Involvement of hDPP III into the oxidative stress
Keap1-hDPP III interaction

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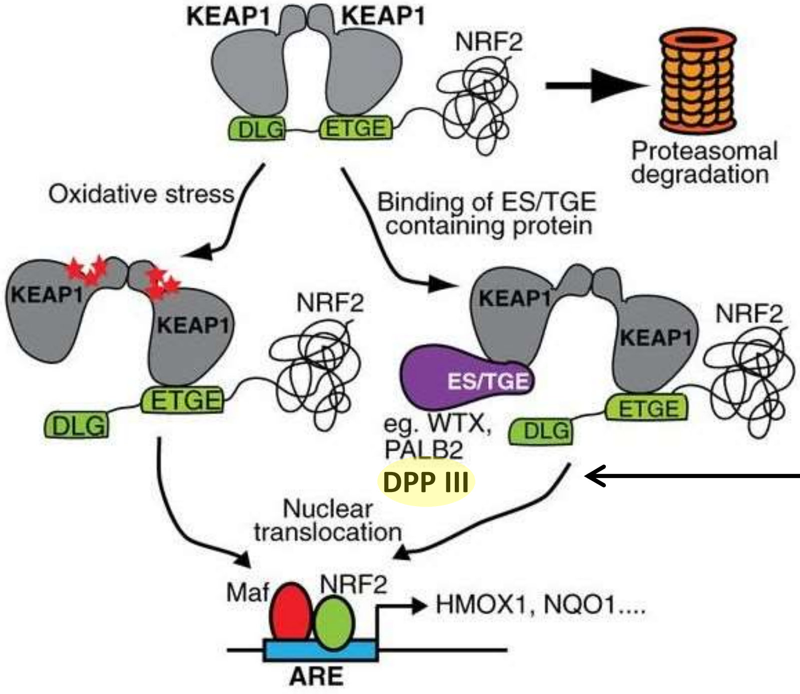
Involvement of hDPP III into the oxidative stress Keap1-hDPP III interaction

Oxidative stress

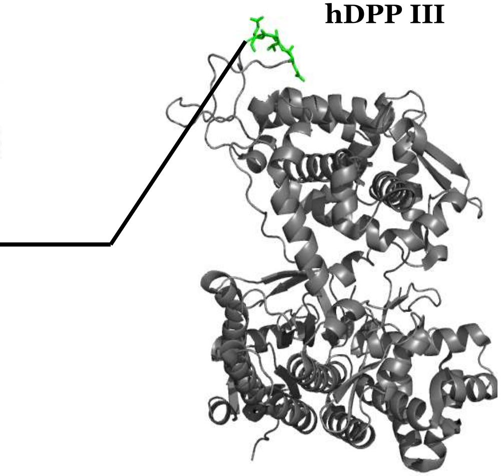
Cancer

Immune Response

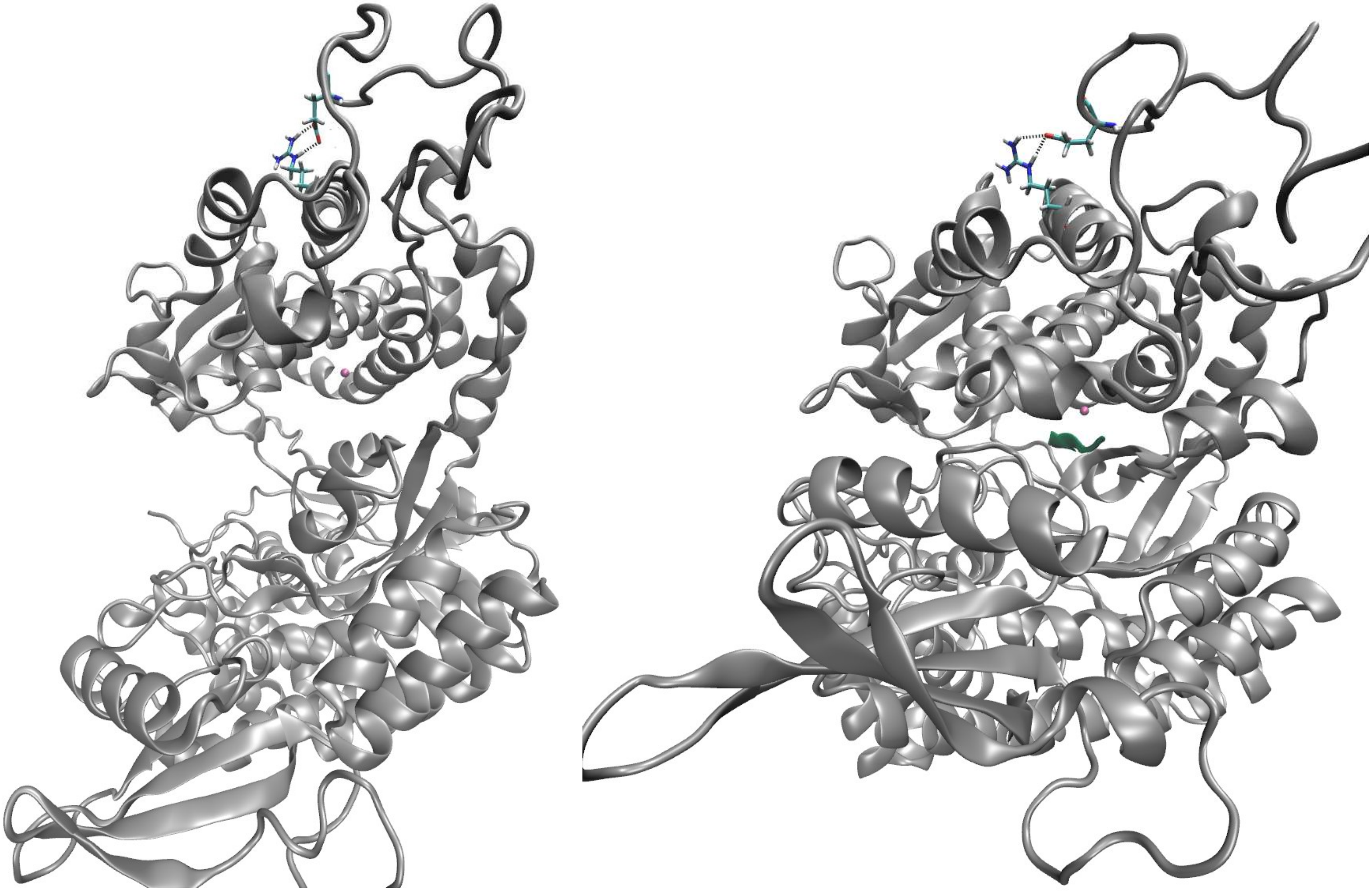
PDB_id **2FLU**



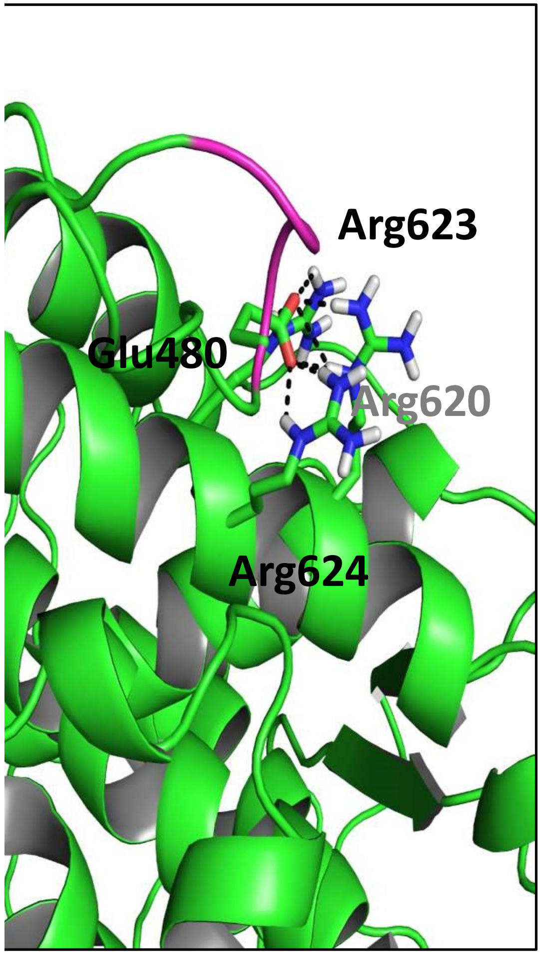
ETGE motif of hDPP III



B.E. Hast et al. *Cancer Res.* **73** (2013) 2199-210
K. Lu et al. *Cancer Res.* **77** (2017) 2881- 92



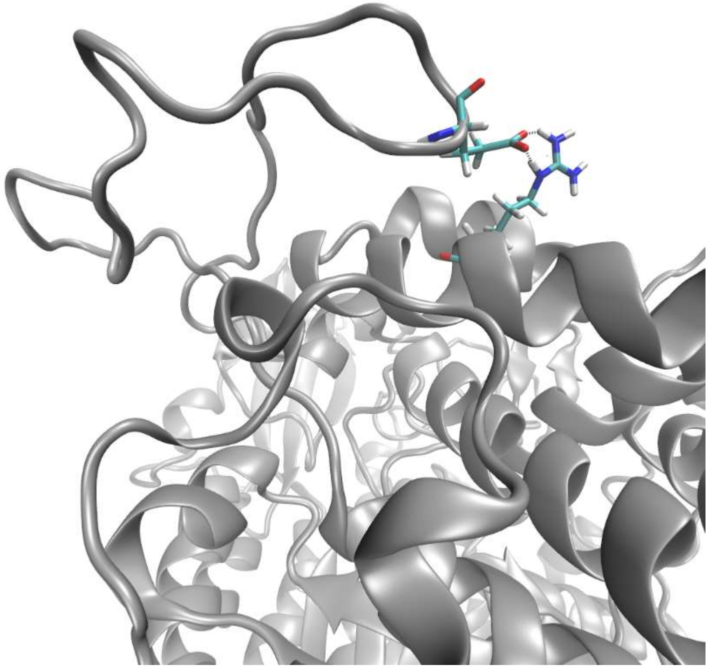
Hydrogen bond analysis

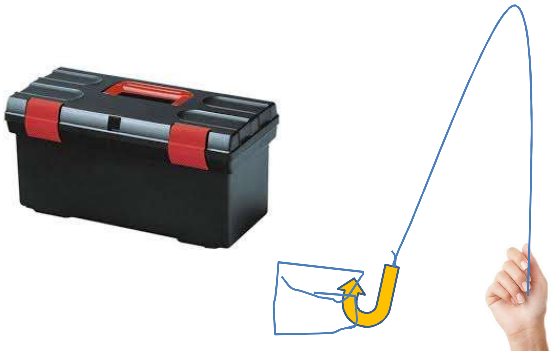


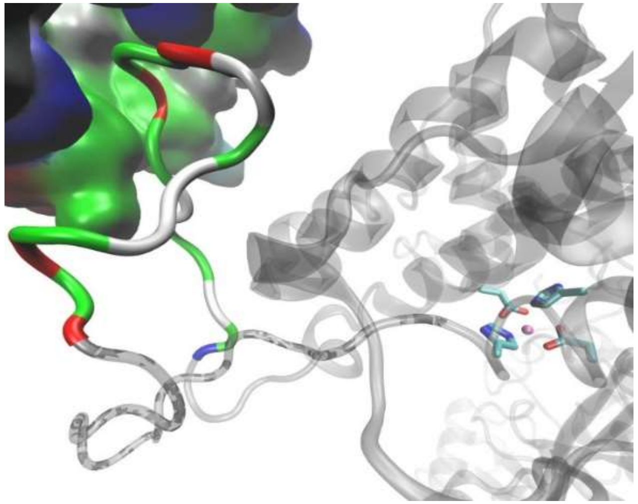
Residue	Electroneutral	0,1M NaCl	0,3M NaCl
Arg620	35.3	24.6	63.8
Arg623	118.3	108.8	139.8
Arg624	134.1	145.0	109.5

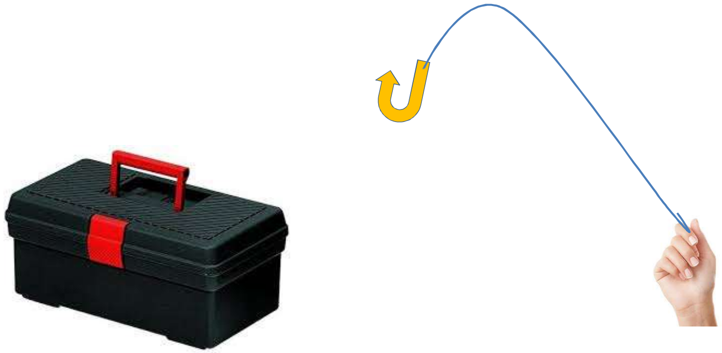
Hydrogen bond occurences (%) between amino acids in the ETGE loop and the rest of the protein during MD simulations

Binding of hDPP III to the Kelch domain

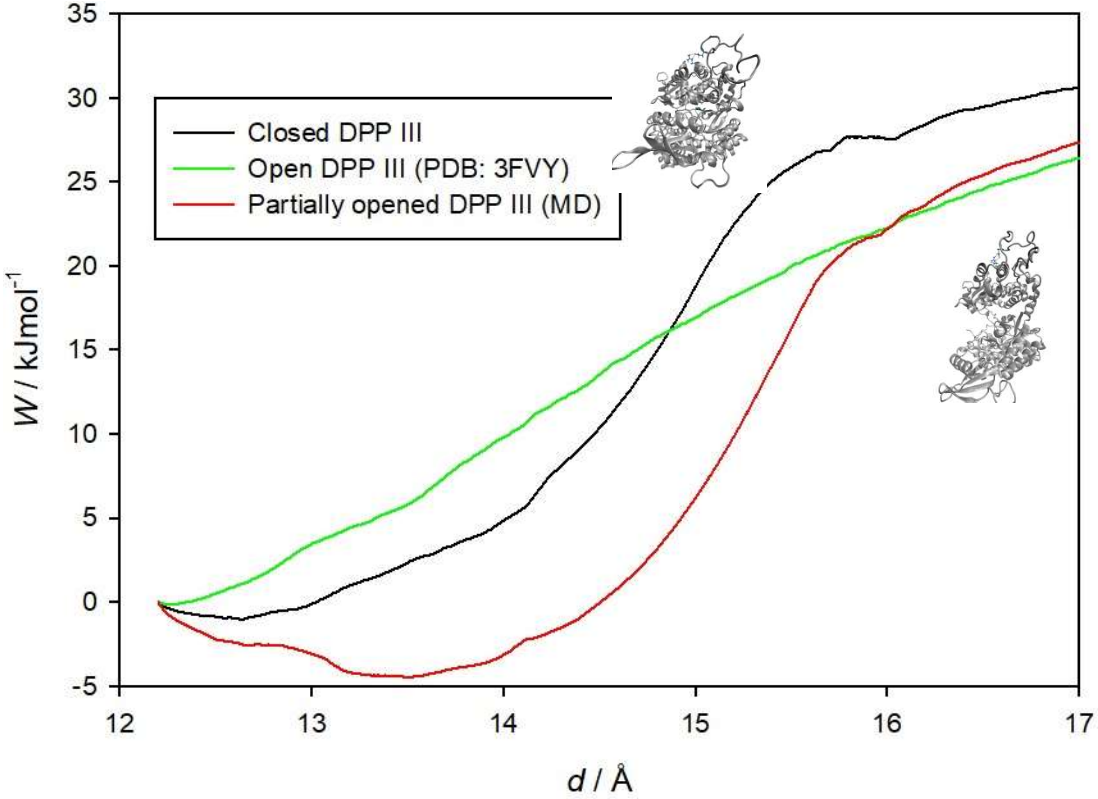






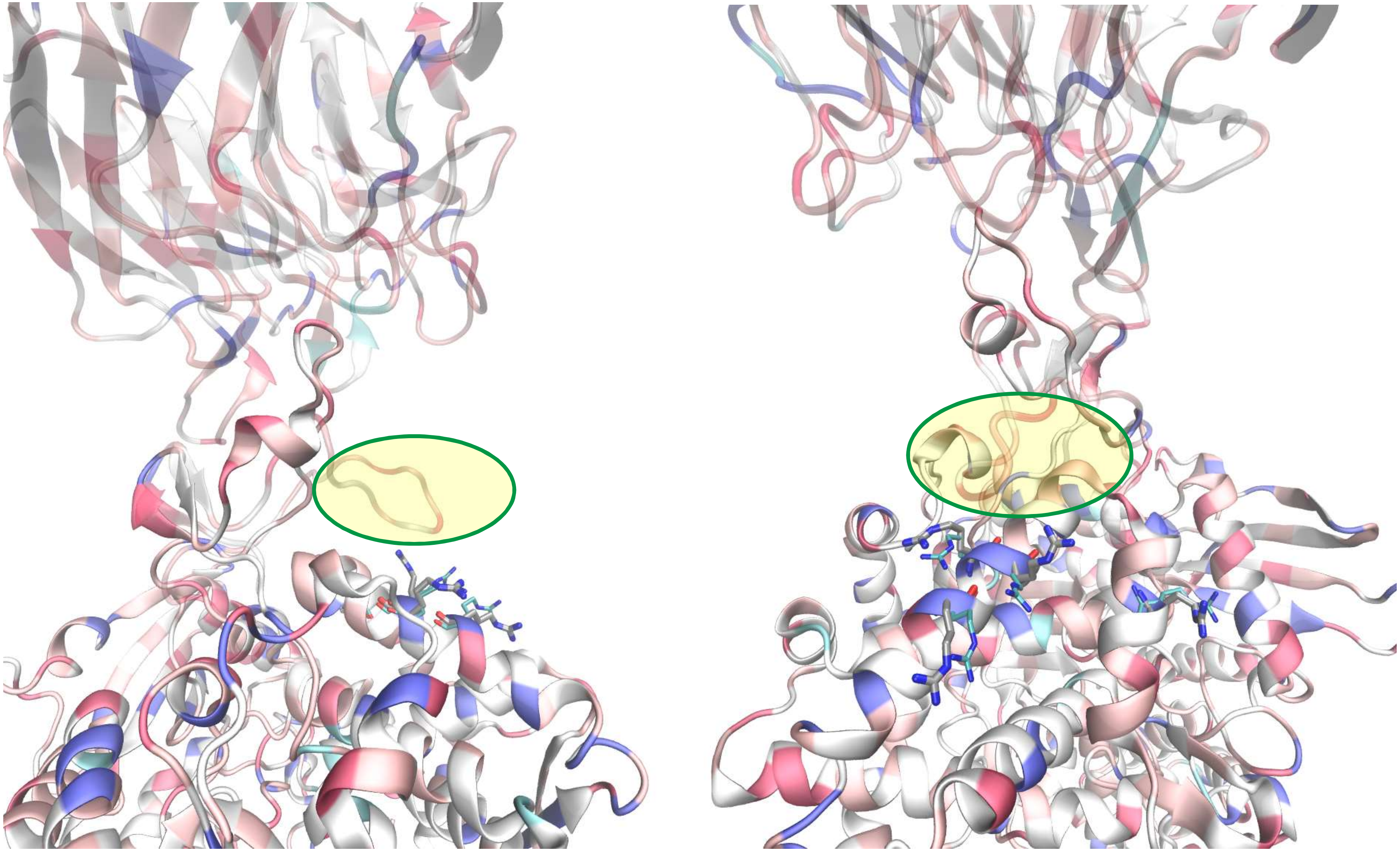


Adaptive steered MD simulations (ASMD)

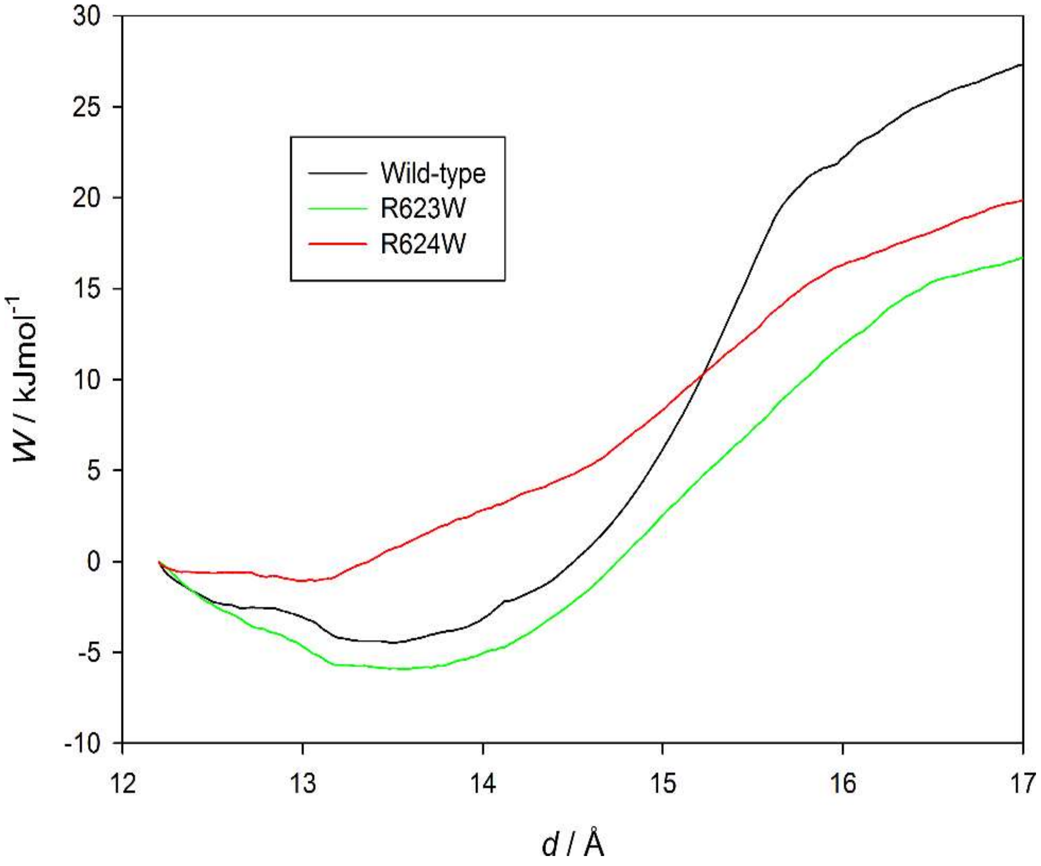


- 10 replicas
- The work needed to releases the loop is lower in the less compact forms of DPP III.

Interactions of the electronegative loop tip with the positively charged arginines

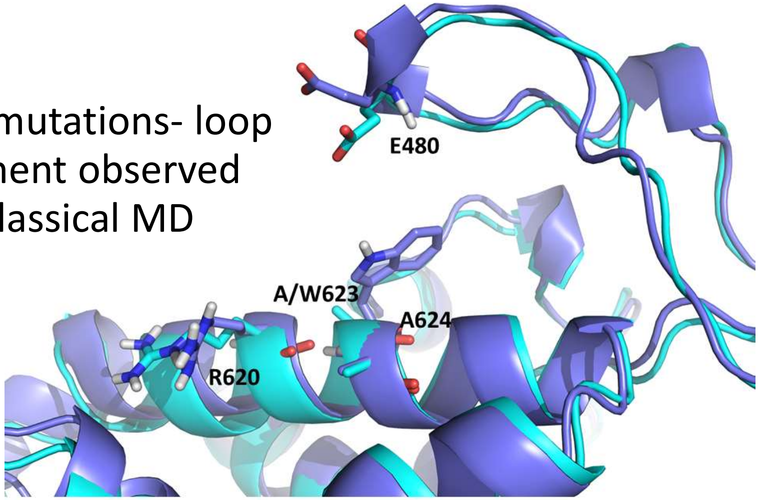


ASMD simulations of the hDPP III mutants



ETGE loop detachment is responsible for a decrease in K_d for DPP III-R623W - Kelch binding

Double mutations- loop detachment observed during classical MD



Significantly lower Kd for R623W mutant measurement of initial fluorescence change

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

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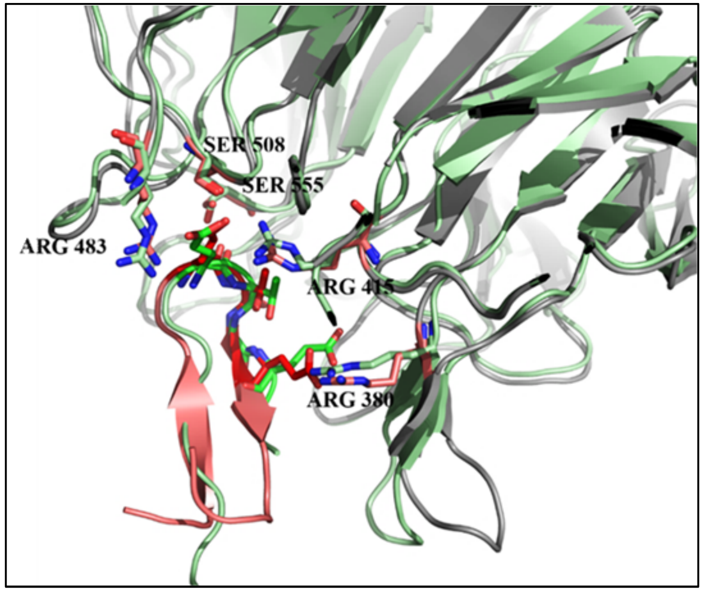
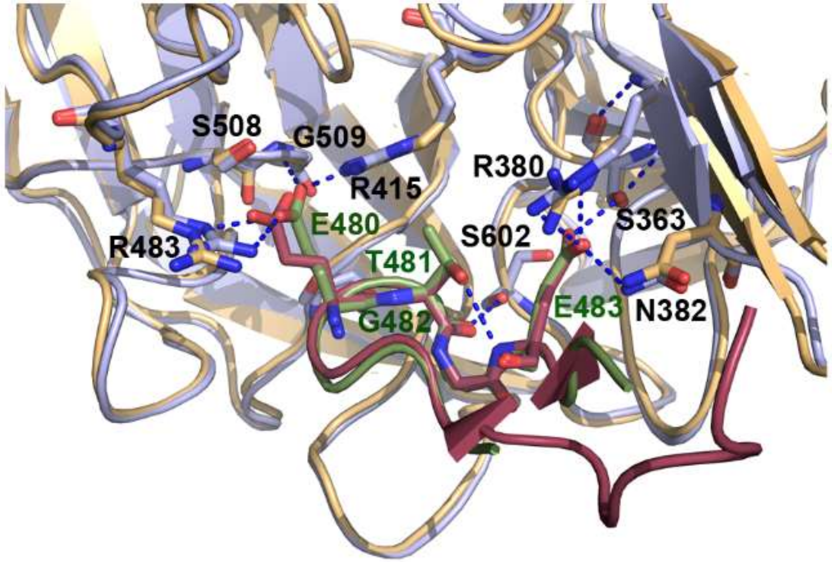
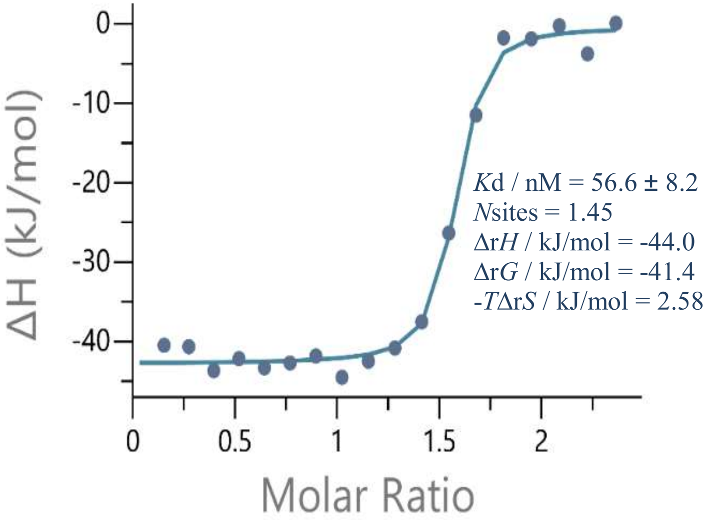
DPP III ETGE loop binding to Kelch domain of KEAP1

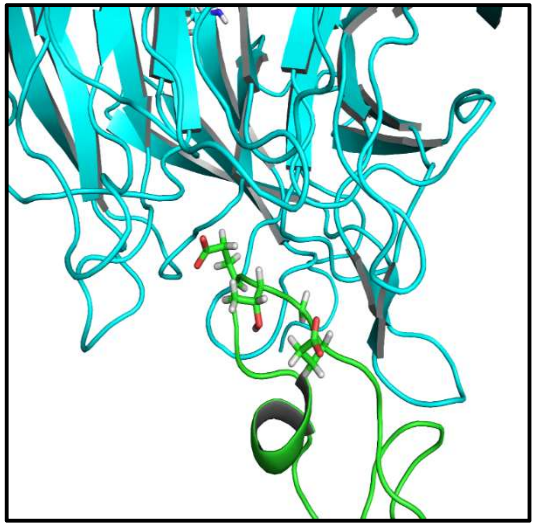






SUMMARY

- ETGE loop crucial for hDPP III binding to the Keap1 Kelch domain.



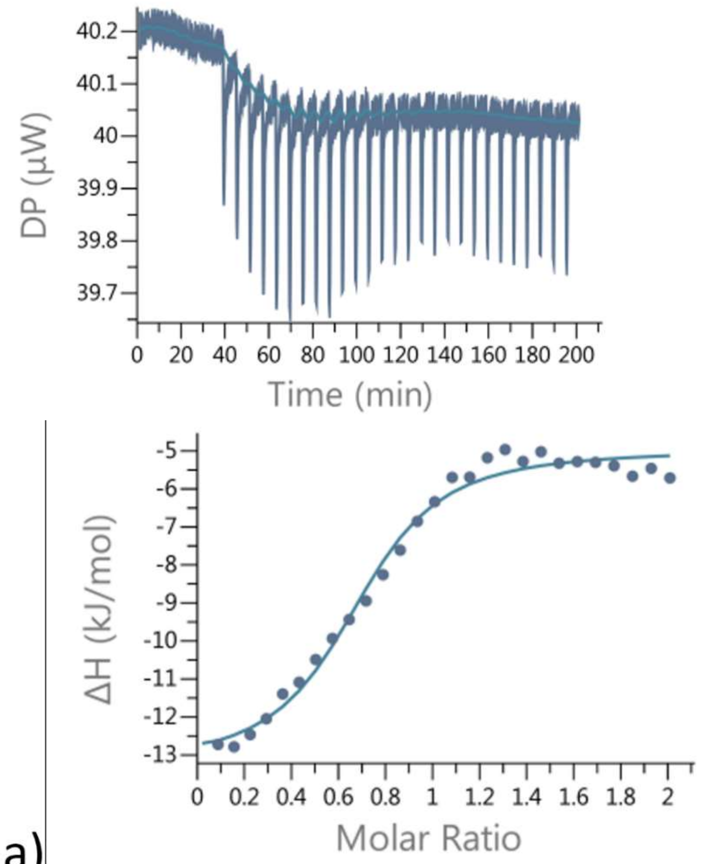
hDPP III - Kelch binding is two step process consisting of:

1. endergonic loop translocation

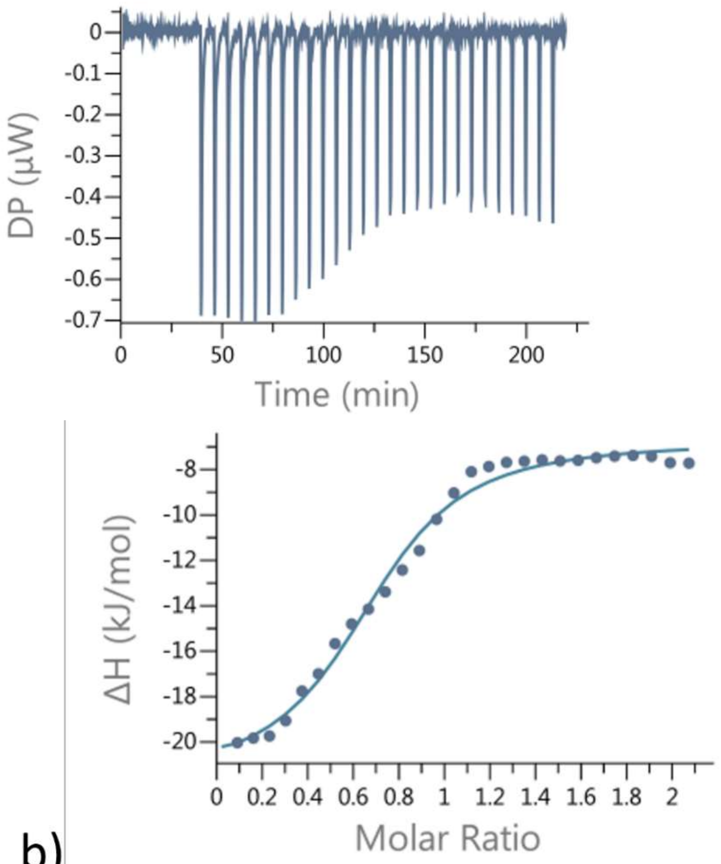
2. exergonic hDPP III – Kelch domain interactions

  - **ΔETGE** decreased binding affinity 1150%
  - Mutation **R623L**, **R623W** increase binding affinity 25 - 90%

ITC titration of DPP III into Kelch domain



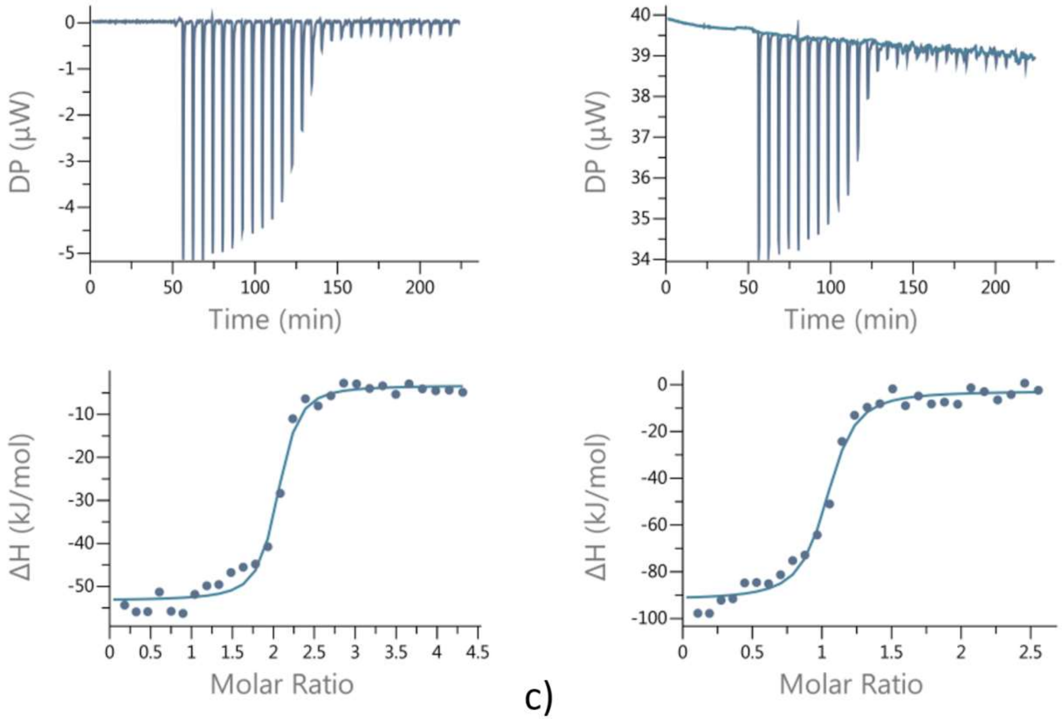
a)



b)

KD (M)	∆H (kJ/mol)	∆G (kJ/mol)	-T∆S (kJ/mol)
0.833E-06	-8.32	-34.7	-26.4
1.13E-06	-12.2	-34	-21.8

ITC titration of the Kelch domain into DPP III

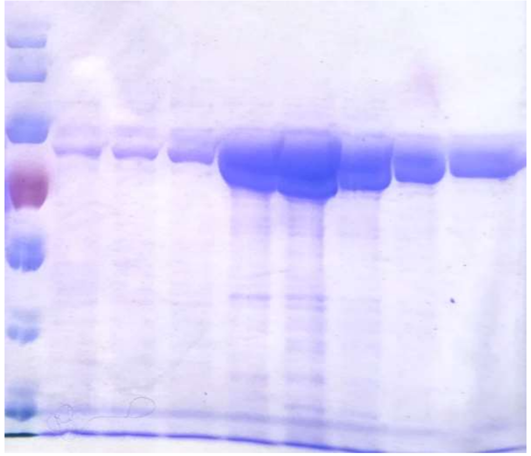


c)

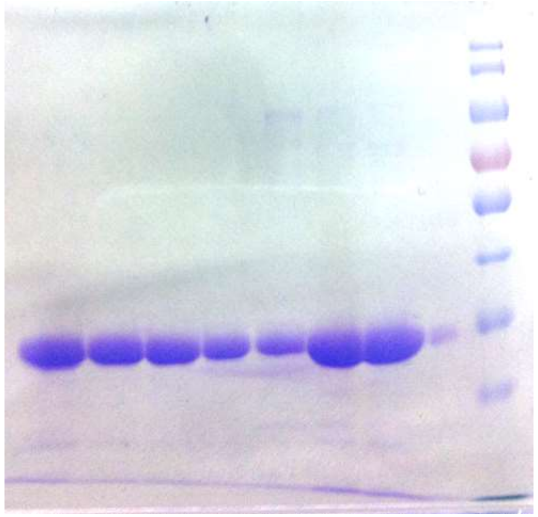
KD (M)	∆H (kJ/mol)	∆G (kJ/mol)	-T∆S (kJ/mol)	Offset (kJ/mol)
1.76E-06	-13.2	-32.9	-19.7	-17.4
8.82E-07	-8.21	-34.6	-26.4	-1.04

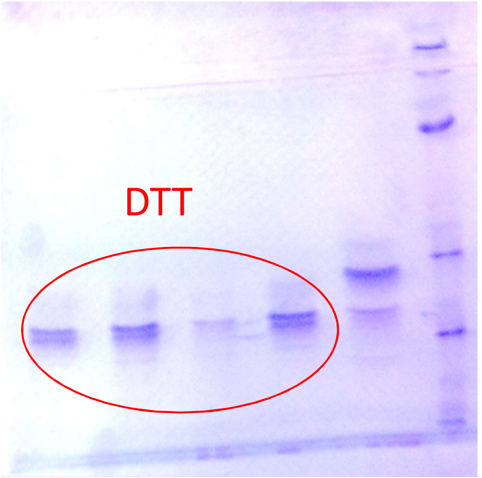
Potential sources of problems

DPP III



Kelch

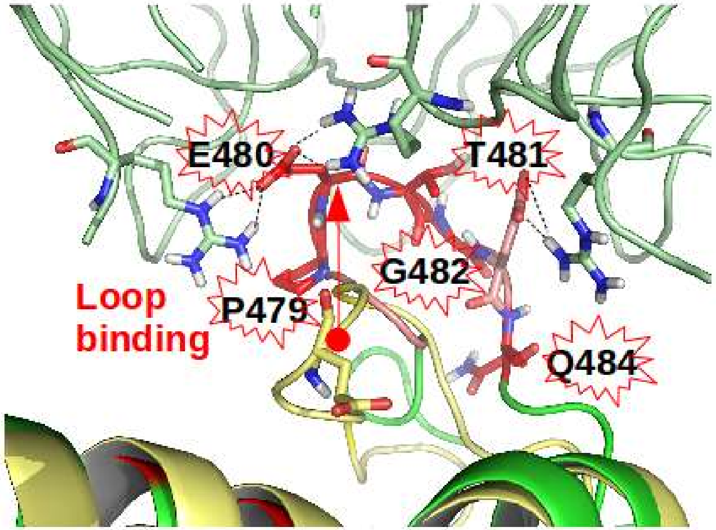




- **Protein purity**
- **Dimerization**

Mutations found in cBioPortalu for cancer genomics

Mutations in the ETGE loop



Kd values determined from initial fluorescence change

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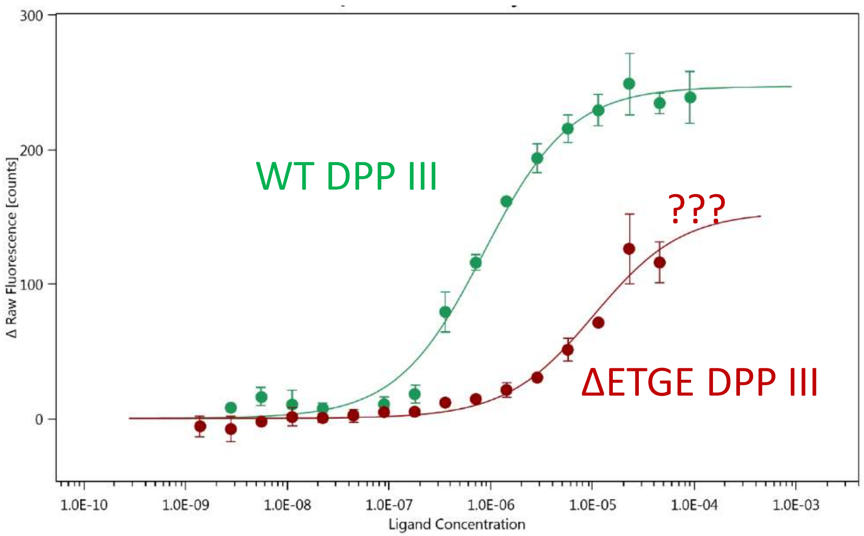
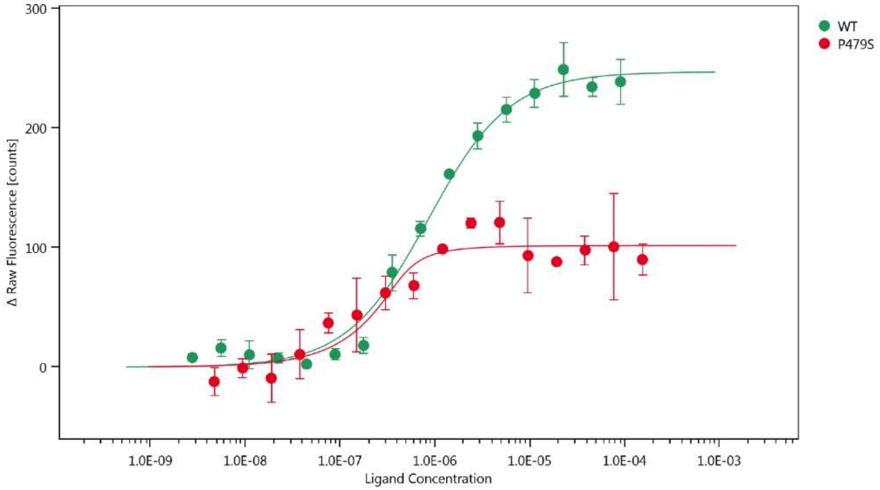
DPP III	Kd (nM)
WT	826 ± 108
P479S	128 ± 47
G482C	1220 ± 450
Q484H	219 ± 181

Initial fluorescence measurement on MST instrument



- **Error**
- **Fluorescence loss**



Next steps

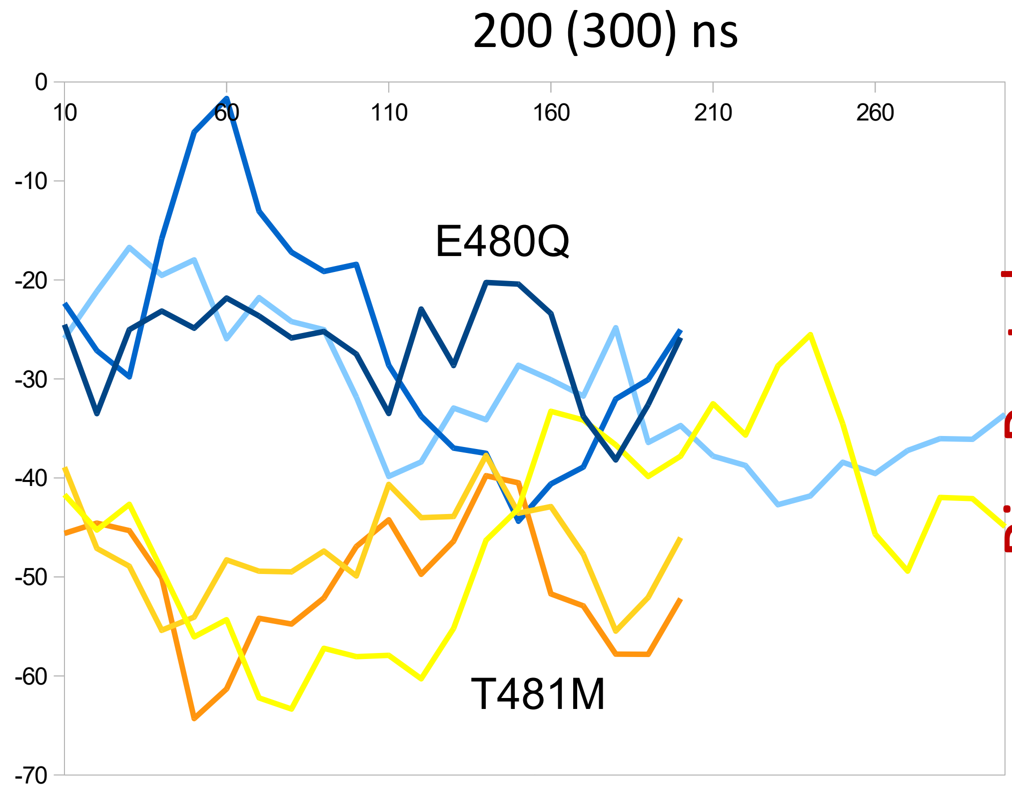
- Produce new wt and mutated proteins and purify by SEC (IEC)
- (crystallization, MST, ITC)
- Label new batch of Kelch
- Measure initial fluorescence change (MST) again – reproducibility
- Repeat ITC (protein purity, buffer, temperature)
- MD simulations analysis to support experimental data

Kelch – DPP III kompleksi

DPP III: WT, E480Q, T481M, P479S, G482C

Simulations – 700 ns = 300 ns + 2·200 ns

MMGBSA calculations



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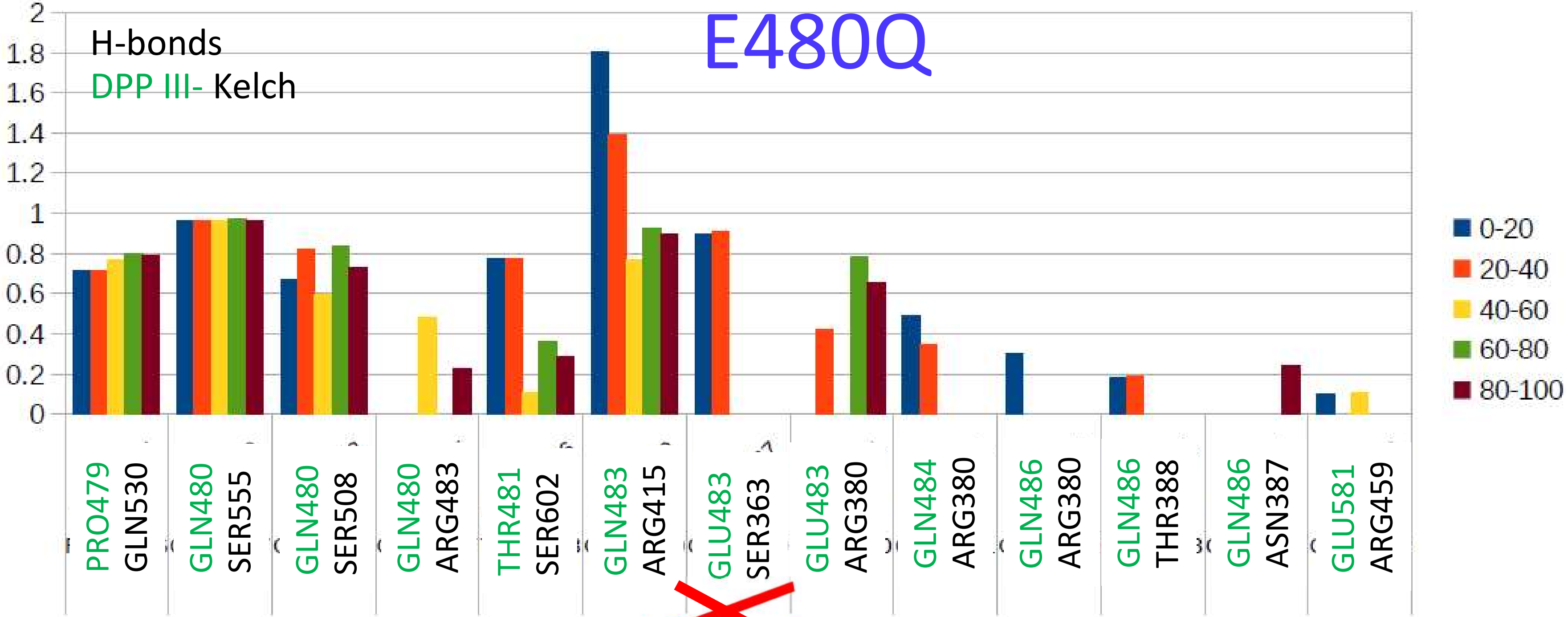
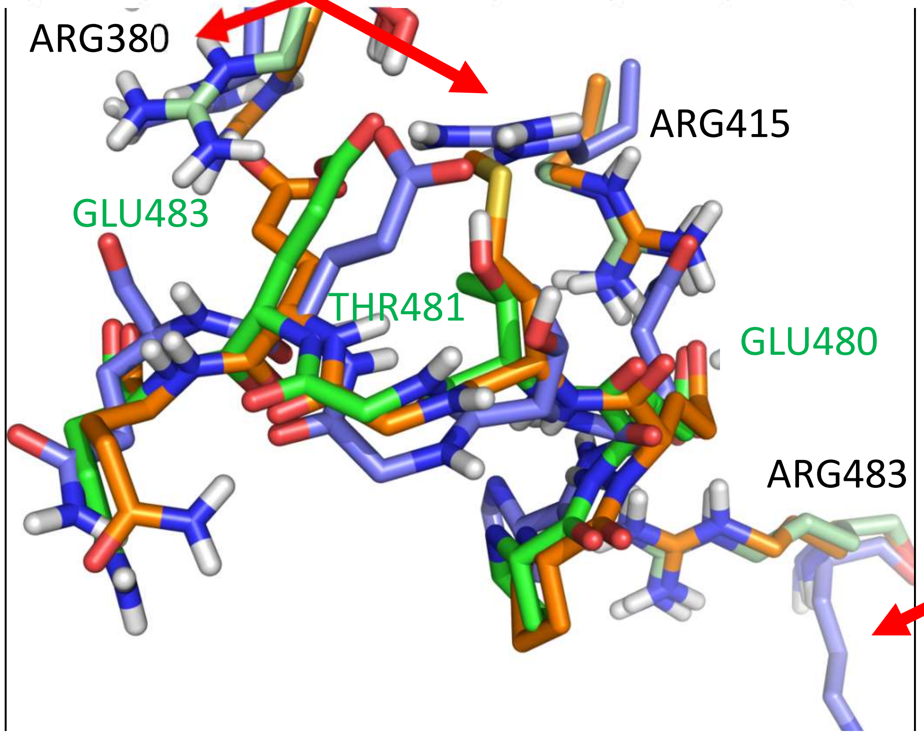
E480Q
ΔG_{MMGBSA}= - 44±6kcal/mol

T481M
ΔG_{MMGBSA}= -63±8kcal/mol

WT
ΔG_{MMGBSA}= -80±9kcal/mol

E480Q





Wt
E480Q
T481M

Alignment to the upper domain (420-660AA)

WT – gray

P479S – green

G482C – yellow

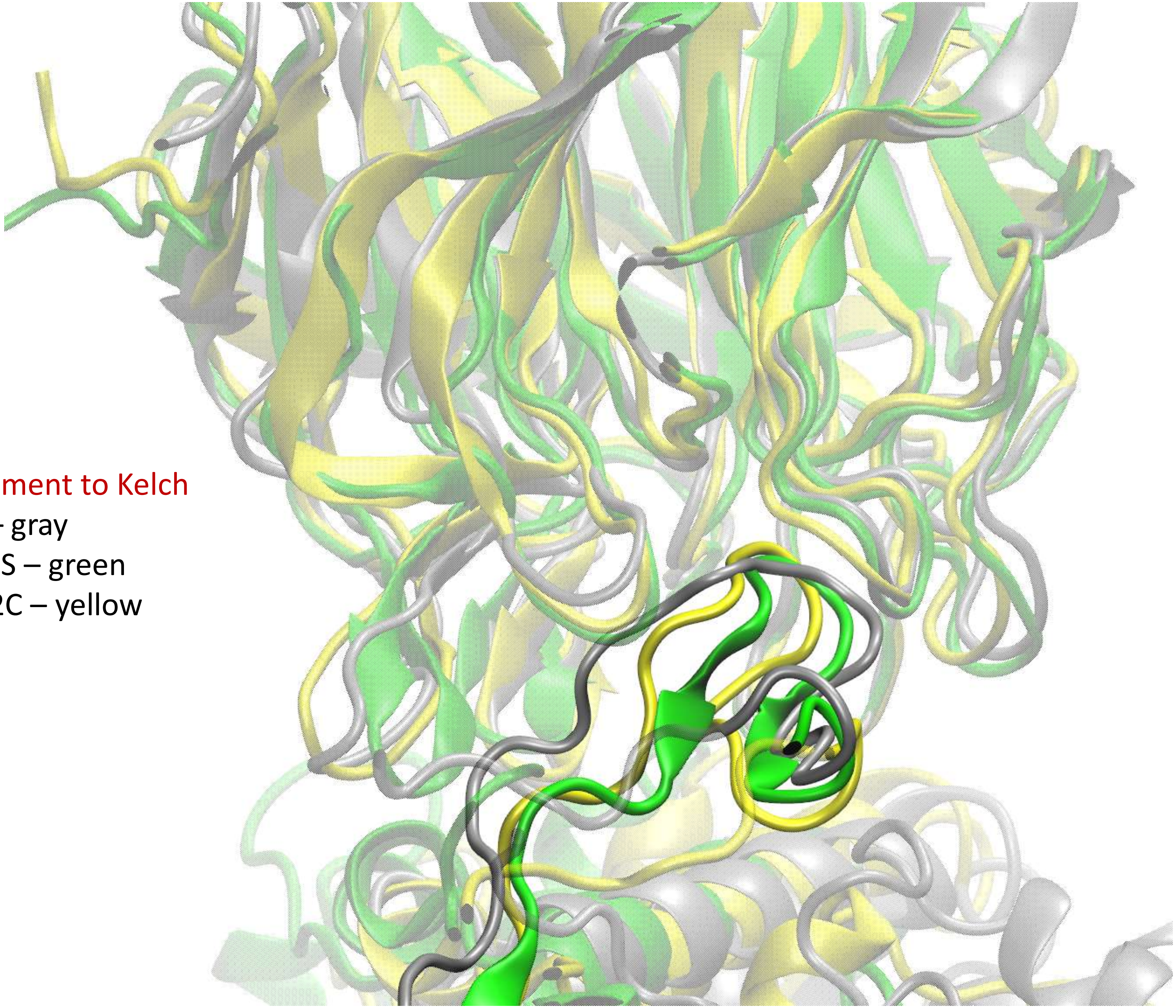
Alignment was performed for the structures sampled at the part of the trajectories for which the lowest MMGBSA energies were determined.

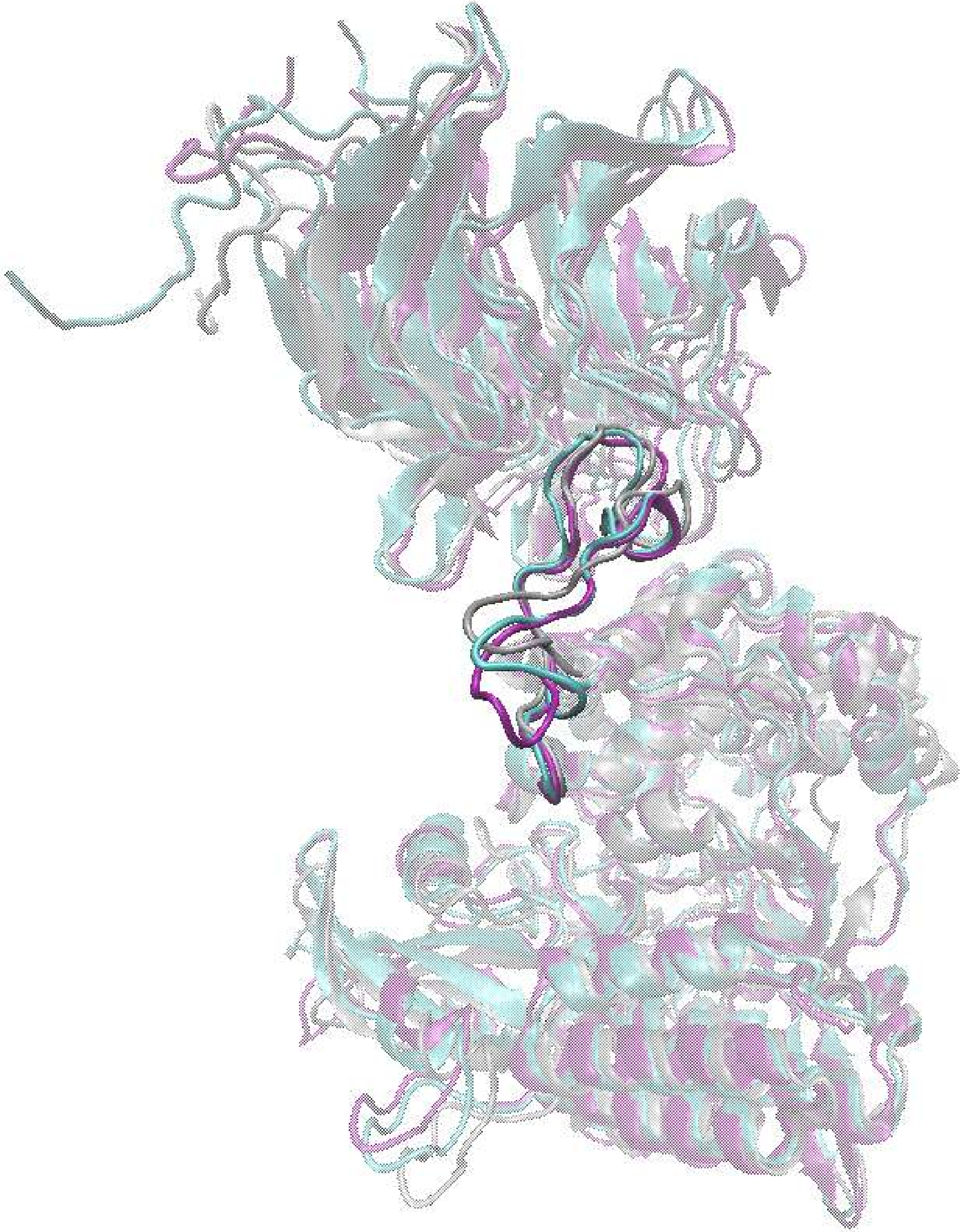
Alignment to Kelch

WT – gray

P479S – green

G482C – yellow





Alignment of several G482C-Kelch complexes structures (from different trajectories). The structure coloured gray is from the 'minimum MMGBSA energy' section. Alignment was performed to the upper hDPP III domain.

l WT-Kelch
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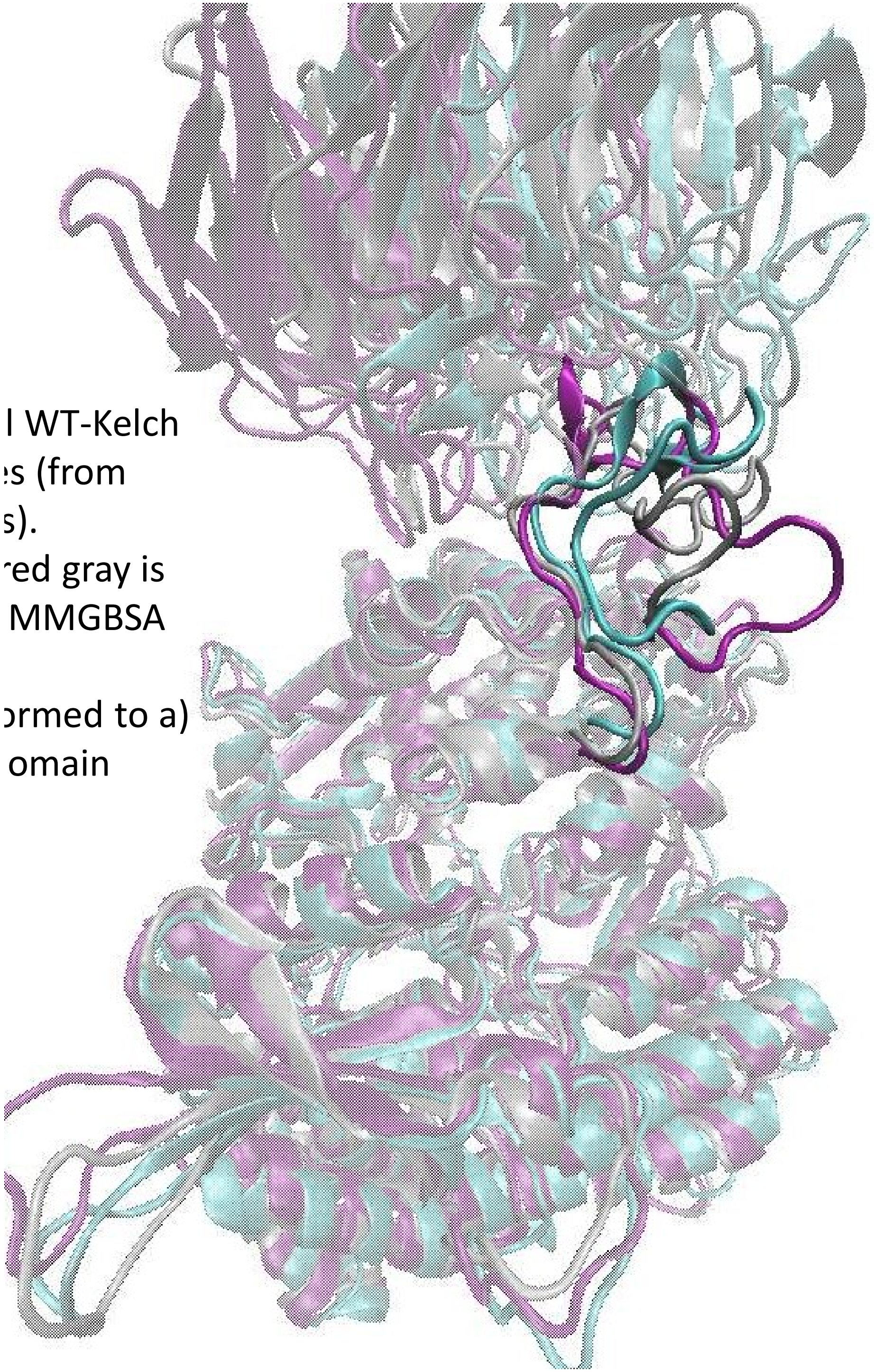
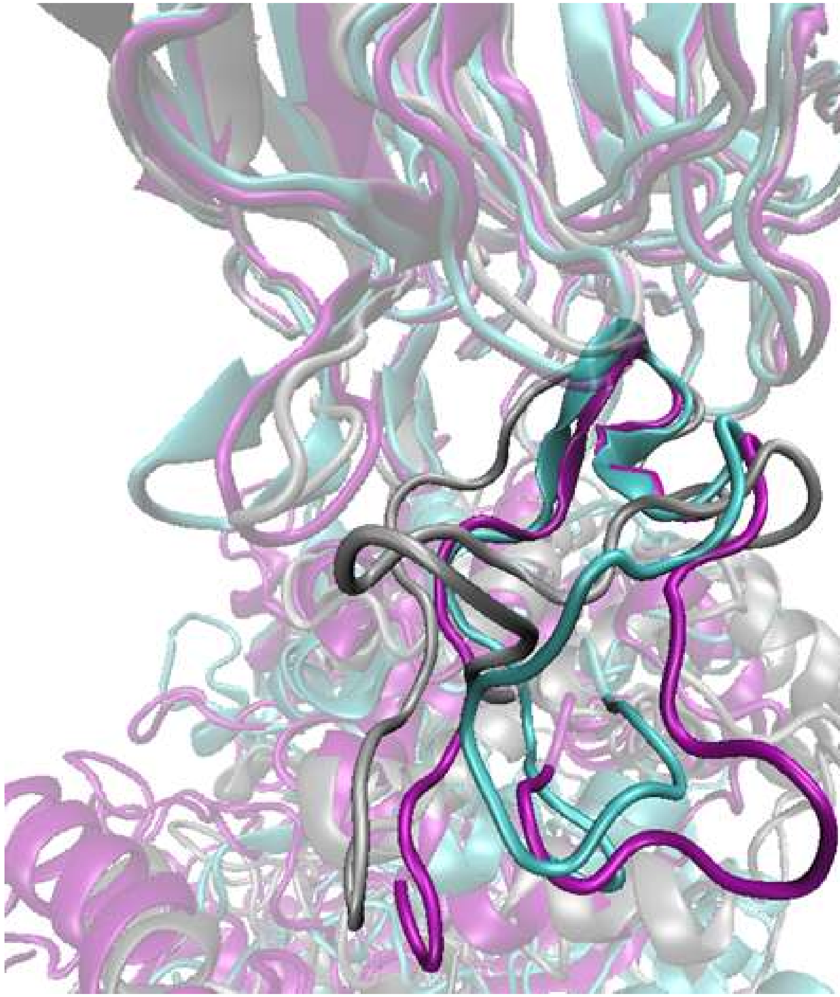
s).
red gray is
MMGBSA

ormed to a)
omain

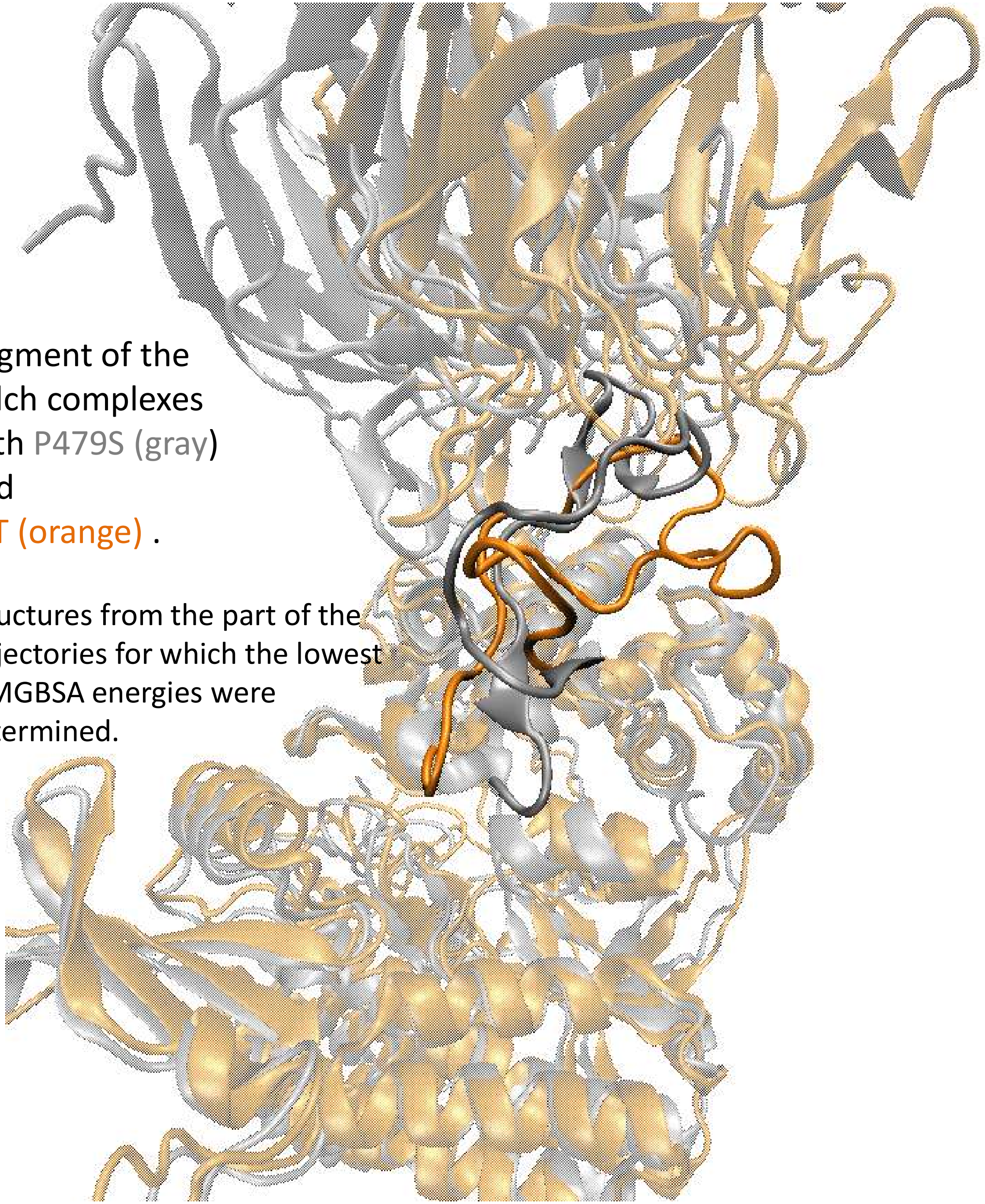


b)

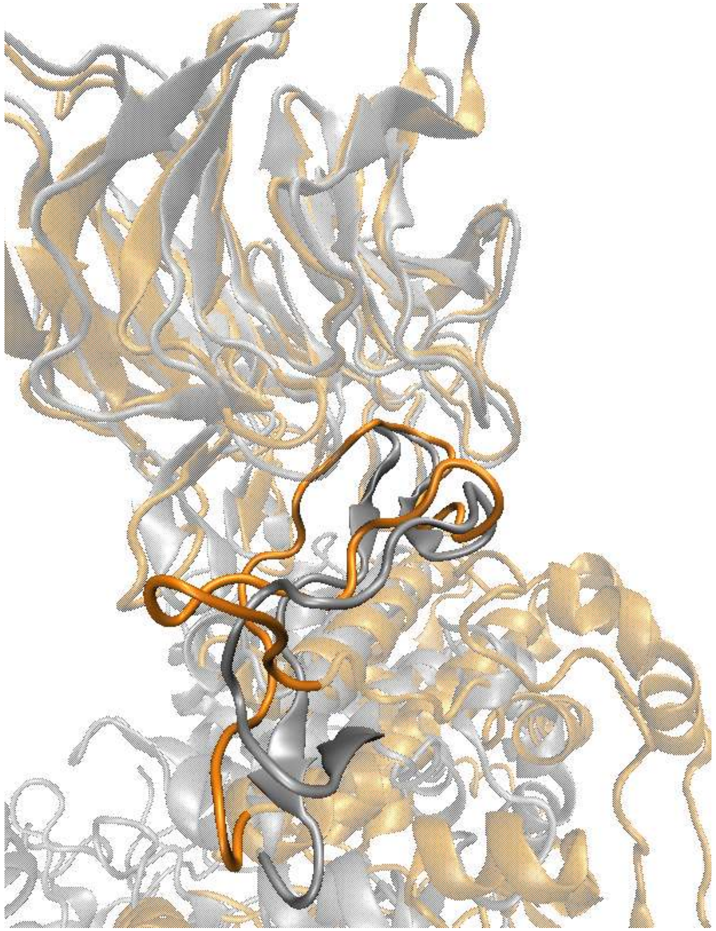


Aligment of the Kelch complexes with P479S (gray) and WT (orange) .

Structures from the part of the trajectories for which the lowest MMGBSA energies were determined.

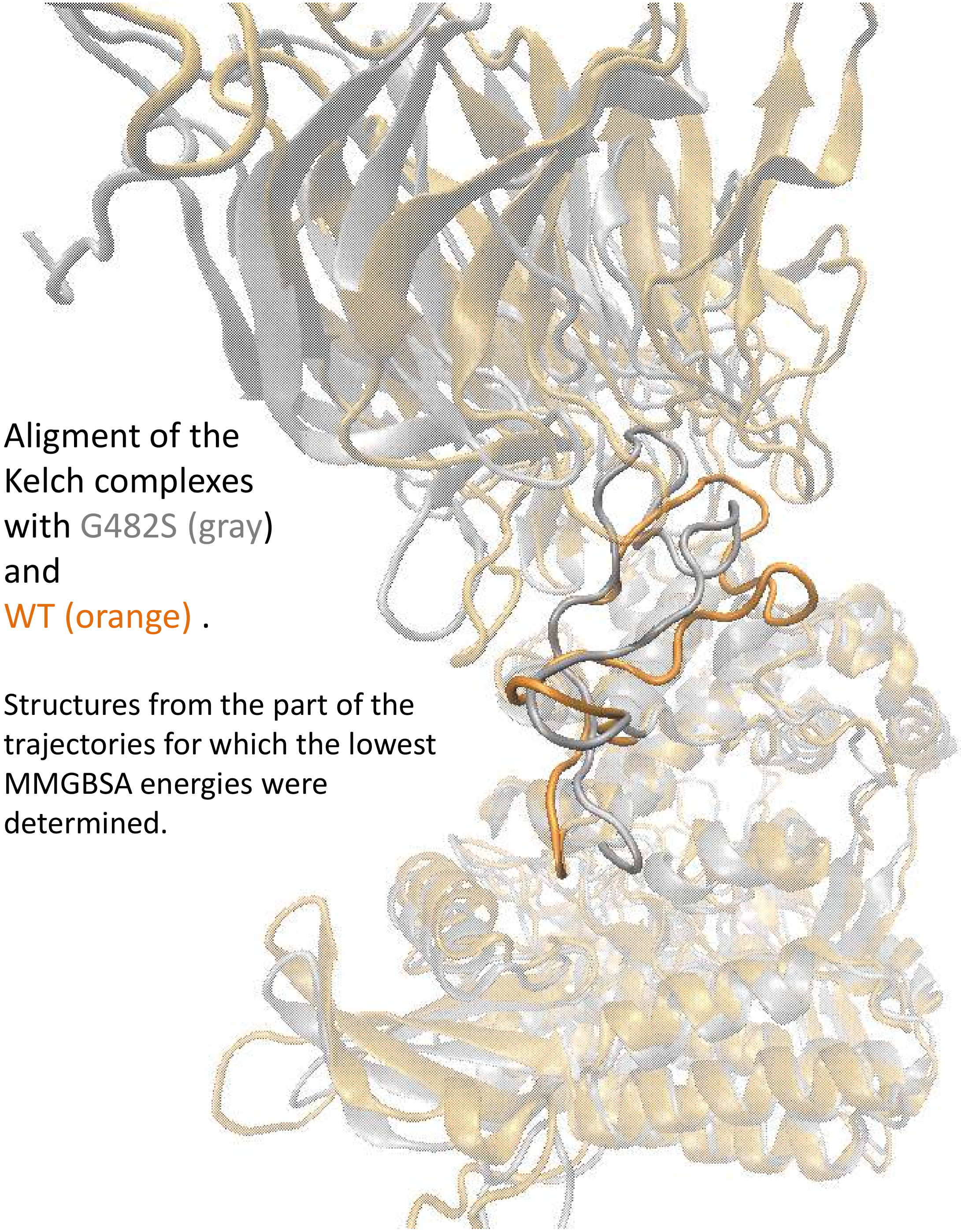


Alignment was performed to the upper hDPP III domain.

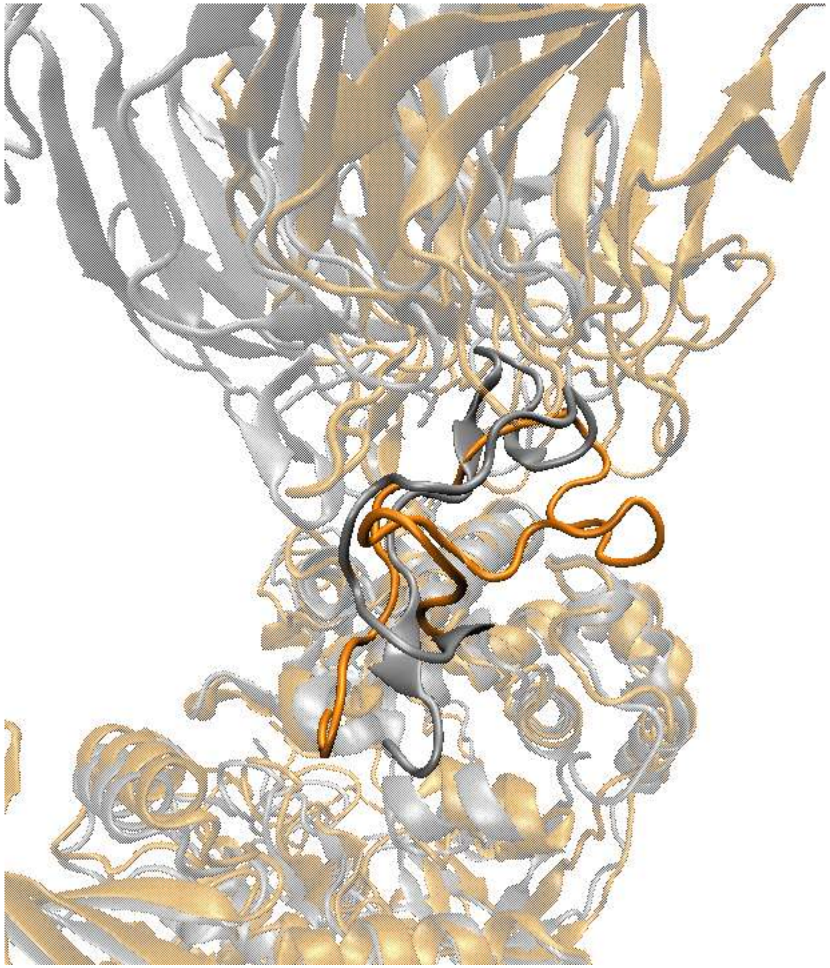


Aligment of the Kelch complexes with G482S (gray) and WT (orange) .

Structures from the part of the trajectories for which the lowest MMGBSA energies were determined.



Alignment was performed to the upper hDPP III domain.



D	A	A	D
E480	S555	R624	C434
	R415	R624	H436
	R483	Q473	G574
	S508	R620	D479
T481	S1006	**0.6**	
Q486	N387		
	R380		
P479	Q530		
E483	R380		
	N387		
	S363		
Q473	Q530		
C482	Y334		
N478	R483		
T481	S602		
10.2			

G482S-Kelch

Intermolecular hydrogen bonds

Hydrogen bond analysis performed for the 20 ns long trajectory for which the lowest MMPBSA energies were determined.

$$\Delta G_{MMGBSA}^{20} = -55\underline{+}7\,\mathrm{kcal/mol}$$

$$\Delta G_{MMGBSA}^{TOT} = -43{,}0\ \mathrm{kcal/mol}$$

D	A	A	D
E480	S555	S487	D385
	R415	Q484	Y334
	R483	R624	C434
	S508	K498	D385
Q486	N387	E474	H575
	Y334	R620	T481
T481	S602	**0.8**	
E483	R380		
S479	Q530		
D472	R363		
T475	R363		
E581	R459		
N470	R363		
11.4			

P479S-Kelch

Intermolecular hydrogen bonds

Hydrogen bond analysis performed for the 20 ns long trajectory for which the lowest MMPBSA energies were determined.

$$\Delta G_{MMGBSA}^{20} = -50 \pm 8 \text{kcal/mol}$$

$$\Delta G_{MMGBSA}^{TOT} = -39{,}6 \text{ kcal/mol}$$

WT DPP III - Kelch

Intermolecular hydrogen bonds

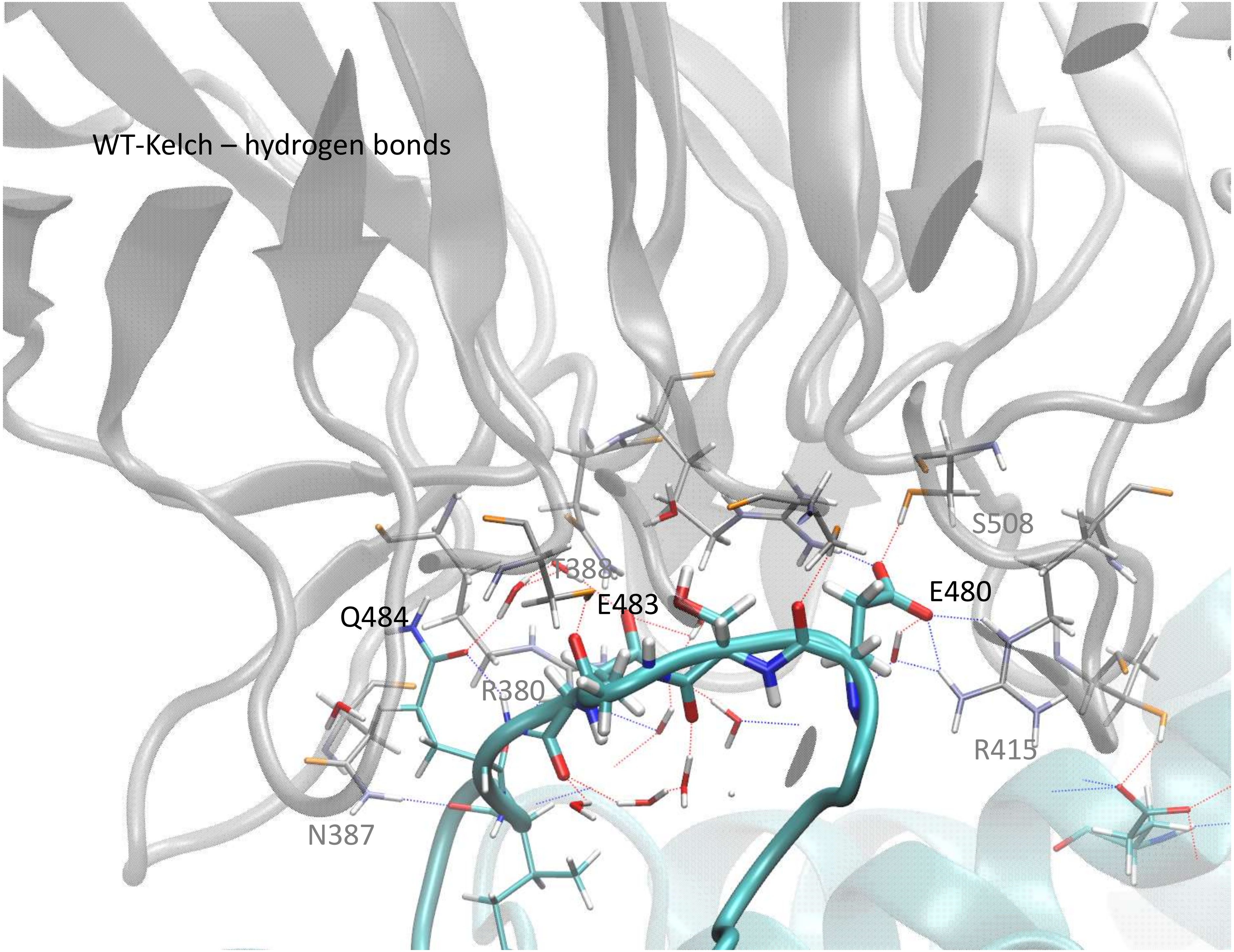
D	A	A	D
E480	S555	Q473	H575
	R415	R620	D479
	R483	Q486	D389
	S508		D387
Q484	R380	R490	P384
	S363		D385
Q486	N387	Q484	N381
P479	Q530		R380
Q473	R336		S363
E483	N416	E474	H393
I485	N387	R624	C434
E619	T481		H436
E474	H575	R623	T481
G482	S602	**5.1**	
10.7			

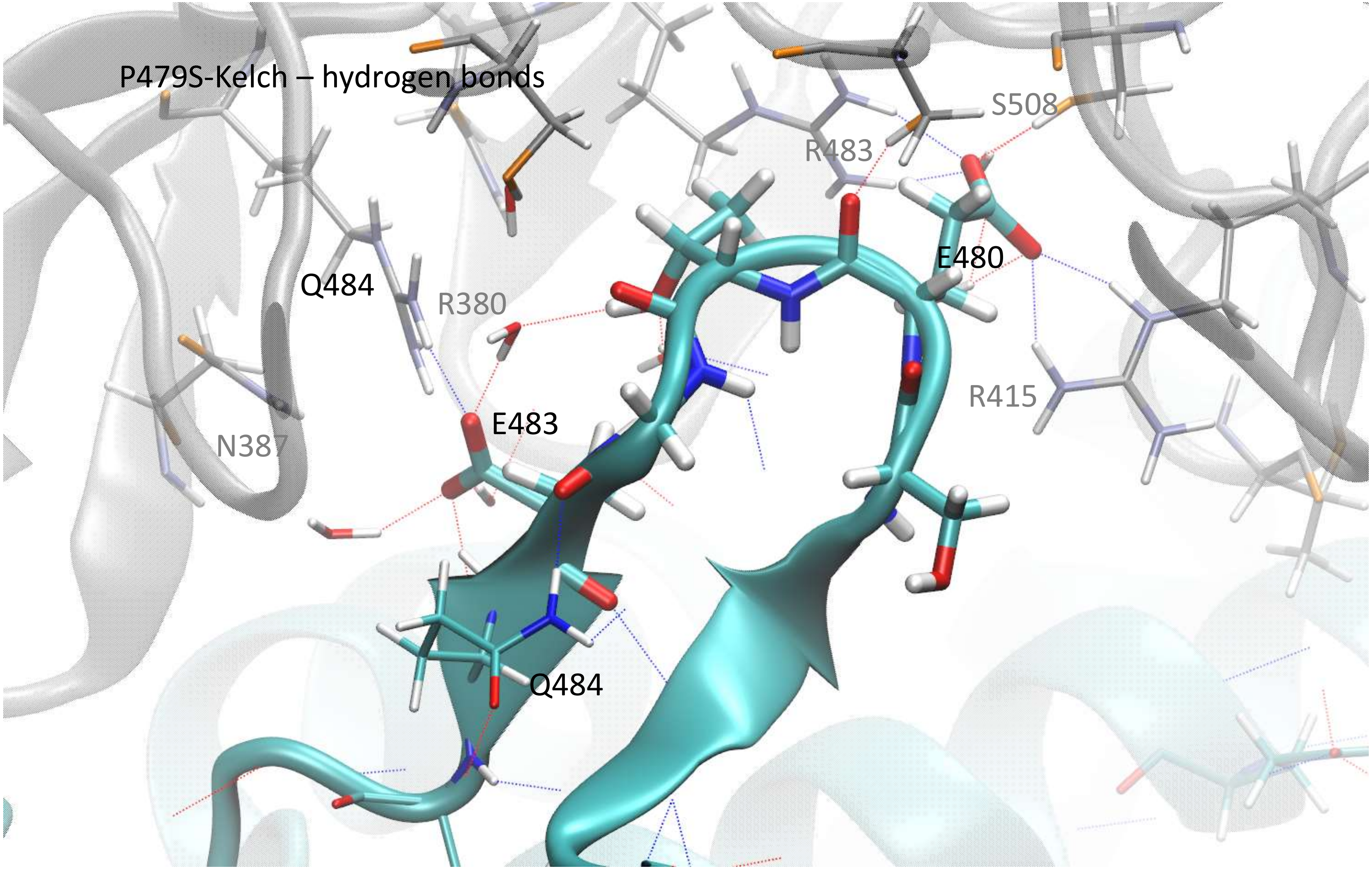
Hydrogen bond analysis performed for the 20 ns long trajectory for which the lowest MMPBSA energies were determined.

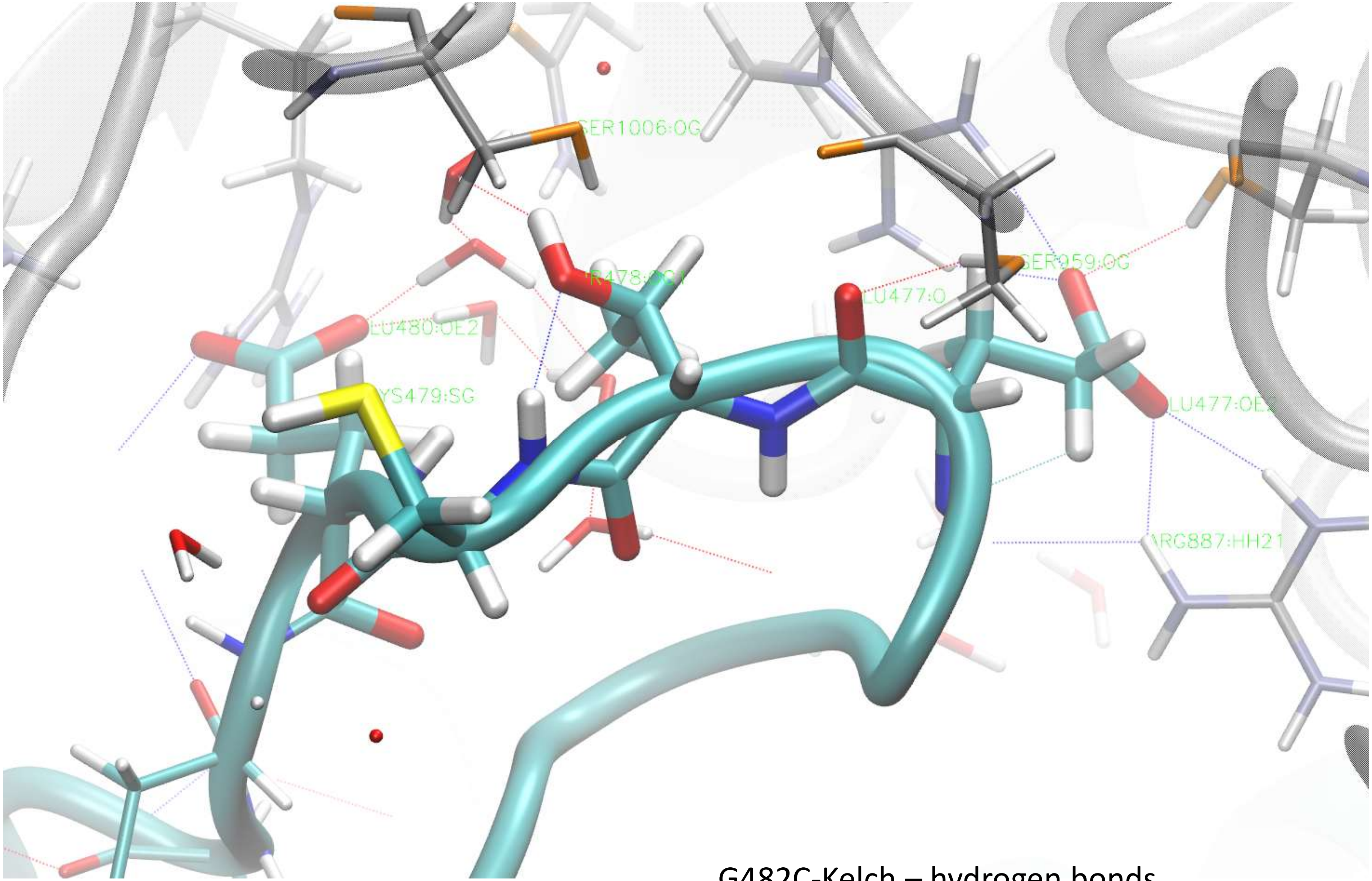
$$\Delta G_{MMGBSA}^{20} = -80\pm9\,\mathrm{kcal/mol}$$

$$\Delta G_{MMGBSA}^{TOT} = -50{,}6\ \mathrm{kcal/mol}$$

WT-Kelch – hydrogen bonds




P479S-Kelch – hydrogen bonds



G482C-Kelch – hydrogen bonds

To be done

Experimental measurements for the computational studied DPP III mutants in cancer (?P479S mutant – resolve discrepancy).

Experimental and theoretcal studies of the ‘**relevant’** Kelch mutants found in cancer and their complexes with DPP III