JOHANNES GUTENBERG UNIVERSITÄT MAINZ

Phase-separation in biology

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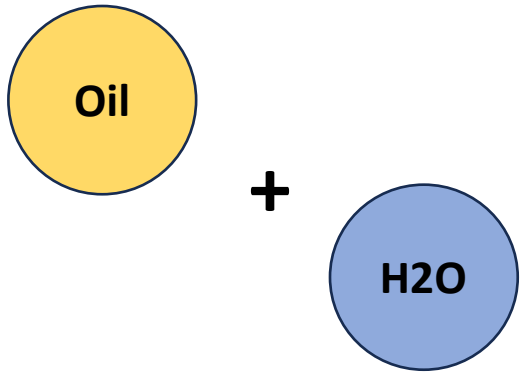
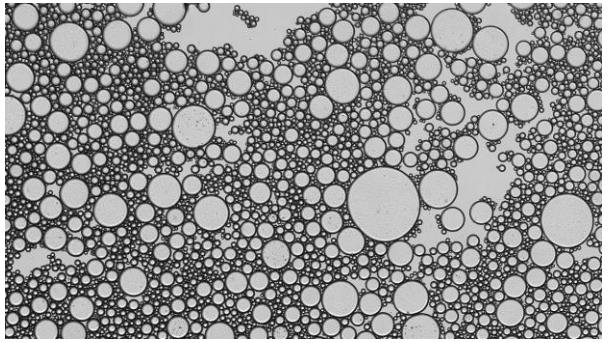
(Liquid-liquid) Phase separation(LLPS) as a concept

In daily life

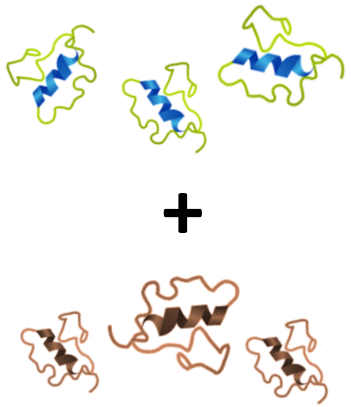




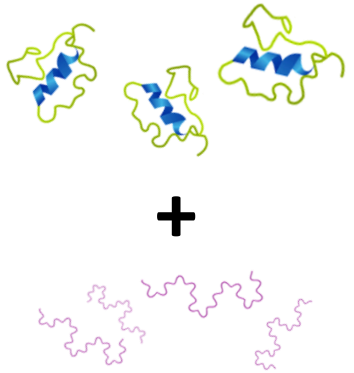
Water/oil emulsion

In biology

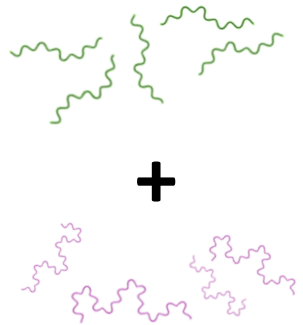
Protein-Protein (I)

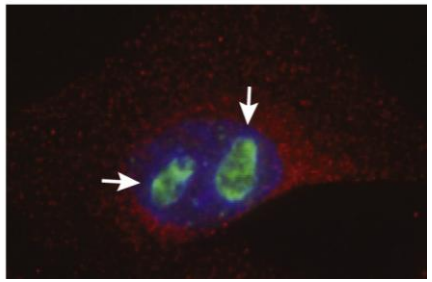


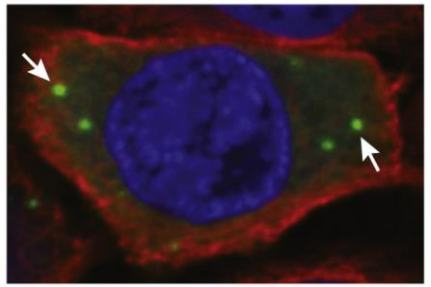
Protein-RNA (II)



RNA-RNA (III)

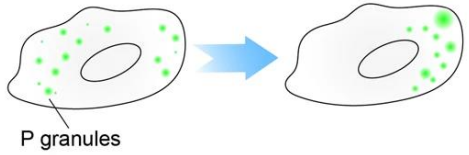






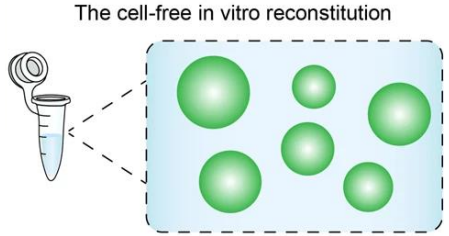
granule
(bio-)condensate
RNP droplet
RNP body
paraspeckle
assembly
…

The young history of LLPS in biology



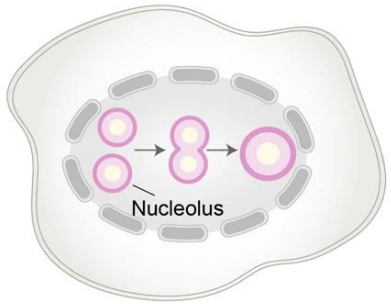
Hyman & Brangwyne:
P-granules exhibits liquid droplet like behaviors and rapidly dissolve and condense!

Steven McKnight:
First in-vitro model for the architecture and formation of RNA granules



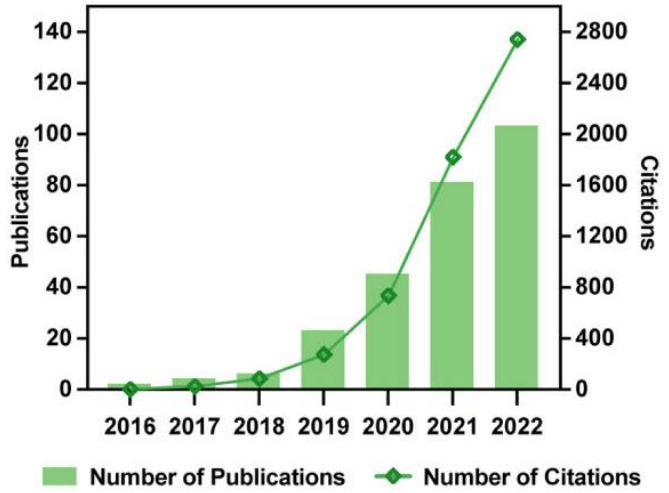
2009 | 2011 | 2012 | 2013 - present

Hyman & Brangwyne:
Nucleolus exhibits liquid droplet like behaviours!

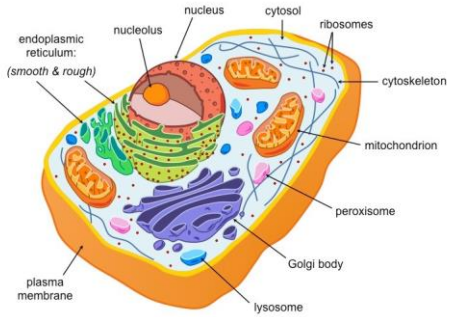


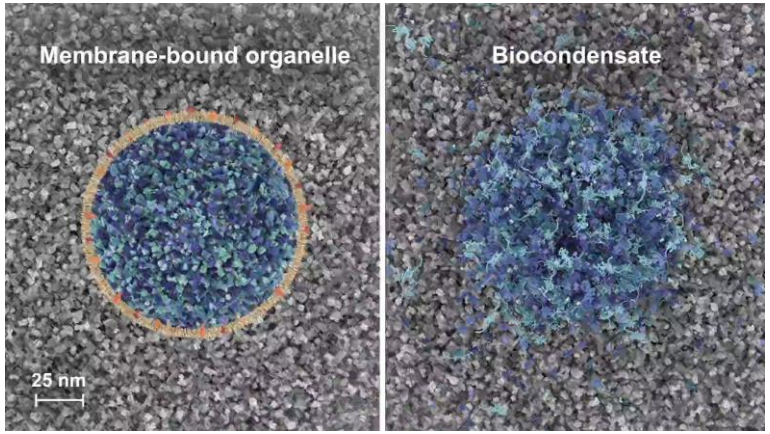
LLPS shown to be involved in many aspects:

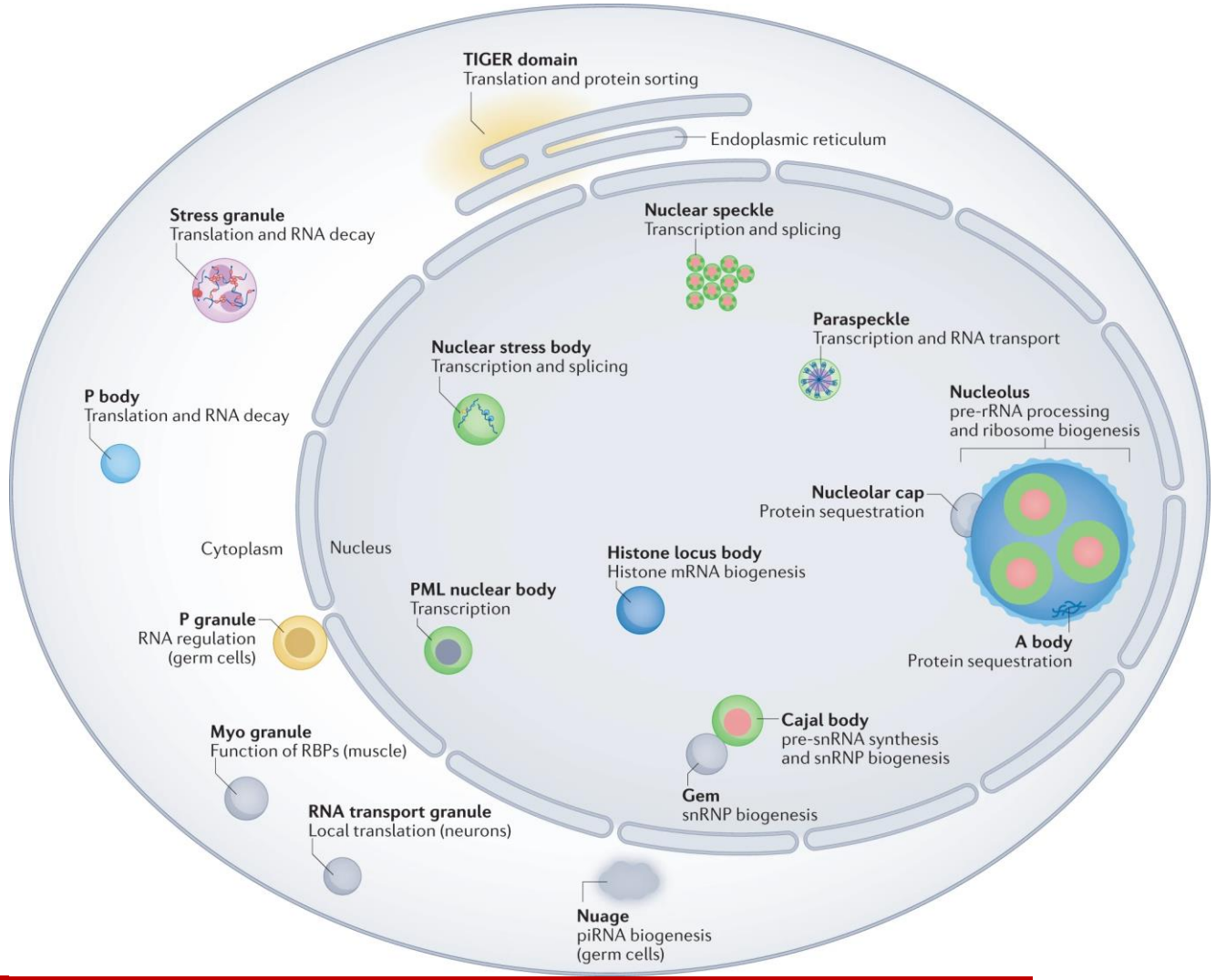
- mediation of the assembly of adhesion complexes
- condensation of cGAS activates innates immune signaling
- LLPS promotes T cell receptor signal transduction
- promotes miRISC assembly
- …



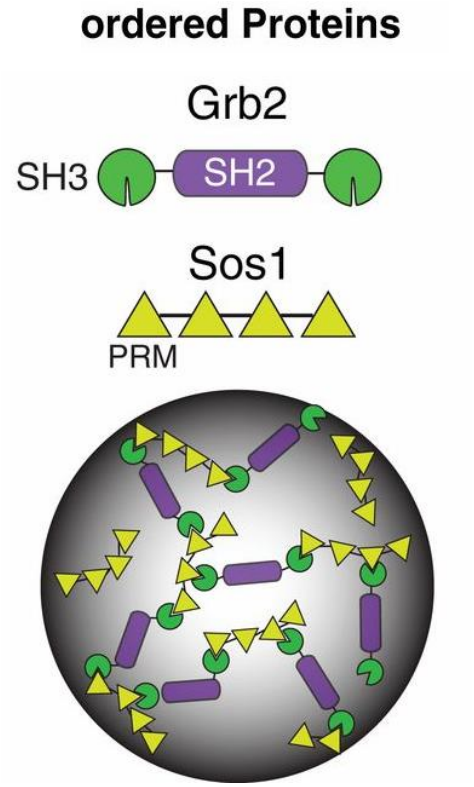
Membrane-less organelles (MLOs)

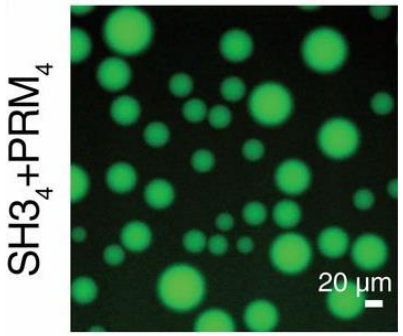




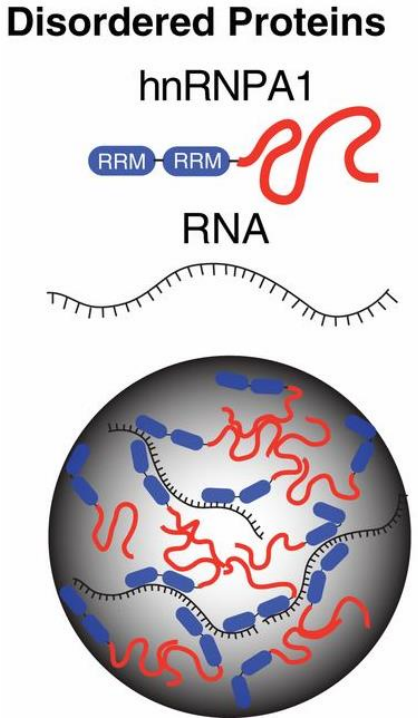


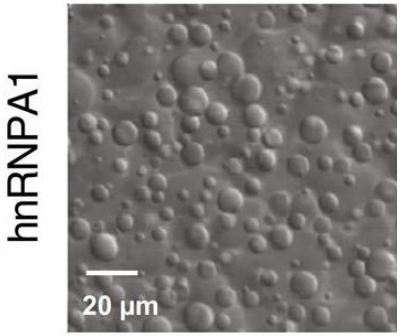
The mechanisms behind LLPS


ordered Proteins
Grb2
SH3
SH2
Sos1
PRM



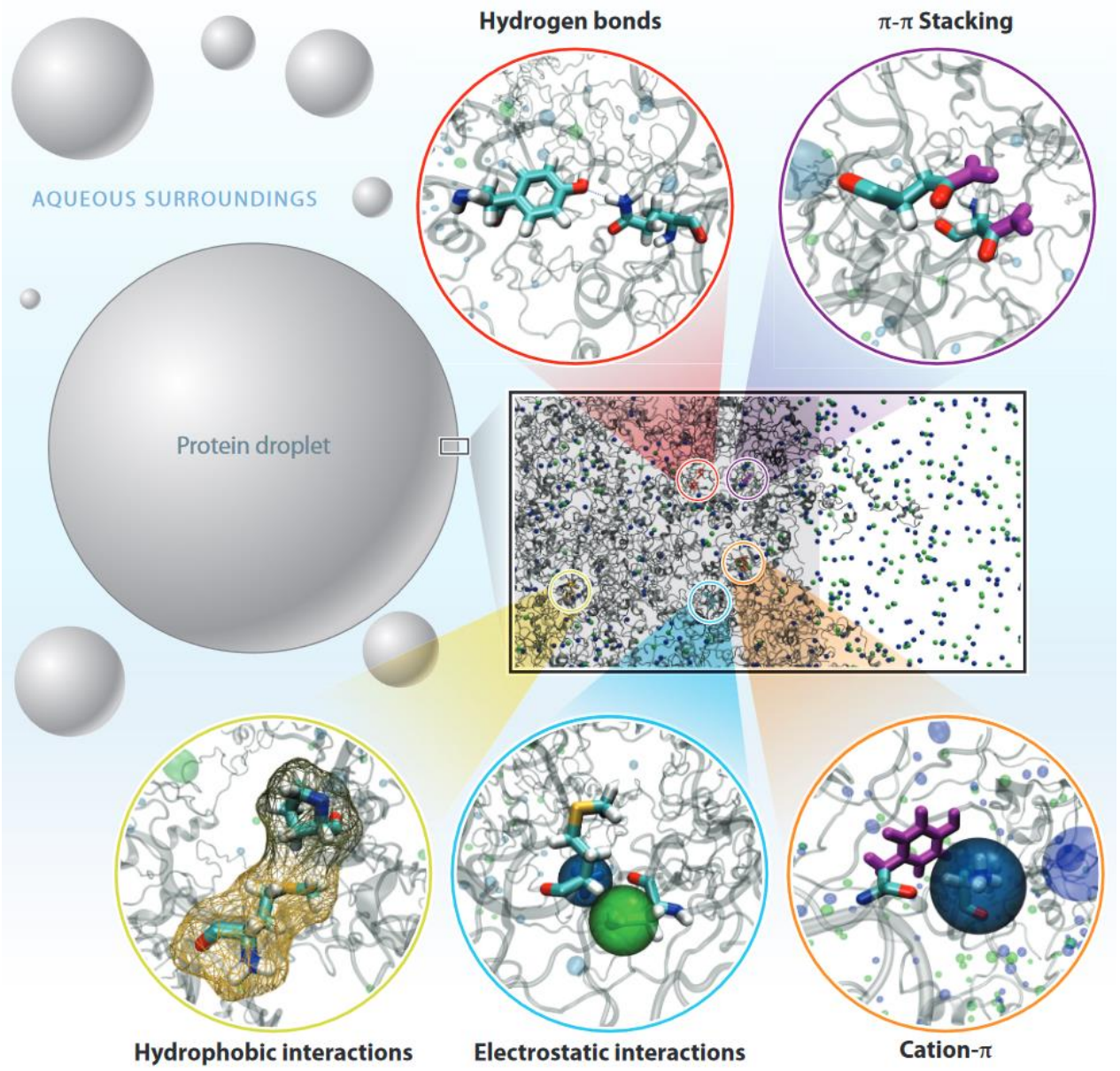
<u>driven by:</u>
- “strong” protein interactions between folded domains





<u>driven by:</u>
- weak, transient **multivalent** interactions between IDRs and/or RNAs:

1. Hydrogen bonds
2. π - π stacking
3. Hydrophobic interactions
4. Electrostatic interactions
5. Cation-π stacking



Hydrogen bonds

- shown to stabilize phase separation
- all amino acids able to participate, but mostly from polar ones
- Likely very important for incorporating RNA/DNA in condensates

Arginine
Arg (R)

Lysine
Lys (K)

Histidin
His (H)

pos. charged

Aspartate
Asp (D)

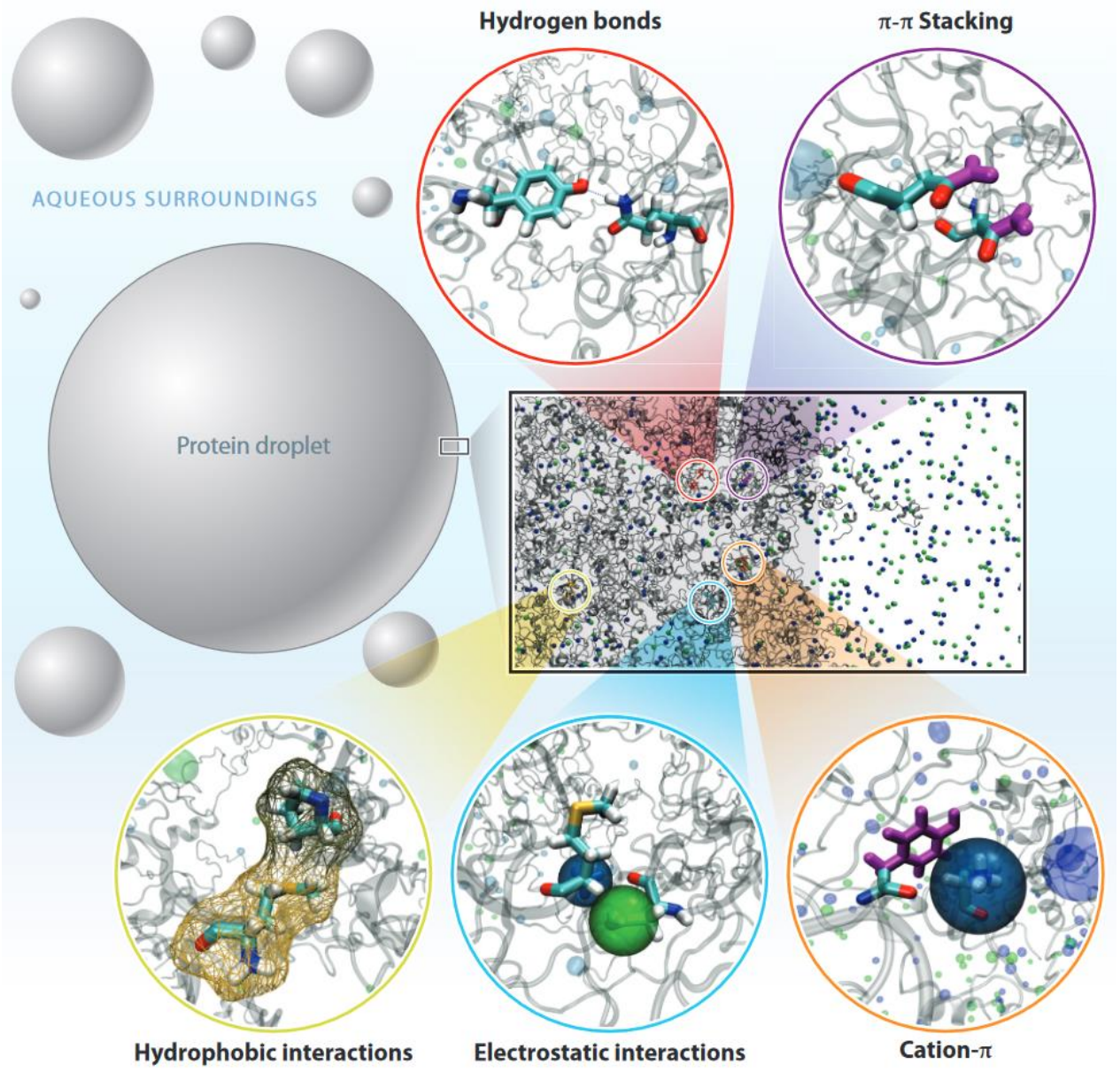
Glutamate
Glu (E)

neg. charged

Asparagine
Asn (N)

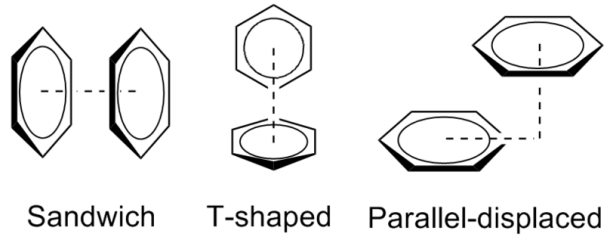
Glutamine
Gln (Q)

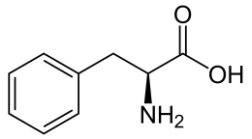
not charged but strong polar



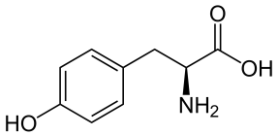
π – π stacking

- interactions between sp^2-hybridized groups
  - mostly **aromatic rings**
  - possibly the strongest of the 5 interaction types

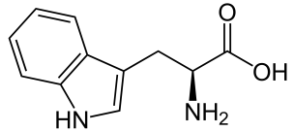




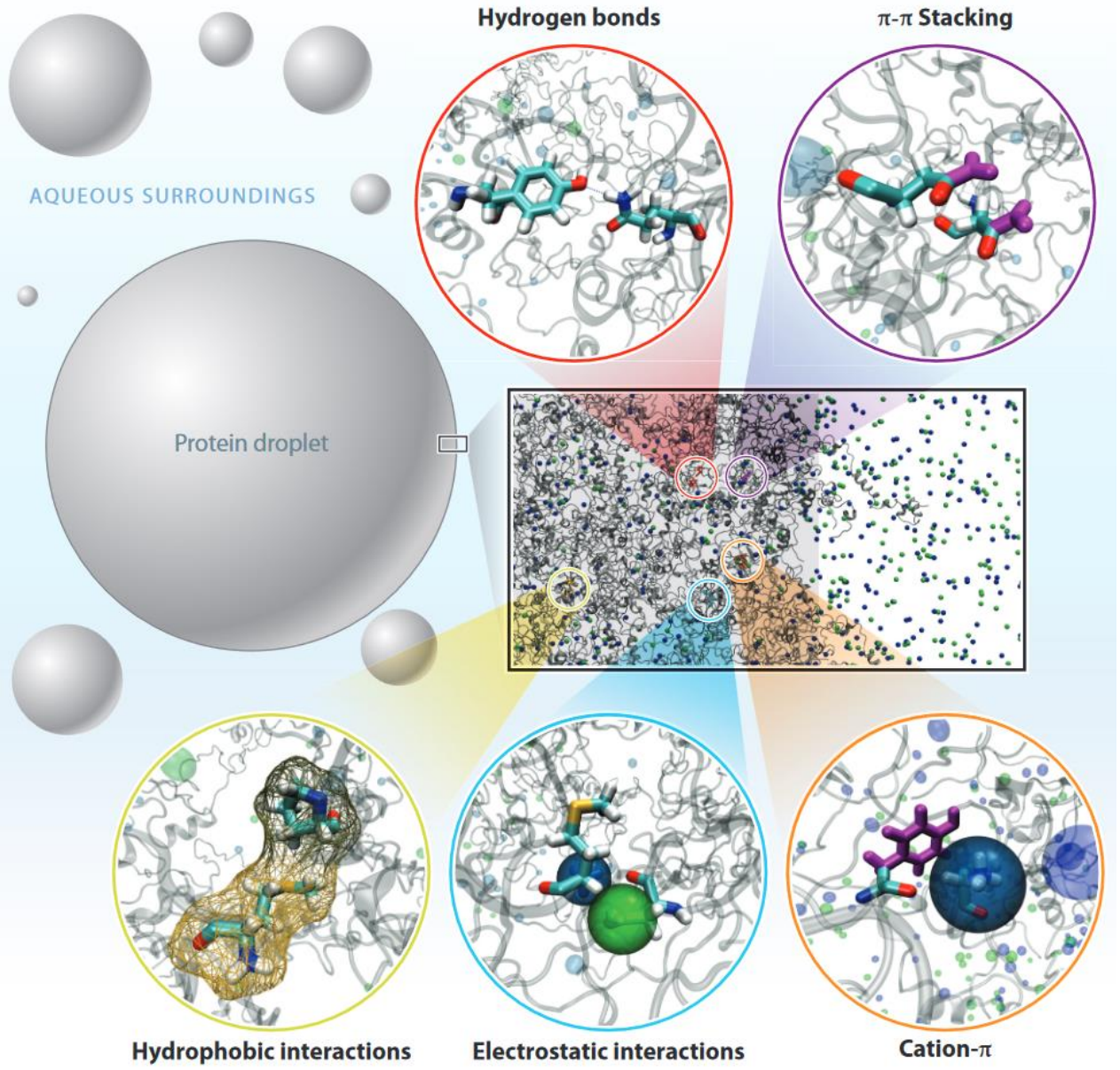
Phenylalanine
Phe (F)



Tyrosine
Tyr (Y)



Tryptophan
Trp (W)

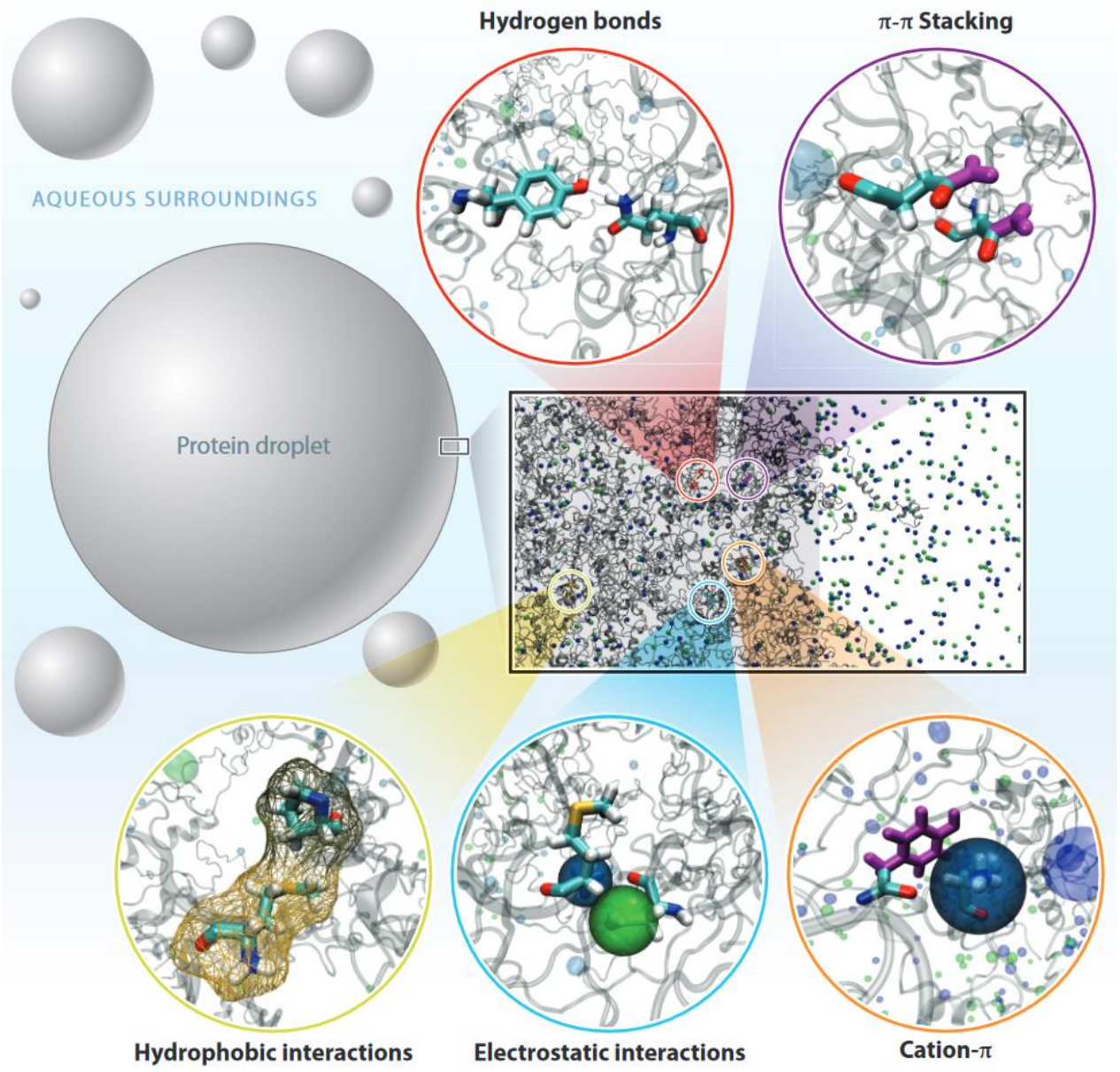


Hydrophobic interactions

- generally a major force of **protein folding**
  - **BUT:**
    - hydrophobic leucine-rich helices in the processing body (MLO) **stabilizes** the formation as condensate
    - important role in recruiting specific ligands into the condensed phase

hydrophobic (aliphatic)

Isoleucine	**Valine**	**Leucine**	**Alanine**	**Glycine**
Ile (I)	Val (V)	Leu (L)	Leu (L)	Leu (L)



Electrostatic interactions

- intrinsically disordered proteins (IDPs) are usually **enriched in charged amino acids**
- strongly affected by pH, salt concentration

Arginine
Arg (R)

Lysine
Lys (K)

Histidin
His (H)

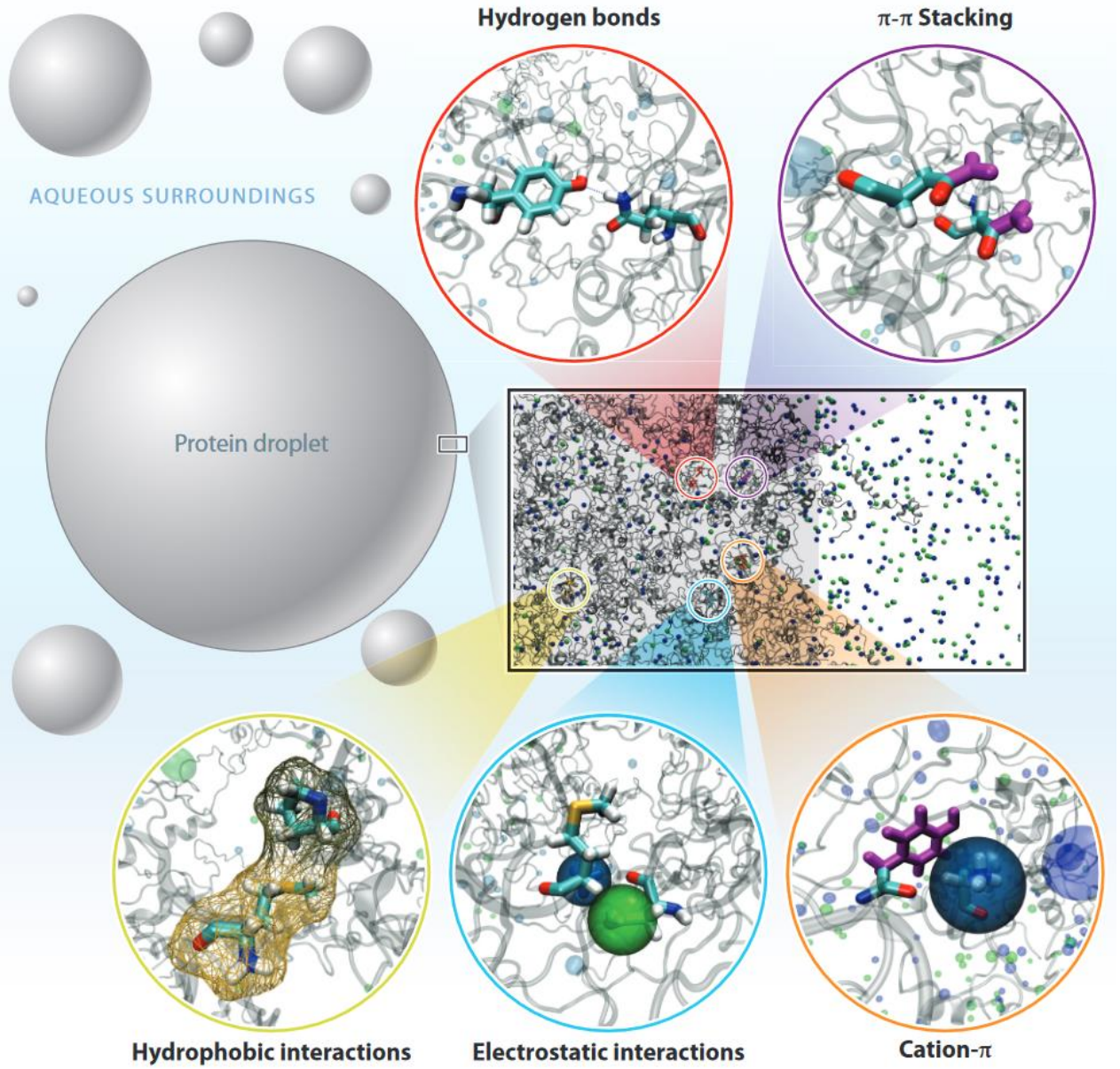
pos. charged

Aspartate
Asp (D)

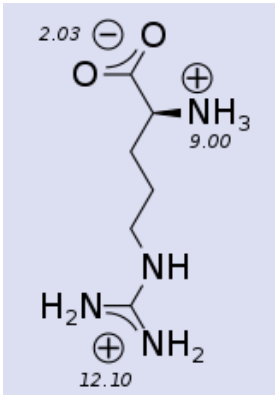
Glutamate
Glu (E)

neg. charged

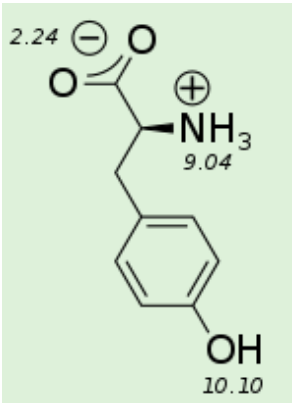
Cation-π interaction



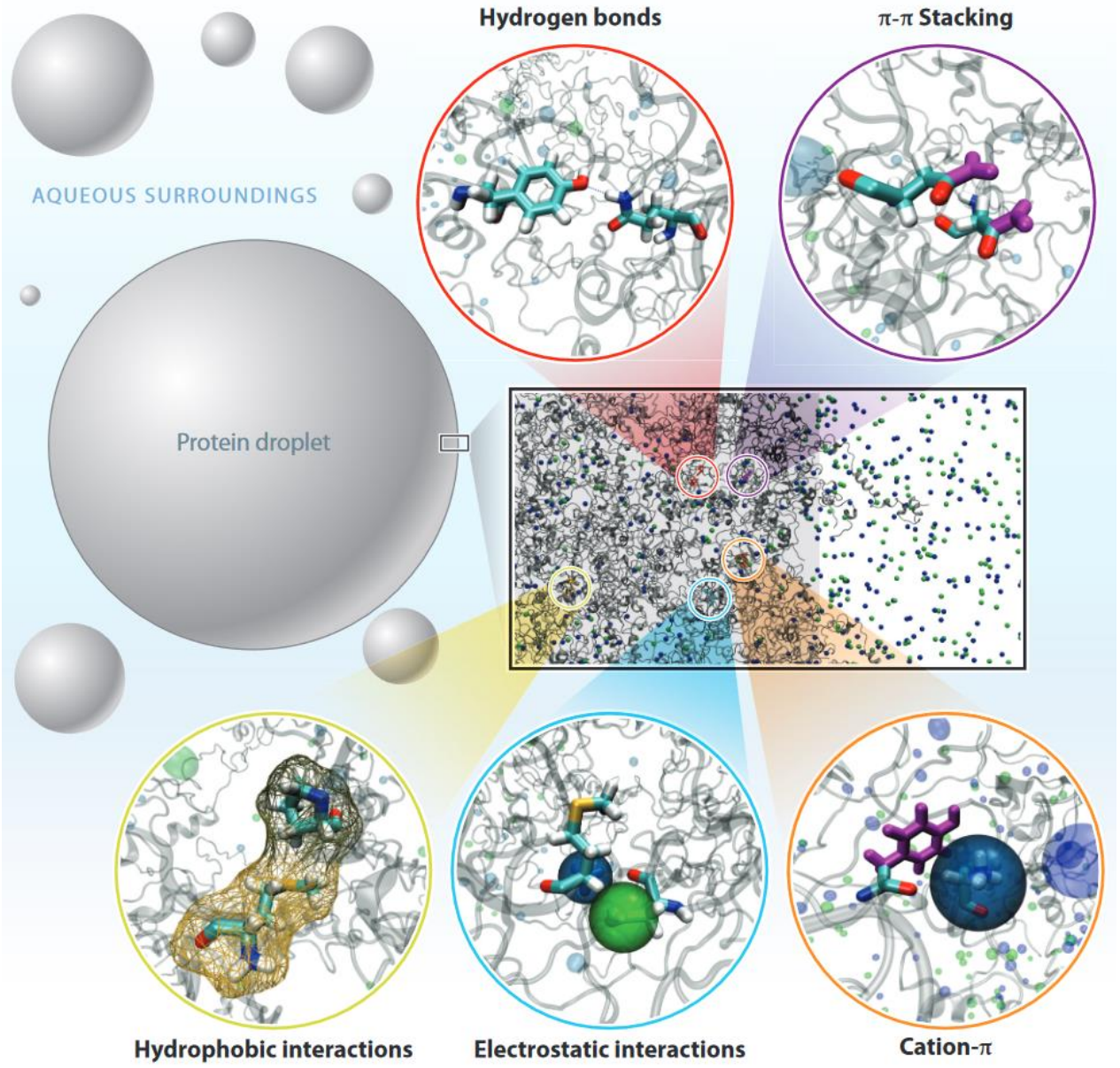
- aromatic rings and cationic residues, particularly between **arginine** and **tyrosine** residues



Arginine
Arg (R)

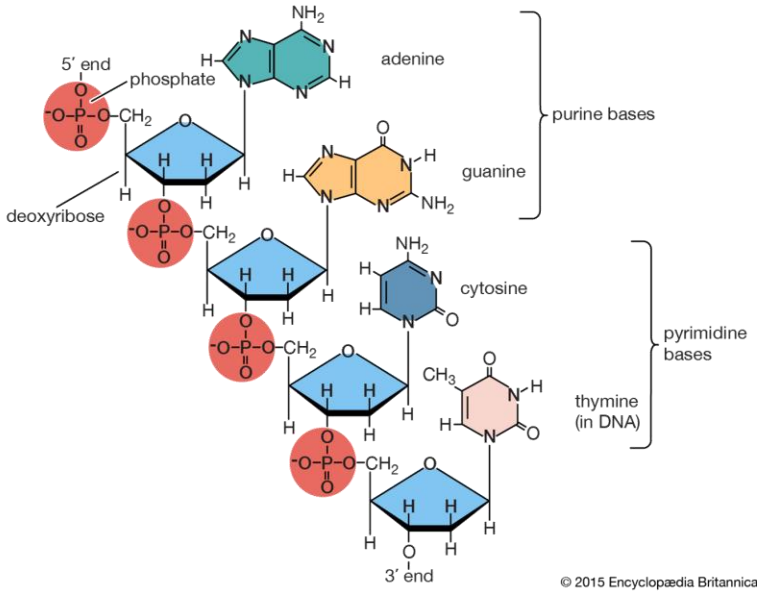


Tyrosine
Tyr (Y)



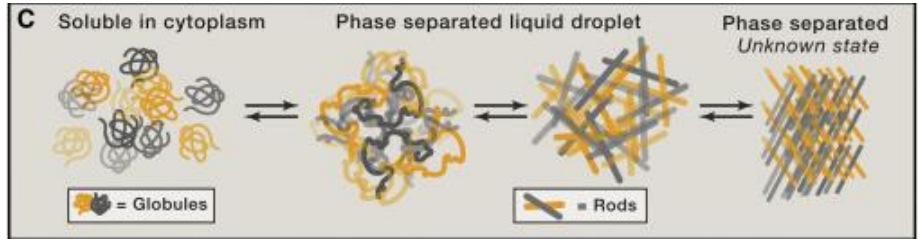
Cation-π interaction

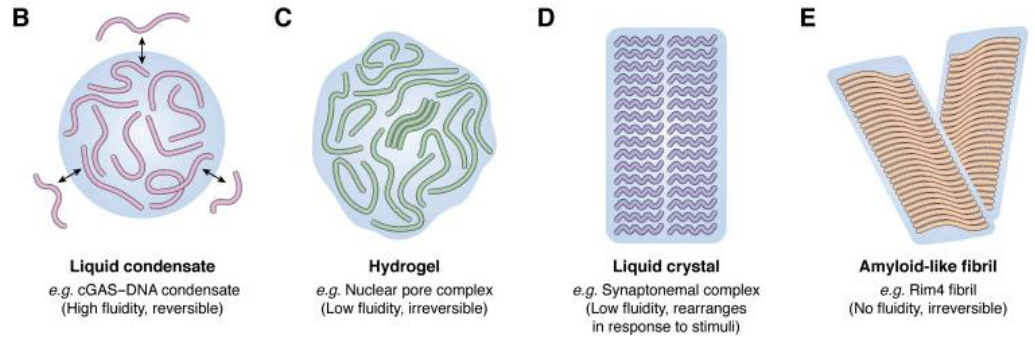
- this and π - π stacking apply also to the aromatic rings of **RNA** and **ssDNA**
  - Why not dsDNA?

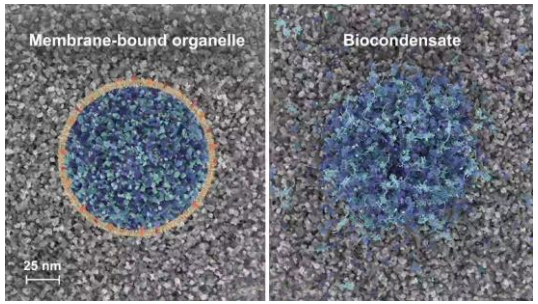


The dynamic nature of phase separation

- organelles with membranes are not dynamic
- MLOs are highly dynamic!
  - quick assembly and disassembly
  - change of state
  - interaction with surrounding phase





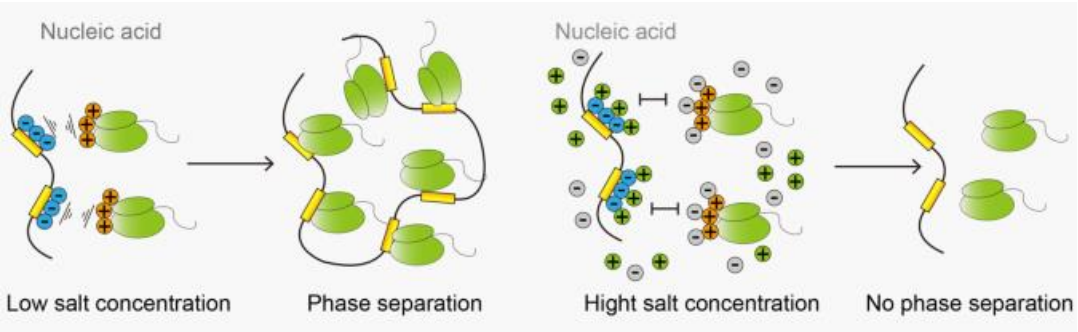


How does the organism "control" LLPS behavior?

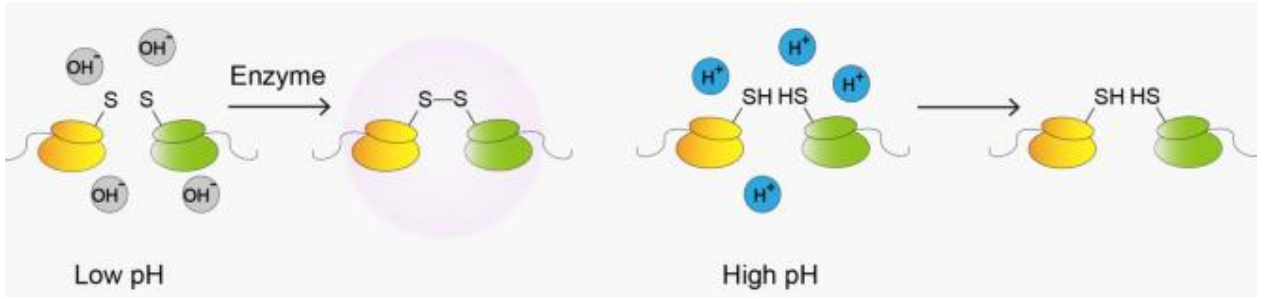
The role of temperature, pH and salt concentration in LLPS

- biocondensates usually have a lower critical solution temperature (LCST) and an upper critical solution temperature (UCST)
  - not that applicable **in-vivo** but important for **in-vitro** studies of LLPS

- salt concentration may induce or prevent phase separation
  - not only explained by charge changes



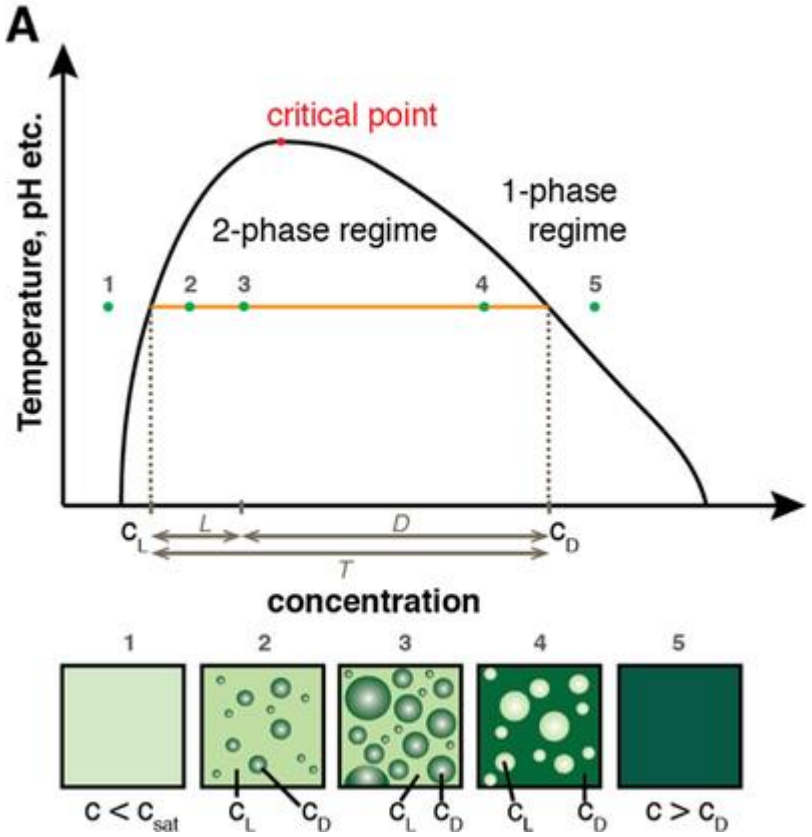
- pH
  - reduced pH causes stress granules (SG) formation
  - but most likely affects LLPS in both direction

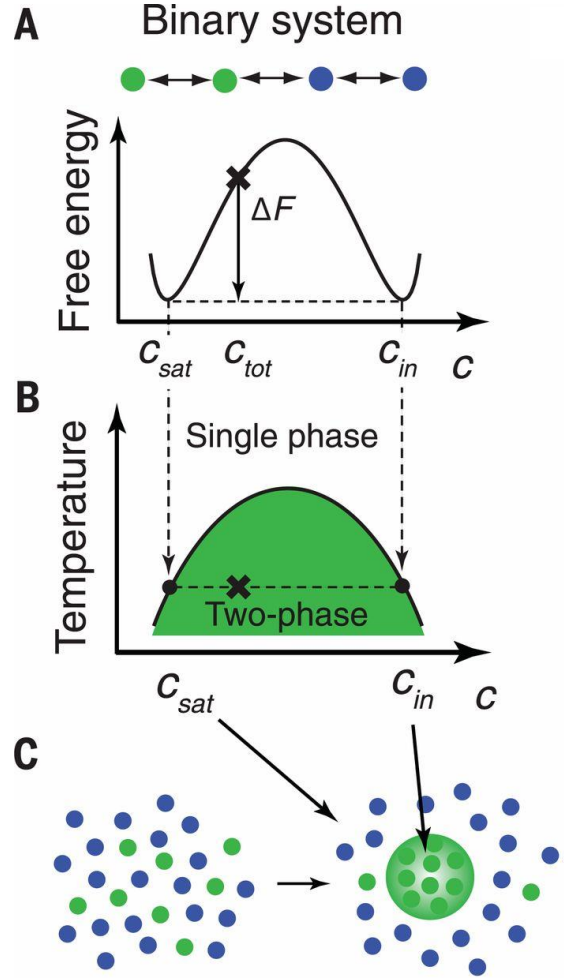


CONTEXT DEPENDENCY

phase diagrams in LLPS

- dilute (c_L or c_{SAT}) & dense (c_D or c_{in}) phase concentrations are **fixed**



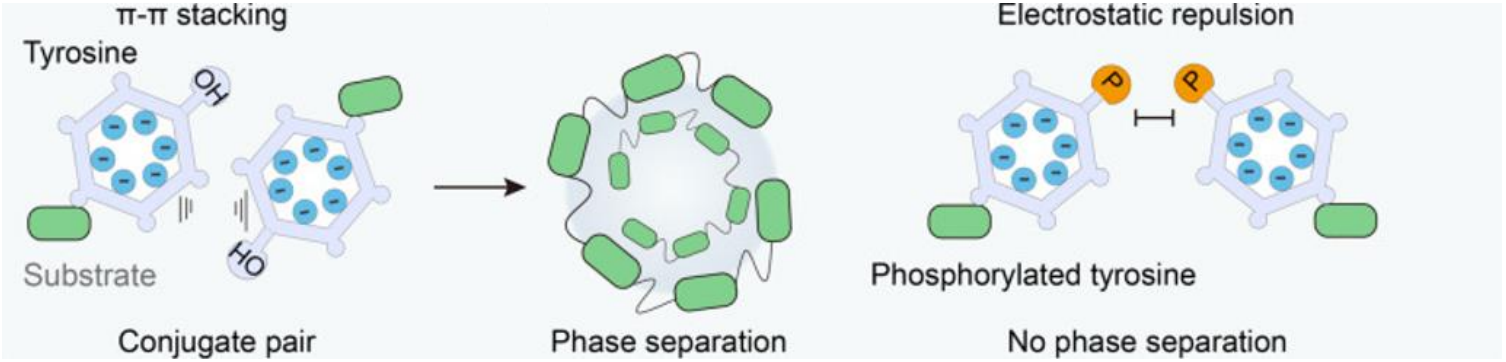


Post-translational modifications (PTMs)

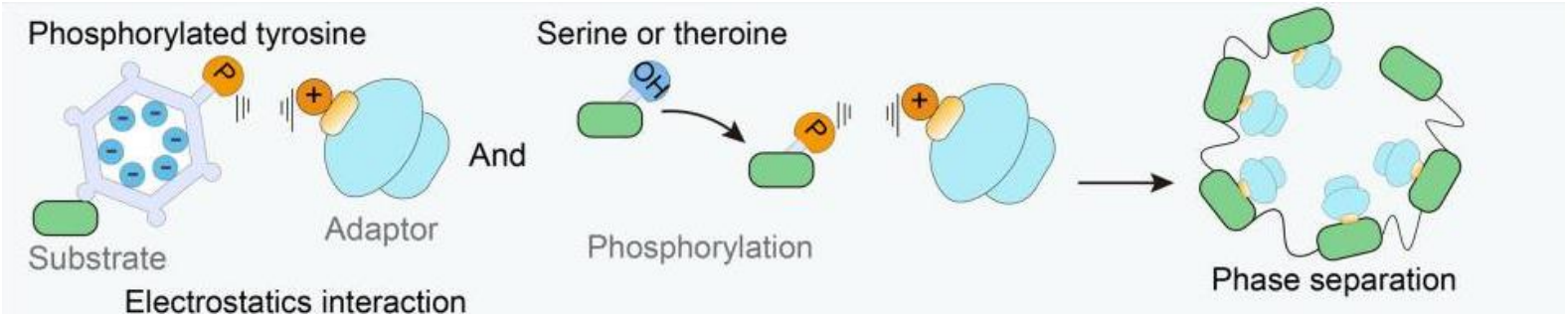
IDRs are more prone to PTMs than folded domains!

PTMs change the chemical properties of the residues → multivalency directly affected

Phosphorylation **hinders** phase separation

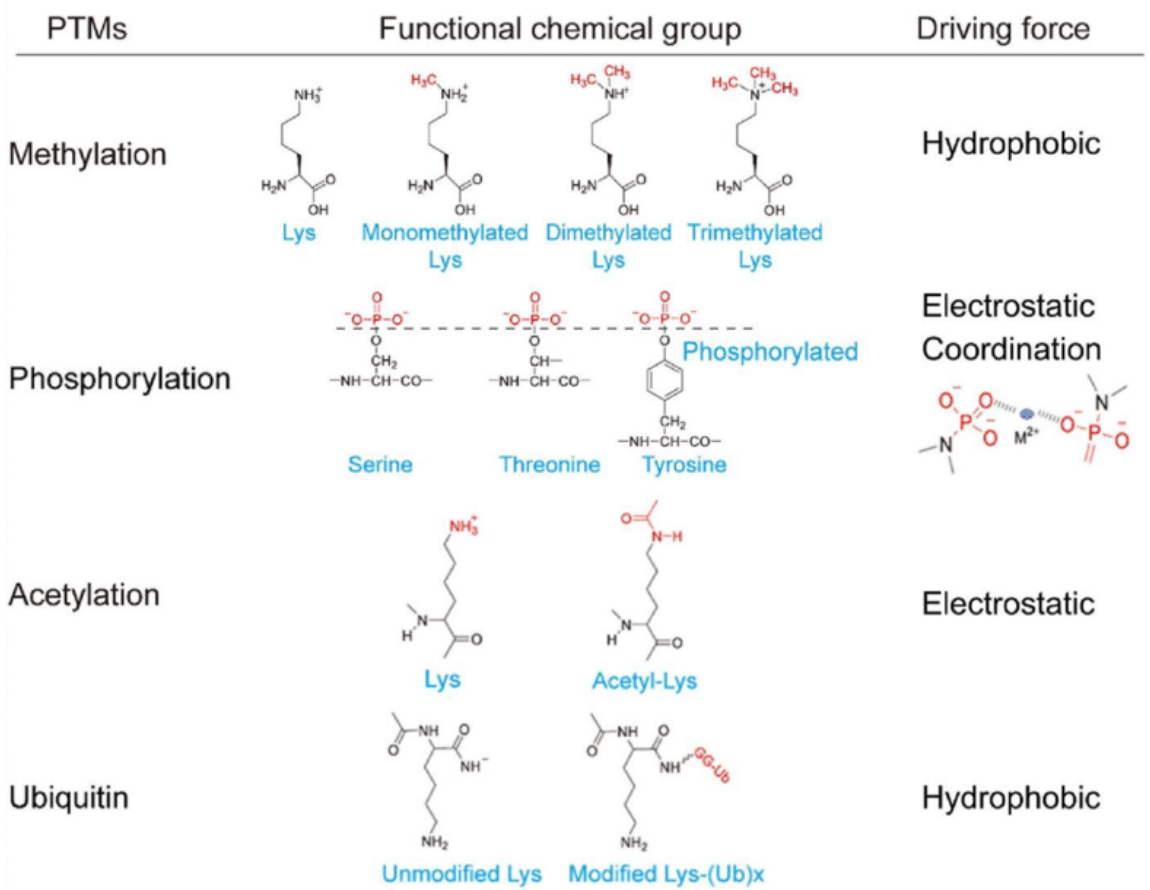


Phosphorylation **facilitates** phase separation



CONTEXT DEPENDENCY

Post-translational modifications (PTMs)

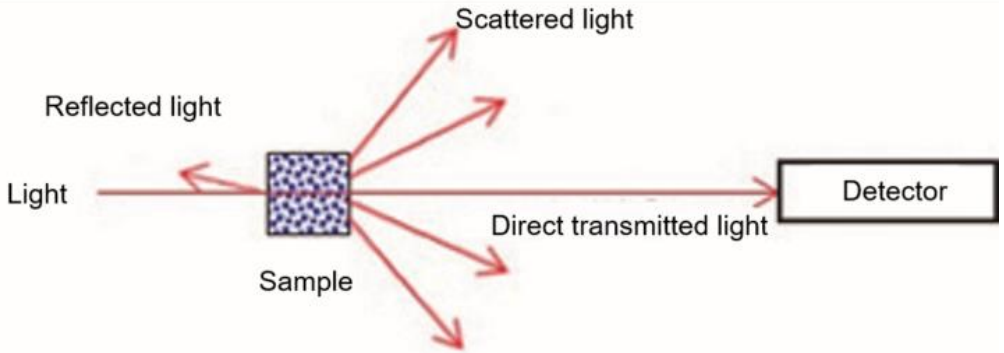


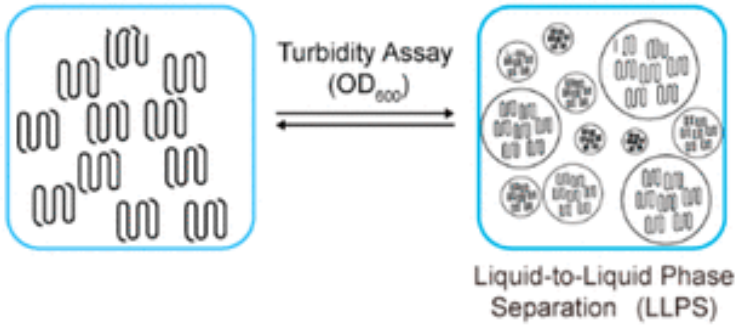
more PTMs suspected to play large roles in LLPS formation!

Methods to study LLPS - Turbidity

no LLPS induced

LLPS induced

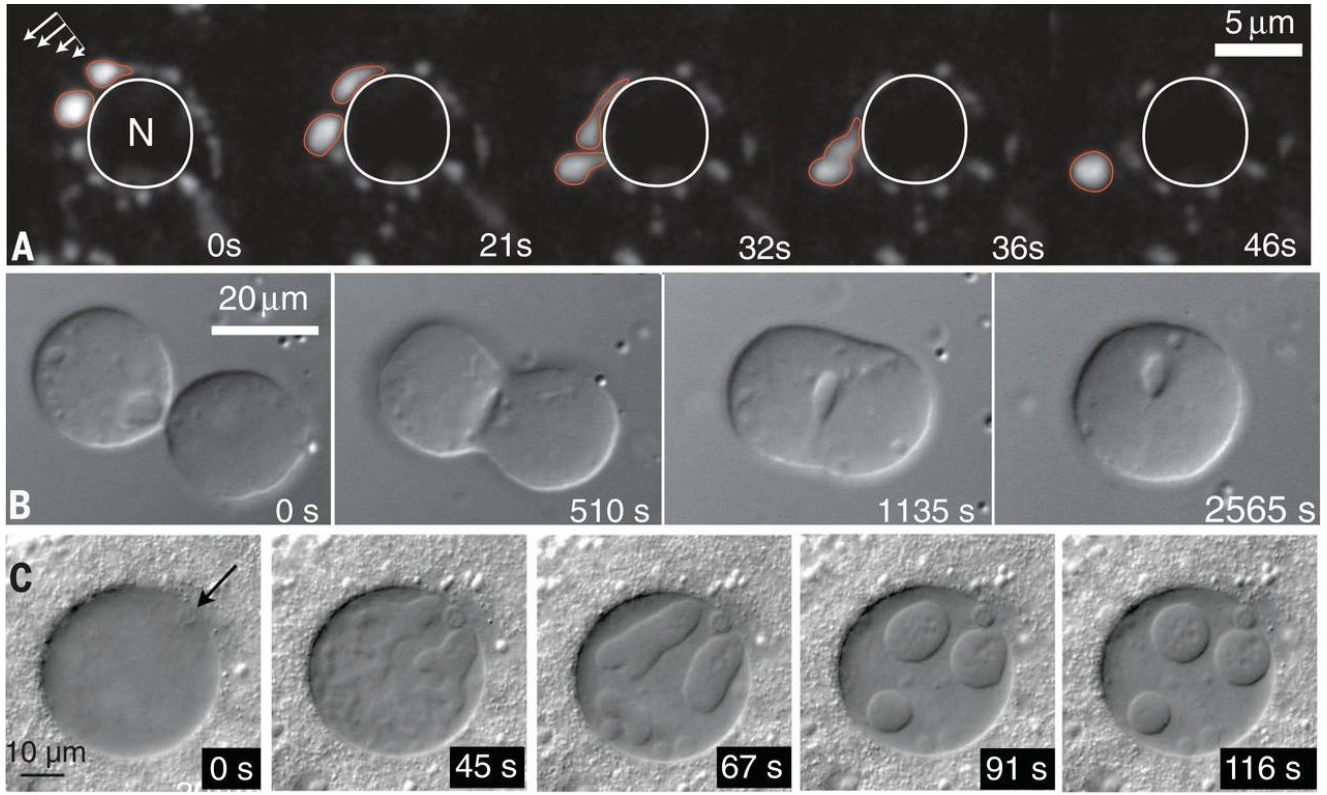




- only in-vitro applicable
- simple, cheap, useful for preliminary experiment

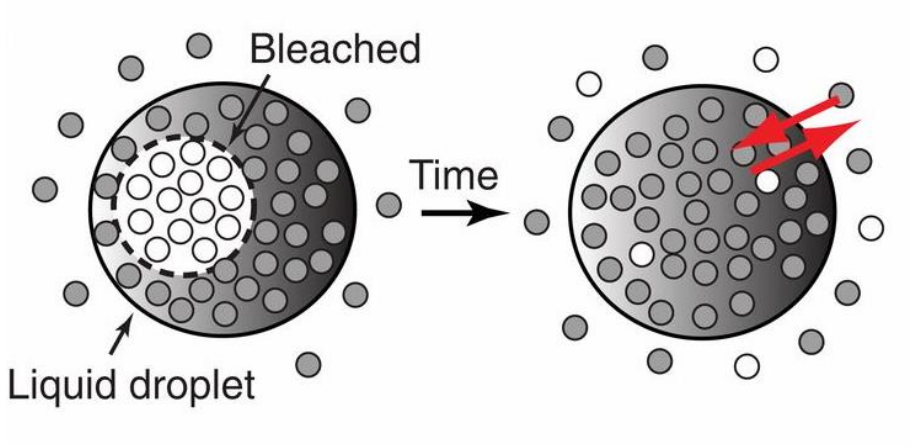
Methods to study LLPS - (Fluorescence) microscopy

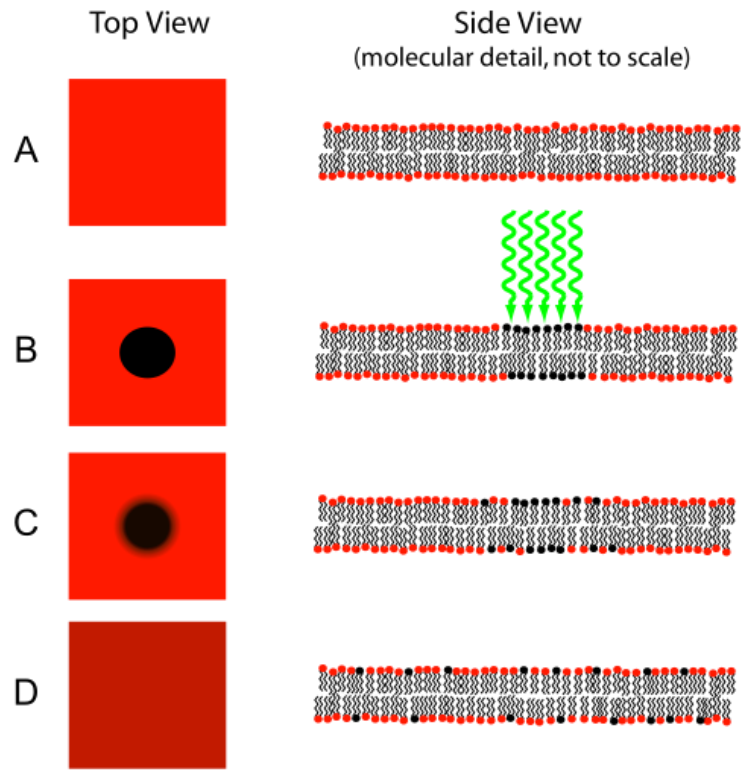
- in-vitro **AND** in-vivo
- common and very flexible
- antibody-staining or fluorescent labeling necessary

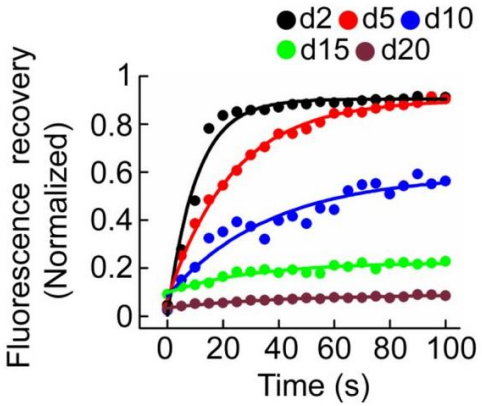


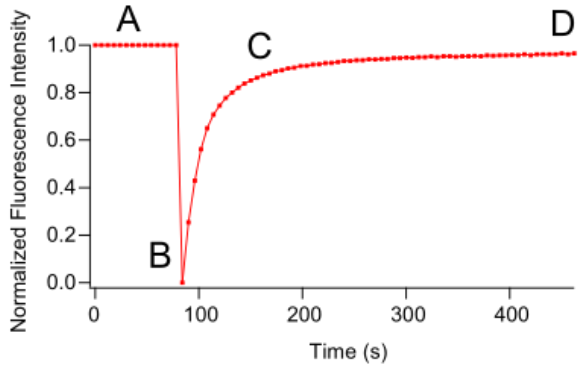
Fluorescence recovery after photobleaching (FRAP)

can quantify the "fluidity" of a biocondensate!

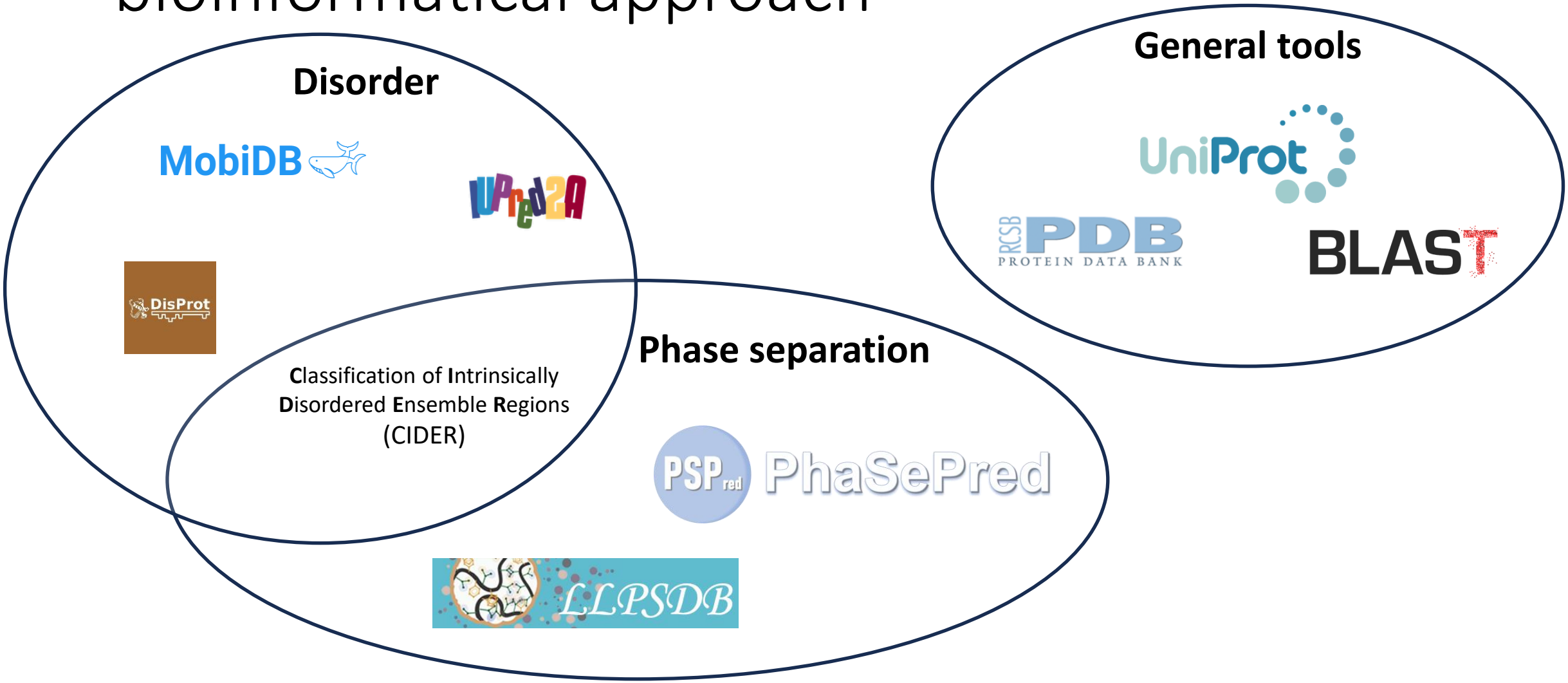








Database/resources and predictors for a bioinformatical approach



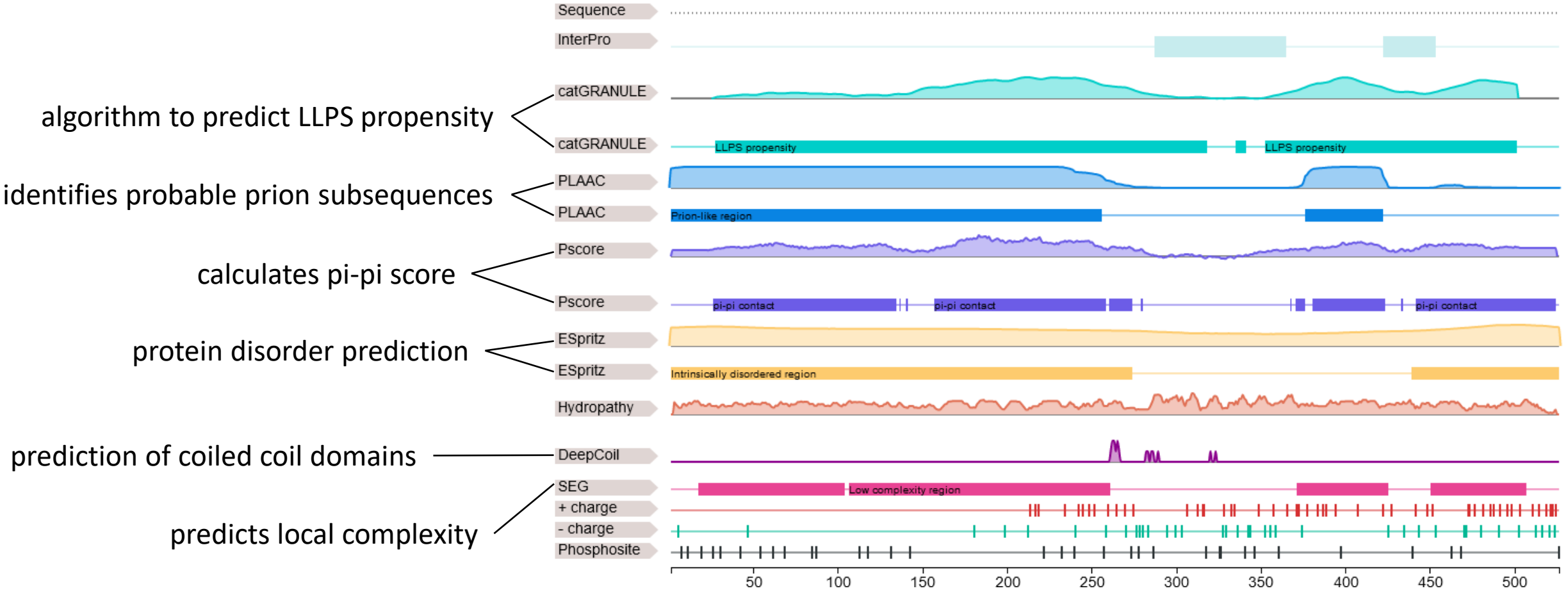
PhaSePred: A Meta-predictor For Phase-Separating Proteins

Protein Information					
Uniprot Entry	P35637	**Entry name**	FUS_HUMAN	**Gene name**	FUS TLS
Length	526	**Status**	reviewed	**Organism**	Homo sapiens (Human)

PhaSePred Scores				
	Score (8-feature)	**Rank (8-feature)**	**Score (10-feature)**	**Rank (10-feature)**
PS-Self score (Proteins that can self-assemble to form condensates)	0.928	0.993	0.930	0.994
PS-Part score (Proteins whose phase separation behaviors are regulated by protein or nucleic acid partner components)	0.817	0.967	0.911	0.981

*** The 8-feature model incorporates Hydropathy, FCR, IDR, LCR, PScore, PLAAC, catGRANULE, and DeepCoil.**
The 10-feature model incorporates the 8 features described above plus Phos frequency and DeepPhase. This model is only available for human proteins.

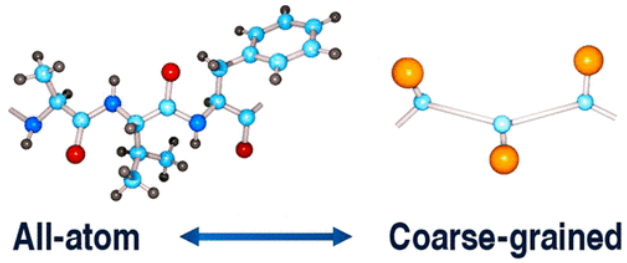
PhaSePred: A Meta-predictor For Phase-Separating Proteins



Molecular dynamics (MD) for LLPS simulation

computer simulation method for analyzing the physical movements of atoms and molecules

- for LLPS used: **coarse-grained** models
  - e.g. MARTINI model
  - each amino acid is one „element“

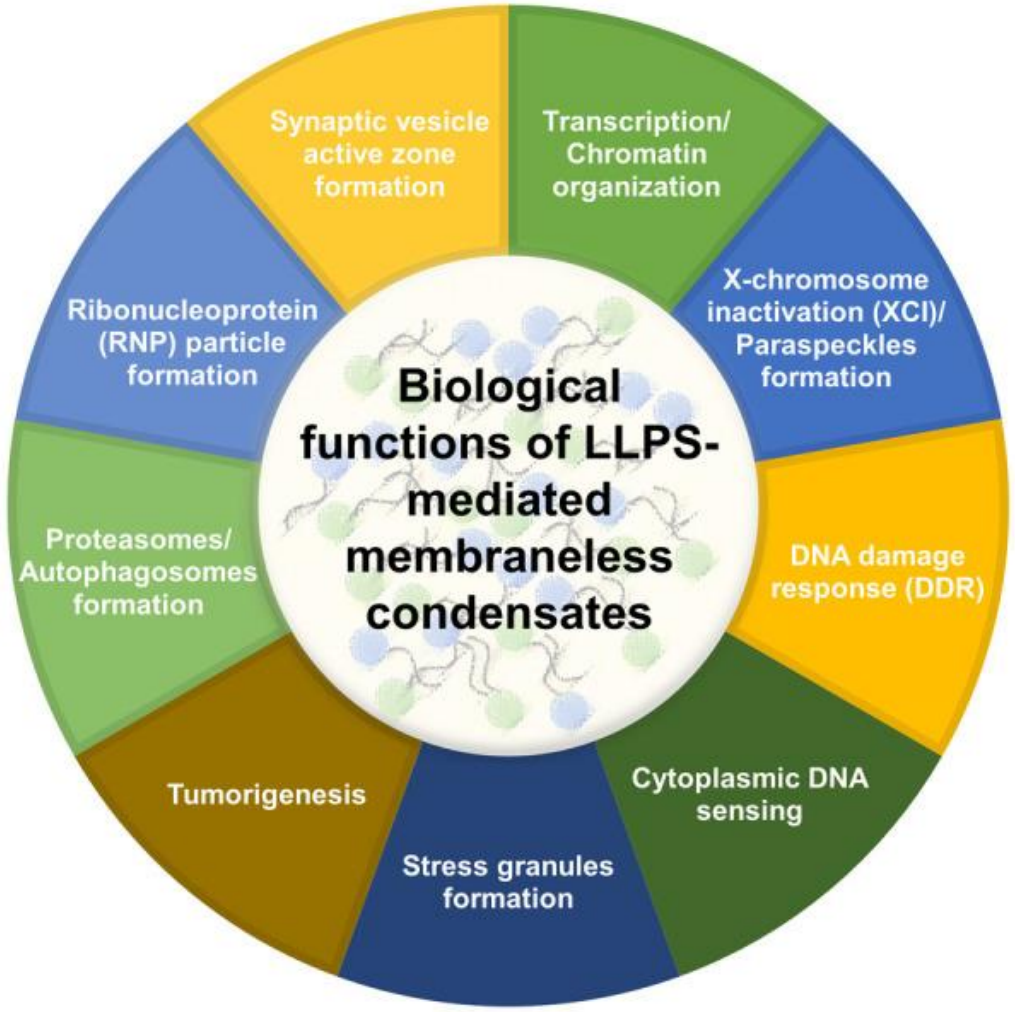


MD simulation promise easy, cheap and fast research on LLPS!

BUT

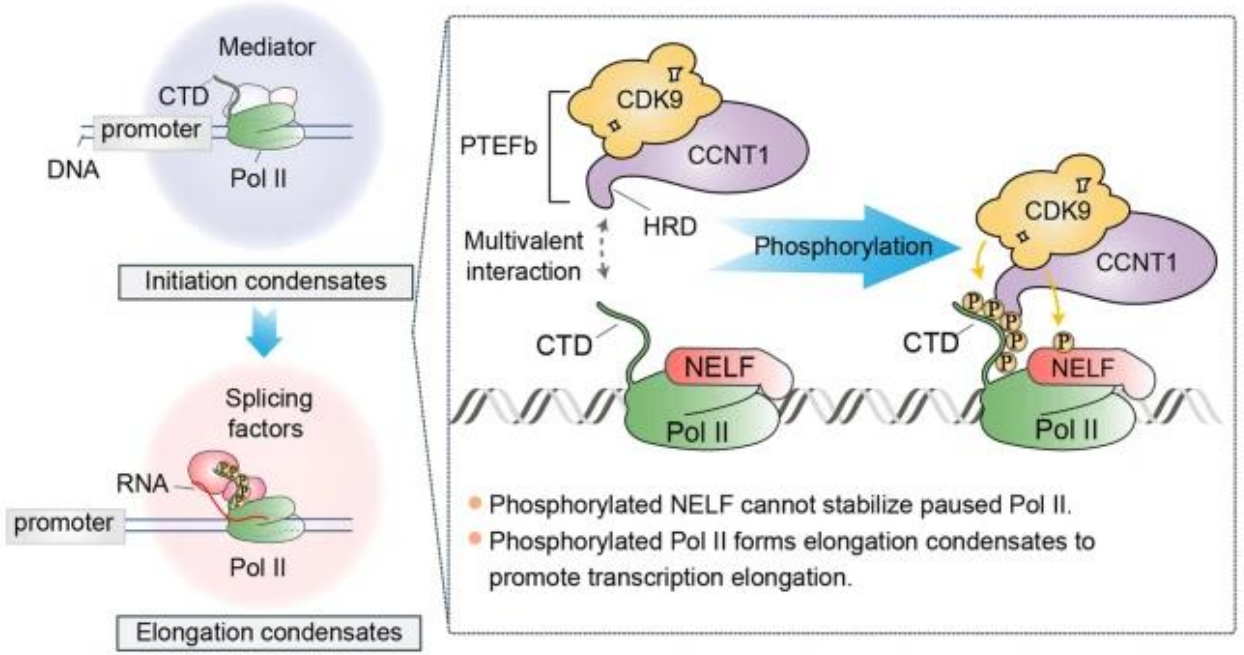
- simplified model loses information (folded domains not considered)
- parametrization of model complicated
- simulation of an artificial subset of the cell
- …

Functions of Phase separation in biology



Understanding function enables us to manipulate LLPS for therapeutic application!

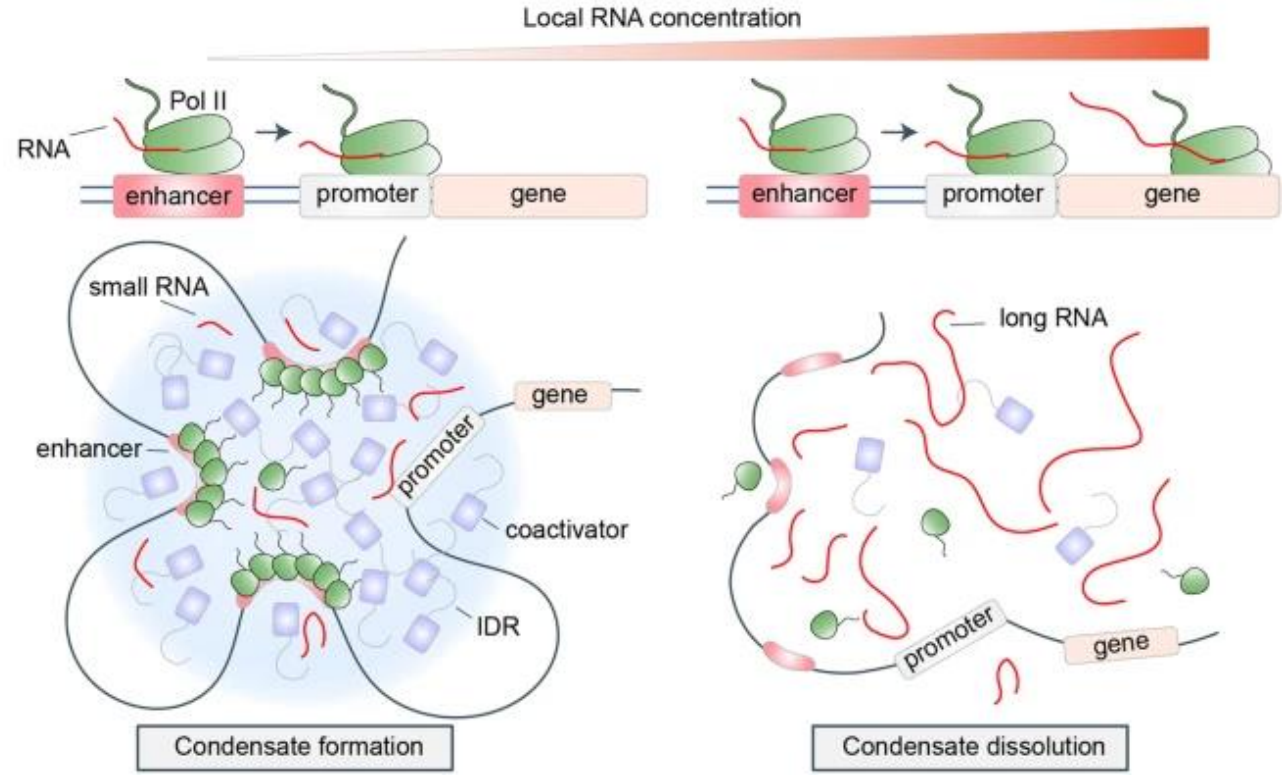
How LLPS regulates transcription



- transcription consists of initiation, elongation and termination
- the initiation complex forms by **condensate** of RNA polymerase II (Pol II), transcription factors and coactivators
- PTEFb recruited through multivalent LLPS mechanics!
- Phosphorylated Pol II (with different multivalency) forms elongation condensates with very different factors (splicing factors)

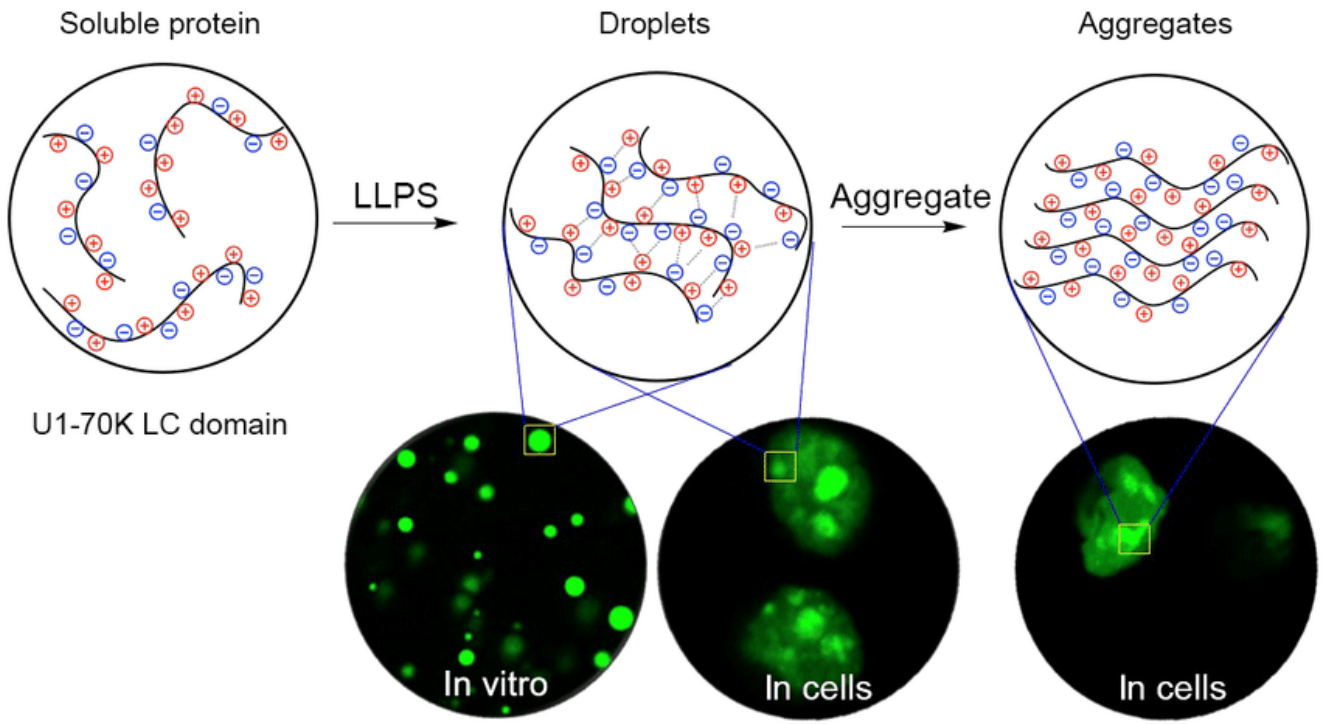
How LLPS regulates transcription

- feedback mechanism in (super)enhancers is controlled by phase separation!
- low levels of RNA **promote** the formation of transcriptional condensates (based around Pol II)
- high levels of RNA can **dissolve** the transcription condensates

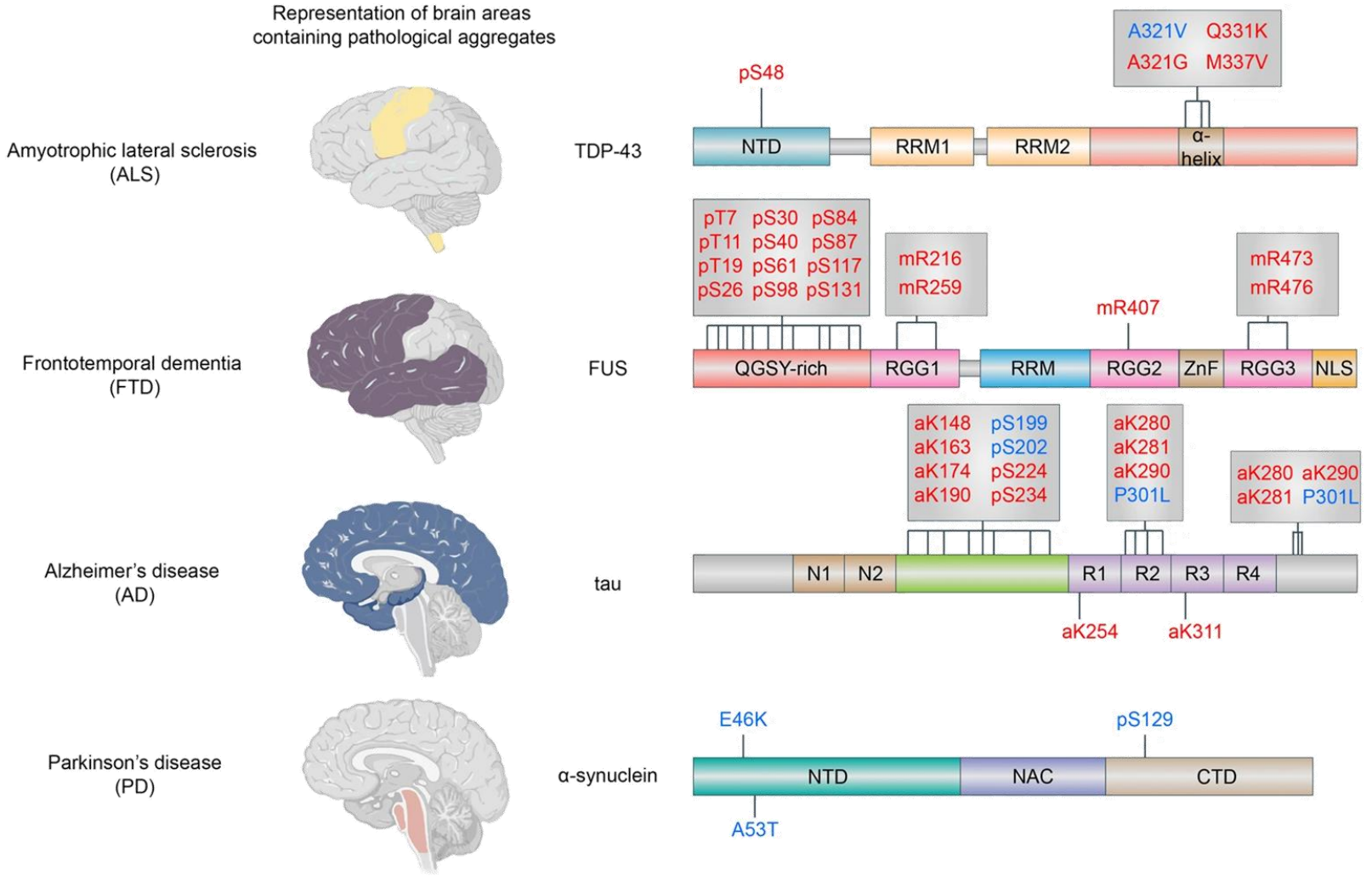


LLPS in neurodegenerative diseases

protein aggregation is largely aberrant phase separation!

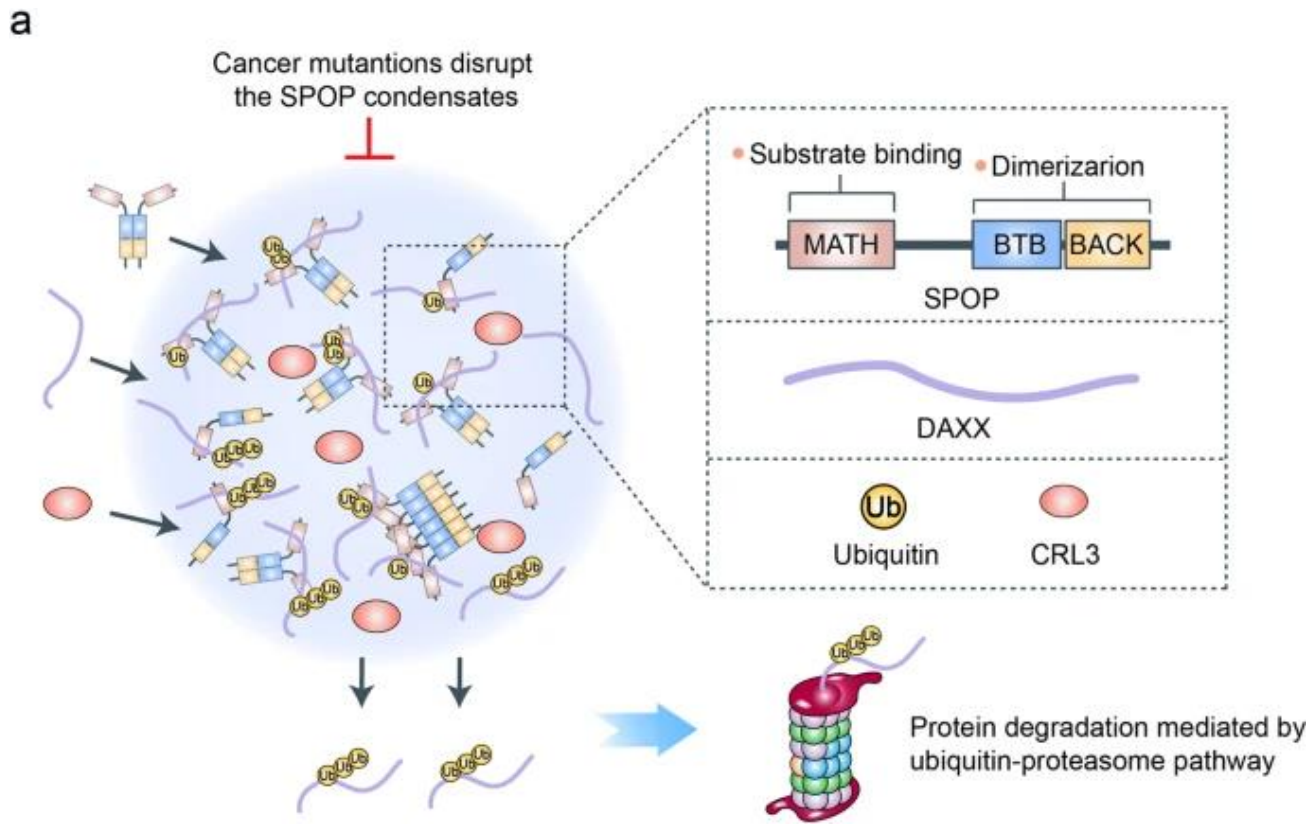


LLPS in neurodegenerative diseases



LLPS in cancer

lots of identifications of specific mutations in proto-oncogenes and tumor-suppressor genes that could cause cancer, but we don't know why!

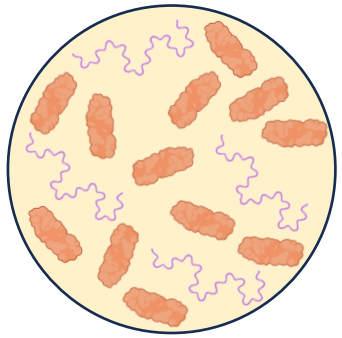


current ongoing RNA therapeutics clinical research to stabilize the condensate even with the mutation!

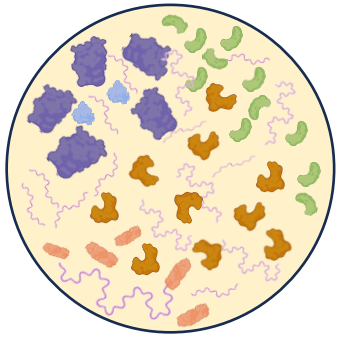
Future challenges and key questions

Complexity of natural condensates

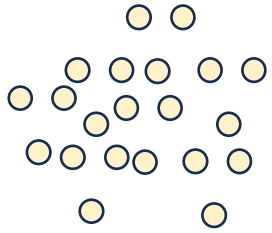
<u>Expectation:</u>
homogenous 1 or 2 phasesystem



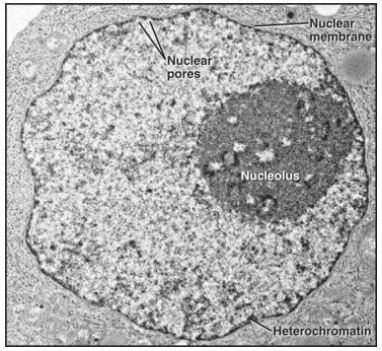
<u>Reality:</u>
up tp 100 different molecules & complex suborganization



The question of size



< 100 nm in size



up to 3,5µm in size!

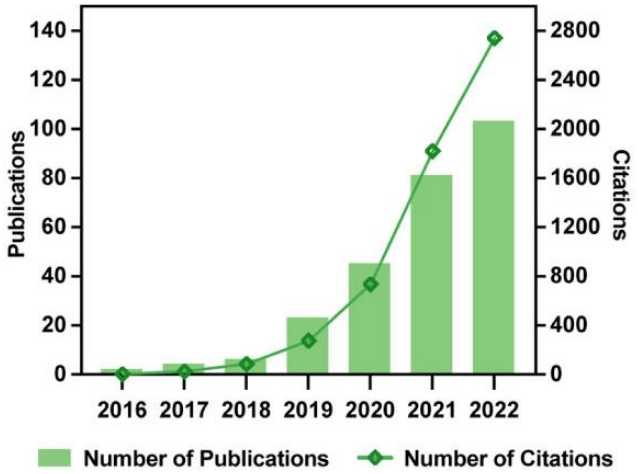
Classifying LLPS sequence features

Arginine & **Glycine**-rich regions

- certain LLPS properties
- RNA binding properties
- more biological functions?

Evolutionary considerations

Conclusions



1. LLPS exists in many biological areas and functions
2. LLPS is extremely complex in its formation and dynamic character and heavily context dependent
3. The importance of LLPS for therapeutic targets

Resources and interesting papers

- Wang, B., Zhang, L., Dai, T. *et al.* Liquid–liquid phase separation in human health and diseases. *Sig Transduct Target Ther* **6**, 290 (2021). https://doi.org/10.1038/s41392-021-00678-1
- Peng PH, Hsu KW, Wu KJ. Liquid-liquid phase separation (LLPS) in cellular physiology and tumor biology. Am J Cancer Res. 2021 Aug 15;11(8):3766-3776. PMID: 34522448; PMCID: PMC8414392.
- Alberti S, Gladfelter A, Mittag T. Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. Cell. 2019 Jan 24;176(3):419-434. doi: 10.1016/j.cell.2018.12.035. PMID: 30682370; PMCID: PMC6445271.
- https://animationlab.utah.edu/phase-separation
- Yongdae Shin, Clifford P. Brangwynne, Liquid phase condensation in cell physiology and disease.*Science***357**,eaaf4382(2017).DOI:10.1126/science.aaf4382
- Louis-Philippe Bergeron-Sandoval, Nozhat Safaee, Stephen W. Michnick, Mechanisms and Consequences of Macromolecular Phase Separation, Cell, Volume 165, Issue 5, 2016, Pages 1067-1079, ISSN 0092-8674, https://doi.org/10.1016/j.cell.2016.05.026.
- Mitrea, D.M., Kriwacki, R.W. Phase separation in biology; functional organization of a higher order. *Cell Commun Signal* **14**, 1 (2016). https://doi.org/10.1186/s12964-015-0125-7
- Lyon, A.S., Peeples, W.B. & Rosen, M.K. A framework for understanding the functions of biomolecular condensates across scales. *Nat Rev Mol Cell Biol* **22**, 215–235 (2021). https://doi.org/10.1038/s41580-020-00303-z
- Chunyu Yu, Boyan Shen, Kaiqiang You, Qi Huang, Minglei Shi, Congying Wu, Yang Chen, Chaolin Zhang, Tingting Li, Proteome-scale analysis of phase-separated proteins in immunofluorescence images, *Briefings in Bioinformatics*, Volume 22, Issue 3, May 2021, bbaa187, https://doi.org/10.1093/bib/bbaa187
- Dignon et al., Biomolecular Phase Separation: From Molecular Driving Forces to Macroscopic Properties (2020), Annual Review of Physical Chemistry, Vol. 71:53-75 (Volume publication date April 2020) https://doi.org/10.1146/annurev-physchem-071819-113553
- Li J, Zhang M, Ma W, Yang B, Lu H, Zhou F, Zhang L. Post-translational modifications in liquid-liquid phase separation: a comprehensive review. Mol Biomed. 2022 May 11;3(1):13. doi: 10.1186/s43556-022-00075-2. PMID: 35543798; PMCID: PMC9092326.
- Mitrea, D.M., Mittasch, M., Gomes, B.F. *et al.* Modulating biomolecular condensates: a novel approach to drug discovery. *Nat Rev Drug Discov* **21**, 841–862 (2022). https://doi.org/10.1038/s41573-022-00505-4