Supporting Information for:

Influence of the Valine Zipper Region on the Structure and Aggregation of the Basic Leucine Zipper (bZIP) Domain of Activating Transcription Factor 5 (ATF5)

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Figure S1. Plot of FTIR absorbance at 1650 cm⁻¹ of reduced and oxidized forms of ATF5

Figure S2. Plot of FTIR absorbance at 1620 cm⁻¹ of reduced and oxidized forms of ATF5

Figure S3. Thioflavin T fluorescence of reduced and oxidized forms of ATF5



Figure S1. FTIR absorption at 1650 cm⁻¹ is plotted for the WT and V257STOP forms of ATF5 under both non-reducing and reducing solution conditions as a function of temperature. Data for the WT form in the absence and presence of 10 mM DTT is illustrated in red and purple, respectively. Data for the V257STOP mutant in the absence and presence of 10 mM DTT is illustrated in blue and green, respectively.



Figure S2. FTIR absorption at 1620 cm⁻¹ is plotted for the WT and V257STOP forms of ATF5 under both non-reducing and reducing solution conditions as a function of temperature. Data for the WT form in the absence and presence of 10 mM DTT is illustrated in red and purple, respectively. Data for the V257STOP mutant in the absence and presence of 10 mM DTT is illustrated in blue and green, respectively.



Figure S3. Thioflavin T fluorescence emission in the presence of the WT and V257STOP forms of ATF5. The WT form in shown in the absence (red) and presence (purple) of 10mM DTT. The V257STOP truncated mutant is also shown in the absence (blue) and presence (green) of 10 mM DTT.

Summary: The structural transitions observed by FTIR appear rather complex for both forms of the protein under the solution conditions tested. In most cases, a sigmoidal fit could not be made and consequently a melting transition temperature (T_m) was not determined. Nonetheless, the data demonstrate the reduction of the disulfide bond in the truncated form has minimal impact on the structure of the protein, whereas a difference in the structure is observed for the wild-type sequence when comparing the reduced, monomeric and oxidized, dimeric forms. C-terminal truncation of the valine zipper region of ATF5 alters the structure and structural transition observed in response to thermal melting compared to the WT. Thioflavin T fluorescence is not observed in the presence of ATF5 at any temperature, indicating no binding occurs despite the presence of aggregates. In contrast, fluorescence is detected in the presence of V257STOP, indicating a structural difference exists between the two protein aggregates at lower temperatures.