A BioID-derived proximity interactome for Protein Phosphatase PP2A

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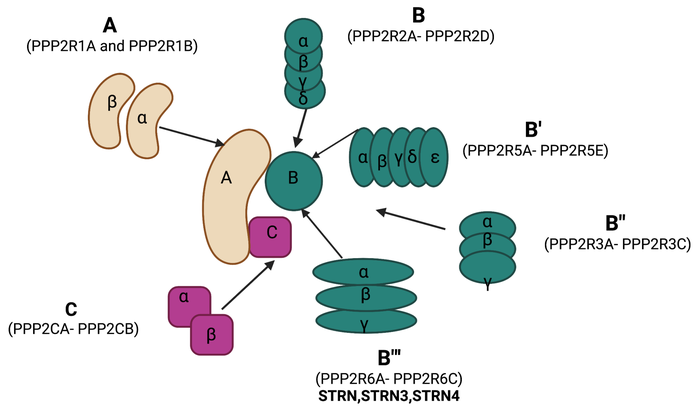
ABSTRACT

Protein Phosphatase 2A (PP2A) is an important and ubiquitously expressed serine threonine phosphatase in brain. To dissect underlying mechanisms of PP2A function, we employed proximity-dependent biotin-identification (Bio-ID) to identify the PP2A interactome. A streptavidin-based enrichment revealed 693 putative interacting proteins but only 4 distinct biotinylated sites were identified. In sharp contrast, we identified total 1438 proteins and 396 Biotin-K and 4 Biotin-N terminal sites identified from 260 biotinylated proteins using the antibody-based-enrichment workflow. Further, pathway analysis showed involvement of PP2A interactors in regulation of phosphatase regulator activity, protein phosphatase 2A binding, microtubule depolymerization and protein folding chaperones.

BACKGROUND

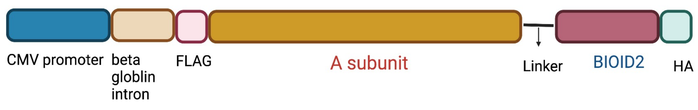
- Protein Phosphatase 2A (PP2A) is an important and ubiquitously expressed serine threonine phosphatase that regulates cell function by dephosphorylating many critical targets such as Akt, p53, c-Myc and β-catenin.
- It plays a critical role in cellular processes, such as cell proliferation, signal transduction and apoptosis.
- Structurally, it is composed of catalytic, scaffold and regulatory subunits as shown below:



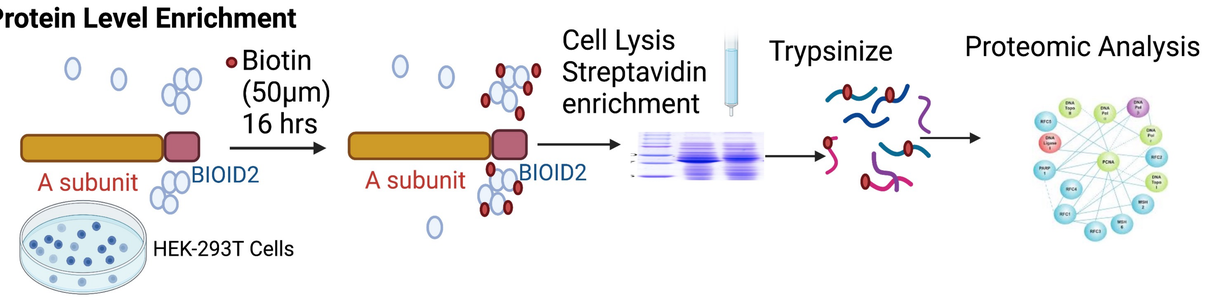
- PP2A imbalance has been reported in neurodegenerative diseases. Summary data for GWAS identifies the first ranked pathway in major depressive disorder (MDD) was “protein phosphatase PP2A regulator activity”.
- The PP2A interactome will identify new molecules and signaling networks involved in MDD and to help identify new therapeutic targets.

METHODOLOGY

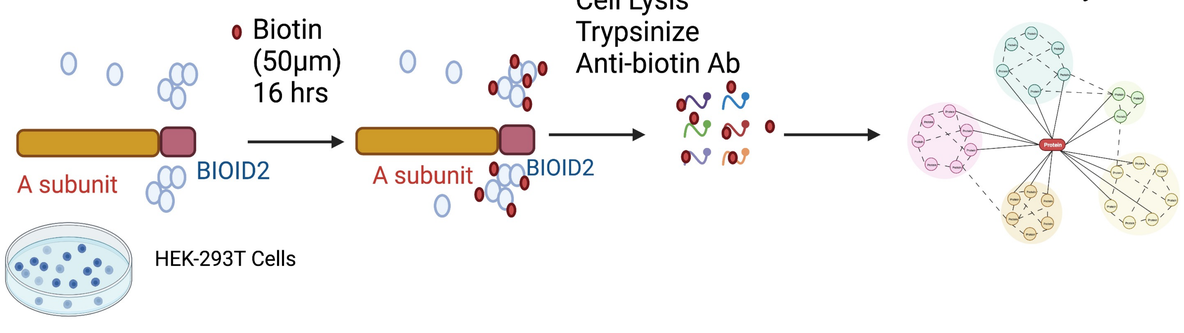
BIOID2-PP2A, A subunit construct



Protein Level Enrichment

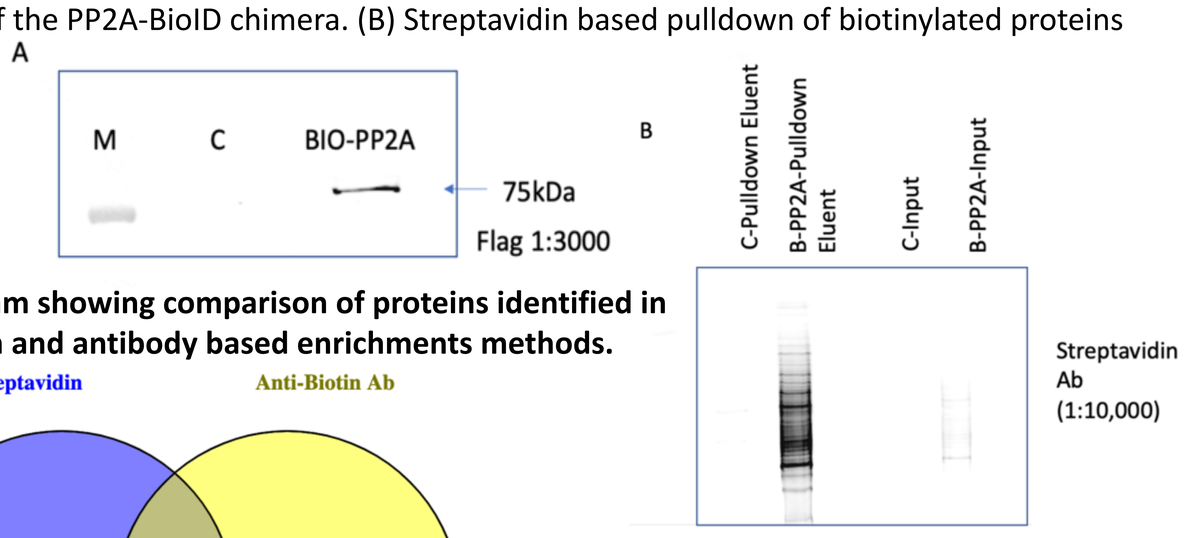


Peptide Level Enrichment

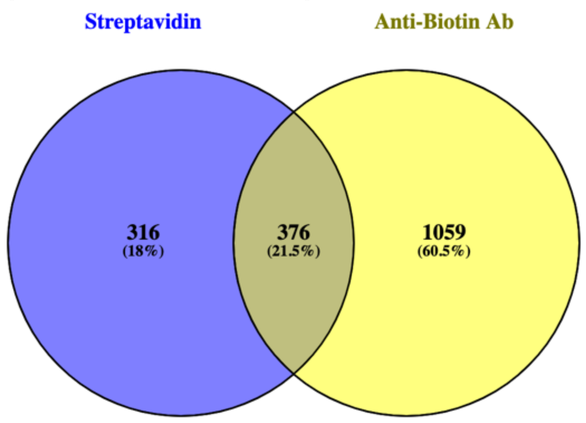


RESULTS

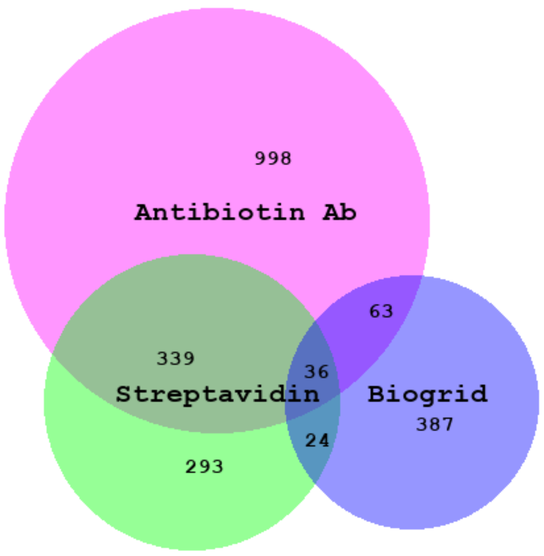
Identification of PP2A expression and biotinylated proteins using western blotting. (A) Immunoblotting with anti-Flag revealed the expression of PP2A. M-Marker showing correct size of the PP2A-BioID chimera. (B) Streptavidin based pulldown of biotinylated proteins



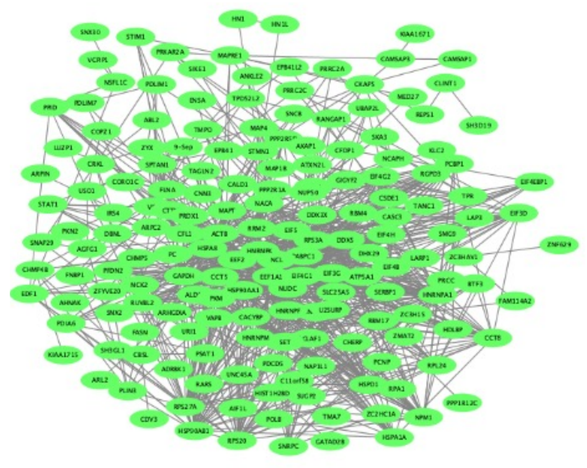
Venn diagram showing comparison of proteins identified in streptavidin and antibody based enrichments methods.



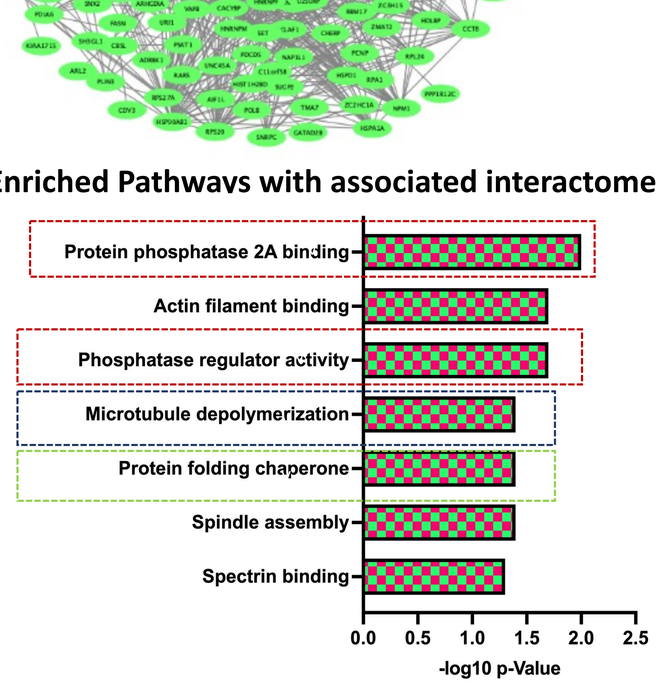
Venn diagram showing comparison of proteins identified in streptavidin, antibody based enrichments methods and PP2A interactors present in the Biogrid database.

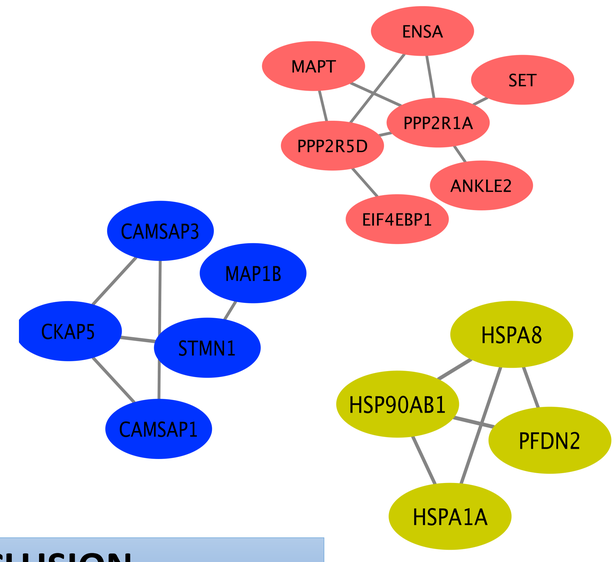


Biotinylated proteins (200) network identified using Anti-Biotin Ab.



Enriched Pathways with associated interactome





CONCLUSION

- ❖ Systematic analysis of the PP2A interactome revealed high density interactions which allows more precise understanding of the phosphatase function.
- ❖ Validation of a few potential interactors is required to better understand the biological significance of the PP2A interactome at a cellular and molecular level.
- ❖ In the future, examination of the PP2A interactome(s) associated with MDD related mutations in cell and animal models may shed light on the pathophysiology of MDD and potentially help to identify new targets for treatment of this highly disabling disorder.

ACKNOWLEGEMENTS

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