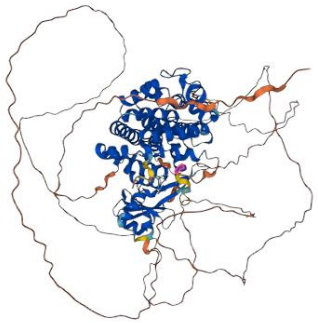
Laboratorij za biokemiju proteina i molekulsko modeliranje
Institut Ruđer Bošković
Ana Tomašić Paić
(viši stručni suradnik)

„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)

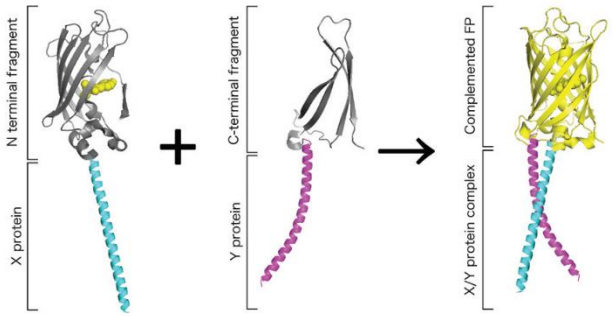


*imena: *Cas/HEF1-associated signal transducer* 1 (Chat-H 1); *SH2 domain-containing Eph receptor-binding protein 1* (SHEP1), *novel Src homology 2-containing protein* (NSP3)
*otkriven analizom interaktoma DPP3 (**SILAC MS**); potencijalni interaktor proteina **DPP3**

*ima **dvije domene**; **SH2** (220-319); **Ras-GEF** (586-854) domenu sličnu domeni faktora razmjene gvanin nukleotida za Ras obitelj GTPaze; te *intrinsically disoredered 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

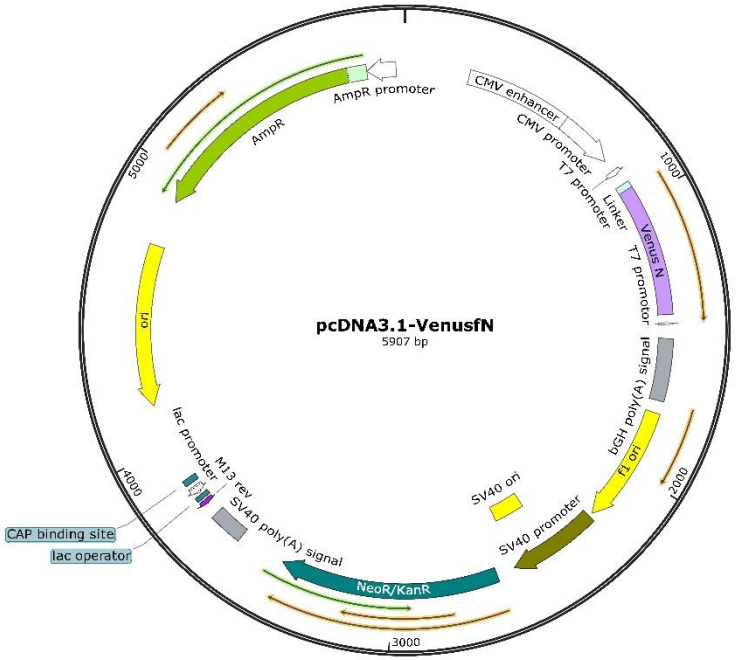
Komplementacija dviju molekula fluorescencijom

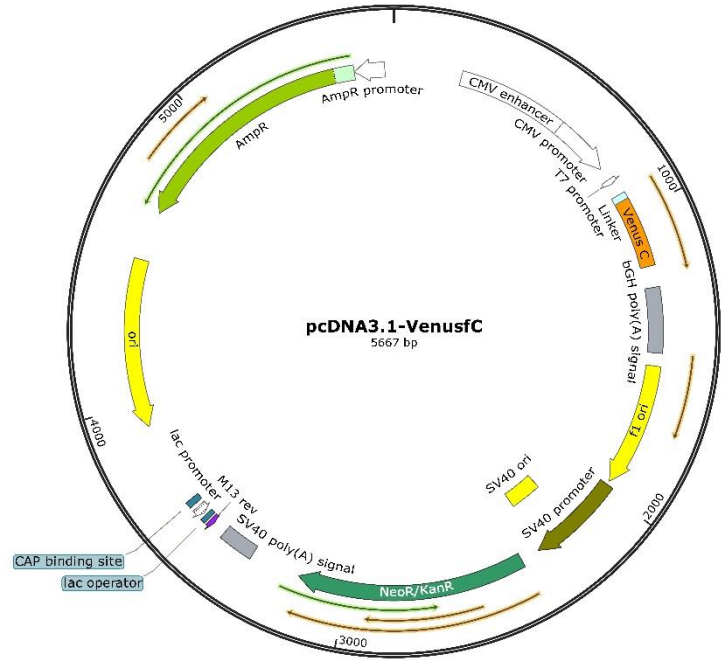
Princip rada BiFC metode



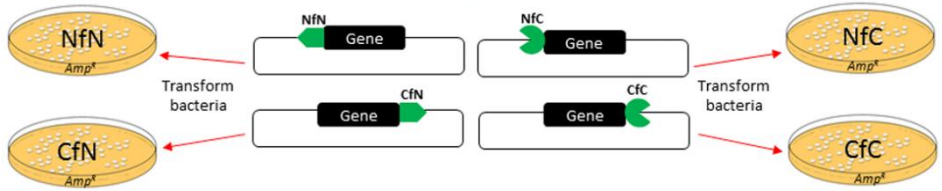
Venus yellow fluorescent protein
(PDB ID: 1MYW from RCSB Protein Data Bank)
Ex=515 nm; Em=528 nm

Ishodišni vektori (za potrebe kloniranja i konstrukciju BiFC plazmida)

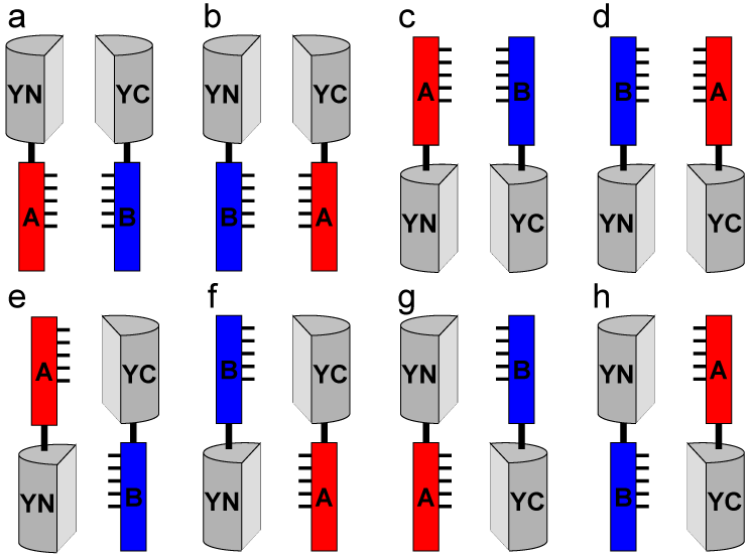




Prikaz mogućih topologija (NfN, NfC, CfN, CfC)



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



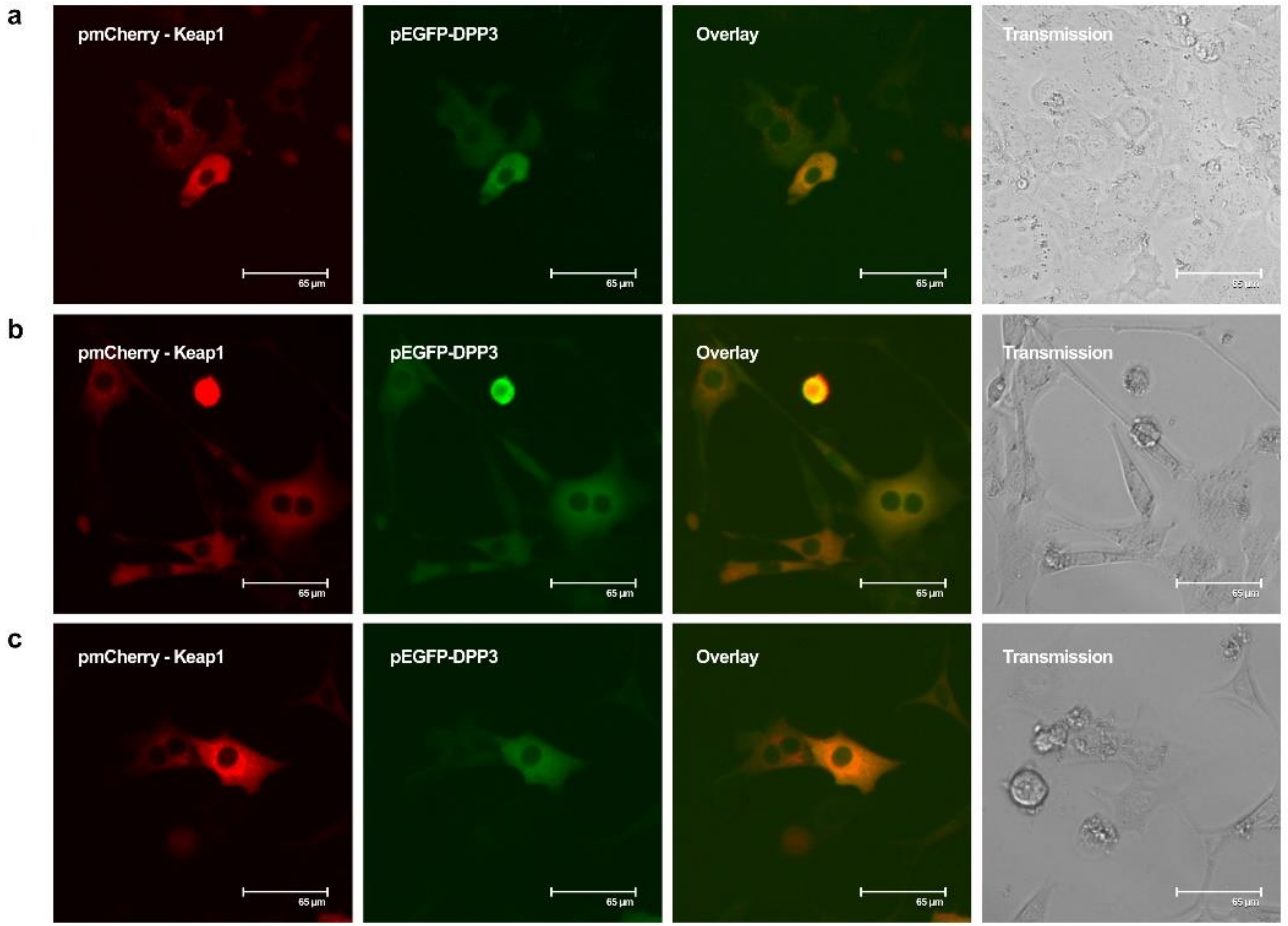
Nat Protoc. 2006 ; 1(3): 1278–1286.

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 Floid uređaja

NIH3T3 cell line



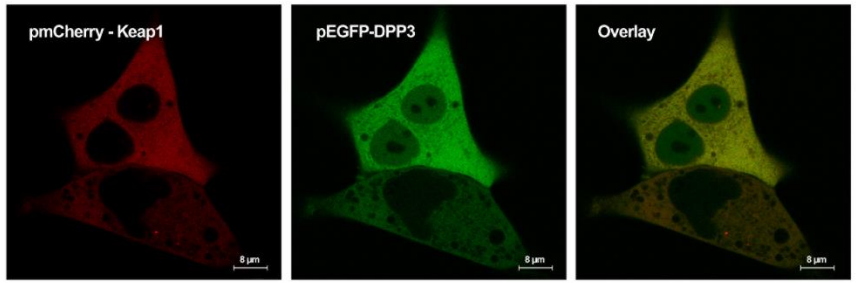
(a) nakon 24 h inkubacije
(b,c) nakon 48 h inkubacije

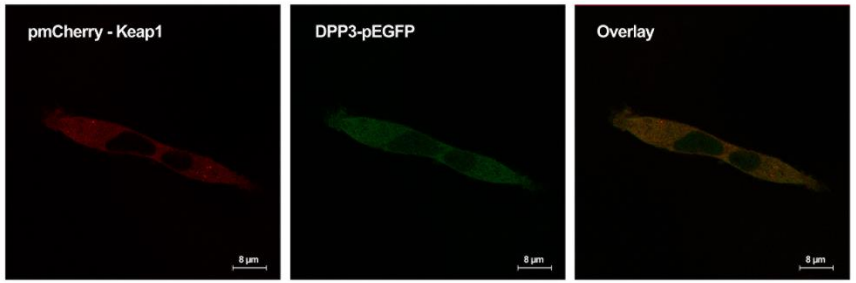
Live-cell istraživanja primjenom SP8 X FLIM

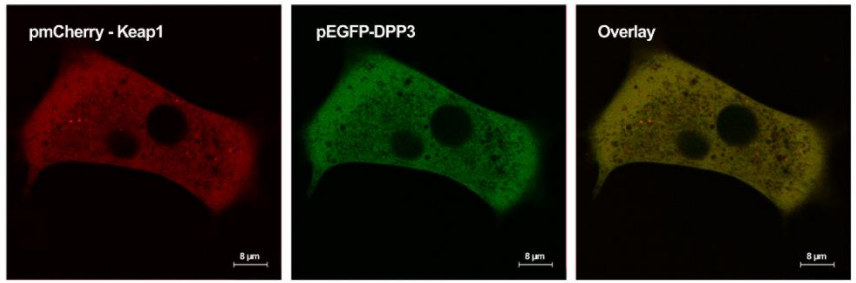
NIH3T3 cell line

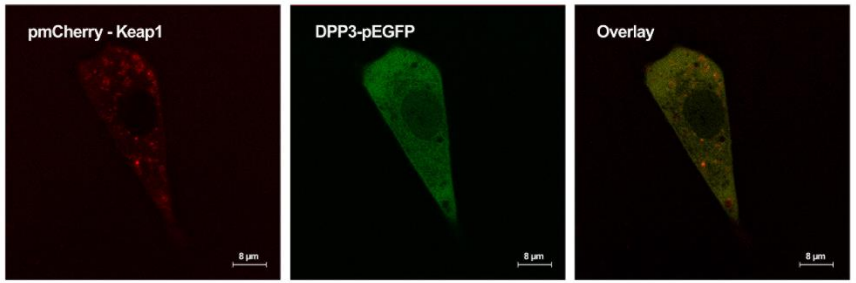
nakon 24 h inkubacije

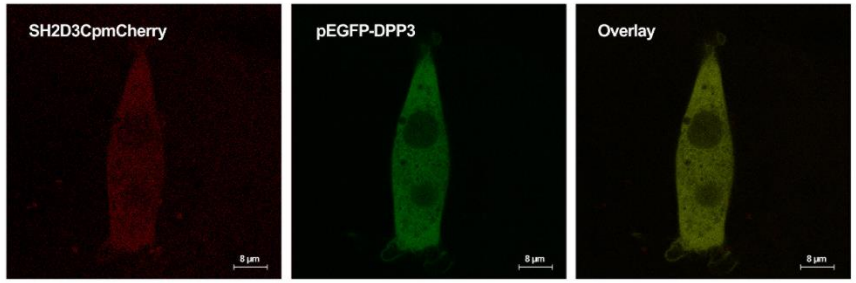
IBL, 4 chamber glass bottom dish, dish size 35mm, well size 20mm

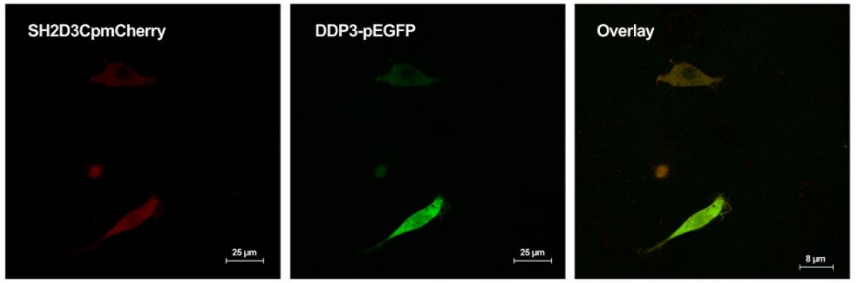


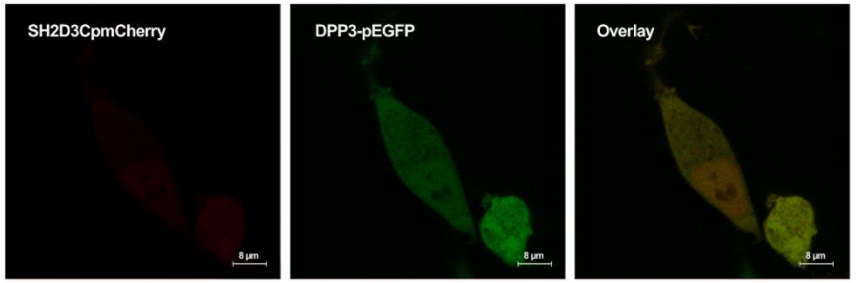




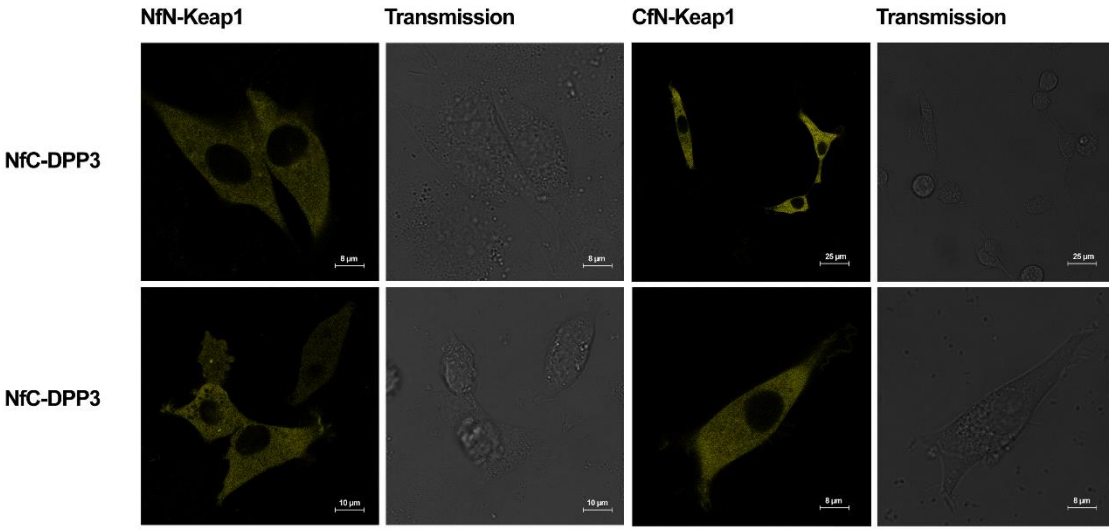


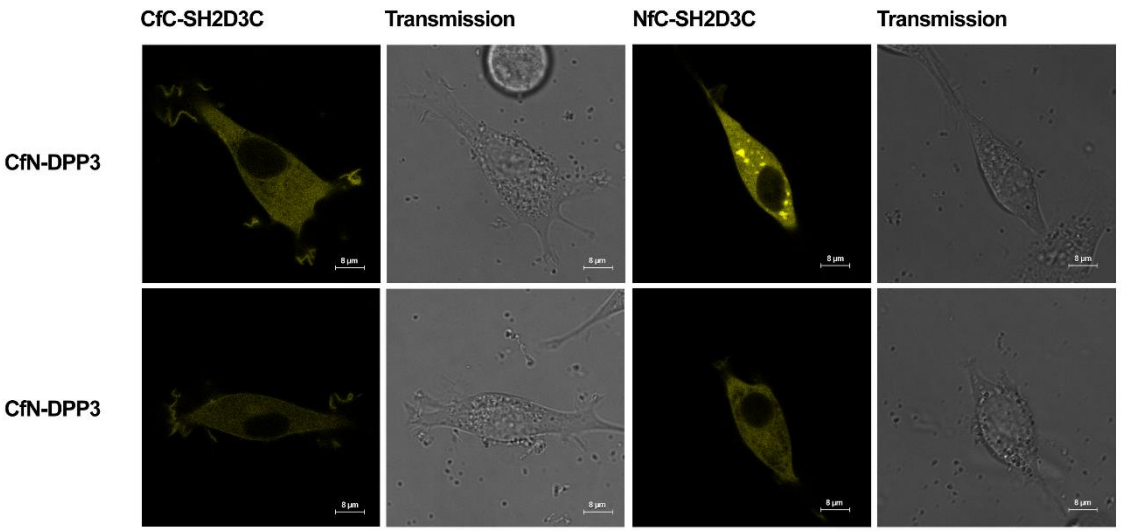


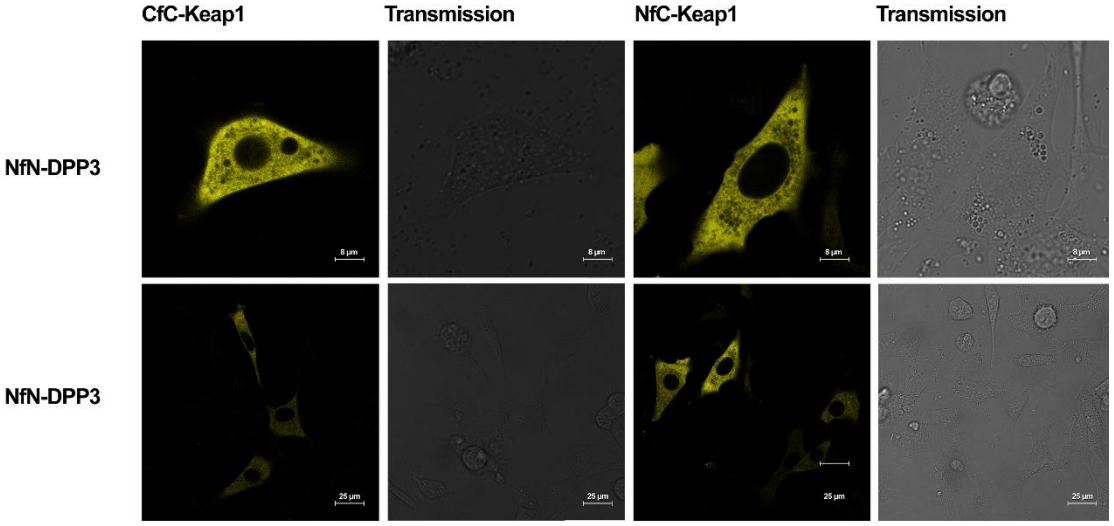


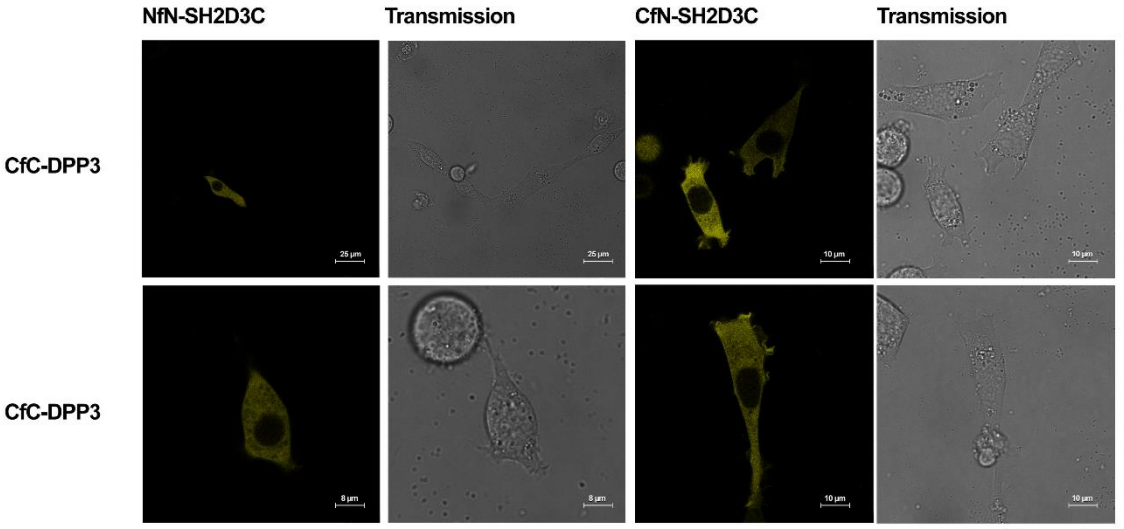


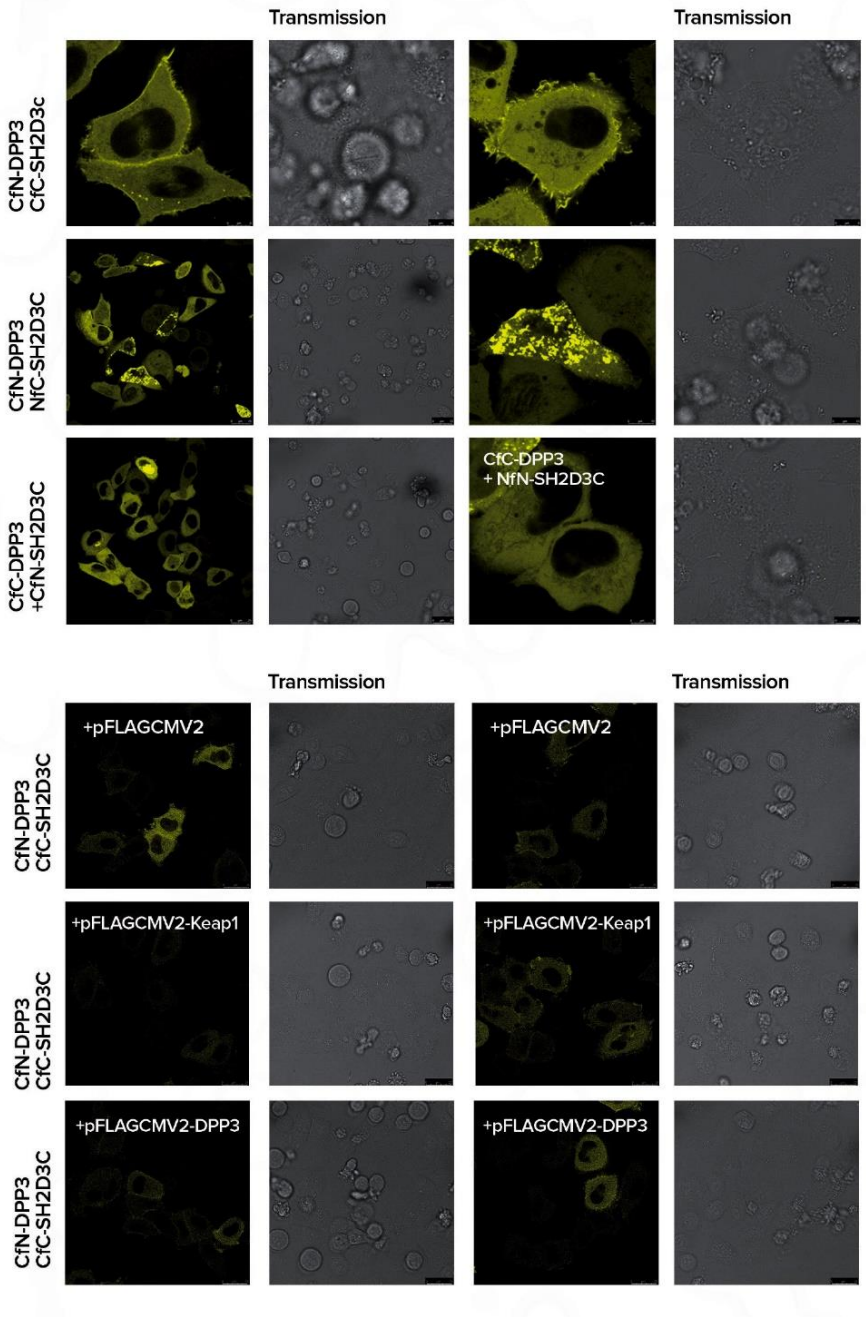
Live-cell istraživanja komplementacije dviju molekula fluorescencijom primjenom SP8 X FLIM











BiFC interakcijska analiza

(kompetitivni eksperiment)

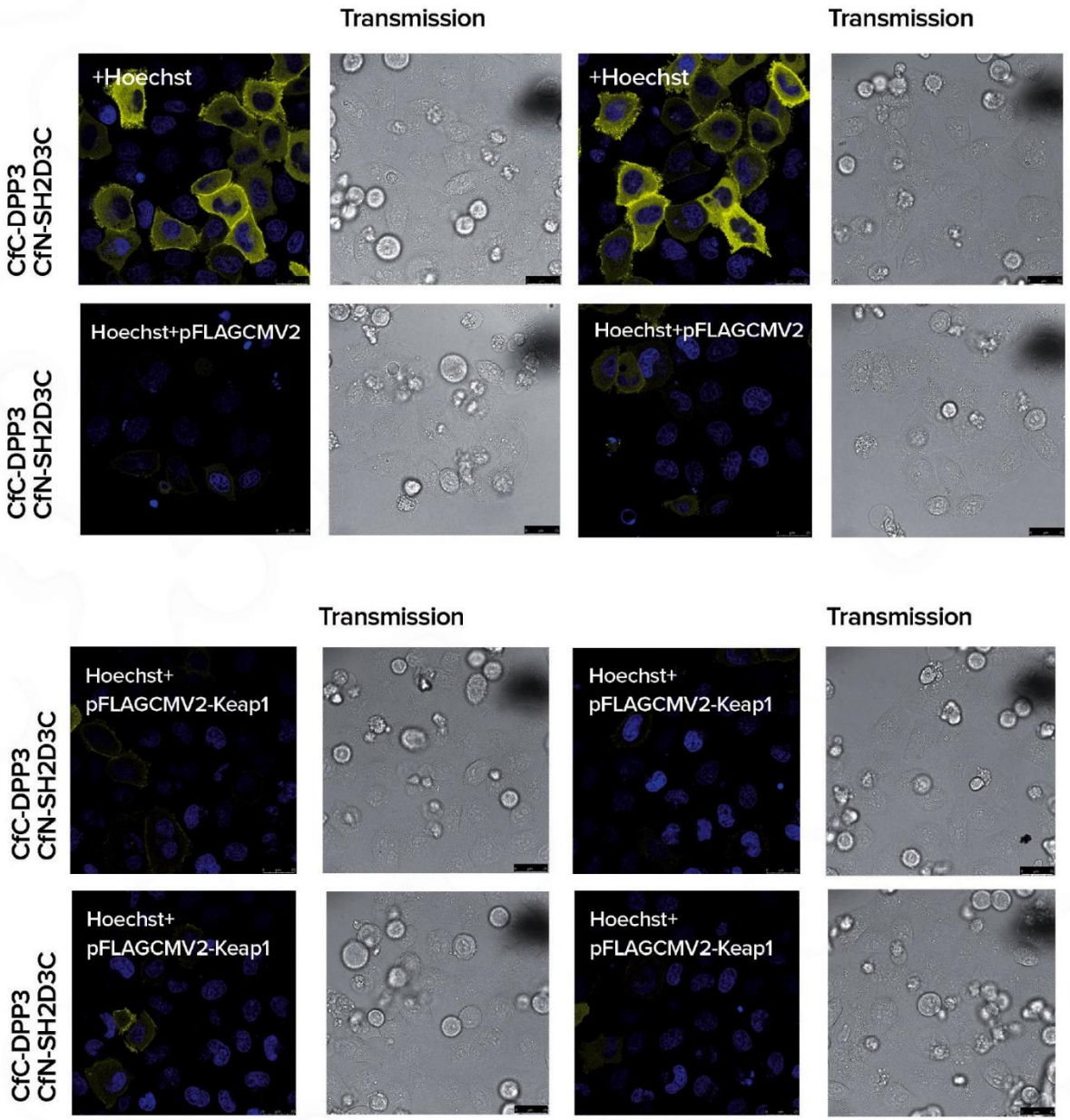
HeLa stanice

Suradnici:

Lucija Horvat

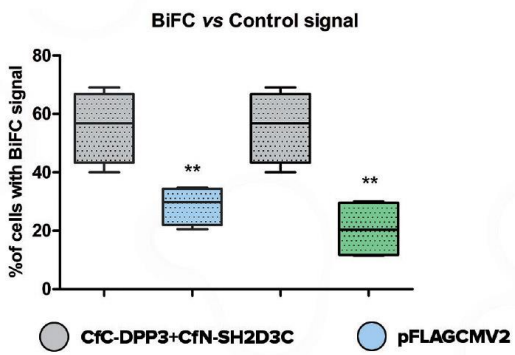
Adriana Lepur

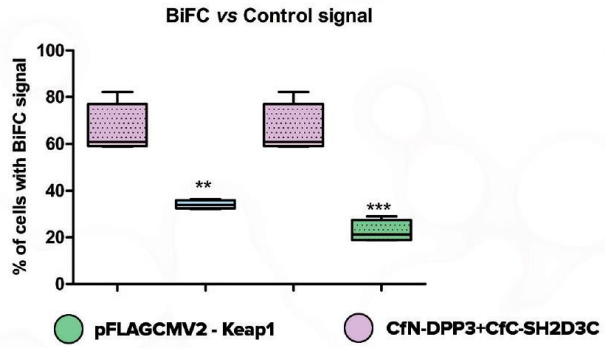
Katja Ester



BiFC screen

(kompeticijski eksperiment)





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6–9 Sep 2022, Florence, Italy

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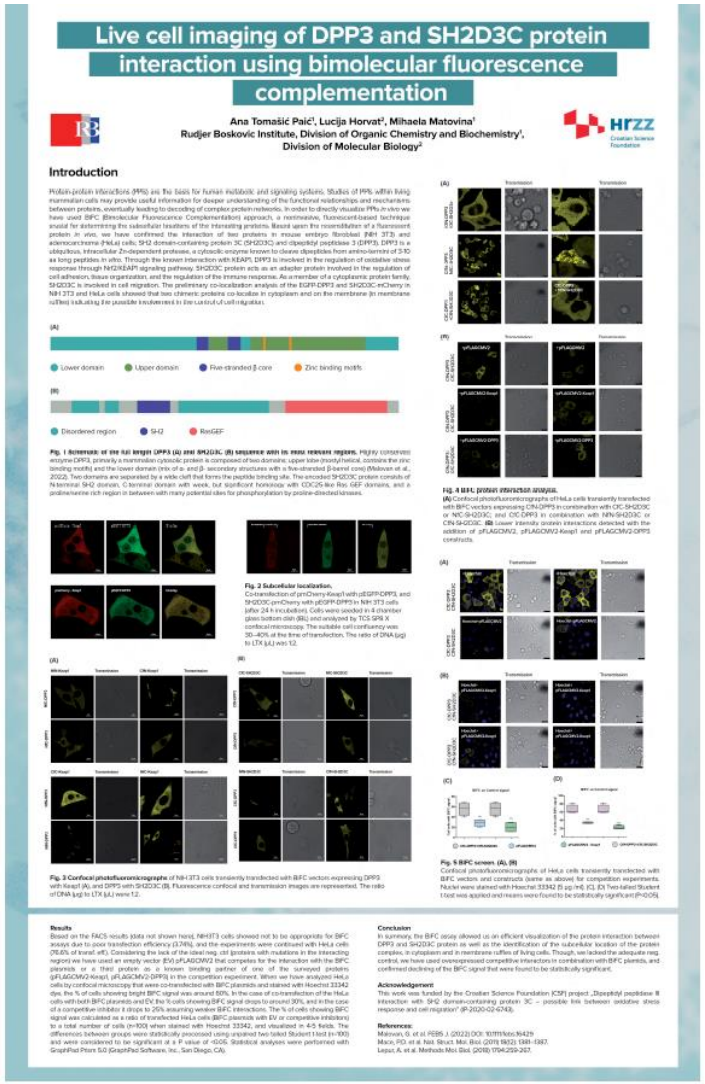

Projekt Mihaele Matovine: OxMiLink

Hvala na pažnji