Structures of substrate and inhibitor complexes of human DPP III

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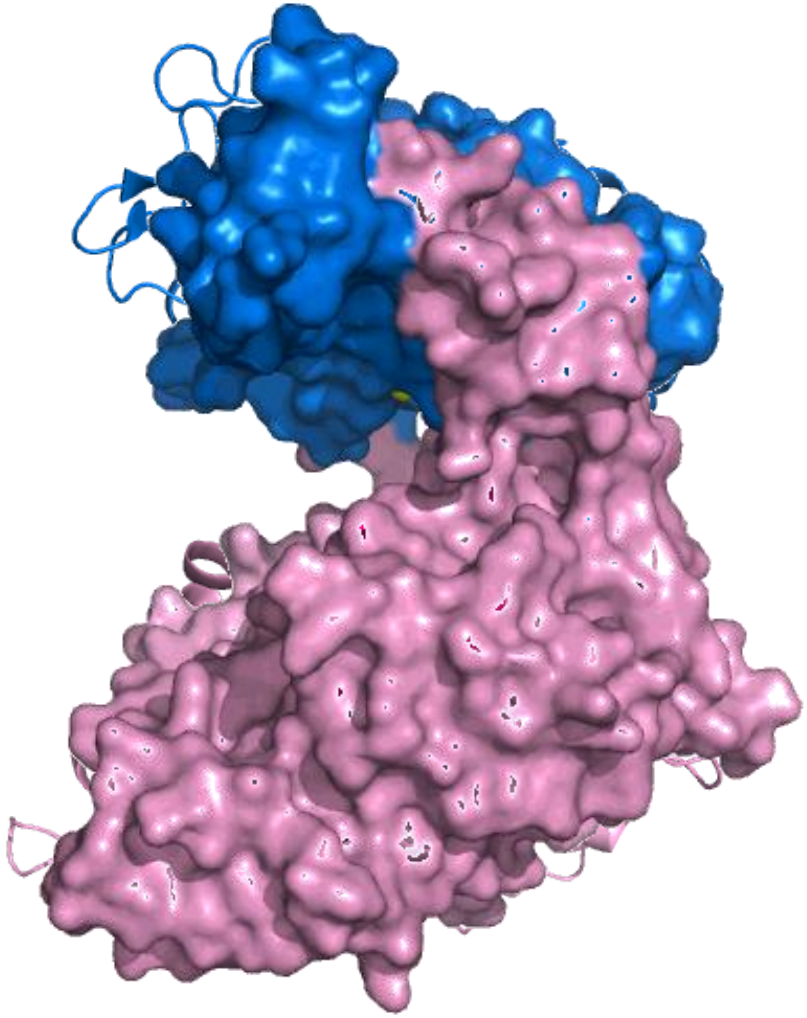
- Pravas Kumar Baral

- Gustavo Arruda Bezerra

- Prashant Kumar

- Altijana Hromic

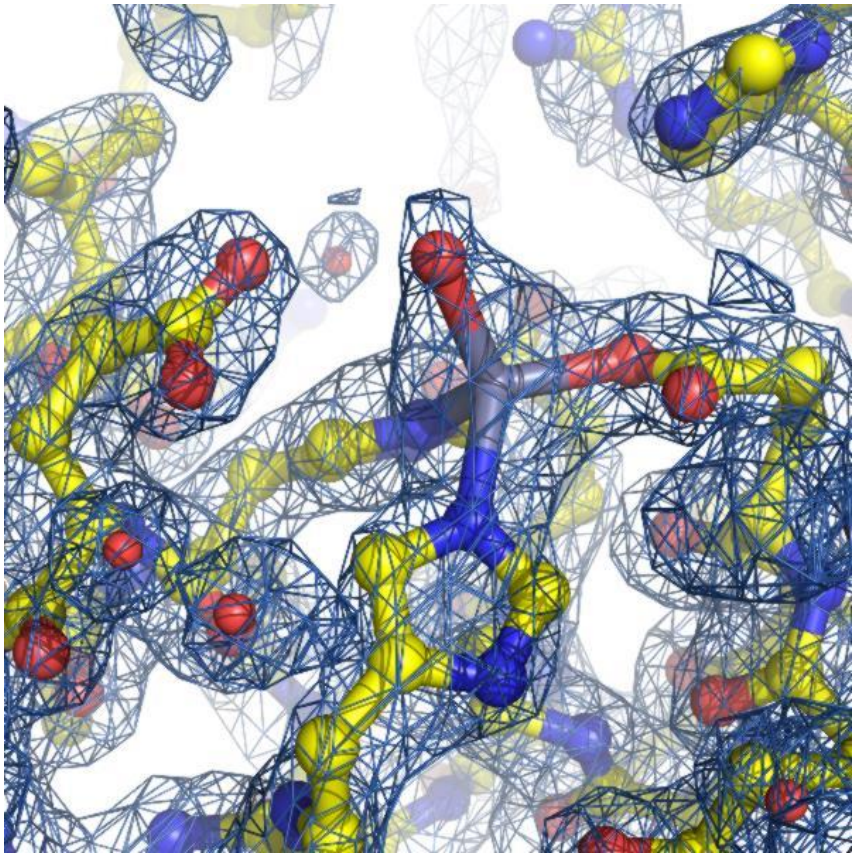
Overall structure of DPP III from yeast



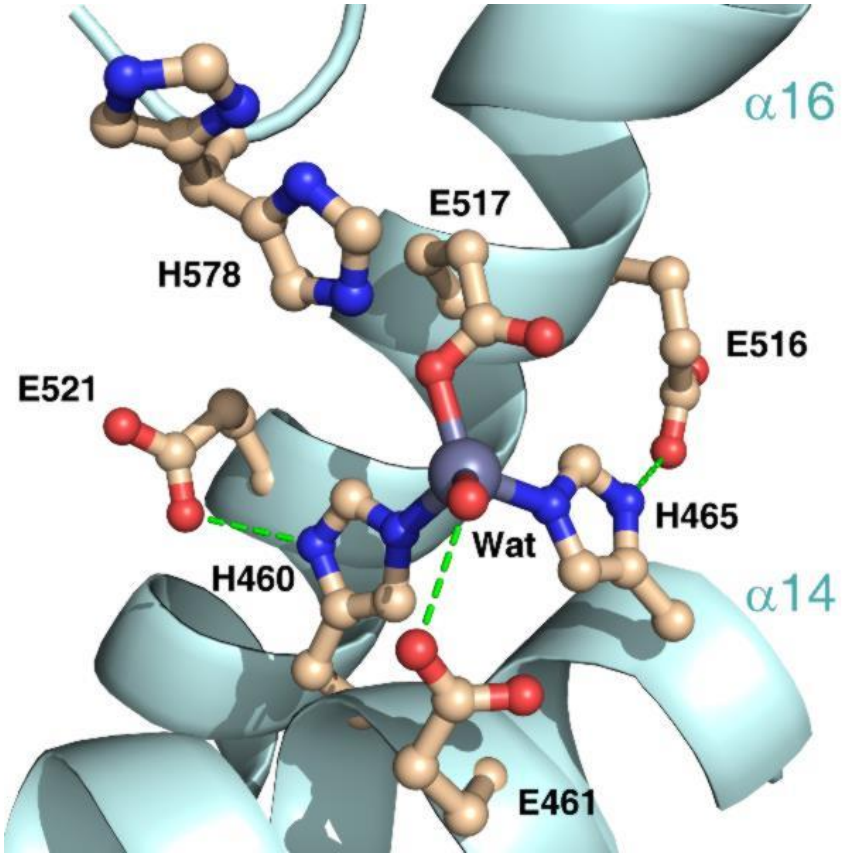
- **1.95 Å resolution**
- **novel fold**
- **two domains**
- **zinc ion bound to the α-helical domain**
- **peptide binding cleft between the two domains**

Baral *et al.*, *J. Biol. Chem.* 2008

Metal-ion coordination



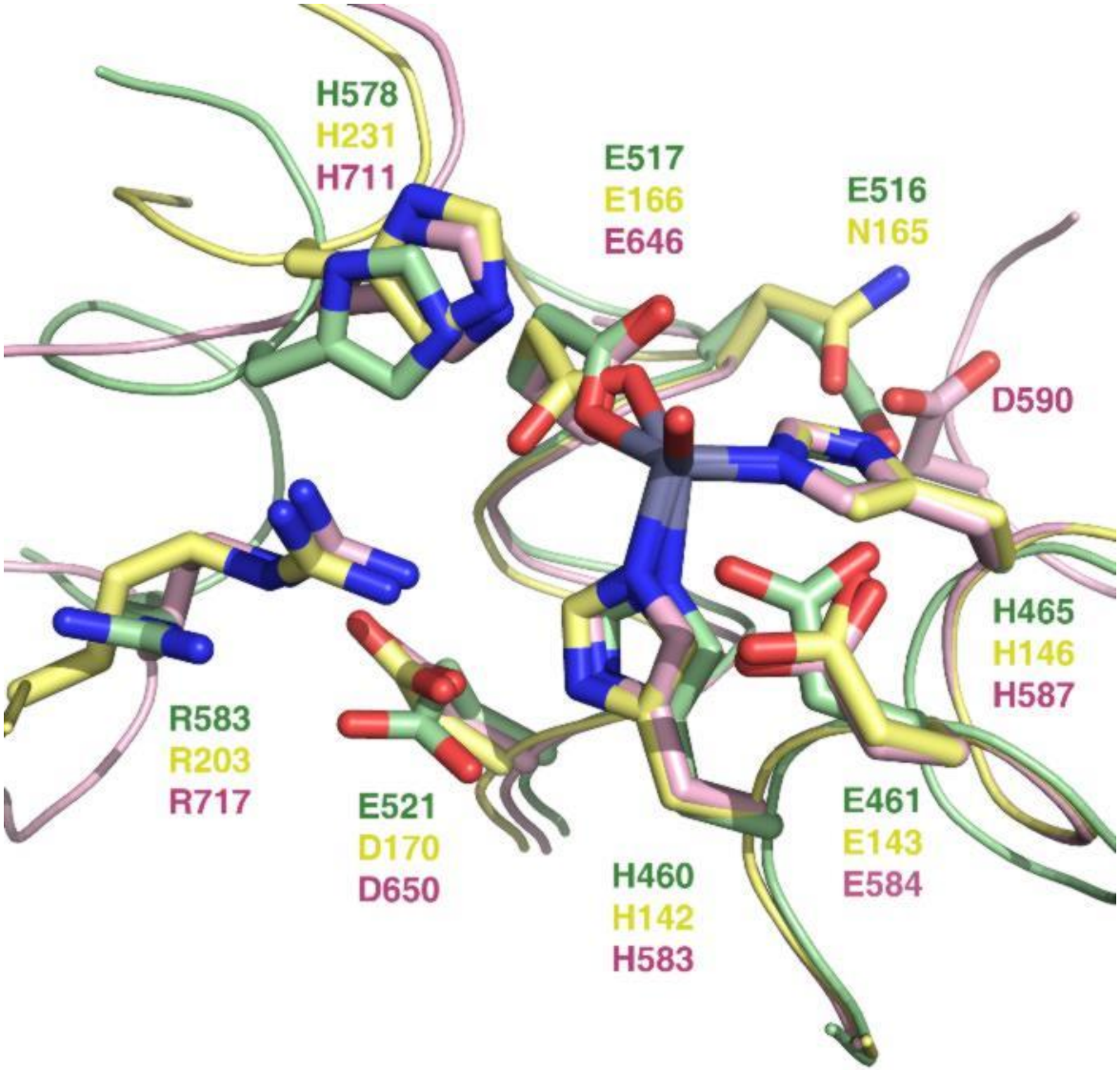
electron density around the Zn-ion



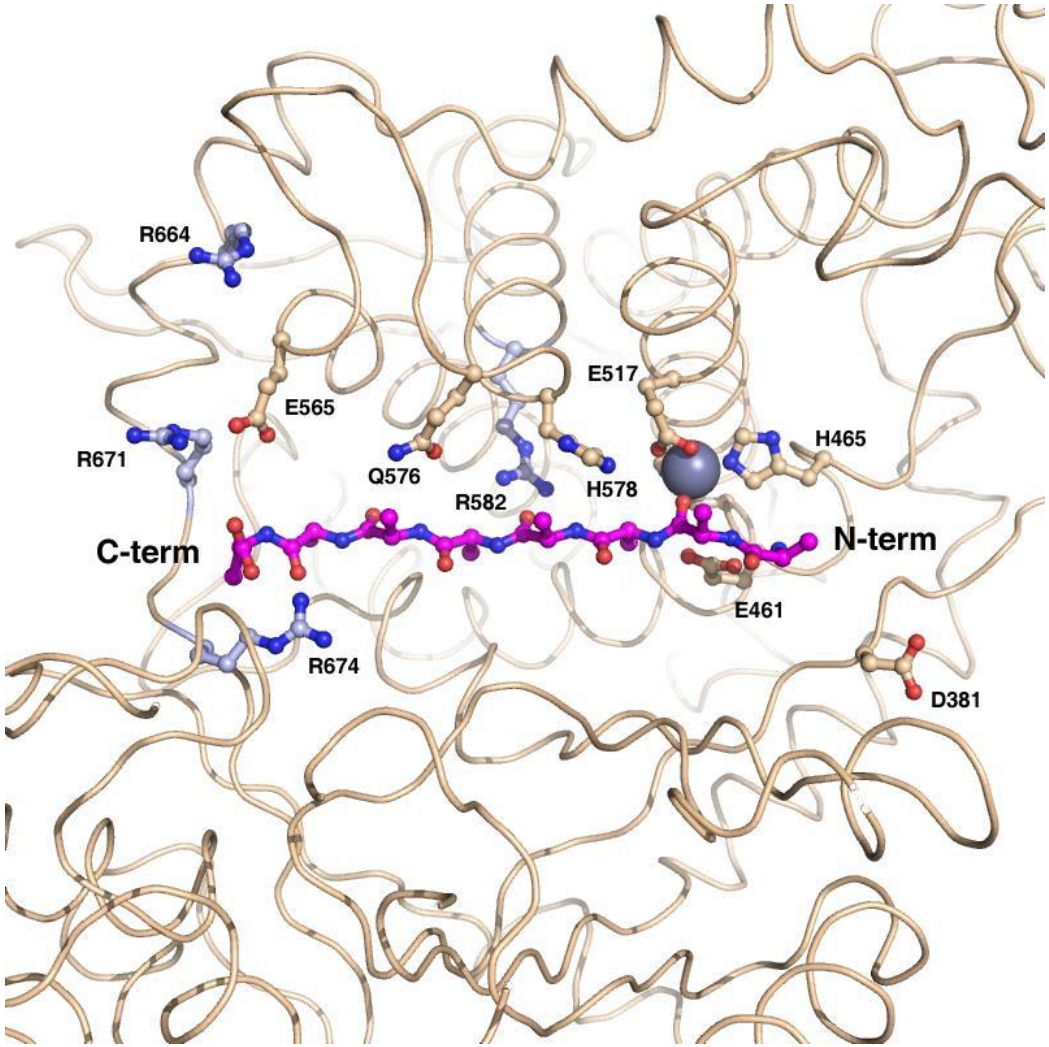
HELLGH, signature motif of the
M49-family of metalloproteases

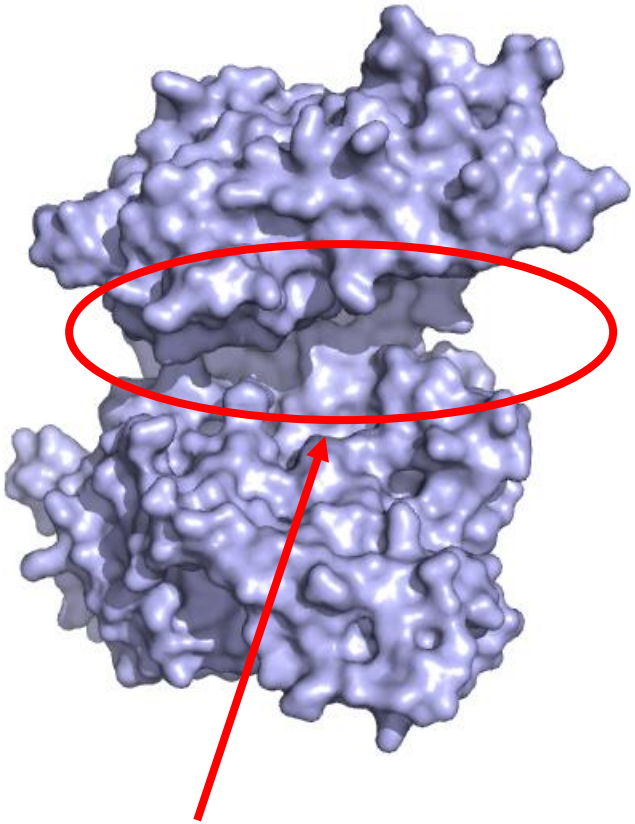
Comparison with other Zn-peptidases

DPP III
thermolysin
neprilysin



How do peptides bind to DPP III?



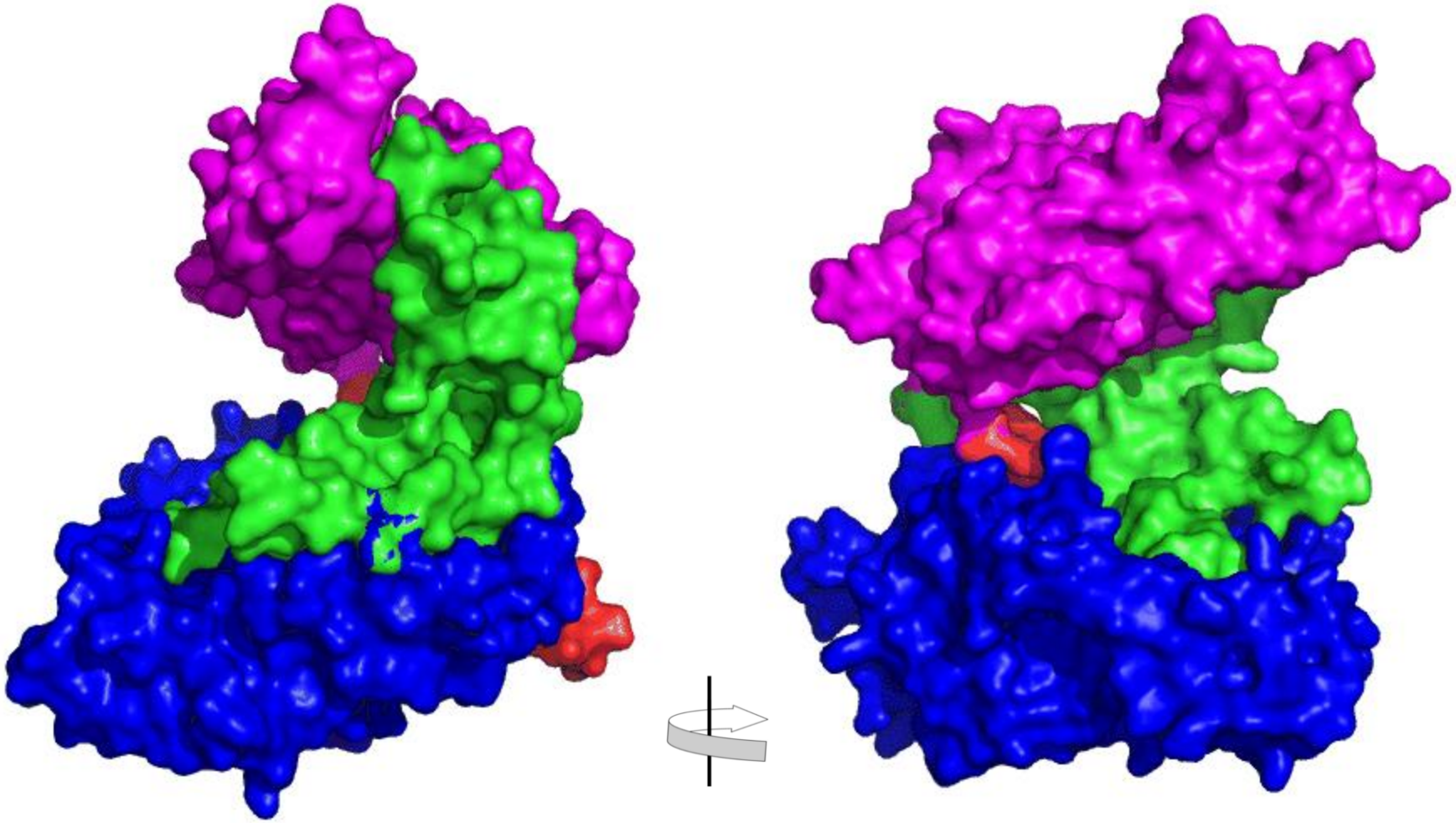


substrate binding cleft

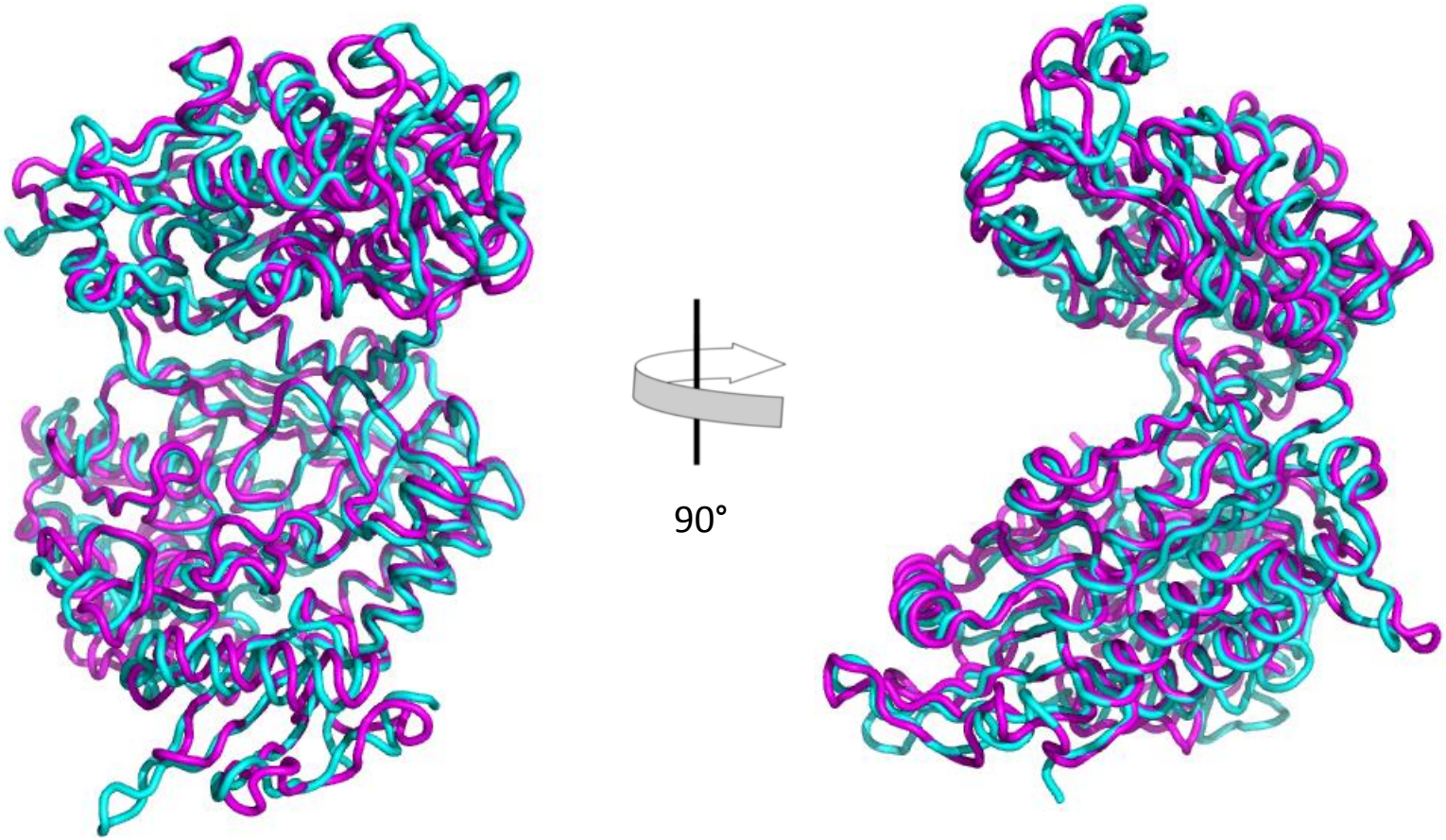
<u>crude model</u> of octa-alanine bound to DPP III

Baral *et al.*, *J. Biol. Chem.* 2008

Domain motion predicted from TLS- analysis



yDPP3 *vs.* hDPP3

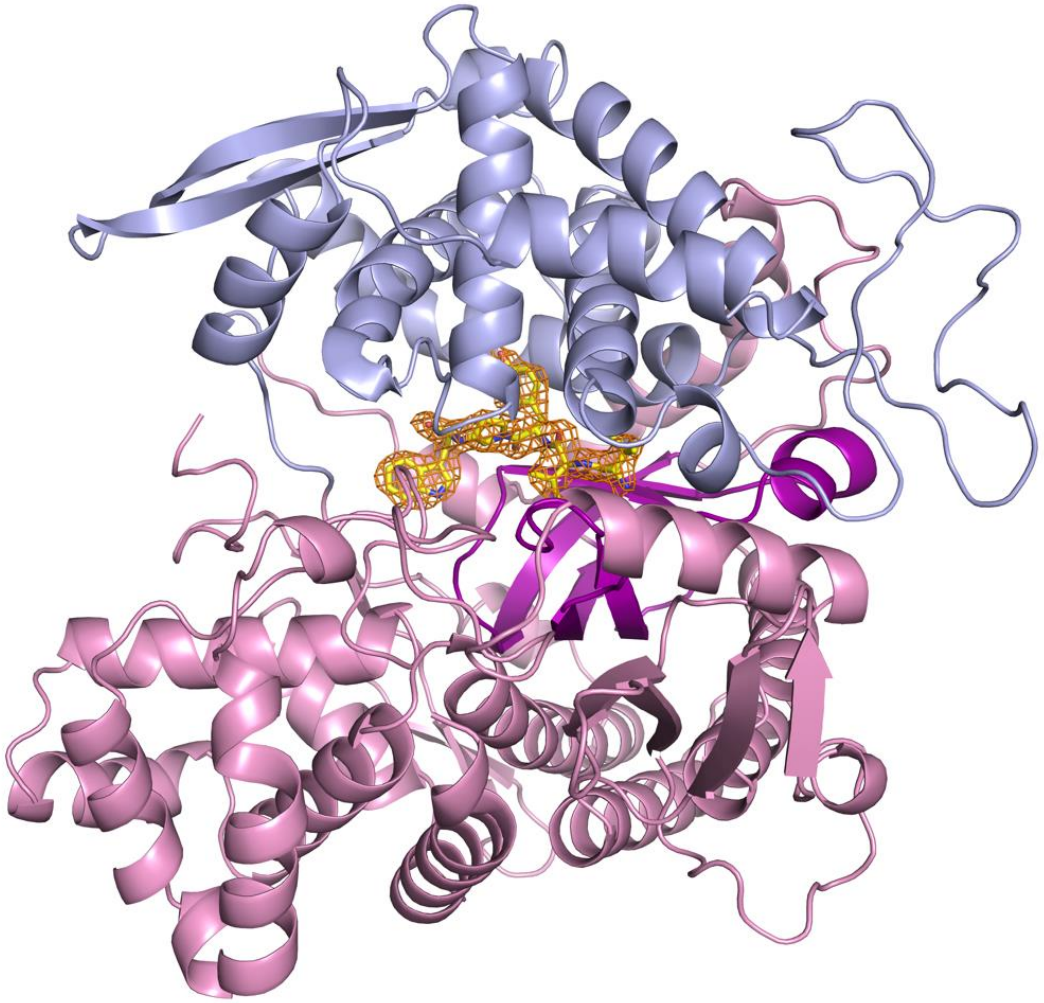


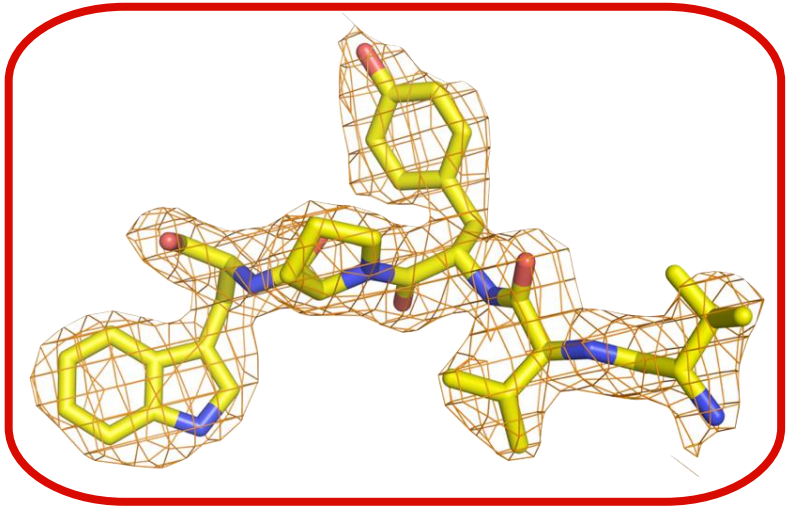
rms-deviation: 1.4 Å

SGC

Sirano Dhe-Paganon

Human DPP III in complex with VVYPW





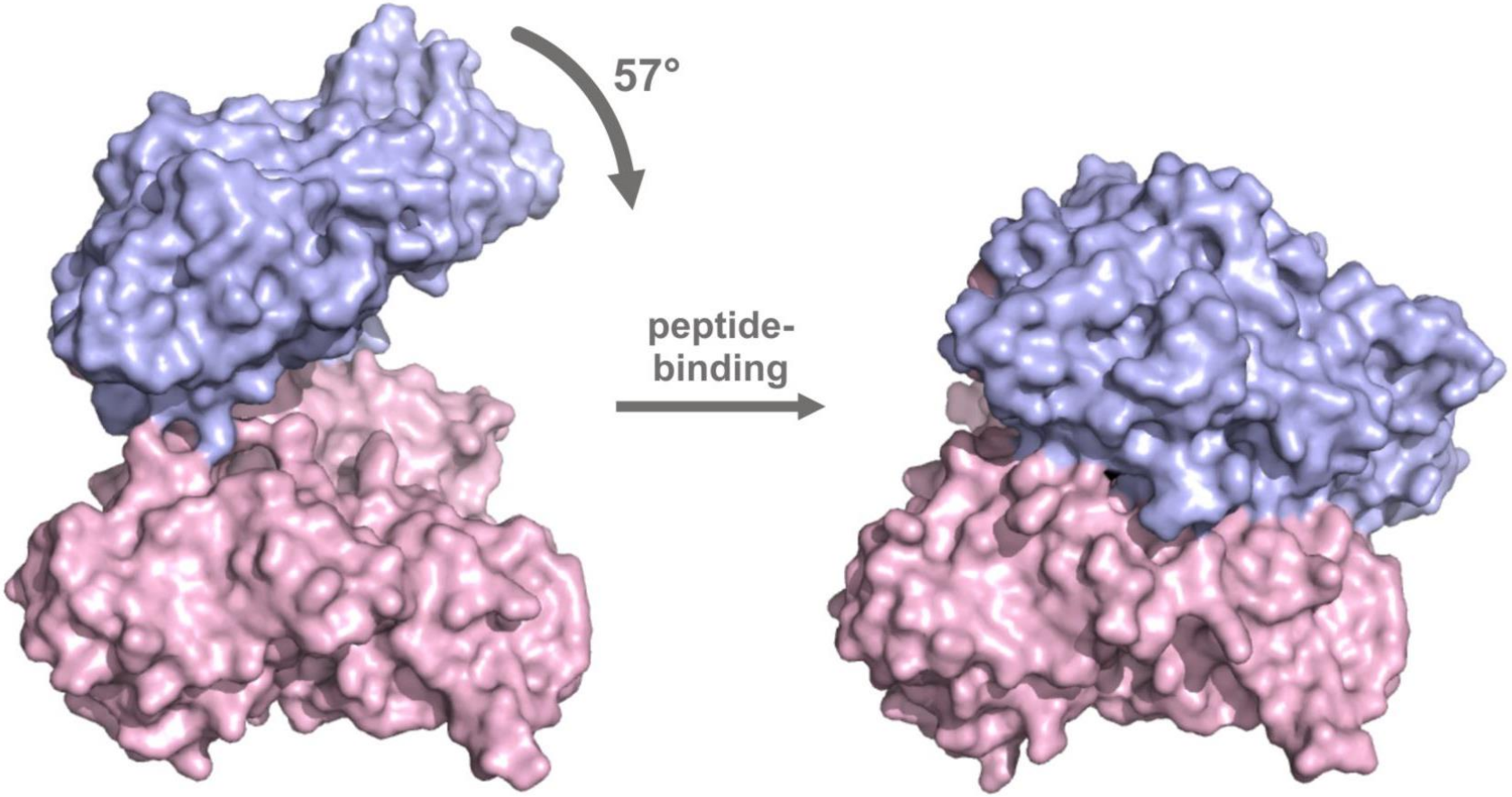
Crystallographic data:

- $P2_1$
- **Resolution: 2.4 Å**
- **R: 0.19**
- **R_{free}: 0.25**

- $C2$
- **Resolution: 3.0 Å**
- **R: 0.23**
- **R_{free}: 0.26**

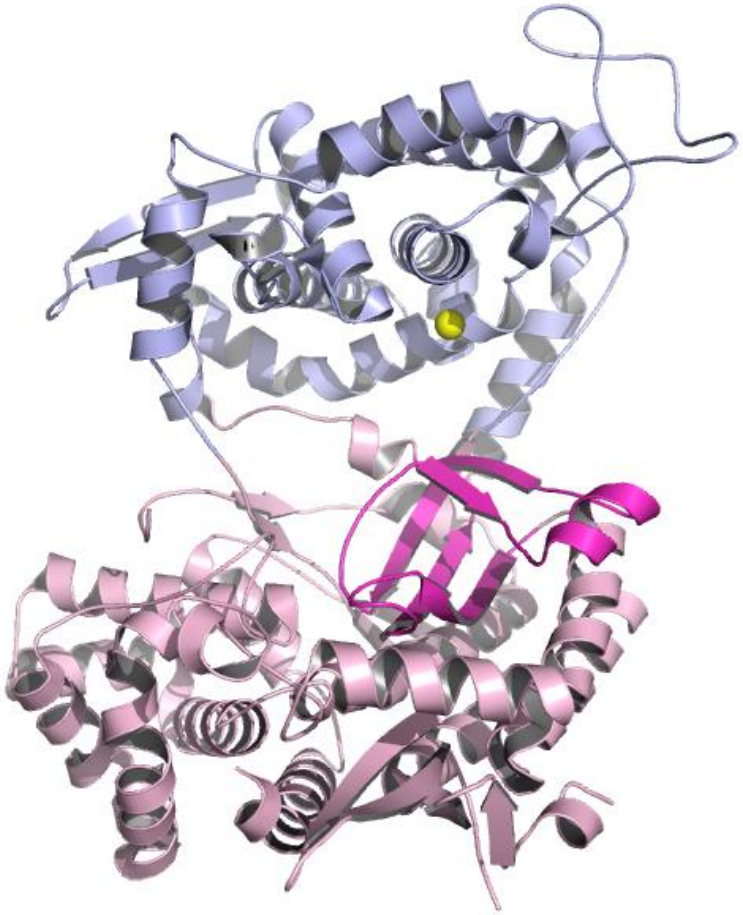
Bezerra *et al.*, *PNAS.* 2012

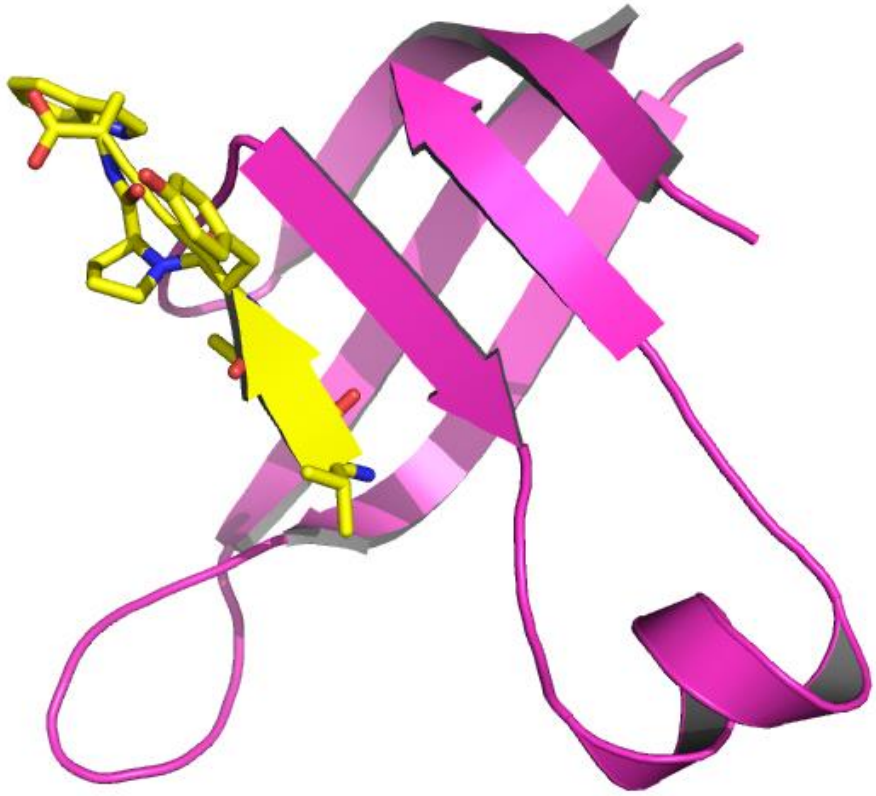
Domain motion upon peptide binding



Total buried surface area (protein + ligand): 3500 Å^2

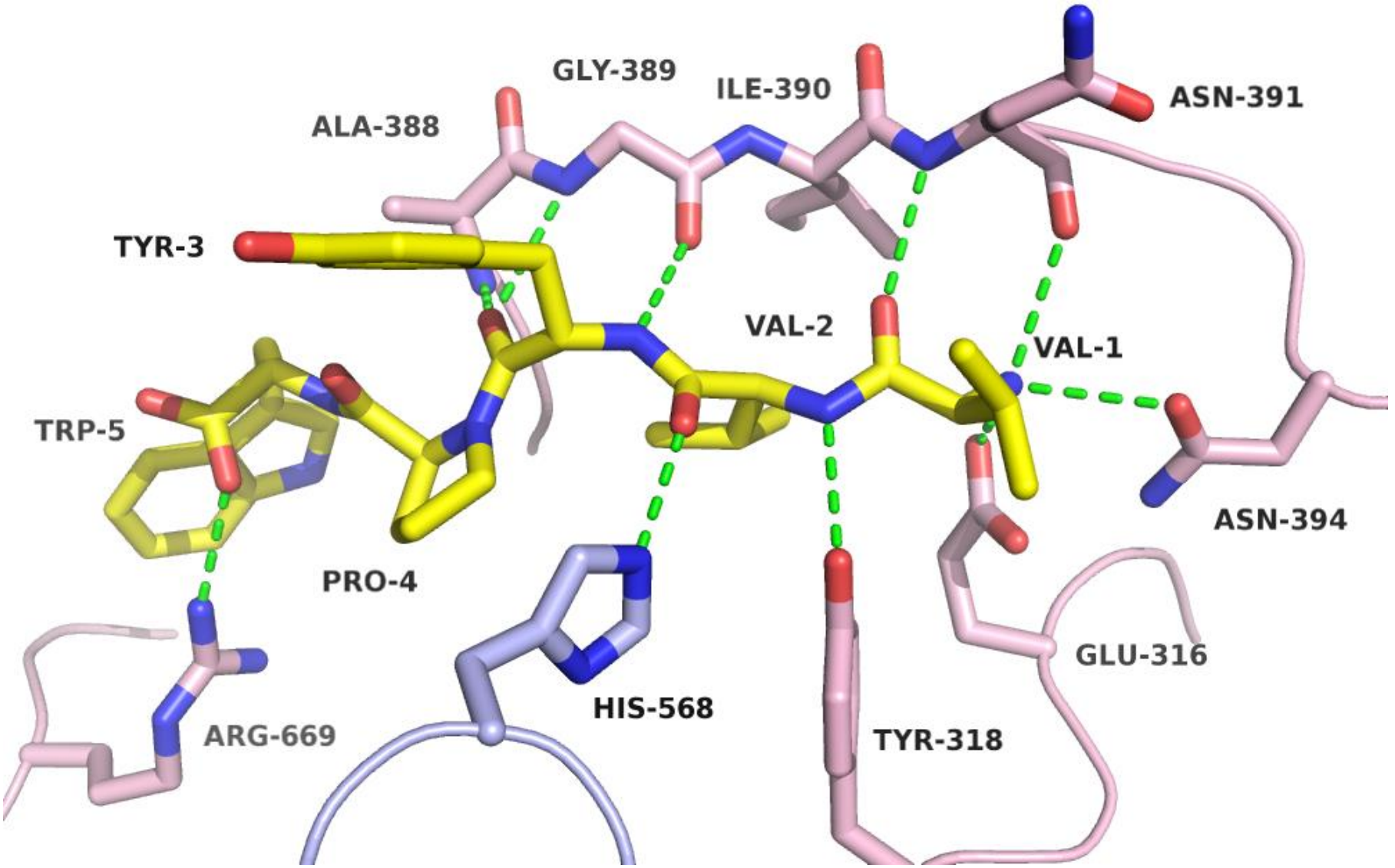
Peptide binding to DPP III



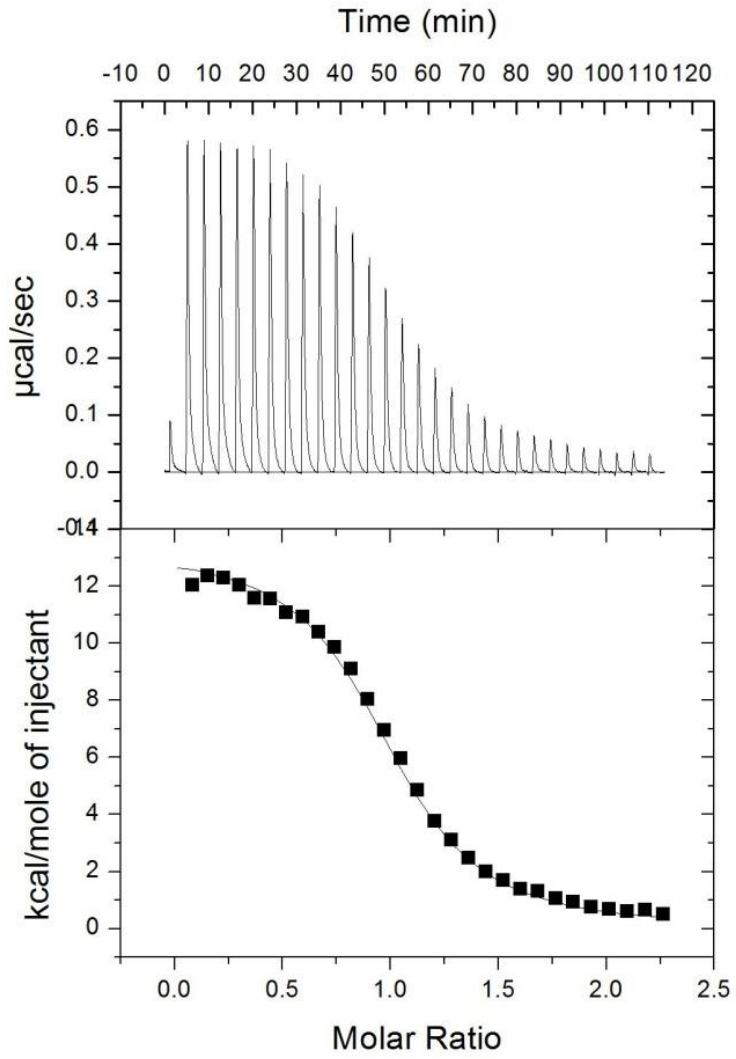


completion of the β-barrel

The peptide interacts primarily with the lower lobe



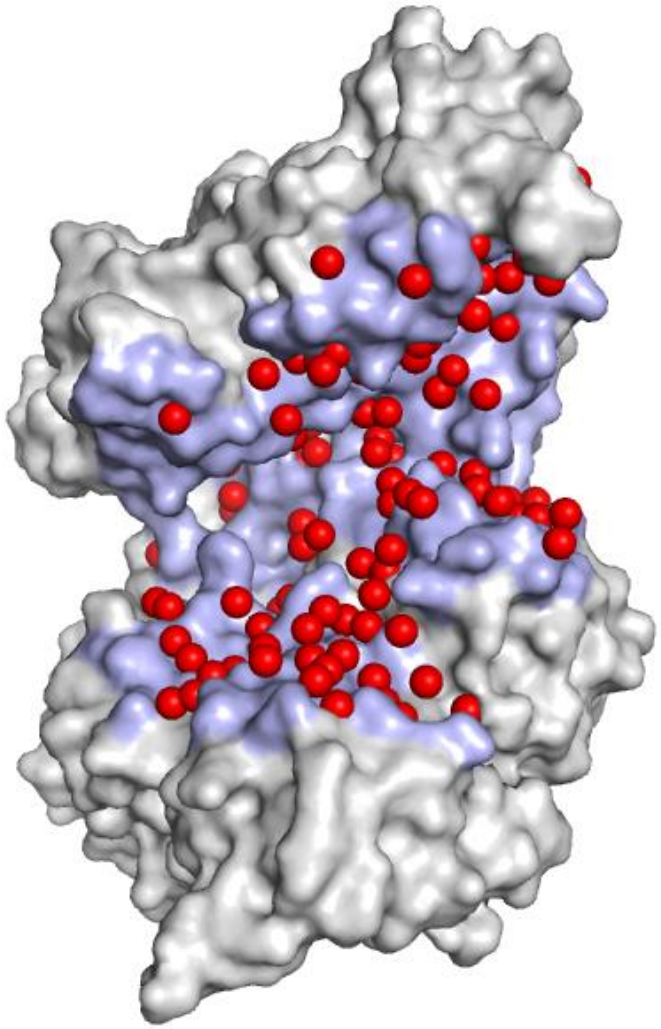
Thermodynamics of peptide binding



- Isothermal titration calorimetry measurements for the binding of VVYPW
- Endothermic process
- K_D=1.18 µM
- Entropy gain as the driving force

Bezerra *et al.*, *PNAS.* 2012

Release of water as entropy source

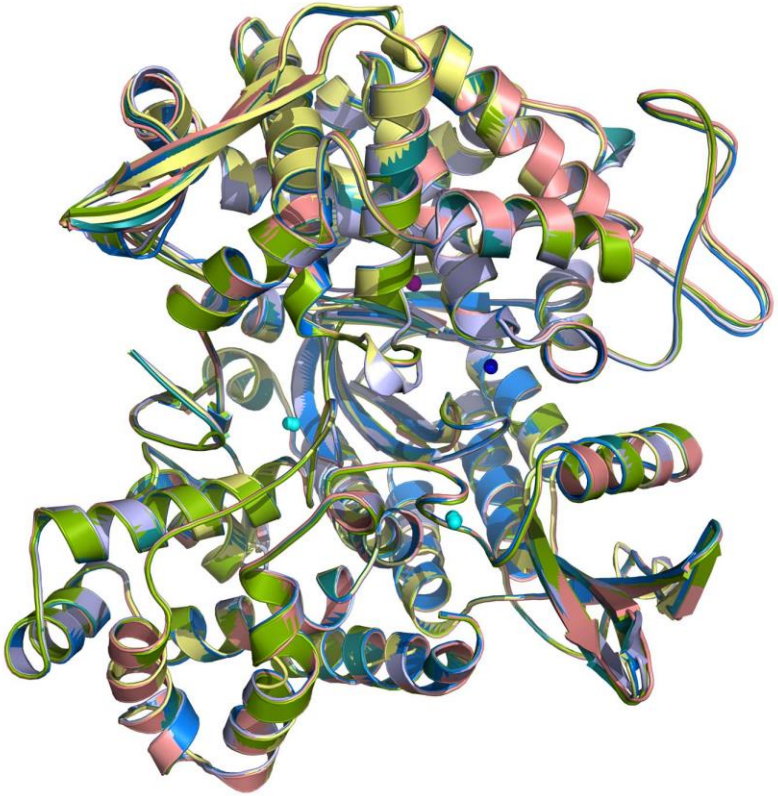


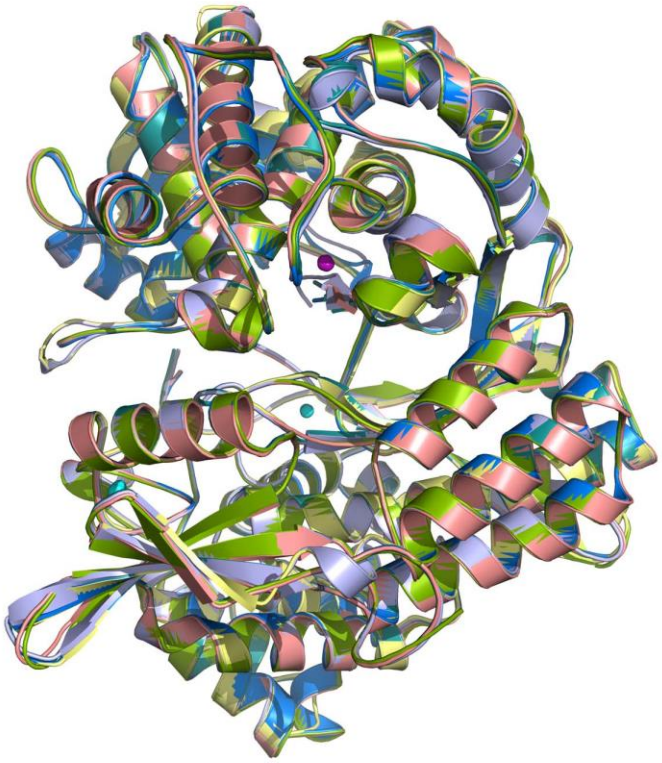
- about 60 ordered water molecules are found in the binding cleft
- from ITC: ΔS = 200-400 J/K/mol
- literature data: 5-30 released water molecules yield a ΔS_{solv} of 100-600 J/K/mol.

Available crystal structures

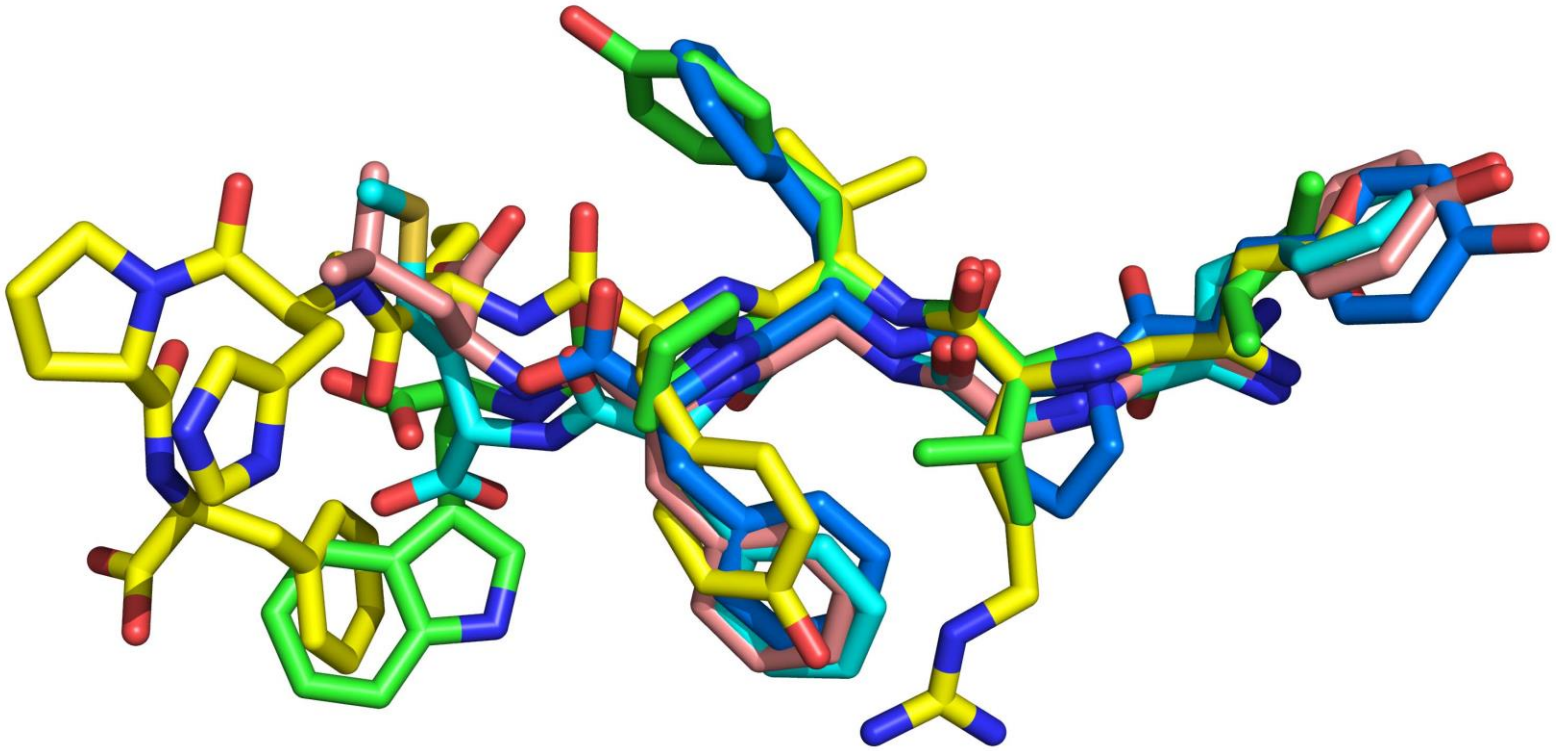
- Complexes with the E451A-variant of human DPP III (smFRET-construct)
- VVYPW
- Angiotensin-II
- Leu-enkephalin
- Met-enkephalin
- Endomorphin-2
- IVYPW
- Synthetic inhibitor ”SHE” (Jakov Ivkovic, Rolf Breinbauer)

The overall structures of the complexes are identical

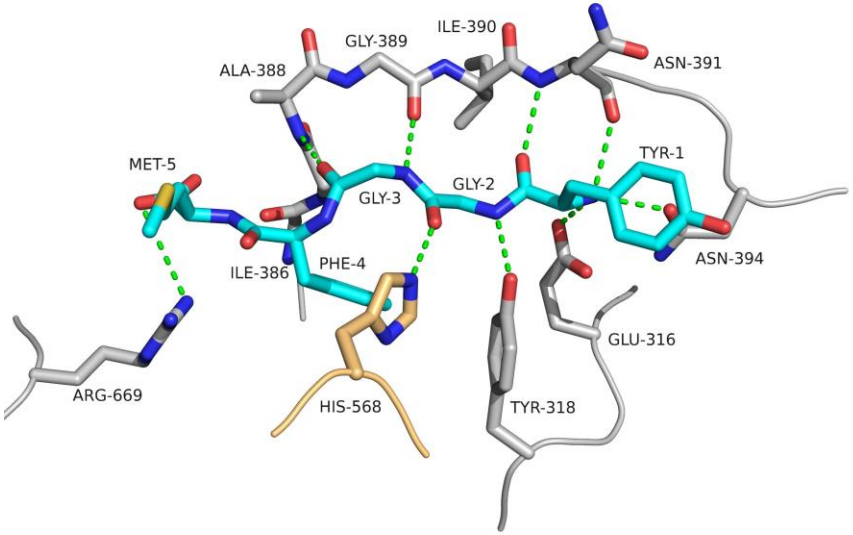




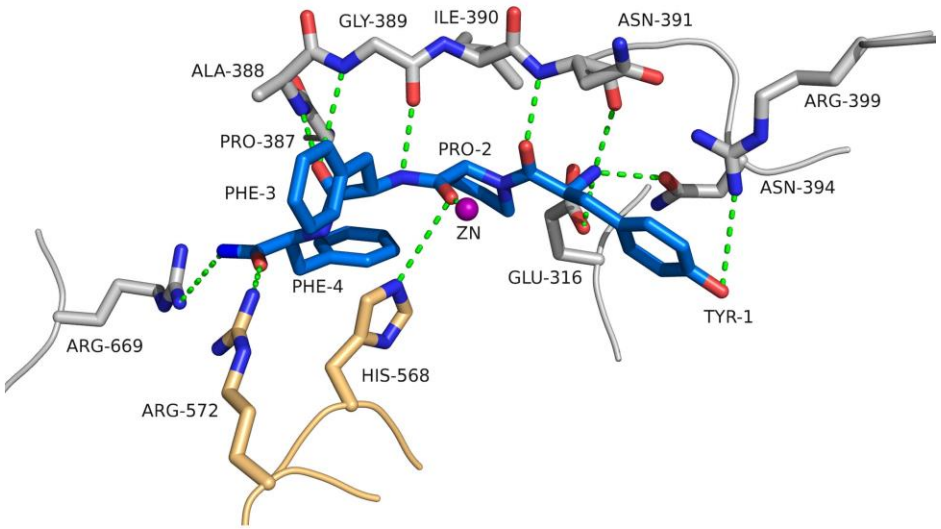
The different peptides bind in the same manner to DPP III

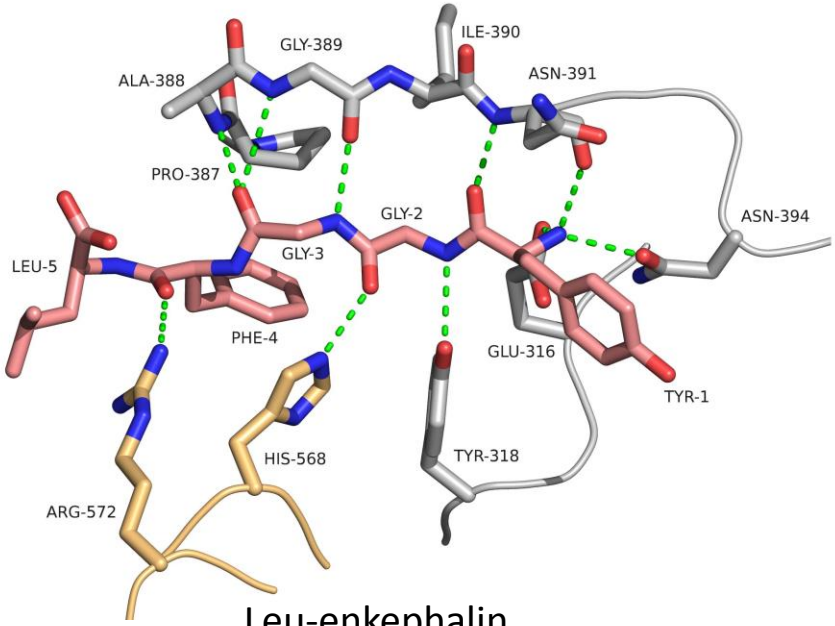


Met-enkephalin

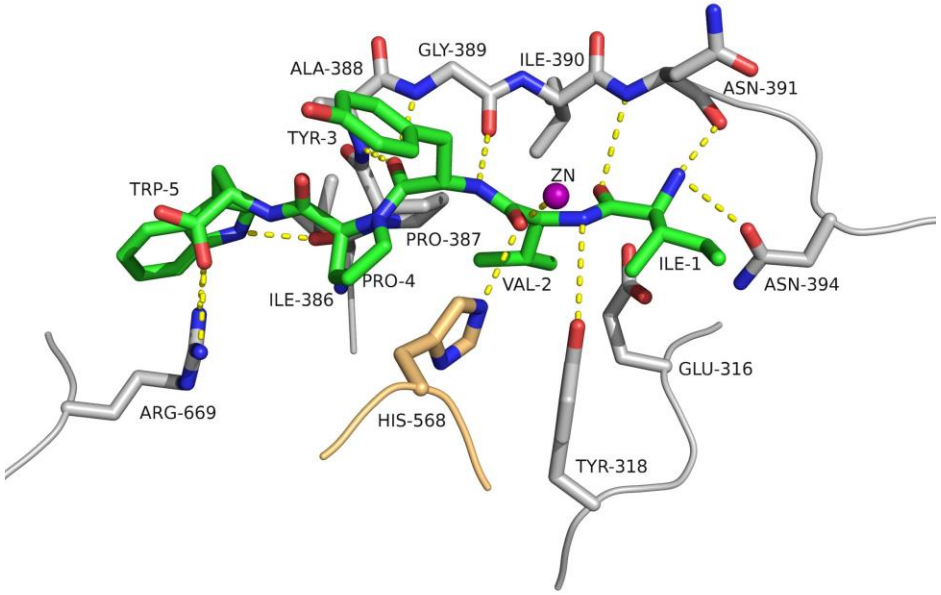


endomorphin-2



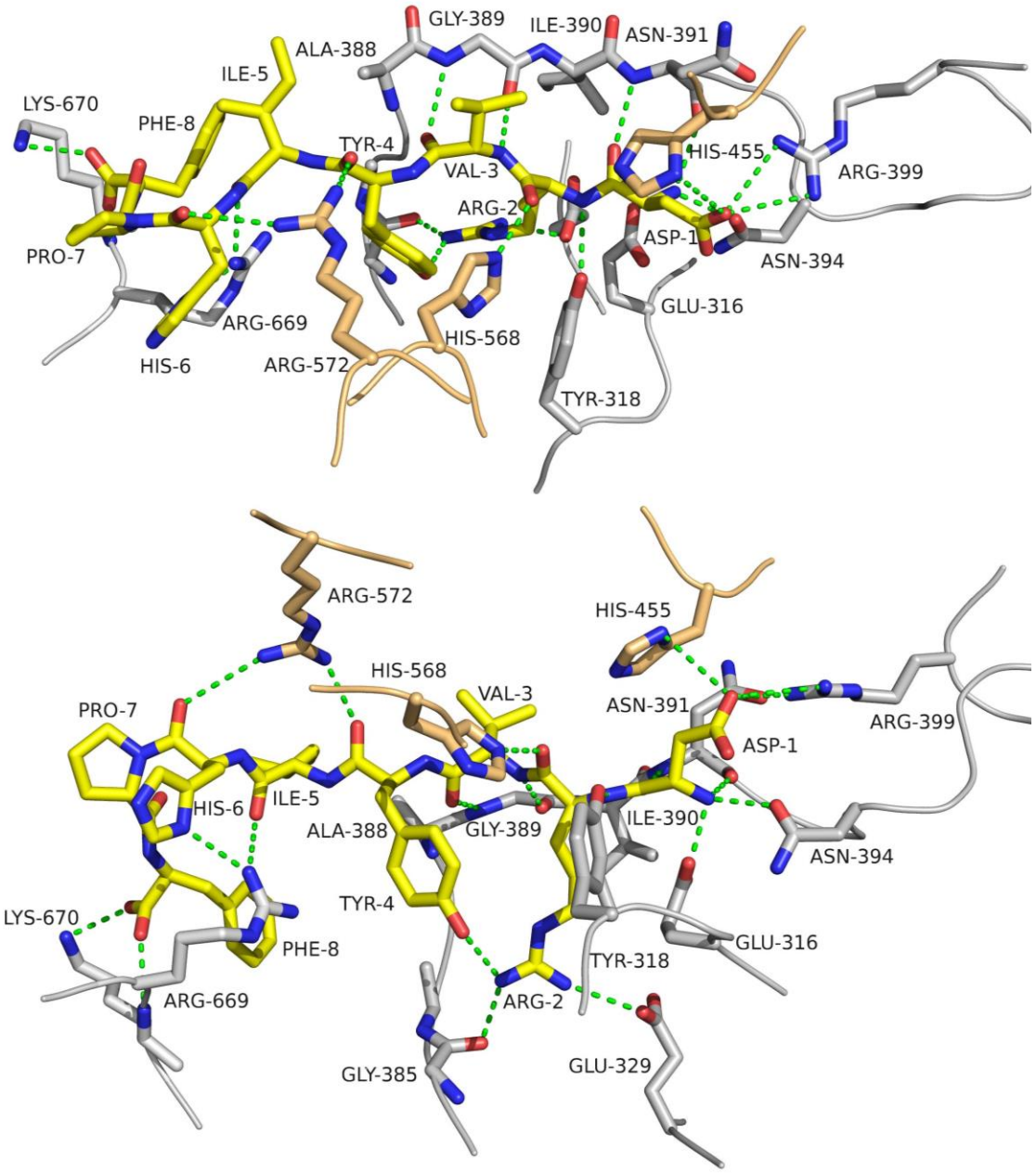


Leu-enkephalin

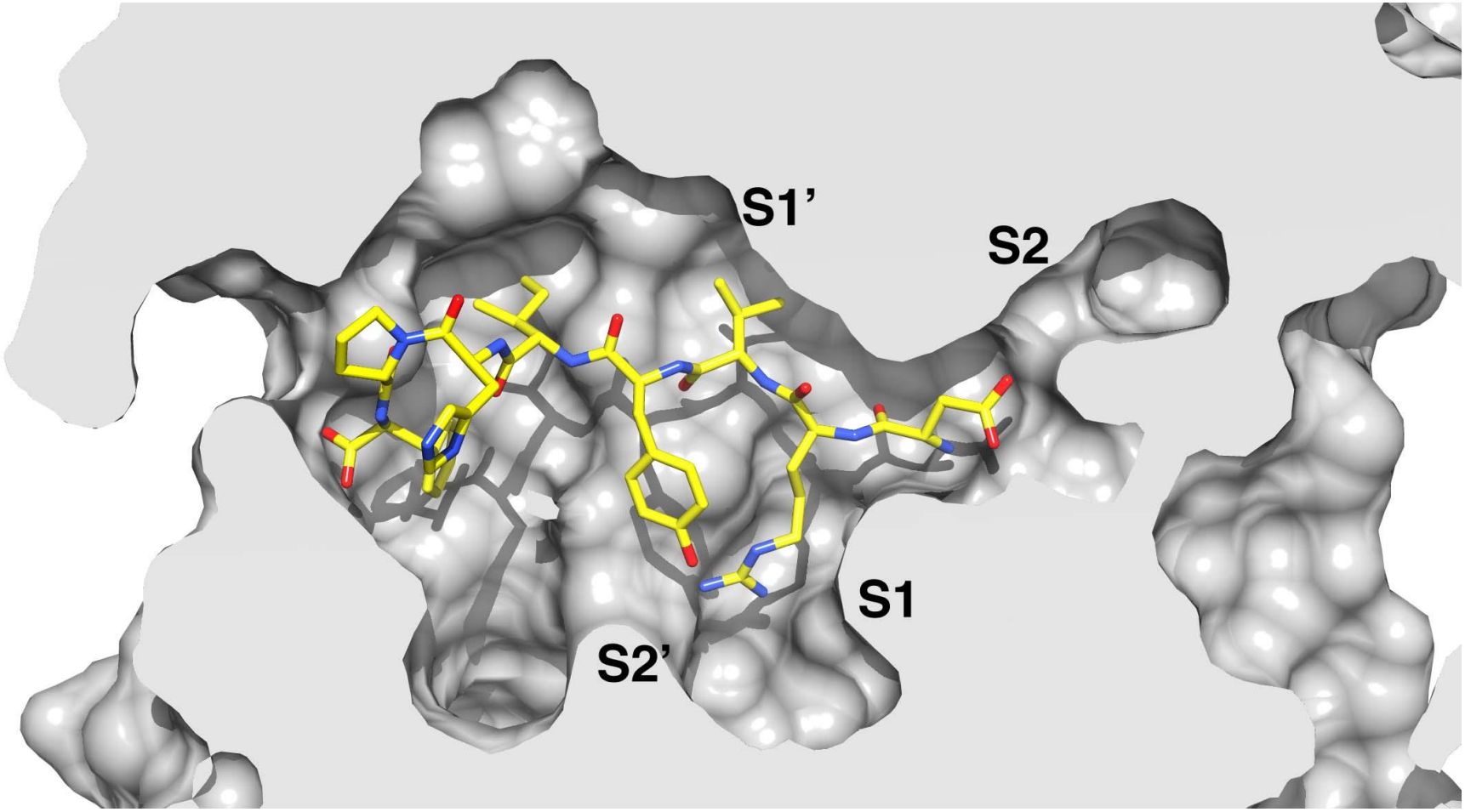


IVYPW

Complex with angiotensin-II

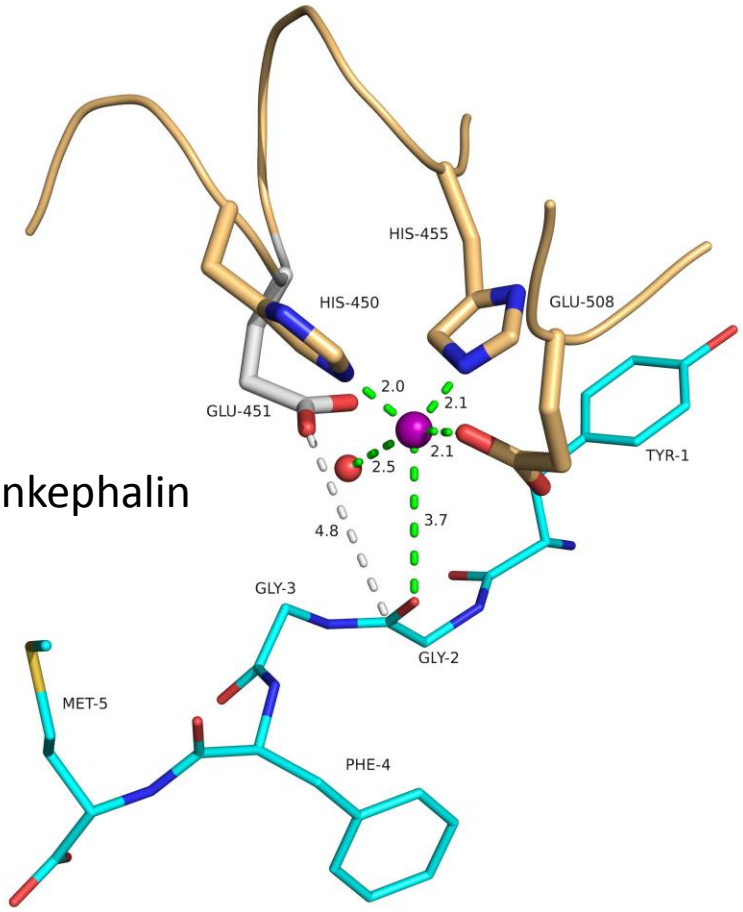


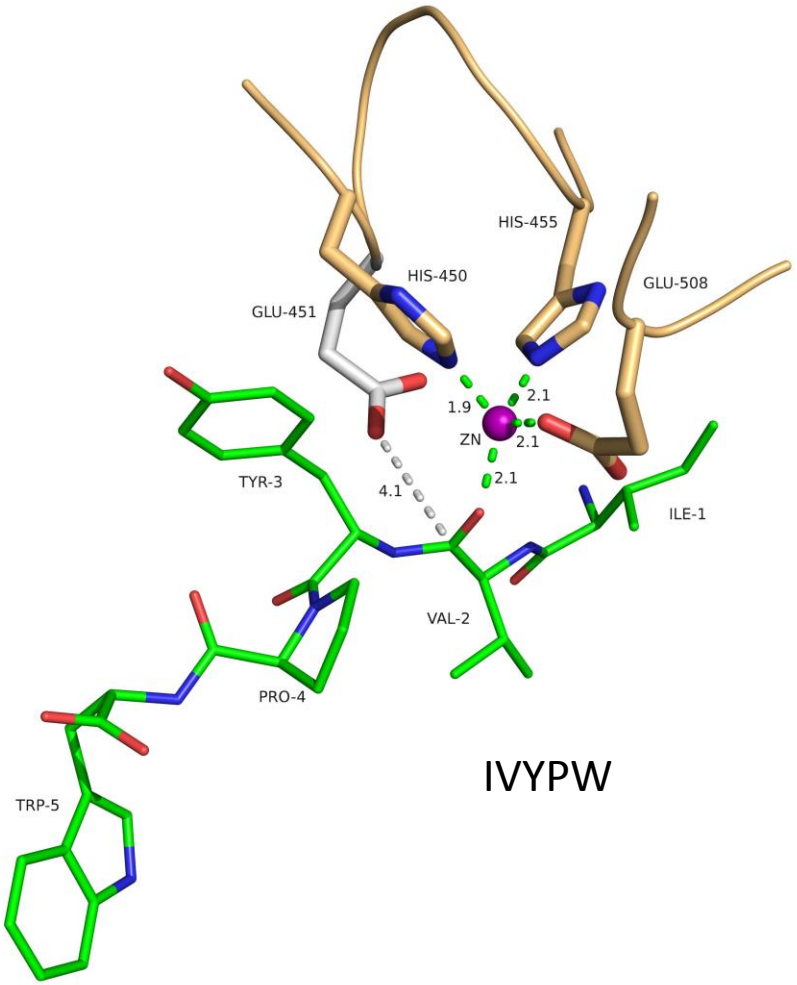
Complex with angiotensin-II



Real substrates *vs.* inhibitors ("slow" substrates)

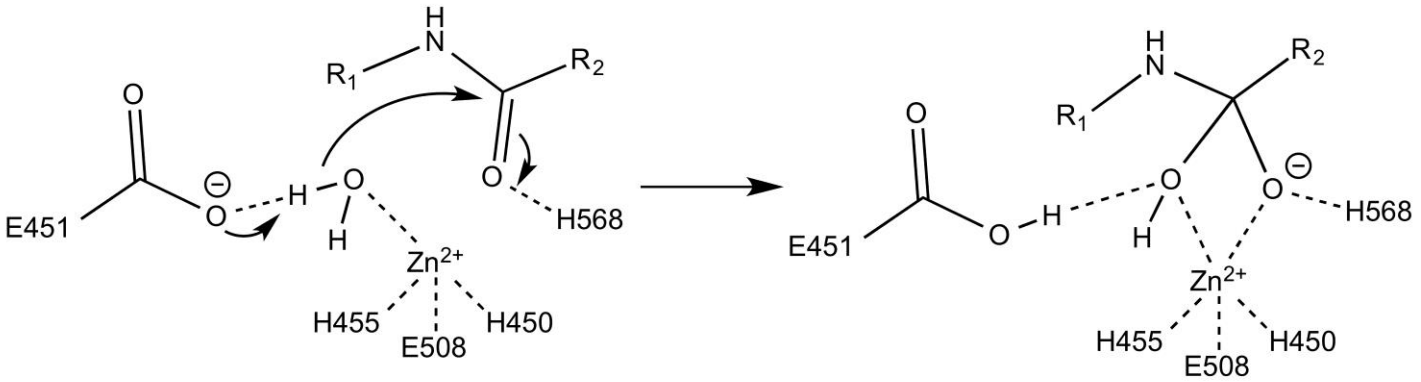
Met-enkephalin



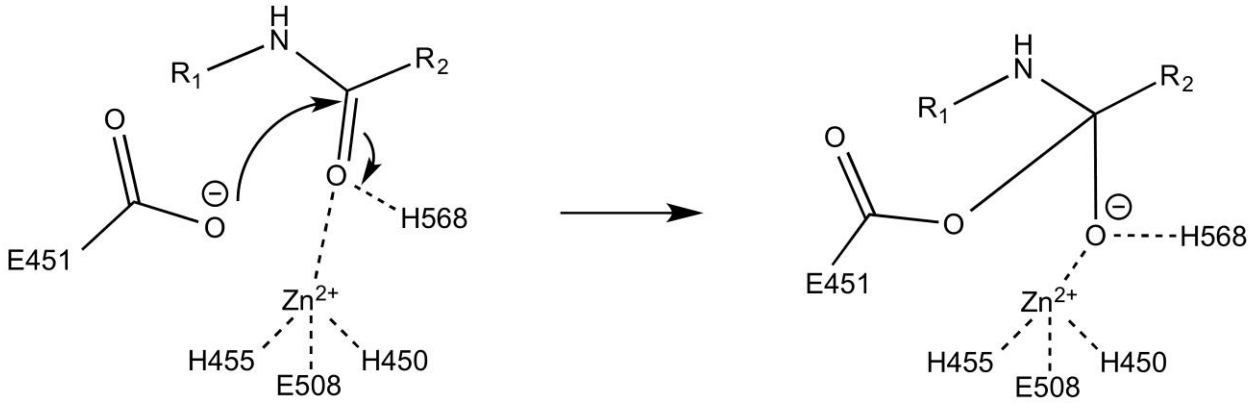


IVYPW

Mechanistic options



"Promoted water"-mechanism



Anhydride mechanism (disfavored, slow)

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