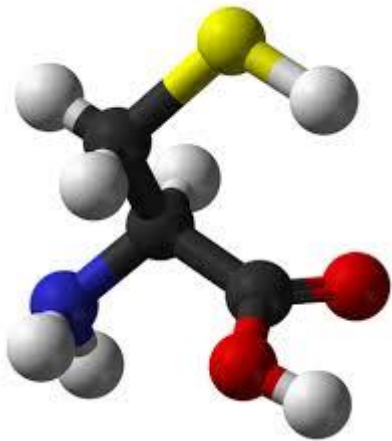


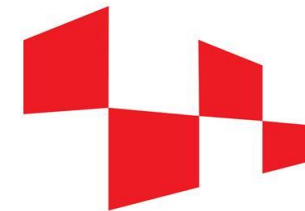
Uloga cisteinskih ostataka u peptidaznoj aktivnosti DPP III iz mahovine



DPP III mikro simpozij , 27.10.2017.

Zrinka Karačić

eksperimentalni rad: Ana Tomašić Paić

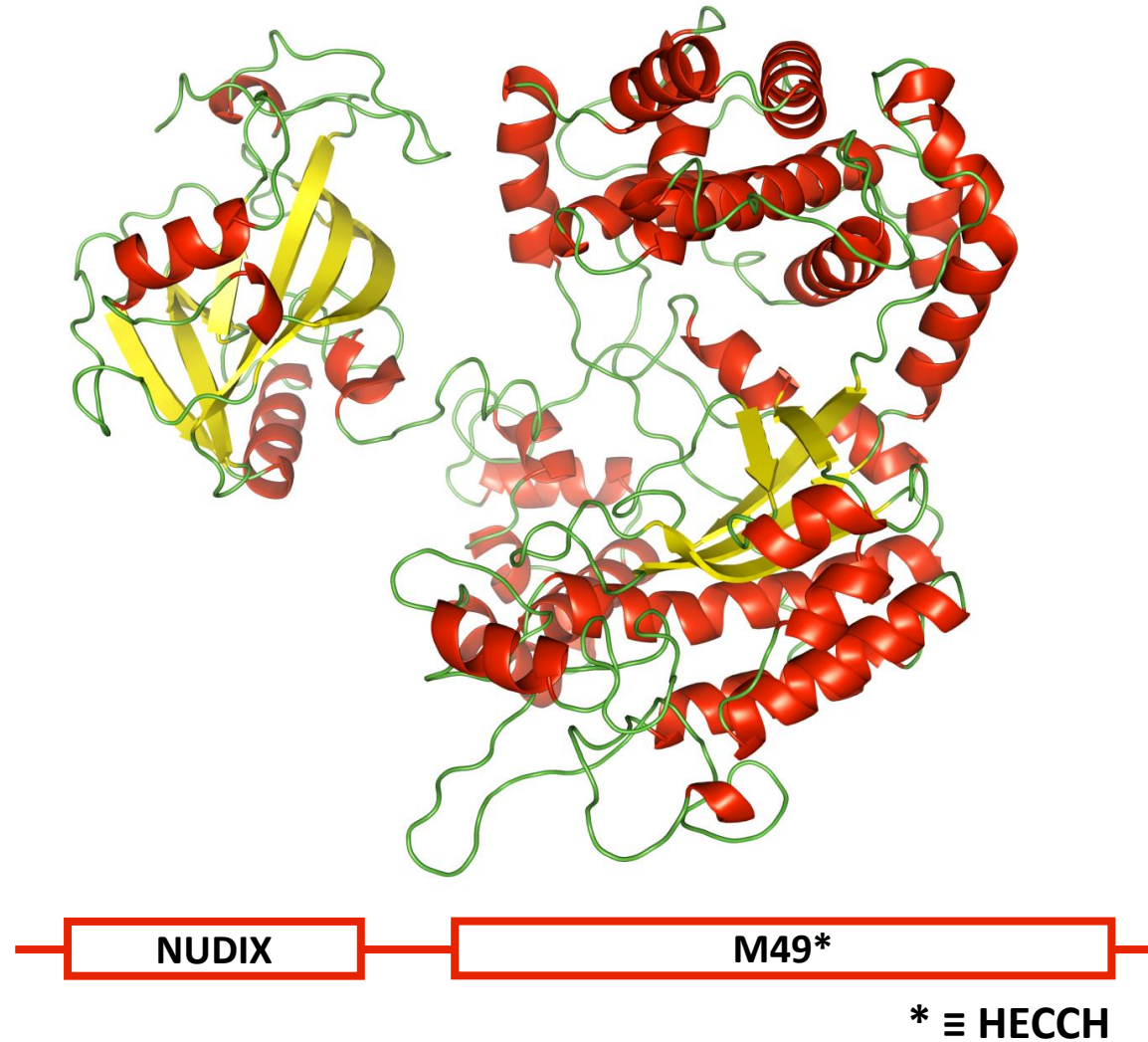


HRZZ

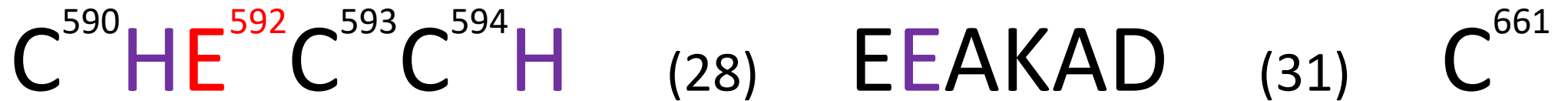
Hrvatska zaklada
za znanost

Biljne DPP III

- fosfataza i peptidaza u istom proteinu
- dva aktivna mjesta: Nudix i DPP III



Očuvani Cys u biljnim DPP III



A9TLP4_PHYPA	590	CHECC-H	595	<i>Physcomitrella patens</i>
H1XW48_9BACT	378	MHEIS-H	383	<i>Caldithrix abyssi</i>
Q8A6N1_BACTN	447	LHECLGH	453	<i>Bacteroides thetaiotaomicron</i>
Q7MX92_PORGI	431	LHECLGH	437	<i>Porphyromonas gingivalis</i>
DPP3_YEAST	459	IHELLGH	465	<i>Saccharomyces cerevisiae</i>
DPP3_DICDI	430	IHELYGH	436	<i>Dictyostelium discoideum</i>
DPP3_DROME	506	LHELLGH	512	<i>Drosophila melanogaster</i>
DPP3_RAT	449	LHELLGH	455	<i>Rattus norvegicus</i>
DPP3_HUMAN	449	LHELLGH	455	<i>Homo sapiens</i>
F2Z4F5_BOVIN	449	LHELLGH	455	<i>Bos taurus</i>

Uloga Cys u proteinima

- jedinstvena tiolna skupina (SH) - može se polarizirati vodikovim vezama, ali i stabilizira hidrofobne interakcije
- uloge:
 - kompleksira metale: Zn^{2+} i Fe (Fe-S klasteri)
 - kao nukleofil u aktivnom mjestu enzima
 - stabilizira strukturu disulfidnim mostovima (ne-citoplazmatski proteini)
- -SH: reaktivna skupina, ali moguće je njome manipulirati mikrookolišem
- Cys visoko očuvani u hidrofobnim regijama, ne na površini proteina

Uloga Cys u DPP III

- ne kompleksira metal
- nije nukleofil
- ne tvori disulfidne mostove?
- „osjeća” redoks stanje??

Očuvani Cys u biljnim DPP III

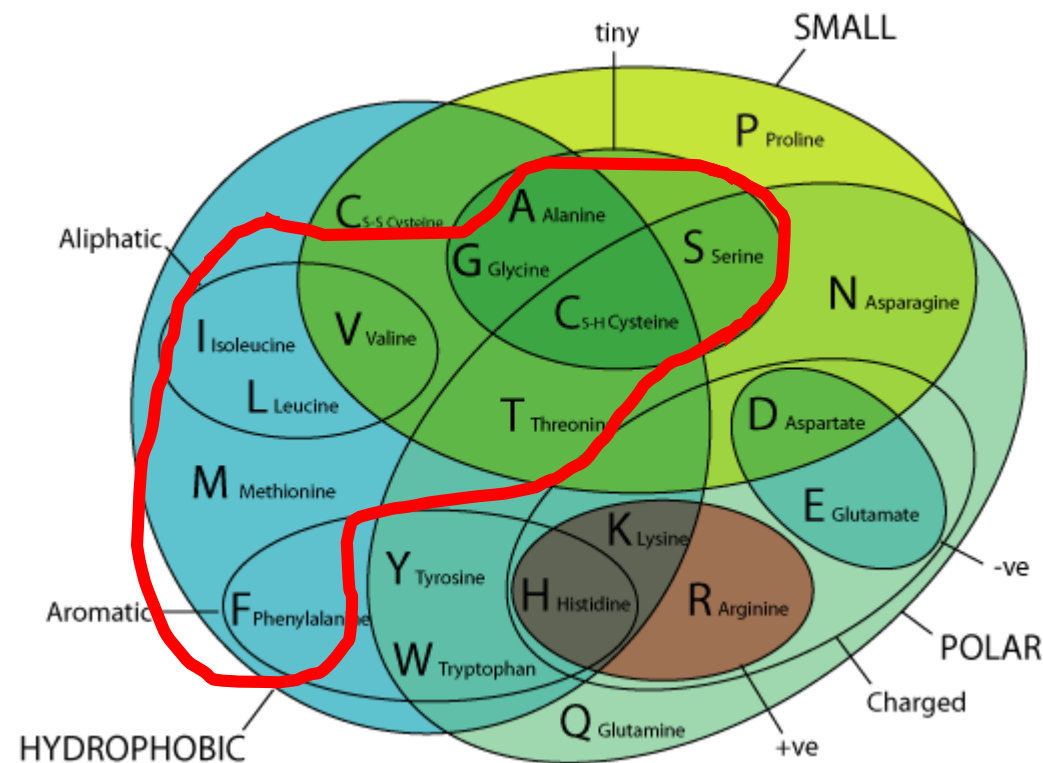
C⁵⁹⁰ H E⁵⁹² C⁵⁹³ C⁵⁹⁴ H (28) E E A K A D (31) C⁶⁶¹

A9TLP4_PHYPA	590	CHECC-H	595	<i>Physcomitrella patens</i>
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A kod *Caldithrix*-sličnih bakterijskih DPP3?

CHECCH
XHEXXH

- isključivo u jednoj sekvenci od 500 (!) samo jedan cistein u motivu
- X = A, S, L, I, V, M, T, F, G, C



Metode

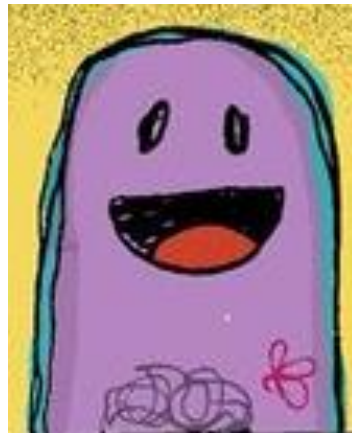
- Mutageneza

- odabrani cisteini pojedinačno zamijenjeni alaninom:

- C590A
- C593A
- C594A
- C661A

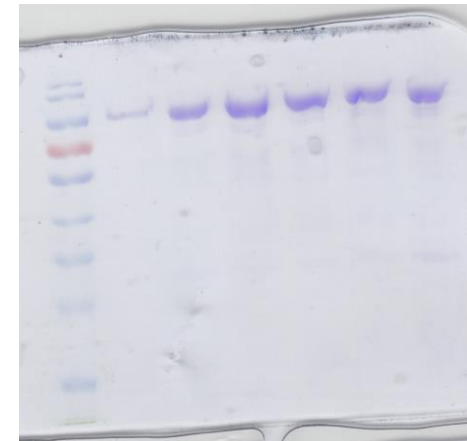
- Ekspresija

- u 0,5 L kulture
- preko noći na 18°C



- Pročišćavanje

- na Ni-NTA koloni
- pročišćeno 2-5 mg proteina

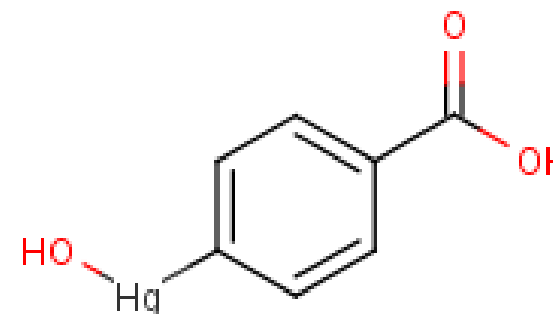


Specifična aktivnost uz Arg₂-2NA

mg/mL	enzim	μL za t.a.	ΔA ₅₃₀	Specifična / min ⁻¹ mg ⁻¹
1,079	wt	5	0.300	3.7
0,985	C590A	30	0.210	0.47
0,814	C593A	15	0.240	1.31
0,720	C594A	2	0.300	13.8
0,269	C661A	20	0.290	3.6

Utjecaj pHMB (c = 0,5 μM)

enzim	% preostale aktivnosti			
wt	41	26	49	39
C590A	84	73	66	74
C593A	95	77	92	88
C594A	41	38	53	44
C661A	33	31	40	35



- usporedba s drugim DPP III

DPP3	c(pHMB) / μM	% akt
Ca	1,0	80
Bt	1,0	20
γ	0,1	40
h	0,01	50

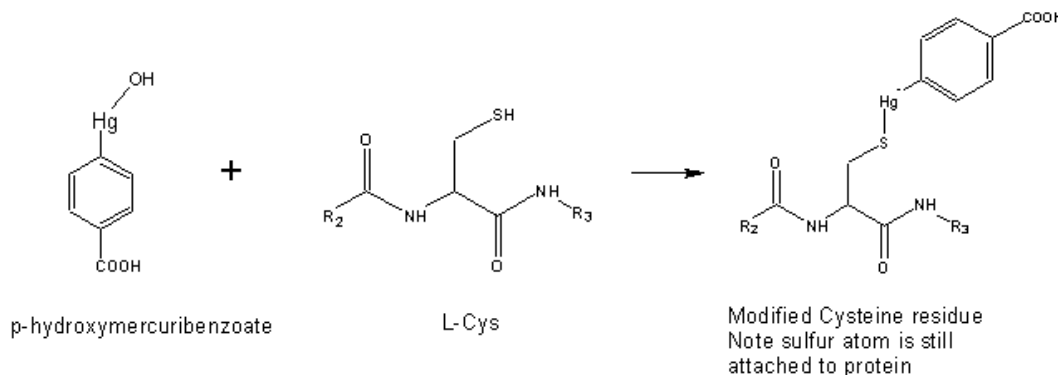
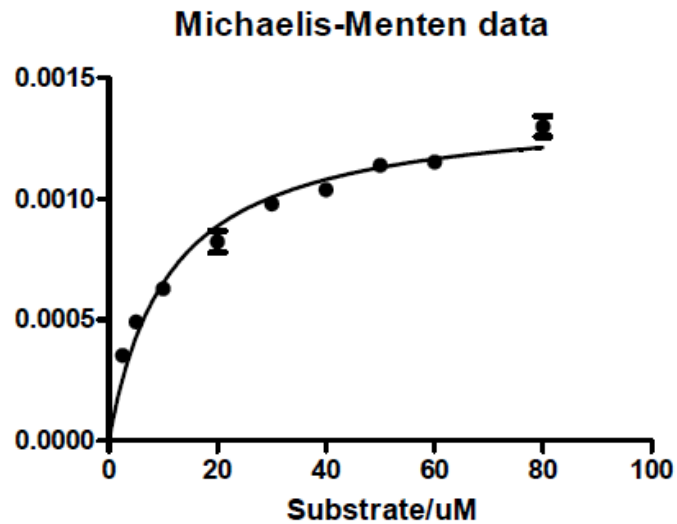


Figure: p-hydroxymercuribenzoate modification of Cys

Kinetika uz Arg₂-2NA



enzim	nM	μL	$K_M / \mu\text{M}$	$k_{\text{cat}} / \text{s}^{-1}$	$k_{\text{cat}}/K_M / \text{M}^{-1} \text{s}^{-1}$
wt	41,3	10	$11,2 \pm 1,2$	0,034	2983
C590A	113	30	$10,7 \pm 1,4$	0,00111	104
C593A	62,3	20	$8,2 \pm 1,0$	0,0074	907
C594A	27,5	10	$16,7 \pm 1,5$	0,271	16218
C661A	30,9	30	$13,2 \pm 2,2$	0,035	2660

