Picosecond to Millisecond Conformational Relaxation and Molecular Oxygen Rebinding in α and β Subunits within Oxy-Cyanomet Valency Hybrids of Human Hemoglobin

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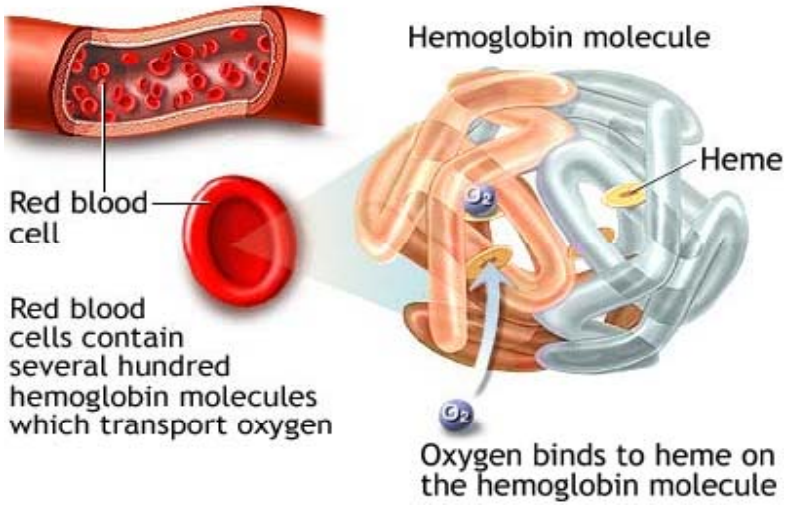
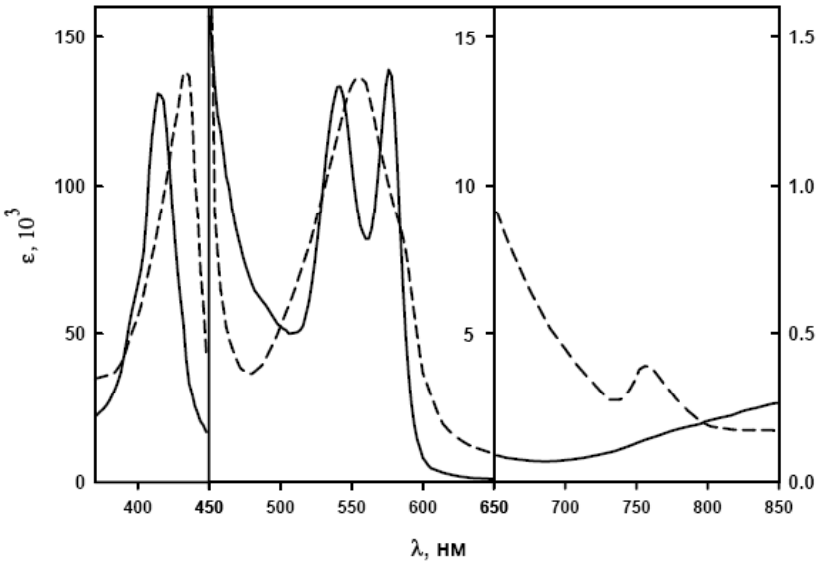
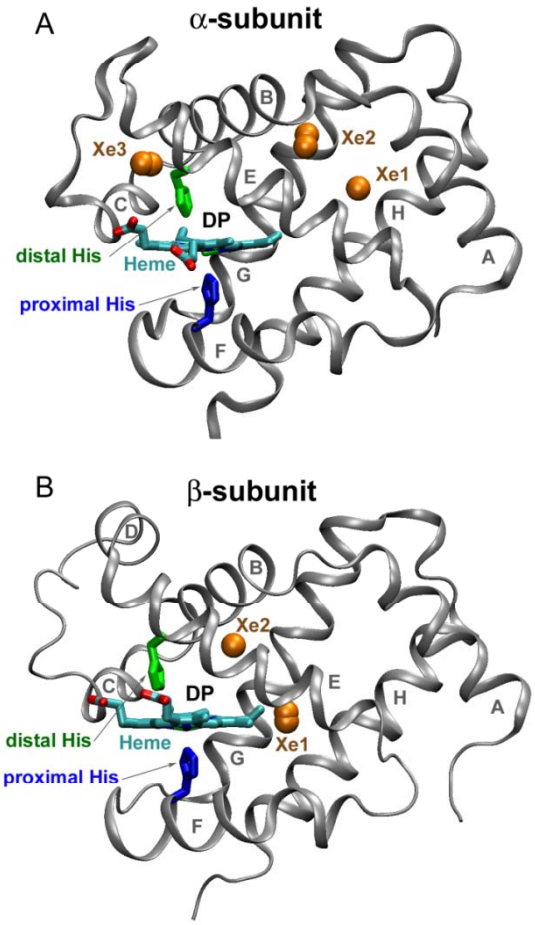




Ground-state absorption spectra for the oxy- (——) and deoxy-myoglobin and hemoglobin (– –)



Structure of the α and β subunits of human hemoglobin (panel A and B, respectively) (PDB entry 2w6v). The distal heme pocket (primary docking site) is labeled as DP. The distal and proximal histidines are labeled in green and blue, respectively. Xe sites are represented by labeled orange spheres.

The principal aim of this study

To determine how ligand-induced conformational changes influence the individual O_2 rebinding properties of the α and β subunits in the R-state hemoglobin (Hb).

Picosecond to millisecond laser time-resolved transient absorption spectroscopy was used to study O_2 rebinding and conformational relaxation following O_2 photodissociation in the α and β subunits within human Hb in the quaternary R-like structure.

Materials

Oxy-cyanomet valency hybrids, $\alpha_2(Fe^{2+}\text{-}O_2)\beta_2(Fe^{3+}\text{-}CN)$ and $\alpha_2(Fe^{3+}\text{-}CN)\beta_2(Fe^{2+}\text{-}O_2)$, were used as models for the oxygenated R-state Hb [1].

Technical details

Time-resolved spectra were measured on the ULTRA apparatus in the Time-Resolved Multiple Probe Spectroscopy mode [2] at CLF.

1 kHz, 100 fs, 543 nm pump pulses; 1 μJ pulse energy; 150 x 150 μm spot.

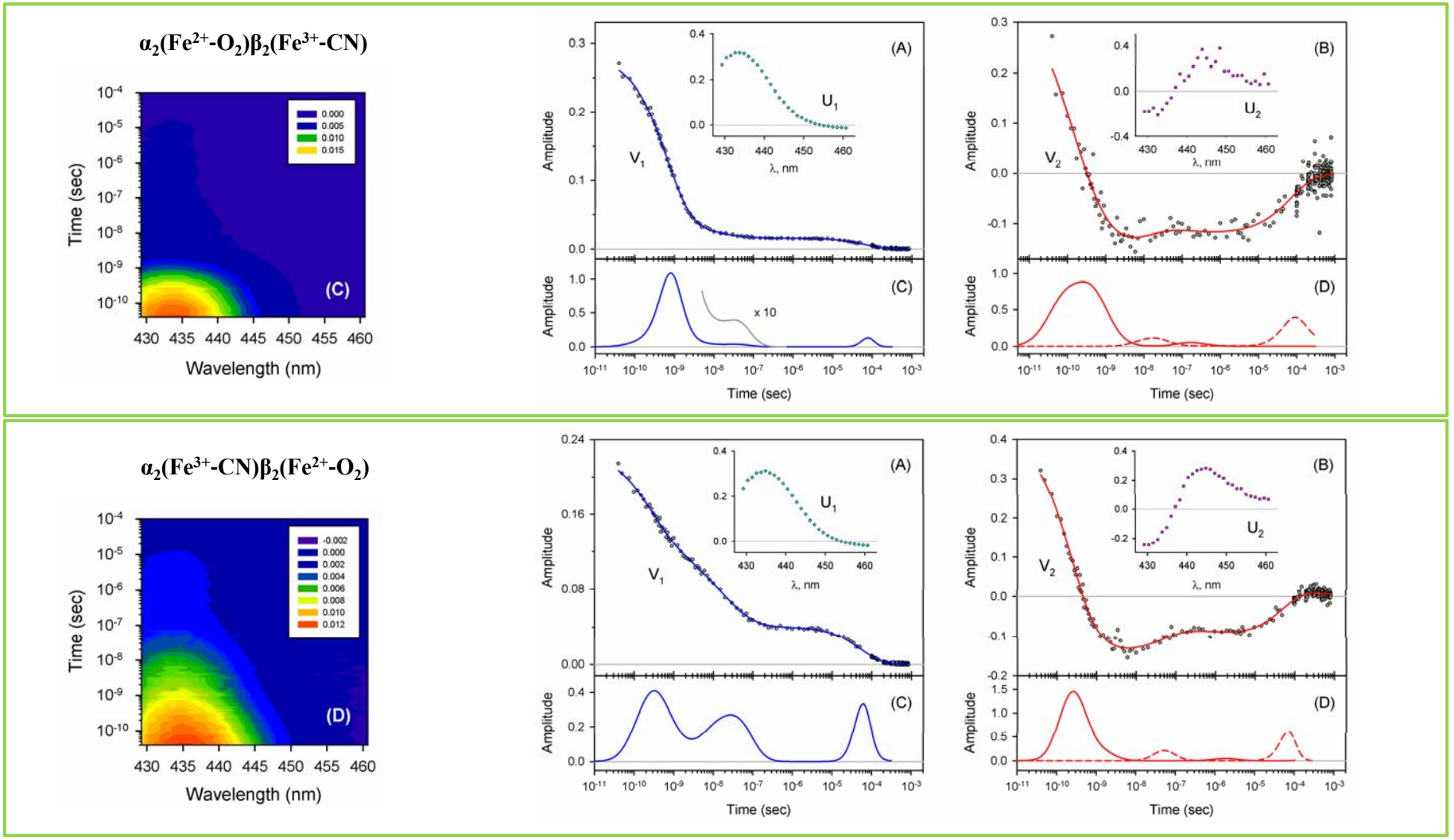
Detection range from about 430 to 460 nm in the time range from 1 ps up to 800 μs after the laser photoexcitation.

References

[1] Lepeshkevich S.V. et al., *Chem. Sci.*, **2021**, DOI:10.1039/D1SC00712B.

[2] Greetham G.M. et al., *Rev. Sci. Instrum.*, **2012**. Vol. 83. P. 103107.

Picosecond to millisecond laser time-resolved transient absorption spectroscopy

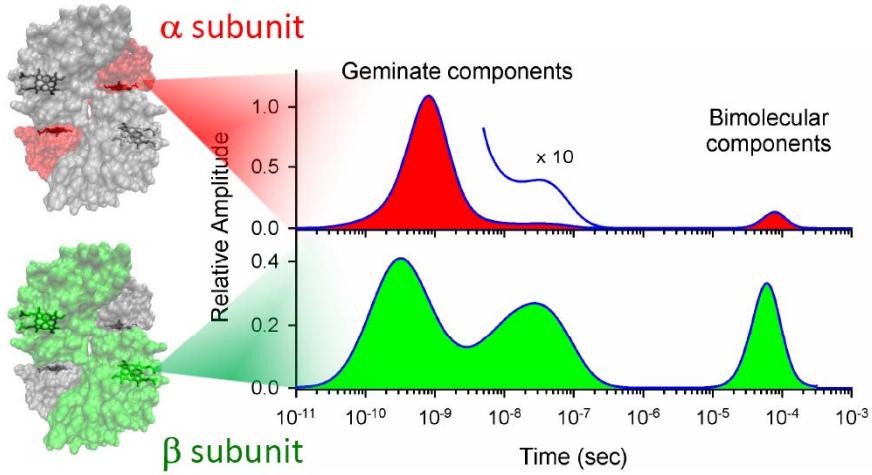


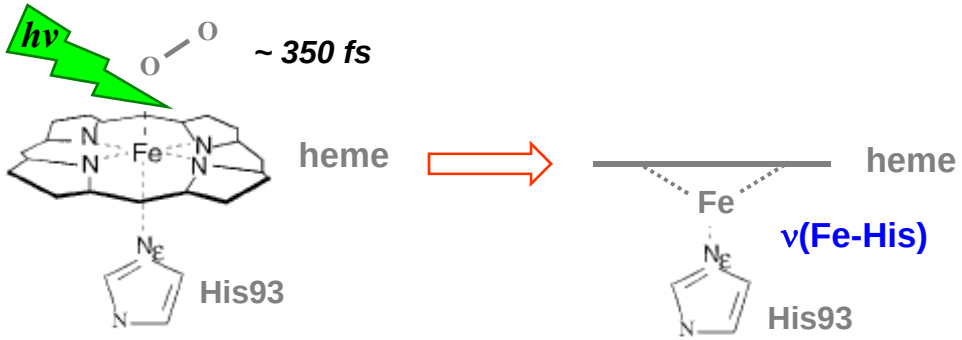
Contour plots of the time-resolved absorption difference spectra measured after O_2 photodissociation

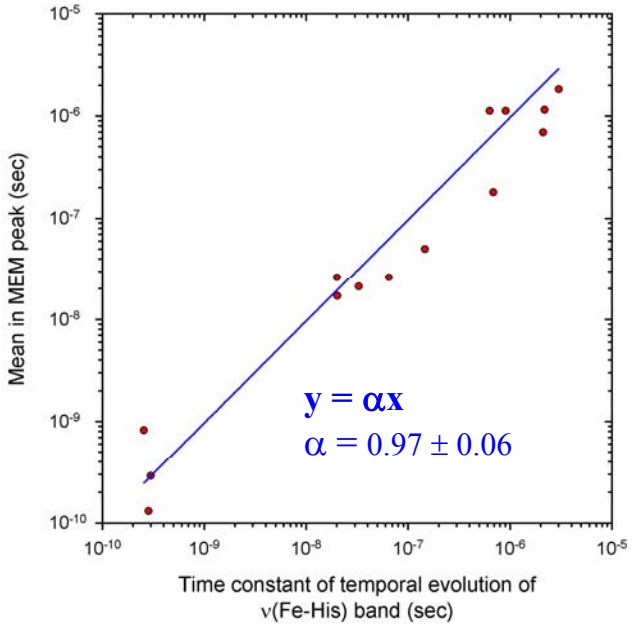
Excitation wavelength: λ_{exc} = 543 nm
Conditions: 50 mM Tris-HCl buffer, pH 8.2, 19°C
Protein concentrations: 130–200 μM in heme

Singular value decomposition of the time-resolved spectra D(λ,t) measured after O_2 photodissociation. **(A)** Time-dependent amplitudes (V_1) of the first basis spectrum. **(B)** Time-dependent amplitudes (V_2) of the second basis spectrum. In panel (A) and (B), the time-dependent amplitudes and their fit obtained with the maximum entropy method analysis are reported as circles and solid lines, respectively. The first and second basis spectra, U_1 and U_2, are shown in the insets to panels (A) and (B), respectively. **(C) Lifetime distribution derived by maximum entropy method (MEM)** from V_1. **(D)** Two lifetime distributions derived by MEM from V_2. The two lifetime distributions, corresponding to decaying and rising kinetics, are presented as solid and dash lines, respectively.

Functional non-equivalence of the α and β subunits within the valency hybrids



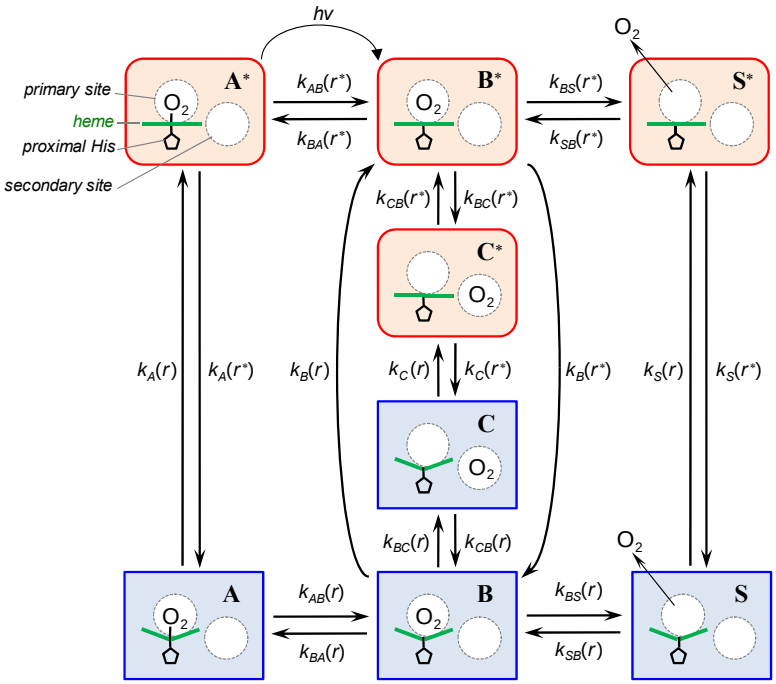




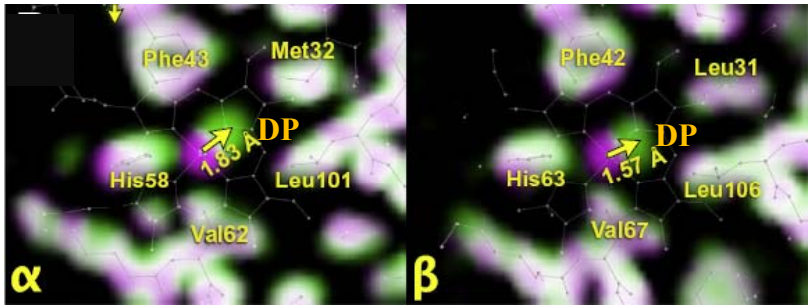
Correlation between time constants of temporal changes in the ν(Fe-His) frequency following the CO photodissociation (taken from literature) and the means of the lifetime distribution peaks extracted by us from the second amplitude vector, V_2

The obtained correlation provided evidence for the modulation of the O_2 rebinding to the individual α and β subunits within human Hb in the R-state structure by **the intrinsic heme reactivity** through a change in proximal constraints upon the relaxation of the tertiary structure on a picosecond to microsecond time scale.

Lepeshkevich S.V. et al., *Chem. Sci.*, **2021**, DOI:10.1039/D1SC00712B.

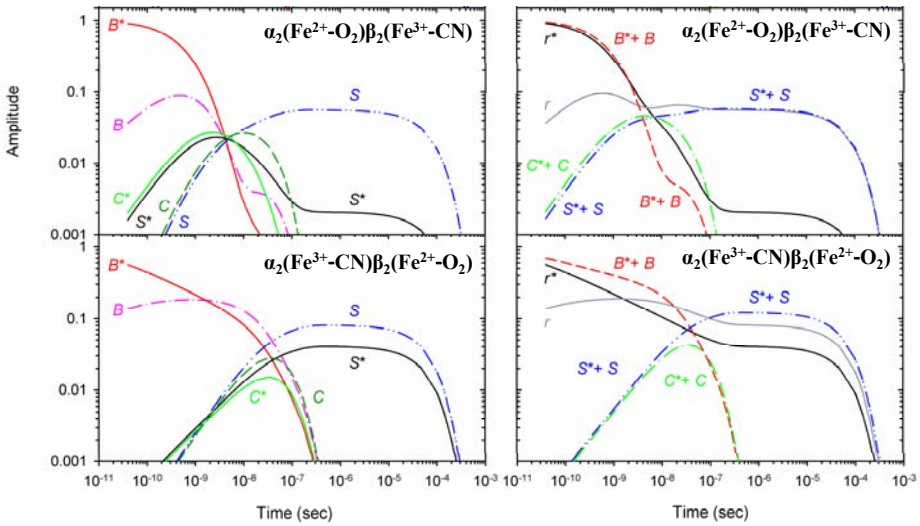


Kinetic model for geminate O_2 rebinding in the ferrous hemoglobin subunits, ligand migration between the primary and secondary docking site(s), and nonexponential tertiary relaxation within the R quaternary structure

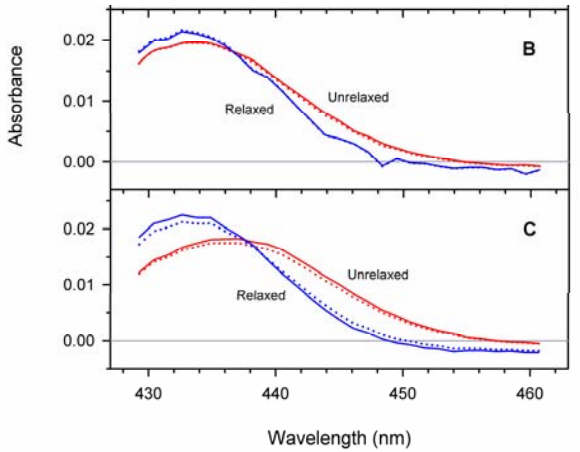


For the β subunits, the larger rate constant $k_{gem}(r^*)$ is explained by the closer location of the primary docking site to its binding site in these subunits compared to that in the α subunits, suggesting **the distal control** of the geminate O_2 rebinding.

(Figure is taken from F. Schotte et al. *Chem. Phys*. 422 (2013) 98–106)



Populations of the unliganded (reagent) states (*left column*) and the unliganded subunits in the unrelaxed r^* and relaxed r tertiary conformations (*right column*) predicted by the kinetic model



Difference spectra between unliganded and O_2-liganded ferrous subunits in each of the two tertiary structures r^* and r (labeled as *unrelaxed* and *relaxed*, respectively) predicted by the kinetic model (*solid lines*)

Conclusions

- Significant functional non-equivalence of the α and β subunits in both the geminate O_2 rebinding and concomitant structural relaxation was revealed.

- For the β subunits, the rate constant for the geminate O_2 rebinding to the unrelaxed tertiary structure and the tertiary transition rate were found to be greater than the corresponding values for the α subunits.

- The conformational relaxation following the O_2 photodissociation in the α and β subunits was found to decrease the rate constant for the geminate O_2 rebinding, this effect being more than one order of magnitude greater for the β subunits than for the α subunits.

- Evidence was provided for the modulation of the O_2 rebinding to the individual α and β subunits within human Hb in the R-state structure by the intrinsic heme reactivity through a change in proximal constraints upon the relaxation of the tertiary structure on a picosecond to microsecond time scale.

- Our results demonstrate that, for native R-state oxyhemoglobin, O_2 rebinding properties and spectral changes following the O_2 photodissociation can be adequately described as the sum of those for the α and β subunits within the valency hybrids.

Acknowledgements

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