May 14th, 10:00 AM - 2:00 PM

Structural Studies of a Circularly Permuted Human Hemoglobin Containing Low O_2-affinity Mutations

Rachel Hubbard
Western Washington University

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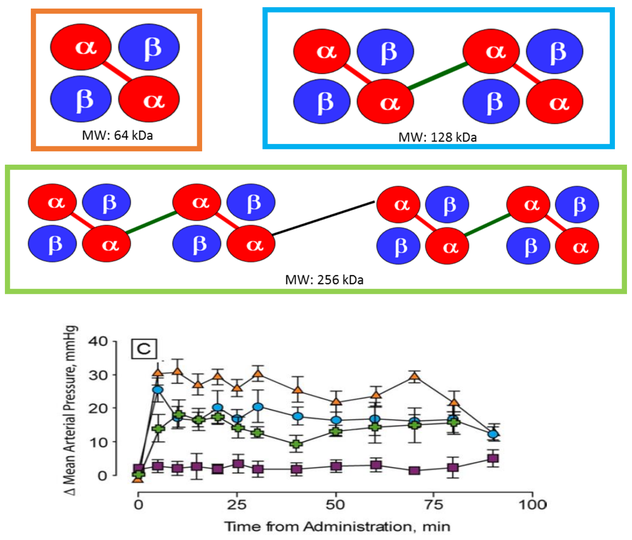
Rachel Hubbard, P. Clint Spiegel and Spencer Anthony-Cahill
Department of Chemistry, Western Washington University

Abstract

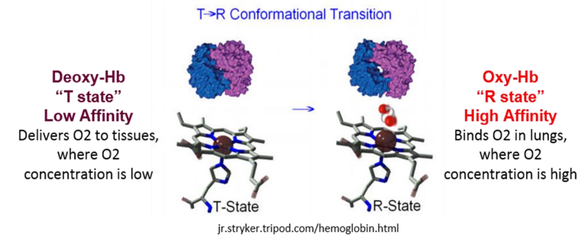
Our research is focused on the production of a hemoglobin-based oxygen carrier (HBOC) which can be used as a therapeutic in the event of acute blood loss. The administration of cell-free hemoglobin is associated with severe adverse effects due to dissociation of the tetrameric $\alpha_2\beta_2$ complex into $\alpha\beta$ heterodimers. Our approach to designing an effective HBOC is based on a recombinant circularly permuted human hemoglobin in which all of the subunits are linked in a single-chain fashion. This design would prevent the dissociation of the tetramer and allow for the biosynthesis of polymeric hemoglobins of defined mass. Preliminary ligand binding data with our permuted hemoglobins indicates that they prefer the high O_2-affinity R-state conformation over the low O_2-affinity T state. The βN108K and αV96W mutations were introduced to restore T state stability. Preliminary studies of the mutants have shown that while the βN108K mutation improved T-state stability, the αV96W mutation displays an unexpected destabilizing effect on the T state. We would like to understand the molecular basis for these surprising results. We intend to determine the X-ray crystal structure of the αV96W mutant as well as the βN108K mutant and the αV96W + βN108K double mutant to gain an atomic-level picture of protein structural differences that could explain these results.

Why sc-Hb?

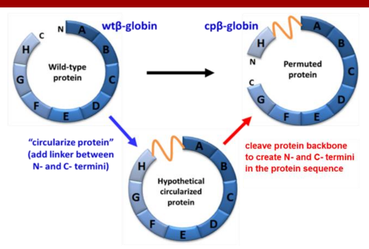


Marquardt, D.A., Doyle, M.P., Davidson, J.S., Epp, J.K., Aitken, J.F., Lemon, D.D. and Anthony-Cahill, S.J. Monodisperse 130 kDa and 260 kDa recombinant human hemoglobin polymers as scaffolds for protein engineering of hemoglobin-based oxygen carriers. *J. Funct. Biomater.* **2011**, *2*, 1-24.

Hemoglobin States

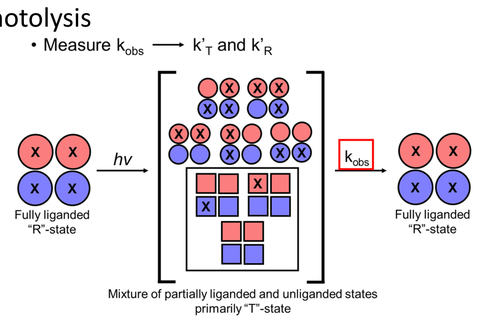


Circular Permutation

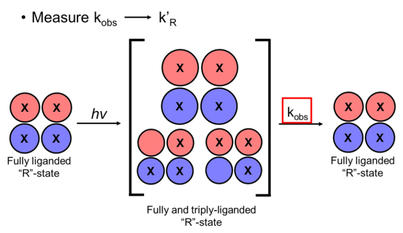


Flash Photolysis

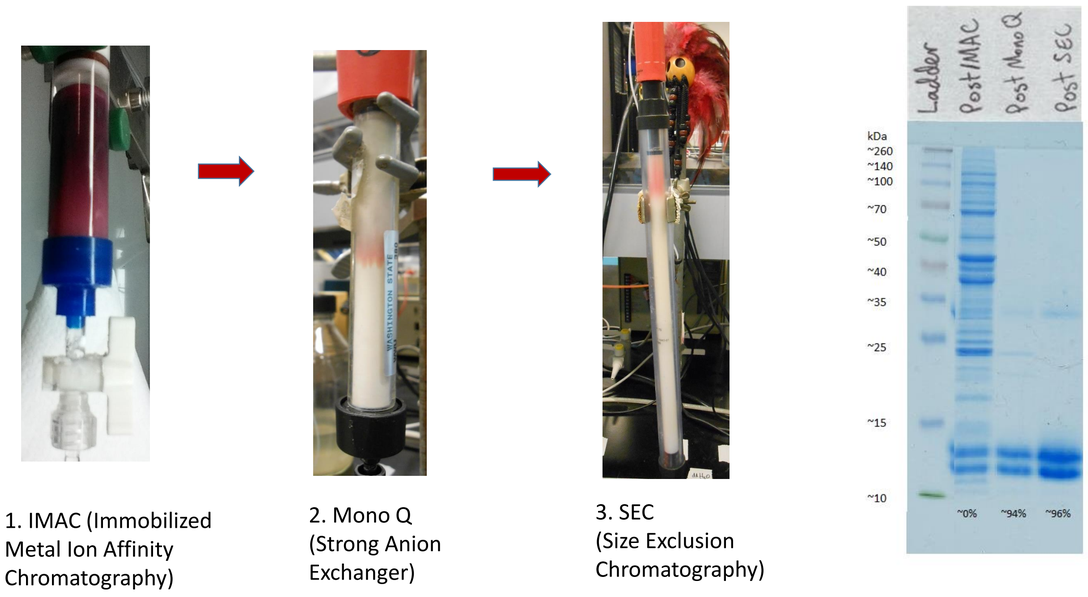
Full Photolysis



Partial Photolysis

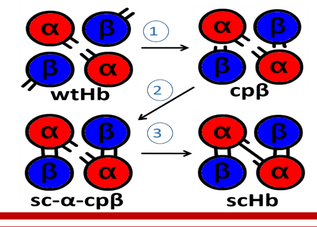


Protein Purification



1. IMAC (Immobilized Metal Ion Affinity Chromatography)
2. Mono Q (Strong Anion Exchanger)
3. SEC (Size Exclusion Chromatography)

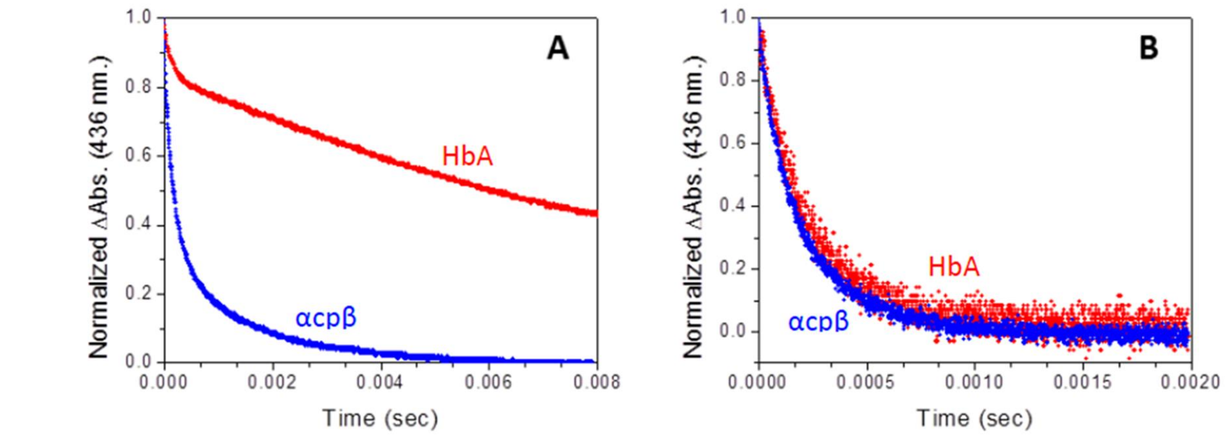
The Approach

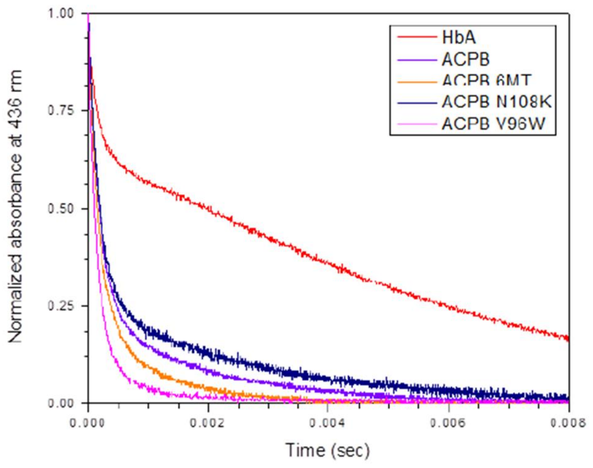


Protein Yields

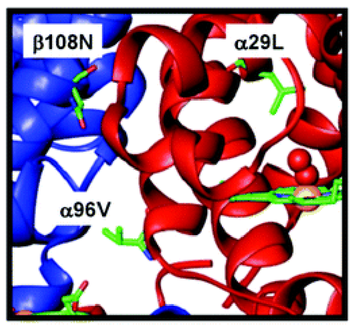
	4 sm (mg/L)	HUG (mg/L)
αcpβ+6M	10.7	-
αcpβ + N108K	21.9	-
scαcpβ	2.7	2.5
scHb	.583	.883
scHb+ N108K	.133	.120

Ligand Binding Studies





Ligand binding data shows distinct stabilization of the the R-state. Several point mutations have been made to enhance T-state stability.
Ligand binding data shows that the βN108K mutation improved T state stability however the αV96W mutation further destabilized the T state.

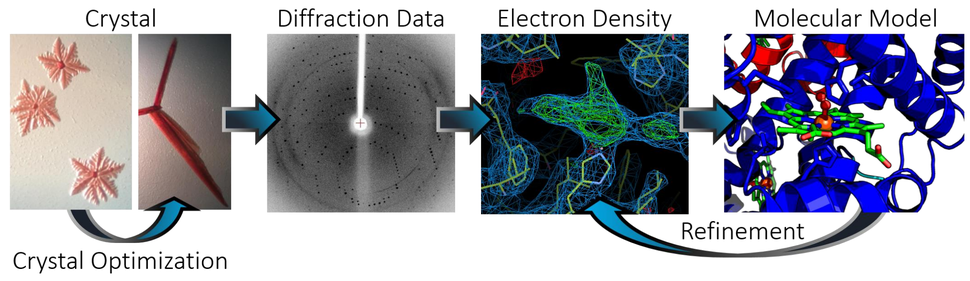


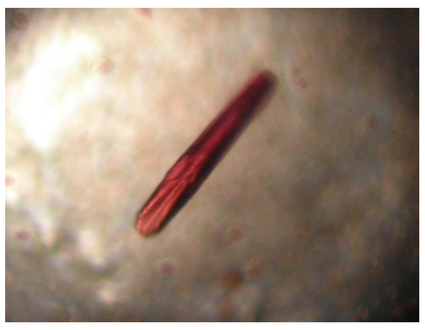
Maillett, David H.; Simplaceanu, Virgil; Shen, Tong-Jian; Ho, Nancy T.; Olson, John S.; Ho, Chien. Interfacial and Distal-Heme Pocket Mutations Exhibit Additive Effects on the Structure and Function of Hemoglobin. *Biochemistry*,**2008,** *47(40),* 10551- 10563

Structural Determination of Permuted Hbs

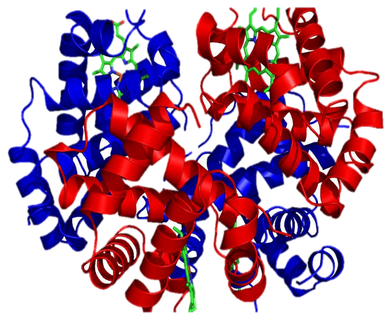
X-ray Crystallography

Functional characterization of the permutiens show a significant reduction in protein stability. Structural models are essential to guide protein engineering efforts aimed at increasing stability and optimizing function. X-ray crystallography was employed to obtain atomic resolution electron density maps of α cpβ and scα-cpβ which were then used to refine models of the proteins and reveal structural changes due to the modifications.



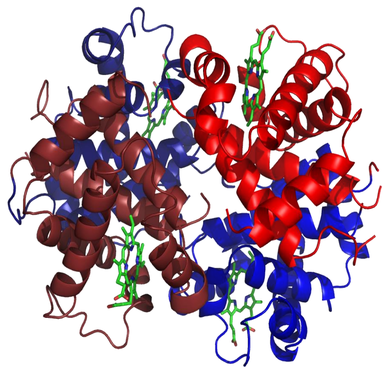


α-cpβ



Crystal structure by Michael Murphy and Clint Spiegel

sc-α-cpβ



Crystal structure by Johann Sigurjonsson and Clint Spiegel

Future Work/ Research Goals

- Obtain crystal structures of
  - αcpβ + V96W + N108K
  - αcpβ + V96W
  - αcpβ + N108K
- Gain understanding of anomalous effects of V96W on T state structure
- Establish reliable bioreactor fermentation protocol to increase yields

Acknowledgments

- Erin Macri (WWU SciTech)
- Harlan Knapp (GE Healthsciences)
- Clint Spiegel
- John Olson Lab (Rice University
- FHCRC
- Past and present lab members

Funding: NIH Grant 2R15HL081068

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74 183.85 W Tungsten | 99 (252) Es Einsteinium | 52 127.60 Te Tellurium | 86 (222) Rn Radon

6 12.011 C Carbon | 2 4.003 He Helium | 74 183.85 W Tungsten | 53 126.9 I Iodine | 16 32.07 S Sulfur | 112 (270) Tr Treatium* | 39 88.91 Y Yttrium