Supporting information for:

An Alternative Strategy for Spectral Tuning of Flavin-binding Fluorescent Proteins

Mohammad Pabel Kabir,[1] Daniel Ouedraogo,[1] Yoelvis Orozco-Gonzalez,[1,*] Giovanni Gadda,[1,2,3*] Samer Gozem[1,*]

[1]Department of Chemistry, Georgia State University, Atlanta, GA 30302, United States.

[2]Department of Biology, Georgia State University, Atlanta, GA 30302, United States.

[3]The Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30302, United States.

Table of content

Table S1: CASPT2//CASSCF/ANO-L-VDZP computed ground (S_0) and first singlet excited (S_1) state energies. Each column refers to a step of the ASEC-FEG protocol. In addition to absolute energies (in Hartree), the S_0-S_1 vertical excitation energies (in eV and nm) are also shown, as well as the shift (in nm) caused by the Q430E mutation.

		Step 1	Step 2	Step 3	Step 4	**Average**
iLOV	S_0 energy (Hartree)	-870.7066891	-870.7208093	-870.7258861	-870.7170955	
	S_1 energy (Hartree)	-870.5992511	-870.6136004	-870.6183912	-870.6093119	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.924 eV (424 nm)	2.917 eV (425 nm)	2.925 eV (424 nm)	2.933 eV (423 nm)	**2.925 eV (424 nm)**
iLOV-Q430E	S_0 energy (Hartree)	-870.6976046	-870.6984876	-870.6965667	-870.6927712	
	S_1 energy (Hartree)	-870.5911651	-870.5919402	-870.590109	-870.5862978	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.896 eV (428 nm)	2.899 eV (428 nm)	2.897 eV (428 nm)	2.897 eV (428 nm)	**2.897 eV (428 nm)**
Shift caused by the Q430E mutation	$\Delta\Delta E_{S1\text{-}S0}$ (nm)					**4 nm**

Table S2: CASPT2//CASSCF/ANO-L-VDZP computed ground (S_0) and first singlet excited (S_1) state energies in the presence of 4 additional Na^+ and Cl^- ions. Each column refers to a step of the ASEC-FEG protocol. In addition to absolute energies (in Hartree), the S_0-S_1 vertical excitation energies (in eV and nm) are also shown, as well as the shift (in nm) caused by the Q430E mutation.

		Step 1	Step 2	Step 3	Step 4	**Average**
iLOV	S_0 energy (Hartree)	-870.7303668	-870.7269008	-870.7253756	-870.708917	
	S_1 energy (Hartree)	-870.623182	-870.6190768	-870.6190198	-870.602247	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.917 eV (425 nm)	2.934 eV (423 nm)	2.894 eV (428 nm)	2.903 eV (427 nm)	**2.912 eV (426 nm)**
iLOV-Q430E	S_0 energy (Hartree)	-870.7099589	-870.7178432	-870.7119662	-870.6988243	
	S_1 energy (Hartree)	-870.603197	-870.6106224	-870.605017	-870.5911968	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.905 eV (427 nm)	2.918 eV (425 nm)	2.910 eV (426 nm)	2.929 eV (423 nm)	**2.915 eV (425 nm)**
Shift caused by the Q430E mutation	$\Delta\Delta E_{S1\text{-}S0}$ (nm)					**0 nm**

Table S3: CASPT2//CASSCF/ANO-L-VDZP computed ground (S_0) and first singlet excited (S_1) state energies at the S_1 minimum geometry. Each column refers to a step of the ASEC-FEG protocol. Note that the calculations needed 3 steps to reach self-consistency, so results from Steps 1-3 were discarded. In addition to absolute energies (in Hartree), the S_0-S_1 vertical excitation energies (in eV and nm) are also shown, as well as the shift (in nm) caused by the Q430E mutation.

		Step 4	Step 5	Step 6	Step 7	**Average**
iLOV	S_0 energy (Hartree)	-870.7135153	-870.7162409	-870.7212094	-870.7294337	
	S_1 energy (Hartree)	-870.6324525	-870.6344888	-870.6396116	-870.6477739	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.206 eV (562 nm)	2.225 eV (557 nm)	2.220 eV (558 nm)	2.222 eV (558 nm)	**2.218 eV (559 nm)**
iLOV-Q430E	S_0 energy (Hartree)	-870.6925643	-870.6866233	-870.7039868	-870.6853863	
	S_1 energy (Hartree)	-870.6124873	-870.6063316	-870.6236473	-870.6050131	
	$\Delta E_{S1\text{-}S0}$ (eV and nm)	2.179 eV (569 nm)	2.185 eV (567 nm)	2.186 eV (567 nm)	2.187 eV (567 nm)	**2.184 eV (568 nm)**
Shift caused by the Q430E mutation	$\Delta\Delta E_{S1\text{-}S0}$ (nm)					**9 nm**

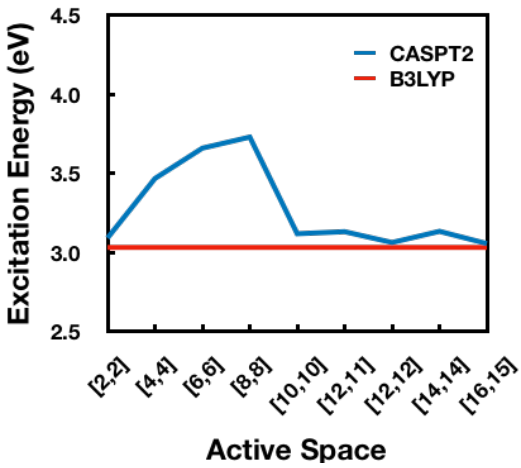

Figure S1: A plot of the S_0-S_1 excitation energy vs active space for CASPT2/ANO-L-VDZP calculations for an MP2-optimized gas-phase geometry of lumiflavin. Only π and π* orbitals were included in the active space in all cases. The first calculation was performed using a [16,15] active space, and the active space was then reduced gradually by removing orbitals based on their occupancies. The calculations indicate that there is a limited benefit to going beyond a [10,10] active space for vertical excitation energies. The TD-DFT/cc-pVTZ excitation energy is added as a reference in red because it was found to reproduce the experimental spectrum of FMN in solution accurately when accounting for Franck-Condon factors (Kabir, M.P. et al., PCCP, 2019, *21*, 16526-16537).

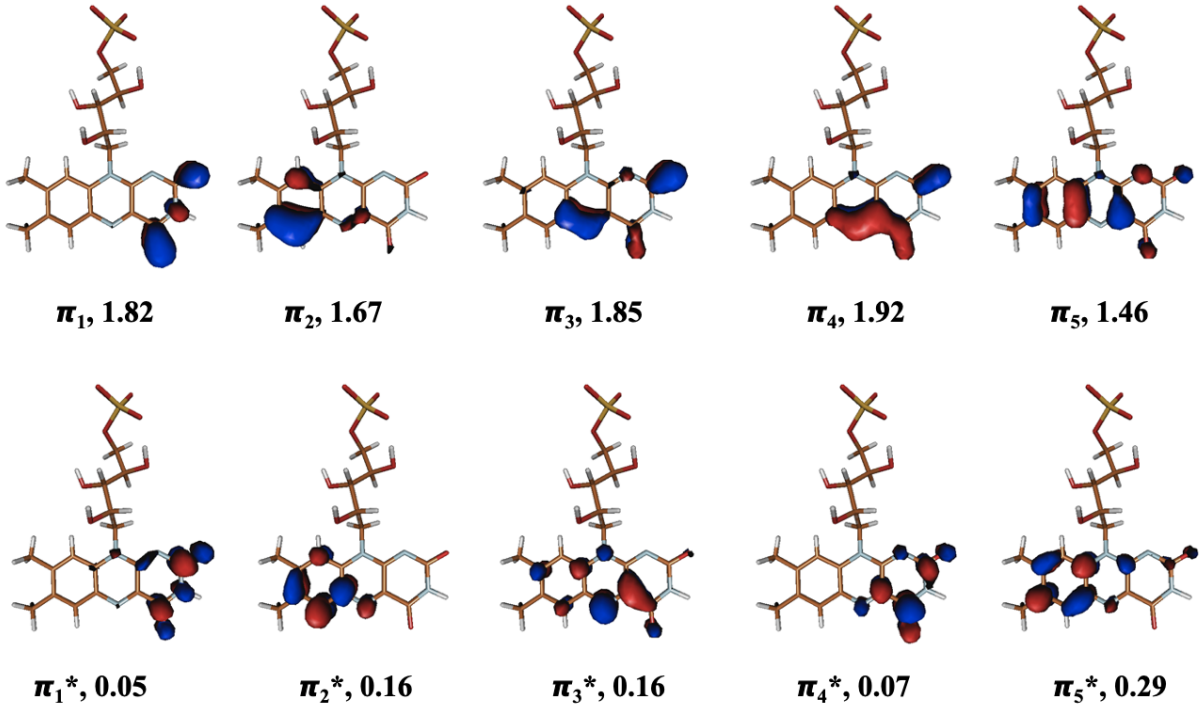

Figure S2: The 10 orbitals included in the CASPT2//CASSCF active space.

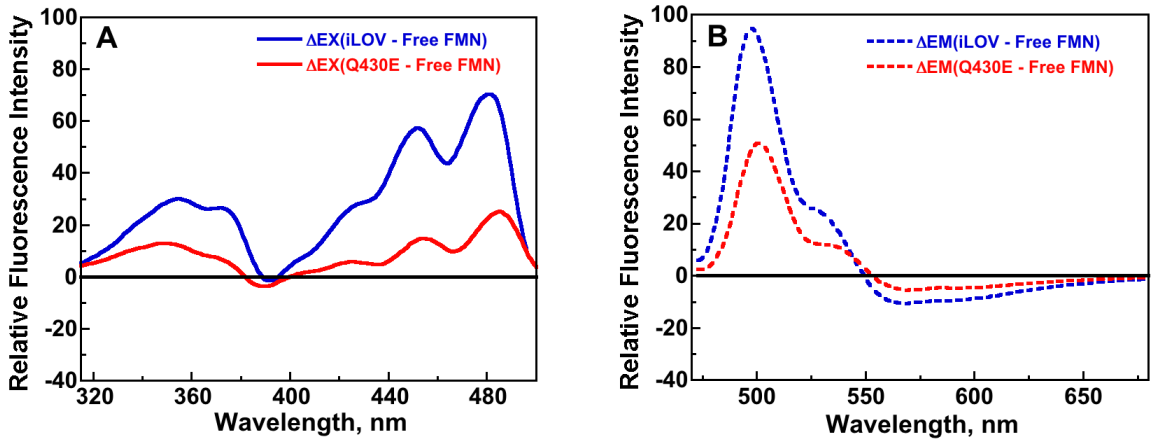

Figure S3: A. Difference excitation spectra of iLOV and iLOV-Q430E minus that of free FMN. **B.** Difference emission spectra of the iLOV and iLOV-Q430E minus that of free FMN. The reference line in panels A and B represents the ΔEX (free FMN-Free FMN) and ΔEM (free FMN-Free FMN), respectively.