

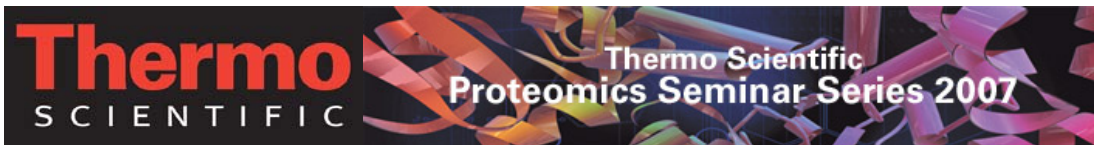
Altered Proteome Biology of Cardiac Mitochondria: Insights of Biomarkers For Cardiac Ischemic Injury

Jun Zhang¹, David Liem¹, Michael Mueller², Xiaohai Li¹, Yueju Wang¹, Ning Deng¹, Thomas M. Vondriska¹, Paavo Korge¹, W. Robb MacLellan¹, James N. Weiss¹, Rolf Apweiler², Peipei Ping¹

¹*UCLA School of Medicine, Los Angeles, CA 90095*

²*EMBL/EBI, Hinxton, UK*

Myocardial ischemia-reperfusion (I/R) induces mitochondrial dysfunction and, depending upon the degree of mitochondrial injury, may lead to cardiac cell death. However, our ability to understand mitochondrial dysfunction has been hindered by an absence of molecular markers defining the various degrees of injury. To address this paucity of knowledge, we sought to characterize the impact of I/R damage on mitochondrial proteome biology. We hypothesized that I/R injury induces differential alterations in various mitochondrial sub-compartments, that these proteomic changes are specific to the severity of injury, and that they are important to subsequent cellular adaptations to myocardial ischemic injury. Accordingly, an *in situ* model of cardiac mitochondria injury was established to examine two stress conditions: *reversible injury* (induced by mild calcium overload, 100 μ M, 5min at room temperature) and *irreversible injury* (induced by hypotonic stimuli, 5mM Tris-HCl, 5min at room temperature). Both forms of injury had a drastic impact on the proteome biology of cardiac mitochondria. Altered mitochondria function was concomitant with significant protein loss/shedding from the injured organelles. LC/MS/MS analysis identified 245 proteins from the mitochondrial milieu in the reversible injury group and 402 proteins from the irreversible injury group, among which 208 proteins were common to the released pool from both conditions. In the setting of calcium overload, mitochondria retained functionality despite the release of numerous proteins, and the majority of mitochondria remained intact. In contrast, hypotonic stimuli caused severe damage to mitochondrial structure and function, induced increased oxidative modification of mitochondrial proteins, and brought about detrimental changes to



the sub proteomes of the inner mitochondrial membrane and matrix. Using an established *in vivo* model of regional myocardial I/R injury in mice, we validated key observations made by the *in situ* model. This pre-clinical investigation provides function and sub-organelle compartment information on a repertoire of cardiac mitochondrial proteins sensitive to ischemia reperfusion stress and highlights protein clusters potentially involved in mitochondrial dysfunction in the setting of myocardial ischemic injury.