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Comment on mr-2020-37

Anonymous Referee #3

Referee comment on "Fragile protein folds: sequence and environmental factors affecting the equilibrium of two interconverting, stably folded protein conformations" by Xingjian Xu et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2020-37-RC3, 2021

In this manuscript, the authors use NMR to examine various factors such as pH, salt, and ligand binding on the equilibrium between two alternate native states of a metamorphic protein (Y456T mutant of an ARNT PAS domain). As the interconversion of these two states involves the rearrangement of a beta-sheet via a 3-residue slip of a beta-strand, the states are referred to as WT and SLIP. Given that this metamorphic protein has great potential as a template for designing switchable proteins, this manuscript would be of interest to the readership of this journal, including the protein folding and engineering communities.

I have a few suggestions for further discussion and/or clarity in this well-written manuscript.

1) Section 3.2, first paragraph: The authors state that “we could lock the protein into the SLIP conformation by adding the F444Q and F446A point mutations on the Hbeta strand to the Y456T background, enabling structural characterization of the SLIP conformation.”

Of the two in silico studies of beta-sheet rearrangements that are mentioned in the manuscript, the Panteva et al., 2011 study has revealed that aromatic residues such as Phe appear to anchor into transient pockets as part of an “aromatic crawling” mechanism for shifting the beta-strand.

Perhaps the authors could mention in the manuscript that the “locking” of the protein into the SLIP conformation via Phe->Ala mutations is consistent with the aromatic crawling mechanism that was previously observed in simulations? I think this is an interesting connection to make between experiment and simulation regarding the rearrangements of beta sheets.

2) Since the beta-sheet shift is a key part of the interconversion between the WT and SLIP states, it would be helpful to include a schematic diagram that shows a) the hydrogen bonds in the beta-sheet that are being broken, and b) the amino acids (perhaps as beads with the one-letter code for amino acids) to highlight the alignment of nonpolar residues across the beta-sheet.

3) Figure 1 is a bit confusing to me as the positions of residues F446 and F444 in panel a) are not indicated in panel b), which is supposed to be a 90-degree rotated view of panel b). Perhaps the suggested figure in my point 2) would be better as a panel b), particularly if the point is to highlight changes in hydrogen bonds that result from the beta-strand slip as well as which nonpolar residues are aligned across the beta-sheet.