The statistical theory of allostery

Michele Vendruscolo

Without tight control on the activity of proteins, it would be impossible to coordinate the elaborate interplay among genetic, biochemical and metabolic networks that maintains homeostasis in living systems. A common way to achieve such control is through binding events in which proteins interact with other molecules capable of activating or deactivating them. When such binding events take place without interfering directly with the active sites of proteins, the regulation is called allosteric. Advances in understanding allosteric mechanisms can be made through the use of NMR spectroscopy, which provides a variety of tools for characterizing the structure and dynamics of proteins. Particularly attractive in this context is the use of chemical shifts, because these parameters can be measured with great accuracy and under a wide variety of different conditions. Two recent studies illustrate just how effective this type of approach is becoming.

It has been very challenging to develop general methods to characterize in detail the molecular mechanisms by which allosteric phenomena take place. The essence of the problem is that proteins are—as it was famously put by Feynman—constantly wiggling and jiggling, and thus they require a description in statistical terms. In this view, an allosteric transition between the active and inactive forms of a protein is a process of non-equilibrium relaxation between two distinct thermodynamic states. Understanding the complex nature of this process, which is crucial to eventually control it, is difficult, as a wide variety of specific molecular mechanisms are at play for different proteins and involve different combinations of conformational and dynamical changes concerning extended networks of residues between the allosteric and active sites (Fig. 1).

This way of looking at allostery, in combination with chemical shift measurements, is revealing the details of intricate allosteric mechanisms. A major step in this direction was made by Zhuravleva and Gierasch, who used chemical shift perturbations to characterize a central aspect of the allosteric activity of Hsp70, a ubiquitous molecular chaperone consisting of an N-terminal nucleotide-binding domain and a C-terminal substrate-binding domain as catalytic tools for directing the folding of designer polyketides both in vitro and in vivo.

Kira J. Weissman is in the Molecular and Structural Enzymology Group, Unité mixte de recherche 7214, Centre National de la Recherche Scientifique–University Henri Poincaré-ARN-RNP, Enzymologie Moléculaire et Structurale, Henri Poincaré University, Vandoeuvre-Les-Nancy, France. e-mail: kira.weissman@maem.ahp-nancy.fr

References

Competing financial interests
The author declares no competing financial interests.
domain connected by a highly conserved hydrophobic linker. Although the central role of this linker in coupling the activity of the nucleotide-binding and substrate-binding sites has been known for a long time, the detailed mechanism by which it operates has remained elusive. Through the work of Zhuravleva and Gierasch, part of the story is now becoming clear. By a careful analysis of the chemical shift changes observed in a series of constructs comprising the nucleotide-binding domain and the flexible linker, they identified a group of residues that make up an allosteric network extending from the ATP binding site in the nucleotide-binding domain to the interdomain linker. Although further studies will be needed to complete the picture for full-length Hsp70, the work by Zhuravleva and Gierasch offers a vivid illustration of the great potential of chemical-shift based approaches.

In a related study, Melacini and co-workers introduced a method in which coupled residues were identified through a covariance analysis of the chemical shift changes caused by a series of covalently modified analogs of the allosteric effectors. They illustrated this approach on the multidomain protein EPAC, which is a guanine nucleotide exchange factor representative of a class of proteins that function as molecular switches and signal transducers, finding also in this case that the change in the state of the protein is associated with a modulation in the structure and dynamics of residues within an extended network. The nub of the method is to identify residue pairs that, following a perturbation, show correlated chemical shifts changes, and then to cluster the residues in groups to obtain the cooperative units that are responsible for giving rise to an allosteric transition. Application of this approach to other systems will undoubtedly enable optimization of technical aspects of the method and full exploitation of the opportunities it offers.

These two studies, together with a series of other recent equally notable ones, show that the goal of accurately describing allosteric mechanisms can be achieved by adopting the view that proteins constantly undergo conformational fluctuations and that such fluctuations can be modulated through specific binding events. Indeed, the accumulated evidence indicates that many aspects of protein behavior, including folding, function and regulation can be effectively explained by statistical theories. This type of conceptual framework in combination with new methods that are emerging in NMR spectroscopy, in particular those that enable the use of chemical shifts for structure determination, provides excellent chances for obtaining detailed descriptions of the ways in which proteins are regulated. These advances will increase our understanding of at least some of the processes responsible for protein homeostasis and create new opportunities to target proteins for therapeutic intervention.

Michele Vendruscolo is at the Department of Chemistry, University of Cambridge, Cambridge, UK.

e-mail: mv245@cam.ac.uk

References

Competing financial interests
The author declares no competing financial interests.

Flipping a switch on huntingtin

Phosphomimetic mutations at huntingtin (Htt) Ser13 and Ser16 within the conserved N-terminal 17-amino-acid domain profoundly suppresses its toxicity in cell and mouse models of Huntington’s disease. New research reveals that cell stress acts as a stimulus for double phosphorylation of endogenous Htt, causing its nuclear translocation, and shows that certain chemicals can target such molecular processes in Huntington’s disease cell models.

Erin R Greiner & X William Yang

Huntington’s disease is caused by the expansion of a polyglutamine (polyQ) repeat near the amino terminus of the mutant huntingtin (mHtt) protein. Despite its monogenetic etiology, the precise molecular pathogenic mechanisms remain poorly understood, and disease-modifying therapies are not yet available. Recent converging evidence reveals that an evolutionarily conserved N terminus of Htt, consisting of only 17 amino acids (N17), is a substantial contributor to a variety of mHtt-associated properties, from subcellular localization and aggregation to toxicity in cells and mice. The impact of the N17 domain in Huntington’s disease pathogenesis was highlighted by recent findings that mimicry of physiological phosphorylation at Ser13 and Ser16 could suppress mHtt toxicity in Huntington’s disease cells and mice. However, the physiological pathways that lead to N17 phosphorylation and the potential for such pathways to be modified by small chemicals remain unclear. In this issue, Atwal et al. provide evidence that cellular stress leads to Htt Ser13 and Ser16 double phosphorylation, which in turn triggers subnuclear targeting of Htt. They also identify several proof-of-concept chemical compounds that can boost neuroprotective Htt modifications in cellular models of Huntington’s disease.

Several independent lines of evidence converge on the crucial role of the Htt N17 domain in Huntington’s disease pathogenesis. Cell biological studies reveal this small N terminus of Htt as a cytoplasmic retention signal, as it can keep fragmented or full-length mHtt in the cytoplasm, physically associate Htt to membranous structures such as the endoplasmic reticulum and mitochondria, and facilitate Htt nuclear export. Also, an in vitro study unexpectedly showed that the presence of N17 can accelerate the mHtt exon1 peptide aggregation, and such aggregation process can be suppressed by an N17 interacting protein, the chaperonin Tcpl (also known as CCT1). Thus, N17 seems to be a small cis-domain that can play a big part in influencing the misfolding and...