



HAL
open science

Unraveling the Speciation of β -Amyloid Peptides during the Aggregation Process by Taylor Dispersion Analysis

Mihai Deleanu, Jean-François Hernandez, Luca Cipelletti, Jean-Philippe Biron, Emilie Rossi, Myriam Taverna, Herve Cottet, Joseph Chamieh

► To cite this version:

Mihai Deleanu, Jean-François Hernandez, Luca Cipelletti, Jean-Philippe Biron, Emilie Rossi, et al.. Unraveling the Speciation of β -Amyloid Peptides during the Aggregation Process by Taylor Dispersion Analysis. *Analytical Chemistry*, 2021, 93 (16), pp.6523-6533. 10.1021/acs.analchem.1c00527 . hal-03359470

HAL Id: hal-03359470

<https://hal.science/hal-03359470>

Submitted on 30 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Unraveling the speciation of β -amyloid peptides
2 during the aggregation process by Taylor dispersion
3 analysis

4 *Mihai Deleanu¹, Jean-François Hernandez¹, Luca Cipelletti^{2,3}, Jean-Philippe Biron¹, Emilie*
5 *Rossi⁴, Myriam Taverna⁴, Hervé Cottet^{*1}, Joseph Chamieh^{*1}*

6 ¹IBMM, Université Montpellier, CNRS, ENSCM, 34095 Montpellier, France

7 ²Laboratoire Charles Coulomb (L2C), Université Montpellier, CNRS, Montpellier, France.

8 ³Institut Universitaire de France (IUF), France

9 ²Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 92296, Châtenay-Malabry, France

10

11 * CORRESPONDING AUTHOR

12 Tel: +33 4 6714 3920, Fax: +33 4 6763 1046. E-mail: joseph.chamieh@umontpellier.fr

13 Tel: +33 4 6714 3427, Fax: +33 4 6763 1046. E-mail: herve.cottet@umontpellier.fr

14

15

16 **ABSTRACT**

17 The aggregation mechanisms of amyloid β peptides depend on multiple intrinsic and extrinsic
18 physico-chemical factors (*e.g.* peptide chain length, truncations, peptide concentration, pH, ionic
19 strength, temperature, metal concentrations...). Due to this high number of parameters, the
20 formation of the oligomers and their propensity to aggregate make the elucidation of this
21 physiopathological mechanism a challenging task. From the analytical point of view, up to our
22 knowledge, few techniques are able to quantify, in real time, the proportion and the size of the
23 different soluble species during the aggregation process. This work aims at demonstrating the
24 interest of modern Taylor dispersion analysis (TDA) performed in capillaries (50 μm i.d.) to
25 unravel the speciation of β -amyloid peptides in low volume peptide samples (~ 100 μL) with an
26 analysis time of ~ 3 min per run. TDA was applied to study the aggregation process of $\text{A}\beta(1-40)$
27 and $\text{A}\beta(1-42)$ peptides at physiological pH and temperature, where more than 140 data points
28 were generated with a total volume of ~ 1 μL over the whole aggregation study (about 0.5 μg of
29 peptide). TDA was able to give a complete and quantitative picture of the $\text{A}\beta$ speciation during
30 the aggregation process, including the sizing of the oligomers and protofibrils, the consumption
31 of the monomer, and the quantification of different early and late-formed aggregated species.

32

33 **KEYWORDS.** Taylor dispersion analysis; peptide aggregation; oligomers; amyloid beta
34 peptides; diffusion coefficient; hydrodynamic radius.

35

36

37 **Introduction**

38 Alzheimer's disease (AD) is the result of a slow degeneration of neurons. It starts in the
39 hippocampus (which lies in the medial temporal lobes of the brain and is responsible for the
40 long-term memory) then extends to the rest of the brain. This fatal neurodegenerative disorder is
41 characterized by progressive cognitive and functional impairment and memory loss¹. Up till
42 today, there is no cure for AD, however, there is extensive research to reveal its risk factors and
43 the mechanisms leading to this dementia. Indeed, more than 95000 articles including more than
44 19000 reviews dealing with Alzheimer's disease were published just in the last decade (number
45 of articles obtained on PubMed between 2010 and 2020 by searching "Alzheimer's disease").

46 For many years, AD was thought to be mainly associated to the formation of extracellular
47 senile plaques composed primarily of amyloid β peptides ($A\beta$) and hyperphosphorylated
48 neurofibrillary tangles of tau protein². Consequently, research toward AD curative treatments has
49 been driven largely by the amyloid cascade hypothesis. This hypothesis developed in the 1990's,
50 relies on the fact that the most hydrophobic $A\beta$ peptides ($A\beta$ 1-40 and mainly $A\beta$ 1-42) released
51 by APP (Amyloid- β Precursor Protein) enzymatic cleavage, readily self-assemble to form
52 amyloid species with evolving morphology and size (oligomers of increasing size, protofibrils
53 and then fibrils) through a highly complicated process, finally accumulating into plaques which
54 were believed to be the major pathogenic forms of $A\beta$ ^{3,4}. More recently, production of soluble
55 amyloid- β oligomers⁵ and inflammation⁶ have also emerged as important early steps in the
56 pathogenesis of Alzheimer's disease. The "amyloid- β oligomer hypothesis", which is still
57 under debate, states that the main reasons behind AD is the formation of soluble oligomers of $A\beta$
58 ⁷⁻¹⁰ considered to be more toxic than plaques and causing selective nerve cell death¹⁰⁻¹². Indeed,
59 soluble $A\beta$ oligomers ($A\beta$ O) are believed to be more toxic¹³ than fibrils, which precipitate as

60 plaques, because they are able to spread across neuronal tissue and they are supposed to mediate
61 neurotoxicity and synaptic loss through binding to membrane receptors, including the prion
62 protein^{14,15}. To assess its validity and to develop new drug candidates against AD targeting the
63 soluble oligomers, new analytical methodologies able to finely monitor, quantify and
64 characterize these oligomeric species are required. However, in contrast to fibrils, which have
65 low solubility and are highly stable, the soluble oligomers are fragile, metastable, transient¹⁶,
66 highly polydisperse in size, and therefore more difficult to detect and study in real time¹⁶.

67 When studied *in vitro*, the aggregation mechanisms leading to the oligomers and then to fibrils
68 depend on multiple physico-chemical factors, that can be intrinsic¹⁷ (*e.g.* chain length,
69 truncations, net charge, hydrophobicity) and extrinsic, such as concentration^{18,19}, pH,
70 temperature, incubation conditions²⁰, buffer ionic strength and salt composition²¹. The influence
71 of metals and other proteins has also been reported.¹⁰ Due to this high number of parameters the
72 elucidation of the aggregation mechanism is a challenging task. The detection of fibrils during
73 the early stages of the aggregation process can be realized by multiple analytical techniques, and
74 specifically by fluorescence using the ThT assay²². However, the ThT assay is mainly insensitive
75 to A β oligomeric species²³. In contrast, other analytical techniques are able to detect the presence
76 of oligomers such as size exclusion chromatography (SEC)^{24,25}, AFM microscopy^{26,27}, capillary
77 electrophoresis²⁸, mass spectrometry²⁹⁻³¹, and dynamic light scattering (DLS)³², to name a few.
78 However, some of the aforementioned methods require large sample volume (*e.g.* SEC), others
79 are very sensitive to the presence of the large fibrils (or particles) making the detection of the
80 small oligomers a difficult task (*e.g.* DLS). Few of these methods are able to follow in real time
81 the aggregation process in a medium representative of the *in vivo* conditions. Furthermore, some
82 of these techniques require a sample pretreatment before the analysis²⁵, which may alter the form

83 of the species present in the sample. Thus, new methods able to rapidly determine the size of
84 aggregates in the range 1-100 nm are highly required to better understand the real-time
85 mechanism of oligomer formation.

86 In this context, Taylor Dispersion Analysis³³⁻³⁵ (TDA) appears as a very promising alternative
87 analytical method. Indeed, TDA is an absolute method (no calibration needed) allowing for the
88 determination of the molecular diffusion coefficient, D , and of the hydrodynamic radius, R_h , of a
89 solute, including for mixtures, without any bias in size, the contribution of the small and the
90 large solutes being proportional to their mass abundance in the mixture³⁶. TDA is based on the
91 dispersion of an injected band under a laminar Poiseuille flow. Its implementation in narrow bore
92 capillaries (typically ~50 μm i.d.) presents several advantages³⁷⁻⁴⁰ such as a low sample
93 consumption, a short analysis time, a wide range of sizing (from angstrom to sub-micron) and a
94 straightforward analysis without any sample pretreatment or filtration⁴¹⁻⁴³.

95 In this work, TDA was applied to study the aggregation process of two A β isoforms A β (1-40)
96 and A β (1-42) at physiological pH (7.4) and temperature (37°C) by providing a direct
97 determination of all possible forms of A β amyloid according to the incubation time. TDA was
98 able to provide a complete picture of the A β speciation during the in-vitro aggregation process,
99 including the consumption of the monomer and the formation of oligomers, protofibrils and
100 fibrils.

101 **Materials and methods**

102 **Materials**

103 Synthesized Amyloid beta (1-40) (denoted A β (1-40) in this work) was prepared as described in
104 the *Solid-phase synthesis of A β (1-40)* section in the supporting information (Figure SI.1 shows a

105 scheme of the synthesis protocol, while Figure SI.2 shows the chromatographic and mass
106 spectrometry analysis of the synthesized peptide). Commercial Amyloid beta 1-40 (batch number
107 1658309, >95%) (denoted cA β (1-40) in this work) was purchased from Anaspec (USA).
108 Amyloid beta (1-42) (A β (1-42), batch number 1071428, >95%) was purchased from Bachem
109 (Bubendorf, Switzerland). Thioflavin T, sodium dihydrogen phosphate,
110 tris(hydroxymethyl)aminomethane, hydrochloric acid fuming 37%, sodium chloride and sodium
111 hydroxide were purchased from Sigma Aldrich (France). *Fmoc* protected amino acids, coupling
112 reagents HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) and PyBOP
113 (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) were purchased from
114 Iris Biotech (Germany). The ChemMatrix H-Val-O-Wang resin was purchased from PCAS
115 Biomatrix (Canada). Dimethylformamide, acetic anhydride, piperidine, dichloromethane,
116 methanol, acetonitrile, trifluoroacetic acid and diethyl ether were acquired from Carlo Erba
117 (Italy), Sigma-Aldrich (Merck, Germany), Acros Organics (Thermo Fisher-Scientific, Germany)
118 or Honeywell-Riedel de Haën (Fisher-Scientific, Illkirch, France), and were all of analytical
119 grade. *N,N*-diisopropylethylamine, 1,1,1,3,3,3-hexafluoro-2-propanol, triisopropylsilane (TIS)
120 and tetra-*n*-butylammonium bromide were purchased from Alfa Aesar (Thermo Fisher-
121 Scientific, Germany) or Fluorochem (UK). The ultrapure water used for all buffers was prepared
122 with a MilliQ system from Millipore (France).

123 **Peptide pretreatment**

124 Both A β (1-40) and A β (1-42) were first pretreated independently as described elsewhere^{28,44}.
125 Briefly, A β (1-40) and A β (1-42) were dissolved in a 0.10 % (m/v) and 0.16 % (m/v) NH₄OH
126 aqueous solution respectively to reach a final peptide concentration of 2 mg/mL. The peptide
127 solutions were then incubated at room temperature for 10 min, separated into several aliquots

128 and freeze-dried. The aliquot volume was calculated in order to obtain 10 nmol of peptide in
129 each Eppendorf tube. The lyophilized peptide aliquots were stored at -20 °C until further use.

130 **ThT fluorescence assay**

131 In order to check the initial state of aggregation of the studied peptides, ThT fluorescence assay
132 was used by adapting the protocol described in⁴⁵. Briefly, peptides were dissolved at a
133 concentration of 1 mM in a 1% NH₄OH aqueous solution, then diluted with 10 mM Tris-HCl +
134 100 mM NaCl buffer (pH 7.4) to a final concentration of 0.2 mM. A volume of 10 μL of the
135 latter peptide solution was withdrawn and put in a Costar® 96-well black polystyrene plate along
136 with 189 μL of 40 μM ThT in the Tris-HCl saline buffer solution. The fluorescence was
137 monitored at room temperature for 24 h using a Berthold TriStar LB 941 instrument (Germany)
138 (excitation wavelength 430 nm and emission wavelength 485 nm). Control wells were prepared
139 by replacing the 10 μL of peptide solution with 10 μL of a 0.2% NH₄OH aqueous solution
140 prepared by diluting a 1% NH₄OH aqueous solution with 10 mM Tris-HCl + 100 mM NaCl
141 buffer (pH 7.4). Five wells were prepared for each solution.

142 **Peptide aggregation study by Taylor dispersion analysis**

143 TDA was performed on an Agilent 7100 (Waldbronn, Germany) capillary electrophoresis system
144 using bare fused silica capillaries (Polymicro technologies, USA) having 40 cm × 50 μm i.d.
145 dimensions and a detection window at 31.5 cm. New capillaries were conditioned with the
146 following flushes: 1 M NaOH for 30 min; ultrapure water for 30 min. Between each analysis,
147 capillaries were rinsed with 20 mM phosphate buffer, pH 7.4 (2 min). Samples were injected
148 hydrodynamically on the inlet end of the capillary (44 mbar, 3 s, injected volume is about 7 nL
149 corresponding to 1% of the capillary volume to the detection point). Experiments were
150 performed using a mobilization pressure of 100 mbar. The temperature of the capillary cartridge

151 was set at 37° C. The vial carousel was thermostated using an external circulating water bath
152 from Bioblock (France). The solutes were monitored by UV absorbance at 191 nm. The mobile
153 phase was a 20 mM phosphate buffer, pH 7.4 (viscosity at 37°C is 0.7×10^{-4} Pa.s)²⁸. Peptide
154 samples were dissolved in 100 μ L of 20 mM phosphate buffer, pH 7.4, to reach a final
155 concentration of 100 μ M and were immediately transferred to a vial and incubated at 37°C in the
156 capillary electrophoresis instrument's carousel. The aggregation was conducted by injecting the
157 sample ($V_{inj} \approx 7$ nL) every 7 min in the case of A β (1-42) and each 30 min in the case of A β (1-
158 40). The total number of TDA runs for each sample was about 150, corresponding to a total
159 sample volume of 1050 nL (1.05 μ L). To avoid sample evaporation, the vial cap was changed
160 three times a day. The taylorgrams were recorded with the Agilent Chemstation software then
161 exported to Microsoft Excel for subsequent data processing.

162 **Dynamic light scattering**

163 Complementary Dynamic Light Scattering data were acquired using a standard setup by
164 Brookhaven Instruments Co. (BI-900AT), equipped with a 150 mW laser with in-vacuo
165 wavelength $\lambda = 535$ nm. Frozen, dehydrated samples were thawed at room temperature. At time
166 $t_{ag} = 0$, a volume of 100 μ L of 20 mM phosphate buffer, pH 7.4, filtered through a 0.22 μ m
167 Millipore filter was added to the thawed powder, setting the A β 42 concentration to 100 μ M. The
168 sample was injected in an NMR tube and placed in the setup immediately after mixing.
169 Measurements were performed as a function of t_{ag} by alternating runs at scattering angles $\theta = 90^\circ$
170 and $\theta = 45^\circ$ (run duration: 240 s and 360 s, respectively). The sample was thermostated at $37.0 \pm$
171 0.1 °C

172 The CONTIN algorithm^{46,47} embedded in the Brookhaven software was used to extract $P_I(D)$, the
173 intensity-weighted distribution of the diffusion coefficients D of the scatterers, which was then

174 converted to the mass-weighted distribution of hydrodynamic radii R_h , $P_M(R_h)$, using custom
175 software. In performing the conversion, it was assumed that the peptides aggregate by forming
176 cylindrical structures resulting from the stacking of dimer units (see the discussion section and
177 Figure 6). This allowed us to calculate the mass and scattered intensity (to within an inessential
178 multiplicative constant) of the aggregates, as a function of their hydrodynamic radius, obtained
179 via the HYDROPRO software⁴⁸. Knowledge of $M(R_h)$ and $I(R_h)$ allowed for re-expressing
180 $P_f(D)$ as $P_M(R_h)$, using standard probability distribution transformation laws and the Stokes-
181 Einstein relationship $R_h = k_b T / (6\pi\eta D)$, with k_b Boltzmann's constant, $T = 310.15$ K, and $\eta = 0.7$
182 mPa s the solvent viscosity.

183 **Results and discussion**

184 **ThT assay and the initial state of the peptide samples**

185 The aggregation of A β peptides is a highly complex process that is dependent on, and very
186 sensitive to, the initial conditions of the peptides preparation. The initial presence of aggregates
187 (or seeds of aggregates) can drastically influence the aggregation process. Therefore, the peptides
188 were treated upon reception with an ammonium hydroxide solution before freeze drying and
189 storage²⁸. The aim of this step is to dissociate any aggregated peptide and to start the kinetic
190 studies from the very early steps, with a non-aggregated sample. To confirm the success of this
191 step, samples were submitted to the ThT fluorescence assay⁴⁹. Figure SI.3 shows the
192 fluorescence kinetic curves of the studied peptide batches: the synthesized A β (1-40), the
193 commercial cA β (1-40), A β (1-42) and a control run. Only the commercial cA β (1-40) was found
194 to be initially aggregated, despite the ammonium hydroxide treatment, since it showed an initial
195 relatively high fluorescence signal and nearly no lag phase. In contrast, the synthesized A β (1-40)

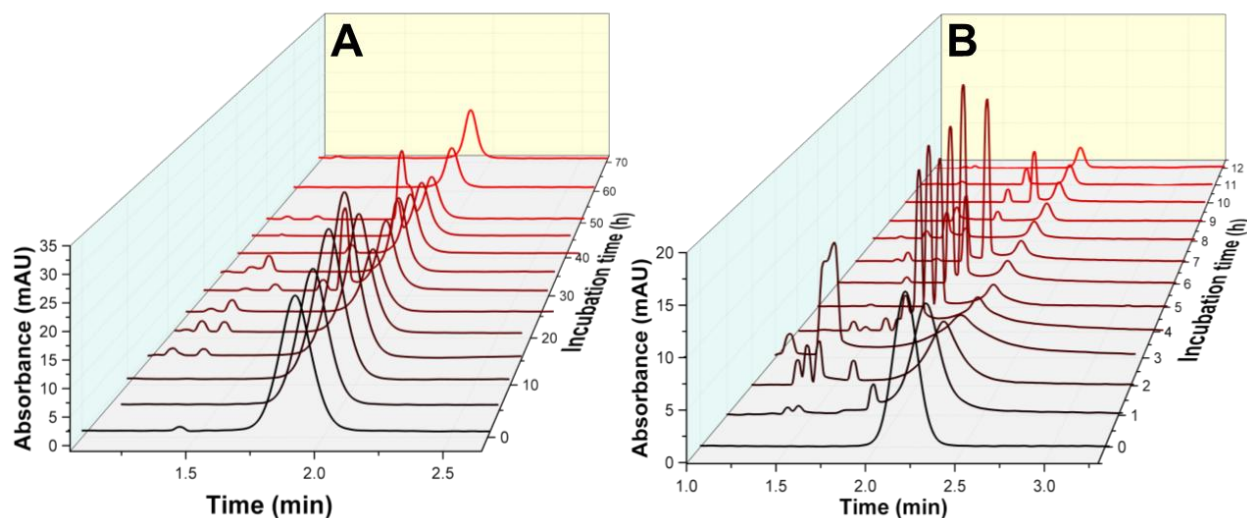
196 and the commercial A β (1-42) peptides were assumed to be free of aggregates since their initial
197 fluorescence intensity was low and in the same order of magnitude as the control run. These
198 results show the importance of using clean (non-aggregated) samples for kinetic studies.

199 **Processing of the taylorgrams**

200 Briefly, the band broadening resulting from Taylor dispersion is easily quantified via the
201 temporal variance (σ^2) of the elution profile. For that, a fit of the experimental peak with a
202 Gaussian function allows for the determination of σ^2 and the calculation of the molecular
203 diffusion coefficient, D , and consequently the hydrodynamic radius, R_h . The reader may refer to
204 the supporting information for the theoretical aspects, equations and more details on the data
205 processing.

206 The peptides were incubated at 37°C in a 20 mM phosphate buffer at pH 7.4. The aggregation
207 was followed for 72 h and 12.5 h for A β (1-40) and A β (1-42), respectively. Figure 1 shows the
208 taylorgrams recorded at selected incubation times for A β (1-40) (Figure 1A) and A β (1-42)
209 (Figure 1B) while all experimental taylorgrams, for all incubations times t_{ag} , are shown in
210 Figures SI.4 and SI.5. Importantly, the elution profile evolved faster in the case of A β (1-42) as
211 compared to A β (1-40), suggesting a faster aggregation kinetics for this peptide. A second
212 observation is that, for both studied peptides, the main peak observed at an elution time $t_0 \approx 2$
213 min, which represents the A β monomer at $t_{ag} = 0$, tended to broaden and to decrease in intensity
214 during the aggregation process. This indicates the appearance of larger species and the decrease
215 in concentration of the soluble species in the sample. At the end of the aggregation experiment,
216 only a small sharp peak was observed (with a size corresponding to a small molecule/ion of
217 about 0.4 nm, smaller than the size of the peptide monomer ~ 1.8 nm), indicating the

218 disappearance of the soluble peptides, probably transformed into insoluble and larger aggregates
219 that were not entering in the capillary, leading to the decrease in the peak area. At intermediate
220 incubation times (*e.g.* t_{ag} between 0.5 h and ~11 h for A β (1-42)) the left side of the elution
221 profile displayed spikes (very sharp peaks appearing before the main elution peak at elution
222 times between 0.9 and 1.7 min), demonstrating the presence of very large species that are out of
223 the Taylor regime^{50,51} and rather belong to the so-called convective regime. In addition to the
224 convective regime⁵⁰, large aggregates such as A β fibrils can also generate spikes, as seen in
225 capillary electrophoresis and/or hydrodynamic flow of bacterial aggregates⁵².



226
227 **Figure 1.** Three-dimensional overview of the obtained Taylorgrams during the aggregation
228 process of A β (1-40) (A) and A β (1-42) (B) at different incubation times. Experimental
229 conditions: Sample: 100 μ M; 20 mM phosphate buffer, pH 7.4. Incubation: quiescent conditions
230 at 37 $^{\circ}$ C. Fused silica capillaries: 50 μ m i.d. \times 40 cm \times 31.5 cm. Mobile phase: 20 mM
231 phosphate buffer, pH 7.4. Mobilization pressure: 100 mbar. Injection: 44 mbar for 3 s, $V_i \approx 7$ nL
232 ($V_i / V_d \approx 1$ %). Analyses were performed at 37 $^{\circ}$ C. UV detection at 191 nm.

233 In general, the obtained elution profiles were not Gaussian meaning that the sample was
234 polydisperse in size. All Taylorgrams were fitted on the basis of the right-side elution profile (*i.e.*

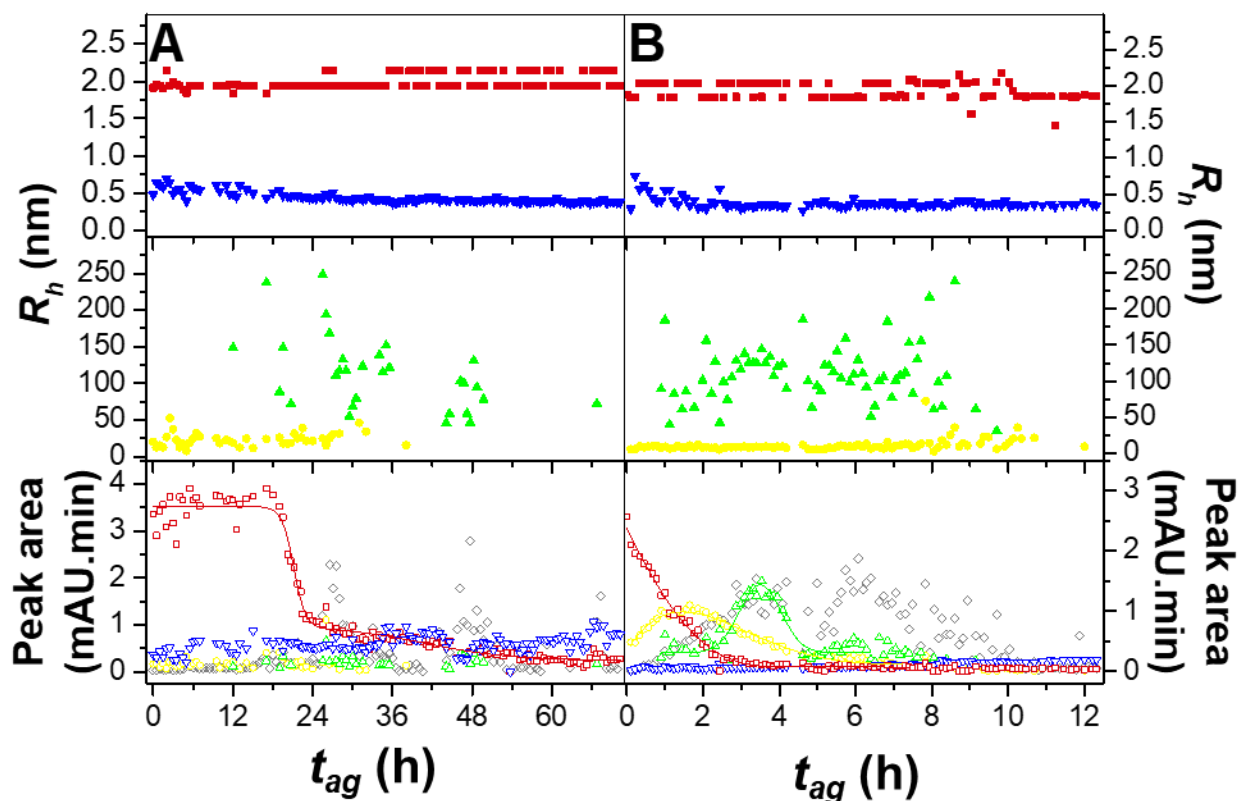
235 $t > t_0$, with t_0 the peak time) to get rid of the spikes that are present on the left side. The
236 deconvolution of the right-side of the taylorgram provides valuable information on the
237 aggregation process. Indeed, a complex mixture of components was obtained, composed of
238 varying proportions of A β monomer, intermediate oligomers (R_h lower than 50 nm), protofibrils
239 (R_h between 50 and 150 nm), small molecules (salts, counter ions...) and fibrils/insoluble
240 aggregates (typical dimensions having an average diameter of approximately 7 – 10 nm and
241 lengths up to several micrometers were reported for fibrils⁵³⁻⁵⁵, they are detected as spikes on the
242 taylorgrams). Except for the fibrils and other insoluble aggregates, all components in the mixture
243 could be sized and quantified by TDA. For that, all the elution profiles were deconvoluted using
244 two different approaches to extract the size and proportion of the different populations. A first
245 fitting approach consisted in using a finite number of Gaussian curves ($n = 1 - 4$). The second
246 fitting approach used the Constrained Regularized Linear Inversion (CRLI) algorithm, which
247 does not require any hypothesis on the number of populations and allows obtaining a continuous
248 distribution of the diffusion coefficient or of the hydrodynamic radius⁵⁶.

249 Figure SI.6 in the supporting information shows two typical examples of deconvolution of a
250 TDA profile for A β (1-40) (Figure SI.6A) and A β (1-42) (Figure SI.6B), at selected incubation
251 times $t_{ag} = 25.52$ h and $t_{ag} = 1.98$ h, respectively. In these examples, four Gaussian functions were
252 used to fit the elution profile, with low residues for the curve fitting on the right side of the
253 profile (see upper part of each Figure). When a lower number of Gaussian functions ($n \leq 3$) was
254 used, the residues were much higher (see Figure SI.7). It is worth noting that a constraint was
255 added to the fitting procedure on the value of the peak variance of the monomer population,
256 allowing it to vary within 5 % with the respect to that at $t_{ag} = 0$ h (initial size of the monomers).
257 Figures SI.8 and SI.9 show the Gaussian peaks extracted from the 4-Gaussian fit for the four

258 populations and for both peptides, together with their respective area as a function of incubation
259 time.

260 **Monitoring A β (1-40) and A β (1-42) aggregation by TDA.**

261 Figure 2 shows the monitoring of A β (1-40) (Figure 2A) or A β (1-42) (Figure 2B) aggregation
262 using the aforementioned data processing. The lower panels of Figure 2 represent the evolution
263 of the peak area of each population (proportional to its mass abundance), while the middle and
264 upper panels represent the evolution in size (R_h) of these populations. The populations were
265 classified by size into four groups. In the first group, some small molecules ($R_h = 0.3 - 0.4$ nm)
266 were detected (blue down triangles). Their size as well as their abundance (peak area) were
267 constant throughout the aggregation, and their presence seems therefore not related to the
268 aggregation process. The second population (red squares) had a size of 1.99 ± 0.09 nm for A β (1-
269 40) and 1.94 ± 0.12 nm for A β (1-42), and was attributed to the monomeric and small oligomeric
270 forms of the peptides (up to dodecamers, see next section). The third population was attributed to
271 higher molar mass oligomers with R_h between 4 and 50 nm. The average size of this population
272 over the whole aggregation process was 24.9 ± 10.3 nm for A β (1-40) and 10.8 ± 6.1 nm for
273 A β (1-42). The fourth population with $R_h > 50$ nm was attributed to soluble protofibrillar
274 structures with an average size of 119 ± 49 nm for A β (1-40) and 110 ± 39 nm for A β (1-42).



275
 276 **Figure 2.** Hydrodynamic radius and peak area evolution of the different populations observed
 277 during the aggregation process of A β (1-40) (A) and A β (1-42) (B) using a 4 Gaussians fitting of
 278 the taylorgrams. Closed symbols are for the hydrodynamic radius: small molecules (B),
 279 monomer and low molar mass oligomers (!), higher molar mass oligomers (.), and soluble
 280 protofibrils (7). Open symbols correspond to the peak area of each species: small molecules (X),
 281 monomer and low molar mass oligomers (∇), higher molar mass oligomers (–), soluble
 282 protofibrils (8) and fibrils (M) (spikes). The straight lines are guides for the eyes. Experimental
 283 conditions as in Figure 1.

284 For the A β (1-40), only the monomeric and low molar mass oligomers populations were
 285 significantly present in the sample (see open red squares in Figure 2A), as compared to the high
 286 molar mass oligomers and protofibrils populations (open yellow circles and open green triangles,
 287 respectively), which were much less abundant. The red traces in Figure 2A showed that A β (1-40)
 288 was essentially in its monomeric form and remained so up to $t_{ag} \sim 18$ h. Afterwards, the peak area
 289 of the monomeric population rapidly dropped to reach a lower plateau at $t_{ag} \sim 24$ h. It is

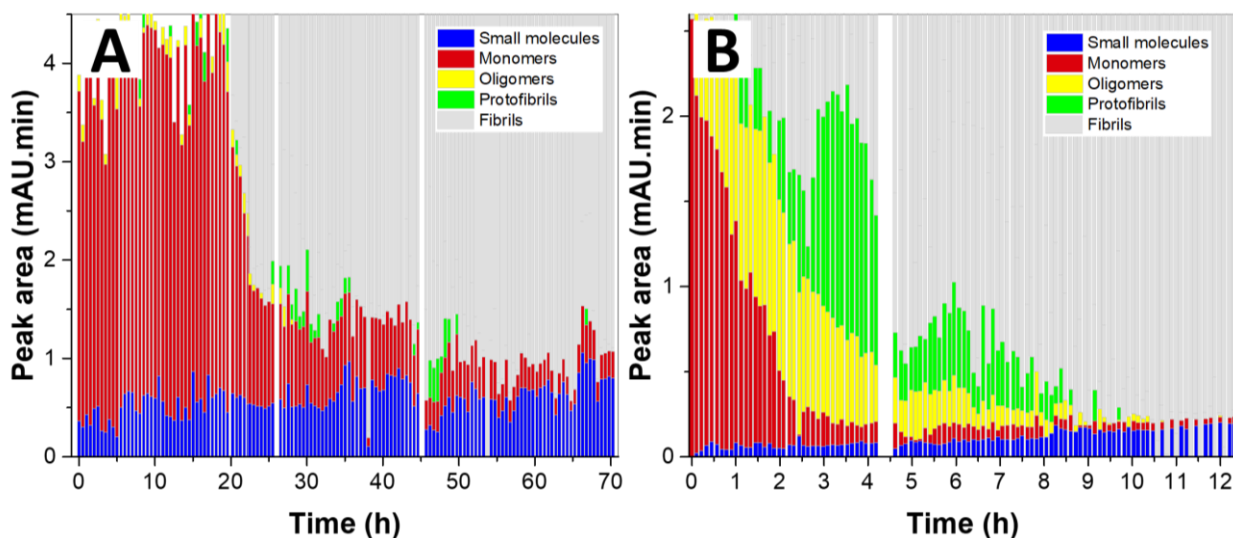
290 important to note that despite the disappearance of the monomeric form, no other soluble species
291 yielded a significant signal in TDA. Indeed, the aggregation of A β (1-40) displayed a threshold-
292 type behavior, which indicates that the rate-determining step for aggregation is the formation of
293 multimeric seeds. In other words, our results seem to indicate that A β (1-40) goes through a
294 secondary nucleation mechanism where monomers add to already present fibrils to elongate
295 them and to produce larger fibrils, without going through intermediate species in accordance
296 with what is discussed in the literature^{57,58}. To confirm this hypothesis, a slight manual shaking
297 of the vial was done at 25 h, 48 h and 70 h, in order to resuspend any precipitate/fibrils that may
298 have sedimented. After each remixing, a significant increase of the peak area of the “spikes”
299 (open grey diamonds in the lower part of Figure 2A) was transiently observed, proving the
300 presence of insoluble species in the sample that suspend upon shaking and then tend to decant.

301 The aggregation process for A β (1-42) displayed a different pathway as compared to that of
302 A β (1-40). For A β (1-42) the proportion of monomeric and low molar mass oligomeric
303 populations decreased rapidly, while the higher molar mass oligomeric species increased to reach
304 a maximum at $t_{ag} = 1.6$ h, after the disappearance of the monomeric species. Subsequently, the
305 protofibrils proportion increased to reach a maximum at $t_{ag} = 3.5$ h, and finally the spikes (non-
306 diffusing species in suspension) increased to reach a maximum at $t_{ag} = 5.6$ h. From these
307 observations, it is evident that TDA experiments gives a clear picture of the early stages of the
308 aggregation process of the A β (1-42) that goes through a primary nucleation leading to
309 intermediate species and successively an elongation step producing protofibrils and then fibrils.

310 The results for A β (1-40) and A β (1-42) obtained by fitting the taylorgram to n Gaussians were
311 compared to the evolution of the R_h distributions obtained by CRLI⁵⁶, as shown in the supporting
312 information (Figures SI.10, SI.11, SI.12 and SI.13). Continuous distributions of the

313 hydrodynamic radius for each run (Figures SI.10 and SI.11) were obtained by CRLI algorithm,
314 allowing for a full and quantitative characterization of the aggregation process. The CRLI
315 analysis confirmed the two different pathways that were inferred for the aggregation of A β (1-40)
316 and A β (1-42) on the basis of the *n*-Gaussians fits.

317 Another way to qualitatively and visually assess the entire aggregation process and the speciation
318 of the amyloid peptides during the aggregation process is shown in Figure 3, which displays a
319 stacked bar representation of the peak area of each population. The grey region represents the
320 insoluble species that can enter the capillary and appear as spikes as well as those that precipitate
321 and no longer enter in the capillary at the injection step, lowering the total observed peak area
322 over incubation time. From this Figure, one can clearly distinguish the two different aggregation
323 pathways^{32,59}.



325 **Figure 3.** Stacked bar graphs showing the speciation of A β (1-40) (A) and A β (1-42) (B) at each
326 analyzed incubation time obtained by TDA. Experimental conditions as in Figure 1. Four
327 populations are distinguished: “small molecules” for $R_h \sim 0.4$ nm; “monomers” for the monomer
328 and the low molar mass oligomers with $R_h \sim 1.9$ nm, “oligomers” for high molar mass oligomers
329 with R_h between 4 and 50 nm; and “protofibrils” for large diffusing soluble species with R_h

330 between 50 and 150 nm. Each population is represented by the corresponding peak area obtained
331 by 4 Gaussian curve fitting. The population in grey represents the fibrils but were not quantified
332 by TDA and are just represented by difference.

333 To confirm these observations, the ThT assay was realized in the same conditions as the TDA
334 analysis. The ThT assay is best known to detect the amyloid fibrillary structures, which are
335 formed at the expense of the soluble ones causing a decrease in their proportion. As seen in
336 Figure SI.14, the ThT assay curve superimpose on the concentration evolution of the insoluble
337 species determined by TDA, demonstrating that TDA faithfully captures the lag phase and the
338 time to reach the plateau of the aggregation process. Additionally, TDA allowed for a
339 quantitative estimation of the intermediate steps of the aggregation , especially in the case of
340 A β (1-42), a feature difficult to obtain⁵⁷ with other techniques such as SEC²⁵.

341 **Discussion on the size of the A β species during the aggregation process.**

342 Regarding the size of the aggregated forms, it was suggested from combined results obtained by
343 NMR, FTIR and AFM, that A β (1-42) rapidly forms low molar mass oligomers upon
344 solubilization⁶⁰. The predominant forms ranged from dimer to dodecamer^{59,61} including some
345 assemblies (from tetramer to octamer) called “paranuclei”³², which were in equilibrium with the
346 lower molar mass oligomers. Several methods were used in the literature to identify the nature of
347 these oligomers. Ion mobility MS allows to get structural information relative to the oligomeric
348 molar mass distribution²⁹, however, the separation is obtained in gas phase which can perturb the
349 oligomeric distribution. Further, ion suppression effect may also occur for quantitative analysis
350 in complex mixtures⁶². Real-time aggregation monitoring methods such as dynamic light
351 scattering (DLS) are very difficult to apply to detect the presence of small oligomers in
352 polydisperse samples, especially in the presence of large aggregates. TDA has the advantage of

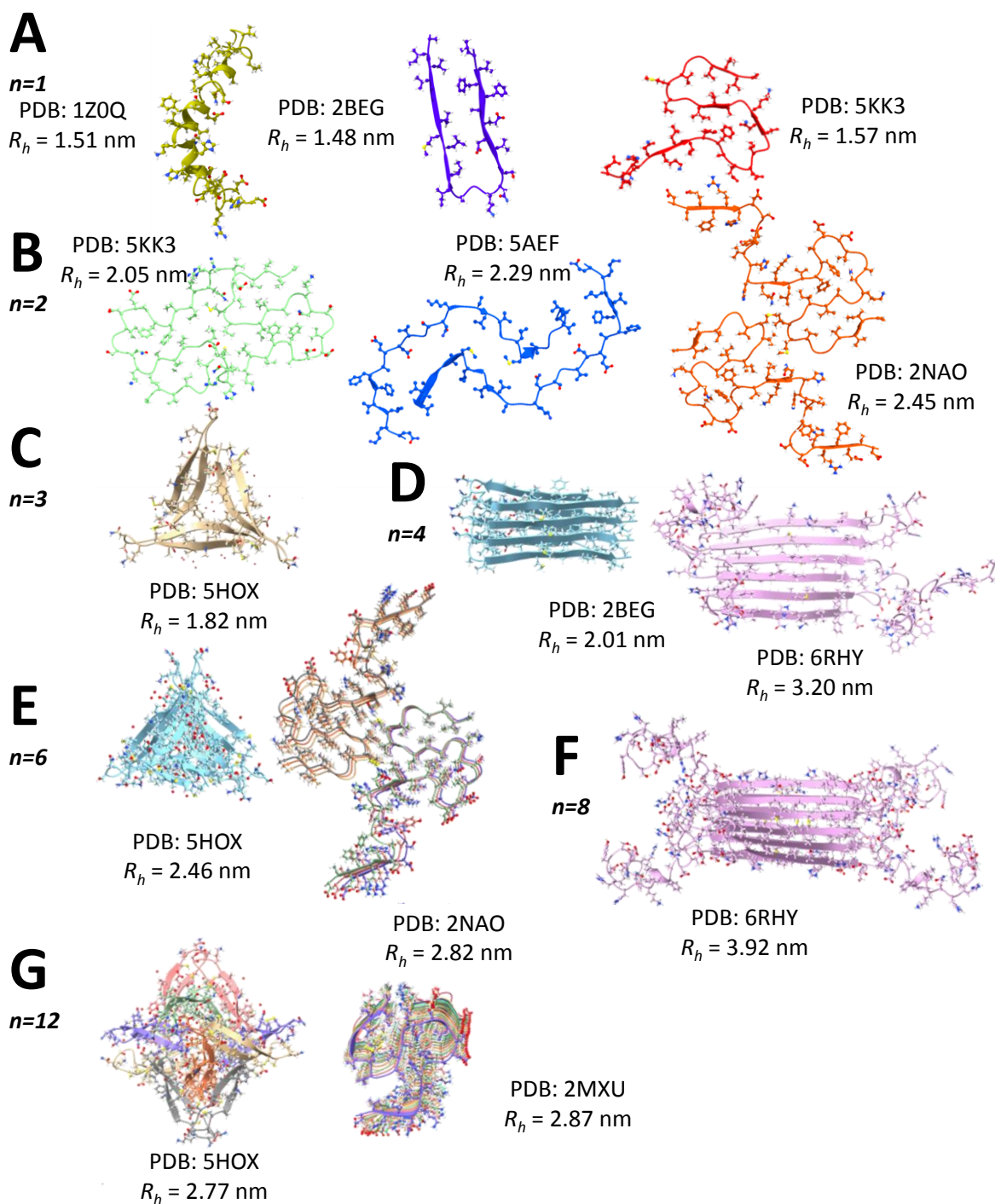
353 being less sensitive to the presence of very large aggregates^{36,63} allowing the detection of the
354 early stage species, without bias in the mass-weighted size distribution. For the sake of
355 comparison, DLS experiments were realized on the A β (1-42) sample in the same conditions as in
356 TDA. From the obtained size distributions, $P_M(R_h)$, we integrated over four intervals, so as to
357 obtain the mass-weighted relative contribution of four classes of aggregates, with $R_h < 5$ nm, 5
358 nm $< R_h < 50$ nm, 50 nm $< R_h < 500$ nm, and $R_h > 500$ nm, respectively (see Methods for
359 details). Figure SI.15 shows the time evolution of the (mass-weighted) fractions of the four
360 classes of aggregates thus obtained. The data shown in the figure correspond to the average of
361 results obtained by processing separately data collected at scattering angles $\theta = 90^\circ$ and $\theta = 45^\circ$;
362 x and y error bars indicate the half-difference between the corresponding pairs of data at 90° and
363 45° . In contrast to TDA results, dimers and small oligomers, corresponding to $R_h < 5$ nm, are not
364 detected by DLS, because their scattered intensity is much weaker than that of larger species. On
365 the other hand, DLS detects large aggregates, including objects up to several hundred nm, which
366 are beyond the range accessible to TDA. Aggregates with $R_h > 500$ nm are detected as early as at
367 $t_{ag} = 500$ s. Their relative contribution increases significantly for $t_{ag} > 1200$ s (0.33 h), at the
368 expenses of both intermediate (50 nm $< R_h < 500$ nm) and smaller (5 nm $< R_h < 50$ nm)
369 aggregates. These results show that DLS is a powerful technique able to follow in real time the
370 evolution of the larger size species. However, in contrast to TDA, the sensitivity of DLS toward
371 the smaller size species is quite limited. Thus, TDA and DLS are complementary methods.

372 In order to propose possible oligomeric structures that fit with the size of each population found
373 by TDA, molecular simulation was performed based on A β (1-42) monomers (folded and
374 unfolded)⁶⁴ and oligomers^{65,66} structures found in the literature (low and high molar mass, from 2
375 to 360 monomer units). Different 3D molecular structures were constructed using the UCSF

376 Chimera X software⁶⁷ that were next loaded into HYDROPRO+⁴⁸ software to calculate the
377 hydrodynamic properties. The 3D structures were adjusted so that the calculated translational
378 diffusion coefficients equals the experimental values obtained by TDA for each population.
379 Figures 4 and 6 display possible conformations for small and large oligomers thus obtained.

380 Different A β (1-42) monomer structures were considered, based on the structures published by
381 Tomaselli *et al.*⁶⁴ (PDB code 1Z0Q), Lührs *et al.*⁶⁸ (PDB code 2BEG) and Colvin *et al.*⁶⁶ (PDB
382 code 5KK3). Results show hydrodynamic radii around 1.5 nm for the different conformations
383 (see Figure 4, monomer structures). The average hydrodynamic radii of the “monomer and small
384 oligomers” population obtained by TDA on all runs over the whole A β (1-42) aggregation study
385 ($t_{ag} = 12.5$ h, $n = 110$ TDA runs) was of 1.94 nm (RSD = 5.9 %) and the initial size at $t_{ag} = 0$ h
386 was 1.84 nm. To correlate the observed experimental size with oligomeric structures, different
387 proposed oligomeric structures from the literature, ranging from dimer to dodecamer with
388 different conformations were used and computed to get the hydrodynamic radii (Figure 4) (PDB
389 codes 5AEF⁶⁹, 2NAO⁷⁰, 5HOX⁷¹, 6RHY⁷² and 2MXU⁷³). The latter structures were determined
390 by electron cryo-microscopy⁶⁹, solid state NMR^{70,73}, X-ray crystallography⁷¹ and NMR⁷². The
391 combination of our results and those from the literature, suggest that the “monomer and small
392 oligomers” population at $t_{ag} = 0$ h was mainly composed of monomers and dimers⁷⁴. The weight-
393 average R_h obtained by TDA is sensitive to the mass proportion of all the soluble species present
394 in the mixture. The CRLI analysis brings additional information about the polydispersity of each
395 population mode (see Figure 5). However, due to the low difference in R_h of the various small
396 species (monomers / dimers / trimers) neither the CRLI nor the Gaussian fitting approaches were
397 able to resolve these small species. CRLI shows that the size distribution of the “monomer and
398 small oligomers” population at $t_{ag} = 0$ h ranges between 1 and 3 nm and is centered around 1.9

399 nm. The polydispersity in size of this mode increases with increasing incubation times. This
400 population becomes negligible after $t_{ag} \sim 2 - 4$ h. Several reports⁷⁵⁻⁷⁷ suggested the presence of a
401 critical nucleus size, which is the minimum size that enables the extension of amyloid fibrils. To
402 our knowledge, no consensus was reached on the exact size of the nuclei, while other reports
403 stated that the nucleation was heterogeneous^{78,79}. However, aggregation numbers between 2 and
404 14 were reported^{75-77,80,81}, which according to this work would correspond to a size distribution
405 between 1.8 and ~ 3 nm, thus the first oligomer size population found by TDA.

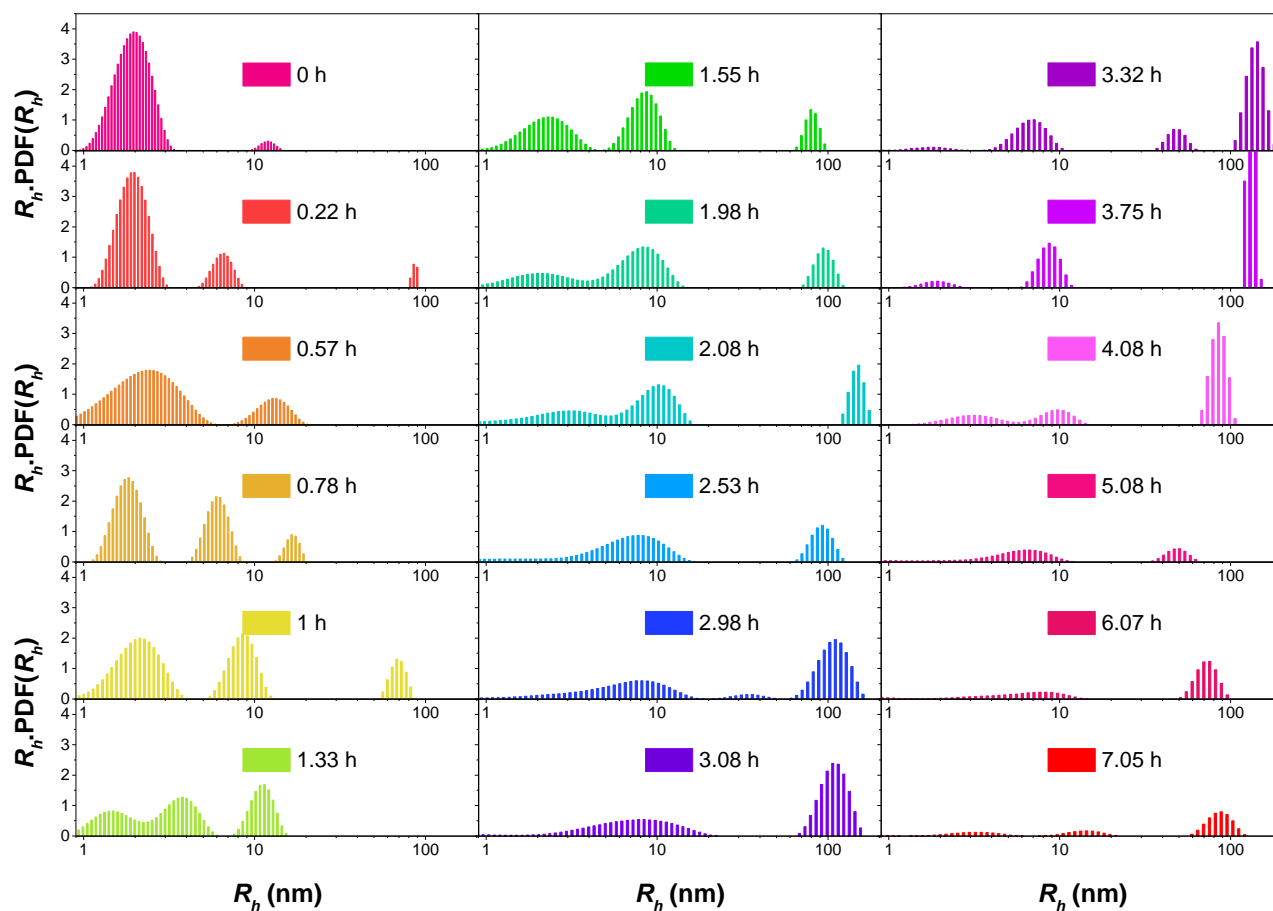


406

407 **Figure 4.** Schematic representation of the monomeric A β (1-42) (A) and small-oligomer
 408 conformations from dimers to dodecamers (B to G represent dimers, trimers, tetramers,
 409 hexamers, octamers and dodecamers respectively). The 3D structures were realized using the
 410 UCSF Chimera X software⁶⁷ and were adapted from the structure found in the literature (PDB
 411 codes 1Z0Q⁶⁴, 2BEG⁶⁸ and 5KK3⁶⁶). The arrangement of the monomers in the oligomeric forms
 412 were adapted from the literature (PDB codes 5AEF⁶⁹, 2NAO⁷⁰, 5HOX⁷¹, 6RHY⁷² and 2MXU⁷³).

413 The R_h were calculated by introducing the generated PDB files for each structure into the
 414 HYDROPRO software⁴⁸. It is worth noting that the R_h calculation takes into account all possible
 415 orientations of the molecular structure relative to the flow direction.

416



417

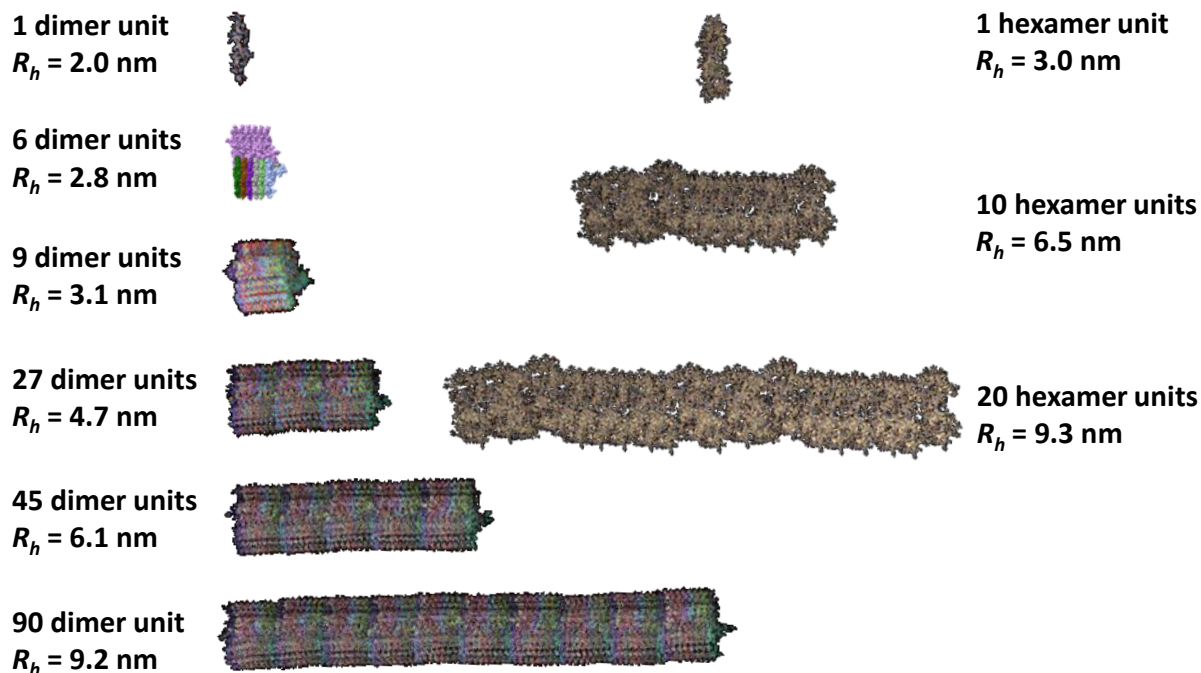
418 **Figure 5.** Size distributions of A β (1-42) obtained by CRLI analysis of the experimental
 419 taylorgrams as a function of incubation time $t_{ag} = 0$ to 7 h. Experimental taylorgrams and
 420 conditions as in Figure 1.

421 In order to identify the structure beneath the distribution of the “high molar mass oligomer”
 422 population, the same approach was applied by constructing 3D models and calculating their
 423 hydrodynamic radii. In fact, several NMR^{66,70} or cryo-EM⁸² studies have shown that the fibril
 424 core of A β (1-42) consisted of a dimer, each monomer containing four β -strands in an S-shaped

425 amyloid fold arrangement (Figure SI.16). On these grounds, protofibrillar and fibrillar structures
426 were constructed, using the PDB file code 5KK3⁶⁶, to get structures having a parallel
427 superposition of dimers and ranging from one dimer unit (disc shaped with a width of ~6.4 nm
428 and a length of ~0.9 nm) up to 720 dimer units (cylinder shaped with a width of ~6.4 nm and a
429 length of ~345 nm). The calculated size for the constructed oligomers is given in Table SI.1 and
430 in Figure 6. From the TDA analysis, the minimum size calculated for the high molar mass
431 oligomers distribution based on the results obtained on the simulated structures in Figure 6 was
432 5.1 nm, and corresponded to an oligomer having 33 dimer units (~300 kDa) and dimensions of
433 17 nm in length and 3.2 nm in radius. The maximum size was 36 nm corresponding to
434 approximately 700 dimer units (~6300 kDa) and dimensions of ~335 nm in length and 3.2 nm in
435 radius. Further, over the whole aggregation process (110 TDA runs), the average size was 10.7
436 nm corresponding to a cylinder-shaped oligomer having ~115 dimer units (~1035 kDa) with a 57
437 nm length and a 3.2 nm radius. The CRLI analysis on the TDA runs of A β (1-42) aggregation
438 (Figure 5) showed that the “high molar mass oligomer” population, centered around 10 nm, was
439 present at $t_{ag} = 0$ h at a very low concentration as compared to that of the “monomer and small
440 oligomers” population. These observations are in agreement with data obtained on A β (1-42) by
441 FCS where stable micelle-like oligomers with a size of $R_h \approx 7 - 11$ nm and having 28 - 88 mers
442 were observed⁸³. The abundance of this population then increased with incubation time to reach
443 a maximum at 2h, and finally became negligible after about 7 h.

444 Further, both the deconvolution using n Gaussian functions and the CRLI analysis showed that a
445 larger sized population, appeared after 1h and reached a maximum at 3h, and then became
446 negligible after 7h. We attributed this population to protofibrils since they are still soluble.
447 Indeed, TDA has shown that this population had a size (R_h) ranging between 50 and 240 nm with

448 an average value of 113 nm over the whole aggregation process ($n = 110$ TDA runs). If the same
 449 calculations were naively applied as done for the “high molar mass oligomers”, structures having
 450 a length between 500 nm and up to 8.5 μm would be obtained, with an average length around 2.2
 451 μm . The number of dimer units in these estimated elongated structures would range between
 452 1200 and 18000 (average of 4650) (with a molar mass per unit length of about 19 kDa/nm).
 453 Nevertheless, one should keep in mind that TDA cannot give reliable information about the
 454 shape of these assemblies, only the R_h distribution is obtained. Other techniques such as AFM
 455 would be more suited for looking at the molecular structure^{84,85}. Despite this limitation, which is
 456 common to all methods based on the determination of the diffusion coefficient (or R_h), the
 457 present work demonstrates that TDA in combination with molecular simulations can rapidly and
 458 advantageously propose a limited number of possible molecular conformations that are
 459 consistent with the experimental data.



460
 461 **Figure 6.** Schematic side view representation of possible conformations for the “high molar
 462 mass oligomeric A β (1-42)” population. The arrangement of the monomers in the oligomeric

463 form was based on the structures described by Colvin *et al.*⁶⁶ and in Tran *et al.*⁶⁵. The 3D
464 structures were realized using UCSF Chimera X software⁶⁷ and were adapted from the structure
465 published in⁶⁴ (PDB code 1Z0Q) for the hexameric structures and in⁶⁶ (PDB code 5KK3) for the
466 dimeric structures. The R_h were calculated by introducing the generated PDB files for each
467 structure into HYDROPRO software⁴⁸. The dimer, the hexamer, the dodecamer and the
468 octadecamer, which size is lower than 4 nm, are represented for the sake of comparison.

469
470 Finally, reports from the literature found that toxic A β oligomers had a molar mass higher than
471 50 kDa^{86,87} which corresponded to oligomers having more than ~11 monomer units. One of the
472 most toxic reported oligomers was identified to be A β *56 (56 kDa)⁸⁸ corresponding to a
473 dodecamer. Based on the calculations described in this work, a dodecamer would have R_h around
474 2.8 nm, if it is formed by the superposition of dimers or of monomers (as depicted in⁷³). The size
475 of the dodecamer would increase to 3.4 nm if constituted by the superposition of two hexamers.
476 According to another report¹⁰, the toxicity of A β (1-42) oligomers decreases with increasing size,
477 and toxic oligomers are likely in the range of 8-24-mers, having an R_h between 3 and 4.2 nm, as
478 calculated in this work. From the CRLI analysis in Figure 5, this fraction of potentially toxic
479 oligomers appears after 30 min, and then tends to decrease in proportion with the aggregation
480 time as the oligomer size is increasing.

481 **Conclusion**

482 This work demonstrates that TDA can be used for the straightforward monitoring of the
483 aggregation of A β amyloid peptides. Further, by using an appropriate data treatment of the
484 taylorgrams, one can assess the aggregation pathway by obtaining quantitative data on the
485 proportion and the size of the different aggregated forms. To our knowledge, there is no other
486 real-time aggregation monitoring method reported in the literature allowing to obtain such

487 information in one single analysis. It is worth noting that low volume was used for each
488 aggregation study (total volume of 1 μ L of a 100 μ M peptide solution) with an unprecedented
489 large number of data points during the aggregation process (about 10 points/h) leading to large
490 amount of valuable data.

491 The results obtained in this work tend to confirm the aggregation pathway of A β (1-40) which
492 goes from the monomeric state directly to a fibrillary structure, in contrast to A β (1-42) which
493 goes through different intermediate states (oligomers and protofibrils) before reaching the fibrils,
494 in agreement with previous work^{58,60}. In addition TDA data gave new insights for the
495 identification of the formed oligomers in the early stages of the aggregation process, including
496 the characterization of the size and abundance evolution of disease-relevant amyloids peptides in
497 solution. The strength of the data processing described in this work lies in its ability to
498 distinguish the small, potentially toxic, oligomers in a polydisperse mixture of larger oligomers,
499 protofibrils and fibrils. In the future, it will be interesting to investigate experimental conditions
500 mimicking the *in vivo* environment, such as lower concentrations of the peptides (in the 100 nM
501 range), mixtures of different amyloid peptides and the physico-chemical properties (ionic
502 strength, pH and composition) mimicking the cerebrospinal fluid.

503

504 **ACKNOWLEDGEMENTS**

505 LC gratefully acknowledges support from the Institut Universitaire de France (IUF)

506

507 **Supporting information**

508 Experimental procedure for the synthesis; Theoretical aspects and data processing of TDA; raw
509 TDA data; ThT assay results; DLS results; modelization data.

510 References

- 511 (1) Association, A. s., 2019 Alzheimer's disease facts and figures; Alzheimer's & Dementia
512 155252602019.
- 513 (2) Folch, J.; Ettcheto, M.; Petrov, D.; Abad, S.; Pedrós, I.; Marin, M.; Olloquequi, J.; Camins,
514 A. Review of the advances in treatment for Alzheimer disease: strategies for combating β -
515 amyloid protein. *Neurología (English Edition)* **2018**, *33*, 47-58.
- 516 (3) Hardy, J. A.; Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science*
517 **1992**, *256*, 184-185.
- 518 (4) Liu, P.-P.; Xie, Y.; Meng, X.-Y.; Kang, J.-S. History and progress of hypotheses and clinical
519 trials for Alzheimer's disease. *Signal Transduction and Targeted Therapy* **2019**, *4*, 29.
- 520 (5) Forloni, G.; Balducci, C. Alzheimer's Disease, Oligomers, and Inflammation. *J. Alzheimers*
521 *Dis.* **2018**, *62*, 1261-1276.
- 522 (6) Heppner, F. L.; Ransohoff, R. M.; Becher, B. Immune attack: the role of inflammation in
523 Alzheimer disease. *Nature Reviews Neuroscience* **2015**, *16*, 358-372.
- 524 (7) Terry, R. D. The Pathogenesis of Alzheimer Disease: An Alternative to the Amyloid
525 Hypothesis. *J. Neuropathol. Exp. Neurol.* **1996**, *55*, 1023-1025.
- 526 (8) Hayden, E. Y.; Teplow, D. B. Amyloid β -protein oligomers and Alzheimer's disease.
527 *Alzheimers Res. Ther.* **2013**, *5*, 60.
- 528 (9) Selkoe, D. J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO*
529 *Mol. Med.* **2016**, *8*, 595-608.
- 530 (10) Sengupta, U.; Nilson, A. N.; Kaye, R. The Role of Amyloid- β ; Oligomers in Toxicity,
531 Propagation, and Immunotherapy. *EBioMedicine* **2016**, *6*, 42-49.
- 532 (11) Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.;
533 Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.;
534 Krafft, G. A.; Klein, W. L. Diffusible, nonfibrillar ligands derived from $A\beta_{1-42}$ are potent central
535 nervous system neurotoxins. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 6448-6453.
- 536 (12) Mastroeni, D.; Nolz, J.; Khdour, O. M.; Sekar, S.; Delvaux, E.; Cuyugan, L.; Liang, W. S.;
537 Hecht, S. M.; Coleman, P. D. Oligomeric amyloid β preferentially targets neuronal and not glial
538 mitochondrial-encoded mRNAs. *Alzheimer's & Dementia* **2018**, *14*, 775-786.
- 539 (13) Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan,
540 M. J.; Selkoe, D. J. Naturally secreted oligomers of amyloid β protein potently inhibit
541 hippocampal long-term potentiation in vivo. *Nature* **2002**, *416*, 535-539.
- 542 (14) Salazar, S. V.; Strittmatter, S. M. Cellular prion protein as a receptor for amyloid- β
543 oligomers in Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **2017**, *483*, 1143-1147.
- 544 (15) König, A. S.; Rösener, N. S.; Gremer, L.; Tusche, M.; Flender, D.; Reinartz, E.; Hoyer, W.;
545 Neudecker, P.; Willbold, D.; Heise, H. Structural details of amyloid β oligomers in complex with
546 human prion protein as revealed by solid-state MAS NMR spectroscopy. *J. Biol. Chem.* **2021**,
547 100499.
- 548 (16) Guerrero-Muñoz, M. J.; Castillo-Carranza, D. L.; Sengupta, U.; White, M. A.; Kaye, R.
549 Design of Metastable β -Sheet Oligomers from Natively Unstructured Peptide. *ACS Chem.*
550 *Neurosci.* **2013**, *4*, 1520-1523.
- 551 (17) Bemporad, F.; Calloni, G.; Campioni, S.; Plakoutsi, G.; Taddei, N.; Chiti, F. Sequence and
552 structural determinants of amyloid fibril formation. *Acc. Chem. Res.* **2006**, *39*, 620-627.
- 553 (18) Ghosh, P.; Kumar, A.; Datta, B.; Rangachari, V. In *BMC Bioinformatics*, 2010, p S24.

554 (19) Jeong, J. S.; Ansaloni, A.; Mezzenga, R.; Lashuel, H. A.; Dietler, G. Novel mechanistic
555 insight into the molecular basis of amyloid polymorphism and secondary nucleation during
556 amyloid formation. *J. Mol. Biol.* **2013**, *425*, 1765-1781.

557 (20) Petkova, A. T.; Leapman, R. D.; Guo, Z.; Yau, W. M.; Mattson, M. P.; Tycko, R. Self-
558 propagating, molecular-level polymorphism in Alzheimer's β -amyloid fibrils. *Science* **2005**, *307*,
559 262-265.

560 (21) Watanabe-Nakayama, T.; Ono, K.; Itami, M.; Takahashi, R.; Teplow, D. B.; Yamada, M.
561 High-speed atomic force microscopy reveals structural dynamics of amyloid β 1-42 aggregates.
562 *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 5835-5840.

563 (22) Vassar, P. S.; Culling, C. F. Fluorescent stains, with special reference to amyloid and
564 connective tissues. *Arch. Pathol.* **1959**, *68*, 487-498.

565 (23) Bieschke, J.; Zhang, Q.; Powers, E. T.; Lerner, R. A.; Kelly, J. W. Oxidative Metabolites
566 Accelerate Alzheimer's Amyloidogenesis by a Two-Step Mechanism, Eliminating the
567 Requirement for Nucleation. *Biochemistry* **2005**, *44*, 4977-4983.

568 (24) Yang, T.; Li, S.; Xu, H.; Walsh, D. M.; Selkoe, D. J. Large Soluble Oligomers of Amyloid
569 β -Protein from Alzheimer Brain Are Far Less Neuroactive Than the Smaller Oligomers to Which
570 They Dissociate. *The Journal of Neuroscience* **2017**, *37*, 152-163.

571 (25) Michaels, T. C. T.; Šarić, A.; Curk, S.; Bernfur, K.; Arosio, P.; Meisl, G.; Dear, A. J.;
572 Cohen, S. I. A.; Dobson, C. M.; Vendruscolo, M.; Linse, S.; Knowles, T. P. J. Dynamics of
573 oligomer populations formed during the aggregation of Alzheimer's A β 42 peptide. *Nat. Chem.*
574 **2020**, *12*, 445-451.

575 (26) Ono, K.; Condrón, M. M.; Teplow, D. B. Structure–neurotoxicity relationships of amyloid
576 β -protein oligomers. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14745.

577 (27) Zhou, Y.; Liu, J.; Zheng, T.; Tian, Y. Label-Free SERS Strategy for In Situ Monitoring and
578 Real-Time Imaging of A β Aggregation Process in Live Neurons and Brain Tissues. *Anal. Chem.*
579 **2020**, *92*, 5910-5920.

580 (28) Brinet, D.; Kaffy, J.; Oukacine, F.; Glumm, S.; Ongeri, S.; Taverna, M. An improved
581 capillary electrophoresis method for in vitro monitoring of the challenging early steps of A β (1-
582 42) peptide oligomerization: Application to anti-Alzheimer's drug discovery. *Electrophoresis*
583 **2014**, *35*, 3302-3309.

584 (29) Brinet, D.; Gaie-Levrel, F.; Delatour, V.; Kaffy, J.; Ongeri, S.; Taverna, M. In vitro
585 monitoring of amyloid β -peptide oligomerization by Electrospray differential mobility analysis:
586 An alternative tool to evaluate Alzheimer's disease drug candidates. *Talanta* **2017**, *165*, 84-91.

587 (30) Iuraşcu, M. I.; Cozma, C.; Tomczyk, N.; Rontree, J.; Desor, M.; Drescher, M.; Przybylski,
588 M. Structural characterization of β -amyloid oligomer-aggregates by ion mobility mass
589 spectrometry and electron spin resonance spectroscopy. *Anal. Bioanal. Chem.* **2009**, *395*, 2509-
590 2519.

591 (31) Hu, J.; Zheng, Q. Applications of Mass Spectrometry in the Onset of Amyloid Fibril
592 Formation: Focus on the Analysis of Early-Stage Oligomers. *Frontiers in Chemistry* **2020**, *8*,
593 324.

594 (32) Bitan, G.; Kirkitadze, M. D.; Lomakin, A.; Vollers, S. S.; Benedek, G. B.; Teplow, D. B.
595 Amyloid β -protein (A β) assembly: A β 40 and A β 42 oligomerize through distinct pathways. *Proc.*
596 *Natl. Acad. Sci. U. S. A.* **2003**, *100*, 330-335.

597 (33) Chamieh, J.; Cottet, H. In *Colloid and Interface Science in Pharmaceutical Research and*
598 *Development*, Ohshima, H.; Makino, K., Eds.; Elsevier: Amsterdam, 2014, pp 173-192.

599 (34) Taylor, G. The Dispersion of Matter in Turbulent Flow through a Pipe. *Proc R Soc Lon Ser-*
600 *A* **1954**, 223, 446-468.

601 (35) Taylor, G. Dispersion of Soluble Matter in Solvent Flowing Slowly through a Tube. *Proc R*
602 *Soc Lon Ser-A* **1953**, 219, 186-203.

603 (36) Cottet, H.; Biron, J. P.; Martin, M. Taylor Dispersion Analysis of Mixtures. *Anal. Chem.*
604 **2007**, 79, 9066-9073.

605 (37) Bello, M. S.; Rezzonico, R.; Righetti, P. G. Use of Taylor-Aris Dispersion for Measurement
606 of a Solute Diffusion Coefficient in Thin Capillaries. *Science* **1994**, 266, 773-776.

607 (38) Sharma, U.; Gleason, N. J.; Carbeck, J. D. Diffusivity of Solutes Measured in Glass
608 Capillaries Using Taylor's Analysis of Dispersion and a Commercial CE Instrument. *Anal.*
609 *Chem.* **2005**, 77, 806-813.

610 (39) Cottet, H.; Martin, M.; Papillaud, A.; Souaïd, E.; Collet, H.; Commeyras, A. Determination
611 of Dendrigrift Poly-L-Lysine Diffusion Coefficients by Taylor Dispersion Analysis.
612 *Biomacromolecules* **2007**, 8, 3235-3243.

613 (40) d'Orlyé, F.; Varenne, A.; Gareil, P. Determination of nanoparticle diffusion coefficients by
614 Taylor dispersion analysis using a capillary electrophoresis instrument. *J. Chromatogr. A* **2008**,
615 *1204*, 226-232.

616 (41) Chamieh, J.; Merdassi, H.; Rossi, J.-C.; Jannin, V.; Demarne, F.; Cottet, H. Size
617 characterization of lipid-based self-emulsifying pharmaceutical excipients during lipolysis using
618 Taylor dispersion analysis with fluorescence detection. *Int. J. Pharm.* **2018**, 537, 94-101.

619 (42) Urban, D. A.; Milosevic, A. M.; Bossert, D.; Crippa, F.; Moore, T. L.; Geers, C.; Balog, S.;
620 Rothen-Rutishauser, B.; Petri-Fink, A. Taylor Dispersion of Inorganic Nanoparticles and
621 Comparison to Dynamic Light Scattering and Transmission Electron Microscopy. *Colloid and*
622 *Interface Science Communications* **2018**, 22, 29-33.

623 (43) Pedersen, M. E.; Østergaard, J.; Jensen, H. Flow-Induced Dispersion Analysis (FIDA) for
624 Protein Quantification and Characterization. *Methods Mol. Biol.* **2019**, 1972, 109-123.

625 (44) Verpillot, R.; Otto, M.; Klafki, H.; Taverna, M. Simultaneous analysis by capillary
626 electrophoresis of five amyloid peptides as potential biomarkers of Alzheimer's disease. *J.*
627 *Chromatogr. A* **2008**, 1214, 157-164.

628 (45) Botz, A.; Gasparik, V.; Devillers, E.; Hoffmann, A. R. F.; Caillon, L.; Chelain, E.; Lequin,
629 O.; Brigaud, T.; Khemtémourian, L. (R)- α -trifluoromethylalanine containing short peptide in the
630 inhibition of amyloid peptide fibrillation. *Peptide Science* **2015**, 104, 601-610.

631 (46) Provencher, S. W. CONTIN: A general purpose constrained regularization program for
632 inverting noisy linear algebraic and integral equations. *Comput. Phys. Commun.* **1982**, 27, 229-
633 242.

634 (47) Provencher, S. W. A constrained regularization method for inverting data represented by
635 linear algebraic or integral equations. *Comput. Phys. Commun.* **1982**, 27, 213-227.

636 (48) Ortega, A.; Amorós, D.; García de la Torre, J. Prediction of Hydrodynamic and Other
637 Solution Properties of Rigid Proteins from Atomic- and Residue-Level Models. *Biophys. J.* **2011**,
638 *101*, 892-898.

639 (49) Naiki, H.; Higuchi, K.; Hosokawa, M.; Takeda, T. Fluorometric determination of amyloid
640 fibrils in vitro using the fluorescent dye, thioflavine T. *Anal. Biochem.* **1989**, 177, 244-249.

641 (50) Chamieh, J.; Leclercq, L.; Martin, M.; Slaoui, S.; Jensen, H.; Østergaard, J.; Cottet, H.
642 Limits in Size of Taylor Dispersion Analysis: Representation of the Different Hydrodynamic
643 Regimes and Application to the Size-Characterization of Cubosomes. *Anal. Chem.* **2017**, 89,
644 13487-13493.

645 (51) Cottet, H.; Biron, J. P.; Martin, M. On the optimization of operating conditions for Taylor
646 dispersion analysis of mixtures. *Analyst* **2014**, *139*, 3552-3562.

647 (52) Zheng, J.; Yeung, E. S. Mechanism of Microbial Aggregation during Capillary
648 Electrophoresis. *Anal. Chem.* **2003**, *75*, 818-824.

649 (53) Schmidt, M.; Sachse, C.; Richter, W.; Xu, C.; Fändrich, M.; Grigorieff, N. Comparison of
650 Alzheimer A β (1–40) and A β (1–42) amyloid fibrils reveals similar protofilament structures.
651 *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 19813-19818.

652 (54) Sachse, C.; Fändrich, M.; Grigorieff, N. Paired β -sheet structure of an A β (1-40) amyloid
653 fibril revealed by electron microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7462-7466.

654 (55) Yusko, E. C.; Prangkio, P.; Sept, D.; Rollings, R. C.; Li, J.; Mayer, M. Single-Particle
655 Characterization of A β Oligomers in Solution. *ACS Nano* **2012**, *6*, 5909-5919.

656 (56) Cipelletti, L.; Biron, J.-P.; Martin, M.; Cottet, H. Measuring Arbitrary Diffusion Coefficient
657 Distributions of Nano-Objects by Taylor Dispersion Analysis. *Anal. Chem.* **2015**, *87*, 8489-8496.

658 (57) Arosio, P.; Knowles, T. P. J.; Linse, S. On the lag phase in amyloid fibril formation. *PCCP*
659 **2015**, *17*, 7606-7618.

660 (58) Chen, Y.-R.; Glabe, C. G. Distinct Early Folding and Aggregation Properties of Alzheimer
661 Amyloid- β Peptides A β 40 and A β 42. *J. Biol. Chem.* **2006**, *281*, 24414-24422.

662 (59) Economou, N. J.; Giammona, M. J.; Do, T. D.; Zheng, X.; Teplow, D. B.; Buratto, S. K.;
663 Bowers, M. T. Amyloid β -Protein Assembly and Alzheimer's Disease: Dodecamers of A β 42, but
664 Not of A β 40, Seed Fibril Formation. *J. Am. Chem. Soc.* **2016**, *138*, 1772-1775.

665 (60) Fu, Z.; Aucoin, D.; Davis, J.; Van Nostrand, W. E.; Smith, S. O. Mechanism of Nucleated
666 Conformational Conversion of A β 42. *Biochemistry* **2015**, *54*, 4197-4207.

667 (61) Bitan, G.; Lomakin, A.; Teplow, D. B. Amyloid β -Protein Oligomerization. *J. Biol. Chem.*
668 **2001**, *276*, 35176-35184.

669 (62) Heyman, H. M.; Zhang, X.; Tang, K.; Baker, E. S.; Metz, T. O. In *Encyclopedia of*
670 *Spectroscopy and Spectrometry (Third Edition)*, Lindon, J. C.; Tranter, G. E.; Koppenaal, D. W.,
671 Eds.; Academic Press: Oxford, 2017, pp 376-384.

672 (63) Hawe, A.; Hulse, W.; Jiskoot, W.; Forbes, R. Taylor Dispersion Analysis Compared to
673 Dynamic Light Scattering for the Size Analysis of Therapeutic Peptides and Proteins and Their
674 Aggregates. *Pharm. Res.* **2011**, *28*, 2302-2310.

675 (64) Tomaselli, S.; Esposito, V.; Vangone, P.; van Nuland, N. A. J.; Bonvin, A. M. J. J.;
676 Guerrini, R.; Tancredi, T.; Temussi, P. A.; Picone, D. The α -to- β Conformational Transition of
677 Alzheimer's A β -(1–42) Peptide in Aqueous Media is Reversible: A Step by Step Conformational
678 Analysis Suggests the Location of β Conformation Seeding. *Chembiochem* **2006**, *7*, 257-267.

679 (65) Tran, L.; Basdevant, N.; Prévost, C.; Ha-Duong, T. Structure of ring-shaped A β 42
680 oligomers determined by conformational selection. *Sci. Rep.* **2016**, *6*, 21429.

681 (66) Colvin, M. T.; Silvers, R.; Ni, Q. Z.; Can, T. V.; Sergeev, I.; Rosay, M.; Donovan, K. J.;
682 Michael, B.; Wall, J.; Linse, S.; Griffin, R. G. Atomic Resolution Structure of Monomorphic
683 A β 42 Amyloid Fibrils. *J. Am. Chem. Soc.* **2016**, *138*, 9663-9674.

684 (67) Goddard, T. D.; Huang, C. C.; Meng, E. C.; Pettersen, E. F.; Couch, G. S.; Morris, J. H.;
685 Ferrin, T. E. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein*
686 *Sci.* **2018**, *27*, 14-25.

687 (68) Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.;
688 Riek, R. 3D structure of Alzheimer's amyloid- β (1–42) fibrils. *Proc. Natl. Acad. Sci. U. S. A.*
689 **2005**, *102*, 17342-17347.

690 (69) Schmidt, M.; Rohou, A.; Lasker, K.; Yadav, J. K.; Schiene-Fischer, C.; Fändrich, M.;
691 Grigorieff, N. Peptide dimer structure in an A β (1–42) fibril visualized with cryo-EM. *Proc. Natl.*
692 *Acad. Sci. U. S. A.* **2015**, *112*, 11858-11863.

693 (70) Wälti, M. A.; Ravotti, F.; Arai, H.; Glabe, C. G.; Wall, J. S.; Böckmann, A.; Güntert, P.;
694 Meier, B. H.; Riek, R. Atomic-resolution structure of a disease-relevant A β (1–42) amyloid fibril.
695 *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E4976.

696 (71) Kreutzer, A. G.; Hamza, I. L.; Spencer, R. K.; Nowick, J. S. X-ray Crystallographic
697 Structures of a Trimer, Dodecamer, and Annular Pore Formed by an A β 17-36 β -Hairpin. *J. Am.*
698 *Chem. Soc.* **2016**, *138*, 4634-4642.

699 (72) Ciudad, S.; Puig, E.; Botzanowski, T.; Meigooni, M.; Arango, A. S.; Do, J.; Mayzel, M.;
700 Bayoumi, M.; Chaignepain, S.; Maglia, G.; Cianferani, S.; Orekhov, V.; Tajkhorshid, E.;
701 Bardiaux, B.; Carulla, N. A β (1-42) tetramer and octamer structures reveal edge conductivity
702 pores as a mechanism for membrane damage. *Nature Communications* **2020**, *11*, 3014.

703 (73) Xiao, Y.; Ma, B.; McElheny, D.; Parthasarathy, S.; Long, F.; Hoshi, M.; Nussinov, R.; Ishii,
704 Y. A β (1–42) fibril structure illuminates self-recognition and replication of amyloid in
705 Alzheimer's disease. *Nat. Struct. Mol. Biol.* **2015**, *22*, 499-505.

706 (74) Cohen, S. I. A.; Linse, S.; Luheshi, L. M.; Hellstrand, E.; White, D. A.; Rajah, L.; Otzen, D.
707 E.; Vendruscolo, M.; Dobson, C. M.; Knowles, T. P. J. Proliferation of amyloid- β 42 aggregates
708 occurs through a secondary nucleation mechanism. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*,
709 9758-9763.

710 (75) Cohen, S. I. A.; Vendruscolo, M.; Welland, M. E.; Dobson, C. M.; Terentjev, E. M.;
711 Knowles, T. P. J. Nucleated polymerization with secondary pathways. I. Time evolution of the
712 principal moments. *J. Chem. Phys.* **2011**, *135*, 065105.

713 (76) Knowles, T. P. J.; Waudby, C. A.; Devlin, G. L.; Cohen, S. I. A.; Aguzzi, A.; Vendruscolo,
714 M.; Terentjev, E. M.; Welland, M. E.; Dobson, C. M. An Analytical Solution to the Kinetics of
715 Breakable Filament Assembly. *Science* **2009**, *326*, 1533-1537.

716 (77) Lee, C.-T.; Terentjev, E. M. Mechanisms and rates of nucleation of amyloid fibrils. *J.*
717 *Chem. Phys.* **2017**, *147*, 105103.

718 (78) Srivastava, A. K.; Pittman, J. M.; Zerweck, J.; Venkata, B. S.; Moore, P. C.; Sachleben, J.
719 R.; Meredith, S. C. β -Amyloid aggregation and heterogeneous nucleation. *Protein Sci.* **2019**, *28*,
720 1567-1581.

721 (79) Shea, D.; Hsu, C.-C.; Bi, T. M.; Paranjpye, N.; Childers, M. C.; Cochran, J.; Tomberlin, C.
722 P.; Wang, L.; Paris, D.; Zonderman, J.; Varani, G.; Link, C. D.; Mullan, M.; Daggett, V. α -Sheet
723 secondary structure in amyloid β -peptide drives aggregation and toxicity in Alzheimer's disease.
724 *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 8895-8900.

725 (80) Ahmed, M.; Davis, J.; Aucoin, D.; Sato, T.; Ahuja, S.; Aimoto, S.; Elliott, J. I.; Van
726 Nostrand, W. E.; Smith, S. O. Structural conversion of neurotoxic amyloid- β 1–42 oligomers to
727 fibrils. *Nat. Struct. Mol. Biol.* **2010**, *17*, 561-567.

728 (81) Ghosh, P.; Vaidya, A.; Kumar, A.; Rangachari, V. Determination of critical nucleation
729 number for a single nucleation amyloid- β aggregation model. *Math. Biosci.* **2016**, *273*, 70-79.

730 (82) Gremer, L.; Schölzel, D.; Schenk, C.; Reinartz, E.; Labahn, J.; Ravelli, R. B. G.; Tusche,
731 M.; Lopez-Iglesias, C.; Hoyer, W.; Heise, H.; Willbold, D.; Schröder, G. F. Fibril structure of
732 amyloid- β (1-42) by cryo-electron microscopy. *Science* **2017**, *358*, 116-119.

733 (83) Novo, M.; Freire, S.; Al-Soufi, W. Critical aggregation concentration for the formation of
734 early Amyloid- β (1–42) oligomers. *Sci. Rep.* **2018**, *8*, 1783.

735 (84) Mastrangelo, I. A.; Ahmed, M.; Sato, T.; Liu, W.; Wang, C.; Hough, P.; Smith, S. O. High-
736 resolution Atomic Force Microscopy of Soluble A β 42 Oligomers. *J. Mol. Biol.* **2006**, *358*, 106-
737 119.

738 (85) Nirmalraj, P. N.; List, J.; Battacharya, S.; Howe, G.; Xu, L.; Thompson, D.; Mayer, M.
739 Complete aggregation pathway of amyloid beta (1-40) and (1-42) resolved on an atomically
740 clean interface. *Sci Adv* **2020**, *6*, eaaz6014.

741 (86) Lacor, P. N.; Buniel, M. C.; Furlow, P. W.; Sanz Clemente, A.; Velasco, P. T.; Wood, M.;
742 Viola, K. L.; Klein, W. L. A β Oligomer-Induced Aberrations in Synapse Composition, Shape,
743 and Density Provide a Molecular Basis for Loss of Connectivity in Alzheimer's Disease.
744 *The Journal of Neuroscience* **2007**, *27*, 796.

745 (87) Velasco, P. T.; Heffern, M. C.; Sebollela, A.; Popova, I. A.; Lacor, P. N.; Lee, K. B.; Sun,
746 X.; Tiano, B. N.; Viola, K. L.; Eckermann, A. L.; Meade, T. J.; Klein, W. L. Synapse-Binding
747 Subpopulations of A β Oligomers Sensitive to Peptide Assembly Blockers and scFv Antibodies.
748 *ACS Chem. Neurosci.* **2012**, *3*, 972-981.

749 (88) Lesné, S.; Koh, M. T.; Kotilinek, L.; Kaye, R.; Glabe, C. G.; Yang, A.; Gallagher, M.;
750 Ashe, K. H. A specific amyloid- β protein assembly in the brain impairs memory. *Nature* **2006**,
751 *440*, 352-357.

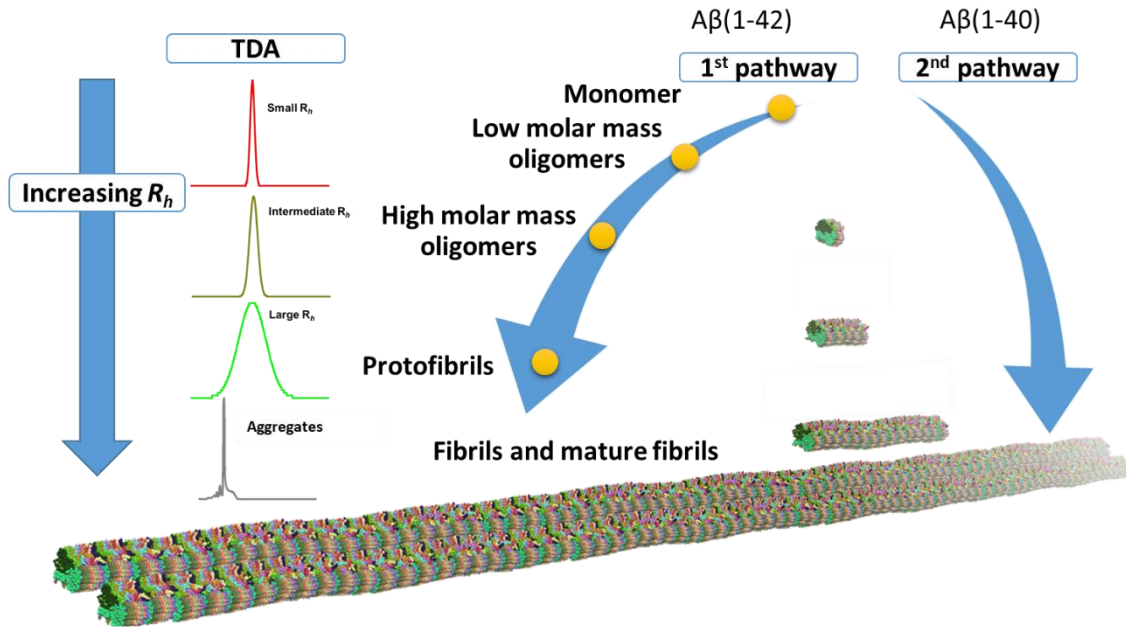
752

753

754

755 for Table of Contents only

756



757

