Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

for

Electronic Spectra of Flavin in Different Redox and Protonation States: A Computational Perspective on the Effect of the Electrostatic Environment

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Calculation of Franck-Condon factors

Franck-Condon (FC) factors are the overlap integrals between ground and excited state vibrational wave functions. In this work, FC factors were computed within the double-harmonic approximation and using Duschinsky rotations in ezSpectrum.[1] The software aligns the ground and excited state geometries to maximize overlap, performs Duschinsky rotations to maximize the overlap between normal modes, and then computes the overlap integral between the vibrational wave functions. This is most successful when there are limited changes in the geometry between the ground state and excited state, such as cases where the geometric changes are restricted to bond length changes. However, large differences in angles or torsions may result in reduced FC overlaps. Those were the issues we encountered when computing FC factors between ground and excited states of flavin in some states. Most often, such issues arose when there were methyl-group rotations, but in the case of the reduced flavin species bending or pyramidalization of the isoalloxazine backbone also complicated the calculation of FC factors. While such changes all occur along low-frequency modes, they still result in a significantly lower FC overlap. To remedy this, there were a number of approximations in the calculation of FC overlaps of some states:

- Excited state geometries were optimized keeping the same methyl group orientations as in the ground state. Sometimes, these methyl conformations were local minima and not global minima on the excited state potential energy surface resulting in small imaginary frequencies associated with methyl group rotations. Those methyl group rotations were then assigned small positive frequencies (10-30 cm^{-1}) in the calculation of FC factors such that they had little to no contribution to vibronic broadening of the electronic transitions[2] (an alternate approach used by Thiel and co-workers is to exclude such modes[3, 4]).

- In the **Fl**, **FlH·**, and **Fl·$^-$** states, flavin remains planar in both the ground and $\pi \rightarrow \pi^*$ excited states, but this is not the case for **FlH_2** and **FlH$^-$**. The hydroquinone ground state is bent at the central ring along a low-frequency (approximately 30-50 cm^{-1}) "butterfly bending" mode.[5-7] However, the relevant $\pi \rightarrow \pi^*$ excited states in **FlH_2** and **FlH$^-$** are planar. This again resulted in low FC overlaps due to the difference in this bending motion. Therefore, to compute FC factors, the ground state hydroquinone was reoptimized in a planar conformation and any imaginary frequencies resulting from this constraint were assigned small positive frequencies (10-30 cm^{-1}) instead.

- In the case of **FlH_2**, even the planar constraint did not yield substantially improved FC overlaps due to methyl rotations. Therefore, only for **FlH_2**, FC factors were computed in a reduced model with no methyl group substituents.

Note that the approximations above were only use for computing the FC factors, while all adiabatic and zero-point vibrational energies were computed using full flavin models that were properly optimized. The FC factors are used in this work to determine the vibronic structure of each electronic transition and may not be quantitative due to the reasons discussed above.

Simulation of experimental spectra using broadening and normalization

The simulation of experimental UV/vis spectra requires us to consider broadening of the computed vibronic excitation energies. In case of **Fl**, **FlH·**, and **Fl·⁻** a full width at half maximum (FWHM) of 0.25 eV is used for all excited states. Relative peak intensities were computed from the relative oscillator strengths and FC overlaps of the corresponding excited states. Peak intensities for these three states were all normalized to the highest peak but were kept at the same relative intensities. In the case of **FlH_2** and **FlH^-**, it was necessary to use different FWHM and renormalize each peak independently to get spectra that agree with experiment. Specifically, a FWHM of 0.80 eV is used for both peaks in the **FlH_2** spectrum while for **FlH^-** FWHM of 0.35 eV and 0.90 eV were used for the second and third excited states, respectively. In both **FlH_2** and **FlH^-**, the first excited state is assumed to be dark. Therefore, the first, second, and third excited state intensities were scaled by factors of 0.00, 0.38, 1.00 and 0.00, 1.00, 0.50, respectively, for **FlH_2** and **FlH^-**, respectively. This tuning of the FWHM and renormalizing that is needed to match experimental data in hydroquinones is likely due to the approximations made in the calculation of the FC factors. However, we note that broadening is expected to be larger in hydroquinones systems than in the quinone and semiquinones because of the low-frequency bending/pyramidalization mode differences in the ground and excited state.

Table of experimental λ_{max} values from literature for oxidized flavin

PDB ID	Organism	Name	1st peak λ_{max} (nm)	2nd peak λ_{max} (nm)	Ref.
	N/A	Aqueous FMN	445	373	8
	N/A	Aqueous FAD	450	375	8
4HIA	*Rhodobacter sphaeroides*	RsLOV	447	380	9
4EES	*Arabidopsis thaliana*	iLOV	447		10
	Arabidopsis thaliana	iLOV-Q489K	440		11
6GPU	*Arabidopsis thaliana*	miniSOG	448		12
4EEU	*Arabidopsis thaliana*	phiLOV2.1	450		13-15
2PR5	*Bacillus subtilis*	EcFbFP	448		14-19
4KUK	*Dinoroseobacter shibae*	DsLOV	449		20
	Chlamydomonas reinhardtii	CreiLOV	450		21
1N9L	*Chlamydomonas reinhardtii*	LOV1	447.0		22
	Chlamydomonas reinhardtii	LOV1 F41Y	445.0	352.0	23
	Chlamydomonas reinhardtii	LOV2	445.5		22
3UE6	*Vaucheria frigida*	VafLOV	450		21, 24
5J3W	*Pseudomonas putida*	Pp1FbFP	450	376	14, 15, 25
	Pseudomonas putida	Pp2FbFP	449		14, 15, 19, 25
	Pseudomonas putida	Pp2FbFP Y112L	449		14, 15, 19, 25
	Pseudomonas putida	Pp2FbFP Q116V	439		14, 15, 19, 25
	Pseudomonas putida	Pp2FbFP F37T/S	450		15, 26
2Z6C	*Arabidopsis thaliana*	LOV1	448.5		22
4HHD	*Arabidopsis thaliana*	LOV2	446.5		22
2Z6D	*Arabidopsis thaliana*	LOV1	447.5		22
4EEP	*Arabidopsis thaliana*	LOV2	445.5		22
	Rice	LOV1	449.5		22
	Rice	LOV2	446.5		22
	Rice	LOV1	448.0		22
	Rice	LOV2	446.5		22
6CNY	*Neurospora crassa*	VVD	450		27
	Oat	LOV1	449		28
	Oat	LOV2	447	378	28

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