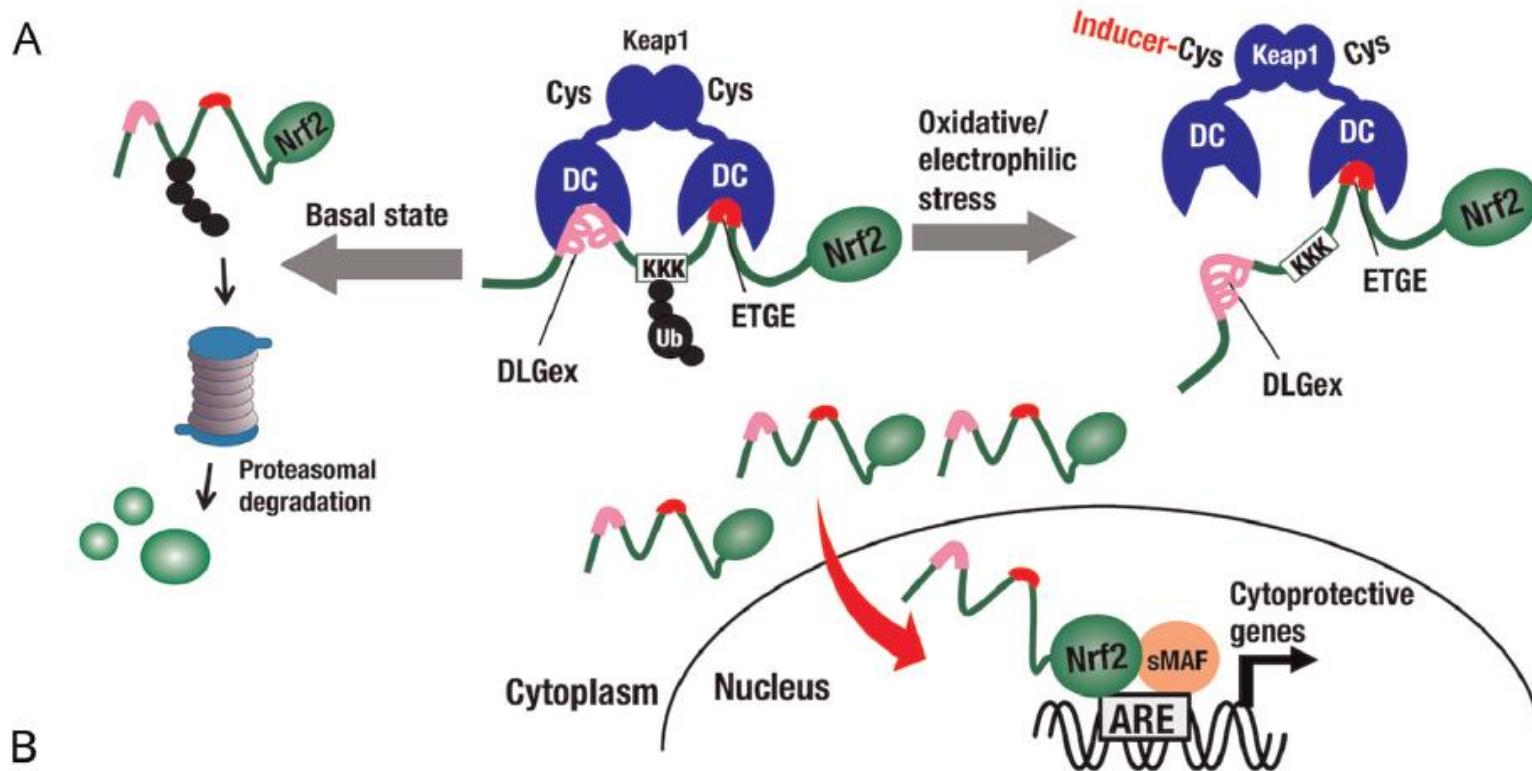


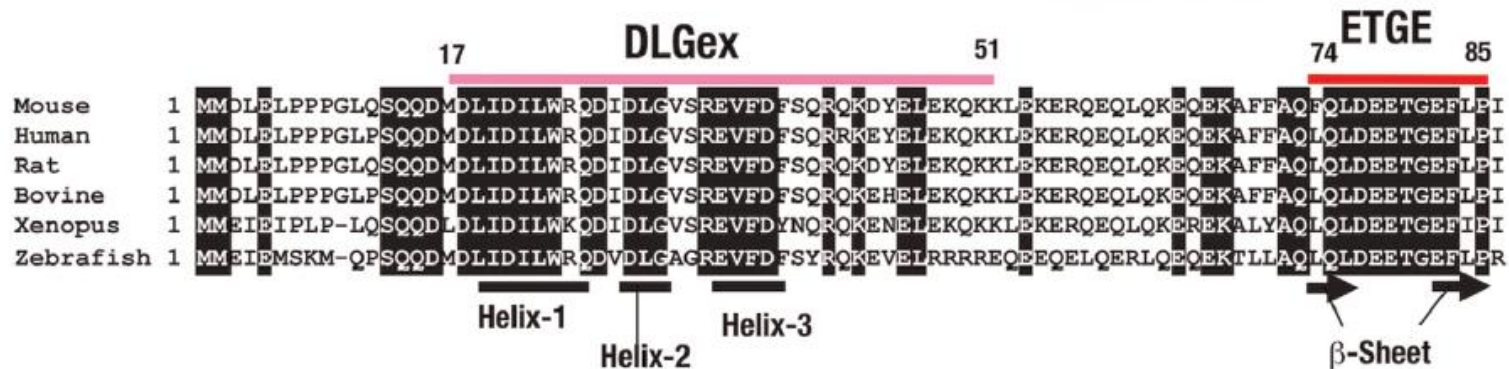
Utjecaj točkastih mutacija na međudjelovanje ljudske DPP III i Keap1 proteina

Saša Kazazić dr. sc. i Sara Matić, mag. mol. biol.



Nrf2

B



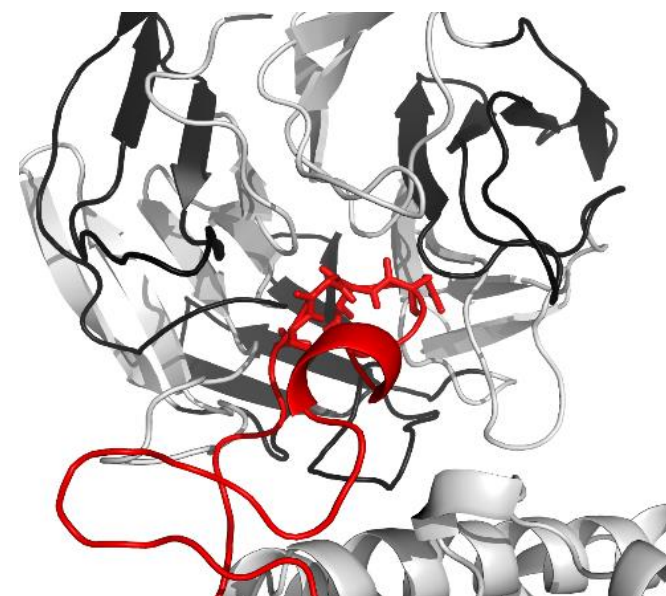
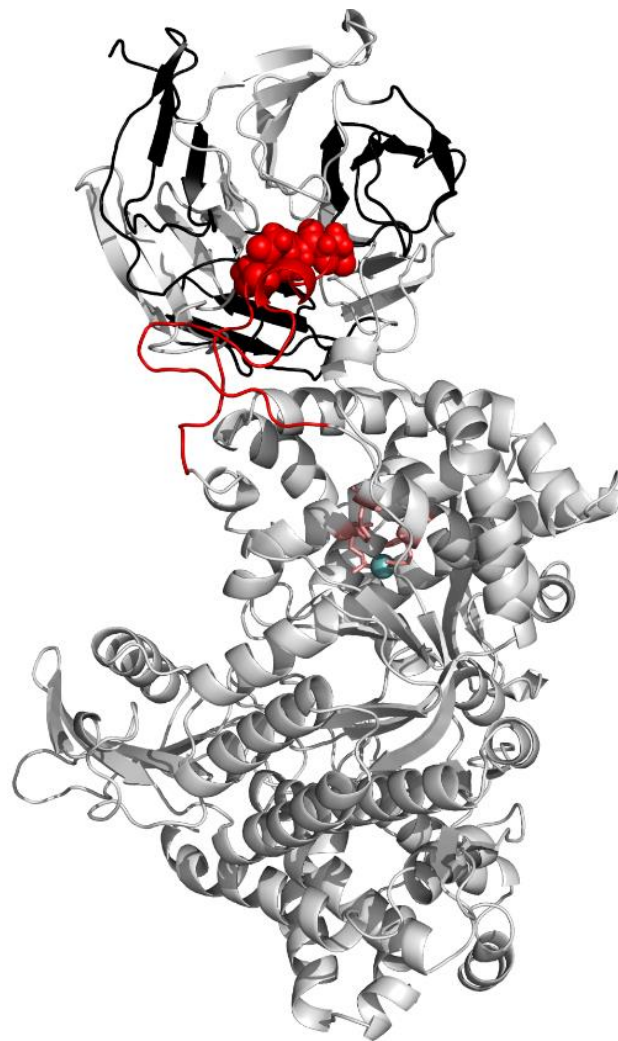
hDPP III

450 460 470 480 490 500 510 520

PSFDVQVGLHELLGHGSGKLFVQDEKGFNFDQETVINP**ETGE**QIQSWYRSGETWDSKFSTIASSYEECRAESVGLYLCL

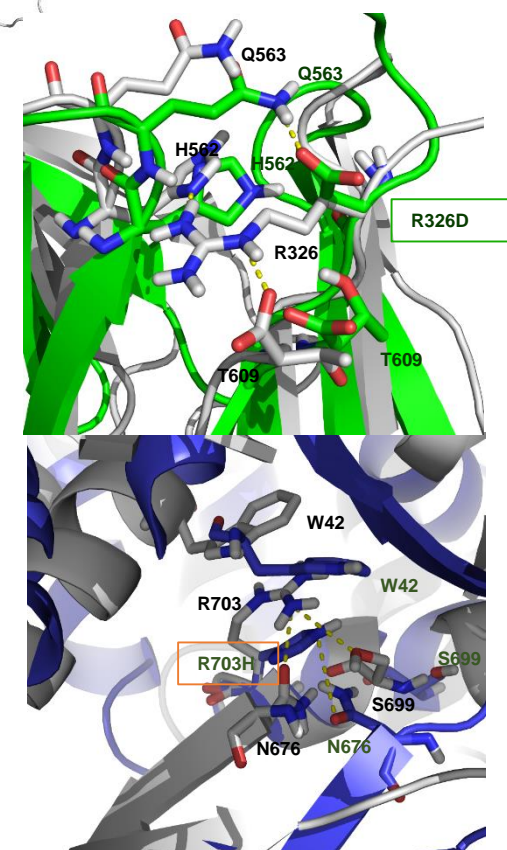
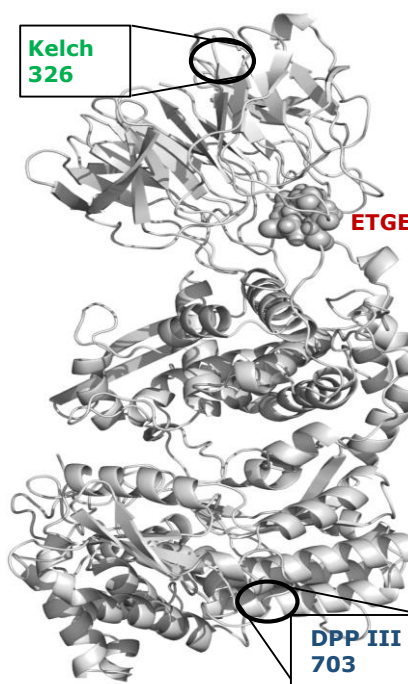
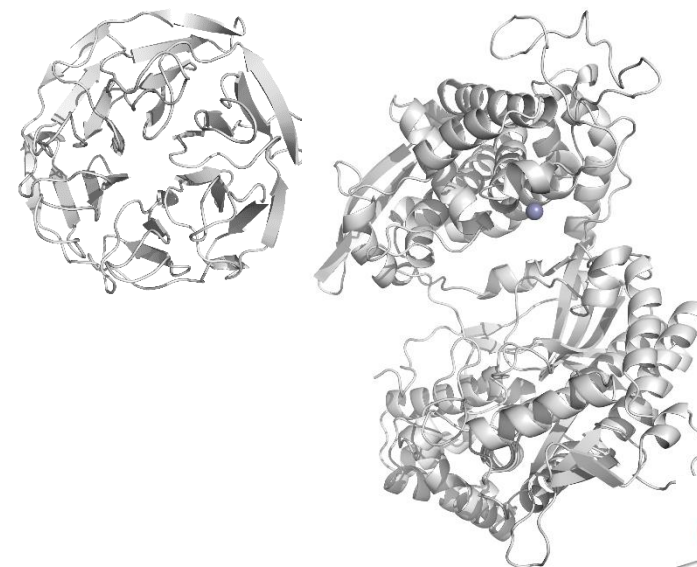
hDPP III

450 460 470 480 490 500 510 520
PSFDVQVGLHELLGHGSGKLFVQDEKGA FNFDQETVINPETGEQIQSWYRSGETWDSKFSTIASSYEECRAESVGLYLCL



- Eksperiment HD izmjene i MD simulacije
- Usporedba divljeg tipa i mutanata DPP III i Kelch domene Keap 1 proteina

- **Apo** : wt DPP III,
wt Klech,
mutanti DPP III R703H i
Kelch R326D
- **Kompleksi** : wt DPP III - wt Klech,
mutanti DPP III R703H - wt Klech, (DPP III R703C - wt Klech)
wt DPP III - Kelch R326D



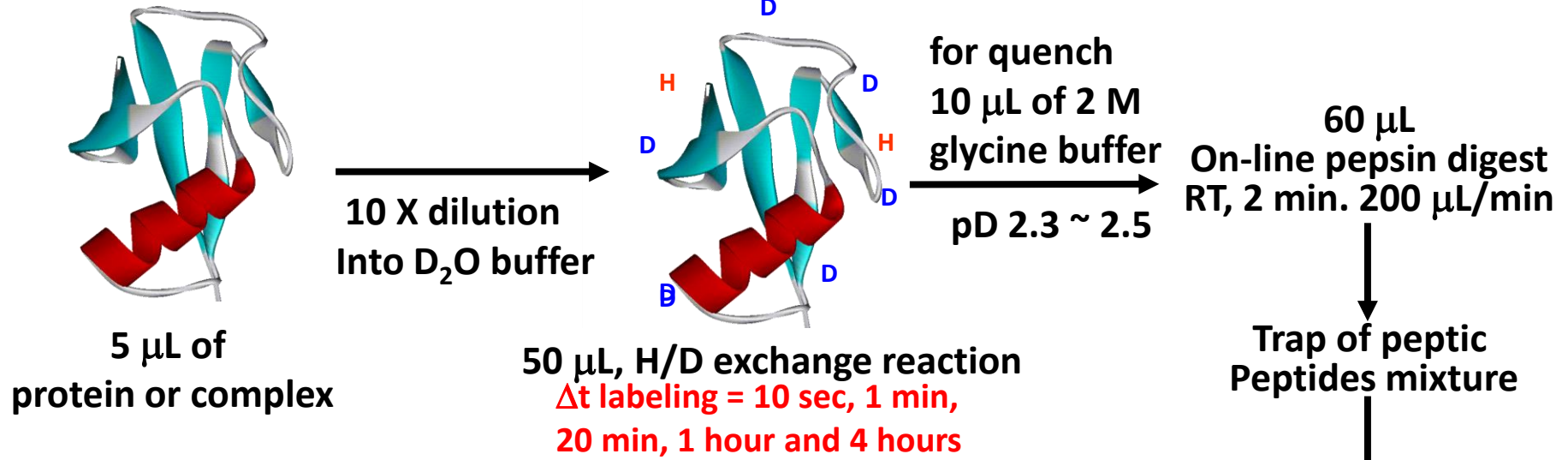
**HDX analiza mjesta interakcije u kompleksu humane
DPP III s Kelch domenom humanog KEAP1 proteina**

- 1) human DPP III wild type + Kelch wild type
- 2) human DPP III wild type + Kelch R27D mutant
- 3) Human DPP III R703 mutant + Kelch wild type

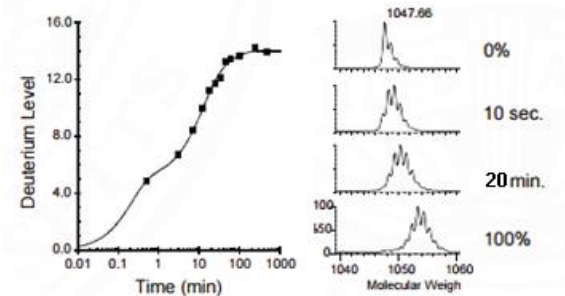
Microscale thermophoresis (MST)
je prosao bez jasnih rezultata za Kd.

		μM	μL
1	Kelch WT	60	130
2	hDPPIII WT	40	130
3	hDPPIII WT + Kelch WT	56	130
4	hDPPIII WT + Kelch R27D	40	130
5	hDPPIII R703H + Kelch WT	38.5	130

Continuous labeling experiment



Electrospray MS



UPLC time 10 min.
8-40% ACN in 8 min. pH 2,5
Temp $\sim 0.5^\circ\text{C}$, flow rate 20 $\mu\text{L}/\text{min}$.



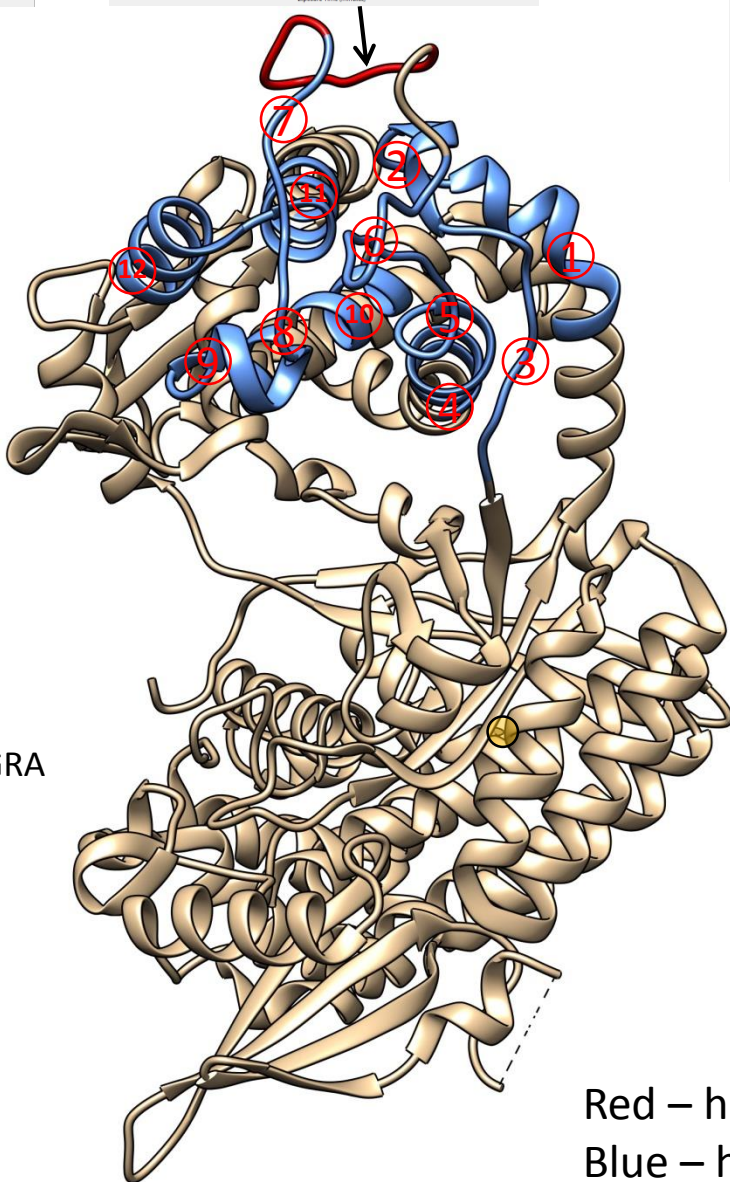
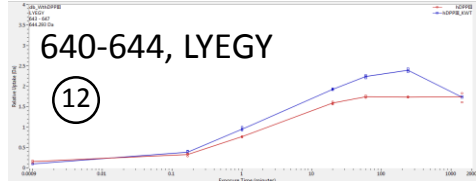
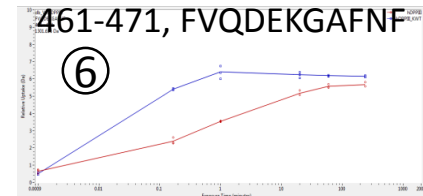
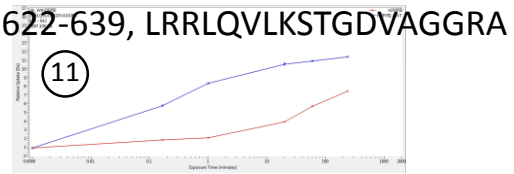
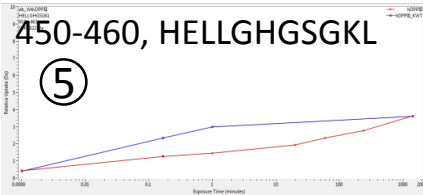
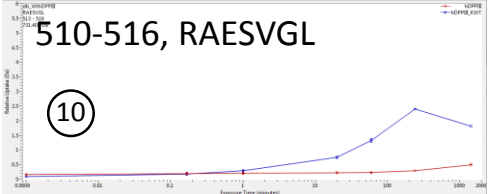
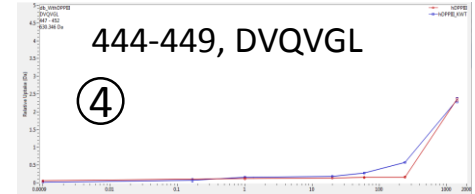
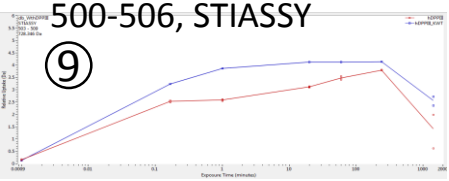
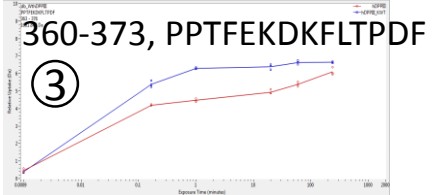
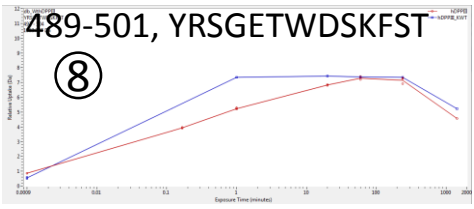
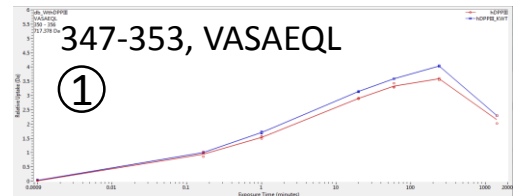
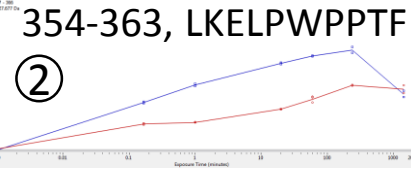
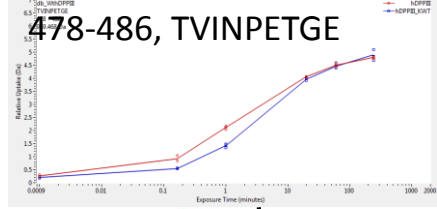
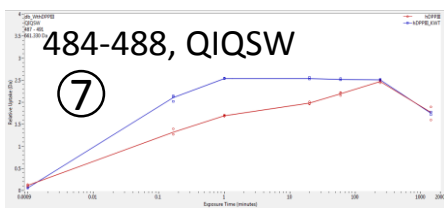
Human DPP III sequence coverage 96.0%



Kelch WT sequence coverage 98.7%

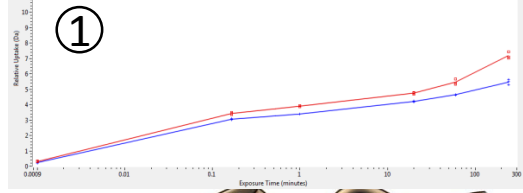


Total: 170 Peptides, 98.7% Coverage, 10.00 Redundancy

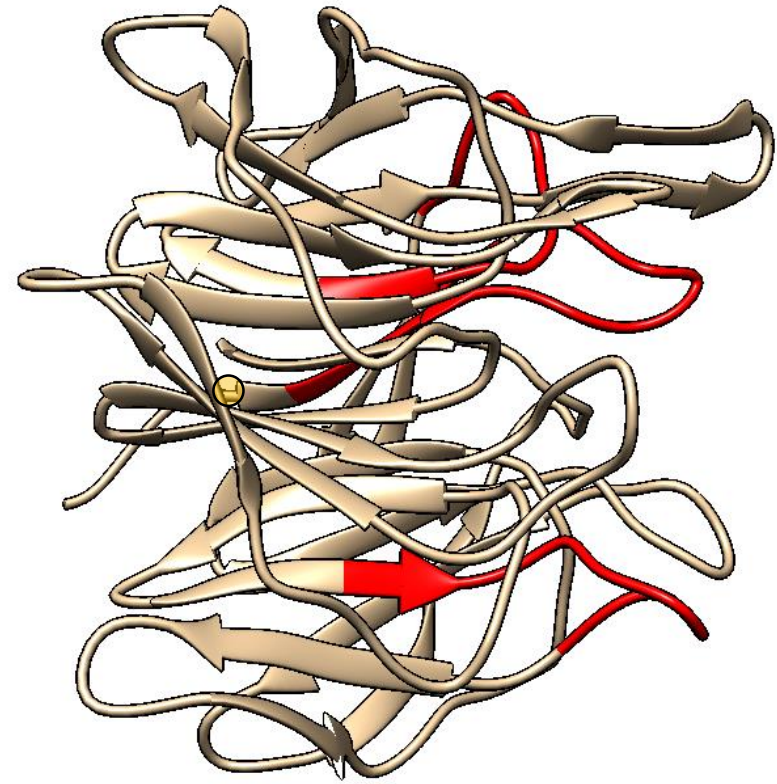
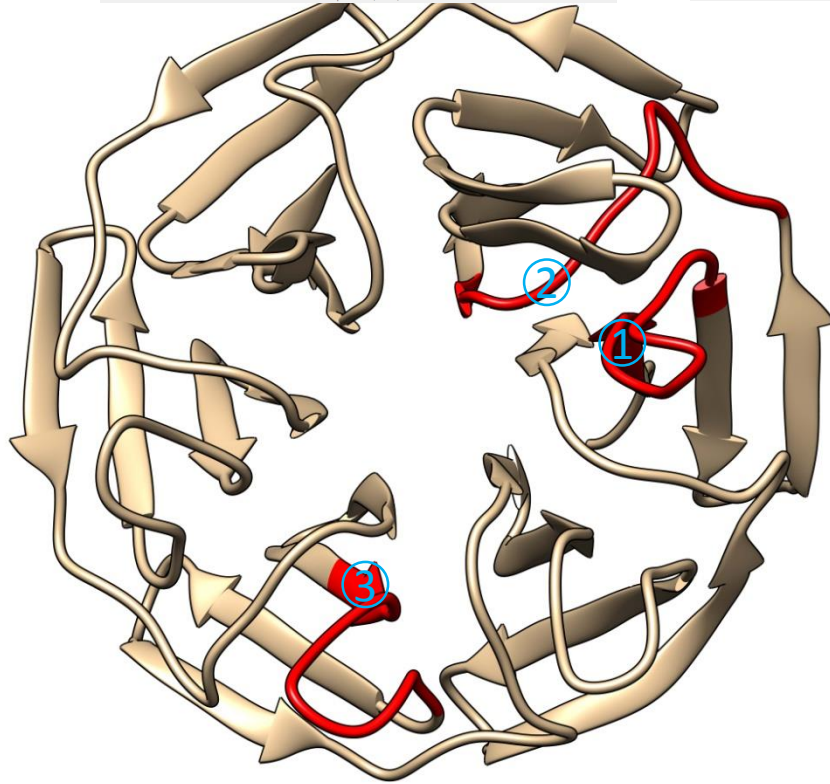
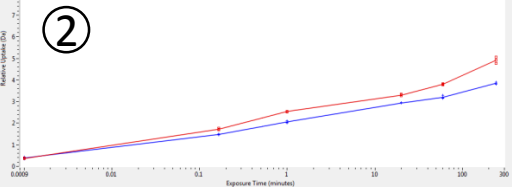


Red – hDPPIII WT free
Blue – hDPPIII WT Kelch WT complex

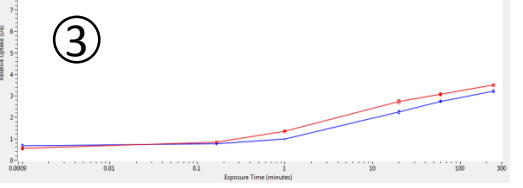
29-43 (328-342), IYTAGGYFRQSLSYL



57-68 (356-367), ADLQVPRSLAGL

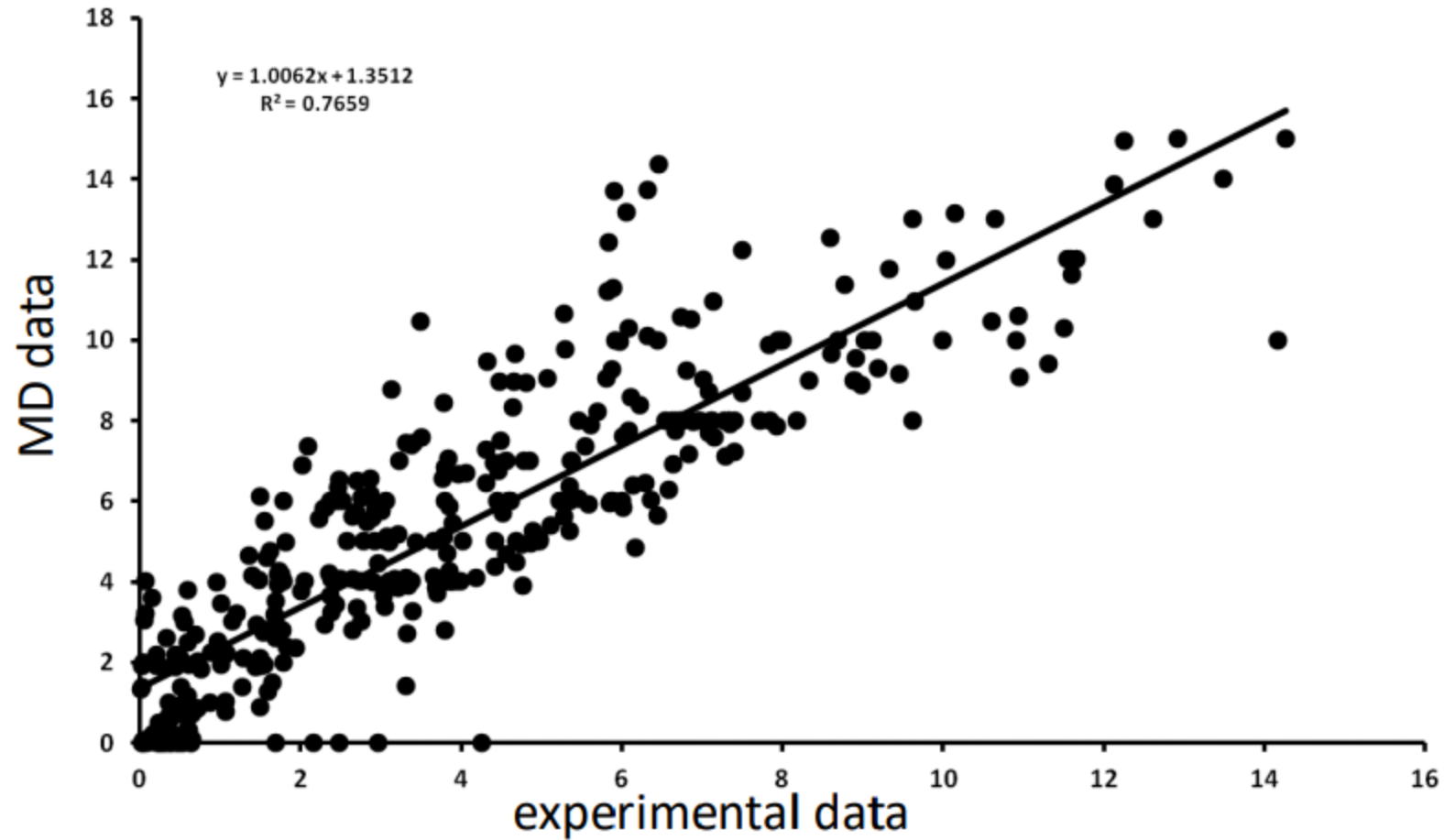


222-232(521-531), AAGGYDGGDQL



Red – Kelch WT free
Blue – hDPPIII WT Kelch WT complex

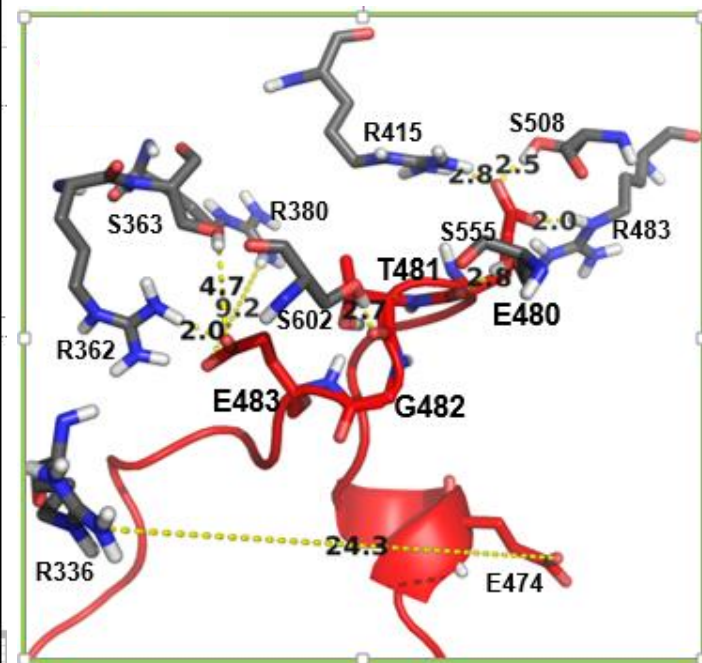
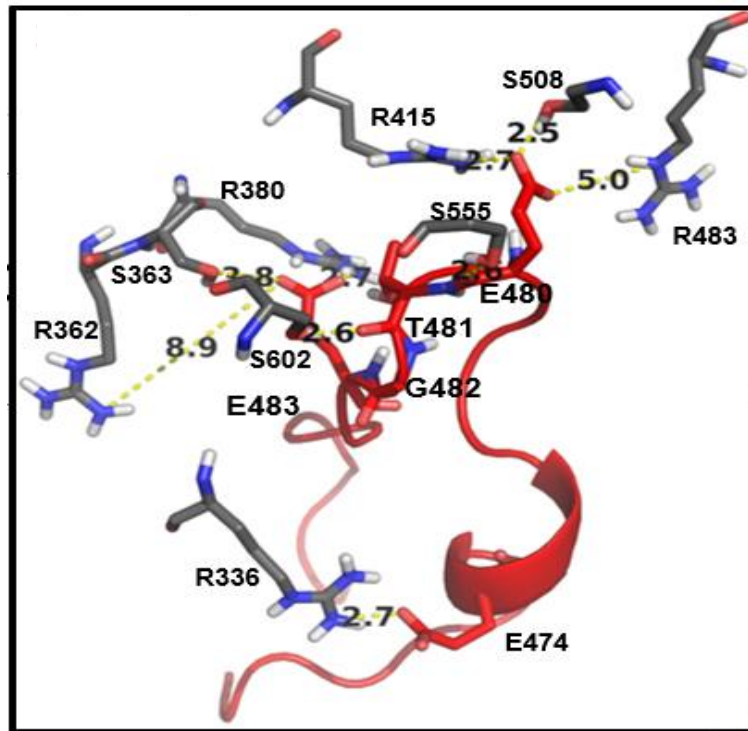
Deuterium uptake, correlation between experimental and data derived from MD simulations.

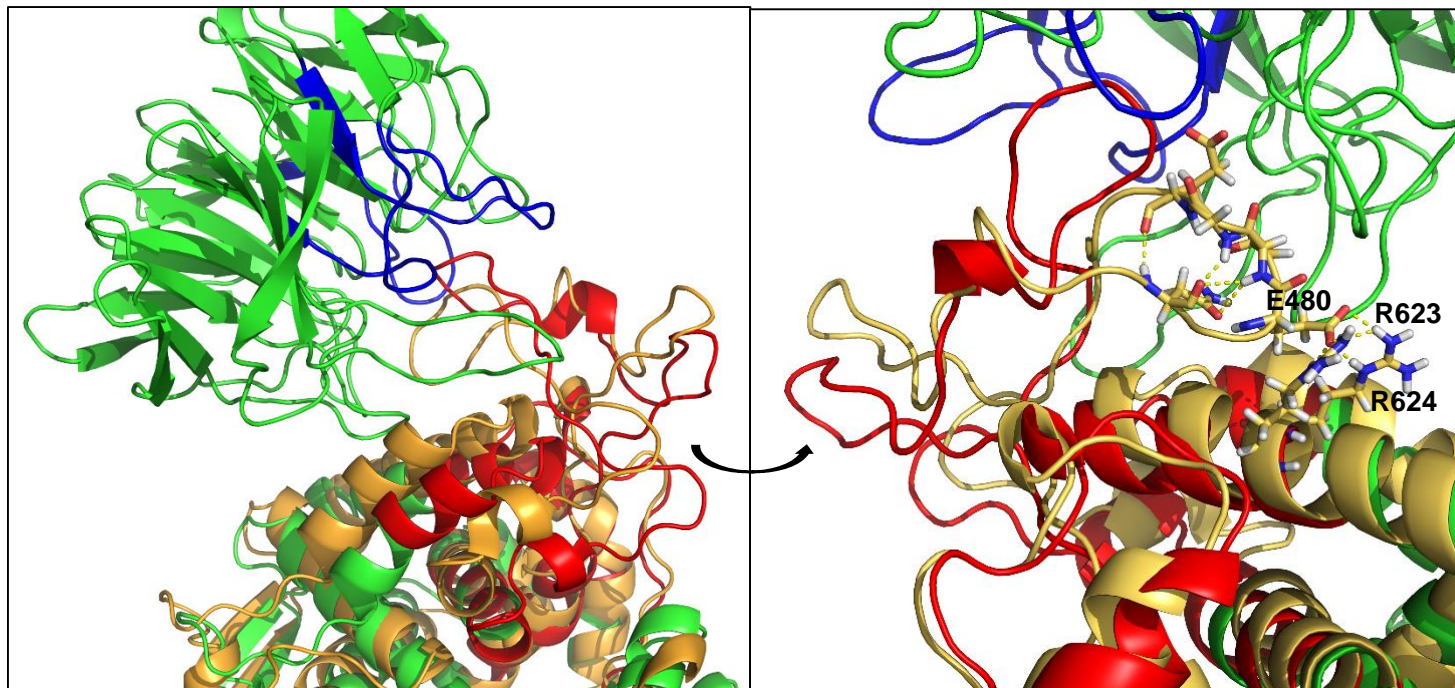
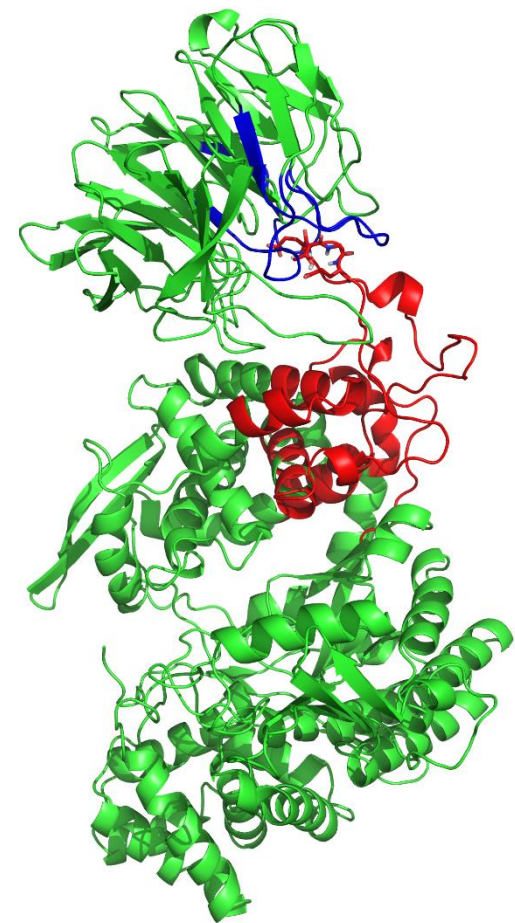


MD simulacije

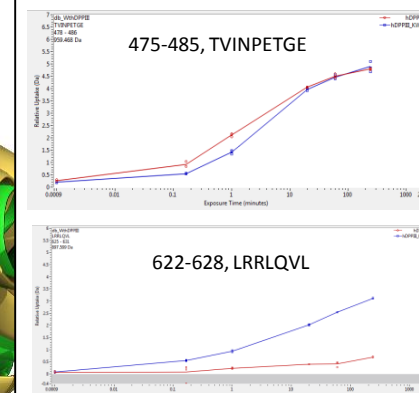
- Apo strukture 300 ns x 1 /Kompleksi 300 ns x 2
- Amber16,
- polje sila ff14SB, parametri za Zn
- Koordinacija Zn
- Analiza interakcija, H-veza ETGE petlje, H-veza okosnice proteina, RMSF, RMSD...

H - veze		wt kompleks	DPP III	DPP III	Kelch
Akceptor (ETGE petlja)	Donor (Kelch)		R703H	R703C	R326D
		Zastupljenost			
GLU 474	ARG 336	0,23	0,24	0,21	0
GLU 480	ARG 415	0,47	0,33	0,35	0,34
GLU 480	ARG 483	0,28	0,42	0,41	0,46
GLU 480	SER 508	0,56	0,59	0,47	0,59
GLU 480	SER 555	0,66	0,50	0,40	0,48
THR 481	SER 602	0,86	0,51	0,30	0,76
GLU 483	ARG 362	0,27	0,47	0,36	0,38
GLU 483	SER 363	0,30	0	0,11	0,17
GLU 483	ARG 380	0,19	0,18	0,42	0,05

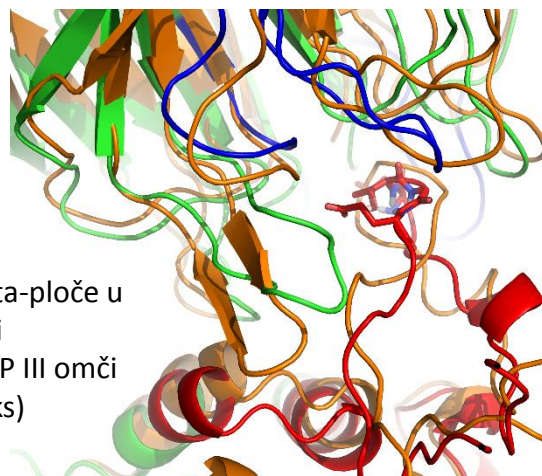




Odmicanje ETGE petlje pri vezanju na Kelch => mala razlika u HD izmjeni u 475-485, TVINPETGE peptidu apo DPP III i kompleksa



Formiranje beta-ploče u Kelch domeni i zavojnice u DPP III omći (apo i kompleks)



- Otvaranje ETGE petlje prilikom stvaranja kompleksa
-manji broj H-veza i povećana HD izmjena

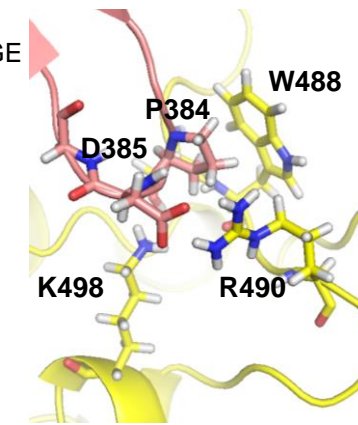
Peptid	wt DPP III	wt kompleks
347-353, VASAEQL	0,310	0,302
354-363, LKELWPPTF	0,192	0,179
360-373, PPTFEKDKFLTPDF	0,096	0,098
444-449, DVQVGL	0,296	0,266
450-460, HELLGHGSGKL	0,379	0,355
452-459, LLGHGSGK	0,363	0,345
454-460, GHGSGKL	0,345	0,331
461-471, FVQDEKGAFNF	0,425	0,355
470-474, NFDQE	0,257	0,111
475-483, TVINPETGE	0,359	0,236
484-488, QIQSW	0,056	0,172
489-501, YRSGETWDSKFST	0,209	0,206
500-506, STIASSY	0,258	0,282
510-516, RAESVGL	0,494	0,444
622-628, LRRQLVL	0,494	0,483
622-639, LRRQLVLKSTGDVAGGRA	0,417	0,411
629-640, KSTGDVAGGRAL	0,373	0,366
640-644, LYEGY	0,325	0,329
Ukupni prosjek	0,311012	0,296



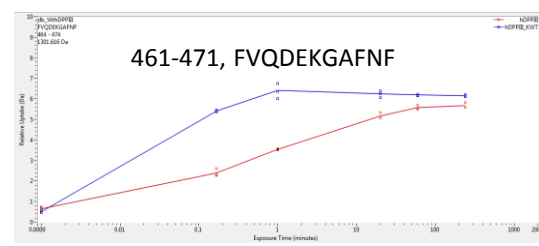
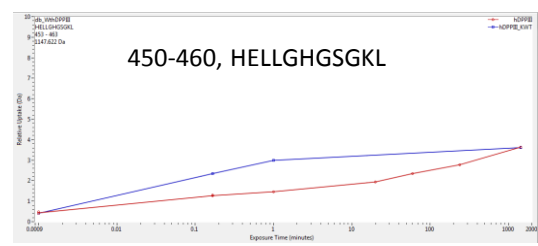
E364*-R336, nastanak zavojnice

QIQSW* u neposrednoj blizini ETGE
W488, s R490 i K498 iz
YRSGETWDSKFST – interakcija s
PD na vrhu petlje (beta ploče)
Kelcha

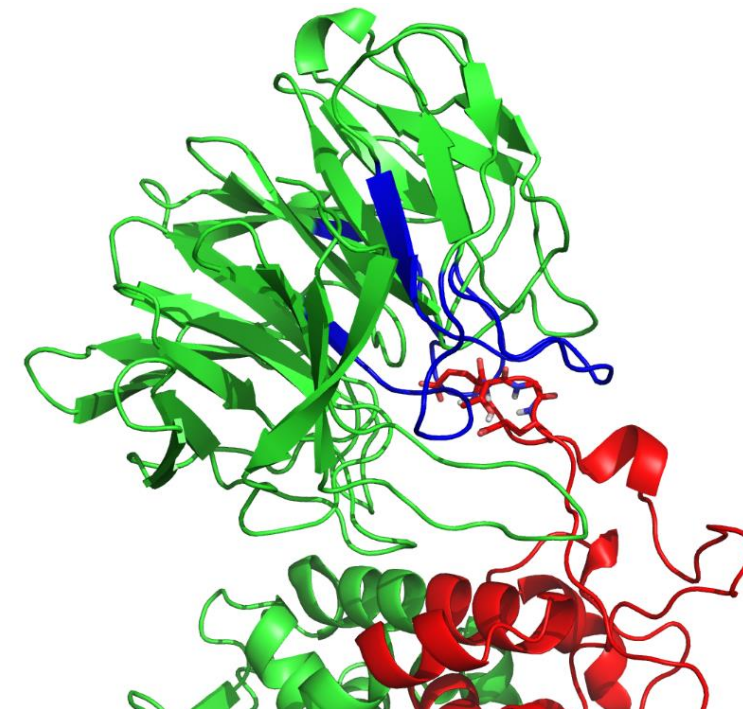
500-506, STIASSY*,
dio zavojnice u blizini
507-512, EECRAE
motiva aktivnog
mjesta



• R703H se ne razlikuje značajno od wt
(potvrđeno HD izmjenom)



- Stabilizacija Kelch peptida u kompleksu
 - veći broj H-veza, smanjena SASA i smanjena HD izmjena
- Razmicanje Kelch petlji prilikom stvaranja kompleksa
 - manji broj H-veza i povećana SASA

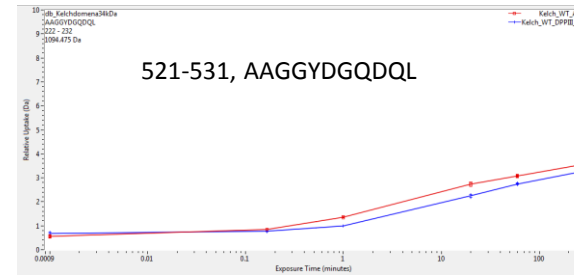
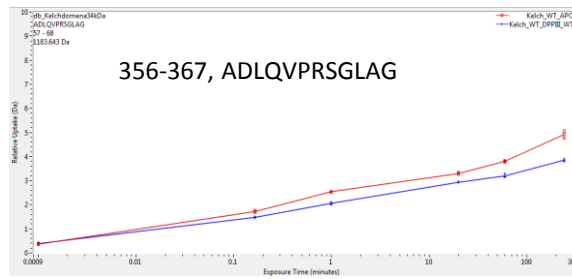
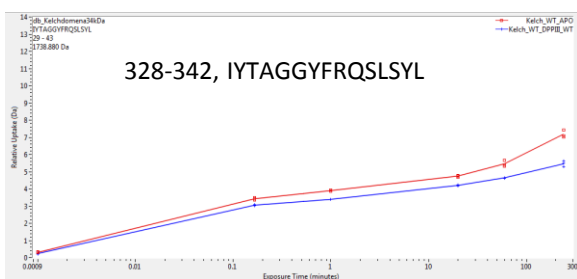


H-veze

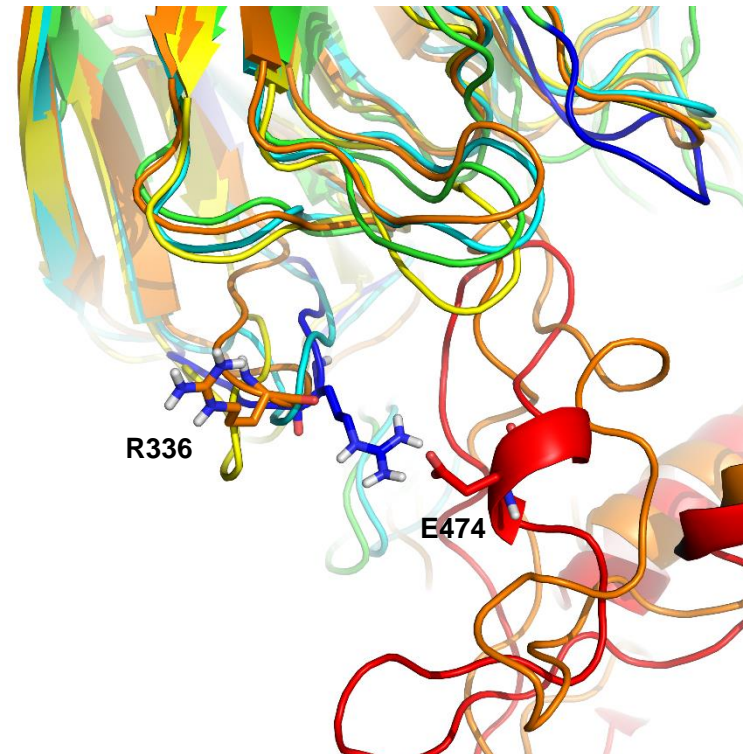
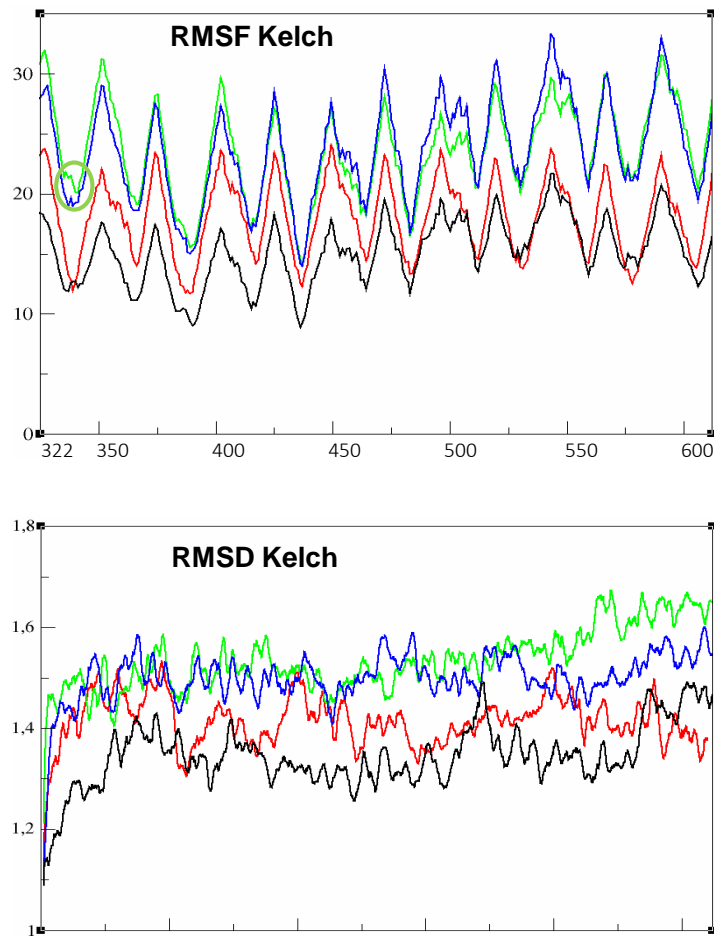
Peptid	wt Kelch	wt kompleks	Kelch R326D	kompleks wt DPP III- Kelch R326D
29-35 (328-334), IYTAGGY	0,453	0,594	0,376	0,499
29-43 (328-342), IYTAGGYFRQSLSYL	0,399	0,336	0,381	0,373
57-68 (356-367), ADLQVPRSLAG	0,225	0,192	0,115	0,176
222-232(521-531), AAGGYDGQDQL	0,601	0,581	0,597	0,566

SASA

Peptid	wt Kelch	wt kompleks	Kelch R326D	kompleks wt DPP III- Kelch R326D
29-35 (328-334), IYTAGGY	20,9	13,025	46,1	17,125
29-43 (328-342), IYTAGGYFRQSLSYL	130,2	163,95	185	147,5
57-68 (356-367), ADLQVPRSLAG	140,9	187,525	213,3	237,8
222-232(521-531), AAGGYDGQDQL	138,6	146,6	154,6	129



Moguć utjecaj mutacije Kelch R326D na destabilizaciju petlje u kojoj se nalazi R336, te na njegovu interakciju s E474 DPP III?



Kratki sažetak

- hDPP III u kompleksu ostvaruje interakcije ETGE motivom s Kelch domenom, kao i Nrf2
- Fleksibilnost pri stvaranju vodikovih veza u skladu s afinitetom prema ETGE i DLG
- Mogućnost interakcija drugim ostacima ETGE petlje i stvaranja sekundarnih struktura u hDPP III i Kelch domeni
- Smanjena zastupljenost vodikovih veza okosnice DPP III u skladu s povećanom HD izmjenom potvrđuje otvaranje ETGE petlje prilikom vezanja
- Smanjena zastupljenost vodikovih veza okosnice Kelch-a i povećana SASA nisu u skladu sa smanjenom HD izmjenom već sugeriraju otvaranje petlji veznog mjesta Kelch-a
- Mutanti DPP III na položaju R703 nisu pokazali značajan učinak na kompleks DPP III – Kelch
- Mutant Kelch R326D potencijalno pokazuje utjecaj na stvaranje kompleksa preko destabilizacije petlje u kojoj se nalazi R336