

TARGETING THE DPP3–KEAP1 INTERACTION: A STRUCTURE-BASED SEARCH FOR SMALL MOLECULE MODULATORS

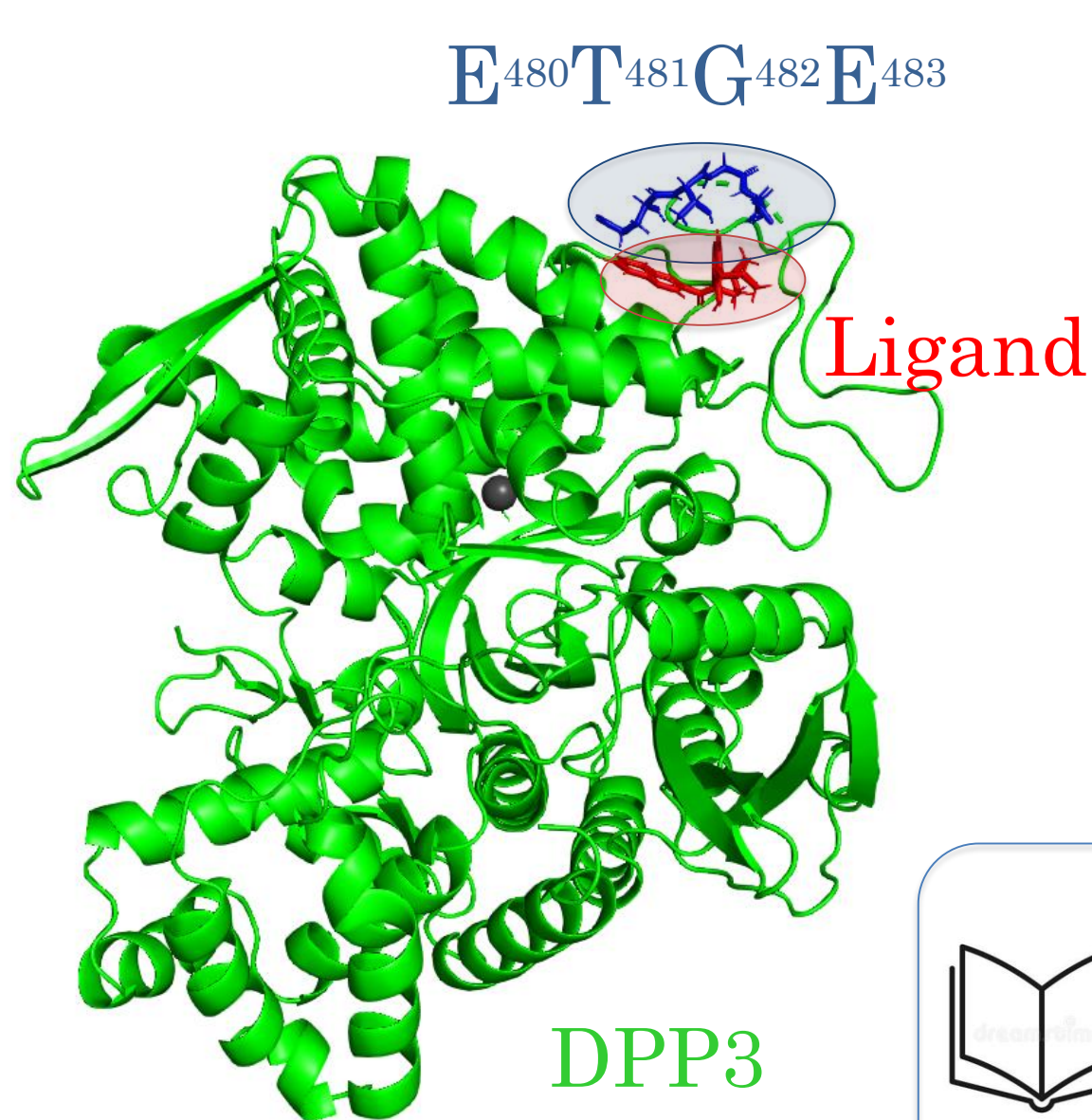
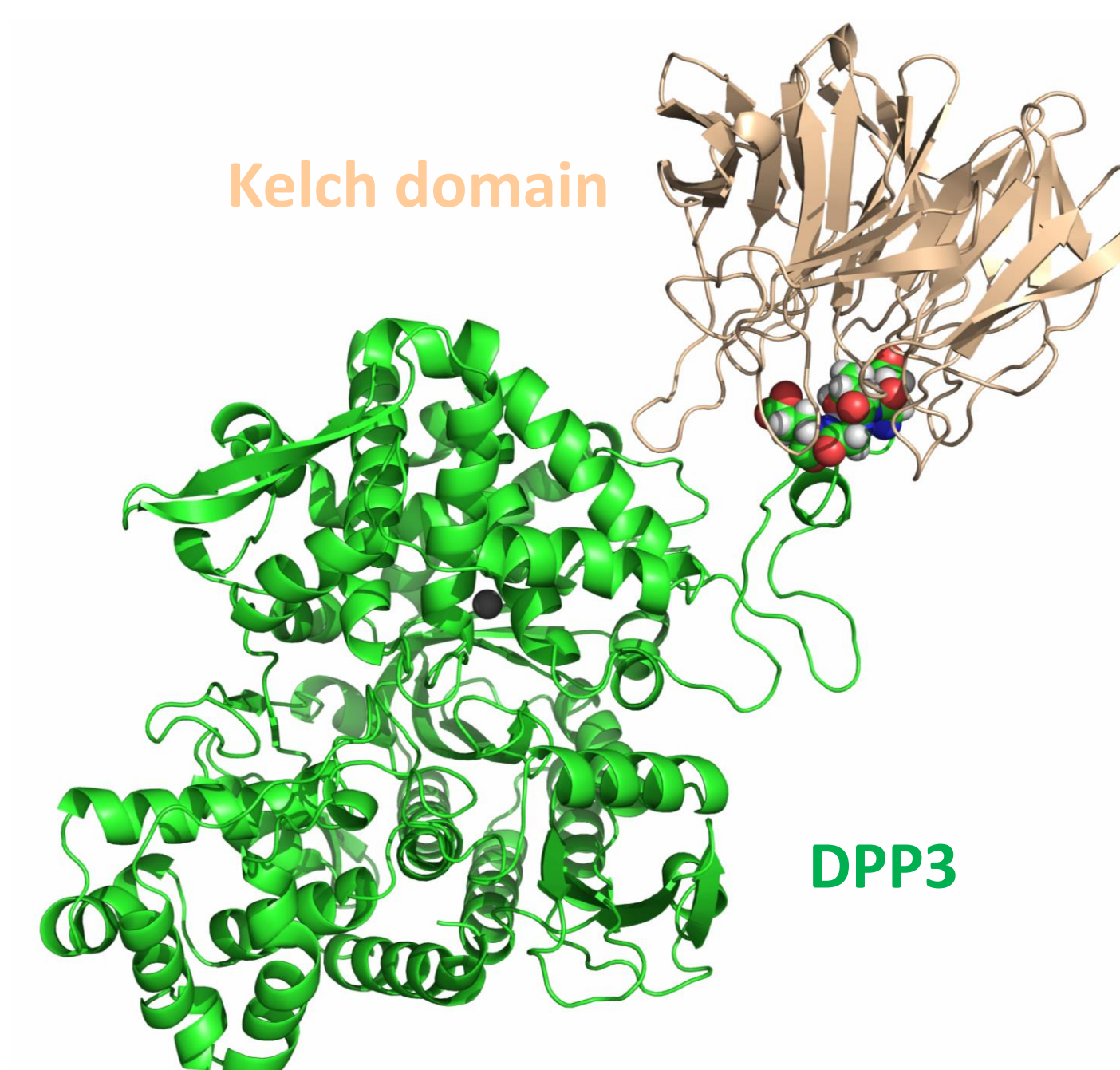
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Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent exopeptidase that, beyond its catalytic function, participates in the regulation of the Keap1–Nrf2 signalling pathway. By binding to the Kelch domain of Keap1 through its ETGE motif, DPP3 disrupts Keap1-mediated Nrf2 degradation, thereby promoting the expression of antioxidant and cytoprotective genes. While transient Nrf2 activation is beneficial for cellular protection, sustained activation has been associated with tumour progression and therapy resistance. Therefore, targeting the DPP3–Keap1 interaction represents a potential strategy for modulating Nrf2 signalling.



Computational Screening of DPP3 Ligands. Selected compounds from the ZINC20, Enamine, and ChEMBL libraries were screened using the Schrödinger Suite, followed by molecular dynamics (MD) simulations to evaluate the stability of the predicted DPP3–ligand complexes. Binding free energies were subsequently estimated using the MM/PBSA approach to prioritize the most promising candidates. 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.

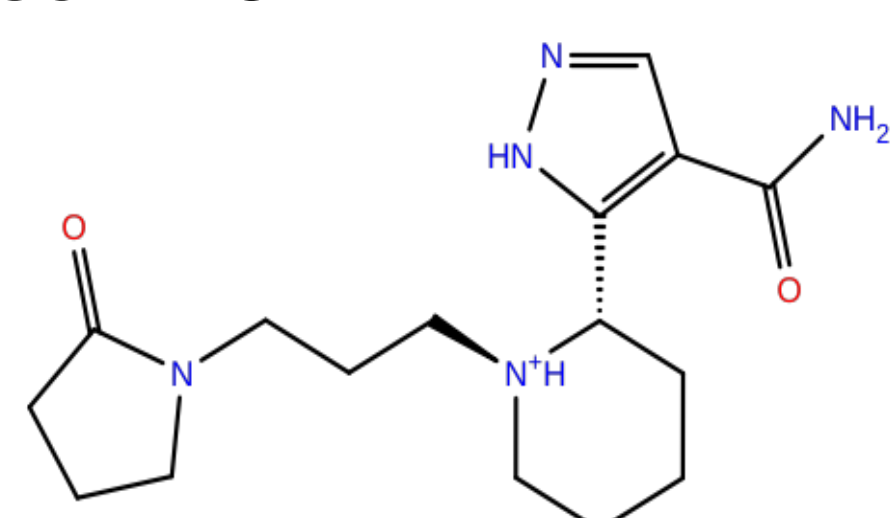


Ligands that maintained stable interactions with the ETGE loop and the rest of the DPP3 structure during MD simulations:

Library	Lig. id	t/ns	Ligand structure	Ligand binding at the end of MD simulation	$\Delta H^{MM/PBSA}/\text{kcal mol}^{-1}$
ZINC20	ZINC001506427316	600			-8.7 ± 3.4
	ZINC000004790416	500			-9.9 ± 5.0
	ZINC000079050977	500			-14.6 ± 3.4
	ZINC000253487775	200			-4.9 ± 3.5
	ZINC000000000000	500			-9.9 ± 5.0
ENAMINE	Z1827470139	400			-9.3 ± 2.5
	Z112760350	600			-8.5 ± 4.7
	Z2099623883	200			-8.7 ± 3.2
	Z1759851743	200			-12.7 ± 4.1
ChEMBL	CHEMBL595084	500			-8.0 ± 3.5

Ongoing experimental validation of candidate ligands. 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.

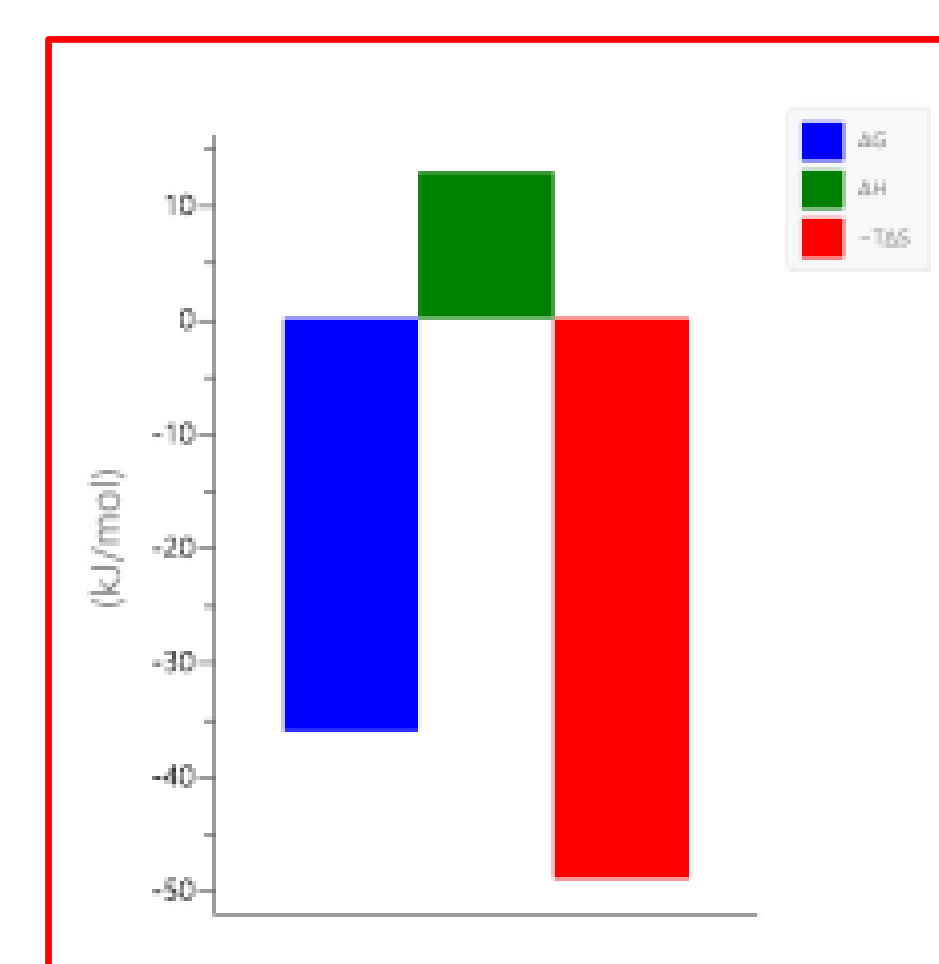
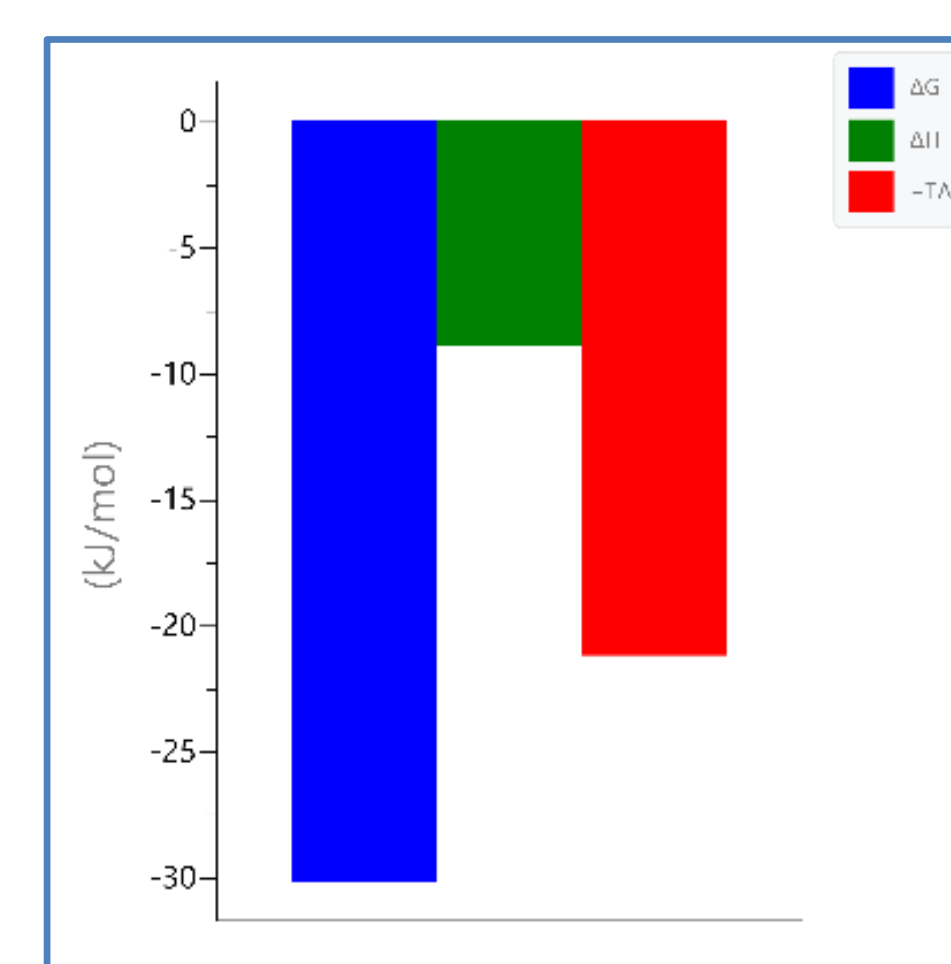
Ligand Z1759851743



$\Delta H^{MM/PBSA}/\text{kcal mol}^{-1} = -12.7 \pm 4.1$

TITRAND	TITRANT	$K_d/\mu\text{M}$	$\Delta_r G/\text{kJ mol}^{-1}$	$\Delta_r H/\text{kJ mol}^{-1}$	$-\Delta_r S/\text{kJ mol}^{-1}$
DPP3	Kelch	4.52 ± 1.83	-30.7 ± 1.1	-8.9 ± 1.0	-21.9 ± 2.2
DPP3	ligand	14.3 ± 6.7	-27.8 ± 1.3	-0.62 ± 0.25	-27.2 ± 1.4
DPP III Δ ETGE	ligand	43.5 ± 25.1	-25.2 ± 1.5	-1.81 ± 0.28	-23.4 ± 1.8
DPP III Δ ETGE-IVYPW	ligand	0.821 ± 0.03	-34.8 ± 0.2	87.4 ± 4.5	-122 ± 4.49
DPP3-ligand	Kelch	0.33 ± 0.22	-37.4 ± 1.8	11.7 ± 1.8	-49.0 ± 0.0

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



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