

Wednesday, June 18th, 2014

LSC 3 - Life Sciences Centre

2350 Health Sciences Mall

12-1pm



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### “Structure and function of recombinant human hemoglobins containing circularly permuted $\beta$ -globin subunits”

We have expressed in bacteria a single-chain human hemoglobin (scHb) composed of two  $\alpha$ -globin subunits and two circularly permuted  $\beta$ -globin subunits fused with various short peptide sequences (1-8 amino acids). The scHb was designed to facilitate the generation of a monodisperse polyhemoglobin that possesses desirable properties for use as a hemoglobin-based oxygen carrier (HBOC) in critical care. We have recently solved X-ray crystal structures at 2.7-2.9 Å resolution for two related hemoglobins: (1) “ $\alpha$ -cp $\beta$ ”, a 64 kDa heterotetramer composed of two  $\alpha$ -globin subunits and two circularly permuted  $\beta$ -globin subunits, and (2) “sc- $\alpha$ -cp $\beta$ ” (single-chain  $\alpha$ -cp $\beta$ ) a 64 kDa dimer composed of two polypeptides in which an  $\alpha$ -globin is covalently linked to a circularly permuted  $\beta$ -globin by two flexible loops located at the  $\alpha_1\beta_1$  interface. scHb is a 64 kDa monomer composed of two sc- $\alpha$ -cp $\beta$  sequences that are linked by a single Gly residue between the  $\alpha$ -globins. Examination of the  $\alpha$ -cp $\beta$  and sc- $\alpha$ -cp $\beta$  crystal structures shows that the permutants fold into tertiary structures that are isomorphic to the wild-type human hemoglobin (HbA) crystal structure. Ligand binding studies have shown that  $\alpha$ -cp $\beta$ , sc- $\alpha$ -cp $\beta$ , and scHb show increased binding affinity and reduced binding cooperativity for CO and O<sub>2</sub> compared to HbA. Incorporation of point mutations ( $\alpha$ G15A,  $\beta$ G16A,  $\beta$ K82D,  $\beta$ H116I, and  $\beta$ N108K) has led to modest increases in  $P_{50}$  and cooperativity for the permuted globins.

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