Quantum-dot-labeled synuclein seed assay identifies drugs modulating the experimental prion-like transmission

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Synucleinopathies are neurodegenerative disorders including Parkinson disease (PD), dementia with Lewy body (DLB), and multiple system atrophy (MSA) that involve deposits of the protein alpha-synuclein (α-syn) in the brain. The inoculation of α-syn aggregates derived from synucleinopathy or preformed fibrils (PFF) formed in vitro induces misfolding and deposition of endogenous α-syn. This is referred to as prion-like transmission, and the mechanism is still unknown. In this study, we label α-syn PFF with quantum dots and visualize their movement directly in acute slices of brain tissue inoculated with α-syn PFF seeds. Using this system, we find that the trafficking of α-syn seeds is dependent on fast axonal transport and the seed spreading is dependent on endocytosis and neuronal activity. We also observe pharmacological effects on α-syn seed spreading; clinically available drugs including riluzole are effective in reducing the spread of α-syn seeds and this effect is also observed in vivo. Our quantum-dot-labeled α-syn seed assay system combined with in vivo transmission experiment reveals an early phase of transmission, in which uptake and spreading of seeds occur depending on neuronal activity, and a later phase, in which seeds induce the propagation of endogenous misfolded α-syn.
Parkinson disease (PD) is a movement disorder characterized by bradykinesia, rigidity, and tremor. The pathological hallmarks of PD include progressive neuronal loss in substantia nigra and Lewy bodies, which are cytoplasmic inclusions composed of abnormally aggregated α-synuclein (α-syn) protein. α-syn is deposited in a phosphorylated form as Lewy bodies and Lewy neurites in PD and dementia with Lewy body (DLB); in multiple system atrophy (MSA), it is deposited as gial cytoplasmic inclusions (GCI) in oligodendrocytes. Disorders with α-syn deposits are called synucleinopathies. Although the pathological process between those α-syn depositions and neurodegeneration remains unclear, other neurodegenerative disorders also show aggregates of misfolded proteins such as amyloid-beta (Aβ) and tau in Alzheimer disease (AD) and TDP-43 in amyotrophic lateral sclerosis (ALS); these misfolded protein aggregates all form amyloid fibrils, which contain highly ordered cross-β sheet structure. These common features suggest that protein misfolding and aggregation could be a primary event in neurodegeneration.

Pathological studies of the PD brain have revealed that α-syn pathology spreads stereotypically from the olfactory bulb and the gut to the brain stem, and it has been postulated that the spreading occurs in connected brain regions over the course of several years. Tau pathology in AD also shows stereotypical spreading occurs in connected brain regions over the course of several years. It was recently reported that neuronal connections are involved in the spread of seeds, yet this has not been demonstrated directly in vivo.

We previously studied the effect of callosotomy (dissection of corpus callosum) on α-syn pathology after the intrastriatal inoculation of α-syn preformed fibril (PFF) seeds, revealing the involvement of corpus callosum in seed spreading to the contralateral hemisphere. Unexpectedly, this spreading of seeds to the contralateral hemisphere occurred within 24 h after the inoculation of the seeds. It was recently reported that neuronal activity modulates α-syn aggregation and spreading in hippocampus. Based on these results, in the current study, we labeled PFF seeds with quantum dots to visualize them directly and developed an acute slice system to observe the movement of seeds along the corpus callosum. Using this system, we could observe the pharmacological effects on the acute phase of seed spreading and found this spreading is dependent on neuronal activity.

**Results**

Quantum dot-labeled α-syn seeds for molecular imaging. To visualize α-syn seeds, we used CdSeTe quantum dot (QD) labeling because it is very bright and shows less photobleaching than other labels. Its emission maximum is ~705 nm, and since fluorescence emission at 700 nm shows little autofluorescence, this condition is suitable for tracking small QD-labeled seeds in thick tissue of the brain slices. To label α-syn seeds with QDs, we coupled α-syn-PFFs and QDs-COOH using the amine coupling reaction. The labeled PFFs were confirmed with electron micrograph (EM) analysis and ThT analysis. After the sonication of QDs-labeled α-syn-PFFs (QD-α-syn-PFFs), they were used for seeds and injected to the mouse striatum of the right hemisphere as described previously. When frozen coronal sections were obtained from seed-injected mouse brain and immunostained with anti-α-syn antibody, all QD-α-syn-seeds in all regions of interest (ROI) at 1 h after injection were positive for α-syn immunoreactivity, confirming that QD fluorescence shows α-syn seeds.

Cytotoxicity of QD-α-syn seeds was tested using neuronal cells. When we administered α-syn seeds or QD-α-syn seeds to neuro2a cells and cell viabilities were examined by MTT assay, there was no statistical difference in their viability between non-labeled α-syn and QD-α-syn seeds, suggesting no effect of QD-labeling on cellular viability.

α-syn seeds are associated with intracellular trafficking-related molecules. We then immunohistochemically examined the association of QD-α-syn seeds with molecular markers for the organelles and intracellular trafficking using frozen sections. Slices from the mice injected with QD-labeled α-syn monomer (QD-sa-α-syn monomer) were used as controls. The early endosome marker EEA1 localized with QD-α-syn seeds after injection (Fig. 1b, Supplemental Fig. S3a). LAMP1 and LAMP2 were used as lysosomal markers and colocalized with QD-α-syn seeds in all ROI, though less in the corpus callosum for LAMP1 (Fig. 1c, d, Supplemental Fig. S3b, c). SNAPE protein synaptobrevin-2/vesicle-associated membrane protein 2 (VAMP2) was used as a synaptic vesicular marker and colocalized with QD-α-syn seeds mainly at the corpus callosum (Fig. 1e, Supplemental Fig. S3d).

We could observe the signals of QD-labeled α-syn monomer only in the ROI including the injected area (Fig. 1a) and the signals show no colocalization with any markers, suggesting QD-labeled monomers did not spread to the other ROI and could not be incorporated into the cell (Supplemental Fig. S4a–f). These results suggest QD-α-syn seeds colocalized and moved with intracellular trafficking-related molecules.

We also investigated whether QD-α-syn seeds could be detected in regions of the contralateral hemisphere, where cell-to-cell transmission is necessary for seeds to be transferred. QD-α-syn seeds appeared in medium spiny neurons in the contralateral hemisphere (Fig. 1f). With these slices, we could observe and record the movement of QD-α-syn seeds around the corpus callosum. After the recording of the QD-α-syn seed movement around the corpus callosum, the QD fluorescence greatly increased at the recording site (Fig. 2a). With these slices, we could observe and record the movement of QD-α-syn seeds. After the recording of the QD-α-syn seed movement around the corpus callosum, the QD fluorescence greatly increased at the recording site (Fig. 2b).

Visualization of QD-α-syn seed dynamics in acute brain slices. Because our previous study suggested that α-syn seeds rapidly disseminate to the contralateral hemisphere through the corpus callosum, we decided to visualize the trafficking of QD-α-syn seeds using acute slices. We prepared living acute brain slices 1 h after injection of QD-α-syn seeds (Fig. 2a). With these slices, we could observe and record the movement of QD-α-syn seeds. After the recording of the QD-α-syn seed movement around the corpus callosum, the QD fluorescence greatly increased at the recording site (Fig. 2b). The speed of QD-α-syn seeds was faster in corpus callosum than in cortex and striatum (Fig. 2d).
and the mean speed was 0.28 ± 0.04 μm/s (Supplemental Fig. S10c). We found that QD-α-syn seeds migrated into the contralateral cortex and striatum (Fig. 2e, right panel), whereas no signal was found when QD only (i.e. without α-syn seeds) was injected (Fig. 2f, right panel). We also tested another labeling method using Qdot 705 streptavidin(sa) conjugate to examine whether there was a difference between QD-sa-α-syn monomer (Supplemental Fig. S5a and b) and QD-sa-α-syn seed migration in the living slice. Tracking analyses for QD-sa-α-syn monomer in the slice showed no migration signals in the contralateral ROI (Supplemental Fig. S5c, upper right panel), whereas α-syn seeds which were labeled with QD by the same method (QD-sa-α-syn seeds) migrated in the contralateral region. (Supplemental Fig. S5c, lower right panel).
Fig. 1 Characterization of QD-α-syn seeds. a Regions of interest (ROI) (white rectangles) for immunohistochemical stainings with antibodies at 1 and 6 h after QD-α-syn-seed or QD-sa-α-syn-monomer injection. iSt ipsilateral striatum (injection side), iCo ipsilateral cortex, CC corpus callosum, cCo contralateral cortex (opposite side of injection), cSt contralateral striatum. Bar: 500 μm (a). b QD-α-syn-seeds (purple) and anti-EEA1 immunoreactivity (green). White: colocalization. Blue: DAPI. c–g Other markers (green): LAMP1 (c), LAMP2 (d), VAMP2 (e), 22C11 for APP (f), LRRK2 (g). Quantitative analyses are shown in Supplemental Fig. S3. h No QD-α-syn monomer in entorhinal cortex (EC). i QD-α-syn seeds in EC. j No QD-sa-α-syn monomer in Amygdala (Amy). k QD-α-syn seeds in Amy. l No QD-sa-α-syn monomer in DARPP32 positive medium spiny neurons in cSt. m Localization of QD-α-syn seeds in DARPP32 positive medium spiny neurons in cSt. n No QD-sa-α-syn monomer in tyrosine hydroxylase positive neuron in SNc. o Localization of QD-α-syn seeds in tyrosine hydroxylase positive neuron in SNc. Yellow arrows: QD-α-syn seeds in cSt (n) and SNc neurons (o). Bar: 5 μm (b–o).

These findings suggest that α-syn seeds are transported from the ipsilateral hemisphere of the injected side to the contralateral cortex and striatum through corpus callosum by axonal transport.

Pharmacological effect on α-syn seed dynamics. To characterize trafficking dynamics of α-syn seeds, we examined the pharmacological effects on QD-α-syn seed numbers counted in the ROI and their trafficking speed (Fig. 3a). Because the ROI was selected at the exit to the contralateral hemisphere of the corpus callosum, we suppose that the number observed reflected the efficiency of the seed spreading to the contralateral hemisphere. Results summary was also shown in Table 1. First, we examined the effect of dynasore, an inhibitor of clathrin and dynamin-mediated endocytosis. The dynasore significantly reduced QD-α-syn seed number (Pre: 100%, Dyn: 25.9 ± 3.6%, reduction rate = seed number reduction after dynasore treatment compared with that of pretreatment (Pre): 74.1 ± 3.58%, p = 0.0001; recovery rate = increased ratio of seed number after washout compared with that of dynasore treatment: 58.5 ± 15.5%, p = 0.042, n = 5 mice) (Fig. 3b), suggesting that α-syn trafficking was increased by the endocytosis of α-syn seeds into neurons. Second, we tested the effect of colchicine, which inhibits microtubule polymerization and fast axonal transport24. The colchicine significantly reduced QD-α-syn seed number (Pre: 100%, Col: 31.6 ± 4.1%, reduction rate: 68.4%, n = 7 mice) and slowed its speed (Fig. 3c). The result suggests that the trafficking of α-syn seeds is dependent on fast axonal transport in association with microtubules.
Third, we examined the effect of botulinum toxin (BoNT), which prevented neurons from releasing synaptic vesicles of neurotransmitters by cleaving SNARE proteins. The BoNT significantly reduced rates of QD-α-syn seed number (Pre: 100%, BoNT: 34.7 ± 5.1%, reduction rate: 65.3%, n = 7 mice Fig. 3d). These results suggest injected α-syn seeds were incorporated into the neurons by clathrin-dependent endocytosis and transported on the fast axonal flow and may be affected by the inhibition of neurotransmitter release.

**Retrograde and anterograde axonal transport is involved in seed spreading.** Dynein and kinesin are key molecules for retrograde and anterograde axonal transport, respectively. To examine which axonal transport is involved in α-syn seed
Neuronal activities are regulated by some clinically available drugs. We tested the effect of the following clinically used drugs on α-syn seed migration: (1) riluzole, which is used for the treatment of ALS, and inhibits NMDA-R and kainate-R as well as voltage-gated sodium channels in damaged neurons, (2) perampanel, which is used for the treatment of epilepsy and is an AMPAR antagonist, (3) sertraline, an antidepressant known as a selective serotonin reuptake inhibitor (SSRI) and reported as an inhibitor of clathrin-dependent endocytosis, and (4) rifampicin, which is an antibiotic used to treat several types of bacterial infections, including tuberculosis, and reported as an inhibitor of oligomer formation and fibrillation of amyloids. Riluzole, perampanel, and sertraline significantly reduced the number of migrating α-syn seeds without changing migration speed (Fig. 5a–c). However, rifampicin did not significantly change the number and speed of α-syn seeds (Fig. 5d). These findings suggest that certain drugs could reduce the dissemination of α-syn seeds, resulting in decreased α-syn pathology.

The reduction of α-syn pathology by clinically available drugs. Because clinically available drugs could reduce migrating α-syn seed numbers, we examined the effect of those drugs on in vivo transmission. As we previously reported, a pathological study was performed 1.5–2 months after the injection of α-syn seeds in the striatum. Each of the four drugs mentioned above was
Fig. 4 QD-\(\alpha\)-syn seed dynamics depend on neuronal activity. Effect of drugs bath-applied on QD-\(\alpha\)-syn seed dynamics in the slice. a GABA, b bicuculline (GABAa receptor antagonist), c ampakine (AMPA receptor agonist), d DNQX(AMPA receptor antagonist), e AP5 (NMDA receptor antagonist), and f TTX, tetrodotoxin. Top panels: number of QD-\(\alpha\)-syn seeds normalized by pretreatment. Bottom panels: mean velocity of QD-\(\alpha\)-syn seeds normalized by that of pretreatment. Experimental procedure and ROI are the same as in Fig. 3a. \(n = 4\text{-}6\) mice. \(p < 0.05\): statistically significant by one-way ANOVA followed by Tukey’s post test.
**Fig. 5** Effect of clinically available drugs on α-syn seed dynamics in the acute slice and transmission experiment. **a-h** The effects on α-syn seed numbers (top) and mean velocity (bottom) in acute slice assay by riluzole (R) (**a**), perampanel (P) (**b**), sertraline (S) (**c**), and rifampicine (Rif) (**d**).  

**e** Experimental procedure for chronic drug effects on phosphorylated α-syn (p-syn) pathology after mouse α-syn seeds injection (in vivo transmission experiment). ROI: iSt ipsilateral striatum, iCo ipsilateral cortex, cCo contralateral cortex, cSt contralateral striatum. Bar: 500 μm.  

**f** Quantitative analyses for p-syn expression in mice (n = 3 mice, respectively). *p < 0.05, **p < 0.01 compared to C. C: control (injection of PBS). Pathology was examined at 1.5 M g and 2.0 M h after mouse α-syn seed injection. Bar: 10 μm.
Amyloids, in general, may spread in the same manner as α-syn seeds. Recently, the prion-like spreading of misfolded protein accumulations was suggested not only for synuclein but also for tau and Aβ. Therefore, we examined whether Aβ1-42-PFF and tau-PFF spread like α-syn seeds did in the acute slices. QD-labeled Aβ1-42-PFF and tau-PFF showed no signal and QD-microtubules showed very few signals compared with pathological Aβ1-42-PFF or tau-PFF (Supplemental Fig. S10a and b). Moving speeds of Aβ1-42-PFF and tau-PFF were similar to α-syn seeds (Supplemental Fig. S10c). Furthermore, riluzole significantly reduced the migrating number of Aβ1-42-PFF and tau-PFF (Supplemental Fig. S10a and d). To confirm that the spreading was not due to the QD-labeling, we examined the spreading of Alexa 488-labeled Aβ1-42-PFF and tau-PFF (Supplemental Fig. S11a and b). Alexa488-labeled Aβ1-42-PFF or tau-PFF also spread to contralateral cortex and striatum, similar to QD-labeled ones. These findings suggest a general mechanism among amyloid proteins for PFF spreading.

Comparison of dynamics for non-pathogenic physiological proteins and α-syn seeds. Finally, we tested whether non-pathogenic physiological proteins showed prion-like transmission. Tubulin and microtubules were used as non-pathogenic physiological proteins in this experiment. Microtubule formation by tubulin polymerization was monitored at OD340 and confirmed (Supplemental Fig. S12a). Microtubules were fixed with glutaraldehyde to prevent depolymerization. Result of western blot showed that fixed microtubules were detected as larger size proteins (>245 kDa) than tubulin monomers (Supplemental Fig. S12b). Then, the tubulin monomers and microtubules were labeled with QD as QD-α-syn seeds and injected into mice striatum (Supplemental Fig. S12c, upper left panel). Compared to the movement of QD-α-syn seeds in the ROI, QD-tubulin showed no signal and QD-microtubules showed very few signals in the ROI (Supplemental Fig. S12c and d). These finding suggest that physiological proteins such as tubulin monomers and fibrils such as microtubules were not incorporated into the cell as α-syn seeds.

**Discussion**

Accumulating evidence suggests that, in neurodegenerative disorders such as synucleinopathy and tauopathy, disease-related proteins exhibit properties of template-driven self-assembly and their aggregates transmit between cells and convert cognate monomers into an ordered assembly. Experimentally, when pathological tau or synuclein protein aggregates derived from diseased brains or formed from synthetic protein are injected into rodent brains, they induce accumulations of the corresponding endogenous proteins. In those experiments, the pathology seems to expand through the brain connectome. However, direct observation of injected protein seed spreading was not performed in vivo.

Our previous study and others suggested that very rapid dissemination of seeds occurs in vivo within 24 h after the seed injection and we thought the spreading process might be observable with labeled seeds. In this study, we labeled α-syn PFFs with QD, which are inorganic fluorescent nanocrystals that provide a useful alternative for studies that require long-term and multicolor imaging of cellular and molecular interactions. We successfully observed α-syn seeds spreading in acute living slices and found regulatory factors for it.

After injection into the striatum, we know that α-syn seeds move to the contralateral hemisphere through the corpus callosum because callosotomy significantly impeded this process, as reported previously. We therefore set an ROI at the exit of the corpus callosum to the contralateral hemisphere. Due to the strong QD fluorescence, we could use thick slices, which keep the neural network structure including corpus callosum. Then, we found that α-syn seeds move on the corpus callosum, which is a
bundle of axons, and the mean speed of α-syn seed movement was 0.28 ± 0.04 μm/s, which is compatible with fast axonal transport. Moreover, colchicine, an inhibitor of fast axonal flow, inhibited the migration of α-syn seeds, supporting the idea that α-syn seeds move on fast axonal transport. A previous study using mouse neurons cultured in microfluidic devices showed that α-syn fibrils were transported by anterograde axonal transport. Our pharmacological study revealed that α-syn seed movement from the ipsilateral to the contralateral hemisphere was on the dynemin-dependent, retrograde transport and its movement in the reverse direction is on kinesin-dependent, anterograde transport. Dynemin and kinesin transport are fast axonal transport and the mean speed is 0.3–0.7 μm/s, compatible with our result. Crossed-corticostralial neurons were reported to and those neurons, though not all, might be involved in those transports. In our study, APP and VAMP2 colocalized with QD-α-syn seeds mainly in the corpus callosum. Previous reports indicated the APP was required for kinesin-mediated axonal transport. However, vesicles containing APP move bi-directionally and require dynemin and kinesin. Although VAMP2 is a member of the vesicle-associated membrane protein (VAMP)/synaptobrevin family and is thought to participate in neurotransmitter release at the synapse, VAMP2-containing carrier vesicles are transported by the anterograde axonal transport motor KIF1A. Thus, the immunohistochemical study also reflects the α-syn seeds on axonal transport machinery.

Because we established a monitoring system for α-syn seeds spreading at the exit of the corpus callosum as an ROI, the migration number of α-syn seeds in the ROI is regulated by entrance of seeds. The entrance of seeds into the ROI could be regulated by the number of seeds taken into neurons. Dynasore, an inhibitor of clathrin and dynamin-mediated endocytosis, reduced the number of α-syn seeds, suggesting the entrance of seeds into neurons could be mediated by clathrin-dependent endocytosis, which is a dominant mode of synaptic vesicle retrieval at physiological stimuli. There is another mode, endocytosis, which is a dominant mode of synaptic vesicle seeds into neurons could be mediated by clathrin-dependent pathway. An inhibitor of clathrin and dynamin-mediated endocytosis, Dynasore, reduced the number of migrating α-syn seeds while the inhibitory effect decreased, revealing that the entrance of seeds into neurons is regulated by neuronal activities and reduced neuronal activity could decrease the entrance of seeds.

Based on those results, we tried to find clinically available drugs that can decrease the spread of α-syn seeds using our system. Riluzole, an inhibitor of NMDA-R and kainate-R, reduced the number of α-syn seeds most effectively. Perampanel, an AMPAR antagonist, and the SSRI sertraline, an inhibitor of clathrin-dependent endocytosis, also reduced the numbers of migrating α-syn seeds in the ROI, but not as effectively. If the dissemination of α-syn seeds is regulated by the endocytosis of the seeds, and that process is dependent on neuronal activity, then activity-dampering drugs should decrease α-syn pathology with chronic administration. As expected, riluzole most effectively suppressed the α-syn pathology detected with an antibody to phosphorylated α-syn. In a previous study, we hypothesized two different modes of α-syn seed transmission and propagation. In the step-by-step mode, misfolded α-syn propagation forms aggregates, and then seeds are released in a neuron, and finally, the seeds are transmitted to a connected neuron via the synapse. In another mode called dissemination mode, seeds are directly disseminated transneuronally and induce the propagation of misfolded α-syn to form aggregates in the disseminated area. In the most prion-like transmission experiments, seeds are injected into a certain region, and the α-syn pathology detected as p-syn deposition was evaluated a few months to several months later. Callosotomy before injection of α-syn seeds reduced the α-syn pathology but not callosotomy 1 day after injection, suggesting the dissemination must have occurred rapidly in our previous experiment. The present study further confirmed that rapid spreading through corpus callosum occurred and reducing this spreading by decreased intake of α-syn seeds with suppression of neuronal activity could alleviate the α-syn pathology. Rifampicin was reported as an inhibitor of oligomer formation and fibrilization of amyloids and no effect was observed in the number of migrating α-syn seeds in our slice experiment. However, the α-syn pathology was reduced by rifampicin to a similar level as sertraline. The result suggests rifampicin works on the propagation of α-syn (amyloid formation), but not on the spreading. Our experiment could dissect the prion-like transmission process into spreading and propagation; only the latter was suppressed by rifampicin (Fig.6). It is noteworthy that the latter phase could be controlled by the degradation of seeds and conversion of endogenous proteins into the misfolded ones.

Previous studies indicate that riluzole improves MPTP-induced movement disorder in a rat model of multiple system atrophy (MSA) and a marmoset model of PD. However, clinical trials of riluzole administration to symptomatic PD and MSA patients showed no significant improvement. In those trials, symptomatic evaluations were performed after short-term administration such as 1 or 4 weeks. Riluzole is an FDA-approved drug for ALS and confirmed to prolong the survival of ALS patients. This effect is thought to be due to the anti-excitotoxic effect of riluzole. However, considering a common feature of misfolding protein aggregation in neurodegenerative disorders, TDP-43 aggregation spreading may be inhibited by riluzole as discussed later for Aβ and tau amyloid. Early-phase and long-term trials are necessary for the evaluation of riluzole on synucleinopathy. Furthermore, the selection of a specific regulator for the activity of the neural networks susceptible to a specific disease should be important for a successful trial. On the other hand, many drugs could have the unexpected effect on the neuronal activities. Drugs discovered as effective in the transmission experiment should be examined whether those are effective on the early phase of transmission or the later phase.

Although we did not use the extracts from the disease brain as seeds for the assay system because the quality of the diseased tissue varies widely across cases, it is important to confirm whether these extracts show spreading based on the same mechanism. We believe that for the drugs discovered by our assay system, it is better to observe their effects on transmission with extracted pathogenic seeds from disease brain to create a foundation for future translational work.

Our results show that α-syn seed trafficking uses a rather non-specific endocytic pathway. Thus, we wondered if other amyloids also spread through this machinery. As expected, QD-Aβ PFFs and QD-tau PFFs are migrating in the ROI as α-syn seeds at the same migration speed. On the other hand, physiological fibrils such as microtubules were much less incorporated than α-syn seeds, suggesting some amyloid-specific mechanism of cellular uptake might exist. Riluzole also decreased the number of Aβ PFFs and tau PFFs in the ROI. Proof-of-concept experiments for other amyloidogenic disease proteins using our system should be possible to screen for effective drugs for those diseases.

Methods

Preparation of preformed fibrils. Preformed fibrils (PFFs) in this study were prepared as follows.

1. Human and mouse α-syn PFFs. Human and mouse α-syn seeds were prepared as described previously. In brief, the Escherichia coli strain BL21(DE3) was transformed with the expression vector PET15b encoding...
human or mouse α-syn. The expression of His-tagged α-syn was induced by the addition of 0.5 mM isopropyl β-D-thiogalactoside at 37 °C for 3 h. Cells were harvested at 1200 rpm in a shaker incubator (8 K MW) tubes containing 4% Triton X-100, centrifuged at 20,000 × g for 30 min, and the supernatant was then loaded on a Ni Sepharose 6 Fast Flow column (1 mL, GE Healthcare). α-syn was eluted with a buffer containing 50 mM Tris–HCl, 100 mM NaCl, and 250 mM imidazole at pH 8.0. The eluted samples were concentrated by centrifugation at 100,000 × g for 15 min using a Vivaspin Turbo (15 mL) with buffer containing 50 mM Tris–HCl and 100 mM NaCl at pH 8.0. Proteins were treated with thrombin (GE Healthcare) to remove the N-terminal His-tag. Purified α-syn monomers in 50 mM Tris–HCl containing 100 mM NaCl (pH 8.0) (100 μM, 150 μL) were incubated at 37 °C at 1200 rpm in a shaking incubator (DWMax M/B-034, Taitec) for 7 days. Measurements at OD 600 (or other wavelengths) were used to check turbidity. α-syn PFFs were pelleted by spinning at 50,000 × g for 20 min and suspended in PBS.

2. Human tau PFFs. Human tau ONAR recombinant protein was prepared as described previously16. In brief, the homogenate of Escherichia coli strain BL21(DE3) expressing human tau in homogenization buffer (50 mM PEPES, ImMEGTA, 1mMDTT, pH6.4) was heated at 95 °C for 5 min and centrifuged at 20,000 × g for 15 min. The resulting supernatant was applied to CellLine Phosphate (INC Corp, cat. 19545) and eluted by 1 M NaCl. The tau containing fractions were precipitated by saturated ammonium sulfate, and the pellets were dissolved in 0.05% formic acid. The solution was fractionated by reverse-phase HPLC and purified tau was obtained. For in vitro aggregation, 10 mM recombinant tau proteins in 10 mM HEPES, 100 mM NaCl (pH 7.4) mixed with 60 mg of hemoglobin was incubated at 37 °C at 1200 rpm in a shaking incubator for 7 days. After washing with PBS, the samples were subjected to gel filtration (Supplemental Fig. S3).

3. Abeta PFFs: Human Aβ1–42 peptides were purchased from AnaSpec (Fremont, CA, USA, cat: A5-24224). The peptides were dissolved in 300 μL 1% NH4Cl for stock solution and then diluted at 1 mg/mL in 50 mM Tris buffered saline (TBS). The peptides were incubated at 1200 rpm in a shaking incubator for 3 days. The concentration of Aβ1–42 was checked by turbidity.

4. Bacterial QD. CdSeTe-COOH, 8.0 μM in borate buffer, cat: Q21361MP, which emits maximum fluorescence intensity at 705 nm, was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Coupling reaction of bacterial QD and PFFs were conducted with Amine Coupling Kit (DOJINDO, Rockville, MD, USA, cat: A351) as shown in Supplemental Fig. S1A. The procedures were conducted according to the manufacturer’s protocols. In brief, the bacterial group of QD was activated with the mixture of reaction buffer: i.e., activated buffer containing of mixture activated dye and activated PFFs were overnight at 4 °C, the non-conjugated QD was blocked by blocking buffer (QD: blocking buffer volume = 1/6). The suspension was spun at 15,000 × g for 10 min. The pellets were resuspended in PBS and checked their fluorescence emission at 795 nm (cy5 filter) for the availability. These QD labeled PFFs were sonicated with BIOUPORTER at ten times of pulses (duration 10 s, interval 10 s) and the QD conjugated PFFs were stored in −80 °C until use.

Transmission electron microscopy (TEM). Samples for TEM were prepared for suspended or fixed sections. Samples were contrasted with 2% uranyl acetate and lead citrate, and the images were recorded with Hitachi HT7700 electron microscopy.

Thioflavin T (ThT) assay. A 96-well plate, 2.5 μL of α-syn seeds, QD or α-syn−QD−Aβ mixtures were applied onto each well precoated with 2% gelatin. The samples were contrasted with 2% uranyl acetate and lead citrate, and the images were recorded with Hitachi HT7700 electron microscopy.

MTT assay. MTT assay in neuro2a cells was conducted using CellQuanti-MTT Cell Viability Assay Kit (Cat: CQMT-500, BioAssay Systems, Hayward, CA, USA) according to the manufacturer’s protocol. In brief, the neuro2a cells were cultured in Dulbecco modified eagle medium (DMEM, Cat: 08435-39, Gibco) supplemented with 10% fetal bovine serum (PBS, cat: 10270, Gibco). After the cells were stably cultured at 37 °C, 5% CO2, 150 μM α-syn−QD−Aβ seeds were placed in PBS containing 2% Triton X-100, centrifuged at 20,000 × g for 30 min, and the supernatant was then loaded on a Ni Sepharose 6 Fast Flow column (1 mL, GE Healthcare). α-syn was eluted with a buffer containing 50 mM Tris–HCl, 100 mM NaCl, and 250 mM imidazole at pH 8.0. The eluted samples were concentrated by centrifugation at 100,000 × g for 15 min using a Vivaspin Turbo (15 mL) with buffer containing 50 mM Tris–HCl and 100 mM NaCl at pH 8.0. Proteins were treated with thrombin (GE Healthcare) to remove the N-terminal His-tag. Purified α-syn monomers in 50 mM Tris–HCl containing 100 mM NaCl (pH 8.0) (100 μM, 150 μL) were incubated at 37 °C at 1200 rpm in a shaking incubator (DWMax M/B-034, Taitec) for 7 days. Measurements at OD 600 (or other wavelengths) were used to check turbidity. α-syn PFFs were pelleted by spinning at 50,000 × g for 20 min and suspended in PBS.

The Aβ1–42 peptides were incubated in blocking one histo (Nacalai, Kyoto, Japan) for blocking the antibodies. After washing with PBS, the samples were subjected to gel filtration.

Fluorescent labeling of PFFs with Alexa Fluor 488. Fluorescent labeling of PFFs was performed with Alexa Fluor 488 carboxylic acid, succinimidyl ester (Alexa Fluor 488 maleimide, cat: A11061, Invitrogen, CA, USA) and Alexa Fluor 488 NHS-ester (Thermo Fisher Scientific, CA, USA) for leucine-rich repeat kinase 2, 22C11 (mouse monoclonal, MAB-348, R&D Systems, CA, USA) for lamin B, 25B5 (mouse monoclonal, MAB-4044, R&D Systems, CA, USA) for synaptophysin, 09671D (mouse monoclonal, 1:100, Pharmingen EEA1 (mouse monoclonal, E41120, 1:100, Transduction laboratories, CA, USA) for early endosome, LAMP1 (mouse monoclonal, 09671D, 1:100, Pharmingen EEA1 (mouse monoclonal, E41120, 1:100, Transduction laboratories, CA, USA) for late endosome, LAMP1 (mouse monoclonal, 09671D, 1:100, Pharmingen EEA1 (mouse monoclonal, E41120, 1:100, Transduction laboratories, CA, USA) for Munc18a (mouse monoclonal, MAB-2821, R&D Systems, CA, USA) for synaptic vesicle associated membrane protein, 3G10 (mouse monoclonal, MAB-326, R&D Systems, CA, USA) for parvalbumin, 12C1 (mouse monoclonal, MAB-326, R&D Systems, CA, USA) for synaptotagmin, K emptor, Keyence, Japan) in optical sectioning mode which is comparable to capture on laser imaging in situ perfusion. The recording of frozen section was performed with Keyence microscope (BZ-X710, Keyence, Japan) in optical sectioning mode which is comparable to capture on laser
confluent microscope. For paraffin-embedded sections, immunostaining of brains injected with non-labeled mouse α-syn seeds were performed with the methods described briefly, the autoclaved paraffin sections were incubated with blocking solution containing 5% skin milk in TBST (20 mM Tris–HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20) for 1 h. Sections were incubated with the primary antibody, phosphorylated α-syn (mouse monoclonal, pS684#6, 1:300, Wako Japan) in TBST overnight at 4 °C, followed by the secondary antibodies. For dianaminobenzidine (DAB) staining, sections were quenched with 3% H2O2/methanol for 30 min before blocking and incubated with the VECTASTAIN Elite ABC Kit reagent (Vector Laboratories) for 30 min after secondary antibody incubation. To count p-syn images, integrals of the whole brain sections were recorded using a Keyence microscope with a bright field for paraffin sections. Multiple fields of parts were captured by a x10 objective lens and stitched together using the Keyence Merge function. The p-syn deposits per area were quantified using Image-J plug-in software, (e.g. due to the drift of slices, etc.), were discarded from data storage. Particle during each step of time lapse recordings. Recording data, which got out of focus alternately. Bright sum, striatum and cortex. Images of zole), Tokyo Chemical Industry Co., Japan, A2423, 10 μM.

alternative. Bright sum, striatum and cortex. Images of zole), Tokyo Chemical Industry Co., Japan, A2423, 10 μM.

S-tryptophan (Sigma-Aldrich, St. Louis, USA). The slices were half-submerged with culture medium containing Neurobasal-A (Gibco) and horse serum (volume, 5:1) with antibiotics, penicillin and streptomycin (100 μg/mL). The 12% syn aggregates were conducted using a HYBRI-DOT MANIFOLD (Cat: 1050MM, BRL Life Technologies Inc.) and cellulose acetate membrane filter with a pore size of 0.2 μm (Cat: C02A1423, Advantec) as described previously. In brief, the samples (5 μL) were mixed in 2% SDS and PBS and applied to apparatus. Soluble proteins were removed by vacuum suction and the SDS-resistant aggregates were trapped at the membrane filter. The filter was washed three times with 2% SDS/PBS solution, and suction was maintained for 20 min. The membrane was subsequently blocked by 5% skin milk. Immunostaining of the filter and detection of signals were performed the same as western blot.

**Tubulin polymerization and labeling with QD.** Formation of microtubule was performed using HTS-Tubulin Polymerization Assay Biochem Kit (Cat: BK004P, Cytoskeleton, Inc., Denver, CO, USA) according to the manufacturer’s protocol. In brief, porcine tubulin monomer (including in kit) was mixed with tubulin glyceral buffer (5% glyceral), 1 mM general tubulin buffer (80 mM PIPES, pH = 6.9, 3 mM MgCl2, 0.5 mM EDTA) and 5% GTP. The mixture solution was incubated in 37 °C and measured OD450nm by SpectraMax M2 (Molecular Devices, CA, USA) for 1 h. The parameter settings were set as follows: (1) measurement type: kinetic, 120 cycles of 1 reading per 30 s, (2) absorbance wavelength: 340 nm, (3) temperature: 37 °C, (4) shaking: once at start of reaction, 5 s medium, orbital. The solution was collected after the reaction, and the concentration was determined by BCA assay kit (Pierce Biotechnology, MA, USA). The microtubules were collected by centrifugation at 100,000 × g for 45 min and the pellet was treated by 5% glutaraldehyde for fixation. For the western blot, anti-β-tubulin (Cat: MAB1637, Chemicon) was used. The microtubules and monomer were labeled with QD by the same method for QD-labeling of PFD. The QD-labeled microtubules were sonicated with BIORUPTOR as QD-labeled PFD were treated. The QD labeled monomer and microtubules were injected to mouse striatum as described for QD-α-syn seed injection and the brains were processed for slice imaging.

**Statistics and reproducibility.** Results were expressed as mean ± SEM. Comparison of two sets of data was analyzed by Student’s t test. Multiple comparisons were conducted by one-way ANOVA followed by Tukey’s post test. p value < 0.05 was evaluated as statistically significant. Data were analyzed by GraphPad Prism 7.0 software (SanDiego, CA, USA).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The dataset used in this study are available on https://doi.org/10.6084/m9.19727125.v2. These Source data files are provided as Supplemental Data 1-11 and Supplemental Movies 1–4.

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Author contributions
Y.I. did all of the experiments. A.O. and N.H. participated in the immunohistochemical and TEM analysis. S.Y. and A.H. performed the preparation of amyloidogenic proteins with the help of T.M. Y.F. performed structural analysis of fibrils. N.N. supervised this project and completed this manuscript with Y.I.

Competing interests
The authors declare no competing interests.

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