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

Ab initio derivation of flavin hyperfine interactions in the protein magnetosensor cryptochrome – Supporting Information.

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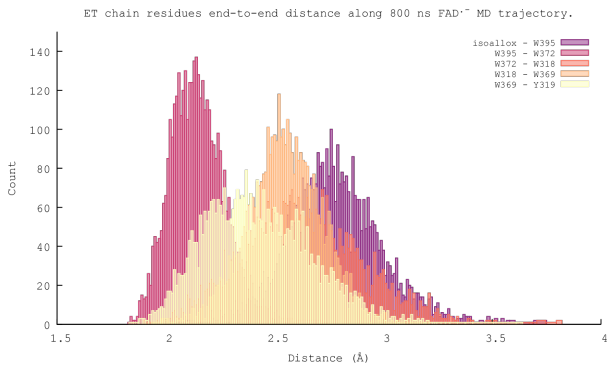
1 Experimental HFCs.

		A_{11}	A_{22}	A_{33}	$A_{\parallel}$	$A_{\perp}$	A_{iso}
		*Dm*Cry[1,2]					
$FAD^{\bullet-}$	N5	-	-	-	54.17	4.20	-
	N10	-	-	-	27.94	0.03	-
	H8α	-	-	-	-	-	12.39
$FADH^{\bullet}$	H8α	9.93	10.40	12.19	-	-	10.84
	H1'	-	-	-	-	-	-
	H5	-	-	-	-	-	-
		A. niger glucose oxidase[3]					
$FAD^{\bullet-}$	H8α	-	-	-	11.45	9.95	-
	N5	-	-	-	53.3	0.3	-
	N10	-	-	-	25.3	0.3	-
$FADH^{\bullet}$	H8α	-	-	-	8.45	6.85	-
	N5	-	-	-	53.5	0.5	-
	N10	-	-	-	30.5	0.5	-
	H5	-10.5	-33.91	-24.11	-	-	-
		X. laevis (6-4) photolyase[1,3]					
$FADH^{\bullet}$	H8α	-	-	-	8.13	6.50	-
	N5	-	-	-	51.2	0	-
	N10	-	-	-	27.2	0	-
	H5	-13.72	-38.41	-26.11	-	-	-
$FADH^{\bullet}$	H8α	6.24	6.59	8.04	-	-	6.96
	H1'	7.05	8.01	11.50	-	-	8.85
	H5	-	-25.90	-38.36	-	-	-

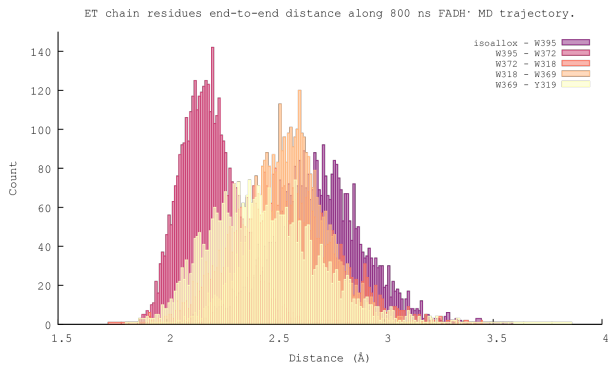
		A_{11}	A_{22}	A_{33}	$A_{\parallel}$	$A_{\perp}$	A_{iso}
		E. coli DNA photolyase[3]					
$FADH^{\bullet}$	H8α	-	-	-	8.66	6.80	-
	N5	-	-	-	50.12	0	-
	N10	-	-	-	31.72	0	-
	H5	-8.50	-37.00	-24.90	-	-	-
		C. reinhardtii Cryptochrome[2]					
$FAD^{\bullet-}$	N5	-	-	-	52.57	2.80	-
	N10	-	-	-	23.12	2.80	-
	H8α	-	-	-	-	-	10.65
		A. thaliana Cryptochrome 1[1]					
$FADH^{\bullet}$	H8α	6.85	7.90	9.00	-	-	7.91
	H1'	8.99	8.99	11.49	-	-	9.82
	H5	-	-25.00	-37.02	-	-	-
		Synechocystis Cryptochrome[1]					
$FADH^{\bullet}$	H8α	6.41	7.01	8.26	-	-	7.23
	H1'	7.84	8.55	11.39	-	-	9.26
	H5	-	-25.53	-38.36	-	-	-
		T. thermophilus CPD photolyase[1]					
$FADH^{\bullet}$	H8α	6.61	6.99	8.47	-	-	7.36
	H1'	6.41	8.70	11.75	-	-	8.62
	H5	-	-25.30	-37.49	-	-	-
		A. thaliana CPD photolyase[1]					
$FADH^{\bullet}$	H8α	6.54	7.21	8.40	-	-	7.38
	H1'	8.41	8.50	11.34	-	-	9.42
	H5	-	-24.60	-37.02	-	-	-

Table S1: Experimental hyperfine couplings of flavin atoms, measured using EPR spectroscopy on various cryptochrome, glucose oxidase and photolyase proteins. All reported values are in MHz.

2 Validation of the MD simulations



(a) $FAD^{\bullet -}$: ET chain inter-residues distances



(b) $FADH^{\bullet}$: ET chain inter-residues distances

Figure S1: Inter-residue distance of the electron-transfer chain (flavin, W395, W362, W318, W369, Y319), where the flavin is (a) $FAD^{\bullet -}$ and (b) $FADH^{\bullet}$.

3 Validation of the aDFT method.

Atom	Δa_{iso} (MHz)	$\Delta\Delta A$ (MHz)
N5	0.1516	0.1642
N10	0.0700	0.0490
H1'a	0.1260	0.0074
H1'b	0.0526	0.0042
H8α	0.0352	0.0094
H7α	0.0326	0.0078
H6	0.0270	0.0078
H5	0.2748	0.1014

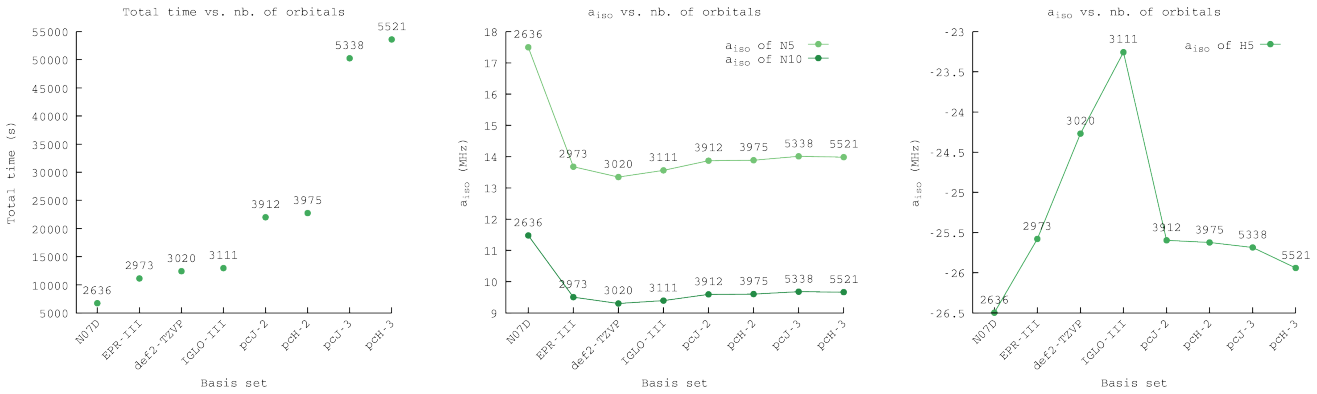
(a) $FADH^{\bullet}$

Atom	Δa_{iso} (MHz)	$\Delta\Delta A$ (MHz)
N5	0.3188	0.1160
N10	0.1282	0.0774
H1'a	0.0708	0.0054
H1'b	0.0394	0.0028
H8α	0.1382	0.0092
H7α	0.0324	0.0034
H6	0.0904	0.0836

(b) $FAD^{\bullet -}$

Table S2: Absolute difference between DFT and aDFT calculation on the same geometry, averaged over 5 **(a)** $FADH^{\bullet}$ and **(b)** $FAD^{\bullet -}$ structures.

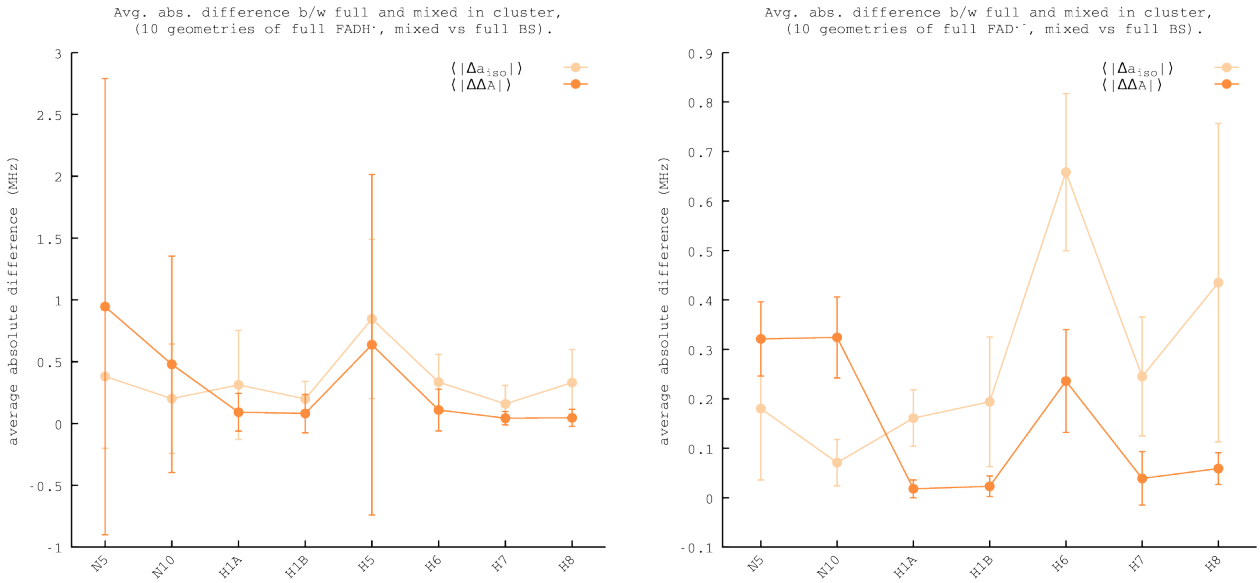
4 DFT basis set benchmark.



(a) Timings (b) a_{iso} of N5, N10 (c) a_{iso} of H5

Figure S2: **(a)** Timings and **(b,c)** a_{iso} benchmarks of various basis sets, using aDFT as implemented in deMon2k. All values calculated on an 295-atoms MD-generated conformation of FADH$^\bullet$ with a cluster of residues around. aDFT specs: B3LYP/basis/GEN-A2* for the flavin, B3LYP/DZVP-GGA/GEN-A2 on the neighbouring residues. The number of orbitals generated for the aDFT calculation are given above data points. Timings are for a single-point calculation parallelised onto 4 nodes (96 cores).

Residues included in the calculations: S250, R291, Y295, H353, R356, D385, D387, I390, N391, N394, W395, and FADH$^\bullet$ (FAH 498).
The basis sets tested here are listed in order of increasing completeness, N07D being the smallest one and pcH-3 the largest. Given that pcH-N and pcJ-N basis sets converge exponentially to the complete basis set limit, we take the a_{iso} calculated with pcH-3 to be the most accurate, and can visually estimate an extrapolation to the CBS. For the three atoms considered here, EPR-III emerged as the best compromise of computational lightness and accuracy.



(a) FADH$^{\bullet}$ (b) FAD$^{\bullet-}$

Figure S3: Mean Absolute Error and standard deviations for a_{iso} and ΔA, calculated between a full and mixed basis set, on a set of 10 MD-generated geometries of (a) FADH$^{\bullet}$ and (b) FAD$^{\bullet-}$ in their explicit cluster of residues. Magnetic properties were calculated using aDFT at the B3LYP/basis/aux-basis level, with the DZVP-GGA/GEN-A2 basis sets on the chemical environment of the flavin. For the "full basis" set, EPR-III/GEN-A2* was applied on all flavin atoms; and for "mixed basis", EPR-III/GEN-A2* was applied only on magnetically active flavin atoms (those listed in Figure S3) and DZVP-GGA/GEN-A2 on the rest.

$\langle|\Delta a_{iso}|\rangle = \frac{\sum_{i=1}^{N=10} \left|a_{iso,i}^{mixed} - a_{iso,i}^{full}\right|}{N}$, and similarly for $\langle|\Delta\Delta A|\rangle$. Both are in MHz. Using a mixed basis set speeds up the calculation by a factor of 1.9, on average.

5 Validation of the minimal cluster.

Atom	minimal cluster		full cluster		$\Delta a = a^{full} - a^{minimal}$	
	a_{iso} (MHz)	ΔA (MHz)	a_{iso} (MHz)	ΔA (MHz)	Δa_{iso} (MHz)	$\Delta\Delta$A (MHz)
H5	-24.920	35.261	-24.768	35.069	-0.152	-0.192
N5	14.161	48.680	14.062	48.395	-0.099	-0.285
N10	7.586	21.240	7.426	20.796	-0.160	-0.444
H1'a	7.407	3.679	7.299	3.630	-0.108	-0.049
H1'b	6.490	4.317	6.386	4.256	-0.104	-0.063
H8α	6.691	1.530	6.592	1.517	-0.099	-0.013
H7α	-0.665	1.222	-0.786	1.209	-0.121	-0.013
H6	1.589	3.434	1.579	3.404	-0.010	-0.030

Table S3: Comparison between HFC tensors on FADH$^{\bullet}$ in full and minimal cluster.

Atom	minimal cluster		full cluster		$\Delta a = a^{\text{full}} - a^{\text{minimal}}$	
	a_{iso} (MHz)	ΔA (MHz)	a_{iso} (MHz)	ΔA (MHz)	Δa_{iso} (MHz)	$\Delta\Delta$A (MHz)
N5	17.437	60.257	17.474	60.473	0.037	0.216
N10	5.827	18.187	5.739	18.003	-0.088	-0.184
H1'a	5.228	3.451	5.173	3.415	-0.055	-0.036
H1'b	3.492	3.869	3.452	3.837	-0.040	-0.032
H8α	8.978	2.371	8.993	2.379	0.015	0.008
H7α	-3.081	1.554	-3.093	1.556	-0.012	0.002
H6	-7.968	5.830	-7.992	5.849	-0.024	0.019

Table S4: Comparison between HFC tensors on $FAD^{\bullet-}$ in full and minimal cluster.

Δa_{iso} and $\Delta\Delta$A are reported in Table S3 for $FADH^{\bullet}$ and in Table S4 for $FAD^{\bullet-}$. They are small in magnitude and typically do not represent more than 2% of the corresponding quantity. The exception is the case of H7α on $FADH^{\bullet}$, whose very small a_{iso} value makes the relative error comparatively large. However, such a small tensor has a correspondingly minuscule impact on the spin dynamics of a magnetosensor, and so increasing the cluster size for this tensor would yield only very marginal accuracy gains.

6 Distribution of spin density in the "minimal cluster" model.

Frame index	% of spin charge delocalised onto cluster atoms	
	$FAD^{\bullet-}$	$FADH^{\bullet}$
1	1.02	0.65
2	1.39	15.01
3	0.69	0.95
4	0.66	0.73
5	0.81	9.56
6	0.95	0.69
7	1.00	1.07
8	0.74	0.54
9	0.90	0.38
10	1.04	0.92

Table S5: Percentage of the total spin charge ($S = 1$ for both FAD^{-} and $FADH^{\bullet}$) localised on non-flavin atoms. The Löwdin atomic spin charges were calculated using aDFT at the B3LYP/EPR-III/GEN-A2* level for magnetically active isoalloxazine atoms and B3LYP/DZVP-GGA/GEN-A2 for the rest, on 10 geometries of the flavin cofactor in its minimal cluster, as defined in the main text, extracted from a 800 ns MD trajectory at 80 ns interval.

7 Autocorrelation times of a_{iso} and ΔA from $FAD^{\bullet -}$ and $FADH^{\bullet}$ atoms.

atom	a_{iso} (MHz)		ΔA (MHz)	
	N_{eff}	τ (ps)	N_{eff}	τ (ps)
N5	101.0	20.0	101.0	20.0
N10	83.7	24.1	44.6	45.3
H1'b	101.0	20.0	101.0	20.0
H1'a	101.0	20.0	101.0	20.0
H5	101.0	20.0	142.4	14.2
H6	101.0	20.0	58.8	34.4
H7α	66.0	30.6	45.8	44.1
H8α	101.0	20.0	101.0	20.0

(a) $FADH^{\bullet}$ in cluster - $N = 101$, $\Delta t = 20$ ps.

atom	a_{iso} (MHz)		ΔA (MHz)	
	N_{eff}	τ (ps)	N_{eff}	τ (ps)
N5	45.0	20.0	45.0	20.0
N10	8.3	108.3	45.0	20.0
H1'b	68.2	13.2	8.7	103.5
H1'a	45.0	20.0	45.0	20.0
H6	45.0	20.0	45.0	20.0
H7α	45.0	20.0	5.0	179.8
H8α	45.0	20.0	23.3	38.6

(b) $FAD^{\bullet -}$ in cluster - $N = 45$, $\Delta t = 20$ ps.

Table S6: Autocorrelation times of a_{iso} and ΔA, calculated on relevant atoms of the (a) semiquinone and (b) radical anion flavin cofactor, in its minimal cluster as defined in the main text. The sample of structures on which these quantities were calculated are extracted from a MD trajectory, at an interval of $\Delta t = 20$ ps. N is the size of the sample; N_{eff} is the effective size of this sample, computed using the R function `coda::effectiveSize(x)`; $\tau = \Delta t \times \frac{N}{N_{eff}}$ is the autocorrelation time of the property considered.

8 RMSD of flavins' isoalloxazine heavy atoms along MD trajectories.

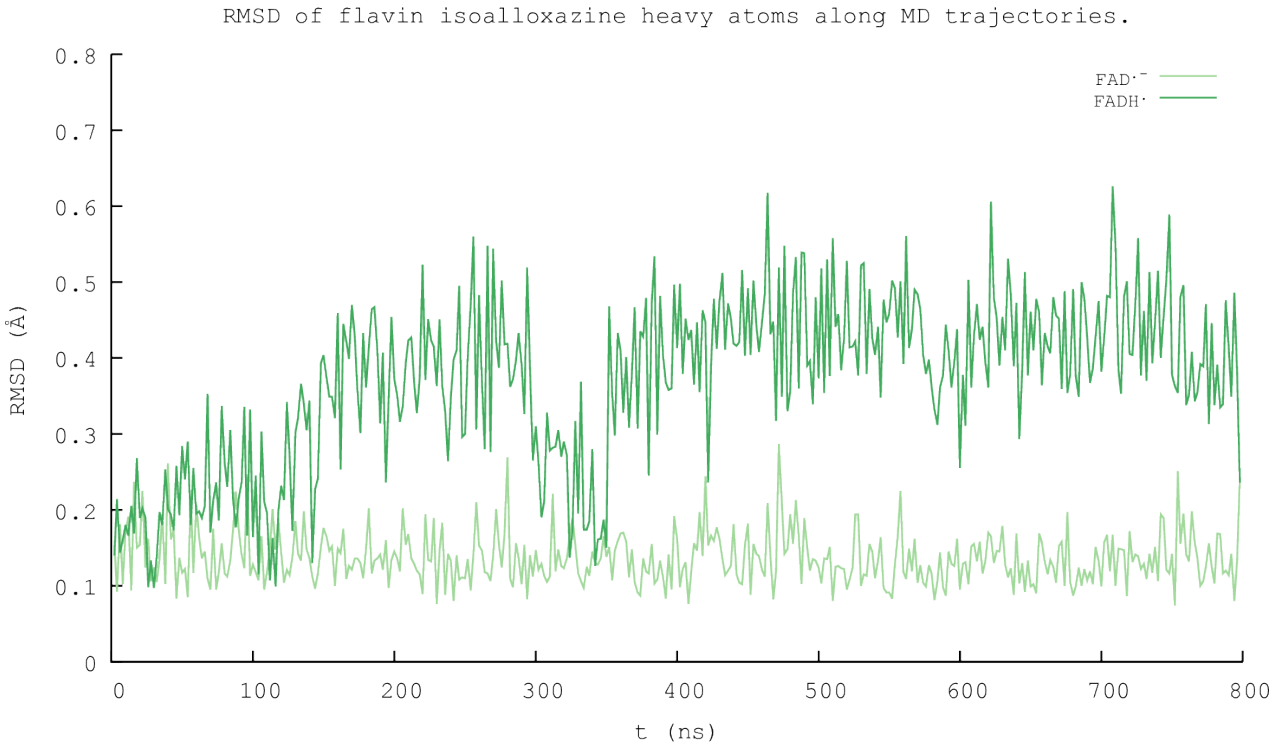


Figure S4: Root-Mean-Square Deviation of the position of flavin atoms along the MD trajectories. For relevance and clarity, only the geometries on which magnetic properties were subsequently computed are included in this analysis. The time interval between two successive data points in therefore 2 ns.

9 The static picture: flavin optimised in vacuum.

atom	A_{YY}	A_{XX}	A_{ZZ}	a_{iso}	ΔA
N5	-2.655	-3.018	50.823	15.050	53.660
N10	-0.423	-0.690	18.861	5.916	19.418
H1'a	1.781	1.284	5.351	2.805	3.819
H1'b	5.519	5.021	9.136	6.559	3.866
H8α	11.516	11.356	14.305	12.392	2.869
H7α	-4.085	-4.433	-2.487	-3.668	1.773
H6	-11.751	-13.647	-4.976	-10.125	7.723

Table S7: Principal elements, isotropic value and anisotropy of the HFC tensors, calculated on a single conformation of $FAD^{\bullet-}$ in vacuum using aDFT. The structure was obtained from a geometry optimisation at the PBEh-3c level, as implemented in Orca. All values are in MHz.

atom	A_{YY}	A_{XX}	A_{ZZ}	a_{iso}	ΔA
N5	-2.428	-2.793	41.182	11.987	43.793
N10	0.244	-0.154	21.537	7.209	21.492
H1'a	2.345	1.911	6.014	3.423	3.886
H1'b	6.555	5.670	10.043	7.423	3.930
H8α	6.924	6.754	8.749	7.476	1.910
H7α	-1.297	-1.542	-0.012	-0.950	1.407
H6	-6.114	-6.460	-1.691	-4.755	4.596
H5	-27.247	-39.191	-1.588	-22.675	31.631

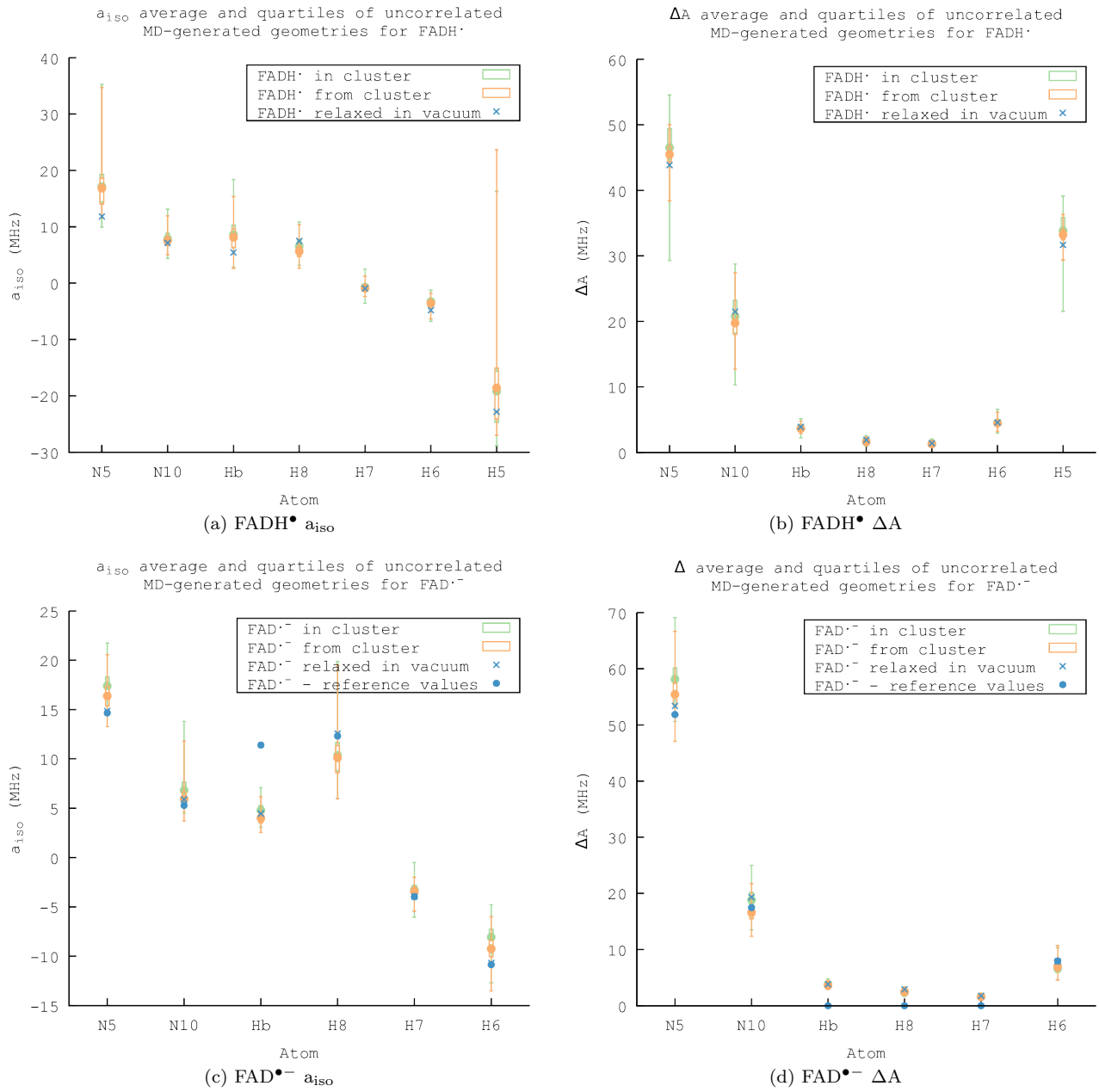
Table S8: Principal elements, isotropic value and anisotropy of the HFC tensors, calculated on a single conformation of $FADH^{\bullet}$ in vacuum using aDFT. The structure was obtained from a geometry optimisation at the PBEh-3c level, as implemented in Orca. All values are in MHz.

10 *In vacuo* lumiflavin vs. in-cluster $FAD^{\bullet-}$: principal components.

atom	A_{YY}		A_{XX}		A_{ZZ}	
	ref	$MD_{cluster}$	ref	$MD_{cluster}$	ref	$MD_{cluster}$
N5	-2.433	-1.827	-2.805	-2.125	49.237	56.176
N10	-0.404	0.666	-0.675	0.436	16.944	19.382
Hb	11.406	3.755	11.406	3.269	11.406	7.334
H8α	12.328	9.691	12.328	9.427	12.328	11.907
H7α	-3.968	-3.663	-3.968	-3.956	-3.968	-2.200
H6	-14.865	-9.769	-12.152	-10.773	-5.538	-3.649

Table S9: Eigenvalues of the diagonalised HFC tensors for atoms of $FAD^{\bullet-}$. "ref" refers to the tensors calculated in [7] ; "$MD_{cluster}$" to the average tensors calculated on the flavin in its cluster. In this latter set, $\overleftrightarrow{\text{Hb}} = (\overleftrightarrow{\text{H1'a}} + \overleftrightarrow{\text{H1'b}})/2$. All values are in MHz.

11 Flavin isotropic HFCs and tensor anisotropy.

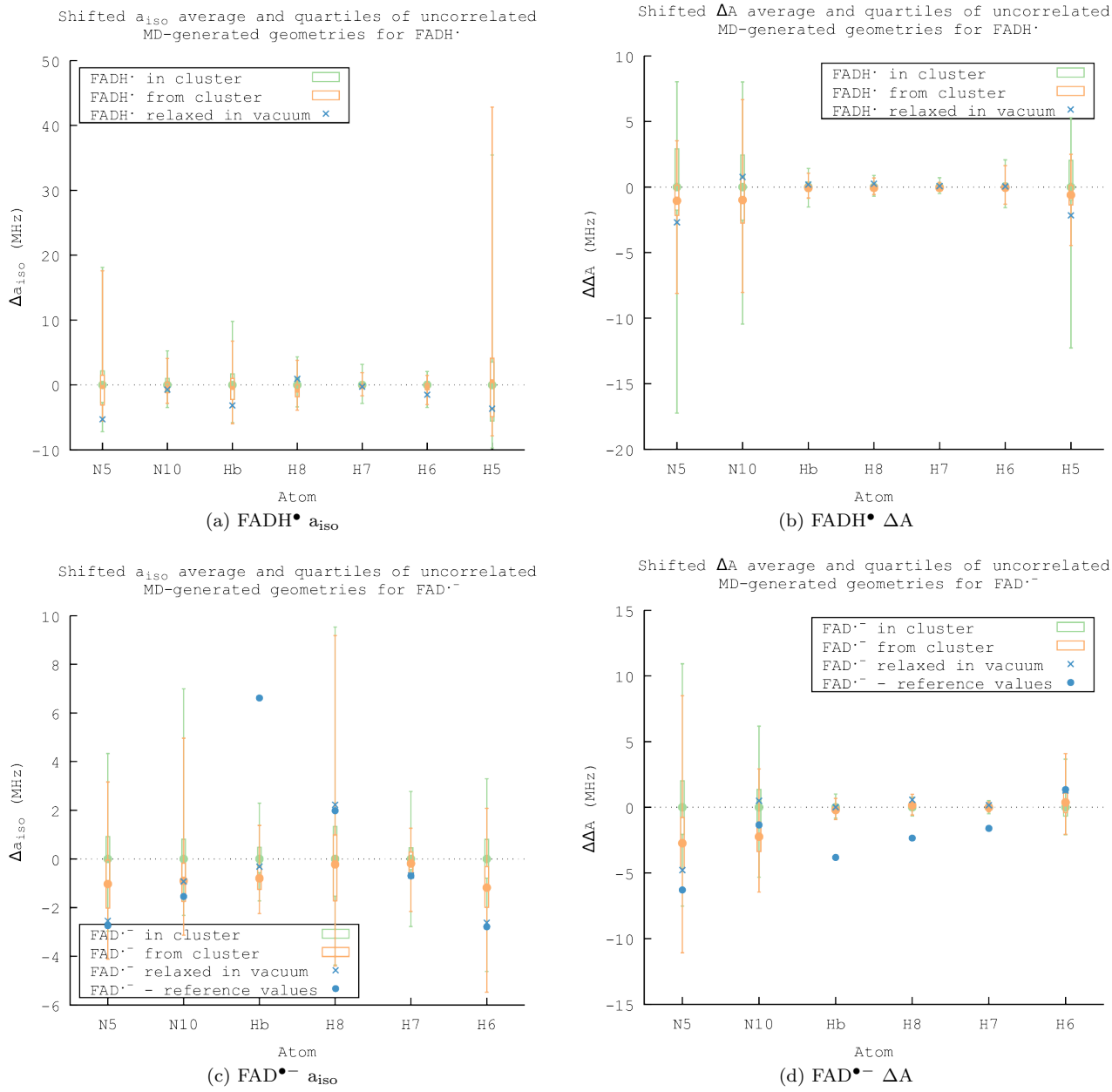


(a) FADH$^{\bullet}$ a_{iso} (b) FADH$^{\bullet}$ ΔA

(c) FAD$^{\bullet-}$ a_{iso} (d) FAD$^{\bullet-}$ ΔA

Figure S5: average (dot) and quartiles (box) of the isotropic HFC and tensor anisotropy for selected atoms on (**a,b**) FADH$^{\bullet}$ and (**c,d**) FAD$^{\bullet-}$. Vertical bars span the entire distribution, locating the minimum and maximum value of a given sample. Green and orange data points were obtained on geometries taken from an MD run, while data points represented by blue crosses were calculated on a flavin optimised in vacuum using the PBEh-3c scheme. The dihedral angle of its ribityl chain with respect to the isoalloxazine moiety was constrained throughout the optimisation. Blue dots correspond to reference values calculated by Hiscock *et al.*[4]

12 Shifted representation of flavin isotropic HFCs and tensor anisotropy.

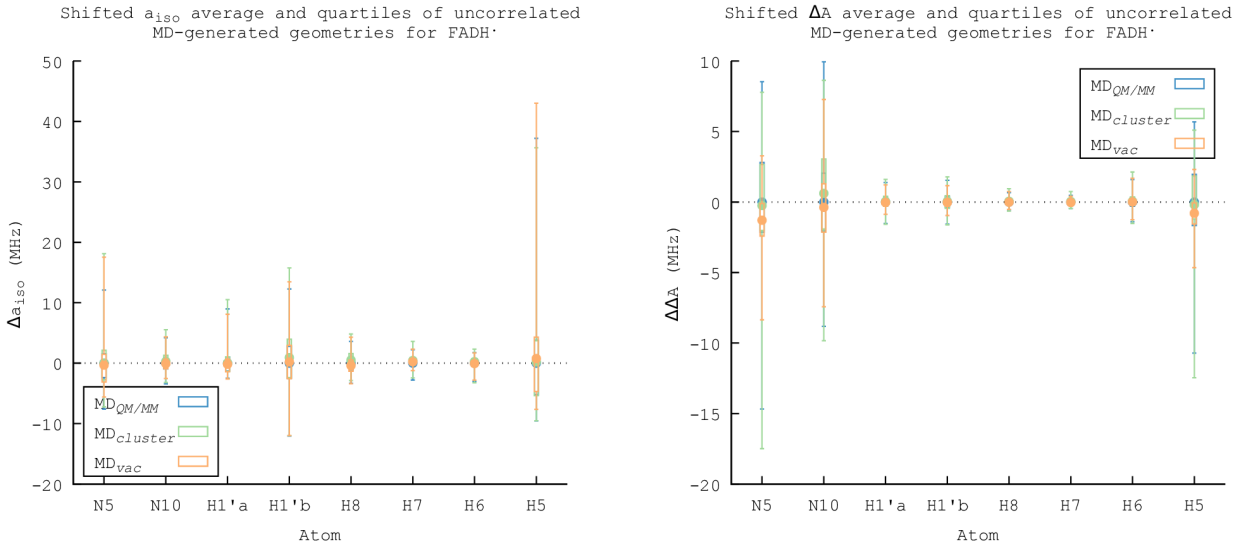


(a) FADH• a_{iso} (b) FADH• ΔA

(c) FAD•⁻ a_{iso} (d) FAD•⁻ ΔA

Figure S6: Shifted average (dot) and quartiles (box) of the **(a,c)** isotropic HFC and **(b,d)** tensor anisotropy for selected atoms on FADH• and FAD•⁻. The average value of the flavin in cluster was subtracted from all values so as to better show the magnitude of the shift induced by polarisation. To regenerate values reported in Figure *S*5, apply: $a_{\text{iso,aniso}} = \text{average}(a_{\text{iso,aniso}}^{\text{atom,in-cluster}}) + \Delta a_{\text{iso,aniso}}$.

13 Shifted representation of flavin isotropic HFCs and tensor anisotropy, including QM/MM tensors.



(a) FADH• a_{iso}

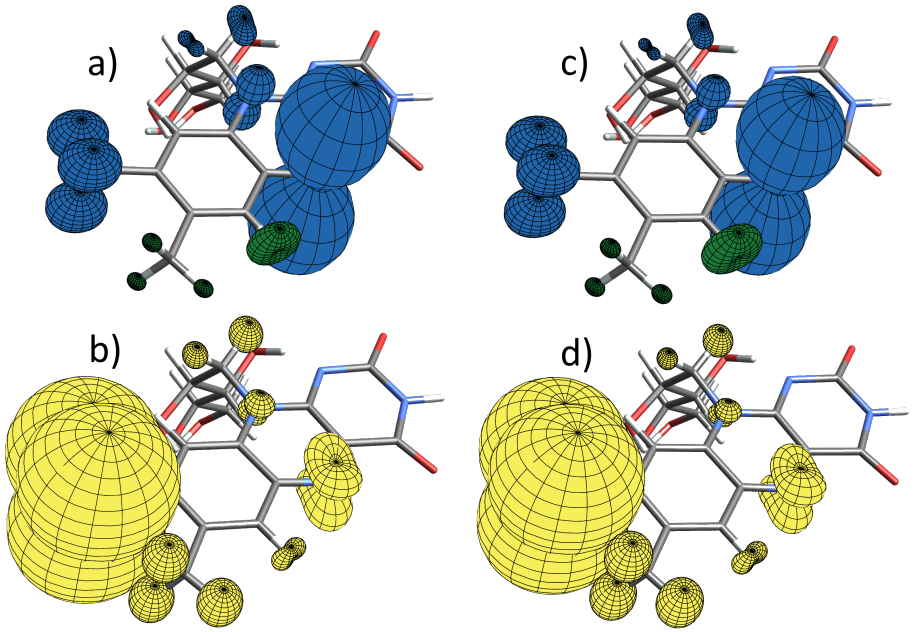
(b) FADH• ΔA

(c) FAD•− a_{iso}

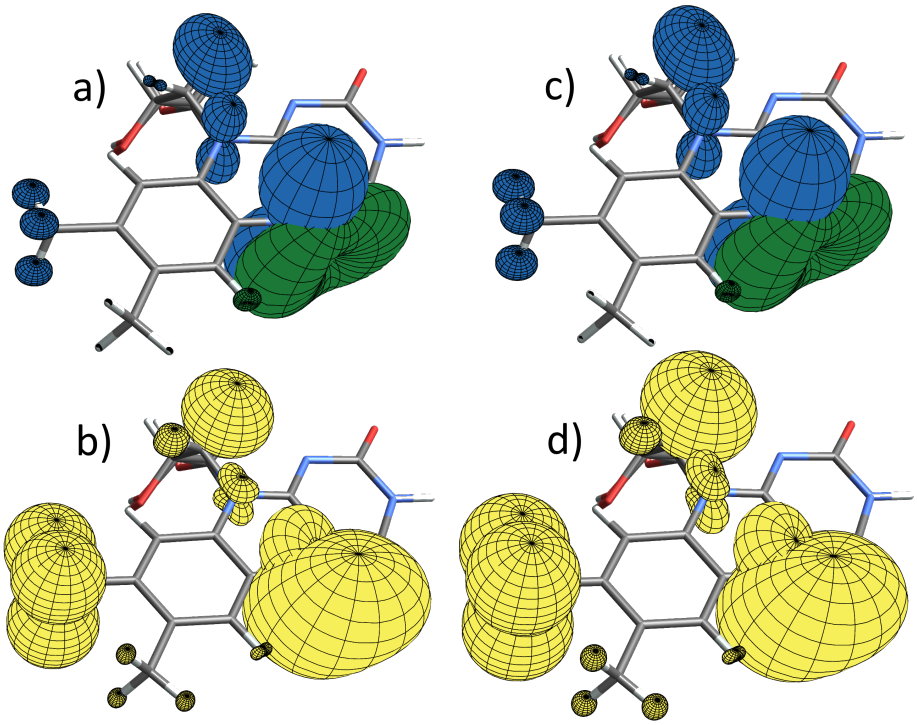
(d) FAD•− ΔA

Figure S7: Shifted average (dot) and quartiles (box) of the **(a,c)** isotropic HFC and **(b,d)** tensor anisotropy for selected atoms on FADH• and FAD•−. The average value of the flavin in cluster was subtracted from all values so as to better show the magnitude of the shift induced by polarisation. To regenerate values reported in Figure *S*5, apply: $a_{\mathrm{iso,aniso}} = \mathrm{average}(a_{\mathrm{iso,aniso}}^{\mathrm{atom,in\text{-}cluster}}) + \Delta a_{\mathrm{iso,aniso}}$.

14 Visual representation of the flavin isotropic HFCs and of its variance, in and out of its minimal cluster.



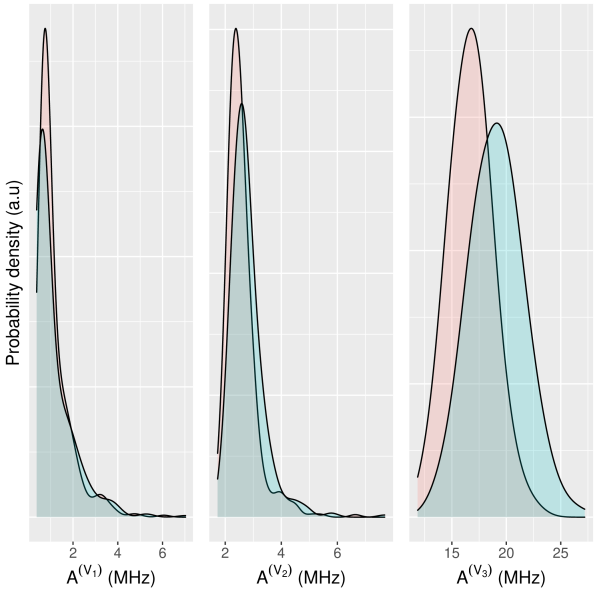
(a) $FAD^{\bullet -}$



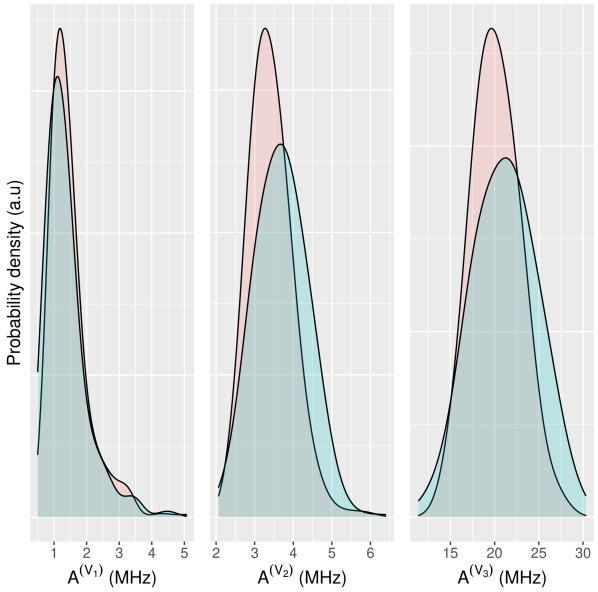
(b) $FADH^{\bullet}$

Figure S8: Graphical representation of average $FAD^{\bullet -}$ and $FADH^{\bullet}$ hyperfine coupling tensors, calculated using aDFT on MD-generated geometries **(a)** without and **(c)** with a minimal chemical environment. The variance of average tensor elements is also plotted in a similar fashion, again **(b)** out of (MD*vac* dataset) and **(d)** in (MD*cluster* dataset) its minimal cluster.

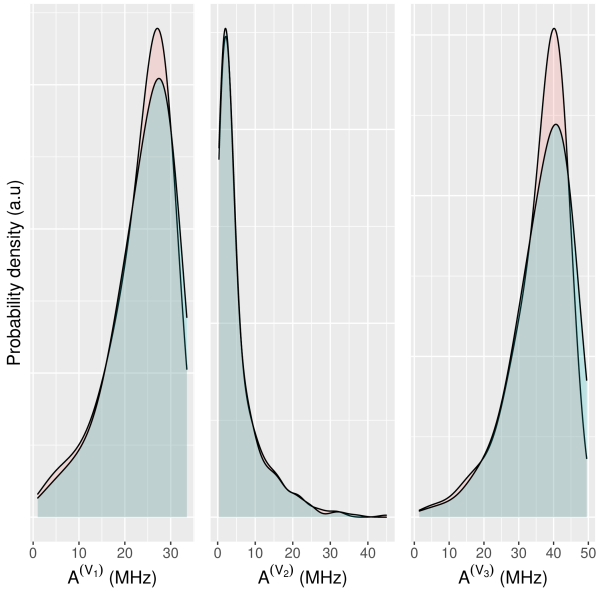
15 Projections of HFC tensors onto average eigenbasis.



(a) $FAD^{\bullet -}$ N10



(b) $FADH^{\bullet}$ N10



(c) $FADH^{\bullet}$ H5

Figure S9: Projection of (a) $FAD^{\bullet -}$ N10, (b) $FADH^{\bullet}$ N10 and (c) $FADH^{\bullet}$ H5 HFC tensors onto their average eigenvectors V_i. Blue (resp. red) histograms are for calculations with (resp. without) inclusion of the environment, *i.e* in the $MD_{cluster}$ (resp. MD_{vac}) dataset.

16 Geometrical origin to extreme a_{iso} values: H5 improper angle.

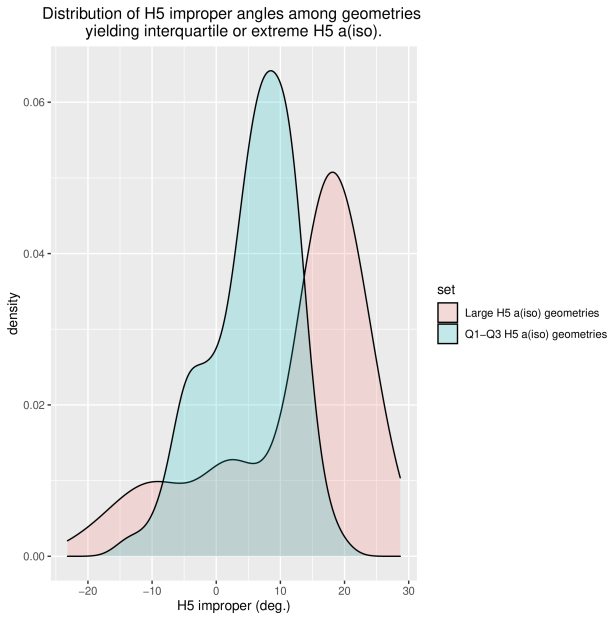


Figure S10: Density plots of H5 improper angle (out-of-planeness) for geometries yielding respectively interquartile (Q1-Q3, in blue) or large (>Q3, in red) a_{iso} values of H5.

17 Statistical uncertainties on a_{iso} and ΔA in $MD_{cluster}$ and $MD_{QM/MM}$ datasets.

Atom	MD_{vac}: $N_{tot} = 400$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	400	0.067	0.069	0.339	0.158	0.068
N10	332	0.105	0.107	0.211	0.141	0.075
H1'a	270	0.105	0.103	0.131	0.113	0.051
H1'b	293	0.179	0.155	0.188	0.174	0.121
H8α	400	0.183	0.182	0.201	0.189	0.142
H7α	400	0.061	0.062	0.07	0.060	0.014
H6	400	0.124	0.182	0.067	0.124	0.059

(a) $FAD^{\bullet -}$ MD_{vac}

Atom	MD_{vac}: $N_{tot} = 400$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	368	0.397	0.397	0.356	0.383	0.587
N10	400	0.074	0.074	0.261	0.136	0.058
H1'a	322	0.181	0.152	0.178	0.170	0.116
H1'b	27	1.502	0.423	1.659	1.195	5.493
H8α	400	0.122	0.119	0.135	0.125	0.063
H7α	400	0.051	0.051	0.052	0.051	0.010
H6	400	0.081	0.075	0.063	0.073	0.021
H5	400	0.717	0.817	0.723	0.752	2.263

(b) $FADH^{\bullet}$ MD_{vac}

Atom	$MD_{cluster}$: $N_{tot} = 386$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	386	0.069	0.071	0.319	0.153	0.066
N10	223	0.114	0.116	0.314	0.181	0.114
H1'a	258	0.150	0.149	0.164	0.154	0.095
H1'b	386	0.172	0.172	0.179	0.175	0.122
H8α	230	0.255	0.253	0.266	0.258	0.266
H7α	386	0.079	0.080	0.071	0.077	0.024
H6	386	0.130	0.165	0.072	0.123	0.057

(c) $FAD^{\bullet -}$ $MD_{cluster}$

Atom	$MD_{cluster}$: $N_{tot} = 389$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	57	0.511	0.512	1.346	0.789	2.183
N10	50	0.076	0.075	0.976	0.376	0.204
H1'a	217	0.198	0.187	0.252	0.212	0.179
H1'b	24	1.918	2.074	2.190	2.061	16.970
H8α	172	0.213	0.206	0.251	0.224	0.199
H7α	201	0.116	0.121	0.110	0.116	0.054
H6	320	0.089	0.093	0.064	0.082	0.027
H5	389	0.705	0.837	0.704	0.749	2.240

(d) $FADH^{\bullet}$ $MD_{cluster}$

Atom	$MD_{QM/MM}$: $N_{tot} = 396$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	326	0.074	0.078	0.326	0.159	0.074
N10	290	0.109	0.111	0.259	0.160	0.092
H1'a	280	0.125	0.122	0.154	0.134	0.071
H1'b	339	0.189	0.189	0.181	0.186	0.139
H8α	396	0.183	0.181	0.199	0.188	0.141
H7α	396	0.072	0.073	0.065	0.070	0.020
H6	396	0.129	0.169	0.072	0.123	0.058

(e) $FAD^{\bullet -}$ $MD_{QM/MM}$

Atom	$MD_{QM/MM}$: $N_{tot} = 369$					
	Lowest N_{eff}	$U(A_{yy})$	$U(A_{xx})$	$U(A_{zz})$	$U(a_{iso})$	$U(\Delta A)$
N5	369	0.371	0.372	0.463	0.402	0.643
N10	199	0.074	0.081	0.429	0.195	0.097
H1'a	369	0.176	0.167	0.179	0.174	0.121
H1'b	22	1.742	1.899	1.888	1.843	13.584
H8α	369	0.138	0.134	0.152	0.141	0.080
H7α	307	0.086	0.088	0.068	0.081	0.026
H6	369	0.088	0.083	0.064	0.078	0.024
H5	369	0.712	0.848	0.704	0.755	2.276

(f) $FADH^{\bullet}$ $MD_{QM/MM}$

Table S10: Statistical uncertainties, in MHz, on the average values of a_{iso} and ΔA for every atom of interest on the flavin, from the MD_{vac}, $MD_{cluster}$ and $MD_{QM/MM}$ datasets. For each table, N_{tot} is the number of data points, *i.e* the number of structures for which the HFC calculation converged successfully. "Lowest N_{eff}" is, for each atom, the smallest N_{eff} among the samples of A_{yy}, A_{xx} and A_{zz} values. This quantity is reported because the uncertainties on a_{iso} and ΔA, are calculated from those on the the tensors' eigenvalues $A_{ii\in\{x,y,z\}}$.

18 Average values of the full HFC tensor.

All values reported below are in MHz.

18.1 $FAD^{\bullet -}$ out of cluster (MD_{vac}).

$$N5 = \begin{pmatrix} -1.904 & 0.038 & -1.140 \\ 0.044 & -1.665 & -2.467 \\ -1.141 & -2.467 & 52.707 \end{pmatrix} \qquad N10 = \begin{pmatrix} 0.976 & -0.073 & 2.398 \\ -0.077 & 0.398 & -0.839 \\ 2.396 & -0.839 & 16.421 \end{pmatrix}$$

$$Hb2 = \begin{pmatrix} 6.965 & -0.532 & -1.236 \\ -0.532 & 4.434 & 0.302 \\ -1.237 & 0.302 & 4.297 \end{pmatrix} \qquad Hb1 = \begin{pmatrix} 4.470 & 0.915 & -1.112 \\ 0.913 & 2.050 & -0.322 \\ -1.113 & -0.323 & 1.742 \end{pmatrix}$$

$$H6 = \begin{pmatrix} -4.931 & -1.282 & 0.217 \\ -1.281 & -11.790 & -0.269 \\ 0.217 & -0.269 & -11.007 \end{pmatrix} \qquad H7\alpha = \begin{pmatrix} -3.598 & -0.533 & -0.001 \\ -0.533 & -2.712 & 0.039 \\ -0.001 & 0.039 & -4.073 \end{pmatrix}$$

$$H8\alpha = \begin{pmatrix} 9.890 & 0.835 & 0.087 \\ 0.835 & 11.010 & 0.158 \\ 0.087 & 0.158 & 9.453 \end{pmatrix}$$

18.2 $FAD^{\bullet-}$ in cluster ($MD_{cluster}$).

$$\text{N5} = \begin{pmatrix} -1.744 & 0.054 & -1.406 \\ 0.059 & -1.555 & -2.688 \\ -1.407 & -2.688 & 55.522 \end{pmatrix} \quad \text{N10} = \begin{pmatrix} 1.133 & -0.098 & 2.528 \\ -0.102 & 0.557 & -0.978 \\ 2.527 & -0.978 & 18.794 \end{pmatrix}$$

$$\text{Hb1} = \begin{pmatrix} 5.410 & 0.996 & -1.160 \\ 0.994 & 2.809 & -0.345 \\ -1.162 & -0.346 & 2.520 \end{pmatrix} \quad \text{Hb2} = \begin{pmatrix} 7.760 & -0.671 & -1.277 \\ -0.671 & 5.192 & 0.384 \\ -1.278 & 0.385 & 5.024 \end{pmatrix}$$

$$\text{H6} = \begin{pmatrix} -4.180 & -1.606 & 0.146 \\ -1.606 & -9.984 & -0.227 \\ 0.146 & -0.228 & -10.028 \end{pmatrix} \quad \text{H7}\alpha = \begin{pmatrix} -3.404 & -0.558 & 0.001 \\ -0.558 & -2.530 & 0.036 \\ 0.001 & 0.036 & -3.886 \end{pmatrix}$$

$$\text{H8}\alpha = \begin{pmatrix} 10.132 & 0.754 & 0.076 \\ 0.754 & 11.155 & 0.139 \\ 0.076 & 0.139 & 9.738 \end{pmatrix}$$

18.3 $FAD^{\bullet-}$ in QM/MM ($MD_{QM/MM}$).

$$\text{N5} = \begin{pmatrix} -1.813 & -0.001 & -1.687 \\ 0.004 & -1.731 & -0.375 \\ -1.689 & -0.375 & 53.680 \end{pmatrix} \quad \text{N10} = \begin{pmatrix} 1.188 & 0.019 & 2.496 \\ 0.016 & 0.591 & -0.205 \\ 2.495 & -0.205 & 18.868 \end{pmatrix}$$

$$\text{Hb1} = \begin{pmatrix} 5.168 & 0.920 & -1.159 \\ 0.918 & 2.642 & -0.342 \\ -1.160 & -0.342 & 2.389 \end{pmatrix} \quad \text{Hb2} = \begin{pmatrix} 8.030 & -0.677 & -1.211 \\ -0.676 & 5.551 & 0.355 \\ -1.212 & 0.356 & 5.326 \end{pmatrix}$$

$$\text{H6} = \begin{pmatrix} -4.420 & -1.419 & 0.218 \\ -1.418 & -10.578 & -0.235 \\ 0.219 & -0.235 & -10.321 \end{pmatrix} \quad \text{H7}\alpha = \begin{pmatrix} -3.362 & -0.554 & 0.024 \\ -0.554 & -2.503 & -0.020 \\ 0.024 & -0.020 & -3.849 \end{pmatrix}$$

$$\text{H8}\alpha = \begin{pmatrix} 9.616 & 0.778 & 0.053 \\ 0.778 & 10.707 & 0.091 \\ 0.053 & 0.091 & 9.218 \end{pmatrix}$$

18.4 $FADH^{\bullet}$ out of cluster (MD_{vac}).

$$\text{N5} = \begin{pmatrix} 2.162 & -0.160 & 2.250 \\ -0.163 & 1.885 & -0.812 \\ 2.250 & -0.812 & 46.440 \end{pmatrix} \quad \text{N10} = \begin{pmatrix} 1.965 & 0.083 & -3.130 \\ 0.080 & 0.864 & -0.032 \\ -3.129 & -0.032 & 19.667 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 16.208 & 0.046 & -0.797 \\ 0.045 & 12.979 & 0.136 \\ -0.797 & 0.136 & 12.819 \end{pmatrix} \quad \text{Hb1} = \begin{pmatrix} 3.800 & 1.258 & 0.457 \\ 1.256 & 1.767 & 0.226 \\ 0.457 & 0.226 & 1.175 \end{pmatrix}$$

$$\text{H5} = \begin{pmatrix} 2.867 & -2.024 & 0.135 \\ -2.016 & -35.803 & -0.136 \\ 0.137 & -0.136 & -22.836 \end{pmatrix} \quad \text{H6} = \begin{pmatrix} -2.184 & -1.928 & 0.003 \\ -1.929 & -3.184 & -0.092 \\ 0.003 & -0.092 & -5.384 \end{pmatrix}$$

$$\text{H7}\alpha = \begin{pmatrix} -1.060 & -0.405 & 0.046 \\ -0.405 & -0.288 & -0.127 \\ 0.046 & -0.127 & -1.354 \end{pmatrix} \quad \text{H8}\alpha = \begin{pmatrix} 5.515 & 0.522 & -0.106 \\ 0.522 & 6.205 & -0.191 \\ -0.106 & -0.191 & 5.247 \end{pmatrix}$$

18.5 $FADH^{\bullet}$ in cluster ($MD_{cluster}$).

$$N5 = \begin{pmatrix} 2.154 & -0.125 & 2.656 \\ -0.127 & 1.839 & -0.842 \\ 2.657 & -0.842 & 47.393 \end{pmatrix} \quad N10 = \begin{pmatrix} 2.080 & 0.053 & -3.477 \\ 0.050 & 0.889 & 0.016 \\ -3.476 & 0.016 & 20.597 \end{pmatrix}$$

$$Hb1 = \begin{pmatrix} 4.062 & 1.245 & 0.502 \\ 1.243 & 1.898 & 0.236 \\ 0.503 & 0.237 & 1.353 \end{pmatrix} \quad Hb2 = \begin{pmatrix} 16.990 & -0.067 & -0.801 \\ -0.067 & 13.653 & 0.142 \\ -0.801 & 0.142 & 13.576 \end{pmatrix}$$

$$H5 = \begin{pmatrix} 2.738 & -1.780 & -0.173 \\ -1.774 & -36.707 & -0.110 \\ -0.171 & -0.111 & -23.508 \end{pmatrix} \quad H6 = \begin{pmatrix} -1.970 & -1.969 & 0.036 \\ -1.969 & -2.769 & -0.124 \\ 0.036 & -0.125 & -5.118 \end{pmatrix}$$

$$H7\alpha = \begin{pmatrix} -0.818 & -0.454 & 0.055 \\ -0.454 & -0.073 & -0.134 \\ 0.055 & -0.134 & -1.160 \end{pmatrix} \quad H8\alpha = \begin{pmatrix} 6.412 & 0.525 & -0.112 \\ 0.525 & 7.086 & -0.201 \\ -0.112 & -0.201 & 6.135 \end{pmatrix}$$

18.6 $FADH^{\bullet}$ in QM/MM ($MD_{QM/MM}$).

$$N5 = \begin{pmatrix} 2.091 & -0.133 & 2.724 \\ -0.134 & 1.787 & -0.880 \\ 2.725 & -0.881 & 47.580 \end{pmatrix} \quad N10 = \begin{pmatrix} 1.965 & 0.059 & -3.344 \\ 0.056 & 0.798 & -0.016 \\ -3.343 & -0.016 & 19.941 \end{pmatrix}$$

$$Hb2 = \begin{pmatrix} 16.053 & -0.031 & -0.757 \\ -0.031 & 12.738 & 0.114 \\ -0.757 & 0.114 & 12.638 \end{pmatrix} \quad Hb1 = \begin{pmatrix} 4.025 & 1.229 & 0.504 \\ 1.227 & 1.861 & 0.235 \\ 0.505 & 0.235 & 1.353 \end{pmatrix}$$

$$H5 = \begin{pmatrix} 2.659 & -1.843 & -0.271 \\ -1.838 & -36.927 & -0.124 \\ -0.269 & -0.124 & -23.830 \end{pmatrix} \quad H6 = \begin{pmatrix} -2.160 & -1.938 & 0.040 \\ -1.939 & -3.101 & -0.092 \\ 0.040 & -0.092 & -5.323 \end{pmatrix}$$

$$H7\alpha = \begin{pmatrix} -1.268 & -0.436 & 0.054 \\ -0.436 & -0.512 & -0.131 \\ 0.054 & -0.131 & -1.586 \end{pmatrix}$$

$$H8\alpha = \begin{pmatrix} 5.911 & 0.522 & -0.110 \\ 0.522 & 6.586 & -0.196 \\ -0.110 & -0.197 & 5.644 \end{pmatrix}$$

19 Standard deviations of the full HFC tensor.

All values reported below are in MHz.

19.1 $FAD^{\bullet -}$ out of cluster (MD_{vac}).

$$N5 = \begin{pmatrix} 0.842 & 0.304 & 4.200 \\ 0.304 & 0.729 & 2.931 \\ 4.201 & 2.932 & 3.442 \end{pmatrix} \quad N10 = \begin{pmatrix} 1.287 & 0.172 & 1.250 \\ 0.172 & 1.108 & 0.995 \\ 1.250 & 0.995 & 1.874 \end{pmatrix}$$

$$Hb2 = \begin{pmatrix} 1.565 & 0.176 & 0.259 \\ 0.176 & 1.546 & 0.104 \\ 0.259 & 0.104 & 1.679 \end{pmatrix} \quad Hb1 = \begin{pmatrix} 1.151 & 0.165 & 0.294 \\ 0.165 & 1.031 & 0.152 \\ 0.294 & 0.152 & 1.049 \end{pmatrix}$$

$$\text{H6} = \begin{pmatrix} 0.572 & 0.509 & 0.605 \\ 0.509 & 2.037 & 0.413 \\ 0.605 & 0.413 & 1.189 \end{pmatrix} \qquad \text{H7}\alpha = \begin{pmatrix} 2.213 & 0.163 & 0.160 \\ 0.162 & 2.380 & 0.264 \\ 0.160 & 0.264 & 2.264 \end{pmatrix}$$

$$\text{H8}\alpha = \begin{pmatrix} 7.028 & 0.335 & 0.246 \\ 0.335 & 7.380 & 0.495 \\ 0.246 & 0.496 & 7.200 \end{pmatrix}$$

19.2 $FAD^{\bullet -}$ in cluster ($MD_{cluster}$).

$$\text{N5} = \begin{pmatrix} 0.852 & 0.322 & 4.339 \\ 0.322 & 0.727 & 3.076 \\ 4.340 & 3.077 & 3.220 \end{pmatrix} \qquad \text{N10} = \begin{pmatrix} 1.355 & 0.188 & 1.391 \\ 0.188 & 1.175 & 1.152 \\ 1.391 & 1.152 & 2.313 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 1.728 & 0.202 & 0.269 \\ 0.202 & 1.698 & 0.122 \\ 0.270 & 0.122 & 1.828 \end{pmatrix} \qquad \text{Hb1} = \begin{pmatrix} 1.371 & 0.183 & 0.301 \\ 0.183 & 1.259 & 0.155 \\ 0.301 & 0.155 & 1.279 \end{pmatrix}$$

$$\text{H6} = \begin{pmatrix} 0.573 & 0.436 & 0.572 \\ 0.436 & 1.939 & 0.377 \\ 0.572 & 0.378 & 1.175 \end{pmatrix} \qquad \text{H7}\alpha = \begin{pmatrix} 2.113 & 0.156 & 0.158 \\ 0.156 & 2.239 & 0.249 \\ 0.158 & 0.249 & 2.177 \end{pmatrix}$$

$$\text{H8}\alpha = \begin{pmatrix} 7.234 & 0.327 & 0.262 \\ 0.327 & 7.607 & 0.507 \\ 0.262 & 0.507 & 7.493 \end{pmatrix}$$

19.3 $FAD^{\bullet -}$ in QM/MM ($MD_{QM/MM}$).

$$\text{N5} = \begin{pmatrix} 0.858 & 0.250 & 4.257 \\ 0.251 & 0.714 & 2.944 \\ 4.258 & 2.944 & 3.302 \end{pmatrix} \qquad \text{N10} = \begin{pmatrix} 1.300 & 0.174 & 1.376 \\ 0.174 & 1.127 & 1.141 \\ 1.376 & 1.141 & 2.182 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 1.763 & 0.188 & 0.259 \\ 0.188 & 1.753 & 0.119 \\ 0.260 & 0.119 & 1.875 \end{pmatrix} \qquad \text{Hb1} = \begin{pmatrix} 1.341 & 0.171 & 0.293 \\ 0.171 & 1.238 & 0.142 \\ 0.293 & 0.142 & 1.254 \end{pmatrix}$$

$$\text{H6} = \begin{pmatrix} 0.602 & 0.463 & 0.567 \\ 0.463 & 1.968 & 0.378 \\ 0.567 & 0.378 & 1.183 \end{pmatrix} \qquad \text{H7}\alpha = \begin{pmatrix} 0.700 & 0.078 & 0.075 \\ 0.078 & 0.693 & 0.122 \\ 0.075 & 0.122 & 0.733 \end{pmatrix}$$

$$\text{H8}\alpha = \begin{pmatrix} 1.898 & 0.102 & 0.097 \\ 0.102 & 1.944 & 0.169 \\ 0.097 & 0.169 & 1.867 \end{pmatrix}$$

19.4 $FADH^{\bullet}$ out of cluster (MD_{vac}).

$$\text{N5} = \begin{pmatrix} 4.007 & 0.304 & 4.443 \\ 0.304 & 4.077 & 2.379 \\ 4.443 & 2.379 & 3.664 \end{pmatrix} \qquad \text{N10} = \begin{pmatrix} 0.919 & 0.260 & 2.722 \\ 0.260 & 0.763 & 1.168 \\ 2.721 & 1.169 & 2.646 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 4.419 & 0.377 & 0.328 \\ 0.377 & 4.154 & 0.160 \\ 0.328 & 0.161 & 4.347 \end{pmatrix} \qquad \text{Hb1} = \begin{pmatrix} 1.596 & 0.172 & 0.695 \\ 0.172 & 1.645 & 0.288 \\ 0.696 & 0.288 & 1.628 \end{pmatrix}$$

$$\text{H5} = \begin{pmatrix} 7.054 & 2.513 & 3.258 \\ 2.514 & 8.336 & 0.823 \\ 3.258 & 0.822 & 7.679 \end{pmatrix} \qquad \text{H6} = \begin{pmatrix} 0.379 & 0.171 & 0.309 \\ 0.171 & 1.162 & 0.306 \\ 0.309 & 0.306 & 0.736 \end{pmatrix}$$

$$\text{H7}\alpha = \begin{pmatrix} 0.828 & 0.086 & 0.100 \\ 0.086 & 0.868 & 0.209 \\ 0.100 & 0.209 & 0.829 \end{pmatrix} \qquad \text{H8}\alpha = \begin{pmatrix} 4.063 & 0.169 & 0.182 \\ 0.170 & 4.243 & 0.325 \\ 0.182 & 0.325 & 4.279 \end{pmatrix}$$

19.5 FADH$^\bullet$ in cluster ($\mathbf{MD}_{cluster}$).

$$\text{N5} = \begin{pmatrix} 3.701 & 0.316 & 4.511 \\ 0.316 & 3.784 & 2.427 \\ 4.510 & 2.427 & 5.300 \end{pmatrix} \qquad \text{N10} = \begin{pmatrix} 0.974 & 0.274 & 2.885 \\ 0.274 & 0.764 & 1.198 \\ 2.884 & 1.198 & 3.296 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 5.505 & 0.395 & 0.313 \\ 0.395 & 5.023 & 0.161 \\ 0.313 & 0.161 & 5.221 \end{pmatrix} \qquad \text{Hb1} = \begin{pmatrix} 1.790 & 0.230 & 0.709 \\ 0.230 & 1.811 & 0.280 \\ 0.709 & 0.281 & 1.802 \end{pmatrix}$$

$$\text{H5} = \begin{pmatrix} 6.843 & 2.578 & 3.253 \\ 2.579 & 8.407 & 0.808 \\ 3.254 & 0.808 & 7.381 \end{pmatrix} \qquad \text{H6} = \begin{pmatrix} 0.399 & 0.239 & 0.308 \\ 0.239 & 1.176 & 0.300 \\ 0.308 & 0.300 & 0.829 \end{pmatrix}$$

$$\text{H7}\alpha = \begin{pmatrix} 1.066 & 0.116 & 0.115 \\ 0.116 & 1.069 & 0.220 \\ 0.115 & 0.220 & 1.077 \end{pmatrix} \qquad \text{H8}\alpha = \begin{pmatrix} 4.715 & 0.193 & 0.213 \\ 0.193 & 4.906 & 0.356 \\ 0.213 & 0.356 & 4.984 \end{pmatrix}$$

19.6 FADH$^\bullet$ in QM/MM ($\mathbf{MD}_{QM/MM}$).

$$\text{N5} = \begin{pmatrix} 3.561 & 0.315 & 4.490 \\ 0.315 & 3.646 & 2.424 \\ 4.490 & 2.424 & 4.665 \end{pmatrix} \qquad \text{N10} = \begin{pmatrix} 0.911 & 0.259 & 2.741 \\ 0.259 & 0.737 & 1.140 \\ 2.740 & 1.140 & 2.994 \end{pmatrix}$$

$$\text{Hb2} = \begin{pmatrix} 4.748 & 0.392 & 0.321 \\ 0.392 & 4.387 & 0.158 \\ 0.321 & 0.159 & 4.558 \end{pmatrix} \qquad \text{Hb1} = \begin{pmatrix} 1.676 & 0.204 & 0.692 \\ 0.204 & 1.717 & 0.271 \\ 0.693 & 0.271 & 1.708 \end{pmatrix}$$

$$\text{H5} = \begin{pmatrix} 6.692 & 2.570 & 3.228 \\ 2.571 & 8.286 & 0.796 \\ 3.229 & 0.795 & 7.210 \end{pmatrix} \qquad \text{H6} = \begin{pmatrix} 0.391 & 0.212 & 0.312 \\ 0.212 & 1.130 & 0.298 \\ 0.312 & 0.298 & 0.799 \end{pmatrix}$$

$$\text{H7}\alpha = \begin{pmatrix} 0.764 & 0.085 & 0.055 \\ 0.085 & 0.734 & 0.105 \\ 0.055 & 0.105 & 0.775 \end{pmatrix} \qquad \text{H8}\alpha = \begin{pmatrix} 1.377 & 0.081 & 0.090 \\ 0.081 & 1.415 & 0.145 \\ 0.090 & 0.145 & 1.355 \end{pmatrix}$$

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