

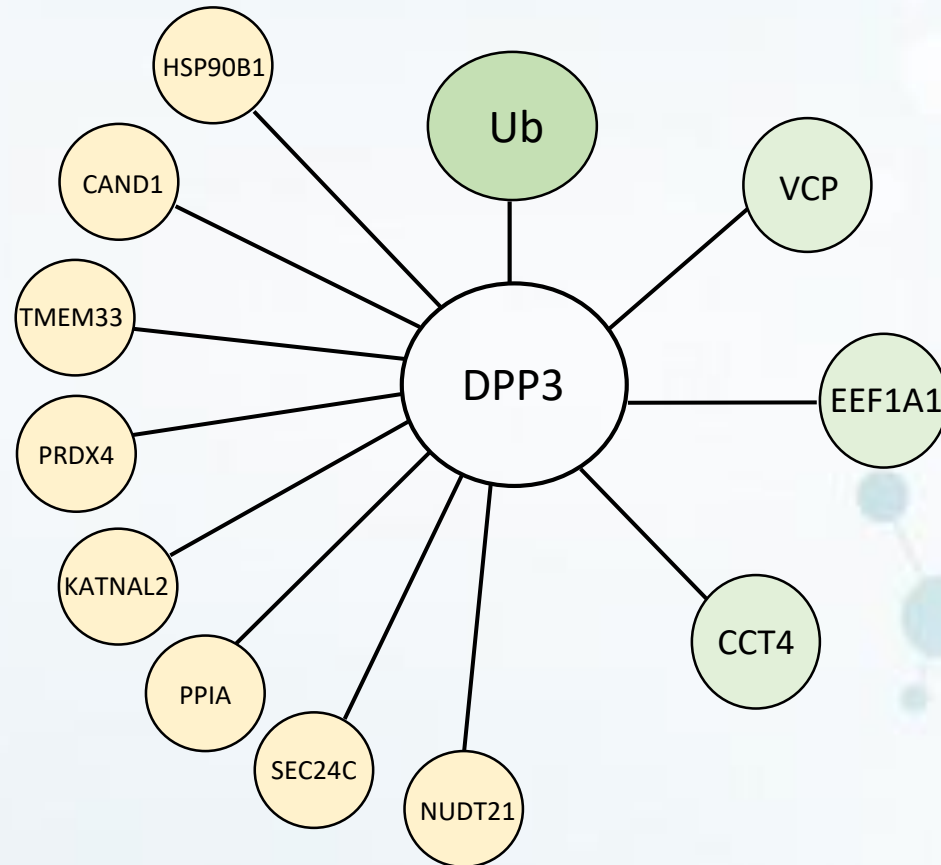
Interakcija proteina DPP III i SH2D3C

Mihaela Matovina



Istraživanje interaktoma humane dipeptidil peptidaze III

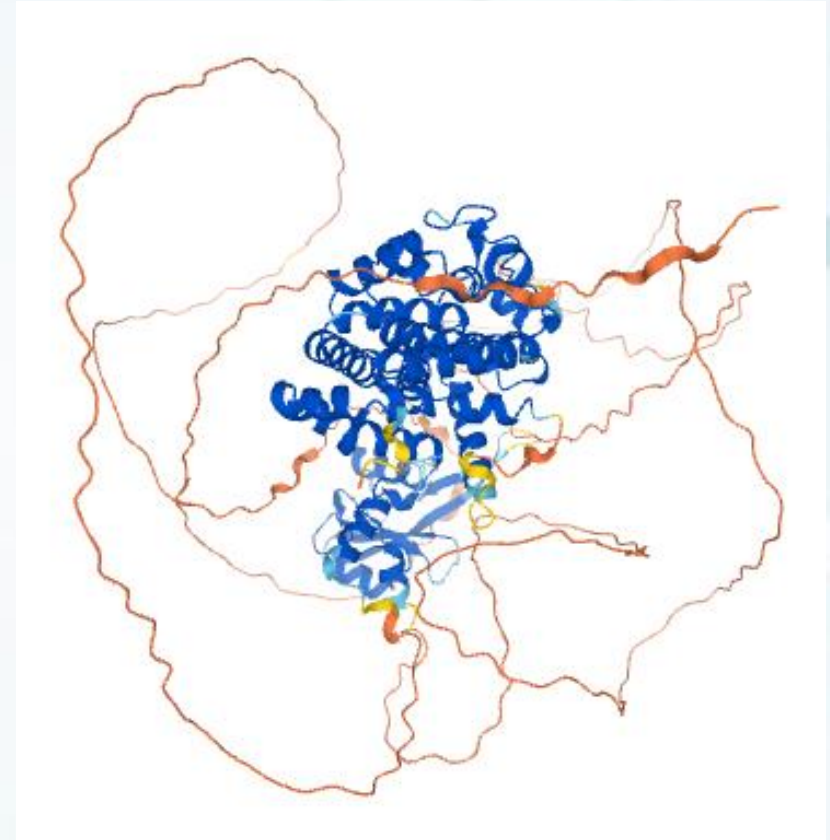
- UKF projekt – SILAC-MS analiza interaktoma DPP III u HEK293T stanicama – DPP III = bait (4 biološka replikata)



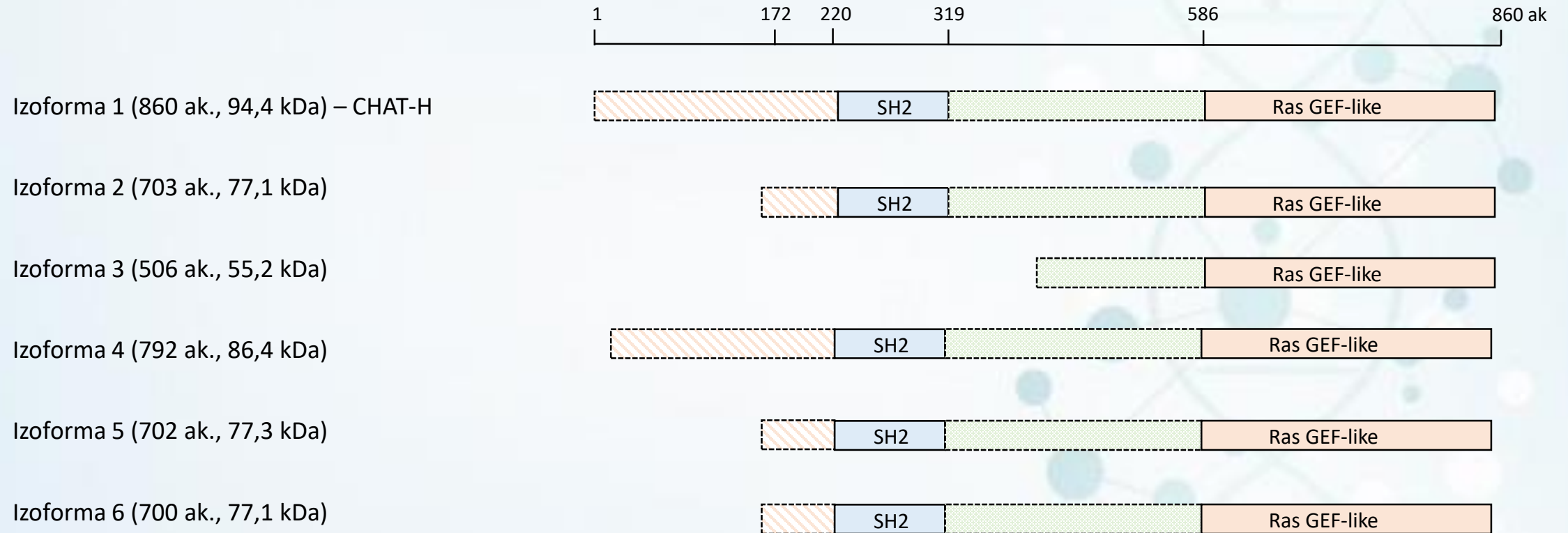
SH2D3C - SH2 domain-containing protein 3C

- SH2D3C – nađen u samo 1 biološkom replikatu (KEAP1 također nađen u samo 1 replikatu)
 - Niska razina ekspresije SH2D3C u HEK293T stanicama
- porodica proteina u kojoj se nalaze još 2 proteina – SH2D3A i SH2D3B (sadrže SH2 i Ras GEF-like domenu)
- nekoliko alt. imena:
 - CHAT/CHAT-H (Cas/HEF1-associated signal transducer)
 - NSP3 (Novel SH2-containing protein 3)
 - SHEP1 (SH2 domain-containing Eph receptor-binding protein 1)
- Uključen u regulaciju adhezije i migracije stanica, tkivne organizacije i imunog odgovora
- Kristalna struktura C-terminalne domene
- Vjerojatno sadrži visok udio nestrukturiranih regija (IDR, engl. *Intrinsically disordered regions*)

Predikcija strukture - Alphafold



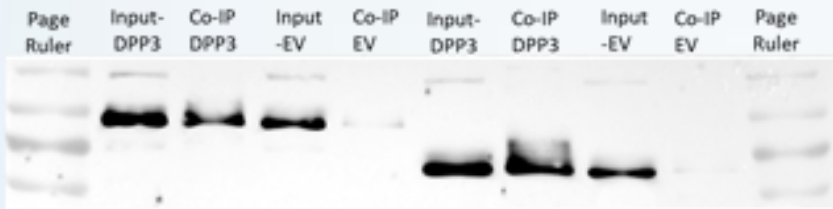
Izoforme SH2D3C eksprimirane u stanicama



Interakcija DPP III i SH2D3C

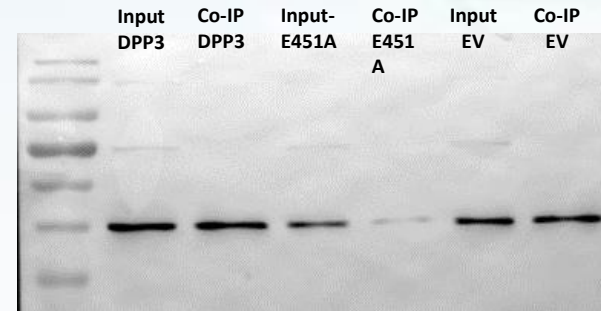
Koimununoprecipitacija

Izoforme 2 i 3



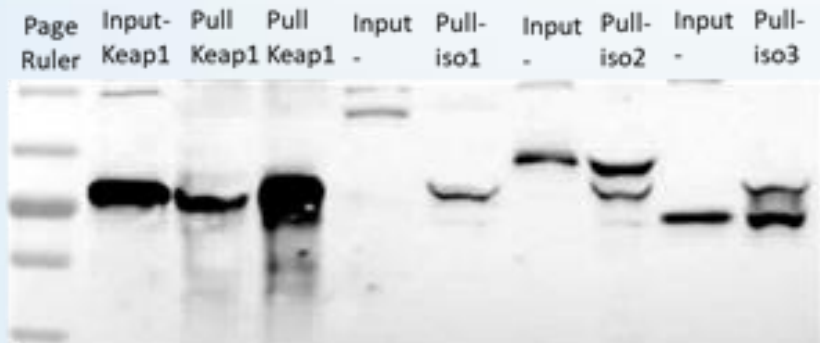
IP: anti-HA
WB: anti-FLAG

C-terminalna domena

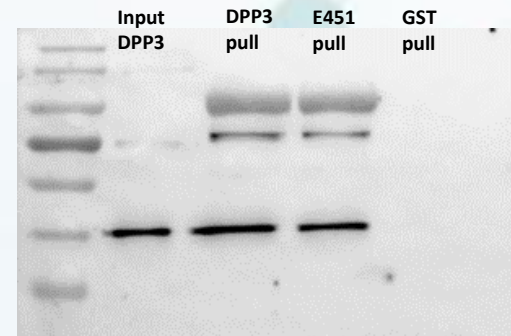


GST-pulldown

Izoforme 2 i 3



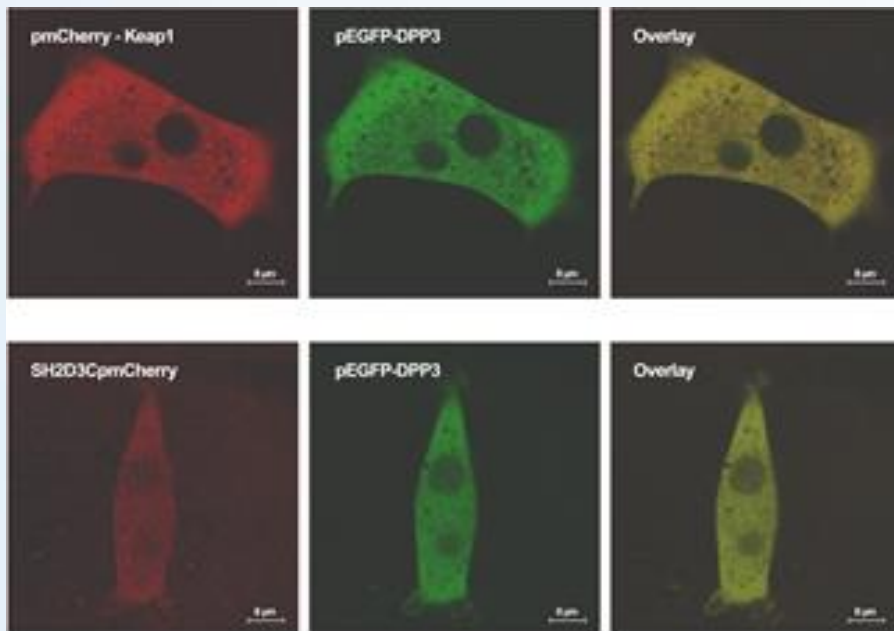
C-terminalna domena



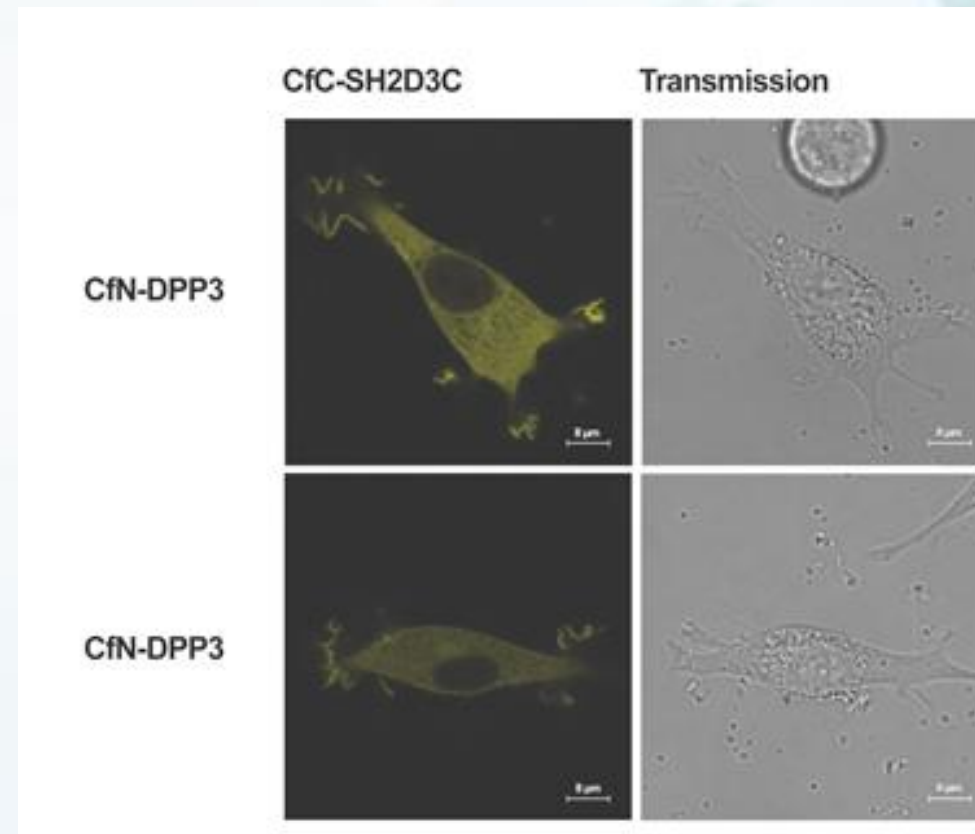
Kolokalizacija/interakcija DPP III i SH2D3C u membranskim naborima (uloga u migraciji stanica) NIH 3T3 stanice

Ana Tomašić Paić i Lucija Horvat

Kolokalizacija: EGFP/mCherry



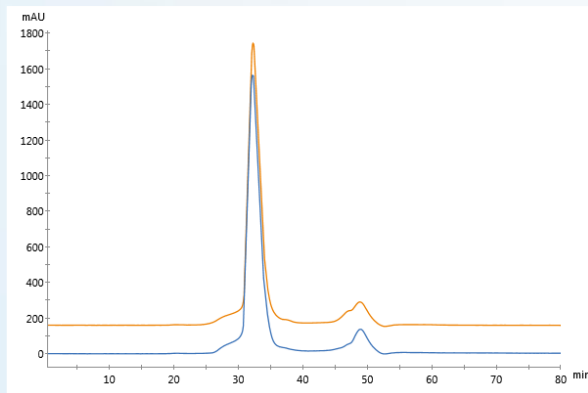
Bimolekularna komplementacija fluorescencije (BiFC)



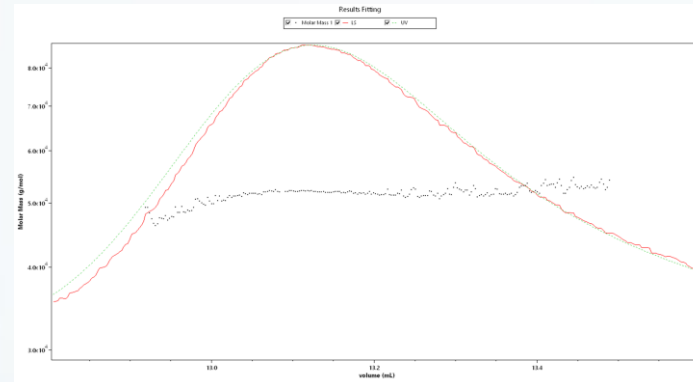
Ekspresija i pročišćavanje proteina SH2D3C u stanicama insekata

- Izoforma 3 SH2D3C (57 kDa) – uspješno eksprimirana i pročišćena u EMBL-u, Heidelberg
- Protein stabilan, ali sadrži visok udio neuređenih regija – oko 42,7 % prema CD mjerenju
- Na SEC-u putuje kao dimer (oko 116 kDa), ali metodom SEC-MALS potvrđeno da se radi o monomeru – nije globularan

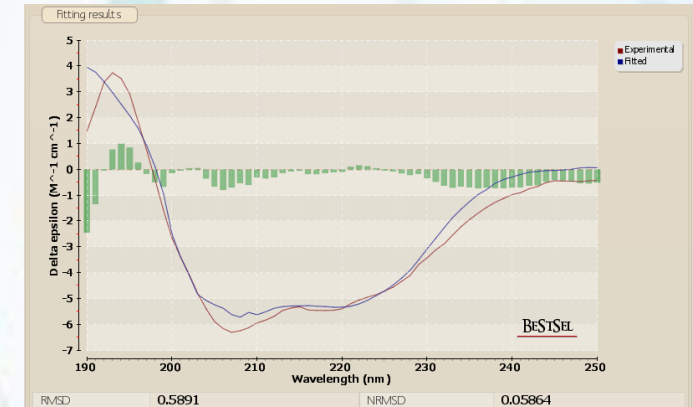
SEC analiza Molekulske mase – 116 kDa



SEC-MALS analiza – 57 kDa



CD analiza

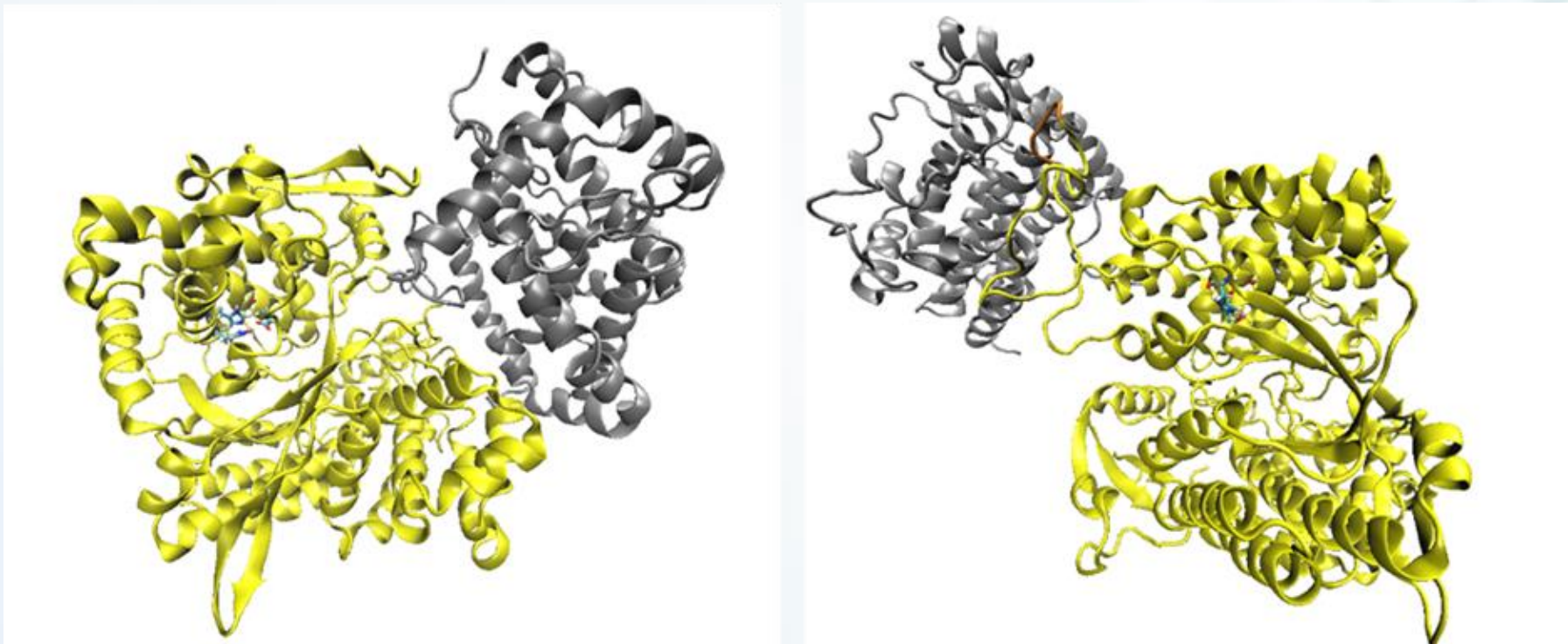


- Helix: 33.8
- Antiparallel: 8.3
- Parallel: 9.1
- Turn: 6.2
- Others: 42.7

Molekulsko dokiranje

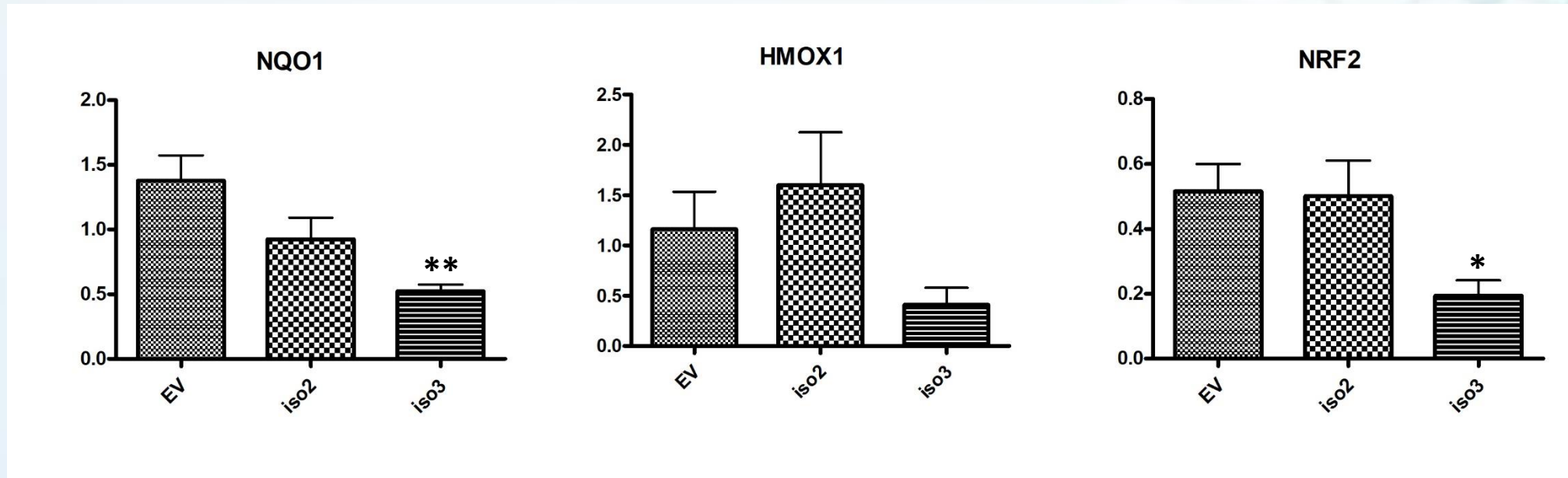
Dva reprezentativna načina vezanja DPP III s C-terminalnom domenom proteina SH2D3C

Sanja Tomić i Hrvoje Brkić



Analiza ekspresije NRF2-kontroliranih gena qPCR-om

- HEK293T stanice transfecirane praznim vektorom (EV), SH2D3C-izofromom 2 i -izofromom 3 (4 biološka replikata)



U tijeku...

- Test GTPazne aktivnosti SH2D3C u prisustvu DPP III
 - Domena Ras GEF SH2D3C inaktivna – zatvorena konformacija (u usporedbi s aktivnim enzimima) → interakcija s DPP III uzrokuje promjenu konformacije i aktivaciju domene Ras GEF
- Ko-immunoprecipitacija endogenih proteina
- Testovi stanične migracije nakon prekomjernje ekspresije SH2D3C i/ili DPP3

HRZZ projekt IP-2020-02-6743 „Interakcija dipeptidil peptidaze III s proteinom SH2 domain-containing protein 3C – moguća veza između odgovora na oksidativni stres i stanične migracije”



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