The review of this manuscript was performed jointly with Dr. Swati Balakrishnan and Dr. Yasiru Perera, who independently generated reviews that were subsequently discussed and edited by the team. These are appended below.

Review #1

This paper uses a previously characterized interconversion of the ARNT PAS domain between two forms resulting from a single point mutation, to study various factors influencing this equilibrium. This study enhances our understanding of 'fragile fold' proteins and how they may be modulated. In general the paper is well written, and the inferences and conclusions drawn by the authors are borne out by the results of the experiments. One general concern is that the reasoning behind carrying out some experiments has not been clearly elaborated.

Specific comments

1) *Materials and methods section 2.5, page 6, line 170:* The authors mention they were unable to match the concentrations of the compounds KG-548 and KG-655 as additional DMSO would be required for the KG-548 sample. Is there some reason more DMSO cannot be added to the other sample in order to match the solvent? Some elaboration would be helpful here.
2) *Results section 3.1, page 8, line 190-195:* Varied individually, the effects of pH and salt concentration are minimal. Was any attempt made to try them in combination with each other, as this might help stabilize alternative conformations? Also why was the pH range chosen as 6.0-9.0? Is the protein unstable outside this range?
3) *Results section 3.2, page 9, line 214-226:* Adding residues to the HI loop region led to the conclusion that loop length plays a role in determining the conformation of the I-beta strand. However, adding a 6-residue TEV cleavage site to a 8 residue loop is a 75% increase in length. The residues added include bulky residues such as Tyr and Phe. It is unclear why multiple constructs were not created to incrementally increase the loop length instead, using residues such as Ala or Gly. Why insert a TEV cleavage site? It is also unclear why they chose to cleave off the I-beta strand, leading to the precipitation of the protein.
4) *Section 3.5, page 13, line 305-313:* The proposed model predicts Kd values assuming that an invisible bound state of ~10% of the protein is in the SLIP conformation. Could this invisible state be quantified more accurately by methods such as CEST, thereby making the calculation of the affinities of these compounds more accurate?

Minor points

1) *Materials and methods section 2.4, page 5, line 138:* chemical shift to be replaced by chemical shift perturbation
2) *Figure 5, page 12:* Panels need to renumbered, the text is referring to panel c as panel b. Panel d also has no error bars.
3) *Section 3.5, page 13, line 284:* 'compare to' to be replaced by 'compared to'

Review #2

This manuscript reports an investigation of the fragile fold of metamophoric protein PAS-B (Pre-ARNT-Sim) domain of human ARNT using solution NMR. ARNT PAS-B interconverts between two structural states, induced by the +3 residue slip of an internal Iβ strand. The two states are named WT and SLIP. The factors affecting the equilibrium of wild-type (WT) and mutant PAS-B were determined using multiple biophysical techniques. Solution NMR was used to measure conformational equilibrium constants and predict the order parameters of the denatured PAS-B. Preferential binding of ligands to the WT and SLIP states was monitored by a thermal shift assay and NMR. These studies concluded that this metamophoric equilibrium could be manipulated and potentially used in biological applications. The approach taken by the authors is sound and experiments appear to be well-designed. While the data supports the general conclusion there are several things to be addressed.

Specific comments

1. Results Page 7

^{15}N-^{1}H HSQC was used to characterize the equilibrirum ratio of WT:SLIP states. It would be useul to show the corresponding NMR spectra of two different states added in the supporting information as it would be more revealing to see the two populations in a single spectrum.

2. Results Page 7

The equilibrium has been monitored over a range of pH (6.0-9.0). It would be interesting to know if the authors tried acidic pH range or if they could speculate about the effects on the equilibrium at lower pH.

3. Results page 7

The authors have stated that mutating P449 to either Ala or Gly produced a shift in the equilibrium. The result for Gly was expanded upon but no comparison was made with respect to the Ala mutant.

4. Results Page 8

The authors report changes in the equilibrium when the length of the HI loop increased. This was studied by adding a TEV cleavage site ENLYFQ. The authors note that the WT:SLIP equilibrium shifted to 20:80 concluding the effect of length. It is important to address the relative contribution of length versus sequence identity since no control experiments were performed. Are any structural changes induced by cleaving the HI loop at the TEV site?

5. Results Page 8

The manuscript would be strengthened by the addition of CPMG or another suitable NMR experiment to more completely characterize exchange dynamics of PAS-B and its mutants. Those data would inform loop motion and secondary structure changes associated with the conformational change. These results would be especially enlightening given the importance of HI loop in modulating the equilibrium. At the least it would be interesting to include this as a future direction.

6. Results Page 12. Figure 5d

No error analysis has been performed and the experiment seems to have been performed only once. If reporting a measure parameter reproducibility and precision should be addressed.

Minor points

1. Line 52 – add the bHLH abbreviation here.
2. Line 77 – Mention HI loop region in pdb structure (Figure 1a)
3. Line 184 – due to
5. Line 264/265 – Figure number 5b and 5c should be swapped.
6. Line 290 – Remove “,” after (0-10mM)
7. Line 344 – Remove “actually”