DPP III mutations affect its binding to KEAP1

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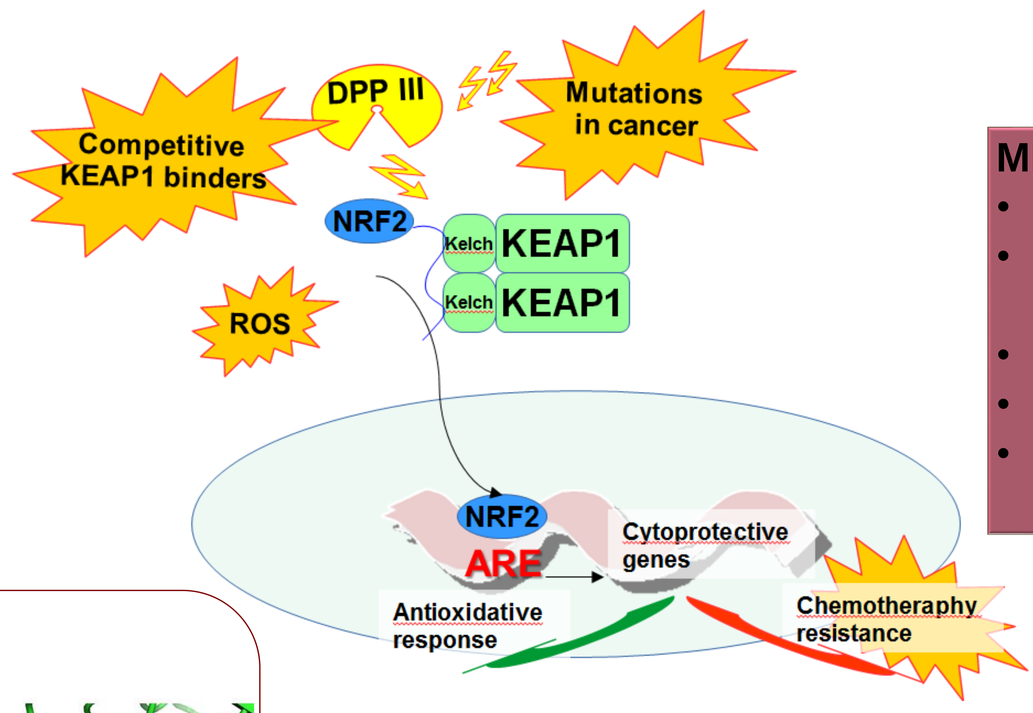
Hrvatska zaklada za znanost

KEAP1-NRF2 signaling pathway

KEAP1 - sensor of oxidative stress, repressor of transcription factor NRF2

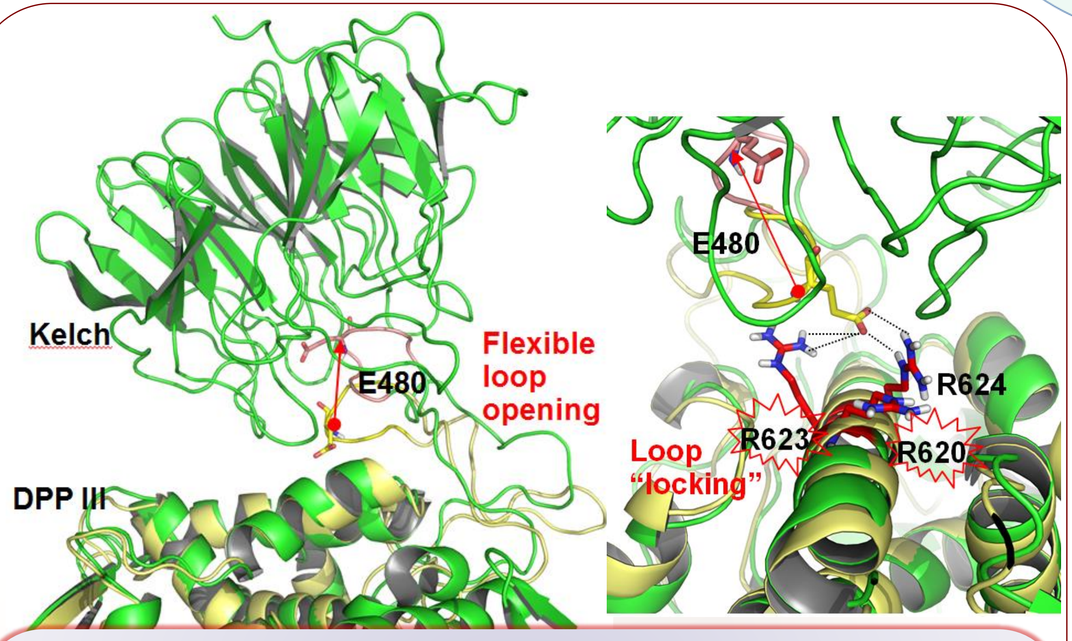
NRF2 - promotes cell survival under oxidative stress conditions

DPP III (dipeptidyl peptidase III) - competitive KEAP1 binder



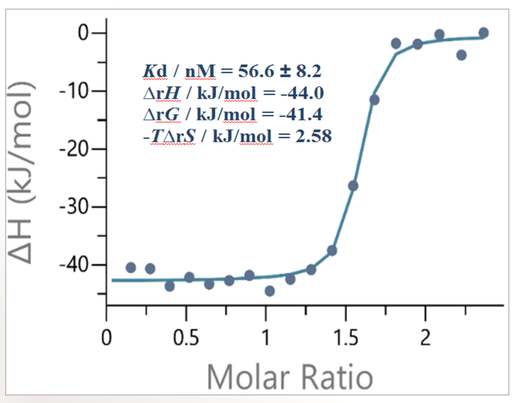
METHODS

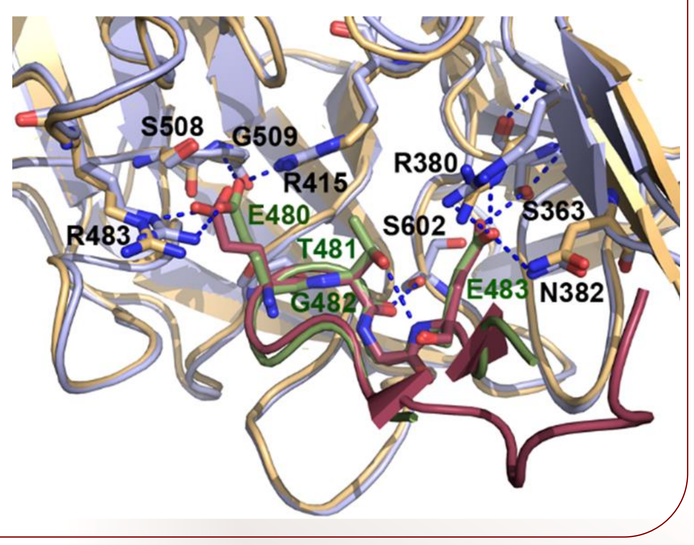
- **molecular modelling**
- **MD and ASMD simulations**
- **protein crystallography**
- **ITC**
- **MST – initial fluorescence change**

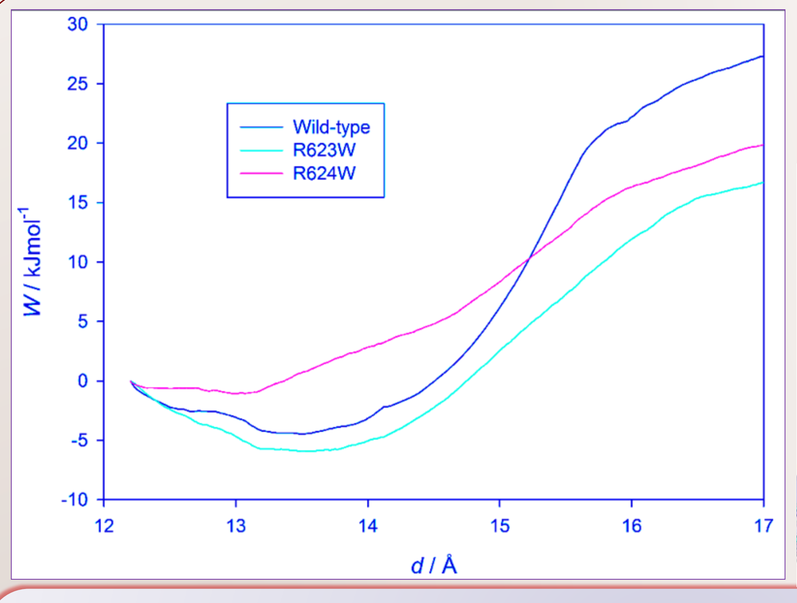


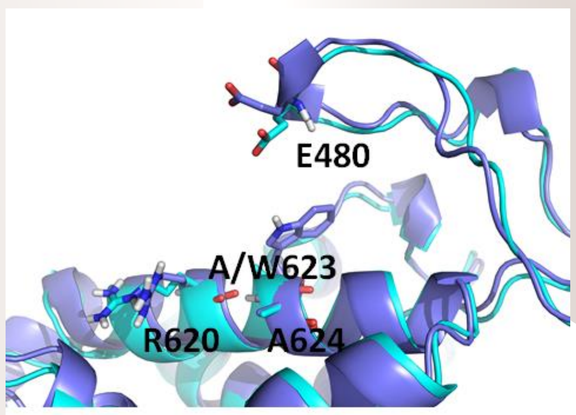
Molecular modelling and MD simulations of DPP III – KEAP1 Kelch domain complex revealed that the release of the ETGE motif, attached to DPP III protein body by H-bonds with arginines, is a requirement for the complex formation.

Binding thermodynamics and crystal structure of the DPP III ETGE peptide – Kelch complex showed similar binding affinity and interactions of NRF2 ETGE peptide to the Kelch domain.









Work required for ETGE motif detachment from the protein body, calculated during AMD simulations, is lower for the R623W and R624W mutants than for the wt DPP III. After double substitutions (R624A, R623A/W) opening is observed during classical MD simulations.

Fluorescence change induced by DPP III binding to Kelch-NT-495 revealed significantly lower Kd for R623W in comparisson to wt and other DPP III mutants selected from cBioPortal.

DPP III	K_d / nM
WT	826 ± 108
R620C	746 ± 194
R623L	394 ± 138
R623W	5 ± 18

CONCLUSIONS

- **residues R623 and R624 are involved in DPP III – KEAP1 binding mechanism acting as a ”lock” for the ETGE motif of DPP III**
- **loop detachment is necessary for DPP III – Kelch binding and likely a rate determining step**
- **mutation R623W, found in cancer, may affect DPP III-KEAP1 binding, and promote oxidative stress and chemotherapy resistance in cancer cells**

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