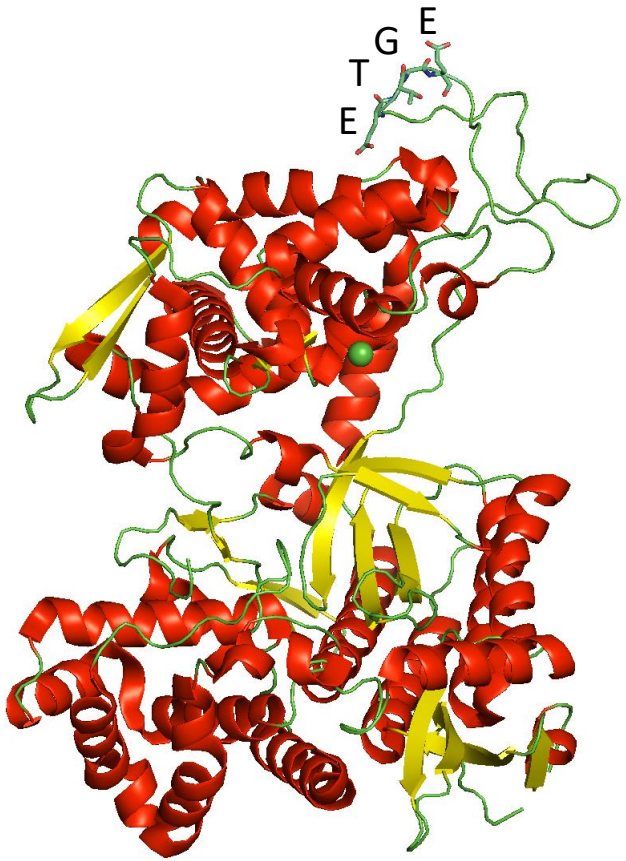
Dipeptidyl peptidase 3, the competitive interactor of KEAP1

Mihaela Matovina
Ruđer Bošković Institute, Zagreb, Croatia

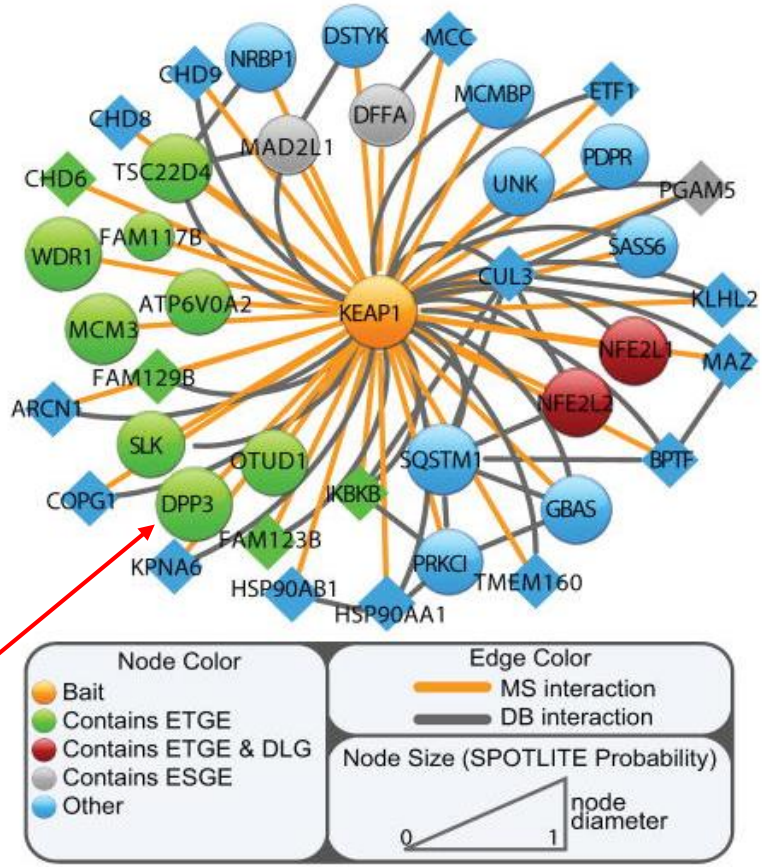
Dipeptidyl peptidase 3

- Zn metallopeptidase
- Several crystal structures – free or in the complex with inhibitors
- Ubiquitously expressed in organisms from bacteria to humans and in almost all human tissues
- Substrates: 3 to 10 amino acids long peptides → protein turnover
- Cleaves bioactive peptides *in vitro*: angiotensins, enkephalines, endorphins → regulation of blood pressure and pain?
- Competitive interactor of KEAP1 → regulation of oxidative stress?
- DPP3 KO mice have impaired oxidative stress response leading to bone loss due to the increased activity of the osteoclasts
- Increased amount (and activity) in cancers of different etiology, ovarian, endometrial, lung, breast…
- Biomarker of cardiogenic shock



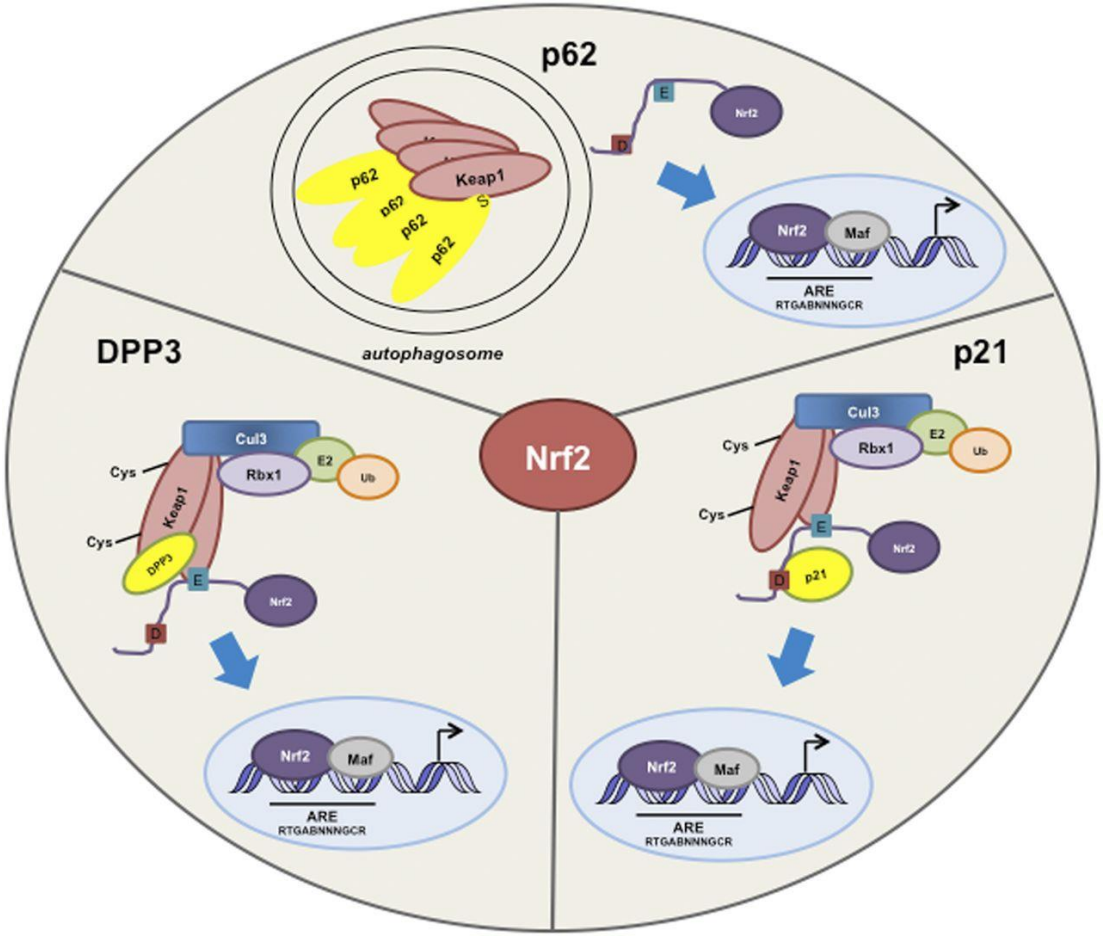
DPP3-KEAP1 interaction

- Hast et al. Cancer Res 2013
  - DPP3 identified in KEAP1 PIN - ETGE containing protein
  - DPP3 ox. activated NRF2-mediated transcription (ETGE-dependant)
  - DPP3 binds (and competes with NRF2) KEAP1 in a similar manner as NRF2
  - DPP3 reduced NRF2 ubiquitination
  - DPP3 mRNA expression and copy number correlate with the NRF2 activity in squamous cell lung carcinoma
- Interaction confirmed by Lu et al. Cancer Res 2017
  - Interaction induced by oxidative stress
  - DPP3 ox. stabilizes KEAP1
  - DPP3 expression correlated with increased expression of NRF2 target genes and poor survival in ER+ breast cancer



NRF2-KEAP1 in cancer

- Jaramillo and Zhang, Genes Dev 2013

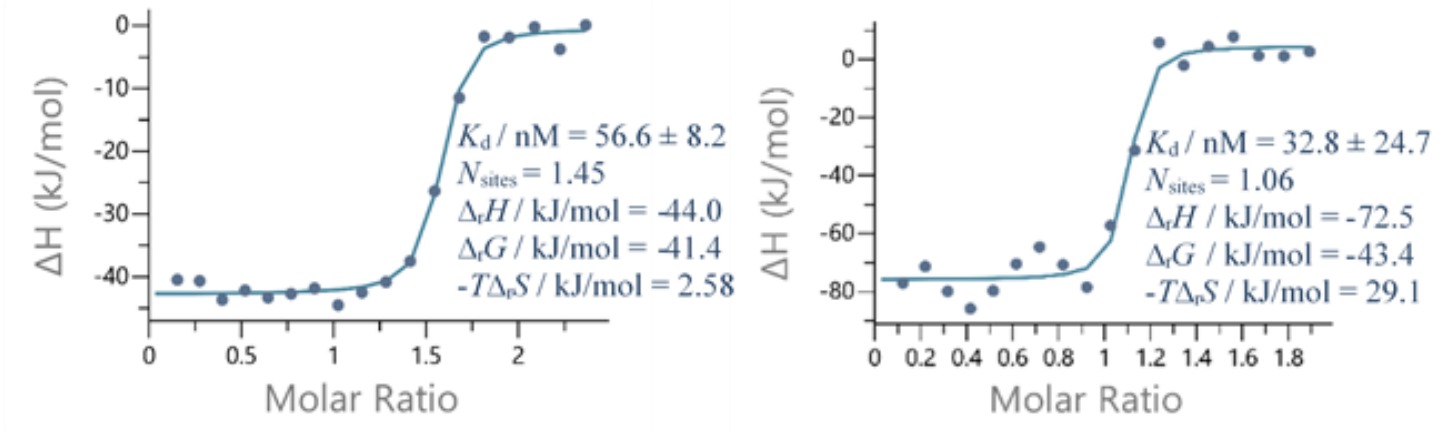


Aims:

1. Analyze the structure of DPP3 and Kelch domain complex and the dynamics of binding
2. Analyze the binding affinities of DPP3 mutant variants found in cancer towards the Kelch domain

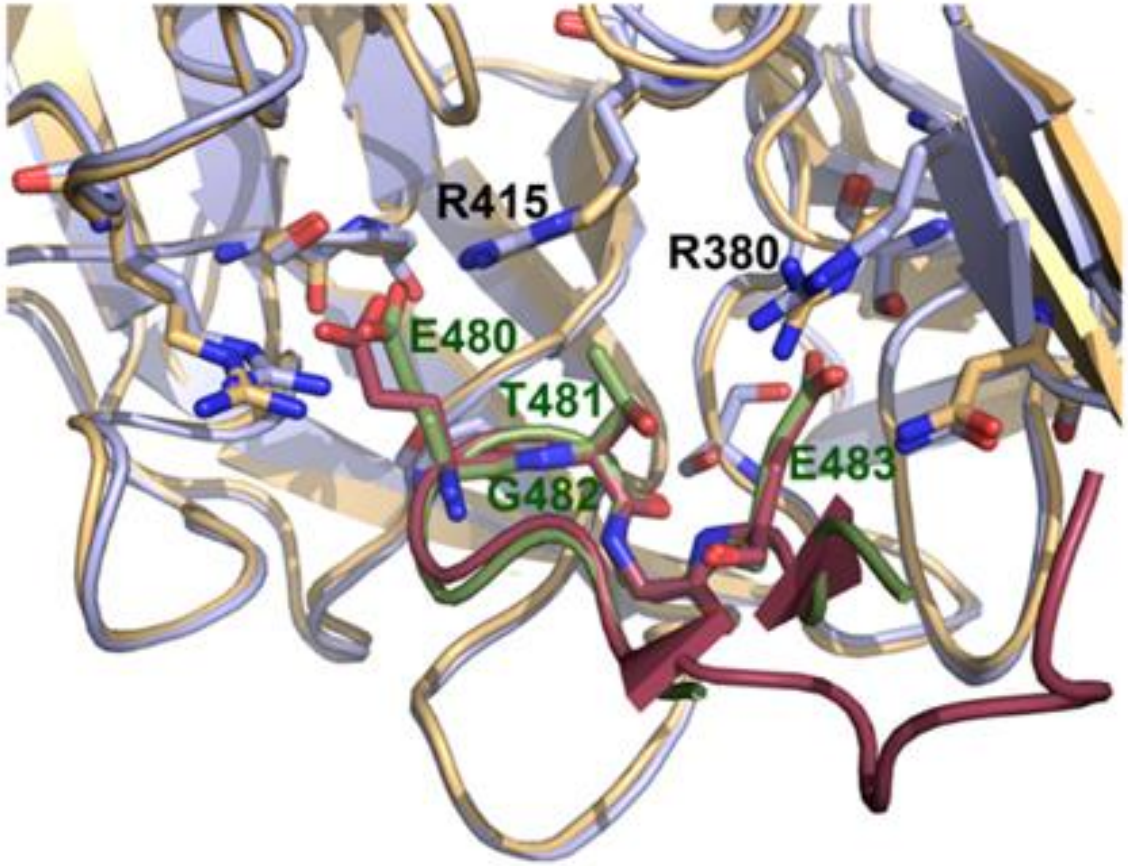
ITC Analysis of DPP3/Kelch interaction

- Isothermal titration calorimetry (ITC) analysis of the thermodynamic parameters of binding of DPP3 peptide (left – 11 aa peptide; right 24 aa peptide) to the Kelch domain of KEAP1



- Slightly lower K_d for the binding of DPP3-ETGE peptide (56.6 ± 8.2 for 11 aa peptide and 32 ± 24.7 for 24 aa peptide) compared to NRF2-ETGE peptide (9-32 nM)

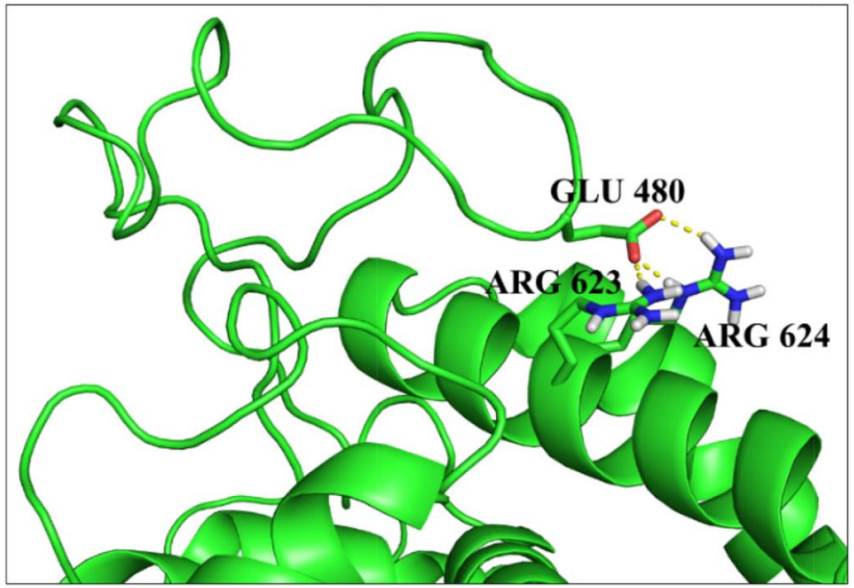
Crystal structure of the Kelch-DPP3-ETGE peptide complex



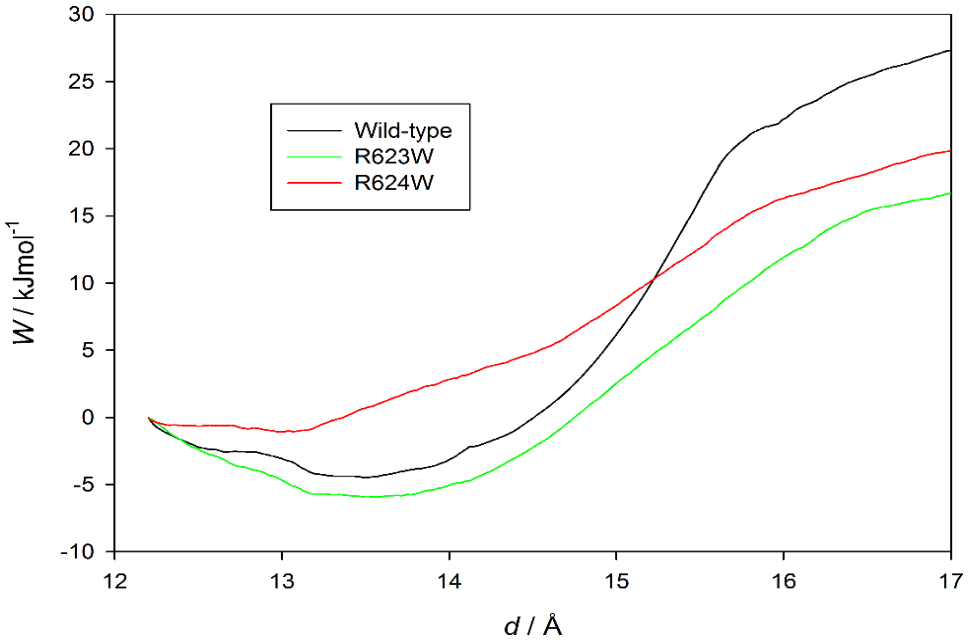
Kelch domain-NRF2 peptide (16 aa) (PDB ID: 2FLU)

Kelch domain-DPP III peptide (11 aa) (PDB ID: 6TG8)

Molecular dynamics analysis of DPP3/Kelch interaction



- Binding of DPP3 to Kelch is preceeded by the release of ^{480}ETGE^{483} loop from DPP3 protein body

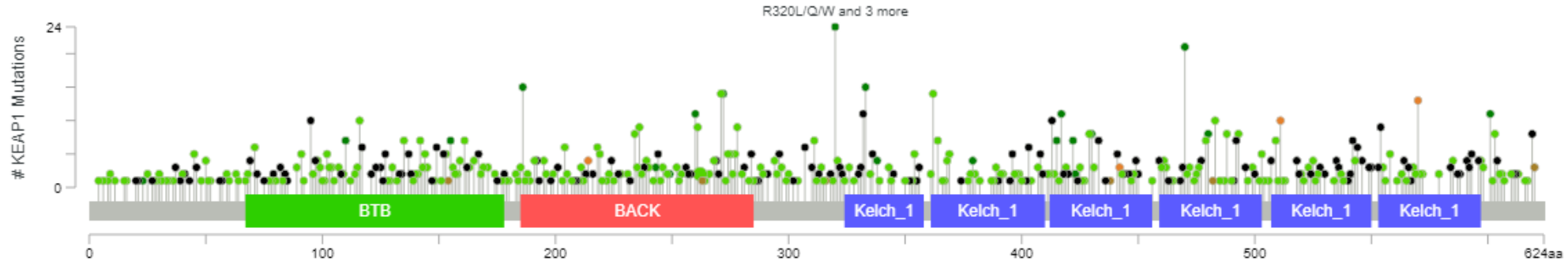


- Work required to detach the ETGE loop is lower in R623W and R624W mutant variants

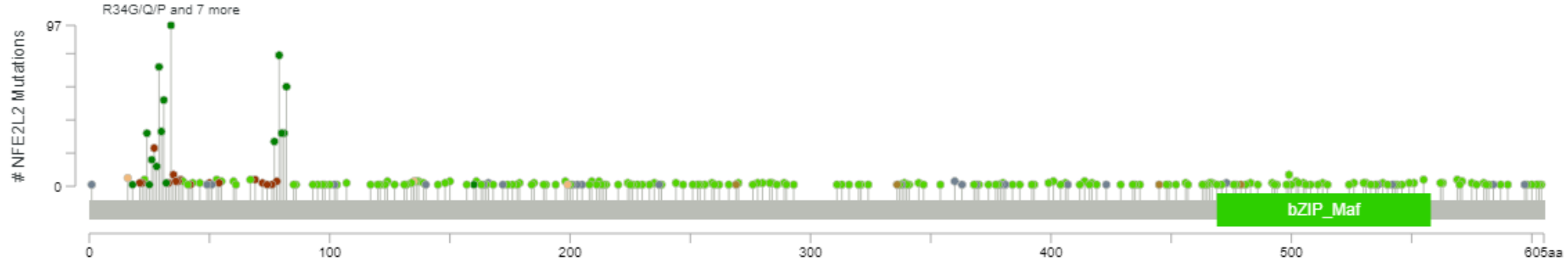
KEAP1/NRF2 mutations in cBioPortal for cancer genomics

Search of curated set of nonredundant studies - 59859 patients/62949 samples in 193 studies

KEAP1 around 1300 mutations (468 driver)

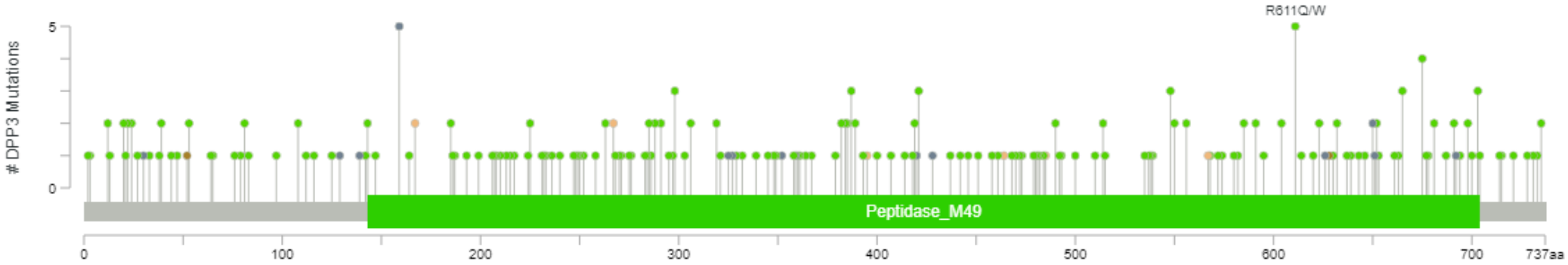


NRF2 around 1000 mutations (602 driver)



DPP3 mutations in cBioPortal for cancer genomics

- DPP3 – around 270 mutations



- enzyme kinetics analysis of mutant variants compared to the WT
  - Most of the variants have the same or lower enzymatic activity than the WT
  - Analysis of the interaction with KEAP1

MST analysis of the binding affinity

DPP3	K_d (WT)/K_d(mut)
WT	1.0
E451K	2.1
P479S	18.4
E480Q	0.1
T481M	0.1
G482C	0.8
Q484H	2.1
R510W	0.3
R623W	160.0[b]
R638L	2.0
R638W	2.0
R703C	1.7

- MST analysis - interaction of DPP3 mutant variants found in cBioPortal with Kelch domain
- P479S around 20 times higher affinity for Kelch than the WT - NRF2 has Glu at the same position
- R623W - more than 100 times higher affinity for the Kelch domain than the WT - confirmes the result of MD analysis of the work needed to release the ETGE loop

Summary

- DPP3 binds to the Kelch domain of KEAP1 protein in a similar manner as NRF2
- DPP3 ETGE motif is located on an unstructured loop that is anchored to the protein body by the Glu480-Arg623 and Glu480-Arg624 H-bonds
- DPP3 binding to Kelch is a 2 step process, the first step being the release of the ETGE loop from the protein body
- MD analysis show that the Arg623Trp Arg624Trp substitutions lower the work needed to release the loop
- MST analysis of the interaction affinity of the DPP3-R623W mutant found in cancer show that it has a more than 100 fold higher affinity for the Kelch domain than the WT
- Experiments are on the way to analyze the effects of DPP3-R623W overexpression on the NRF2-controlled gene expression in the cell culture

Tools for NRF2 research

- Plasmids for the expression of Kelch domain of KEAP1 protein in E. coli - His and GST-tag
- Plasmids with KEAP1 insert for Y2H
- Plasmids for the expression of KEAP1 in mammalian cells:
  - FLAG-KEAP1
  - mCherry-KEAP1
  - Plasmids with KEAP1 insert for BiFC
  - qPCR primers for NRF2, NQO1, HMOX1, PRDX1, SOD1, GCLM, SLC7A11, GPX1

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