1529-Pos  Board B421  
Screening for SERCA Activators using a High-throughput Time-Resolved FRET Assay

Ji Li1, Holly Langer1, Kurt C. Peterson2, Joseph M. Muretta3, Gregory D. Gillipis1, Razvan L. Corna1, David D. Thomas1

1University of Minnesota, Minneapolis, MN, USA, 2Fluorescence Innovations, Inc., Minneapolis, MN, USA, 3Loyola University, Chicago, IL, USA.

We used a prototype time-resolved fluorescence lifetime microplate reader to carry out a high-throughput screen designed to identify compounds that interact with the sarcoplasmic reticulum calcium ATPase (SERCA). SERCA is essential for the Ca homeostasis in many cell types. Insufficient SERCA activity leads to cardiovascular disease, muscular dystrophy, skin disease, and diabetes. Our goal is to discover activators of SERCA that can be developed into drugs to treat diseases in which Ca transport is deficient. The fluorescence lifetime plate reader was made possible by our recent development of fast time-resolved fluorescence by direct waveform recording, which achieves 105 higher throughput than the conventional single-photon counting technology. By using this plate reader, we detected fluorescence resonance energy transfer (FRET) between IAEDANS-labeled SERCA and nucleotide analog TNPADP in native sarcoplasmic reticulum membranes. Initial hit compounds were further analyzed in functional assays. Upon screening a small (1300 compound) library, we determined that the time-resolved microplate reader has at least 10x higher precision than a conventional intensity-based microplate reader, raising the quality index (z’) of our assay from marginal, to the intensity reader, to excellent. A 384-well plate is read with high precision in 2 min, which allows screening of thousands of compounds/day. An important advantage of the time-resolved fluorescence measurement is that it provides detailed structural information, thus enabling discovery of multiple classes of compounds during the primary screen.

1530-Pos  Board B422  
In-Cell FRET as a Tool to Develop SERCA Activators for Drug or Gene Therapy

Simon J. Gruber1, Kurt C. Peterson1, Bengt Svensson1, Seth L. Robia2,3, David D. Thomas3

1University of Minnesota, Minneapolis, MN, USA, 2University of Minnesota: Twin Cities, Minneapolis, MN, USA.

We are using in-cell FRET methods (Photobleaching, FLIM, time-resolved FRET in a plate reader) to study the relationships among structure, dynamics, and function of the sarcoplasmic reticulum Ca-ATPase (SERCA) and its cardiac regulator phospholamban (PLB), with the goal of designing activators of SERCA for treatment of heart failure (HF) and muscular dystrophy (MD). Ca2+ drives muscle contraction, and relaxation is accomplished by the sequestration of Ca2+ by the sarcoplasmic reticulum Ca-ATPase (SERCA), which is inhibited by phospholamban at submicromolar [Ca2+] in cardiac muscle. SERCA activity is frequently reduced in HF, and many current therapeutic strategies aim to increase cardiac Ca2+ cycling activity. We are designing LOF-PLB mutants (PLBM) that can compete with WT-PLB (PLBW) and thus relieve SERCA inhibition. Our ideal mutant is partial loss-of-function that interact with SERCA and modify either the enzyme’s structure or the binding affinity of TNP-ADP. Initial hit compounds were further analyzed in functional assays. Upon screening a small (1300 compound) library, we determined that the time-resolved microplate reader has at least 10x higher precision than a conventional intensity-based microplate reader, raising the quality index (z’) of our assay from marginal, to the intensity reader, to excellent. A 384-well plate is read with high precision in 2 min, which allows screening of thousands of compounds/day. An important advantage of the time-resolved fluorescence measurement is that it provides detailed structural information, thus enabling discovery of multiple classes of compounds during the primary screen.

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EPR Detects Changes in the Transmembrane Region of the SERCA-Phospholamban Complex upon Ser16 Phosphorylation

Zachary M. James, Jesse E. McCaffrey, Christine B. Karim, David D. Thomas.

University of Minnesota, Minneapolis, MN, USA.  
We have used site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to investigate the effects of Ser16 phosphorylation on phospholamban’s interaction with the sarcoplasmic reticulum Ca-ATPase (SERCA). Muscle contraction is signaled by the release of Ca from the sarcoplasmic reticulum (SR), while SERCA mediates muscle relaxation by actively pumping Ca back into the SR lumen. Cardiac SERCA is regulated by phospholamban (PLB), a single-pass transmembrane protein that inhibits the Ca pump unless phosphorylated at Ser16.

We have shown that Ser16 phosphorylation does not dissociate PLB from SERCA to relieve inhibition, but instead induces a structural change in PLB’s transmembrane (TM) helix that restores SERCA Ca sensitivity. To characterize this change, we have attached spin labels along the TM helix and performed EPR accessibility measurements to determine the effects of Ser16 phosphorylation on TM helix topology within the regulatory complex. Our results show that upon phosphorylation, the TM helix undergoes a vertical shift that could break inhibitory interactions between SERCA and PLB. Complementarity studies employ these spin-labeled PLB constructs to determine changes in (a) the orientation of PLB and (b) distances to probes on SERCA, with the goal of obtaining a detailed structural model for the regulatory transitions within the SERCA-PLB complex.