(1) The triangulation approach of using the intersections of three PCS isotherms has been reported before in other papers (e.g. J Biomol NMR 71, 27, 2018), so it is not clear why the current approach is not compared with published methods.

There seems to be a typo in the page number - we presume that the reference referred to is J Biomol NMR 71, 271, 2018 (Lescanne et al.), which is indeed relevant.
Using different paramagnetic metal ions and paramagnetic metal tags attached to proteins at two or more sites (one at a time) is a well-established strategy for pinpointing the location of protein sidechains, bound ligand molecules or proteins, as well as for 3D structure determinations of proteins. We propose to include the following (broadly representative though still incomplete) list of references in the introduction.

For side chain conformations:
Pearce, B. J. G. Jabar, S., Loh, C.-T., Szabo, M., Graham, B. and Otting, G.: Structure restraints from heteronuclear pseudocontact shifts generated by lanthanide tags at two different sites, J. Biomol. NMR, 68, 19–32, https://doi.org/10.1007/s10858-017-0111-z, 2017.

Lescanne, M., Ahuja, P., Blok, A., Timmer, M., Akerud, T. and Ubbink, M. Methyl group reorientation under ligand binding probed by pseudocontact shifts, J. Biomol. NMR, 71, 275–285, https://doi.org/10.1007/s10858-018-0190-5, 2018.

For protein-ligand complexes:
Guan, J.-Y., Keizers, P. H. J., Liu, W.-M., Löhr, F., Skinner, S. P., Heeneman, E. A., Schwalbe, H., Ubbink, M. and Siegal, G.: Small-molecule binding sites on proteins established by paramagnetic NMR spectroscopy, J. Am. Chem. Soc., 135, 5859–5868, https://doi.org/10.1021/ja401323m, 2013.

Chen, W.-N., Nitsche, C., Pilla, K. B., Graham, B., Huber, T., Klein, C. D. and Otting, G.: Sensitive NMR approach for determining the binding mode of tightly binding ligand molecules to protein targets, J. Am. Chem. Soc., 138, 4539–4546, https://doi.org/10.1021/jacs.6b00416, 2016.

Zimmermann, K., Joss, D., Müntener, T., Nogueira, E. S., Schäfer, M., Knörr, L., Monnard, F. W. and Häussinger, D.: Localization of ligands within human carbonic anhydrase II using ^{19}F pseudocontact shift analysis, Chem. Sci., 10, 5064–5072, https://doi.org/10.1039/c8sc05683h, 2019.

For protein-protein complexes:
Pintacuda, G., Park, A. Y., Keniry, M. A., Dixon, N. E. and Otting, G.: Lanthanide labeling offers fast NMR approach to 3D structure determinations of protein-protein complexes, J. Am. Chem. Soc., 128, 3696–3702, https://doi.org/10.1021/ja057008z, 2006.

Keizers, P. H. J., Mersinli, B., Reinle, W., Donauer, J., Hiruma, Y., Hannemann, F., Overhand, M., Bernhardt, R. and Ubbink, M.: A solution model of the complex formed by adrenodoxin and adrenodoxin reductase determined by paramagnetic NMR spectroscopy, Biochemistry, 49, 6846–6855, https://doi.org/10.1021/bi100598f, 2010.

de la Cruz, L., Nguyen, T. H. D., Ozawa, K., Shin, J., Graham, B., Huber, T. and Otting, G.: Binding of low molecular weight inhibitors promotes large conformational changes in the dengue virus NS2b-NS3 protease: fold analysis by pseudocontact shifts, J. Am. Chem. Soc., 133, 19205–19215, https://doi.org/10.1021/ja208435s, 2011.

Kobashigawa, Y., Saio, T., Ushio, M., Sekiguchi, M., Yokochi, M., Ogura, K. and Inagaki, F.: Convenient method for resolving degeneracies due to symmetry of the magnetic susceptibility tensor and its application to pseudo contact shift-based protein-protein complex structure determination, J. Biomol. NMR, 53, 53–63, https://doi.org/10.1007/s10858-012-9623-8, 2012.

Brewer, K. D., Bacaj, T., Cavalli, A., Camilloni, C., Swarbrick, J. D., Liu, J., Zhou, A., Zhou, P., Barlow, N., Xu, J., Seven, A. B., Prinslow, E. A., Voleti, R., Häussinger, D., Bonvin, A. M. J. J., Tomchick, D. R., Vendruscolo, M., Graham, B., Südhof and T. C., Rizo, J.: Dynamic binding mode of a synaptotagmin-1-SNARE complex in solution. Nat. Struct. Mol. Biol., 22, 555–564, https://doi.org/10.1038/nsmb.3035, 2015.

For 3D protein structure determination:
Yagi, H., Pilla, K. B., Maleckis, A., Graham, B., Huber, T. and Otting, G.: Three-dimensional protein fold determination from backbone amide pseudocontact shifts generated by lanthanide tags at multiple sites, Structure, 21, 883–890, https://doi.org/10.1016/j.str.2013.04.001, 2013.

Crick, D. J., Wang, J. X., Graham, B., Swarbrick, J. D., Mott, H. R. and Nietlispach, D.: Integral membrane protein structure determination using pseudocontact shifts. J. Biomol. NMR, 61, 197–207, https://doi.org/10.1007/s10858-015-9899-6, 2015.

Pilla, K. B., Otting, G. and Huber, T.: Protein structure determination by assembling super-secondary structure motifs using pseudocontact shifts, Structure, 25, 559–568, https://doi.org/10.1016/j.str.2017.01.011, 2017.

(2) In the discussion (l. 479 – 486), the point of not using different metals in the same tag but multiple orthogonal sites has been made by other studies, so references are required there.

We agree that the discussion needs to acknowledge previous findings regarding the importance of isosurfaces intersecting in an orthogonal manner. The comment raises an interesting question: are the tensor axes also aligned, when comparing tensors generated by paramagnetic lanthanoid ions and transition metal ions such as Co(II)? We remember having observed this once:

Man, B., Su, X.-C., Liang, H., Simonsen, S., Huber, T., Messerle, B.A. and Otting, G.: 3-Mercapto-2,6-pyridinedicarboxylic acid, a small lanthanide-binding tag for protein studies by NMR spectroscopy, Chem. Eur. J., 16, 3827–3832, https://doi.org/10.1002/chem.200902904, 2010.

but there may be more examples.

We suggest the following new paragraph:

It has been pointed out previously that the accuracy with which localisation spaces can be determined is best when PCS isosurfaces intersect in an orthogonal manner (Pintacuda et al., 2006; Lescanne et al., 2018; Zimmermann et al., 2021). In the present work, we found that, counterintuitively, the provision of additional data can considerably degrade the accuracy of the localisation space. This effect arises when PCS isosurfaces intersect at a shallow angle, as the location of these intersections becomes very sensitive with regard to small errors in the relative orientations of the underpinning Dc tensors. Shallow intersection angles of PCS isosurfaces routinely occur, when two PCS datasets are from tags and tagging sites that differ only in the identity of the paramagnetic metal ion in the tag. This situation commonly generates Dc tensors of different magnitude and sign, but closely similar orientation (Bertini et al., 2001; Su et al., 2008; Keizers et al., 2008; Man et al., 2010; Graham et al., 2011; Joss et al., 2018; Zimmermann et al., 2021). Therefore, while the use of Tm^{3+} and Tb^{3+} tags is helpful for assigning the cross-peaks in the paramagnetic state, it is safer to use only one of these data sets for calculating the localisation space. Good localisation spaces were thus obtained by using only PCSs measured for Tb^{3+} tags (Fig. 6) or only PCSs measured for Tm^{3+} tags (Fig. S12). In contrast, however, very different tags attached at the same site, such as the C2 and C12 tags installed in the mutant N172C, produced independent Dc-tensor orientations and therefore contributed positively to localising the Trp28 H^{e1} atom.

(3) In line 246 it is mentioned that double peaks are observed for the Trp NHe groups. That could mean that the indoles are in different conformations in slow exchange. It is not discussed whether these could be the other conformations observed in the crystal structures. Could the PCS analysis be done for these minor peaks to exclude that possibility? In that case this work only yields the position of the major form, not the only form.

We do not understand the origin of the minor species and could not assign these weak cross-peaks sequence-specifically, also not with the help of PCSs. In previous work, we discovered that IMP-1 readily installs a Fe(III) ion one of the Zn(II) sites (Carruthers et al., 2014), which explained some of the additional peaks observed in early preparations. Our current protein purification protocol carefully excludes iron. As the cross-peaks of the remaining minor species varied in intensity between different

sample preparations and following long NMR measurements, we believe that they arise from some process of sample degradation.

(4) The mass spectrometry shown in Fig. S2 and the yields mentioned give rise to questions. In line 204 the efficiency of 90% is mentioned. However, using the information in the caption of Fig. S2, a different result is suggested: The masses in the figure are about 9 Da lower than expected for 100% labelling (given the mentioned masses), which 25% of the expected 36 Da extra (in the caption it says +6 Da/Trp), so labelling efficiency would be 75%. However, after converting the indole to tryptophan, one deuteron is removed, so the expected mass increase is 4XD + 1 13C = 5 Da per Trp, not 6. That would result in a labelling of 90%, agreeing with the main text, but suggesting that the masses mentioned in the caption are too high. Please check.

Thank you for alerting us to the error. Following double-checking, the calculated masses for the spectra of Figure S2a-c should have been reported as 25234.88 Da, 25191.86 Da and 25218.88 Da, respectively, in agreement with 90 % incorporation efficiency of the isotope-labelled indole. We also noticed that the spectra shown in Figure 2d and f had accidentally been swapped and that the tag used increased the mass by 858 Da (not 832 Da).

(5) In section 2.2, mention the protein concentration(s) used for the NMR samples and indicate the tube type (3 mm, 5 mm, Shigemi), to know how much sample was used. Also mention the protein concentration in the captions of the NMR spectra figures in the supplementary material.

The spectra were recorded using 3 mm tubes. The requested information will be provided in Section 2.2. The protein concentrations were already given in the captions of the overview NMR spectra shown in the supplementary material.

(6) l. 213, how were the assignments for the diamagnetic protein obtained? If from previous reports, give the reference and the BMRB entry.

The assignments of the diamagnetic protein were already released in 2014 in BMRB entry 25063. We will refer to this entry in Section 2.3.

(7) Thank you for pointing out these oversights, which we will fix in the revised version.