Supporting Information

Secondary Structure in the Core of Amyloid Fibrils Formed from Human β_2m and Its Truncated Variant ΔN6

Yongchao Su [a, ‡], Claire J. Sarell [b, ‡], Matthew T. Eddy [a], Galia T. Debelouchina [a, §], Loren B. Andreas [a], Clare L. Pashley [b], Sheena E. Radford [b], Robert G. Griffin [a,*]

[a] *Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology Cambridge, MA, 02139, USA*

[b] *Astbury Centre for Structural Molecular Biology and School of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom*

Running title: Backbone Conformation of different hβ_2m fibrils

* Corresponding author: Robert G. Griffin, Tel: 617-253-5597, Fax: 617-253-5404, E-mail: rgg@mit.edu

‡ Y.S. and C.J.S. have equal contributions.

§ Current address: Department of Chemistry, Princeton University, Princeton, NJ 08544

Experiment details of MAS NMR

Approximately 45 mg of hydrated uniformly ^{13}C, ^{15}N-labeled fibrils were sufficient to fill a thin-wall Bruker rotor for 3D solid-state MAS NMR experiments. For specifically labeled samples, approximately 25-35 mg of hydrated fibrils was packed into a 3.2 mm rotor for 2D experiments.

All spectrometers are equipped with triple-resonance $^{1}H/^{13}C/^{15}N$ 3.2 mm MAS Bruker E^{free} probes (Bruker BioSpin, Billerica, MA). Typical $\pi/2$ pulse lengths were 2.5-3 μs for ^{1}H, 3-5 μs for ^{13}C, and 5-6 μs for ^{15}N. Unless otherwise indicated, an 83.3 or 100 kHz ^{1}H TPPM decoupling with carefully optimized phase and pulse length was applied during indirect evolution and acquisition periods for all experiments [1]. Chemical shifts were calibrated relative to DSS, using adamantine as a secondary standard. The temperature of approximately 275 K for all experiments was maintained by a stream of nitrogen gas from either the Kinetics Thermal System XR air-jet system (Stone Ridge, NY) on the 750 MHz spectrometer or the Bruker BCU system (Bruker BioSpin, Billerica, MA) on the 800 MHz and 900 MHz spectrometers.

For 1D experiments, a ^{13}C-^{1}H CP contact time of ~ 1.5 ms was used. For DP and INEPT, a recycle delay of 5-5.5 s was used to allow sufficient relaxation.

For 2D ^{13}C-^{13}C experiments, broadband RFDR spectra were acquired at 18 kHz or 20 kHz spinning frequency. A 6 μs π pulse was placed in the middle of each rotor cycle of the rotor-synchronized RFDR mixing period to recouple the ^{13}C-^{13}C dipolar coupling. A 100 kHz continuous-wave (CW) decoupling was applied to the ^{1}H channel during the RFDR period. A total mixing time, τ_{RFDR}, of 1.6 ms was used to establish adjacent ^{13}C-^{13}C correlations. In the case of band-selective RFDR, a relatively weak 12.5 μs π pulse was used to excite the aliphatic resonances and a long τ_{RFDR} of 16.2 ms was used for the neighboring Cα-Cα correlations. A 32-step phase cycling was used to compensate the chemical shift offsets and rf inhomogeneity for weak π pulses.

For 2D ^{15}N-^{13}C experiments, the ZF TEDOR is a modified TEDOR experiments consisting of two z-filter periods, removing unwanted multiple-quantum and anti-phase spin coherences from spin evolution ^{13}C-^{13}C J couplings. All ZF TEDOR experiments were conducted at $\omega_r/2\pi$ of 12.5 kHz and 18 kHz spinning frequencies on 750 MHz and 800 MHz spectrometers (^{1}H frequency), respectively. Two π

pulses (a pulse length of 12 μs for each one) per rotor period were applied on the ^{15}N channel and an xy-4 phase cycling scheme was used. The total dipolar recoupling time (τ_{TEDOR}) of 1.6 ms and 6.4 ms was used for one-bond and multi-bond ^{15}N-^{13}C correlations. ^{1}H TPPM decoupling at 95 kHz was used during τ_{TEDOR}. The PAIN-CP experiment was performed with $\omega_r/2\pi$ of 20 kHz MAS on the 900 MHz ^{1}H frequency. A mixing time of approximately 8 ms was used. ^{15}N-^{1}H CP contact time was 1.6 ms to 2.5 ms for all ZF TEDOR and PAIN-CP experiments.

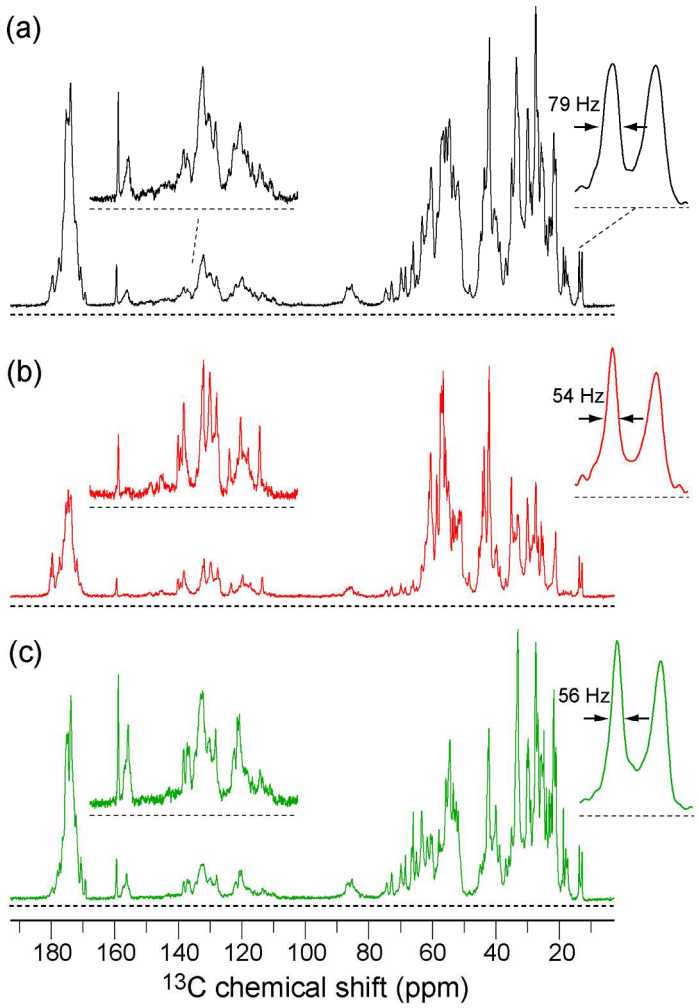
Two kinds of 3D experiments are carried out for inter- or intra-residue correlations. Inter- or intra-residue N-C transfer was achieved by utilizing the band-selective SPECIFIC-CP in conventional N-C-C pulse sequences. Sampling of t1 and t2 dimensions was rotor-synchronized with the spinning frequency to fold the rotational sidebands. For the NCOCX experiment, the ^{15}N and ^{13}CO spectral width were 4.2 kHz and 6.3 kHz, respectively. 64 t1 points and 88 t2 points were used, giving the maximum evolution time of 7.7 and 7.0 ms, respectively. For NCACX experiments, the ^{15}N and ^{13}CO spectral width were 4.2 kHz and 12.5 kHz, respectively. 48 t1 points and 80 t2 points were used, giving the maximum evolution time of 5.8 and 3.5 ms, respectively. The ^{13}C-^{13}C mixing time of 60 ms and 80 ms was used for NCOCX and NCACX experiments, respectively. For CONCA experiments, the ^{15}N and ^{13}CO spectral width were 6.3 kHz and 4.2 kHz, respectively. The maximum t1 and t2 evolution time was 5.1 ms and 7.8 ms, respectively. All these SPECIFIC-CP based experiments were conducted under 12.5 kHz spinning frequency on the 750 MHz spectrometer (^{1}H frequency). The magnetic field lock was achieved using a home-built field mapping unit (FMU) system by monitoring the ^{1}H signal of H_2O. Each experiment has 8 transients and two consecutive experiments were conducted and added for better signal average, giving a total of ~ 7-10 days measurement time for each 3D spectrum.

In contrast, simultaneous ^{15}N-^{13}CO and ^{15}N-$^{13}C\alpha$ transfers in TEDOR-CC were achieved using ZF TEDOR [2,3]. This pulse program was designed as described in our recent work and was successfully applied for the assignment of drug-resistant S31N M2 proton transporter from influenza A [4]. After one-bond ^{15}N-^{13}C transfer, ^{13}C–^{13}C magnetization transfer was achieved through a RFDR recoupling period of 4.8 ms in the current study. Phase alternations of xy-4 and xy-16 were used for TEDOR mixing and RFDR pulses, respectively. We measured TEDOR-CC spectra under 20 kHz MAS on the 900 MHz spectrometer. Optimized linear field compensation was applied to correct the field drift. The ^{15}N spectral width was 6.7 kHz and the maximum t_1 evolution time was 11.1 ms, corresponding to 148 t_1 points. The

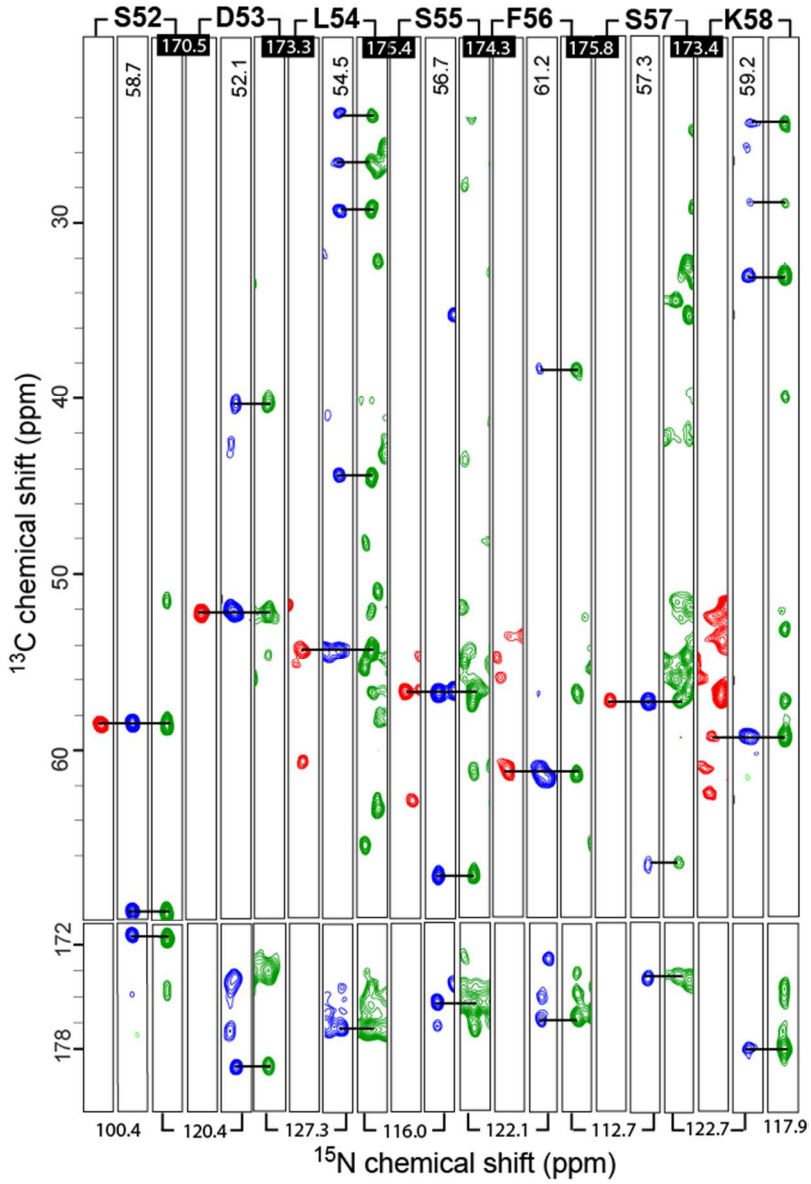
^{13}C spectral width was 20 kHz and the number of t_2 points was 354, giving a maximum t_2 of 8.9 ms. An 83 kHz ^{1}H decoupling field was used during TEDOR mixing and detection. A 100 kHz CW ^{1}H decoupling was used in the period of the 6-ms π pulse in RFDR mixing. The total experiment time was approximately 5 days. NCOCX and NCACX regions were extracted respectively from the single TEDOR-CC spectrum.

SI Table 1. NMR chemical shifts of P32 and C80 of $h\beta_2m$ and ΔN6 in native and fibril forms. Chemical shift of native $h\beta_2m$ and ΔN6 proteins were taken from [5].

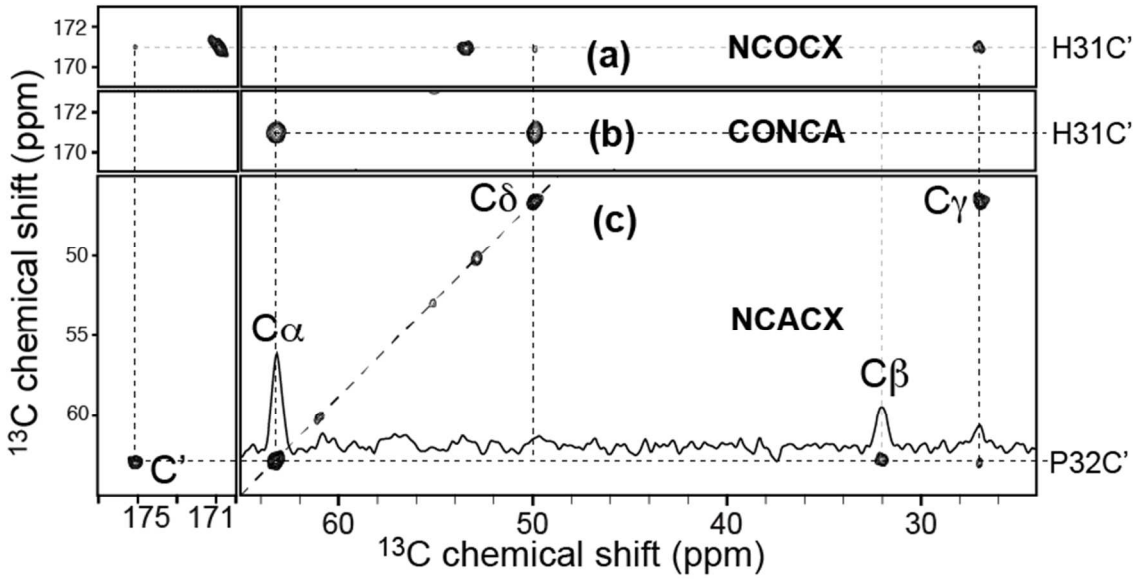
Protein	Residue	Form	N	C'	C^{α}	C^{β}	C^{γ}	C^{δ}
$h\beta_2m$	P32	*native*	-	176.7	62.6	35.0	25.0	50.5
		fibril	130.9	176.4	63.6	32.0	27.7	51.7
	C80	*native*	119.9	171.2	52.9	43.5	-	-
		fibril	120.1	173.1	55.1	43.1	-	-
ΔN6	P32	*native*	103.9	178.5	64.5	31.5	27.2	50.3
		fibril	133.4	175.1	63.4	32.7	27.8	50.3
	C80	*native*	120.0	171.1	53.1	43.3	-	-
		fibril	117.4	173.7	60.4	42.1	-	-



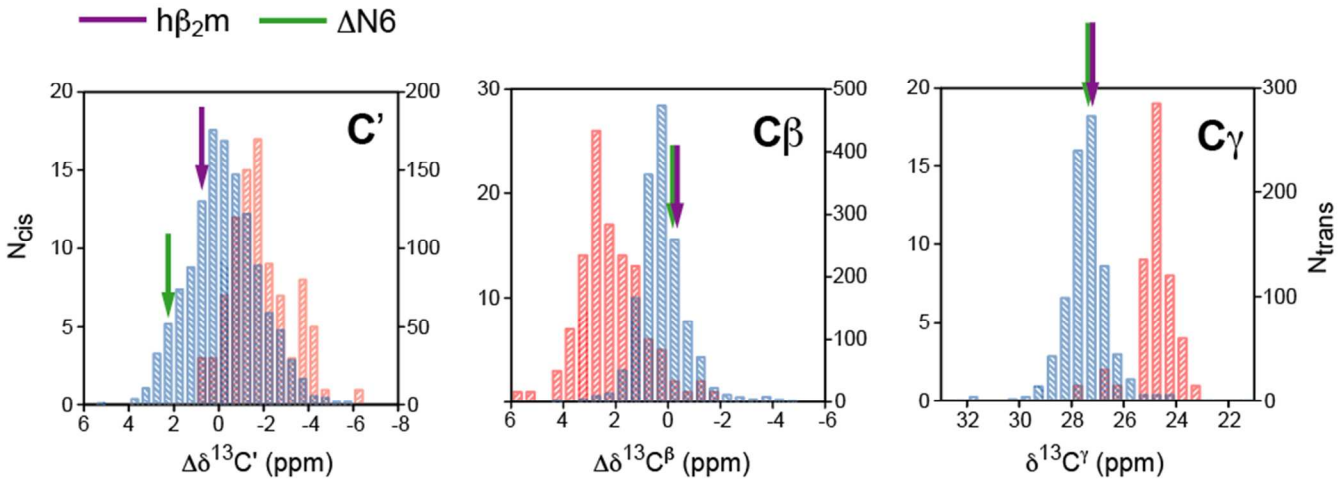
SI Figure 1. Comparison of 1D ^{13}C spectra of ΔN6 fibrils recombinantly expressed using different ^{13}C sources, showing the high quality of the samples. (a) U-ΔN6, (b) 2-ΔN6 and (c) 1,3-ΔN6 fibrils. All spectra were collected under 20 kHz MAS frequency, 100 kHz ^{1}H TPPM decoupling, at approximately 270 K on a 900 MHz spectrometer.



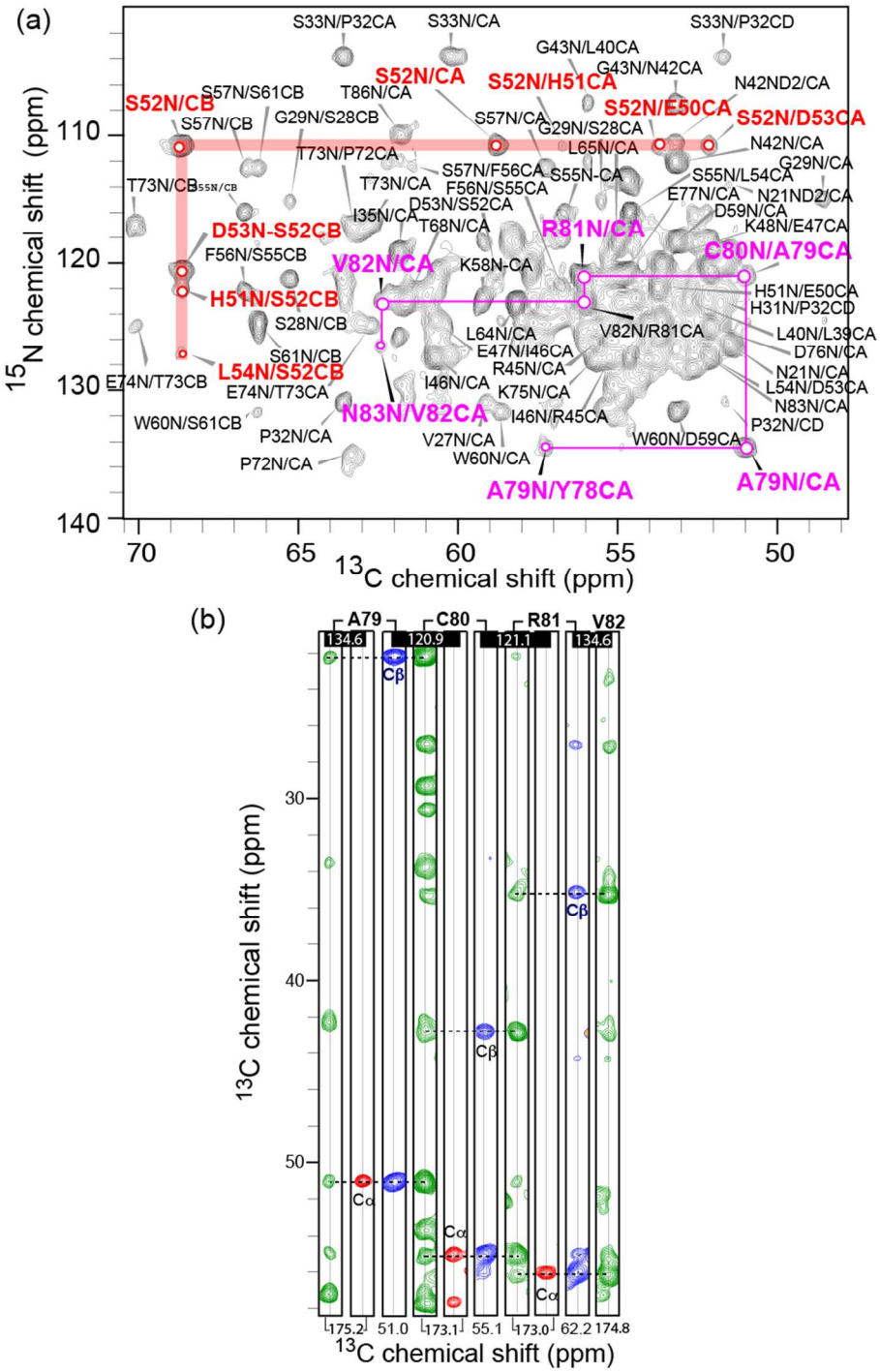
SI Figure 2. Representative sequential backbone walks from S52 to K58 in 3D correlation experiments (NCOCX, CONCA and NCACX) of hβ$_2$m fibrils. CO chemical shifts are shown in black squres. ^{15}N chemical shifts are shown at the bottom. Cα chemical shifts are shown inside the spectra. The spectra were recorded from a 45-mg [U-^{13}C, ^{15}N-labeled]-hβ$_2$m fibril sample at 750 MHz and show a similar resolution and dispersion to the ΔN6 spectra (**Fig. 5**, main text).



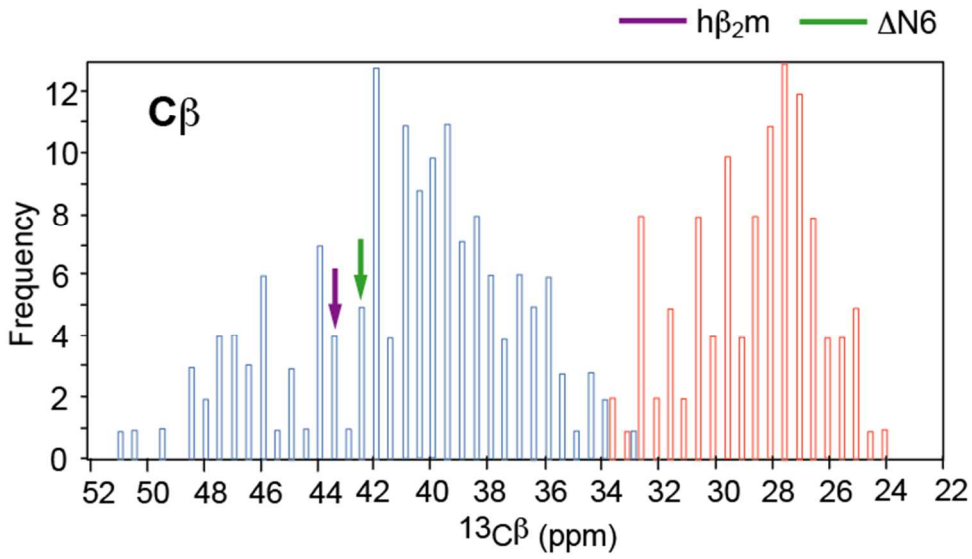
SI Figure 3. Assignment of H31-P32 from 3D correlation experiments. Efficient ^{15}N-^{13}C-^{13}C correlations of P32 of ΔN6 fibrils in the 3D spectra: (a) NCOCX, (b) CONCA and (c) NCACX. The ^{13}C-^{13}C planes at ^{15}N of P32 from ΔN6 fibrils (133.4 ppm) are shown. All experiments utilize ^{13}C-^{1}H CP to generate the initial magnetization, which significantly enhance the intensity of proline. NCOCX and NCACX were obtained using the TEDOR-CC experiment, which utilizes TEDOR scheme for N-C magnetization transfer. The obtained chemical shift identified its trans-conformation.



SI Figure 4. Identification of bond conformation of H31-P32 in hβ$_2$m and ΔN6 fibrils by comparing the C'/Cβ/Cγ chemical shift to folded proteins from the biological magnetic resonance bank (BMRB). Histograms show secondary chemical shift of C' and Cβ and chemical shift of Cγ for Pro residues preceded by a cis (red) or trans (blue) bond conformation. The histogram is reproduced with permission from Shen and Bax [6]. Secondary chemical shifts of C' and Cβ are calculated relative to the random coil values used in TALOS+ program. As indicated by violet and green arrows, respectively, for hβ$_2$m and ΔN6, H31-P32 adopts a trans-conformation for both fibril types.



SI Figure 5. Representative 2D and 3D spectra of hβ$_2$m fibrils to show the assignment of C80. (a) PAIN-CP spectra of [U-^{15}N- and 1,3-$^{13}C_2$-glycerol]-labeled hβ$_2$m. Violet solid lines guide the sequential connectivity of Y78-A79-C80-R81-V82. Pink stripes show the connectivity between S52 with its neighboring residues, E50, H51, D53 and L54, indicating the efficient correlation of the PAIN-CP experiment. (b) Backbone walks from A79 to V82. 2D and 3D spectra were acquired on 900 and 750 MHz spectrometers, respectively.



SI Figure 6. Identification of redox state of C80 in fibrils formed from hβ$_2$m and ΔN6 by analysis of their Cβ chemical shifts. Histograms show the distribution of Cβ chemical shifts of oxidized (red) and reduced (blue) cysteines in different proteins. The histogram is reproduced with permission from Sharma and Rajarathnam [7]. As indicated by violet and green arrows for hβ$_2$m and ΔN6 fibrils, respectively, the chemical shifts of Cβ for C80 are consistent with an oxidized state, suggesting that the disulfide bond between C25 and C80 is intact in both fibril types.

Supplemental References

(1) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951.

(2) Hing, A. W.; Vega, S.; Schaefer, J. *J. Magn. Reson.* **1992**, *96*, 205.

(3) Hing, A. W.; Vega, S.; Schaefer, J. *Journal of Magnetic Resonance Series A* **1993**, *103*, 151.

(4) Andreas, L. B.; Eddy, M. T.; Chou, J. J.; Griffin, R. G. *J. Am. Chem. Soc.* **2012**, *134*, 7215.

(5) Eichner, T.; Kalverda, A. P.; Thompson, G. S.; Homans, S. W.; Radford, S. E. *Mol. Cell* **2011**, *41*, 161.

(6) Shen, Y.; Bax, A. *Journal of biomolecular NMR* **2010**, *46*, 199.

(7) Sharma, D.; Rajarathnam, K. *J. Bio. NMR* **2000**, *18*, 165.