ASEC 2025 Conference

The 6th International Electronic Conference on Applied Sciences

09–11 December 2025 | Online

MDPI

Determination of Carbon Monoxide Dynamics in Hemoglobin Subunits Using Singular Value Decomposition and Maximum Entropy Method

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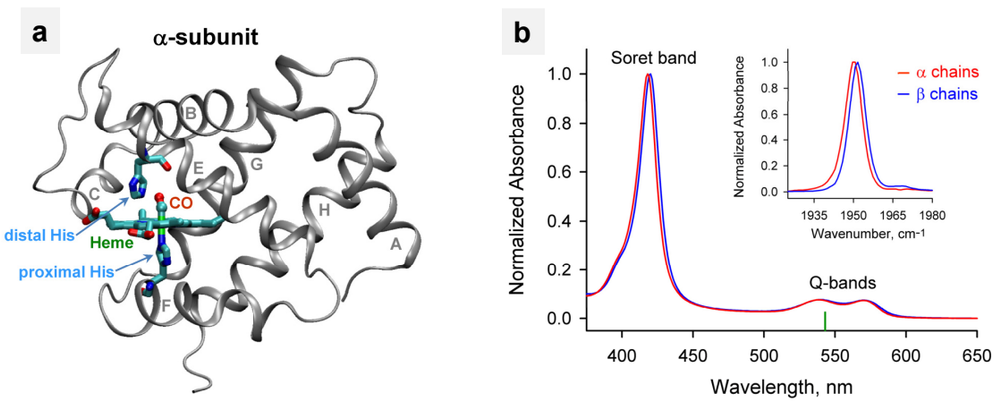
INTRODUCTION & AIM

Determining dynamics of bond breaking between carbon monoxide (CO) and heme proteins is essential to understand the interplay between protein function and dynamics, which is one of the fundamental challenges of physical biology. There is an ongoing debate about the mechanism of CO photodissociation from the heme iron. Here we use picosecond to millisecond transient mid-infrared spectroscopy to determine the dynamics of CO photodissociation from the isolated human hemoglobin chains.



(a) Structure of the α subunit of human carbonmonoxy hemoglobin (HbCO)
(b) Visible (the main figure) and mid-infrared absorption spectra (the inset) of the isolated carbonmonoxy α and β chains.
The absorbance in the visible region is due to the heme group present in the proteins. The excitation wavelength, λ exc = 543 nm, is indicated as the vertical green mark.

METHOD

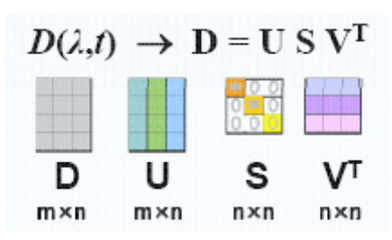
Photoinduced absorption changes of the isolated carbonmonoxy hemoglobin subunits were measured after photoexcitation at 543 nm into the Q bands of the heme moiety.

Time-resolved spectra in the mid-IR region were measured on the ULTRA apparatus at the Central Laser Facility (Harwell, UK).

All the experiments were performed in deuterated 50 mM Tris buffer, pD 8.2, at 19°C.

Time evolution of the vibrational spectra of the coordinated as well as photodissociated CO molecule was monitored in the spectral range between 1900 and 2180 cm^{-1}. The mid-IR spectrum of the liganded subunits shows discrete CO stretch bands, denoted A_0 (~1,968 cm^{-1}) and A_1 (~1,950 cm^{-1}). The distinct stretch bands for CO photolyzed and temporarily trapped in the protein matrix are detected in the region of 2,090–2,160 cm^{-1}.

Singular value decomposition (SVD)
The measured transient mid-IR spectra were analyzed using SVD.



Maximum entropy method (MEM) analysis
The time-dependent amplitudes, V_i, obtained in the course of the SVD analysis, were subsequently subjected to the MEM analysis which extracts two model-independent distributions of the effective log-lifetimes, *g(log τ)* and *h(log τ),* from the data.

$$F(t) = F_0 \int_{-\infty}^{+\infty} (g(\log\tau) - h(\log\tau)) e^{-t/\tau} d\log\tau$$

CONCLUSION

We find that the breaking of the Fe–CO bond is not a single-step process as was commonly accepted, but rather at least a two-step process, which includes both the known prompt sub-50-fs CO dissociation event and the additional slower, ~15 ps CO dissociation process discovered in this study.

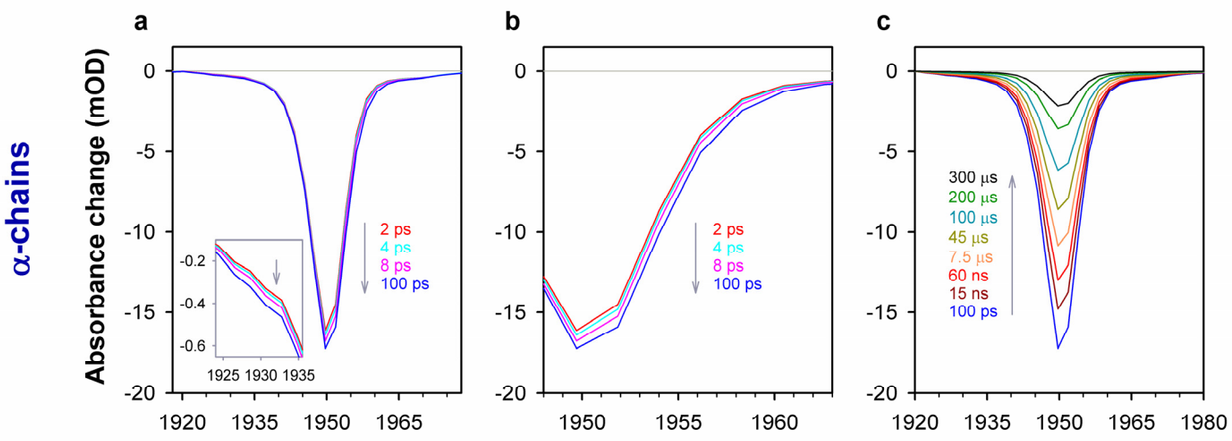
These results offer the first direct experimental proof of the CO photodissociation mechanism containing several dissociative states.

REFERENCES

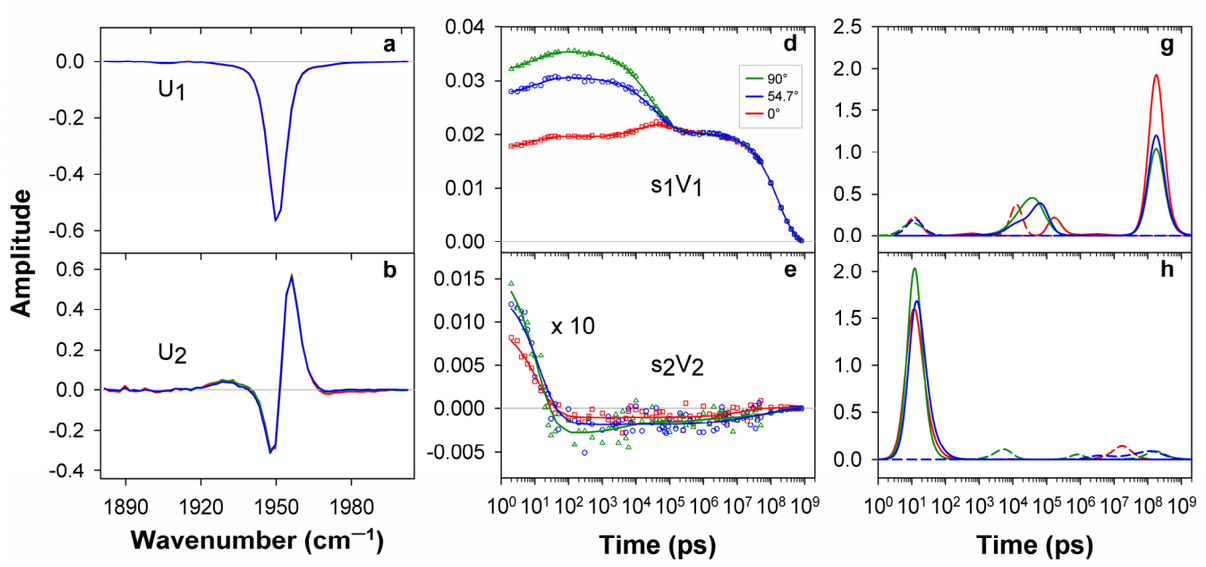
S.V. Lepeshkevich, I.V. Sazanovich, M.V. Parkhats, S.N. Gilevich, A.V. Yantsevich, J.A. Weinstein, M. Towrie, B.M. Dzhagarov. Direct observation of two-channel photodissociation of carbon monoxide from the hemoglobin subunits // *Nature Communications*. 2025. V. 16, P. 7746.

The authors thank STFC for the beamtime on CLF Ultra facility (App. 16130005).

RESULTS & DISCUSSION

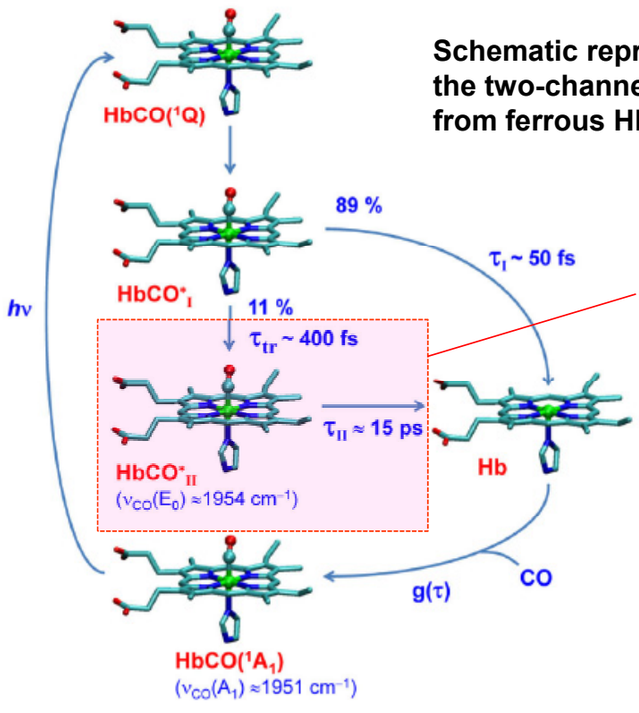


Time-resolved transient mid-IR absorption changes of the isolated carbonmoxy α chains of human HbCO in the region of ground-state bleach



SVD and MEM of the measured time-resolved polarized transient IR absorption spectra after the photoexcitation of the isolated carbonmonoxy α chains

The first two SVD components that make the main contribution to the observed spectra are shown. **a, b** Orthonormal basis spectra (columns of U). **d, e** Time-dependent amplitudes (columns of V) multiplied by the corresponding singular values (elements of the diagonal matrix S). The time-dependent amplitudes and the fit of those obtained with the MEM analysis are reported as symbols and solid lines, respectively. **g** and **h** Lifetime distributions derived by MEM from V_1 and V_2, respectively. The lifetime distributions, corresponding to decaying and rising kinetics, are presented as solid and dash lines, respectively. The data obtained at the three polarization settings are colour-coded: 0° (red), 54.7° (blue), and 90° (green).



Schematic representation of the two-channel photodissociation of CO from ferrous HbCO

the discovered dissociative excited state

The excited-state species $HbCO^*_I$ and $HbCO^*_{II}$ are assumed to be formed sequentially from the ground-state species $HbCO(^1A_1)$ after the photoexcitation to the $^1\pi\pi^*$(Q) state, HbCO(^{1}Q), of the CO-bound protein.