# Characterization and Use of Genetically Encoded Fluorescent Heme Sensors to Interrogate Heme Trafficking, Dynamics, and Signaling

David A. Hanna, Liu Liu, Hyojung Kim, Matt Torres, Iqbal Hamza, Angela Fleischhacker, Stephen Ragsdale, Amit Reddi

Email: dhanna7@gatech.edu

*School of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, GA 30332, USA*

Heme is an essential yet cytotoxic iron containing metallonutrient. Well recognized for its role as a protein prosthetic group, more recent genetic and biochemical evidence indicate heme can act as a dynamic signaling molecule. Due to the cytoxicity associated with free or misregulated heme, the bioavailable heme pool utilized for signaling is tightly regulated and buffered to low levels, making heme acquisition and heme dependent signaling reliant on the ability to safely mobilize heme. However, the factors involved in mobilizing heme have remained poorly understood. Recently, utilizing our novel heme sensor technology, we revealed that heme is a highly dynamic molecule that is regulated by cell cycle and that nitric oxide (NO), a well-established and ubiquitous signaling molecule, mobilizes cytosolic and nuclear heme pools. Additionally, we discovered that under Pb stress the regulatory heme pool increases while total heme is diminished. Having identified several physiological and pathophysiological conditions that mobilize labile heme, in collaboration with Prof. Matt Torres, we are now developing mass spectrometry-based techniques to identify proteins that bind and release heme in these contexts to define new heme signaling networks. Further, using our heme sensors in HEK293 cells we reveal a heme sequestering role for HO2 that is independent of its catalytic function.

**References:**

[1] Hanna DA, Harvey RM, Martinez-Guzman O, Yuan X, Chandrasekharan B, Raju G, Outten FW, Hamza I, and Reddi AR. Heme Dynamics and Speciation Revealed by Genetically Encoded Fluorescent Heme Sensors. *Proc Natl Acad Sci,* **2016**;113:7539-7544.

[2] Hanna DA, Martinez-Guzman O, and Reddi AR. Heme Gazing: Illuminating Eukaryotic Heme Trafficking, Dynamics, and Signaling with Fluorescent Heme Sensors. *Biochemistry Article ASAP,* **2017**; DOI: 10.1021/acs.biochem.7b00007

[3] Yuan X, Rietzschel N, Kwon H, Nuno Da Silva ABW, Hanna DA, Phillips J, Raven E, Reddi AR and Hamza I. Regulation of intracellular heme trafficking revealed by subcellular reporters. Proc Natl Acad Sci, 2016; 113(35): E5144-E5152.

[4] Mense, S. M. and L. Zhang. Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. Cell Res, **2006**; 16(8): 681-692.