Supporting information for:

Assigning Flavin's Difference-FTIR Spectral Bands in Solution: Frequency and Intensity Shifts in Flavin's 1-electron and 2-electron Reduced States

D.P. Ngan Le,[†] Gary Hastings,*[,§,†] Samer Gozem*[,†]

[†] Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, United States
[§] Department of Physics and Astronomy, Georgia State University, Atlanta, Georgia 30303, United States

Email: ghastings@gsu.edu, sgozem@gsu.edu

Table of content

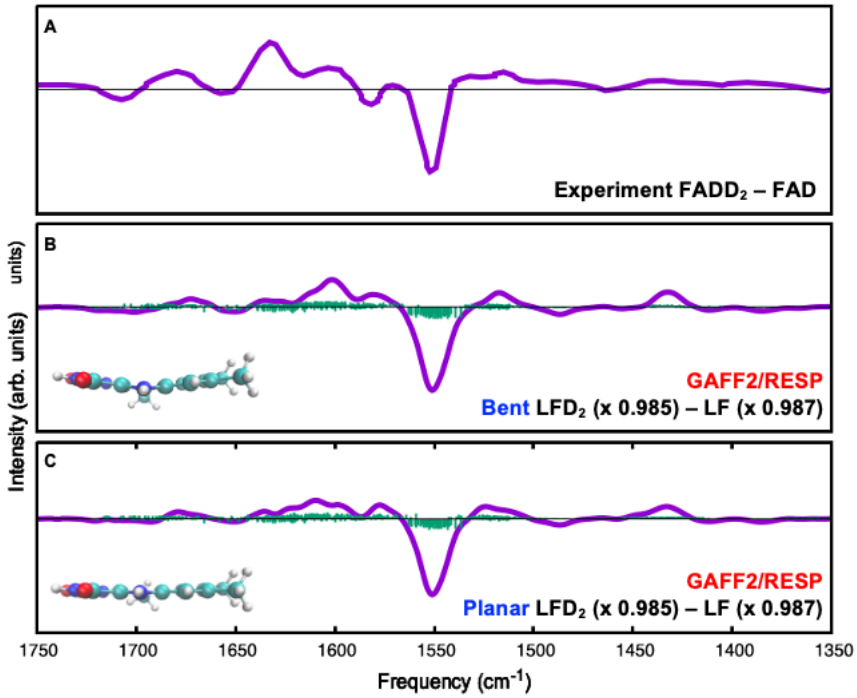


Figure S1: Comparison between experimental **(A)** $FADD_2$ – FAD[1] in D_2O and computational FTIR difference spectra in two different force field **(B)** bent LFD_2 – LF using GAFF2/RESP, and **(C)** planar LFD_2 – LF using GAFF2/RESP. The x-axis represents frequency (cm^{-1}), while the y-axis indicates intensity (arbitrary units). The horizontal black line helps distinguish positive from negative peaks.

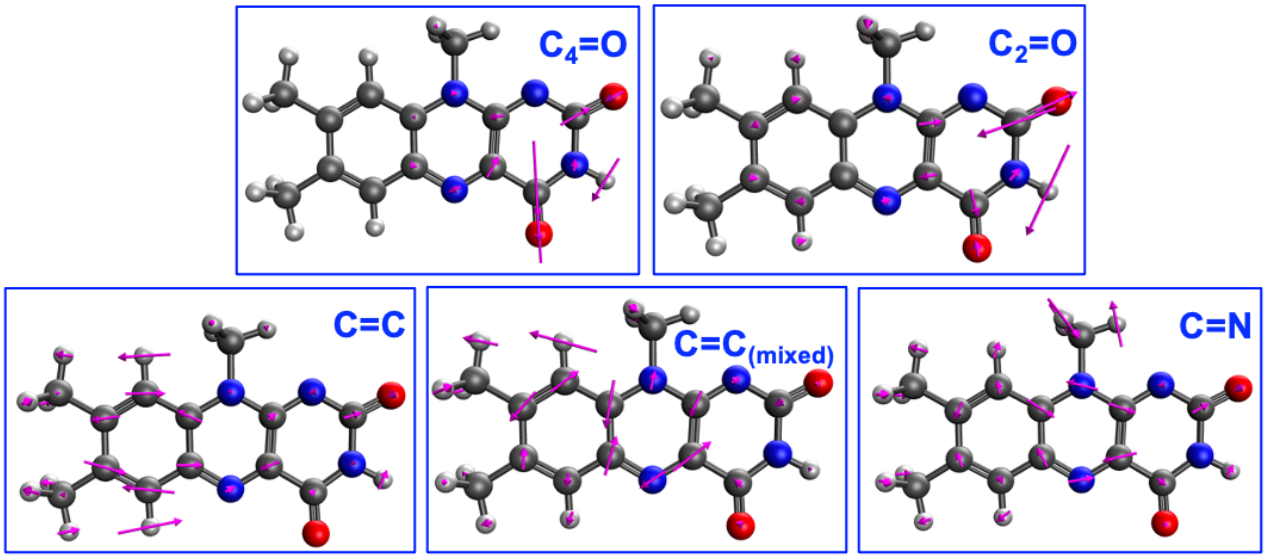


Figure S2: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the protonated LF.

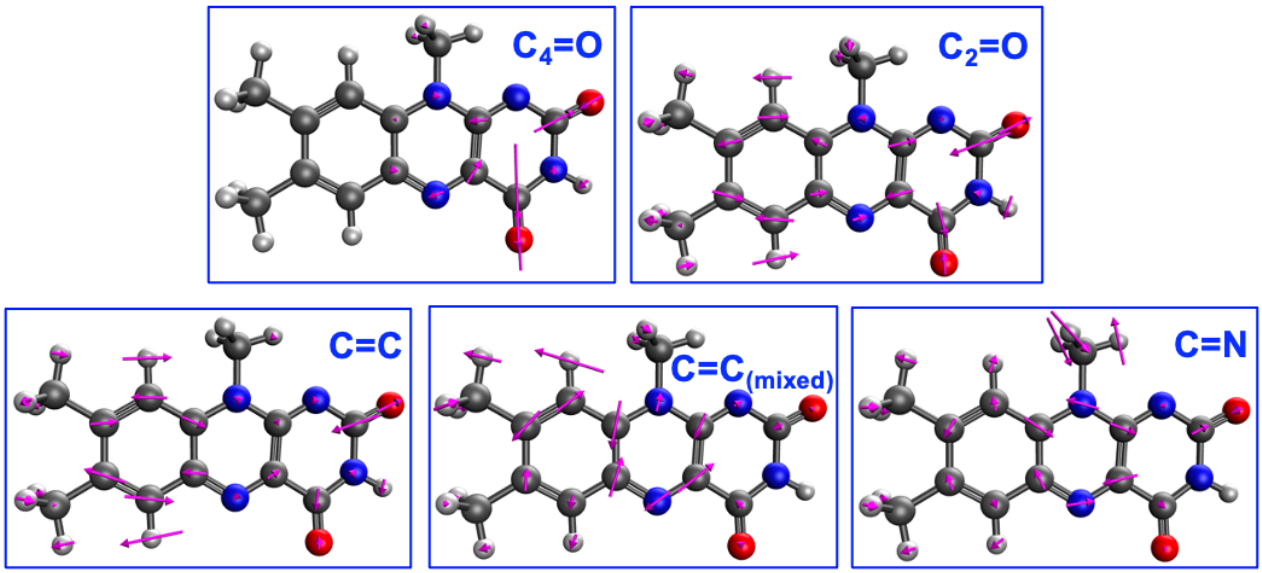


Figure S3: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the deuterated LF.

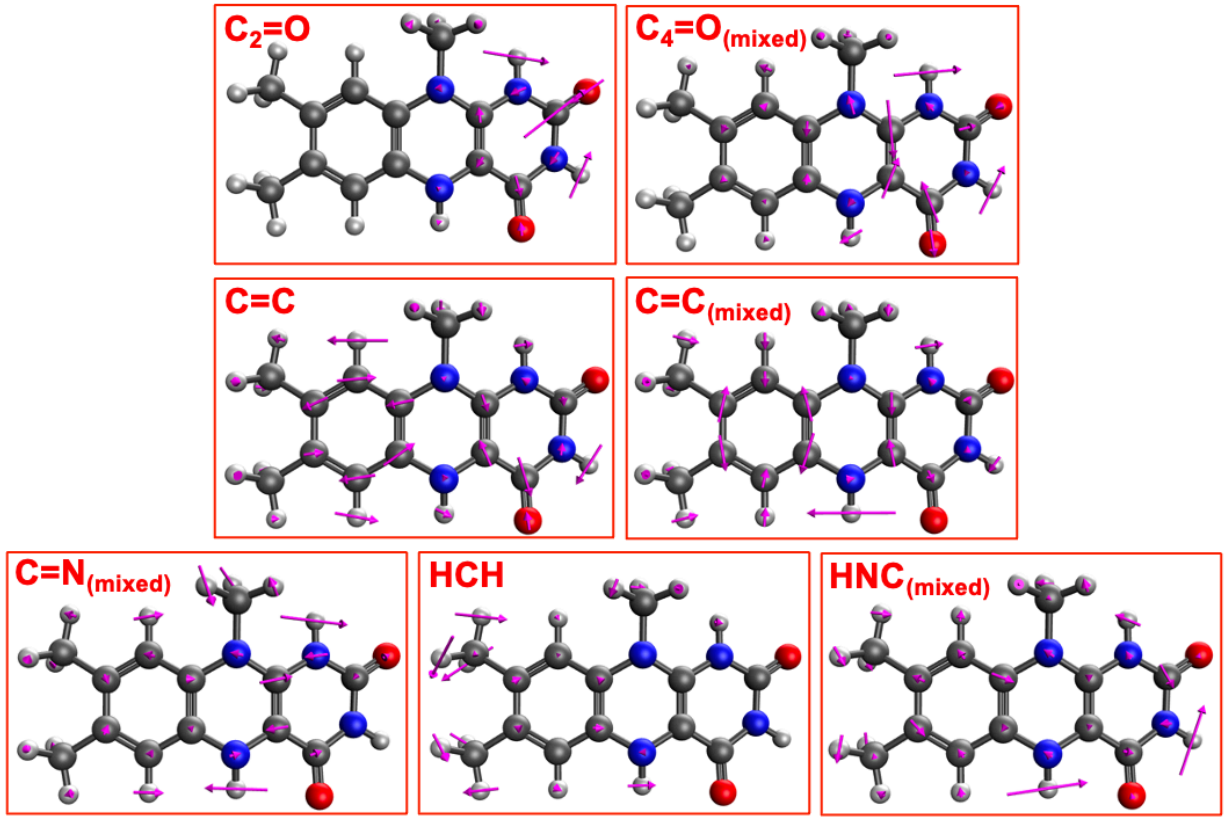


Figure S4: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the protonated LFH_2.

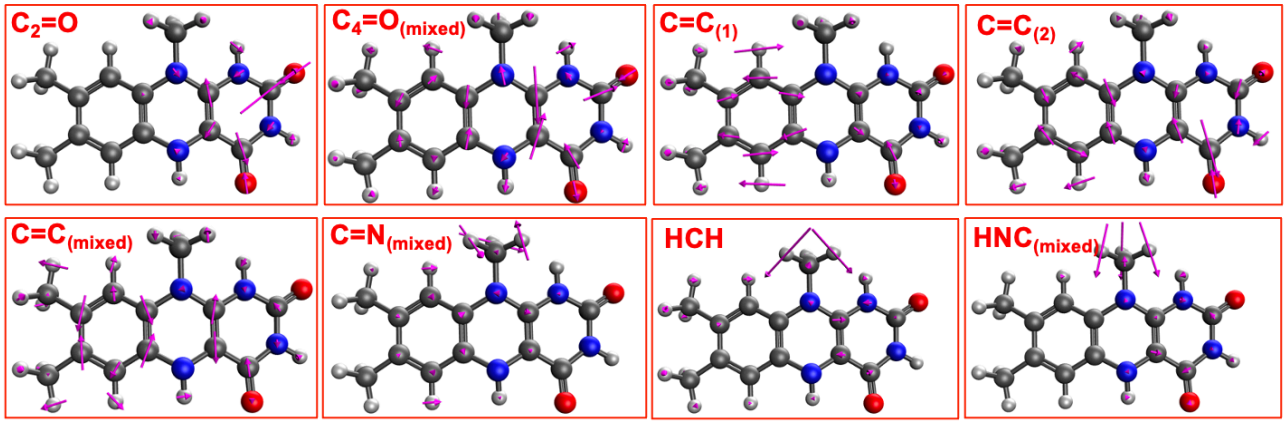


Figure S5: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the deuterated LFD_2.

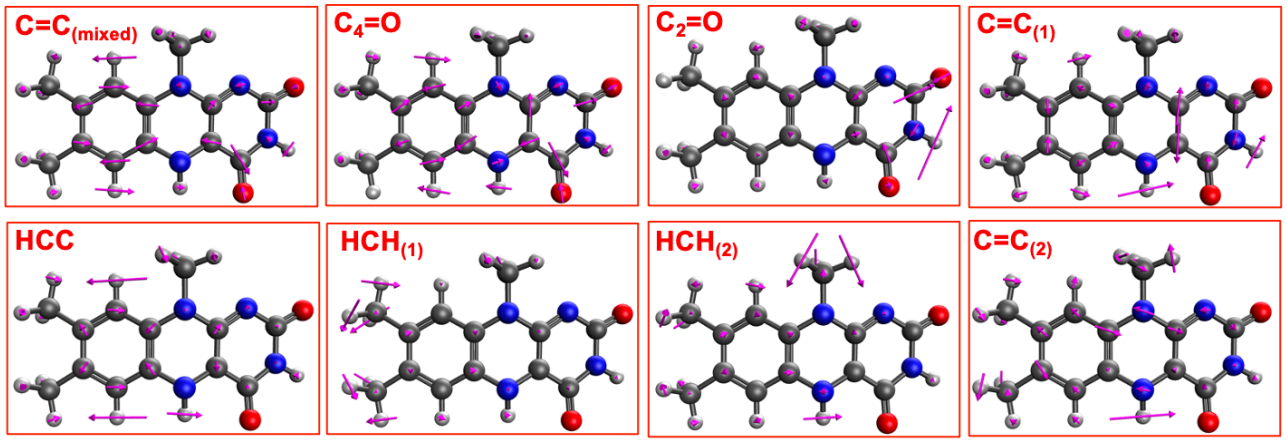


Figure S6: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the protonated LFH^-.

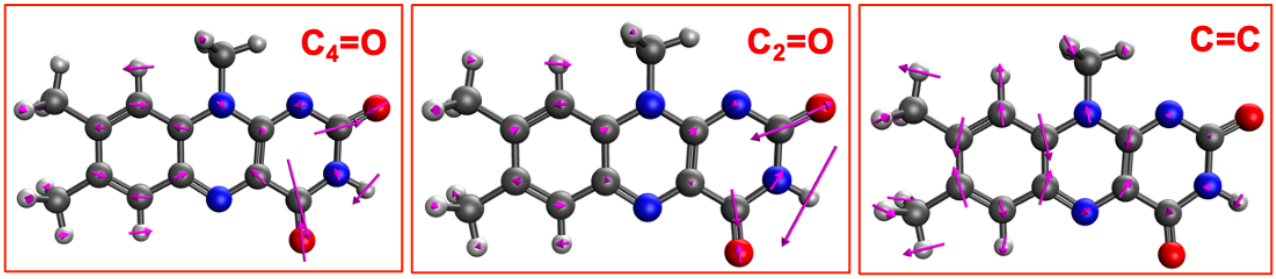
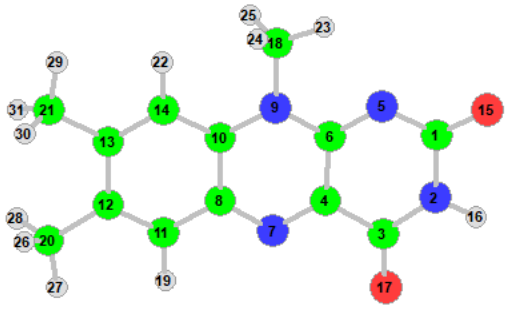


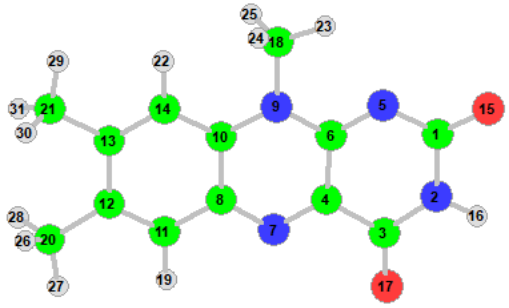
Figure S7: Normal modes computed with the B3LYP/6-31+G*/IEF-PCM model for the protonated $LF^{\cdot-}$.

Table S1: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 0.988 factor followed by % contribution of specific modes based on VEDA analysis for protonated LF.



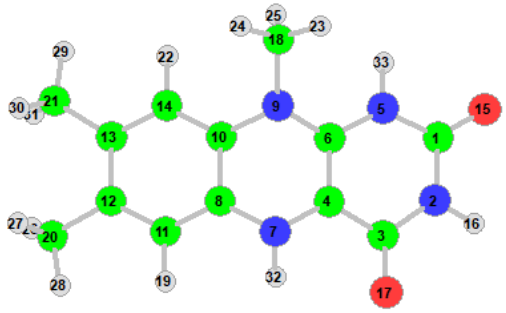
Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1714	OC stretch	15 – 1	14
		17 – 3	
	OC stretch	15 – 1	60
		17 – 3	
1669	OC stretch	15 – 1	-54
		17 – 3	
	OC stretch	15 – 1	13
		17 – 3	
1650	CC stretch	11 – 8	-55
		12 – 11	
		13 – 14	
		14 – 10	
	HCC bend	19 – 11 – 12	11
		22 – 14 – 13	
1589	NC stretch	5 – 6	-20
		7 – 4	
	CC stretch	8 – 10	24
1551	NC stretch	5 – 6	14
		7 – 4	
	NC stretch	2 – 1	14
		2 – 3	
		9 – 6	
		9 – 10	

Table S2: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 0.988 factor followed by % contribution of specific modes based on VEDA analysis for deuterated LF.



Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1711	OC stretch	15 – 1	14
		17 – 3	
	OC stretch	15 – 1	60
		17 – 3	
1656	OC stretch	15 – 1	-54
		17 – 3	
	OC stretch	15 – 1	13
		17 – 3	
1647	CC stretch	11 – 8	-55
		12 – 11	
		13 – 14	
		14 – 10	
	HCC bend	19 – 11 – 12	11
		22 – 14 – 13	
1589	NC stretch	5 – 6	-20
		7 – 4	
	CC stretch	8 – 10	24
1551	NC stretch	5 – 6	14
		7 – 4	
	NC stretch	2 – 1	14
		2 – 3	
		9 – 6	
		9 – 10	

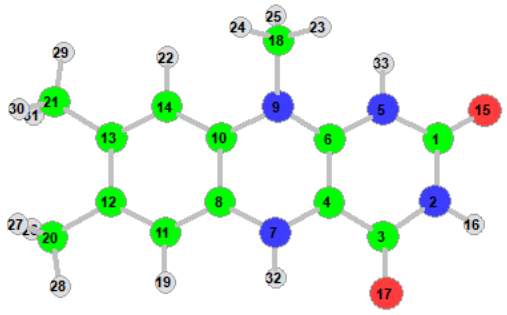
Table S3: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 0.965 factor followed by % contribution of specific modes based on VEDA analysis for protonated LFH_2.



Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1673	OC stretch	15 – 1	17
		17 – 3	
	OC stretch	15 – 1	54
		17 – 3	
	NC stretch	2 – 1	10
		5 – 1	
		5 – 6	
		9 – 6	
1629	OC stretch	15 – 1	23
		17 – 3	
	CC stretch	6 – 4	-10
		8 – 11	
		10 – 14	
		12 – 11	
	HNC bend	16 – 2 – 3	-10
		32 – 7 – 4	
		33 – 5 – 6	
1605	CC stretch	6 – 4	-12
		8 – 11	
		10 – 14	
		12 – 11	
	CC stretch	6 – 4	14
		8 – 11	
		10 – 14	
		12 – 11	
1567	CC stretch	6 – 4	-23
		8 – 11	
		10 – 14	
		12 – 11	

	CC stretch	13 – 12	20
		14 – 13	
	CCC bend	8 – 11 – 12	11
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	
	CCC bend	8 – 11 – 12	12
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	
1497	NC stretch	2 – 1	-20
		5 – 1	
		5 – 6	
		9 – 6	
	HNC bend	16 – 2 – 3	20
		32 – 7 – 4	
		33 – 5 – 6	
1461	HCH bend	26 – 20 – 28	-26
		27 – 20 – 26	
		28 – 20 – 27	
		29 – 21 – 31	
	HCH bend	30 – 21 – 29	27
		31 – 21 – 30	
	HCCC torsion	30 – 21 – 13 – 12	-12
1362	CC stretch	6 – 4	-15
		8 – 11	
		10 – 14	
		12 – 11	
	HNC bend	16 – 2 – 3	-17
		32 – 7 – 4	
		33 – 5 – 6	

Table S4: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 0.975 factor followed by % contribution of specific modes based on VEDA analysis for deuterated LFD_2.

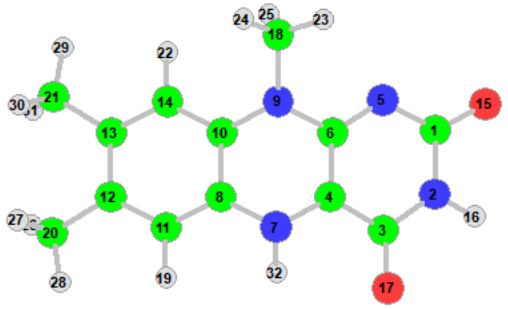


Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1681	OC stretch	15 – 1	17
		17 – 3	
	OC stretch	15 – 1	54
		17 – 3	
	NC stretch	2 – 1	10
		5 – 1	
		5 – 6	
		9 – 6	
1628	OC stretch	15 – 1	23
		17 – 3	
	CC stretch	6 – 4	-10
		8 – 11	
		10 – 14	
		12 – 11	
	HNC bend	16 – 2 – 3	-10
		32 – 7 – 4	
		33 – 5 – 6	
1618	CC stretch	6 – 4	-12
		8 – 11	
		10 – 14	
		12 – 11	
	CC stretch	6 – 4	14

		8 – 11	
		10 – 14	
		12 – 11	
1598	CC stretch	6 – 4	-30
		8 – 11	
		10 – 14	
		12 – 11	
1571	CC stretch	6 – 4	-23
		8 – 11	
		10 – 14	
		12 – 11	
	CC stretch	13 – 12	20
		14 – 13	
	CCC bend	8 – 11 – 12	11
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	
	CCC bend	8 – 11 – 12	12
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	
1495	NC stretch	2 – 1	-20
		5 – 1	
		5 – 6	
		9 – 6	
	HNC bend	16 – 2 – 3	20
		32 – 7 – 4	
		33 – 5 – 6	
1482	HCH bend	23 – 18 – 25	58
		24 – 18 – 23	
		25 – 18 – 24	
	HCNC torsion	23 – 18 – 9 – 10	-11
		24 – 18 – 9 – 10	
		25 – 18 – 9 – 10	

1437	NC stretch	2 – 1	10
		5 – 1	
		5 – 6	
		9 – 6	
	NC stretch	2 – 3	-11
		7 – 4	
		7 – 8	
	HNC bend	16 – 2 – 3	12
		32 – 7 – 4	
		33 – 5 – 6	
	HNC bend	16 – 2 – 3	12
		32 – 7 – 4	
		33 – 5 – 6	

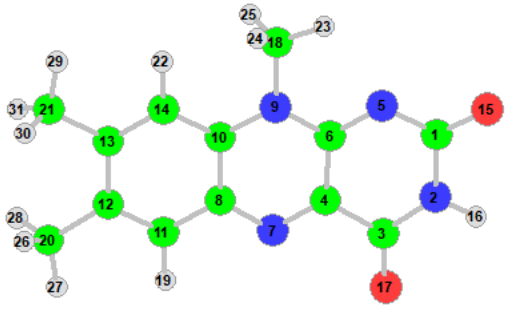
Table S5: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 1.01 factor followed by % contribution of specific modes based on VEDA analysis for protonated LFH^{-}.



Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1674	OC stretch	15 – 1	13
		17 – 3	
	CC stretch	4 – 6	-23
		8 – 11	
		11 – 12	
		14 – 10	
1665	OC stretch	15 – 1	26
		17 – 3	
1621	OC stretch	15 – 1	41
		17 – 3	
	HNC bend	16 – 2 – 1	10
		32 – 7 – 4	

	NCN bend	2 – 1 – 5	10
		9 – 6 – 5	
1602	CC stretch	4 – 6	13
		8 – 11	
		11 – 12	
		14 – 10	
	CC stretch	4 – 6	28
		8 – 11	
		11 – 12	
		14 – 10	
1564	CC stretch	4 – 6	14
		8 – 11	
		11 – 12	
		14 – 10	
	HCC bend	19 – 11 – 12	39
		22 – 14 – 13	
1528	HCH bend	26 – 20 – 28	-31
		27 – 20 – 26	
		28 – 20 – 27	
		29 – 21 – 31	
	HCH bend	30 – 21 – 29	27
		31 – 21 – 30	
	HCCC torsion	30 – 21 – 13 – 12	-12
		31 – 21 – 13 – 12	
1489	HCH bend	23 – 18 – 25	37
		24 – 18 – 23	
		25 – 18 – 24	
1419	CC stretch	4 – 6	-15
		8 – 11	
		11 – 12	
		14 – 10	
	CC stretch	4 – 6	-11
		8 – 11	
		11 – 12	
		14 – 10	

Table S6: B3LYP/6-31+G*/PCM computed vibrational frequencies (cm^{-1}) after scaling by a 0.9925 factor followed by % contribution of specific modes based on VEDA analysis for protonated $LF^{\bullet -}$.



Frequency (cm^{-1})	Vibrational Mode	Atoms involved in internal coordinates	Contribution (%)
1632	OC stretch	15 – 1	52
		17 – 3	
1610	OC stretch	15 – 1	51
		17 – 3	
	HNC bend	16 – 2 – 3	-18
1566	CC stretch	8 – 11	-15
		10 – 14	
		12 – 11	
		14 – 13	
	CC stretch	13 – 12	29
	CCC bend	8 – 11 – 12	12
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	
	CCC bend	8 – 11 – 12	12
		10 – 14 – 13	
		13 – 12 – 11	
		14 – 13 – 12	

Table S7: Mean signed errors (cm^{-1}) between B3LYP and B3LYP-D3 frequencies for ten LF structures.

	C_4=O	**C_2=O**	**C=C**	**C=N**
Mean signed error (cm^{-1})	+4.3	+5.8	+2.2	+2.5

B3LYP/6-31+G* Optimized Structure of LF with IEF-PCM

C	3.754453	-0.629817	-0.000243
N	3.734663	0.781001	-0.000106
C	2.622534	1.589446	0.000083
C	1.342269	0.826756	0.000097
N	2.567956	-1.304323	-0.000200
C	1.434351	-0.623714	0.000010
N	0.219366	1.494310	0.000175
C	-0.948343	0.797622	0.000140
N	0.241265	-1.305410	0.000216
C	-0.967555	-0.626358	0.000142
C	-2.172337	1.507729	0.000054
C	-3.391876	0.858626	-0.000044
C	-3.403087	-0.572924	-0.000022
C	-2.207985	-1.287447	0.000030
O	4.852704	-1.194038	-0.000369
H	4.640954	1.240897	-0.000121
O	2.701711	2.815225	0.000252
C	0.241759	-2.778835	0.000329
H	-2.116778	2.592569	0.000052
C	-4.681658	1.641339	-0.000325
C	-4.711574	-1.319344	-0.000089
H	-2.251763	-2.369359	-0.000060
H	1.272962	-3.117568	0.001466
H	-0.268709	-3.142625	-0.894703
H	-0.270647	-3.142435	0.894301
H	-5.291983	1.408621	-0.881926
H	-4.484516	2.716980	-0.000035
H	-5.292692	1.408251	0.880711
H	-4.553472	-2.400954	-0.000110
H	-5.313543	-1.060687	-0.880353
H	-5.313588	-1.060727	0.880151

B3LYP/6-31+G* Optimized Structure of LFH_2 with IEF-PCM

C	-3.758535	0.580856	0.374839
N	-3.690655	-0.789630	0.302359
C	-2.549687	-1.564908	0.019426
C	-1.370737	-0.813935	-0.254263
N	-2.557639	1.219265	0.110233
C	-1.384441	0.560227	-0.202073
N	-0.182946	-1.472193	-0.662782

C	1.017694	-0.805133	-0.333086
N	-0.244005	1.279800	-0.464632
C	1.000052	0.599711	-0.246591
C	2.220731	-1.481839	-0.137209
C	3.420214	-0.800942	0.126602
C	3.400740	0.602795	0.222843
C	2.181127	1.280317	0.046804
O	-4.784256	1.202897	0.654690
H	-4.550427	-1.294908	0.491249
O	-2.628597	-2.806564	-0.005527
C	-0.268326	2.729665	-0.634883
H	2.225617	-2.567748	-0.207290
C	4.702991	-1.579297	0.307268
C	4.659777	1.386712	0.516021
H	2.171107	2.361031	0.143215
H	-1.180004	3.024605	-1.158985
H	0.573808	3.021561	-1.265044
H	-0.200253	3.268344	0.319804
H	5.145437	-1.405390	1.296967
H	5.460357	-1.286630	-0.431880
H	4.530556	-2.654898	0.201960
H	4.453324	2.460581	0.564039
H	5.425838	1.228252	-0.254783
H	5.109819	1.089670	1.472598
H	-0.198139	-2.463666	-0.443045
H	-2.567103	2.222480	0.247455

B3LYP/6-31+G* Optimized Structure of LFH$^-$ with IEF-PCM

C	3.711807	0.651567	-0.332304
N	3.688150	-0.745018	-0.316119
C	2.573414	-1.539675	-0.034121
C	1.404487	-0.799868	0.240710
N	2.554455	1.312243	-0.087231
C	1.449392	0.592200	0.192982
N	0.210788	-1.465167	0.648913
C	-0.986734	-0.803419	0.322504
N	0.264086	1.286846	0.467727
C	-0.966728	0.606831	0.247804
C	-2.190473	-1.481843	0.127608
C	-3.397088	-0.805734	-0.119491
C	-3.382417	0.597941	-0.197453
C	-2.162461	1.278447	-0.024272
O	4.800735	1.227188	-0.583312
H	4.560512	-1.225774	-0.506979
O	2.669571	-2.798075	-0.024394
C	0.268357	2.742726	0.506763
H	-2.189546	-2.569078	0.186409
C	-4.677798	-1.589070	-0.300104
C	-4.647056	1.382164	-0.469270
H	-2.163554	2.360465	-0.102599
H	1.247292	3.080435	0.840926

H	-0.493216	3.086379	1.212962
H	0.063815	3.189165	-0.478049
H	-5.130151	-1.408544	-1.284555
H	-5.432999	-1.311236	0.447498
H	-4.498286	-2.665152	-0.208179
H	-4.443624	2.457437	-0.505304
H	-5.407355	1.213415	0.305614
H	-5.106495	1.098043	-1.425785
H	0.228582	-2.454399	0.418529

B3LYP/6-31+G* Optimized Structure of $LF^{\bullet-}$ with IEF-PCM

C	3.766637	-0.647109	0.000272
N	3.750908	0.749668	0.000304
C	2.621506	1.564018	0.000153
C	1.365928	0.840552	-0.000148
N	2.578975	-1.297617	0.000037
C	1.443294	-0.582337	-0.000164
N	0.201295	1.550561	-0.000372
C	-0.953787	0.827370	-0.000316
N	0.248984	-1.287696	-0.000370
C	-0.976623	-0.599905	-0.000130
C	-2.198671	1.503032	-0.000342
C	-3.421624	0.838072	-0.000046
C	-3.428095	-0.579291	0.000332
C	-2.206372	-1.269752	0.000247
O	4.874499	-1.230010	0.000473
H	4.650288	1.219512	0.000462
O	2.760231	2.805015	0.000160
C	0.243315	-2.749228	-0.000929
H	-2.168459	2.590432	-0.000548
C	-4.715532	1.620172	-0.000171
C	-4.725960	-1.352425	0.000906
H	-2.233219	-2.353717	0.000557
H	1.272534	-3.096100	-0.001722
H	-0.271158	-3.125749	-0.892210
H	-0.269983	-3.126556	0.890694
H	-5.329355	1.388557	-0.880744
H	-4.522571	2.697662	-0.000385
H	-5.329414	1.388949	0.880465
H	-4.542508	-2.431594	0.001554
H	-5.339696	-1.117982	-0.879487
H	-5.339518	-1.116895	0.881121

References

(1) Wille, G.; Ritter, M.; Friedemann, R.; Mäntele, W.; Hübner, G. Redox-Triggered FTIR Difference Spectra of FAD in Aqueous Solution and Bound to Flavoproteins. *Biochemistry* **2003**, *42* (50), 14814-14821. DOI: 10.1021/bi035219f.