PhasAGE
EXCELLENCE HUB ON PHASE TRANSITIONS IN AGING AND AGE-RELATED DISORDERS

TRAINING SCHOOL 1

24 -28 MAY 2021

Computational methods to study Phase Separation

Lecture: Phase separation and emergent functions of intrinsically disordered proteins –

Peter Tompa

This project received funding from European Union's Horizon 2020 research and innovation programme under grant agreement No 952334.

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AND AGE-RELATED DISORDERS

TRAINING SCHOOL

Computational Methods to Study Protein Phase Separation

24-28 May 2021 - Online event

Monday May 24th, 14:00 - 18:00 CEST | 13:00 - 17:00 GMT+1

Time (CEST)	Session title	Speaker	Type
14:00 - 15:00	**PhasAGE: Excellence Hub on phase transitions in aging and age-related disorders**	Sandra Macedo Ribeiro	L
15:00 - 16:45	**Phase separation and emergent functions of Intrinsically Disordered Proteins**	Peter Tompa	L
16:45 - 17:00	*Break*		
17:00 - 18:00	**Group activity**		G

1) How it started: three stories
2) An outline of LLPS and emergent functions
3) LLPS and disease
4) Experimental techniques to study LLPS
5) Databases and bioinformatics tools

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1) How it started: three stories

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Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation

Clifford P. Brangwynne,[1,2,3] Christian R. Eckmann,[1] David S. Courson,[3] Agata Rybarska,[1] Carsten Hoege,[1] Jöbin Gharakhani,[2,3] Frank Jülicher,[2,3] Anthony A. Hyman[1,3*]

the embryo posterior; however, close to the cortex there was a flux of P granules into the anterior that was of similar magnitude to the posteriorly directed flux (Fig. 1, D to F). This behavior closely matched the overall flow behavior of cytoplasmic material such as yolk granules (*6*), quantified by particle imaging velocimetry (PIV) (Fig. 1, B and C) (*9*). P granules cannot preferentially localize to the posterior by convection in the surrounding cytoplasm alone. Thus, flows have little or no role

In sexually reproducing organisms, germ cells generate sperm and eggs. In *C. elegans*, the first germ cell is established when RNA and protein-rich P granules localize to the posterior of the one-cell embryo. It was shown that P granules exhibit liquid-like behaviors, including fusion, dripping, and wetting, and arise by condensation (phase separation). Polarity of the embryo is marked by biased localization at the posterior.

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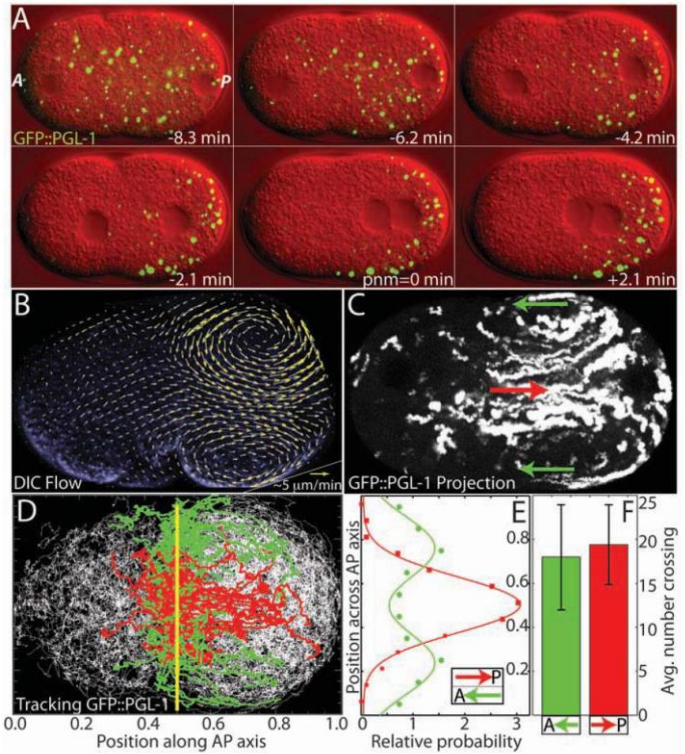
Brangwynne… Hyman (2009) *Science* 324: 1729

Preferential localization and movement of PGs

- GFP-PGL1, posterior end of embryo -



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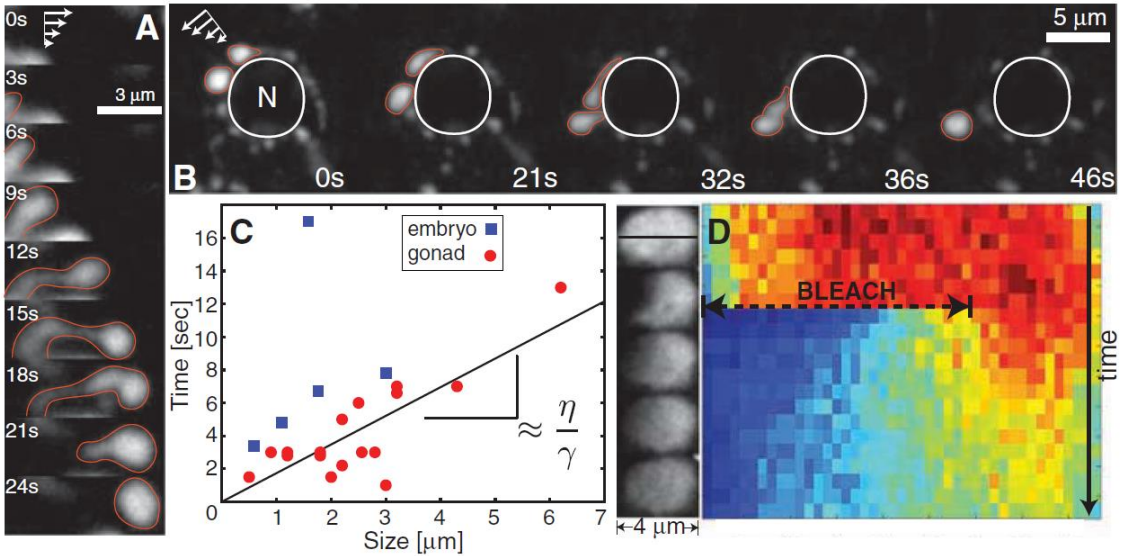
Brangwynne… Hyman (2009) *Science*

GFP-PGL-1

Liquid behavior of PGs



(A) jetting from nucleus, (B) dripping and fusion, (C) fusion and viscosity, (D) FRAP

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Brangwynne… Hyman (2009) *Science*

LETTER

doi:10.1038/nature10879

Phase transitions in the assembly of multivalent signalling proteins

Pilong Li[1]*, Sudeep Banjade[1]*, Hui-Chun Cheng[1]*, Soyeon Kim[1], Baoyu Chen[1], Liang Guo[2], Marc Llaguno[3], Javoris V. Hollingsworth[4], David S. King[5], Salman F. Banani[1], Paul S. Russo[4], Qiu-Xing Jiang[3], B. Tracy Nixon[6] & Michael K. Rosen[1]

It is shown *in vitro* that mixtures of signaling proteins Wiskott-Aldrich syndrome protein (N-WASP), NCK and phosphorylated nephrin1 (complex for actin cytoskeletal rearrangements) phase separate by liquid-liquid demixing, generating μm-sized liquid droplets. The concentrations needed are directly related to the valency of the interacting species. The phase transition is regulated by nephrin phosphorylation and corresponds to a sharp increase in activity towards an actin nucleation factor, the Arp2/3 complex.

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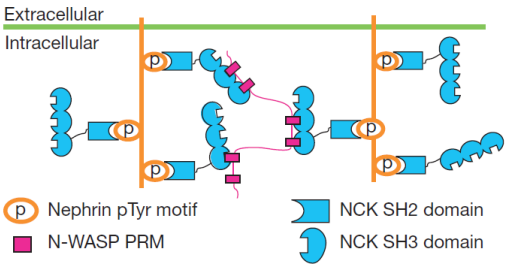
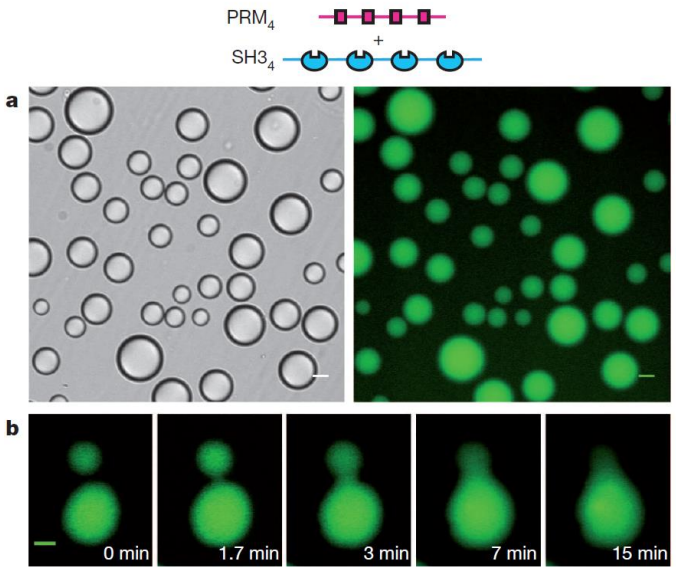
Li… Rosen (2012) *Nature* 483: 336

Nephrin-NCK-N-WASP system in cytoskeleton remodeling





Multivalency
SH2 - pTyr, SH3 - PRM interactions: signaling through Arp 2/3 to actin

(A) Differential interference contrast (DIC) microscopy
(B) Wide-field fluorescence microscopy
(C) time-lapse imaging of fusion events

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Li… Rosen (2012) *Nature* 483: 336

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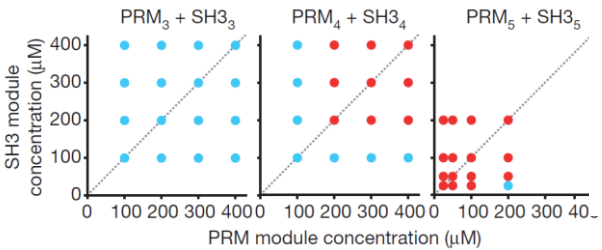
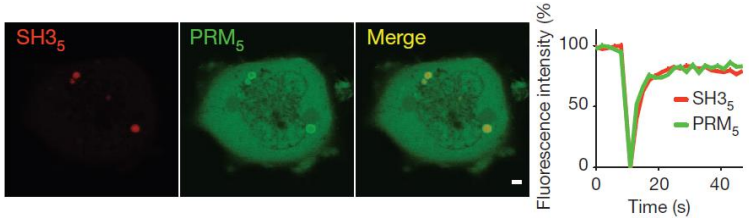
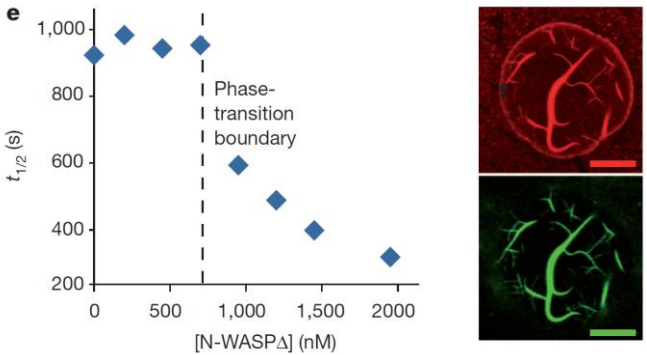
Nephrin-NCK-N-WASP system in cytoskeleton remodeling



Valency and phase diagram



Colocalization and FRAP



$t_{1/2}$ of actin polymerization
(Arp2/3 and rhodamine-actin added)

Li… Rosen (2012) *Nature* 483: 336

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Cell

Cell-free Formation of RNA Granules: Low Complexity Sequence Domains Form Dynamic Fibers within Hydrogels

Masato Kato,[1] Tina W. Han,[1] Shanhai Xie,[1] Kevin Shi,[1] Xinlin Du,[1] Leeju C. Wu,[1] Hamid Mirzaei,[1] Elizabeth J. Goldsmith,[1] Jamie Longgood,[1] Jimin Pei,[1,3] Nick V. Grishin,[1,3] Douglas E. Frantz,[4] Jay W. Schneider,[2] She Chen,[5] Lin Li,[5] Michael R. Sawaya,[6] David Eisenberg,[6] Robert Tycko,[7] and Steven L. McKnight[1,*]

Eukaryotic cells contain assemblies of RNAs and proteins termed RNA granules. Many proteins within RNA-binding domains and LCDs. Exposure of cell lysates to a biotinylated isoxazole (b-isox) precipitated hundreds of RNA-binding proteins with significant overlap to the constituents of RNA granules. LCDs are both necessary and sufficient for b-isox-mediated aggregation and can undergo a concentration-dependent phase transition to a hydrogel-like state. X-ray diffraction and EM showed that hydrogels are composed amyloid-like (but highly dynamic) fibers.

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Kato… McKnight (2012) *Cell* 149: 753

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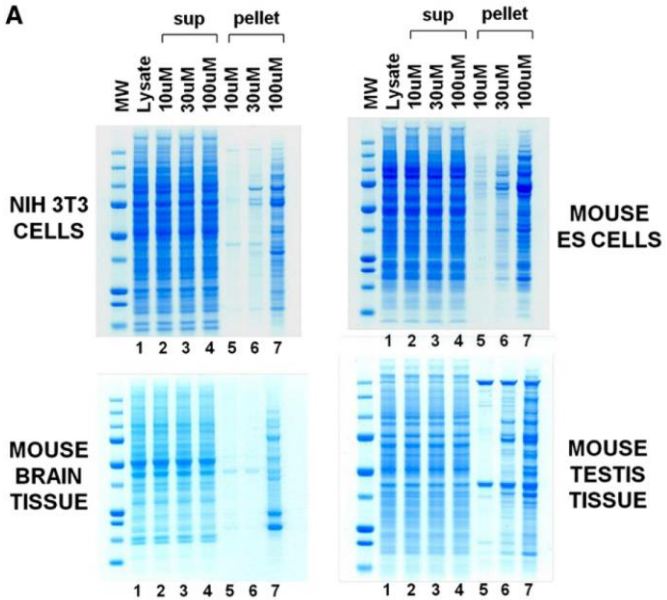
Cellular extracts treated with b-isox

A high-throughput drug screen for chemicals that promote mouse embryonic stem (ES) cell differentiation toward cardiomyocytes lead to the discovery of 5-aryl-isoxazole-3-carboxyamide. Neuronal progenitors further progressed to morphologically mature neurons. The biotinylated derivative (b-isox) was used to find its targets: it selectively precipitated proteins.



Kato… McKnight (2012) *Cell* 149: 753

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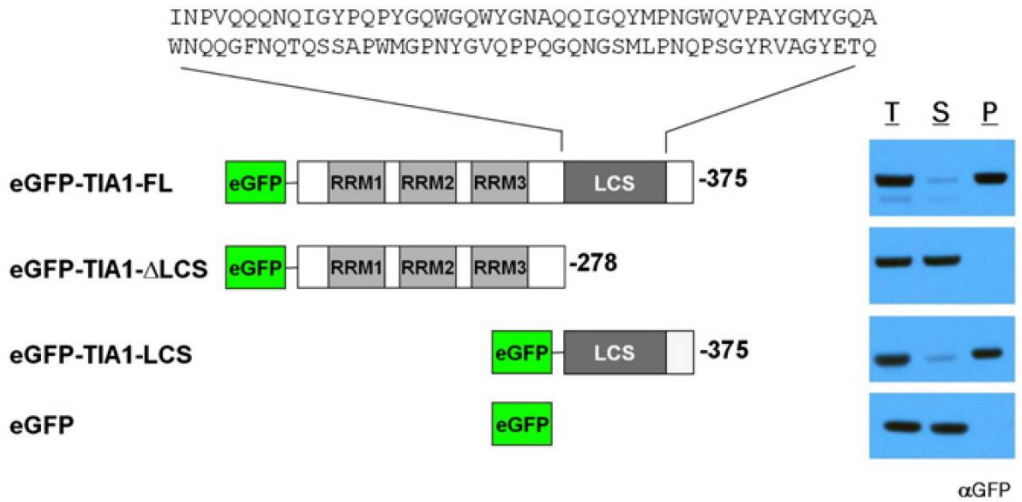
Enrichment for RNA granule proteins

Of 106 proteins found, 53 are among canonical RNA granule proteins (FUS, RBM3, hnRNPA2, CPEB2, TIA1, hnRNPA1, Atxn2, Ddx1, G3BP1, Npm1, Tdp-43, Caprin1). LCD drives b-isox-mediated precipitation.



S: supernatant
P: pellet

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Kato… McKnight (2012) *Cell* 149: 753

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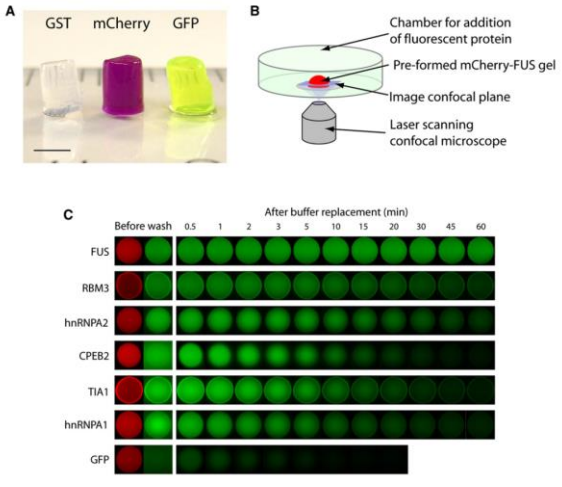
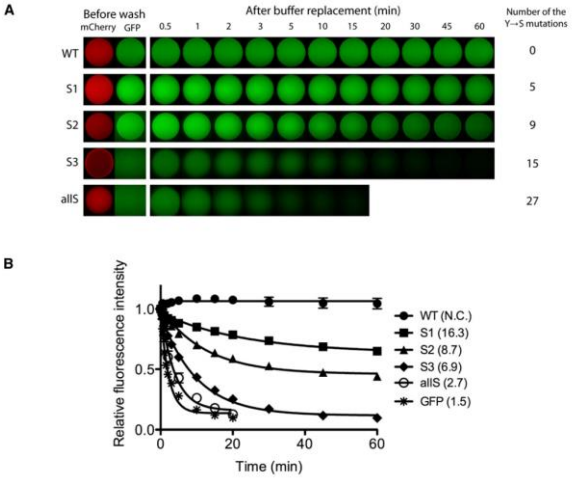
LCDs undergo gelation (without b-isox)



FUS LCD hydrogel
Retention of other GFP-LCDs



FUS LCD hydrogel, GFP-FUS LCD retention
Mutation of Tyr residues

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Kato… McKnight (2012) *Cell* 149: 753

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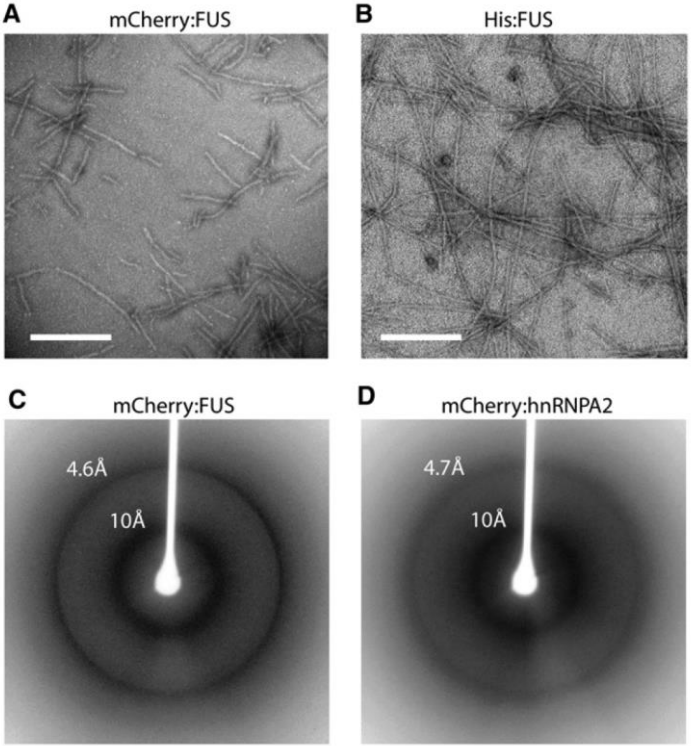
b-isox causes reversible amyloid formation



hydrogel droplets visualized by TEM

Kato… McKnight (2012) *Cell* 149: 753

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2) An outline of LLPS and emergent functions

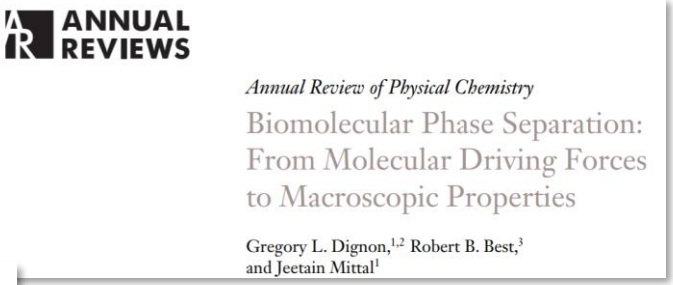

REVIEW

CELLULAR BIOPHYSICS

Liquid phase condensation in cell physiology and disease

Yongdae Shin and Clifford P. Brangwynne*

Shin, Brangwynne (2017) *Science* 357: eaaf4382

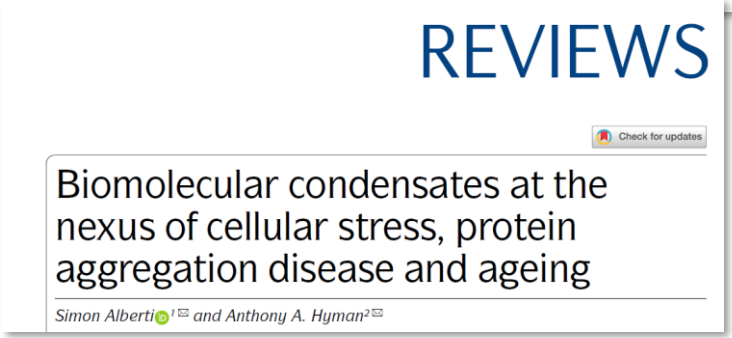

ANNUAL REVIEWS

Annual Review of Physical Chemistry

Biomolecular Phase Separation: From Molecular Driving Forces to Macroscopic Properties

Gregory L. Dignon,[1,2] Robert B. Best,[3] and Jeetain Mittal[1]

Dignon… (2020) *Annu. Rev. Phys. Chem.* 71: 53


REVIEWS

Check for updates

Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing

Simon Alberti[1] and Anthony A. Hyman[2]

Alberti, Hyman (2021) *Nat. Rev. MCB* 22: 196

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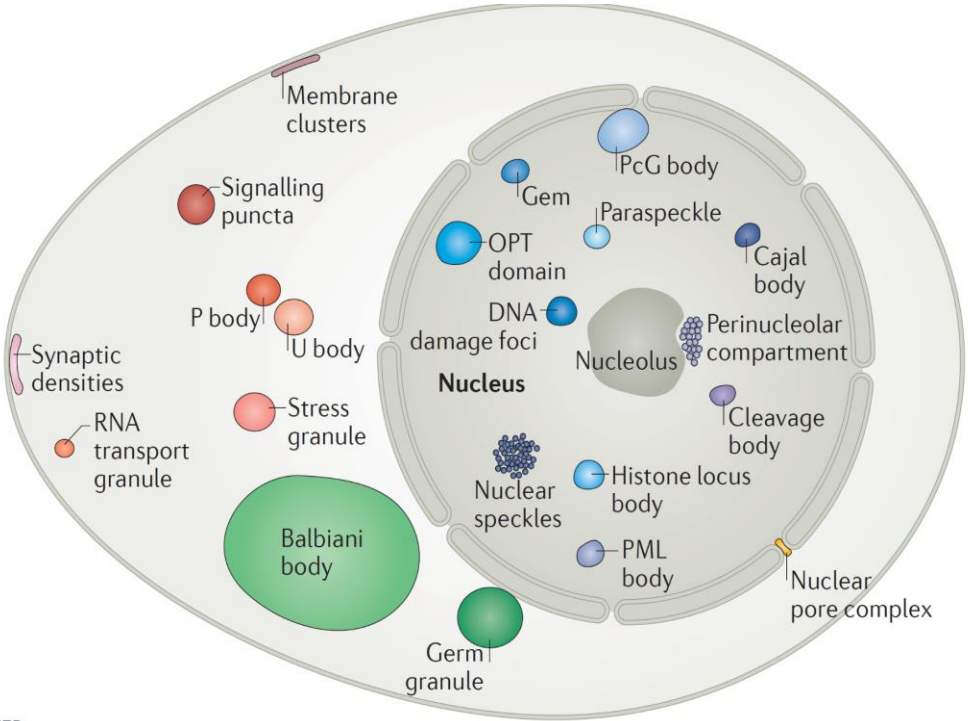
Membraneless organelles

- biomolecular condensates, RNP bodies, LLPS -

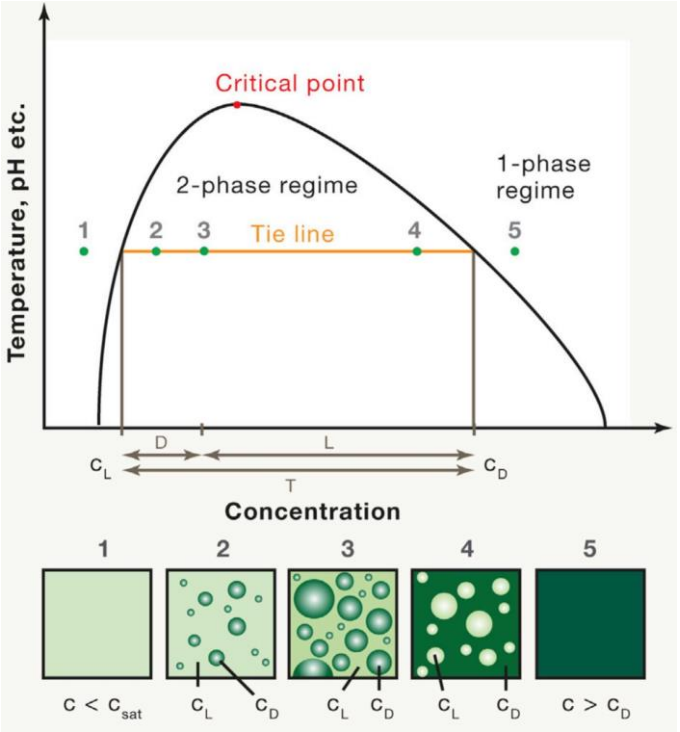


Banani et al. (2017) *Nat. Rev. Mol. Cell Biol.*

Their mechanism of formation

- spontanous demixing, LLPS -

1) phase diagram
2) binodal/coexistence line
3) saturation concentration (C_{sat})



Alberti (2019) *Cell* 176: 419

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Polymer physics (thermodynamics) of LLPS

- e.g. Flory-Huggins formalism -

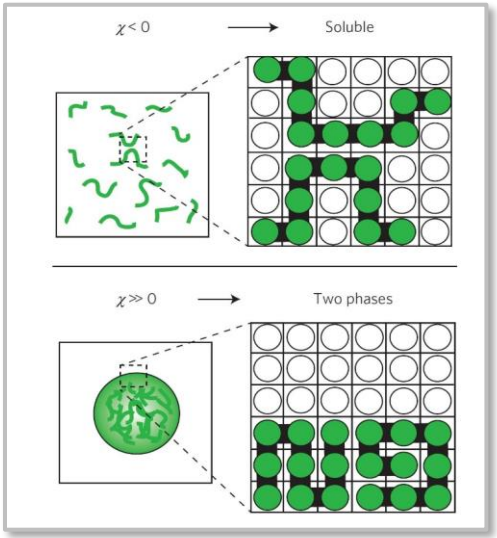
Free energy of mixing per lattice site (Φ - volume fraction)

Entropy Enthalpy

$$\frac{F}{k_B T} = \frac{\phi}{N}\ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi)$$

Chain-chain vs. chain-solvent interaction (χ - Flory prmt.)

$$\chi = \frac{z}{k_B T}\left[u_{ps} - \frac{1}{2}(u_{pp} + u_{ss})\right]$$



Homogeneous homopolymers

Homotypic, homopolymer

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Brangwynne, Tompa, Pappu (2015) *Nature Phys*. 11: 899

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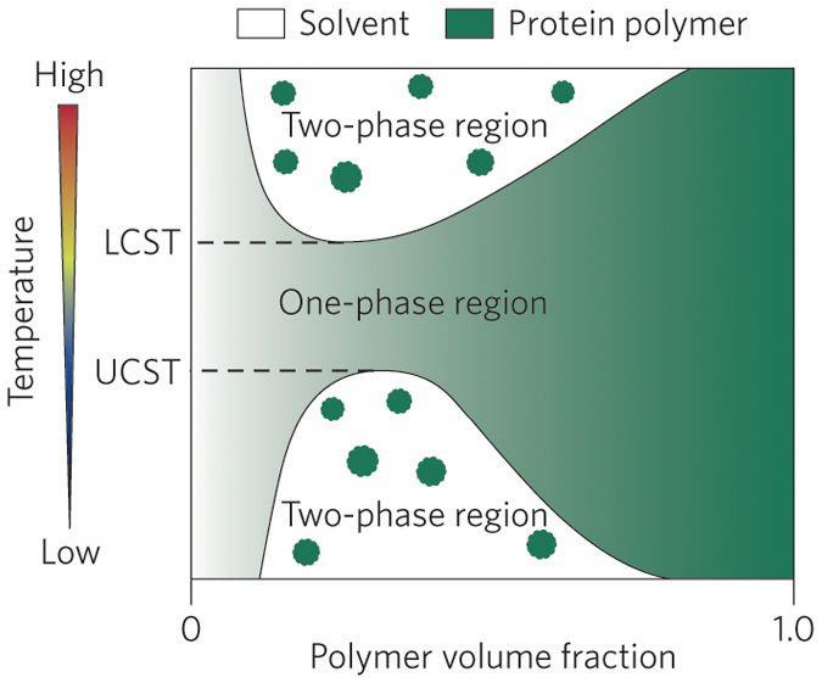
Sometimes opposite behavior

Temperature dependence of attractive term



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Holehouse, Pappu (2015) *Nat. Materials* 14: 1083

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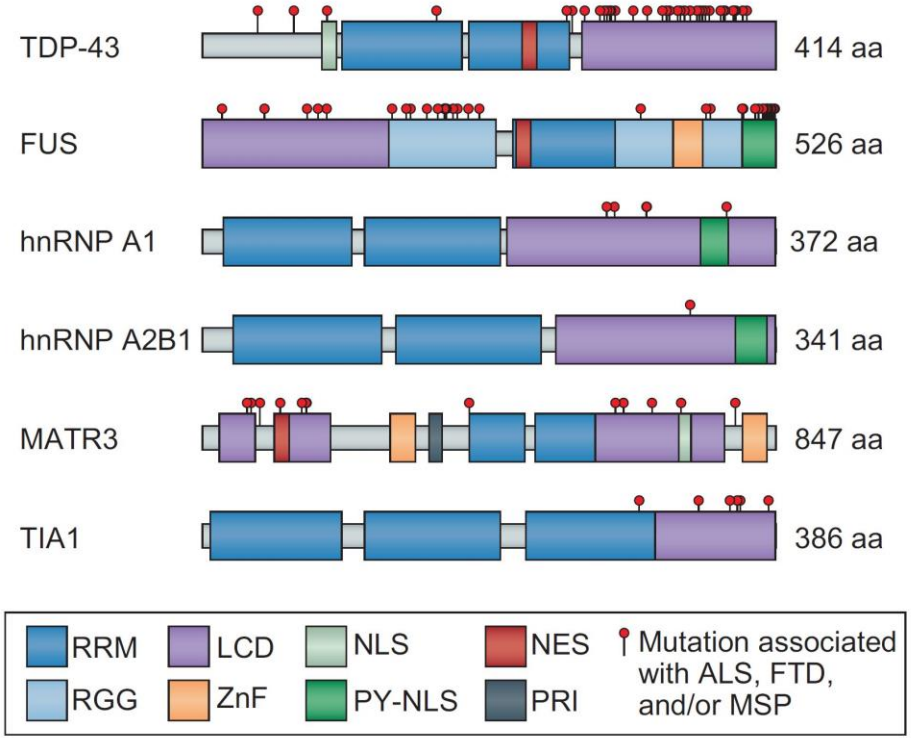
Structural disorder and RNA binding in LLPS proteins

- LCD: low-complexity IDR -



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Purice and Taylor (2018) *Front Neurosci*. 12: 326

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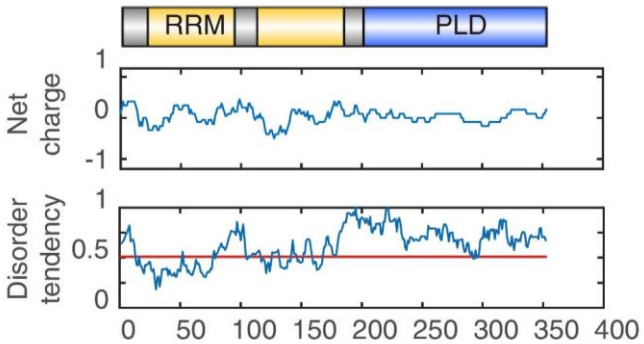
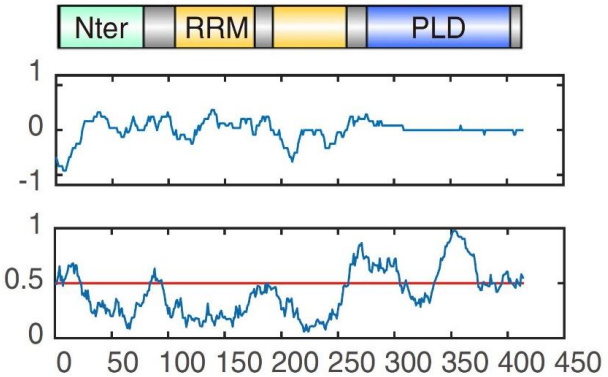
Structural disorder in LLPS proteins

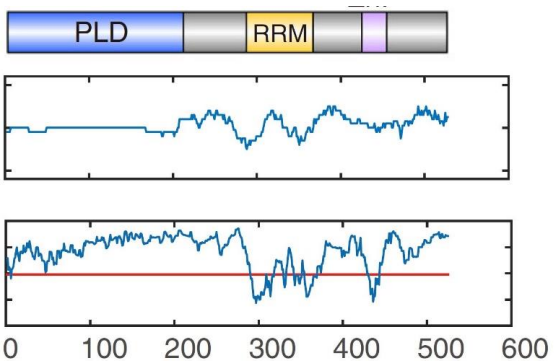
hnRNP A2B1



TDP-43



FUS



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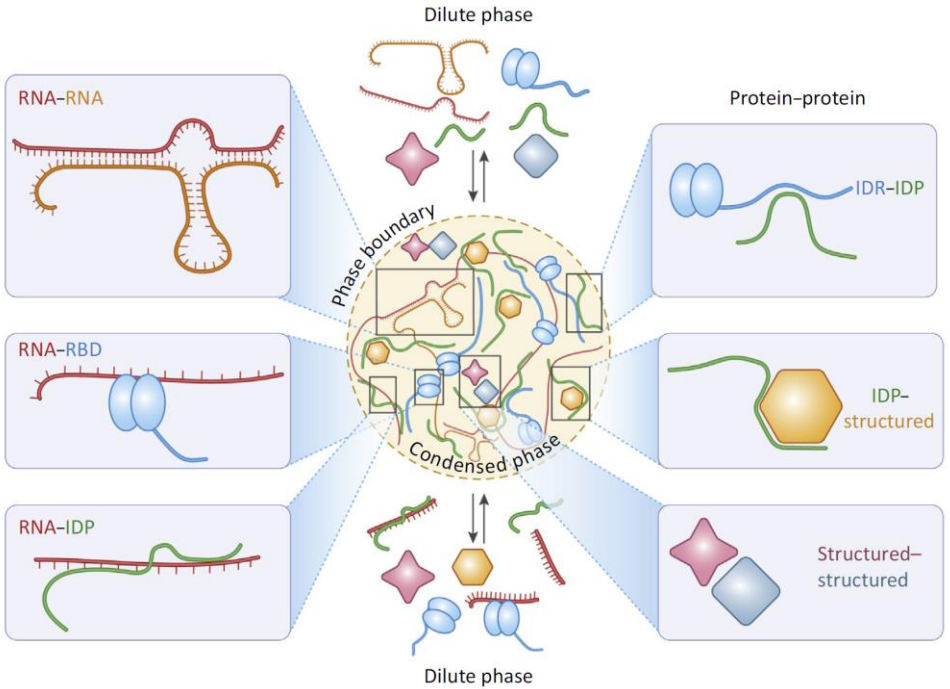
Purice and Taylor (2018) *Front Neurosci*. 12: 326

Different types of interactions driving LLPS



hydrophobic
cation-pi
pi-pi
elecrostatic
H-bond

Features: (i) dynamics, (ii) strength, (iii) specificity, (iv) stoichiometry, (v) structure

Ryan and Fawzi (2019) *TiNS* 42: 693

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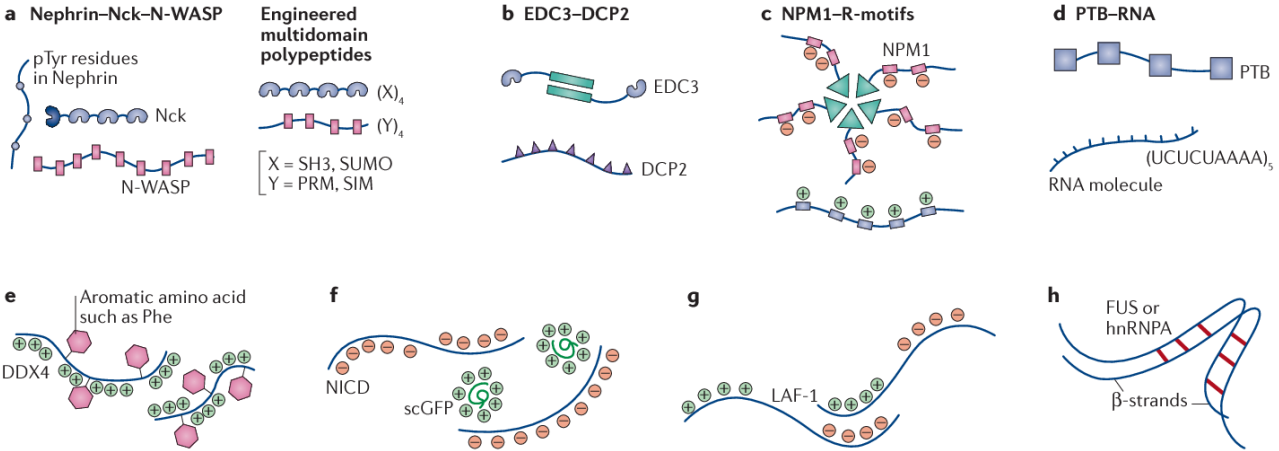
Multivalency is basic to LLPS



b) Enhancer of mRNA-decapping protein 3 (EDC3) decapping enzyme subunit 2 (DCP2)
d) polypyrimidine tract (RNA) binding protein (PTB)
f) nephrin intracellular domain (NICD) and supercharged GFP
g) P-granule LAF-1

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Banani et al. (2017) *Nat. Rev. MCB* 18: 285

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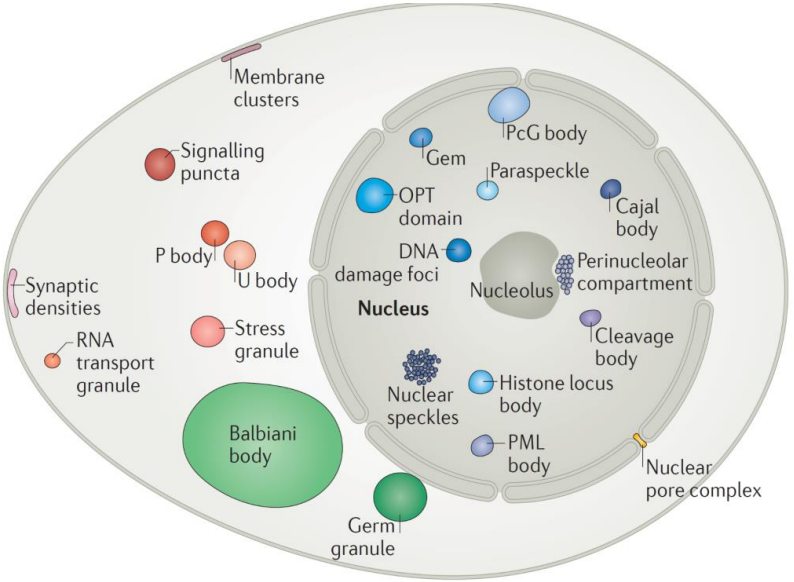
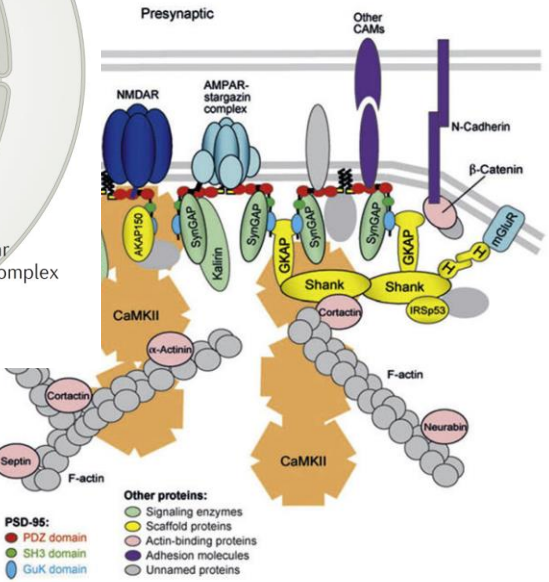
Not every cellular assembly is LLPS though



Banani et al. (2017) *NRMCB*



Pancsa (2019) *BBA*

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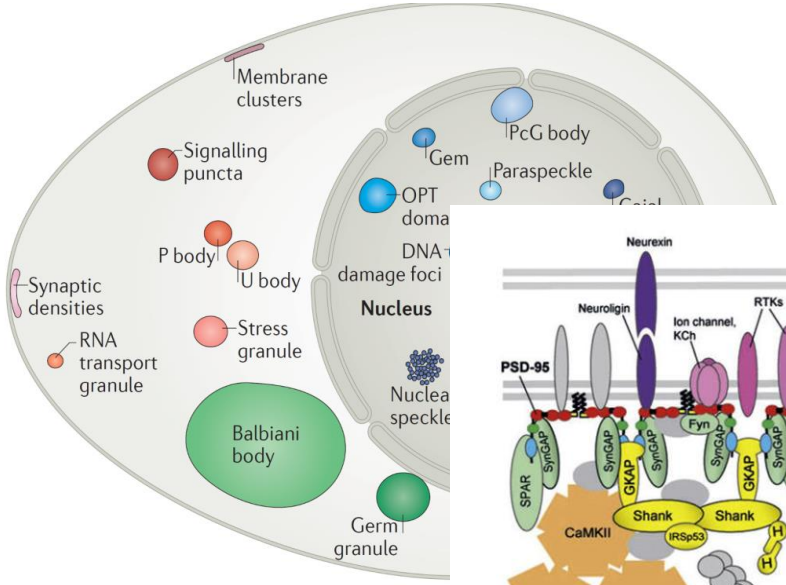
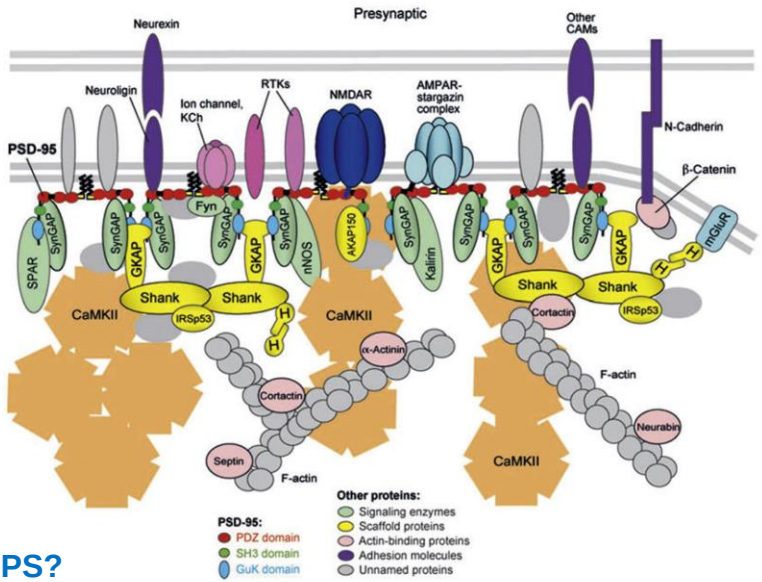
Not every cellular assembly is LLPS though



Banani et al. (2(



Pancsa (2019) *BBA*

1) Assembly by PS?
2) Cooperativity
3) Structure (beyond 1st neighbor)

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Functional consequences of LLPS

1) an emergent property, might not manifest at the level of individual proteins

2) means two different things (remember Gene Ontology MF, BP and CC)

- the way an LLPS droplet functions (MF)
- what it does in the cell (BP)

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Pancsa… (2019) *BBA* 1867: 970

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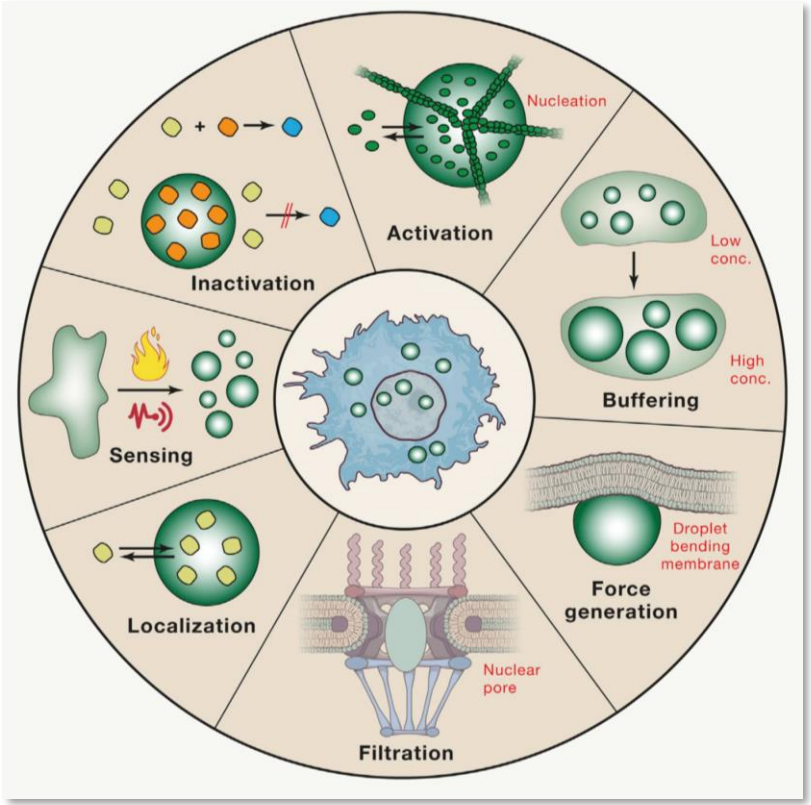
Functional consequences of LLPS

(Molecular Function ontology)



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Alberti (2019) *Cell* 176: 419

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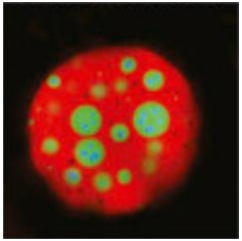
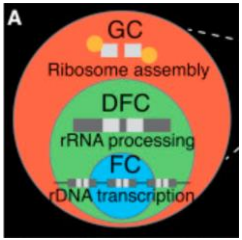
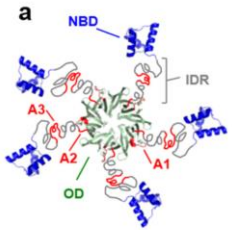
Function of nuceolus

- ribosome biogenesis -

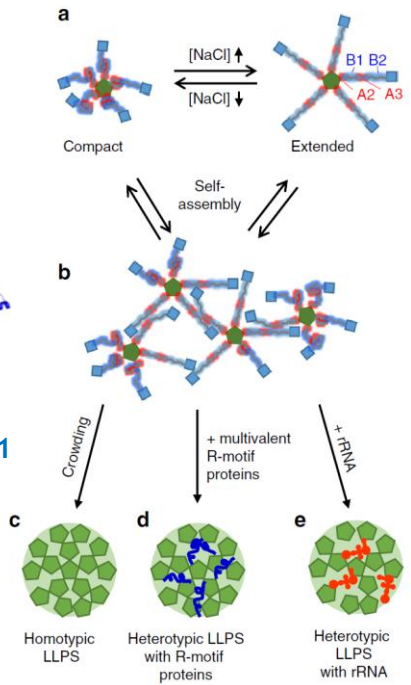


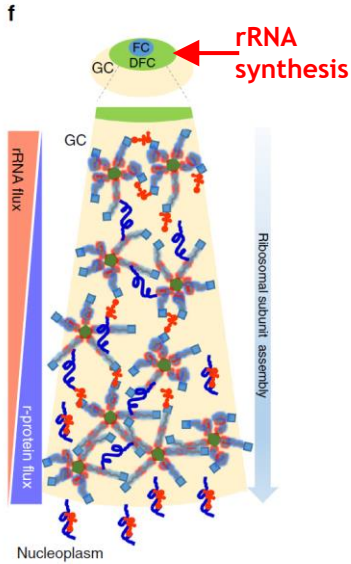


fibrillar center (FC)
dense fibrillar component (DFC)
granular component (GC)



Nucleophosmin 1





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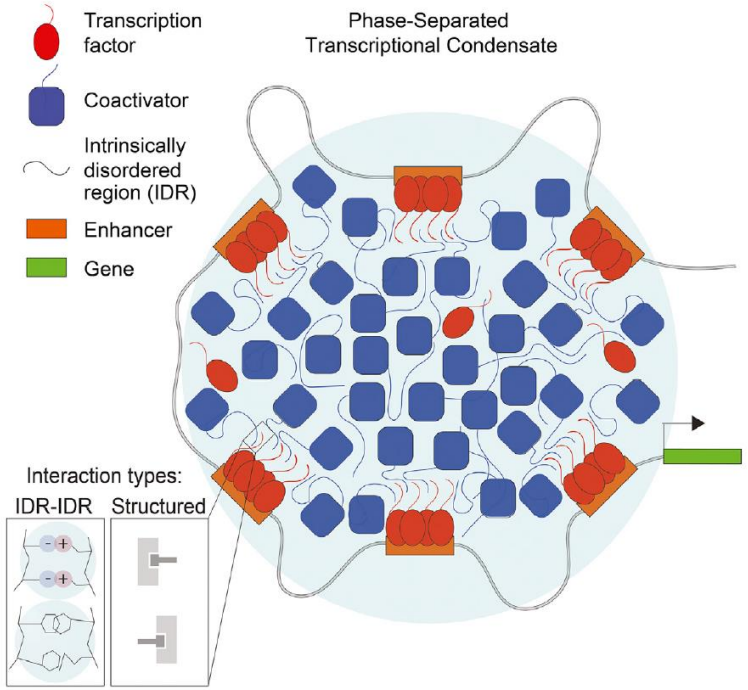
Feric et al. (2016) *Cell* 165: 1686

PhasAGE

Function of super-enhancers (SEs) in transcription regulation



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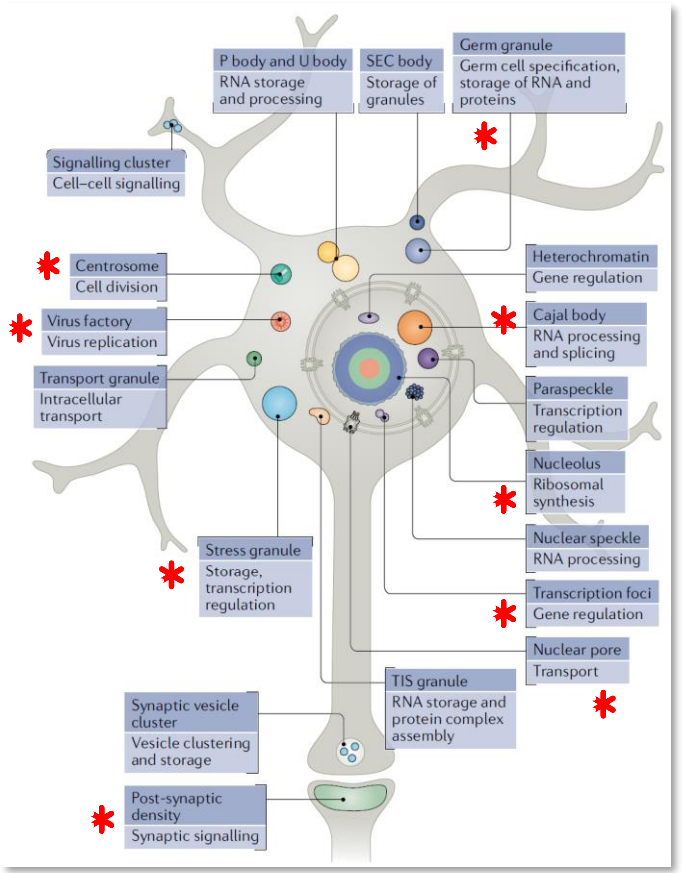
Boija… (2018) *Cell* 175: 1842

PhasAGE

Functional consequences of LLPS

(Biological Process ontology)



Alberti, Hyman (2019) *Nat. Rev. MCB* 22:196

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PhasAGE

3) LLPS and disease

LLPS may lead to disease
- ALS/FTD, Lou Gehrig's disease

Lou Gehrig

Stephen Hawking

- Progressive loss (atrophy) of muscles
- Survival after first symptoms: 2 – 5y
- No cure

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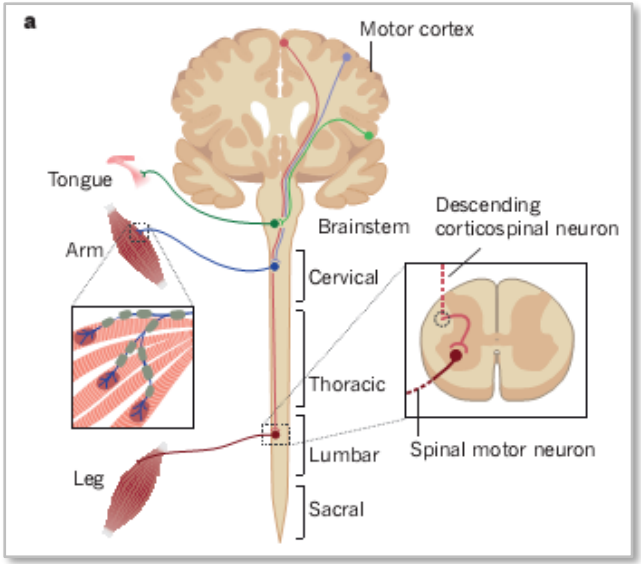
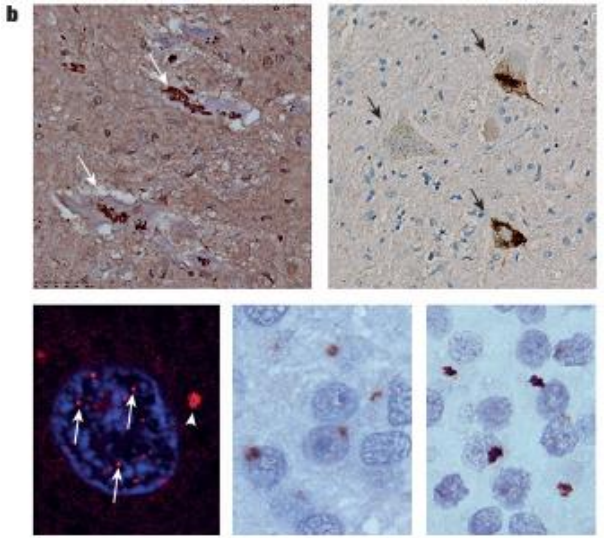
PhasAGE

ALS is motor neuron disease





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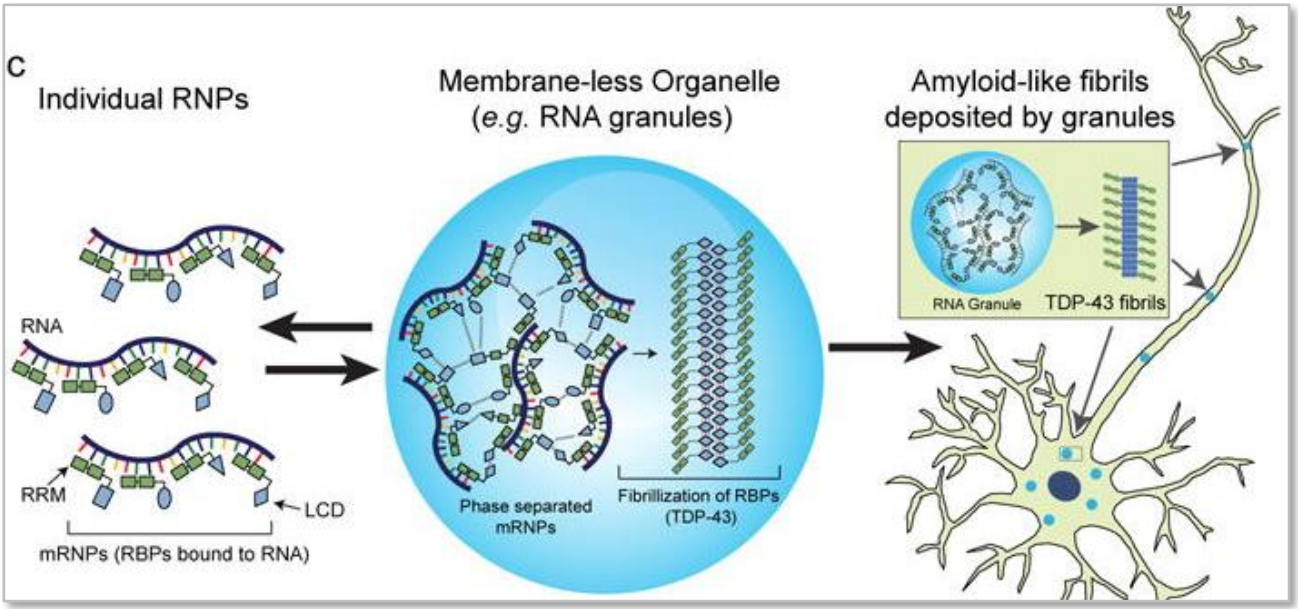
Taylor P. (2016) *Nature* 539: 197

PhasAGE

LLPS may lead to disease

- stress granules in ALS (?) -



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Taylor P. (2016) *Nature* 539: 197

PhasAGE

Many neurodegenerative proteins phase separate

Table 1. Summary of Neurodegenerative Diseases and LLPS/MLOs

Protein	Associated diseases	Evidence for disease association	Granule types	Evidence for *in vitro* LLPS	Evidence for *in vivo* LLPS or granules
FUS	ALS/FTD	Point mutations (NLS and others)	Stress	[18,44,89]	[44,89]
TDP-43	ALS/FTD, AD	Point mutations, truncations	Stress, transport	[19,90]	[47,48,90,105]
hnRNPA1	ALS/FTD/multisystem proteinopathy (MSP), MS	Point mutations in ALS/FTD/MSP, mislocalized in MS [64]	Stress, transport	[6]	[6]
hnRNPA2	ALS/FTD/MSP	Point mutations in ALS/FTD/MSP	Stress, transport	[17]	[106]
C9ORF72	ALS/FTD, SCA	G4C2 expansion in ALS/FTD, SCA [72]	Stress	[45,46,52]	[45,46,51,52]
UBQLN2	ALS/FTD	Point mutations in ALS/FTD	Stress	[54,107]	[54]
TIA1	ALS/FTD, AD, MS, SMA	Point mutations in ALS/FTD; mislocalized in AD [61], MS [64], and SMA [77]	Stress	[55,56]	[55,56]
Profilin	ALS/FTD, HD	Point mutations in ALS/FTD; modifies HD aggregation *in vitro* [66]	Stress		[57]
Ataxin-2	ALS/FTD, SCA	PolyQ expansion, 32 or more in SCA, 29–32 is a risk factor for ALS [73]	Stress, transport	[38]	[38]
Tau	AD	Mutated in AD	Stress	[29,60,108]	[29]
DJ-1	PD	Mutated in PD	Stress (P bodies)		[67]
Huntingtin	HD	PolyQ expansion in HD	Stress	[65,66]	[65]
Staufen-1	SCA	Modifies SCA; recruited to aggregates and increased expression [74]	Transport		[74]
SMN	SMA	Mutated/reduced levels in SMA	Stress, transport		[77,78]
FMRP	Fragile X, FXTAS	5′-UTR repeat expansion in fragile X, FXTAS	Stress, transport	[22,80]	[8,13]

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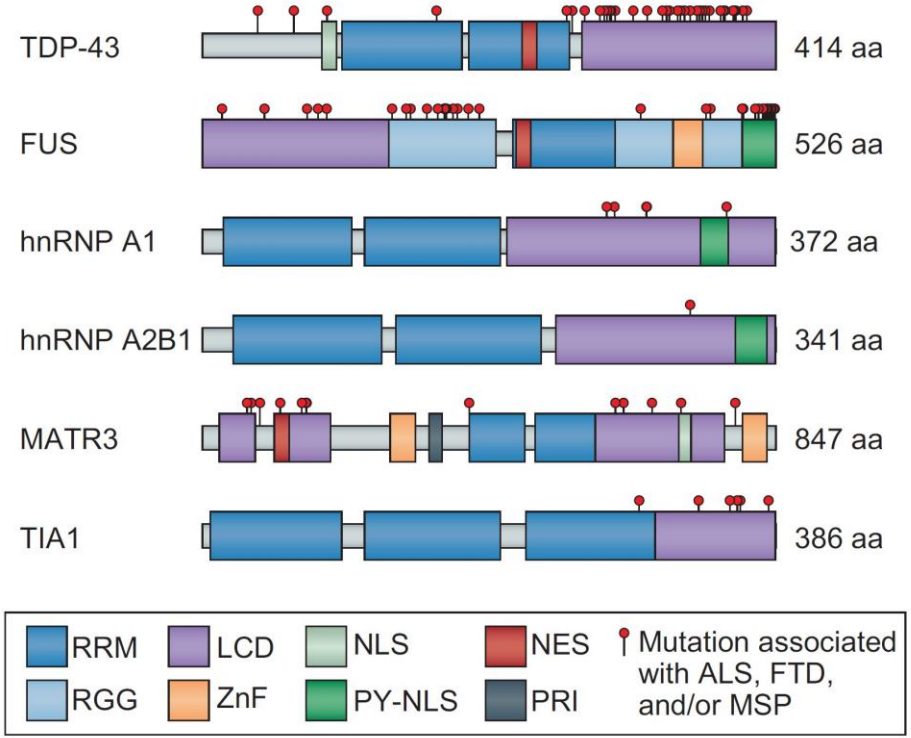
Ryan and Fawzi (2019) *Trends Neurosci*

And carry disease-specific mutations



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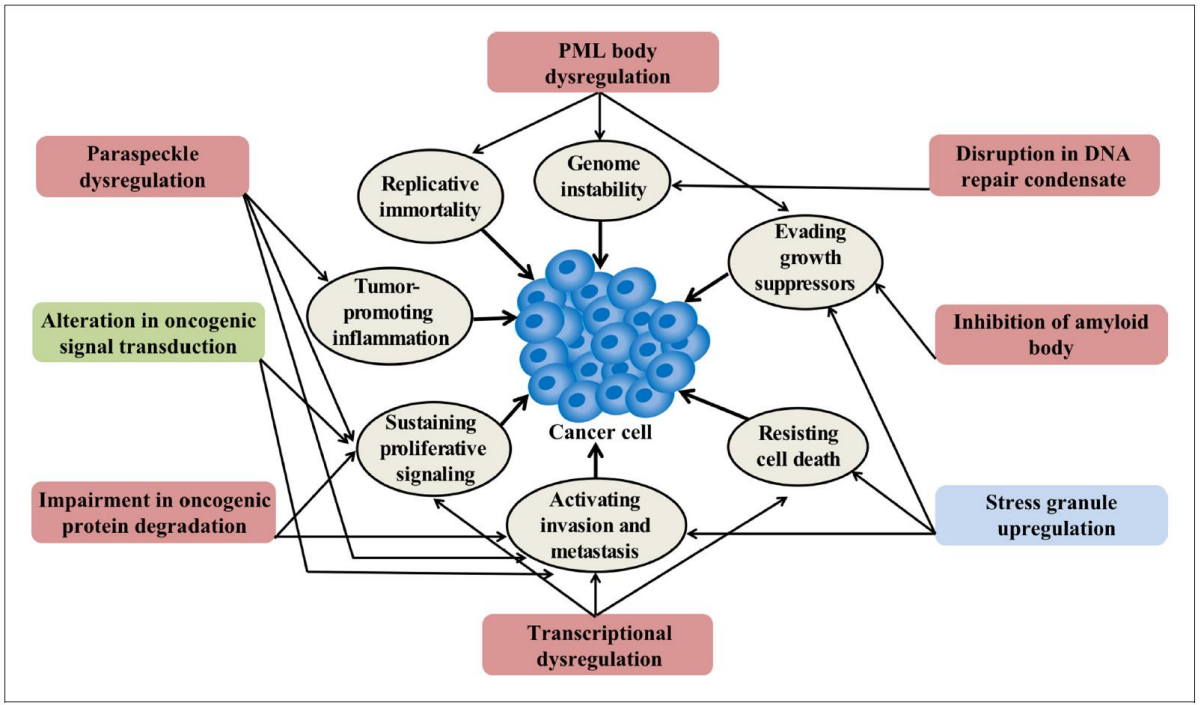
Purice and Taylor (2018) *Front Neurosci*. 12: 326

PhasAGE

Break (?)

LLPS might also be involved in cancer

- “hallmarks of cancer" -



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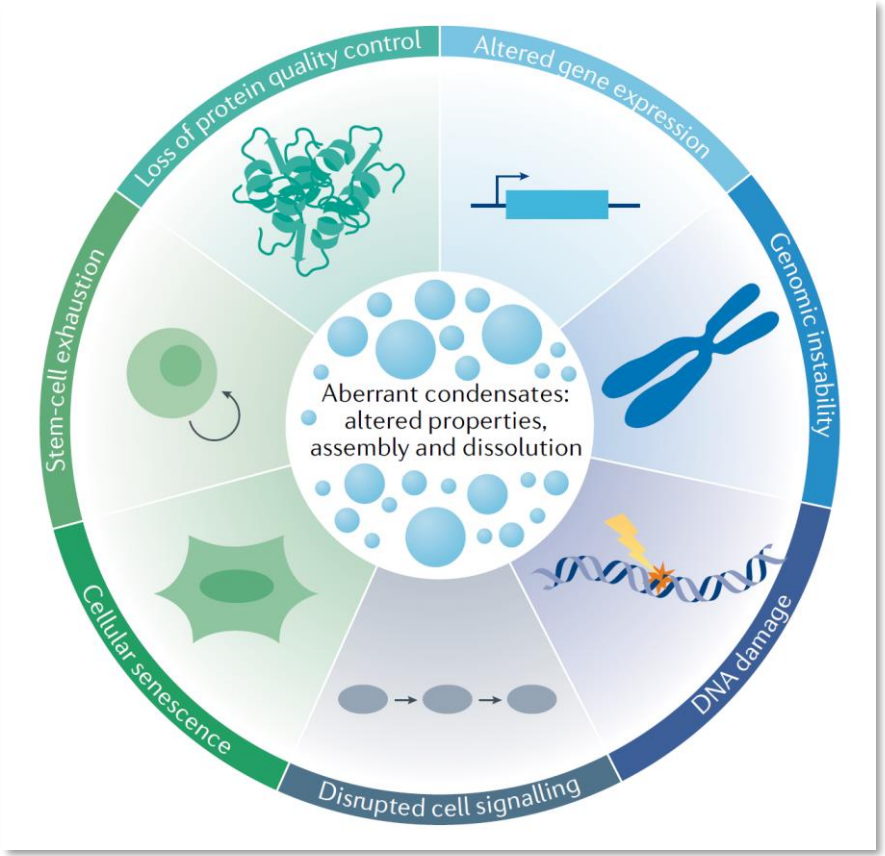
Jiang et al. (2020) *eLife* 9: e60264

LLPS might also be involved in ageing (!)

- "hallmarks of ageing" associated with ageing -

PhasAGE



Alberti, Hyman (2021) *Nat. Rev. MCB*

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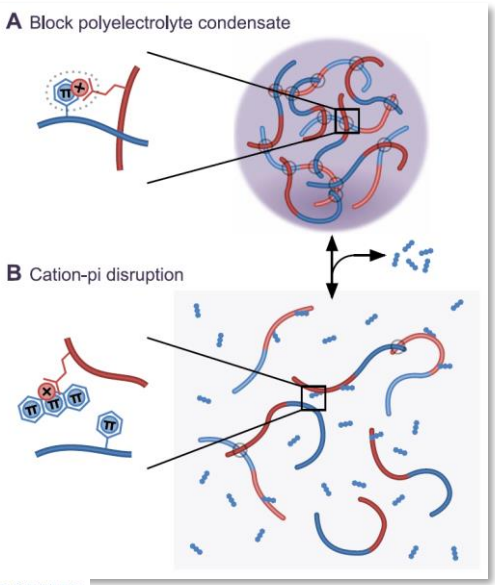
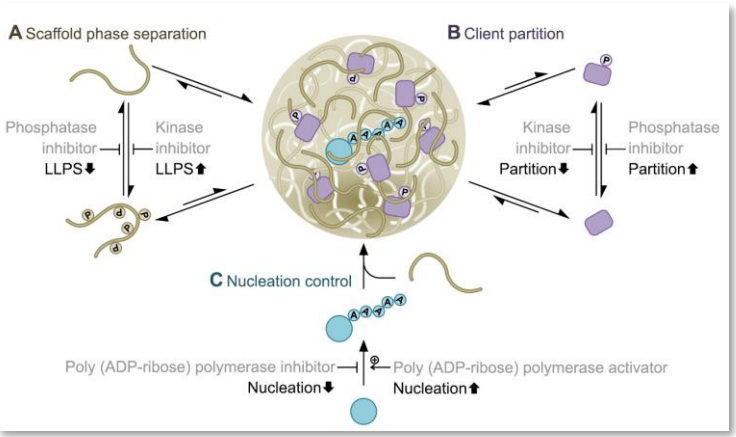
Targeting LLPS?

Targeting LLPS and aggregation



Targeting LLPS via regulation



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Wheeler RJ (2020) *Emerging Topics Life Sci.* 4: 331

PhasAGE

“Competitive landscape”

dewpointx

Tony Hyman, Rick Young
$ 60M Series A, $ 77M Series B
Merck: $ 305M (HIV)
Bayer: $ 77M (cardiovascular, gynecology)
Pfizer: $ 239M (myotonic dystrophy type 1)
oncology, neuro, metabolic, immunology

nereid

Cliff Brangwynne
$50M Series A
neurodegeneration, cancer

FAZE MEDICINES

Michael Rosen
$81M Series A
ALS

TRANSITION BIO

David Weitz, Tuomas Knowles, Peter St George-Hyslop
$9M Seed

PhasAGE

4) Experimental techniques to study LLPS

A User's Guide for Phase Separation Assays with Purified Proteins

Alberti… (2018) *J. Mol. Biol.* 430: 4806

Simon Alberti†, Shambaditya Saha†, Jeffrey B. Woodruff, Titus M. Franzmann, Jie Wang and Anthony A. Hyman

Methods for Physical Characterization of Phase-Separated Bodies and Membrane-less Organelles

Mitrea… (2018) *J. Mol. Biol.* 430: 4773

Diana M. Mitrea[1], Bappaditya Chandra[1,†], Mylene C. Ferrolino[1,†], Eric B. Gibbs[1,†], Michele Tolbert[1,†], Michael R. White[1,†] and Richard W. Kriwacki[1,2]

Leading Edge

Primer

Cell

Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates

Alberti… (2019) *Cell* 176: 419

Simon Alberti,[1,2,*] Amy Gladfelter,[3,4,*] and Tanja Mittag[5,*]

PhasAGE

Protein purification

LLPS proteins are often aggregation prone, frequently found in inclusions in neurodegenerative diseases

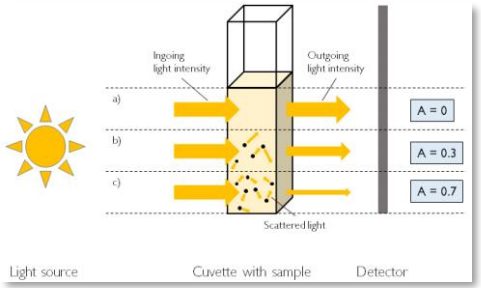
1) Often require a trick to keep them in solution: (i) solubility tag (MBP, GST, GFP), (ii) non-native/denaturing conditions (e.g. urea, pH away from pI), (iii) additives disfavoring LLPS (high salt, high Arg)

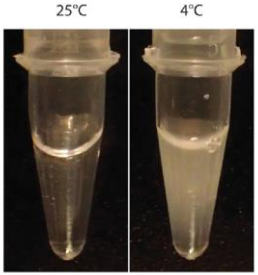
2) Purification not only from *E. coli* (insect cell, mammalian cells)

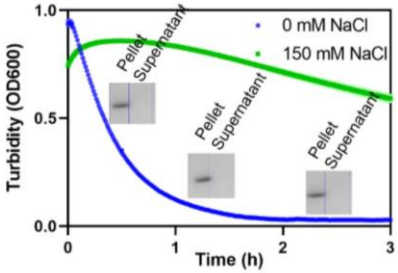
3) Advised to be aliquoted, only used once (no repeated freeze-thaw cycles)

Turbidity

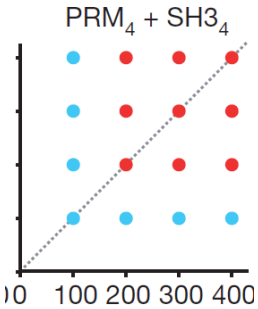
- OD_{340}, OD_{400}, OD_{600} -







hnRNPA2 LCD LLPS



Neph. - Nck - WASP (Rosen 2012)

1) fast
2) easy control (of conditions, T)
3) appropriate for determining C_{sat}
4) can be made highly parallel (phase diagram)
5) but: convolution of number and size of scattering particles

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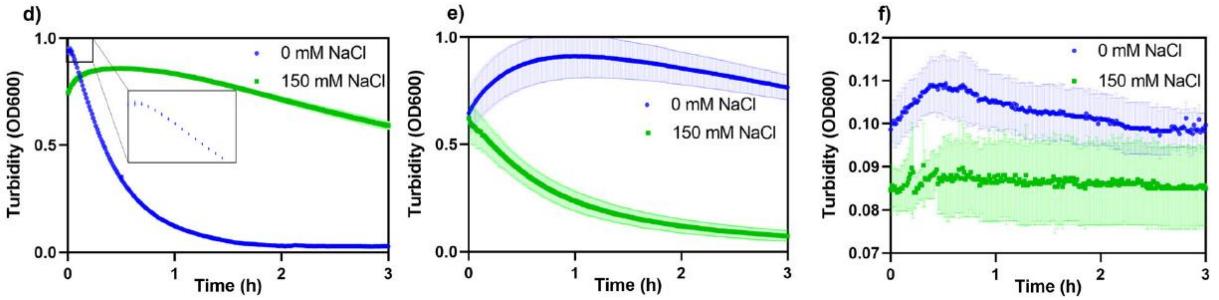
Turbidity

- hnRNPA2 LCD LLPS initiated by: -

pH jump: 11.0 → 7.5

Urea: 8M → 80mM

Cleavage of MBP tag



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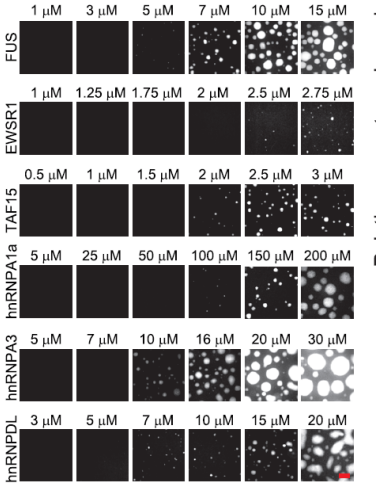
Van Lindt… (2021) *Comm. Biol.* 4: 77

PhasAGE

Microscopy

A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins

Jie Wang,[1] Jeong-Mo Choi,[2] Alex S. Holehouse,[2] Hyun O. Lee,[1] Xiaojie Zhang,[1] Marcus Jahnel,[1] Shovamayee Maharana,[1] Régis Lemaitre,[1] Andrei Pozniakovsky,[1] David Drechsel,[3] Ina Poser,[1] Rohit V. Pappu,[2] Simon Alberti,[1,*] and Anthony A. Hyman[1,4,*]



Here: FUS family of proteins

1) direct visualization
2) confirmation of spherical shape
3) shape change, fusion, colocalization
4) targeted FRAP
5) cellular applications

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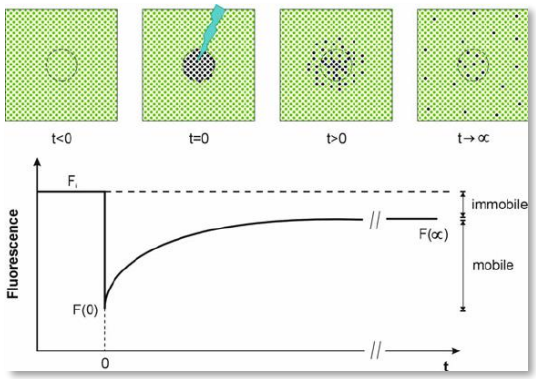
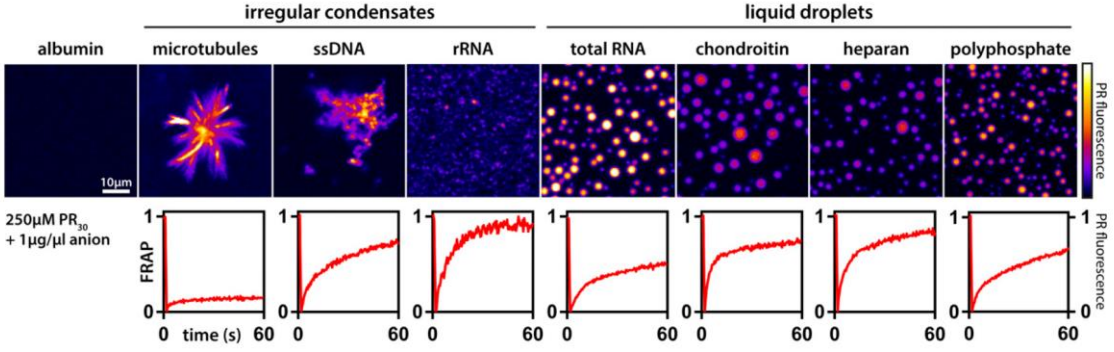
Wang… (2018) *Cell* 174: 688

PhasAGE

Fluorescence recovery after photobleaching (FRAP)





Morphologies and dynamics of condensates of PR30/various anions

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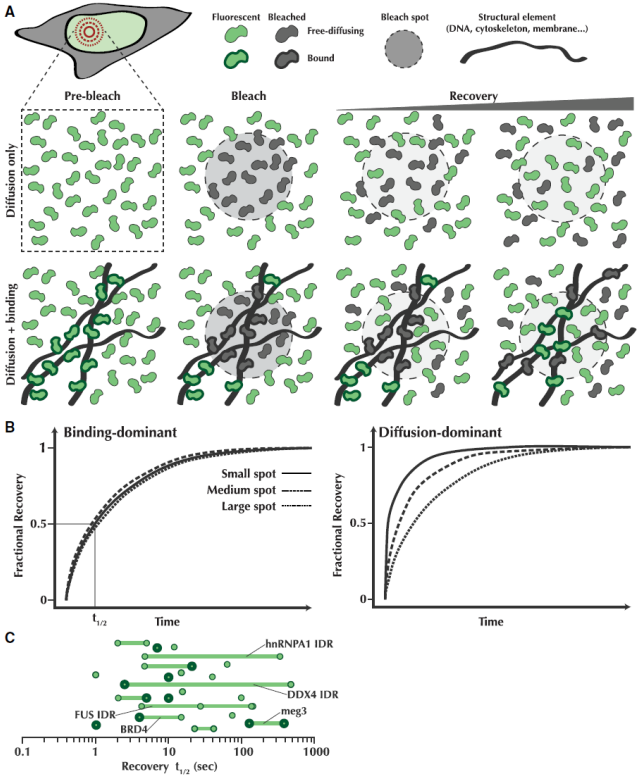
Boeynaems… (2019) *PNAS* 116: 7889

PhasAGE

Interpretation of FRAP: model dependent



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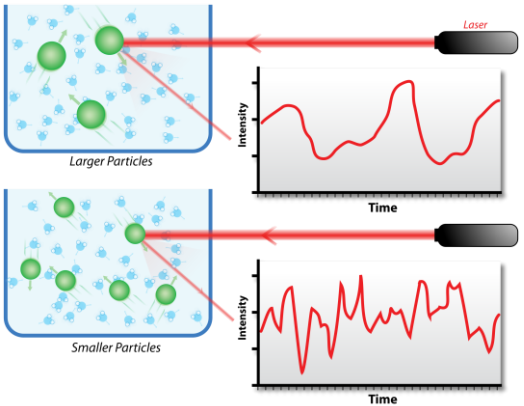
McSwiggen... (2019) *Genes Dev* 33: 1619

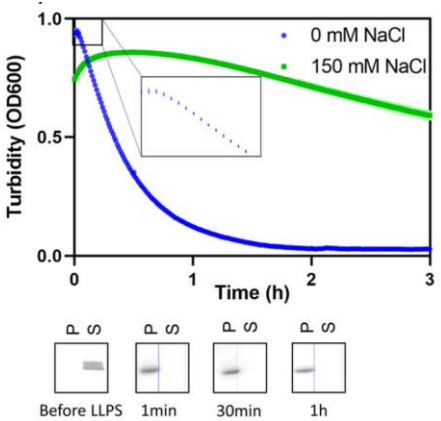
PhasAGE

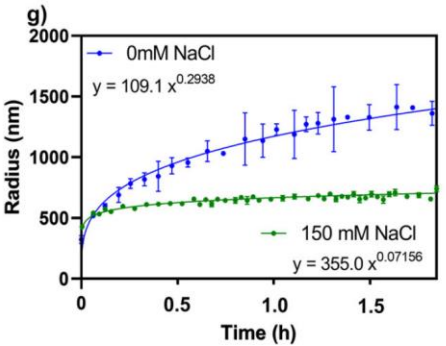
Dynamic light scattering (DLS)

- measuring size distribution profile of droplets -



Analyzing temporal fluctuations (autocorrelation) of light intensity





hnRNPA2 LCD LLPS

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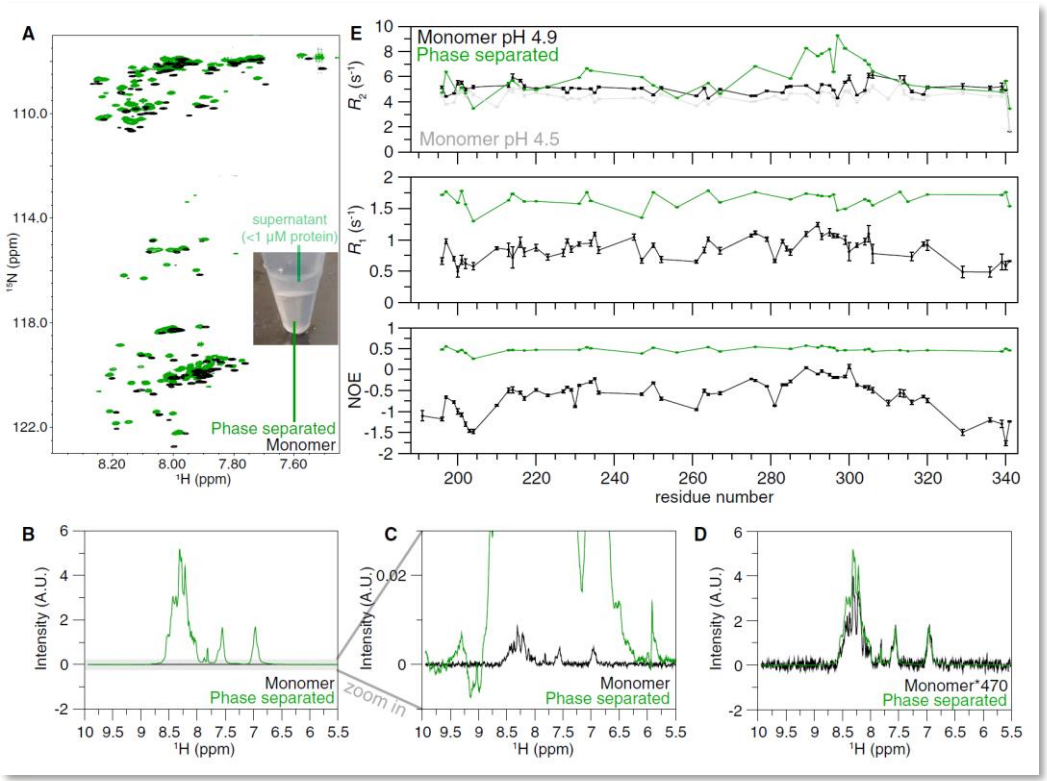
Van Lindt… (2021) *Comm. Biol.* 4: 77

PhasAGE

Nuclear magnetic resonance (NMR)

- hnRNPA2 LCD remains disordered in the droplet -



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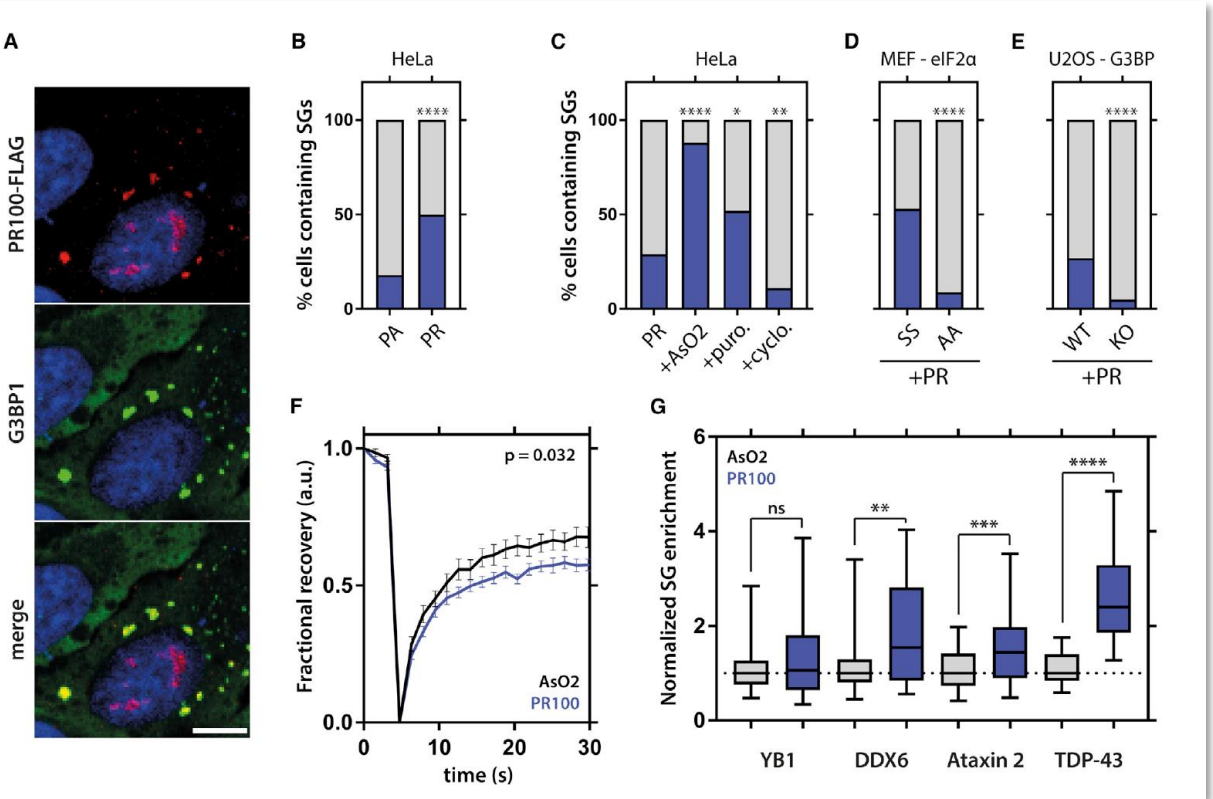
Ryan… Fawzi (2018) *Mol Cell* 69:565

PhasAGE

Cellular: overexpression

- better: driver/modifier -



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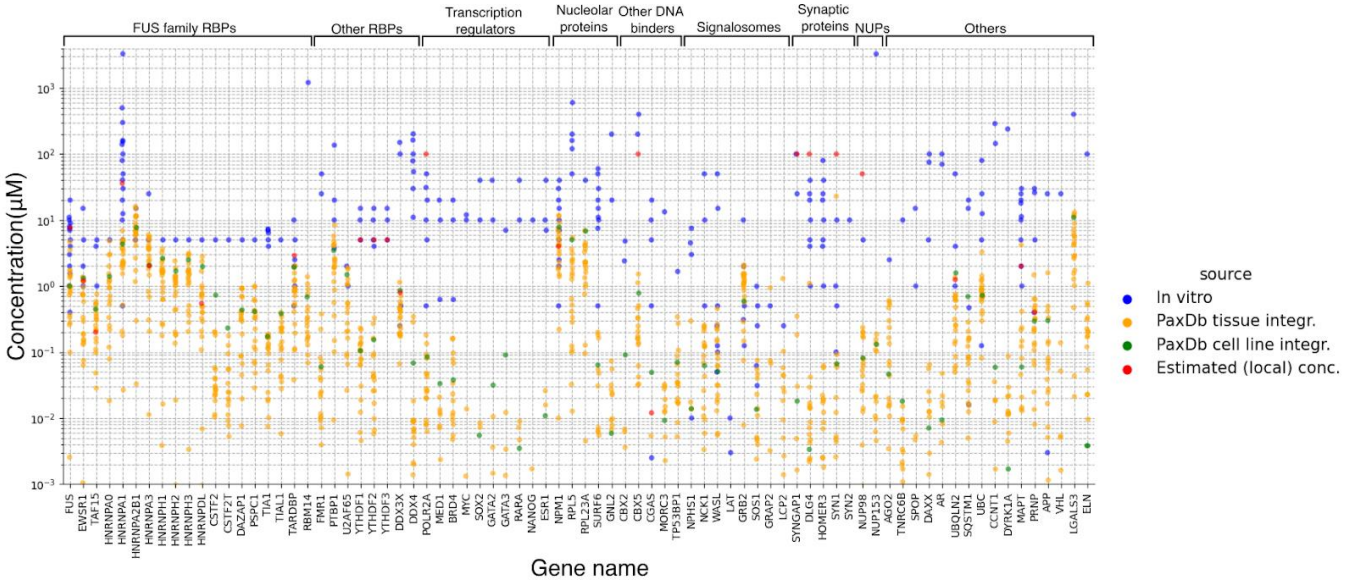
Boeynaems… (2017) *Mol. Cell* 65: 1044

PhasAGE

Issue with concentrations



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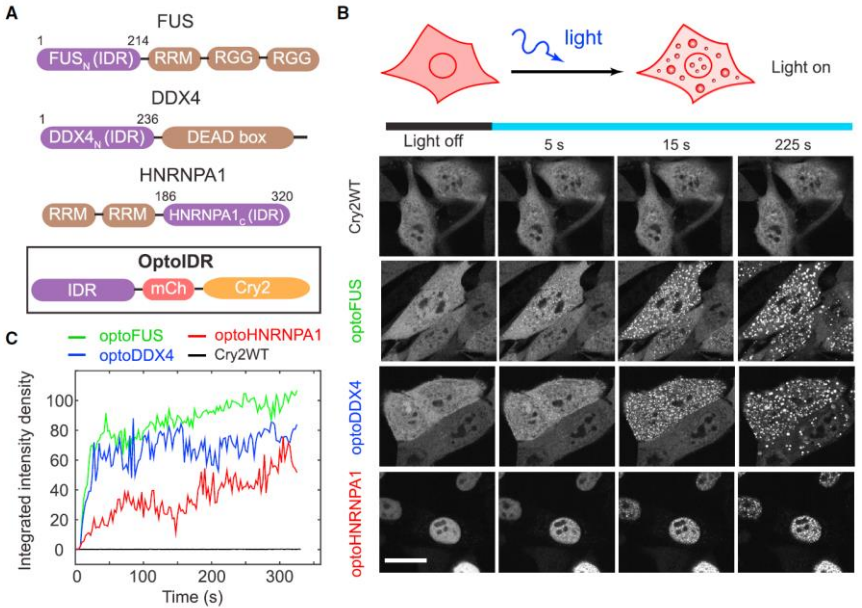
Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

PhasAGE

Cellular: optogenetis
- Cry2 (cryptochrome) based -

Photolyase homology region (PHR) of *A. thaliana* Cry2, a light-sensitive protein that dimerizes upon blue light exposure



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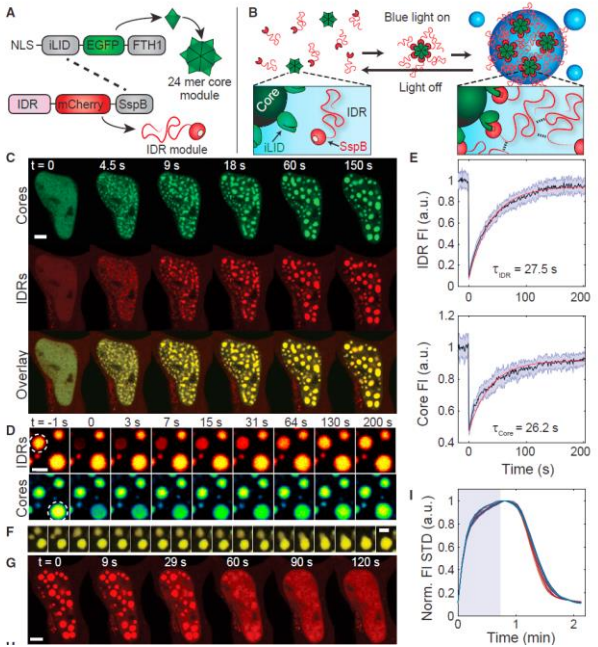
Shin… Brangwynne (2017) *Cell* 168: 159

PhasAGE

Cellular: optogenetis
- Corelets -

24-mer ferritin (FTH1) derivatized by photoactivatable iLID domain, plus its cognate partner, SspB



Shin… Brangwynne (2017) *Cell* 168: 159

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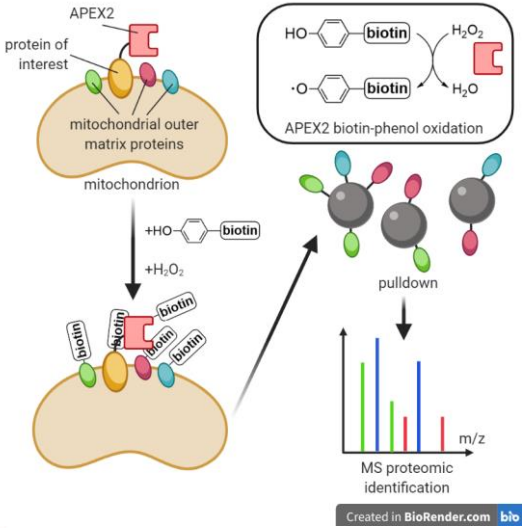
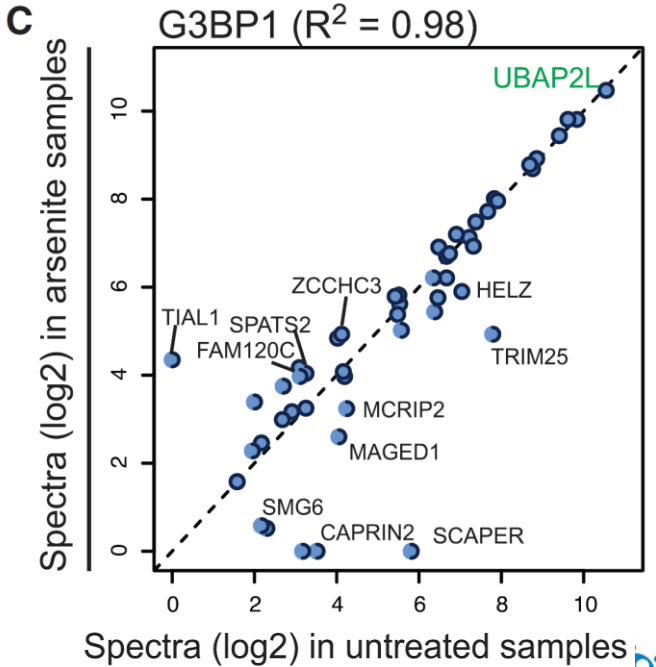
Cellular: proximity tagging

Protein of interest carries an enzyme fused (biotin ligase, BirA* or ascorbate peroxidase, APEX) that can biotinylate nearby proteins in the cell.





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Youn... (2018) *Mol. Cell* 69: 517

PhasAGE

5) Databases and bioinformatics tools

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Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

First-generation predictors of biological protein phase separation

Robert M Vernon[1] and Julie D Forman-Kay[1,2]

Vernon, Forman-Kay (2019) *COStBi* 58: 88

Computational resources for identifying and describing proteins driving liquid–liquid phase separation

Rita Pancsa, Wim Vranken and Bálint Mészáros

Pancsa... (2021) *Brif. Bioinfo.* 1-20

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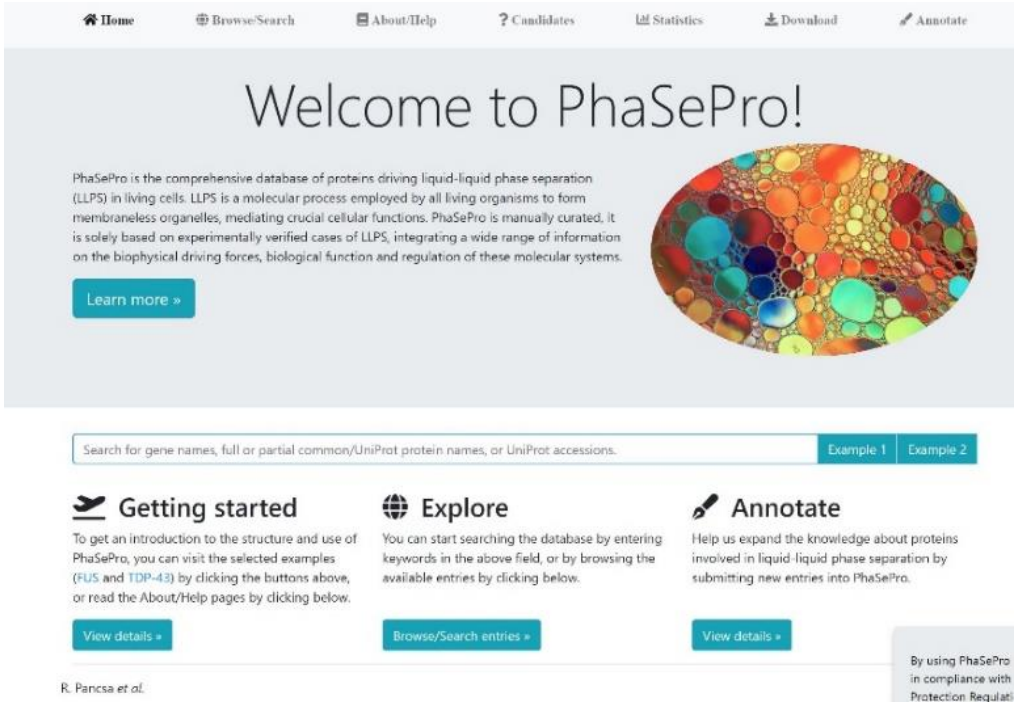
PhasAGE

LLPS databases

- https://phasepro.elte.hu -



Manually curated for LLPS drivers that has both *in vitro* and *in vivo* relevance

Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

LLPS databases

- http://bio-comp.org.cn/llpsdb/home.aspx -

In vitro LLPS experiments as entries, containing both natural designed proteins (the role of protein is not defined).

Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

LLPS databases

- http://db.phasep.pro/ -

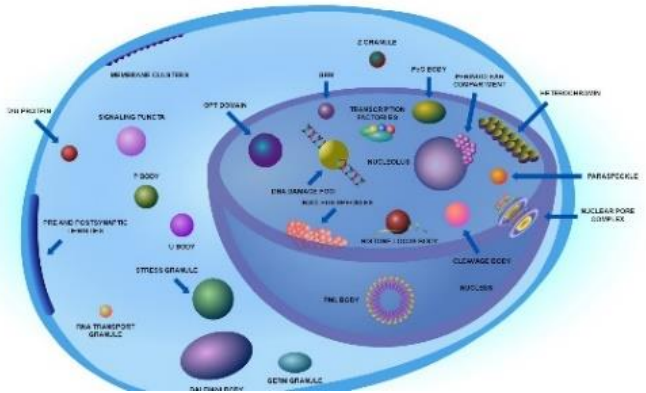
What is PhaSepDB?

PhaSepDB is a novel database that provides a collection of phase separation related proteins manually curated from publications and public database.

As of October 2019, this database includes 2957 eligible proteins. 2303(77.9%) of the proteins were localized in different organelles.
Click a body to browse by organelles →

While 861(22.1%) of them were not associated with known organelles.
Click here to browse these proteins.

In addition to curated proteins, *PhaSepDB* also provides the researchers with molecular signatures that may facilitate phase separation related proteins identification for all human proteins.
Click here to browse human proteins.



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Annotations based on MLO localization (literature evidence, UniProt annotations, and HTS localization experiment).

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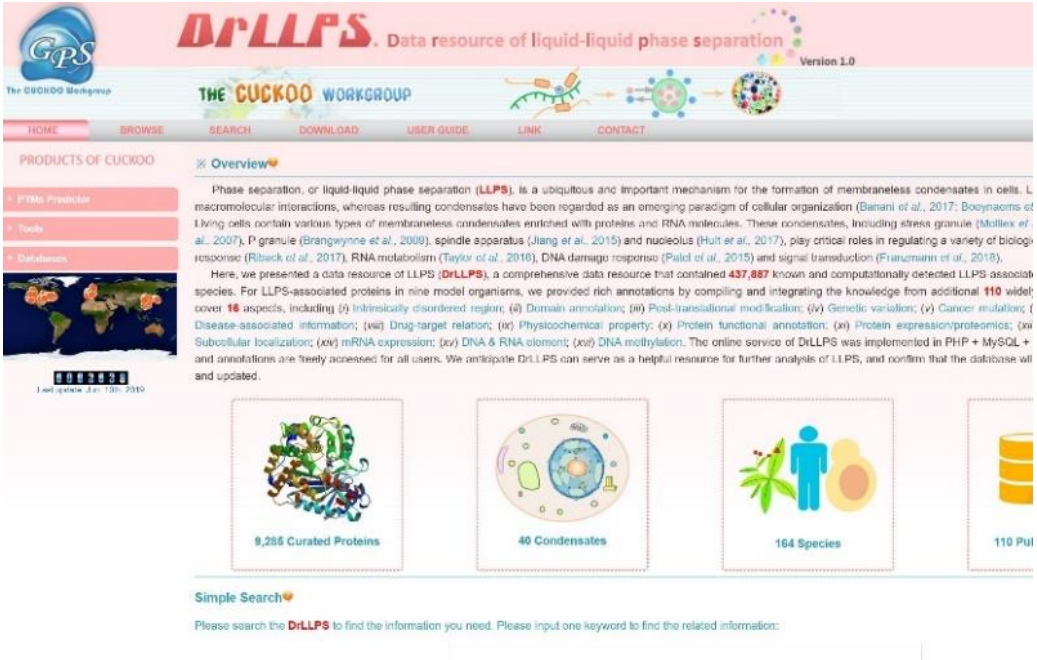
Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

LLPS databases

- http://llps.biocuckoo.cn -



MLO-associated proteins classified as scaffolds, regulators, and clients as assessed by automated text mining, followed by curator assessment. *In vitro*, *in vivo* and computational evidence is accepted (HTS, LTS), such as KO, silencing, overexpression.

Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

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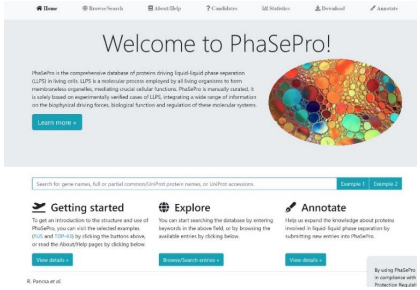
What does “LLPS protein” mean?

PhaSePro: 120 proteins



LLPSDB: 1200

PhaSepDB: 3000

DrLLPS: 9300

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Farahi et al. (2021) *Int J Mol Sci*. 22: 3017

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Why is not straightforward to call an LLPS protein?

1) the capacity to phase separate is not a binary classifier (not intrinsic but contextual property of the protein and its environment)

2) phase separation depends on the concentration of the protein (physiological?)

3) proteins have distinct roles in phase separation

4) LLPS is not equivalent to biomolecular condensation (which includes gelation, crystallization, clustering, pleiomorphic assembly, polymerization and amorphous or amyloid aggregation) or templated assembly.

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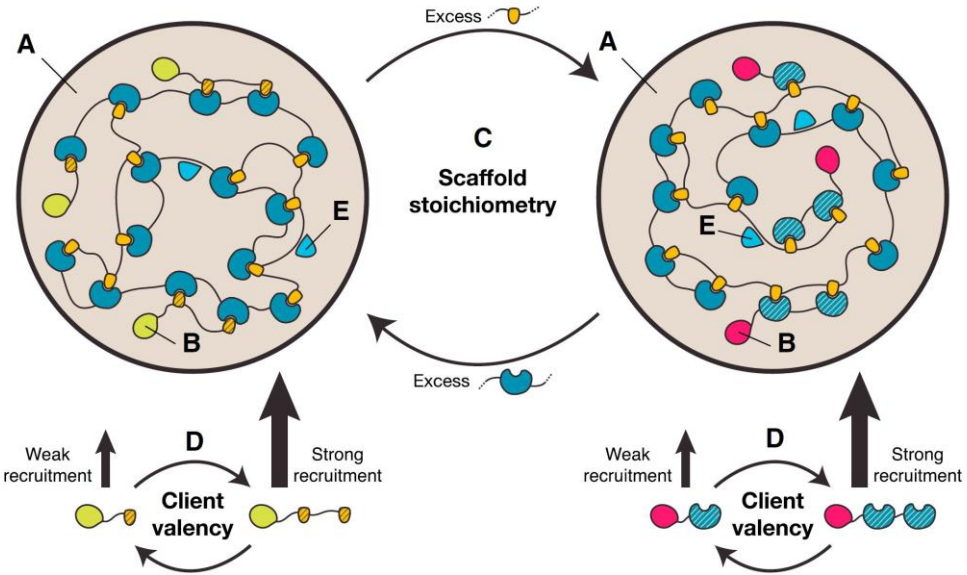
Scaffolds and clients



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Banani et al. (2016) *Cell* 166, 651

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Different roles of proteins in LLPS.

1) Driver (scaffold): can phase separate on their own. If RNA is mandatory, we consider it as a "co-driver". Small molecules (and crowder) are "condition".

2) Co-driver: a macromolecule (protein, RNA or DNA) that strictly requires another macromolecule for phase separation (then both are "co-drivers")

3) Regulator: its presence/activity is required for LLPS, but no part of condensate (modifying enzyme, transport protein, transcription factor, etc...)

4) Client: not required for and has no effect on LLPS, but localizes to the condensate formed (through interactions with driver/co-driver.

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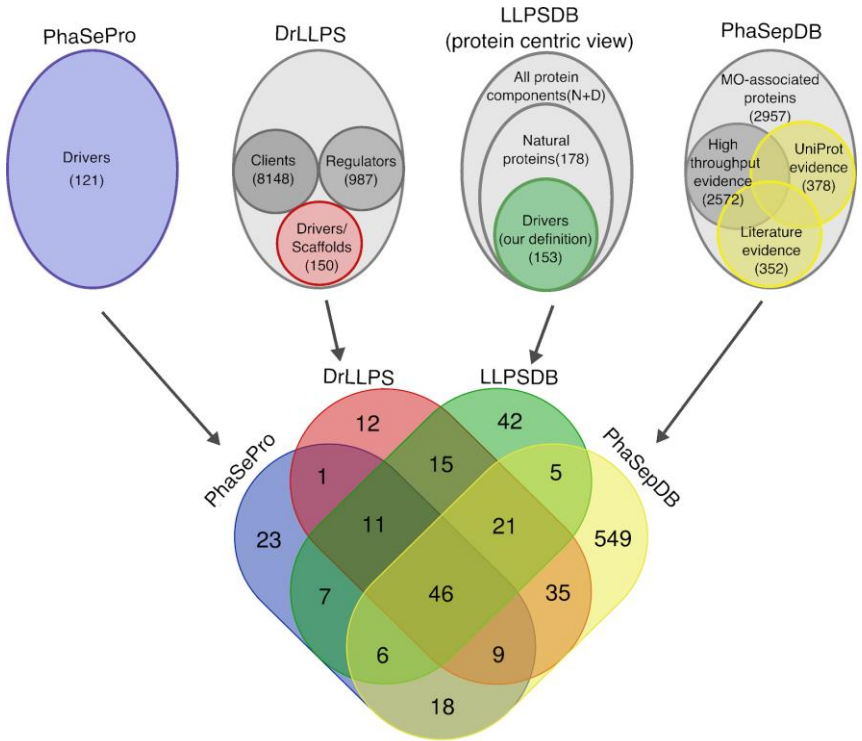
Different databases contain different type (and amount) of data



PhaSePro: 120
LLPSDB: 1200
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A primary goal: develop LLPS predictors

Table 1

Phase-separation prediction methods

Method name	Description	Residue # information	Availability	Ref.
PLAAC	Prion-like domain prediction by hidden Markov model	Yes	plaac.wi.mit.edu	[43,46]
LARK	Energetic compatibility with aromatic-rich kinked amyloid structures, by threading sequences and modeling with Rosetta	Specific regions	In supplement of original paper	[23••]
R + Y	Critical concentration prediction based on number of arginine and tyrosine residues, extrapolated from FET family proteins	No	In supplement of original paper	[50••]
DDX4-like	Sequence composition and residue spacing similarity to DDX4	Specific regions	From authors	[51]
CatGranule	Composition weighted by sequence length, R/G/F content, and amino acid propensity for nucleic acid binding and disorder	Yes	tartaglialab.com	[52]
PScore	Prediction based on expected numbers of long-range planar sp2 pi-pi contacts	Yes	abragam.med.utoronto.ca/~JFKlab/	[42••]
CRAPome	Empirical measurement related to non-specific interactions and *in vivo* concentration, taken from the frequency at which each human protein is identified by affinity purification mass spectrometry negative controls	No	crapome.org	[54]

PSPredictor machine learning based on LLPSDB database

http://www.pkumdl.cn:8000/PSPredictor/

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Vernon, Forman-Kay (2019) *COStBi* 58: 88

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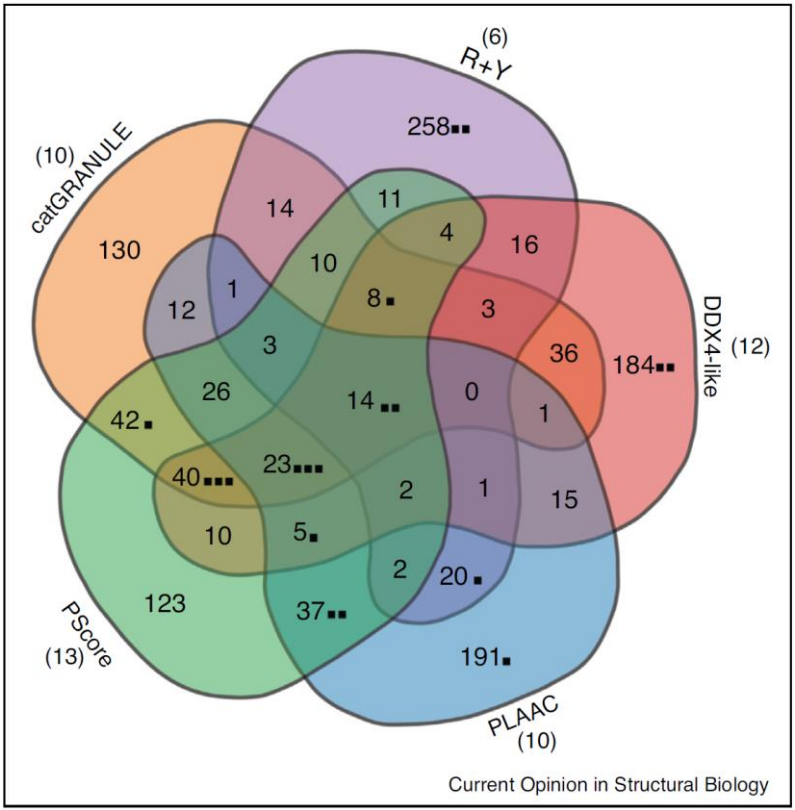
Prediction of LLPS proteins in human proteome



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Vernon, Forman-Kay (2019) *COStBi* 58: 88

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Thank you