DIFFERENCES IN DPP III SPECIFICITY TOWARD NEUROPEPTIDES

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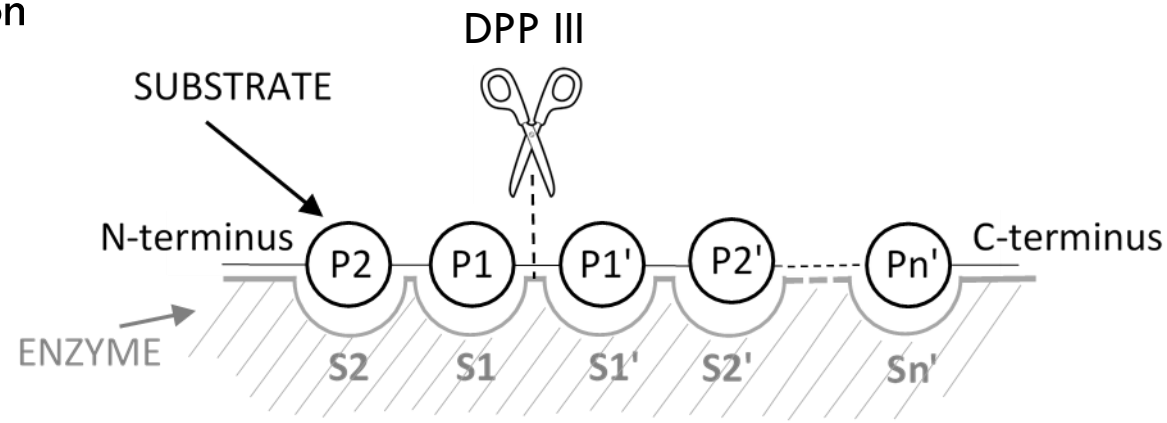
23.09.2022, IRB

BROAD SUBSTRATE SPECIFICITY

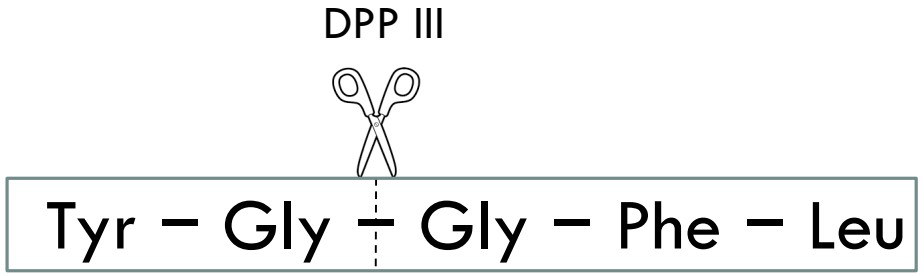
A preference for (*in vitro*):

- a positively charged N-terminus,
- the ability of the substrate to form β-sheet secondary structure
- hydrophobic AA residues at the P1' position
- a PRO residue at the P1 position



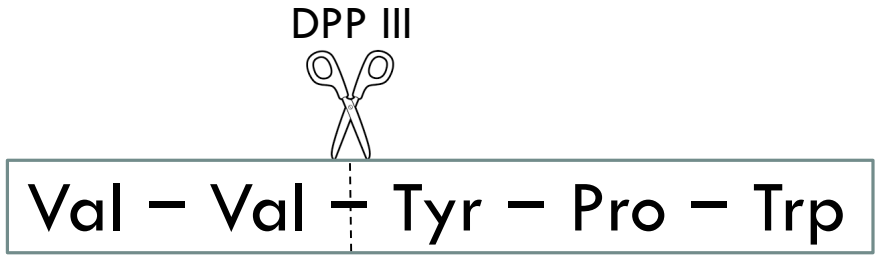
1. MECHANISM OF HYDROLYSIS

LEU-ENKEPHALIN
(„GOOD" SUBSTRATE)



- Baršun et al., *Biol. Chem.* 388 (**2007**)
  K_m = 6.5 μM
  k_{cat} = 9.0 s^{-1}

TYNORPHIN
(„SLOW" SUBSTRATE)

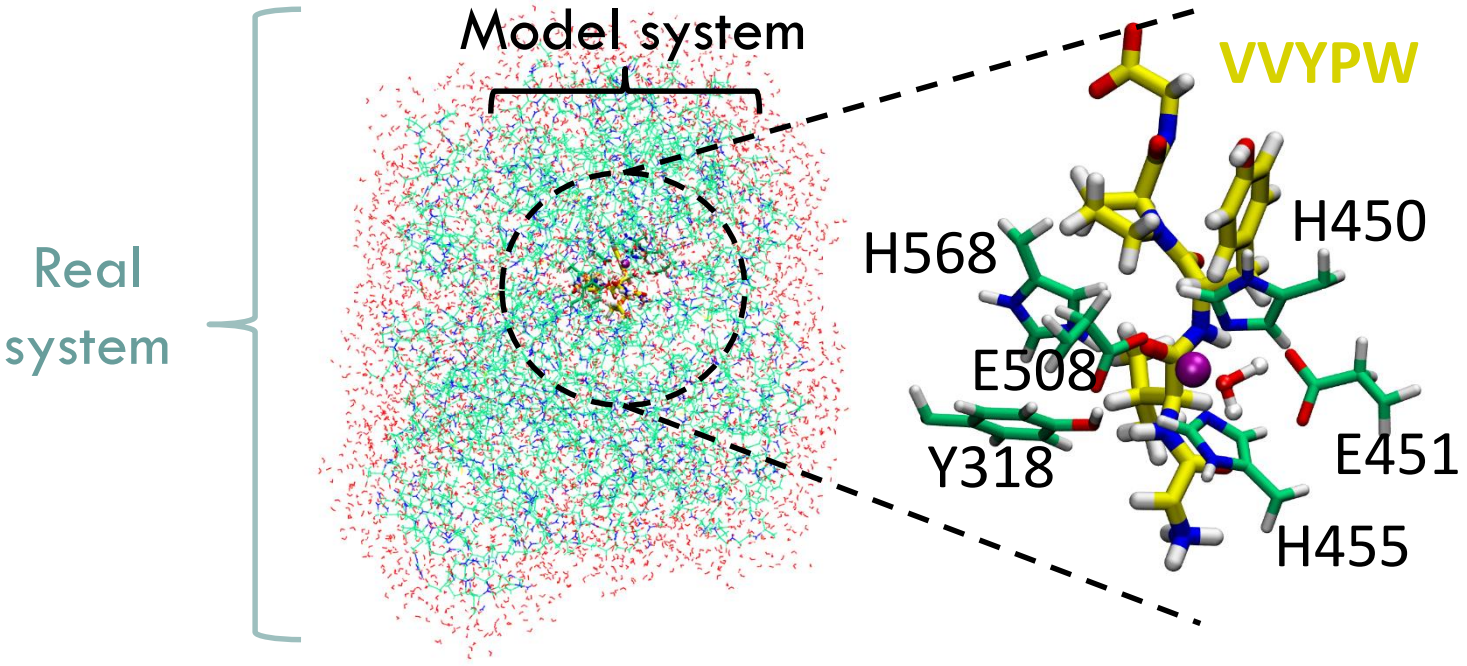


- Jha et al. JBC **2020** → mice DPP III
- Y. Yamamoto et al . *Peptides* **2000** → DPP III from a rat brain K_i (VVYPW)= 7.5 × 10^{-8} mol L^{-1}
- T. Chiba et. al. *Peptides* **2003** → recombinant DPP III
  K_i(VVYPW) = 2.67 ± 0.58 μM
  K_i(IVYPW) = 0.100 ± 0.011 μM
  K_i(WVYPW) = 0.126 ± 0.015 μM

1. MECHANISM OF HYDROLYSIS

$$E_{high,real} \approx E_{ONIOM} = E_{low,\ real} + E_{high,\ model} - E_{low,\ model}$$

QM/MM (2-layer ONIOM) CALCULATIONS (Gaussian 09)



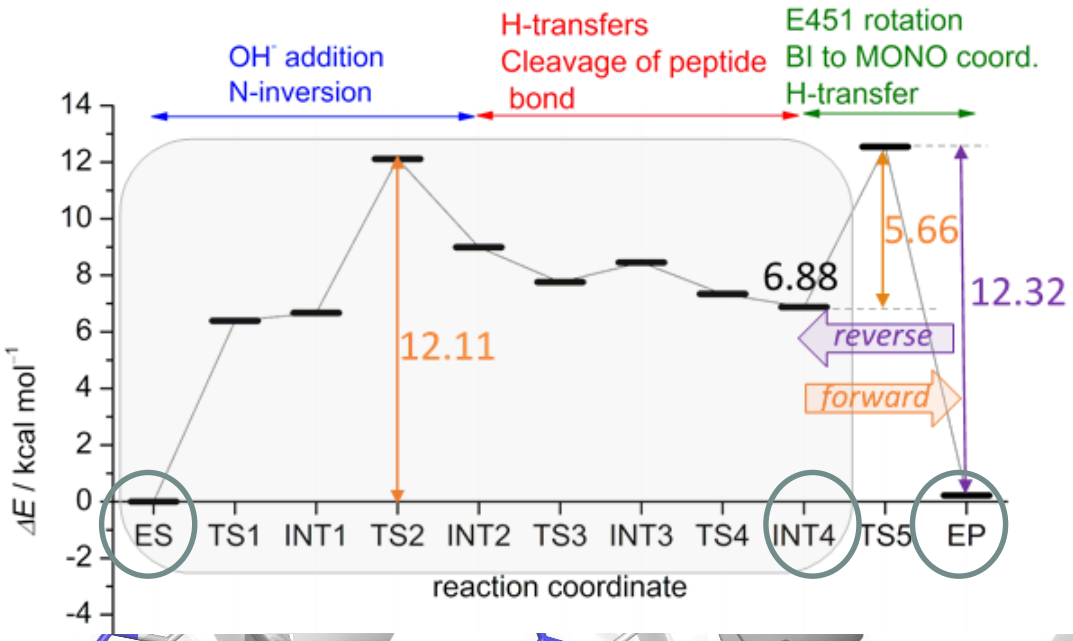
COMPLEX + 1st and 2nd enzyme solvatation sphere

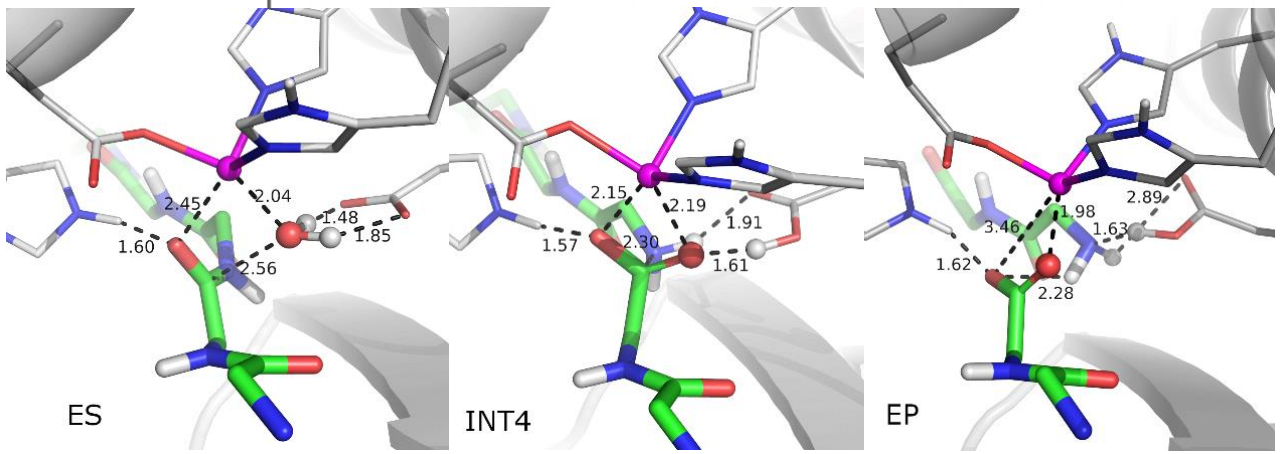
High-level: B97D/[6-31G(d) + LANL2DZ-ECP]
Low-level: parm96 AMBER force field

- FIX protein residues and water molecules > 8 Å from the substrate
- VIBRATIONAL ANALYSIS - minima and saddle points

DPP III

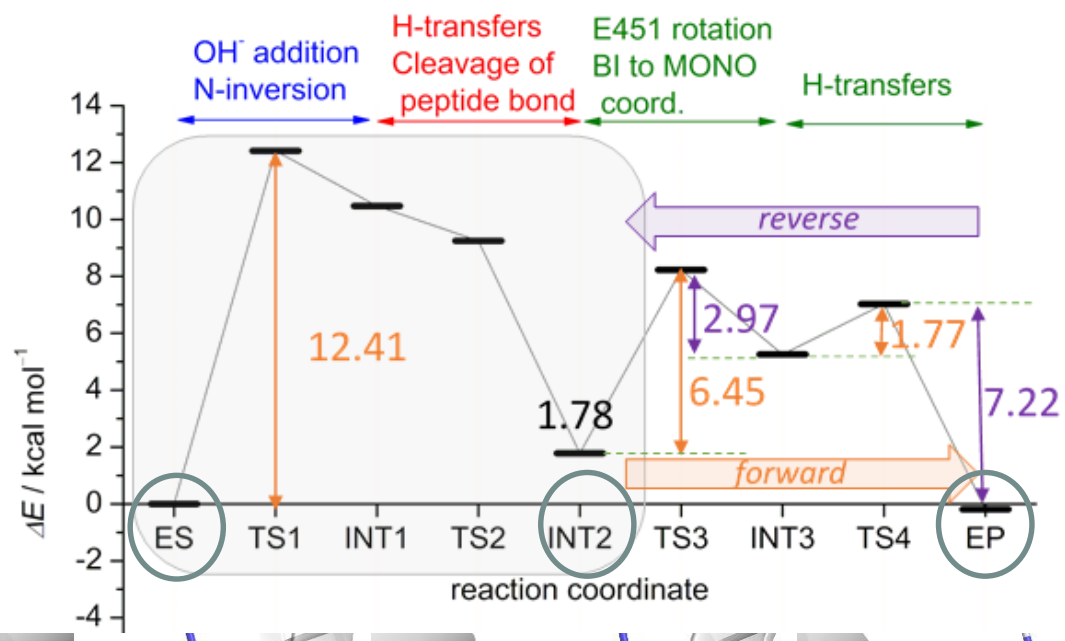
Tyr – Gly ÷ Gly – Phe – Leu

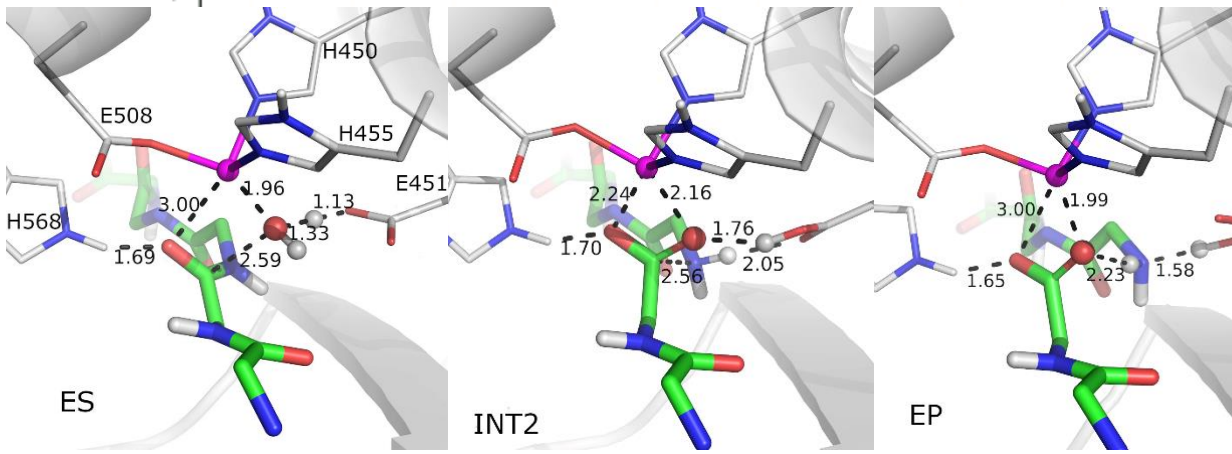




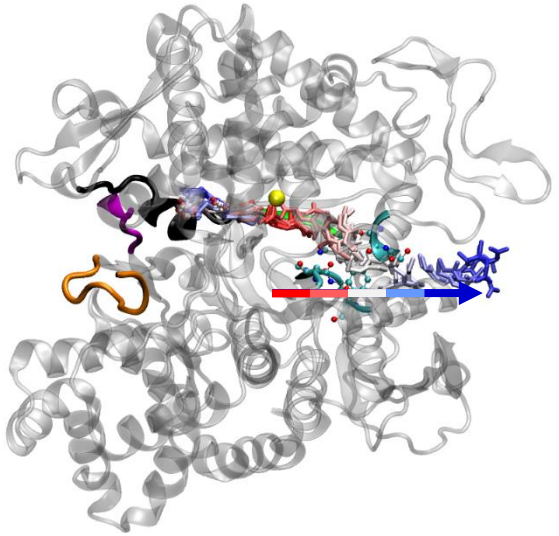
DPP III

Val – Val ÷ Tyr – Pro – Trp



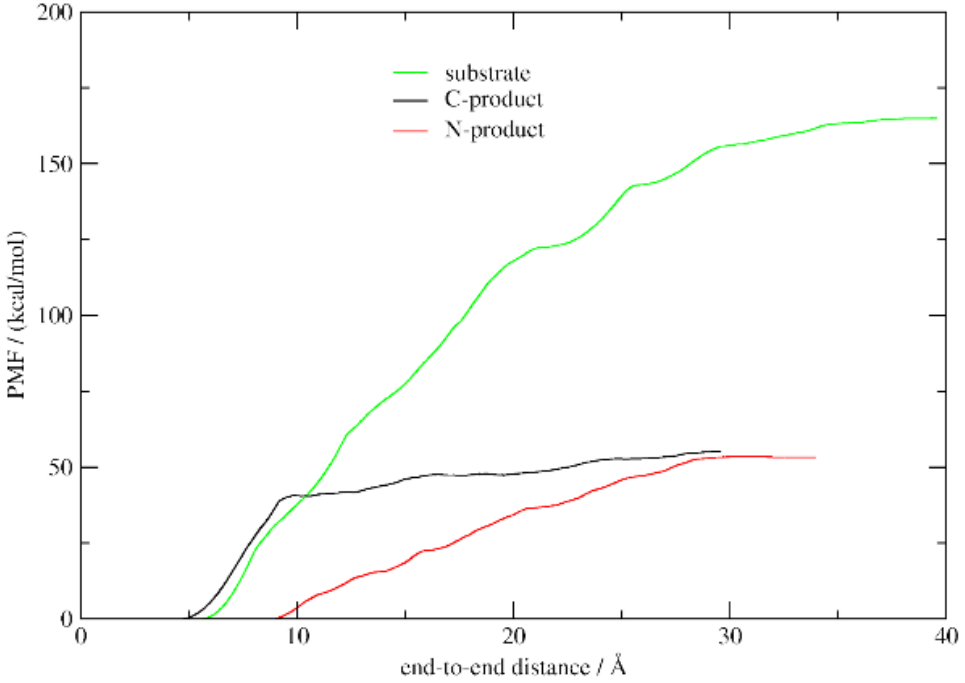


ADAPTIVE STEERED MD SIMULATIONS

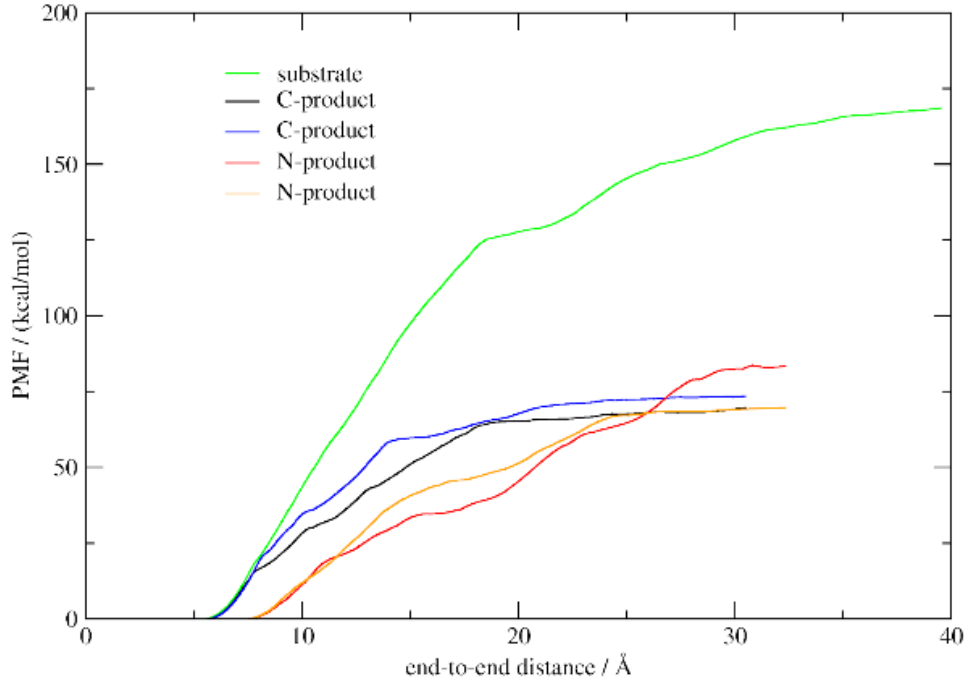


- force constant of 5 kcal mol^{-1} Å^{-2} and pulling velocity of 0.5 or 1 Å/ns
- reaction coordinate was partitioned into 25 equal segments (each 1 Å in long) and either 25 (each 2 ns long) or 50 (each 1 ns long) trajectories were simulated per stage

DPP III – Leu-enkephalin Y G G F L

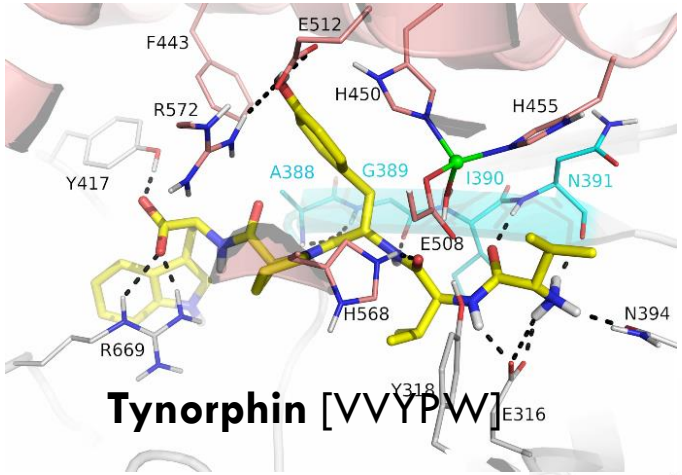


DPP III – tynorphin V V Y P W

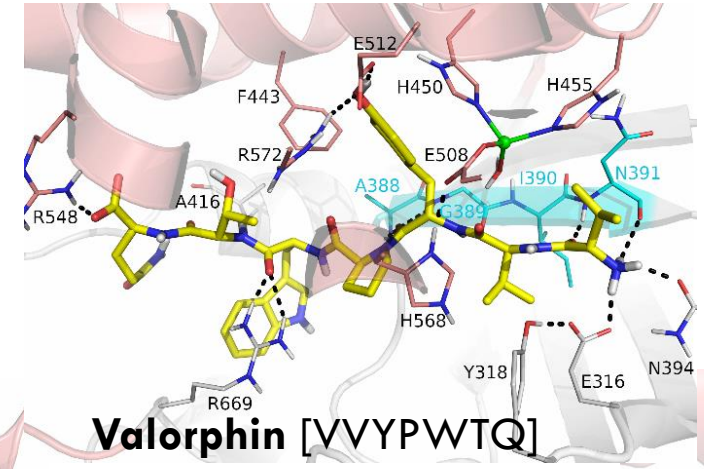


2. NEUROPEPTIDE BINDING

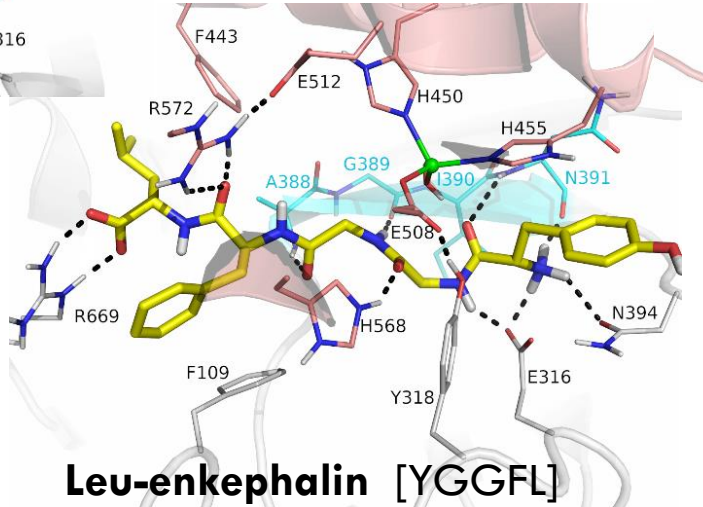
*AMBER 20, **2 x 1µs**, NpT ensamble, ff19SB force field, OPC water model, extended 4-ligand hybrid bonded/non-bonded parameters for Zn(II)*



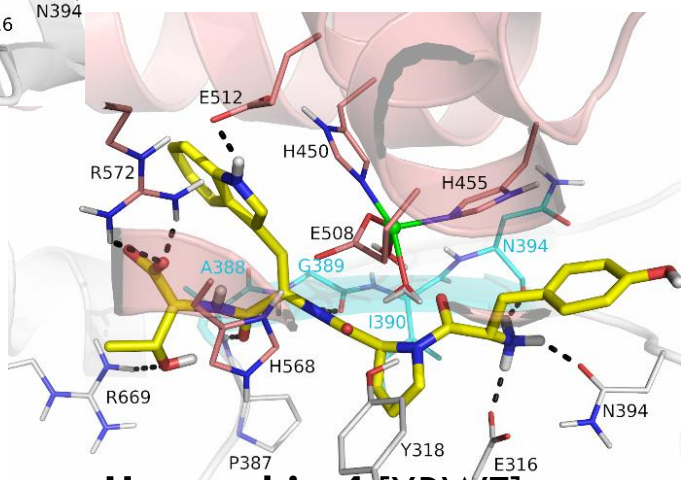
Tynorphin [VVYPW]



Valorphin [VVYPWTQ]

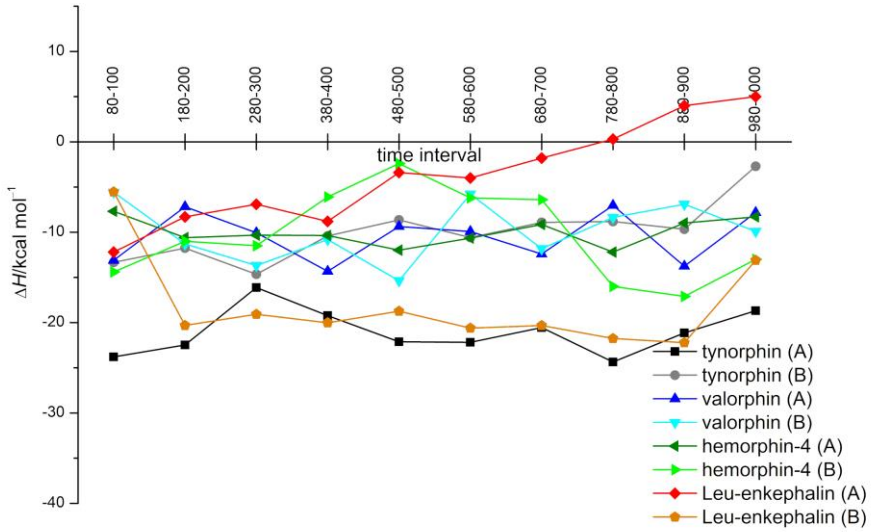


Leu-enkephalin [YGGFL]



Hemorphin-4 [YPWT]

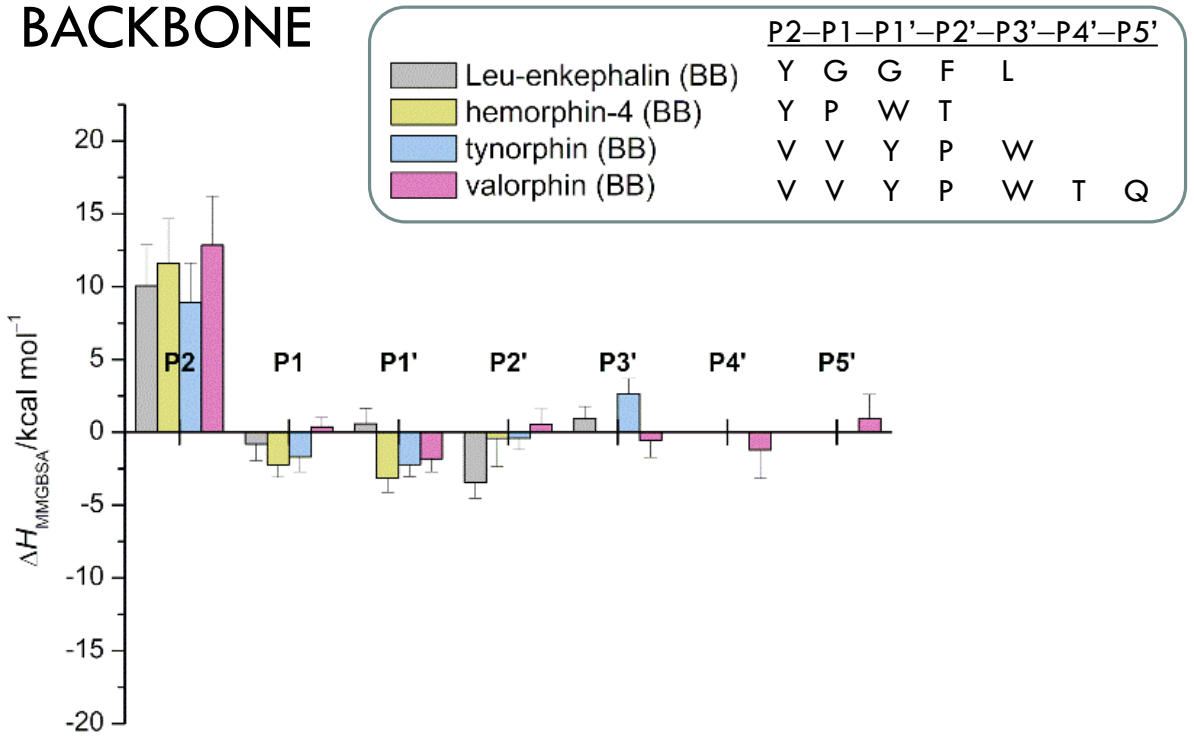
MM-PBSA CALCULATIONS



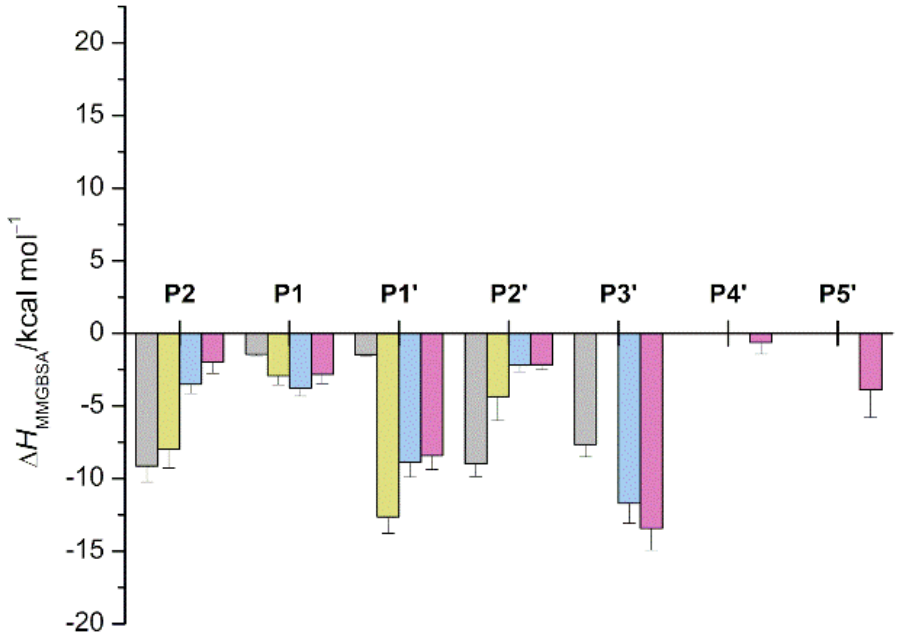
Ligand in complex with DPP III	Sim.	[<ΔH> ± SD]/kcal mol^{-1}
tynorphin	A	−21.1 ± 2.5
	B	−10.0 ± 3.2
valorphin	A	−10.5 ± 2.7
	B	−10.0 ± 3.3
hemorphin-4	A	−10.0 ± 1.5
	B	−10.4 ± 4.9
Leu-enkephalin	A	−3.6 ± 5.6
	B	−18.2 ± 5.1

MM-GBSA CALCULATIONS

BACKBONE

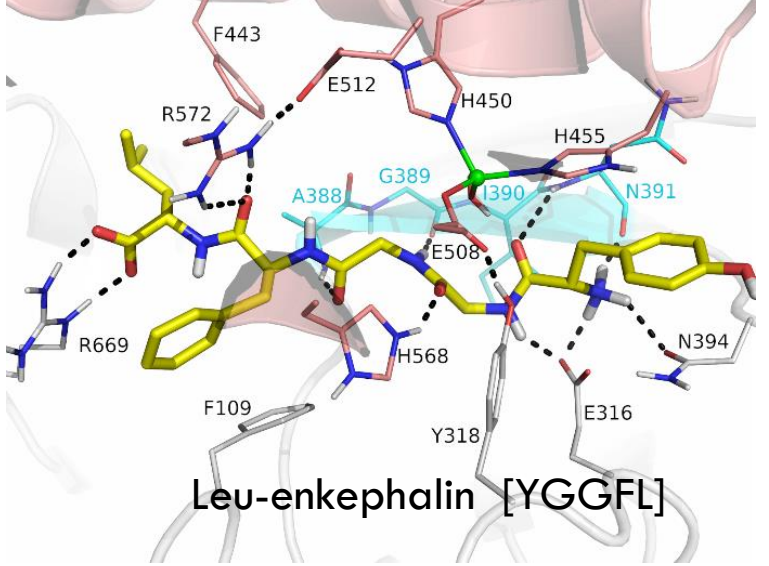


SIDE CHAIN

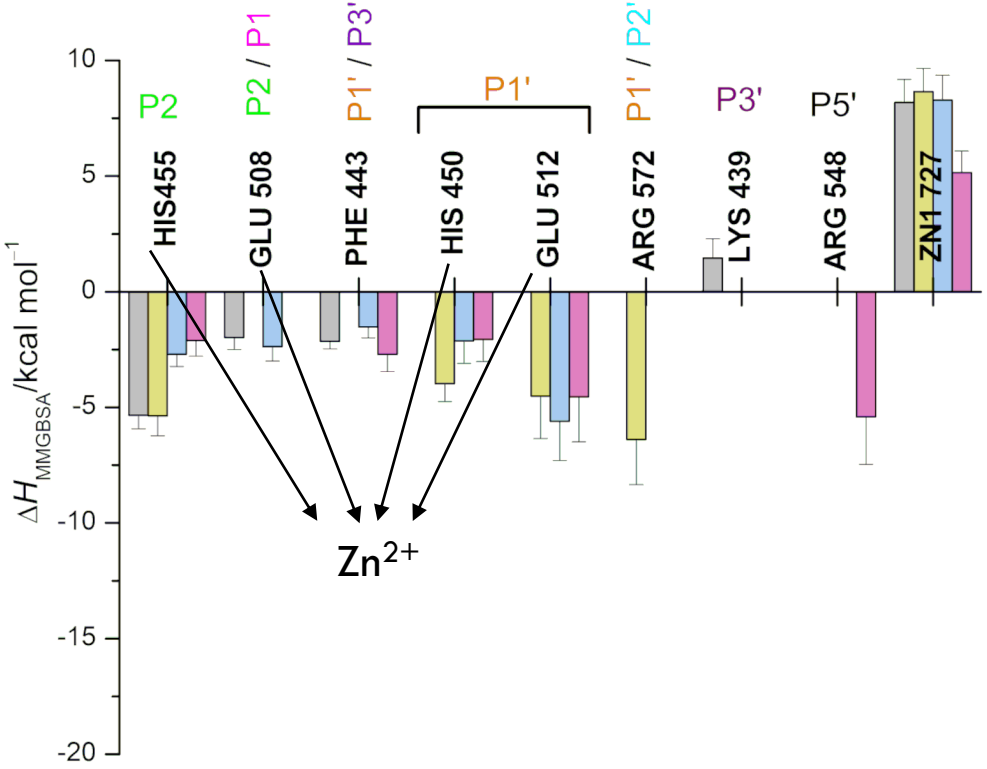


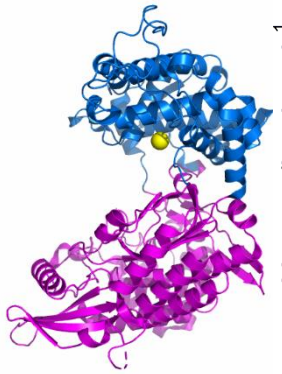
MM-GBSA CALCULATIONS

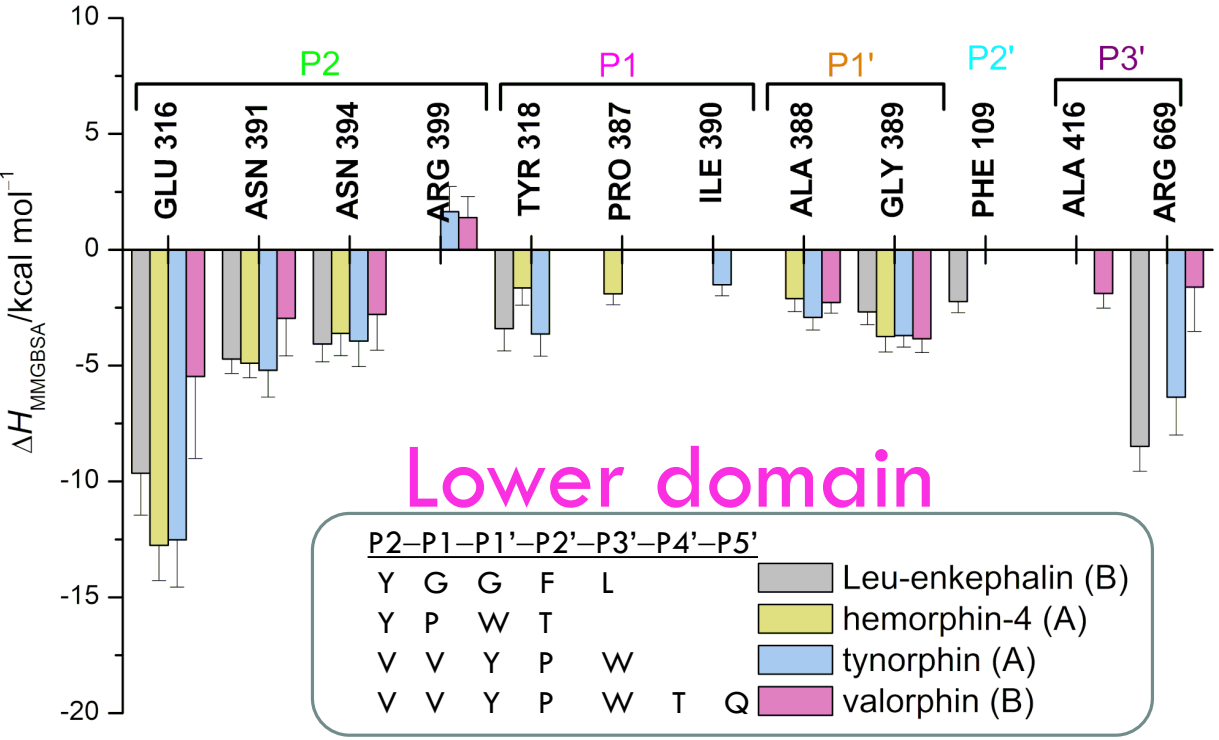
Upper domain



Leu-enkephalin [YGGFL]



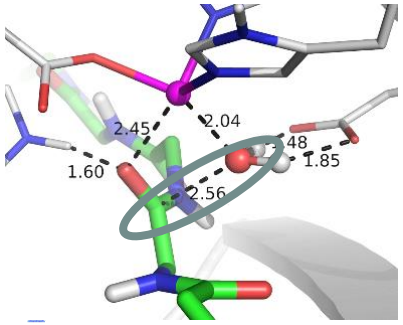


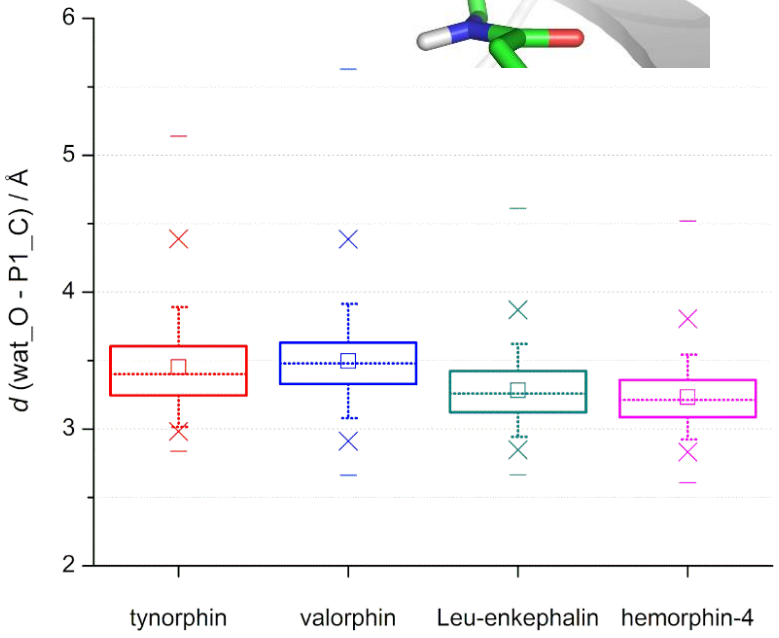


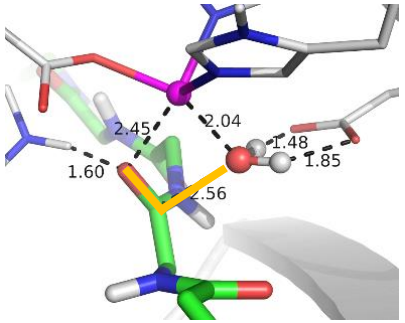
Lower domain

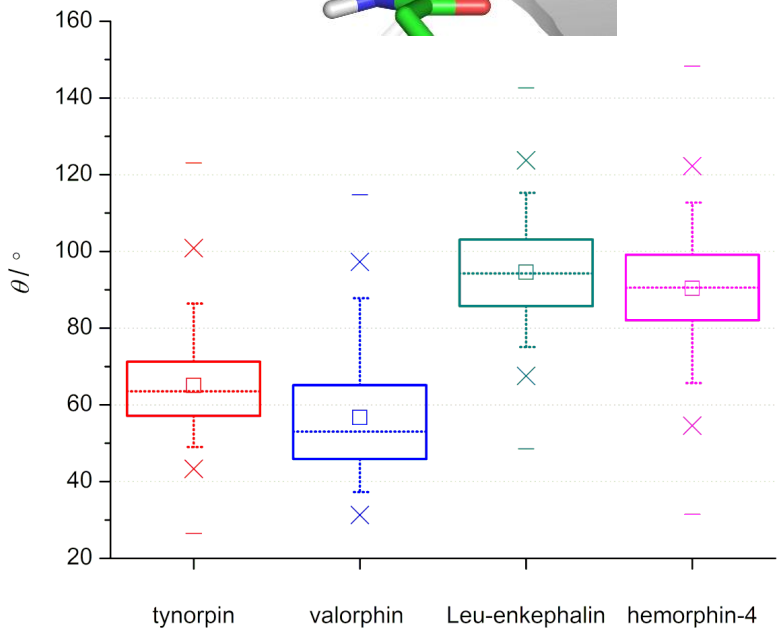
P2	P1	P1'	P2'	P3'	P4'	P5'	
Y	G	G	F	L			Leu-enkephalin (B)
Y	P	W	T				hemorphin-4 (A)
V	V	Y	P	W			tynorphin (A)
V	V	Y	P	W	T	Q	valorphin (B)

GEOMETRICAL PARAMETERS









Bürgi–Dunitz attack angle of θ≈107°