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:

1. 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?

   The four mutation sites (R14C, T150C, E326C and S376C) have been used in a previous study (Kaiser et al. Nature (2006) 444 455-460) to investigate TF binding to the ribosome and nascent chains using site specific labeling of fluorescent dyes, which are similarly bulky as the MTSL spin label. The biological functions of the single cysteine variants were considered to be similar to that of the wild type. According to the 15N-1H and 13Cm-1Hm correlation spectra of the MTSL-labeled single cysteine TF variants in their reduced states, the MTSL labeling only introduced localized chemical shift perturbations (data not shown), indicating that the introductions of the spin labels did not introduce significantly structural perturbations.

2. 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?

   As we mentioned in the figure caption of Figure 2, the fitting of IcdH5 was indeed not very reliable due to the lack of plateau resulting in large errors. In the case of IcdH3 and IcdH4, although the plateaus have not been reached, the nonlinear regression of the dataset yielded reasonably small error estimates as shown in Table 1 and Figure 2c. We therefore considered the fitting results to be relatively reliable.

3. 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.

   We did not characterize the structures of the peptides spectroscopically as we did not expect well-defined secondary structures to be populated due to their short lengths. Furthermore, most of the selected sequences correspond to loop regions in the reported crystal structure. We have now included a statement when describing the choice of the peptide sequences in line 230, page 8, that reads:

   “Except for IcdH3 and IcdH4 whose sequences are partly helical in the crystal structure (Bolduc *et al.*, 1995), all the remaining sequences correspond to loop regions that do not adopt particular secondary structures.”

   We hope the reviewer will find this acceptable.

4. 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?

   To clarify how we chose the peptide sequences for this study, we have revised the description in line 224, which now 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 description acceptable.

5. 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?

   As commented by Reviewer #1, we cannot unambiguously determine the dimeric state of the chemically cross-linked TF. We therefore removed the data and discussion about the cross-linking experiment.

6. 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.

   When discussing the simultaneous binding of multiple TF to the same unfolded polypeptide, we were referring to the work by Saio et al. who showed by NMR that multiple TF can bind to unfolded PhoA.

Minor comments:

1. A domain map for the protein (in SI or in main) would have been helpful in understanding the individual domain lengths and relative positions.

   We have now included the domain organization as a schematic drawing in Fig. 1d.

2. Line 27: Spelling mistake for 'isomerization'

   We have corrected this typo.

3. Line 47: Sentence needs to be corrected. It reads: ….corrected sorted by….

   It should be "correctly sorted by…", which is now corrected.

4. Line 112: Missing word after 'eight-channel'.

   We have added 'pipette' after 'eight-channel'.

5. No gap between units and corresponding numbers at several instances in the manuscript.

   We have carefully checked and added the missing gap between units and corresponding numbers.