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Interactive comment on "DeerLab: A comprehensive toolbox for analyzing dipolar EPR spectroscopy data" *by* Luis Fábregas Ibáñez et al.

Anonymous Referee #2

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In this paper the authors present an exciting new Matlab toolbox for the analysis of pulsed dipolar EPR data. DeerLab contains many notable improvements and enhancements over DeerAnalysis, previously available from the Jeschke lab, and should prove to be a valuable resource for the EPR community. I have the following specific comments that should be addressed prior to final publication.

1) How were all of the data in the paper simulated? How were the P(R) generated? Are they derived from the Edwards and Stoll test data set? What is the noise level added to each data set?

2) The authors do an excellent job of contrasting the advantages and disadvantages of using parametric and parameter-free models for distance distributions. Ultimately, however, they favor the parameter-free approach.

(171-173) "In contrast, regularization approaches use parameter-free distributions and are less affected by these biases. Therefore, it is recommended to use parametric models only when there are strong reasons to prefer them over parameter-free models. Even then, the results should always be contrasted, and presented along with a parameter-free analysis."

(425-427) "This is our rational for recommending routine comparison of results obtained with parametrized models to those obtained by parameter-free analysis, as the latter imposes the least constraints on the shape of the distribution."

These statements are, however not supported by any evidence or examples in the paper. I am confident that all of the examples presented in this paper would be satisfactorily fit using parametric models. On the other hand, as the authors note, (133) "The outcomes of regularization analysis depend strongly on the choice of penalty norm, regularization operator, and alpha." Also, issues using TR when the ground truth distribution contains multiple components with varying widths is well-known. Considerable more research involving comparisons between different methods on a variety of different data sets is needed before drawing conclusions about best practices. DeerLab will certainly facilitate such comparisons.

3) In my mind, the statement (140-141) "The reduced dimensionality of the theta-space compared to P-space often stabilizes the solution of the ill-posed inverse problem to a sufficient extent, without the need of regularization." is misleading as fitting with parametric models is not an inverse problem.

4) The development of a one-step approach to analysis using TR is welcomed. The authors should note that results very similar to those in their Figure 5 were presented in Figure 5 of Brandon et al. (2012) and Figure 11 of Stein et al (2015). These signals have long been analyzable using a parametric approach.

5) The development of an approach to global analysis using parameter-free distance distributions is very exciting. I suggest the authors provide some additional detail about

how this is accomplished. It is not evident to me from Equations 22-26. For example, does this involve separable non-linear least squares? How robust is the non-parametric approach when one or both of the extremes of the binding curve are not available experimentally (fully bound or fully unbound).

6) Repeating the optimization process with different initial parameter values was used in DEFit (Sen et al, 2007).

7) Showing 50% confidence level in Figure 11 is somewhat unusual. A better choice might be the 1 sigma confidence level.

8) The authors do not mention the calculation of 'confidence intervals' as implemented in Brandon et al. and Stein et al., an approach that determines parameter uncertainties without the use of the covariance matrix and the assumption of symmetry. Also, Hustedt et al. (2018) describe the use of a covariance matrix based approach to estimate a 'confidence band' for P(R).

9) Earlier the authors note that the calculation of an effective number of free parameters for a non-parametric model using tr{KK ÌĚ } is problematic (337). Is this how the number of parameters is estimated for the calculation of AICc values in Figure 12B? This should be noted.

10) Figure 9B legend references 'black'. Should this be blue? Figure 9C, the unbound state is in red not blue.

11) Line 181, comma should be replaced with 'and'.

12) Line 339, Hansen et al. is inside comma.

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