Cellular thermal shift assay: Case studies of membrane proteins

Aarti Kawatkar, Juhi Sardana, Thomas Lundbäck, Andrew X. Zhang, M.Paola Castaldi
Mechanistic Biology & Profiling, R&D, AstraZeneca, Gothenburg, Sweden

Target engagement is an important parameter for successful drug discovery, hence it is applied as an integral component of most screening cascades. The cellular thermal shift assay (CETSA) is a powerful new method for assessing target engagement in the physiological environment of living cells. A unique advantage is the possibility to run this assay without labels on either compound or proteins, allowing studies also in patient-derived cells. Originally, CETSA was based on extraction of soluble target proteins in a detergent free setting, thus it was not applicable to membrane-bound targets. This isn't sufficient as a significant portion of drug targets are integrated with cellular membranes. Later protocols included detergents to allow analysis also of membrane-bound proteins. Here we further extend these results by showcasing CETSA data for multipass membrane spanning proteins, with particular focus on the unique challenges associated with the establishment of thermal aggregation curves and interpretation of observed shifts thereof.

Our case studies for various transmembrane proteins showcases unique challenges in establishing protocols for successful melt curves. Different membrane proteins have different CETSA behaviors and some are not amenable to the approach despite optimized conditions. Sample preparation strongly influences the melting behavior. Our Work provides the drug discovery community with a broad picture of what to expect when establishing target engagement using CETSA for complex membrane-bound proteins.