Comment on mr-2021-2
Anonymous Referee #1

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-RC1, 2021

In this report Camponeschi et al. present NMR assignments of the dimeric membrane-anchored human CDGSH protein ("mitoNEET"). Each subunit contains a Fe$_2$S$_2$ cluster and the current study aims at investigating its electronic properties in both the oxidized and the reduced states. Due to the paramagnetism of the cluster the resonance assignment requires separate sets of experiments for residues located outside a ~ 10 Å sphere and residues near the cluster. The former involves standard 3D backbone triple-resonance and side-chain experiments. In order to reduce the “blind sphere” around the cluster and observe very fast relaxing resonances 2D $^{15}$N-IR-HSQC-AP, developed a couple of years ago by one of the authors, and protonless $^{13}$C-detected CON experiments were carried out. Finally, a number of protons from Fe-coordinating residues were assigned using 1D NOE experiments in conjunction with X-ray structure derived distances. Conclusions about the electron distribution within the Fe$_2$S$_2$ cluster were drawn from the envelope of the hyperfine-shifted spectral region, which has some functional implications.

Although I am not an expert for iron-sulfur proteins it appears to me that the system studied here is of high biological relevance (nice literature overview in the Introduction, by the way). Overall, the manuscript is very well-written, except for paragraph 3.2.2 (see below). It describes sound experimental work and comprehensible interpretation of the results. However, it mostly represents an application of established techniques to a well-studied protein. Considering its editorial policy the current paper falls outside the scope of Magnetic Resonance.

Paragraph 3.2.2 ("Paramagnetic experiments on [Fe$_2$S$_2$]-mitoNEET reduced and oxidized") is to a large extent phrased in a lab-style language and should be rewritten.

Some examples:

Heading: are the experiments paramagnetic or the samples ?

Lines 190, 194, 200: “Spectra were recorded on a Bruker Av600 MHz” (spectrometer ?)

Line 198: “Each experiment consisted from ~ 300k up to ~900k scans.”

Line 202: “…using 16.5 ms and 13.7 ms as acquisition and a $t_{1\text{max}}$ delay....”
Line 209: “...2048 scans each fid were collected...”

Line 211: “...the IPAP approach was used for homodecoupling...” Does that mean virtual decoupling of $^1J_{C'Ca}$?

Further minor issues:

According to the Material and Methods section $^{15}$N relaxation experiments ($R_1$, $R_2$, hetNOE) were performed for the diamagnetic part of the protein. The purpose and result of these experiments is not reported at any point in the manuscript.

Proton 1D spectra were recorded with a spectral width of 320 ppm, much wider than required for spectral range observed here. Were any specialized wide-band pulses employed that would be able to excite a ~ 200-kHz region?

It is mentioned that the CON experiment was optimized for paramagnetic systems (section 4.2.3). Which modifications were applied? Simply shorter magnetization transfer periods? An INEPT delay of 8 ms is specified in the experimental section, which is shorter than $1/(2*^1J_{C'Ca})$. Is that sufficient to incorporate the IPAP module?