OVEREXPRESSION AND PURIFICATION OF THE C-TERMINAL DOMAIN OF THE PROTEIN SH2D3C IN *ESCHERICHIA COLI*

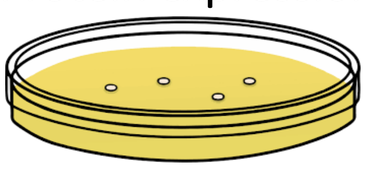
Lea Barbarić[1], Ana Tomašić-Paić[1], Sara Matić[1], Mihaela Matovina[1]

[1] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb

HrZZ Croatian Science Foundation

Protein SH2D3C is one of the three members of the family of proteins which contain both SH2 domain and a C-terminal domain similar to guanine nucleotide exchange factor domains for Ras family GTPases (Ras GEF-like domain).
SH2D3C acts as an adapter protein in signaling pathways involved in cell adhesion and migration, tissue organization, and regulation of the immune response.[1] Although the protein contains a Ras GEF-like domain, it has no significant GEF activity,[2] but may interact with other proteins. Analysis of the cellular proteome revealed a potential interaction of SH2D3C with dipeptidyl peptidase III (DPP III) involved in the regulation of oxidative stress.
To confirm the interaction, the C-terminal domain of SH2D3C was overexpressed in *E. coli* and purified with two different tags, GST and MBP.

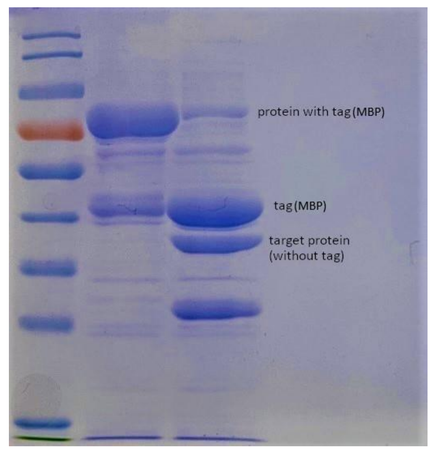
Protein expression



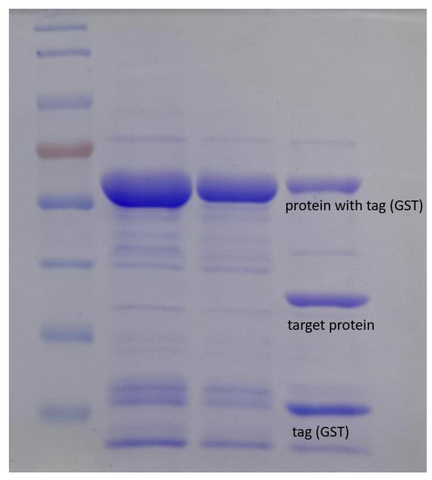
E. coli BL21(DE3)Ril+ cells + plasmid DNA

Purification of the target protein using affinity chromatography

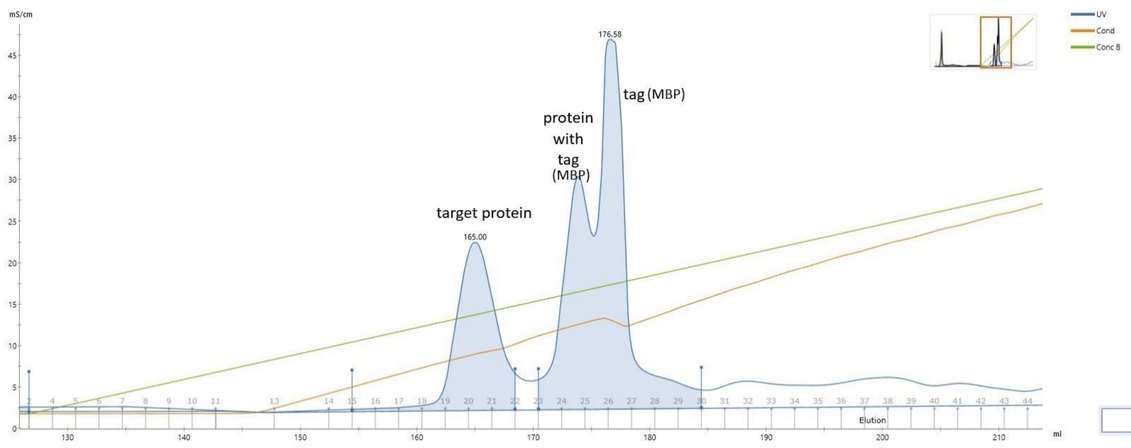
Removal of the tag by cleavage with TEV/HRV-3C protease

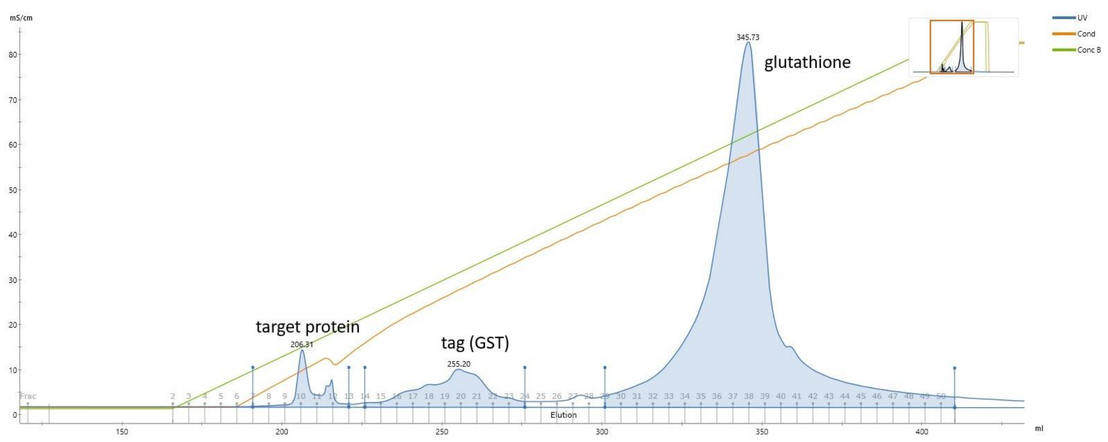


Detection of the cleaved protein by SDS-PAGE before and after incubation with TEV/HRV-3C protease

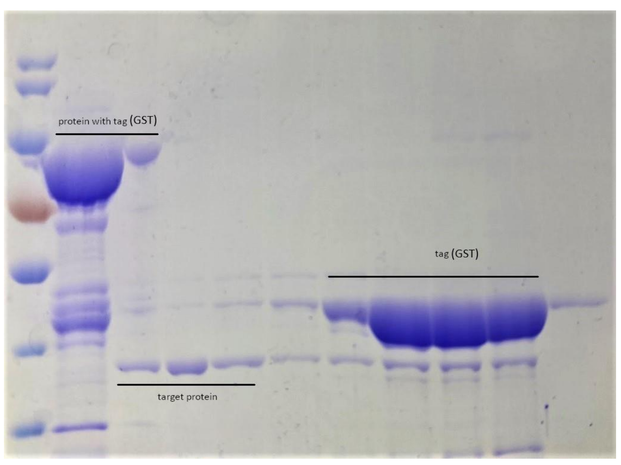


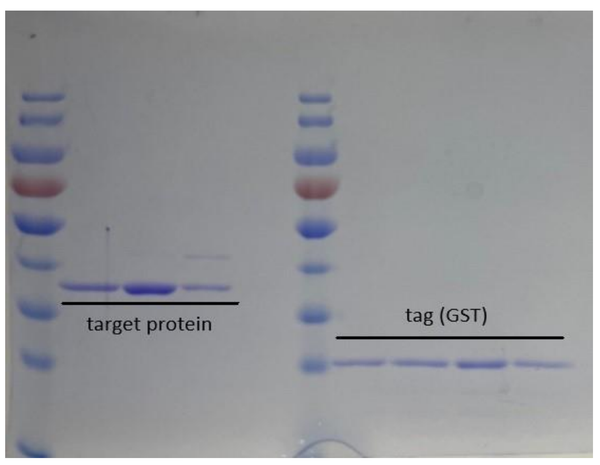
Purification of the target protein from the cleaved tag and other impurities by FPLC





Detection of the target protein in the fractions by SDS-PAGE





FPLC *(fast protein liquid chromatography)*
The target protein is additionally purified from its tag and other impurities by anion-exchange chromatography using the column **HiPrep Mono Q HP 16/10** (Cytiva).

Mixture:
- Target protein (without tag)
- Tag
- Protein with tag (uncleaved)
- Other impurities + protease

Start buffer: 20 mM Tris, 1 mM TCEP, pH = 8.0

Elution buffer: 20 mM Tris, 1 M NaCl, 1 mM TCEP, pH = 8.0

Resin:
the functional group is a **quaternary amino group** coupled to the matrix via chemically stable ether linkages

Starting from 2 L of *E. coli* bacterial culture, we purified as little as 0.5 mg of the target protein with both GST and MBP tags.
With the removal of the tag, the protein becomes unstable and precipitates.
For the upcoming experiments, we will try to isolate the protein from greater volumes of bacterial culture and proceed without the removal of the tag.

References:
1. A. Sakakibara, Y. Ohba, K. Kurokawa, M. Matsuda, S. Hattori, J. Cell Sci. **115** (2002) 4915-4924.
2. V. C. Dodelet, C. Pazzagli, A. H. Zisch, C. A. Hauser, E. B. Pasquale, Int. J. Biol. Chem. **274** (1999) 31941-31946.

Acknowledgements: This work was funded by the Croatian Science Foundation (CSF) project „Dipeptidyl peptidase III interaction with SH2 domain-containing protein 3C – possible link between oxidative stress response and cell migration" (IP-2020-02-6743)