HOT-ROXS: An integrated platform for identifying activators of non-druggable targets using biophysical screening, x-ray solution scattering and high-throughput co-crystallization

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HOT-ROXS: An integrated platform for identifying activators of non-druggable targets using biophysical screening, x-ray solution scattering and high-throughput co-crystallization

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Research Abstract
? DESCRIPTION (provided by applicant): Enzyme activators are significantly underrepresented as therapeutic agents versus enzyme inhibitors. There are only about a dozen examples of activator discovery in the scientific literature. The lack of activator therapeutics is not from a ack of targets for diverse unmet medical needs. Diseases such as neurodegeneration, cancer and type 2 diabetes could all benefit from an activator therapeutic. This application introduces a new platform, HOT-ROXS, for discovery of therapeutics for this class of “”non-druggable”” targets. HOT-ROXS addresses three of the common issues in activator discovery: a library rich in activators, a generally applicable assay directed at activator identification and structural characterization of the activators to drive medicinal chemistry optimization of the hits. To date, most activator discovery has been through high-throughput screening (HTS). HTS libraries are typically composed of large complex molecules. Probability calculations indicate that complex molecules are much less likely to bind to a target than a smaller simpler compound (fragments of drugs). Furthermore, the ligand binding efficiency (binding energy per atom) is typically much lower for HTS hits versus fragment hits. This confounds medicinal chemistry optimization and can lead to flat SAR. Here, activators are defined as compounds that bind directly to the target of interest and stabilize it in the active conformation. In HOT-ROXS, potential fragment activators are ideally identified as compounds that stabilize the active conformation of the protein by a positive shift in protein melting temperature. In cases where the active conformation cannot be screened, the inactive conformation is screened and the effect of activators on the melting temperature characterized early in the program by parallel activity screens. Protein structure for the activators is initially measured in solution using Wide Angle X-ray Scattering (WAXS). WAXS provides the molecular envelope for the protein-ligand complex and is very sensitive to conformational shifts. Changes as small as loop shifts can be detected by this method. WAXS is used as part of an iterative process with single crystal x-ray diffraction. The initial x-ray structure (maybe apo or a ligand complex) is fit to the WAXS pattern and changes upon activator binding identified. The x-ray structure may be remodeled to fit the new WAXS pattern. WAXS is also very sensitive to conformational uniformity which is also a key characteristic for protein crystallization. Activators are thought to increase the flexibility of proteins which would make crystallization of the complex more challenging. So, the WAXS pattern also identifies and prioritizes complexes for high-throughput co-crystallization studies. WAXS may also identify different protein conformation classes which may streamline the co-crystallization process or potentially provide for soaking of activators into pre-formed crystals. HOT-ROXS has been used to identify activators for a high priority Parkinson’s disease target and the method will be further developed and refined using this model system.

Further information available at:

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