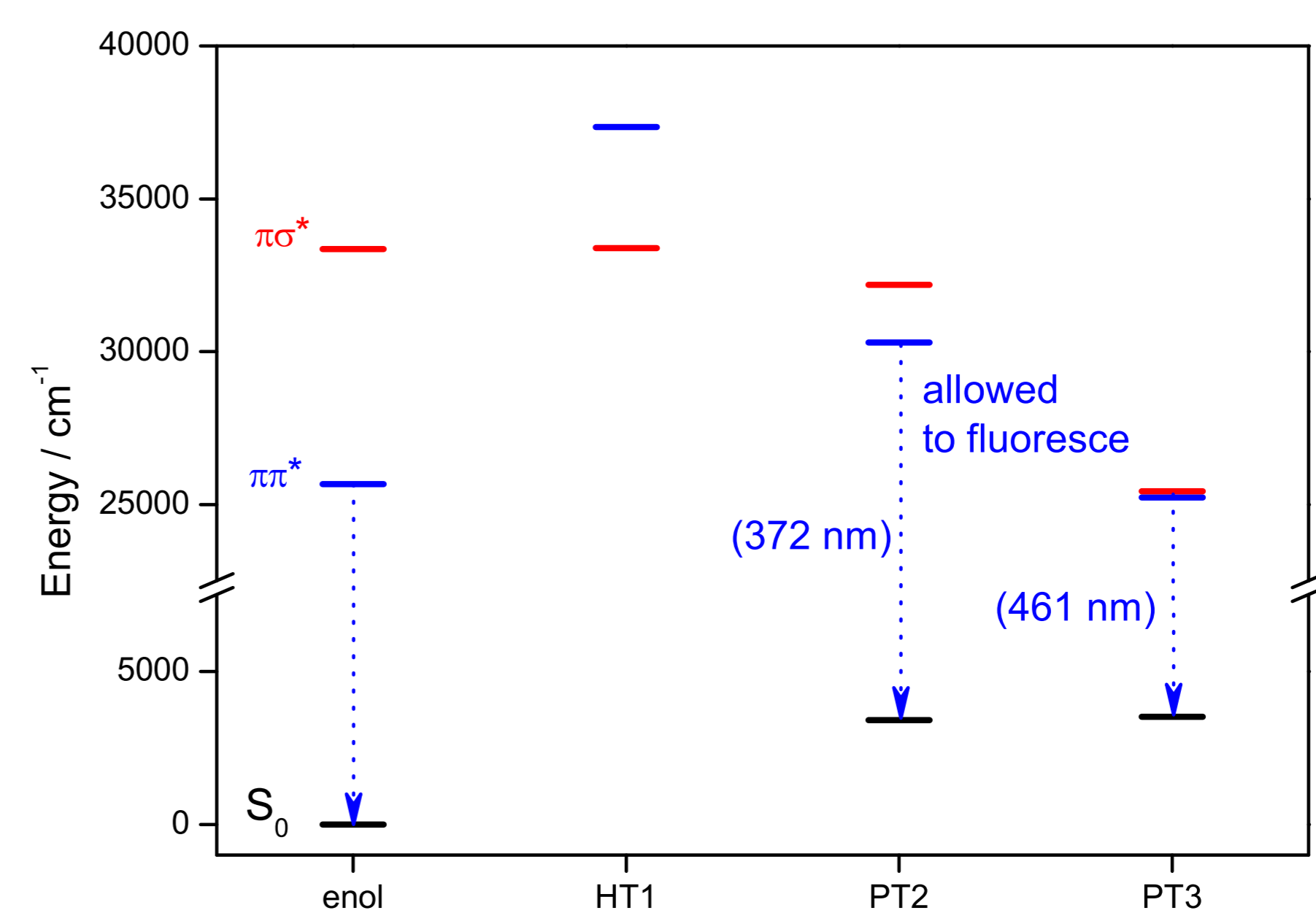


- The $\pi\pi^*-\pi\sigma^*$ crossing is an intrinsic property of the chromophore itself \Rightarrow dissociation when stretching the O-H bond.



- This model chemistry is capable to qualitatively reproduce the CASSCF results from ref. [7].
- Increasing the hydrogen bonded wire length does not prevent the $\pi\pi^*-\pi\sigma^*$ crossing (no proton transfer), but the cluster dissociation (analog to 7HQ-(NH₃)₃).

Schematic path for the System C



all the stationary points are offset so that the enol $\pi\pi^*$ excitation matches the experimental value (≈ 400 nm).

- The HT1 form in its lowest singlet excited state ($\pi\sigma^*$) is not allowed to fluoresce
- Back-crossing during the second step of the reaction (transfer from H₃O to Ser205): the reaction is now a proton transfer and the PT2 in its lowest singlet excited state ($\pi\pi^*$) is allowed to fluoresce

Calculations

- **CIS/6-31G(d,p)** with diffuse functions on H atoms involved in H bonds
- **Newton paths:** The distance between the transferring H⁺/H and the donor O is constrained at different values; the remaining coordinates are optimized.
- **Simplification of the system:** Ser205 modelled by methanol, Glu222 modelled by acetate

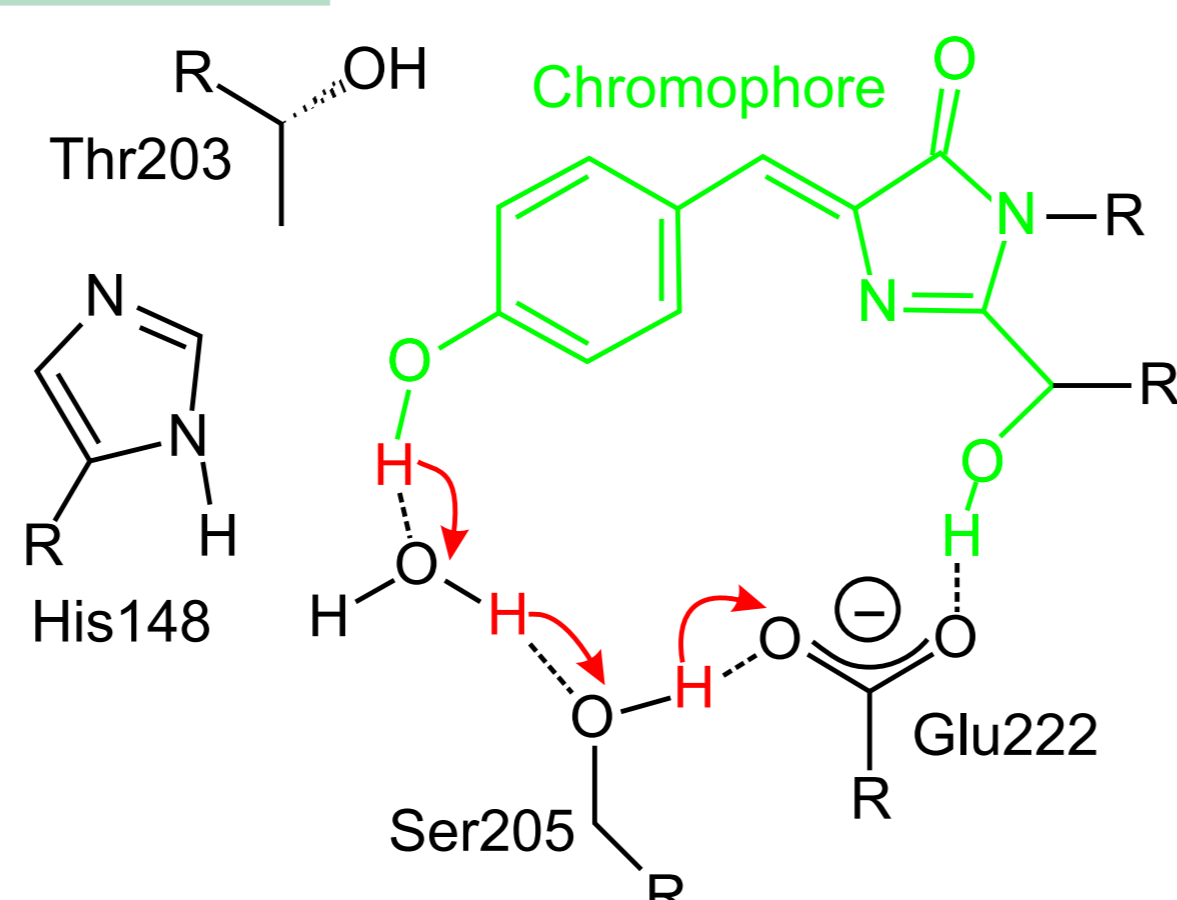
Systems studied

System A: Chromophore

System B: Chromophore + water molecule

System C: Chromophore + Hydrogen bonded wire

System D: Chromophore + Hydrogen bonded wire + His148

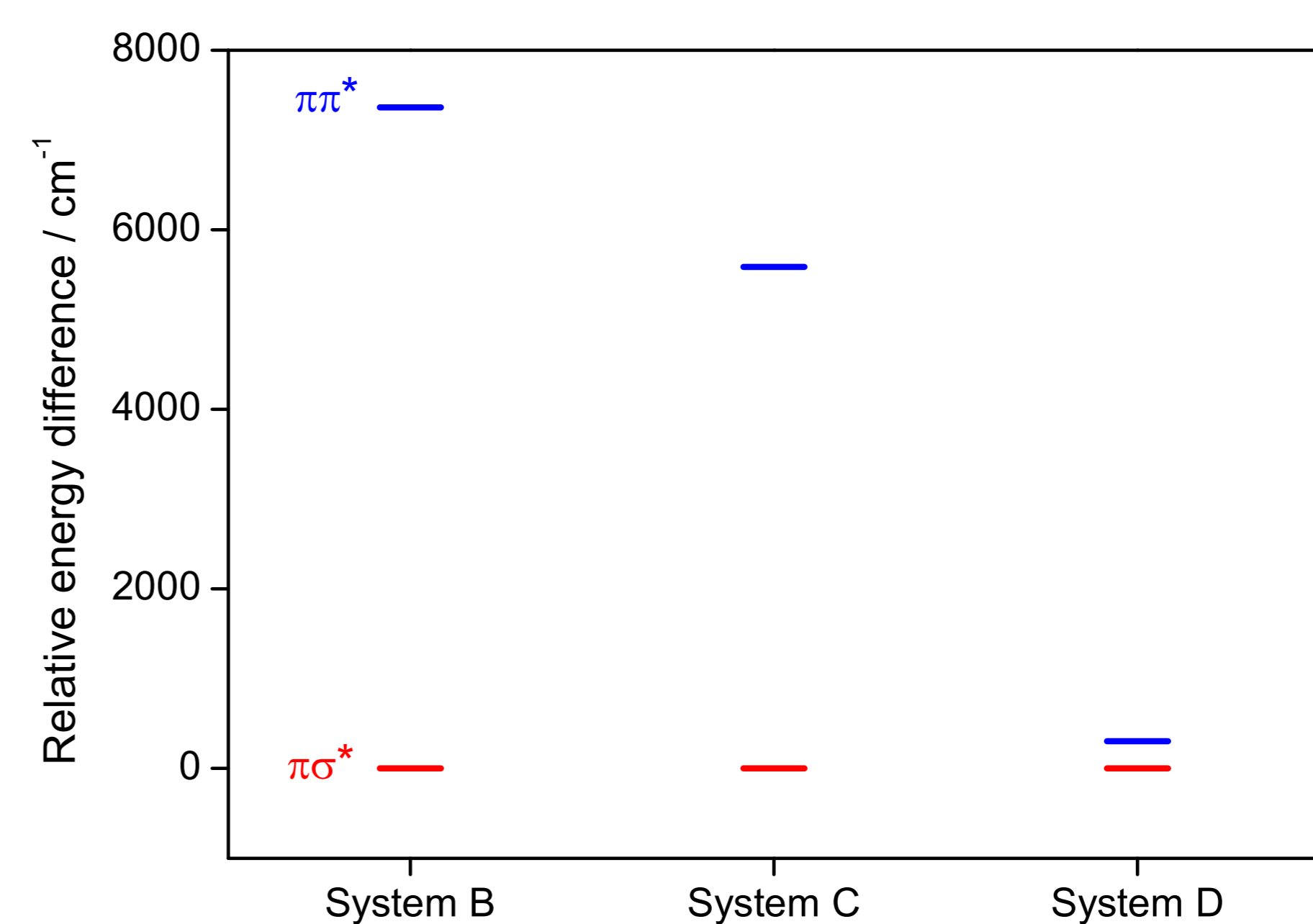


References

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Effect of the environment

The optimized $\pi\pi^*$ and the $\pi\sigma^*$ states of the HT1 intermediate for several systems.



- Increasing the H bond wire (System B \rightarrow System C) has low effect on the energy in its lowest singlet excited state $\pi\sigma^*-\pi\sigma^*$ energy difference
- Further solvation on the chromophore itself (System C \rightarrow System D) stabilizes the $\pi\pi^*$ state and can prevent the $\pi\sigma^*-\pi\pi^*$ state crossing \Rightarrow competition between H atom- and Proton-transfer reactions.

Conclusion

- CIS calculations with an appropriate basis set can qualitatively reproduce the $\pi\pi^*-\pi\sigma^*$ state crossing; their "low cost" allows the comparison with larger systems and the study of solvation effect on the chromophore
- System A: $\pi\sigma^*$ state crosses the S_0 state for larger O-H distances
- System C: back crossing $\pi\sigma^*-\pi\pi^* \Rightarrow$ PT2 is allowed to fluoresce.
- solvation effect: competition between H-Atom- and Proton transfer only when the chromophore itself is more solvated