Supporting Information

Structural basis for the formation of soy protein nanofibrils

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Table S1. List of the chosen parent ions for MS/MS analysis from the spectra shown in Fig. S2 for HCCA and DBH matrices, respectively.

HCCA

m/z	Intensity	S/N	Resolution	Area
1777.073	23248.041	94.617	11440.506	11550.949
1891.114	22101.473	92.449	11896.028	11819.713
1963.138	17720.256	77.956	12030.325	9769.858
2005.156	13914.157	62.629	12590.619	7401.657
2077.185	16062.022	77.002	11930.455	9512.688
2122.062	13330.304	66.012	12356.862	7788.914
2167.293	5568.540	26.729	13667.438	2832.156
2191.222	9387.085	48.640	13219.050	5172.552
2237.087	4197.435	21.110	11758.122	2594.563

DHB

m/z	Intensity	S/N	Resolution	Area
1896.842	29951.482	38.246	9149.521	19804.315
2010.878	24719.168	33.628	9884.904	15817.651
2121.916	119140.219	191.075	9544.002	91663.185
2236.932	20557.604	32.878	9610.288	15465.109
2257.027	20615.116	33.528	8837.897	16766.186
2372.041	20919.756	37.221	9603.280	17045.658
2377.993	17539.128	30.688	10047.793	13658.426
2970.714	8730.050	23.613	9454.797	9222.154

Table S2. Secondary structure content of the peptide samples derived from the CD spectra (see Fig. S9) using the BeStSel algorithm.[1]

Peptide	Time (days)	Helix	Antiparallel	Parallel	Turn	Others	RMSD	NRMSD
GG1	0	11.4	20.4	3.0	15.8	49.4	0.0796	0.02223
	1	4.6	33.4	0.0	15.0	47.1	0.0624	0.02858
	2	12.0	16.8	5.4	15.4	50.4	0.0805	0.02764
GG2	0	46.6	4.1	49.3	0.0	0.0	1.9098	0.05137
	1	0.0	91.7	0.0	8.3	0.0	1.6439	0.06553
	2	0.0	89.5	0.0	10.5	0.0	1.4763	0.06768
BA1	0	27.5	21.6	7.3	3.7	40.0	0.3711	0.05451
	1	31.8	16.1	12.1	3.4	36.6	0.3564	0.05296
	2	24.7	26.0	10.6	0.0	38.6	1.409	0.14956
BB1	0	11.2	30.1	2.8	4.7	51.2	0.6059	0.06408
	1	10.2	32.0	0.0	5.2	52.6	0.5851	0.06029
	2	10.4	31.8	0.4	5.4	52.0	0.5998	0.06125
BB2	0	2.9	30.4	0.0	17.4	49.3	0.0710	0.01986
	1	23.6	18.7	2.0	12.3	43.4	0.1049	0.01953
	2	28.7	21.5	5.7	0.0	44.0	0.9829	0.92820

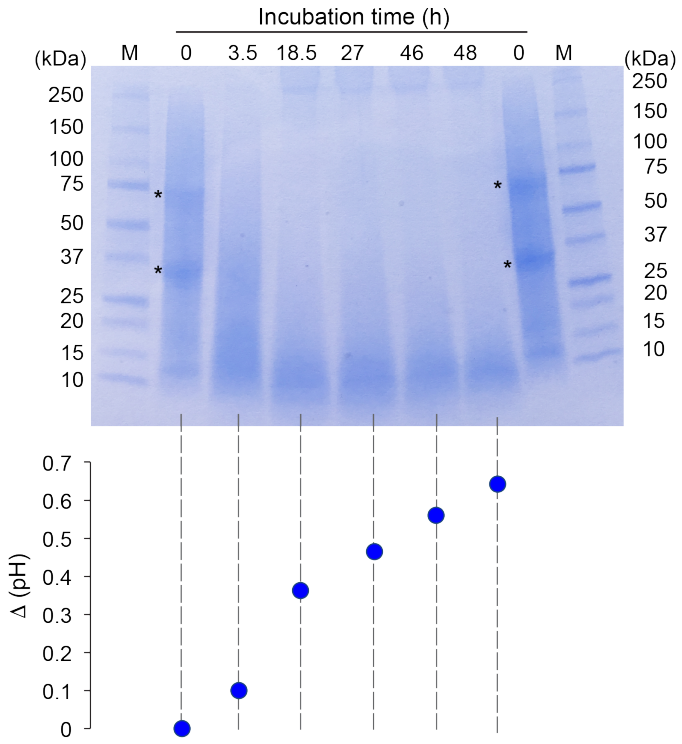

Figure S1. Hydrolysis of soy proteins during fibrillation. (Top) SDS-PAGE shows that the proteins in SPI are hydrolyzed upon incubation at 90 °C. In the starting sample (0 h) the glycinin and β-conglycinin subunits are visible at *ca* 35 kDa and *ca* 70 kDa, respectively (indicated by *). Notably, at longer incubation times, the appearance of high molecular weight bands (> 250 kDa) indicates the formation of supramolecular protein aggregates. (Bottom) Progressive changes in the pH value of the SPI solution provide further support for the hydrolysis process.

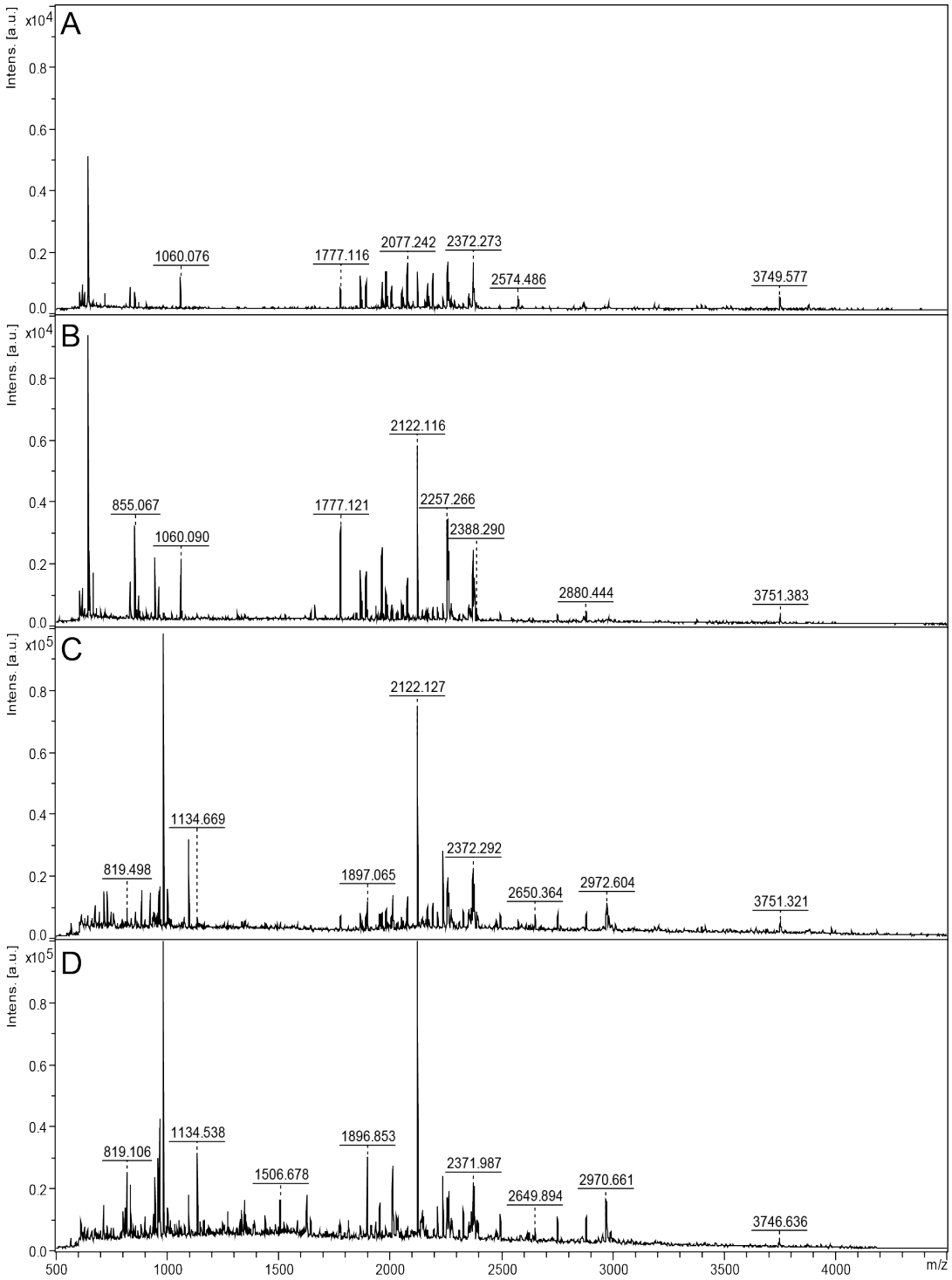

Figure S2. Mass spectrum of disaggregated soy PNF samples with different incubation (fibrillation) times and/or different matrices. (A) 3 days with HCCA matrix. (B) 5 days with HCCA matrix. (C) 3 days with DHB matrix. (D) 5 days with DHB matrix. The spectra illustrate the differences between having the sample incubated for 3 days compared to 5 days. The selected parent ions for further MS/MS analysis are listed in Table S1.

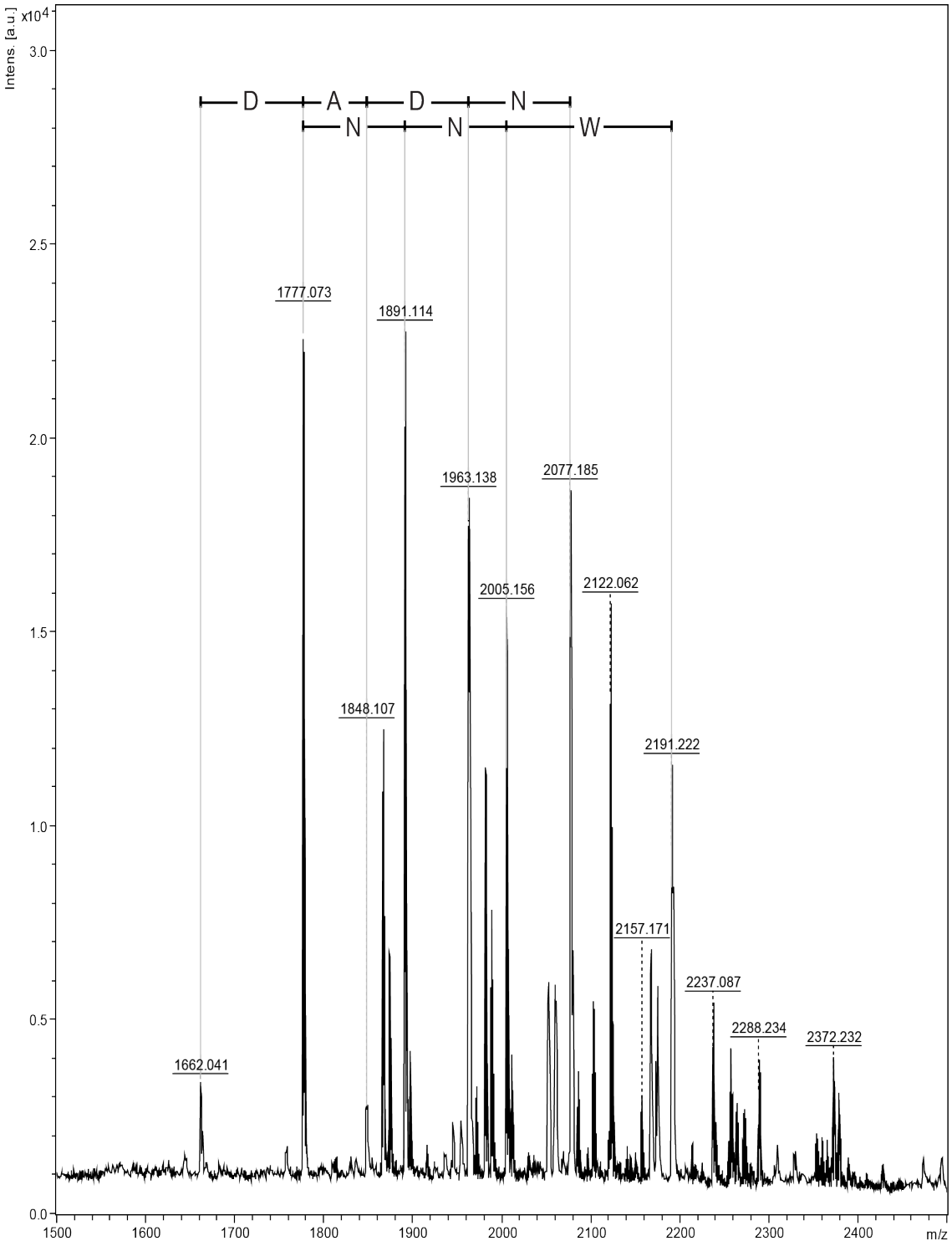

Figure S3. Mass spectrum of disaggregated soy PNFs (3 days incubation) with HCCA matrix. The potential amino acid sequences DADN and NNW are indicated.

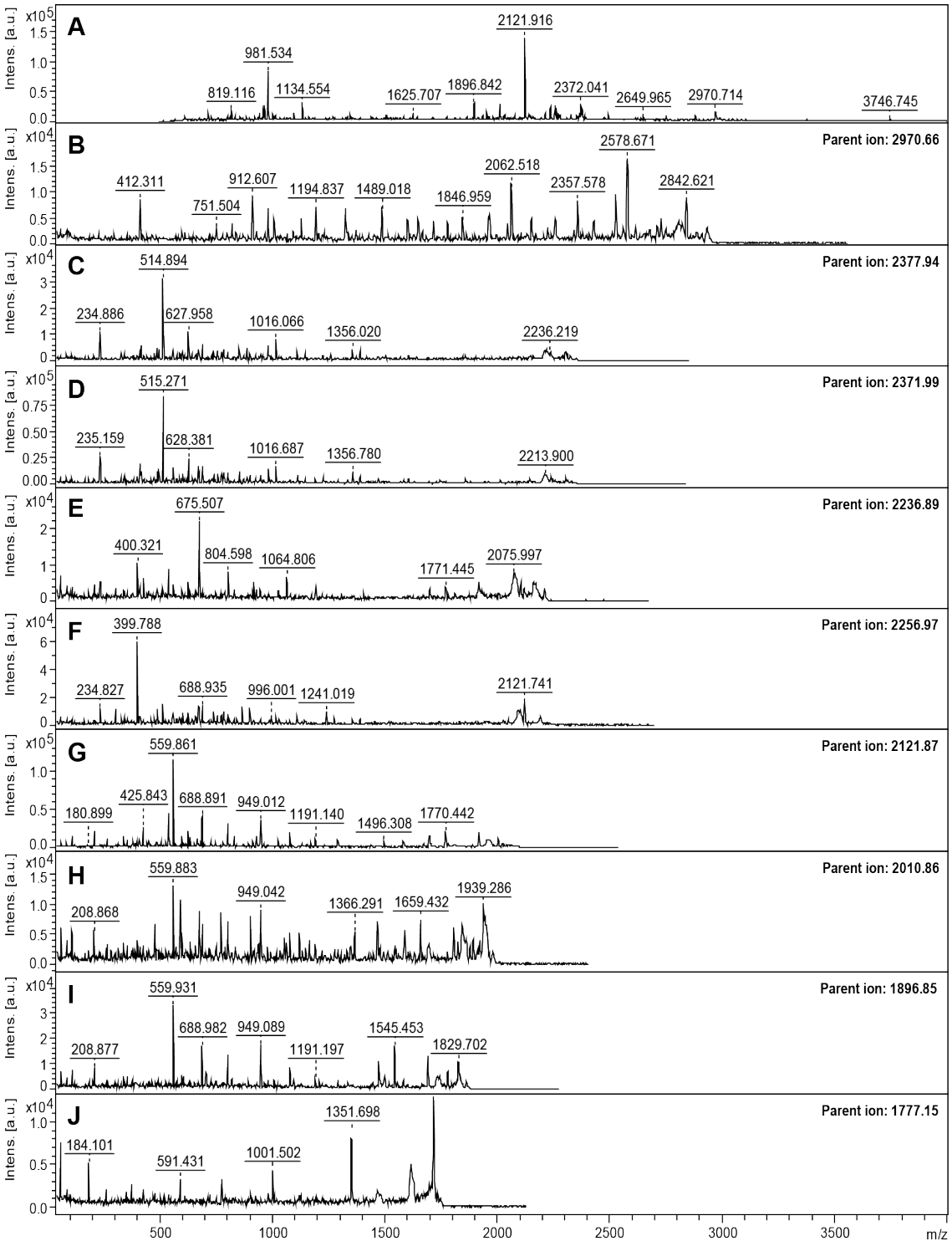

Figure S4. Mass spectrum of disaggregated soy PNFs. (A) 5 days incubation and DHB matrix. (B-J) MS/MS analysis of the indicated parent ions.

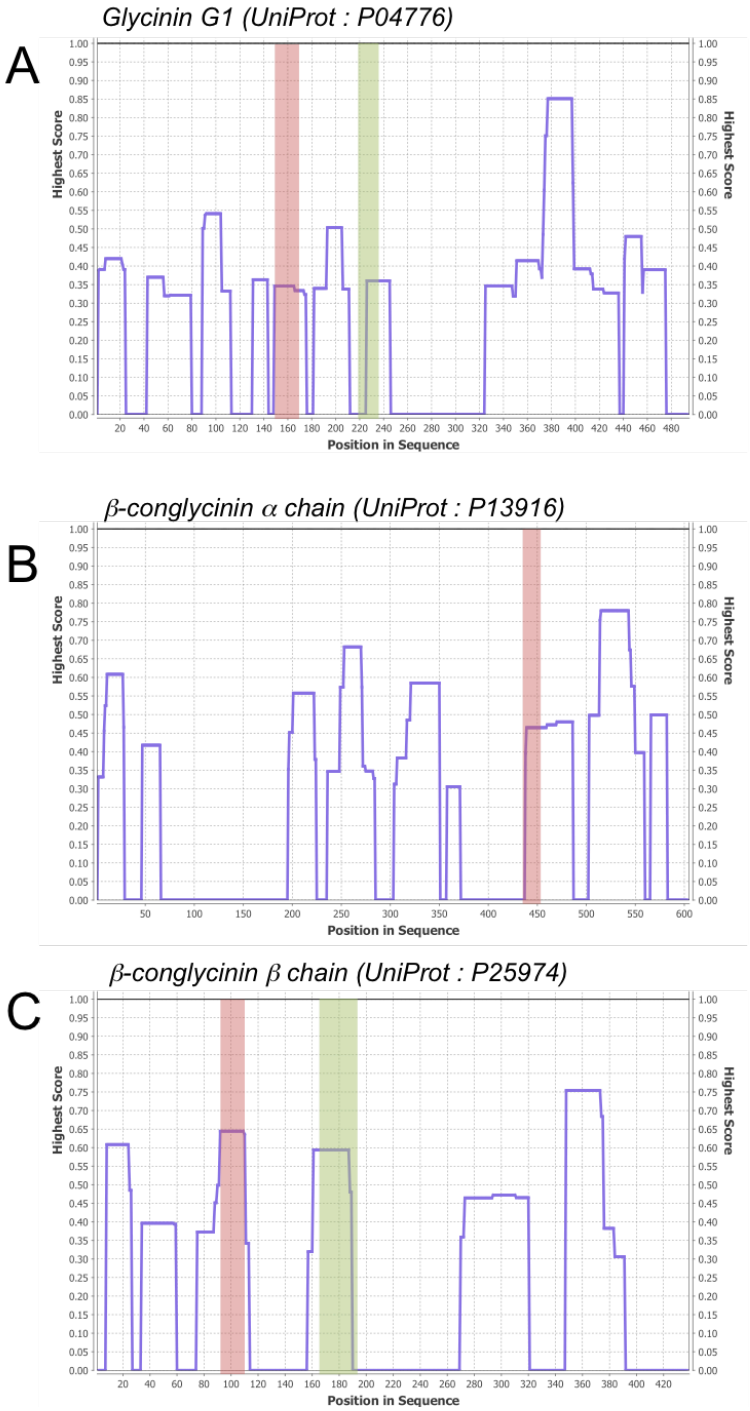

Figure S5. Predictions of "β-arch" propensities in glycinin (A) and the α- and β-chains of β-conglycinin (B and C, respectively) using the software ArchCandy.[2] The regions indicated with red are the PNF-forming segments with native β-structure while the PNF-forming peptides with native helical structure are indicated in green.

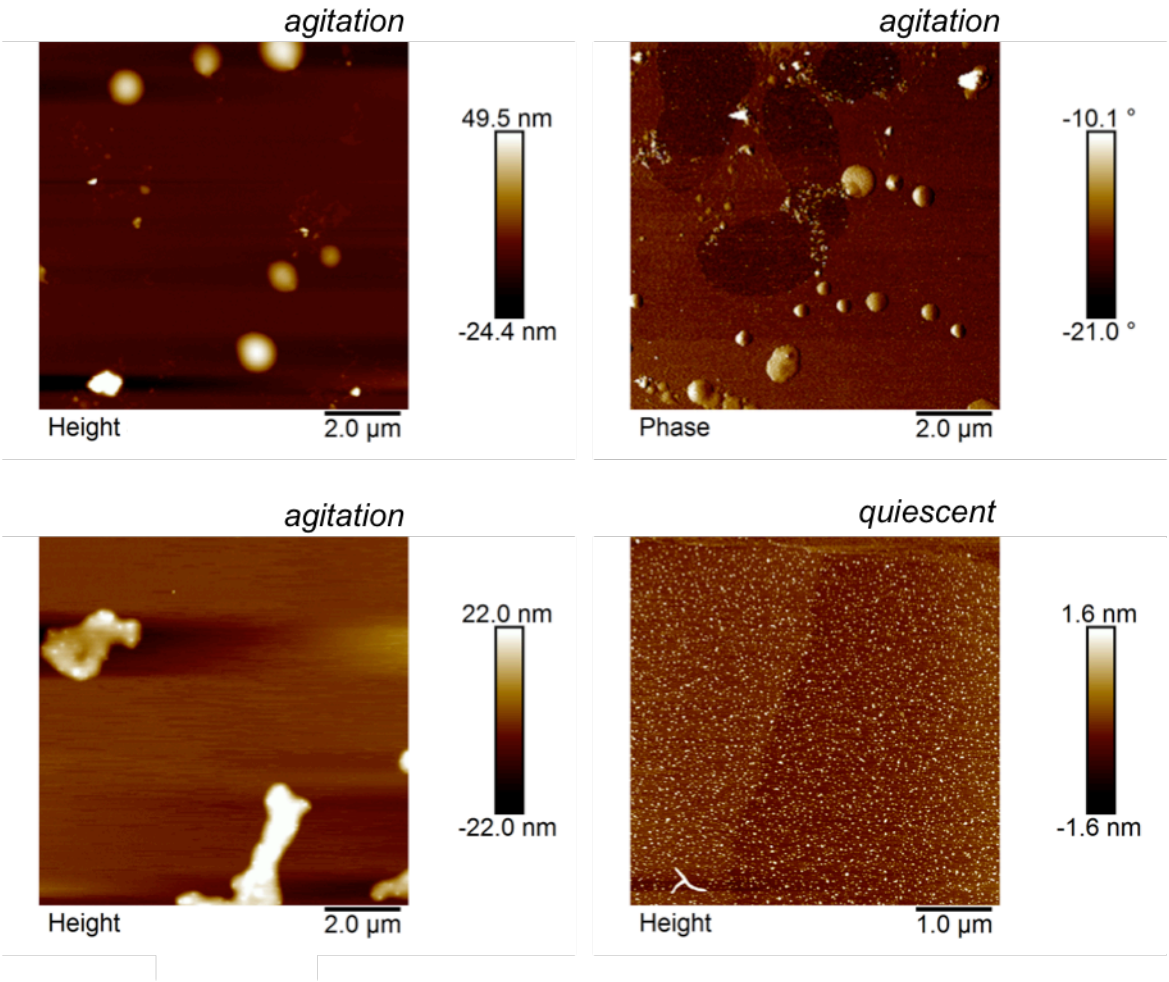

Figure S6. AFM images of BB1 peptide samples incubated at 50 °C with or without agitation. The samples mainly contain particulates and large aggregates. Only one fibril-like structure was observed (bottom, right image).

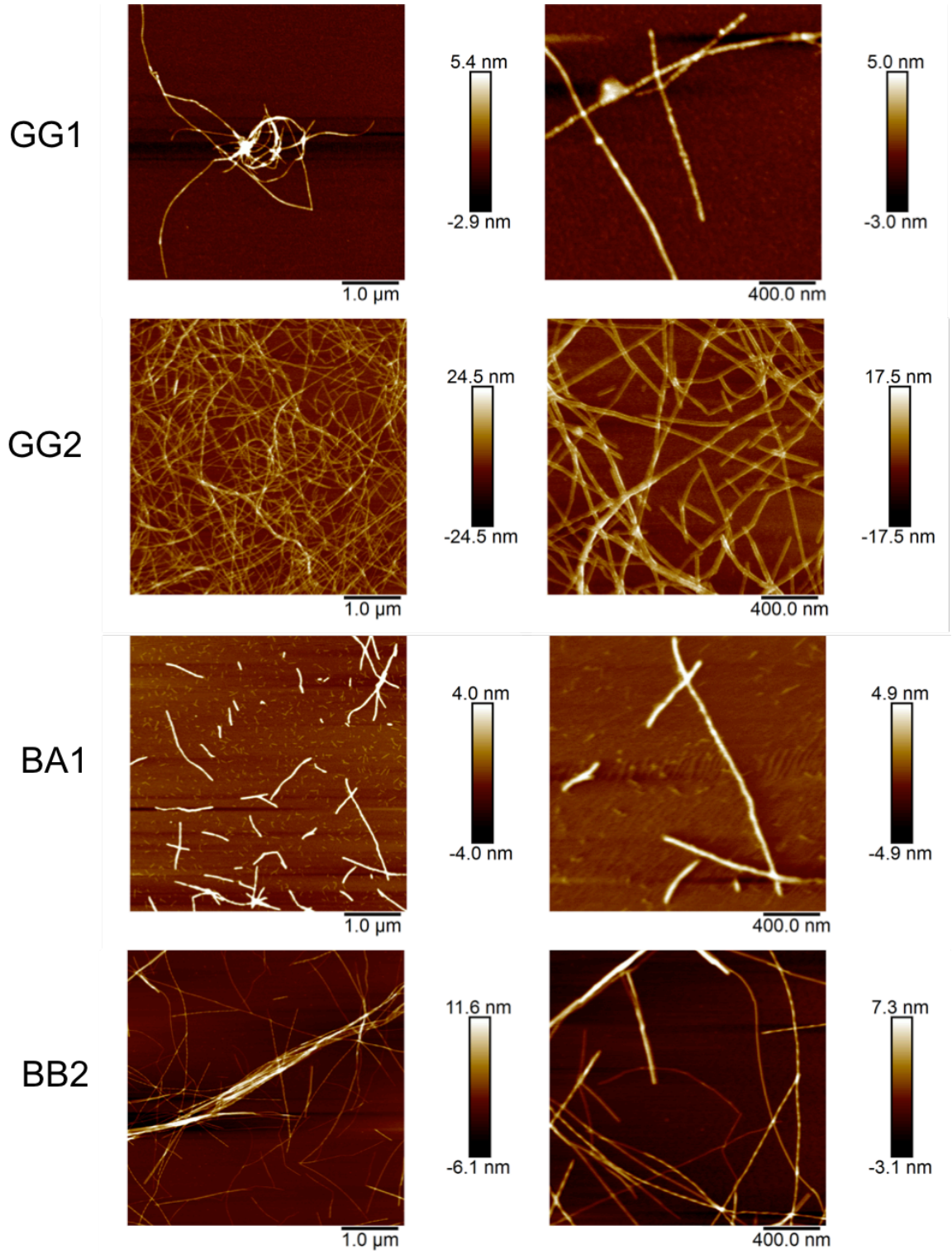

Figure S7. AFM images of peptide samples incubated at 50 °C with agitation.

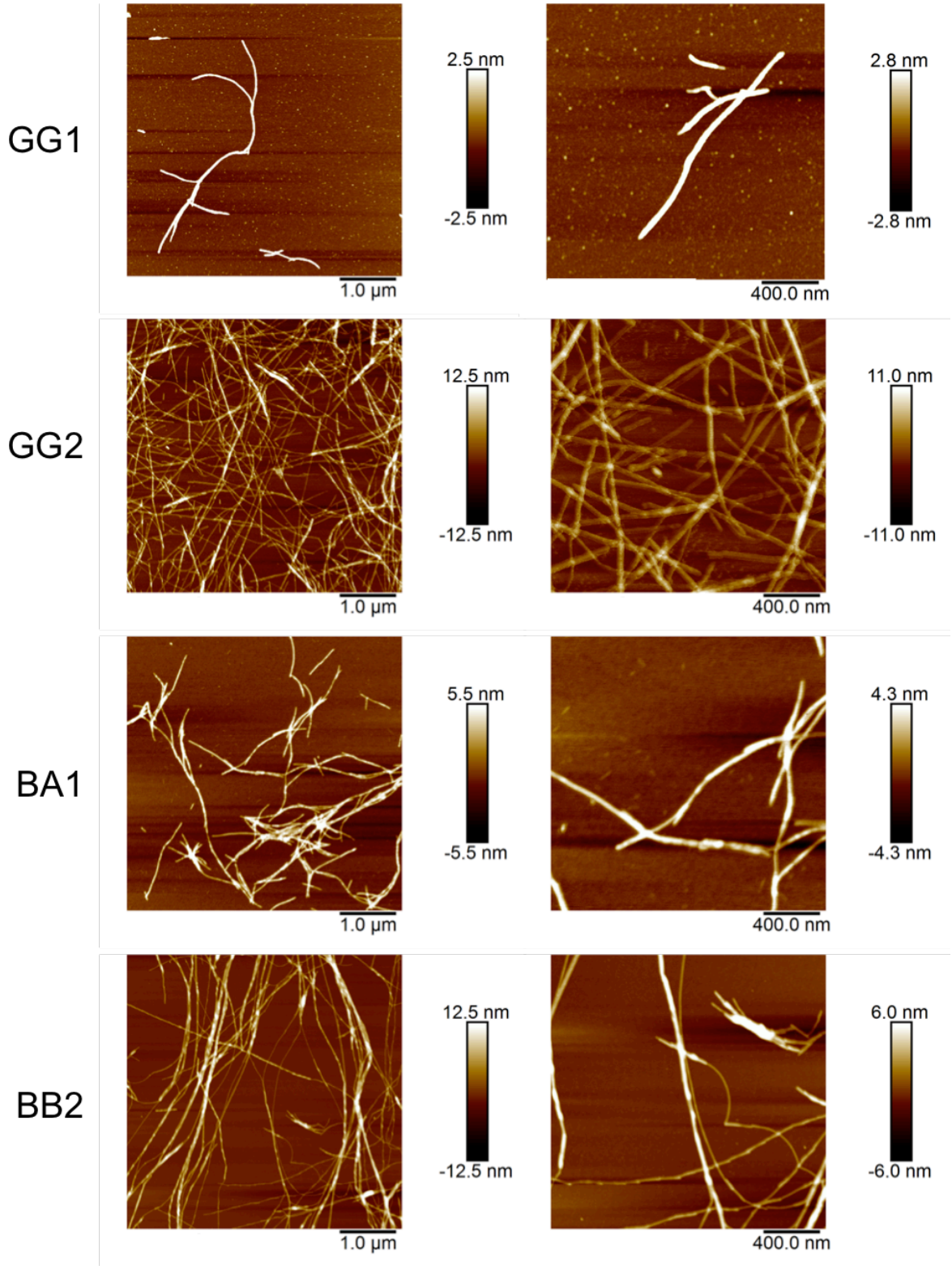

Figure S8. AFM images of peptide samples incubated at 50 °C under quiescent conditions.

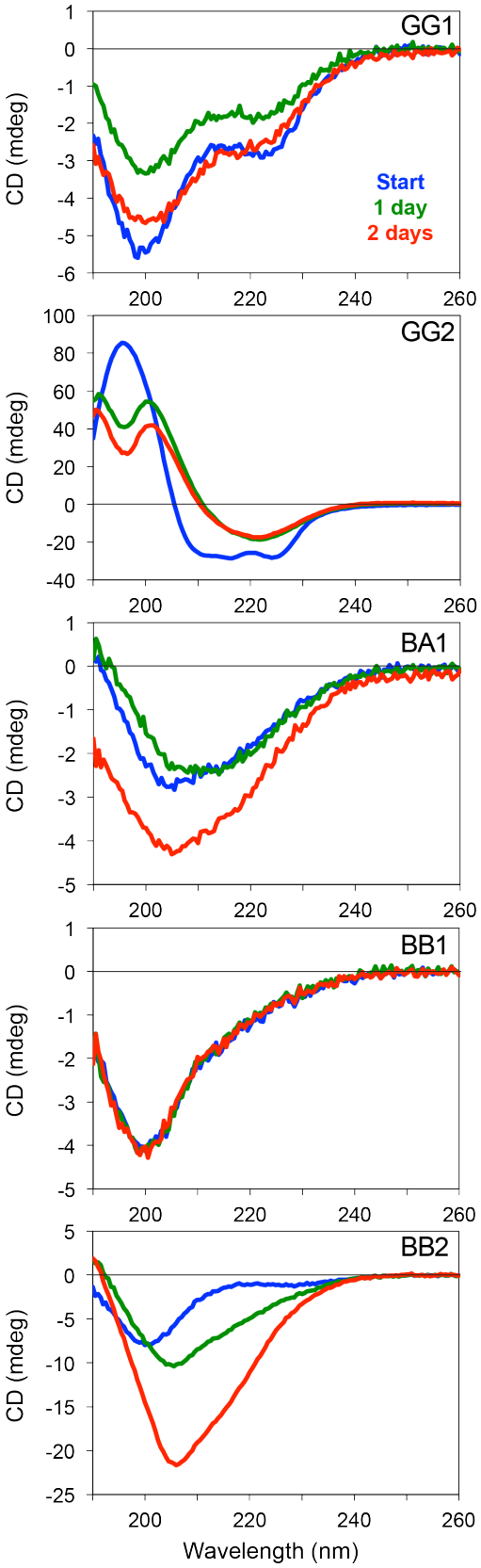

Figure S9. CD spectra of peptide samples before (blue) and after incubation at 50 °C with agitation for 1 day (green) or two days (red).

References

1. A. Micsonai, F. Wien, L. Kernya, Y.-H. Lee, Y. Goto, M. Réfrégiers and J. Kardos, *Proc. Natl. Acad. Sci. U.S.A.* **2015,** 11: E3095-E3103

2. A. B. Ahmed, N. Znassi, M. T. Chateau and A. V. Kajava, *Alzheimers Dement.* **2015,** 11, 681-690.