Zuiderweg-MR-2021-30

This paper has an ambitious objective: avoid all the niceties of measuring various ^{15}N relaxation rates, on the grounds that these sophisticated experiments do not offer sufficient sensitivity for large biomolecules in low concentrations, and use only the proton linewidths in HSQC spectra, supplemented by semi-selective T_{1rho} measurements.

The author is far from naïve and discusses most hurdles in lucid detail. "Amide proton linewidths may be affected by a plethora of mechanisms, which we will try to unravel in this work." The crucial questions are indeed: "is a narrow line narrow because it has few dipolar neighbors, or is it motionally narrowed? Is a broad line broad because of conformational exchange, or because it has a dense proton environment?"

Unfortunately, the outcome of the analysis is disappointing. The problems are summarized in the conclusions: "The theory of 1HN R2 for proteins is not iron-clad; issues such as "like" and "unlike" R2, "in-phase/antiphase" relaxation, "selective" and "unselective" R1 rates and cross-correlated R2 relaxation all play roles in these issues."

I cannot agree more. The main problem is that these issues cannot be resolved by establishing clear boundaries. Thus, one cannot easily choose between "like" and "unlike" R2, since the question if two chemical shifts can be considered to be degenerate depends on the linewidths. There is of course a grey zone between "like" and "unlike"; indeed, an equation describing a smooth transition between identical and non-identical spins has been given (Goldman, 1988). The relative weights of "in-phase" and "antiphase" contributions to transverse relaxation depend on the scalar couplings, the lifetimes of the signals, and on truncation of the signals if the observation ("acquisition time") has a limited length. The distinction between "selective" and "unselective" R1 rates of neighboring scalar-coupled protons (that contribute to transverse relaxation of antiphase terms) depends on the degree of saturation, the breadth of the rf irradiation (the statement "hence the 5 kHz r.f. field "hits" those HA whereas the 500 Hz r.f. field does not" does not leave any room for a grey area.) The spin temperature of the surrounding bath ("hot" if saturated, or "cold" if in thermal equilibrium) is not uniform since there must be an offset-dependent grey zone between "hot" and "cold". Methods designed to return the water magnetization to +z during the FID are never perfect. Contributions of a manifold of neighboring protons (as many as "40 protons in a 6 Å sphere around an amide proton"!) are certainly not additive, but it is tricky to extend consideration of cross-correlated fluctuations to a manifold of densely packed neighboring protons.

To our relief, the author frankly admits that he has not solved all problems: "There is hardly a correlation between experiment and calculation – the calculated values all lie around 7 Hz, while the experimental values vary almost a factor of two. At the moment we have no explanation for this …"

Statements like "Experimental data points larger than computed ones are harder to explain" amount to admitting a failure of the analysis.

It is therefore surprising to read "With this, we have arrived at our goal: we show that we *can* extract motional information from the 1HN linewidths in a HSQC spectrum by making simple calculations based on a crystal structure."

To my regret, I cannot recommend acceptance of this paper in “Magnetic Resonance”, nor in any other journal. It seems useful however that it will remain accessible on “Magnetic Resonance Discussions”, along with this critical review, since “Interactive comments are posted alongside the preprint and will remain permanently archived, publicly accessible, and fully citable.”

A few details:

The scalar couplings, whether resolved or not, should not be “subtracted” but must be properly de-convoluted.

The assumption that anisotropy of rotational diffusion can be neglected because “relaxation vectors” point in many directions seems a bit superficial.

I would prefer the use of indices like in $^{15}N^{H}$ and $^{1}H^{N}$ or, since isotopes are obvious, simply N^{H} and H^{N} rather than the ambiguous notation NH and HN

Since indices on indices are hard to print, why not use $^{3}J(H^{N}H^{A})$ rather than $^{3}J_{HNHA}$?

Likewise, it would be better to use symbols such as “$R_1(H^{A})$ contributing to $R_2(H^{N})$” rather than long-winded phrases like “R1 relaxation rate for 1HA contributing to the effective R2 relaxation rate of 1HN.”

It would be better to speak about “internuclear vectors” instead of “relaxation vectors”

There are a few minor spelling errors like: offfeset, KHz for kHz

What is meant by “Nevertheless, the protein is not perdeuterated”?

What is meant by “three of five *excessively* broadened resonances”?

Do SeM and Sem both mean seleno-methionine?