Supporting information for

Investigations of the Low Frequency Spectral Density of Cytochrome c upon Equilibrium Unfolding

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Vibrational Coherence of Cytochrome c

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Soret band absorption spectra and fits for equilibrium unfolding of hh Cyt c.

a) Serial model

The fitting of the Soret band during the equilibrium unfolding of Cyt c is performed using a three-state model proposed by Latypov et al.(1). In addition to the native (N) and unfolded (U) states, an intermediate (I) state was introduced by Latypov and coworkers in order to account for the unfolding equilibrium spectral changes. The following scheme is assumed:

$$N \overset{K_{NI}}{\Longleftrightarrow} I \overset{K_{IU}}{\Longleftrightarrow} U$$

where K_{NI} and K_{IU} are equilibrium constants that can be expressed as $K = \mathrm{e}^{-\frac{\Delta G}{RT}}$. We assume that the free energy for each transition varies linearly with the denaturant concentration, c : $\Delta G_i(c) = m_i(C_{m_i} - c)$, where C_{m_i} and m_i are the midpoint concentration and slope, respectively, and the index i stands for either NI or IU.

The extinction coefficient of the superposition of unfolding states, $\varepsilon(\nu, c)$ is given by:

$$\varepsilon(\nu, c) = \varepsilon_N(\nu) f_N + \varepsilon_I(\nu) f_I + \varepsilon_U(\nu) f_U \qquad (1)$$

This is a superposition of the absorption from the native (N), unfolded (U), and intermediate (I) states with fractional populations (f_N, f_I, f_U):

$$f_N = \frac{1}{1 + K_{NI} + K_{NI}K_{IU}}, \quad f_I = K_{NI} f_N, \quad f_U = K_{NI}K_{IU} f_I \qquad (2)$$

The global fitting procedure is performed using IGOR Pro (WaveMetrics, Inc.). The program simultaneously optimizes the fit to the absorption spectrum at each wavelength and GdHCl concentration using 7 common fitting parameters: $C_{m_{NI}}$, $C_{m_{IU}}$, m_{NI}, m_{IU}, and three coefficients to construct the I-state absorption by a linear combination of the spectra at 0M, 2M and 5M GdHCl.

The fitting results are summarized in Table S1. The fitted absorption spectra are shown in Fig. S1, the spectra of the three states are summarized in Fig. S2, and the population of each state as a function of GdHCl concentration is depicted in Fig S3.

Table S1. Thermodynamic parameters obtained by global fitting to the absorption spectra of hh Cyt c at pH 7.

C_{mNI} (M)	m_{NI} (kcal mol^{-1} M^{-1})	ΔG_{NI} (0) (kcal mol^{-1})	C_{mIU} (M)	m_{IU} (kcal mol^{-1} M^{-1})	ΔG_{IU}(0) (kcal mol^{-1})
2.13±0.02	2.17±0.12	4.62±0.26	2.94±0.01	3.95±0.12	11.61±0.35

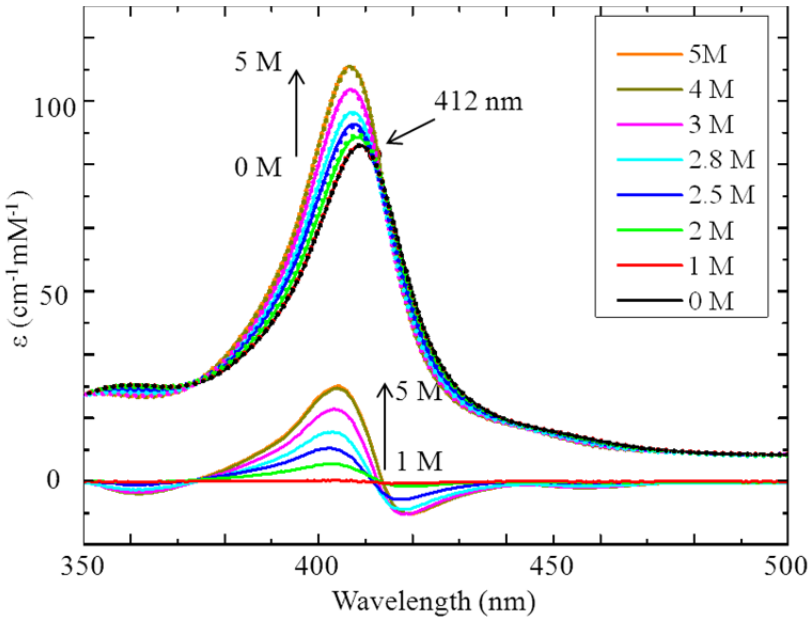

Figure S1. Absorption spectra of hh cyt c (dotted line) and their fitted spectra (solid line) at pH 7 and [GdHCl] = 0 M (dark yellow), 1 M (yellow), 2 M (magenta), 2.5 M (cyan), 2.8 M (blue), 3 M (green), 4 M (red), and 5 M (black), by three-state global fits. The lower part shows the spectral difference with respect to the native cyt c absorption spectrum at 0 M GdHCl.

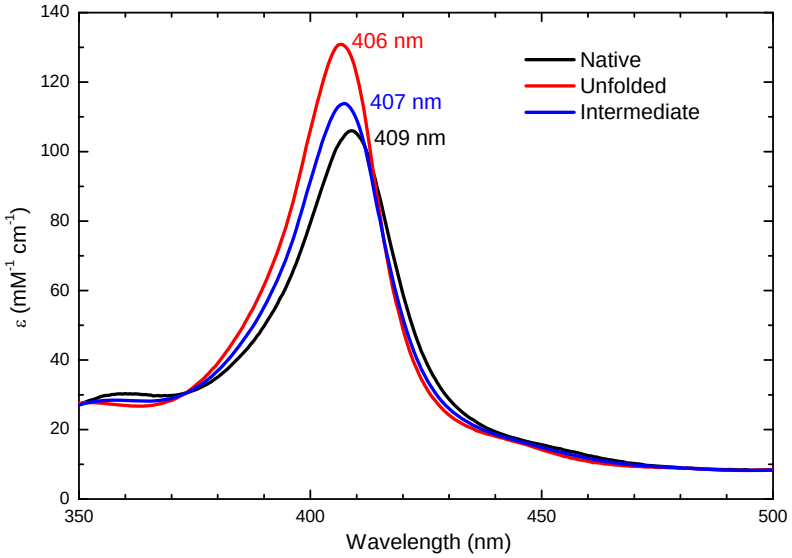

Figure S2. Absorption spectra obtained from three-state global fits, native (black), intermediate (blue) and unfolded (red).

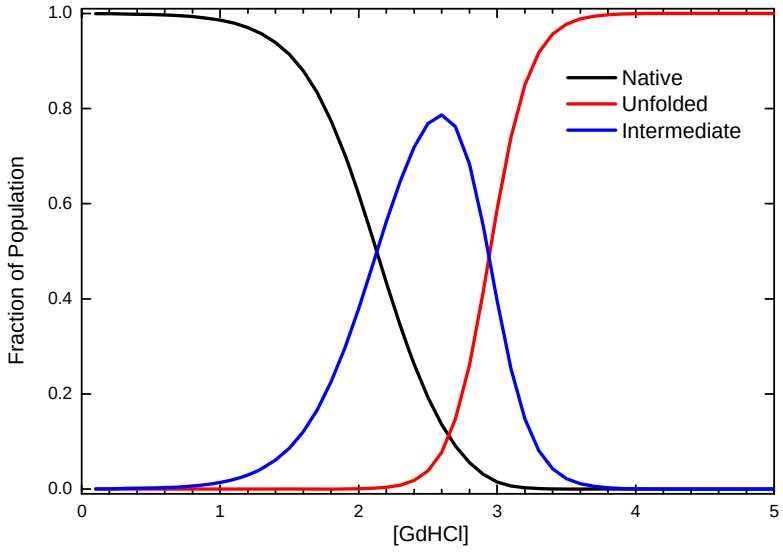

Figure S3. Population of each state as function of GdHCl concentration obtained from three-state global fits. Native (black), intermediate (blue) and unfolded (red).

b) Parallel model

The three-state unfolding process can also be explained in the context of a "parallel" scheme, which includes a direct N to U transition (global unfolding) in parallel

to the N to I transition. This, in effect, closes the "thermodynamic loop" so the direct N to U transition is equivalent to N to I followed by I to U and the thermodynamic cycle links the equilibrium states (i.e., $K_{NU}=K_{NI}K_{IU}$). The "parallel" scheme is often used in NMR studies for the unfolding of cyt c.(2-6) The populations can be written in terms of the free energies connecting N-I and N-U states, denoted by ΔG_{NI} and $\Delta G_{NU}(=\Delta G_{total})$, respectively. K_{NU}, can be written as $K_{NU} = \exp(-\Delta G_{NU}/RT) = \exp(-\Delta G_{NI}/RT)\exp(-\Delta G_{IU}/RT) = K_{NI}K_{IU}$, so Eq. 2 still holds to describe the population of each state. The m-value, m_{NU}, and midpoint concentration, C_{NU}, for the N to U transition can be derived using:

$$\Delta G_{total} = \Delta G_{NU} = m_{NI}(C_{NI} - c) + m_{IU}(C_{IU} - c)$$
$$= (m_{NI} + m_{IU})\left((m_{NI}C_{NI} + m_{IU}C_{IU})/(m_{NI} + m_{IU}) - c\right),$$

So that, when written in terms of the parameters for 3-state scheme, we have:

$$m_{NU} = m_{NI} + m_{IU}$$
$$C_{NU} = (m_{NI}C_{NI} + m_{IU}C_{IU})/m_{NU} \quad (3)$$

Thus, in this study, m_{NU} = 6.12±0.17 kcal mol^{-1} M^{-1}, $\Delta G_{NU}(0)$=16.23±0.44 kcal mol^{-1}, and C_{NU}=2.65±0.15 M. Table S2 summarizes some ΔG_{NI}, ΔG_{NU}, m_{NI}, and m_{NU} values. The unfolding free energy and m-value obtained at 20 °C and pH 7 is higher than what is obtained under less thermodynamically stable conditions.

Table S2. Comparison of unfolding free energies and m-values. Eq.3 is used to derive the N-U-parameters from ref 45 and this work. The unfolding of the Met80 loop in ref. 64 is taken to correspond to N-I. The reference numbers refer to the main text.

Solution condition	m_{NI} (kcal mol^{-1}M^{-1})	ΔG_{NI} (0) (kcal mol^{-1})	m_{NU} (kcal mol^{-1}M^{-1})	ΔG_{NU} (0) (kcal mol^{-1})	Reference
Urea, pH 7.0, 30 °C	0.5	4.0	1.5	11.2	Ref. 71
GdHCl, pH 5.0, 20 °C	1.3	2.0	4.4	9.8	Ref. 45
GdHCl, pH 7.0, 30 °C	1.5	6.0	4.7	12.8	Ref. 64
GdHCl, pH 7.0, 20 °C	2.2	4.6	6.1	16.2	This work

Imidazole-Cytochrome c complex and NSD analysis

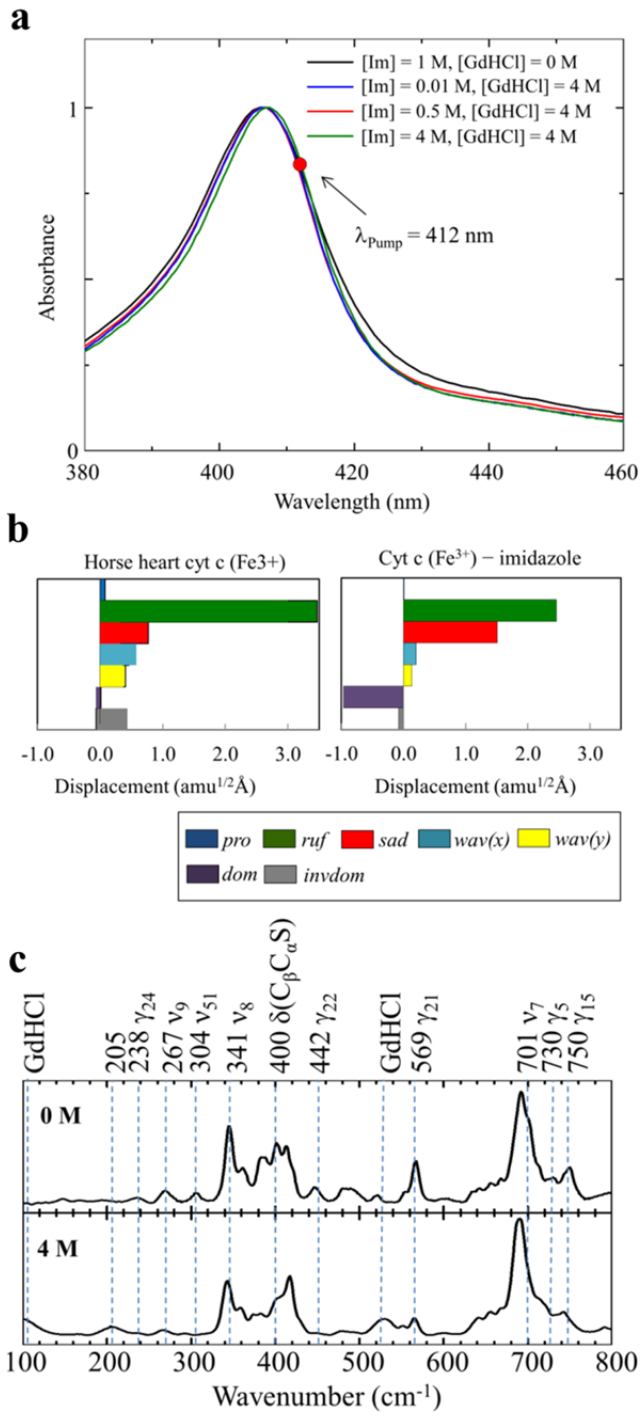

Figure S4. (a) The Soret band absorption spectra of folded cyt c and the GdHCl unfolded ferric cyt c-imidazole complex at different concentrations of GdHCl and

imidazole. All spectra are normalized to O.D. = 1 at the peak of Soret band. (b) NSD analysis of the heme core distortions for hh cyt c (crystal structure) and the hh cyt c-imidazole complex (NMR structure). A displacement of 1 $amu^{1/2}$Å represents the square root of the sum of squares of the mass weighted displacements of the Fe and the 24 (4N, 20C) porphyrin atoms. The color coding for the modes is propelling (blue), ruffling (green), saddling (red), waving (light blue), waving (yellow), doming (purple), inverse doming (gray). The minus sign of displacement is defined only for doming and inverse doming to indicate the direction of Fe displacement (positive is proximal; negative is distal). The PDB IDs are 1AKK and 1FI7 for hh cyt c and cyt c-imidazole. (c) The Raman spectra in the low frequency region (100 – 800 cm^{-1}) of the folded (upper panel) and GdHCl-induced unfolded (lower panel) ferric cyt c imidazole complex.

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