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Interactive comment on "Phosphoserine for the generation of lanthanide binding sites on proteins for paramagnetic NMR" *by* Sreelakshmi Mekkattu Tharayil et al.

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This manuscript describes a new way of tagging proteins with lanthanoids. It is an original new approach yielding excellent results on some systems. It is highly appreciated that the authors also discuss the systems for which the method does not work, describing its limitations as well as its advantages. The methods are described in sufficient detail and all results appear sound. Thus, the work is a useful addition to the field of Magnetic Resonance.

There are only a few comments that the authors may address for further improvement:

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Discussion paper

l. 100: A 100-fold excess of TEV was used, that seems an awful lot for an enzyme. How was it removed? Was second NTA column used?

l. 108, 109: The Bruker line of consoles is called Avance, not Advance

Table 1: It would be useful to add the number of PCS used in each calculation in an extra column. Also, the tensors for Tm3+ are very low indeed compared to those for Tb3+. Other tags, such as CLaNP give very high values for Tm3+ ($\sim$ 55 x 10ˆ-32). Do the authors know why these differ so much? The Q-values are very low, as mentioned, and all but one are 0.03, yet looking at the plot for Ubi E16Q/E18Sep(Tm3+) the spread looks clearly larger than for others (Fig. 2). How can that be?

Fig. S1: Can you indicate the fitted parameters for the ITC? Were n, Delta-H and K(D) all fitted? What are the results?

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