

Magn. Reson. Discuss., referee comment RC3
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Comment on mr-2020-38

Anonymous Referee #3

Referee comment on "Protein dynamics insights from 15N-1H HSQC" by Erik R. P. Zuiderweg, Magn. Reson. Discuss., <https://doi.org/10.5194/mr-2020-38-RC3>, 2021

The manuscript proposed by Erick Zuiderweg presents a valuable attempt to rationalize the intensities measured on protein 1H-15N correlation spectra in order to get a qualitative description of the backbone dynamics at different timescales. The different parameters affecting the 2D correlation peak's intensities are exhaustively listed and their values estimated from a structural model of the protein. The concept is tested on the BPTI protein and, as noted by the author, modelling the different known contributions to the signal intensities fails to reproduce the experimental measurements. The relevance of the approach is defended by the observation that large deviations from the modelled intensities do cluster in regions of BPTI where specific dynamical features were previously reported from 15N relaxation measurements. A graphic based clustering of these "model deviating" signals is therefore proposed as a fast approach to get qualitative insights on the protein dynamics. The approach is applied to a large protein (Hsc70) enabling some observations to be made on its dynamical properties. As stated by the author in his introduction, such an approach would be very valuable in the field of protein NMR, as we do share the general feeling that the information content of 15N-HSQC or TROSY are under-exploited, and the author's attempt to address this task is interesting. However, my general opinion is that the current state of this development is far too preliminary and would deserve more work to be published. The general applicability of such a method should be assessed by probing the concept on different class of proteins that display distinct and well documented dynamical features (depending on their size, geometry, experimental conditions of the study T° pH ...). The "correlation" approach is indeed the only way to go when the model fails to reproduce the experiment. Anyway, some statistical assessment is necessary for the applicability of the proposed approach on other systems: for instance, what criteria should be used to identify a residue with abnormal intensities? A quantitative description of the deviating between the theoretical model and the experimental values is clearly missing here.

Some of the hypothesis made by the author are questionable. In particular, the assumption that intrinsic exchange rates are identical for all amide protons is probably not true since local chemical environment at the protein surface do modulate these exchanges with water. Such information may be obtained by simple 2D experiments such as the Het-SOFAST proposed by Paul Shanda and Bernhard Brutscher.

Relaxation mechanisms different from the dipole-dipole interaction may also contribute for

the amide transverse relaxation: can we fully discard scalar relaxation ?

Small points:

- Equation 1 contains some mistakes:

I guess the two factors in the denominator are line-width ($\Delta\nu$) and not frequencies (Δ missing)

The term describing the nitrogen relaxation doesn't make sense to me: why is the average $\langle t_1 \rangle$ considered ?

The amplitude of the peak on the F1 (15N) dimension depends on the magnetisation's level at the end of the t_1 increment.

-140 Please describe how the amide value of CSA is derived ? Is it reasonable to assume the same CSA for all amides ? could this not be one major reason of the observed deviations, since the amide may be engaged within a hydrogen bond modulating the distribution of electrons.

- 145 The author should mention the model they used to derive the linewidths from Sparky (Lorentzian fitting ? gaussian ?)

- Figure 4: There are some discrepancies between the text and the figure:

- orange points are below 11 Hz for Reduced Experimental Line width
- What is meant by "at opposite side of the diagonal ?" I suggest using "Upper triangle" and lower triangle regions

- Figure 6:

- Legend : plain circle and squares

- Figure 9 : Labelling the different domains of Hsc70 would be helpful to follow the dynamical description.