Mechanism of the human DPP III catalysed tynorphin hydrolysis

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Substrate complexes of human dipeptidyl peptidase III reveal the mechanism of enzyme inhibition

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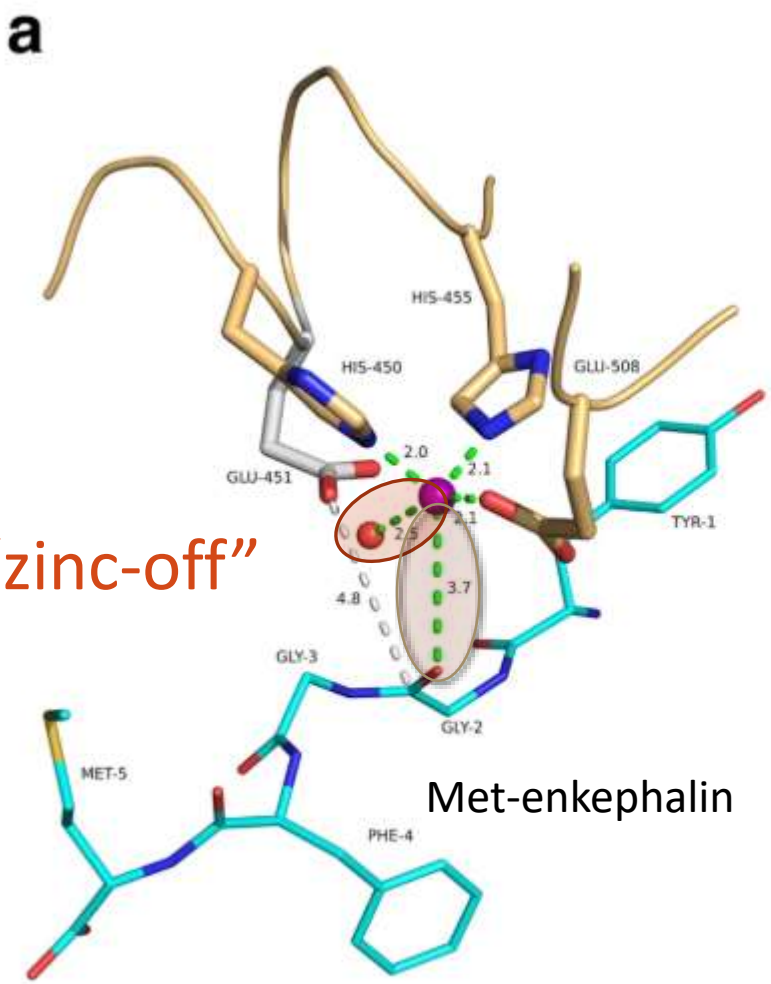
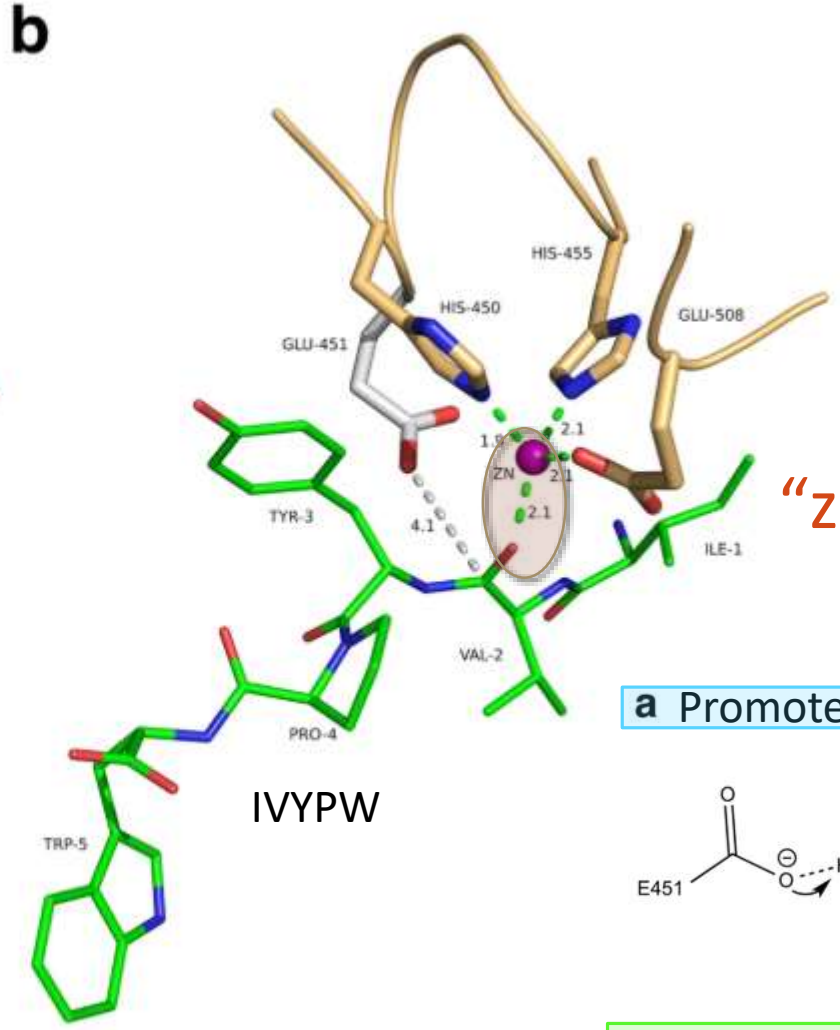
Human dipeptidyl-peptidase III (hDPP III) is a zinc-dependent hydrolase cleaving dipeptides off the N-termini of various bioactive peptides. Thus, the enzyme is likely involved in a number of physiological processes such as nociception and is also implicated in several forms of cancer. We present high-resolution crystal structures of hDPP III in complex with opioid peptides (Met-and Leu-enkephalin, endomorphin-2) as well as with angiotensin-II and the peptide inhibitor IVYPW. These structures confirm the previously reported large conformational change of the enzyme upon ligand binding and show that the structure of the closed conformation is independent of the nature of the bound peptide. The overall peptide-binding mode is also conserved ensuring the correct positioning of the scissile peptide bond with respect to the catalytic zinc ion. The structure of the angiotensin-II complex shows, how longer peptides are accommodated in the binding cleft of hDPP III. Differences in the binding modes allow a distinction between real substrates and inhibitory peptides or "slow" substrates. The latter displace a zinc bound water molecule necessitating the energetically much less favoured anhydride mechanism as opposed to the favoured promoted-water mechanism. The structural data also form the necessary framework for the design of specific hDPP III inhibitors.



" "zinc-off"

Met-enkephalin

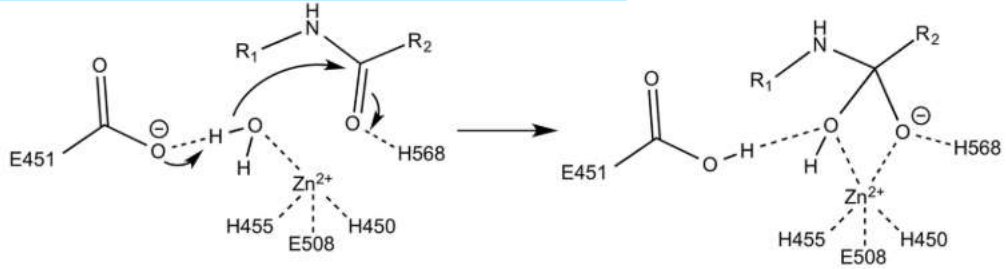


"zinc-on"

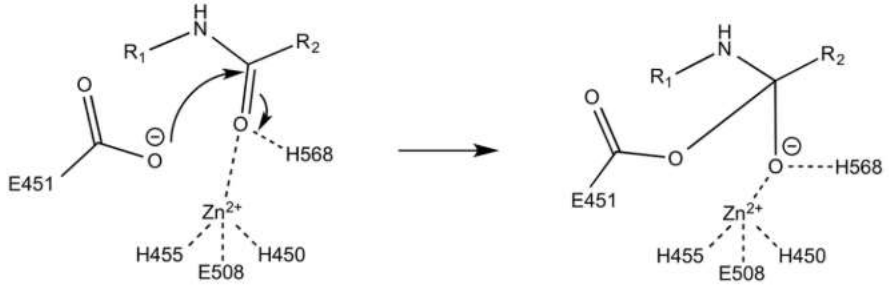
IVYPW

Coordination of the catalytic zinc ion in substrate and inhibitor complexes.

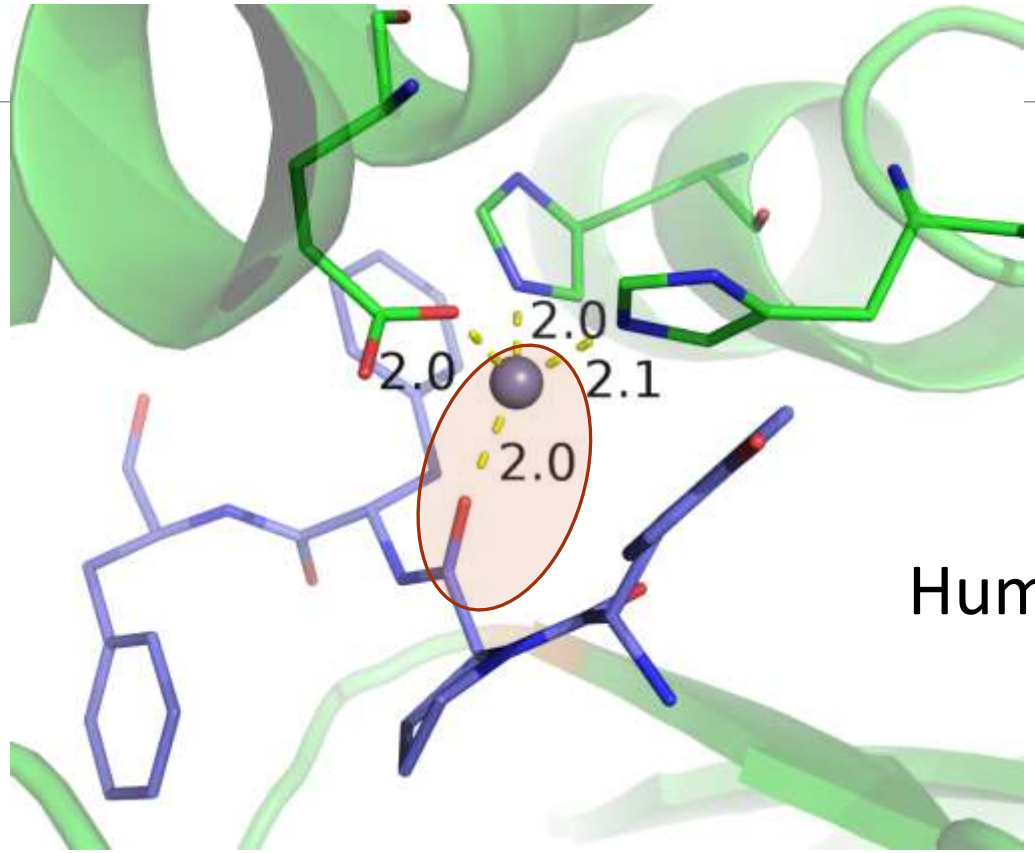
a Promoted-water mechanism



b Anhydride mechanism



Possible mechanisms of peptide hydrolysis catalysed by hDPP III.

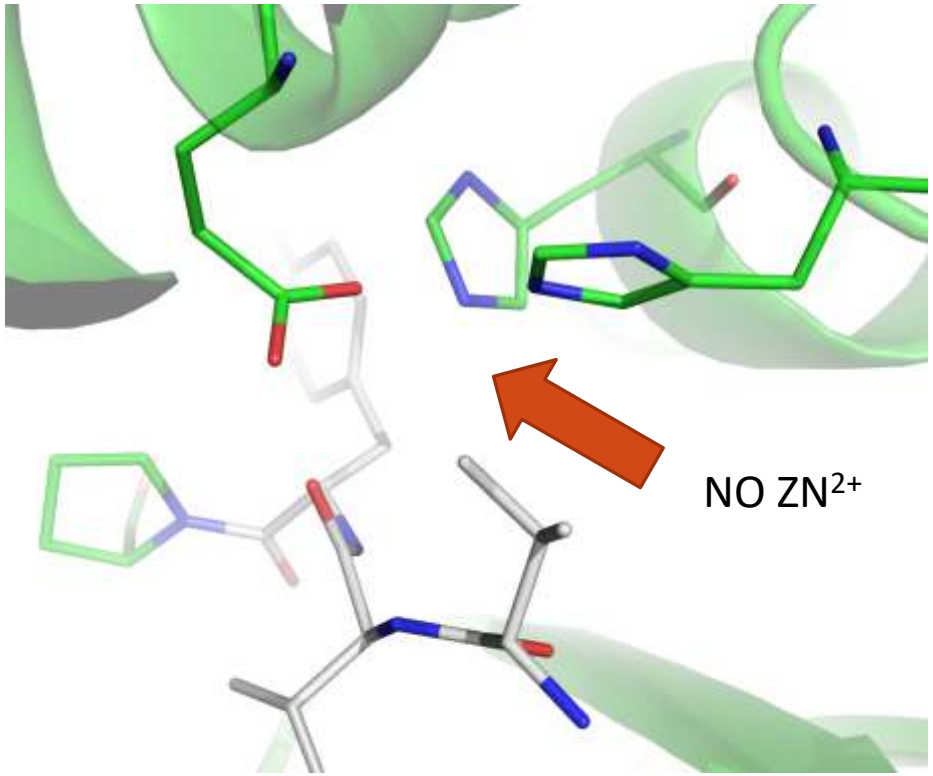


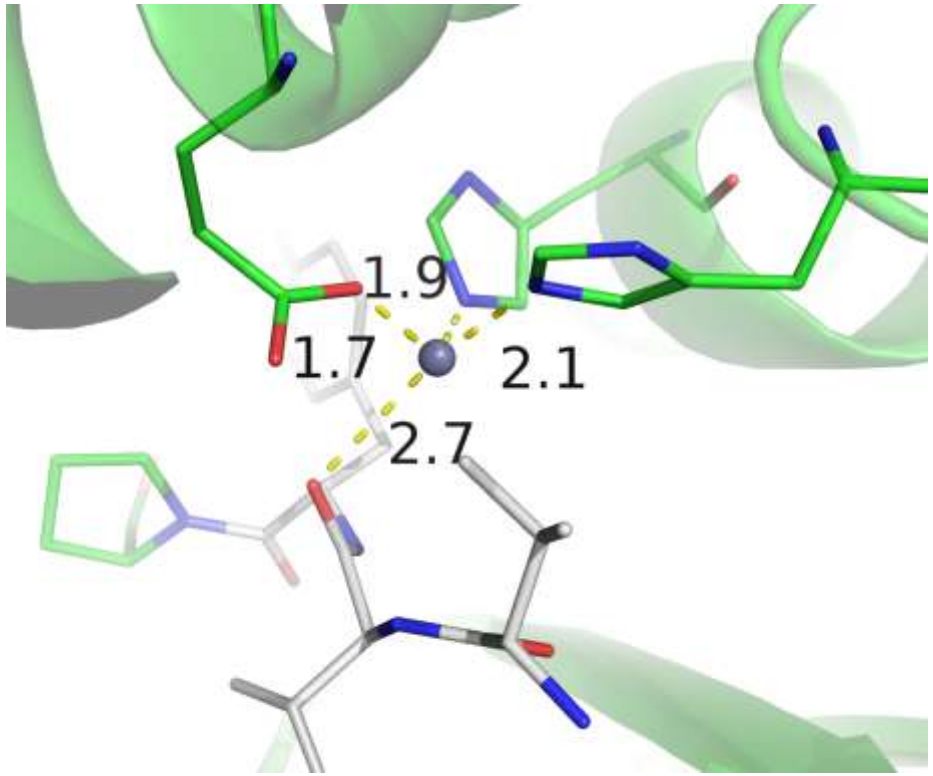
! PDB code: 5EHH

Human DPP III – endomorphin-2 complex

Human DPP III – tynorphin complex

pdb codes: 3T6B i 3T6 (Bezzera et. al. Proc.Natl.Acad.Sci.USA (**2012**) 109: 6525-6530)





Zn position (in 3T6B) determined using the complex with Leu-enkephalin (5E3A) as a template (alignement of H450, H455 and E508)

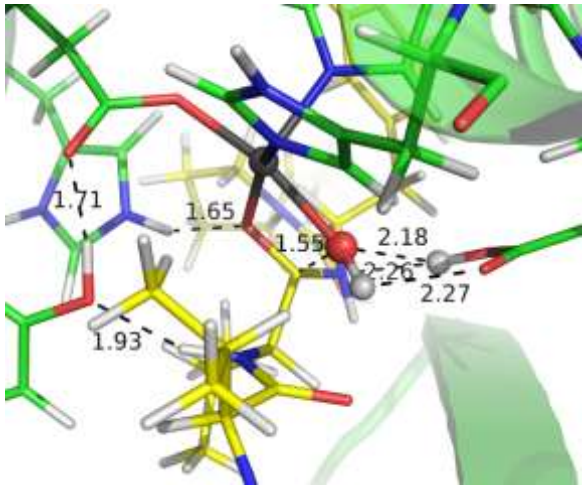
MOLECULAR MODELING STUDY

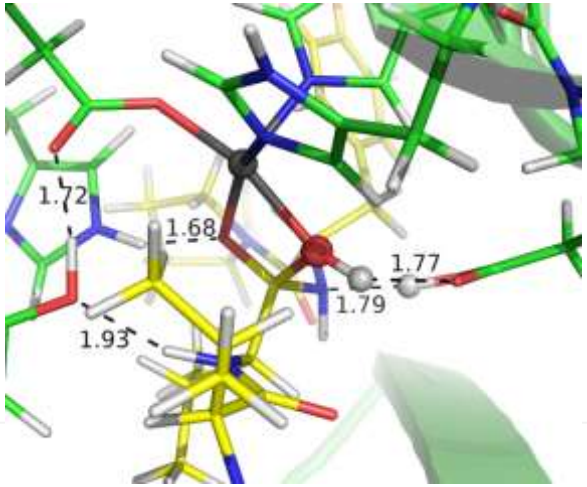
- **3T6B starting complex structure** (Zn position determined using DPP III – Leu-enkephalin complex structure, 5E3A, as a template)
- Complex structure obatined after **10 ns of MD simulation** (AMBER14, ff14sb, nonbonding zinc parameters) was used to determine water molecules in the 1st and 2nd protein solvatation sphere.
- In the minimized structure obtaind after 10 ns of MD simulations positions of **Y318, H450, E451, H455, E508, H568, Zn and tynorphin** were adjusted using the X-ray structure (3T6B) as a template.
- The **water molecule coordinated to the zinc ion** was added manually based on its position in the Michaelis complex structrue obtained by QMMM optimization of the hDPP III – Leu-enkephalin complex.

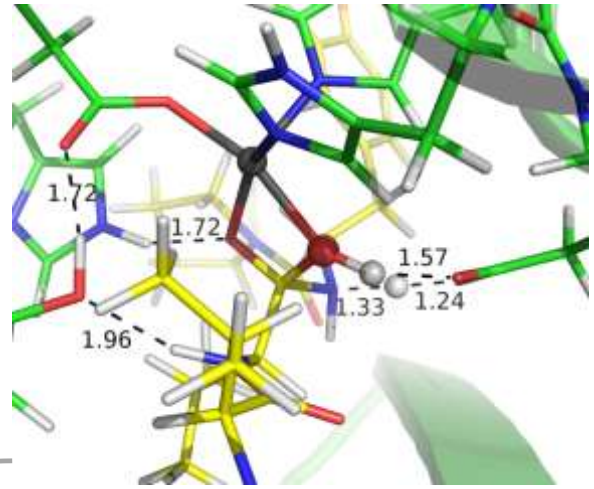
QMMM calculations

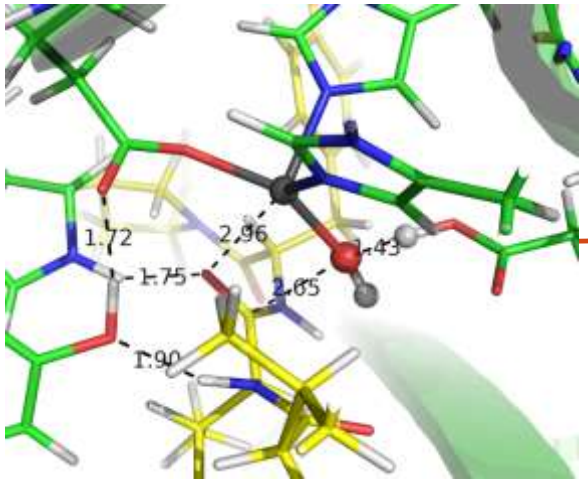
- **2-layer ONIOM calculations** (Gaussian 09)

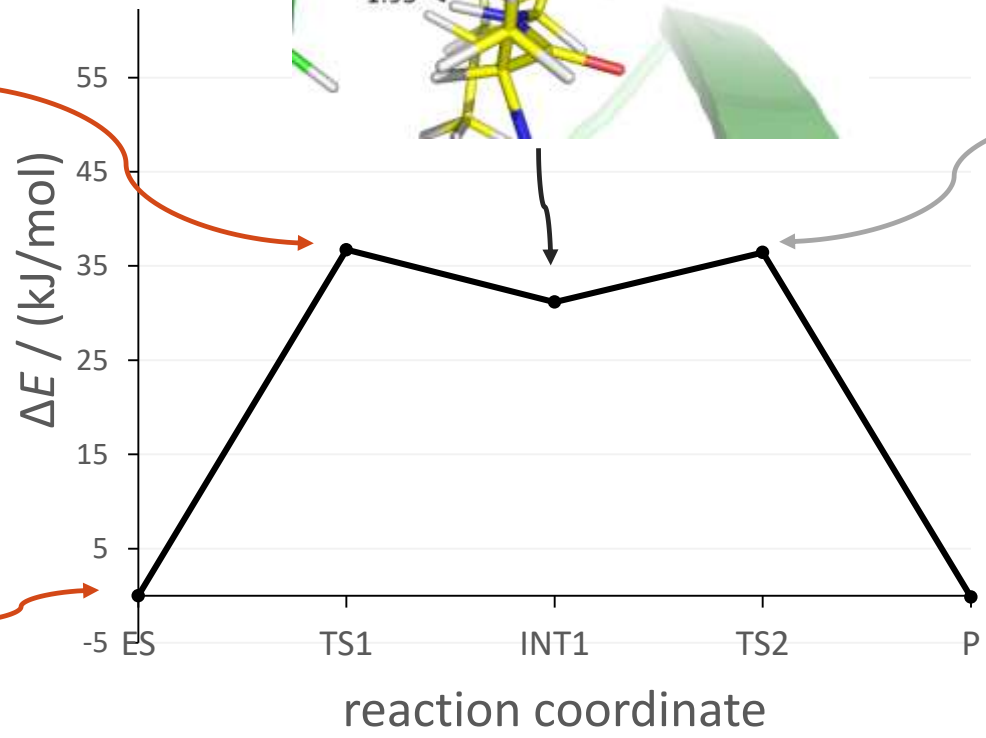
  - **QM**: Y318, H450, E451, H455, E508, H568, Zn, zinc-coordinated water and tynorphin
  - **MM**: rest of the protein and waters
  - Optimization, scan, TS calculations, IRC→ B97D/[6-31G(d) + LanL2DZ-ECP] +parm96 force field
  - Charge embedding

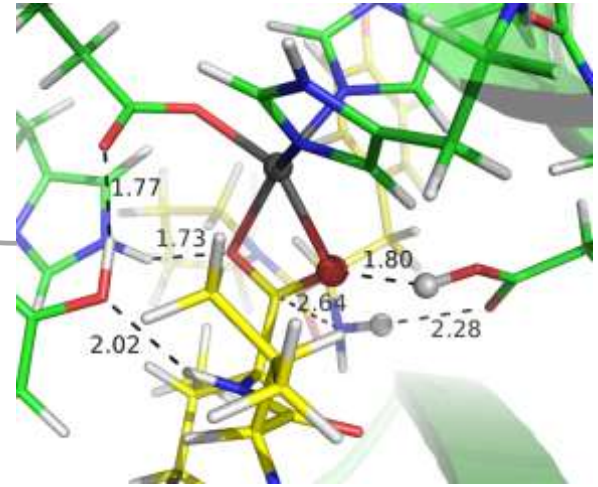






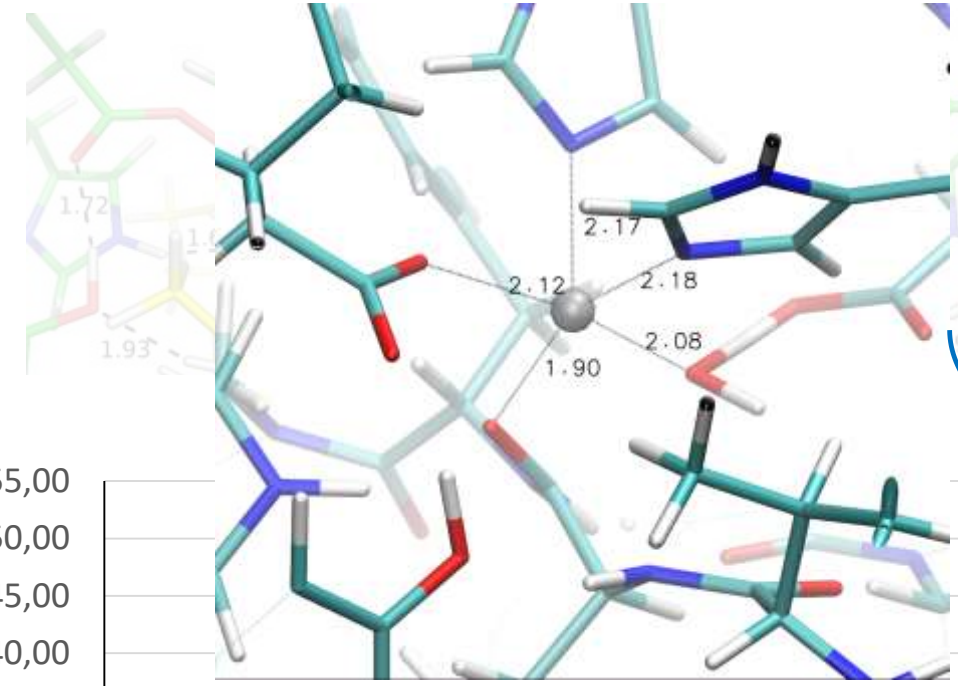




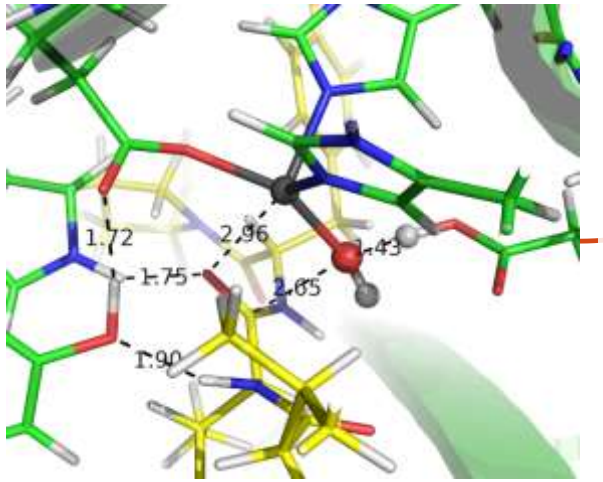


B97D/[6-31G(d) + LanL2DZ-ECP] + $ZPVE_{\text{B97D/[6-31G(d) + LanL2DZ-ECP]}}$

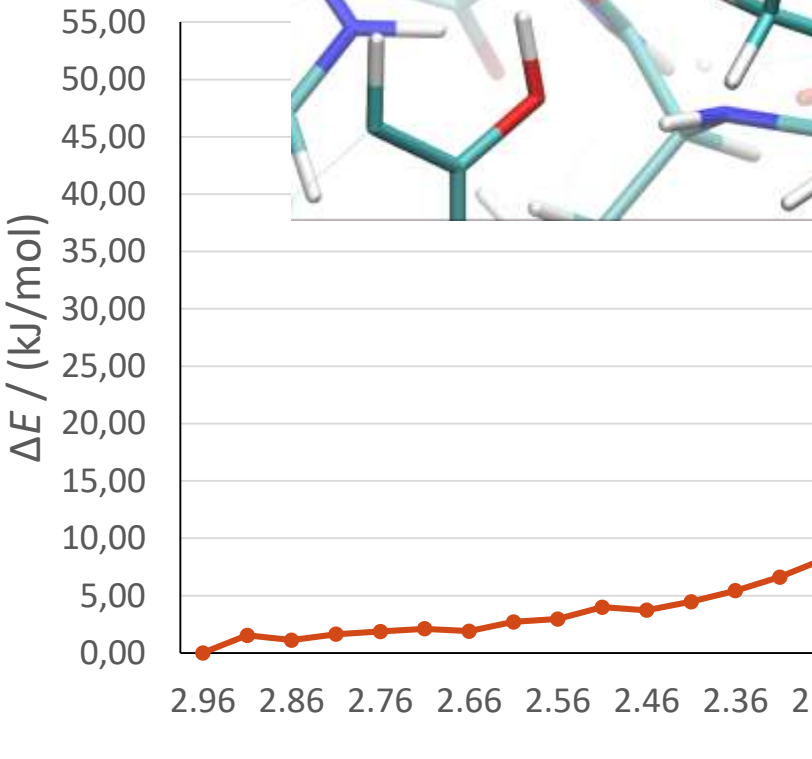
1.71
1.65
1.55
2.18
2.20
2.27
1.93


2.17
2.12
2.18
2.08
1.90


1.72
1.57
1.33
1.24

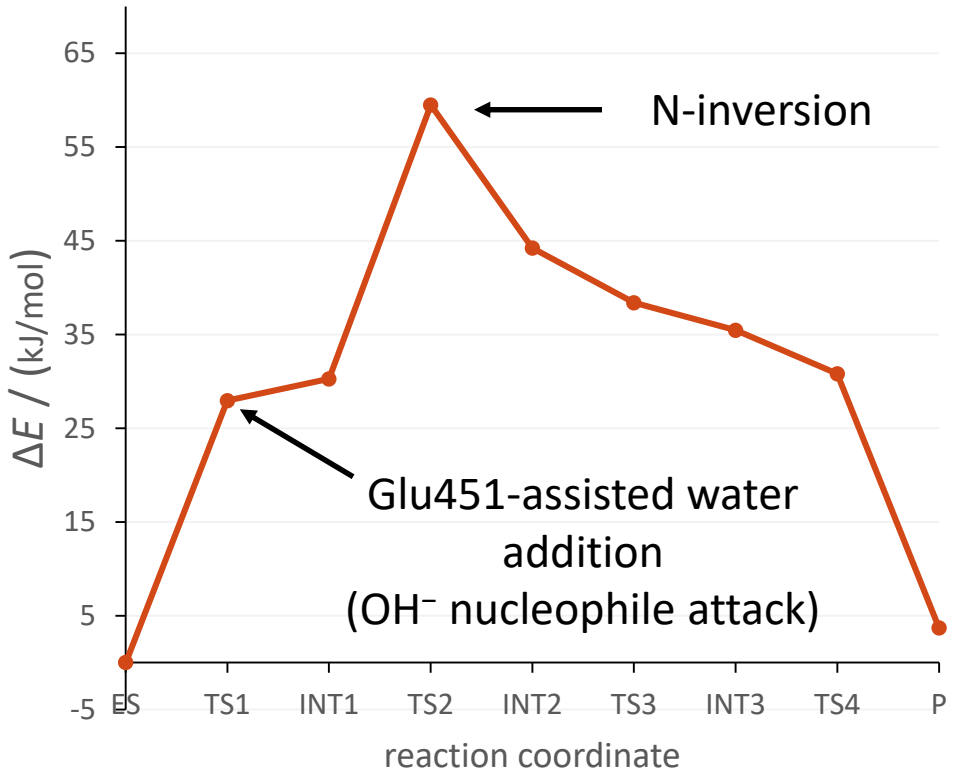

1.72
2.96
1.75
2.65
1.90

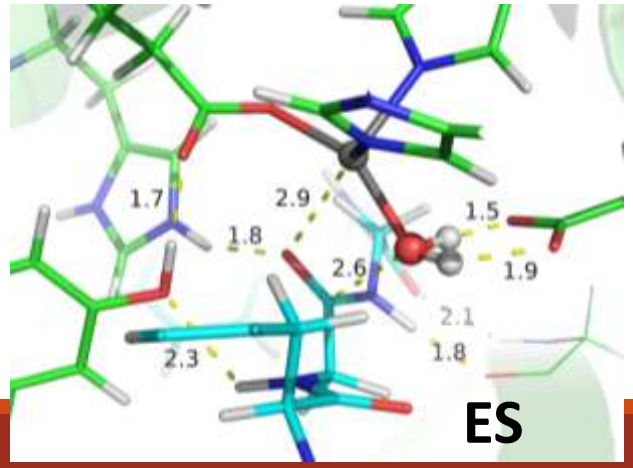
1.80
2.64
2.28

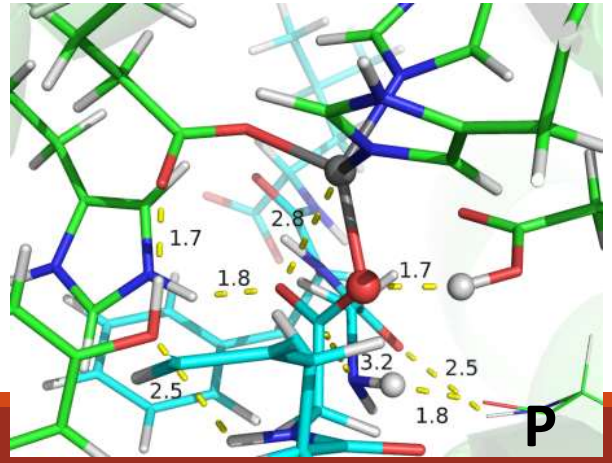

ΔE / (kJ/mol)
B97D/[6

55,00
50,00
45,00
40,00
35,00
30,00
25,00
20,00
15,00
10,00
5,00
0,00
ΔE / (kJ/mol)
2.96
2.86
2.76
2.66
2.56
2.46
2.36
2.26
2.16
2.06
1.96
1.86
d (Zn-Os) / Å

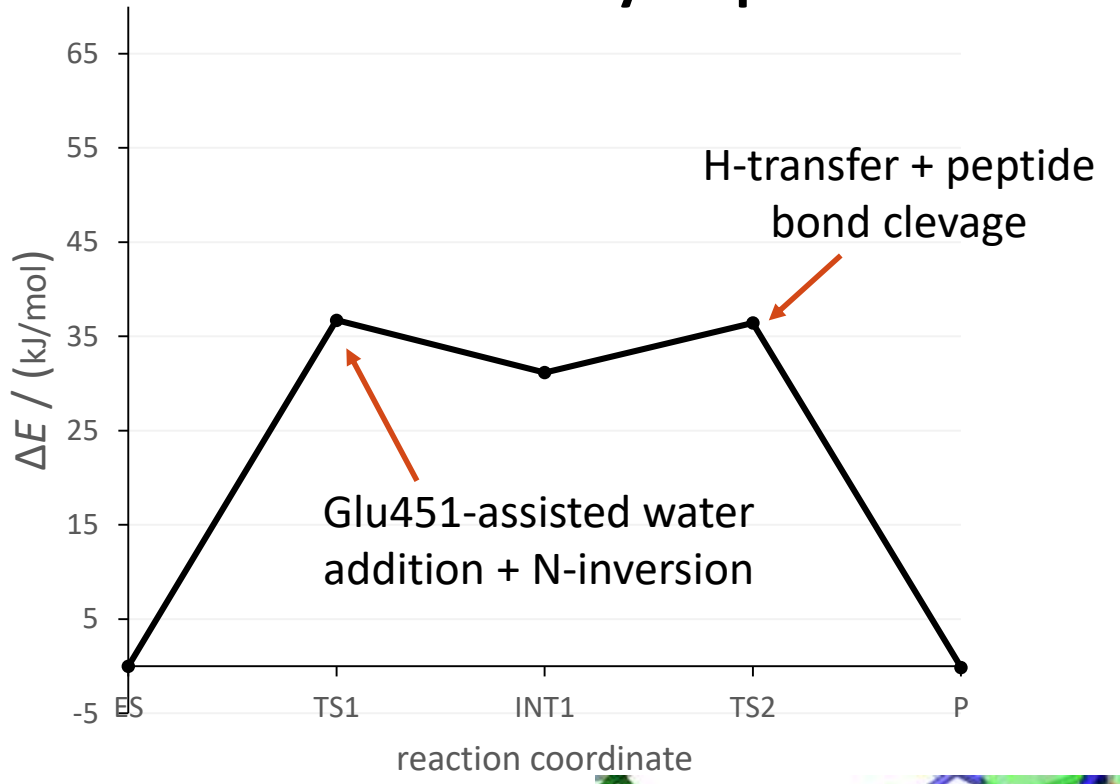
hDPP III – Leu-enkephalin

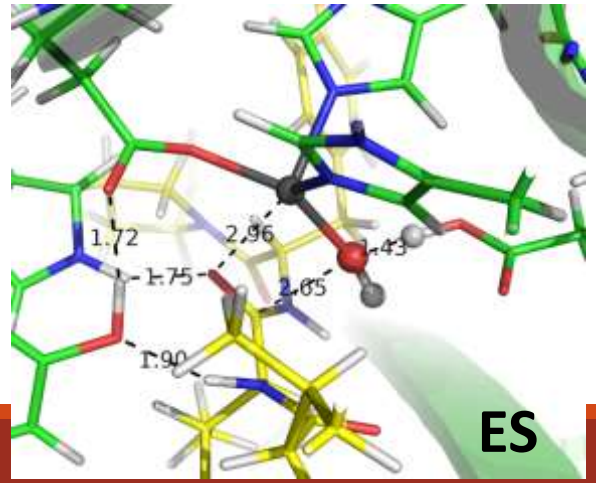


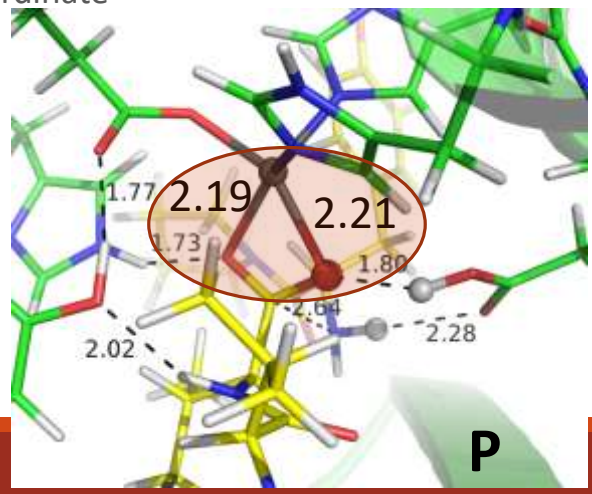




hDPP III – tynorphin



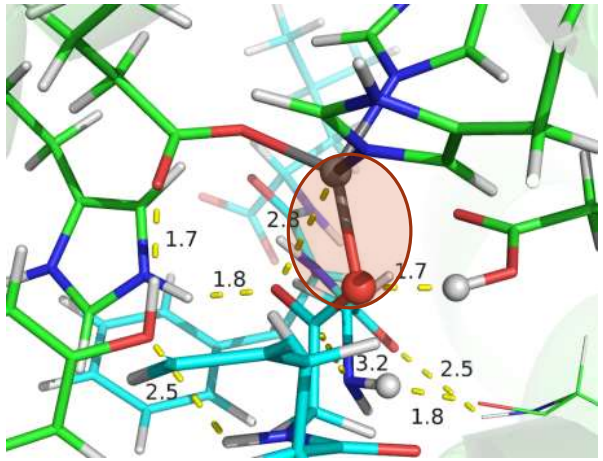




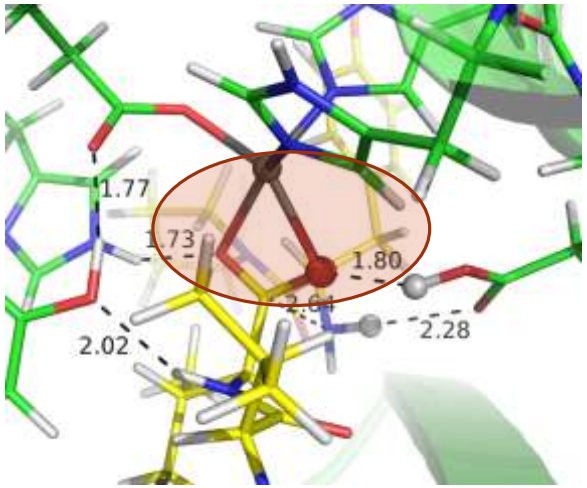
FUTURE STEPS?

- Release of the product?

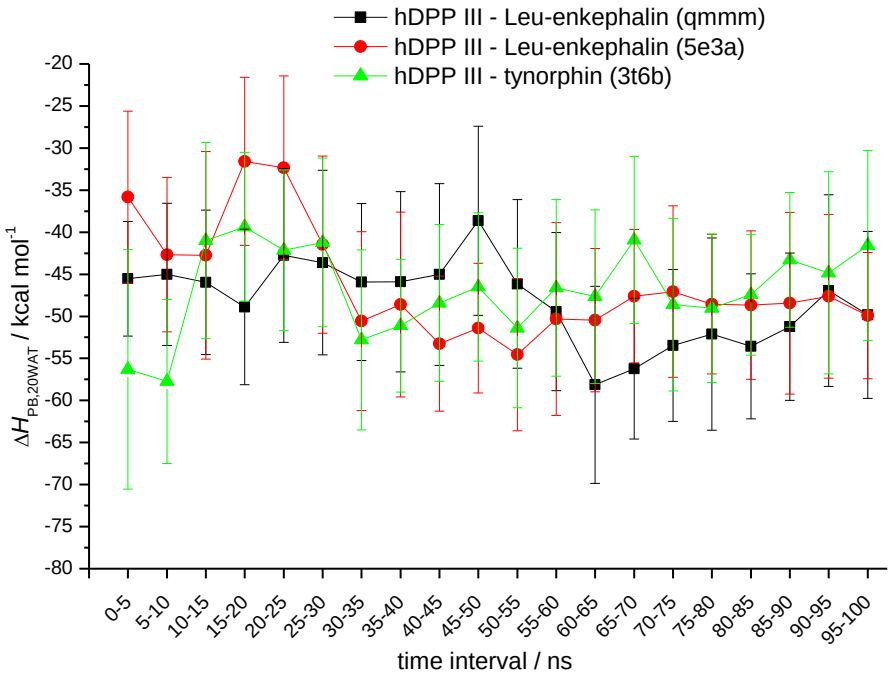
Leu-enkephalin - MONO



Tynorphin - BI



- Ligand binding affinities?



100 ns explicit water *NpT* MD simulations (AMBER16, ff14sb, hybrid bonded/nonbonded zinc parameters)

HVALA ;)