# "Inside-Out" Site Directed PEGylation of Cross-Linked Hemoglobin

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An urgency to create an effective blood substitute comes from the lack of blood supply from human donors and the risk of blood pathogens. A hemoglobin-based oxygen carrier (HBOC) from a non-human source would carry oxygen to hypoxic tissue treating hemorrhagic trauma and other distresses that provoke oxygen deprivation of vital organs and tissue. However, injecting unmodified hemoglobin (Hb) into a living system can cause severe complications such as nephritic toxicity and vascular constriction. Nephritic toxicity is caused by the dissociation of the hemoglobin tetramer into two alpha-beta dimers [1]. Thus far, two approaches have been used to prevent such toxicity. Both approaches utilize a 3,5-dibromosalicylic fumarate (DBSF) to crosslink the bovine hemoglobin preventing tetramer dissociation. The first approach crosslinks the hemoglobin via the alpha subunits under deoxy conditions producing a low oxygen affinity product. The other approach crosslinks the hemoglobin via beta subunits under oxy conditions producing a high oxygen affinity product. In addition to nephritic toxicity, vascular constriction is a concern when hemoglobin tetramers are used to replace blood [2]. This can be prevented by producing a high molecular weight complex via "Inside-out PEGylation" in which multiple hemoglobins have been attached to an eight-arm PEG molecule (Figure 1). For the high affinity product, sulfhydryl-maleimide chemistry was used to attach the PEG to the Hb; while for the low affinity product, alkyne-azide chemistry was used to create better results. SDS-PAGE, gel filtration and analytical ultracentrifugation studies demonstrate that 3-4 hemoglobins are attached per PEG molecule. With the successful characterization results, we believe this is the first step to creating a viable blood substitute.

## *References*

[1] Hu et al. (2011) Increased Inter Dimeric Inter
action of Oxy Hemoglobin is Necessary for Attenuation of Reductive Pegylation Promoted Dissociation of Tetramer. Artificial Cells, Blood Substitutes, and Biotechnology. *Informa Healthcare: USA, Vol. 39: 69-78.*

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