Supplementary Information

Effect of Differences in the Primary Structure of the A-chain on the Aggregation of Insulin Fragments

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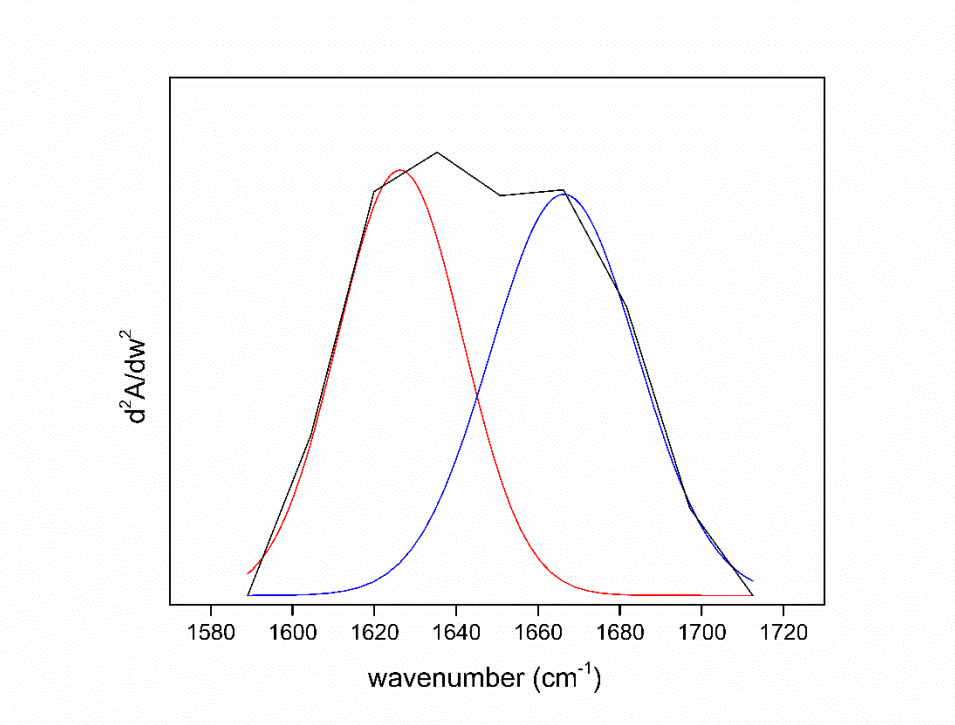


Figure S1. Second-derivative spectra for native BIF (—) and its curve-fitted peaks.

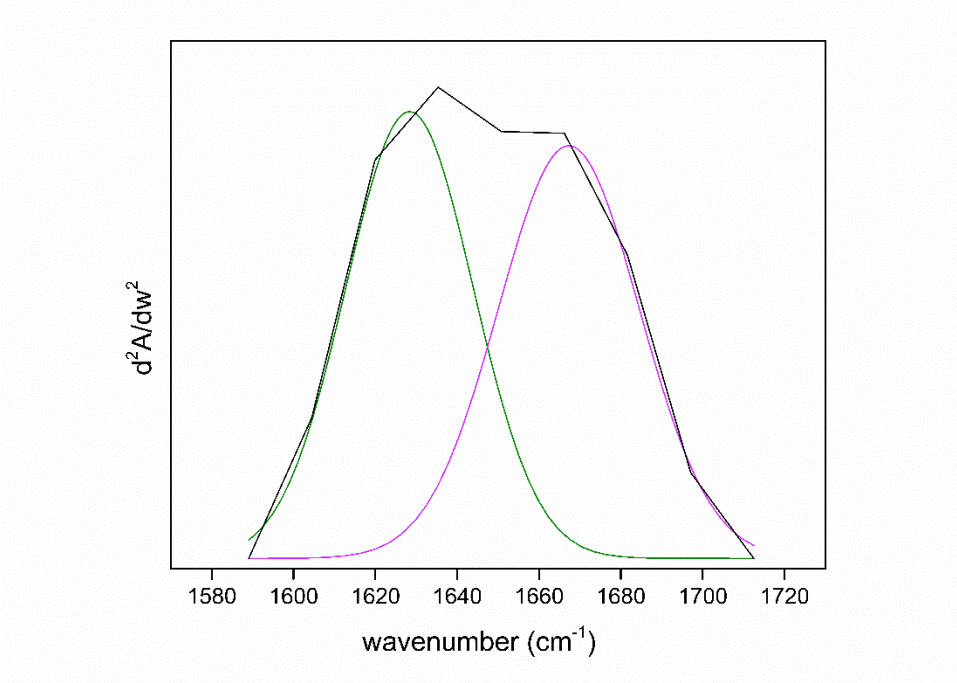
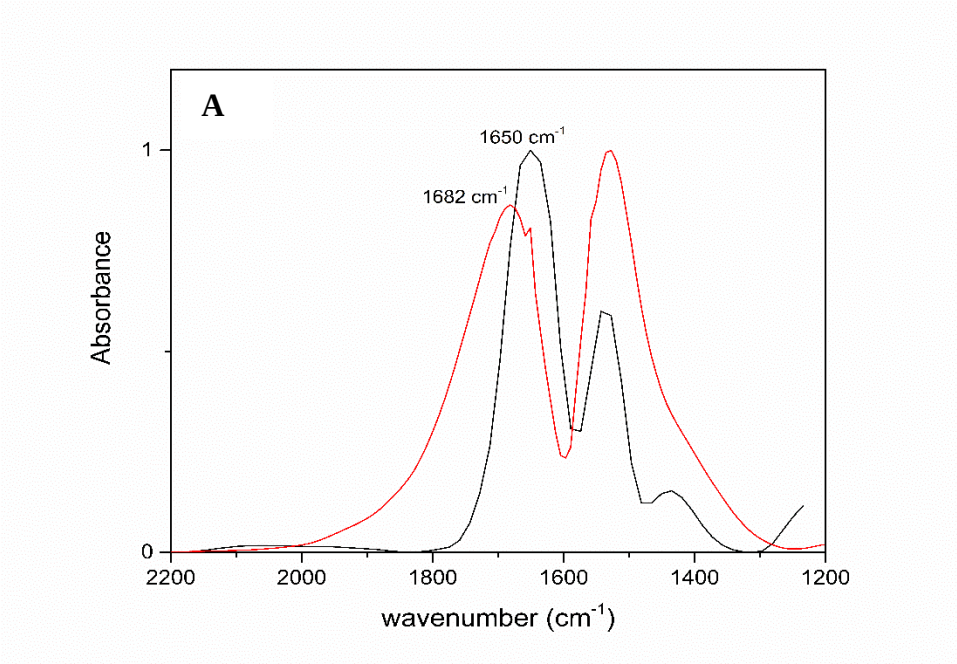


Figure S2. Second-derivative sp



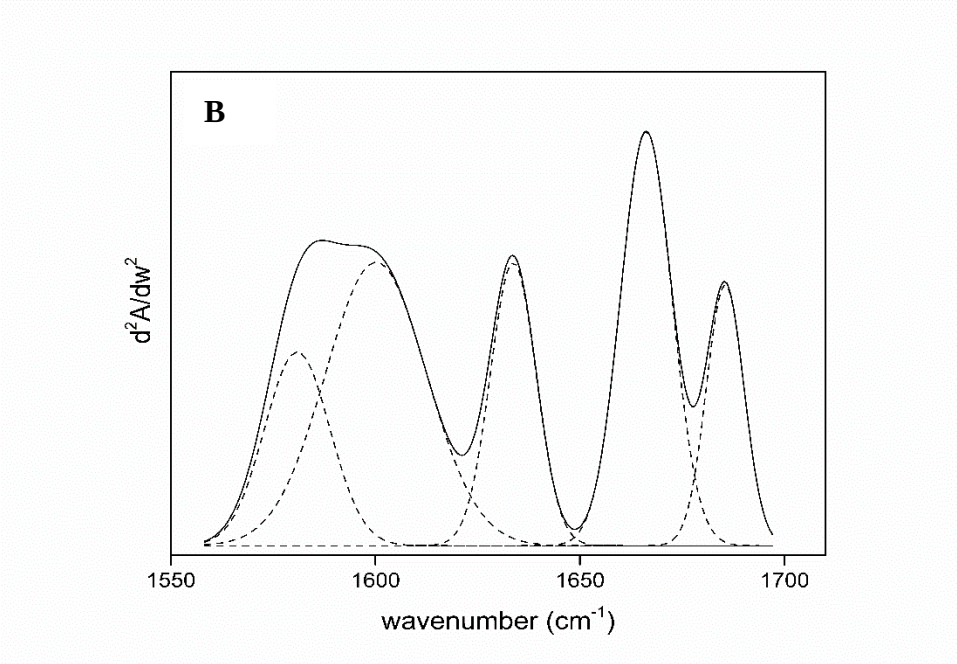
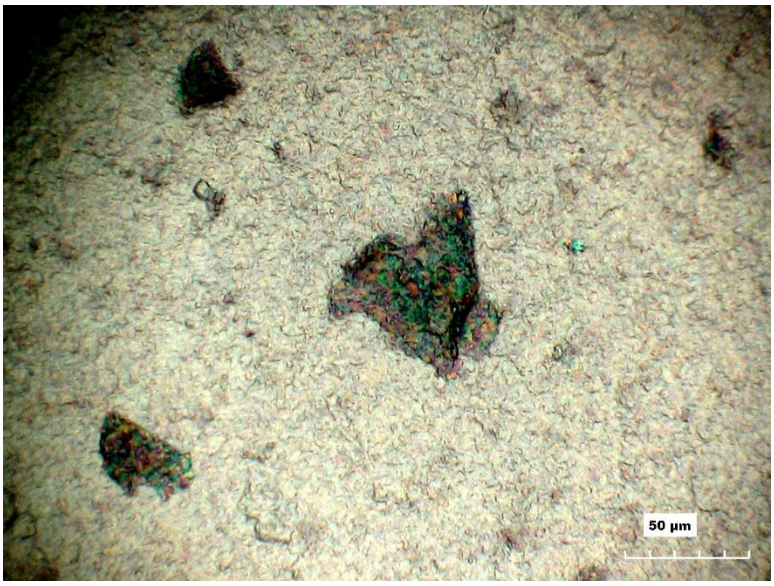


Figure S3. A) FTIR spectra for HIF at pH 1.6, 60°C, 1M urea, 0.02M NaCl collected at t = 0 days (—) and t = 42 days (—) showing the shift in the amide I region. B) Second-derivative spectra for HIF at pH 1.6, 60°C, 1M urea, 0.02M NaCl collected at the end of week-1 (—) and its curve-fitted peaks.

A



B

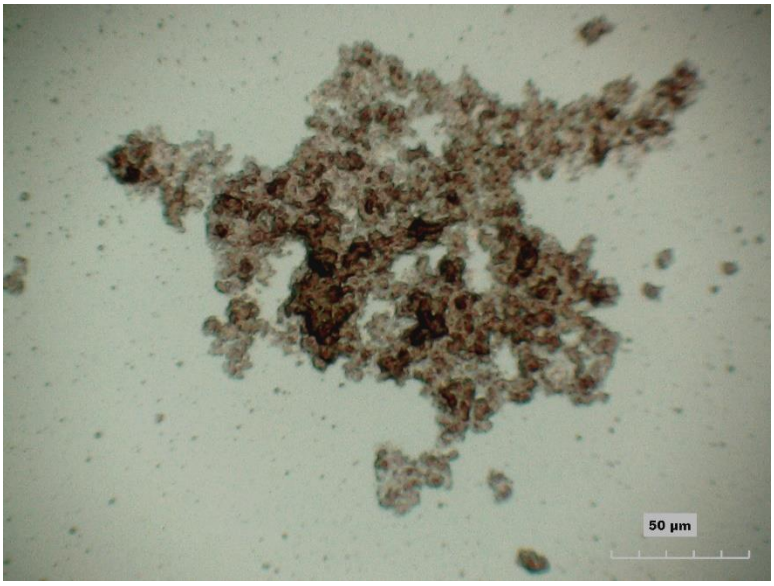
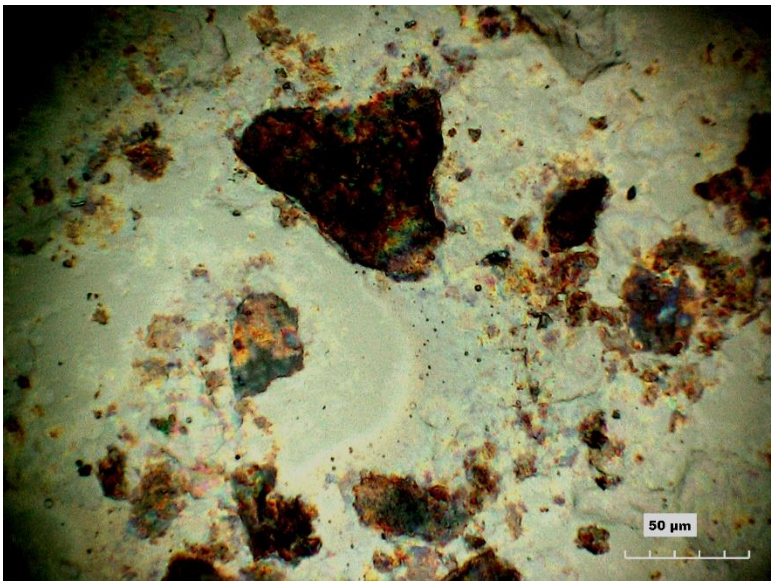


Figure S4. Micrographs of the samples stained with Congo Red for A) BIF at pH 1.6, 25°C, 0M urea, 0.02M NaCl B) HIF at pH 1.6, 25°C, 0M urea, 0.02M NaCl.

A



B

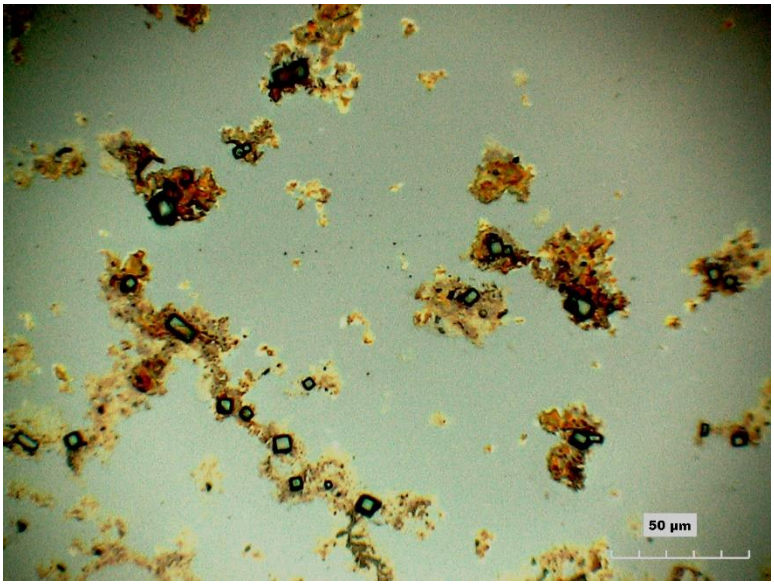
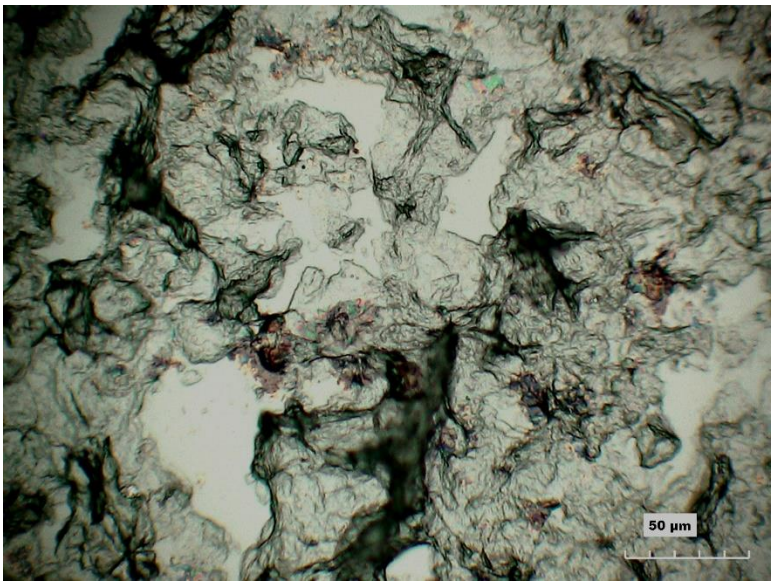


Figure S5. Micrographs of the samples stained with Congo Red for A) BIF at pH 1.6, 25°C, M urea, 1M NaCl B) HIF at pH 1.6, 25°C, 1M urea, 1M NaCl.

A



B

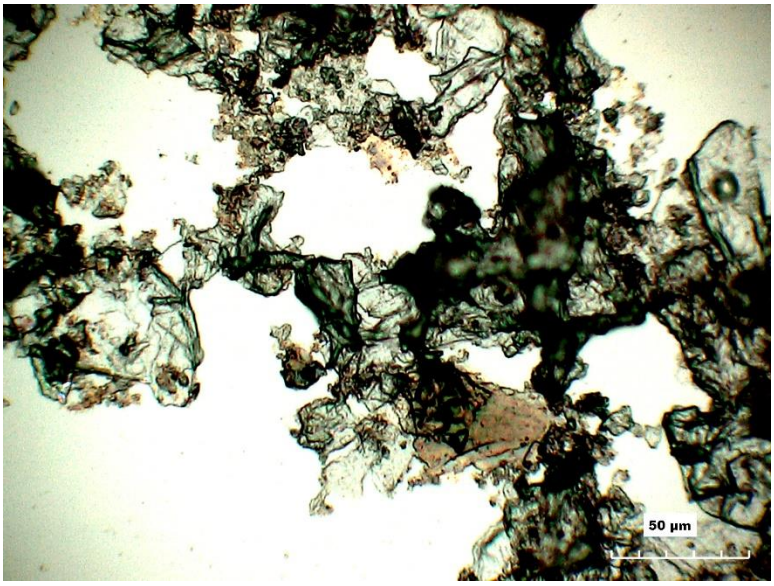


Figure S6. Micrographs of the samples stained with Congo Red for A) BIF at pH 1.6, 60°C, 0M urea, 1M NaCl B) HIF at pH 1.6, 60°C, 0M urea, 1M NaCl.

A

B

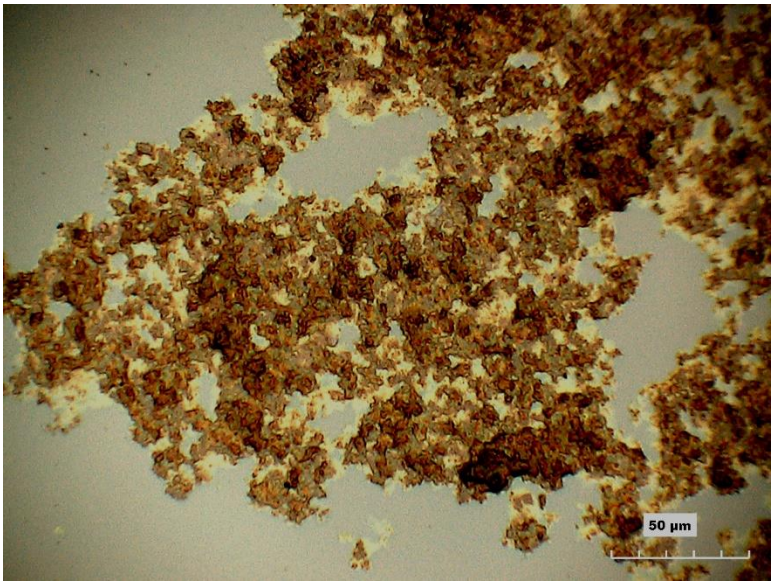
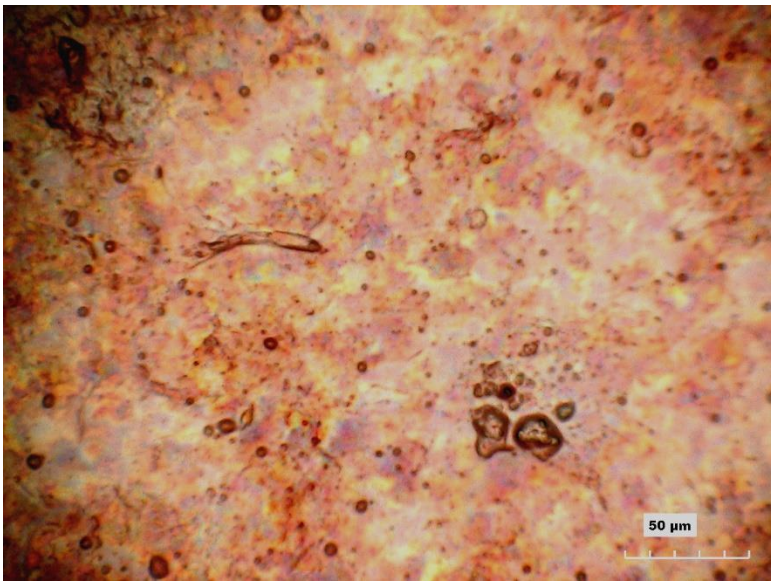


Figure S7. Micrographs of the samples stained with Congo Red for A) BIF at pH 5, 25°C, 0M urea, 1M NaCl B) HIF at pH 5, 25°C, 0M urea, 1M NaCl.

A



B

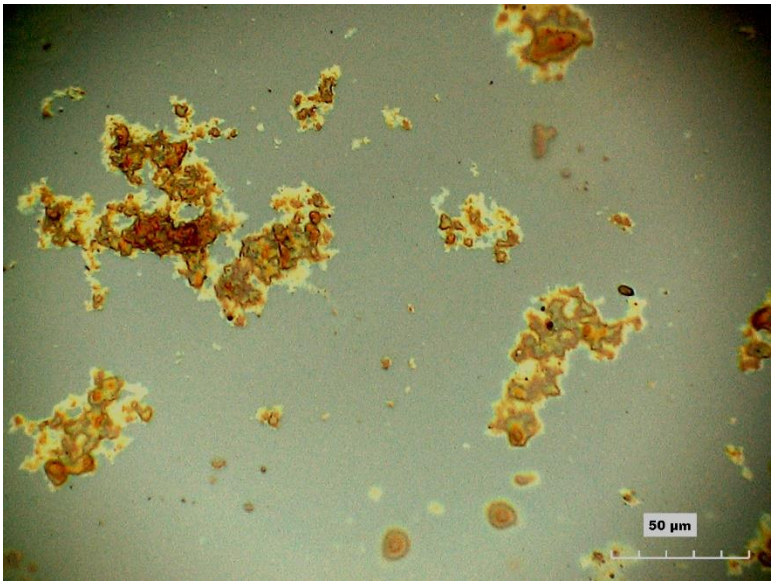


Figure S8. Micrographs of the samples stained with Congo Red for A) BIF at pH 5, 25°C, 1M urea, 0.02M NaCl B) HIF at pH 5, 25°C, 1M urea, 0.02M NaCl.

A

B

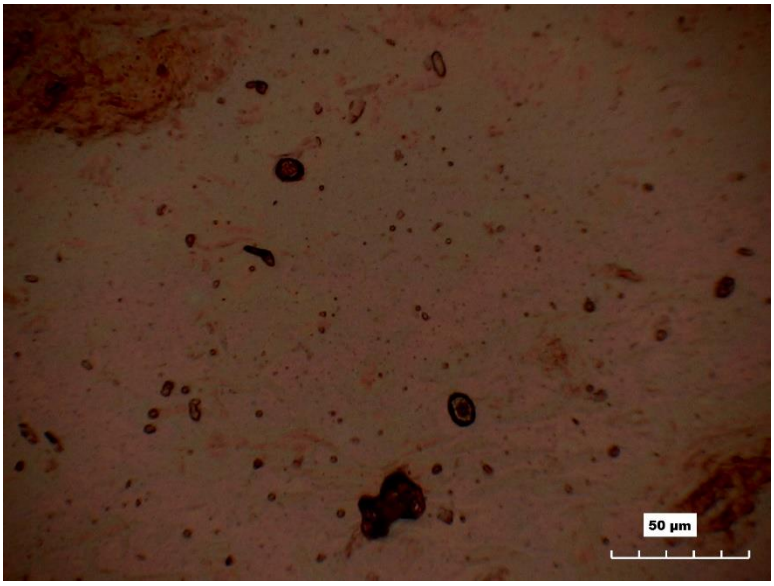


Figure S9. Micrographs of the samples stained with Congo Red for A) BIF at pH 5, 60°C, 1M urea, 1M NaCl B) HIF at pH 5, 60°C, 1M urea, 1M NaCl.

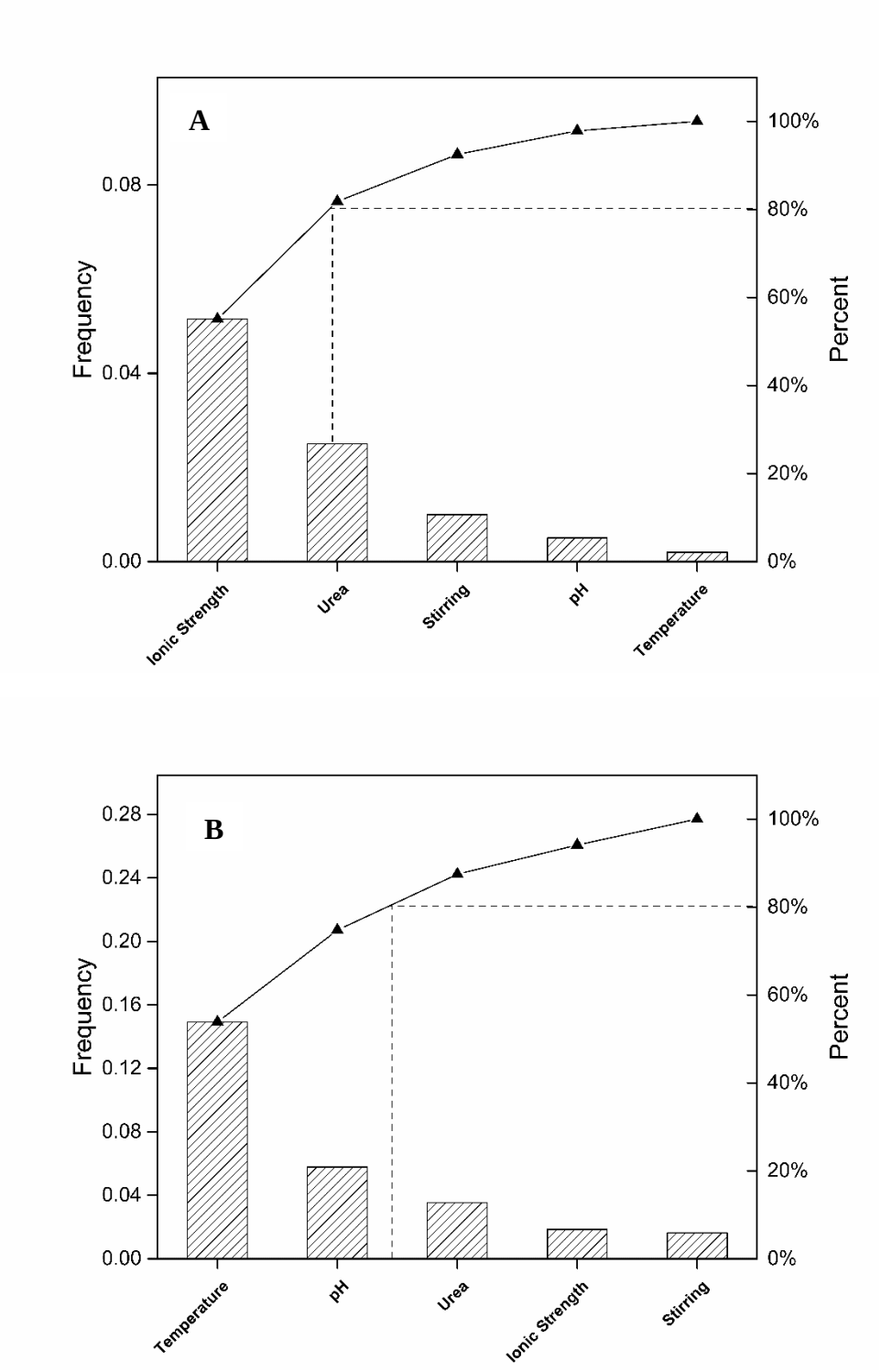


Figure S10. Pareto charts showing the effect of various factors based on K_β as an outcome from the experimental design FF0508 for A) BIF and B) HIF.

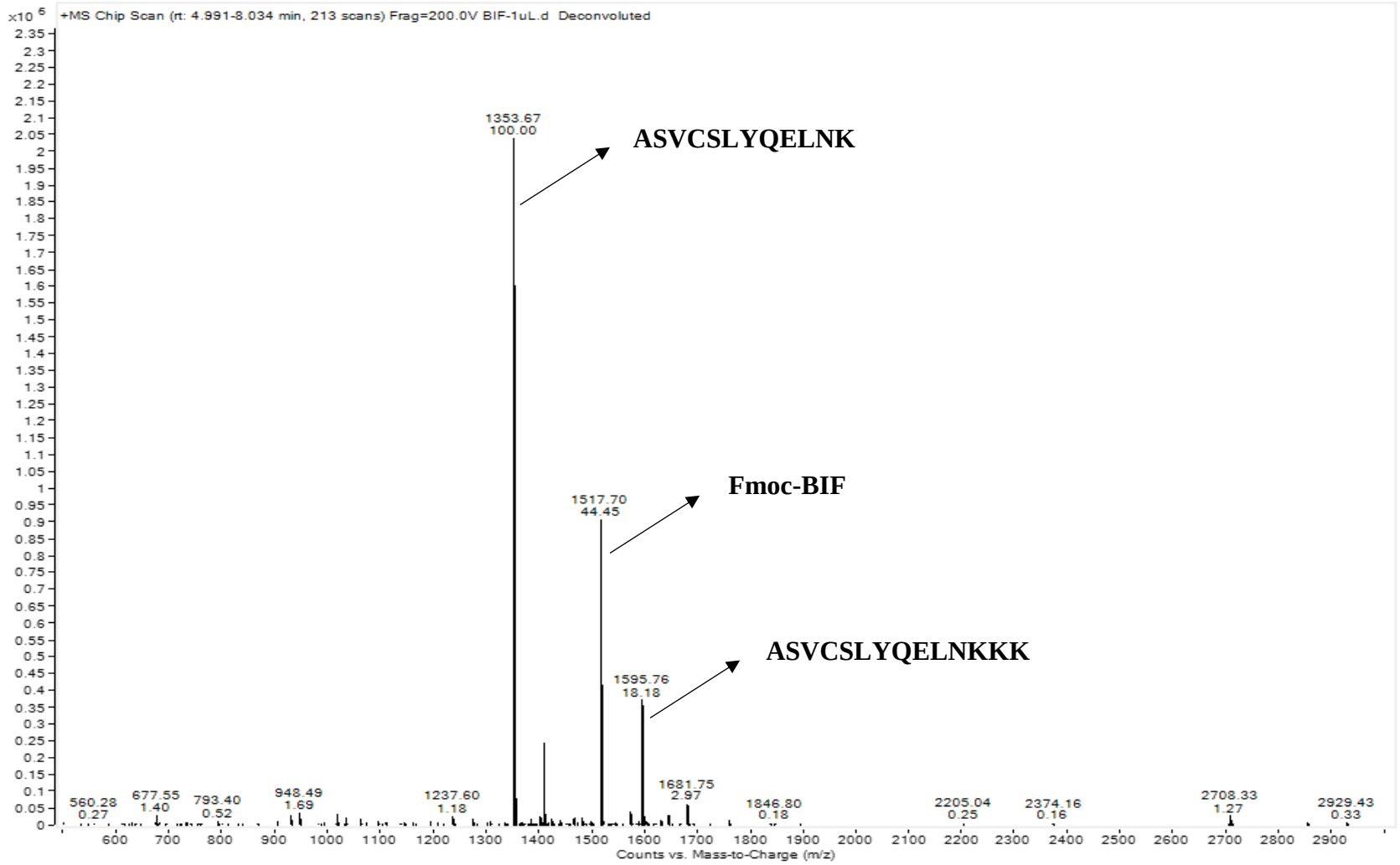


Figure S11. LC-MS of native BIF showing the native BIF (with 1 and 3 Lys tail) and impurity (Fmoc-BIF) with their respective mass (top) and relative abundance (below) shown over each peak.

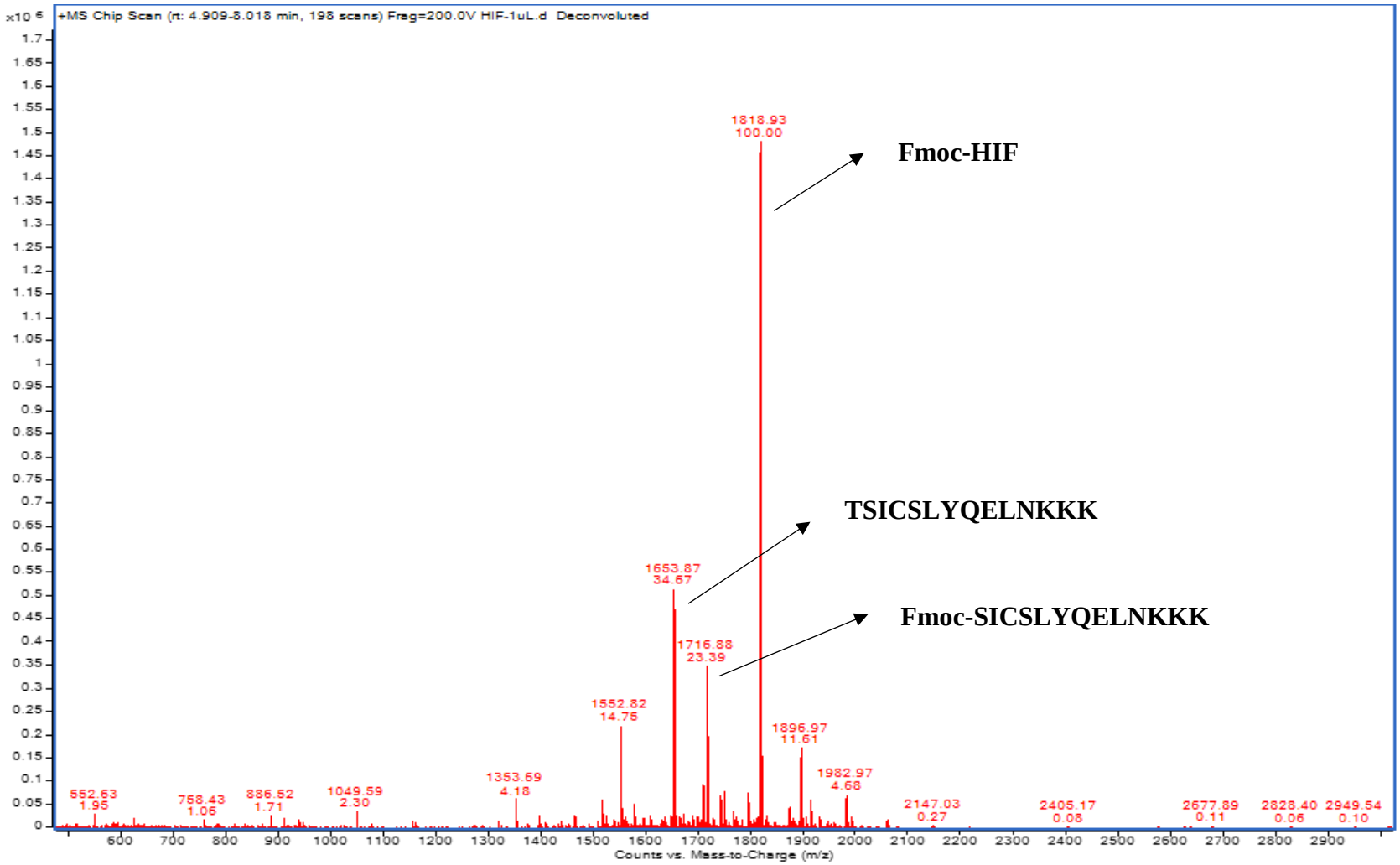


Figure S12. LC-MS of native HIF showing the native HIF, truncated HIF (with Fmoc) and impurity (Fmoc-HIF) with their respective mass (top) and relative abundance (below) shown over each peak.

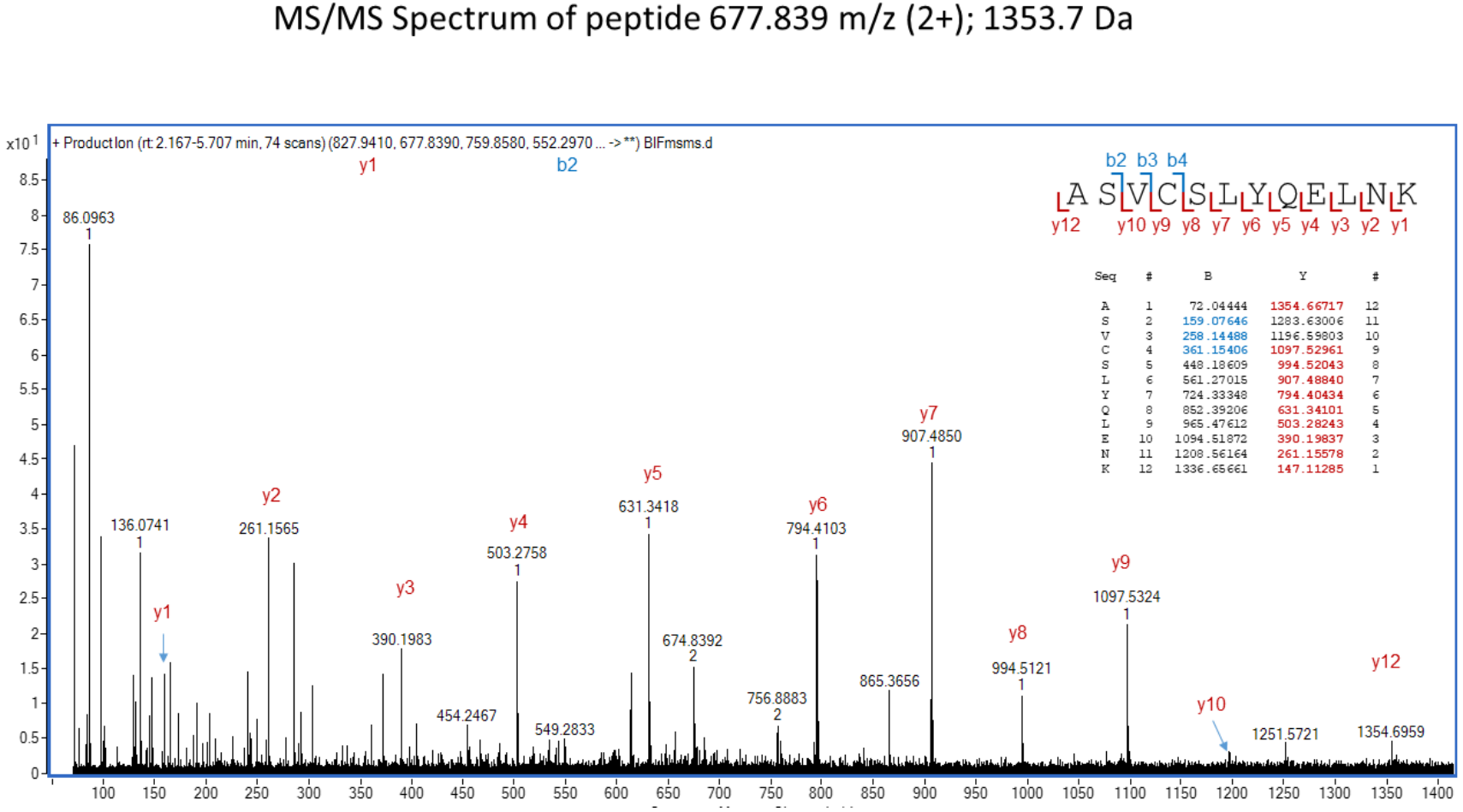


Figure S13. LC-MS/MS spectrum of native BIF (1353.7 Da) showing detailed sequence analysis.

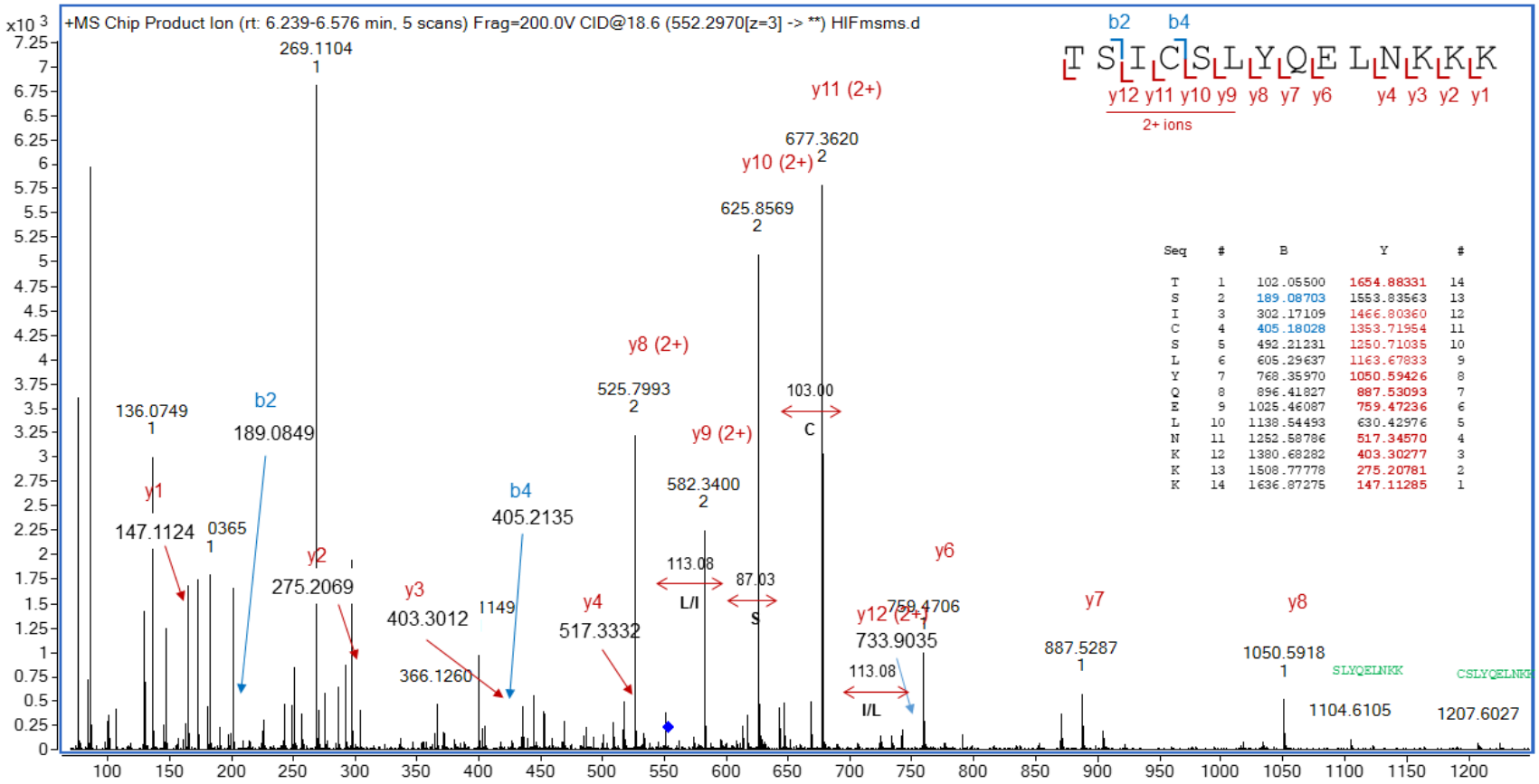


Figure S14. LC-MS/MS spectrum of native HIF (1654.88 Da) showing detailed sequence analysis.

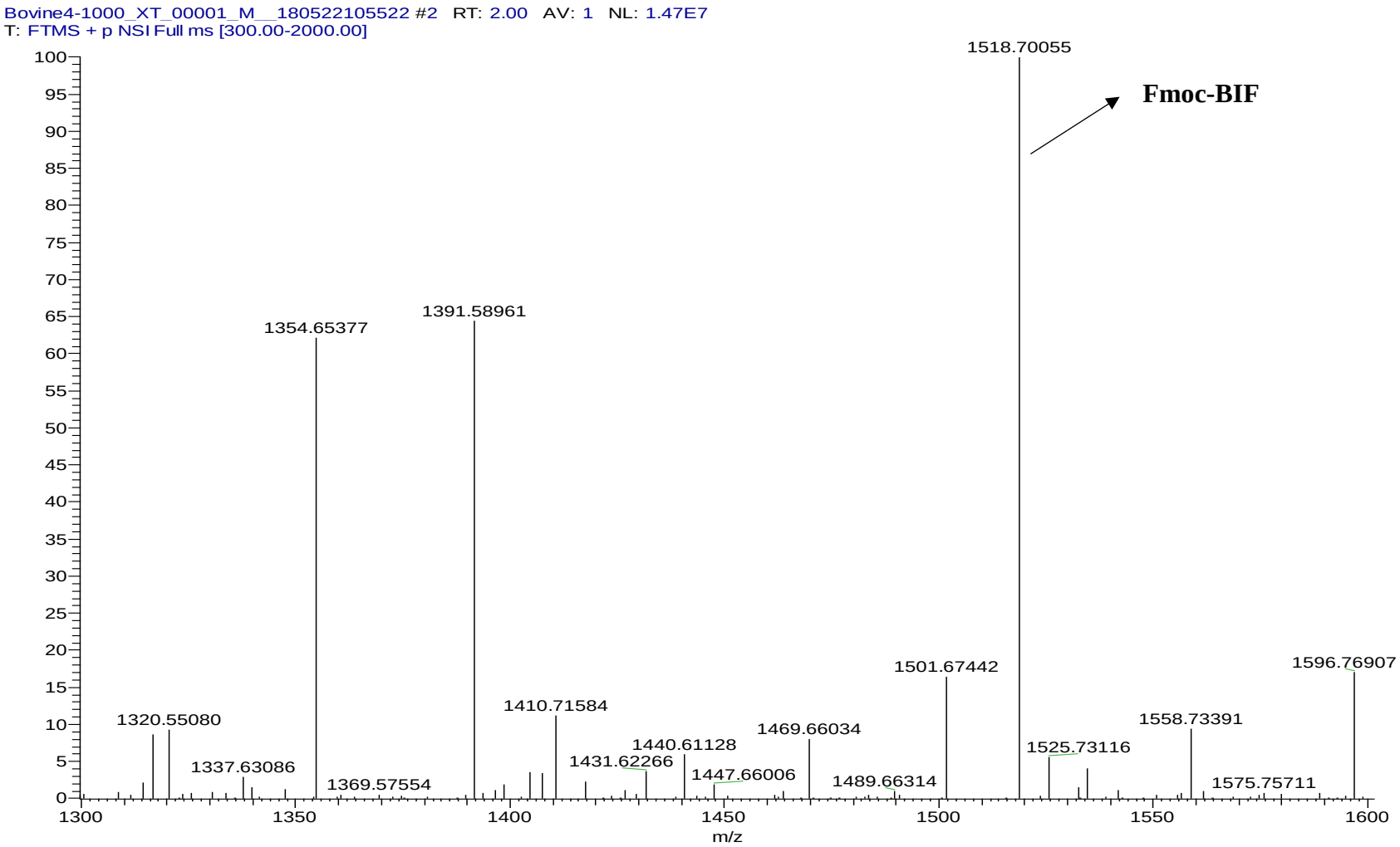


Figure S15A. LC-MS graph of fibrils obtained after the experiment for BIF at pH 1.6, 60°C, 1M urea, 0.02M NaCl showing the relative abundance of Fmoc-BIF.

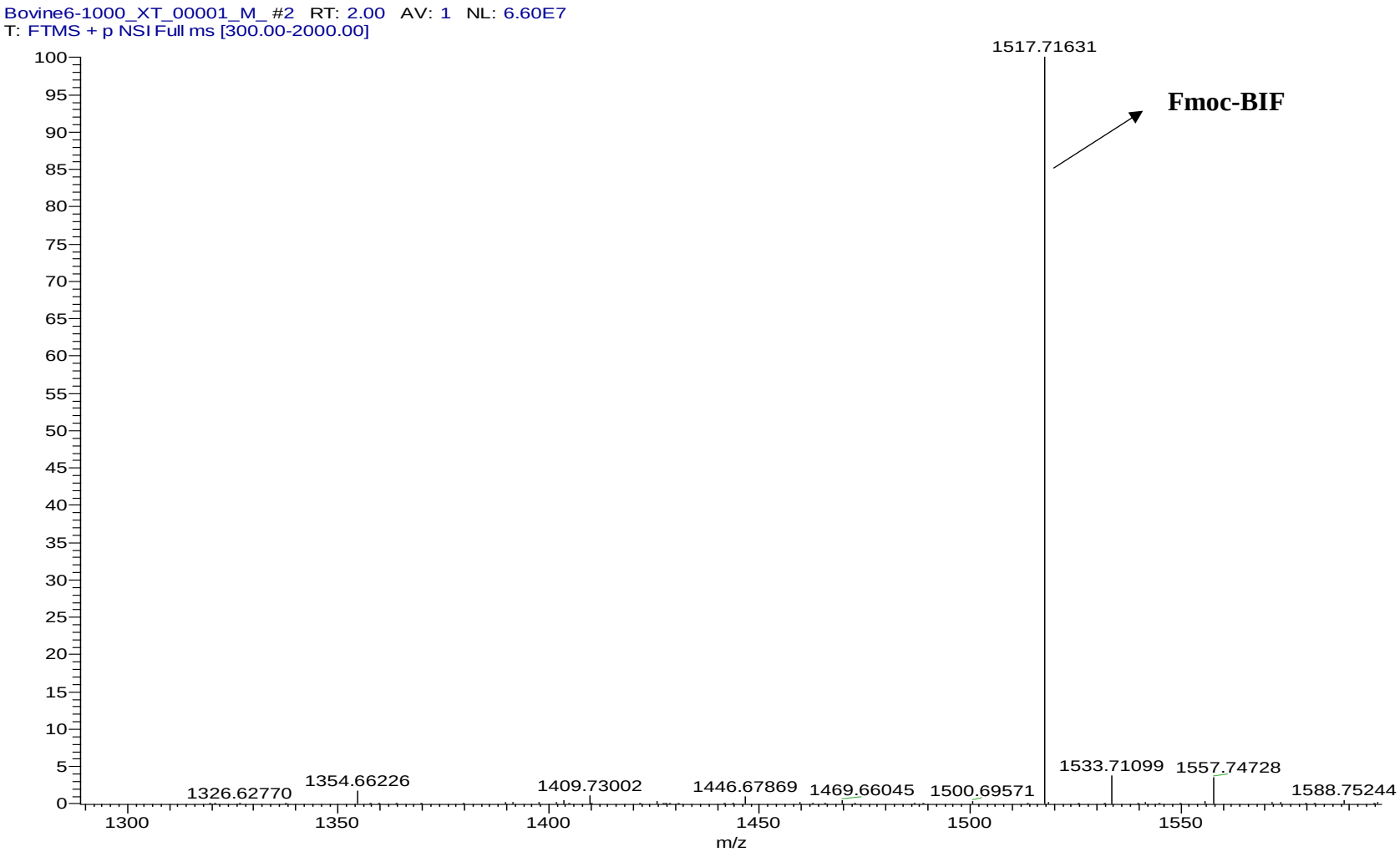


Figure S15B. LC-MS graph of fibrils obtained after the experiment for BIF at pH 5, 25°C, 1M urea, 0.02M NaCl showing the relative abundance of Fmoc-BIF.

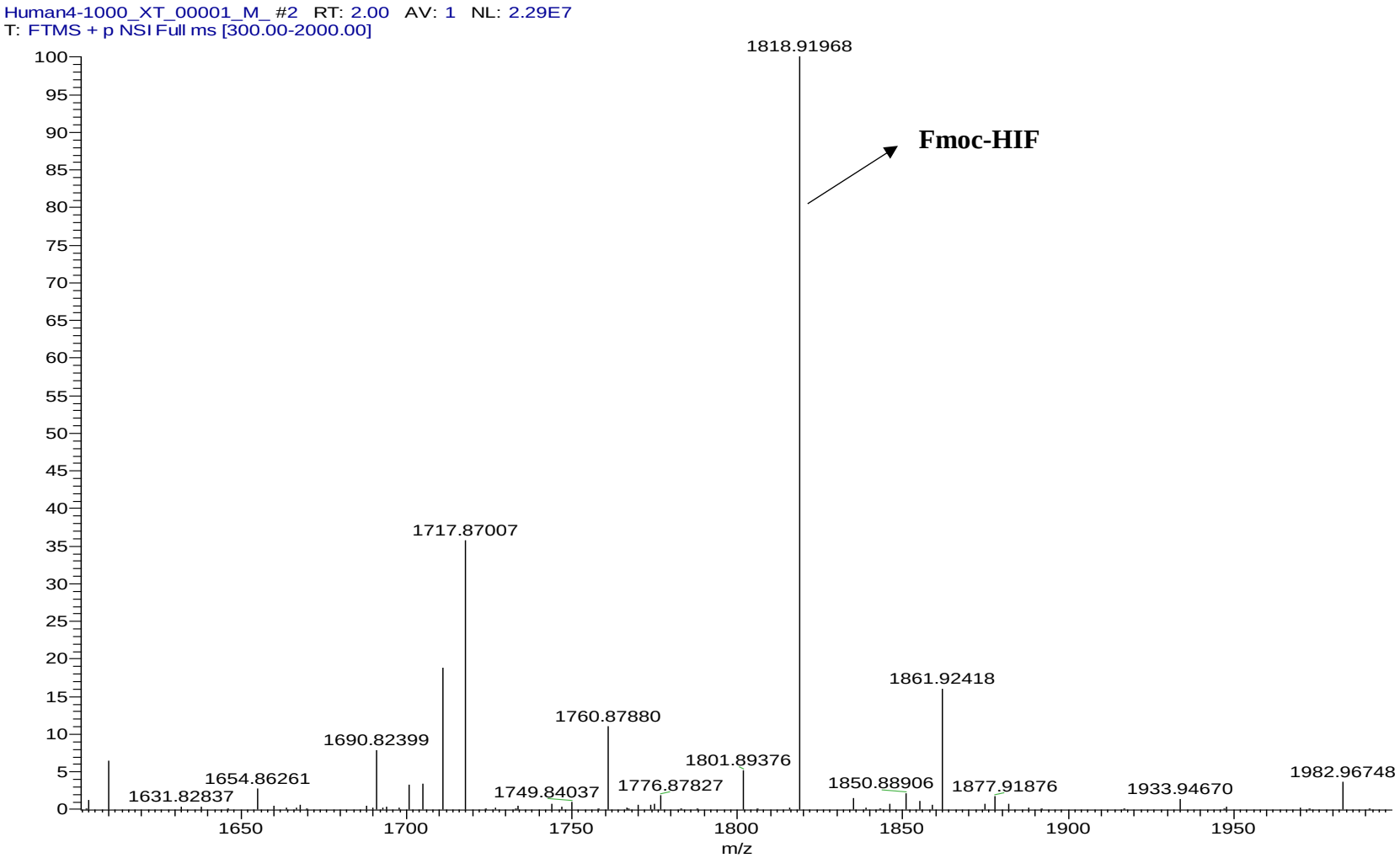


Figure S16A. LC-MS graph of fibrils obtained after the experiment for BIF at pH 1.6, 60°C, 1M urea, 0.02M NaCl showing the relative abundance of Fmoc-HIF.

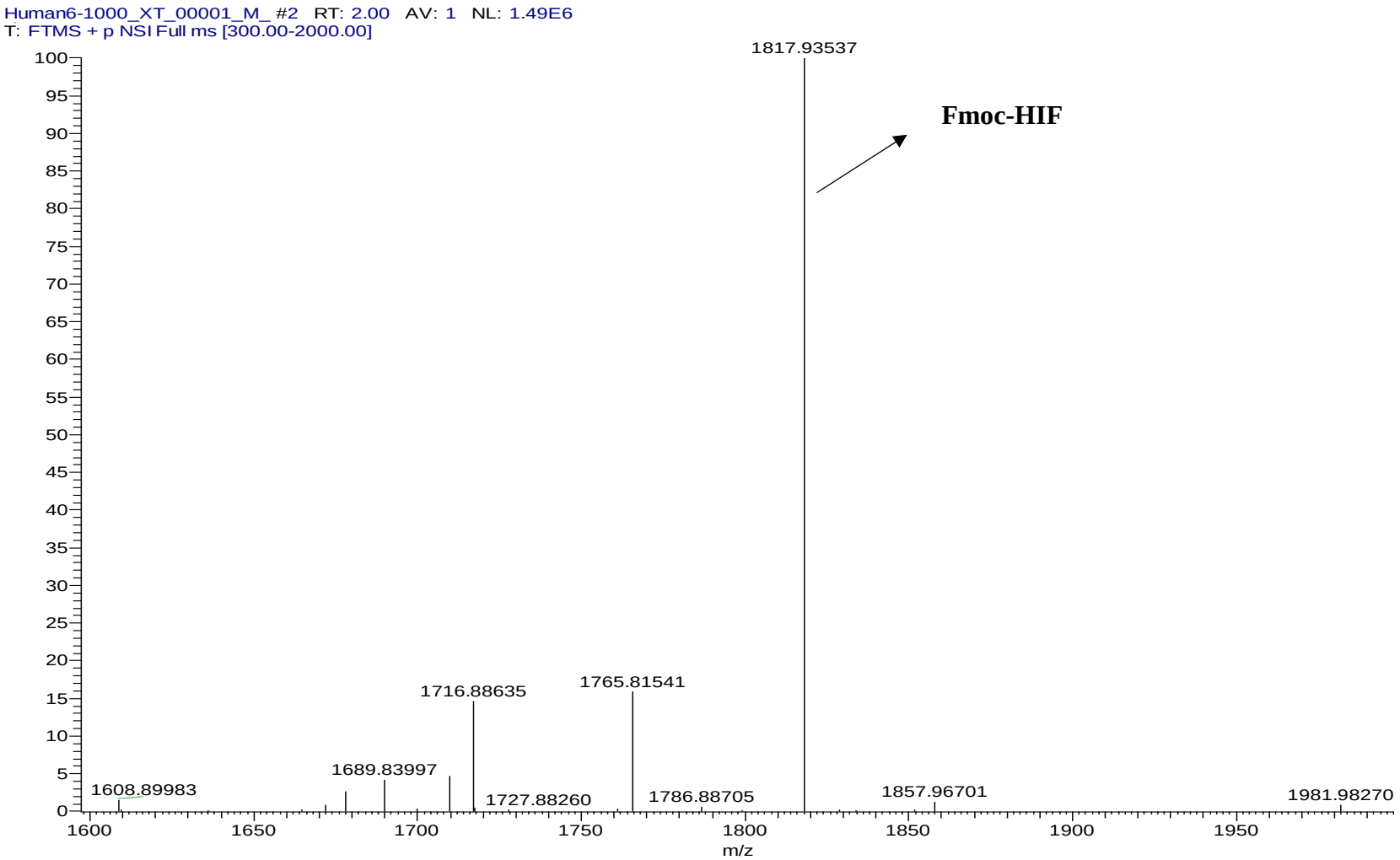


Figure S16B. LC-MS graph of fibrils obtained after the experiment for BIF at pH 5, 25°C, 1M urea, 0.02M NaCl showing the relative abundance of Fmoc-HIF.

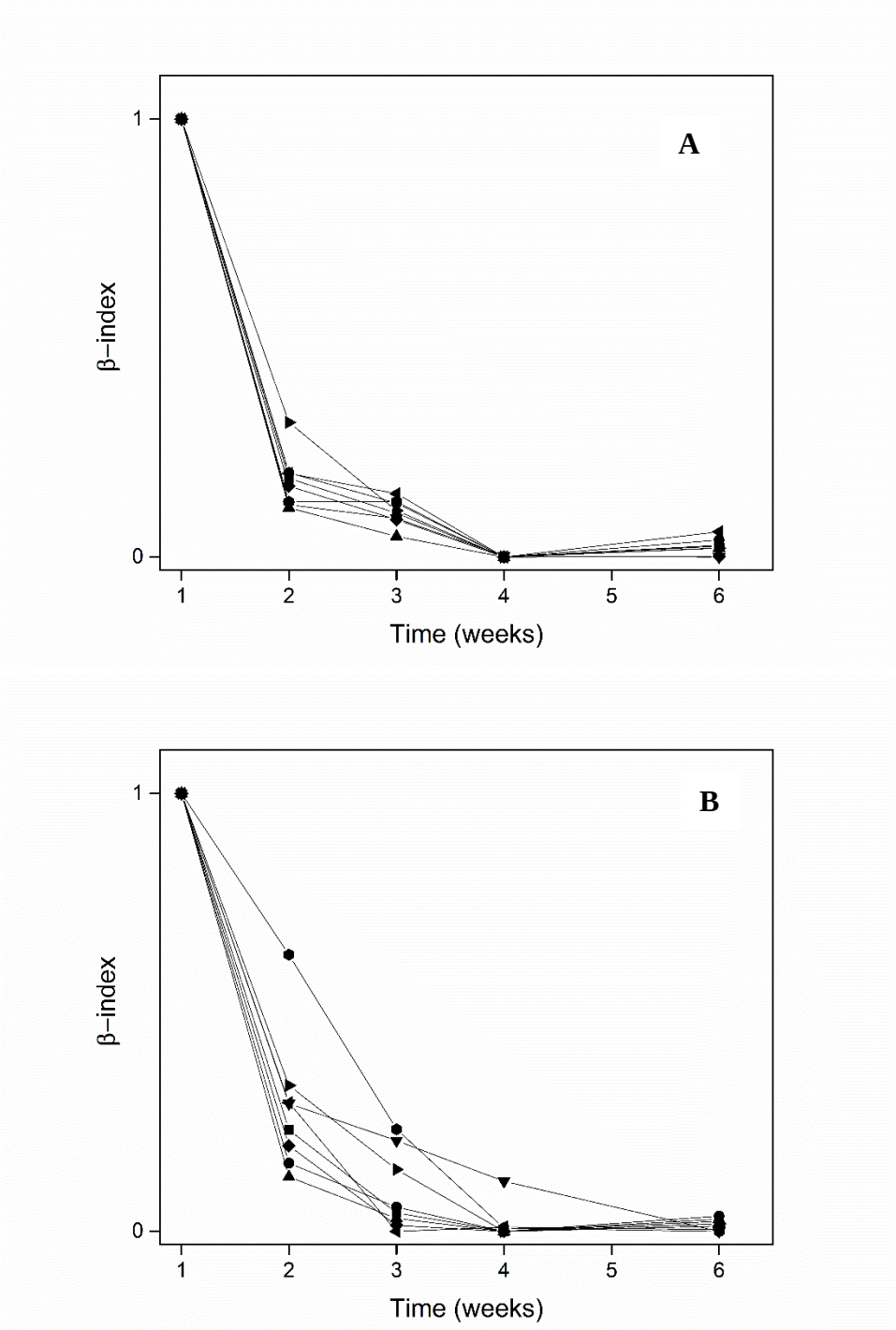


Figure S17. β-index for A) BIF and B) HIF at pH 1.6, 25°C, 0M urea, 0.02M NaCl (—■—); pH 1.6, 25°C, 1M urea, 1M NaCl (—●—); pH 1.6, 60°C, 0M urea, 1M NaCl (—▲—); pH 1.6, 60°C, 1M urea, 0.02M NaCl (—▼—); pH 5, 25°C, 0M urea, 1M NaCl (—◆—); pH 5, 25°C, 1M urea, 0.02M NaCl (—◀—); pH 5, 60°C, 0M urea, 0.02M NaCl (—▶—); pH 5, 60°C, 1M urea, 1M NaCl (—⬢—)

Table S1. ^{1}H NMR chemical shifts for BIF.

Proton chemical shift (ppm)					
Amino Acid	H_α	H_β	H_γ	H_δ	H_ε
Ala	4.25	1.35	-	-	-
Ser	4.46	3.65	-	-	-
Val	4.17	2.28	0.96	-	-
Cys	4.62	3.32	-	-	-
Leu	4.35	1.77	-	0.66	-
Tyr	-	2.84	-	7.07,6.59	-
Gln	4.21	2.09	2.5	-	6.67
Glu	4.01	1.9	2.24	-	-
Asn	-	2.76	-	-	-
Lys	4.29	1.81	1.39	1.58	2.88

Table S2. ^{1}H NMR chemical shifts for HIF.

Proton chemical shift (ppm)					
Amino Acid	H_α	H_β	H_γ	H_δ	H_ε
Thr	4.38	4.11	1.09	-	-
Ser	4.46	3.65	-	-	-
Ile	3.99	1.78	1.21	0.67	-
Cys	4.62	3.32	-	-	-
Leu	4.35	1.77	-	0.66	-
Tyr	-	2.84	-	7.07,6.59	-
Gln	4.21	2.09	2.5	-	6.67
Glu	4.01	1.9	2.24	-	-
Asn	-	2.76	-	-	-
Lys	4.29	1.81	1.39	1.58	2.88

Table S3. FTIR deconvoluted peaks corresponding to HIF at pH 1.6, 60 °C, 1M urea, 0.02M NaCl.

Wavenumber (cm^{-1})	Week-1	Week-6
HIF	1633	1619
	1667	1649
	1686	1696