Supplementary information (Lysozyme paper)

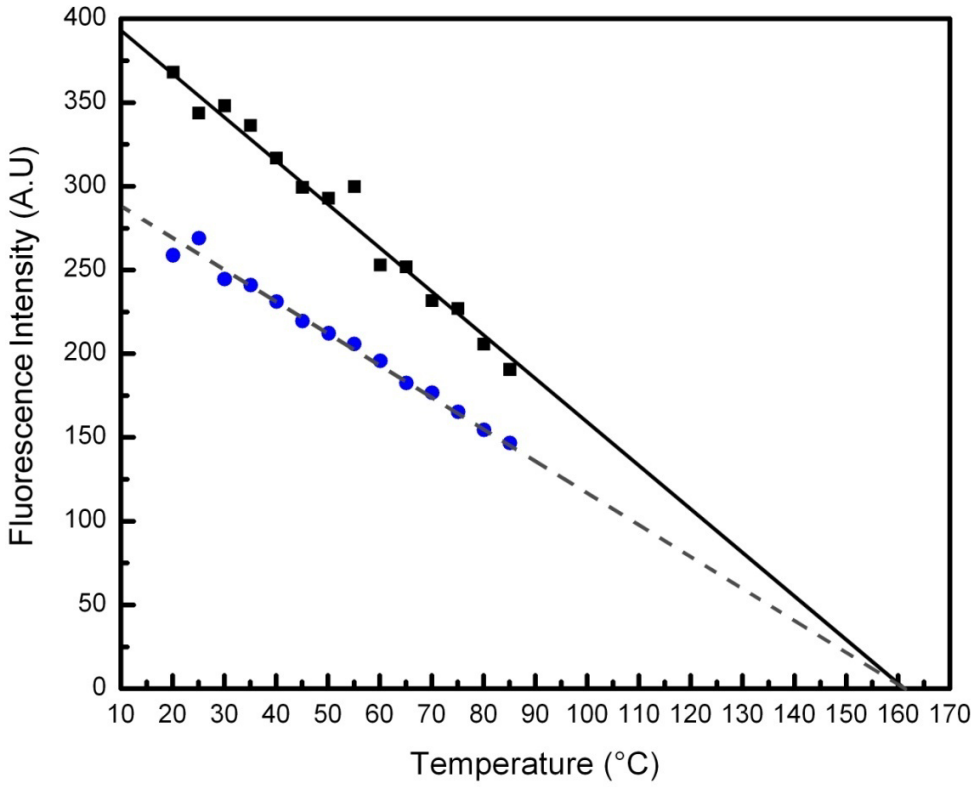

Figure S1: ThT fluorescence at various temperatures for two HEWL samples of different intensities including line of best fit to a common point on the X axis. The fluorescence emission intensity of the two samples is shown as crosses for the first set and diamonds for the second set. A common point at 161°C is observed as the approximate interception point of the lines.

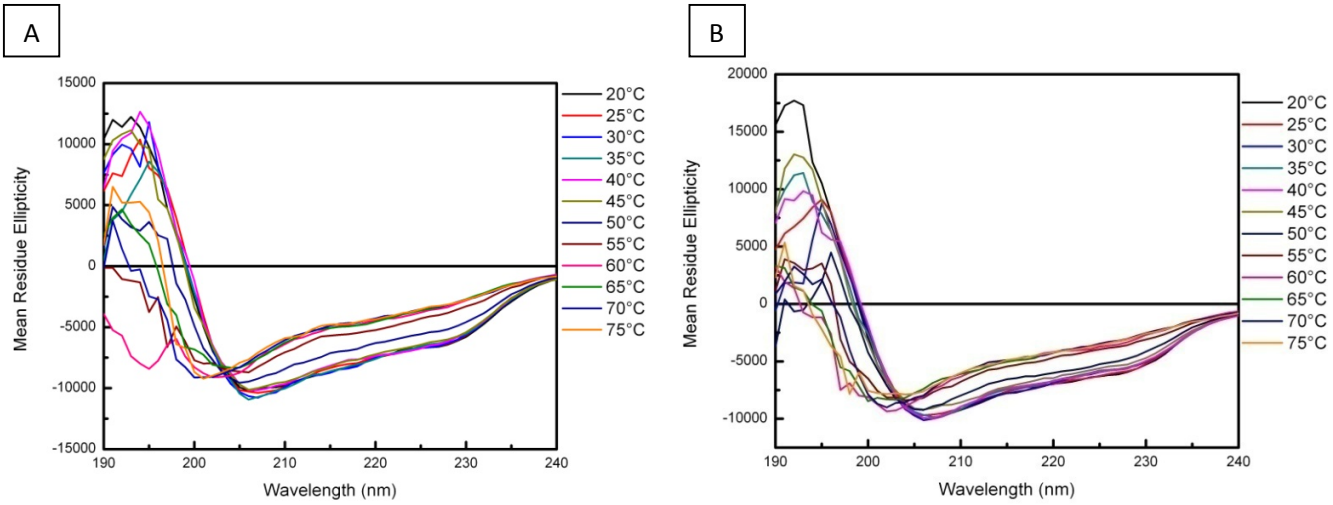

Figure S2: Graphs showing the CD spectra at various temperatures for the denaturation (A) and renaturation (B) or HEWL Major changes in the CD spectra are observed between 50 and 55°C

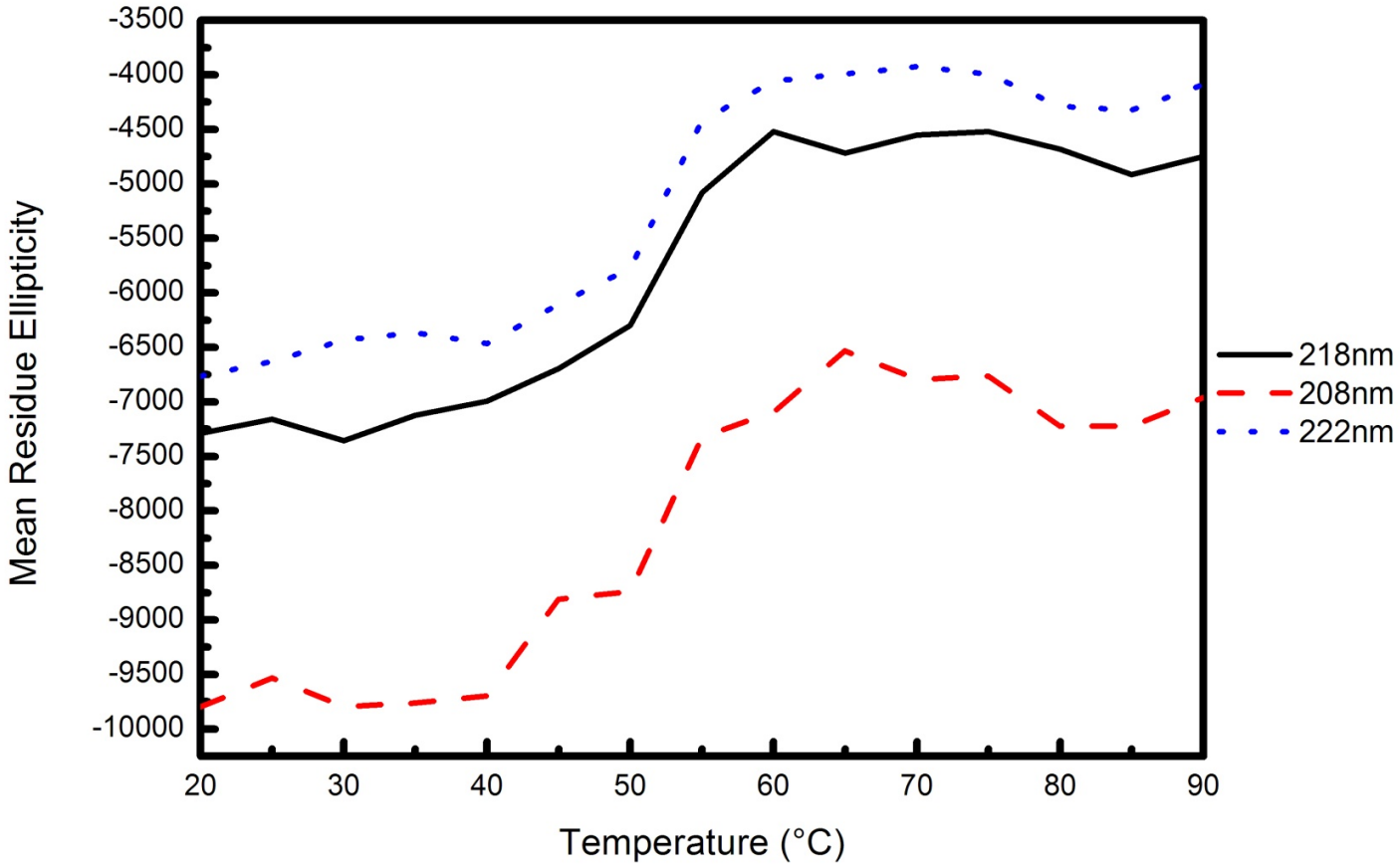

Figure S3: Mean Residue Ellipticity of CD spectra at various wavelengths of the renaturation of HEWL at various temperatures. Figure shows a major change in the alpha and beta secondary structure occurs between 40 and 65°C

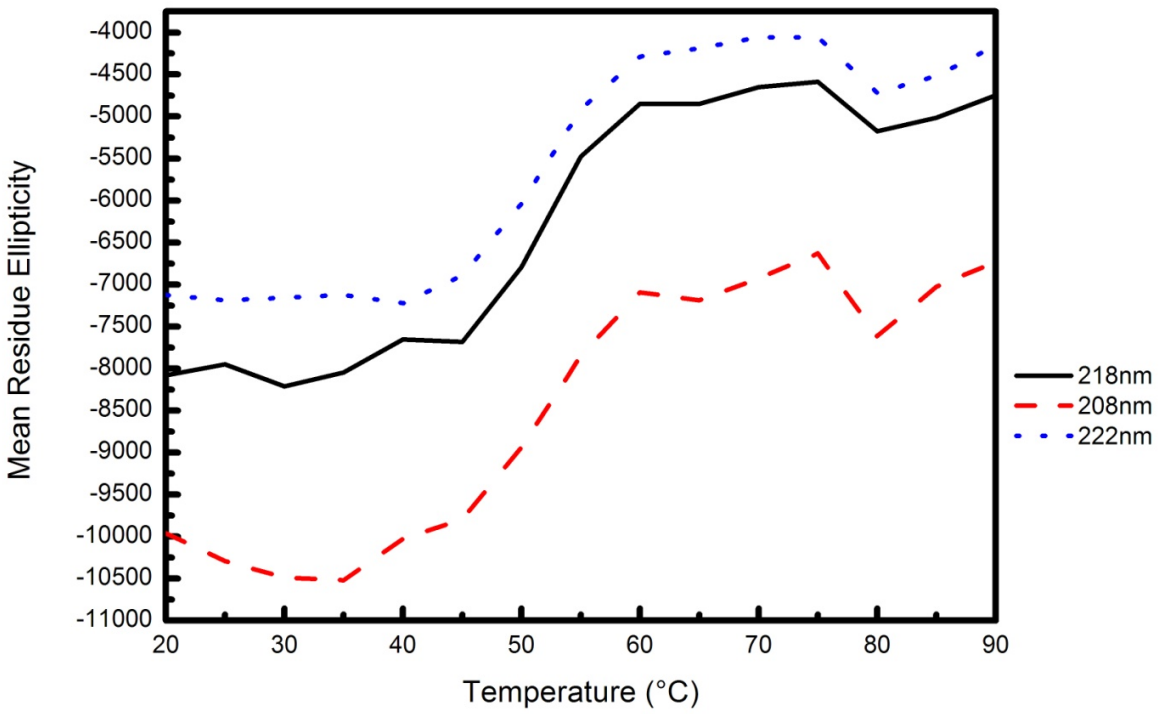

Figure S4: Mean Residue Ellipticity of CD spectra at various wavelengths of the denaturation of HEWL at various temperatures. Figure shows a major change in the alpha and beta secondary structure occurs between 35 and 60°C

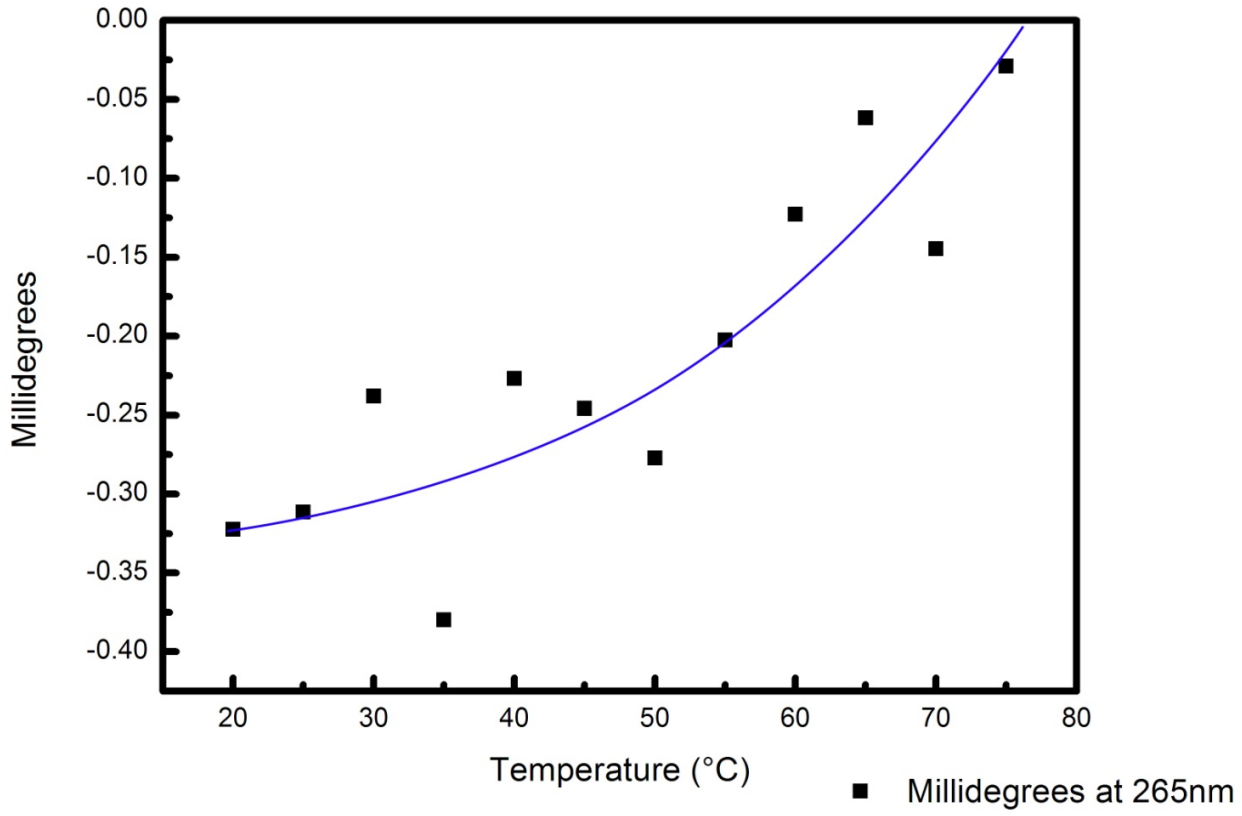

Figure S5: Figure showing the CD trace in millidegrees at 265nm indicating a gradual loss of tertiary protein structure through the experiment. Tertiary structure loss is more pronounced beyond 50°C

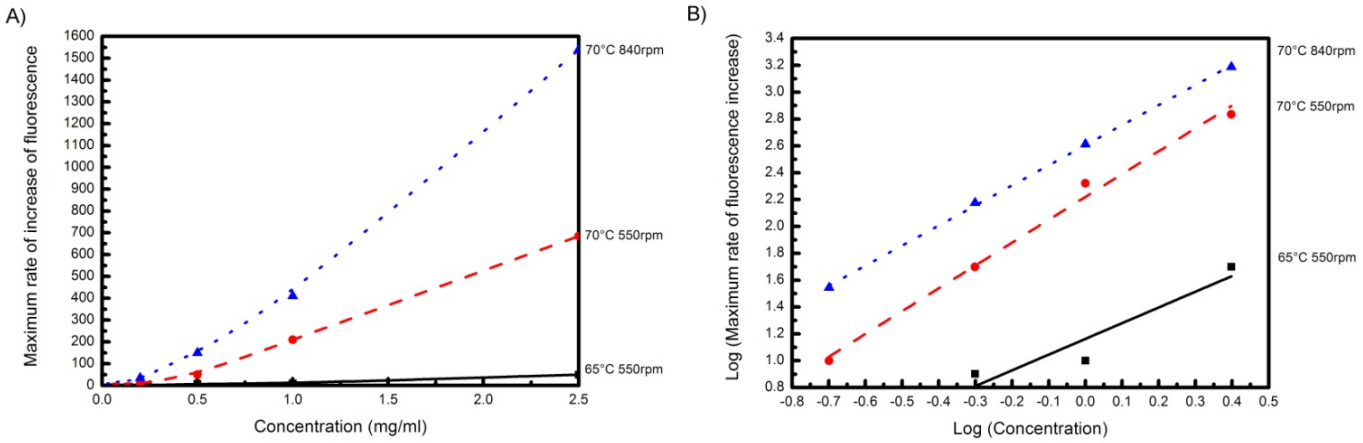

Figure S6: maximum gradients of HEWL amyloid fibril formation at different heat and stirring conditions. Conditions are: 65°C 550rpm (black line), 70°C 550rpm (dashed line) and 70°C 840rpm (dotted line) stirring speed plotted in (A) normal and (B) log values. Gradients of lines of best fit in (B) are 1.49, 1.70 and 1.17 for 70°C 840rpm, 70°C 550rpm and 65°C 550rpm respectively. Gradients and derivative values determined by Origin8.

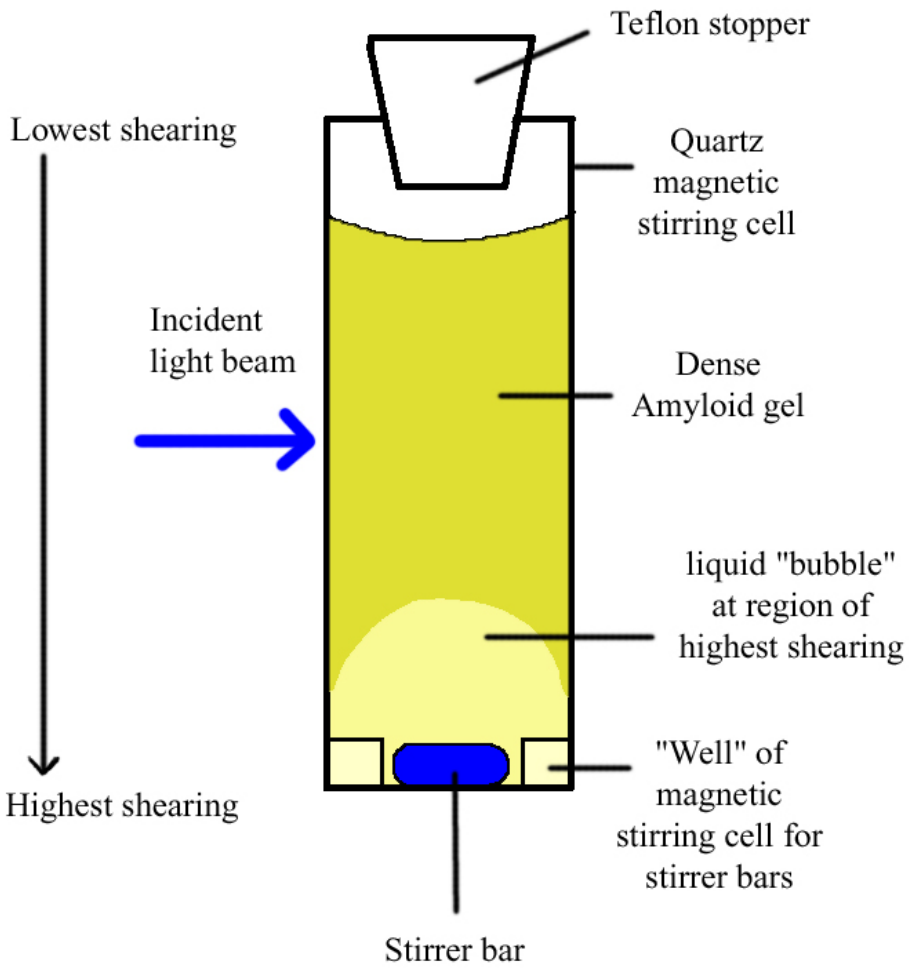

Figure S7: Figure showing gel formation at 30mg/ml HEWL incubated at 65°C at 550rpm stirring speed showing the distribution of the gel and liquid phases due to the heterogeneous shearing in the magnetic stirring quartz cell.

Concentration (mg/ml)	Parameter	Estimate	Standard Error	Confidence interval
0.2	k(1)	0.00300466	0.000208777	{0.00259483, 0.00341448}
	A(0)	11.371	0.100035	{11.1747, 11.5674}
	τ	678.744	14.3625	{650.551, 706.937}
0.5	k(1)	0.00110269	0.0000187407	{0.00106587, 0.00113951}
	A(0)	173.417	1.02101	{171.411, 175.423}
	τ	208.107	4.45857	{199.348, 216.867}
1.0	k(1)	0.000664884	9.433771×10^{-6}	{0.00064635, 0.000683418}
	A(0)	257.117	1.78614	{253.607, 260.626}
	τ	153.971	3.35328	{147.383, 160.558}
2.5	k(1)	0.00137124	8.32645×10^{-6}	{0.00135488, 0.0013876}
	A(0)	625.646	1.085	{623.514, 627.778}
	τ	77.8157	1.62093	{74.6309, 81.0004}

Table S1: Table showing estimates for parameters for aggregation of HEWL at 65°C 550rpm from a MATLAB algorithm to fit the data to a modified first order rate equation

Concentration (mg/ml)	Parameter	Estimate	Standard Error	Confidence interval
0.2	k(1)	0.0022868	0.0000178245	{0.00225178, 0.00232183}
	A(0)	129.556	0.192568	{129.178, 129.934}
	τ	137.316	1.76256	{133.853, 140.779}
0.5	k(1)	0.0034922	0.0000737531	{0.0033473, 0.00363711}
	A(0)	394.656	1.17775	{392.342, 396.97}
	τ	109.895	3.61275	{102.797, 116.993}
1.0	k(1)	0.00287262	0.0000192079	{0.00283488, 0.00272404}
	A(0)	1236.52	1.30614	{1233.95, 1239.09}
	τ	58.1813	1.33413	{20.3054, 26.7901}
2.5	k(1)	0.00268254	0.0000211236	{0.00264103, 0.00272404}
	A(0)	2743.78	3.54868	{2736.81, 2750.75}
	τ	23.5478	1.65025	{20.3054, 26.7901}

Table S2: Table showing estimates for parameters for aggregation of HEWL at 70°C 550rpm from a MATLAB algorithm to fit the data to a modified first order rate equation

Concentration (mg/ml)	Parameter	Estimate	Standard Error	Confidence interval
0.2	k(1)	0.00191423	0.0000171089	{0.00188055, 0.0194791}
	A(0)	340.596	1.08383	{338.462, 342.73}
	τ	64.0601	1.29861	{61.5036, 66.6166}
0.5	k(1)	0.0036277	0.0000360494	{0.00355673, 0.00369867}
	A(0)	1048.16	2.21361	{1043.81, 1052.52}
	τ	82.7186	1.31211	{80.1355, 85.3017}
1.0	k(1)	0.00322506	0.0000277166	{0.0031705, 0.00327963}
	A(0)	2195.06	4.32366	{2186.55, 2204.57}
	τ	52.339	1.20317	{49.9704, 54.7077}
2.5	k(1)	0.00408656	0.0000702731	{0.00394821, 0.0042249}
	A(0)	4713.79	15.1934	{4683.88, 4743.7}
	τ	18.9528	2.19175	{14.638, 23.2676}

Table S3: Table showing estimates for parameters for aggregation of HEWL at 70°C 840rpm from a MATLAB algorithm to fit the data to a modified first order rate equation

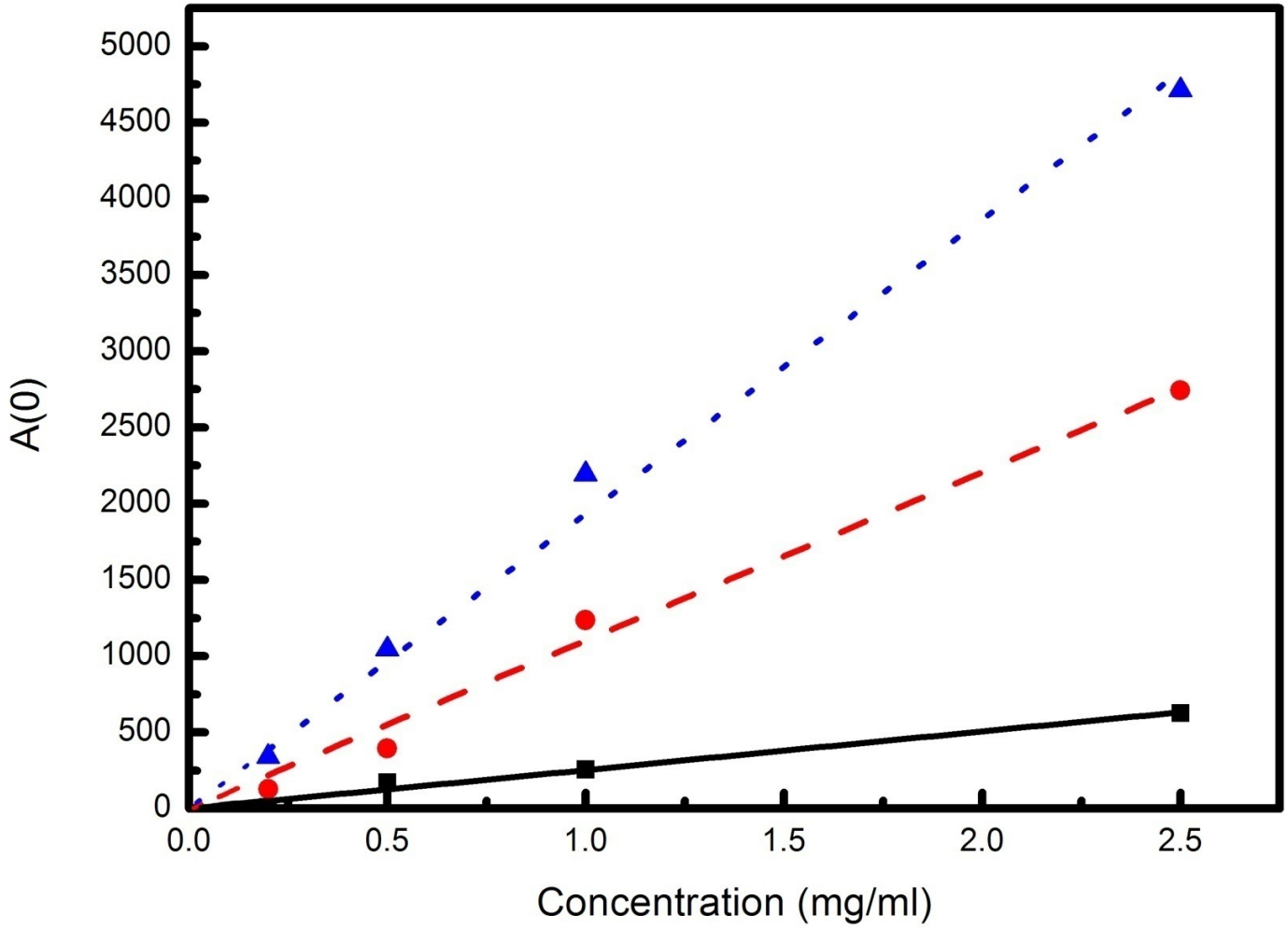

Figure S8: Figure showing the values of A(0) against temperature for HEWL incubated at 65°C 550rpm (Black line and squares), HEWL incubated at 70°C 550rpm (Red dashed line and circles) and HEWL incubated at 70°C 840rpm (Blue dotted line, Triangles). The error bars for each data point in table S1-S3 were not included as they are too short to be noticeable for the given scale. Graph shows a linear relationship between A(0) and protein concentration, indicating a good fit of the data to the suggested equation.

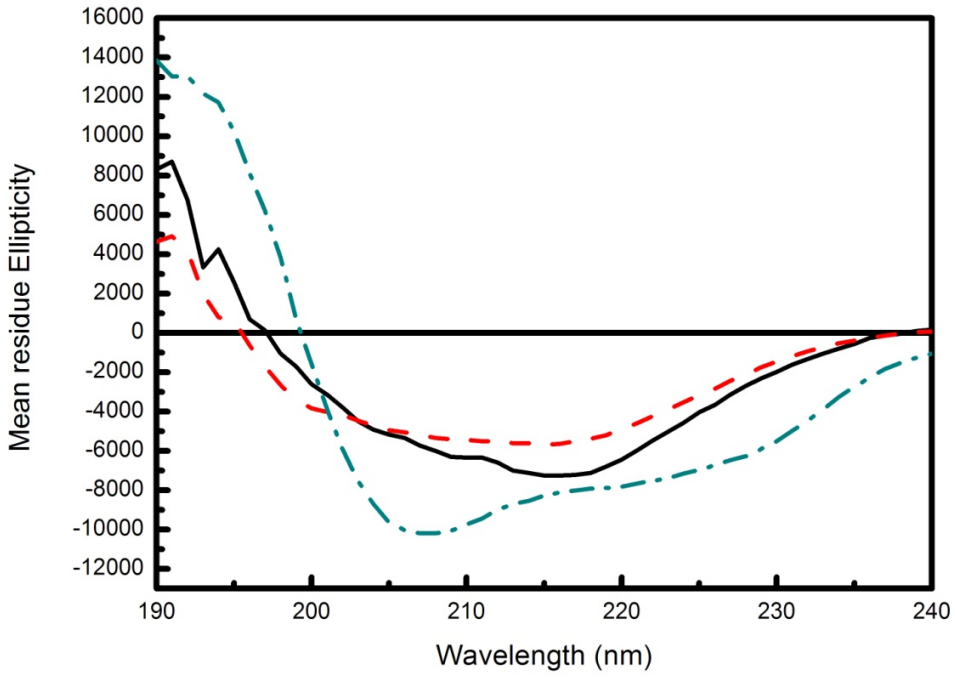

Figure S9: CD spectra of freshly dissolved HEWL (grey dash and dotted line) in as well as Lysozyme fibrils grown in 0.5 (black line) and 1(red dashed line) HEWL at 70°C, 840 RPM stirring speed. Graphs show a distinct shift to a more β-sheet rich structure after incubation.

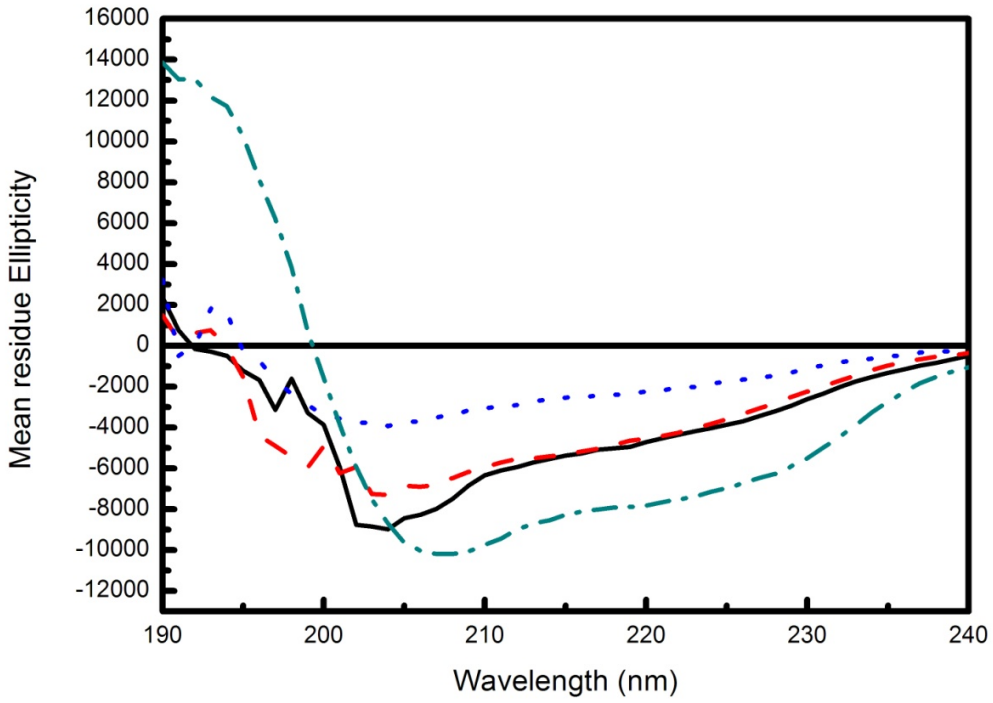

Figure S10: CD spectra of freshly dissolved HEWL (grey dash and dotted line) in as well as Lysozyme fibrils grown in 0.5 (black line), 1(red dashed line) and 2.5 mg/ml (blue dotted line) HEWL at 65°C, 550 RPM stirring speed. Graphs show a distinct shift to a more β-sheet rich structure after incubation.

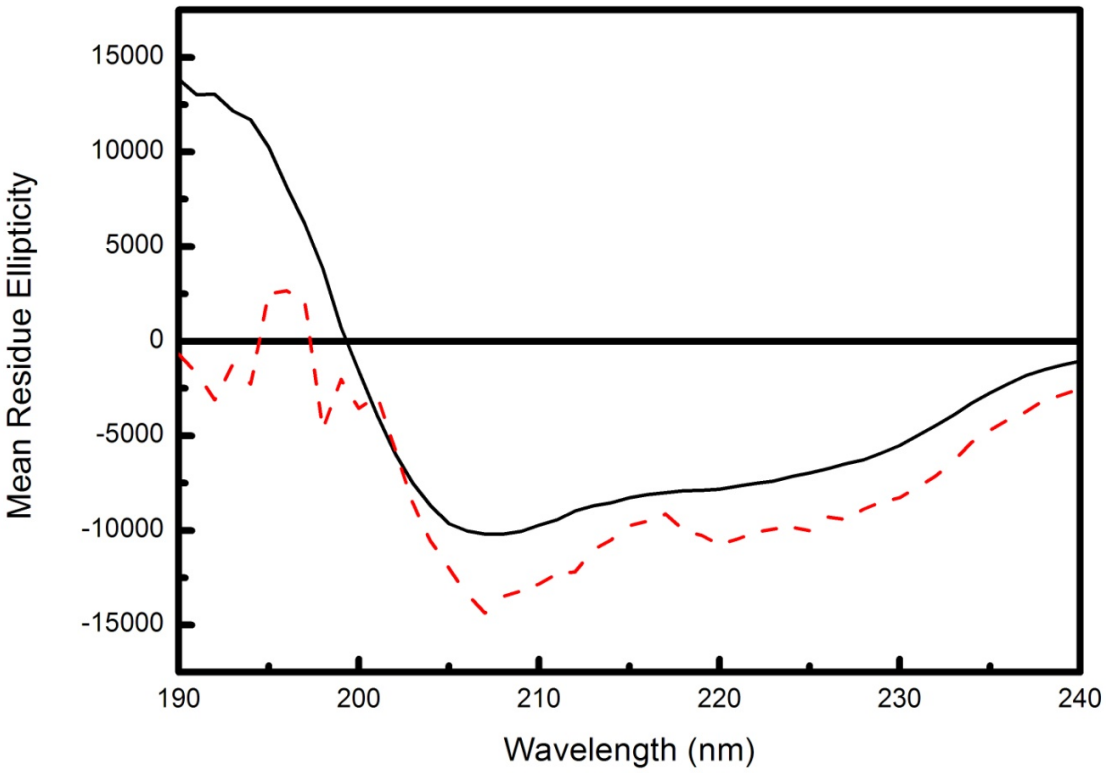

Figure S11: CD spectra of freshly dissolved HEWL in 0.1% HCl pH 1.5 (black solid line) and in pH 7.2, 0.0646M Phosphate Buffer (red dashed line). Graphs show a distinct decrease in α-helical content from a reduction in the negative peak at 220nm from pH 7.2 to pH 1.5.