A STUDY OF MOSAIC SPREADS IN MEMBRANE PROTEIN STRUCTURE DETERMINATION BY SOLID STATE NMR

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Introduction

Solid state NMR experiments on membrane proteins, such as PISEMA, are performed on aligned samples, and are subject to errors in alignment. The traditional method of orienting samples by depositing them on glass or mica substrates has been known to produce a significant "mosaic spread" of orientations [1]. There are various reasons for such misalignment [2] that influence the resonance frequency determinations.

We model the mosaic spread of a membrane protein as a probability distribution on the set of rotation matrices, each rotation representing a perturbation of the sample in the lab frame. The same rotation is applied to each of the molecular frames in the sample [3].

The mosaic spread of a sample could be thought of as a small circle of radius ε on the unit sphere representing a range of values for the unit magnetic field vector ($\mathbf{B_0}$). When a circle of radius ε is mapped onto the frequency plane the footprint of the lineshape has a nearly elliptical image with varying eccentricities. The difference between the maximum and minimum values of σ, chemical shift, and ν, dipolar interaction, respectively represent the linewidth, or in this case the range in spectral intensity, in each dimension caused by the mosaic spread.

Results and Discussion

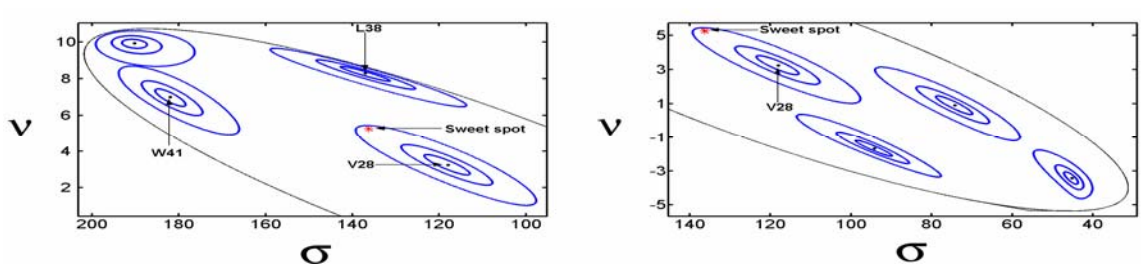

Figure 1: Circles of radii 2º, 4º and 8º on the $\mathbf{B_0}$ unit sphere are mapped into the PISEMA frequency plane for V28, L38 and W41 data points from M2-TMD, as well as a few simulated points in the top half and bottom half of the PISEMA ellipse. These show the effect of mosaic spread due to variations in sample alignment. The 'sweet spot' is the coordinates of maximum frequency sensitivity.

Preliminary investigations using PISEMA data from the transmembrane domain of M2 protein suggest that the contribution to linewidths by mosaic spread scale approximately linearly with the degree spread in the spectral region. An illustration involving three experimental data points and four simulated data points is shown in Fig. 1. While few observed PISEMA resonances have displayed the intensity distributions that we have obtained, there have been very few examples of high signal to noise spectra. Higher magnetic field strengths and optimized radio frequency probes are dramatically improving PISEMA sensitivity, and consequently for those samples with significant mosaic spread it can be anticipated that such lineshape footprints will be observed in the future. In such cases this mosaic spread analysis will be a useful tool in analyzing PISEMA spectra of aligned samples.

References

[1] Arnold, K., *et al.*, Stud.Biophys., **75**, 189 (1979).
[2] Moll, F., *et al.*, Biophys. J., 57, **351** (1990).
[3] Nevzorov, A.A., *et al.*, J. Am. Chem. Soc., **121**, 7636 (1999).