IMPERIAL

LEVERHULME TRUST

Tracking Fluidity Changes During Neurodegenerative Disease: Protein Phase Separation or Aggregation?

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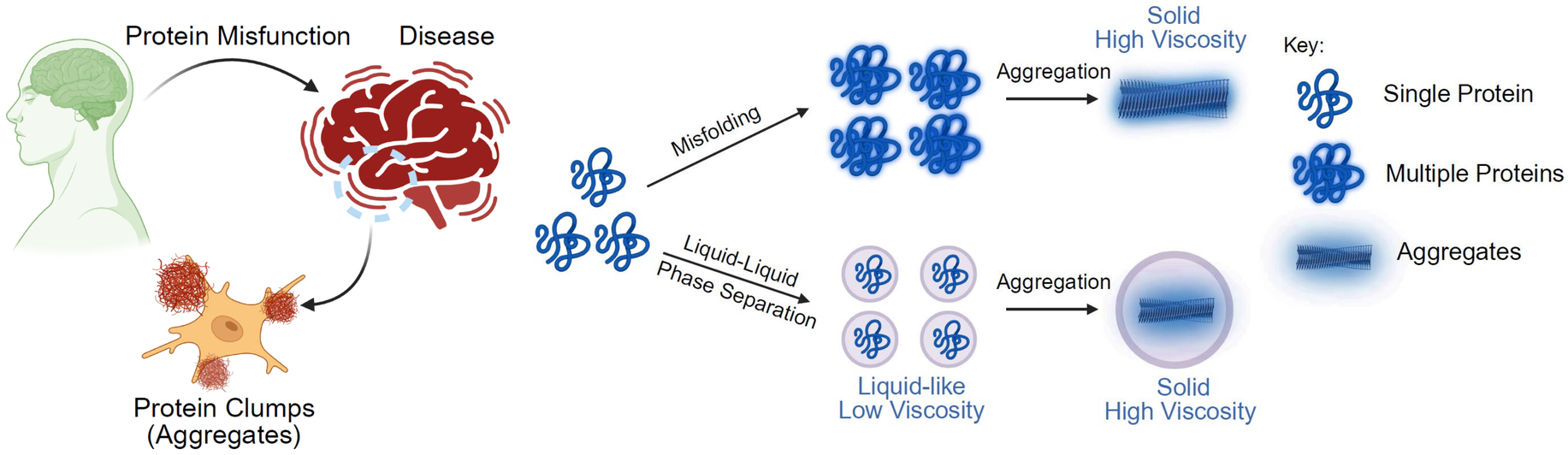
Motivation

I



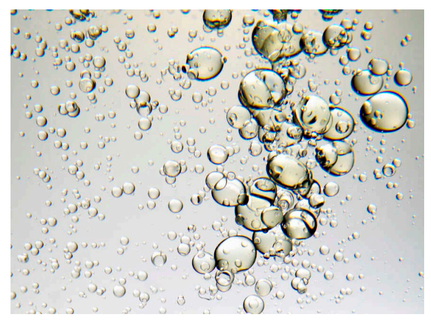
Neurodegenerative diseases (e.g. Parkinson's, PD) involve loss of protein function.

This leads to formation of protein clumps (aggregates) and disease.

There is no cure for PD, and new tools are needed for drug discovery and diagnosis.

Aggregates form through: i) misfolding or ii) liquid-liquid phase separation (LLPS).

Liquid-liquid Phase Separation



Example: Oil droplets in water

Leads to two phases (yellow, clear)

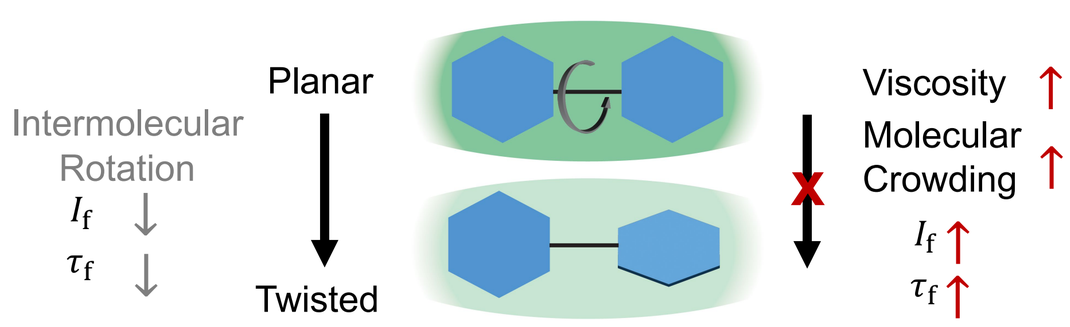
Phases have different fluidity

Currently no suitable probes image droplets formed by LLPS and distinguish them from aggregates.

Solution: Use viscosity sensitive probes to detect LLPS

Molecular Rotors and Fluorescence Lifetime Imaging Microscopy

II



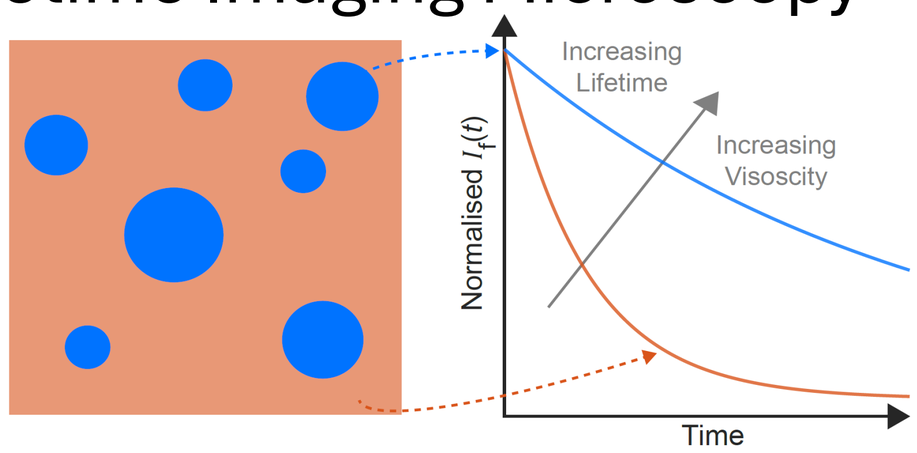
Molecular rotors (MRs) are fluorescent environmentally sensitive probes.

As viscosity increases, rotation about a central bond is hindered.

During aggregation MRs also become increasingly crowded by protein molecules.

Both processes lead to an increase in fluorescence
i) Intensity (I_f) - the amount of light produced when the MR is excited.
ii) Lifetime (τ_f) - how quickly the light decays after excitation.

MRs should show sensitivity to LLPS/aggregation



FLIM differentiates between objects based on lifetime.

Can obtain high contrast images of different structures during protein aggregation.

New MRs were synthesised for high LLPS sensitivity.

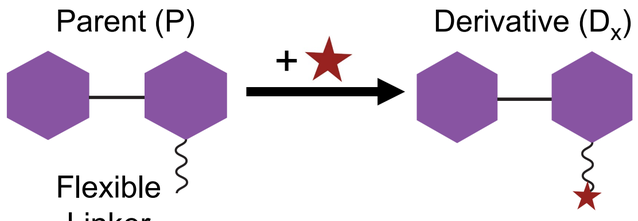
Lifetime values could provide quantification on the effect of drug molecules on LLPS and aggregation.

Ability to pick out droplets and aggregates in the same sample

Improving MR Sensitivity

Patent filed 10/11/25

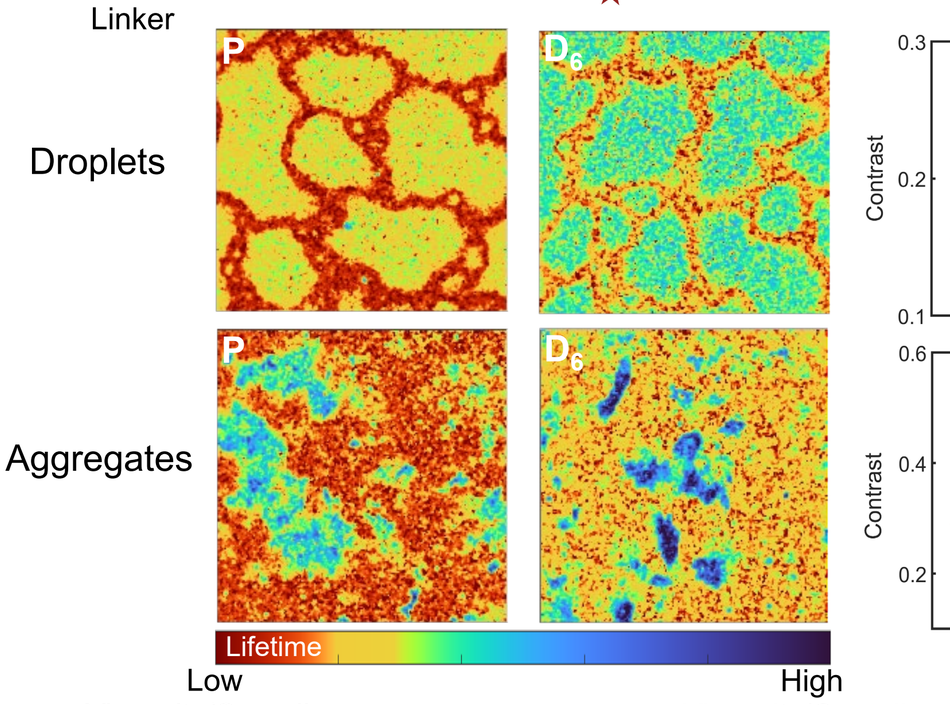
III



MR derivative library screened.

Informs on key design rules for LLPS probes.

x = {1-6}



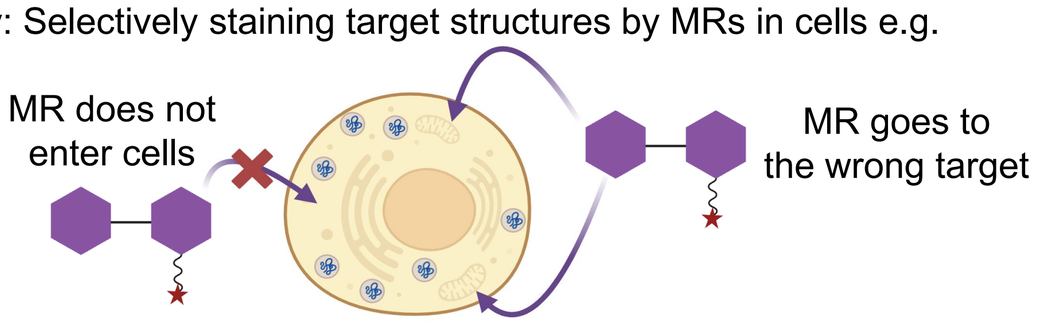
Derivatives show improved MR sensitivity to droplets/aggregates

Towards in-cell Imaging

IV

More information on disease mechanism is obtained from in-cell imaging.

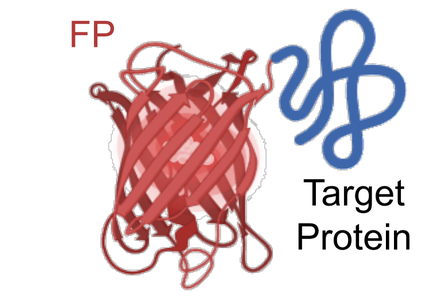
Difficulty: Selectively staining target structures by MRs in cells e.g.

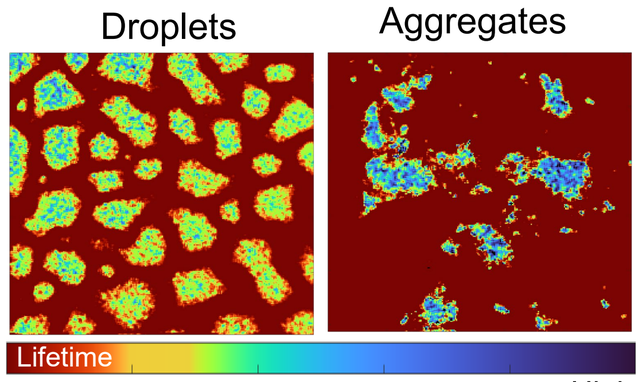


Alternative: Use fluorescent proteins (FPs) fused to target protein

✓ Cell expresses probe ✓ FP localises with protein of interest

in vitro proof of concept:





FPs could be used to monitor protein aggregation in cells