SUPPLEMENTARY MATERIAL

METHODS

Expression and purification of recombinant α-synuclein

Expression of human wild-type and the A30P, E46K and A53T disease mutants of α-synuclein protein was performed in *E.coli* Bl21(DE3) in a pT7-7 based expression system. After IPTG induction bacterial cell pellets were harvested by centrifugation and resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM Pefabloc protease inhibitor cocktail. Cell lysis was carried out by sonication and followed by centrifugation at 10,000 x g for 30 min. at 4° C. Streptomycin sulfate precipitated DNA was removed by centrifugation at 13,500 x g for 30 min. at 4° C. An ammonium sulfate precipitation step was performed to selectively precipitate the α-synuclein protein. After centrifugation at 13,500 x g for 30 min. at 4° C the pellet was dissolved in 10 mM Tris-HCl, pH 7.4, 1 mM Pefabloc and 0.2 µm filtered. The resulting solution was loaded onto a Resource Q anion exchange column on an Åkta Basic chromatography system (GE Healthcare Biosciences, Little Chalfont, UK). Fractions collected during elution with a salt gradient were assayed for the presence of α-synuclein protein by SDS-PAGE followed by Coomassie staining. Fractions containing α-synuclein were pooled, dialyzed against 10 mM HEPES, 50 mM NaCl, pH 7.4 and concentrated to 250 µM. Protein concentrations were determined by measuring the absorbance at 275 nm using an extinction coefficient of 5,600 M^{-1} cm^{-1}. Purified protein was stored at -75° C in 0.5 ml aliquots.

Induction of aggregation

Monomeric α-synuclein (wildtype and the three mutants A30P, E46K and A53T) at an initial concentration of 100 µM was incubated at 37 ºC in 10 mM HEPES, 50 mM NaCl, pH 7.4 under constant stirring at 300 rpm in glass vials. The volume per vial was 500 µl. All aggregations were performed in triplicate. Aliquots of 20 µl were withdrawn at 24h intervals to accommodate for both ThioT and AFM measurements. While ThioT measurements were made immediately, samples for AFM imaging were stored at 4 ºC in 16 µl aliquots before measurement. No change in aggregate morphology was observed between fresh samples and samples stored at 4 ºC (unpublished data).

Kinetics of aggregation monitored by thioflavin T fluorescence

The fluorescence increase of thioflavin T upon binding to a β-sheet secondary structure was used as an indicator of protein aggregation state. At time points chosen 24h apart, 4 µl aliquots of aggregate mixture were added to 2 ml of a 5 µM ThioT solution in 50 mM sodium glycinate, pH 8.2. Fluorescence was excited at 457 nm in a fluorescence spectrophotometer (Cary Eclipse, Varian Inc, Palo Alto, CA, USA), and emission spectra were recorded from 477-600 nm. The entrance and exit slit widths were set at 10 nm. Thioflavin T reference spectra were subtracted from every measurement (Fig. S1). The intensity quoted in Fig. S1 is the peak intensity of a skewed Gaussian function fitted to the ThioT spectrum.

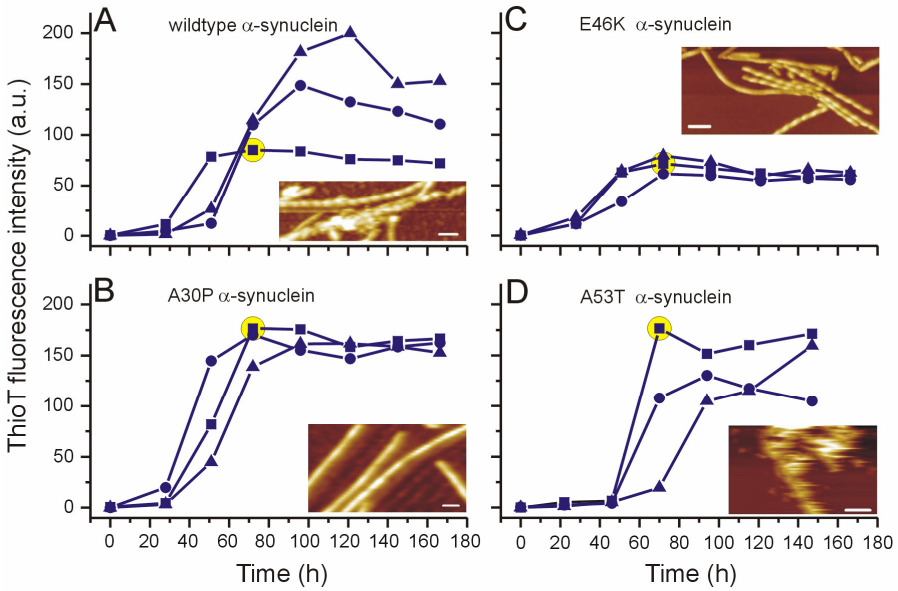

FIGURE S1 Kinetics of (A) wildtype, (B) A30P, (C) E46K, and (D) A53T human α-synuclein aggregation, measured by Thioflavin T fluorescence emission. Each experiment was performed in triplicate (squares, circles, and triangles in each of the graphs) and all experiments were performed using the same environmental parameters. The *insets* show parts of atomic force micrographs of the aggregates at t = 72h (as indicated by the highlighted data point in each graph). Scale bars 100 nm, except A53T scale bar 500 nm.

Morphology of aggregates is measured by atomic force microscopy

Atomic force microscopy images were made on a custom built instrument (1-3) using Si_3N_4 tips (Veeco Instruments, Woodbury NY, USA, type MSCT-AUHW) with a spring constant of 0.5 N/m and a nominal tip radius of 10 nm. The measurements were made in tapping mode in liquid. The tapping amplitude was less than 4 nm and tip-sample contact time was minimal, such as to minimize the force exerted on the sample and therefore the influence of the scan on the aggregate morphology. All AFM images have 512x512 pixels and a lateral resolution of 11 nm/pixel or better. Images were made at a typical scan speed of 40 μm/s.

Buffers were based on Milli-Q water (resistivity > 18 MΩcm) and 0.22 μm filtered to remove any impurities.

Samples for AFM were prepared as follows: 4 μl of protein sample was placed on unmodified freshly cleaved mica and allowed to adsorb in a humid environment for 2 minutes. Unbound protein was gently washed off with 100 μl of buffer (10 mM HEPES, 50 mM NaCl, pH 7.4). The sample was then mounted on the AFM stage and 100 μl of fresh buffer was applied. The samples remained in buffer throughout the experiment to maintain as much of a physiologically relevant environment as possible and to avoid drying artifacts in the fibril morphology and deposition of buffer salts on the mica.

Fibril characterization was done on unprocessed AFM height images. In images used for presentation purposes, height discontinuities between subsequent scan lines were removed by 0^{th} order line-wise leveling, and piezo drift was compensated by a 3^{rd} order least-mean-squares average profile using SPIP software (Image Metrology A/S, Lyngby, Denmark).

Calculation of tip radius and expected trough depth

See the legend to Fig. 4 for the definition of the symbols and Fig 4 *b* for the definition of the coordinates (x,y). The tip center *c* has coordinates (x_c, y_c); the point of contact between tip and sample *0* has coordinates (x_0, y_0); the trough location *t* has coordinates (x_t, y_t). The tip radius r_t is computed from the triangle equation (see Fig. 4 *a*), given the measured sample radius r_s and apparent width w:

$$(r_t + r_s)^2 = (r_t - r_s)^2 + (w/2)^2$$

Solving for r_t gives

$$r_t = w^2/(16 \cdot r_s)$$

To compute the expected trough height (Fig. 4 *b*), we define the tip as a circle, with

$$r_t^2 = (x - x_c)^2 + (y - y_c)^2$$

where the tip x position x_c is at $p/2$, as can be seen from Fig. 4 *b*. The bottom half of the circle is then described by rearranging the above equation into

$$y_{tip} = y_c - \sqrt{r_t^2 - (x - p/2)^2}$$

The top contour of the sample, which in the twisted protofibril model is a double helical structure, is given by

$$y_{sample} = \frac{3}{2} r_s + \frac{1}{2} r_s \cos(\pi x / p)$$

where $x = 0$ to $p/2$. At the contact point, their derivatives with respect to x are equal:

$$\frac{dy_{sample}}{dx} = \frac{dy_{tip}}{dx}$$

or

$$-\frac{1}{2} r_s \sin(\pi x / p)\pi / p = (x - p/2)/\sqrt{r_t^2 - (x - p/2)^2}$$

This equation can be solved numerically for x to yield the contact point x position x_0. Substitution of x_0 into the equation for the sample then yields the y contact coordinate y_0, from which tip center height y_c and trough height $y_t = y_c - r_t$ follow.

The effect of tip-sample convolution is an essential issue in atomic force microscopy (4,5). For calculation of the tip radius, we validated the model presented here with an alpha-synuclein sample exhibiting spherical oligomer and fibrillar features with heights ranging from 2 to 14 nm. The tip radii calculated from the heights and associated lateral widths from each feature yielded self-consistent values within the range of measurement error.

REFERENCES AND FOOTNOTES

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