Interaction of human dipeptidyl peptidase III and Keap1

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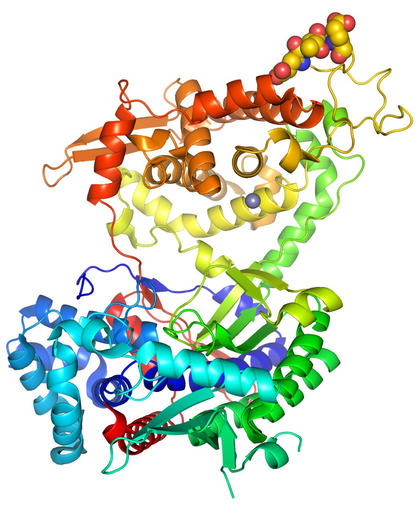
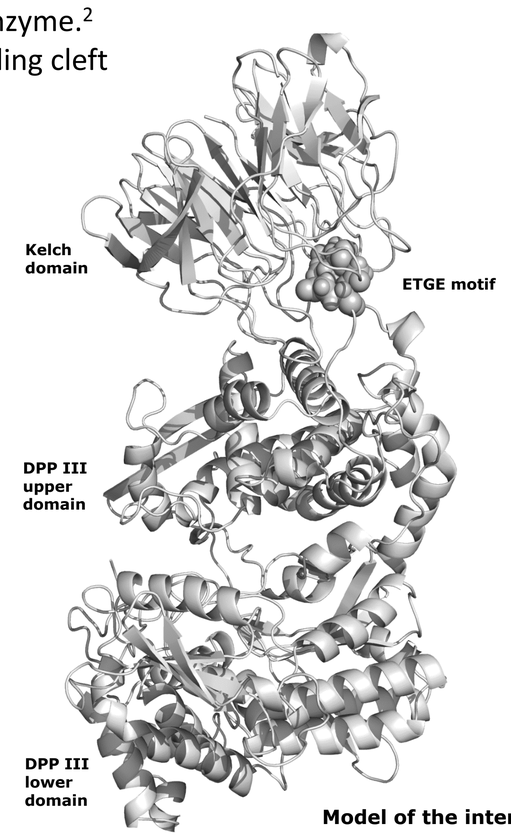
Human dipeptidyl peptidase III

Dipeptidyl peptidase III (DPP III) is a metallo-exopeptidase suspected to be involved in various physiological processes: protein catabolism, blood pressure regulation, pain modulation, inflammation, and oxidative stress response, and it is overexpressed in several different tumor types.[1] DPP III cleaves N-terminal dipeptides off oligopeptides 4-8 amino acids long. Structural and enzymatic investigations have described the mechanism and mode of action of this enzyme.[2] Peptide hydrolysis involves closing of the substrate binding cleft through domain movement.

The only well documented case of protein-protein interaction is the interaction with Keap1. The interaction is independent from DPP III peptidase activity.[3]



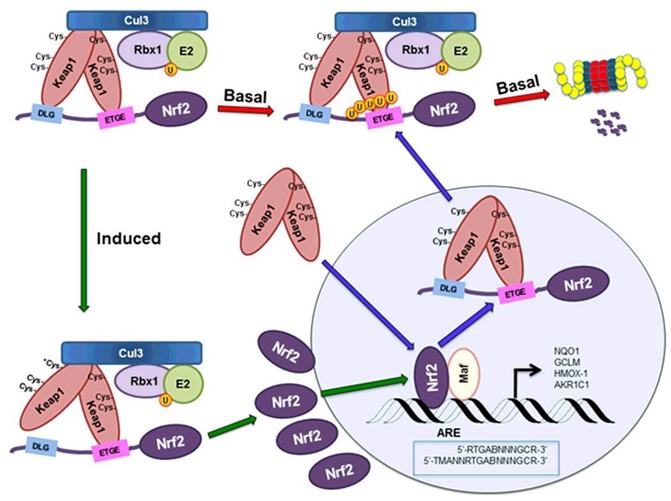
Keap1 interacts with DPP III through its Kelch domain, and the interaction is mediated through the ETGE motif of DPP III, located on a loop between two conserved regions that build the peptidase active site.



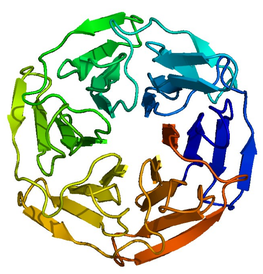
Model of the interaction

Keap1

Keap1 is a cytoplasmic sensor for oxidative stress, regulating expression of hundreds of cytoprotective genes through the release of a transcription factor Nrf2.[4] Nrf2 binds to Keap1 through DLG and ETGE motifs.[5]

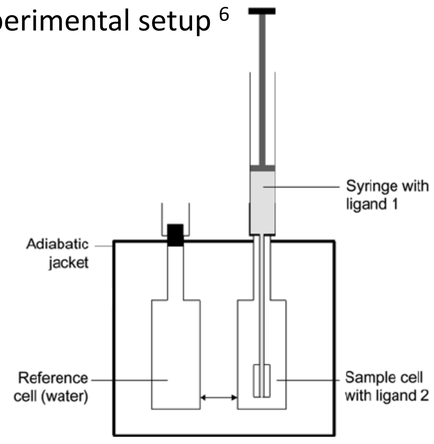


The structure of the Nrf2-interaction domain, Kelch, has been solved. All proteins interacting through the ETGE motif are presumed to bind in a way similar to Nrf2.

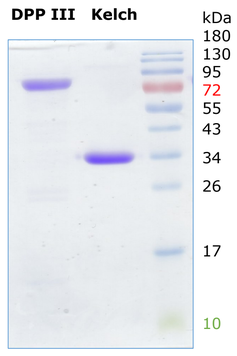


Isothermal titration calorimetry (ITC)

Experimental setup [6]



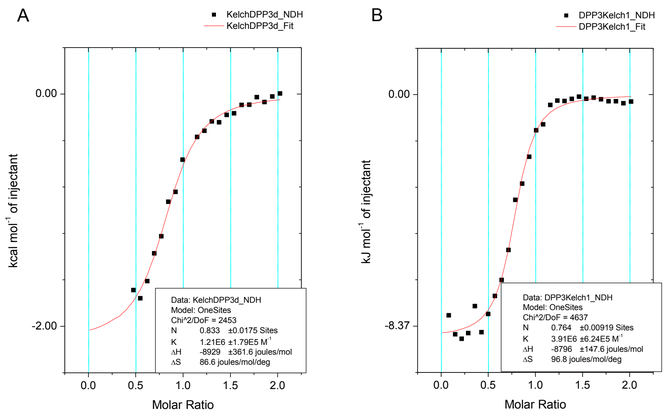
Purified proteins



We are currently using ITC to determine the thermodynamic parameters of the interaction between DPP III and Kelch domain of Keap1. In the future, we plan to use it to determine binding affinities of other possible interactors of DPP III.

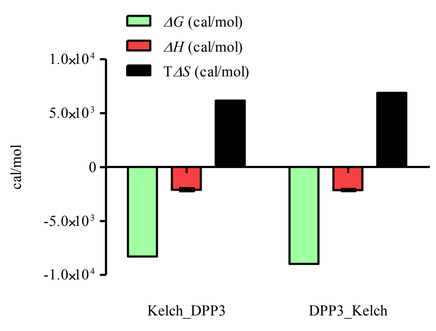
Results

Binding isotherms from ITC titrations: A) Kelch in the syringe (176 µM) and hDPP3 in the cell (18 µM); B) hDPP3 in the syringe (156 µM) and Kelch in the cell (16 µM).



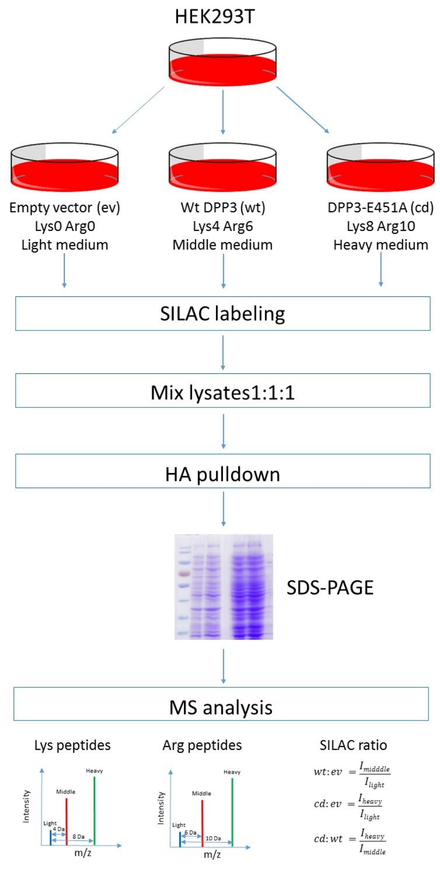
The results show relatively high affinities in both setups: A) K_D=0.8 µM; B) K_D=0.3 µM.

Calculated thermodynamic parameters show considerable entropic contribution to the free energy. This indicates that the more pronounced driving forces are hydrophobic interactions.



SILAC approach to identify new DPP III interactors

New candidates will be obtained using the SILAC mass spectrometry approach.



References

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Acknowledgements

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