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Weak protein complexes: challenging to study but essential for life

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The human genome encodes thousands of proteins that are crucial for life. These proteins function by interacting with a variety of targets, including other proteins, nucleic acids, carbohydrates, lipids, metabolites, small molecules and metals, etc. For more than a century, researchers have attempted to understand the nature of these protein–ligand interactions and how they regulate cellular events. Although the current scientific literature contains thousands of articles devoted to protein–ligand interactions (e.g. searching PubMed with the term protein interactions yields > 250 000 articles), the majority of these studies focus on high-affinity complexes (typically with a $K_D < 10^{-6}$ M) that are readily detectable and therefore amenable to a variety of techniques for analysis. When an interaction is weak or very weak (e.g. $K_D > 10^{-4}$ M), many conventional approaches fail or become unreliable. Thus, compared to the large database of the tight protein–ligand complexes, information about weak protein–ligand complexes is still scarce, and few of these have been thoroughly investigated or structurally characterized. Indeed, a significant bias still exists toward treating weak protein–ligand interactions as nonspecific and physiologically irrelevant. Such a bias mainly stems from considering the mean concentration of a particular protein in the cell, which typically lies in the nanomolar to micromolar range. At such concentrations, weak interactions are expected to have no consequence.

This simple view, however, is fundamentally flawed. Certain subcellular compartments are significantly enriched in certain proteins; thus, their local concentration can be high, as is the case for proteins involved in the assembly of focal adhesions, actin filaments or proteins assembling into viral capsids [1,2]. Indeed, in these scenarios, ultra-weak protein–ligand interactions become biologically important. Other examples of low-affinity complexes are those formed transiently by proteins involved in electron transfer or multi-enzyme complexes [3,4]. The transient nature and low stability of such noncovalent assemblies is such that, *in vitro*, the complexes usually dissociate, rendering detailed structural studies by most common techniques (e.g. X-ray crystallography) challenging. Because of these problems, weak interactions have, until now, received comparatively little attention, despite the fact that weak and transient complexes are extremely important with respect to the regulation of biochemical pathways, allosteric regulation and signaling cascades in cells. There are many other examples where weak interactions play crucial roles, often providing effective mechanisms for the cell to quickly respond to temporary stimuli. For a complete elucidation of life processes, it appears necessary to investigate both strong and weak protein–ligand interactions.

Accordingly, the first international conference on the use of targeted biophysical techniques and their application to the study of weak protein–ligand interactions was held in Beijing in the autumn of 2012 (https://www.biophysics.org/2012china/). Almost every methodology was represented: (a) three-dimensional structure analysis methods, such as X-ray crystallography, NMR spectroscopy, cryo-electron microscopy, small angle X-ray scattering (SAXS) and computational docking; (b) biochemical approaches for the identification of complexes in the cell, including yeast two-hybrid, co-immunoprecipitation and chemical cross-linking; and (c) other biophysical methods, such as MS, surface plasmon resonance, isothermal titration calorimetry, fluorescence spectroscopy and analytical ultracentrifugation, etc. The meeting generated an overall consensus that many of these techniques, if developed further, have enormous potential for the investigation and characterization of dynamic interactions in hitherto unimagined detail, even if they are technically challenging. There is no doubt that they hold great promise for deciphering the multitude of complex protein networks regulating cellular events.

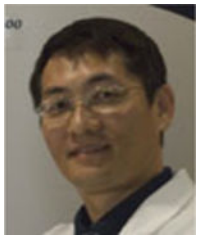
This series of minireviews by prominent experts provides glimpses into current and future possibilities. Schmidt and Robinson [5] eloquently illustrate how MS can be used to analyze a large variety of dynamic protein–ligand interactions. The technique is extremely powerful for large-scale proteomic studies of transient protein–ligand encounters. It is also able to provide detailed information on binding site, shape, size and the stoichiometry of components in such protein assemblies. The review by Schmidt and Robinson emphasizes the breadth of the approach when combined with other complementary techniques such as hydrogen–deuterium exchange, hydroxy radical footprinting, chemical cross-linking and computational docking. In another review, Luna *et al.* [6] provide an overview of the unique capabilities of NMR spectroscopy to structurally characterize weak (and even ultra-weak) protein interactions during translation initiation. Compared to other techniques, NMR is indeed one of the most useful tools for investigating weak protein interactions at the atomic level, and chemical shift perturbation assays are especially effective for rapidly delineating binding interfaces on proteins [7]. Tuukkanen and Svergun [8] report on both the theory and application of SAXS for the analysis of weak protein complexes in solution. Scattering methods are becoming increasingly popular as an alternative structural approach, given the limitations of high-resolution techniques; indeed, it is difficult to crystallize weak protein assemblies without loss of components, and solution NMR has well-known limitations in terms of molecular mass and sample concentration. For these reasons, combinations of methodologies, in hybrid approaches, are gaining momentum. For example, high-resolution domain/subunit structures, as determined by X-ray or NMR, provide valuable information that can be used to fit SAXS data. Finally, the review by Rodrigues and Bonvin [9] illustrates how computational docking can be applied for the analysis of weak protein complexes. These computational methods are extremely useful when only sparse experimental data are available. Docking approaches often provide rapid results that can be validated and refined iteratively by directed mutagenesis.

In summary, all of the above approaches are undergoing constant improvements and refinement, and they are becoming increasingly powerful and versatile for applications in biology. More and more weak protein assemblies are anticipated to be characterized in the near future, ultimately providing an unbiased and comprehensive view of the complex

structural and temporal interplay between the cellular components that govern biological function.

Biographies

Jun Qin studied proteins via NMR spectroscopy during his thesis work with Gerd LaMar at the University of California, Davis. His postdoctoral research in the Clore/Gronenborn laboratory at NIH dramatically expanded his interest in protein– ligand interactions. He has been on the faculty at the Lerner Research Institute and a Professor at Case Western Reserve University since 1996. His principal research interests are to determine the protein–ligand interactions involved in cell adhesion and migration, which comprise many dynamic and weak protein complexes.



Angela M. Gronenborn started to use NMR on small molecules during her thesis work with Harald Günther at the University of Cologne. Her postdoctoral training at the National Institute for Medical Research in Mill Hill, London, introduced her to protein NMR and the analysis of dihydrofolate reductase complexes with a variety of ligands. Throughout her career, she has studied a large number of protein–nucleic acid, protein–protein and protein–carbohydrate complexes, characterizing interactions at the atomic level by NMR and crystallography. She currently holds the UPMC Rosalind Franklin Chair in Structural Biology at the University of Pittsburgh, School of Medicine. A major research focus is centered on HIV–cellular protein interactions and her work on lectin–carbohydrate complexes provides illustrative examples for weak protein interactions.

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