Proučavanje vezanja metalnih iona (Zn^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}) na humu DPPIII

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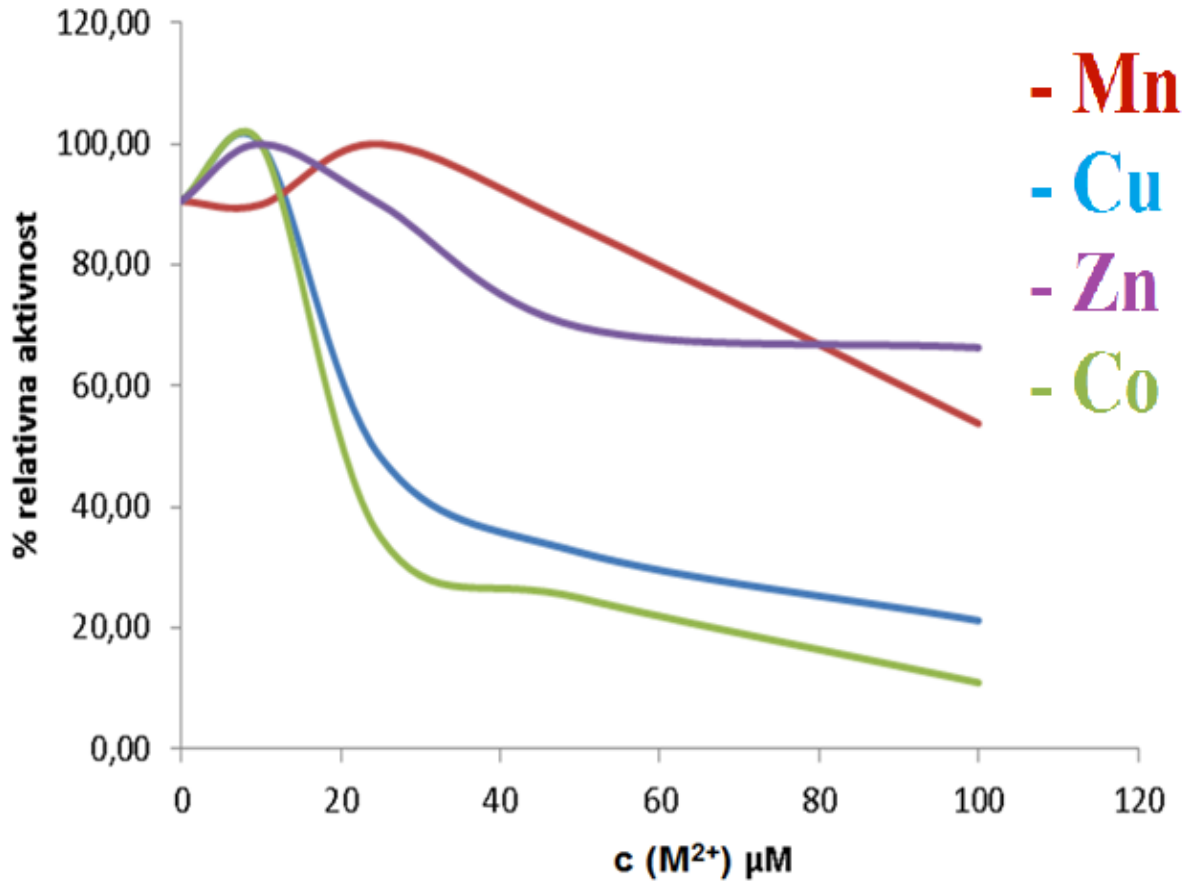
Utjecaj metalnih dikationa na aktivnost divljeg tipa hDPP III.



ICP-MS (IMI)

IZMJERENO IMI:									
UZORCI hDPP3 Mw 81636	umol/L S	umol/L Zn*	%	umol/L Cu*	%	umol/L Mn*	%	umol/L Co*	%
1	39,30	0,12	4,43	0,02	0,56	0,00	0,05	0,03	1,16
2	19,18	0,04	2,60	0,00	0,35	0,00	0,03	0,02	1,54
3	25,14	0,04	2,39	0,00	0,11	0,00	0,12	0,03	1,80
4	37,66	2,57	95,6	0,01	0,55	0,00	0,08	0,05	1,91
5	21,22	0,01	0,50	2,99	196,9	0,00	0,11	0,01	0,64
6	29,14	0,51	24,7	0,01	0,64	0,00	0,08	0,68	32,7
7	29,52	0,63	30,1	0,02	0,85	0,04	1,73	0,03	1,60

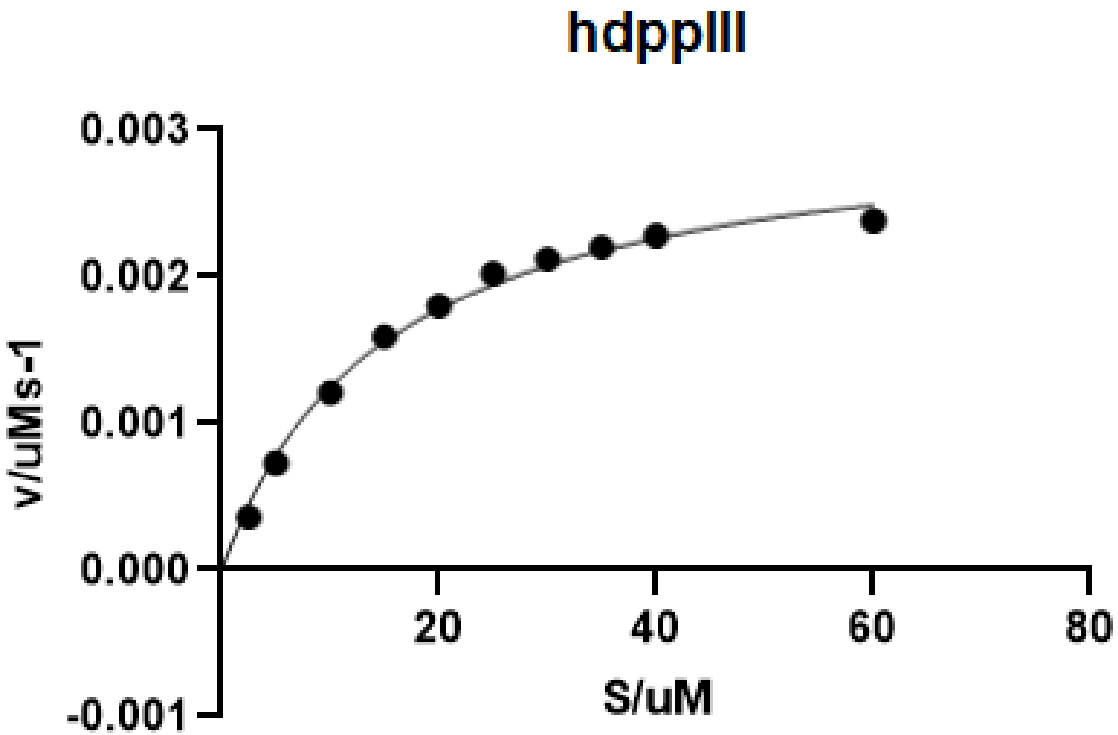
Opis uzorka
1.nativni protein hDPP3
2.hDPP3 dijalizirani uzorak (apo-bez metala-stari)
3.hDPP3 dijalizirani uzorak (apo-bez metala)
4.hDPP3 kojoj je nakon dijalize dodan Zn 6 x ekvivalentan
5.hDPP3 kojoj je nakon dijalize dodan Cu 6 x ekvivalentan
6.hDPP3 kojoj je nakon dijalize dodan Co 6 x ekvivalentan
7.hDPP3 kojoj je nakon dijalize dodan Mn 6 x ekvivalentan

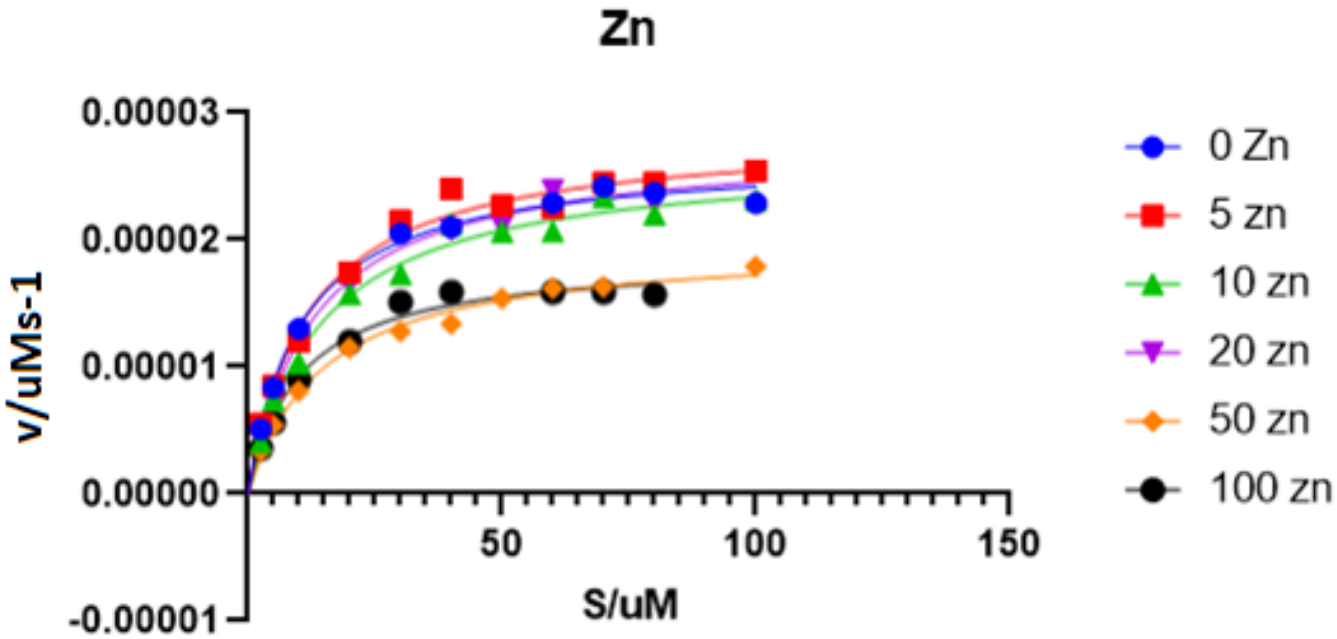
IZMJERENO IMI:													
UZORCI hDPP3 Mw 81636	umol/ L Zn*	%	umol/ L Cu*	%	umol/L Co*	%	umol/ L Mn*	%	**Opis uzorka**				
1	0,45	9,27	0,06	1,14	0,07	1,43	0,01	0,12	**nativni protein hDPP3**				
2	0,12	10,05	0,01	0,80	0,03	2,17	0,00	0,10	**hDPP3 dijalizirani uzorak (apo- bez metala)**				
3	0,72	117,9	0,02	2,99	0,01	0,89	0,00	0,27	**hDPP3 kojoj je nakon dijalize dodan Zn 6 x ekvivalentan**				
4	0,07	8,05	1,69	209,50	0,02	2,41	0,00	0,14	**hDPP3 kojoj je nakon dijalize dodan Cu 6xekvivalentan**				
5	0,39	**48,61**	0,04	4,46	0,27	32,9	0,00	0,07	**hDPP3 kojoj je nakon dijalize dodan Co 6 x ekvivalentan**				
6	0,49	**34,14**	0,04	2,78	0,01	0,74	0,05	3,22	**hDPP3 kojoj je nakon dijalize dodan Mn 6xekvivalentan**				

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

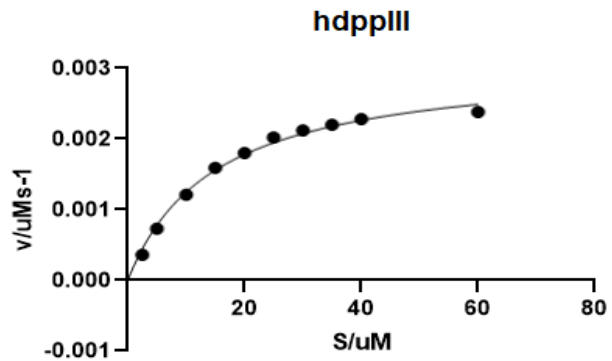
Kinetika na florimetru

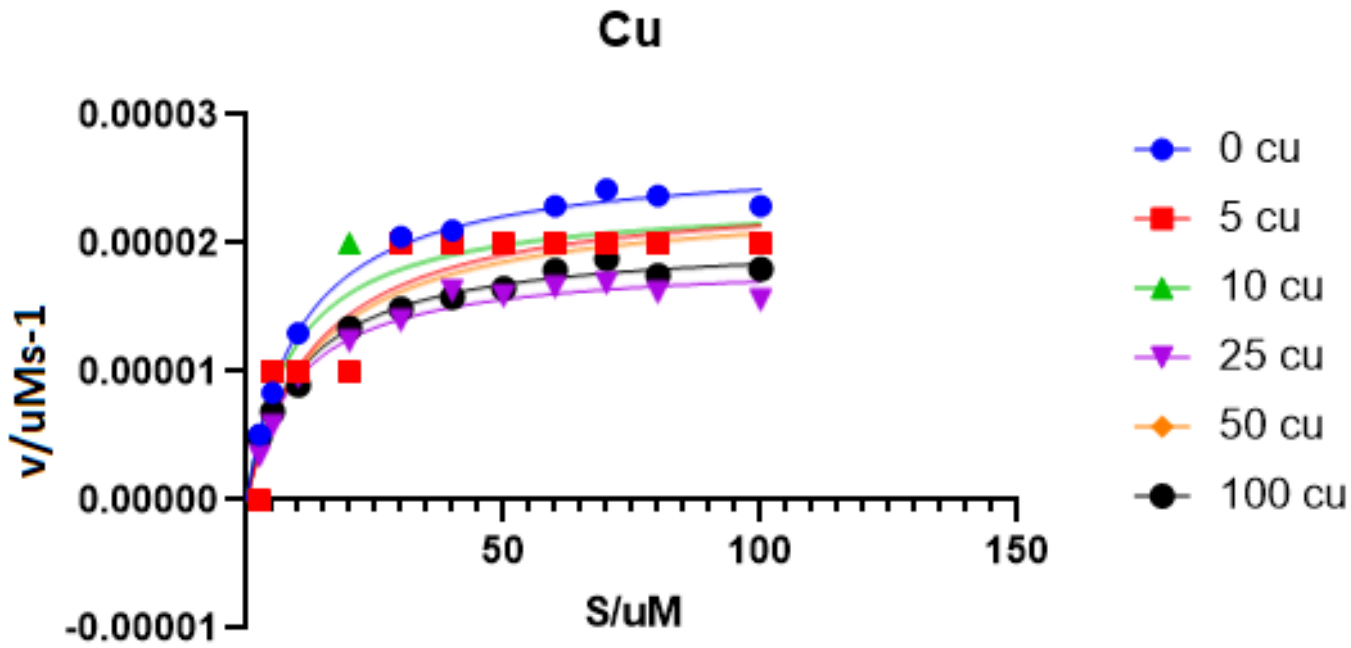
- Wt hDPPIII 20nM
- supstrat ArgArg-2NA

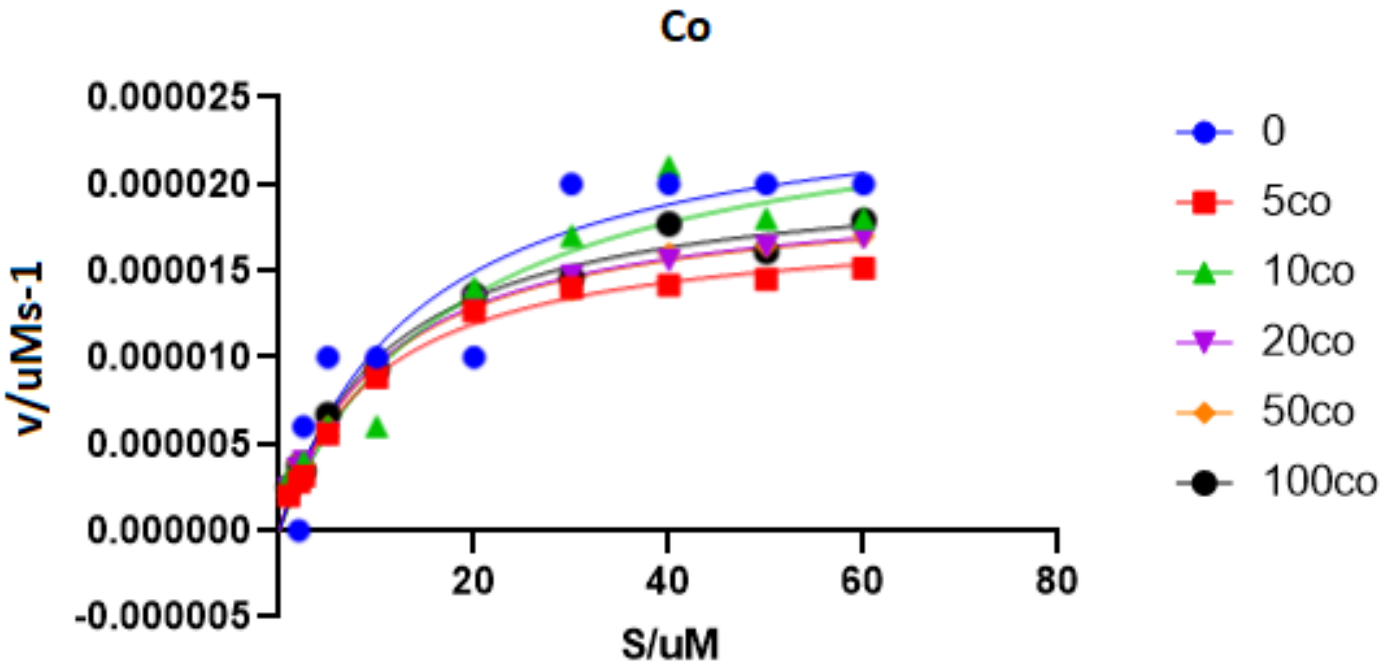


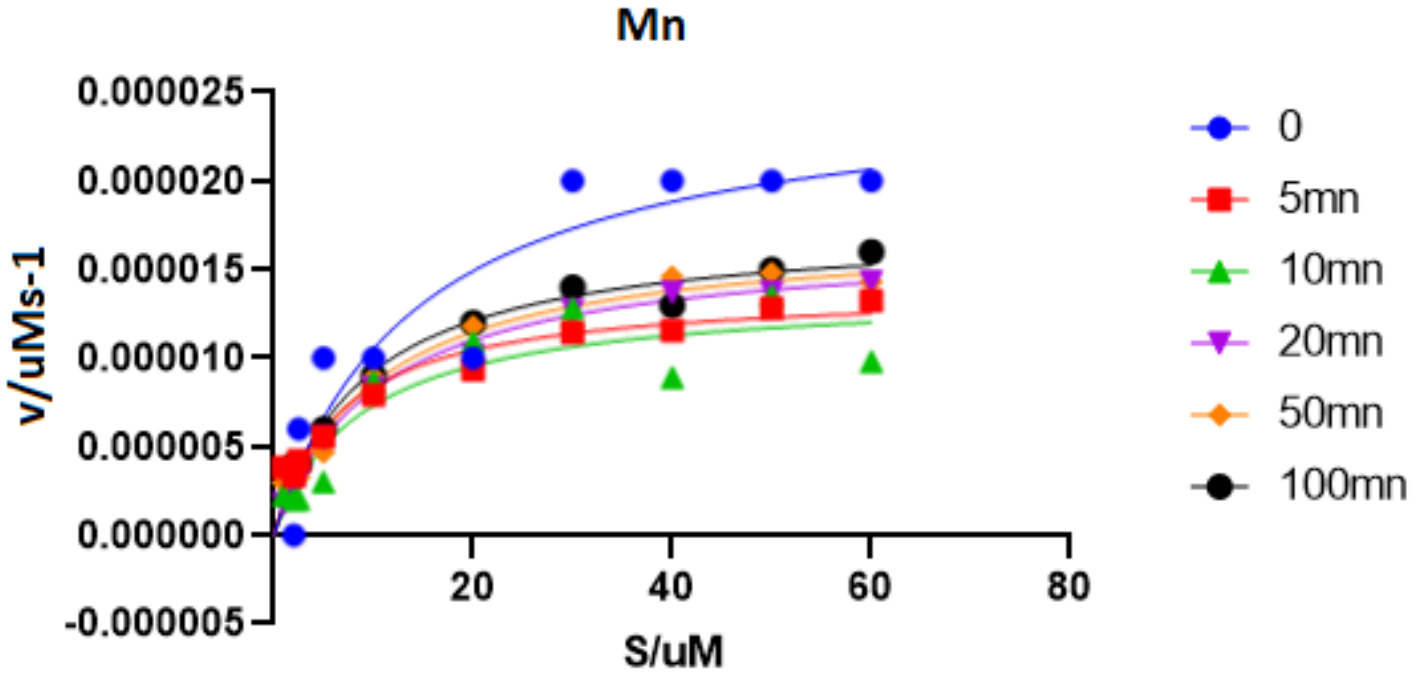

Zn
0.00003
0.00002
0.00001
0.00000
-0.00001
v/uMs-1
50
100
150
S/uM

0 Zn
5 zn
10 zn
20 zn
50 zn
100 zn

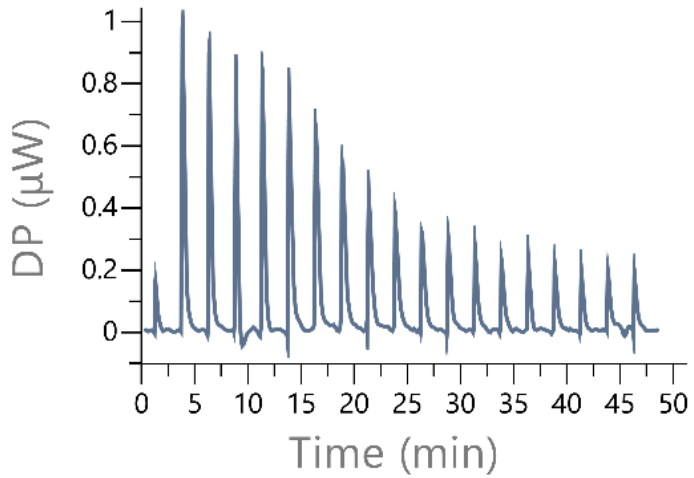

hdpplll
0.003
0.002
0.001
0.000
-0.001
v/uMs-1
20
40
60
80
S/uM

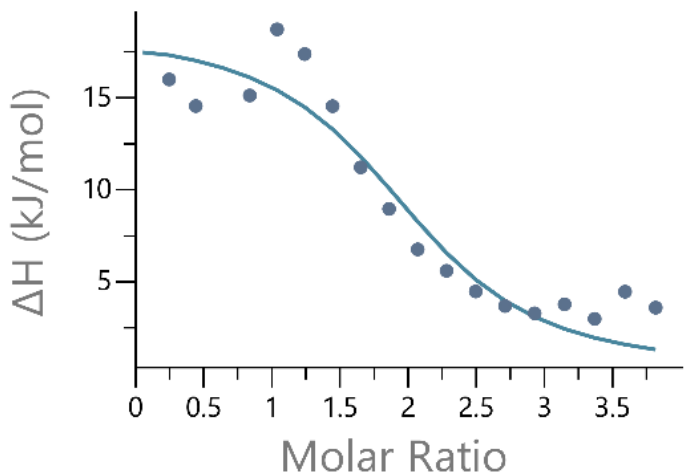

Cu
0.00003
0.00002
0.00001
0.00000
-0.00001
v/uMs-1
50
100
150
S/uM
0 cu
5 cu
10 cu
25 cu
50 cu
100 cu

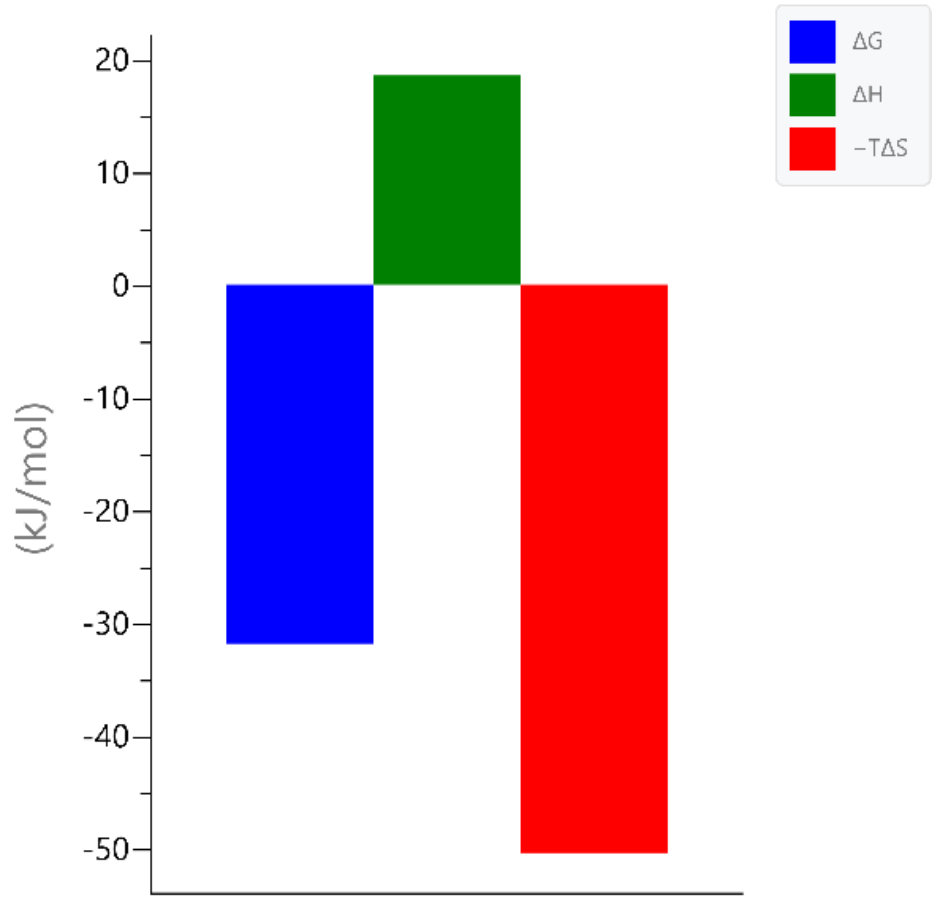

Co
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
-0.000005
v/uMs-1
20
40
60
80
S/uM
0
5co
10co
20co
50co
100co


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

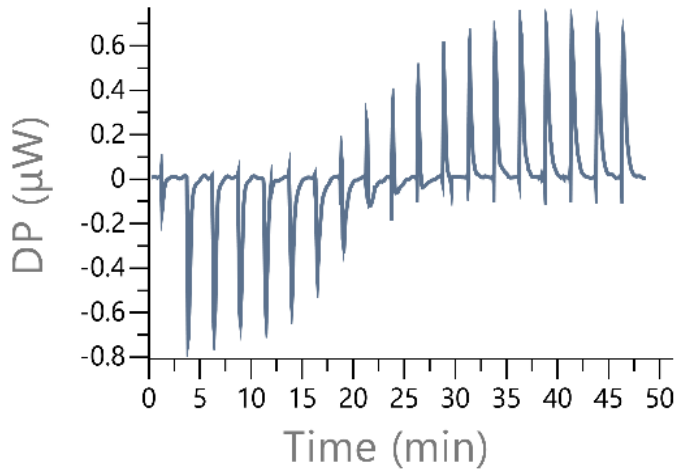
ITC (cink)

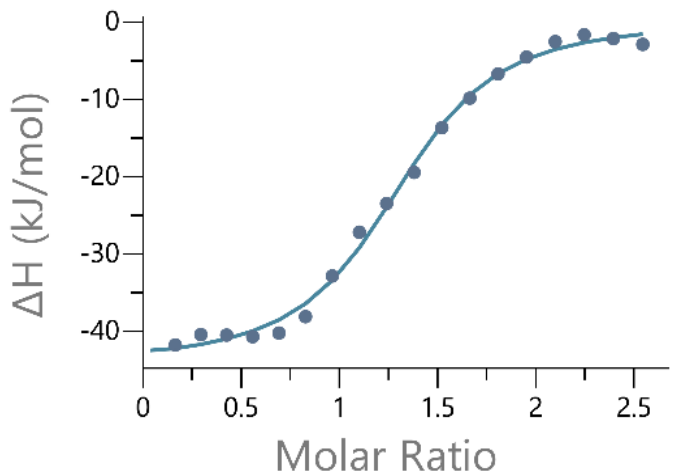

DP (µW)
1
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45 50
Time (min)

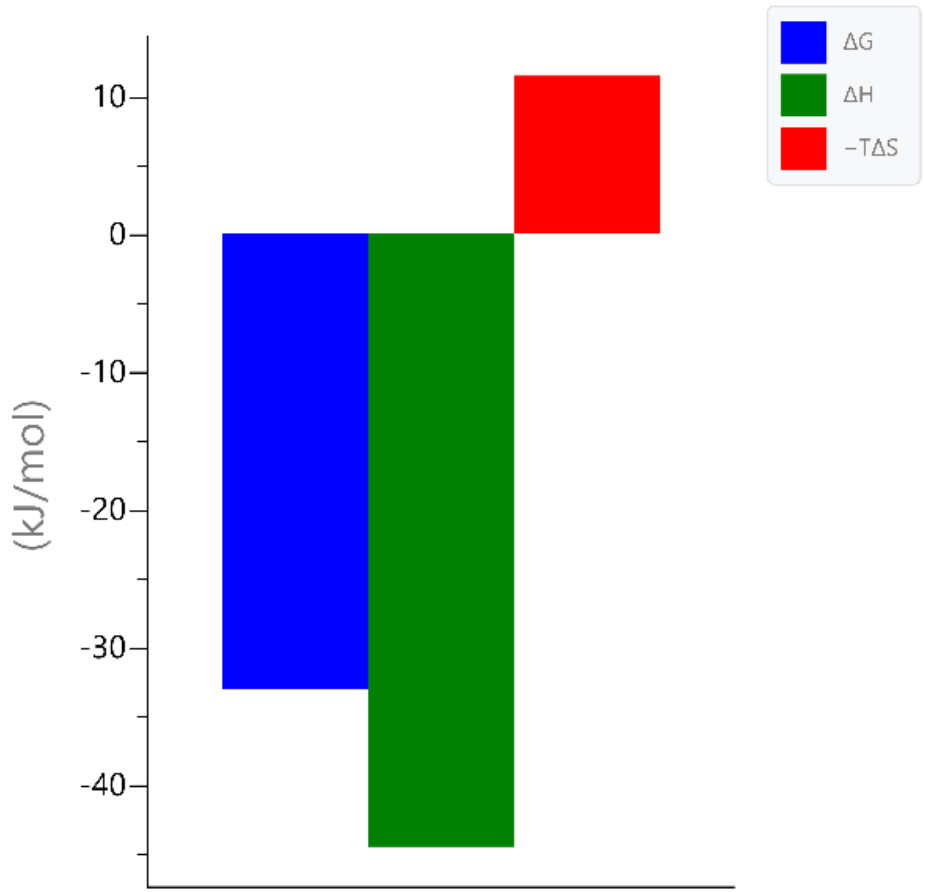

ΔH (kJ/mol)
15
10
5
0 0.5 1 1.5 2 2.5 3 3.5
Molar Ratio


ΔG
ΔH
−TΔS
(kJ/mol)
20
10
0
-10
-20
-30
-40
-50

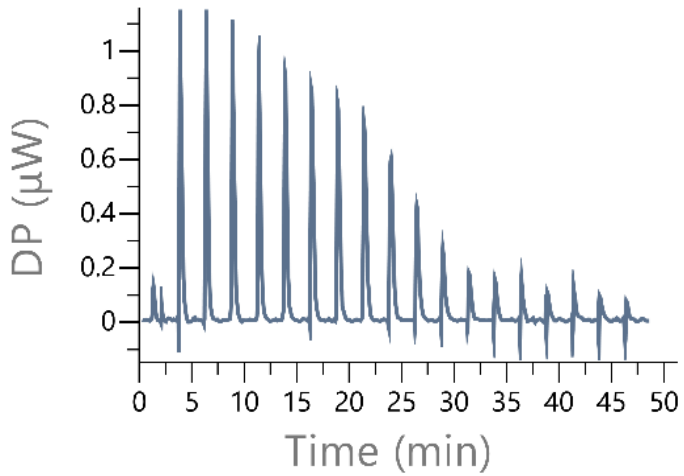
Bakar

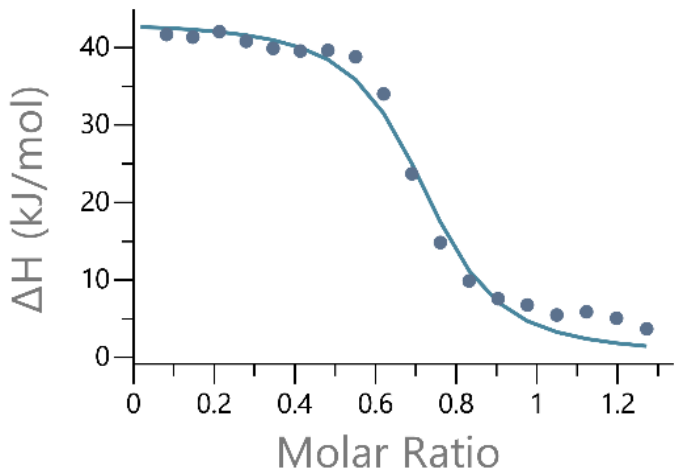

DP (µW)
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
0 5 10 15 20 25 30 35 40 45 50
Time (min)

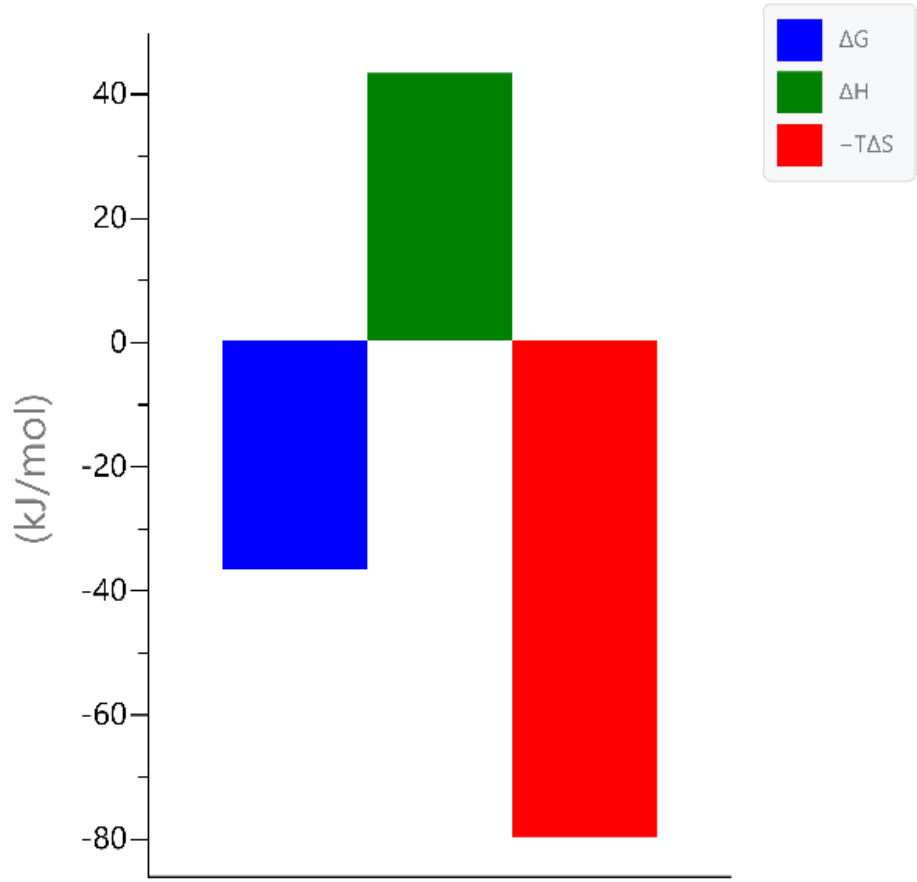

ΔH (kJ/mol)
0
-10
-20
-30
-40
0 0.5 1 1.5 2 2.5
Molar Ratio


ΔG
ΔH
−TΔS
(kJ/mol)
10
0
-10
-20
-30
-40

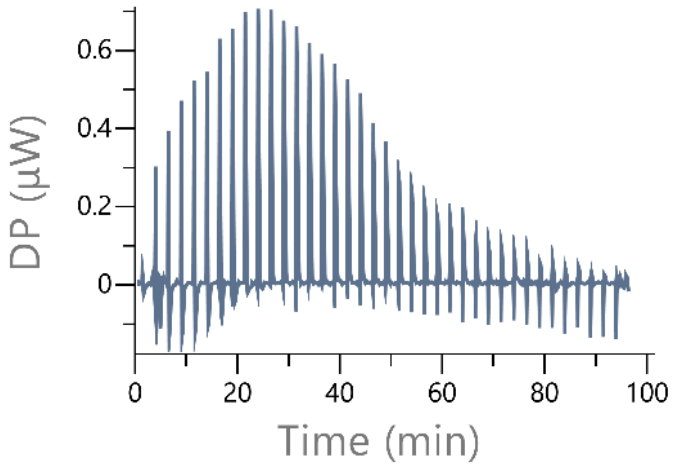
Kobalt

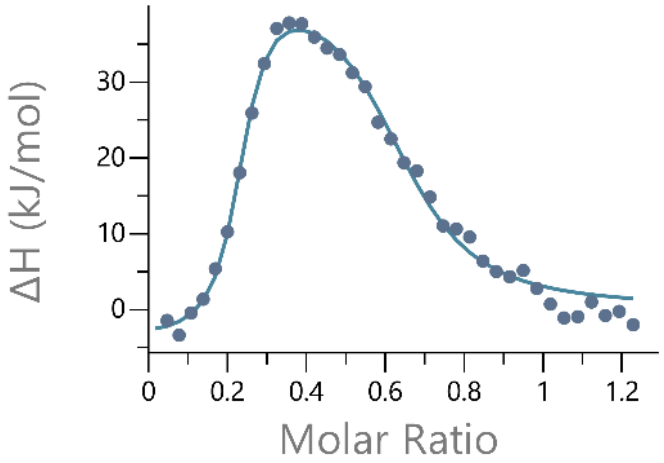

DP (µW)
1
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45 50
Time (min)


ΔH (kJ/mol)
40
30
20
10
0
0 0.2 0.4 0.6 0.8 1 1.2
Molar Ratio


ΔG
ΔH
−TΔS
(kJ/mol)
40
20
0
-20
-40
-60
-80

Mangan

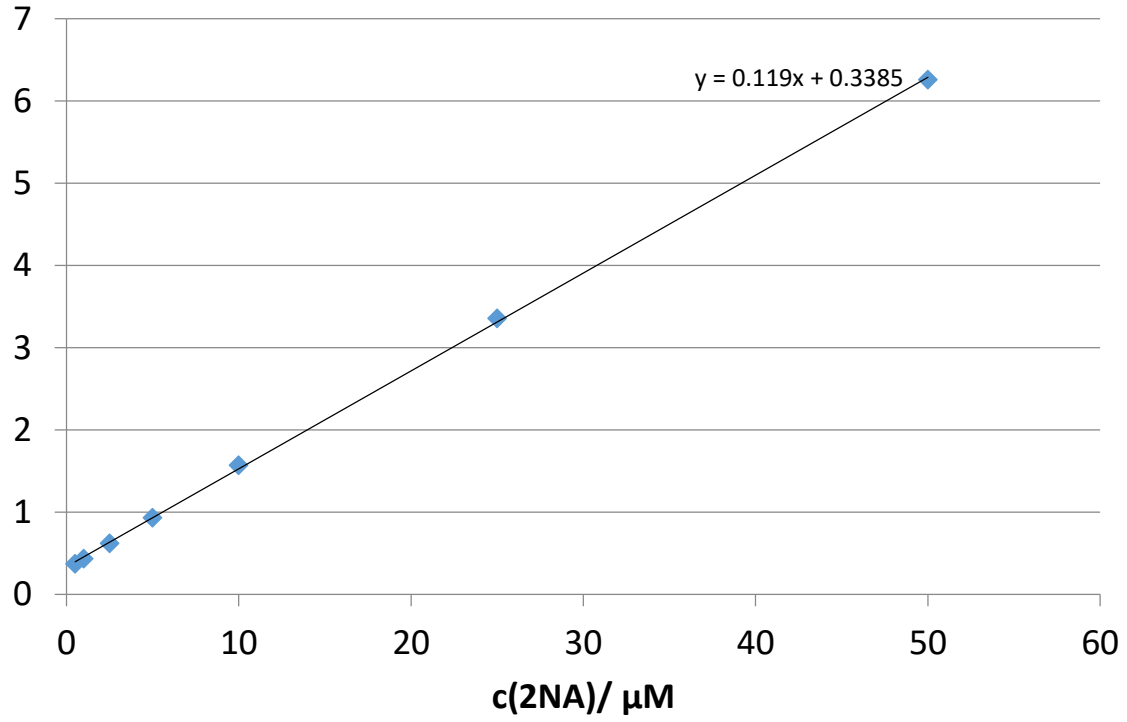

DP (µW)
0.6
0.4
0.2
0
0
20
40
60
80
100
Time (min)


ΔH (kJ/mol)
30
20
10
0
0
0.2
0.4
0.6
0.8
1
1.2
Molar Ratio

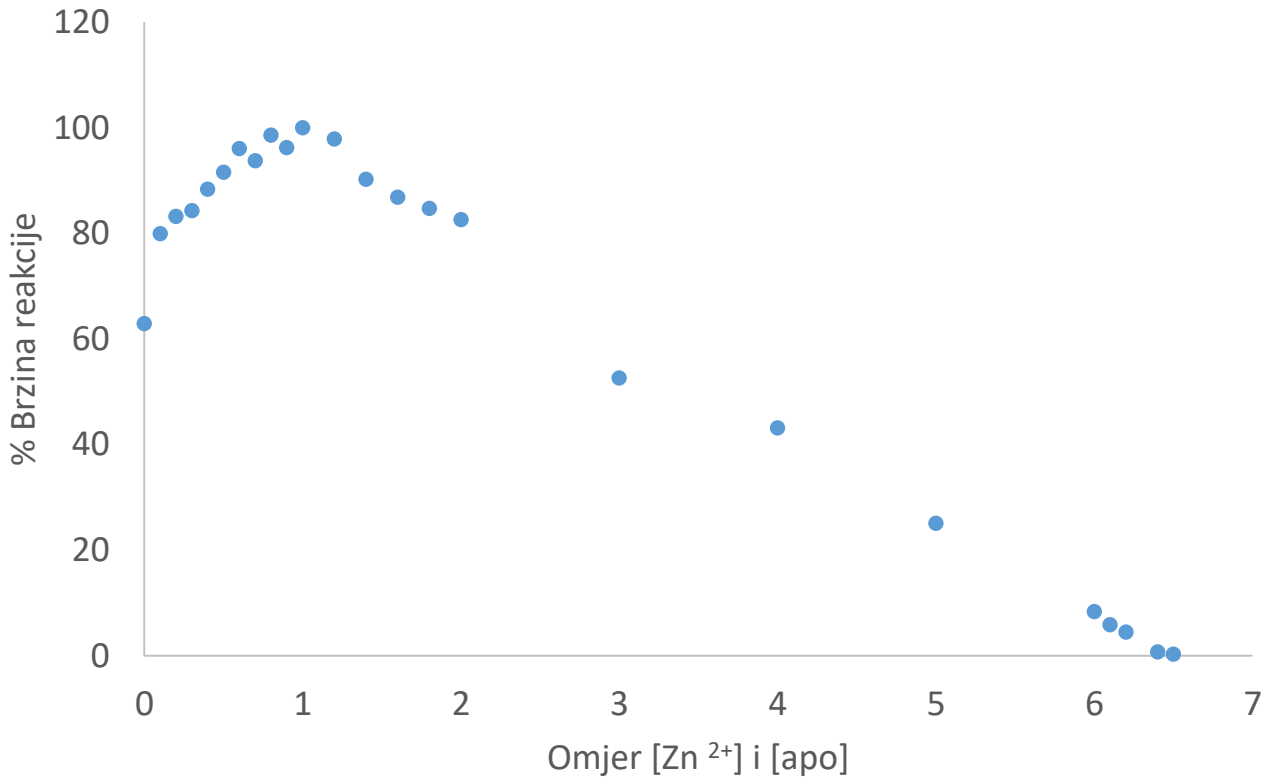
	N	Kd/ uM
Zn	2,00+/-0,08	5,29+/-2,36
Cu	1,2+/-0,10	1,65+/-0,04
Co	0,663+/-0,013	0,383+/-0,055
Mn	0,213+/-0,008	0,0127+/-0,0036
	0,435+/-0,021	0,984+/-0,141

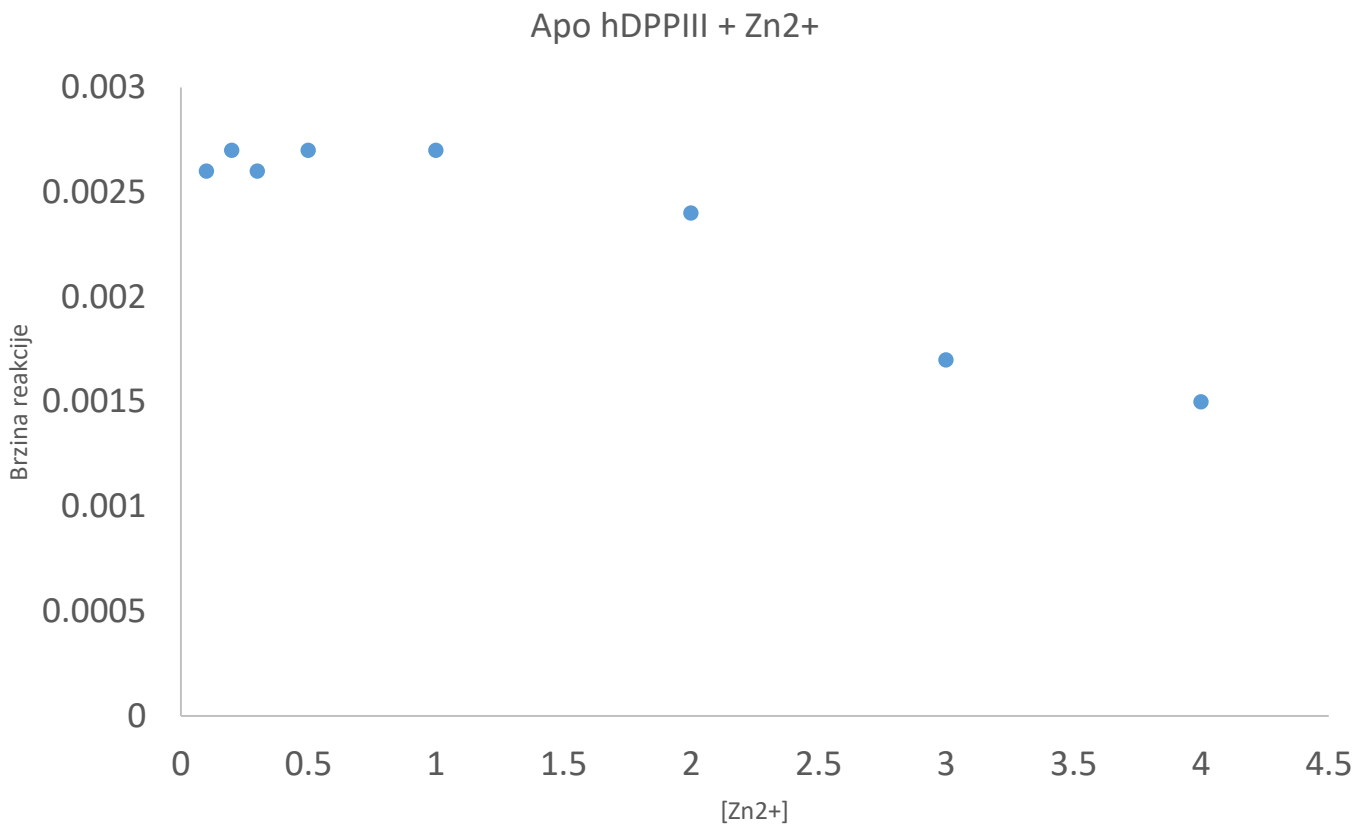
Stopped flow (PMF)

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




Apo hDPPIII + Zn2+
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
Brzina reakcije
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
[Zn2+]