BIOCHEMICAL CHARACTERISATION OF THE DPP3–KEAP1 INTERACTION AND ITS ROLE IN NRF2 PATHWAY REGULATION

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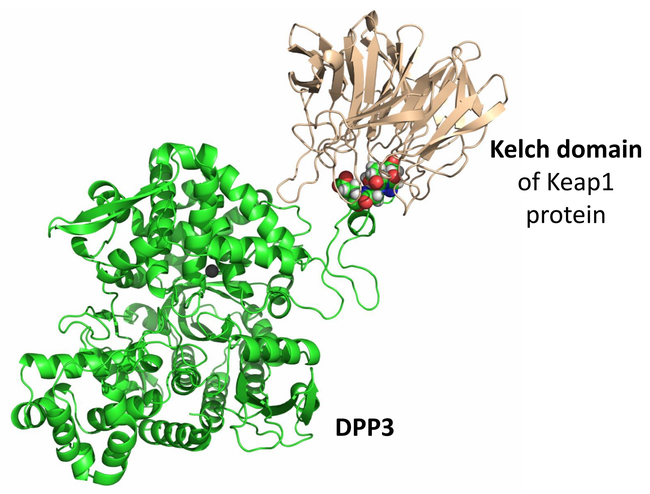
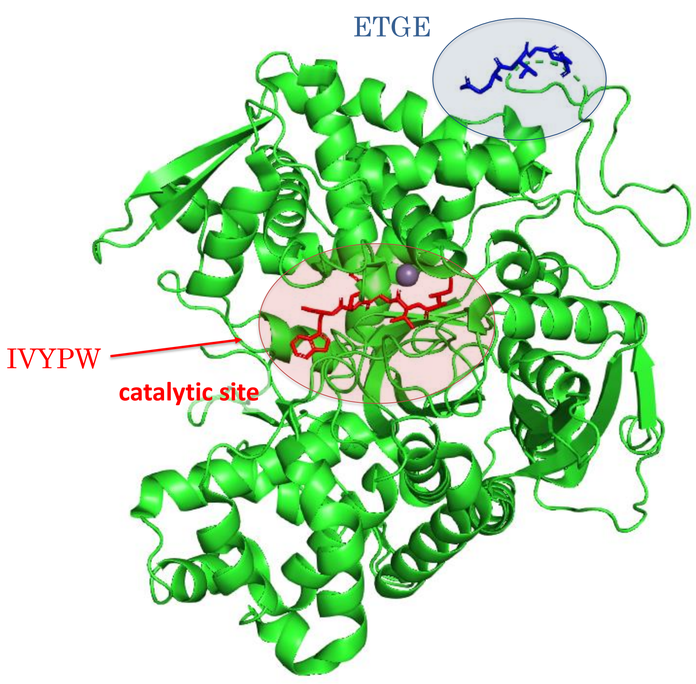
Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent exopeptidase involved in peptide degradation and various physiological processes, including the regulation of oxidative stress. Following its identification as a component of the Keap1 (Kelch-like ECH-associated protein 1) interactome, DPP3 was shown to modulate the Keap1–Nrf2 signalling pathway through competitive binding to the Kelch domain of Keap1 via its ETGE motif [1,2]. By disrupting the Keap1–Nrf2 interaction, DPP3 promotes Nrf2 activation, thereby enhancing the cellular antioxidant response. While this mechanism is protective under physiological conditions, elevated DPP3 expression and sustained Nrf2 activation have been associated with tumor progression and resistance to therapy in several cancers, including breast, lung, and colorectal cancer [1,3].

Scheme of DPP3 binding to the Kelch domain of Keap1

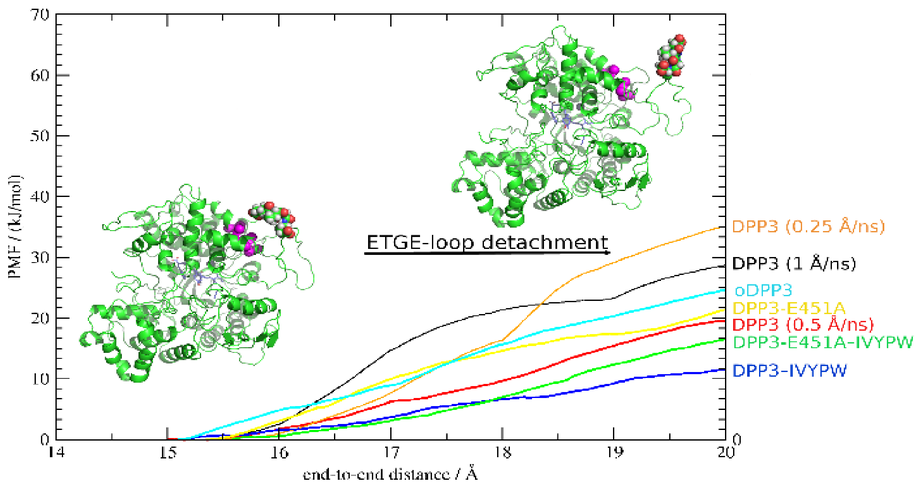


Characterization of DPP3–Keap1 Complex Formation. Binding affinities of the studied systems were first characterized by isothermal titration calorimetry (ITC), providing thermodynamic insights into DPP3–Kelch complex formation. To further elucidate the underlying molecular mechanisms, adaptive steered molecular dynamics (ASMD) simulations were performed in the *NVT* ensemble to examine the initial, rate-determining step of complex formation. Potential of mean force (PMF) profiles were generated to quantify the energy required for detachment of the ETGE-loop from the upper domain of DPP3. In parallel, conventional 1-μs molecular dynamics (MD) simulations in the *NpT* ensemble were used to identify structural and dynamic differences between the bound and unbound states of wild-type (WT) and E451A-mutated DPP3. All simulations were carried out using the AMBER22 software package with the ff19SB force field and explicit OPC water model.

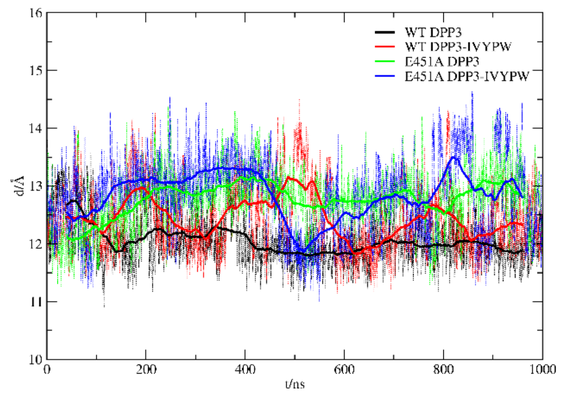
DPP3 (PDB: 5E3C)



PMF profiles for the detachment of the ETGE-loop from the upper domain of DPP3, obtained from ASMD simulations.



IVYPW binding lowers the energetic barrier for ETGE-loop detachment, facilitating Keap1 recognition.



Distance between centers of mass of Cα atoms from the ETGE and L622-S630 motifs during 1-μs MD simulations.

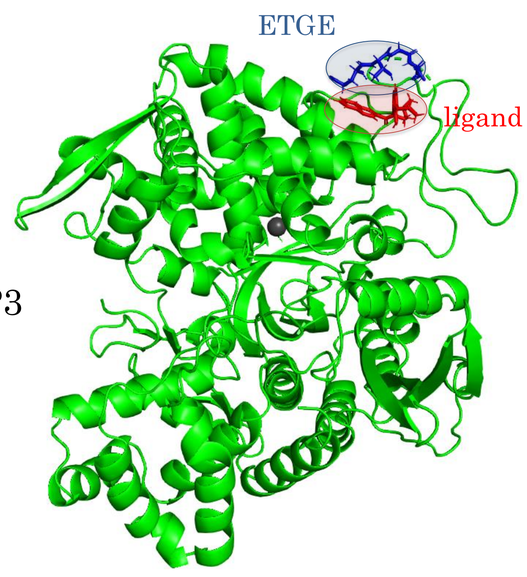
ITC (Isothermal Titration Calorimetry) experiments were performed on the Malvern PEAQ-ITC microcalorimeter to characterize protein-protein or protein-ligand interactions at 25°C and pH = 7.5 in 20 mM Tris buffer with 150 mM NaCl and 1 mM TCEP.*

TITRAND	TITRANT	K_d/μM	$\Delta_r G$/kJ mol^{-1}	$\Delta_r H$/kJ mol^{-1}	$-T\Delta_r S$/kJ mol^{-1}
WT DPP3	**Kelch**	4.52 ± 1.83	−30.7 ± 1.1	−8.9 ± 1.0	−21.9 ± 2.2
Kelch	**WT DPP3**	20.3 ± 12.0	−27.3 ± 2.2	−15.6 ± 5.2	−11.7 ± 7.0
DPP3 E451A	**Kelch**	0.834 ± 0.039	−34.7 ± 0.2	−27.4 ± 4.5	−7.29 ± 4.49
DPP3 E451A	**IVYPW**	0.319 ± 0.085	−37.2 ± 0.7	20.8 ± 0.4	−57.9 ± 0.3
DPP3 E451A –IVYPW	**Kelch**	0.222 ± 0.039	−38.1 ± 0.4	−28.2 ± 3.2	−9.82 ± 3.53

*The results are mean of three independent experiments with standard deviation. The stoichiometry of the interactions was fixed (*N* = 1) based on previous studies

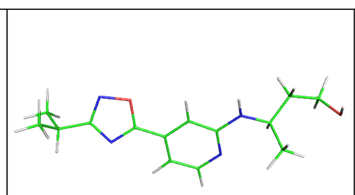
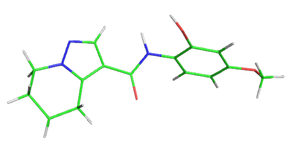
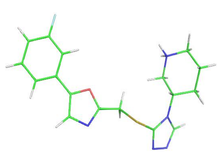
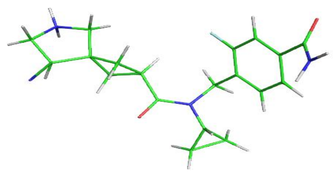
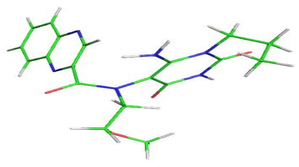
Screening for small molecules that impede the dissociation of the ETGE-loop. Small ligands were docked to DPP3 in the vicinity of the ETGE-loop, followed by molecular dynamics (MD) simulations. MM/PBSA binding free energies were calculated for ligands that remained associated with the ETGE-loop throughout the simulations. The aim was to identify ligands capable of stabilizing the ETGE-loop of DPP3, thereby potentially preventing DPP3–Keap1 complex formation. Such inhibition could indirectly enhance Keap1–Nrf2 binding and consequently reduce Nrf2 activation and the cellular antioxidant response.

DPP3



MD simulations:
- AMBER22, *NpT*, 300K, ff19SB, OPC.

MM-PBSA binding energies of DPP3–Ligand complexes:

Ligand					
$\Delta H^{MM/PBSA}$/ kcal mol^{-1}	−4,68 ± 2,48	−4,80 ± 3,31	−11,27 ± 3,17	−11,53 ± 3,10	−12,62 ± 2,69

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[1] B.E. Hast et al., Cancer Res. 2013, 73, 2199–2210; Hast et al., Cancer Res. 2014, 74, 808–817.
[2] Matić S, Kekez I, Tomin M, et al. J Biomol Struct Dyn. 2021;39:6870–6881.
[3] Oskomić M, Tomić A, Barbarić L, et al. Cancers. 2025;17:447.

ACKNOWLEDGMENTS
This work was funded by the European Union's NextGenerationEU program and by Croatian Science Foundation under the project IP-2025-02-7100.