Supplementary Information

Revisiting the nitrosyl complex of myoglobin by high-field pulse EPR spectroscopy and quantum mechanical calculations.

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Table S1. Computed spin populations of rhombic models ($\mathbf{R_{M1}}$ and $\mathbf{R_{M2}}$) using PBE functional.

	$\mathbf{R_{M1}}$				$\mathbf{R_{M2}}$			
	Gas	COSMO	NOSOL	PROTEIN	Gas	COSMO	NOSOL	PROTEIN
Fe	0.662	0.665	0.661	0.702	0.694	0.697	0.694	0.733
N_{NO}	0.184	0.183	0.186	0.165	0.172	0.172	0.173	0.153
O_{NO}	0.133	0.129	0.133	0.118	0.119	0.115	0.119	0.103
$^{\varepsilon 2}N_{His93}$	0.026	0.027	0.025	0.022	0.028	0.029	0.026	0.023
$^{\varepsilon 2}N_{His64}$	-0.001	-0.001	0.000	-0.001	-0.001	-0.001	-0.001	-0.001
$^{\varepsilon 2}H_{His64}$	0.021	0.020	0.019	0.020	0.021	0.019	0.019	0.020

***Table S2*.** Computed g-tensor of rhombic models ($\mathbf{R_{M1}}$ and $\mathbf{R_{M2}}$) using PBE functional.

	$\mathbf{R_{M1}}$				$\mathbf{R_{M2}}$				***Exp***
	Gas	COSMO	NOSOL	PROTEIN	Gas	COSMO	NOSOL	PROTEIN	
g_1	1.984	1.983	1.984	1.984	1.982	1.981	1.983	1.983	1.987
g_2	2.001	2.001	2.001	2.003	2.000	2.000	2.000	2.001	2.008
g_3	2.028	2.027	2.028	2.030	2.039	2.039	2.039	2.042	2.075
g_{iso}	2.004	2.004	2.004	2.006	2.007	2.007	2.007	2.009	

Table S3. Computed ^{14}N HFC's (in MHz) of rhombic models ($\mathbf{R_{M1}}$ and $\mathbf{R_{M2}}$) using PBE functional. Experimental values from this work are listed as well.

		$\mathbf{R_{M1}}$				$\mathbf{R_{M2}}$				***Exp***
		Gas	COSMO	NOSOL	PROTEIN	Gas	COSMO	NOSOL	PROTEIN	
N_{NO}	A_1	21.9	21.9	23.6	21.6	19.8	19.8	21.4	19.1	
	A_2	26.2	26.4	27.8	26.8	24.5	24.6	25.9	24.8	
	A_3	59.1	59.8	60.7	58.1	55.1	55.7	56.5	53.7	
	a_{iso}	35.7	36.1	37.4	35.5	33.1	33.4	34.6	32.5	38.0[a]
$^{\varepsilon 2}N_{His93}$	A_1	17.0	17.0	17.0	16.0	18.0	18.0	17.9	17.0	
	A_2	17.3	17.3	17.2	16.3	18.4	18.3	18.3	17.3	
	A_3	21.6	21.7	21.5	20.2	23.0	23.1	22.7	21.5	
	a_{iso}	18.6	18.7	18.6	17.5	19.8	19.8	19.6	18.6	18.6
$^{\varepsilon 2}N_{His64}$	A_1	4.3	4.1	4.0	4.1	3.8	3.6	3.5	3.6	
	A_2	4.4	4.1	4.0	4.2	3.9	3.6	3.5	3.7	
	A_3	6.0	5.7	5.6	5.7	5.3	5.0	5.0	5.1	
	a_{iso}	4.9	4.6	4.5	4.7	4.3	4.1	4.0	4.1	
$^{A}N_{heme}$	a_{iso}	5.3	5.2	5.5	4.9	1.0	0.9	1.1	0.6	
$^{B}N_{heme}$	a_{iso}	-2.5	-2.5	-2.4	-2.6	-3.8	-3.7	-3.8	-3.8	
$^{C}N_{heme}$	a_{iso}	-3.8	-3.8	-3.8	-3.9	-2.4	-2.4	-2.3	-2.7	
$^{D}N_{heme}$	a_{iso}	2.1	1.9	2.1	1.6	4.5	4.4	4.6	4.2	3.8
$^{\delta 1}N_{His93}$	A_1	1.3	1.2	1.1	1.1	1.3	1.1	1.1	1.1	
	A_2	1.4	1.2	1.2	1.2	1.3	1.2	1.1	1.1	
	A_3	1.7	1.6	1.5	1.5	1.7	1.6	1.5	1.5	

[a]R. LoBrutto, Y. H. Wei, R. Mascarenhas, C. P. Scholes and T. E. King, *J. Biol. Chem*., 1983, **258**, 7437.

T***able S4***. Calculated nuclear quadruple coupling constants (e^2Qq/h) in MHz, and asymmetry parameter η using PBE functional.

		$\mathbf{R_{M1}}$				$\mathbf{R_{M2}}$				***Exp***
		Gas	COSMO	NOSOL	PROTEIN	Gas	COSMO	NOSOL	PROTEIN	
$\mathbf{N_{NO}}$	e^2qQ/h	-2.269	-2.326	2.224	-2.319	-2.218	-2.270	-2.180	-2.274	
	η	0.928	0.889	0.956	0.862	0.943	0.910	0.969	0.876	
$\mathbf{^{\varepsilon 2}N_{His93}}$	e^2Qq/h	-2.473	-2.415	-2.615	-2.701	-2.478	-2.422	-2.620	-2.702	2.6±0.1
	η	0.515	0.540	0.471	0.428	0.510	0.534	0.466	0.425	0±0.1
$\mathbf{^{\varepsilon 2}N_{His64}}$	e^2Qq/h	-2.406	-2.435	-2.462	-2.427	-2.447	-2.465	-2.501	-2.467	
	η	0.230	0.184	0.168	0.226	0.204	0.166	0.142	0.203	
$\mathbf{^{\varepsilon 2}H_{His64}}$	e^2Qq/h	0.219	0.220	0.220	0.219	0.219	0.219	0.220	0.218	
	η	0.122	0.124	0.122	0.120	0.122	0.123	0.121	0.121	

Table S5. Computed ^{1}H hyperfine couplings (in MHz) of $\mathbf{R_{M2}}$ model at the QM/MM level using PBE functional compared with experimental results reported in this work for $\mathbf{^{\varepsilon 2}H_{His64}}$ and those reported in ref. 1 for other protons.

		$\mathbf{^{\varepsilon 2}H_{His64}}$	$\mathbf{^{\varepsilon 1}H_{His64}}$	$\mathbf{^{\delta 1}H_{His93}}$	$\mathbf{^{\varepsilon 1}H_{His93}}$	$\mathbf{^{C\gamma 2I}H_{Val68}}$	$\mathbf{^{C\gamma 2II}H_{Val68}}$	$\mathbf{^{C\gamma 2III}H_{Val68}}$	$\mathbf{^{A,(C)}H_{heme}}$	$\mathbf{^{B,(D)}H_{heme}}$
$\mathbf{R_{M2}}$	A_1	-4.9	-0.7	0.6	-0.9	-1.6	-1.3	-0.9	-0.7 (-0.6)	-0.5 (-0.6)
	A_2	-6.0	-0.9	0.8	-1.2	-1.8	-1.8	-0.9	-0.8 (-0.8)	-0.6 (-0.7)
	A_3	11.9	1.5	2.3	3.9	3.3	3.6	1.6	1.3 (1.5)	1.4 (1.5)
Exp	A_1	-5.1 (1.3)[a]	0.68	-0.9	-4.4	1.47		-0.35	1.9	-0.71
	A_2	-6.0 (2.6)	-0.38	-0.25	1.13	-0.73	--	0.7	-0.6	-0.71
	A_3	8.0 (-4.2)	-0.36	-2.07	2.0	1.47		0.7	-0.95	1.5

[a] the values in parenthesis are from ref 1.

1. Flores, M.; Wajnberg, E. ; Bemski, G. *Biophysical J.* **2000**, *78*, 2107-2115.

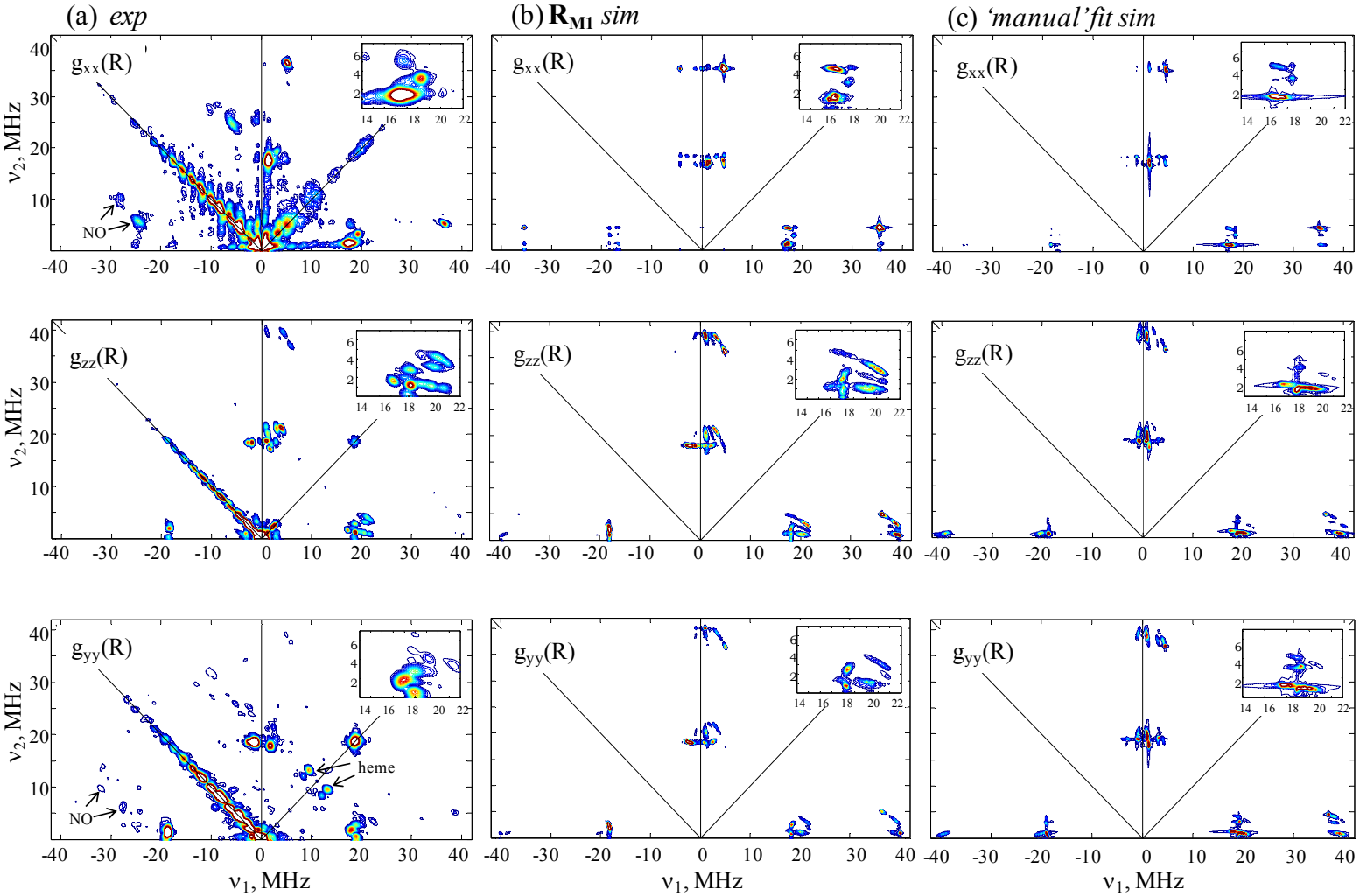

Figure S1. W-band HYSCORE experimental (a), simulated using the R_{M1} parameters (b) and simulated using 'manual' fit (c) spectra of MbNO pH 7.0. The g-values corresponding to the field at which the measurements were carried out are given on the Figure.