Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Photoreduction of ferricytochrome c in the presence of potassium ferrocyanide

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Supporting Information

1. Analysis of photoreduction data

For our analysis, we have assumed that the obtained photoreduction process is at equilibrium all the time. We recall eq. (2) from the main manuscript which reflects the law of mass action. We use a somewhat more abbreviated notation to write this equation as follows

$$k_{p,obs}\left[c^{3+}\right]\cdot\left[f^{4-}\right]=k_0\left[c^{2+}\right]\cdot\left[f^{4-}\right] \qquad \text{(S1)}$$

where *f(P)* is a linear function of the laser power and contains the Einstein coefficient of absorption and a constant describing the proportionality between laser power and spectral energy density. $k_{p,obs} = k_p \cdot f(P)$ and k_0 are the rate constants of cytochrome c reduction and oxidation, respectively, while *c* and *f* denote cytochrome and electron donor concentration. The respective oxidation states are indicated. With $R=c^{3+}/c^{2+}$, we rewrite eq. (S1) as follows:

$$\frac{k_{p,obs}}{k_0}\cdot\frac{f^{4-}}{f^{3-}}=R^{-1} \qquad \text{(S2)}$$

Now we consider the law of mass conservation for cytochrome c"

$$c_T = c^{2+} + c^{3+} = c^{2+}\left(1+R\right) \qquad \text{(S3)}$$

where $c_0 = c^{3+}{}_0$ denotes the total cytochrome c concentration, which equals the initial ferricytochrome concentration. We now assume that prior to the investigated electron transfer reaction $f^{3-} = 0$, so that its concentration at equilibrium can be equated with c^{2+}. Solving eq. (S3) for the latter and inserting the result in S2 thus yields

$$\frac{k_{p,obs}}{k_0} \cdot \frac{f^{4-}}{c_0}(1+R) = R^{-1} \qquad \text{(S4)}$$

f^{4-} can be expressed in terms of the total concentration $f_T = f^{4-}_0$:

$$f^{4-} = f_T - f^{3-} = f_T - c^{2+} = f_T - \frac{c_T}{1+R} \qquad \text{(S5)}$$

which we utilize in eq. (S4) to yield:

$$\frac{k_{p,obs}}{k_0}\left[\frac{f_T}{c_T}(1+R) - 1\right] = R^{-1} \qquad \text{(S6)}$$

which corresponds to equation S6 in the main manuscript.

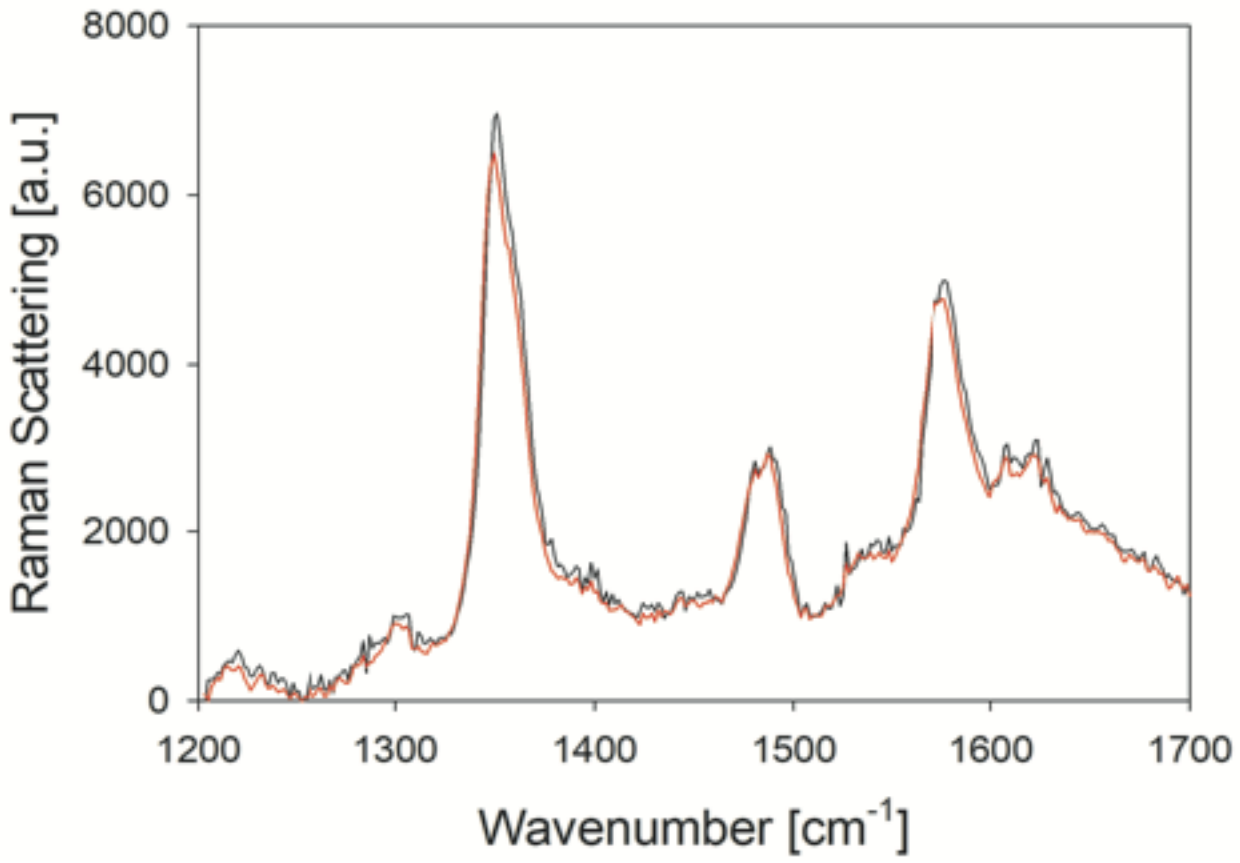

Figure S1: 442 nm Resonance Raman spectra of cytochrome c recorded for two different samples with different cytochrome c concentrations (black: 100µM**,** red: 25µM) and a constant stoichiometric ratio of four ferrocyanide to one cytochrome. The spectra are the average over multiple measurements (2 for 100µM, 6 for 25µM). Their intensities were corrected to reflect the different protein concentrations.

2. Ferrocyanide binding to cytochrome c

We consider two binding sites for ferrocyanide to ferricytochrome c the equilibrium constants of which are denoted as K_1 and K_2. The respective binding isotherms read as:

$$Y_i = \frac{k_i f^{4-}}{1 + k_i f^{4-}} \qquad \text{(S7)}$$

where $i=1,2$. The concentration of unbound ferrocyanide can be derived from the law of mass conversation. This yields

$$f^{4-} = -\alpha + \sqrt{\alpha^2 + \beta}$$
with
$$\alpha = \frac{1}{2}\left(\frac{1}{K_1 + K_2} + c_T - f_T \right) \qquad \text{(S8)}$$
$$\beta = \frac{f_T}{K_1 + K_2}$$

We calculated Y_1 and Y_2 as a function of f_T with $K_1 = 8.3 \cdot 10^4 M^{-1}$ and $K_2 = 2.6 \cdot 10^2 M^{-1}$.[1,2] The calculated binding isotherms are shown in Figure S1.

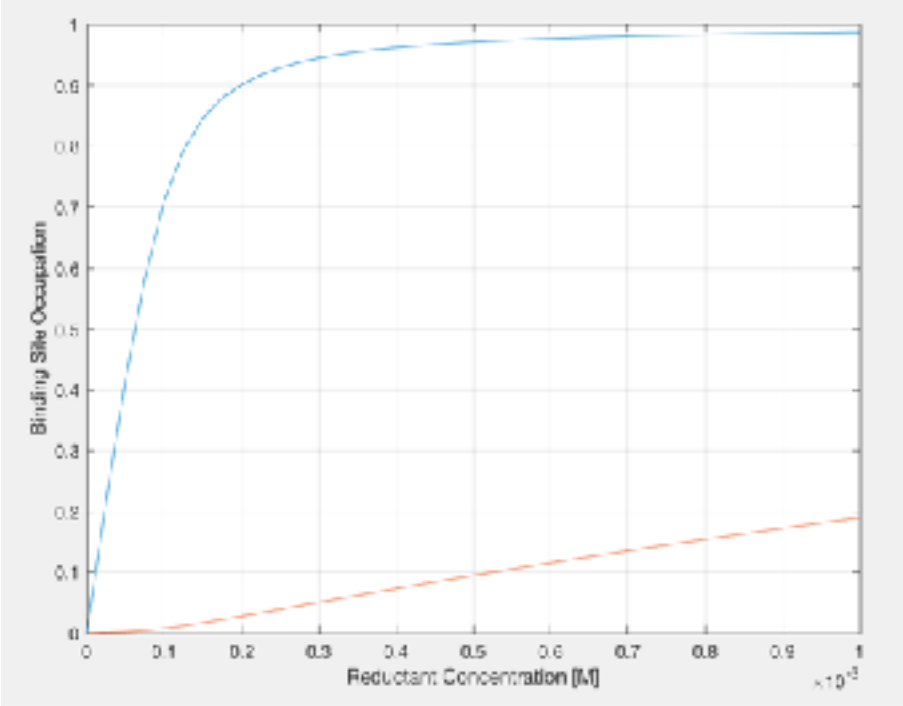

Figure S2: Calculated binding isotherms for the two identified binding sites of ferricytochrome c for ferrocyanide. The blue curve represents binding to the high affinity site in the heme pocket.

References

1. N. Ohno and A. Cusanovich, M, Reaction of C-type Cytochromes with the Iron Hexacyanides, *Biophys. J.*, 1981, **36**, 589–605.
2. E. Stellwagen and R. D. Cass, Complexation of Iron Hexacyanides by Cytochrome c, *J. Biol. Chem.*, 1975, **250**, 2095–2098.