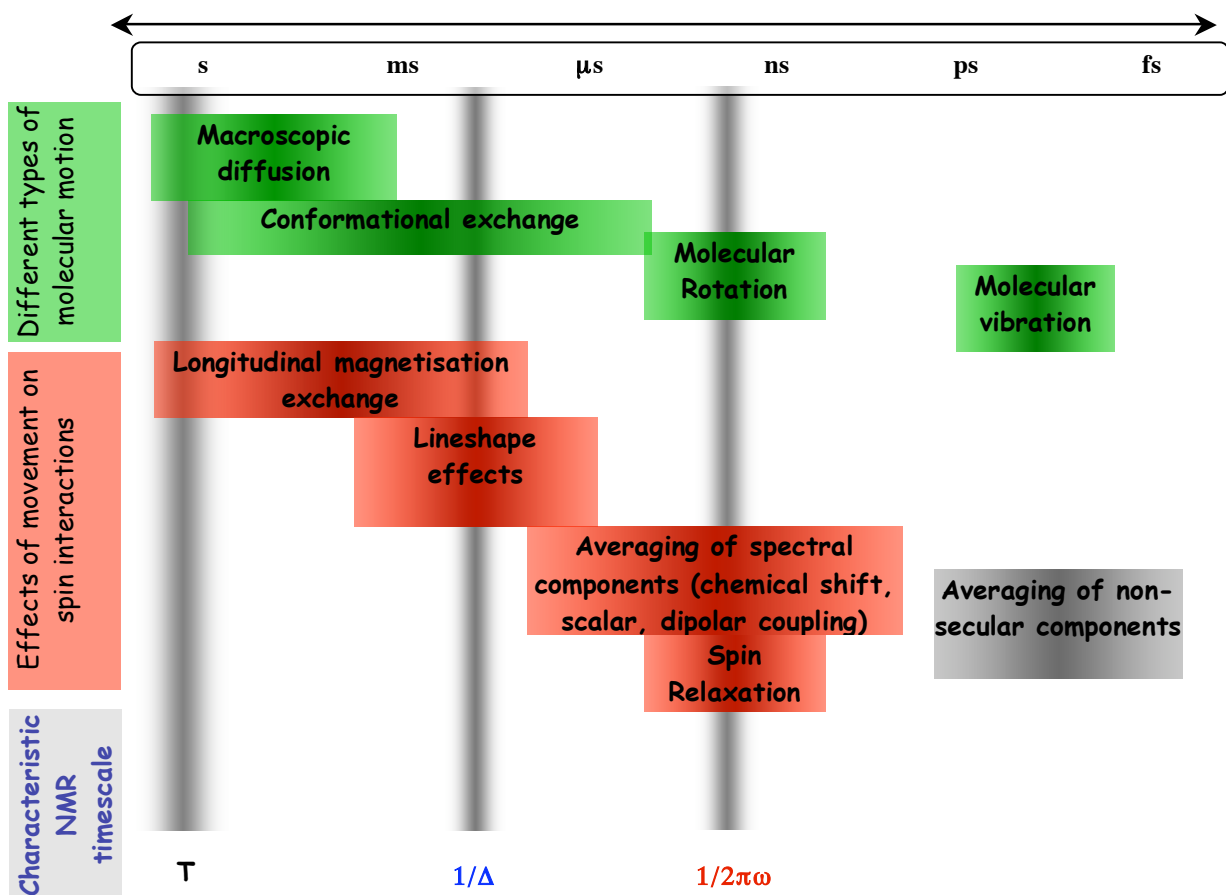# STUDYING PROTEIN DYNAMICS USING NMR

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Biological function relies on a complex spectrum of conformational dynamics occurring in biomolecules. Nuclear magnetic resonance (NMR) is exquisitely sensitive to protein dynamics on a vast range of timescales and therefore provides access to motional parameters governing the function of biological systems. In particular spin relaxation measurements provide precise information about local dynamics on picosecond to nanosecond timescales. During the first part of this course we will briefly present the physical basis of these phenomena, covering the general concepts required to understand spin relaxation, including some analytical formulations, and in particular introducing the relevance of the reorientational correlation and spectral density functions (see Blackledge1.pdf). We will then spend some time describing the analytical tools commonly used to interpret these data, namely explicit mapping of the spectral density function, and the so-called model-free analysis developed by Lipari and Szabo (Blackledge2.pdf). These techniques will be illustrated by examples taken from the literature, and part of the practical course in the afternoon will be based on the use of these approaches to use ^{15}N relaxation to study protein backbone dynamics (see Relaxation_TD1.pdf and Relaxation_TD2.pdf).

Dynamics occurring on slower time-scales are also of particular interest because many biologically important processes, such as enzymatic catalysis, signal transduction, ligand binding and allosteric regulation are expected to occur in this range. Relaxation dispersion experiments have successfully extended the range of timescales to identify conformational exchange between states in proteins, relating microsecond molecular motion to enzymatic function, and resolving thermodynamic, and even structural descriptions of very weakly populated excited states. The second part of the course will describe

experimental, theoretical and analytical aspects of rotating frame relaxation measurements (see CvHeijenoort1.pdf). Further examples will be encountered in the afternoon practical session.

Residual dipolar couplings (RDCs) have emerged as a powerful addition to these techniques. The sensitivity of RDCs to the relative orientation of a pair of coupled spins has led to their routine use in protein structure determination. However it is in terms of molecular dynamics that a second, equally important aspect of RDCs has recently become apparent. RDCs represent averages over all orientations of the dipolar interaction sampled up to the time-scale defined by the inverse of the measured dipolar coupling (up to the millisecond range), and thereby hold great promise for the description of slower motion in proteins. This sensitivity of RDCs to local conformational fluctuation has recently been exploited to examine the extent and nature of local dynamic behaviour present in proteins over this time range. A brief description of this recently developing field will be presented if time permits (Blackledge3.pdf).

s   ms   μs   ns   ps   fs

Different types of molecular motion

Macroscopic diffusion

Conformational exchange

Molecular Rotation

Molecular vibration

Effects of movement on spin interactions

Longitudinal magnetisation exchange

Lineshape effects

Averaging of spectral components (chemical shift, scalar, dipolar coupling)

Spin Relaxation

Averaging of non-secular components

Characteristic NMR timescale

T $1/\Delta$ $1/2\pi\omega$

Finally, Molecular Dynamics (MD) simulation enables the exploration of conformational energy landscapes accessible to proteins and therefore represents a powerful tool for understanding and interpreting experimental NMR data in terms of molecular motion. We will show briefly how MD simulation can be employed to predict fast stochastic motions responsible for spin relaxation, and how these techniques can be extended to explore longer timescale motions.