Supporting Information

Understanding the Formation of Apoferritin Amyloid Fibrils

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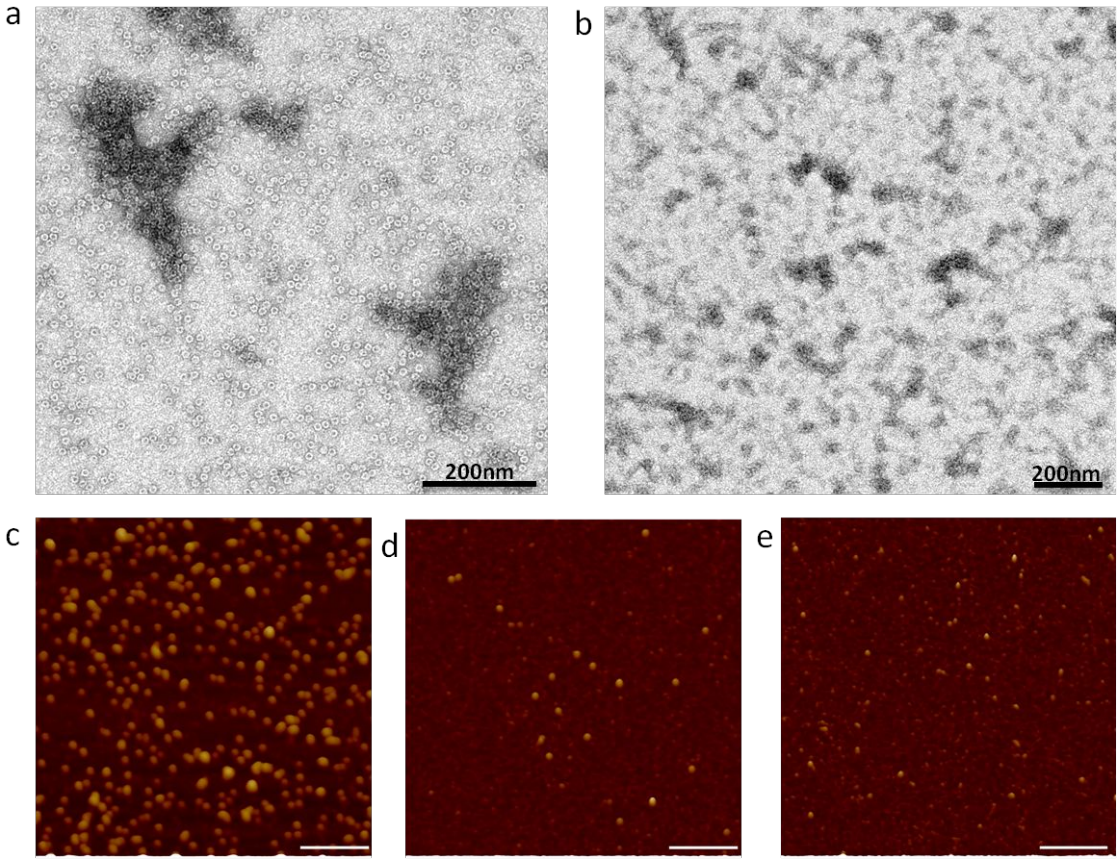


Figure S1. TEM images of (a) native APO at pH 7, and (b) at pH 2 after dilution. 3D AFM images of (c) native APO at pH 7, (d) at pH 2 after dilution, and (e) at pH 2 incubated for 120 h. Scale bars represent 200 nm.

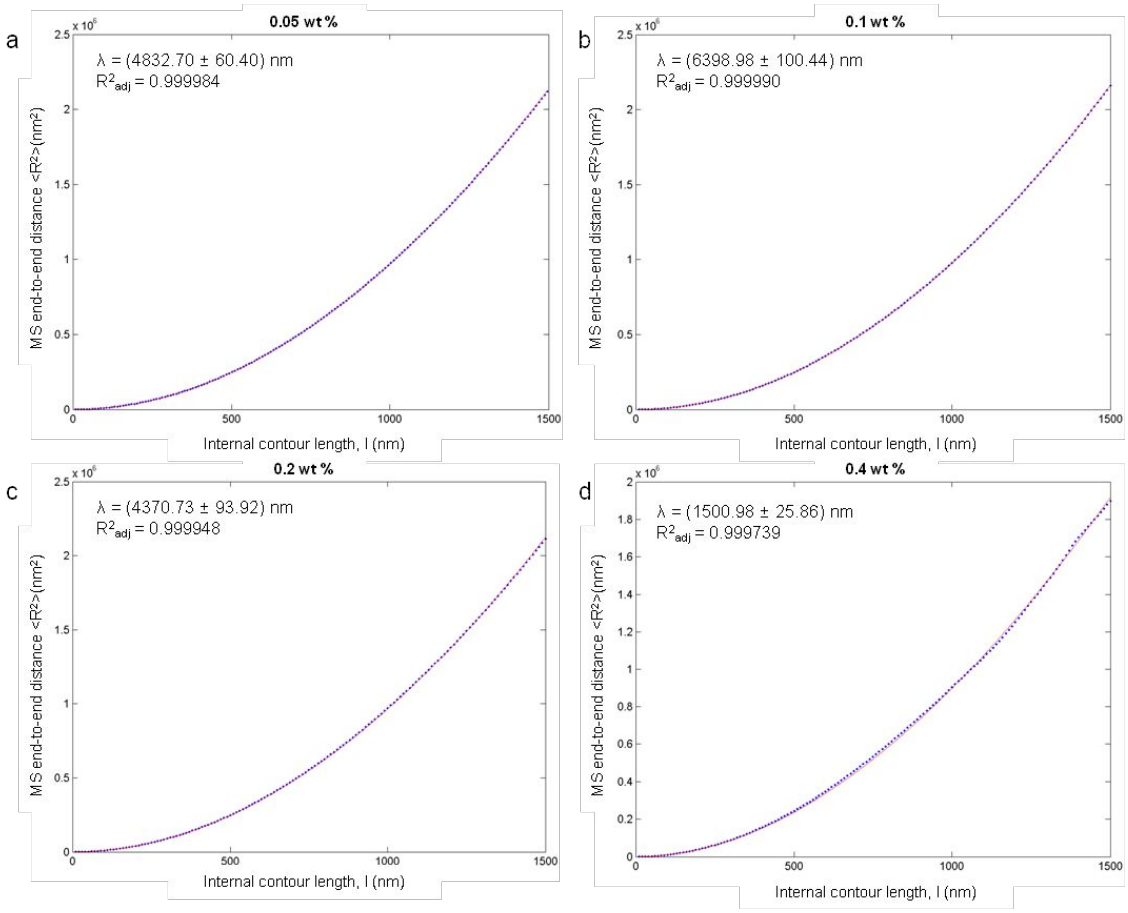


Figure S2. Calculated persistence length for (a) 0.05 wt.%, (b) 0.1 wt.%, (c) 0.2 wt.%, and (d) 0.4 wt.% APO after 9 h of incubation at 90 °C.

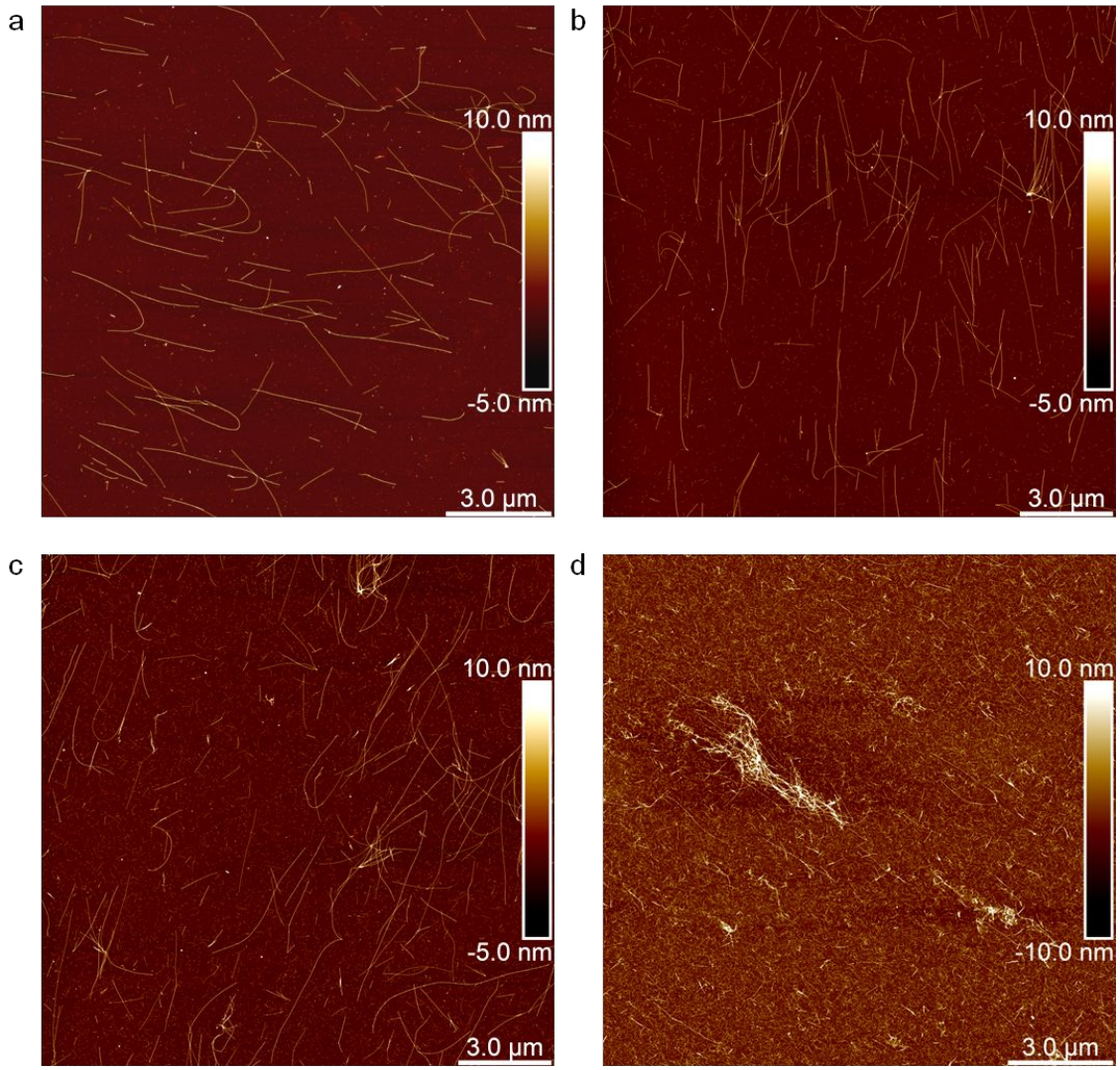


Figure S3. AFM images of (a) 0.05 wt %, (b) 0.1 % wt, (c) 0.2 wt %, and (d) 0.4 wt % APO heated at 90 °C after 9 h.

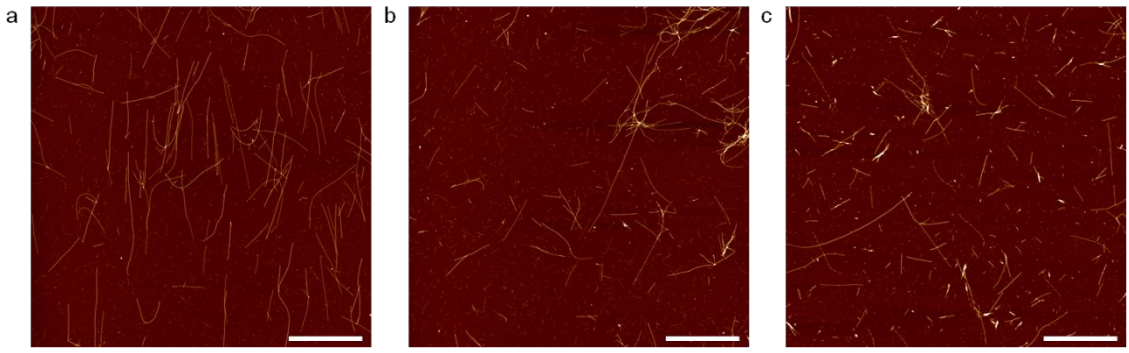


Figure S4. AFM images of APO heated at 90 °C (a) after 9 h at 90 rpm, (b) after 24 h at 90 rpm, and (c) after 9 h at 220 rpm. Scale bars represent 3 µm.

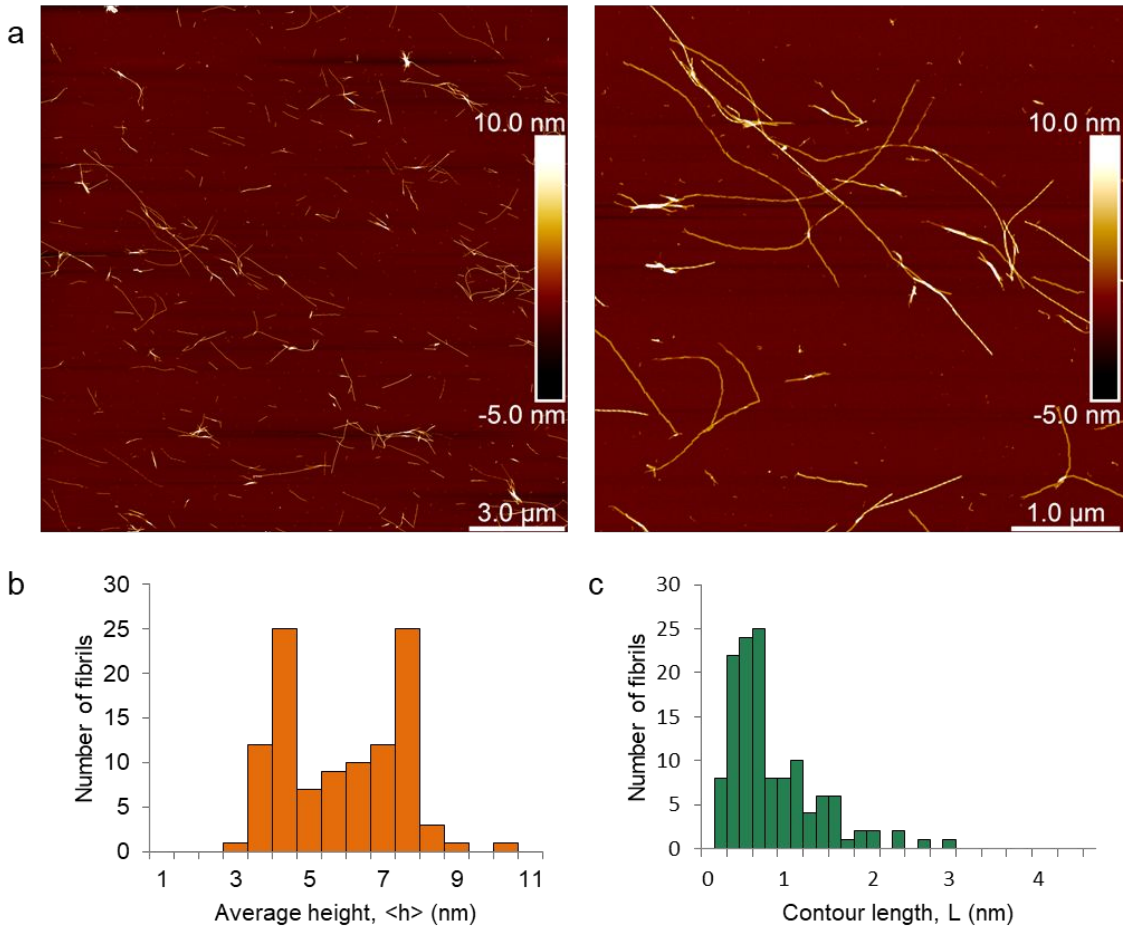


Figure S5. (a) AFM images, (b) average height distribution, and (c) contour length distribution of APO heated at 90 °C after 24 h at 220 rpm.

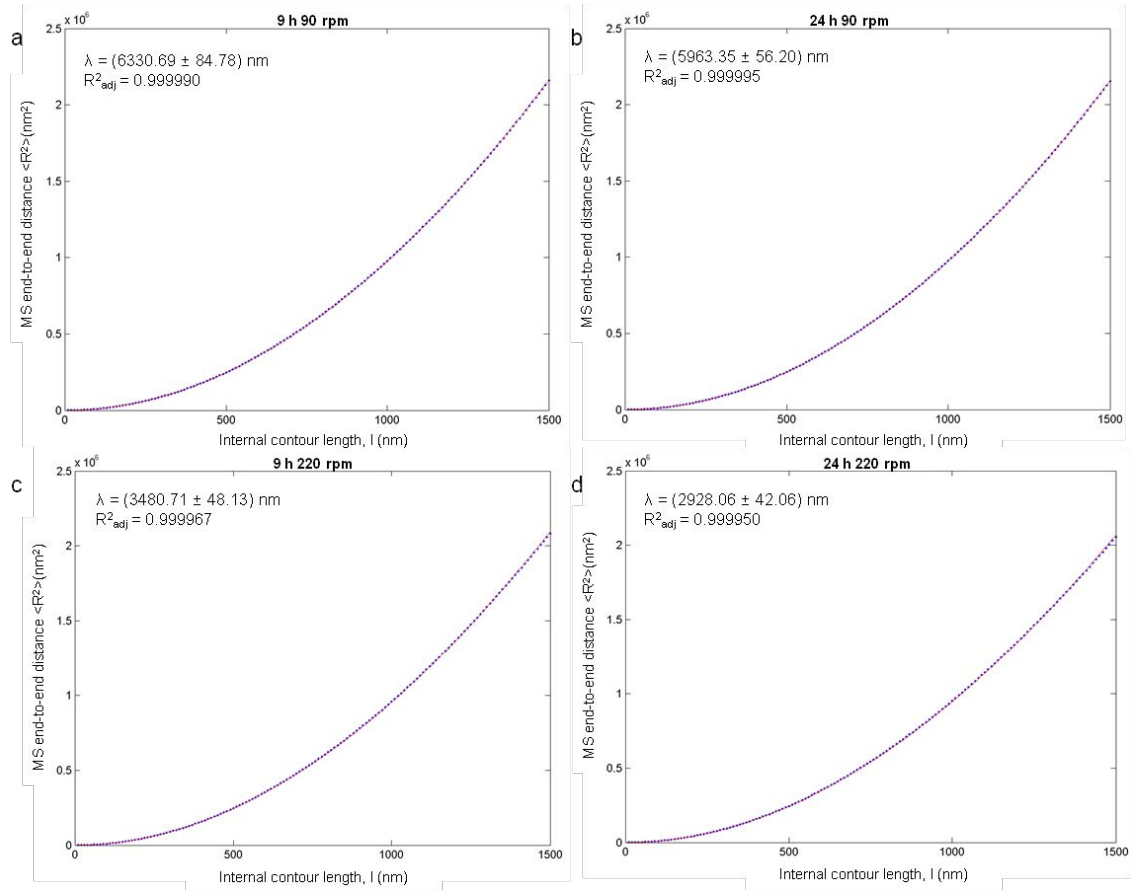


Figure S6. Calculated persistence length for APO heated at 90 °C: (a) after 9 h of incubation at 90 rpm, (b) after 24 h at 90 rpm, (c) after 9 h at 220 rpm, and (d) after 24 h at 220 rpm.

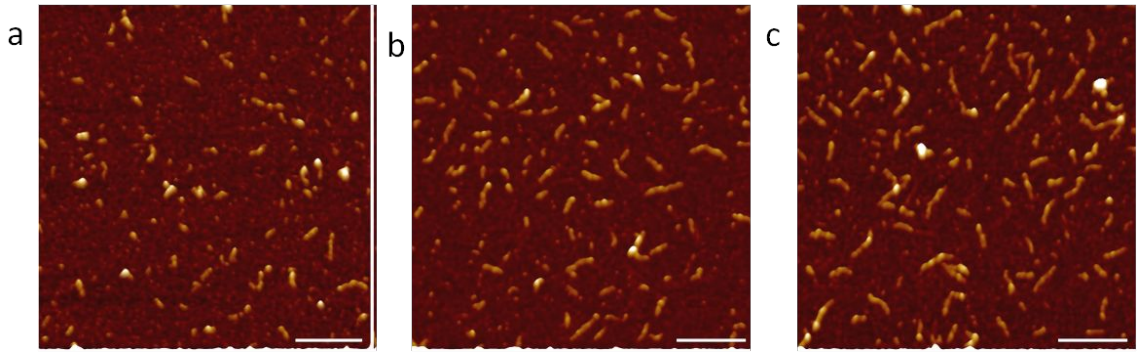


Figure S7. 3D AFM images of APO heated at 90 °C after (a) 5 min, (b) 45 min, and (c) 1 h. Scale bars represent 200 nm.

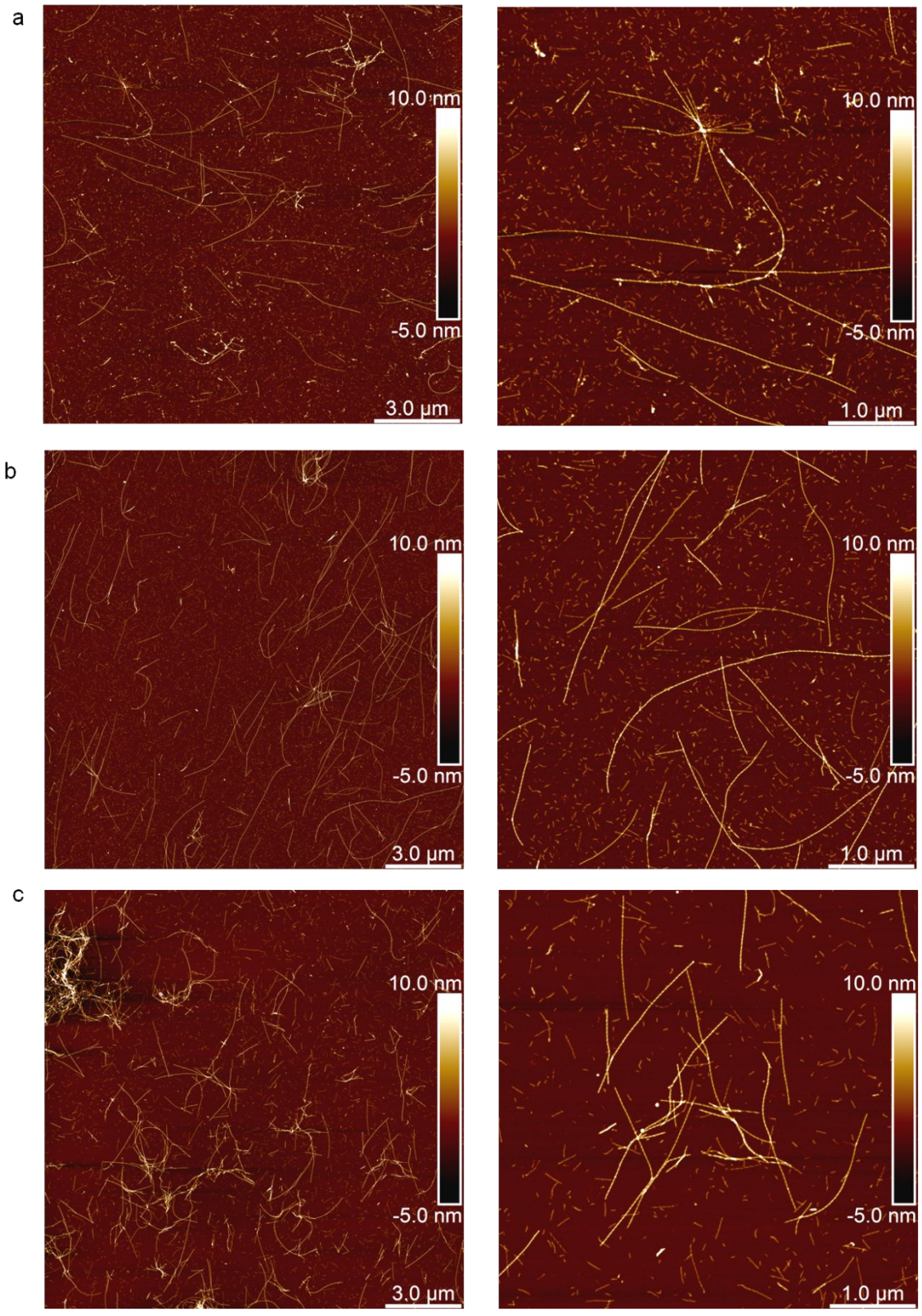


Figure S8. AFM images of APO heated at 90 °C after (a) 5 h, (b) 9 h, and (c) 24 h. It can be observed small and medium

aggregates coexisting together with long fibrils in the three cases.

	Small aggregates			Long fibrils			
Time	**N**	**Height**	**Length**	**N**	**Height**	**Length**	**Pitch**
3h	500	2,75 ± 0,88	72,05 ± 24,2	57	5,28 ± 1,29	1631,4 ± 1216,8	53,7
5 h	500	3,49 ± 0,88	68,45 ± 19,8	92	6,89 ± 1,34	1573,83 ± 1174,1	52,1
9 h	500	3,80 ± 0,98	49,48 ± 15,2	257	6,92 ± 1,24	1888,33 ± 1346,1	47,8
24 h	500	3,06 ±1,04	67,54 ± 28,9	628	6,57 ± 1,60	1013,23 ± 756,9	47,8

Table S1. Basic structural information about APO small aggregates and long fibrils at diferent incubation times. N=number of filaments tracked. All measurements are expressed in nanometers.

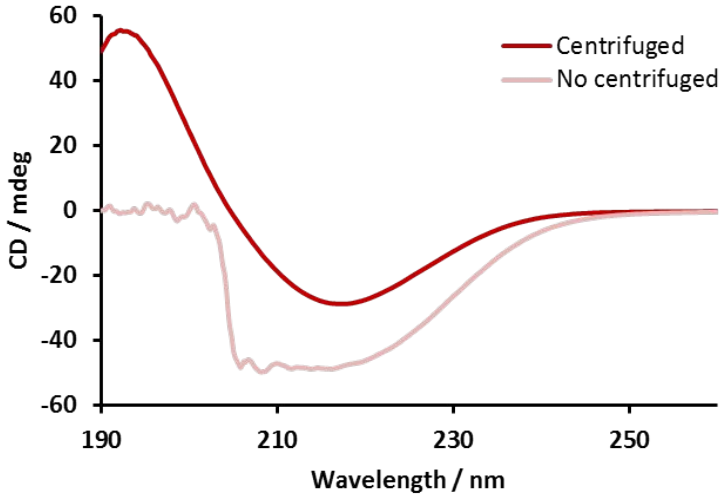
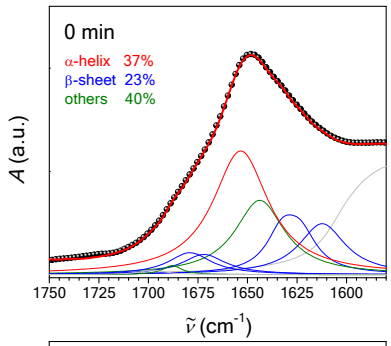
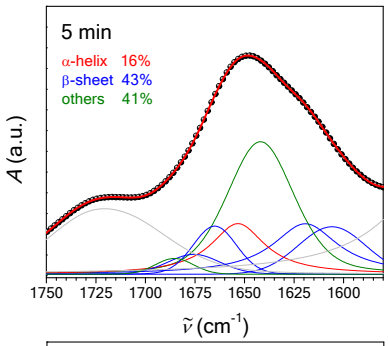


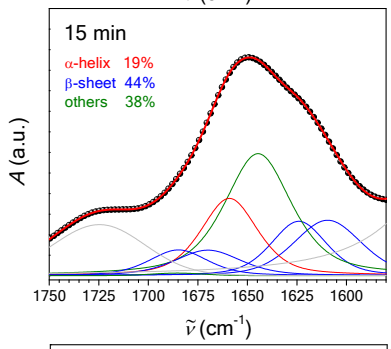
Figure S9. Far-UV circular dichroism of APO fibrils heated for 3 h, before and after being centrifuged.

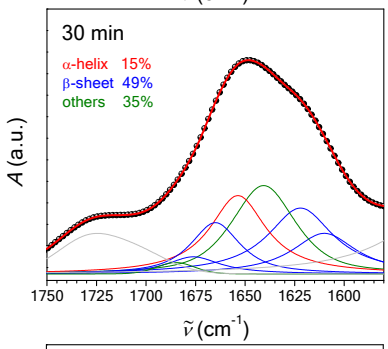
Time	α-helix	Strand	turns	unordered
0 min	35.1	18.6	18.4	27.9
5min	15.4	31.9	21.4	31.2
15min	8.5	37.7	21.1	32.7
30min	7.8	40.0	20.8	31.4
45min	6.0	42.6	21.4	30.0
1h	6.0	42.2	21.4	32.1
3h	5.7	39.3	22.4	32.6
5h	4.6	40.6	22.2	32.6
9h	3.7	41.9	21.8	32.4
24h	3.6	41.7	21.4	33.3

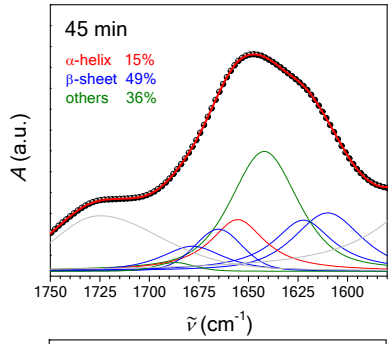
Table S2. Estimation of protein secondary structure of APO after different incubation times at pH 2 and 90 °C from the CD analysis. The content is expressed in %.

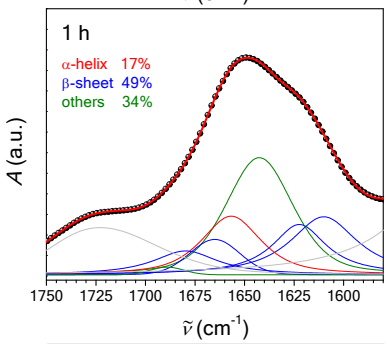

0 min
α-helix 37%
β-sheet 23%
others 40%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)

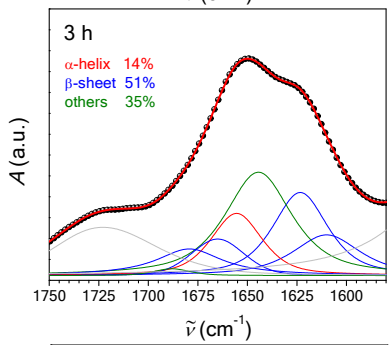

5 min
α-helix 16%
β-sheet 43%
others 41%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)

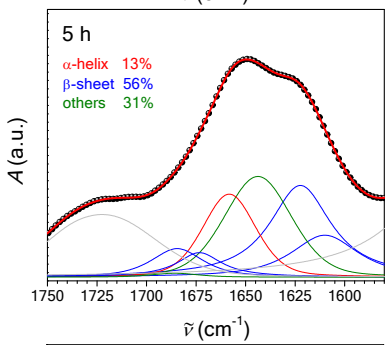

15 min
α-helix 19%
β-sheet 44%
others 38%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)

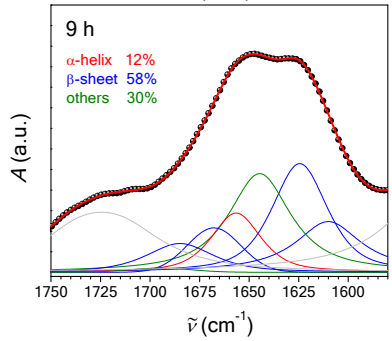

30 min
α-helix 15%
β-sheet 49%
others 35%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)


45 min
α-helix 15%
β-sheet 49%
others 36%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)


1 h
α-helix 17%
β-sheet 49%
others 34%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)


3 h
α-helix 14%
β-sheet 51%
others 35%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)


5 h
α-helix 13%
β-sheet 56%
others 31%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)


9 h
α-helix 12%
β-sheet 58%
others 30%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)

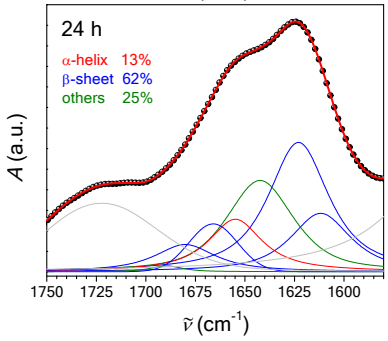

24 h
α-helix 13%
β-sheet 62%
others 25%
A (a.u.)
1750 1725 1700 1675 1650 1625 1600
ν̃ (cm-1)

Figure S10. FTIR spectra with the corresponding deconvolution analysis for the APO heated at 90 °C and pH 2 at different incubation times.

time	α-helix (%)	β-sheet (%)	random (%)
0	37.1	23.4	39.5
5 min	15.6	43.1	41.3
15 min	18.5	43.9	37.5
30 min	15.4	49.3	35.3
45 min	14.6	48.9	36.5
1 h	16.5	49.1	34.4
3 h	14.2	51.3	34.5
5 h	13.0	56.2	30.8
9 h	12.4	58.0	29.6
24 h	12.6	62.4	24.9

Table S3. Estimation of the protein secondary structure of APO after different incubation times at 90 °C and pH 2 from the FTIR analysis.