# Heterologous expression and purification of SH2 domain containing protein 3C, protein with a predicted high content of intrinsically disordered regions

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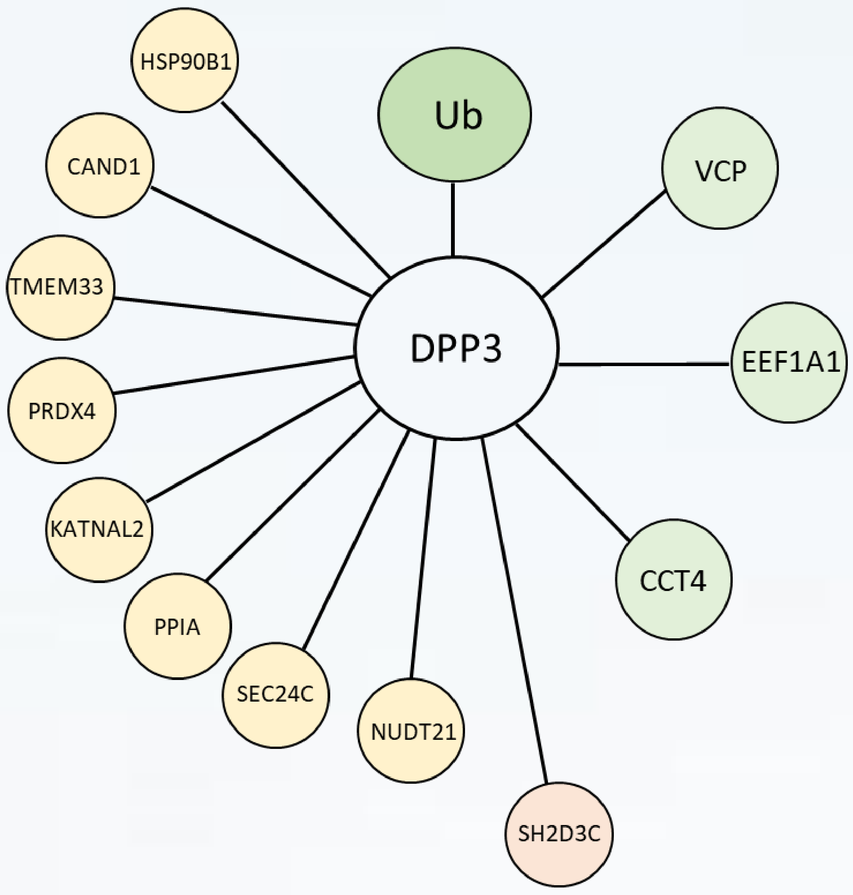
HrZZ Croatian Science Foundation

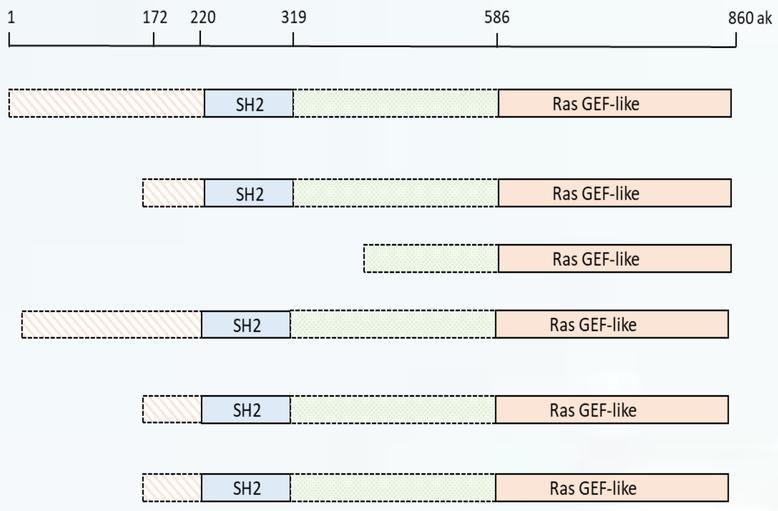
## SILAC-MS DPP3 interactome



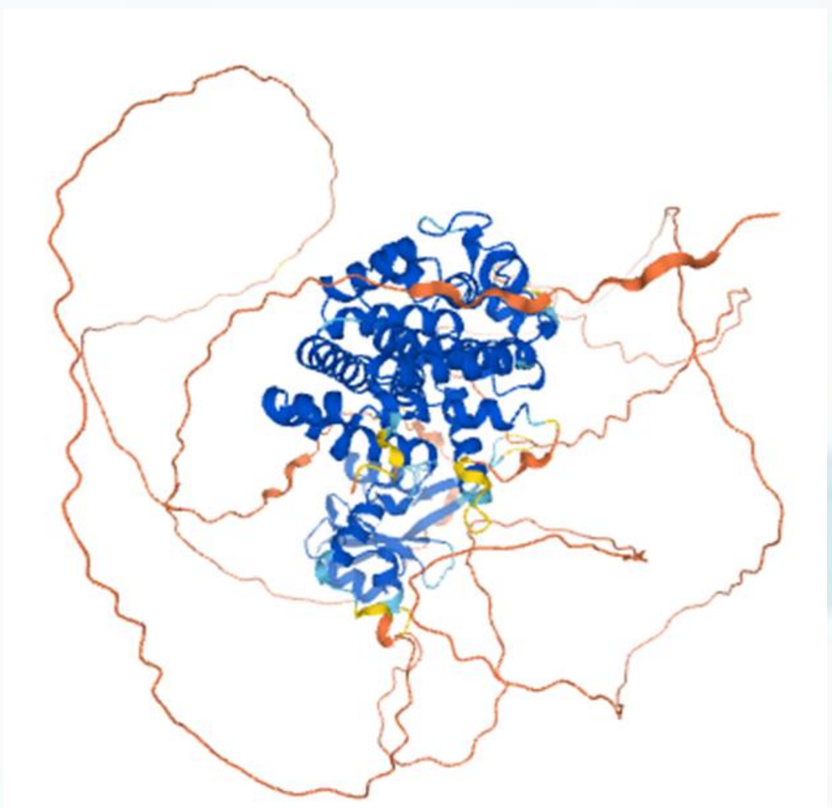

**SH2 domain-containing protein 3C (SH2D3C) (alt. names CHAT, NSP3, SHEP1)**

- 1 out of 4 SILAC-MS replica (Ratio WT/EV 8,5492)
- contains SH2 and Ras GEF-like domain
- acts as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response

## SH2D3C isoforms

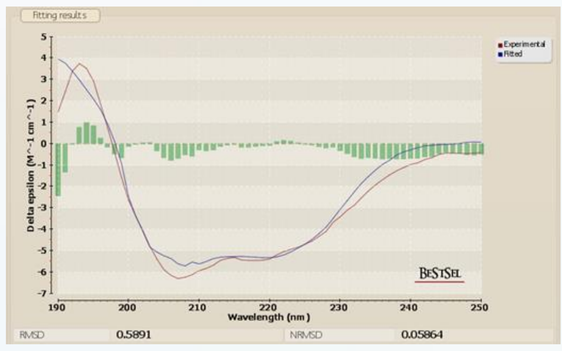



## Alphafold structure prediction – isoform 1



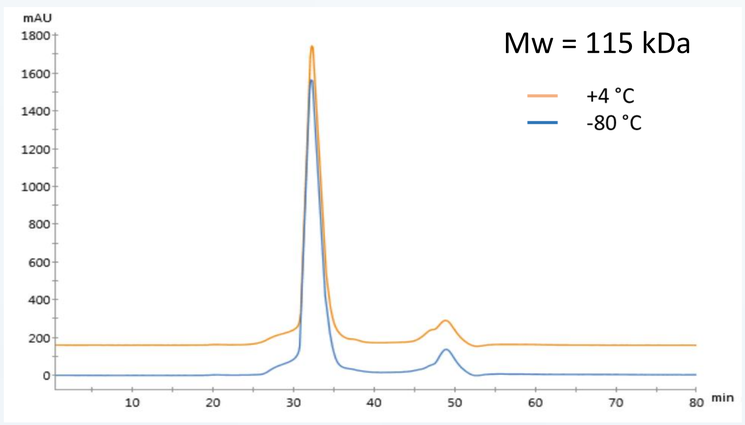
## Purification of isoform 3


## CD

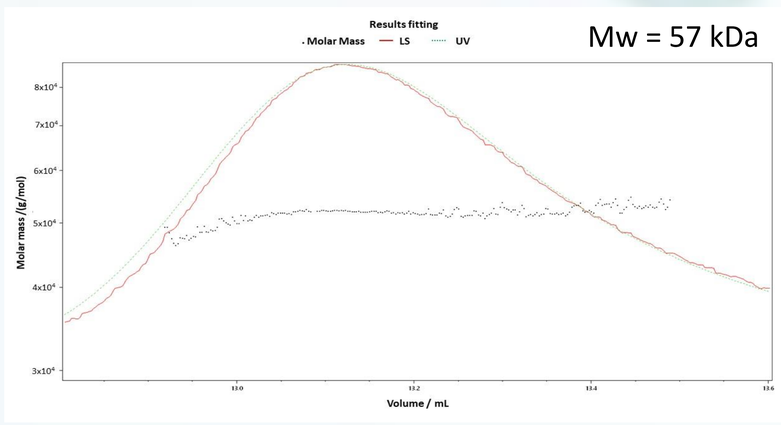



| | |
|---|---|
| Helix | 33.8 % |
| Antiparallel | 8.3 % |
| Parallel | 9.1 % |
| Turn | 6.2 % |
| Others | 42.7 % |

## SEC




## SEC-MALS




## CONCLUSION

SH2D3C was identified as a putative interactor of DPP3 by SILAC-MS approach. DPP3 is a peptidase, biochemically and structurally well characterized, while data about SH2D3C structure are scarce. To date, only the crystal structure of the C-terminal Ras-GEF-like domain of SH2D3C in the complex with BCAR1 (alt. name p130Cas) protein was determined (PDB: 3T6G). AlphaFold structure predictions of the canonical isoform 1 indicate that SH2D3C contains large proportion of internally disordered regions (IDRs), while further biochemical and biophysical investigations of SH2D3C protein are hindered by the difficulties with its heterologous expression in *E. coli*. Our efforts to express SH2D3C in *E. coli* were largely unsuccessful, however, isoform 3 with GST-tag was successfully expressed by baculovirus-mediated protein expression in the insect cells, and it was confirmed that more than 40 % of the protein is disordered, but it is stable and is being used for further biochemical, biophysical and structural analysis.

**Reference**: Mace, Peter D., Yann Wallez, Małgorzata K. Dobaczewska, Jeongeun J. Lee, Howard Robinson, Elena B. Pasquale, and Stefan J. Riedl. 2011. "NSP-Cas Protein Structures Reveal a Promiscuous Interaction Module in Cell Signaling." Nature Structural and Molecular Biology 18 (12): 1381–87. https://doi.org/10.1038/nsmb.2152.

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