Dear Stephan,

In response to your comments, we have made the following changes. We refer to the original manuscript as ms_orig and the current revised version as ms_current.

1. The major goal of the manuscript is to define the conformation/dynamics around W28 in solution. However, this is not very much worked out. I found the description on lines 401–409 very confusing. Apparently, the PCS of Trp28 Heps1 is scaled down without captopril (Fig. S5b, line 403). Contrary to what is stated on lines 404, 405 I could not see the backbone amide PCS in Fig. S11. Why would this suggest different loop conformations for differently labeled samples (line 406)? In my understanding, the reduced PCS of Trp28 Heps1 in the absence of captopril may very well indicate flexibility. This would also agree with the observed flexibility of the L3 loop and the Trp28 sidechain in the mentioned NMR relaxation study (line 71). The entire discussion on lines 401–409 should be clarified and the comparison to the earlier relaxation data should be made. Eventually this should rather be a key point of the conclusion section.

Response:
The goal was to define the possible change in conformation of an active-site loop of IMP-1 in response to inhibitor binding. As we were unable to pin down conformational changes in an unambiguous manner, we feel that this cannot be a key point of the conclusions. NMR work with this protein is difficult due to the occurrence of peak doubling as well as missing cross-peaks. Missing cross-peaks probably arise from conformational exchange in the μs–ms time regime in parts of the protein. Peak doubling, however, behaves in more mysterious ways, as minor additional peaks of residues near the active site tend to vanish with time. This observation (reported in the PhD thesis by T. J. Carruthers in 2014) was only made for the [ZnZn] complex, but not for the [FeZn] complex, which produces cleaner NMR spectra. We now illustrate minor peaks in the ^{15}N-HSQC spectrum of the [ZnZn] complex of wild-type IMP-1 selectively labelled with ^{15}N-tryptophan (Fig. S6). At the same time, sample degradation is an issue. (For example, IMP-1 cannot be stored frozen without irreversible unfolding and precipitation.) As minor peaks arise from different species, do not appear in all spectra (apparent from a comparison of Figs S5 and S6) and were too weak to measure PCSs, we cannot assign and characterize in terms of structure. We feel that an extensive discussion of minor species detracts from the main conclusions of our work, namely the capacity of PCSs to extract structural information for difficult samples, the advantages gained from selectively ^{13}C-labelled indoles and the effect that localisation spaces can be determined with greater accuracy by omitting data from similar $\Delta\chi$ tensors.

Regarding the scaling down of the PCSs of Trp28 $H^{\varepsilon 1}$ in the presence of captopril (line 403 of ms_orig), this effect was not observed in all spectra. It was also small. To recapitulate, we basically have two sets of spectra. Set I comprises the NOE-relayed ^{13}C-HSQC spectra of ^{13}C-indole-labelled protein (Figs 2 and 3). Set II comprises the ^{15}N-HSQC spectra of uniformly ^{15}N-labelled protein (Figs S5 and S8). The PCSs of the backbone amides, as far as they could be assigned, were practically the same with and without inhibitor (shown in Fig. S12), indicating conservation of the $\Delta\chi$ tensors. This allows attributing changes in PCSs displayed by the side-chain of the tryptophan in the loop of

interest (Trp28 in the L3 loop) to differences in its location rather than changes in $\Delta\chi$ tensor (as described in the first paragraph of Section 3.5).

In the spectra of Set I, the PCSs of Trp28 $H^{\varepsilon 1}$ were practically the same with and without inhibitor. In Set II, however, they were slightly smaller in the absence of inhibitor (most apparent in Fig. S5b). As the localisation spaces calculated with either set of PCSs overlapped, the significance of these differences is limited. We now write (lines 478–481 of ms_current): "We attribute the differences in PCSs observed between the selectively ^{13}C-labelled and uniformly ^{15}N-labelled samples to differences in sample preparation of unknown origin, which are also reflected by different numbers of weak unassigned cross-peaks (Figs 2, 3, S5 and S6)."

Relaxation studies have never been performed of IMP-1 (only of the related metallo-β-lactamases referenced on line 415 of ms_orig and line 487 of ms_current), but IMP-1 almost certainly behaves the same, i.e. the L3 loop is probably relatively mobile. We pointed out that the presence of captopril caused an increase in the peak intensities of Trp28. Therefore, the localization spaces determined for Trp28 atoms are time averages. This was discussed in the second paragraph of Section 3.5.

Any attempt to interpret the PCSs in terms of a range of models would present a difficult inverse problem that cannot be addressed without additional assumptions. One approach, termed 'maximum allowed probability', was developed by the Florence group to identify a small number of conformations, which explain the PCS data observed for calmodulin labelled with paramagnetic lanthanide ions (Bertini et al., PNAS, 101, 6841-6846, https://doi.org/10.1073/pnas.0308641101, 2004). We are reluctant to follow this approach not only because any differences in PCSs with and without ligand are very much smaller in the case of IMP-1, but also because the PCSs were not fully reproduced in different sample preparations.

With the supply of selectively labelled indole exhausted, we are limited in the means to explore the biochemical features of IMP-1 further.

2. The minor species in the spectra seem a major concern. This problem should be explained more carefully with respect to the origin of the degradation or minor conformations and the interpretation of the data. The description in Lines 243-251 should be expanded and the interpretation should also be taken up again in the discussion, e.g. why should these minor peaks not be subconformations that correspond to some of the X-ray observations. In this respect all the additional peaks (minor species or unassigned) should be properly labeled (asterisks etc) in all the spectra, i.e. Figure 3, S3, S5, S6, S7. In this respect 'S5' on lines 245, 248 should probably be 'S6'.

Response:
As per our response to query 1, the minor peaks arise from different species, do not appear in all spectra and their cross-peaks were too weak to measure PCSs. We do not know how to prevent degradation of the protein. Unavoidably, the tagging reactions take some time and require sample handling. The legend of the new Fig. S6 states: "Stars identify weak cross-peaks arising from sample heterogeneity. They are of unknown origin and were not reproduced between different sample preparations."

For the mutant N172C with C2 tags, PCSs > 1 ppm are expected for Trp28 $H^{\varepsilon 1}$ regardless of its conformation, including the extreme conformation in the crystal structure 1DDK (green in Fig. 1). We observed no PCS of this magnitude for any of the unassigned peaks in the spectra of Fig. S5b and therefore have no evidence for alternative subconformations of the L3 loop that would be in slow exchange. We now write in the discussion (lines 423–427): "None of the minor additional cross-peaks observed in any of the sample preparations could be attributed to alternative conformations of Trp28 either. In particular, the most extreme conformation observed in the crystal structure 1DDK (green in Fig. 1) predicts PCSs > 1 ppm for Trp28 $H^{\varepsilon 1}$ in the mutant N172C with C2 tags, but we observed no PCS of this magnitude for any of the unassigned peaks."

We labelled the spectra of Fig. S5 as requested. Comparison with the selectively ^{15}N-Trp-labelled sample of wild-type IMP-1 (now provided in Fig. S6) clearly identifies peaks originating from tryptophan. As expected, the ^{15}N-HSQC spectra of the uniformly ^{15}N-labelled protein are more confusing because they contain cross-peaks not only for tryptophan (Fig. S5).

The reference to Fig. S5 in lines 245-248 of ms_orig (now line 276 of ms_current) was correct. We now also refer to the new Fig. S6.

Minor:

1. It is very confusing that the number notation and the IUPAC notation are used interchangeably for the isotope labeling of the Trp sidechain and of indole. My personal preference would be IUPAC. Of course, this is your decision, but please use only one consistently. In any case, it would be very helpful to show the Trp/indole chemical structure with suitable numbering in one of the main figures.

Response:

Different IUPAC nomenclature applies to chemicals (such as indoles) and amino acids (tryptophan). To limit confusion, we added the chemical structures and their nomenclatures (as far as needed in the present manuscript) in Figure 1.

2. The labeling of all spectral figures is not very reader-friendly. It would very helpful to label the subpanels with experimental conditions such that one can immediately understand the differences. E.g. Figure 2: add mutations and experiments (NOE-relay, HSQC) to subpanel rows and columns as well as color legend. This applies to all others as well: Figure 3, S3, S4, S5, S6, S7.

Response:

We added labelling as requested.

3.line 283: the Q factors should be put into Table S7 and this Table should be referenced in the main text.

Response:

We added a column with the Q factors. Table S7 was referenced in the main text (line 273 of ms_orig and line 309 of ms_current).

4. line 302: '[13C,1H]-HSQC spectra with 150 ms NOE' seems a misnomer.

Response:
Fixed (line 336 of ms_current).

5. line 344: 'Fig. 5' is apparently 'Fig. 6'
Response:
Line 402 in ms_current: Thank you for pointing out the typo!

6. Figures S3, S4, S6: please indicate Trp sidechain assignments where available.
Response:
Figs S3, S4 and S7 in ms_current serve as overviews of the ^{15}N-HSQC spectra. To assign all the Trp sidechain resonances in these figures would require a minute font size. Therefore why we provided the assignments of the relevant regions of the same figures in enlarged versions displayed in Figs S5 and S8. A careful comparison of the zoomed regions with the overview figures revealed an error in spectral calibration, which we amended throughout.

7. Figure S13: There is no red and blue points in the figure. They are rathe green and magenta in my PDF. Also in my impression, the localization spaces indicated by these points don't seem to be the same on left and right side.

Response:
We changed naming of the colours to magenta and cyan to be consistent with the colours in Fig. S15. (The figure is Figure S14 in ms_current.)

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

Best regards,
Gottfried