A Multidisciplinary Approach to Understanding How DPP3 Catalytic Activity Influences Its Interaction with Keap1 and Vice Versa

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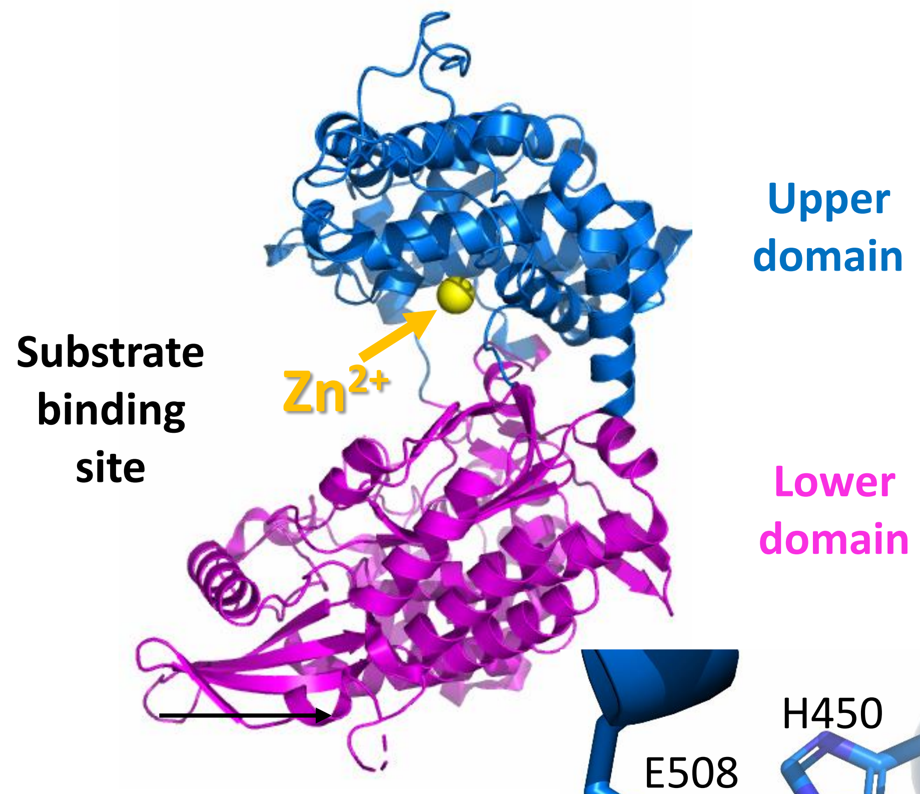
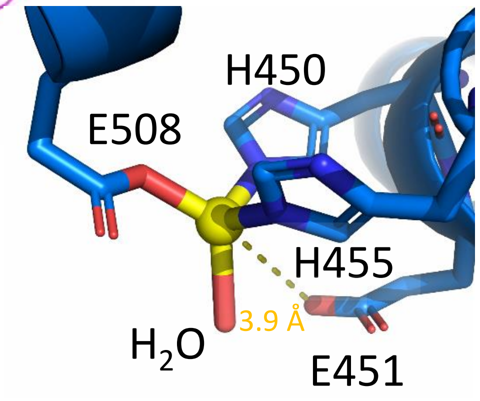
May 9th 2026, Zagreb

COMPUTATIONAL CHEMISTRY DAY 2026

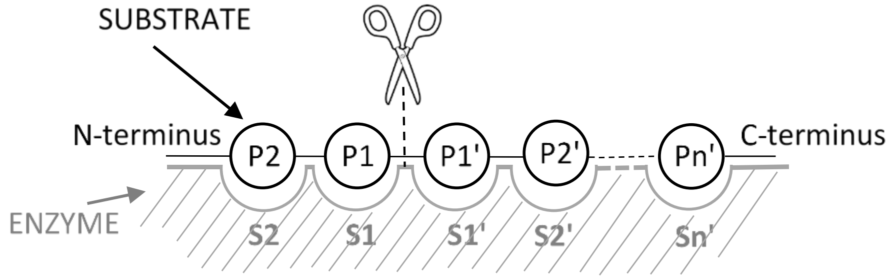
Dipeptidyl peptidase 3 (DPP3)



2009. human DPP3



- **ubiquitously** expressed peptidase (in prokaryotes and eukaryotes)
- **zinc-dependent exopeptidase** (M49 family)
- **hydrolyzes dipeptides** from the N-terminus of 4–8 amino acid-long oligopeptides



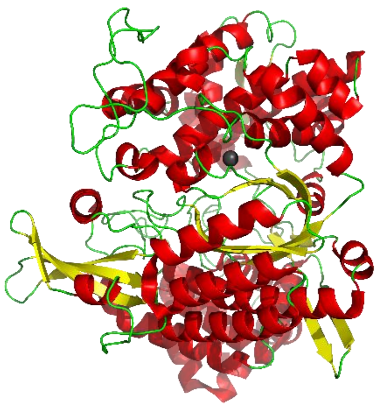
I. Schechter, A. Berger, *Biochem. Biophys. Res.* **27** (1967) 157-162.

Conformational flexibility

X-ray structures:

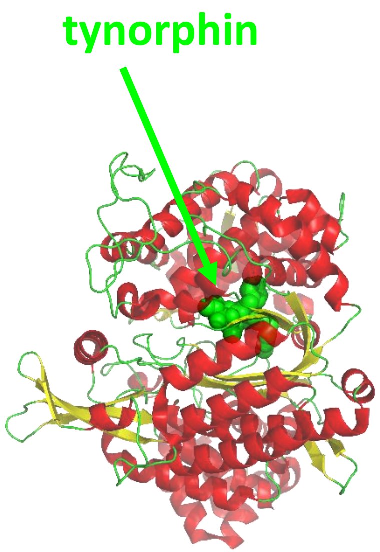
OPEN form
in ligand-free state

2009. **WT** human DPP3
(3FVY)



CLOSED form
in ligand-free state

2016. ligand-free **DPP3-E451A**
(5EGY)

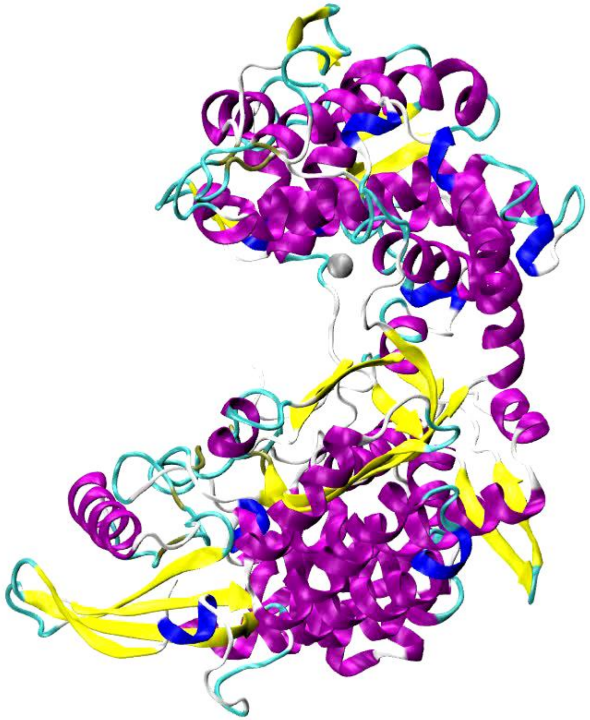


CLOSED form
in ligand-bound state

2012. human **DPP3-E451A** – tynorphin complex
(3T6B, 3T6J)

A. Tomić *et. al.*, *J. Chem. Inf. Model.* **52** (2012) 1583

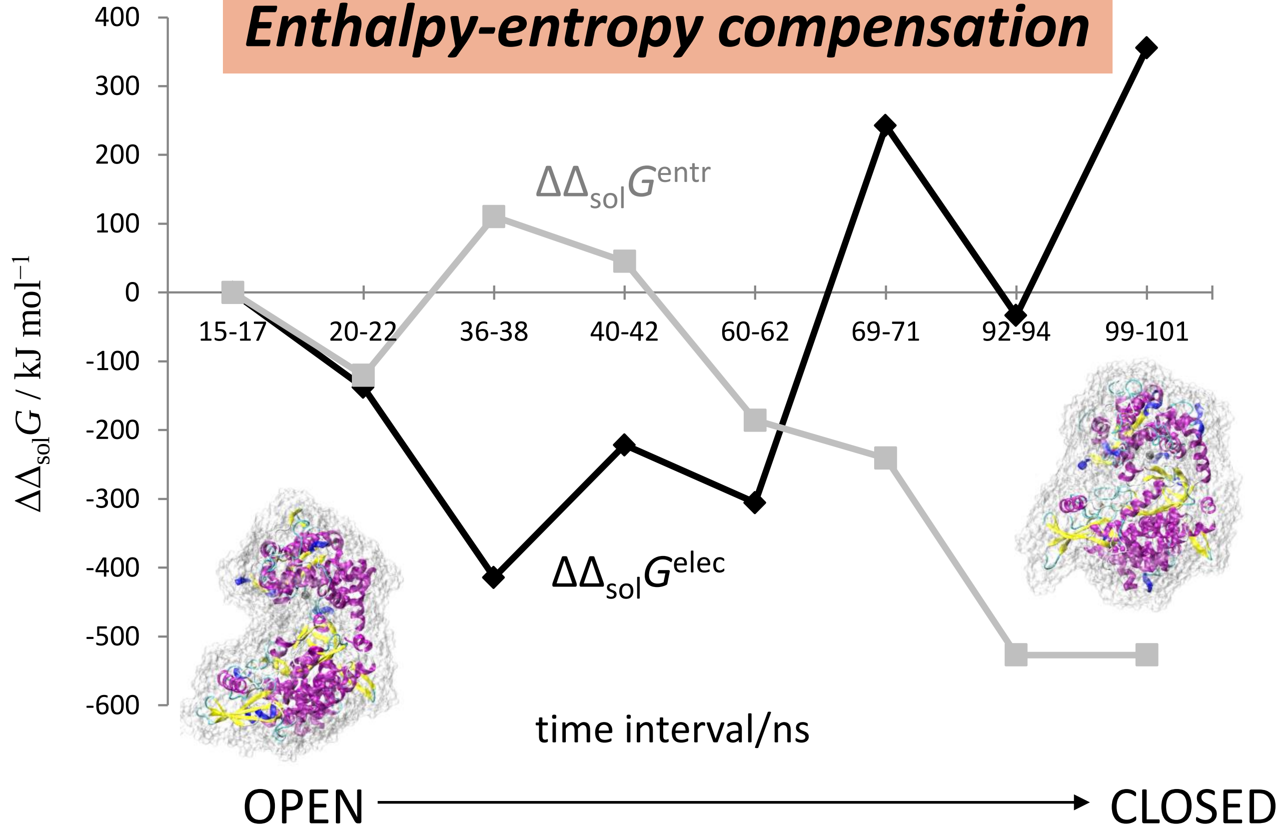
Standard 100 ns-long MD simulations of ligand-free DPP3 in explicit waters (program AMBER)



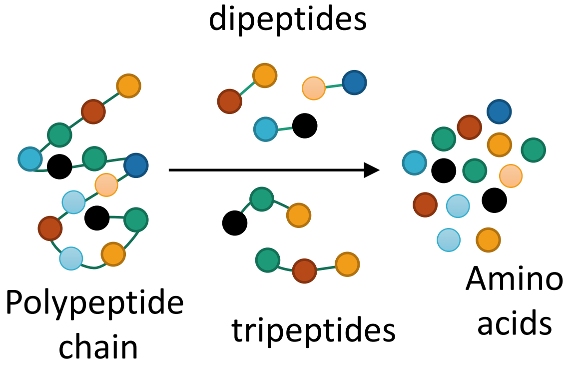
MM-PBSA calculations

(*Molecular Mechanics Poisson-Boltzmann Surface Area*)

Enthalpy-entropy compensation



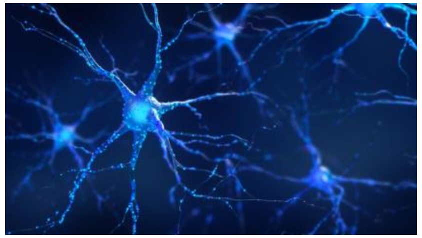
- DPP3 shows **broad substrate specificity** (*in vitro*)
- DPP3 has implied ROLE in:



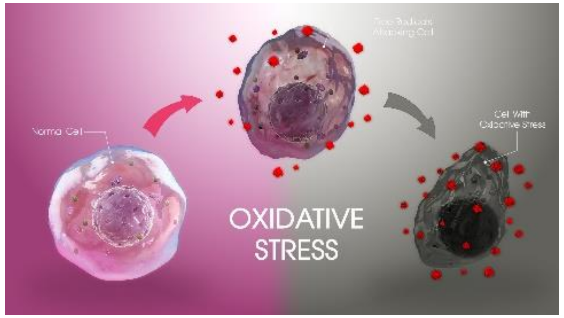
➢ **protein catabolism**

➢ **blood pressure regulation**
(hydrolyzes Ang II, Ang(1–5) and Ang(1–7))

➢ **pain modulation**
(*in vitro* hydrolyzes a number of biologically active (neuro)peptides, like enkephalins, endomorphins etc.)



➢ (cancer cell) **defense against oxidative stress**

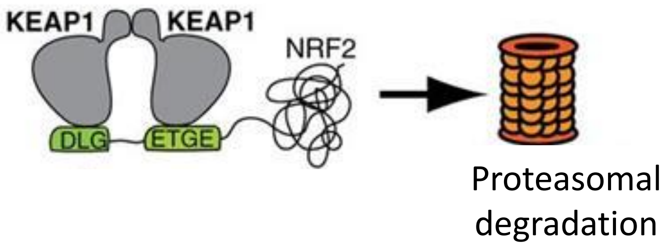


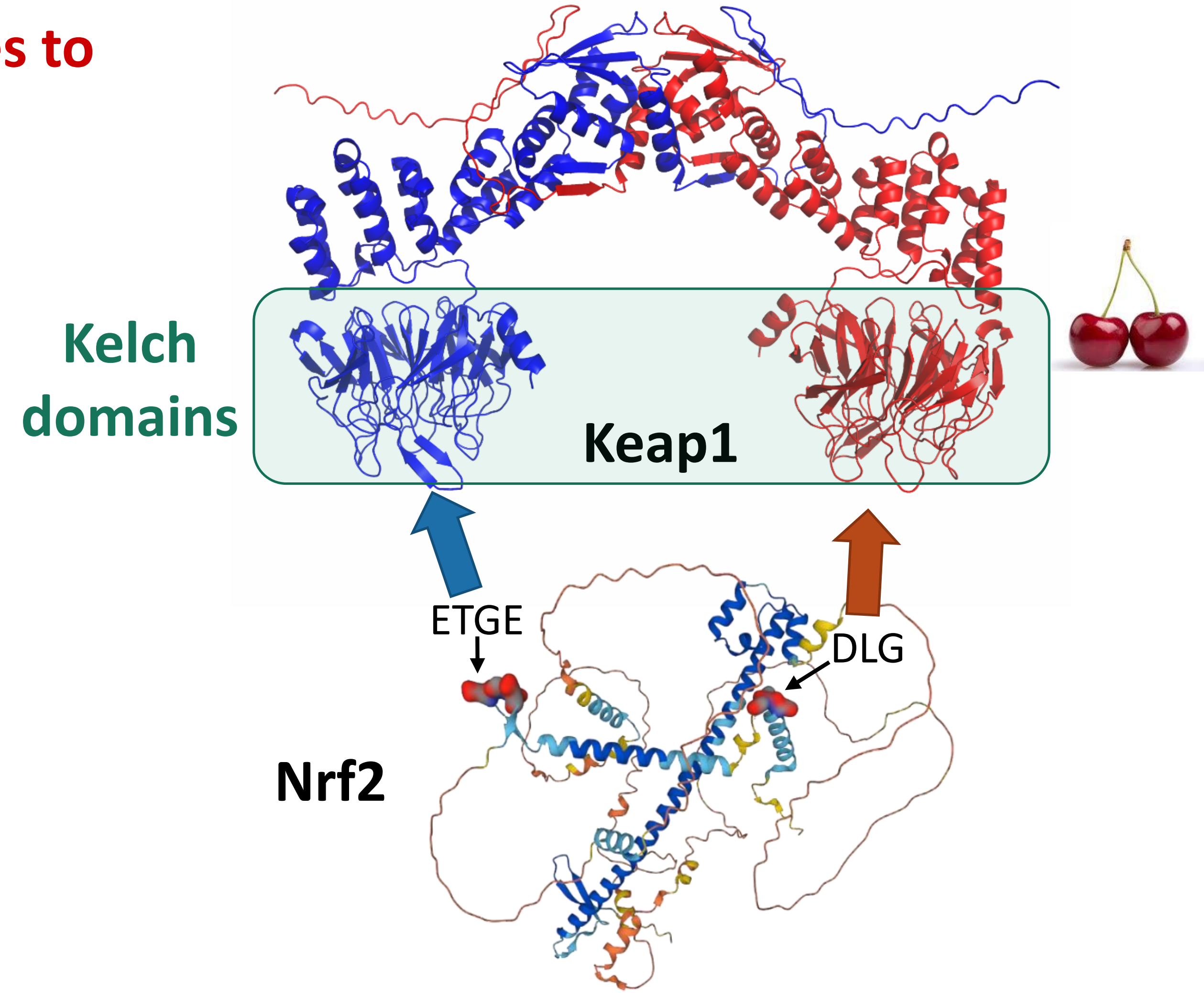
➢ **...**

Keap1 – Nrf2 signalling pathway

Kelch-like ECH-associated protein 1 – Nuclear factor erythroid 2-related factor 2

The main regulator of cellular responses to oxidative and electrophilic stresses





Hast et al. *Cancer Res*; 73(7) April 1, **2013**

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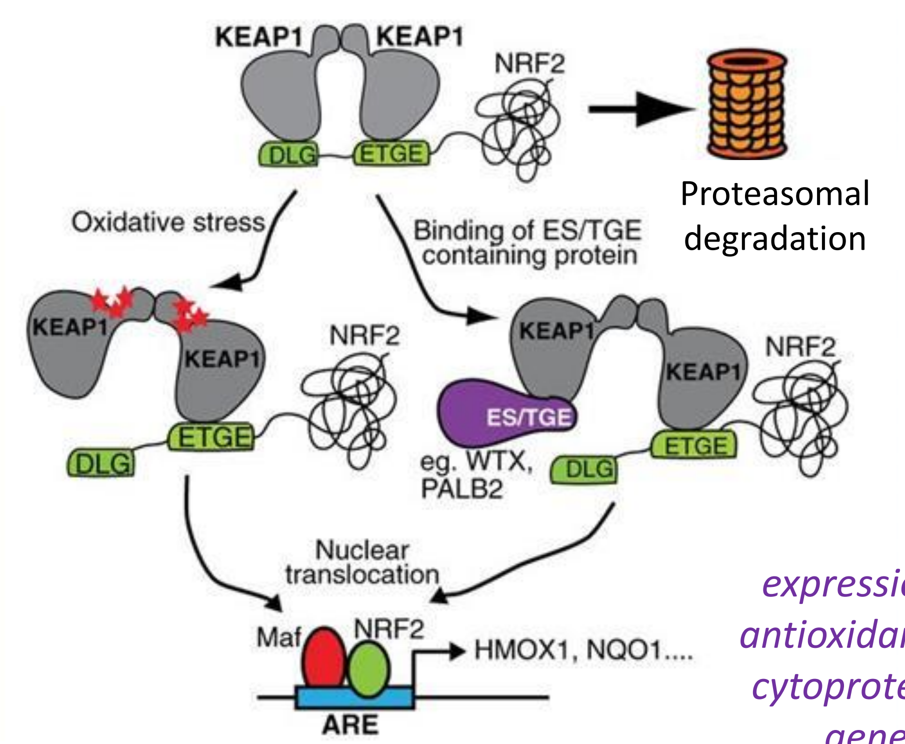
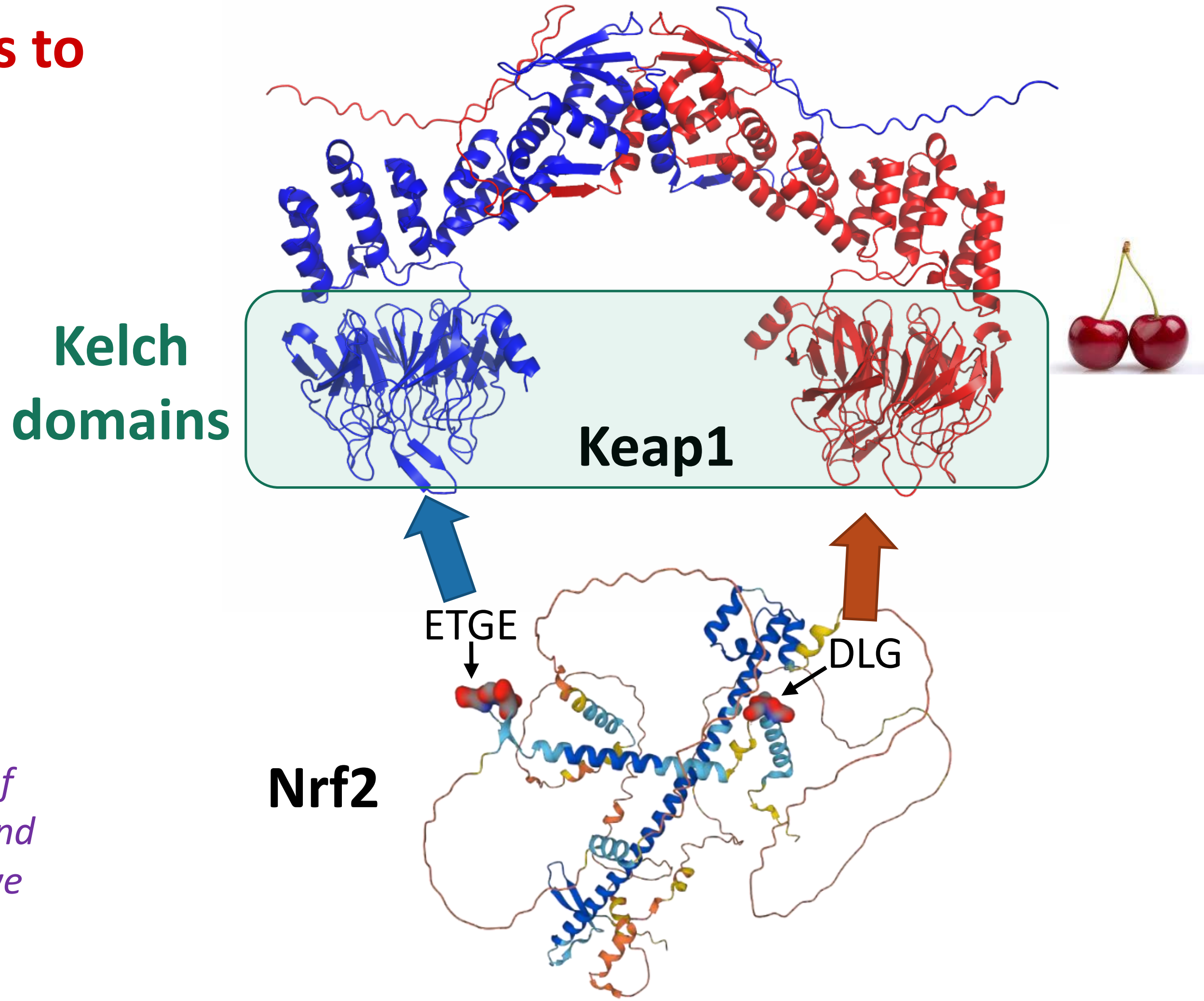


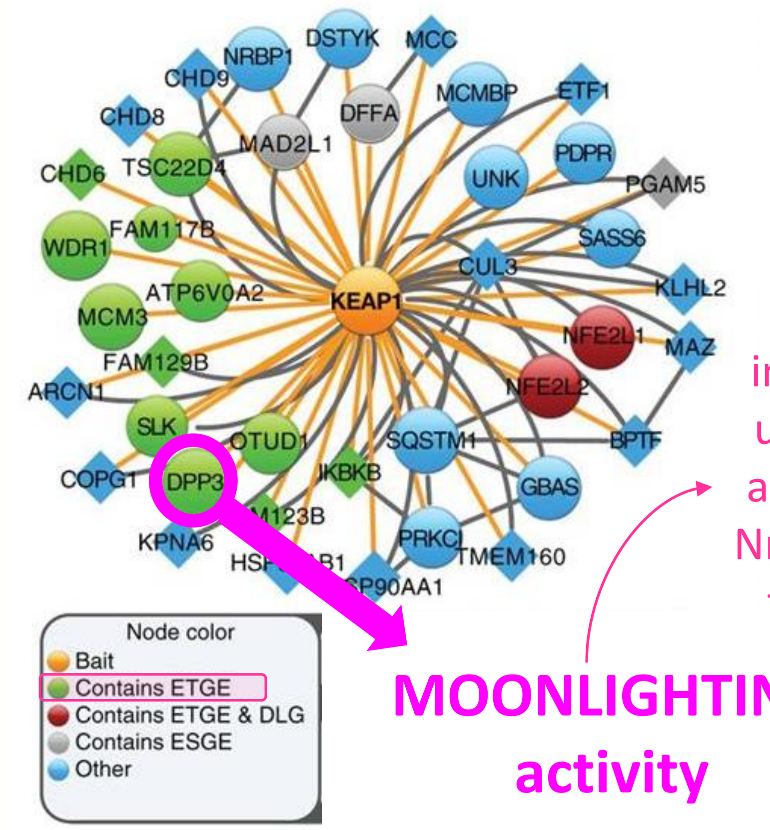
expression of antioxidant and cytoprotective genes

Hast et al. *Cancer Res*; 73(7) April 1, **2013**



Keap1 protein interaction network

Hast et al. *Cancer Res*, **2013**, 73(7)



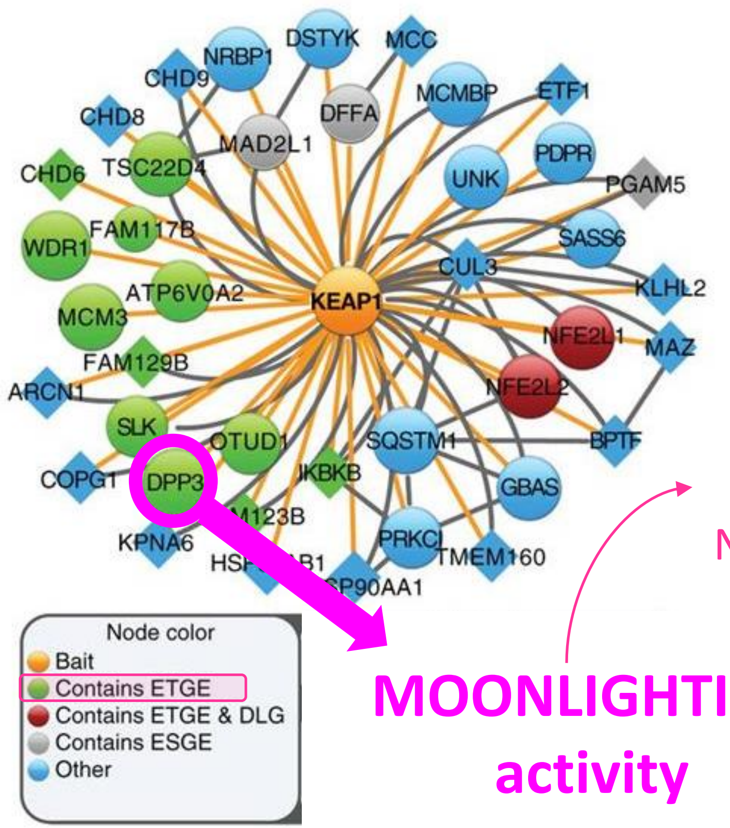
MOONLIGHTING activity

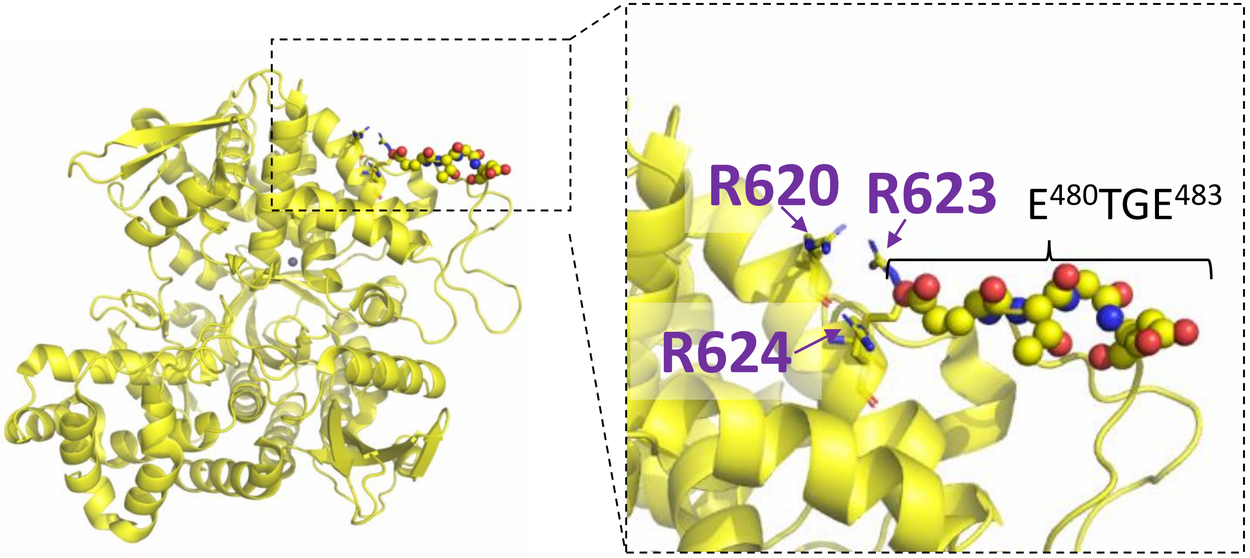
inhibiting Nrf2 ubiquitination and promoting Nrf2-dependent transcription

Nrf2 ACTIVATION

Keap1 protein interaction network

Hast et al. *Cancer Res*, **2013**, 73(7)





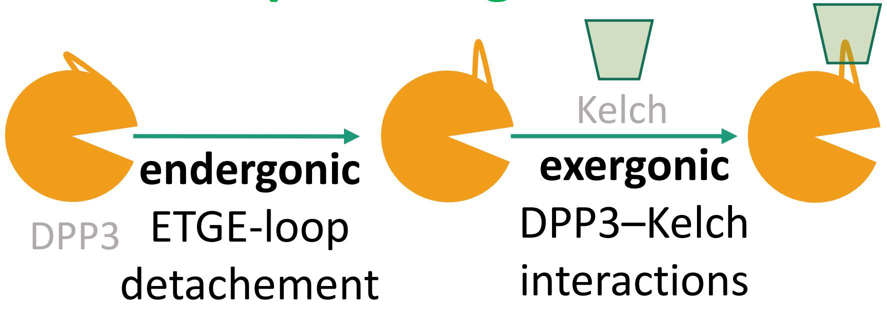
MOONLIGHTING activity

inhibits Nrf2 ubiquitination and promotes Nrf2-dependent transcription

Nrf2 ACTIVATION

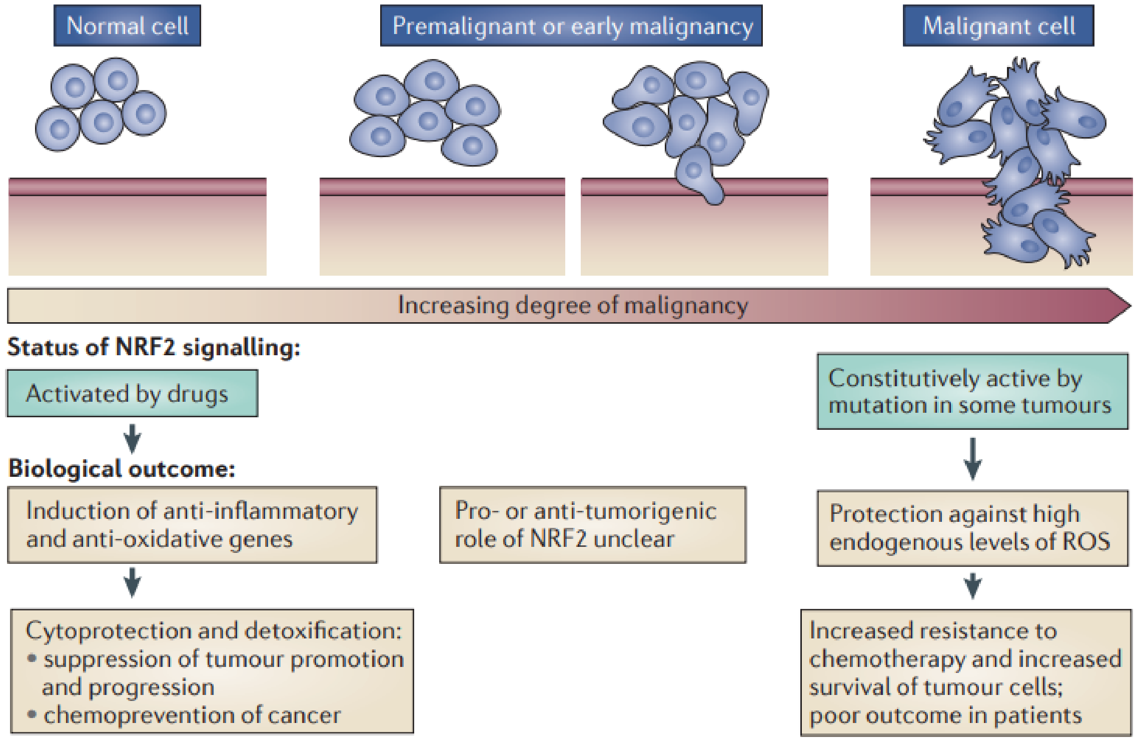
S. Matić, *J Biomol Struct Dyn*, **2021**,39(18)

Two-step binding mechanism



Dark side of Nrf2

A model for the importance of the context of tumour stage for the biological consequences of NRF2 activation



Sporn, M., Liby, K. Nat Rev Cancer, **2012**, 12, 564–571

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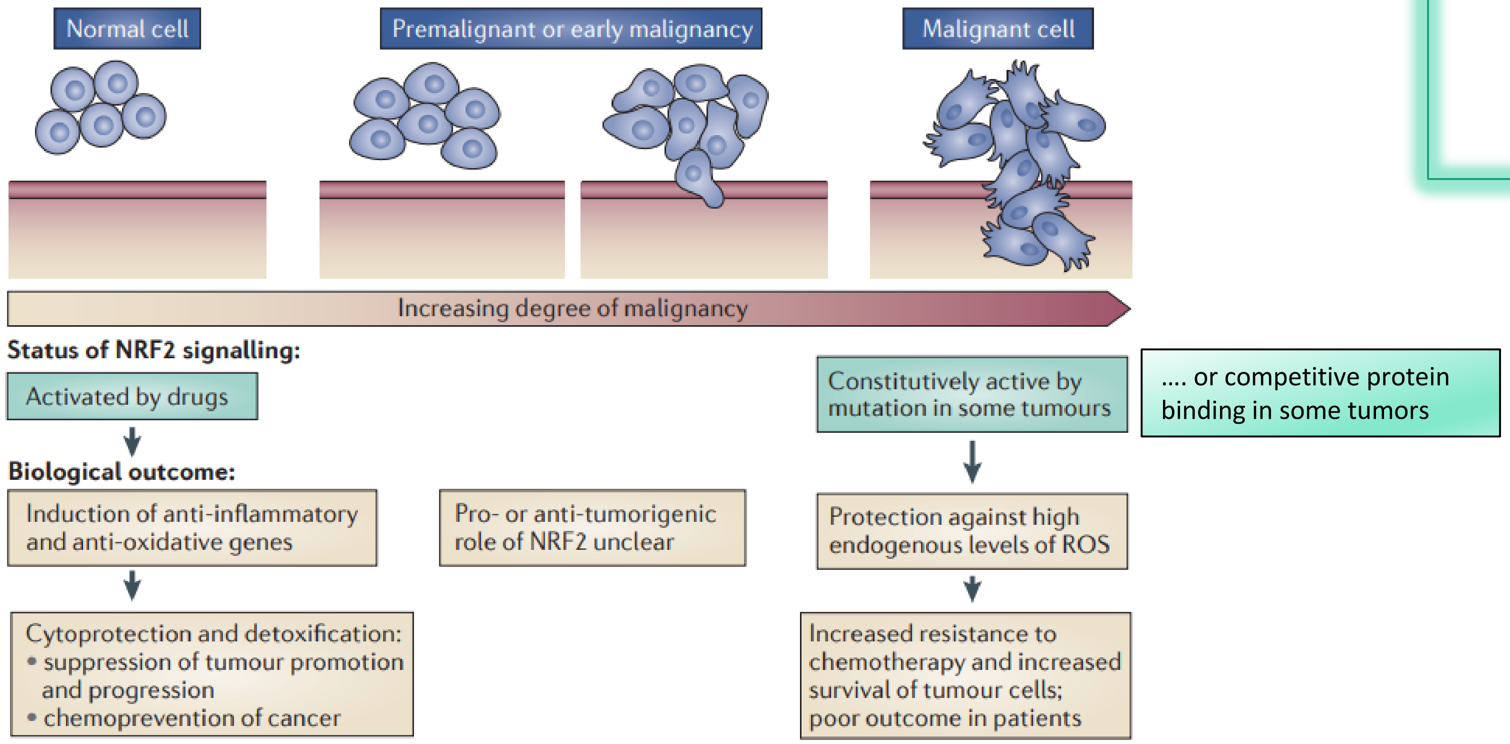


Is there a cross-talk between catalytic and moonlighting functions of DPP3?

Regulation of the moonlighting activity of DPP3 by targeting its catalytic function?

- Increased expression of DPP3 in **breast, colorectal and lung** cancer was correlated with significantly increased expression of genes controlled by Nrf2

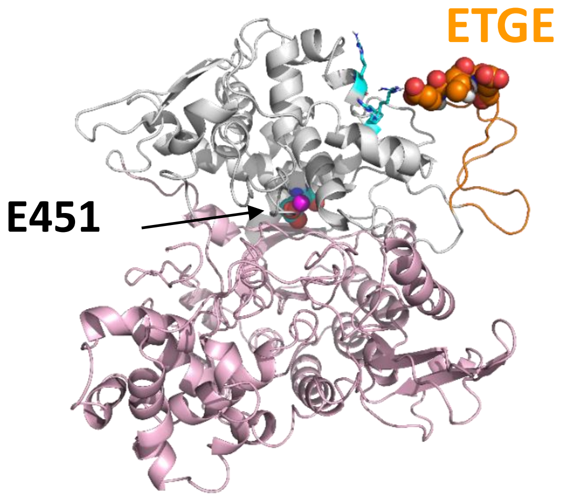
Sporn, M., Liby, K. Nat Rev Cancer, **2012**, 12, 564–571

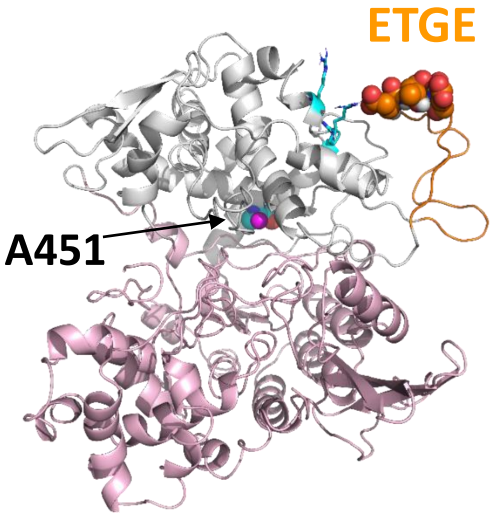
Computational study – MD simulations

WT
(different conformations)

catalytic mutant

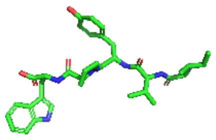
DPP3:

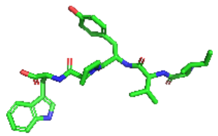




Catalytic ligand:

I-tynorphin

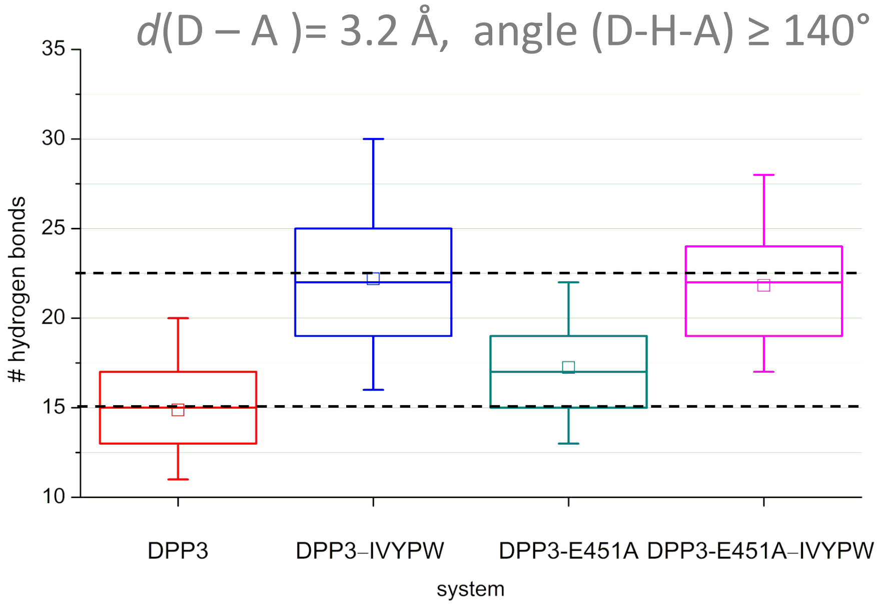




Standard MD simulations of DPP3 and its complexes

- 1 μs-long MD simulation of WT and E451A-mutated DPP3 (both ligand-free and IVYPW-bound):
  - *NpT* ensamble, ff19SB, OPC water model, 4-ligand hybrid bonded/non-bonded parameters for Zn^{2+}, d*t*=2fs, PBC, PME, AMBER 22

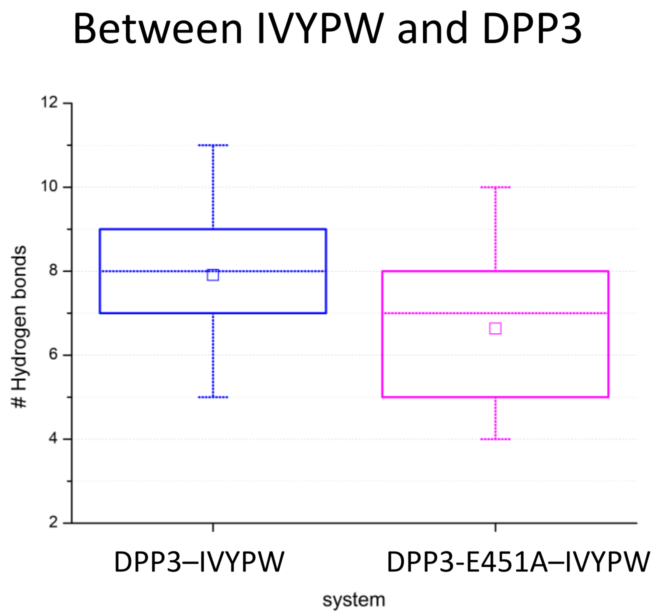
Hydrogen bonds between domains

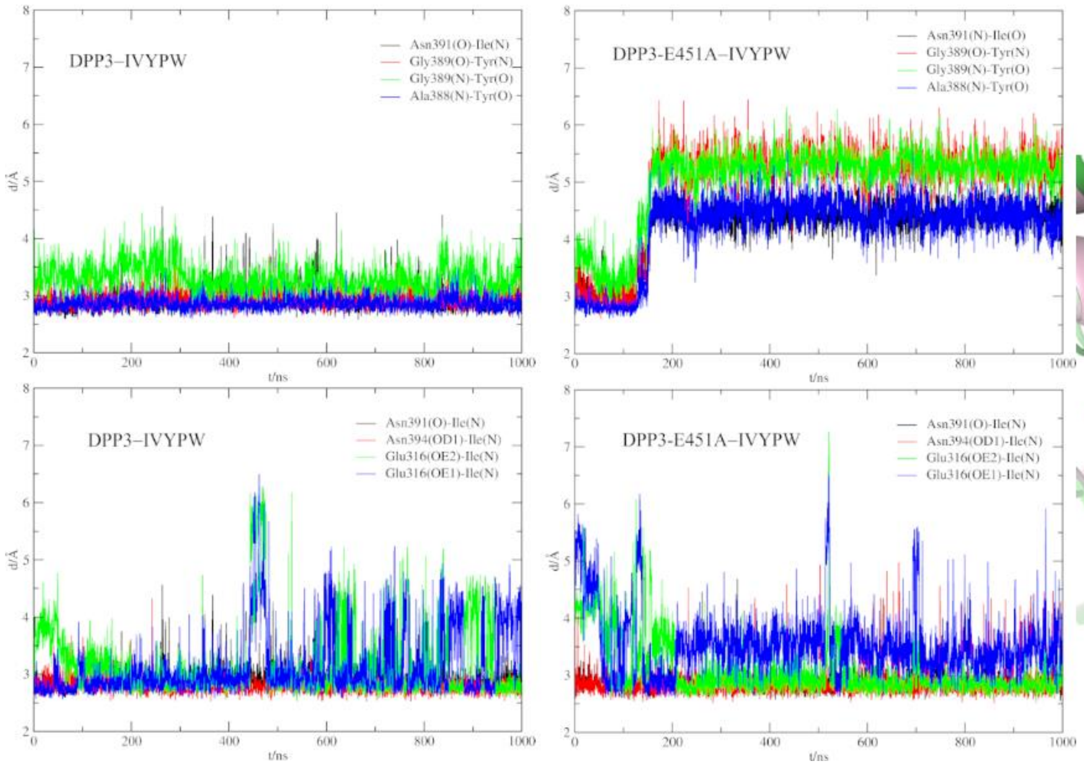


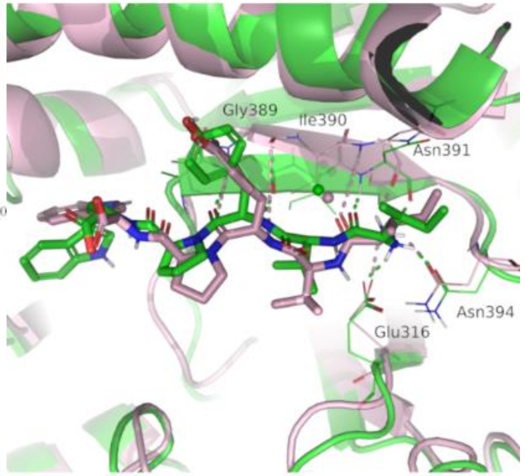
A higher overall number of H-bonds in the region between protein domains in:

- **complexes**, both WT and E451A mutant, compared to their ligand-free forms
- **the E451A mutant** compared to WT DPP3

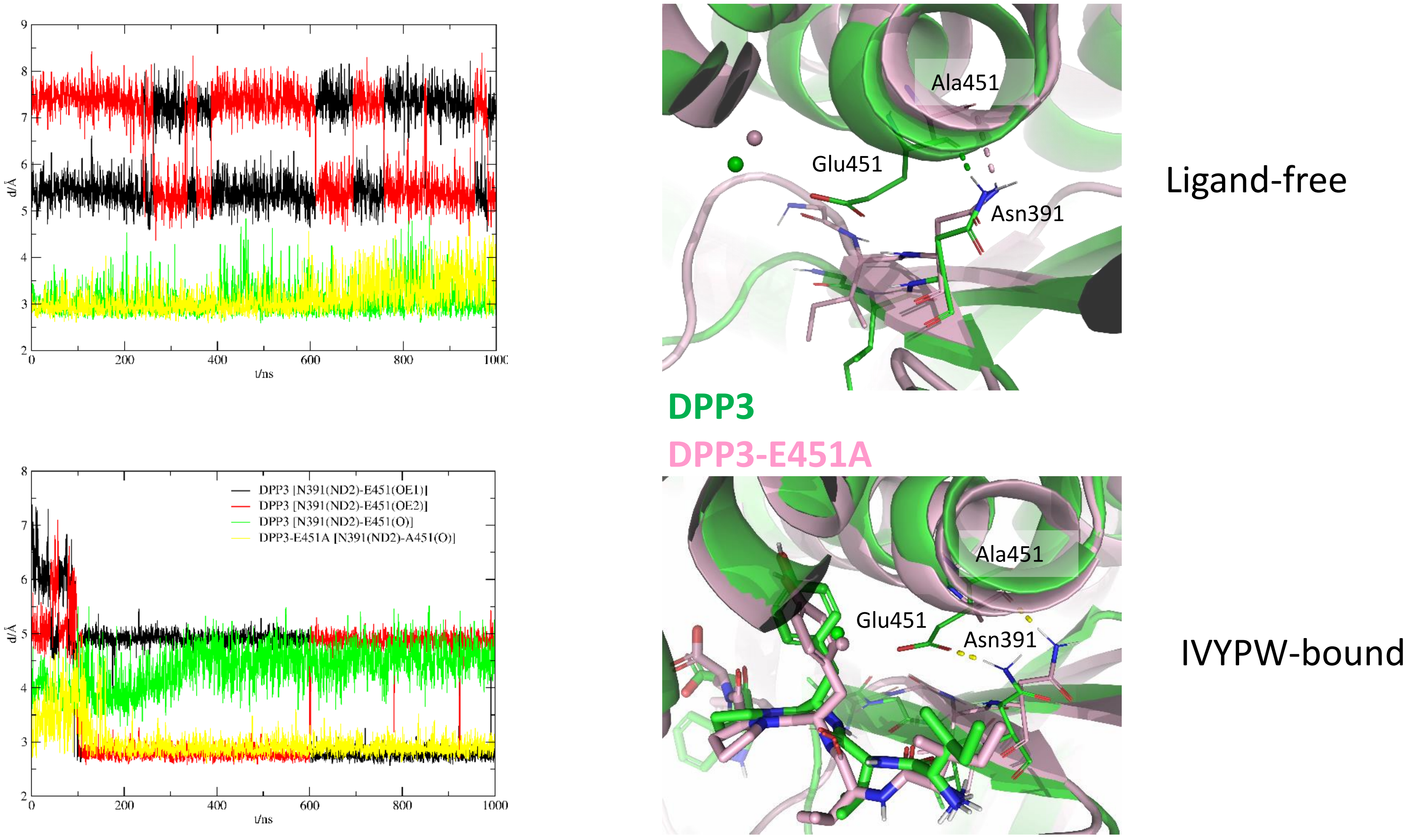
HIGHER NUMBER OF H-bonds → STABILIZING CLOSED ENZYME FORM







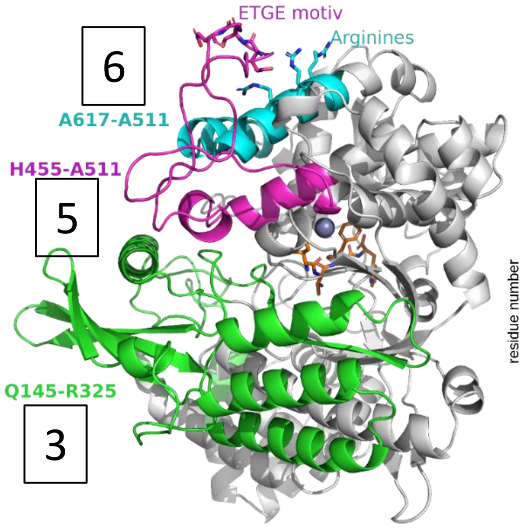
The E451A mutation disrupts the antiparallel binding of I-tynorphin to the β-sheet in the lower protein domain

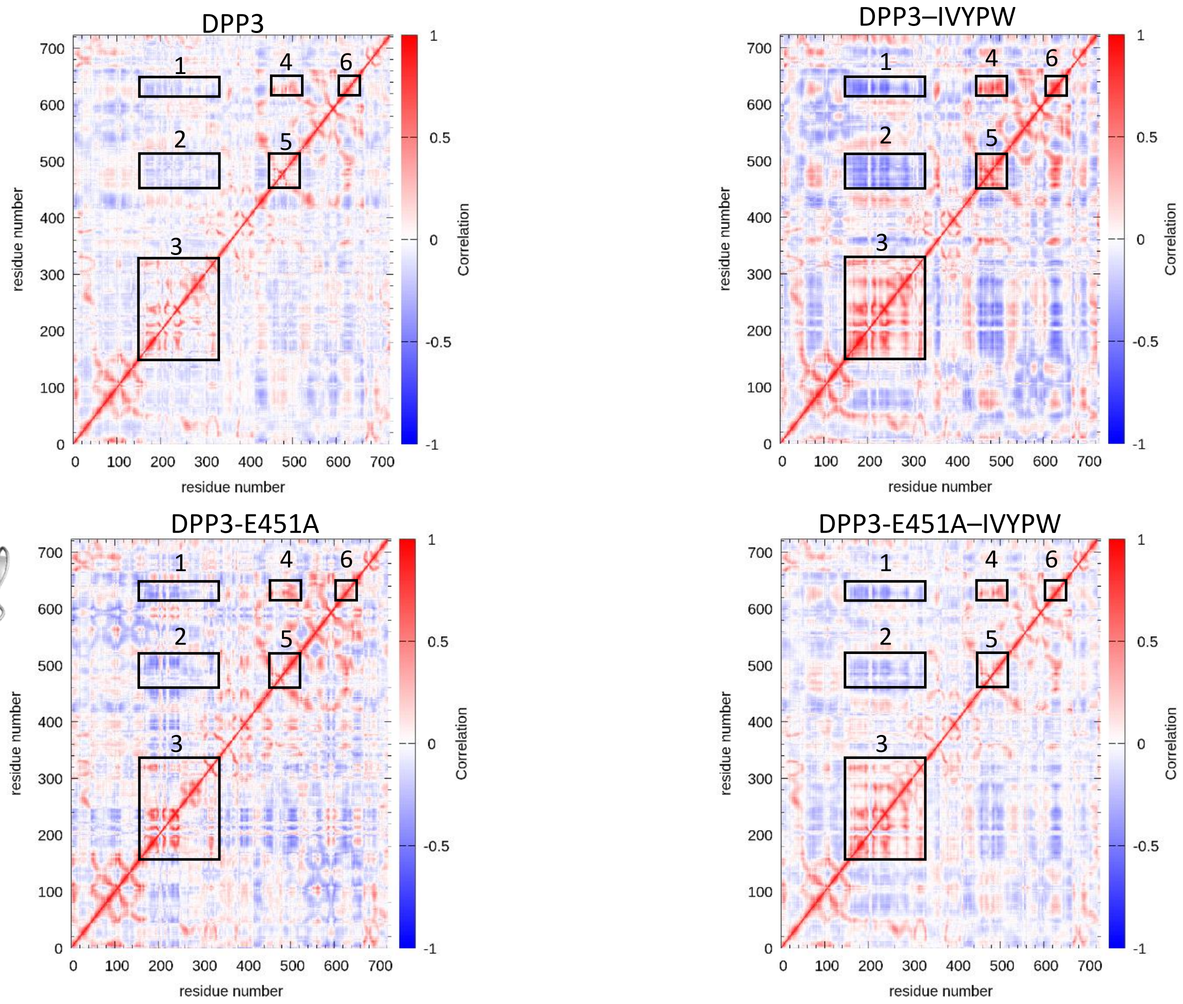


d/Å
t/ns
DPP3 [N391(ND2)-E451(OE1)]
DPP3 [N391(ND2)-E451(OE2)]
DPP3 [N391(ND2)-E451(O)]
DPP3-E451A [N391(ND2)-A451(O)]
Ala451
Glu451
Asn391
Ligand-free
DPP3
DPP3-E451A
Ala451
Glu451
Asn391
IVYPW-bound

Dynamic cross-correlation analysis of the motion during last 0.4 µs MD simulations

Regions with highly correlated and anticorralated motion are colored red and blue, respectively.





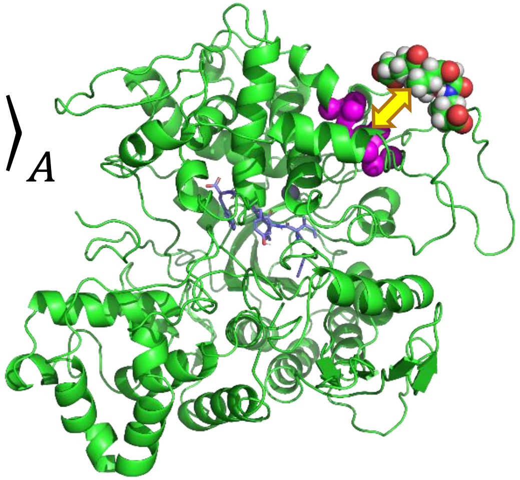
- The ligand binding **promotes coordinated local and interdomain dynamics** and **reduces conformational heterogeneity** in DPP3
- More importantly, rectangles 4 & 5 suggest possible dynamic coupling between residues in the ETGE-loop and those coordinating the catalytic zinc ion (H455 and E508)

possibility that inhibitor binding at the catalytic site may exert an allosteric effect on DPP3 involvement in the Keap1–Nrf2 pathway

The effect of inhibitor binding on the energy required for the ETGE-loop detachment?

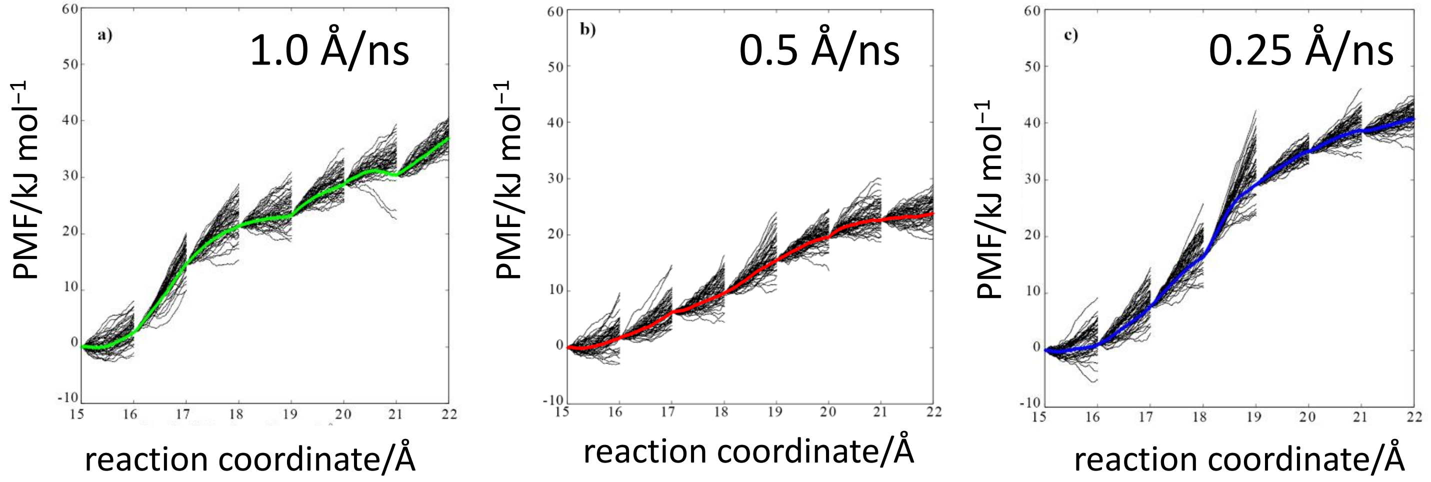
ADAPTIVE STEERED MD simulations

$$G_B = G_A - \frac{1}{\beta}\ln\langle e^{-\beta W_{A\to B}}\rangle_A$$

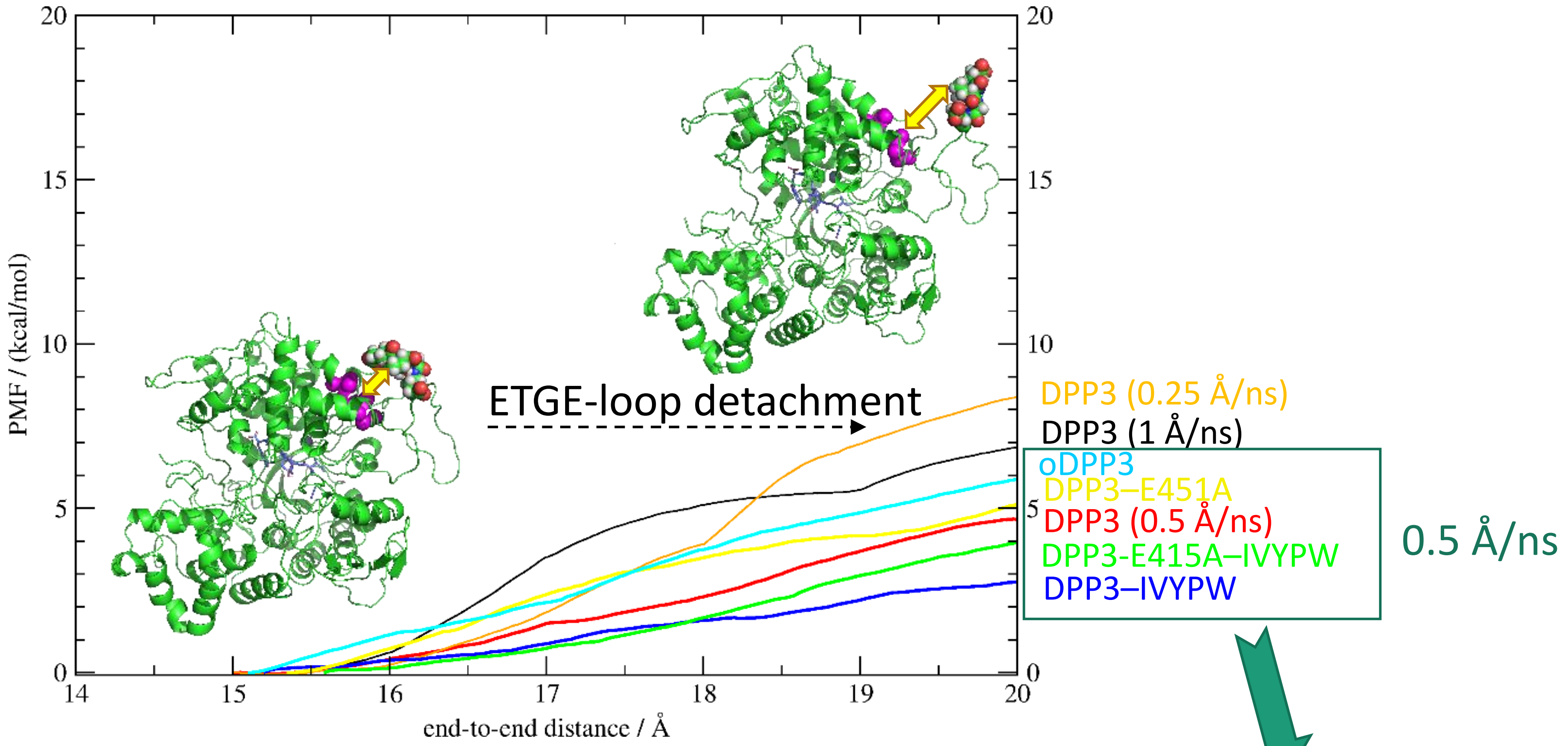


- AMBER 22 program package
- structures equilibrated in *NVT* ensemble
- force constant of **5 kcal mol⁻¹ Å⁻²**
- reaction coordinate was partitioned into 7 equal segments (each 1 Å in long) **50** trajectories were simulated per stage with different pulling velocities (**1.0**, **0.5** and **0.25** **Å/ns)** tested **in closed WT DPP3**:

Luka Petohleb,
diploma thesis, Faculty of Science



oDPP3 > DPP3, DPP3 E451A > DPP3 E451A–IVYPW > DPP3–IVYPW



distance between the center of the Cα atoms in the $E^{480}TGE^{483}$ motif and the center of the Cα atoms in the L622-S630 motif

IVYPW binding at the catalytic site may facilitate DPP3 interaction with the Kelch domain

Luka Petohleb, Dr. Antonia Matić (IRB)

Experimental study

- **Enzyme expression and purification:**

Dr. Antonia Matić, Division of Organic Chemistry and Biochemistry, RBI, Zagreb

- **Isothermal titration calorimetry (ITC):**

Dr. Filip Šupljika, assistant professor, Faculty of food technology and biotechnology, University of Zagreb

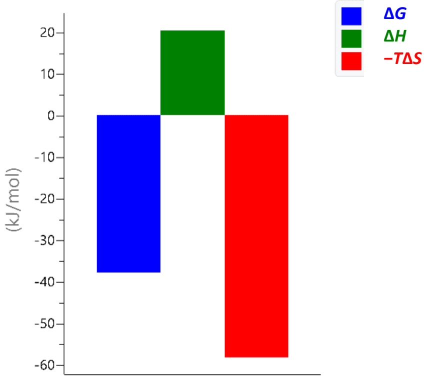
  - Malvern PEAQ-ITC microcalorimeter (MicroCal, Inc. Northampton, MA, USA)
  - Experiments were performed in 20 mM Tris buffer, pH = 8.0 with 150 mM NaCl and 1 mM TCEP at 25.0 °C
  - Experiments to correct for heat of dilution (buffer-buffer, peptide-buffer, buffer-protein) were performed for all experiments
  - MicroCal PEAQ-ITC analysis software

- **Kinetic Assay:**

Domagoj Christian Kindl, RBI, Zagreb

- Varian Cary Eclipse Fluorescence Spectrophotometer (Kinetics Application) (Agilent Technologies, Santa Clara, CA, USA)

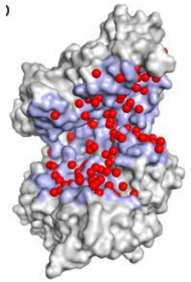
DPP3-E451A + IVYPW



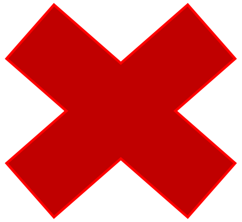
$-T\Delta_r S < 0$

$\Delta_r H > 0$

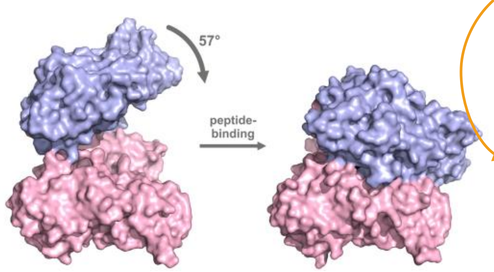
$-T\Delta_r S > \Delta_r H$



Kelch + IVYPW



TITRAND	TITRANT	$K_d/\mu M$	$\frac{\Delta_r G}{\text{kJ mol}^{-1}}$	$\frac{\Delta_r H}{\text{kJ mol}^{-1}}$	$\frac{-T\Delta_r S}{\text{kJ mol}^{-1}}$
DPP3-E451A	IVYPW	0.319 ± 0.085	−37.2 ± 0.7	20.8 ± 0.4	−57.9 ± 0.3

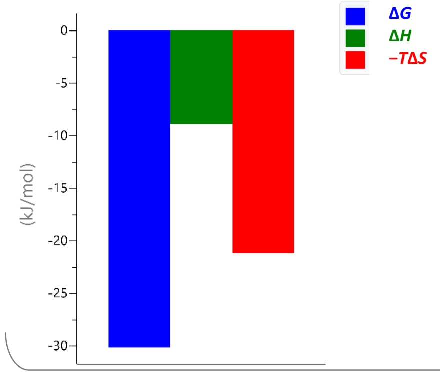


K_d= 0.23 ± 0.02 μM
reported by Bezerra et al.
PNAS 109 (2012)

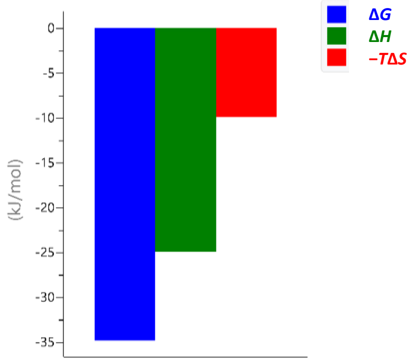
Strongly endothermic binding is counteracted by a large gain in entropy, resulting in an exergonic binding proces

Inhibition of DPP3 facilitates the formation of a complex with the Kelch domain

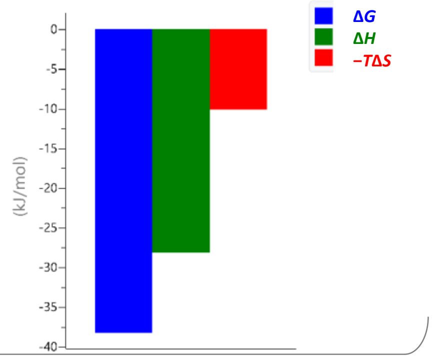
DPP3 + Kelch



DPP3-E451A + Kelch



DPP3-E451A – IVYPW + Kelch



$-T\Delta_r S$, $\Delta_r H < 0$

$-T\Delta_r S > \Delta_r H$

How to explain differences in thermodynamic signature plots?

$-T\Delta_r S < \Delta_r H$

TITRAND	TITRANT	K_d/µM	$\frac{\Delta_r G}{\text{kJ mol}^{-1}}$	$\frac{\Delta_r H}{\text{kJ mol}^{-1}}$	$\frac{-T\Delta_r S}{\text{kJ mol}^{-1}}$
DPP3	Kelch	4.52 ± 1.83	−30.7 ± 1.1	−8.9 ± 1.0	−21.9 ± 2.2
DPP3-E451A	Kelch	0.834 ± 0.039	−34.7 ± 0.2	−27.4 ± 4.5	−7.29 ± 4.49
DPP3-E451A–IVYPW	Kelch	0.222 ± 0.039	−38.1 ± 0.4	−28.2 ± 3.2	−9.82 ± 3.53

N (stoichiometry) was fixed to 1

X-ray structures:

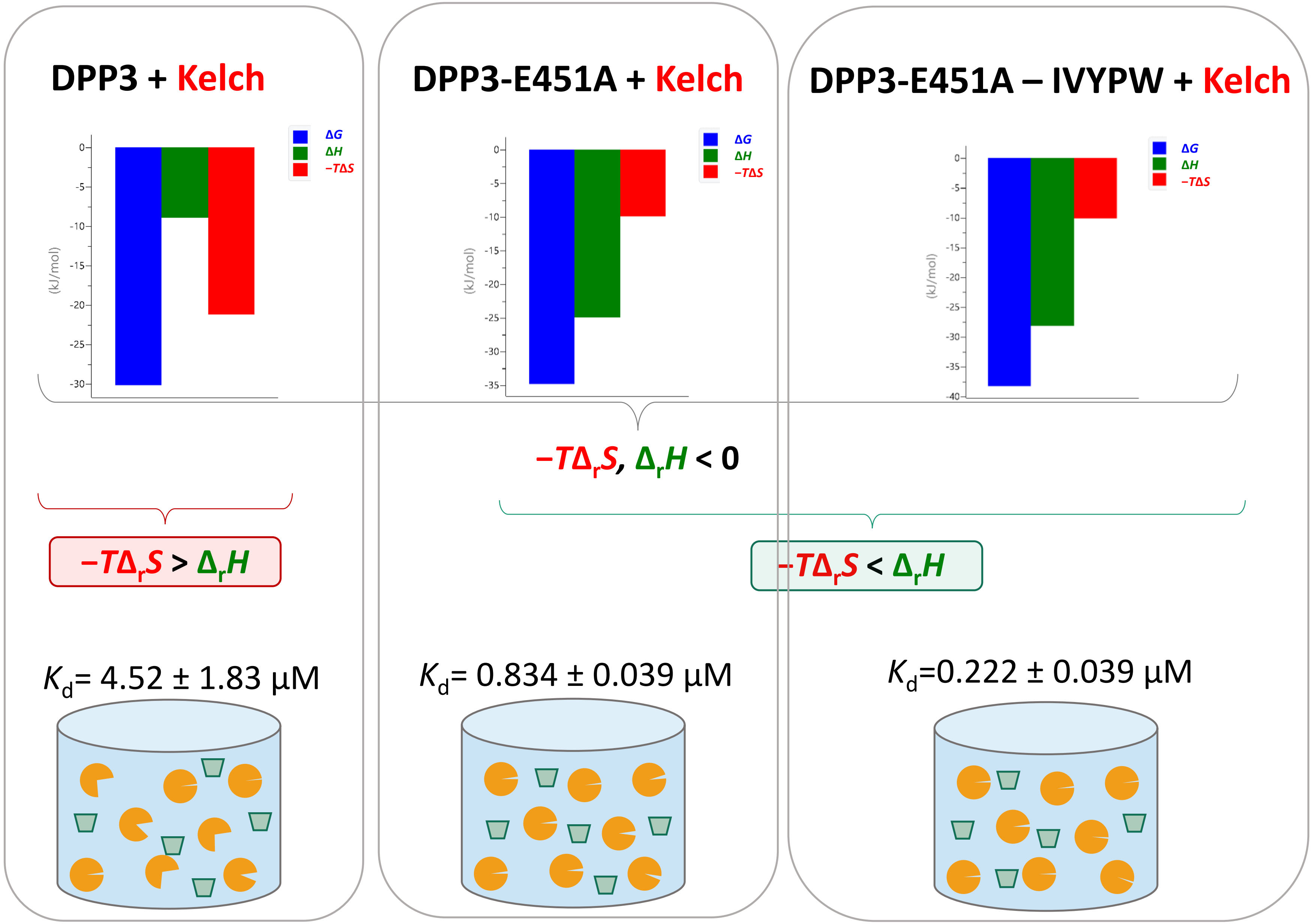
- ✓ E451A mutation and ligand binding induce enzyme closure

MD SIMULATIONS:

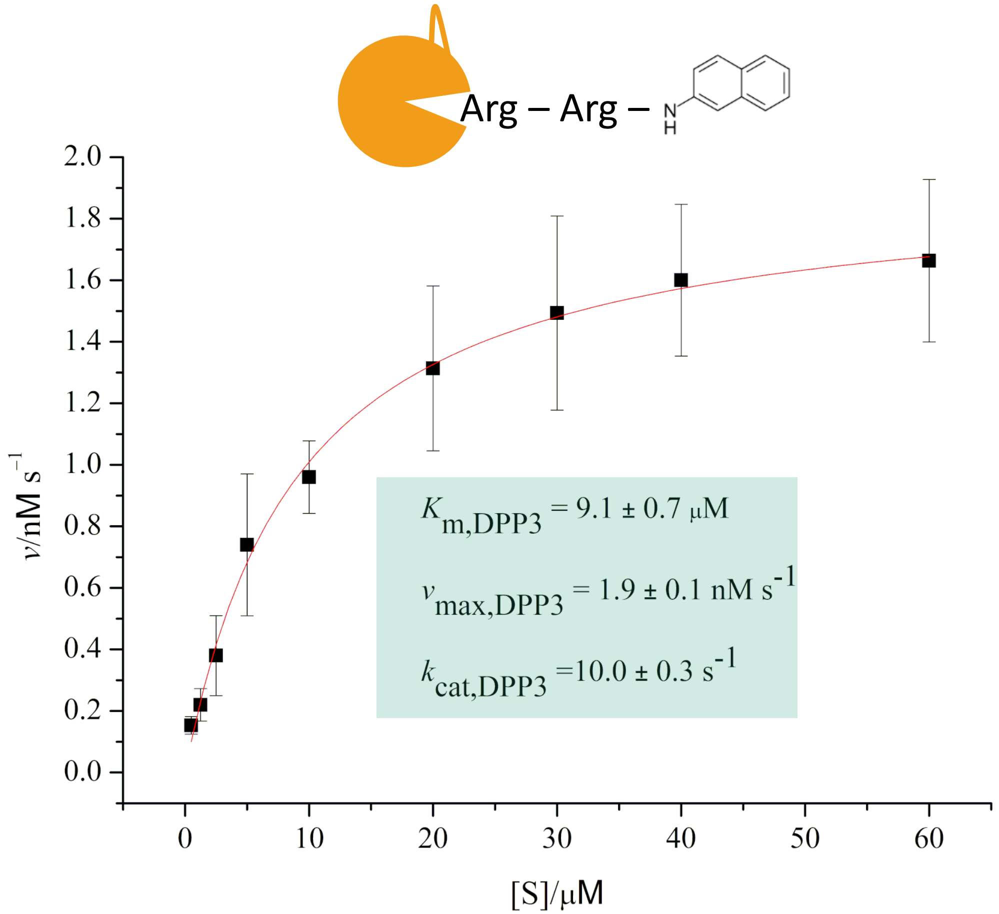
- ✓ Energy for the ETGE-loop detachment:

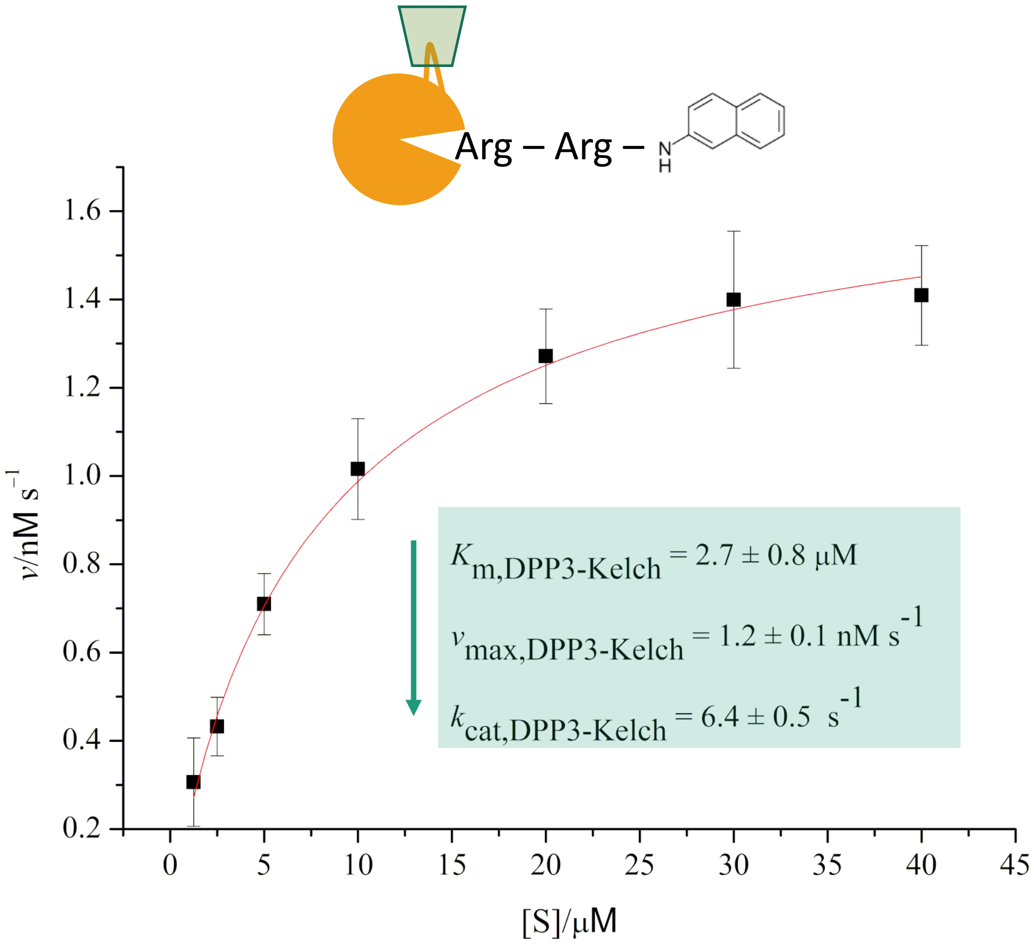
oDPP3 > DPP3, DPP3-E451A > DPP3-E451A–IVYPW > DPP3–IVYPW

- ✓ E451A mutation and IVYPW binding increases the number of H-bonds in the interdomain cleft stabilizing the closed enzyme form



Kinetic analysis of Arg_2-2NA hydrolysis





$$\frac{k_{cat}}{K_m} = 1.099 \text{ x } 106 \text{ M}^{-1} \text{ s}^{-1}$$

approximately twofold greater catalytic efficiency

$$\frac{k_{cat}}{K_m} = 2.344 \text{ x } 106 \text{ M}^{-1} \text{ s}^{-1}$$

CONCLUSION

➢ ***Crosstalk between the catalytic and moonlighting functions of DPP3***

Catalytic inactivation of DPP3 enhances its binding to Kelch

Kelch binding increases catalytic efficiency of DPP3

➢ ***This crosstalk could have a dual effect on cancer progression:***

in addition to its moonlighting role in activating Nrf2-dependent cytoprotective gene expression via Keap1 binding, interaction with Keap1 also enhances the catalytic efficiency of DPP3, which in turn increases the availability of amino acids that could support rapid cellular proliferation and/or reduce tumor immunogenicity

Acknowledgments:

- Laboratory for protein biochemistry and molecular modeling, Ruđer Bošković Institute
- Dr. Filip Šupljika, Faculty of Food Technology and Biotechnology, University of Zagreb
- Luka Petohleb, Faculty of Science
- srce | University of Zagreb University Computing Centre

- **FUNDING:**

Hrzz Croatian Science Foundation

This work is funded by Croatian Science Foundation under the project IP-2025-02-7100

Funded by the European Union NextGenerationEU

This work was funded by the European Union – NextGenerationEU, under the project titled ”Preliminary study of inhibiting NRF2-dependent transcription by preventing the DPP III – KEAP1 interaction for more effective cancer treatment”.