

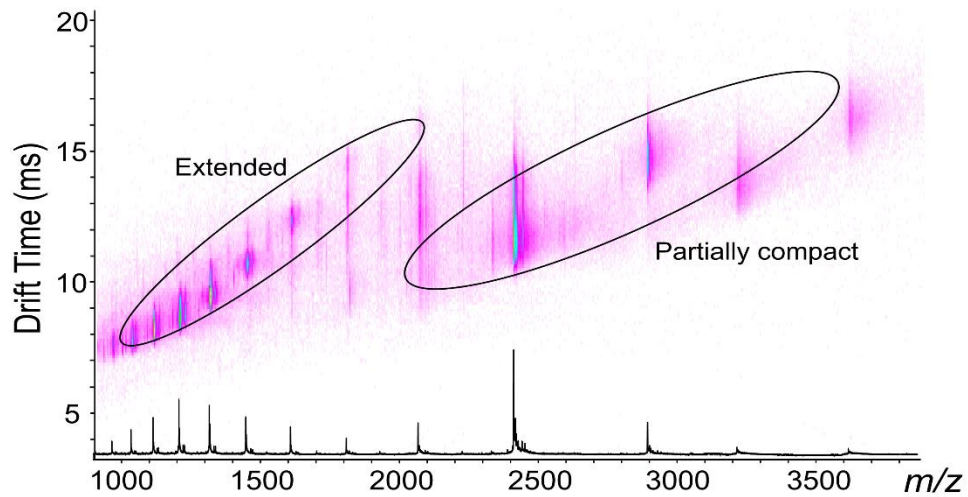
**Supplementary Information for  
Brazilin Removes Toxic alpha-Synuclein and Seeding Competent Assemblies from  
Parkinson Brain by Altering Conformational Equilibrium**

George R. Nahass<sup>1,2,3,7</sup>, Yuanzi Sun<sup>2</sup>, Yong Xu<sup>4</sup>, Mark Batchelor<sup>2</sup>, Madeleine Reilly<sup>2</sup>, Iryna Benilova<sup>2</sup>, Niraja Kedia<sup>3</sup>, Kevin Spehar<sup>3</sup>, Frank Sobott<sup>4,5</sup>, Richard B. Sessions<sup>6</sup>, Byron W. Caughey<sup>7</sup>, Sheena E. Radford<sup>4</sup>, Parmjit Jat<sup>2</sup>, John Collinge<sup>2</sup>, Jan Bieschke<sup>2,3\*</sup>

1. Colorado College, Colorado Springs, CO, USA
2. University College London Institute of Prion Diseases / MRC Prion Unit, London, UK
3. Washington University in St. Louis, St Louis, MO, USA
4. Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds, LS2 9JT UK
5. Biomolecular and Analytical Mass Spectrometry group, University of Antwerp, Antwerp, Belgium
6. Biomedical Sciences Building, University Walk, Bristol, BS8 1TD, UK
7. Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT, USA.

## Supplementary Figures

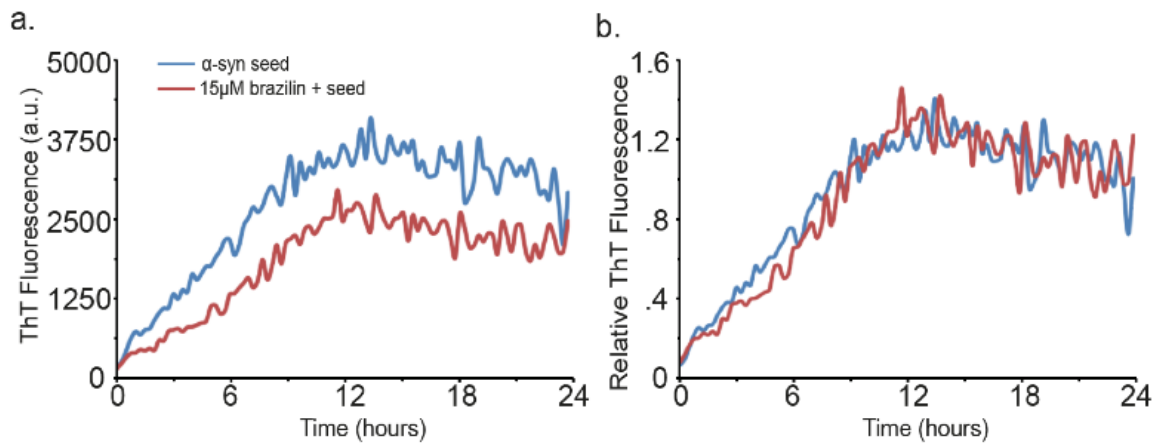
### Supplementary Figure S1



**Supplementary Figure 1:** ESI-IM-MS driftscope plot of different charge states of  $\alpha$ -syn alone.

ESI-IM-MS driftscope shows IMS drift time versus  $m/z$ , and the corresponding ESI mass spectrum is shown at the bottom.

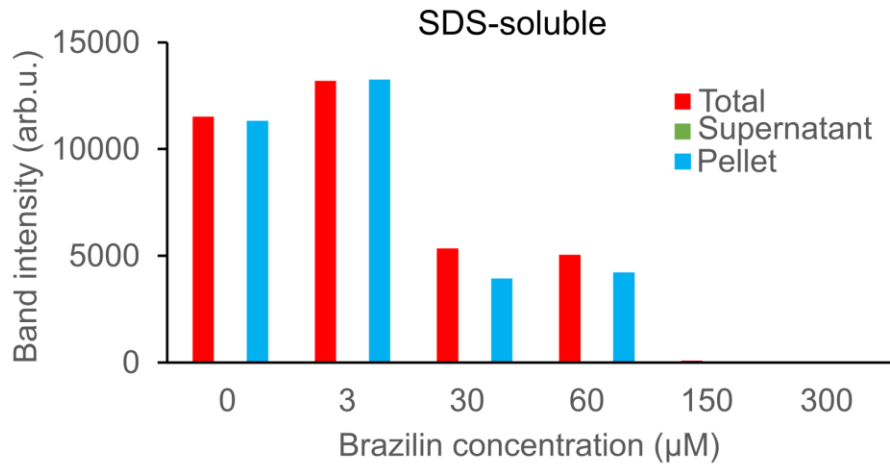
## Supplementary Figure S2



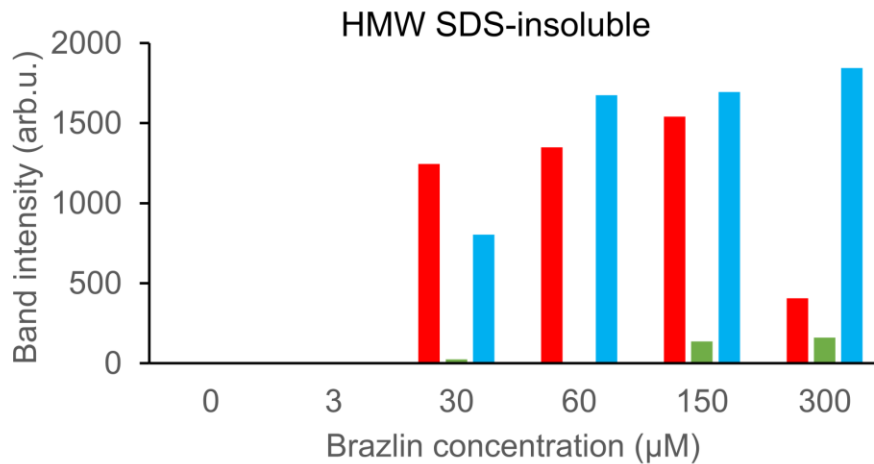
**Supplementary Figure 2:** a) Buffer subtracted kinetic aggregation data of  $\alpha$ -syn and  $\alpha$ -syn in the presence of 15  $\mu$ M Brazilin in ThT Buffer. b) ThT signals were normalized to post-aggregation amplitudes. Aggregation was seeded by 5% (w/w) sonicated  $\alpha$ -syn fibrils. ThT fluorescence was measured in a Tecan Infinite F200 microplate reader at 436 nm excitation and 482 nm emission wavelength. Graphs represent averages of triplicate curves.

## Supplementary Figure S3

a.

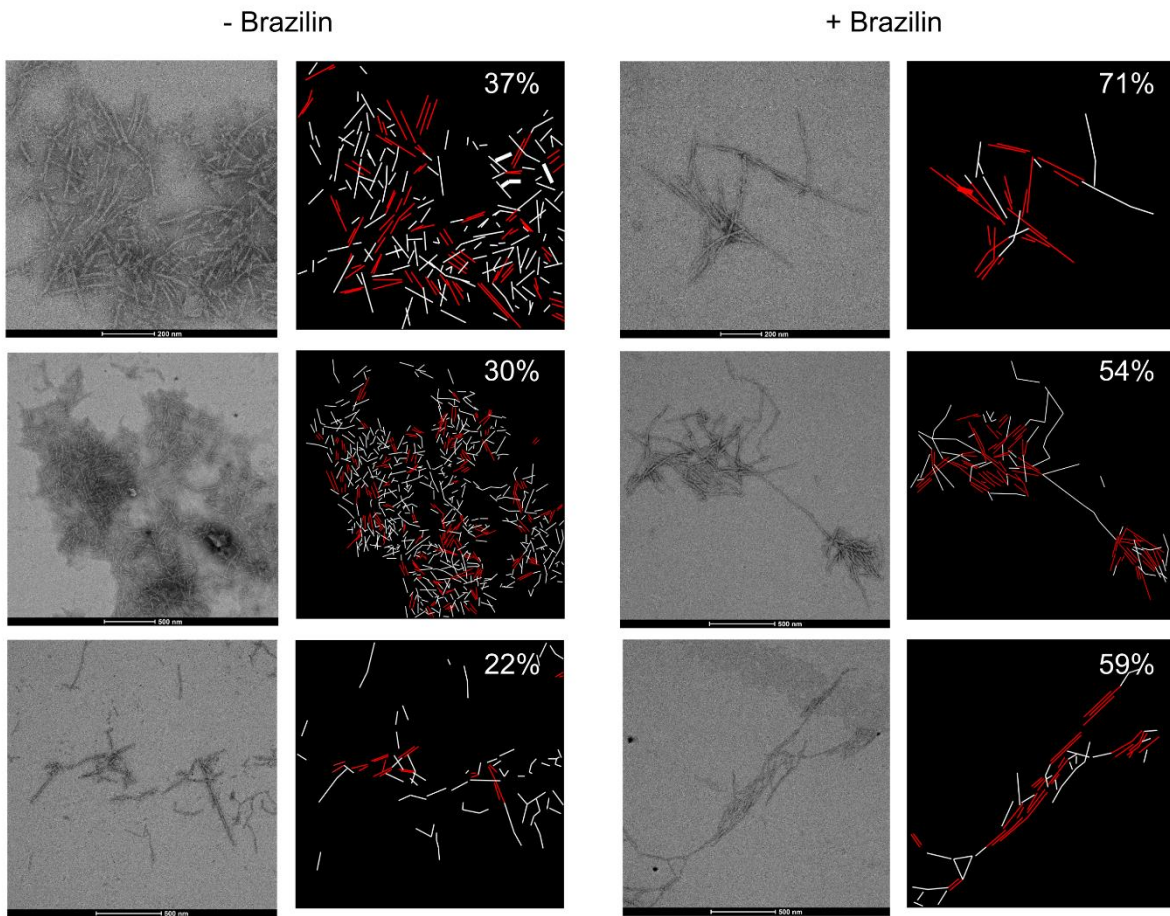


b.



**Supplementary Figure 3:** Densitometric quantification of SDS-PAGE bands from total (T) supernatant (S) and pellet (P) fractions SDS PAGE of mature  $\alpha$ -syn fibrils remodeled with various concentrations of Brazilin shown in Fig. 6c. a) Quantification of SDS-soluble protein monomer bands; b) quantification of SDS-insoluble high molecular weight protein aggregates left in the gel loading pockets.

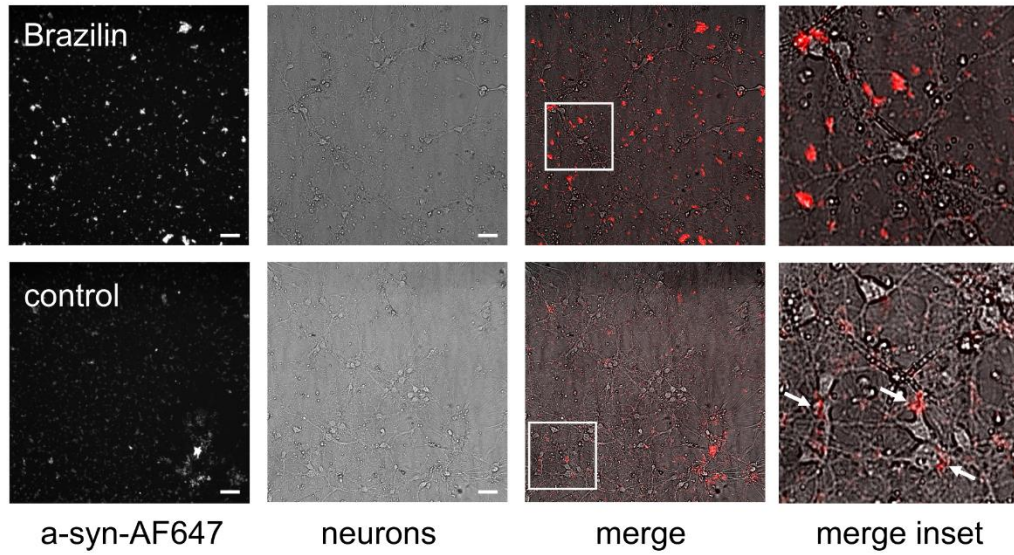
## Supplementary Figure S4



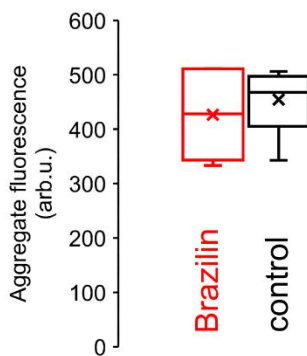
**Supplementary Figure 4:** d) Negative stain TEM images of mature  $\alpha$ -syn fibrils treated with 0  $\mu$ M Brazilin or 300  $\mu$ M Brazilin for 24 h each with its respective fibril clustering analysis plot. Clustered fibrils are shown in red, non-clustered fibrils in white. The percentage scores represent the fraction of fibrils present in a clustered state in each image.

# Supplementary Figure S5

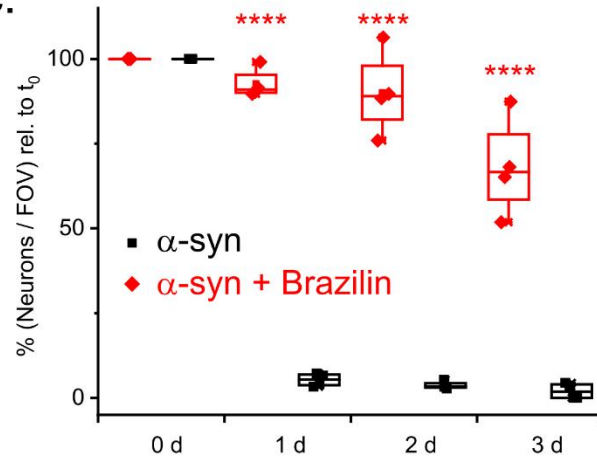
a.



b.



c.



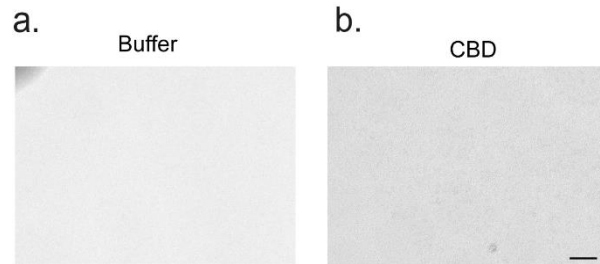
## Supplementary Figure 5:

a) Fluorescence and DIC images from neurons incubated with labeled  $\alpha$ -syn fibrils (30  $\mu$ M monomer, 5%  $\alpha$ -syn-AF647) treated with Brazilin (60  $\mu$ M) before addition to neurons or untreated control fibrils. Aggregates were added to primary neuronal culture at 0.1  $\mu$ M monomer equivalent and incubated for 5d; arrows indicate intracellular  $\alpha$ -syn aggregates. b) Quantitative

analysis of total amounts of  $\alpha$ -syn aggregates. Total fluorescence from five images was quantified in ImageJ under each condition. No significant difference in total aggregate signal was observed between Brazilin treated and untreated fibrils.

c) Cell counts of primary mouse hippocampal neurons monitored by live cell imaging after incubation with  $\alpha$ -syn fibrils (1  $\mu$ M monomer equivalent), or Brazilin remodeled  $\alpha$ -syn fibrils (1  $\mu$ M monomer equivalent, 2  $\mu$ M Brazilin) for 3 days. Four fields of view from four independent samples were analyzed for each condition. All data were normalized to the number of neurons in the FOV at the time of  $\alpha$ -syn addition, corresponding to 80-150 neurons per FOV; \*\*\*\* denotes  $p \leq 0.0001$ .

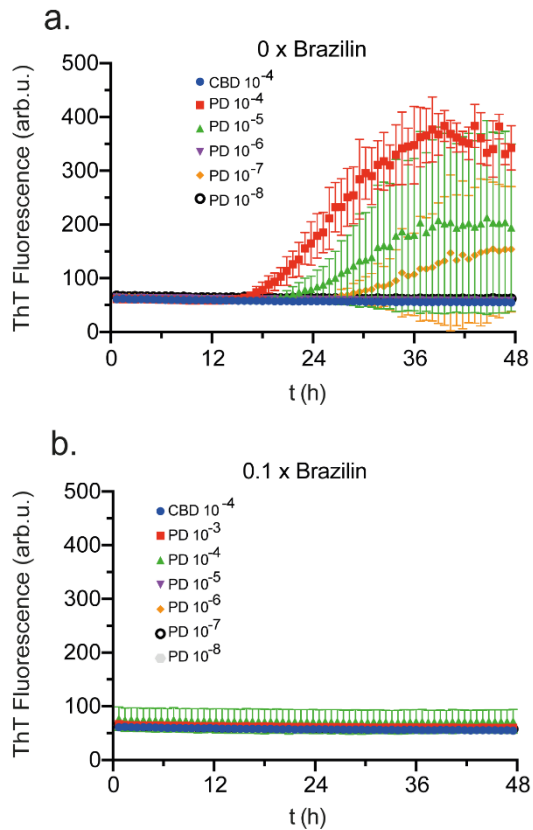
## Supplementary Figure S6



**Supplementary Figure 6:** TEM images of a) Rt-QulC aggregation buffer (40 mM NaP, 170 mM NaCl) and b) Rt-QulC end product when K23Q  $\alpha$ -syn when seeded with CBD patient brain homogenate; scale bar = 200 nm.

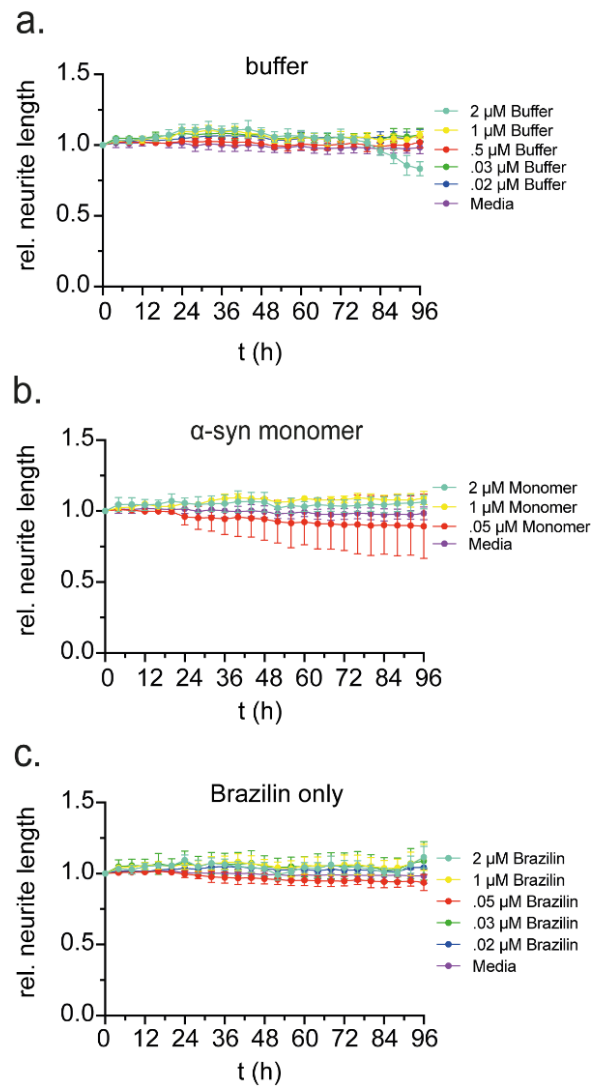


## Supplementary Figure S7



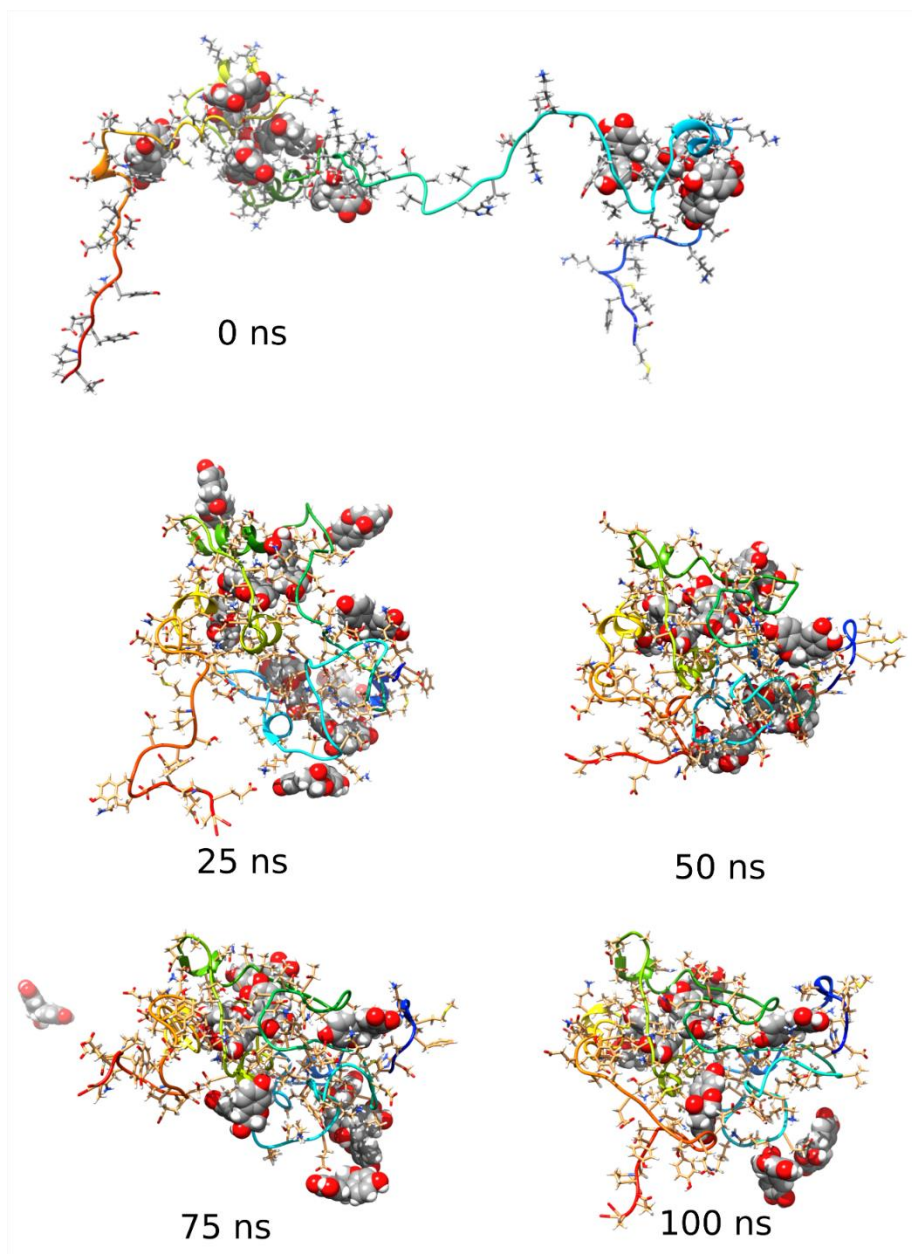
**Supplementary Figure 7:**  $\alpha$ -syn RT-QulC performed using K23Q  $\alpha$ -syn as substrate, PD brain 3089 homogenate was used as seed, and CBD brain as a negative control seed. A full dilution series of brain homogenate was tested in the a) absence or b) presence of 0.1x (0.6 $\mu$ M) Brazilin.

## Supplementary Figure S8



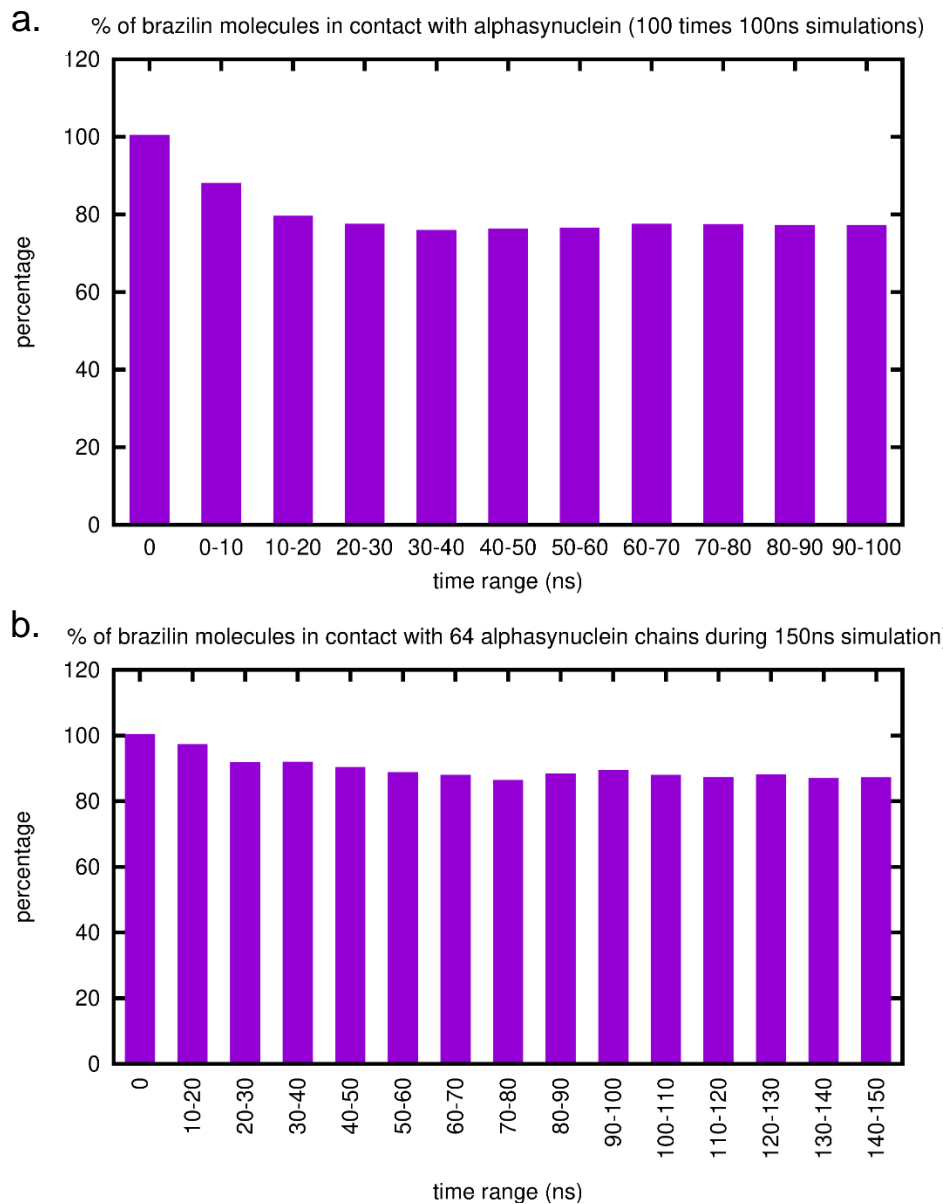
**Supplementary Figure 8:** Neurite length of primary mouse hippocampal neurons monitored by live cell imaging after incubation with a) buffer, b) varying concentrations of  $\alpha$ -syn monomer (0.05 – 2  $\mu\text{M}$ ) or c) varying concentrations of Brazilin (0.02 – 2  $\mu\text{M}$ ). Buffer dilutions are equivalent to the  $\alpha$ -syn concentrations 0.02 – 2  $\mu\text{M}$ ; means  $\pm$  SD, n = 4.

## Supplementary Figure S9



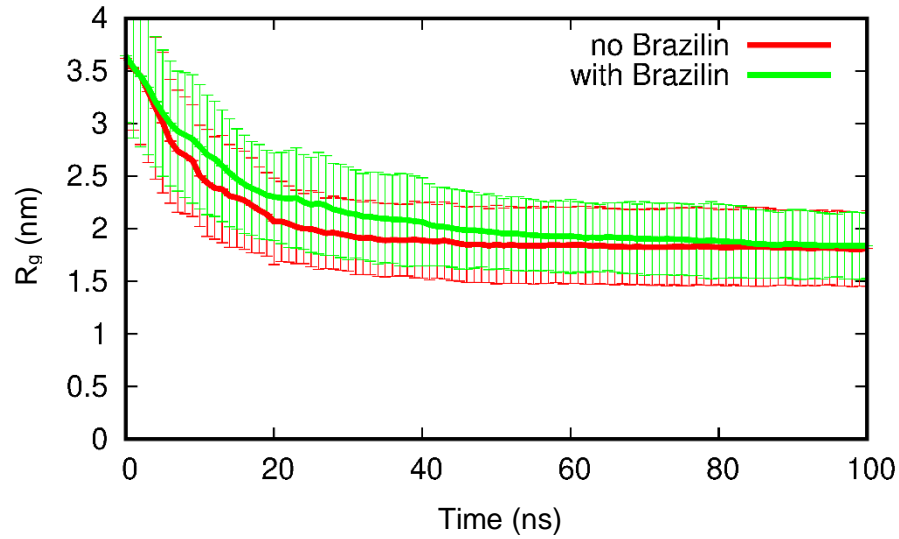
**Supplementary Figure 9:** Structures shown every 25 ns from one 100 ns monomer simulation with Brazilin. Brazilin shown as space-filling atoms,  $\alpha$ -syn monomers shown as rainbow ribbons (blue, N-term; to red, C-term).

## Supplementary Figure S10



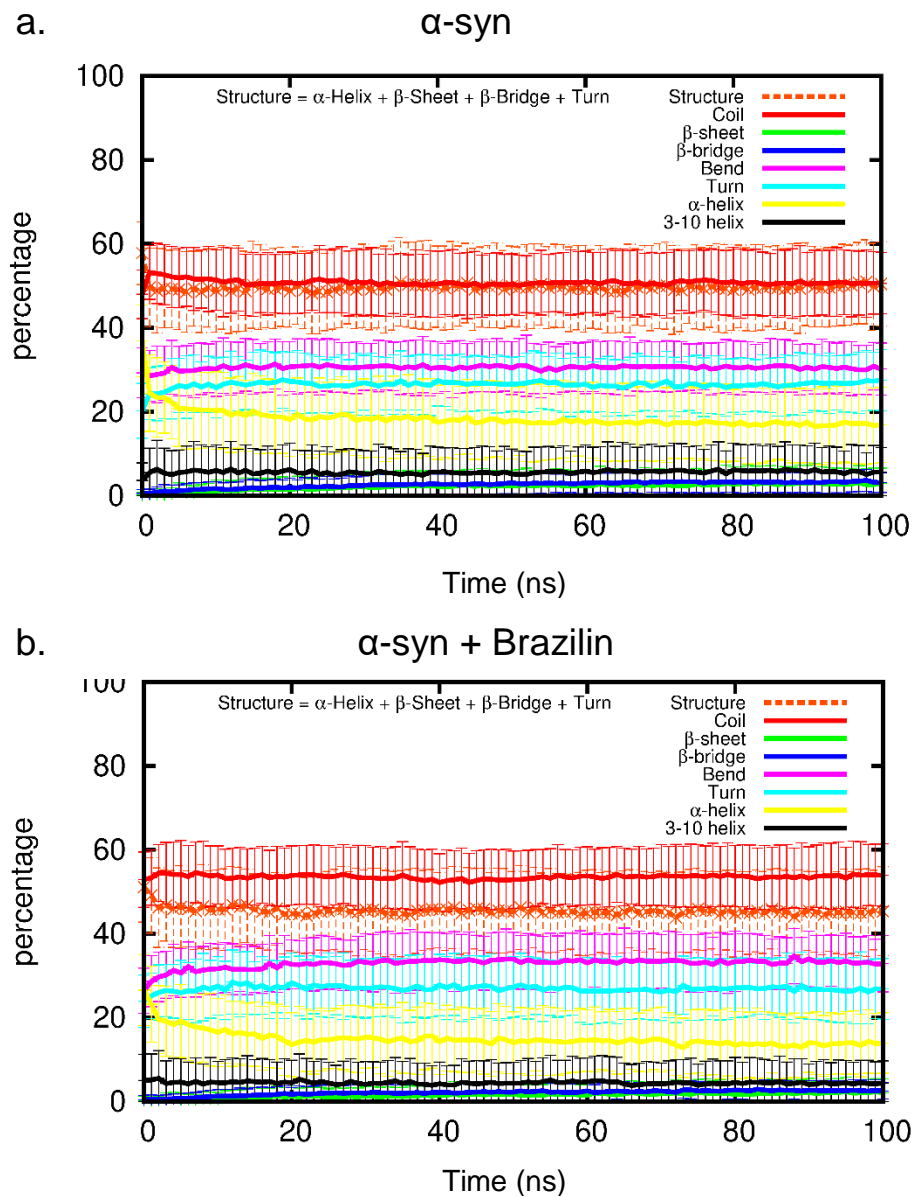
**Supplementary Figure 10:** Bar charts showing the equilibration of Brazilin binding to  $\alpha$ -syn monomers. The simulations start from a state where each  $\alpha$ -syn monomer is bound to 10 Brazilin molecules; a) combined data from 100 trajectories of 100 ns of a single  $\alpha$ -syn protein; b) data from the first 150 ns of the single 200 ns trajectory of 64  $\alpha$ -syn monomers in a single simulation box.

## Supplementary Figure S11



**Supplementary Figure 11:** Graph showing the evolution of the radius of gyration of  $\alpha$ -syn monomers over the trajectories. Combined data from 100 trajectories of 100 ns of a single  $\alpha$ -syn protein. Error bars are 1 SD from the average. Red,  $\alpha$ -syn monomers only. Green, with 10 Brazilin molecules initially bound to each  $\alpha$ -syn monomer.

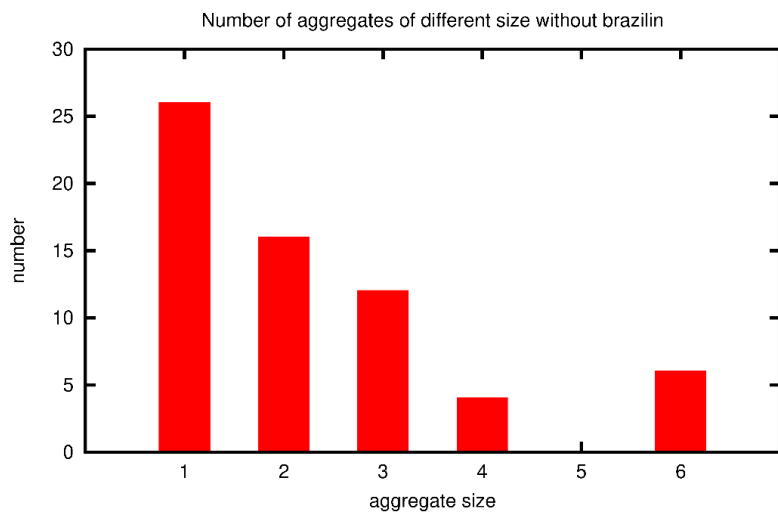
## Supplementary Figure S12



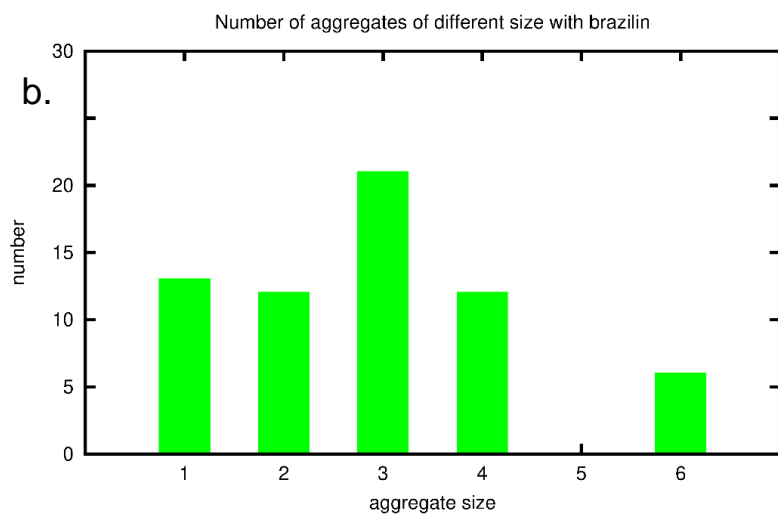
**Supplementary Figure 12:** Graphs showing the evolution of secondary structure in  $\alpha$ -syn monomers over the trajectories. Combined data from 100 trajectories of 100 ns of a single  $\alpha$ -syn protein. Error bars are 1 SD from the average; a)  $\alpha$ -syn monomers only; b) with 10 Brazilin molecules initially bound to each  $\alpha$ -syn monomer.

## Supplementary Figure S13

a.

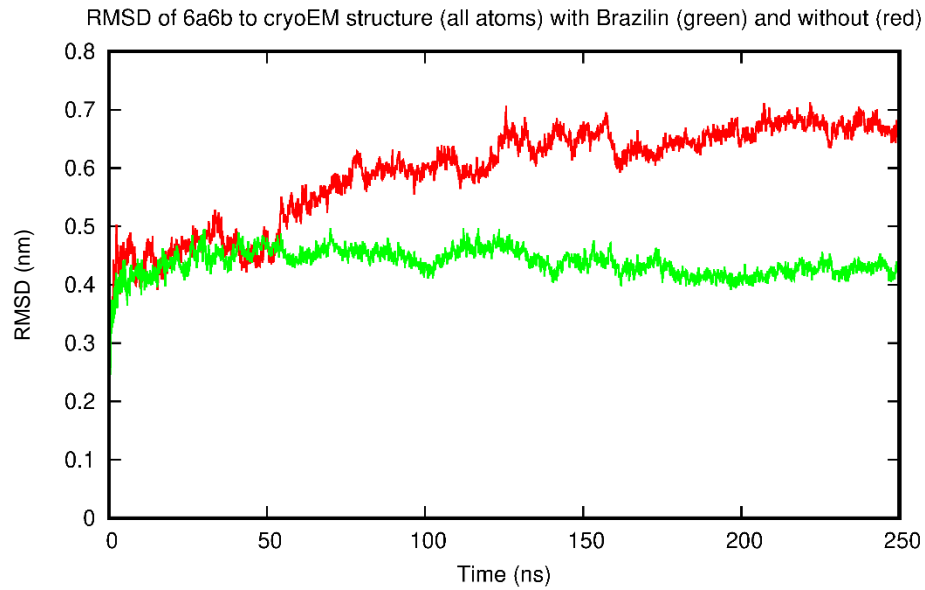


b.



**Supplementary Figure 13:** Bar charts showing the distribution of oligomeric states of  $\alpha$ -syn at the end (200 ns) of the simulations comprised of 64 monomers in a single simulation box. a)  $\alpha$ -syn monomers only, b) with 10 Brazilin molecules initially bound to each  $\alpha$ -syn monomer.

## Supplementary Figure S14

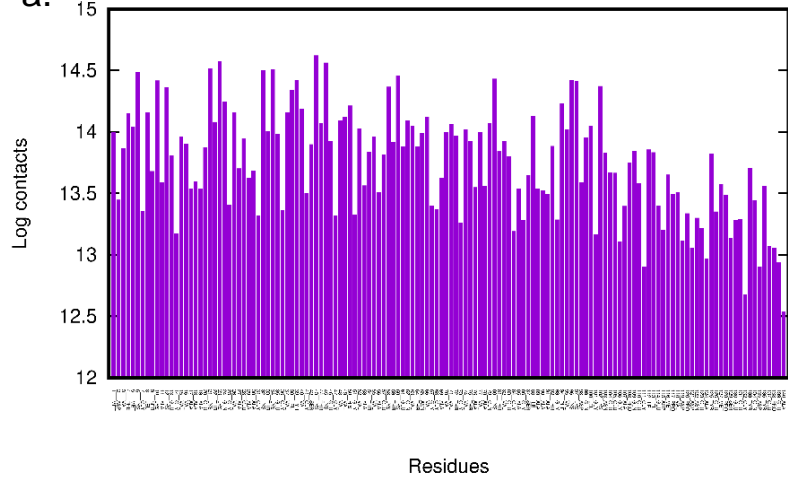


**Supplementary Figure 14:** Graph showing the root-mean squared deviation of the protein atoms with respect to the cryoEM structure (6a6b) over the simulations. Red,  $\alpha$ -syn fibril fragment only. Green, with 222 Brazilin molecules included in the simulation box.

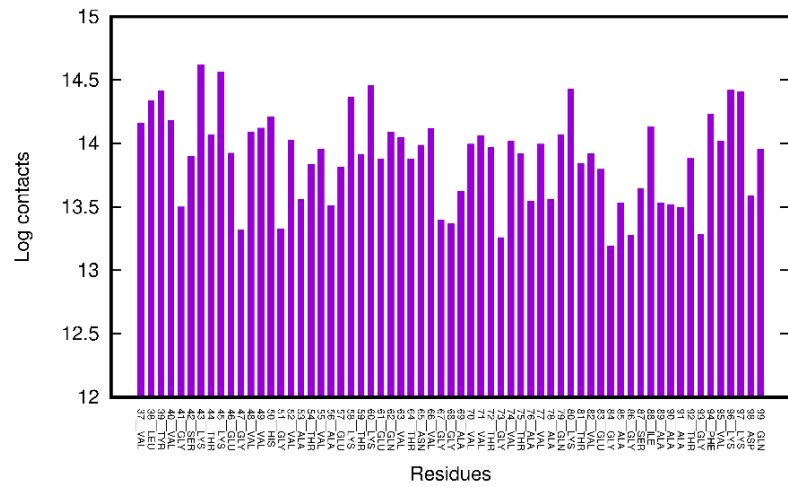


# Supplementary Figure S15

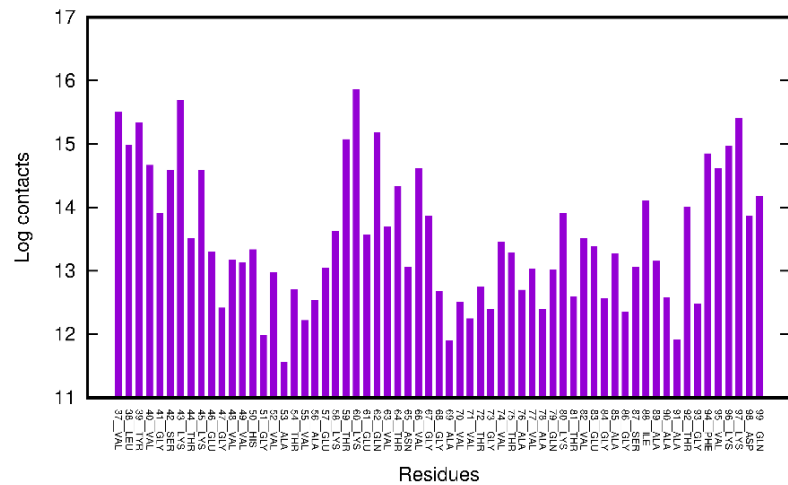
a. Brazilin/ $\alpha$ -syn monomer contacts by residue over 100 trajectories (residues 1–140)



b. Brazilin/ $\alpha$ -syn monomer contacts by residue over 100 trajectories (residues 37–99)



c. Brazilin/ $\alpha$ -syn fibril (6a6b) contacts by residue (residues 37–99)



**Supplementary Figure 15:** Bar charts depicting the number of contacts made between each  $\alpha$ -syn residue and a Brazilin molecule over the simulations. While the absolute numbers of  $\log(N_{\text{contacts}})$  has no significance, each log interval approximates  $1.4 \text{ kcal mol}^{-1}$  in binding energy. a) combined data from 100 trajectories of 100 ns of a single  $\alpha$ -syn protein for the whole sequence, residues 1-140, b) expanded interval, residues 37-99, of (a) for comparison with (c), c) data from the 250 ns simulation of the fibril fragment in the presence of 222 Brazilin molecules.

### **Supplementary Movies**

**Movie1:** Depicts rotating the 100 ns structure from Figure S9 and illustrates typical binding of Brazilin to the surface and interior of the collapsed  $\alpha$ -syn monomer.

**Movie2a:** Depicts rotating the 64-monomer simulation box  $360^\circ$  in the vertical axis. A) shows the initial disposition of the monomers and also depicts the case with 10 Brazilin molecules bound per monomer;

**Movie2b:** shows the final box with Brazilin present;

**Movie2c:** shows the final box when Brazilin is absent from the simulation.