Controlling cells with optogenetics

Mathieu COPPEY

KITP july 29th 2019

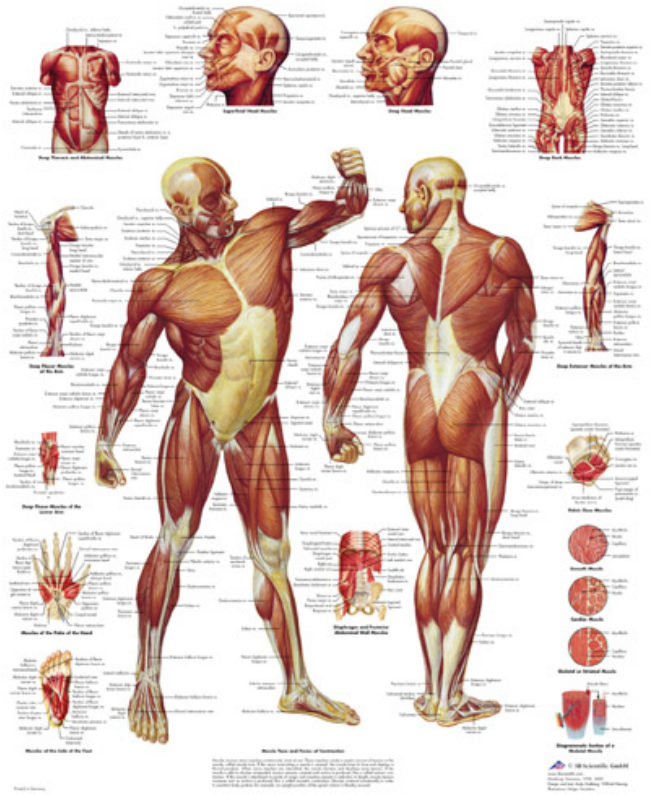
institutCurie

cnrs

Search for principles

Genome

1 Gb



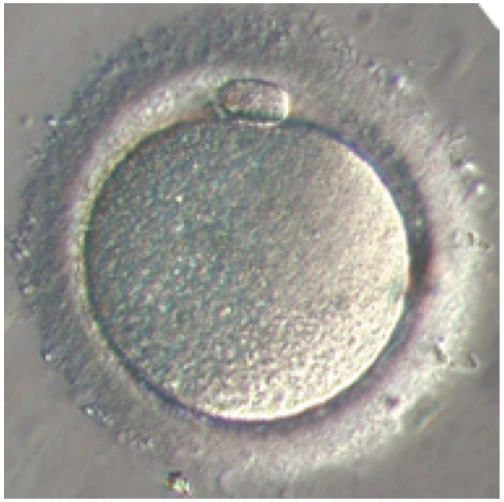
… Gb?

Search for principles

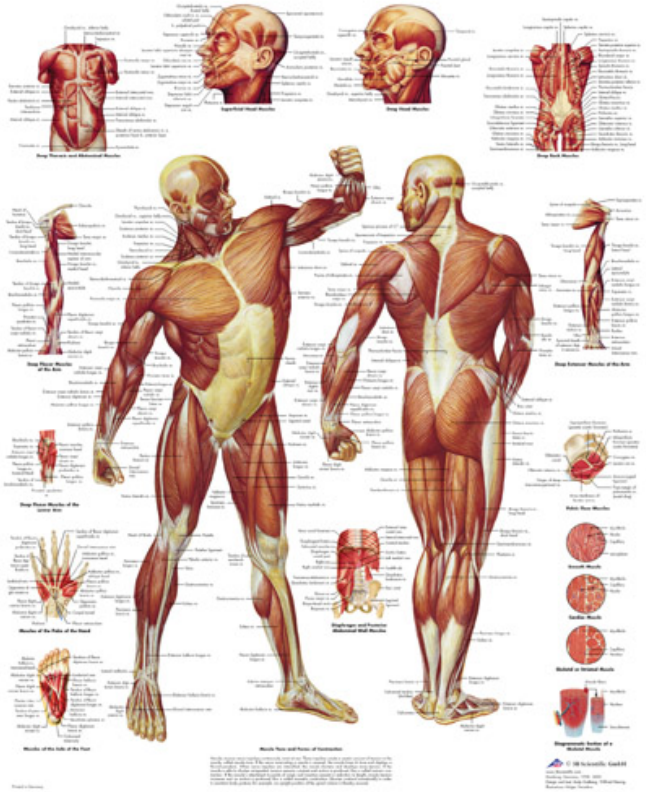
Genome

1 Gb

+

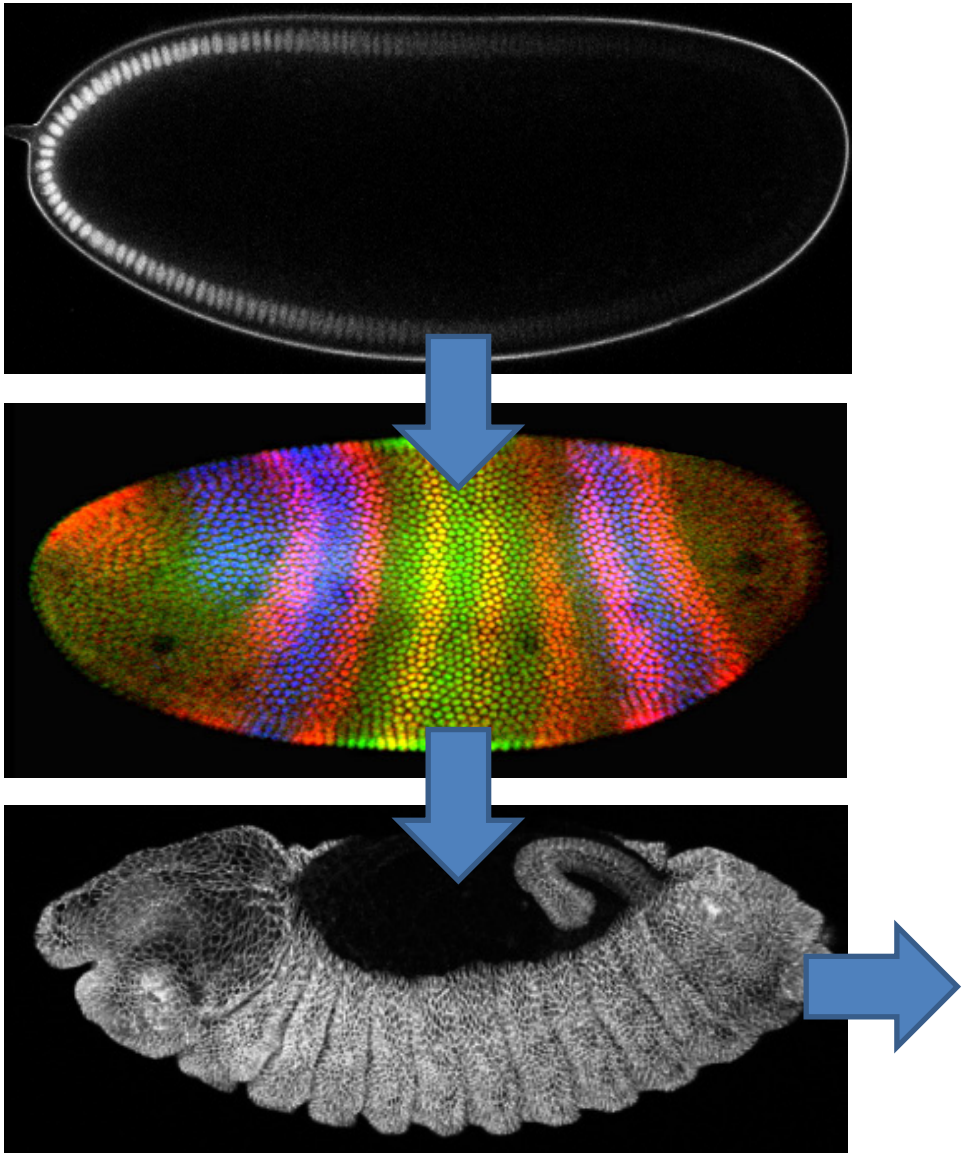


"Template"



… Gb?

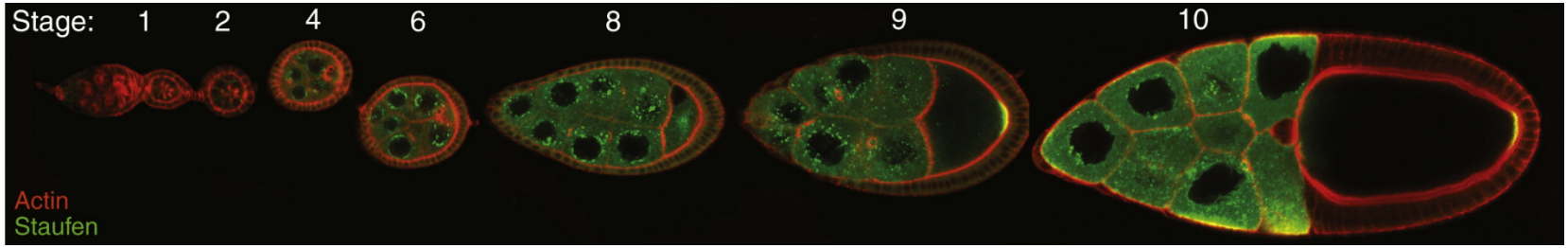
Ex of a template: the morphogen gradient

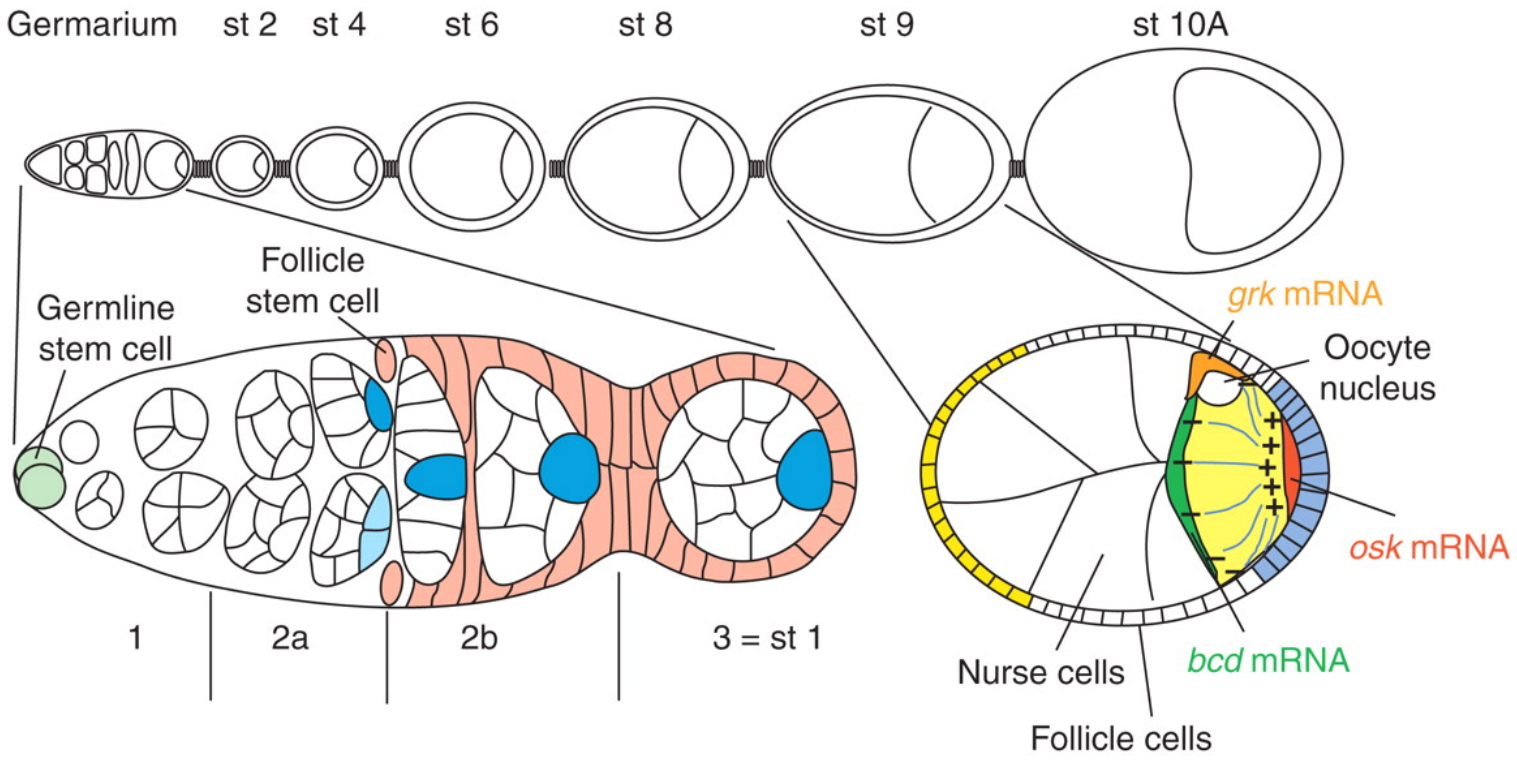


Work from Thomas Gregor et al.

- Enough bits in maternal gradients to specify gap gene domains with cellular resolution
- Positional information persist up to the final organism

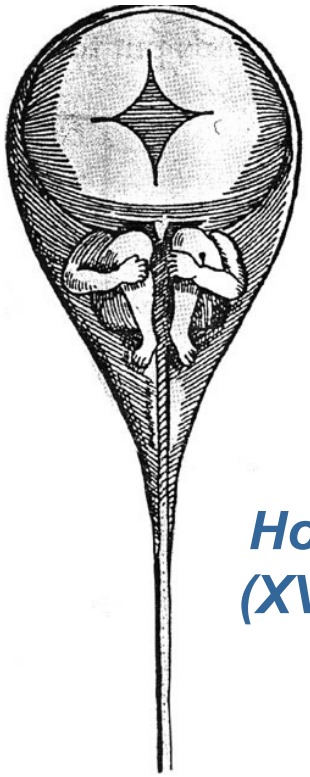
Where the template comes from?





Genome + templates are enough?

- **Homunculus: preformation**, miniature prefiguration of the future organism in the semen

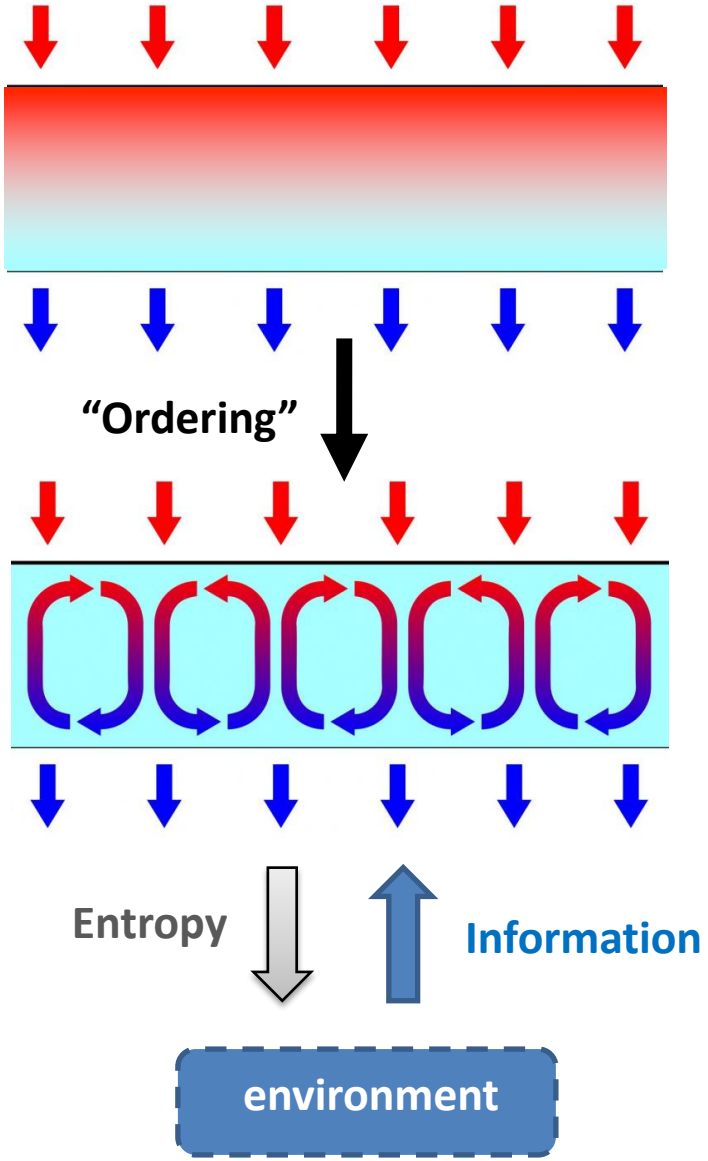


Homunculus (XVII century)

- **Aristotle: Epigenesis,** synthetic creation, progressive formation of new material from unformed matter

Self-organization as a source of information

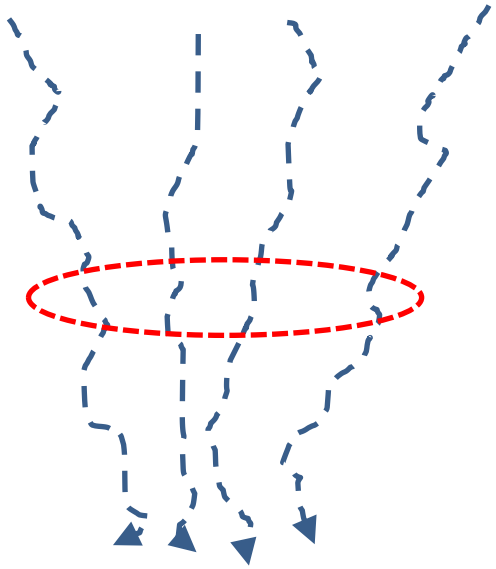
Convection

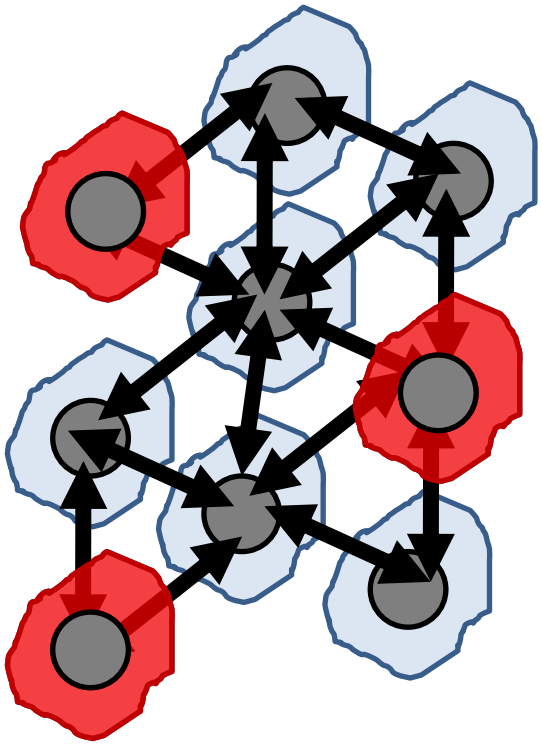


Morphogenesis: searching for principles

Templates
-few bits-

Self-organizing system
-many bits-





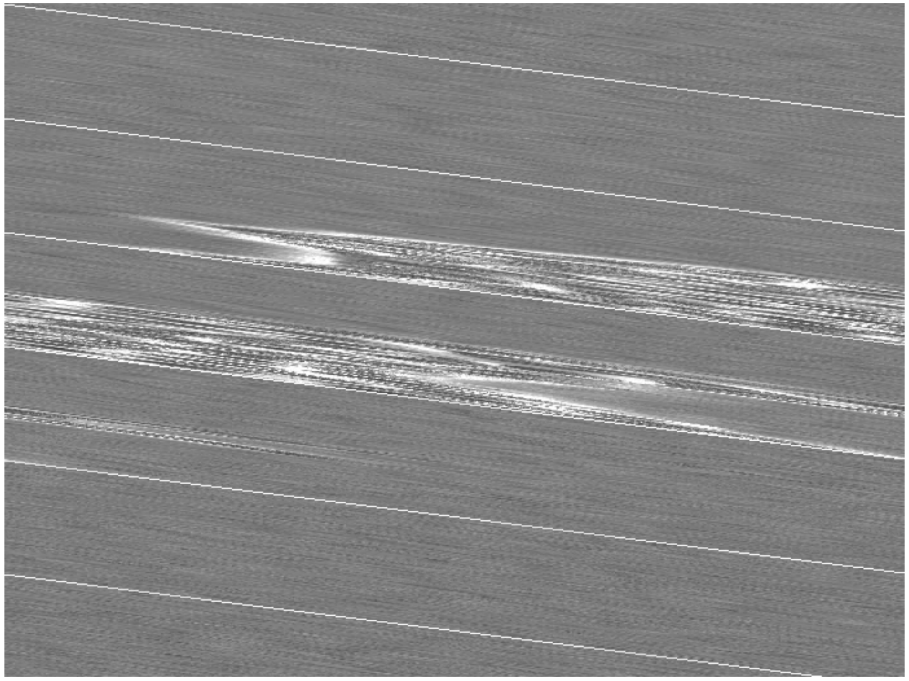
What are the *control principles* of self-organized matter?

… of collective systems?

cell migration

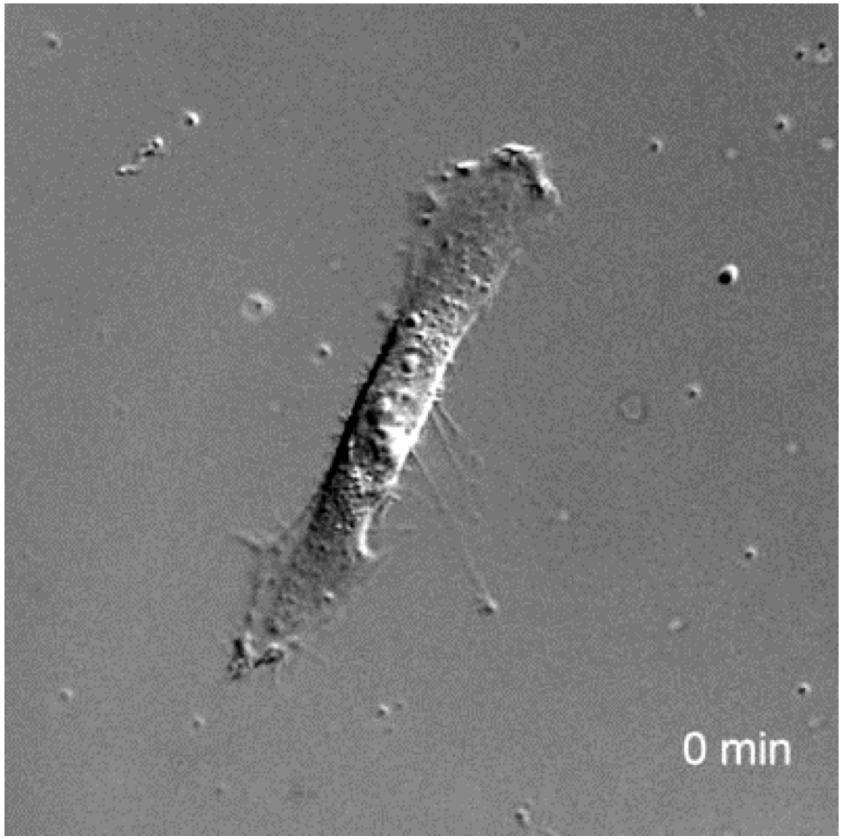
Cell migration

Random collective migration



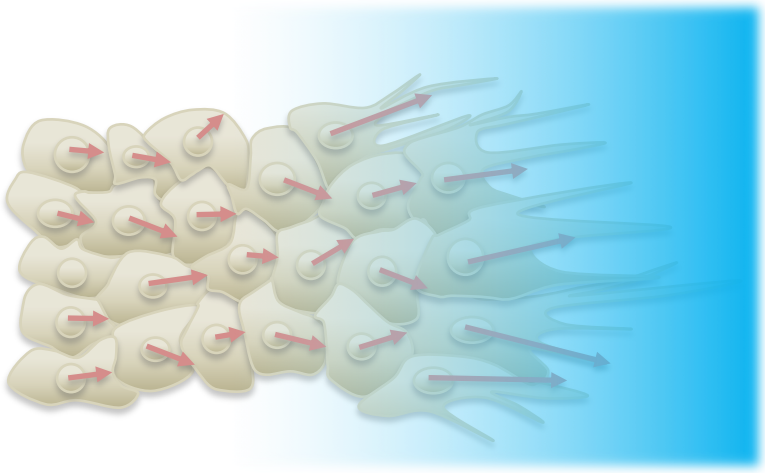
Yamaguchi et al, Scientific Reports 2015

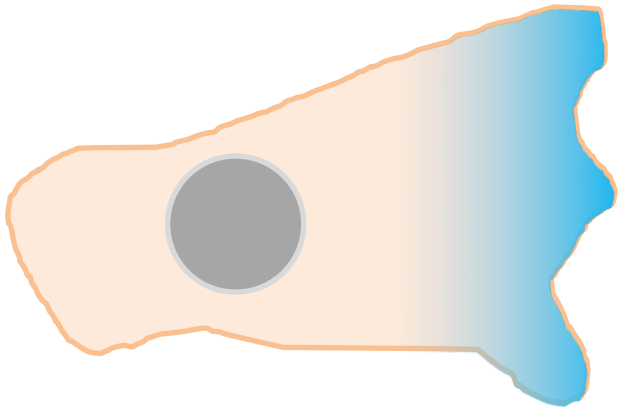
Random single cell migration



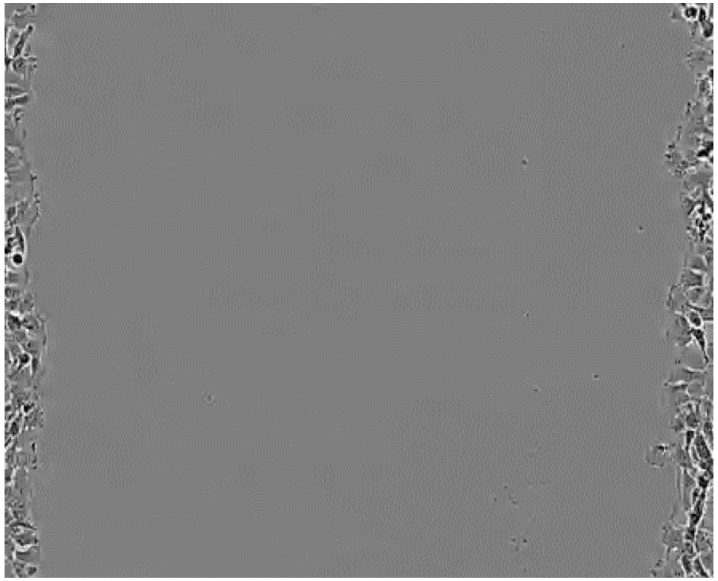
From Kotryna

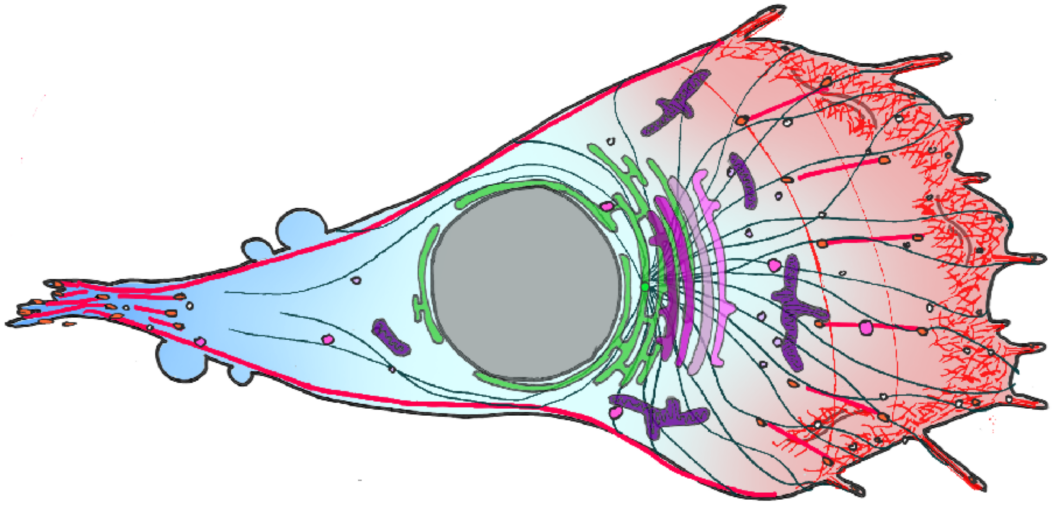
Control by system-scale gradients

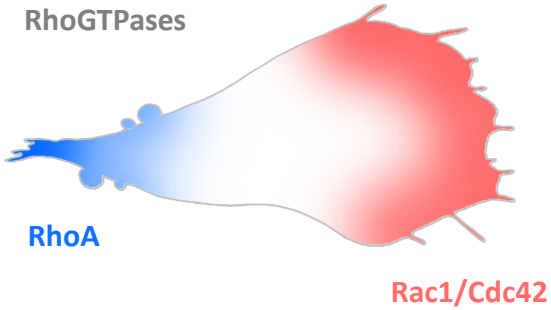


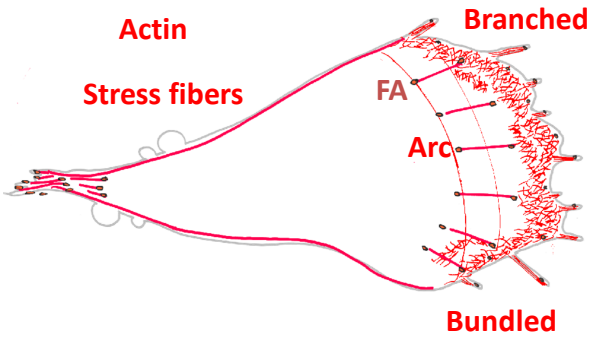


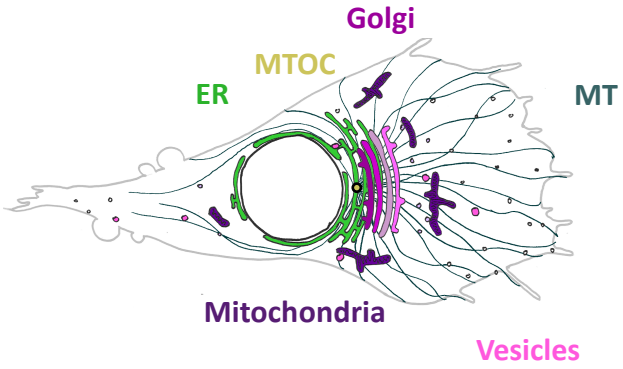
Eukaryotic cell migration



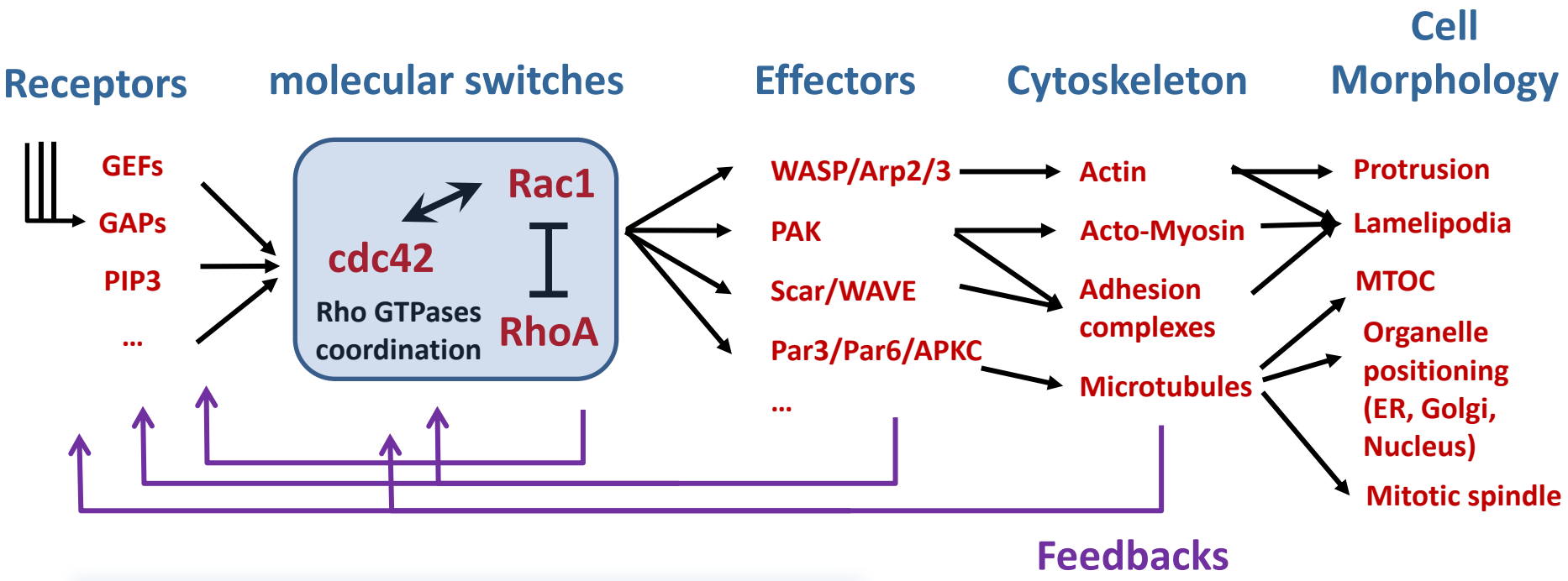


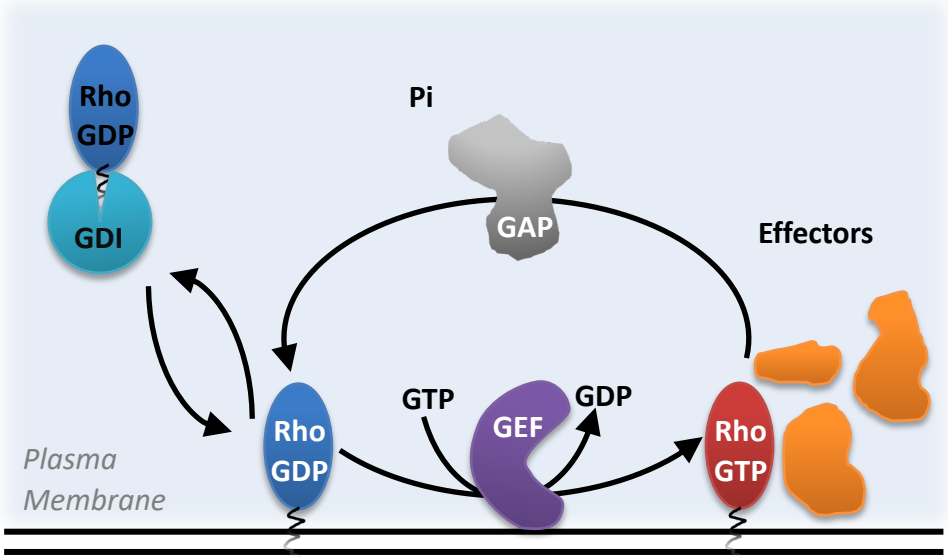


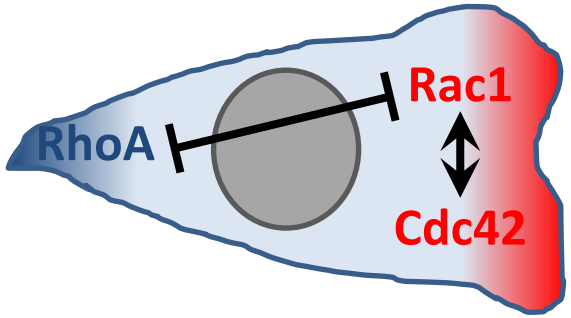




Rho GTPases signaling

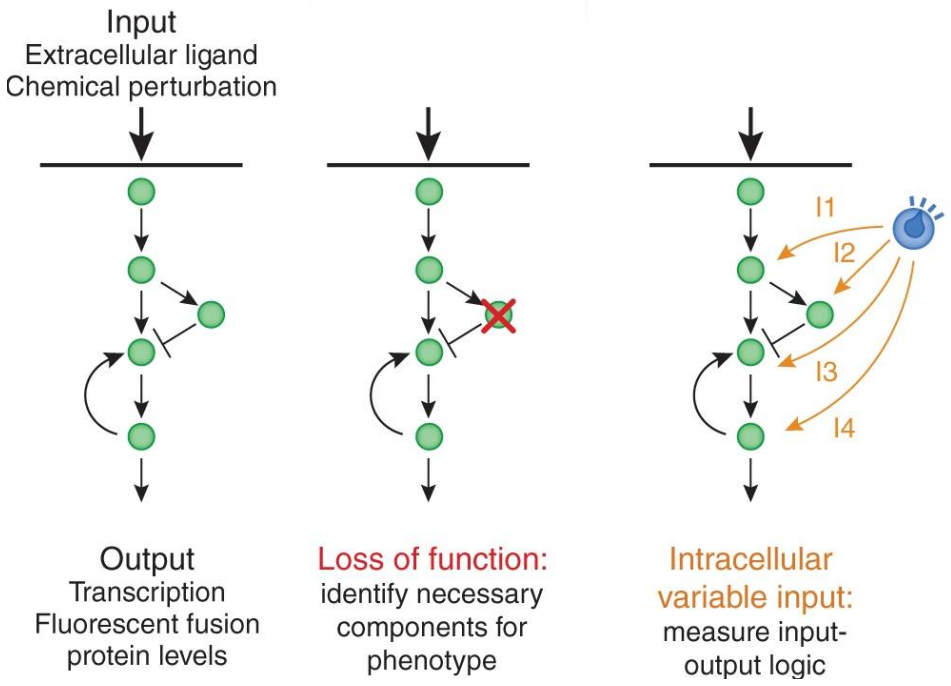


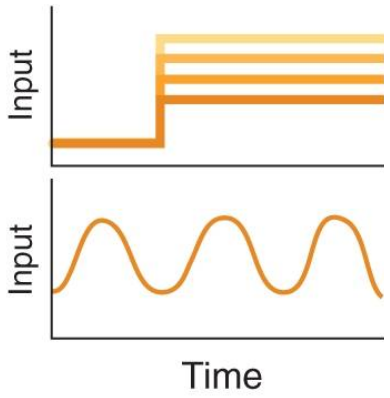




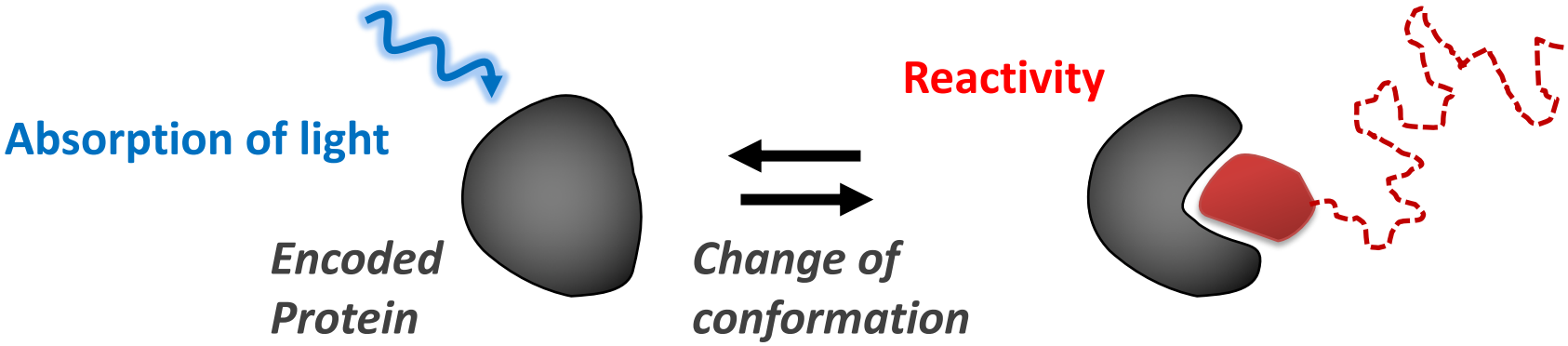
optogenetics

Optogenetic dissection

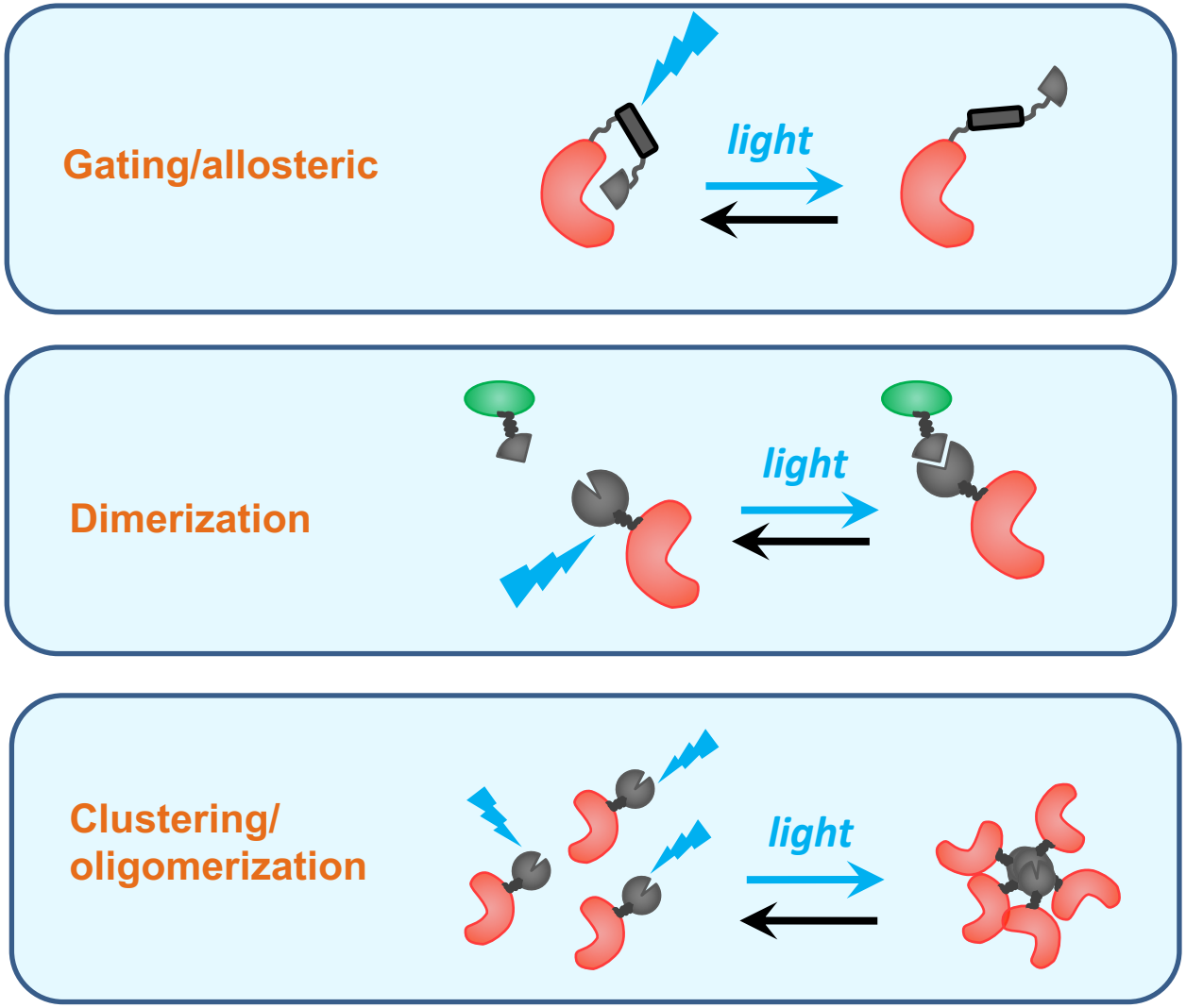




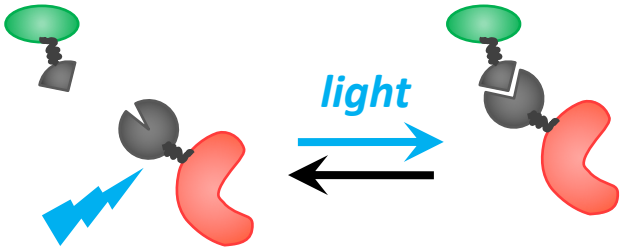
J. Toettcher et al., Nature Methods 2011

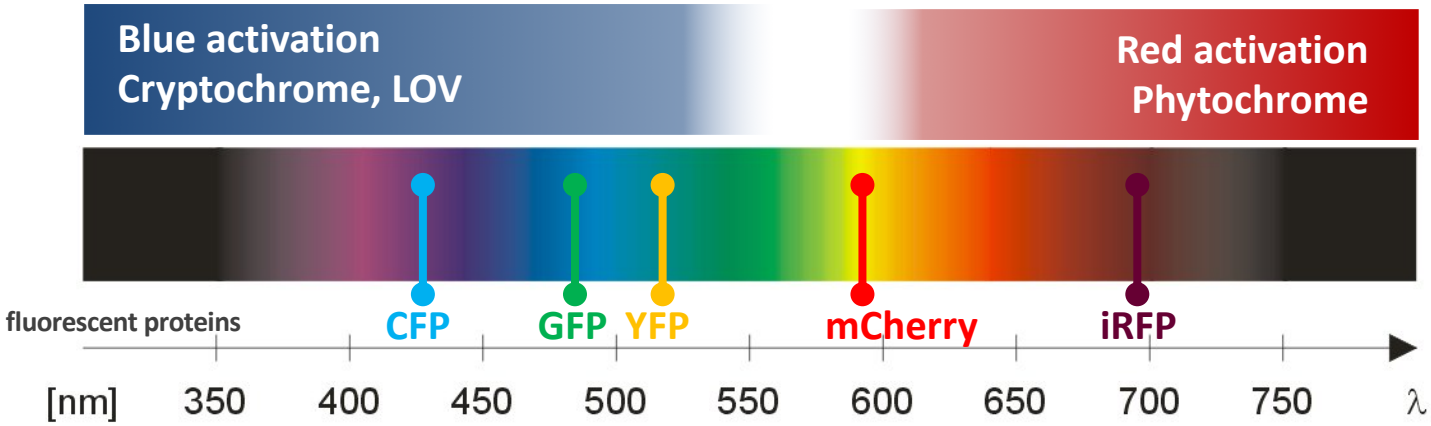


Main modalities for controlling cells



Light activated proteins for heterodimerization

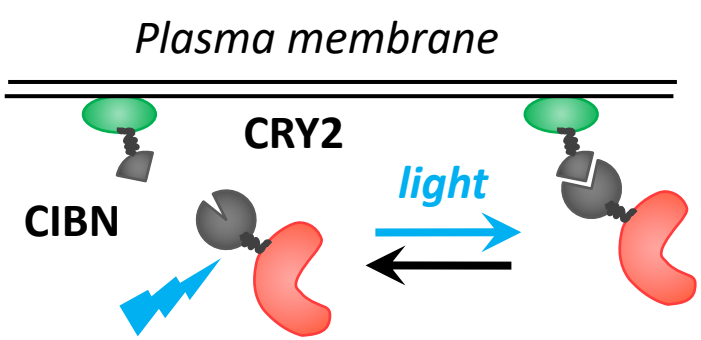




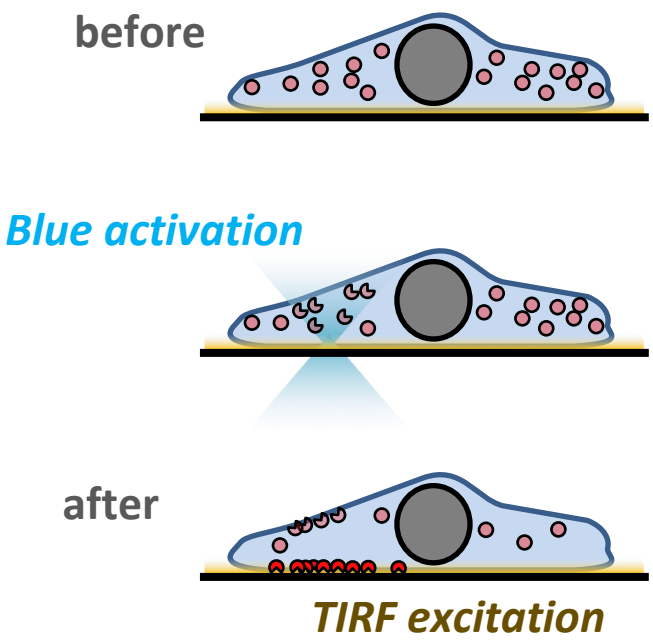
Photosensitive protein	Turn-on speed	Turn-off speed ($t_{1/2}$)	Chromophore requirement	Compatible imaging wavelengths (nm)	λ_{on} (nm)	λ_{off} (nm)	Effector affinity
PHYB	Seconds	• Seconds (illuminated at 750 nm) • Hours (dark reversion)	PCB; exogenous or synthesized *in situ*	≤514	650	750	• <100 nM (post 650 nm) • >100 µM (post 750 nm)
CRY2	Seconds	5 minutes	Flavin; endogenous	≥561	405–488	NA	Not determined
LOV	Seconds	Tens of seconds to minutes	Flavin; endogenous	≥514	440–473	NA	• 1 µM (dark) • 100 µM (light)

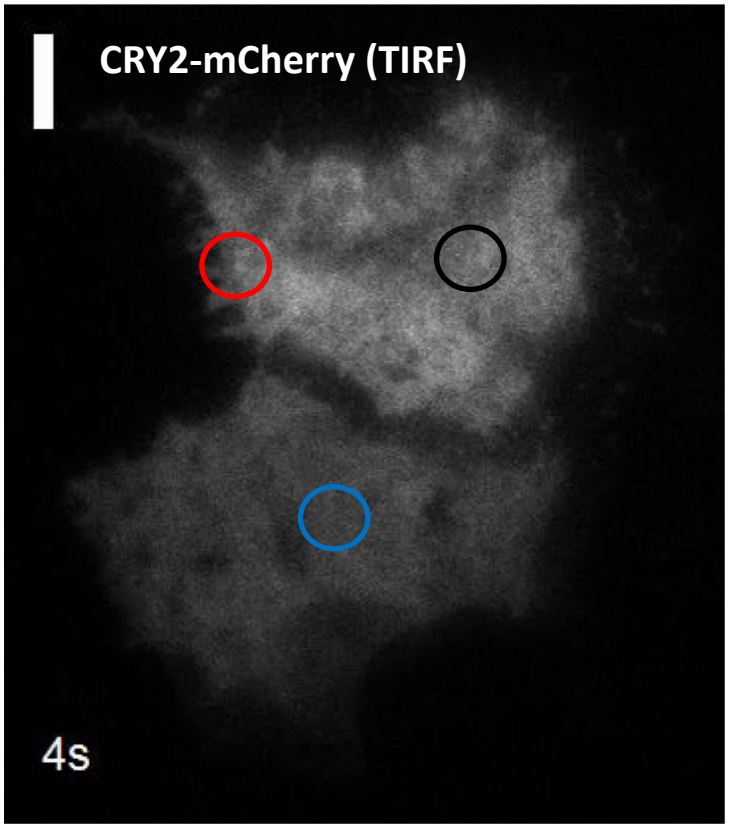
Illuminating cell signalling with optogenetic tools, ***Doug Tischer and Orion D. Weiner Molecular Cell Biology 2014***

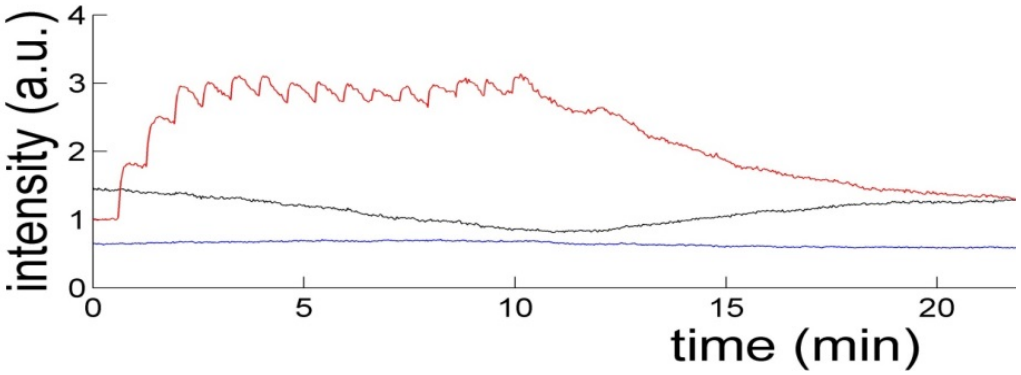
Manipulating protein localization



Predictive Spatiotemporal Manipulation of Signaling Perturbations Using Optogenetics
Leo Valon **Biophysical Journal 2015**

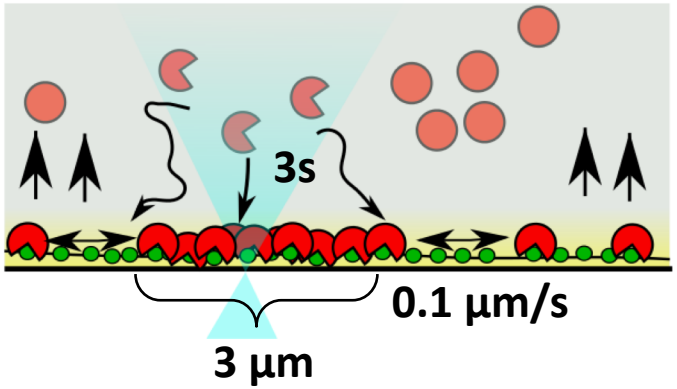




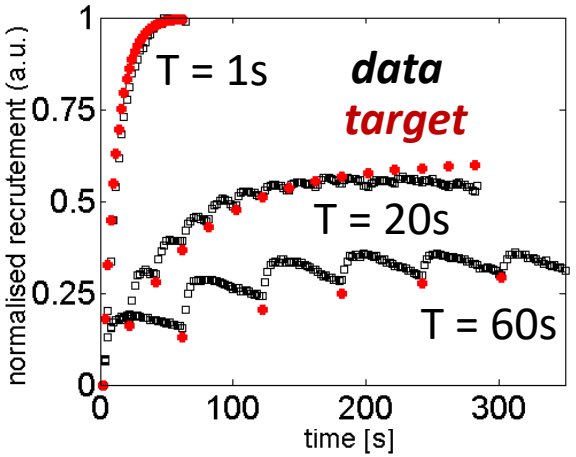


Predictive quantitative control

Biophysical picture



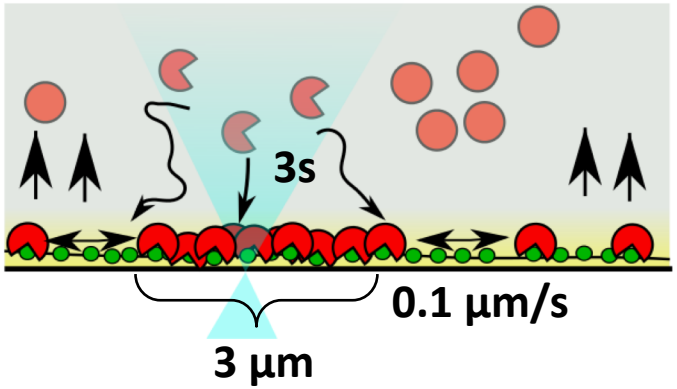
➢ level control



T : pulse frequency

Predictive quantitative control

Biophysical picture

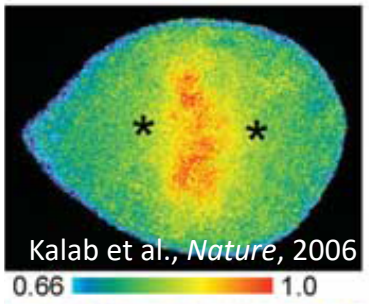


➢ **Spatial control**

$$C_{\text{steady state}} \sim e^{-\frac{x}{\lambda}}$$

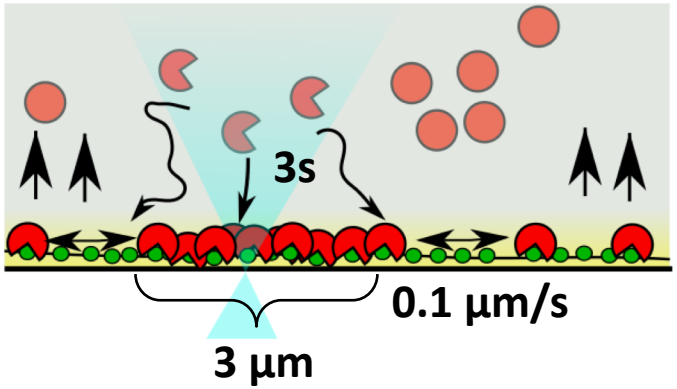
$$\lambda = \sqrt{D\tau_{off}} \sim 5\mu\text{m}$$

Ran activity gradient



Predictive quantitative control

Biophysical picture

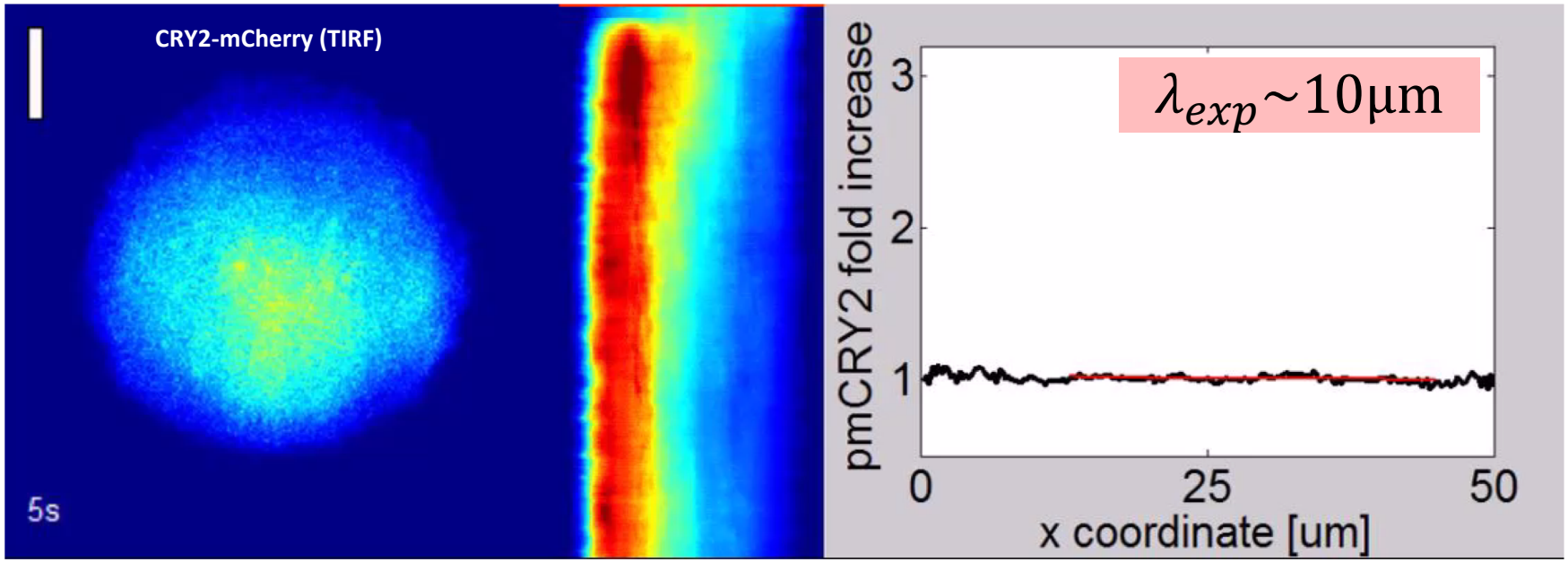


3min

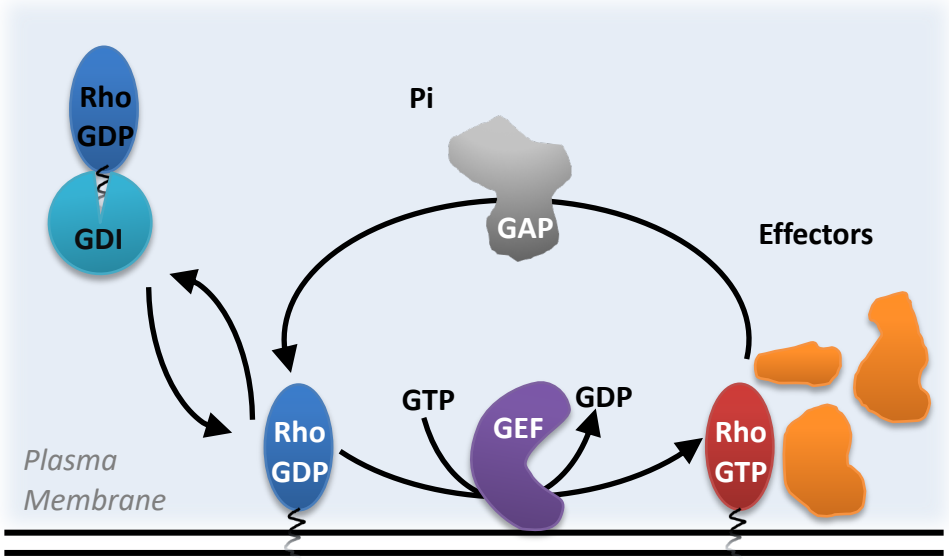
➢ **Spatial control**

$$C_{\text{steady state}} \sim e^{-\frac{x}{\lambda}}$$

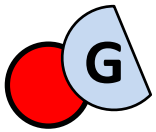
$$\lambda = \sqrt{D\tau_{off}} \sim 5\mu m$$



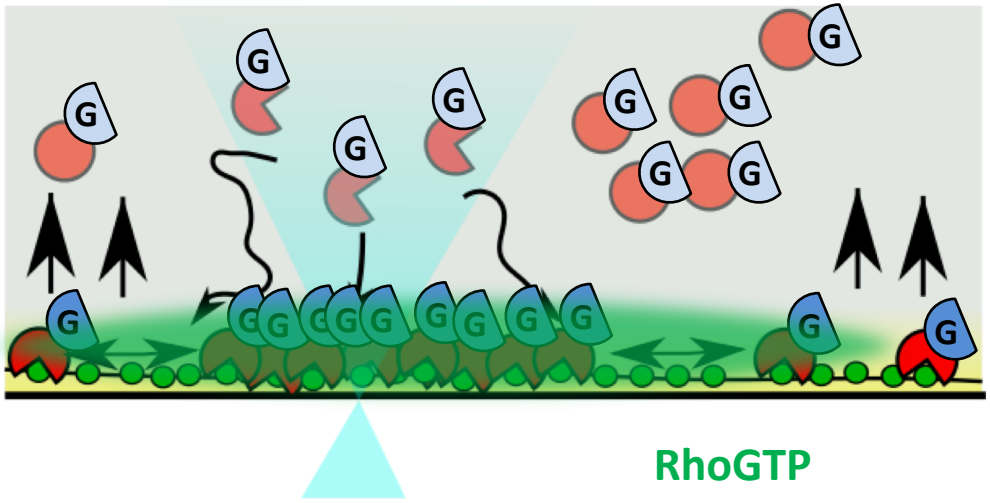
Light-gated RhoGTPase activation



Truncated GEF (catalytic domain) fused to CRY2

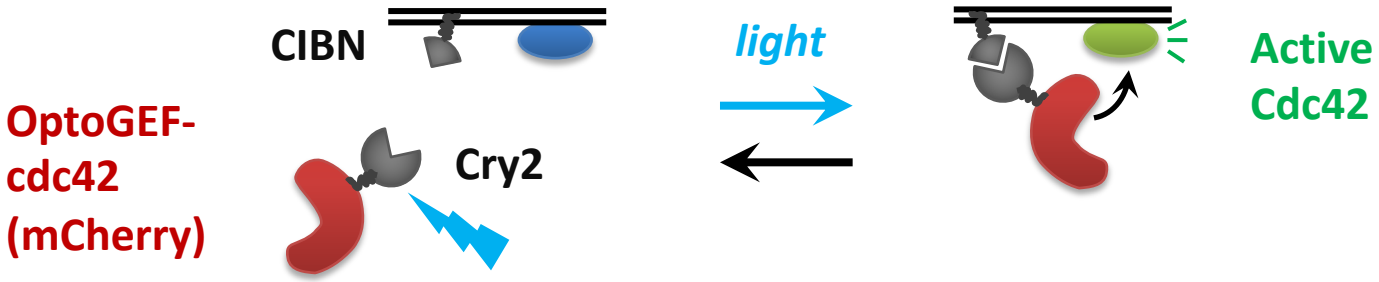


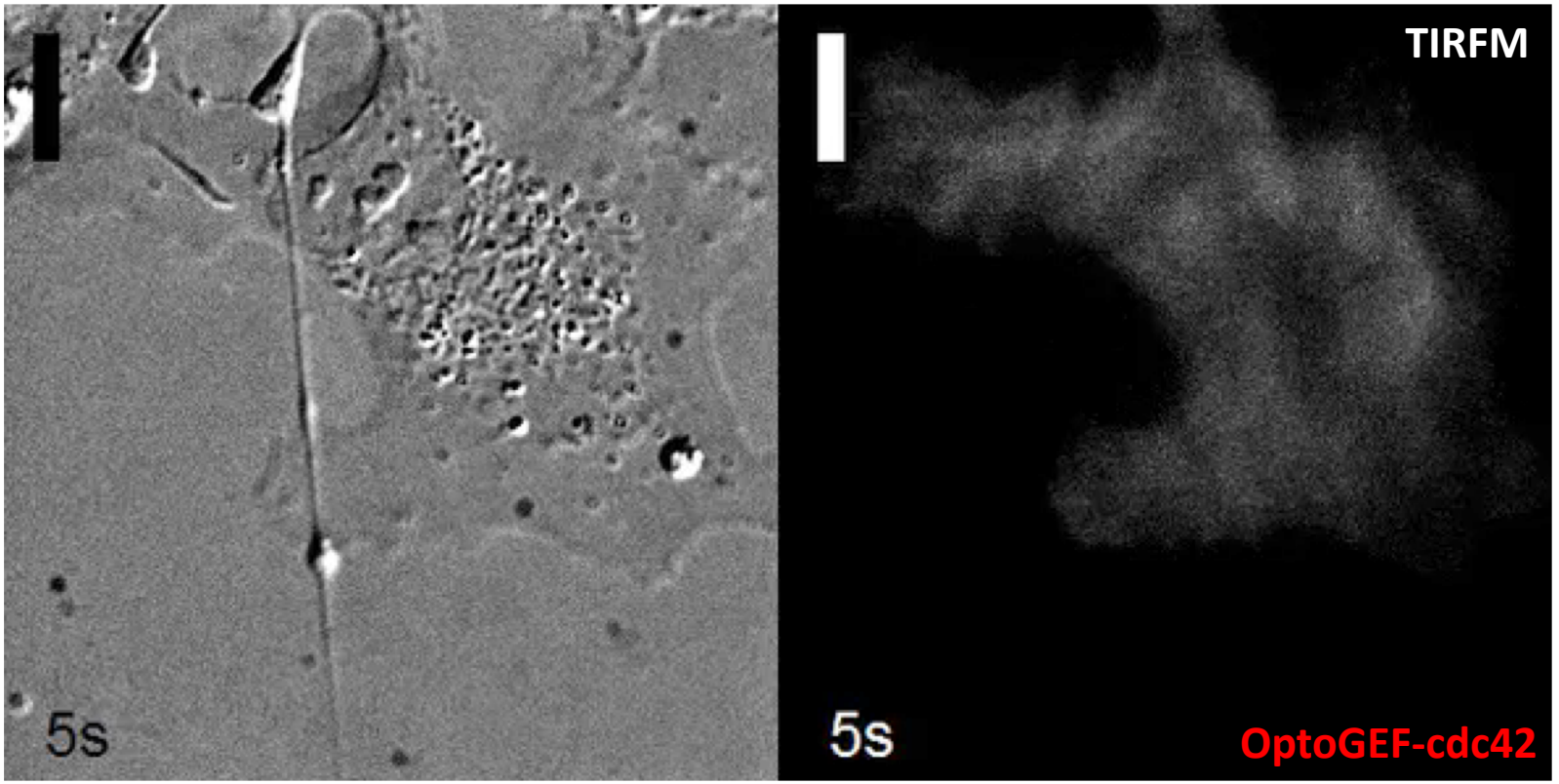
Local activation of the **endogenous** pool of RhoGTPases



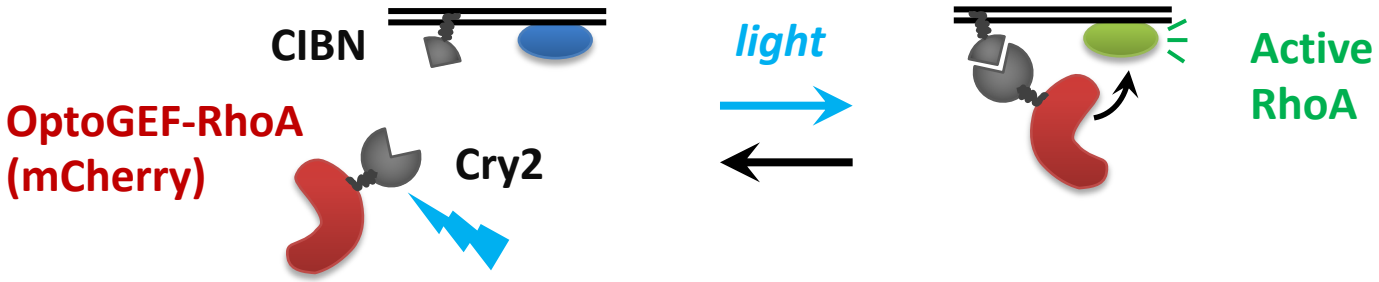
TIAM1 → OptoGEF-Rac1
INTERSECTIN → OptoGEF-cdc42
ARH11 → OptoGEF-RhoA

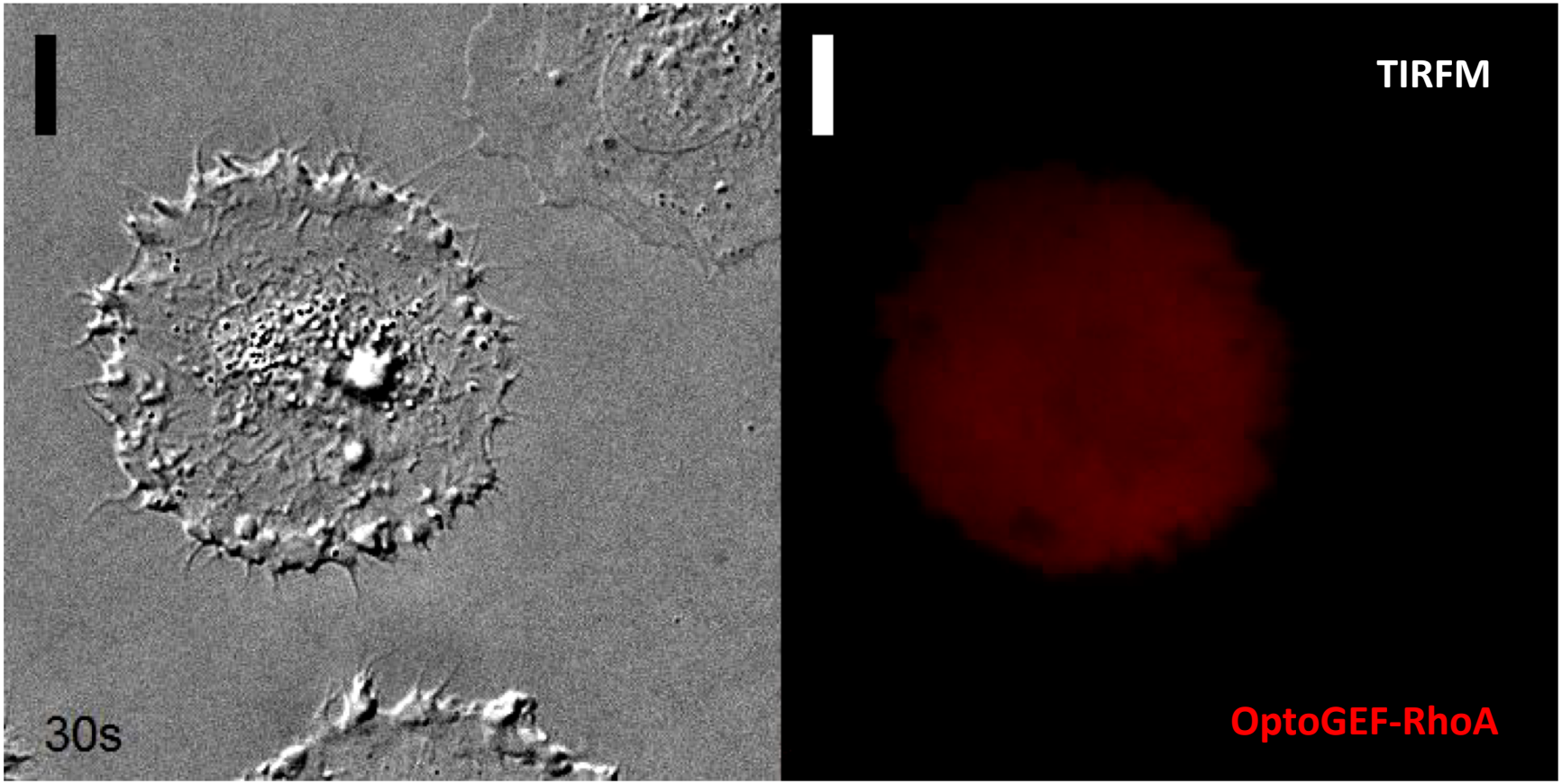
Local cdc42 perturbation induces migration





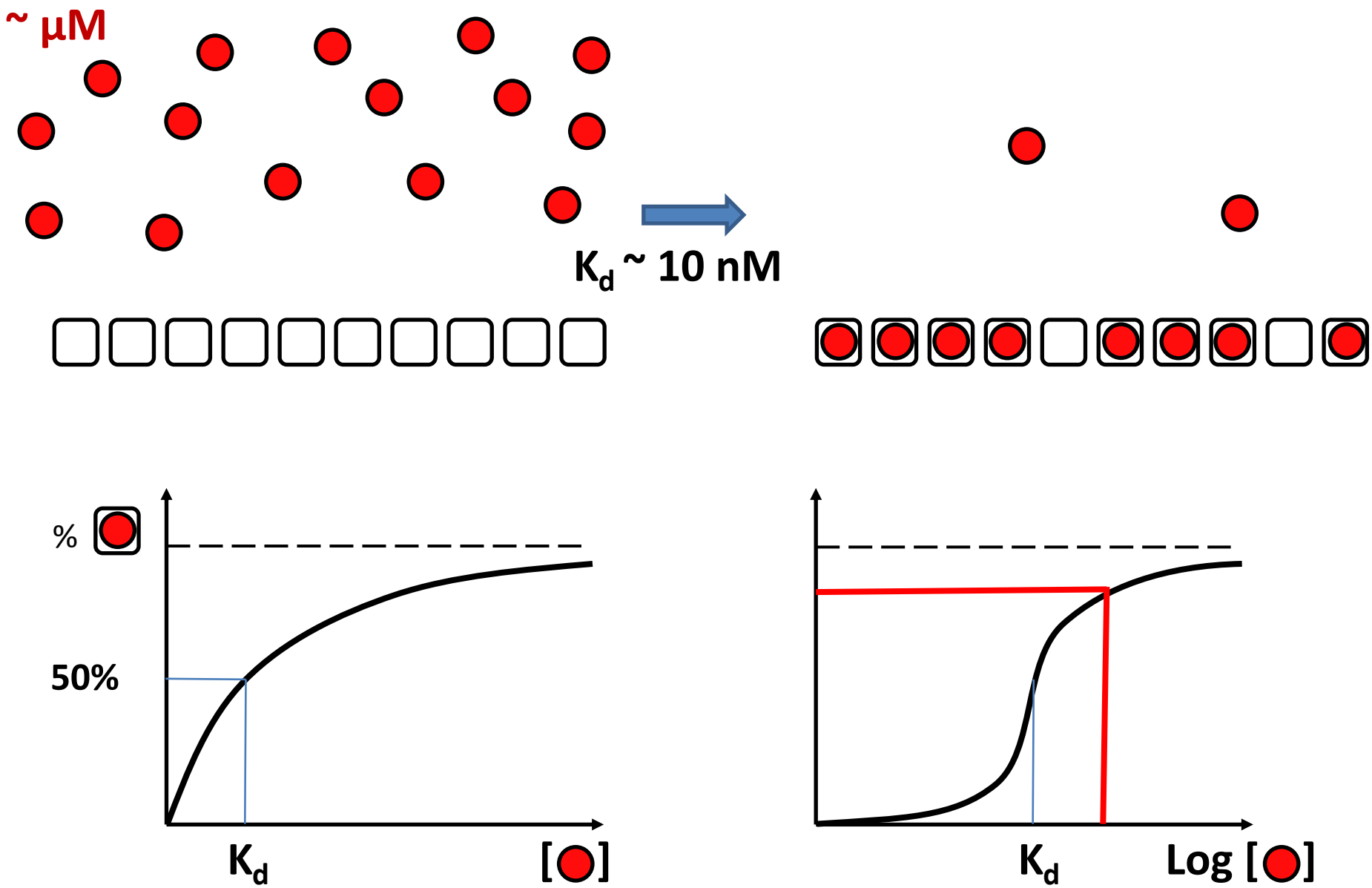
Local RhoA perturbation induces *reverse* migration



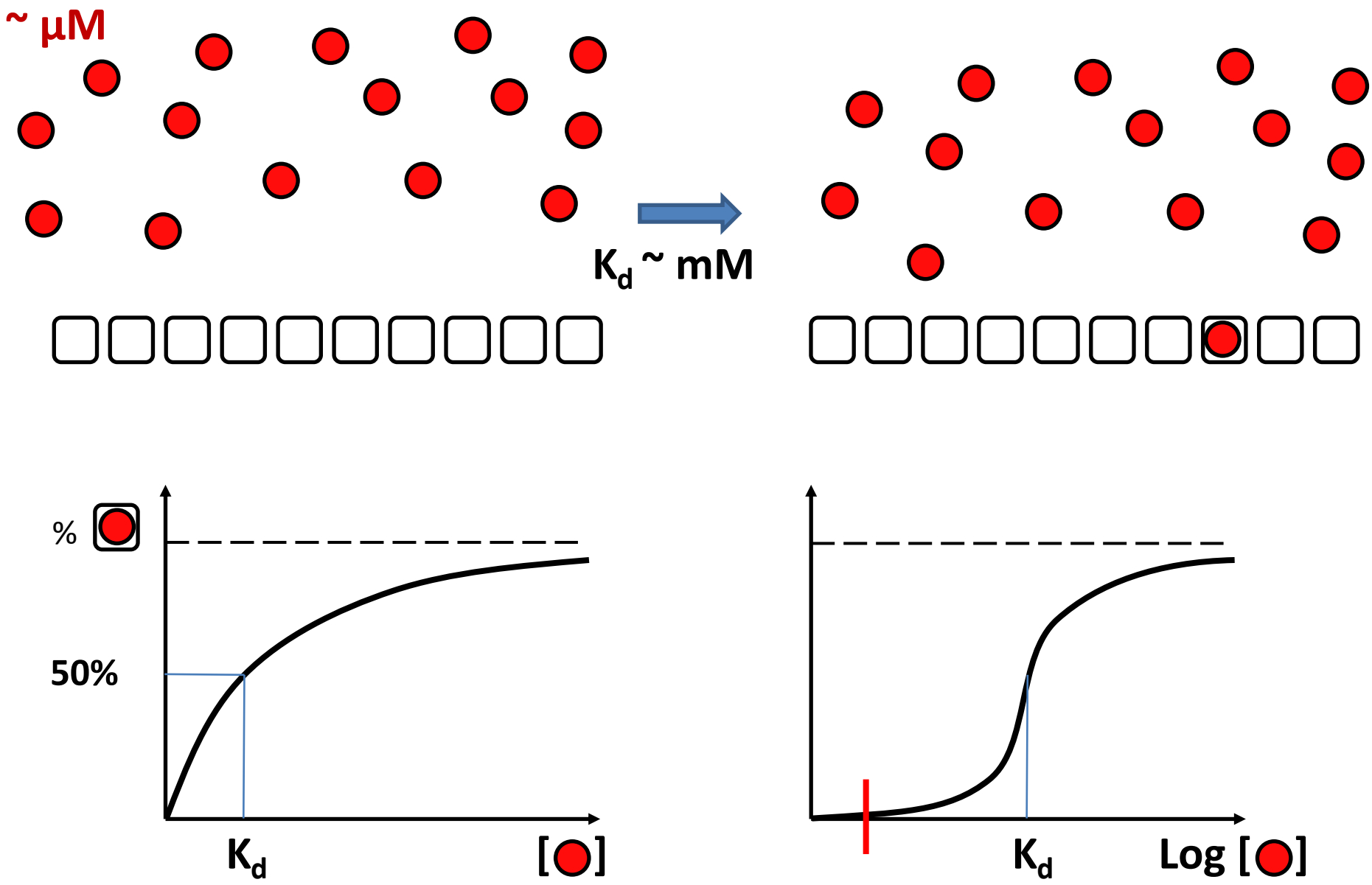


a quick comment

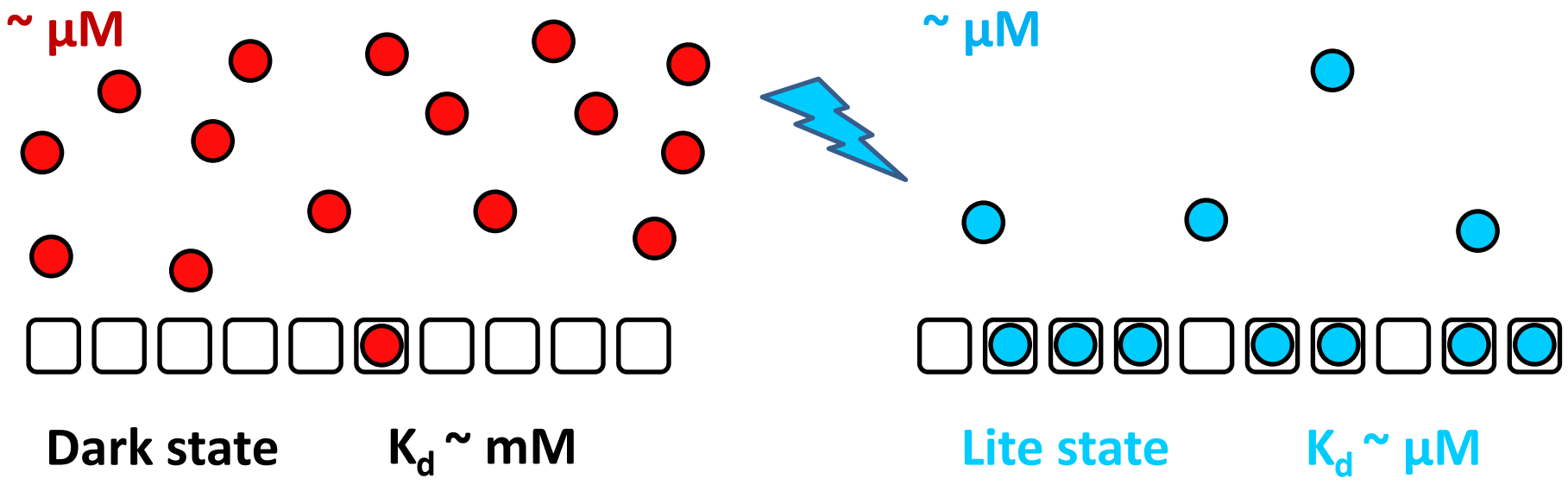
Recall: first order kinetics

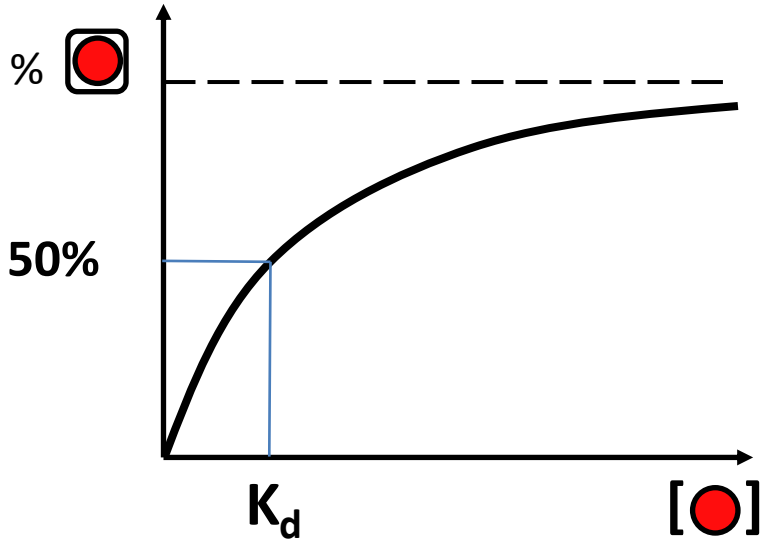


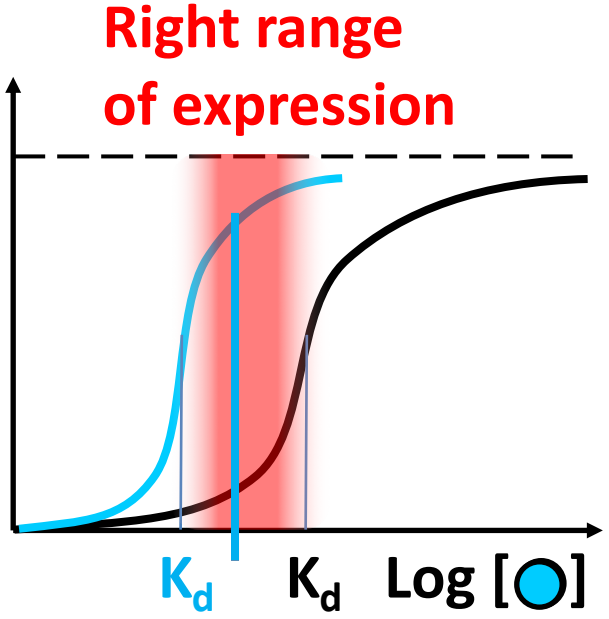
Recall: first order kinetics



Recall: first order kinetics



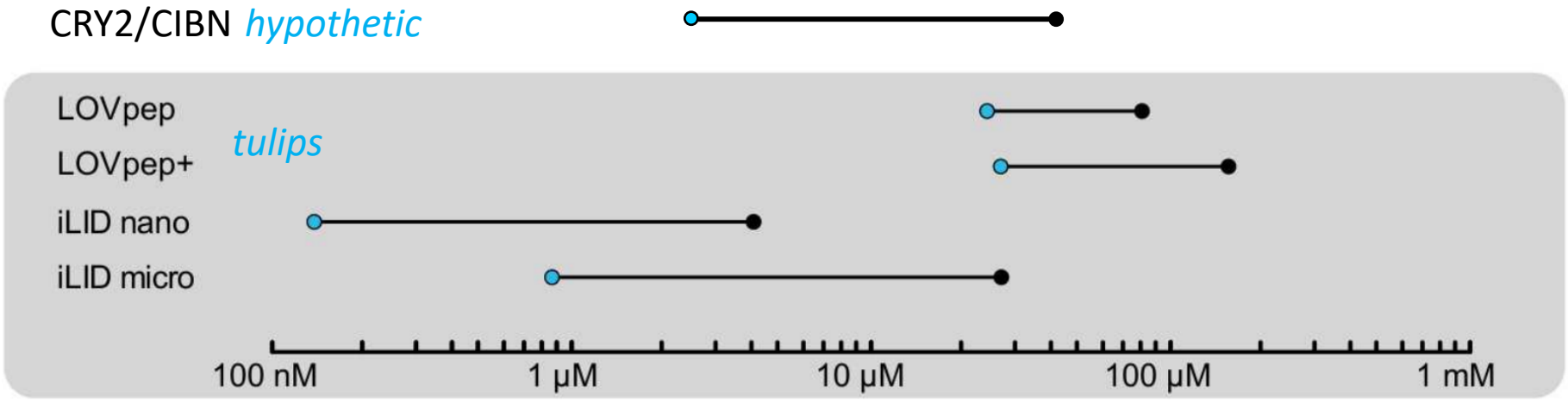




Choosing the right dynamic range

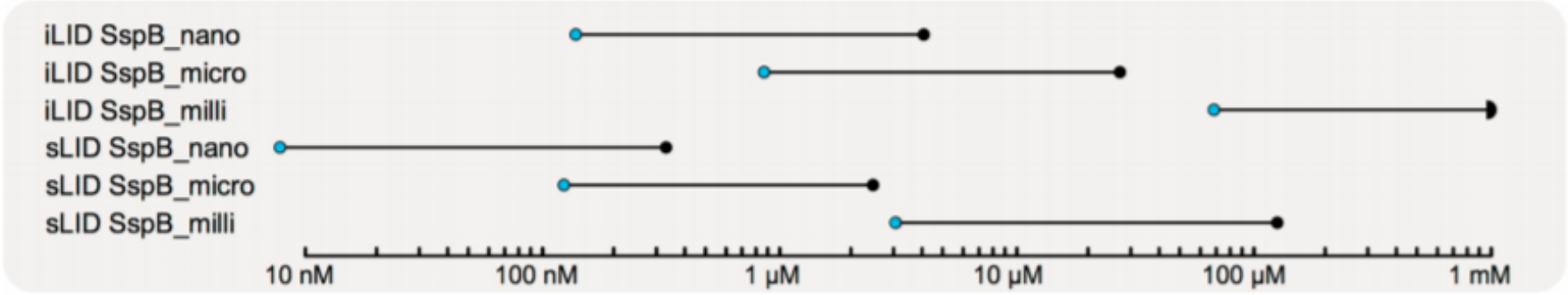
Benchmarking dimerizers

Correlating in vitro and in vivo Activities of Light Inducible Dimers: a Cellular Optogenetics Guide
Brian Kuhlman **ACS synthetic biology 2015**



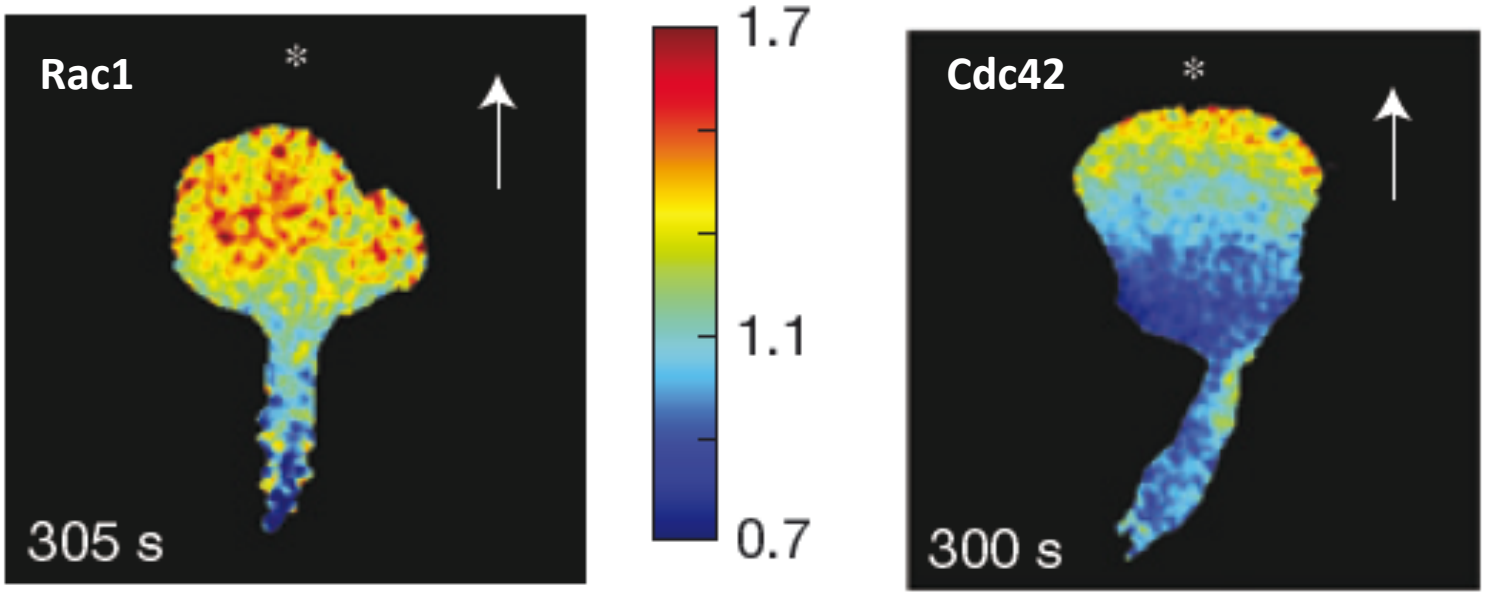
New variants

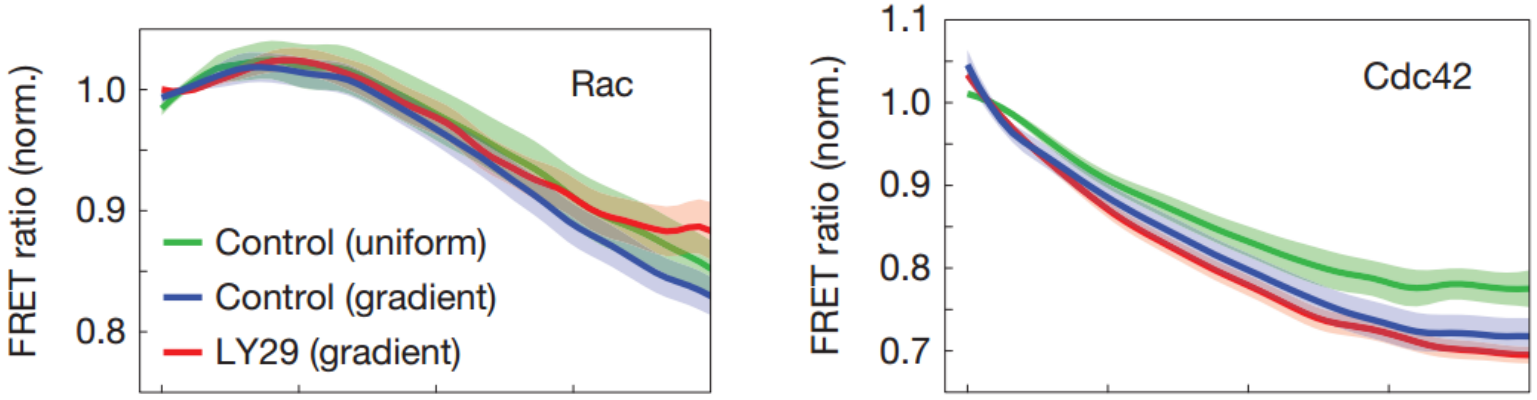
Tuning the Binding Affinities and Reversion Kinetics of a Light Inducible Dimer Allows Control of Transmembrane Protein Localization
Brian Kuhlman **Biochemistry 2016**



Engineered RhoGTPase gradients

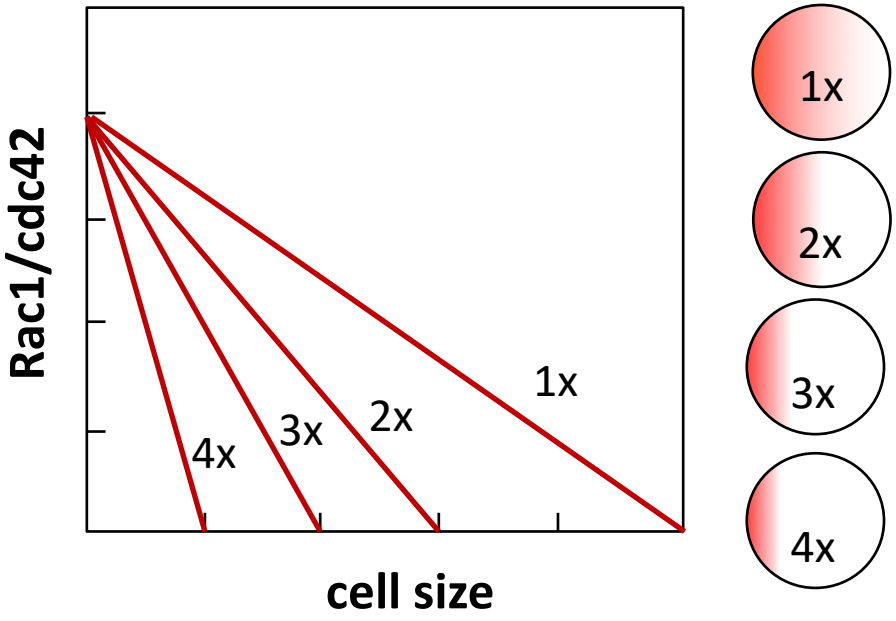
Shape of RhoGTPase gradients

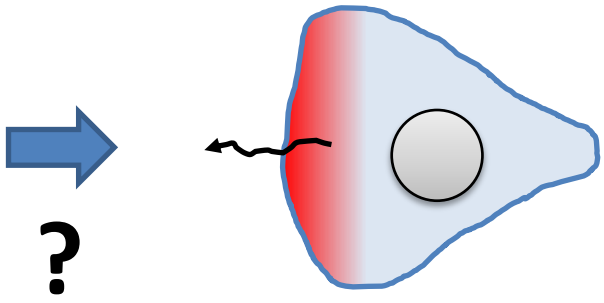


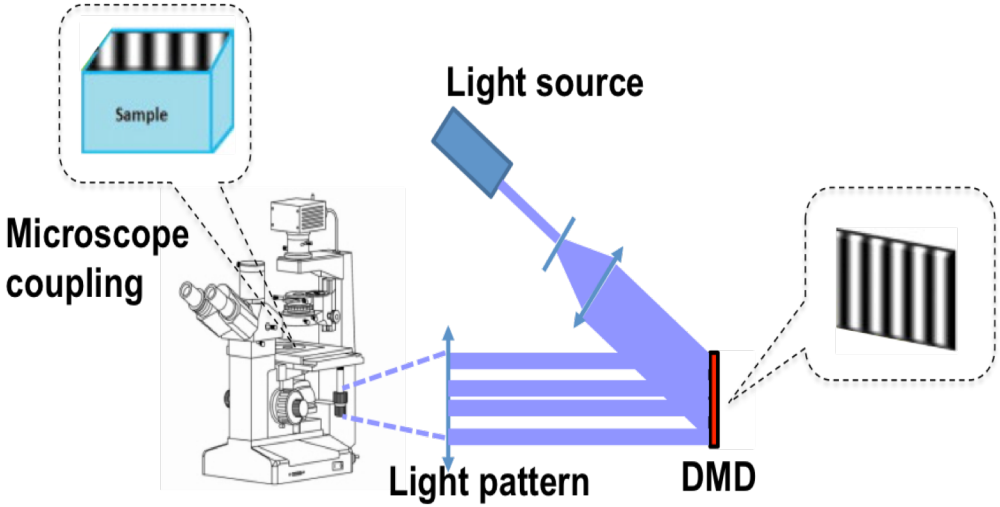


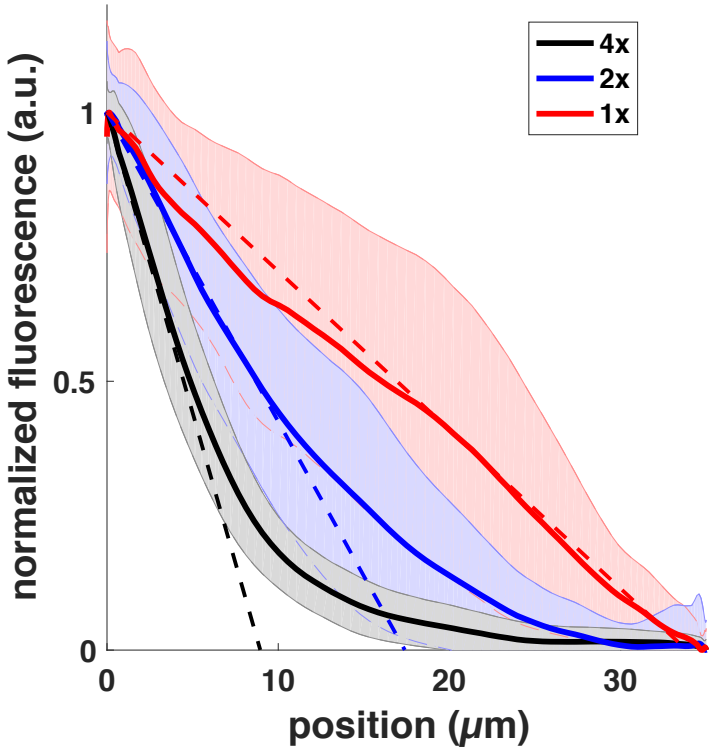
Yang et al., Nat Cell Biol 2015

Engineered gradients





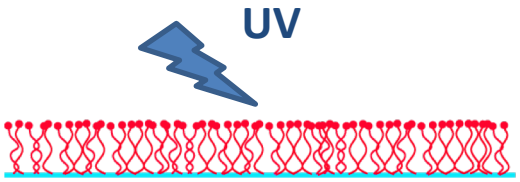




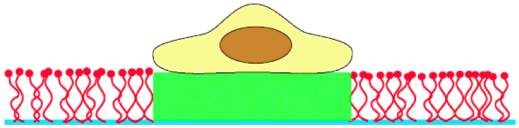
Controlled initial conditions

APP

Cell repellent coating

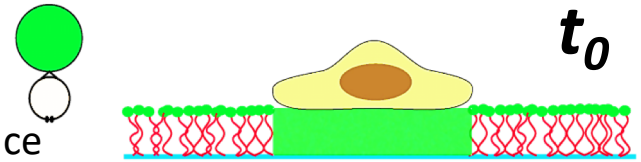


Isotropic confined cells

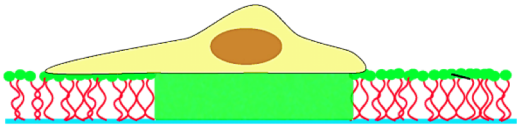


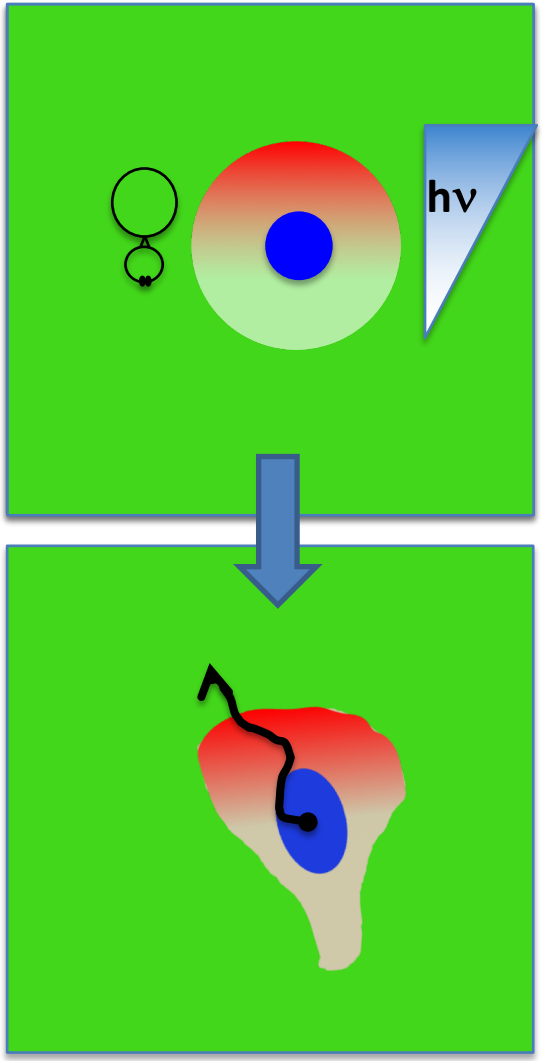
BCN-RGD

Fully adhesive surface



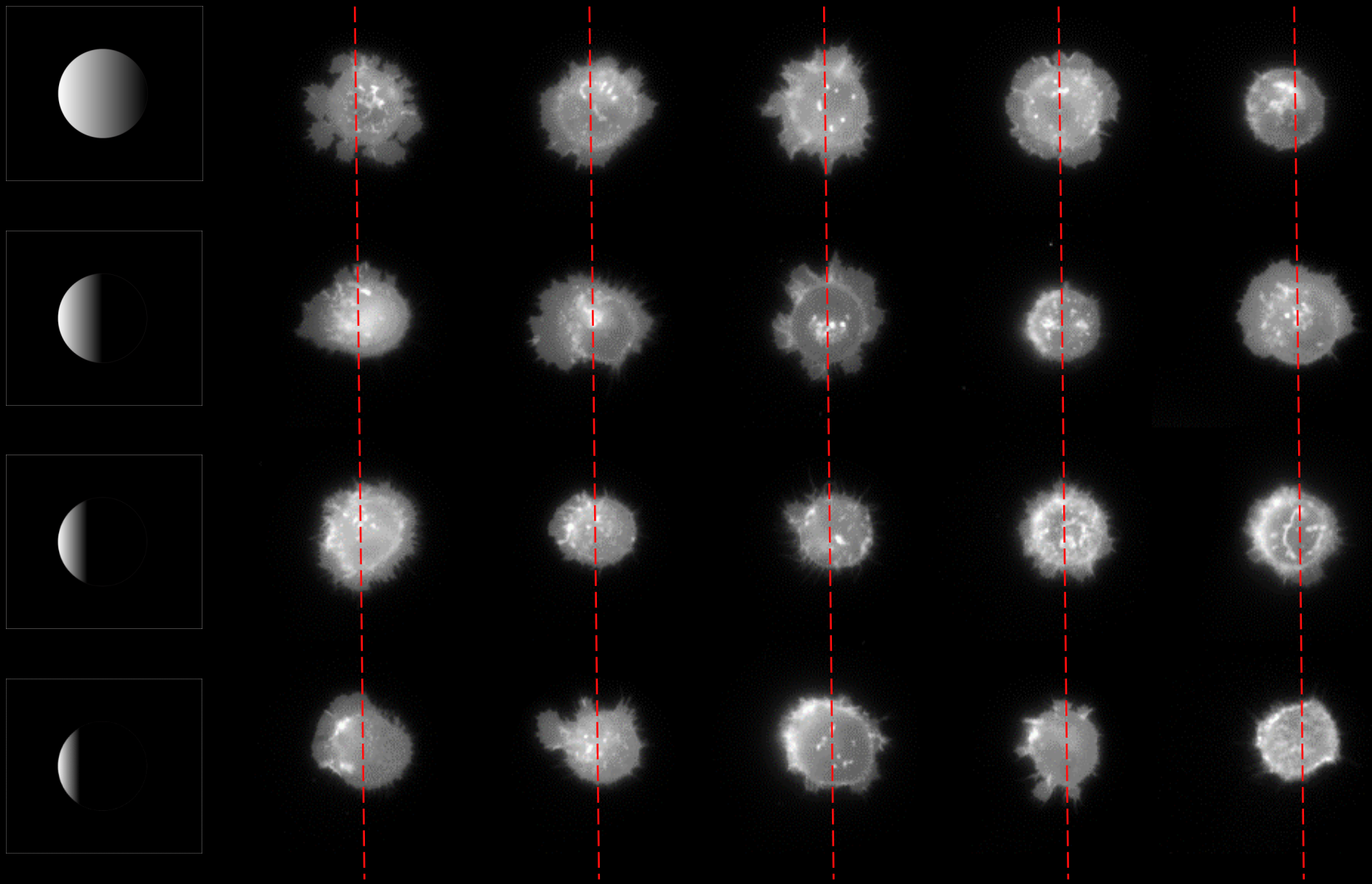
Released migration



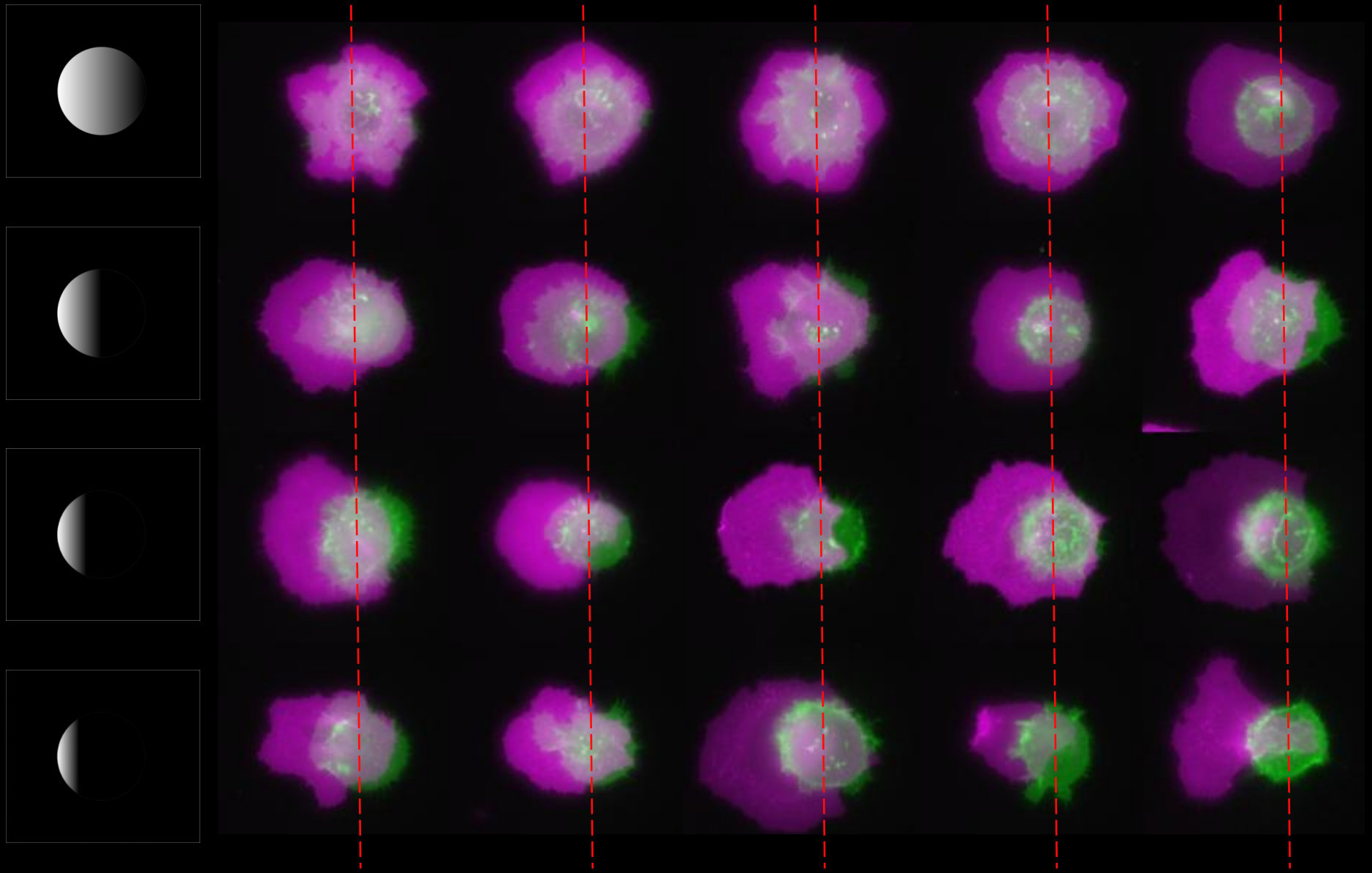


Adapted from Van Dongen et al. Advanced Material 2013

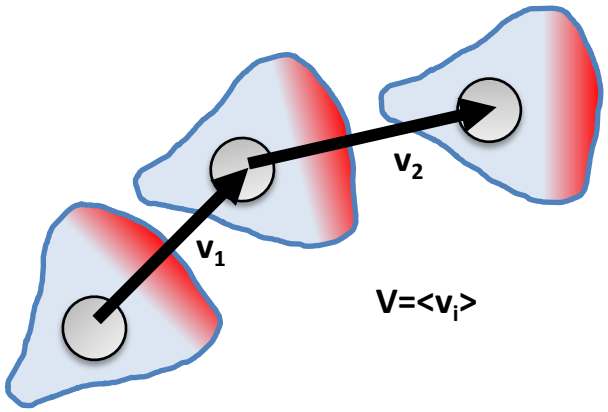
TIAM (Rac1)

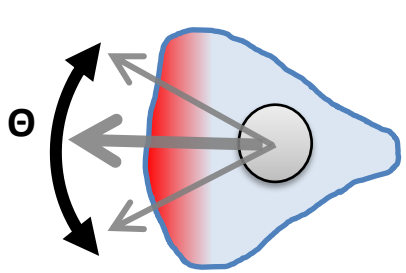


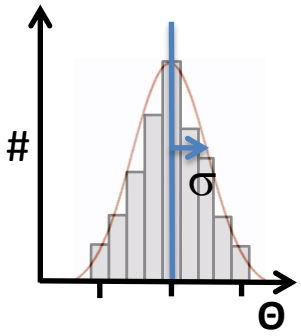
TIAM (Rac1)

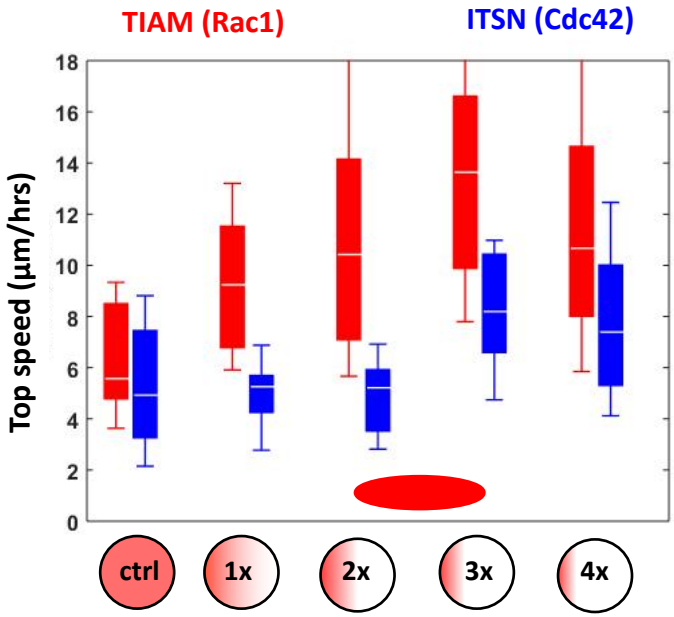


How gradients drive migration

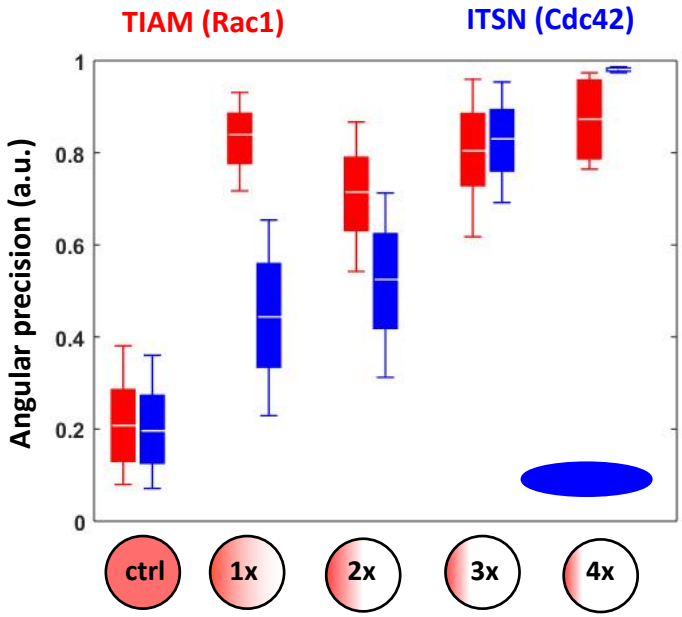






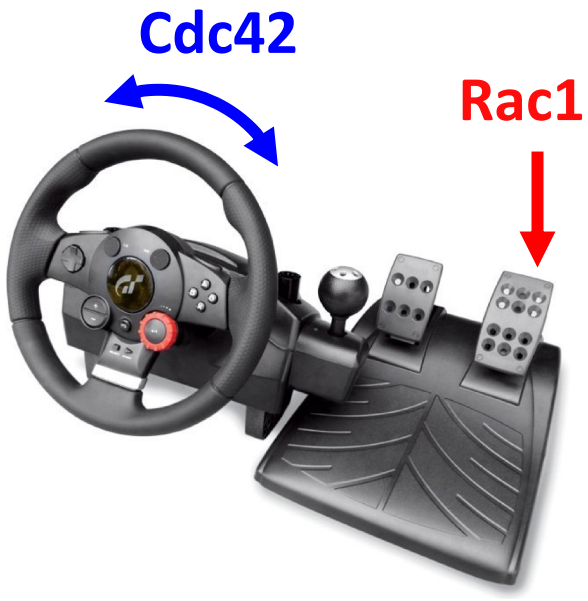


Rac1 → speed

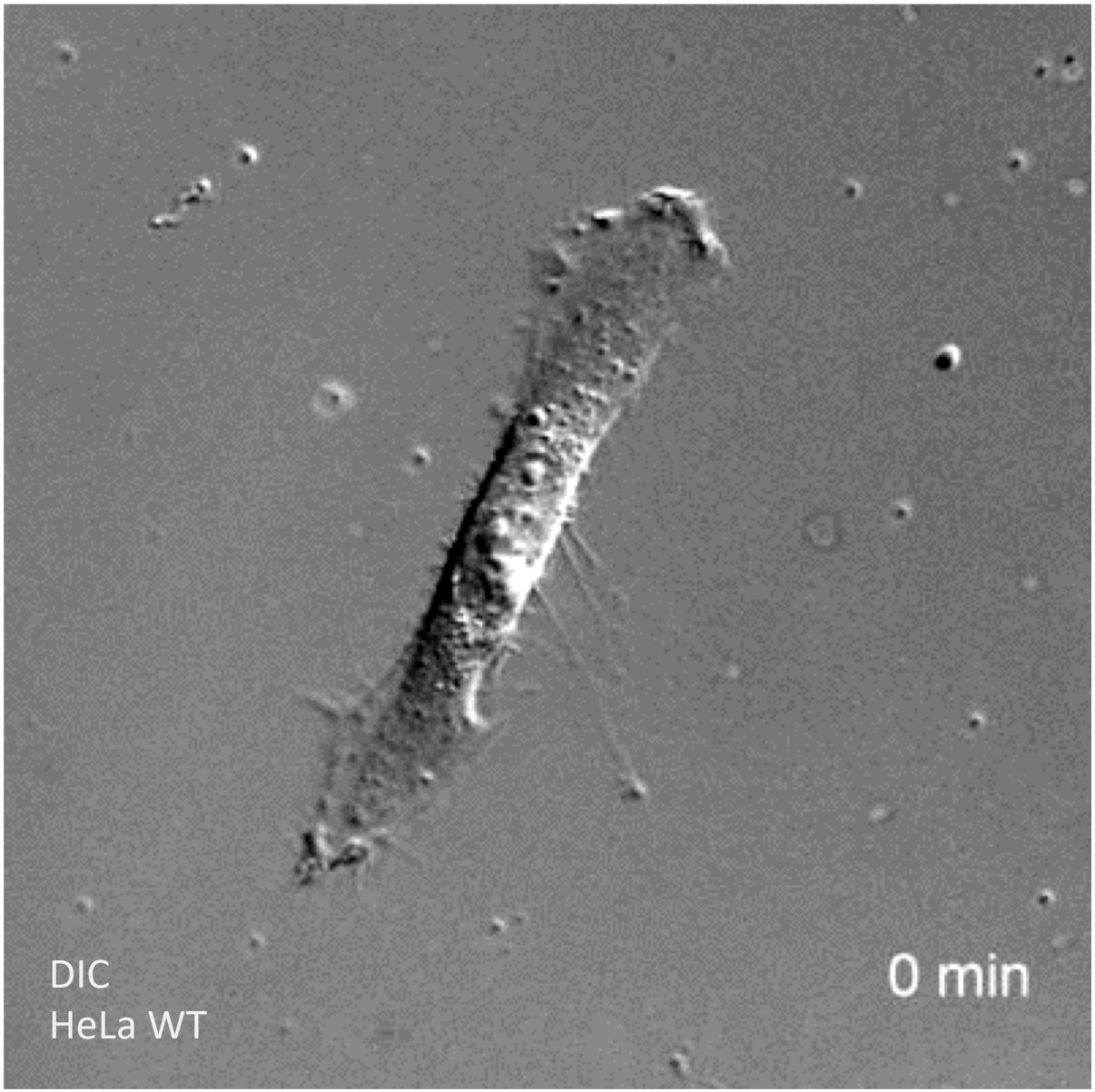


Cdc42 → orientation

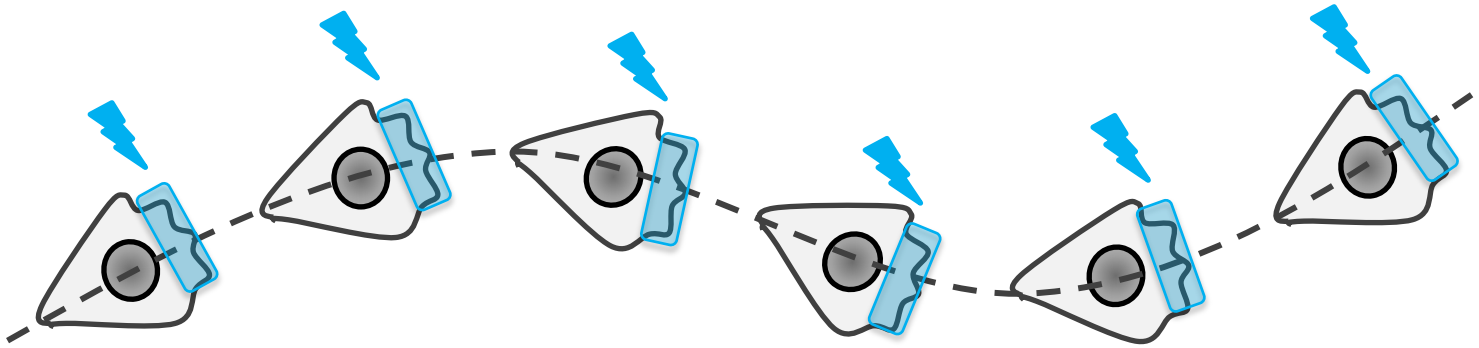
How gradients drive migration

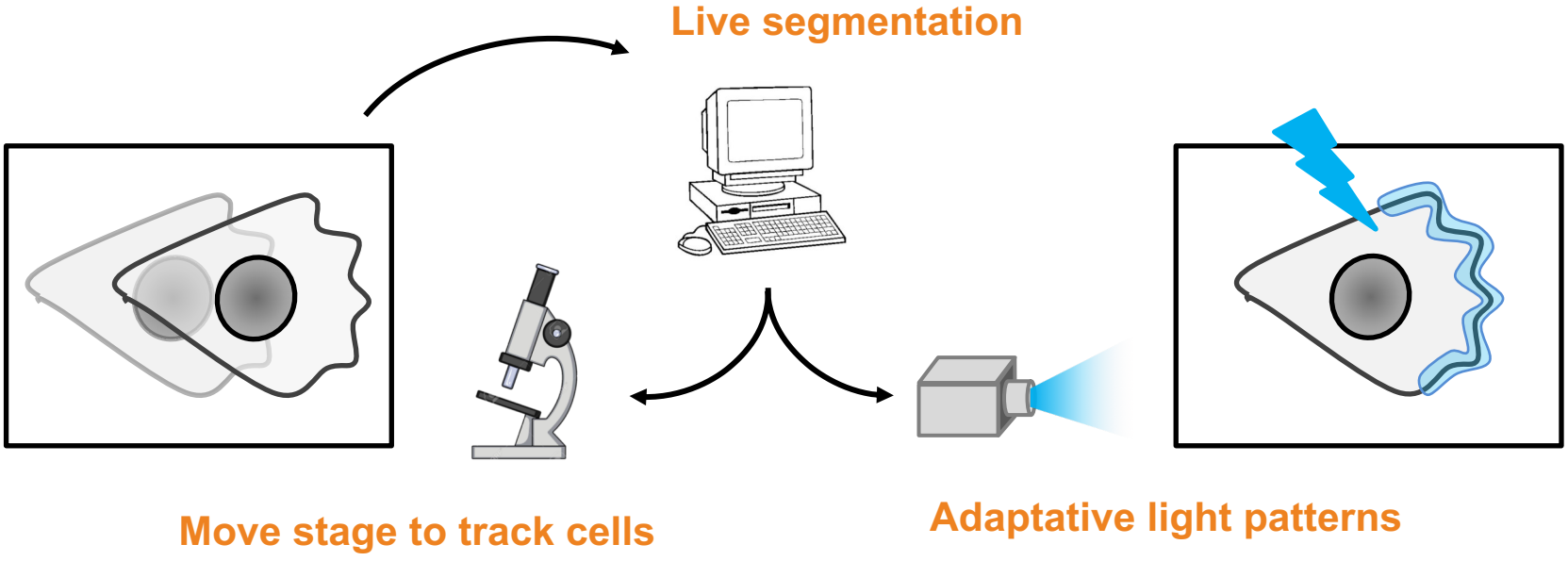


Eukaryotic random cell migration

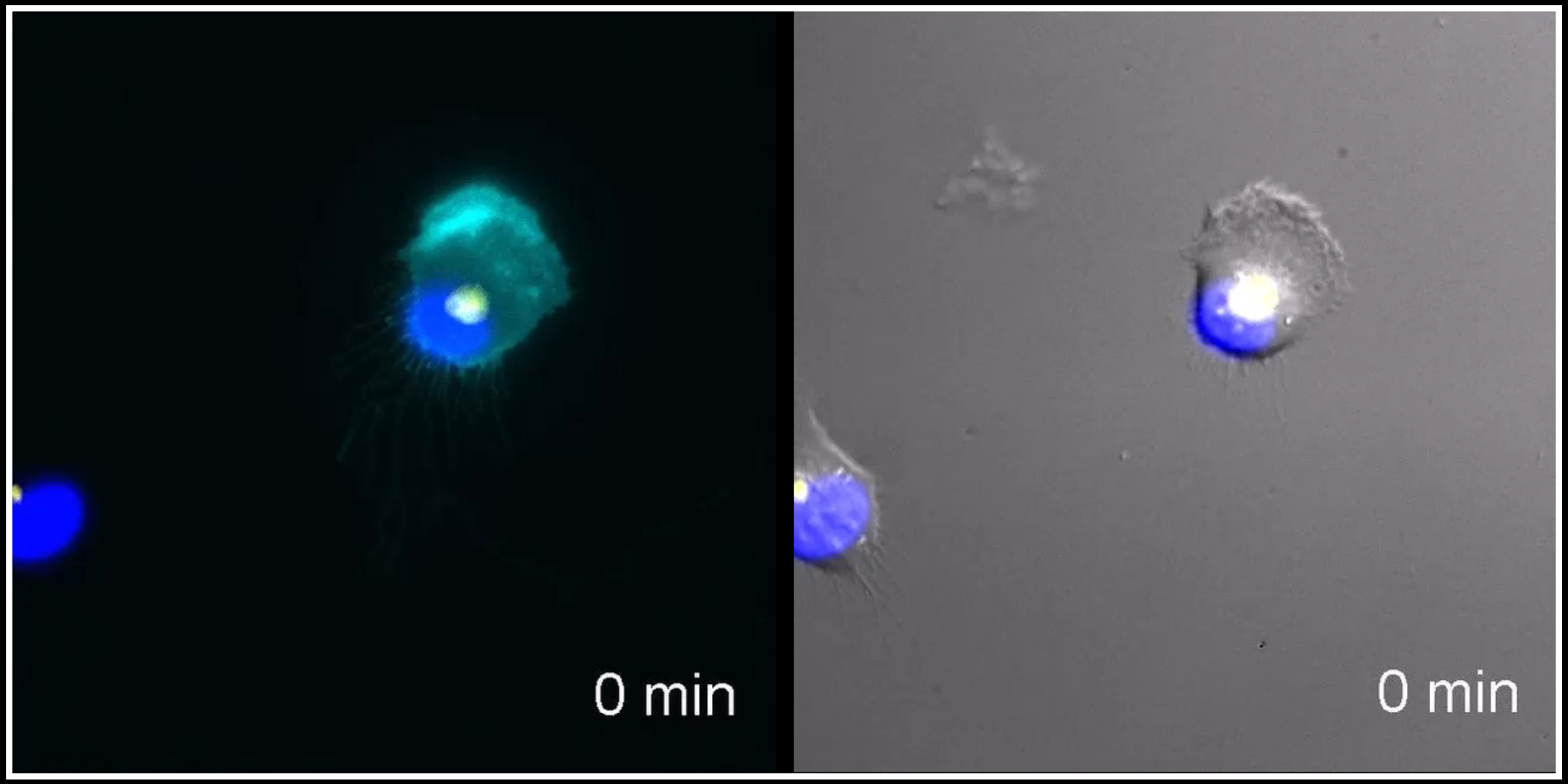


Ongoing: optogenetic feedback



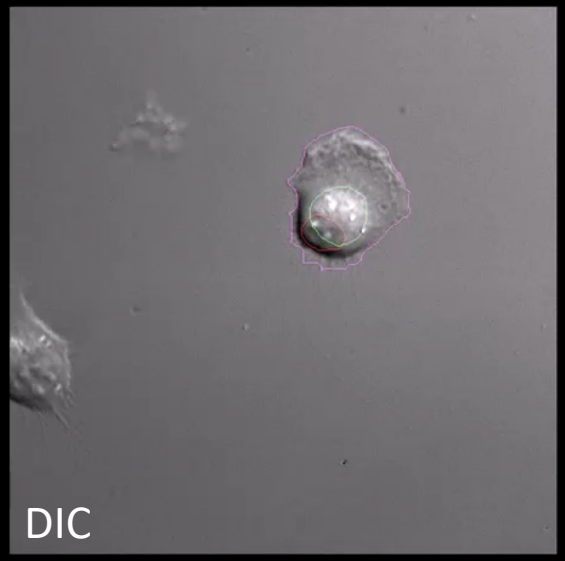


Live cell tracking and imaging



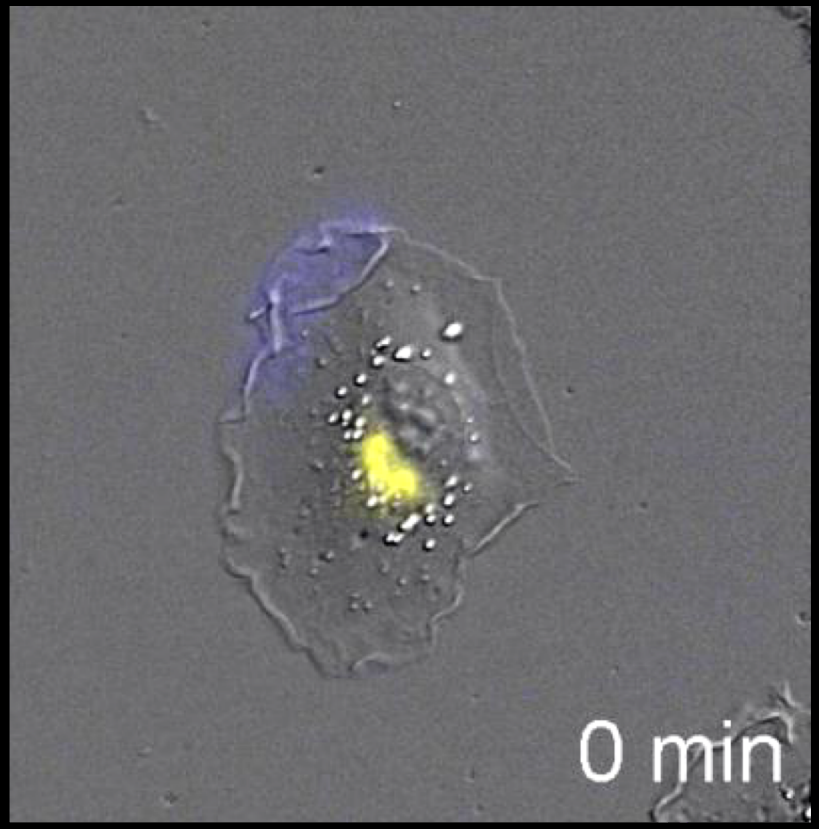
Membrane label - myr-iRFP
Golgi label - GFP-Rab6A
Nuclear label - Hoechst 33342

Live cell tracking and imaging

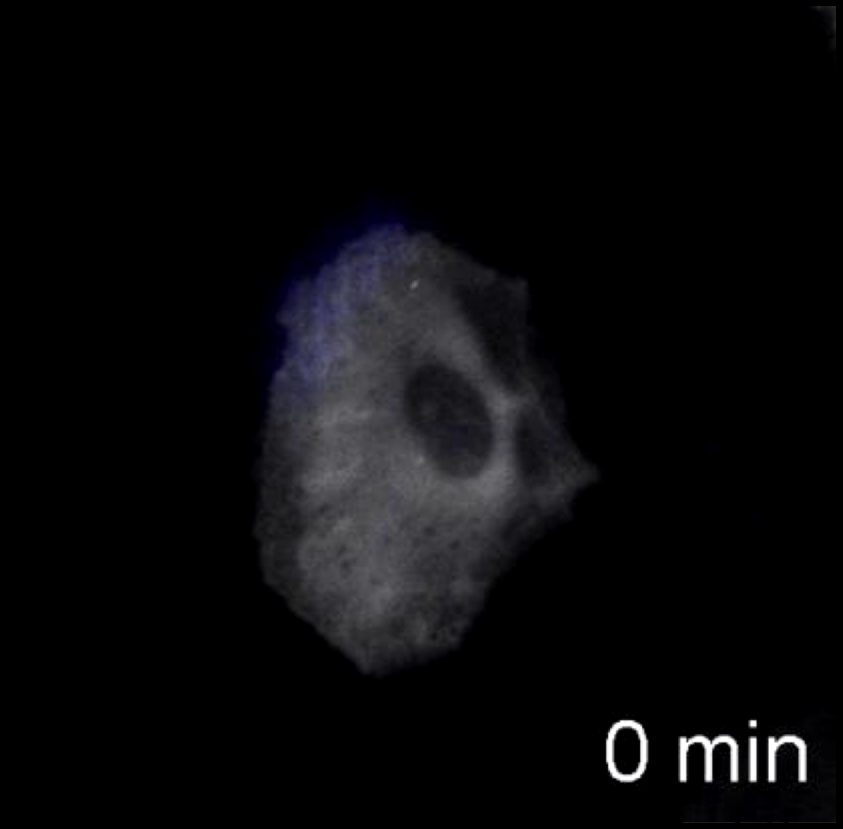


Duration - 15h in total

Imposed Cdc42 gradient reorient the Golgi

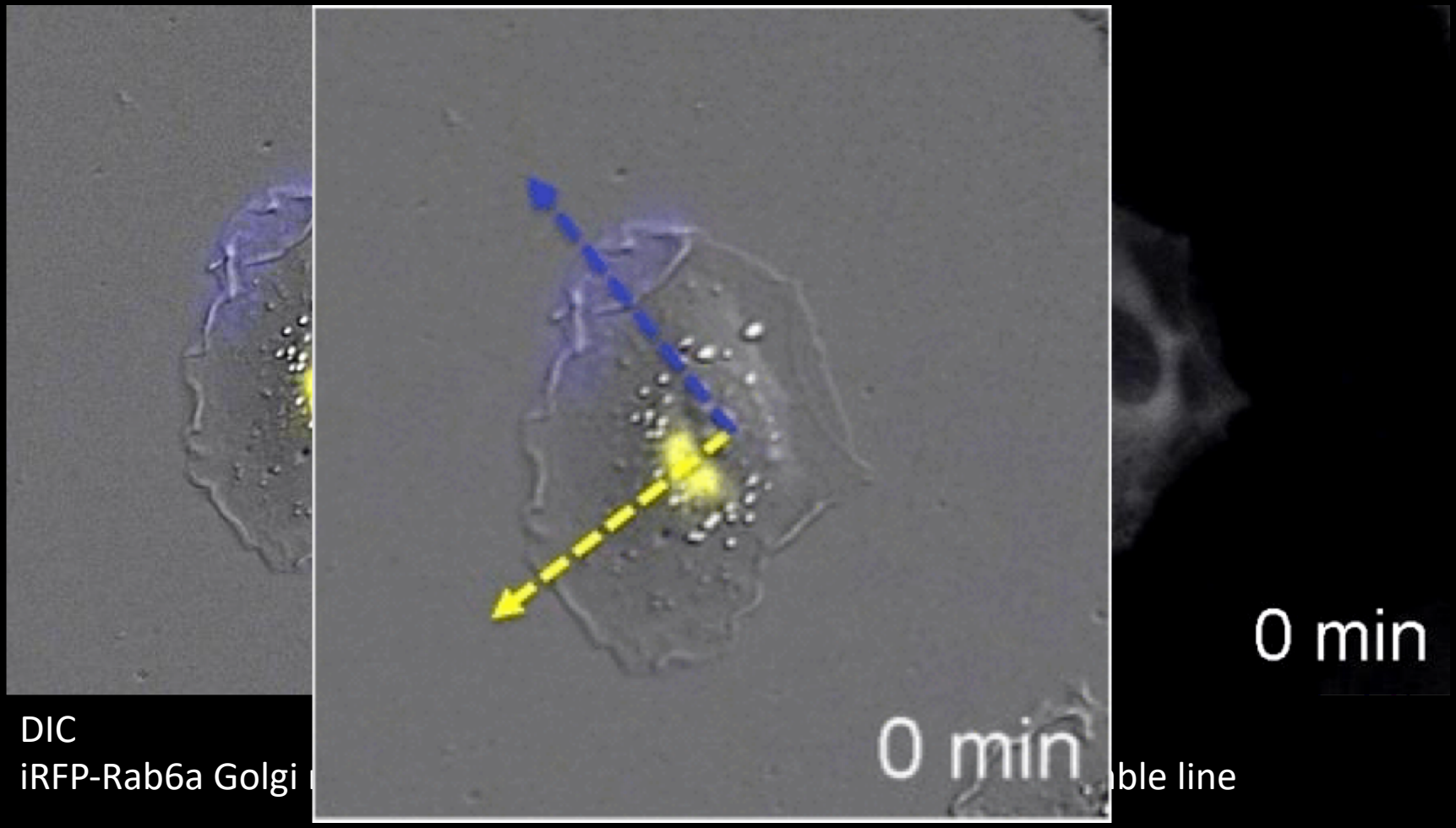


DIC
iRFP-Rab6a Golgi reporter



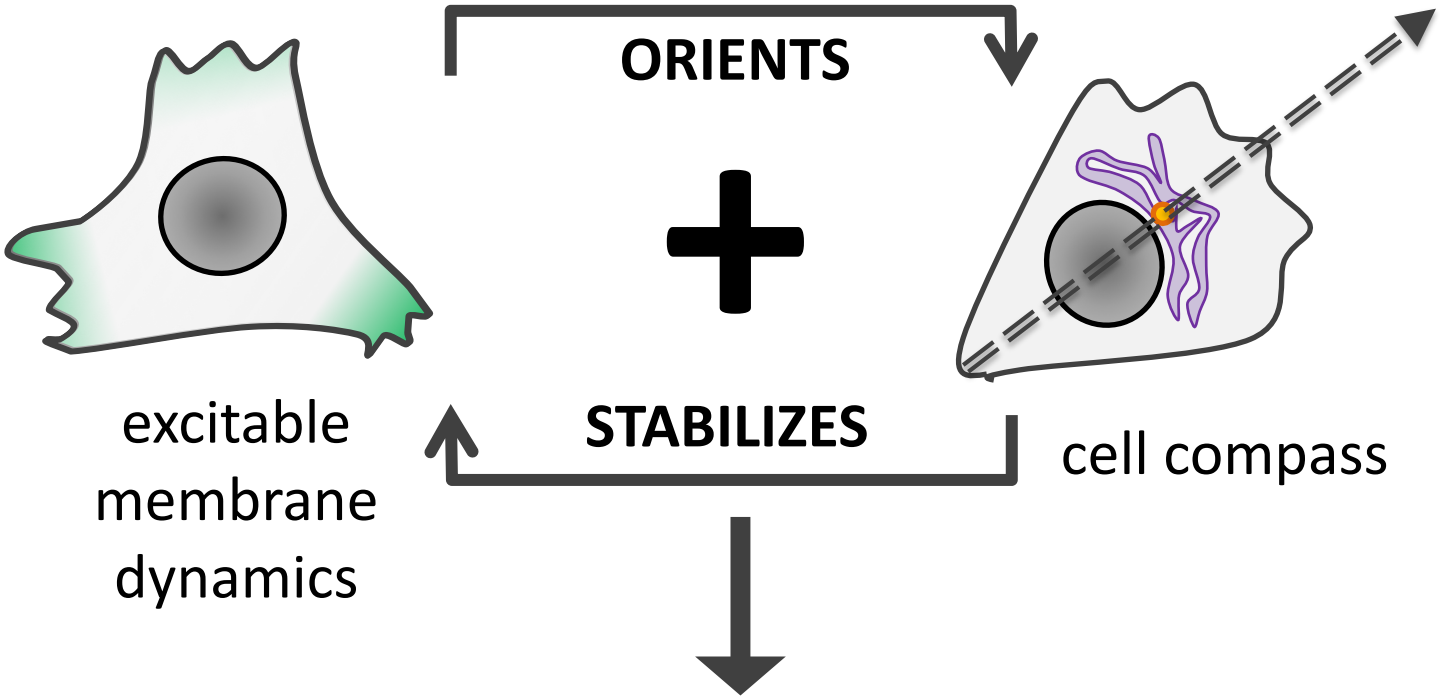
TIRF 561nm
RPE1-ITSNwt-iLID stable line

Imposed Cdc42 gradient reorient the Golgi



DIC
iRFP-Rab6a Golgi
ble line

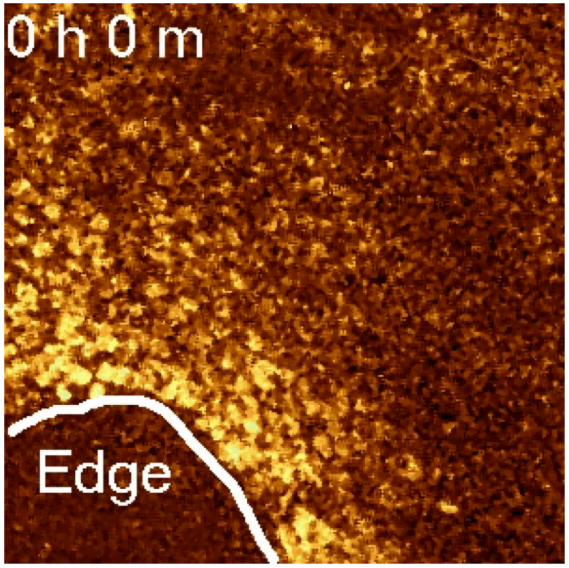
Current working model

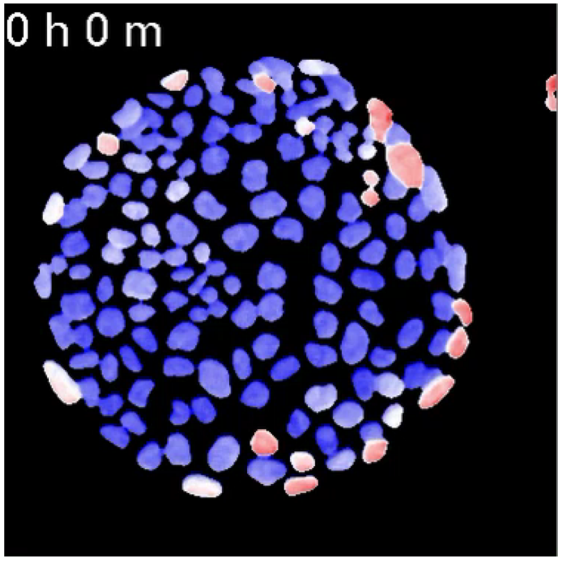


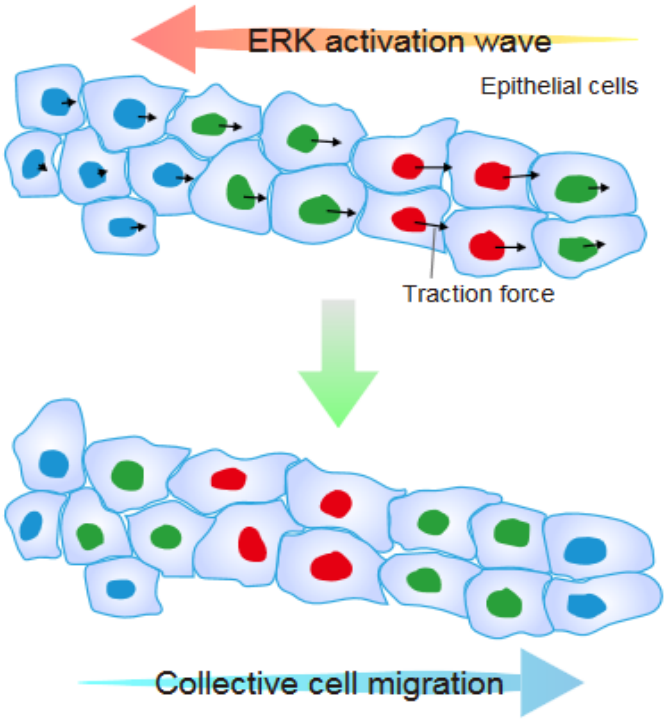
Oriented migration

This week project(s)

Project 4a: ERK signaling Waves

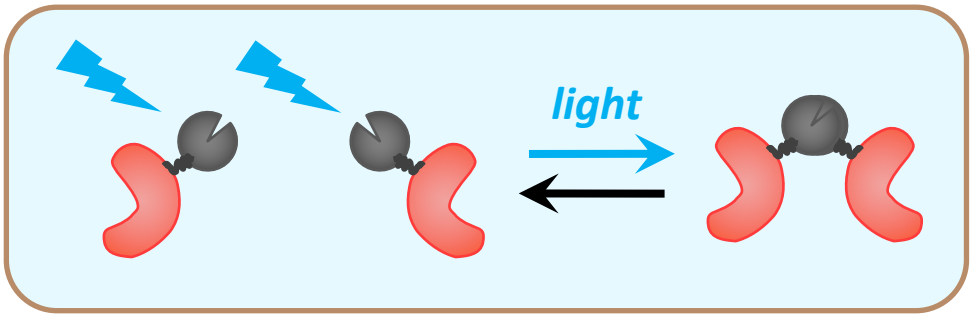




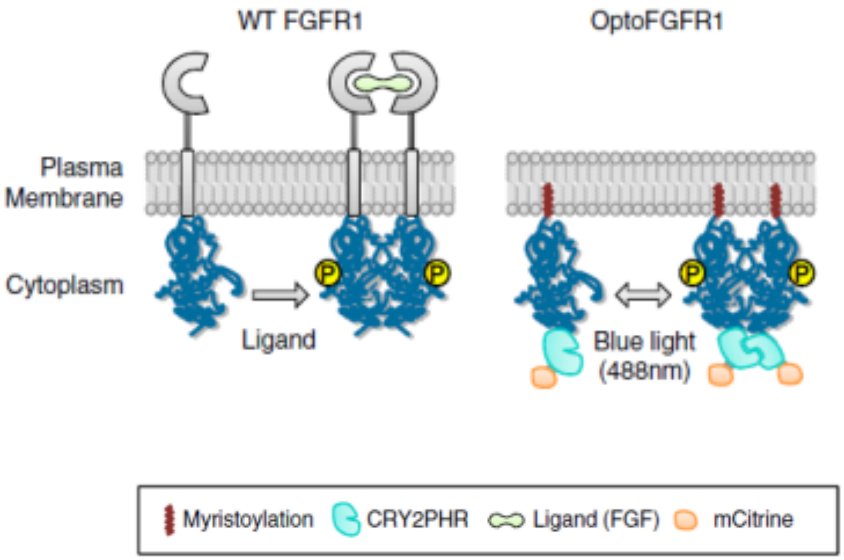


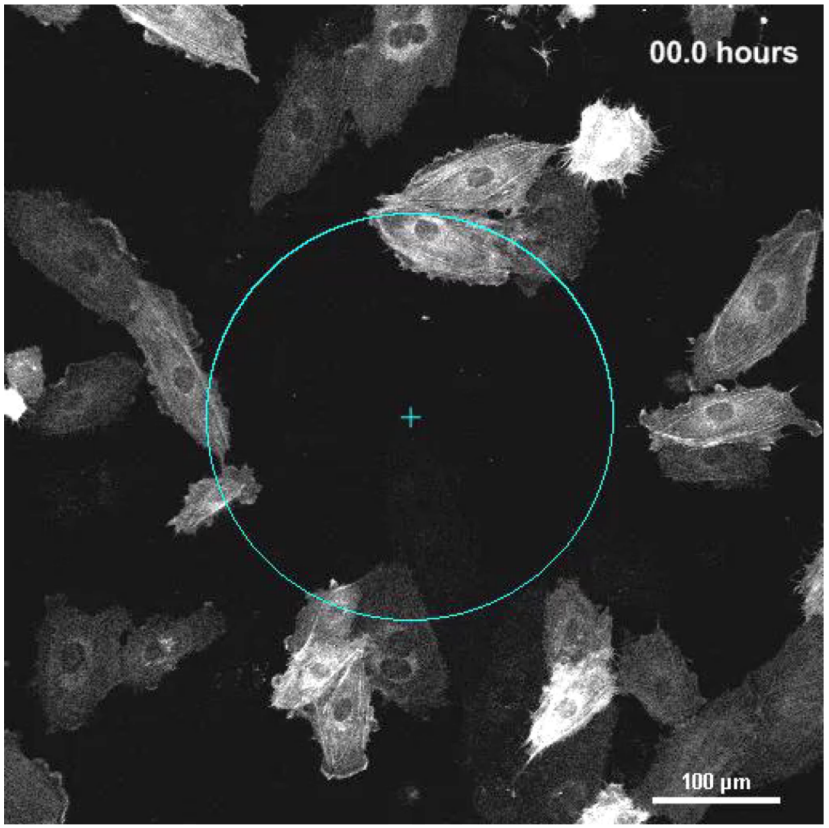
Aoki et al, **Dev Cell 2017**

Intracellular control of receptors



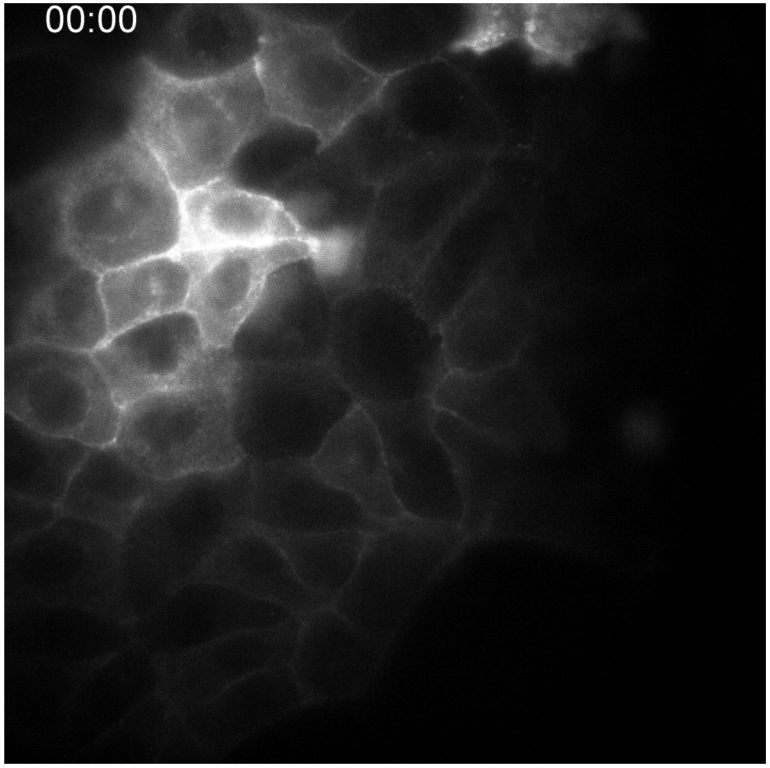
Spatiotemporal Control of Fibroblast Growth Factor Receptor Signals by Blue Light
Won Do He **Cell 2014**



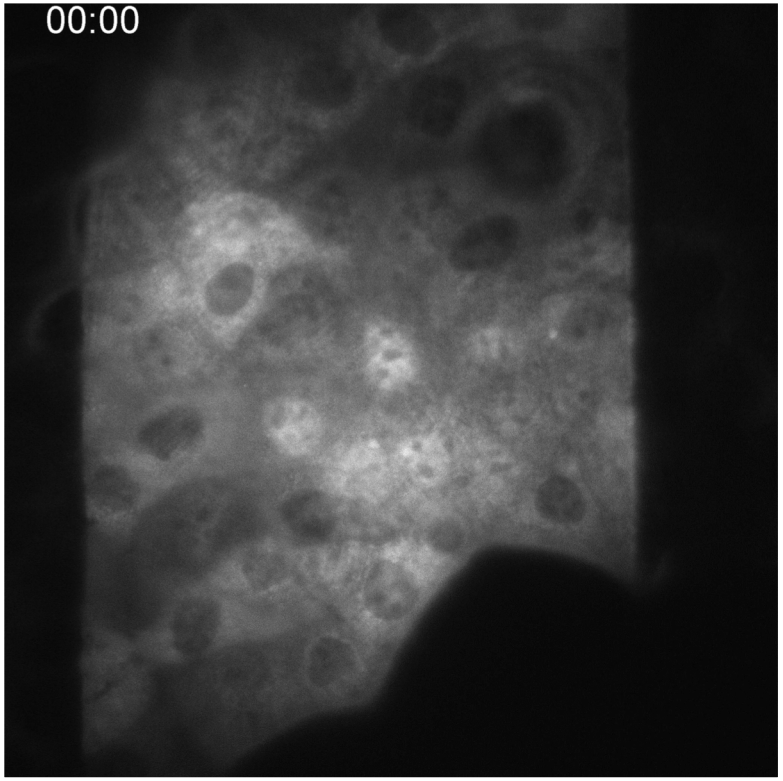


Engineered epithelial MDCK cells

optoFGFR1

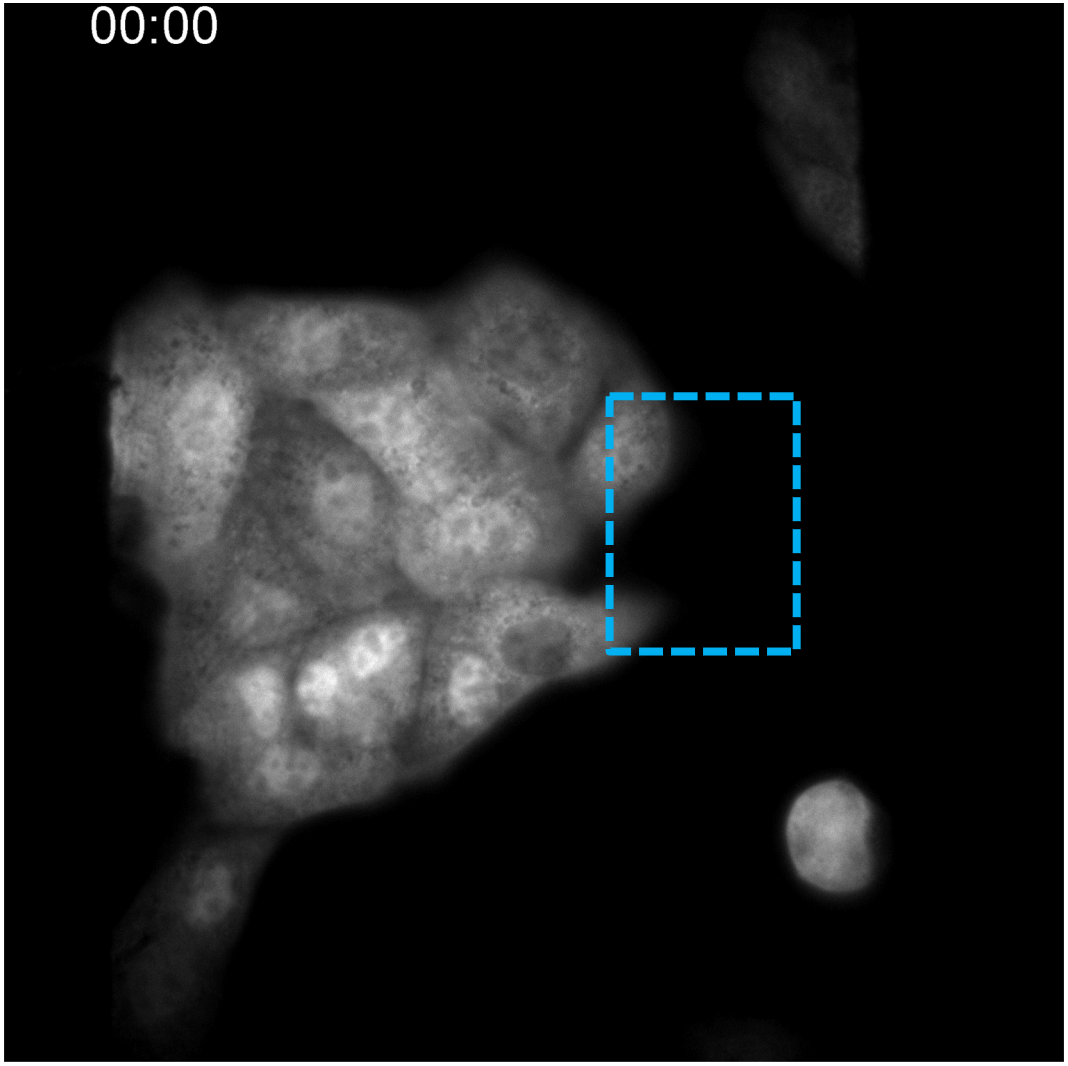


ERK biosensor



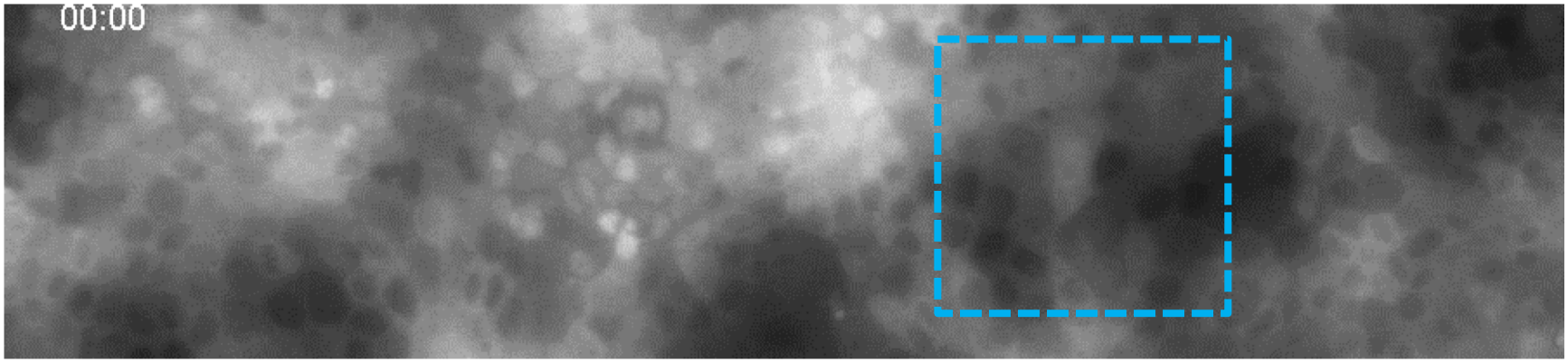
TIRFM

FGFR1 local activation induces ERK waves



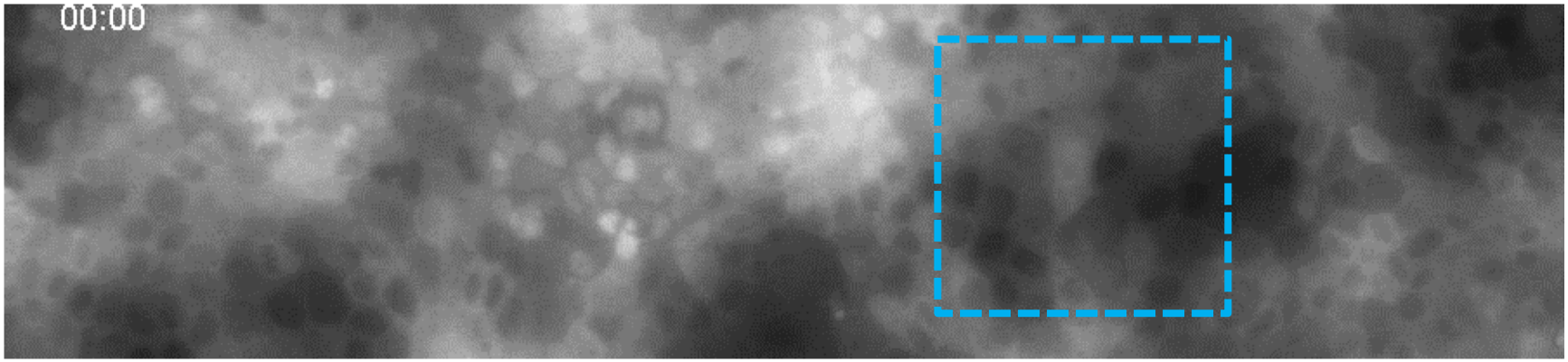
Possible aims

- **Analyze and dissect mechanisms of wave propagation**

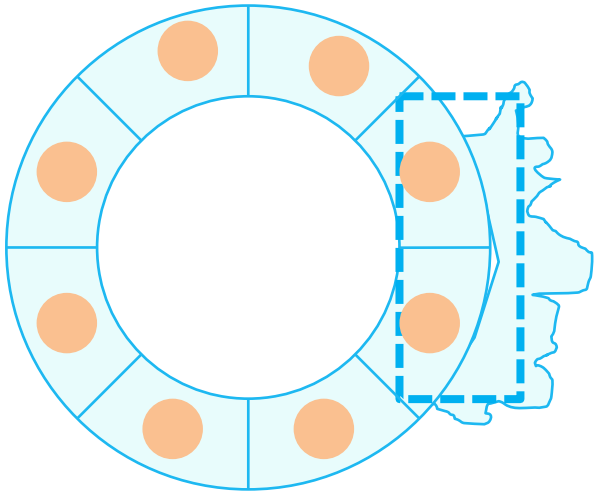


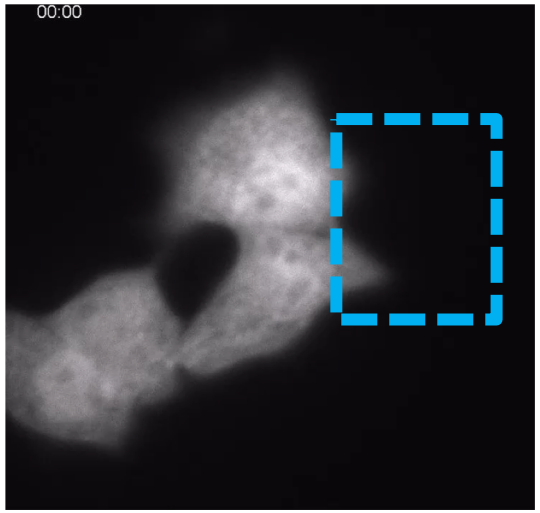
Possible aims

- **Analyze and dissect mechanisms of wave propagation**



- **Induce EMT/Gastrulation in cyst**





LOC²O: Light-based Observation and Control of Cell Organization

Bassam Hajj & Mathieu Coppey

Alicia Damm
Aude Battistella
Elie Balloul
Jean De Seze
Koceila Aizel
Kotryna Vaidžiulytė
Laura Caccianini
Laurence Vaslin
Lorena Kolar-Znika
Maud Bongaerts
Mohamed El Beheiry
Tommaso Galgani
Veer Keizer

Leo Valon (ex PhD)

Simon De Beco (ex Pdoc)

Kotryna Vaidžiulytė (PhD)

Collaboration

Kristine Schauer Curie UMR144