

Regarding general matter (1), as referee #1 has pointed out as well, our study is indeed predictive and conceptual in nature. In our paper, we want to acknowledge Geoffrey Bodenhausen's contributions to the field by discussing how CCR experiments developed in his group over the years can potentially be used to investigate and characterize local (segmental) dynamics in (partially) disordered proteins.

Let us clarify: We do not claim nor intend to provide a thorough and general description of IDP dynamics. While segmental motions are clearly present in IDPs (Rezaei-Ghaleh et al. 2018, Parigi et al. 2014), it is obvious that the concept of diffusion anisotropy cannot be expected to rigorously apply. Still, this somewhat elusive concept has been invoked in previous studies even for proteins such as  $\alpha$ -Synuclein (Mantsyzov et al. 2014). This leads us to the central question of our study: If the segmental motion of IDPs exhibited features/trends associated with anisotropic tumbling, how could we best detect it? Can we define a sensitive and unambiguous experimental measure?

Complementing  $\text{NH}^{\text{N}}$  CCR, we show that  $\text{C}'\text{C}^{\alpha}$  CCR allows to probe the peptide plane quite literally from a different angle. The isolated zero frequency components are straightforward to compare and particularly sensitive for larger correlation times, allowing to probe the presence or lack(!) of anisotropic dynamics in IDPs on the local scale of the peptide unit. To assess the combined information content of the CCR rates, we build on the previously invoked and simplified model of a tumbling symmetric top. The "dampening" effect of local motions is approximated as simply as possible using a single exponential decay with equal weight for both  $\text{NH}^{\text{N}}$  and  $\text{C}'\text{C}^{\alpha}$ . We do not claim this treatment is rigorous nor do we imply that features of this MF-like anisotropic tumbling should be expected for all protein systems. The experiments were conceptualized and designed to assess the presence of features/trends associated with this simplified dynamic model.

In the following paragraphs, we will address the questions and remarks of referee #2. First, we see fit to discuss the formal objections.

- Eq. (2) (mr-2021-35) is very general. We cannot follow how it might be invalid. In fact, the study of (Tjandra et al. 1996) highlighted as counter-argument features this very expression already in Eq. (2) as well as in the appendix Eq. (A.1).
- The same holds true for the objected expression (3) (mr-2021-35). Eq. (3) is a very general form of a TCF. It only implies that the decay can be modeled as a superposition of exponential decays. All commonly employed analytical models and even MD-extracted TCFs adhere to this general shape. We believe sufficient references have been provided.
- From (2) and (3) follows (4) (mr-2021-35), so we must disagree with the objections raised. With the angle between  $\mathbf{u}$  and  $\mathbf{v}$  fixed, the same expression is also found in (Tjandra et al. 1996) between (A.1) and (A.2).
- We do not understand in what way Eqs. (6) and (7) (mr-2021-35) are confusing. Again, they are taken from the highlighted study of (Tjandra et al. 1996), see Appendix (A.14). The expression suggested by referee #2 makes use of the auto-correlated expression (A.15). We already commented on the possibility to approximate the entire cross-correlated TCF by an auto-correlated TCF on page 6. As the angle between  $\text{C}'\text{C}^{\alpha}$  and  $\sigma_{yy}$  is rather large, we prefer to model  $C_{\text{tumb}}(t)$  according to (A.14). Referenced in the paper, a different representation has been derived by (Deschamps & Bodenhausen, 2001). Can referee #2 clarify, is the validity of Eqs. (6) and (7) (mr-2021-35) being questioned or the combination with a fourth Lorentzian in Eq. (10)?
- From (2) to (9) (mr-2021-35) we simply establish the effect of anisotropic diffusion on the TCF which depends on the relative orientations of  $\mathbf{u}$  and  $\mathbf{v}$ . For  $\text{H}^{\alpha}\text{H}^{\text{N}}$  intraresidual and sequential NOEs, this model has been invoked to rationalize unexpected variations in  $\alpha$ -

Synuclein (Mantsyzov et al. 2014, p. 1281-1282). However, as  $H^a H^N$  distances vary with  $\phi$  and  $\psi$ , the observed effects were ultimately considered to be dominated by distance variations (p. 1286).  $C^a C^a CCR$  would not suffer from this ambiguity, which is why we propose it as an alternative.

- In addition, to **assess** the effect of isotropic local motions, we simply introduce an additional exponential decay / Lorentzian. As we said in the manuscript “While the fast isotropic motions could be modeled in more detail to better fit the shape of the TCF using e.g. the extended MF approach (Clare et al., 1990) or correlation time distributions (Hsu et al., 2018), we only intend to divide  $J(0)$ , i.e. the TCF’s enclosed area, into contributions with and without orientational biases.” (page 6). Eq. (10) (mr-2021-35) is a rough MF-like approximation,  $(1-S^2)\tau_3$  is simply the contribution to  $J(0)$  attributed to isotropic motions. While in principle arbitrary how this contribution is denoted,  $S^2$  and  $\tau_{int}$  tend to provide a better “feel” for many. Including additional and/or differently termed isotropic terms would not change the behavior of  $Q$ , only their cumulative size is relevant.

Our approximation can be justified from various angles which we have sketched in the paper. While one can argue about the physical meaning of MF-type models, two different approaches connecting  $\tau_{int}$  with  $\tau_0$   $\tau_1$   $\tau_2$  were highlighted. We agree that the word “coupling” is a poor choice. We were referring to how the factorization (which generally implies **no** dynamic coupling)  $C_{tumb}(t)C_{int}(t)$  is handled. It should be noted that already this product form is not strictly applicable in case of anisotropic tumbling. (Kroenke et al. 1998) keep  $C_{tumb}(t)$  anisotropic, Eq. (1), which yields a  $\tau_3$ ,  $\tau_4$  and  $\tau_5$  and retains orientational biases even with  $S^2=0$ . We follow (Barbato et al. 1992), Eqs. (6a) and (6b) / (Tjandra et al. 1995), sec. Theory, who approximate  $\tau_3$  assuming an effective isotropic  $C_{tumb}(t)$ , see below.  $C_{int}(t)$  decays from  $P_2(\mathbf{u} \cdot \mathbf{v})$  towards  $S^2_{uv}$  which for the approximated isotropic tumbling can be expressed as  $S^2_{uv} = S^2 P_2(\mathbf{u} \cdot \mathbf{v})$  (Ghose et al., 1998), Eq. (19), Appendix, or (Fischer et al. 1997), Eq. (29), which yields the “second term of eq 3”.

The MF approach of (Halle 2009), which does not assume the factorization of  $C_{tumb}(t)C_{int}(t)$ , was highlighted as well. The auto-correlated expression for anisotropic diffusion is found in Eq. (2.40). Treatment of cross-correlations are described in section D. Replacing 1 in Eq. (2.40) with  $\kappa_{uv} = P_2(\mathbf{u} \cdot \mathbf{v})$ , Eq. (2.59), and again approximating  $S^2_{uv} = S^2 \kappa_{uv} = S^2 P_2(\mathbf{u} \cdot \mathbf{v})$ , the expressions of (Tjandra et al. 1996), Eqs. (3), (4) and (6) are obtained. These correspond to Halle’s Eq. (2.64) for isotropic internal mobility which relates the cross-correlated TCF to the auto-correlated TCF as suggested by referee #2. The chosen exponential form of  $C_{int}(t)$  and the choice of  $\tau_{eff}$  can be motivated as above. If the angular dependencies of  $C_{tumb}(t)$  are encoded by Eqs. (6) and (7) (mr-2021-35) instead of  $S^2_{uv} = S^2 P_2(\mathbf{u} \cdot \mathbf{v})$ , Eq. (10) (mr-2021-35) is obtained. The expression suggested by (Ghose et al., 1998), Eq. (7), is very similar but lacks the prefactor  $P_2(\mathbf{u} \cdot \mathbf{v})$  for the internal TCF, which is problematic for large angles (e.g. between  $C^a C^a$  and  $\sigma_{yy}$ ). Regarding the necessity of various assumptions (including frame transformation properties) as well as the general validity of different MF approaches, we find Halle’s remarks in VI.A worth highlighting.

We feel Eq. (10) (mr-2021-35) adheres closely to conventional descriptions of MF-adjusted diffusion anisotropy. Again, we are not claiming this is how an IDP will realistically behave. Rather, what signature would the simplified image of anisotropic tumbling imply? And to what extent could we still detect it if we include faster isotropic motions in a simplified manner?

- “Why should the effective correlation time for global motion be equal to  $\tau_2^{-1}$ ?” It appears referee #2 is mistaken,  $\tau_{eff} = (4D_{\perp} + 2D_{\parallel})^{-1} \neq (4D_{\parallel} + 2D_{\perp})^{-1} = \tau_2$ . As we referenced

(Barbato et al. 1992, Tjandra et al. 1995), it is calculated from the trace of the diffusion tensor

$$\tau_{eff} = 6D^{-1} = 6 \frac{1}{3} (D_x + D_y + D_z)^{-1} = 6 \frac{1}{3} (D_{\perp} + D_{\perp} + D_{\parallel})^{-1} = (4D_{\perp} + 2D_{\parallel})^{-1}$$

## General matters

(2) As stated before, the concept of anisotropic tumbling of  $\alpha$ -helical and chain-like elements has been invoked in that very same paper (Mantsyzov et al 2014), pages 1281-1282. It was speculated that the local orientation of the spin pairs with respect to the  $C^{\alpha}C^{\alpha}$  vector (in part) explains the variations of intraresidual and sequential  $^1H^{\alpha}H^N$  NOEs.

(3) Following (Mantsyzov et al 2014), we assume that the peptide plane is embedded within the same diffusion tensor. Its unique axis is assumed to lie in the peptide plane such that the sketched edge cases (parallel/perpendicular) are covered. The details are described in the Methods section.

(4) As we mentioned before, we do not expect Eq. (10) (mr-2021-35) to apply in any strict sense. It is indeed the spectral density for MF-like anisotropic tumbling of a (sufficiently) rigid symmetric top. That being said, the general form of Eq. (10), i.e. a weighted sum of Lorentzians, can be expected to describe the TCFs of virtually any protein system in isotropic solution.

(5) We agree that fitting experimental relaxation parameters with only few Lorentzians has its limitations. The number of parameters and consequential statistical uncertainties are problems in their own right. In fact, Crawley and Palmer address this issue in this Festschrift (mr-2021-28). In our study, we have referenced different possibilities ranging from correlation time distributions to spectral density visualizations. Again, we are interested in detecting effects of anisotropic dynamics in  $J(0)$ . We do not intend nor suggest to fit experimental relaxation parameters of IDPs using Eq. (10) (mr-2021-35).

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