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

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Motivation
Sirtuin2 is an NAD+-dependent protein deacetylase and is part of family of highly conserved enzymes. It plays a critical role to regulate several biological processes and some epigenetic mechanisms based on genome integrity-stability. Its misregulation is often related to mutations that can disrupt the deacetylase activity, making Sirtuin2 an attractive drug target for chemoprevention of some human diseases. E. coli CobB is a bacterial Sirtuin2 homologue characterized by a smaller zinc-binding domain (critical point for substrate recognition in bacteria). It was previously suggested that 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 [1].
The structural and functional analogies between Sirtuin2 and CobB suggest that a general mechanism of allosteric regulation could be common for Sirtuin2 proteins. However, the molecular mechanism involved in this process is still unknown [2].
Here 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. Using a comparative approach, we infer functional properties in Sirtuin2 and unveil analogies in the mechanisms of allosteric communication of the two proteins that could explain a conserved role of distal molecular interactions [3].
Methods
The study involved three methodological steps: a) residue coevolution analysis; b) molecular dynamics simulations; c) comparative study of coevolved motions.
a. A multiple sequence alignment for the sequence portion of the PDB structure 1S5P (CobB) was obtained through the MetaPSICOV webserver and used for all the analysis. Residue coevolution analysis was performed using MetaPSICOV [4] and DCA [5]. Conserved and coevolved rigid domains were detected with SPECTUS-Evo [6].
b. Molecular Dynamics (MD) simulations were performed using ARCHER (UK National Supercomputing Service). Three replicas of 500ns were generated for 1S5P

(CobB) and 1J8F (Sirtuin2) using GROMACS 2016.2 [7]. 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 [8].

c. Network and pathways 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. The structure alignment between 1S5P and 1J8F was used as a reference to identify equivalent positions between the two proteins. Finally, a mapping of pathogenic mutations from the literature was added to the pathway.

Results

Our primary biological drug target is human Sirtuin2, but current residue coevolution methods are only suitable for bacterial proteins with a large pool of annotated sequences. To overcome this challenge, we considered the bacterial homologue CobB as computational model. It was possible to successfully transfer the knowledge obtained on CobB to the human system: the two proteins show a clear conservation of domain structure and allosteric wiring. While fold conservation is expected, the presence of similar dynamic domains and allosteric communication pathways is surprising below 40% sequence identity. The three identified dynamic domains (zinc-binding domain, core domain and NAD-binding domain) are linkedthrough a set of multiple pathways that have consistent overlap with known location of pathogenic mutations.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 [9] and detection of transient pocket using MDpocket, we will identify putative binding sites for compensatory drugs.

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