UNDERSTANDING THE HUMAN DPP III SUBSTRATE SPECIFICITY - QM/MM and MD calculations

Antonija Tomić,[a] and Sanja Tomić[b]

[a]*Institute Ruđer Bošković, Bijenička cesta 54, 10 000 Zagreb, atomic@irb.hr*

[b]*Institute Ruđer Bošković, Bijenička cesta 54, 10 000 Zagreb, Sanja.Tomic@irb.hr*

Dipeptidyl-peptidase III (**DPP III**; EC 3.4.14.4) is a two-domain monozinc exopeptidase of the peptidase family M49 that hydrolyzes dipeptides from the unsubstituted N-terminus of its substrates. It is considered to be an enzyme with broad substrate specificity with studies showing that tetrapeptides to octapeptides are the best substrates.

(BIOACTIVE) PEPTIDE SUBSTRATES

DPP III hydrolyzes biologically active peptides such as: angiotensins (angiotensin II, angiotensin III, angiotensin IV, angiotensin-(1-7) and angiotensin (3-7)), proctolin, α-melanocyte-stimulating hormone, dynorphin A(1-8), enkephalins (Leu-enkephalin and Met-enkephalin)) and endomorphins (endomorphin-1 and endomorphin-2), as well as some hemorphins (valorphin) and exorphins (β-casomorphin).

PEPTIDE INHIBITORS

Tynorphin (VVYPW), the truncated form of spinorphin, showed inhibitory activity towards DPP III isolated from monkey brain. Examination of the inhibitory activities of various synthetic hemorphin-like peptides revealed that the tynorphin analogs IVYPW and WVYPW showed the strongest inhibitory activity for recombinant DPP III.

QM/MM calculations

2-layer ONIOM calculations in the program Gaussian 09:
QM-layer: B97D20 density functional method and 6-31G(d) basis set for the H, N, C and O atom and LANL2DZ-ECP basis set for the Zn^{2+} ion
MM-layer: parm96 AMBER force field

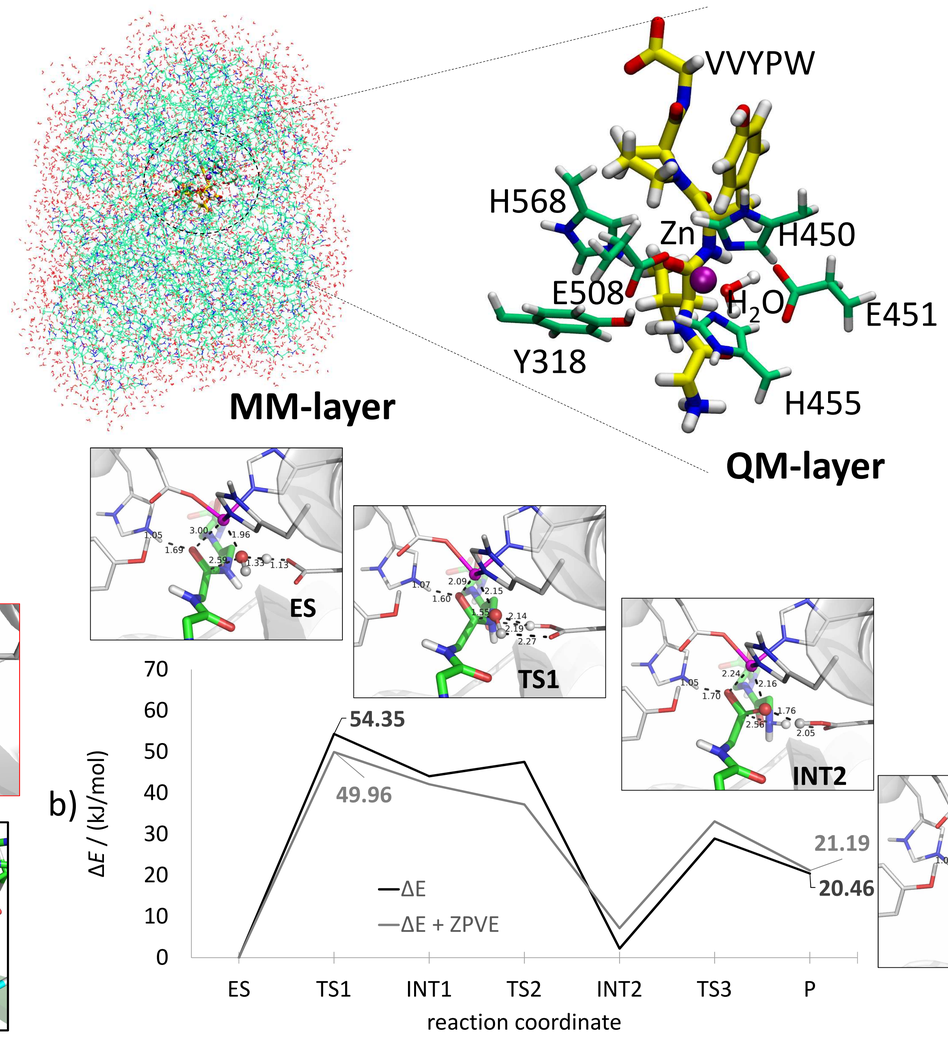

Figure 1. Energy profiles for the amide bond hydrolysis in: a) Leu-enkephalin and b) tynorphin bound in the active site of DPP III. Calculations were performed at: B97D/[6- 31G(d)+LanL2DZ-ECP] (black and red lines) and B97D/[6-31G(d)+ LanL2DZ-ECP] + $ZPVE_{B97D/[6\text{-}31G(d)+LanL2DZ\text{-}ECP]}$ (gray and pink lines) levels of theories. The most interesting structures are shown. For comparison, for reaction with Leu-enkephaline energy profile determined in 2016. (A. Tomić *et al.* Phys. Chem. Chem. Phys., 2016, 18, 27245) is shown as well (black and gray lines).

Adaptive Steered MD simulations

External force was applied to system in order to calculate the Potential of Mean Force (PMF) for extraction of the hydrolysis product from the enzyme active site.

The reaction coordinate was the end-to-end distance between the product (heavy atoms of the carboxylate, R-COO^-, or amino, R-NH_3^+, part of the product) and the zinc.

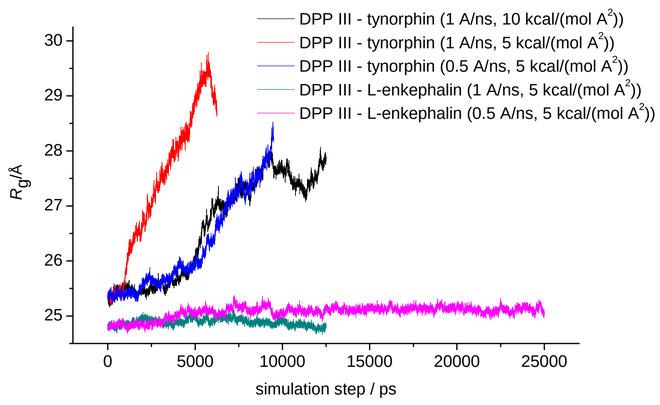

Figure 2. Radius of gyration profile of the trajectories closest to Jarzynski Average. The carboxylate part of the product was pulled with the pulling velocity and the force constant listed in paranthesis.

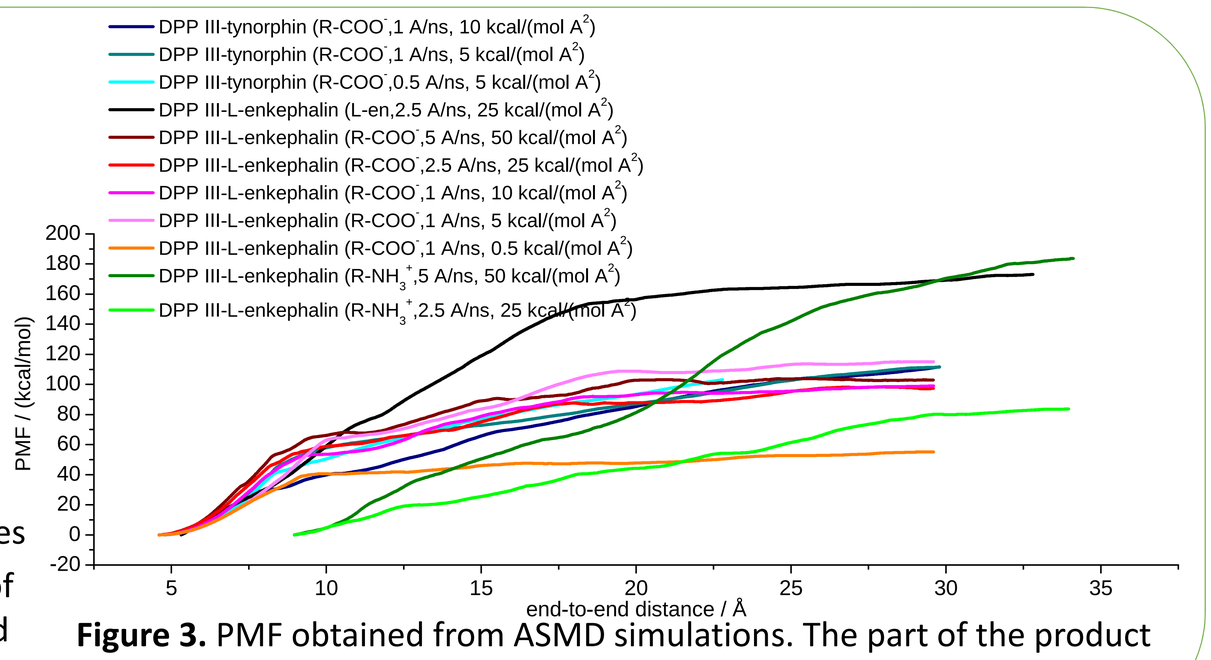

Figure 3. PMF obtained from ASMD simulations. The part of the product pulled, the pulling velocity and the force constant are listed in paranthesis.