**Thioflavin T Assay**

Adapted from Alex Crowe, Jing Guo, Dustin Covell 032012 protocol, Mian Horvath Updates

1. Resuspend fibril reaction. Fibrils will settle over time. Dilute 1.5 µL of 5 mg/mL fibrillization reaction 1:50 with PBS (total 75µL).

\* Ideally, the same dilution of monomer, PBS alone and a previous batch of PFFs should be run in parallel. Mouse PFFs have low ThT fluorescence. In this case 5 µL PFFs can be diluted 1:10.

1. Assay each fibrillization in triplicate on the 96 well black assay plate. Dispense 20 µL of diluted α-synuclein fibrils per well.
2. Dilute 1mM Thioflavin T stock into PBS 1:1000 to obtain the required volume of Thioflavine T solution at a concentration of 1 µM.
3. Dispense 180 µL of 1 µM Thioflvain T per well.
4. Maintain plate at room temperature for 1 hour in the dark.
5. Read plate on a Spectrophotometer excitation 450 nm, emission 510 nm, cutoff 475 nm.

Consumables:

1 mM Thioflavin T in MilliQ

PBS

Equipment needed: Spectrophotometer

Black 96 well plate