Supplementary Information

Phosphite binding by the HtxB periplasmic binding protein depends on the protonation state of the ligand

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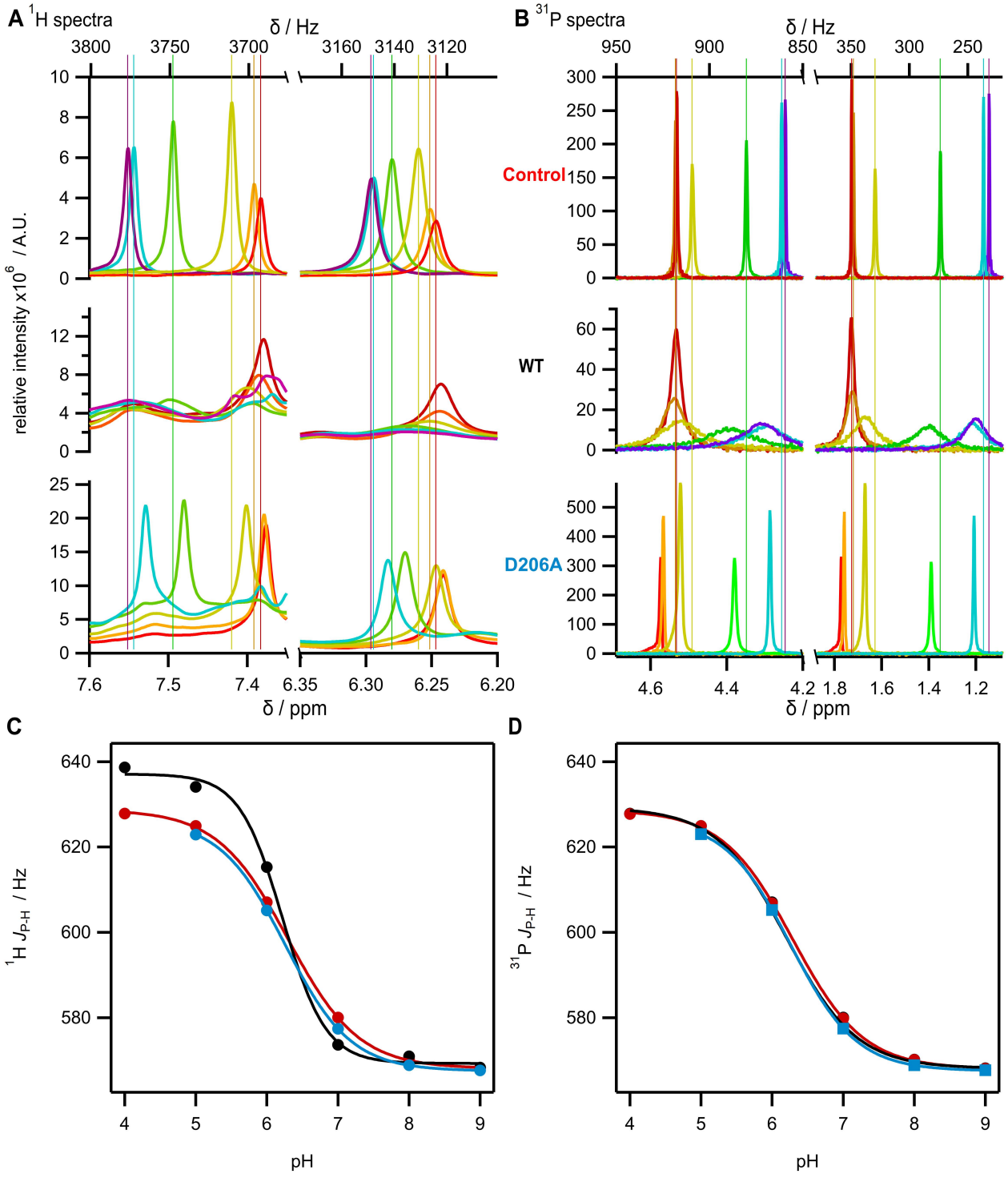

Figure S1. ^{1}H and ^{31}P spectra and coupling constant data from NMR pH titrations. (A) ^{1}H and (B) ^{31}P spectra of phosphite control, HtxB WT with 10 mM phosphite, and HtxB D206A, showing regions measured for coupling constants. Colors indicate pH of measurement: Red, 9; Orange, 8; Yellow, 7; Green, 6; Blue, 5; Violet, 4. pH dependence of $^{1}J_{HP}$ coupling constants; (C)^{1}H reporting and (D) ^{31}P reporting. Red, no protein; black, HtxB WT; blue, HtxB D206A. The lines are theoretical and are fitted to a sigmoid relationship (equation 4), with calculated pK_a values reported in table 2.

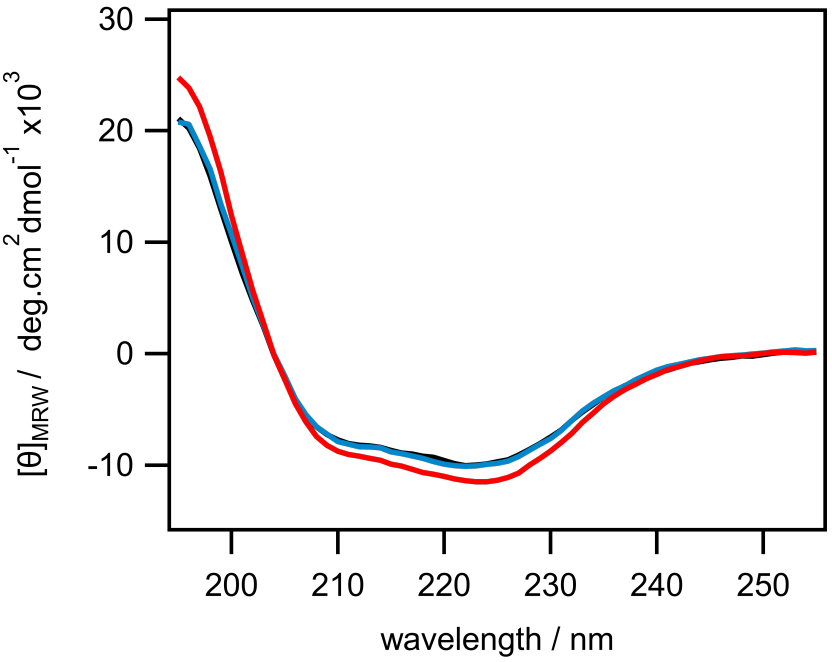

Figure S2. D206A and D206N mutations cause no gross changes in the secondary structure of HtxB. CD Spectra (mean residue elipticity) measured at 25 °C in 5 mM sodium phosphate, pH 7.4. Black, WT; blue, D206A; red, D206N.

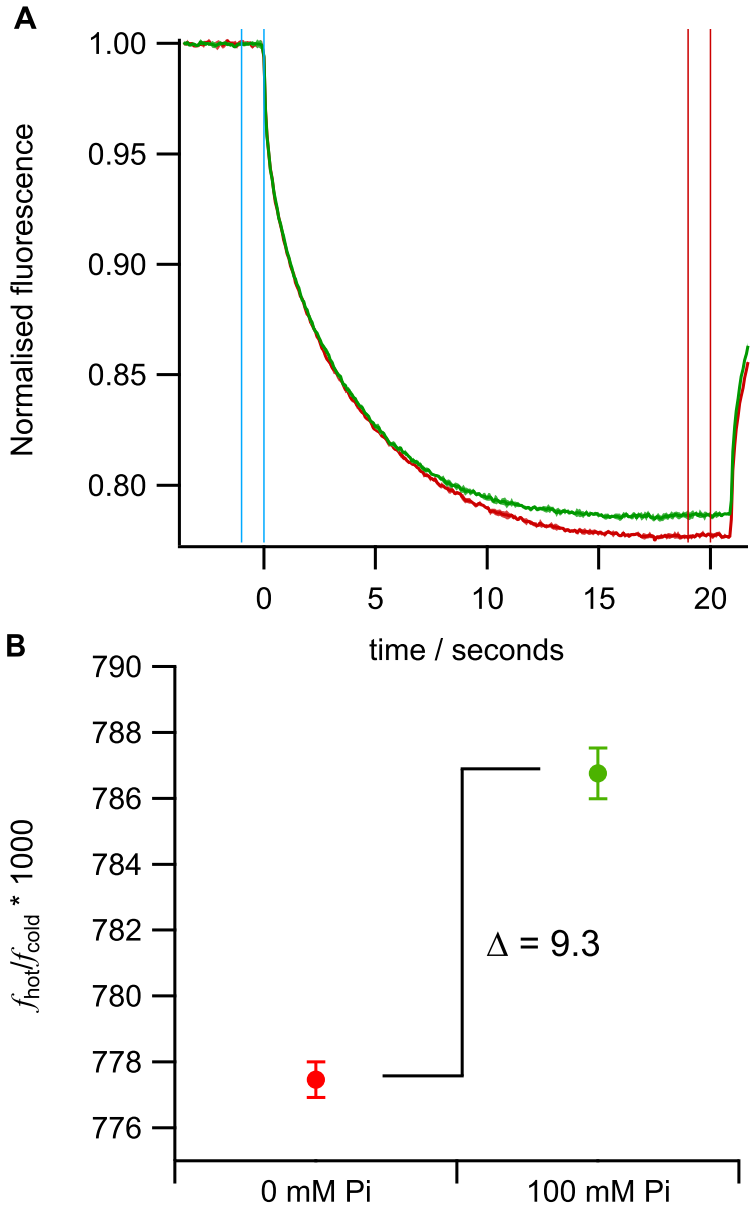

Figure S3. HtxB D206N binds phosphate weakly. (A) Normalized thermophoresis traces of HtxB D206N subjected to 22 seconds of thermophoresis in 50 mM HEPES pH 7.4, 250 mM NaCl, 0.05 % Tween-20 (red), and mixed with either 100 mM phosphate (green). Lines are mean fluorescence of 4 independent measurements (standard deviation shown by shading). Blue and red vertical lines indicate the time periods where the average of f_{hot}/f_{cold} ratio is calculated. (B) Category plot highlighting the difference in thermophoresis between apo-HtxB and HtxB mixed with 100 mM phosphate. The difference between plus and minus 100 mM phosphate is above the signal to noise ratio (S/N) of the Monolith NT.115 machine and binding can be concluded. Error bars indicate the standard deviation from the mean of four independent experiments. When a full titration was performed, protein was not saturated at concentrations $>$ 100 mM phosphate.