Supplementary Information for:

Repurposing doxycycline for synucleinopathies: remodelling of α-synuclein oligomers towards non-toxic parallel beta-sheet structured species

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1. Analysis of doxycycline binding to α-synuclein by NMR

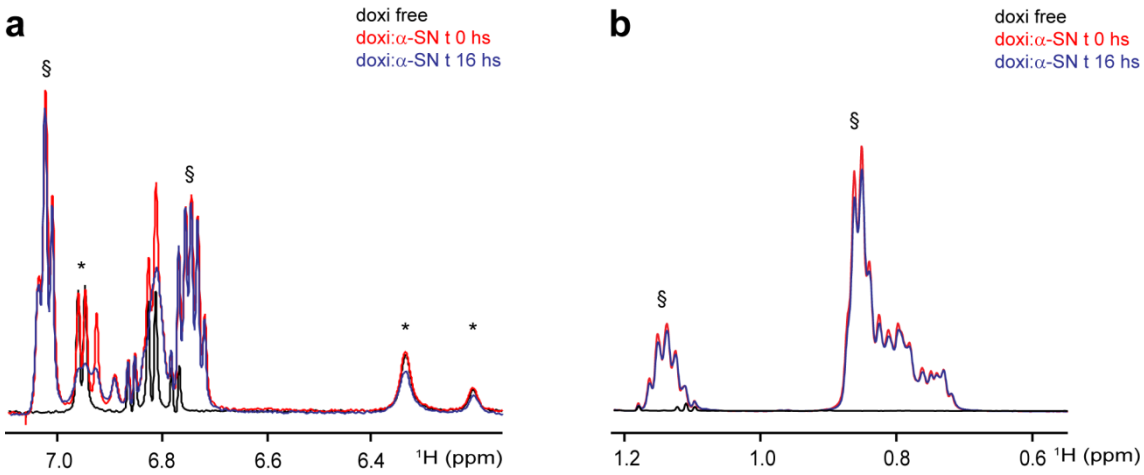

Figure S1. Analysis of doxycycline binding to α-synuclein by NMR. ^{1}H 1D NMR spectra in the aromatic **(a)** and methyl region **(b)** of 200 µM doxycycline alone (black line) and upon addition of 100 µM monomeric α-synuclein (red line) or α-synuclein that was aged for 16 hs (blue line). Before preparing the mixtures, the α-synuclein samples were spun down to remove insoluble particles that might have formed during this time frame. Asteriks denote isolated doxycycline ^{1}H NMR signals that broaden upon binding to α-synuclein oligomers or protofibrils. The symbols § indicate isolated clusters of α-synuclein ^{1}H NMR signals. We did not detect major changes in α-synuclein signals between time 0 and 16 hs indicating that slow-tumbling, highly ordered structures such as amyloid fibrils or large protofibrils were not formed at great extents. NMR spectra were acquired at 25^{o}C. Samples were dissolved in 20 mM HEPES supplemented with 150 mM NaCl and 10% D_2O.

2. Dose:response effect of doxycycline on α-synuclein aggregation

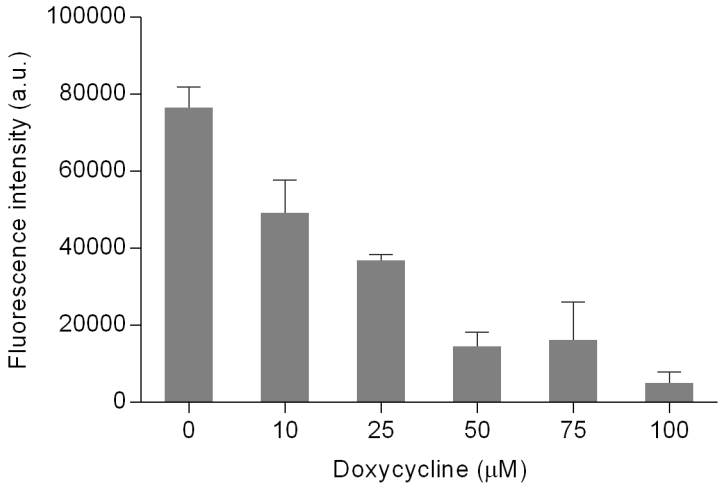

Figure S2. Dose-response effect of doxycycline on the α-synuclein fibrillation process. A solution containing 70 μM α-synuclein in 20 mM HEPES buffer, 150 mM NaCl, pH 7.4 was incubated at 37ºC under orbital agitation for 48 h in the presence of 0 μM, 10 μM, 25 μM, 50 μM, 75 μM and 100 μM doxycycline as described in Methods. On each sample Thioflavin T was added to a final concentration of 25 μM and the formation of cross-beta structure was estimated by the increase in fluorescence at λ_{exc}=450 nm, λ_{em}=482 nm.