Electronic Supplementary Material (ESI) for Soft Matter.

SUPPLEMENTAL MATERIAL FOR:

Role of protein aggregation on the strength and underwater performance of barnacle-inspired adhesives

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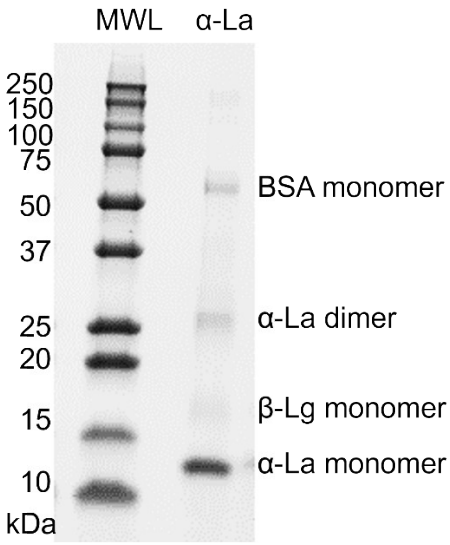
KEYWORDS. Bioinspired Adhesive, Amyloid, Adhesion, Protein Aggregation



Protein band	Amount (%)
α-La monomer	69.6%
β-Lg monomer	10.5%
α-La dimer	16.9%
BSA monomer	1.3%
Other	1.8%

Figure S1: SDS-PAGE results to determine purity of α-Lactalbumin. Data shown next to molecular weight ladder (MWL). The intensity of each band is calculated for calibration of results using Analyze Gel in ImageJ. Table indicates average of two samples.

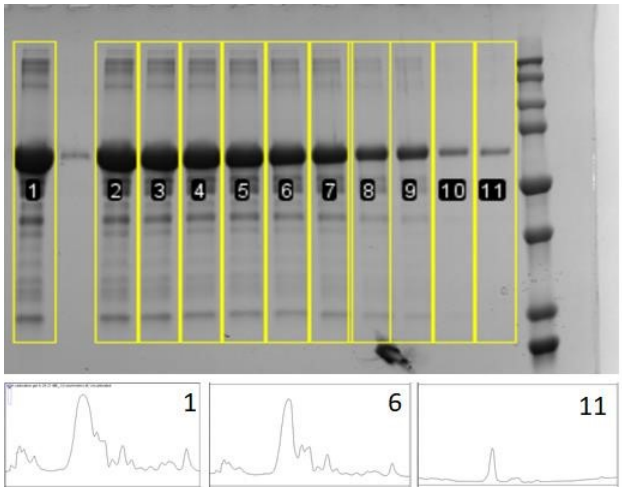


Figure S2: SDS-PAGE results for concentrations of BSA. The intensity of each lane is calculated for calibration of results using Analyze Gel in ImageJ.

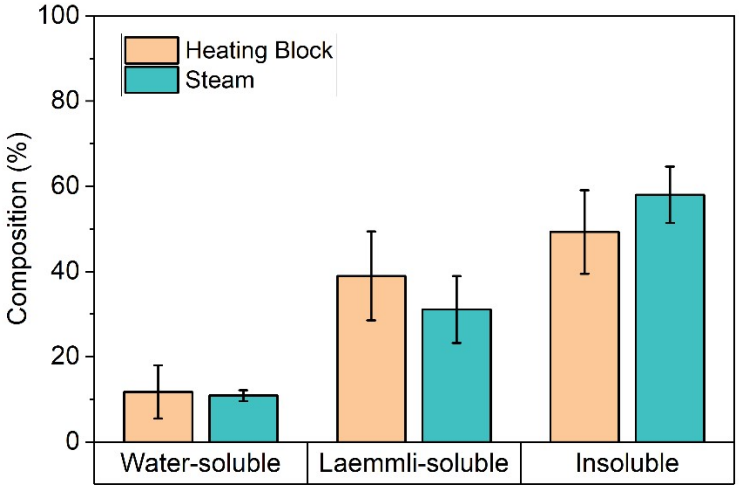


Figure S3: Composition of β-Lg heated at 90 °C in a heating block or in the steaming chamber.

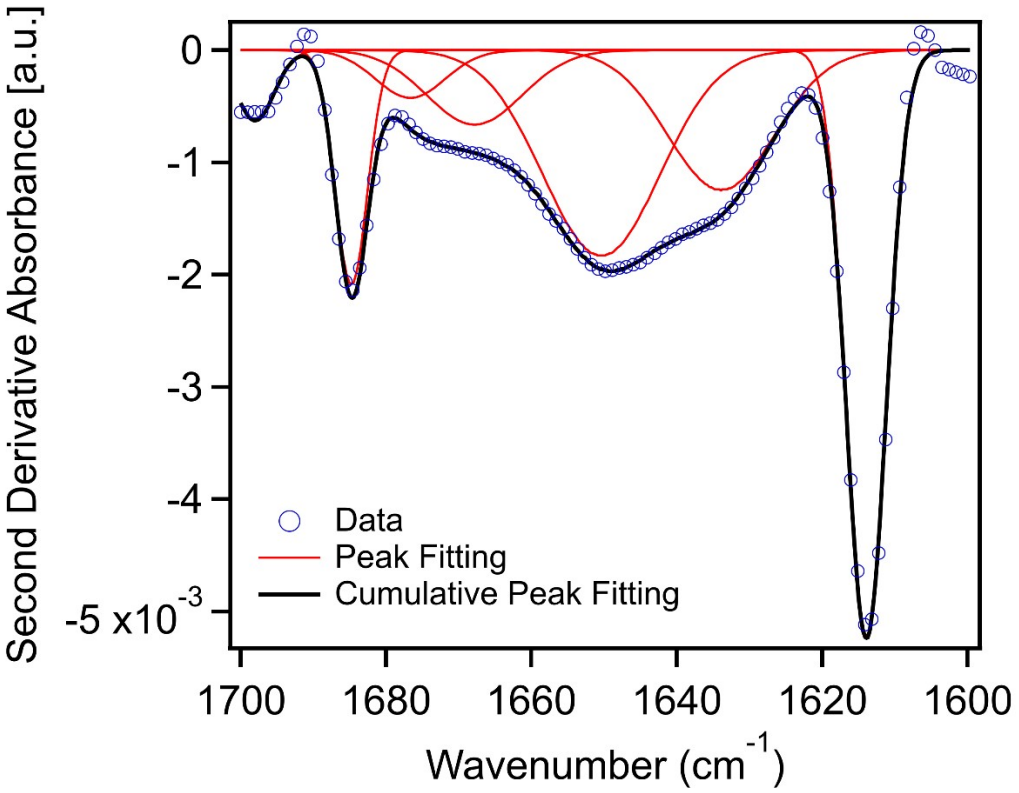


Figure S4: Example fitting of second derivative of amide I absorbance from transmission FTIR experiments. Sample is BSA cured at 90 °C.

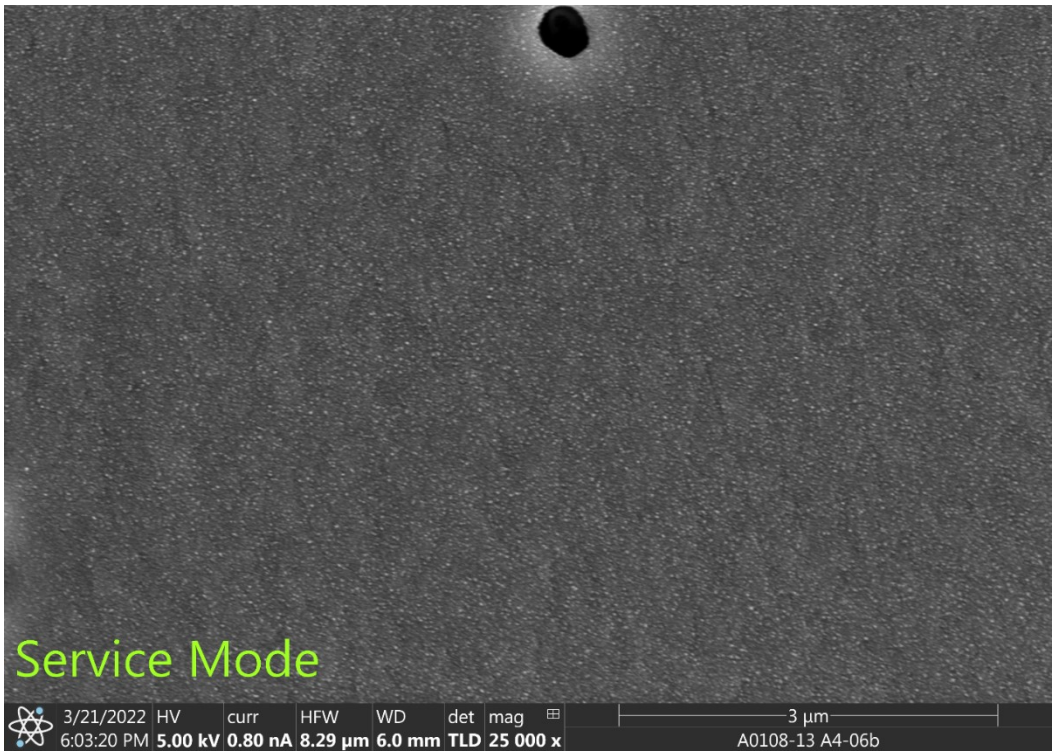


Figure S5: Scanning electron microscopy of β-Lg gel steam-cured at 70 °C

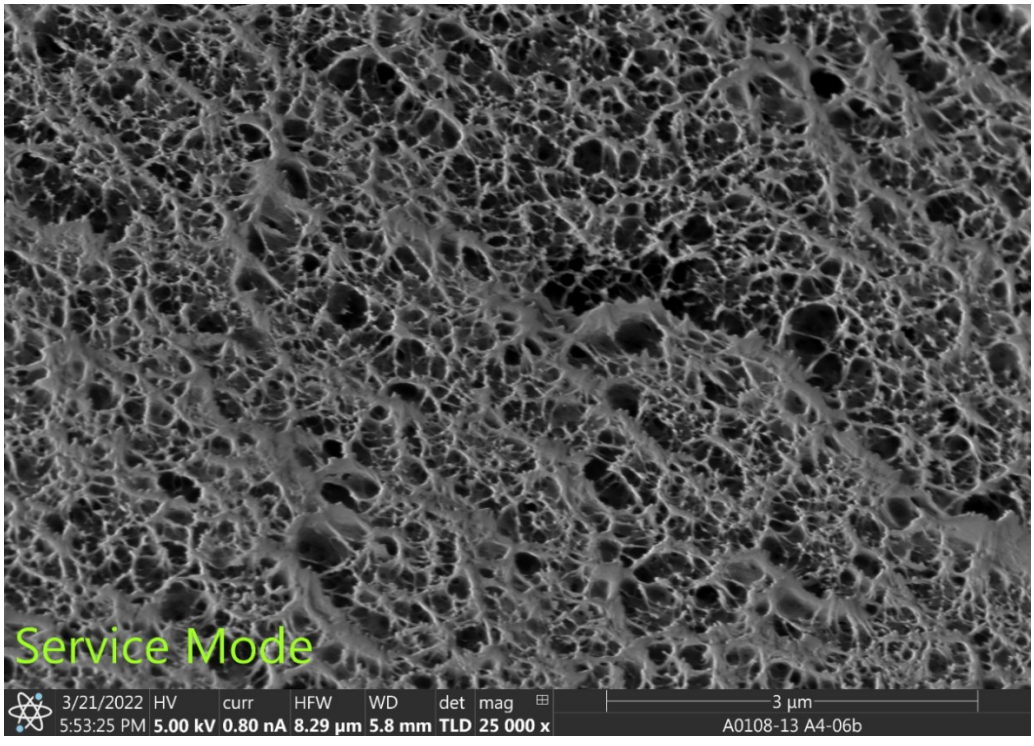


Figure S6: Scanning electron microscopy of β-Lg gel steam-cured at 90 °C

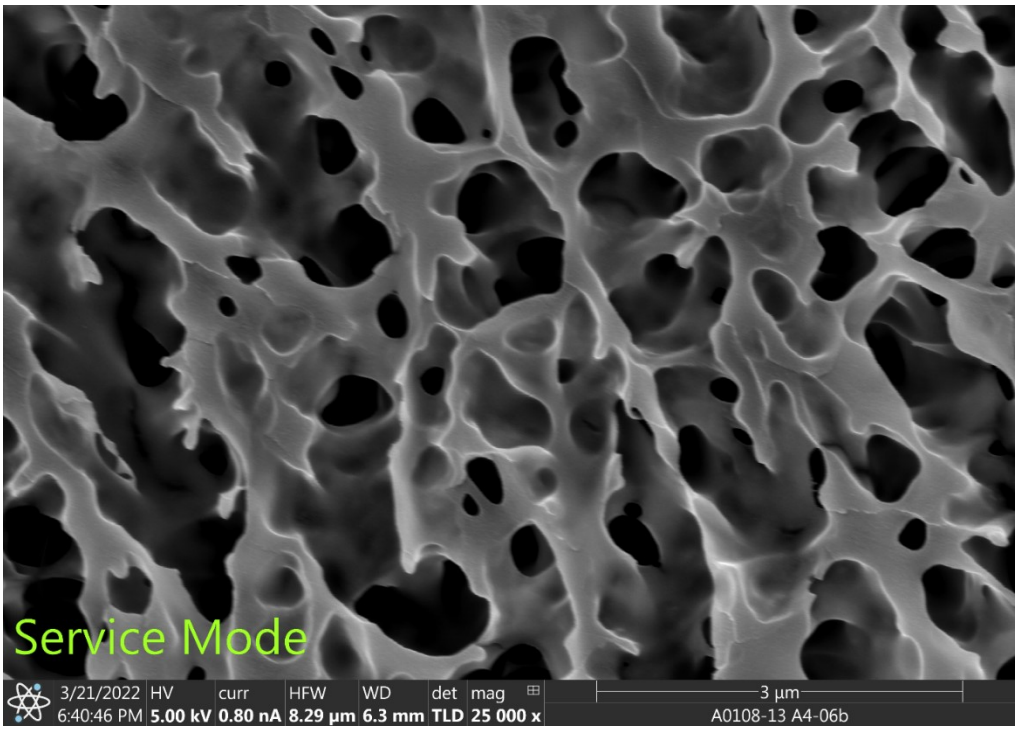


Figure S7: Scanning electron microscopy of BSA gel steam-cured at 60 °C

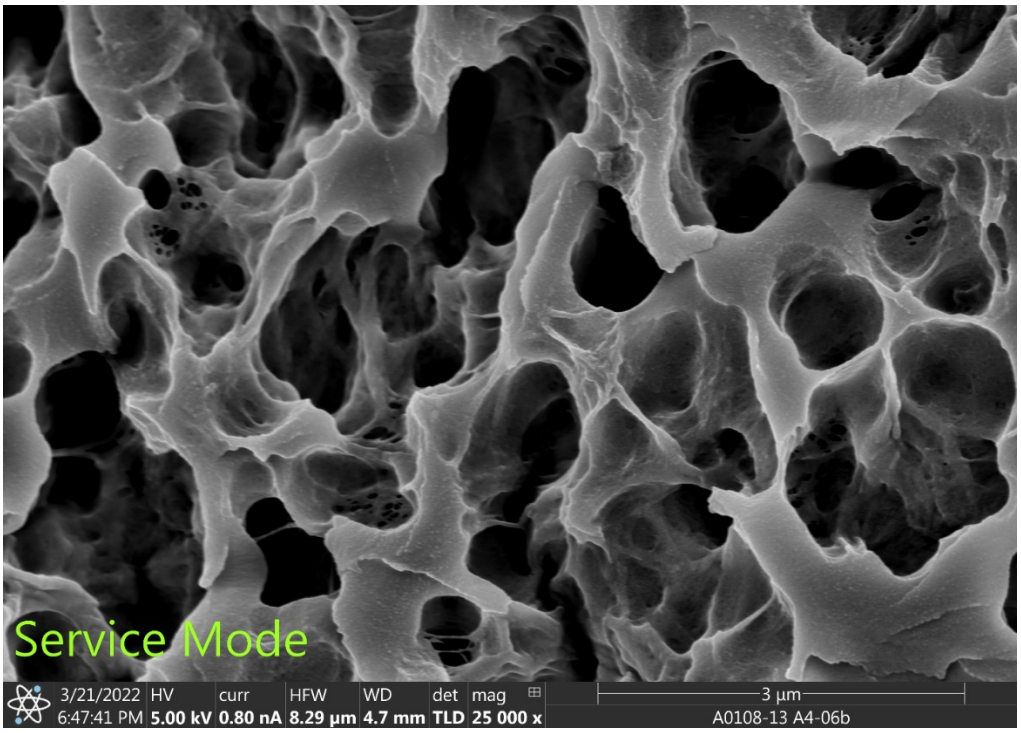


Figure S8: Scanning electron microscopy of BSA gel steam-cured at 90 °C

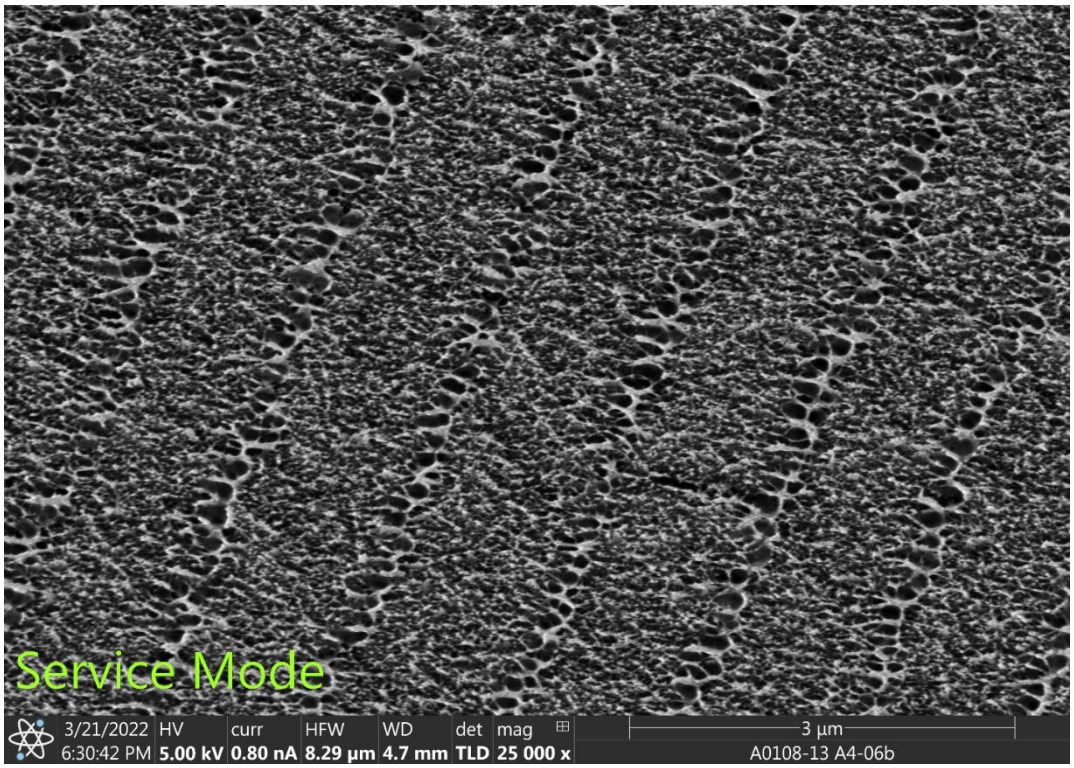


Figure S9: Scanning electron microscopy of α-La gel steam-cured at 60 °C

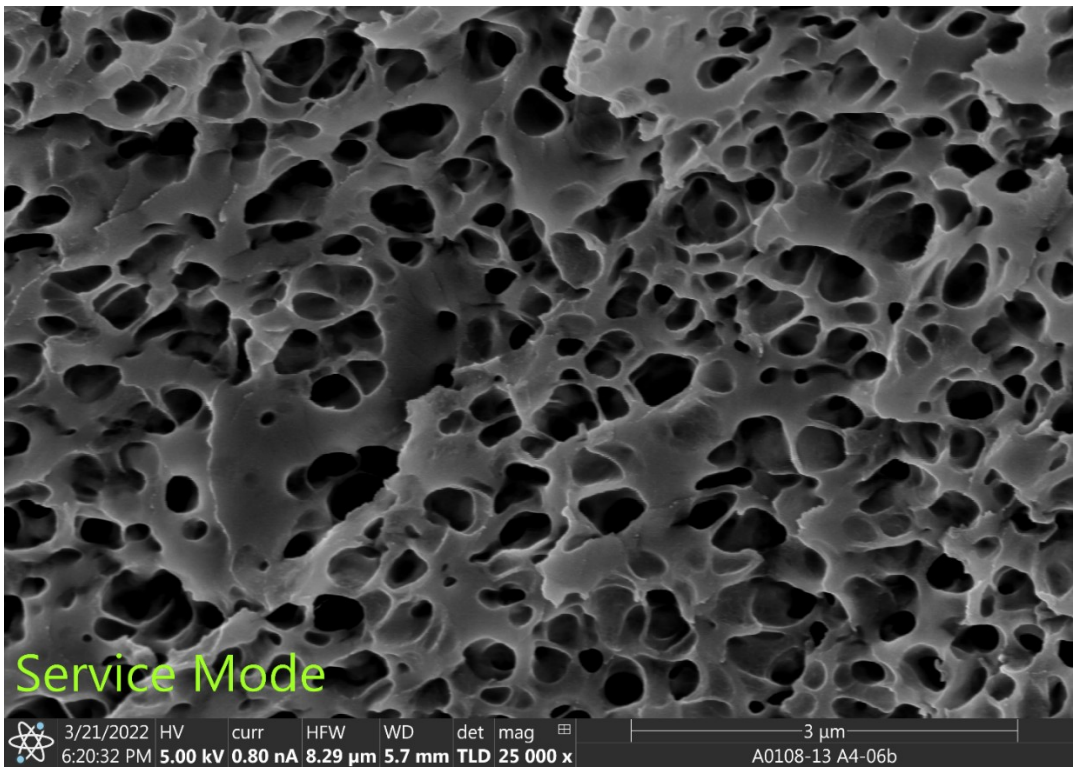


Figure S10: Scanning electron microscopy of α-La gel steam-cured at 90 °C

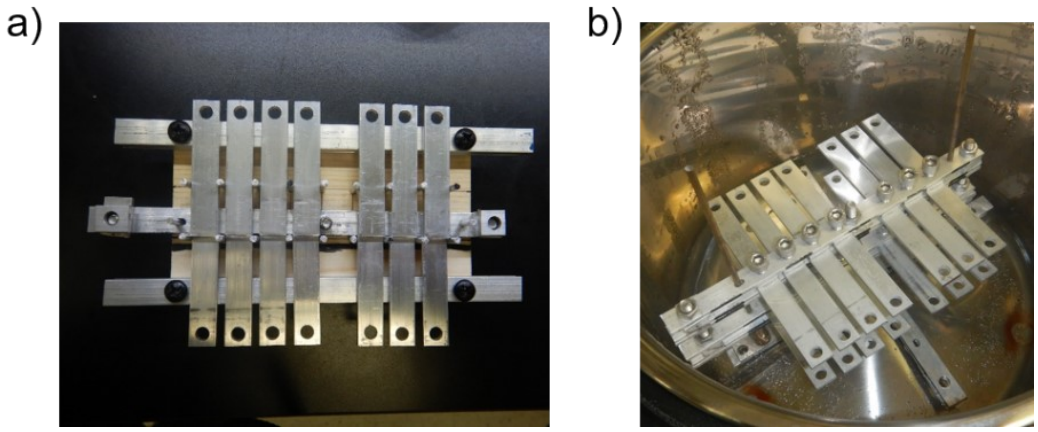


Figure S11: Pictures of lap shear sample fabrication. (a) Alignment of samples on a frame. (b) Samples held in place by screws and mounted on two brass rods.

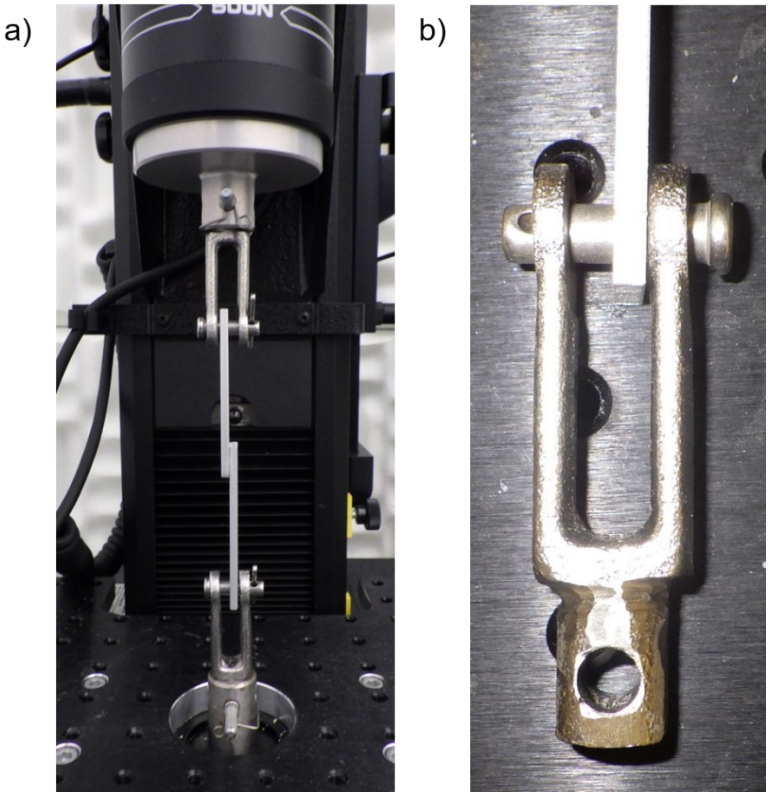


Figure S12: Lap shear experimental setup on clevis rod mounts.

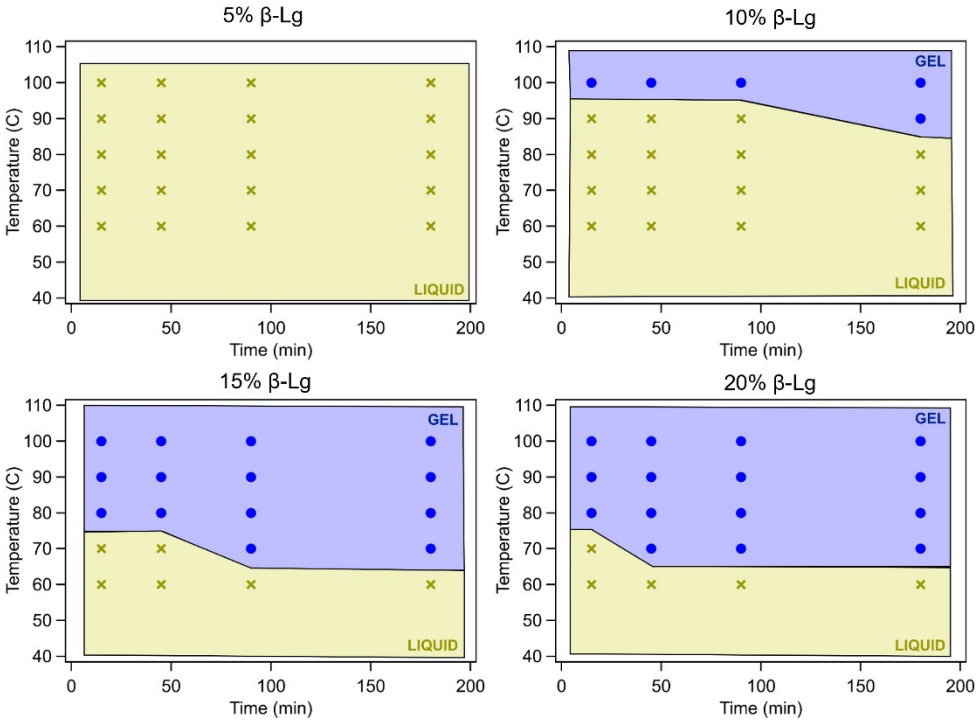


Figure S13: Processing diagram for various concentrations of β-Lg solutions heated in a heating block. Material state determined by inversion testing.

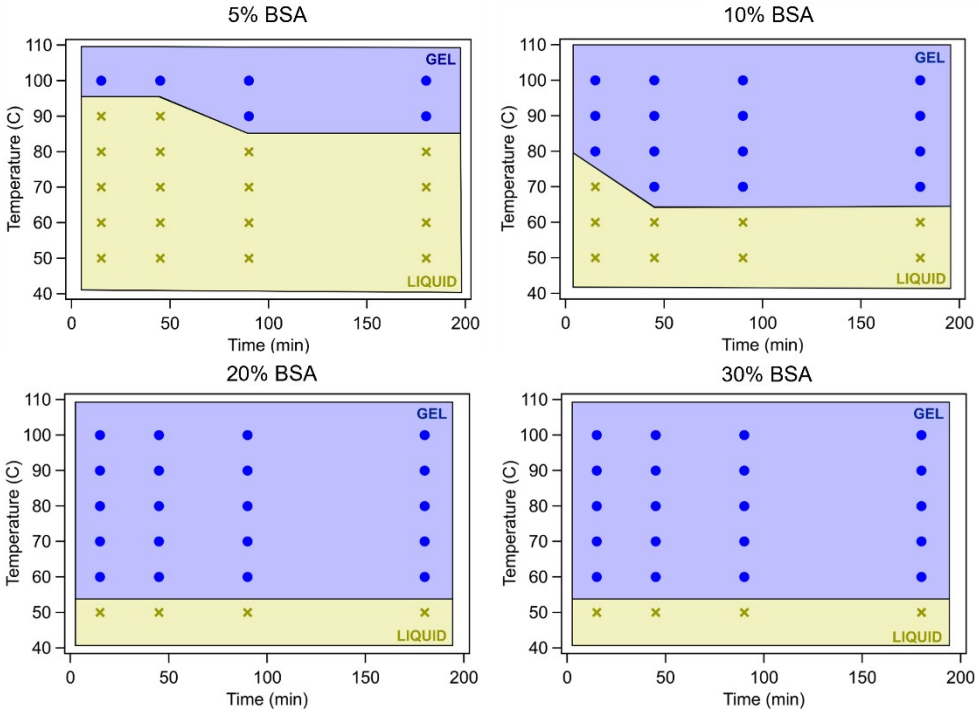


Figure S14: Processing diagram for various concentrations of BSA solutions heated in a heating block. Material state determined by inversion testing.

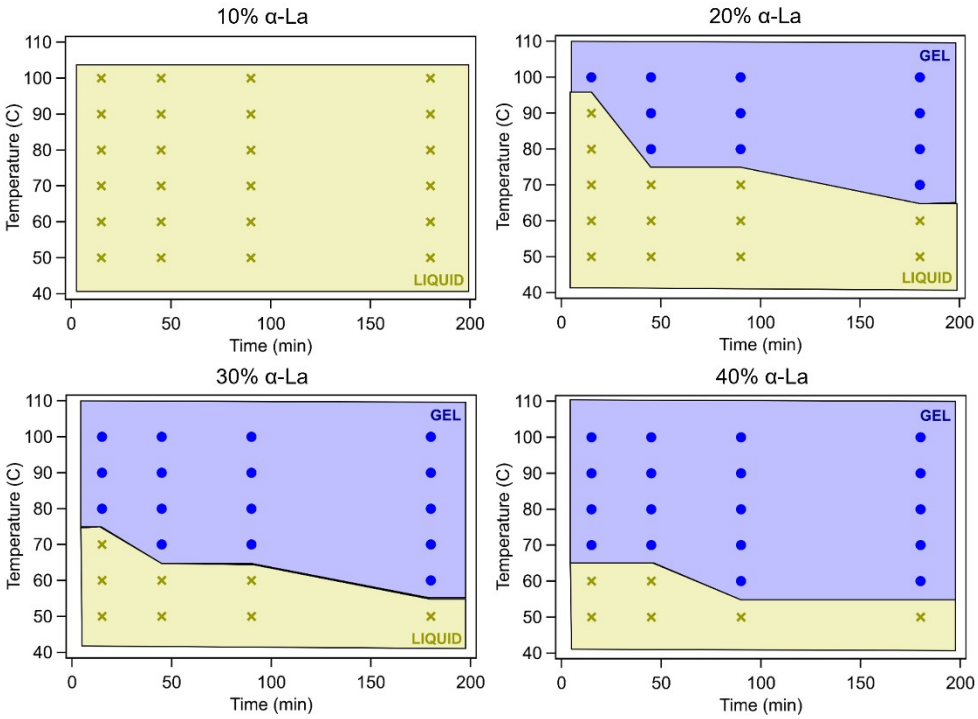


Figure S15: Processing diagram for various concentrations of α-La solutions heated in a heating block. Material state determined by inversion testing.

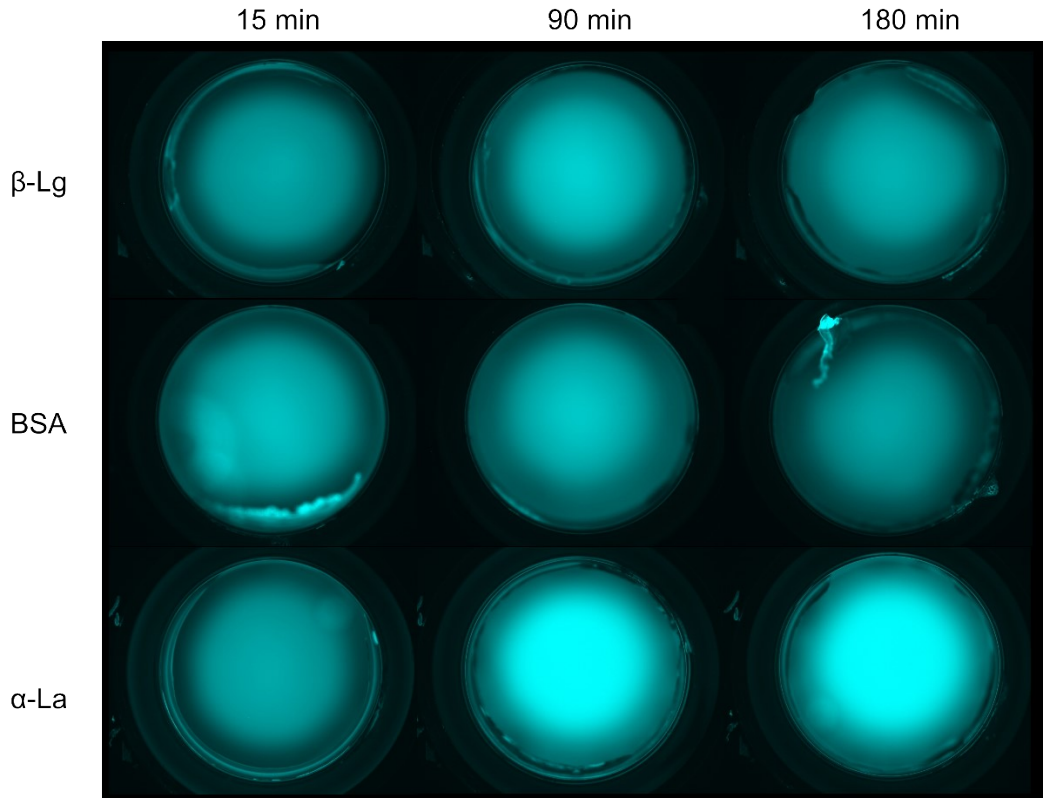


Figure S16: Microscope images of ThT fluorescence of protein solutions heated at 90 °C in a heating block for 15 min (left column), 90 min (center column) and 180 min (right column).

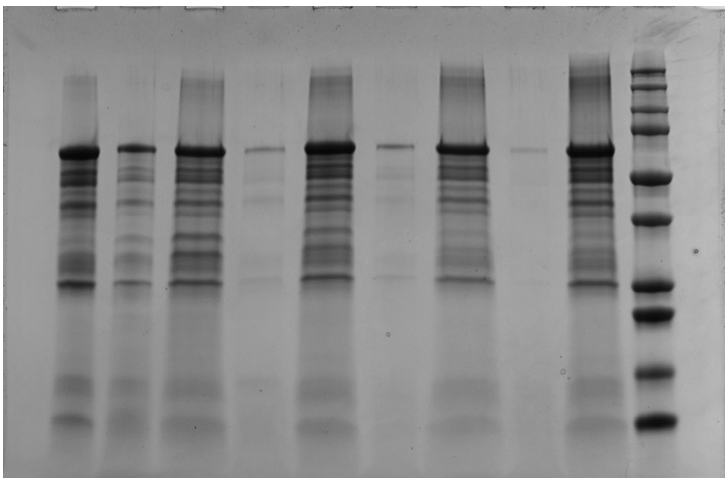


Figure S17: SDS-PAGE for 30% BSA cured at different temperatures. Lanes (from left to right) 60 °C Laemmli extracted, 60 °C water extracted, 70 °C Laemmli extracted, 70 °C water extracted, 80 °C Laemmli extracted, 80 °C water extracted, 90 °C Laemmli extracted, 90 °C water extracted, BSA internal standard, molecular weight markers.

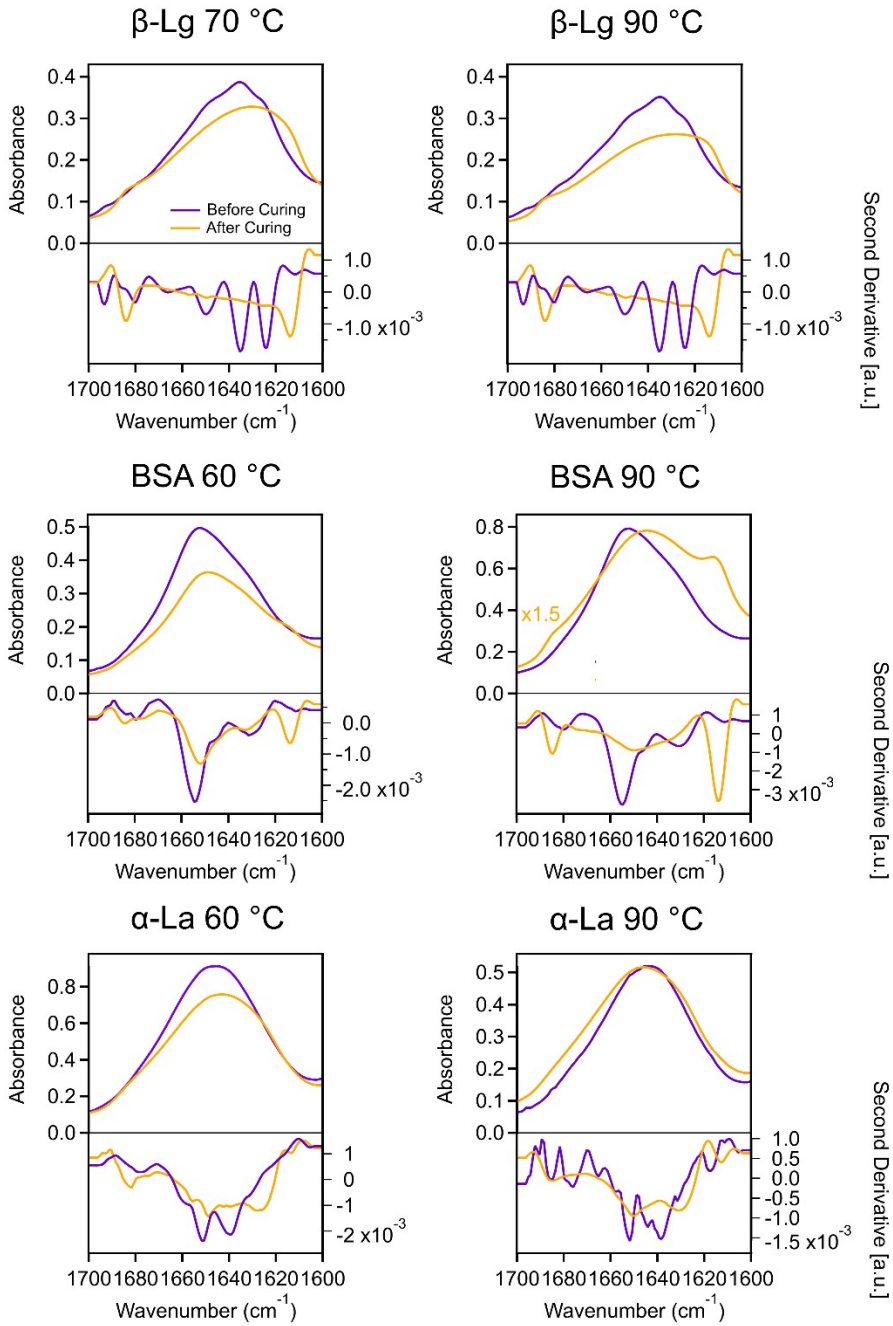


Figure S18: Example transmission FTIR and second derivative for each protein at high and low curing temperatures. All spectra were collected at room temperature.

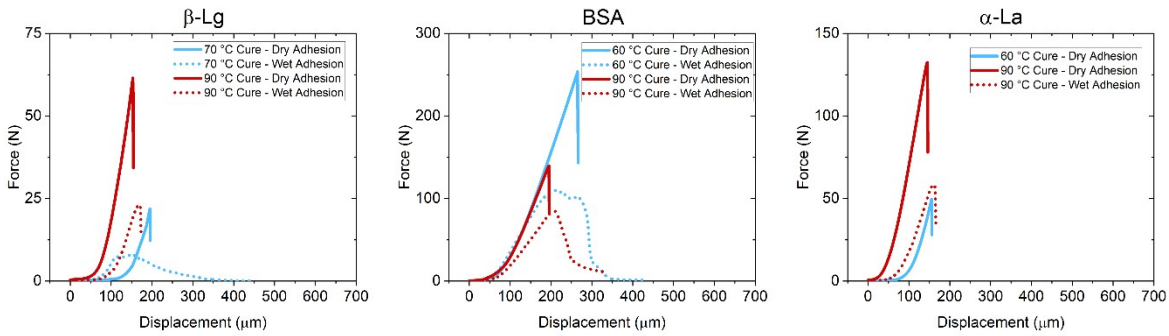


Figure S19: Example force-displacement curves for lap shear testing of each protein and curing condition. α-La cured at 60 °C did not provide measurable results in nearly all cases, so no example curve is given. Note: The y-axis scale differs for each protein.

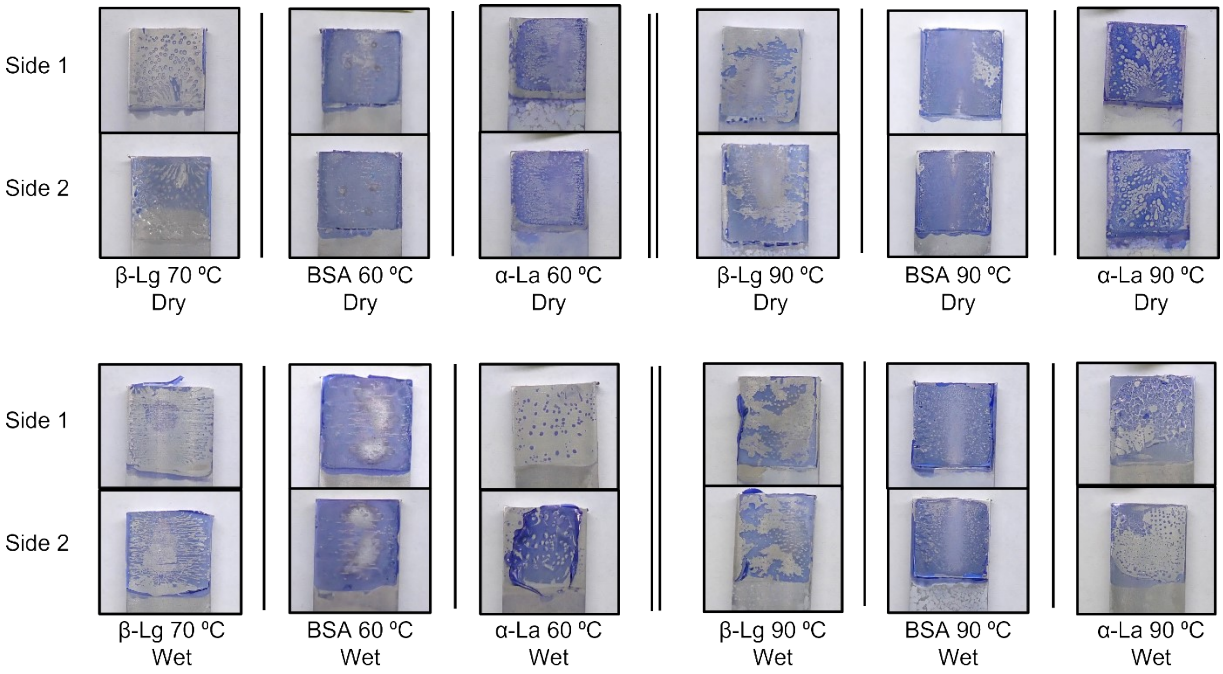


Figure S20: Coomassie-stained lap shear samples of short-term conditioning to depict failure mode.

Table S1: Lap shear sample size, average force, and average adhesive area.

Sample Set	Sample Size	Force (N)[a]	Area (mm^2)[a]
Short-Term			
β-Lg 70 °C dry	5	27 ± 5	169 ± 5
β-Lg 70 °C wet	7	15 ± 4	155 ± 4
β-Lg 90 °C dry	7	58 ± 11	150[c]
β-Lg 90 °C wet	7	20 ± 2	160 ± 2
BSA 60 °C dry	7	247 ± 30	155 ± 4
BSA 60 °C wet	7	121 ± 26	160 ± 4
BSA 90 °C dry	7	158 ± 18	147 ± 3
BSA 90 °C wet	7	80 ± 8	153 ± 5
α-La 60 °C dry	7	64 ± 13	142 ± 7
α-La 60 °C wet	7	3 ± 3	142 ± 3[b]
α-La 90 °C dry	7	201 ± 32	144 ± 4
α-La 90 °C wet	7	58 ± 6	144 ± 4
Accelerated Degradation			
β-Lg 70 °C wet	5[d]	12 ± 2	158 ± 2
β-Lg 90 °C wet	6[d]	10 ± 4	156 ± 1
BSA 60 °C wet	7	16 ± 2	140 ± 8
BSA 90 °C wet	7	77 ± 12	151 ± 2
α-La 90 °C wet	5	34 ± 7	138 ± 3

[a]Reported as mean ± std. err.
[b]Calculated from 6 measurements. One area from a sample with zero force was unmeasurable, so adhesion strength is unaffected.
[c]Calculated from the average of all samples (149.9 mm^2) .
[d]Not including outlier samples with >150 N force.

Table S2: Composition of heated protein solutions across compositions that form gels determined by gel electrophoresis.

Protein	Concentration (w/v)	Curing Temp (°C)	Water-soluble fraction (%)[a]	Laemmli-soluble fraction (%)[a,b]	Laemmli-insoluble fraction (%)[a]
β-Lg	10	70	54 ± 12	36 ± 9	10 ± 4
β-Lg	10	80	21 ± 13	53 ± 15	26 ± 4
β-Lg	10	90	23 ± 16	51 ± 8	25 ± 9
β-Lg	15	70	47 ± 8	44 ± 14	8 ± 6
β-Lg	15	80	26 ± 17	50 ± 15	24 ± 7
β-Lg	15	90	20 ± 14	40 ± 12	40 ± 13
β-Lg	20	70	27 ± 2	64 ± 5	9 ± 4
β-Lg	20	80	12 ± 6	48 ± 11	40 ± 10
β-Lg	20	90	11 ± 6	36 ± 5	52 ± 5
β-Lg	25	70	48 ± 8	36 ± 6	16 ± 3
β-Lg	25	80	14 ± 4	36 ± 2	50 ± 5
β-Lg	25	90	12 ± 6	39 ± 10	49 ± 10
BSA	10	60	59 ± 4	14 ± 7	26 ± 3
BSA	10	70	30 ± 4	39 ± 6	32 ± 2
BSA	10	80	25 ± 6	50 ± 6	25 ± 6
BSA	10	90	7 ± 2	37 ± 4	56 ± 6
BSA	20	60	49 ± 9	25 ± 11	26 ± 6
BSA	20	70	30 ± 12	44 ± 10	27 ± 6
BSA	20	80	16 ± 12	38 ± 10	46 ± 6
BSA	20	90	4 ± 1	31 ± 9	65 ± 9
BSA	30	60	45 ± 11	27 ± 12	29 ± 4
BSA	30	70	30 ± 14	36 ± 10	34 ± 5
BSA	30	80	13 ± 4	41 ± 8	46 ± 6
BSA	30	90	1 ± 0	26 ± 9	73 ± 9
BSA	40	60	35 ± 5	17 ± 6	47 ± 11
BSA	40	70	8 ± 5	26 ± 16	66 ± 14
BSA	40	80	5 ± 3	17 ± 8	77 ± 10
BSA	40	90	3 ± 2	16 ± 7	81 ± 7
α-La	15	60	67 ± 1	15 ± 2	18 ± 1
α-La	15	70	54 ± 7	36 ± 9	10 ± 3
α-La	15	80	41 ± 11	42 ± 5	17 ± 7
α-La	15	90	47 ± 7	37 ± 6	15 ± 5
α-La	25	60	59 ± 5	27 ± 5	14 ± 6
α-La	25	70	38 ± 11	21 ± 3	41 ± 9
α-La	25	80	32 ± 11	24 ± 2	44 ± 13
α-La	25	90	40 ± 8	29 ± 3	31 ± 5
α-La	40	60	51 ± 4	33 ± 4	16 ± 8
α-La	40	70	39 ± 8	26 ± 5	34 ± 4
α-La	40	80	31 ± 11	26 ± 7	43 ± 5

α-La	40	90	27 ± 3	33 ± 2	40 ± 3
α-La	50	60	39 ± 6	37 ± 6	25 ± 10
α-La	50	70	23 ± 9	39 ± 5	38 ± 12
α-La	50	80	22 ± 8	39 ± 5	39 ± 13
α-La	50	90	16 ± 5	34 ± 3	51 ± 7

[a]Reported as mean ± std. err.
[b]Water insoluble; Laemmli buffer consists of 2% SDS, 10% glycerol, 5% beta-mercaptoethanol, 0.5% bromophenyl blue

Table S3: Wavenumber and Area from peak fitting for β-sheet/extended structures from transmission FTIR.

Protein	Curing Temp (°C)	Average Wavenumber (cm^{-1})	Average Peak Area (%)
β-Lg	RT	1612 ± 0.1	0.7 ± 0.2
		1624 ± 0.4	23.5 ± 1.3
		1635 ± 0.2	30.1 ± 1.5
		1680 ± 0.4	8.5 ± 0.8
β-Lg	70	1613 ± 0.1	13.0 ± 0.1
		1620 ± 0.7	18.4 ± 1.4
		1632 ± 1.2	19.7 ± 0.5
		1677 ± 1.6	10.4 ± 0.2
β-Lg	90	1613 ± 0.0	15.9 ± 0.3
		1620 ± 0.0	15.5 ± 0.7
		1632 ± 0.4	18.3 ± 0.4
		1674 ± 0.7	10.2 ± 0.1
BSA	RT	1611 ± 0.1	0.2 ± 0.1
		1631 ± 0.3	21.7 ± 0.3
		1679 ± 0.4	6.3 ± 0.8
BSA	60	1614 ± 0.0	16.3 ± 0.8
		1632 ± 0.2	18.8 ± 1.5
		1678 ± 0.5	4.4 ± 0.2
BSA	90	1614 ± 0.2	27.3 ± 1.2
		1634 ± 0.3	19.5 ± 1.4
		1674 ± 2.3	8.5 ± 4.5
α-La	RT	1619 ± 1.1	5.7 ± 1.1
		1629 ± 0.7	12.2 ± 3.6
		1639 ± 0.4	27.2 ± 5.0
		1676 ± 0.4	8.3 ± 0.6
α-La	60	1613 ± 0.0	1.4 ± 0.5
		1626 ± 0.4	20.8 ± 3.6
		1637 ± 1.7	23.5 ± 0.9
		1675 ± 2.1	11.3 ± 1.0
α-La	90	1612 ± 0.4	3.1 ± 0.2
		1627 ± 0.7	12.5 ± 1.4
		1635 ± 0.2	25.7 ± 0.1
		N/A[a]	N/A

[a]Closest peak position at 1682 cm^{-1}, assigned to β-turn.