Exploring the effect of mutations on interactions between Keap1 and DPP III by MD simulations

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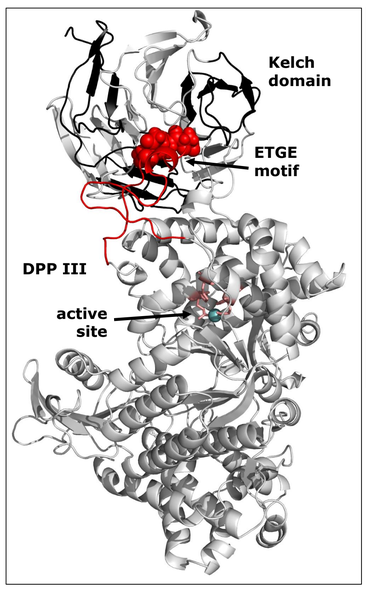
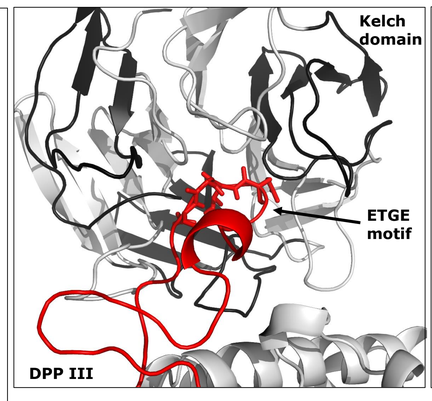
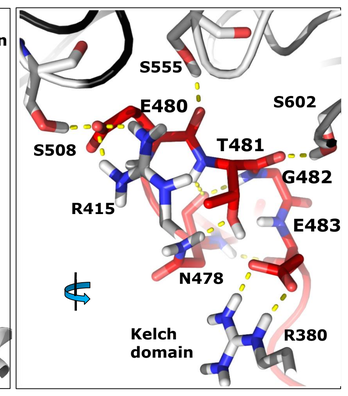
Dipeptidyl peptidase III (DPP III) is a zinc-exopeptidase that hydrolyses dipeptides from the N-termini of short peptides. It cleaves bioactive peptides, such as angiotensin, Leu-enkephalin and Met-enkephalin, and it is involved in the final steps of intracellular protein catabolism [1,2]. In addition to its catalytic activity, DPP III possesses the "ETGE" motif, which is one of the NRF2 transcription factor motifs responsible for its binding to Keap1 followed by NRF2 ubiquitination and degradation. According to the experimental results, competitive binding of DPP III to Keap1 resulted with increased downstream activity of NRF2 [3,4] leading to enhanced antioxidative response and resistance to anti-cancer treatment.

The large-scale cancer genomics data offers insights into mutations found in cancer cells, which could help predicting possible 'functional' mutations in DPP III and Keap1, potentially responsible for the variations in their interaction affinity. In this work, molecular dynamics (MD) simulations were used to study how the point mutations of both, DPP III (R703H and R703C), and Keap1 (R326D), influence the structure and dynamics of the Keap1-DPP III complex.



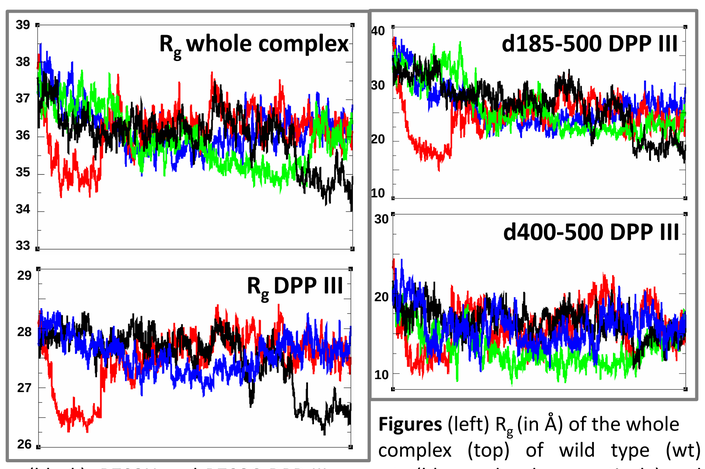




Figures (left) Structure of DPP III – Keap1 Kelch domain complex, (middle) position of the DPP III loop with ETGE motif in the binding site of Kelch and (right) the main interactions established during complex formation.

Radius of gyration (R_g)

Radius of gyration $R_g = \sqrt{\sum m_i \, r_i^2 / \sum m_i}$, was used to measure the compactness of DPP III- Kelch complex, as well as each protein individually. The compactness of the complex mainly correlated with the compactness Of DPP III. Therefore, the degree of DPP III closure was evaluated by measuring the distances between pairs of Cα atoms at opposite sides of the DPP III inter-domain cleft, d185-500 and d400-500. Our previous study showed that the closed DPP III conformation is the active one [5].



Figures (left) R_g (in Å) of the whole complex (top) of wild type (wt) (black), R703H and R703C DPP III mutants (blue and red, respectively) and the R326D Kelch mutant (green) and the DPP III R_g for wt and the DPP III mutants (bottom), (right) distances (in Å) of residue pairs in lower and upper domain of DPP III during 300 ns MD simulations.

Final complexes

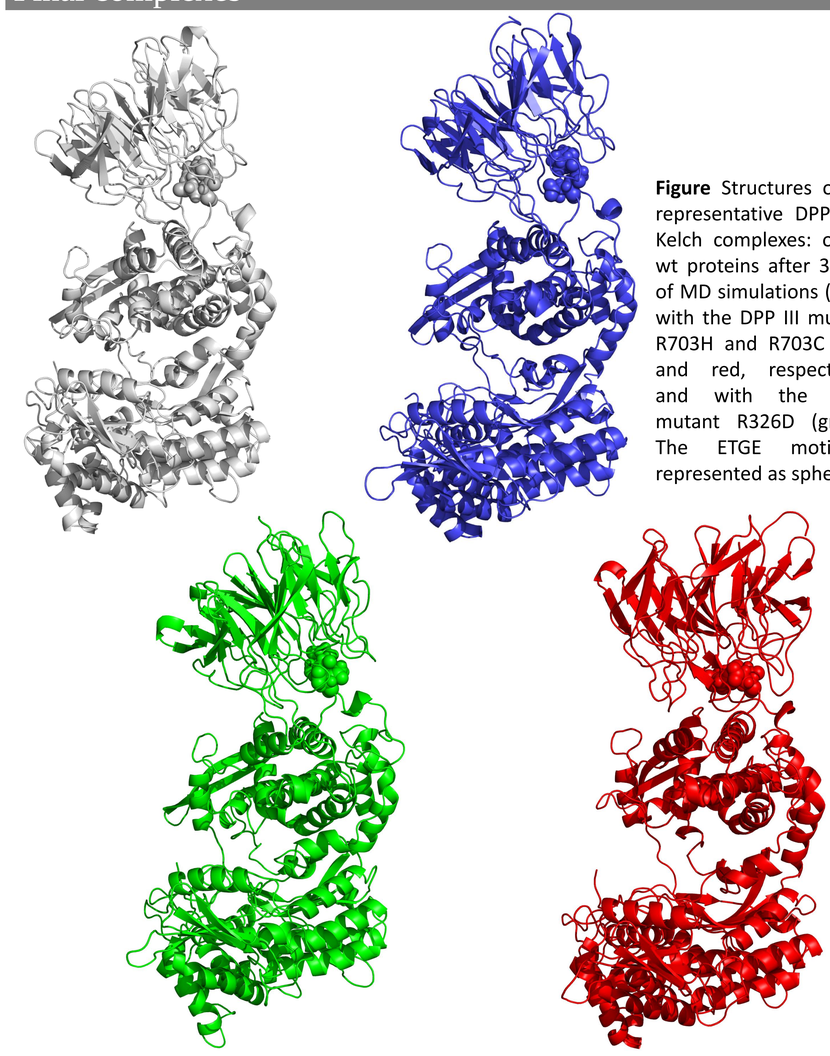
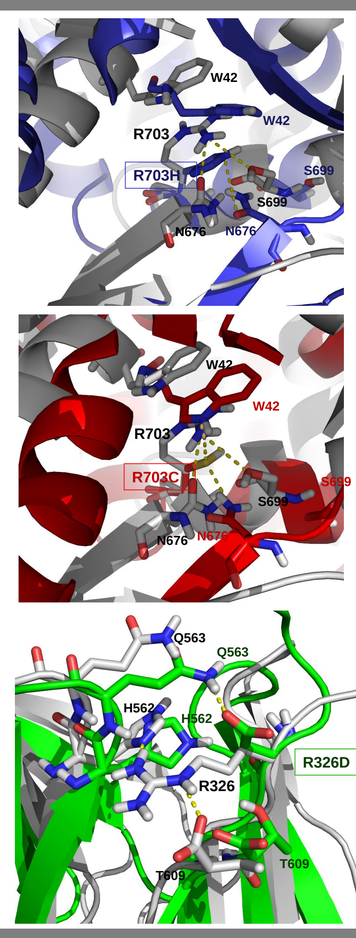
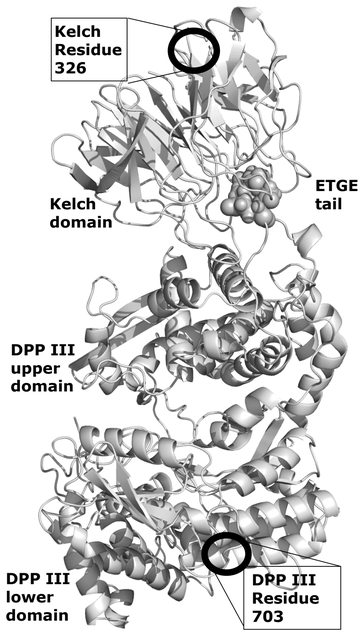


Figure Structures of the representative DPP III – Kelch complexes: of the wt proteins after 300 ns of MD simulations (gray), with the DPP III mutants R703H and R703C (blue and red, respectively) and with the Kelch mutant R326D (green). The ETGE motif is represented as spheres.

Environment of mutated residues



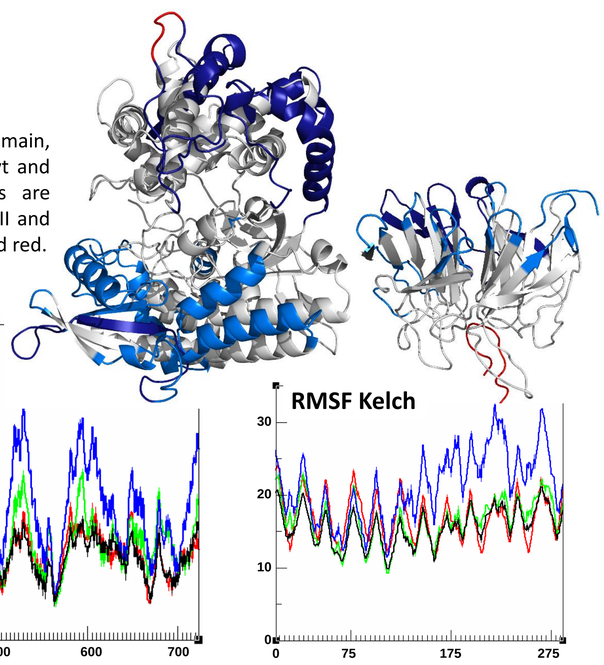
Figures (left) Position of the mutated residues in the wt (gray) DPP III – Kelch complex with marked positions of mutated residues. (right) Interactions of wt DPP III R703 and Kelch R326 comparing to mutated residues R703H, R703C and R326D .



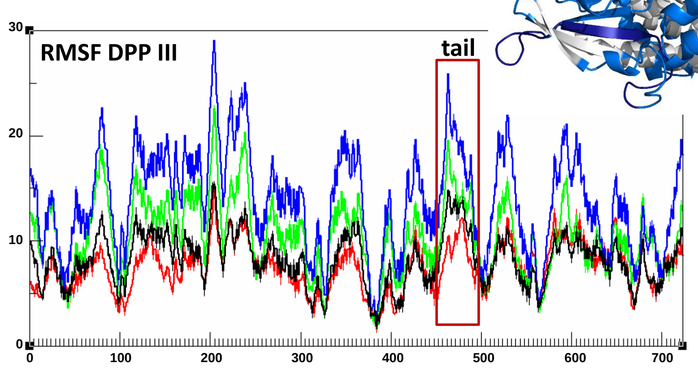
Root mean square fluctuation (RMSF)

RMSF, computed as $RMSF = \sqrt{1/T \sum_{t_j=1}^{T} (x_i(t_j) - \tilde{x_i})^2}$, from the atomic coordinates of C_a, describes the fluctuation of their position during the simulation time T.

The highest fluctuations are observed for the simulation of the DPP III mutant R703H, followed by the Kelch mutant R326D. In all systems the fluctuations of the loop region containing ETGE motif are above average. In the Kelch domain, fluctuations of the outer region, opposite to the binding site, are the highest.



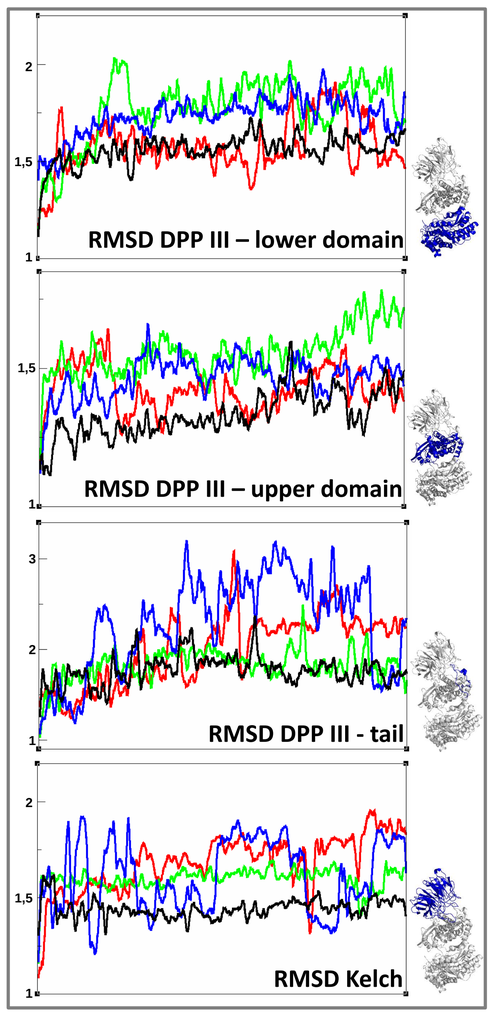
Figures RMSF (in Å) of the DPP III and Kelch domain, per residue, during 300 ns MD simulations of wt and mutant complexes. The most flexible regions are coloured blue in PyMol representations of DPP III and Kelch, and the tail containing ETGE motif is marked red.



Root mean square deviation (RMSD)

RMSD ($\sqrt{1/n \sum_{i=1}^{n} \|x_i - \tilde{x_i}\|^2}$) was used to compare structures sampled during the simulations to the initial one, in order to quantify conformational changes in each segment of the complex.

Figure RMSD (in Å) of the DPP III lower and upper domain, its tail region and the Kelch domain during 300 ns MD simulations of wt and mutant complexes.

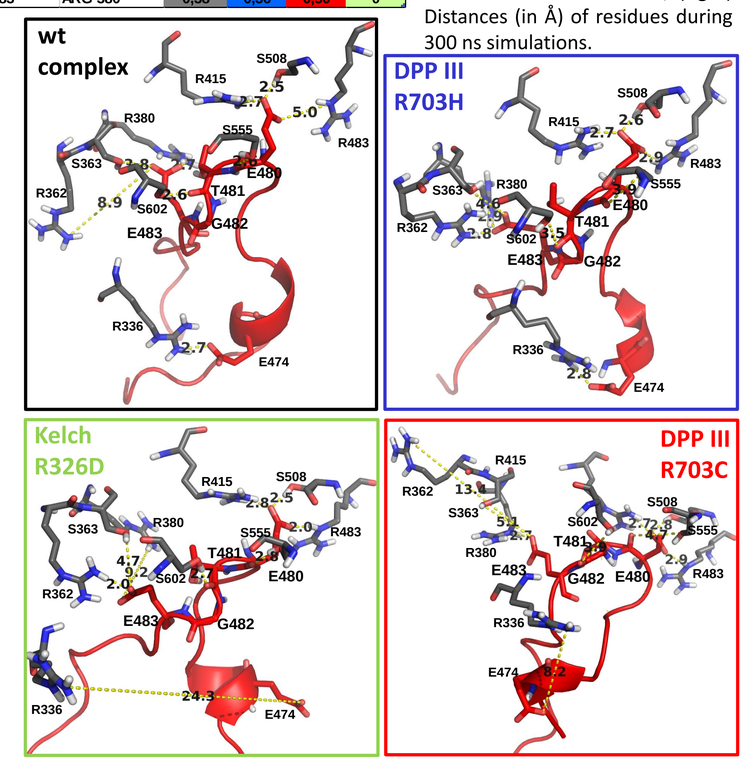


Interactions of ETGE motif and the tail with Kelch domain

The nine residues of the Kelch domain listed in Table below were found to form the most frequent interactions with the DPP III tail, especially with the ETGE motif. Four arginines (R415, R483, R363 and R380) and two serines (S508 and S363, respectively) are involved in interactions with the $E^{480}TGE^{483}$ glutamates side chains. Serines (S555 and S602) stabilize the ETGE backbone, and the additional contacts are established between the adjacent glutamate E474 and arginine R336.

Hydrogen bonds		wt complex	DPP III R703H	DPP III R703C	Kelch R326D
Acceptor (tail)	Donor (Kelch)	Fraction of time			
GLU 474	ARG 336	0,46	0,26	0,12	0
GLU 480	ARG 415	0,44	0,34	0,38	0,44
GLU 480	ARG 483	0,28	0,33	0,51	0,20
GLU 480	SER 508	0,49	0,59	0,47	0,58
GLU 480	SER 555	0,82	0,58	0,07	0,84
THR 481	SER 602	0,86	0,42	0,25	0,92
GLU 483	ARG 362	0,14	0,50	0,31	0,49
GLU 483	SER 363	0,23	0	0,22	0,35
GLU 483	ARG 380	0,38	0,36	0,50	0

Table Fraction of simulation time during which the listed residues are hydrogen bonded.



Figures (left) Positions and distances between residues in the wt and mutant complexes after 300 ns of MD simulations, (right) Distances (in Å) of residues during 300 ns simulations.

Summary

Using MD simulations of wild type and mutant variants of DPP III-Keap1 complexes, we were able to follow fluctuations and conformational changes of their structures, and to study the interactions between two proteins on molecular level. This preliminary study will be continued by performing further simulations, and the results will be compared to the experimental data obtained by hydrogen exchange mass spectrometry and isothermal titration calorimetry.

References

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5) Tomić and Tomić. Dalton Trans. 2014;43(41):15503-14.

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