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Comment on mr-2021-2

Anonymous Referee #3

Referee comment on "The long-standing relationship between paramagnetic NMR and iron–sulfur proteins: the mitoNEET example. An old method for new stories or the other way around?" by Francesca Camponeschi et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-2-RC3, 2021

Camponeschi and her co-workers described a synergic application of paramagnetic and diamagnetic NMR techniques on protein mitoNEET, a dimer iron-sulfur protein, in both oxidation states. The NMR signals from residues surrounding the metal cofactor is usually crucial for understanding the structure-function in Fe-S proteins and is also challenging to detect due to the paramagnetic cluster. The authors demonstrate how to combine different paramagnetic NMR methods including 1D NOE, paramagnetism-tailored HSQC experiments, 13C detection experiments to reveal the information of protons as close as 4-5 Å around the paramagnetic cluster. The information obtained offers insights into the unique electronic properties of mitoNEET, which help to understand the role of the electronic structure in the biological function of NEET protein. The work in fact provides a potential general protocol that could be applied on many other similar challenging systems. The author gave a nice introduction on the history of NMR study of Fe-S protein started from 1970, and one that of paramagnetic NMR applications. The NMR data were elucidated and presented clearly; the manuscript is well written as well.

One concern is, what is new here for those paramagnetic techniques? The author may want to make it clearer in the paper. I recommend the paper to be published with changes to emphasize more on technical advances that applied here.

Specific comments:

1. Line 230, Figure 1(b), The chemical shift differences are all positive, it looks the chemical shift differences are absolute values of amide H only? The author may describe how to obtain these values. It might also be interesting to map these residues with significantly shift difference to the structure.

2. Line 256: Figure 2, right, it might be better to label the cluster binding residues in figure.

3. 346, "peak labelled with asterisk" are difficult to recognize in the figure, some are labeled "+" or "x".

4. Some minor format issues:

a. Line 172, D_2O

b. Line 174, 177,…, name of experiments not consistent: eg. HNCACO or HN(CA)CO?

c. Line 210, T_{1max}