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Electronic supplementary information: Ergodicity breaking of iron displacement in heme proteins.

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I. SIMULATION PROTOCOL

The simulation setup mainly follows the previous work[1] in setting up two oxidation states of the cytochrome c (Cyt-C). The NAMD package[2] was used for simulations. Initial structure was taken from 1GIW PDB file and CHARMM 27[3] was used for the peptide chain, while heme parameters were adopted by combining atomic charges from Leu et al[4] with the bonded and van der Waals parameters from Kaszuba et al.[5] The Fe-His bond was modified with applying a Morse potential[1] to model a weaker bond for Fe-His compared to Fe-Met.[6] After taking crystallographic water from 1YCC PDB file and alignment, 150 ns wetting procedure was applied[1] and then a box of total 101440 atoms was created. NPT simulations were performed to relax the box and then different temperature were achieved by NVT simulations of 1 ns long for each 1 K temperature step. Rigid hydrogen bonds were used for simulations with 2 fs step, while all protein bond constrains were relaxed for simulations with 0.25 fs step. For all simulations particle mesh Ewald was applied to long range electrostatics with a 12.0 Å cutoff. The production runs were 250 ns long for temperatures 280 K and above, and 135 ns for lower temperatures.

The calculations of the force acting on the heme involved all atoms of the protein and water except the following atoms closest to the heme: SG on the SYS, SG on the CYS and SD on the MET. For the force variance of the Ox state, the expected linear temperature scaling of $\langle \delta \mathbf{F}^2 \rangle$ was not followed for the variances obtained from 250 ns simulation trajectories. Therefore, the force variance was calculated from 50 ns segments and then averaged over 5 such values to obtain the data shown in Fig. S8. These results were used to produce Fig. 3 in the main text.

II. DATA ANALYSIS

A. Force acting on the heme

The force autocorrelation function

$$C_F^a(t) = \langle \delta \mathbf{F}^a(t) \cdot \delta \mathbf{F}^a(0) \rangle \tag{S1}$$

was calculated from MD trajectories in Red and Ox states of Cyt-C. The component $a = \mathrm{El}, \mathrm{Tot}$ here indicate either the electrostatic force or total force acting on the heme of Cyt-C. $\mathbf{F}^a(t)$ in this equation is therefore the sum of all forces acting on the atoms of the heme. The calculations of the autocorrelation function were done in the time interval from 0.2 ps to 10–40 ns by sliding the averaging window along the trajectory of 250 ns. The normalized correlation function was fitted to five decaying exponents ($\sum_{n=1}^{5} A_n = 1$)

$$S_F^a(t) = C_F^a(t)/C_F^a(0) = \sum_{n=1}^{5} A_n e^{-t/\tau_n} \tag{S2}$$

with the fitting parameters listed in Tables S1–S4. The average relaxation time, also listed in the tables, was calculated according to the relation

$$\langle \tau \rangle = \sum_{n=1}^{5} A_n \tau_n. \tag{S3}$$

An alternative fitting procedure employing a sum of one exponent and a stretched-exponential relaxation was also attempted. The corresponding fitting function is given as

$$S_F^a(t) = A_1 e^{-t/\tau_1} + (1 - A_1) e^{(-t/\tau_2)^\beta} \tag{S4}$$

We consistently find that the quality of the fit to Eq. (S2) is superior to that done with Eq. (S4) (Fig. S2) and, therefore, the five-exponential fitting ("Exp5" in Figs. S2 and S3) in chosen for the analysis presented in the main text. Nevertheless, the temperature dependence of the average relaxation time from Eq. (S3) and from the stretched-exponential fit are very close to each other (Fig.

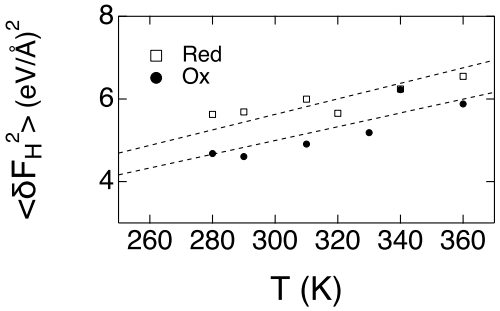

Figure S1. Variance of the force acting on the heme $\langle \delta F_H^2 \rangle$ vs T. The dashed lines are regressions through the points, $\langle \delta F_H^2 \rangle = cT$.

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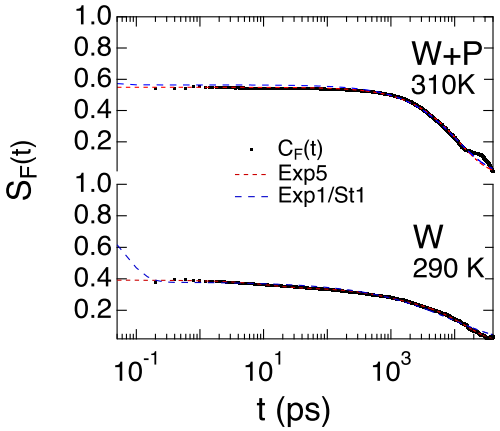

Figure S2. Fitting of the normalized force-force time correlation function with 5 decaying exponents ("Exp5", Eq. (S2)) and with exponent plus stretched exponent ("Exp1/St1", Eq. (S4)). The top panel shows $S_F(t)$ for the force arising from water and protein (310 K), while the bottom panel displays the force arising from water molecules (290 K). Both correlation functions refer to Red state of Cyt-C.

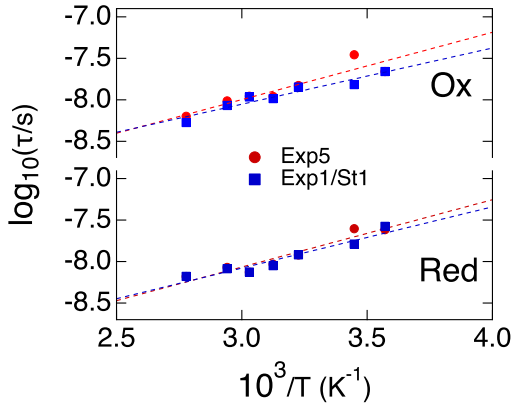

Figure S3. Comparison of the long-time component of 5-exponent fit ("Exp5", Eq. (S2)) to the results of using stretched exponential fit ("Exp1/St1", Eq. (S4)) for the Ox (top) and Red (bottom) states of Cyt-C. The slopes of the linear fits for "Exp1/St1" fits are 1559 K for the Ox and 1701 K the Red state. The fits of both the Red and Ox states give 1868 K in the "Exp5" approach.

S3). The average time for the stretched-exponential fits was determined according to the relation

$$\langle\tau\rangle = A_1\tau_1 + (1-A_1)\Gamma(1+\beta^{-1})\tau_2 \qquad \text{(S5)}$$

here the $\Gamma(x)$ is the gamma function.

Figure S4 shows the temperature dependence of the average long relaxation time, $\Gamma(1+\beta^{-1})\tau_2$, vs $1/T$ for the protein and water components of the solution. The results for water do not generally fall on a straight line in either exponential of stretched+exponential fits. The issue is related to the difficulty of converging the corresponding correlation functions since water dynamics are affected by slow motions of the protein.

In contrast to $S_F(t)$, which effectively averages over vibrations of the individual atoms in the heme, the force-force time auto-correlation function of the force acting on the heme iron is highly oscillatory (Fig. S5). This correlation function is difficult to analyze in terms on the long-time dynamics required for the dynamical transition and the force acting on the entire heme was chosen for that reason.

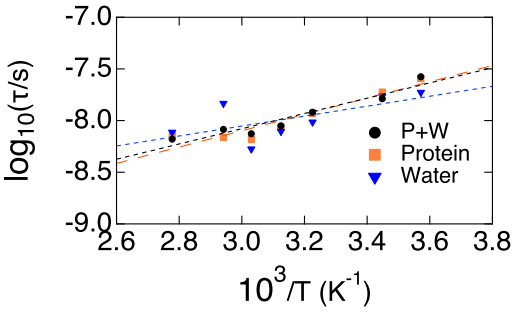

Figure S4. Average slow relaxation time $\Gamma(1+\beta^{-1})\tau_2$ for the protein (P) and water (W) components of the force. The slopes of linear fits are: 1701 K (P+W), 1819 K (P) and 1108 K (W). The results for water, which do not fit well to Eq. (S4), were also fitted with 3 exponents and one stretched exponential, which gave better fits with the slope of 1155 K (not shown in the graph).

B. MSF of Cyt-C

The modeling of the MSF of the heme iron requires calculating the overall force variance acting on the heme and the nonergodicity parameter $f_{\rm ne}(T)$ in Eq. (16) in the main text. The variance of the total force acting on the heme depends on temperature. Based on the expectations from the fluctuation-dissipation theorem, we approximated the simulation results at different temperatures by a linear function, $\langle\delta F_H\rangle = cT$. The coefficient c for two oxidation states is $c_{\rm Red} = 0.018$ and $c_{\rm Ox} = 0.017$ $(\text{eV/Å})^2\text{K}^{-1}$. The simulation points and the linear fit are shown in Fig. S1

C. MSF of Hydration Shell

The self intermediate scattering function (ISF) was calculated separately for translations and rotations of the water molecules in the hydration shell of Cyt-c. The

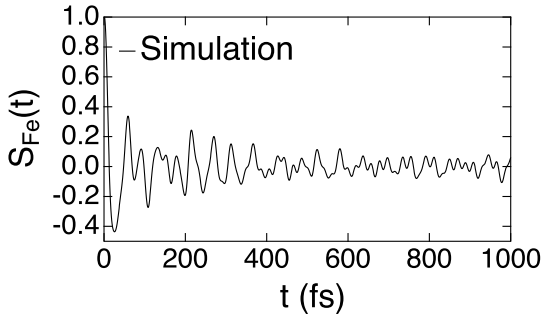

Figure S5. Normalized time auto-correlation function of the force acting on the Fe atom $S_{\rm Fe}(t) = C_{\rm Fe}(t)/C_{\rm Fe}(0)$, where $C_{\rm Fe}(t) = \langle \delta\mathbf{F}_{\rm Fe}(t) \cdot \delta\mathbf{F}_{\rm Fe}(0)\rangle$.

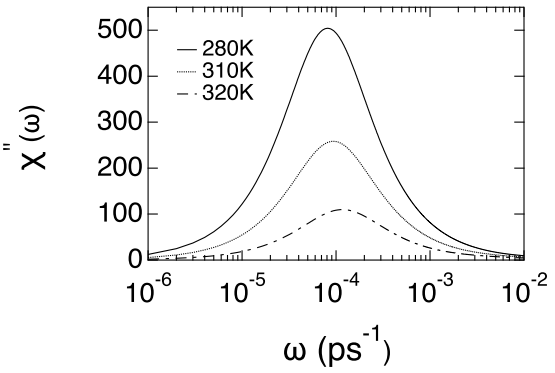

Figure S6. $\chi''(\omega)$ for $T = 280$, 310 and 320 K. The thickness of the water shell is 6 Å.

translational ISF is

$$F_s(k,t) = N^{-1} \sum_j \left\langle e^{i\mathbf{k}\cdot\Delta\mathbf{r}_j(t)} \right\rangle, \qquad \text{(S6)}$$

where $\Delta\mathbf{r}(t) = \mathbf{r}_{\rm O}(t) - \mathbf{r}_{\rm O}(0)$ for oxygen atoms counted within the shell 6 Å thick. A similar function was calculated replacing $\Delta\mathbf{r}(t)$ in Eq. (S6) with $\Delta\mathbf{r}_{\text{O-H}}(t)$ for the vector connecting the oxygen and hydrogen atoms. For each function $-\ln[F_s(k,t)]$ vs k^2 in the range of small k-values ($k < 0.13$ Å^{-1}) was fitted wit a linear function to produce the corresponding MSFs. Time values of $t = 100$ ps and $t = 1$ ns were used in the calculations shown in Fig. 11 of the main text.

D. Dynamics of the susceptibility

The calculations of the static dipolar susceptibility of the hydration shell are shown in Fig. 10 of the main text. The dynamic version of the susceptibility function requires calculating the time correlation function of the shell dipole moment, $\langle \delta\mathbf{M}(t) \cdot \delta\mathbf{M}(0)\rangle$. The imaginary part of the frequency Fourier transform of the susceptibility is the loss function $\chi''(\omega)$ shown in Fig. S6. The calculations are performed for the water shell with the thickness of 6 Å around the van der Waals surface of the protein.

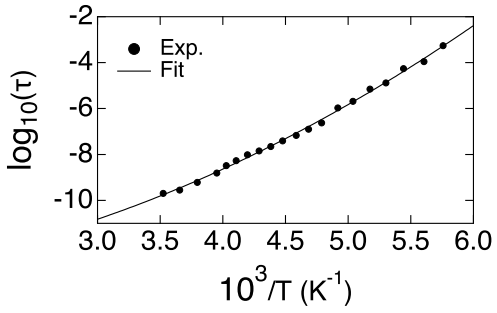

Figure S7. Relaxation time of the ν-process reported from broad band dielectric spectroscopy of hydrated myoglobin powders (points, $h = 0.36$ g of water/g of prot.).[8] The solid line is regression through the points with the equation: $\log_{10}[\tau(\text{ps})] = -1.5974 + 314348/T^2 - 17.757/T$.

E. Vibrational density of states

Vibrational density of states of the Fe atom in the heme was calculated from the velocity correlation function

$$Z(t) = \tfrac{1}{3}\langle \mathbf{v}_{\rm Fe}(t) \cdot \mathbf{v}_{\rm Fe}(0)\rangle. \qquad \text{(S7)}$$

It is connected to the vibrational density of states (VDOS) $D(\omega)$ by the relation[7]

$$Z(t) = \frac{k_{\rm B}T}{2m_{\rm Fe}} \text{Re} \int_{-\infty}^{\infty} D(\omega) e^{i\omega t} d\omega, \qquad \text{(S8)}$$

where $m_{\rm Fe}$ is the mass of the Fe atom.

The Fourier transform was calculated numerically by multiplying $Z(t)$ with a Gaussian function with FWHM = 1 meV. The resulting VDOS presented in the text was produced from 1 ns NVE simulation with non-rigid protons and 0.25 fs simulation step (1 fs saving frequency). Since the trajectory length limits the range of low frequencies, quadratic extrapolation to zero was applied below 10 cm^{-1}.

III. ANALYSIS OF EXPERIMENTAL RESULTS

The relaxation time for myoglobin (Fig. 9 in the main text) was taken from dielectric measurements of protein powders by Nakanishi and Sokolov.[8] The process named as "main" in Ref. 8 is highly stretched, with the high-frequency wing of the dielectric loss following the power law decay, $\epsilon'' \propto \omega^{-\alpha}$. The stretching exponent α changes

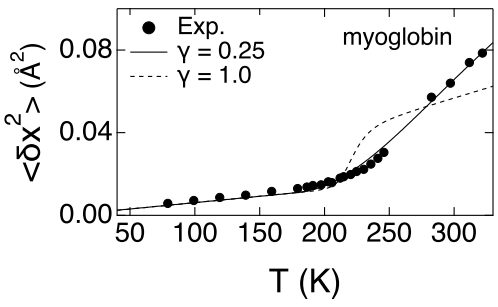

Figure S8. MSF of heme iron in oxidized myoglobin. Points indicate experimental results,[9] solid line refers to the fit to Eqs. (1) and (16) in the main text with the nonergodicity factor $f_{\rm ne}(T)$ determined from stretched dynamics [Eq. (20) in the main text] with $\gamma = 0.25$ (solid line) and $\gamma = 1.0$ (dashed line). The nonergodic force variance is determined as $\beta\langle\delta F^2\rangle_r = A f_{\rm ne}(T)$ with the fitting constant $A = 2.5$ nN/Å ($\gamma = 0.25$) and 1.53 nN/Å ($\gamma = 1.0$). The relaxation time $\tau(T)$ is from the broad band dielectric spectroscopy of hydrated myoglobin powders[8] as shown in Fig. S7.

from 0.24 at $T = 163$ K to 0.17 at $T = 143$ K (lysozyme). The dielectric loss was fitted to the Cole-Cole dispersion function with the resulting relaxation time shown by points in Fig. S8. These data, fitted to a function shown by the solid line in Fig. S8, were used in producing the nonergodic variance of the force acting on the Fe atom in the heme of myoglobin.

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Table S1. Total time correlation function for Cyt-c (Red). The relaxation times are in ps. The units of the force are eV/Å.

T	A_1	A_2	A_3	A_4	A_5	τ_1	τ_2	τ_3	τ_4	τ_5	$\langle\tau\rangle$	$\langle\delta\mathbf{F}_H^2\rangle$
280	0.518	0.045	0.002	0.012	0.423	24273	1228	0.008	0.005	0.008	12634	5.63
290	0.44	0.11	0.45	0.00	0.00	24929	2350	0.006	0.008	0.005	11240	5.69
310	0.527	0.021	0.036	0.009	0.406	12026	1228	0.008	0.010	0.005	6369	5.99
320	0.493	0.017	0.002	0.035	0.451	9259	50.40	0.009	0.007	0.01	4567	5.65
330	0.566	0.000	0.202	0.000	0.232	7444	0.026	0.009	0.020	0.008	4212	8.32
340	0.475	0.016	0.485	0.00	0.02	8501	62.65	0.009	0.62	0.01	4038	6.24
360	0.500	0.000	0.003	0.000	0.497	6603	0.009	0.008	0.006	0.007	3303	6.54

Table S2. Electrostatic component of the force-force correlation function for Cyt-c, Red. The relaxation times are in ps. The units of the force are eV/Å.

T	A_1	A_2	A_3	A_4	A_5	τ_1	τ_2	τ_3	τ_4	τ_5	$\langle\tau\rangle$	$\langle\delta\mathbf{F}_H^2\rangle$
280	0.861	0.027	0.072	0.010	0.03	16229	4.733	0.002	0.4	0.05	13979	4.05
290	0.85	0.02	0.11	0.02	0.00	15443	35.34	0.07	2.940	0.06	13169	4.14
310	0.79	0.07	0.057	0.05	0.032	8333	0.038	0.03	0.1	77	6585	2.75
320	0.76	0.04	0.002	0.08	0.12	10529	1175	0.01	1.5	0.00	8038	3.45
330	0.81	0.03	0.00	0.01	0.14	7962	67.52	0.00	0.06	0.00	6483	3.73
340	0.77	0.06	0.13	0.02	0.02	10938	1081	0.077	2.98	8.12	8517	4.02
360	0.82	0.01	0.11	0.02	0.033	7837	45.2	0.00	4.52	0.585	6427	4.39

Table S3. Total force-force time correlation function for Cyt-c, Ox. The relaxation times are in ps. The units of the force are eV/Å.

T	A_1	A_2	A_3	A_4	A_5	τ_1	τ_2	τ_3	τ_4	τ_5	$\langle\tau\rangle$	$\langle\delta\mathbf{F}_H^2\rangle$
280	0.580	0.168	0.002	0.028	0.373	21944	338.959	0.007	0.004	0.007	12730	7.09
290	0.15	0.41	0.43	0.008	0.0	35007	11053.6	0.004	29.39	0.008	9884	6.15
310	0.550	0.029	0.191	0.023	0.207	14854	78.834	0.002	0.158	0.004	8164	6.25
320	0.57	0.00	0.003	0.059	0.362	10973	0.007	0.009	0.733	0.01	6318	6.64
330	0.558	0.013	0.021	0.0	0.408	11047	198	0.004	0.005	0.007	6167	6.33
340	0.48	0.038	0.190	0.03	0.25	9712	149.6	0.003	0.183	0.004	4688	6.97
360	0.550	0.010	0.005	0.003	0.432	6331	0.250	10.0	0.005	0.009	3482	6.30

Table S4. Electrostatic component of the force-force correlation function for Cyt-c, Ox. The relaxation times are in ps. The units of the force are eV/Å.

T	A_1	A_2	A_3	A_4	A_5	τ_1	τ_2	τ_3	τ_4	τ_5	$\langle\tau\rangle$	$\langle\delta\mathbf{F}_H^2\rangle$
280	0.87	0.00	0.00	0.024	0.10	16917	111.7	0.002	19.72	0.003	14697	4.99
290	0.9	0.02	0.04	0.045	0.00	12124	1.86	0.001	0.051	0.018	10912	6.01
310	0.81	0.08	0.08	0.02	0.01	16048	1451	0.00	0.78	0.7	13202	6.29
320	0.9	0.00	0.03	0.00	0.067	10553	0.327	0.00	0.01	0.00	9486	6.47
330	0.852	0.017	0.015	0.092	0.023	11171	164	7.68	0.060	1.076	9526	6.26
340	0.738	0.15	0.04	0.04	0.03	27377	1727	0.745	0.01	0.01	20461	6.97
360	0.861	0.002	0.11	0.03	0.0	7028	64.34	0.00	0.37	0.00	6054	6.30