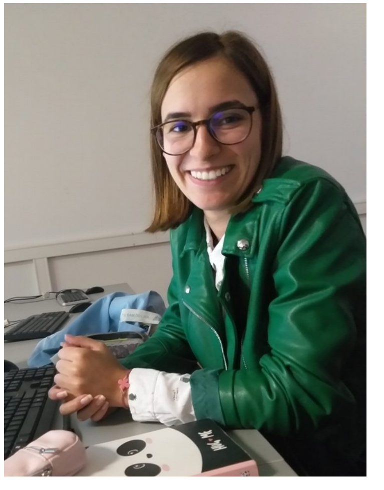
Student seminar as a one (wo)man show

Obligate course seminar – How to make it fun?

Martina Manenica

CONNECT2020

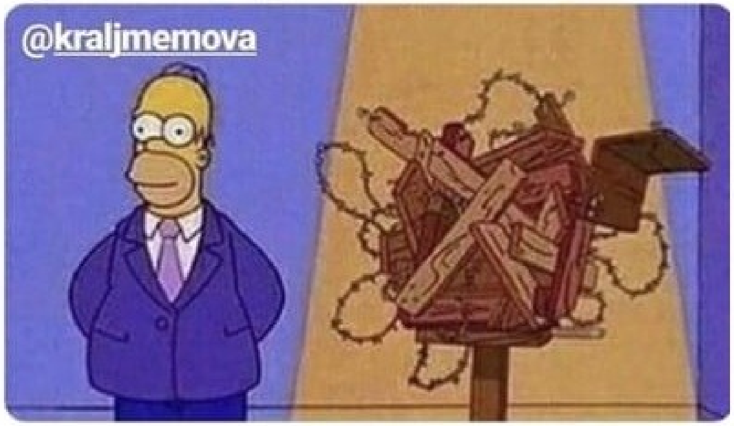
Neum, August 2020



There are two ways to make a presentation…

1.) Just to do it…

Giving a presentation that you made at 2 am



- 2.) To make an impact

Liquid-Liquid Phase Separation in Biology

Student seminar

Class: Physical chemistry of macromolecules

Zagreb, 2019.

Science AAAS

Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation
Clifford P. Brangwynne *et al.*
Science **324**, 1729 (2009);
DOI: 10.1126/science.1172046

Letter | Published: 07 March 2012

Phase transitions in the assembly of multivalent signalling proteins

nature International journal of science

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

Cell

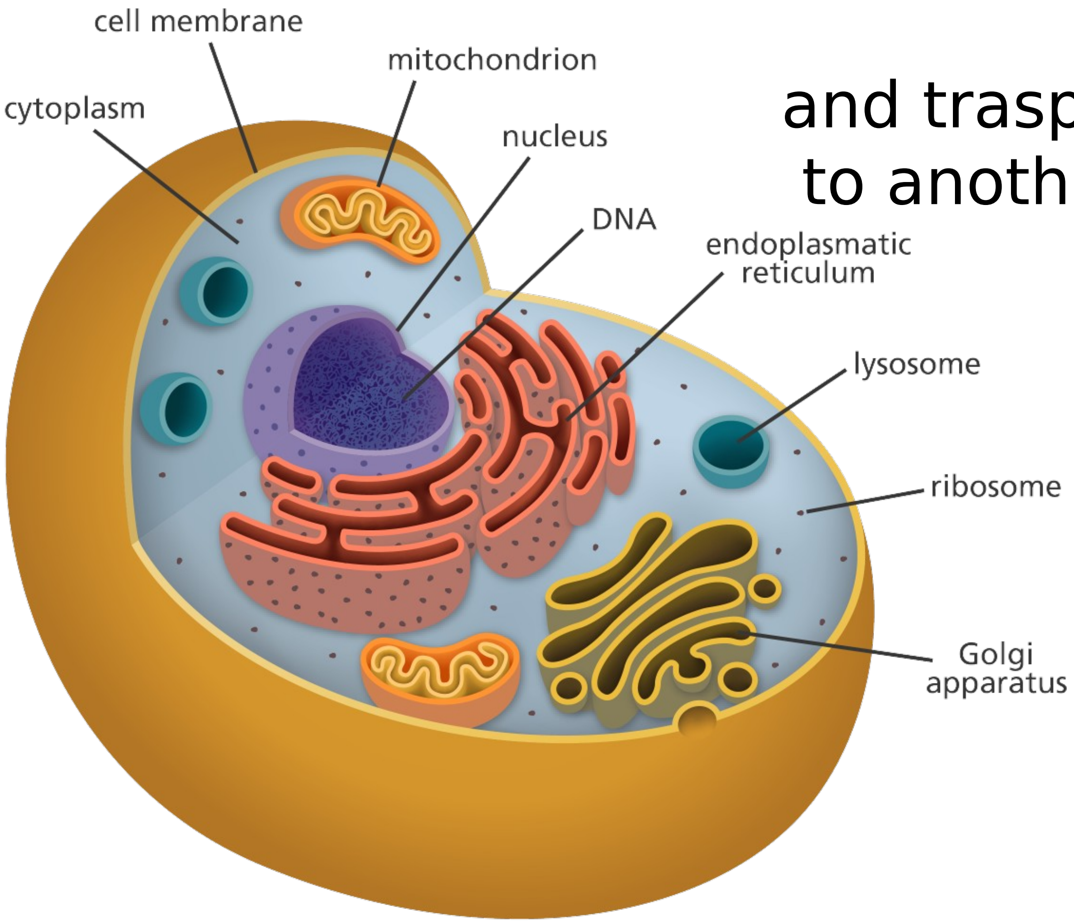
Dual Specificity Kinase DYRK3 Couples Stress Granule Condensation/Dissolution to mTORC1 Signaling

2013.

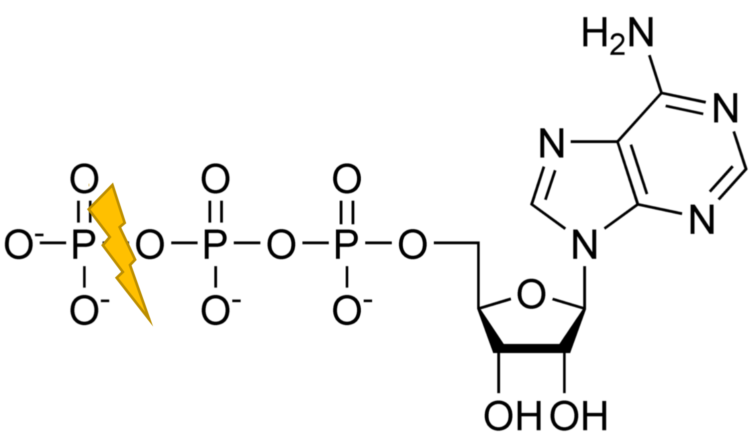
Frank Wippich,[1,3] Bernd Bodenmiller,[1] Maria Gustafsson Trajkovska,[1] Stefanie Wanka,[1] Ruedi Aebersold,[2,3] and Lucas Pelkmans[1,*]

Eukaryotic cell

mpartments are separated by membranes…

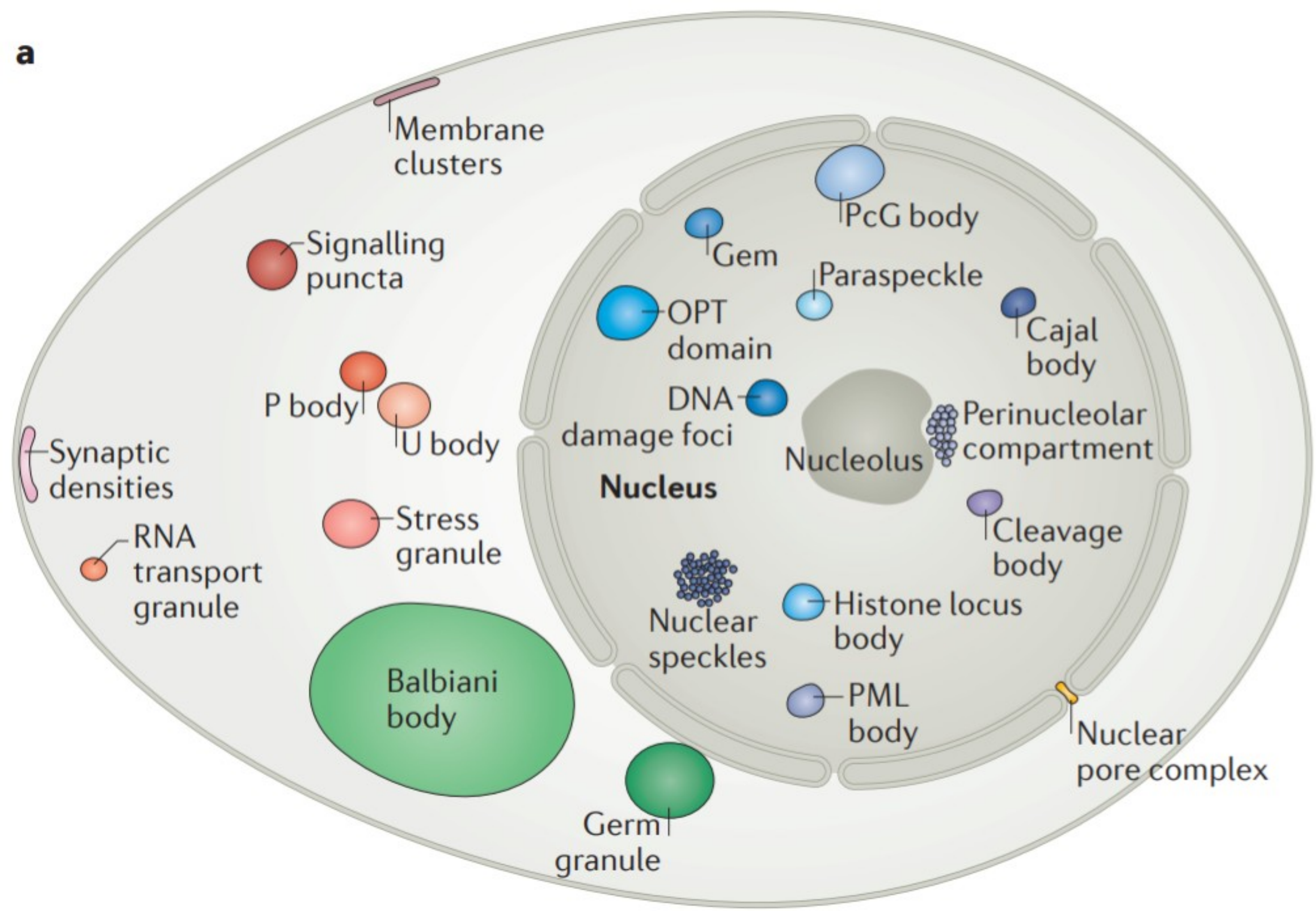


and trasport from one compartmen
to another is payed in ATP ‘curren



ttps://www.yourgenome.org/facts/what-is-a-cell

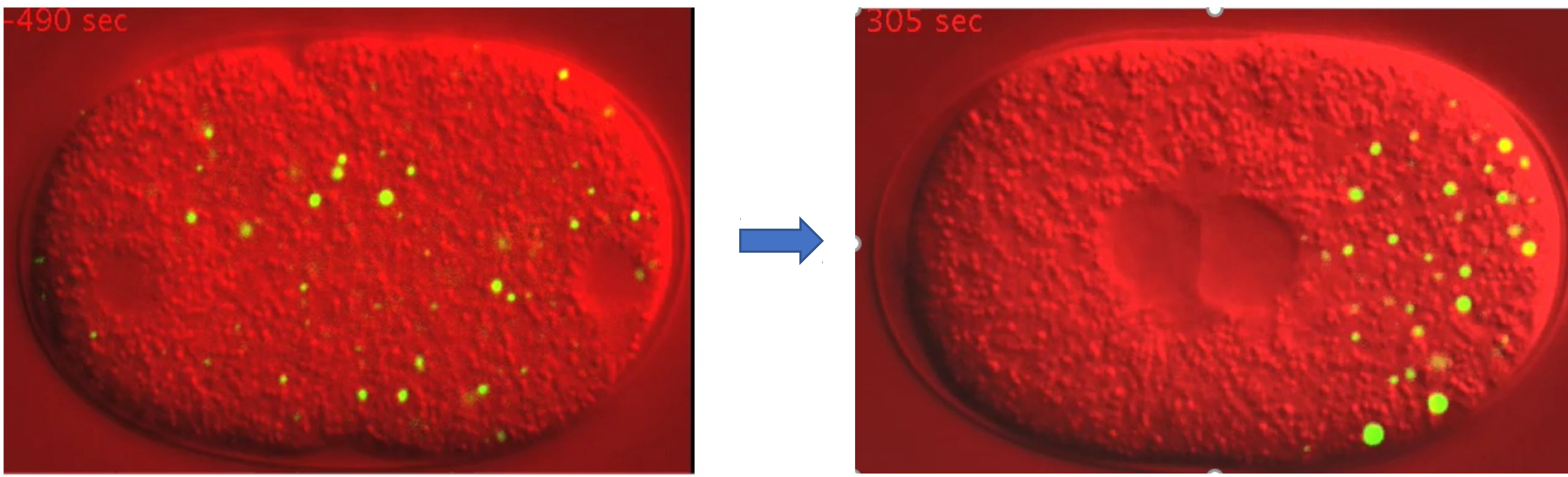
https://commons.wikimedia.org/wiki/File:ATP_chemical_structure.png



S. F. Banani, H. O. Lee, A. A. Hyman M. K. Rosen, *Mol. Cell Biol.* **18** (2017) 85-88.

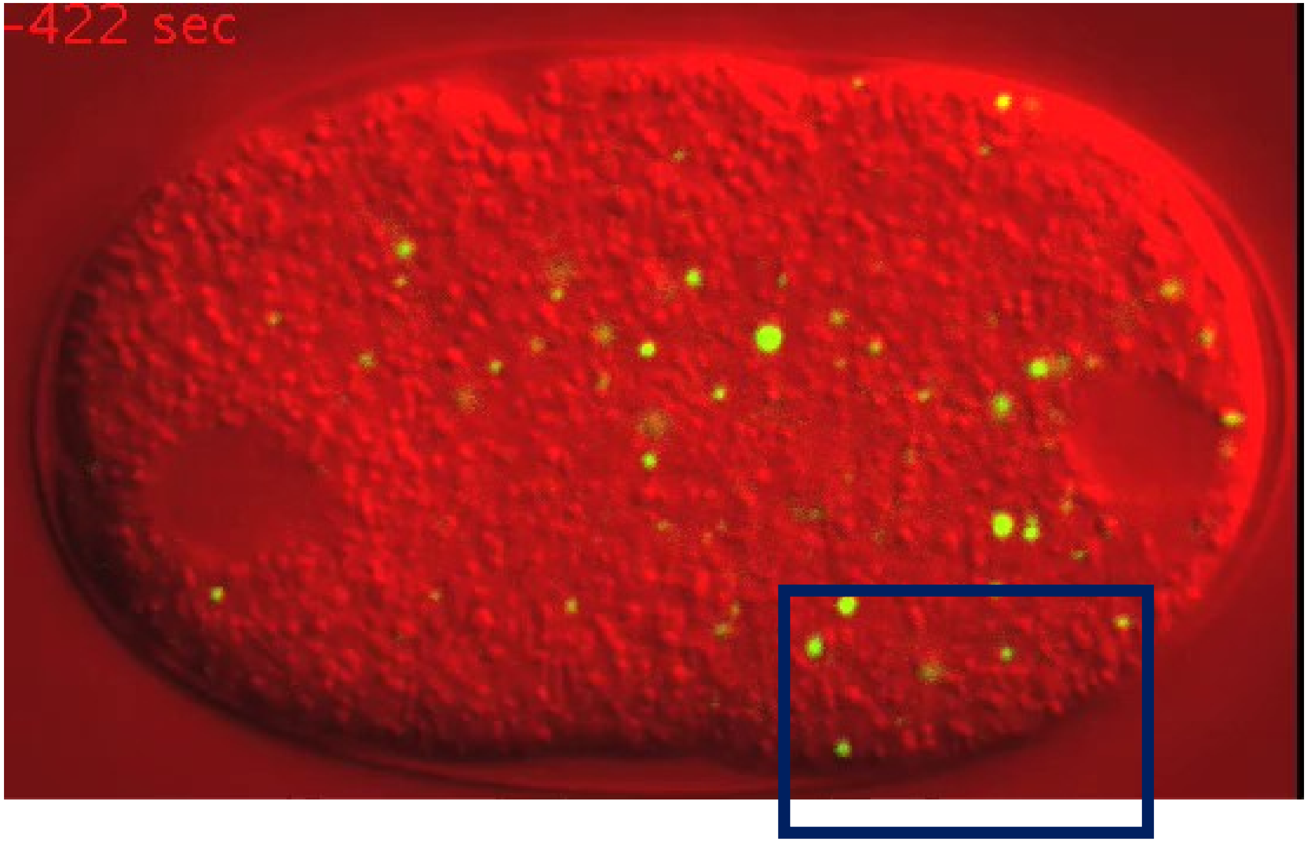
Discovery of phase separation in biology - P granules

- RNA rich granules
- Important for specification of cells in germ embrio



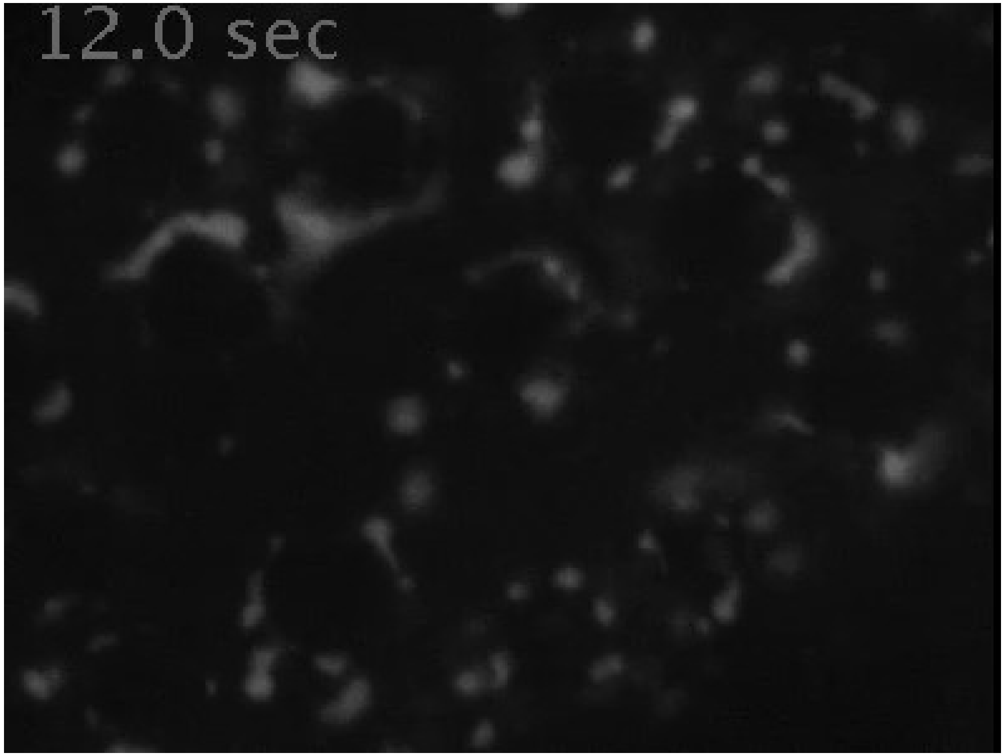
C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, A. A. Hyman, *Science* **324** (2009) 1729−1732 .

Discovery of phase separation in biology - P granules



C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, A. A. Hyman, *Science* **324** (2009) 1729−1732 .

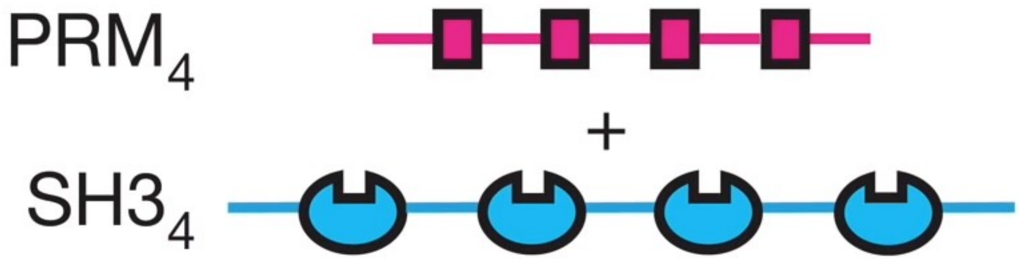
Otkriće fazne separacije u biologiji – P granule



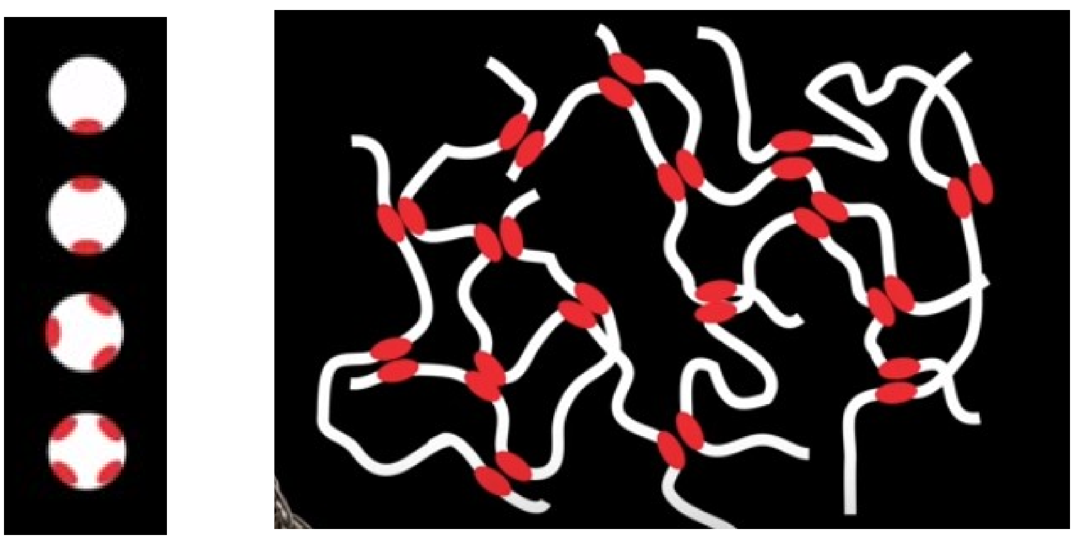
reuzeto: C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege
J. Gharakhani, F. Jülicher, A. A. Hyman, *Science* **324** (2009) 1729−1732 .

Which proteins take part in LLPS?

1. Multivalent proteins with ‘’sticky patches’’



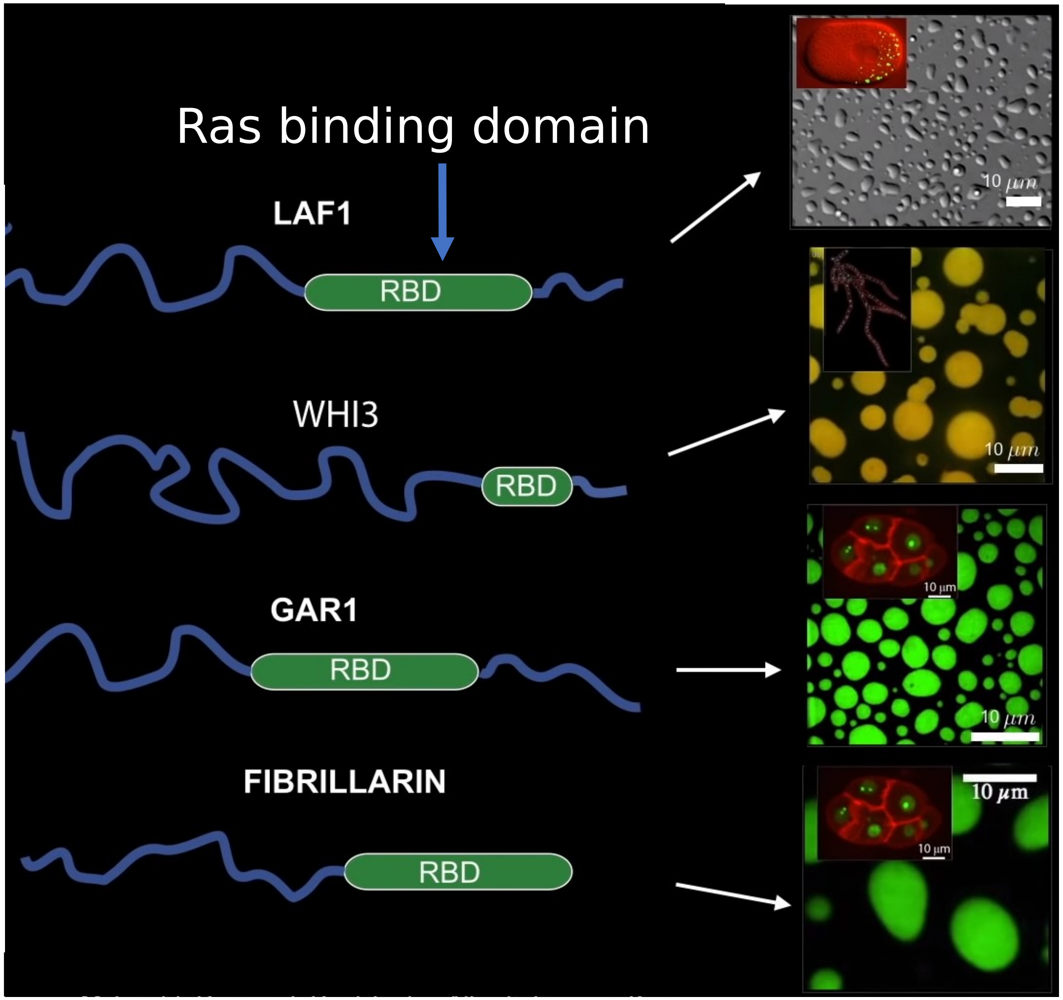
Preuzeto: P. Li, S. Banjade, H.-C. Cheng, S. Kim, B. Chen, L. Guo, M. Llaguno, J. V. Hollingsworth, D.S. King, S.F. Banani, *Nature* **483** (2012) 336–340.



Preuzeto: https://www.ibiology.org/biophysics/liquid-phase-separation-in-living-cells/

Which proteins take part in LLPS?

Proteins with intrinsically disordered domains



1. Gly/Ser-Phe sequence
2. Tyr-Gly-Ser sequence
3. poly-Gln i poly-Asn
4. – or + -charge on side chains

S. F. Banani, H. O. Lee, A. A. Hyman M. K. Rosen, *Mol. Cell Biol.* **18** (2017) 85-88.

www.ibiology.org/biophysics/liquid-phase-separation-in-living-cells/

$p,T)=U+pV-TS$

$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{T,p,N_{j \neq i}}$

Ideal mixture (no interactions)

$\Delta H^{mix} = 0$

- Mixture with interactions

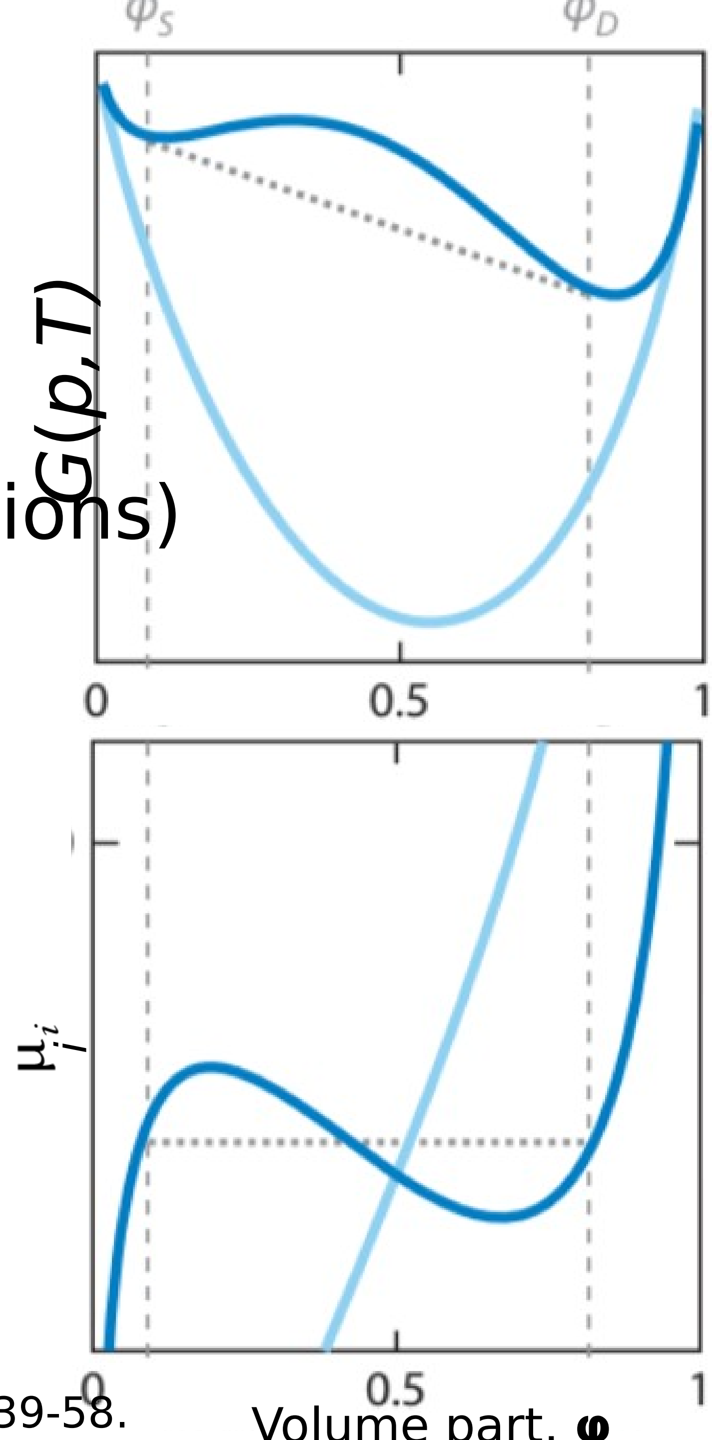
  $\Delta H^{mix} \neq 0$

  $\Delta H^{mix} = V(\delta_2 - \delta_1)^2 \phi_1 \phi_2$

  Flory-Huggins

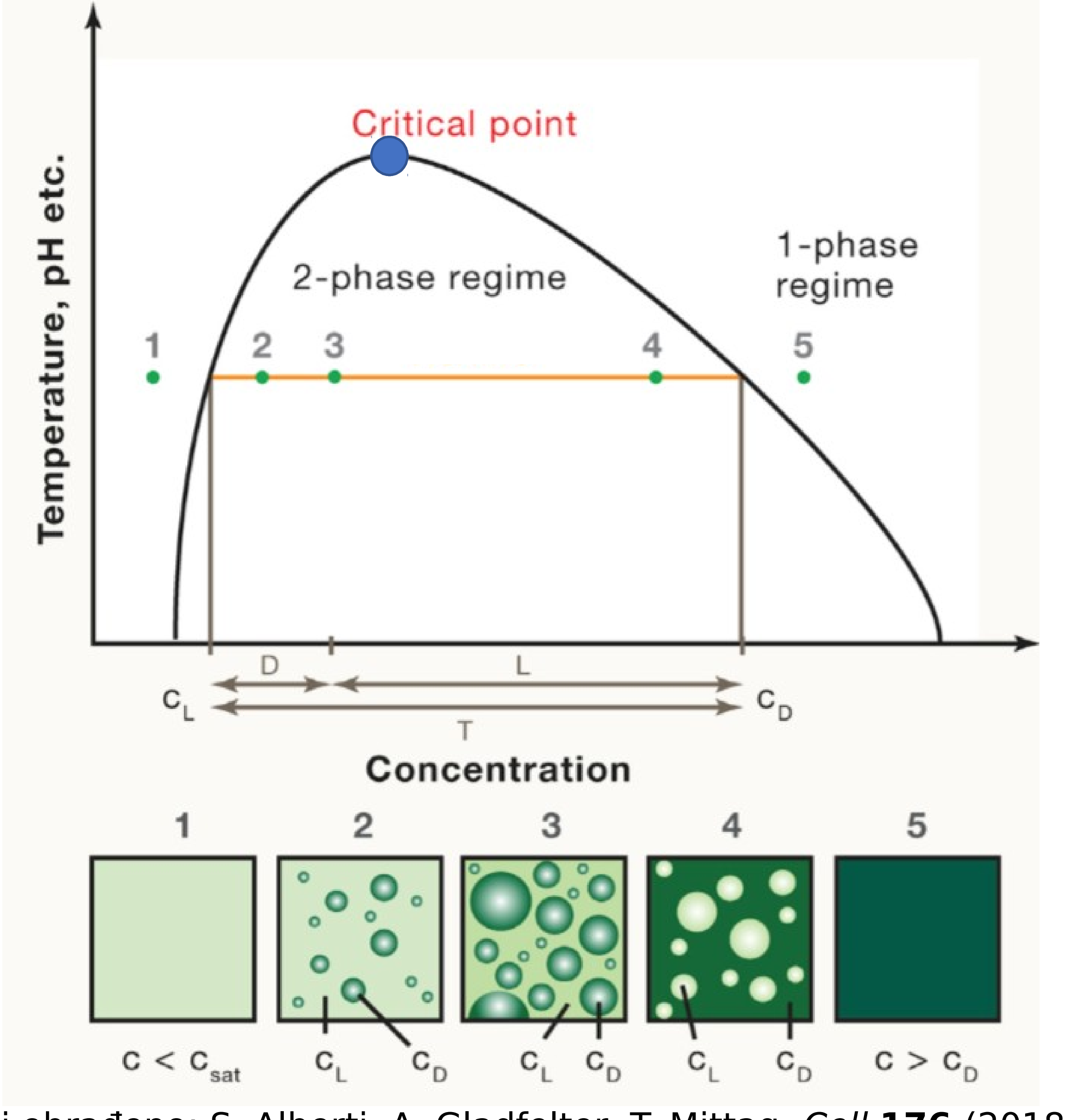
  δ – Solubility

  ϕ – Volume part in the mixture



A.A. Hyman, C.A. Weber, F. Jülicher, *Annu. Rev. Cell Dev. Biol.* **30** (2014) 39-58.

Goal: Phase diagram *in vitro* ...

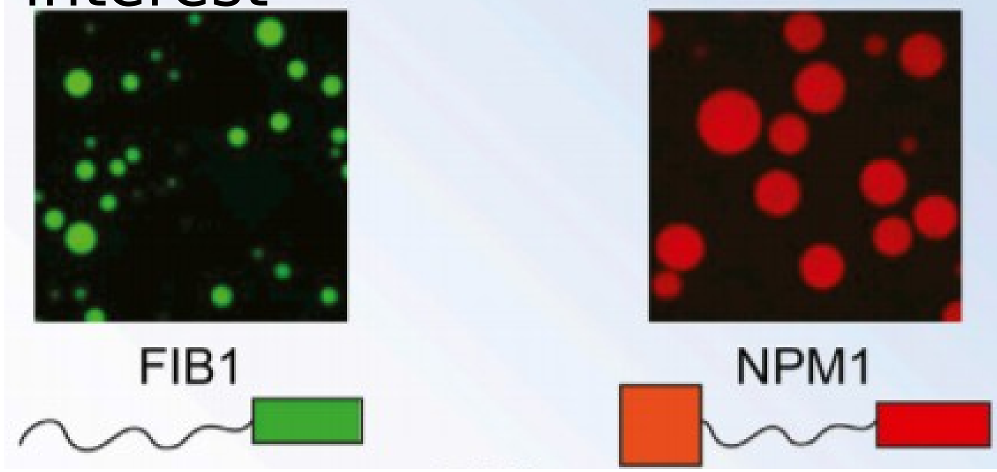


Preuzeto i obrađeno: S. Alberti, A. Gladfelter, T. Mittag, *Cell* **176** (2018) 419-434.

...that explains *in vivo* behavior.

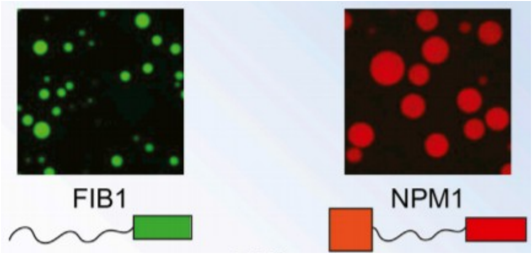
How to find proof for LLPS in a cell?

1. Proteins of interest

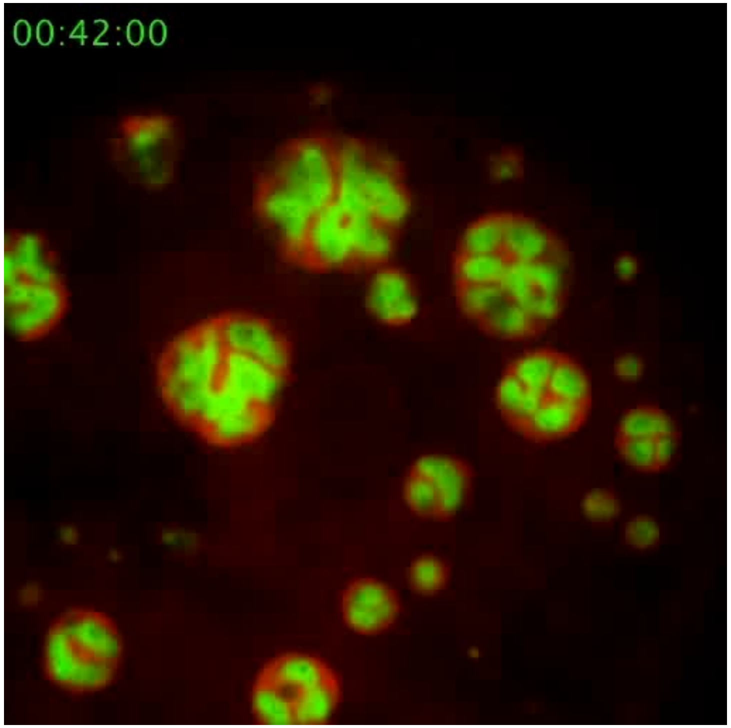


M. Feric, N. Vaidya, T.S. Harmon, D. M. Mitrea, L. Zhu, T.M. Richardson, C.P. Brangwynne, *Cell* **165(7)** (2016) 1686–1697.

1. Proteins of interest



2. Behavior in the cell



M. Feric, N. Vaidya, T.S. Harmon, D. M. Mitrea, L. Zhu, T.M. Richardson, C.P. Brangwynne, *Cell* **165(7)** (2016) 1686–1697.

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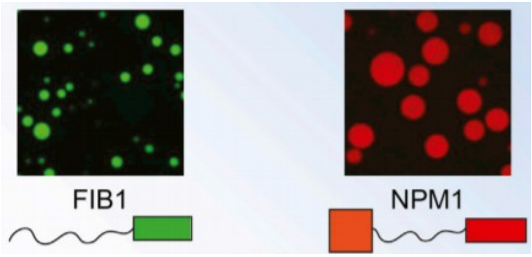
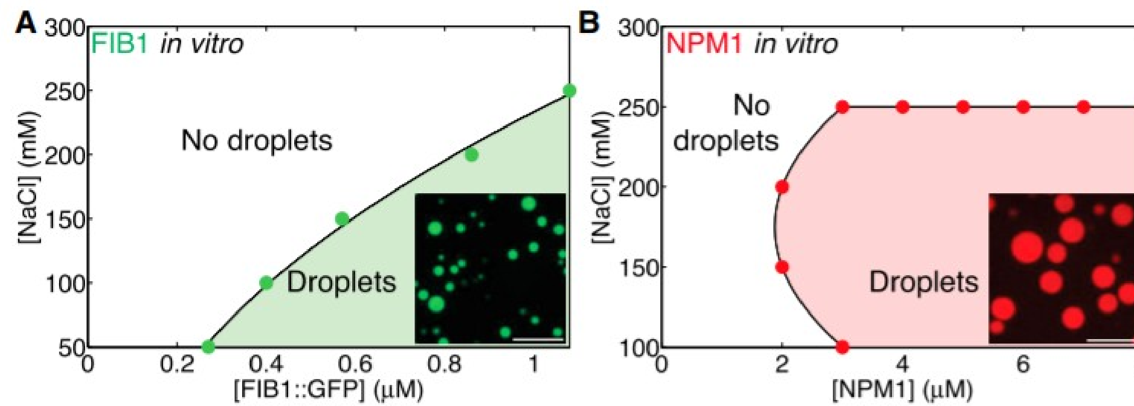
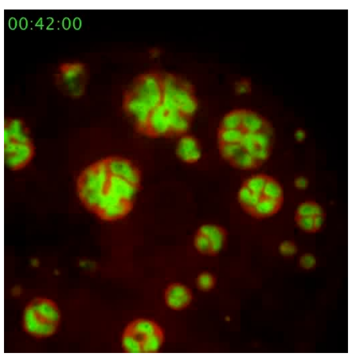
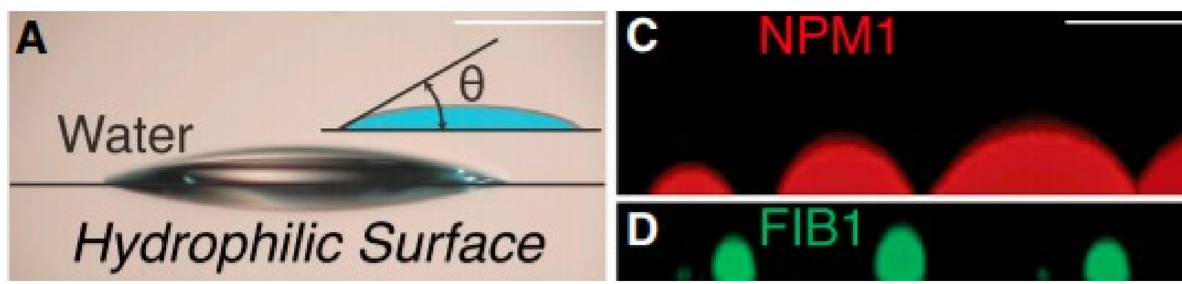


3. Phase diagram and physical properties



2. . Behavior in the cell





M. Feric, N. Vaidya, T.S. Harmon, D. M. Mitrea, L. Zhu, T.M. Richardson, C.P. Brangwynne, *Cell* **165(7)** (2016) 1686–1697.

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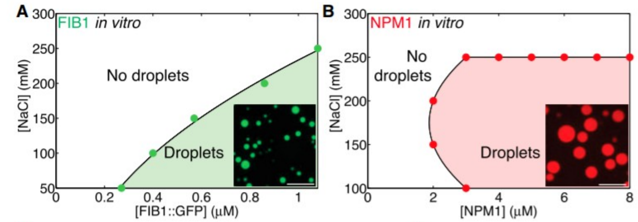

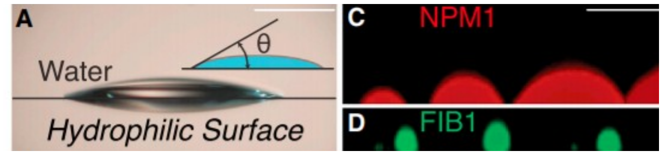

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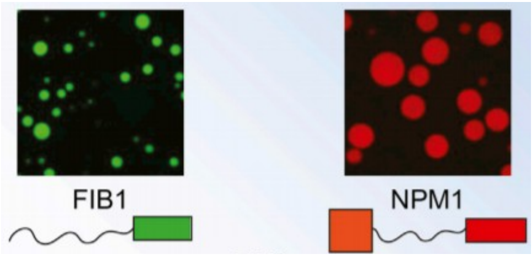
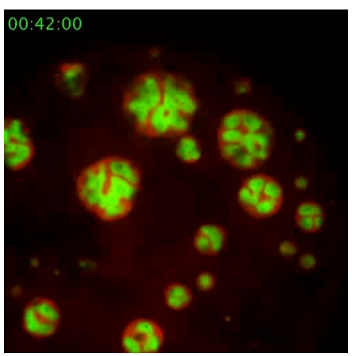
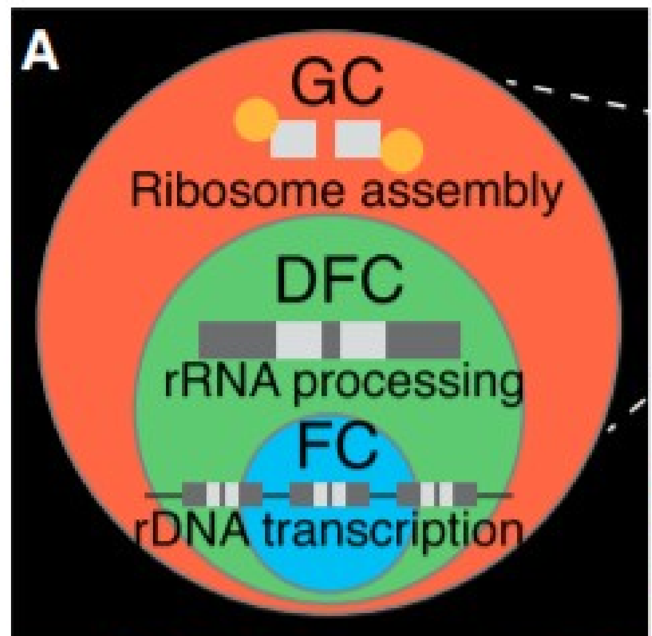


4. Biological significance

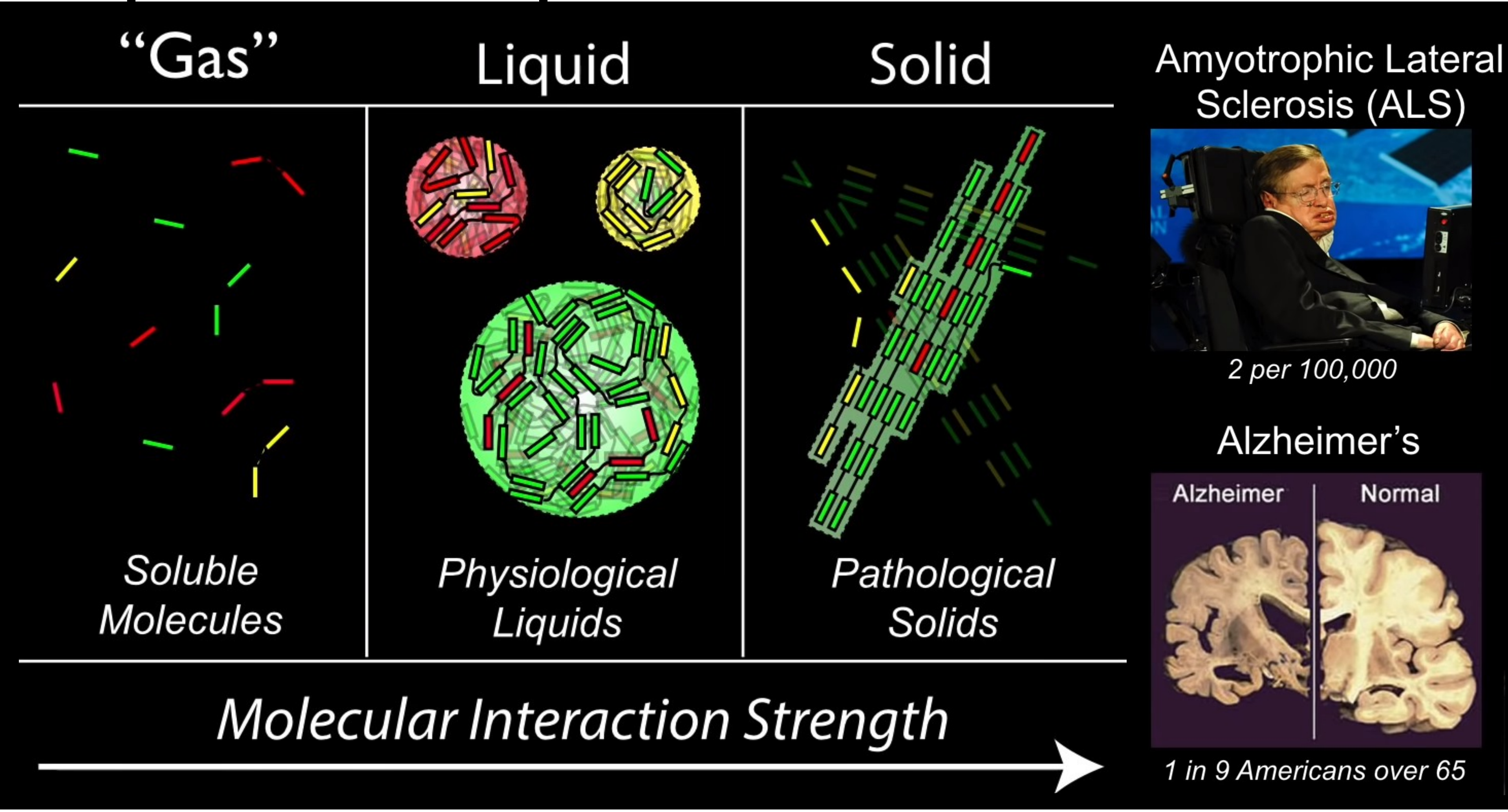
2. . Behavior in the cell





M. Feric, N. Vaidya, T.S. Harmon, D. M. Mitrea, L. Zhu, T.M. Richardson, C.P. Brangwynne, *Cell* **165(7)** (2016) 1686–1697.

Biological significance of phase separation



https://www.quantamagazine.org/phase-separating-proteins-may-protect-and-regulate-cells-20181126/

Thank you!

Photo by: ZNANSTVENE ČAROLIJE

Some tips ⏪

- 1. Literature
- 2. Time management
- 3. Cookie ‘Madeleine’

1. Literature

- Scientific paper

Cell

Article

Coexisting Liquid Phases Underlie Nucleolar Subcompartments

Marina Feric,[1,5] Nilesh Vaidya,[1,5] Tyler S. Harmon,[2,3] Diana M. Mitrea,[4] Lian Zhu,[1] Tiffany M. Richardson,[1] Richard W. Kriwacki,[4] Rohit V. Pappu,[3] and Clifford P. Brangwynne[1,*]

[1]Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, USA
[2]Department of Physics, Washington University in St. Louis, St. Louis, MO 63130, USA
[3]Department of Biomedical Engineering and Center for Biological Systems Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA
[4]Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN 38103, USA
[5]Co-first author
*Correspondence: cbrangwy@princeton.edu
http://dx.doi.org/10.1016/j.cell.2016.04.047

Here, we combine in vivo and in vitro studies, together with computational modeling, to show that subcompartments within the nucleolus represent distinct, coexisting liquid phases. Consistent with their in vivo immiscibility, purified nucleolar proteins phase separate into droplets containing distinct non-coalescing phases that are remarkably similar to nucleoli in vivo. This layered droplet organization is caused by differences in the biophysical properties of the phases—particularly droplet surface tension—which arises from sequence-encoded features of their macromolecular components. These results suggest that phase separation can give rise to multilayered liquids that may facilitate sequential RNA processing reactions in a variety of RNP bodies.

INTRODUCTION

organization and the spontaneous formation of micron-scale membrane-less organelles. Such behavior is reminiscent of well-known in vitro observations in protein crystallization, where soluble proteins are observed to condense into concentrated liquid phases or crystalline solid phases. A number of recent papers suggest that intrinsically disordered proteins or low complexity sequences (IDP/LCS) drive phase transitions underlying assembly of the nucleolus (Berry et al., 2015), stress granules (Wippich et al., 2013; Molliex et al., 2015; Patel et al., 2015), P granules and nuage bodies (Elbaum-Garfinkle et al., 2015; Wang et al., 2014), and nuclear speckles (Hennig et al., 2015).

The liquid-like nature of the nucleolus may facilitate its function in ribosome biogenesis. The nucleolus forms around regions of chromosomes containing stretches of tandem rDNA gene repeats, known as nucleolar organizer regions (NORs). In most eukaryotes (including human, *Xenopus laevis*, and *Caenorhabditis elegans*), a precursor rRNA transcript is generated from the rDNA gene and contains each of the co-transcribed 18S, 5.8S, and 28S rRNA subunits, separated by two intervening transcribed sequences (Figure 1A). The nucleolus may facilitate

Google the boss!

Clifford P. Brangwynne review

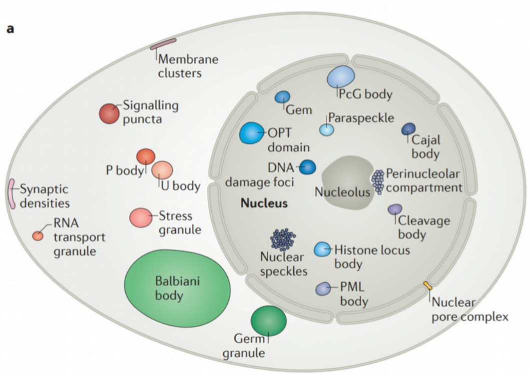
Clifford P. Brangwynne review - Google Pretraživanje

reviews

Published: 06 September 2017

Cell organization by liquid phase separation

Daniel W. Gerlich



S. F. Banani, H. O. Lee, A. A. Hyman M. K. Rosen, *Mol. Cell Biol.* **18** (2017) 85-88.

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The Company of Biologists

CELL SCIENCE AT A GLANCE

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

The liquid nucleome – phase transitions in the nucleus at a glance

Amy R. Strom and Clifford P. Brangwynne*

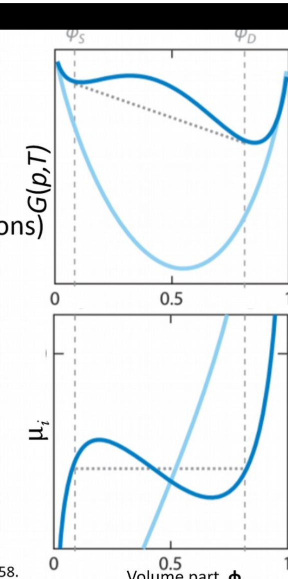
$G(p,T)=U+pV-TS$

$\mu_i=\left(\frac{\partial G}{\partial N i}\right)_{T,p,N j_{\neq i}}$

Ideal mixture (no interactions)
$\Delta H^{mix} = 0$

Mixture with interactions
$\Delta H^{mix} \neq 0$
$\Delta H^{mix} = V(\delta_2 - \delta_1)^2 \phi_1 \phi_2$

Flory-Huggins
δ – Solubility
ϕ – Volume part in the mixture



A.A. Hyman, C.A. Weber,
F. Jülicher, *Annu. Rev. Cell Dev. Biol.* **30** (2014) 39-58.

Use all possible sources

YouTube HR

Clifford P. Brangwynne

FILTAR

Cliff Brangwynne (Princeton & HHMI) 1: Liquid Phase Separation in Living Cells

iBiology • 18 tis. pregleda • prije 1 godinu

https://www.ibiology.org/biophysics/liquid-phase-separation-in-living-cells Liquid-liquid phase separation drives the formation of ...

Biophysical Engineer Clifford Brangwynne | 2018 MacArthur Fellow

macfound • 2,9 tis. pregleda • prije 1 godinu

Clifford Brangwynne is a biophysical engineer using the principles of soft matter physics and cell biology to illuminate novel ...

Titlovi

Intracellular Liquid Condensates: Cliff Brangwynne

APS Physics • 1,2 tis. pregleda • prije 1 godinu

Cliff **Brangwynne** (Princeton University) presents at the Fred Kavli Special Symposium: From Unit Cell to Biological Cell at the ...

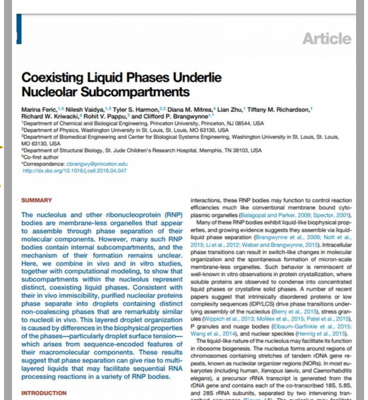
2. Time Management

15 min presentation

7 min INTRODUCTION	3 min RESOULTS	5 min IMPACT

(Reviews, YT, Twitter…)

1. Fun intro with something familiar
2. from general knowledge to specific concepts


Article

Coexisting Liquid Phases Underlie Nucleolar Subcompartments

Marina Feric,[1,5] Nilesh Vaidya,[1,5] Tyler S. Harmon,[2,3] Diana M. Mitrea,[4] Lian Zhu,[1] Tiffany M. Richardson,[1] Richard W. Kriwacki,[4] Rohit V. Pappu,[3] and Clifford P. Brangwynne[1,*]

[1]Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, USA
[2]Department of Physics, Washington University in St. Louis, St. Louis, MO 63130, USA
[3]Department of Biomedical Engineering and Center for Biological Systems Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA
[4]Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN 38103, USA
[5]Co-first author
*Correspondence: cbrangwy@princeton.edu

http://dx.doi.org/10.1016/j.cell.2016.04.047

SUMMARY

The nucleolus and other ribonucleoprotein (RNP) bodies are membrane-less organelles that appear to assemble through phase separation of their molecular components. However, many such RNP bodies contain internal subcompartments, and the mechanism of their formation remains unclear. Here, we combine in vivo and in vitro studies, together with computational modeling, to show that subcompartments within the nucleolus represent distinct, coexisting liquid phases. Consistent with their in vivo immiscibility, purified nucleolar proteins phase separate into droplets containing distinct non-coalescing phases that are remarkably similar to nucleoli in vivo. This layered droplet organization is caused by differences in the biophysical properties of the phases—particularly droplet surface tension—which arises from sequence-encoded features of their macromolecular components. These results suggest that phase separation can give rise to multilayered liquids that may facilitate sequential RNA processing reactions in a variety of RNP bodies.

INTRODUCTION

interactions, these RNP bodies may function to control reaction efficiencies much like conventional membrane bound cytoplasmic organelles (Balagopal and Parker, 2009; Spector, 2001).

Many of these RNP bodies exhibit liquid-like biophysical properties, and growing evidence suggests they assemble via liquid-liquid phase separation (Brangwynne et al., 2009; Nott et al., 2015; Li et al., 2012; Weber and Brangwynne, 2015). Intracellular phase transitions can result in switch-like changes in molecular organization and the spontaneous formation of micron-scale membrane-less organelles. Such behavior is reminiscent of well-known in vitro observations in protein crystallization, where soluble proteins are observed to condense into concentrated liquid phases or crystalline solid phases. A number of recent papers suggest that intrinsically disordered proteins or low complexity sequences (IDP/LCS) drive phase transitions underlying assembly of the nucleolus (Berry et al., 2015), stress granules (Wippich et al., 2013; Molliex et al., 2015; Patel et al., 2015), P granules and nuage bodies (Elbaum-Garfinkle et al., 2015; Wang et al., 2014), and nuclear speckles (Hennig et al., 2015).

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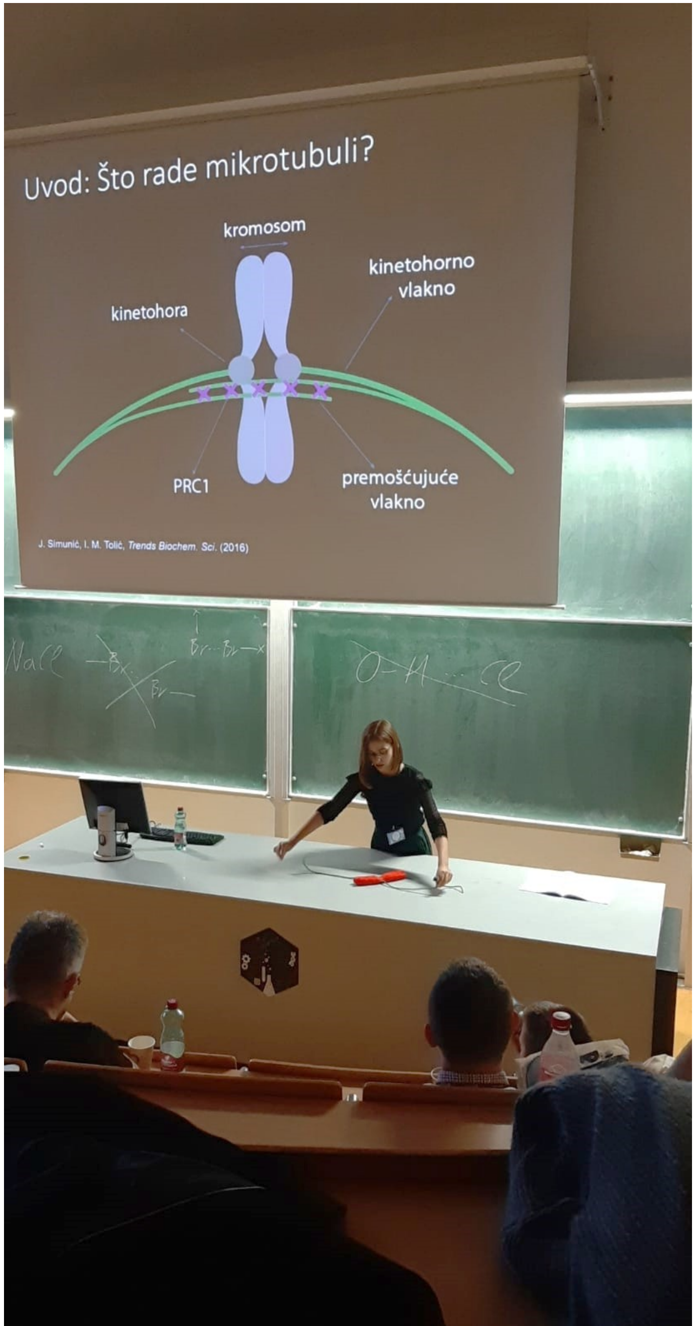
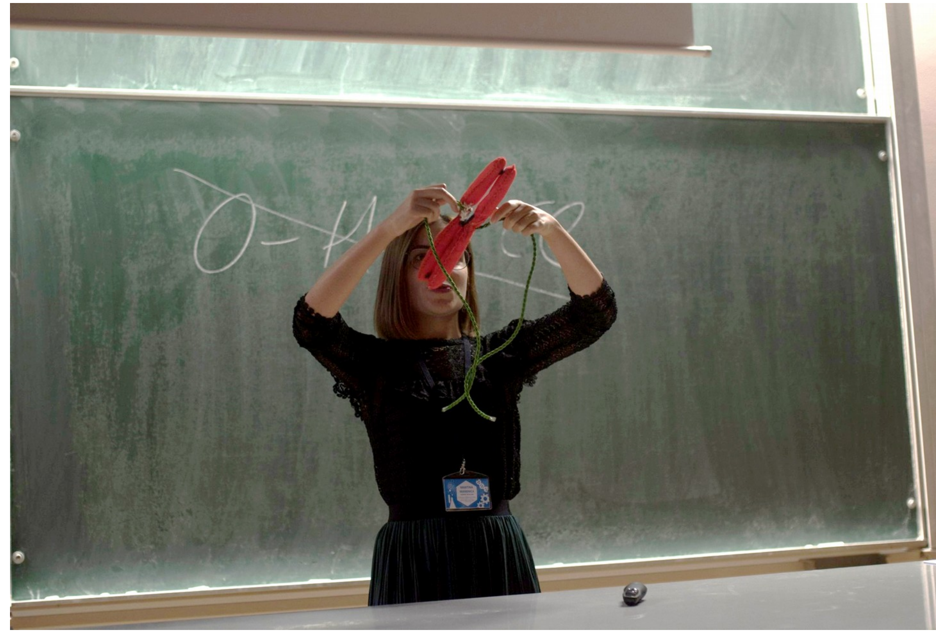
1. Applications, contribution…
2. Round out the story

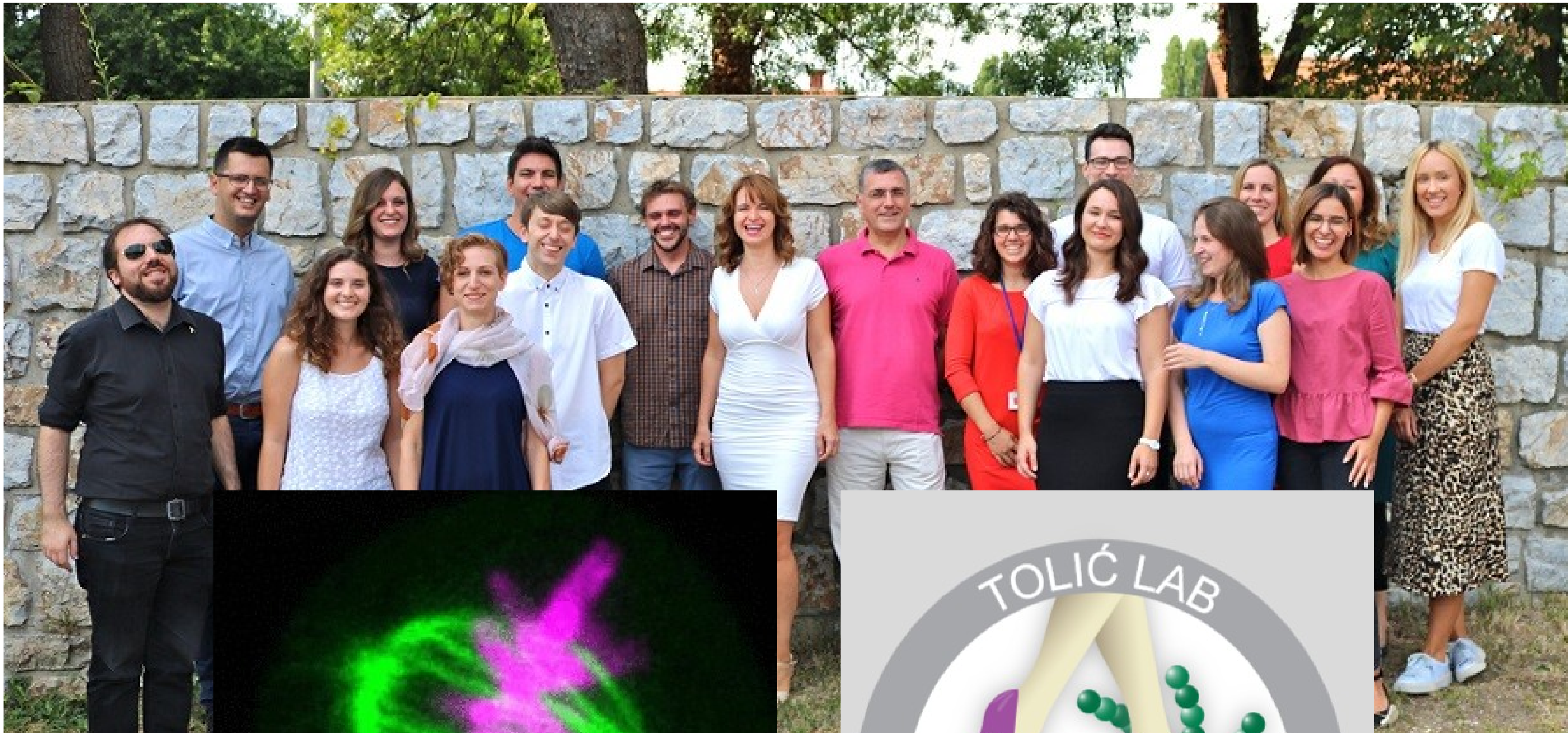
3. Cookie ‘Madeleine’

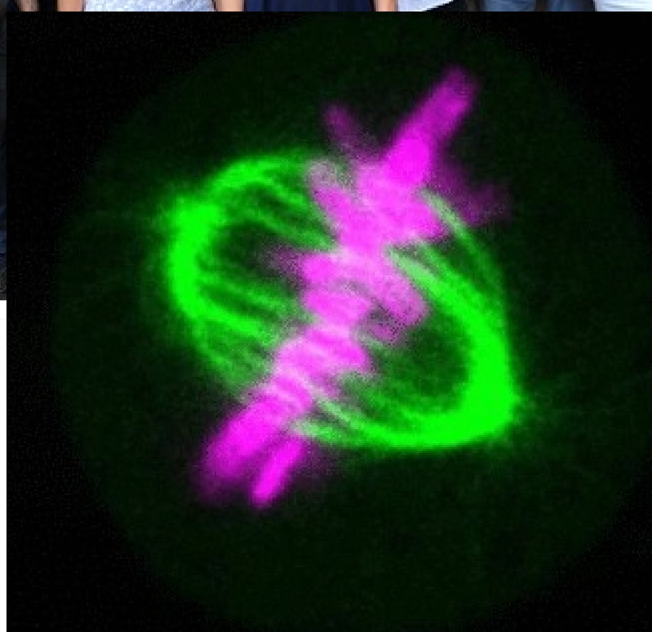
Simple, memorable, part of your personality


Uvod: Što rade mikrotubuli?
kromosom
kinetohorno
vlakno
kinetohora
PRC1
premošćujuće
vlakno
J. Simunić, I. M. Tolić, Trends Biochem. Sci. (2016)



Cookie ‘Madeleine’ – Pro version





Cookie ‘Madeleine’ – EXTRAPro version


- * CHEERING *
- THANK YOU, FOLKS.