

Genetic circuits for monitoring dynamic changes in post-translational modifications.

Kevin G. Hoff^{1*}, Jonathan J. Silberg², and Christina D. Smolke¹

1. Department of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, 2. Department of Biochemistry and Cell Biology, Rice University, Houston, TX, 77251

[*Kevin_g_hoff@yahoo.com](mailto:Kevin_g_hoff@yahoo.com)

Diverse cellular networks such as energy metabolism, transcriptional and translation regulation, and redox sensing utilize proteins that contain iron-sulfur (Fe/S) metalloclusters. However, both free iron and sulfur are toxic and defects in Fe/S-cluster biogenesis leads to several pathologies including aging, cardiac disease, neurodegeneration, and increased formation of reactive oxygen species (ROS) known to cause cancer. Despite their importance and a plethora of biochemical information regarding individual Fe-S proteins, the general mechanisms by which metalloclusters are synthesized and relayed to most apo-acceptor proteins are not well understood. To this end, we have begun to develop tools for elucidating the network of assembly and regulatory proteins responsible for Fe/S-cluster biogenesis and monitoring defects in the function of these proteins. Bacterial Fe/S-cluster dependent transcriptional repressors have been used to construct eukaryotic genetic circuits that respond to metallocluster homeostasis. Initial studies examining the transcriptional repression activity of *Escherichia coli* IscR in *Saccharomyces cerevisiae* provided evidence that eukaryotes assemble metalloclusters on bacterial transcriptional repressors. A circuit built using native IscR fused to the VP-16 transcriptional activator exhibited 80-fold activation of reporter constructs and a 6-fold greater activation than a circuit that used a Cys-less mutant of IscR that cannot coordinate a 2Fe-2S cluster. Directed evolution is currently being used to extend the sensitivity and the dynamics of this circuit. These circuits provide a new tool for studying how toxic elements (iron and sulfur) are relayed in higher eukaryotes and should be sensitive to the ROSs that underlie many basic cellular functions (apoptosis, signaling, and the immune response) and disease states.