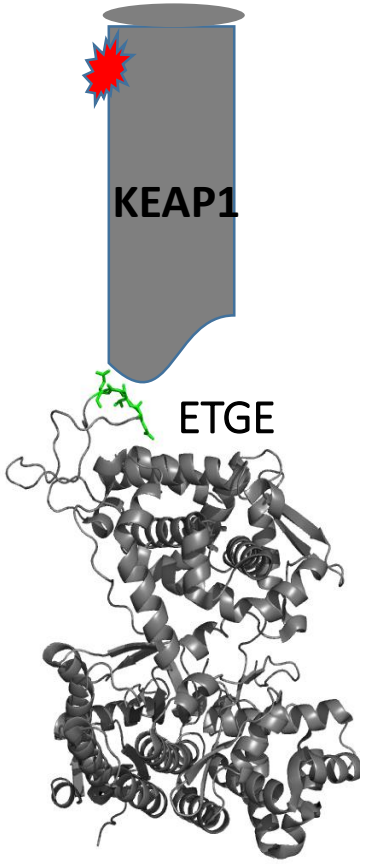
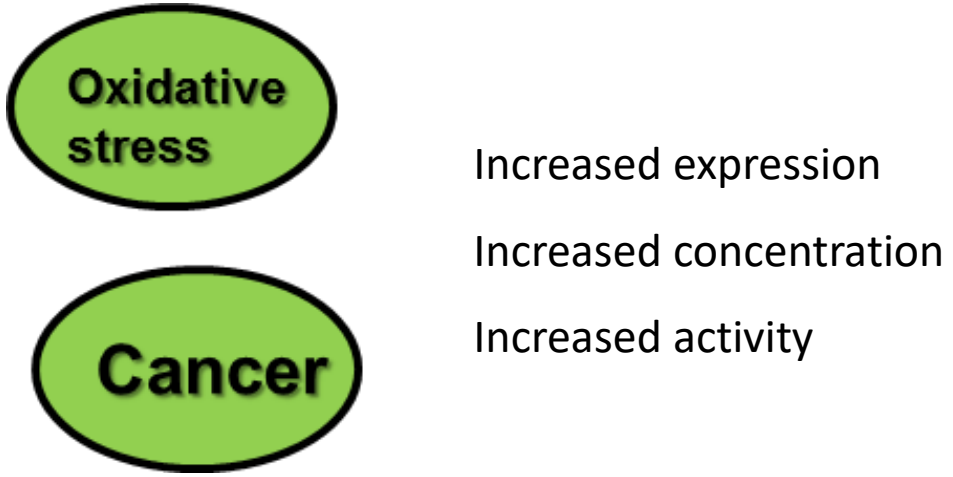
Influence of the Cancer Mutations of DPP III on Its Interactions with KEAP1

Dipeptidyl Peptidase III possible target for improving efficiency of chemotherapy





Increased expression

Increased concentration

Increased activity

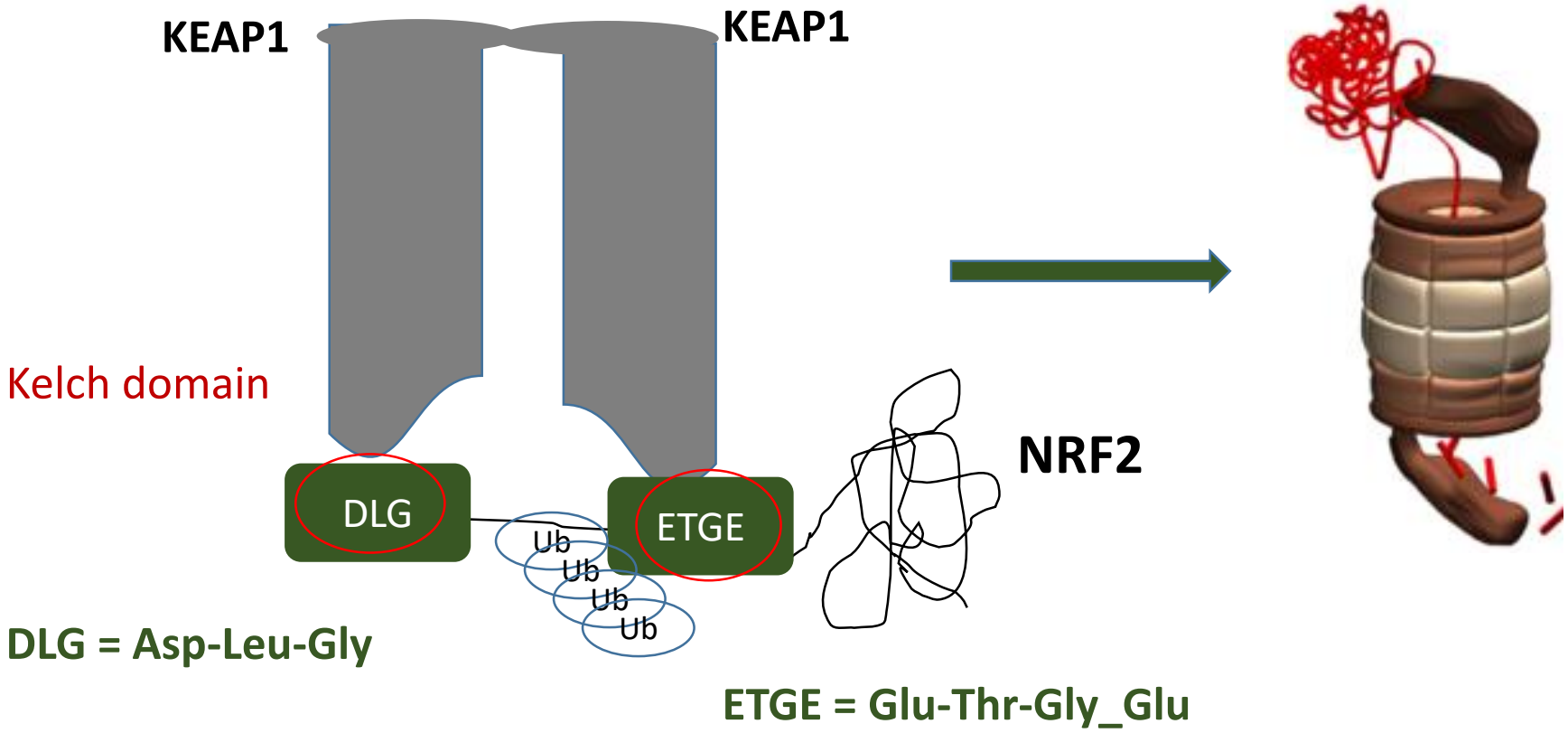
Šimaga et al. (*Eur. J. Cancer* **1998**, *34*, 399) detected increased levels and activity of DPP III in malignant endometrial tissue
Šimaga et al. (*Gynecol. Oncol.* **2003**, *91*, 194) found that expression of DPP III has been positively correlated with ovarian cancer aggressiveness

Gamrekelashvil et al. (*Cell. Mol. Life Sci.* **2015**, *72*, 273) showed that DPP III is epigenetically induced in liver cancer cells by promoter hypomethylation, while DPP III and thimet oligopeptidase-1 (TOP-1) decrease the immunogenicity of necrotic tumor cells by blocking antigen cross-presentation

Miettinen *et al.* (*Cancers (Basel).* **13**, (2021). DOI: 10.3390/cancers13071527) found that **higher expression of DPP III correlates with shorter survival of patients with multiple myeloma**, and the increased level of DPP III in patients with relapsed multiple myeloma compared to newly diagnosed patients suggests that it may be involved in cancer

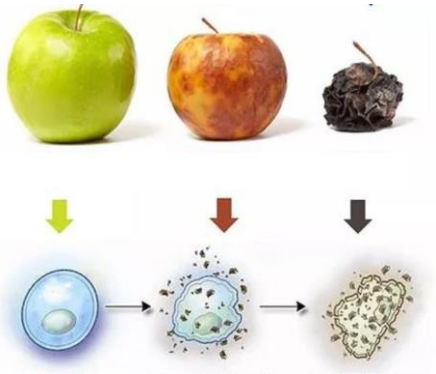
KEAP1-NRF2 – pathway in regular conditions

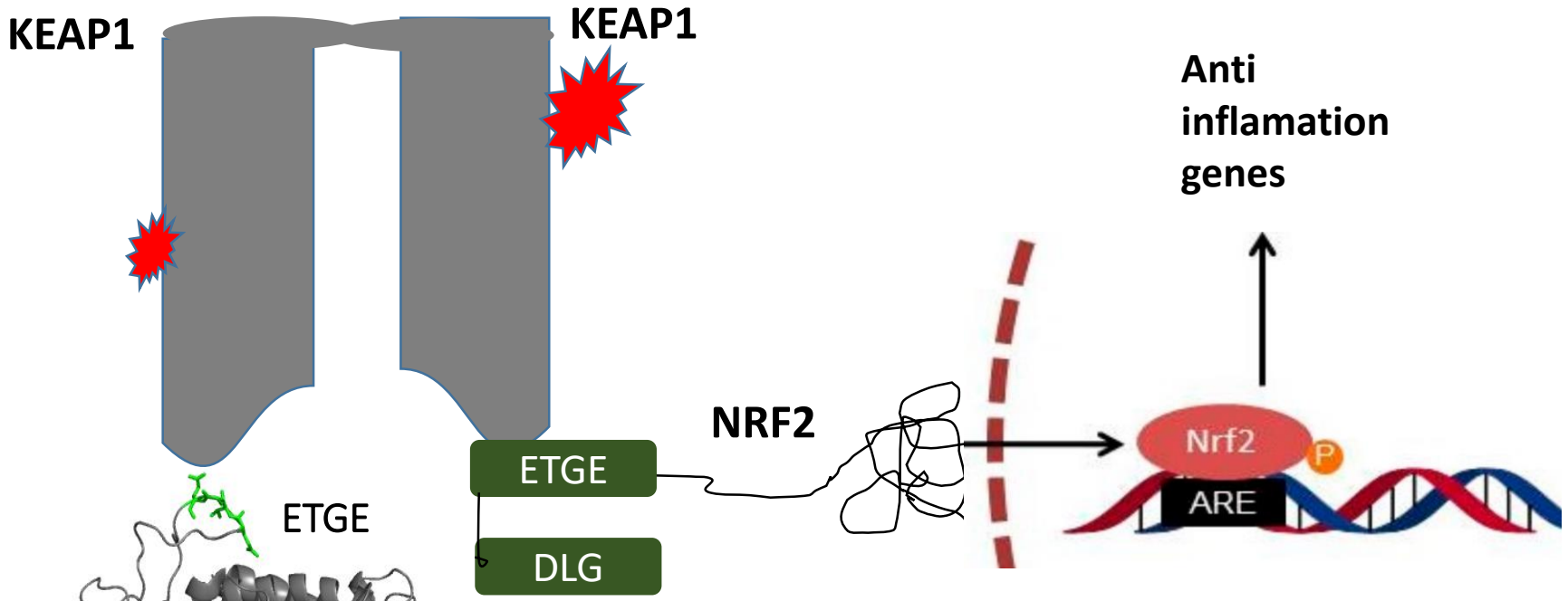
Kelch-like ECH-associated protein 1 (KEAP1) – NRF2 (Nuclear factor [erythroid-derived 2]-like 2 protein)

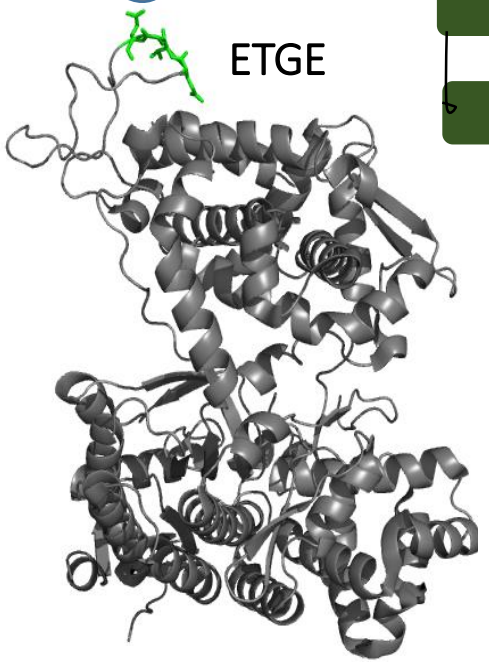


KEAP1 mediated degradation of NRF2 *via* the ubiquitin-proteosome pathway

KEAP1-NRF2 – pathway under the oxidative stress



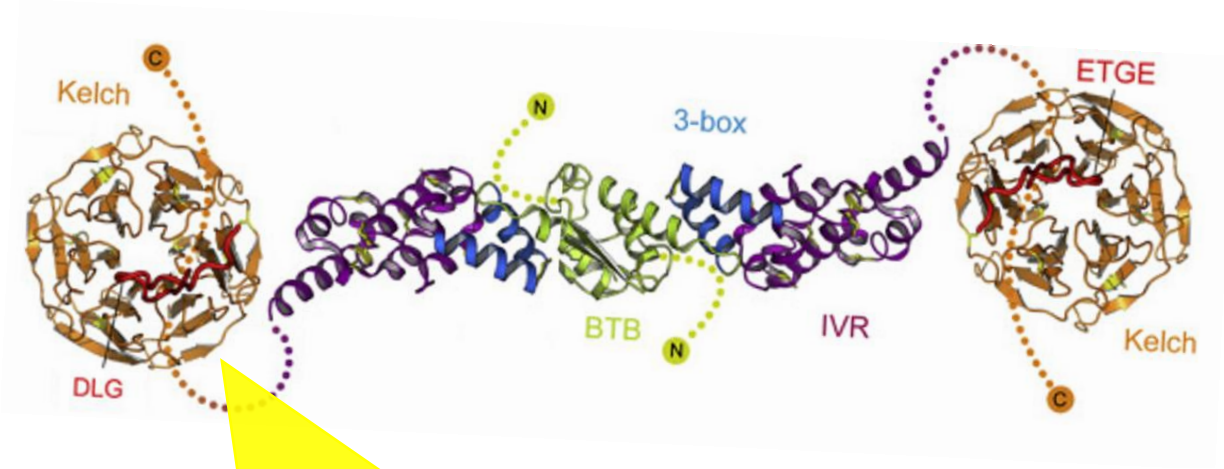


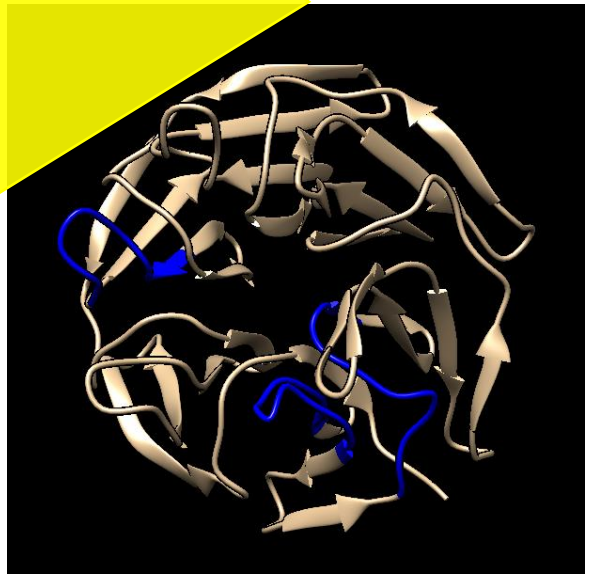


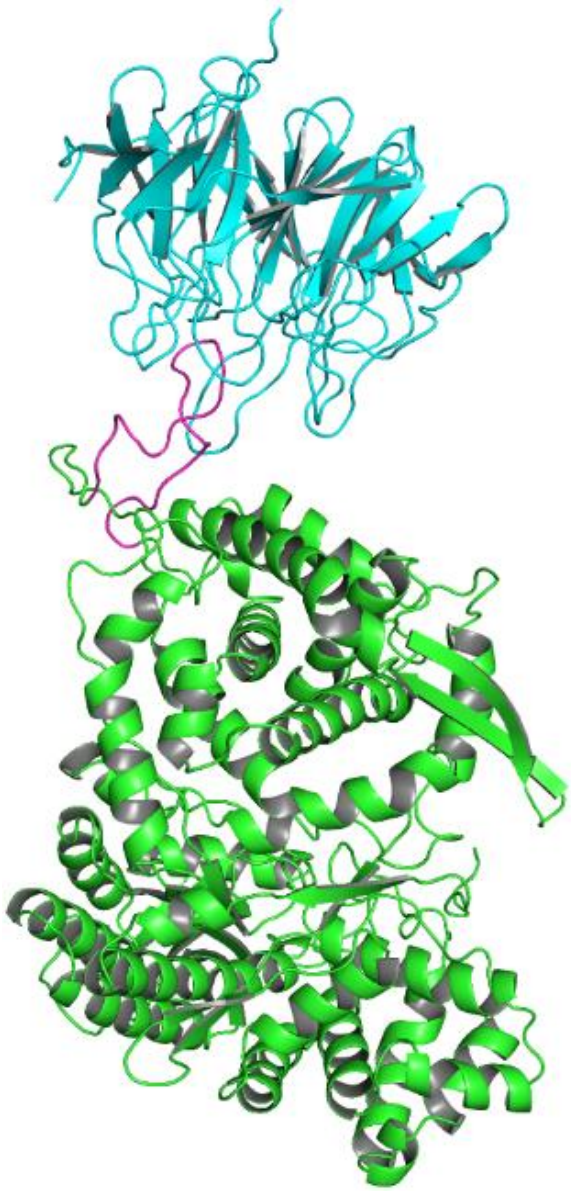
Hast et al. *(Cancer Res.* ***2013****;73(7), 2199)* determined DPP III as a KEAP1 interactor and showed that the ETGE motif is crucial for the formation of the DPP III–KEAP1 complex

Lu et al. (*Cancer Res.* **2017**, *77*, 2881) found correlation between DPP III overexpression and NRF2 activation in the breast cancer, and high DPP III expression was in the strong correlation with the progression of the disease, especially in the estrogen-positive breast cancer

KEAP1-DPP III interactions

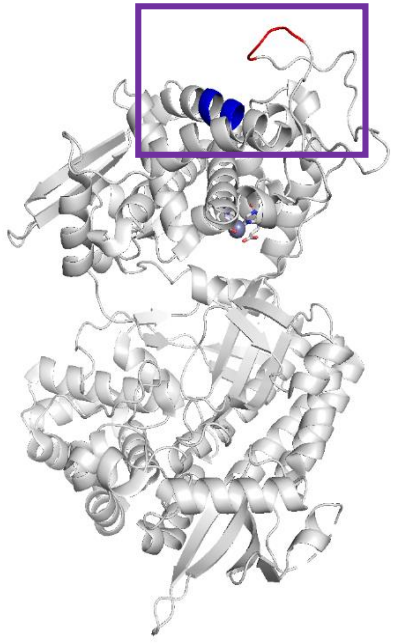


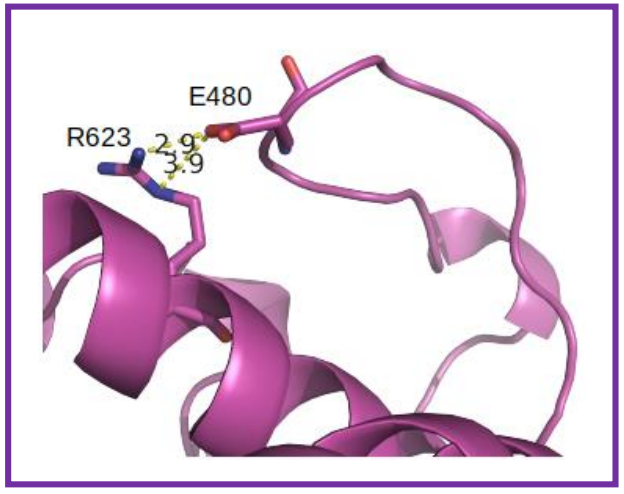


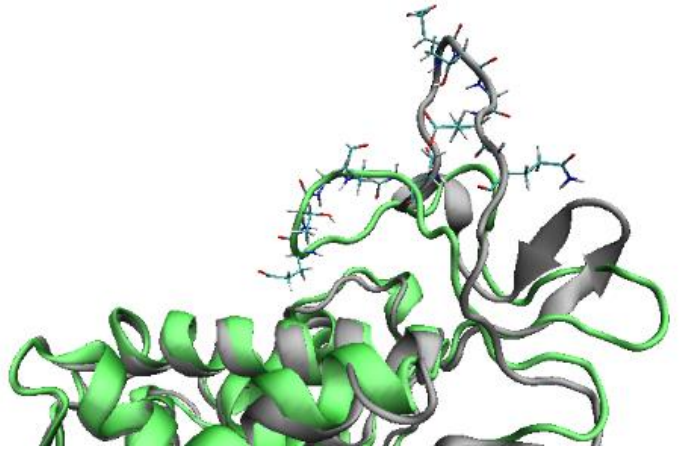


Dipeptidyl peptidase III – Kelch domain

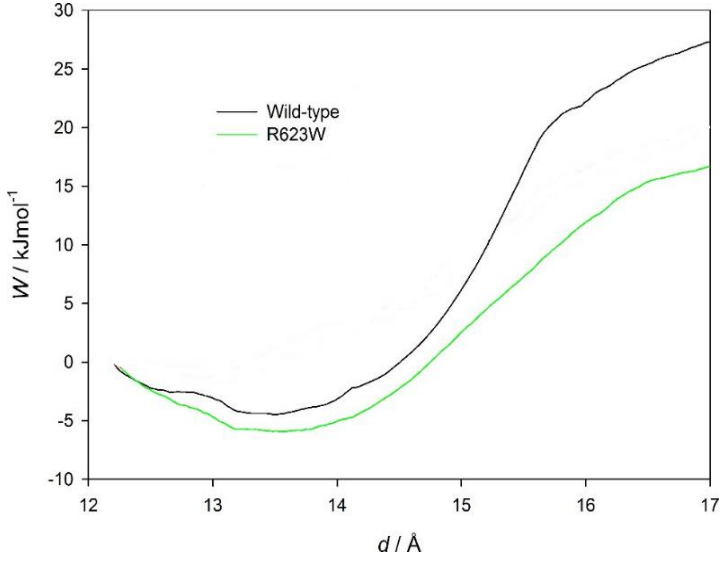
^{480}ETGE^{483} - R620, R623, R624 interactions





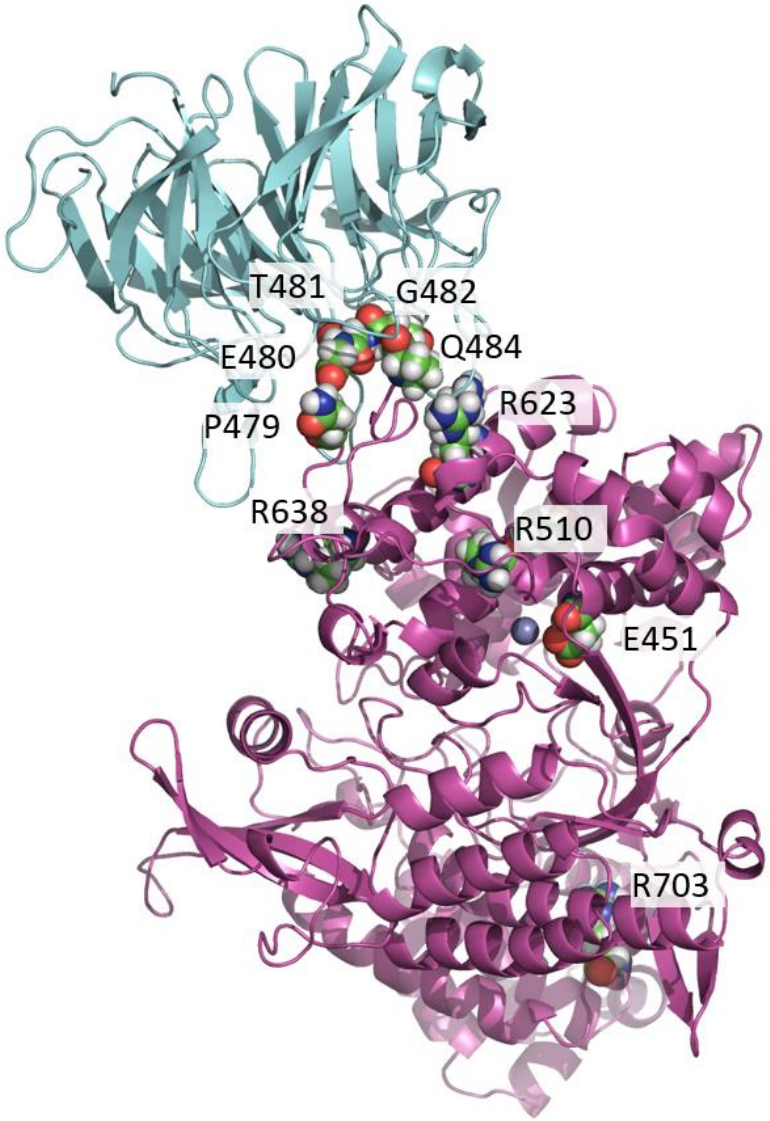


The ETGE motif detachment is an endergonic process



J. Biomol. Struct. Dyn. **2021**, *39*, 6870–6881

DPP III cancer mutations: impact on the affinity for the Kelch domain



MST measurements. Binding affinity of DPP III mutants for the Kelch domain compared to the affinity of the wild-type protein, expressed as the ratio K_d(WT)/K_d(mutant).

DPP III	K_d(WT)/K_d (M)
WT	1.0
P479S	**18.4**
E480Q	**0.1**
T481M	**0.1**
G482C	0.8
Q484H	2.1
R510W	**0.3**
R623W	**160.0**
R638L	**2.0**
R638W	**2.0**
R703C	1.7

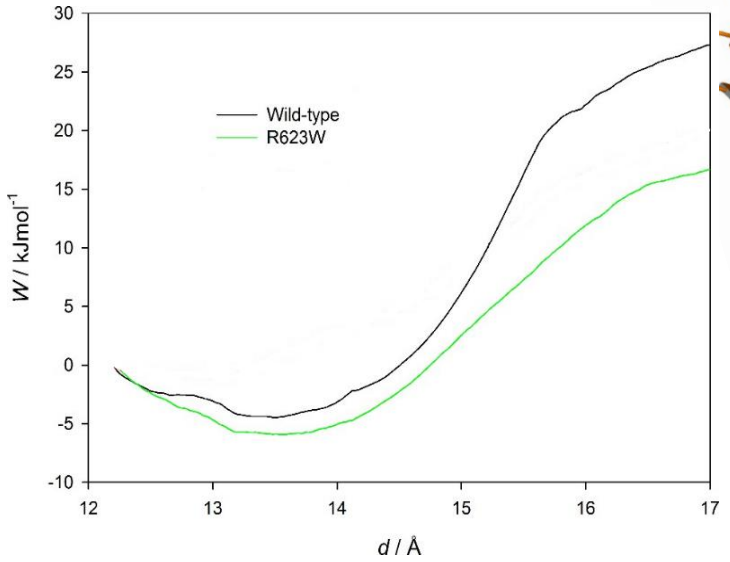
K_d of R623 ~ 5×10^{-9} mol dm^{-3}
K_d of WT DPP III ~ 8×10^{-7} mol dm^{-3}

Such a significant increase in affinity for the variant is consistent with our proposed mechanism of binding of DPP III to KEAP1.

Int. J. Mol. Sci. **2022**, 23(4), doi:10.3390/ijms23041994

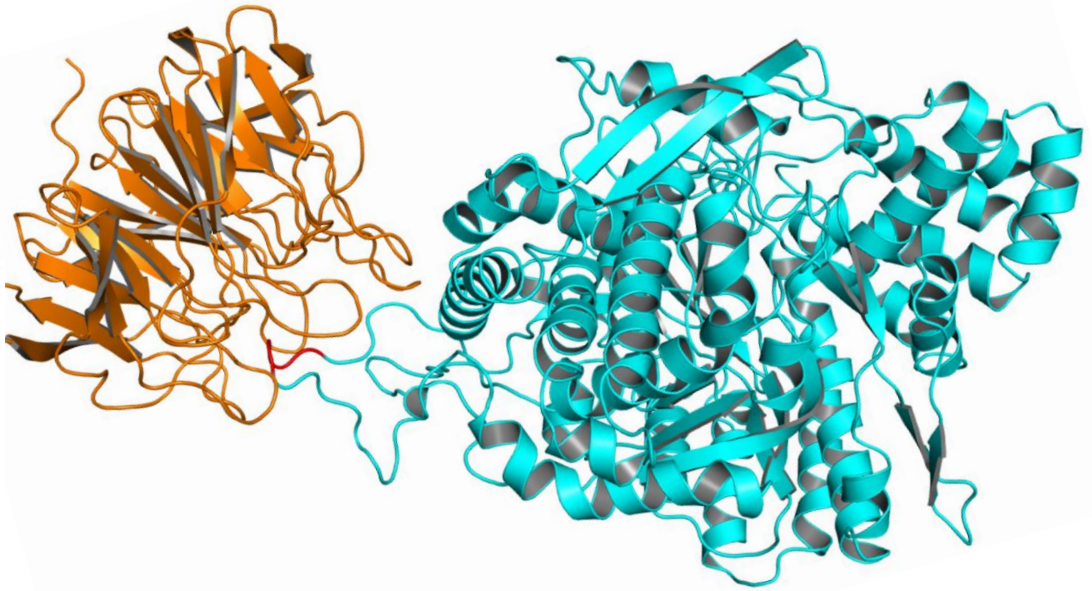
MM/GBSA energies calculated during 700 ns of MD simulations

DPP III	MM/GBSA (kcal/mol)
WT	**-80**
P479S	**-116**
E480Q	**-42**
T481M	**-63**
G482C	**-56**
R510W	**-69**
R623W	**-65***



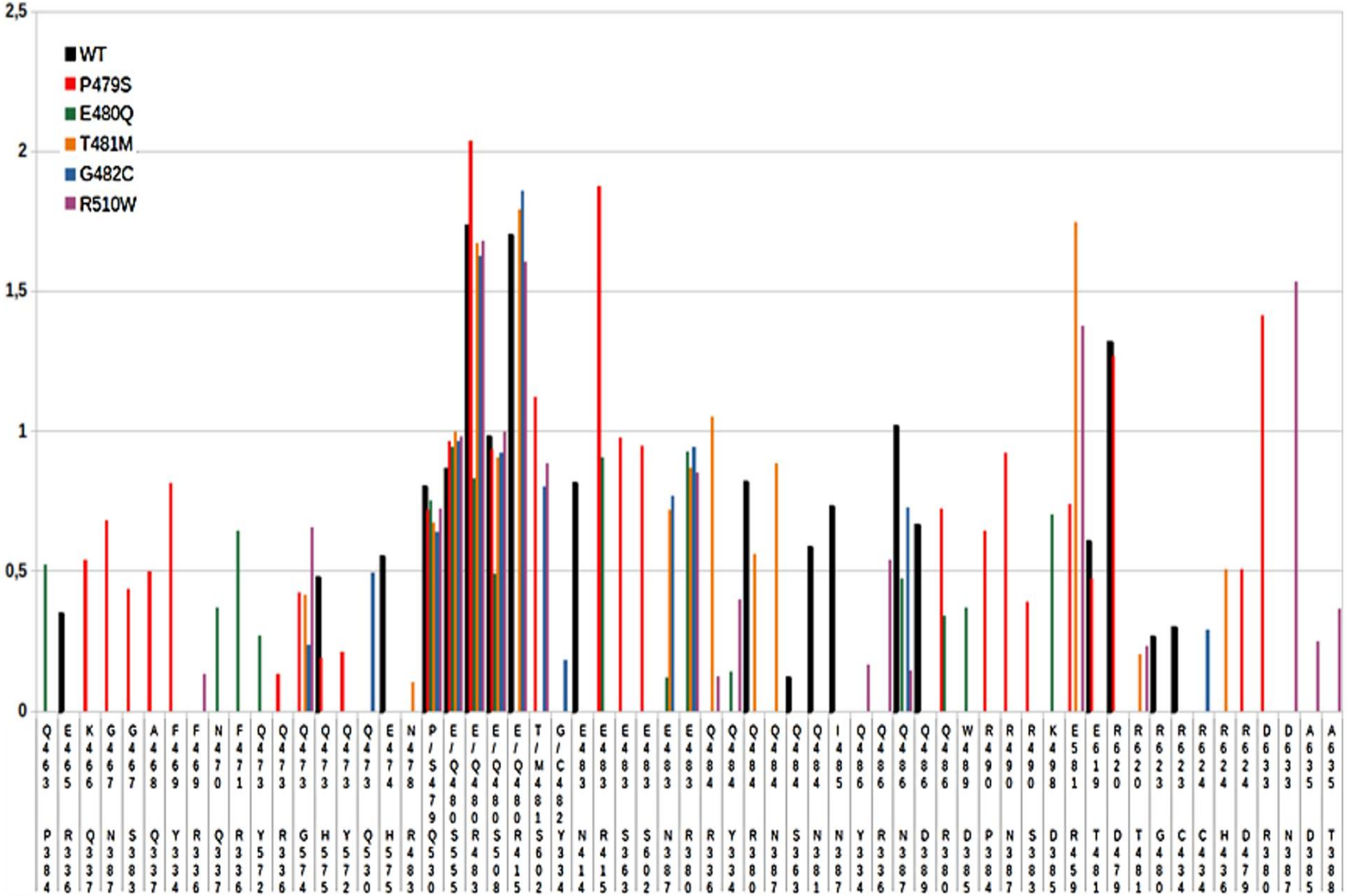
*The signifficantly lower binding affinity measured by MST is due to the easier release of the flexible loop from the protein body, the binding affinity increased.

The lowest-energy structure of the DPP III (cyan) – Kelch (orange) complex with the ETGE motif in red.



Binding of DPP III to KEAP1 is a two-step process involving endergonic translocation of the loop, followed by exergonic interactions between DPP III and the Kelch domain.

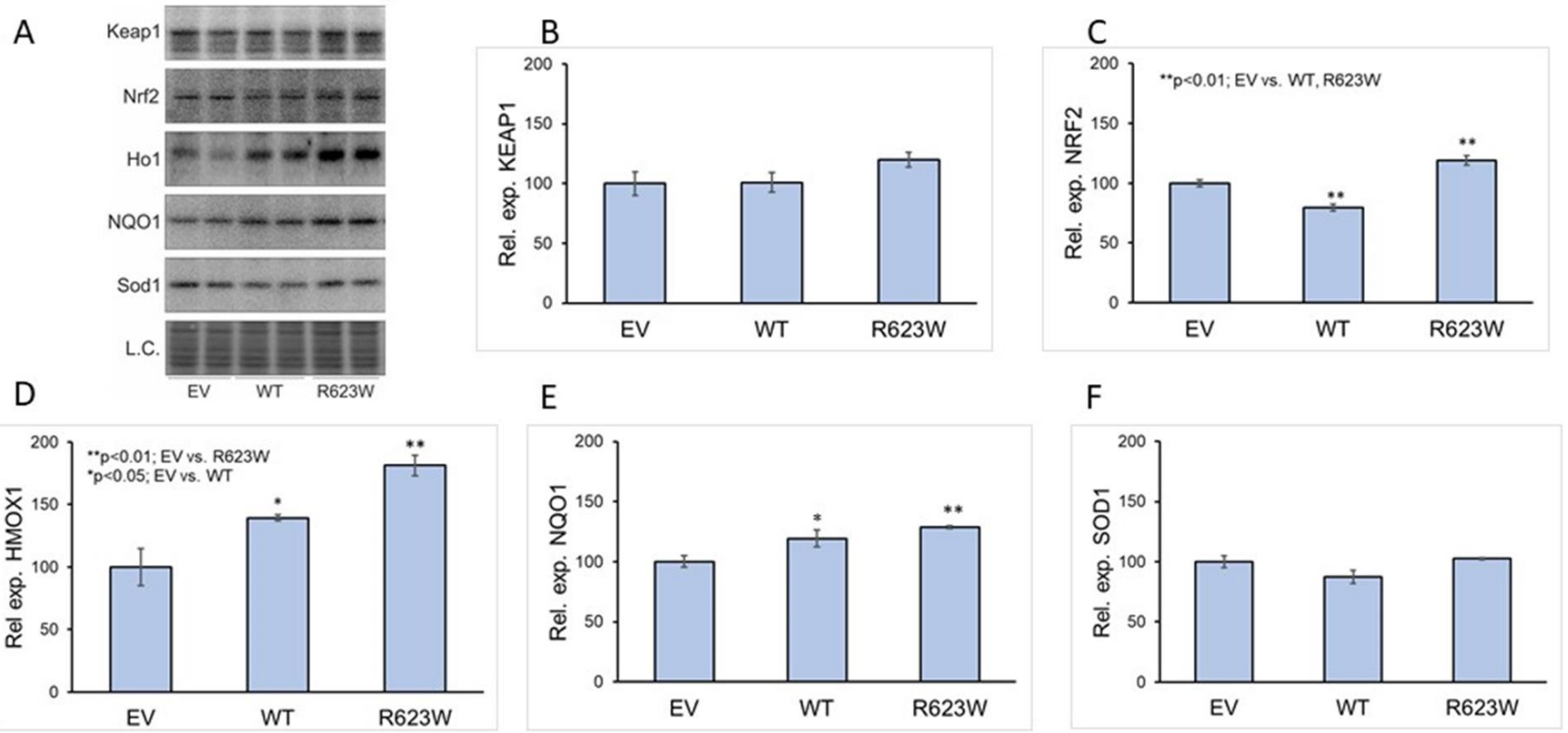
Effect of DPP III mutations on the Kelch – DPP III structure

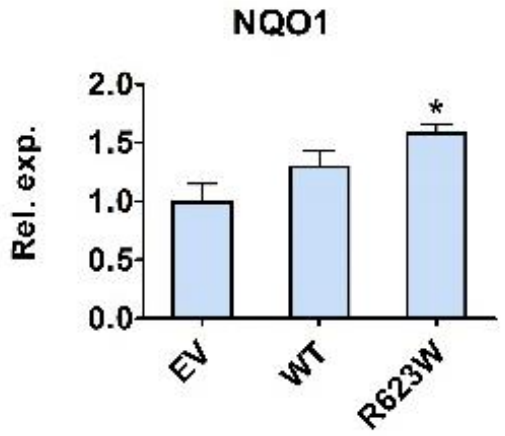


Distribution of the intermolecular hydrogen bonds determined in the 20 ns long interval with the lowest MM/GBSA energy.

Effect of DPP III mutations on expression of the NRF2 controlled genes

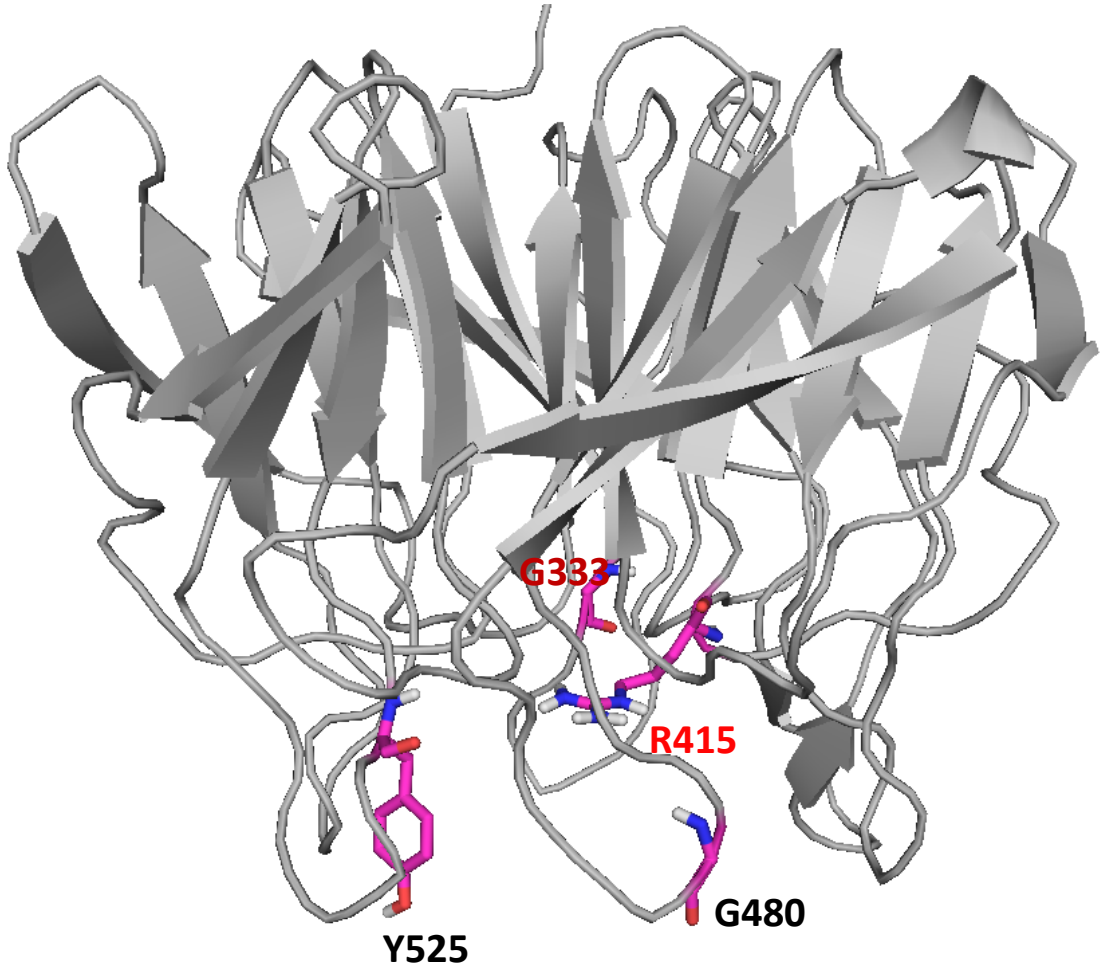
Western blot analysis of the relative expression of KEAP1, NRF2, HMOX1 (Ho1), NQO1 and SOD1 in the cells transfected with EV, WT and R623W, respectively. R623W was shown to increase the expression of some NRF2-controlled genes.

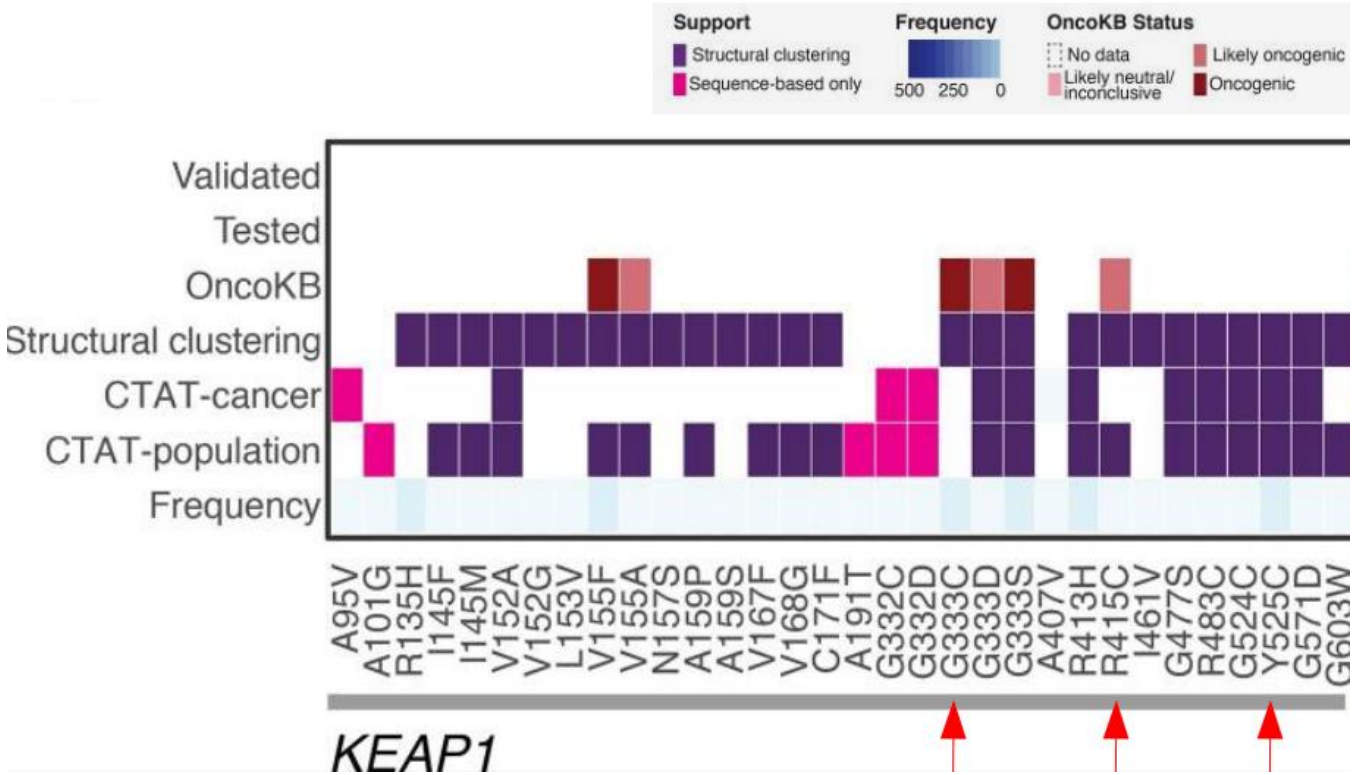




Relative expression of NQO1 mRNA in HEK293T cells

Influence of the Cancer Mutations of the KEAP1 protein on Its Interactions with DPP III





Influence of the Cancer Mutations of the KEAP1 protein on Its Interactions with DPP III

Interaction with the representative DPP III peptide (24 AA long)

Kelch	ITC K_d(M)	MMGBSA (kcal/mol)^{min}
DT	$(3 \pm 2)10^{-8}$	-19
G333C	NB	-1
G480W	$(2 \pm 1)10^{-7}$	-13
R415C	$(3 \pm \mathbf{3})10^{-6}$	21
Y525C	$(1.2 \pm 0.3)10^{-6}$	-20

Interaction with DPP III

Kelch	MST K_d(M)	MMGBSA (kcal/mol)^{min}
DT	$(5 \pm 4)10^{-7}$	-80
G333C	$(1 \pm 25)10^{-3}$	-51
G480W	$(5 \pm 2)10^{-6}$	-65
R415C	$(4 \pm \mathbf{5})10^{-8}$	-51
Y525C	$(4 \pm 2)10^{-6}$	-59

CONCLUSIONS

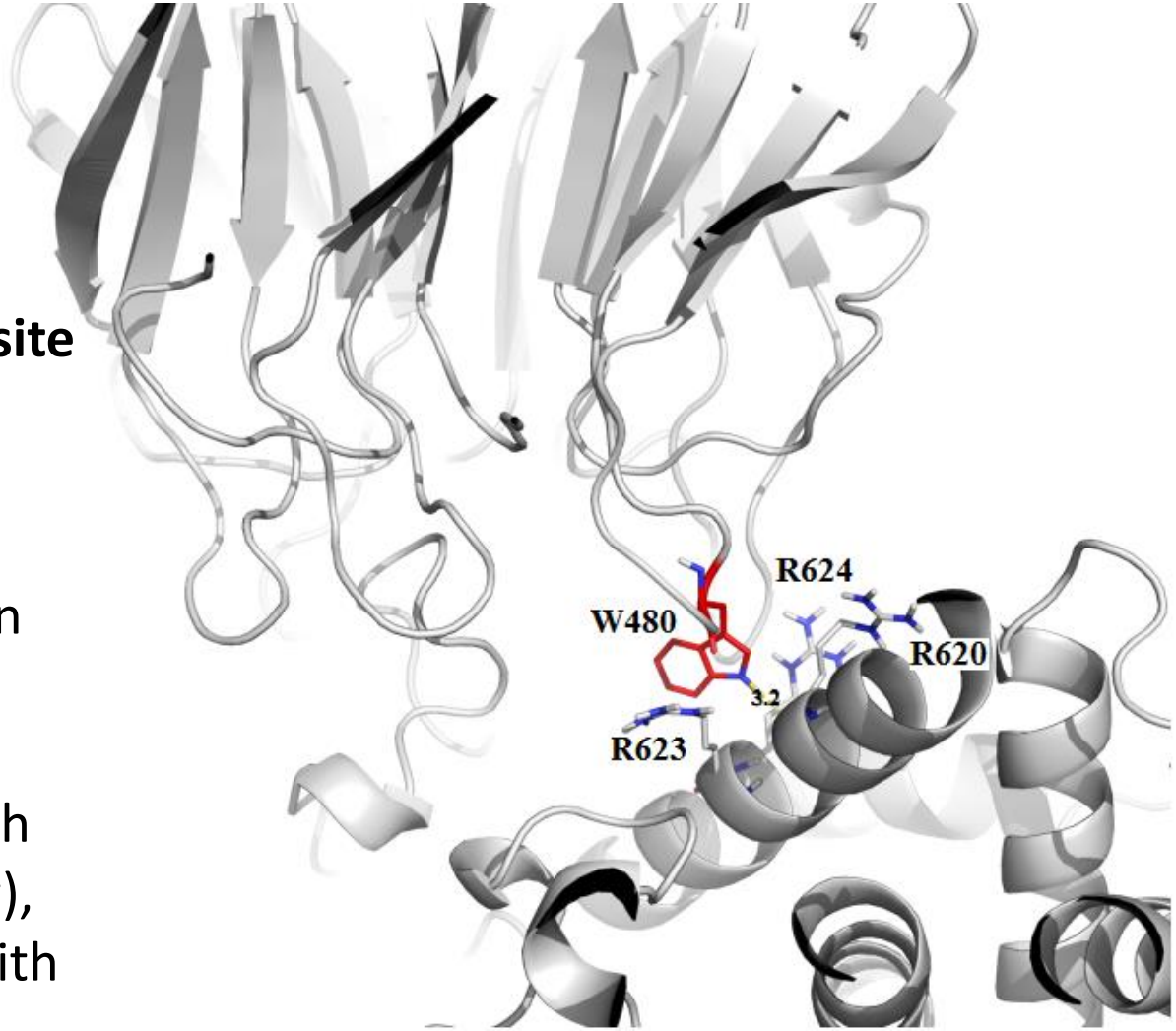
ITC measurements of Kelch-peptide interactions are in good agreement with computational data

G333C likely destabilises the structure of the binding site

R415 is important for binding of the ETGE motif

The impact of the Y525C mutation is better predicted in simulations with DPP III than with peptide

The G480W mutation decreases the affinity of the Kelch domain for the ETGE loop (destabilises peptide binding), but W480 interacts favourably with R620 in complex with DPP III.



Summary

- The influence of different mutations of DPP III, present in human cancer, on the affinity of DPP III for Kelch was investigated, and it was found that the R623W and P479S mutations significantly improved this affinity. The R623W mutation was found to increase the expression of some NRF2 regulating genes in the cell.

Acknowledgement

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Thank you!

Questions?

Sugestions?