In response to the evaluation by Marcellus Ubbink:

(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.

Response: The reference referred to (Lescanne et al., J Biomol NMR 71, 271, 2018) is indeed relevant, as are several others, the most important ones we have now attempted to include in the list of references (cited on lines 104-110). We now explicitly state that 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 also made sure to avoid any impression of a claim that the strategy per se is novel. Instead, our contribution is to (i) illustrate the accuracy with which structural details in a solvent-exposed loop can be elucidated in this way and (ii) highlight the finding that PCSs from two paramagnetic metal ions (rather than one) in the same tag at the same site can produce a worse result than reducing the PCS data set to include only data from a single paramagnetic metal ion per tag and tagging site (now pointed out more clearly in the Discussion and Conclusion sections).

(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.

Response: We now cite previous work pointing out the importance of isosurfaces intersecting in an orthogonal manner more comprehensively (Section 3.3 and the Discussion, lines 498-513). Nonetheless, some success has been had in the past with different metal ions in the same site, and this is now discussed too. We believe that it has not been reported previously that more data can give objectively worse results.

(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.

Response: 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 (Carruthers 2014). 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. We now point out that IMP-1 is a protein of limited stability.

(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.

Response: Unfortunately, the spectra shown in Figure S2d and f had accidentally been swapped, and the mass increase by the tag had been incorrect too. We double-checked all mass spectrometry data carefully and concluded that they do not allow claiming >80 % incorporation efficiency of the isotope-labelled indole (line 218). To allow the reader to check the calculated mass, we specify the exact amino acid sequence of our construct in Section 2.1.1.

(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.

Response: The spectra were recorded using 3 mm tubes. The requested information is now 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.

Response: The assignments of the diamagnetic protein were released in 2014 in BMRB entry 25063. This is now referred to in Section 2.3.

(7) Some supplementary figures have the wrong numbers in the text:
line 165, Fig S1 > S8;
line 170, Fig. S2 > S9;
line 212, Fig. S2-S5, S3-S6 (?)
Tables S1 – S6 are not mentioned in the text.

Response: Thank you for pointing out these oversights, which we corrected in the revised version.

In response to the evaluation by Daniel Häussinger:

1) The procedure of the triangulation by PCS is not new and earlier work by some of the authors and others should be referenced accordingly.

Response: We agree and included a set of references in the introduction (as per our response to comment 1 by Marcellus Ubbink).

2) The finding, that two sets of PCS created at the same tagging site and by the same tag, but different lanthanoids give less accurate results, is not new but certainly remarkable – it would be tempting to quantify this finding by elucidating the "angle score" parameter for these data sets as suggested by Joss et al. (Chem. Sci., **2019**, 10, 5064-5072.)

Response: The angle score parameter is useful. We are working on a more comprehensive comparison of different metrics for the identification of localisation spaces in a separate publication. In the present context, we refer to our response to comment 2 by Marcellus Ubbink.

3) The authors refer only to proton PCS despite the fact, that they obtained also PCS data from ^{15}N and ^{13}C – could you comment on that?

Response: We use only ^{1}H PCSs because nuclear spins with large CSA tensors display residual anisotropic chemical shift (RACS) effects due to weak paramagnetic alignment of the molecule in the magnetic field. The mixture of alignment and PCS information is cumbersome to disentangle. Furthermore, paramagnetic shifts measured of heteronuclei in the indirect dimension tend to come with greater uncertainties. We now point this out in the Discussion section of the revised version (lines 523-558).

4) The description of the MS instrumentation and conditions is missing.

Response: We now added the information in the supporting information (page S3).

5) The suggested incorporation level of the ^{13}C-indole is not in accordance with the presented MS spectra in Fig S2, panels a-c.

The figure should be reproduced with better resolution of the individual spectra to allow judgement of the incorporation and a deconvolution of the different isotopomers should be provided.

Response: Some of the data in Figure S2 were swapped by mistake, see our response to point 4 by Marcellus Ubbink. We are not entirely happy with the accuracy of our mass spectrometric measurements, which seem to be no better than 1 Da. We amended the claim of 90 % incorporation rate to 80 % (line 218). Compared to the tallest mass peak, we feel that expanded versions, as provided in our response during the discussion phase, do not offer a more accurate determination of the incorporation rate of the deuterated and 13C-labelled indoles.

6) It would be useful to have the individual metal – cysteine-sulphur distances for each tensor included in table S7

Response: We have provided the information on metal-to-beta-carbon distances in Table S7. The distances vary between 8.2 and 12.1 Å.

7) In the experimental section it is reported that the conjugation of the tags to the protein was performed in the presence of 100 uM $ZnSO_4$ (buffer A), does this not interfere with the cysteines, given the nM Kd values of $Zn(Cysteine)_2$ complexes?

Response: The presence of zinc was necessary as IMP-1 binds traces of iron more tightly (Carruthers et al., Angew. Chem. Int. Ed. 2014, 53, 14268). A single sidechain thiol group doesn't bind zinc very tightly. The solutions contained no free cysteine and there was no evidence for zinc interfering in the ligation reaction.

8) minor typos:
line 142: should read "preparation of each sample"
line 165 should read "Fig. S8"
line 170 should read "Fig. S9"
line 204 c.f. 5)
line 207: "100%" on a deconvoluted mass spectrum is probably stretching the significance a bit – how about "virtually quantitative" or " > 95%"?
line 346: the "of" in "isosurfaces of associated" seems superfluous to me.

Response: We are grateful for the careful evaluation and fix these issues in the revised version. We accept that it is rarely justified to talk of 100 % yield and changed the sentence accordingly (line 221).