Detection of the impairment of allosteric regulation in Sirtuin2 proteins through molecular dynamics and residue coevolution analysis

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Introduction

Sirtuin2 is an NAD+-dependent protein deacetylase involved in several biological processes based on genome integrity. Its misregulation can disrupt the deacetylase activity, making Sirtuin2 an attractive drug target for chemoprevention of human diseases. E. coli **CobB** is a bacterial Sirtuin2 homologue characterized by a smaller zinc-binding domain ***(Fig1)***. Selective substrate-binding in CobB is mediated by distal molecular interactions between the zinc-binding domain and the pocket residues around the acetyl-lysine modified residue on the substrate. This supports the hypothesis of an allosteric regulation of Sirtuin2 proteins. We present a computational study based on the combined use of molecular simulations and co-evolution analysis of CobB to unveil Sirtuin2 mechanisms of substrate recognition and of allosteric communication that could explain a conserved role of distal molecular interactions.

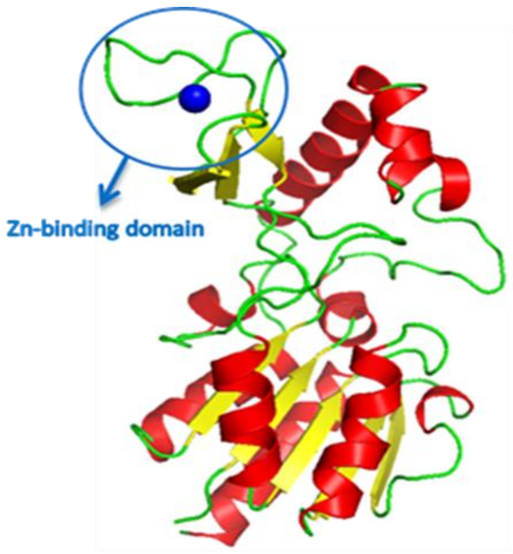

Fig1. CobB

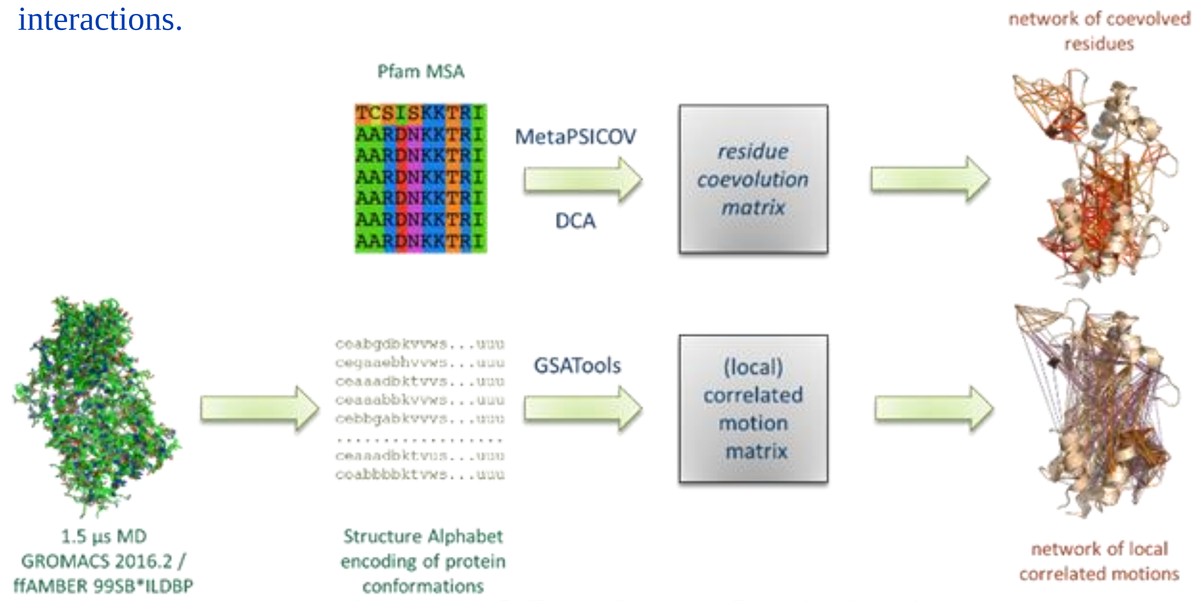

Fig2. Workflow for study of Sirtuin2

Methods

The study consists of three methodological steps:

a) **Residue coevolution analysis.** A multiple sequence alignment for CobB and residue coevolution analysis was performed. Conserved and coevolved rigid domains were detected with SPECTRUS-Evo.

b) **Molecular dynamics simulations.** Molecular Dynamics simulations were performed using ARCHER Supercomputing. Three replicas of 500ns were generated using GROMACS 2016.2. Critical residues and functional local correlated motions involved in the signal allosteric transmission between zinc-binding region and core domain acetylated position in CobB were detected using GSATools.

c) **Comparative study of coevolved motions.** Networks of signal transmission between the two domains were extracted and annotated with the location of coevolved positions for CobB. For Sirtuin2, critical coevolved residues along signal communication were detected by comparison with the list of functionally analogous coevolved residues in CobB. Finally, a mapping of pathogenic mutations from the literature was added to the pathway ***(Fig2).***

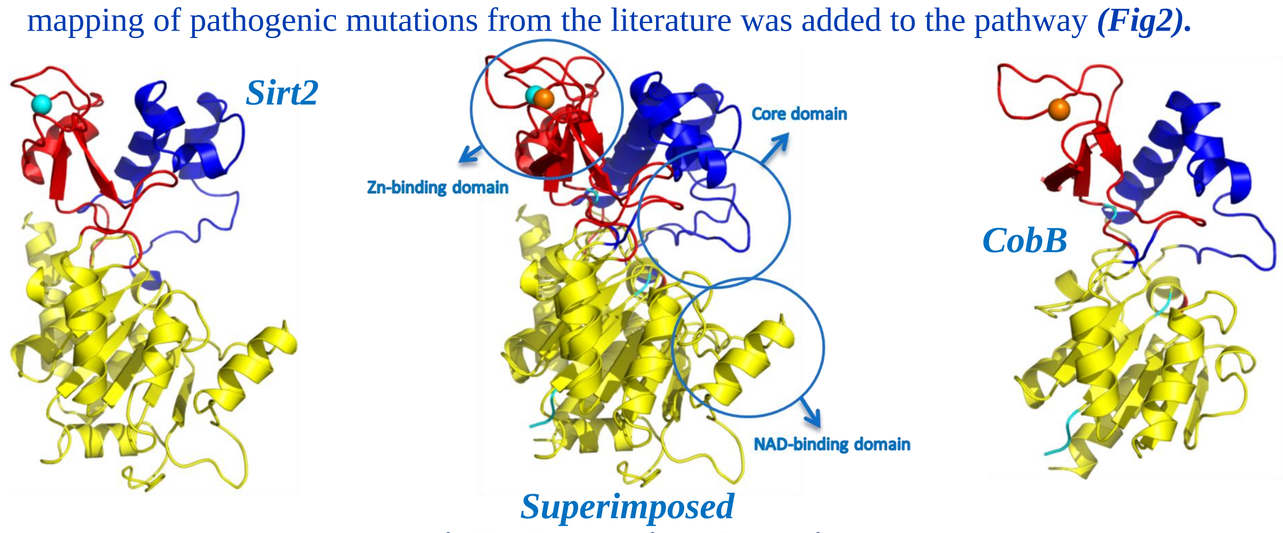

Fig3. Dynamics Domains

Results

CobB and Sirtuin2 show a clear conservation of domain structure and allosteric wiring. We have shown that exists a strong conservation of three identified **dynamic domains (zinc-binding domain, core domain and NAD-binding domain)** and overlapping them correspond exactly to the three functional domains ***(Fig3).*** This information is consistent with another result based on identification of **evolutionary domains** calculated on CobB in direct way to investigate the coevolved pairs of contacts and we have transferred this information on Sirt2 to obtain indirectly its coevolved pairs of residues ***(Fig4)***. We have shown the conservation of dynamics and evolutionary domains, but it is present also an **allosteric communication** between three dynamics and evolutionary domains inside of two proteins, and some of these relations are stronger than others ***(Fig5)***. It was possible to evaluate other important properties of our systems. For example we have analyzed the RMSF profile calculated on PCA to investigate the **flexible regions** ***(Fig6)*** involved in dynamics motions excluding all the others. After that, we have explored the **energy minima** and the hypothetical transition sampled during the simulations ***(Fig7)***. Finally, it was possible to map the pathogenic mutations on minima structures obtained by simulations trying to outline a new strategy to design new drugs.

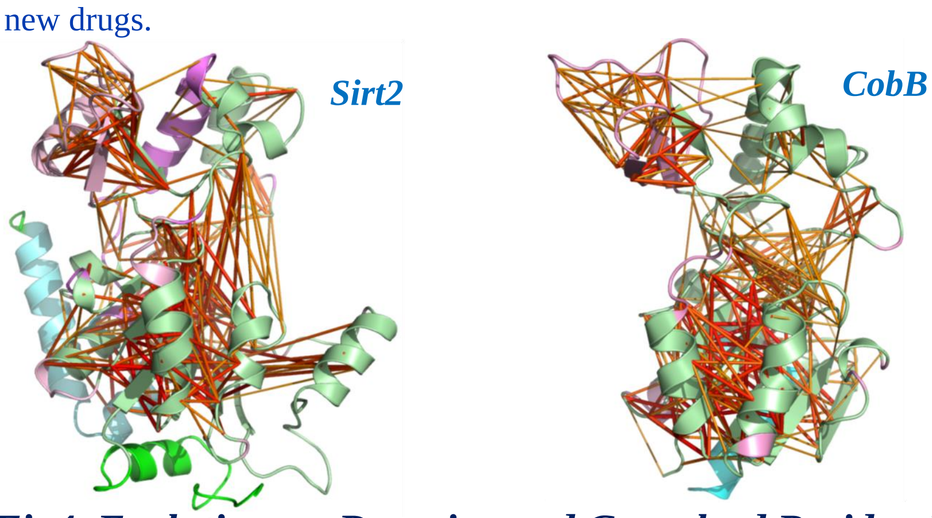

Fig4. Evolutionary Domains and Coevolved Residue Pairs

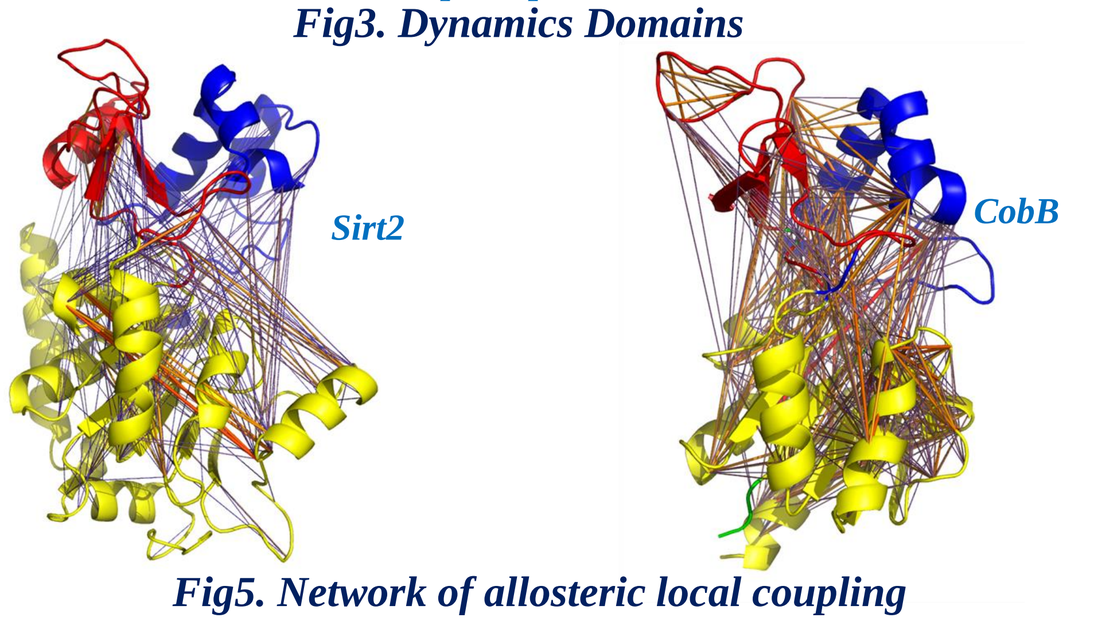

Fig5. Network of allosteric local coupling

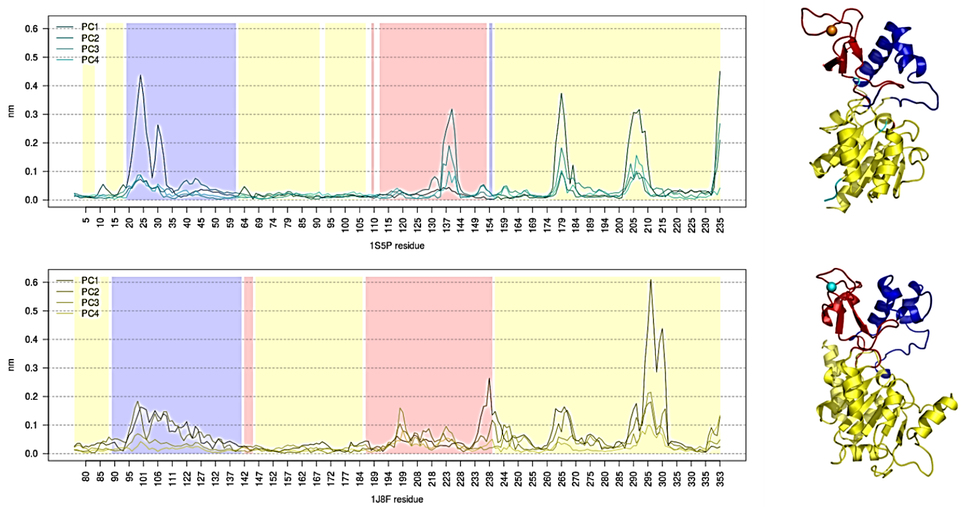

Fig6. Flexible Regions

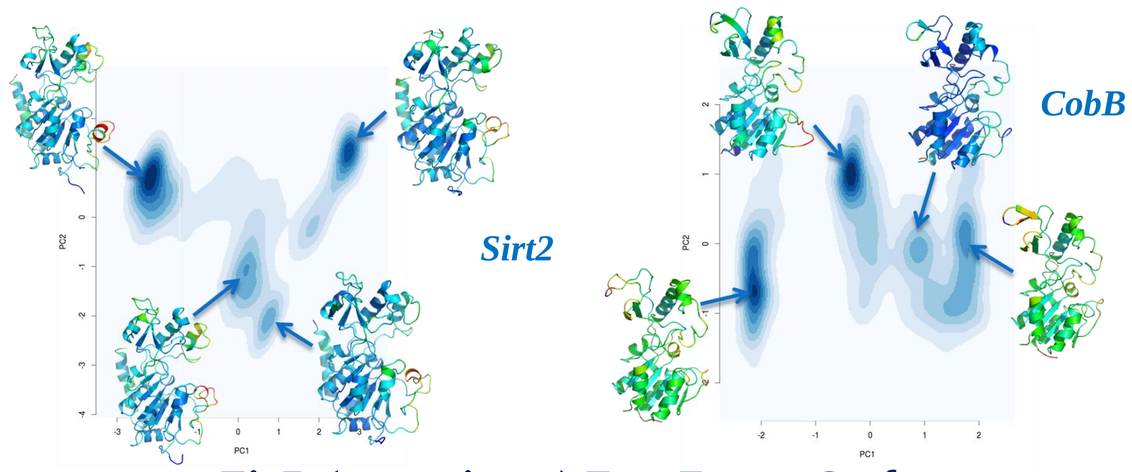

Fig7. (approximate) Free Energy Surface

Conclusions and Future Work

This information confirms our hypothesis of a conserved allosteric regulation mechanism in both systems at the domain and residue level. The outcomes of this study shed light on a possible role of pathogenic mutations in impairing Sirtuin2 function by disruption of allosteric communication. This opens the direction for a preliminary drug discovery study. We planned to investigate the holo form of Sirtuin2 and the dynamics of its pathogenic mutants. Through annotation of candidate compensatory mutations with DFS and detection of transient pocket using MDpocket, we will identify putative binding sites for compensatory drugs.

References

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