Experimental report

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Proposal: 8-03-999 **Council:** 10/2019

Title: Destruction of synaptic vesicles by alpha-synuclein

Research area: Biology

This proposal is a new proposal

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Samples: D2O
alpha synuclein proteins
synaptic vesicles

Instrument	Requested days	Allocated days	From	To
D33	5	3	21/08/2020	24/08/2020
S18	2	0		

Abstract:

The neurodegenerative disorder Parkinson's disease is becoming progressively more widespread as our population ages, resulting in thousands of deaths globally each year. The cause of this disease is poorly understood, however onset is associated with an accumulation of the protein, a-synuclein, in the brain. In its monomeric form, a-synuclein is a small (<10 nm), intrinsically disordered soluble protein that is involved in neurotransmitter release. However, it has the propensity to aggregate into large, rigid structures known as fibrils that have a characteristic beta-sheet structure and are insoluble in water. a-synuclein is known to bind to synaptic vesicles and modulate vesicle recycling and homeostasis. In a-synuclein overexpression models synaptic vesicles become clustered, rendering them unavailable for release. As such, release of the neurotransmitter dopamine contained within these vesicles is also reduced, as well as the number of vesicles themselves. A current gap in our understanding of the role of a-synuclein in disease is whether its accumulation in cells directly leads to the degradation of synaptic vesicles, and if so, what is the mechanism.

Experimental Report

Fibrillar alpha-synuclein leads to synaptic vesicle destruction

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During Parkinson's Disease the protein alpha-synuclein (aSyn) which is usually a soluble monomeric protein becomes aggregated and forms into insoluble structures called Lewy bodies and Lewy neurites. The neurons affected during PD release less dopamine which is retained in and released from synaptic vesicles. The study of synapse structure in mice with an over expression of aSyn shows a reduced number of synaptic vesicles. We investigate whether the presence of excess and aggregated aSyn can lead to the intracellular destruction of synaptic vesicles which in turn would lead to less dopamine release. Preliminary microscopy-based data showed synaptic vesicles associating to fibrillar, aggregated aSyn and over time some potential destruction of the vesicles (Figure 1). SANS experiments aimed to determine whether the association of synaptic vesicles to aSyn fibrils lead to the destruction of the synaptic vesicles. We also investigate whether vesicles in the presence of monomeric aSyn become destroyed, or as shown in our previous work that the vesicles become more clustered, supporting its presumed function in aiding synaptic vesicle release and recycling at the presynapse[1,2].

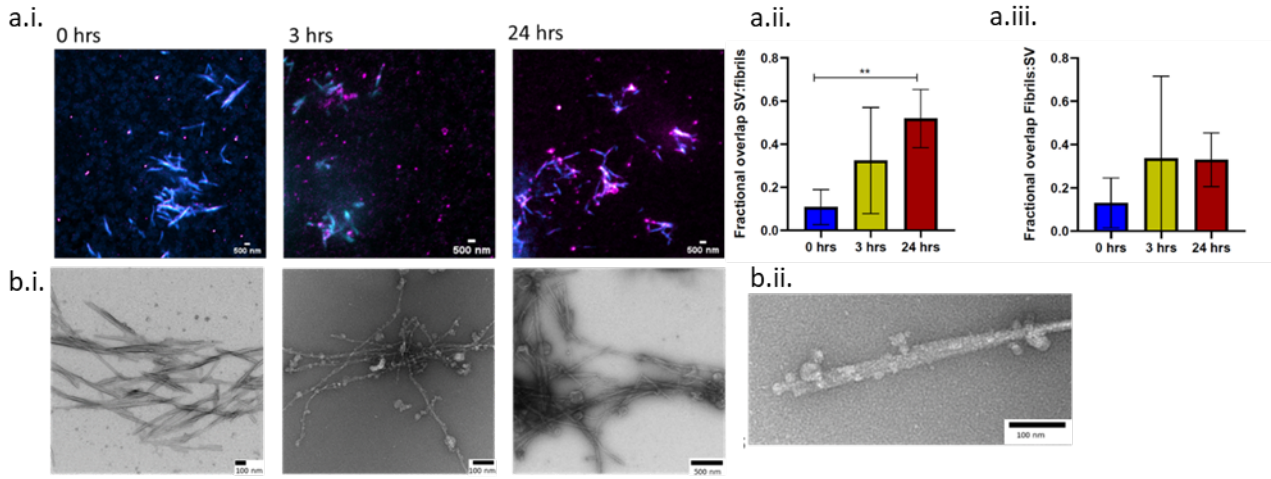

Figure 1. Vesicles associate to aSyn fibrils over time. (a.i.) Stimulated emission depletion (STED) microscopy shows increased association over time of mCLING-ATTO647N labelled synaptic vesicles (pink) to aSynC141-ATTO595 (blue) fibrils at 0, 3 and 24 hrs. (a.ii.) Fractional overlap analysis shows it is the vesicles that associate to the fibrils over time, (a.iii.) on the other hand fibrils do not significantly associate to vesicles over time. (b.i) Transmission electron microscopy (TEM) shows increased clustering of unlabelled vesicles and aSyn fibrils over 0, 3 and 24 hrs. (b.ii.) Small blebs of vesicles appear along fibrils after incubation for 24 hrs indicating vesicle destruction occurring.

SANS results

To observe only the lipid signal from the synaptic vesicles, the aSyn and vesicles were prepared in 42% D_2O to contrast match the protein present on the vesicles and the aSyn fibrils.

We show the incubation of the vesicles with fibrillar aSyn lead to a loss of lipid signal over 45 hrs and therefore destruction of the lipid bilayer of the synaptic vesicles (Figure 2a.). Yet synaptic vesicles incubated with monomeric aSyn lead to an increase in signal, possibly due to clustering of the vesicles, but not destruction of the vesicles even after 45 hours (Figure 2b.).

Fitting and analysis of the data using the Gunier-Porod model [3] gives the radius of gyration (R_g) of the synaptic vesicles and a dimensionality parameter, where s = 0 represents spheres or globules, while s = 1 represents cylinders or rod-like structures (Table 1). The vesicle only sample had an average R_g of 70.1 ± 6.1 nm which did not significantly change over 45 hrs. The dimension variable reduced from 0.31 to 0.13 suggesting they became more spherical. For vesicles incubated with aSyn fibrils and monomer at 0 hrs the R_g was not dissimilar to the vesicle only sample, yet the s component had reduced to 0.25 for vesicles incubated with fibrils suggesting they became more spherical in the presence of fibrils. The Gunier-Porod model could not be fitted to the vesicles with aSyn fibrils after 45 hrs due to lack of signal. However, for vesicles incubated with monomeric aSyn the R_g increased to 97.9 ± 11.8 nm suggesting clustering or merging of the vesicles. The s component increased to 0.48 indicating they became more rod-like in shape, possibly due to merging of vesicles together.

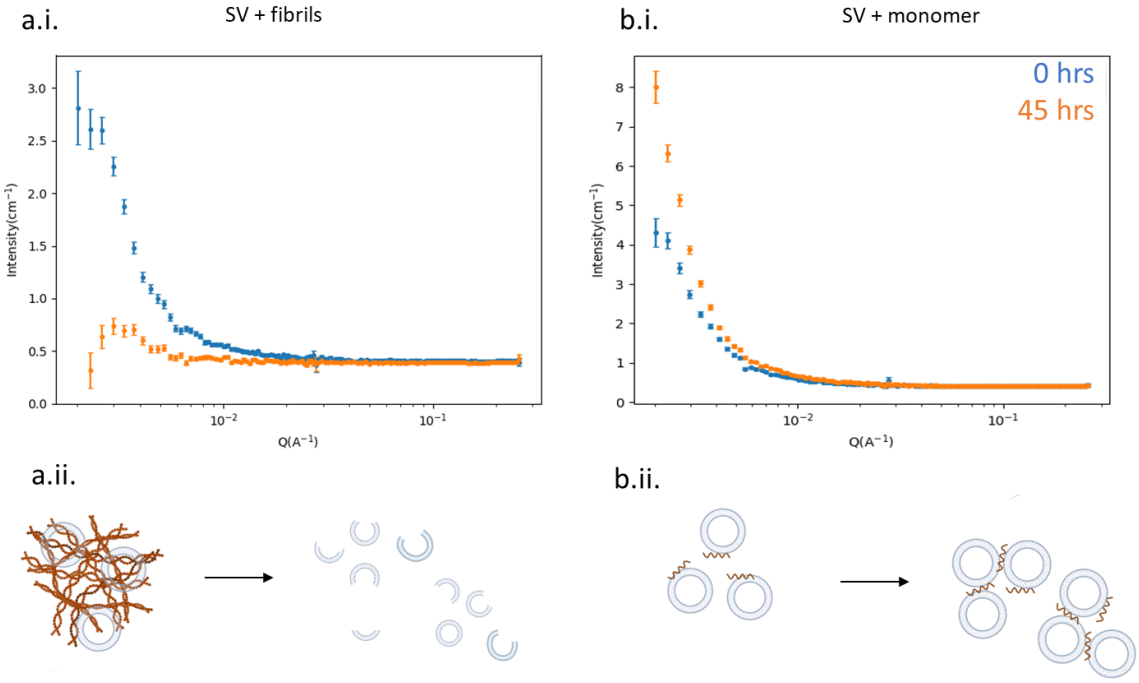

Figure 2. Over time synaptic vesicles become destroyed in the presence of aSyn fibrils, but clustered in the presence of aSyn monomer. (a.i.) The intensity of the SANS signal from lipids of the synaptic vesicles incubated with aSyn fibrils is reduced after 45 hours (orange)

compared to the initial signal at 0 hrs (blue). (a.ii.) The SANS data suggests that as vesicles associate to the fibrils, over time the lipid bilayer becomes destroyed. (b.i) The intensity of the SANS signal from lipids of the synaptic vesicles incubated with monomeric aSyn increases after 45 hours (orange) compared to the initial signal at 0 hrs (blue). (b.ii.) The SANS data suggests that over time the monomeric aSyn is clustering the vesicles together leading to a stronger lipid signal.

Table 1. Parameters of vesicles from SANS data fitted using the Guinier-Porod model

	Incubation time (hrs)	$R_{gyration}$ (nm)	Dimension variable (s)
Vesicles only	0	70.1 ± 6.1	0.31
	45	59.9 ± 6.8	0.13
Vesicles + aSyn fibrils	0	59.4 ± 4.6	0.25
	45	N/A	N/A
Vesicles + aSyn monomer	0	61.3 ± 3.8	0.32
	45	97.9 ± 11.8	0.48

1. Lautenschläger, J. *et al.* C-terminal calcium binding of α-synuclein modulates synaptic vesicle interaction. *Nat. Commun.* **9**, 1–13 (2018).
2. Fusco, G. *et al.* Structural basis of synaptic vesicle assembly promoted by α-synuclein. *Nat. Commun.* **7**, 12563 (2016).
3. Hammouda, B. A new Guinier-Porod model. *J. Appl. Cryst* **43**, 716–719 (2010).