Architecture of Autoinhibited and Active BRAF-MEK1-14-3-3 Complexes

Repligen Corporation now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Researchers at the Dana-Farber Cancer Institute and Harvard Medical School leveraged the PATsmart™ ZipChip® in a combined structural biology-phosphoproteomic study to decipher key topological features and post-translational modifications on the kinase BRAF. Like most cellular proteins, BRAF does not work alone, but rather assembles with other proteins into "molecular machines" which work in concert to orchestrate the cellular "circuitry" required for normal physiology. However, genetic mutation can commandeer and re-direct the activity of these protein machines leading to human tumors. In fact, BRAF mutations contribute to a large number of different human cancers.

Unfortunately, the myriad of structural features, interaction surfaces, and post-translational modifications on BRAF and its associated proteins make it challenging to understand how the kinase functions in normal physiology and how genetic mutations hijack the kinase and drive human cancer.

Contact

Repligen Corporation
685 Route 202/206
Bridgewater, NJ, USA 08807

analytics-support@repligen.com

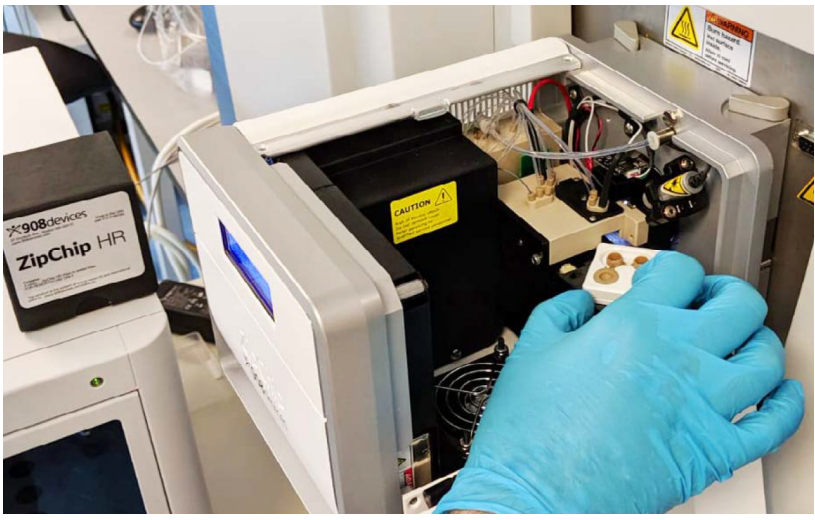
(908) 707-1009

Technical Note

The Dana-Farber team generated purified forms of normal and mutant BRAF in addition to its key protein partners typically found in cells, and then assembled these to produce detailed molecular structures by cryo-electron microscopy. In addition, the researchers digested small aliquots of these complexes with a combination of proteolytic enzymes and then used the ZipChip on a Thermo Q-Exactive HF mass spectrometer to identify phosphorylation sites on BRAF and its co-regulatory protein partners. The rapid-fire, 10-minute injection-to-injection throughput provided by the ZipChip enabled the researchers to analyze hundreds of samples spanning different BRAF mutations, protein partners, and proteolytic enzymes. The multitude of "targeted" and "unbiased" CE-MS/MS data sets enabled the researchers to read out phosphorylation stoichiometry at key sites on BRAF which control its kinase activity and interaction with other co-regulatory proteins.

The Team combined their proteomic and structural data to better understand how BRAF mutations disrupt its normal cellular function. These results provide important insight to guide development of new, precision drugs which may improve clinical outcomes for patients with BRAF-driven cancers.

To view the full article published in Nature Magazine click here.



The rapid-fire, 10-minute injection-to-injection throughput provided by the ZipChip enabled the researchers to analyze hundreds of samples spanning different BRAF mutations, protein partners, and proteolytic enzymes.

REPLIGEN