**Structural Determinants for the Activation of Soluble Guanylyl Cyclase**

Kenneth Childers University of Maryland, Baltimore County kchilde1@umbc.edu
Sam Giannakoulias
Elsa Garcin

Soluble guanylyl cyclase (GC-1) plays a vital role in vascular health by cyclizing GTP into cGMP, a potent vasodilator and inhibitor of platelet aggregation and adhesion. Nitric oxide (NO) binds to a heme cofactor at the GC-1 N-terminus and stimulates activity 100-200 fold. Chronic oxidative stress, a common feature in cardiovascular diseases, reduces NO bioavailability and oxidizes GC-1 heme, thus reducing cGMP levels and leading to systemic vasoconstriction. How the NO binding event is transduced to the C-terminal catalytic domain and increases cGMP turnover remains unknown, making drug design challenging. We hypothesized that NO stimulation relies on **L**ong-range **I**nteraction **N**etwor**Ks** (abbreviated LINKs) that allow for communication between the heme pocket and GTP-binding site. Identification of amino acids that make up LINKs will help us understand the mechanism of NO stimulation and pave the way for the discovery of novel small molecules that enhance GC-1 activity.

We have designed, optimized, and validated a novel luciferase reporter assay to identify activating GC-1 mutations. This assay uses a cGMP-dependent promoter upstream of a luciferase gene and a separate plasmid encoding GC-1. Expressed GC-1 converts intracellular GTP to cGMP, thus inducing luciferase expression. Luciferase activity is measured in cell lysates and used to infer GC-1 activity. *E. coli* cells lack endogenous cGMP, making them an ideal host for luciferase expression.

We have measured significantly higher luciferase activities in cells overexpressing full-length wild-type GC-1 over uninduced cells at varying time points. In addition, the only two known activating GC-1 mutants - αCys595Ser/βGC-1 and αGC-1/βMet537Asn – show significantly higher luciferase activities compared to wild-type and elevated extracellular cGMP levels, confirming the validity of the assay. We hypothesized that these residues participate in a network of hydrogen bonds at the inter-subunit interface of the catalytic domain. To verify our hypothesis, we attempted to solve the structure of the mutant catalytic domains ($\alpha\beta GC^{cat}$) via x-ray crystallography. However, crystallization trials have yielded inactive $\beta GC^{cat}$ homodimers thus far.

Using the luciferase reporter assay, we are currently testing novel mutations in full-length GC-1 and a truncated construct containing only the catalytic domains, to identify amino acids involved in LINKs.