The evolution of allosteric networks. How do polypeptides function?

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ABSTRACT

Proteins are responsible nearly for all processes occurring within cells. Thus, protein malfunction represents the molecular etiology of diseases and death. To accomplish their biological function, proteins need to be able to: **i.** Fold and acquire a well-defined three-dimensional shape, **ii.** Execute specific biochemical activities, but also **iii.** Adapt to a continuously fluctuating chemical environment. The above-mentioned properties rely on the capacity of proteins to vary their shape or structure over time. These variations are finely-regulated through the interaction of proteins with small molecules and/or other biopolymers present in their chemical environment, and termed Structural Dynamics[1]. The means by which the interactions occurring at specific sites (protein clefts) signal events at remote locations to trigger Structural Dynamics represents a long-standing research topic in life sciences. In 1904 it has been termed the "Bohr effect" and only ~ half a century later Allostery, defined also as the "*second secret of life*".

In my talk I will be describing the statistical tools[2] developed to reveal the networks of molecular interactions responsible to transmit allosteric signals from the site of the perturbation to distal locations. To verify if the identified networks are truly allosteric determinants, we disrupted them and followed the repercussions on the Structural Dynamics. The ability to monitor Structural Dynamics is extremely challenging, as it is needed to follow biological systems at length- (Å to nm) and time- (ns to min) scales that vary several orders of magnitude. Moreover, as biological systems are heterogeneous, it is needed to adopt ensemble but also tools with single-molecule resolution. To address the aforementioned challenges, we used a wide range of biophysical tools, the main of which are: *i.* single-molecule Förster Resonance Energy Transfer to probe large-scale Structural Dynamics, *ii.* Hydrogen Deuterium Exchange - mass spectrometry to detect localized Dynamics and *iii.* Molecular Dynamic simulations to explore fast Structural fluctuations that are beyond the time resolution of any experimental method.

Our results[3] reveal that latent allosteric networks are expanded during evolution, conceding functional nascency and are critical to understand the molecular basis of diseases, and pivotal to protein engineering and drug development.

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