OIC Course, Functional Imaging and Super Resolution 2026, Optogenetics

From observing to controlling biology with light



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Erasmus MC

Why optogenetics?

Rapid, reversible and spatial control

- To study highly dynamic systems (e.g. cytoskeleton, cell migration, mitotic spindle)

  (KO is slow compared to the timescale at which MT dynamics are controlled.. Cells can adapt)

  - Where in the cell is a certain activity required?
  - When is a certain protein activity required?
  - Timed perturbation of essential protein activities (situations where KO = lethal)

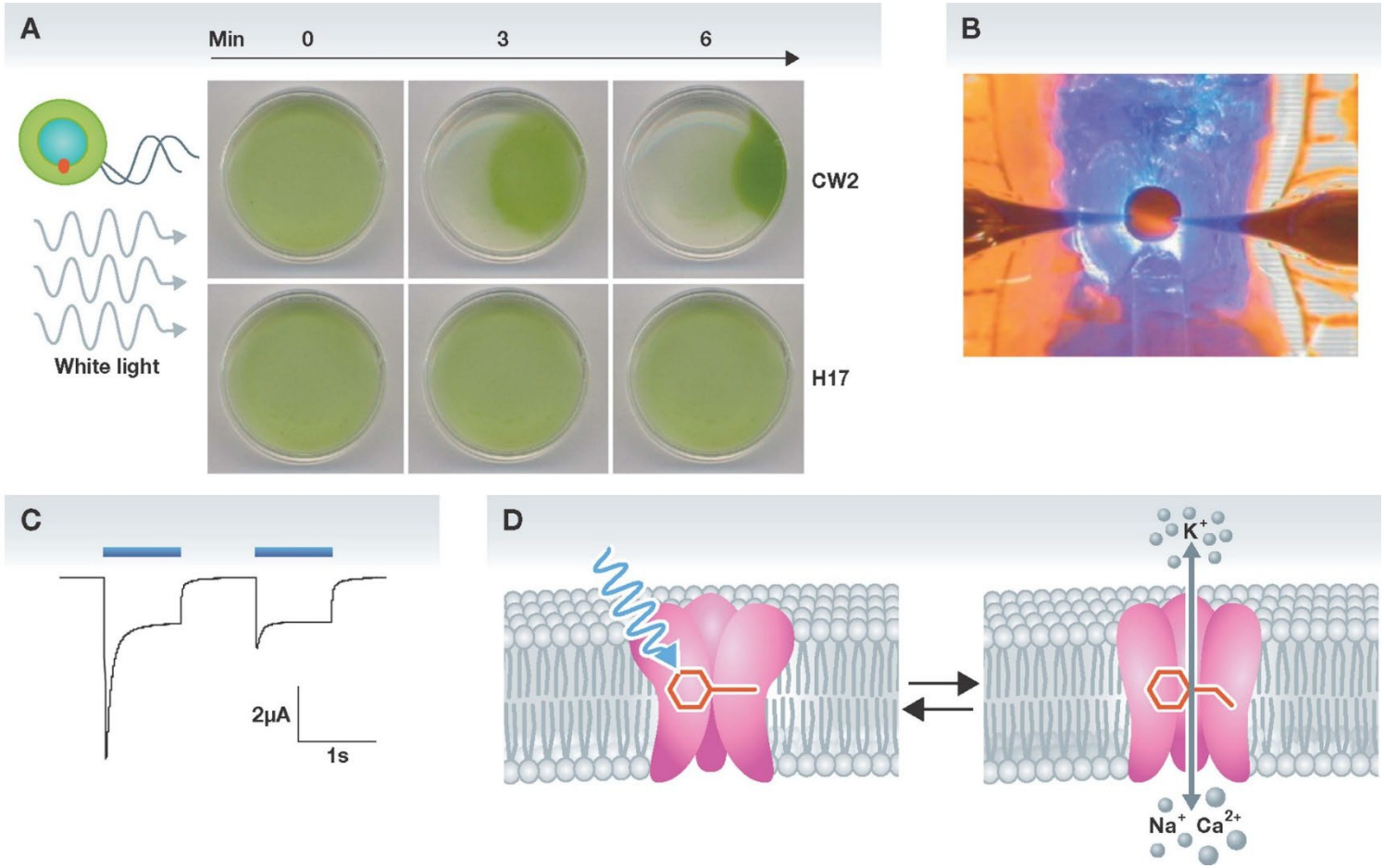
- Tissue engineering purposes

local cell fate specification, polarization, cell adhesion/sorting, tissue folding, directed cell movement

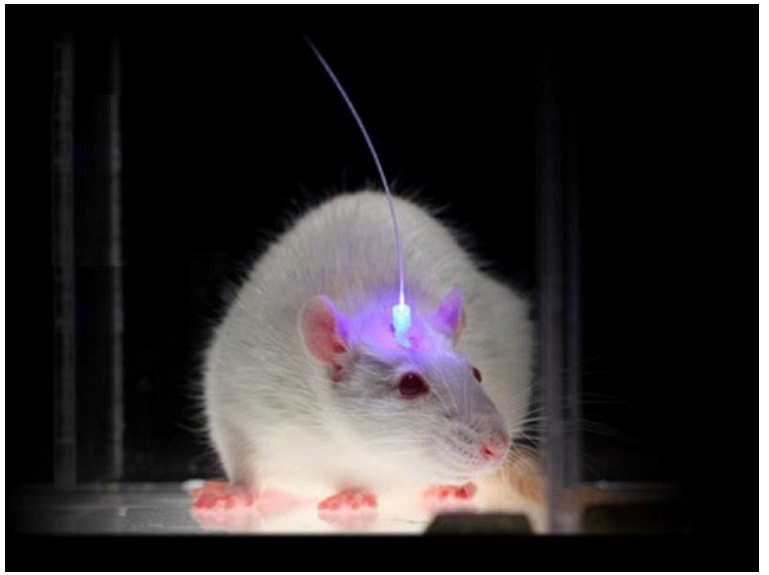
Classical Optogenetics, light controlled ionchannels to control neuronal activity

Chlamydomonas: motile unicellular green algae

Channelrhodopsins clustered in eyespot, allows Chlamy to detect light and move towards or away from it…



Opsins, light controlled ionchannels



Deisseroth lab

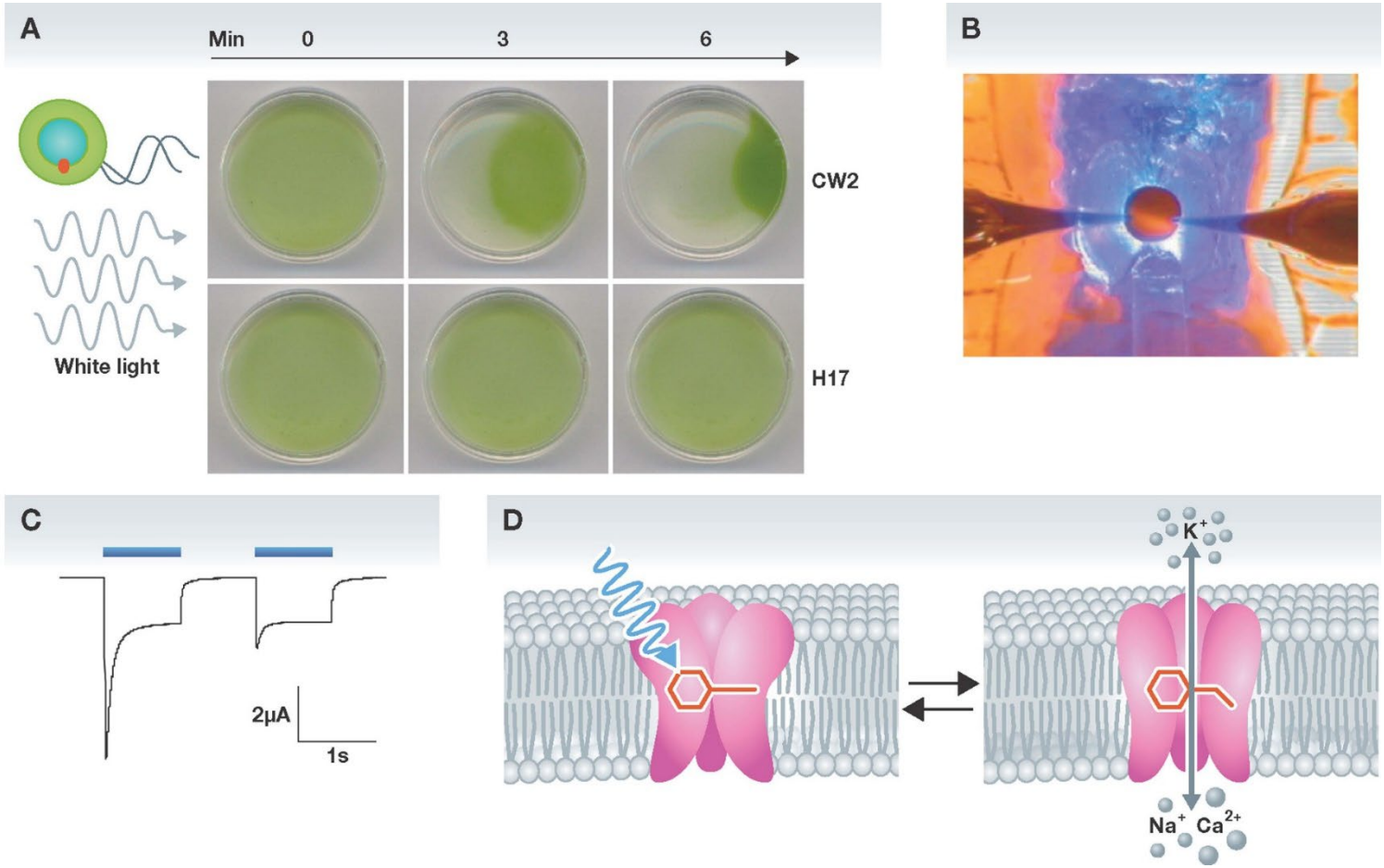
From channelrhodopsins to optogenetics, Hegemann and Nagel, EMBO 2013

The form and function of channelrhodopsin, Deisseroth and Hegemann, Science 2017

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Chlamydomonas: motile unicellular green algae

Channelrhodopsins clustered in a so called eyespot, allow Chlamy to detect light and move towards or away from it…



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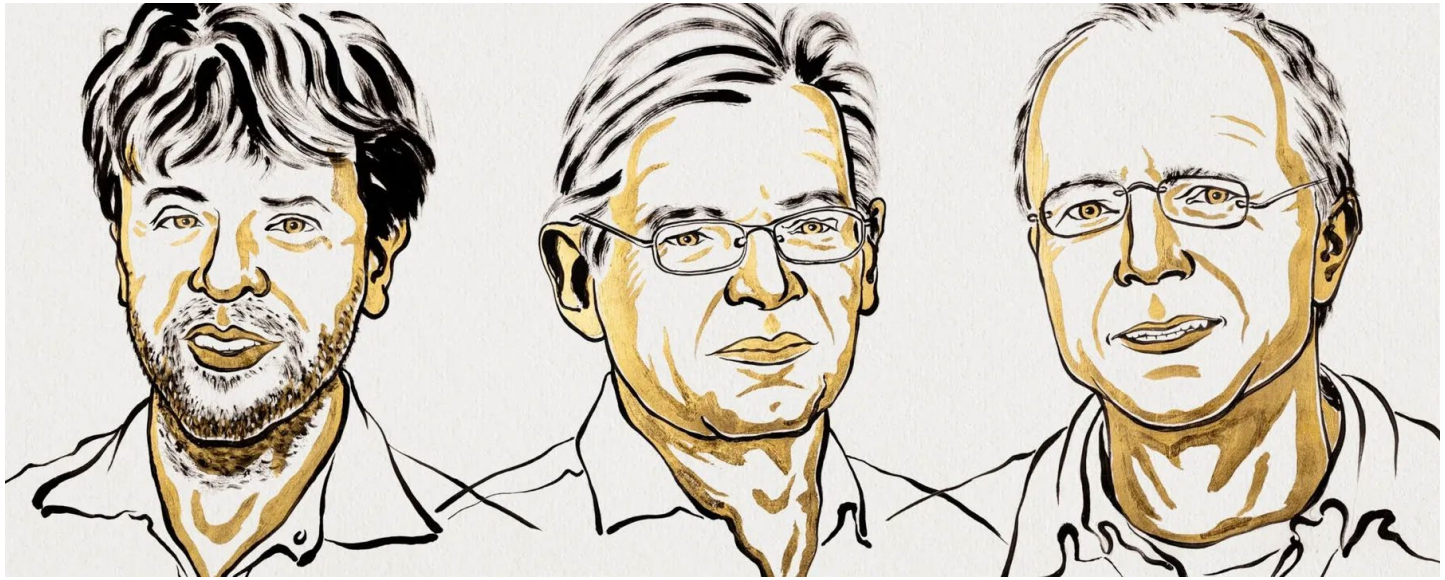
Opsins, light controlled nematodes



ChR2 expr. cholinergic motor neurons

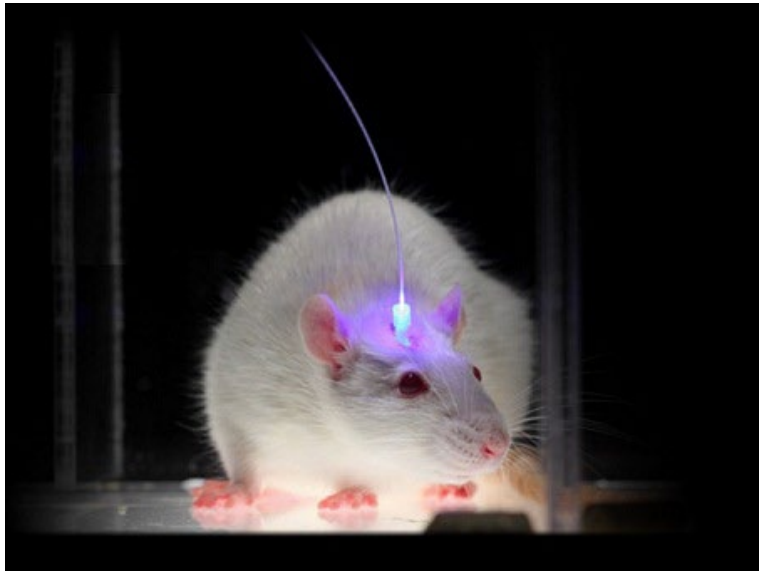
Stirman, J., Crane, M., Husson, S. *et al.* Real-time multimodal optical control of neurons and muscles in freely behaving *Caenorhabditis elegans*. *Nat Methods* **8**, 153–158 (2011). https://doi.org/10.1038/nmeth.1555

Light-controlled ion channels to control neuronal activity



Karl Deisseroth, Peter Hegemann, Georg Nagel

Opsins, light controlled ionchannels



Deisseroth lab

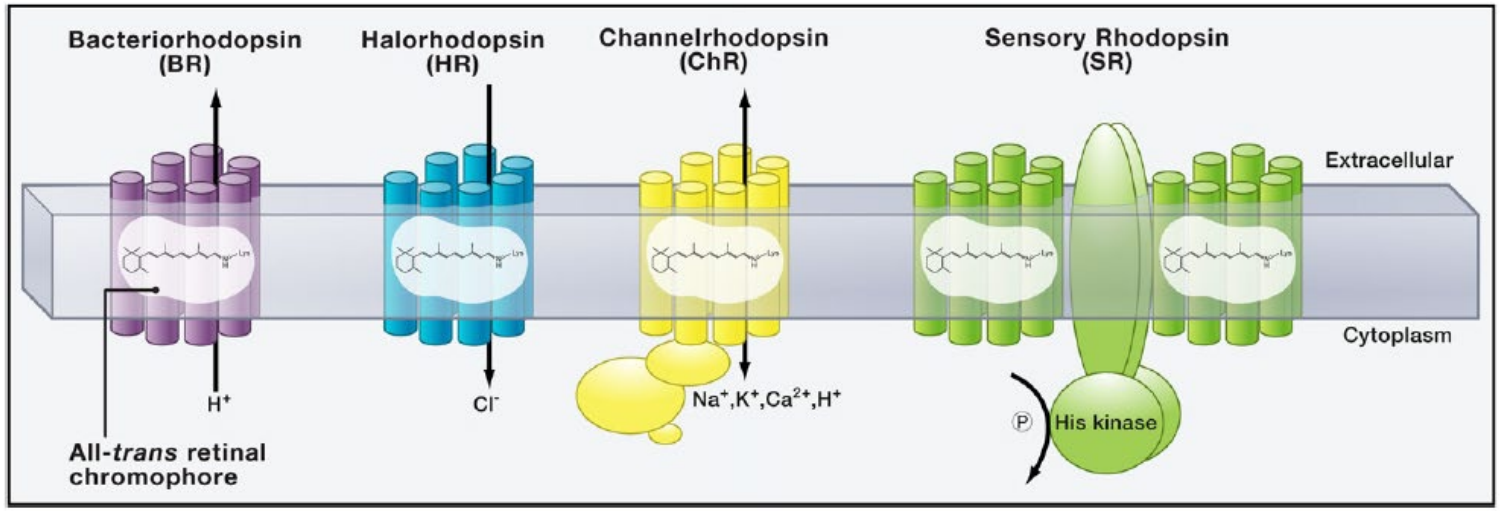
2026 Nobel Prize in Physiology or Medicine “for their discoveries concerning light-gated ion channels and optogenetics”

Many different light activated ion channels are found in nature



Halobacterium salinarium
(halophilic marine archaeon)

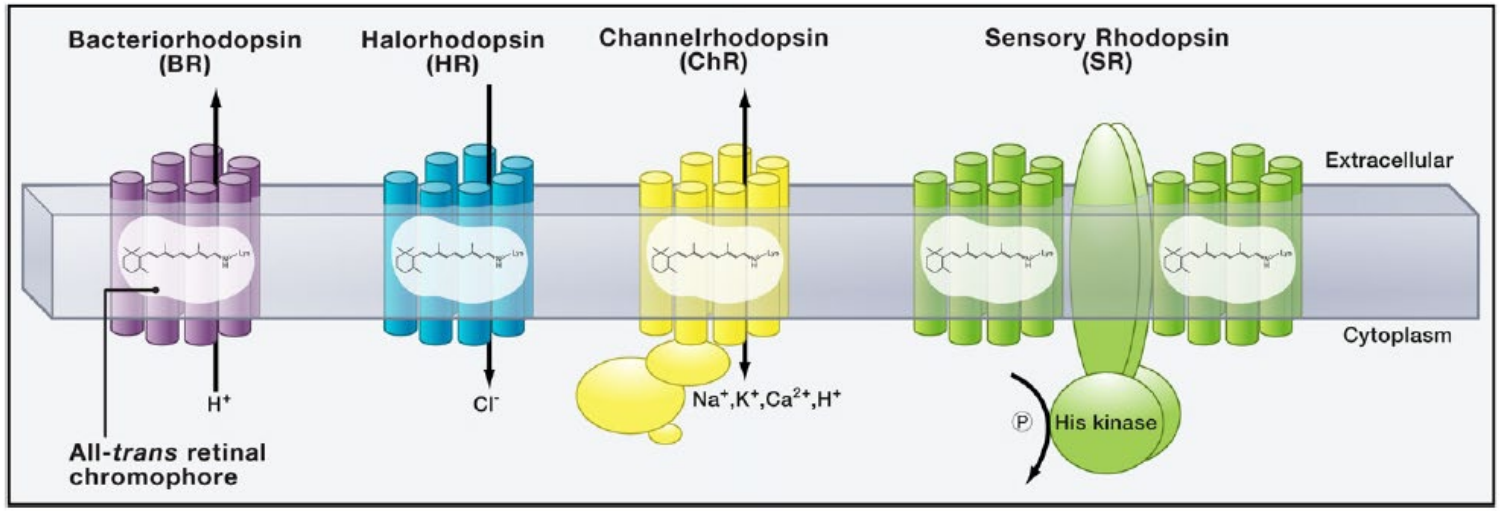
Extremophiles: abundant source of light responsive ionchannels



Many different light activated ion channels are found in nature



Halobacterium salinarium
(halophilic marine archaeon)



Very powerful tools!

But of limited use for cell biologists….. We want to control our favorite protein/pathway of interest…

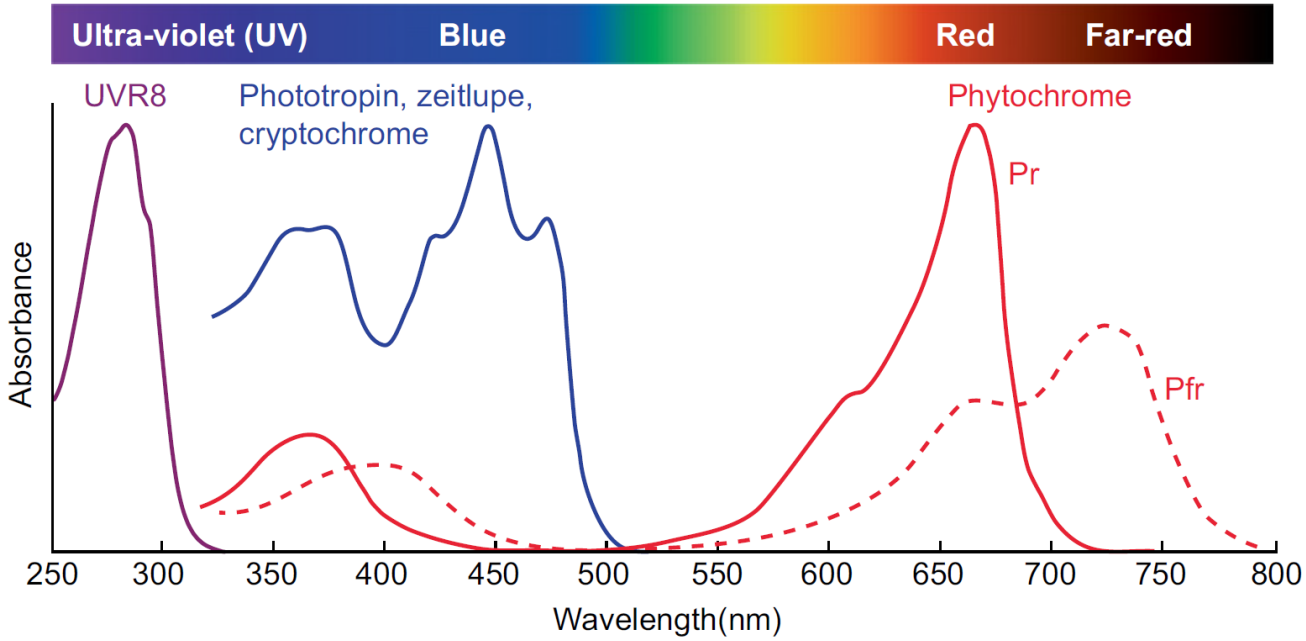
From plant light receptor to optogenetic switch

Phototropism



mixed vegetables growing in our kitchen
(J.V.H, A.R)

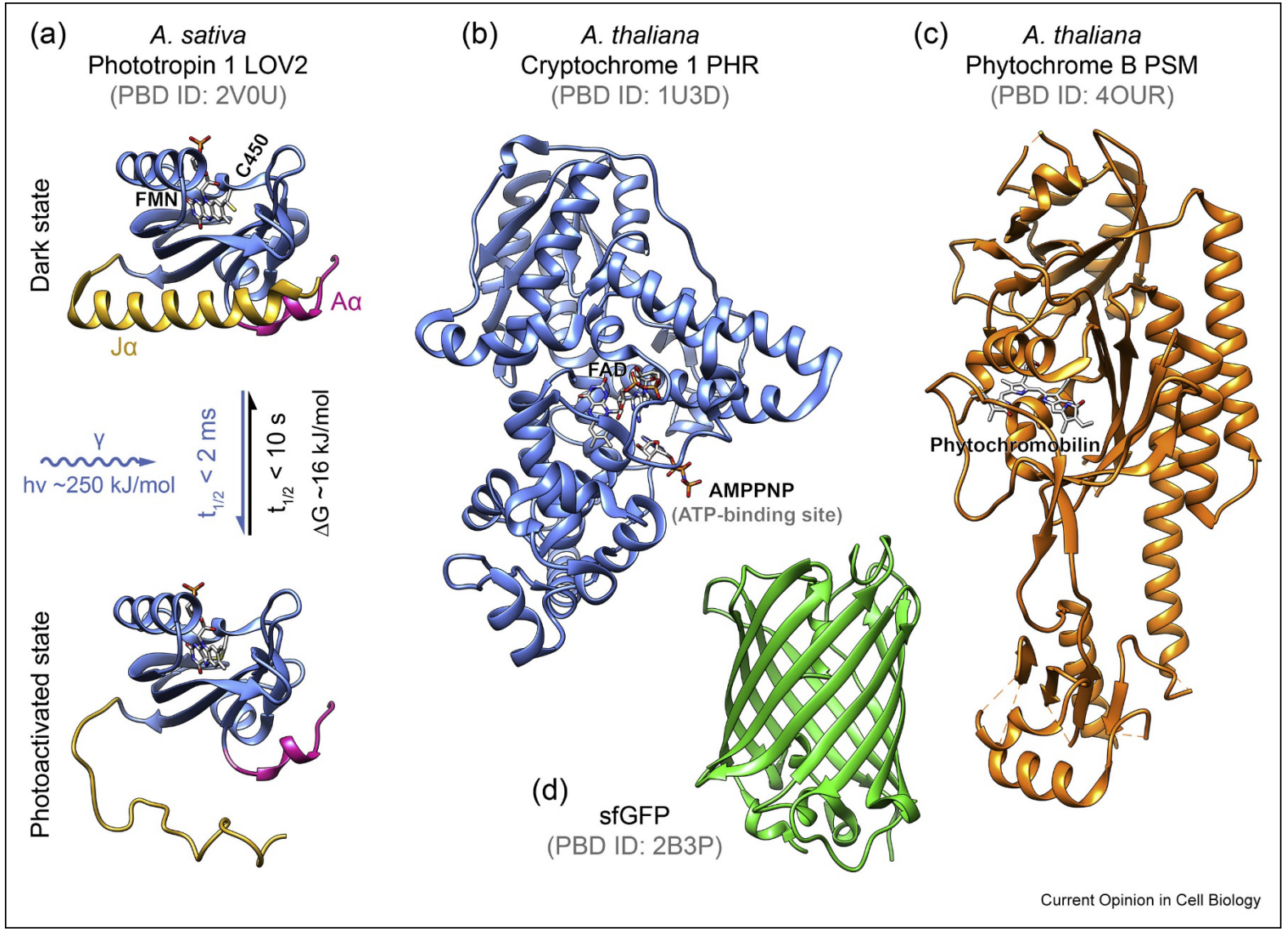
Absorbance spectra of various plant light receptors



Plant light receptors mediate many aspects of plant life:
e.g. phototropism, heliotropism, flowering, chloroplast positioning, leaf positioning

Christie J.M., Zurbriggen M.D. Optogenetics in Plants, New Phytologist 2020, https://doi.org/10.1111/nph.17008

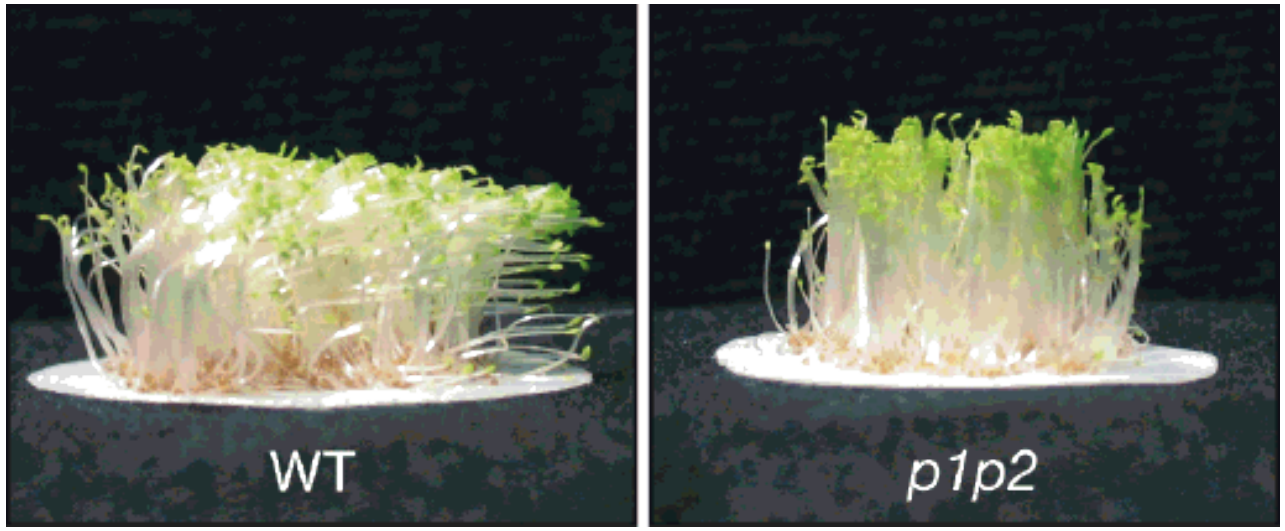
Examples of plant light receptor domains

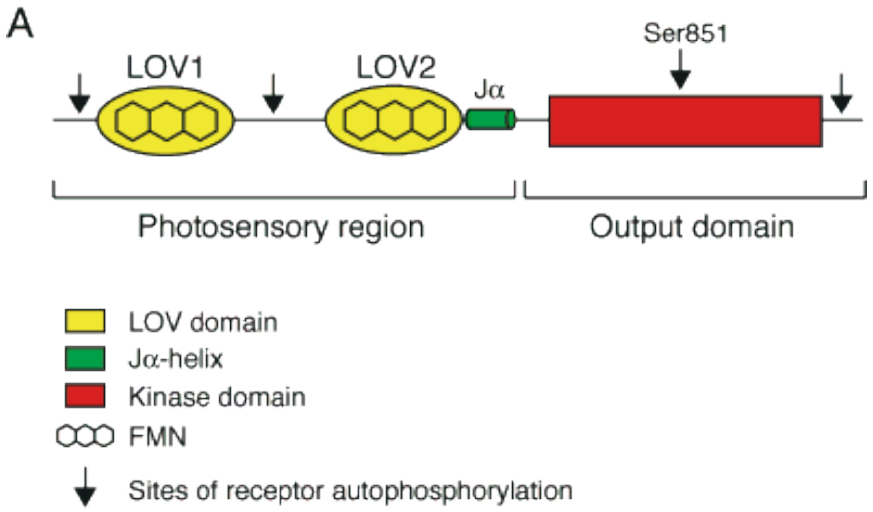


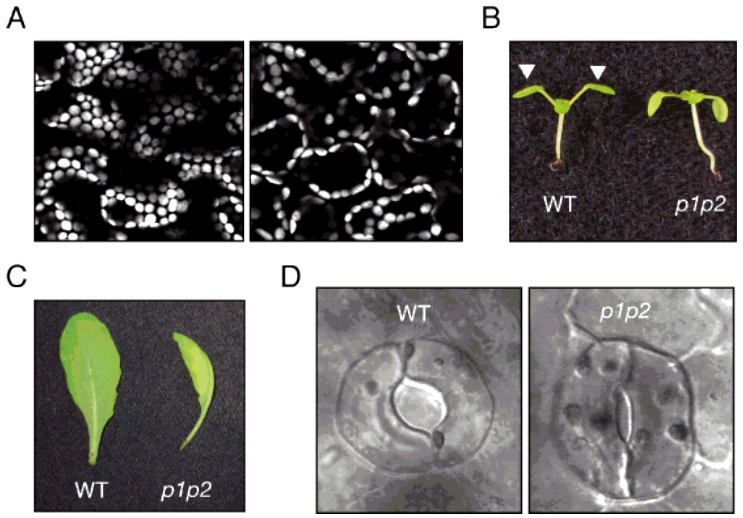
Wittmann T, van Haren J. “Lights, Cytoskeleton, Action: Optogenetic Control of Cell Dynamics” 2020, Curr. Op. in Cell Bio.

Plant blue light receptors

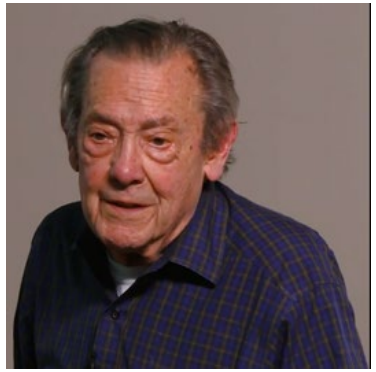
Phototropins are a blue light activated kinase that control various light responses in all land plants







Liscum and Briggs, 1995, Plant Cell
Briggs and Christie, 2002, Trends in Plant. Sc.



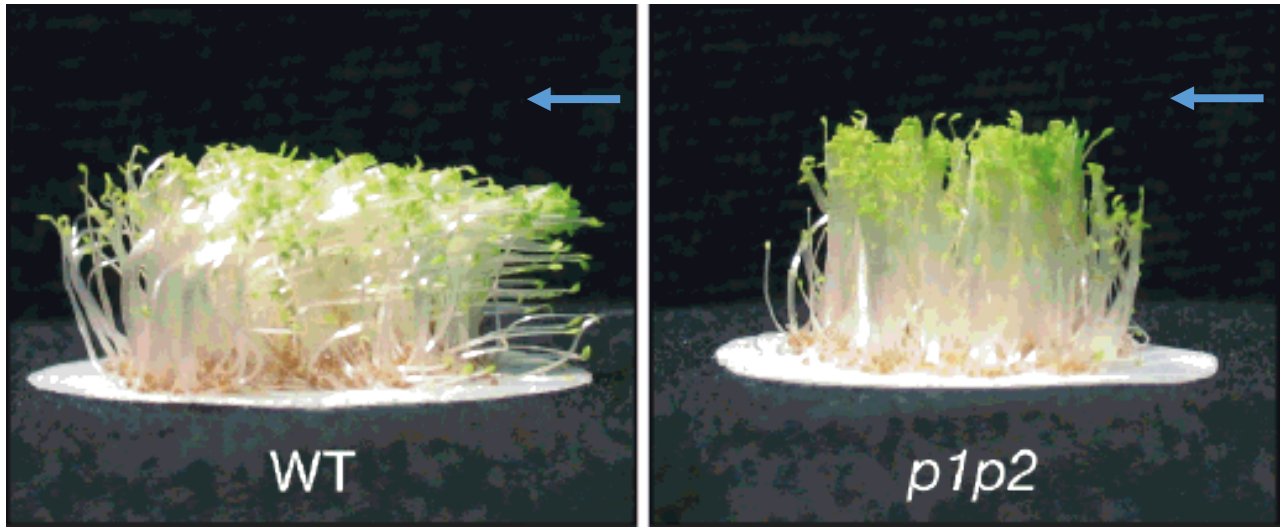
Winslow Briggs

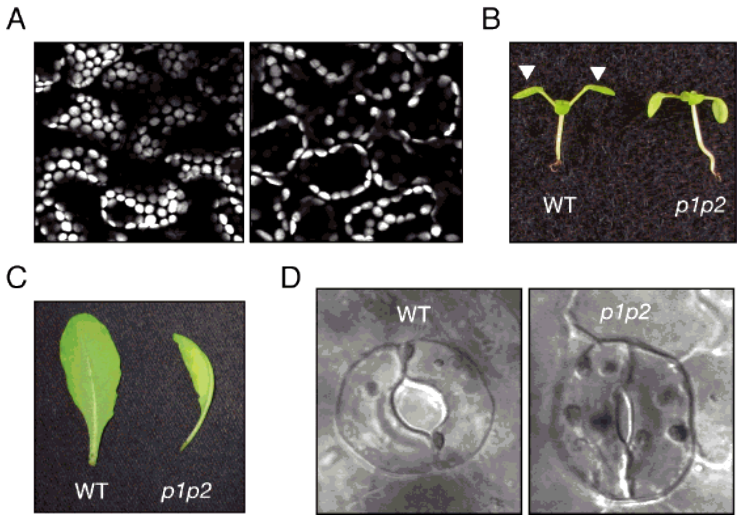
(LOV, CRY, Vivid)

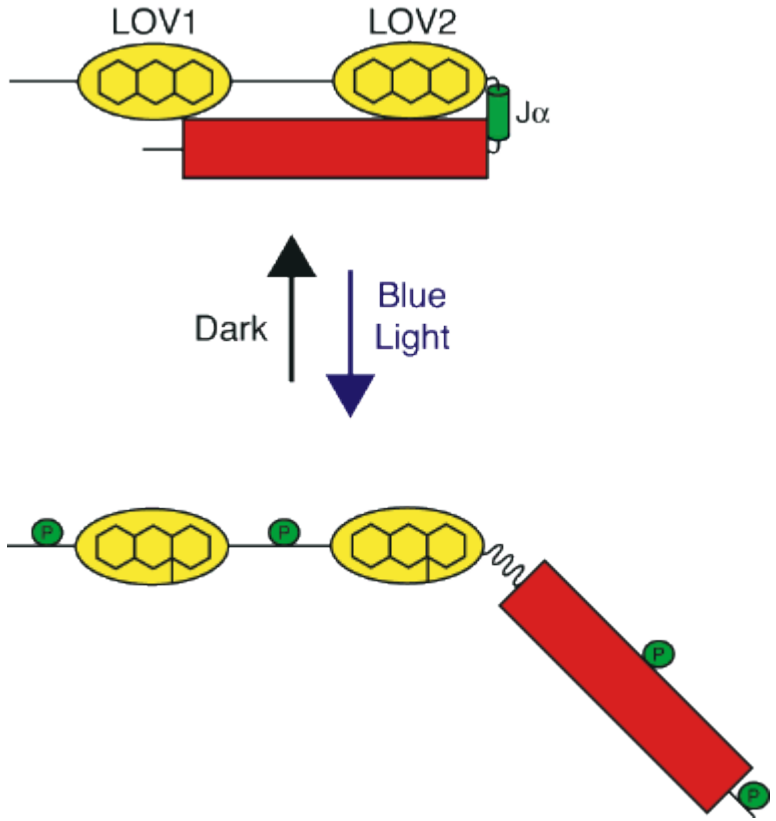
http://photobiology.info/Christie.html

Plant blue light receptors

Phototropins are a blue light activated kinase that control various light responses in all land plants



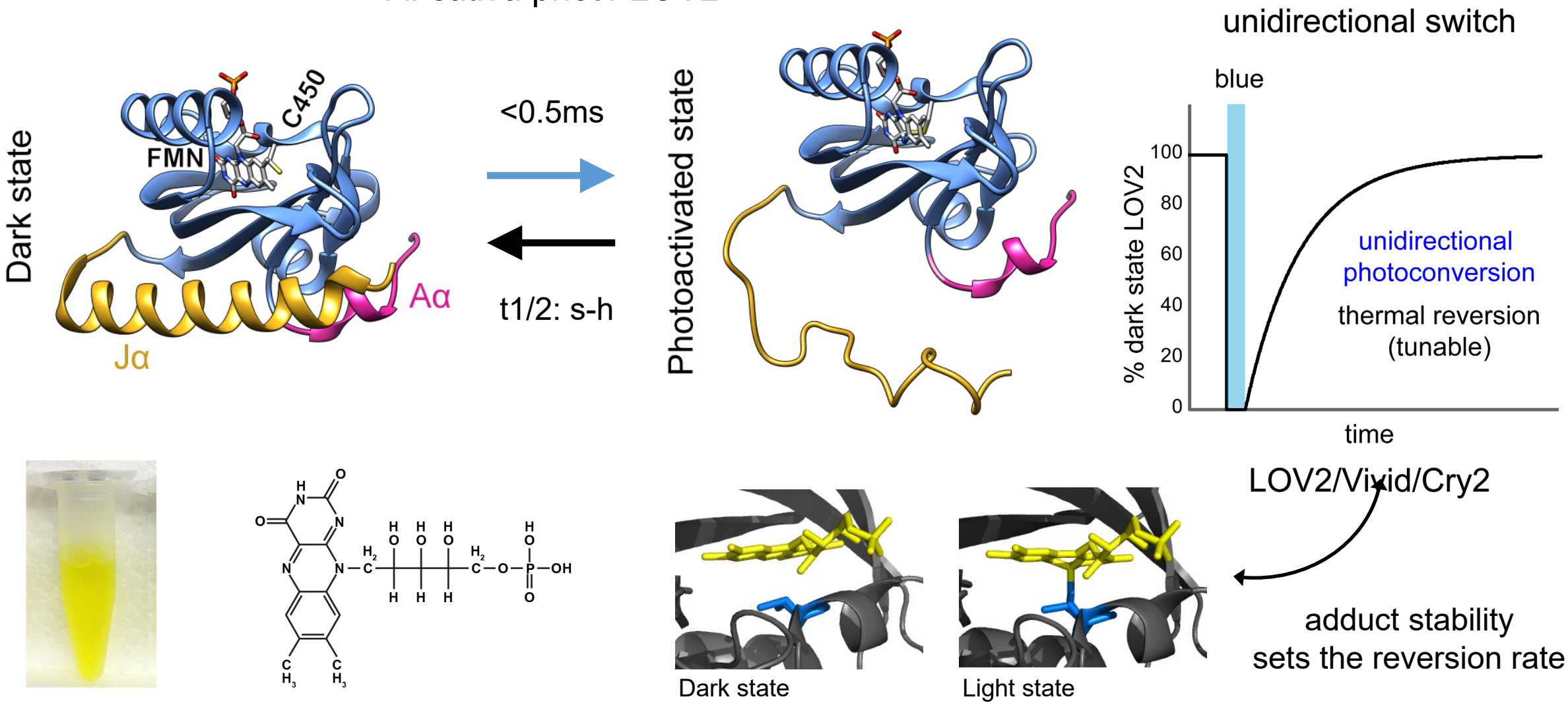




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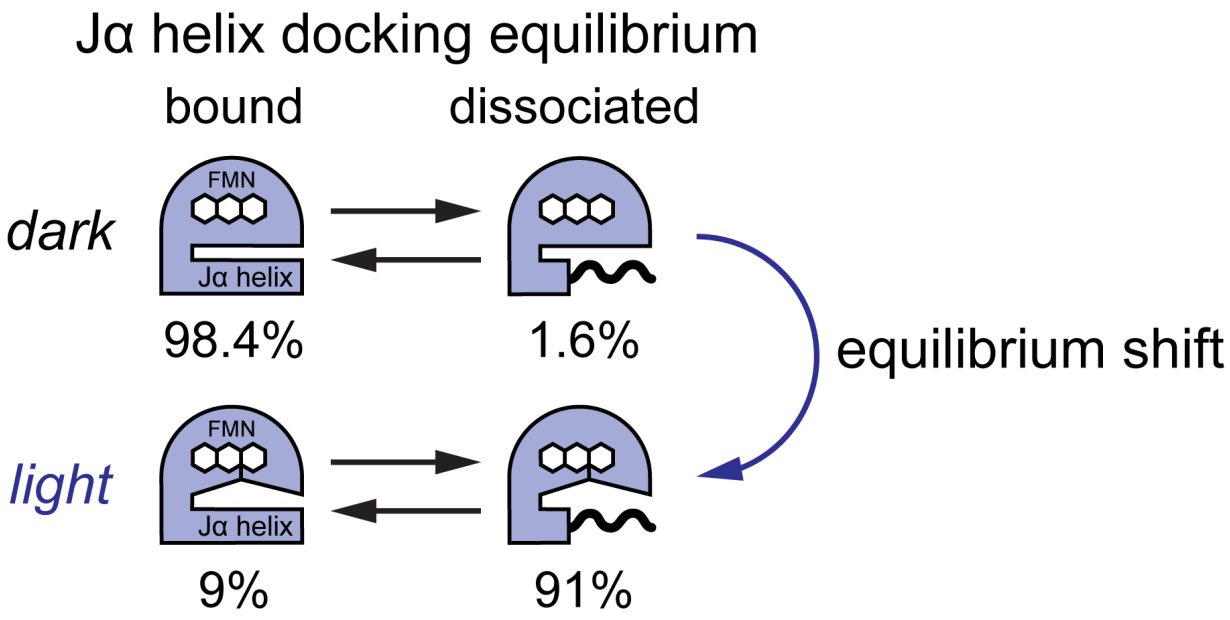
Properties of the phot1 LOV2 light receptor domain

A. sativa phot1 LOV2



A.s. LOV2 flavin mononucleotide (FMN) flavin-cysteinyl adduct

Properties of the phot1 LOV2 light receptor domain



~57 fold increase in the “active” form

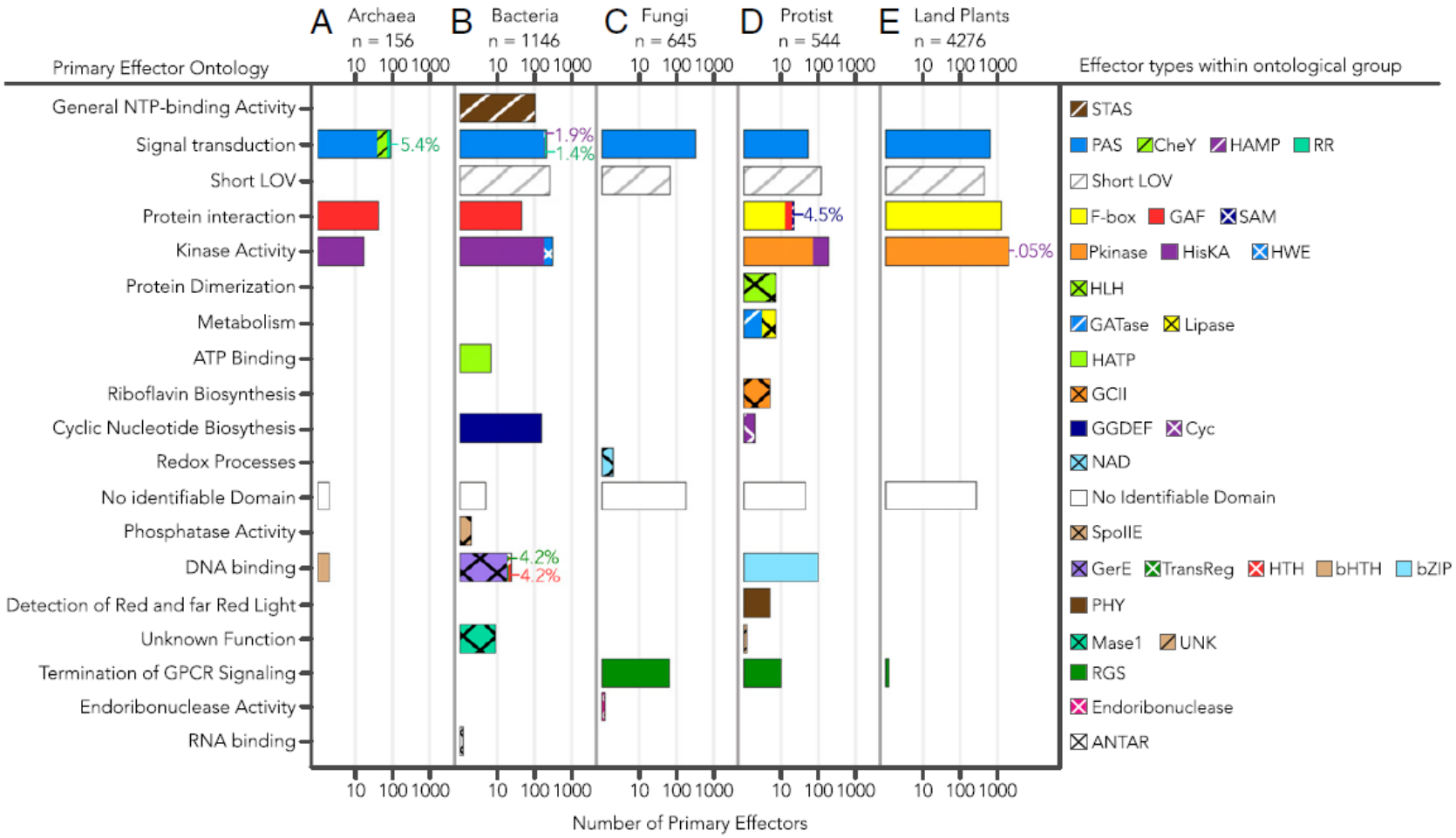
Ultimately this is an important determinant
of the dynamic range
of engineered photoswitches

However, probably very strongly dependent on the context
(some proteins might stabilize the docked or undocked conformation)

Yao and Gardner 2008, Nat.Chem.Biol. Estimation of Available Free Energy in a LOV2-Jα Photoswitch

LOV is everywhere!

LOV domains are commonly used in nature:
e.g. Circadian rythms, virulence, phototropism, stress responses



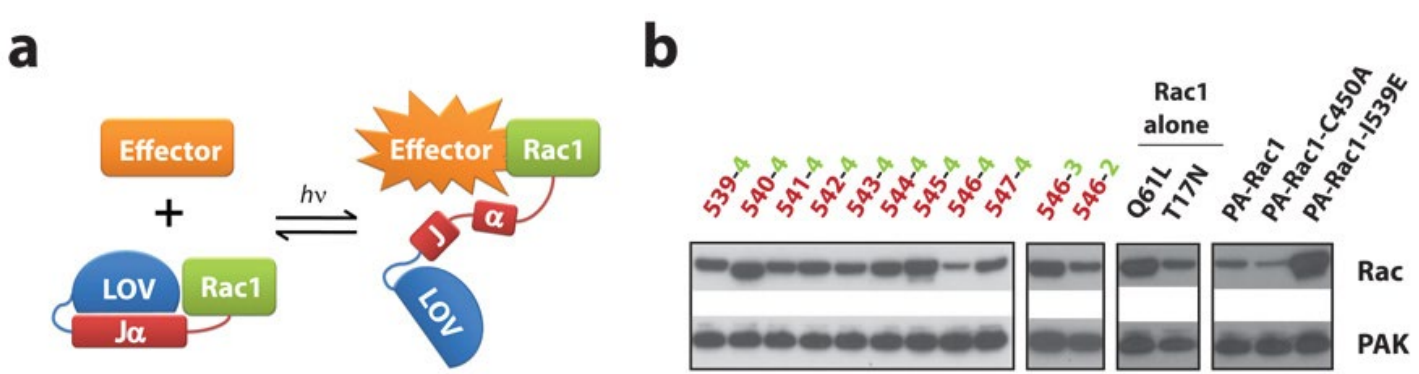
-This paper identified >4000 LOV domain containing proteins in various kingdoms of life

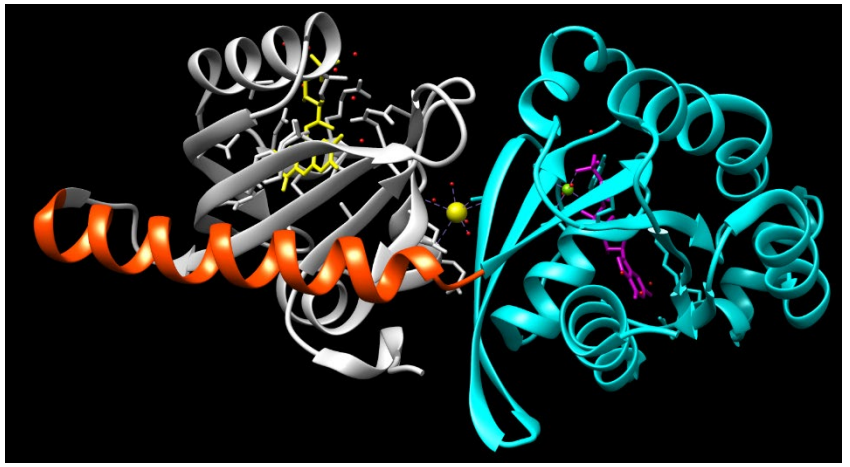
-Sensor-Effector topology
-LOV domains connected to a variety of effector domains, highlighting the versatility of LOVs

Glantz S.T. Functional and topological diversity of LOV domain photoreceptors, 2016 PNAS

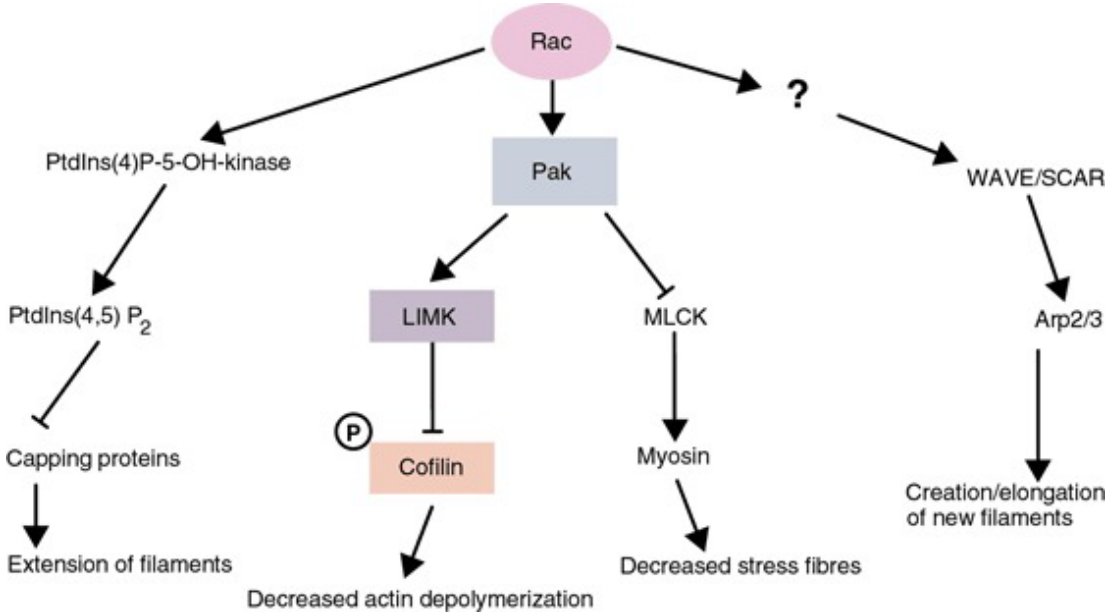
Utilizing light receptor domains to construct synthetic optoswitches

Photoactivatable-RAC1 (PA-RAC1)

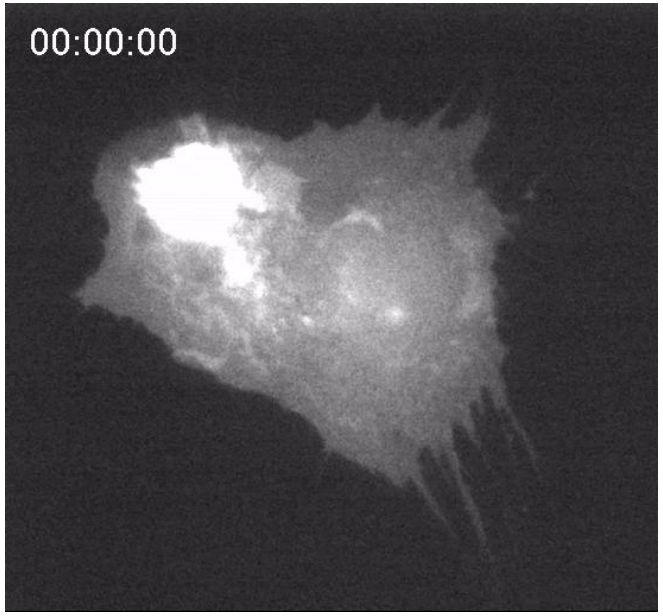




LOV2-RAC (Q61L, E91H, N92H), Constitutively active form of RAC1



Various RAC effectors



Controlling cell protrusion formation

Types of optogenetic switches

Light induced multimerization

PhyB/PIF

Cry2/CIBN

LOV2 based (TULIPs, iLID)

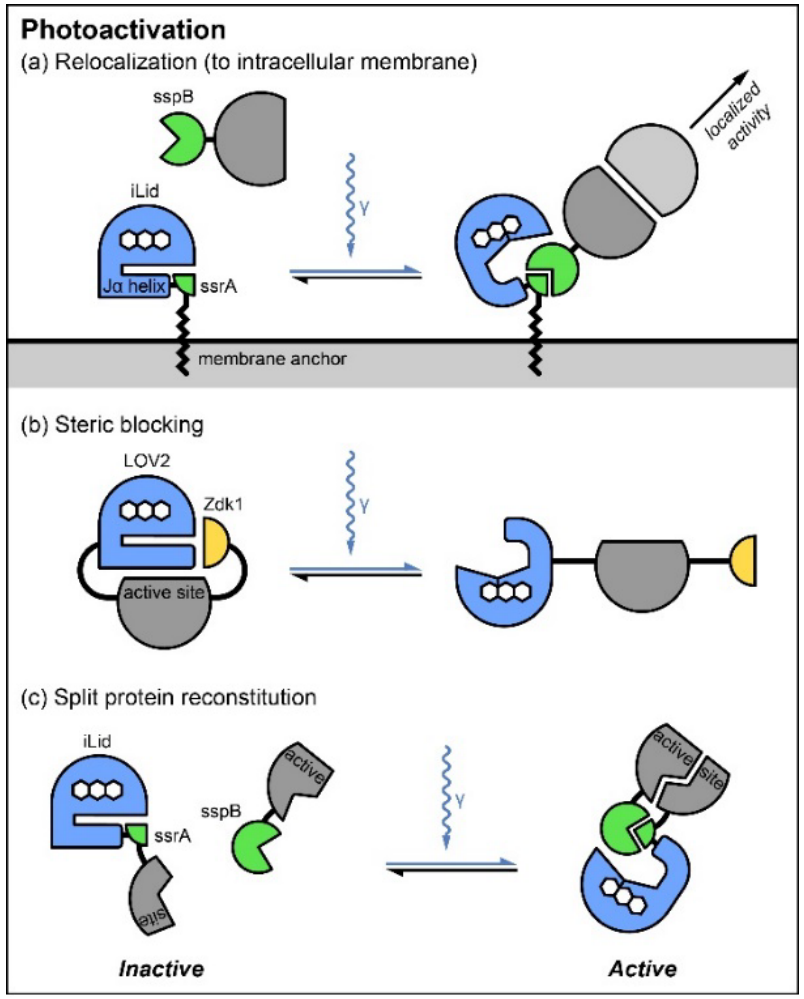
Cry2 clust

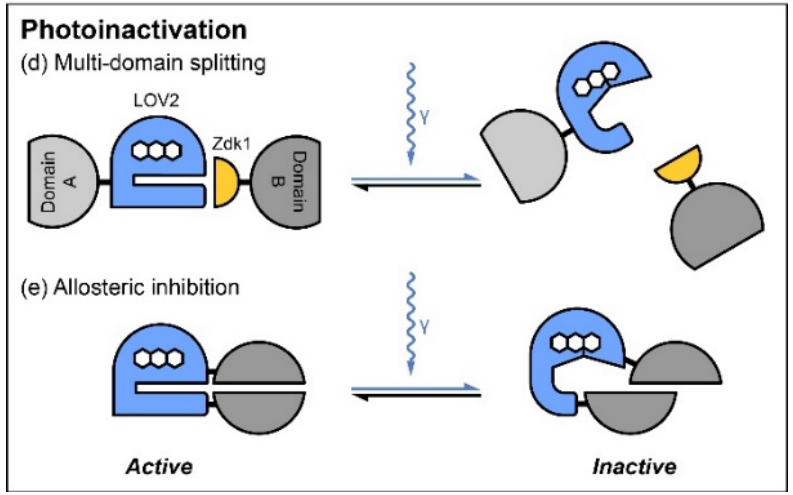
Light induced dissociation

LOVTRAP

Single-chain switches

LOV2, DRONPA



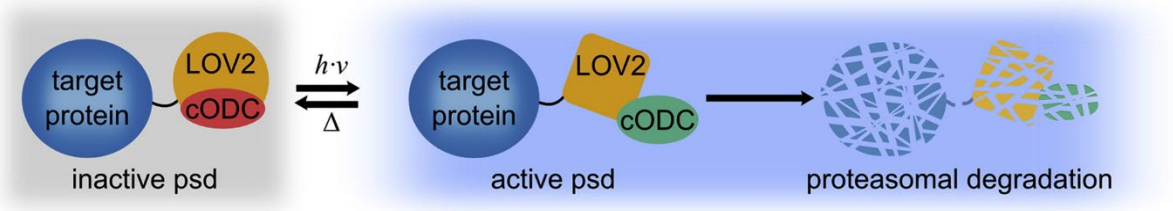


Wittmann T, van Haren J. “Lights, Cytoskeleton, Action: Optogenetic Control of Cell Dynamics” 2020, Curr. Op. in Cell Bio.

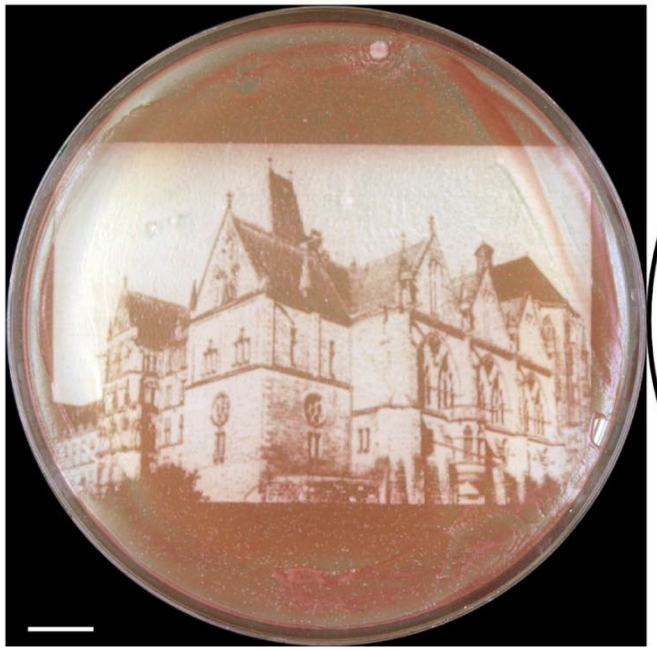
LOV based optogenetic switches

Hiding short protein motifs in the LOV2 j-alpha helix

Photo-switchable degron (PSD)



"Yeast photography" using a LOV2 based light-activated degron (PSD)



dark regions: exposed to light
(mRFP-PSD degraded)



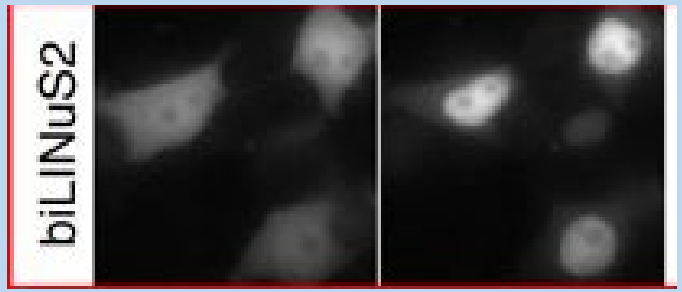
photomask

Renicke et al. Chem. and Bio. (2013)

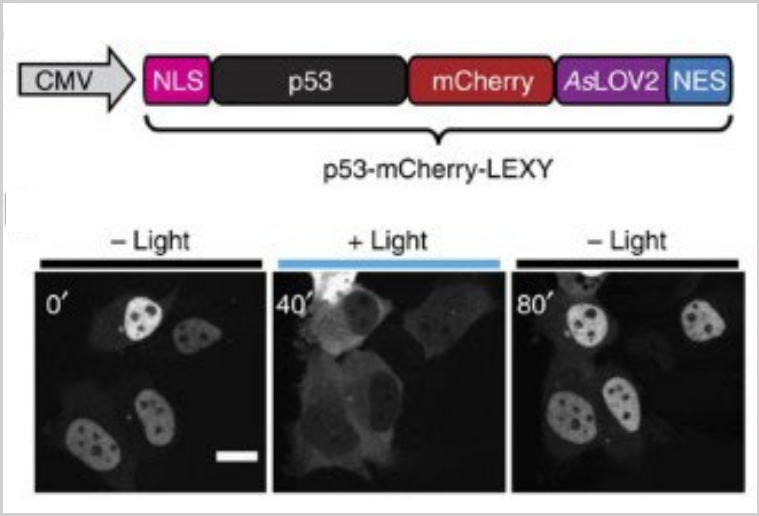
Photo-switchable NLS/NES



NLS

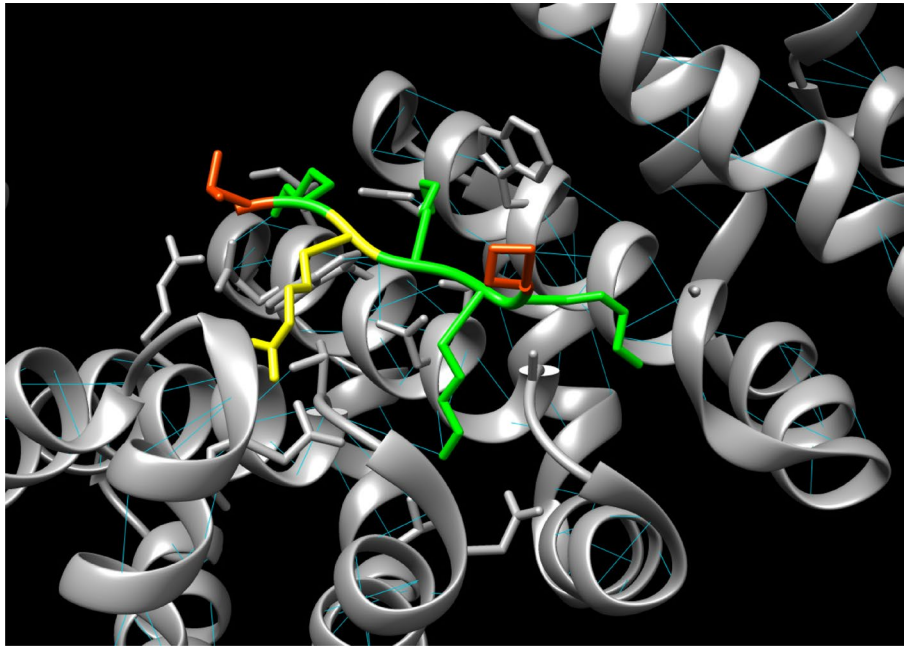


NES



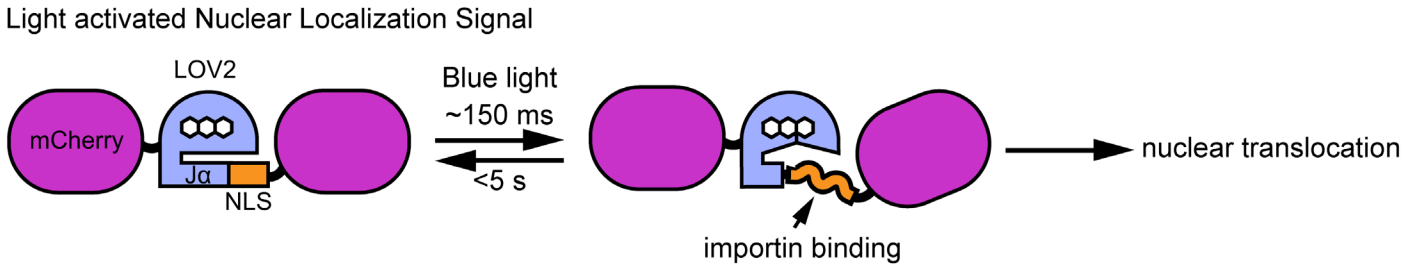
Niopek et al. Nat.Com. (2014, 2016)

Our own attempt at making a light controllable NLS



M.m. importin-α/ SV40_LTA-NLS (crystal structure)

mCherry-LOV2-NLS-mCherry



NLS unstructured
K/R sidechains oriented towards importin

Light activated nuclear localization signals

K-1: LAAALERIE---AENIDEAAKELRAAKRKRAG

1-1: LATTLERIE---AEEIDEAAKEERLARAAKRKRAG

2-2: LATTLERIE---AEEIDEAAKEERARAAKRKRAG

3-1: LATTLERIE---AEEIDEAAKEEARAAKRKRAG

4-2: LATTLERIE---AEEIDEAAKEARAAKRKRAG

5-3: LATTLERIE---AEEIDEAAKARAAKRKRAG

6-1: LATTLERIE---AEEIDEAAARAAKRKRAG

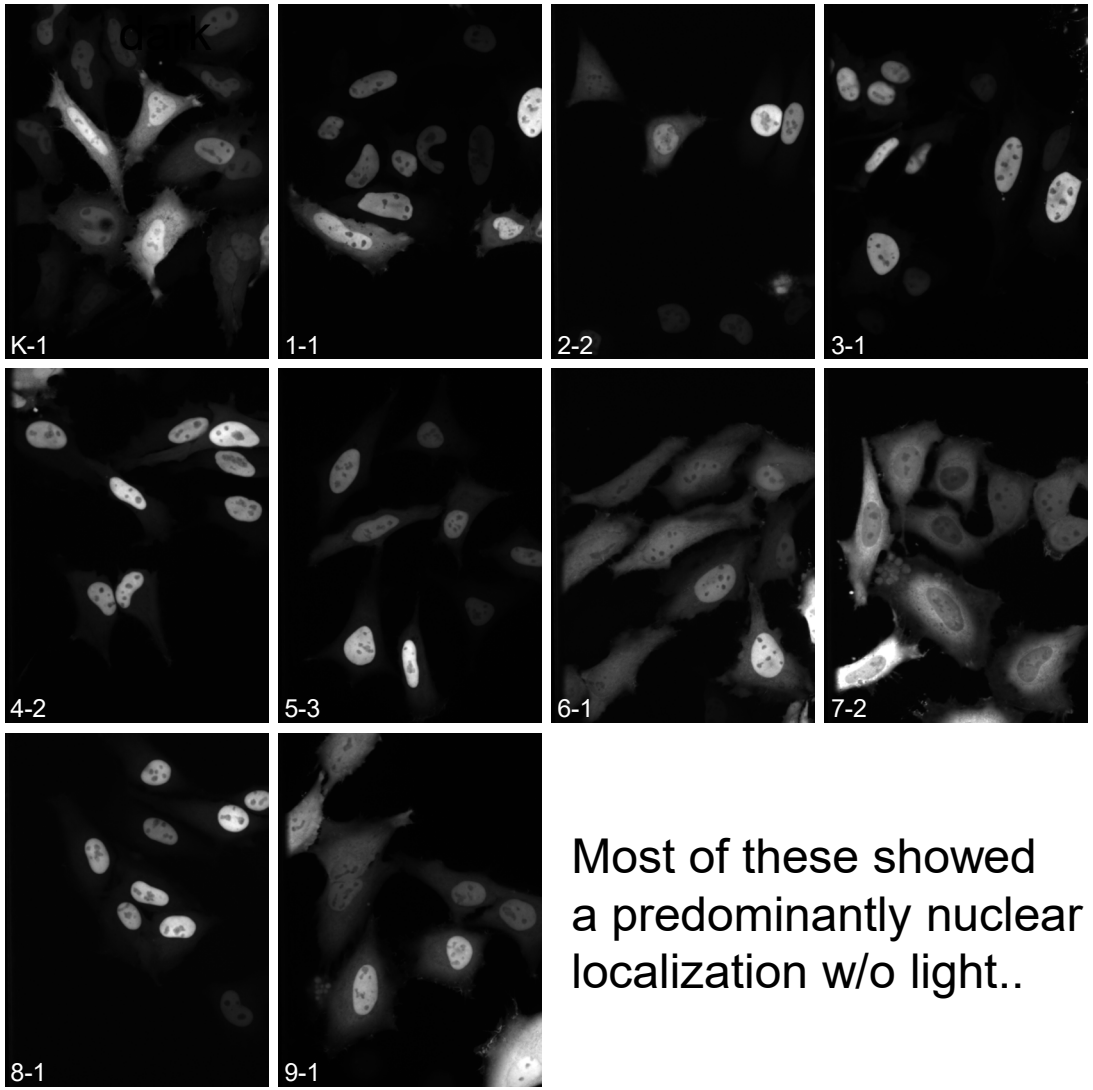
7-2: LATTLERIE---AEEIDEAARAAKRKRAG

8-1: LATTLERIE---AEEIDEARAAKRKRAG

9-1: LATTLERIE---AEEIDARAAKRKRAG

k-1: WT c-terminus, altered N-terminus LAAA
1-1_9-1:WT N-terminus, g528a,n538e altered C-termini
(helix stabilizing mutations)

Localization of LOV2-NLS fusions in the dark



Most of these showed a predominantly nuclear localization w/o light..

Light activated nuclear localization signals

K-1: LAAALERIE---AENIDEAAKELRAAKRKRAG

1-1: LATTLERIE---AEEIDEAAKEERLARAAKRKRAG

2-2: LATTLERIE---AEEIDEAAKEERARAAKRKRAG

3-1: LATTLERIE---AEEIDEAAKEEARAAKRKRAG

4-2: LATTLERIE---AEEIDEAAKEARAAKRKRAG

5-3: LATTLERIE---AEEIDEAAKARAAKRKRAG

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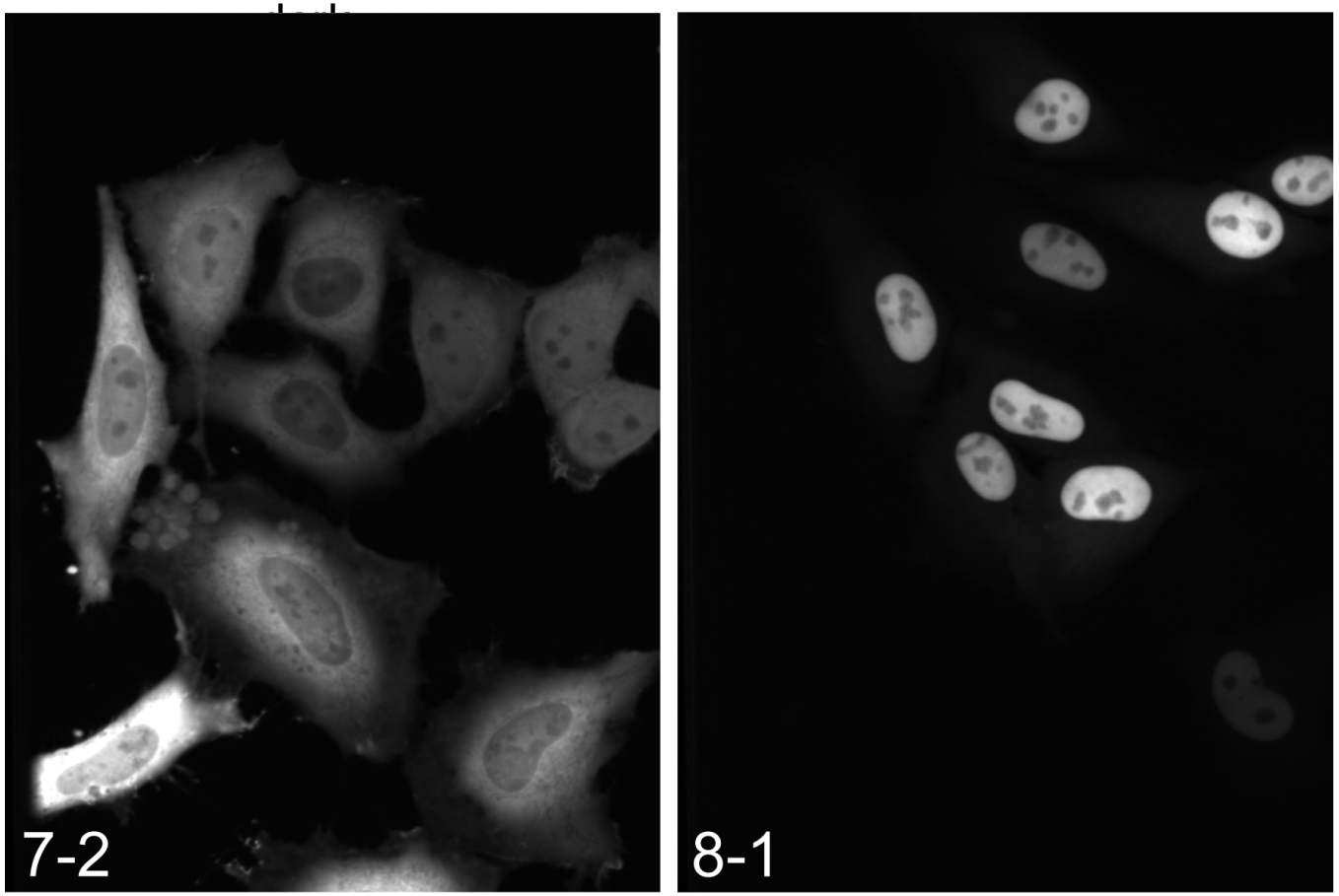
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Localization of LOV2-NLS fusions in the



Light activated nuclear localization signals

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1-1: LATTLERIE---AEEIDEAAKEERLARAAKRKRAG

2-2: LATTLERIE---AEEIDEAAKEERARAAKRKRAG

3-1: LATTLERIE---AEEIDEAAKEEARAAKRKRAG

4-2: LATTLERIE---AEEIDEAAKEARAAKRKRAG

5-3: LATTLERIE---AEEIDEAAKARAAKRKRAG

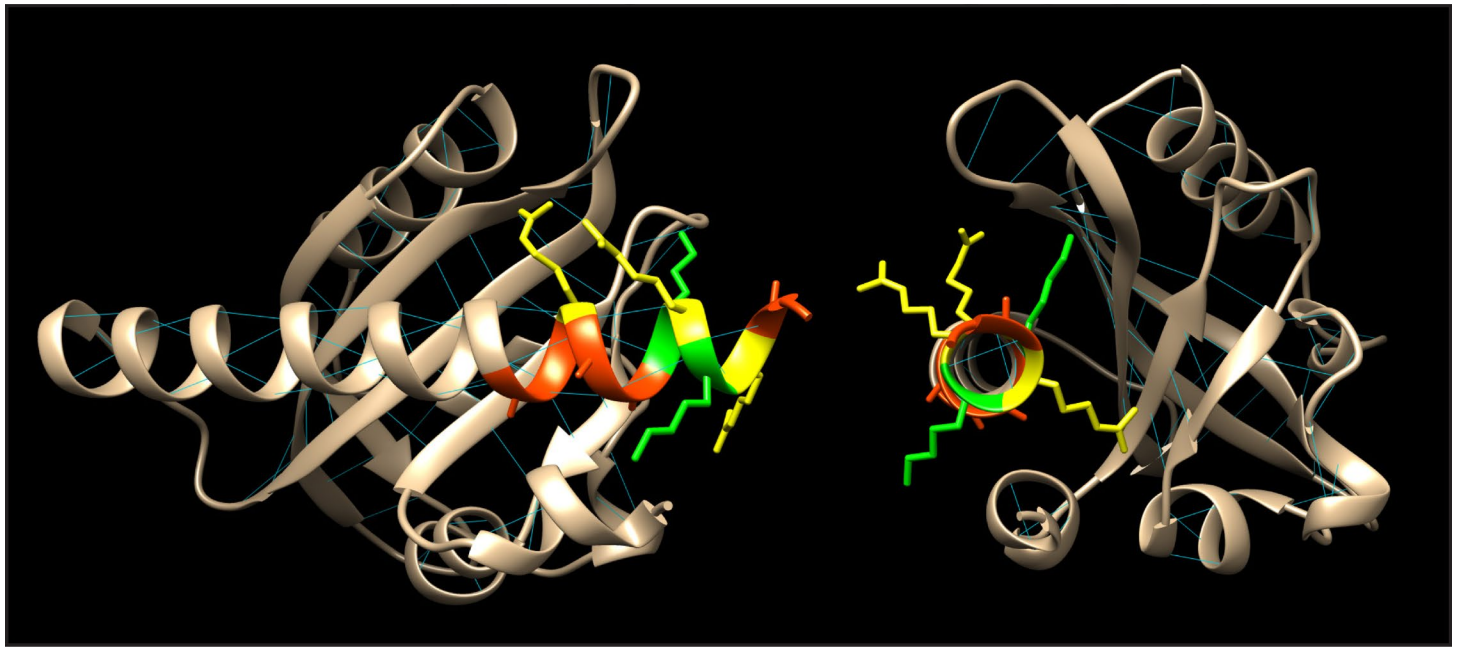
6-1: LATTLERIE---AEEIDEAAARAAKRKRAG

7-2: LATTLERIE---AEEIDEAARAAKRKRAG

8-1: LATTLERIE---AEEIDEARAAKRKRAG

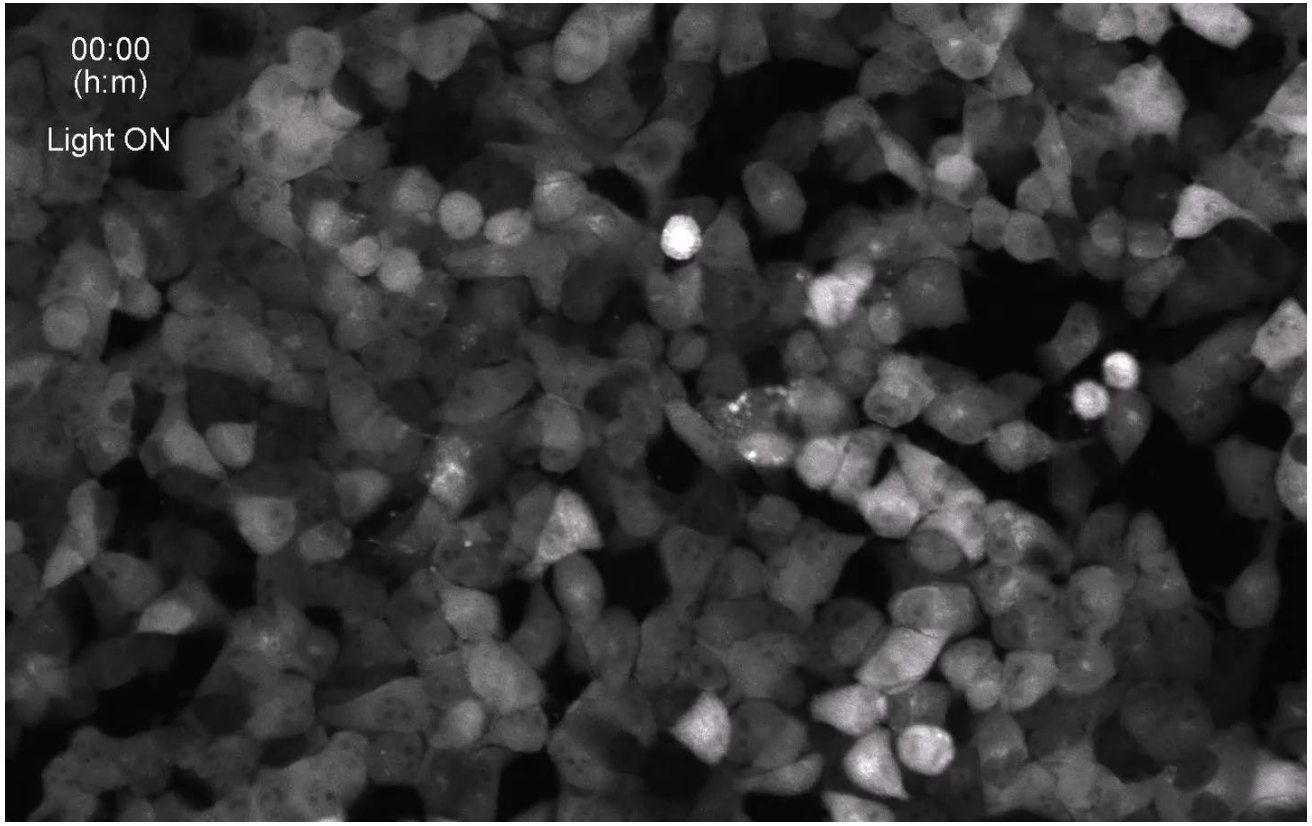
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(helix stabilizing mutations)



A.s. LOV2-synNLS-7-2 (Alphafold 2.0 structure prediction)

Light activated nuclear localization signals

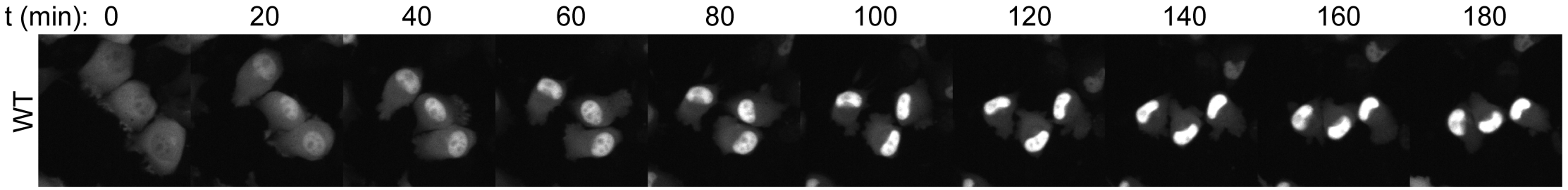


It's a relatively slow switch
(takes about two hours to reach plateau)

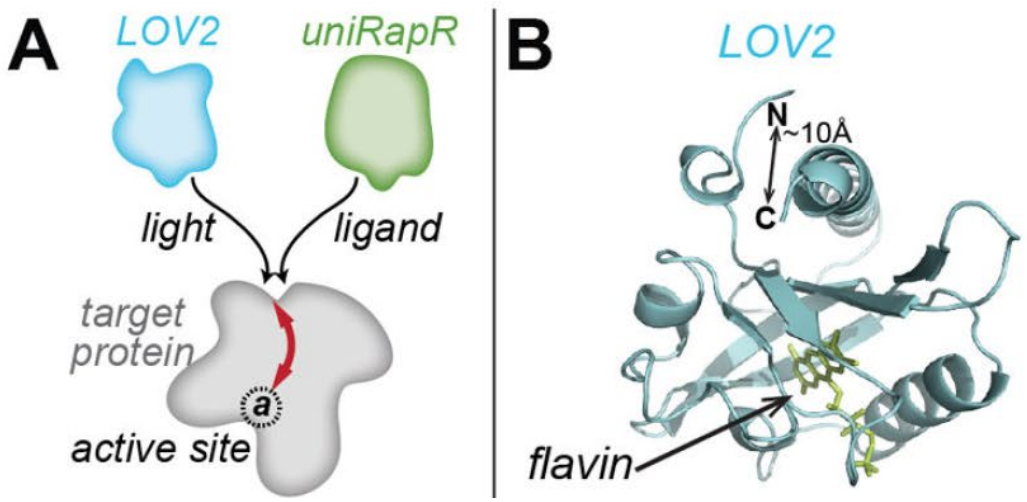
Perhaps the NLS itself is relatively weak.
Incomplete unfolding of J-alpha-NLS?

But the dynamic range of the switch is quite good.

The next step step is where we got stuck.
Light controlled import of TFs.
(opto-NLS dynamics were strikingly different when
We attached it to TFs…)



Loop-insertion-strategy (Loopology)

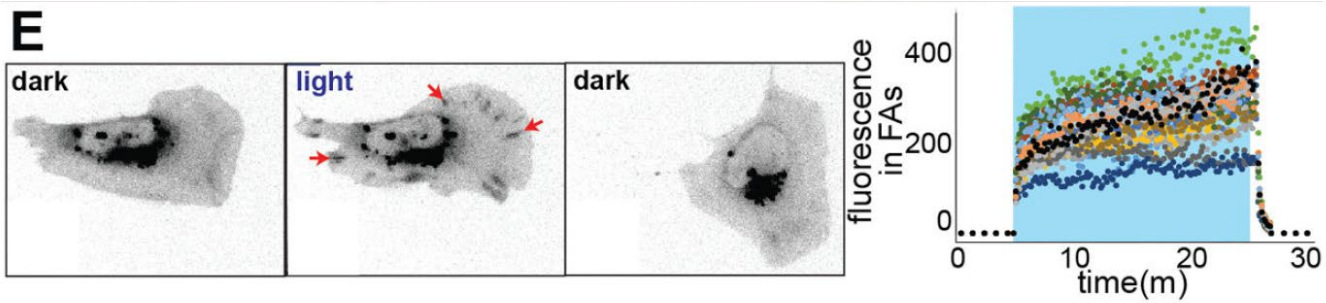


Criteria for determining loop insertion site:

-Surface-exposed and evolutionarily non-conserved loops to avoid possible structural perturbations

-Loops mechanically coupled to the active site?

-Loop tightness (tight loops are preferred)



Loopology requires protein structures,
This limits applicability…

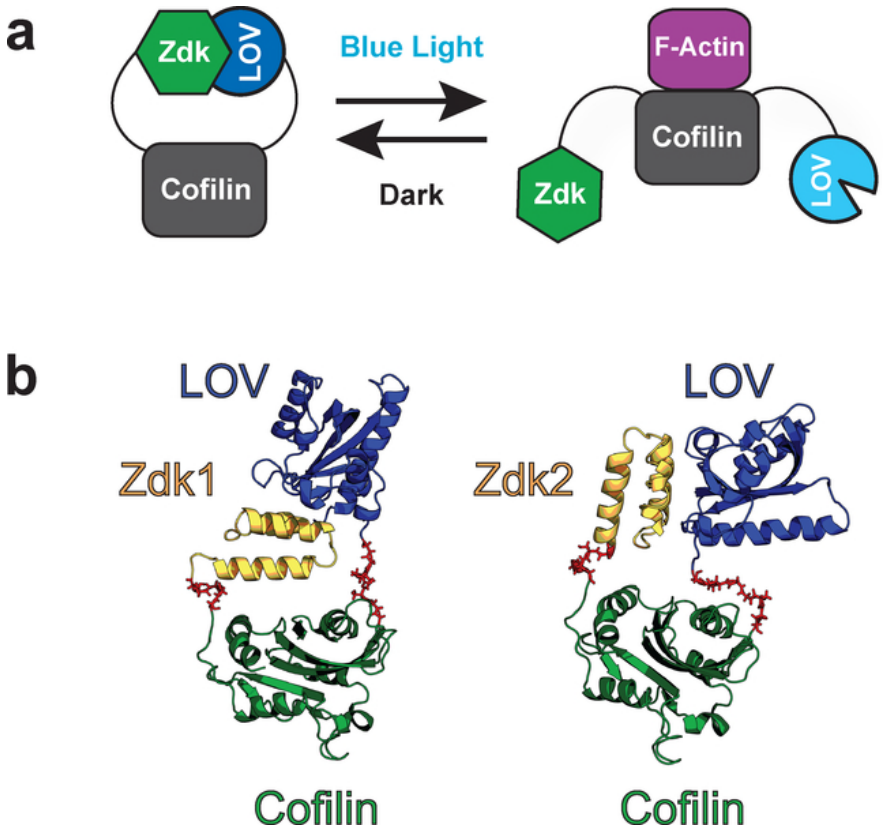
(Alphafold2.0 might help)

Loopology broadens our ability to directly target proteins of interest

Dagliyan et al. Engineering extrinsic disorder to control protein activity in living cells, Science 2017 june

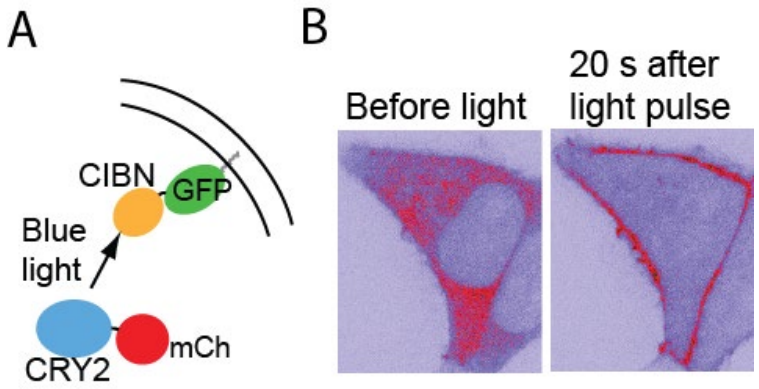
DRONPA, Z-lock strategy

- Caging of an active site through intramolecular interactions

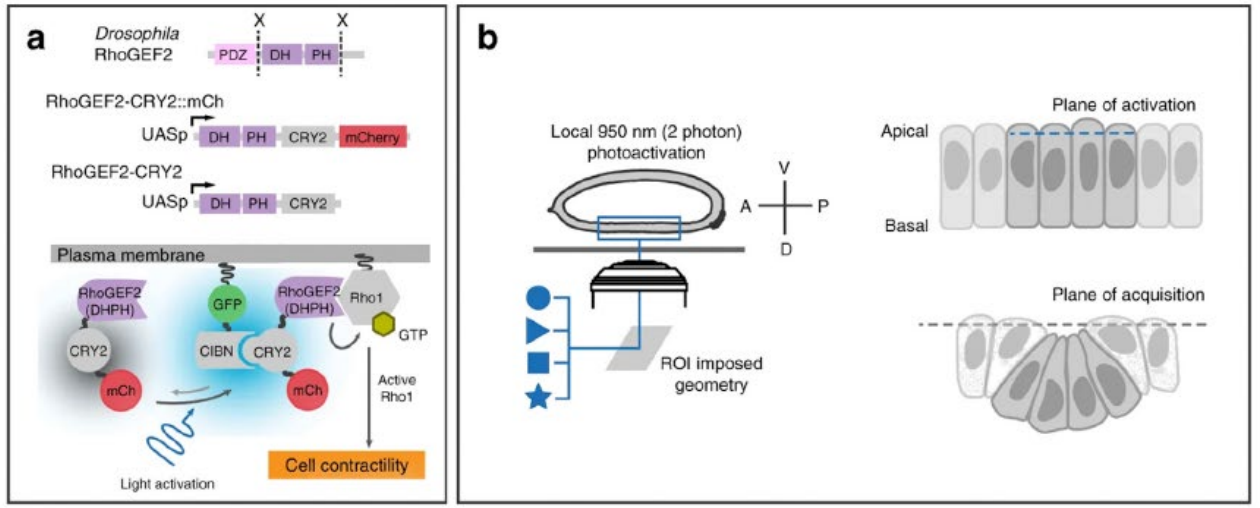


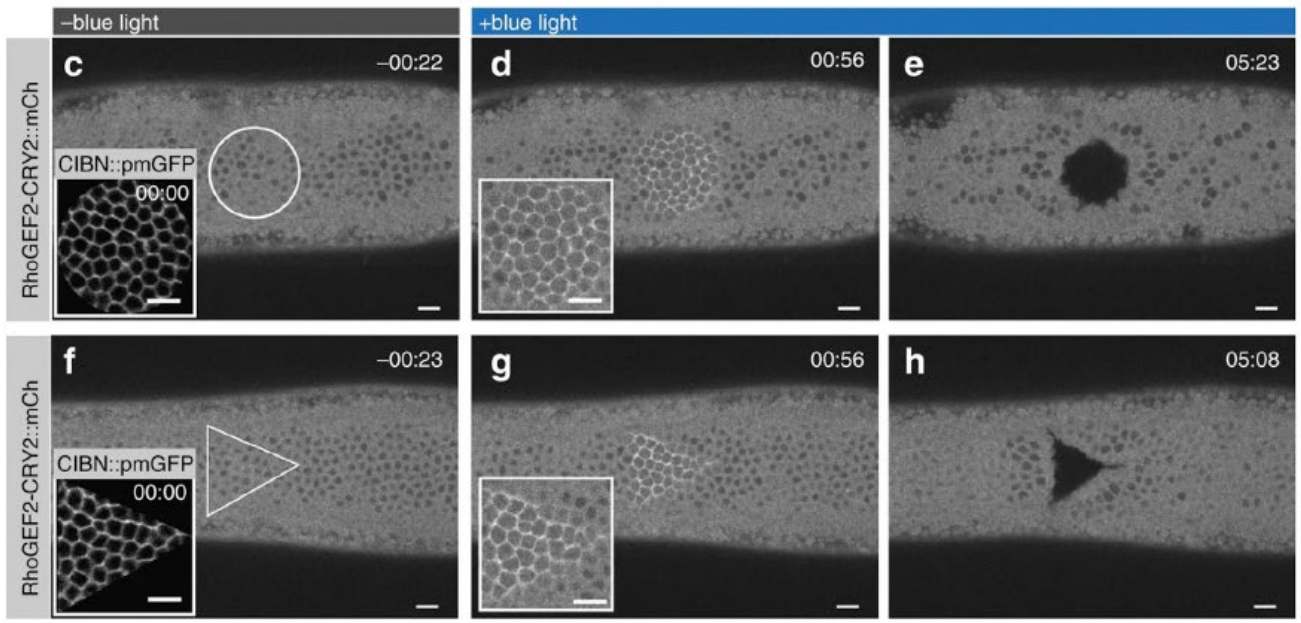
Effective conc of LOV2-Zdk1/2 is extremely high
in such fusion proteins
(so affinity needs to be tweaked for this to work well)

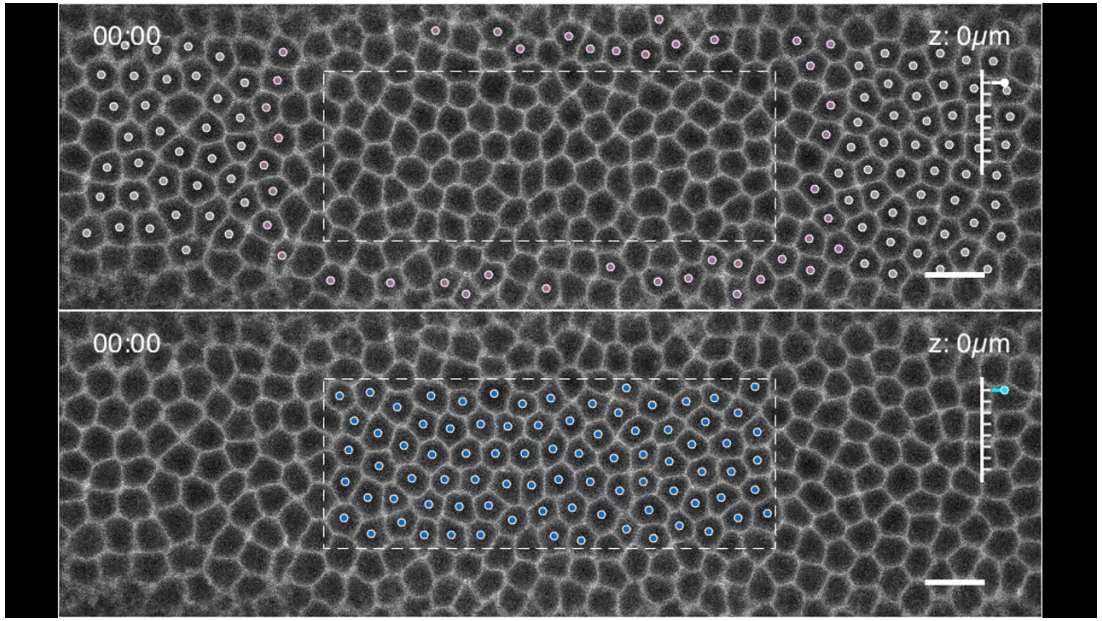
Light induced dimerization by CRY2/CIB



Kennedy et al. Nat. Meth. (2010)





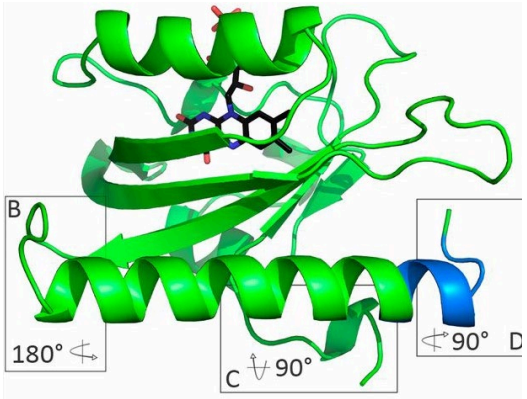


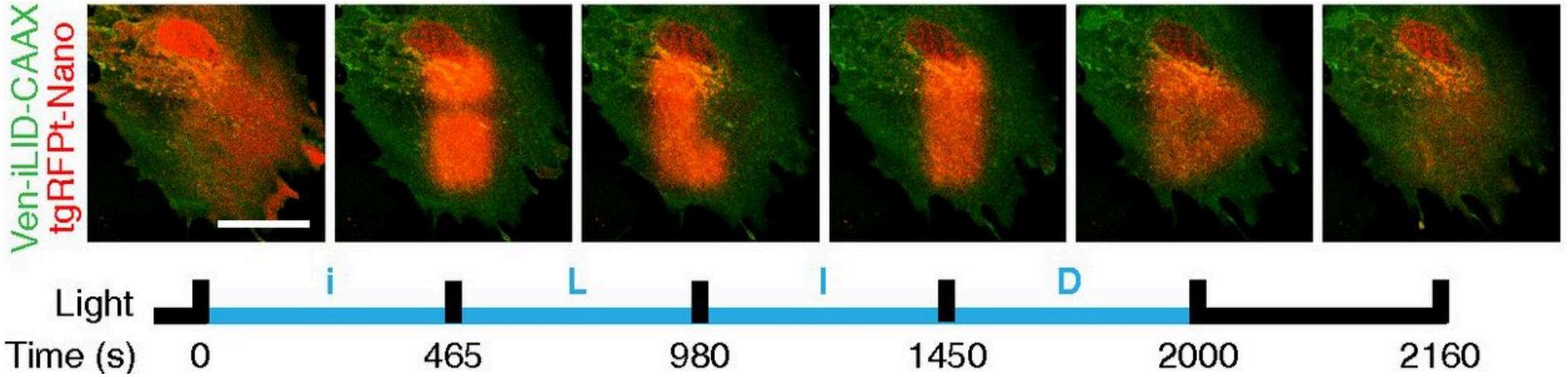
Guided morphogenesis through optogenetic activation of Rho signalling during early *Drosophila* embryogenesis. Izquierdo et al. Nat. Com. (2018)

TULIPS and iLID Light induced dimerization systems also based on LOV2-J-α helix fusions

ILID/ nano system uses *E. Coli* SsrA and SspB proteins

-LOV2-SsrA peptide fusion (7 aa)
-SspB





Binding affinity switches from 130nM under blue light to 4.7uM in the dark

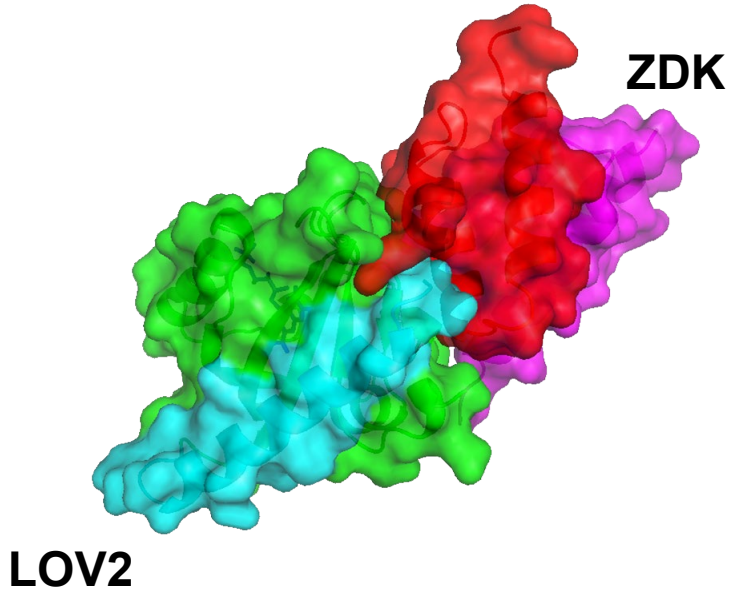
Guntas et al. 2015, PNAS, doi.org/10.1073/pnas.1417910112

Light induced dissociation: LOV2 Trap and Release of proteins

Blue light induces dissociation of LOV2 and Zdk1 (the only blue light dissociated complex)

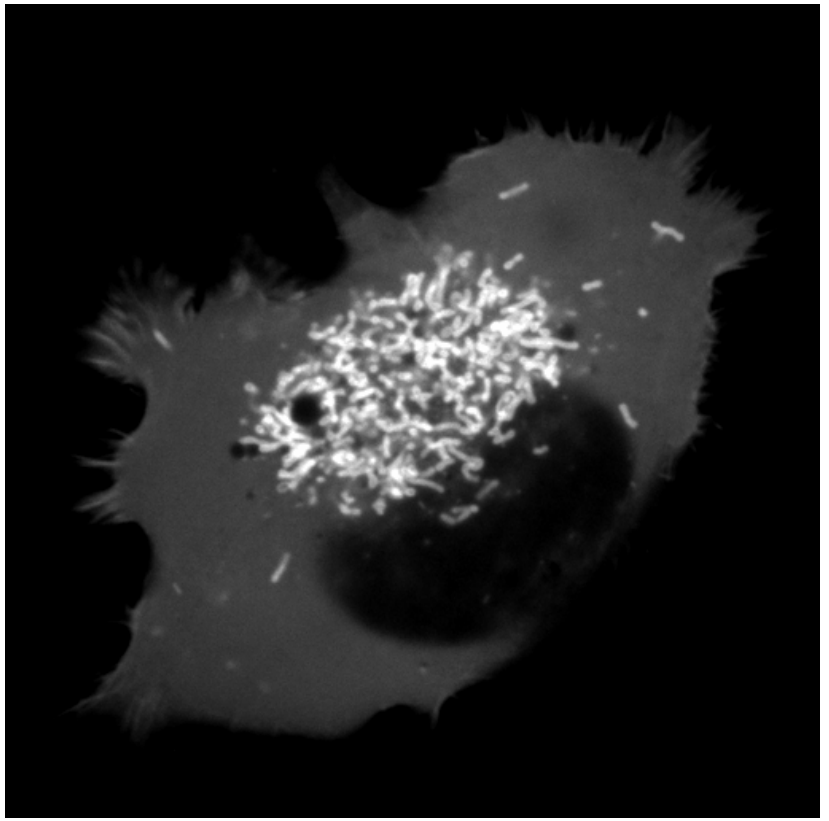
jvh

LOVTRAP



from Hui Wang & Klaus Hahn @ UNC

ZDK binds the LOV2 dark state
with Kd ~ 20 nM



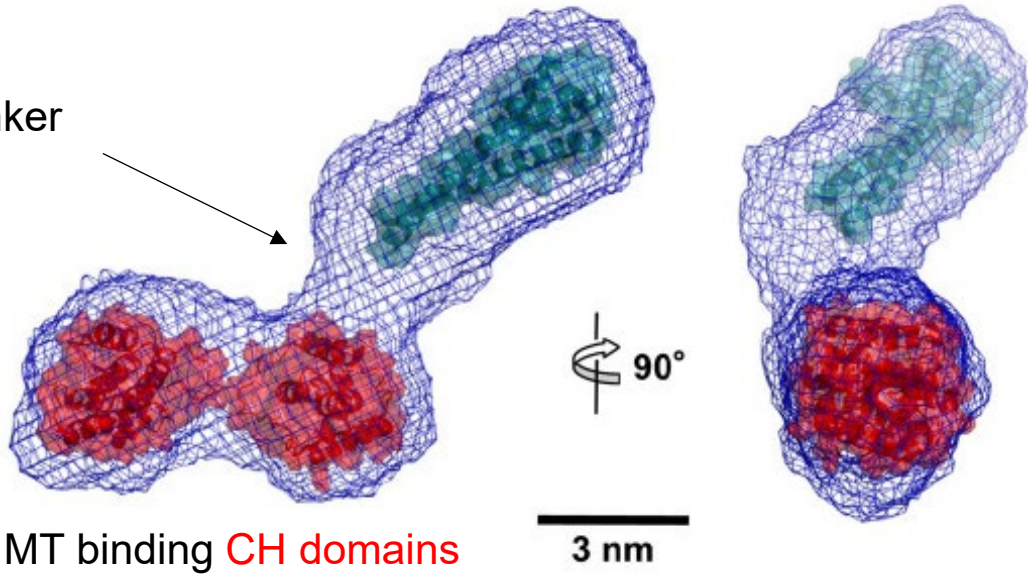
LOV2 attached on Mitochondrial surface
Traps mCherry-Zdk1 in the dark, releases it when illuminated

Wang et al. Nature Methods July 2016, doi:10.1038/nmeth.3926

Light induced “domain splitting”, design of a photo-inactivated EB1

+TIP adapter domain
(recruits >40 other +TIPs to MTs)

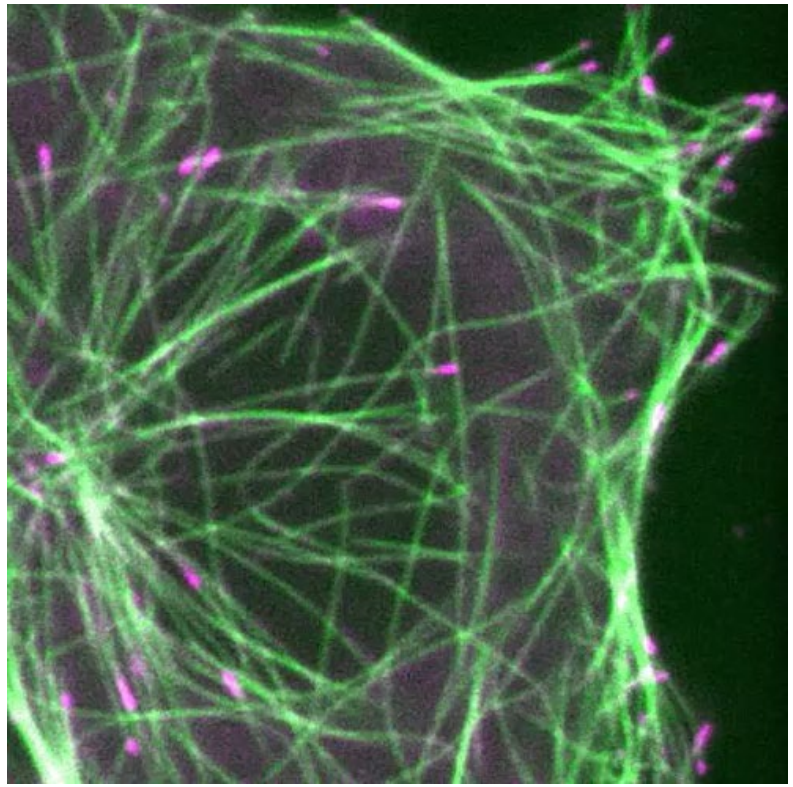
flexible linker



MT binding CH domains

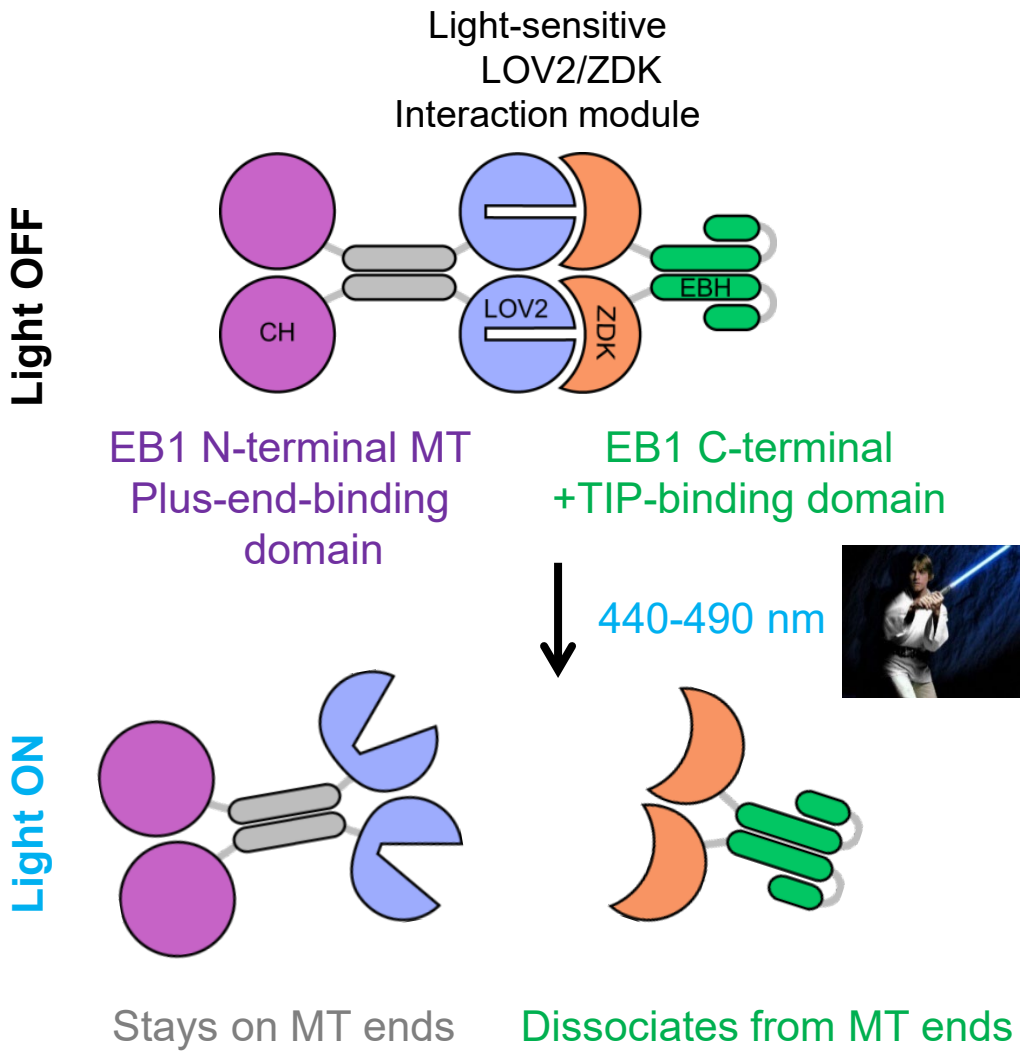
SAXS envelope of EB1, crystal structures of CH and EBH domains

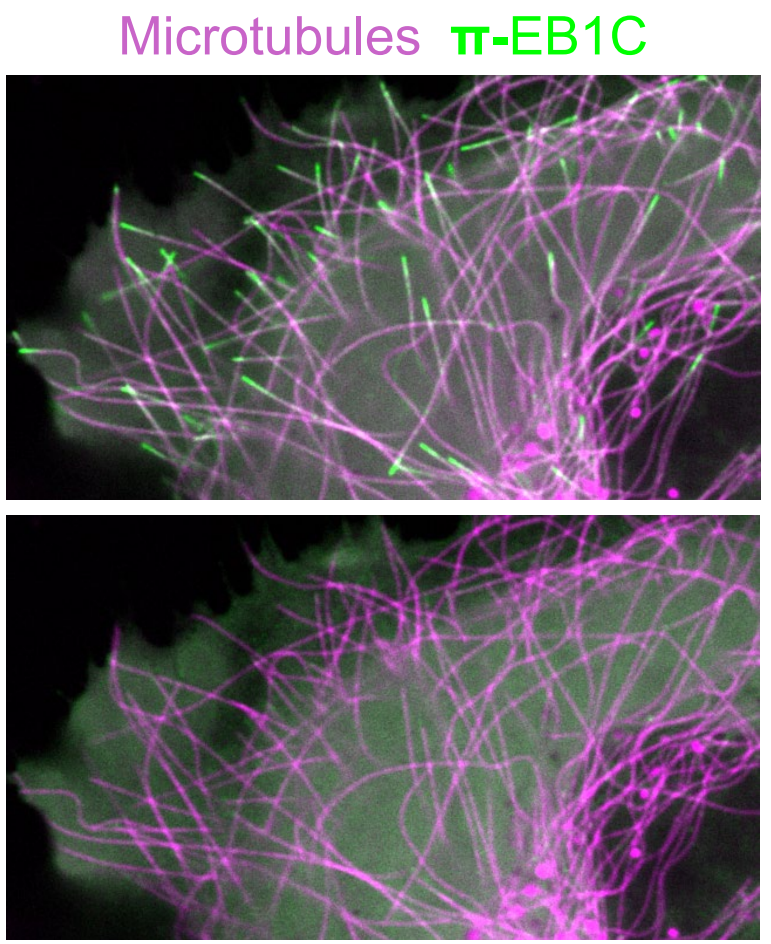
Microtubules EB1



EB1 associates with growing microtubule ends

Light induced “domain splitting”, design of a photo-inactivated EB1

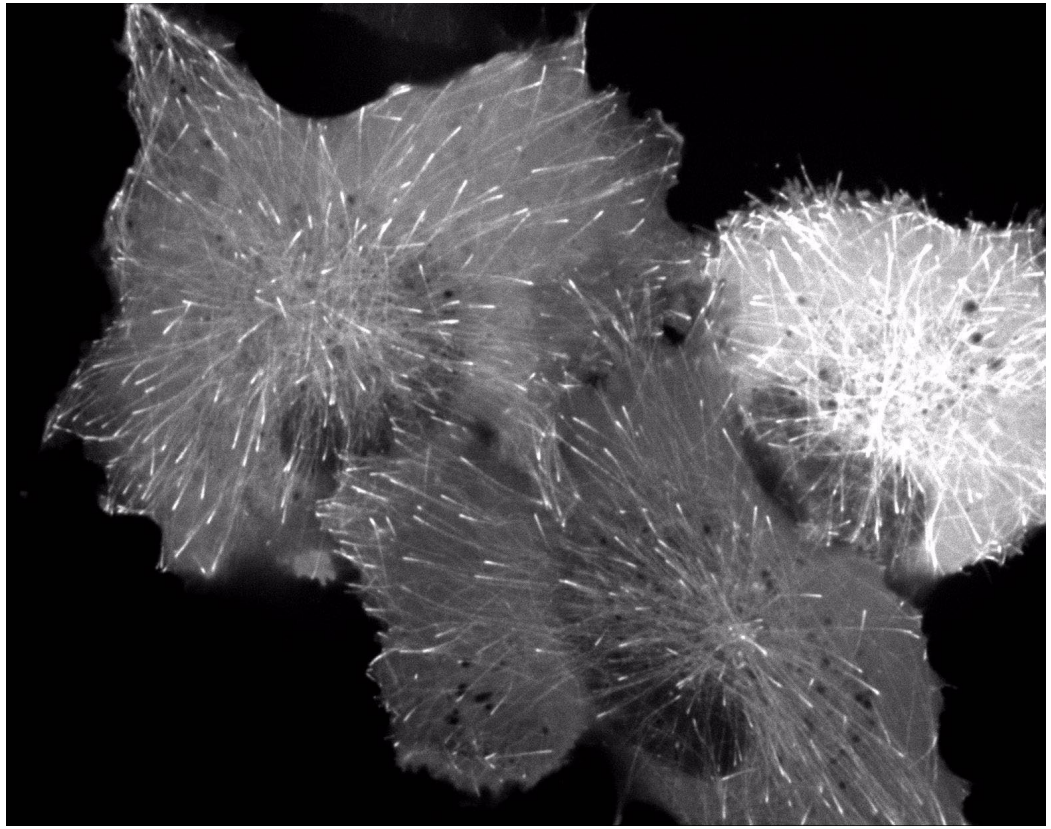




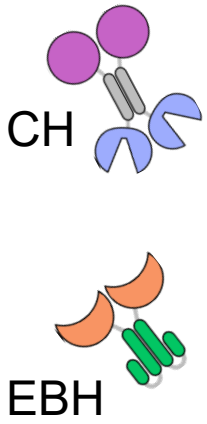
Blue light triggers π-EB1C dissociation from growing MT ends

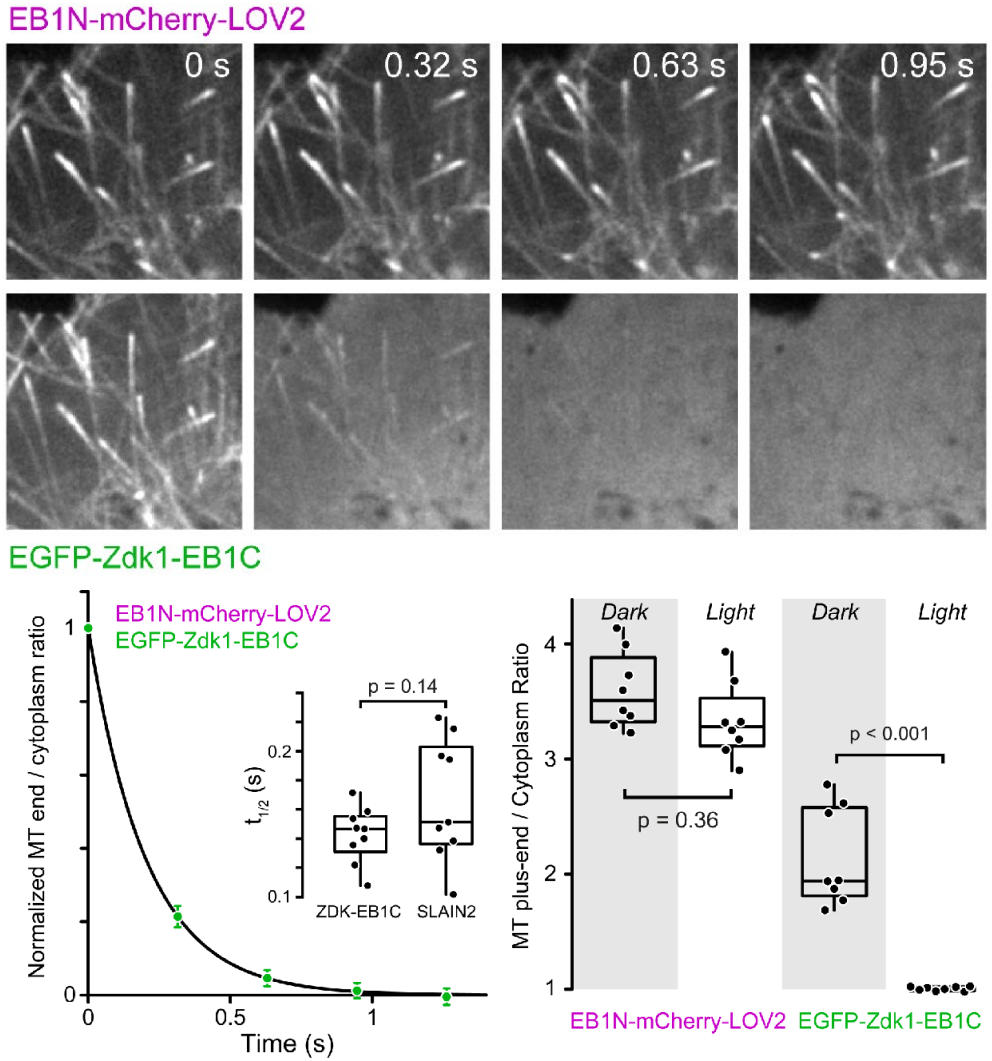
van Haren J. et al. Nature Cell Biology 2018, doi:10.1038/s41556-017-0028-5

Rapid and reversible inactivation of π-EB1



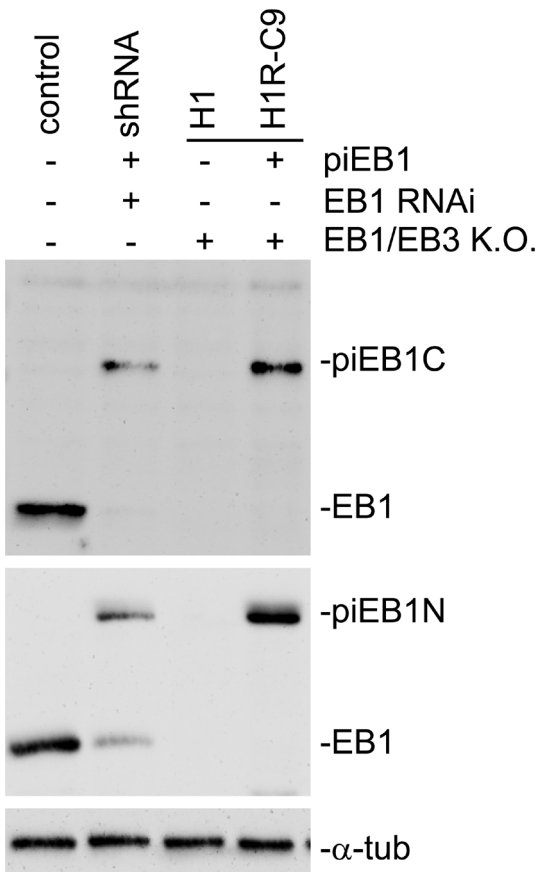
mCherry-ZDK-EB1C



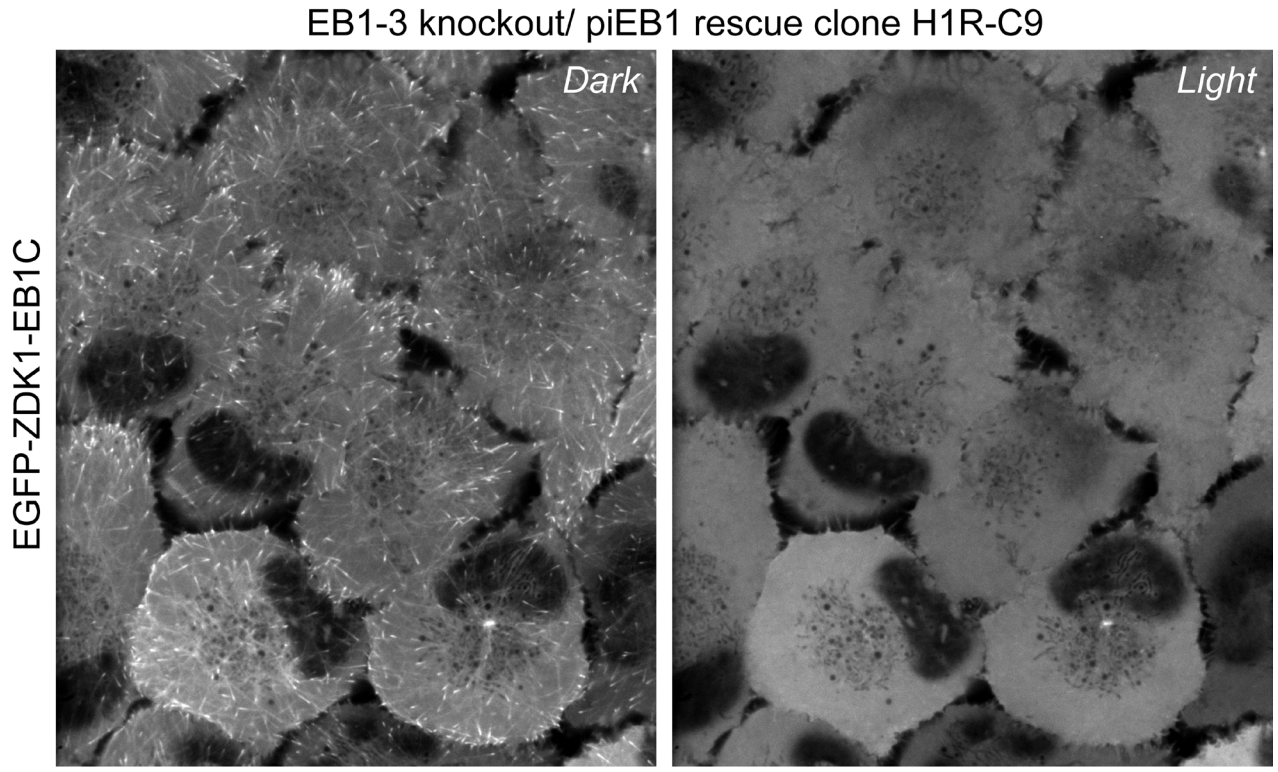


van Haren J. et al. Nature Cell Biology 2018, doi:10.1038/s41556-017-0028-5

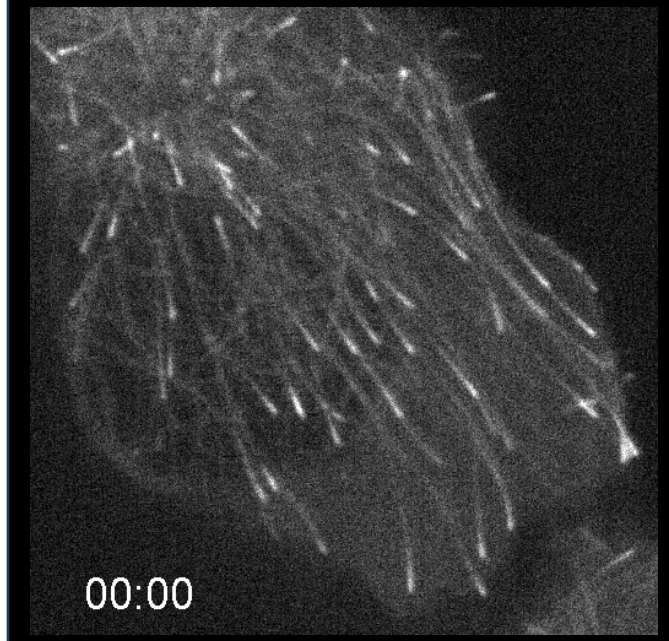
Replacing wt EB1 by pi-EB1



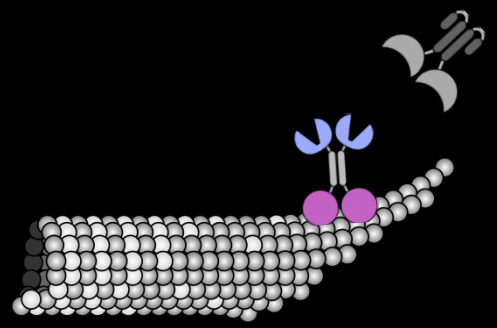
CRISPR/Cas9 knockout of EB1-3
pi-EB1 Rescue

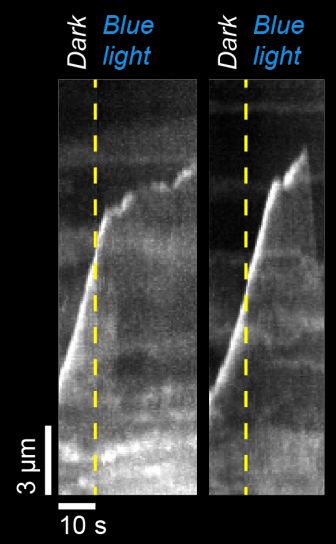


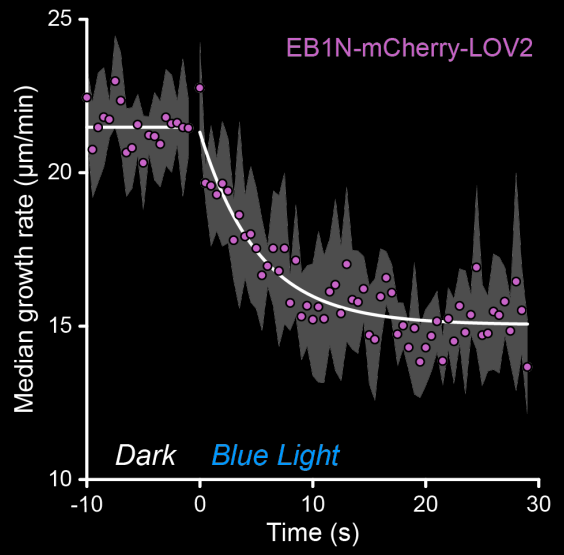
Does π-EB1 inactivation impact MT growth?

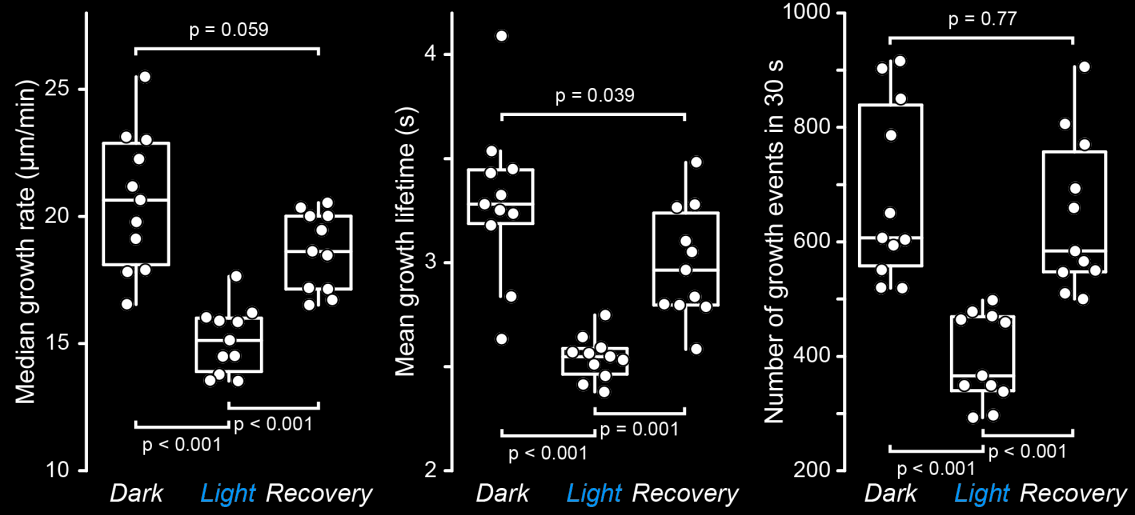


EB1N-mCherry-LOV2

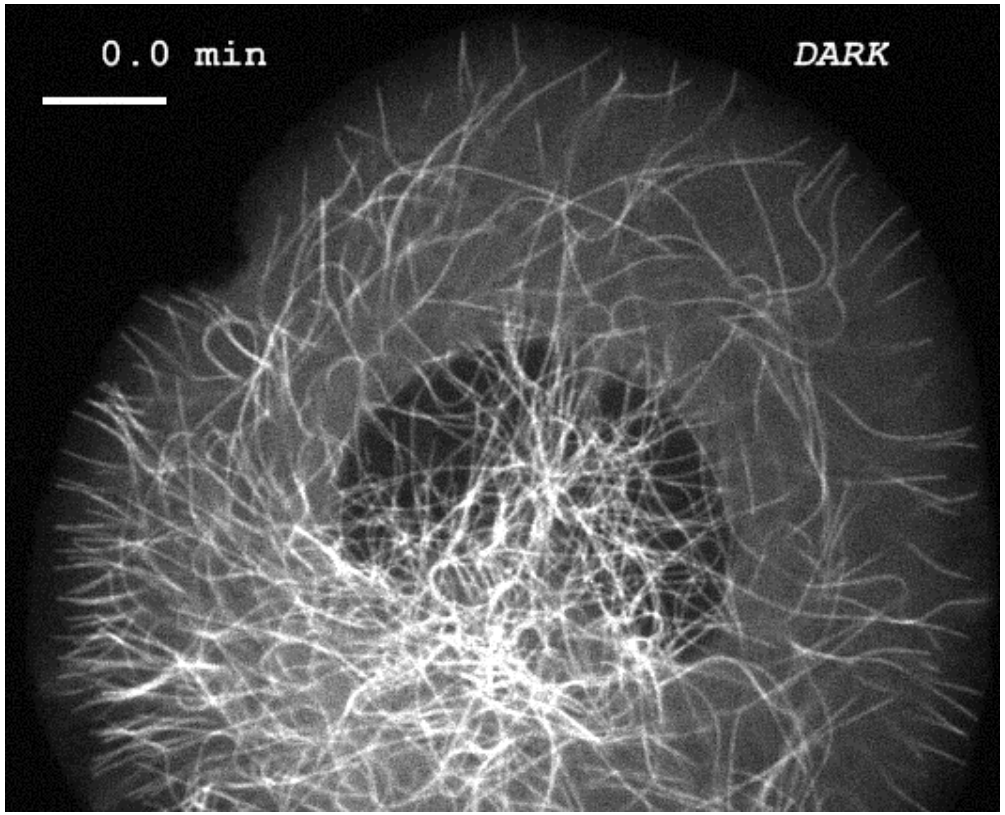






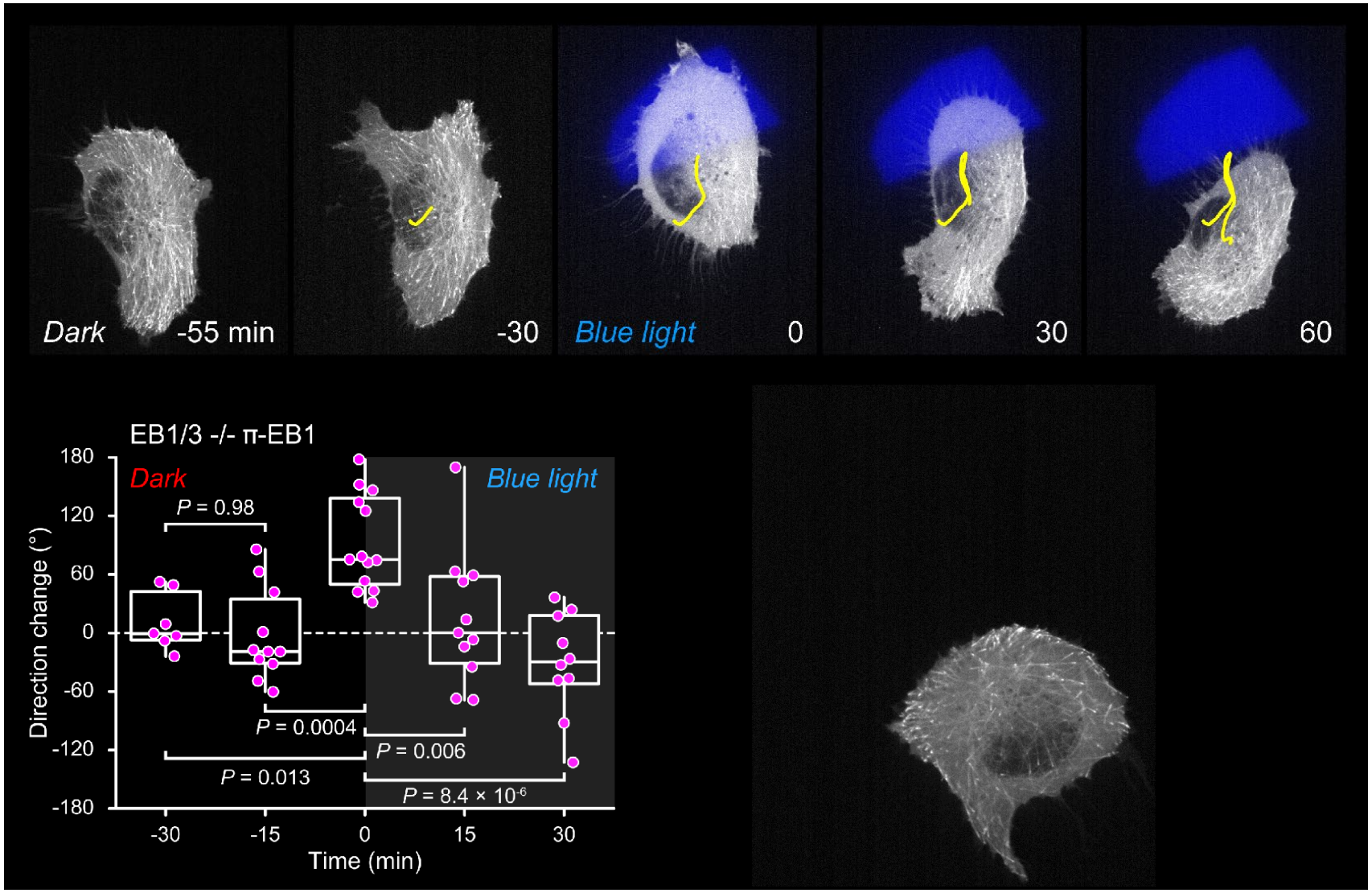


Spatial control of MT growth



pi-EB1 allows for instant and precise spatial manipulation of MT growth dynamics (cell expressing CA-RAC)

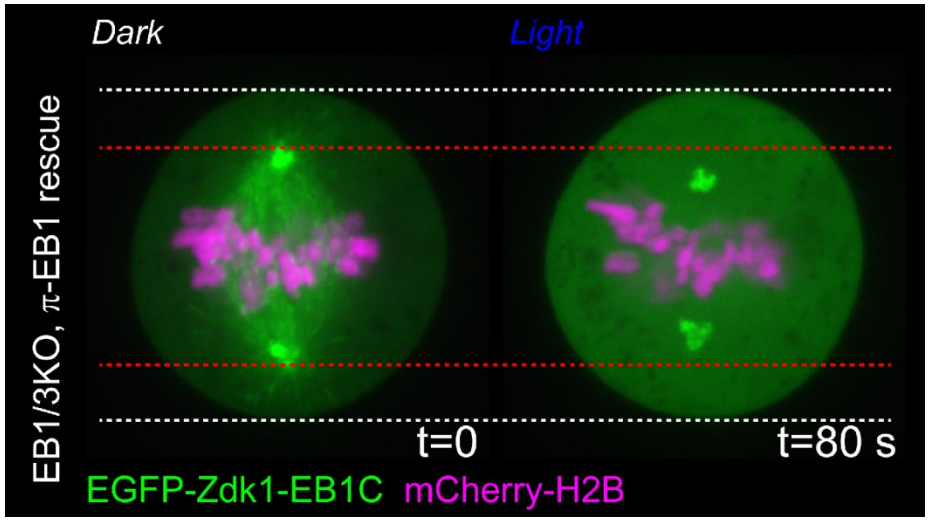
Do local MT dynamics control directional migration?

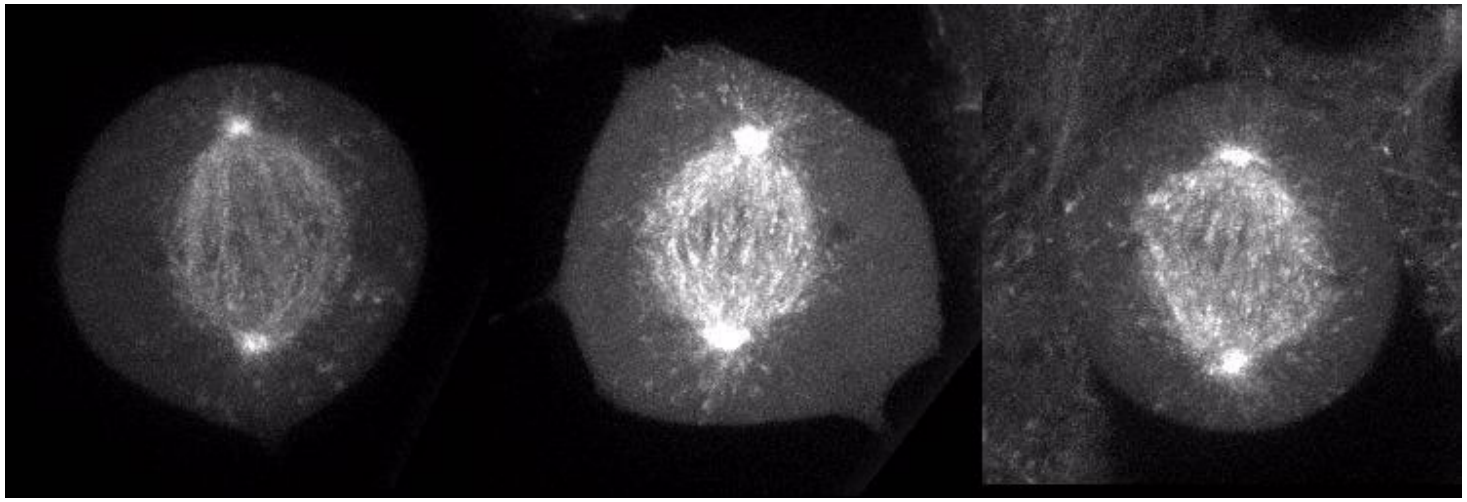


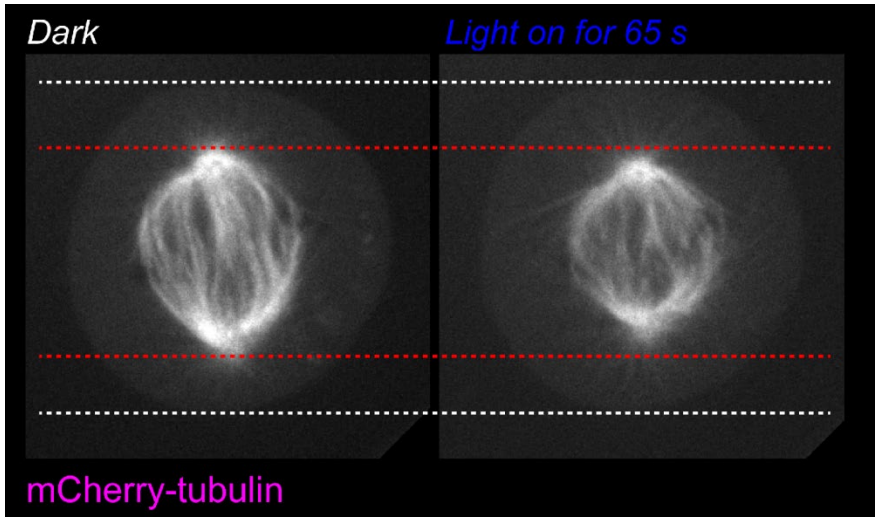
Local inactivation of π-EB1 results in an immediate turning response

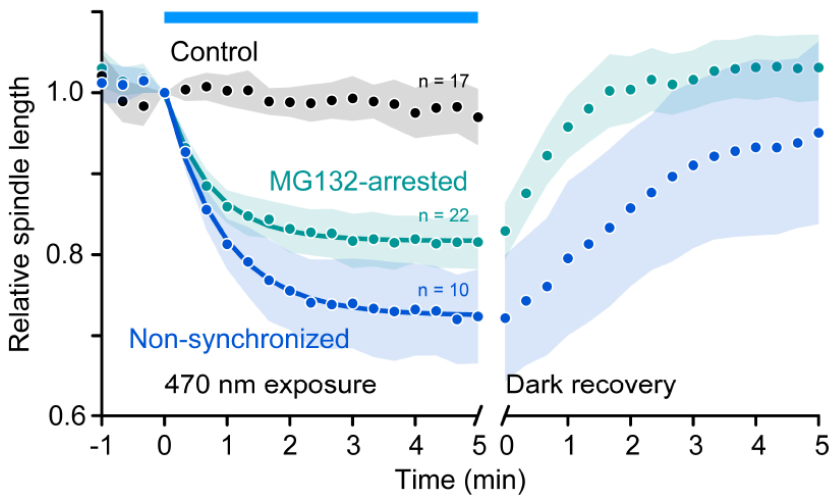
van Haren J. et al. Nature Cell Biology 2018, doi:10.1038/s41556-017-0028-5

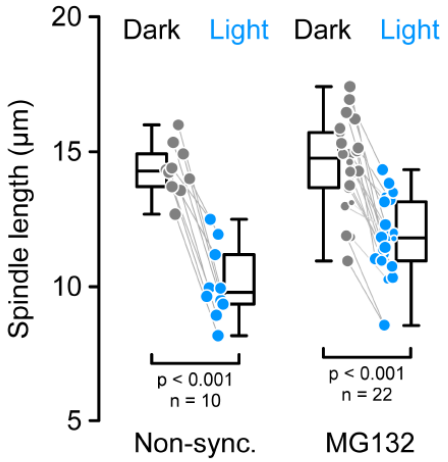
Optogenetic EB1 inactivation shortens metaphase spindles





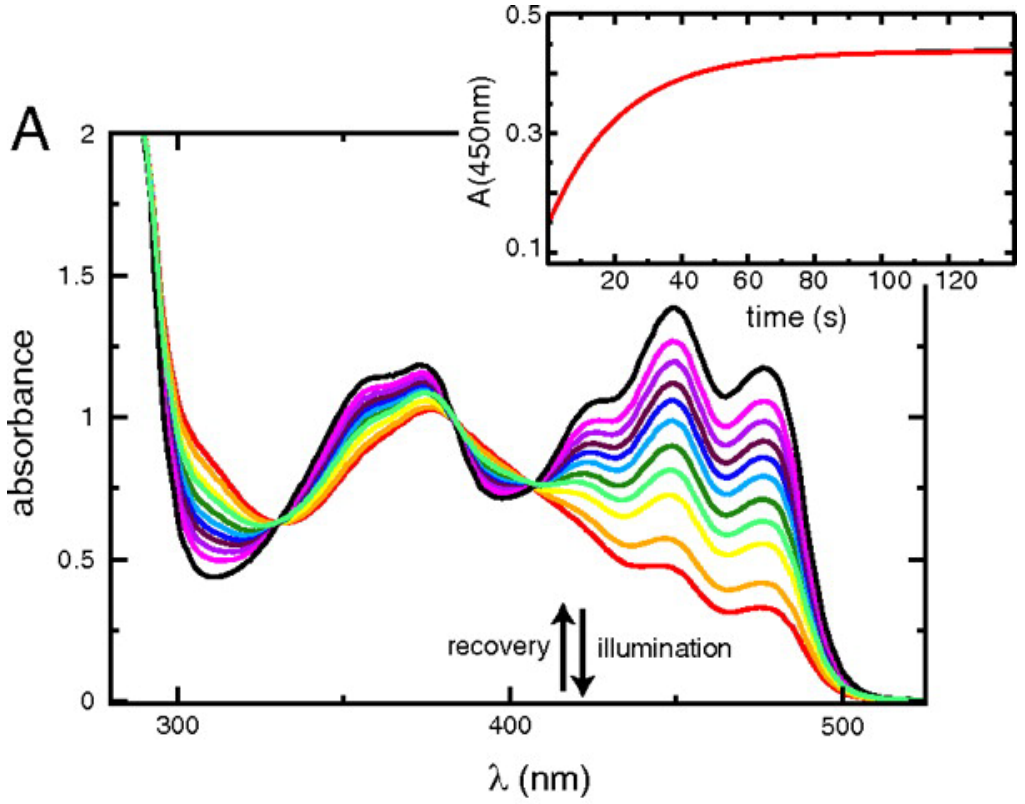




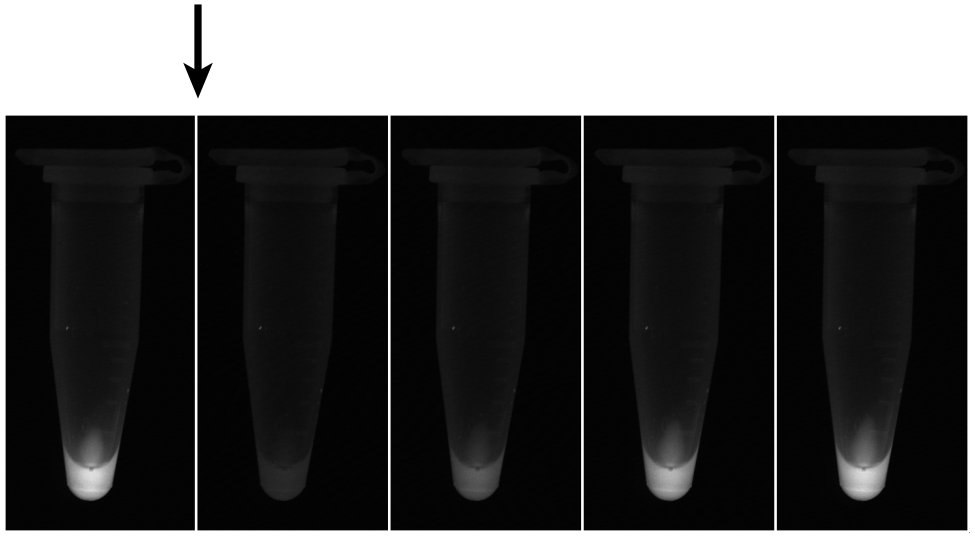


Dema A, van Haren J, Wittmann T. Optogenetic EB1 inactivation shortens metaphase spindles by disrupting cortical force-producing interactions with astral microtubules. March 2022. doi: 10.1016/j.cub.2022.01.017 Current Biology

Tuning the LOV2 photocycle



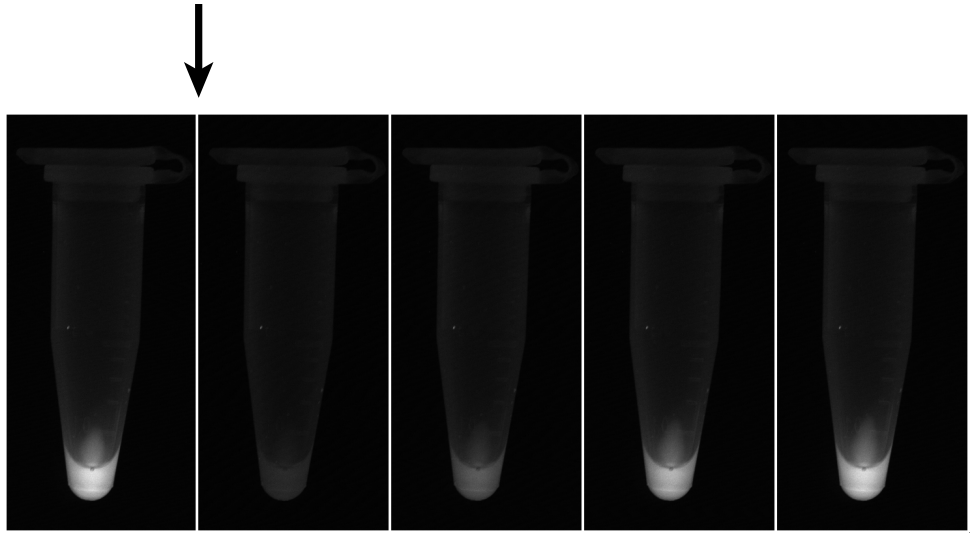
Nash et al 2011, PNAS, EL222 (a LOV-HTH DNA binding protein)



GST- A.s.LOV2 bound to glutathione-sepharose

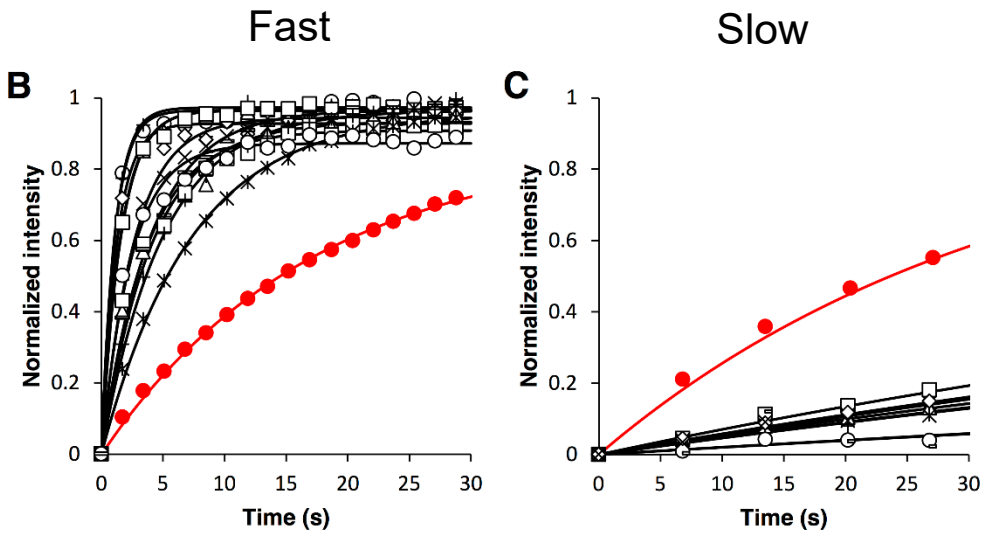
flavin-cysteinyl adduct formation decreases flavin-fluorescence
(in response to blue excitation light)

Fast and slow LOV2 photocycle variants found by directed and random mutagenesis



GST- A.s.LOV2 bound to glutathione-sepharose

flavin-cysteinyl adduct formation decreases flavin-fluorescence
(in response to blue excitation light)

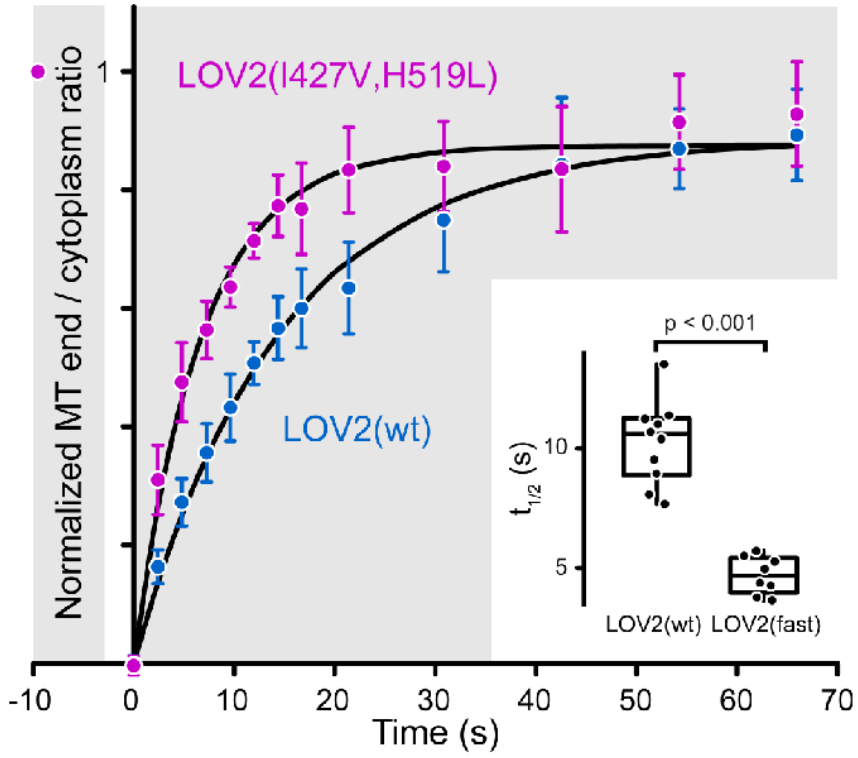


Kawano F, Aono Y, Suzuki H, Sato M. Fluorescence imaging-based high-throughput screening of fast- and slow-cycling LOV proteins. PLoS One. 2013 Dec 18;8(12):e82693. doi: 10.1371/journal.pone.0082693. PMID: 24367542; PMCID: PMC3867380.

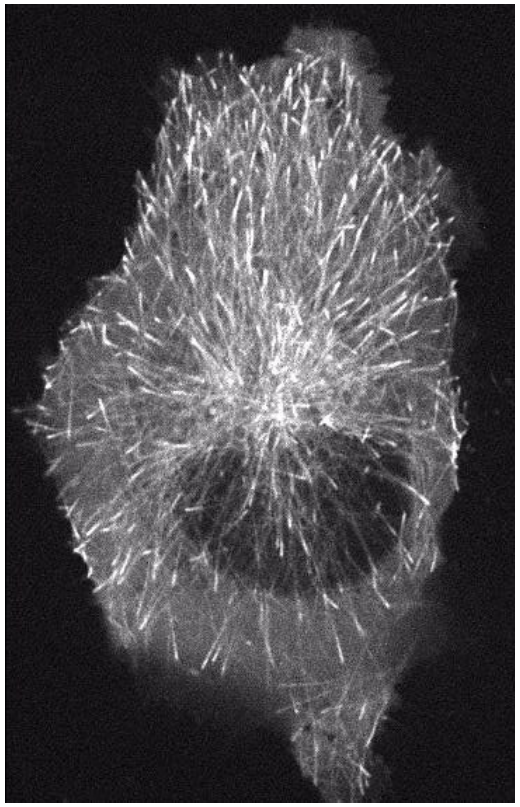
Zayner JP, Sosnick TR. Factors that control the chemistry of the LOV domain photocycle. PLoS One. 2014 Jan 27;9(1):e87074. doi: 10.1371/journal.pone.0087074.

To keep a large fraction of LOVs in a population in the activated state:
- FAST LOVs require very frequent illumination
- SLOW LOVs require very infrequent illumination

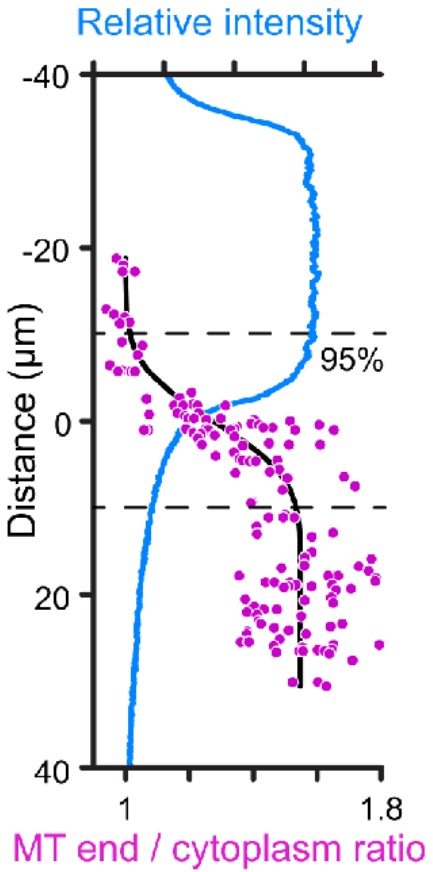
Fast LOV2 enables precise spatial control of π-EB1

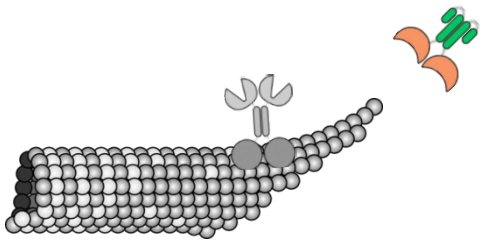


EB1N-LOV2 (fast)



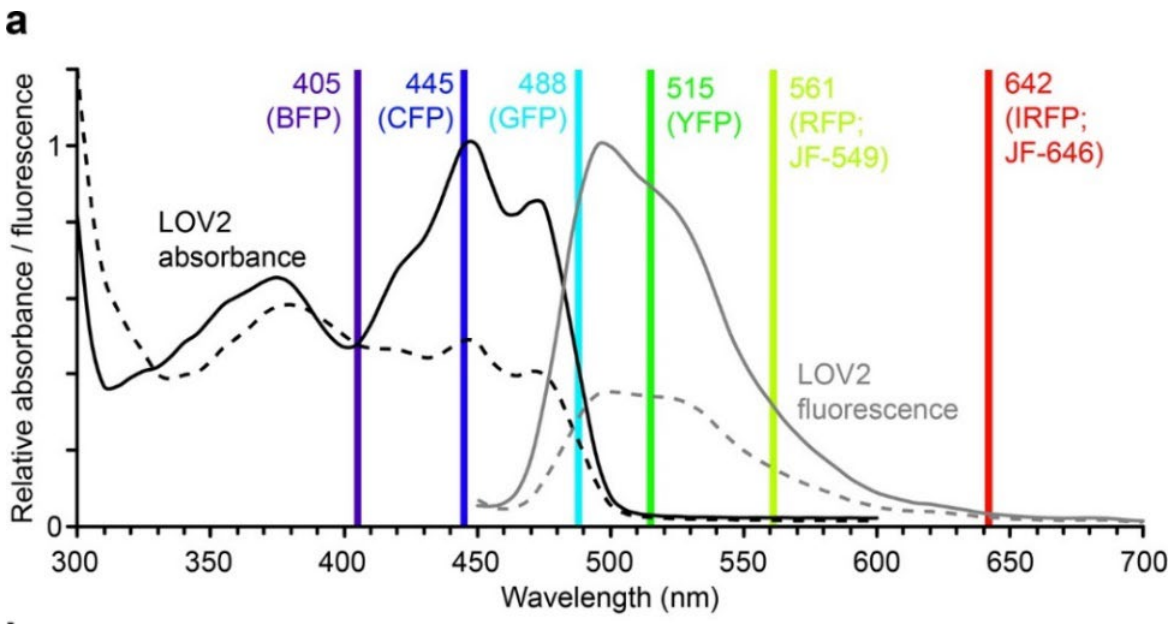
mCherry-ZDK-EB1C

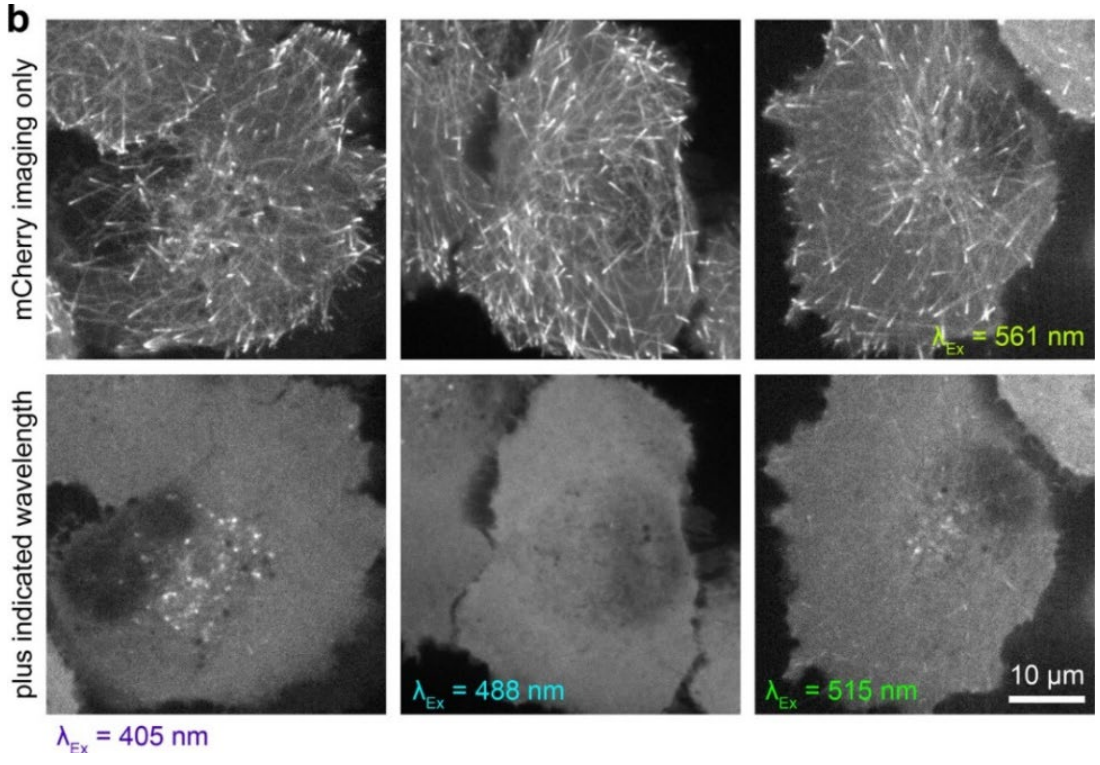




Fast dissociation/re-association kinetics enable precise local control

How to illuminate cells/samples?

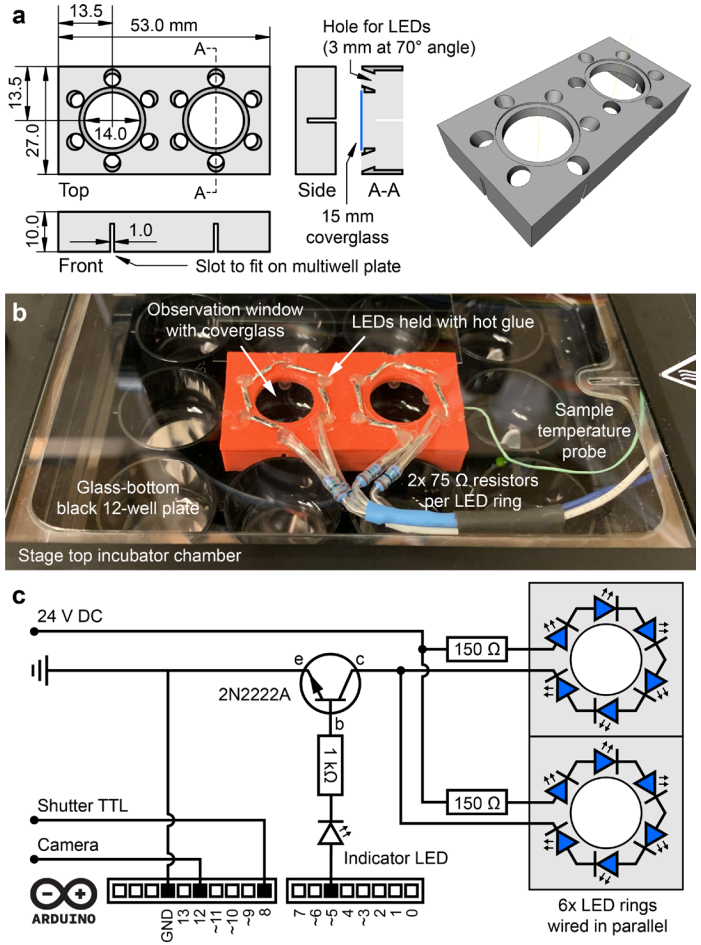




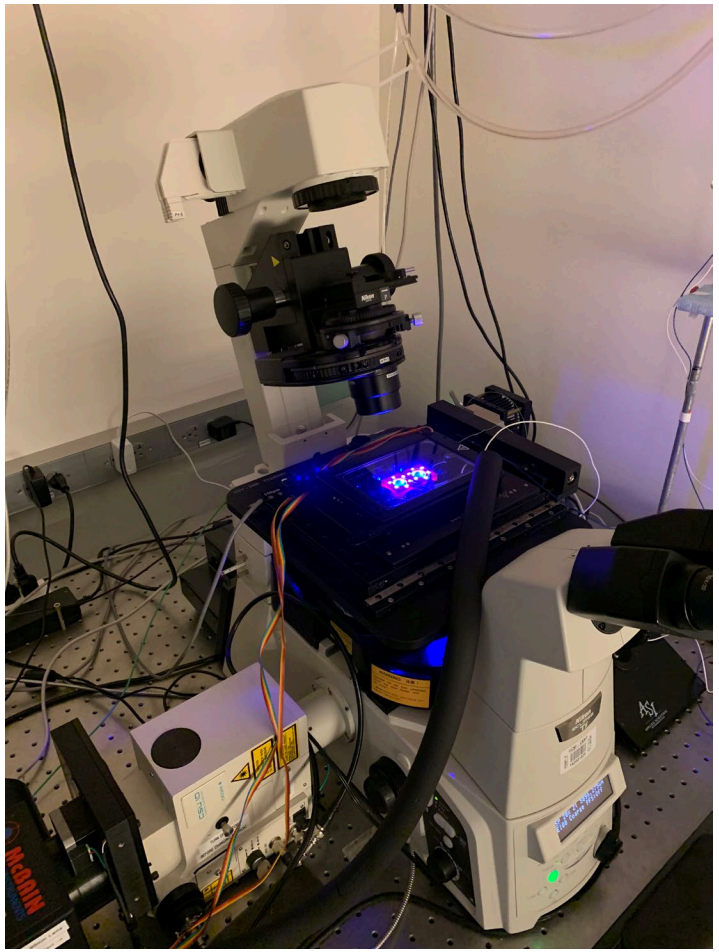
Almost all fluorescence microscopes have lightsources/filtersets for visualizing GFP
and can also be used for activating plant blue light receptors
(limited field of view/ photoactivation determined by objective mag)

Illumination strategies to control LOV2

3D printed LED-cube



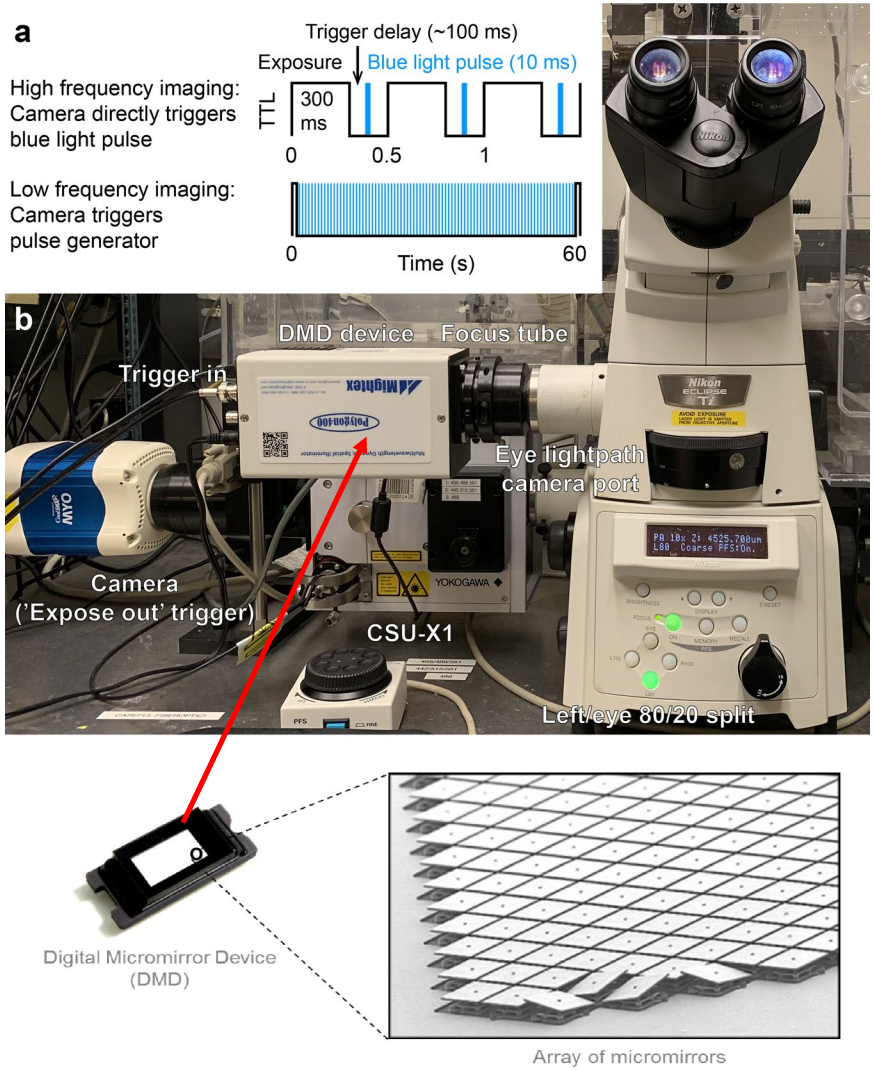
LED-cube in action

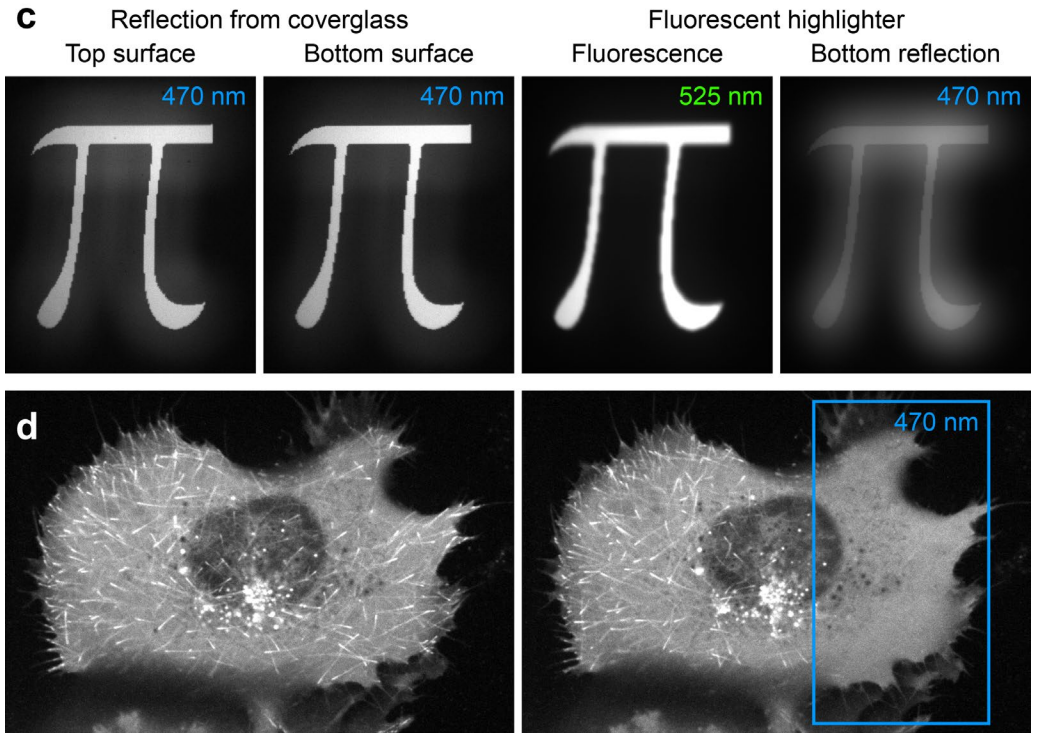


For illuminating large areas, one can use an external light source, such as this LED-cube

Jeffrey van Haren, Lauren S. Adachi and Torsten Wittmann. Optogenetic Control of Microtubule Dynamics, **Methods in Molecular Biology, Cytoskeleton Dynamics** 2020

A DMD allows for very precise spatial control





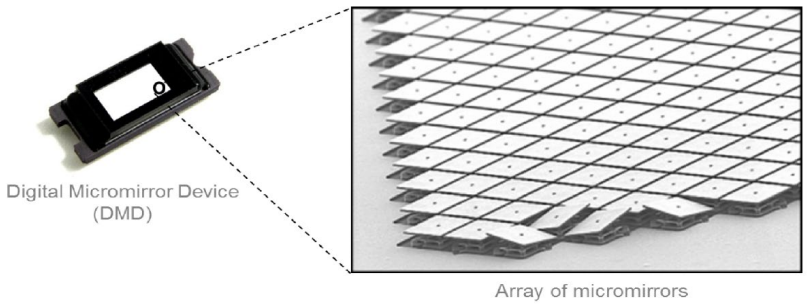
Jeffrey van Haren, Lauren S. Adachi and Torsten Wittmann. Optogenetic Control of Microtubule Dynamics, **Methods in Molecular Biology, Cytoskeleton Dynamics** 2020

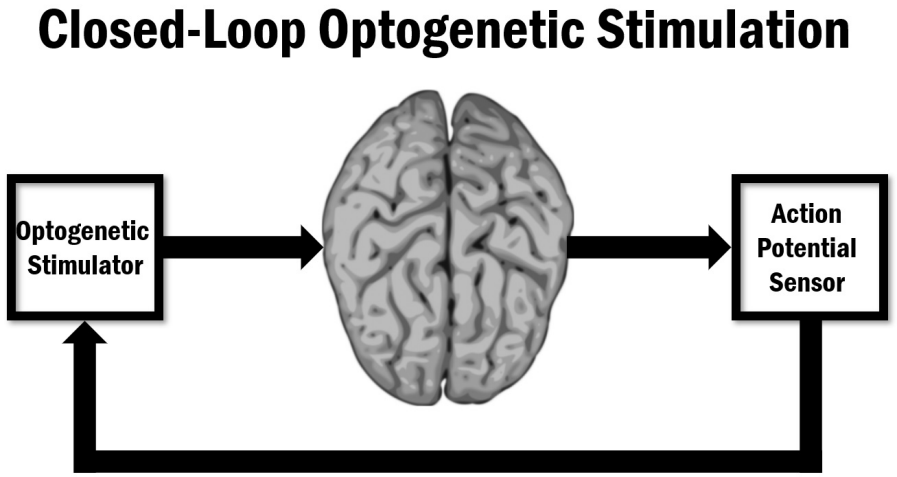
Latest generation DMDs enable multicolor/gradient patterning and…

Older DMDs, such as the one shown earlier (Polygon 400) are quite slow.
(No gradients patterns, and only one color at a time)

Newer generation of DMDs are however much faster (PG-1000: 396 fps)
and these allow for near-simultaneous:

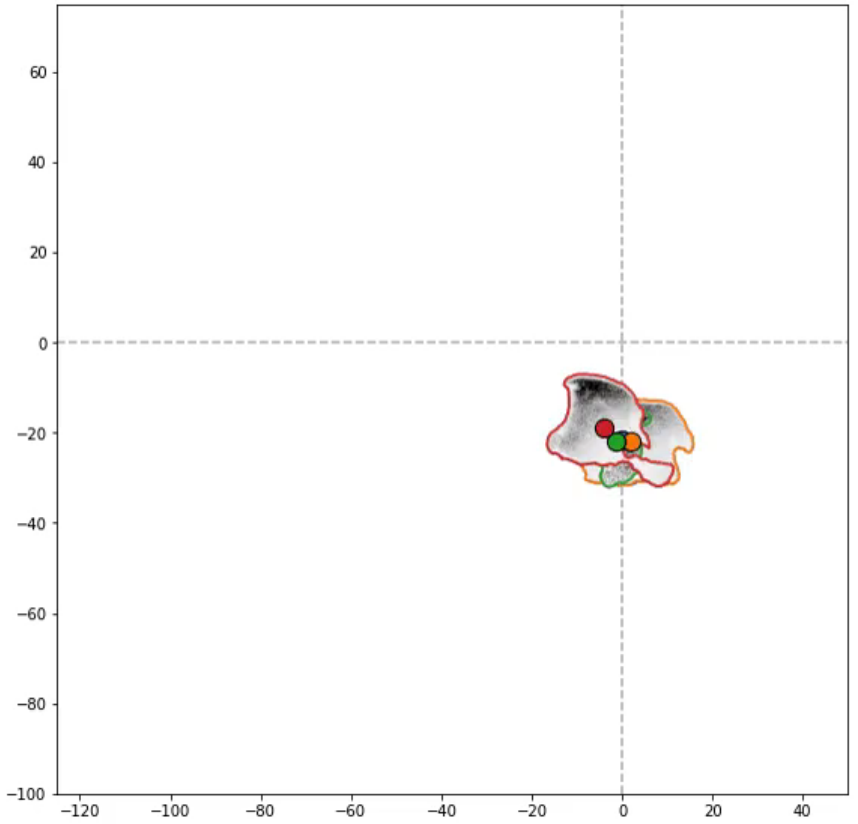
- **Multicolor patterning** (rapid alternation of patterns and excitation light)
- **Gradient patterning** (by rapidly changing the illumination pattern)
- **"Closed-loop" control**





Closed-Loop Optogenetic Cell Control

Example of feedback control by Jason Town, Orion Weiner (PLOS Biology 2023)

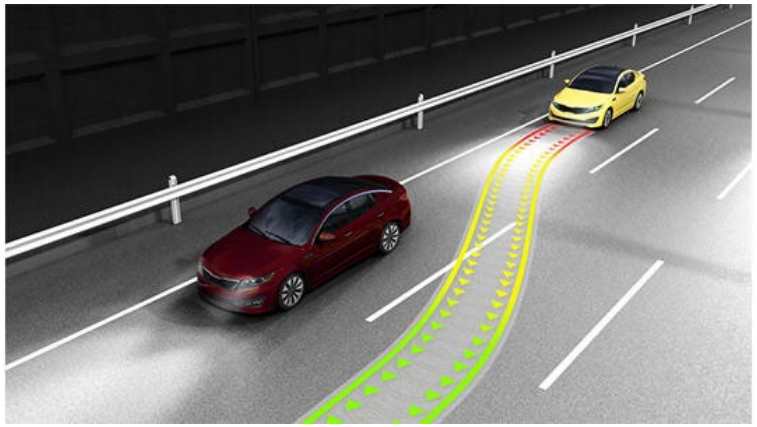


Cells moving in circles, adaptive optogenetic stimulus to locally activate Rac (through PI3K)

Closed-loop optogenetic control of cell biology enables outcome-driven microscopy

Supplementary Video 3

Outcome-driven control of directed cell migration
Multiple cells with active avoidance



collision avoidance system

Passmore, J.B., Rates, A., Schröder, J. *et al.* Closed-loop optogenetic control of cell biology enables outcome-driven microscopy. *Nat Commun* **17**, 1087 (2026). https://doi.org/10.1038/s41467-025-67848-5

summary

Many optogenetic switches/modules have been developed already (many based on LOV2). Good starting point for building your own?

-Specific optoswitches: LOV2 fusion, loop-insertion, allosteric control of POI.
-General relocalization modules: recruitment, delocalization.

Optogenetics enables:

- -Dissecting protein functions
  - Precise temporal control
  - Precise spatial control
  - Feedback control
- -Potential for guiding/controlling biology: tissue engineering?

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questions?