Mapping Pathways and Ontologies for PamChip® data using Enrichr, a free online tool

Phosphosites on the PamChip (PTK and STK) can be phosphorylated by a number of kinases. The Upstream Kinase Tool is a functional scoring method that ranks the Top kinases differentially activated comparing 2 conditions. Pathway and Ontology analysis of phosphosite level data and/or kinase level data is useful for further biological interpretation

Phosphosite Level data:

Comparative analyses using T-tests or ANOVA-Dunnett tests, result in Tables with Log2Fold Change (LFC) and p-values for each Test Condition compared to Control (T vs C), per Phosphosite (UniPROT ID) for each of the Protein Tyrosine Kinase (PTK) and Ser/Thr Kinase (STK) assays. Data extracted from the Tables are used for Enrichr analysis.

- If applicable, combine the PTK and STK Tables
- Select a p-value threshold (recommended <0.05), to select the significant UP or DOWN phosphosites
- Order the Table by LFC, to separate the UP and DOWN regulated Phosphosite UniPROT IDs

Kinase Level data:

Kinase Score Tables are generated from PamChip Data (PamApp Tool, Upstream Kinase Analysis/UKA), for each comparison, for each assay. The prediction of Top kinases differentially activated between 2 conditions can provide biological insights, especially when analyzed in the context of ontologies and pathways. Data extracted from the Tables are used for Enrichr analysis.

- If applicable, combine the PTK and STK Tables
- Select a threshold cut-off (recommended Median Final Score >1.2)
- Order the Table by Median Kinase Statistic, to separate the UP and DOWN regulated Kinase UniPROT IDs

Combining data:

- If applicable, for each comparison, combine the pre-selected, significantly UP or DOWN regulated UniPROT IDs from the Phosphosite level and/or Kinase level data
- Make a list of either UP or DOWN UniPROT IDs per comparison.

Converting UniPROT to Gene names:

- UniPROT IDs need to be converted to Gene names before adding to Enrichr input list.
- For conversion: https://www.uniprot.org/uploadlists/
- Save the output Table of Gene names

Data analysis with Enrichr:

- Pathway or Gene Ontology analysis can be done using ENRICHR (https://maayanlab.cloud/Enrichr/)
- The UP or DOWN lists of the Gene names for each comparison, can be copy-pasted to Enrichr where indicated
- Pressing the Submit button will result in several Results TABs that you explore
- The Pathways and Ontology TABs can provide interesting data from different tools/ sources (Click on each source), that can be downloaded and saved, as Figures or Tables.