

Excited State Resonance Raman of Flavin Mononucleotide: Comparison of Theory and Experiment.

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Introduction

The varied redox chemistry and light sensing abilities of flavoproteins are employed in a range of photobiological functions and optogenetics applications.¹ There is, therefore, great interest in the excited state structure and dynamics of their cofactors, which feature a common isoalloxazine chromophore. Femtosecond stimulated Raman spectroscopy (FSRS) gives well-resolved Raman spectra of excited states with ultrafast resolution, overcoming issues of background noise and competing solvent/protein contributions which blight other methods. But the clarity of FSRS spectra is due to *resonance enhancement*, where peak intensity is dependent on higher, resonant excited states. There have been a number of FSRS studies of flavoproteins where assignments are based upon *off-resonance* spectra from TD-DFT calculations.²⁻⁴ Here, we refine and improve the assignment of FSRS bands made in an earlier study by our group by calculating *resonance* Raman spectra using the time-dependent gradient approximation.

Isotopologue Study

Previously, we modelled the cofactor flavin mononucleotide (FMN) as lumiflavin solvated by 4 water molecules, representing the hydrogen bonding environment.⁴ The S_1 and T_1 excited states were optimized using TD-DFT and unrestricted DFT, respectively. Experimental femtosecond stimulated Raman spectra (FSRS) for a series of 4 isotopologues of FMN were then assigned by comparing the mode frequency shifts with calculated off-resonance spectra; but these neglect crucial resonance enhancements involved in FSRS.

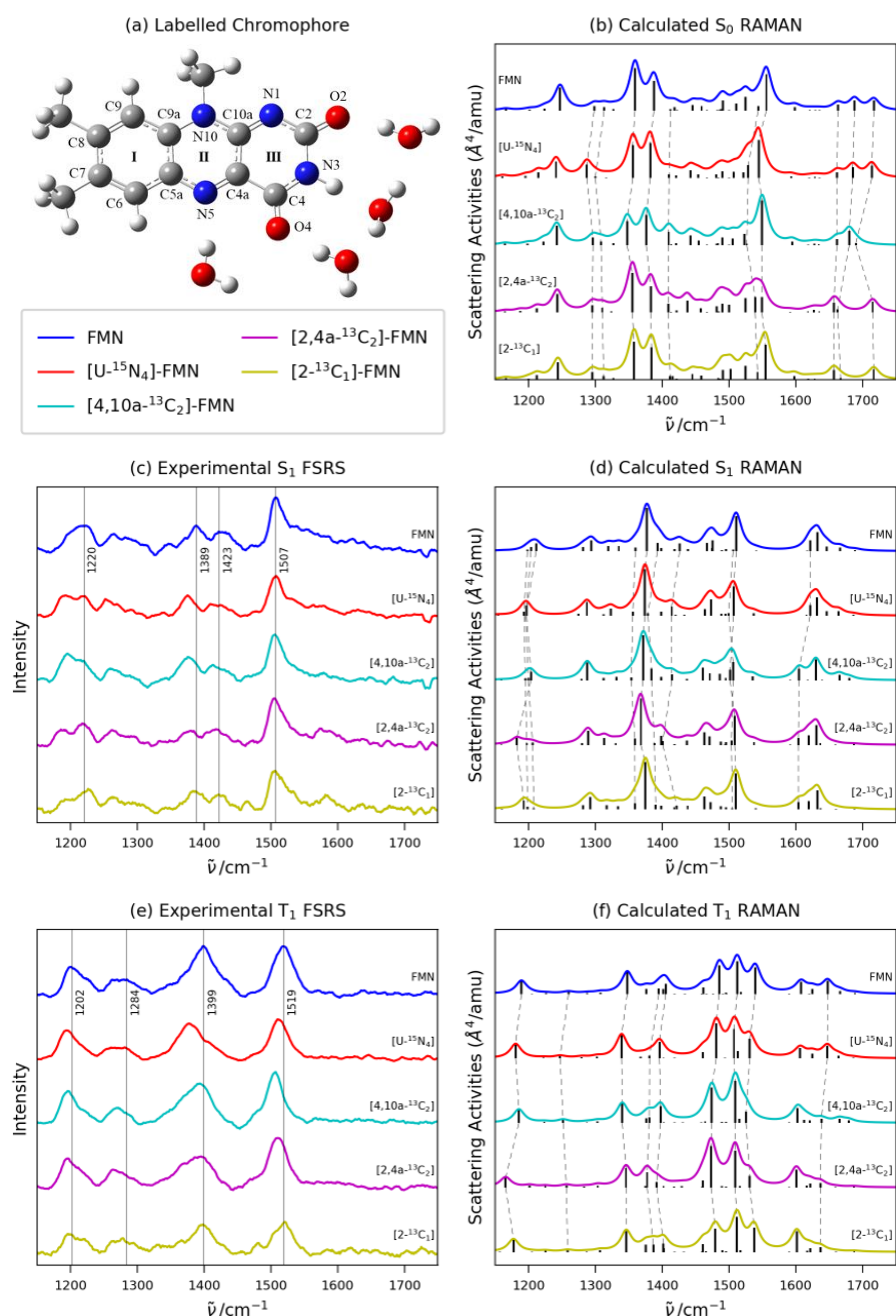


Figure 1: (a) Ground state geometry of lumiflavin, with atom designations, solvated by 4 water molecules and a PCM using B3LYP/TZVP. (b), (d) and (f), calculated off-resonance Raman spectra for (b) S_0 , (d) S_1 , (f) T_1 . (c) and (e), measured FSRS spectra of FMN for (c) S_1 , (e) T_1 . Spectra are arbitrarily offset for clarity. Dashed lines track the shift in frequency of key modes on isotopic substitution. Adapted with permission from ref. 4. Copyright 2020 American Chemical Society.

Theory

Strong enhancement in a resonance Raman spectrum is a result of the equilibrium geometry of the resonant, upper, electronic state, $|N\rangle$, being displaced relative to the lower electronic state, $|I\rangle$.⁵ A larger displacement corresponds to a greater gradient of the upper state potential with respect to the vibrational coordinate of the lower electronic state. The intensity of the k th mode of the lower electronic state, I_k , is approximated as,⁶

$$I_k \propto \omega_p(\omega_p - \omega_k)^3 |a_{fi}^k|^2 \approx \omega_p(\omega_p - \omega_k)^3 \frac{|\mu_{NI}|^4}{2\omega_k} \left(\frac{\partial V_N}{\partial q_k} \right)^2, \quad (1)$$

where ω_k is the mode frequency, q_k is the normalised mode coordinate, ω_p is the excitation (pump) frequency and μ_{NI} is the electric transition dipole moment between the lower and upper electronic states.

Methods

Resonance Raman spectra were calculated by repeated TD-DFT at $q_k = 0, \pm 0.1, \pm 0.2$ for all 31 modes in the range 1150-1750 cm^{-1} of the lower, S_1 and T_1 states. The upper, resonant state was then chosen as the greatest oscillator strength transition nearest to 750 nm experimental pump wavelength. The resonant state potential energy surface (S_8 or T_8) for each mode, v_k , was then fit with a cubic function to yield the resonance Raman intensity proportional to the gradient at $q_k = 0$, as in eq. 1.

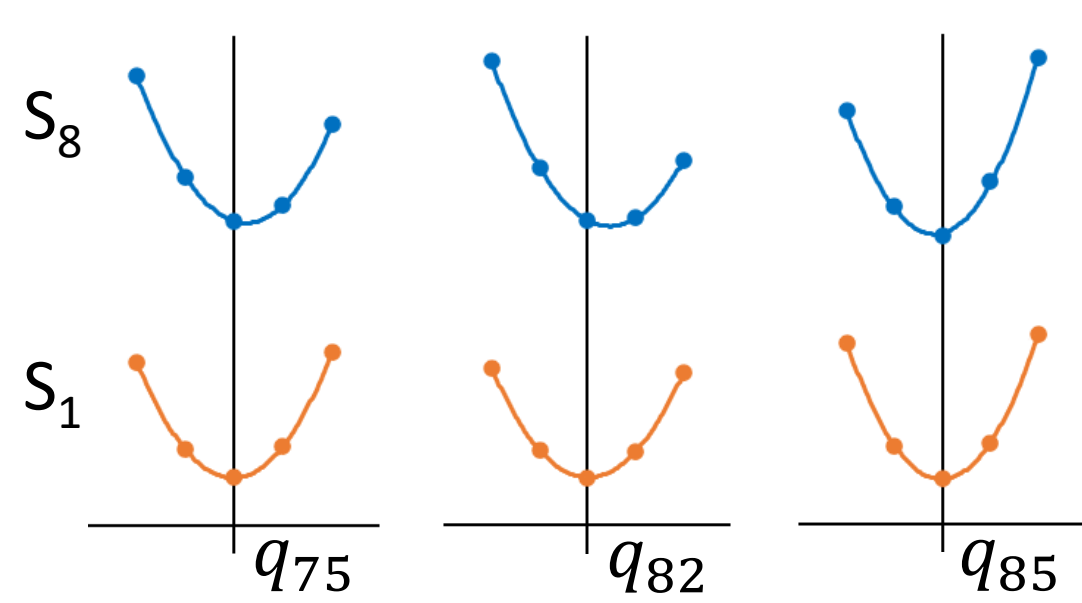


Figure 2: Potential energy surfaces of S_1 and S_8 for modes v_{75} , v_{82} and v_{85} .

Resonance Raman Spectra

