

High-throughput FRET-based screening to identify small-molecule effectors of SERCA

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ABSTRACT

A robust high-throughput screening (HTS) strategy has been developed to discover small-molecule effectors targeting the sarco/endoplasmic reticulum calcium ATPase (SERCA), based on a fluorescence microplate reader that records both the nanosecond decay waveform (lifetime mode) and the complete emission spectrum (spectral mode), with high precision and speed. This spectral unmixing plate reader (SUPR) was used to screen libraries of small molecules with a fluorescence resonance energy transfer (FRET) biosensor expressed in living cells. Ligand binding was detected by FRET associated with structural rearrangements of green (GFP, donor) and red (RFP, acceptor) fluorescent proteins fused to the cardiac-specific SERCA2a isoform. The results demonstrate accurate quantitation of FRET along with high precision of hit identification. Fluorescence lifetime analysis resolved SERCA's distinct structural states, providing a method to classify small-molecule chemotypes on the basis of their structural effect on the target. The spectral analysis was also applied to flag interference by fluorescent compounds. FRET hits were further evaluated for functional effects on SERCA's ATPase activity via both a coupled-enzyme assay and a FRET-based calcium sensor. Concentration-response curves indicated excellent correlation between FRET and function. These complementary spectral and lifetime FRET detection methods offer an attractive combination of precision, speed, and resolution for HTS.

