

## Introduction

### Description

The elucidation of the 3D structures and dynamics properties of oligosaccharides and glycoconjugates, both in the free state and bound to proteins, is a prerequisite for a better understanding of the molecular basis of their associations and interactions, and the relationships between structures and functions, which are involved in the biochemistry of recognition processes and the subsequent rational design of carbohydrate-derived drugs. These have been claimed to be the main challenges in structural glycoscience (Woods et al., 2010) and many efforts in this direction still have to be done.

A large variety of biophysical techniques have been developed to characterize protein–ligand complexes, e.g., surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), fluorescence polarization assay (FP), fluorescence resonance energy transfer (FRET), enzyme-linked immunosorbent assay (ELISA), differential scanning fluorimetry (DSF), microscale thermophoresis (MST) and electrospray ionization mass spectrometry (ESI-MS) to cite the most popular methods (Arkin et al., 2004 Morelli et al., 2011 Renaud et al., 2009). SPR and MST techniques permit to obtain kinetic interaction parameters, whereas ITC measures the thermodynamic properties of binding in solution. *In silico* approaches have been also applied to search for ligands for a protein target (virtual screening) or to propose 3D models of protein–ligand complexes (docking calculations) (Trott et al., 2010 Huang et al., 2010) whereas X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are both experimental techniques for resolving atomic structures (Kay et al., 2011 Joachimiak, 2009).

When the scientific interests are focused on the structural studies of protein-carbohydrate complexes at atomic resolution, X-ray crystallography and NMR spectroscopy are the methods of choice. One of the disadvantages of X-ray crystallography applied to carbohydrates is that the oligosaccharides, either in their free form or as part of glycoconjugates, are inherently difficult to crystallize, and structural data from X-ray studies are sparse (Imberty & Perez, 2000). Even when succeeding in crystal formation, part or the whole glycan is, in most cases, not observed in the high-resolution electron density map (Wormald et al., 2002 Petrescu et al., 2002) due to the intrinsic high flexibility of carbohydrates. Furthermore, the experimental assessment of carbohydrate recognition by X-ray crystallography is impeded by difficulties of co-crystallizing proteins and carbohydrates. To overcome this limitation, it has been tried, for instance, to stretch the polysaccharide into an oriented fibre (Chandrasekaran et al., 1997). Also, it has been employed electron diffraction to study very small crystals, or needles, that can be obtained from polysaccharides (Perez & Chanzy, 1989). In any case, the amount of data collected to date is that small that building a model by molecular mechanics is necessary to resolve the 3D structure.

On the other hand, high-field NMR spectroscopy in solution state is one of the most important techniques for probing intermolecular interactions. NMR spectroscopy detects and reveals protein–ligand interactions with a large range of affinities, and it is widely used in pharmaceutical research to identify hits from compound library screening in drug discovery (Pellechia et al., 2008 Peng et al., 2004 Goldflam et al., 2012).

---

Protein–ligand complexes are analysed using the so-called protein-observed and ligand-observed NMR experiments in which the NMR parameters of the protein and the ligand, respectively, are compared in their free and bound states. In particular, ligand-observed methods are not limited by the protein molecular size and therefore have great applicability for analysing protein–ligand interactions. The use of these NMR techniques has considerably expanded in recent years, both in chemical biology and in drug discovery.

In protein-observed methods, the chemical shift perturbations of the protein resonances observed upon ligand addition are identified to localize the ligand binding site. This enables one to immediately distinguish specific from non-specific binding. The 3D structure of the protein-ligand complex can be resolved via heteronuclear experiments performed on isotopically labelled ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^2\text{H}$ ) protein samples. The structure resolution requires molecular dynamics calculations with experimental NMR restraints resulting from chemical shifts, scalar couplings, nuclear Overhauser effects (NOEs), paramagnetic interactions or residual dipolar couplings (Kay, 2011 Billeter et al., 2008 Cavanagh et al., 1995).

The major drawbacks are the experimental time and the need for a highly stable and soluble protein. In addition, these methods are limited in routine practice to proteins with low molecular masses (less than 30 kDa) to avoid great effort with regard to both labelling strategies and resonance assignment.

NMR parameters such as transverse, longitudinal, and cross-relaxation rates strongly depend on the molecular rotational correlation time  $\tau_c$ , which is directly related to the molecular weight. Ligand-based NMR experiments rely on the modification of such size-sensitive NMR parameters for the ligand in the presence of a protein receptor (Peng et al., 2004 Meyer & Peters, 2003).

Considering a diffusion controlled protein-ligand binding of weak to moderate affinity (dissociation constant,  $K_D$ , typically between  $10^{-8}$  and  $10^{-3}$  M), the association-dissociation process is fast within the chemical shift time-scale, so that the NMR parameters observed are a simple population-weighted average between the free and bound states. In contrast to protein-observed experiments, ligand-observation is more sensitive with larger receptors and do not require the use of isotopically labelled proteins. Ligand-based methods can be used for the detection of interactions and the measurement of protein–ligand affinities, and can also provide pertinent structural information on the protein–ligand complexes.