# IPD Project Details

**Project ID:** IPD5120

**Project Title:** Characterization of the novel broad-spectrum kinase inhibitor CTx-0294885 as an affinity reagent for mass spectrometry-based kinome profiling

**Description:** Kinome profiling of human basal breast cancer cell line MDA-MB-231 using CTx-0294885 or mixture of 4 broad-spectrum kinase inhibitor (Purvalanol B, SU6668, VI16832 and CTx-0294885). Kinase enrichment utilizing broad-spectrum kinase inhibitors enables the identification of large proportions of the expressed kinome by mass spectrometry. However, the existing inhibitors are still inadequate in covering the entire kinome. Here, we identified a novel bis-anilino pyrimidine, CTx-0294885, exhibiting inhibitory activity against a broad range of kinases in vitro, and further developed it into a Sepharose supported kinase capture reagent. Use of a quantitative proteomics approach confirmed the selectivity of CTx-0294885-bound beads for kinase enrichment. Large-scale CTx-0294885-based affinity purification followed by LC-MS/MS led to the identification of 235 protein kinases from MDA-MB-231 cells, including all members of the AKT family that had not been previously detected by other broad spectrum kinase inhibitors. Addition of CTx-0294885 to a mixture of three kinase inhibitors commonly used for kinase-enrichment increased the number of kinase identifications to 261, representing the largest kinome coverage from a single cell line reported to date. Coupling phosphopeptide enrichment with affinity purification using the four inhibitors enabled the identification of 799 high confidence phosphosites on 183 kinases, approximately 10 % of which were localized to the activation loop, and included previously unreported phosphosites on BMP2K, MELK, HIPK2 and PRKDC. Therefore, CTx 0294885 represents a powerful new reagent for analysis of kinome signalling networks that may facilitate development of targeted therapeutic strategies. Data processing and bioinformatics: Raw files were processed with MaxQuant (version 1.1.1.25) for feature detection, protein identification and quantification, using the Andromeda search engine integrated into the MaxQuant environment for database searching. Extracted peak lists were searched against the UniProtKB/Swiss-Prot Homo sapiens database (Uniprot_human_2010_10) containing 35052 entries and a separate reverse decoy database for controlling the false discovery rate (FDR). The following search parameters were selected; fixed cysteine carbamidomethylation modification; variable methionine oxidation modification, variable protein N-acetylation, variable phosphorylation of serine, threonine and tyrosine; minimum peptide length of 6 amino acids and up to 2 missed cleavages were allowed. In addition, for SILAC experiments, the SILAC labels Arg10 and Lys8 were selected as modifications, and minimum peptide

count for protein quantification was set to 1. The initial first search mass tolerance was 20 ppm for precursor ions and 0.5 Da for fragment ions, with individualized peptide mass tolerances used for the subsequent searches. The ‘match between runs’ option in MaxQuant was used to transfer identifications between runs based on matching of precursors with high mass accuracy. The FDR was limited to 1 % for both protein and peptide identifications. Peptides with posterior error probability greater than 10 % were removed and protein identification required a minimum of 1 unique peptide. For phosphopeptides, those exhibiting a phosphosite localization probability (LP) > 0.75 were included in further analyses.

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**Sample Preparation:** MDA-MB-231 cells were cultured in RPMI1640 (Invitrogen) supplemented with insulin at 0.25 IU/mL and 10% fetal bovine serum (Invitrogen).See details in reference(s):

**Peptide Separation:** Tryptic digestion of the reduced and alkylated proteins was performed at 37 °C overnight using sequencing grade modified porcine trypsin (Promega) at a protein-to-enzyme ratio of 1:100.See details in reference(s):

**Protein Characterization:** Raw files were processed with MaxQuant (version 1.1.1.25) for feature detection, protein identification, and quantification using the Andromeda search engine integrated into the MaxQuant environment for database searching.See details in reference(s):

**Experiment Type:** Bottom-up

**Species:** Data in species_details  No Data

**Tissue:** Unknown  No Data

**Cell Type:** Unknown  No Data

**Disease:** Unknown  No Data

**Instrument Details:** Data in instrument_details  Data in instrument_details

**Protein Modifications:** monohydroxylated residue, phosphorylated residue, mono N-acetylated residue, iodoacetamide derivatized residue

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