



Laboratorij za biokemiju proteina i molekulsko modeliranje

Institut Ruđer Bošković

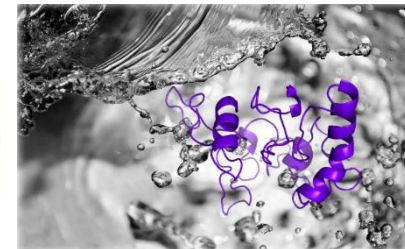
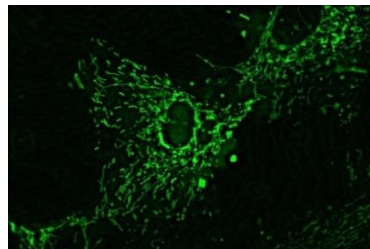
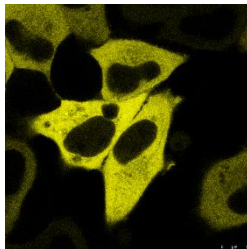
Ana Tomašić Paić

(viši stručni suradnik)

„Bioimaging of DPP3-SH2D3C Protein Interactions in Living Cells Using BiFC Method”

Projekti: „DPP3 BioRe” (Sanja Tomić)

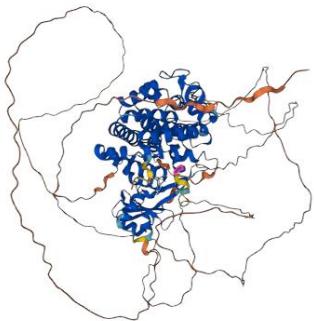
„OxMiLink” (Mihaela Matovina)



SH2 domain-containing protein 3C · Homo sapiens (Human) · Q8N5H7; 860 aa



Predviđena struktura SH2D3C
prema AlphaFold-u
(AF-Q8N5H7-F1, UniProt)



*imena: *Cas/HEF1-associated signal transducer 1 (Chat-H 1)*; *SH2 domain-containing Eph receptor-binding protein 1 (SHEP1)*, *novel Src homology 2-containing protein (NSP3)*

*otkriven analizom interaktoma DPP3 (SILAC MS); potencijalni interaktor proteina DPP3



*ima **dvije domene**; **SH2** (220-319); **Ras-GEF** (586-854) domenu sličnu domeni faktora razmjene gvanin nukleotida za Ras obitelj GTPaze; te *intrinsically disordered regions* (51-117; 130-180; 335-537); **regiju bogatu prolinom/serinom** s potencijalnim mjestima fosforilacije pomoću prolin-usmjerenih kinaza

*6 isoformi /UniProt

*proteini iz **NSP porodice**: SH2D3A, BCAR3 (SH2D3B), SH2D3C ulaze u interakciju s proteinima iz porodice Cas putem svoje RasGEF slične domene

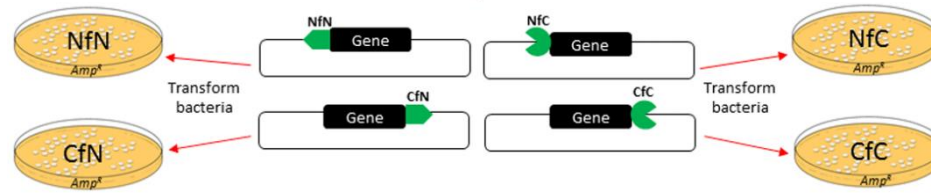
***SH2D3C** ulazi u interakciju s EphB2 fosforil. domenom proteina te s proteinima R-Ras i Rap1A (porodica malih Ras GTPaza), no ne pokazuje GTPaznu aktivnost *in vitro*

***BCAR3 i SH2D3C**: inaktivna Ras GTPaza

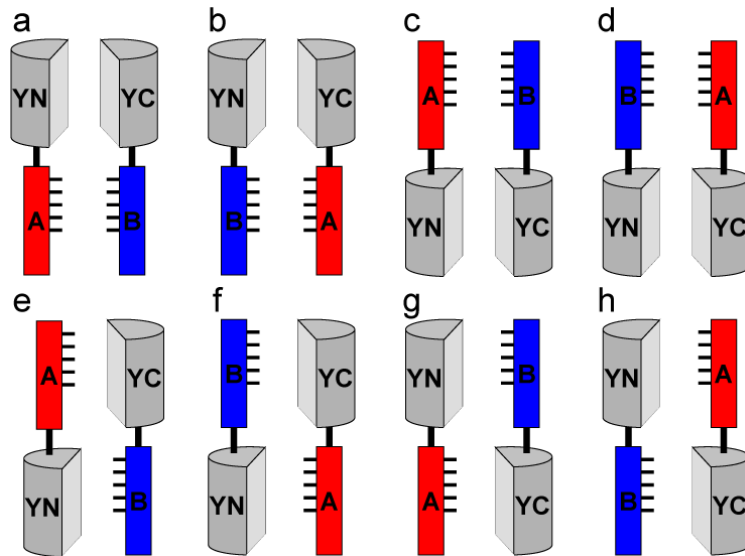
*sudjeluje u regulaciji stanične migracije i adhezije, organizacije tkiva i regulaciji imunog odgovora

*SH2D3C protein kodira SH2D3C gen smješten na 9. kromosomu

Prikaz mogućih topologija (NfN, NfC, CfN, CfC)



*Lepur A. et al. (2016) *Journal of Biomolecular Screening*
DOI: 10.1177/1087057116659438



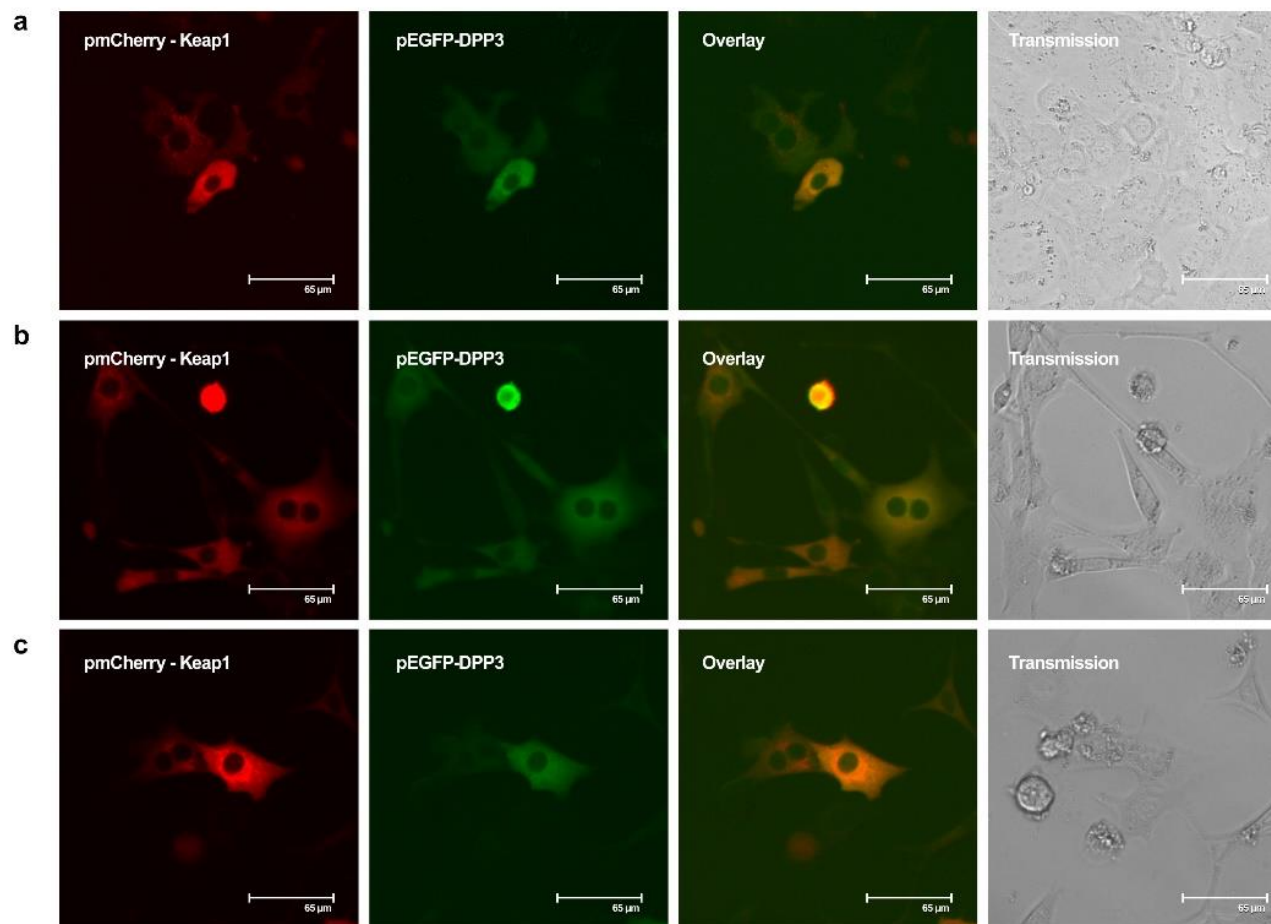
**Nat Protoc.* 2006 ; 1(3): 1278–1286.

Kombinacije potencijalnih interakcija (pozitivna kontrola):

- | | |
|----------------------|-----------------------|
| VenusfN- DPP3 | VenusfC- Keap1 |
| | Keap1 -VenusfC |
| VenusfC- DPP3 | VenusfN- Keap1 |
| | Keap1 -VenusfN |
| DPP3 -VenusfN | VenusfC- Keap1 |
| | Keap1 -VenusfC |
| DPP3 -VenusfC | VenusfN- Keap1 |
| | Keap1 -VenusfN |

**Live-cell istraživanja substanične (ko)-lokalizacije
pmCherry-Keap1 i pEGFP-DPP3 plazmida
primjenom EVOS Fluid uređaja**

NIH3T3 cell line

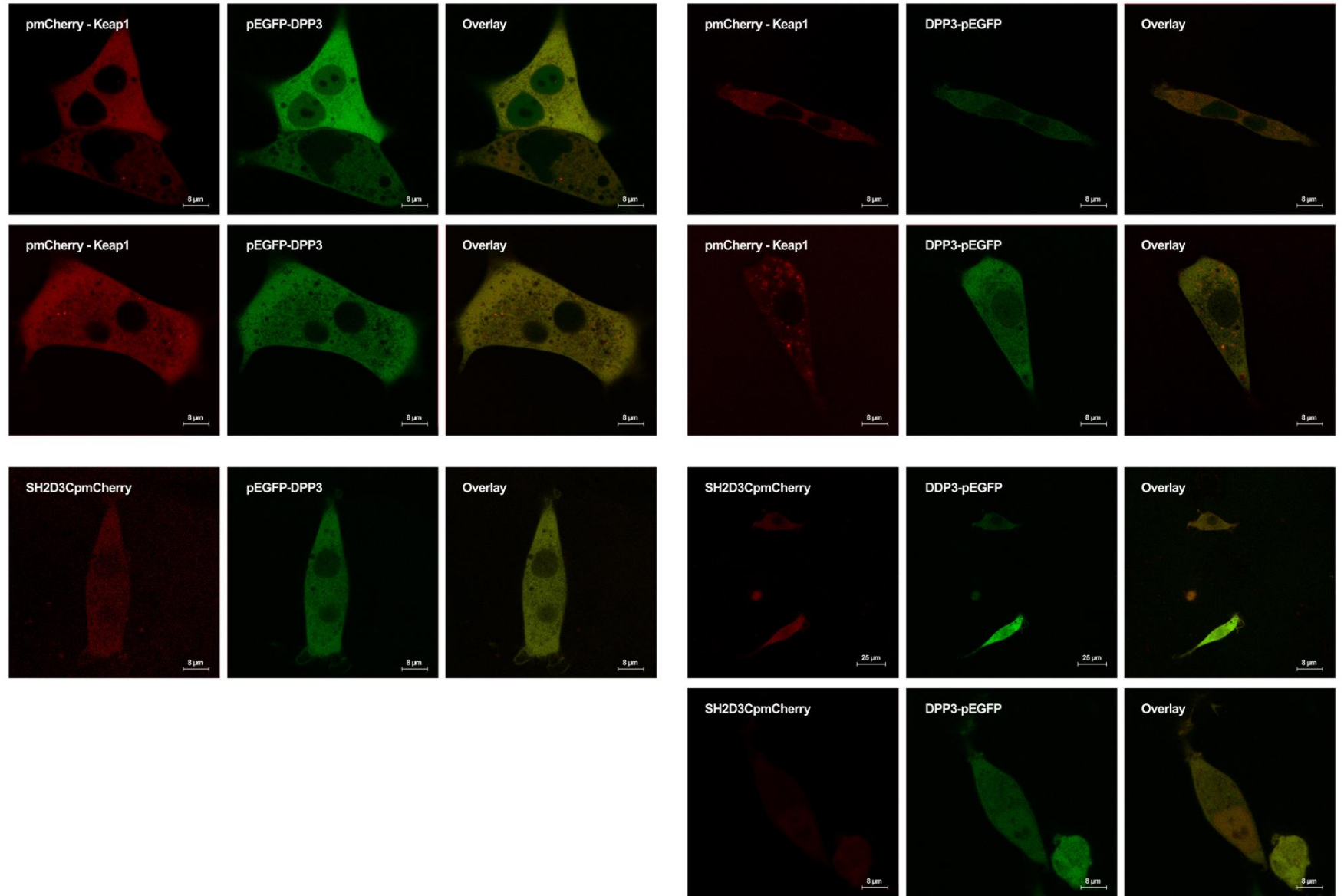


(a) nakon 24 h inkubacije
(b,c) nakon 48 h inkubacije

Live-cell istraživanja
primjenom SP8 X FLIM

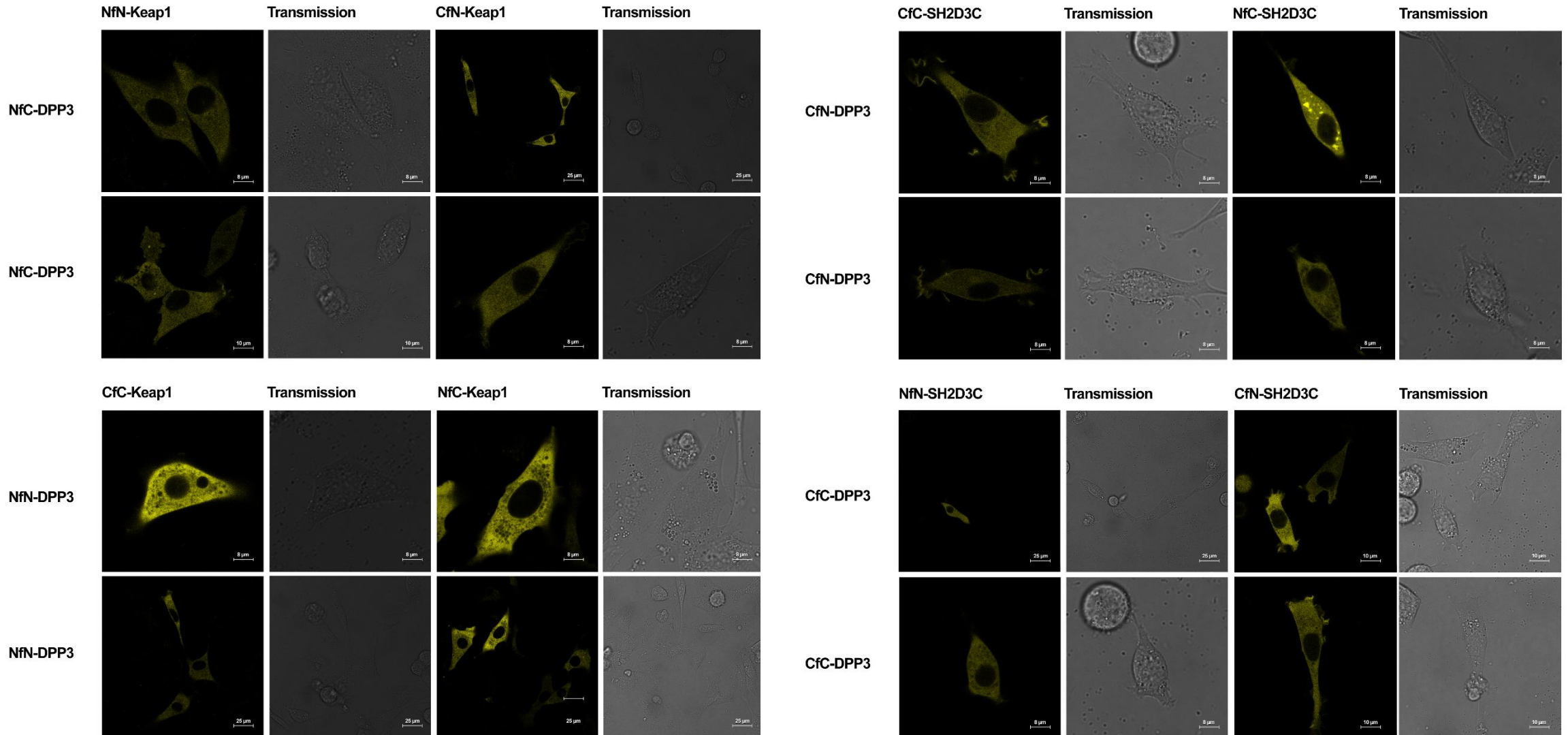
NIH3T3 cell line

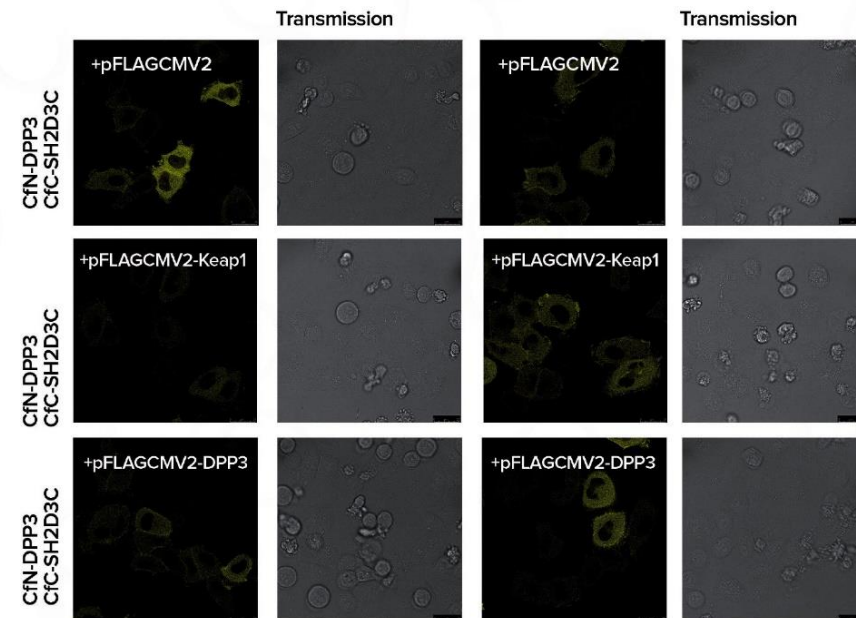
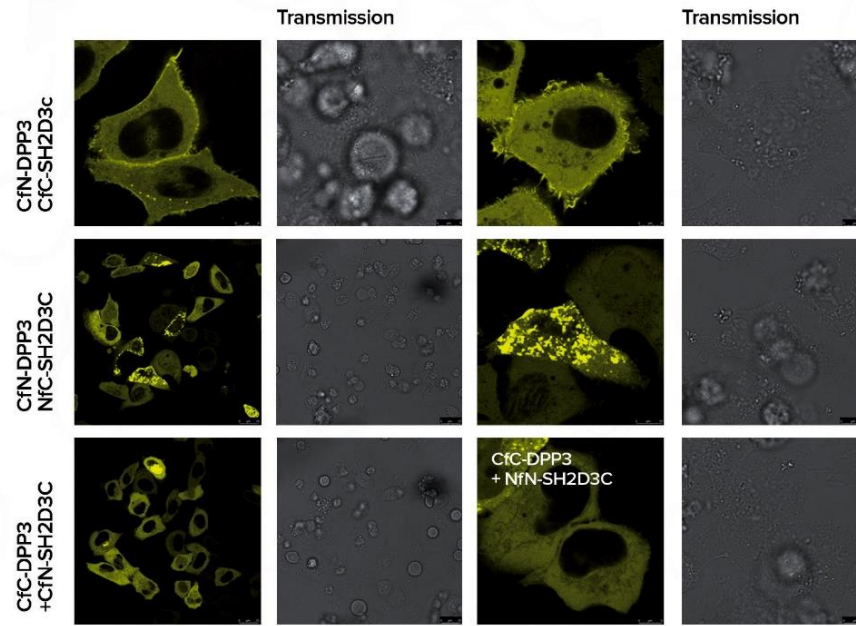
nakon 24 h
inkubacije



IBL, 4 chamber glass
bottom dish, dish size
35mm, well size 20mm

Live-cell istraživanja komplementacije dviju molekula
fluorescencijom primjenom SP8 X FLIM

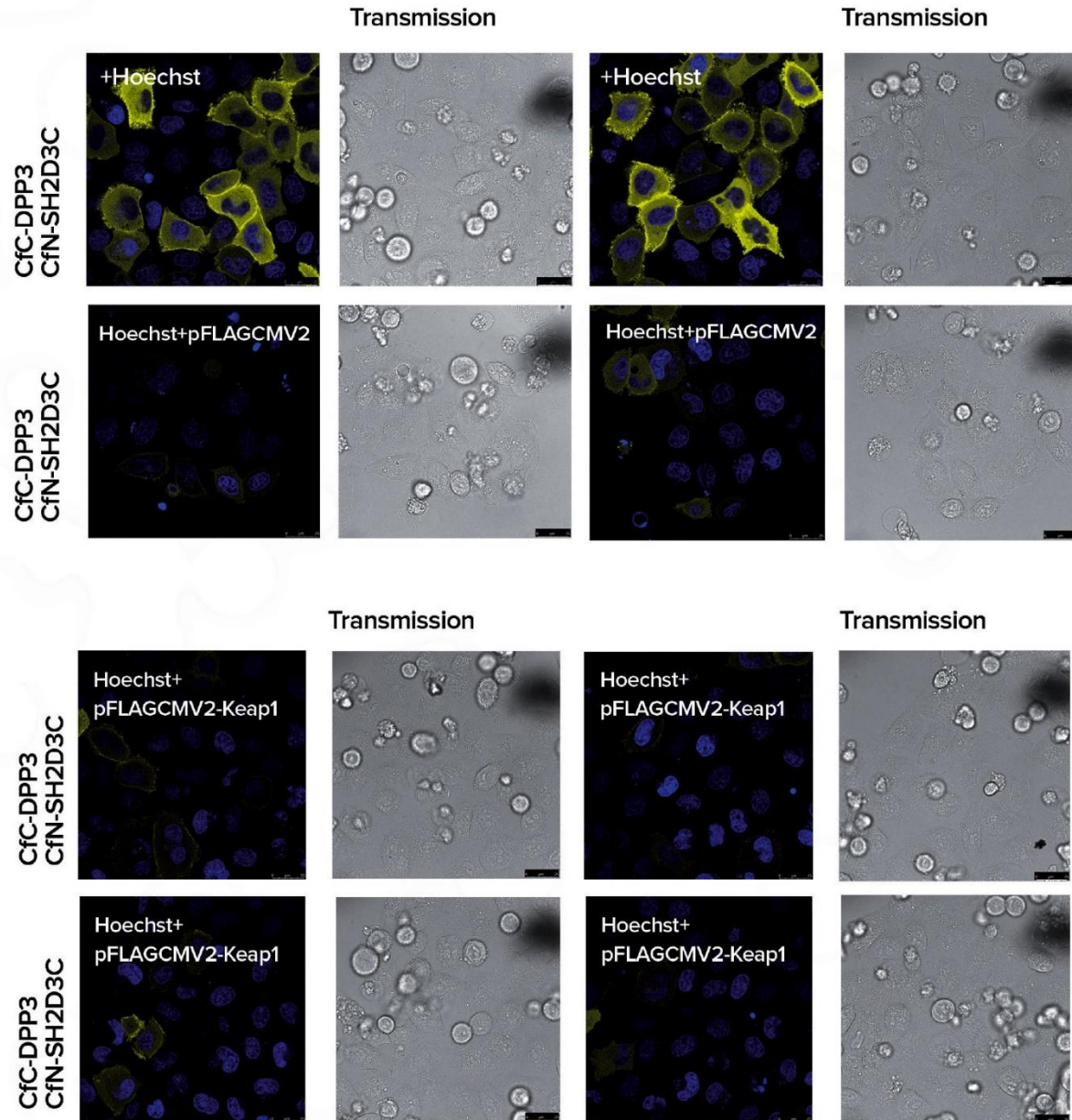




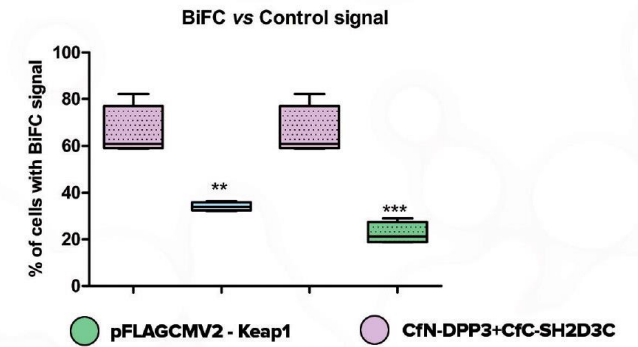
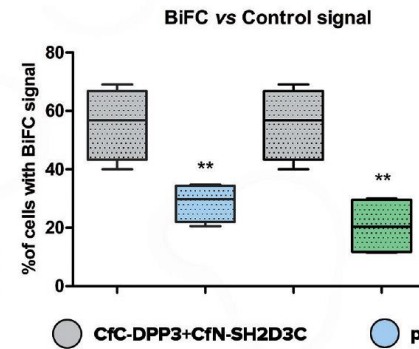
BiFC interakcijska analiza
(kompetitivni eksperiment)
HeLa stanice

Suradnici:
Lucija Horvat
Adriana Lepur
Katja Ester





BiFC screen
(kompeticijski eksperiment)





4th ISFMS—Biochemistry, Molecular Biology and Druggability of Proteins 6–9 Sep 2022, Florence, Italy



Live cell imaging of DPP3 and SH2D3C protein interaction using bimolecular fluorescence complementation

Ana Tomasić Pačić, Lucija Horvat, Mihaela Matovina, Rudjer Bosković Institute, Division of Organic Chemistry and Biochemistry, Division of Molecular Biology



Introduction

Protein-protein interactions (PPIs) are the basis for human metabolic and signaling systems. Studies of PPIs within living mammalian cells may provide useful information for deeper understanding of the functional relationships and mechanisms...

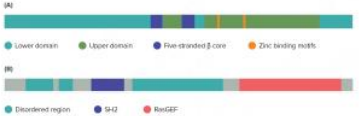


Fig. 1. Schematic of the full length (PPI3) and SH2D3C (SH2) sequences with its most relevant regions. PPI3 is composed...

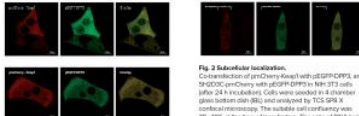


Fig. 2. Subcellular localization. Co-transfection of pEGFP-N1-Flag and SH2D3C-His6 in HeLa cells... Confocal microscopy. The subcellular colocalization was 30–40% in the five different fractions...

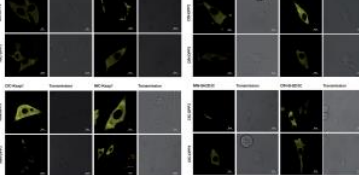


Fig. 3. Confocal (pseudo)fluorescence images of HeLa cells transiently transfected with BFC vectors expressing DPP3...

Results: Based on the FACS results (data not shown here), HEK293T cells showed not to be appropriate for BFC assays...

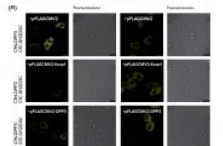
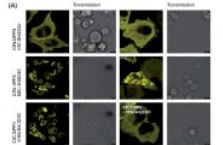


Fig. 4. BFC protein interaction assays. (A) Confocal fluorescence images of HeLa cells transiently transfected with BFC vectors expressing SH2D3C...

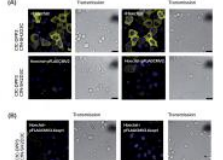


Fig. 5. BFC screen. (A) Confocal fluorescence images of HeLa cells transiently transfected with BFC vectors and constructs...

Conclusion: In summary, the BFC assay allowed us to efficiently visualize the protein interaction between DPP3 and SH2D3C protein...

Acknowledgment: This work was funded by the Croatian Science Foundation (CSP) project "Disorderly proteome II... References: Matovina, M. et al. PDB: 1D023-D001 (10/10/06); H415...



UNDER CONSTRUCTION

Hvala na pažnji