This work studies the chaperone trigger factor (TF) by NMR and EPR spectroscopy. The study addresses two topics. (1) What is the structure of the trigger factor dimer? (2) How do certain peptides interact with TF? Both these questions are of high biological / biophysical impact and have been previously addressed by other studies, as correctly cited by the authors. While the present study is following those previous works, it has nonetheless the potential to contribute valuable information on both topics and should therefore eventually be published. It requires however major revisions.

On the one hand, I support the technical issues raised by the other two referees, without wanting to rephrase them here. These should be addressed.

On the other hand, the authors should substantially strengthen the interpretation and discussion part of their data such that additional insights can be gained. Contrasts and communalities to prior work are to be spelled out explicitly.

Regarding topic 1, the structure of the TF dimer has been studied previously by two independent studies (Morgado et al. Nat Comm 2017 and Saio et al. eLife 2018). Interestingly, while both studies identified the same global arrangements of domains, they came to opposite results regarding the dynamics of the complex. The Morgado study comes up with the finding that the dimer forms a multi-conformational complex. The Saio study finds that TF dimer is a single conformer. The data presented here can contribute to distinguish between the two scenarios. The authors should revise their manuscript to introduce this question in detail and to come up with an analysis as to which scenario is better (or completely) supported by their data.

We appreciate the reviewer's suggestion to clearly state the contrasts and communalities to the prior work, namely the two NMR studies of Morgado et al. that reported two conformations of TF dimer and Saio et al that reported anther conformation of TF. Through the newly included ESR DEER measurements, which report on the pair-wise distance distributions between two spin labels, we were able to demonstrate how the three NMR structures fit to the conformational space sampled by the DEER measurements.

The differences between the prior work and our current study were detailed from line 207 to line 215.

They were further discussed in the Discussion section: last paragraph of page 10.

Regarding point 2, it seems that the peptides bind in a multi-conformational ensemble at multiple sites, as evidenced by the PRE data in Figures 4 and 5. This finding should be discussed with regard to the functionality of the chaperone and contrasted more clearly to the study Saio et al 2014, where other peptides bind in a single conformation.

To address the reviewer's comment, Saio et al. also observed multiple substrate binding sites for shorter peptides. When a longer peptide fragment of PhoA that contain multiple TF binding motifs was used, the binding affinity was increased due to multivalency and a single set of binding mode was observed. We now elaborate this point in the Discussion section (line 290, page 11) that reads

"The authors also reported multiple binding sites within the PPI and SBD when short peptides were used to map the binding sites by chemical shift perturbations and intermolecular NOEs. When a longer peptide fragment of PhoA is used as a substrate, each of the substrate binding sites within the PPI and SBD is occupied by a specific TF binding motif thereby leading to a unique binding mode that enables structure determination of the substrate-bound TF. By determining the microscopic K_d values for individual binding sites, which fall within the low µM range, the authors demonstrate by relaxation dispersion analysis that the multivalency of substrate recognition significantly increases the binding affinity to a nM range."

We hope this will help clarify the discussion of in relation with the prior work.

Minor point:

- The first abstract of the discussion is essentially an introduction. It should be moved to and merged with the introduction.

We thank the reviewer's suggestion. The first paragraph of the Discussion section is now merged into the second paragraph of the Introduction (line 46, page 2).

- Figure 7 is unsystematic, mixing affinities and lifetimes. Also, the present study adds no new insight to this Figure. It should be removed.

We have removed Figure 7 as suggested by the reviewer.