Nanoscale Characterization of Parallel and Anti-parallel β-Sheet Amyloid Beta 1-42 Aggregates

Kiryl Zhaliazka[1] and Dmitry Kurouski[1,2]*

1. Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, United States
2. Department of Biomedical Engineering, Texas A&M University, College Station, Texas, 77843, United States

Corresponding author: dkurouski@tamu.edu

Supporting Information

Table S1. Content table of conformations in percentage of Amide I area in selected by PCA groups of Ab_{1-42} aggregates in each time points.

Structure	Wavenumber	Time of incubation																					
		oh	4 h			24 h					72 h								168 h				
		monomer	oligomers			oligomers			protofibrils		oligomers			protofibrils			fibrils		oligomers		protofibrils		fibrils	
		groups																					
		—	1	2	3	1	2	3	1	2	1	2	3	1	2	3	1	2	1	2	1	2	1	2
		% in Amide I region																						
↑↑ β-sheet	≈ 1625-1636 cm^{-1}	16	81	76	85	63	79	44	83	47	45	38	34	45	39	37	45	32	74	78	54	70	69	77
β-turn	≈ 1667 cm^{-1}	84	14	15	12	15	14	24	9	32	25	23	26	28	30	30	24	30	14	13	23	18	7	13
↑↓ β-sheet	≈ 1694 cm^{-1}	—	5	9	3	22	8	32	8	20	30	39	40	27	31	33	31	38	12	9	23	11	24	10

Table S2. Bonferroni corrected Post Hoc T-test and ANOVA results of variance in toxicity exerted by Ab_{1-42} aggregates formed at different time periods of protein aggregation according to the LDH assay.

Asterisk (*) show 95% confidence interval for the true value of median for each compared group of spectra; NS is non-significant difference.

	P-value	
0h to 4h	0.01	NS
0h to 24h	0.0001	*
0h to 72h	0.000001	*
0h to 168h	0.00019	*
4h to 24h	0.00036	*
4h to 72h	0.000003	*
4h to 168h	0.0002	*
24h to 72h	0.0005	*
24h to 168h	0.0007	*
72h to 168h	0.0018	*
Contr. to 0h	0.02	NS
Contr. to 4h	0.04	NS
Contr. to 24h	0.0012	*
Contr. to 72h	0.0000023	*
Contr. to 168h	0.000056	*

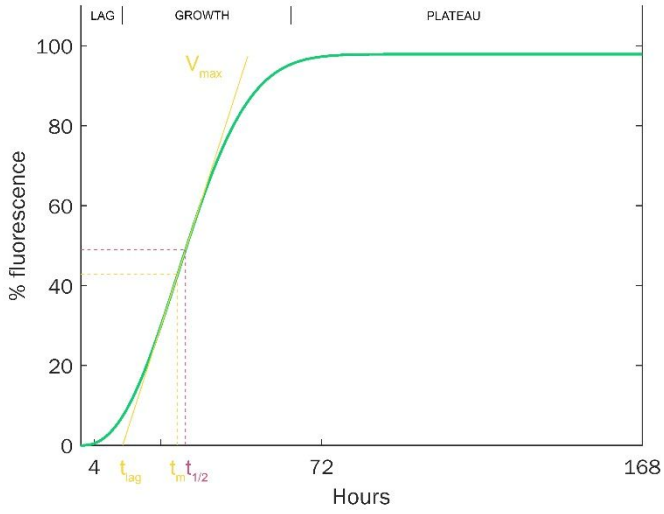

Figure S1. Fitted kinetic curve of thioflavin T fluorescence using modified version of the Avrami equation. The aggregation kinetics observed in Tht analysis has a typical sigmoid trend of the thioflavin T fluorescence signal.

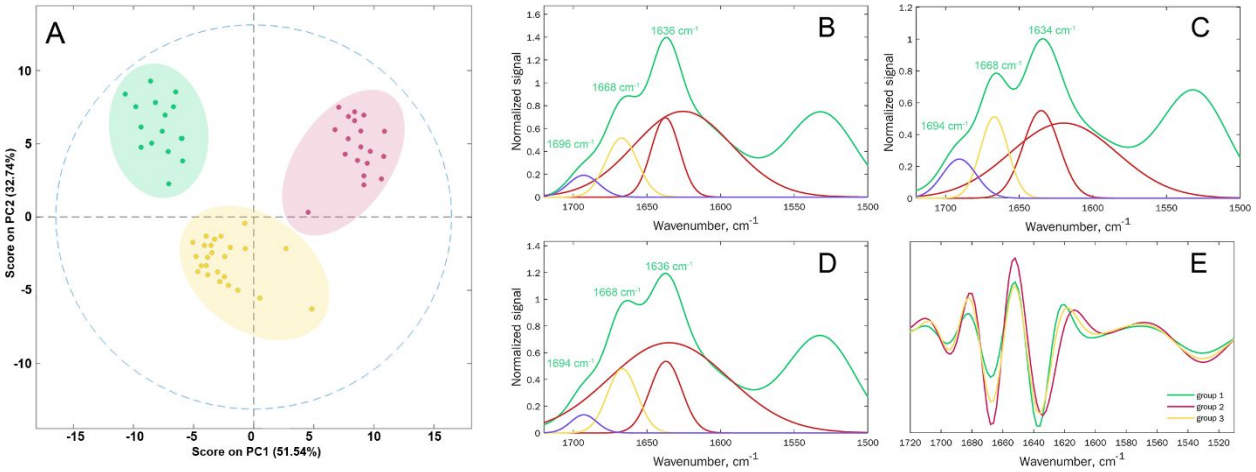

Figure S2. (A) PCA of spectra of $A\beta_{1-42}$ oligomers at 4-hour incubation time point, group 1 (green), group 2 (red), group 3 (yellow). Fitted average IR-spectrum from group 1(B), group 2(C), group 3(D) of $A\beta_{1-42}$ oligomers at 4-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (E) Second derivative spectra of Amide I and Amide II region of 3 groups of aggregates.

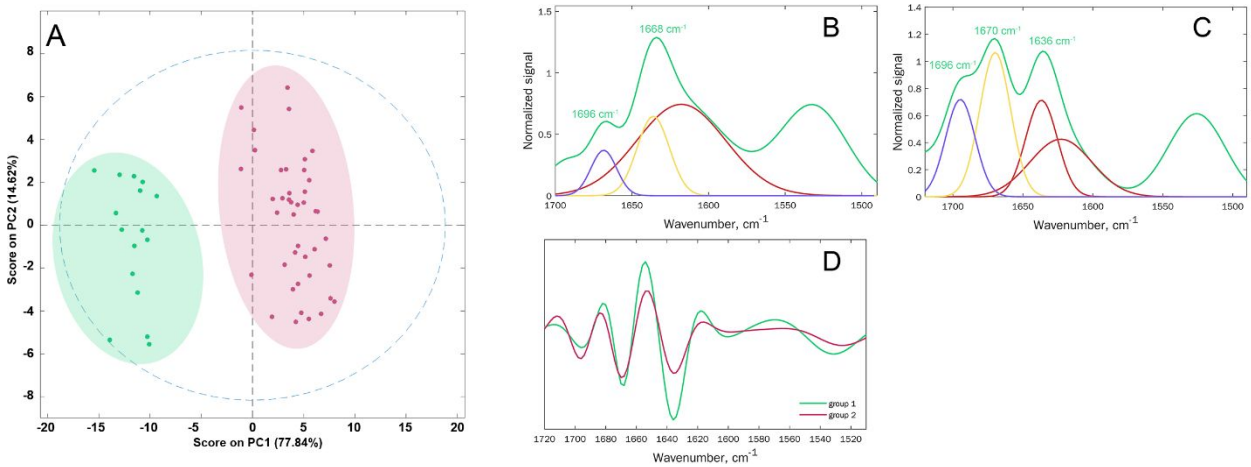

Figure S3. (A) PCA of spectra of $A\beta_{1-42}$ protofibrils at 24-hour incubation time point, group 1 (green), group 2 (red). Fitted average IR-spectrum from group 1(B), group 2(C) of $A\beta_{1-42}$ protofibrils at 24-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (D) Second derivative spectra of Amide I and Amide II region of 2 groups of aggregates.

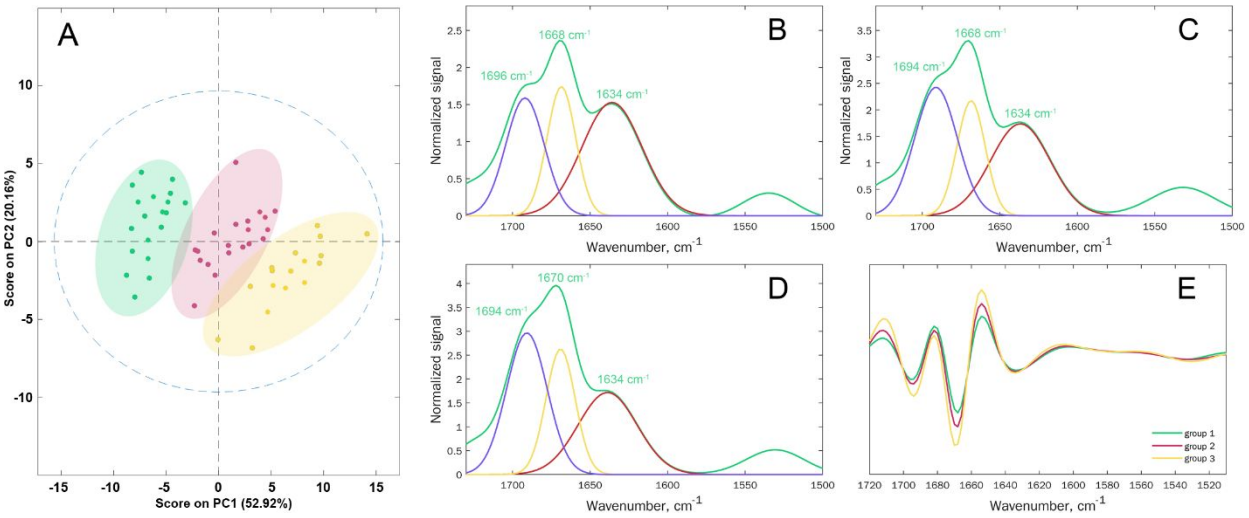

Figure S4. (A) PCA of spectra of $A\beta_{1-42}$ oligomers at 72-hour incubation time point, group 1 (green), group 2 (red), group 3 (yellow). Fitted average IR-spectrum from group 1(B), group 2(C), group 3(D) of $A\beta_{1-42}$ oligomers at 72-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (E) Second derivative spectra of Amide I and Amide II region of 3 groups of aggregates.

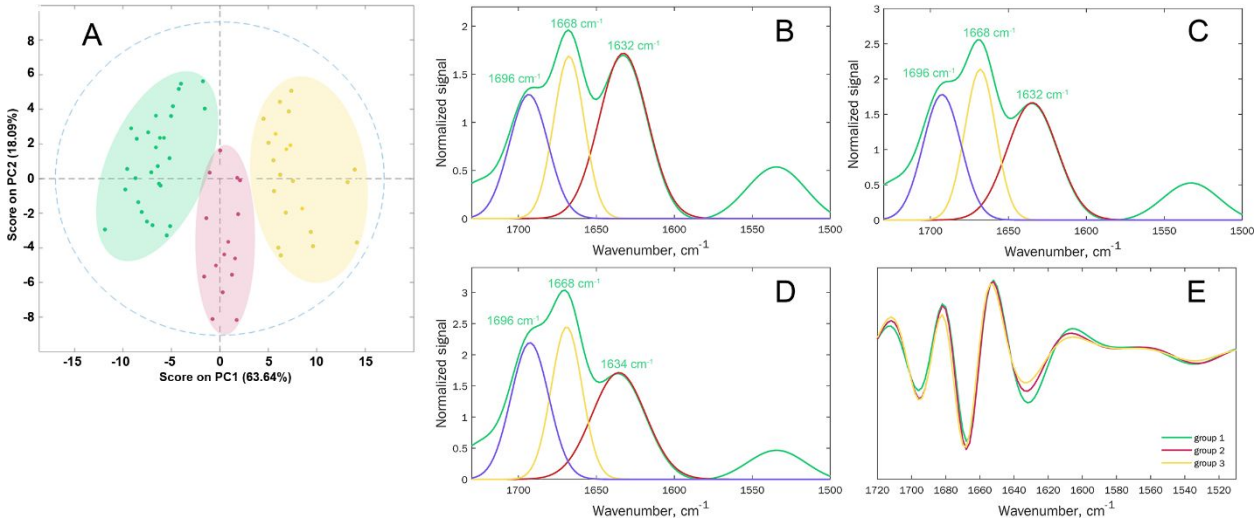

Figure S5. (A) PCA of spectra of $A\beta_{1-42}$ protofibrils at 72-hour incubation time point, group 1 (green), group 2 (red), group 3 (yellow). Fitted average IR-spectrum from group 1(B), group 2(C), group 3(D) of $A\beta_{1-42}$ protofibrils at 72-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (E) Second derivative spectra of Amide I and Amide II region of 3 groups of aggregates.

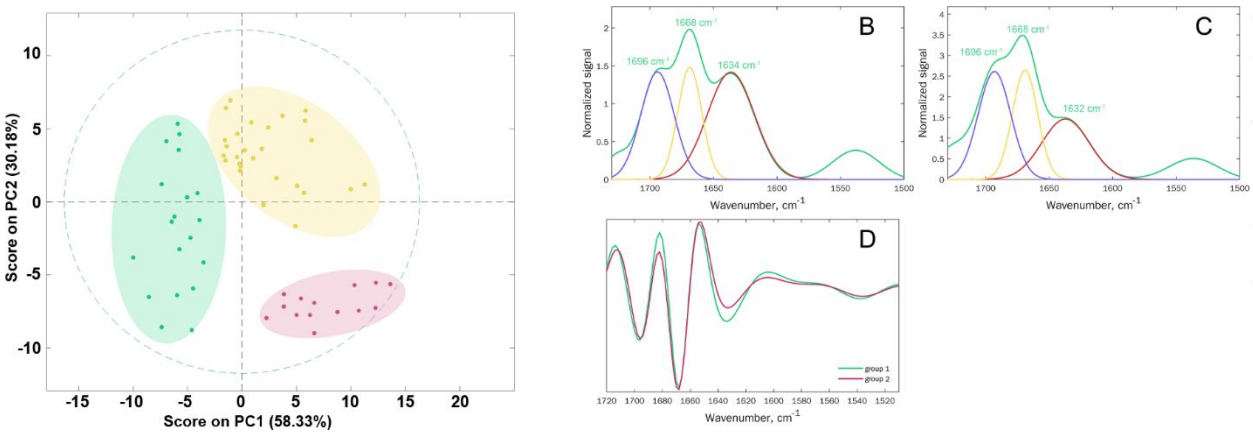

Figure S6. (A) PCA of spectra of $A\beta_{1-42}$ fibrils at 72-hour incubation time point, group 1 (green), group 2 (red). Fitted average IR-spectrum from group 1(B), group 2(C) of $A\beta_{1-42}$ protofibrils at 72-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (D) Second derivative spectra of Amide I and Amide II region of 2 groups of aggregates.

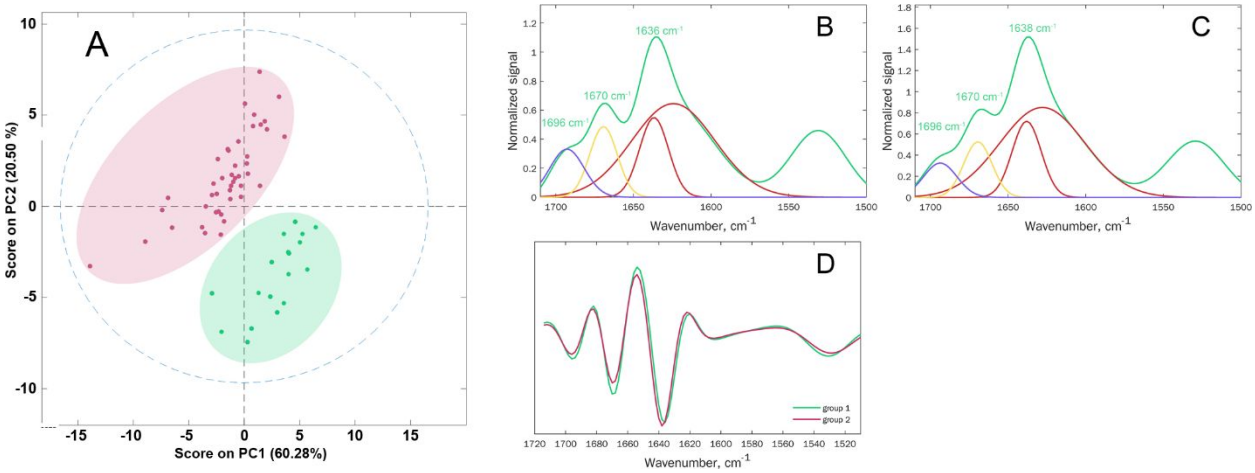

Figure S7. (A) PCA of spectra of $A\beta_{1-42}$ oligomers at 168-hour incubation time point, group 1 (green), group 2 (red). Fitted average IR-spectrum from group 1(B), group 2(C) of $A\beta_{1-42}$ oligomers at 168-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (D) Second derivative spectra of Amide I and Amide II region of 2 groups of aggregates.

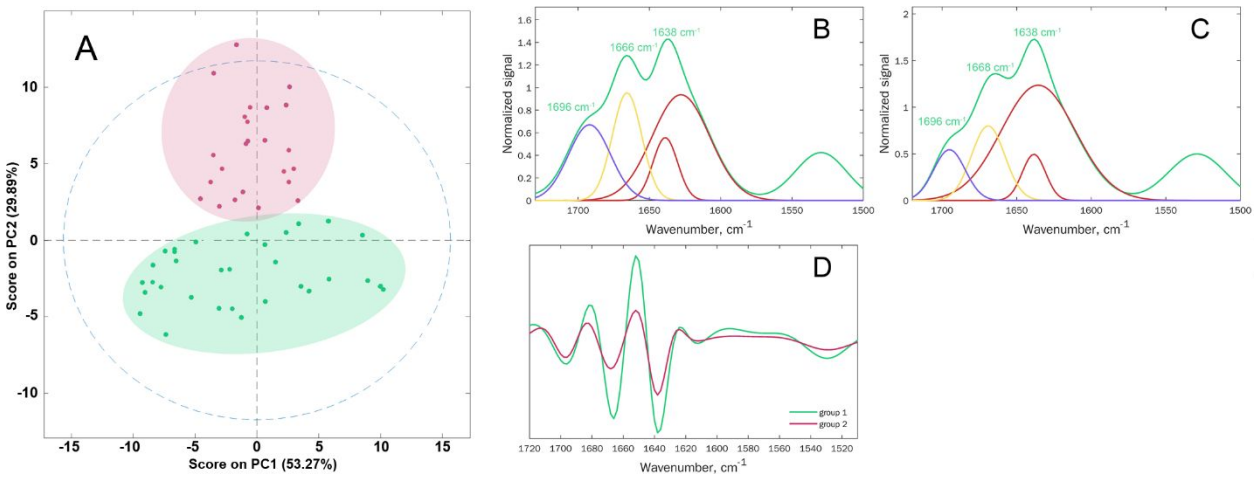

Figure S8. (A) PCA of spectra of $A\beta_{1-42}$ protofibrils at 168-hour incubation time point, group 1 (green), group 2 (red). Fitted average IR-spectrum from group 1(B), group 2(C) of $A\beta_{1-42}$ protofibrils at 168-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (D) Second derivative spectra of Amide I and Amide II region of 2 groups of aggregates.

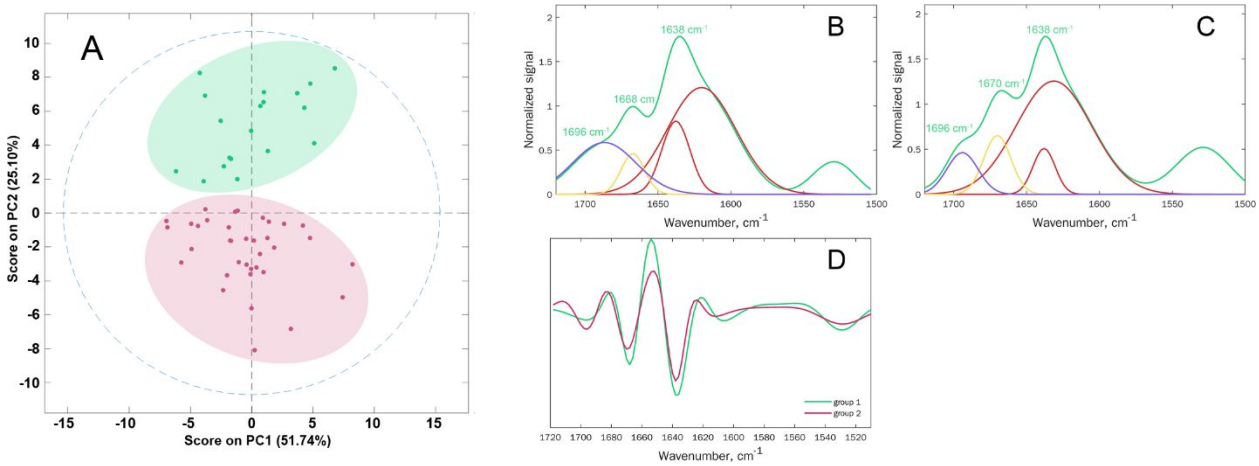

Figure S9. (A) PCA of spectra of $A\beta_{1-42}$ fibrils at 168-hour incubation time point, group 1 (green), group 2 (red). Fitted average IR-spectrum from group 1(B), group 2(C) of $A\beta_{1-42}$ fibrils at 168-hour incubation time point and corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered, violet line – anti-parallel beta-sheet. (D) Second derivative spectra of Amide I and Amide II region of 2 groups of aggregates.

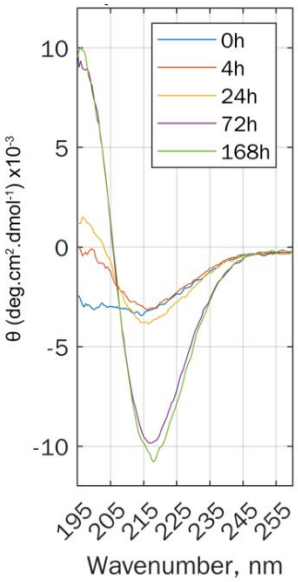

Figure S10. CD spectra of $A\beta_{1-42}$ collected at different time points of protein aggregation.

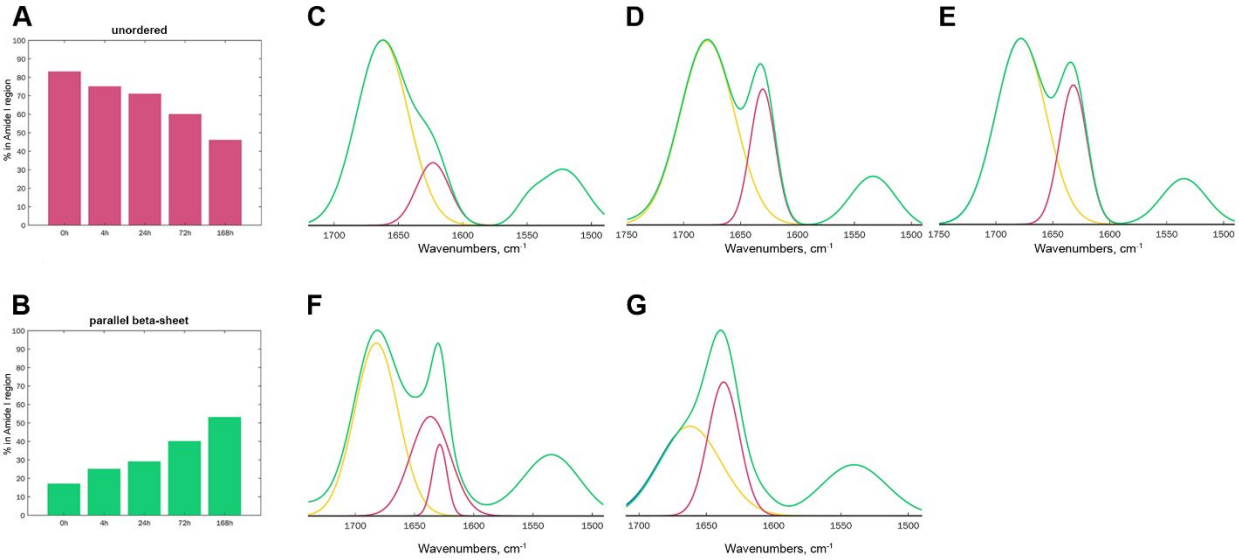

Figure S11. Content of unordered (1667 cm^{-1}) (A), parallel beta-sheet (1625-1636 cm^{-1}) (B) from FTIR-spectra. Fitted FTIR-spectrum of Ab_{1-42} aggregates in 0-hour time point (C), 4-hour time point (D), 24-hour time point (E), 72-hour time point (F), 168-hour time point (G), with corresponded structures: green line – fitted spectrum, red line – parallel beta-sheet, yellow line – unordered.

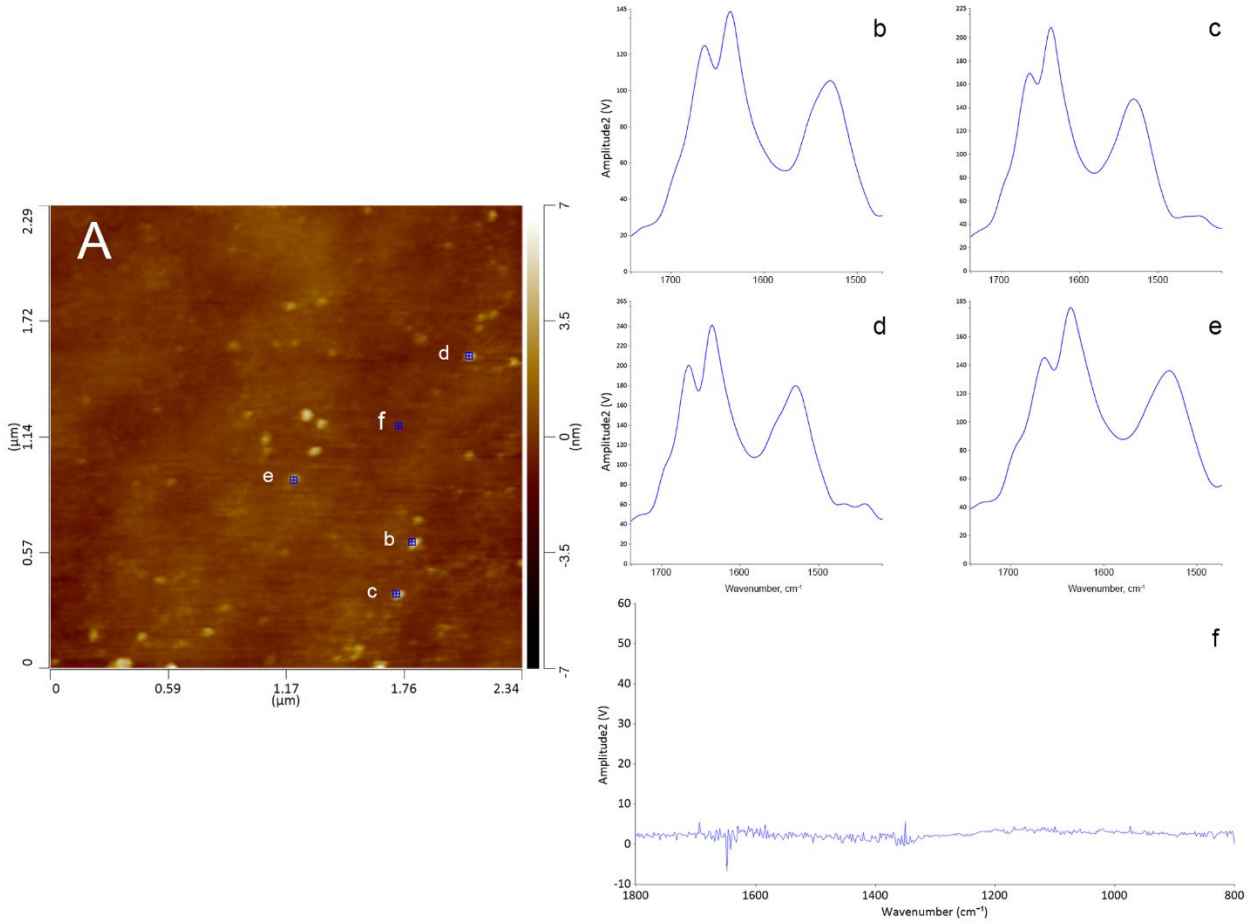

Figure S12. (A) AFM map of single oligomers of $A\beta_{1-42}$ formed at 4h after initiation of protein aggregation. (b-e) Spectra from single oligomers (smoothing filter 10 points) and (f) background spectrum.

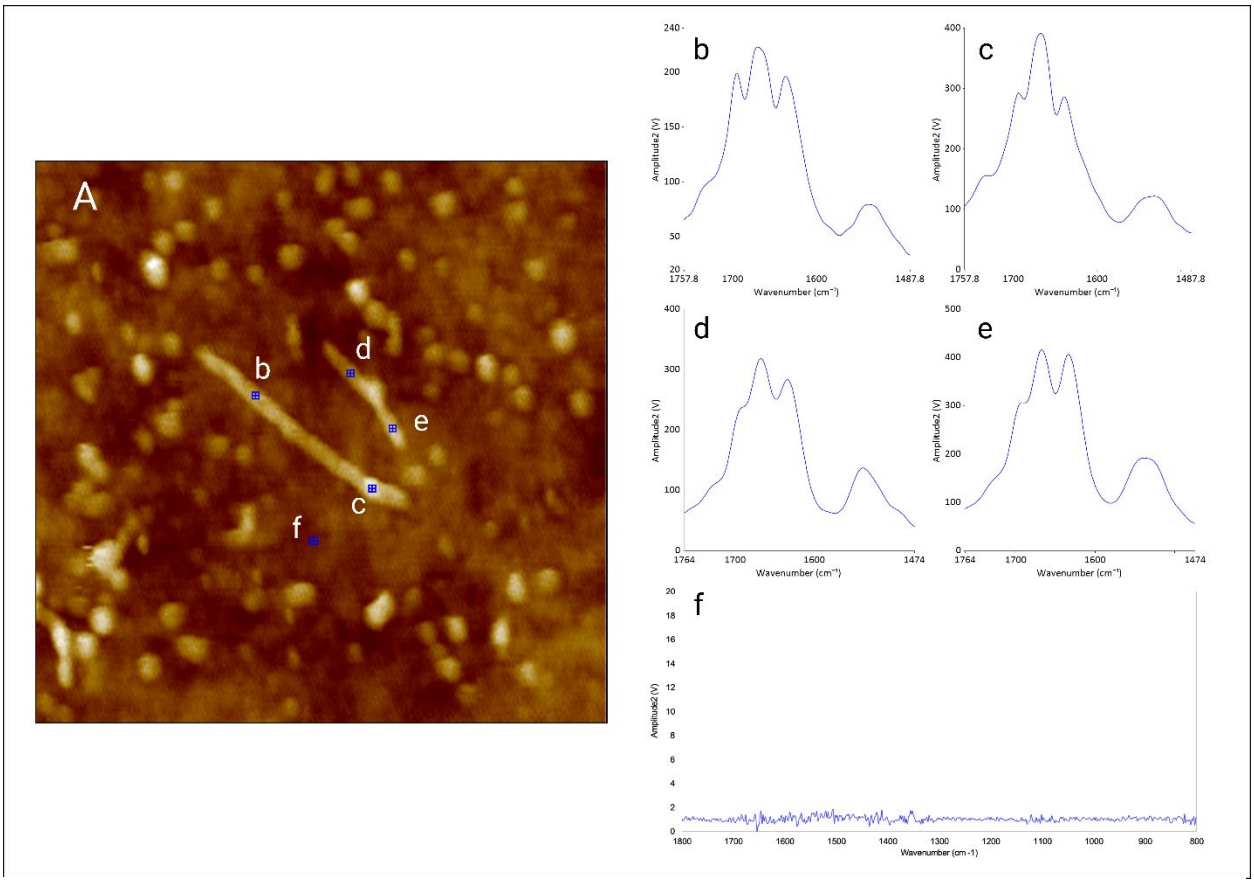

Figure S13. AFM map (left) of single proto-fibrils and fibrils of $A\beta_{1-42}$ formed at 168h after initiation of protein aggregation with the corresponding AFM-IR spectra (a-d).