Phase separation: an emergent function of disordered proteins

Peter Tompa

PhasAGE

EXPERT SEMINAR SERIES

26 APRIL 2021 – 3PM CEST

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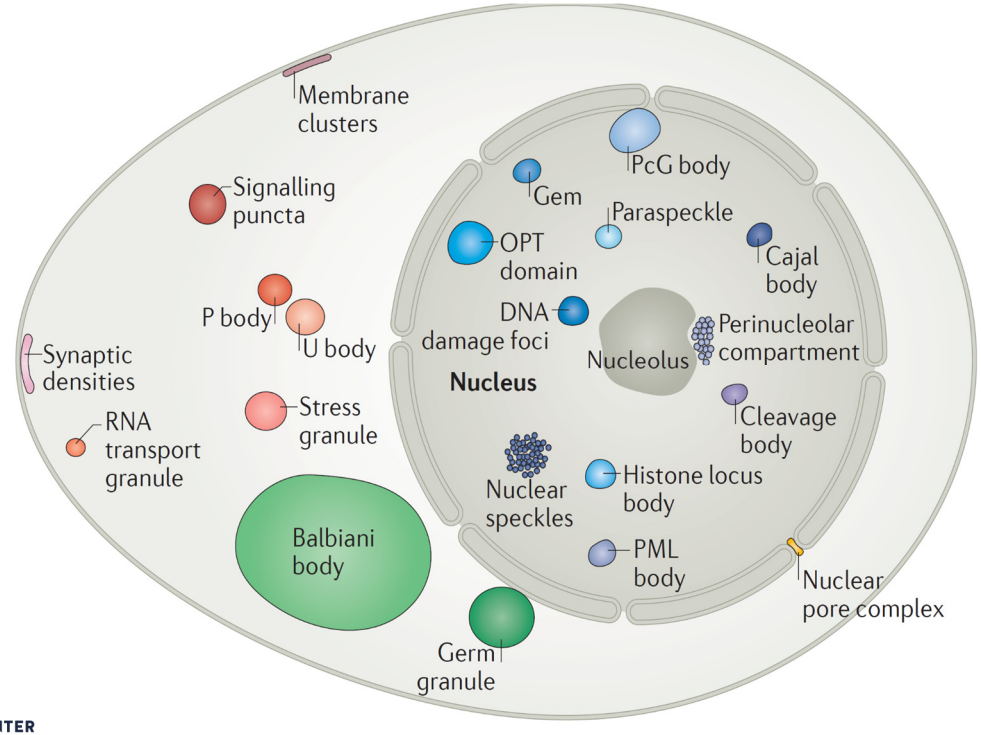
EXCELLENCE HUB ON PHASE TRANSITIONS IN AGING AND AGE-RELATED DISORDERS

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

Membraneless organelles

- biomolecular condensates, RNP bodies, LLPS -



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

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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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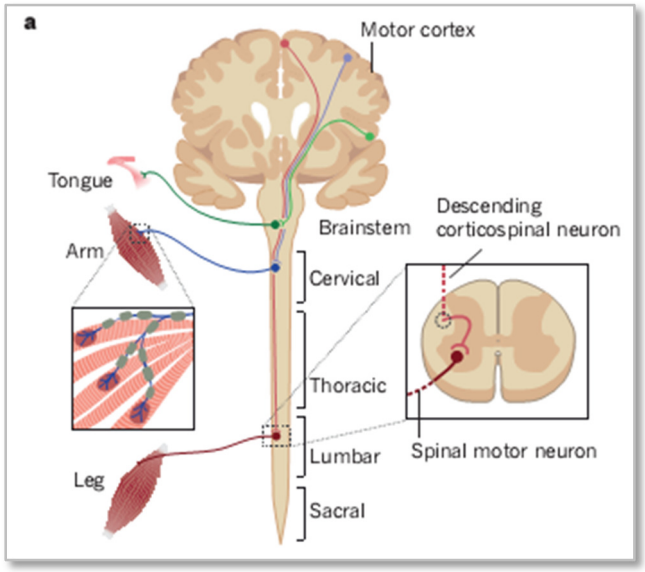
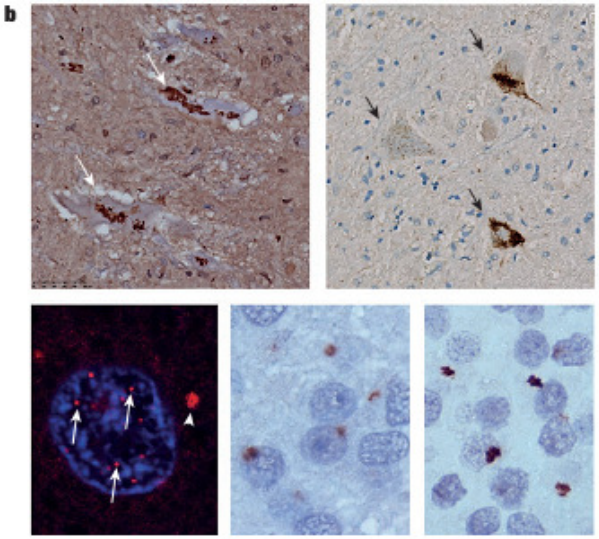
ALS is motor neuron disease





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

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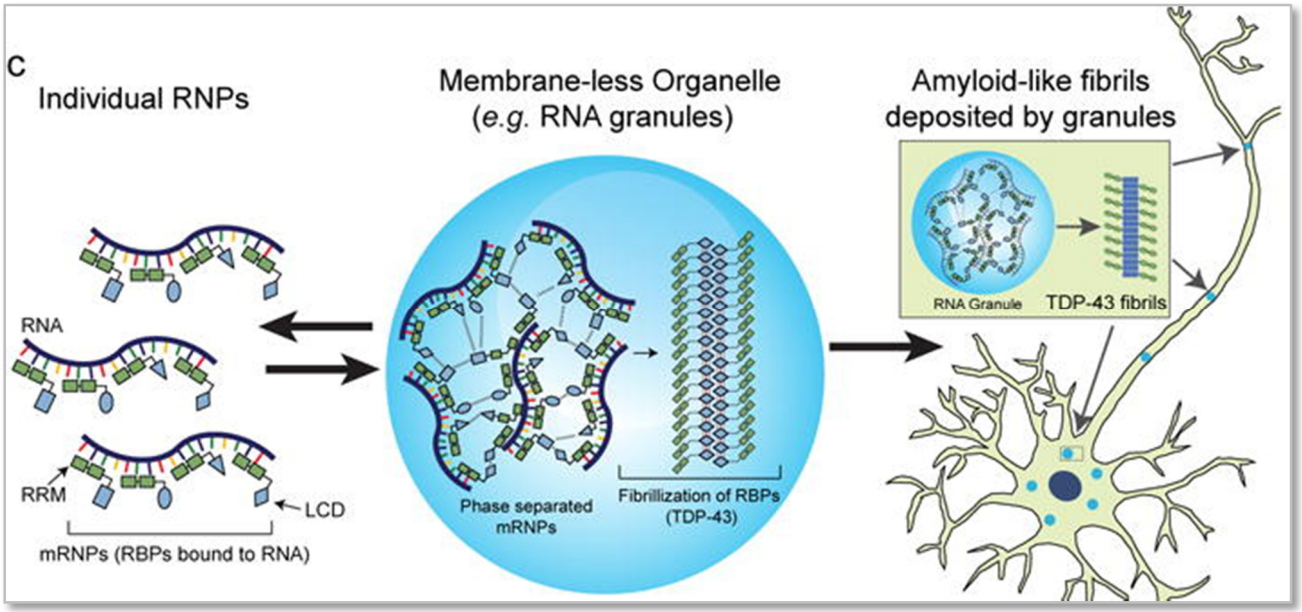
LLPS may lead to disease

- stress granules in ALS (?) -



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

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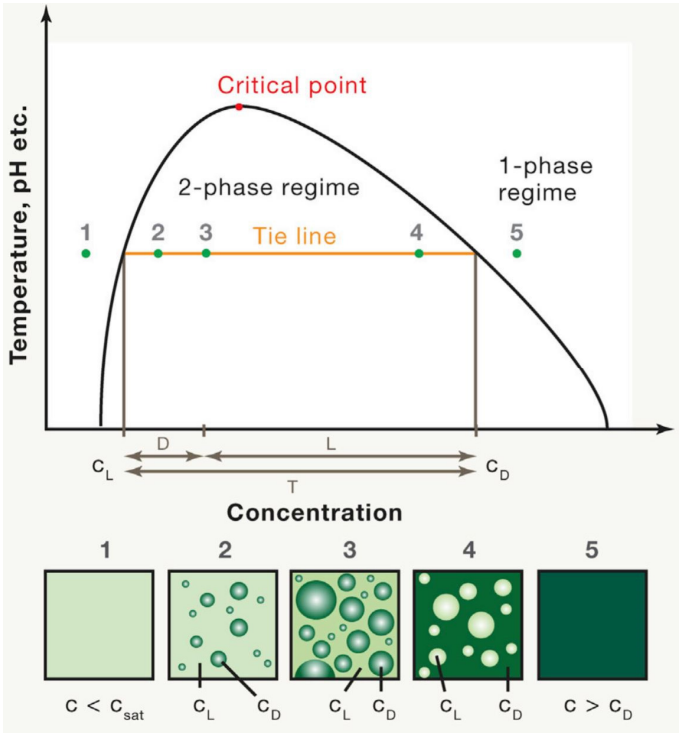
Their mechanism of formation

- spontanous demixing -

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

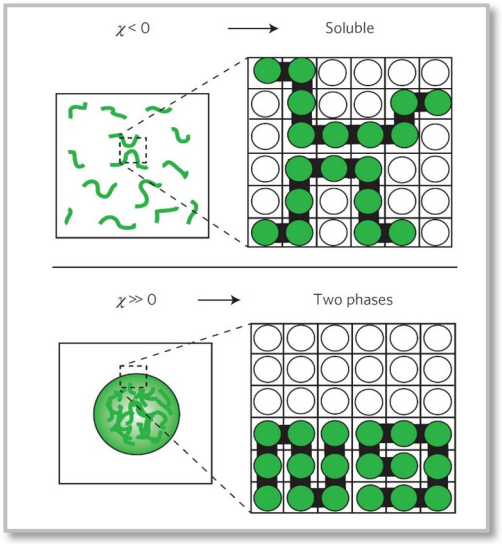
- e.g. Flory-Huggins formalism -

Free energy of mixing (Φ - volume fraction)

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

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

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



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Brangwynne (2015) *Nature Phys.* 11: 899

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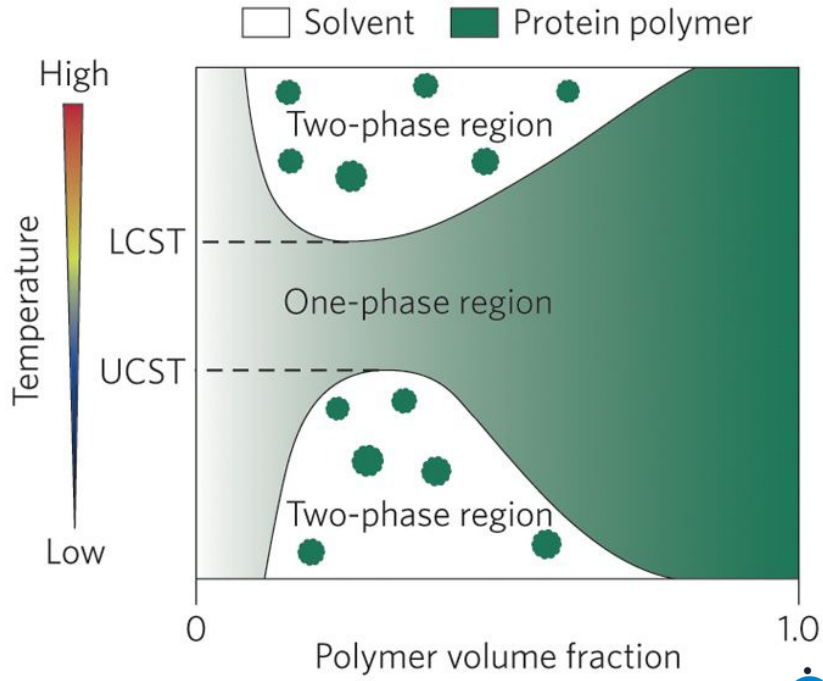
Sometimes opposite behavior

Temperature dependence of attractive term



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

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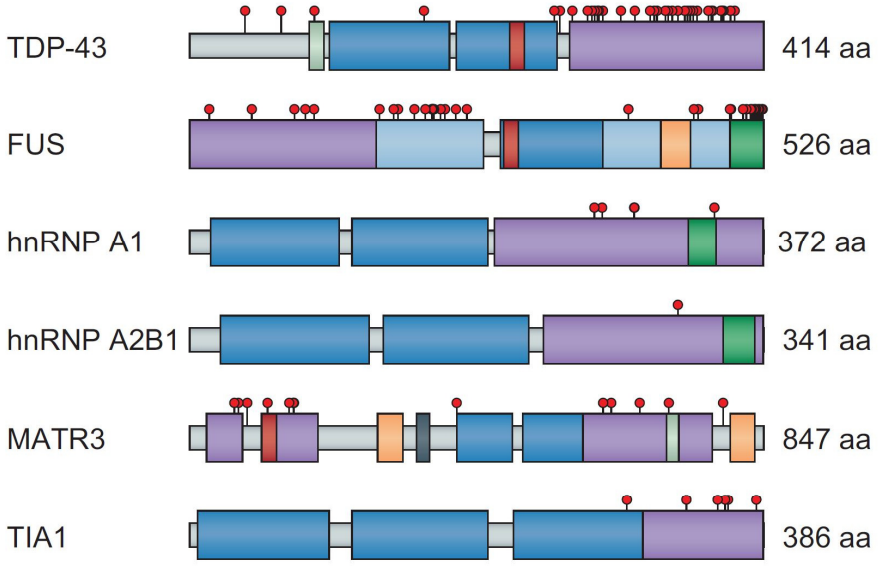
Structural disorder in LLPS proteins

- LCD: low-complexity IDR -



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

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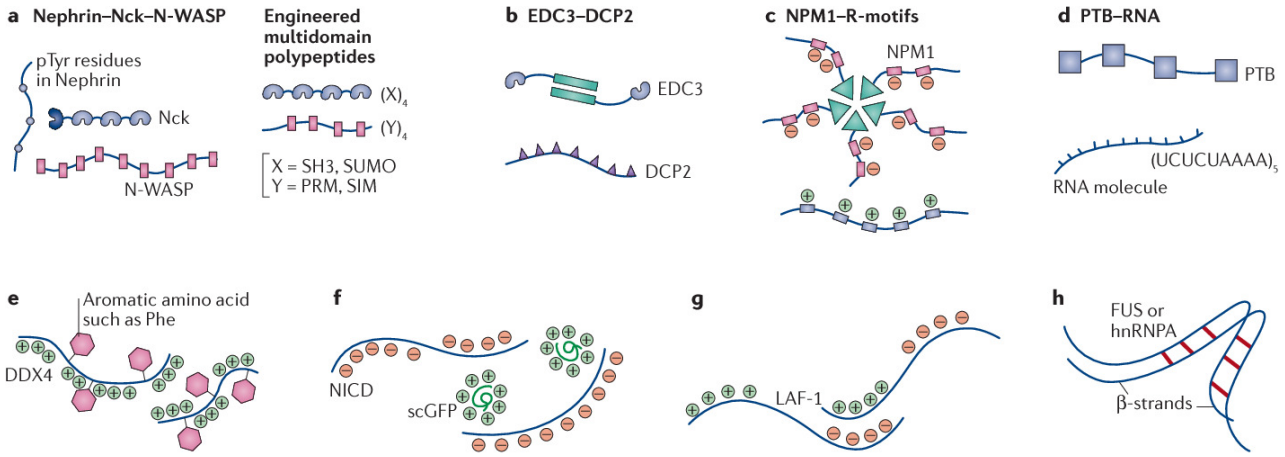
Multivalency is basic to LLPS



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

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

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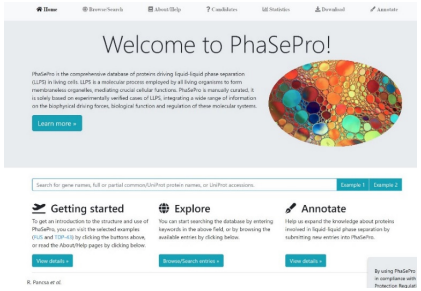
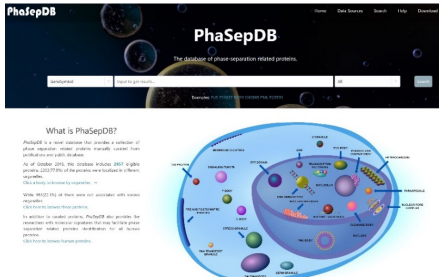
What does “LLPS protein” mean?

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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“Problems” with LLPS (1)

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

2) proteins have distinct roles in phase separation

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

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

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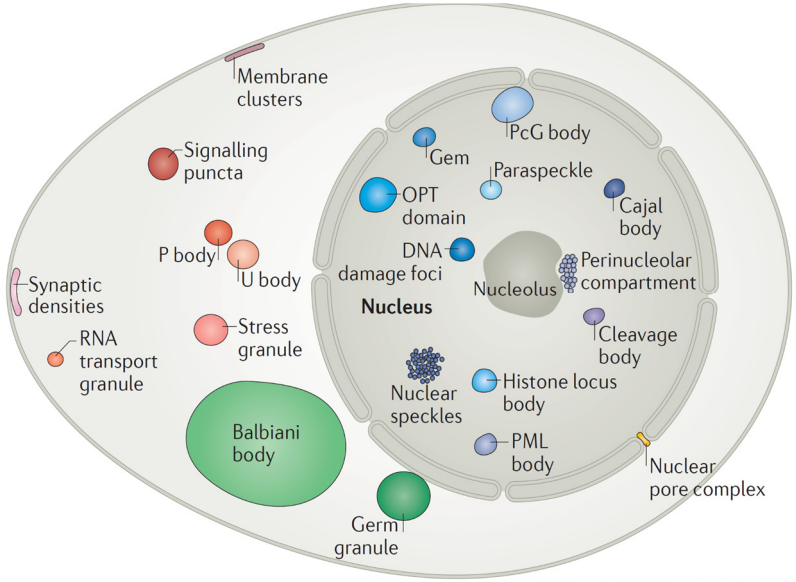
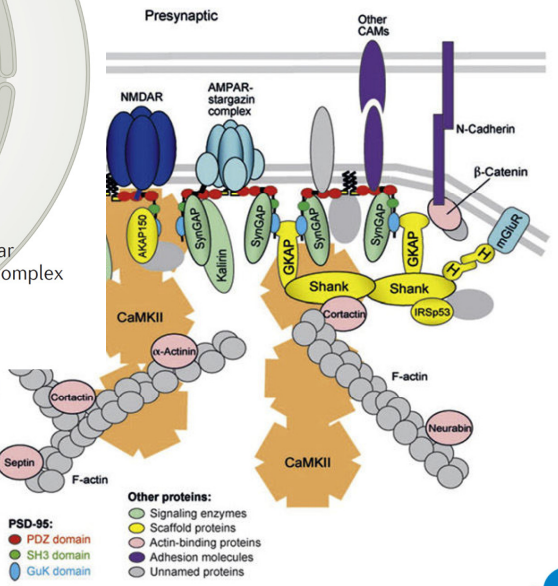


Banani et al. (2017) *NRMCB*



Pancsa (2019) *BBA*

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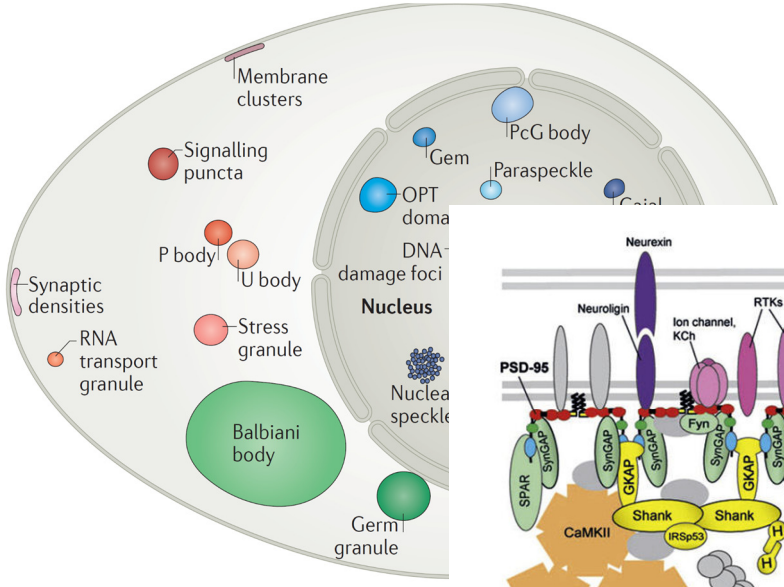
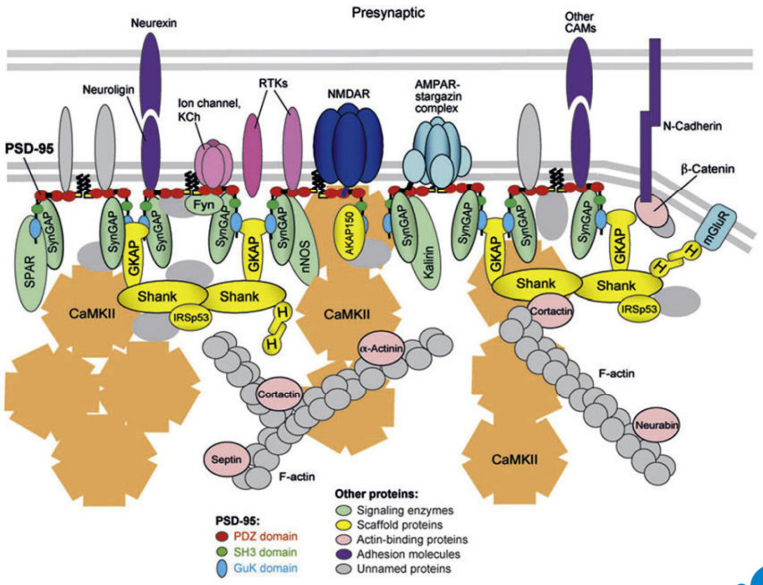
“Problems” with LLPS (1)



Banani et al. (2



Pancsa (2019) *BBA*

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“Problems” with LLPS (2)

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.

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

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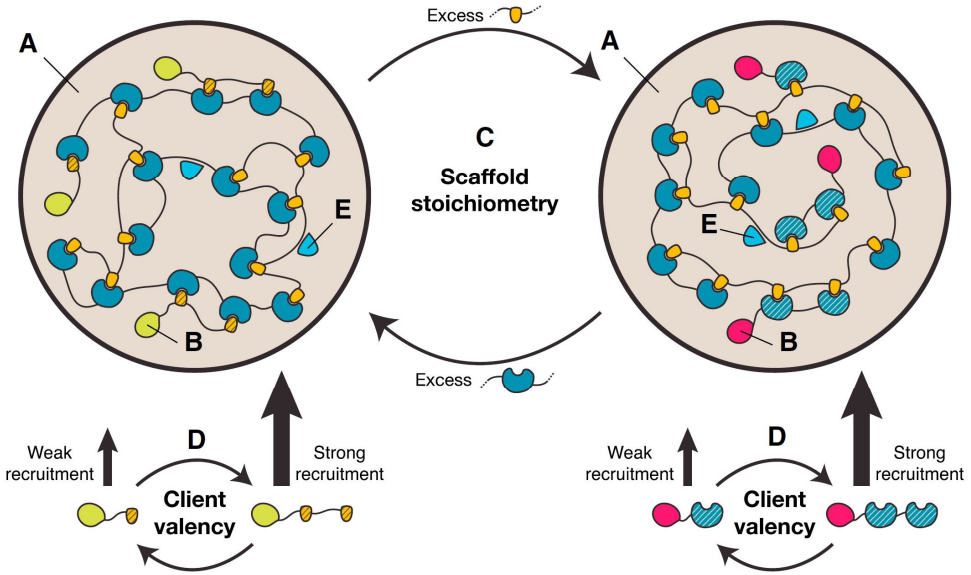
Scaffolds and clients



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

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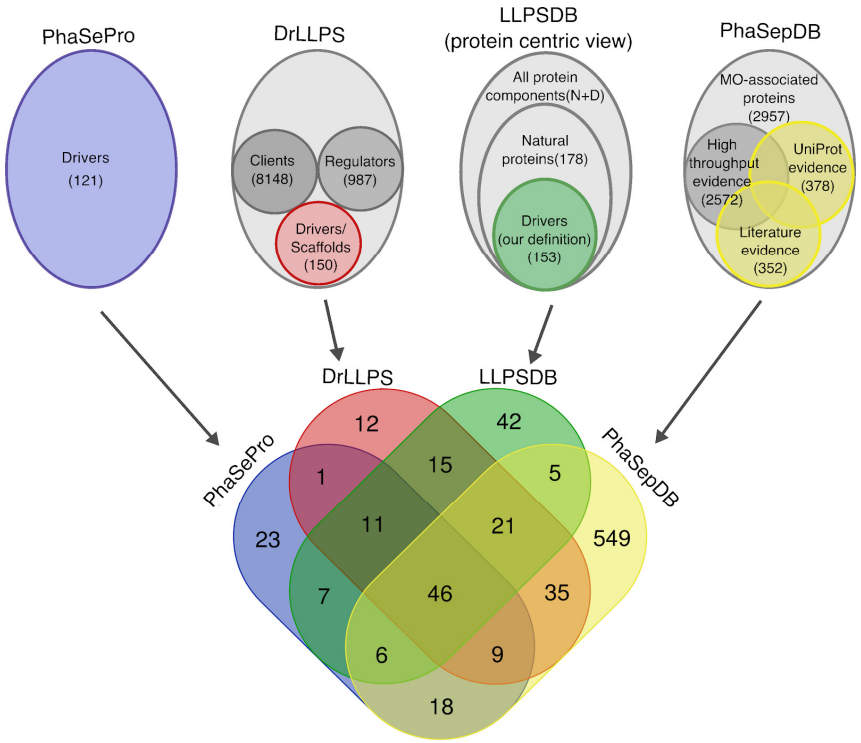
Different databases contain different type of data



PhaSePro: 120
LLPSDB: 1200
PhaSepDB: 3000
DrLLPS: 9300

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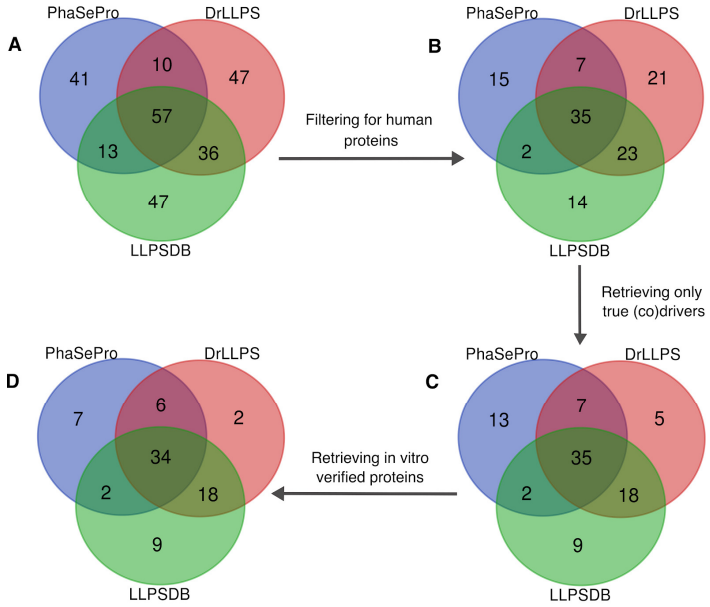
Filtering for high-confidence “drivers”

89 human high-confidence drivers



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

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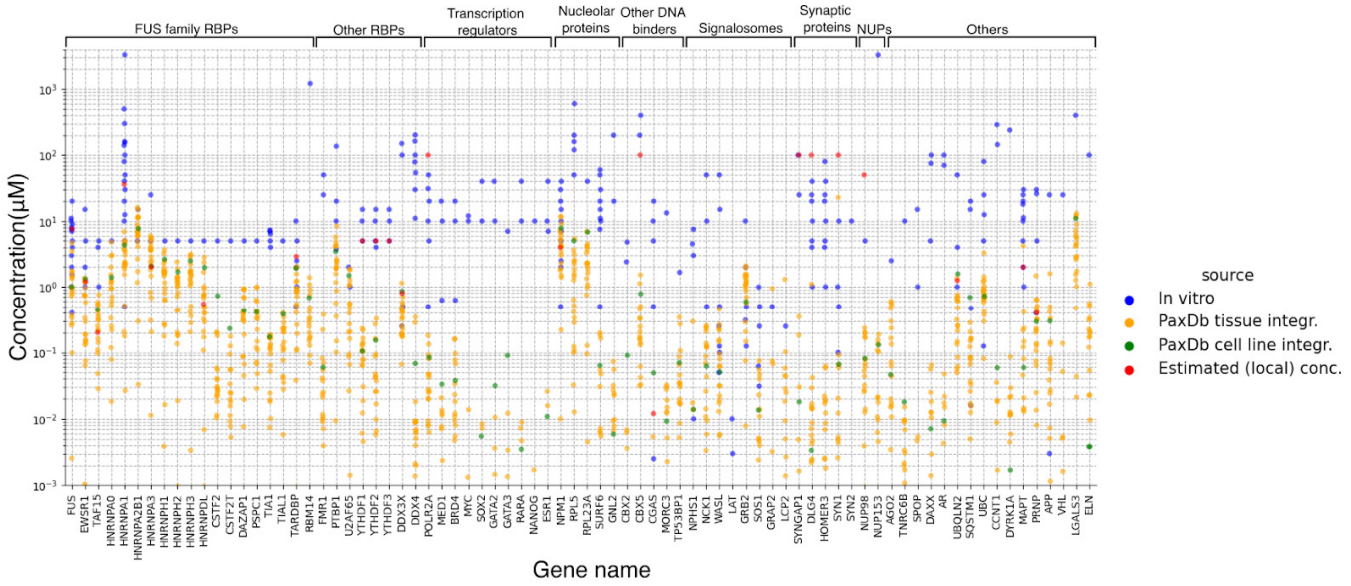
Issue with concentrations



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

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Three (four) basic types of protein-protein interactions in LLPS

1) IDP-IDP (transient, dynamic, non-specific, non-stoichiometric, distributed)

(Motif-motif (dynamic, semi-stoichiometric))

2) Domain-motif (strong, specific, stoichimetric)

3) Domain-domain (strong, specific, stoichiometric)

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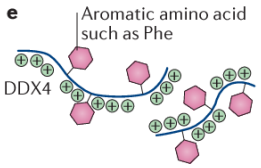
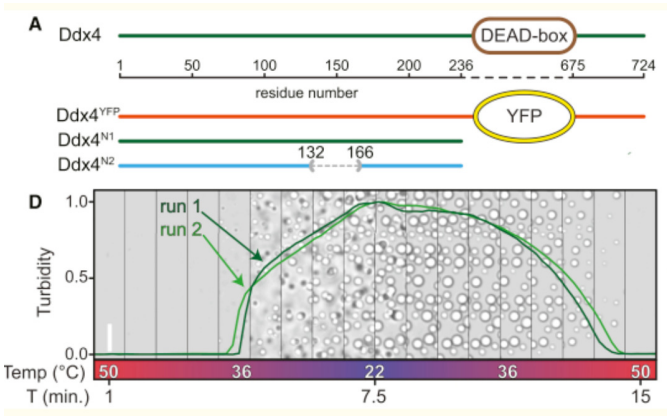
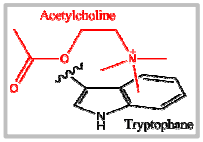
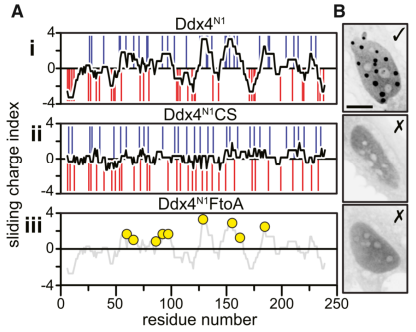
IDP-IDP (e.g. cation-pi, charge)

- Dead-box helicase, germ-granule (nuage) - regulation of translation in germ cells









Nott et al. (2015) *Mol. Cell* 57: 936

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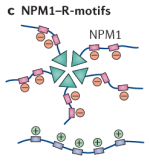
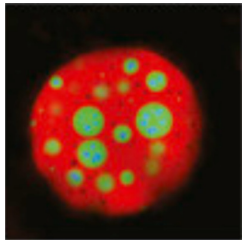
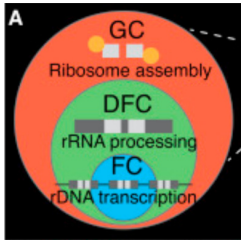


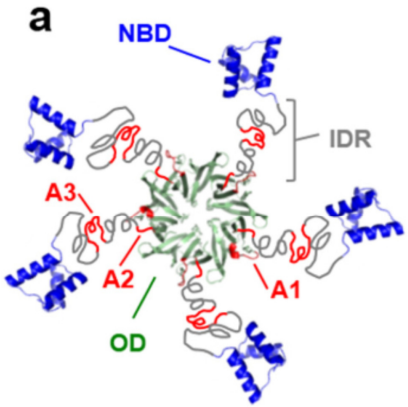
Domain-domain

- Nucleophosmin 1 (NPM1), nucleolus - ribosome biogenesis





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



Oligomerization domain (OD)
Acidic tracts (A1, A2, A3)
Nucleic-acid binding domain (NBD)

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

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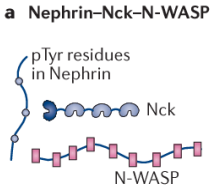
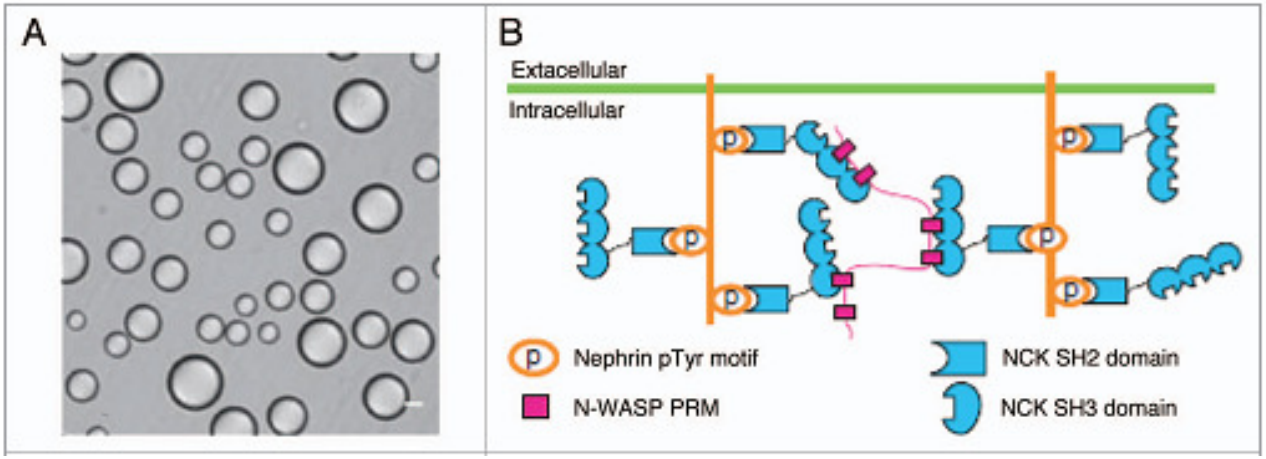


Domain-motif

- SH2 - pTyr, SH3 - PRM -

signaling complex in cytoskeleton remodeling



… Rosen (2012) *Nature* 483: 336

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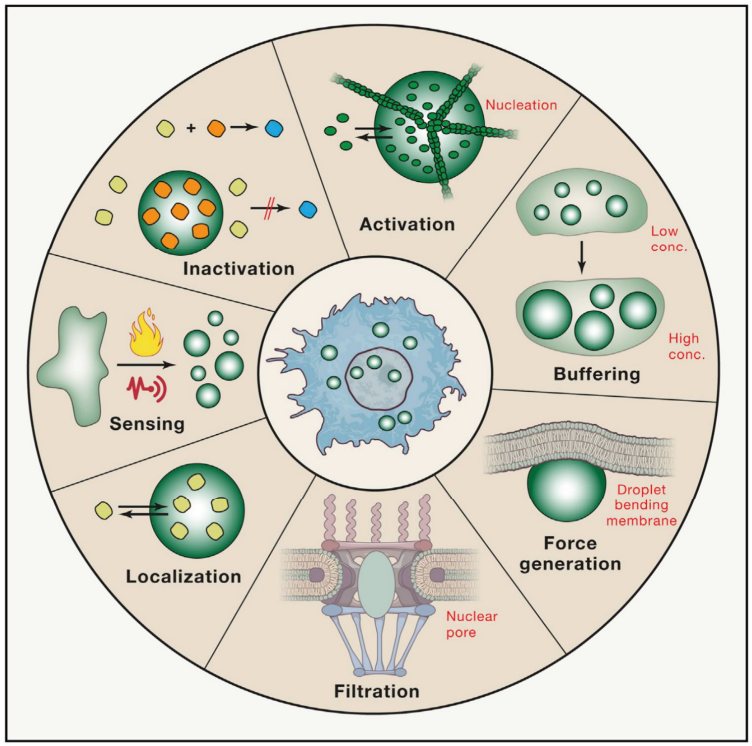
Functional consequences of LLPS

- is an emergent property -



Alberti (2019) *Cell* 176: 419

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