Tetracycline prevents Aβ oligomer toxicity through an atypical supramolecular interaction

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Supplementary Information

S1 – NMR Characterization of Aβ1-40 and Aβ1-42 assemblie

Figure 2 (main text) shows ^{1}H-NMR spectra acquired on the same samples of Aβ1-40 or Aβ1-42 in PBS at pH 7.4 or pH 12, 5°C. For both peptides, S/N ratios were higher and ^{1}H-NMR signals sharper in the spectrum recorded at pH 12, in agreement with higher relaxation times (Table 1S and 2S). In addition, several differences in proton resonances were seen. These findings are consistent with the diffusion coefficients reported in Table 1 (main text) and demonstrate oligomerization of Aβ peptides at pH 7.4. In addition, they support a higher level of oligomerization for Aβ1-42 versus Aβ1-40.

Table 1S. T1 values of His residues for Aβ1-40 and Aβ1-42 peptide in solution at 5°C, pH 7.4 or pH 12.

Aβ sequence	pH	T1 value[a]
1-40	7.4	1.964 s
1-40	12	3.178 s
1-42	7.4	1.717 s
1-42	12	2.733 s

[a] T1 values were calculated as exponential decay of the average integral of His 6, His 13 and His 14.

Table 2S. Selective T1 values of some aromatic signals for Aβ1-40 and Aβ1-42 peptide in solution at 5°C, pH 7.4 or pH 12.

Aβ sequence	pH	signal	selT1 value[a]
1-40	7.4	His (2H)	0.680 s
		His (4H)	0.569 s
		Tyr (3H, 5H)	0.469 s
1-40	12	His (2H)	1.734 s
		His (4H)	1.385 s
		Tyr (3H, 5H)	1.143 s
1-42	7.4	His (4H)	0.296 s
		His (4H)	0.307 s
		Tyr (3H, 5H)	0.249 s
1-42	12	His (4H)	1.042 s
		His (4H)	0.866 s
		Tyr (3H, 5H)	0.896 s

[a] Selective T1 values were calculated as exponential decay of isolated signal from the aromatic region of Aβ peptide ^{1}H spectra.

Figure 1S reports DOSY spectra obtained for peptide Aβ1-40, pH 7.4, at 5, 25 or 37°C.

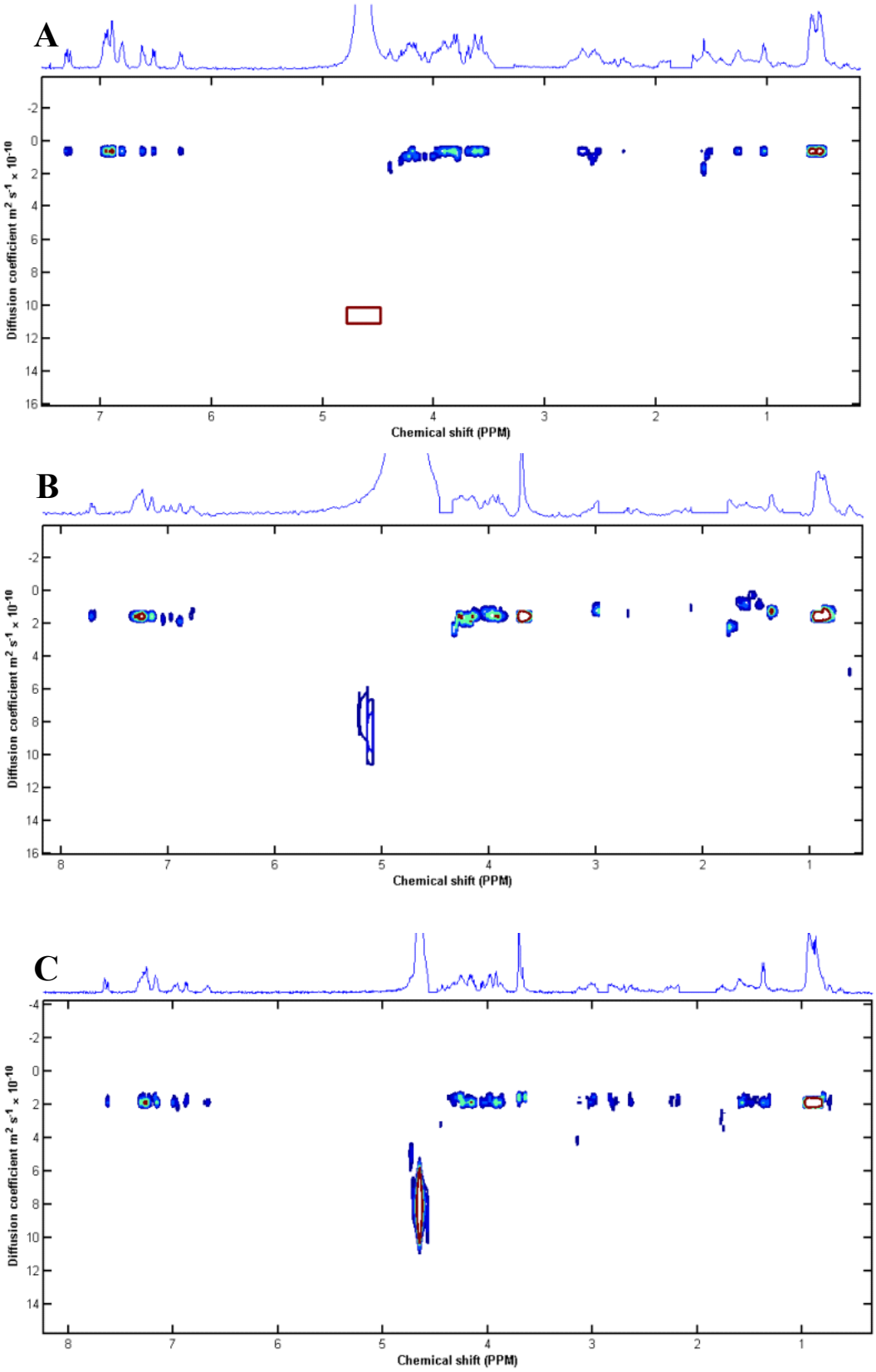

Figure 1S. 2D-DOSY spectra of Aβ1-40, 0.5 mM, at 5 (**A**), 25 (**B**) or 37°C (**C**), pH = 7.4.

S2 - Aβ1-40 and Aβ1-42 STD-NMR

STD-NMR experiments of Aβ peptide-tetracycline mixtures were carried out for both peptides at different temperatures, at several peptide:drug molar ratios, and with different frequencies for the protein envelope saturation pulse. Peptides dissolved in PBS, pH 7.4, provided clear evidence of tetracycline binding. The first example is shown in Figure 4 (main text); further experiments are illustrated in Figures 2S-6S.

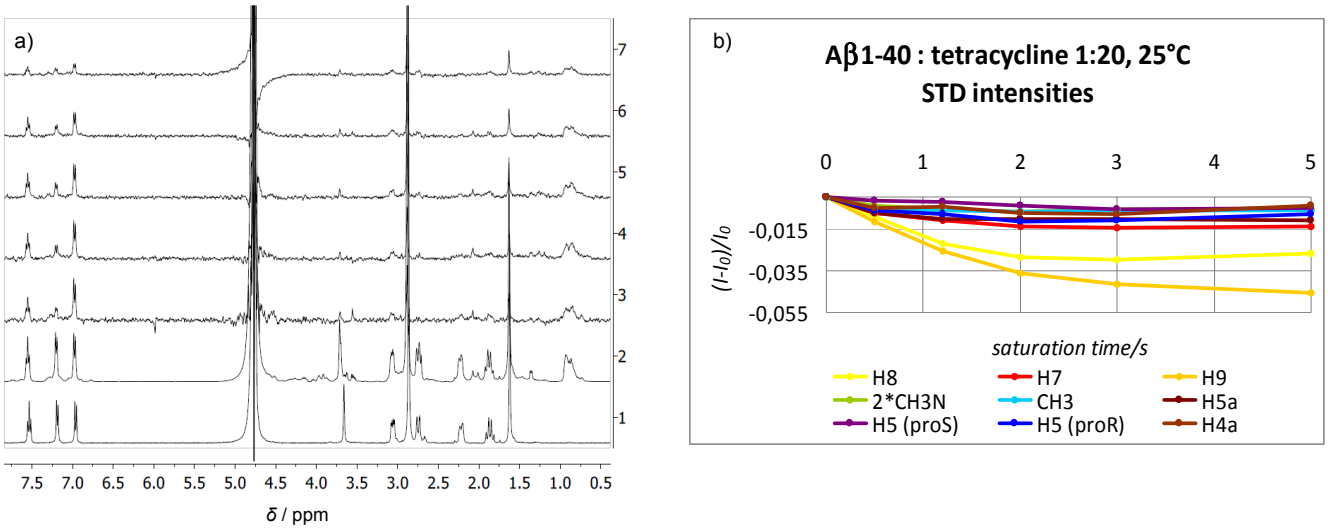

Figure 2S. a) ^{1}H NMR spectra of tetracycline, NS=32 (1), Aβ1-40-tetracycline mixture, at 1:20 molar ratio, NS=128 (2), STD spectra of the mixture recorded with different peptide saturation times (3, 5 s; 4, 3 s; 5, 2 s; 6, 1.2 s; 7, 0.5 s; enhanced 3x), NS=1792, on-resonance frequency= -1.0 ppm, off-resonance frequency=40 ppm. Spectra 2-7 were recorded on the same sample; all samples were dissolved in PBS, pH 7.4, 25°C. b) Fractional STD effects for each tetracycline proton, calculated by $(I-I_0)/I_0$, where $(I-I_0)$ is the peak intensity in the STD spectrum and I_0 is the peak intensity of an unsaturated reference spectrum.

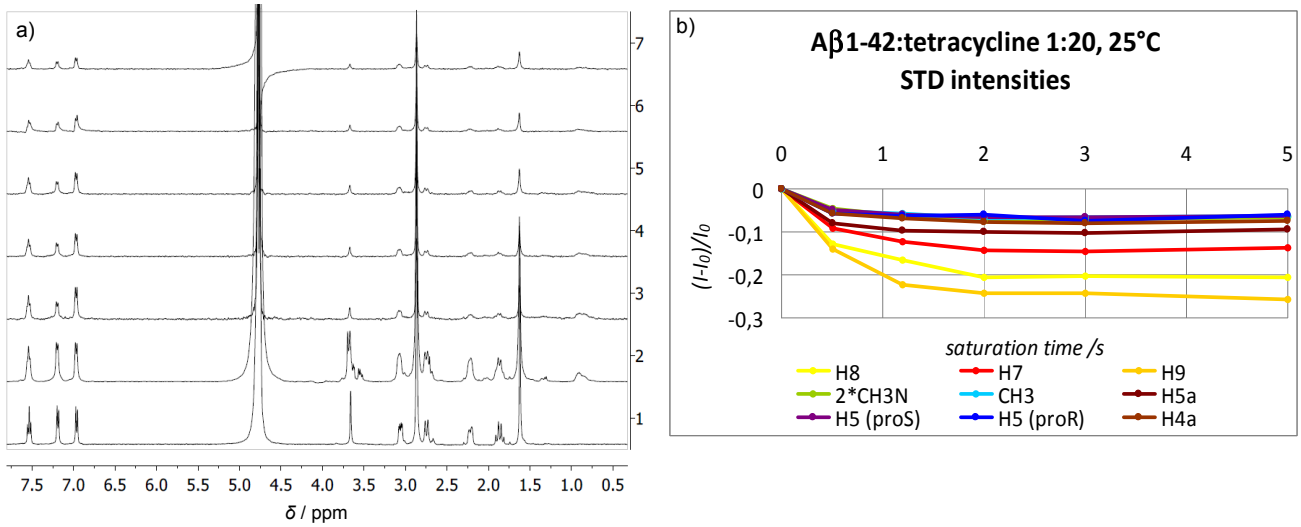

Figure 3S. a) ^{1}H NMR spectra of tetracycline, NS=32 (1), Aβ1-42-tetracycline mixture, at 1:20 molar ratio, NS=128 (2), STD spectra of the mixture recorded with different peptide saturation times (3, 5 s; 4, 3 s; 5, 2 s; 6, 1.2 s; 7, 0.5 s; enhanced 3x), NS=1792, on-resonance frequency= -1.0 ppm, off-resonance frequency=40 ppm. Spectra 2-7 were recorded on the same sample; all samples were dissolved in PBS, pH 7.4, 25°C. b) Fractional STD effects for each tetracycline proton, calculated by $(I-I_0)/I_0$, where $(I-I_0)$ is the peak intensity in the STD spectrum and I_0 is the peak intensity of an unsaturated reference spectrum.

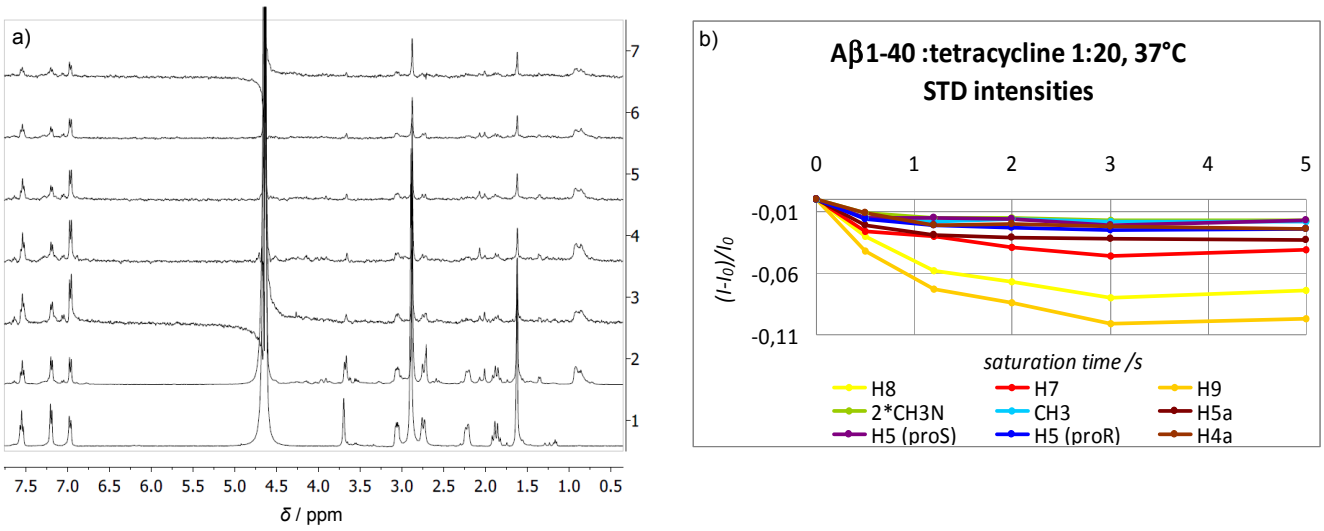

Figure 4S. a) ^{1}H NMR spectra of tetracycline, NS=32 (1), Aβ1-40-tetracycline mixture, at 1:20 molar ratio, NS=128 (2), STD spectra of the mixture recorded with different peptide saturation times (3, 5 s; 4, 3 s; 5, 2 s; 6, 1.2 s; 7, 0.5 s; enhanced 3x), NS=1792, on-resonance frequency= -1.0 ppm , off-resonance frequency=40 ppm. Spectra 2-7 were recorded on the same sample; all samples were dissolved in PBS, pH 7.4, 37°C. b) Fractional STD effects for each tetracycline proton, calculated by $(I-I_0)/I_0$, where $(I-I_0)$ is the peak intensity in the STD spectrum and I_0 is the peak intensity of an unsaturated reference spectrum.

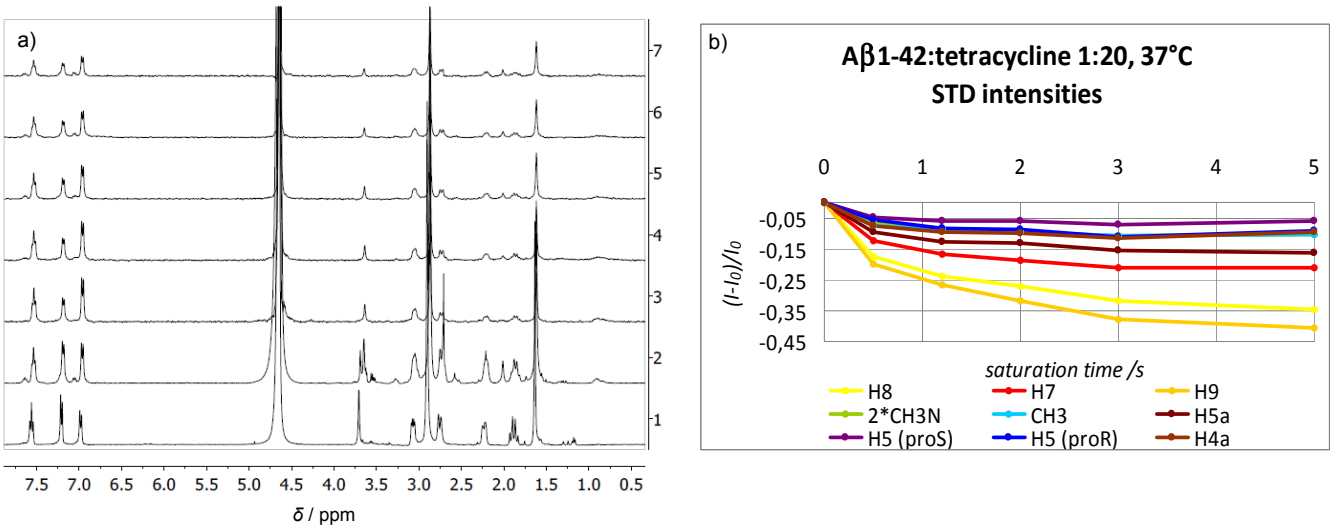

Figure 5S. a) ^{1}H NMR spectra of tetracycline, NS=32 (1), Aβ1-42-tetracycline mixture, at 1:20 molar ratio, NS=128 (2), STD spectra of the mixture recorded with different peptide saturation times (3, 5 s; 4, 3 s; 5, 2 s; 6, 1.2 s; 7, 0.5 s; enhanced 3x), NS=1792, on-resonance frequency= -1.0 ppm, off-resonance frequency=40 ppm. Spectra 2-7 were recorded on the same sample; all samples were dissolved in PBS, pH 7.4, 37°C. b) Fractional STD effects for each tetracycline proton, calculated by $(I-I_0)/I_0$, where $(I-I_0)$ is the peak intensity in the STD spectrum and I_0 is the peak intensity of an unsaturated reference spectrum.

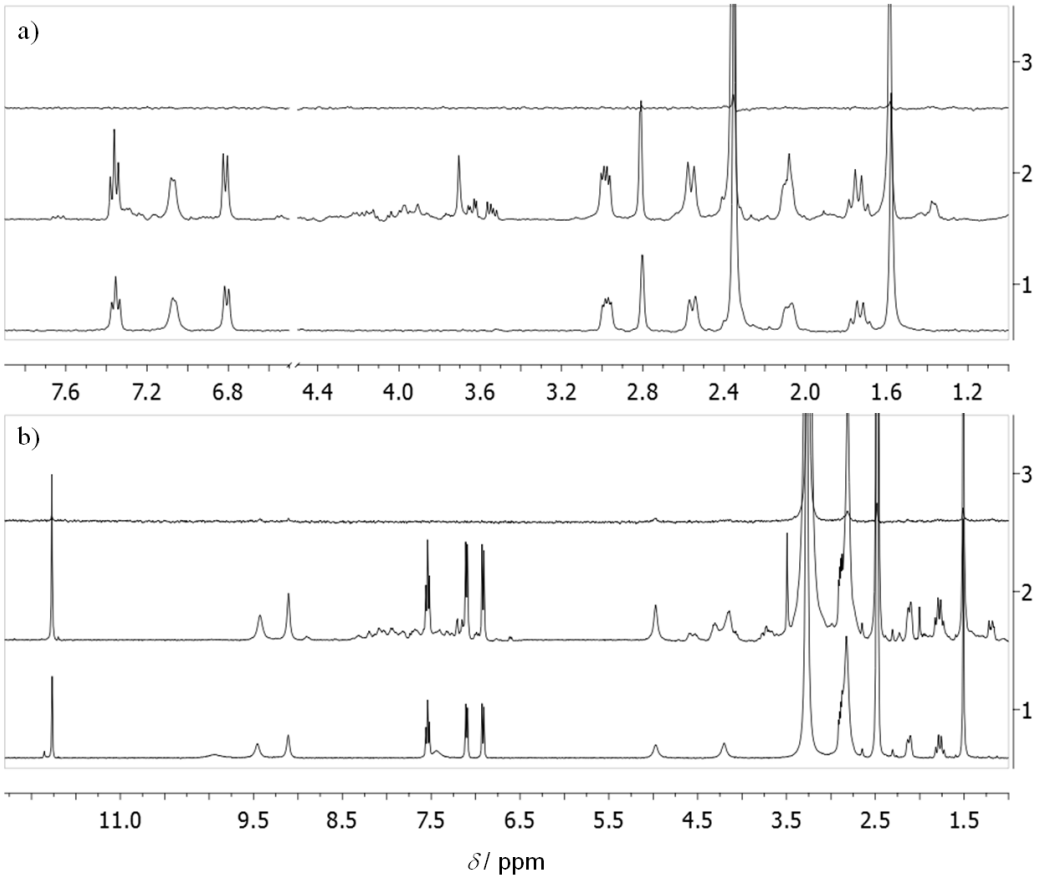

Figure 6S. a) ^{1}H NMR spectra of tetracycline, NS=32 (1), Aβ1-40-tetracycline mixture, at a 1:2 molar ratio, NS=128 (2), and STD spectrum of the mixture recorded with saturation times=2 s, NS=512 (3), in PBS, pH 12, 5°C. b) ^{1}H NMR spectra of tetracycline NS=32, (1), Aβ1-40-tetracycline mixture, at a 1:2 molar ratio, NS=128 (2) and STD spectrum of the mixture recorded with saturation times=2s, NS=512 (3) in DMSO, 25°C, on-resonance frequency= -1.0 ppm, off-resonance frequency=40 ppm.

S3 – Diffusion data deconvolution

The analysis of diffusion experiments acquired on a sample containing Aβ1-40 and tetracycline (1:2 molar ratio) and performed with SCORE, DECRA and MCR are reported in the following figures (7S-18S). HDO signal region was excluded to avoid complications associated with its spectral overlap with peptide and tetracycline resonances. As expected, the best results were obtained with SCORE. It was possible to separate Aβ1-40 and tetracycline spectra (and diffusion coefficients, reproducing values obtained with DOSY analysis) by increasing the number of components imposed for the deconvolution; in fact, the best fitting with results produced by DOSY were obtained by SCORE for 5 components. Nevertheless, two of the additional components identified showed diffusion coefficients higher than those calculated for tetracycline and Aβ1-40, and they clearly could not represent the oligomer diffusion coefficient, while one component presented a negative diffusion coefficient associated with an exponential increase of signal intensities vs the gradient amplitude, and the corresponding spectrum is mostly dominated by noise. We can conclude that this kind of analysis was not effective for the separation of diffusion coefficients of monomers and oligomers.[i]
The following figures (7S-10S) report the deconvolution of diffusion data performed by SCORE for 2, 3, 4 or 5 components.

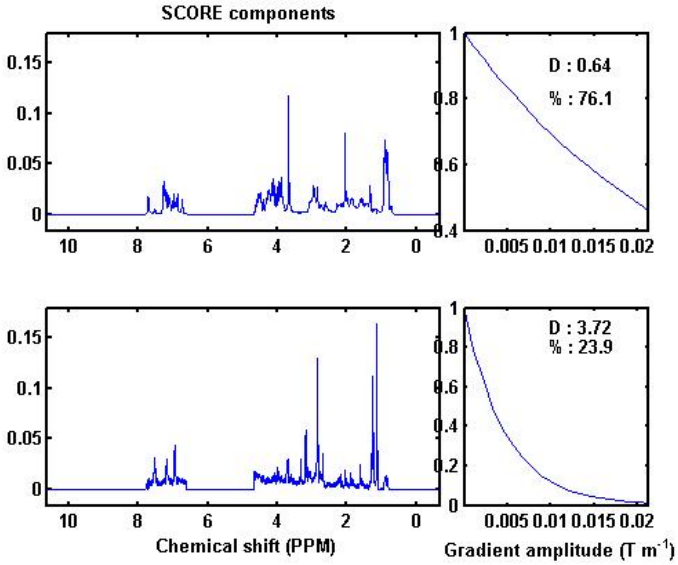

Figure 7S. Deconvolution of diffusion data performed by SCORE for 2 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

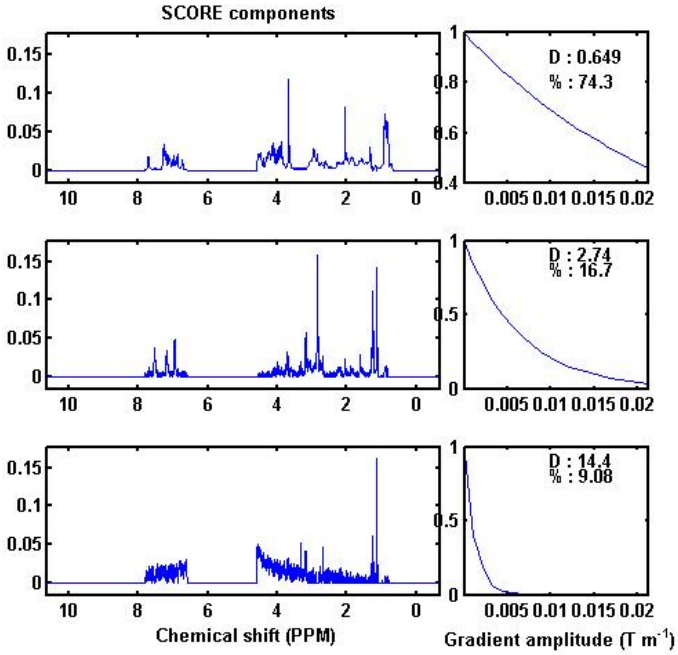

Figure 8S. Deconvolution of diffusion data performed by SCORE for 3 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

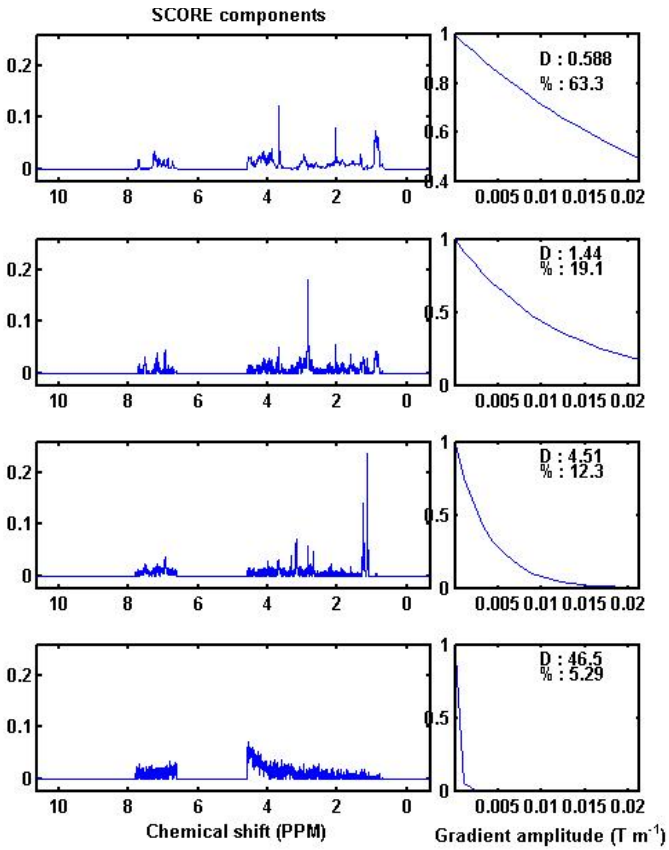

Figure 9S. Deconvolution of diffusion data performed by SCORE for 4 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

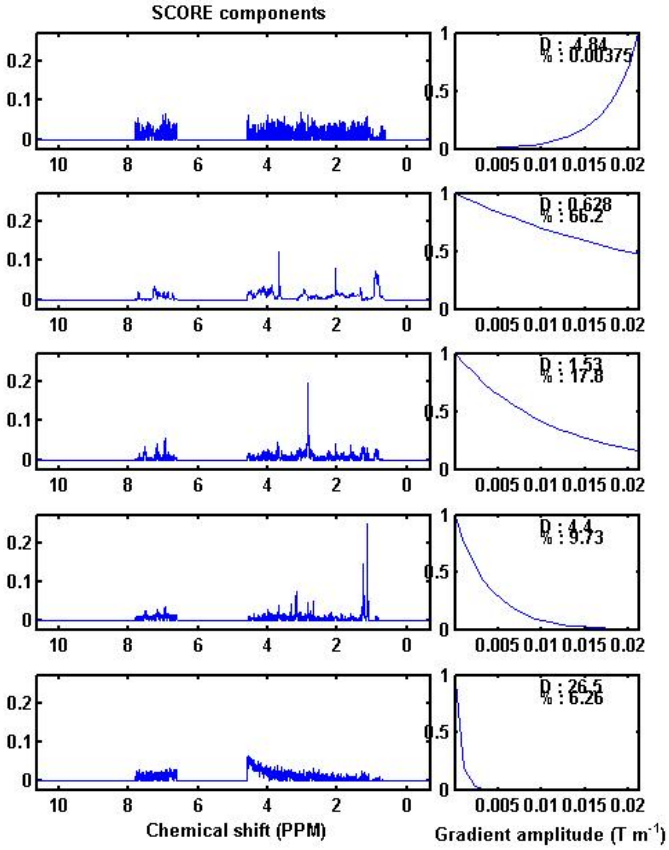

Figure 10S. Deconvolution of diffusion data performed by SCORE for 5 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

The following figures (11S-14S) report the deconvolution of diffusion data performed by DECRA for 2, 3, 4 or 5 components

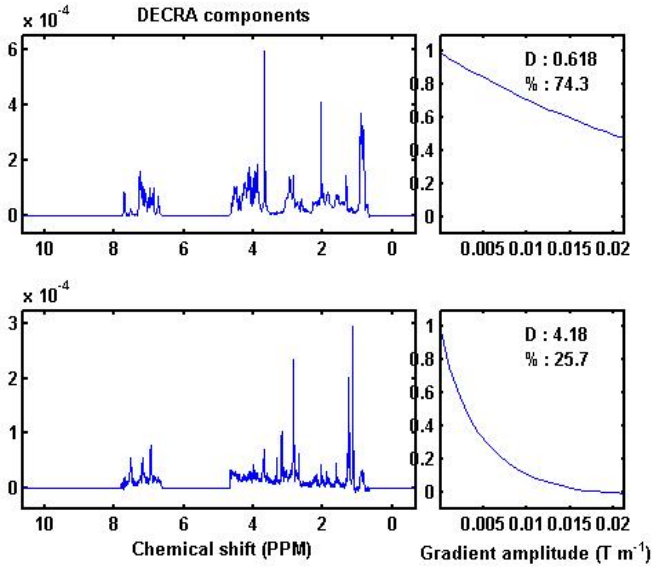

Figure 11S. Deconvolution of diffusion data performed by DECRA for 2 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

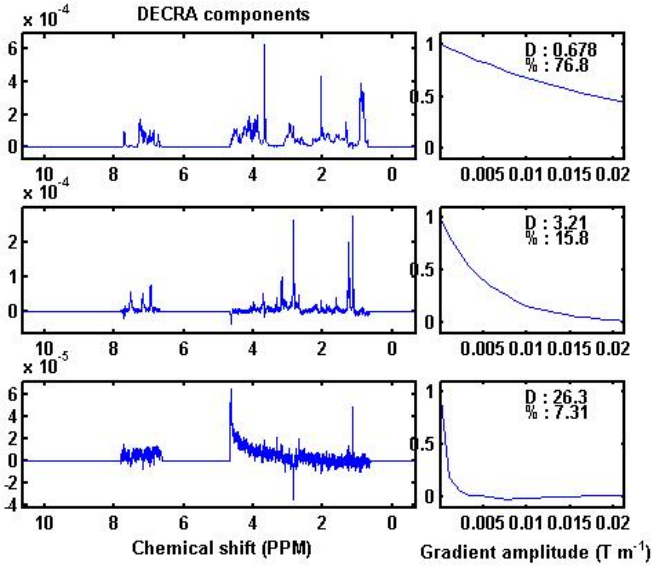

Figure 12S. Deconvolution of diffusion data performed by DECRA for 3 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

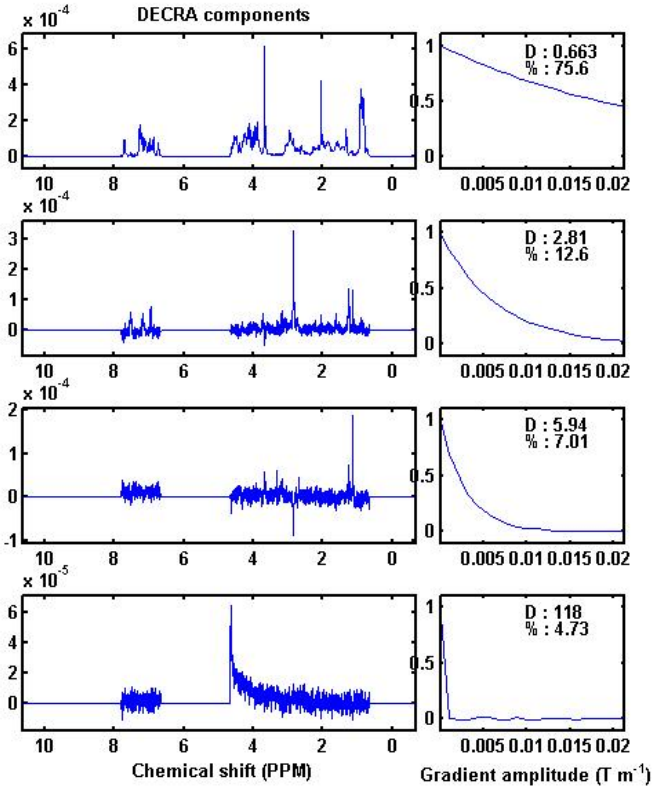

Figure 13S. Deconvolution of diffusion data performed by DECRA for 4 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

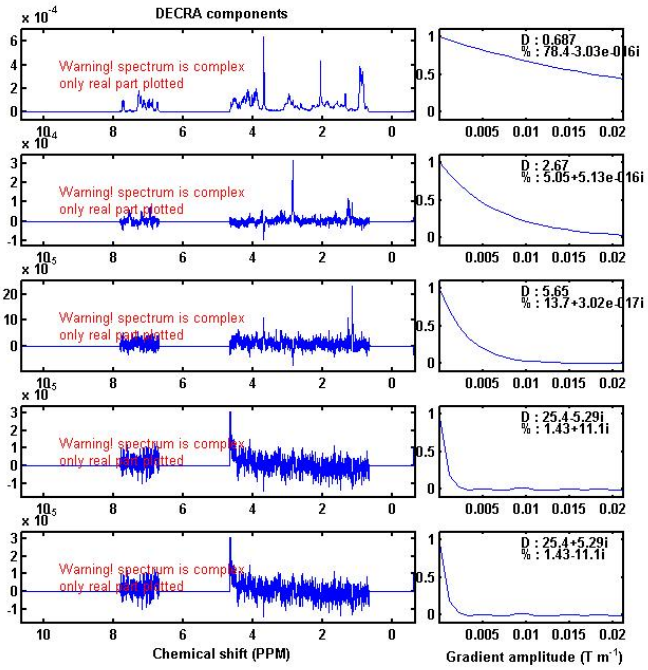

Figure 14S. Deconvolution of diffusion data performed by DECRA for 5 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

The following figures (15S-18S) report the deconvolution of diffusion data performed by MCR for 2, 3, 4 or 5 components.

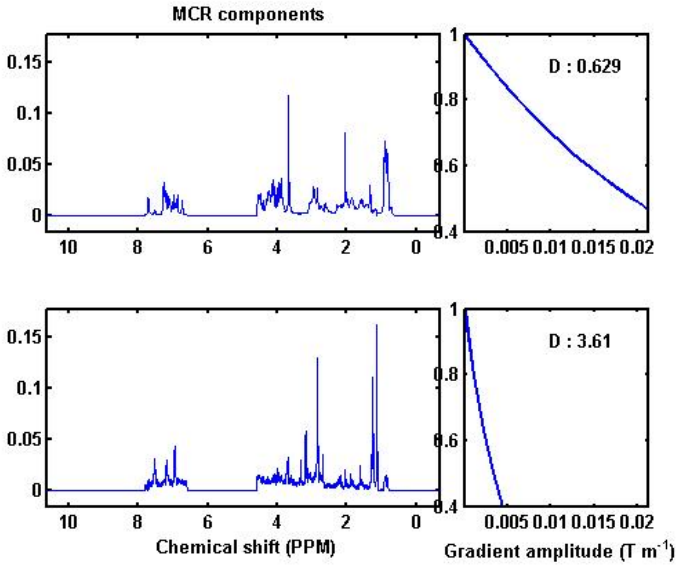

Figure 15S. Deconvolution of diffusion data performed by MCR for 2 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

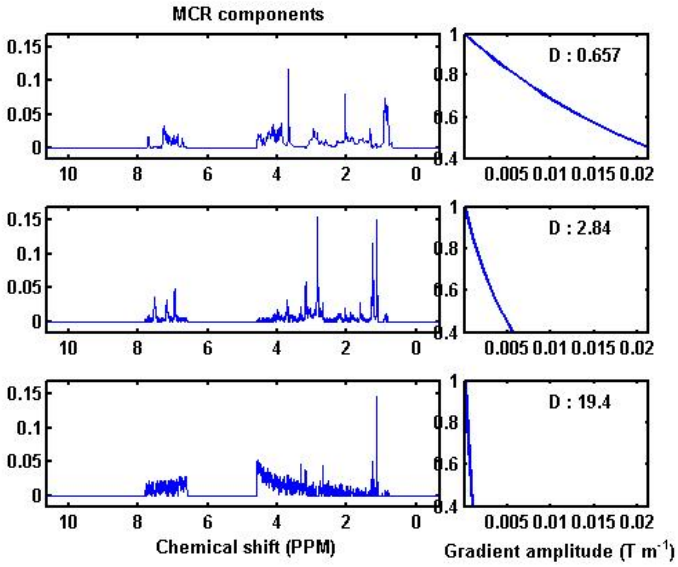

Figure 16S. Deconvolution of diffusion data performed by MCR for 3 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

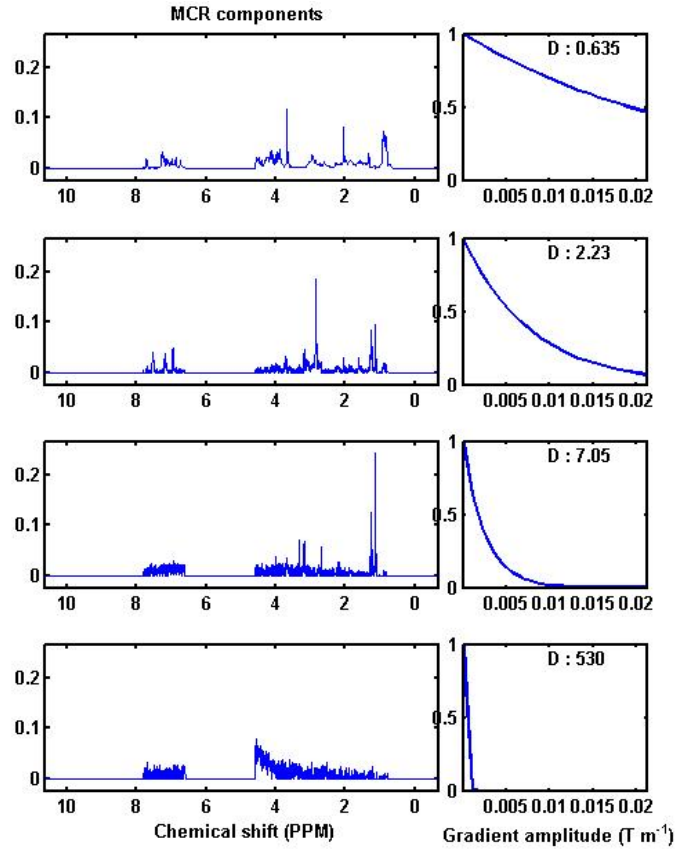

Figure 17S. Deconvolution of diffusion data performed by MCR for 4 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

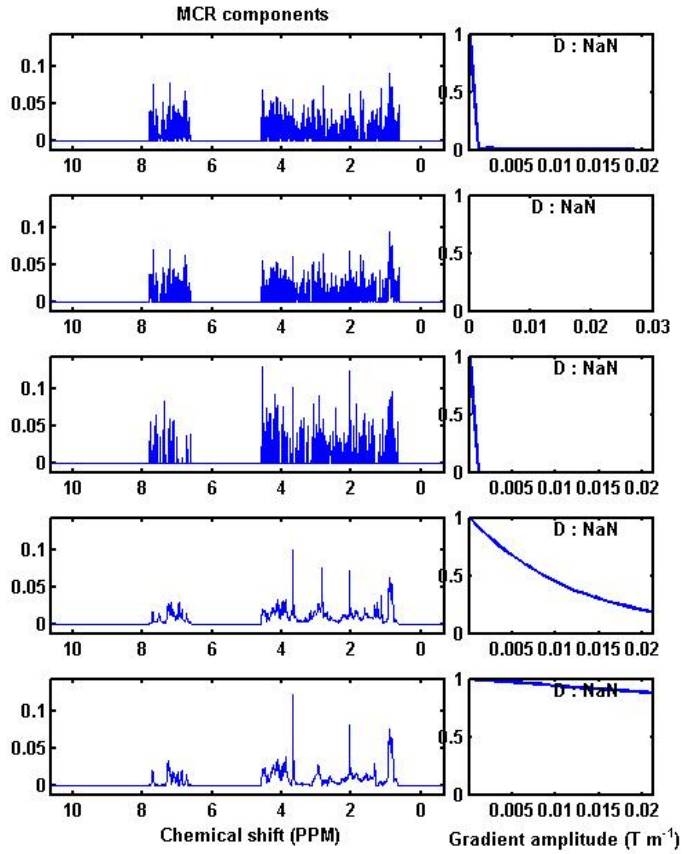

Figure 18S. Deconvolution of diffusion data performed by MCR for 5 components (sample: Aβ1-40:tetracycline 0.5mM:1mM, pH 7.4, 5°C).

S4 - Aβ1-42 trNOESY

trNOESY spectra confirmed tetracycline interaction with both Aβ1-40 and Aβ1-42, as deduced by drug cross-peak change of sign (trNOE). Figure 19S-b shows the trNOESY spectrum of Aβ1-42:tetracycline at a 1:4 molar ratio.

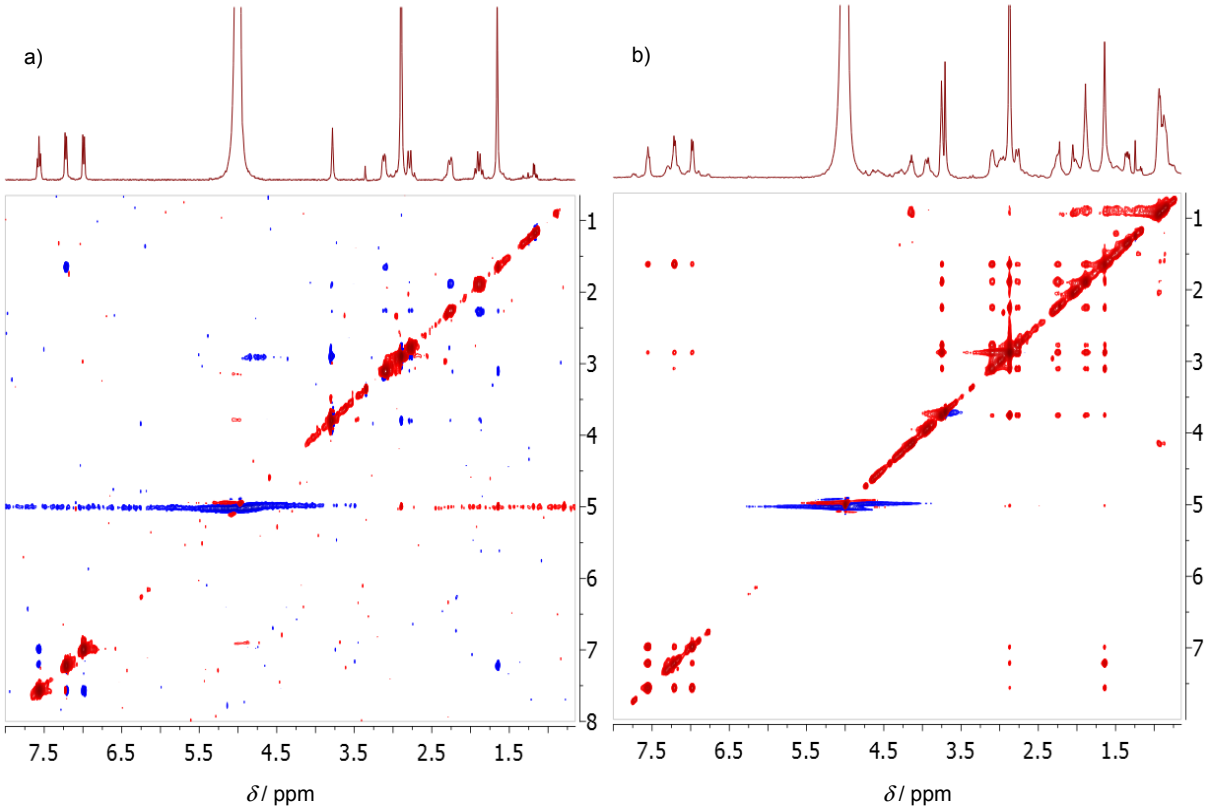

Figure 19S. a) 2D-NOESY spectrum of tetracycline, mix 650 ms; b) 2D-NOESY spectrum of Aβ1-42:tetracycline, at a 1:4 molar ratio, mix 300 ms. Samples were dissolved in PBS, pH 7.4, 5°C.

S5 - Aβ1-40 and Aβ1-42 titration with tetracycline

^{1}H titration experiments of Aβ1-40 and Aβ1-42 (Figure 20S) were performed to study the chemical shift changes induced in the presence of tetracycline. No significant changes in peptide chemical shifts were observed after tetracycline addition (up to a 1:8 molar ratio). Only a negligible downfield shift of about 0.02 ppm was observed for 2H protons of the three His residues (His6, His13, His14). These data were confirmed by analysis of the corresponding 2D-TOCSY spectra (data not shown).

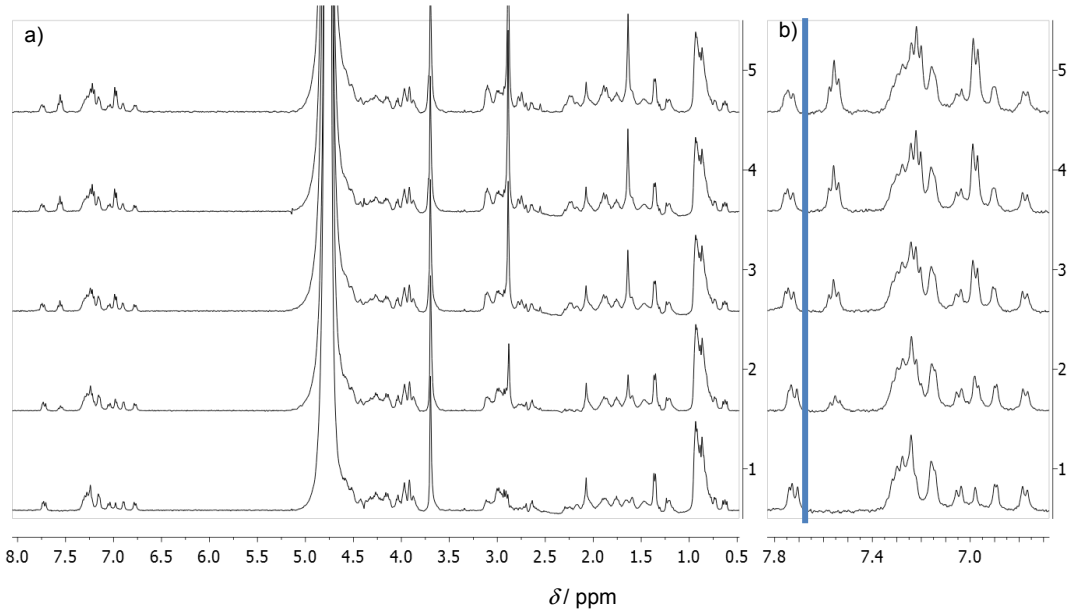

Figure 20S. a) ^{1}H NMR spectra of Aβ1-42 (0.25mM) (1), Aβ1-42-tetracycline mixture, at a 1:1 molar ratio (2), Aβ1-42-tetracycline mixture, at a 1:2 molar ratio (3), Aβ1-42-tetracycline mixture, at a 1:3 molar ratio (4), Aβ1-42-tetracycline mixture, at a 1:4 molar ratio (5). b) Expansion of the 7.9-6.8 ppm region of the spectra reported in (a). NS=128, PBS, pH 7.4, 25°C.

S6 – CD spectra of Aβ1-42 in the presence of Tetracycline

The effect of tetracycline on the secondary structures of Aβ1-42 was studied also by CD in the far-UV region. CD spectra of Aβ1-42 in the absence and in the presence of tetracycline are almost super imposable and indicate a β-sheet secondary structures,[ii] in agreement with the FTIR results.

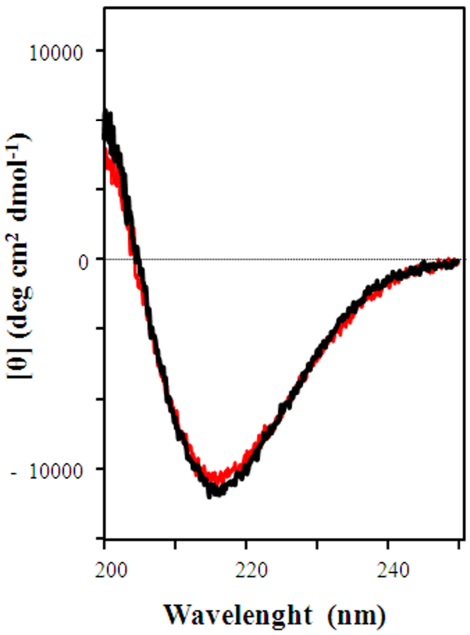

Figure 21S. CD spectra of Aβ1-42 (25 μM concentration) in the absence (black) and in the presence of 50 μM tetracycline (red). The samples were prepared at 0.25 mM peptide concentration – plus 0.5 mM tetracycline when required- as reported for the other analyses in the paper and diluted of a factor 10 to perform CD measurements.

S7 – Atomic Force Microscopy. Analysis of aggregate height formed following co-dissolution of Aβ peptides with tetracycline.

The aggregate height was determined with a Research NanoScope 7.20 (Veeco) software after analysis of 50 independent spots in three different areas of 5 μm^2 (Figure 22S). Statistical analysis (Tables 3S-5S) was carried out by t-test. Aβ1-42, time 0, mean and confidence interval, peptide alone 1.56 nm, 1.23-1.90 nm *vs* peptide-tetracycline 2.94 nm, 2.54-3.34 nm, $p<0.0001$. Aβ1-42; time 120 h, peptide alone 3.42 nm, 2.76-4.09 nm *vs* peptide-tetracycline 16.96 nm, 13.99-19.92 nm, $p<0.0001$. Aβ1-40; time 120 h, peptide alone 0.38 nm, 0.33-0.44 nm *vs* peptide-tetracycline 1.66 nm, 1.38-1.95 nm, $p<0.0001$.

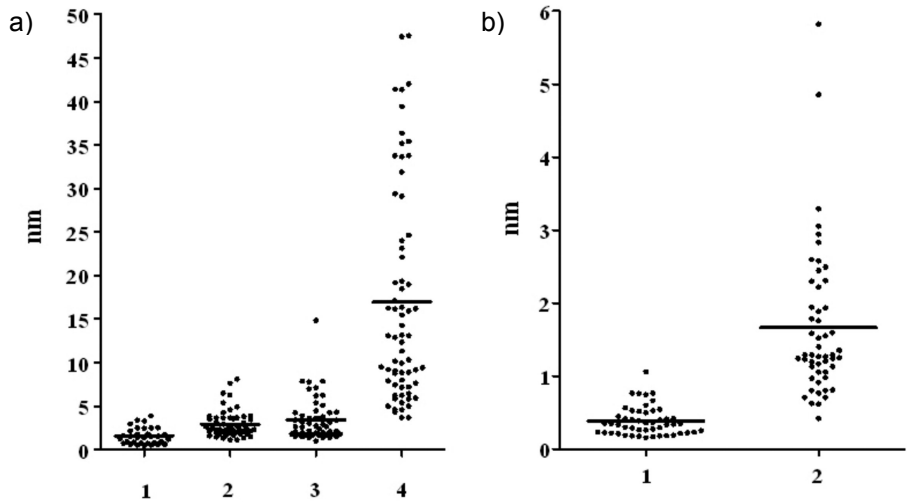

Figure 22S. Scattered plot of aggregate height distribution. Panel a. (1) Aβ1-42 time zero, (2) Aβ1-42-tetracycline, time zero; (3) Aβ1-42, time 120 h, (4) Aβ1-42-tetracycline, time 120 h. Panel b. (1) Aβ1-40 time 120 h, (2) Aβ1-40-tetracycline, time 120 h.

	Parameter	A Value
		Y
1	Table Analyzed	Data 1
2	Column A	1-42 0hrs
3	vs	vs
4	Column B	1-42 0hrs tetra
5		
6	Unpaired t test	
7	P value	P<0.0001
8	P value summary	***
9	Are means signif. different? (P	Yes
10	One- or two-tailed P value?	Two-tailed
11	t, df	t=4.724 df=88
12		
13	How big is the difference?	
14	Mean ± SEM of column A	1.564 ± 0.1629 N=50
15	Mean ± SEM of column B	2.940 ± 0.2002 N=57
16	Difference between means	-1.376 ± 0.2912
17	95% confidence interval	-1.956 to -0.7961
18	R squared	0.2023
19		
20	F test to compare variances	
21	F,DFn, Dfd	2.609, 56, 32
22	P value	0.0045
23	P value summary	**
24	Are variances significantly diff	Yes

Table 3S. Statistical analysis (t test) of scattered Aβ1-42 vs Aβ1-42-tetracycline, time zero.

	Parameter	A Value
		Y
1	Table Analyzed	Data 1
2	Column A	1-42 120hrs
3	vs	vs
4	Column B	1-42 120hrs tetra
5		
6	Unpaired t test	
7	P value	P<0.0001
8	P value summary	***
9	Are means signif. different? (P < 0.05)	Yes
10	One- or two-tailed P value?	Two-tailed
11	t, df	t=7.993 df=120
12		
13	How big is the difference?	
14	Mean ± SEM of column A	3.424 ± 0.3320 N=54
15	Mean ± SEM of column B	16.96 ± 1.484 N=68
16	Difference between means	-13.53 ± 1.693
17	95% confidence interval	-16.88 to -10.18
18	R squared	0.3475
19		
20	F test to compare variances	
21	F,DFn, Dfd	25.16, 67, 53
22	P value	P<0.0001
23	P value summary	***
24	Are variances significantly different?	Yes

Table 4S. Statistical analysis (t test) of scattered Aβ1-42 vs Aβ1-42-tetracycline, time 120 hours.

	Parameter	A Value
		Y
1	Table Analyzed	Data 1
2	Column A	1-40 120hrs
3	vs	vs
4	Column B	1-40 120hrs tetra
5		
6	Unpaired t test	
7	P value	P<0.0001
8	P value summary	***
9	Are means signif. different? (P < 0.05)	Yes
10	One- or two-tailed P value?	Two-tailed
11	t, df	t=8.714 df=100
12		
13	How big is the difference?	
14	Mean ± SEM of column A	0.3839 ± 0.02781 N=50
15	Mean ± SEM of column B	1.664 ± 0.1416 N=52
16	Difference between means	-1.280 ± 0.1470
17	95% confidence interval	-1.572 to -0.9885
18	R squared	0.4316
19		
20	F test to compare variances	
21	F,DFn, Dfd	26.94, 51, 49
22	P value	P<0.0001
23	P value summary	***
24	Are variances significantly different?	Yes

Table 5S. Statistical analysis (t test) of scattered Aβ1-40 vs Aβ1-40-tetracycline, time 120 hours.

S8 – Tetracycline - Thioflavin T competitive binding studies

trNOESY spectra confirmed tetracycline and ThT binding to Aβ1-40 and Aβ1-42, as deduced by compound cross-peak change of sign (trNOE) (Figure 23S).

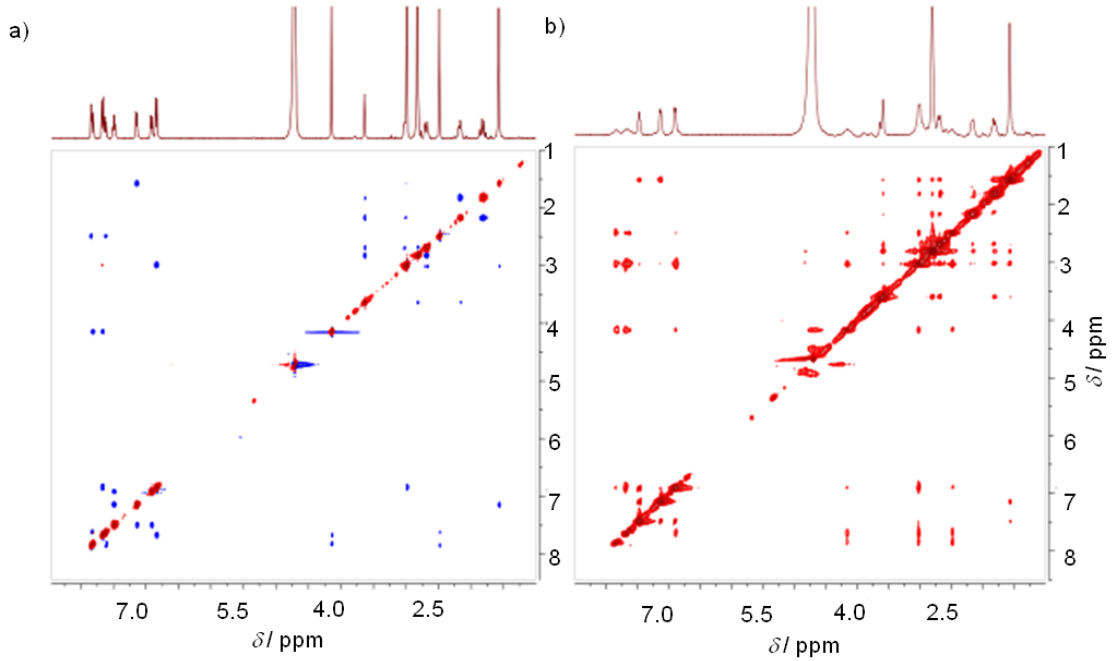

Figure 23S. NOESY spectrum of tetracycline:ThT mixture, at a 1:1 molar ratio, mix 800 ms (a), trNOESY of Aβ1-42:tetracycline:ThT mixture, at tetracycline:ThT 2:1 molar ratio, mix 250 ms b). Peptide:tetracycline at a 1:15 molar ratio. Samples were dissolved in PBS, pH 7.4 25°C.

S9 – FTIR spectra of Aβ peptides in presence of thioflavin T

The effect of ThT on the structural properties of Aβ1-40 and Aβ1-42 was examined by their infrared absorption in the Amide I region. The second derivative spectra of the peptides are reported in Figure 24S in presence and absence of ThT. The spectra are dominated by the two intermolecular β-sheet bands, which are signature of Aβ peptide oligomers (see Results and Discussion in the main text). In addition the spectra display a band around 1,605 cm^{-1} due to a ThT absorption, reported in the bottom of the figure. It can be seen that ThT does not induce appreciable differences in the intermolecular β-sheet bands of the two peptides, similarly to what observed for tetracycline. Moreover, the FTIR response of ThT around 1605 cm^{-1} is very similar in the absence and in the presence of Aβ oligomers, displaying only a minor downshift. It seems that this band is not very sensitive to the ThT interaction.

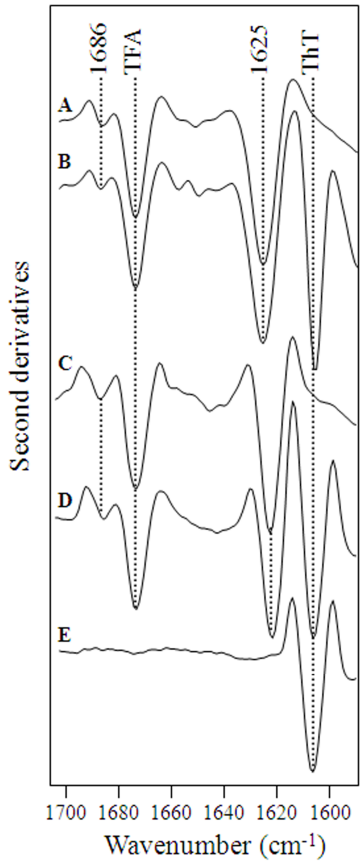

Figure 24S. FTIR second derivative spectra of Aβ peptides in presence of ThT. Spectra of Aβ1-42 (in D_2O PBS buffer, pH 7.4) in the absence (A) and in the presence (B) of ThT at a 1:4 molar ratio. Spectra of Aβ1-40 (in D_2O PBS, pH 7.4) in the absence (C) and in the presence (D) of ThT at a 1:4 molar ratio. The spectrum of free ThT in the same buffer is also reported (E).

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