Supporting Information for

Fast helix formation in the B domain of protein A revealed by site-specific infrared probes

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1. Temperature dependent FTIR spectra of I32M and A47M BdpA mutants were recorded to confirm that the peptide was folded. The FTIR spectroscopy methodology is given in the experimental section of the full paper. Temperature dependent FTIR of I32M and A47M BdpA mutants are reported in Figure 1S.

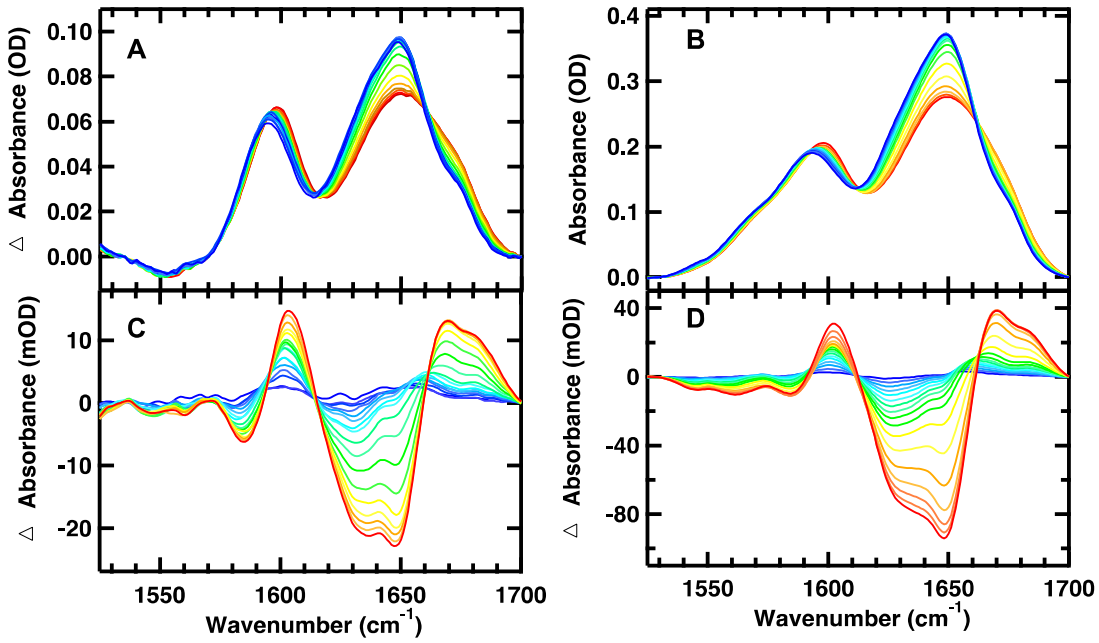

Figure 1S. Temperature dependent FTIR spectra of 2 mg/ml I32M BdpA (A, C) and 8 mg/ml A47M BdpA (B, D) in 25 mM potassium phosphate and 50 mM NaCl (pH 6.8). (A, B) Absorbance spectra in Amide I' region; the temperatures of the individual traces varies from 25 to 100 °C in 5 °C intervals. (C, D) Difference spectra obtained by subtracting the spectrum at 25 °C from the spectra at higher temperatures.

2. Protein was treated with $NiCl_2$ to remove EDTA contamination Supernatent harvested during the removal of the histidine tag from contained some residual EDTA, which interfered in the infrared. The supernatant was treated with a 1:1 ratio of $NiCl_2$ to EDTA in order to remove any EDTA in the solution. The solution was dialyzed with water overnight to remove the NiEDTA product and buffer solution. Dialyzed sample was lyophilized and exchanged in D_2O. The Y15M mutant pre and post cleaning is reported in Figure 2S. The IR peaks are more easily distinguished in the second derivative spectra (Figure 3S A). IR peaks assigned to solvated (1632 cm^{-1}) and buried (1648 cm^{-1}) helix and the labeled position (1560 cm^{-1}) can be identified in both the pre and post cleaned Y15M sample. The peak arising from residual EDTA is centered ~20 cm^{-1} away from the labeled position, so it does not interfere in the region of interest. A normalized melt of the labeled position pre and post purification reveals that the melting temperature and cooperativity of folding is unchanged (Figure 3S B).

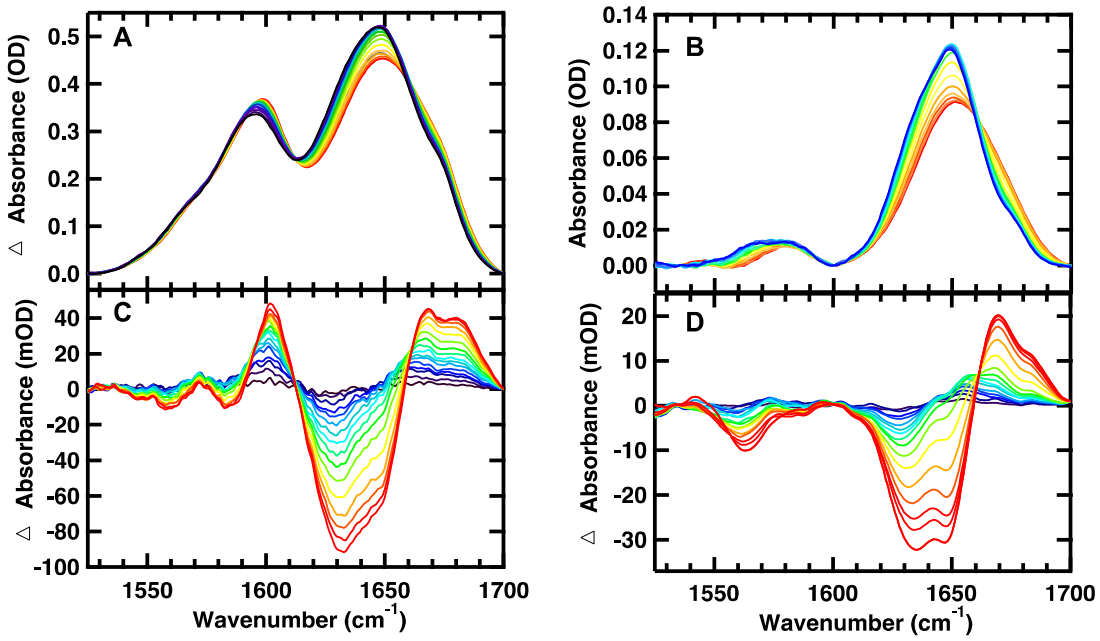

Figure 2S. Temperature dependent FTIR spectra of 8 mg/ml Y15M BdpA with EDTA contamination (A, C) and 3 mg/ml Y15M BdpA without EDTA contamination (B, D) in 25 mM potassium phosphate and 50 mM NaCl (pH 6.8). (A, B) Absorbance spectra in Amide I' region; the temperatures of the individual traces varies from 25 to

100 °C in 5 °C intervals. (C, D) Difference spectra obtained by subtracting the spectrum at 25 °C from the spectra at higher temperatures.

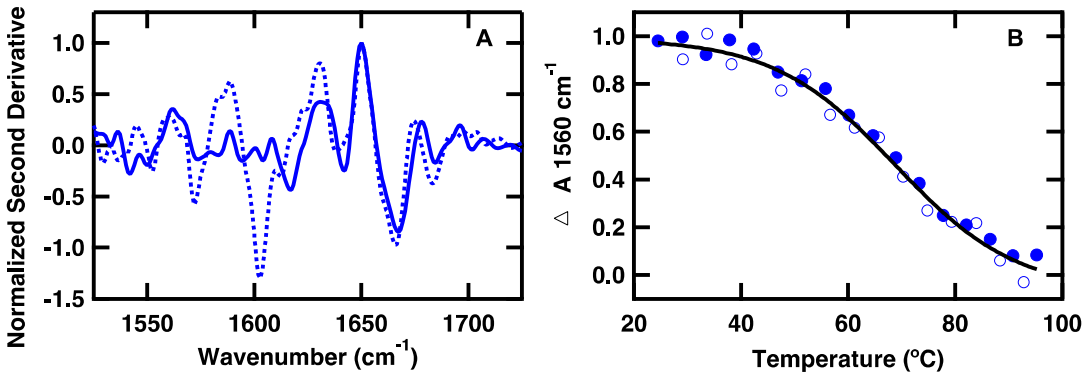

Figure 3S. Data are normalized at the maximum for clarity. **(A)** Second derivative of the FTIR difference spectrum (100-25 °C) of Y15M BdpA pre (dashed line) and post (solid line) removal of EDTA. **(B)** FTIR melt curves of Y15M pre (open circles) and post (closed circles) removal of EDTA obtained by plotting the change in IR difference spectra at 1560 cm^{-1} versus temperature.

3. Temperature dependent FTIR spectra of A47M and $^{13}C{=}^{18}O$ were collected to confirm the position of the labeled peak. The FTIR spectroscopy methodology is given in the experimental section of the full paper. Temperature dependent FTIR of His-Tagged A47M and His-Tagged $^{13}C{=}^{18}O$ A47M BdpA mutants are reported in Figure 4S. A second derivative of the lowest temperature absorbance data (Figure 5S) reveals that there is good agreement in the folded structure of the His-Tagged A47M, wildtype and $^{13}C{=}^{18}O$ His-Tagged A47M. There are no differences in the side chain contributions from the folded His-Tagged A47M mutant compared to the wildtype mutant.

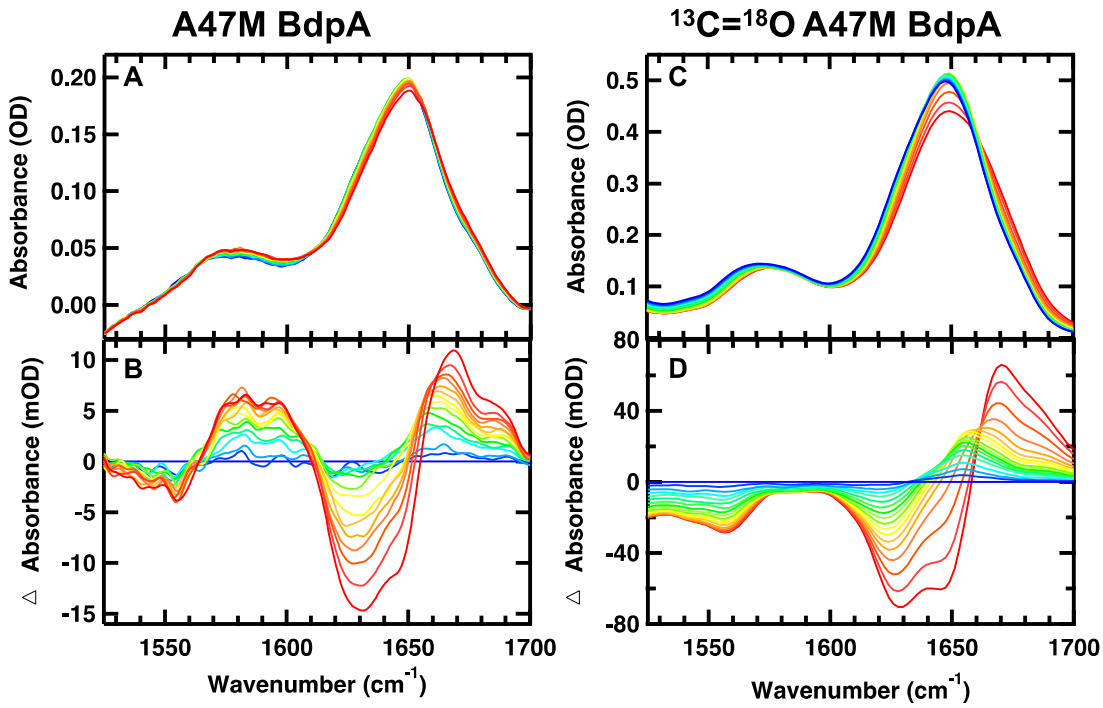

Figure 4S. Temperature dependent FTIR spectra of His-Tagged A47M BdpA (A,C) and His-Tagged $^{13}C=^{18}O$ A47M BdpA (B, D) in 25 mM potassium phosphate and 50 mM NaCl (pH 6.8). (A, B) Absorbance spectra in Amide I' region; the temperatures of the individual traces varies from 25 to 100 °C in 5 °C intervals. (C, D) Difference spectra obtained by subtracting the spectrum at 25 °C from the spectra at higher temperatures

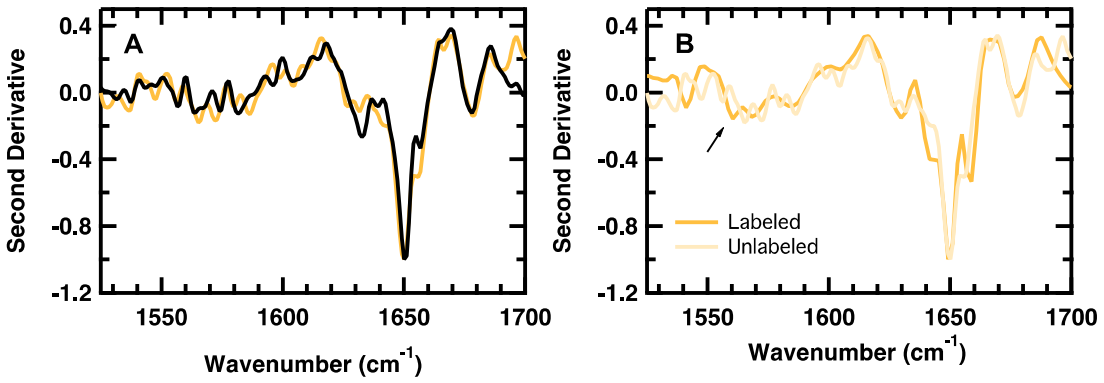

Figure 5S. Second derivative of the FTIR spectra collected at 25 °C. (A) Comparison of WT (black) and His-Tagged A47M (Yellow). (B) Comparison of His-Tagged $^{13}C=^{18}O$ A47M (Dark Yellow) and His-Tagged A47M. Arrow points to 1560 cm^{-1} peak.

4. IR T-jump of the wildtype, Y15M and A47M BdpA were collected to determine the dynamics of the systems. The IR T-jump methodology is given in the methods section of the full paper. Wavelength dependent IR T-jump measurements were collected at ~1648, 1632 and 1560 cm^{-1}. Representative IR T-jump relaxation kinetics of wildtype, I32M and A47M BdpA are reported in Figure 6S-8S.

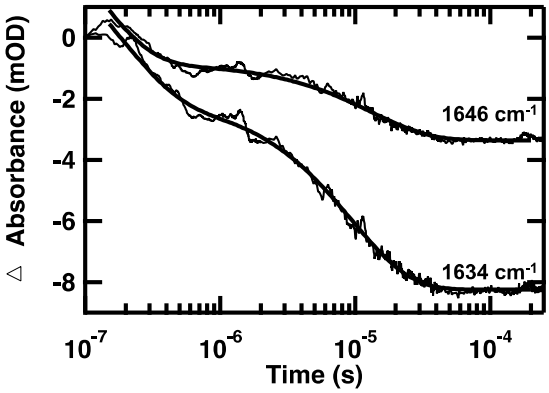

Figure 6S. Representative IR T-jump relaxation kinetics of wildtype BdpA monitored in the amide I' spectral region at 1646 and 1634 cm^{-1} following a T-jump from 50 to 60 °C. A double exponential fit is overlaid over each kinetic trace (black solid line).

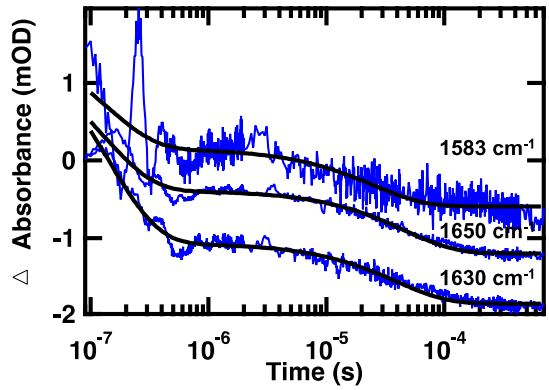

Figure 7S. Representative IR T-jump relaxation kinetics of Y15M BdpA monitored in the amide I' spectral region at 1650, 1630 and 1583 cm^{-1} following a T-jump from

20 to 30 °C. A double exponential fit is overlaid over each kinetic trace (black solid line).

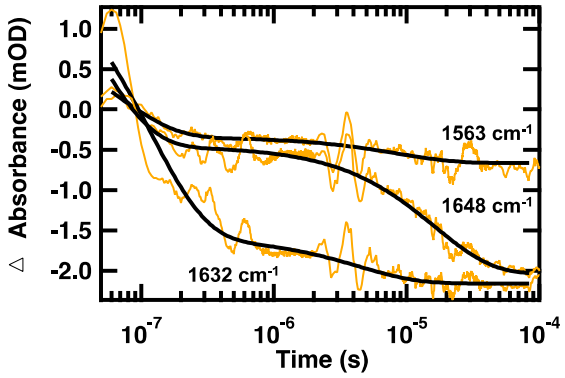

Figure 8S. Representative IR T-jump relaxation kinetics of A47M BdpA monitored in the amide I' spectral region at 1648, 1632 and 1563 cm^{-1} following a T-jump from 50 to 60 °C. A double exponential fit is overlaid over each kinetic trace (black solid line).