DPP III Minisymposium, Zagreb March 21st 2016

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Hrvatska zaklada za znanost

Human DPP III-Keap1 complexes computational study

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WHY?

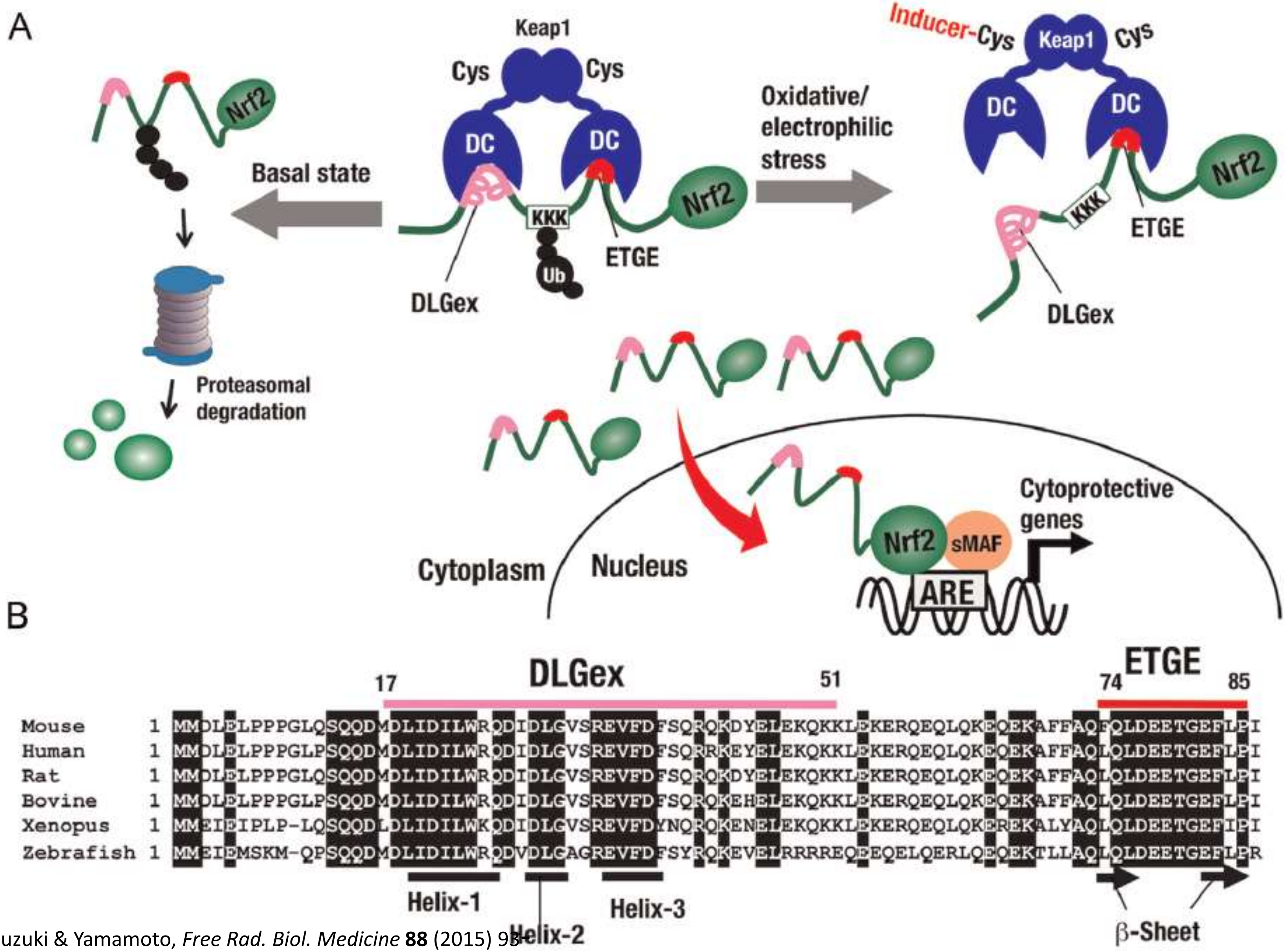
DPP III pathophysiological and physiological roles**?**

DPP III binds to Keap1

↓

activation of transcription factor Nrf2.[1]

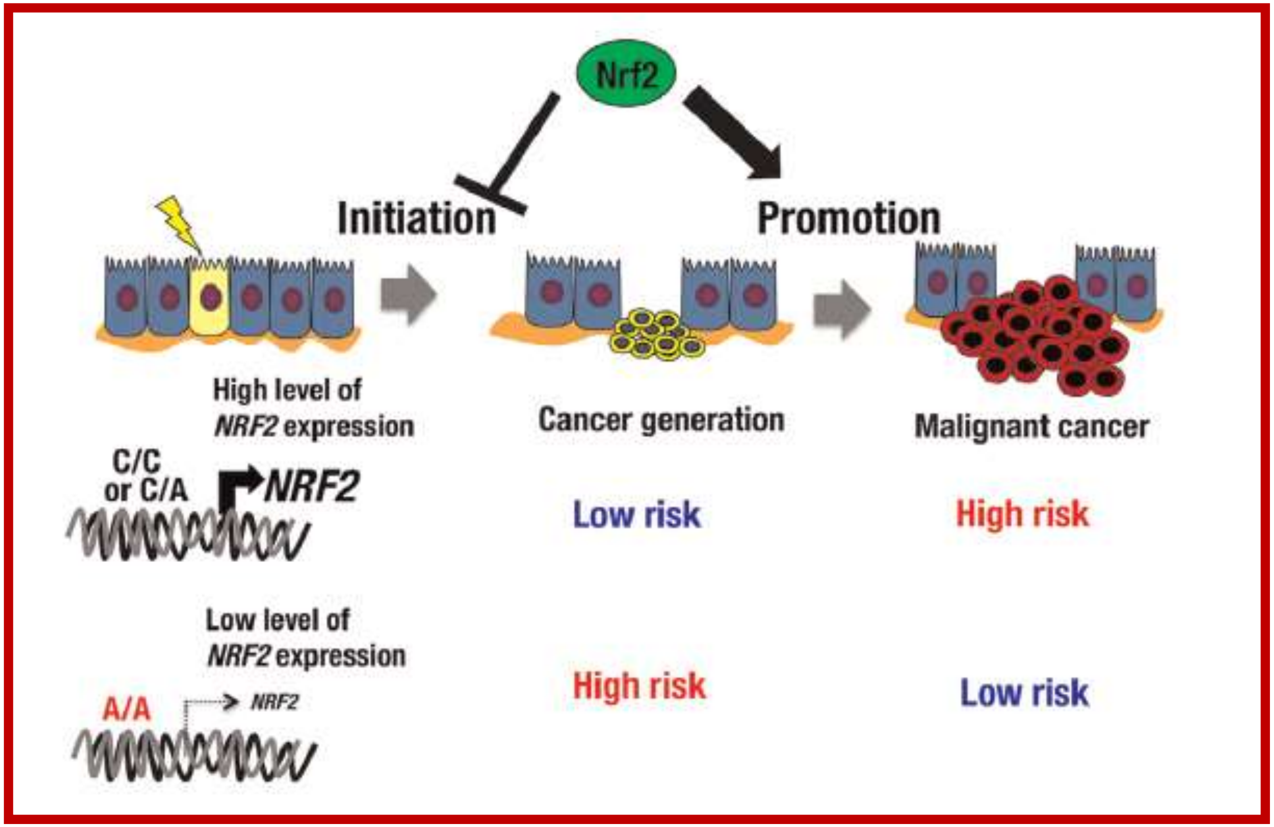
[1] Hast B.E., Goldfarb D., Mulvaney K.M., Hast M.A., Siesser P.F., Yan F., Hayes D.N. and Major M.B. 2013 *Cancer Res.* **73**; 2199 -2210.



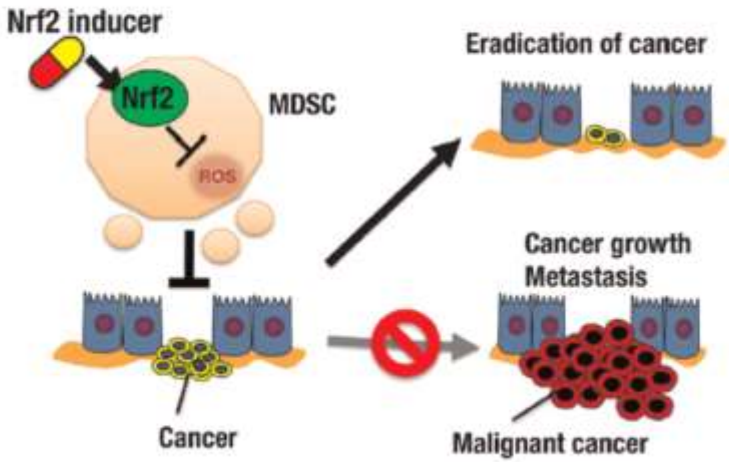
Suzuki & Yamamoto, *Free Rad. Biol. Medicine* **88** (2015) 93-

Oxydation and NRF2 and KEAP1 mutations activate NRF2 (regulates transcription of cytoprotective genes).

However, many tumors display high NRF2 activity in the absence of mutation, supporting the hypothesis that alternative mechanisms of pathway activation exist



High Nrf2 activity is common in cancer cells with adverse outcomes



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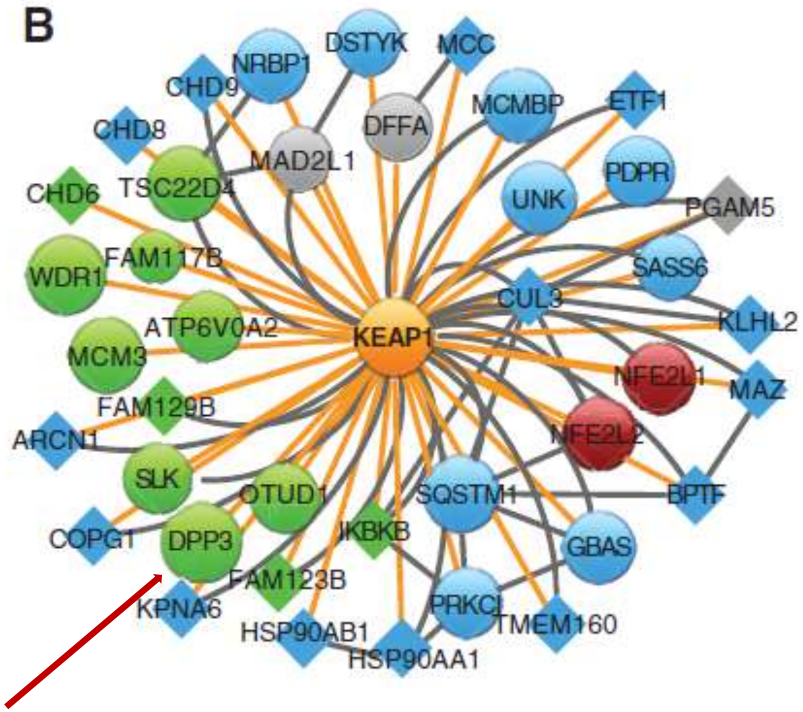
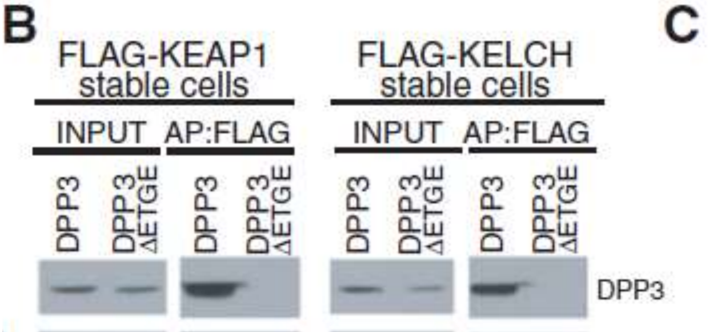
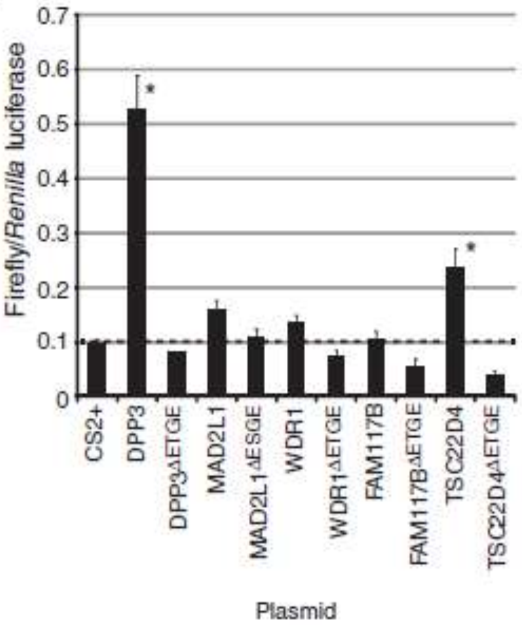


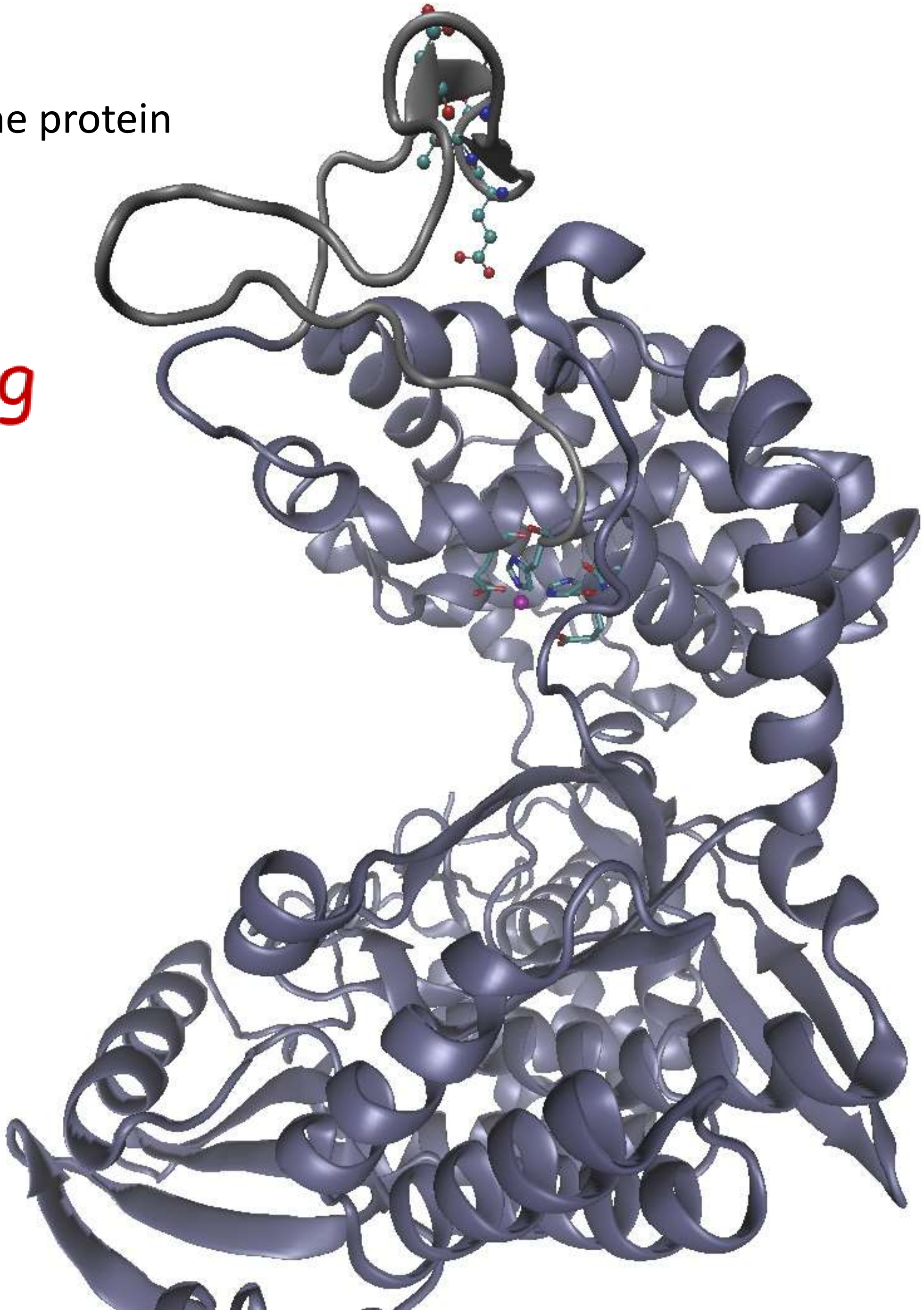




Schematic representation of the KEAP1 protein interaction network as defined by affinity purification and mass spectrometry. DPP III had the greatest impact on NRF2-dependent transcription and was the most abundant protein within the KEAP1 PIN.

[1]Hast et. al. 2013 *Cancer Res.* **73**; 2199 -2210.

The ETGE motif in DPP3 lies on an unstructured loop on the surface of the protein



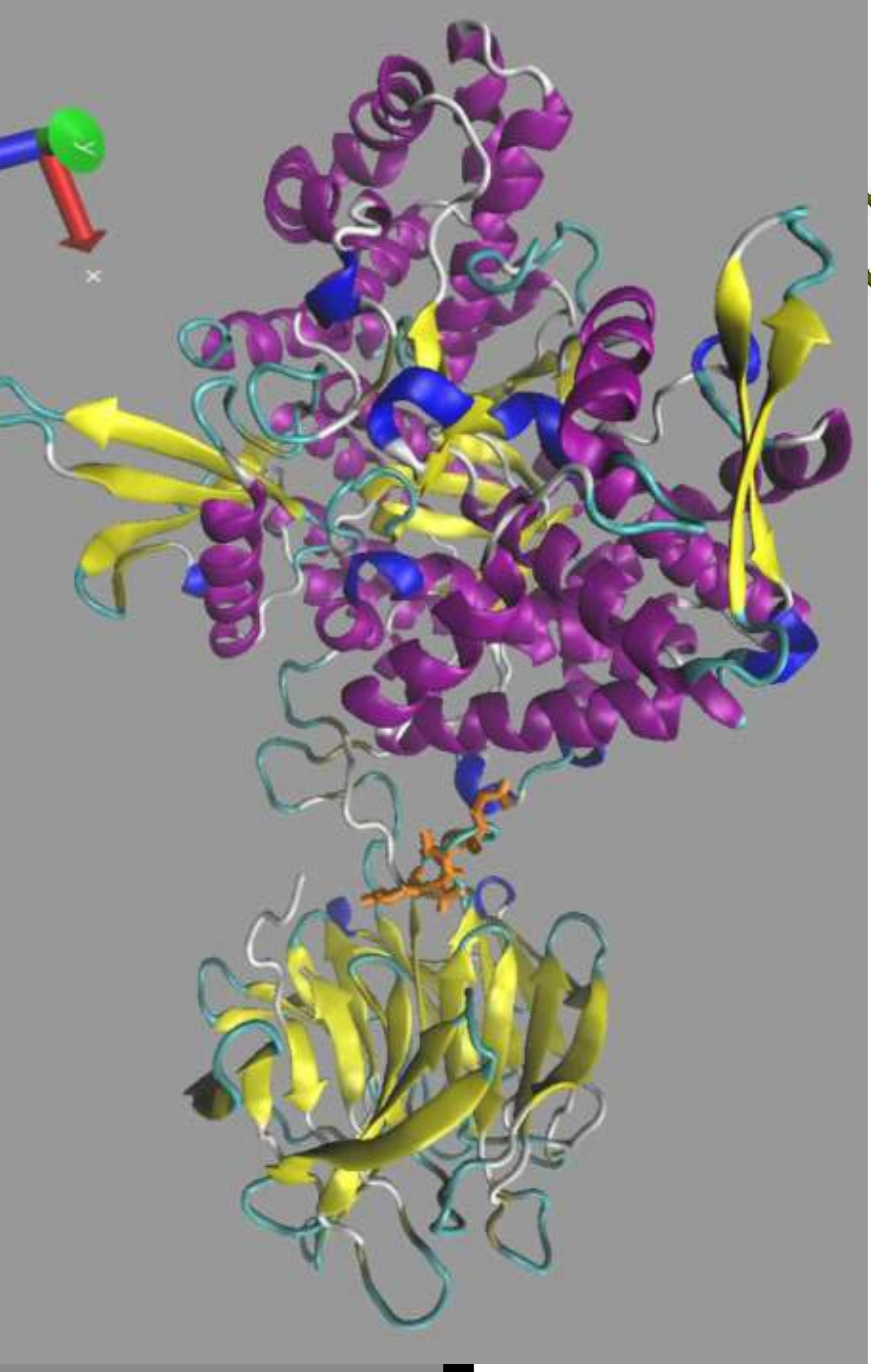
Complex building

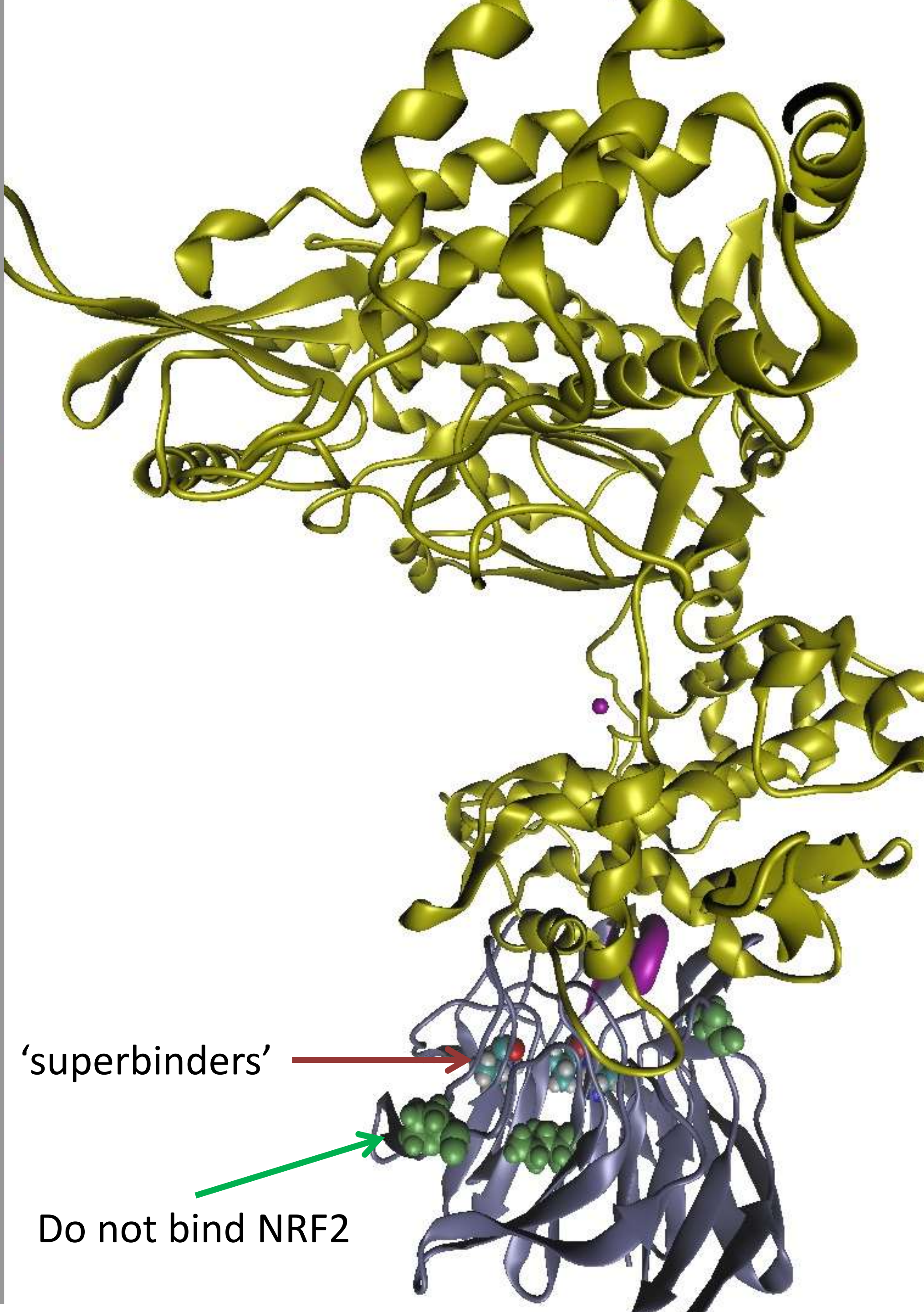
Protein-Protein Docking Web Servers

- **GRAMM-X**
- **ZDOCK**
- **Cluspro 2.0**
- **HADDOCK**
- **SDA**

Computational study of the h.DPP III-Keap1 complexes objectives

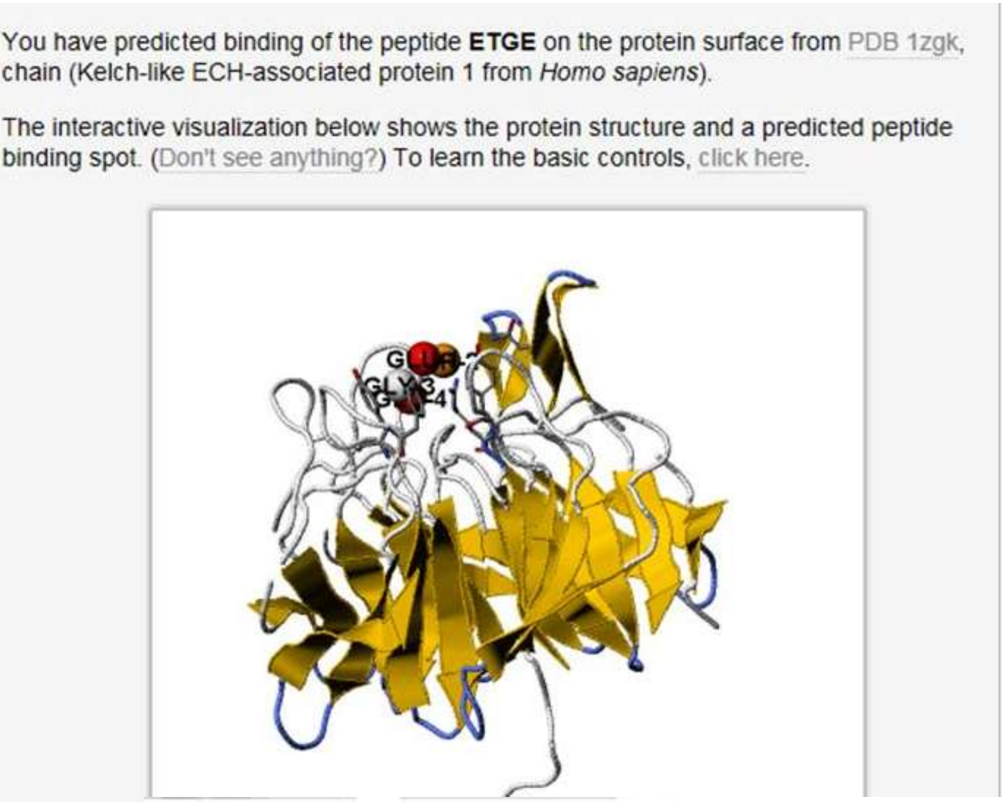
- Complex structure – inteaction patches
- Complex stability (DPP III closed/open)
- Influence of the Keap1 Kelch domain on protein structure and dynamics
- Influence of point mutations on DPP III affinity towards Keap1 Kelch domain





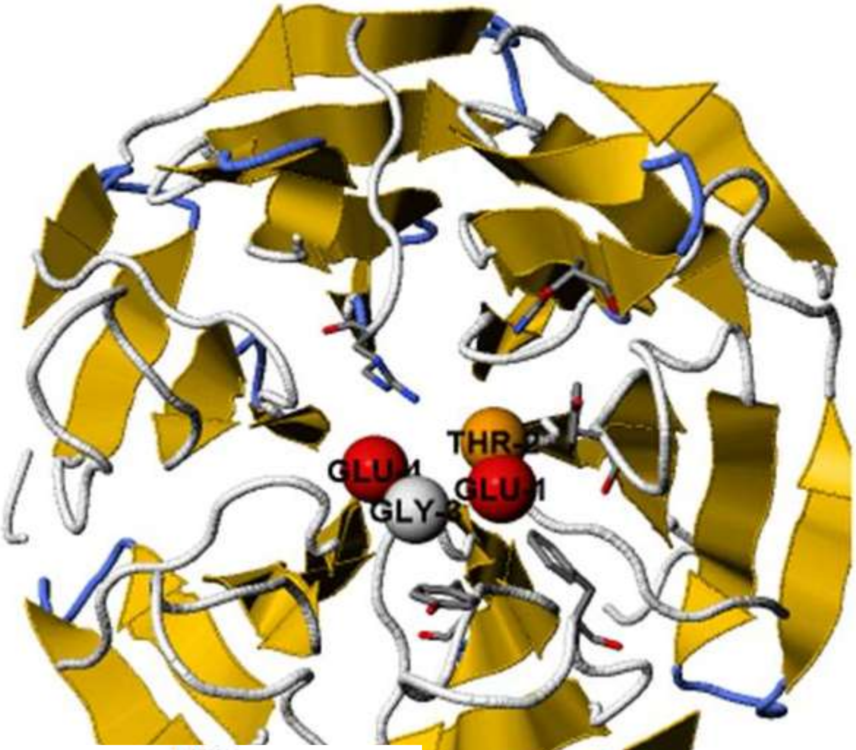
‘superbinders’

Do not bind NRF2

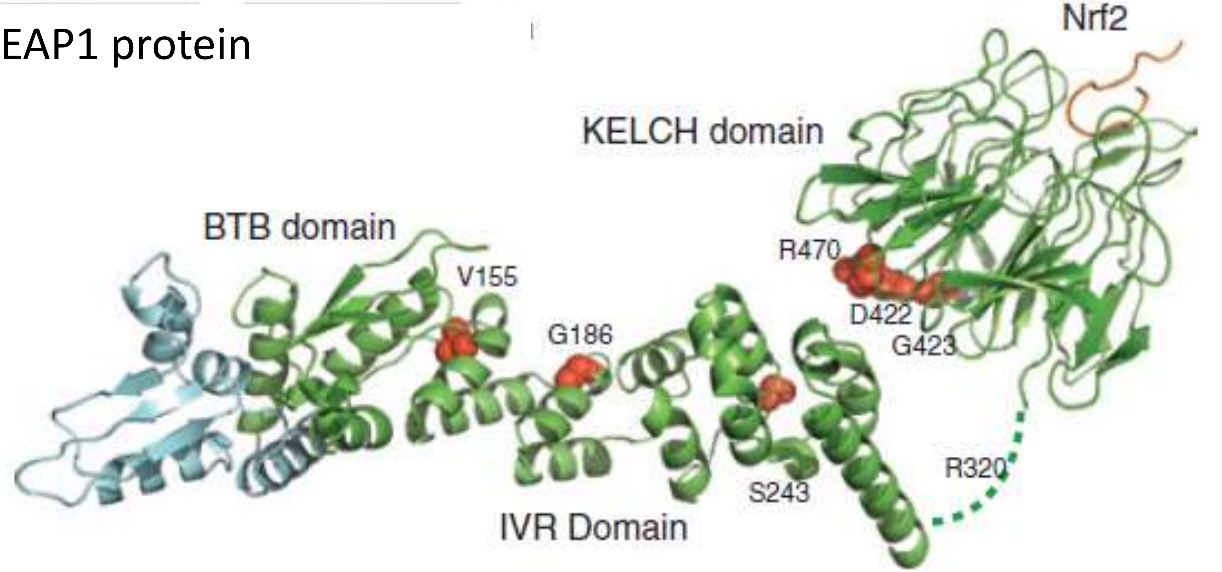


You have predicted binding of the peptide **ETGE** on the protein surface from PDB 1zgk, chain (Kelch-like ECH-associated protein 1 from *Homo sapiens*).

The interactive visualization below shows the protein structure and a predicted peptide binding spot. (Don't see anything?) To learn the basic controls, click here.



KEAP1 protein



Simulations

> Orientation 1

All atom (AMBER14) **DPP III^{o}** (200+40) ns

DPP III^{c} (40) ns

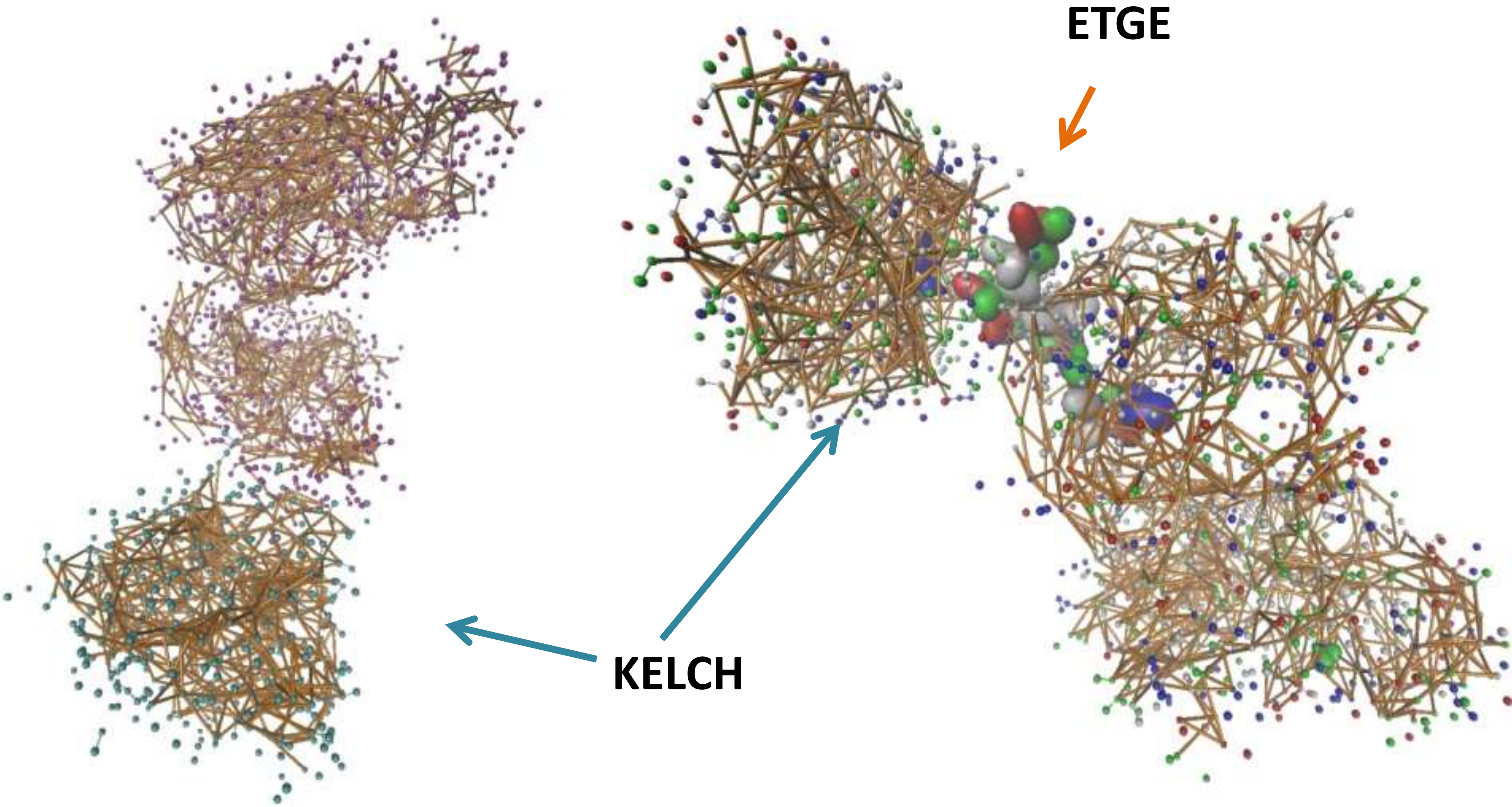
Coars grained (Martini/Gromacs) **DPP III^{o}** 20 µs

DPP III^{c} 6 µs

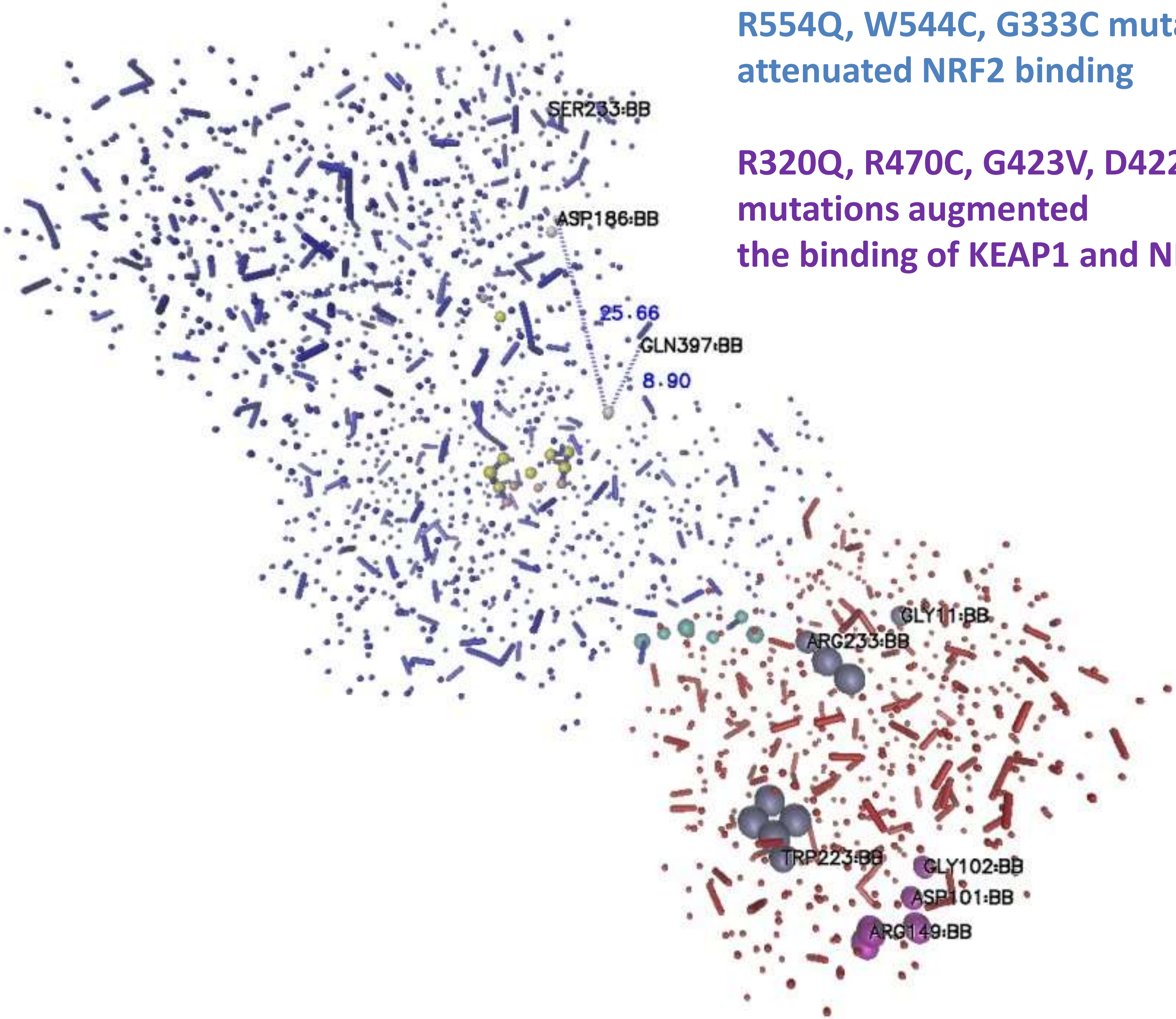
> Orientation 2

All atom (AMBER14) **DPP III^{o}** (100) ns

DPP III^{c} (72) ns



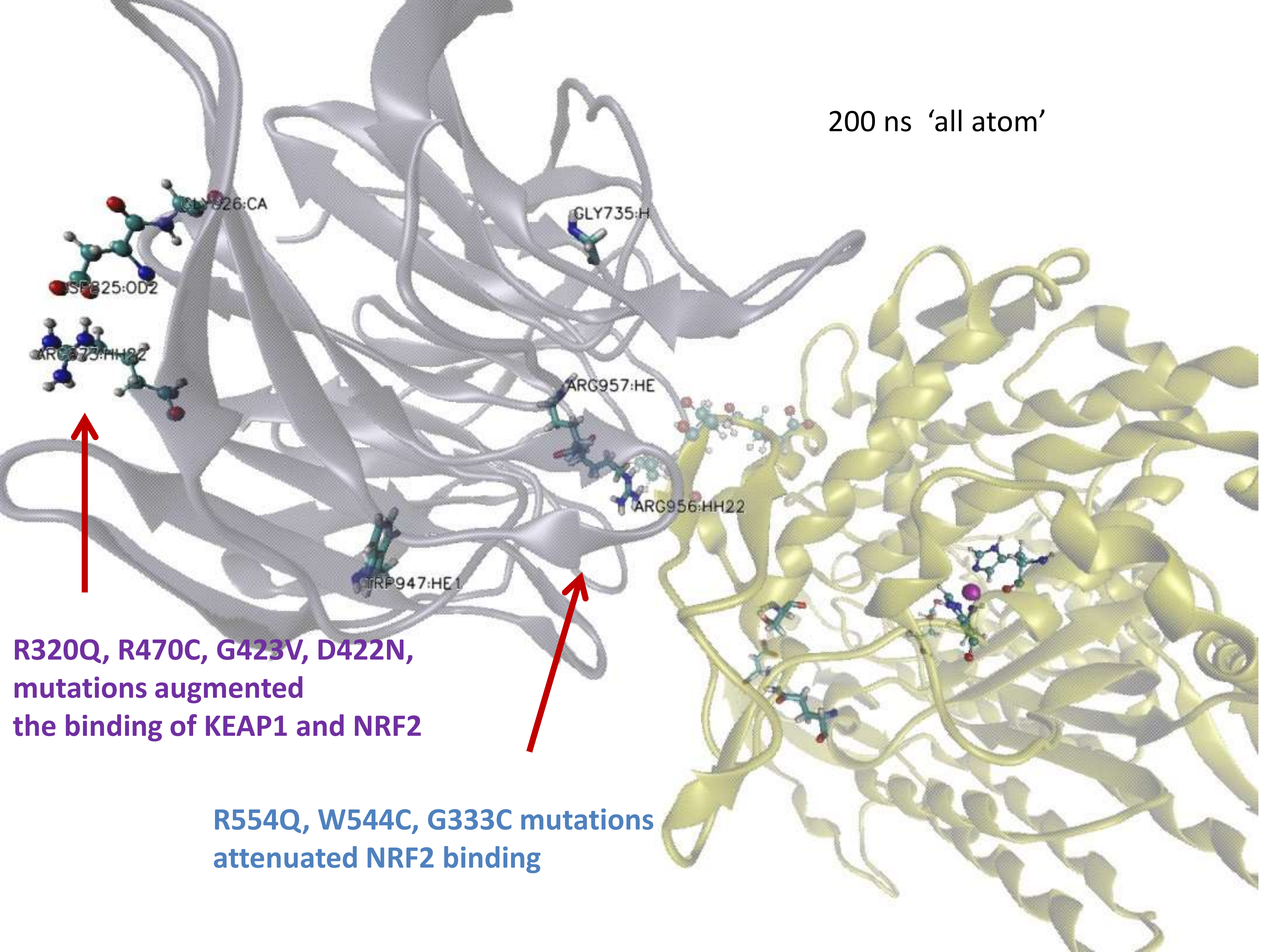
ETGE
KELCH



R554Q, W544C, G333C mutations attenuated NRF2 binding

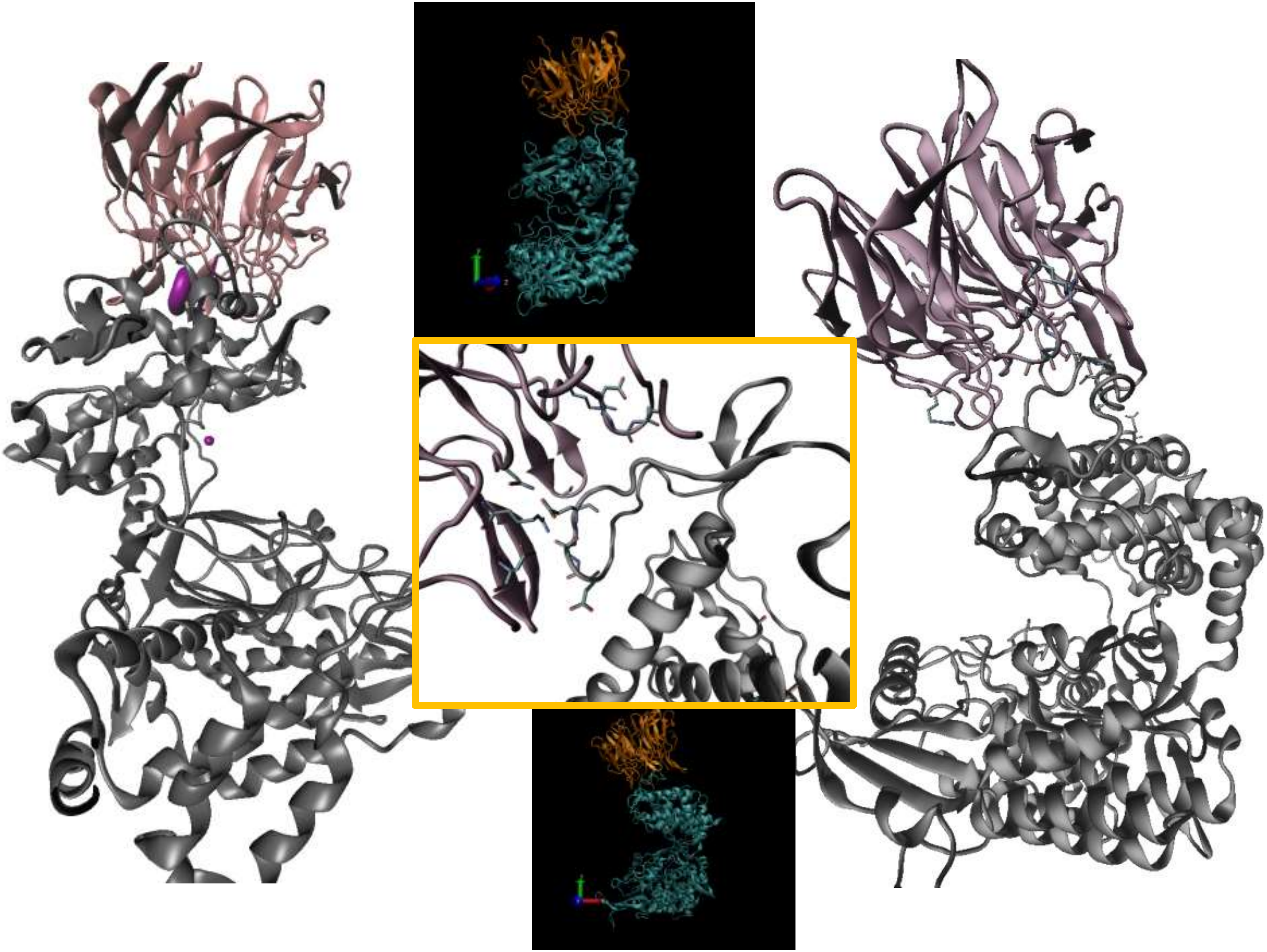
R320Q, R470C, G423V, D422N, mutations augmented the binding of KEAP1 and NRF2

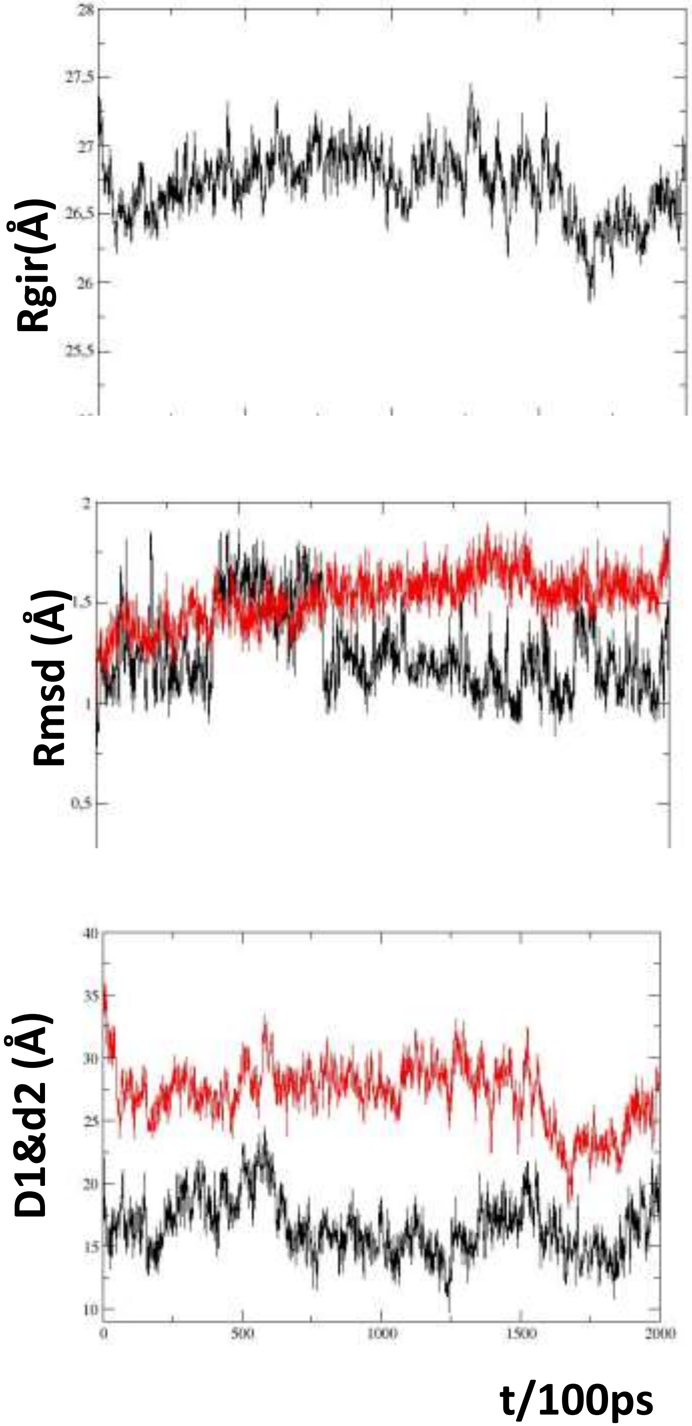
200 ns ‘all atom’



R320Q, R470C, G423V, D422N, mutations augmented the binding of KEAP1 and NRF2

R554Q, W544C, G333C mutations attenuated NRF2 binding




Rgir(Å)
28
27,5
27
26,5
26
25,5
Rmsd (Å)
2
1,5
1
0,5
D1&d2 (Å)
40
35
30
25
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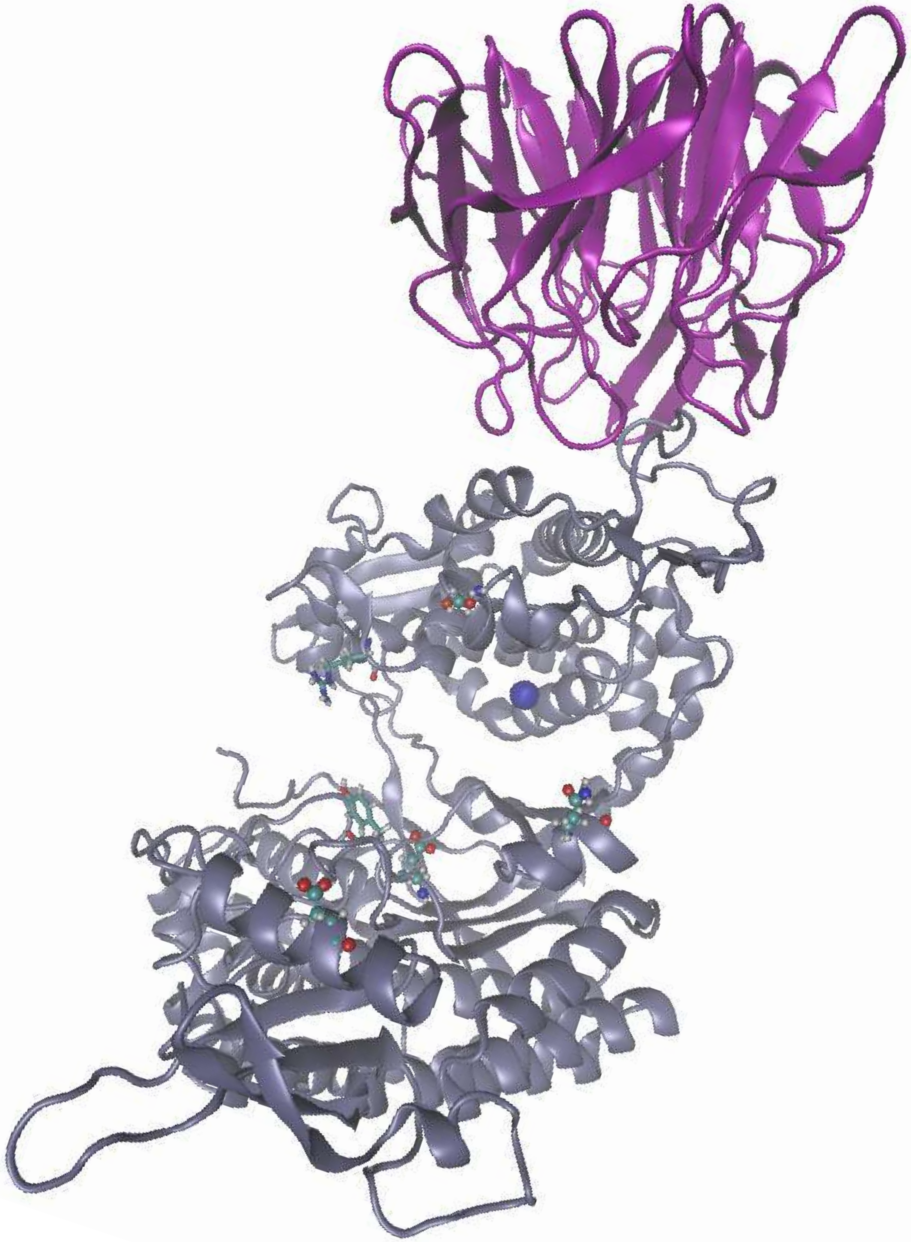
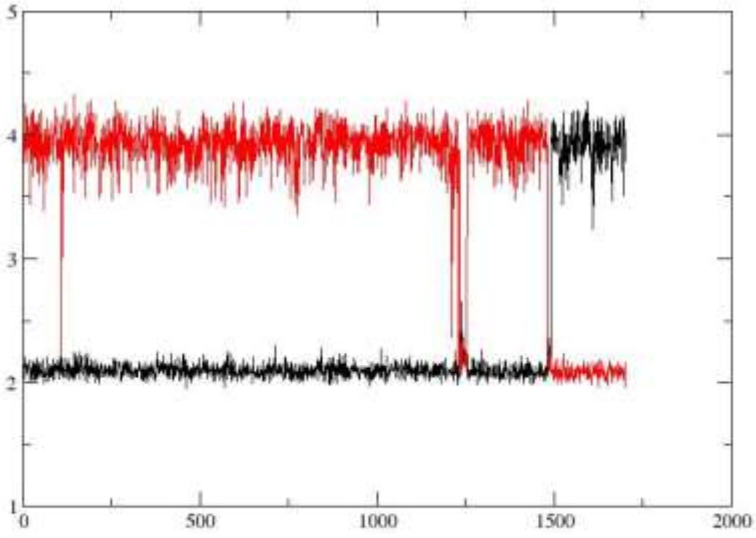
2000
t/100ps



d(Å)



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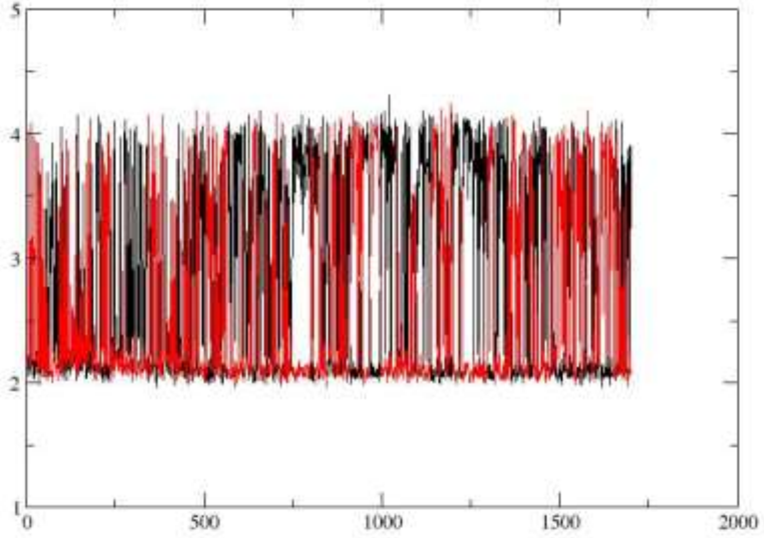

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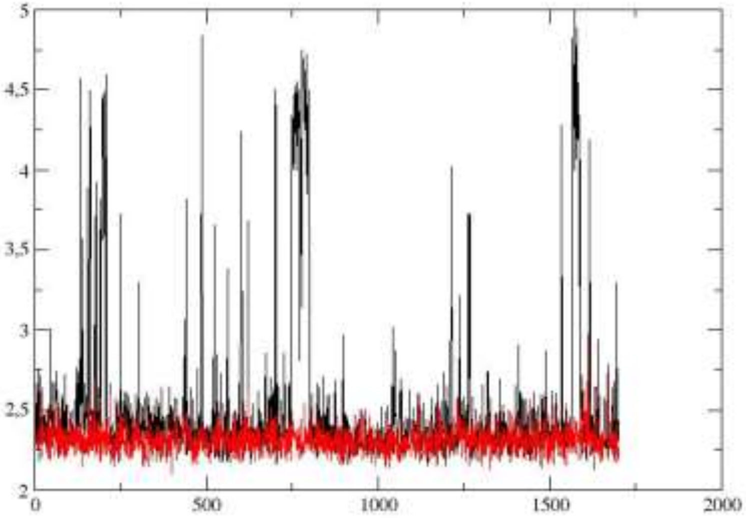

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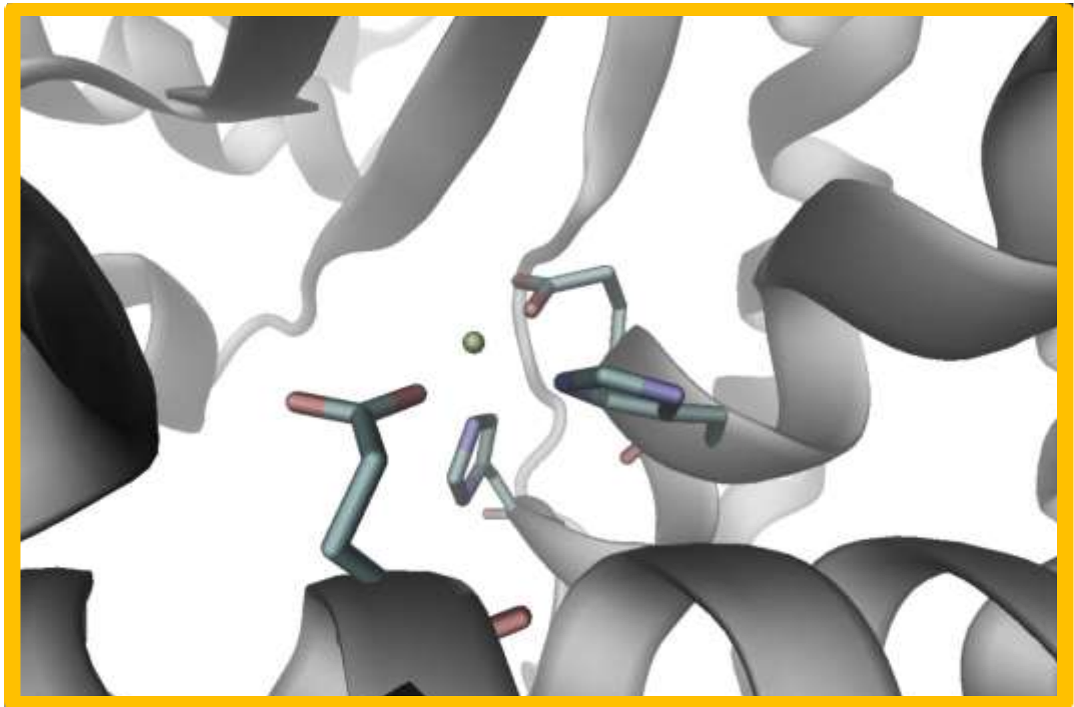
t/100ps



INSTEAD CONCLUSION

- ***To be done***

- Thermophoresis
- H/D exchange
- Additional simulations for the WT complexes and mutants
- ITC measurements ???

THANK YOU FOR YOUR VALUABLE SUGGESTIONS