

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

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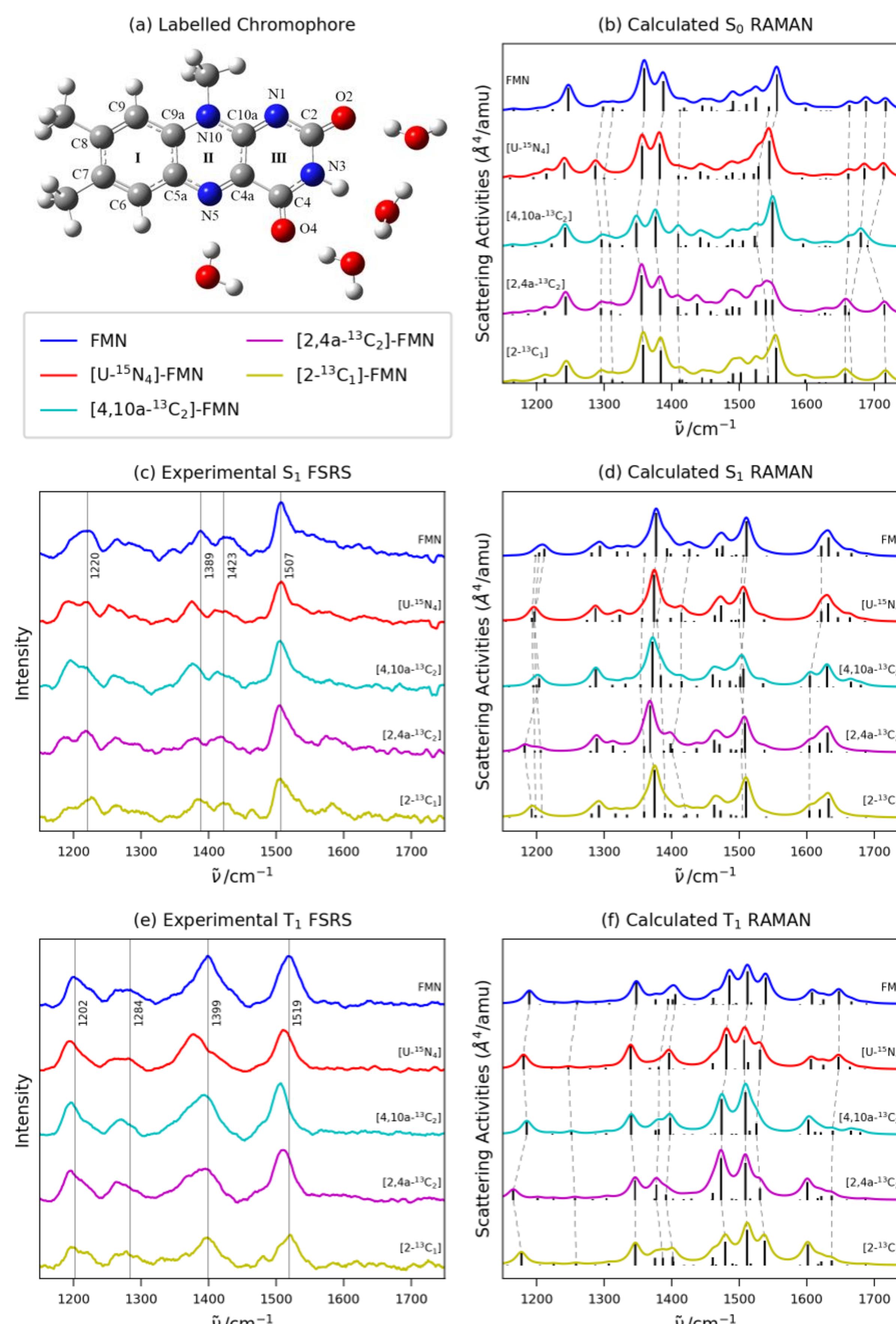
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Introduction

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.



Theory

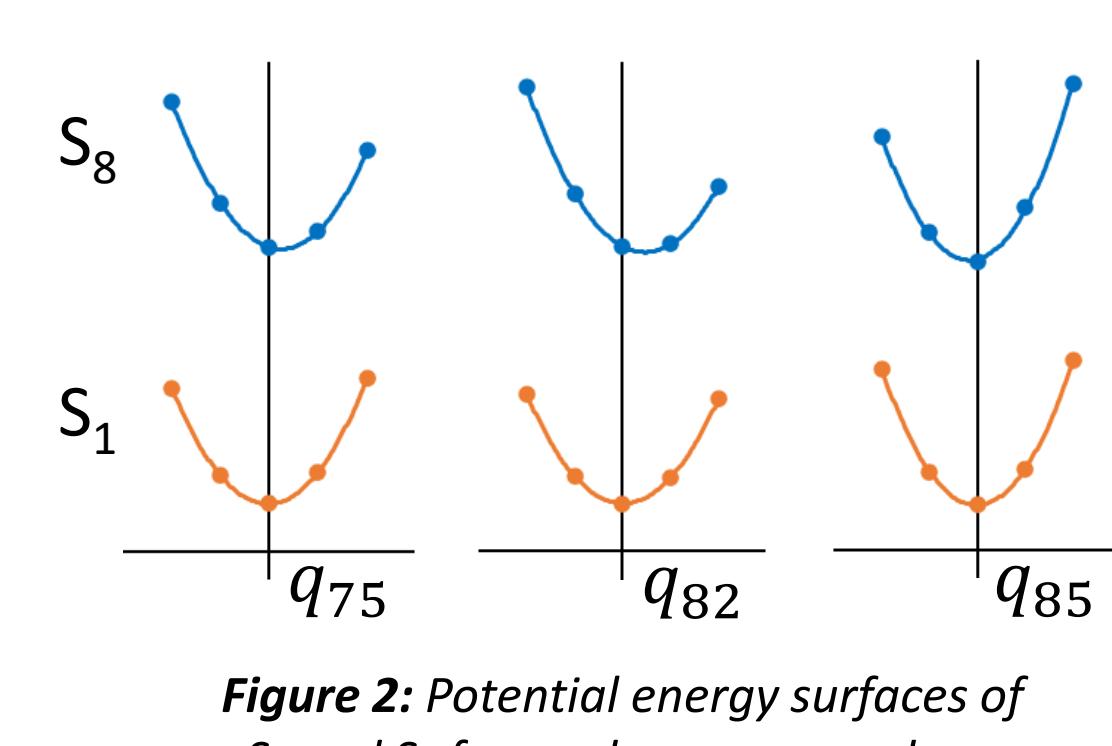
Strong enhancement in a resonance Raman spectrum is a result of the equilibrium geometry of the resonant, upper, electronic state, $|N\rangle$, being displaced relative to the lower electronic state, $|I\rangle$.⁵ A larger displacement corresponds to a greater gradient of the upper state potential with 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

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

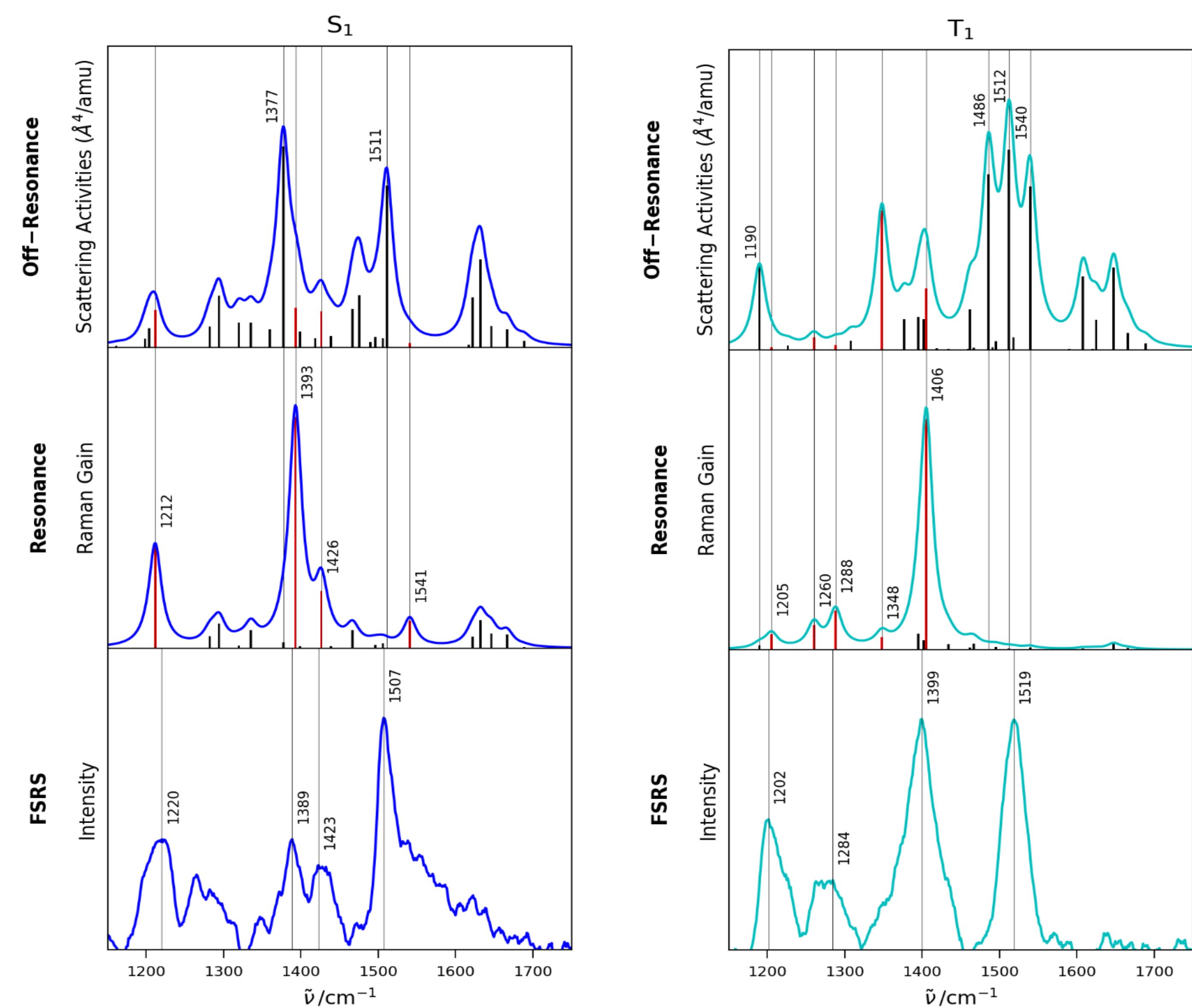
where ω_k is the mode frequency, q_k is the normalised mode coordinate, ω_p is the excitation (pump) frequency and μ_{NI} is the electric transition dipole moment between the lower and upper electronic states.

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_5) 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.



Resonance Raman Spectra



Refined Assignment of Resonance Enhanced Modes

Clear enhancement of fewer modes in the calculated resonance Raman spectra improves the assignment of all FSRS bands, except for the strong band at ca. 1500 cm^{-1} for both S_1 and T_1 which is curiously absent.

Table 1: Assignment of FSRS peaks for S_1 based on the calculated off-resonance and resonance Raman spectra. Reprinted with permission from ref. 7. Copyright 2021 American Chemical Society.

FSRS / cm^{-1}	Off-Resonance Raman / cm^{-1}	Resonance Raman / cm^{-1}	Mode	Assignment*
1220	1198	1212	73	$sN5-C5a, sN3-C4, sC6-C7, wC6-H, wC9-H$
	1204		74	$sC2-N3, ssN5-C4a-C10a, sC9-C9a, sC6-C7, wC6-H, wC9-H, wN10-Me$
1389	1360	1393	75	$osC10a-N1-C2, sN3-C4, sC4a-N5, sC6-C7$
	1377		81	$sN10-C10a, sN3-C4, sC7-C8-C9, sC6-C5a-C9a, bN10-Me, wC6-H$
1423	1426	1426	82	$sC4a-N5, sN10-C10a, sN1-C2, ssC8-C9-C9a, wC6-H, bN10-Me, wC8-Me, wC7-Me$
	1507		95	$sC4a-N5, sN1-C10a, sC7-C8, sC5a-C9a, wN3-H, bC7-Me, bC8-Me, bN10-Me, wC9-H, wC6-H$

*s: stretch (a: antisymmetric, s: symmetric), w: wag, sc: scissor, b: bend. Three atom stretches are described with respect to the centre atom.

Table 2: Assignment of FSRS peaks for T_1 based on the calculated off-resonance and resonance Raman spectra. Notation as per Table 1. Reprinted with permission from ref. 7. Copyright 2021 American Chemical Society.

FSRS / cm^{-1}	Off-Resonance Raman / cm^{-1}	Resonance Raman / cm^{-1}	Mode	Assignment
1202	1190	1205	73	$asN1-C2-N3, asC4-C4a-C10a, asC5a-C6-C7, wC6-H, ssC8-C9-C9a$
	1204		74	$sN3-C4, sC5a-N5, sC9-C9a, wC6-H, wC9-H, wN10-Me$
1284	1260	1260	76	$asC2-N3-C4, sN10-C10a, sC4a-N5, sC6-C7, wC6-H, wN10-Me$
	1288		77	$sC7-Me, sC5a-N5, sN10-Me, sN1-C2, wC6-H, wC9-H$
1399	1348	1406	79	$sN10-Me, sN1-C10a, ssC2-N3-C4, asC6-C7-C8, asC9-C9a-C5a, wC6-H, wC9-H$
	1395		81	$sN1-C10a, sC4a-N5, ssC2-N3-C4, sC9-C9a, bC7-Me, wN3-H$
1406	1406	1406	83	$sN10-C10a, sC4a-N5, sN1-C2, ssC8-C9-C9a, wC7-Me, wC8-Me, wN10-Me$
	1406		83	$sN10-C10a, sC4a-N5, sN1-C2, ssC8-C9-C9a, wC7-Me, wC8-Me, wN10-Me$
1519	1486	1512	90	$sC4a-N5, sN1-C10a, asC2-O2, C4=O4, wN3-H$
	1512		94	$sC4a-N5, sN1-C10a, ssC5a-C6-C7, sC8-Me, sC9a-N10, wN3-H, bN10-Me, bC7-Me$
1540	1540	1540	96	$sC4a-N5, sN1-C10a, sC2=O2, sN3-C4, ssC7-C8-C9, wC6-H, wC9-H, wN3-H$

Conclusion

FSRS assignments based upon off-resonance Raman spectra neglect the essential contribution of the resonant excited state. Here, the time-dependent gradient approximation has been used to model resonance Raman spectra for the S_1 and T_1 states of FMN. The calculated spectra confirm or refine the assignment of all FSRS peaks, except for prominent bands at ca. 1500 cm^{-1} which are absent, likely due to the neglect of vibronic couplings in this theory and the inadequacy of TD-DFT to correctly account for double-excitation character in higher energy, resonant excited states.

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