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Seed-dependent deposition behavior of A β peptides studied with wireless quartz-crystal-microbalance biosensor

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Abstract

Real-time monitoring of deposition processes of A β 1-40 and A β 1-42 peptides on various seeds has been performed using 55-MHz wireless quartz-crystal microbalance (QCM) over long-time periods (\sim 40 h). Dissolved peptide solutions were stirred for nucleation and growth of seeds at pH=7.4 and 4.6, which were immobilized on the sensor chips. The isolated A β peptides were then flowed at the neutral pH, focusing on the interaction between the seeds and the monomers (or small multimers), excluding other interactions among seeds and other aggregates. The thioflavin-T fluorescence assay and atomic-force microscopy were used for evaluating structures of the seeds and deposited aggregates. The deposition rate, determined by the frequency decrease, is about 100 monomers/nm²/year, in the case of fibril formation.

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The notable deposition behavior was observed in the deposition of A β 1-40 peptide on A β 1-42 seeds grown at the lower pH, which can be an important model for Alzheimer's disease.

Introduction

Aggregation process of amyloid fibrils have received intensive studies in the last decade, because it is deeply involved in pathogenic mechanisms of Alzheimer's disease (AD).¹⁻³ Principal targets are the full-length peptides, A β 1-40 (A β 40) and A β 1-42 (A β 42), which cause proteinaceous deposits in central nervous systems.^{4,5} They are extracted from the parental amyloid precursor proteins by enzymes or enzyme complexes,⁶ and their concentrations are normally controlled by specific enzymes and antibodies in the cortical area of the brain.⁵ A β 40 is predominantly produced, but A β 42 is reported to be more hydrophobic to cause fibril formation⁷ and more neurotoxic.⁸ Thus, understanding of the aggregation mechanism of the full-length peptides remains a central issue in the peptide polymerization study.

Most previous works studied their aggregation process in bulk solutions.^{7,9-12} However, interactions among seeds, aggregates, and monomers dominate the polymerization process for oligomers and fibrils in this case, blinding the most important interaction between the nuclei and surrounding peptides. In-situ monitoring of a single-fibril growth has been recently made possible.¹³⁻¹⁸ These studies provided essential properties in the kinetics of the fibril formation, including the critical concentration for the fibril growth and nonmonotonic growth behavior (stop-and-go growth).^{14,17,18} However, these methods fail to estimate macroscopic aggregation rate, including aggregations for oligomers and other amorphous phases: It is important to quantitatively know the aggregation rate of the peptides on various seeds, including oligomeric-phase growth as well as the fibril growth, because of higher neurotoxicity of oligomers than fibrils.^{19,20}

Macroscopic and quantitative analysis of interactions between proteins has been made possible with high-frequency quartz-crystal microbalance (QCM) biosensors.²¹⁻²⁶ They use change in the resonance frequency of quartz plate for the mass detection, which is caused by the additional

inertia effect by the adsorbed proteins on the quartz surfaces. Although the viscosity effect would participate into the frequency change, high-frequency oscillator can make this effect negligible.²⁶

In this study, we systematically investigate the deposition behaviors of A β 40 and A β 42 peptides on various seeds using the homebuilt wireless quartz-crystal microbalance (W-QCM) biosensor with fundamental resonance frequency about 55 MHz.²⁷ First, we investigate the binding reaction between A β 42 peptide and anti-A β antibody to confirm the hydrophobicity of immobilized peptides and to show the sensitivity of our W-QCM. Then, we monitor the deposition behaviors of the peptides at a neutral pH on different seeds. The nucleation and growth of the seed is performed by stirring the isolated peptide solution in two different-pH solutions (pH=7.4 and 4.6) with different stirring time (0-168 h). The β -sheet contribution is evaluated by the thioflavin T (ThT) fluorescence. Structures of the seeds and those of deposits are observed by atomic-force microscopy (AFM). The most important observation is that A β 40 peptide shows significantly high deposition rate on the A β 42 seeds grown at the low pH.

Experimental Section

Wireless QCM and flow-injection system

We used the originally developed wireless QCM system, whose details appear in previous studies.²⁷ 30- μ m-thick AT-cut blank quartz plates with 3 mm diameter were used. The surface modified sensor crystals were set in the homebuilt flow-injection system.²⁶ A micropump ran the carrier solution of phosphate buffered saline (PBS; pH=7.4) solution with 100-mM NaCl at a flow rate of 500 μ l/min. When the A β peptide solution flowed, it returned to the injection vial after the flow in the QCM cell; the peptide solution was circulated for a long-time monitoring of the deposition reaction. The temperature of the QCM cell was maintained at $37\pm0.05^\circ\text{C}$. The minimum meaningful frequency shift is about 2 Hz, and the Q factor of the system is between 1000 and 2500 in the solution flow, depending on the gripping condition of the sensor chip. (Note that very thin quartz resonators were manually set in the sensor cell.)

Preparation of seeds and flowing solution

The lyophilized A β peptides were dissolved in dimethyl sulfoxide (DMSO), and diluted to a final concentration of 12 μ M either by the PBS solution or by an acetic acid-sodium acetate buffer solution (ABS) to prepare two different pH values of 7.4 or 4.6, respectively. The prepared solutions were stirred to form the seeds, whose configurations were varied by the stirring time. We used ThT fluorescence to evaluate the degree of the β -sheet structure in the seeds.

The flowing solutions were prepared by the same procedure shown above, using the PBS buffer with 100 mM NaCl (pH=7.4). Thus, depositions proceed at the neutral pH.

Immobilization of seeds on sensor chips

We deposited 1-nm Cr thin film and then 9-nm Au film on both surfaces of the quartz plates to immobilize the seeds via the gold-alkanethiol binding reaction. The AFM observation confirmed a flat Au surface with a roughness of $R_a=0.4$ nm. The quartz crystals were cleaned in a piranha solution (98% H_2SO_4 :33% $H_2O_2=4:1$) for 10 min, and after rinsing with ultrapure water several times, they were immersed in a 10 μ M 10-carboxy-1-decanethiol/ethanol solution for 24 h. The sensor surfaces were then activated using a 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (EDC) solution for 1 h at 37 $^{\circ}C$. The crystals were then immersed in the seed solution for 24 h at 4 $^{\circ}C$, and after the rinsing procedure with the PBS, they were immersed in a 10 mM aminoethyl-polyethylene glycol(NH_2 -PEG)/PBS solution to block the remaining activated sites to avoid nonspecific binding with flowing peptides. The sensor crystals were then set in the handmade sensor cell.

Thioflavin T fluorescence measurement

The benzothiol-dye ThT specifically binds to β -sheets constructing amyloid fibrils and produces enhanced light emission.^{28,29} The ThT fluorescence assay has been then used for evaluating formation of protofibrils and their extension.^{14–16,30} We measured the ThT fluorescence level in the

seed solutions and in the flowing solutions: The stock solution for the ThT assay was prepared by dissolving ThT in a glycine/sodium-hydroxide buffer solution (pH=8.5) with a final concentration of 5 μ M. A 100- μ l sample solution was mixed with 1-ml stock solution, and the mixture solution was poured into a quartz-glass cell for the fluorospectrophotometer. The ThT fluorescence level was measured as the fluorescence at 490 nm with the excitation at 450 nm.

AFM measurement

The AFM observation was performed to evaluate the morphology on sensor chips before and after the deposition reaction. The tapping-mode measurement was adopted with a silicon cantilever with the stiffness of 40 N/m, showing the resonance frequency near 300 kHz. The scan frequency was 0.5 Hz.

Materials and Instruments

The lyophilized A β peptides were obtained from Peptide Institute (4307-v for A β 40 and 4349-v for A β 42). The monoclonal anti-human A β antibody was from Sigma (A 1349). The NH₂-PEG (molecular weight: 3,000) was from Fluka (No. 07969). The 10-carboxy-1-decanethiol and EDC were from Dojindo (C385 and W001, respectively). PBS, ABS, DMSO, ThT were from Wako Pure Chemical Industries, Ltd. The AFM system was produced by Shimadzu Co. Ltd. (SPMAJ9600). ThT fluorescence was measured by a fluorescence spectrometer by JASCO Corporation (FP-6200).

Results

Monitoring of binding reaction between A β 42 and anti-A β antibody

Figure 1 shows the change in the amount of frequency decrease during binding reaction for various concentrations of the antibody solutions injected.

Deposition on homogeneous peptide seeds

Figure 2 shows frequency evolutions during the deposition reactions of the peptides on their homogeneous seeds grown at pH=7.4. (The time for the seed nucleation is shown inside the frames.) In this study, we evaluate the deposition rate from the amount of the deposited monomer by 40 h in unit area in unit time, which was calculated by the Sauerbrey equation³¹ and the effective area of the sensor chip, assuming purely mass loading effect on the frequency shift. This result is shown in Figs. 3(a) and (b) for homogenous depositions. (For example, the deposition rate of 100 monomers/nm²/year corresponds that 100 A β peptides are deposited in the area of 1 nm² every year.) The deposition behavior of A β 40 peptide is nearly independent of the seed structure, while that of A β 42 peptide is significantly dependent on the time for seed nucleation.

The AFM micrographs on the sensor chips before and after the deposition reactions are shown in Figs. 4 and 5. We observe similar structures among seeds of A β 40 grown for different stirring times; we fail to observe any fibril structures on the sensor chip but find spherical aggregates (Figs. 4 (a)-(c)). Also, we principally observe fibril structures after the deposition reaction for 40 h, being independent of the time for seed nucleation (Figs. 4(d)-(f)).

On the other hand, we see significant difference in the seed structure in the case of A β 42. As the time for seed nucleation increases, the diameter of the spherical aggregate increases (Figs. 5(a)-(c)). However, a longer nucleation time (\sim 90 h or longer) caused smaller acute seeds (\sim 50 nm) and their beaded seeds (Fig. 5(d)). The deposited structures are dependent on the seeds: The spherical seeds yielded oligomeric deposits involving no fibrils (Figs. 5(e)-(g)), whereas the acute and beaded seeds caused well grown fibril structures (Fig. 5(h)).

Deposition on heterogeneous peptide seeds

Figures 3(c) and (d) show the deposition rates of the peptides on the seeds grown at pH=7.4 and 4.6, respectively. Notable observation is that A β 40 peptide shows a high deposition rate on the A β 42 seeds nucleated at the lower pH (40/42 in Fig. 3(d)), and that the deposited structure shows oligomeric conformation without fibrils (Fig. 6 (b)), unlike the other cases of the A β 40-peptide

deposition. This high deposition behavior is also confirmed by our multichannel QCM,²⁷ where we can monitor the deposition behaviors on different sensor chips simultaneously with the same flowing solution. Figure 7 shows the result, confirming the high deposition rate of A β 40 on A β 42 seeds grown at the lower pH. The A β 42 seeds grown at pH=4.6 (Fig. 6(a)) are non-spherical aggregates and their diameter (\sim 50 nm) is smaller than those grown at pH=7.4 (Fig. 5 (c)).

ThT fluorescence measurement

Figure 8 shows the change in the ThT fluorescence intensity caused by the stirring procedure for nucleation of seeds. The ThT fluorescence level appears to increase with the increase in the stirring time, indicating formation of β -sheet structures in the seeds, except for the A β 42 seed grown at pH=4.6: The ThT fluorescence in this case remains lower, close to the baseline. We also monitored the change in the ThT fluorescence levels in the flowing solutions, which were nearly the baseline value.

Discussion

Recognition of hydrophobic amino acids by antibody

We immobilized the peptide seeds on the sensor surfaces covalently using the self-assembled monolayer via their amino terminals. Such a tight binding may affect the hydrophobic nature of the peptide. Because major hydrophobic amino acids are located from middle part to the carboxyl end, the hydrophobicity of the immobilized peptide can be confirmed using an antibody, which recognizes the middle-part hydrophobic amino acids as the epitope. We then used the monoclonal anti-A β antibody, which recognizes the middle part hydrophobic amino acids between 17 and 24. Figure 1 exhibits significant frequency change by the injection of the monoclonal antibody. Because the antibody recognizes the middle-part amino acids, the hydrophobic nature of amino acids from middle to the carboxyl end remains active, which is important for the peptide

deposition. Thus, we expect that the deposition behavior on immobilized peptides and seeds will proceed without being affected by the covalent immobilization treatment.

We evaluate the affinity between the immobilized peptide and the monoclonal antibody: In a flow-injection system, the frequency change obeys the exponential law with the exponential coefficient $\alpha = C_A k_a + k_d$, where k_a and k_d denote the association and dissociation velocity constants, respectively, and C_A is the concentration of the analyte injected.^{32,33} Thus, the equilibrium constant K_A is given by $K_A = k_a/k_d$. By fitting the exponential function to the observed frequency change, we obtained the exponential constant with various analyte concentrations, and determined k_a and k_d , and then K_A value from their relationship as $K_A = 3.5 \times 10^7 \text{ M}^{-1}$. This value is acceptable as a binding affinity between antigen-antibody reactions and is significantly higher than that of nonspecific binding ($K_A < \sim 10^5 \text{ M}^{-1}$),³⁴ confirming that the antigen-antibody reaction was monitored. The result in Fig. 1 shows the successful detection of a low concentration analyte ($\sim 7 \text{ pM}$), demonstrating the high sensitivity of the W-QCM. This supports the high capability of monitoring the A β peptide deposition, whose molecular mass ($\sim 4500 \text{ Da}$) is relatively smaller and difficult to be detected with a conventional low-frequency QCM.

Homogenous deposition

First, we discuss importance of our success in the long-time monitoring over 40 h of the deposition reaction of A β peptides on immobilized nuclei, which has never been achieved previously with a QCM. In the past, in-situ AFM studies provided essential aggregation behavior of peptides in early stages.^{13,17} However, because of the short time monitoring ($< \sim 2 \text{ h}$) focusing only on a measurable target, the global aggregation rate cannot be determined. The direct monitoring on a single fibril by a total reflection microscopy with the fluorescence assay^{14–16} revealed very important characteristics of the fibril growth, including the stop-and-go growth. This approach is, however, unavailable to monitoring of aggregation behavior of non- β sheet structures because of insensitivity of the low affinity of ThT to non- β structures. Thus, macroscopic and quantitative deposition monitoring for a long time has been required to systematically investigate the deposition behavior of peptides on

seeds without involving interactions between seeds. The deposited structures on seeds are actually different from those formed solely by stirring the solution. For example, in Fig. 4(c), we observe spherical aggregates of A β 40 in the seed solution stirred for 168 h, but the deposits onto 6-h and 48-h seeds predominantly consist of fibrils by 40 h. (Total incubation times are 46 and 88 h, respectively, shorter than 168 h.) Therefore, the aggregated structure in the bulk solution will be governed by interactions among aggregates and ever-nucleating seeds, and not by interacting between specific seeds and monomers.

The seed-nucleation process for A β 40 causes spherical aggregates with diameters of 30-100 nm, and their forms are apparently independent of the nucleation time (Figs. 4(a)-(c)), except for the zero-seeding-time case (in this case, unnoticeable structure was observed with AFM because of smaller peptides immobilized). Because the ThT fluorescence increases (Fig. 8), the A β 40 seeds should involve the β -sheet structures, potentializing the fibril nuclei. The resultant deposits by the peptide-solution flow principally consist of fibrils (Figs. 4(d)-(f)). Pathway for fibril formation from monomer peptides is complicated in bulky solutions and experiences multistep aggregates, including oligomers,^{35,36} non- β seeds,¹² and protofibrils.^{10,11,13} Thus, it has never been straightforward to evaluate the aggregation rate with previous methods because different mechanisms for the fibril formation could be involved. In contrast, the deposition monitoring with our QCM can narrowly focus on the mechanism of the single-peptide deposition on the nuclei, excluding other interactions. The initial dissolving preparation of the peptide would, however, involve smaller aggregates of multimers as well as monomers.¹¹ We therefore centrifuged the dissolved solutions before the injection using a 10-kDa filtration tube for 30 min with 8000g to remove the initial aggregates (if any), which yielded identical results to those in Fig. 2. Thus, the peptide dissolving solutions mainly contain monomers and dimers. The ThT fluorescence level also remained the baseline value in the flowing solution (broken line in Fig. 8), indicating insignificant growth of seeds in the flowing solution. Observations in Fig. 3(a) and Figs. 4(d)-(f) show that both the deposition rate and deposited structures are nearly independent of the seed, including the deposition on immobilized monomers (0 h seed-nucleation time). In this case, the hydrophobic amino acids

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faced outward can be the seed source eventually for fibrils. We expect that protofibrils are involved in the A β 40 seeds, and the peptides accumulate to grow the fibrils structures. Thus, nearly constant deposition rate of ~ 100 monomers/nm²/year (Fig. 3(a)) is expected to represent the rate of an A β 40 peptide to be adsorbed on the nuclei to grow the fibril.

In the case of the A β 42 peptide, paranuclei and oligomeric aggregates are immediately formed after the peptide dissolution.¹¹ In our observation, the diameter of the spherical seed increases (Figs. 5(a)-(c)) and the deposition rate raises (Fig. 3(b)) as the time for seed nucleation increases. The oligomeric aggregates enlarge by adsorbing monomers, multimers, and paranuclei in the flowing solution (Figs. 5(e)-(g)), and the number of the adsorption site on their surfaces will be increased as their growth, resulting in the increase in the deposition rate. Because the ThT fluorescence increases as the seed-nucleation time at pH=7.4 (Fig. 8), the immobilized seeds are expected to involve β -sheet structures. However, the resultant deposited structures are apparently oligomeric aggregates, and we cannot see any fibrils when the seed-nucleation time is shorter than ~ 60 h. On the other hand, when the seed-nucleation time is long enough, fibril structures are clearly observed after the deposition reaction (Fig. 5(h)), and the deposition rate becomes as high as that in the deposition of A β 40 for fibrils. In this case, the immobilized seeds consist of smaller (~ 50 nm) and acute aggregates and their beaded structures, which are similar with structures reported previously.^{10,11} We consider that they are protofibrils or related structures and significantly adsorb monomers and multimers in the flowing solution for the growth of the fibril structures (Fig. 5(h)). Therefore, a long-time incubation procedure is needed to engender protofibrillar structures, which is in agreement with previous reports.^{10,13} It is important to note the close deposition rates in achieving fibril structures (~ 100 monomers/nm²/year) both for A β 40 and A β 42 peptides (Figs. 3(a) and 90 h in (b)), indicating that the kinetics of the peptide deposition purely for fibril growth is similar between the two peptides.

Heterogeneous deposition

The most important observation in this study is the result in Fig. 3(d) and Fig. 7, where A β 40 peptides significantly accumulate on the A β 42 seeds grown at pH=4.6, resulting in oligomeric deposits (Fig. 6(b)). This deposition rate is much higher than those in other cases for seeds grown at the lower pH and is comparable with those of the homogeneous depositions for the fibril formation (~ 100 monomers/nm²/year). The seed structure in this case (Fig. 6(a)) is significantly different from that grown at pH=7.4 (Fig. 5(c)), consisting of smaller non-spherical aggregates. The ThT fluorescence keeps low level, close to the baseline, during the nucleation of the A β 42 seed at pH=4.6 (Fig. 8). Previous work shows that non- β structures formed during incubation can transform into β -sheet fibril structures.¹² However, we cannot see such a transformation in our result: The non- β seeds of A β 42 grow oligomeric structures by adsorbing A β 40 peptides and fail to form any fibrils despite the long deposition time (~ 40 h).

The deposition of A β 40 peptides on A β 42 seeds grown at pH=7.4 also caused oligomeric structures (40/42 in Fig. 3(c)). In this case, the seeds should involve β structures because of their higher ThT fluorescence level, but the deposition rate is smaller than that on the seeds grown at pH=4.6. Jarrett et al.⁷ indicated that small amounts of A β 42 seeds could enhance fibril formation in A β 40 peptides, which disagrees with our result for the A β 42 seeds grown at pH=7.4, yielding the smaller deposition rate. We attribute this discrepancy to the bulk solution used in the previous study, where interactions among aggregates of A β 40 peptides, involving the A β 42 core, would cause transformation of the non- β structure aggregates into protofibrils β structures,¹² enhancing the fibril formation. Our method here monitors nearly pure interaction between monomers (and small multimers) and seeds, revealing that deposition of A β 40 peptides onto A β 42 seeds forms oligomeric structures without fibrils.

The deposition of A β 42 peptide on A β 40 seeds grown at pH=7.4 caused fibril structures, and its deposition rate is larger (42/40 in Fig. 3(c)). The ThT fluorescence level of the A β 40 seed is higher, and we consider that the protofibrils in the A β 40 seeds yielded the fibril structure even for the deposition of A β 42 peptide, unlike the oligomeric structure on A β 42 seeds with the same

nucleation time (42/42 in Fig. 3(c)).

The deposition rates on seeds grown at pH=4.6 are significantly smaller than those in other cases. This will be explained by relatively lower ThT fluorescence level of the seeds. Wood et al.⁹ reported that aggregation of A β 40 peptide onto A β 40 seeds grown at a low pH was decelerated, which agrees with our observation (40/40 in Fig. 3(d)). Thus, seeds formed at lower pH shows low activity for the peptide adsorption, except for the special case of the deposition of A β 40 on A β 42 seeds as described above.

We consider that this special case can be an important model for AD. Oligomers and related structures are more neurotoxic than fibrils,^{1,19,20,37} and significant aggregation behavior of oligomeric structures are required for an AD model. A β 42 is more hydrophobic and will be self-associate just after its production from the amyloid precursor protein by the cleaving enzymes (β and γ secretases⁶), which show high activity at lower pH. Therefore, the isolated A β 42 peptides can form the seeds immediately at lower pH environment, and they diffuse inside and outside cells for neutral pH environments, with which A β 40 peptides interact more frequently, because most of the produced peptides is A β 40 (\sim 90 %).^{38–40} Thus, the large amount of A β 40 peptides deposits on the A β 42 seeds to cause and grow oligomeric structures.

Conclusion

We achieved following four principal conclusions in this study: (i) The wireless QCM successfully monitors the long-time deposition behavior of A β peptides on their nuclei over 40 h. (ii) A β 40 peptide shows nearly constant deposition rate on A β 40 seeds grown at the neutral pH. The deposition rate is about 100 monomers/nm²/year. The deposits show fibril structures. (iii) A β 42 peptides show significantly seed-dependent deposition behavior on A β 42 seeds grown at the neutral pH. The deposition rate increases as the seed-nucleation time increases. It becomes higher for well-grown seed (\sim 100 monomers/nm²/year), yielding fibril structures, otherwise the deposition caused oligomeric structures. (iv) A β 40 peptide shows notable deposition behavior on the A β 42

seeds grown at a low pH(=4.6), resulting in oligomeric structures. The deposition rate is as high as those on seeds grown at the neutral pH for fibril structures and is much higher than other cases for depositions on seeds grown at the low pH.

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Figure Caption

Fig. 1 Binding curves between immobilized A β 42 peptides and the monoclonal anti-A β antibody to recognize the middle-part hydrophobic amino acids. The concentration of the antibody is varied between 6.7 pM and 67 nM.

Fig. 2 (Color) Real-time monitoring of deposition behaviors of (a)A β 40 and (b)A β 42 peptides on their homogenous seeds. The frequency change Δf is proportional to the deposited mass on the sensor chips. The peptide concentrations are 12 μ M for both cases. The times for nucleation of seeds are shown.

Fig. 3 The deposition rates (monomers/nm²/year) for depositions of (a)A β 40 on A β 40 seeds, (b)A β 42 on A β 42 seeds, (c)A β 40 and A β 42 on seeds grown at pH=7.4, and (d)A β 40 and A β 42 on seeds grown at pH=4.6. Symbols [F] and [O] mean that deposited structures are principally fibrils and oligomeric structures, respectively. The notation 40/42, for example, denotes the deposition of A β 40 peptide on A β 42 seed.

Fig. 4 (Color) AFM images (2 μ m \times 2 μ m) for A β 40 seeds grown at pH=7.4 for (a)6 h, (b)48 h, and (c)168 h and for resultant structures by deposition of A β 40 peptide for 40 h ((d), (e), and (f), respectively).

Fig. 5 (Color) AFM images (2 μ m \times 2 μ m) for A β 42 seeds grown at pH=7.4 for (a)6 h, (b)24 h, (c)48 h, and (d)90 h and for resultant structures by deposition of A β 42 peptide for 40 h ((e), (f), (g), and (h) respectively).

Fig. 6 (Color) AFM images ($2\mu\text{m} \times 2\mu\text{m}$) for (a) $\text{A}\beta_{42}$ seed grown at $\text{pH}=4.6$ for 40 h and (b) resultant structure by deposition of $\text{A}\beta_{40}$ peptide at $\text{pH}=7.4$ for 40 h.

Fig. 7 Deposition behaviors of $\text{A}\beta_{40}$ peptides on $\text{A}\beta_{42}$ seeds grown at $\text{pH}=7.4$ and 4.6 monitored by the multichannel wireless QCM simultaneously.

Fig. 8 Evolutions of ThT fluorescence intensity by stirring the peptide solution. In the flowing solution, the ThT fluorescence remained the baseline level.

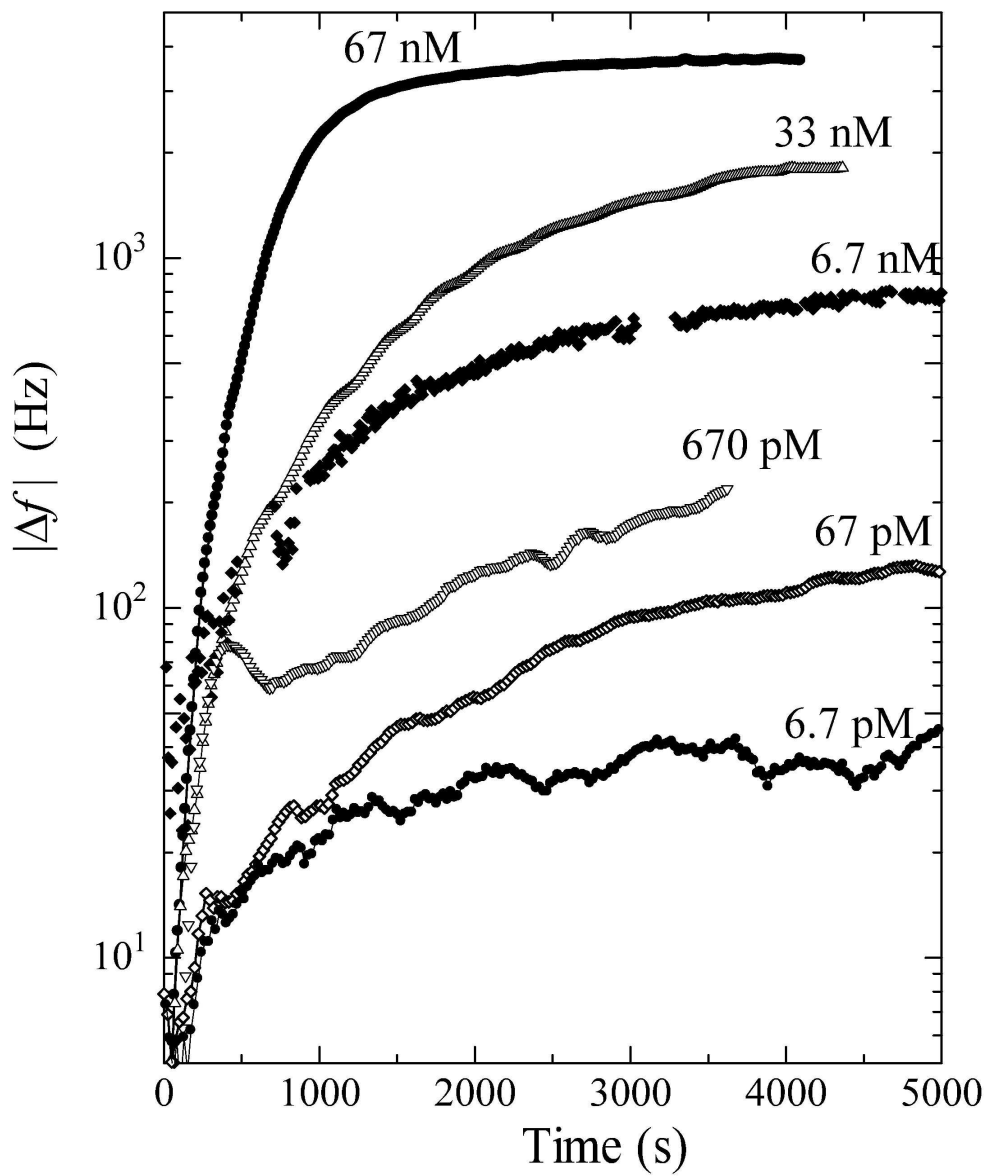


Fig. 1
188x224mm (600 x 600 DPI)

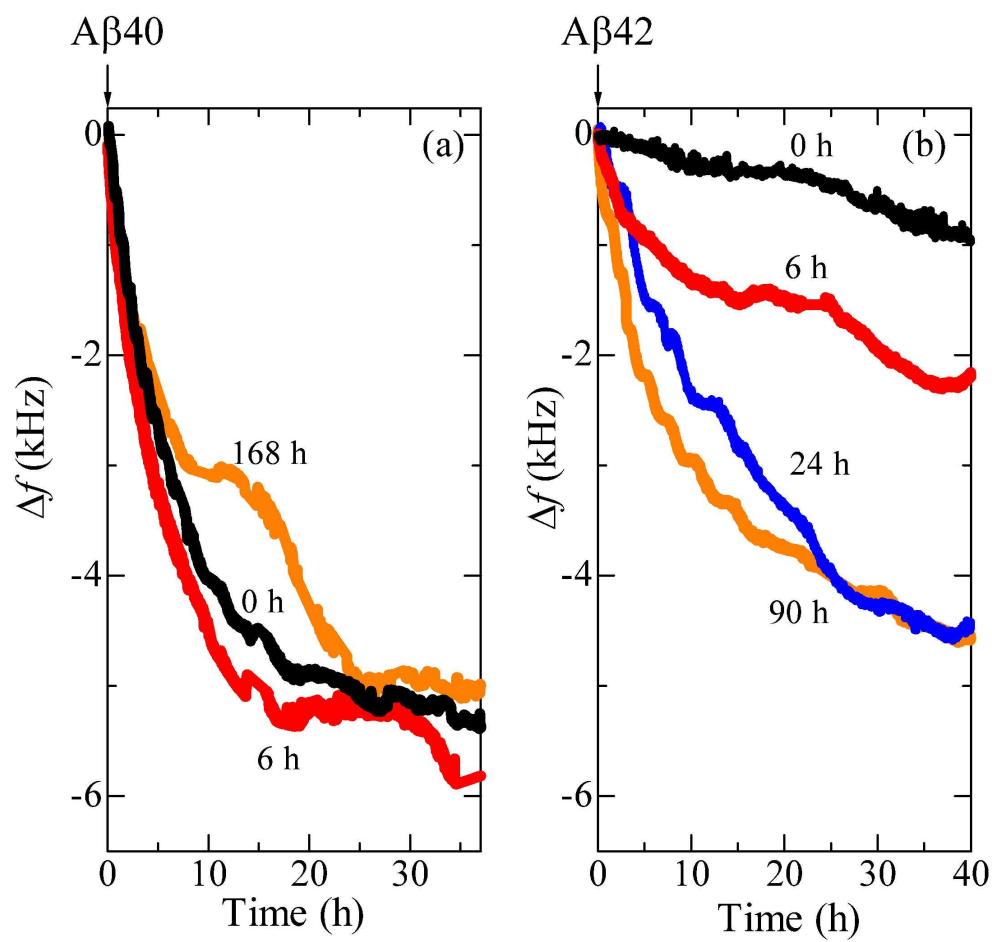


Fig. 2
188x179mm (600 x 600 DPI)

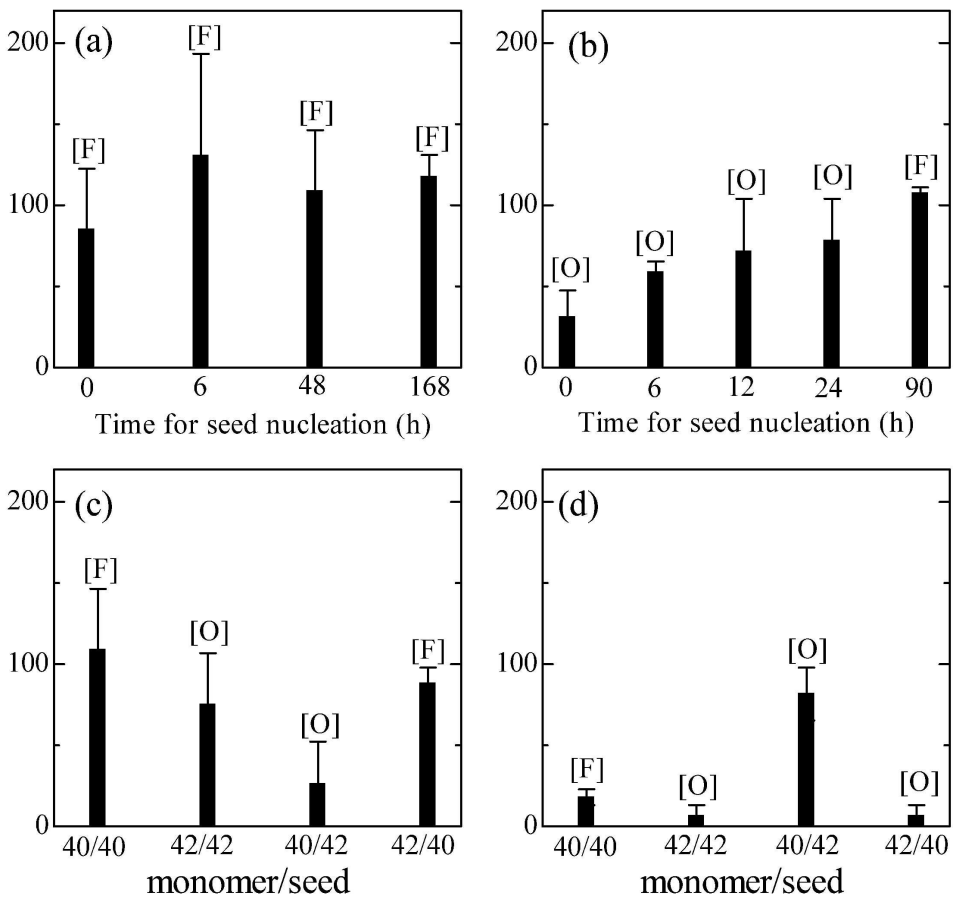


Fig. 3
196x181mm (600 x 600 DPI)

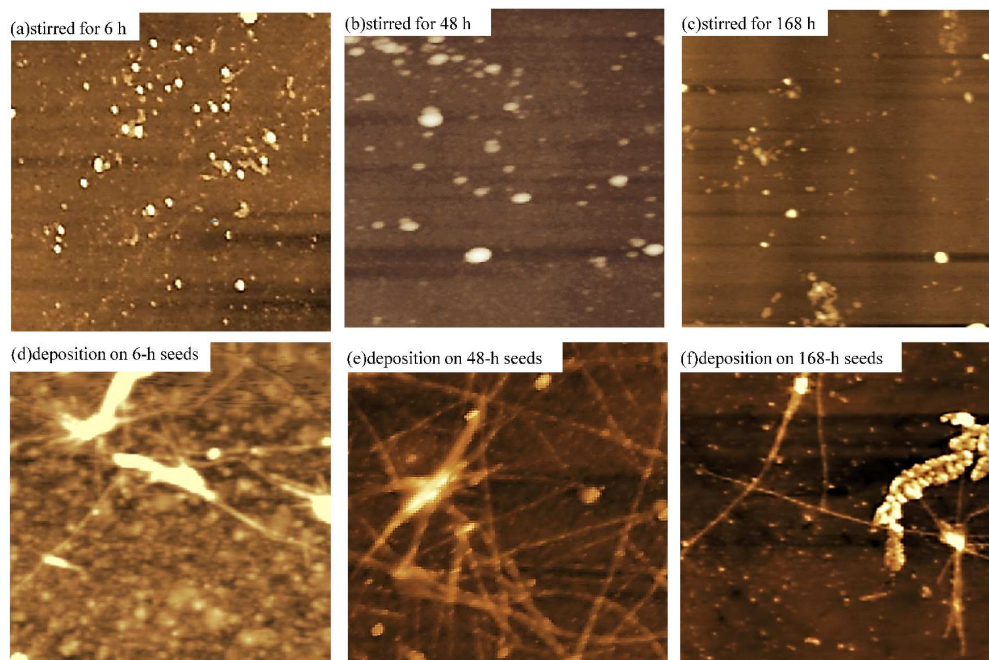


Fig. 4
298x196mm (600 x 600 DPI)

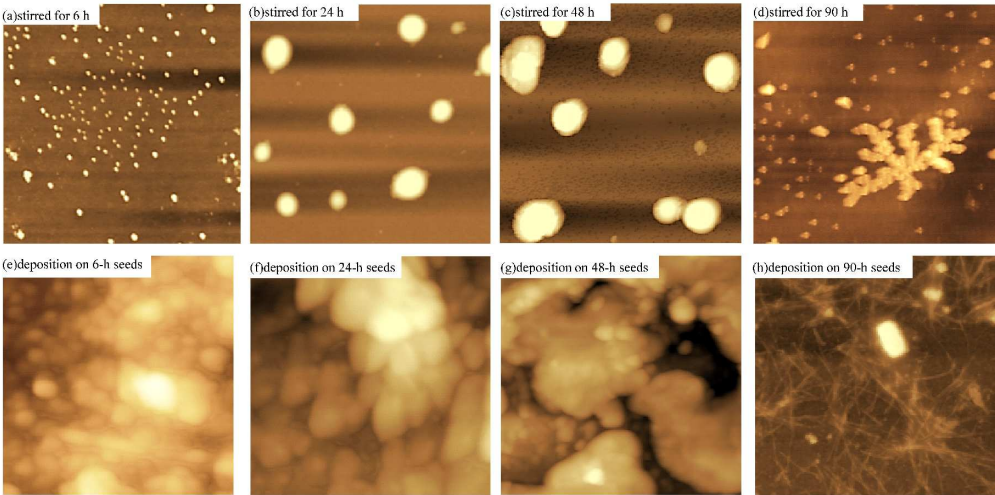


Fig. 5
386x192mm (600 x 600 DPI)

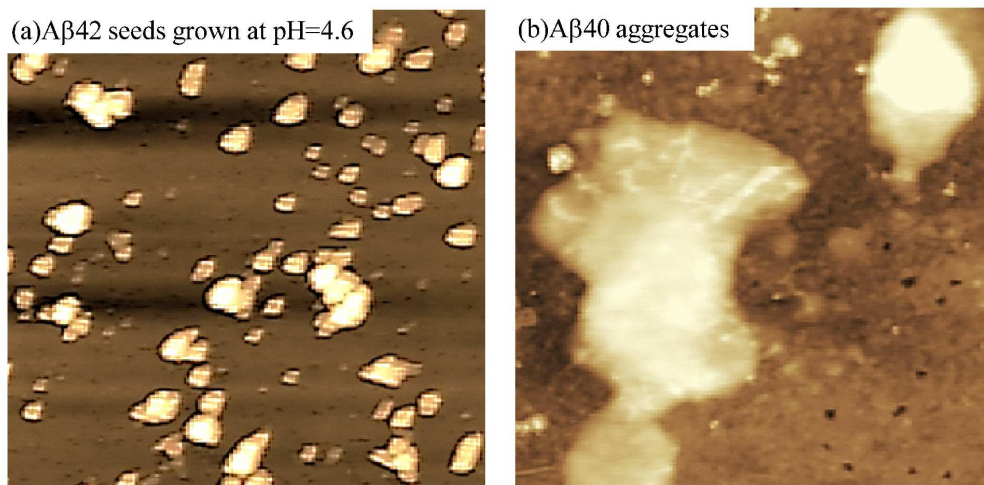


Fig. 6
245x119mm (600 x 600 DPI)

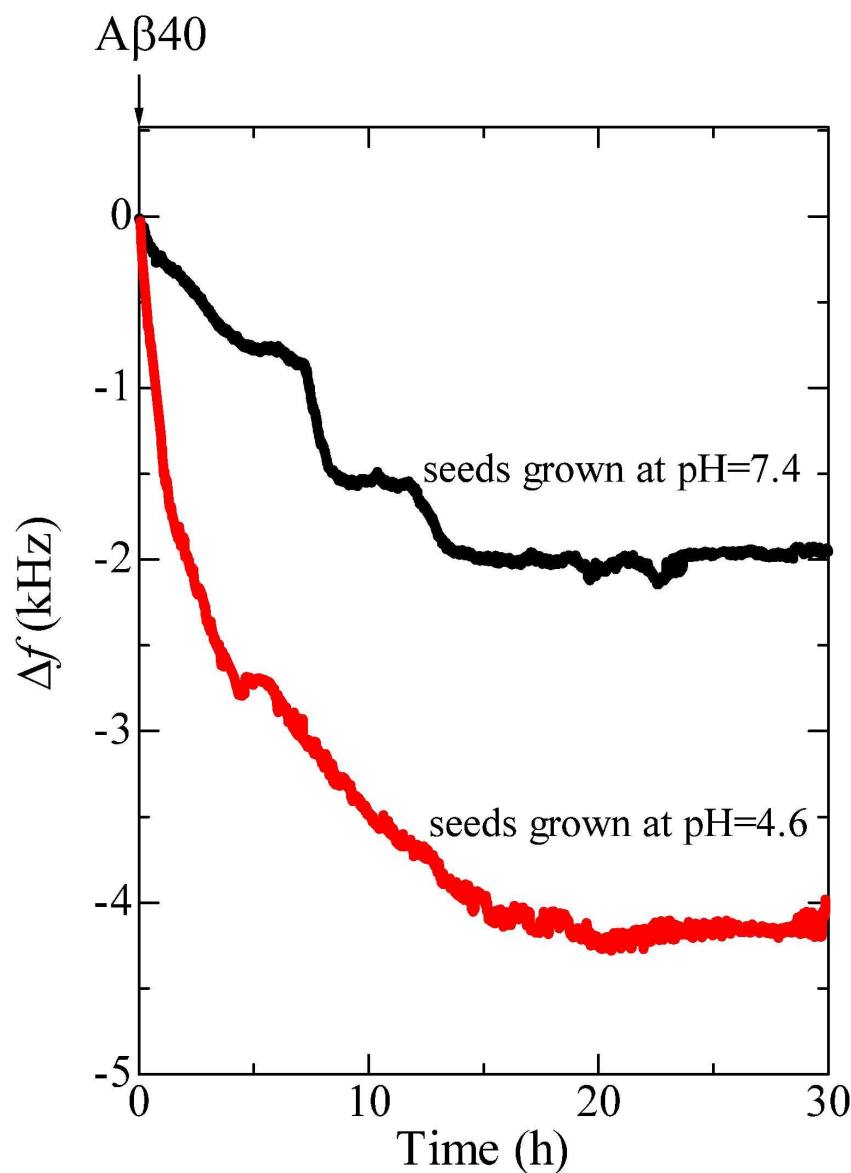


Fig. 7
137x186mm (600 x 600 DPI)

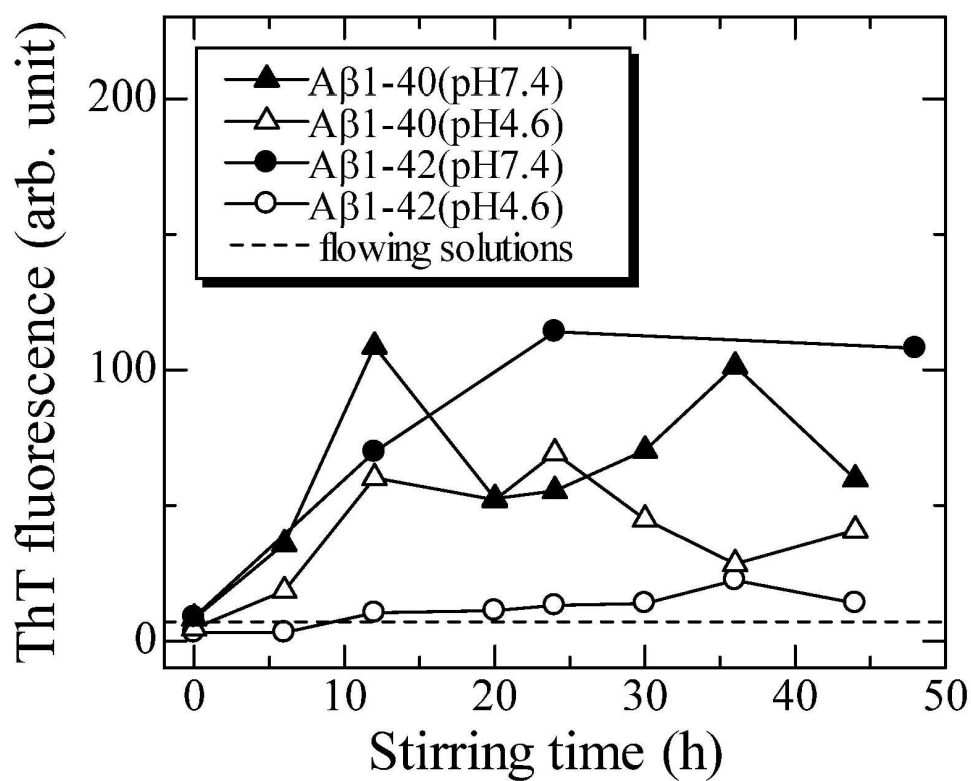
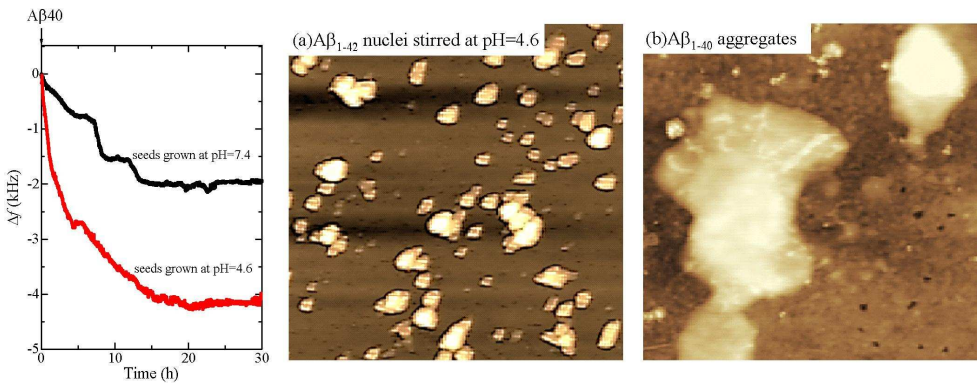


Fig. 8
137x109mm (600 x 600 DPI)

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for TOC only
91x38mm (600 x 600 DPI)