In the present manuscripts the authors aim at characterising the chaperone trigger factor (TF) regarding the dimerisation of TF and its implication on interaction with substrates. If the topic is interesting and trying to answer biologically relevant questions, the overall quality of the paper appears poor and too preliminary for publication.

1. There is a lengthy discussion about TF assignments. As presented here, RDB and PPI domains were assigned previously by the authors, SBD and TF113-432 by Dyson's group and the full length by Kalodimos and Hiller groups. It thus seems to me that all this constructs were assigned before. Please clarify this section and state explicitly your contribution to the field.

   As per the reviewer's suggestion, we have substantially shortened the literature review of the previous NMR work, but still acknowledge their contributions that enable us to carry out the NMR PRE analyses as described in this work.

2. For characterising the dimer interface and dynamics, the authors use a combination of NMR line-widths analysis and EPR. This is an interesting approach, however the obtained data remain low resolution and do not allow for a clear description of the dimer properties. This section is thus not very conclusive and as the authors have all the tools in hand to label with MTSL each domain of TF, I am wondering why they do not prepare mixture of isotopically labelled TF with each of the MTSL constructs successively to answer which domains of TF are in fact interacting or in close proximity. Extra EPR measurements could be used to derive informations about the domain even if more distant than the PRE distance range.

   We appreciated the reviewer's suggestion, and have now included the DEER measurements that indeed provide additional structural information that is not accessible by NMR-based analysis. The ESR-based DEER results showed that the previously reported TF dimer structures reported by Kalodimos and Hiller's groups sample part of the conformational space that is probed by ESR. The description of the DEER analysis is included in lines 196-215.

3. In the section regarding interaction with peptides, the authors aims at verifying the quality of a theoretical model model regarding TF-target interactions. This is an interesting question however the current approach lack rigorous testing. In fact the selection of the 5 constructs remains unclear to me. For exemple why is the site around 290 not chosen? It seems from the figure to be a potentially better site than 5. Also no testing on a site which is predicted to not or poorly interact is done.

   The five peptide sequences were selected not only based on the predicted binding scores but also based on the previously reported peptide array data by Deuerling *et al.*, 2003. Visual inspection of the blotting intensities of individual peptide fragments showed that the site around residue 290 has little TF binding. So we did not choose this region for peptide synthesis. To clarify this issue, we included a statement in line 224 that reads:

   "As a model system, we correlated the previously reported peptide array data of TF binding to ICDH (Deuerling et al., 2003) and the predicated TF binding score as a function of ICDH sequence (Fig. 3a). By visual inspection of the blotting densities of the peptide array, we identified five segments within the ICDH sequence that showed strong TF binding and fulfilled the requirement of peptide length and composition (Table 1). We adjusted the window sizes of the selected sequences to maximize the amount of preferred amino acid types and chemically synthesized these peptides."

   We hope the reviewer will find this explanation acceptable.

4. In the comparison of the interaction between IcdH2 and IcdH3, in the actual format it is almost impossible to assess the quality and relevance of the data and analysis. The PRE figures are extremely hard to read. Please adjust those figures, possibly with a multiple panel organisation so that the data readable and easily comparable between the different considered systems/probes. Please also indicate where each domain is in the sequence. For exemple, when the authors indicate that "the loss of PRE was much more pronounced for IcdH3 compared to that of IcdH2", I couldn't find any quantitative comparison or direct comparison of the experimental data. The

conclusion of this section regarding TF dimerisation seems to me quite speculative regarding the current data. It might be possible however to better reach those conclusions if the data where more adequately presented.

We appreciate the reviewer's critic of our data presentation. The PRE data are replotted individually for the backbone amide and side-chain methyl groups in Figures 4 and 5.

5. In the cross-linking section, the authors aims at distinguishing the effect of TF dimerisation on substrate recognition. In Fig 6, the gel used to control the cross-linking data show a band at 50kDa for TF in absence of BS3, while in the presence of BS3 the band is a blurry broad band at very high molecular weight (>130kDa and much higher). This indicate that the form obtained by cross-linking is not a dimer but probably a set of oligomers of TF (with 3, 4 or more TF units). To test the role of dimerisation in the interaction process the cross-linking should have given a dimer and not an oligomer. To me, this render this part of the study inconclusive.

   We agree with the reviewer's comment that the dimeric assembly of TF may be affected by the chemical cross-linking, but we do not have a robust method at the moment to verify the native-like conformation of the cross-linked TF. We therefore decide to remove this part of the experimental data and the corresponding discussion to avoid misinterpretation of our data.

Considering the points above, I consider it difficult to obtain strong conclusions from this study. In the current state I do not see the added value of the current study for the state of the art of the research in the field. The last figure is quite striking to me, only the (b) part seems to be a novel contribution from the group which I believe is lagging behind current structural studies of TF already existing. I would thus not recommend this paper for publication.

Additional extra minor points

1. For readability I would strongly encourage the authors to homogenise the nomenclature regarding TF constructs and domains.

   We now use RBD, PPI and SBD to refer to the individual domains and their combinations.

2. Figure S3 is missing in the file I could download

   We have reorganized the figures, and double checked the cross-references in the revised manuscript.

3. Figure 3 could be moved to the SI

   We have moved Figure 3 to the SI as Supplement Fig. S

4. Some references are incomplete regarding, e.g. doi numbers

   We have included all the doi numbers for the references.