Supporting Information

Title: Cleavage profile of protein substrates by ClpXP reveals deliberate starts and pauses

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Table of Contents

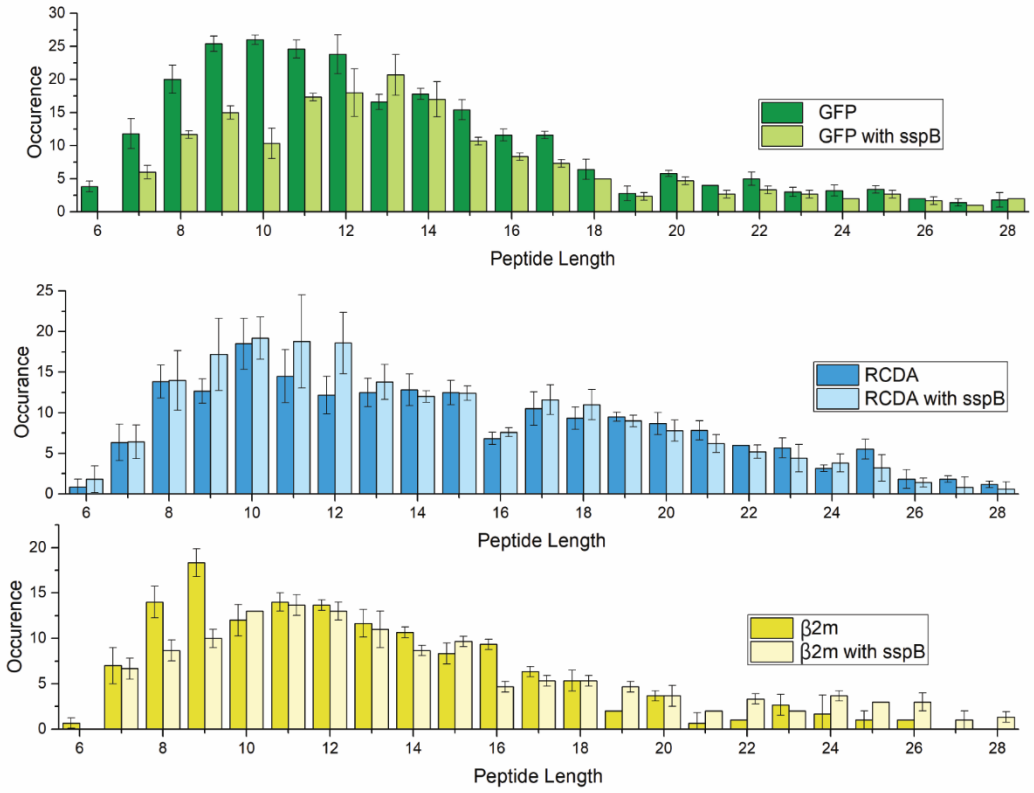

Figure S1: Peptide length histogram for standard digestion of ClpXP and digestion with the sspB adapter. (A) GFP, (B) RcdA, and (C) disulfide reduced β2m.

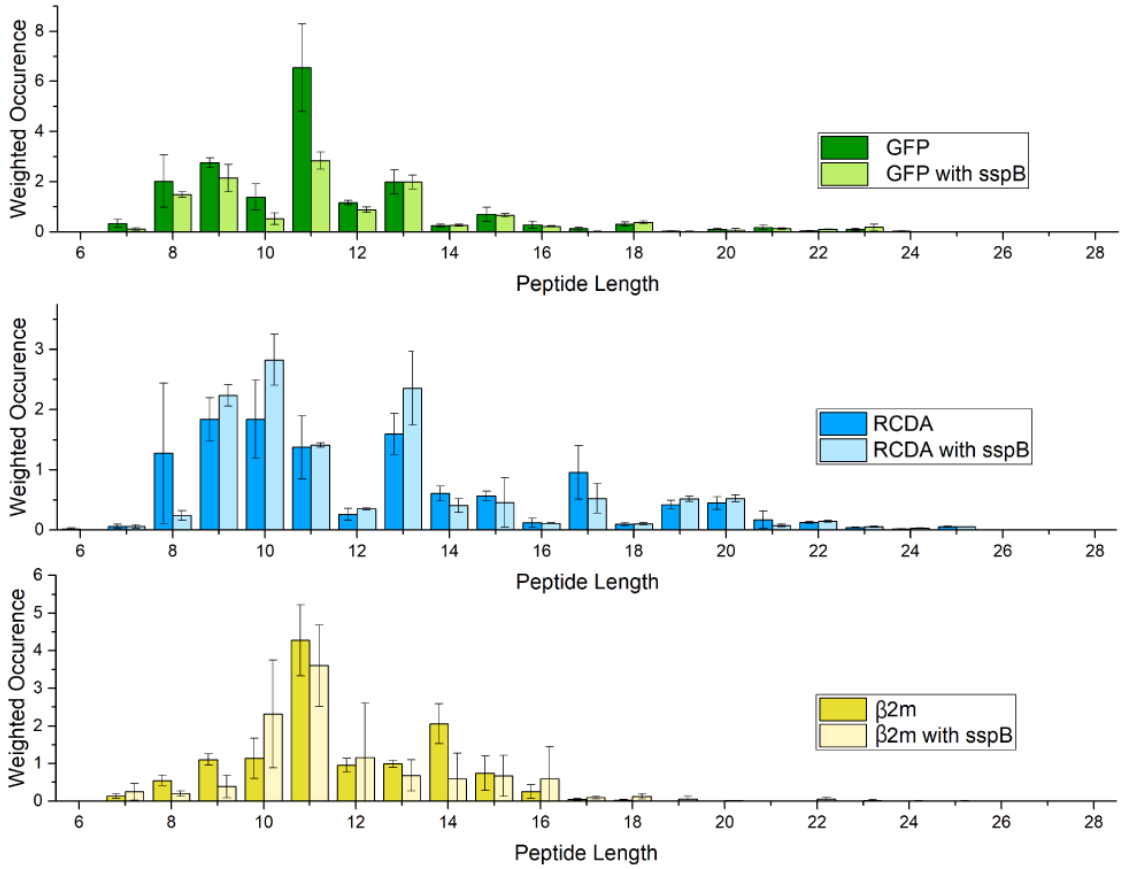

Figure S2: Peptide length histogram weighted for mass spectral intensity for standard digestion of ClpXP and digestion with the sspB adapter. (A) GFP, (B) RcdA, and (C) disulfide reduced β2m

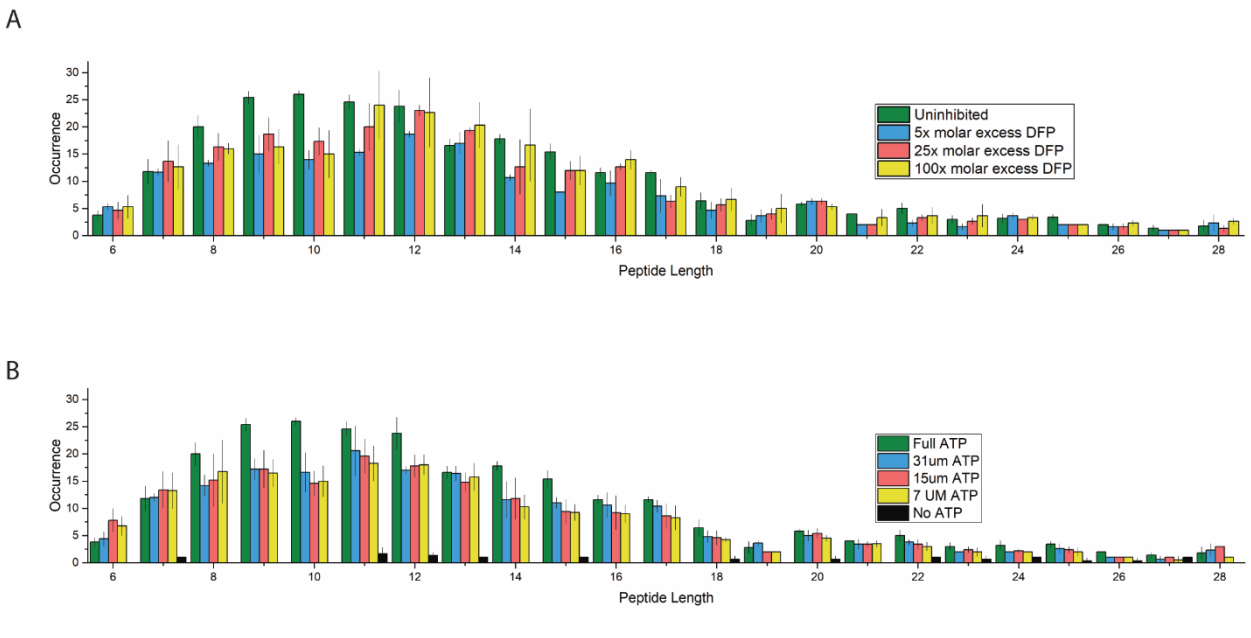

Figure S3: peptides length histogram for digestion of GFP under (A) DFP inhibited degradation and (B) ATP limited degradation

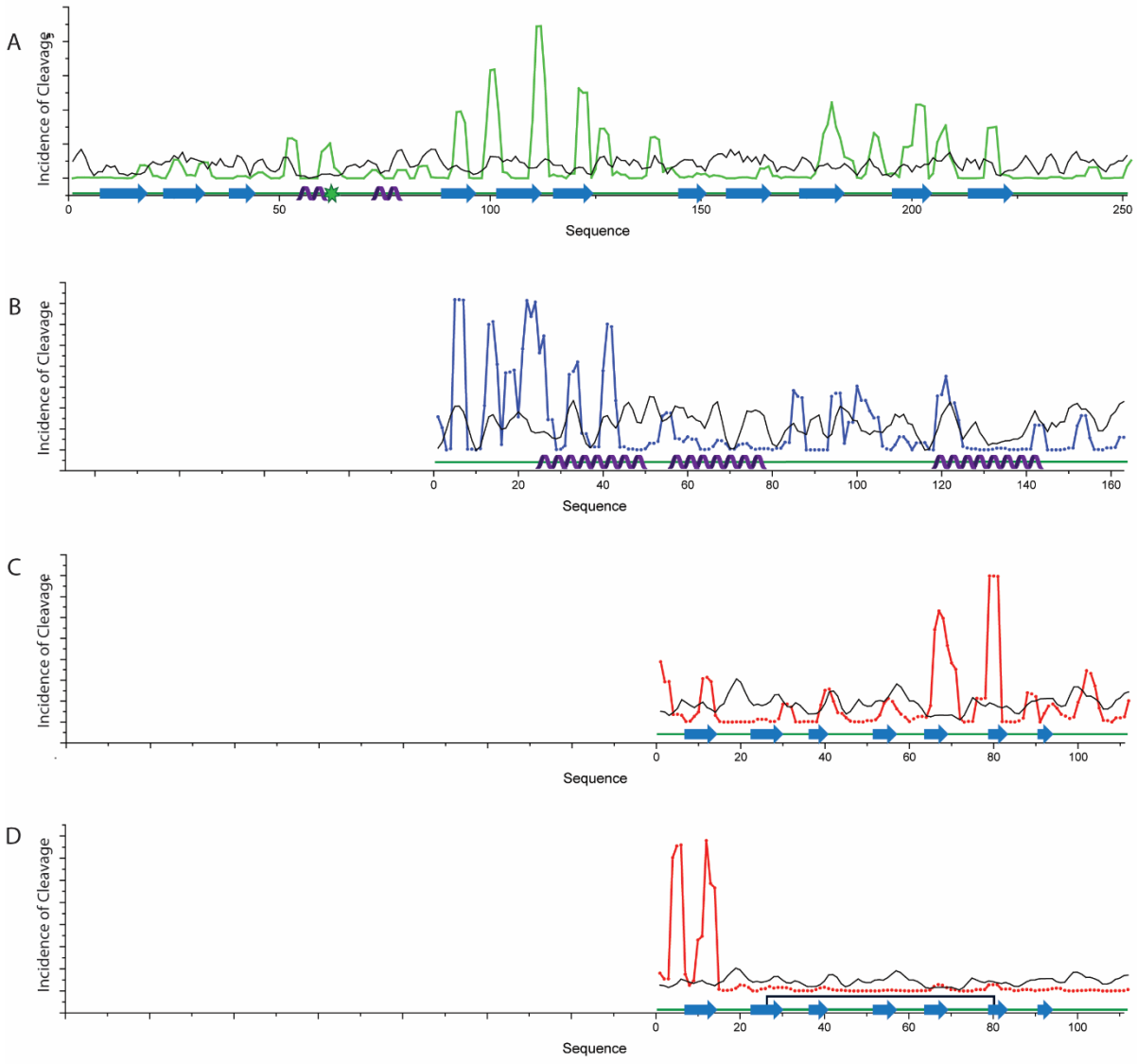

Figure S4: Cleavage incidence locations on the studied substrates weighted for mass spectral intensity. (A) GFP, (B) RcdA, (C) reduced β2m and (D) oxidized β2m. Secondary structure is indicated underneath with blue arrows representing beta sheets, purple helices representing alpha helices and the chromophore of GFP represented as a star. The location of the disulfide bond is depicted as a black line. Data was subject to a moving average smoothing over 3 data points. The black line on each graph represents primary sequence cleavage specificity.

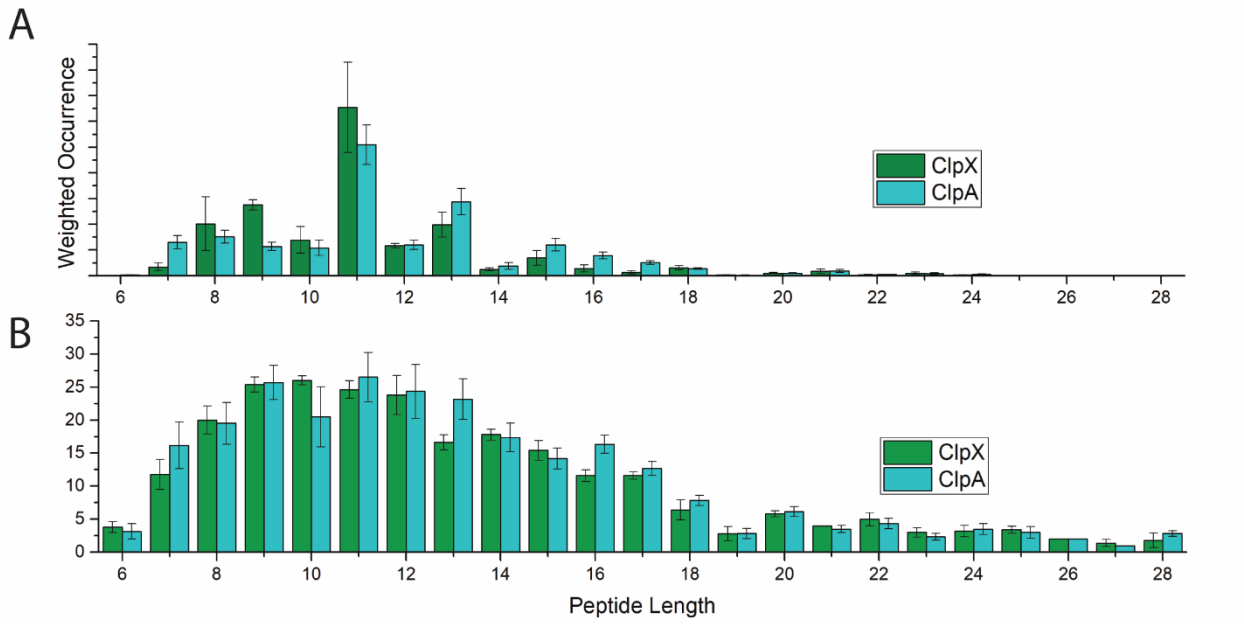

Figure S5: Peptide length distribution for ClpA degradation compared to ClpX, (A) weighted for mass spectral intensity and (B) unweighted.

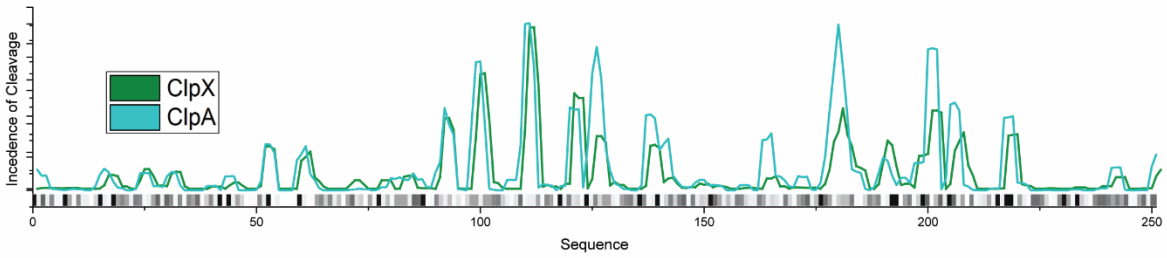

Figure S6: Cleavage incidence locations on the GFP weighted for mass spectral intensity for ClpX and ClpA. Data were subject to a moving average smoothing over 3 data points. The black and gray bars on each graph represent primary sequence cleavage specificity, where darker colors indicate higher likelihood of cleavage.

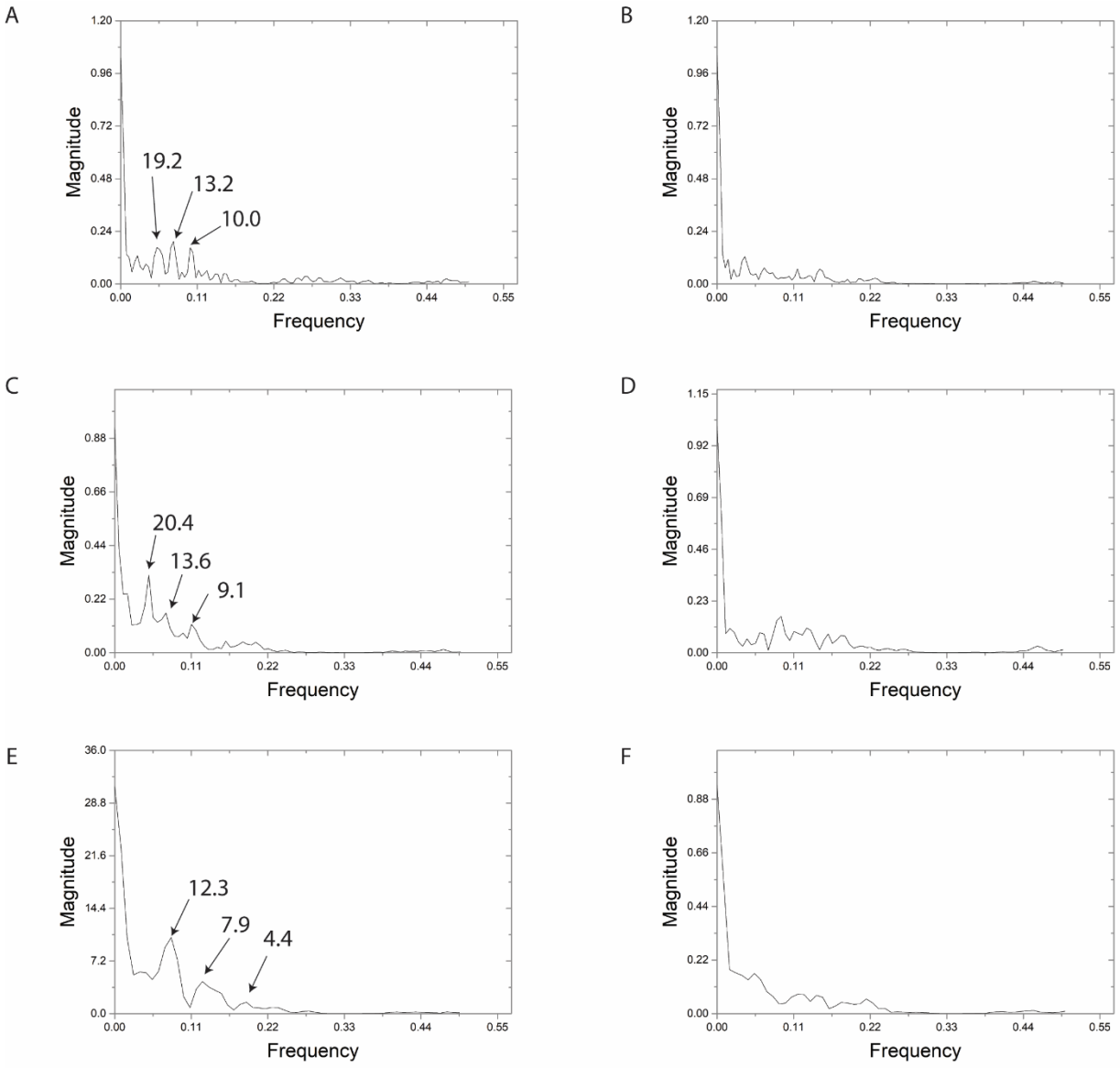

Figure S7: Fast Fourier transform (FFT) of cleavage incidence of (A) GFP observed cleavage locations, (B) GFP amino acid sequence preference, (C) RcdA observed cleavage locations, (D) RcdA amino acid sequence preference and (E) reduced β2m overserved cleavage locations, and (F) β2m amino acid sequence preference. FFT analysis was done using the Origin FFT function with default setting with the exception of the blackman window which was used to exclude the C-terminus. FFT analysis confirms periodicity in cleave location of each substrate that does not exist if simply the primary sequence is considered. Peptide length values were calculated using the reciprocal of the frequency.