Microsecond Protein Dynamics Observed at the Single-molecule Level

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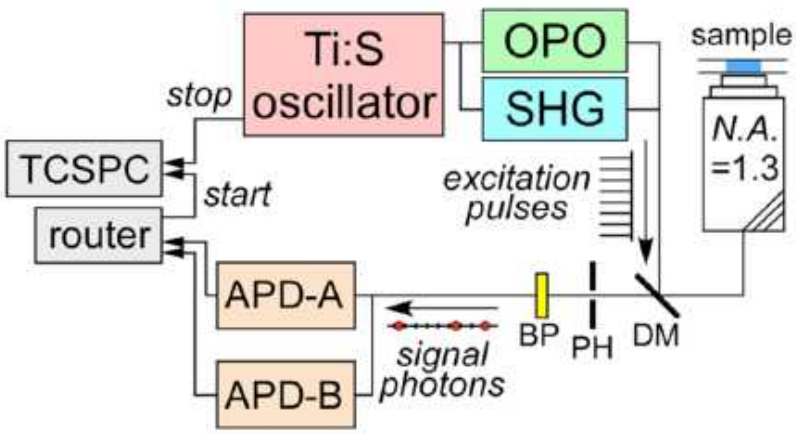
Motivation – Protein dynamics

- "In fact, the **'speed limit' of protein folding is supposed to be ~1 μs** and it closely relates to the roughness of the folding free energy landscape as well as to the internal friction of the polypeptide chain in the unfolded state."

- "In spite of the importance of microsecond dynamics of proteins, application of the **conventional smFRET technique is limited to dynamics slower than ~100 μs** because of the difficulty in collecting a sufficient number of photons to evaluate the FRET efficiency in a short bin time."

Setup: 2D Fluorescence Lifetime Correlation Spectroscopy (2D-FLCS)



1. Ti:Sa oscillator: Coherent Mira 900-F, **76 MHz (~ 13 ns in pulse interval)** centered at **775 nm**

2. OPO: Coherent Mira- OPO with intracavity frequency doubling
tuned to **540 nm**, **~40 μW** at the entrance of microscope.

3. SHG (optional and not used in this exp)

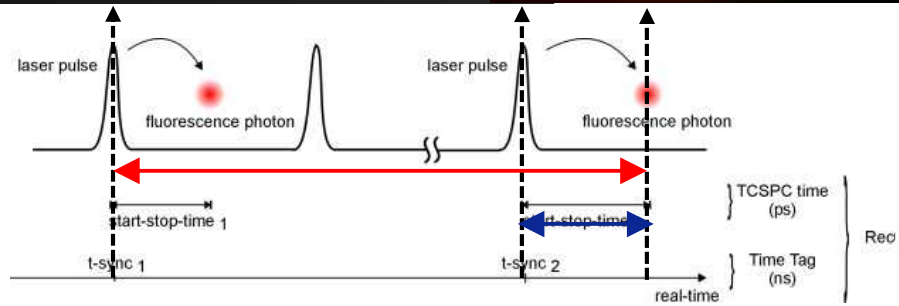
4. Objective lens: Nikon S Fluor 100×H, N.A. = 1.3 (mounted in Nikon TE-2000U microscope)

5. DM: Chroma Technology 585/40m

6. APD: id Quantique id100-20-ULN (~50 ps fwhm in instrumental response)

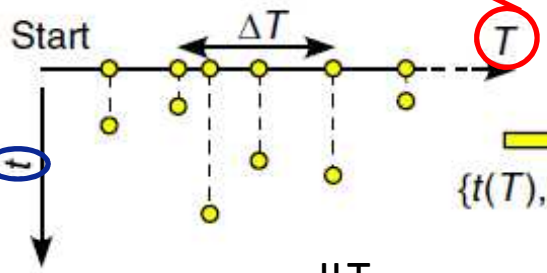
7. TCSPC module: Becker & Hickl SPC 140

Method: 2D Fluorescence Lifetime Correlation Spectroscopy (2D-FLCS)

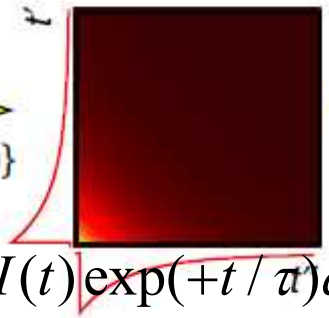


Absolute arrival time of photon

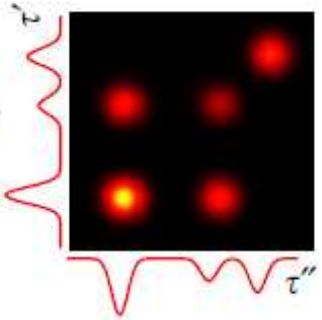
Delayed emission time



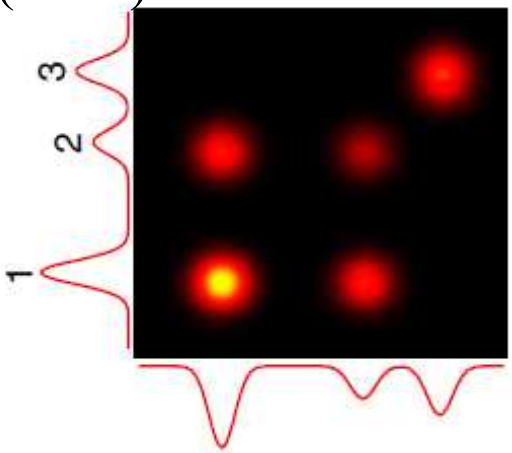
$\{t(T), t(T+\Delta T)\}$



ILT



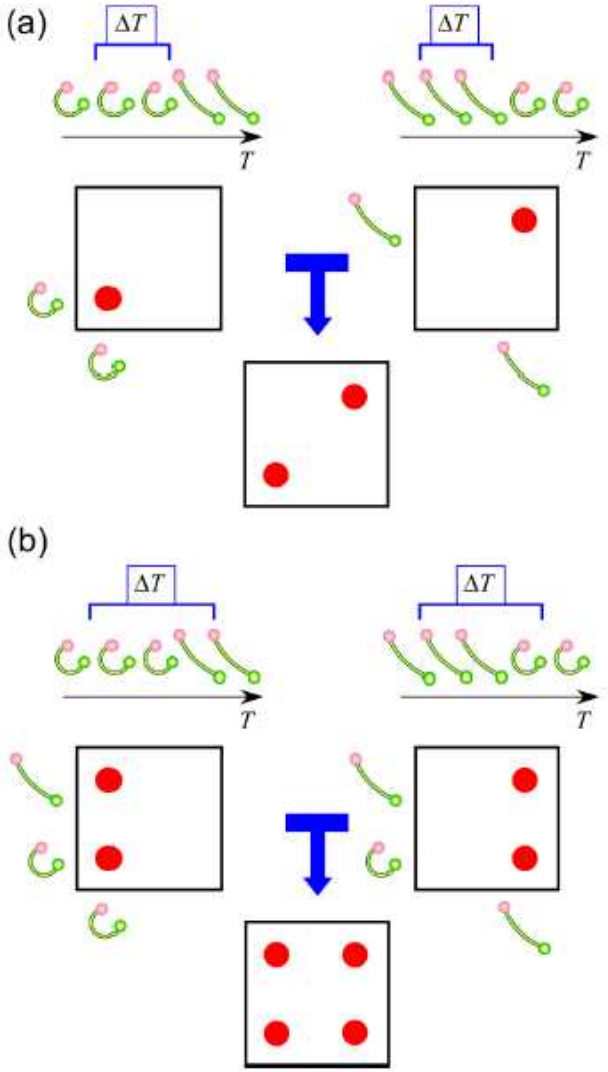
$$I(t) = \int \alpha(\tau)\exp(-t/\tau)d\tau \quad \overset{\text{ILT}}{\longleftrightarrow} \quad \alpha(\tau) = \int I(t)\exp(+t/\tau)dt$$

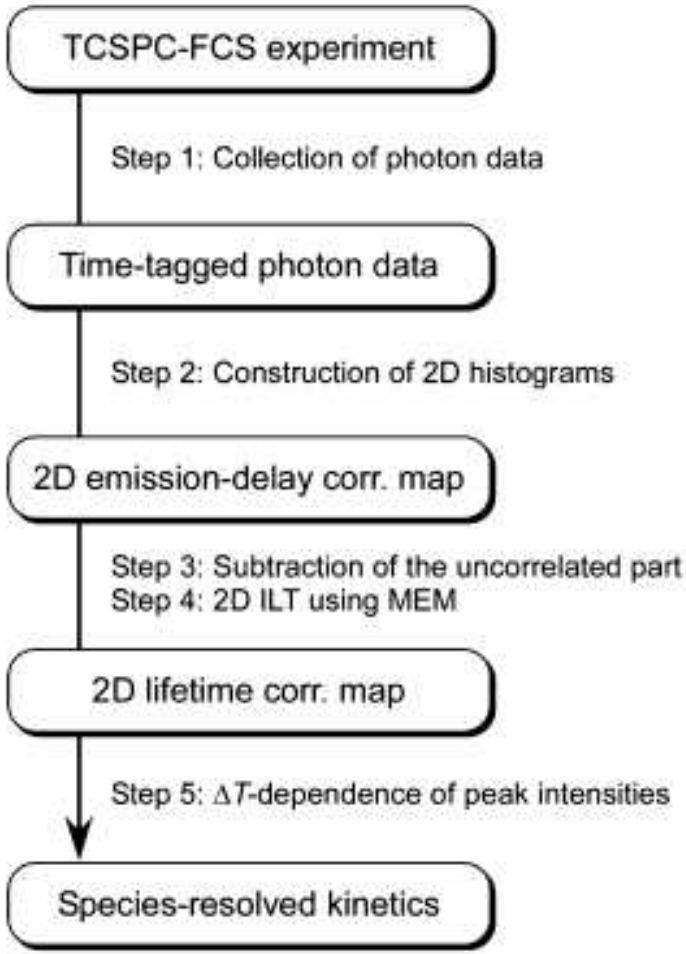


$$(k_{12}+k_{21})^{-1} < \Delta T < (k_{23}+k_{32})^{-1}$$

$$1 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} 2 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} 3$$

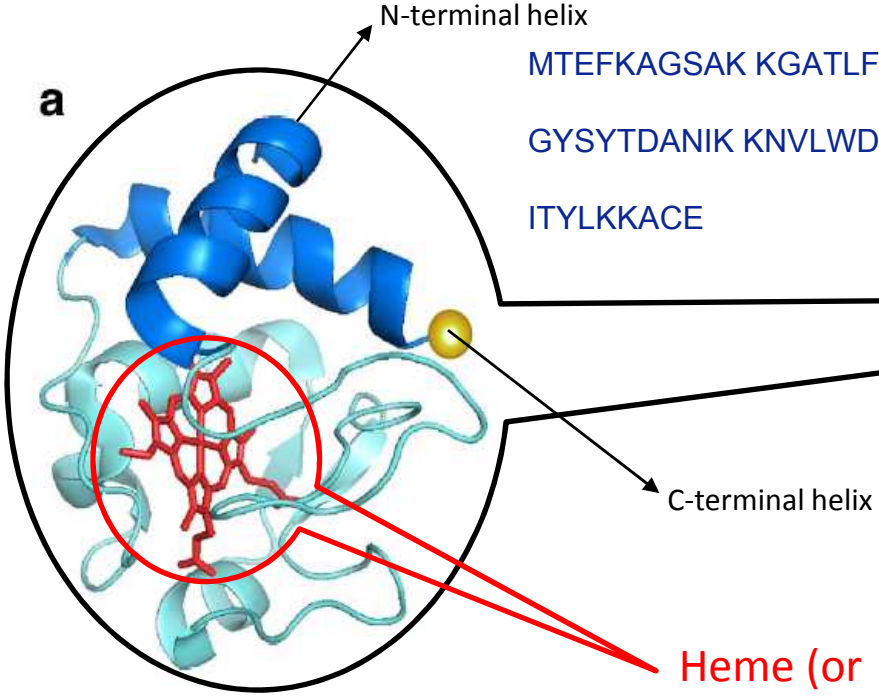
Method: 2D Fluorescence Lifetime Correlation Spectroscopy (2D-FLCS)





Sample – Cyt c protein

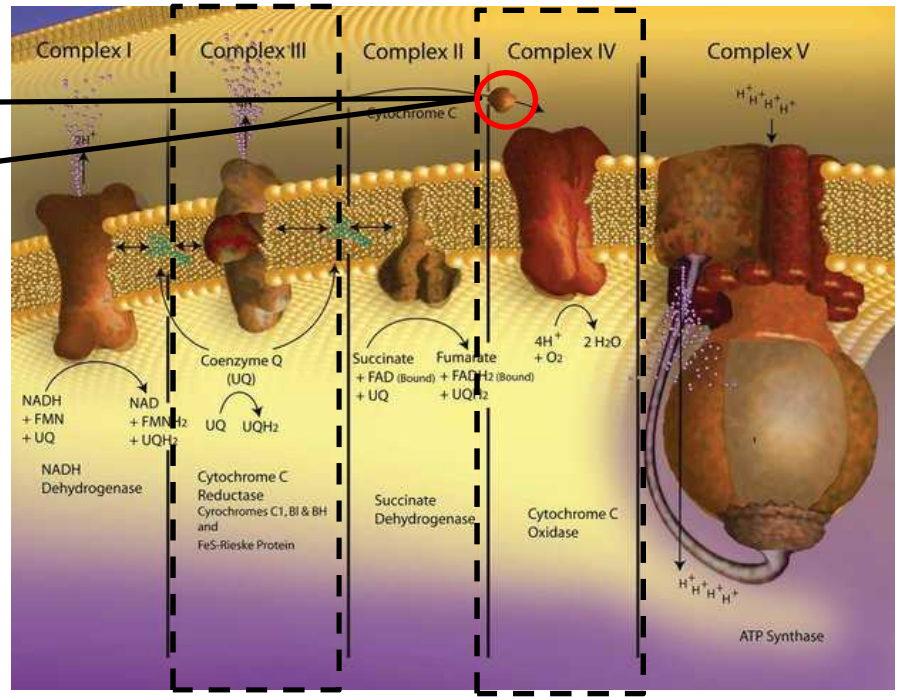
*Yeast iso-1-cytochrome c (cyt c): electron transporting protein



MTEFKAGSAK KGATLFKTRC LQCHTVEKGG PHKVGPNLHG IFGRHSGQAE

GYSYTDANIK KNVLWDENNM SEYLTNPKKY IPGTKMAFGG LKKEKDRNDL

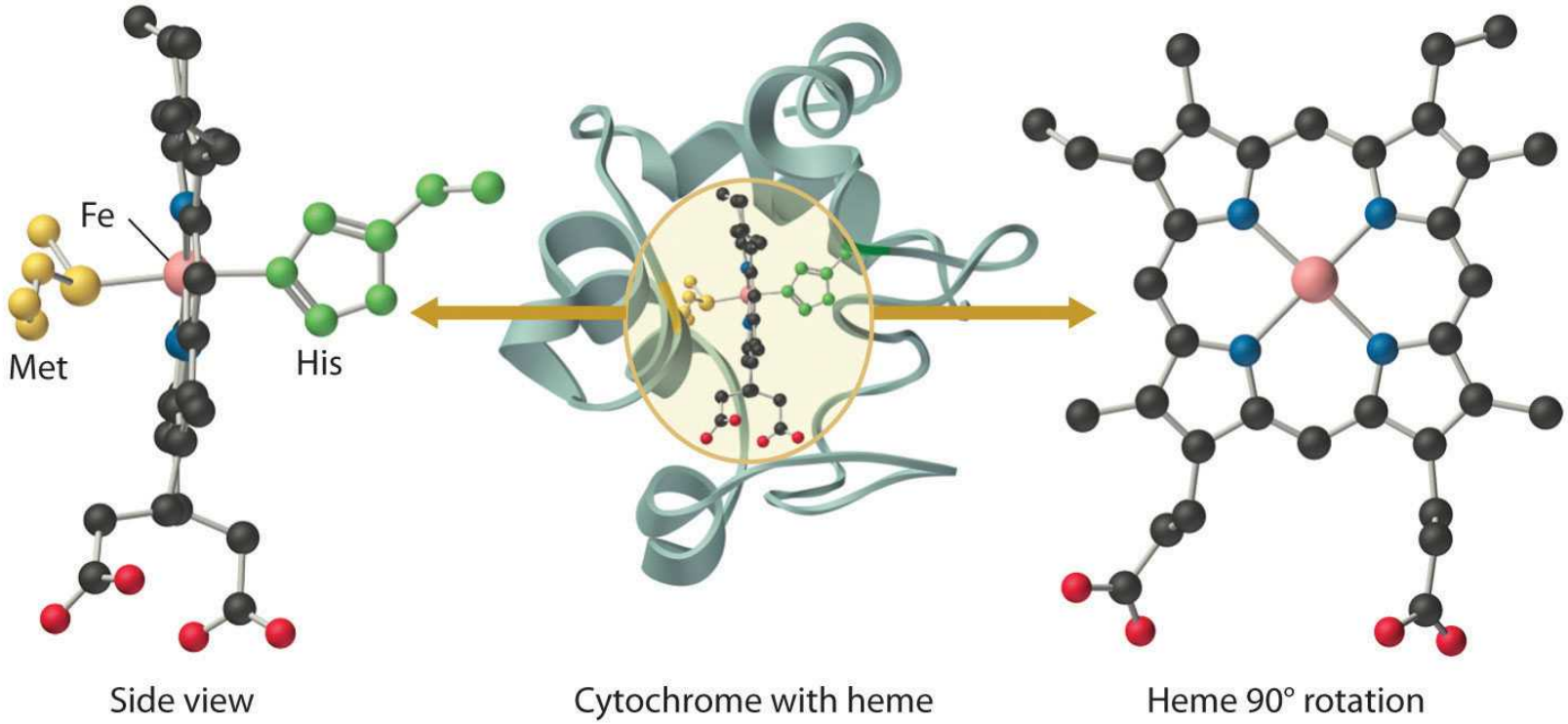
ITYLKKACE



Conformation change occurs with pH variation.

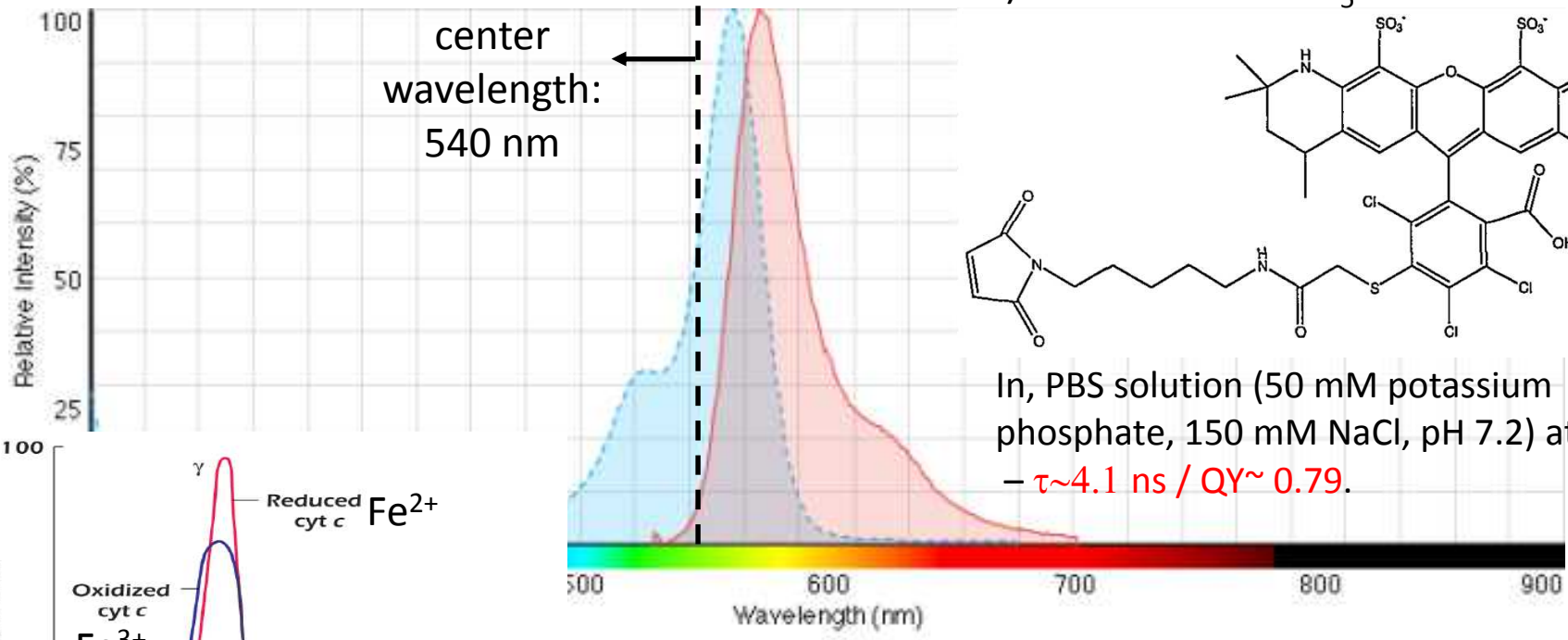
Sample – Cyt c protein

*Yeast iso-1-cytochrome c (cyt c): electron transporting protein



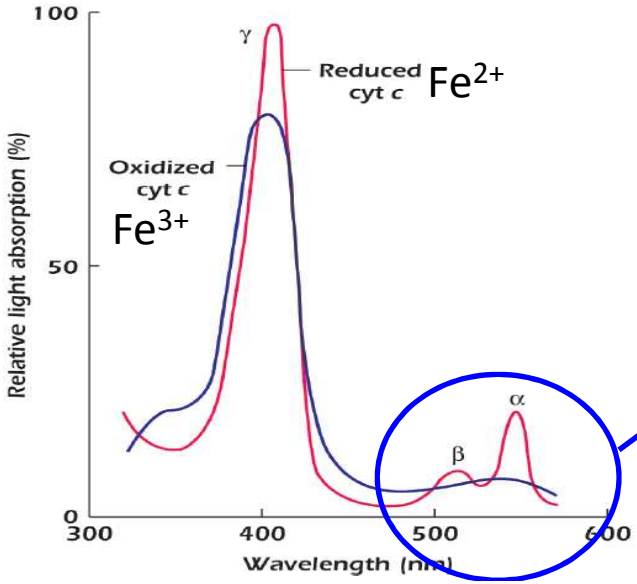
Donor dye – Alexa Fluor 546

Excitation center wavelength: 540 nm



Ex) Alexa Fluor 546 C_5

In, PBS solution (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) at 22°C.
– τ~4.1 ns / QY~ 0.79.



Overlap btw absorption band of heam in cyt c and Alexa 546 emission band.

Experimental Results

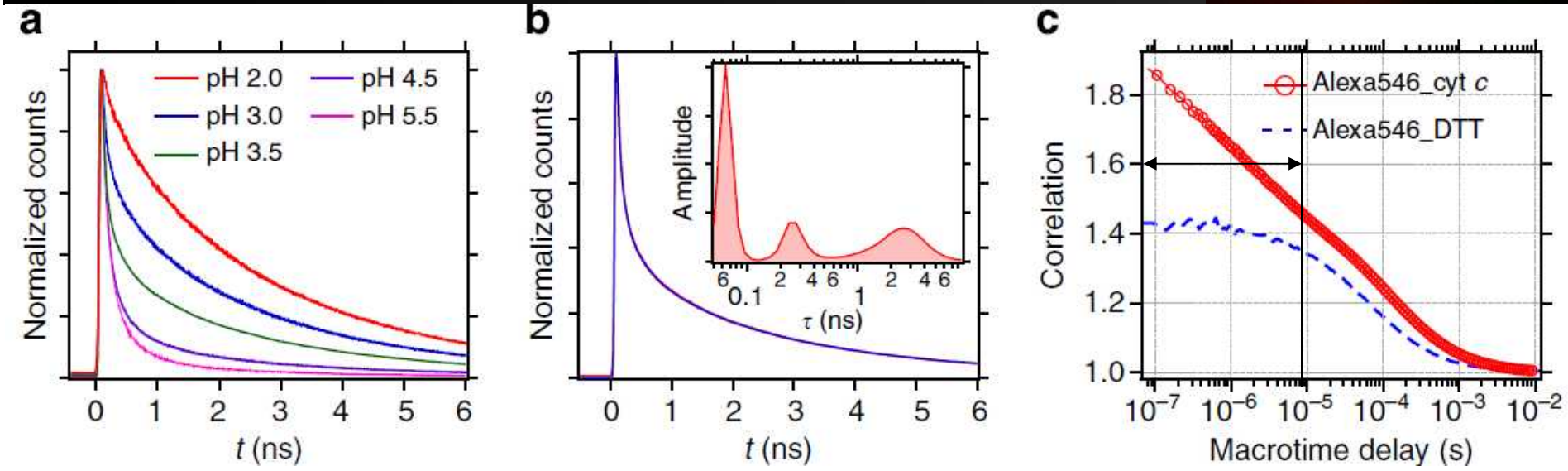
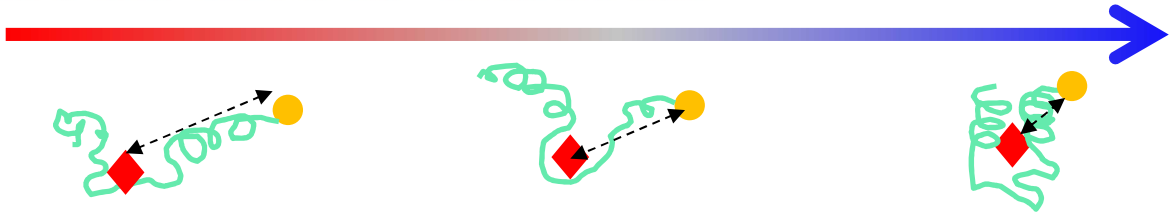
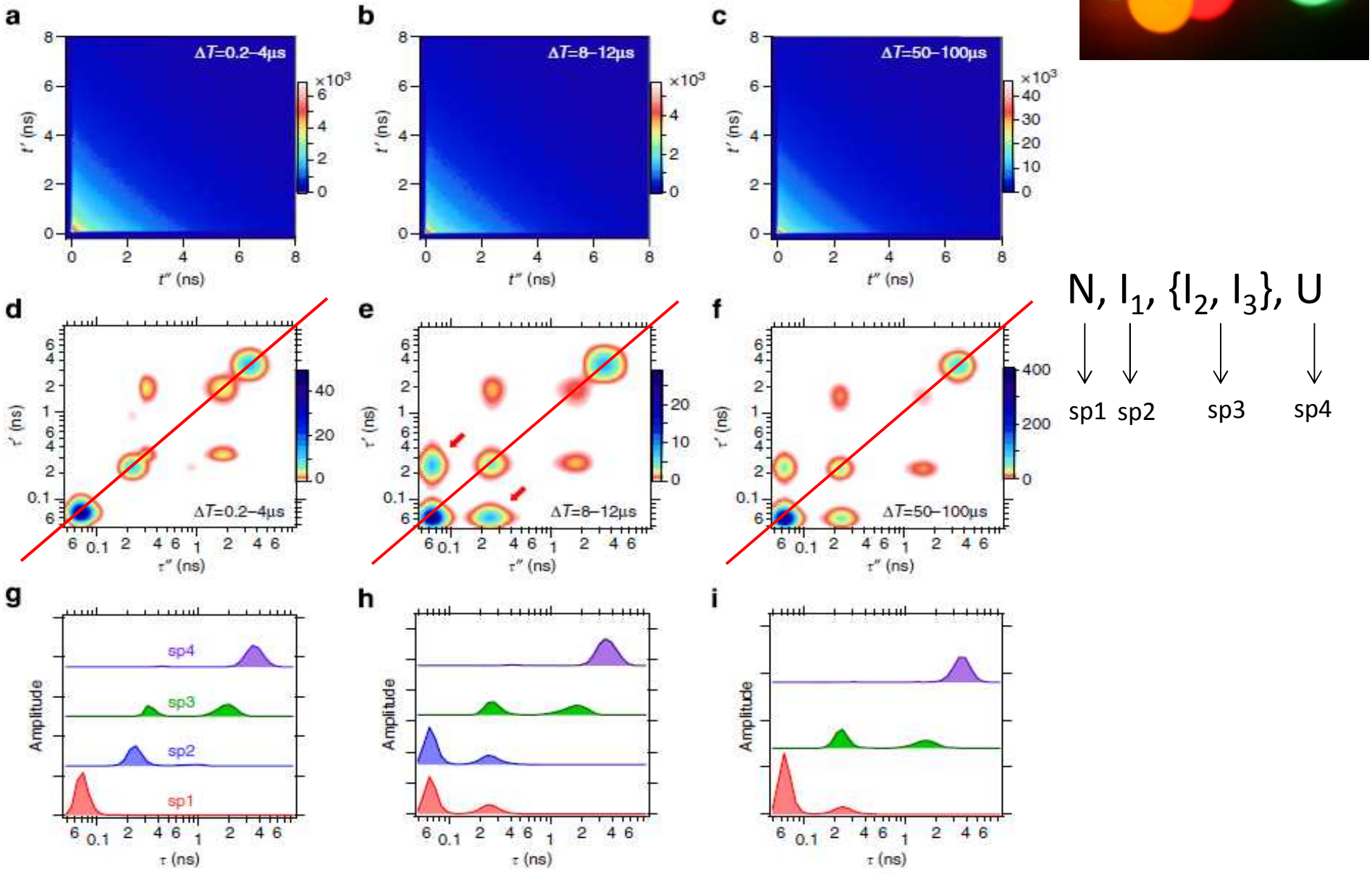


Figure 2 | Fluorescence decay and fluorescence correlation curves. (**a**) pH-dependent fluorescence decay curves of Alexa546_cytochrome *c*. (**b**) Fluorescence decay curve of Alexa546_cytochrome *c* at pH 3.5 (red solid line). Inverse Laplace transform was performed and the result is shown in the inset. The decay curve calculated by Laplace transform from the lifetime distribution is also shown (blue solid line). Decay curves in **a**,**b** are normalized for the intensity at $t = 0$. (**c**) Fluorescence correlation curve (red circle) of Alexa546_cytochrome *c* at pH 3.5. The data obtained from Alexa546_DTT at the same pH is also shown for comparison (blue broken line).



$$I(t) = \int \alpha(\tau)\exp(-t/\tau)d\tau \quad \xleftrightarrow{\text{ILT}} \quad \alpha(\tau) = \int I(t)\exp(+t/\tau)dt$$

Experimental Results – 2D FLCS



$N, I_1, \{I_2, I_3\}, U$

sp1 sp2 sp3 sp4

Experimental Results
-Autocorrelation peak vs ΔT

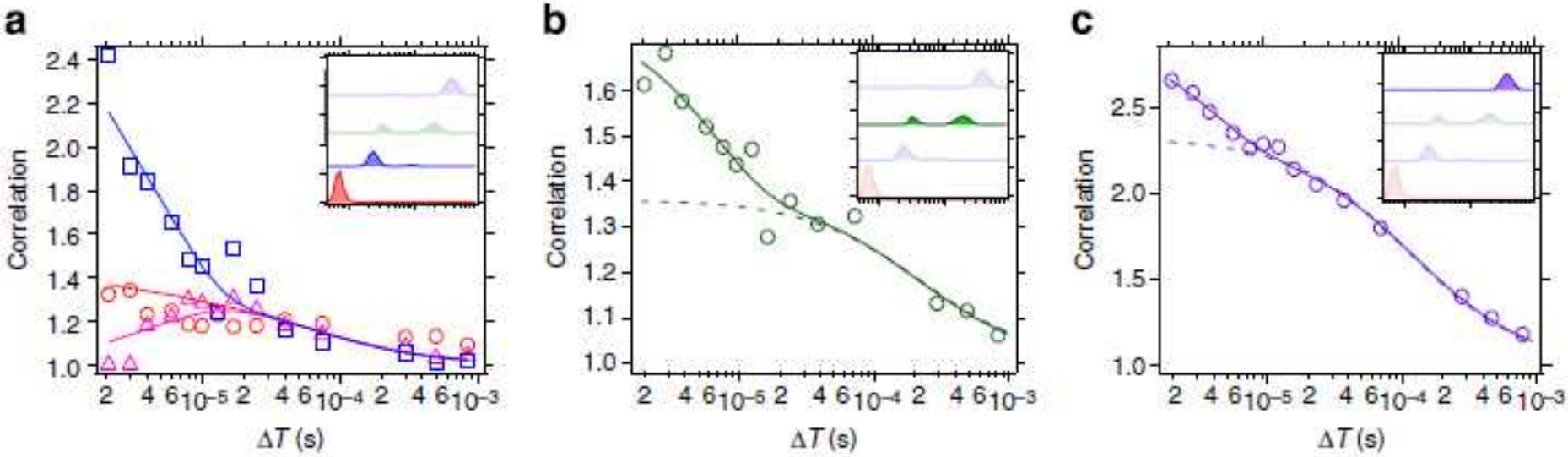
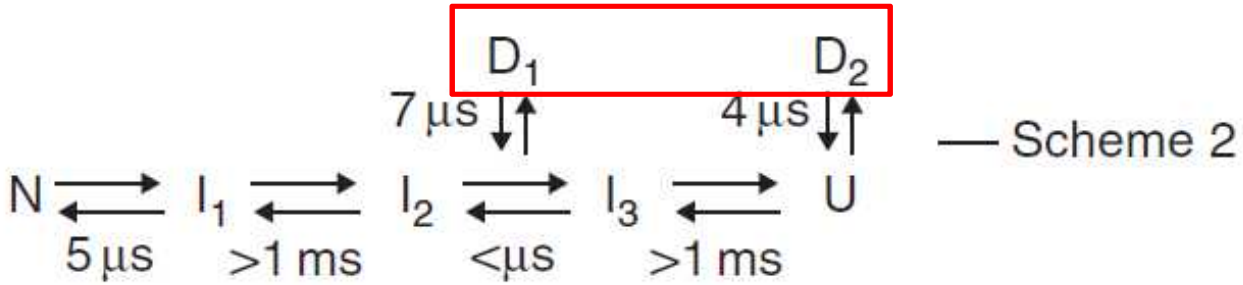


Figure 5 | Correlation curves of substates of cytochrome c. (**a**) Autocorrelations of N (red circles), I_1 (blue squares), and the cross-correlation between N and I_1 (pink triangles). (**b**) Autocorrelation of I_{en} (green circles). (**c**) Autocorrelation of U (purple circles). The corresponding lifetime component is highlighted in the inset of each figure. The correlation curves in **a** were fitted with theoretical equations (20)–(22), and the best fits are shown with solid lines. For the data shown in **b**,**c**, each data set was fitted both with equations (25) and (26), and the fits are shown with broken and solid lines, respectively.



Experimental Results
-Autocorrelation peak vs ΔT

For fitting N, I_1 auto and cross correlation

$$G_{NN}(\Delta T) = \frac{1}{N_{(N+I_1)}} g_D(\Delta T)(1 + K\exp(-\Delta T/\tau_R)) + 1, \quad (20)$$

$$G_{II}(\Delta T) = \frac{1}{N_{(N+I_1)}} g_D(\Delta T)\left(1 + \frac{1}{K}\exp(-\Delta T/\tau_R)\right) + 1, \quad (21)$$

$$G_{NI}(\Delta T) = \frac{1}{N_{(N+I_1)}} g_D(\Delta T)(1 - \exp(-\Delta T/\tau_R)) + 1, \quad (22)$$

$$g_D(\Delta T) = \left(\frac{1}{1+\Delta T/\tau_D}\right)\left(\frac{1}{1+w^2\Delta T/\tau_D}\right)^{1/2}, \quad (23)$$

$$\tau_R = 1/(k_{NI} + k_{IN}). \quad (24)$$

For fitting I_{en}, U auto correlation

$$G(\Delta T) = \frac{1}{N} g_D(\Delta T) + 1, \quad (25)$$

$$G(\Delta T) = \frac{1}{N} g_D(\Delta T) g_R(\Delta T) + 1, \quad (26)$$

$$g_R(\Delta T) = 1 + K\exp(-\Delta T/\tau_R). \quad (27)$$

Energy landscape

$$r = R_0 \left(\frac{\tau_{DA}}{\tau_D - \tau_{DA}} \right)^{1/6}, \qquad (3)$$

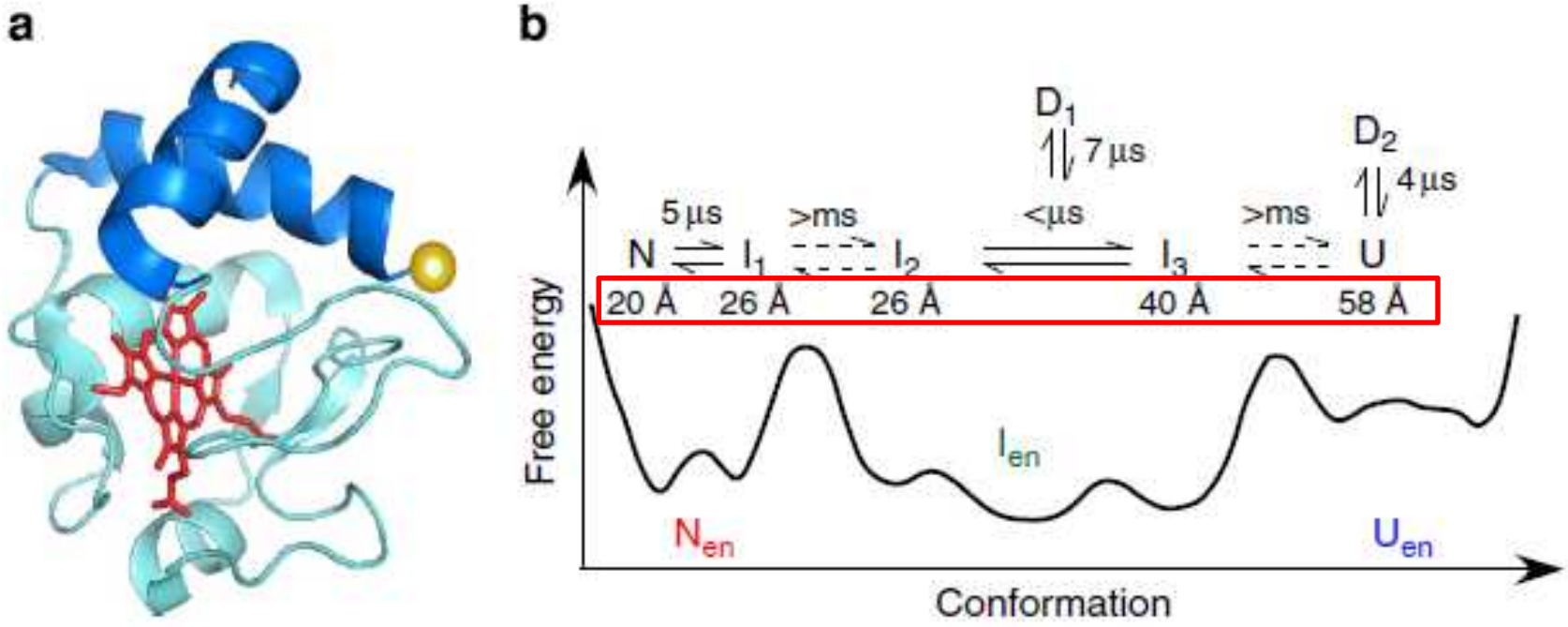


Figure 7 | Conformational transition of cytochrome *c*. (**a**) Native-state structure of cytochrome *c* (PDB ID:1YCC). N- and C-terminal helices are highlighted with blue. The position of the donor dye is shown by a yellow sphere. (**b**) Schematic free energy landscape and relevant conformational dynamics of cytochrome *c* (pH 3.5). The equilibration times among conformers and the donor-haem distances evaluated in the present work are given.

Summary

1.Few μs conformation change between sub states can be resolved by 2D-FLCS.

2. Auto correlation curves of sub states U and I_{en}, decaying profile can only be explained by introducing another state (dark states).

3. Relationship energy landscape and conformation change is described.