

# Evaluation of PEGylated and UnPEGylated *Chelonia mydas* and *Caretta caretta* hemoglobin: development of a hemoglobin based oxygen carrier

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With increased blood donor dependency, development of a novel blood substitute has become imperative. A hemoglobin based oxygen carrier could fulfill this need, and meet the demand for transfusions in underdeveloped areas and combat zones. The proposed nitric oxide generating capability of hemoglobins from the sea turtles *Caretta caretta* and *Chelonia mydas* play a major role in reoxygenation injury protection. This project evaluates relevant properties of the sea turtle hemoglobins with respect to potential for nitric oxide generation and seeks to PEGylate the sea turtle hemoglobins to increase suitability for in vivo animal studies. Initial tests compared PEGylated and UnPEGylated turtle hemoglobins to human hemoglobin (HbA) for colloid oncotic pressure (COP), viscosity, p50, and auto-oxidation. To understand the role of nitric oxide reduction rates, nitrite reductase reactions were performed. Further investigation of rebinding kinetics identified the hemoglobin equilibrium state. Results suggest that the turtle hemoglobins have higher viscosity and COP and lower oxygen affinity in comparison to HbA. Additionally, PEGylation increased the viscosity and COP while reducing methemoglobin percentages in turtle Hb. Turtle hemoglobin reduction ( $\text{Fe}^{+3} \rightarrow \text{Fe}^{+2}$ ) occurs quickly and more efficiently than human hemoglobin as well. Ultimately this examination furthers understanding of the structure and mechanisms used by sea turtle hemoglobins. Development of a blood substitute that models the unique characteristics of these hemoglobins could simultaneously mitigate reperfusion injury complications after surgery and help meet the high demand for blood transfusions.