Dear Stephan,

In response to your comments and the comments made by Nico Tjandra, we have made the following changes.

I don't see that the lettering of Trp sidechain assignments in Figures S3, S4, S7 would have to be any smaller than for the other annotated resonances. There is plenty of space and the annotation will make the data easier to understand for readers.

Response:
We have added the assignments of the Trp sidechains in Figures S3, S4 and S7 for easier comparison with the zoomed in regions shown in Figures S5 and S8.

In response to Nico Tjandra's comments, we have made the following changes:

1. I suspect that the errors in the tensor parameters are rather small since a large number of backbone PCS were used to fit the tensor to the structure s of the protein in various forms. In fact the authors did a 20% random deletion to estimate the error. Can this standard of deviation of the parameters be added to the table S7.

Response:
The uncertainty in chemical shift measurement is rather small and, in the case of IMP-1, PCSs are more likely to be affected by variable sample conditions. We added the following footnote to Table S1: "Estimating the uncertainty of peak positions, σ, with the equation $\sigma = 0.66N/(S \times t_{2max})$, where S/N is the signal-to-noise ratio and t_{2max} the acquisition time (Kontaxis et al., 2000), suggests uncertainties of the PCSs much smaller than 0.001 ppm for all PCSs measured in the present work. More critically, the chemical shifts (in particular of amide protons) are sensitive to minor differences in sample conditions between paramagnetic and diamagnetic samples, the impact of which is difficult to predict."

To obtain an estimate of minimal uncertainty ranges of the Δχ-tensor parameters of Table S7, we produced families of tensors by random omission of 20 % of the data (as described in lines 366–368 of the main text). Table S7 is pretty congested as it is and we therefore reported the uncertainties obtained in this way in a separate table underneath (Table S8).

2. Line 436 the authors state "within the uncertainty of the experiments". So what is the uncertainty? Note that PCS as low as 1-4 ppb are listed in Table S3-S5.
I think quantifying this is quite relevant as the authors discuss the PCS analysis for W28 He1 in N172C mutant. The PCS value for that specific proton apparently is different in the two (H-C or H-N) spectra and yet using either value doesn't seem to alter the final position of that proton in the structure. This suggests to me that for that particular orientation and distance combination, large deviation in the PCS value doesn't translate to large spatial change. Yet in line 487, the authors concluded that W28 indole side chain can be determined "with remarkable accuracy". As written these sections are contradictory to me. What is the spatial accuracy?

Response:
To capture the uncertainties, we varied the $\Delta\chi$ tensors. We found the resulting localisation spaces remarkable in that they were able to distinguish clearly between different crystal structures, as pointed out in the sentences following line 487. To address the question of uncertainties, we added a new footnote to Table S1 as spelled out in our response to point 1.

Uncertainties in the $\Delta\chi$ tensors also affect the spatial accuracy, which we addressed by using a range of $\Delta\chi$ tensors fitted to variable data sets as described in lines 366 to 369. We highlight this now a bit more clearly in line 364. Furthermore, we discuss the sources of errors in a new paragraph in the discussion section as described in our response to point 3 below.

3. The authors correctly stated on line 361 that experimental error affect the PCS isosurface in a non-isotropic way. In fact, I would argue that it is really a shell with varying thickness (due to experimental error) rather than a 2D iso-surface that should be considered due to experimental error. Choosing Ln tagging site such that the area of interest is within the smallest volume of that "iso-shell" can be very important as exemplified by comment 2 above.

Response:
We thank the reviewer for the clarifying comment. We added the following paragraph to the discussion section:
"The accuracy, with which localisation spaces can be determined, further depends on the accuracy with which PCSs can be measured (which critically depends on the reproducibility of the sample conditions between the paramagnetic and diamagnetic states), the accuracy of the protein structure used to fit the $\Delta\chi$ tensors and the angle with which PCS isosurfaces of different tensors intersect. To take into account the uncertainties associated with the PCS isosurfaces, it is useful to think of each of them individually as a shell of a certain thickness (rather than a surface) that represents a compatible localisation space. Two shells of a given thickness share a smaller common space if they intersect orthogonally than if they intersect at a shallow angle."

Thank you again for considering our manuscript for publication in Magnetic Resonance.

Best regards,
Gottfried