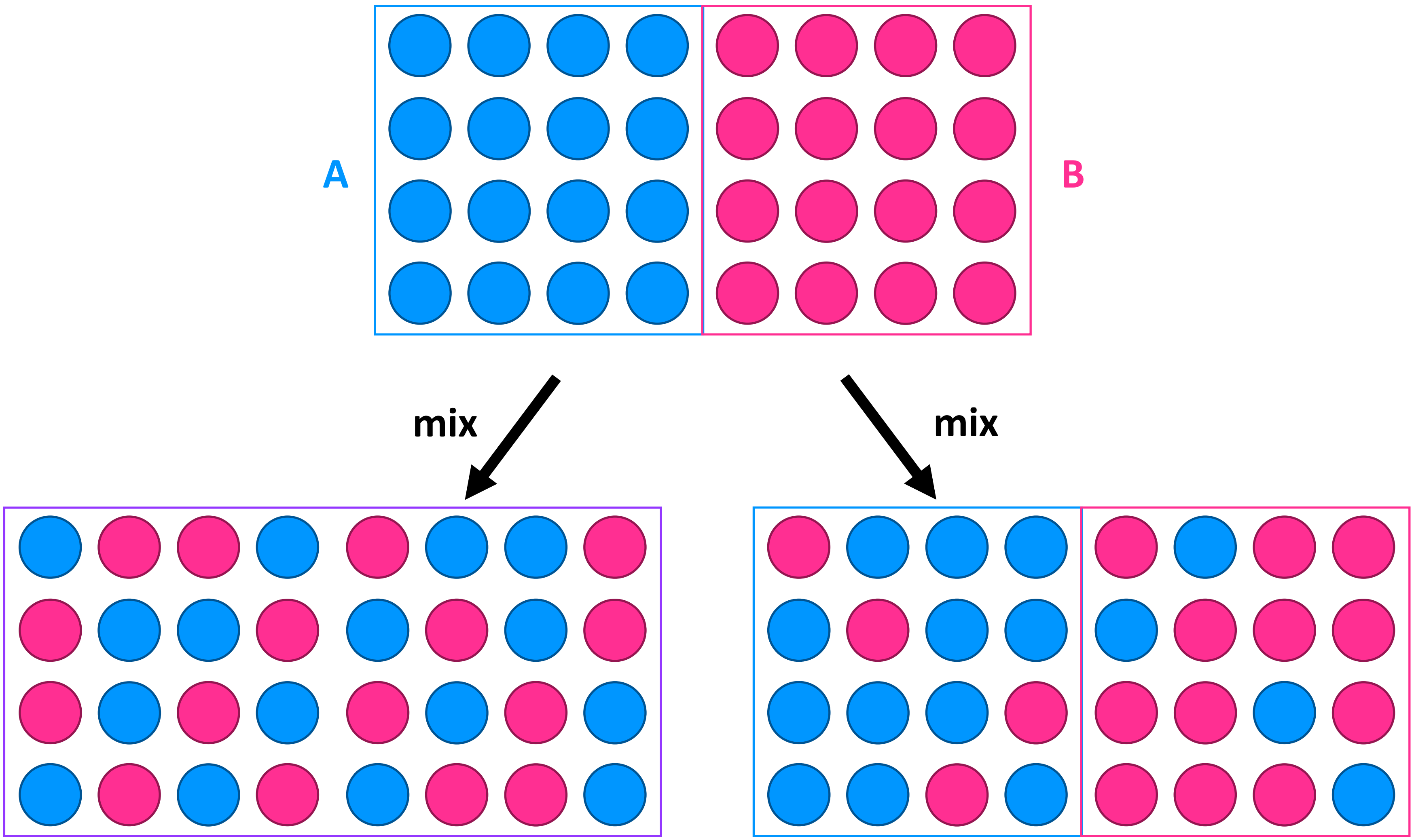
Liquid-Liquid Phase Separation of α-Synuclein

Literature Seminar

2021/07/08

M2 Atsushi Iwai

Liquid - Liquid Phase Separation



Homogeneous solution

Phase separation

LLPS in Eukaryotic Cells

Liquid-liquid phase separation

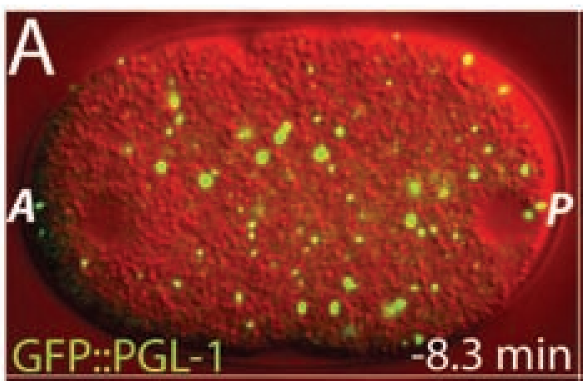
×

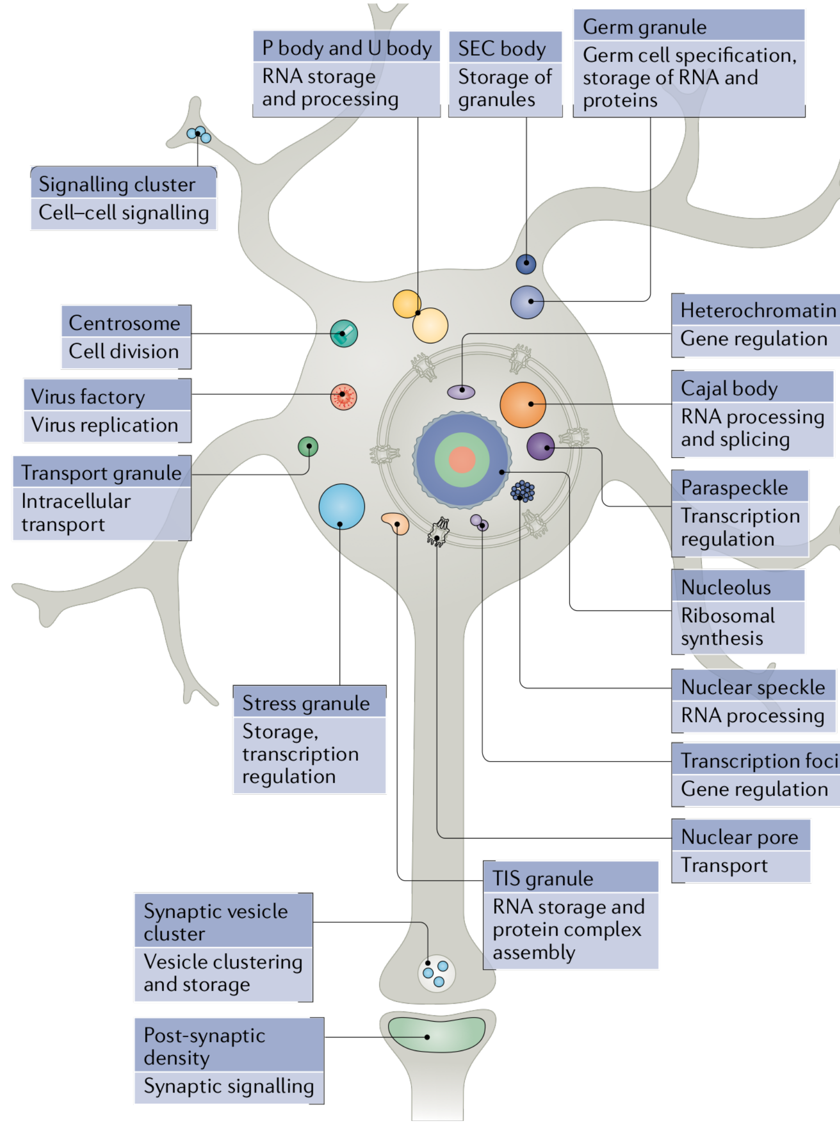
Membraneless organelles

↓

Germline P granules are liquid droplets.

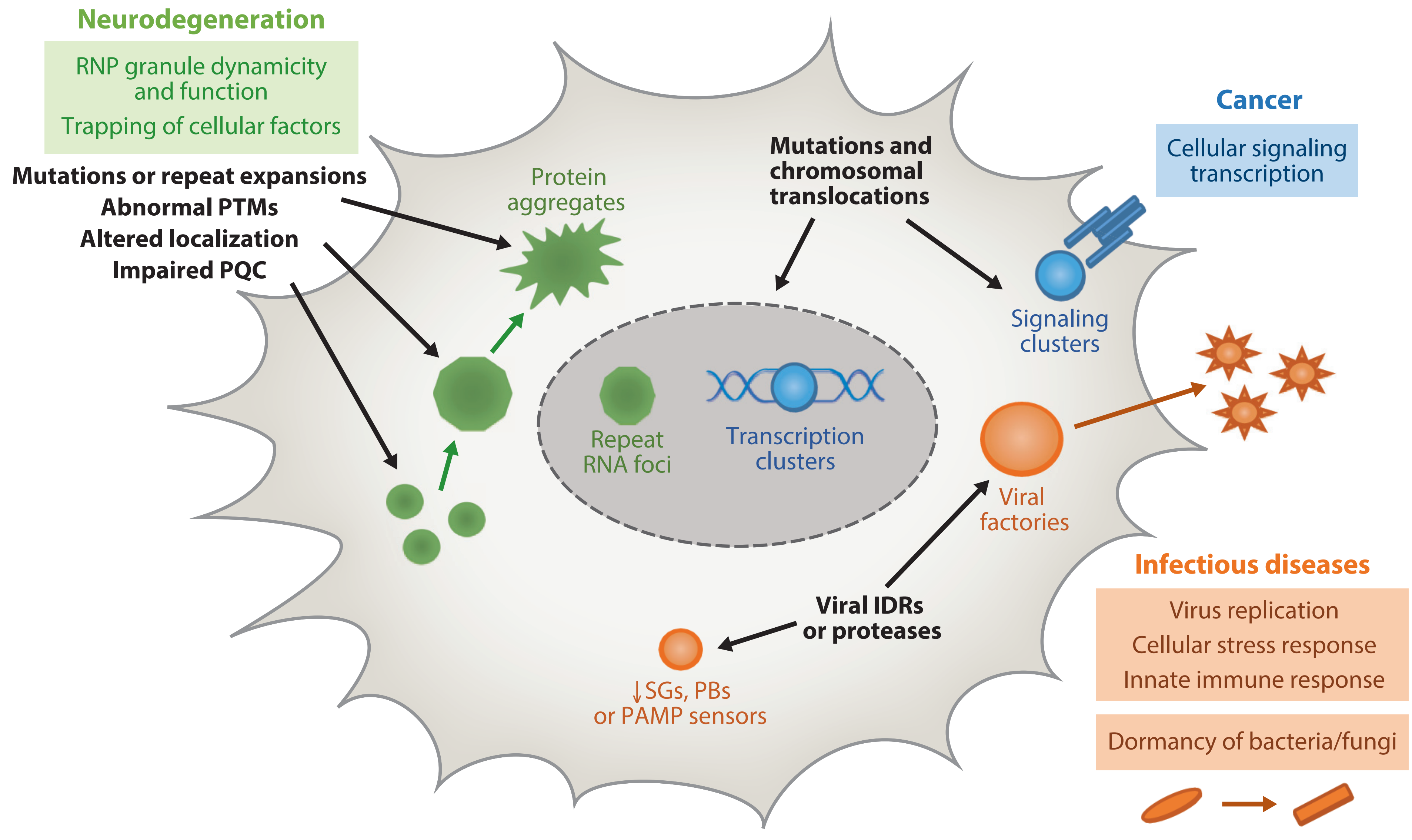
(A. A. Hyman et al. *Science* **2009**, *324*, 1729.)





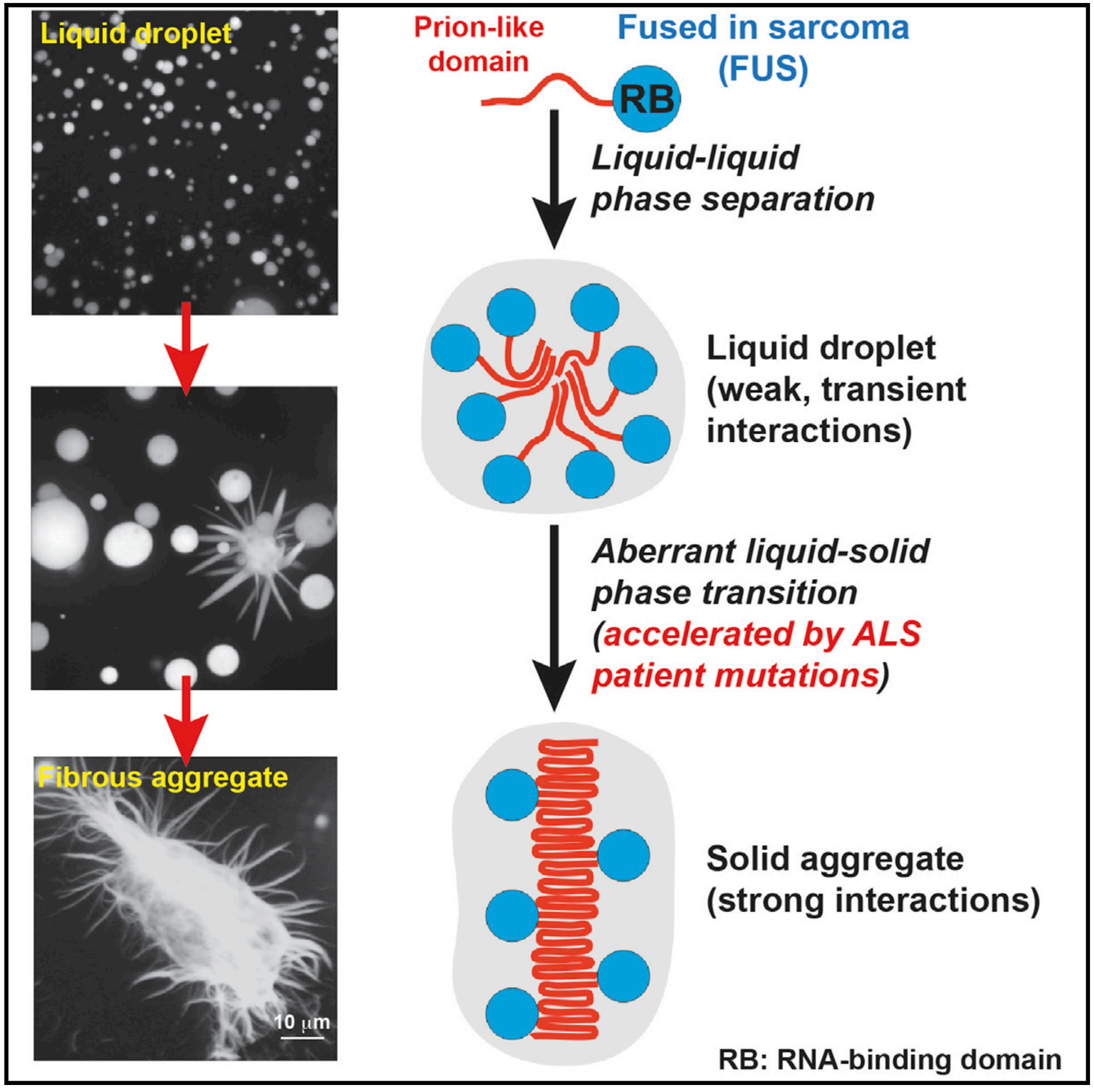
S. Alberti & A. A. Hyman *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 196.

LLPS & Disease



S. Alberti & D. Dormann *Annu. Rev. Genet.* **2019**, *53*, 171.

Droplet-forming Amyloid : FUS



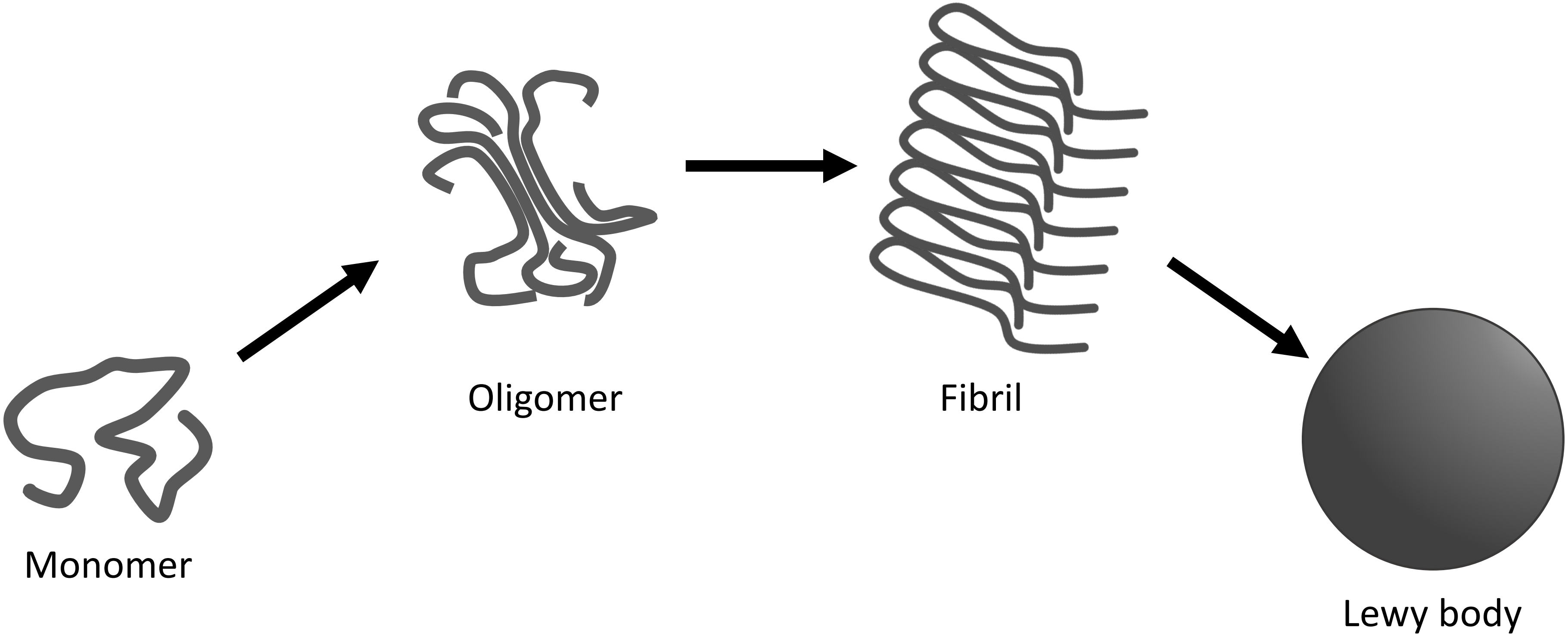
FUS: ALS-associated protein

S. Alberti et al. *Cell*, **2015**, *162*, 1066

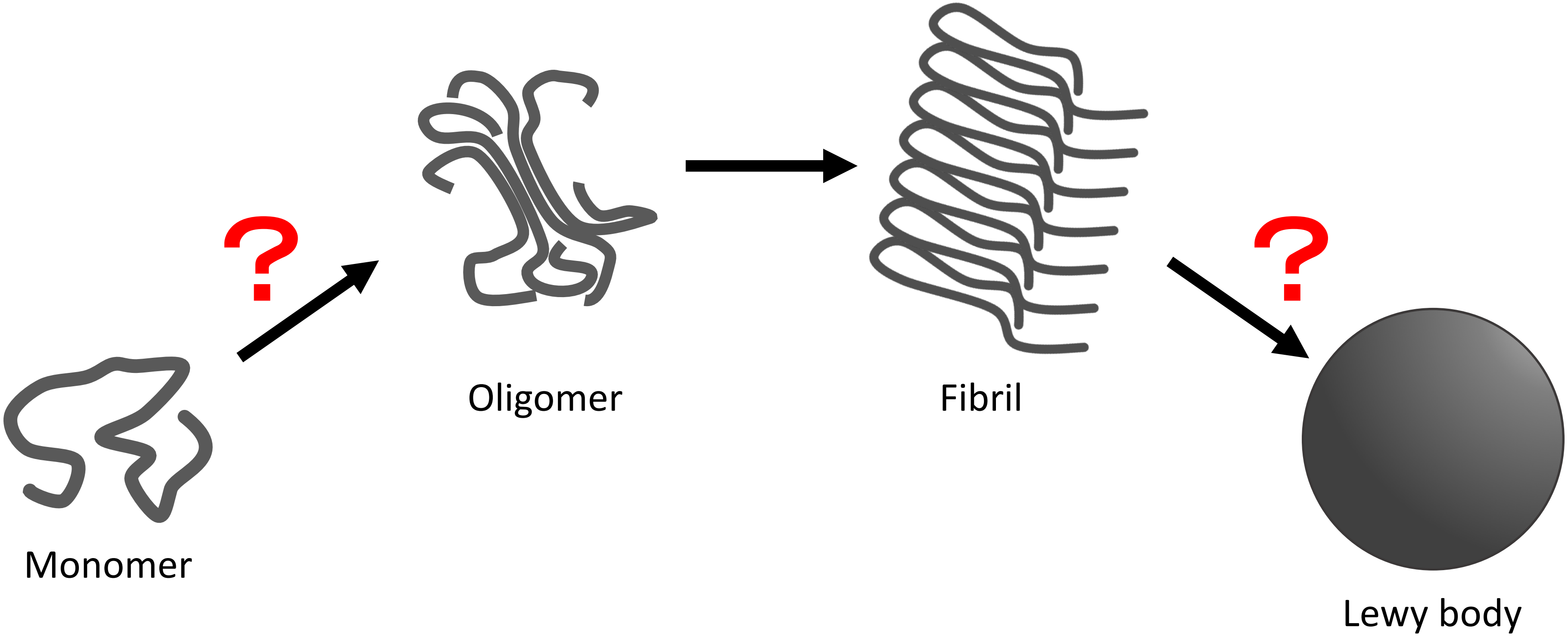
Majima–san's literature seminar about ALS-associated protein

https://gousei.f.u-tokyo.ac.jp/seminar/pdf/Lit_Sohei_Majima_M2.pdf

α-Synuclein Aggregation Mechanism

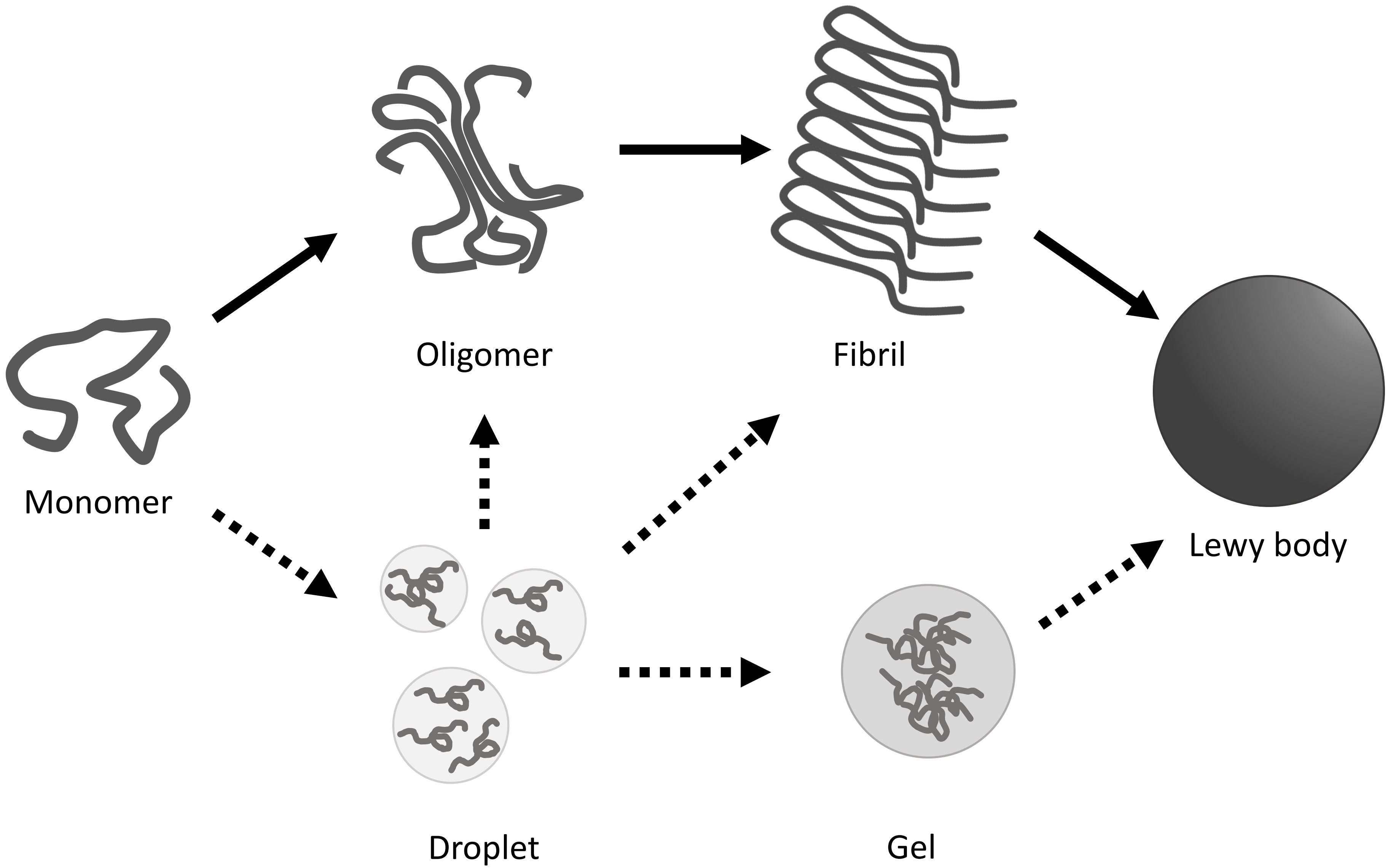


α-Synuclein Aggregation Mechanism



How does α-synuclein aggregation start?
Ordered amyloid fibrils ↔ Highly complex and heterogeneous Lewy bodies

α-Synuclein Aggregation through Droplet Formation



Amino Acid Sequence

MDVFM KGLSK AKEGV VAAAE KTKQG VAEAA GKTKE GVLYV GSKTK EGVVH GVATV AEKTK EQVTN VGGAV VIGVT AVAQK TVEGA GSIAA ATGFV KKDQL GKNEE GAPQE GILED MPVDP DNEAY EMPSE EGYQD YEPEA

N-terminal region

Amphiphilic
Common sequence KTKEGV
Adopt an alpha-helix structure

non-amyloid component (NAC)

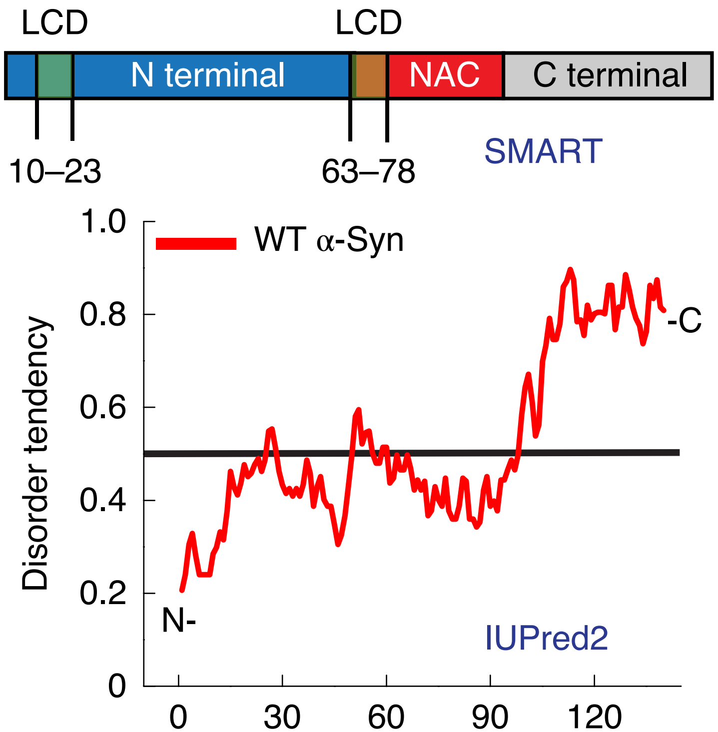
Hydrophobic
Aggregation center

C-terminal region

Highly acidic
Proline-rich region
Flexible

Amino Acid Sequence

MDVFM KGLSK AKEGV VAAAE KTKQG VAEAA GKTKE GVLYV GSKTK EGVVH GVATV AEKTK EQVTN VGGAV VIGVT AVAQK TVEGA GSIAA ATGFV KKDQL GKNEE GAPQE GILED MPVDP DNEAY EMPSE EGYQD YEPEA



IDR: intrinsically disordered regions

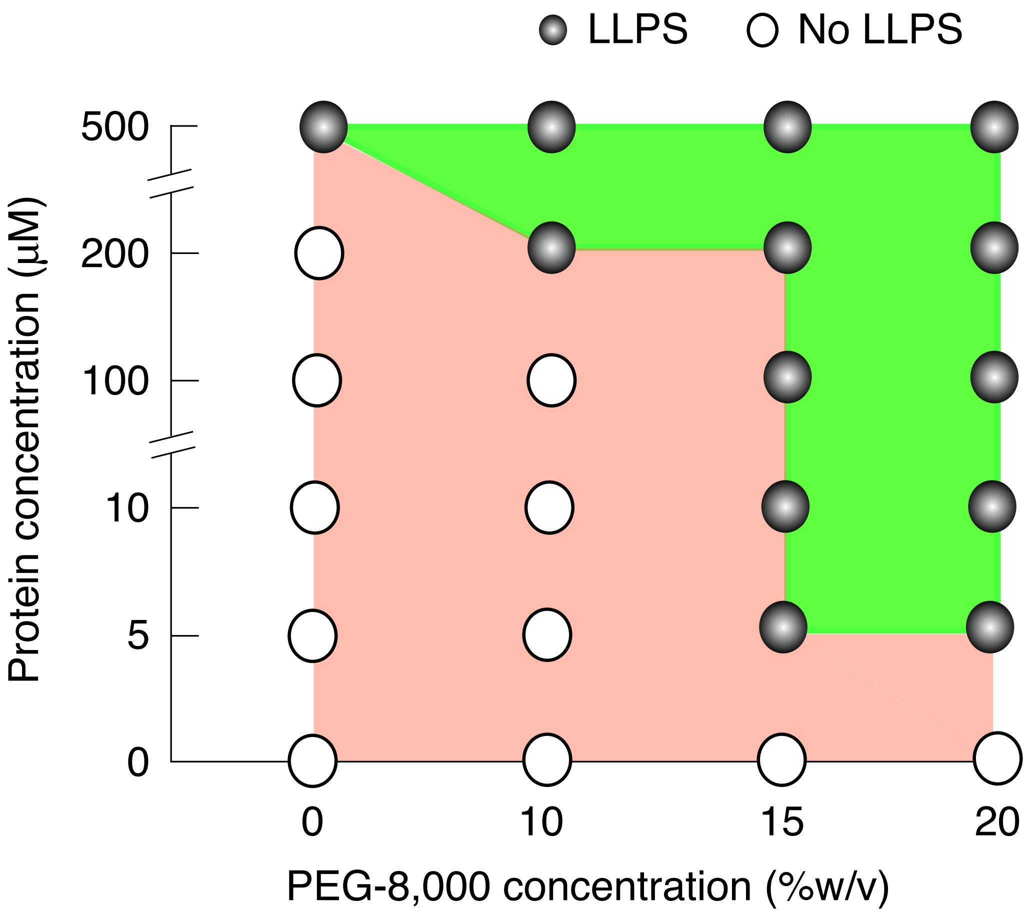
A region that cannot have a stable secondary structure by itself.

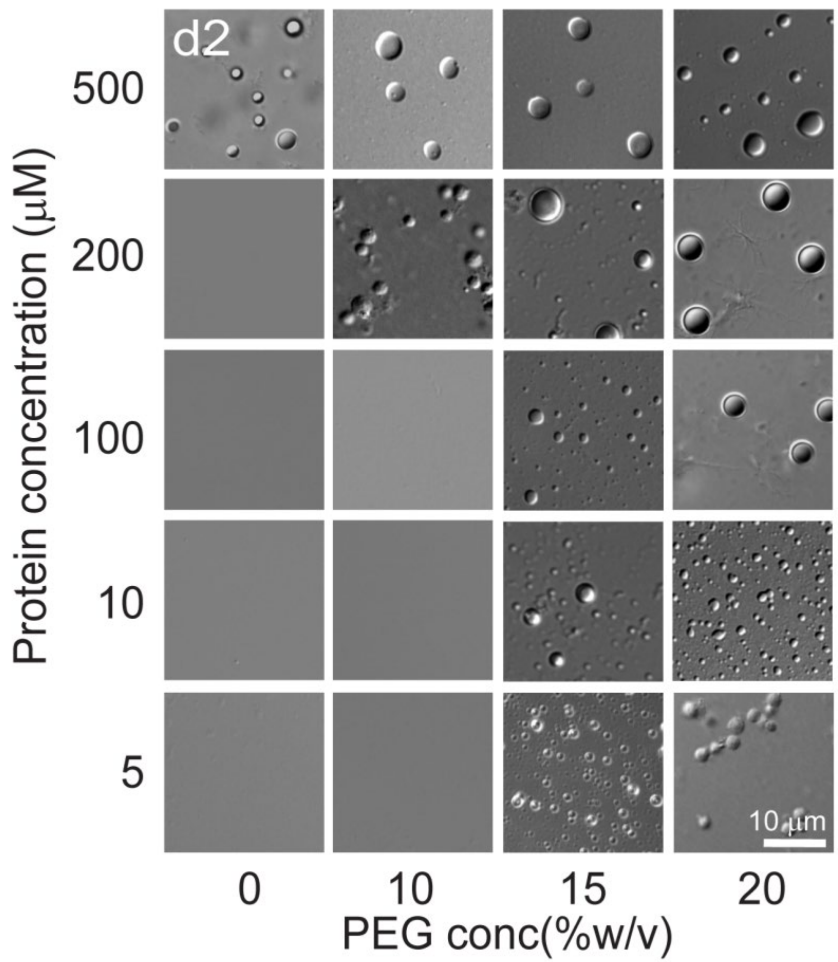
LCD: low complexity domain

A domain consisting of a limited number of amino acids

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

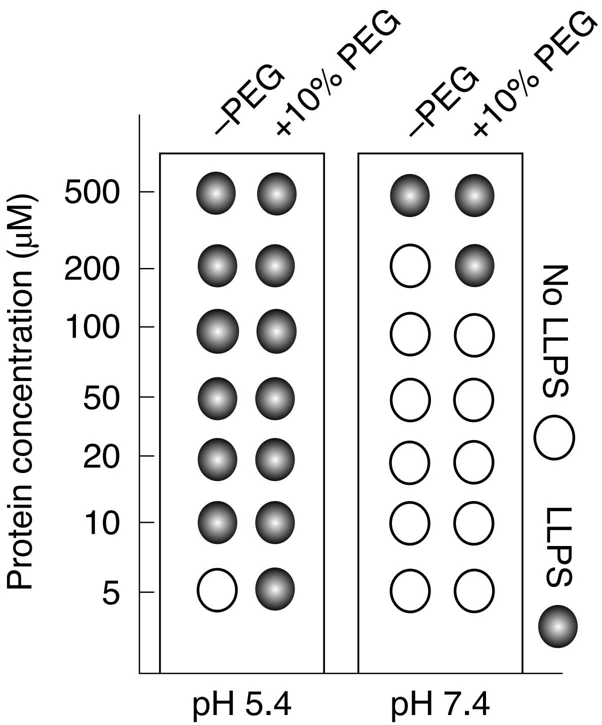
Droplet formation in the Presence of a Molecular Crowder

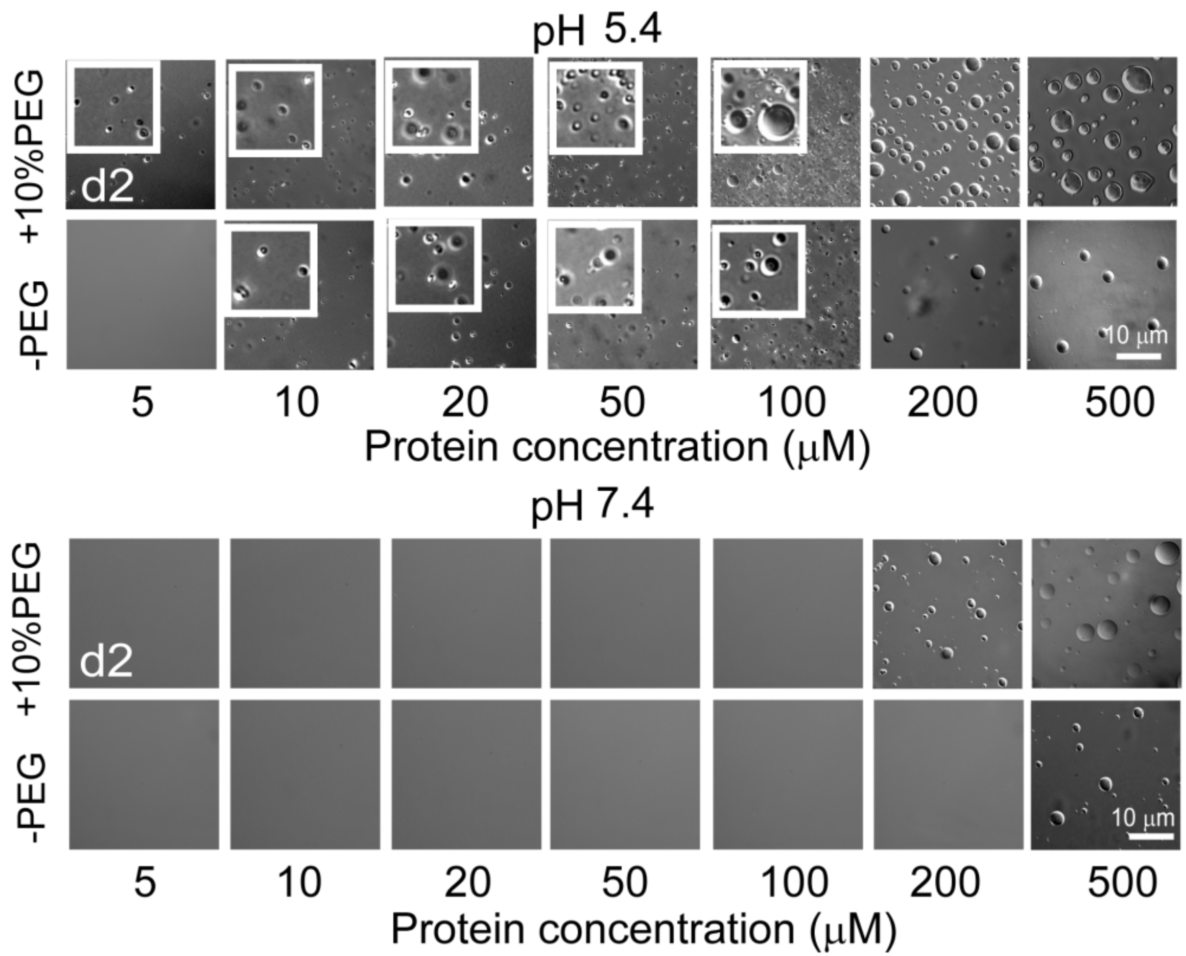




S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

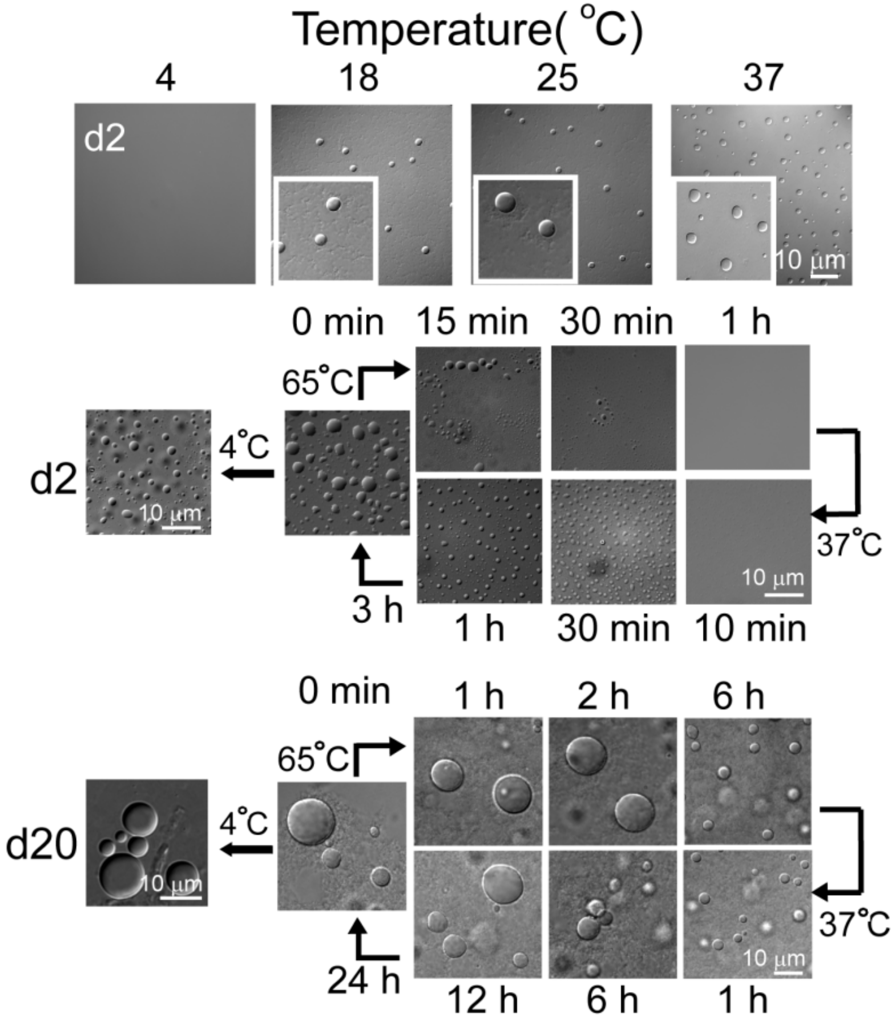
Droplet formation at Different pH Values

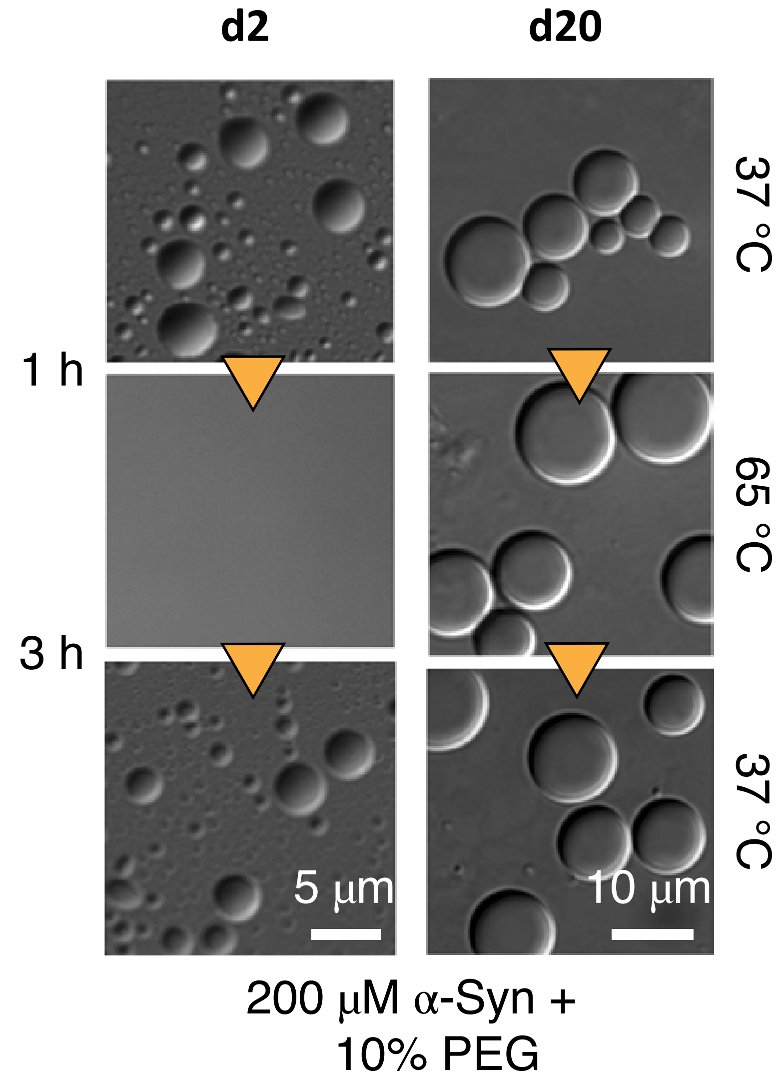




S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

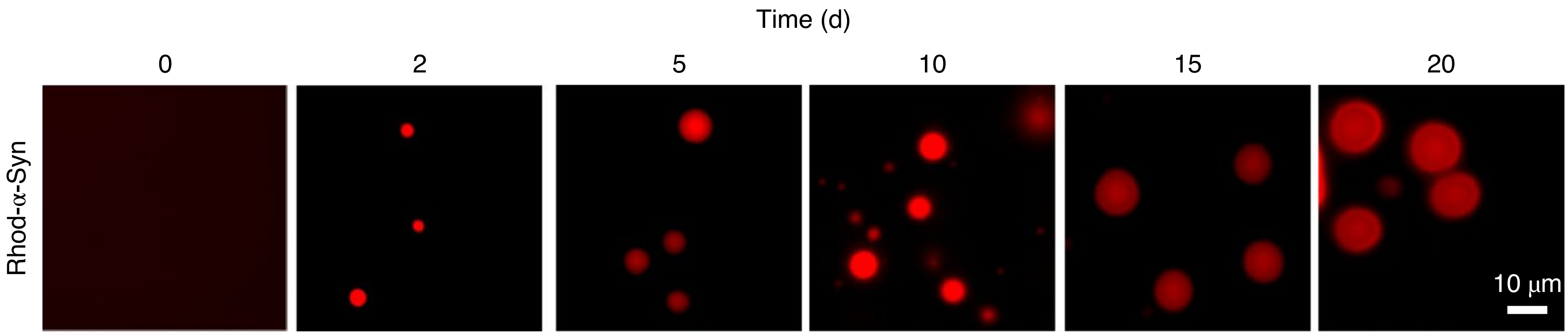
Droplet formation at Different Temperature

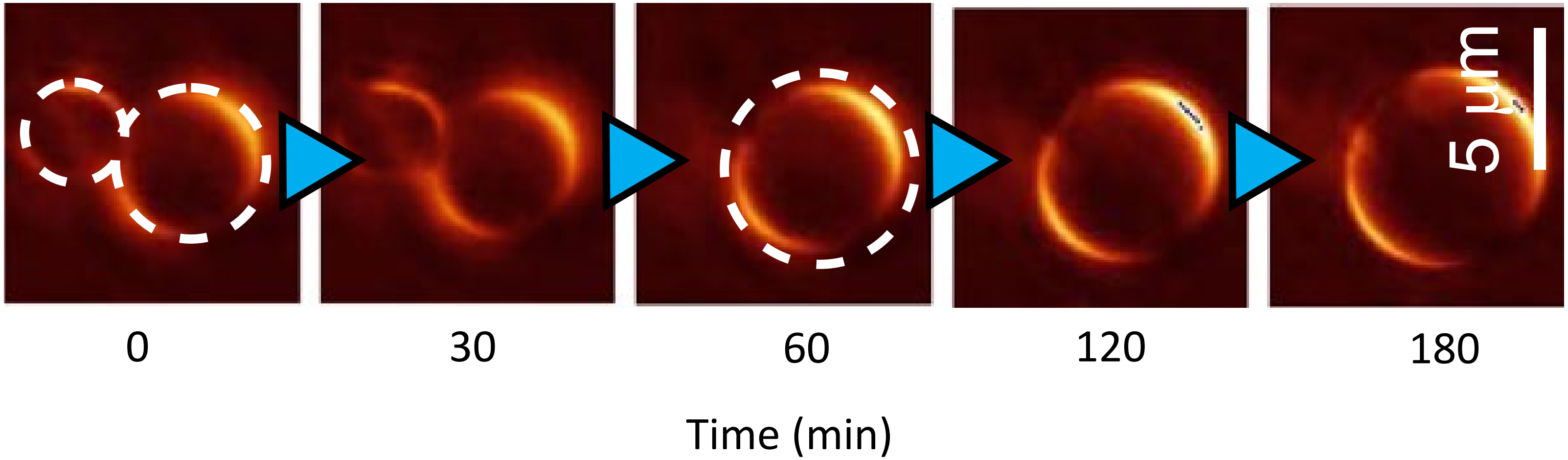




S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

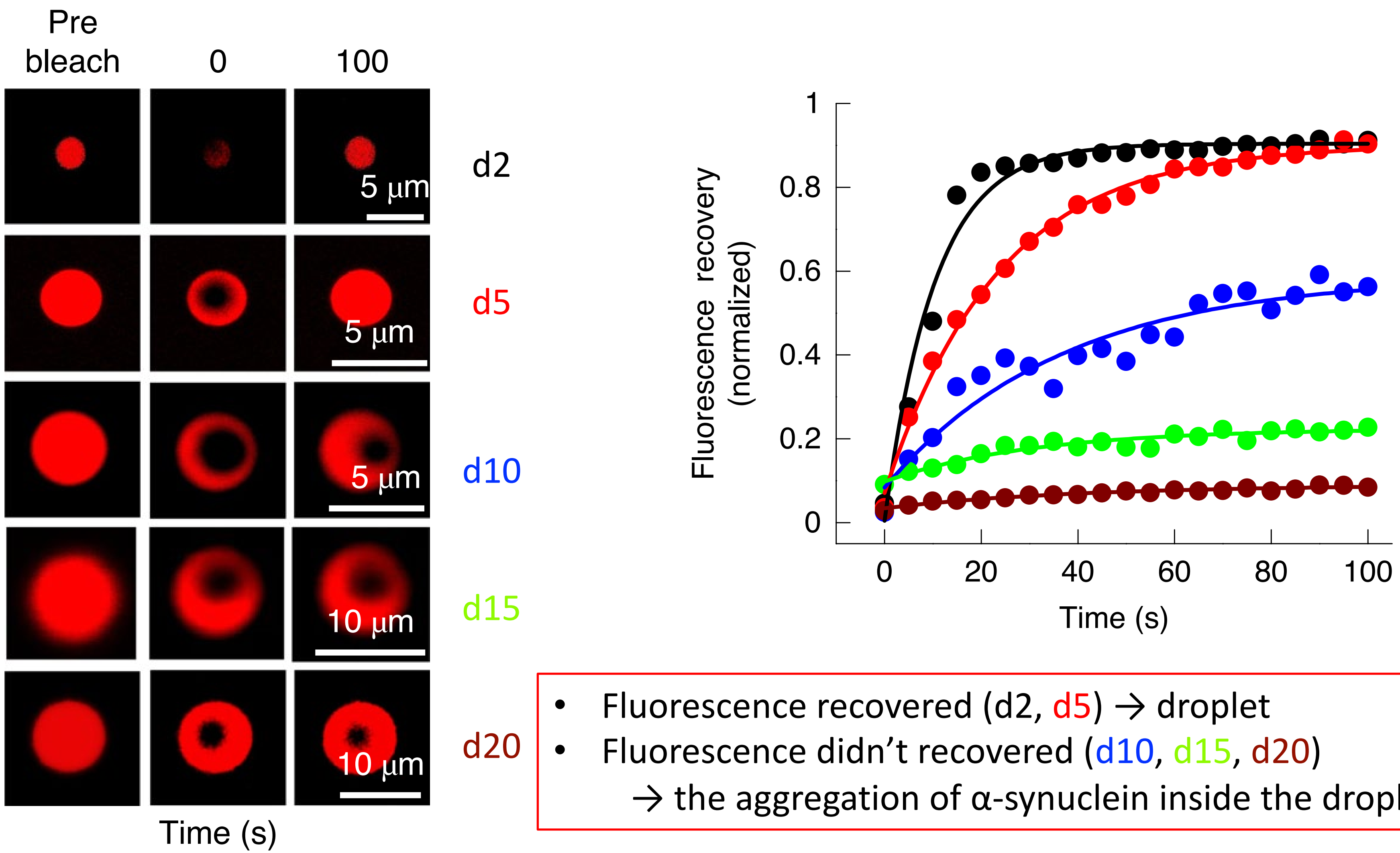
The Growth of α-Synuclein Droplets





S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

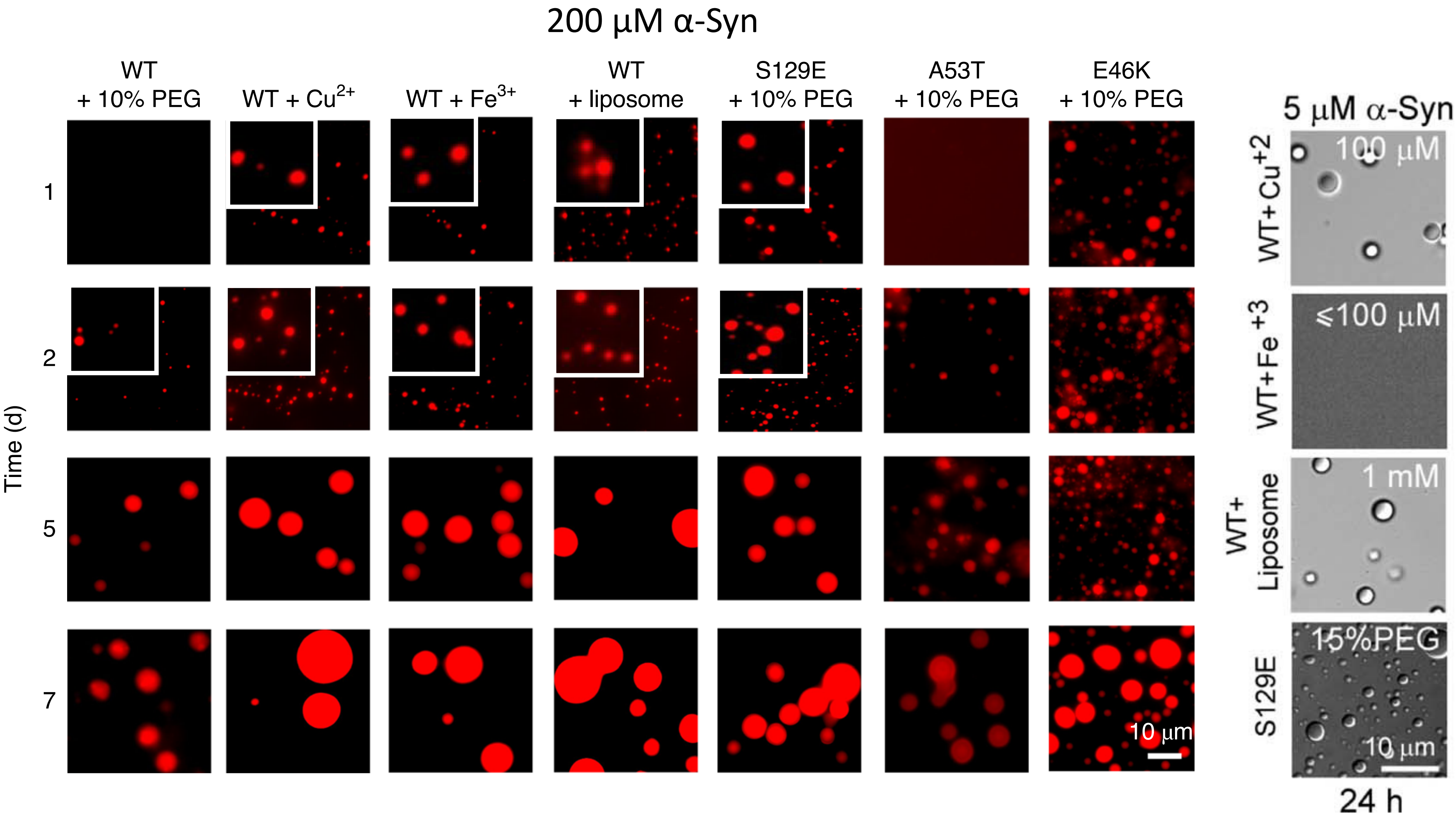
Fluorescence Recovery after Photobleaching (FRAP)



- Fluorescence recovered (d2, d5) → droplet
- Fluorescence didn't recovered (d10, d15, d20)
  → the aggregation of α-synuclein inside the droplets

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

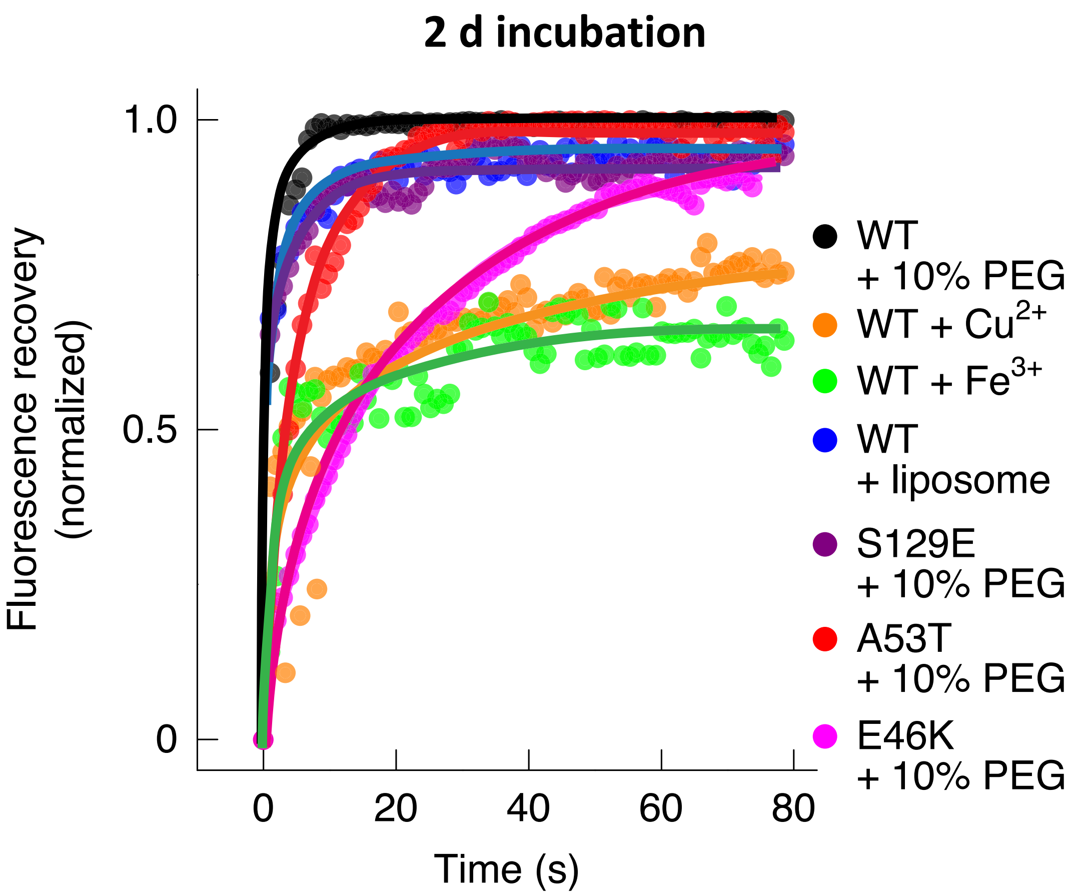
Droplet formation with PD-Associated Factors



PD-associated factors promote droplet formation. → Aggregation occurs via droplet formation.

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

FRAP with PD-Associated Factors



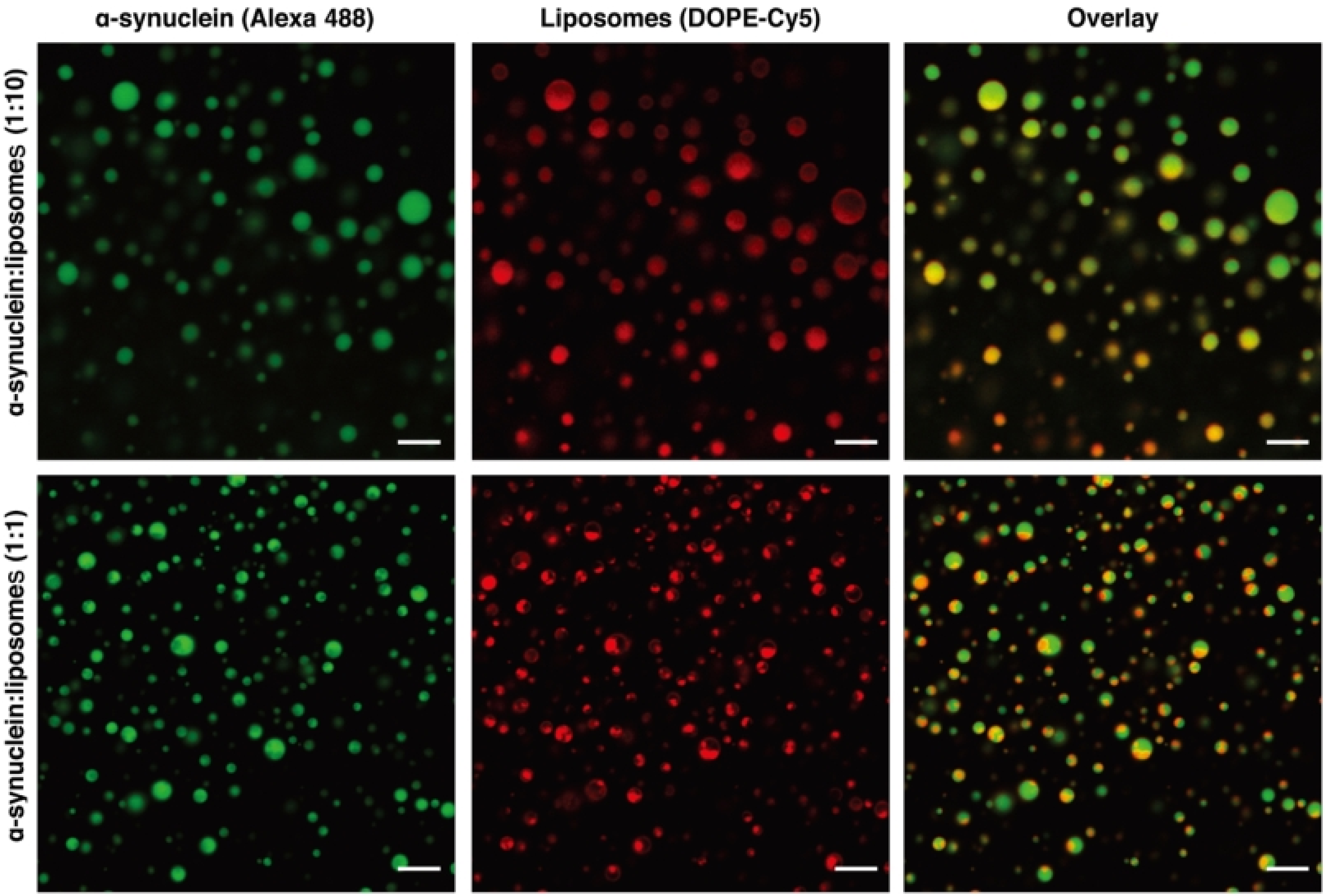
- Slow fluorescence recovery rate
- Small fluorescence recovery value

↓

PD-associated factors speed up the aggregation of α-synuclein.

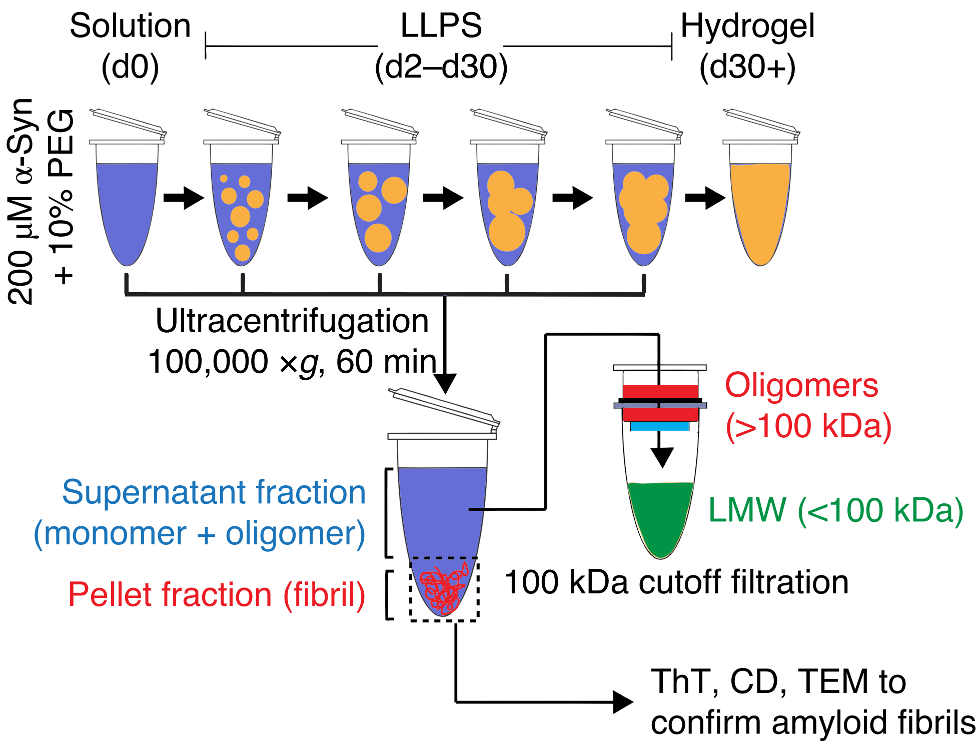
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

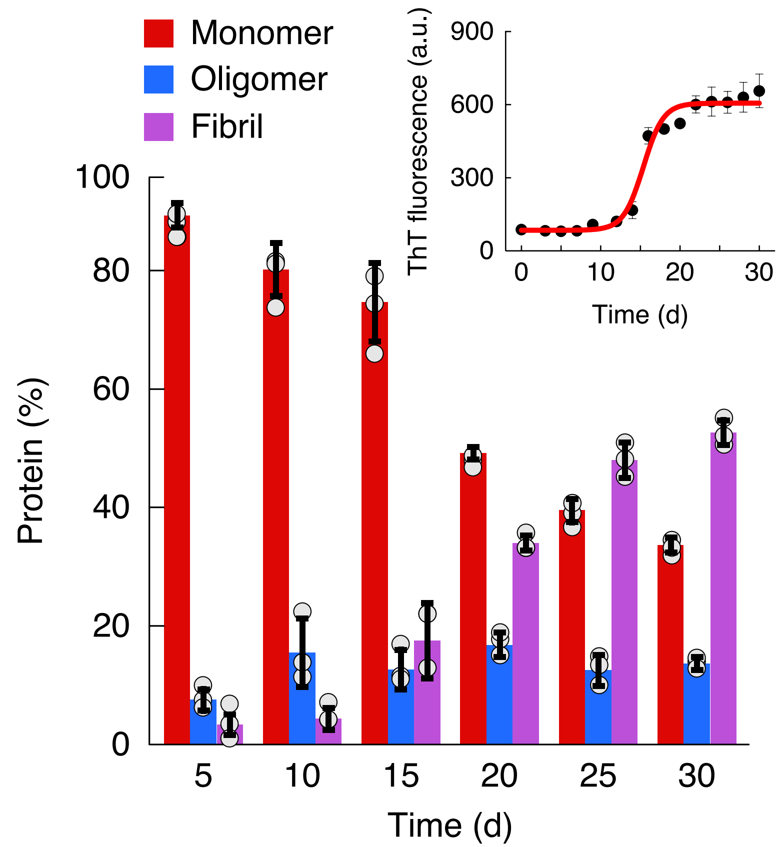
Liposomes Are Localized in Droplets



M. Vendruscolo et al. *J. Mol. Cell. Biol.* **2021**, mjaa075

Droplet & Aggregation

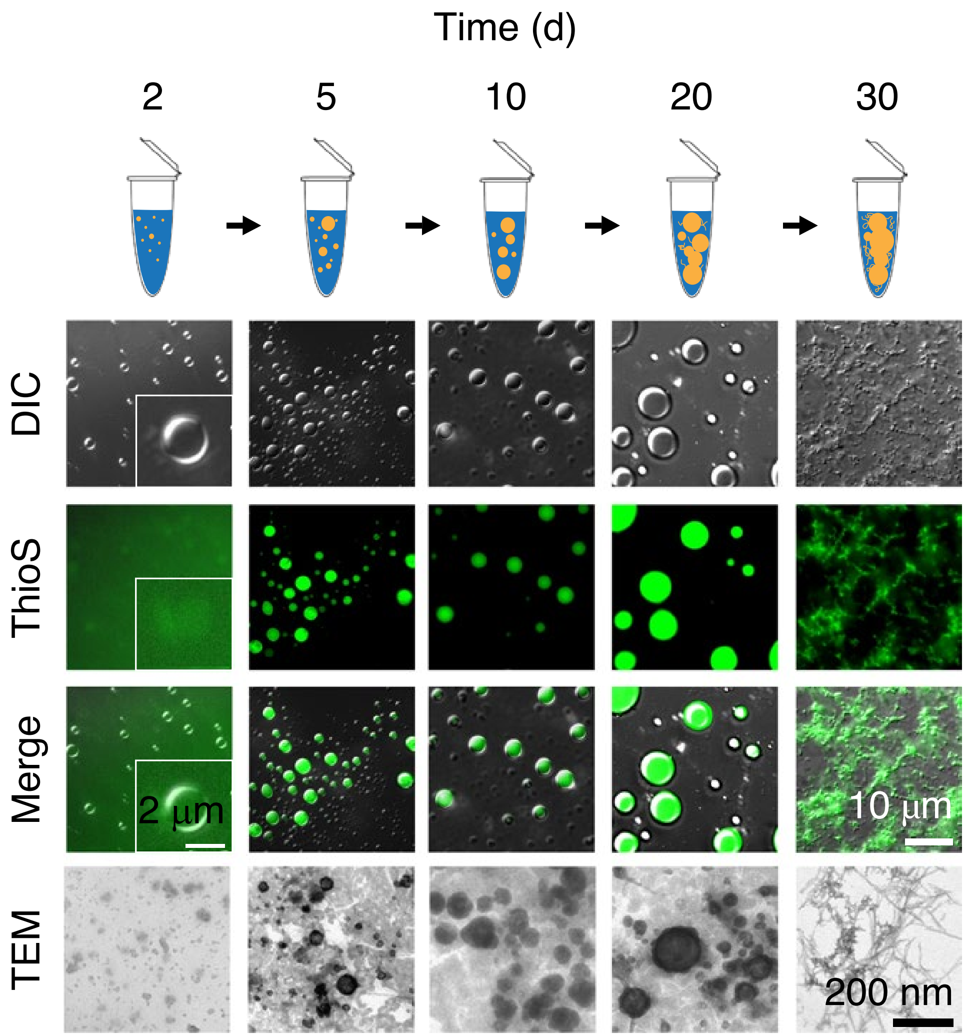


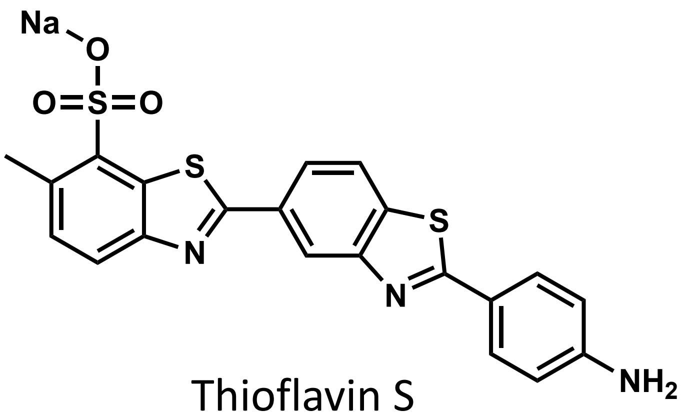


- The initial droplets have a high percentage of monomer
- The percentage of aggregates increases with time.

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

Droplet & Aggregation



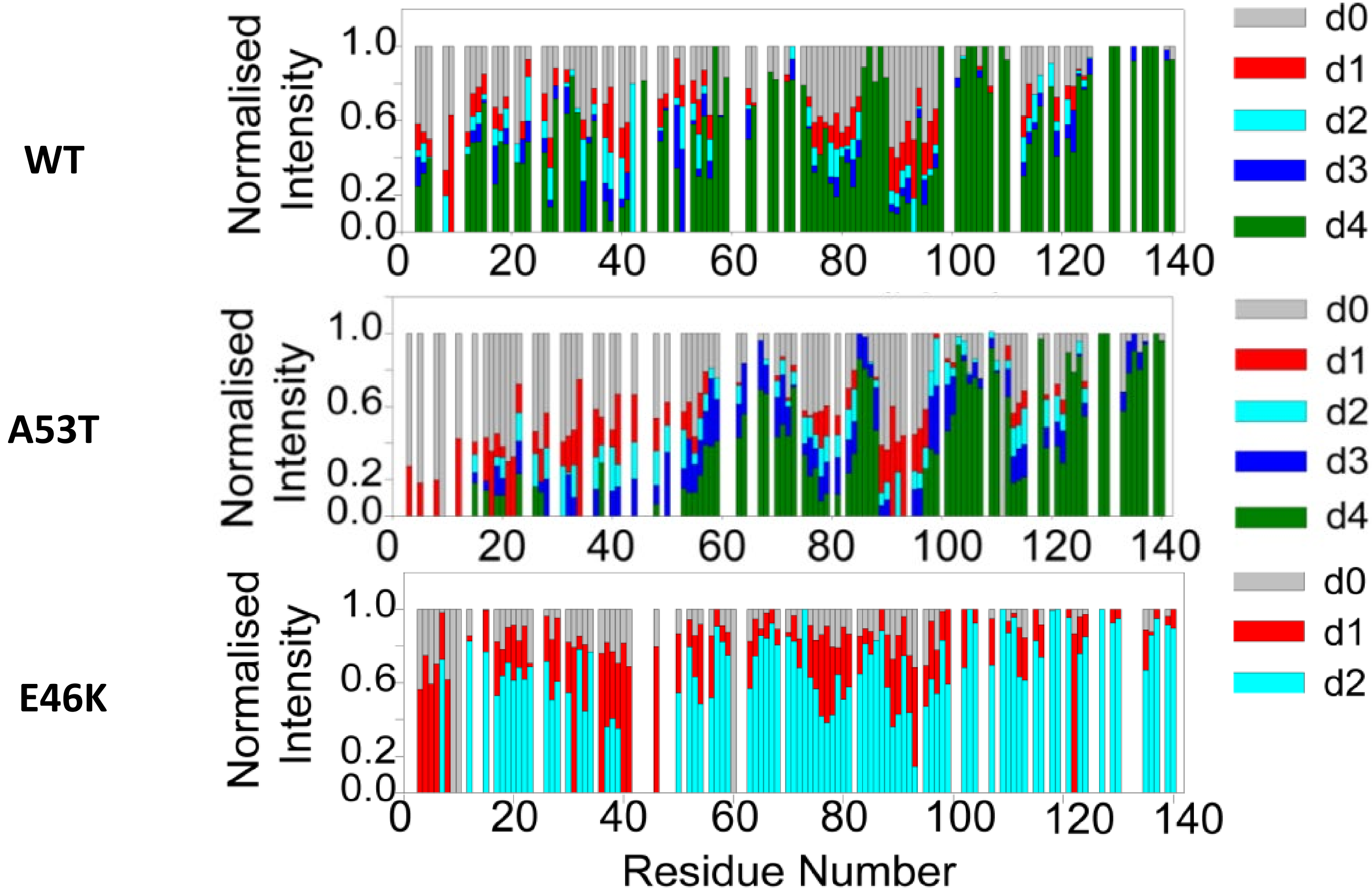


Thioflavin S
(Detect amyloid fibrils)

Presence of α-synuclein aggregates in the droplet

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

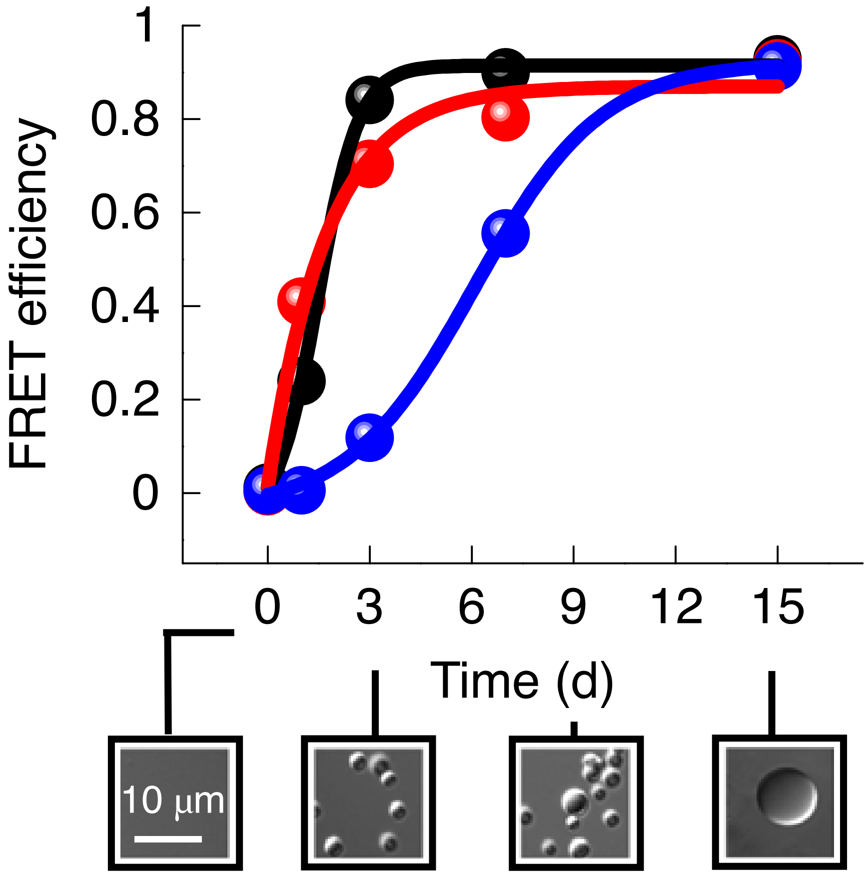
The Changes in the Intensity Profile of Amide Cross-Peaks



- A gradual decrease in the intensities of the residues at the N terminus (V3-A27, V37-K43 and H50-E57) and NAC region (V74-V82 and A89-K97).
- The residues in the C terminus (I112–N122) showed a comparatively lower reduction in the intensity.

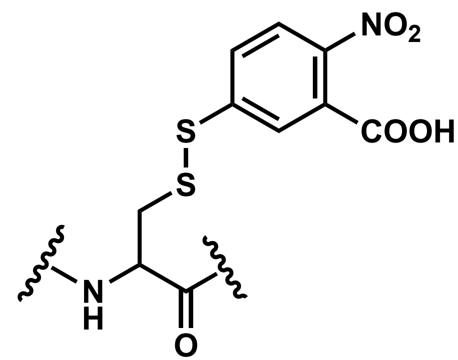
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

FRET Efficiency between Trp & Cys-DTNB



α-syn. 3W +α-syn. 3C-DTNB
α-syn. 71W +α-syn. 74C -DTNB
α-syn. 124W +α-syn. 124C-DTNB

Cys-DTNB

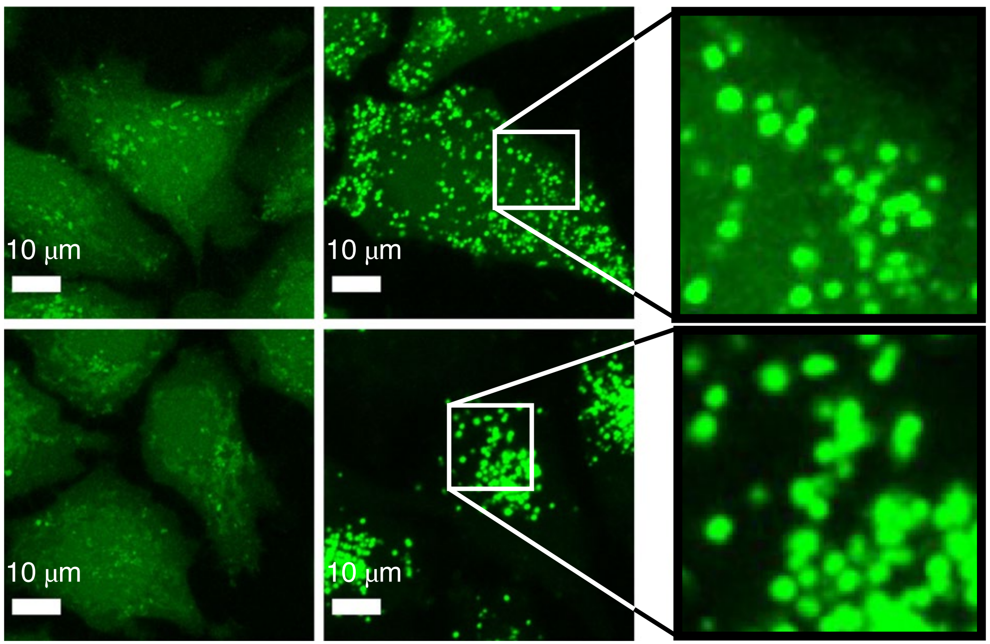


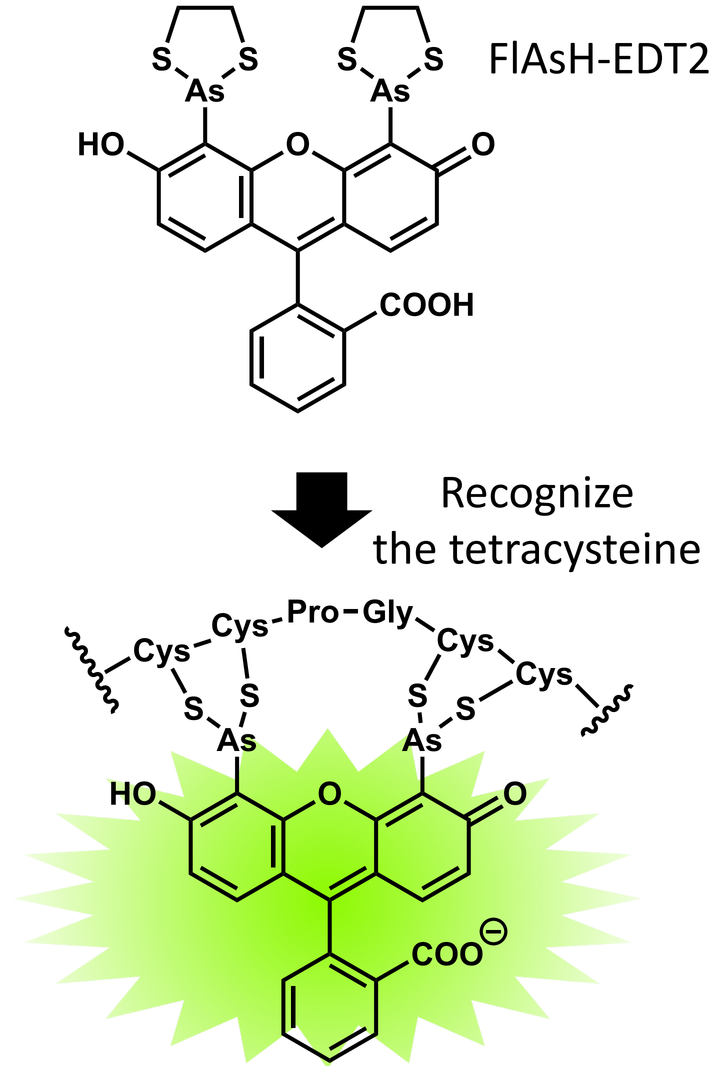
High FRET efficiency → Close distance → Interaction
N terminus & NAC region interact from the early stage of droplet formation.

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

Droplet in Cell

C_4 - α-synuclein **(FLNCCPGCCMEP - α-syn.)**
10 mM ferric ammonium citrate

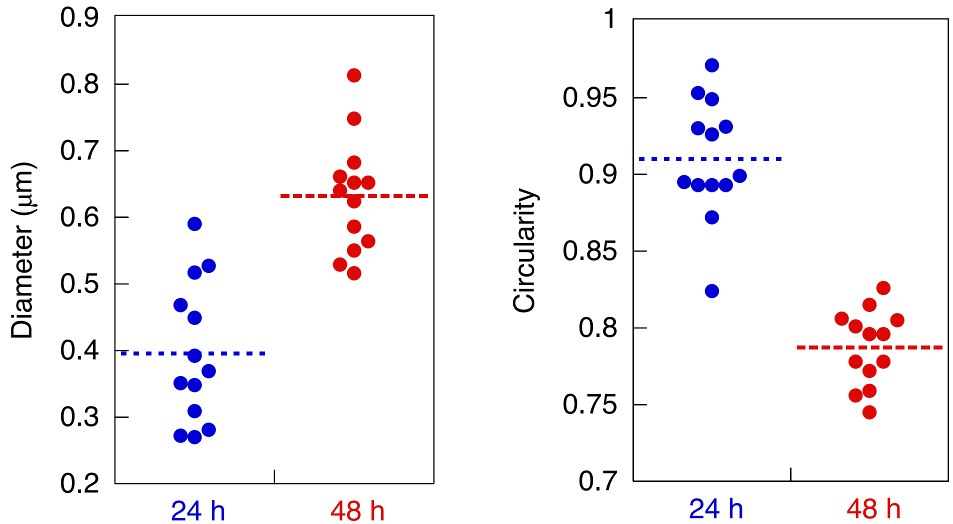




T. M. Jovin et al. *Nat. Methods*, **2007**, *4*, 345.
J. Levin et al. *J. Parkinson. Dis.* **2011**, *1*, 205
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

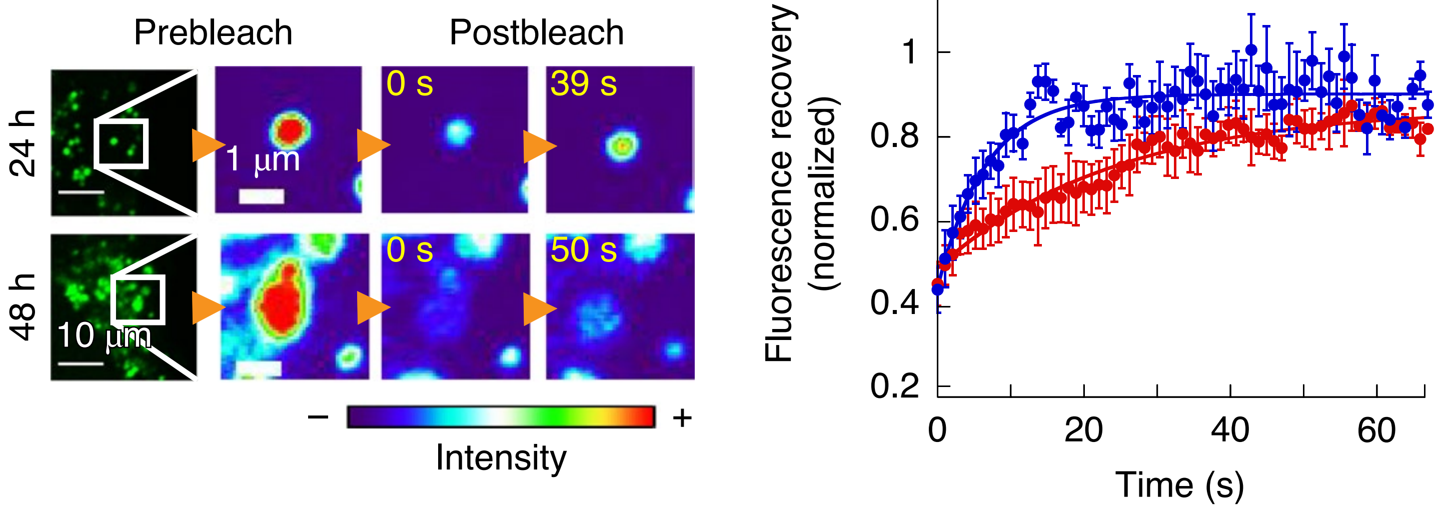
Droplet in Cell

- **Size & surface tension of droplet**



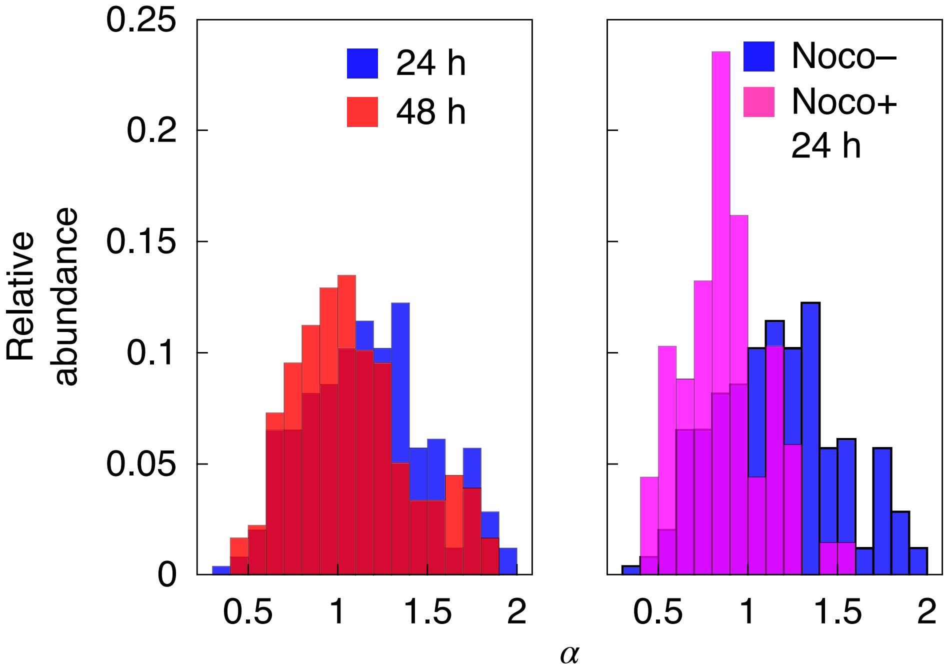
- Droplets at 24 hours have the properties of a liquid.
- Droplets at 48 hours have reduced liquid properties.

- **FRAP**



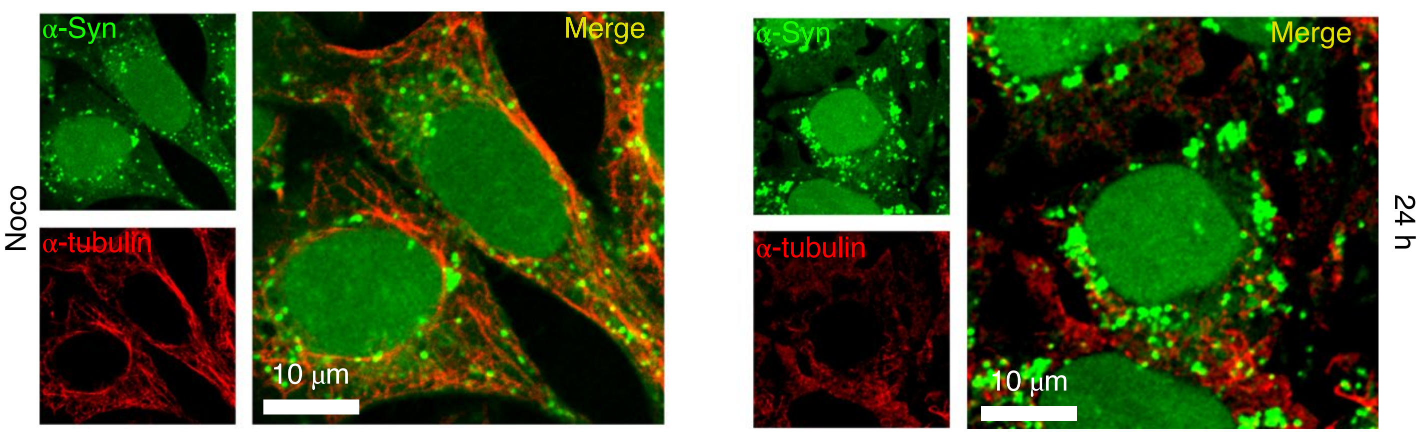
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

Diffusion Dynamics of Droplets



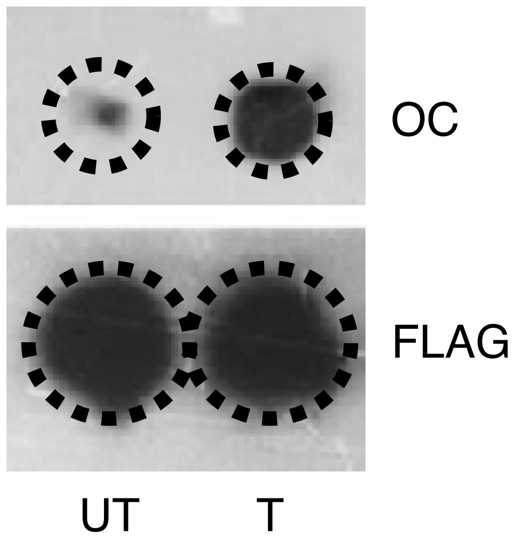
Nocodazole (NOCO):
microtubule-depolymerizing agent

- Movement of the liquid-like α-Syn droplets is initially much more directed with the assistance of the microtubules
- Movement of the liquid-like α-Syn droplets reduced upon liquid-to-solid transition

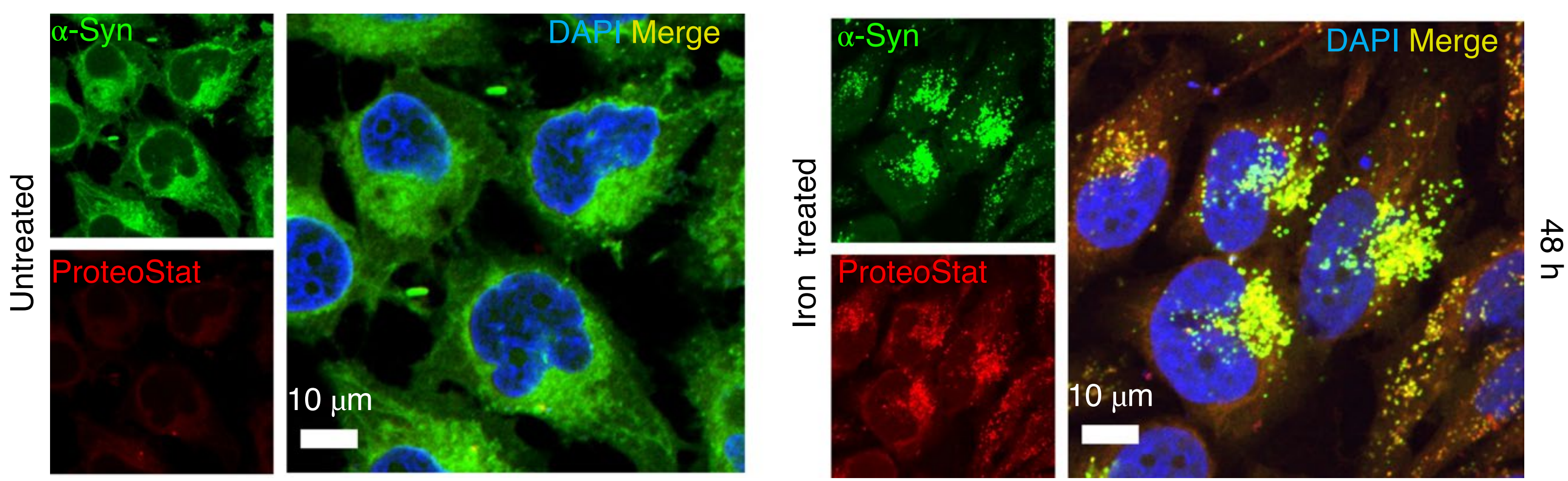


S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

Droplet & Aggregation in Cell



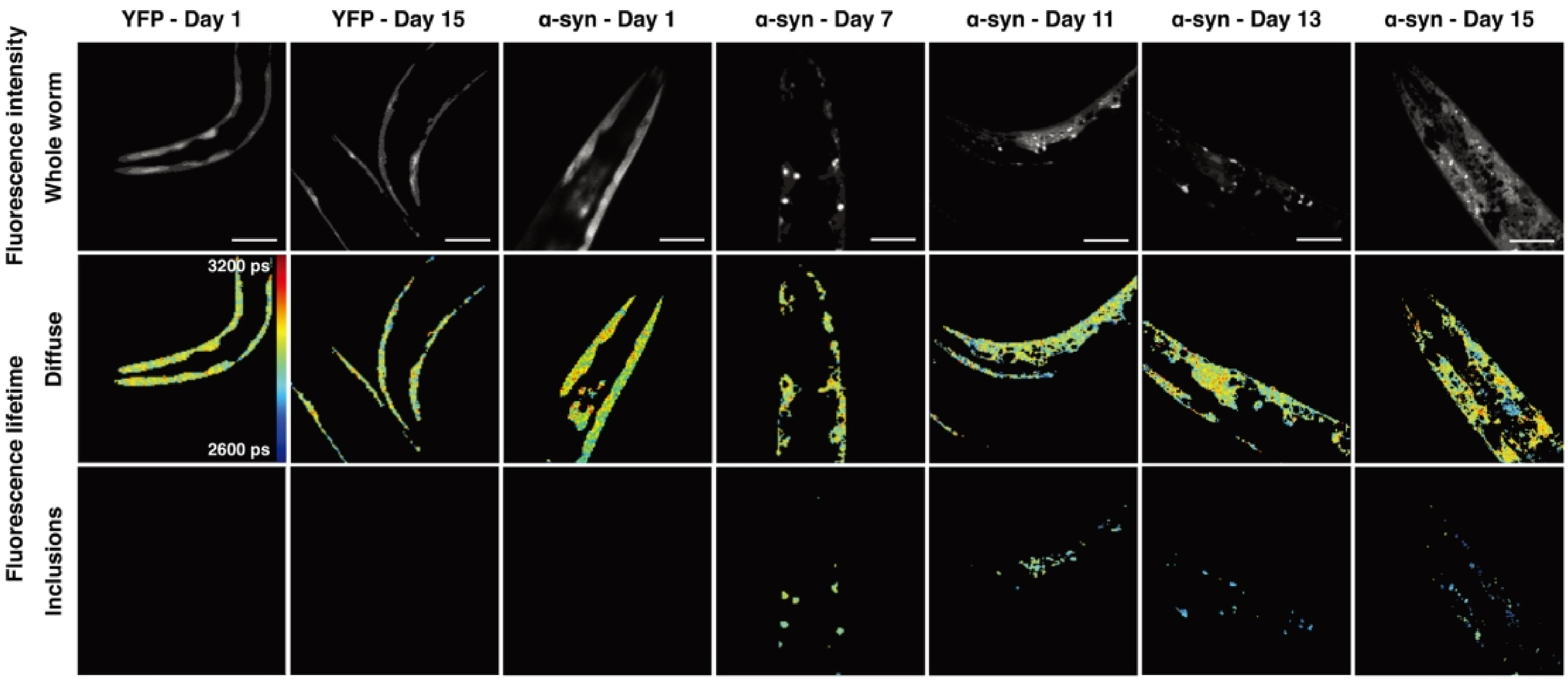
T (Treated) : W/ Ferric ammonium citrate
UT (Untreated) : W/O Ferric ammonium citrate
OC : Amyloid-specific antibody
FLAG : Total protein
ProteoStat : Aggresome detection



S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

α-Synuclein in C. elegans.

α-Syn. – YFP (yellow fluorescent protein)

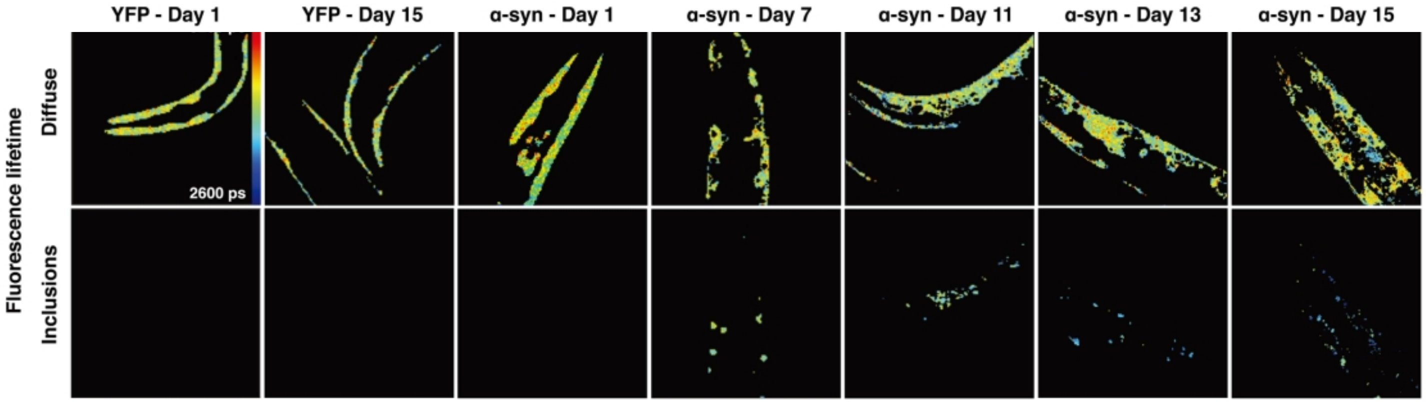


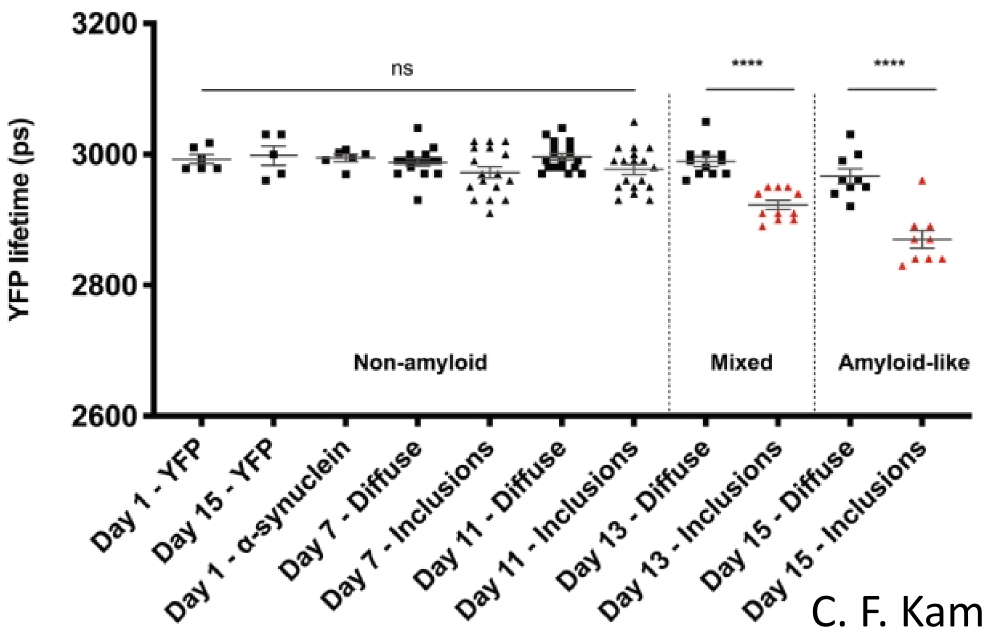
M. Vendruscolo et al. *J. Mol. Cell. Biol.* **2021**, mjaa075

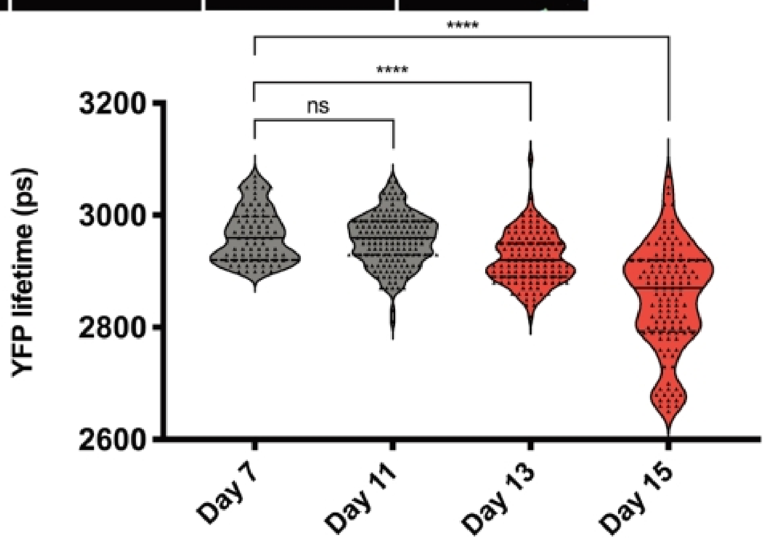
α-Synuclein in C. elegans.

- **Fluorescence lifetime imaging**

FRET between YFP and α-synuclein aggregates reduces the fluorescence lifetime of YFP.



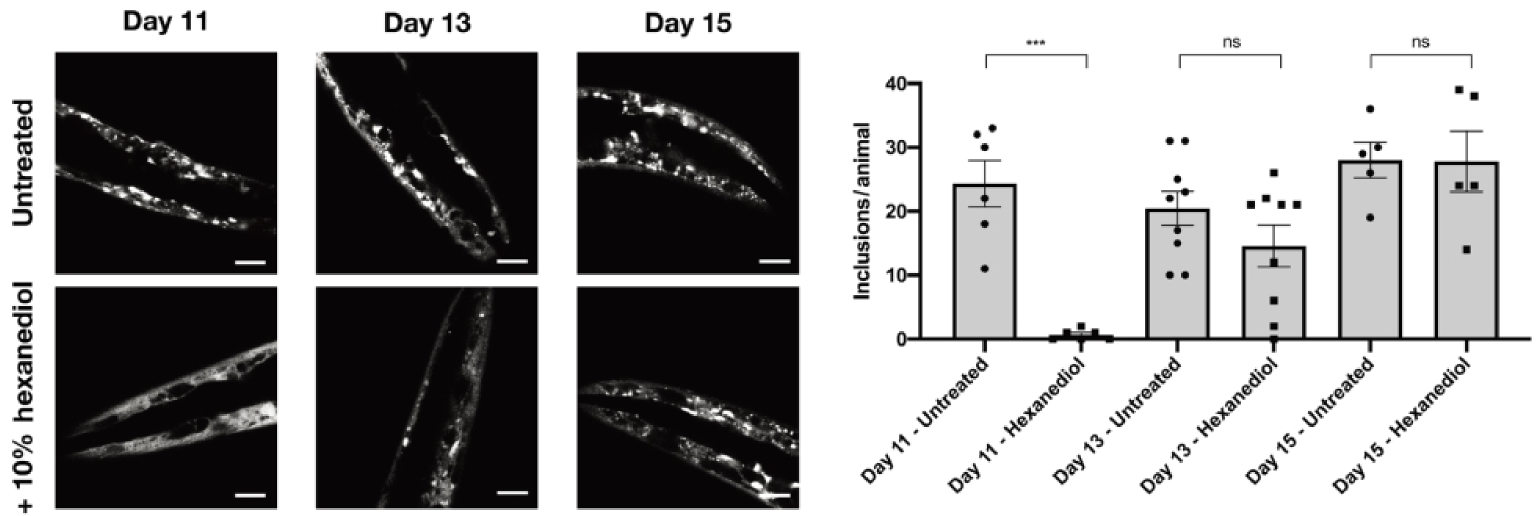


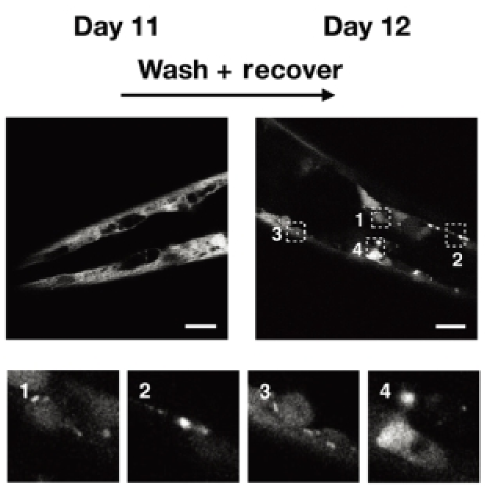


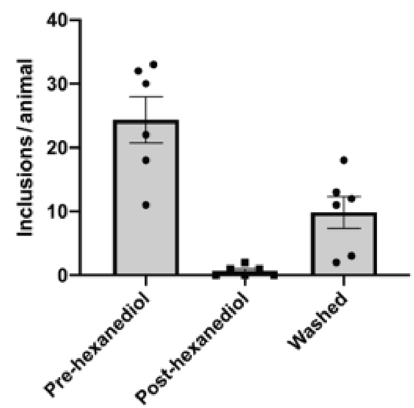
C. F. Kaminski et al. *ChemPhysChem* **2011**, *12*, 673.
M. Vendruscolo et al. *J. Mol. Cell. Biol.* **2021**, mjaa075

Droplet in *C. elegans.* with 1,6-Hexanediol

Hexanediol : dissolves droplets



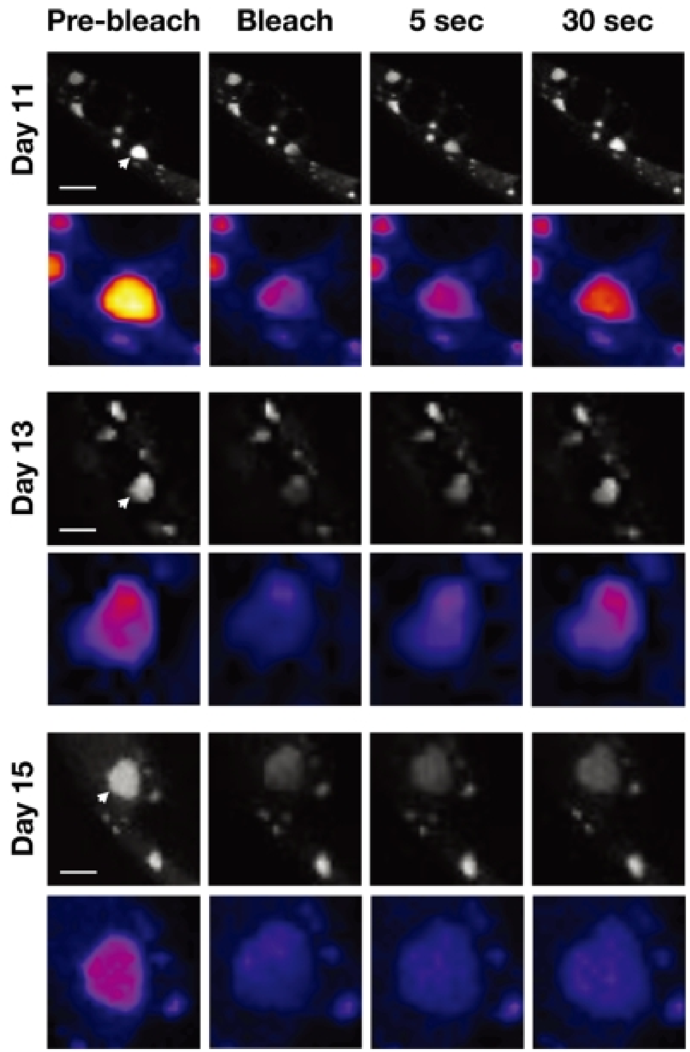


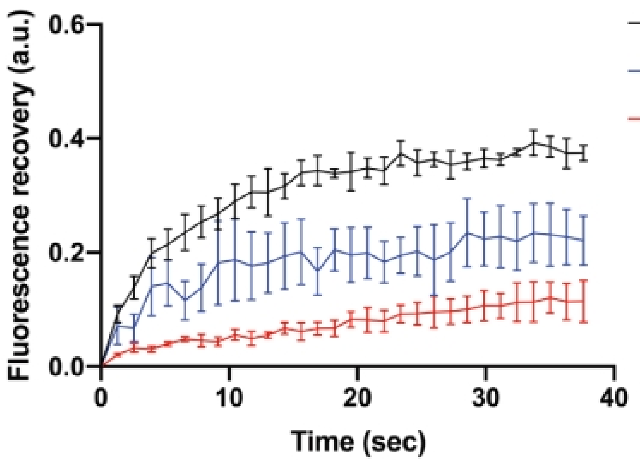


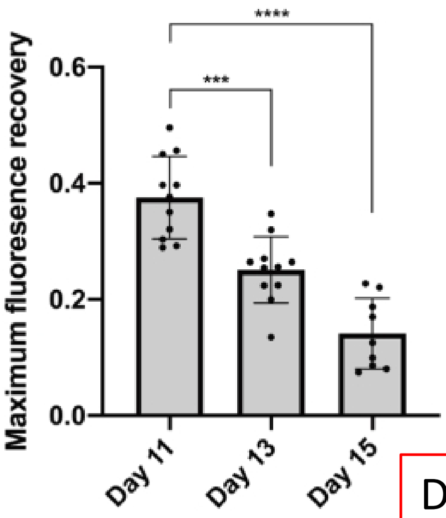
Day-11: droplet
Day-15: aggregates

M. Vendruscolo et al. *J. Mol. Cell. Biol.* **2021**, mjaa075

FRAP Analysis of Droplet in C. elegans.



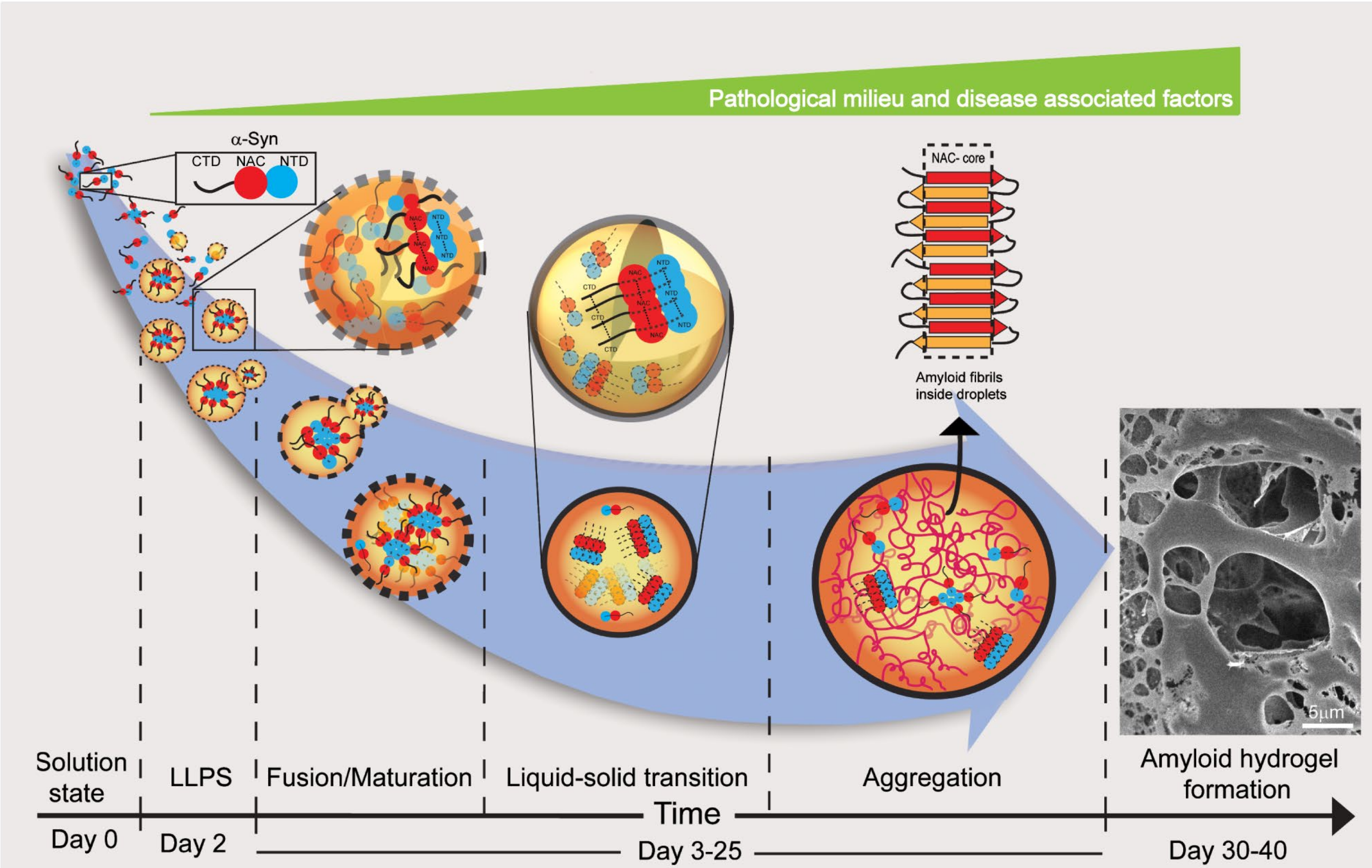




Day-11: droplet
Day-15: aggregates

M. Vendruscolo et al. *J. Mol. Cell. Biol.* **2021**, mjaa075

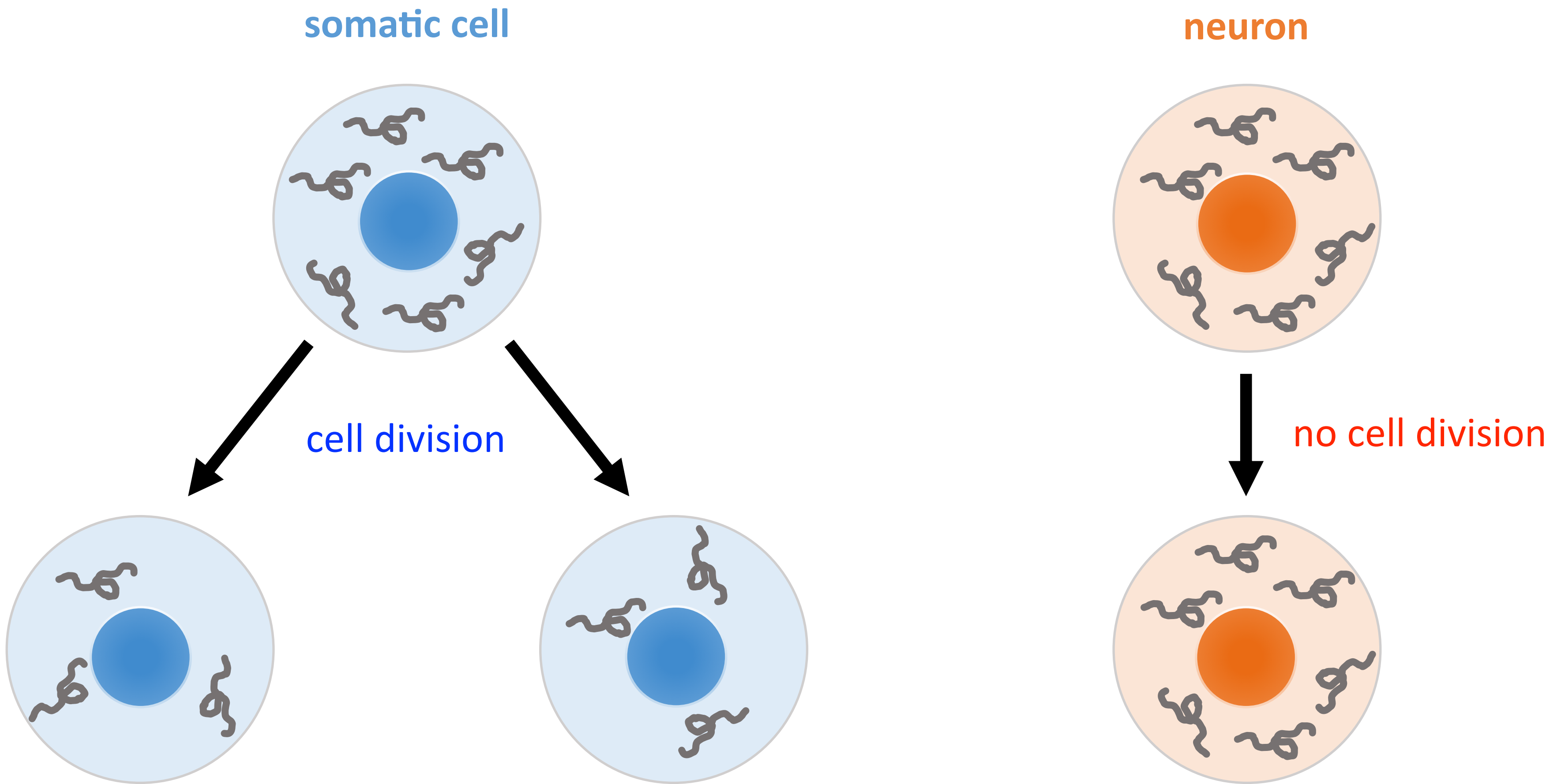
Summary



- α-Synuclein forms droplets in vivo, in cell & in C. elegans.
- α-Synuclein aggregates from droplets.
- PD-associated factors & familial mutation promote droplet formation.

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

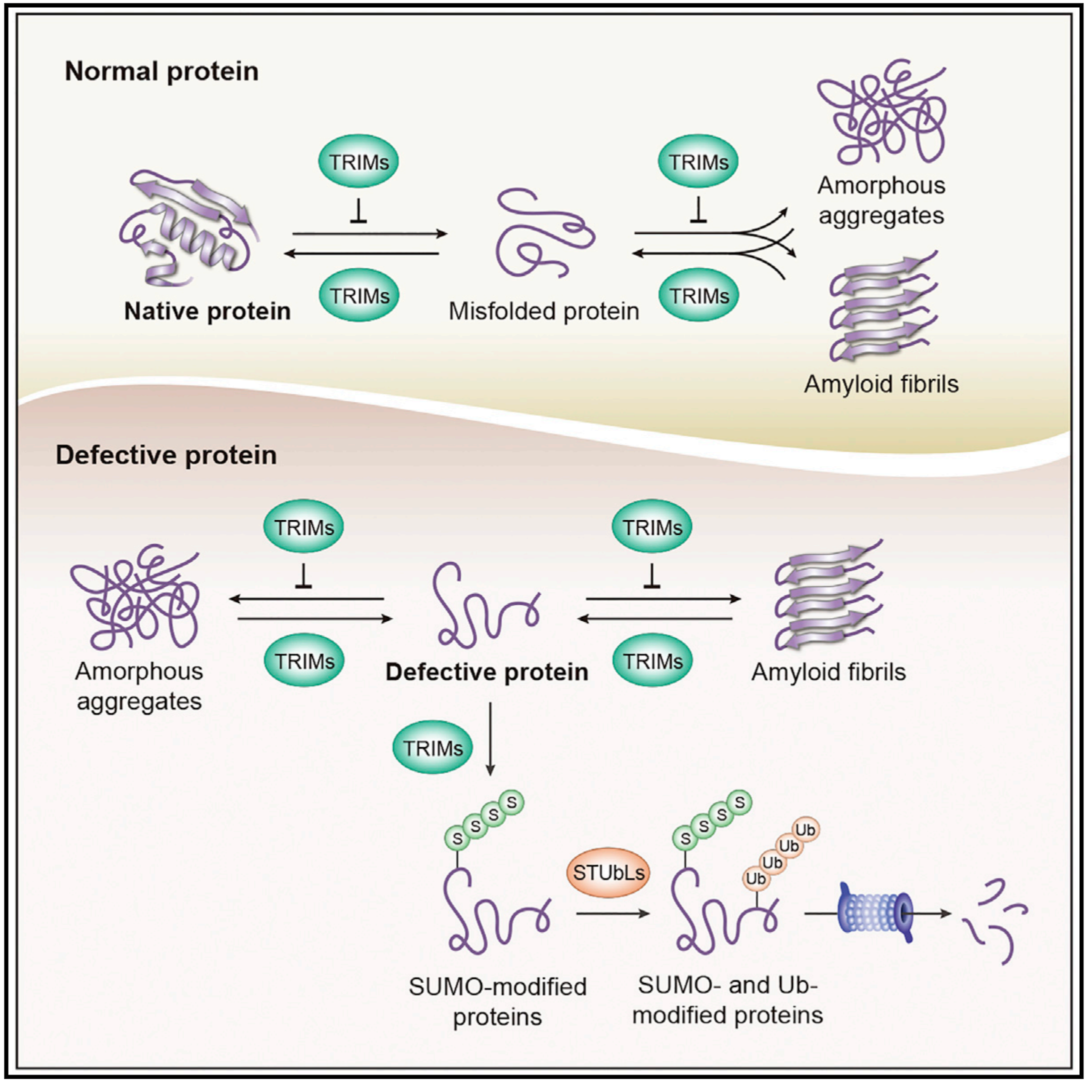
Toxic Misfolded Conformers Are Diluted by Cell Division

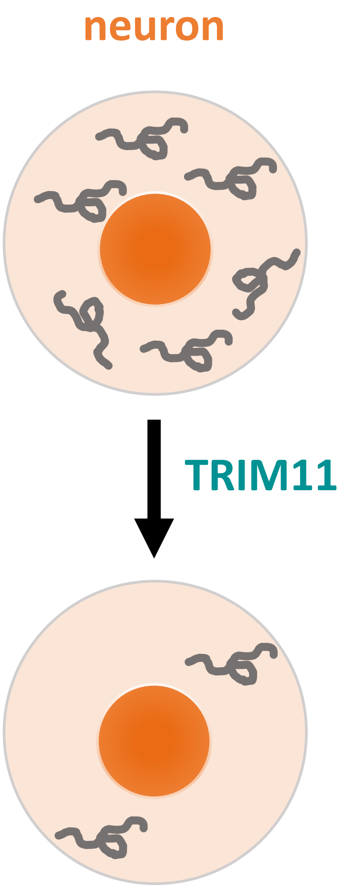


Toxic misfolded conformers are diluted.

Toxic misfolded conformers are not diluted.

Molecular Chaperones & Protein Disaggregases



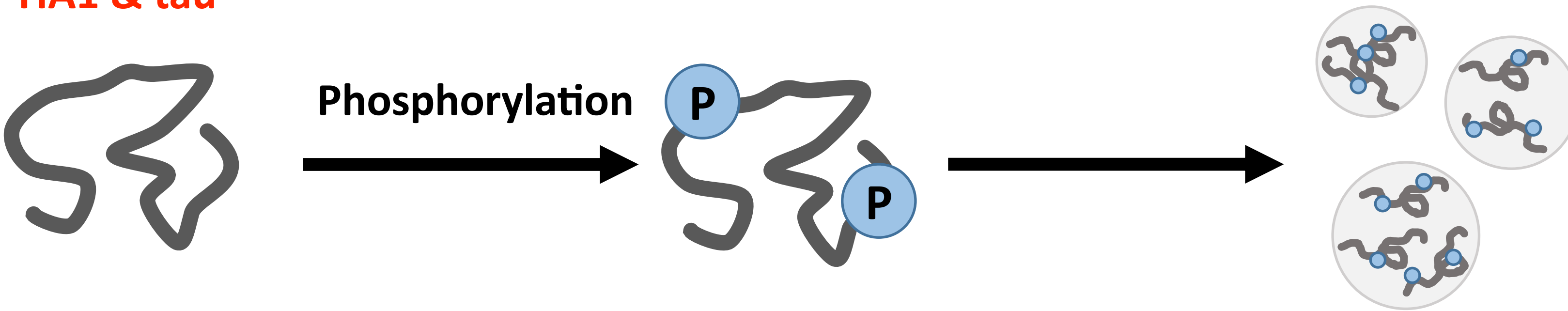


X. Yang et al. *Cell Rep.*, **2020**, *33*, 108418.

Post-Translational Modifications : Phosphorylation

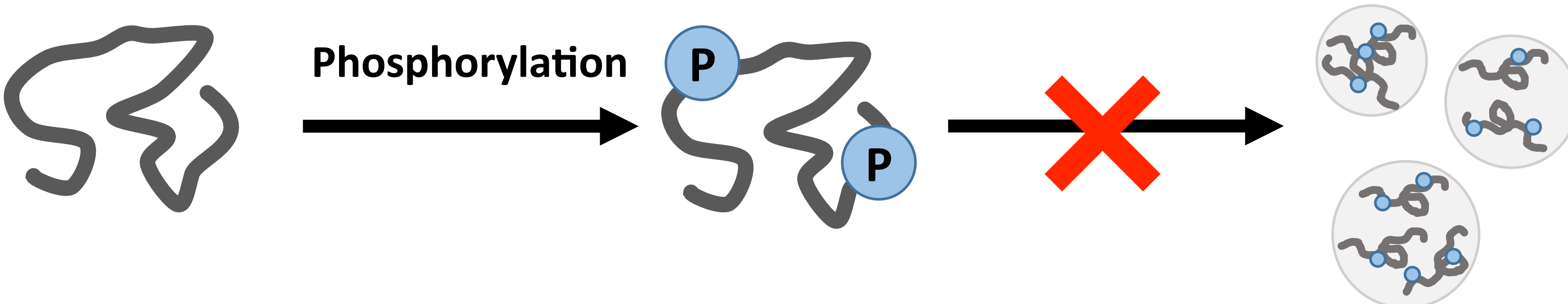
- **Phosphorylation promotes droplet formation & aggregation.**

TIA1 & tau



- **Phosphorylation suppresses droplet formation & aggregation.**

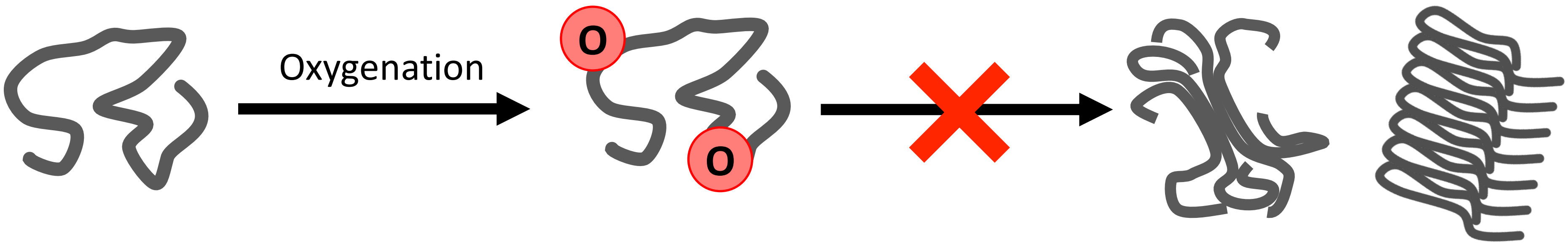
FUS & TDP-43



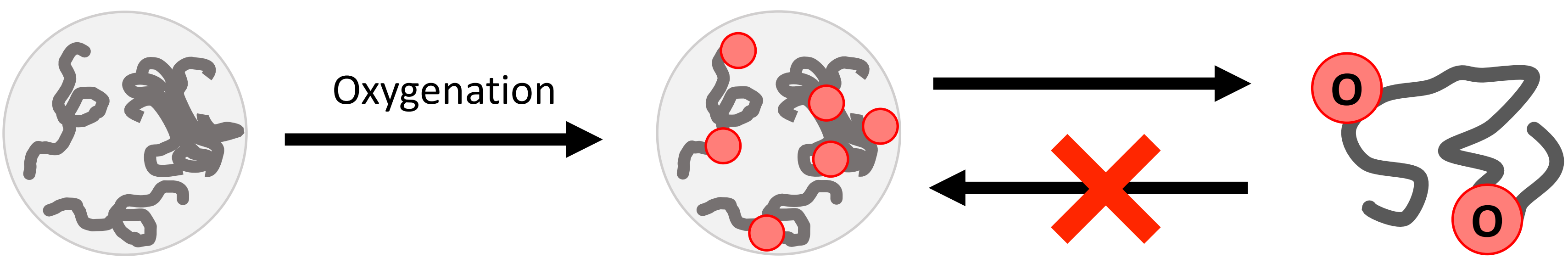
A. L. Darling & J. Shorter, *Biochim. Biophys. Acta, Mol. Cell Res.*, **2021**, *1868*, 118984.
TIA1 : Y. Jin, et al. *Neuron*, **2019**, *104*, 290.
tau : B.T. Hyman et al. *Embo J.* **2018**, *37*, e98049.
FUS : N.L. Fawzi et al. *Embo J.* **2017**, *36*, 2951.
TDP-43 : N.L. Fawzi et al. *Embo J.* **2018**, *37*, e97452

Post-Translational Modifications : Oxygenation

- **Photo-oxygenation suppresses α-synuclein aggregation.**



- **Photo-oxygenation suppresses α-synuclein droplet formation (?)**



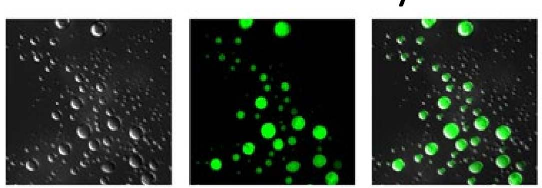
Day 5 droplets

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

FRAP analysis

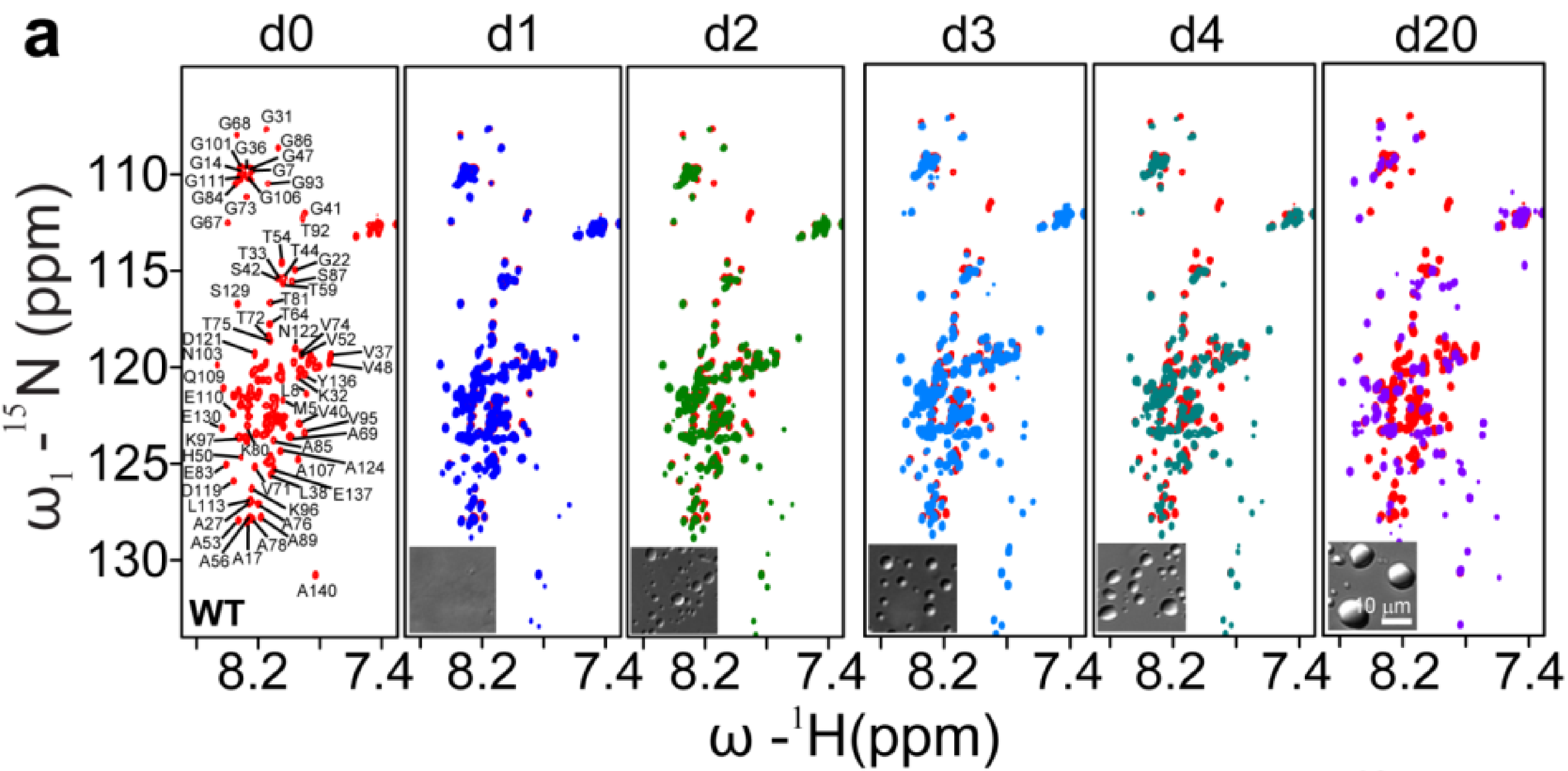
Pre 0 s 100 s

Thioflavin S analysis



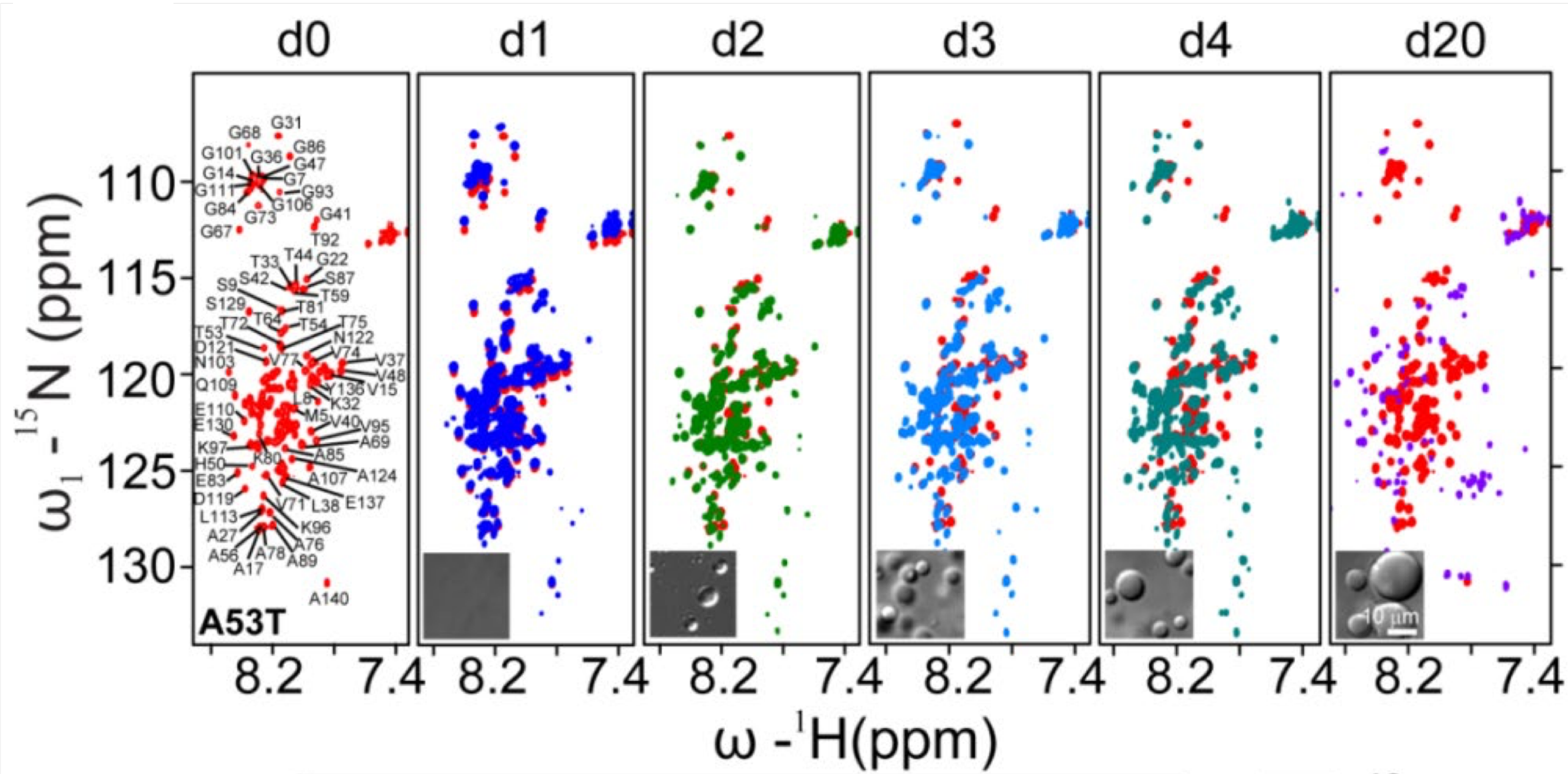
DIC ThioS Merge

HSQC Spectra : WT



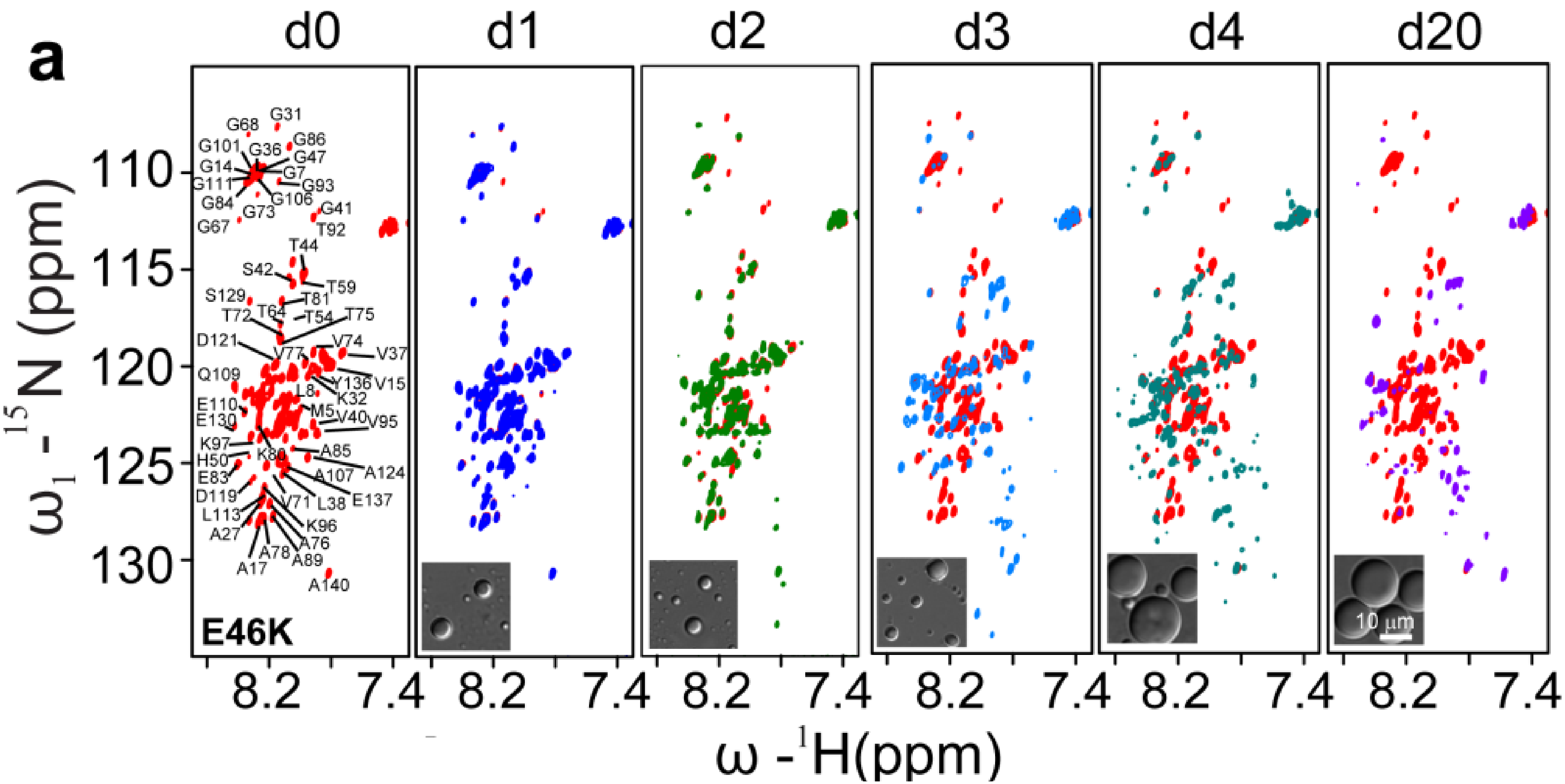
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

HSQC Spectra : A53T



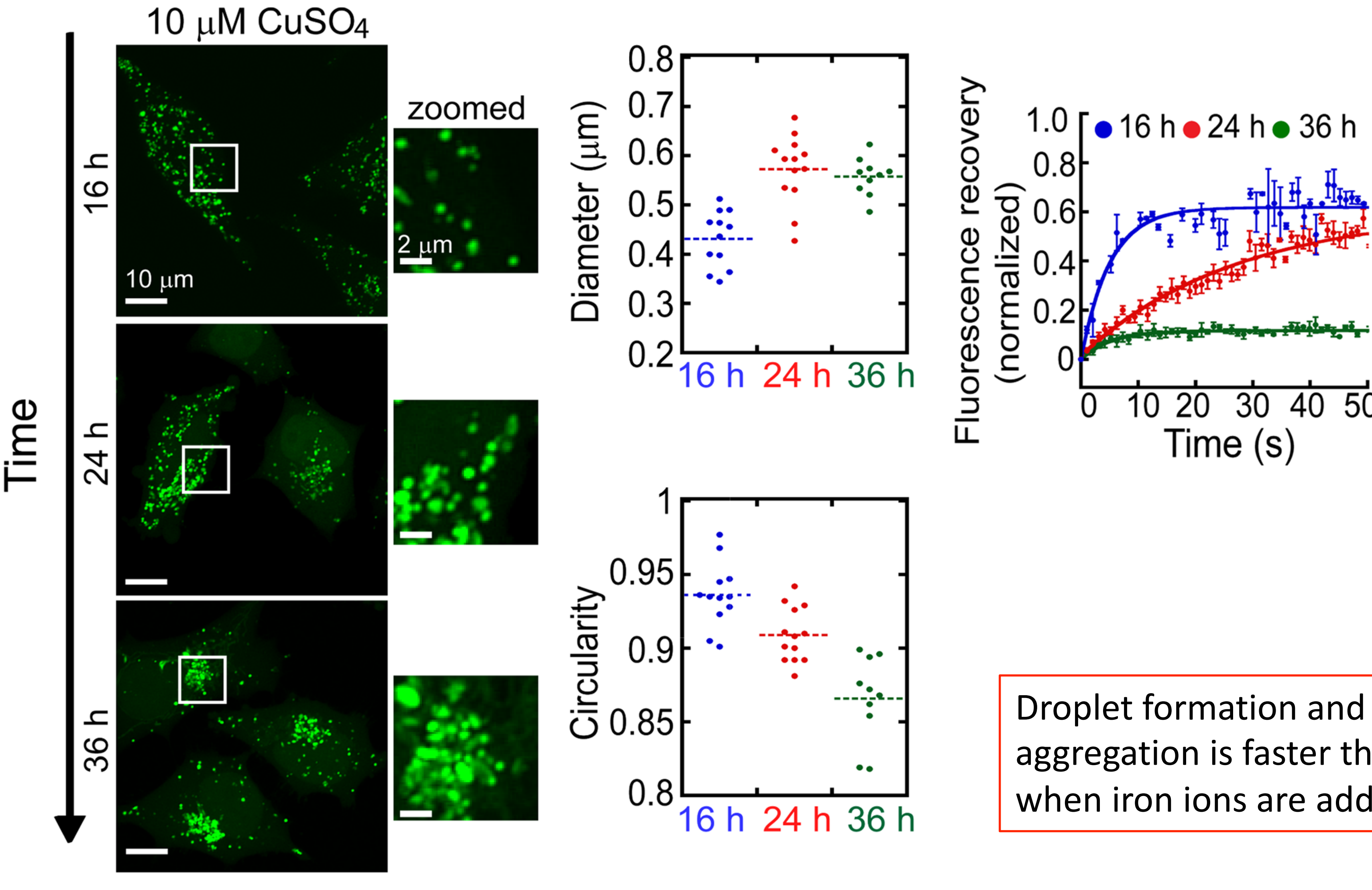
S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

HSQC Spectra : E46K



S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.

$CuSO_4$ Treated Droplet in Cell



Droplet formation and aggregation is faster than when iron ions are added.

S. K. Maji et al. *Nat. Chem.* **2020**, *12*, 705.