An Investigation of Rheo-NMR Techniques to Improve the Capture of Residual Dipolar Couplings

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Abstract

Residual Dipolar Couplings (RDCs) are an increasingly important structural restraint that can be used to help generate high quality structural models of proteins by Nuclear Magnetic Resonance (NMR) methods. They are captured with the aid of an alignment medium that imposes some anisotropy to the protein's tumbling. Current methods for the capture of multiple sets of these couplings are tedious, expensive, and do not always result in unique sets being captured. This thesis set out to investigate whether multiple RDC sets could be captured from a single sample by controllably shearing the liquid crystal alignment medium used.

Initial experiments focused on the ability to controllably realign a number of different nematic phase liquid crystals. These experiments found that controlling the director angle of the liquid crystal is possible, and that a number of stable alignments can be achieved through the application of different shear stresses.

The application of RDCs to small molecules is a very young field that is still developing and finding potential uses. In this thesis a small molecule system of (+)-isopinocampheol ((+)-IPC) was investigated with RDCs being collected from this molecule within a liquid crystal phase with the director at a number of different orientations relative to the external magnetic field. The fitting of these captured RDCs to a structural model of the (+)-IPC was not able to generate a high quality fit for any of the RDC sets collected, leading to some puzzling results. It is hypothesized that inhomogeneity of the alignment phase was responsible for these difficulties.

As the application of RDCs is so heavily dominated by protein structure studies, a small protein was investigated. The protein of choice, ubiquitin, has been heavily investigated in the past, and is often used as a demonstrator protein for new NMR techniques. This work presents several RDC data sets measured from ubiquitin which were successfully captured at a variety of different director orientations of the alignment media. These RDC sets were all successfully fitted to a previously known X-Ray crystallographic structure of ubiquitin, and unique alignment tensors for each RDC data set were extracted.

Finally, structure calculations were carried out incorporating these captured ubiquitin RDC data sets with the goal of investigating how the variation in the ensembles of structures generated was modified. The results from these calculations showed that the addition of RDC data (over and above NOE constraints) to the simulated annealing process results in ensembles of higher quality structures being obtained. However, the addition of multiple sets of RDC data (collected with different director alignments) did not appear to cause any further improvement.

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Nomenclature

(+)-IPC (1S, 2S, 3S, 5R)-(+)-Isopinocampheol

1D One Dimension

2D Two Dimensions

3D Three Dimensions

B_{eff} Effective Magnetic Field

CD Circular Dichroism

COSY Correlation Spectroscopy

CTAB Cetyltrimethylammonium Bromide

D Dipolar Coupling

FID Free Induction Decay

HSQC Hetronuclear Single Quantum Coherence

I Spin Quantum Number

INEPT Insensitive Nuclei Enhanced by Polarization Transfer

IPAP In-phase Anti-phase Heteronuclear Single Quantum Correlation Spectroscopy

K Degrees Kelvin

Nomenclature

k Boltzmann Constant

MAS Magic Angle Spinning

MHz Megahertz

MW Molecular Weight

NMR Nuclear Magnetic Resonance

NOE nuclear Overhauser effect

NOESY Nuclear Overhauser Effect Spectroscopy

PBLG Poly-γ-Benzyl-L-Glutamate

PEEK Polyether Ethyl Ketone

ppm Parts per Million

P_α Population of alpha spins

P_β Population of beta spins

Q Quality factor

RDC Residual Dipolar Coupling

RF Radio Frequency

RMSD Root-Mean-Square Deviation

S_{xx} Saupe Tensor xx component

S_{yy} Saupe Tensor yy component

S_{zz} Saupe Tensor zz component

TMS Tetramethylsilane

T_2	Transverse relaxation time
β-LG	β-Lactoglobulin
ΔE	Change in Energy
δ	Chemical Shift
γ	Gyromagnetic Ratio
$\hat{A}$	Alignment Tensor
$\hat{P}$	Probability Tensor
$\hat{S}$	Saupe Tensor
$\hbar$	Reduced Planck Constant
μ_0	Vacuum Permeability Constant
ν_{ref}	Resonant Frequency of a Reference Nuclei
$\nu_{spectrometer}$	Operating Frequency of Spectrometer
ν_o	Lamor Precession Frequency
ω_0	Zero Quantum Transition
ω_1	Single Quantum Transition
ω_2	Double Quantum Transition
$\vec{r}$	Internuclear Unit Vector
^1J	One Bond J-Coupling
^3J	Three Bond J-Coupling
$\mathbf{B}_0$	Spectrometer's Magnetic Field Vector

M	Net Magnetization Vector
h	Planck's Constant
m	Magnetic Quantum Number
t_1	Spin-lattice relaxation time