

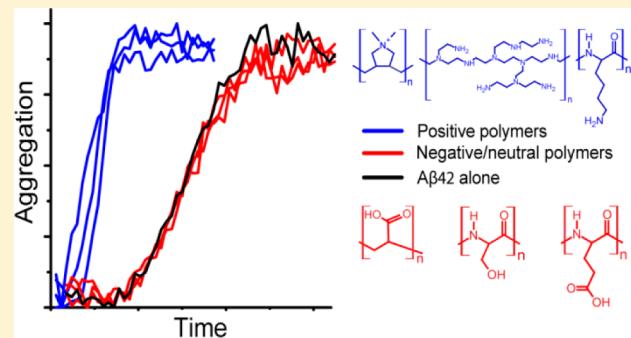
## Effects of Polyamino Acids and Polyelectrolytes on Amyloid $\beta$ Fibril Formation

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 Supporting Information

**ABSTRACT:** The fibril formation of the neurodegenerative peptide amyloid  $\beta$  ( $A\beta$ 42) is sensitive to solution conditions, and several proteins and peptides have been found to retard the process.  $A\beta$ 42 fibril formation was followed with ThT fluorescence in the presence of polyamino acids (poly-glutamic acid, poly-lysine, and poly-threonine) and other polymers (poly(acrylic acid), poly(ethylenimine), and poly(diallyldimethylammonium chloride). An accelerating effect on the  $A\beta$ 42 aggregation process is observed from all positively charged polymers, while no effect is seen from the negative or neutral polymers. The accelerating effect is dependent on the concentration of positive polymer in a highly reproducible manner. Acceleration is observed from a 1:500 polymer to  $A\beta$ 42 weight ratio and up. Polyamino acids and the other polymers exert quantitatively the same effect at the same concentrations based on weight. Fibrils are formed in all cases as verified by transmission electron microscopy. The concentrations of polymers required for acceleration are too low to affect the  $A\beta$ 42 aggregation process through increased ionic strength or molecular crowding effects. Instead, the acceleration seems to arise from the locally increased  $A\beta$ 42 concentration near the polymers, which favors association and affects the electrostatic environment of the peptide.



### INTRODUCTION

According to the amyloid cascade hypothesis, the formation of fibrils from  $A\beta$  monomers plays a causative role in Alzheimer's disease.<sup>1,2</sup>  $A\beta$  is a naturally occurring peptide of variable length due to a variation in enzymatic cleavage sites.<sup>3</sup> Forty amino acids is the most common length, but a variant with a C-terminal extension of two amino acids is more amyloidogenic and disease-relevant. The fibrils formed by  $A\beta$  have a cross- $\beta$  structure common to amyloids, where  $\beta$ -strands are stacked perpendicular to the fibril axis.<sup>4</sup> Each  $A\beta$  peptide contributes two  $\beta$  strands to the fibril core.<sup>5</sup>

The aggregation of  $A\beta$ , here defined as the process whereby fibrils are formed from monomers via various oligomeric states, has been extensively studied, and the rates of the underlying microscopic events such as nucleation and elongation have been determined.<sup>6,7</sup> The process is dominated by secondary nucleation, and the concentration of oligomers is low throughout the process.<sup>7,8</sup> In the final state, the fibrils are in equilibrium with a very low concentration of soluble  $A\beta$ .<sup>9</sup>

The aggregation process is sensitive to pH, temperature, and ionic strength as well as additives such as proteins,<sup>10–13</sup> nanoparticles,<sup>14–16</sup> and surfactants.<sup>17</sup> Proteins generally retard aggregation whereas diverse effects have been seen for nanoparticles. Retarding effects of proteins have been seen for biologically relevant proteins that interact with  $A\beta$  in vivo but also for completely unrelated proteins.<sup>10,11</sup>

A number of short peptides with various sequences have been reported to inhibit  $A\beta$  aggregation, for example,  $A\beta$ -derived KLVFF,<sup>18</sup> RGKLVFFGR,<sup>19</sup> transthyretin-derived DTK-SYWKALG, and PRRYTIAALLSPYWS peptides.<sup>20</sup> A particular class of peptides is polyamino acids, in which a single kind of amino acid residue is repeated. Polyamino acids have similar compositions to peptides or proteins but not a globular structure. Luo et al.<sup>21</sup> found that small cellular polyamines (spermine and spermidine) accelerate aggregation and change the aggregation pathway of  $A\beta$ 40.

Several other polymers have been investigated for their effect on  $A\beta$  aggregation. Lysine dendrimers have been found to retard  $A\beta$  aggregation and reduce its cell toxicity.<sup>22</sup> PAMAM dendrimers have also been reported to retard the aggregation of  $A\beta$  fragments and to prompt the dissolution of  $A\beta$  aggregates.<sup>23</sup> Different forms of glycosaminoglycans have been found to promote fibril formation in vitro in addition to associating with  $A\beta$  plaques and neurofibrillary tangles in vivo.<sup>24</sup> Polymer effects on amyloid formation are not unique to  $A\beta$ . Biological polyanions such as heparin and DNA have been found to accelerate the aggregation of acylphosphatase,<sup>25</sup> and positively charged polymers (poly-Lys, PEI, and poly-arginine) have been found to accelerate  $\alpha$ -synuclein aggregation.<sup>26</sup>

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Table 1. Properties of the Polymers Used

Polymer	PDPA	PEI <sup>a</sup>	poly-Lys	PAA	poly-Glu	poly-Thr
Structure						
pK <sub>a</sub>	+	8.4 <sup>27</sup>	9.8±0.2 <sup>28</sup>	5-6.5 <sup>29</sup>	5.4 <sup>30</sup>	
Molecular weight (g/mol)	200-350000	25000	>15000	14000	50-15000	7600
Weight of repeating unit (g/mol)	162	42 <sup>b</sup>	146.2	95	147	119

<sup>a</sup>Schematic representation of the structure. <sup>b</sup>Based on the charge-bearing subunit ( $\text{NCH}_2\text{CH}_2$ ).

In the current work, we have investigated the effect of polyamino acids and other polymers on the aggregation kinetics of amyloid  $\beta$  peptide 1-42 with an extra methionine at the N-terminus, hereafter referred to as  $\text{A}\beta42$ . To distinguish between the effects of side chains versus the peptide backbone, other polymers with similar chemical groups were included in the study. We observe significant catalytic effects on  $\text{A}\beta42$  aggregation from several of the investigated polymers. The main determining factor in this study was found to be the charge of the repeating units of the polymer. To investigate the role of electrostatic interactions further, the experiments were repeated in a series of salt concentrations.

## EXPERIMENTAL SECTION

**Materials.** Poly(acrylic acid sodium salt) (PAA), poly(diallyldimethylammonium chloride) (PDPA), poly-lysine hydrobromide (poly-Lys), poly(ethylenimine) (PEI), poly-threonine (poly-Thr), and poly-glutamic acid sodium salt (poly-Glu) were purchased from Sigma. The polymers were dissolved at 2 mg/mL in 20 mM phosphate buffer with 200  $\mu\text{M}$  EDTA, and the pH was set to 8.

**Aggregation Kinetics.**  $\text{A}\beta42$  was recombinantly produced in *E. coli* as previously described.<sup>31</sup> The initial methionine has no significant effect on the aggregation rate.<sup>31</sup> Aliquots of the purified peptide were freeze-dried and stored at  $-20^\circ\text{C}$ . On the day of the kinetic experiment the peptide was subjected to gel filtration as previously described.<sup>10</sup> The concentration of  $\text{A}\beta42$  in the monomer fraction from the column was determined through peak integration at 280 nm. The sample was normally diluted to 2.5  $\mu\text{M}$   $\text{A}\beta42$  in 20 mM degassed and filtered sodium phosphate buffer, pH 8, supplemented with 200  $\mu\text{M}$  EDTA. Thioflavin T (ThT) was added to the  $\text{A}\beta42$  solution to a final concentration of 14  $\mu\text{M}$  from a 1.4 mM stock solution.

The  $\text{A}\beta42$  solution was then added, 50  $\mu\text{L}$  per well, to a 96-well half area plate of black polystyrene with a clear bottom and a nonbinding surface (Corning 3881) on ice. Before  $\text{A}\beta42$  was added, each well had been provided with a solution of polymer or buffer to a total volume of 50  $\mu\text{L}$ . All concentrations of  $\text{A}\beta42$ , NaCl, and polymers given in the Results section and figure legends are the final values. Before incubation in the plate reader, the plate was sealed with a plastic film (Sigma-Aldrich).

For experiments with different salt concentrations, the  $\text{A}\beta42$  peptide was diluted in Milli-Q water after gel filtration. The wells were filled with 25  $\mu\text{L}$  of NaCl solution or water and 25  $\mu\text{L}$  of polymer solution in 3 mM sodium phosphate buffer, pH 8, with 30  $\mu\text{M}$  EDTA before the addition of 50  $\mu\text{L}$  of the  $\text{A}\beta42$  solution. The final salt

concentration in the reaction wells was 0–300 mM, and the buffer concentration was approximately 2 mM phosphate and 20  $\mu\text{M}$  EDTA.

The aggregation of  $\text{A}\beta42$  was followed by fluorescence spectroscopy with a 440 nm excitation filter and a 480 nm emission filter in a plate reader (Fluostar Omega or Fluostar Optima, BMG Labtech, Offenburg, Germany). The fluorescence intensity of ThT was read every 180 s, and the temperature was  $37^\circ\text{C}$ .

The half time of the aggregation ( $t_{1/2}$ ) was calculated by fitting the following equation to the kinetic data in OriginPro (OriginLab corporation, Northampton, MA):

$$y = A_2 \left( 1 - \frac{1}{1 + e^{(t-t_{1/2})/dx}} \right) \quad (1)$$

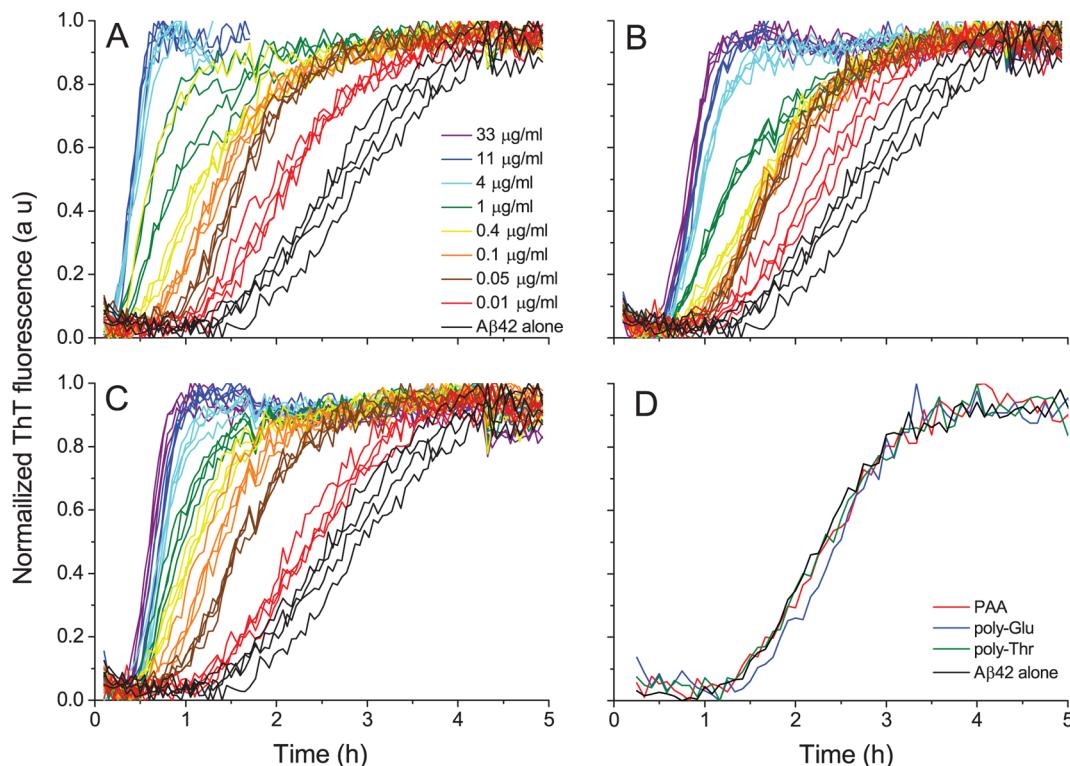
$A_2$  is the maximum fluorescence,  $t_{1/2}$  is the half time of the reaction, and  $dx$  is the inverse apparent elongation rate.

**Transmission Electron Microscopy.** Freshly fibrillated samples (according to the ThT fluorescence) of  $\text{A}\beta42$  with PDPA, PEI, poly-Lys, PAA, poly-Glu, or poly-Thr were spotted on 300 mesh formvar carbon film grids (Electron Microscopy Sciences, Hatfield, PA). Five microliters of the sample was placed on the grid for 3–6 min, blotted, stained on a drop of 1.5% uranyl acetate (Merck) for another 1 min, and rinsed on 2 drops of water (Milli-Q). The samples were analyzed in a Philips CM120 BioTWIN cryoTEM at 6200 $\times$  and 31 000 $\times$  magnifications.

**Circular Dichroism Spectroscopy.** Samples of 0.25 mg/mL of the polyamino acids in 2.5 mM sodium phosphate buffer, pH 8, with 25  $\mu\text{M}$  EDTA were analyzed in a Jasco J-815 spectrometer. CD spectra were recorded at  $37^\circ\text{C}$  in a quartz cuvette with a 1 mm path length. Far-UV spectra were recorded at 1 nm intervals between 190 and 250 nm using a scan rate of 20 nm/min with a response time of 4 s and a band pass of 1 nm.

## RESULTS

We have studied the aggregation of  $\text{A}\beta42$  in the presence of polymers with different charges, lengths, and backbone structures. The effect from the polymers has been further analyzed through morphological studies of the fibrils and the salt dependence on the aggregation reaction. The formation of fibrils has been followed by fluorescence spectroscopy by monitoring the intensity from ThT, the quantum yield of which increases upon binding to the stacked  $\beta$ -sheets that form the core of the amyloid fibril.<sup>32</sup> The aggregation process normally follows a sigmoidal curve with a lag phase, a steep growth phase, and a plateau phase. The steep phase is often called the elongation phase, although all underlying microscopic processes



**Figure 1.** Aggregation of 1.25  $\mu\text{M}$  A $\beta$ 42 in the absence and presence of PDDA (A), PEI (B), and poly-Lys (C) followed by ThT fluorescence intensity with four replicates of each polymer concentration. In panel D, 37  $\mu\text{g}/\text{mL}$  of PAA (red), poly-Glu (blue), and poly-Thr (green) is added to the aggregation reaction.

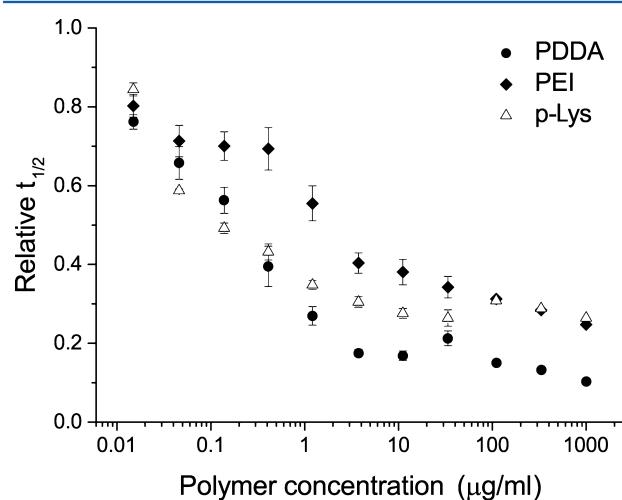
(primary and secondary nucleation and elongation) are in operation in all three phases, albeit at different rates.<sup>33</sup> To compare the effect from the different polymers, we extracted  $t_{1/2}$ , which is the time when the fluorescence intensity has reached half of its maximal value and approximately half of the monomers have formed fibrils.<sup>7</sup>

**Polymer Characterization.** Our set of polymers consists of polyamino acids (poly-Lys, poly-Glu, and poly-Thr), linear polymers (PDDA and PAA), and a branched polymer (PEI; polymer properties in Table 1). The polyamino acids have the ability to form both  $\beta$ -sheets and  $\alpha$ -helices based on the solution conditions.<sup>30,34,35</sup> The polyamino acids were mostly unstructured in pH 8 phosphate buffer as seen by CD spectroscopy (Figure S1). No increased ThT fluorescence intensity was observed for samples with 1 mg/mL of the polymers after 5 h at 37 °C (data not shown), indicating no formation of amyloid-like structures from the polymers without A $\beta$ 42 under the experimental conditions.

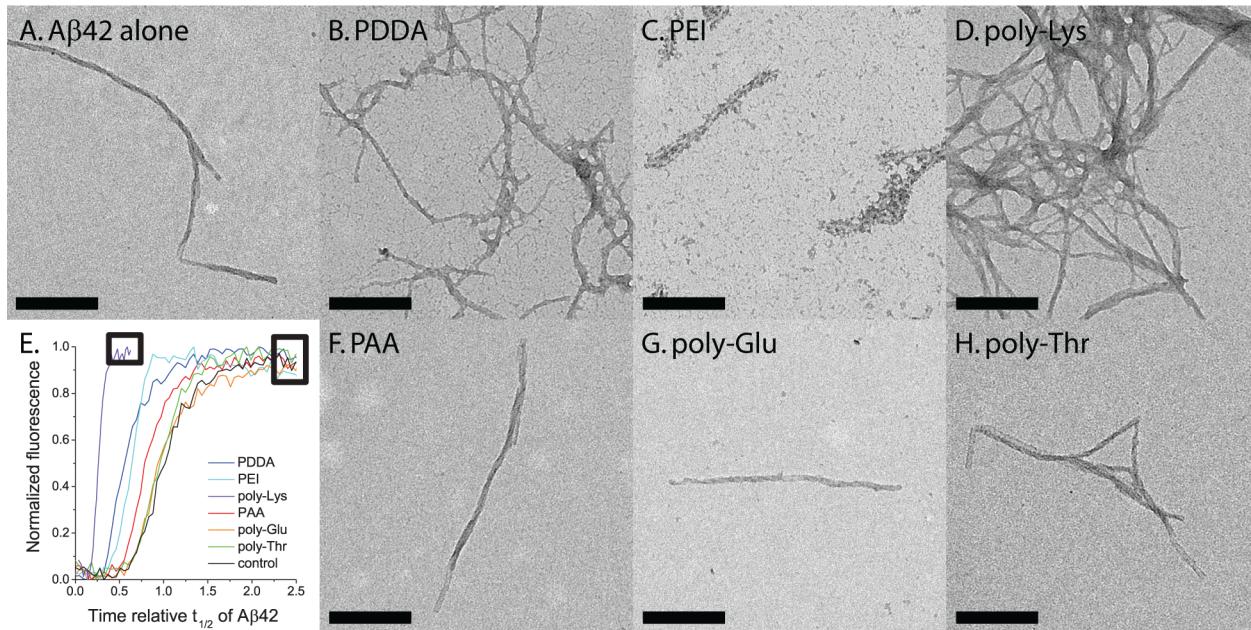
**Effects of Polymers on the Aggregation Kinetics.** We have followed the A $\beta$ 42 aggregation process with ThT fluorescence in the presence and absence of a total of six different polymers. PDDA, PEI, and poly-Lys have a positive net charge from amine groups at the pH of the aggregation reaction (pH 8). These polymers all accelerate A $\beta$ 42 aggregation in a concentration-dependent manner (Figure 1A–C). The lag times are shorter and the slopes steeper compared to those of A $\beta$ 42 aggregated in the absence of polymers, whereas the final plateau values display no systematic variation (Figure S2). On the other hand, PAA, poly-Glu, and poly-Thr do not affect the aggregation process for polymer concentrations up to 1 mg/mL (Figures 1D and S3). The concentration of A $\beta$ 42, 1.25  $\mu\text{M}$ , corresponds to 5.4  $\mu\text{g}/\text{mL}$ , so

even at a 1:200 weight ratio the negative and neutral polymers have no effect on the length of the lag phase, slope, or final plateau value of the fluorescence. Thus, polymers with an opposite net charge compared to that of A $\beta$ 42 (roughly  $-3$  at pH 8) accelerate the aggregation, whereas there is no effect from negative and neutral polymers.

Figure 2 shows the relative  $t_{1/2}$  extracted from the progression curves (Figure 1) of the aggregation for the



**Figure 2.** Half time,  $t_{1/2}$ , of A $\beta$ 42 aggregation in the presence of PDDA, PEI, or poly-Lys compared to the  $t_{1/2}$  of A $\beta$ 42 aggregation without additives. The error bars indicate the standard error of the mean from two to seven replicates from several independent experiments. Data for a concentration of 110  $\mu\text{g}/\text{mL}$  and higher are singles. The concentration of A $\beta$ 42 was 1.25  $\mu\text{M}$ .

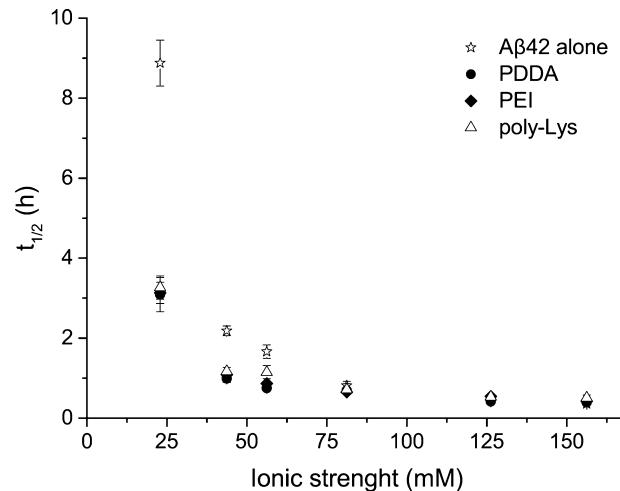


**Figure 3.** TEM images of A $\beta$ 42 fibrils formed in the absence (A) and presence (B–D, F–H) of the polymers indicated. In panel E, the corresponding ThT fluorescence intensity curves are shown with the boxes indicating the time points of the sampling at the fluorescence plateau. The polymer concentration is 1 mg/mL for the negative and neutral polymers (F–H), 0.066  $\mu$ g/mL for PDDA (A) and PEI (B), and 0.011  $\mu$ g/mL for poly-Lys (C). In panels A–C and F–H, the concentration of A $\beta$ 42 is 2.5  $\mu$ M, and in panel D, the concentration is 1.25  $\mu$ M. The scale bar represents 200 nm.

different polymer concentrations. All three polymers display a decrease in  $t_{1/2}$  with increasing polymer concentration. PDDA displays the largest accelerating effect, where at high concentrations  $t_{1/2}$  is reduced to only about 10% of  $t_{1/2}$  of A $\beta$ 42 alone.

**Fibril Morphology.** The ThT fluorescence intensity at the plateau of the A $\beta$ 42 aggregation kinetic curves is not systematically affected by the presence of polymers (Figure S2). To verify the presence of fibrils and to study the morphology of the fibrils, grids were prepared for transmission electron microscopy (TEM) with freshly formed fibrils based on the ThT fluorescence intensity level. Fibrils with a diameter of roughly 15 nm were detected in all samples (Figure 3). This is in agreement with previous studies on A $\beta$ 42 morphology.<sup>5</sup> The A $\beta$ 42 fibrils formed in the presence of the negative and neutral polymers are short and quite dispersed just as for the A $\beta$ 42 fibrils formed without additives. In the case of PDDA and poly-Lys, the fibrils are longer and a more extensive fibril network is observed. The morphology of the individual A $\beta$  fibrils formed with PEI are more diffuse and decorated compared to that of the other fibrils. The difference can originate from the polymer altering the fibril structure or interfering with the staining procedure.

**Salt Effects.** The effect of polymers on A $\beta$ 42 aggregation is clearly dependent on the charge of the polymer, whereas the size, structure of the charged groups, or the backbone seems to have less impact. Therefore, we wanted to investigate the effect of the electrostatic environment on the interaction. The A $\beta$ 42 aggregation is known to be heavily dependent on the ionic strength of the solution and is accelerated at higher salt concentrations.<sup>36,37</sup> We followed the aggregation of A $\beta$ 42 in the presence of the positive polymers at different ionic strengths (Figure 4 and S3) by additions of NaCl. The selected polymer concentration (0.05  $\mu$ g/mL) was expected to lead to a slight acceleration of A $\beta$ 42 aggregation. The main contributors to the



**Figure 4.** Salt dependence of A $\beta$ 42 aggregation with and without 0.05  $\mu$ g/mL PDDA, PEI, or poly-Lys followed by fluorescence spectroscopy of the ThT fluorescence intensity.  $t_{1/2}$  of the aggregation reaction is plotted against the ionic strength. The concentration of A $\beta$ 42 was 1.6  $\mu$ M, and the concentration of polymer was 0.05  $\mu$ g/mL. The error bars show the standard error of the mean of three replicates.

ionic strength are the salt and the 2 mM phosphate buffer. The polymers make negligible contributions if the charged groups act as individual units and not as macroions. At low ionic strength there is acceleration from A $\beta$ 42 with polymers compared to A $\beta$ 42 alone, and the difference decreases as the ionic strength increases. At high polymer concentration (11  $\mu$ g/mL), the variation in ionic strength has no effect on the aggregation process (Figure S4).

## ■ DISCUSSION

In this study, we find distinct effects of polymers on  $\text{A}\beta_{42}$  aggregation dependent on the polymer charge. The negative or neutral polymers have no effect on the aggregation of  $\text{A}\beta_{42}$  based on the measurements of ThT fluorescence intensity as a function of time. However, the three polymers with positive charge, PDDA, PEI, and poly-Lys, all accelerate the  $\text{A}\beta_{42}$  aggregation process in a concentration-dependent manner. These polymers affect the overall aggregation process in terms of decreased lag time and increased slope of the growth phase, whereas the final fluorescence plateau value is approximately the same at all polymer concentrations. Since the ThT concentration was chosen in a range where the fluorescence intensity varies in a linear manner with the fibril concentration,<sup>7</sup> this indicates that fibrils are formed to a similar extent in all cases.

In agreement with the enhanced ThT fluorescence, fibrils were observed by TEM for  $\text{A}\beta_{42}$  in the presence of all polymers. No alteration in the morphology was observed in cases where no effect on the aggregation kinetics is seen ( $\text{A}\beta$  with PAA, poly-Glu, or poly-Thr). The TEM images of  $\text{A}\beta_{42}$  fibrils formed in the presence of positive polymers are not identical to those formed by  $\text{A}\beta$  alone. The morphology of  $\text{A}\beta$  fibrils, in terms of, for example, the length distribution and node-node distance, is sensitive to solution conditions such as the pH, salt,<sup>37</sup> shaking,<sup>7</sup> and presence of accelerating compounds such as polyamines.<sup>21</sup> On the other hand, PEI and poly-Lys have no effect on the  $\alpha$ -synuclein morphology.<sup>26</sup>

There are several possible explanations of the acceleration effect of the positive polymers on  $\text{A}\beta_{42}$  aggregation. The polymers might affect the  $\text{A}\beta_{42}$  aggregation through increased ionic strength, through electrostatic interactions with charged groups due to the increased local  $\text{A}\beta_{42}$  concentration near the polymers, by direct binding, or by crowding through an increased total molarity of the solution. Polymers are commonly used to study the effect of molecular crowding on amyloid aggregation as a way to mimic amyloid formation in vivo. While the polymer concentrations used in this study are too low to affect the crowding of the solution through excluded volume effects or increased viscosity, other investigators<sup>38–40</sup> have found accelerated aggregation for amyloidogenic proteins from crowding agents at higher concentrations.

This study includes three positive polymers, one neutral polymer, and two negative polymers. Among the polymers are three polyamino acids with a peptide backbone and three nonpeptide polymers. We see no difference between the polyamino acids and the other polymers, which indicates that the backbone structure is unrelated to the effect on  $\text{A}\beta_{42}$  aggregation, at least in comparison to the effect of the charged groups. The three positive polymers have similar effects on  $\text{A}\beta_{42}$  aggregation, although at higher polymer concentration PDDA has a larger effect on  $t_{1/2}$  compared to the other polymers. The PDDA polymer chain used in this work is more than 10 times longer than PEI and poly-Lys. This means that the effect per polymer molecule is much larger in the PDDA case. At the lowest concentration tested, the molar ratio is roughly 0.001 PEI or poly-Lys per  $\text{A}\beta_{42}$  molecule or 0.00001 PDDA. However, all polymers are significantly longer than the  $\text{A}\beta_{42}$  chain, and a comparison at the level of the total concentration of repeating units is most likely more relevant. The weight of the repeating unit is similar for PDDA and poly-Lys and smaller for PEI. Taking that into account, we find that

the concentration of repeating units is still more relevant for the effect on the aggregation than the molar concentration of polymer.

PDDA is a quaternary amine and therefore always positively charged. Poly-Lys has a  $\text{p}K_a$  value of 9.8 and a steep pH dependence<sup>28</sup> and thus a high degree of ionization at pH 8.0. PEI is a mix of primary, secondary, and tertiary amines in a 1:1:1 ratio<sup>27</sup> and has a  $\text{p}K_a$  value near the pH of the buffer.<sup>27</sup> PEI has a weak pH dependence, and the degree of ionization decreases with polymer concentration.<sup>41</sup> This might explain the weak dependence of  $t_{1/2}$  with polymer concentration at low concentrations of PEI compared to poly-Lys and PDDA (Figure 2).

The charge dependency in the effect of polymers on  $\text{A}\beta_{42}$  makes it interesting to explore the effects of ionic strength. The aggregation rate of  $\text{A}\beta$  is increased at increased salt concentration. The effect can to large extent be explained by increased ionic strength which reduces the  $\text{A}\beta-\text{A}\beta$  self-repulsion through a decreased Debye screening length.<sup>36</sup> Other explanation models include amyloid charge neutralization by the ions/nonspecific ion binding and altered water structure at the protein surface as discussed for  $\text{A}\beta_{40}$ <sup>37</sup> and other amyloids<sup>42,43</sup> in studies with different kinds of salts.

Increased ionic strength decreases the electrostatic interaction between polymers and  $\text{A}\beta_{42}$ , but the properties of the polymers themselves are also changed by the ionic strength. Both the degree of ionization and the radius of gyration are affected by the screening of electrostatic repulsion. Both salt and polymers accelerate the aggregation, and the relative effect of polymers is the largest at low salt concentration. At high salt concentration,  $t_{1/2}$  is lower and the aggregation is faster both for  $\text{A}\beta_{42}$  alone and  $\text{A}\beta_{42}$  with polymers, and the additional effects of polymers are small.

The polymers contribute to the ionic strength through the charges on the polymer itself and the counterions. The concentration of counterions is approximately 6 mM for PDDA and poly-Lys at the highest polymer concentration. At 0.05  $\mu\text{g}/\text{mL}$ , the counterion concentration is 0.3  $\mu\text{M}$  for PDDA and poly-Lys assuming full ionization. For PEI, the concentration of counterions is low but hard to estimate since the degree of ionization is not easy to verify. The acceleration effect from 0.05  $\mu\text{g}/\text{mL}$  polymer is equivalent to the effect seen from 15 mM salt, as estimated from Figure 4. This is a lot more than the contribution of the polymers to the ionic strength from counterions. The contribution to the ionic strength from the polymer itself depends on the flexibility of the chain. Dos et al.<sup>28</sup> have investigated poly-Lys with NMR and found that the ionized groups move freely, but in the cases of PDDA and PEI, it is more likely that the charges cannot move independently of each other. This means that PEI and PDDA partially function like a macroion, which can have a substantial impact on the ionic strength.

The effect from increased ionic strength would be practically the same for the positively and negatively charged polymers. The lack of an acceleration effect from PAA and poly-Glu indicates that increased ionic strength is not a major explanation of the acceleration effect from the positive polymers. The lack of an effect on aggregation from poly-Glu has been seen for  $\alpha$ -synuclein as well.<sup>26</sup> In addition, no interaction between  $\text{A}\beta_{42}$  and poly-glutamic acid was found by Chauhan et al.<sup>44</sup> through dot blot analysis. The requirement of opposite charges seems to be important since poly-Lys has a

retarding effect on the aggregation of acylphosphatase, while polymers with negative charge accelerate the process.<sup>25</sup>

Even if the increased ionic strength cannot explain all of the effect from the positive polymers, as is the case at least for poly-Lys, electrostatic interactions seem to play a role. The partitioning of A $\beta$ 42 to the polymer coils will lead to an increased local concentration, which might favor nucleation.<sup>45</sup> Moreover, such partitioning leads to modulation of pK<sub>a</sub> values of both A $\beta$ 42 and the polymer,<sup>46–48</sup> leading to lower self-repulsion of A $\beta$ 42, again promoting the nucleation of aggregation. To analyze if there is any strong direct interaction between A $\beta$ 42 and poly-Lys, we immobilized poly-Lys on an SPR sensor chip and injected A $\beta$ 42 at increasing concentrations (Supporting Information). Small differences in signal from A $\beta$ 42 injection are detected (Figure S5), but there is no strong binding between poly-Lys and monomeric A $\beta$ 42. The low signal suggests that the interaction is too weak ( $K_D > 1 \mu\text{M}$ ) or too short-lived ( $k^{\text{off}} > 10^{-3} \text{ s}^{-1}$ ) to be quantified with this method. Another option is that poly-Lys interacts with an oligomeric form of A $\beta$ 42 that is present at very low concentrations on the chip.<sup>10</sup>

It has been proposed that A $\beta$  interacts with polymers in a highly specific manner, for example, with glycosaminoglycans where the distance between sulfate groups is an important determinant of the effect on A $\beta$  aggregation.<sup>24,49</sup> Residues 13–16 in A $\beta$  (HHQK) have been suggested to interact with the sulfate groups on the glycosaminoglycans.<sup>24</sup> An organized stacking of A $\beta$  peptides is unlikely for the branched PEI, and the effect is similar to that of the other positive polymers, suggesting that the effects we observe are due to general electrostatic interactions rather than any specific structural rearrangement.

The effect of positive polymers on A $\beta$ 42 aggregation resembles the effects observed from increasing peptide concentration, with a shorter lag phase and steeper slopes.<sup>7,9</sup> The locally increased A $\beta$ 42 concentration near the fibrils is thus a plausible explanation of our result. The same explanation model has been used for the accelerating effect on  $\alpha$ -synuclein aggregation from PEI and poly-Lys in combination with the screening of negative charges on  $\alpha$ -synuclein.<sup>26</sup> The locally increased concentration has also been used to explain interactions between amyloids and nanoparticles.<sup>15,45,50</sup>

## CONCLUSIONS

Positively charged polymers (PEI, poly-Lys, and PDDA) accelerate A $\beta$ 42 aggregation in a similar manner based on the total concentration of repeating units. The effect on aggregation kinetics is rationalized in terms of the localized higher A $\beta$ 42 concentration close to the polymer, with the modulation of A $\beta$ 42 charge leading to decreased self-repulsion with both factors favoring association and increasing the rate of aggregation. Moreover, these polymers affect the morphology of the formed A $\beta$ 42 fibrils. On the other hand, no effect on A $\beta$  aggregation is seen from PAA, poly-Thr, and poly-Glu.

## ASSOCIATED CONTENT

### Supporting Information

Details of the SPR experiment, figures from complementary experiments, and raw data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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