Comment on "The physical basis of model-free analysis of NMR relaxation data from proteins and complex fluids" [J. Chem. Phys. 131, 224507 (2009)]

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In a recent article,[1] Halle was critical of the use of the slowly relaxing local structure (SRLS) model[2,3] for the analysis of NMR spin relaxation in proteins.[4,5] Although he cited private communications with us, he did not discuss the contents of his article prior to its publication, nor were we aware of his intentions. We are in disagreement with a number of aspects of this article, but confine ourselves just to a brief rebuttal of his negative comments about SRLS and its use. We do, however, respect Halle's efforts to provide a physical basis for the model-free (MF) approach.

SRLS is a relatively new approach to NMR spin relaxation in proteins,[4,5] which involves the calculation of second rank time correlation functions for the orientation of a magnetic nucleus residing on the protein. The traditional approach is MF.[6–8] Enhanced versions of MF were presented by Halle.[1] We first note a fundamental difference between the two approaches: While MF is based on the (*a priori* mathematical) assumption of statistical independence among relaxation processes occurring in the protein without regard to a precise physical model, the SRLS approach is based on a clearly defined physical model. Both approaches are mesoscopic in spirit since they do not attempt – nor should they attempt – to overinterpret the limited experimental data in terms of atomic details. MF simplifies the ensuing treatment, whereas SRLS employs specific models with clear physical and geometric implications that can be analyzed rigorously, including whether statistical independence is justified. This approach of SRLS is, in fact, within the classical tradition of formulating NMR relaxation in terms of simple but well-defined models.[9]

The version of SRLS which has been applied in protein NMR studies[4,5,10] represents the simplest form of the more general approach of employing multidimensional Fokker–Planck (FP) equations, whereby the fundamental physics representing the "relevant" degrees of freedom is rigorously incorporated, and then stochastic assumptions are introduced in a manner subject to basic statistical-mechanical requirements, such as detailed balance.[2,11,12] This current model consists of two rotors, coupled by an orientational potential; one rotor represents the local reorientational motion of the probe (e.g., the $^{15}N-^{1}H$ bond), which is coupled to the overall reorientation of the protein, i.e., the second rotor. These are exactly the same degrees of freedom that are relevant in the MF approach,[1,6–8] which avoids precisely formulating the physics of the model. The current version of SRLS for NMR relaxation and MF apply to overdamped motions (i.e., the Smoluchowski limit), or to related simplified dynamical motions. More generally, the full physics of rotating bodies should not only include their orientational relaxation but also their respective angular momentum relaxation. Instead, the latter is assumed to be overdamped (thus extremely rapid) for simplicity in the current version of SRLS; it cannot be accounted for in MF. This neglect of "inertial" effects is clearly a limitation, which can lead under certain conditions, noted below, to unphysical conclusions; yet it does greatly simplify the analysis. For SRLS, we will refer to this overdamped limit as Smoluchowski-SRLS.

However, the procedures for formulating the full coupled two-rotor FP equations, which we refer to as FP-SRLS, do exist;[2] results for reorientational (and angular momentum) correlation functions for cases of simple symmetry have been presented.[2] Efforts are underway to treat the more general cases which we have found are required[10] for optimum analysis of the experimental NMR relaxation data.[13,14]

There are two issues of importance requiring the inclusion of inertial effects. Smoluchowski-SRLS successfully treats a probe diffusing (or "jumping" in the limit of high potentials) between multiple potential minima, as we have previously shown for the case of overdamped motion (cf. Fig. 4 of Ref. 3) despite Halle's claims to the contrary. This theory,[3] was shown to be directly applicable to proteins.[15] More realistically one has rapid, although damped, torsional oscillations within each potential well with occasional transitions over the barrier to another potential well. In fact, such behavior has been shown to be naturally included within the context of the two-body FP-SRLS approach.[2,16–18]

The second issue is the so-called backreaction of the probe motion on the global motion of the protein via the coupling potential, as required by detailed balance (as well as Newton's laws). In the context of Smoluchowski-SRLS this effect is negligible for the usual case when the diffusion rate for the local motion is faster than that of the overall motion. In special cases the two rates could become comparable; the backreaction can then affect the overall motion leading to what we have referred to as "mode coupling" (although this expression has been used in very different contexts by others). In our extensive analyses of NMR data, we found that such cases of slow local motion are typically as-

sociated with mobile domains or relatively large loops. For these heavier probes, Smoluchowski-SRLS is reasonably adequate.[10] Clearly, in the inertial limit, the local motion of a small probe moiety (e.g., a N–H bond), with its much smaller moment of inertia, should not affect the overall protein motion significantly. An adequate model must thus contain both inertial and damping effects to more rigorously describe this form of mode coupling, as implemented in the FP-SRLS model. The asymmetry in the motions of the two bodies arises naturally from their relative diffusion rates and/or their relative moments of inertia, while both follow the same laws of physics. Halle misinterprets this point when he complains that it is inappropriate to treat the two bodies symmetrically. Furthermore, as we just discussed, the torsional oscillations and transitions between potential wells also occur naturally in the FP-SRLS framework. Finally, we note that Smoluchowski-SRLS for the limit of overdamped motions, and the more general FP-SRLS models, are appropriate whether or not there is a time scale separation; no assumption is made to invalidate this, contrary to Halle's claim.

A major advantage in an approach based on well-defined stochastic processes lies in being able to improve the model by including key additional features, consistent with the properties of the physical system investigated. Care must be taken that the model devised, despite its limited degrees of freedom, has physical relevance and is flexible enough. We have already noted that jump motions, as well as inertial effects, are included in the SRLS model. Additionally, terms that account for restructuring of the local torque acting on the probe can be conveniently and properly introduced as needed *via* master equations.[18] It was shown[18] that the mere presence of local strong collision-like relaxation processes, or jump motions (referred to by Halle as "intermittent" motions) is not *per se* a cause for statistical independence between local and global motions, contrary to Halle's claim. Rather, time scale separation is needed, leading to the conclusion that only for very slow or very fast local jumps no correlation with the global reorientation exists.[18] Furthermore, hydrodynamic coupling, which Halle stresses (but which cannot be included in MF treatments), can readily be included into the two-body FP models, as we previously showed.[2] This is the case where there are coupling terms in the generalized friction tensor of the combined global and local systems.[2] A general method for calculating friction tensors of large flexible molecules treats this feature explicitly.[19] This method was used recently within the scope of a stochastic model for treating the dynamics of a flexible saccharide.[20] Thus, unlike the MF approach, well-defined hydrodynamic coupling can readily be included in our stochastic modeling approaches.

Improved stochastic modeling based on multidimensional FP equations does require more sophisticated computations, but these have become much more accessible with the newer generation of computer systems and effective programs.[21] Thus, one need not be constrained to the simplest modeling. Halle is critical of the numerical, as opposed to the more transparent analytical MF forms for the time correlation functions that he strives for. The latter requires utilizing formal constructs in an effort to achieve apparent generality. On the other hand, we utilize clearly defined physical models, which do not include *a priori* assumptions of statistical independence, and which rigorously treat the well-defined but complex geometric features of the magnetic, local diffusion, local ordering, and overall diffusion tensors, and the Euler angles between these three-dimensional tensors. In fact, it is even possible to develop analytical approximations to the FP equations in special cases, such as the limit of a strong potential between an inertial local body coupled to a slowly diffusing global protein.[14] Comparison with the full numerical results can establish the range of validity of the approximate analytic expressions.

To summarize, the current version of the Smoluchowski-SRLS is a well-defined stochastic model (without the need for *a priori* assumptions and formal constructs as in MF) that helps to clarify the complex details of NMR relaxation in proteins. Methods exist, and are currently being utilized, to improve upon this model to better represent the physical details of protein dynamics within the context of mesoscopic models.[2,13,14] Further aspects of the comparison between SRLS and MF in the analysis of the NMR relaxation in proteins are described in a recent review.[10]

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