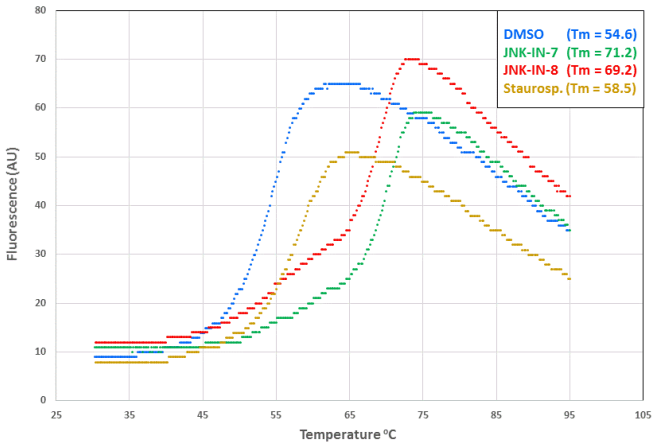
The use of biophysical methods/assays in drug discovery has increased dramatically in the last 10 years ranging from use in Hit characterization to Hit identification by high concentration fragment screening. Although many biophysical approaches are currently available including Surface Plasmon Resonance, NMR spectroscopy and Isothermal Calorimetry, Protein Thermal Shift (PTS) assays using differential scanning fluorimetry (DSF) to measure the melting temperature of proteins in solution affords a highly robust, cost efficient technique that uses negligible amounts of protein in contrast to the methods previously mentioned. **Revolution Biosciences** has developed 384-well DSF assays for assessing ligand/protein interactions by detecting shifts of the protein melting temperature (T_m) as measured by changes in fluorescence of the indicator dye SYPRO Orange on an ABI 7900HT real-time PCR instrument.



MAPK10 melt curves after treatment with JNK-IN-7, JNK-IN-8, Staurosporine & DMSO.

MAPK10 Differential Scanning Fluorimetry Assay

As a validation of our Protein Thermal Shift assay approach we developed a MAPK10 (JNK3) DSF assay based on the discovery of the covalent inhibitors JNK-IN-7 and JNK-IN-8; Zhang, T. et. al. *Chem. Biol.* 2012, 19(1), 140-154. The figure above demonstrates that both covalent MAPK10 inhibitors dramatically stabilize the protein resulting in >14°C increase in MAPK10's T_m versus only a ~4°C increase for the reversible inhibitor Staurosporine. The DSF assay's 384-well format allows testing from hundreds of compounds in dose response to thousands in primary screening per run.

About Us

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Revolution Biosciences' collaborative R&D discovery model leverages the power of chemical biology to discover and develop transformative medicines in partnership with the life science community by enabling access to cutting edge technologies (MALDI-MS, High Content Image Screening & Differential Scanning Fluorimetry) through consulting agreements, collaborative partnerships and contract research.

We develop new bioanalytical methods to directly identify proteins, peptides and small molecules and their molecular interactions in all types of biological samples. Our small molecule drug discovery expertise and application of advanced proteomics technologies accelerates drug discovery research and our state of the art bioanalytical laboratory on the UMass Boston campus is equipped to handle bioanalytical studies from assay development through high throughput screening.