We thank the reviewer for thoughtful comments.

−   Was it always possible to achieve saturation of the various proteins with the lanthanide metals? For example, in Figure 1 (a) the complex is in slow exchange and a fraction of the peaks still appear at the (assumed) unbound position. This is also observed for other spectra in this paper e.g. Figure 3, GB1. For many applications e.g. observation of PCSs on binding partners, complete saturation with metal ions is necessary to accurately interpret the PCSs. Is this affected by the choice of metal?

Response: Indeed, hitting exact lanthanide:protein ratios in titration experiments can be quite tricky due to inaccuracies in concentration measurements (ubiquitin, for example, is devoid of a tryptophan or tyrosine residue, giving rise to low UV absorption). In addition, we experimentally observed that titration with lanthanides delivered PCSs more readily after we had treated the proteins with EDTA, but any incompletely removed traces of EDTA would compete with metal binding to the protein. We therefore adopted an operational approach to establishing the lanthanoid-protein complexes, namely by titrating until the original signal of the protein had vanished or, at least, substantially decreased (to avoid over-titration and potential binding to other sites). This is now described in lines 114-118 and 223, and discussed in a new paragraph on page 21. In addition, to avoid possible overstatements, we removed all references to exact titration ratios.
    While the paramagnetic peaks are well resolved due to PCSs, incomplete saturation with diamagnetic yttrium could indeed be problematic for signals that are significantly shifted by diamagnetic metal ions. In practice, this is not a serious problem, because PREs in the paramagnetic samples anyway prevent the observation of amide protons that are located near the metal binding site and thus most likely to shift upon titration with metals. This is now discussed on page 21. We assumed that the binding affinities of different lanthanoid ions are very similar. The close similarity in their chemical properties is well documented in the literature.

−   The authors say that the proteins were titrated with paramagnetic lanthanide metals. In some cases they mention that a 1:1 ratio of lanthanide:protein was used. Was this used in all cases? How did the authors avoid free Ln3+ in solution potentially creating non-specific bleaching due to the PRE component of the lanthanides ("solvent PRE"). It would be useful to comment on this.

Response: We rather under-titrated the proteins with metal ions (see response to the query above). In this way we not only avoided non-specific PREs, but also the possibility of generating PCSs from alternative metal binding sites. If a significant degree of binding at other sites had occurred, it would have been manifested in a decreased quality of the fit of a single DeltaChi tensor – we detected no sign of this.

−   An impressive range of different proteins and mutants is tested in this manuscript. It would be helpful to include a supplementary table comparing all the different mutants studied in terms of number of phosphoserines, other mutations, expression level, metal binding etc.

Response: we already report all the different failed mutants in Figures S3, S6 and S8 and see no advantage in repeating this information in an additional table.

–   The rather low binding affinity for the lanthanides has potential disadvantages and would for example prohibit the use in combination with nucleic acids as free lanthanide ions will bind and potentially cleave the nucleic acid. But free lanthanides may also interfere with other regions of a given protein. The authors may want to comment and discuss this.

Response: The phosphodiester backbones of DNA and RNA are known to be sensitive to hydrolysis by free lanthanides as well as metal ions that are in complexes with remaining accessible ligand binding sites. As phosphoserine residues also change the overall charge of the protein, the lanthanide-binding strategy presented in this manuscript would quite clearly not be a good choice for studies of protein-DNA or protein-RNA complexes. We feel that discussion of these effects in the main text goes beyond the scope of the present work.

Technical comments
Line 62: "only *a* few" ("a" missing).

Response: fixed.

Line 112 (Methods): Were the lanthanide stock solutions for NMR titration also prepared in NMR buffer as for the ITC experiments?

Response: The stock solutions were in water (unadjusted pH = 5.7). As all protein solutions were in 20 mM HEPES buffer and the lanthanoid concentrations did not exceed 0.5 mM, any change in pH of the final solutions would have been minimal. If a change in pH had had a significant effect on the chemical shifts, this would have become apparent in worse quality factors of the DeltaChi-tensor fits.

Line 245: "The difficulties to express most of the double-phosphoserine mutants was not due to expression into insoluble inclusion bodies, as we did not find the proteins in the insoluble fraction after cell lysis." → The difficulties *in* expressing most of the double-phosphoserine mutants *were* not …. Also in line 250.

Response: fixed.

NMR spectra: The ^{15}N axis label looks like it is divided by ppm.

Response: that's correct. Chemical shifts are numbers with the unit ppm. Divide by the unit and the result gives the unitless numbers as they are displayed along the axes.

Figure 2: It would be useful to provide a key to the colours on the graphs or maybe to choose different colours – blue and red could be confused with the blue and red lobes of the PCS tensors shown on the right hand side. This is true in some of the other figures too.

Response: we have used this colour standard in many other articles. The figure legends state the colour code clearly.

Figure 5: It would be helpful to mark the distances to the lanthanide metal – in particular in (a) Glu26 does not appear to be close to the metal position, whilst in (b) the proximity is evident.

Response: The distance between metal ion and the nearest sidechain oxygen of Glu26 is 4.9 Å in Figure 5a and the corresponding distance to the nearest oxygen of Glu56 is 3.6 Å in Figure 5b. We now write in the main text that these glutamate side chains are potentially within reach of the metal ion (line 270). Notably, Figure 5 displays the crystal structure conformation for these glutamate residues without trying to explore alternative side chain conformations. This is now also mentioned in the figure legend. As we already stated in line 274, mutation of Glu26 to an uncharged residue abolished the observation of PCSs.

Figure 6: It would be useful to mark the proposed interactions as discussed in the text.

Response: salt bridges are rarely manifested in crystal structures by obvious proximity between the charged groups of different amino acid side chains. As part of the discussion section, the figure only means to highlight the possibility of salt bridge formation. Inspection of the full 3D structure of the proteins is required to appreciate this possibility. The figure legend gives the colour code to identify the amino acid side chains of interest.