Structural basis for regulation of Ca^{2+} ions in cardiomyocytes by HAX-1

Erik Larsen[1], Cristina Olivieri[2], Jonggul Kim[3], Gianluigi Veglia[1,2]

[1]Department of Chemistry and [2]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455

[3]Department of Biophysics, University of Texas Southwestern, Dallas, TX 75390

Calcium cycling in cardiomyocytes is the critical signaling component that governs heart contraction and relaxation. Relaxation is initiated by the transport of Ca^{2+} from the cytosol into the sarco/endoplasmic reticulum (SR) by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). The activity of SERCA is regulated by multiple peptides. Phospholamban (PLN) is the inhibitory peptide most common in cardiomyocytes. Normally, PLN inhibits SERCA by reducing its apparent affinity for Ca^{2+}. Extensive research has shown that disruption of this fine-tuned regulation leads to depressed calcium cycling and, ultimately, heart failure. As a result, understanding the regulation of PLN and SERCA has been of clinical importance for understanding the molecular drivers for heart failure. Recently, Vafiadaki et al. identified hematopoietic – substrate – 1 associated protein X-1 (HAX-1) as a binding partner to PLN. They hypothesized that HAX-1 could play an integral role in the signaling cascade which ultimately controls the Ca^{2+} cycling within cardiomyocytes. Originally thought to have a role strictly in apoptosis, HAX-1, a 35 kDa, ubiquitously expressed mitochondrial protein, is now known to also partake in the cascade of signals regulating Ca^{2+} circulation in cardiomyocytes, specifically with SERCA within the sarcoplasmic reticulum (SR).

The aim of this project is to understand the structural basis for the regulation of SERCA through the PLN/HAX-1 complex. To do so, NMR spectroscopy will be coupled with biochemical experimental methods. Preliminary NMR experiments carried out have demonstrated that HAX-1 does not have a well-defined secondary structure in solution. In addition, NMR titration experiments have shown that HAX-1 interacts with PLN, Ca^{2+}, and unfolds in the presence of urea. With these results, we propose that HAX-1 is intrinsically disordered in solution which contributes to its role in regulation of the Ca^{2+} homeostasis in the sarcoplasmic reticulum.