Math/Chem/Comp 2022 – 33rd MC2 Conference
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COMPUTATIONAL STUDY OF THE HUMAN DPP III CATALYZED PEPTIDE HYDROLYIS – DIFFERENCE BETWEEN "GOOD" AND "SLOW" SUBSTRATES

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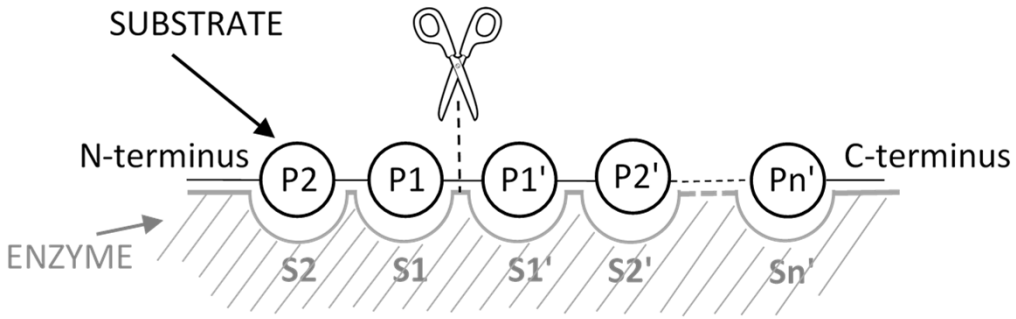
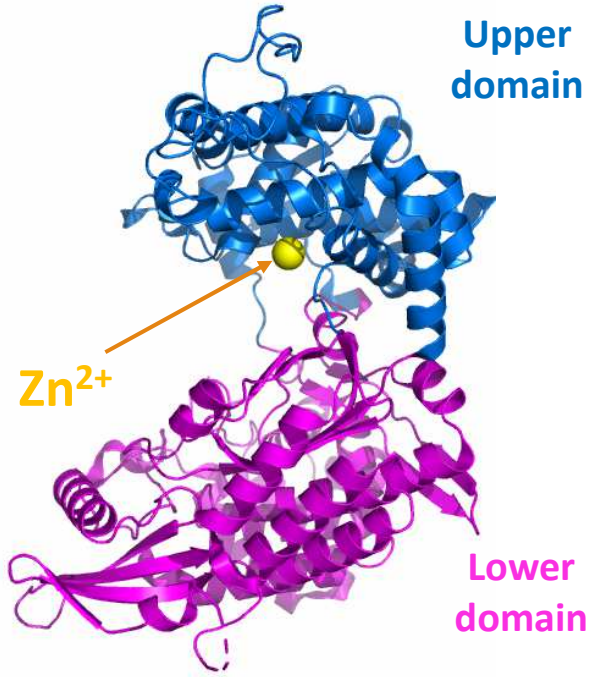
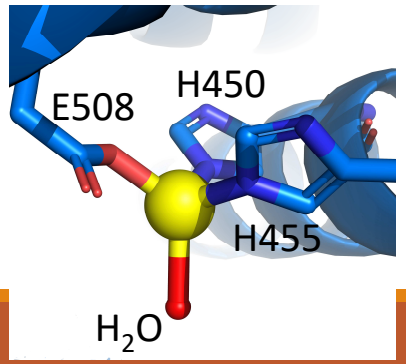
Dipeptidyl peptidase III (DPP III)

- Expressed in **prokaryotes** and **eukaryotes**
- Two-domain **zinc**-exopeptidase (M49 family)
- Hydrolyzes dipeptides from the N-terminal of its substrates



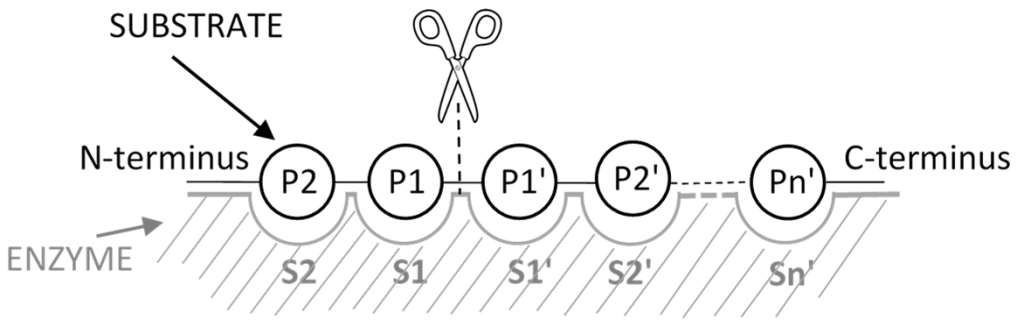




I. Schechter and A. Berger, Biochem. Biophys. Res. 27 (1967) 157-162.

IMPLIED ROLE IN:

- ✓protein catabolism
- ✓blood pressure regulation
- ✓pain modulation (*in vitro* hydrolyzes a number of biologically active (neuro)peptides)
- ✓defense against oxidative stress
- ✓…



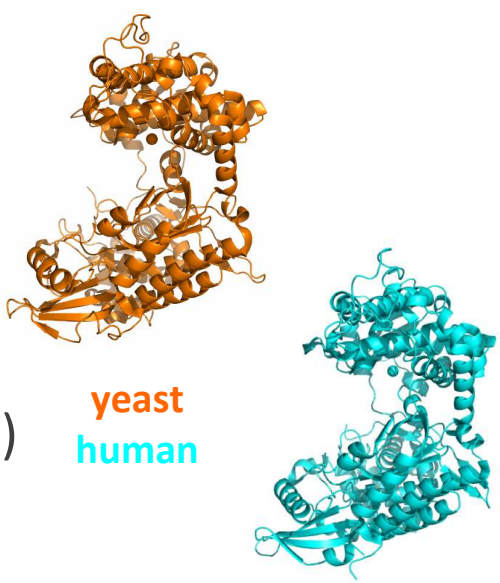
BROAD SUBSTRATE SPECIFICITY (*in vitro*)
- a preference for:

- ❖ a positively charged N-terminus,
- ❖ the ability (propensity) of the substrate to form β-sheet secondary structure
- ❖ hydrophobic AA residues at the P1’ position
- ❖ a proline residue at the P1 position

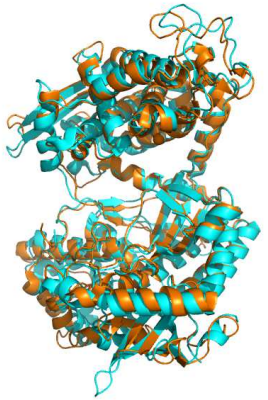
RCSB PDB
PROTEIN DATA BANK

2008. → yeast DPP III (3CSK)

2009. → human DPP III (3FVY)



sequence identity = 35%
RMSD = 2.17 Å



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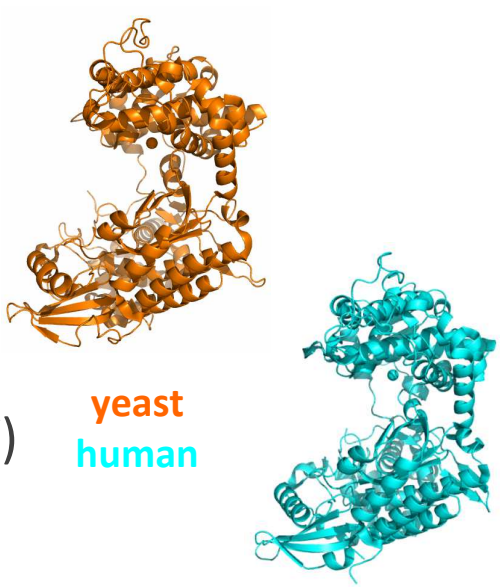

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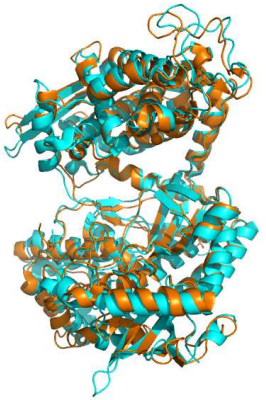
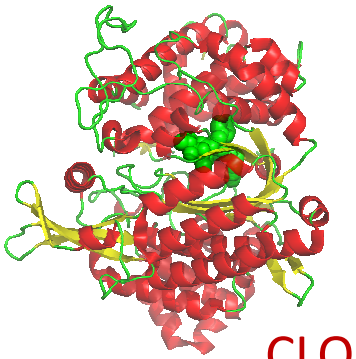
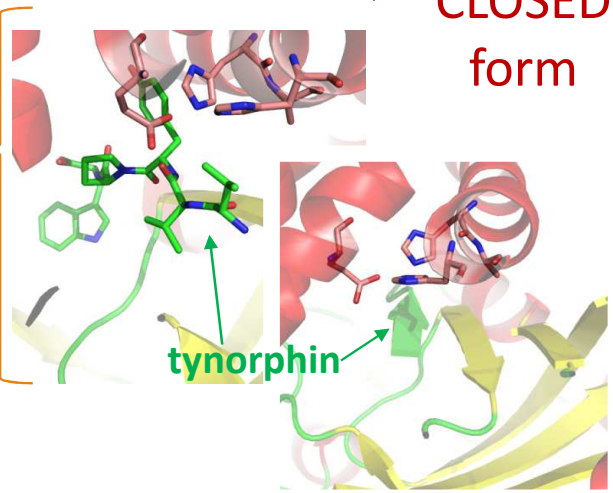




CLOSED form

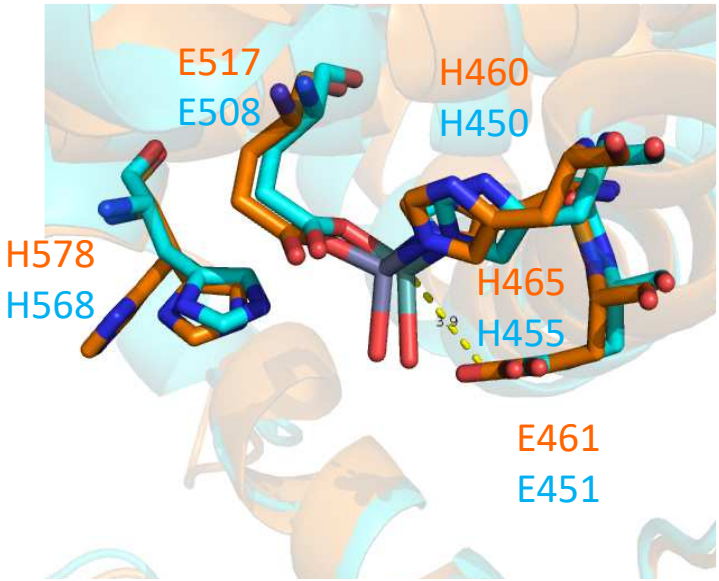
2012. → human E451A DPP III – tynorphin (3T6B and 3T6J)



2016. → human E451A DPP III peptide complex (5E33, 5E3A, 5E2Q, 5EHH, 5E3C) and ligand-free (5EGY)

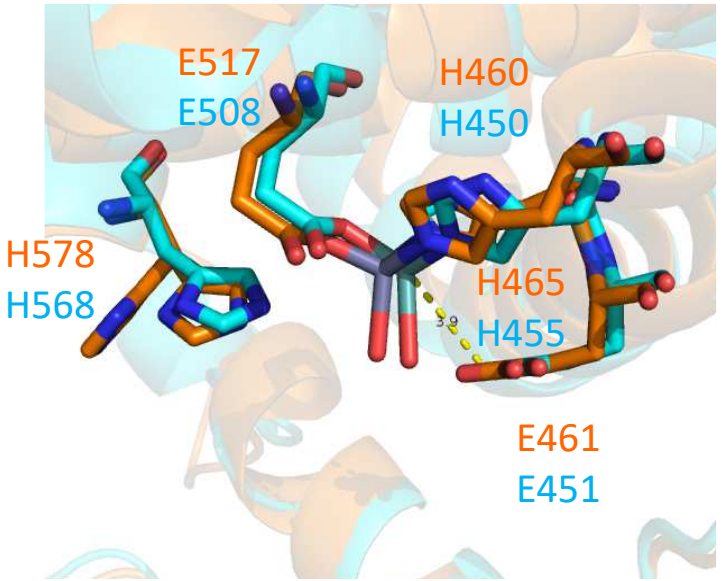
…..

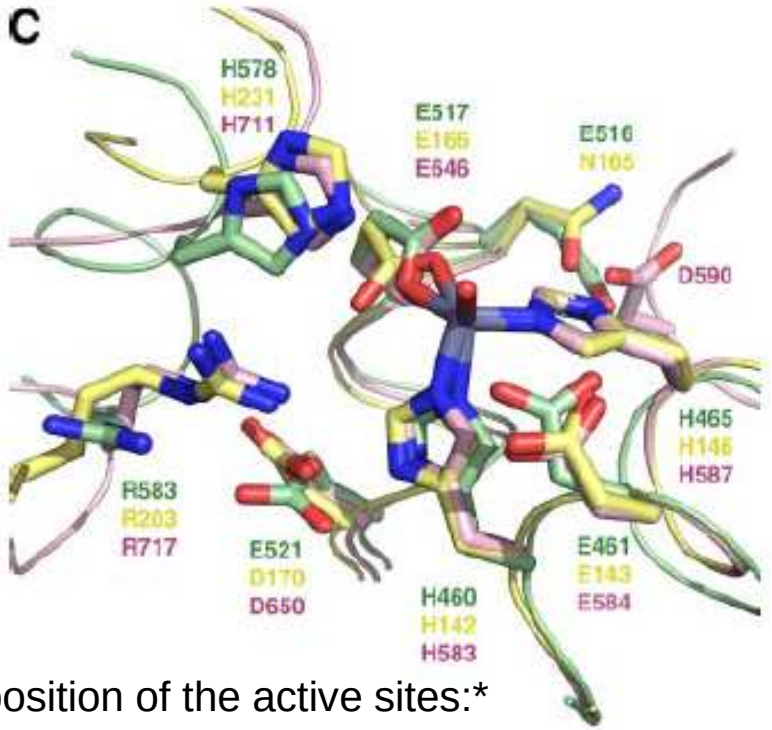
Active site



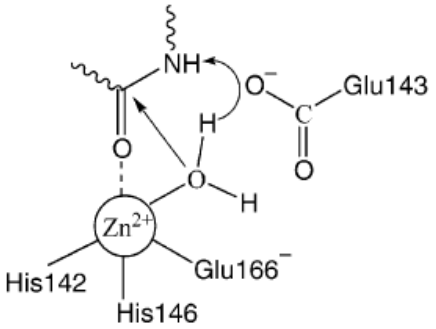
yeast
human

Active site



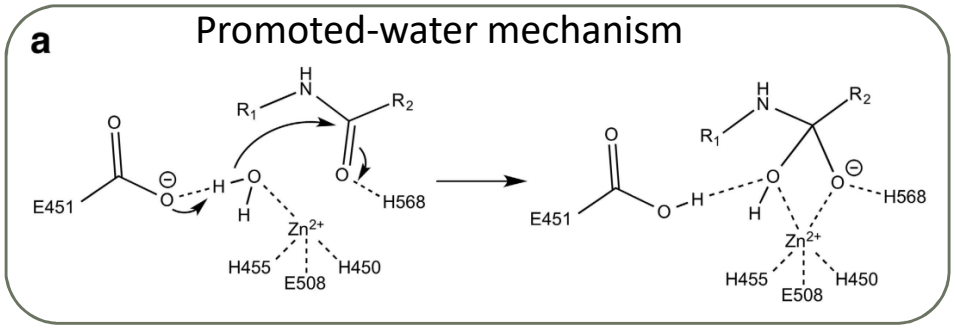


Superposition of the active sites:*
yeast DPP III, thermolysin and neprilysin



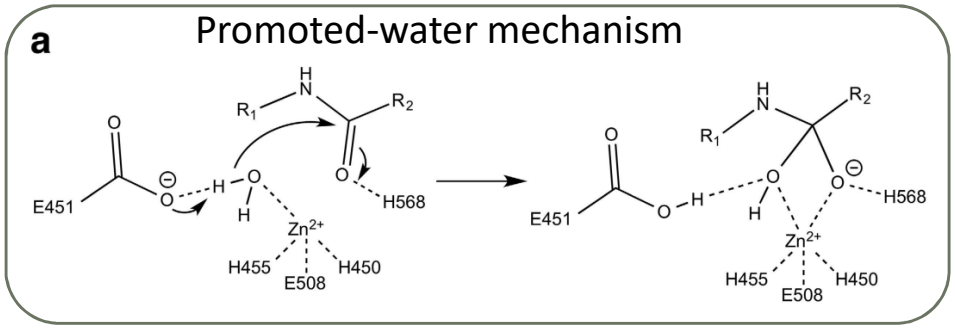
Pelmenschikov *et. al.* 2002.
Blumberger *et. al.* 2007

*P. Kumar Baral et al, The Journal of Biological Chemistry, 283 (2008) 32, 22316



P. Kumar et al. *Sci Rep.* **2016**

- Met-enkephalin (5E33)
- Leu-enkephalin (5E3A)
- Angiotensin-II (5E2Q)
- Endomorphin-2 (5EHH)
- IVYPW (5E3C)
- Unbound (5EGY)



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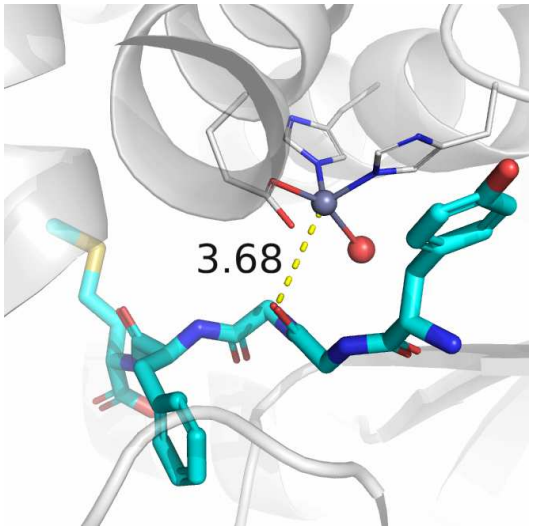
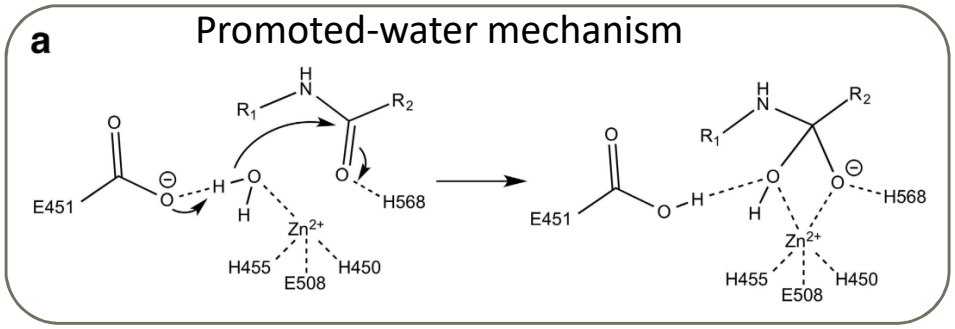


DPP III – Met-enkephalin
(pdb: 5E3A)



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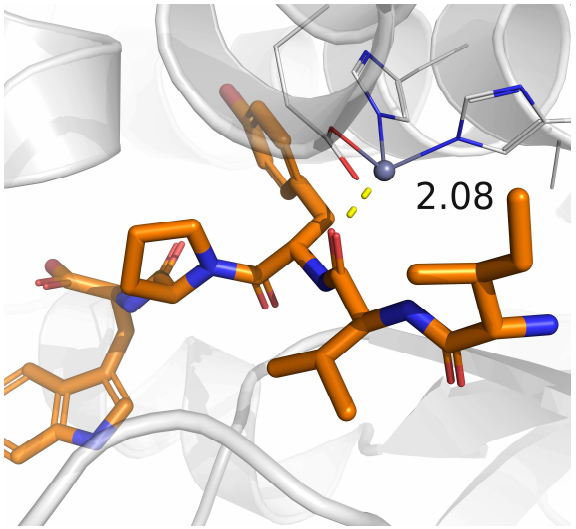
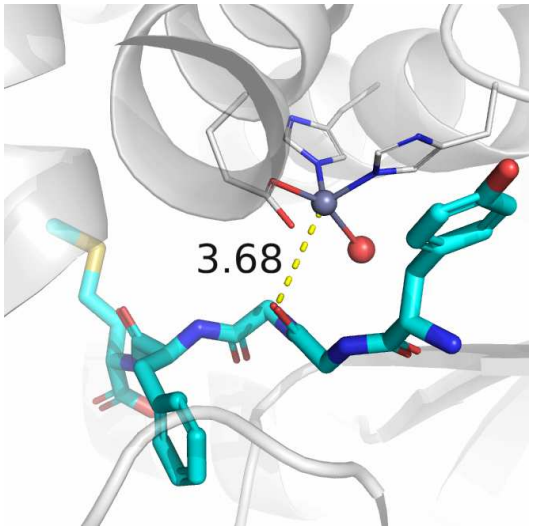


DPP III - IVYPW
(pdb: 5E3C)



DPP III – Met-enkephalin
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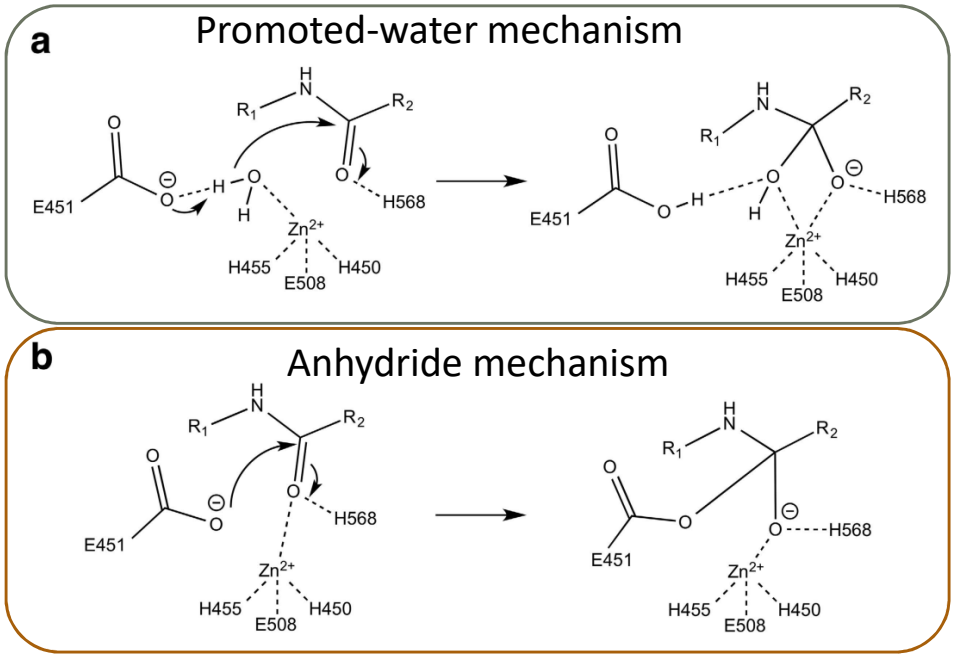

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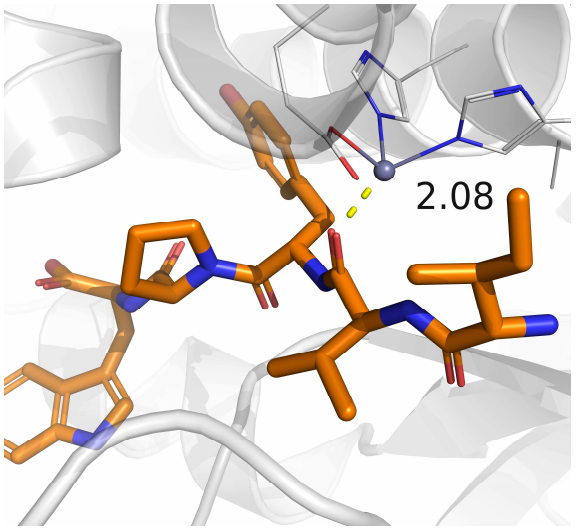
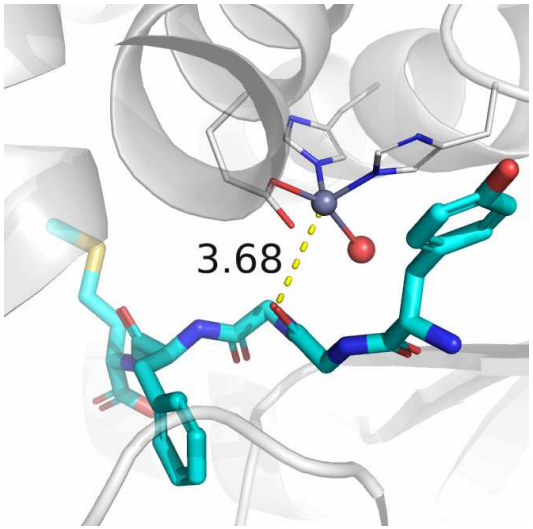
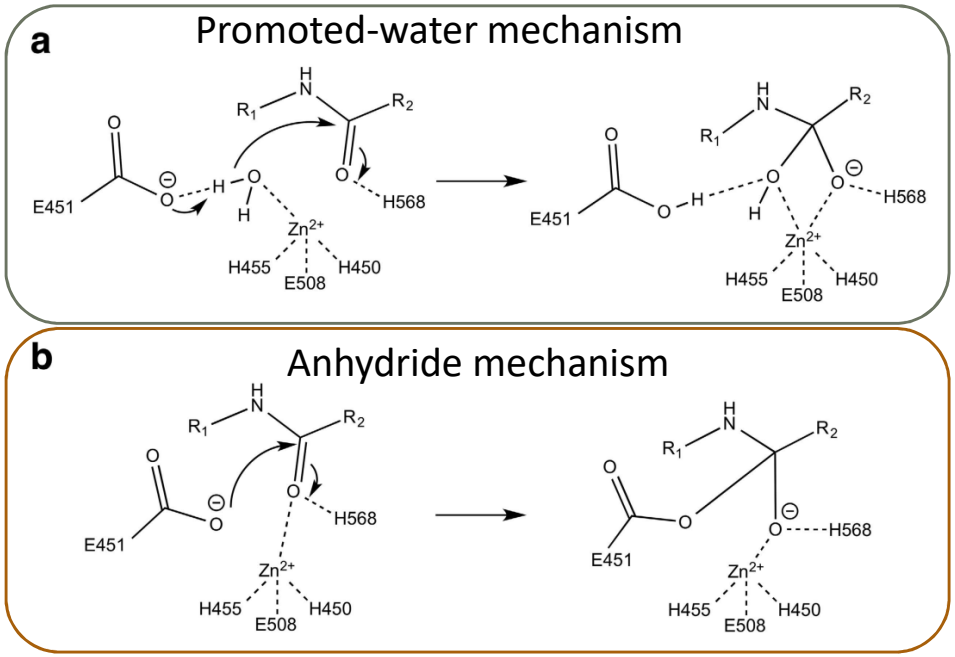


DPP III - IVYPW
(pdb: 5E3C)



DPP III – Met-enkephalin
(pdb: 5E3A)



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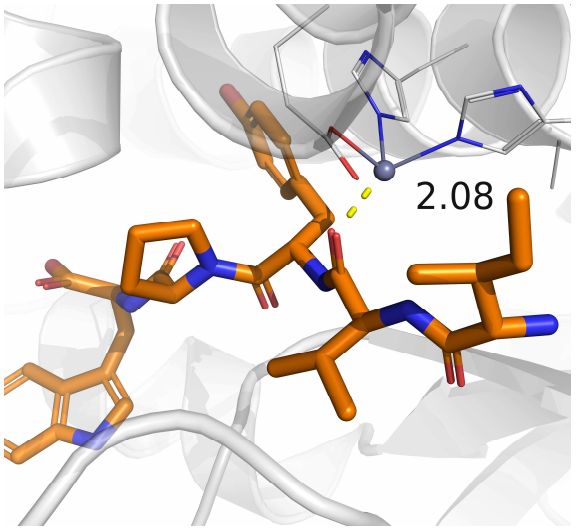
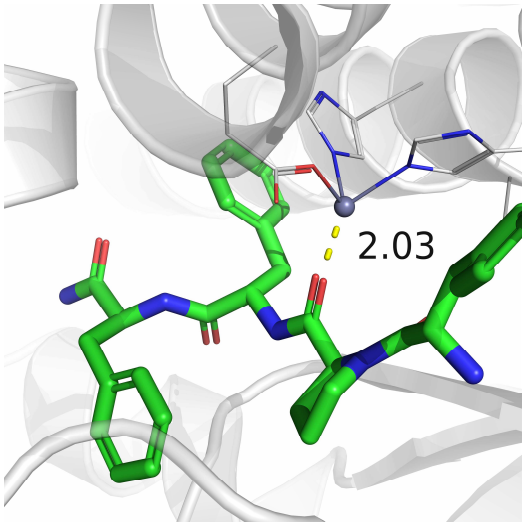


DPP III - IVYPW
(pdb: 5E3C)



DPP III – endomorphin2
(2016. pdb: 5ehh)

MECHANISM OF HYDROLYSIS

LEU-ENKEPHALIN

(„GOOD" SUBSTRATE)

DPP III

Tyr – Gly ÷ Gly – Phe – Leu

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

TYNORPHIN

(„SLOW" SUBSTRATE)

DPP III

Val – Val ÷ Tyr – Pro – Trp

- 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

MECHANISM OF HYDROLYSIS

MOLECULAR MECHANIC - QUANTUM MECHANIC (**QM/MM**) CALCULATIONS

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

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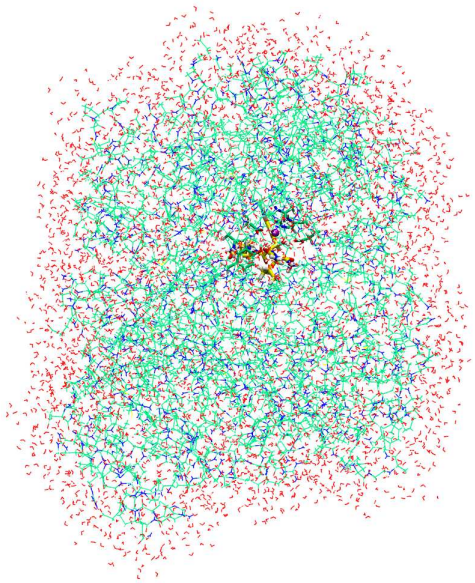
2-layer **ONIOM** calculations (Gaussian 09)

Real system



COMPLEX + 1st and 2nd enzyme solvatation sphere

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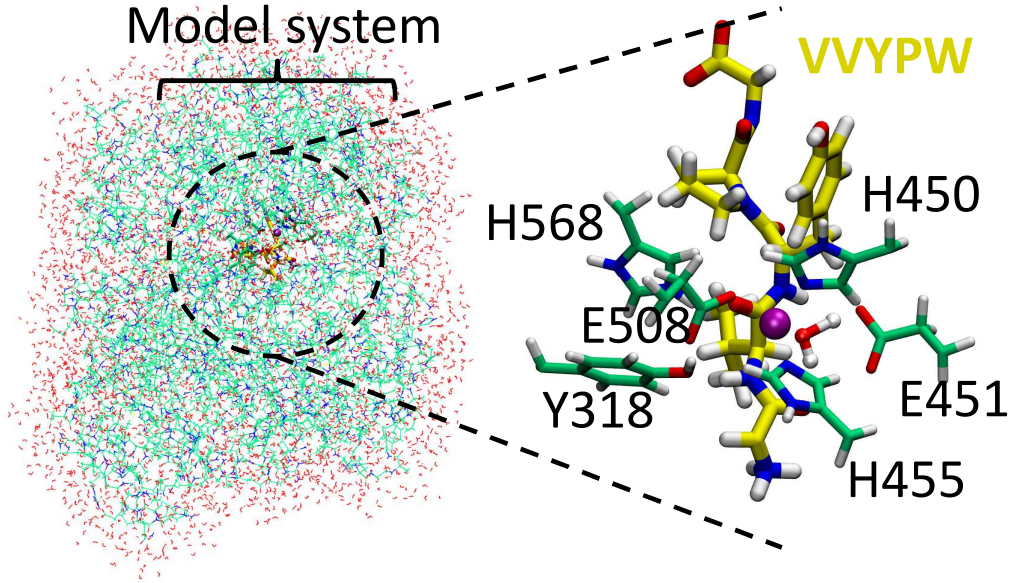

COMPLEX + 1st and 2nd enzyme solvatation sphere

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

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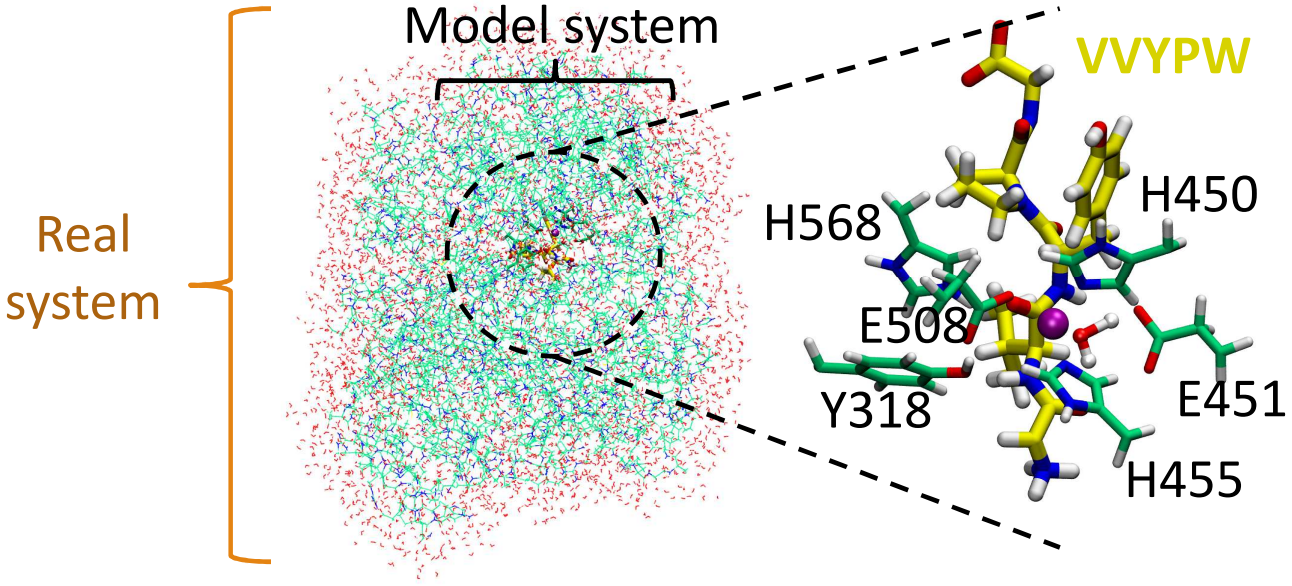


COMPLEX + 1st and 2nd enzyme solvatation sphere

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

LEU-ENKEPHALIN hydrolysis

DPP III

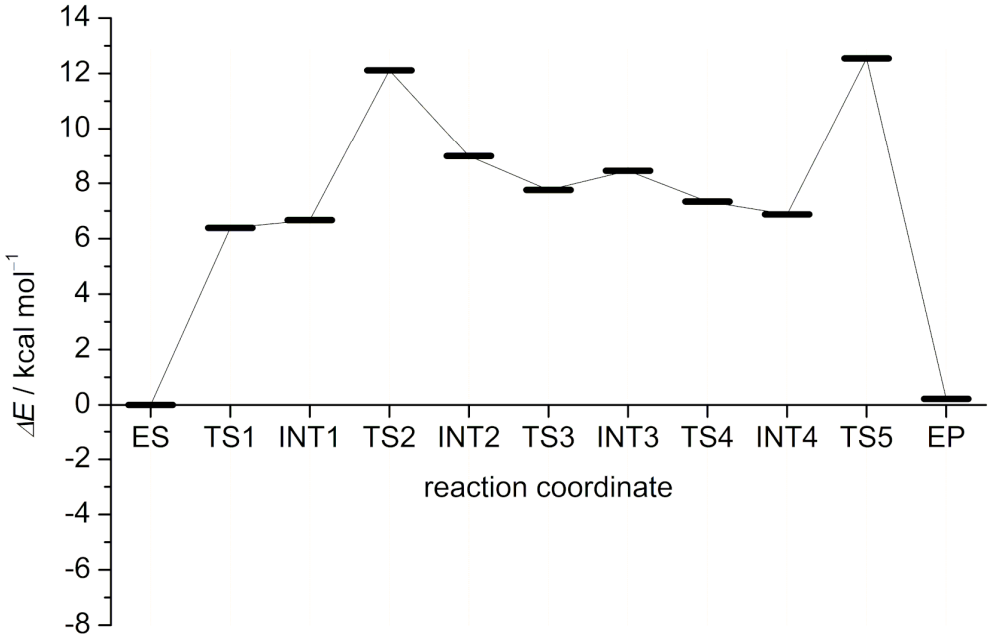
Tyr – Gly – Gly – Phe – Leu

LEU-ENKEPHALIN hydrolysis

DPP III

Tyr – Gly – Gly – Phe – Leu

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

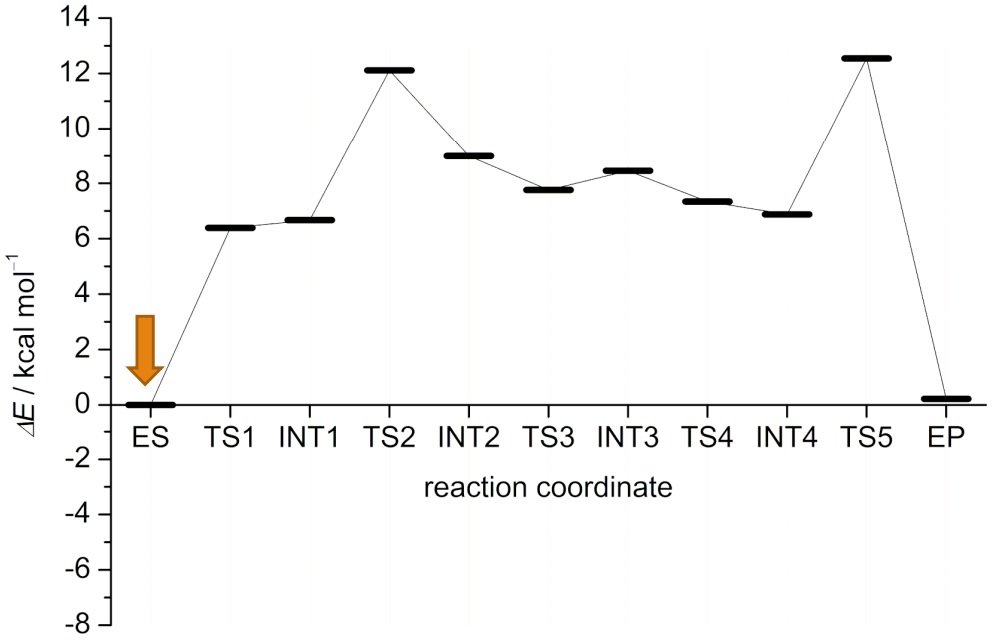


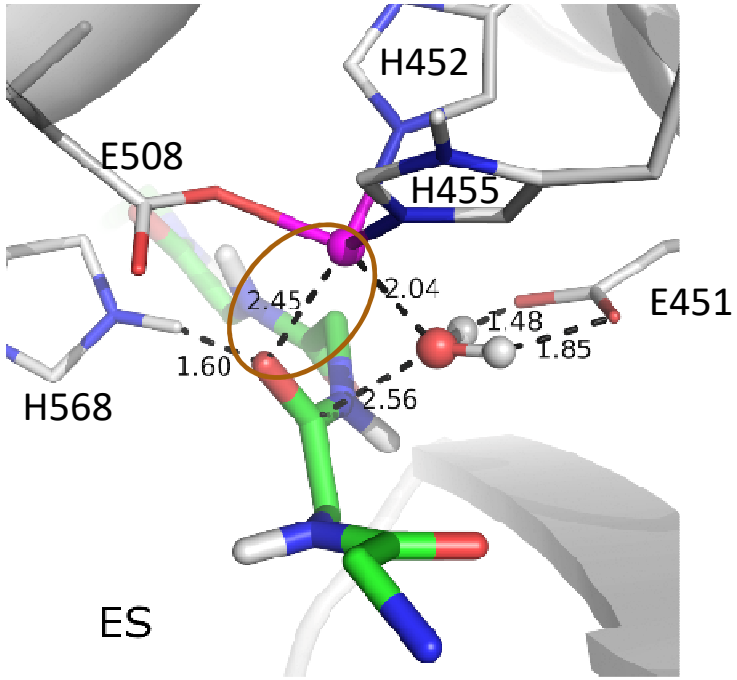
LEU-ENKEPHALIN hydrolysis

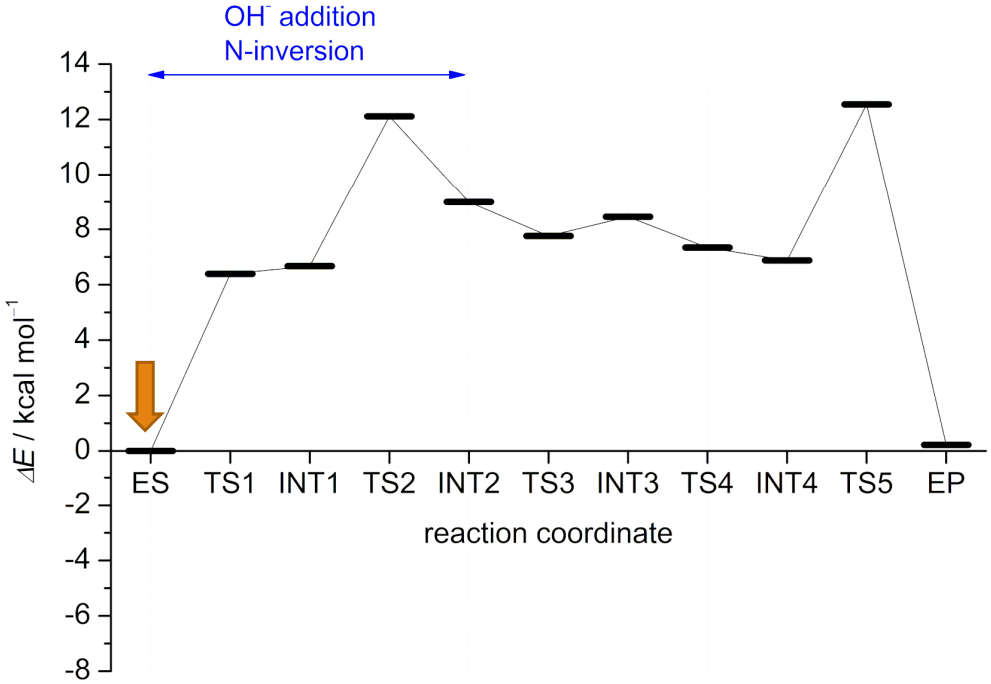
DPP III

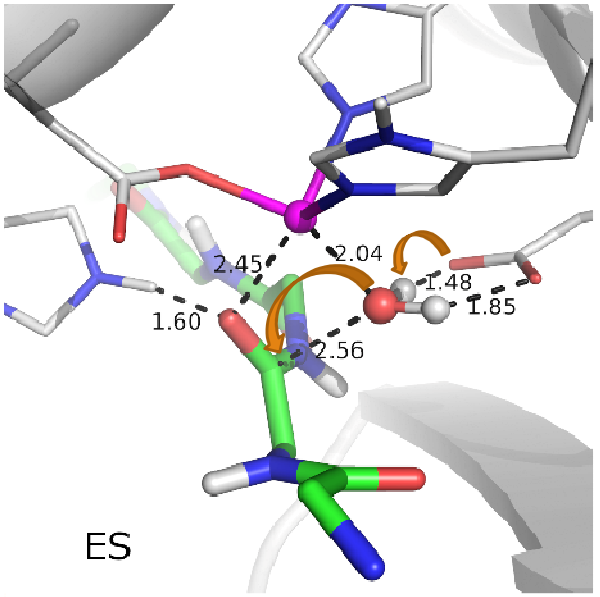
Tyr – Gly – Gly – Phe – Leu

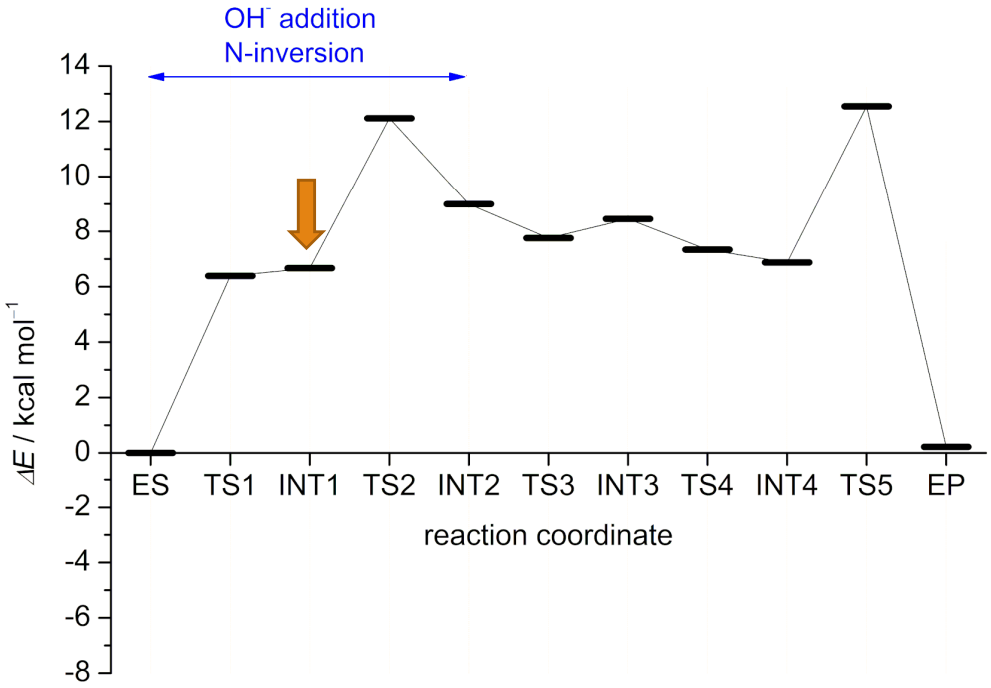
B97D/[6-31G(d)+LanL2DZ-ECP] + ZPVE$_{\text{B97D/[6-31G(d)+LanL2DZ-ECP]}}$






OH- addition
N-inversion
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
INT4
TS5
EP
reaction coordinate


2.45
2.04
1.48
1.85
1.60
2.56
ES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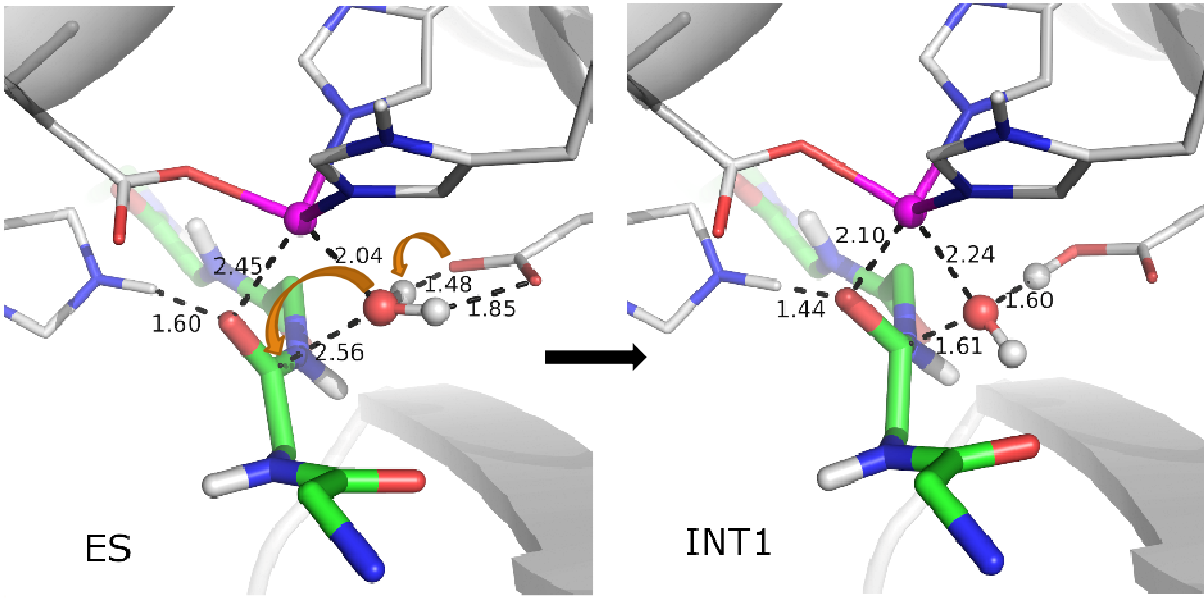
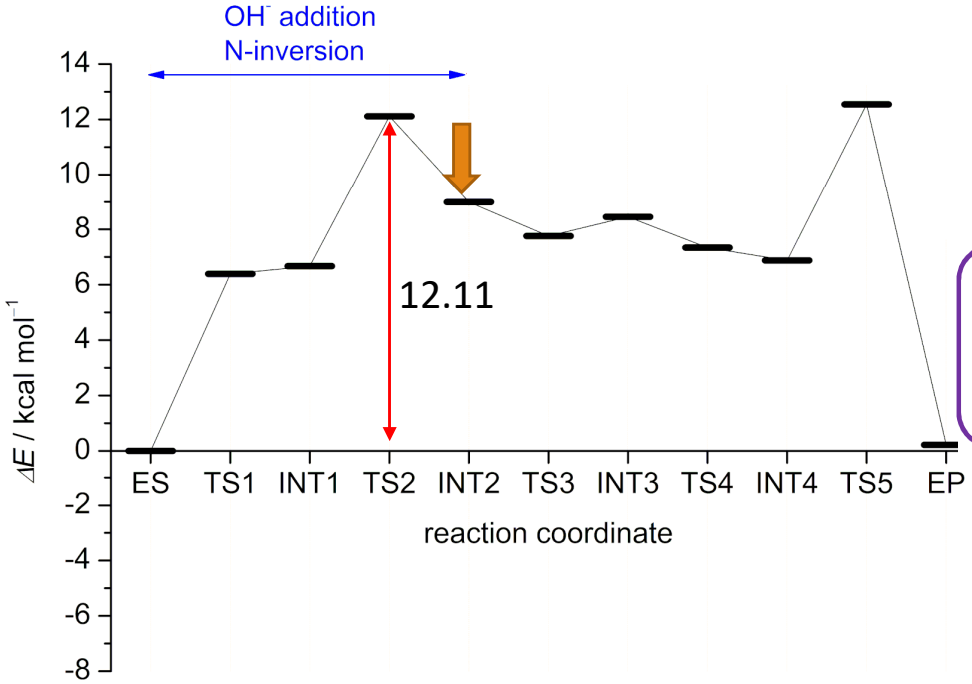
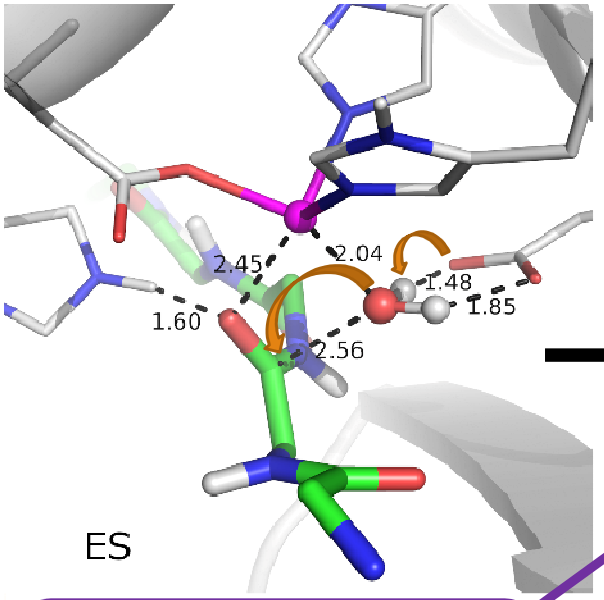
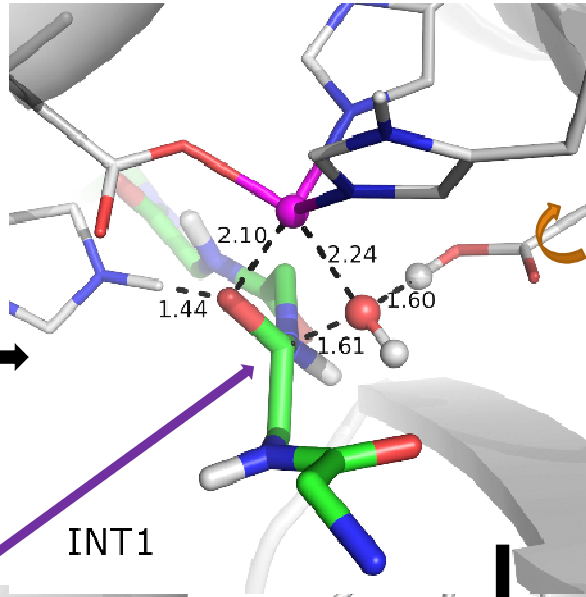
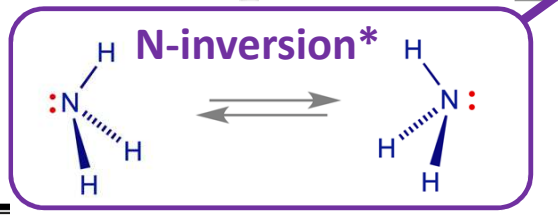
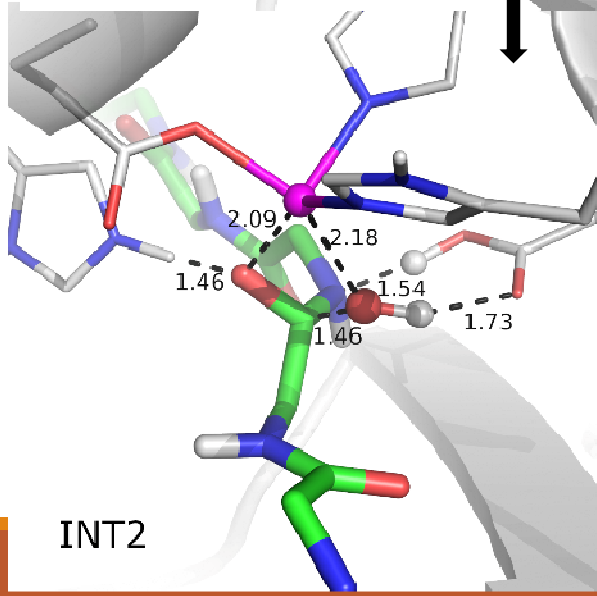

2.45
2.04
1.48
1.85
1.60
2.56
ES
2.10
2.24
1.44
1.60
1.61
INT1



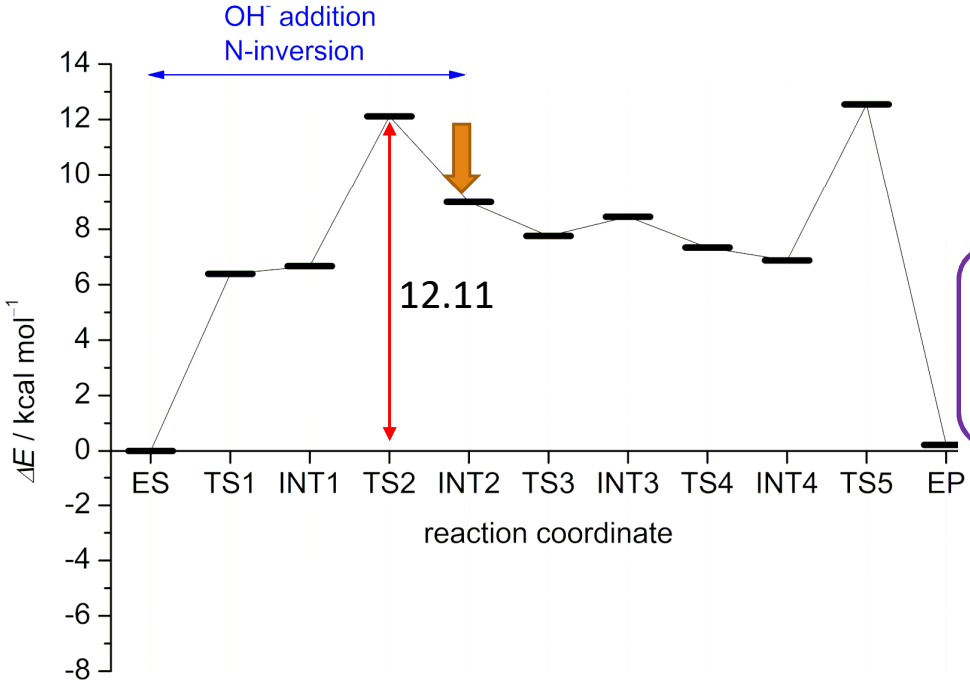


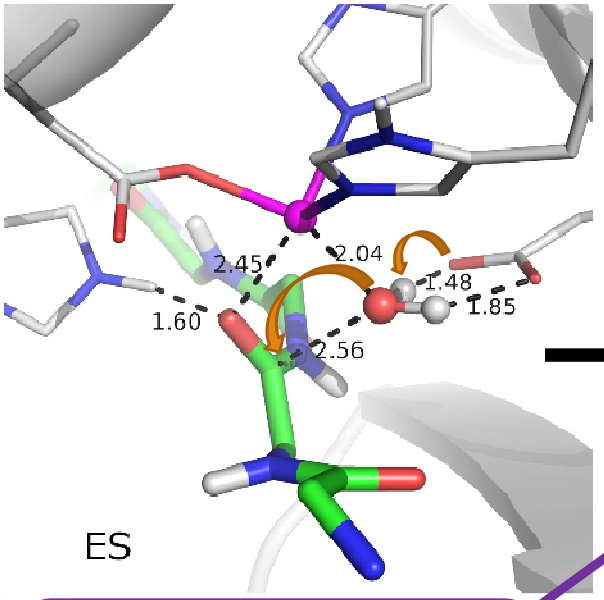


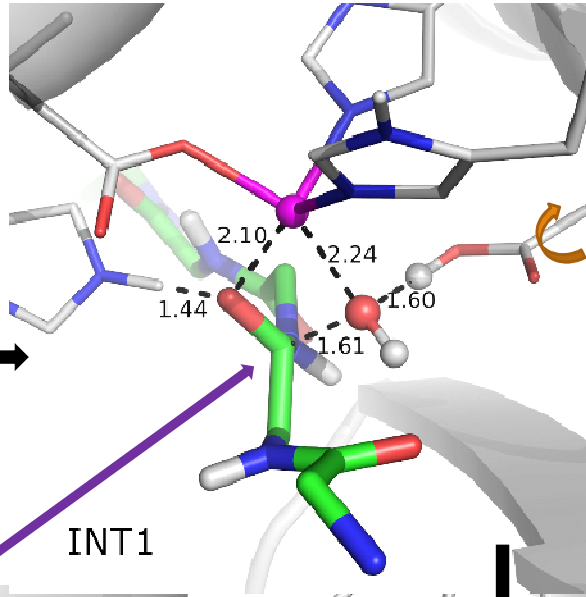


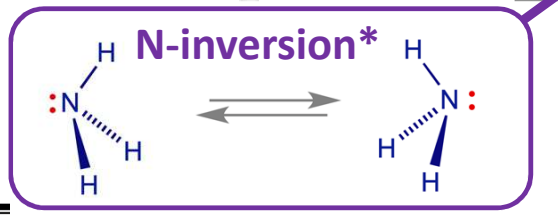


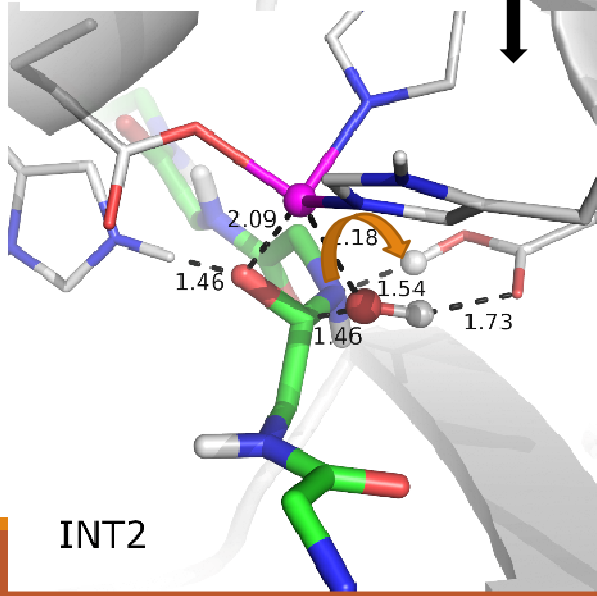
*https://www.stereoelectronics.org/webSC/SC_04.html



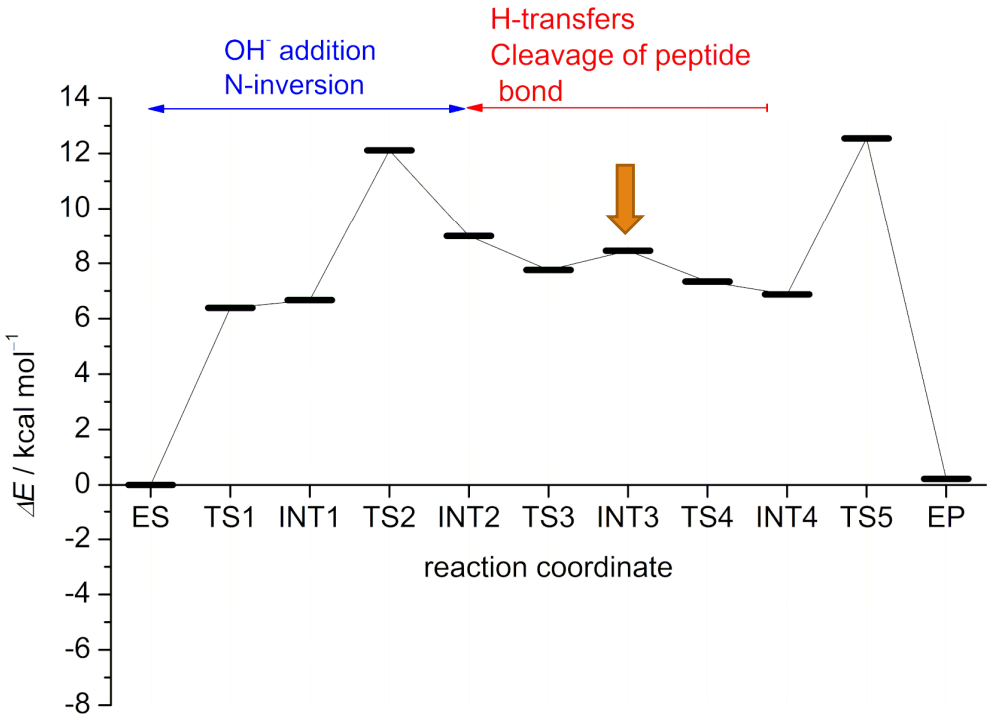


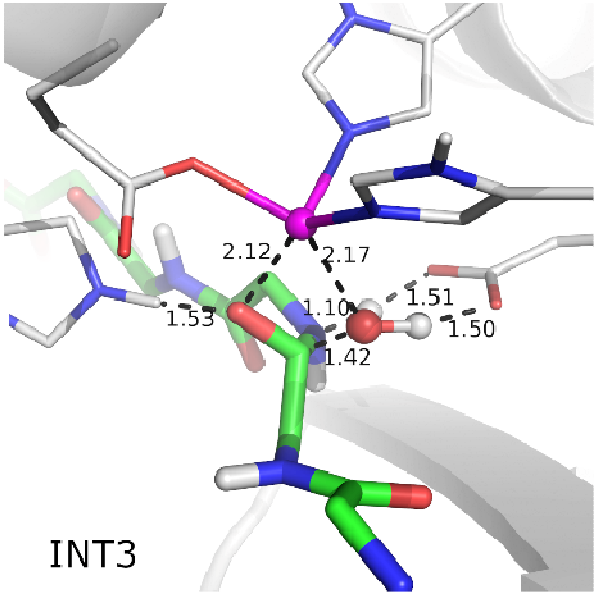


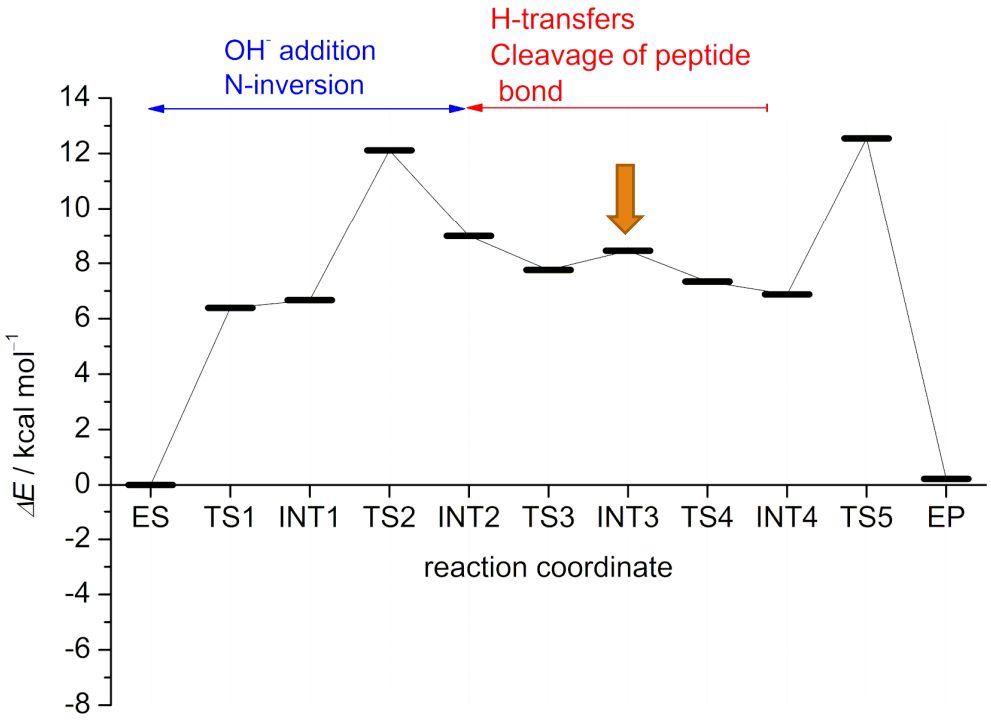


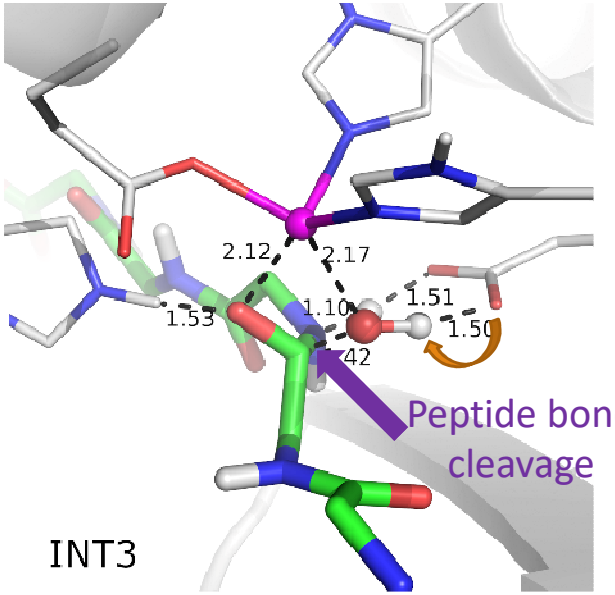


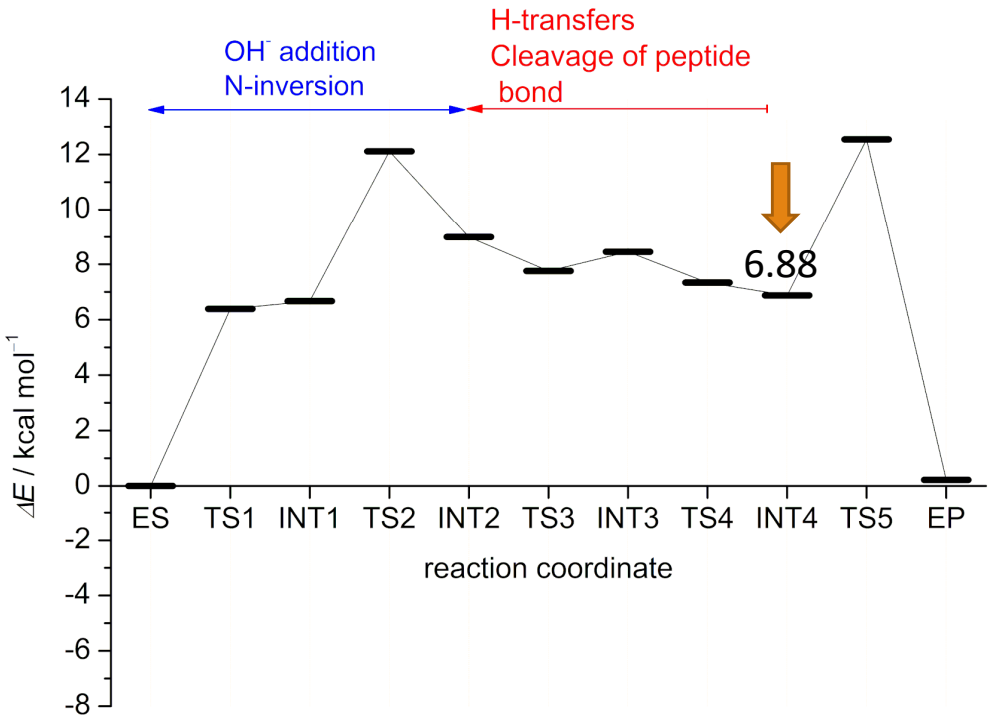
*https://www.stereoelectronics.org/webSC/SC_04.html

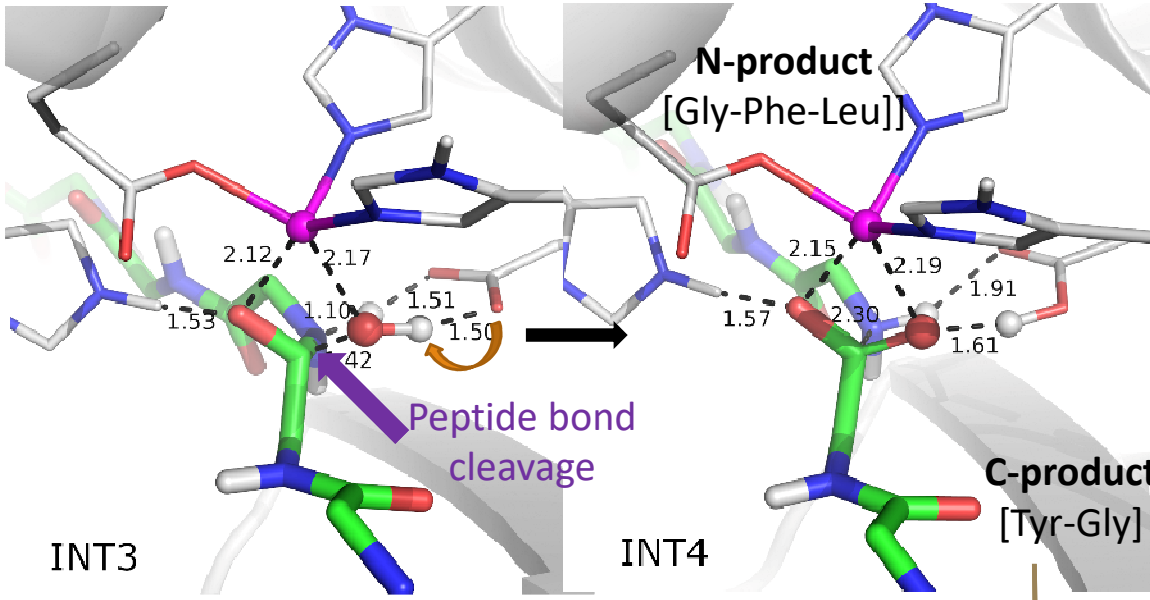

OH- addition
N-inversion
H-transfers
Cleavage of peptide
bond
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
INT4
TS5
EP
reaction coordinate

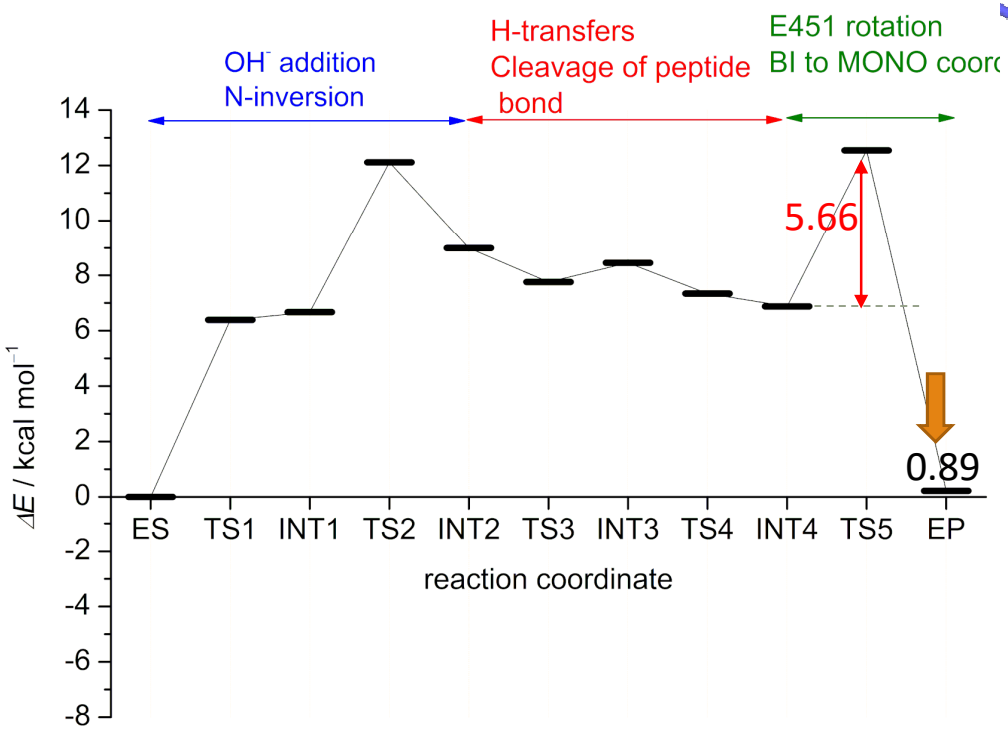

2.12
2.17
1.53
1.10
1.51
1.50
1.42
INT3

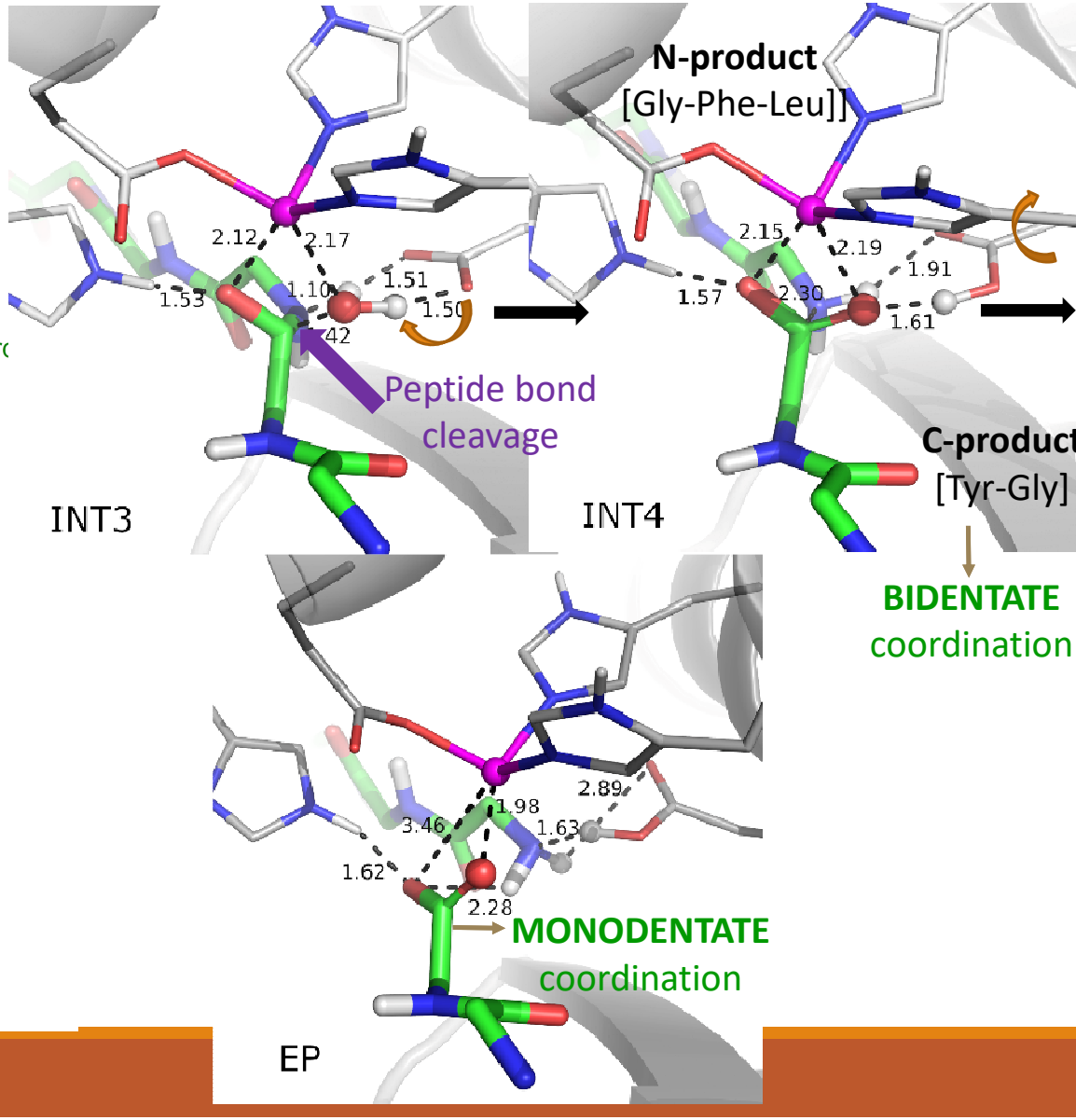

OH- addition
N-inversion
H-transfers
Cleavage of peptide
bond
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
INT4
TS5
EP
reaction coordinate


2.12
2.17
1.53
1.10
1.51
1.50
42
Peptide bond
cleavage
INT3

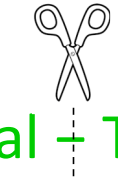

OH- addition
N-inversion
H-transfers
Cleavage of peptide
bond
6.88
ΔE / kcal mol-1
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
INT4
TS5
EP
reaction coordinate


N-product
[Gly-Phe-Leu]]
2.12
2.17
1.53
1.10
1.51
1.50
42
2.15
2.19
1.91
1.57
2.30
1.61
Peptide bond
cleavage
INT3
INT4
C-product
[Tyr-Gly]
BIDENTATE
coordination


OH⁻ addition
N-inversion
H-transfers
Cleavage of peptide bond
E451 rotation
BI to MONO coord
5.66
0.89
ΔE / kcal mol⁻¹
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
INT4
TS5
EP
reaction coordinate


N-product
[Gly-Phe-Leu]]
2.12
2.17
1.53
1.10
1.51
1.50
42
Peptide bond
cleavage
INT3
INT4
2.15
2.19
1.91
1.57
2.30
1.61
C-product
[Tyr-Gly]
BIDENTATE
coordination
2.89
1.98
3.46
1.63
1.62
2.28
MONODENTATE
coordination
EP

TYNORPHIN hydrolysis

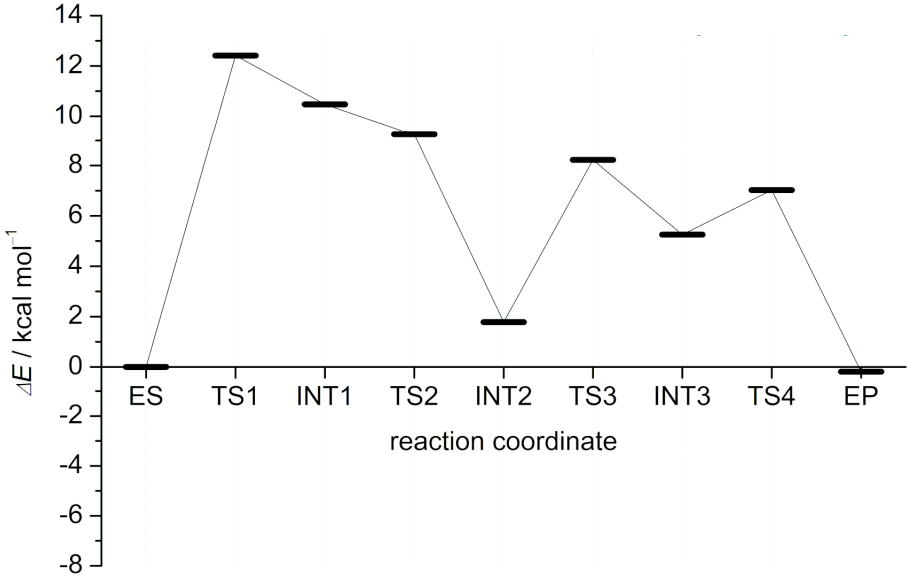


Val – Val – Tyr – Pro – Trp

TYNORPHIN hydrolysis

Val – Val – Tyr – Pro – Trp

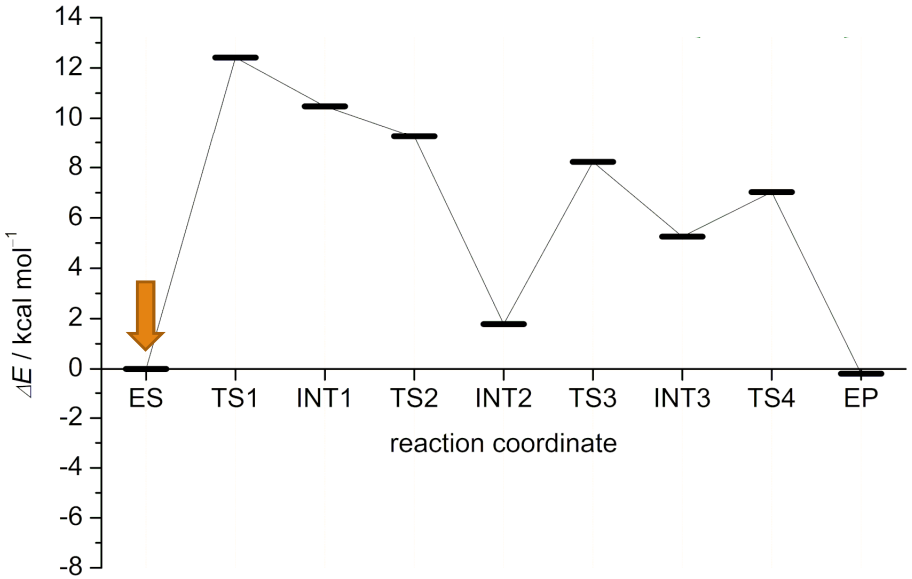
B97D/[6-31G(d)+LanL2DZ-ECP] + $ZPVE_{B97D/[6\text{-}31G(d)+LanL2DZ\text{-}ECP]}$

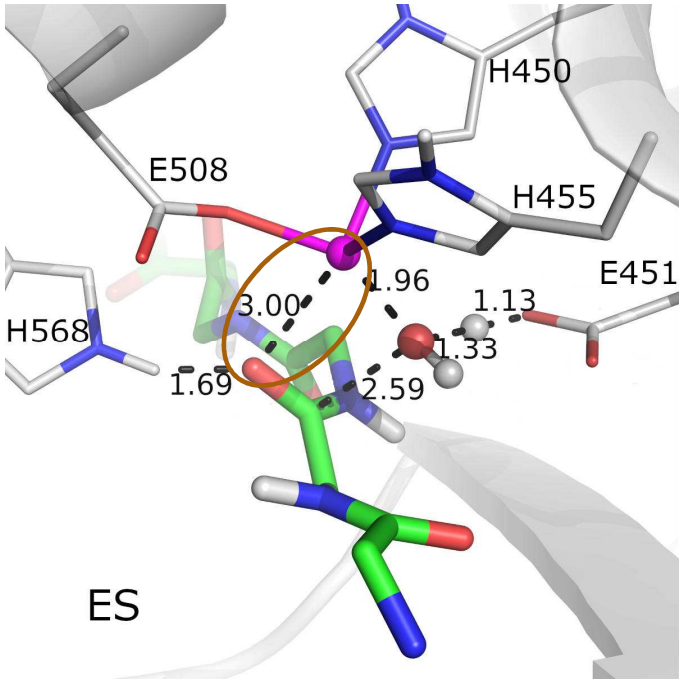


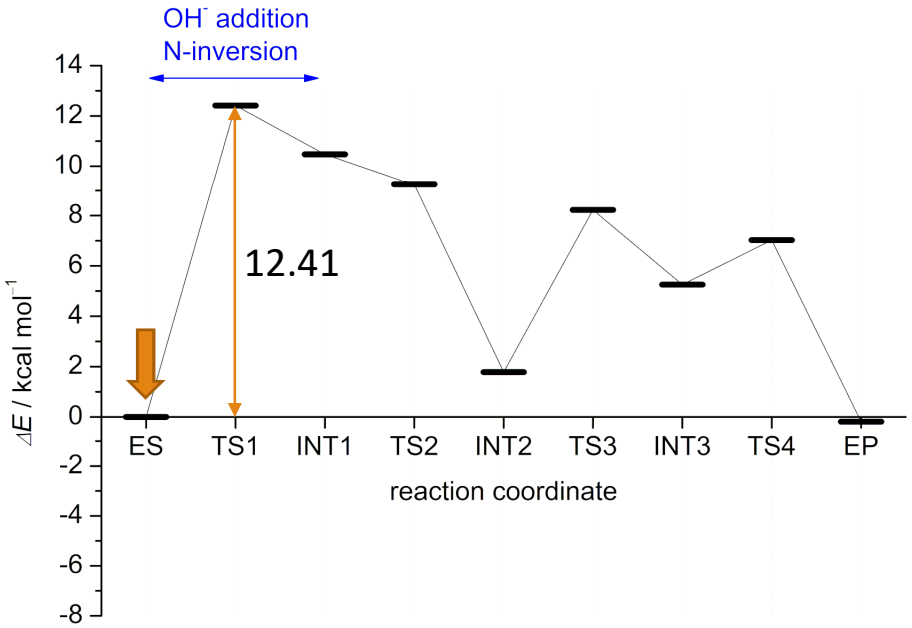
TYNORPHIN hydrolysis

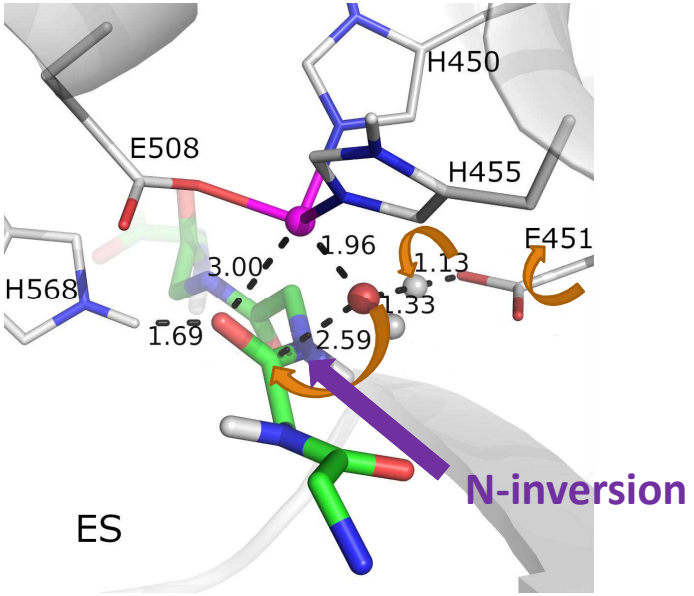
Val – Val – Tyr – Pro – Trp

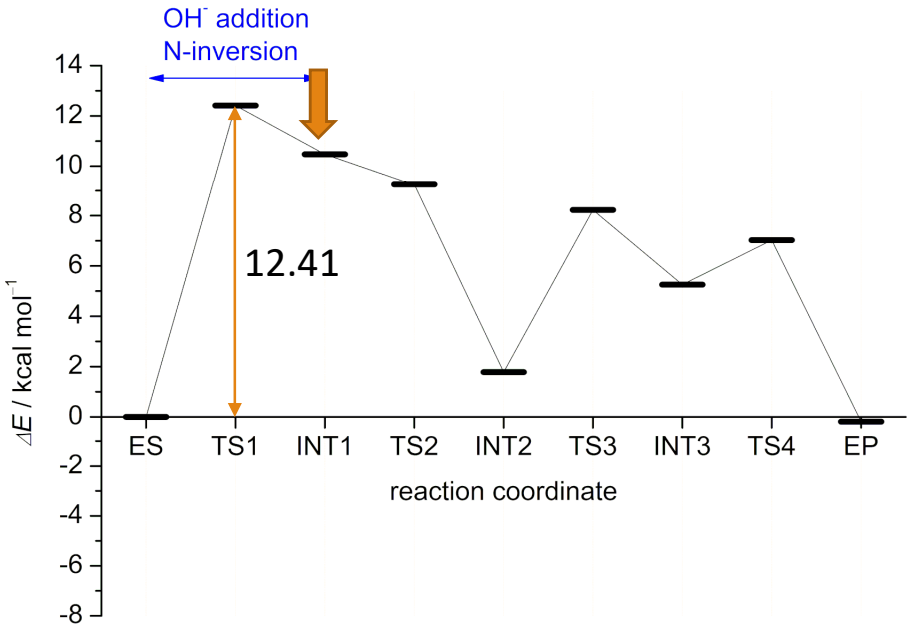
B97D/[6-31G(d)+LanL2DZ-ECP] + ZPVE$_{\text{B97D/[6-31G(d)+LanL2DZ-ECP]}}$

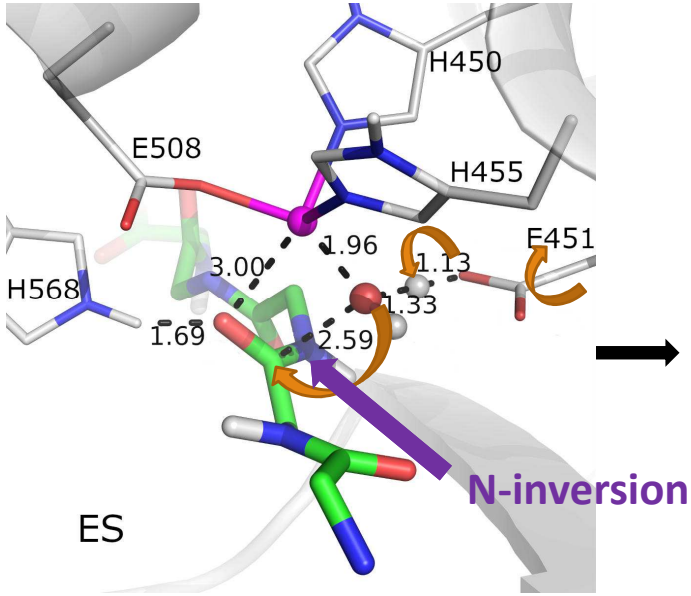


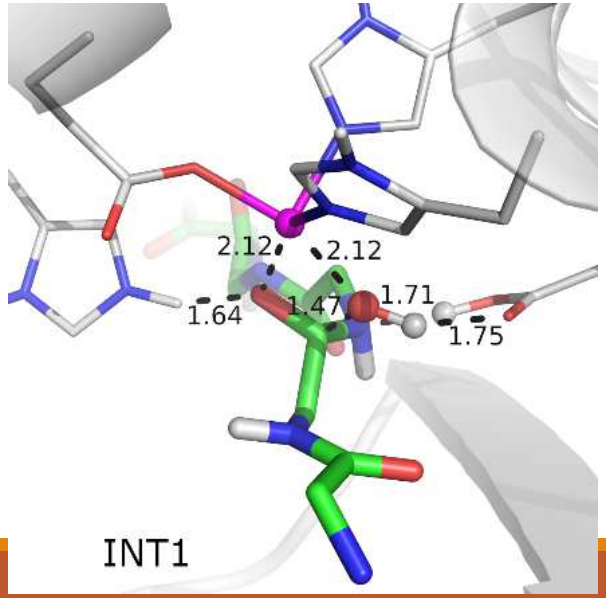


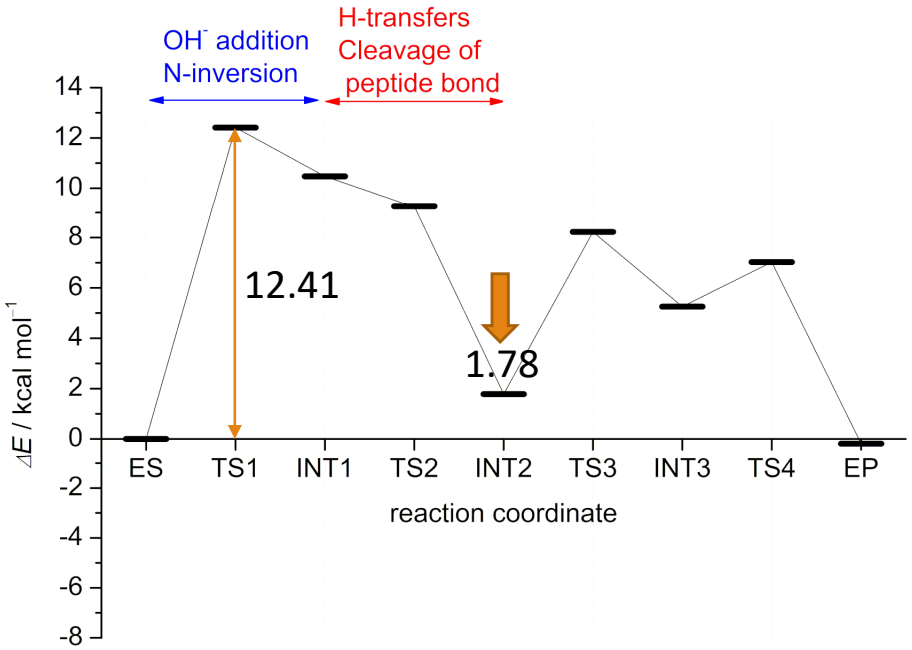

OH- addition
N-inversion
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
12.41
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate

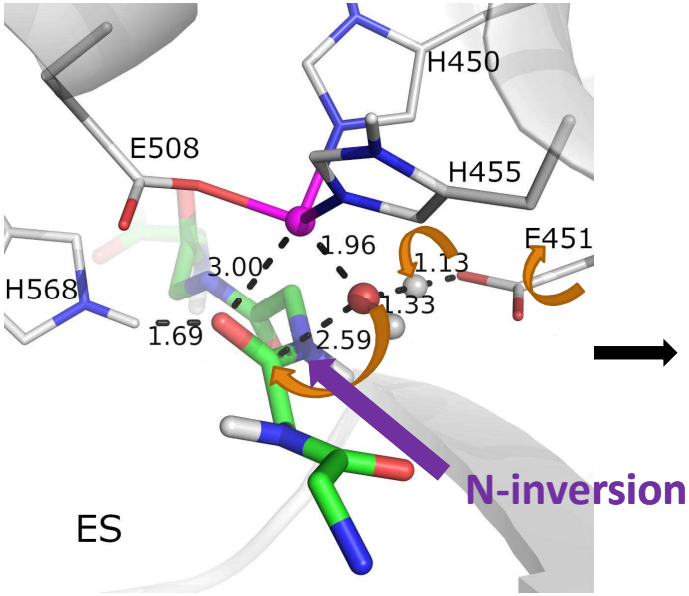

H450
E508
H455
E451
H568
1.96
3.00
1.13
1.33
1.69
2.59
N-inversion
ES

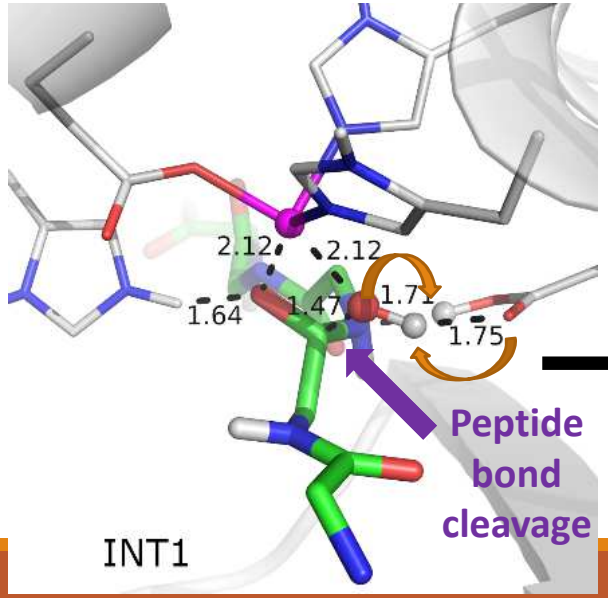

OH- addition
N-inversion
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
12.41
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate

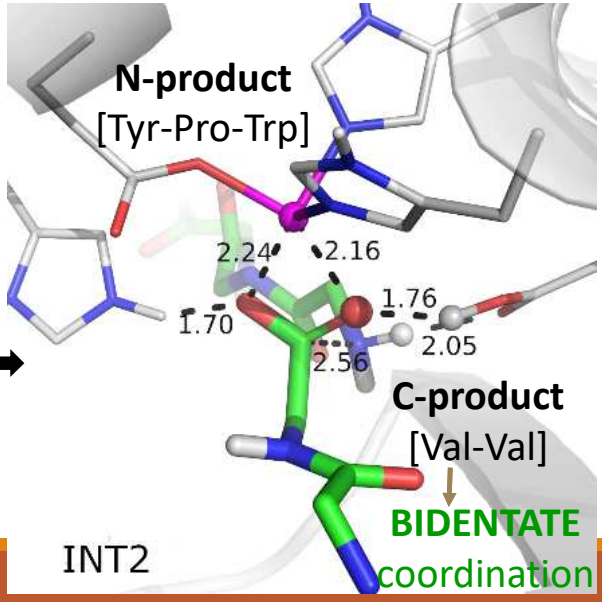

H450
E508
H455
E451
H568
3.00
1.96
1.13
1.33
1.69
2.59
N-inversion
ES

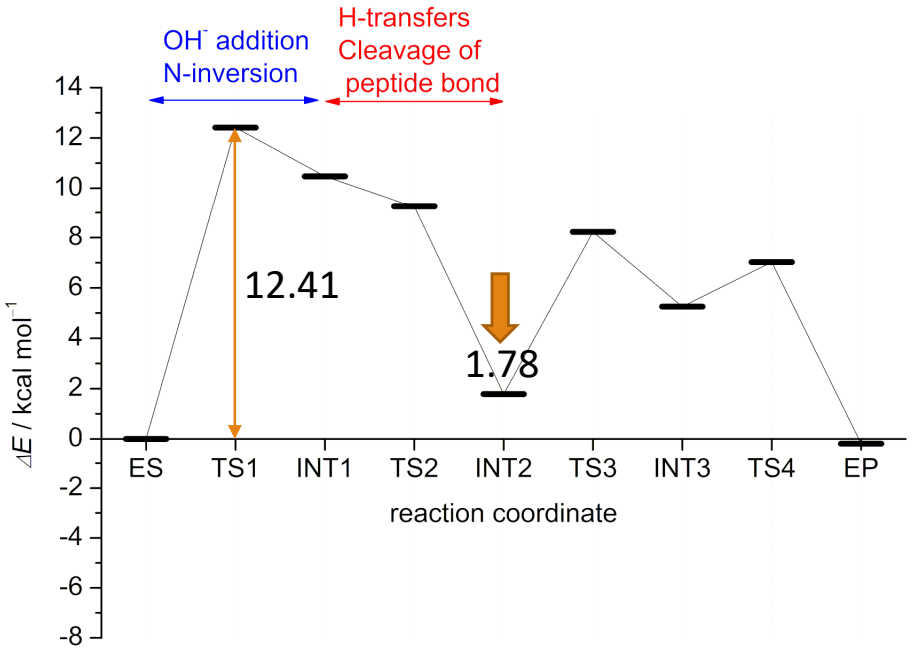

2.12
2.12
1.64
1.47
1.71
1.75
INT1

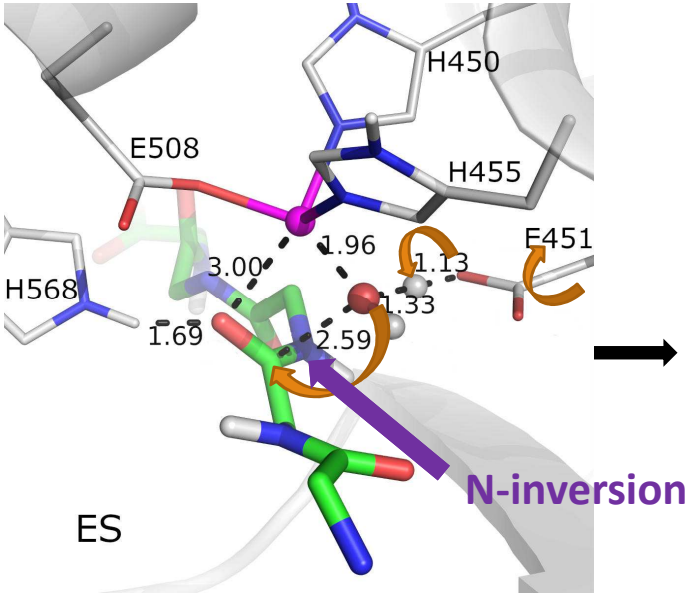

OH⁻ addition
N-inversion
H-transfers
Cleavage of
peptide bond
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol⁻¹
12.41
1.78
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate

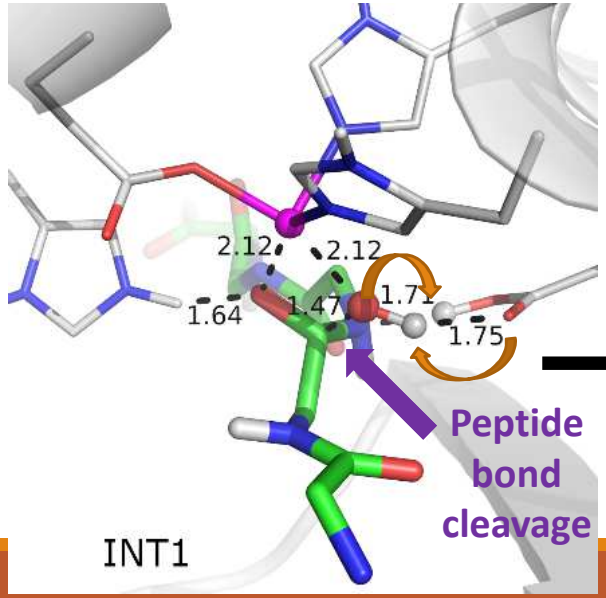

H450
E508
H455
E451
H568
3.00
1.96
1.13
1.33
1.69
2.59
N-inversion
ES

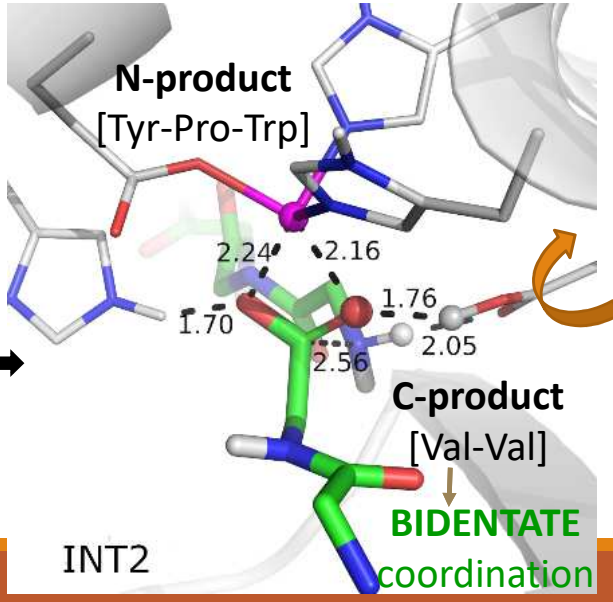

2.12
2.12
1.64
1.47
1.71
1.75
Peptide
bond
cleavage
INT1

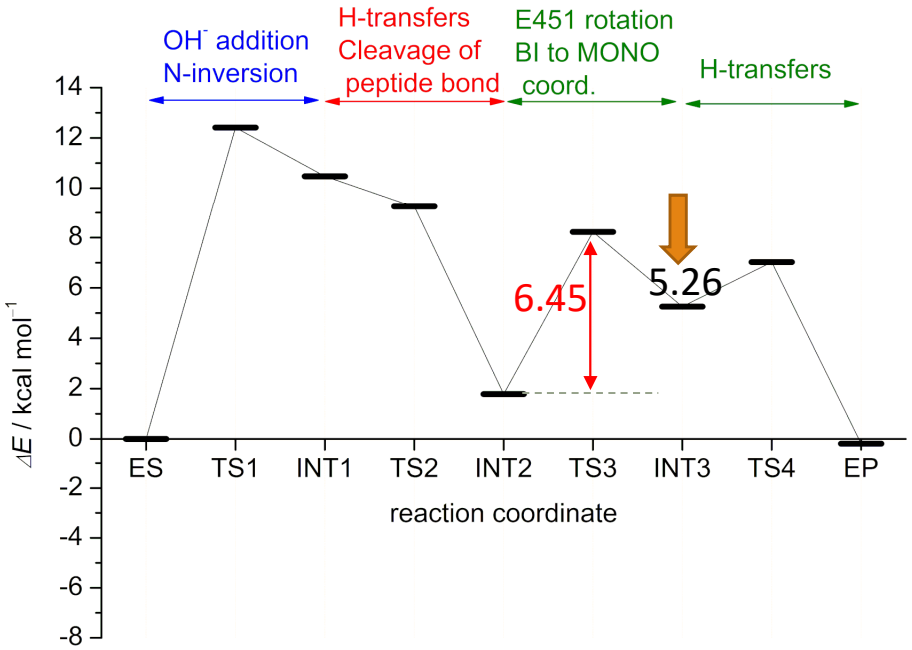

N-product
[Tyr-Pro-Trp]
2.24
2.16
1.76
1.70
2.05
2.56
C-product
[Val-Val]
BIDENTATE
coordination
INT2

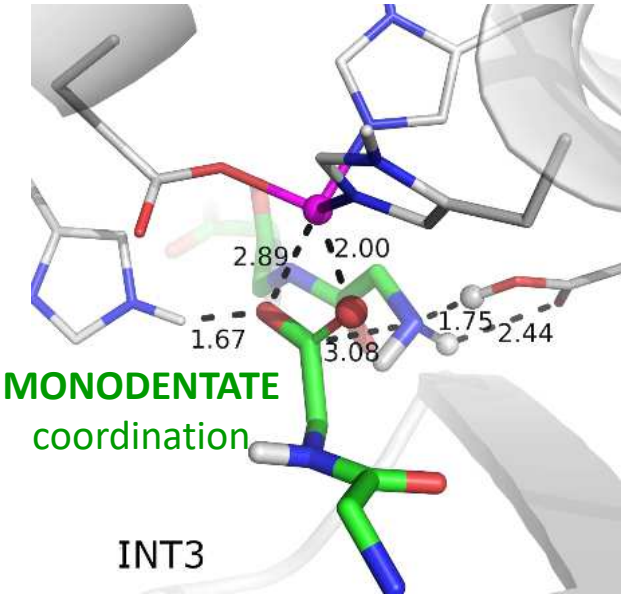

OH⁻ addition
N-inversion
H-transfers
Cleavage of
peptide bond
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol⁻¹
12.41
1.78
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate

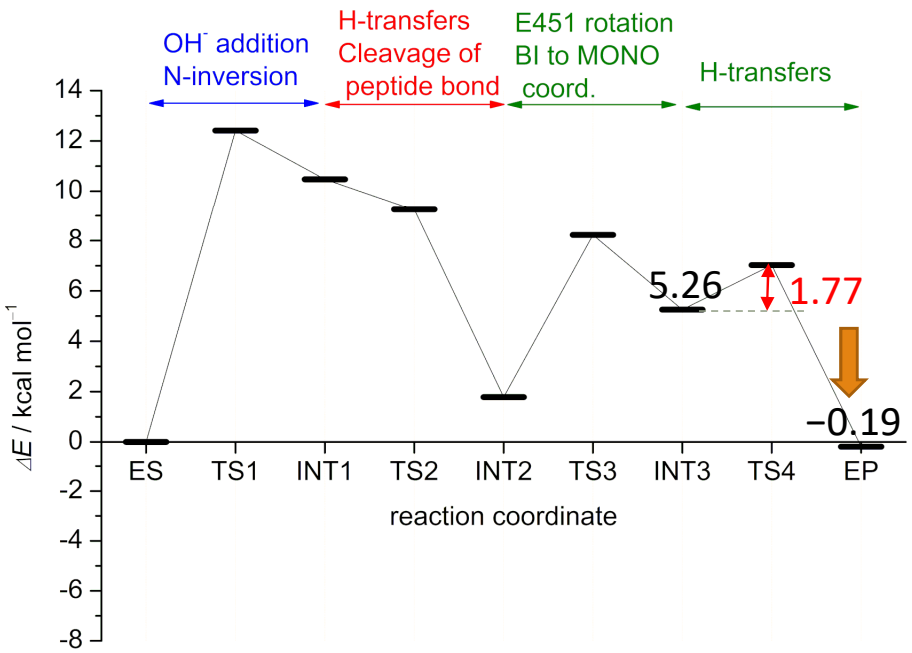

H450
E508
H455
E451
H568
3.00
1.96
1.13
1.33
1.69
2.59
N-inversion
ES

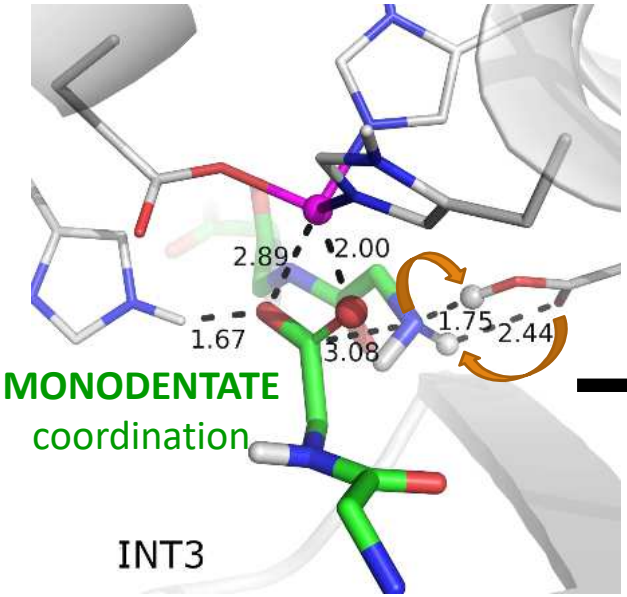

2.12
2.12
1.64
1.47
1.71
1.75
Peptide
bond
cleavage
INT1

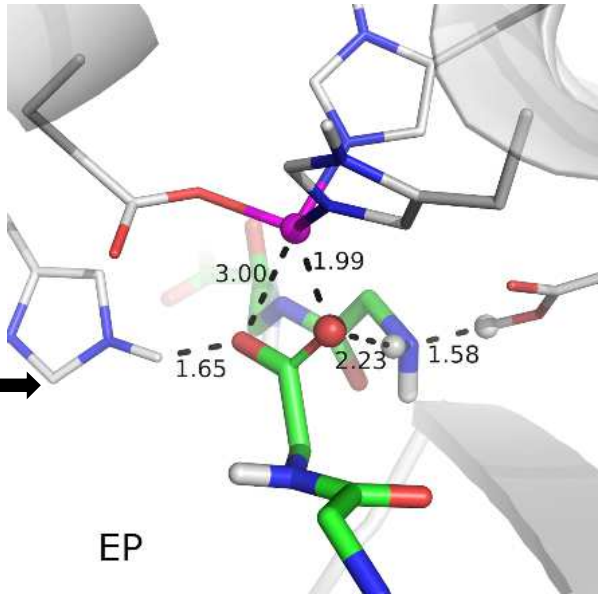

N-product
[Tyr-Pro-Trp]
2.24
2.16
1.76
1.70
2.05
2.56
C-product
[Val-Val]
BIDENTATE
coordination
INT2


OH- addition
N-inversion
H-transfers
Cleavage of
peptide bond
E451 rotation
BI to MONO
coord.
H-transfers
14
12
10
8
6
4
2
0
-2
-4
-6
-8
ΔE / kcal mol-1
6.45
5.26
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate


2.89
2.00
1.67
3.08
1.75
2.44
MONODENTATE
coordination
INT3

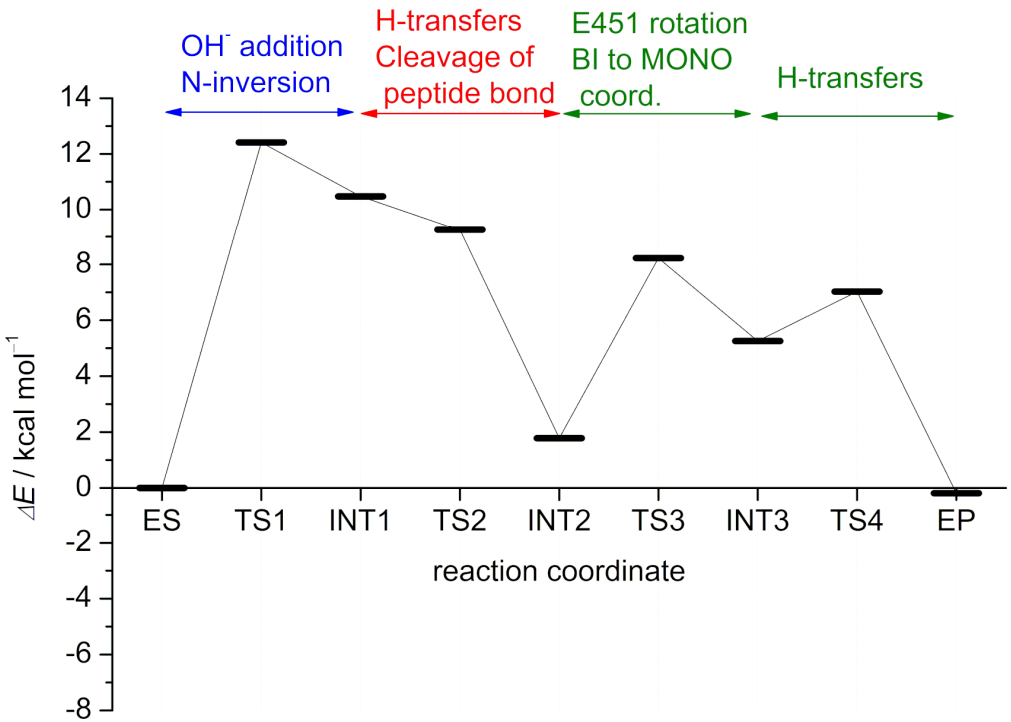

OH⁻ addition
N-inversion
H-transfers
Cleavage of
peptide bond
E451 rotation
BI to MONO
coord.
H-transfers
ΔE / kcal mol⁻¹
14
12
10
8
6
4
2
0
-2
-4
-6
-8
5.26
1.77
−0.19
ES
TS1
INT1
TS2
INT2
TS3
INT3
TS4
EP
reaction coordinate


2.89
2.00
1.67
3.08
1.75
2.44
MONODENTATE
coordination
INT3


3.00
1.99
1.65
2.23
1.58
EP

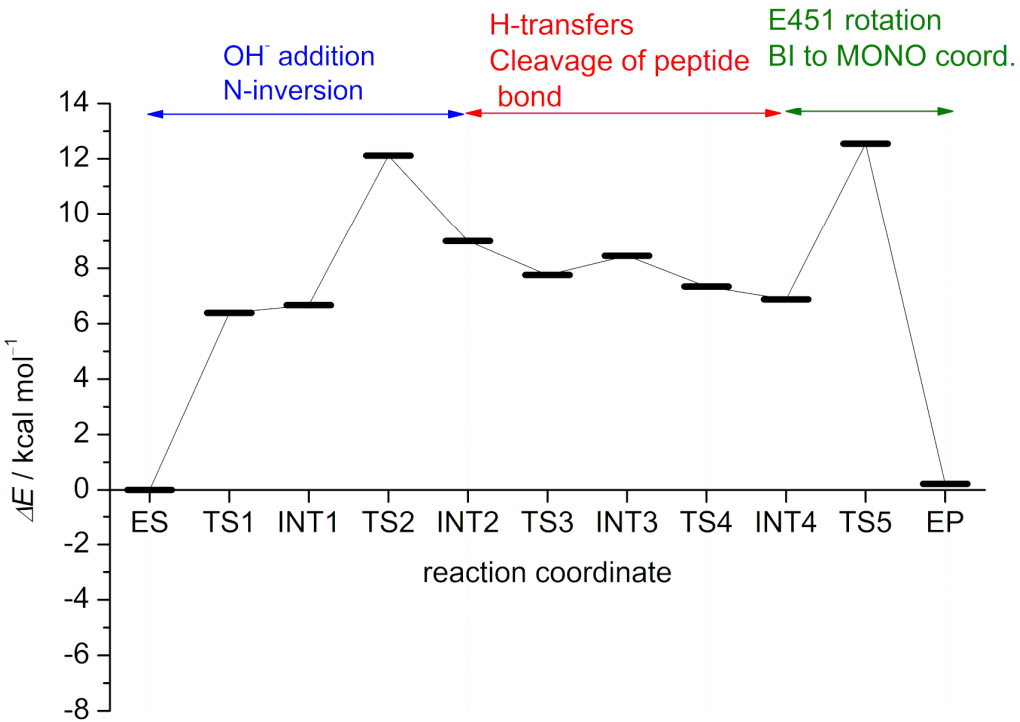
„SLOW" SUBSTRATE

DPP III - tynorphin



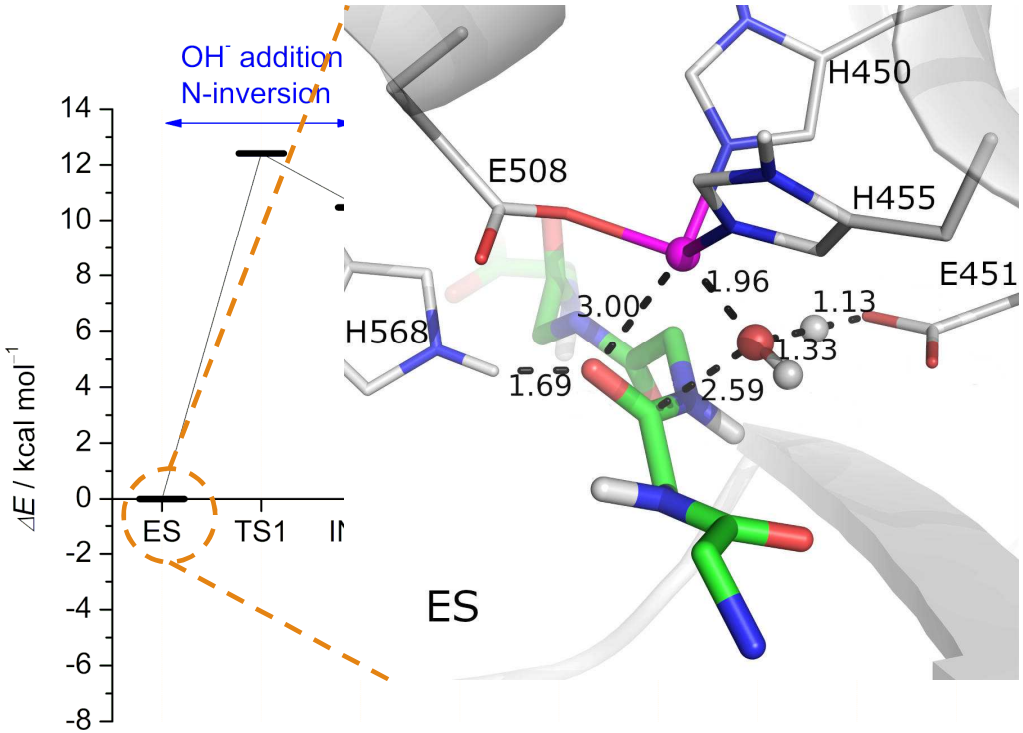
„GOOD" SUBSTRATE

DPP III – Leu-enkephalin



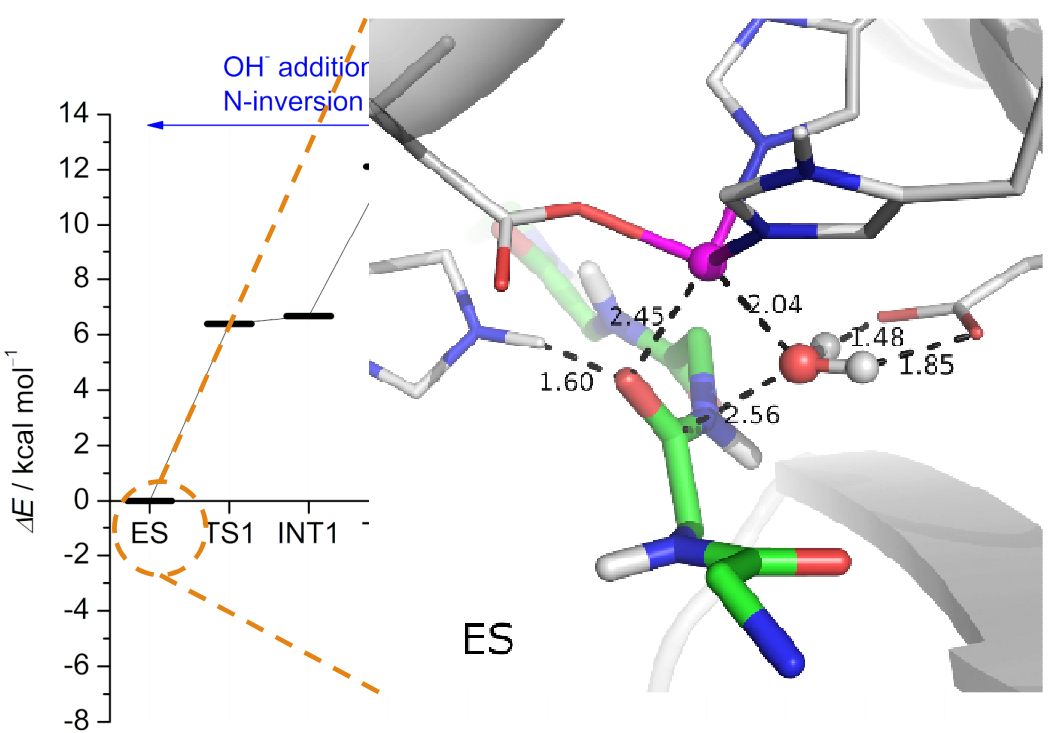
„SLOW" SUBSTRATE

DPP III - tynorphin



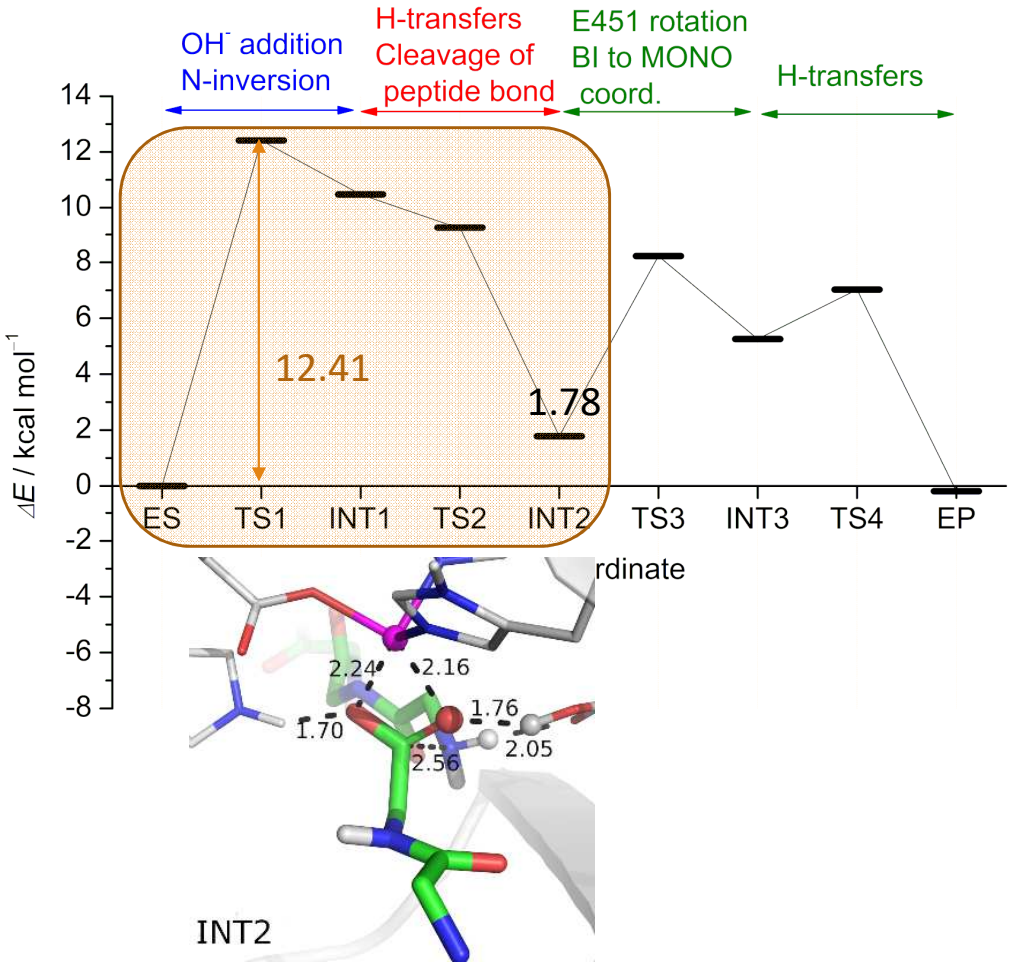
„GOOD" SUBSTRATE

DPP III – Leu-enkephalin



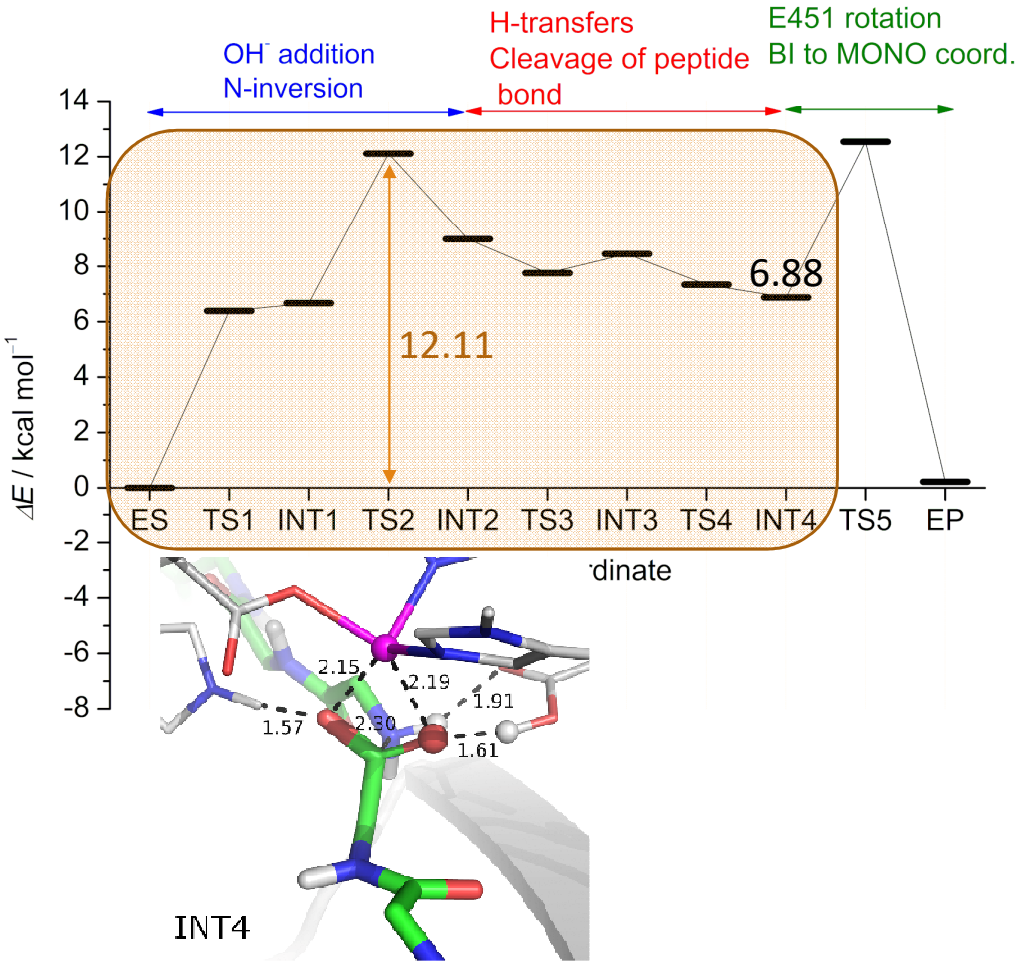
„SLOW" SUBSTRATE

DPP III - tynorphin



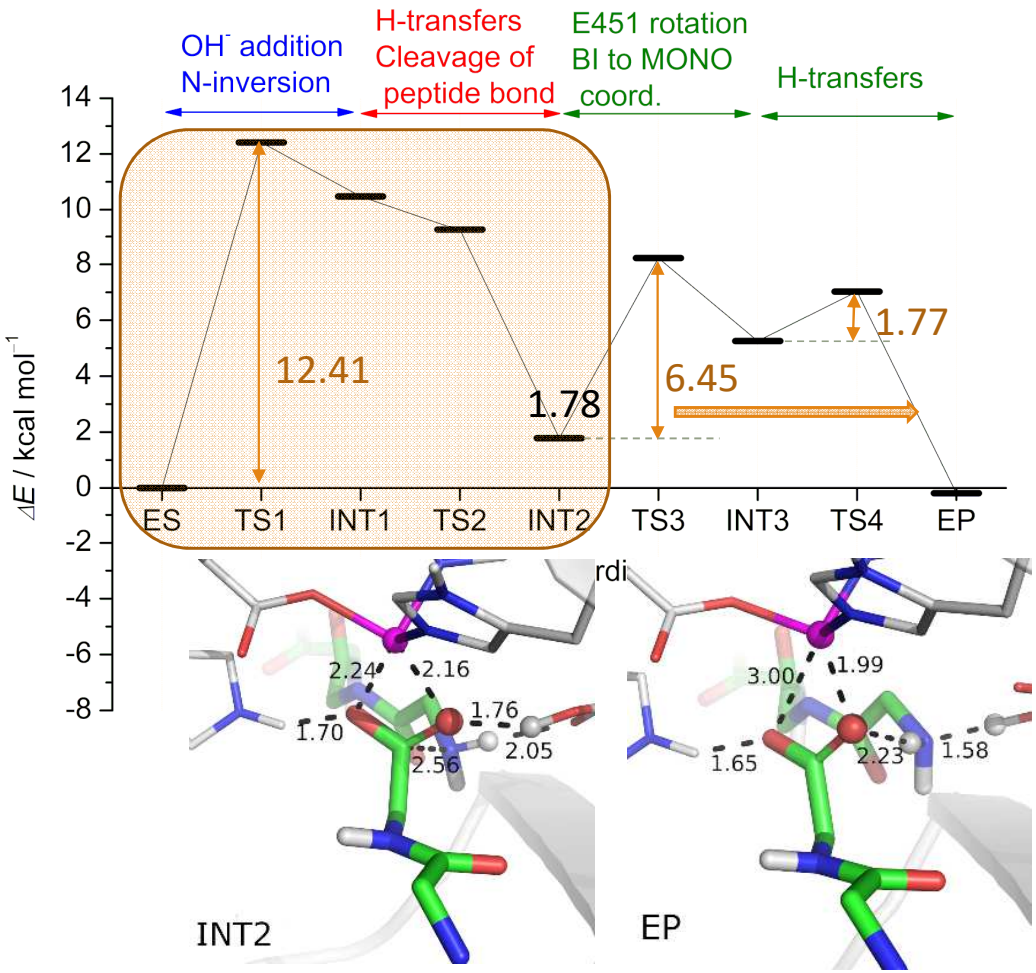
„GOOD" SUBSTRATE

DPP III – Leu-enkephalin



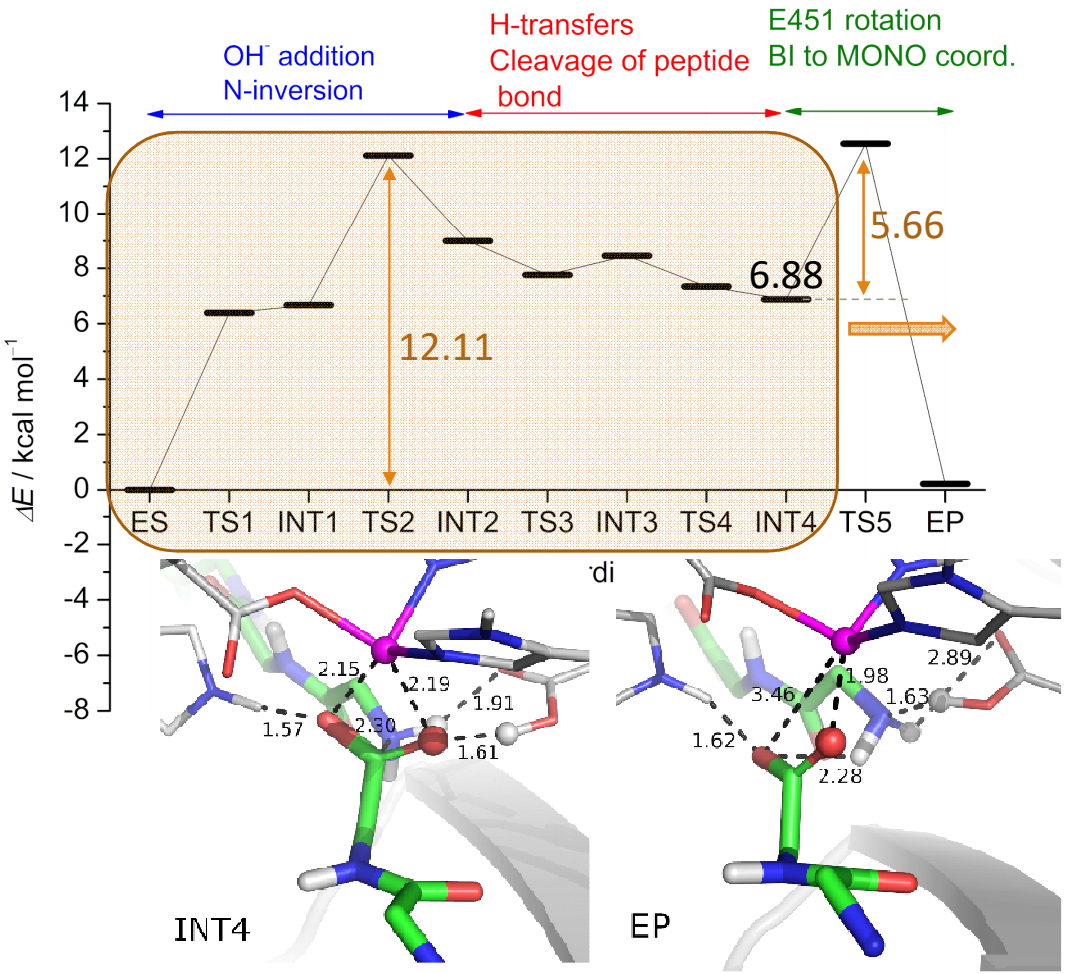
„SLOW" SUBSTRATE

DPP III - tynorphin



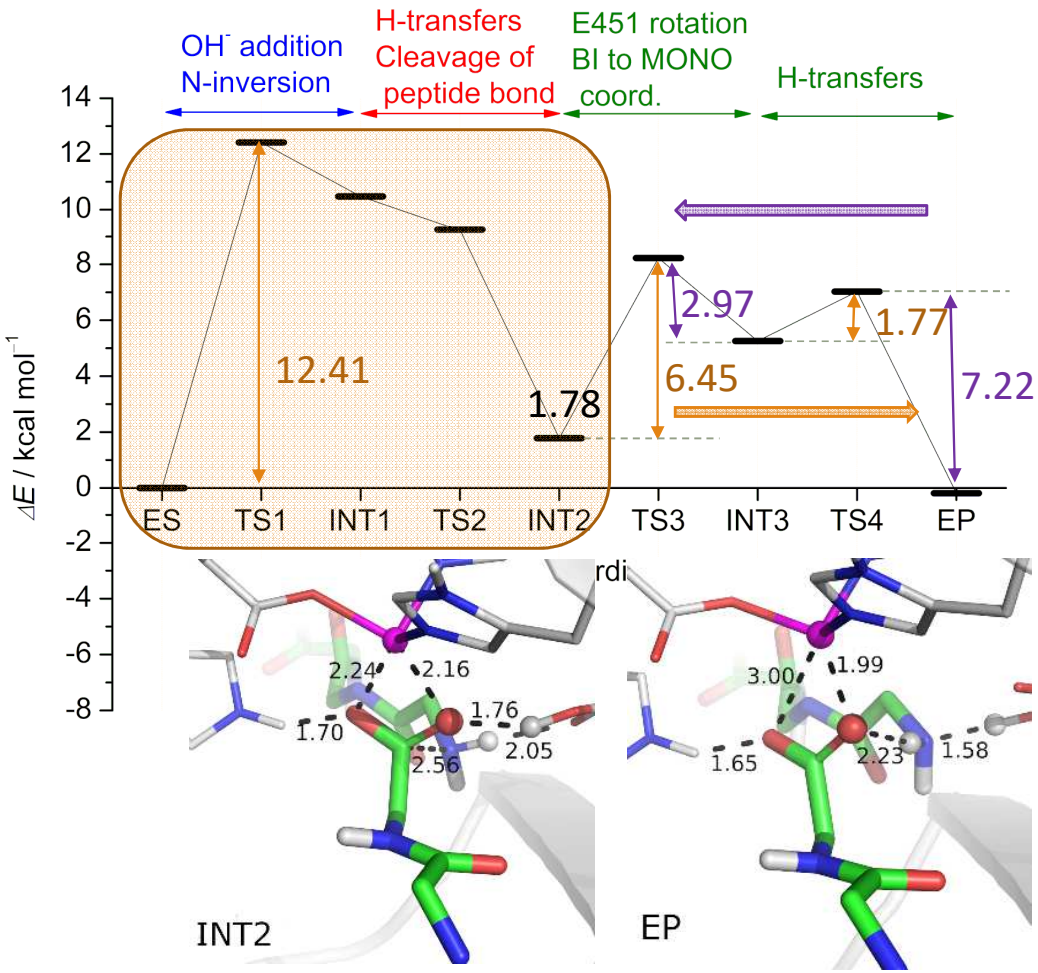
„GOOD" SUBSTRATE

DPP III – Leu-enkephalin



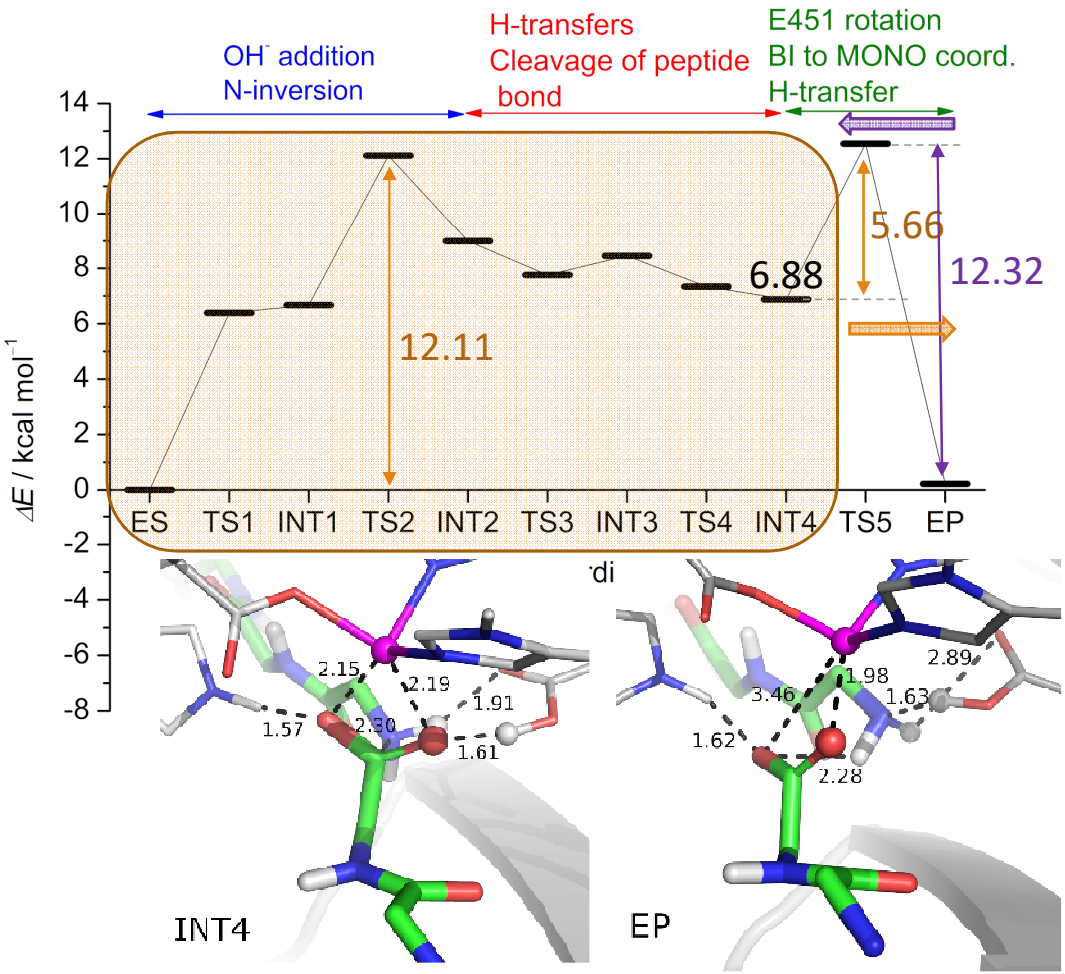
„SLOW" SUBSTRATE

DPP III - tynorphin



„GOOD" SUBSTRATE

DPP III – Leu-enkephalin



Molecular Dynamics (MD) simulations

DPP III – tynorphin; DPP III – tynorphin product; DPP – Leu-enkephalin; DPP III – Leu-enkephalin product

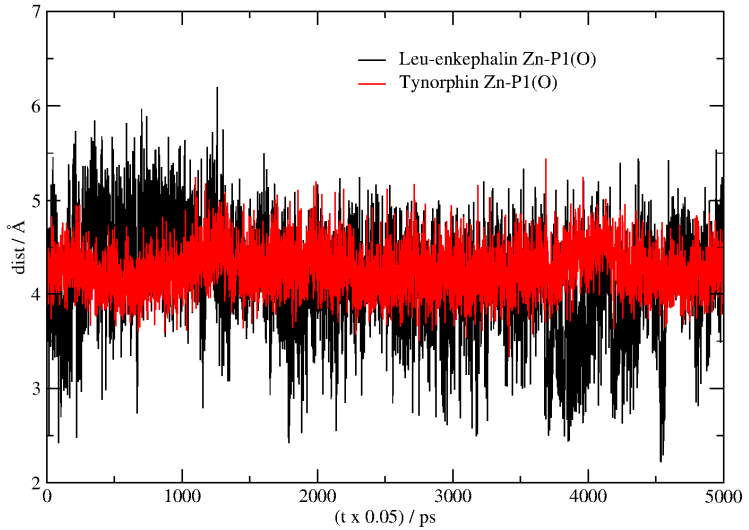
program AMBER 20 → 100 ns, *NpT*, 300 K, TIP3P, ff14SB, hybrid bonded/nonbonded parameters for Zn(II)*

*A. Tomić et. al., *J. Chem. Inf. Model*. 2019, 59, 8, 3437–3453

Molecular Dynamics (MD) simulations

DPP III – tynorphin; DPP III – tynorphin product; DPP – Leu-enkephalin; DPP III – Leu-enkephalin product; program AMBER 20 → 100 ns, *NpT*, 300 K, TIP3P, ff14SB, hybrid bonded/nonbonded parameters for Zn(II)*

DPP III – substrate



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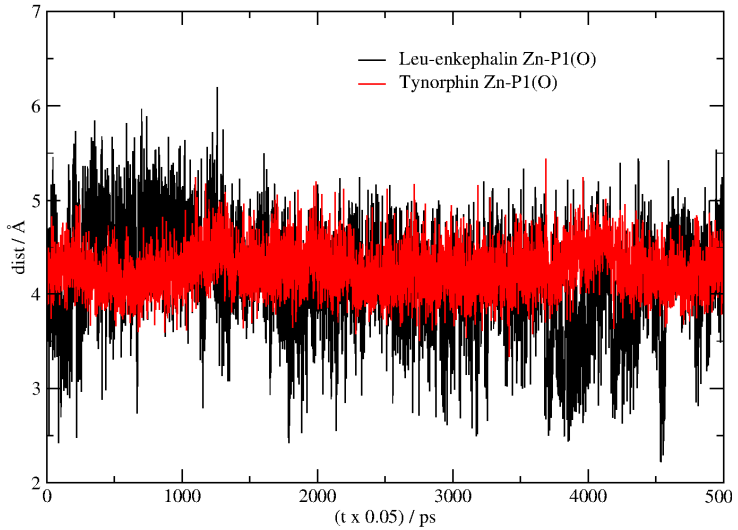
Molecular Dynamics (MD) simulations

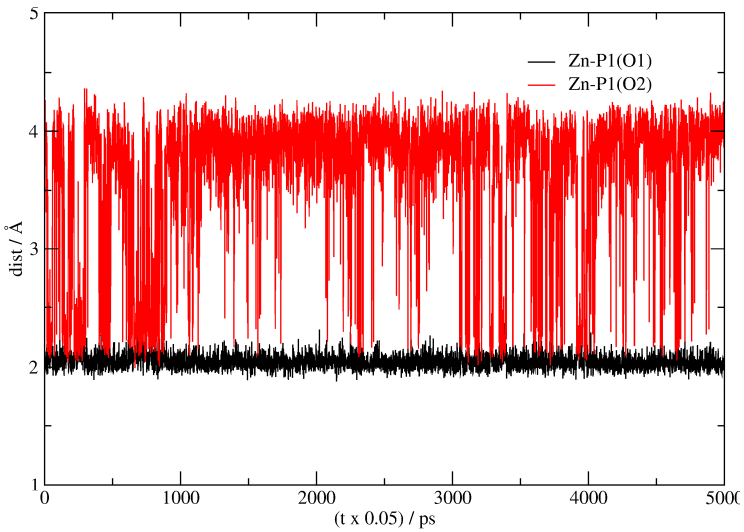
DPP III – tynorphin; DPP III – tynorphin product; DPP – Leu-enkephalin; DPP III – Leu-enkephalin product

program AMBER 20 → 100 ns, *NpT*, 300 K, TIP3P, ff14SB, hybrid bonded/nonbonded parameters for Zn(II)*

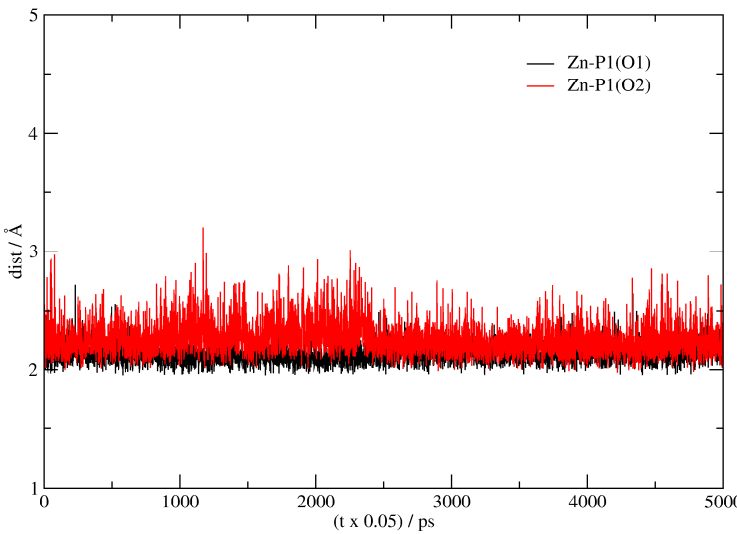
DPP III – substrate



DPP III – [YG + GFL]



DPP III – [VV + YPW]



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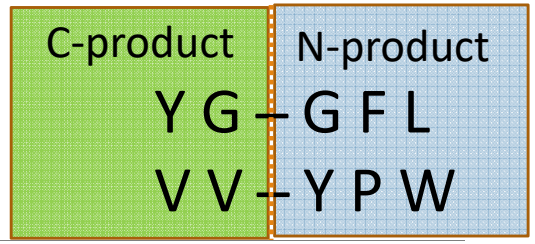
MM/PBSA binding energy

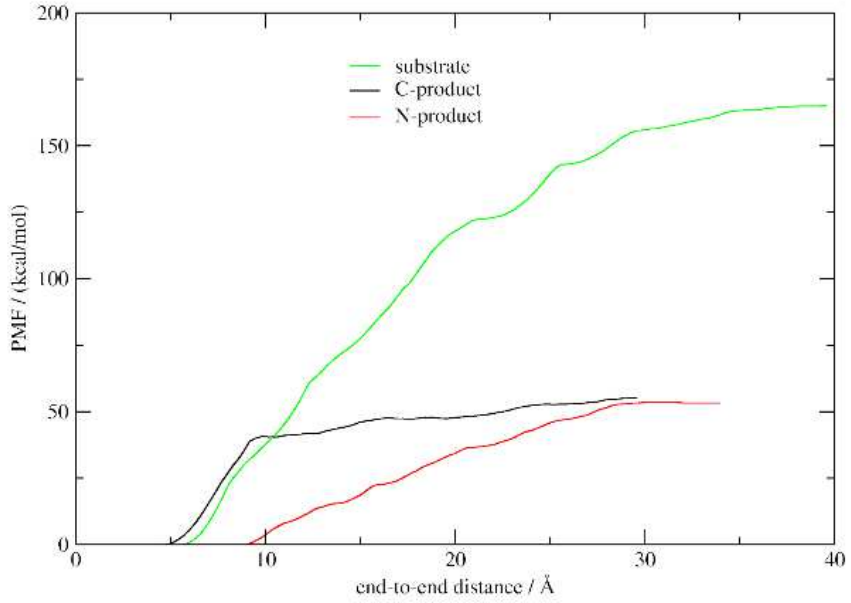


MM/PBSA	complex	receptor	ligand	ΔH/ (kcal/mol)	SD/ (kcal/mol)
Complex with SUBSTRATE	DPP III – Leu-enkephalin	DPP III + WAT	Leu-enkephalin	−17.73	4.83
	DPP III – tynorphin	DPP III + WAT	tynorphin	−26.56	5.33
Complex with PRODUCT	DPP III – Leu-enkephalin	DPP III + C-prod	N-prod	−6.97	3.80
	DPP III – tynorphin	DPP III + C-prod	N-prod	−27.88	2.93
	DPP III – Leu-enkephalin	DPP III + N-prod	C-prod	−18.13	4.56
	DPP III – tynorphin	DPP III + N-prod	C-prod	−34.40	3.43

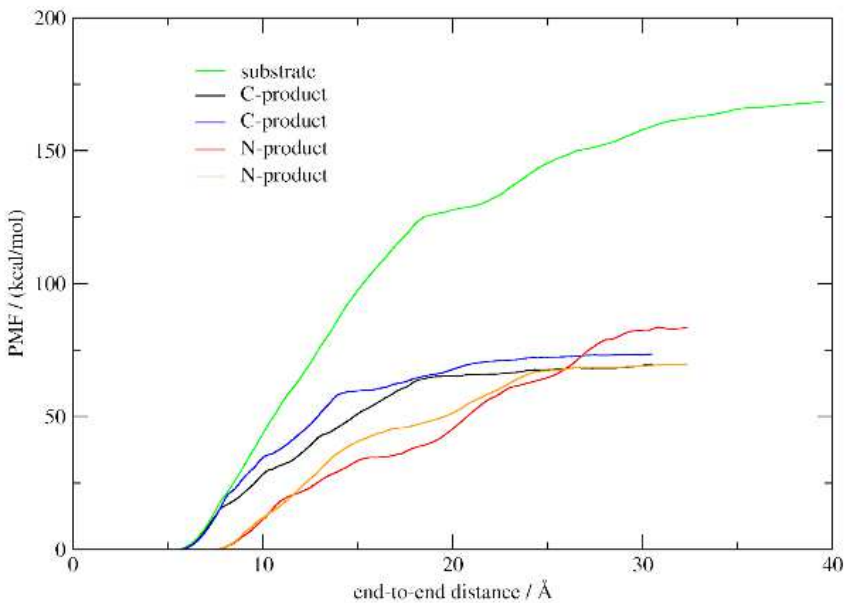
Adaptive steered MD simulations

- 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

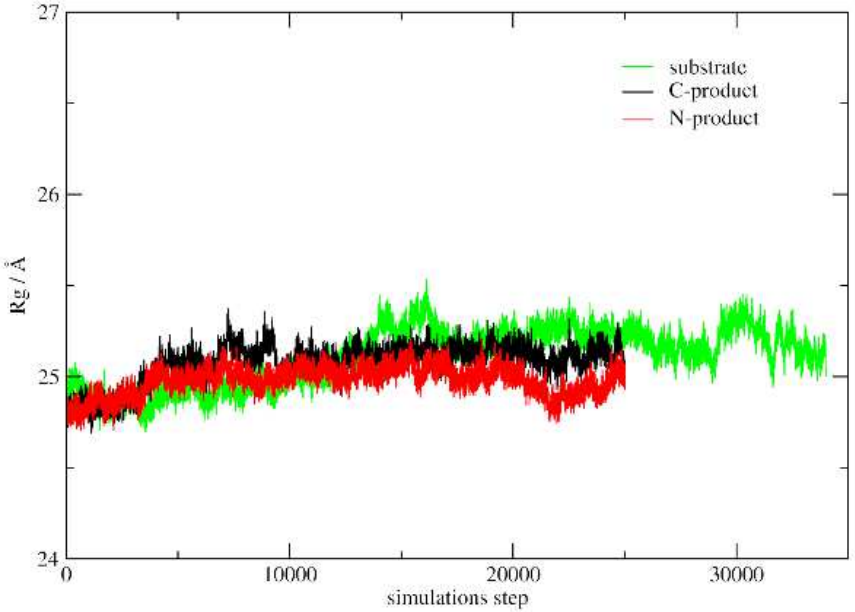
DPP III – Leu-enkephalin Y G G F L



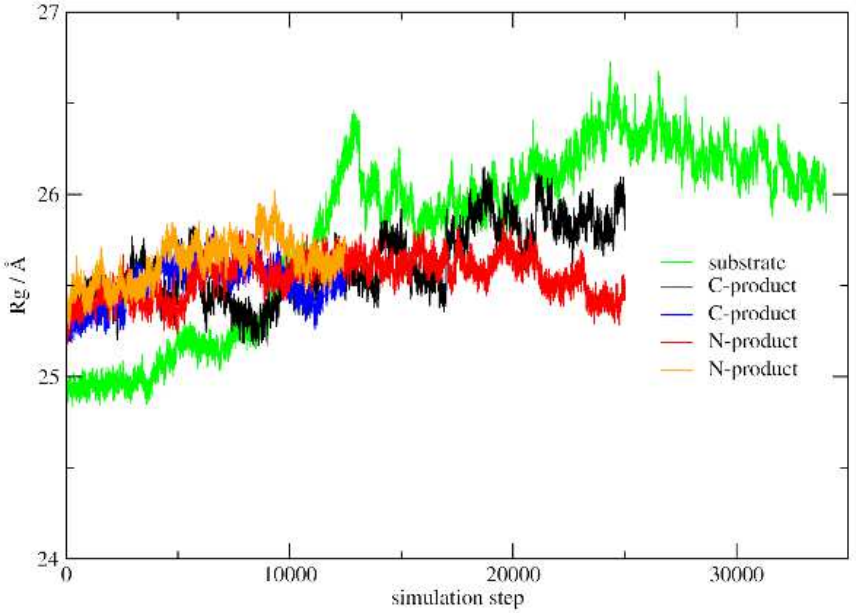
DPP III – tynorphin V V Y P W



DPP III – Leu-enkephalin Y G G F L



DPP III – tynorphin V V Y P W



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THANK YOU FOR YOUR ATTENTION!