

Structural Studies on *Cerebratulus lacteus* Mini-Hb K(E10)W and L(G12)A Mutants

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A very short hemoglobin (CerHb; 109 amino acids) binds O₂ cooperatively in the nerve tissue of the nemertean worm *Cerebratulus lacteus* to sustain neural activity during anoxia. The structure of oxygenated wild-type CerHb displays a substantial editing of the globin fold which makes CerHb unique among the known globin fold evolutionary variants [1].

Here we present the crystal structures of two CerHb mutants: Lys(E10)Trp (at 2.3 Å resolution) and Leu(G12)Ala (at 1.6 Å resolution) and its complex with xenon atoms (at 2.3 Å resolution).

The single mutation Lys(E10)Trp, intended to perturb the protein heme binding, has also a dramatic and unexpected effect on the H-bond network stabilizing the O₂ ligand, and it makes the protein more susceptible to heme-iron oxidation.

The Leu(G12)Ala mutant and its complex with xenon atoms map a wide protein matrix tunnel connecting the distal site to a surface cleft between the E and H helices, thus suggesting a novel ligand access to heme.

[1] Pesce A., Nardini M., Dewilde S., Geuens E., Yamauchi K., Ascenzi P., Riggs A.F., Moens L., Bolognesi, M., *Structure*, 2002, **10**, 725.

Keywords: hemoglobin, mutagenesis, Xe binding