Generation and sonication of α-synuclein fibrils

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We use this protocol and it's working

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Sonication of α-synuclein Fibrils for injection into the mouse brain

Forked from Generation and Sonication of α-synuclein Fibrils

Vijay Singh[1], Marta Castellana-Cruz[2], Nunilo Cremades[3], Laura Volpicelli-Daley[1]

[1]Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.;
[2]Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, CB2 1EW, UK.;
[3]Institute for Biocomputation and Physics of Complex Systems (BIFI)-Joint Unit BIFI-IQFR (CSIC), University of Zaragoza, Zaragoza 50018, Spain.

Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

Saroj Kumar Sah

ABSTRACT

Animal models that accurately recapitulate the accumulation of alpha-synuclein (α-syn) inclusions, progressive neurodegeneration of the nigrostriatal system and motor deficits can be useful tools for Parkinson's disease (PD) research. The preformed fibril (PFF) synucleinopathy model in rodents generally displays these PD-relevant features, however, the magnitude and predictability of these events is far from established. We therefore have optimized the synthesis generation of α-syn fibrils to ensure reliable, robust results. These fibrils can be added to neurons in culture, differentiated iPSCs, or injected into mice or rats. The protocol includes steps for fibril synthesis as well as sonication for fibril fragmentaion which is a critical step for inducing formation of α-syn inclusions.

ATTACHMENTS

fibril_prep_protocol_060920_(Autosaved).docx

GUIDELINES

This protocol is a modification from previously published manuscripts (Patterson et al., 2019; Polinski et al., 2018; Stoyka et al., 2020; Volpicelli-Daley, Luk, & Lee, 2014).

For safe handling of fibrils please read Bousset L et al. (2016) An Efficient Procedure for Removal and Inactivation of alpha-Synuclein Assemblies from Laboratory Materials *J Parkinsons Dis.*6:143-51 https://pubmed.ncbi.nlm.nih.gov/26639448/

Keywords: monomeric α-synuclein fibrils, in vitro primary neuron, human recombinant, iPSCs

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.

References:

CITATION

Patterson, J. R., Polinski, N. K., Duffy, M. F., Kemp, C. J., Luk, K. C., Volpicelli-Daley, L. A., . . . Sortwell, C. E. (2019). Generation of Alpha-Synuclein Preformed Fibrils from Monomers and Use In Vivo. J Vis Exp(148).
LINK
10.3791/59758

CITATION

Polinski, N. K., Volpicelli-Daley, L. A., Sortwell, C. E., Luk, K. C., Cremades, N., Gottler, L. M., . . . Dave, K. D. (2018). Best Practices for Generating and Using Alpha-Synuclein Pre-Formed Fibrils to Model Parkinson's Disease in Rodents. J Parkinsons Dis, 8(2), 303-322.
LINK
10.3233/JPD-171248

CITATION

Stoyka, L. E., Arrant, A. E., Thrasher, D. R., Russell, D. L., Freire, J., Mahoney, C. L., . . . Volpicelli-Daley, L. A. (2020). Behavioral defects associated with amygdala and cortical dysfunction in mice with seeded alpha-synuclein inclusions. Neurobiol Dis, 134, 104708.
LINK
10.1016/j.nbd.2019.104708

CITATION

Volpicelli-Daley, L. A., Luk, K. C., & Lee, V. M. (2014). Addition of exogenous alpha-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous alpha-synuclein to Lewy body and Lewy neurite-like aggregates. Nat Protoc, 9(9), 2135-2146.
LINK
10.1038/nprot.2014.143

MATERIALS

Equipment:

- Spectrophotometer
- Shaker at 37 °C
- -80 °C freezer
- Dynamic light scattering detector such as Dynapro Nanostar (WDPN; Wyatt Technology) or access to transmission electron microscopy
- Benchtop centrifuge
- Qsonica 700W cup horn sonicator with chiller at 15 °C (other labs use Bioruptor Plus (Diagenode; Denville, NJ) with success)
  OR
  Probe tip sonicator (our lab uses Fisher FB12011).**

> **Note**
>
> **This is not recommended for in vivo work. If it is used for cell culture, use it in the BSL2 hood. Wear disposable lab sleeves over lab coat, Filtering Facepiece Respirator (Fisher 19-002-711), goggles. Clean hood with 1% SDS followed by water followed by 70% ethanol.

- 1.5 mL sonication tube (cat# NC0869649 Fisher Scientific)

Materials:

- Monomeric α-synuclein

Note

Monomeric α-synuclein. For *in vitro* primary neuron experiments, fibrils made using mouse or human recombinant α-synuclein will work. For differentiated human iPSCs, use human α-synuclein. For in vivo mouse models in which α-synuclein is endogenously expressed, use recombinant mouse α-synuclein because human α-synuclein is not as efficient in seeding α-synuclein inclusions from endogenously expressed mouse α-synuclein.

We recommend expressing and purifying α-synuclein from *Escherichia coli* as described (Volpicelli-Daley et al., 2014) or obtaining purified α-synuclein. For best results the α-synuclein should not have a tag (His, HA, GFP etc.). Our lab has not had success making fibrils from commercial sources.

Remove endotoxin using Pierce High Capacity Endotoxin Removal Spin columns (PI88276). Most other endotoxin removal kits have detergent which can be toxic in neuron assays.

Determine endotoxin levels using LAL endotoxin assay kit (GenScript catalog number L00350). Our values are <0.05 endotoxin units per 1 µg of protein.

Store purified α-synuclein in 10 mM Tris, pH 7.5 at >10 mg/mL at -80 °C.

- 8M guanidinium chloride
- 1.5 mL sterile LoBind microcentrifuge tubes
- 500 mM KCl; sterile filtered
- 500 mM Tris, pH 7.5; sterile filtered
- Ice
- LAL endotoxin assay kit (GenScript catalog number L00350)
- PBS
- Uranyl acetate solution
- Deionized water
- Cuvettes
- 1% SDS

SAFETY WARNINGS

Please see the Safety Data Sheet (SDS) for safety warnings and hazards before start.

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.

BEFORE START INSTRUCTIONS

Sonicating Fibril

Proper sonication is a key step for the fibril model to work. For all *in vivo* work which involved injecting fibrils into mice or rats, we use the QSonica 700 sonicator with cup horn and tube rack for 1.5 mL polypropylene tubes with a chiller at 16°C. The cup horn sonication produces short fragments which maintain their morphology for 6-8 hours (at least) and can be stored in dry ice overnight, thawed and maintained at room temperature, and therefore remain active after overnight shipments.We found that over time, the heat generated by a probe tip sonicator causes the fibrils to form amorphous aggregates (Figure 1). This is a problem because stereotaxic surgeries can take several hours and the amorphous aggregates that form while the fibrils sit on the bench causes variability and reduces the concentration of seeding competent fragments. Another advantage of using the cup horn sonicator over probe tip is that 25 µL of fibrils can be sonicated, reducing the volume needed. This is also a closed tube system which increases safety. For neuron or cell culture work in which the fibrils are added to media and then the cells immediately after sonication, a probe tip sonicator is okay. Again, this should be performed in a BSL2 hood with all proper PPE (nanoparticle respirator, goggles, gloves etc.). The volume of fibrils to be sonicated cannot be less than 100 µL.

In all cases, we wear PPE when working with fibrils. We clean any spills with 1% SDS.

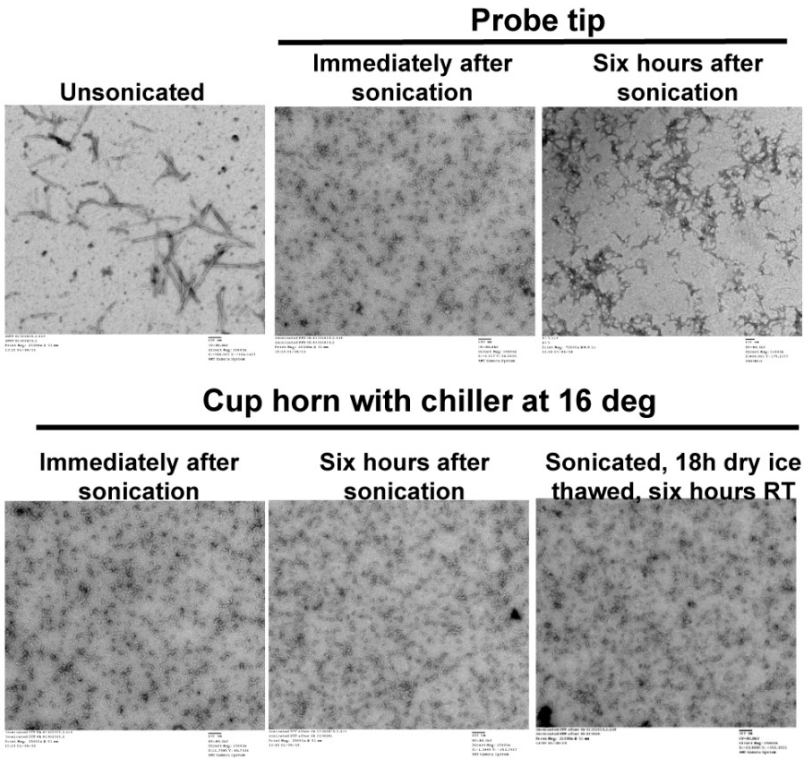

Figure 1: Transmission electron microscopy of α-synuclein fibrils. Immediately after probe tip or cup horn sonication, long fibrils are broken into small fragments. However, after six hours at room temperature, probe tip sonicated fibrils begin to form amorphous aggregates. With cup horn sonication performed at 16°C, the fragments after 6 hours appear similar in morphology compared to immediately after sonication. When the sonicated fibrils are placed in dry ice overnight, thawed and left at room temperature for 6 hours, the fragments appear similar to immediately after sonication, indicating that overnight shipments will maintain active fragments.

Sonicating Fibrils

1 Fill Qsonica water reservoir with about 900 mL water.

Note

Make sure the reservoir water level height is around 7 cm.

2 Attach cooling system to Qsonica and set the temperature at 10 °C.

3 Thaw and pipet 25-22 µL fibrils (5 mg/mL) in 1.5 mL polystyrene sonication tube.

4 Place 1.5 mL, therefore,sonication tube with fibrils in Qsonica tube holder.

> **Note**
>
> The water level inside the reservoir should be at or above 5 mm from the level of the sample inside the sonication tube.

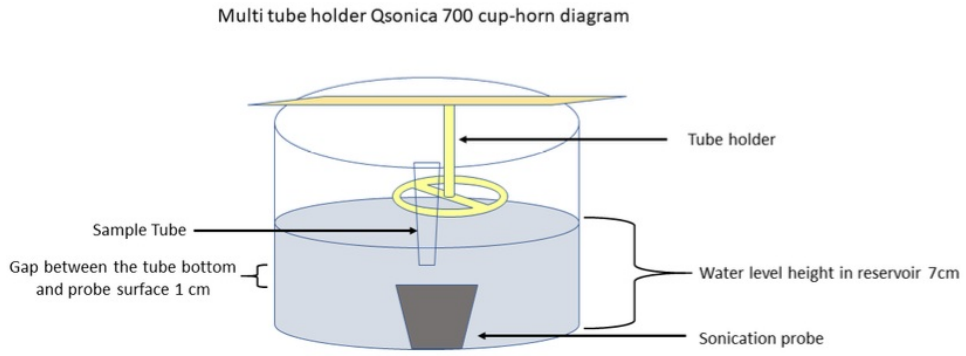

Figure 2. Diagram of Qsonica700 with multi-tube holder

Note

Sonication cycle parameters:
We initially spent time optimizing the parameters for sonication using our Qsonica 700 system. The sonication parameters we are using are as follows: Amplitude at 45%, pulse on and off durations of 3 and 2 seconds respectively, and power for each pulse set at around 110 watts. However, these parameters may not be universally ideal and can vary depending on the specific sonicator used in an individual lab. Therefore, we recommend performing personal optimization of the conditions first. The primary objective is to consistently obtain α-synuclein fragments with an average length of 50 nm. In cases where the fibrils are not adequately fragmented, there might be a low and highly variable production of α-synuclein inclusions.

5 Total sonication time: 00:15:00 .

6 Sonication pulse on for 00:00:03 and off for 00:00:02 .

7 Amplitude 45%.

Note

After sonication sometimes, there are few droplets inside the sample tube and sometimes not. If droplets are there take out tube and spin it at 1000 rpm, 00:00:10 . Take the sample tube in safety hood and mix the PFF sample with pipette 5 times in and out (avoid introducing bubbles while pipetting).

8 Sonicate sample for another 07:30:00 .

Note

After sonication, PFF sample is used on the same day

Confirming Fragmentation

9

Note

Before injecting fibrils into a large cohort of mice and waiting several months for results, researchers should ensure that their sonication protocol results in sufficient fibril fragmentation. We use a dynamic light scattering detector as a quick and reliable method to check for fragmentation. Transmission electron microscopy is another method that can be used.

Dynamic Light Scattering:

10

Pipet 1 µL sonicated PFF sample and dilute with 4 µL 1x PBS (filtered with 0.22 µm filter).

11

Put 5 µL diluted PFF sample in disposable microcuvette (WYATT Technology) for DLS.

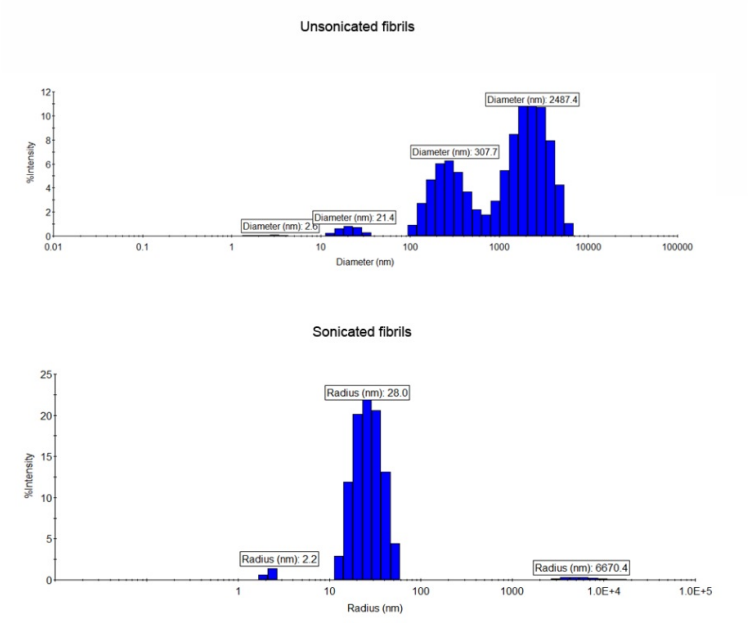

Figure 3. Example of DLS profiles of fibrils before and after sonication. A radius of 50-70nm with minimal variability is optimal.

When done, fill cuvette with 2% SDS to decontaminate fibrils (using a squirt bottle).

12

13 Let sit for at least 00:30:00.

14 Use squirt bottle to rinse several times with DI water.

15 Use filtered water for last rinse.

16 Make sure dry before next use.

> **Note**
>
> The important point is to make sure the cuvette remains dust free.

> **Note**
>
> In the next step we perform our transmission electron microscopy with the help of our High Resolution Core facility. Many universities offer an EM core.

Transmission Electron Microscopy

17 Immediately after sonication, dilute 2 µL of sonicated fibrils with 98 µL of PBS.

18 Spot 5 µL of diluted fibrils on glow discharged EM grid.

19 Wait for 00:00:20 and remove extra buffer by allowing it to wick onto a kimwipe.

20 

Add 8 µL uranyl acetate solution.

21 Remove extra uranyl acetate solution after 00:00:10.

22 

Let dry and image.