Supporting Information

Protein Charge Transfer Absorption Spectra: An Intrinsic Probe to Monitor Structural and Oligomeric Transitions in Proteins

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Figure S1 Disorder prediction plots

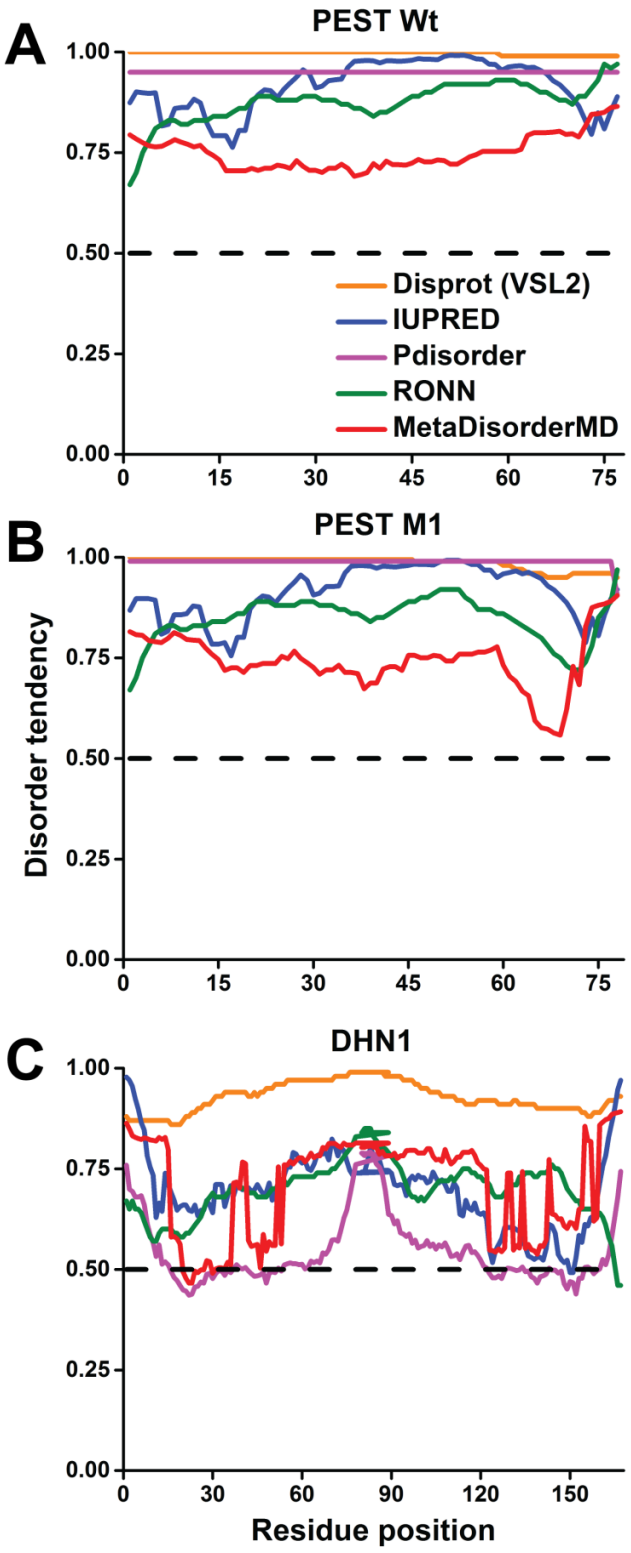

Figure S1: Intrinsic disorder prediction plots of the proteins for **[A]** PEST Wt; **[B]** PEST M1 and **[C]** DHN1 as obtained by various predictors. Disorder prediction was determined using Genesilico MetaDisorder server. (L. P. Kozlowski and J. M. Bujnicki, *BMC Bioinformatics*, 2012, 13, 111.)

Figure S2 Purification of PEST Wt and PEST M1

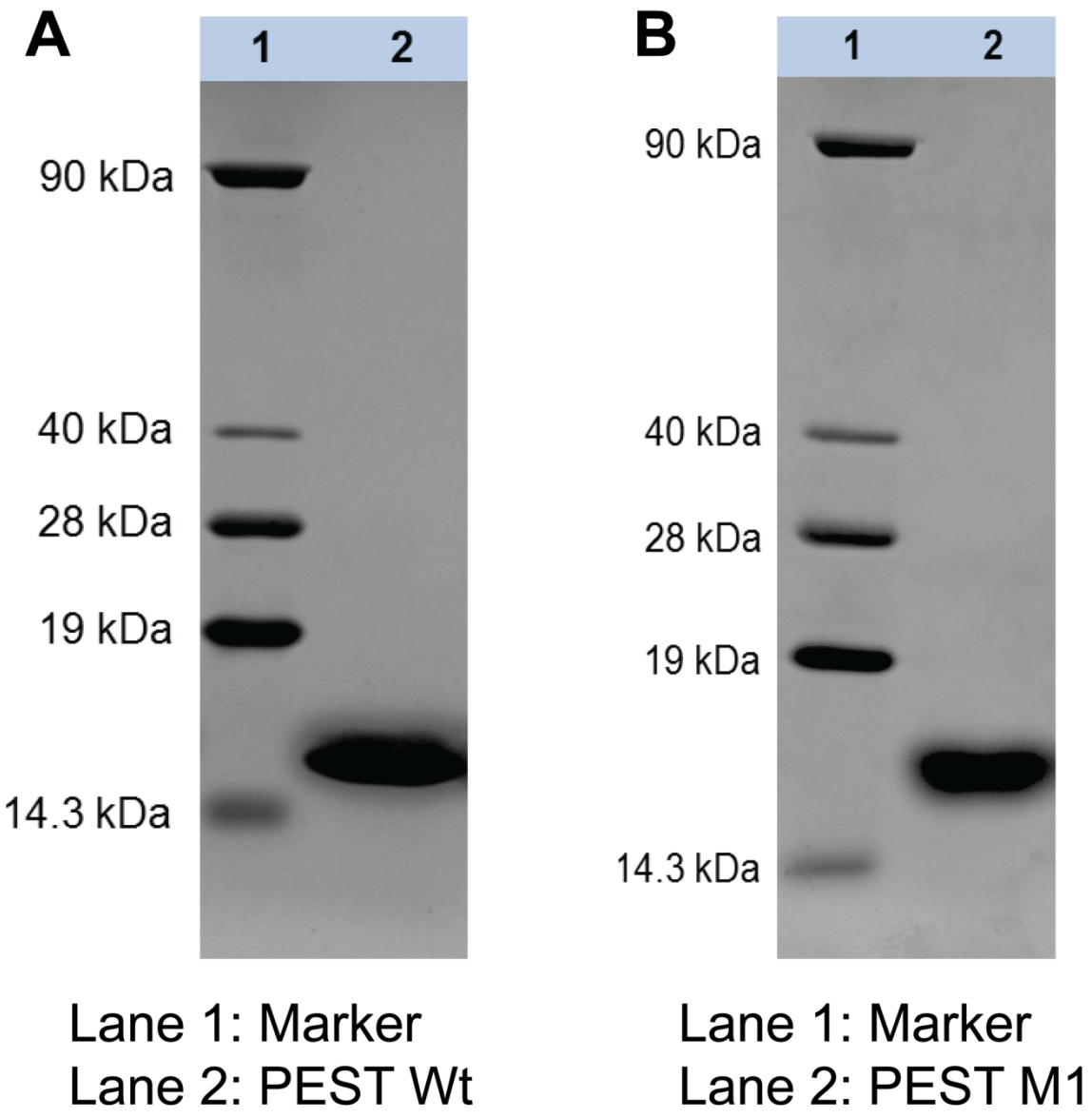

Figure S2: 15 % Reducing SDS-PAGE showing a single band of purified proteins for **[A]** PEST Wt and **[B]** PEST M1. Apparent molecular weight of PEST proteins on SDS-PAGE is approximately 15 kDa because of its anomalous mobility.

Figure S3 Mass Spectra of PEST Wt and PEST M1

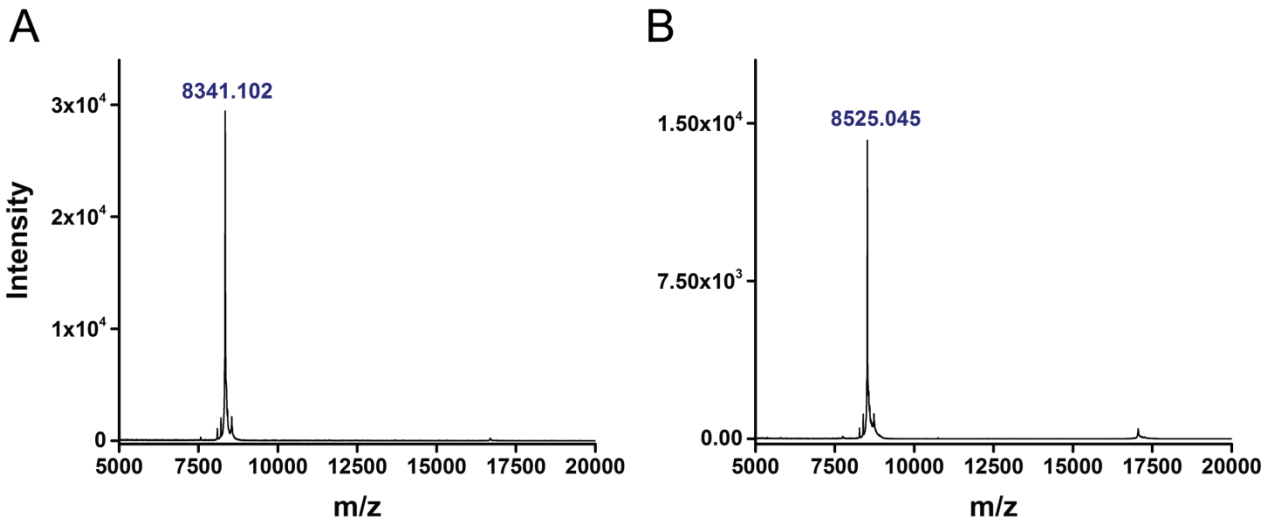

Figure S3: Mass spectra of [A] PEST Wt and **[B]** PEST M1. The mass calculated from sequence were 8341.61 and 8527.82 Da.

Figure S4 Purification of DHN1

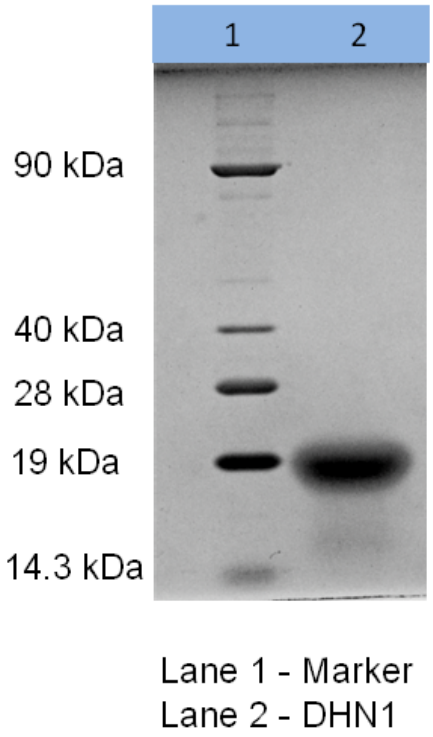

Figure S4: 15% Reducing SDS-PAGE shows the single band of purified DHN1 at 19 kDa. Higher molecular weight of DHN1 on SDS-PAGE is observed due to its anomalous mobility.

Figure S5 Mass Spectrum of DHN1

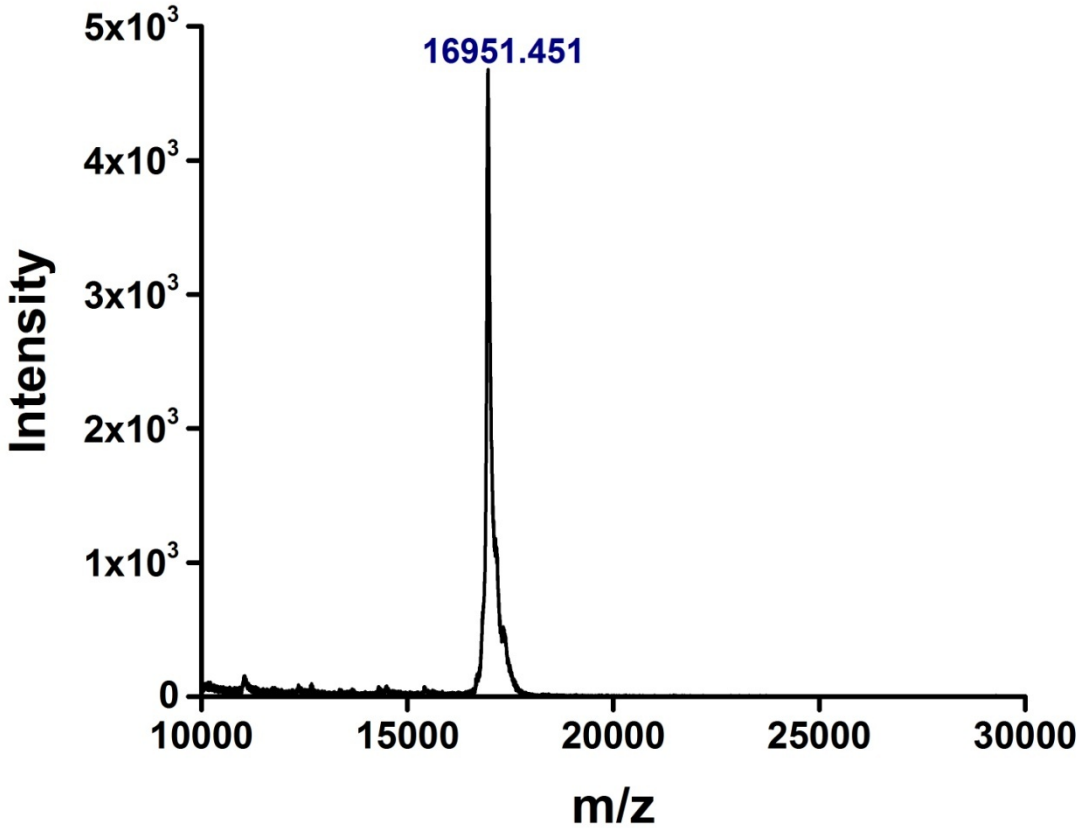

Figure S5: Mass spectrum of Dehydrin (DHN1). The mass calculated from sequence was 16955.33 Da.

Figure S6 Comparison of absorption spectra with simulated scatter

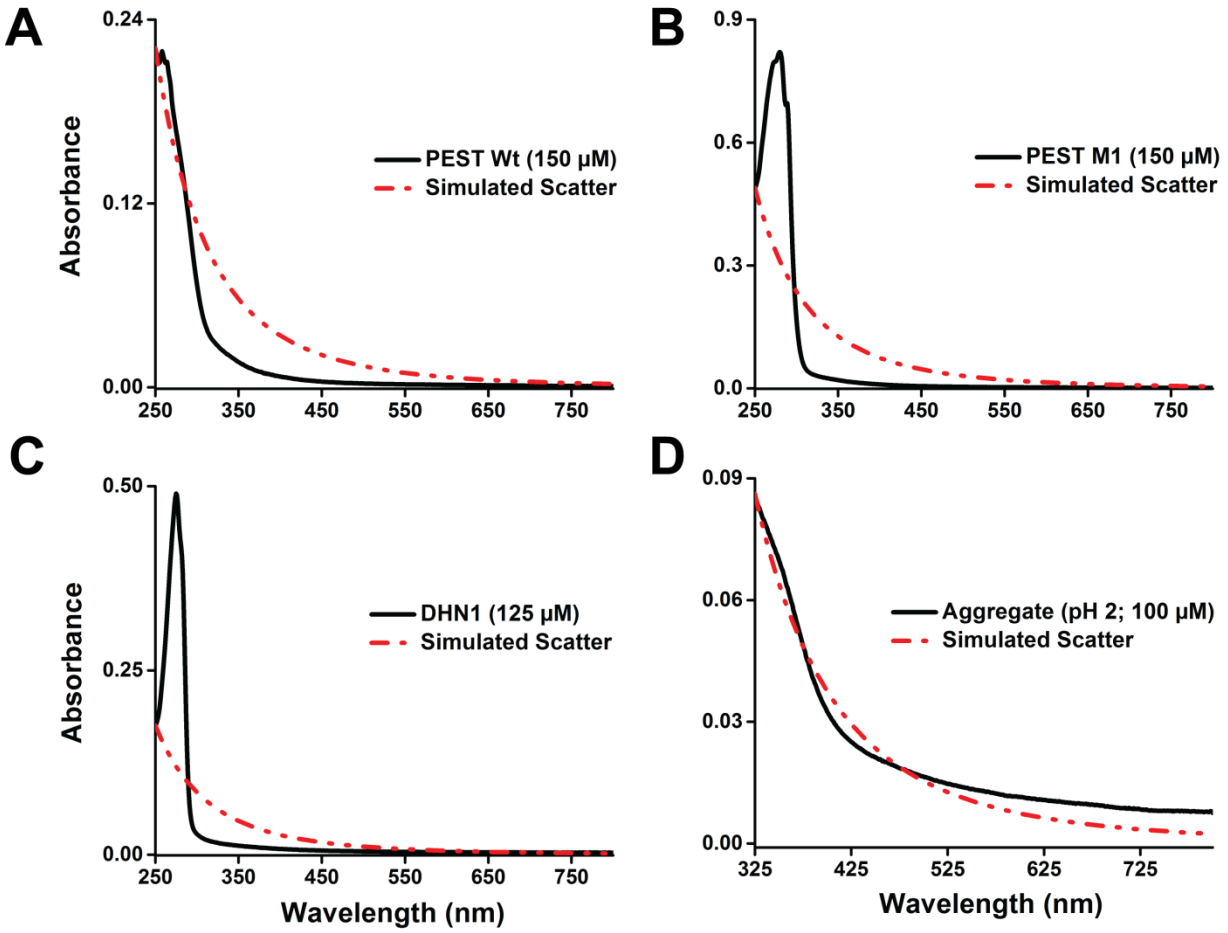

Figure S6: Comparision of absorbance spectra with simulated Rayleigh scatter (using $1/\lambda^4$ dependence) for **[A]** PEST Wt; **[B]** PEST M1; **[C]** DHN1 and **[D]** HEWL aggregate formed in Glycine buffer (pH 2.0).

Figure S7 PEST Wt, PEST M1 and DHN1 exist as a monomer in solution

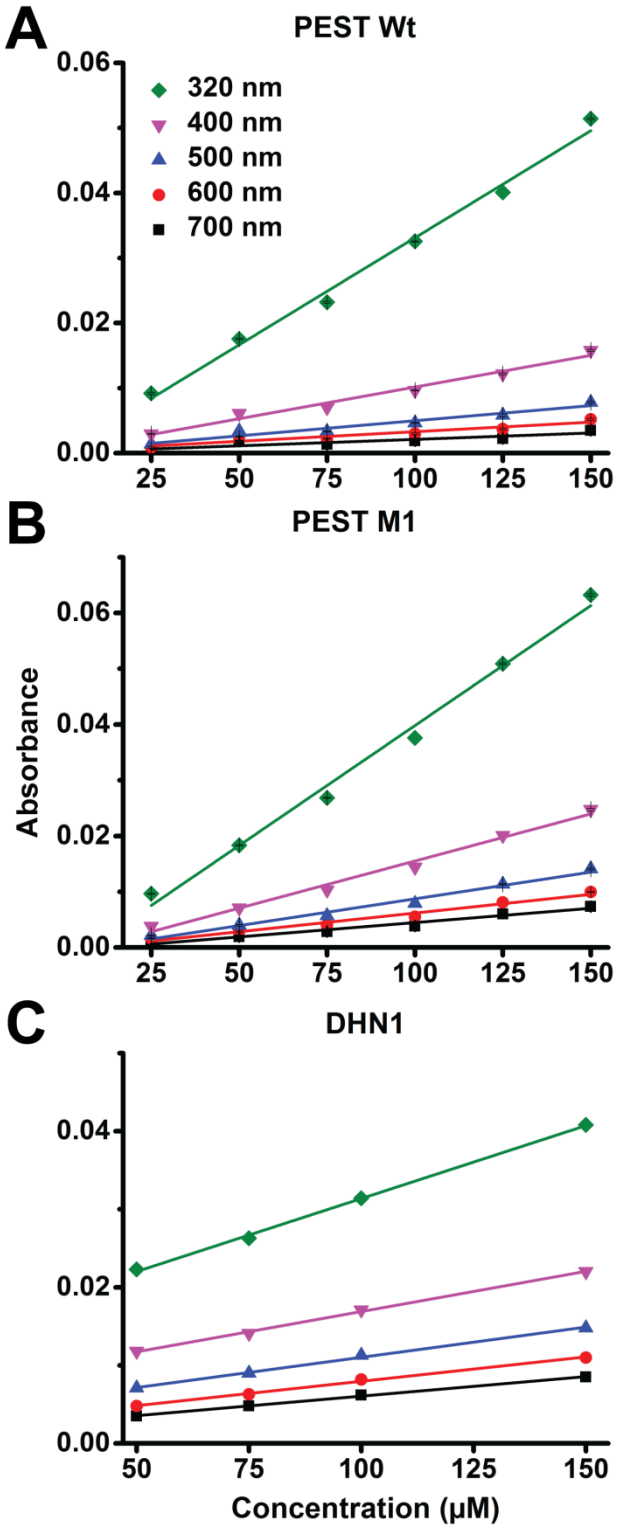

Figure S7: Variation of ProCharTS absorbance with protein concentration for **[A]** PEST Wt; **[B]** PEST M1 and **[C]** DHN1 at chosen wavelengths.

Figure S8 Absorption spectrum of DHN1 in 0.1 N NaOH

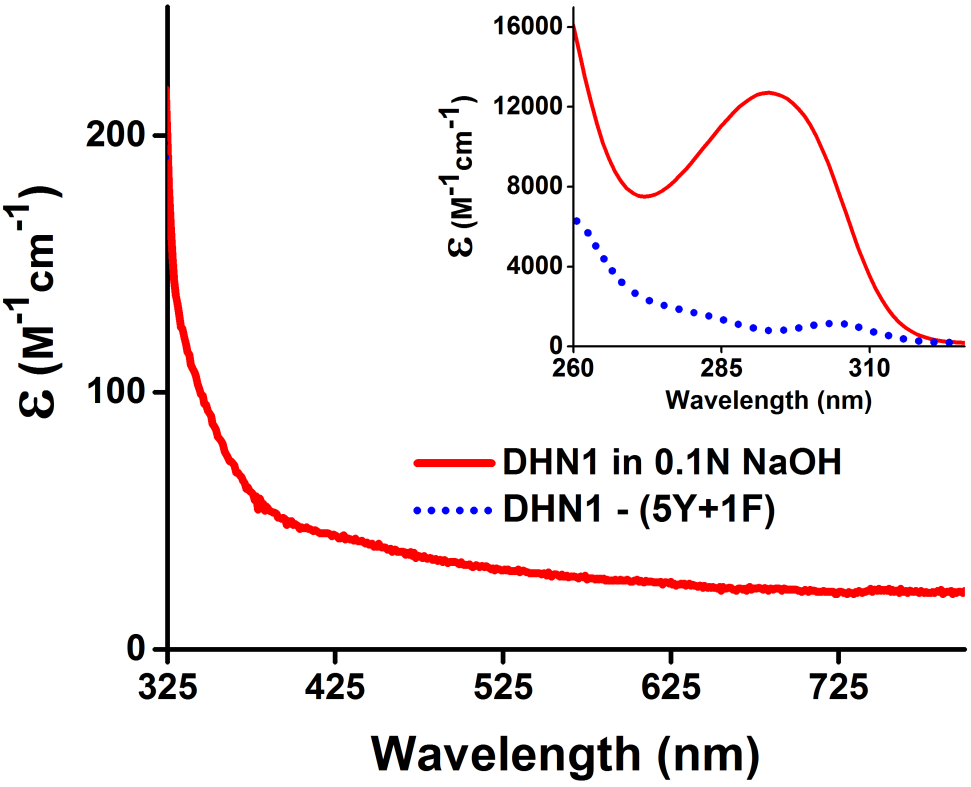

Figure S8: Absorption spectrum of DHN1 in 0.1 N NaOH. Inset shows DHN1 spectrum after subtracting contribution of Phe and Tyr (260 to 326 nm).

Figure S9 Percent change in absorbance at different pH with respect to pH 7

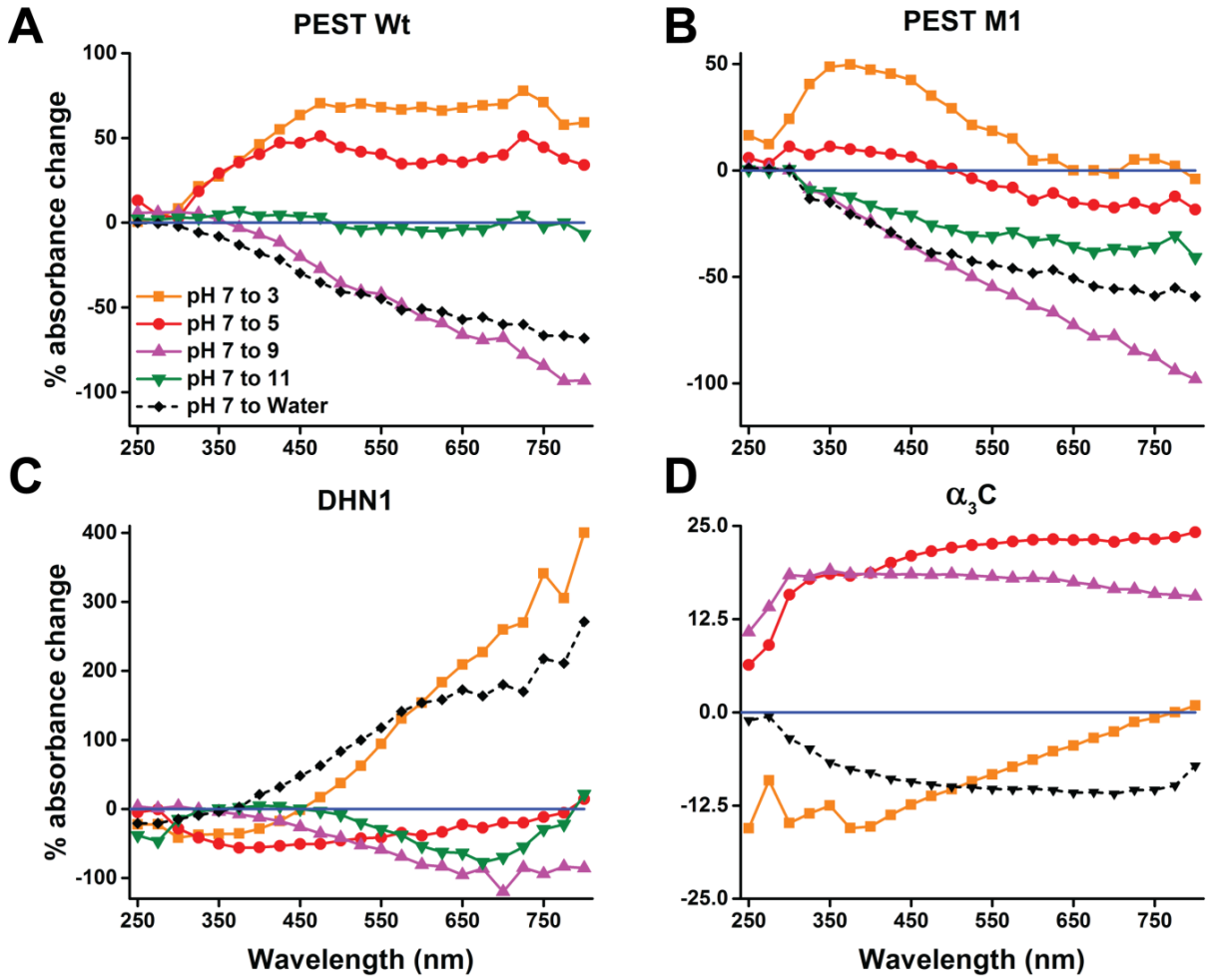

Figure S9: Percent change in absorption intensity measured every 25 nm at specified pH among the proteins **[A]** PEST Wt; **[B]** PEST M1; **[C]** DHN1 and **[D]** α_3C with respect to pH 7. The change in absorbance at selected wavelengths were calculated as [(Absorbance at chosen pH – Absorbance at pH 7)/Absorbance at pH 7]×100.

Figure S10 Secondary structure content of PEST Wt and M1 at various pH

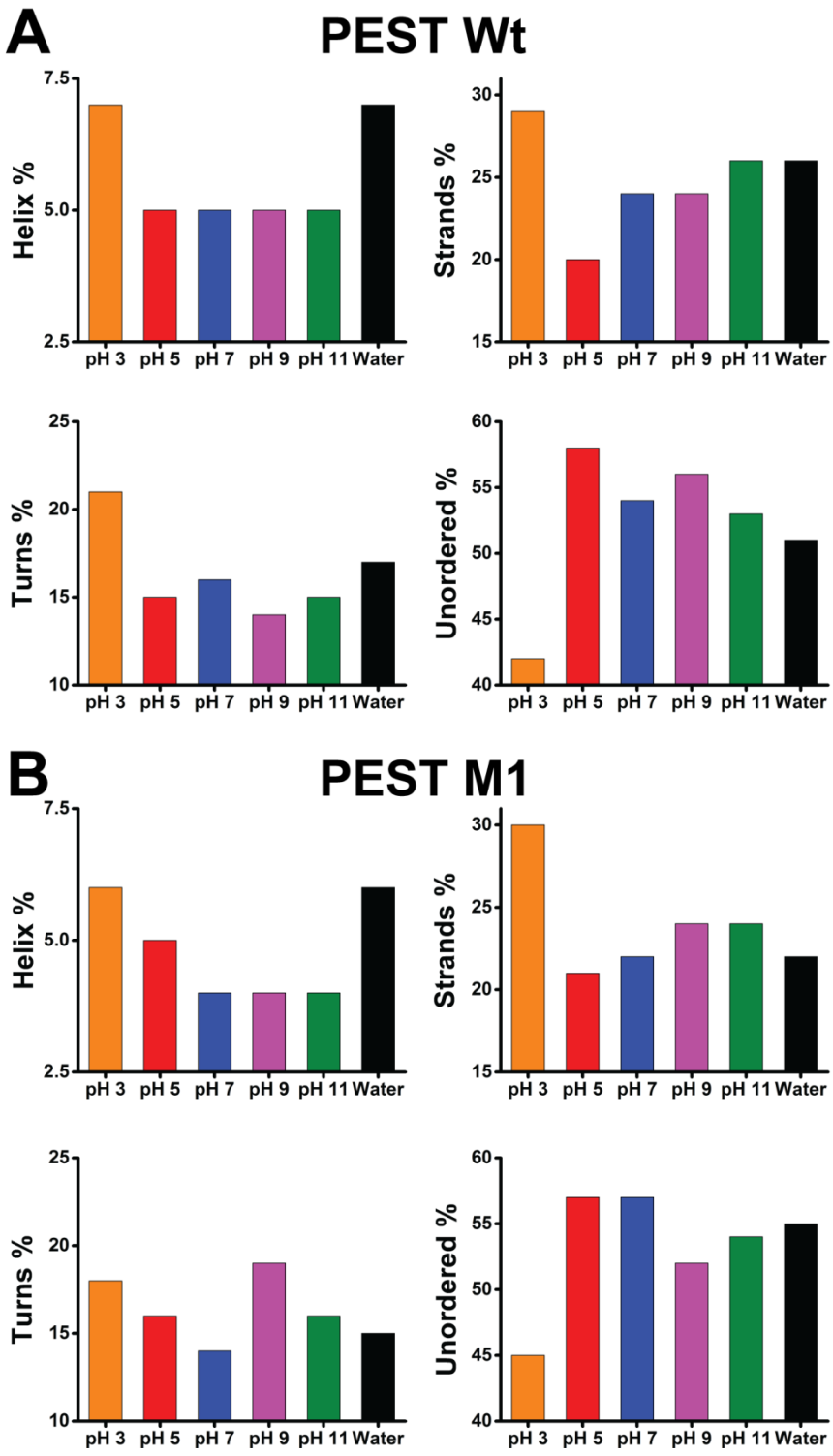

Figure S10: Change in secondary structure content in **[A]** PEST Wt and **[B]** PEST M1 at various pH and in deionized water.

Figure S11 Fitted CD Spectra of PEST Wt and M1 at various pH

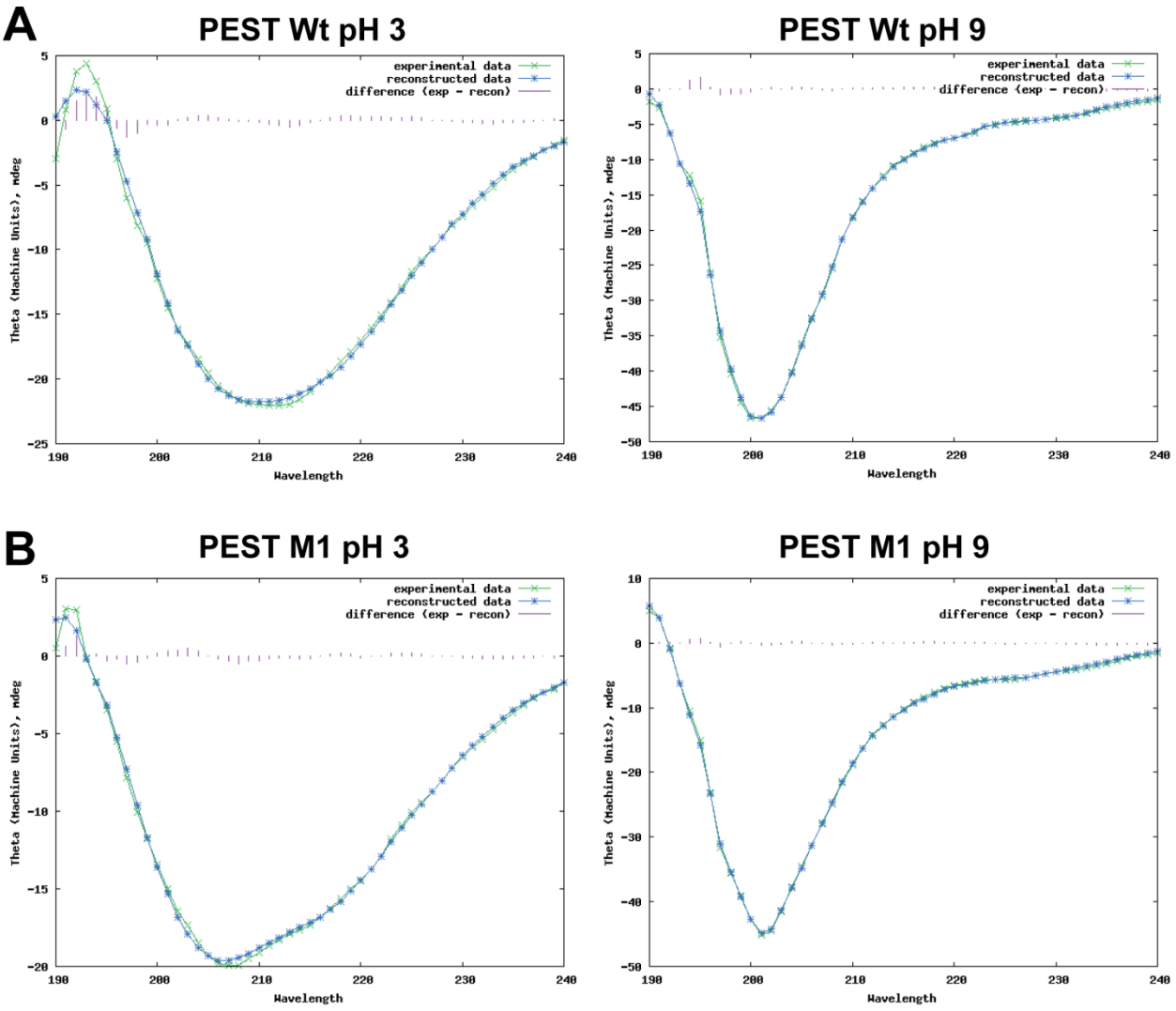

Figure S11: Fitted CD spectra of **[A]** PEST Wt and **[B]** PEST M1 by using DichroWeb server at pH 3 and 9.

Figure S12 CD spectra of DHN1 and α_3C at various pH

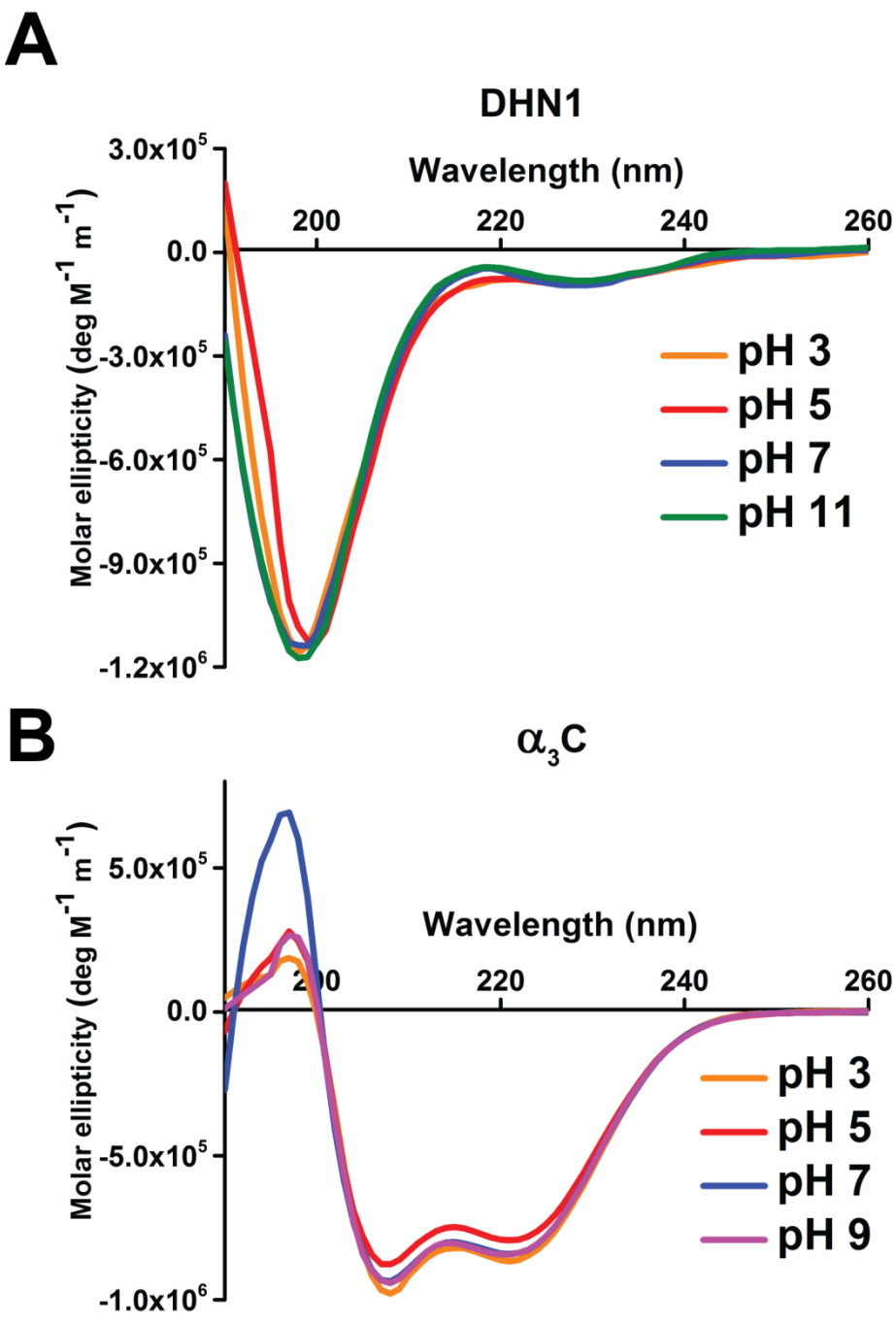

Figure S12: CD spectra of **[A]** 12.5 μM DHN1 and **[B]** 30 μM α_3C at various pH.

Figure S13 Percent change in absorbance at different temperatures with respect to room temperature (25 °C)

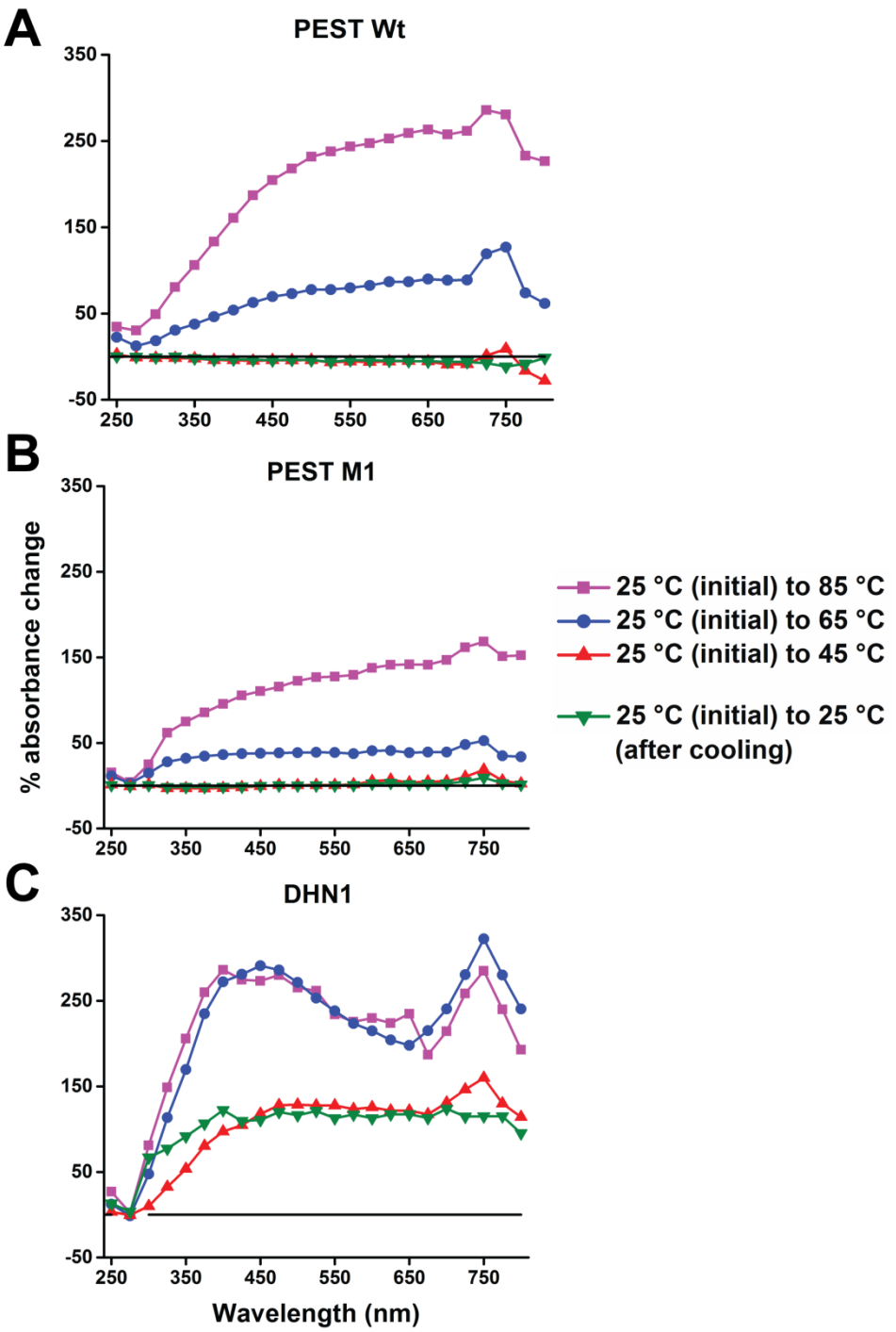

Figure S13: Percent change in absorption intensity measured every 25 nm at specified temperature among the proteins **[A]** PEST Wt; **[B]** PEST M1 and **[C]** DHN1 with respect to room temperature (25 ˚C). The change in absorbance at selected wavelengths were calculated as [(Absorbance at chosen temperature – Absorbance at 25°C)/Absorbance at 25 °C]×100.

Figure S14 Secondary structure content of PEST Wt and M1 at various temperatures

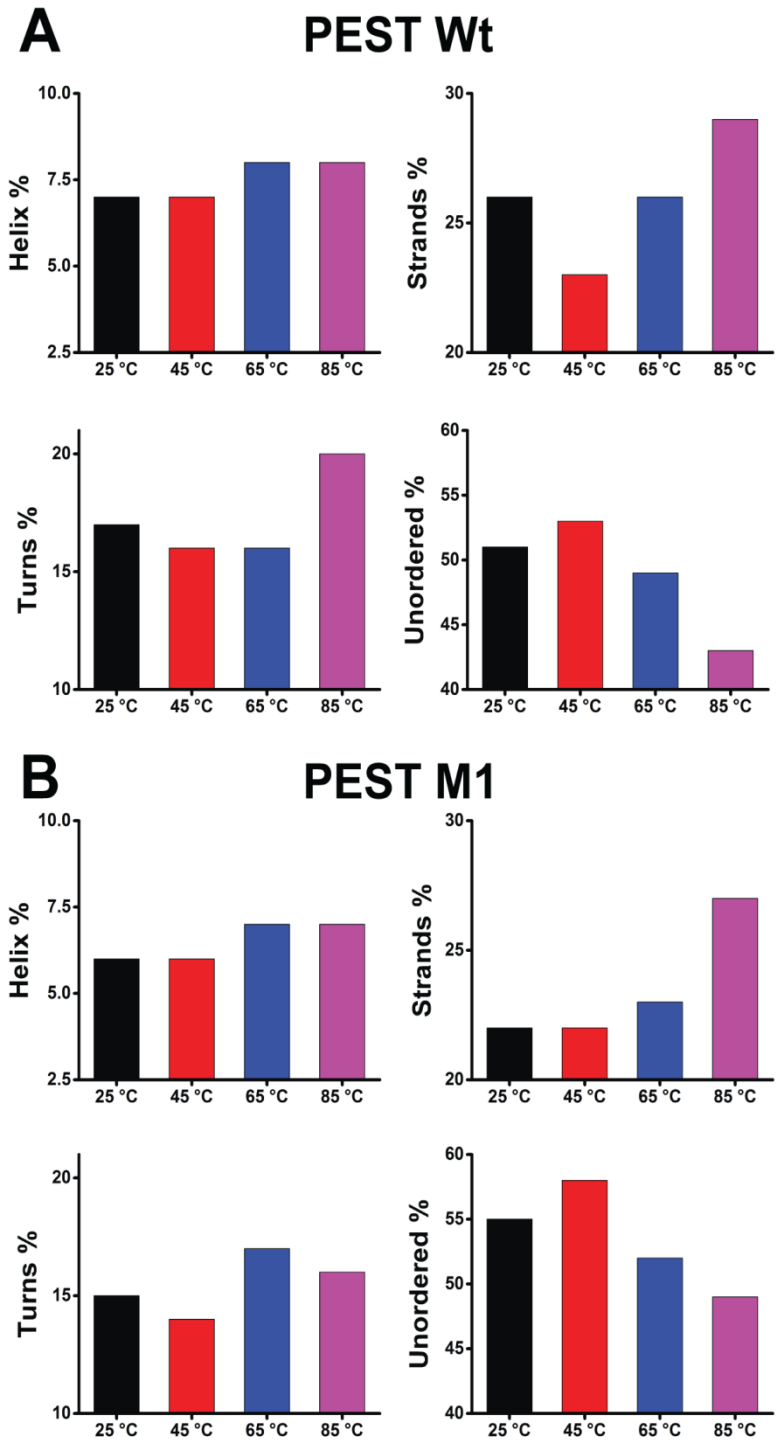

Figure S14: Change in secondary structure content in **[A]** PEST Wt and **[B]** PEST M1 at different temperatures.

Figure S15 Fitted CD Spectra of PEST Wt and M1 at various temperatures

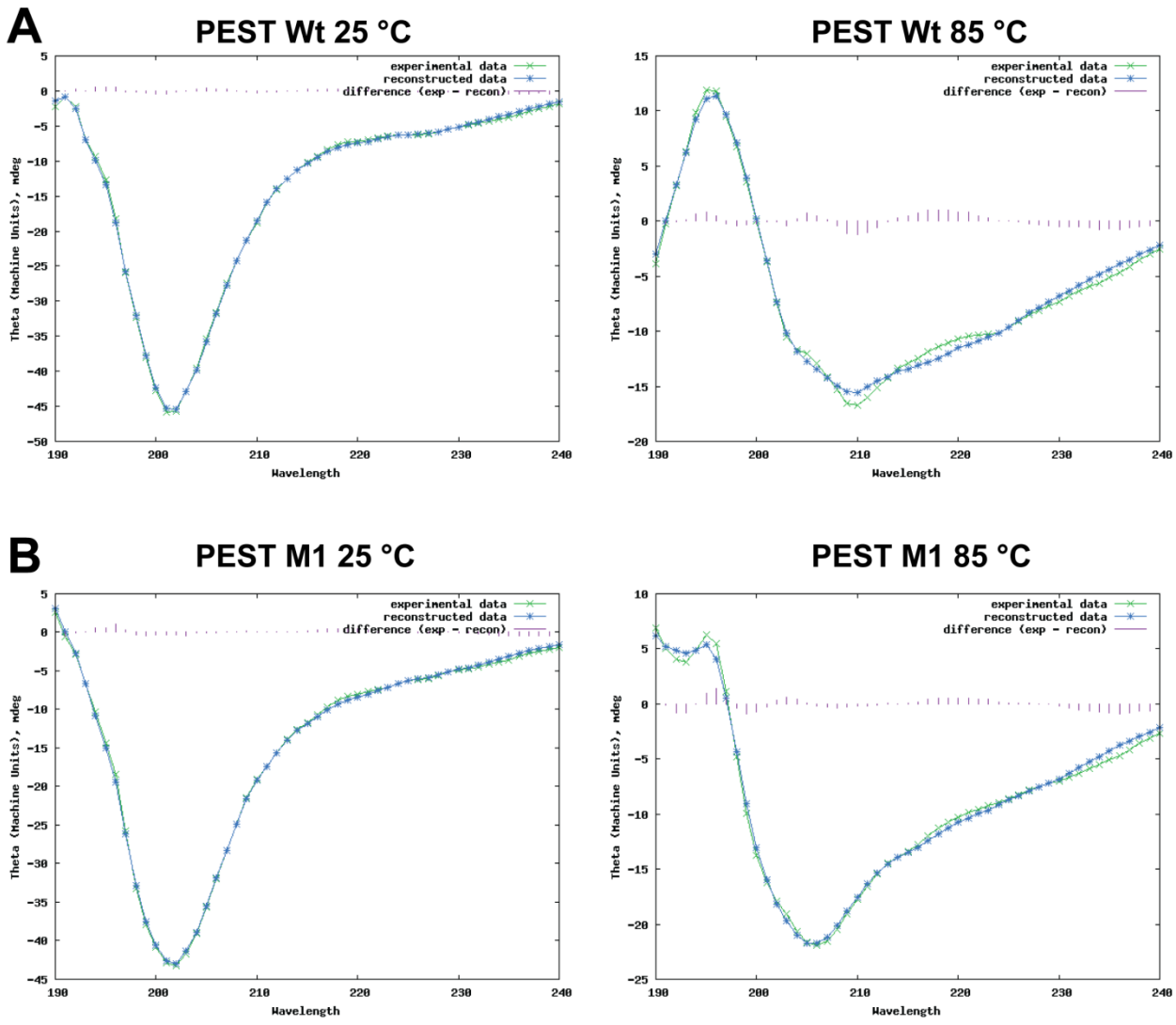

Figure S15: Fitted CD spectra of **[A]** PEST Wt and **[B]** PEST M1 by using DichroWeb server at 25 and 85 °C.

Figure S16 Percent change in absorbance with NaCl and KCl with respect to water

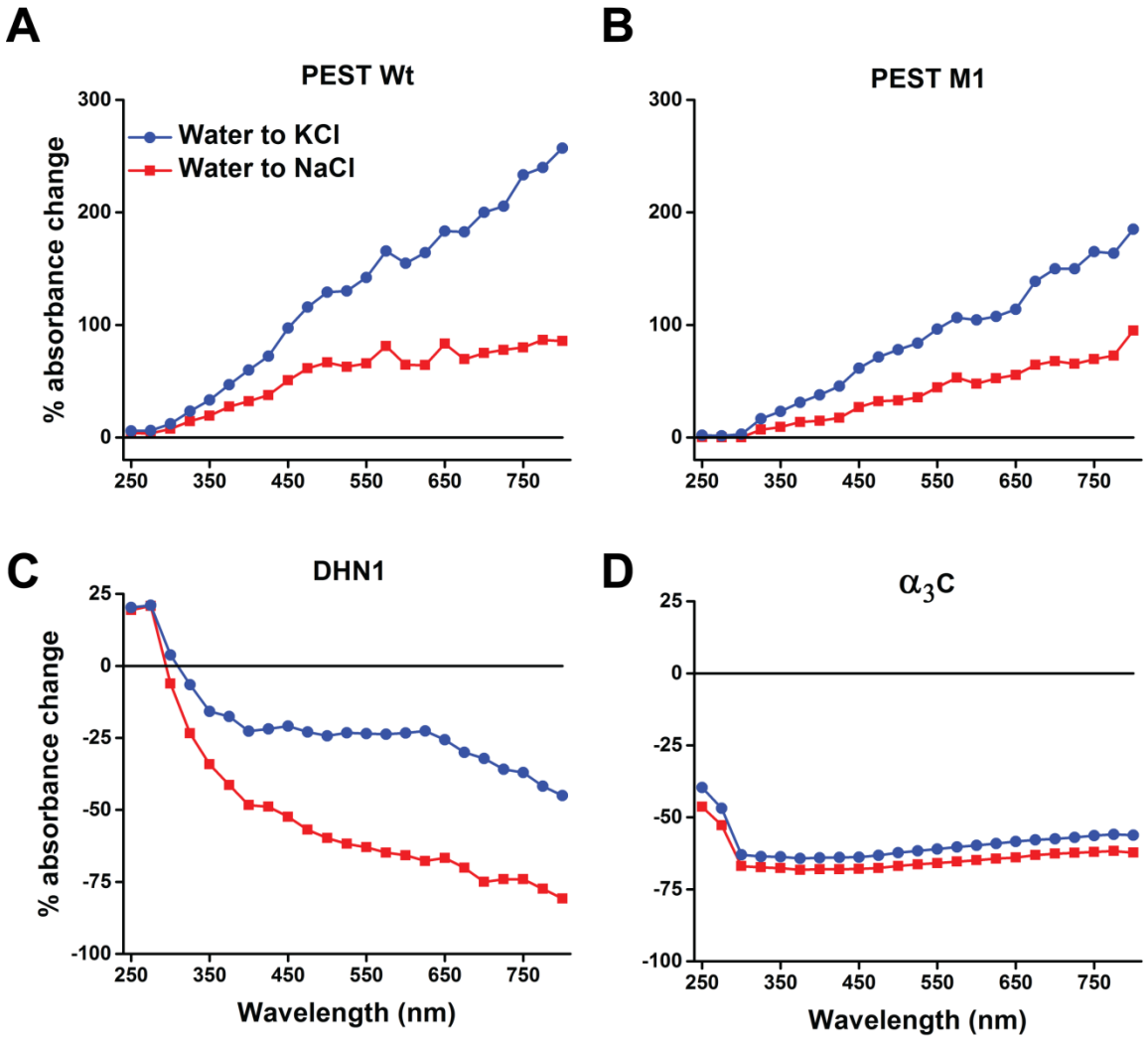

Figure S16: Percent change in absorption intensity measured at 25 nm intervals among the proteins **[A]** PEST Wt; **[B]** PEST M1; **[C]** DHN1 and **[D]** α_3C in 250 mM NaCl and 250 mM KCl with respect to absorption of proteins in deionised water. The change in absorbance were calculated at selected wavelengths as [(Absorbance in salt – Absorbance in water)/Absorbance in water]×100.

Figure S17 Concentration dependence of ProCharTS on concentration of HEWL aggregates

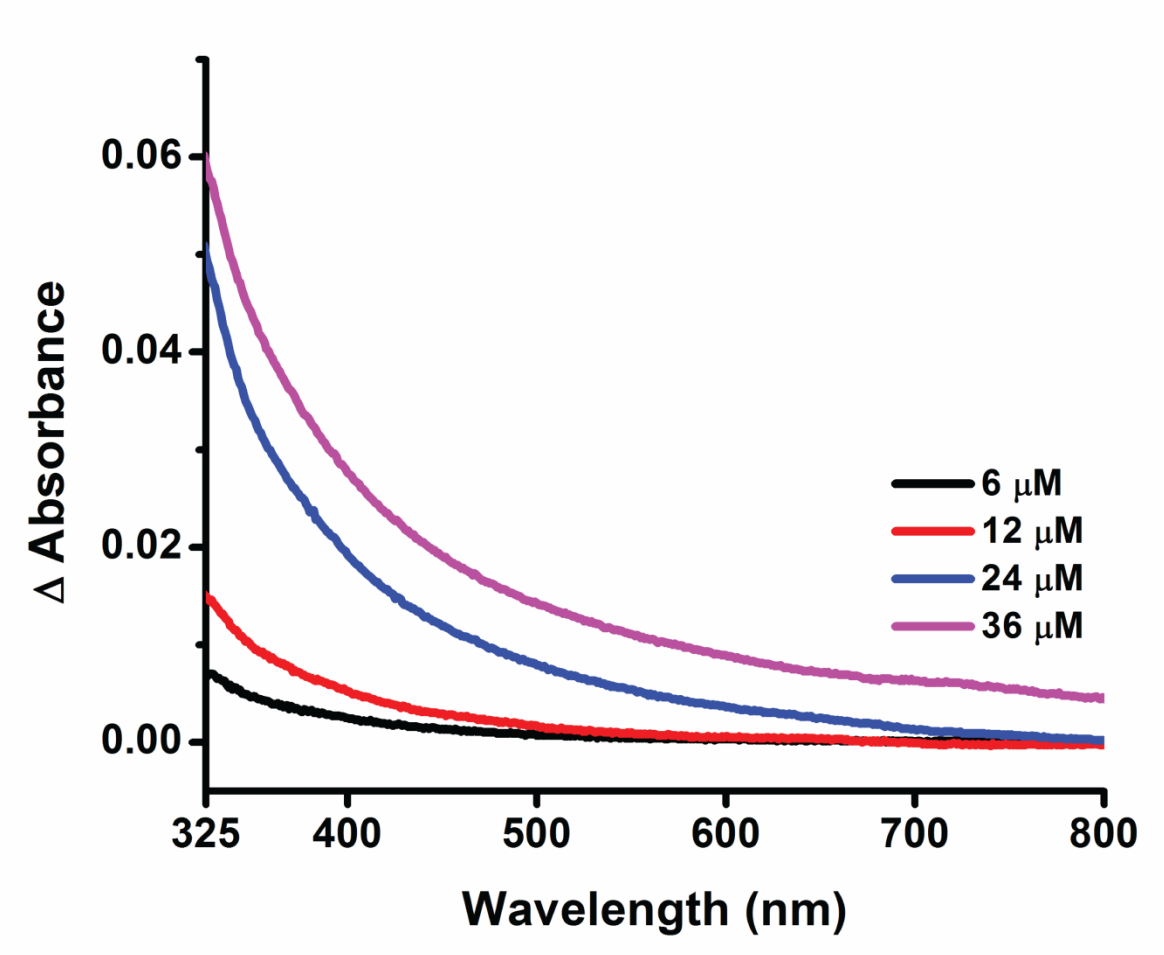

Figure S17: ProCharTS of HEWL aggregates formed at pH 12.2: Difference absorption spectra of 10 day old HEWL aggregates for different monomer concentrations is shown. All absorbance measurements were done immediately after transfer in 0.1 M sodium bicarbonate buffer (pH 9.3). Difference spectra are generated by subtracting the spectra of fresh HEWL monomer of same concentration in the same buffer as the transferred aggregates.