

Nikita Rozanov^{a, b, 1}, Miles Taylor^a, Liangdong Zhu^{a, b}, Kenneth Stout^a, and Chong Fang^{a, b, 2}

^aOregon State University, Department of Chemistry, 263 Linus Pauling Science Center (lab), 153 Gilbert Hall (Office), Corvallis, OR 97331, USA; and ^bDepartment of Physics, 301 Weniger Hall, Corvallis, OR 97331, USA. ¹E-mail: rozanov@oregonstate.edu; ²E-mail: Chong.Fang@oregonstate.edu.

Experimental

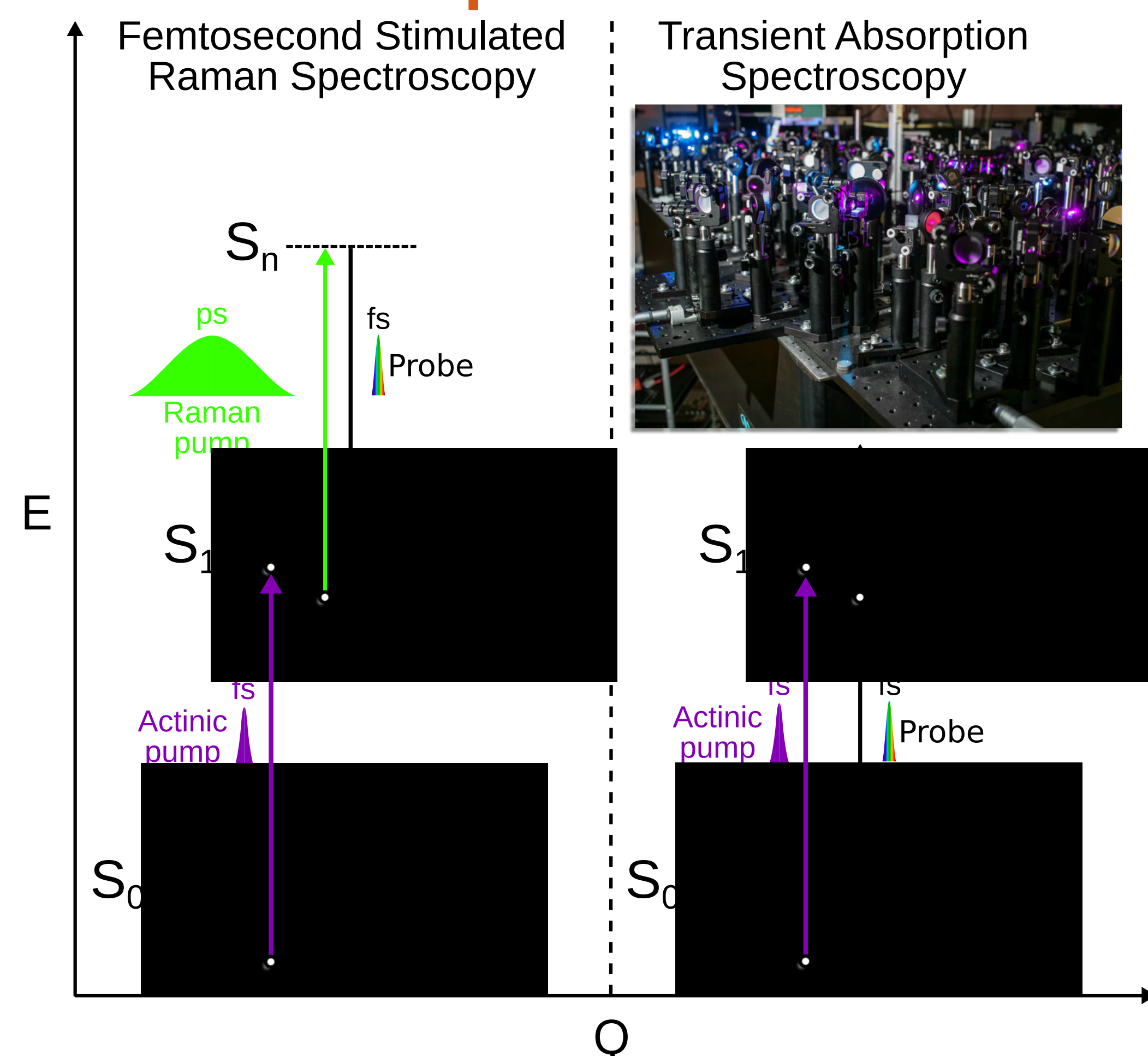


Figure 1. Schematic for the laser pulse sequence in femtosecond stimulated Raman spectroscopy (FSRS) and transient absorption (TA) spectroscopy. In both methods, a femtosecond (fs) actinic pump initiates photochemistry, while an fs white light probe collects the molecule's response. In FSRS, the addition of a picosecond (ps) Raman pump allows for probing the excited state stimulated Raman spectrum. FSRS relies on a varying pulse durations to provide excellent frequency and temporal resolution.

Anharmonic Coupling

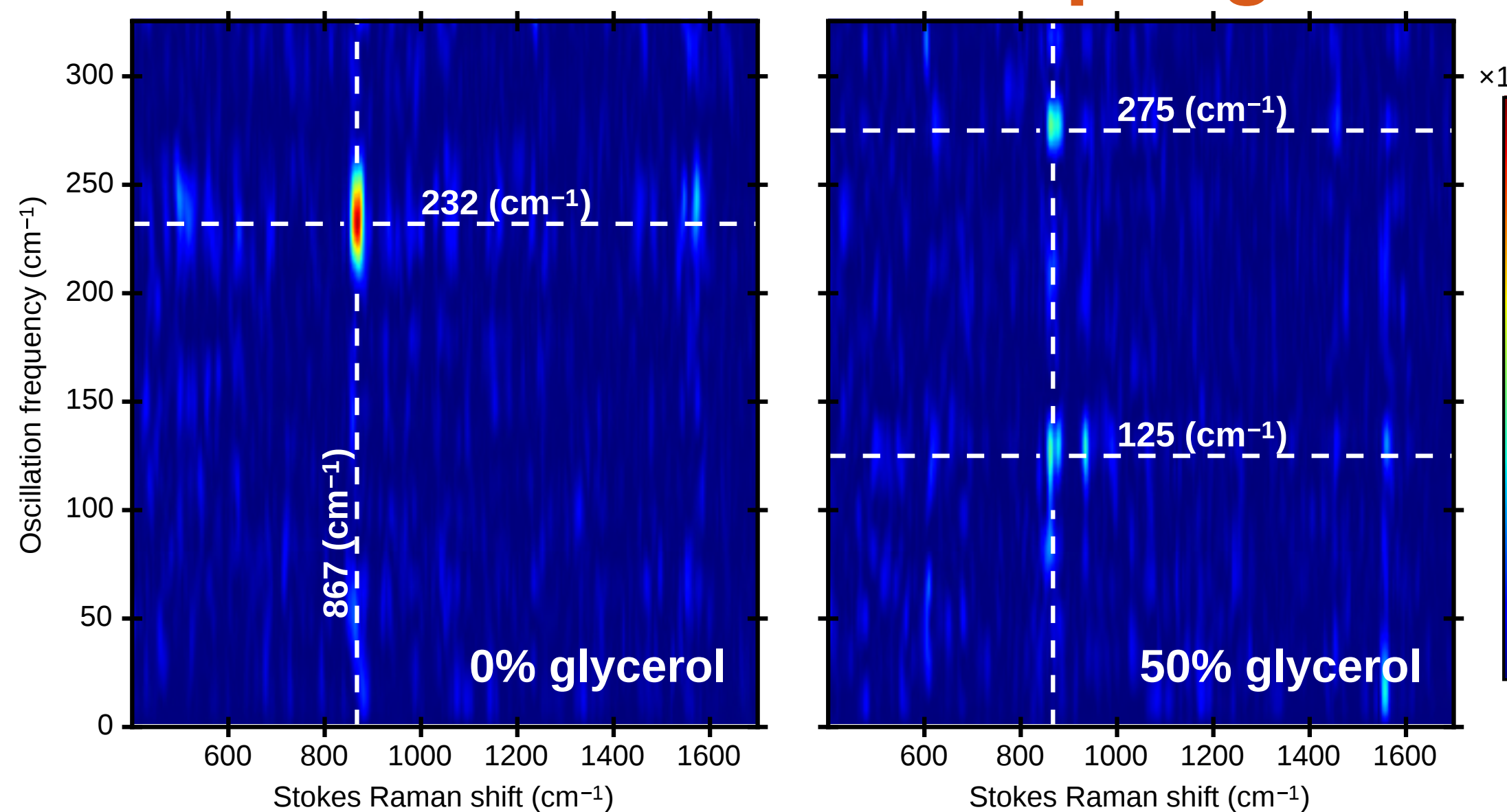


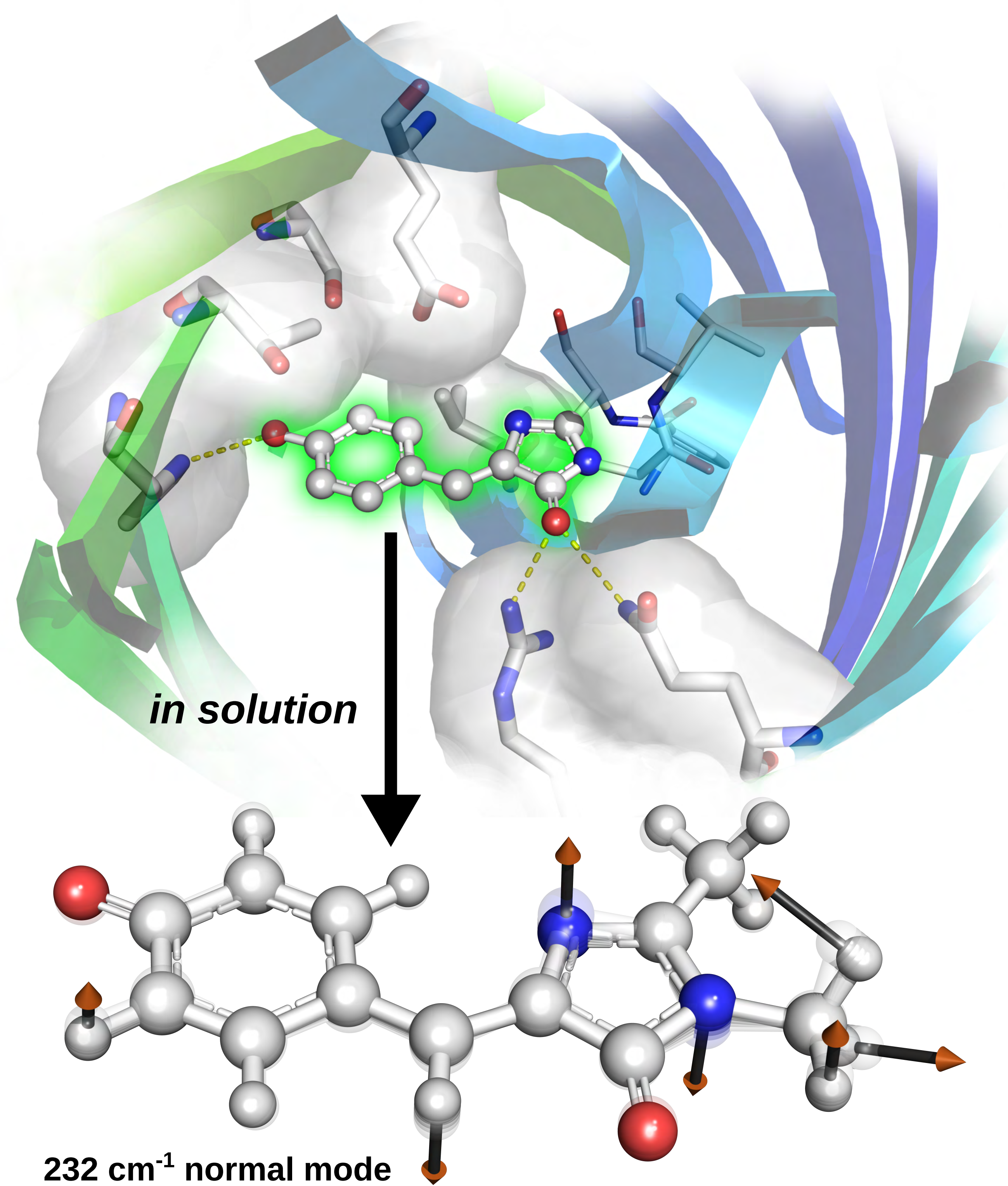
Figure 4. Frequency analysis of the FSRS data reveals the presence of anharmonic coupling between normal modes in HBDI. In 0% glycerol, a 232 cm⁻¹ out-of-plane bending mode modulates a 867 cm⁻¹ in-plane phenol hydrogen out-of-plane (HOOP) mode. Remarkably, this modulation disappears upon addition of glycerol, giving way to a 125 cm⁻¹ phenolic out-of-plane bending and a 275 cm⁻¹ in-plane ring deformation. It is likely that, owing to its strong out-of-plane character, the activation energy for the 232 cm⁻¹ mode becomes prohibitively large in glycerol, whereas this is not true for the two other modes. Changes in the anharmonic coupling matrix reveal how increasing steric hindrance significantly inhibits the efficacy of nonradiative energy dissipation in HBDI.

References

- Oscar, Breiland G., et al. "Ultrafast intermolecular proton transfer to a proton scavenger in an organic solvent." *Physical Chemistry Chemical Physics* 18.37 (2016): 26151-26160.
- Vengris, Mikas, et al. "Ultrafast excited and ground-state dynamics of the green fluorescent protein chromophore in solution." *The Journal of Physical Chemistry A* 108.21 (2004): 4587-4598.

Introduction

Fluorescent proteins as biomarkers have provided decades of valuable research insights for the scientific and engineering community. Recently, a bonanza of new fluorescent proteins has emerged to fuel the growing demand for bioimaging. Many of these new proteins are green fluorescent protein (GFP) derivatives. Using ultrafast spectroscopy, we investigate the excited state structural motions that occur in lieu of fluorescence when the GFP model chromophore 4-hydroxybenzylidene-1,2-dimethylimidazolinone (HBDI) is in solution. This investigation provides insights into key structural motions that govern the fate of fluorescence in the model chromophore, providing crucial information for engineering new fluorescent proteins.



Conclusion

New spectroscopic data reveal the molecular motions that compete with fluorescence when the GFP model chromophore is in solution. TA and FSRS peak dynamics illustrate the potential energy landscape of photoexcited HBDI, particularly the presence of an intermediate CS state. FSRS frequency analysis reveals a critical 232 cm⁻¹ out-of-plane mode anharmonically coupled to a HOOP motion. Additionally, pronounced changes in the anharmonic coupling matrix upon addition of glycerol reveal how altering the hydrogen bonding network around HBDI can impact its excited state structural motions and resulting reaction pathways.

Spectroscopic Investigations

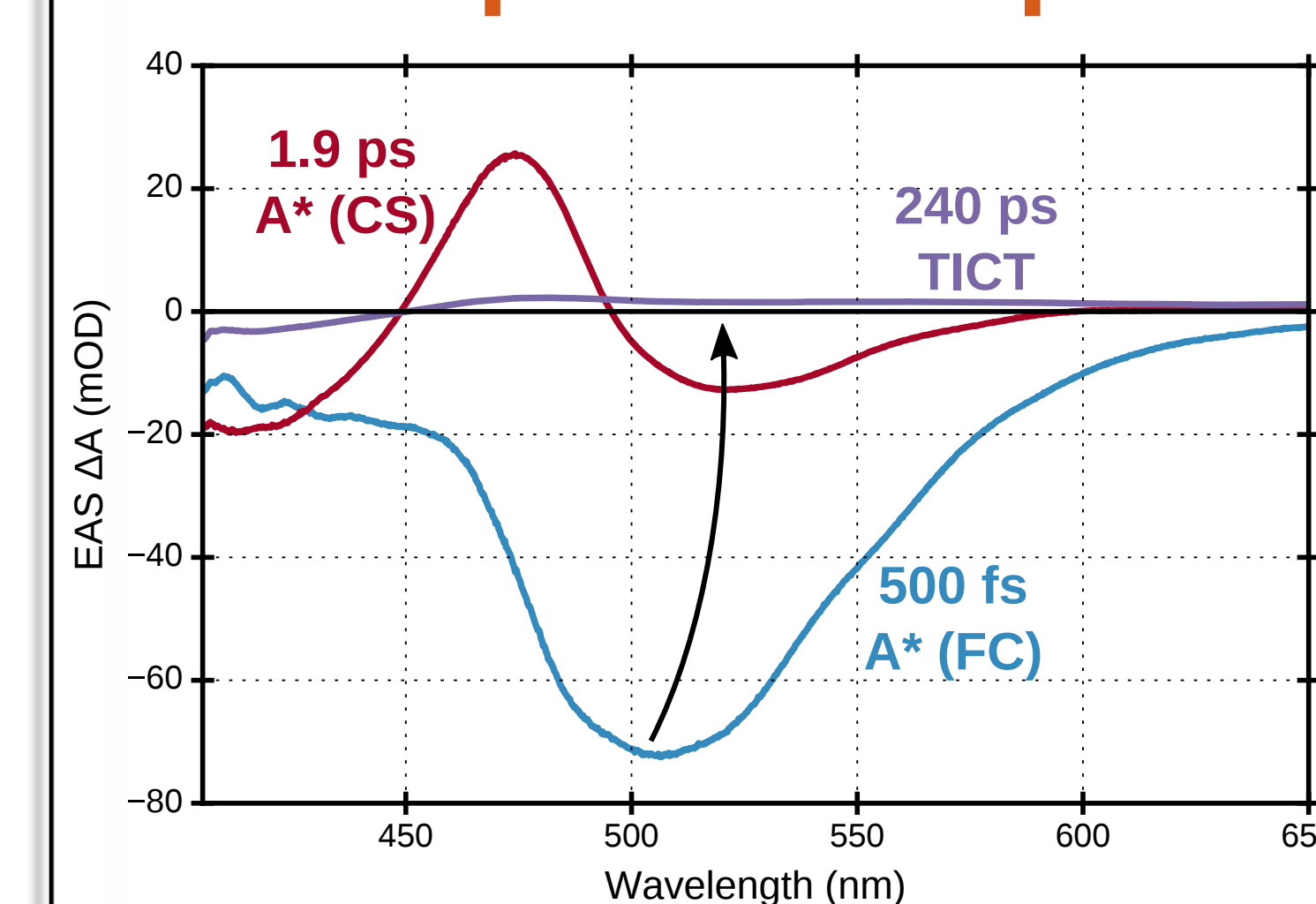


Figure 2. Global analysis of 0% glycerol TA data reveals that photoexcited HBDI evolves through three electronic states, including an intermediate. A blueshift of the the stimulated emission (SE) peak reports on vibrational cooling out of the Franck-Condon (FC) region. The excited state absorption (ESA) indicates the presence of an intermediate.

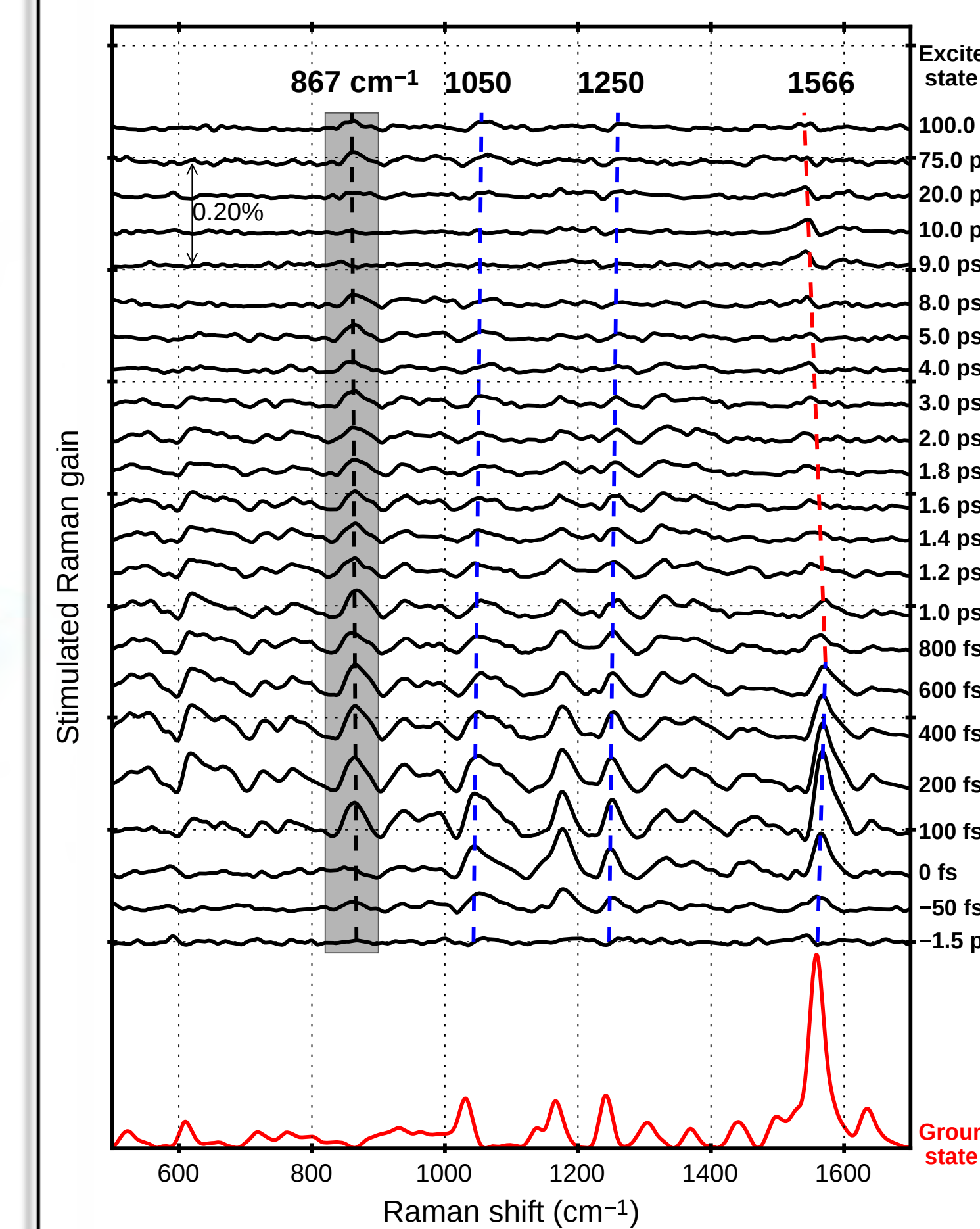


Figure 3. FSRS time traces for HBDI in 0% glycerol, along with a ground state Raman spectrum, reveal excited state structural changes. Observed time dynamics are consistent with TA data. Quantum mechanical calculations for the colored frequency shifts indicate that HBDI undergoes a twisting motion. Additionally, the shaded 867 cm⁻¹ mode exhibits oscillations during the first ~2 ps after photoexcitation.

Potential Energy Surface (PES)

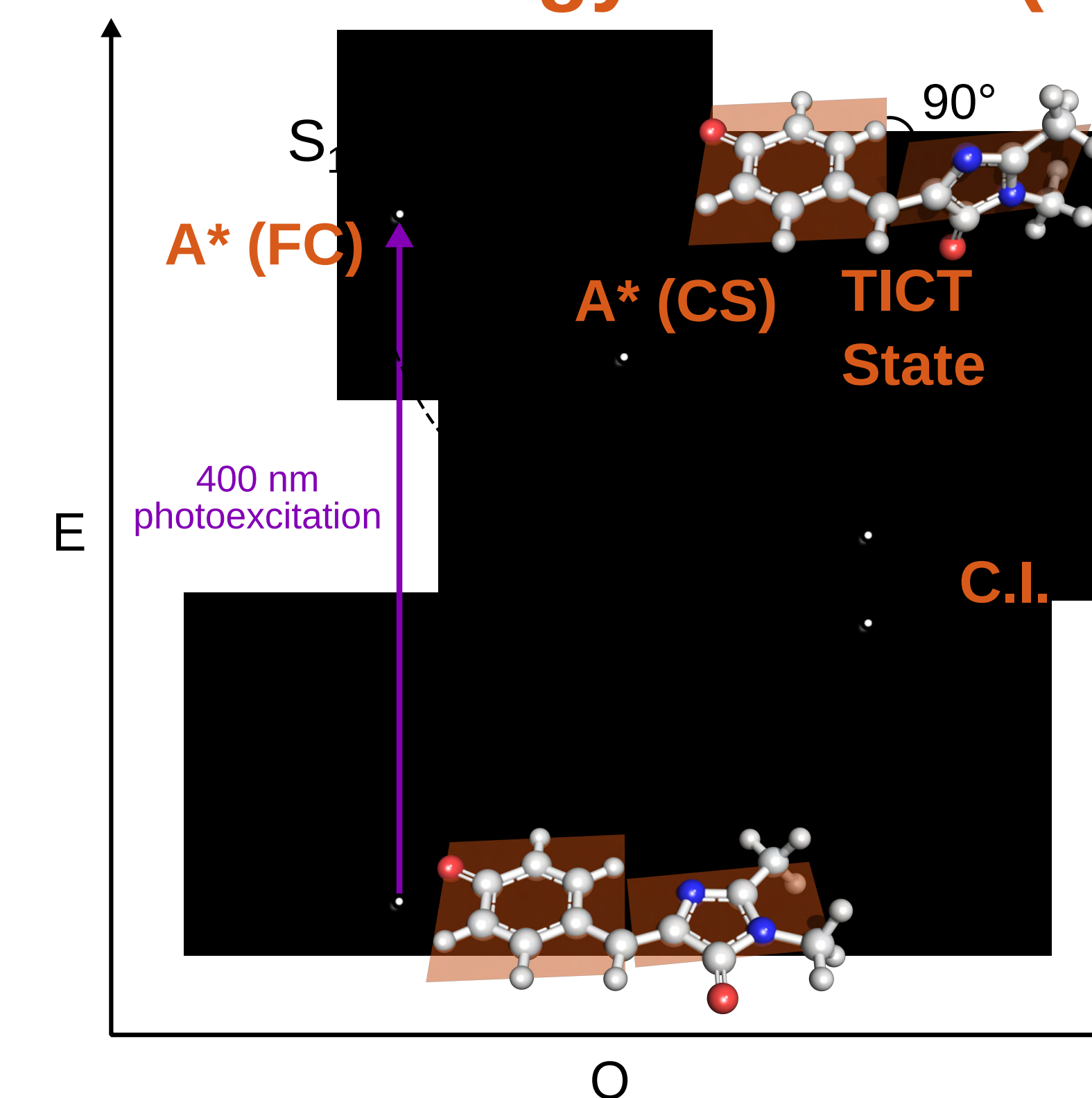


Figure 5. Proposed potential energy surface for HBDI in solution. After a 400 nm photoexcitation, HBDI vibrationally cools and rapidly transitions to a charge separated (CS) state. The molecule then proceeds to a twisted internal charge transfer (TICT) state and crosses a conical intersection (C.I.) on a 1.9 ps timescale. Finally, the molecule relaxes in the ground state.

- Berera, Rudi, Rienk van Grondelle, and John TM Kennis. "Ultrafast transient absorption spectroscopy: principles and application to photosynthetic systems." *Photosynthesis research* 101.2-3 (2009): 105-118.

Acknowledgments

This work was supported in part by the NSF-CAREER Award grant (CHE-1455353) to C.F.

- Kukura, Philipp, David W. McCamant, and Richard A. Mathies. "Femtosecond stimulated Raman spectroscopy." *Annu. Rev. Phys. Chem.* 58 (2007): 461-488.
- Snellenburg, Joris, et al. "Glotaran: a Java-based graphical user interface for the R package TIMP." *Journal of Statistical Software* 49.3 (2012).
- Frisch, M. J. E. A., et al. "Gaussian 09, Revision A. 02, Gaussian, Inc., Wallingford, CT 200 (2009).
- Yang, Fan. *The molecular structure of green fluorescent protein*. Diss. Rice University, 1997.