SynAggreg : a high-throughput screening assay to discover aggregation modulators of amyloid disease proteins

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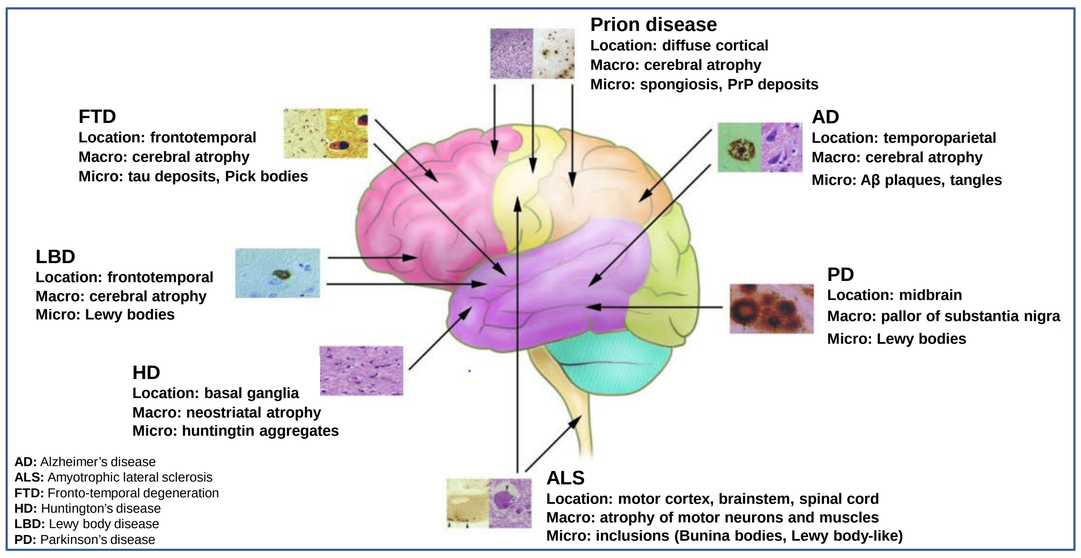
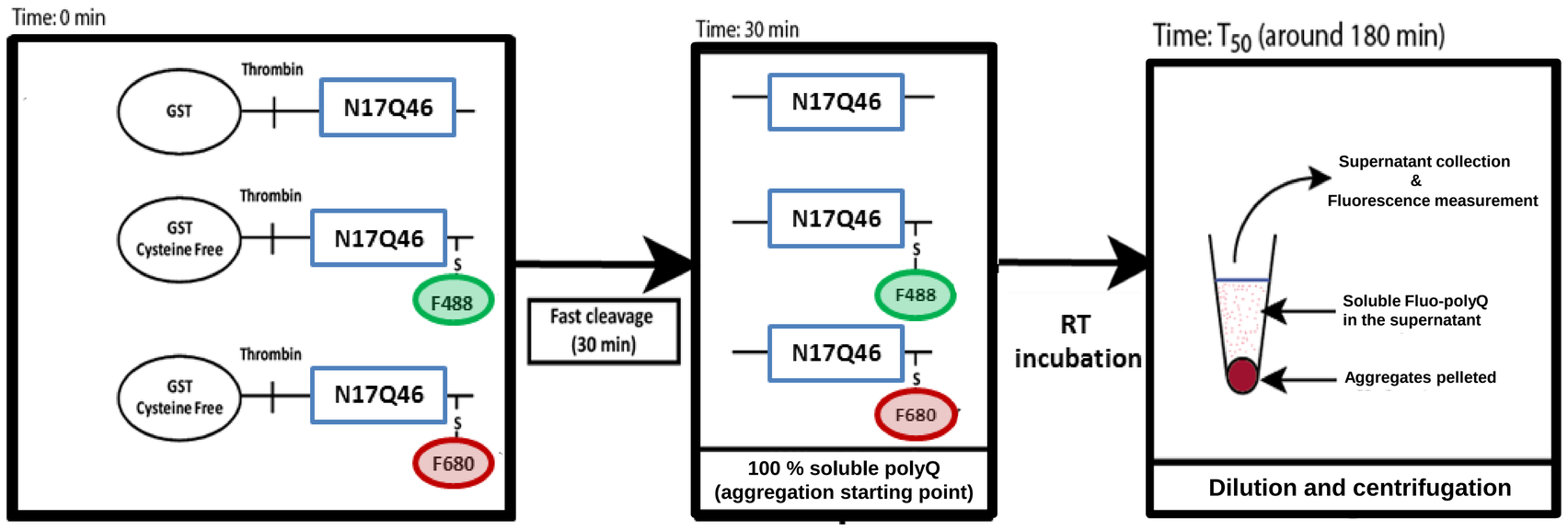
- **Protein aggregation is the hallmark of numerous amyloid diseases (amyloidoses)**
- **Modulating the amyloid aggregation = therapeutic strategy**
- ***SynAggreg*** **(1) : High throughput assay to identify aggregation modulators**
- **N17Q46: a fragment of the main amyloid that accumulates in Huntington's disease (HD)**
- **Our Goals :**
  - **Show that *SynAggreg* is adapted to screen N17Q46**
  - **Scale-up our assay by performing a larger screen**
  - **Identify novel aggregation modulators of N17Q46**

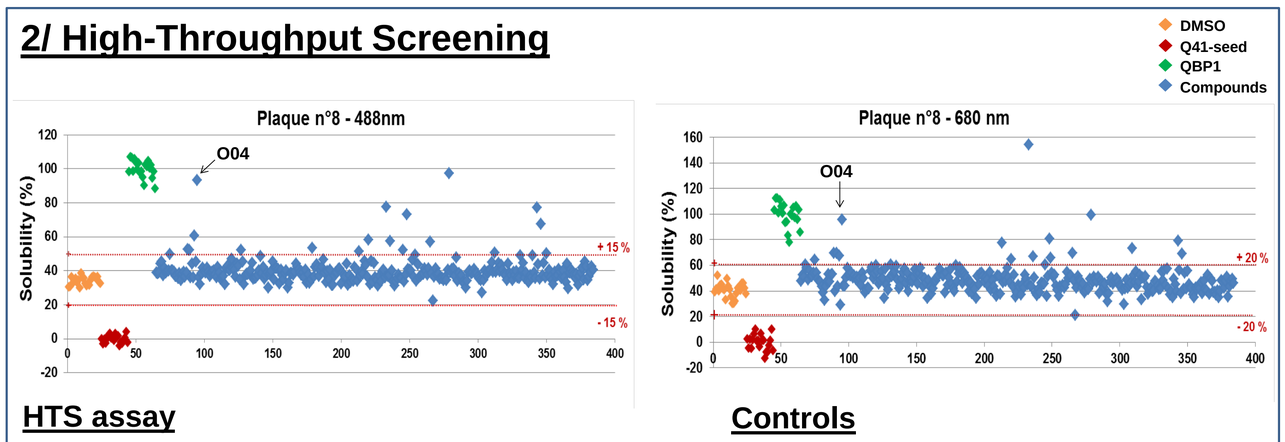


1/ *SynAggreg* : Principle



SynAggreg uses a mixture of unlabelled N17Q46 that drives the aggregation, and traces of N17Q46 coupled with fluorophores for quantification (AlexaFluo 488 & AlexaFluo 680). After thrombin proteolysis of the carrier protein (GST), the chemical compounds are added and the screening plates incubated 3 hours to let N17Q46 partially aggregate. The aggregation is then stopped by dilution and by collecting the soluble phase after centrifugation. Fluorescence measurements allow quantifying the amounts of soluble N17Q46 after incubation. Aggregation modulators affect N17Q46 solubility, and by consequence the fluorescence intensity. The double-fluorescence readout (AF488 & AF680) allows identifying false positive and false negative compounds.

2/ High-Throughput Screening



HTS assay

- Miniaturization to 10 µL in 384-well plate
- Throughput: up to 2,560 molecules/day
- Identification of both aggregation inhibitors and accelerators in a same assay and at the same time
- Hits selection: threshold corresponding to a variation of +/- 15 % of solubility at 488 nm, and +/- 20 % of solubility at 680 nm
- Double fluorescent aggregation assay : identify false positives and false negatives

Controls

- DMSO: no effect, 65 % of N17Q46 is aggregated (in orange)
- QBP1: aggregation inhibitor, N17Q46 remains fully soluble (in green)
- Q41-seed: aggregation accelerator, N17Q46 is fully aggregated (in red)
- Low variability: CV < 5 %
- 0.21 < Z' < 0.74

3/ Results of the screening

Primary screening — 7360 molecules (including the Prestwick Chemical Library®) were screened at 160 µM on N17Q46 (8 µM)

428 primary hits

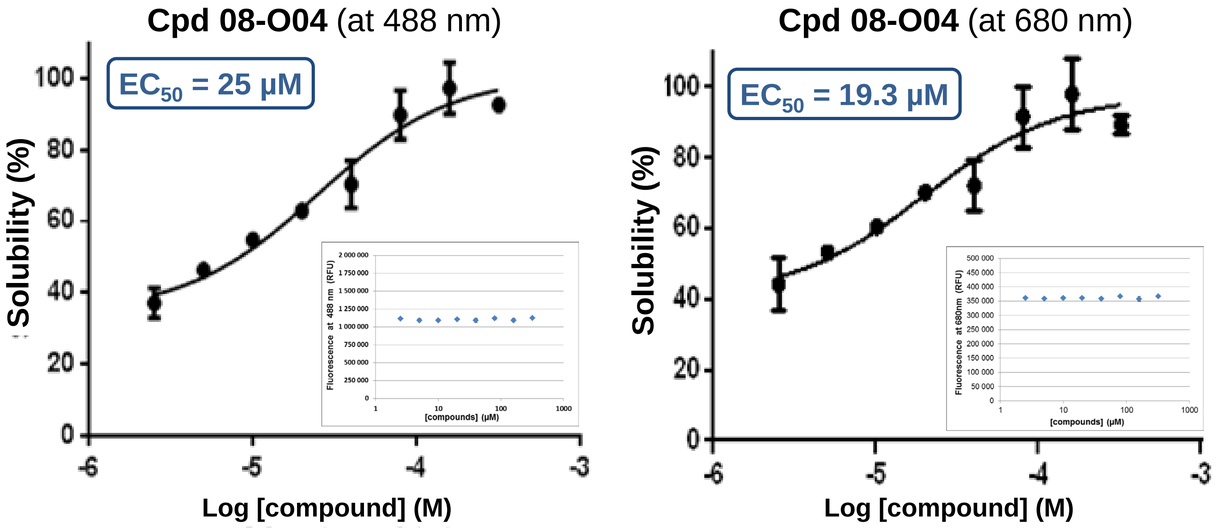
Confirmation assay — 400 primary hits were tested in duplicate at two concentrations (80 µM and 160 µM) in the confirmation assay

135 Hits confirmed at 160 µM

Validation assay — 35 molecules selected for dose-response experiment in triplicate (selection based on compound efficacy, purity and availability of the compound)

28 hits validated

4/ Aggregation modulators



The compound Cpd 08-O04 inhibits the aggregation of N17Q46 in a dose-dependent manner. Similar results were obtained with both AF488 and AF680 readouts.
Additional controls (insets) show that Cpd 08-O04 does not affect the fluorophores properties (no autofluorescence or quenching effect).
The EC_{50} were determined by modelization of the curves with GraphPad Prism® software.

5/ Conclusions

- ***SynAggreg*** **is adaptable to numerous amyloids**
- **Robustness of the results for the study of amyloid aggregation and for the discovery of aggregation modulator compounds**
- **Scale-up of the assay to perform a larger screen (> 7000 molecules)**
- **28 compounds were validated to be an aggregation modulators of N17Q46**
- **Most efficient hits mainly belong to two chemical families**

6/ Perspectives

- **Structure-activity relationship (SAR) studies**
- **Combinatorial screening ⇔ looking for synergistic effects (1)**
- **Study the effect of our most promising hits or combinations of hits in *in vivo* models of HD**

(1) Aviolat H, Nominé Y, Gioria S, Bonhoure A, Hoffmann D, Rulmann C, Nierengarten H, Ruffenach F, Villa P, Trottier, Y* and Klein F.A.C.* " SynAggreg: A Multifunctional High-Throughput Technology for Precision Study of Amyloid Aggregation and Systematic Discovery of Synergistic Inhibitor Compounds". J Mol Biol. 2018 Dec 7;430(24):5257-5279, PMID 30266595.