NEUROPEPTIDI KAO POTENCIJALNI SUPSTRATI I INHIBITORI LJUDSKE DPP3

Zrinka Karačić, 23. rujna 2022.

Minisimpozij DPP3

NAŠ NOVI RAD PRIHVAĆEN!

ELSEVIER

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Neuropeptides, substrates and inhibitors of human dipeptidyl peptidase III, experimental and computational study — A new substrate identified

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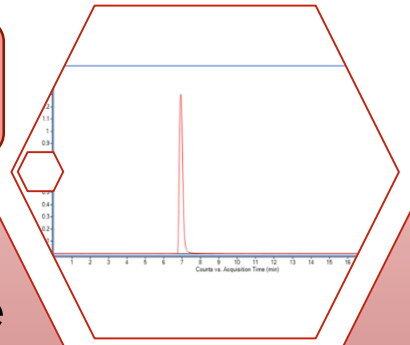
METODE

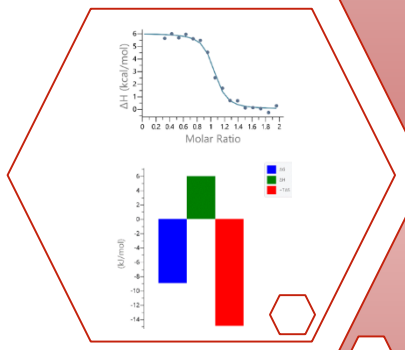
Ana Tomašić Paić
Lidija Brkljačić



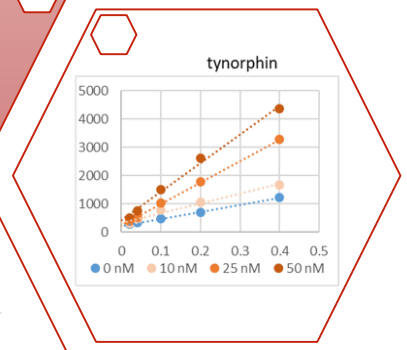
Cijepanje
HPLC-MS

Inhibicija
FLUO

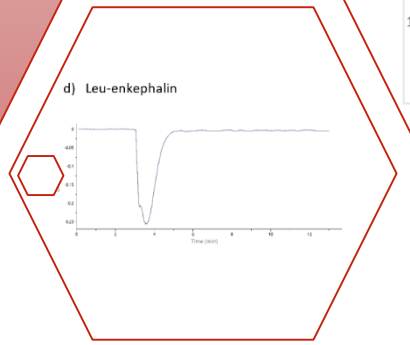
Mirsada Ćehić



Kinetika
ITC



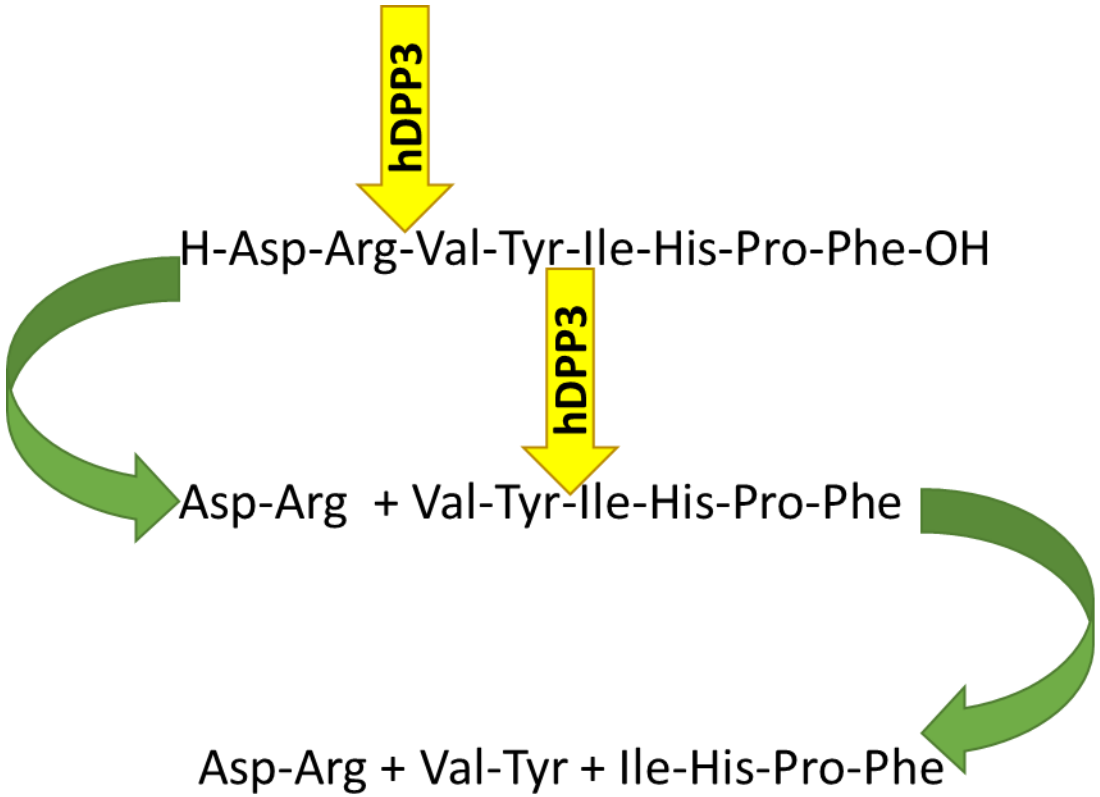
Vezanje
ITC



Filip
Šupljika

CIJEPANJE

- 1 mM peptidi inkubirani uz veliku koncentraciju enzima (0,18 µM) 2h (ili manje) i 24h
- HPLC-MS korišten za određivanje količine preostalog peptida, kao i detekciju produkata odgradnje
- U 200 µL reakcijske smjese dodano 100 uL acetonitrila za zaustavljanje enzimske reakcije, pa pohranjeno na -20°C
- Primjer: angiotenzin II



INHIBICIJA

- Inhibicija peptidima kao kompetitivnim inhibitorima – mjerenje brzine reakcije cijepanja supstrata Arg_2-2NA uz 3 različite koncentracije inhibitora
- Određivanje K_I nelinearnom regresijom
- Primjer: inhibicija nM tinorfinom

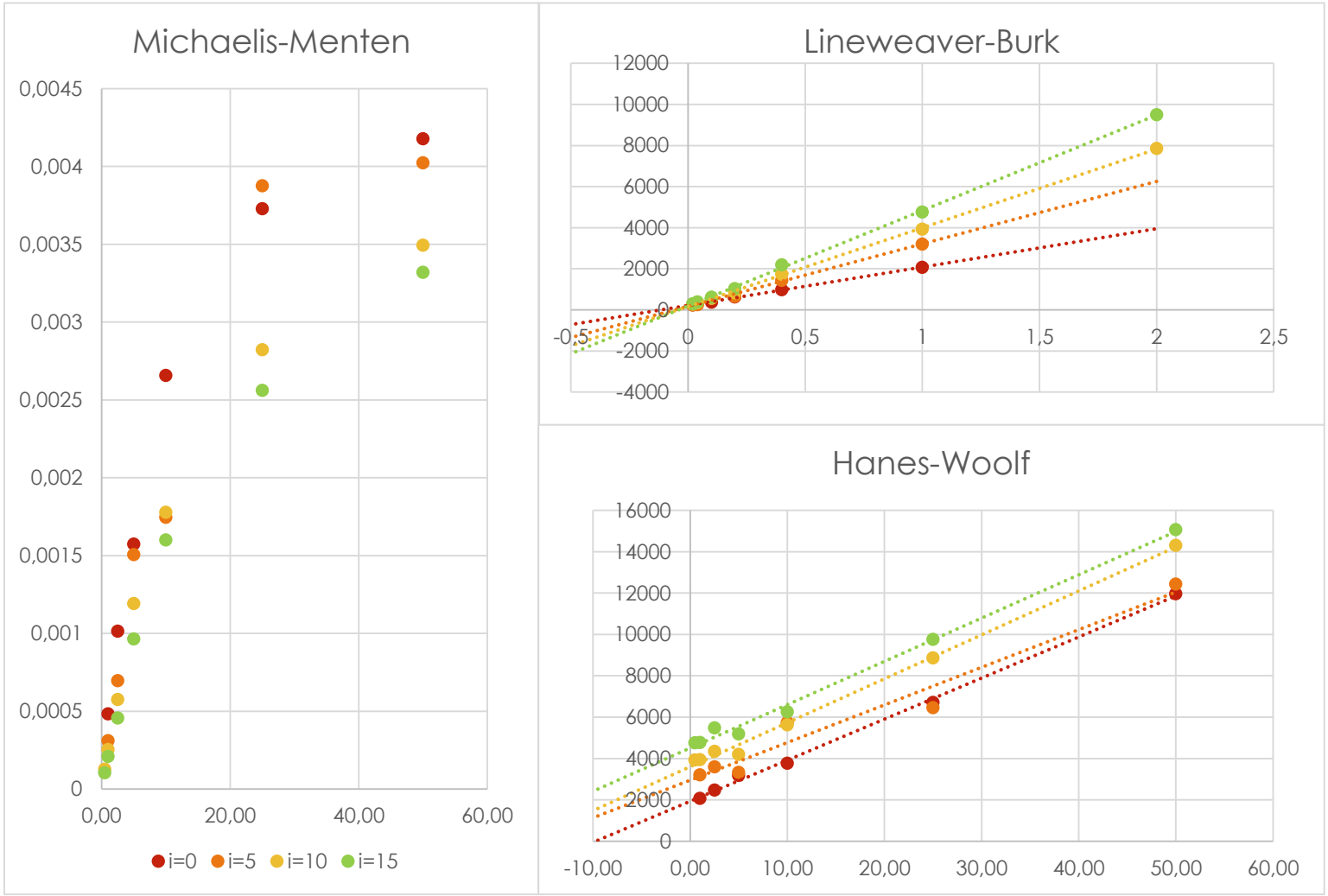


Table 1

Interaction of human DPP III with neuropeptides: peptide degradation was studied using HPLC-MS, and inhibition constants K_i were measured for inhibition of human DPP III in reaction with a fluorescent substrate analogue. The peptides are sorted according to increasing K_i.

Peptide	Sequence	Cleaved[a]	K_i/μM[b]
I-tynorphin	IVYPW	Y	0.00045 ± 0.00005
S-tynorphin	SVYPW	Y	0.0077 ± 0.0007
Tynorphin	VVYPW	Y	0.0112 ± 0.0008
Valorphin	VVYPWTQ	Y	0.0365 ± 0.0029
β-Casomorphin	YPFVEPI	Y	1.0 ± 0.1
Angiotensin II	DRVYIHPF	Y	4.4 ± 0.5
Leu-valorphin-Arg	LVVYPWTQR	Y	5.2 ± 0.5
Hemorphin-4	YPWT	Y	6.5 ± 0.7
Endomorphin-2	YPFF	Y	10.4 ± 1.0
Leu-enkephalin	YGGFL	Y	10.4 ± 1.4
Arg-vasopressin	C*YFQNC*PRG	N	n.d.[c]
Hemopressin	PVNFKFLSH	N	n.d.
β-Neoendorphin	YGGFLRKYP	N	n.d.

* Denoting a disulfide bridge.

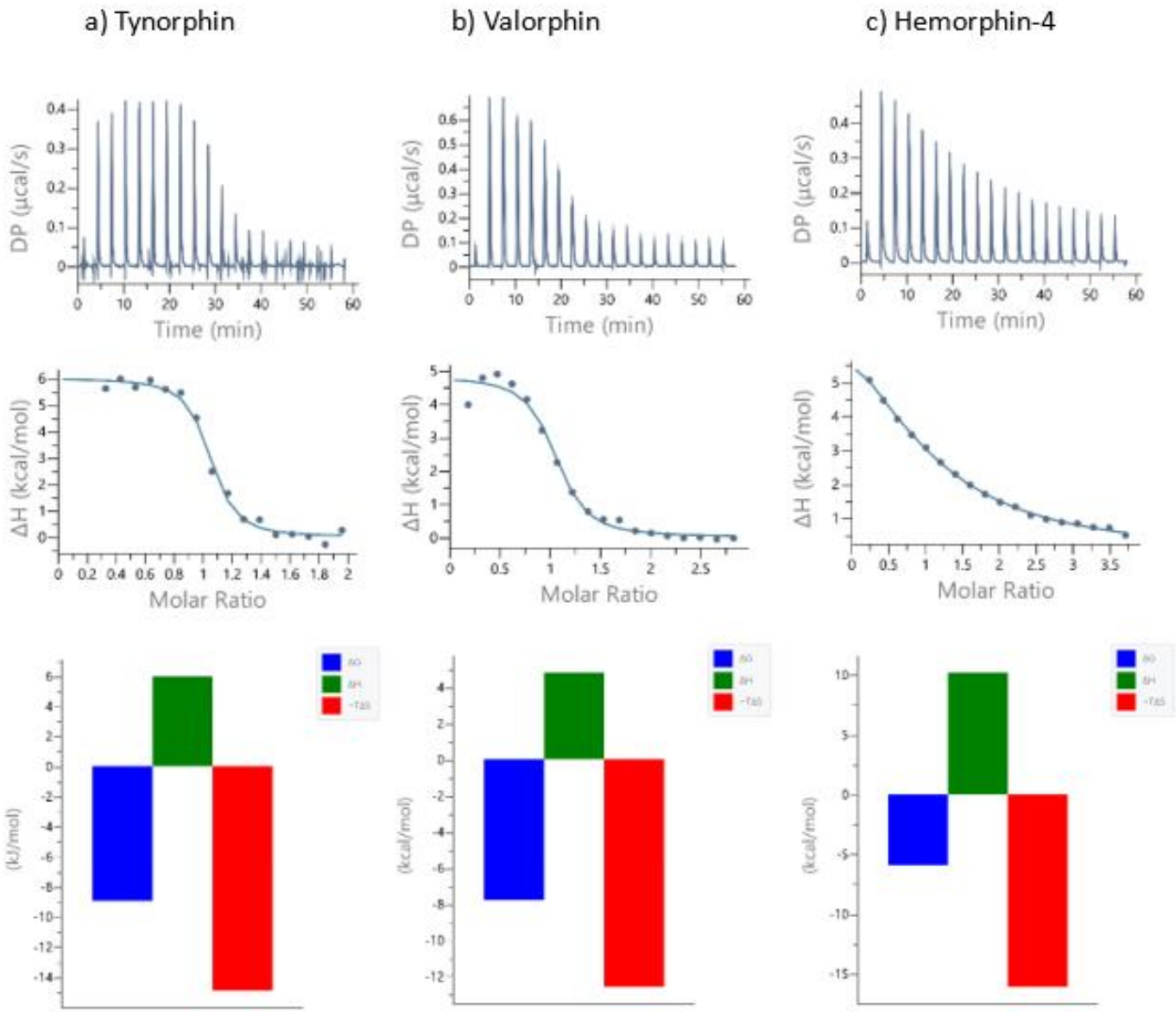
[a] Cleavage (Y yes or N no) determined by HPLC-MS as reduction of peptide amount after incubation of 1 mM peptide with 0.18 μM enzyme after 24 h at 25 °C in ammonium bicarbonate buffer pH = 7.4.

[b] Inhibition constant for inhibition of enzyme-catalyzed cleavage of artificial substrate Arg_2–2NA at 25 °C in 20 mM TrisHCl buffer pH = 7.5.

[c] No inhibition trend detected with peptide in the range of 1–50 μM.

VEZANJE

- Mikrokalorimetrijska mjerenja (ITC = izotermalna titracijska kalorimetrija) interakcije peptida i inaktivnog mutanta E451A
- Određeni parameti: konstanta vezanja, reakcijska Gibbsova energija, entalpija i entropija
- Pretpostavka N=1
- Endotermni proces, entropijski vođen



ITC VEZANJE

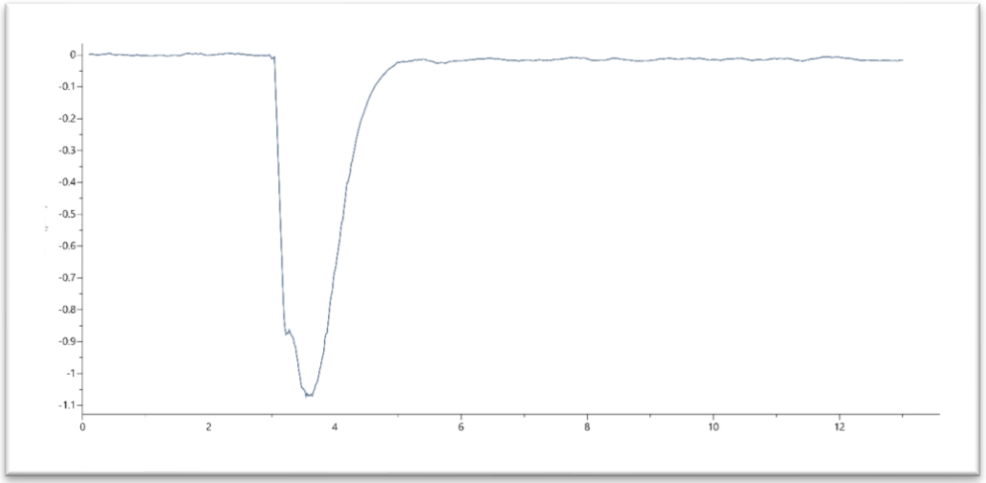
Table 2
Thermodynamic parameters of peptide binding to human DPP III at 25 °C and pH = 7.5 in 20 mM TrisHCl buffer.

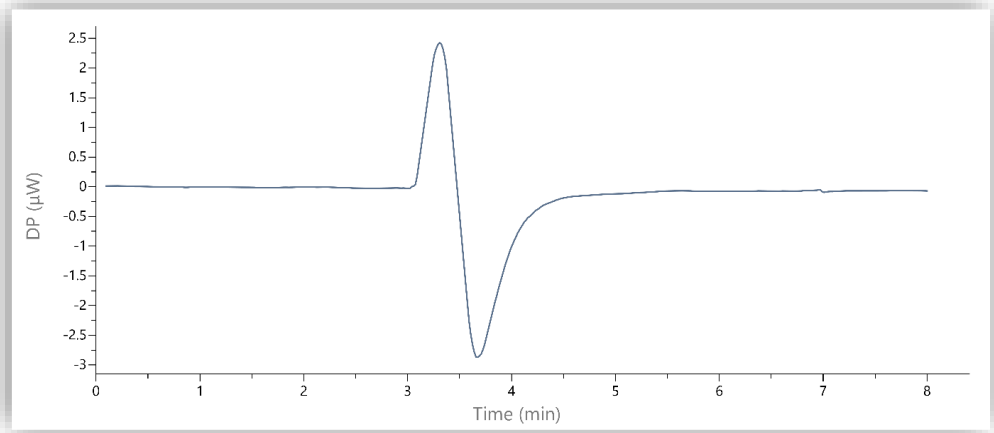
Peptide	K_d/μM	$\Delta_r H$/ kcal mol^{-1}	$\Delta_r G$ / kcal mol^{-1}	$-T^*\Delta_r S$ / kcal mol^{-1}
I-tynorphin	0.0973 ± 0.0091	8.01 ± 0.24	−9.58 ± 0.05	−17.6 ± 0.2
S-tynorphin	.298 ± 0.061	5.69 ± 0.24	−8.91 ± 0.12	−14.7 ± 0.4
tynorphin	0.386 ± 0.127	6.19 ± 0.29	−8.77 ± 0.19	−15.0 ± 0.1
Valorphin	1.78 ± 0.21	4.64 ± 0.19	−7.86 ± 0.07	−12.5 ± 0.1
Angiotensin II	2.22 ± 0.24	6.17 ± 0.72	−7.72 ± 0.07	−13.9 ± 0.6
Leu-valorphin-Arg	2.50 ± 1.92	4.61 ± 2.15	−7.77 ± 0.45	−12.4 ± 1.8
Hemorphin-4	9.4 ± 14.6	8.70 ± 1.82	−6.05 ± 0.24	−14.7 ± 1.6
Endomorphin-2	0.1 ± 4.8			
Leu-enkephalin	118 ± 39			
β-Casomorphin	130 ± 87			
Arg-vasopressin	n. d.			
Hemopressin	n. d.			
β-Neoendorphin	n. d.			

n.d. – binding not detected.

KINETIKA

- Kalorimetrijski - ITC
- SIM – single injection – potpuna konverzija supstrata u produkt
- Primjer: SIM Leu-enkefalin i tinorfin





Spori supstrati:

- I/S/-tinorfin
- valorfin
- β-kazomorfin

Table 3

Kinetic parameters of peptide degradation as measured by ITC using SIM at 25 °C in 50 mM Tris-HCl buffer with 100 mM NaCl and pH = 8.0.

Peptide	$\Delta_r H$/kcal mol^{-1}	K_M/µM	k_{cat}/s^{-1}	(k_{cat}/K_M)/s^{-1} M^{-1}
Leu-valorphin-Arg	−1.53 ± 0.07	33.9 ± 6.4	0.35 ± 0.09	1.03···10^4
Leu-enkephalin	−1.57 ± 0.02	34.7 ± 5.7	1.08 ± 0.12	3.11···10^4
Hemorphin-4	−1.79 ± 0.17	55.1 ± 13.1	6.11 ± 0.96	1.11···10^5

KINETIKA

- MIM – multiple injection – djelomična pretvorba do 5% supstrata u produkt tijekom svake injekcije supstrata u otopinu enzima
- Primjer: MIM Leu-enkefalin

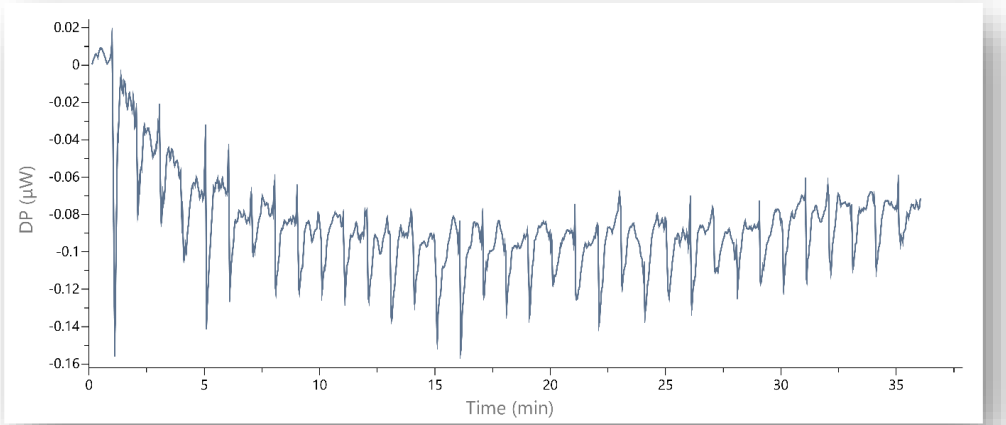


Table S2. Kinetic parameters of peptide degradation as measured by ITC using MIM at 25 °C in 50 mM TrisHCl buffer with 100 mM NaCl and pH = 8.0

peptide	K_M / µM	k_{cat} / s^{-1}	(k_{cat}/K_M) / $s^{-1} M^{-1}$
Leu-valorphin-Arg	27.3	0.54	$1.98 \cdot 10^4$
Leu-enkephalin	33.7	2.38	$7.06 \cdot 10^4$
Hemorphin-4	75.6	5.21	$6.89 \cdot 10^4$

ZAKLJUČCI

- Uspješno uvedene nove metode:
  - Mikrokalorimetrija za praćenje klinetike peptidazne enzimske reakcije – SIM i MIM
  - HPLC-MS za ispitivanje peptida kao supstrata
- Postojeće metode nadopunjuju se s novima
- Potvrđen hemorfin-4 kao do sada nepoznati supstrat hDPP III

ŠTO DALJE?

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ORIGINAL PAPER

A Group of Weakly Bound to Neurons Extracellular Metallopeptidases (NEMPs)

Ekaterina S. Kropotova[1,2] · Mark I. Mosevitsky[1,2]

- Endogeni inhibitori enkefalinaza:
- **Opiorfin** i sialorphin: **QRFSR** i QHNPR
  - inhibitor NEP i APN

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Dipeptidylamino-tripeptidylcarboxypeptidase NEMP3 and DPP3 (DPP III) are the same protein

Ekaterina S. Kropotova [a, b], Ekaterina N. Pavlova [b], Stanislav N. Naryzhny [a], Mark I. Mosevitsky [a, b, *]