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Comment on mr-2021-2

Anonymous Referee #2

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-RC2, 2021

In this study, Camponeschi et al use NMR to characterize mitoNEET, a mitochondrial Fe2S2 protein. By using 1D NOE experiments, 13C direct-detected experiments, and the optimization of NMR experiments for paramagnetic systems, the authors show significantly reduction of the “blind” sphere of the protein around the paramagnetic cluster, thus allowing the detection of residues possibly involved in the biological function of mitoNEET. The study has significant implications in the fields of paramagnetic NMR and FeS proteins. Some revisions are recommended.

In details:

1. I have some general questions about the mitoNEET protein I hope the authors can help answer.
a) If mitoNEET can repair Fe-S proteins by donating its own Fe2S2 cluster, how does it reacquire the Fe2S2 cluster? Can the authors comment on the source of its Fe2S2 cluster?

b) The redox states of mitoNEET are crucial for its function and stability. How are the redox states of mitoNEET regulated in cells?

2. Some experimental details are needed.
a) For M9 media growth, how much (15NH4)2SO4 and 13C-glucose were supplemented?
b) What kind of anaerobic environment was used?
c) Does the phosphate buffer contain any NaCl?
d) I assume there were additional steps to remove the extra K4Fe(CN)6 or sodium dithionite?

2) What's the Fe2S2: protein ratio 'as purified'? It would be helpful to include UV data to show the load of Fe2S2 on the protein in both redox states.

3) The authors purified the protein in an anaerobic environment, I assume it's because the Fe2S2 is susceptible to oxidative damage. Would addition of 10mM K4Fe(CN)6 to the protein solution damage the Fe2S2 cluster?

4) Is the purified mitoNEET protein a homodimer as shown in Fig. 1A?

5) In Fig. 1A, can the authors highlight the residues that are affected by different redox states?

6) Fig 1B, how were the chemical shift differences between two redox states calculated?

7) It's intriguing to me that the redox state change would mainly affect the regions involved in inter-subunit contacts. Do the authors have any hypothesis why?

8) There is no mention of Fig. 1C in the text. The author might add some.

9) Can the authors provide some explanations why no hyperfine shifted signals were observed for the reduced [Fe2S2]+-bound form of mitoNEET?

10) The authors should provide the data showing the broadening of signal B collected in D2O.

11) The authors might want to highlight the additional residues assigned by 15N-IR-HSQC-AP in the structure of mitoNEET.

12) The labels in Fig.3 are too small to read, the authors might want to improve that.