Utjecaj metalnih dikationa na strukturu i funkciju hDPP III

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HrZZ
Hrvatska zaklada za znanost
IP-2018-01-2936

Eksperimentalno

ICP-MS (IMI) 25mM amonijev acetat

IZMJERENO IMI:					
UZORCI hDPP3 Mw 81636	umol/L S	Zn* %	Cu* %	Mn* %	Co* %
1 nativni	39,30	4,43	0,56	0,05	1,16
2 apo-stari	19,18	2,60	0,35	0,03	1,54
3 apo	25,14	2,39	0,11	0,12	1,80
4 apo + Zn (6 mol ekv)	37,66	**95,6**	0,55	0,08	1,91
5 apo + Cu (6 mol ekv)	21,22	0,50	**196,9**	0,11	0,64
6 apo + Co (6 mol ekv)	29,14	**24,7**	0,64	0,08	**32,7**
7 apo + Mn (6 mol ekv)	29,52	**30,1**	0,85	**1,73**	1,60

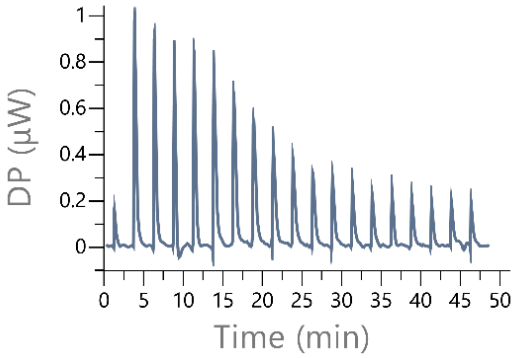
IZMJERENO IMI:				
UZORCI hDPP3 Mw 81636	Zn* %	Cu* %	Co* %	Mn* %
1 nativni	9,27	1,14	1,43	0,12
2 apo	10,05	0,80	2,17	0,10
3 apo + Zn (6 mol ekv)	117,9	2,99	0,89	0,27
4 apo + Cu (6 mol ekv)	8,05	209,50	2,41	0,14
5 apo + Co (6 mol ekv)	**48,61**	4,46	32,9	0,07
6 apo + Mn (6 mol ekv)	**34,14**	2,78	0,74	3,22

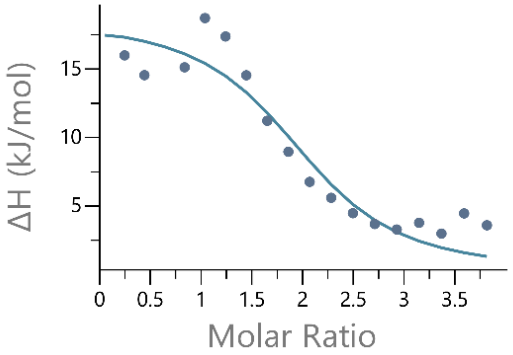
standardi	mg/L	Mn	Fe	Co	Cu	Zn	Ba	Al
Cu(NO3)2	1000	0,00	11,6	0	978	9,3	63,1	5,5
Mn(NO3)2	1000	1032,9	3,1	0	0,3	6,1	11	1
Co(NO3)2	1000	0.0	2,1	1018,3	0,4	2,6	6,6	0,9

ITC (cink)

50mM Natrijev kakodilat

apo hDPPIII titriran s metalom





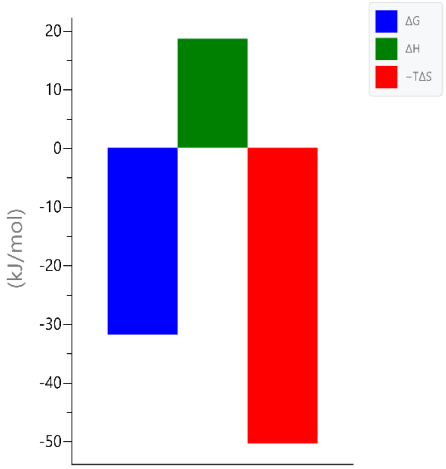
N = (2.32 ± 0.28)

Kd = (2.29 ± 0.73) · 10^{-6} mol dm^{-3}

ΔrH = (13.9 ± 0.3) kJ mol^{-1}

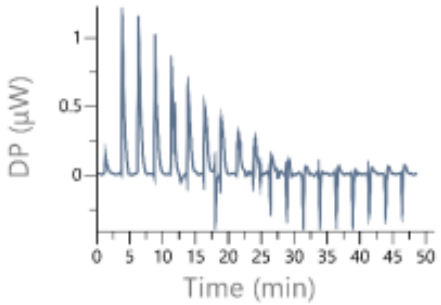
ΔrG = (-32.3 ± 0.8) kJ mol^{-1}

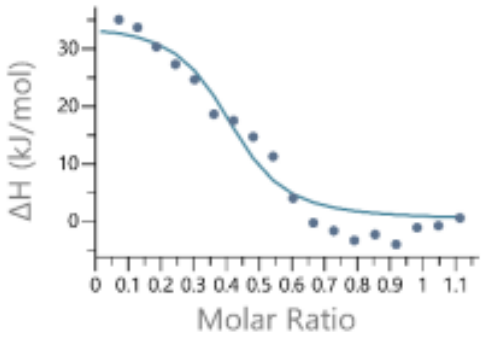
$-T$ ΔrS = (-46.2 ± 1.1) kJ mol^{-1}



reverzna

metal titriran s apo hDPPIII





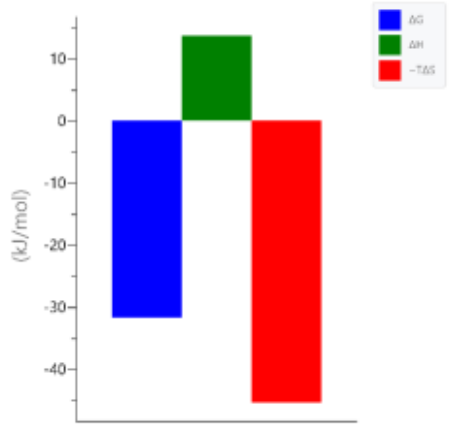
N = (2.00 ± 0.08)

Kd = (5.29 ± 2.36) · 10^{-6} mol dm^{-3}

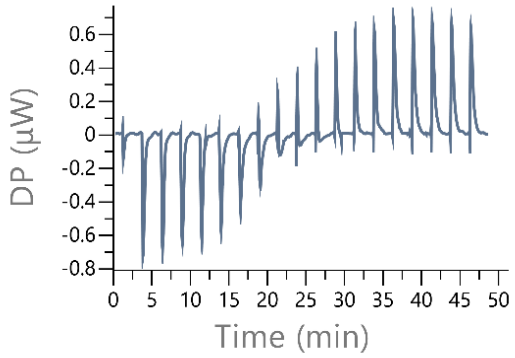
ΔrH = (20.0 ± 1.2) kJ mol^{-1}

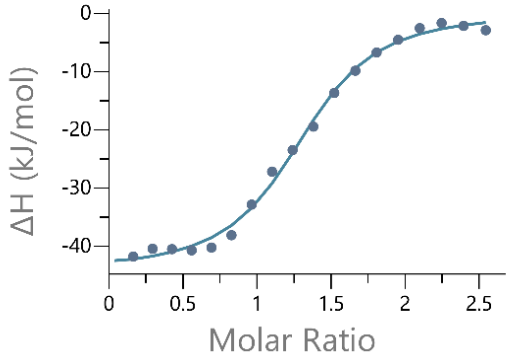
ΔrG = (-30.3 ± 1.3) kJ mol^{-1}

$-T$ ΔrS = (-50.3 ± 0.5) kJ mol^{-1}

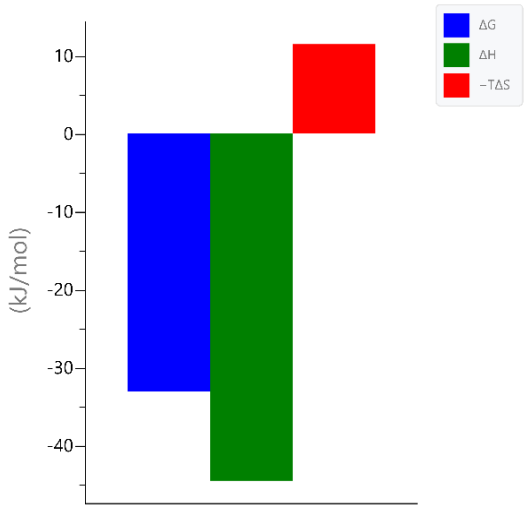


Bakar

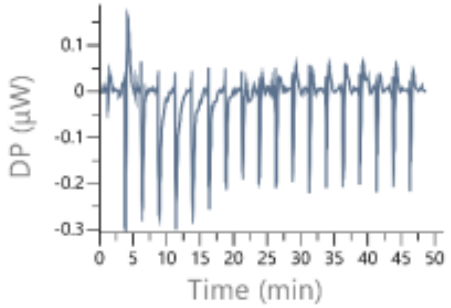


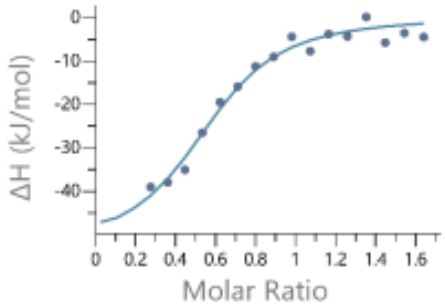


N = (2.03 ± 0.37)
Kd = (1.90 ± 0.04) · 10^{-6} mol dm^{-3}
ΔrH = (-29.3 ± 1.6) kJ mol^{-1}
ΔrG = (-32.7 ± 0.1) kJ mol^{-1}
-T ΔrS = (-3.4 ± 1.5) kJ mol^{-1}

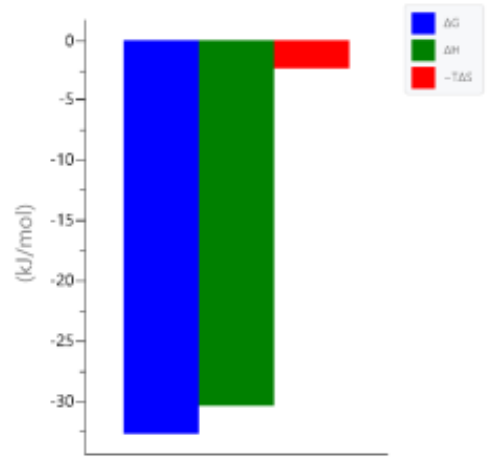


reverzna

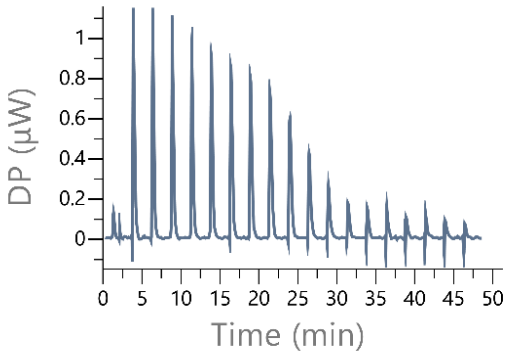


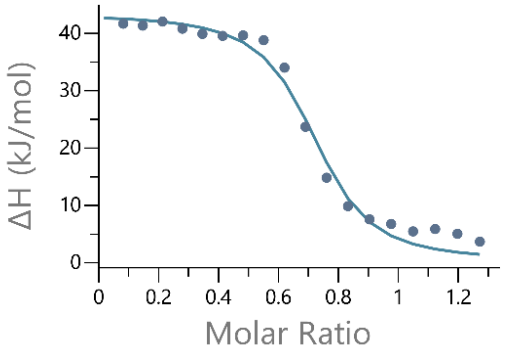


N = (1.21 ± 0.10)
Kd = (1.65 ± 0.04) · 10^{-6} mol dm^{-3}
ΔrH = (-44.5 ± 2.0) kJ mol^{-1}
ΔrG = (-33.0 ± 0.1) kJ mol^{-1}
-T ΔrS = (11.5 ± 2.0) kJ mol^{-1}

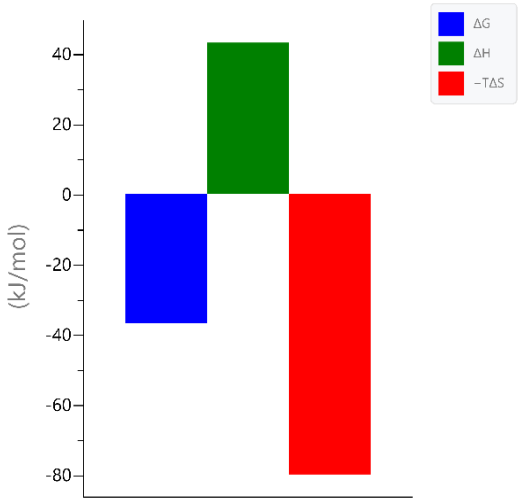


Kobalt



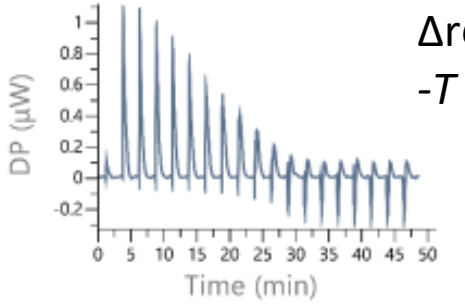


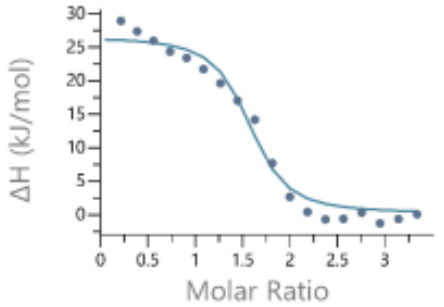
N = (0.644 ± 0.030)
Kd = (3.17 ± 0.23) · 10^{-7} mol dm^{-3}
ΔrH = (37.8 ± 2.7) kJ mol^{-1}
ΔrG = (-37.2 ± 0.2) kJ mol^{-1}
-T ΔrS = (-74.9 ± 2.5) kJ mol^{-1}

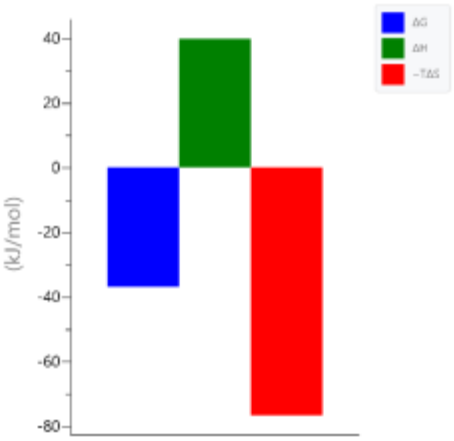


reverzna

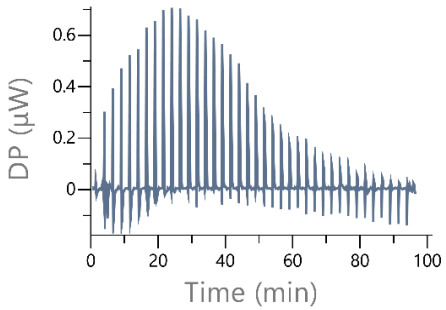
N = (0.663 ± 0.043)
Kd = (3.83 ± 0.55) · 10^{-7} mol dm^{-3}
ΔrH = (44.7 ± 1.5) kJ mol^{-1}
ΔrG = (-36.7 ± 0.4) kJ mol^{-1}
-T ΔrS = (-81.4 ± 1.3) kJ mol^{-1}

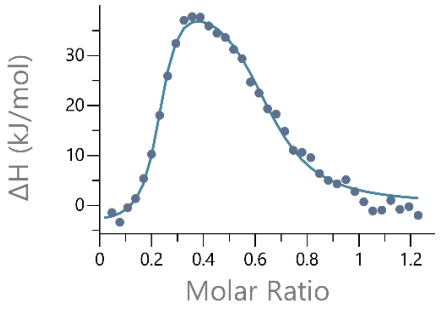






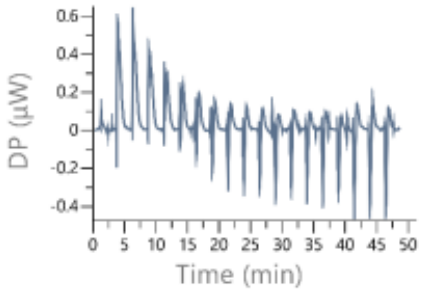
Mangan

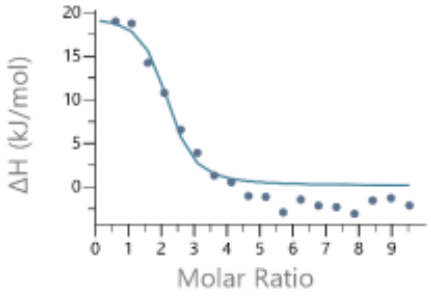




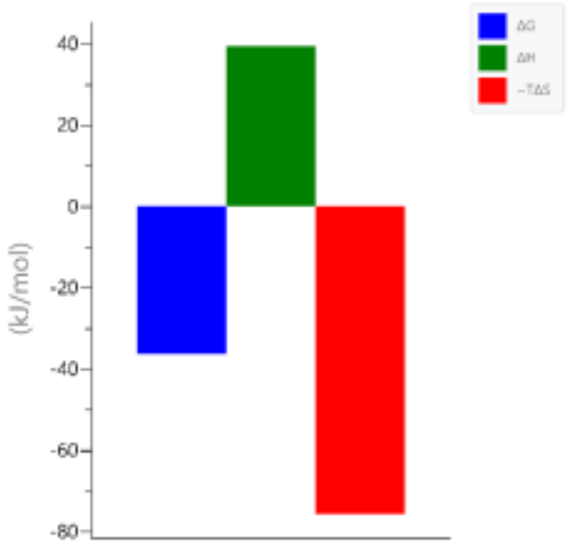
N_1 = (0.213 ± 0.008)
$Kd_{,1}$ = (1.27 ± 0.36) · 10^{-8} mol dm^{-3}
ΔrH_1 = (-4.3 ± 0.2) kJ mol^{-1}
ΔrG_1 = (-45.2 ± 0.8) kJ mol^{-1}
$-T$ ΔrS_1 = (-40.9 ± 0.9) kJ mol^{-1}

N_2 = (0.435 ± 0.021)
$Kd_{,2}$ = (9.84 ± 1.41) · 10^{-7} mol dm^{-3}
ΔrH_2 = (41.1 ± 2.4) kJ mol^{-1}
ΔrG_2 = (-34.3 ± 0.4) kJ mol^{-1}
$-T$ ΔrS_2 = (-75.4 ± 2.0) kJ mol^{-1}





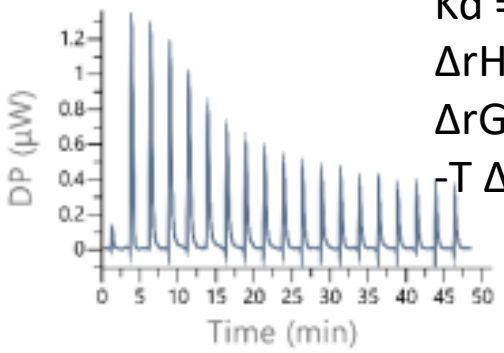
N = (0.496 ± 0.011)
Kd = (3.94 ± 0.25) · 10^{-7} mol dm^{-3}
ΔrH = (38.7 ± 0.8) kJ mol^{-1}
ΔrG = (-36.6 ± 0.1) kJ mol^{-1}
$-T$ ΔrS = (-75.3 ± 0.7) kJ mol^{-1}

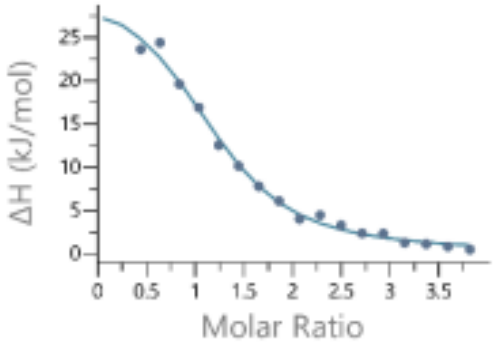


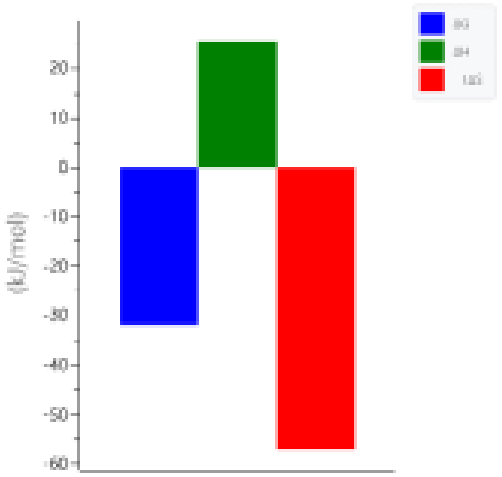
	N	Kd/ uM	reverzna	N	Kd/ uM
Zn	2,00+/-0,08	5,29+/-2,36		2,32+/-0,28	2,29+/-0,73
Cu	1,2+/-0,10	1,65+/-0,04		2,03+/-0,37	1,90+/-0,4
Co	0,663+/-0,013	0,383+/-0,055		0,644+/-0,03	31,7+/-0,23
Mn	0,213+/-0,008	0,0127+/-0,0036		0,496+/-0,011	39,4+/-0,25
	0,435+/-0,021	0,984+/-0,141			

Cink mut(E508D)

N = (1.43 ± 0.26)
Kd = (2.93 ± 0.64) ·10^{-6} mol dm^{-3}
ΔrH = (26.3 ± 4.7) kJ mol^{-1}
ΔrG = (-31.7 ± 0.5) kJ mol^{-1}
-T ΔrS = (-58.0 ± 4.2) kJ mol^{-1}

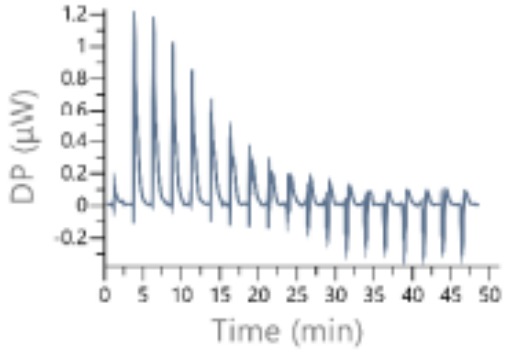


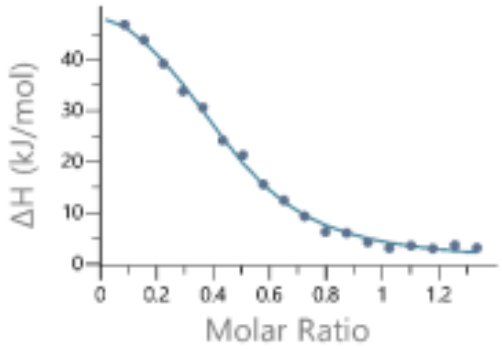


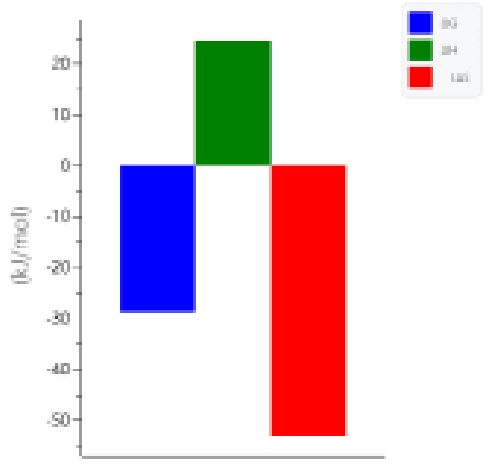


reverzna

N = (2.23 ± 0.09)
Kd = (9.1 ± 1.3) · 10 $^{-6}$ mol dm $^{-3}$
ΔrH = (25.3 ± 1.2) kJ mol $^{-1}$
ΔrG = (-28.8 ± 0.4) kJ mol $^{-1}$
-T ΔrS = (-54.2 ± 1.0) kJ mol $^{-1}$

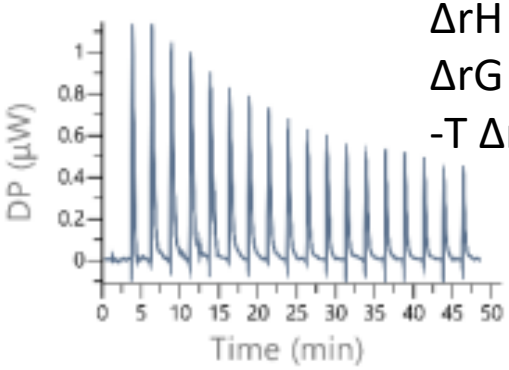


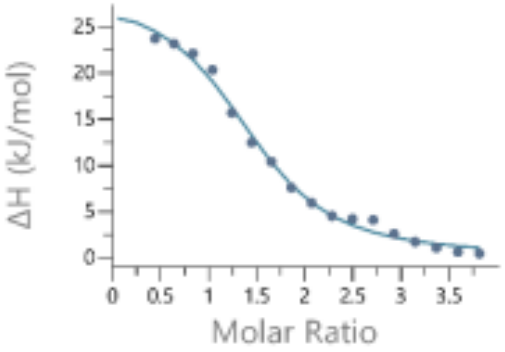


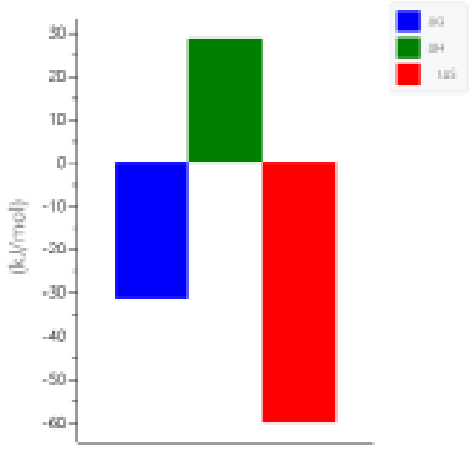


Cink mut(H455Y)

N = (1.49 ± 0.10)
Kd = (3.79 ± 0.88) · 10 $^{-6}$ mol dm $^{-3}$
ΔrH = (28.7 ± 6.5) kJ mol $^{-1}$
ΔrG = (-31.0 ± 0.6) kJ mol $^{-1}$
-T ΔrS = (-59.7 ± 6.1) kJ mol $^{-1}$

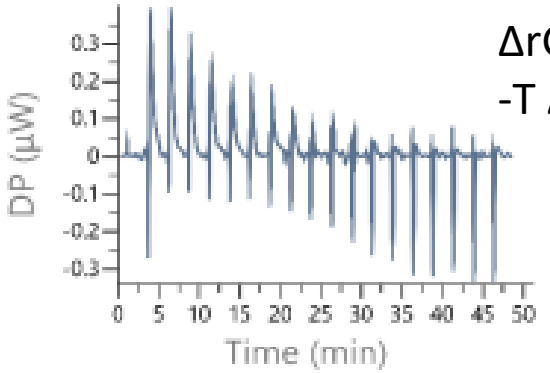


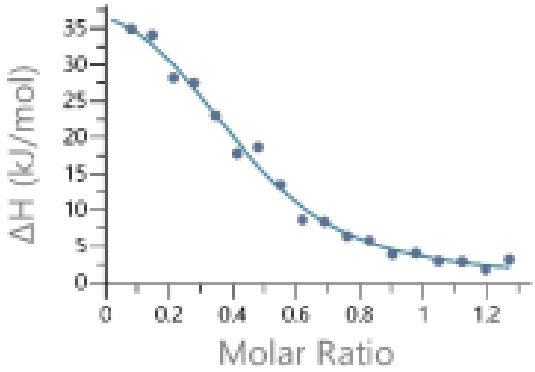


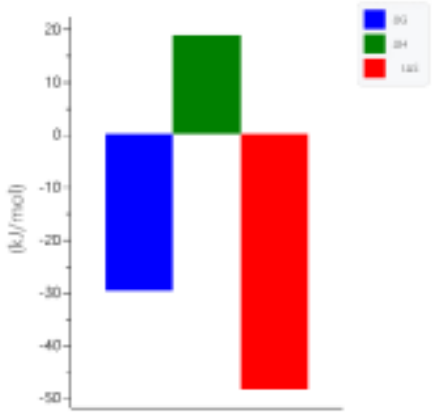


reverzna

N = (2.37 ± 0.34)
Kd = (8.98 ± 3.93) · 10 $^{-6}$ mol dm $^{-3}$
ΔrH = (18.2 ± 2.8) kJ mol $^{-1}$
ΔrG = (-29.0 ± 1.0) kJ mol $^{-1}$
-T ΔrS = (-47.1 ± 2.3) kJ mol $^{-1}$

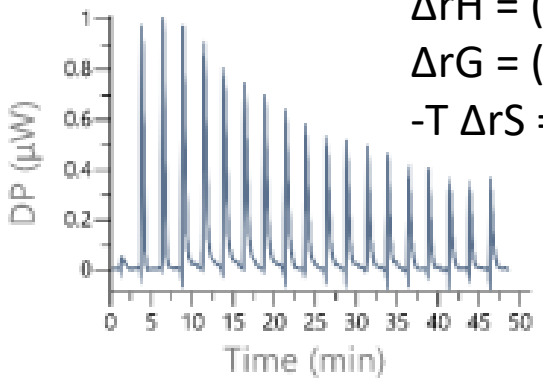


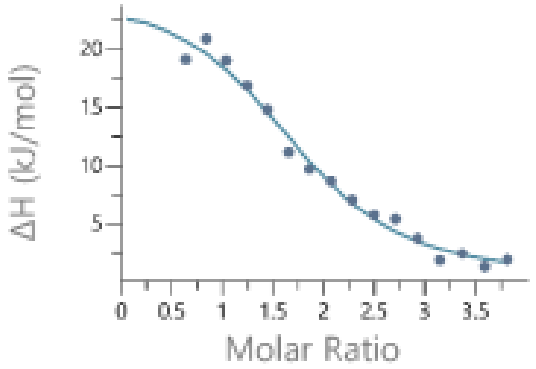


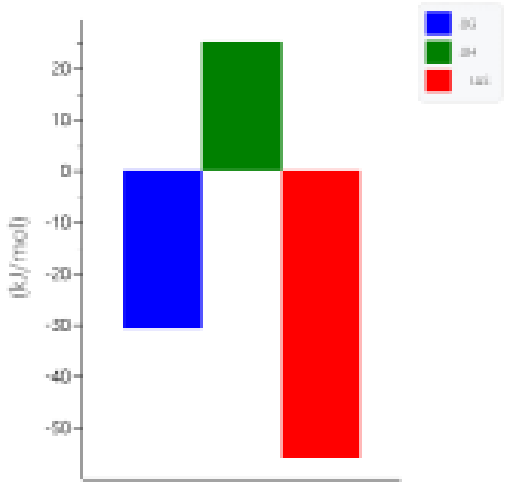


Cink (H568y-E316H)

N = (1.83 ± 0.09)
Kd = (2.92 ± 0.96) · 10^{-6} mol dm^{-3}
ΔrH = (21.4 ± 3.6) kJ mol^{-1}
ΔrG = (-31.7 ± 0.8) kJ mol^{-1}
-T ΔrS = (-53.0 ± 3.0) kJ mol^{-1}

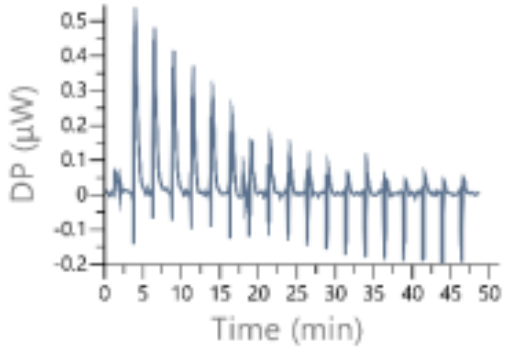


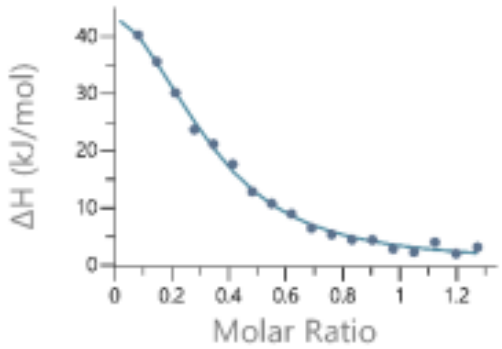


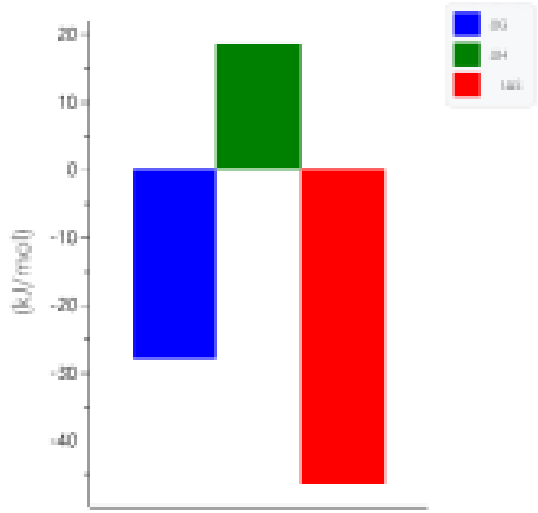


reverzna

N = (3.39 ± 0.09)
Kd = (1.21 ± 0.42) · 10^{-5} mol dm^{-3}
ΔrH = (18.5 ± 2.6) kJ mol^{-1}
ΔrG = (-28.2 ± 0.9) kJ mol^{-1}
-T ΔrS = (-46.8 ± 1.6) kJ mol^{-1}







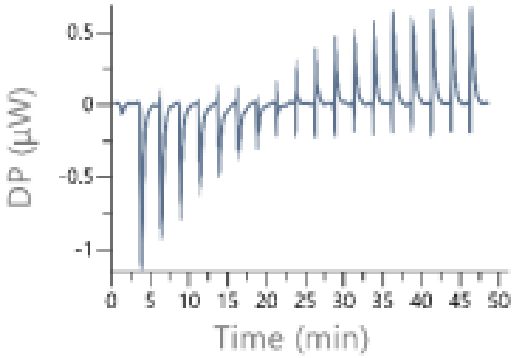
Cink

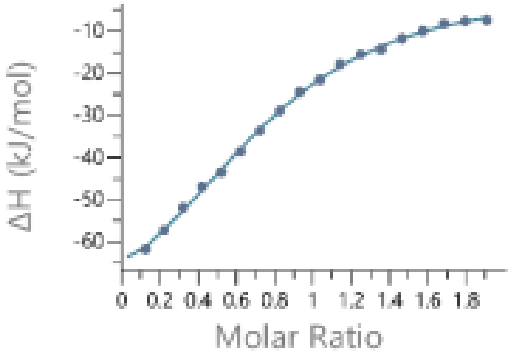
mutanti	N	Kd/ uM	reverzna	N	Kd/ uM
E508D	1,43+/-0,26	2,93+/-0,64		2,23+/-0,9	9,1+/-1,3
H455Y	1,49+/-0,1	3,79+/-0,88		2,37+/-0,34	8,98+/-3,93
H568Y-E316Y	1,83+/-0,09	2,92+/-0,96		3,39+/-0,09	0,121+/-0,042

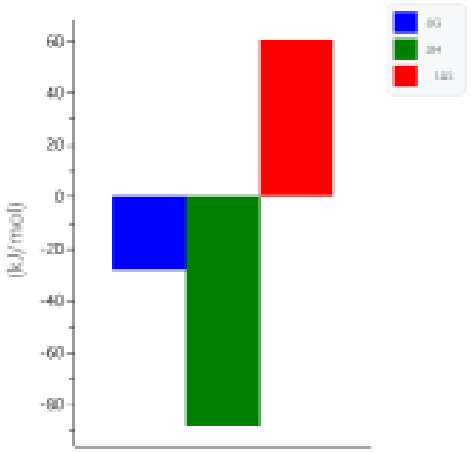
	N	Kd/ uM	reverzna	N	Kd/ uM
Apo hDPPIII	2,00+/-0,08	5,29+/-2,36		2,32+/-0,28	2,29+/-0,73

Bakar (E508D)

N = (0.66 ± 0.13)
Kd = (6.77 ± 4.12) · 10 $^{-6}$ mol dm $^{-3}$
ΔrH = (-79.0 ± 9.7) kJ mol $^{-1}$
ΔrG = (-29.8 ± 1.5) kJ mol $^{-1}$
-T ΔrS = (49.1 ± 10.7) kJ mol $^{-1}$

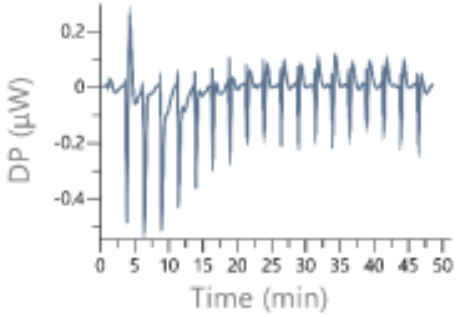


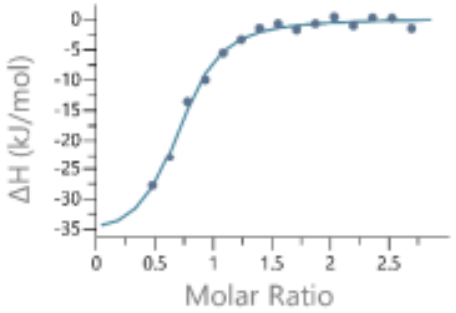


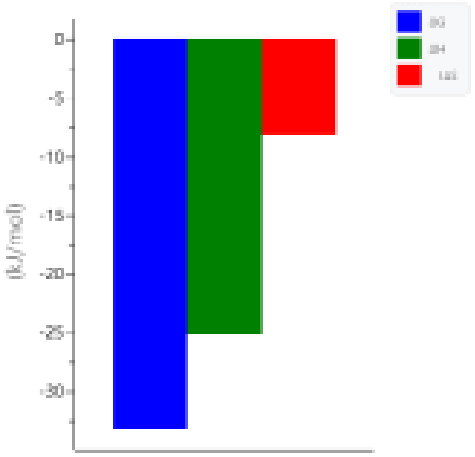


reverzna

N = (1.39 ± 0.30)
Kd = (1.40 ± 0.07) · 10 $^{-6}$ mol dm $^{-3}$
ΔrH = (-23.5 ± 1.5) kJ mol $^{-1}$
ΔrG = (-33.5 ± 0.2) kJ mol $^{-1}$
-T ΔrS = (-10.0 ± 1.6) kJ mol $^{-1}$



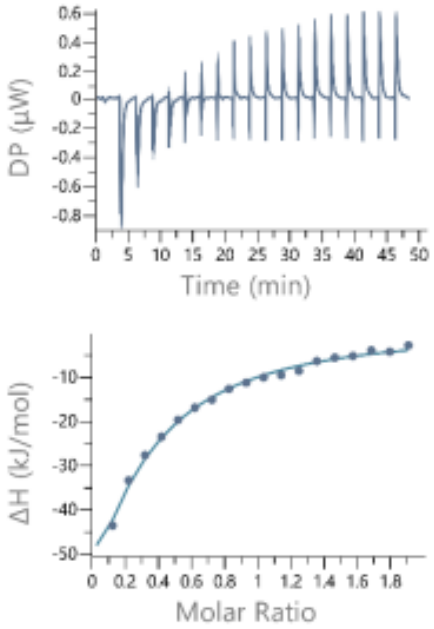




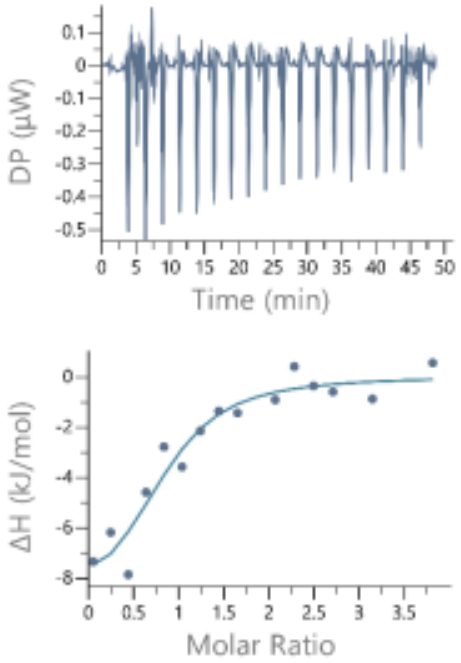
Cu (H455Y)

N=?

Kd = (2.57 ± 0.26) · 10^{-5} mol dm^{-3}



reverzna



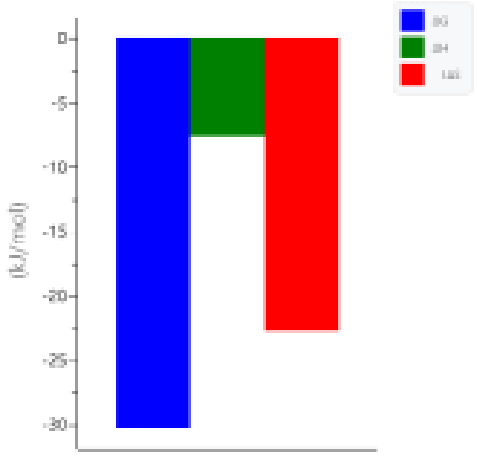
N = (1.25)

Kd = (4,93) · 10^{-6} mol dm^{-3}

ΔrH = (-7,6) kJ mol^{-1}

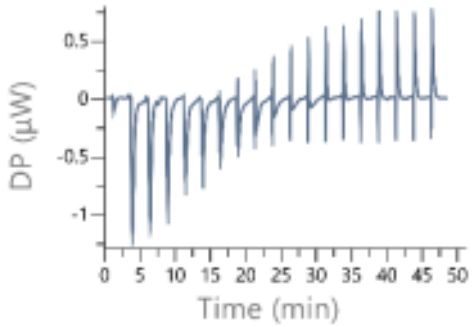
ΔrG = (-30,3) kJ mol^{-1}

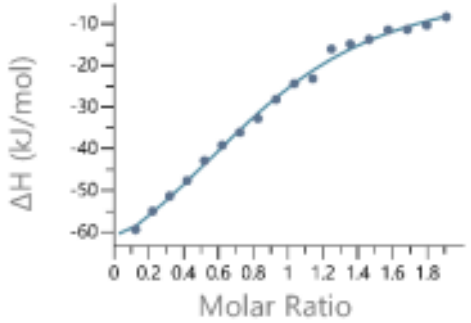
-T ΔrS = (-22,7) kJ mol^{-1}

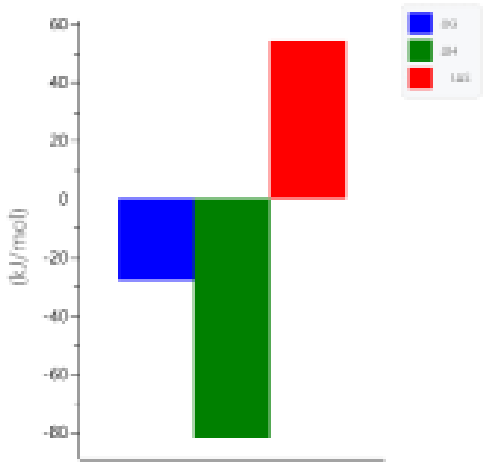


Cu (H568Y-E316H)

N = (1.03 ± 0.13)
Kd = (1.23 ± 0.20) · 10 $^{-5}$ mol dm $^{-3}$
ΔrH = (-72.9 ± 14.4) kJ mol $^{-1}$
ΔrG = (-28.1 ± 0.4) kJ mol $^{-1}$
-T ΔrS = (44.8 ± 14.7) kJ mol $^{-1}$

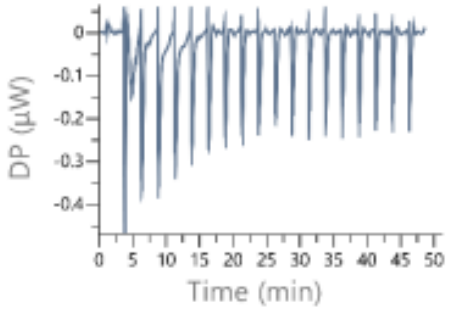


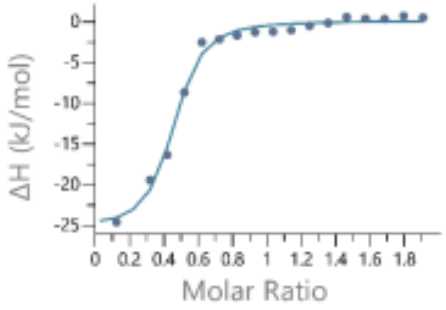


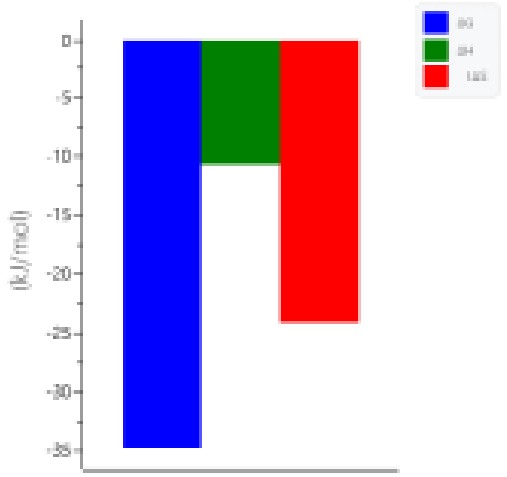


reverzna

N = (2.36 ± 0.03)
Kd = (8.50 ± 0.80) · 10 $^{-7}$ mol dm $^{-3}$
ΔrH = (-10.6 ± 0.1) kJ mol $^{-1}$
ΔrG = (-34.7 ± 0.3) kJ mol $^{-1}$
-T ΔrS = (-24.1 ± 0.1) kJ mol $^{-1}$







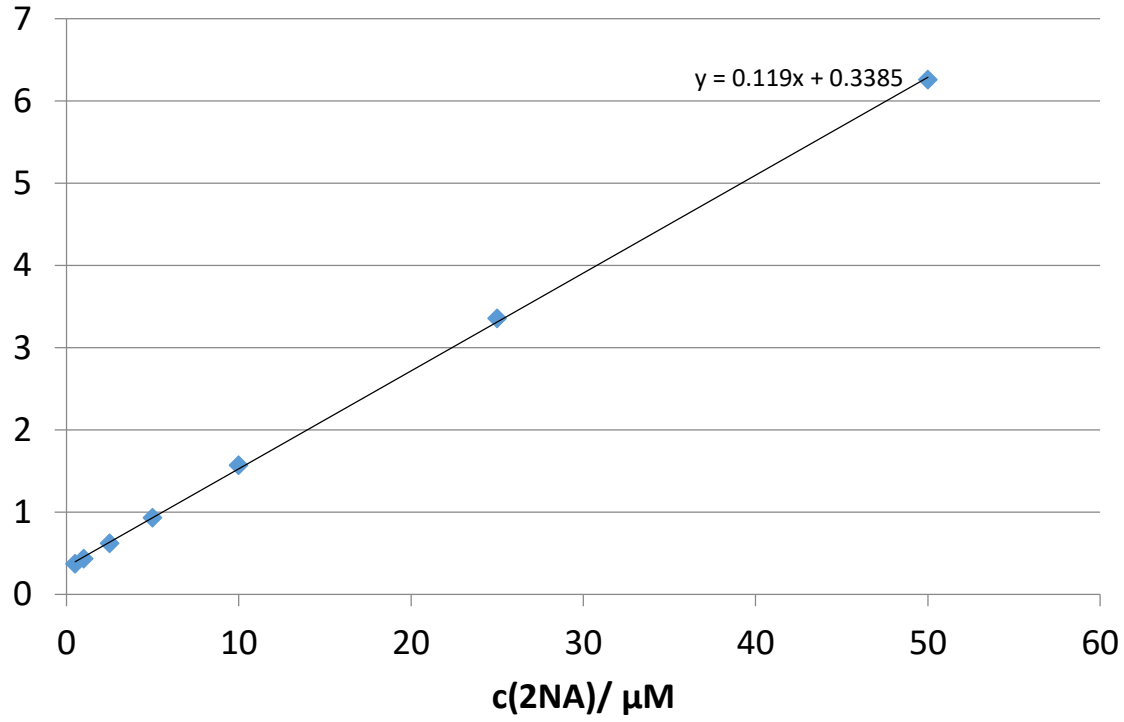
bakar

mutanti	N	Kd/ uM	reverzna	N	Kd/ uM
E508D	0,66+/-0,13	6,77+/-4,12		1,39+/-0,3	1,4+/-0,07
H455Y	-	0,257+/-0,026		2,37+/-0,34	8,98+/-3,93
H568Y-E316Y	1,03+/-0,13	0,123+/-0,02		2,36+/-0,03	85+/-8

	N	Kd/ uM	reverzna	N	Kd/ uM
Apo hDPPIII	1,2+/-0,10	1,65+/-0,04		2,03+/-0,37	1,90+/-0,4

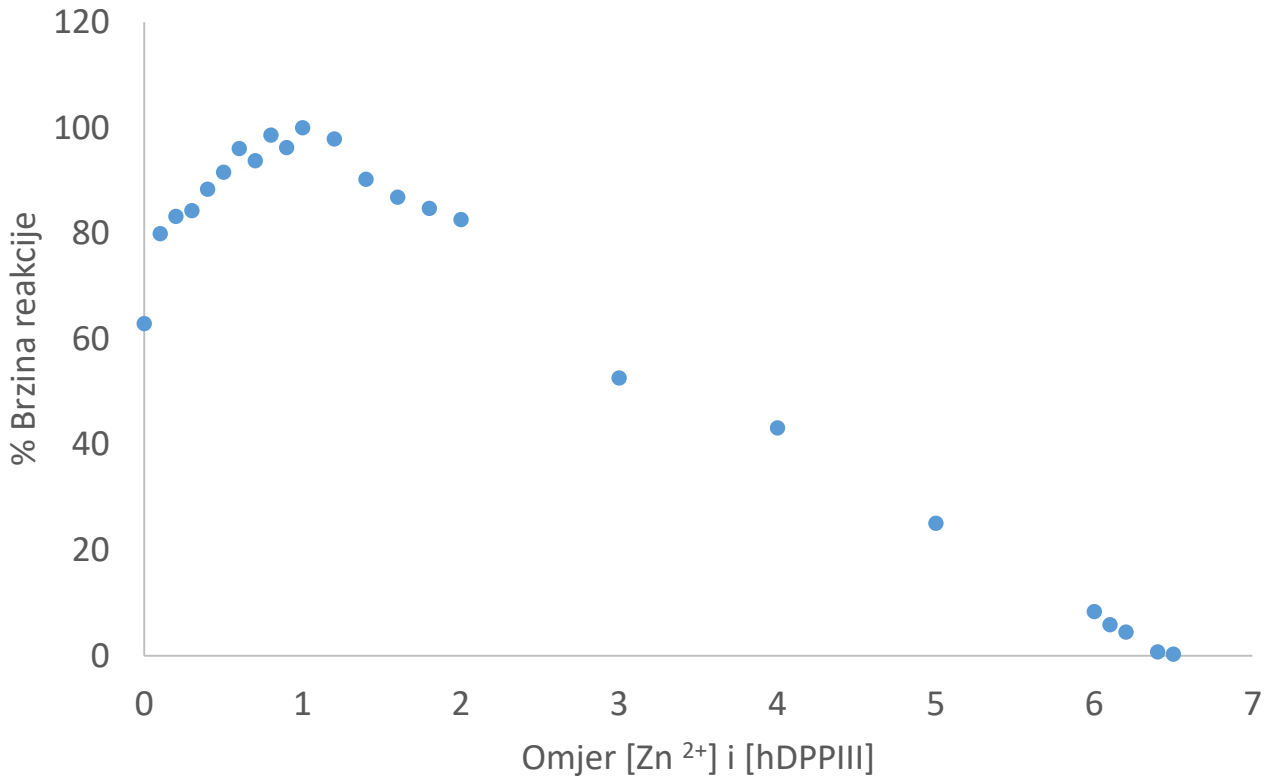
Stopped flow (PMF)

produkt/uM	nagb/s-1
0.5	0.373803
1	0.437
2	0.622
5	0.933
10	1.574
25	3.358
50	6.260



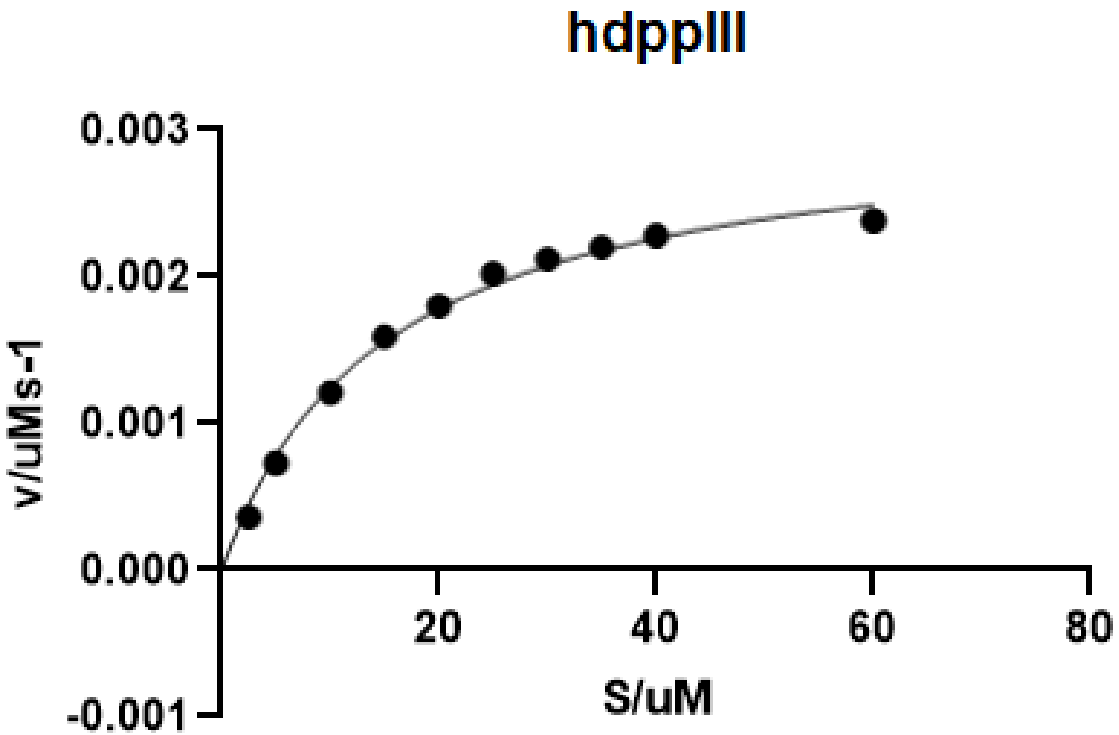
- Koncentracije wt hDPPIII 10nM
- supstrat ArgArg-2NA

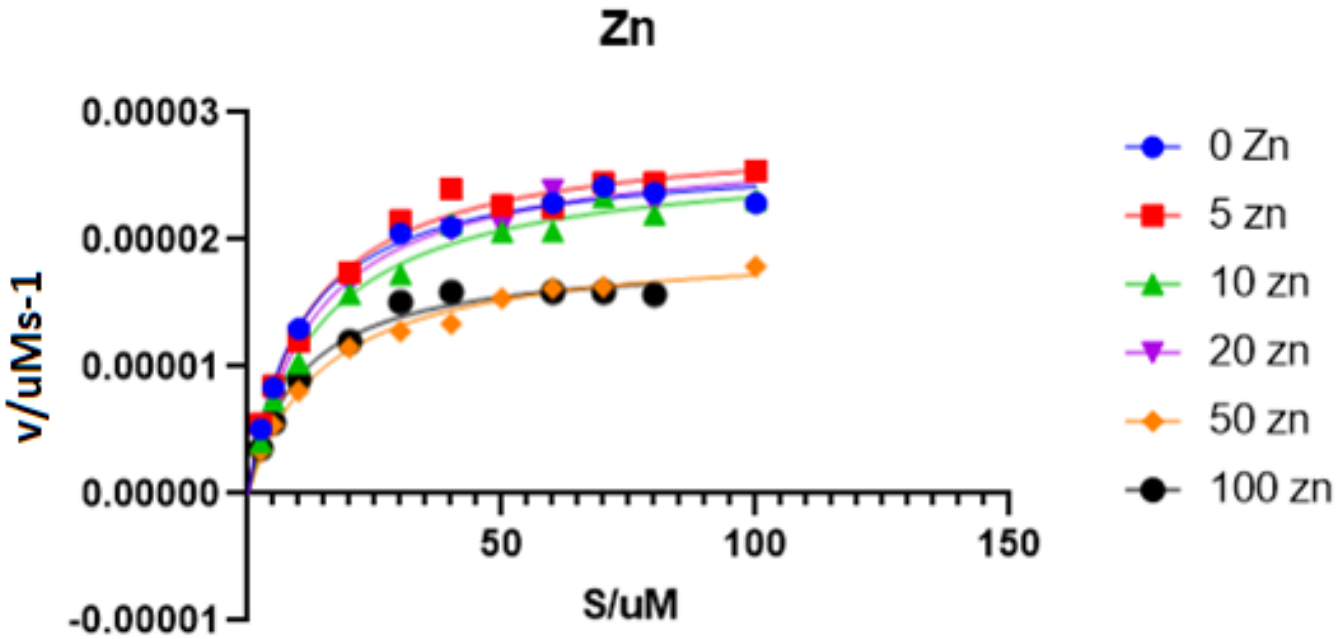
- Mjerenja su izvršena na 332nm i 320V
- **Predinkubacija**

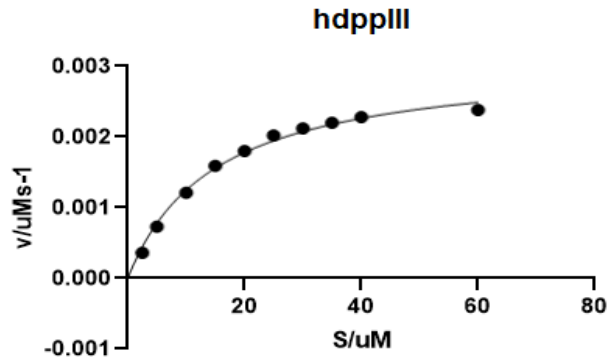


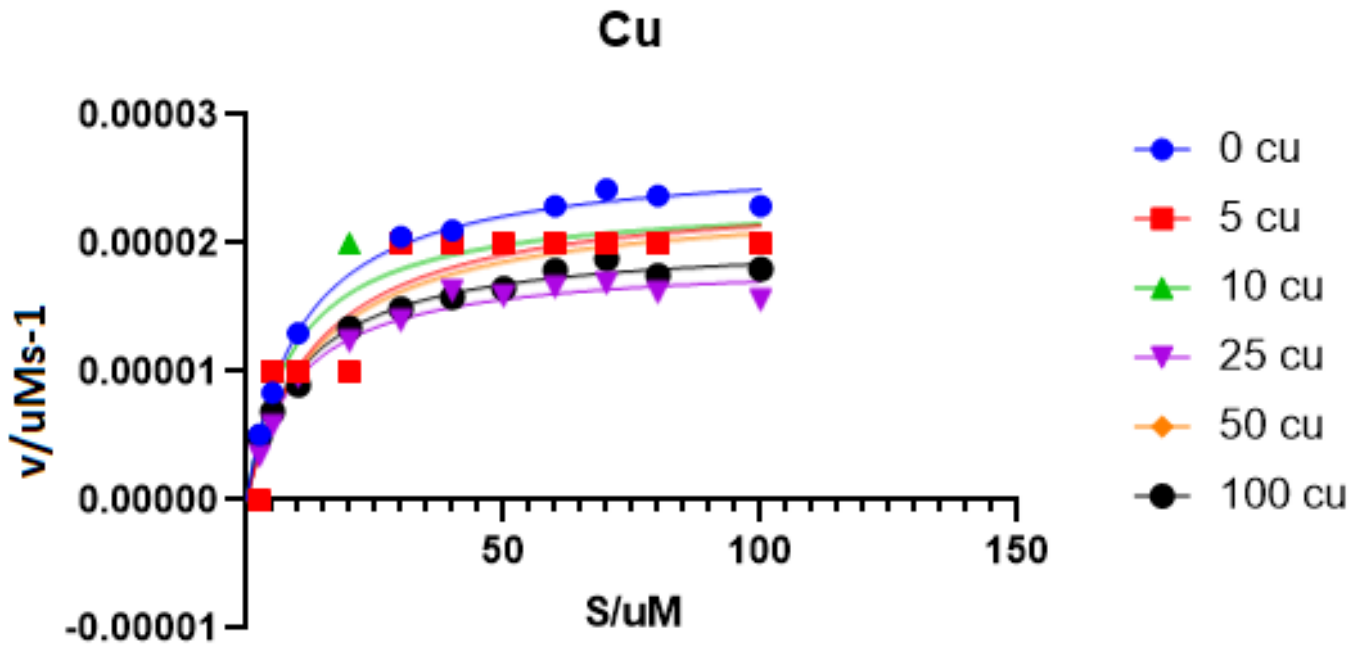
Kinetika na florimetru

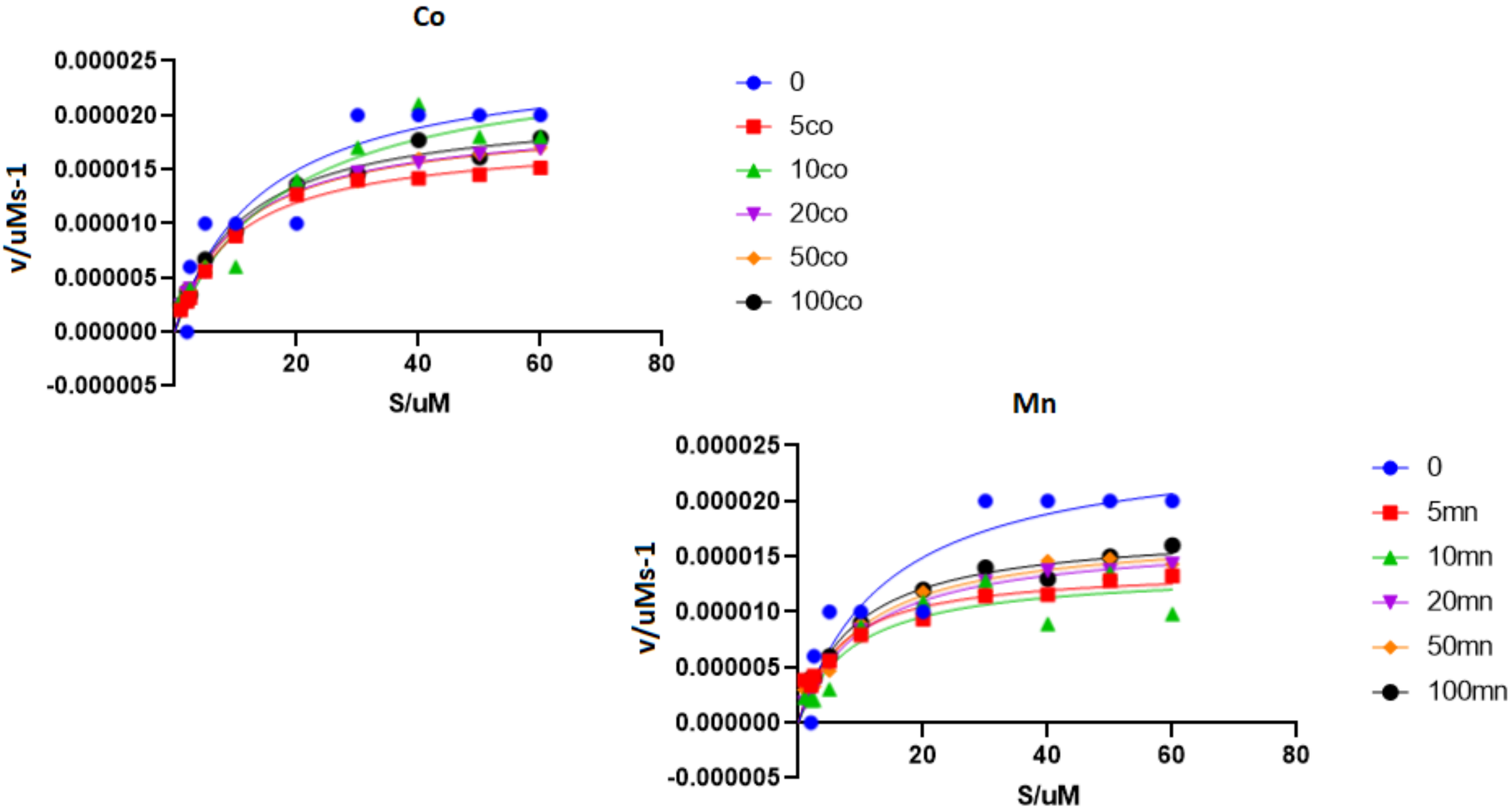
- Wt hDPPIII 20nM
- Pufer TrisHCl
- supstrat ArgArg-2NA




Zn
0.00003
0.00002
0.00001
0.00000
-0.00001
v/uMs-1
50
100
150
S/uM
0 Zn
5 zn
10 zn
20 zn
50 zn
100 zn


hdpplll
0.003
0.002
0.001
0.000
-0.001
v/uMs-1
20
40
60
80
S/uM


Cu
0.00003
0.00002
0.00001
0.00000
-0.00001
v/uMs-1
50
100
150
S/uM
0 cu
5 cu
10 cu
25 cu
50 cu
100 cu


Co
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
-0.000005
v/uMs-1
20
40
60
80
S/uM
0
5co
10co
20co
50co
100co
Mn
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
-0.000005
v/uMs-1
20
40
60
80
S/uM
0
5mn
10mn
20mn
50mn
100mn

(FBF) 10 nM **apo** hDPPIII.

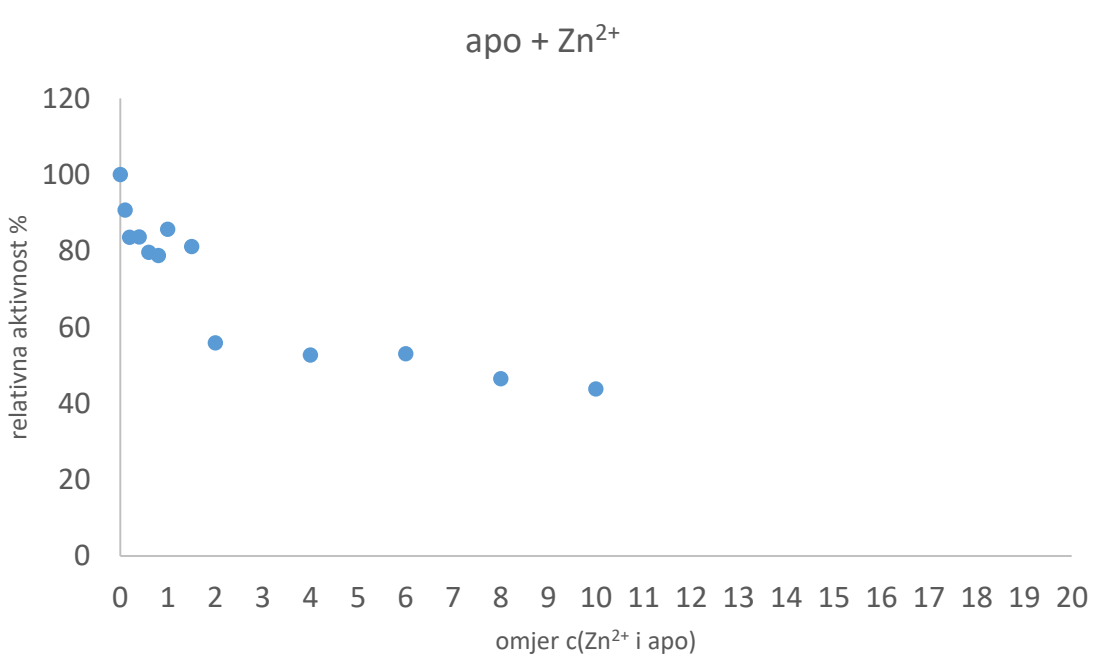
Pufer:50 mM natrijev kakodilat, pH = 7,4.

Standardna otopina metalnih iona je nitratna.

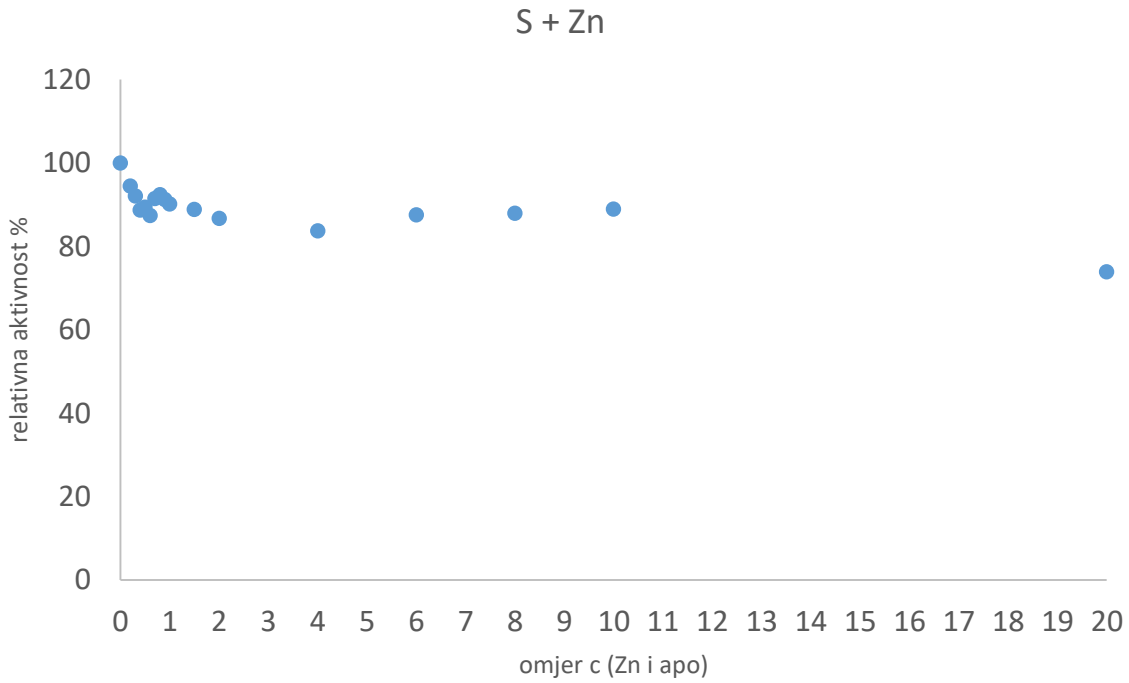
Supstrat: Arg-Arg-2NA.

Zn^{2+}

Predinkubacija

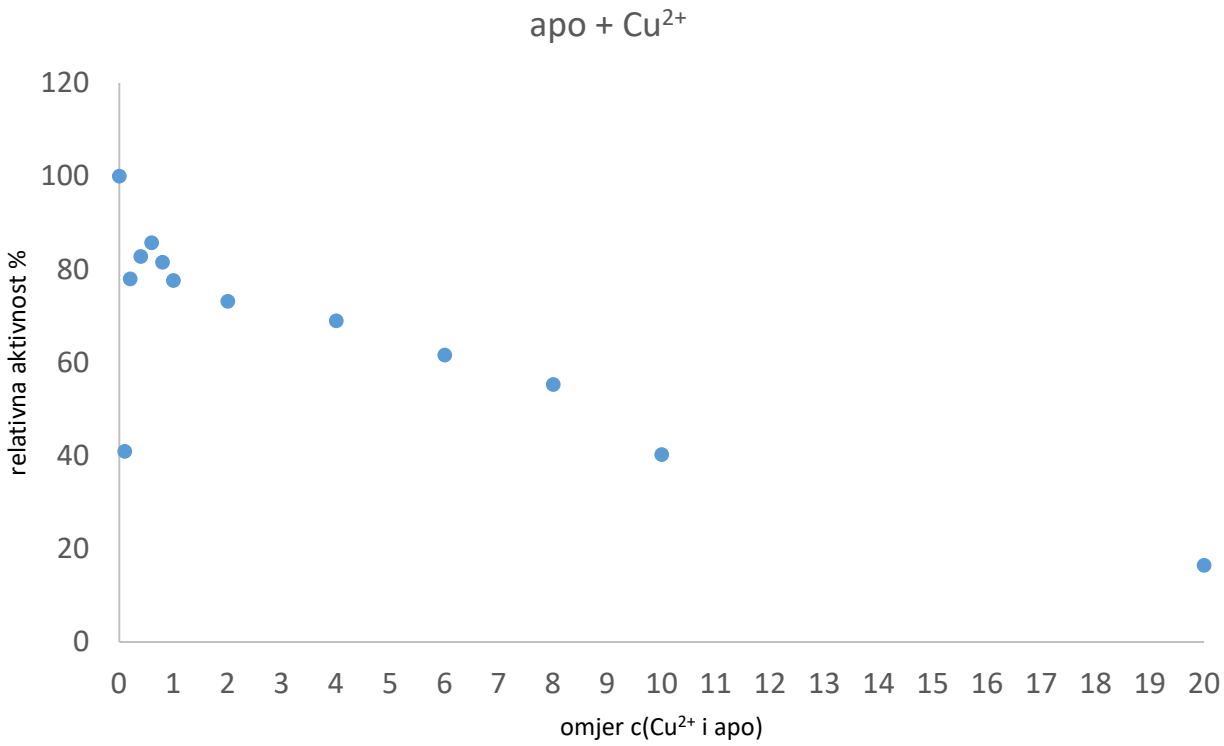


Bezinkubacije

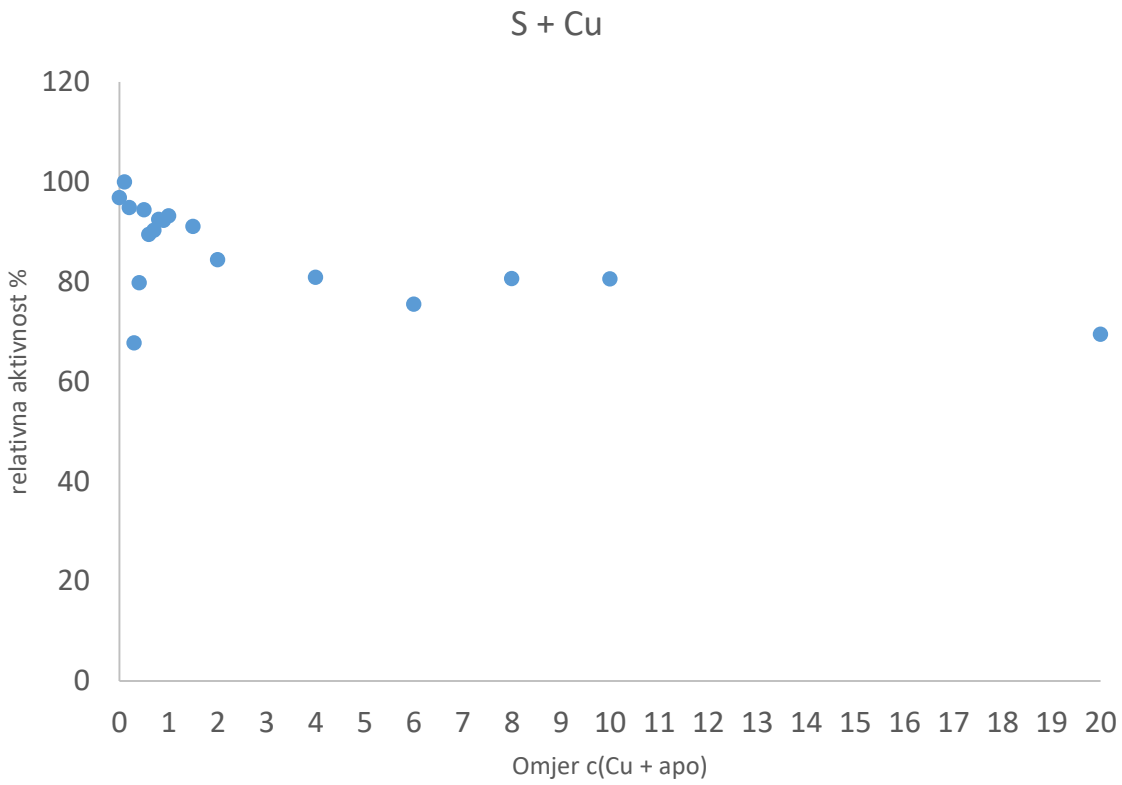


Cu^{2+}

Predinkubacija

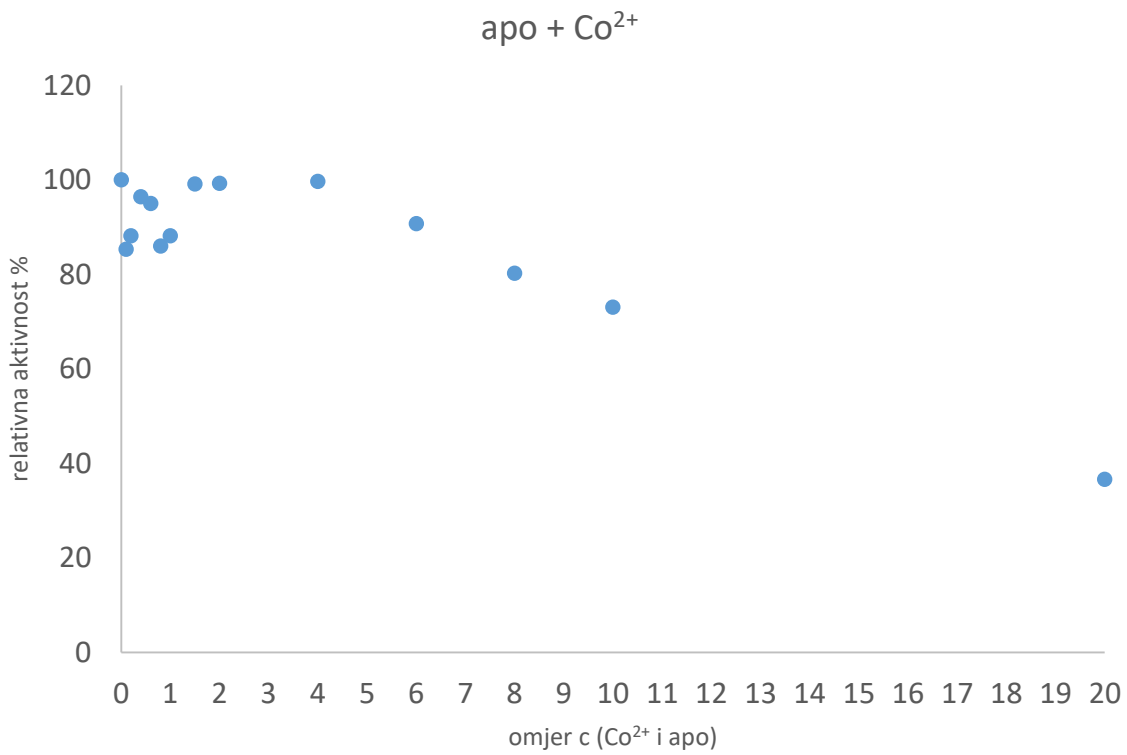


Bezinkubacije

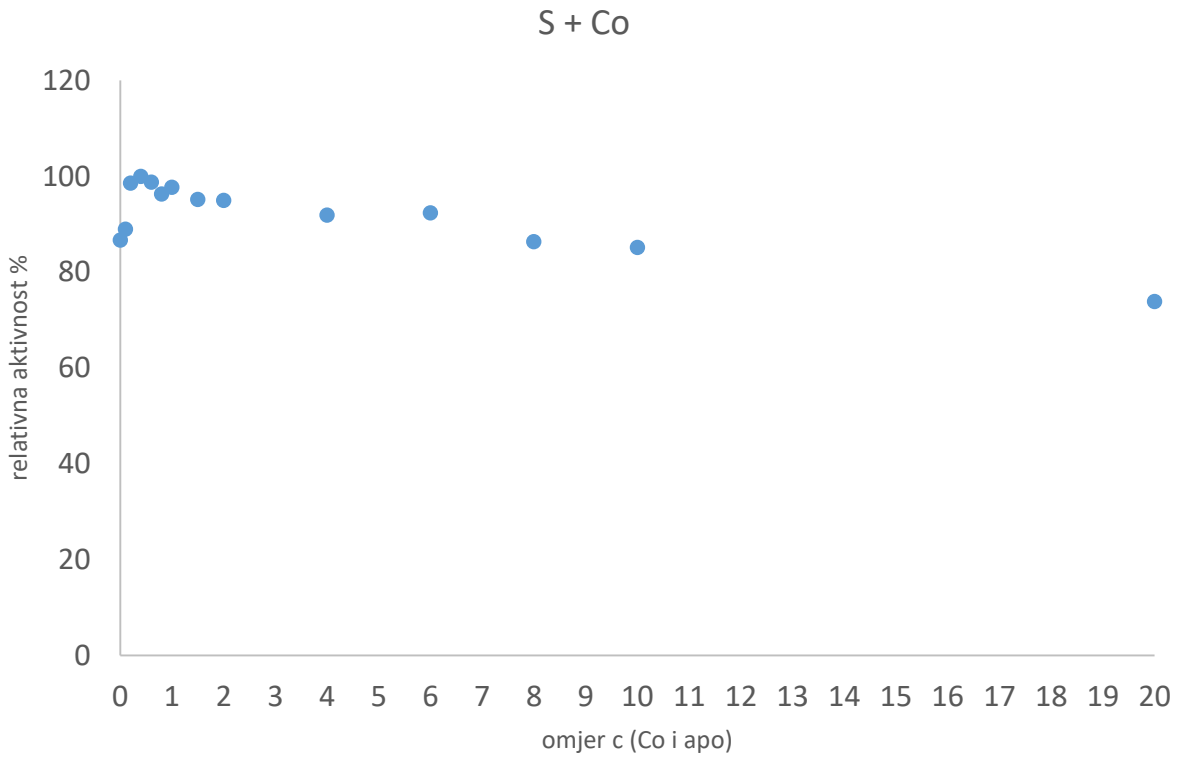


Co^{2+}

Predinkubacija

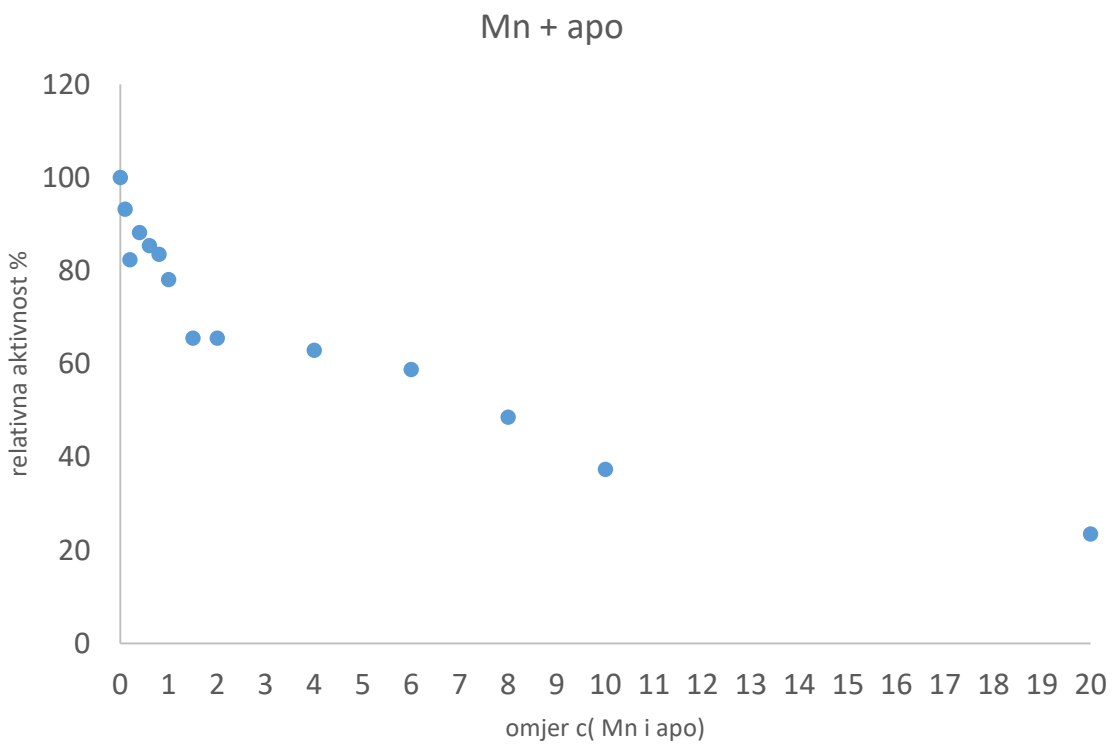


Bezinkubacije

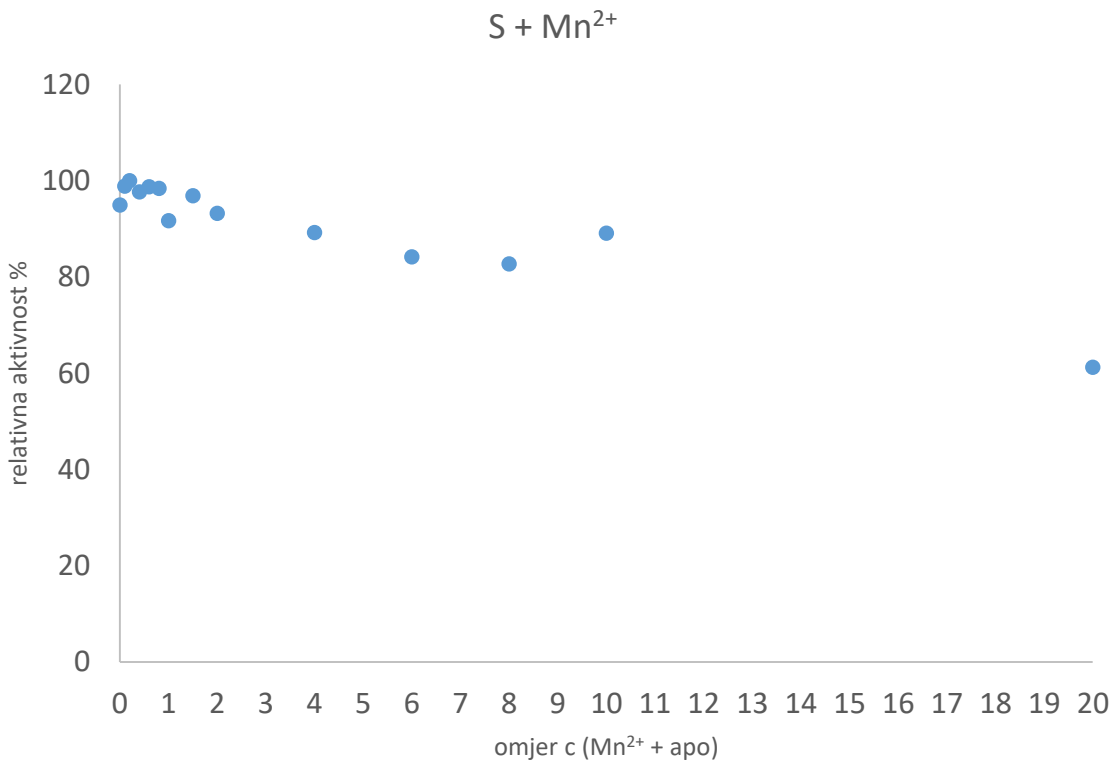


Mn^{2+}

Predinkubacija



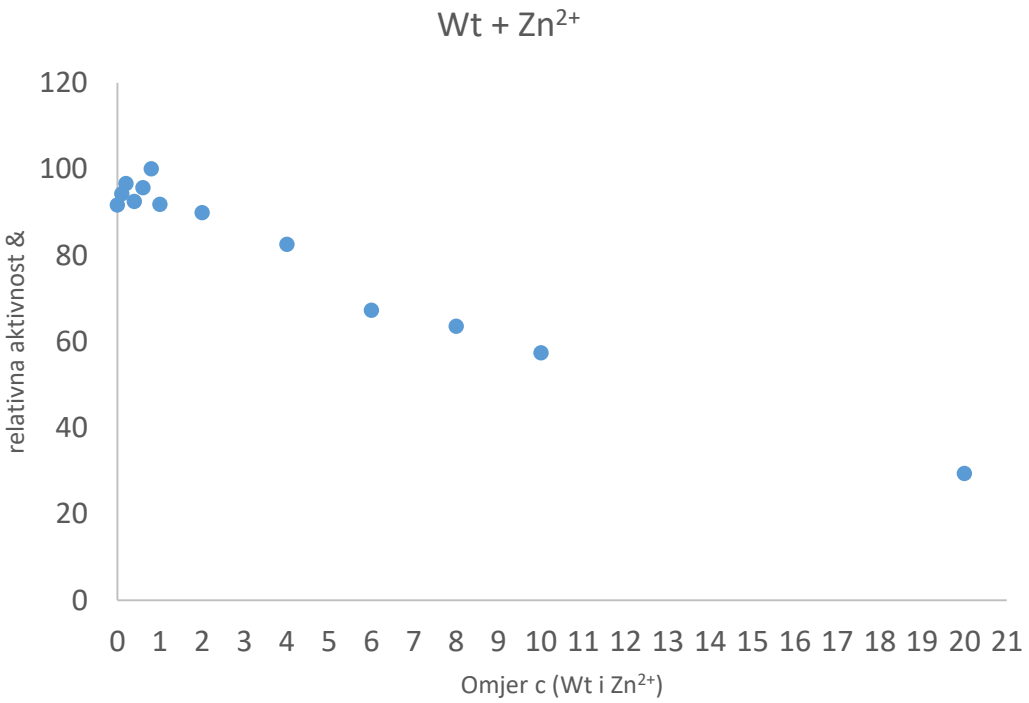
Bezinkubacije



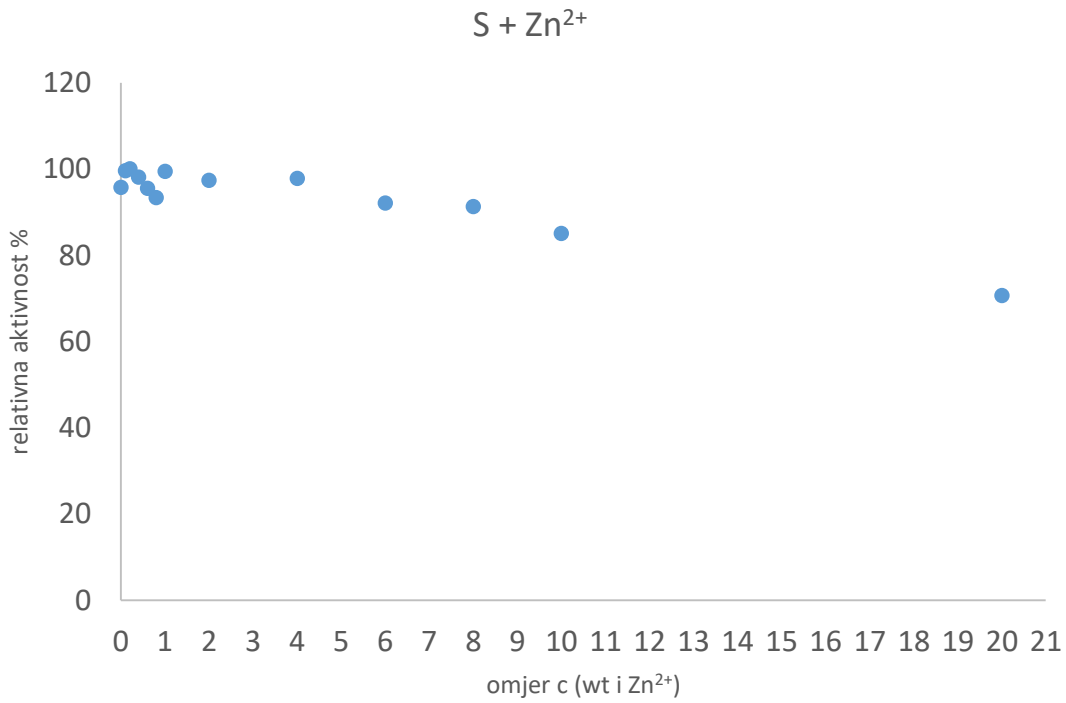
WT hDPPIII (Wt)

Zn^{2+}

Predinkubacija

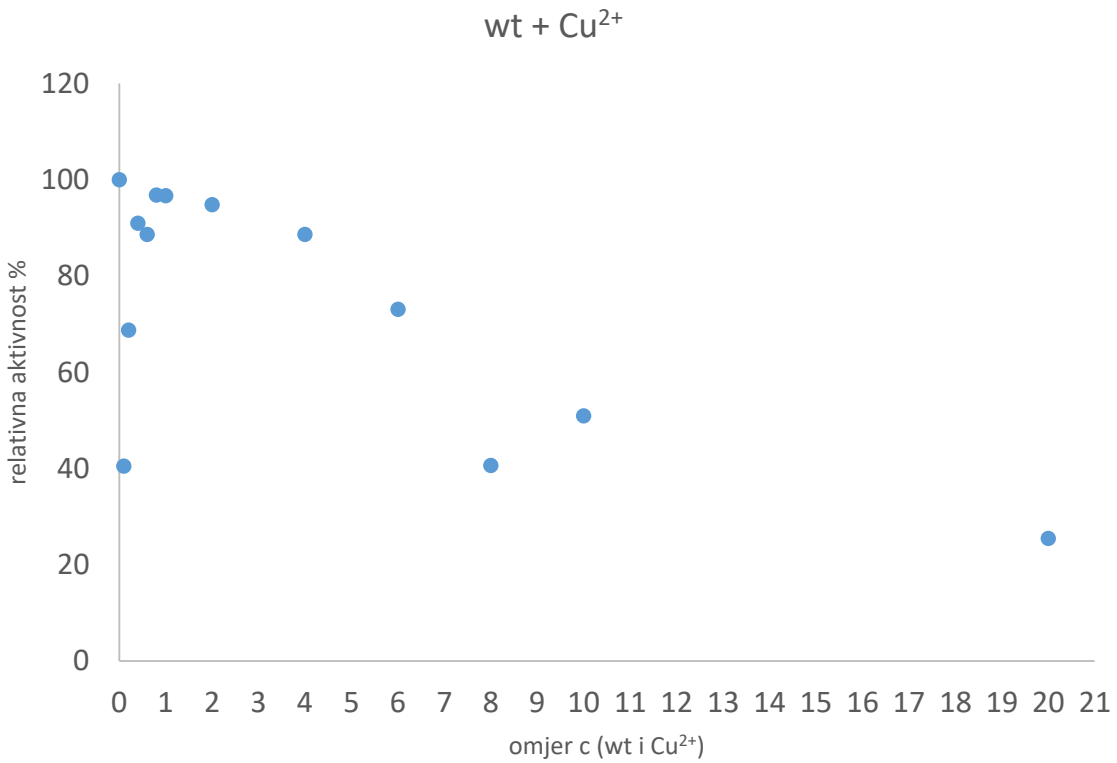


Bezinkubacije

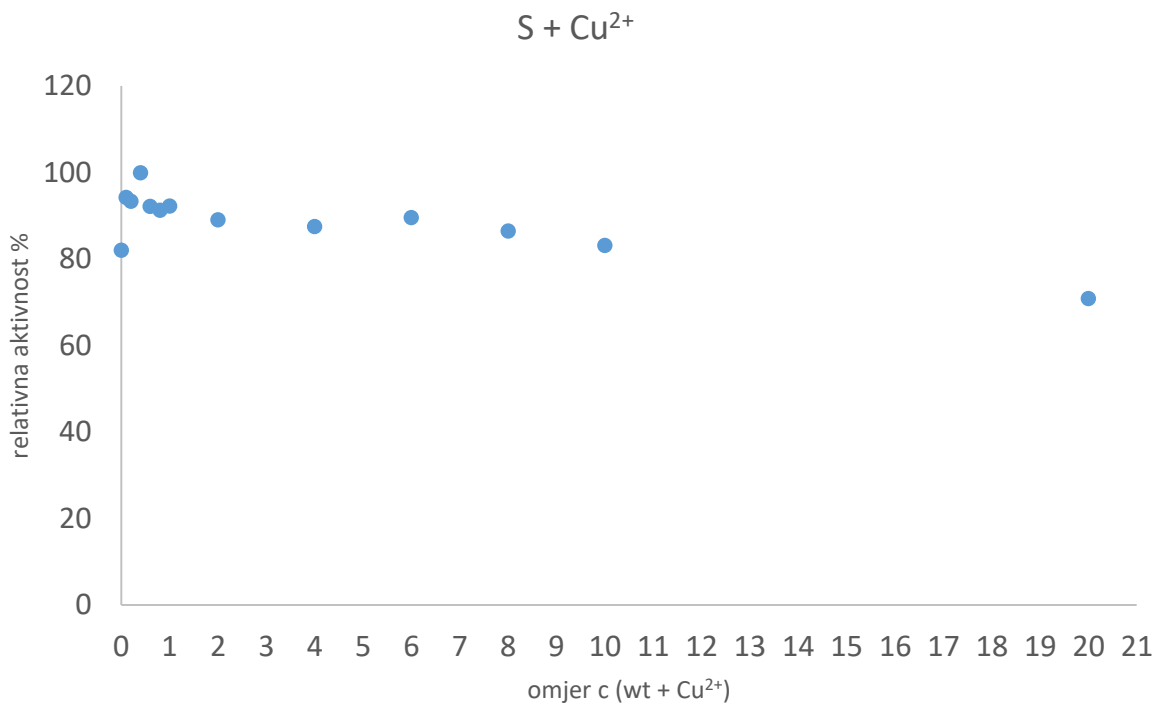


Cu^{2+}

Predinkubacija

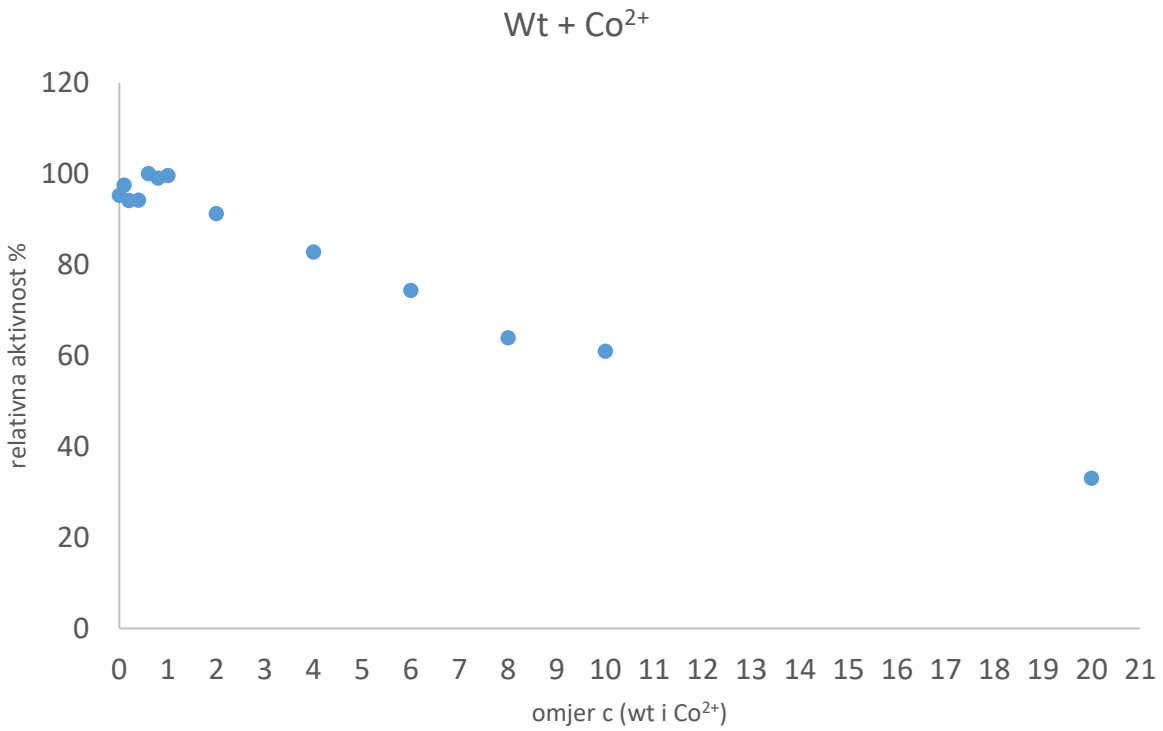


Bezinkubacije

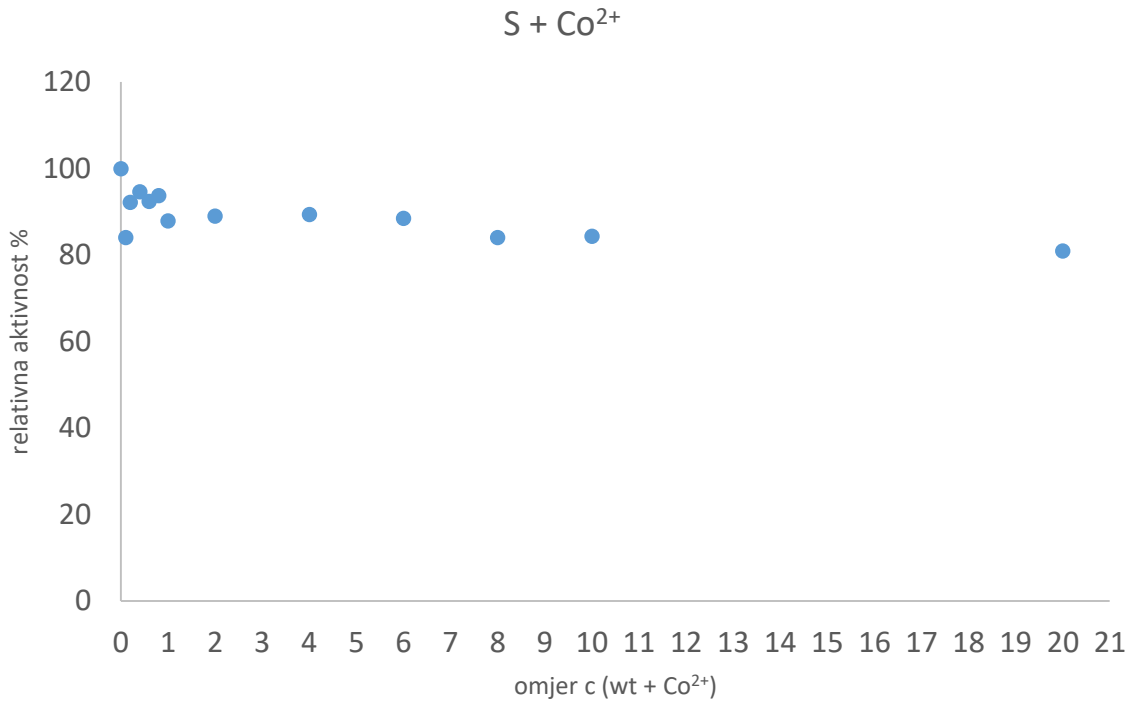


Co^{2+}

Predinkubacija

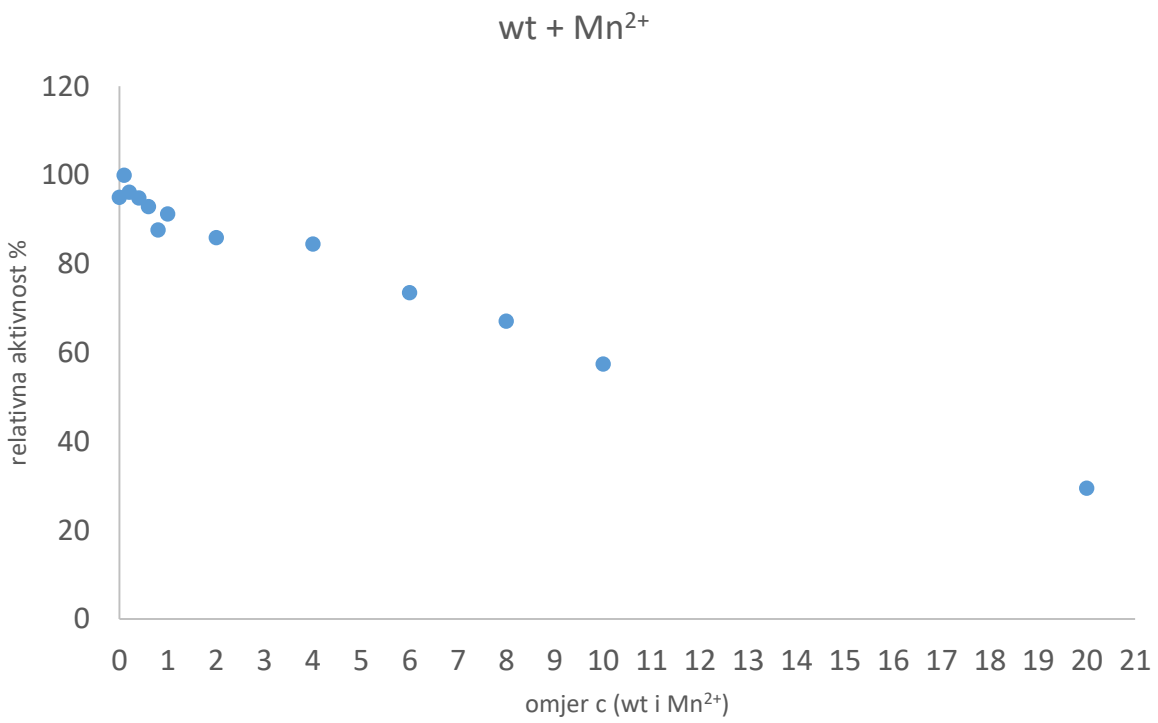


Bezinkubacije

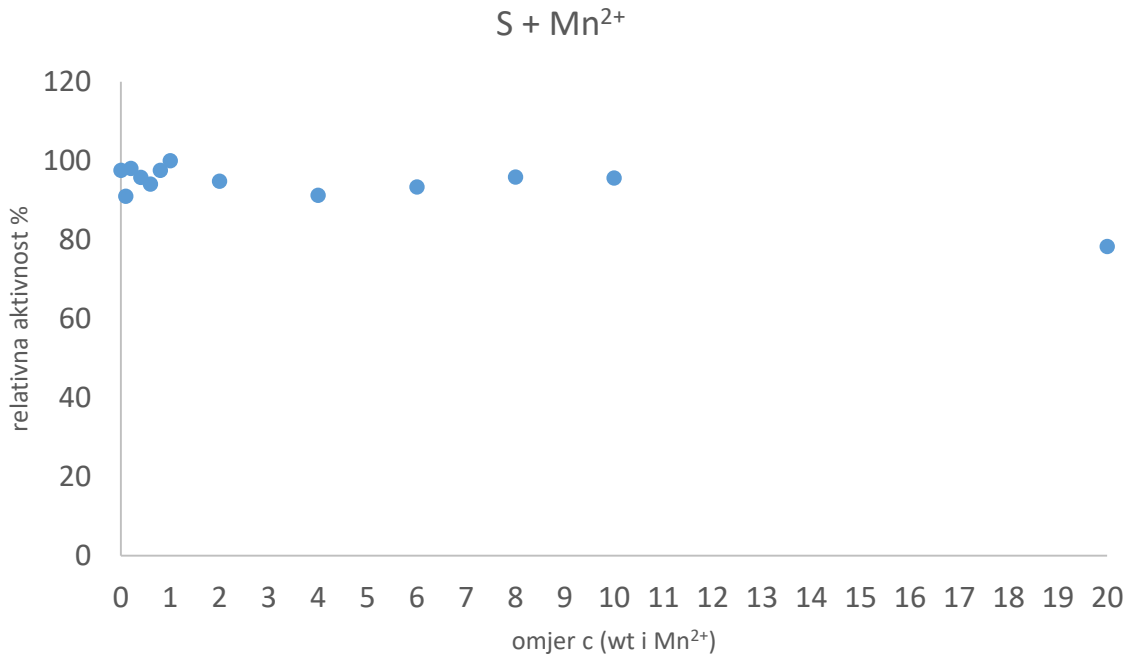


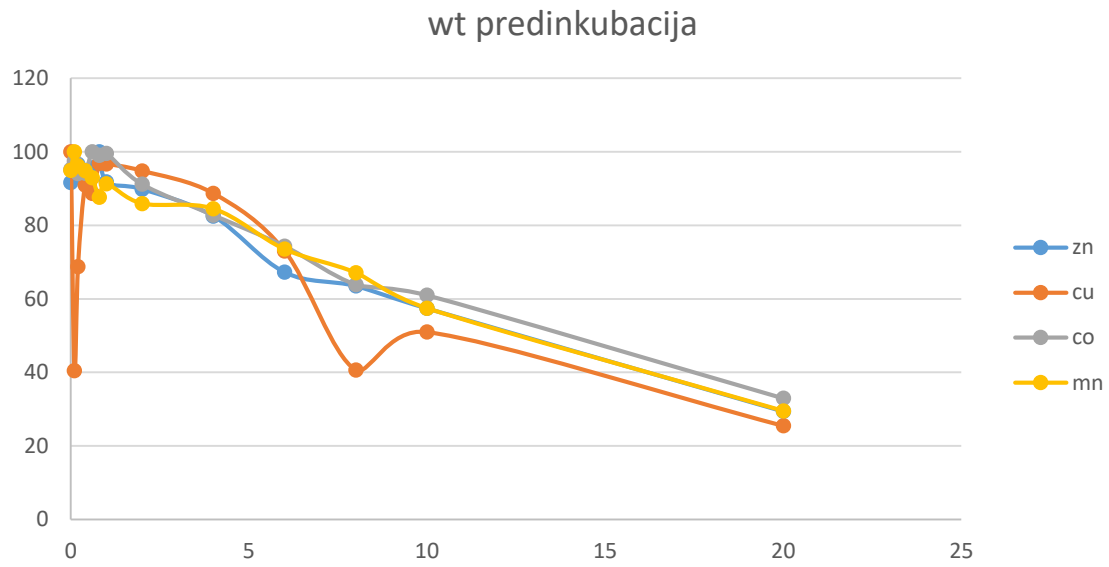
Mn^{2+}

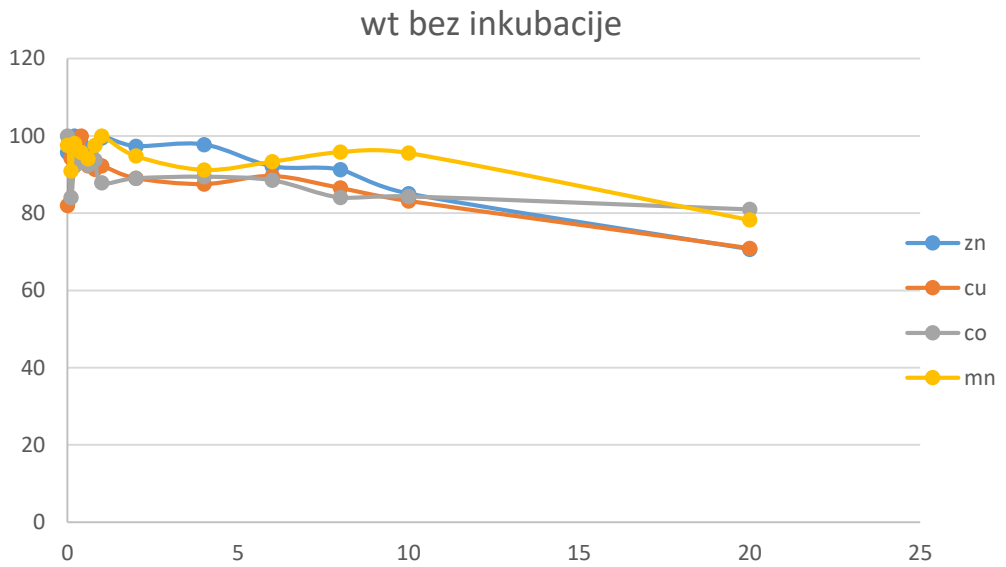
Predinkubacija

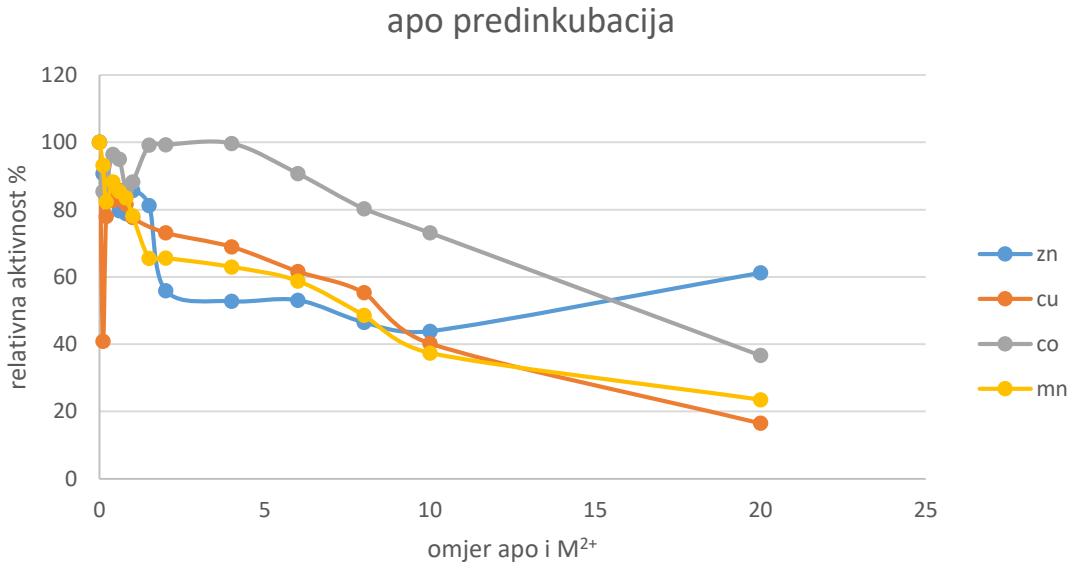


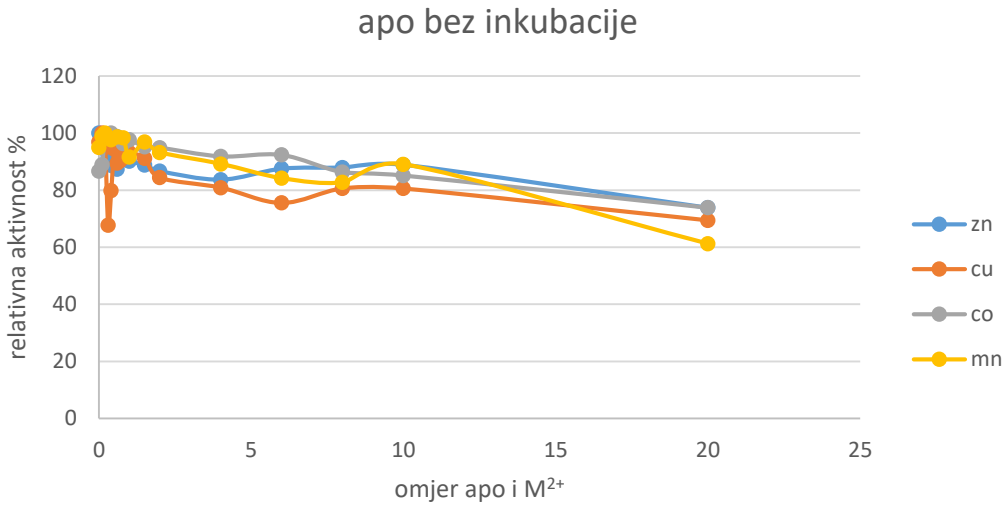
Bezinkubacije




wt predinkubacija
120
100
80
60
40
20
0
0
5
10
15
20
25
zn
cu
co
mn


wt bez inkubacije
120
100
80
60
40
20
0
0
5
10
15
20
25
zn
cu
co
mn


apo predinkubacija
relativna aktivnost %
120
100
80
60
40
20
0
0
5
10
15
20
25
omjer apo i M2+
zn
cu
co
mn


apo bez inkubacije
relativna aktivnost %
120
100
80
60
40
20
0
0
5
10
15
20
25
omjer apo i M2+
zn
cu
co
mn

WT hDPPIII za cink

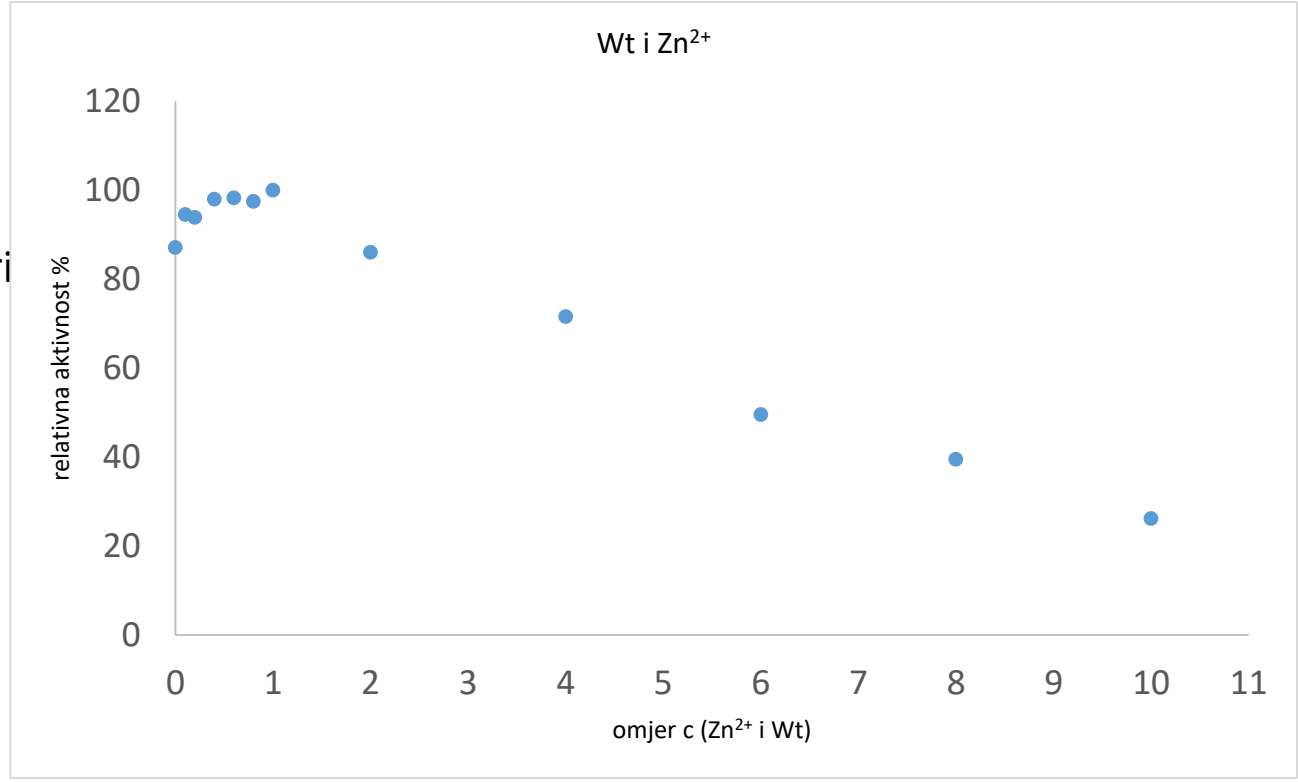
Pufer: 40mM TrisHCl, pH 7,5.

Supstrat: ArgArg-2NA.

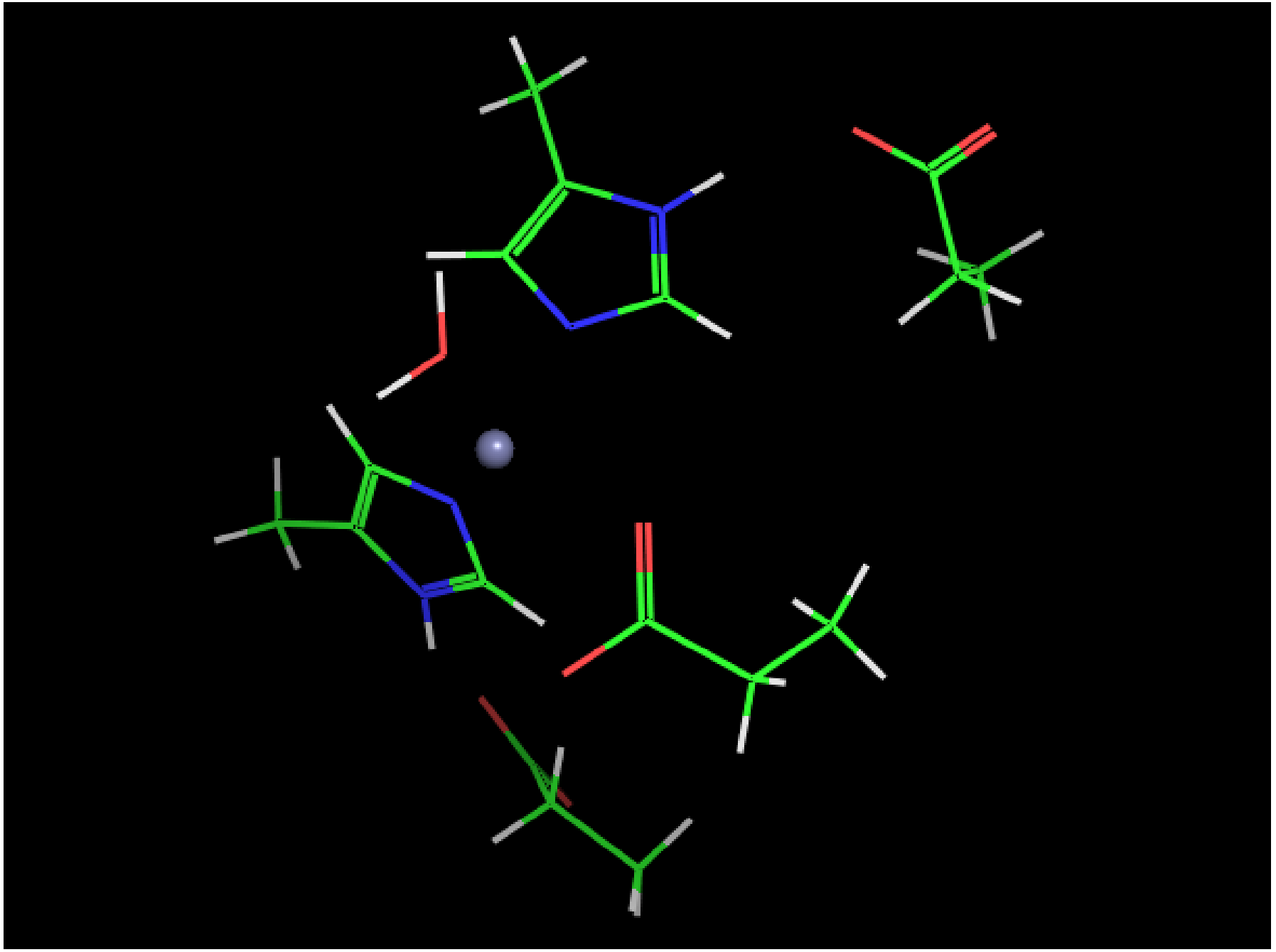
Standardne otopine metalnih iona su nitrati.

Mjerenja su izvršena na 332nm i na sobnoj temperaturi

Predinkbacija



Računalno



	Cu	Co	2HE	Zn	dva Zn	Mn
Optim.	+	+	+	+	+	-
freq	+	+	+	negativna	negativna	-
Zero-point correction (Hartree/Particle)	0.446599	0.447025	0.298605			
Thermal correction to Energy	0.479116	0.478399	0.319970			
Thermal correction to Enthalpy	0.480061	0.479343	0.320915			
Thermal correction to Gibbs Free Energy	0.379395	0.380217	0.246943			
Zero-point vibrational energy	1172544.4 (J/mol)	1173663.6 (J/mol)	783986.2 (J/mol)			
	280.24483 (Kcal/Mol)	280.51233 (Kcal/mol)	187.37720 (Kcal/Mol)			
Sum of electronic and zero-point Energies	-3051.179	-2793.555	-2654.278			
Sum of electronic and thermal Energies	-3051.147	-2793.5234	-2654.2567			
Sum of electronic and thermal Enthalpies	-3051.146	-2793.5225	-2654.2558			
Sum of electronic and thermal Free Energies	-3051.247	-2793.622	-2654.3298			