



Supporting Information

© Wiley-VCH 2008

69451 Weinheim, Germany

# Polymorphism in an amyloid-like fibril forming model peptide.

*René Verel, Ivan T. Tomka, Carlo Bertozzi, Riccardo Cadalbert, Richard A. Kammerer, Michel O. Steinmetz, and Beat H. Meier*

## **Experimental Details**

cc $\beta$ -p peptide with natural isotopic abundance and two specifically  $^{15}\text{N}/^{13}\text{C}$  labeled variants were synthesized on an Applied Biosystems 433A automated batch peptide synthesizer starting from a Fmoc-PAL-PEG-PS resin using standard protocols for fmoc synthesis and cleavage<sup>[1]</sup>. One of the labeled cc $\beta$ -p peptides (Compound I) contained a  $^{15}\text{N}$  label (98%) at the amide nitrogen of Ala7 and a  $^{13}\text{C}$  label (99%) at the carbonyl carbon of Leu14. The other labeled cc $\beta$ -p peptide (Compound II) contained a  $^{15}\text{N}$  label (98%) at the amide nitrogen of Ile2 and a  $^{13}\text{C}$  label (99%) at the carbonyl carbon of Leu14. Isotopically labeled, protected amino acids were obtained from Cambridge Isotopes Laboratories. The raw product was dried and dissolved in trifluoroethanol. Purification was done on a Merck/Hitachi HPLC system using a LiChrospher 100 RP-18 reversed phase C-18 column (Merck KGaA, Darmstadt Germany). A linear gradient from 100% H<sub>2</sub>O/0.2% TFA to 57% Acetonitrile/0.2% TFA was used. The fraction with the pure cc $\beta$ -p was lyophilised and stored at  $-18^{\circ}\text{C}$  prior to use. Product mass of the product was determined to be within 0.1% of the expected mass by MALDI-TOF mass spectrometry. In addition the purity was above 95% pure as determined by analytical HPLC.

Fibrils were prepared by dissolving the lyophilised cc $\beta$ -p in water of  $4^{\circ}\text{C}$  to a concentration of approximately 5 mM. The resulting solution had a pH of approximately 3.5. The pH was adjusted to the desired value by adding 0.1 M NaOH or 0.1 M HCl. All samples were prepared in pure water as a solvent except for the samples prepared at pH=7.3 where a 20 mM Sodium Phosphate buffer was used to aid with the adjustment of the pH. After adjustment of the pH the solution was centrifuged in an Eppendorf 5403 at 5000 RPM for 10 minutes to separate any undissolved material. The supernatant was transferred to a new tube and incubated at  $43\pm 3^{\circ}\text{C}$  for at least 6 hours. Subsequently the sample was heated to  $90^{\circ}\text{C}$  for 3 minutes to fibrillise any remaining material. The fibrils were sedimented in a Beckman-Coulter XLA bench top ultra centrifuge (Beckmann Coulter Inc., Fullerton, CA, USA) for 3 hours at 55000 RPM. Finally the pellet was dried under a gentle stream of N<sub>2</sub>(g). TEM micrographs of cc $\beta$ -p fibrils prepared at pH=7.4 and pH=2.0 are shown in Figure S1. The same temperatur protocol was used for other characterizations (ref 11 and 12 of main text).

The dry samples were packed into a 4mm outer diameter, ZrO Varian/Chemagnetics type pencil rotor. Typically 10 mg of material was restricted to a length of 3 mm in the central part of the rotor by means of Teflon spacers. 1D CP MAS  $^{13}\text{C}$  and  $^{15}\text{N}$  spectra acquired at  $-80^{\circ}\text{C}$ ,  $6000\pm 2$  Hz spinning frequency and with TPPM decoupling with an amplitude of  $\sim 115$  kHz are shown in Figures S2 and S3. The spectra were processed without apodisation and show a dominant and rather broad signal caused by the  $^{13}\text{C}$  and  $^{15}\text{N}$  label respectively. The FWHM of the peaks are 3.4 ppm for both  $^{13}\text{C}$  spectra and 10.5 ppm for the  $^{15}\text{N}$  spectrum.

The pulse sequence used for the REDOR experiments (Figure S4) employed an initial APHH cross polarisation period<sup>[2-4]</sup> from  $^1\text{H}$  to  $^{13}\text{C}$  and TPPM decoupling<sup>[5]</sup> with an rf amplitude of approximately 115 kHz on the proton channel during both the REDOR and acquisition periods. A REDOR pulse sequence with a single  $\pi$  pulse per rotor period on both the  $^{15}\text{N}$  and  $^{13}\text{C}$  channels was used to recouple the dipolar interaction.<sup>[6, 7]</sup> The

pulses on both channels were offset by half a rotor period with respect to each other and were phase cycled according to a XY-8 scheme<sup>[8]</sup> to reduce the effect of pulse errors. The duration of the  $\pi$  pulse on the  $^{13}\text{C}$  channel was 10  $\mu\text{s}$  and on the  $^{15}\text{N}$  channel 12.5  $\mu\text{s}$ . The REDOR dephasing period was incremented from 0 to 74.67 ms ( $448\tau_r$ ) in steps of 5.33 ms ( $32\tau_r$ ) for a total of 15 points. Free induction decays for the dephased and non-dephased ( $^{15}\text{N}$  pulse amplitude set to zero) signals were acquired in an interleaved manner with a recycle delay between scans of 3 s. A total of 2048, 4096 and 8192 scans were co-added for REDOR points 1 through 5, 6 through 10 and 11 through 15 respectively for all samples.

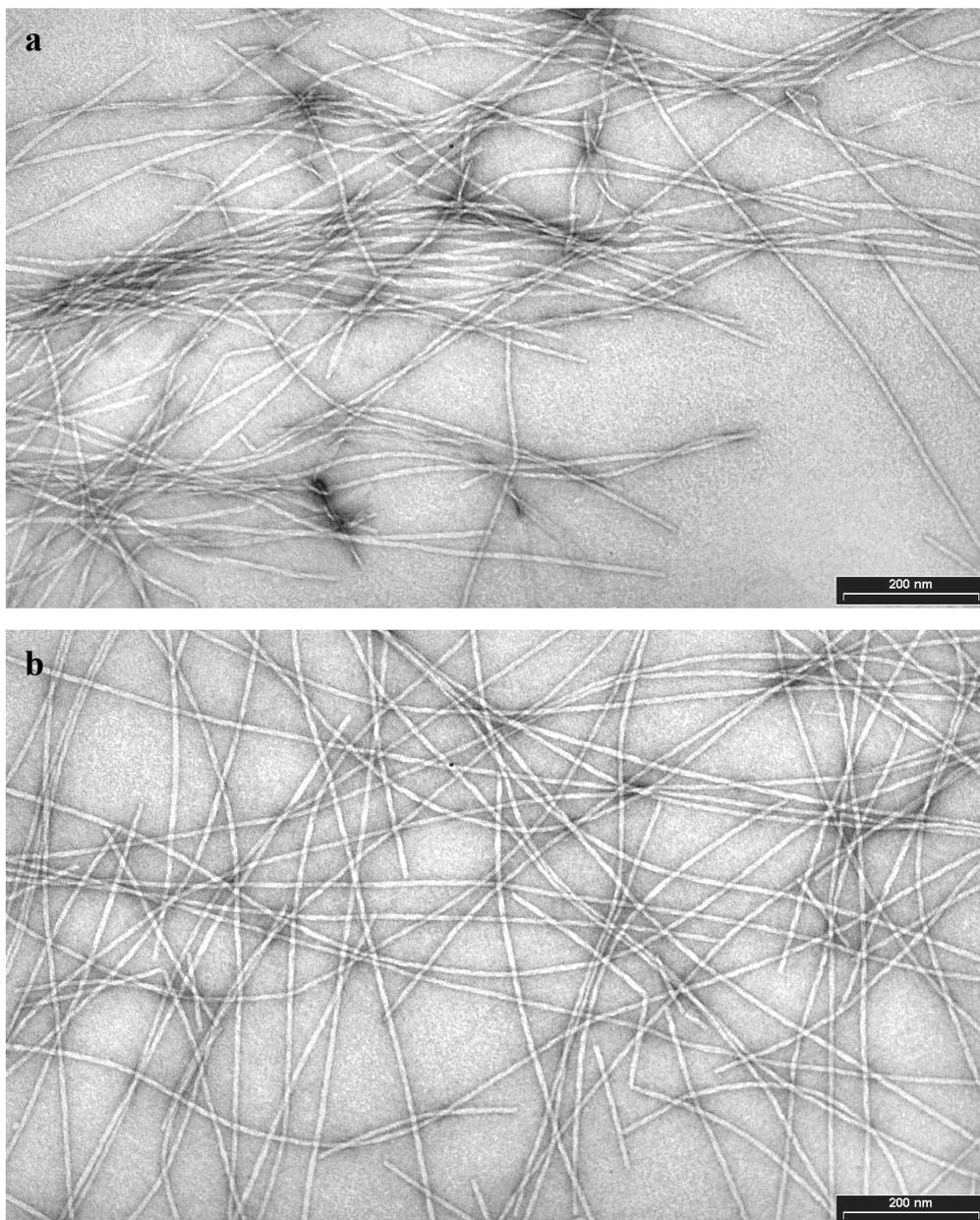
Processing of the data was done with custom written Matlab scripts. The experimental REDOR points were calculated by summation of the intensities of the data points in a region of interest which included all carbonyl resonances (but did not cover the Arginine  $\zeta$  peak around 156 ppm). Simulations were calculated using a combination of Matlab scripts and C++ programs using the GAMMA environment<sup>[9]</sup>. Model structures on which the simulations are based were generated with CYANA<sup>[10]</sup>. Fitting of the simulated to experimental data was done with Matlab Scripts.

The calculated REDOR dephasing curves were based on idealised model structures of the two  $\beta$ -sheet registries. The contribution to the signal of each carbonyl found in cc $\beta$ -p was taken into account (22 in total; 17 amide CO, 4 glutamic acid CO and 1 Acetyl CO). The contributions were weighted by the amount of  $^{13}\text{C}$  (21 at 1.1% natural abundance level and one selectively labelled at 99%). In addition for each carbonyl position the configuration and probability of having one or more  $^{15}\text{N}$  in close proximity was analysed and the corresponding REDOR dephasing signal was calculated. No dephasing by  $^{15}\text{N}$  was assumed if the distance between the relevant  $^{13}\text{C}$  and  $^{15}\text{N}$  was larger than 7  $\text{\AA}$ . Finally, contributions by configurations which contained two or more nuclei at natural abundance level were neglected. For structures in which the labels are close together (e.g. as depicted in Figure 1b and 1c), the dominant contribution to the dephasing curve (statistical weight of  $\sim 75\%$ ) is caused by the three spin system consisting of the specifically labelled  $^{13}\text{C}$  and two specifically labelled  $^{15}\text{N}$ 's on the previous and next  $\beta$ -strand. To improve the quality of the simulation the local geometry of this three spin system was optimised against the experimental data of figure 2a to produce an optimal fit. This produced small changes in the distance ( $\sim 0.5$   $\text{\AA}$ ) compared to the idealised structure. For the structures in which the distance between the labels is big (e.g. as depicted in Figure 1a and 1d) no local optimisation was performed. The experimental data at various values of pH were fitted by a linear combination of the simulated dephasing curves of the two registers.

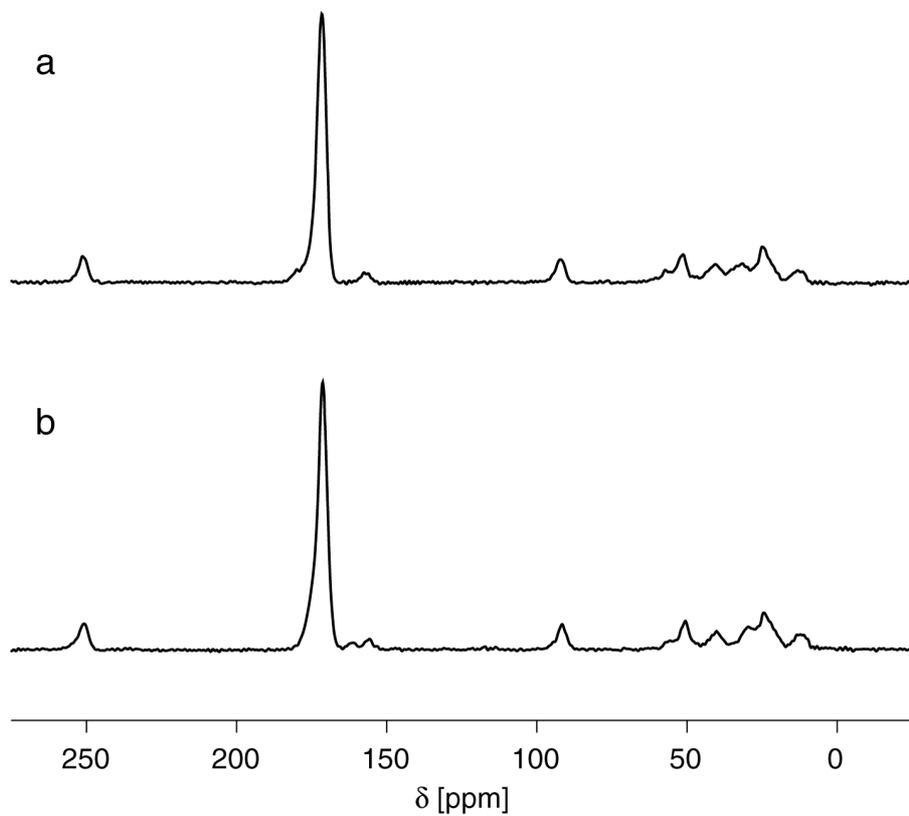
## References

- [1] W. C. Chan, P. D. White, *Fmoc solid phase peptide synthesis: a practical approach*, Oxford University Press, Oxford/New York, **2000**.
- [2] S. R. Hartmann, E. L. Hahn, *Phys. Rev.* **1962**, *128*, 2042.
- [3] S. Hediger, B. H. Meier, R. R. Ernst, *Chem. Phys. Lett.* **1995**, *240*, 449.
- [4] S. Hediger, B. H. Meier, N. D. Kurur, G. Bodenhausen, R. R. Ernst, *Chem. Phys. Lett.* **1994**, *223*, 283.
- [5] A. E. Bennett, C. M. Rienstra, M. Auger, K. V. Lakshmi, R. G. Griffin, *J. Chem. Phys.* **1995**, *103*, 6951.
- [6] T. Gullion, J. Schaefer, *J. Magn. Reson.* **1989**, *81*, 196.
- [7] T. Gullion, J. Schaefer, *Adv. Magn. Reson.* **1989**, *13*, 57.
- [8] T. Gullion, D. B. Baker, M. S. Conradi, *J. Magn. Reson.* **1990**, *89*, 479.

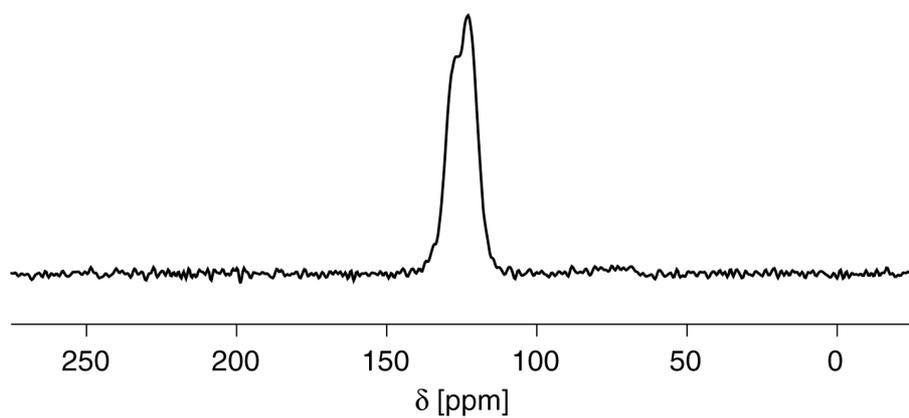
- [9] S. A. Smith, T. O. Levante, B. H. Meier, R. R. Ernst, *J. Magn. Reson., Ser A* **1994**, *106*, 75.
- [10] P. Guntert, C. Mumenthaler, K. Wuthrich, *J. Mol. Biol.* **1997**, *273*, 283.
- [11] W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graphics* **1996**, *14*, 33.



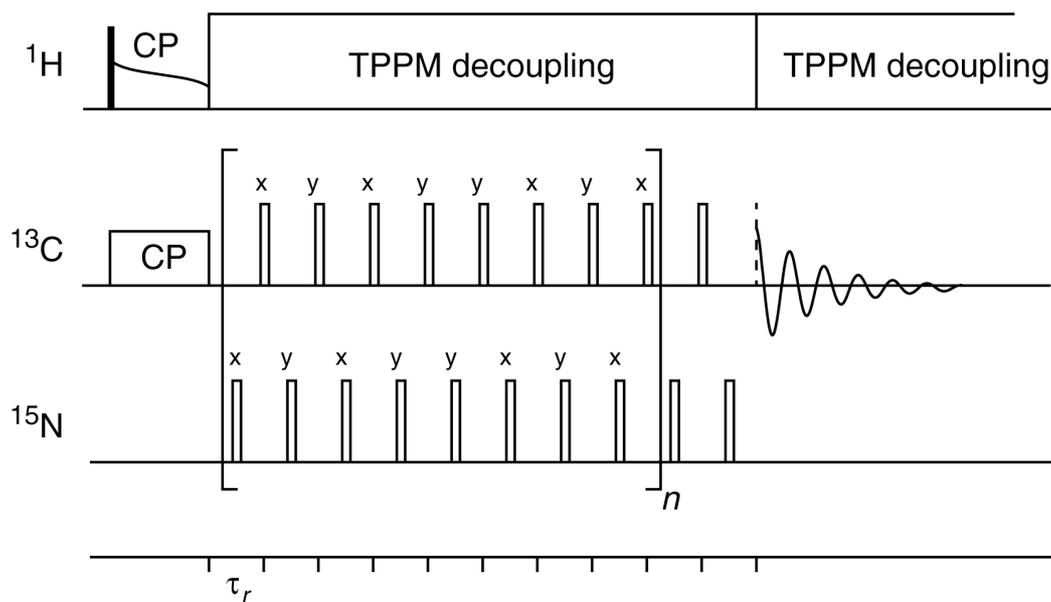
**Figure S1.** TEM micrographs of negatively stained cc $\beta$ -p fibrils obtained at pH 7.4 (a) and at pH 2.0 (b).



**Figure S2.**  $^{13}\text{C}$  CP spectra of cc $\beta$   $^{15}\text{N}$ -Ala7  $^{13}\text{C}$ -Leu14 fibrils prepared at pH=7.3 (a) and pH=2.0 (b)



**Figure S3.**  $^{15}\text{N}$  CP spectrum of cc $\beta$   $^{15}\text{N}$ -Ala7  $^{13}\text{C}$ -Leu14 fibrils prepared at pH=7.3



**Figure S4.** REDOR pulse sequence with APHH CP and TPPM  $^1\text{H}$  decoupling. XY-8 phase cycling is used on  $^{13}\text{C}$  and  $^{15}\text{N}$  REDOR  $\pi$  pulses. The final  $\pi$  pulse on  $^{13}\text{C}$  is used to generate a Hahn echo at the start of the acquisition period.