# Non-equivalence of α and β subunits within human hemoglobin in conformational relaxation and molecular oxygen rebinding

**S.V. Lepeshkevich[a], I.V. Sazanovich[b], M.V. Parkhats[a], S.N. Gilevich[c], B.M. Dzhagarov[a]**

*[a]B.I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, 68 Nezavisimosti Ave, Minsk 220072, Belarus,*
*e-mail: s.lepeshkevich@ifanbel.bas-net.by*
*[b]Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, OX11 0QX, UK*
*[c]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 5 Academician V. F. Kuprevich Street, Minsk 220141, Belarus*

Picosecond to millisecond laser time-resolved transient absorption spectroscopy was used to study molecular oxygen ($O_2$) rebinding and conformational relaxation following $O_2$ photodissociation in the α and β subunits within human hemoglobin in the quaternary R-like structure. Oxy-cyanomet valency hybrids [1] were used as models for oxygenated R-state hemoglobin. An extended 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, was introduced and discussed. 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 hemoglobin 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.

**REFERENCE(S)**

1. **S.V. Lepeshkevich, I.V. Sazanovich, M.V. Parkhats, S.N. Gilevich, B.M. Dzhagarov**. Chem. Sci., **12** (2021) 7033–7047.