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NMR studies of weak protein–protein interactions

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ABSTRACT

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1. Introduction

1.1. Weak protein–protein interactions

Interactions between proteins are fundamental to life since proteins rarely act in isolation and very little happens in a cell without one protein making contacts with another. Speed and specificity are two opposing features of biological interactions. Protein–protein interactions are either stable or transient [1]. A stable

Abbreviations: CPMG, Carl–Purcell–Meiboom–Gill; CSP, chemical shift perturbation; ETDA, ethylenediaminetetraacetic acid; HADDOCK, High Ambiguity Driven protein–protein DOCKing; nOe, nuclear Overhauser effect; HSQC, heteronuclear single-quantum coherence; IDIS-NMR, isotope-discriminate NMR; ITC, isothermal titration calorimetry; NOESY, nuclear Overhauser effect spectroscopy; PCS, psuedocontact shift; PRE, paramagnetic relaxation enhancement; RDC, residual dipolar coupling; TROSY, transverse relaxation optimised spectroscopy.

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interaction is when the protein exists only in its complexed form and is, hence, a strong interaction. On the other hand, transient interactions are temporary in nature and require a set of conditions, such as conformational changes, phosphorylation or colocalisation within the cell, to promote the interactions. When in complex with interacting partners, transiently interacting proteins are involved in many cellular processes such as folding, electron transfer, protein modification, cell cycling and signalling.

It is now clear that many protein assemblies are weak and/or transient for biological reasons; complexes need to break and reform with specificity and at appropriate rates as part of their biological function and it is energetically preferable if the affinities of these complexes are relatively low. Transient interactions can be strong or weak; strong transient associations require a trigger to shift the equilibrium to the stable state, such as binding of cofactors, whereas in weak interactions, a dynamic oligomeric equilibrium exists with the interaction being broken and formed continuously. Examples of common biological and cellular processes where weak interactions are desirable include reversible cell–cell contacts, rapid enzymatic turnover, electron transfer, transient assembly/reassembly of large, and multiprotein complexes in which target proteins are modified, regulated or translocated to other cellular compartments [1]. In addition, productive, thermodynamically stable specific protein–protein complexes are often formed through the initial assembly of low-affinity, diffusion-controlled complexes.

Weak interactions are characterised by small protein–protein interfaces. Typically, protein–protein interactions interfaces are over 1500 Å^2; for weak complexes, however, the interface area can be as low as 500 Å^2 [2]. In terms of strengths of interactions, K_D values ($K_D = k_{off}/k_{on}$) less than 10^{-9} M are described as strong interactions, and K_D greater than 10^{-4} M, weak interactions. In protein–protein interactions, k_{on} is approximately 10^5–10^6 M^{-1} s^{-1} [3]; hence, for transient interactions, k_{off} can be as fast as 10^4 s^{-1}. In the cell, a majority of the protein–protein interactions occur with K_D less than 10^{-6} M.

Being able to detect and characterize these weak complexes is crucial for understanding biological processes, mechanisms and pathways. The common biophysical techniques for studying weak protein–protein interactions include hydrodynamic methods, surface plasmon resonance, isothermal titration calorimetry, nuclear magnetic resonance spectroscopy (NMR) and optical techniques such as fluorescence resonance energy transfer and bioluminescence resonance energy transfer [4]. However, NMR spectroscopy, is the most versatile and information-rich for reasons given below.

Central to the function of a protein and its interactions are the dynamics of the protein and, hence, methods such as NMR that are able to detect and characterise these are particularly powerful [5]. In addition, interaction effects are not restricted to the localised, contact interface between two proteins; rather information can be transmitted throughout the molecule [6]. NMR is able to provide such information for the entire protein at atomic detail. It is undoubtedly one of the best techniques for measuring dynamics and conformation changes. The method is suited for interactions associated with the very rapid dissociation rates (k_{off}) that are often found in weak protein–protein complexes [7–14]. The types of information obtainable from NMR studies of weak complexes range from complete structure determination to examinations of low resolution datasets which simply highlight the possible regions involved in intermolecular interactions. Between these extremes, other types of structural and dynamics information can be obtained including providing affinity constants [15], and detecting binding intermediates [16]. To obtain high resolution structures of a complex, NMR-derived restraint-driven docking and energy minimisation methods are used, provided that the structures of the individual components are known [17–20]. For weak interactions, combined NMR and functional studies, often involving mutagenesis, have been shown to be one of the most effective approaches for detecting otherwise undetectable interactions.

One main drawback of the NMR method is the low sensitivity; in very weak interactions the population of molecules in the complexed forms are often very low, posing a significant practical challenge in NMR studies. Two recent approaches have been particularly useful for detecting lowly populated species which may also be transient. These are paramagnetic relaxation enhancements (PREs) [21,22] and relaxation dispersion spectroscopy [23]. Furthermore, because many of the NMR experiments are conducted under equilibrium conditions, it is often possible to increase the population of complexed forms by increasing the initial concentrations of the interacting proteins.

The focus of this review is on the use of NMR to study weak heterotypic protein–protein interactions in which complexes are formed between different proteins rather the homotypic interactions which lead to the multimerisation of a particular protein. Section 2 describes methods for preparing weak protein complexes for NMR studies, and includes the different types of isotope labelling that are required, as well as the practicalities of making protein–protein complexes that are functionally relevant. Sections 3–6 describe the common NMR techniques that can be used to study weak protein–protein complexes; although many of these are similar to those used for the investigation of tight complexes, some are particularly relevant for the weaker complexes. Furthermore, in studies of weak interactions, the different techniques can be used in the 'titration' mode where the NMR characteristics are observed as a function of increasing concentrations of a partner protein, rather than as a single equilibrium mix with only one protein:protein concentration ratio; in this mode special considerations must be given as to how the parameters in the fully bound forms are derived [24,25]. Section 8 describes how NMR-derived information can be integrated with docking software to enable the determination of the structures of protein complexes; this latter hybrid method is proving to be a very powerful approach for the weakly-interacting protein complexes. Many of the techniques and approaches described here can also be found in Ref. [26] and Table 1 summarises examples of the weak complexes which have been studied in some detail.

1.2. NMR timescales

The success of an NMR method is dependent on the compatibility of the particular chosen experiment with the dynamics of the system under consideration. This is particularly pertinent to weak protein interactions where the populations of some of the states of interests are very low.

The term exchange rate between species, k_{ex}, is often used in NMR. It is related to the commonly used term k_{off} or k_{-1}(off rate) for interactions between protein E and ligand L by the equation $k_{ex} = k_{off}/p_L$ (when detecting the ligand signal) and $k_{ex} = k_{off}/p_E$ (when detecting the protein signal) where mole fraction $p_L = [L]/L_T$ (and $L_T = [L] + [EL]$, $[L]$ is the concentration of ligand in the free state) and mole fraction $p_E = [E]/E_T$ (and $E_T = [E] + [EL]$) [26]. The exchange rate is defined on the NMR timescale by considering the lifetimes of each state relative to the difference in the NMR parameters of chemical shift, scalar coupling or relaxation rate. The chemical shift is the most common parameter, although exchange regimes on the relaxation timescales are becoming important especially when using relaxation-based characteristics such as PRE and relaxation dispersion experiments. When using the chemical shift timescale, in a second-order exchange between two molecules A and B as shown in Eq. (1), *slow exchange* is defined by $k_{ex} \ll |\delta_A - \delta_B|$ where δ_A and δ_B are the chemical shifts in Hertz (Hz) in the two states, *intermediate exchange* defined by