Organisation spatiale et temporelle à l'échelle mésoscopique d'une protéine de signalisation cellulaire

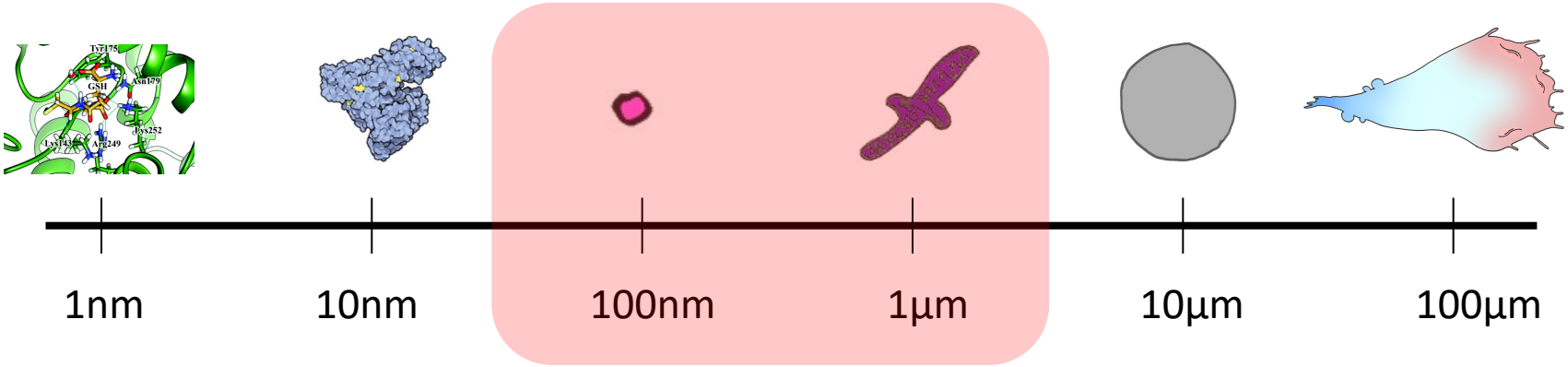
Mathieu COPPEY

UMR168 Physics and chemistry departments Curie Institute

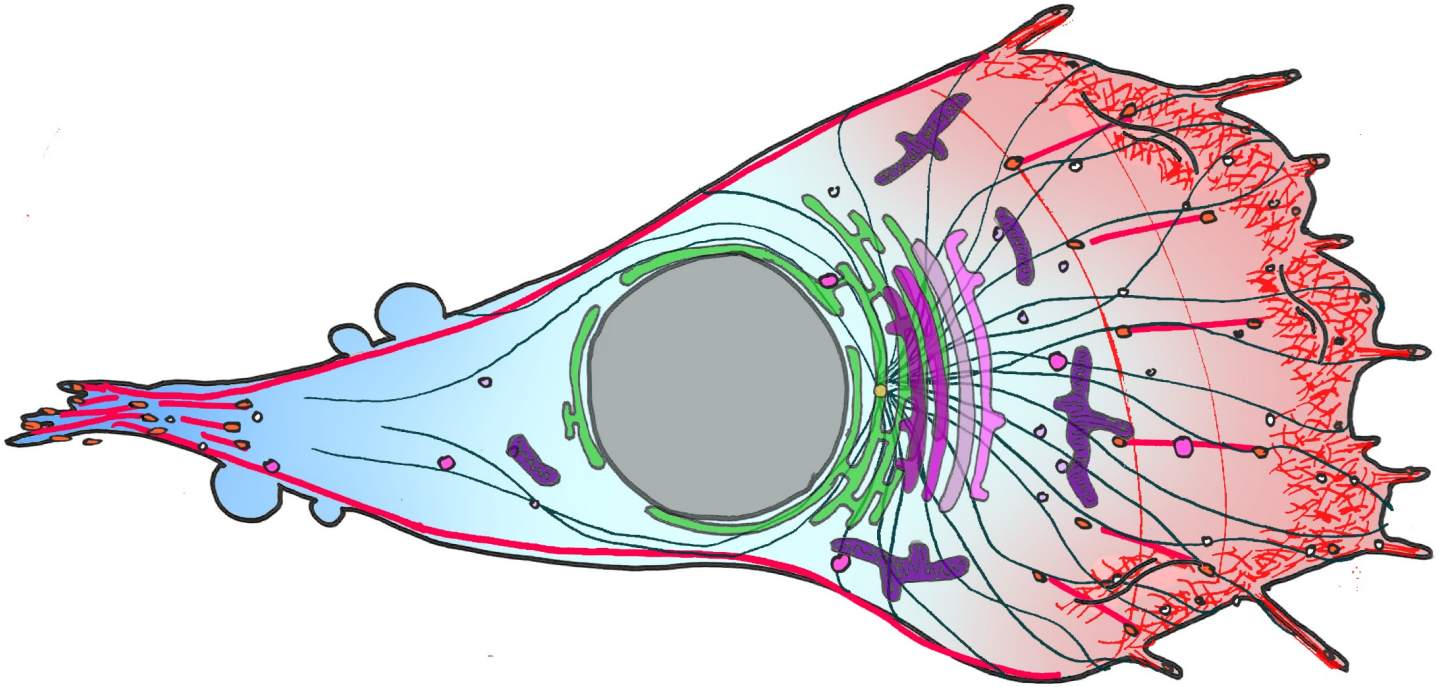
PARIS

Cell at the mesoscopic scale

The meso scale for eukariotic cells



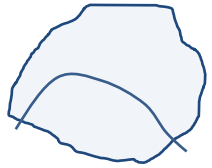
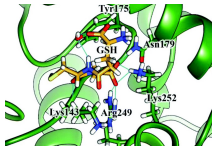
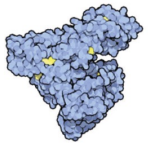
Meso scale

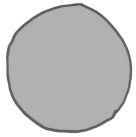
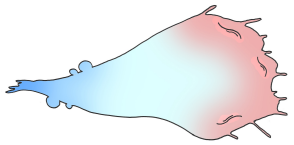


The meso scale for eukariotic cells





1nm
0,1m

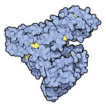
10nm
1m

100nm
10m

1µm
100m

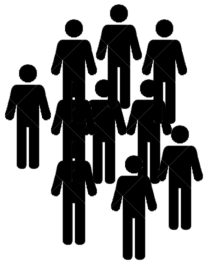
10µm
1km

100µm
10km



N ~ 2.10^9

V ~ 2000µm^3

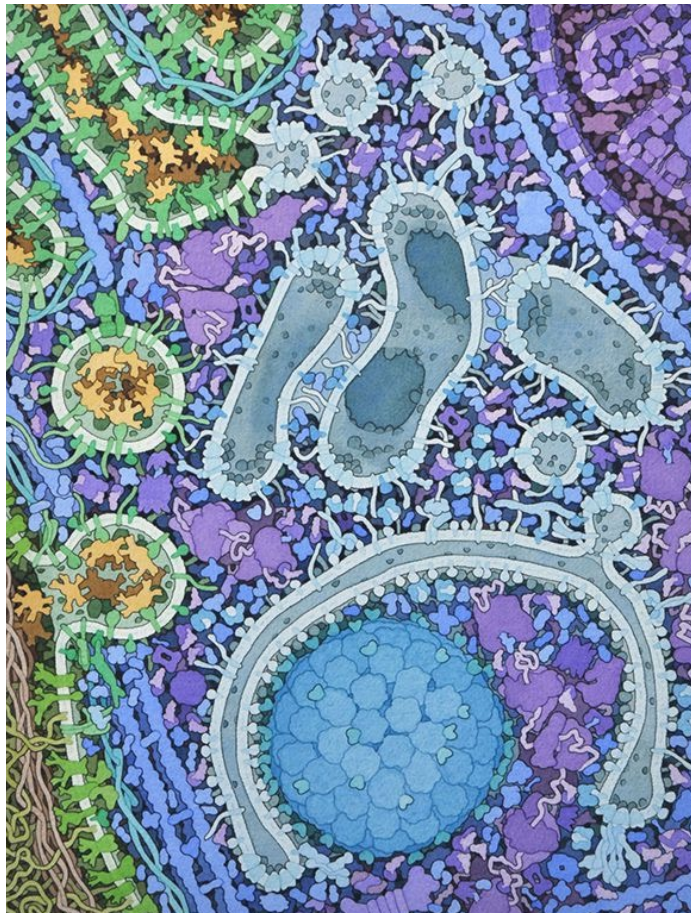


100 millions

What is found at the meso scale?

Macromolecular complexes, supramolecular assemblies of ~10-100 molecules

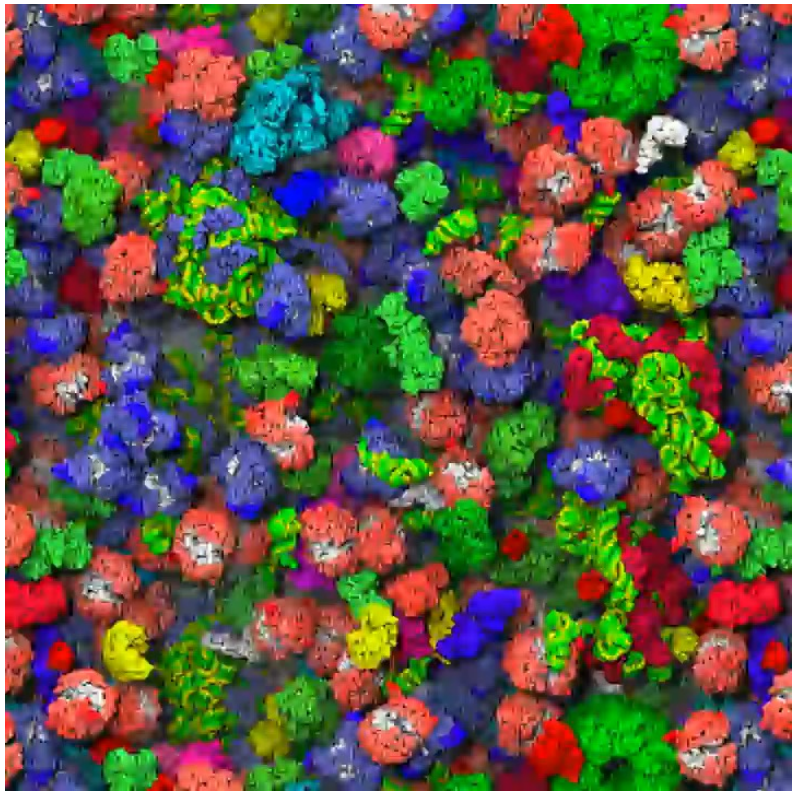
100nm



100nm

David Goodshell

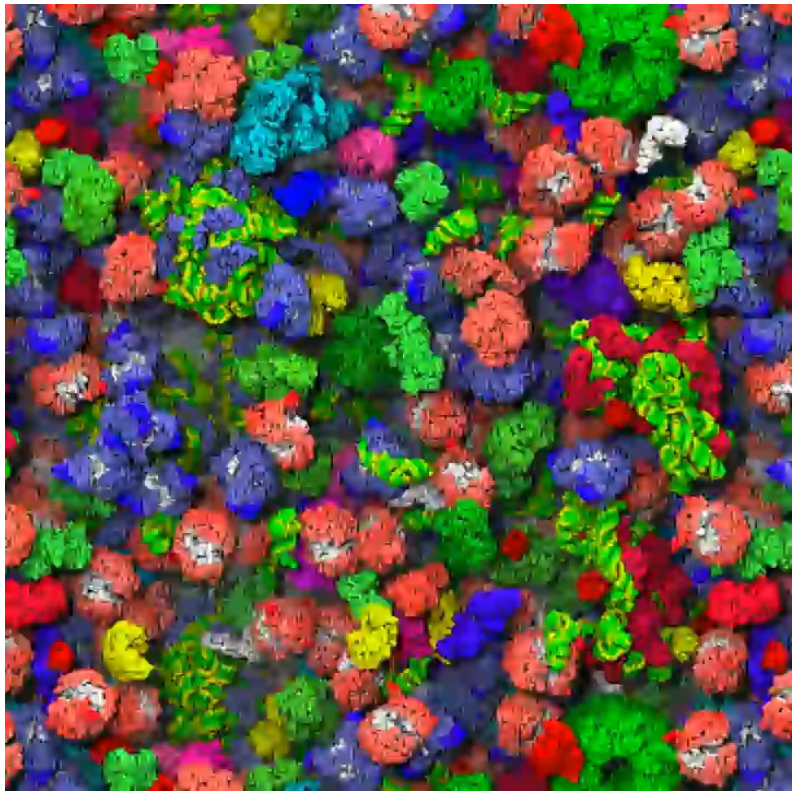
Why the meso scale is a challenge?



MD simulation of the Ecoli cytoplasm (100nm, 1µs)

McGuffee, and Elcock 2010

Why the meso scale is a challenge?



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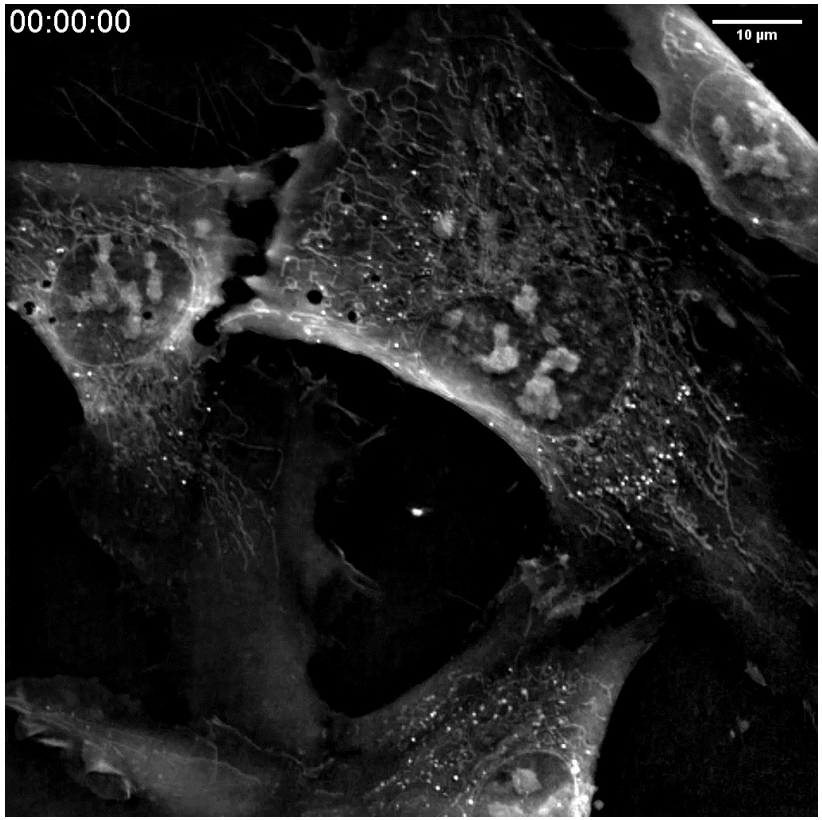
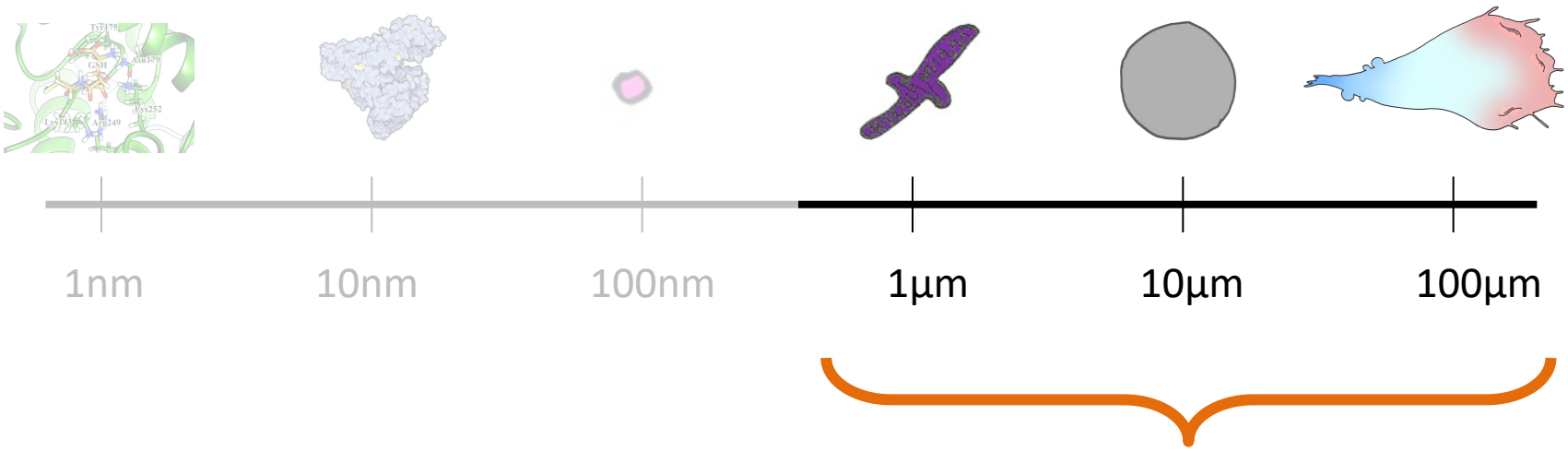
?



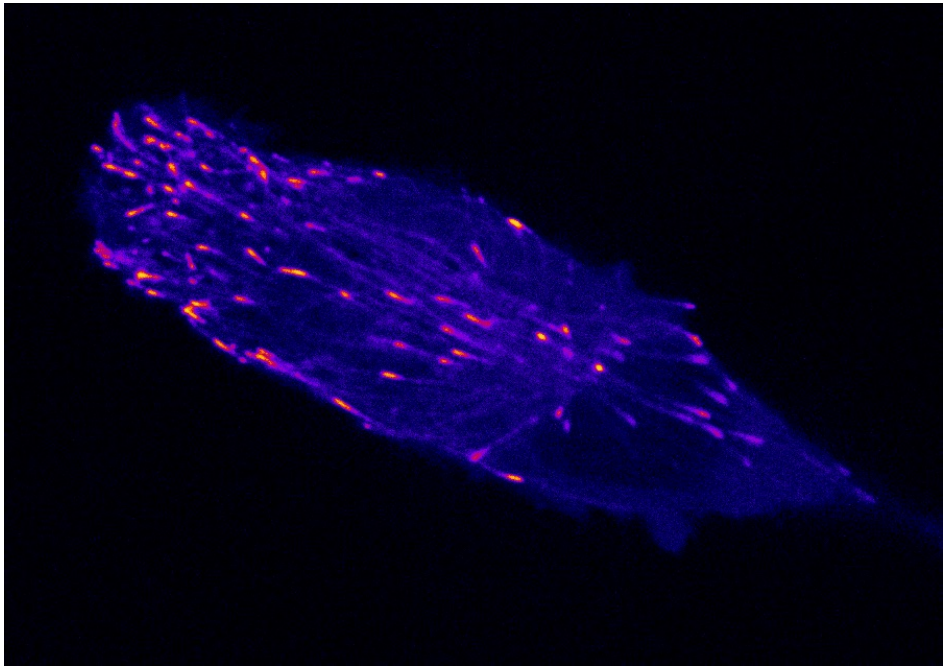
Dividing mammalian cell (100µm, 1hrs)

www.nanolive.ch

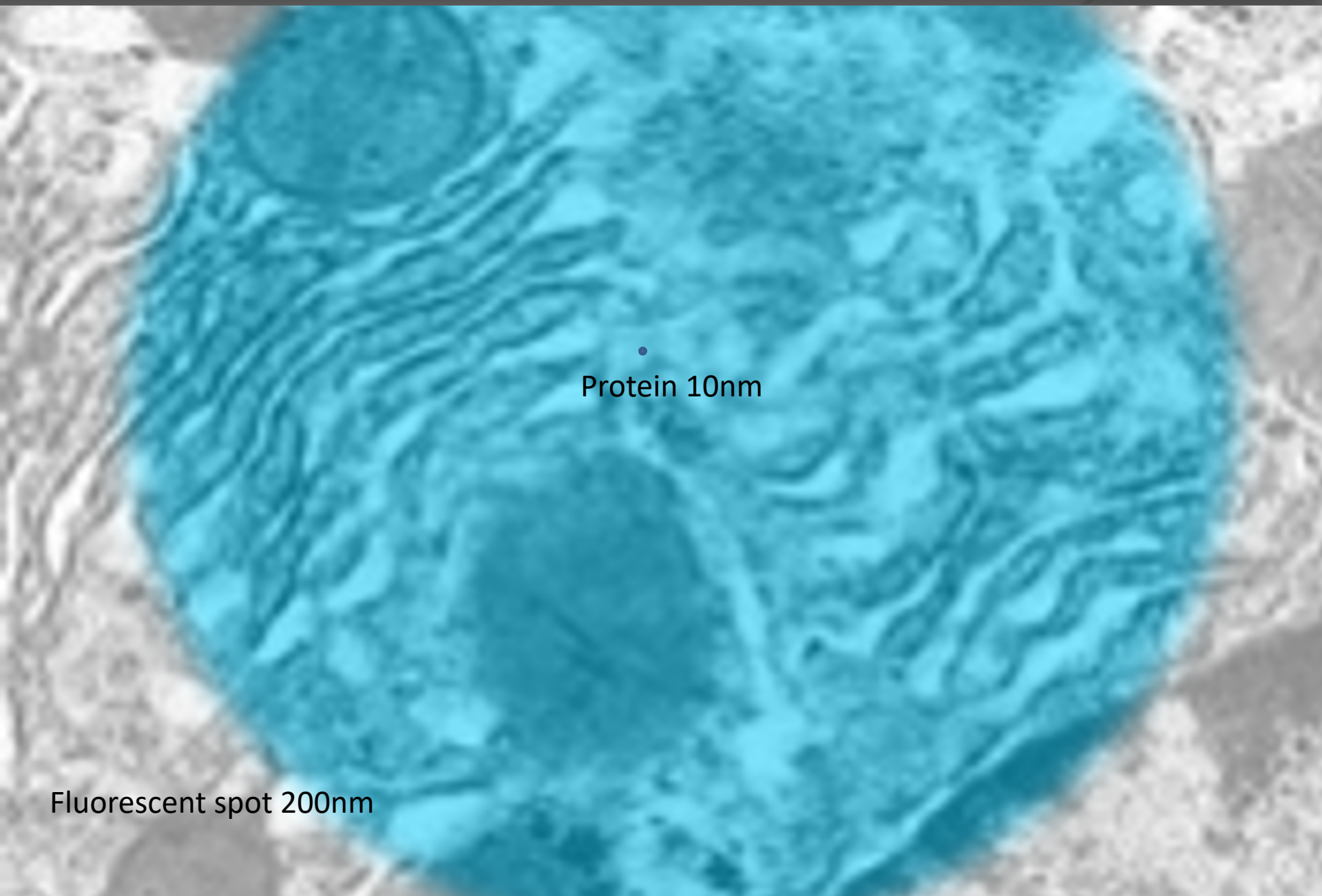
Why the meso scale is a challenge?



Fluorescent microscopy to follow biomolecules

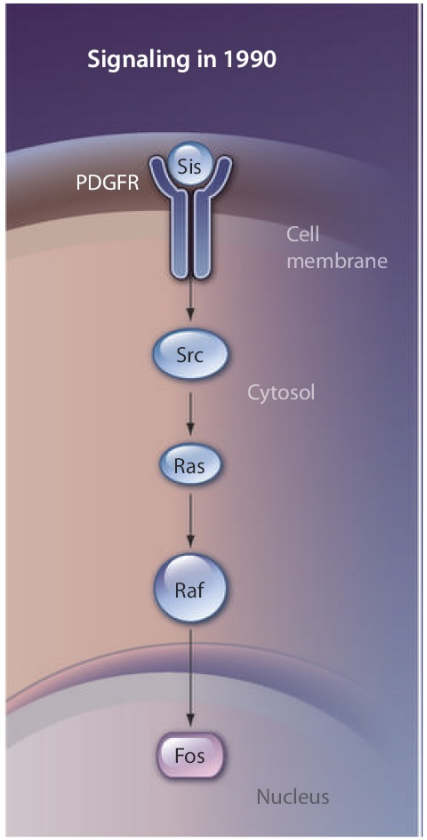


Diffraction limit



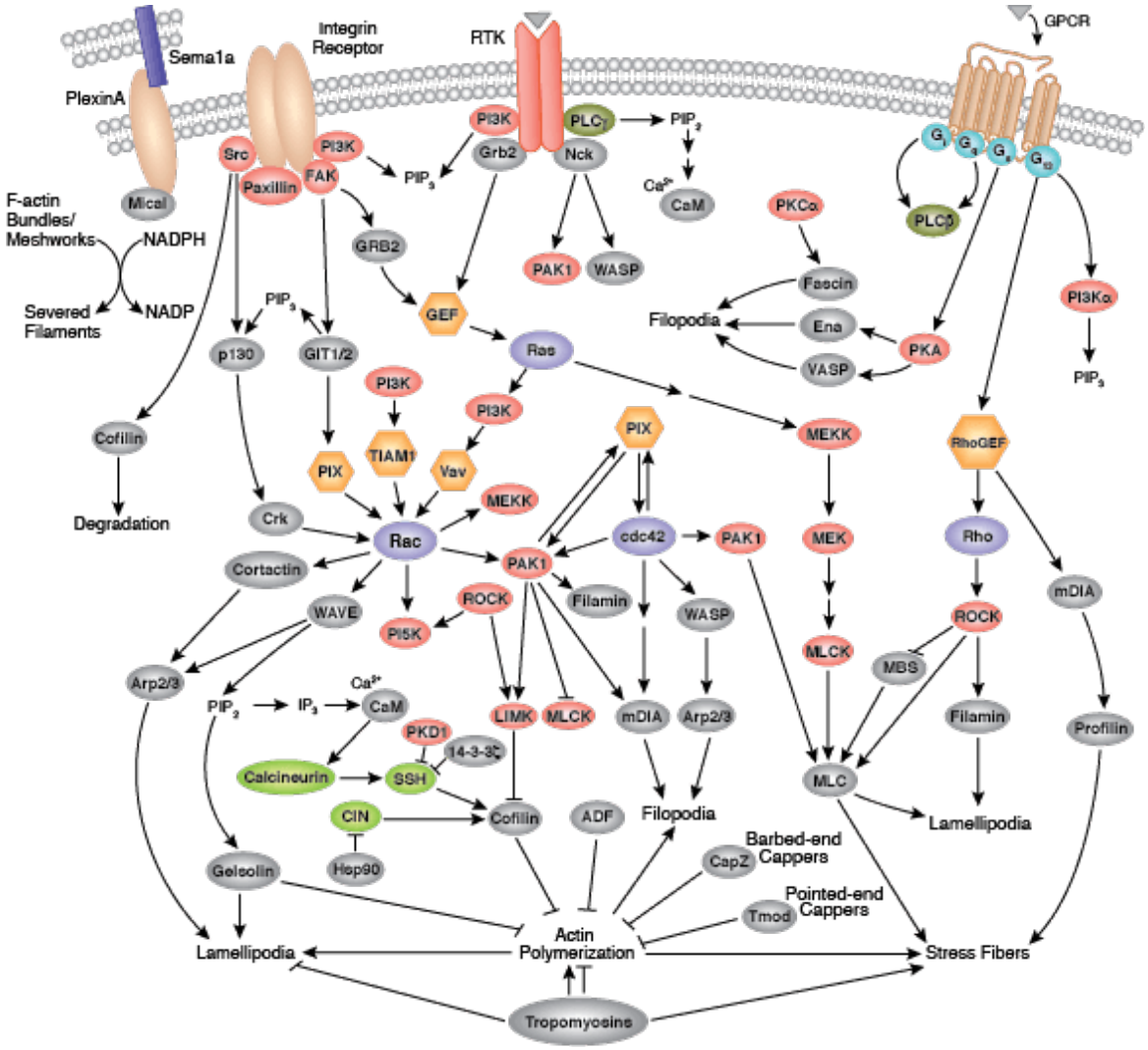
Signaling in cells

Signal transduction

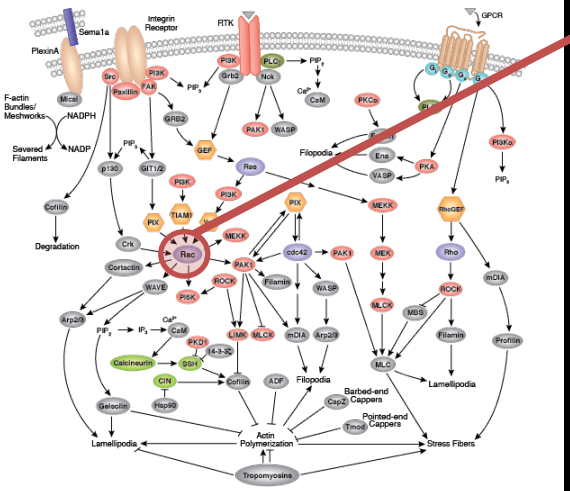


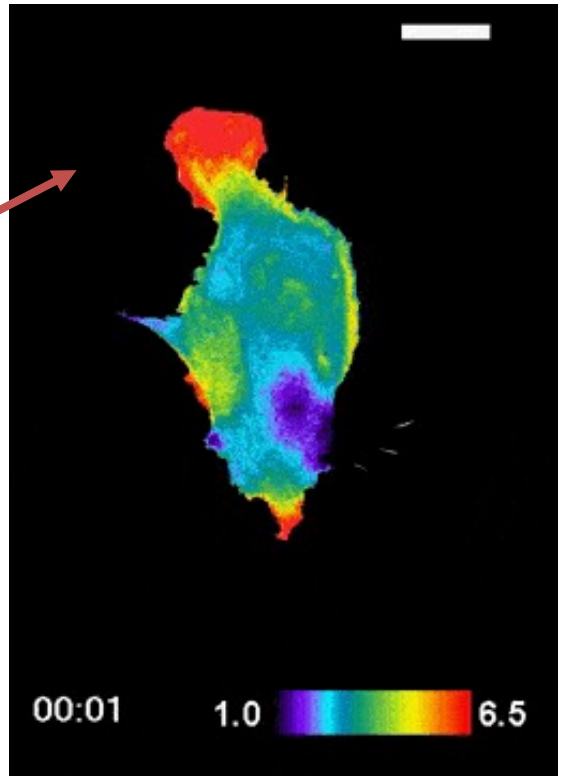
Signaling pathways

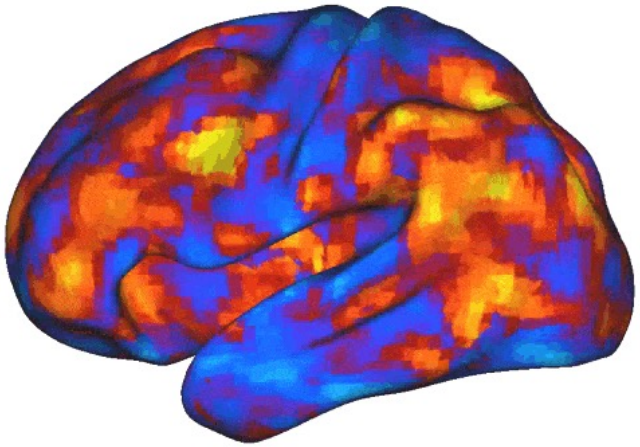
Signaling network controlling actin cytoskeleton dynamics



Signaling in space and time





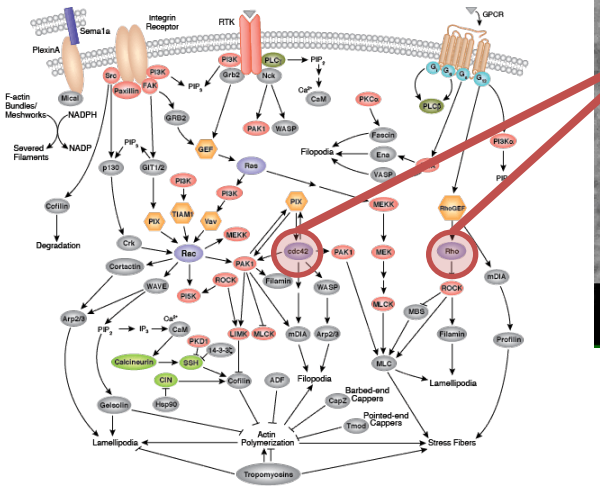


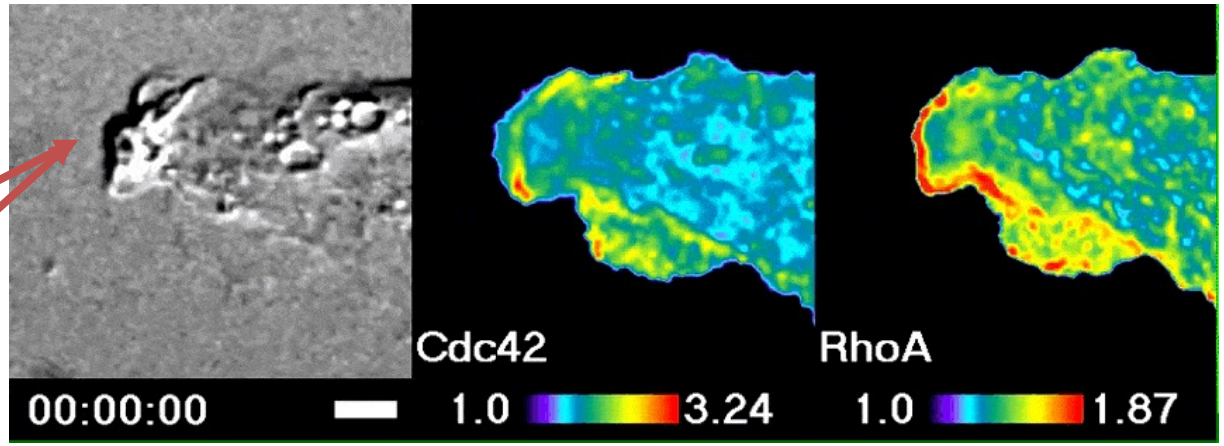
Functional MRI
Spontaneous brain activity
J. Vincent

nm-µm
s-min

FRET Biosensor of Rac1 activity
Spontaneous intracellular activity
K. Hahn

Collective protein dynamics





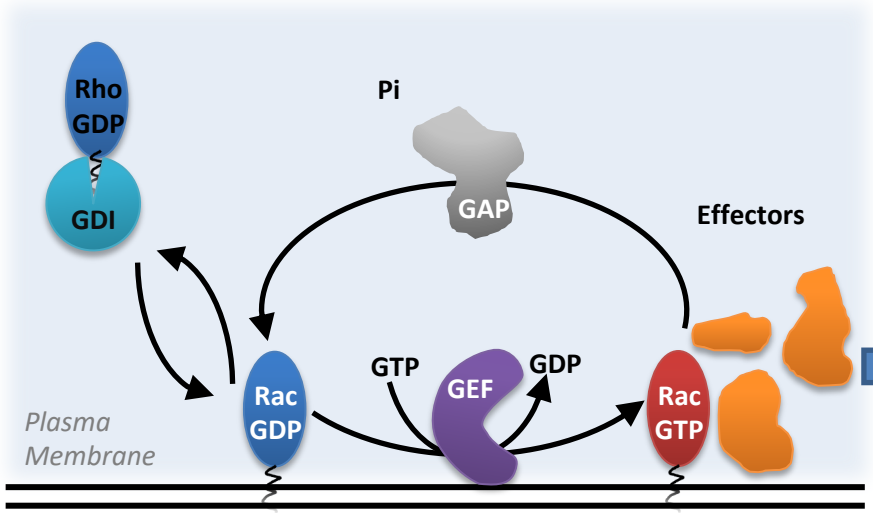
Kraynov, V. S. et al, Science, 2000

Rac1 nanoclusters

Remorino A. et al, Cell Report, 2018

Rac1 signaling protein

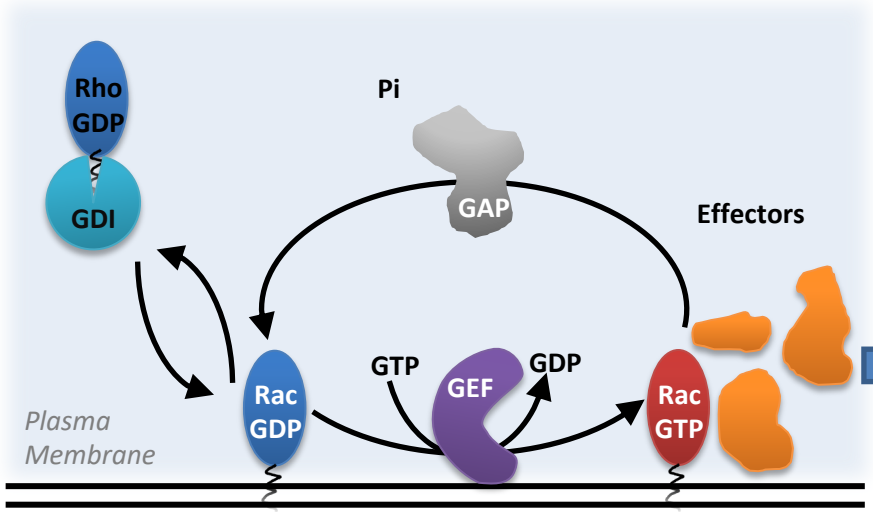
RhoGTPase switch and shuttling activity cycle



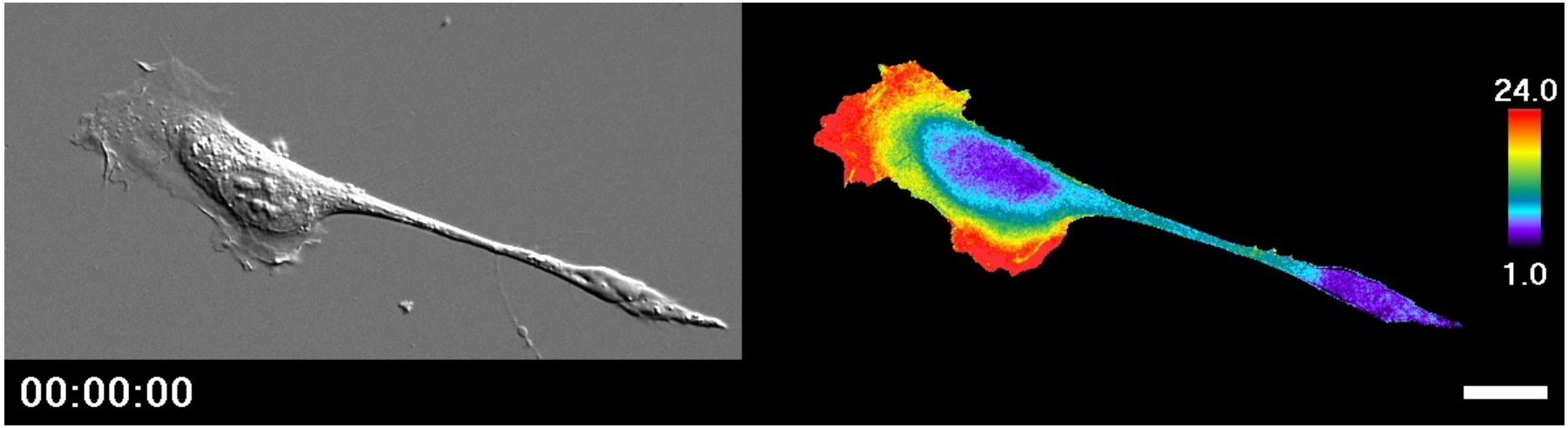
Actin polymerization
Lamellipodia

Rac1 signaling protein

RhoGTPase switch and shuttling activity cycle



Actin polymerization Lamellipodia



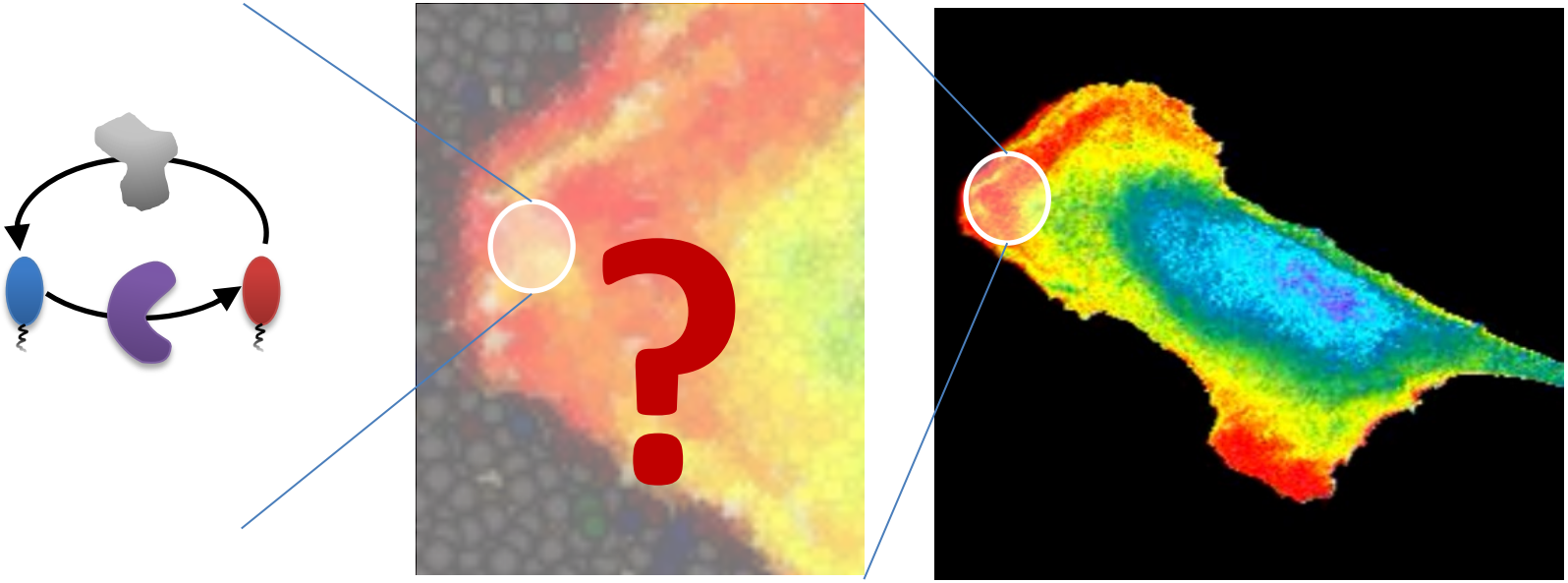
K. Hahn, 2009

How signal is regulated at the meso scale?

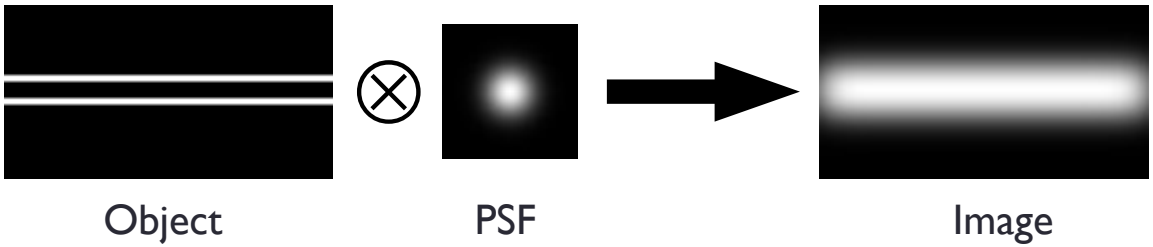
Molecular scale

Meso scale

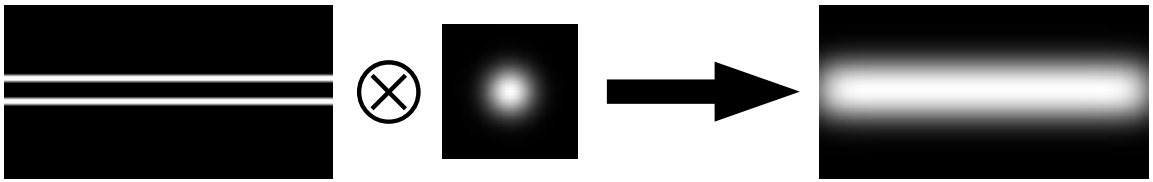
Cellular scale



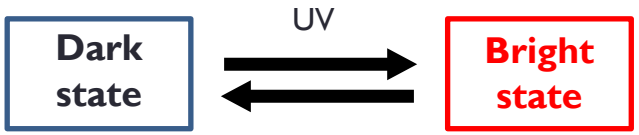
Diffraction limit

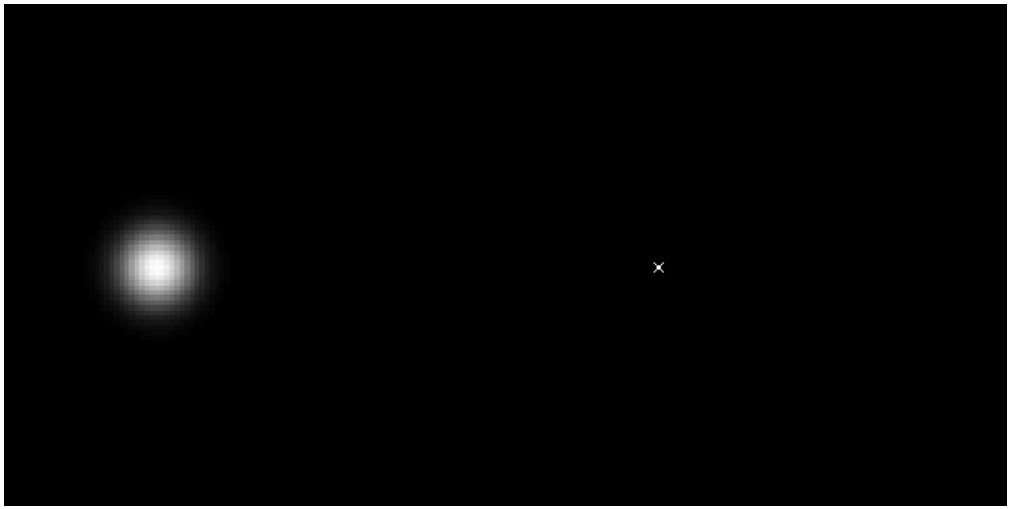


Pointillist super-resolution

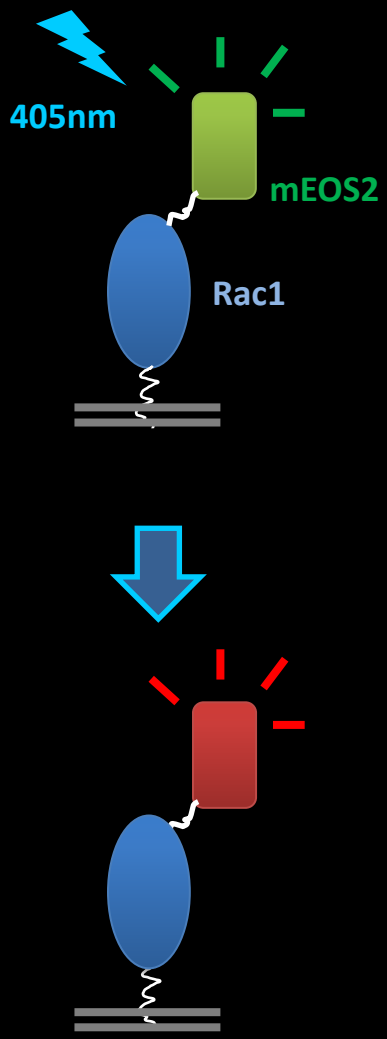


Object PSF Image



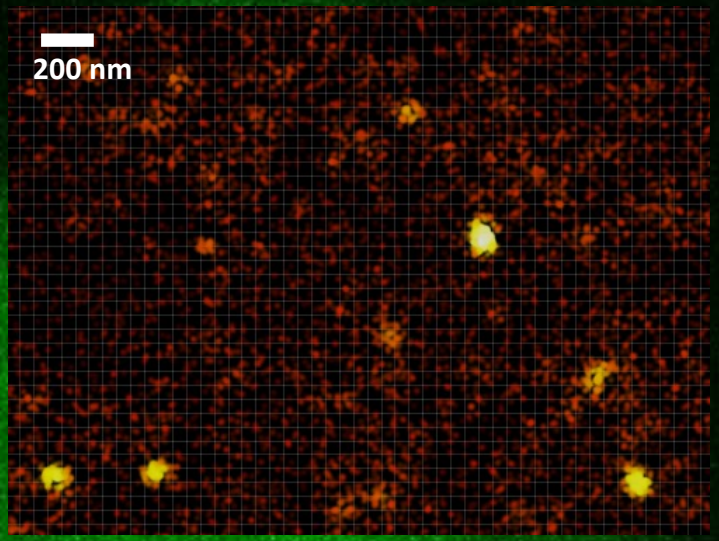


Single molecule imaging of Rac1



TIRFM

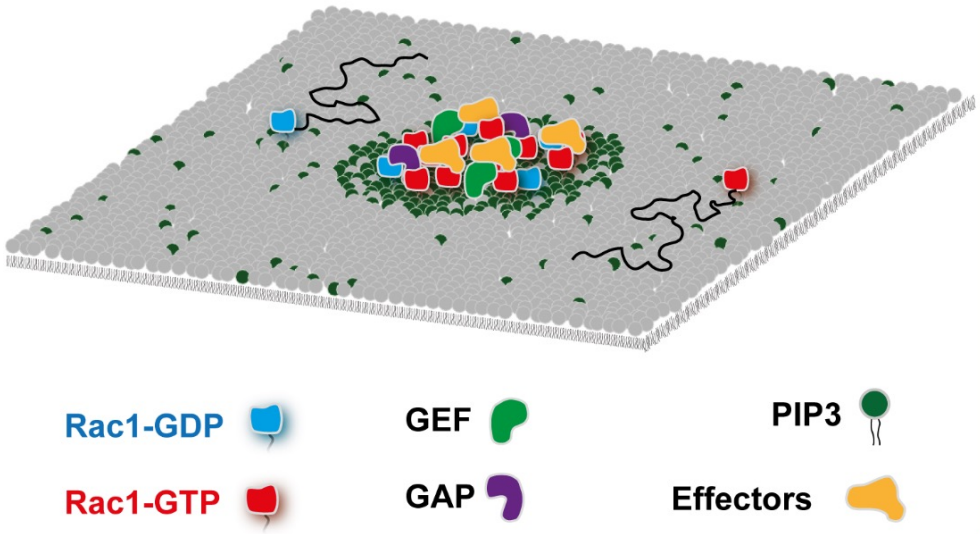
PALM image of Rac1 distribution

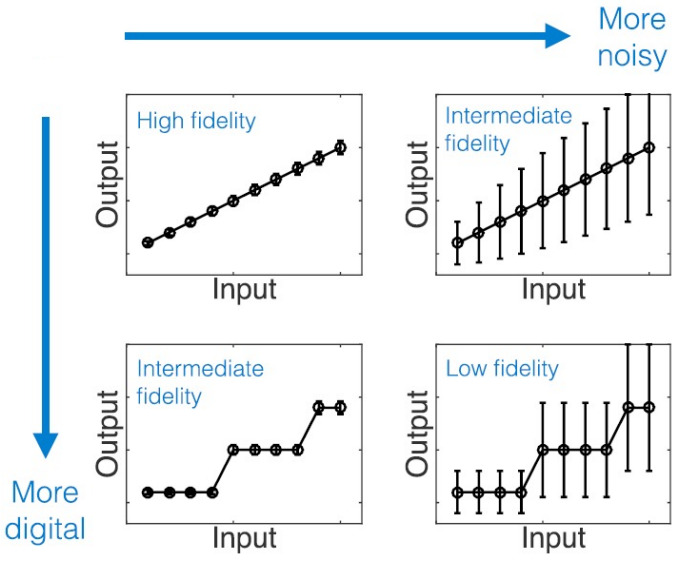


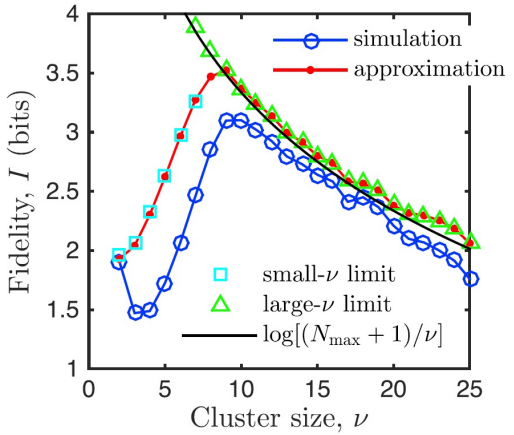
- 70 nm nanoclusters
- ~50 molecules
- Localized in active regions

Functional role of nanoclusters?

Signaling quanta for analog-digital-analog processing?



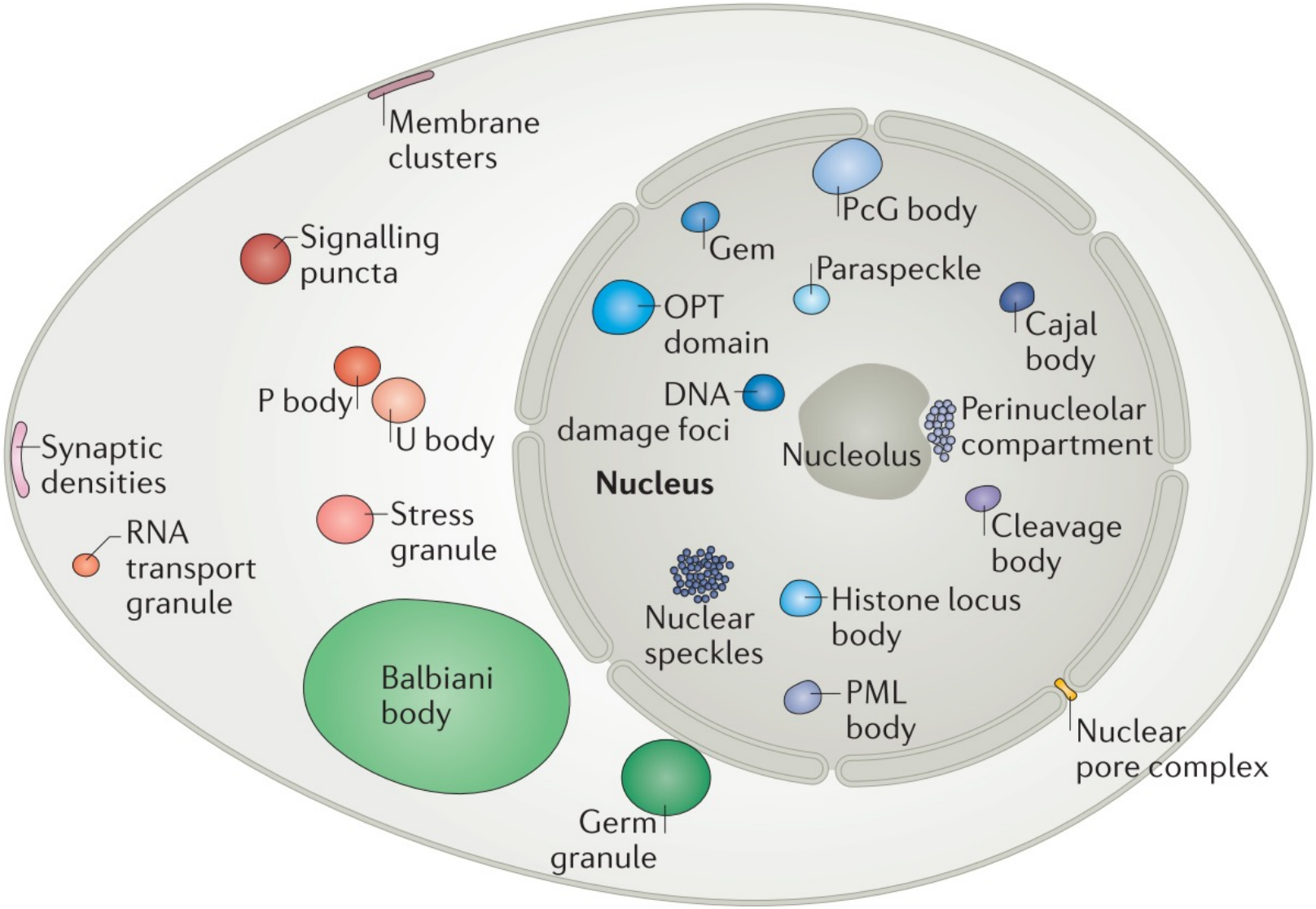




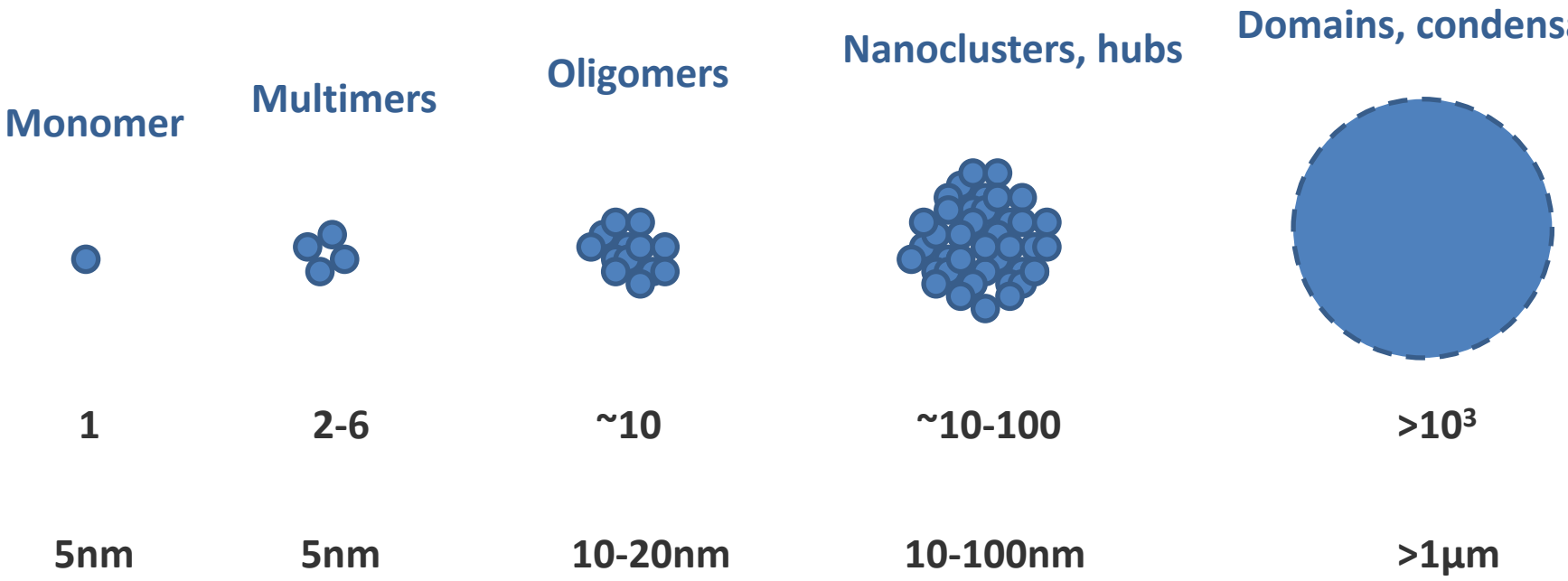
Roob et al, 2019

A new physics for the meso scale?

Membraneless organelles



Biological meso-objects

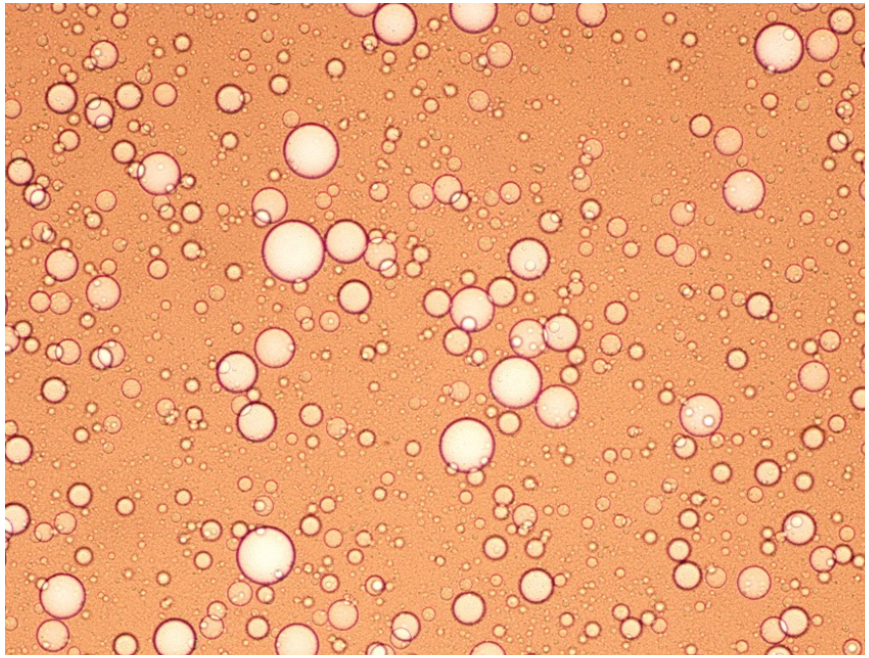


➢ **Weak and multivalent interactions**

Two key physical concepts for meso-assemblies

- **Avidity**

- **Liquid-liquid phase separation**



Weak interactions in living systems

kT is the measure of thermal energy

Hydrogen-bond ~10 kT
Covalent bonds ~ 100–200 kT

Many bonds in biological systems are weak

"The reason for the weakness and short lifetimes of LR bonds is that nature does not actually want all of its bonds to be long-lived, just long enough for them to perform some function that requires a certain time—not less, but not more either."

« lock-and-key » interactions in biology

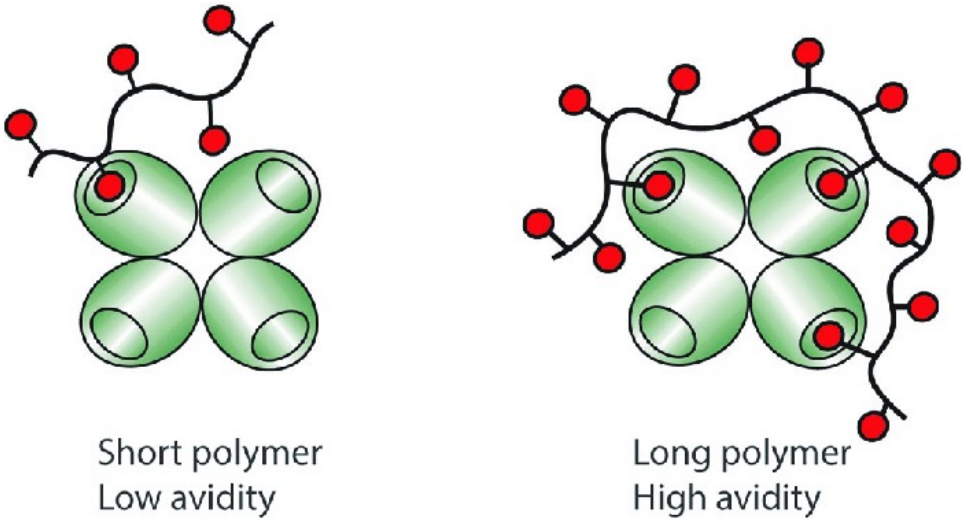
Table 21.2. Bond Energies and Lifetimes of LR Bonds[1]

Binding Protein (Receptor, R)	Target (Ligand, L)	Affinity [K_D (M)]	Energy[2] (kT)	Lifetimes of Bonds[3]
Avidin	Biotin	10^{-15}	35	months
Antibody	Antigen	10^{-7}–10^{-11}	16–25	seconds–hours
Receptor	Hormone	10^{-9}	21	seconds
Enzyme	Substrate	10^{-3}–10^{-9}	7–21	μs–seconds
Transport protein	Hormone	10^{-6}–10^{-8}	14–18	<seconds
Lectins[4]	Glycoconjugates	10^{-3}–10^{-5}	7–12[5]	μs–ms[5]

Intermolecular and Surface Forces
Jacob N. Israelachvili

How to build robust structures with weak interactions?

- **Multimers increase the lifetime of interaction**
  ex: the two strands of DNA, Transcription factors, …



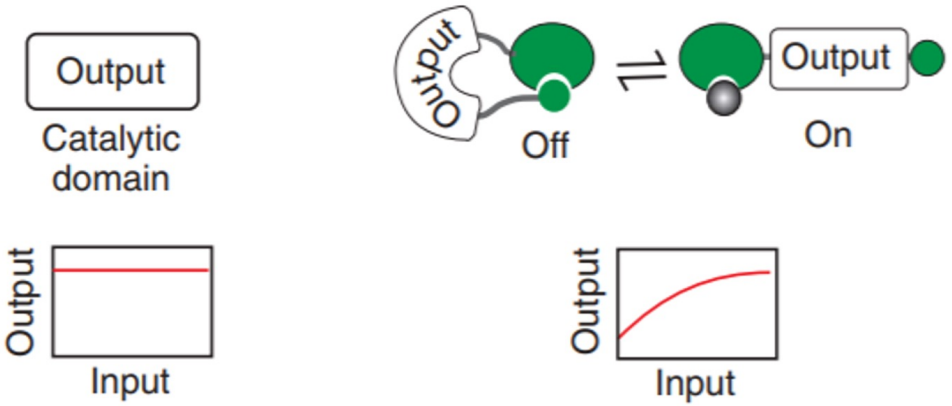
"because individual binding events increase the likelihood of other interactions to occur (i.e. increase the local concentration of each binding partner in proximity to the binding site), ***avidity should not be thought of as the mere sum of its constituent affinities but as the combined effect of all affinities participating in the biomolecular interaction"***
Wikipedia

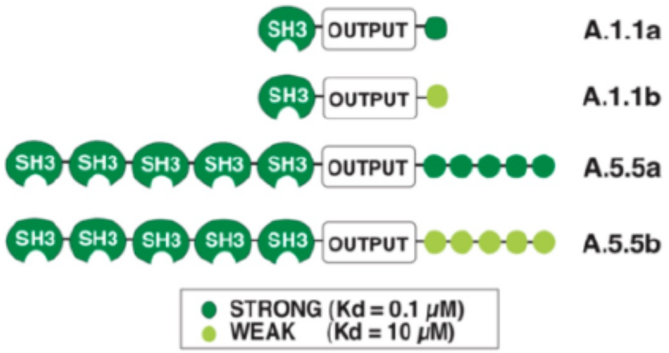
Avidity

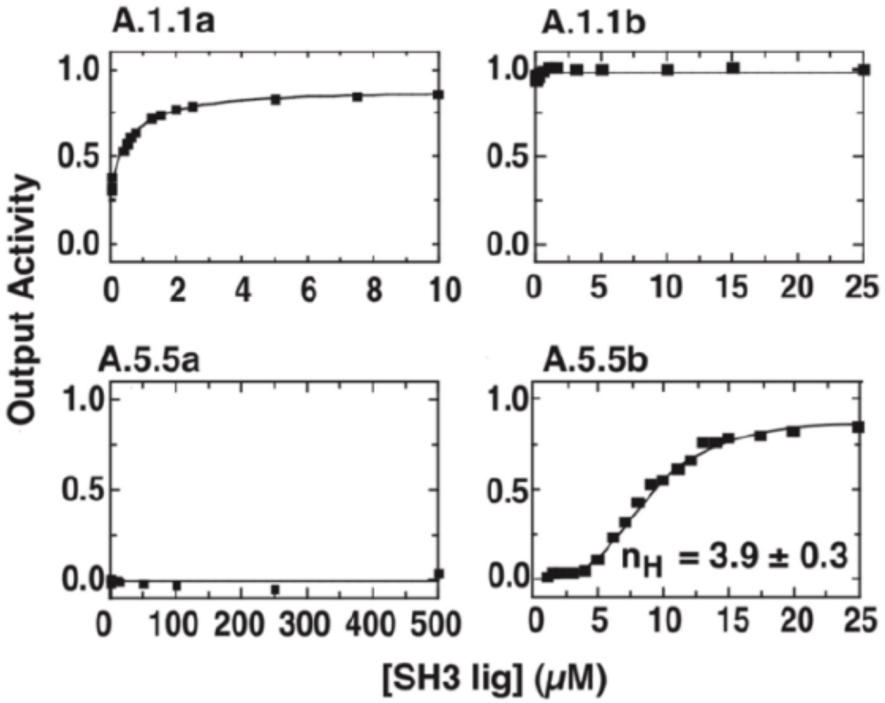
Example: switch like response of a biomolecule

Engineering synthetic signaling proteins with ultrasensitive input/ output control

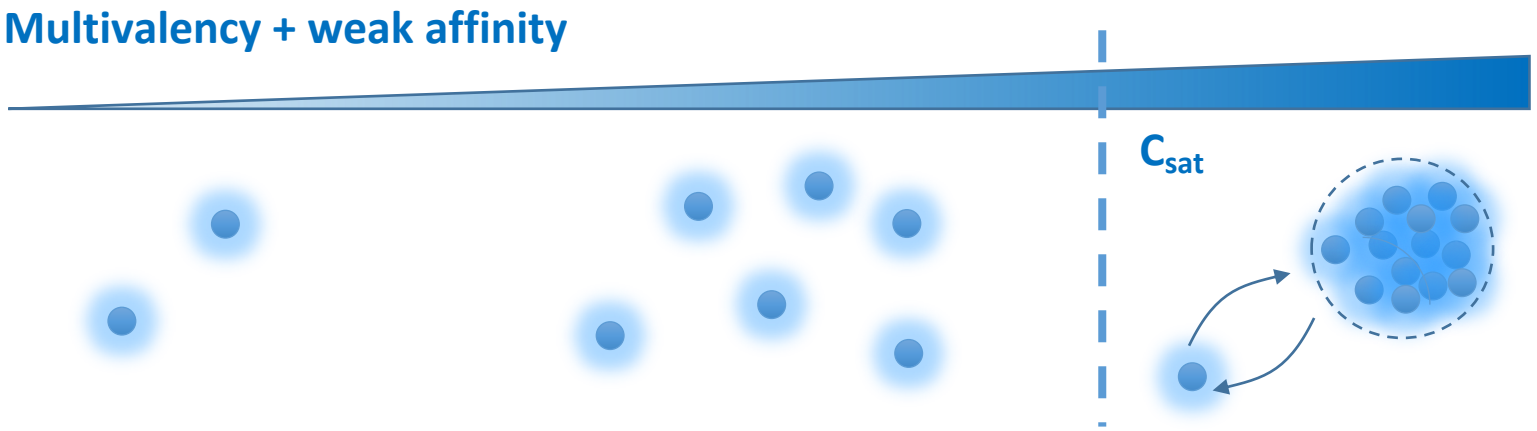
John E Dueber[1,5,6], Ethan A Mirsky[2,5] & Wendell A Lim[3–5]





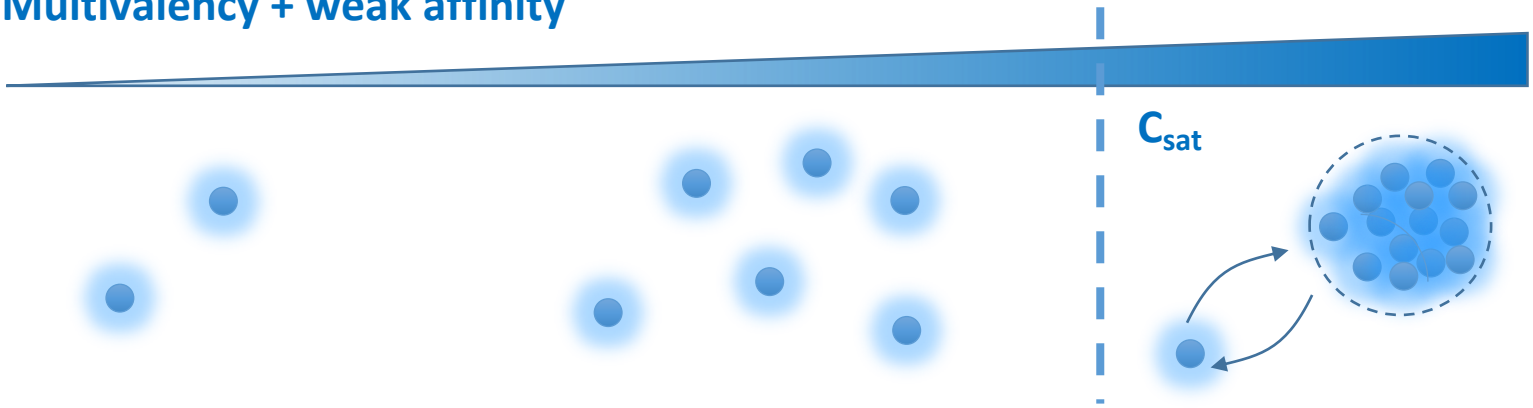


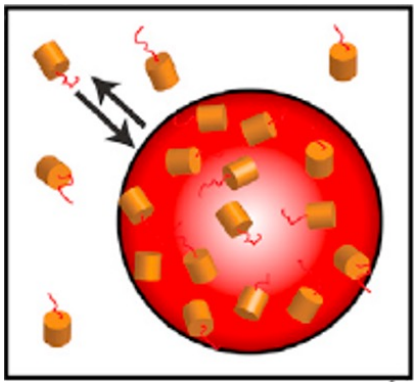
Phase separation



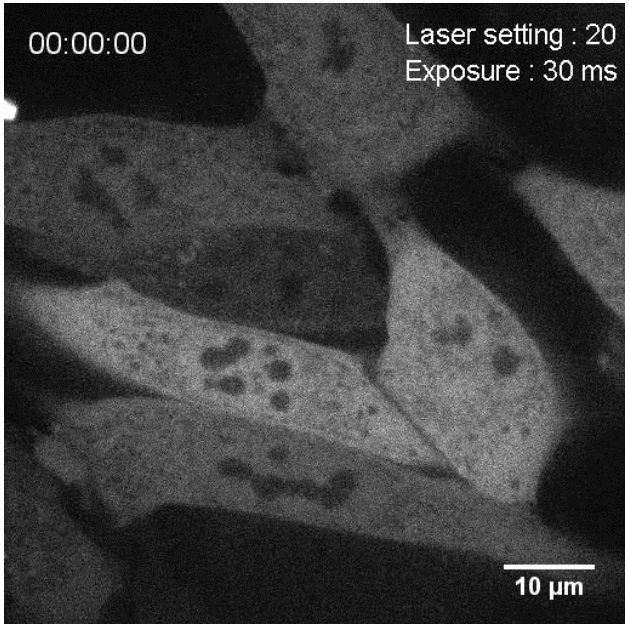
Phase separation

Multivalency + weak affinity



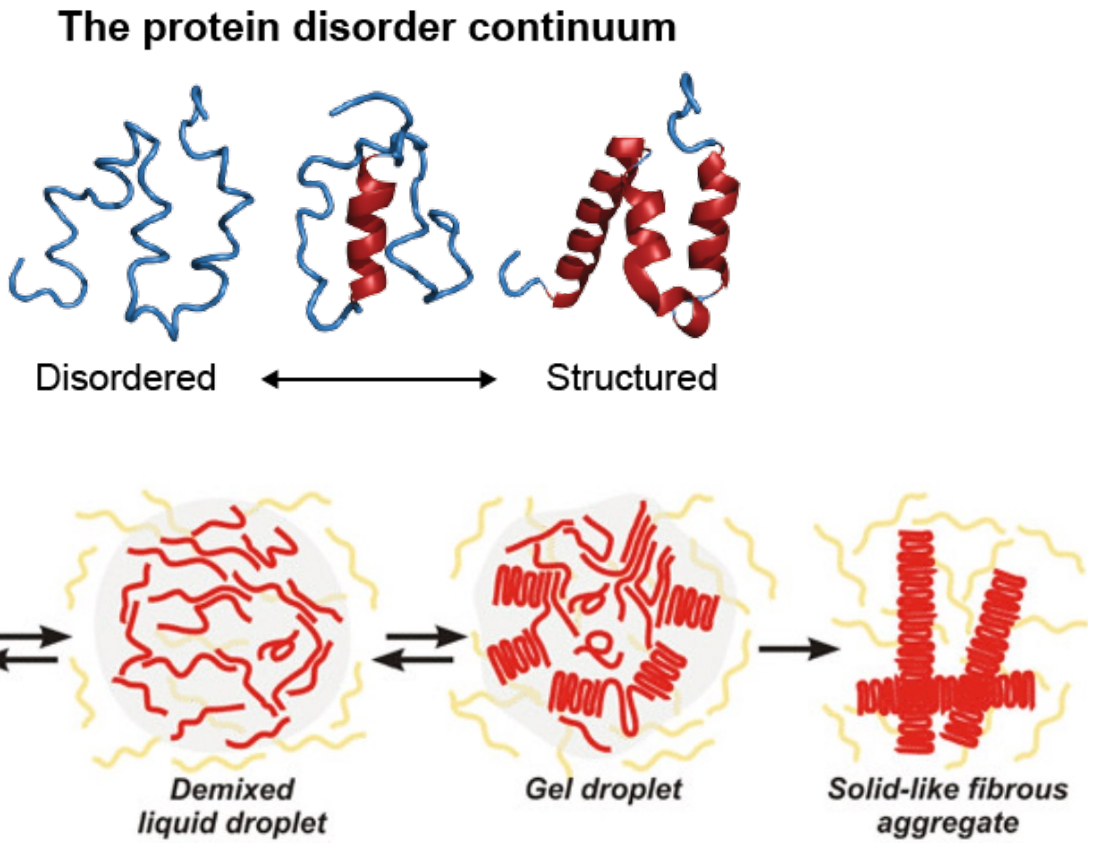


Liquid droplets
Toettcher **Cell**
2017



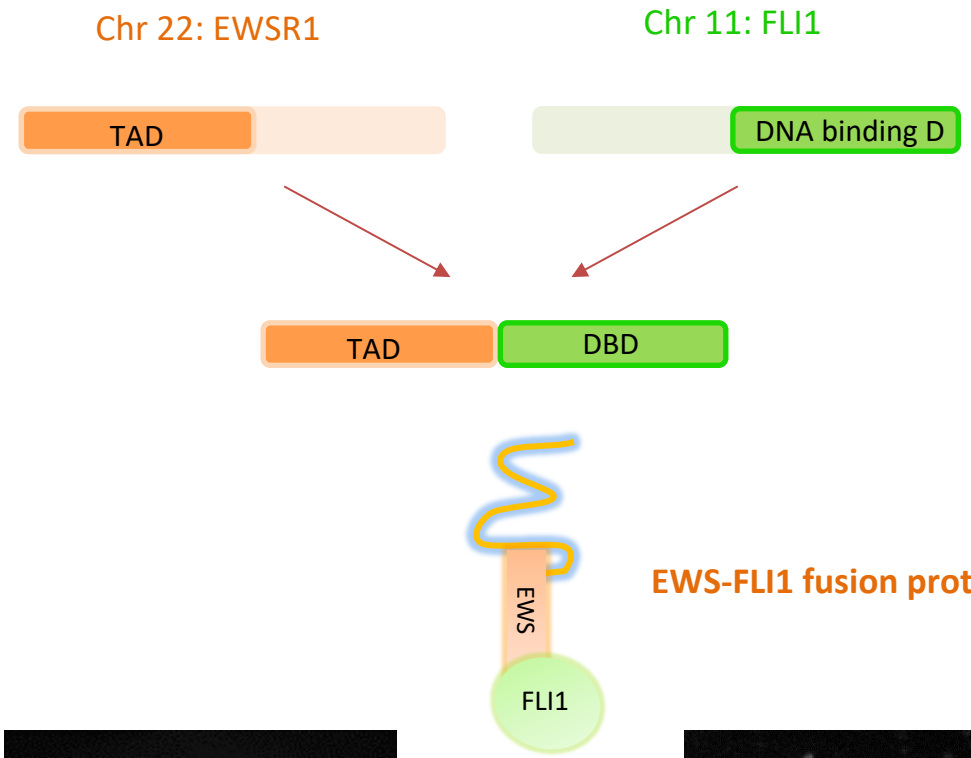
LCD, IDR, Prion like domains

More than 40% of the proteome is made of disordered regions

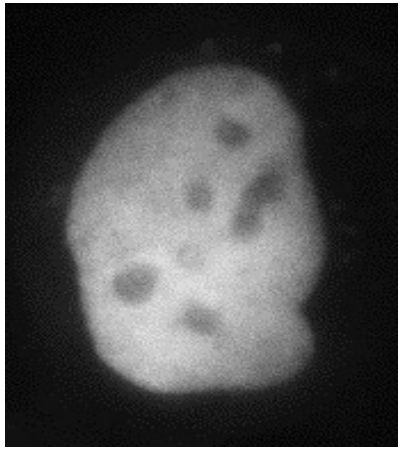


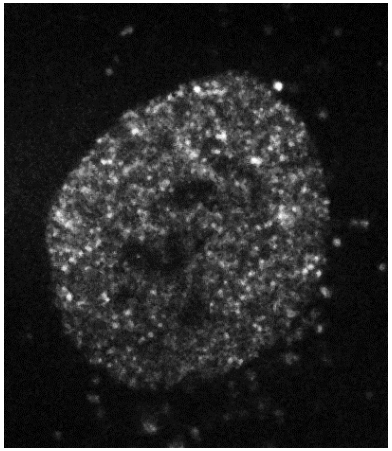
Ongoing project: Ewing Sarcoma

Olivier Delattre (Curie)

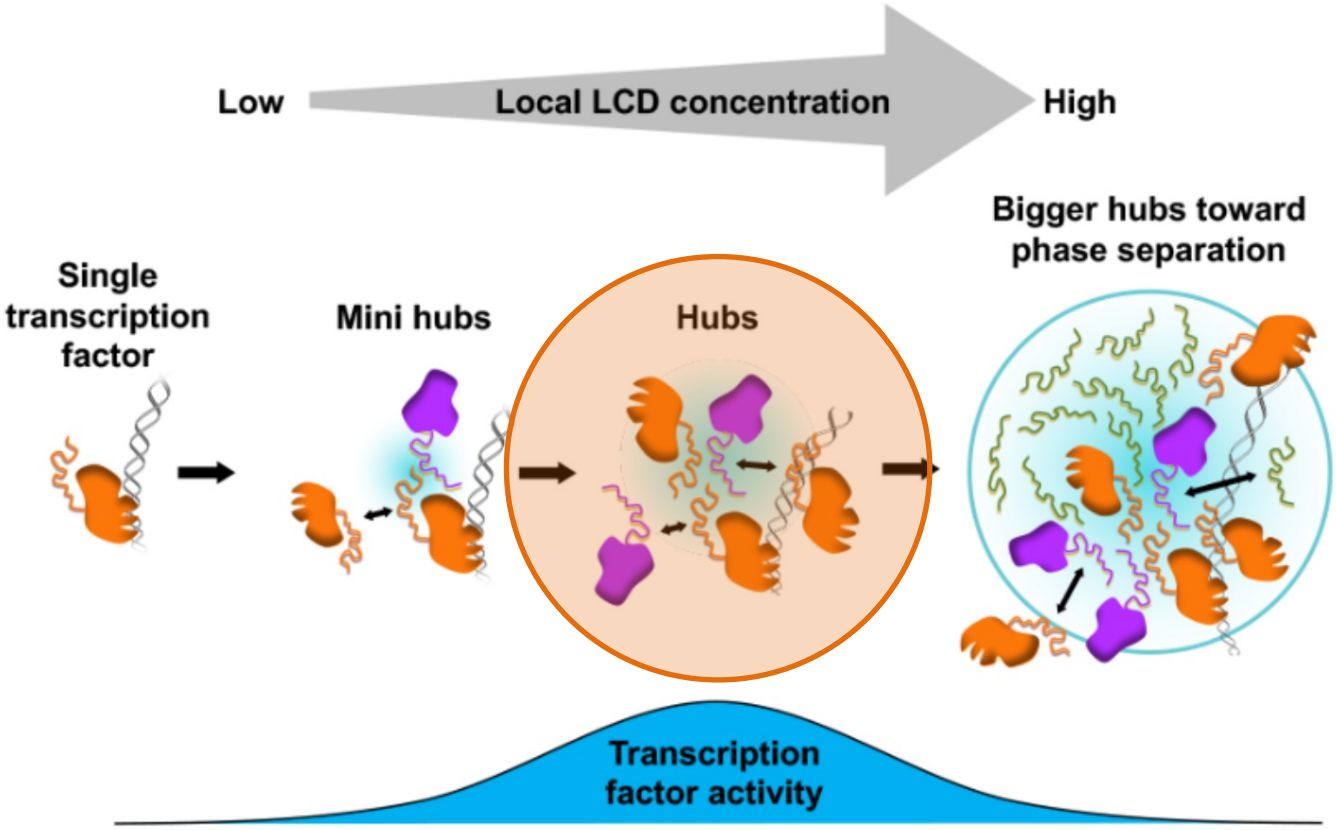


EWS-FLI1 fusion protein (EF1)





Open questions



Chong S. et al, Biorxiv 2021

LOC²O: Light-based Observation and Control of Cell Organization

LOCCO Team

Mathieu Coppey
Bassam Hajj
Patrick Keller
Tommaso Galgani
Lorena Kolar-znika
Maud Bongaerts
Brieuc Chauvin
Thomas Blanc
Jean De Seze
Koyomi Nakazawa
Mirna Kramar
Louise Regnier
Anumita Jawahar

Past members:
Kotryna Vaidziulyte
Simon De Beco
Leo Valon

ANR

FONDATION PIERRE-GILLES DE GENNES POUR LA RECHERCHE

FONDATION RECHERCHE MÉDICALE

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EMBO

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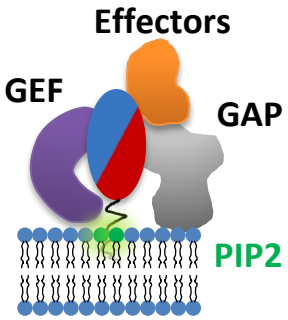
Clustering due to the charged protein tail

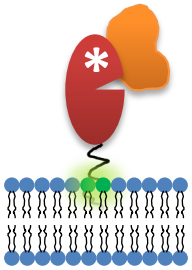
Rac1 WT

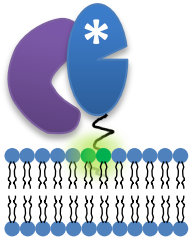
Rac1^{Q61L} active

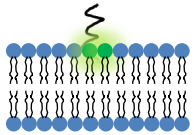
Rac1^{T17N} inactive

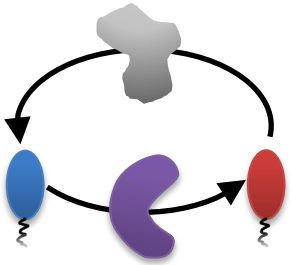
CAAX membrane anchor (PB)

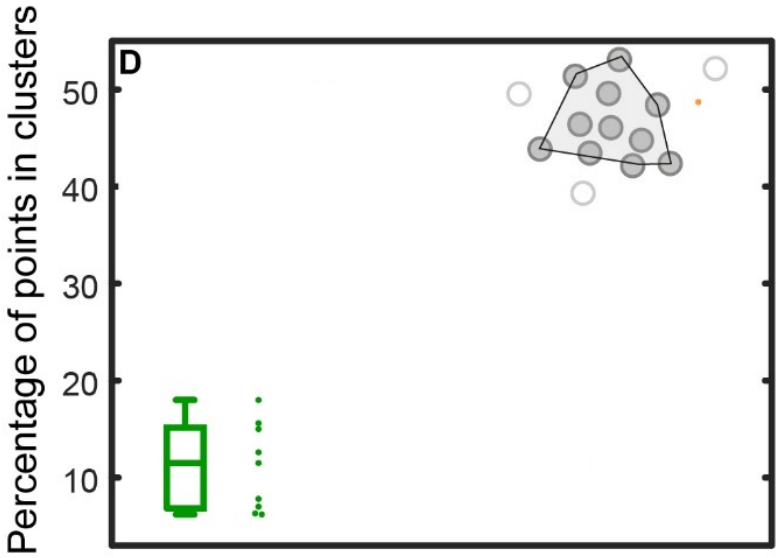




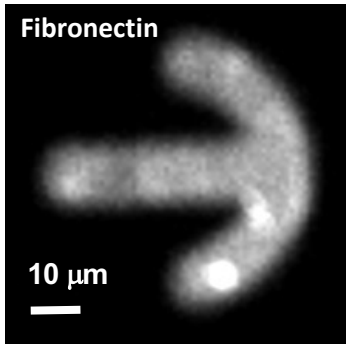


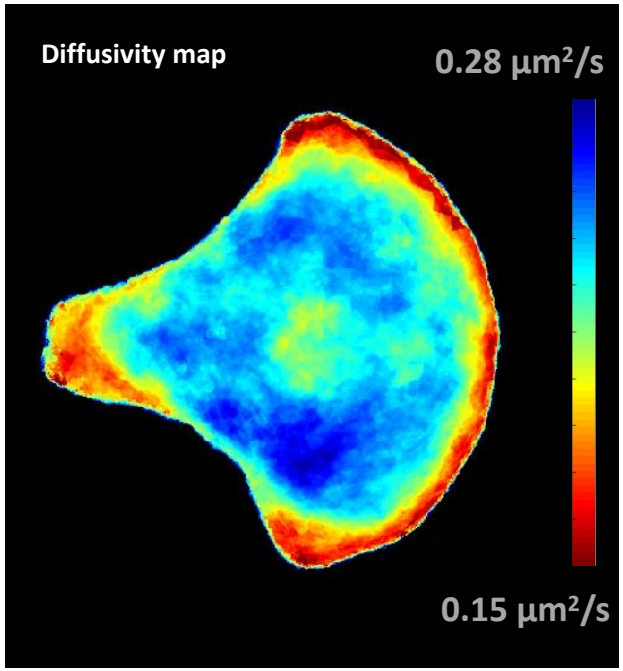


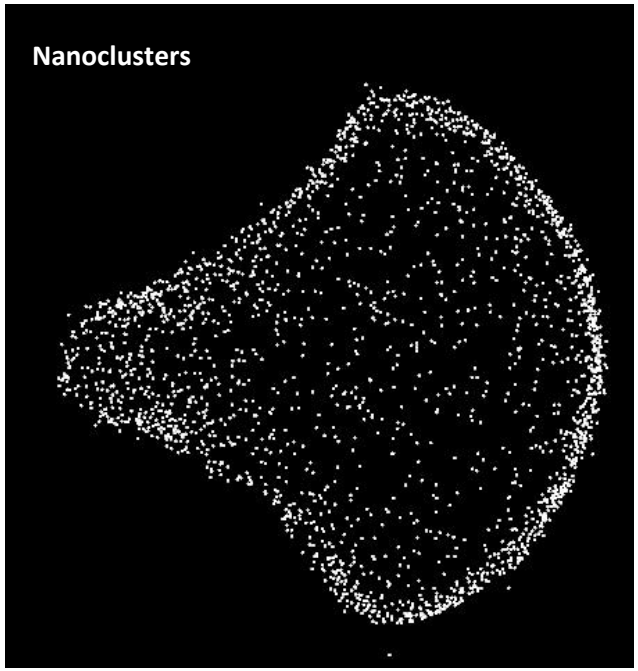




Nanoclusters immobilize Rac1







And are enriched by molecular interactions