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1 **Taylor dispersion analysis discloses the**
2 **impairment of A β peptide aggregation by the**
3 **presence of a fluorescent tag**

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6

7 **Abstract**

8 The use of fluorescently tagged amyloid peptides, implicated in Alzheimer's disease, to study
9 their aggregation at low concentrations is a common method, however the fluorescent tag
10 should not introduce a bias in the aggregation process. In this work, native amyloid peptides
11 A β (1-40) and A β (1-42) and Fluorescein-5-isothiocyanate (FITC) tagged ones, were studied
12 using Taylor dispersion analysis (TDA) coupled with a simultaneous UV and Light Emitting
13 Diode Induced Fluorescence (LEDIF) detection, to unravel the effect of FITC on the
14 aggregation process. For that, a total concentration of 100 μ M of peptides consisting of a
15 mixture of native and tagged ones (up to 10% in moles), was applied. Results demonstrated
16 that FITC had a strong inhibition effect upon the aggregation behavior of A β (1-42), while for
17 A β (1-40), only a retardation in kinetics was observed. It was also shown that when mixed
18 solutions of A β (1-40) and A β (1-42) are used, the A β (1-42) alloform was the leading peptide
19 in the aggregation process, and when the latter was tagged the aggregation kinetics decreased
20 but the lifetime of potentially toxic oligomers was drastically increased. These results
21 confirmed, that the hydrophilicity of the *N*-terminus part of the peptide plays a major role in
22 the aggregation process.

23

24 1. Introduction

25 Alzheimer's disease (AD) is a neurodegenerative pathology inducing damage to the patient's
26 brain and translating in memory loss and behavioural impairment. One of the main hallmarks
27 of AD is the presence of amyloid β peptide ($A\beta$) plaques in the brain of patients and several
28 evidence points that $A\beta$ peptides are one of the main reasons behind the symptoms of AD either
29 in its oligomeric form (thought to be neurotoxic)[1, 2] or in its fibrillary form[3].

30 In all cases, there is a necessity to better understand the aggregation mechanism of $A\beta$ peptides.
31 However *in vitro* studies are often hampered by the detection limits of the used methods which
32 often impose the use of concentrations (μ M range) several tens of times higher than the *in vivo*
33 ones (nM range[4]). Studying the aggregation process of amyloid peptides in detail is a
34 challenging task especially at concentrations in the order of magnitude of physiological ones.
35 For that, the use of selective and sensitive detection methods such as fluorescence based ones
36 is highly desirable to study peptides at lower concentrations[5]. This strategy implies a
37 modification of the peptide structure by adding a label often on its *N*-terminal end. However,
38 the attached tag may affect the physical and chemical properties of the amyloid peptides[6] and
39 their propensity to form beta-sheets and thus fibrillar structures. Wägele *et al.*[6] studied by
40 fluorescence correlation spectroscopy (FCS) the effect of four fluorescent tags (HiLyte 647,
41 HiLyte 488, Atto 655 and Atto 488) on the oligomer distribution of both $A\beta(1-40)$ and $A\beta(1-$
42 $42)$. They concluded that HiLyte 647 and Atto 655, compared to the other two tags, had high
43 peptide-tag interactions which promoted the formation of the high molecular weight oligomers
44 of $A\beta(1-40)$ and consequently the fibrillation step. In this experiment, Atto 488 seemed to be
45 the most suitable as no significant interaction was noted. Quinn *et al.* [7] demonstrated that
46 HiLyte fluor 555-labelling of $A\beta$ peptides on its *N*-terminus did not alter the self-assembly
47 kinetics or the resulting aggregated structures. On the contrary, Taverna *et al.* demonstrated
48 that a fluorescent tag (Fluoprobe 488 NHS Ester) attached on a side-chain of amyloid $A\beta(1-$
49 $42)$ (on the amines of Lys-16, Lys-28 and/or *N*-terminus Asp residues) inhibited the
50 aggregation [8]. In another study by Zheng *et al.* [9], the effect of five different dyes introduced
51 in the form of *N*-hydroxysuccinimidyl esters (BP, RB and 5-SFX) or as isothiocyanates (RITC
52 and 5-(6)-FITC) was evaluated. The authors concluded that 5-SFX and 5-(6)-FITC inhibited
53 the aggregation, since no change in the R_h was observed during the incubation period of 4 h,
54 while the other three promoted it. However, this study showed limitations as a concentration

55 of 5 μM in peptide was used and the aggregation was monitored for only few hours at 25 $^{\circ}\text{C}$.
56 Under these conditions the aggregation of the $\text{A}\beta$ peptides would take a few days (even
57 weeks)[10] to start and it seems difficult to judge on the inhibition effect of these dyes. In our
58 opinion, in order to study the aggregation process of amyloid peptides at relevant physiological
59 concentrations one might consider the use of preformed seeds known to accelerate the
60 aggregation process at such low concentrations [11]. Eventhough, when labelled peptides are
61 used, it is important to understand if the attached label is not introducing a bias in the
62 aggregation process.

63 Amyloid peptides exist under different isoforms, the most common ones being $\text{A}\beta(1-40)$ and
64 $\text{A}\beta(1-42)$, which differ from the former by two extra amino acids at the C-terminus, making it
65 more hydrophobic. These co-existing isoforms aggregate differently when separate[12, 13].
66 However, *in vivo* these isoforms coexist and it is not clear how they influence each other. In
67 fact, it was found that the amyloid plaques in the brains of AD patients were mainly, and
68 sometimes exclusively, constituted of $\text{A}\beta(1-42)$ [14], despite that $\text{A}\beta(1-40)$ concentration is
69 several-fold higher than $\text{A}\beta(1-42)$ one. The co-aggregation of these two isoforms was
70 intensively studied over the last decade[15, 16]. Some authors claimed that the peptide mixing
71 only influences the elongation phase by seeding the fibrillation but no heteromolecular fibrils
72 are formed[16]. But other reports suggested that mixed fibrils could be formed[15, 17].

73 In previous works, we showed the potential of Taylor dispersion analysis (TDA) to speciate
74 the aggregation process of $\text{A}\beta(1-40)$ and $\text{A}\beta(1-42)$, as pure or mixed peptide solutions[13, 18].
75 In this work we present the use of TDA coupled with simultaneous UV and LEDIF detections
76 to study the influence of the fluorescent tag FITC on the $\text{A}\beta$ peptide aggregation and to unravel
77 if heteromolecular species are formed along the aggregation course.

78 **2. Materials and methods**

79 **2.1. Materials**

80 $\text{A}\beta(1-40)$ was prepared by fast conventional SPPS using a *Fmoc* orthogonal strategy, as
81 described elsewhere[13]. $\text{A}\beta(1-42)$ (batch number 100002591, >95% purity by HPLC), were
82 purchased from Bachem (Bubendorf, Switzerland). Both FITC peptides were tagged on their
83 *N*-terminal side, and the molar mass of the tagged peptides was confirmed by MALDI-MS
84 (provider) and HPLC-ESI-MS in this work (4790 g/mol and 5016 g/mol for tagged $\text{A}\beta(1-40)$)

85 and tagged A β (1-42) respectively). Sodium dihydrogen phosphate and sodium hydroxide
 86 were purchased from Sigma Aldrich (France). All buffers were prepared using ultrapure water
 87 obtained from a MilliQ system (Millipore, France).

88 2.2. Peptide pretreatment and sample preparation

89 Both native (nA β) and tagged (tA β) peptides were pretreated independently using the protocol
 90 previously described[13, 19]. Briefly, the peptides were first dissolved at a concentration of 2
 91 g/L in an ammonium hydroxide solution (NH₄OH) which concentration differed between the
 92 two studied peptides (0.10 % (m/v) for A β (1-40), 0.16 % (m/v) for A β (1-42)). The peptide
 93 solutions were then incubated for 10 minutes at room temperature, then aliquoted and freeze-
 94 dried. The stock aliquots contained 10 nmol of peptide, and lyophilized peptide aliquots were
 95 stored at -20 °C until use. The pure tagged or native peptide solutions were prepared directly
 96 from the aliquoted fractions and dissolved in the adequate amount of buffer to reach the desired
 97 final peptide concentrations of 1, 10 and 100 μ M.

98 The mixed aliquots containing both native and tagged A β were prepared so that the final sample
 99 contained either 7% of tA β (1-40) or 10% of tA β (1-42). First stock aliquot of native or tagged
 100 peptides was dissolved in 100 μ L of 0.16% (m/v) NH₄OH, then appropriate volumes were used
 101 to obtain the desired combinations having a total A β content of 10 nmol, as detailed in table 1.
 102 Figures 1, 2 and 5 show pie charts with the proportion of the native and tagged peptides in the
 103 samples. The final aliquots were immediately subjected to freeze-drying and then stored at -
 104 20°C before being dissolved in 20 mM phosphate buffer at pH 7.4 to perform the aggregation
 105 study.

106 **Table 1:** Peptide quantities (nmol) and proportions in the mixed native/tagged samples

Sample	Peptide quantities (nmol) and (proportions)			
	nA β (1-40)	nA β (1-42)	tA β (1-40)	tA β (1-42)
A β (1-40) with tA β (1-40)	9.3 (93 %)		0.7 (7 %)	
A β (1-42) with tA β (1-42)		9 (90 %)		1 (10 %)
3:1 A β (1-40):A β (1-42)	7.5 (75 %)	2.5 (25 %)		
3:1 A β (1-40):A β (1-42) with tA β (1-40)	6.8 (68 %)	2.5 (25 %)	0.7 (7 %)	
3:1 A β (1-40):A β (1-42) with tA β (1-42)	7.5 (75 %)	1.5 (15 %)		1 (10 %)

107

108 2.3. Study of A β aggregation by Taylor dispersion analysis

109 TDA was performed using an Agilent 7100 (Waldbronn, Germany) capillary electrophoresis
110 system with bare fused silica capillary (Polymicro technologies, USA), having 60 cm \times 50 μ m
111 i.d. dimensions and a UV detection window at 51.5 cm from the inlet end. The system was
112 coupled with a Zetalif light-emitting diode induced fluorescence (LEDIF) detector
113 (Picometrics, Toulouse, France) with a window at 33 cm from the inlet end. The capillary was
114 conditioned with the following flushes: 1 M NaOH (30 min) followed by a flush with ultrapure
115 water (30 min). Between each analysis, the capillary was rinsed with a 20 mM sodium
116 phosphate buffer at pH 7.4 for 2 min. Samples were injected hydrodynamically on the inlet end
117 of the capillary (44 mbar, 4 s) and the injected volume was about 6.5 nL, corresponding to 1%
118 of the capillary volume to the LEDIF detection point and 0.64% with respect to the UV
119 detection window. In the case of the 1 μ M, 10 μ M and 100 μ M tA β (1-42) samples, the solutions
120 were injected hydrodynamically by applying for 3 s a pressure of 33, 55 and 6 mbar
121 respectively. The injection volumes were modified to prevent detector saturation.

122 Experiments were performed at a mobilization pressure of 100 mbar. The temperature of the
123 capillary cartridge was set at 37 $^{\circ}$ C and the vial carousel was thermostated using an external
124 circulating water bath 600F from Julabo (Germany). The solutes were simultaneously
125 monitored by UV with an absorbance measured at 191 nm and by fluorescence with an
126 excitation at 480 nm. Emission light was collected through a ball lens and a high-pass filter in
127 the wavelength range from 515–760 nm. The proportion of tagged peptide in the mixtures was
128 chosen so that the obtained LEDIF signal was as high as possible without saturating the
129 detector. The higher fluorescence intensity observed for tA β (1-40) presumably stems from the
130 presence of unreacted FITC during synthesis, as observed for these samples from the
131 taylorgrams (see supporting information Figures SI.1 to SI.8). The mobile phase was a 20 mM
132 sodium phosphate buffer (pH 7.4; viscosity at 37 $^{\circ}$ C: 0.7×10^{-4} Pa.s). Peptide aliquots were first
133 dissolved in 100 μ L of a 20 mM sodium phosphate buffer solution (pH 7.4) to reach a final
134 concentration of 100 μ M and then immediately transferred to a vial to be incubated at 37 $^{\circ}$ C in
135 the capillary electrophoresis carousel. The aggregation was conducted by injecting the sample
136 ($V_{inj} \approx 7$ nL) every 11 min for 100% A β (1-42) experiments, and each 30 min for all the
137 experiments containing A β (1-40). The total average of TDA injections was between 124 and
138 185 TDA runs per sample, corresponding to a total sample volume consumption between 870
139 to 1300 nL (0.87 and 1.3 μ L). Finally, to prevent sample evaporation, the vial cap was changed
140 three times a day. All taylorgrams were recorded using Agilent Chemstation software and then

141 exported to Microsoft Excel for subsequent data processing. The data obtained from the UV
142 signal were treated on the right side of the taylorgram to avoid the spikes detected on the left
143 part, as described elsewhere[13, 18].

144 **3. Results and discussion**

145 **3.1. Influence of the peptide concentration**

146 The effect of the peptide concentration on the kinetics of the aggregation process was first
147 investigated by TDA at different tagged peptide (tA β (1-42)) concentrations ranging from 1 to
148 100 μ M. As can be seen from Figure 1A, the total peak area, obtained by integrating the
149 taylorgrams from the LEDIF detection, did not evolve significantly in the case of the lowest
150 concentrations (1 and 10 μ M), over the whole studied incubation time (352 h for the 1 μ M
151 sample and 162 h for the 10 μ M sample), suggesting that the kinetics of the aggregation at
152 these concentrations is very slow, as shown in the literature the kinetics of the aggregation
153 increase with increasing concentration[20]. The normalization of the peak area in Figure 1A
154 was realized for the sake of comparison and was obtained by dividing each peak area of each
155 run by the peak area obtained at $t_{ag} = 0$ h.

156 In the case of the highest concentration (*i.e.* 100 μ M), the peak area decreased to reach a plateau
157 after ~ 3 h of incubation. The comparison with a sample having the same 100 μ M total peptide
158 concentration but with a mixing of 90 μ M nA β (1-42) with 10 μ M of tA β (1-42), demonstrated
159 similar kinetics of aggregation from the LEDIF peak area. In addition, Figure 1.B shows the
160 average R_h obtained for the 100 μ M samples by integration of the LEDIF elution profiles as
161 described elsewhere[21]. At $t_{ag} = 0$ h and during the first hour of incubation the average R_h was
162 around 1.9 nm (corresponding to the size of the A β (1-42) monomer and small oligomer
163 population, in good agreement with previously published works[13, 18]). After about 1 h, the
164 R_h value decreased to reach a plateau around 0.8 nm, which most likely corresponds to the free
165 fluorescent tag or free tagged aminoacids (FITC, theoretical R_h obtained from modelization[13]
166 using HydroPro software[22] on a energy minimized 3D structure is 0.64 nm). In the case of
167 the low concentration samples, the R_h at $t_{ag} = 0$ h was of 1.95 and 1.85 nm for the 1 μ M and
168 the 10 μ M samples respectively. The average R_h value decreased very slowly with the
169 appearance of the small sized population ($R_h \sim 0.8$ nm) and reached the plateau after two weeks
170 for the 1 μ M sample (see supporting information figure SI.9). Eventhough it is theoretically

171 possible to follow the aggregation process at these two low concentrations and for a relatively
172 long period of time, it is not possible to do a control run without the fluorescent tag at these
173 concentrations because of the low sensitivity of the UV detection as compared to fluorescence.
174 Therefore in order to study the impact of the fluorescent tag it seems important to apply
175 conditions where both native and tagged peptides could be followed simultaneously.

176 In conclusion of this first part, it was found that a high concentration of typically 100 μM was
177 recommended to study the impact of the fluorescent tag on the aggregation process within a
178 reasonable time range (few days instead of weeks). Therefore, if fluorescent detection
179 combined with a low peptide concentration (below 1 μM) seems hardly applicable for
180 monitoring amyloid peptide aggregation because of slow kinetics, the impact of the fluorescent
181 tag on the aggregation process may however be studied on mixed mixtures of native and tagged
182 peptides, at 100 μM total peptide concentration. To increase the aggregation kinetics for low
183 concentration samples, the use of preformed seeds might also be a suitable option.

184 **3.2. Monitoring the aggregation process of A β peptides by TDA-UV**

185 In this work, we used a proportion of tagged peptide between 7 and 10% (see pie charts in
186 Figure 2), keeping constant the total peptide concentration at $\sim 100 \mu\text{M}$. Simultaneous UV and
187 fluorescent detections could be compared using these mixed non-tagged and tagged mixtures.
188 The UV detection was used to determine the kinetics of aggregation which can be compared in
189 presence or in absence of tagged peptide. In a recent work[13], we showed by TDA that the
190 products of A β peptide aggregation could be divided into four main populations differing by
191 their size. The first population is that of monomeric and small oligomeric forms of the peptides
192 having an $R_h < 5 \text{ nm}$. The second population corresponds to higher molar mass oligomers with
193 R_h between 4 and 50 nm. The third population shows $R_h > 50 \text{ nm}$ and corresponds to soluble
194 protofibrillar structures. Finally the fourth one is that of insoluble fibrils which size is not
195 accessible by TDA. Figure 3 displays one example of Taylorgram deconvolution with the
196 contributions of the different populations to the total UV signal. The residual trace shown in
197 the upper part of Figure 3 (difference between the experimental data and the theoretical fit)
198 represents the soluble fibril fraction.

199 In Figure 2 are presented the area of the monomer and small oligomers population obtained by
200 deconvolution of the elution profile by UV detection over the whole aggregation process for
201 all the studied systems. The evolution of this population was found to be representative of the

202 aggregation kinetics[13, 18] via the consumption of the monomeric units. As can be seen from
203 Figure 2A, nA β (1-40) showed a lag phase of about 18 h before a rapid decrease in the
204 monomeric population to reach a final lower plateau after ~26 h. A β (1-40) is thought to follow
205 a secondary nucleation mechanism, where monomers add to already existing fibrils to produce
206 larger fibrils, without going through intermediate states as discussed in the literature[20, 23,
207 24]. Conversely, the nA β (1-42) (Figure 2C) decreased rapidly (less than 1h) without showing
208 any lag phase. The 3:1 nA β (1-40):nA β (1-42) mixture showed a similar trend as for the nA β (1-
209 42) sample but with slower kinetics (the plateau was reached after 16 h). As displayed in
210 Figure 2D, the addition of 7% of tA β (1-40) to nA β (1-40) did not seem to affect the lag phase,
211 however it affected the kinetics of the monomer consumption which reached a plateau after
212 ~60 h instead of 26 h for the 100 % native sample. This observation suggests that the tagged
213 peptide influenced the elongation phase of the aggregation mechanism but not the nucleation
214 step. When the tA β (1-42) (10%) was added to the nA β (1-42) sample (see Figure 2G), the
215 kinetics of the aggregation decreased as well and the time to reach the final lower plateau
216 changed from less than 1 h to about 3 h. Similarly, in the case of the 3:1 mixture of A β (1-
217 40):A β (1-42), when 7% of tagged A β (1-40) were added to the sample, the aggregation pathway
218 was not significantly changed compared to the native mixture. However slower kinetics were
219 observed (lower plateau reached after 36 h instead of 16 h for the native mixture; see Figure
220 2E compared to Figure 2B). These results suggest that the nA β (1-42) peptide is driving the
221 aggregation process: it rapidly formed the nucleus (no lag phase) and the elongation step was
222 slowed by the tA β (1-40). Likewise, when a fraction of tA β (1-42) was added to the mixture the
223 aggregation mechanism resembled to the aggregation mechanism of A β (1-40) solutions, *i.e.* a
224 lag phase was observed up to about 30 h and then the monomer population proportion
225 decreased until it reached a final plateau at about 60 h. These observations confirmed that the
226 tA β (1-42) delayed the formation of the A β (1-42) nucleus serving as a seed for the elongation
227 of A β (1-40). In a study by Zheng *et al.*[9], the effect of several fluorescent tags, among which
228 FITC tag used in this work, on the aggregation process of tA β (1-42) was investigated using
229 fluorescence correlation spectroscopy. The authors concluded that the FITC tag decreased the
230 propensity of oligomers to aggregate and they attributed this effect to the increase of the peptide
231 hydrophilicity by the fluorescent tag. Further, it was reported that the native A β (1-42) monomer
232 and its fibrillary structures adopt compacted conformations[25], in which the *N*-terminus is
233 supposedly water-shielded. In this work, the FITC tag can change in the hydrophilic-
234 hydrophobic balance of the peptides, can induce steric hindrance or may change the peptide
235 conformation, leading to a lower propensity for aggregation. For instance, it was reported in

236 the literature that the *N*-terminally truncated amyloid peptides were more prone to aggregation
237 than the full length ones [26-29] because the truncated peptides would become more
238 hydrophobic, which supports that an increase in hydrophilicity at the *N*-terminus region would
239 impair the aggregation.

240 Figure 4 shows the hydrodynamic radii obtained for all studied samples and for all populations
241 obtained by deconvolution of the UV elution profile. The monomers and low molar mass
242 oligomers have an R_h of 1.60 – 2.25 nm which was relatively constant during the whole
243 aggregation process (Figure 4, monomers). According to our previous study, these values
244 suggest that this population is mainly comprised of monomers and dimers[13]. As for the
245 higher mass oligomer population, it varied from ~4 to ~30 nm for all studied samples with an
246 average value around 10 nm. Then the R_h of protofibrils ranged from 50 to 250 nm for all
247 studied systems. These results suggest that the fluorescent tag did not influence the size of the
248 different populations when compared to native samples.

249

250 When a deeper look is taken at the higher mass oligomer population for the 3:1 mixtures with
251 and without tagged peptides (Figure 5), the following observations could be made: first, in the
252 native peptides mixture, this population increased in proportion and reached a maximum value
253 after 6h incubation and then decreased and reached a lower plateau after 15 h. Then, the
254 addition of tA β (1-40) did not seem to significantly influence the evolution of this population,
255 which seemed to disappear after 24 h in both cases (with and without tA β (1-40)). Finally, when
256 tA β (1-42) was added to the mixture, the bell shaped distribution extended from 0 h up till 60 h
257 with a maximum reached around 30 h, suggesting that the oligomers are more stabilized under
258 these conditions.

259 The comparison of the evolution of the monomer and small oligomer populations obtained
260 from the elution profiles from the UV and the LEDIF detections (Figure 6), showed no
261 difference in the pathway between the tagged peptide (selectively detected by LEDIF) and the
262 total peptides (detected by UV; composed of tagged and non-tagged peptides), suggesting that
263 the aggregation involves all the different peptides at all steps and that the formed assemblies
264 might be heteromolecular (mixture of A β (1-40) and A β (1-42)). Figure 6C helps supporting this
265 claim since a rapid decrease of tA β (1-42) is observed during the first hours of incubation,
266 probably corresponding to the formation of a nucleus, followed by a plateau. Then, another

267 decrease in the tA β (1-42) fraction is observed after about 24 h suggesting the onset of the
268 elongation phase with the participation of the A β (1-40) (predominantly present in the sample)
269 where the decrease of the monomer population in UV is observed as well.

270 **4. Conclusion**

271 In this work, we showed the ability of TDA to follow the aggregation process of labelled A β
272 peptides by fluorescence detection and to study the effect of the label on the aggregation
273 propensity of amyloid peptides. It was found that at very low concentrations (lower than 10
274 μ M) the aggregation kinetics were drastically decreased and the monitoring of the aggregation
275 at these concentrations would be a long process (days or weeks). To increase the kinetics and
276 the experimental throughput, the concentration was increased up to 100 μ M with a certain
277 proportion of fluorescent-tagged peptides. At this concentration, it was possible to monitor the
278 aggregation process of A β peptide mixtures in a reasonable amount of time. Further, it was
279 reported that the aggregation of A β (1-40) and A β (1-42) was affected by a small proportion of
280 FITC label. The effect of the fluorescent tag was observed on the elongation phase rather than
281 on the nucleation one. Further, in the case of A β (1-40):A β (1-42) mixtures, when the tagged
282 peptide was A β (1-42), it was observed a higher influence of the FITC label on the aggregation
283 mechanism than with the tagged A β (1-40). As a consequence, the formed oligomers in A β (1-
284 40):A β (1-42) mixtures (with a proportion of tA β (1-42)) had a substantial increase of their
285 lifetime and potentially of their toxicity. Finally, the comparison of both detection modes
286 allowed to assume that the aggregation mechanism was a heteromolecular process involving
287 both peptide isoforms and both tagged and untagged peptides in the aggregates. In perspective,
288 the study of seeding at low A β concentrations and the effect of the truncation on the aggregation
289 process, monitored and speciated by TDA would be an important input into the huge amount
290 of literature dealing with the aggregation of amyloid peptides.

291

292 **Data availability statement:** “The data that support the findings of this study are available
293 from the corresponding author upon reasonable request.”

294

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297

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Figure Captions

377 **Figure 1.** (A) Evolution of the peak area (normalized with the peak area at $t_{ag} = 0$ h) obtained by integration of
378 the elution profiles with the LEDIF detection for all analyzed incubation times for 100 % tA β (1-42) solutions at
379 1 μ M (squares), 10 μ M (circles) and 100 μ M (triangles), and for a 100 μ M A β (1-42) solution constituted of 90%
380 nA β (1-42) and 10% tA β (1-42) (inversed triangles). (B) Comparison of the average hydrodynamic radii (obtained
381 by integrating the LEDIF elution profile as described in[21]) over the whole aggregation study for the 100 μ M
382 A β (1-42) solutions (100 % tA β (1-42) (triangles) and the mixture of 90% nA β (1-42) and 10% tA β (1-42) (inversed
383 triangles)).

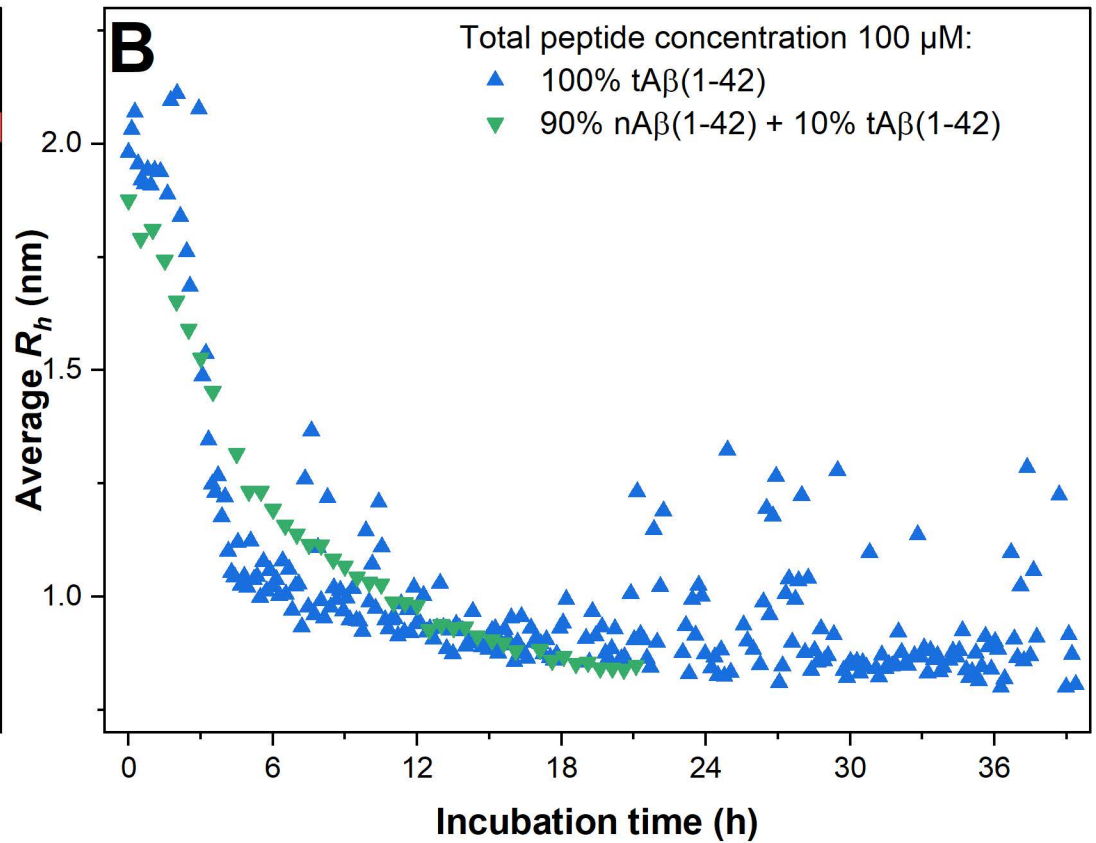
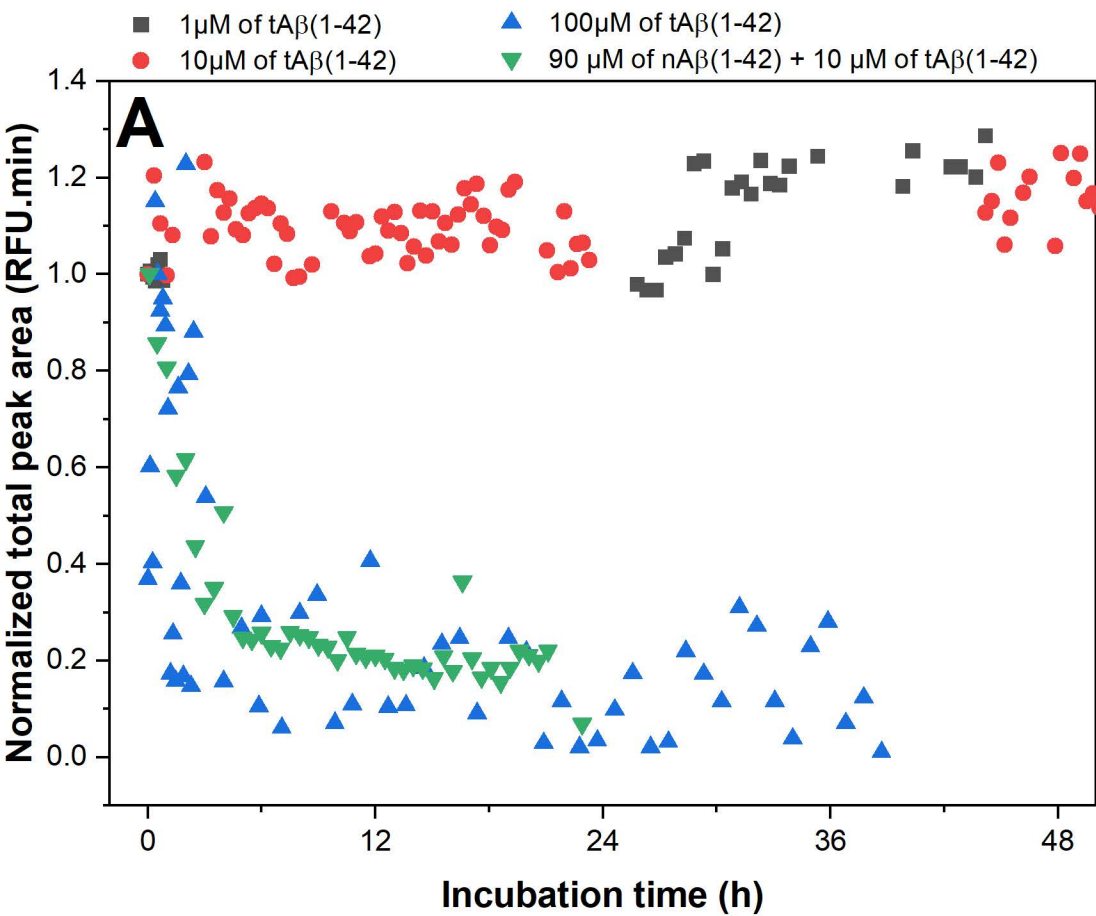
384 **Figure 2.** Peak area evolution of the monomer and small oligomer populations obtained for both nA β + tA β
385 systems and 100 % nA β experiments at 100 μ M total peptide concentration (except for 3:1 native peptides mixture
386 where the concentration was of 133 μ M) as obtained from the analysis of the UV-TDA elution profile: (A) 100%
387 nA β (1-40), (B) 75% nA β (1-40) + 25% nA β (1-42), (C) 100% nA β (1-42), (D) 93% nA β (1-40) + 7% tA β (1-40),
388 (E) 68% nA β (1-40) + 7% nA β (1-40) + 25% nA β (1-42), (F) 75% nA β (1-40) + 15% nA β (1-42) + 10% tA β (1-42)
389 and (G) 90% nA β (1-42) + 10% tA β (1-42). Peak area was normalized to obtain a better comparison of the species
390 evolution, by dividing the area of each data point to the total peak area obtained for the first run at $t_{ag} = 0$ h. The
391 inserted pie charts denote the proportion of the nA β and tA β content in the studied samples.

392 **Figure 3:** Example of data processing of the experimental taylorgrams. The lower graph represents the
393 experimental data (black) fitted with the sum of four Gaussian peaks (dashed orange) which are individually
394 represented on the graph. The residuals plot in the upper part of the graphs is the difference between the
395 experimental data and the theoretical fit. Experimental conditions: Sample: 100 μ M of 3:1 A β (1-40):A β (1-42)
396 mixture (68% nA β (1-40) + 7% tA β (1-40) + 25 % nA β (1-42)) at $t_{ag} = 16$ h; 20 mM phosphate buffer pH 7.4.
397 Incubation: quiescent conditions at 37 °C. Fused silica capillaries: 50 μ m i.d. \times 60 cm \times 51.5 cm. Mobile phase:
398 20 mM phosphate buffer, pH 7.4. Mobilization pressure: 100 mbar. Injection: 44 mbar for 4 s. Analyses were
399 performed at 37 °C. UV detection at 191 nm. Baseline treatment was performed in Microcal Origin. The
400 experimental fitting of the taylorgrams was performed by using the sum of four Gaussian functions in Microsoft
401 Excel.

402 **Figure 4.** Hydrodynamic radius evolution of the species obtained for both nA β + tA β systems and 100 % nA β
403 experiments during the UV analysis: (A) 100% nA β (1-40), (B) 3:1 A β (1-40):A β (1-42) - 75% nA β (1-40) + 25%
404 nA β (1-42), (C) 100% nA β (1-42), (D) 93% nA β (1-40) + 7% tA β (1-40), (E) 3:1 A β (1-40):A β (1-42) - 68% nA β (1-
405 40) + 7% tA β (1-40) + 25 % nA β (1-42), (F) 3:1 A β (1-40):A β (1-42) - 75% nA β (1-40) + 15% nA β (1-42) + 10%
406 tA β (1-42), (G) 90% nA β (1-42) + 10% tA β (1-42). The species are represented as follows: monomer and low molar
407 mass oligomers (squares, ■), higher molar mass oligomers (circles, ●) and protofibrils (triangles, ▲).
408 Experimental conditions: Sample: 100 μ M total A β ; 20 mM phosphate buffer, pH 7.4. Incubation: quiescent
409 conditions at 37 °C. Fused silica capillaries: 50 μ m i.d. \times 60 cm \times 51.5 cm. Mobile phase: 20 mM phosphate
410 buffer, pH 7.4. Mobilization pressure: 100 mbar. Injection: 44 mbar for 4 s, $V_i \approx 7$ nL ($V_i/V_d \approx 0.6$ %). Analyses
411 were performed at 37 °C. UV detection at 191 nm.

412 **Figure 5.** Peak area evolution of the higher molar mass oligomer population obtained from the UV analysis of the
413 TDA elution profile for 3:1 A β (1-40):A β (1-42) - 75% nA β (1-40) + 25% nA β (1-42) (squares); 3:1 A β (1-
414 40):A β (1-42) - 68% nA β (1-40) + 7% nA β (1-40) + 25% nA β (1-42) (circles); and 3:1 A β (1-40):A β (1-42) - 75%
415 nA β (1-40) + 15% nA β (1-42) + 10% tA β (1-42) (triangles). Peak area was normalized, to obtain a better
416 comparison of the species evolution, by dividing the area of each data point to the total peak area obtained for the
417 first run at $t_{ag} = 0$ h.

418 **Figure 6.** Comparison of the peak area evolution of the monomer and small oligomer populations obtained by
419 UV detection (closed symbols) and LEDIF detection (open symbols) for nA β + tA β systems: (A) 93% nA β (1-40)
420 + 7% tA β (1-40), (B) 3:1 A β (1-40):A β (1-42) - 68% nA β (1-40) + 7% nA β (1-40) + 25% nA β (1-42), (C) 3:1 A β (1-
421 40):A β (1-42) - 75% nA β (1-40) + 15% nA β (1-42) + 10% tA β (1-42) and (D) 90% nA β (1-42) + 10% tA β (1-42).
422 Peak area was normalized to obtain a better comparison of the species evolution by dividing the area of each data
423 point to the total peak area obtained for the first run at $t_{ag} = 0$ h. The inserted pie charts denote the proportion of
424 the nA β and tA β content in the studied samples.



■ nA β (1-40)
 ■ tA β (1-40)
 ■ nA β (1-42)
 ■ tA β (1-42)

