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Magn. Reson. Discuss., referee comment RC2
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Comment on mr-2021-9

Anonymous Referee #2

Referee comment on "Structural polymorphism and substrate promiscuity of a ribosome-associated molecular chaperone" by Chih-Ting Huang et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-9-RC2, 2021

In this study, the authors have measured the structural plasticity of TF domains by carrying out the ESR studies with spin-label placed at different domains in the protein. T2 relaxation is also measured to highlight the domain dynamics. Fluorescence polarization studies were done to measure the apparent binding constant of TF with five peptides labeled with FITC. The binding affinities were correlated with TF binding scores. PREs were measured on various TF variants bound to two peptides. Crosslinked dimer of TF was tested for multiple site binding to urea-denatured MBP to propose that dimerization leads to sequestering of binding sites available in SBD. Although the manuscript discusses an important aspect of protein folding, and the work is carried out meticulously, but it lacks an explanation of various points mentioned below and I, therefore, recommend the manuscript for major revision.

Major comments:

- The basis for choosing the positions, where four amino acids (which ones?) are mutated to Cysteine, in TF for the spin-labeling is not mentioned in the manuscript. Also, are there any structural perturbations due to Cysteine mutations?
- Figure 2b shows FP data of TF binding to five peptides. Some of the binding curves do not go all the way to saturation. How reliable is such a fitting to estimate Kd values?
- What are the structures of the peptides used for binding? Is there any correlation between structure of the peptide and the binding affinity to TF? It is important to look

at this aspect as it will shed light on selective recognition of client proteins by TF.

- It is mentioned in the manuscript that an ideal TF binding motif should be at least eight residues long, and rich in aromatic residues and positively charged lysine or arginine. But that combination may give rise to a gigantic number of peptide sequences. What is the basis for choosing the peptide sequences used in the study?
- What is the structural evidence that the crosslinked-dimer of TF sequesters the binding sites within SBD and the change in Kd (with respect to Native TF) is not due to loss of binding sites due to some other perturbation in structure originated due to crosslinking?
- Authors proposed a model where a long nascent chain can be occupied by multiple TF molecules, however, this would be strongly dependent on peptide sequence as the number of favorable binding residues, the 3D structure of the peptide chain, the folding kinetics would all dictate the binding to TF and is dependent on primary sequence. No experimental data has been provided to support the claim and the statement is highly speculative.

Minor comments:

- A domain map for the protein (in SI or in main) would have been helpful in understanding the individual domain lengths and relative positions.
- Line 27: Spelling mistake for 'isomerization'
- Line 47: Sentence needs to be corrected. It reads: ….corrected sorted by….
- Line 112: Missing word after 'eight-channel'.
- No gap between units and corresponding numbers at several instances in the manuscript.