How to Catalyze the Development of Drugs Targeting Protein Aggregation for Neurodegenerative Disorders

Ncardia

Capture the real therapeutic potential of your candidates with the **Proteinopathy Exploration Package** that provides a holistic view of key neurodegenerative diseases quickly and efficiently

Unveiling therapies for tomorrow's minds

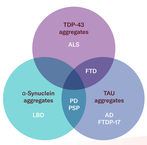
The mislocalization and accumulation of protein aggregates is a shared hallmark for multiple neurodegenerative disorders (NDDs). Production of abnormal proteins is an important driver of Alzheimer's, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) as well as other NDDs categorized as proteinopathies.

Many therapies aiming to prevent or eliminate protein aggregation looked quite promising but failed in the clinic. In those failures lie the possible blueprint to unlocking a deeper understanding of those devastating diseases.

A key learning from past failures - it is essential to target early stages of protein aggregation.

ALS Amyotrophical lateral sclerosis
FTD Frontotemporal dementia
PSP Progressive supranuclear palsy
LBD Lewy body dementia
PD Parkinson's disease
AD Alzheimer's disease
FTDP-17 Frontotemporal dementia and parkinsonism linked to chromosome 17

Shared underlying mechanisms in neurodegenerative disorders

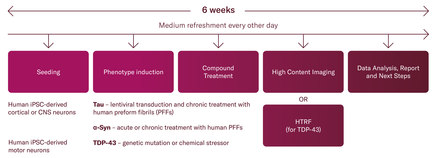


We have developed three robust assays that allow you to examine both early and late stages of aggregation and the impact on additional disease-linked phenotypes.

Purpose-built to provide a holistic view on the efficacy of your drug candidates to combat Tau, α-Syn and TDP-43 aggregation

The Proteinopathy Exploration Package is a suite of drug discovery assays developed by Ncardia using human induced pluripotent stem cell (iPSC) technology. This suite of assays is designed to simultaneously study the impact of drug candidates in the aggregation of Tau, α -Synuclein and TDP-43, catalyzing the identification of therapeutics with a real potential to treat proteinopathies.

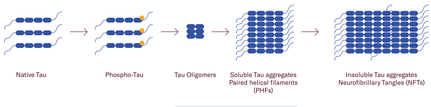
- **6 weeks turnaround time**
- **Fully automated**
- **High robustness: Z factor > 0.5 and %CV < 10%**
- **Available in 96 or 384 well plate formats, HTS compatible**
- **Multiplexing possibilities**
- **Automated data analysis with Ncardia's in-house developed algorithm**

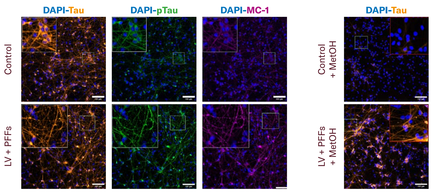


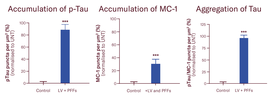
Tau aggregation assay

Tau protein is abundantly expressed in axons of central nervous system neurons with a primary role of promoting assembly and stabilization of microtubules. Mutation and/or abnormal post translational modification of TAU lead to its dissociation from the microtubules and its aggregation. Tau aggregates occur in multiple neurodegenerative disorders referred as tauopathies.

Tau overexpression and chronic treatment with Tau PFFs in Ncardia's human iPSC-derived cortical neurons induces a notable increase in MC-1 and phospho-Tau signals, the initial stages of protein aggregation. As expected, treatment with methanol, removes the phospho-Tau and MC-1 signals in control neurons. However, despite adding methanol in the Tau aggregation model, Tau aggregates are still present, indicating that this model also recapitulates the formation of insoluble Tau aggregates.





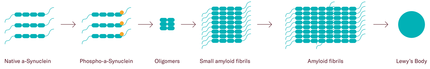


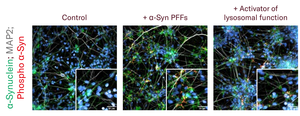
Quantification of the normalized parameters of untransduced cells (control) compared to cells transduced and treated with PFFs, expressed as % to condition TR+ PFFs set to 100%.

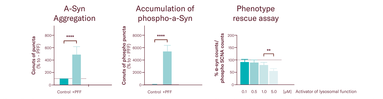
α-Synuclein aggregation assay

Patients with Parkinson's Disease (PD) show elevated levels of α-Synuclein accumulation and aggregation in the form of Lewy Bodies and neurites. Soluble oligomeric α-Synuclein conformations may contribute to PD pathology by disrupting synaptic function and consequently causing neuronal cell death.

Acute treatment of Ncardia's human iPSC-derived cortical neurons with PFFs induces a significant increase in the formation of puncta phospho- α-Syn when compared to untreated neurons. Chronic treatment can further increase the phenotype. To show the potential of the assay, diseased neurons were treated with inhibitors of the protein degradation pathway and activators of lysosomal activity. Both treatments showed the expected phenotype rescue effect.







Normalized parameters (puncta counts, area, counts of phosphorylated puncta and their intensity, respectively) of cells without PFFs (control) compared to cells treated with a-syn PFFs

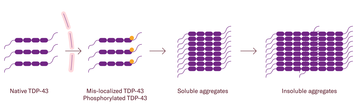
Normalized SCNA/phospho SCNA ratio of a-syn aggregation model treated with a lysosomal activator, expressed as % to cells with PFFs set to 100%.

TDP-43 aggregation assay

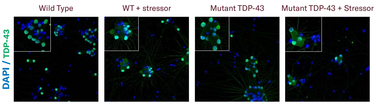
Majority of ALS cases and approximately 50% of FTD cases (both familial and sporadic) present pathological aggregates of TDP-43. Mutations of TDP-43 has been reported to have multitude of effects on important neuronal functions. One direct consequence of TDP-43 aggregation is the mis-splicing of STMM2, which leads to axonal degeneration.

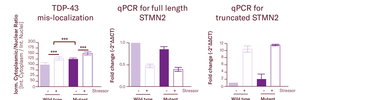
We have developed a protocol for the maturation of human iPSC-derived motor neurons with both wild type and TDP-43 CRISPR engineered lines*. After maturation, mutant TDP-43 motor neurons show mis-localization and aggregation of TDP-43. Addition of a chemical stressor can further enhance the phenotype and induce TDP-43 aggregation. Based on this model, we optimized a HTRF assay to quantify the aggregation of TDP-43 as part of the Proteinopathy Exploration Package. Additionally, TDP-43 mis-localization and STMM2 mis-splicing assays are available at Ncardia to provide a complete understanding of drugs' effect on ALS and related diseases.

* iCell(R) Motor Neurons, 01279 from FUJIFILM Cellular Dynamics, Inc.



Quantification of the normalized parameter (TDP-43 aggregation) by HTRF of wild type and mutated TDP-43 motor neurons, compared to wild type neurons and expressed as % to condition set to 100%.





Quantification of the normalized parameter (ratio of cytoplasmic/nuclear TDP43) of wild type and mutated TDP-43 (M337V mutation) motor neurons, compared to wild type neurons (in purple) and expressed as % to condition set to 100%.

Quantification of RNA levels of STMN2 and truncated STMN2 by qPCR. Data was normalized to wild type neurons. Upon treatment with the stressor, STMN2 levels significantly decreased while there was a significant increase in truncated STMN2 levels.

For all figures in this poster: Plotted values are averages ±SD. Statistical significance calculated with unpaired t-test * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0000.1

Summary

We have developed a suite of robust drug discovery assays using the latest nderstanding of disease mechanisms to facilitate the identification of drug candidates with a real potential to treat one or more proteinopathies.

This assay suite evaluates clinically relevant readouts on human in vitro models, providing information about early and late stages of protein aggregation. Additional disease-linked readouts are also available at Ncardia and can be combined in a bespoke project.

By screening your drug candidates in the three models simultaneously you can have a holistic view on their impact for several neurodegenerative disorders linked to Tau, α-Syn and TDP-43 aggregation.

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