

Influence of the LOV domain on low-lying excited states of flavin: A combined quantum-mechanics / molecular-mechanics investigation

Supporting Information

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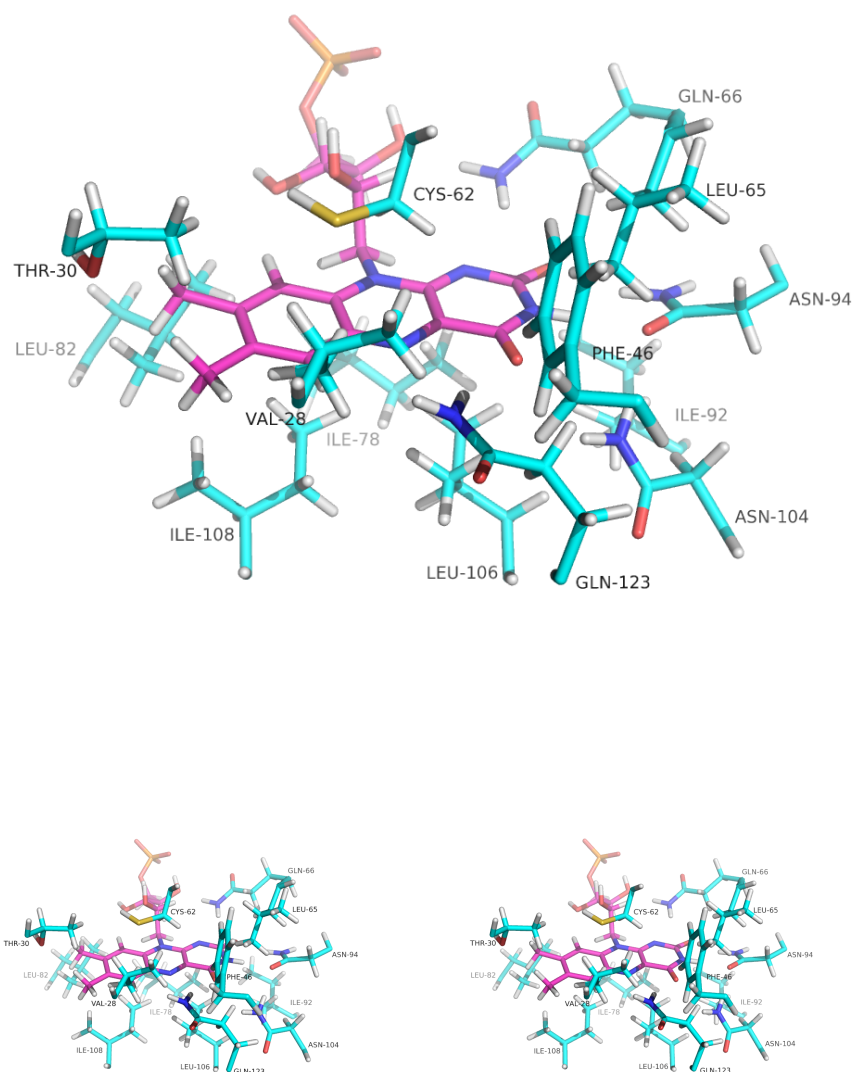
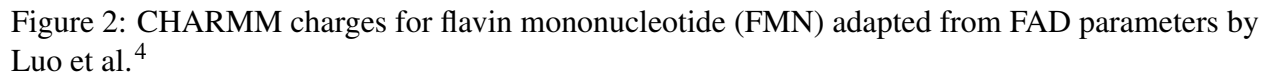


Figure 1: Graphical representation of the flavin binding pocket (top). For a better 3D impression of the binding pocket the scene is shown in stereo representation (bottom). (Figures of protein molecules were created using PyMol (www.pymol.org)).

Preparation of the system The initial geometry was based on an X-ray structure (PDB code 2PR5).¹ For this investigation, only the core LOV domain (residue 25-126) was considered in our calculations. One monomer from the crystallographic dimer was used with its FMN cofactor, which corresponds to the A chain of the PDB structure. 240 associated crystal water molecules (chain A) were included in the model. Apart from CYS62 there are four different residues with alternate atom positions, namely THR54, LYS60, THR100 and ASN107, all located outside the FMN binding pocket. Upon visible inspection of their environment, conformation B was chosen for LYS60 due to favorable hydrogen bonds with crystal water w874 and w908, and conformation A was taken for the remaining residues. Protein hydrogen positions were built with CHARMM.² The assignments of the protonation state of ionizable groups were cross-checked with the empirical pK_a prediction program propKa.³ On the basis of this evaluation, the only histidine residue (HIS69) was protonated due to favorable hydrogen bonding with w851 and w852. Note that this residue is found to be outside the binding pocket. The system was neutralized with 9 sodium ions and solvated in a 35 Å water sphere. The force-field parameters for the FMN cofactor were adapted from published FAD parameters.⁴ The charges of the phosphate atoms (PB, O3A, O3B) in the ribityl chain were manually adjusted with the constraint that the total charge of the FMN cofactor remains -2 . The resulting values given in Figure 2 are comparable to MM charges adopted in other recent studies.^{5,6} Minor variations in the chosen force-field parameters for FMN are not expected to be critical for our purposes, since a full MM description of the cofactor is used only during the setup phase; in the QM/MM calculations, the crucial chromophore part of the cofactor is always included in the QM region. During system setup, all (non-hydrogen) atoms of protein and cofactor were frozen to preserve the X-ray structure, and only hydrogen atoms and water molecules were allowed to move during the various minimization steps (1000 steps of steepest descent (SD) minimization followed by 1000 steps of adopted basis Newton-Raphson (abNR) minimization). In order to retain the two CYS62 conformations, we refrained from performing MM molecular dynamics simulations.



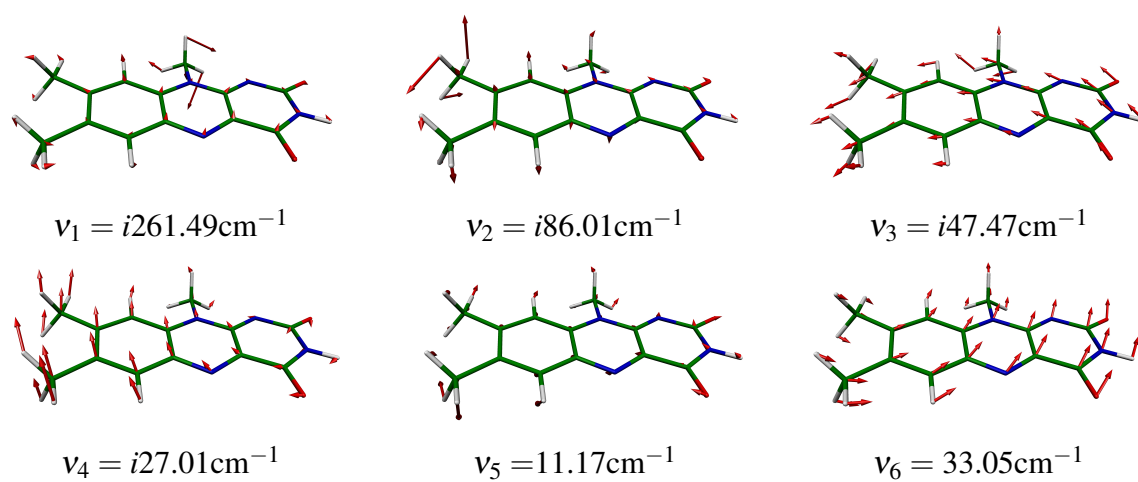


Figure 3: Distortions along low-energy vibrational normal modes at the S_1 geometry. For the nuclear arrangements of the other states a qualitatively similar picture is found.

Table 1: Selected ground-state bond lengths [pm] of LF for the four QM regions in confA and confB, respectively. Atomic labels are displayed in Figure 2 of the main paper.

	LOV (Konf. A)				LOV (Konf. B)			
	QM-1	QM-2	QM-3	QM-4	QM-1	QM-2	QM-3	QM-4
N ₁ -C ₂	136.4	136.0	136.3	136.1	136.2	135.9	136.2	135.9
C ₂ -N ₃	140.9	140.4	141.1	140.4	141.0	140.3	140.9	140.2
N ₃ -C ₄	137.0	136.9	136.8	136.9	136.9	136.9	137.0	137.0
C ₄ -C _{4a}	148.3	148.1	148.0	148.1	148.2	148.3	148.2	148.3
C _{4a} -N ₅	130.1	130.2	130.1	130.2	130.1	130.2	130.0	130.1
N ₅ -C _{5a}	135.1	135.0	134.9	134.9	135.2	135.2	135.2	135.2
C _{5a} -C ₆	140.8	140.9	140.9	140.9	140.9	140.9	141.0	141.0
C ₆ -C ₇	137.1	137.0	137.0	137.0	137.2	137.2	137.2	137.2
C ₇ -C ₈	142.7	142.8	142.7	142.7	142.8	142.8	142.8	142.8
C ₈ -C ₉	138.7	138.6	138.7	138.7	138.4	138.5	138.5	138.5
C ₉ -C _{9a}	139.9	139.8	139.9	139.8	139.9	139.9	139.9	139.9
C _{9a} -N ₁₀	139.5	139.2	139.3	139.3	138.9	138.9	139.0	138.9
N ₁₀ -C _{10a}	137.4	137.1	137.3	137.1	137.2	137.0	137.3	137.0
C _{10a} -N ₁	131.6	131.8	131.7	131.8	131.5	131.7	131.6	131.7
C _{5a} -C _{9a}	142.2	142.3	142.2	142.2	142.1	142.1	142.1	142.1
C _{4a} -C _{10a}	145.0	144.6	144.8	144.5	144.8	144.5	144.9	144.6
C ₂ -O	122.7	123.5	122.6	123.4	122.7	123.4	122.7	123.4
C ₄ -O	122.1	122.5	122.4	122.5	122.3	122.4	122.2	122.3

Table 2: Selected excited-state bond lengths [pm] of LF for region QM-1 in confA and confB, respectively. The data of LF in vacuum is taken from.⁷ Atomic labels are displayed in Figure 2 of the main paper.

	$^1(\pi\pi^*) \text{ S}_1$			$^3(\pi\pi^*) \text{ T}_1$			$^3(\pi\pi^*) \text{ T}_2$		
	LF	confA	confB	LF	confA	confB	LF	confA	confB
N ₁ -C ₂	139.9	138.5	138.7	139.1	138.1	138.1	137.9	136.2	136.1
C ₂ -N ₃	137.8	137.8	137.6	138.4	138.2	138.0	140.8	140.3	140.4
N ₃ -C ₄	141.4	139.7	139.9	139.8	138.9	139.1	138.9	137.6	137.7
C ₄ -C _{4a}	145.5	144.6	144.5	147.0	145.7	145.6	147.1	145.7	145.7
C _{4a} -N ₅	135.5	134.0	134.1	136.5	133.9	134.0	136.3	136.9	136.9
N ₅ -C _{5a}	134.3	136.7	136.5	133.8	136.2	136.2	133.2	132.8	132.8
C _{5a} -C ₆	140.6	138.8	138.9	141.7	138.4	138.6	143.2	144.0	144.1
C ₆ -C ₇	139.0	141.8	141.6	137.7	140.5	140.3	143.6	140.0	140.4
C ₇ -C ₈	142.4	140.5	140.6	143.2	142.3	142.4	137.3	138.6	138.5
C ₈ -C ₉	138.9	139.3	139.1	138.8	138.0	137.9	144.7	145.4	145.2
C ₉ -C _{9a}	140.3	142.3	142.0	139.7	141.5	141.3	140.0	138.9	139.0
C _{9a} -N ₁₀	138.7	138.9	138.7	138.7	137.7	137.6	137.7	140.1	139.6
N ₁₀ -C _{10a}	137.6	139.4	139.0	140.0	142.4	141.9	141.6	139.1	139.0
C _{10a} -N ₁	133.3	131.6	131.7	132.4	131.4	131.4	130.5	131.8	131.8
C _{5a} -C _{9a}	144.1	141.5	141.8	144.9	144.6	144.6	142.1	141.9	142.0
C _{4a} -C _{10a}	142.5	143.7	143.5	141.3	141.9	141.7	142.5	142.8	142.6
C ₂ -O	121.9	122.9	123.0	121.9	123.1	123.1	121.5	123.1	123.1
C ₄ -O	121.7	122.9	123.1	121.5	122.9	122.8	121.9	123.2	123.1

Table 3: confA:Vertical singlet excitation energies ΔE [eV] for different QM regions. In addition to the dominant excitations, oscillator strengths $f(r)$ of optically bright states are given.

	electronic structure	$\Delta E(f(r))$			
		QM-1	QM-2	QM-3	QM-4
S ₀	ground state	0.00	0.00	0.00	0.00
S ₁	$\pi_H \rightarrow \pi_L^*$	2.84 (0.2804)	2.79 (0.2974)	2.82 (0.2321)	2.77 (0.2438)
S ₂	$\pi_{H-1} \rightarrow \pi_L^*$	3.40 (0.2926)	3.31 (0.2500)	3.21 (0.2367)	3.14 (0.2470)
S ₃	$n_N \rightarrow \pi_L^*$	3.46	3.35	3.45	3.34
CT	$p_S \rightarrow \pi_L^*$			3.65	3.57
S ₄	$n_O \rightarrow \pi_L^*$	3.87	3.71	3.89	3.71

Table 4: confB:Vertical singlet excitation energies ΔE [eV] for different QM regions. In addition to the dominant excitations, oscillator strengths $f(r)$ of optically bright states are given.

	electronic structure	$\Delta E(f(r))$			
		QM-1	QM-2	QM-3	QM-4
S ₀	ground state	0.00	0.00	0.00	0.00
S ₁	$\pi_H \rightarrow \pi_L^*$	2.86 (0.2935)	2.82 (0.3089)	2.85 (0.2718)	2.83 (0.2880)
S ₂	$\pi_{H-1} \rightarrow \pi_L^*$	3.46 (0.2701)	3.38 (0.2892)	3.39 ^a (0.2253)	3.37 ^a (0.1928)
S ₃	$n_N \rightarrow \pi_L^*$	3.47	3.34	3.45 ^a (0.0422)	3.31 ^a (0.0833)
S ₄	$n_O \rightarrow \pi_L^*$	3.87	3.69	3.88	3.70
CT	$p_S \rightarrow \pi_L^*$			3.92	3.83

^a The $\pi_{H-1} \rightarrow \pi_L^*$ and $n_N \rightarrow \pi_L^*$ excitations mix.

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