Regulating the Antioxidative Response by Designing Kelch Domain Mutants to Inhibit DPP III Activity in the Keap1–Nrf2 Signaling Pathway

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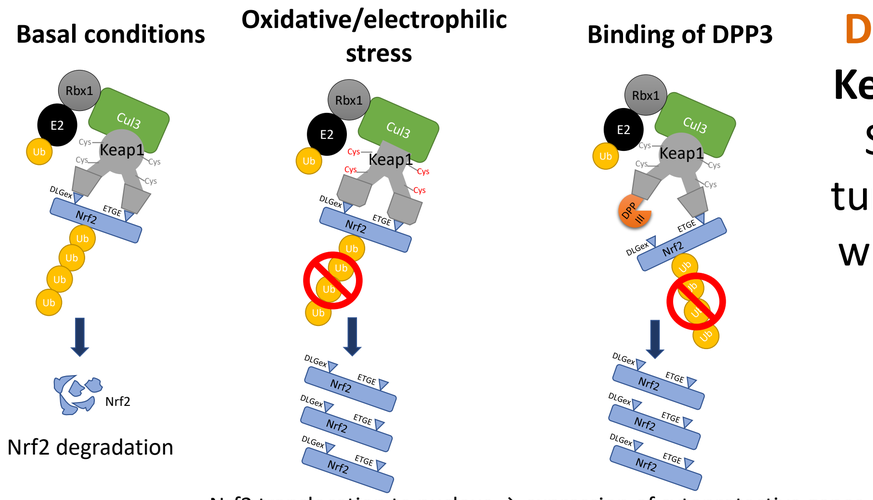
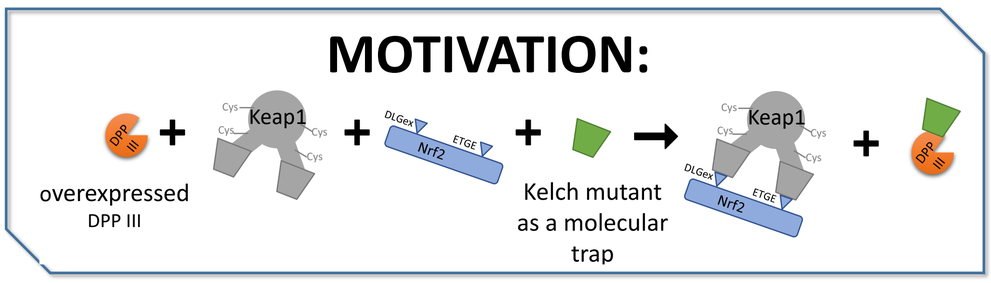
CASE STUDY

The Keap1 – Nrf2 signalling pathway is the main regulator of the oxidative and electrophilic stress response in the cell



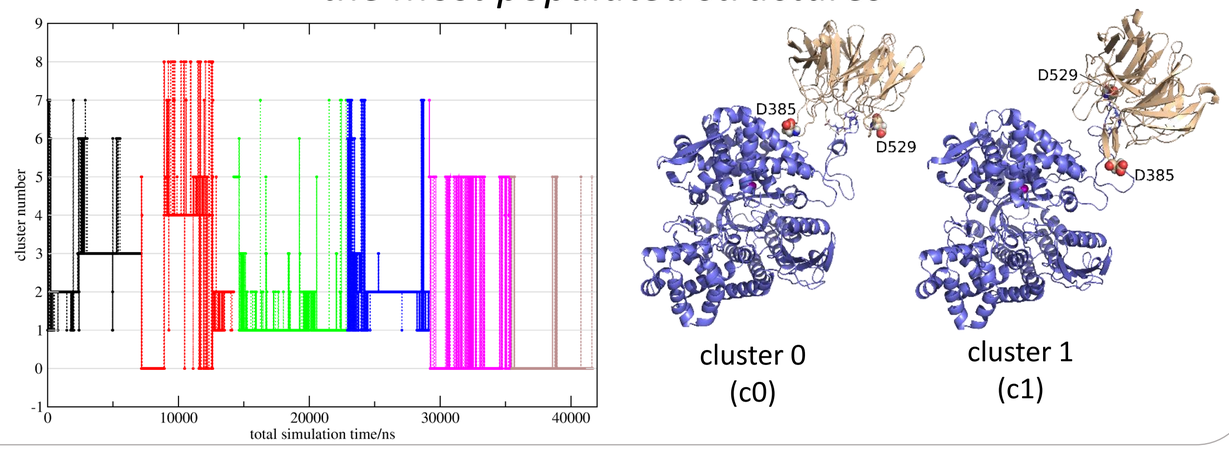
Dipeptidyl peptidase III (DPP III) competes with Nrf2 for Keap1 binding, promoting Nrf2-dependent transcription. Studies have linked elevated DPP III gene expression to tumorigenesis, particularly in breast and colorectal cancer, where overexpression of DPP III enhances the antioxidant response, supporting cancer cell growth through competitive binding to Keap1.



Complex determination I – X-ray structures & MD simulations

- Starting structures: DPP III (pdb: 5EGY) + Kelch domain of Keap1 (pdb: 2FLU)
- In three independent steered MD simulations (A, B, and C), the ETGE motif of DPP III — responsible for binding to the Kelch domain—was detached from the protein surface.
- The complexes were prepared by aligning the ETGE-containing peptide from Nrf2 bound to the Kelch domain with the ETGE motif of DPP III
- A total of 16 μs of MD simulations (*NpT*, 300K, ff18SB, OPC) in AMBER22:
  2,5 μs[A], 2,5 μs[A], 3,5 μs[B], 2,5 μs[B], 2,5 μs[C], 2,5 μs[C]

Cluster analysis of the MD trajectories was performed to identify the most populated structures



PEAQ-ITC results for Kelch (WT and mutants) binding with DPP III at 25 °C, pH = 7.5 and 20 mM Tris-HCl buffer.

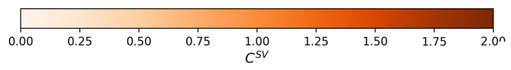
TITRAND	TITRANT	*N**	K_d/μM	$\Delta_r G$/kJ mol^{-1}	$\Delta_r H$/kJ mol^{-1}	$-T\Delta_r S$/kJ mol^{-1}
DPP III	WT Kelch	1	4.52 ± 1.83	−30.7 ± 1.1	−8.9 ± 1.0	−21.9 ± 2.2
DPP III	D385L Kelch	1.26 ± 0.08	3.55 ± 0.44	−31.2 ± 0.4	−10.9 ± 1.1	−20.3 ± 1.5
DPP III	D529L Kelch	1	2.54 ± 0.63	−32.0 ± 0.6	−19.0 ± 1.4	−13.1 ± 2.1

A per-residue MM-GBSA energy decomposition analysis helped to determine amino acids that make unfavorable energetic contributions to the binding interaction

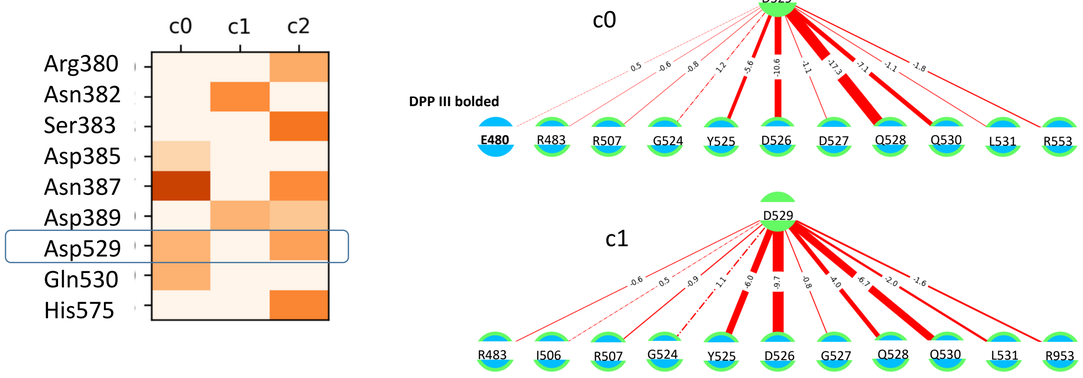
cluster 0		cluster 1	
residue	<Δ*H* ± SD> / kcal mol^{-3}	residue	<Δ*H* ± SD> / kcal mol^{-3}
ASN 387	0.38 ± 1.23	ASP 385	0.33 ± 1.12
PHE 335	0.39 ± 0.27	PHE 335	0.44 ± 0.19
ASP 529	0.41 ± 0.24	ASP 529	0.33 ± 0.18
ARG 362	0.43 ± 0.22	ARG 554	0.36 ± 0.08
ASN 414	0.43 ± 0.20	ASN 414	0.33 ± 0.18
ASN 381	0.51 ± 0.25		

Singular value centrality (CSV) calculations were used for ranking the residue importance, combined with fragment molecular orbital calculations to obtain the PIEs (pair interaction energies; DFTB/PA/3ob) between residues in the protein–protein interaction interface

C^{SV} from repulsive PIEs (E_{lim} = +0.5 kcal/mol) for residues in Kelch



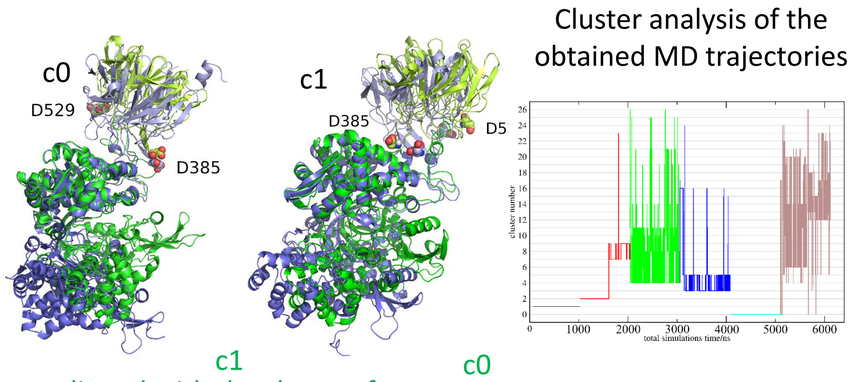
Interaction partners of D529 (Kelch) in c0 and c1 with E_{lim} = +/– 0.5 kcal/mol



Complex determination II – AlphaFold3 & MD simulations

Six structures predicted by AF3 were subjected to MD simulations

Detachment of the ETGE loop from DPP III in the complex with Kelch was achieved by applying restrictive settings in AF3: limiting the template search space, reducing the depth of paired and unpaired MSAs, and specifying the ETGE-loop residues as ambiguous.



Cluster analysis of the obtained MD trajectories

Complex determination III – X-ray crystallography

Determination of the DPP III-E451A-IVYPW – Kelch complex structure using X-ray crystallography

WORK IN PROGRESS