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Using chemical shift perturbation to characterise ligand binding

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ABSTRACT

Chemical shift perturbation (CSP, chemical shift mapping or complexation-induced changes in chemical shift, CIS) follows changes in the chemical shifts of a protein when a ligand is added, and uses these to determine the location of the binding site, the affinity of the ligand, and/or possibly the structure of the complex. A key factor in determining the appearance of spectra during a titration is the exchange rate between free and bound, or more specifically the off-rate k_{off}. When k_{off} is greater than the chemical shift difference between free and bound, which typically equates to an affinity K_d weaker than about 3 μM, then exchange is fast on the chemical shift timescale. Under these circumstances, the observed shift is the population-weighted average of free and bound, which allows K_d to be determined from measurement of peak positions, provided the measurements are made appropriately. ^{1}H shifts are influenced to a large extent by through-space interactions, whereas $^{13}C\alpha$ and $^{13}C\beta$ shifts are influenced more by through-bond effects. ^{15}N and $^{13}C'$ shifts are influenced both by through-bond and by through-space (hydrogen bonding) interactions. For determining the location of a bound ligand on the basis of shift change, the most appropriate method is therefore usually to measure ^{15}N HSQC spectra, calculate the geometrical distance moved by the peak, weighting ^{15}N shifts by a factor of about 0.14 compared to ^{1}H shifts, and select those residues for which the weighted shift change is larger than the standard deviation of the shift for all residues. Other methods are discussed, in particular the measurement of $^{13}CH_3$ signals. Slow to intermediate exchange rates lead to line broadening, and make K_d values very difficult to obtain. There is no good way to distinguish changes in chemical shift due to direct binding of the ligand from changes in chemical shift due to allosteric change. Ligand binding at multiple sites can often be characterised, by simultaneous fitting of many measured shift changes, or more simply by adding substoichiometric amounts of ligand. The chemical shift changes can be used as restraints for docking ligand onto protein. By use of quantitative calculations of ligand-induced chemical shift changes, it is becoming possible to determine not just the position but also the orientation of ligands.

Contents

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1. Introduction

Chemical shift perturbation (CSP, also known as chemical shift mapping or complexation-induced changes in chemical shift, CIS) is a very simple experimental technique for studying binding to a protein. In the standard experiment, one needs an ^{15}N-labelled protein plus an unlabelled ligand, which can be a small molecule or another macromolecule. The ligand is titrated into the protein, monitored at each stage of the titration by acquiring a 2D HSQC spectrum [1]. Using a moderately highfield spectrometer with a cryocooled probe, one can acquire HSQC spectra in about 30 min for proteins at concentrations of 200 μM or more. This means that one can acquire a complete titration in about a day. With TROSY and perdeuterated protein [2] one can observe proteins of several hundred kDa, so that (for a well-behaved protein, and with a bit of effort) one can acquire CSP data on most targets of interest. If one is studying the binding of two proteins to each other, then each protein can be labelled in turn, providing information about both partners: indeed, by use of ^{13}C labelling on one protein but not the other, one can observe both ^{15}N-labelled proteins separately and simultaneously, in relatively small systems at least [3].

The chemical shift change is very sensitive to structural changes, and can be measured very accurately, meaning that almost any genuine binding interaction will produce CSPs. The analysis is also simple, at least in its basic form: measure the chemical shifts at each titration point, follow the movement of peaks, and measure how each peak moves throughout the titration. The peaks that move the most are very likely to map to the binding site for the ligand. Moreover, the shape of the titration curve (chemical shift vs. concentration of ligand) can often be fitted straightforwardly to obtain a value for the dissociation constant of the ligand, K_d. CSP is the only technique that can directly provide both a K_d value and a binding site from the same set of measurements [4]. The only important caveat, as with any quantitative measurement, is that during the titration, it is important to keep experimental conditions as consistent as possible. In particular, it is important to use the same buffer for protein and ligand, because small changes in pH or salt concentration can alter protein signals and confuse the analysis. Similarly, if the ligand is only soluble in an organic solvent such as DMSO, then the titration must be set up in such a way as to keep the DMSO concentration constant.

The technique can be useful even without a chemical shift assignment of the HSQC spectrum. Most usefully, if a ligand does not bind, then there will be no chemical shift changes seen. CSP is widely used in drug discovery for this reason: many other techniques such as spectrophotometry, calorimetry or enzyme assay are prone to giving false positive results, whereas CSP in general does not. It is thus a useful and moderately high-throughput method for checking whether potential ligands really do bind, and forms the basis for the 'SAR by NMR' methodology [5]. Furthermore, CSP can be used to obtain K_d values in the absence of assignments; and one can compare which signals move on addition of different ligands, and thus ascertain whether different ligands bind in the same binding site or not. CSP is however much more powerful when the assignments are known. Fortunately, triple resonance techniques mean that backbone assignments are often obtainable quickly and even automatically [6]. CSP can be used with solid-state spectra as well as solution, making it even more versatile [7].

CSP is remarkably reliable as a guide to interaction sites, both of ligand with protein and of protein with protein. Provided that the crystal structure of the protein is known and the spectrum assigned, a big advantage of this method is that it is not necessary to calculate an NMR structure; one can use the crystal structure and simply map chemical shift changes onto it. Alongside this, the increased number of assignments of proteins with known structures, and the vastly increased speed of computers, has also meant that we are now better able to understand the origins of chemical shifts in proteins. CSPs are thus entering an exciting new phase, in which we can make quantitative use of the shift changes to probe the geometry of the interactions.

We therefore start with a brief discussion of the origins of chemical shifts in proteins, and go on to consider how CSPs can be applied. Because CSPs are experimentally and conceptually simple, there has been surprisingly little analysis of their application: remarkably, this is the first article specifically on CSPs to appear in *Progress in NMR Spectroscopy*. Hiding behind the simplicity, there are a range of issues that one needs to be aware of, most importantly to do with multiple binding modes, as discussed below; and with the problems arising when the system is not in fast exchange – a situation not always easy to spot.

2. Origins of chemical shift effects in proteins

2.1. Calculation of chemical shifts

There are two main approaches towards the calculation of chemical shifts in proteins. One is to use quantum chemical methods, most commonly standard packages such as Gaussian 98 [8], which calculate the electron densities in molecules, and therefore allow calculations of the shielding of nuclei from the external magnetic field by their electrons, which is what ultimately is responsible for the observed chemical shifts. In the past, the problems with these methods have been that their accuracy (by which we mean the agreement with experimentally determined shifts) is questionable, and they are slow, implying that they can only be applied to very small molecular fragments. There is a more subtle difficulty with quantum chemical methods, in that the result is essentially just a calculated shift for a given molecular configuration, which offers little help in understanding what aspect of the structure has produced the calculated shift. The user therefore has to select his or her structural models carefully, in order to gain useful insight. The methods are improving rapidly, as are the computers that they run on, and quantum chemical calculations are now the method of choice for ^{15}N and ^{13}C′ nuclei, and probably for other carbon nuclei also. For ^{1}H, the difficulty is that chemical shift effects arising from through-space interactions are just as important as through-bond interactions; and that the shielding of the proton by its surrounding electron is weaker than that of heavier nuclei, implying that the chemical shift range for ^{1}H is smaller than it is for ^{15}N or ^{13}C, and thus calculations need to be relatively more accurate. Chemical shifts in ^{1}H therefore are harder to calculate