IMAGING MOLECULAR PROCESSES IN CELLS BY POLARIZATION-RESOLVED FLUORESCENCE MICROSCOPY

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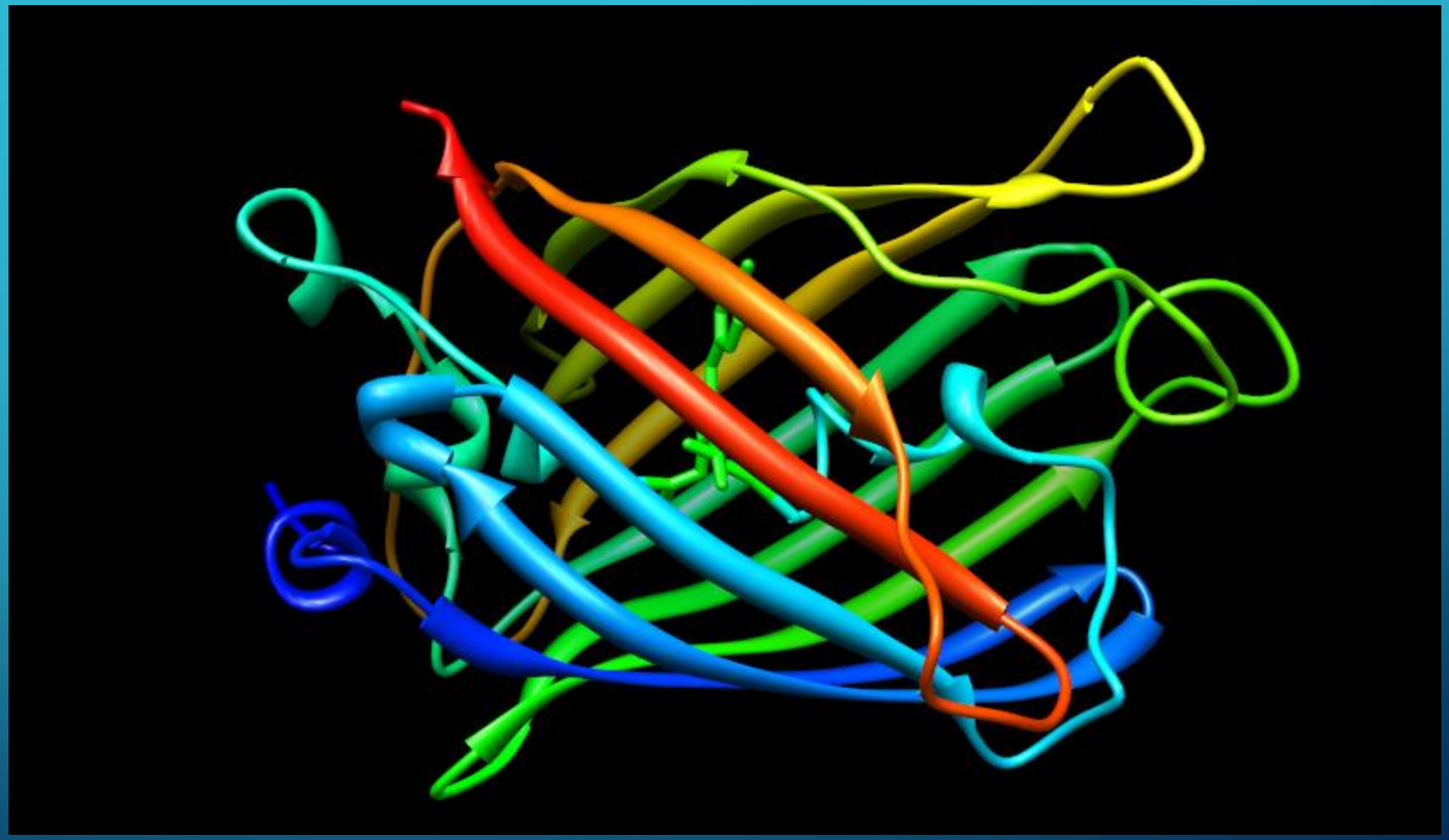
THE GREEN FLUORESCENT PROTEIN



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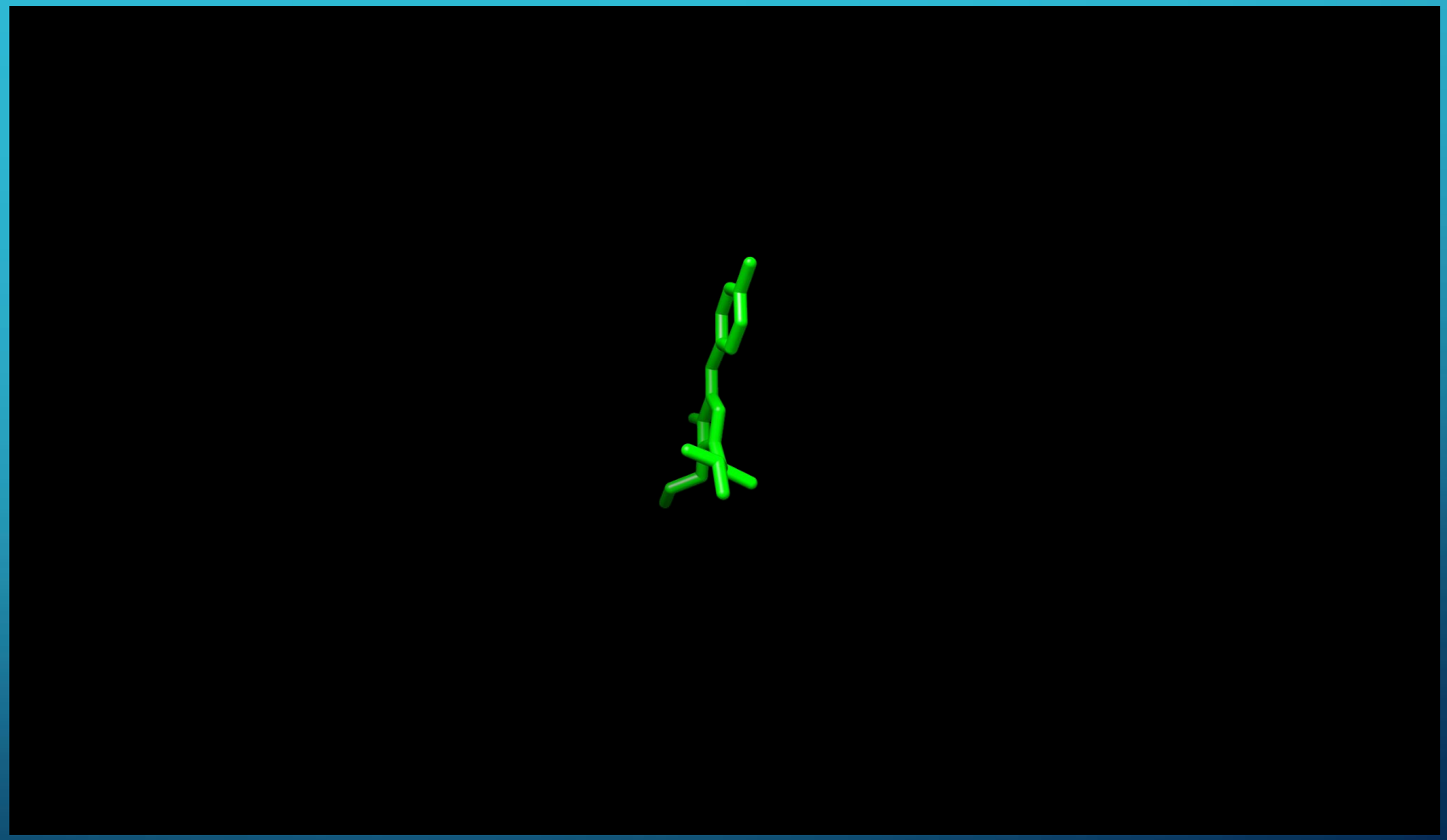

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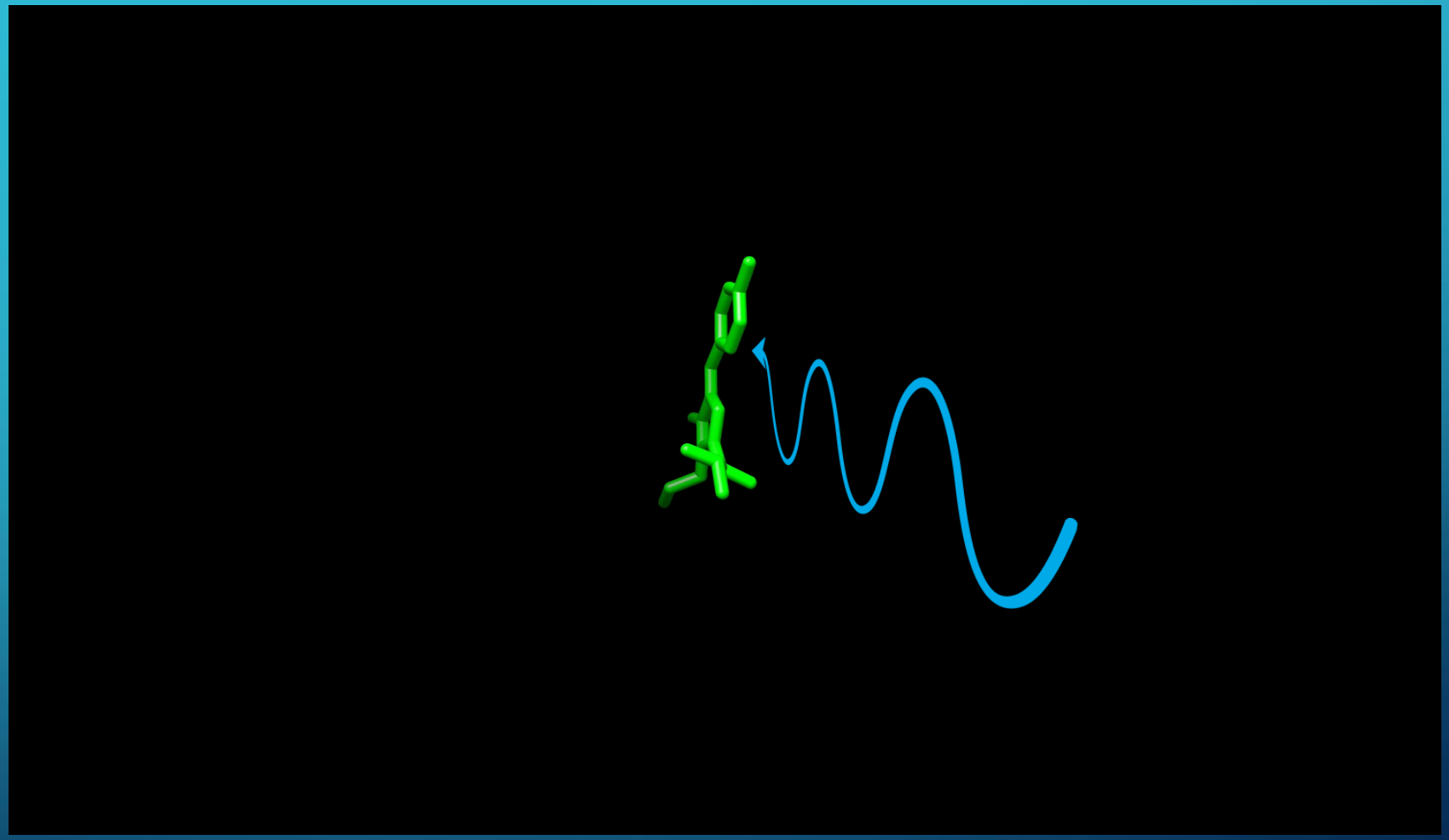

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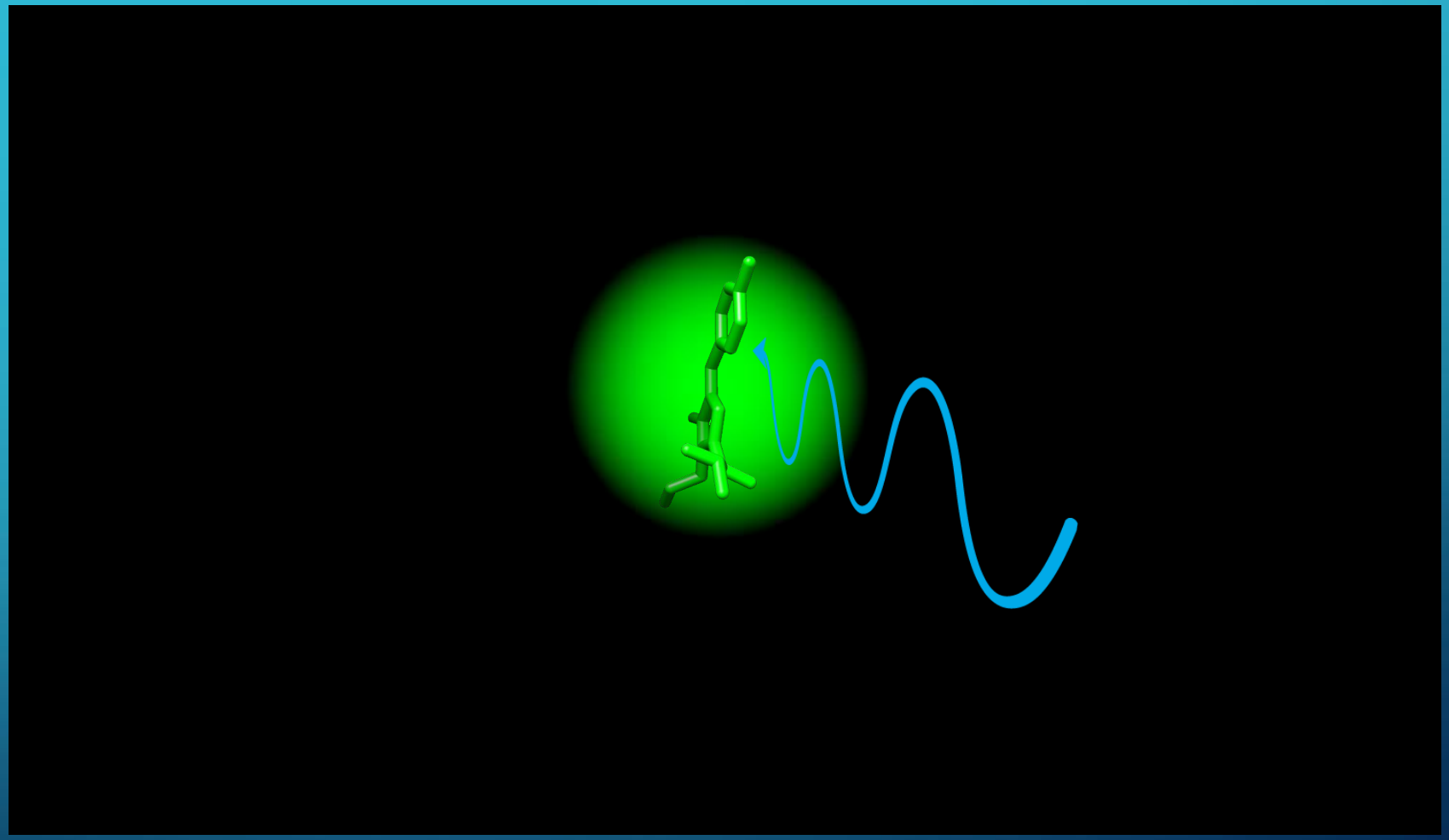

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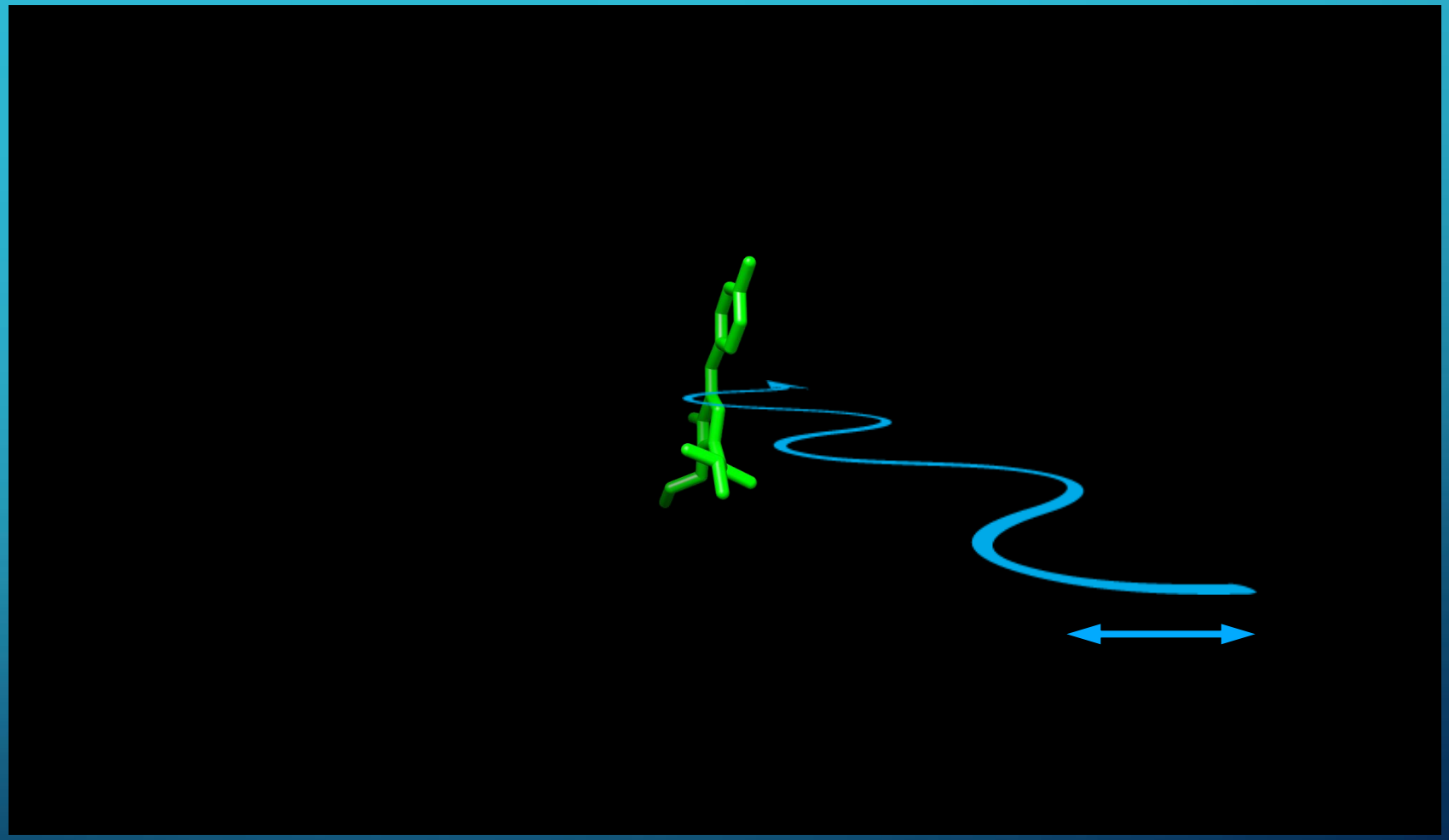

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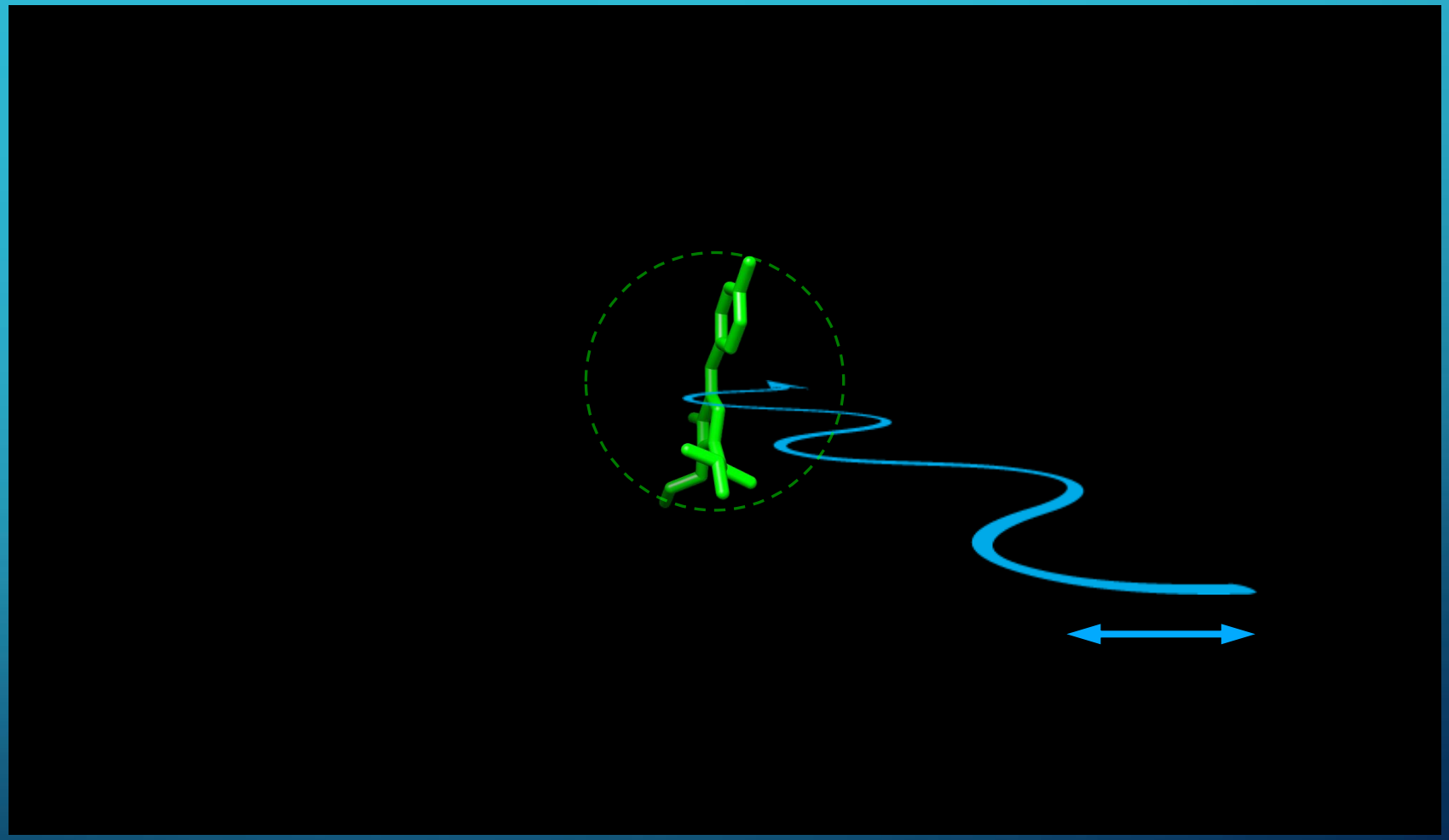
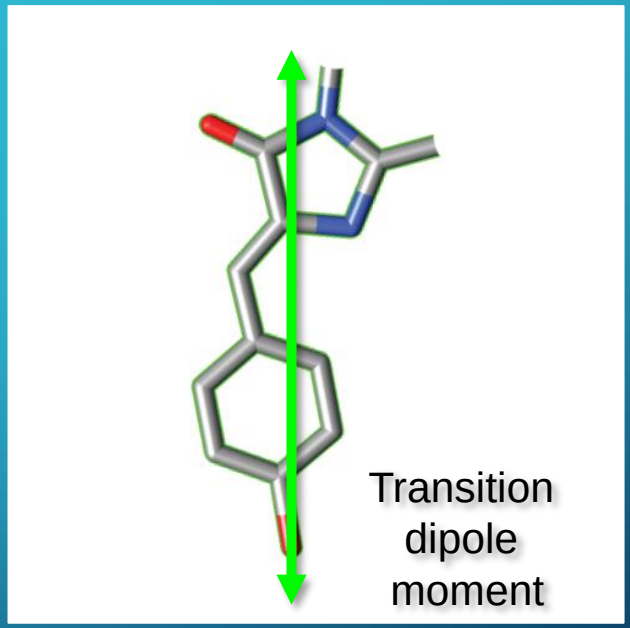
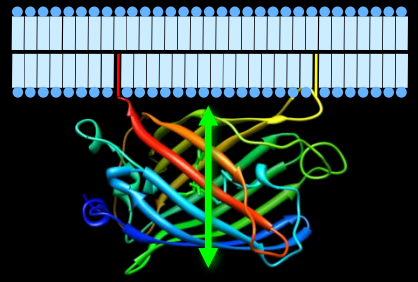


MOLECULES ARE LIKE ANTENNAS



DOUBLY LIPIDATED eGFP



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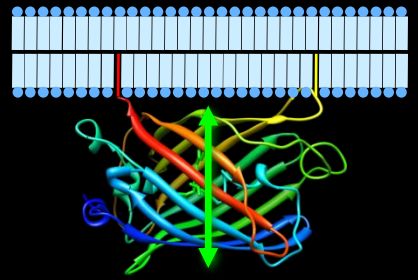

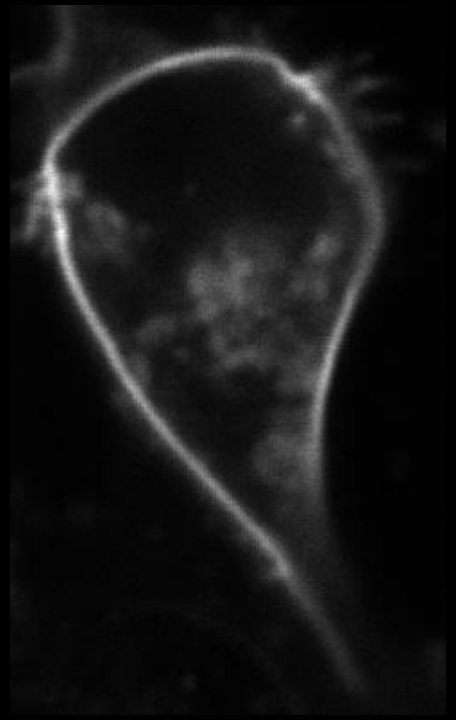

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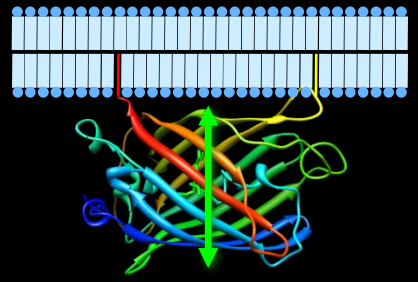
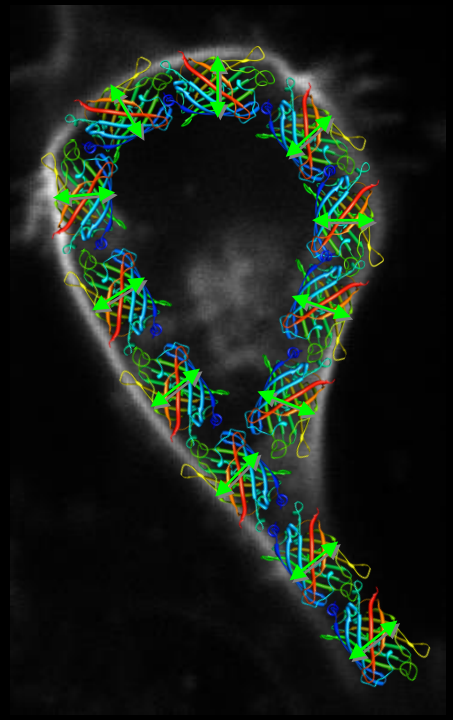
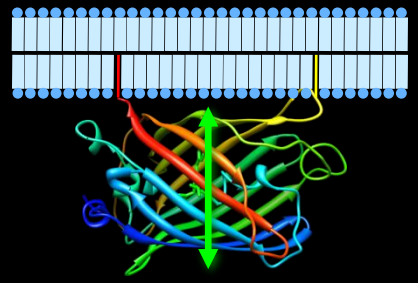
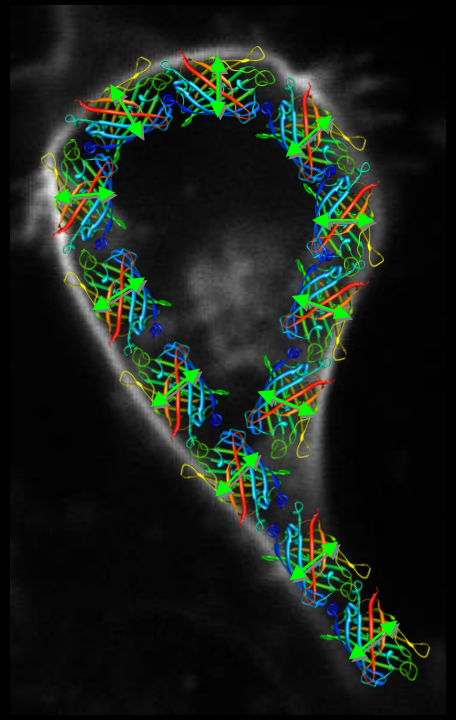
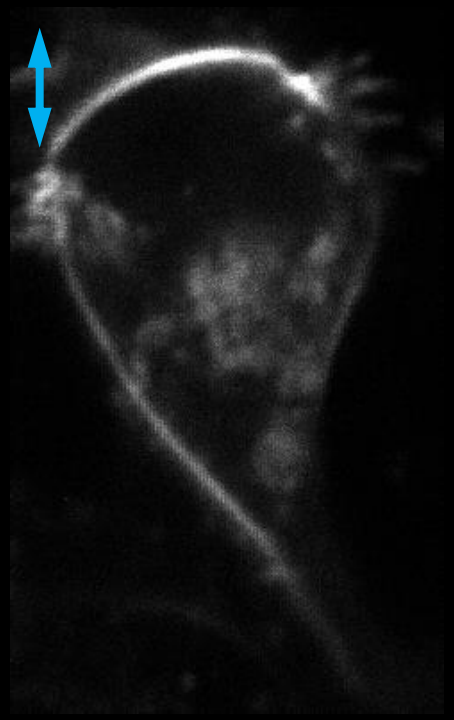


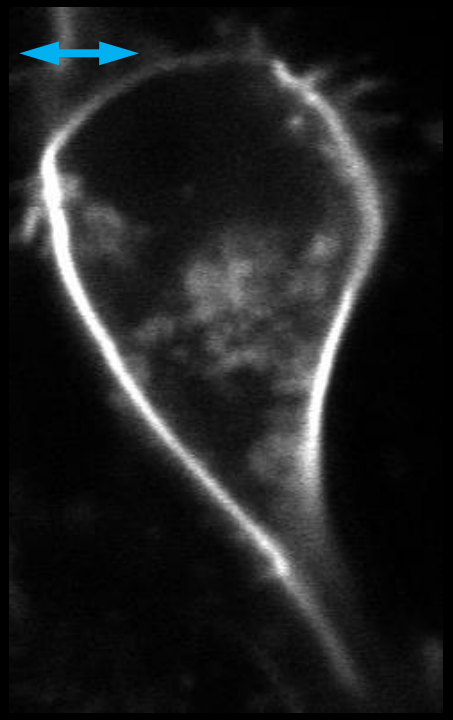


DOUBLY LIPIDATED eGFP









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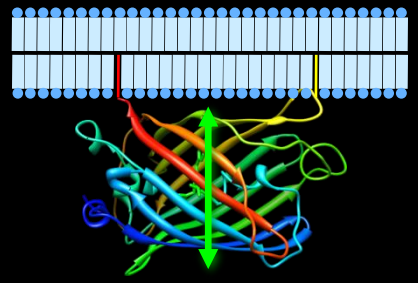

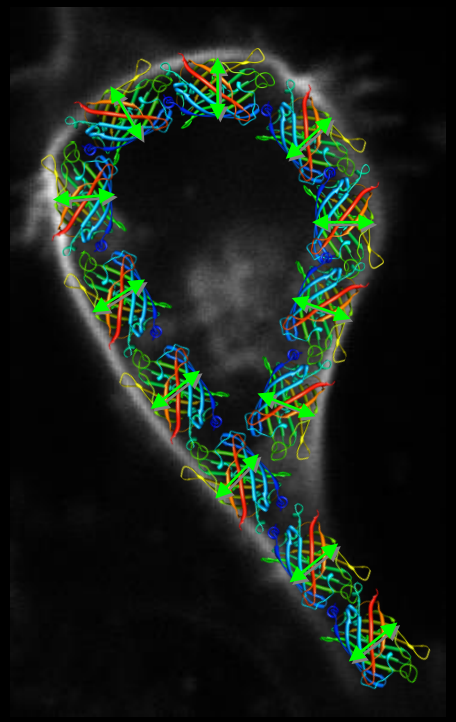

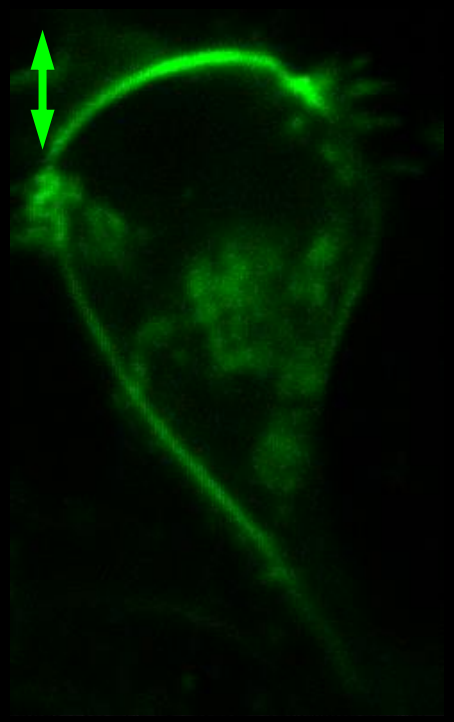

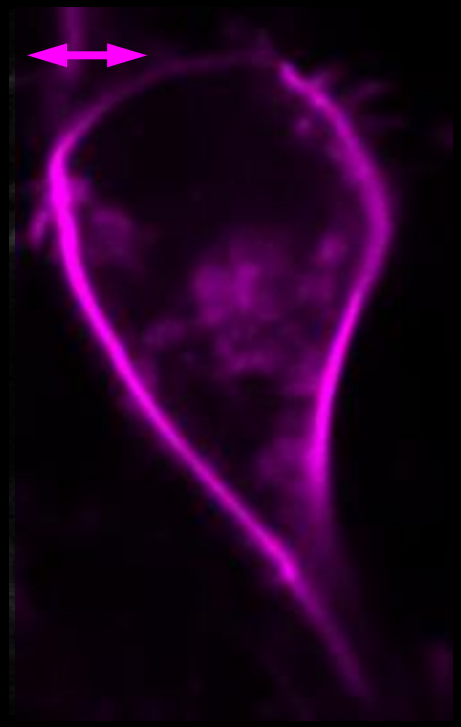

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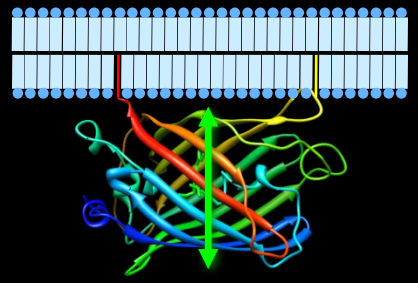
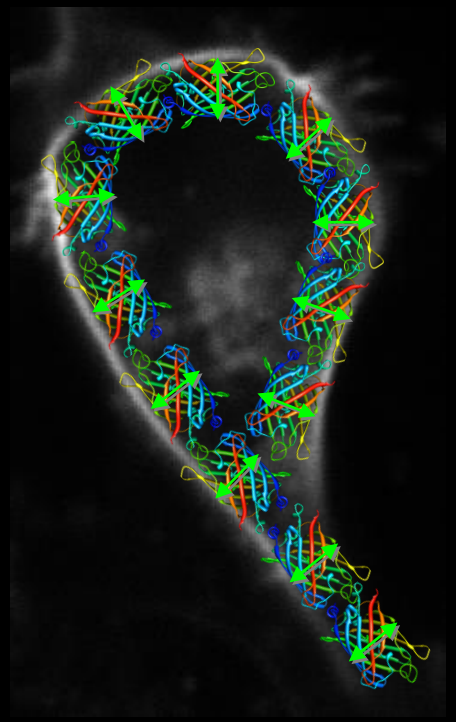
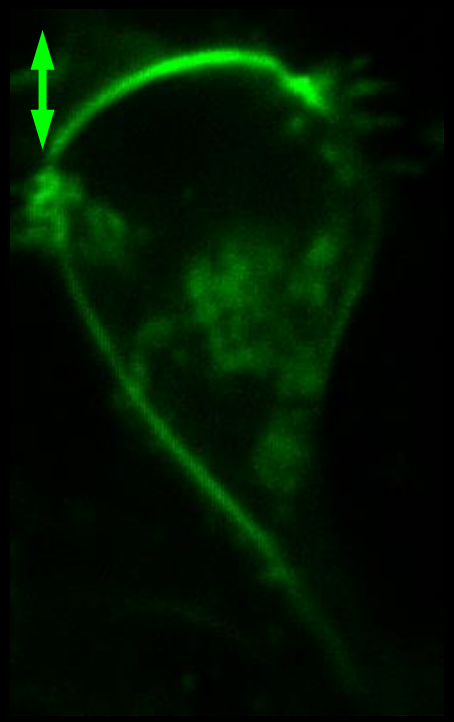
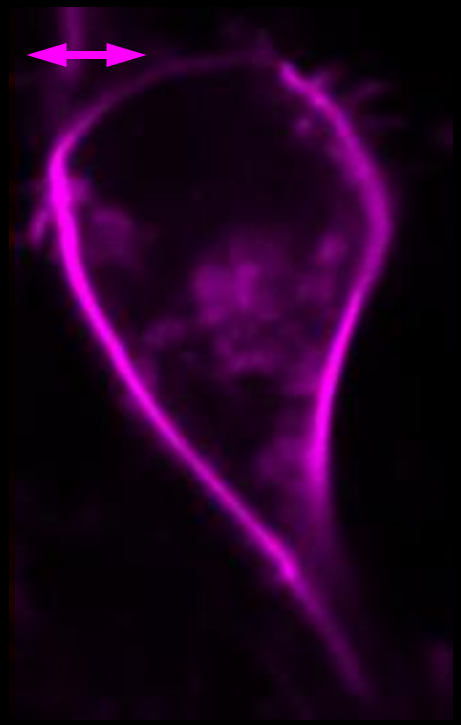
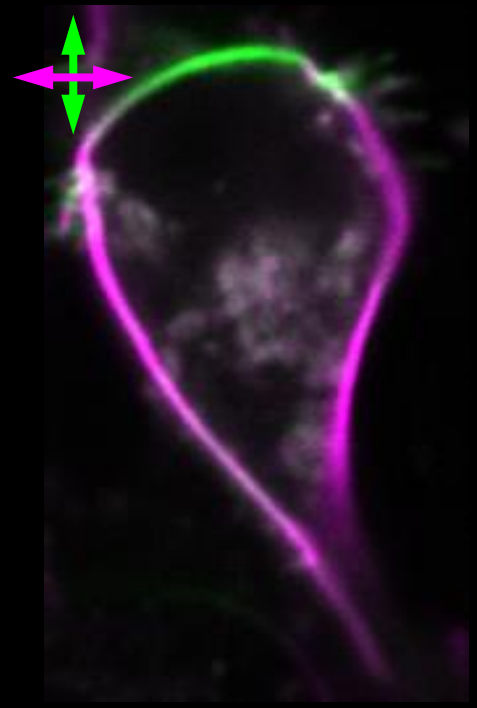


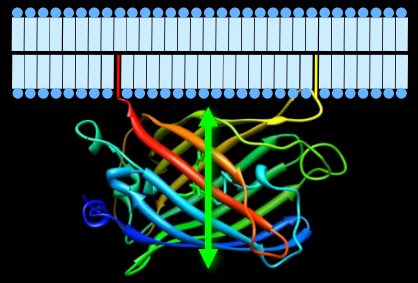


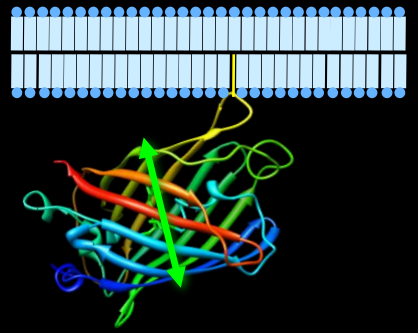


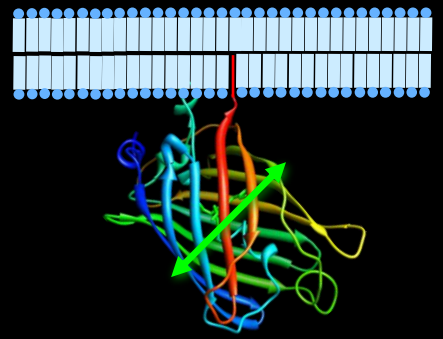


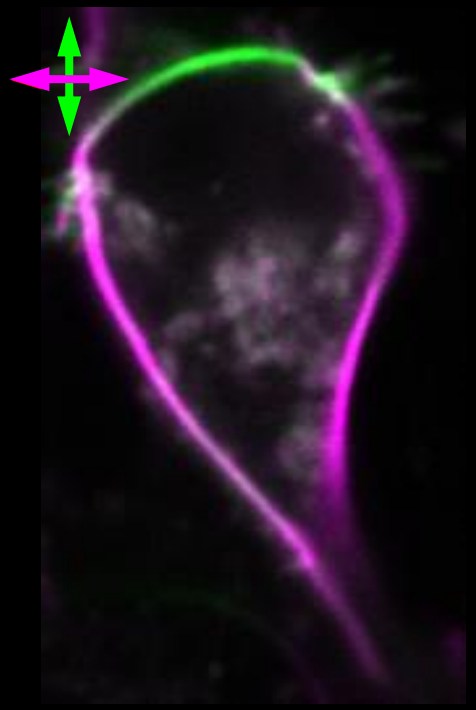


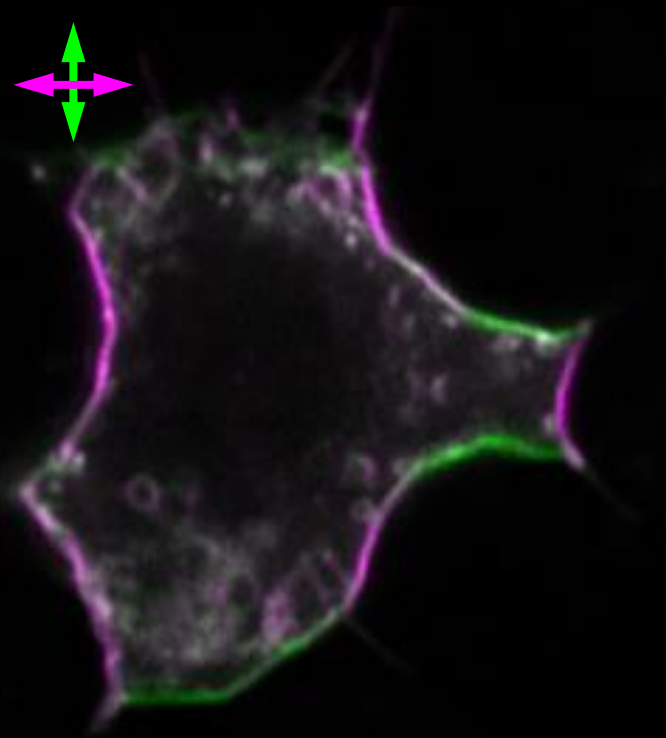
LINEAR DICHROISM REPORTS ON MOLECULAR ORIENTATION

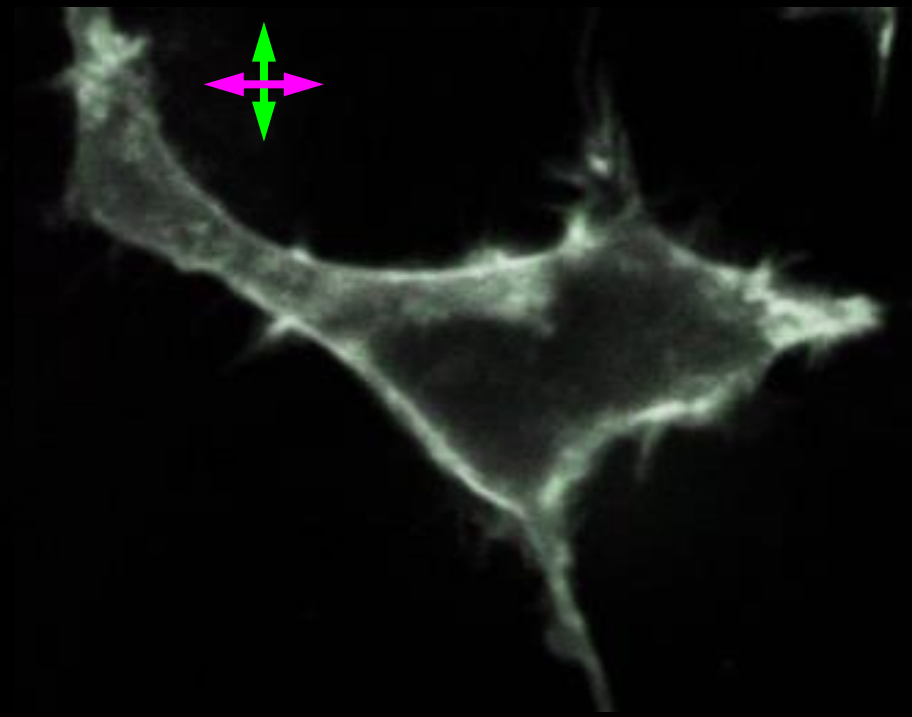




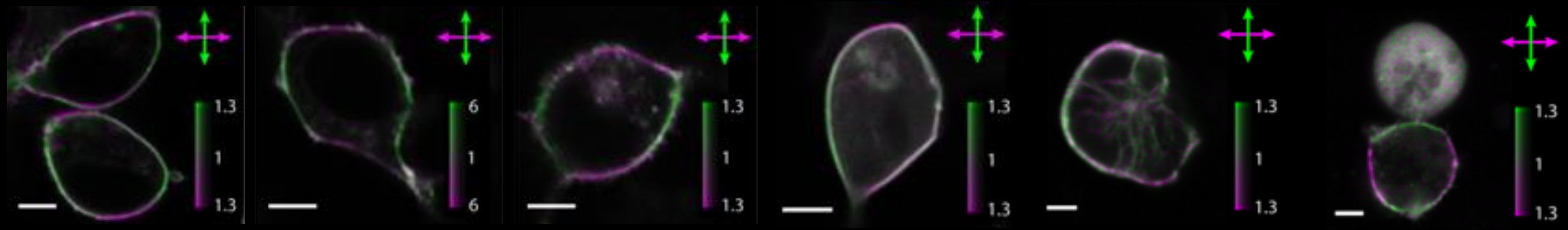




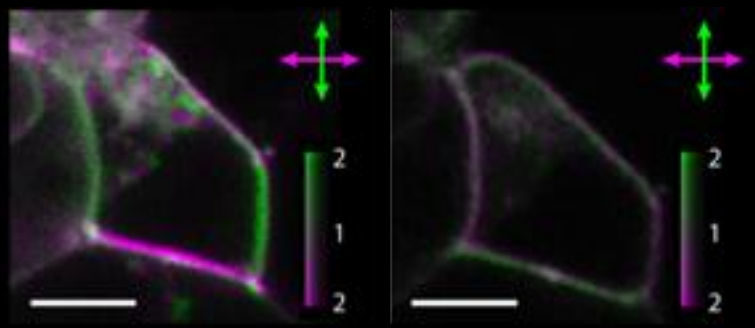




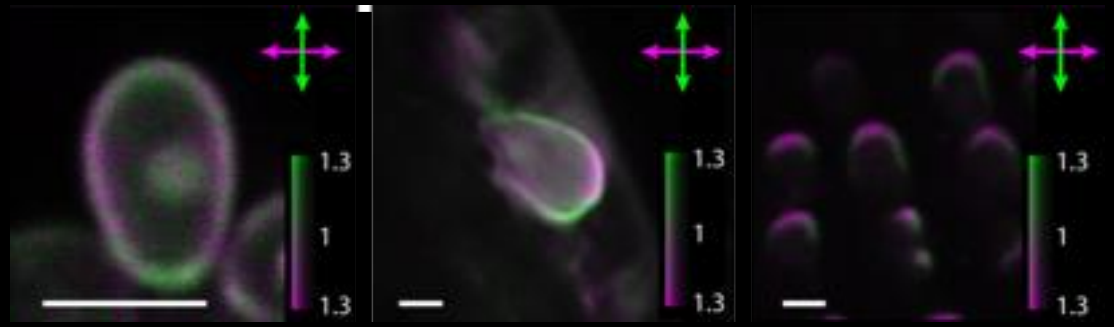
LINEAR DICHROISM IS UBIQUITOUS



a2A receptor-YFP mGluR1a-YFP α2AR-CFP channelrhodopsin-GFP tau-GFP PKC-dsRed



Gα-CFP Gβ-YFP

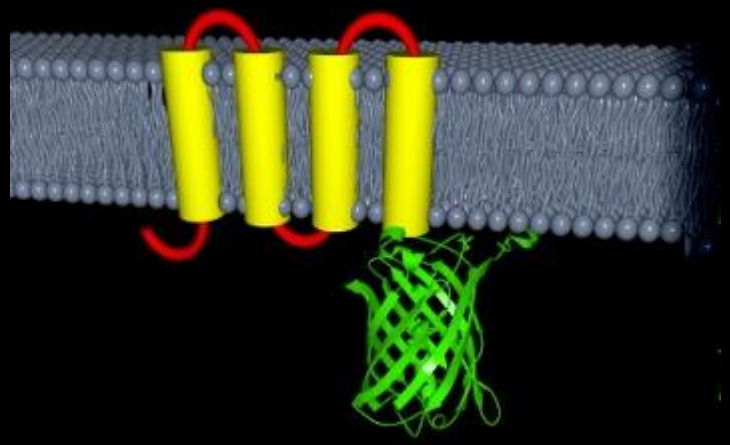


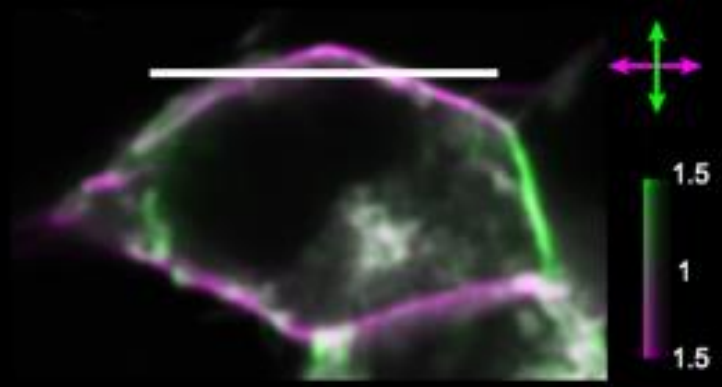
S. cerevisiae C. elegans D. melanogaster

Lazar J. & al., Nature Methods 2011

IMAGING CONFORMATIONAL CHANGES IN MEMBRANE PROTEINS

ArcLight: a genetically encoded fluorescent voltage sensor



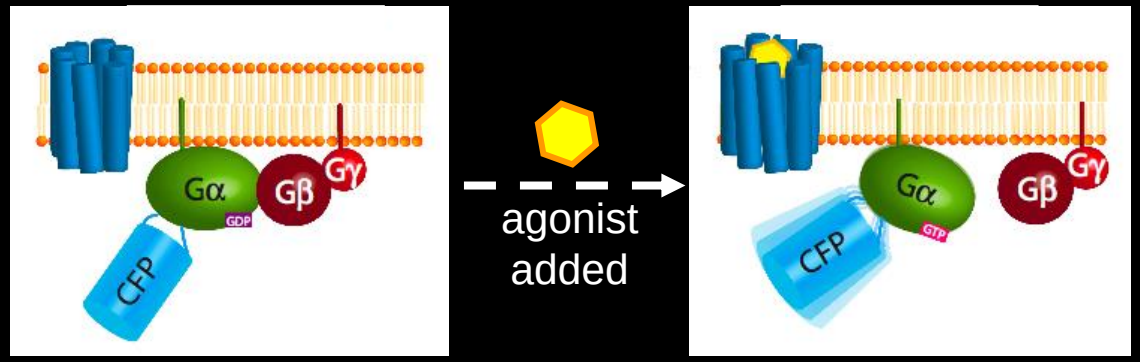


- a ratiometric voltage sensor, should allow determining absolute voltages

Lei J. & al., PLoS One 2013

IMAGING PROTEIN-PROTEIN INTERACTIONS

Activation of heterotrimeric G-proteins:

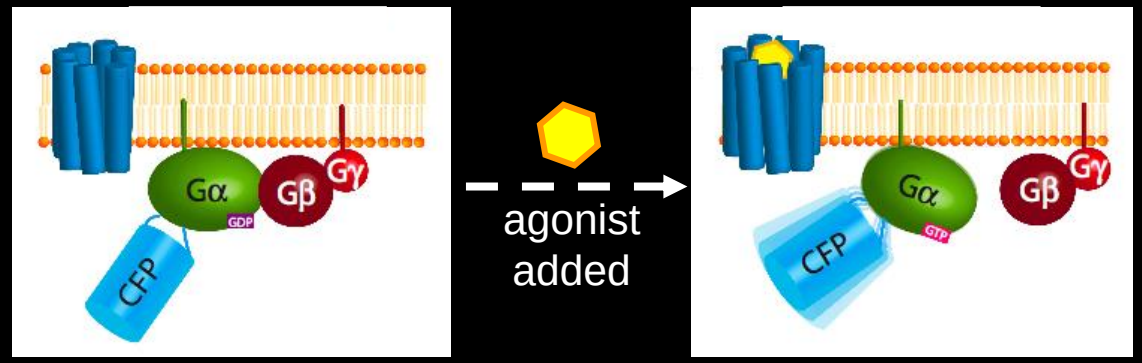


resting state

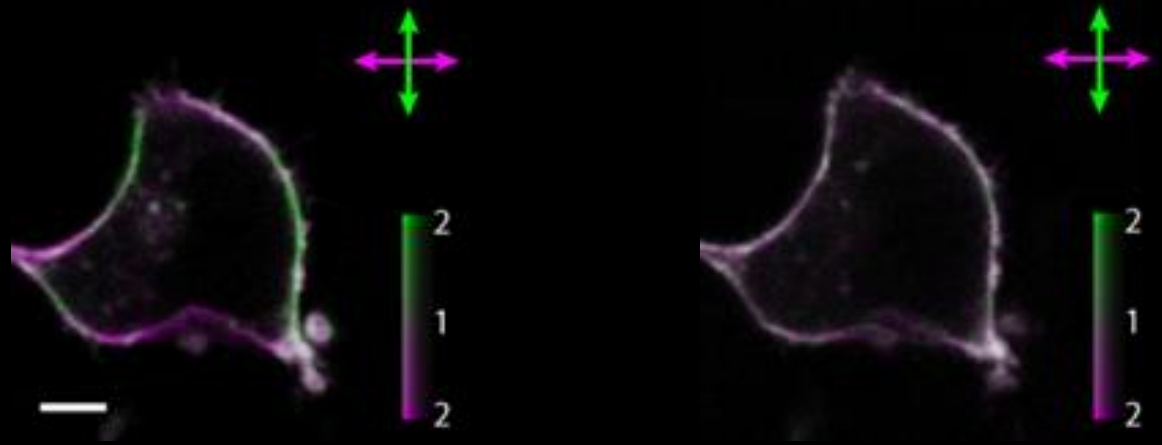
activated state

IMAGING PROTEIN-PROTEIN INTERACTIONS

Activation of heterotrimeric G-proteins:



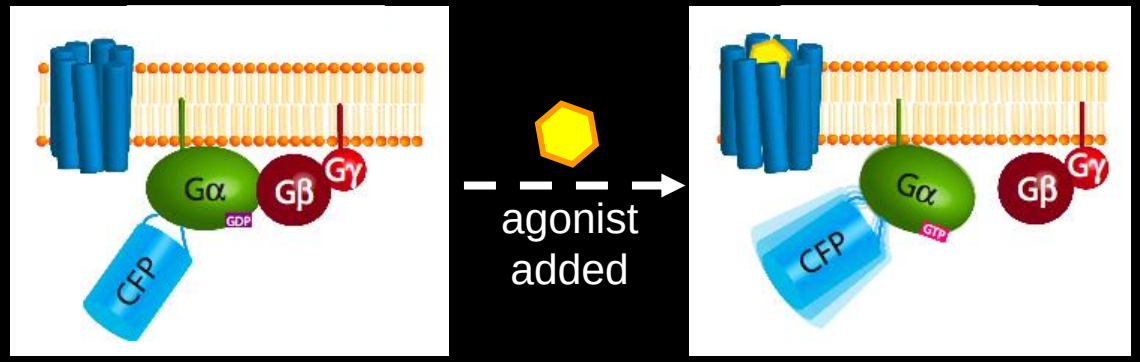
resting state activated state



Gαi2 (GAP43-CFP-Gαi2, Gβ1, Gγ2, α2-AR-YFP)

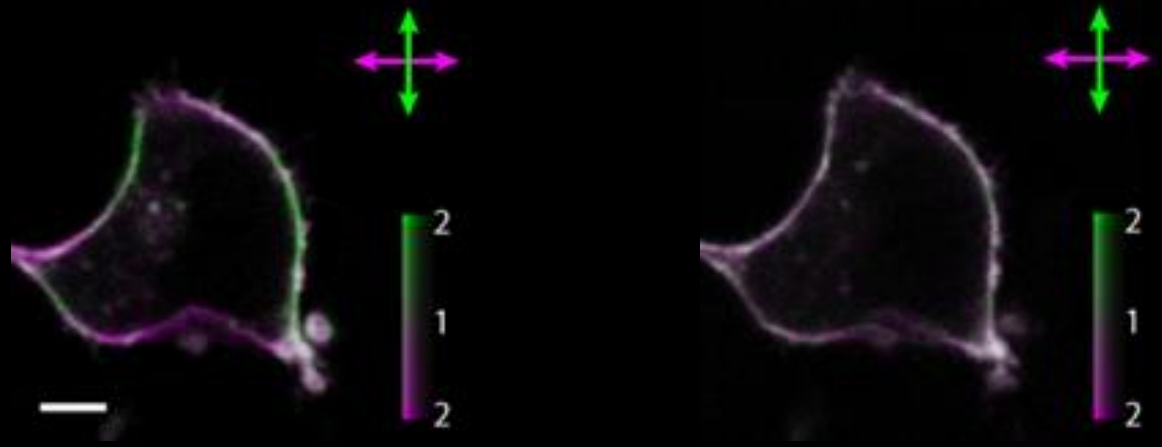
IMAGING PROTEIN-PROTEIN INTERACTIONS

Activation of heterotrimeric G-proteins:



resting state activated state

- A single fluorescent label
- Off-the-shelf constructs
- Imaging an inhibitory signal

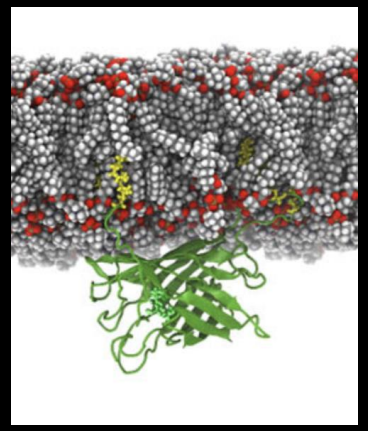


Gαi2 (GAP43-CFP-Gαi2, Gβ1, Gγ2, α2-AR-YFP)

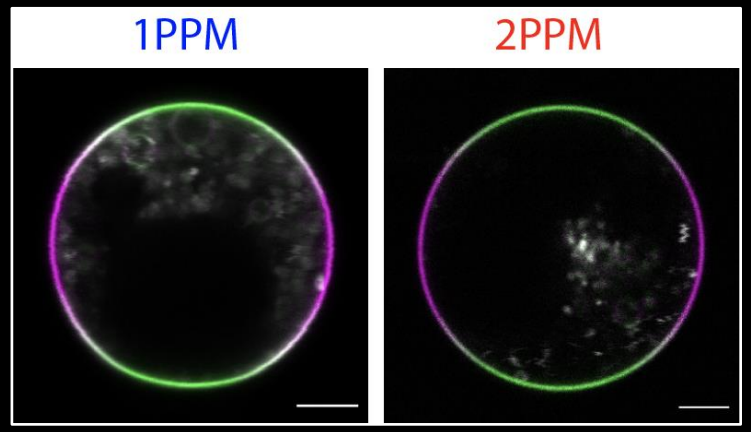
A. Bondar & al., JBC 2015 (Gαi dissociation vs. rearrangement)
A. Bondar & al., JBC 2017 (Receptor/G-protein pre-coupling)

QUANTITATION OF LINEAR DICHROISM

ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS

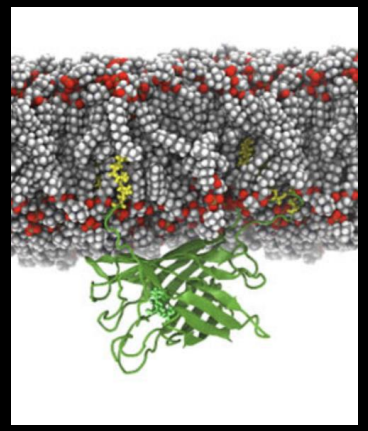


Doubly
lipidated eGFP



Bondar A. & al., Communications Biology 2021

ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS



Doubly
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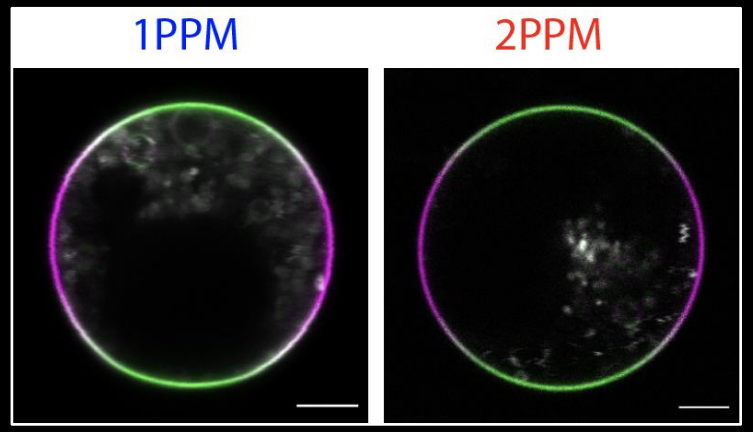
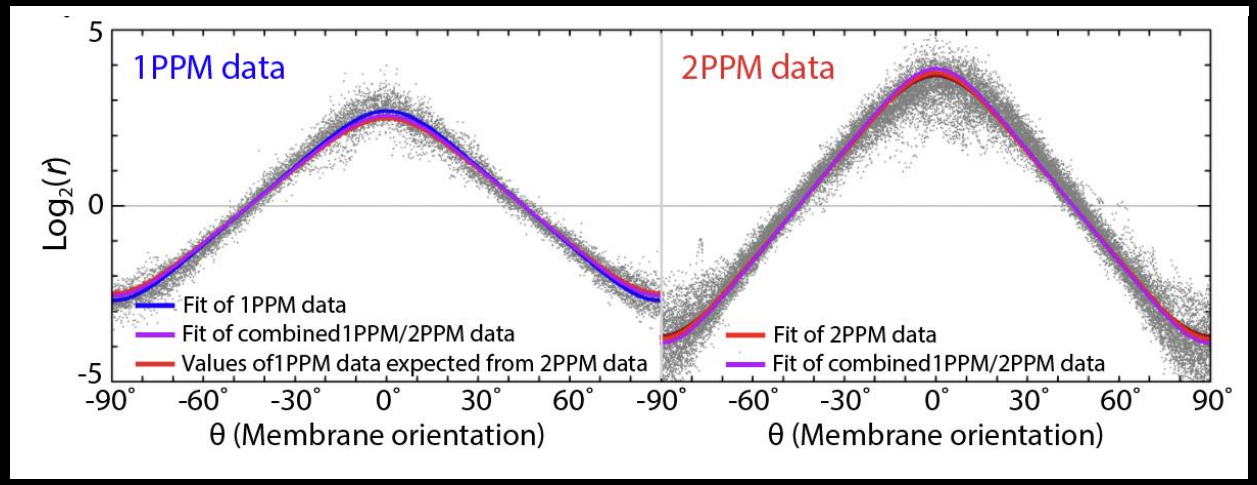
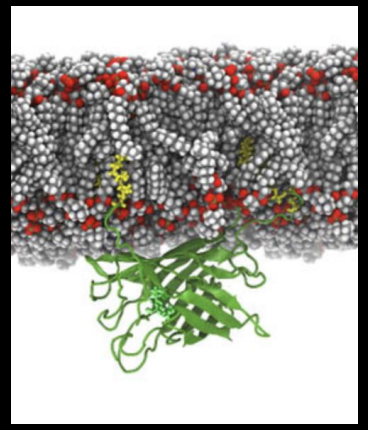


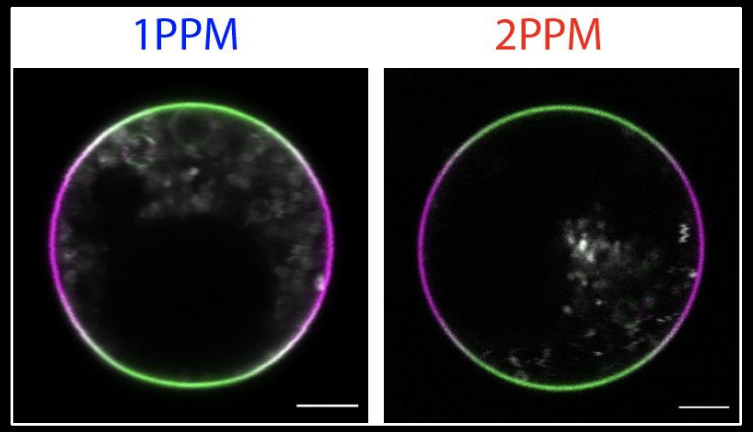


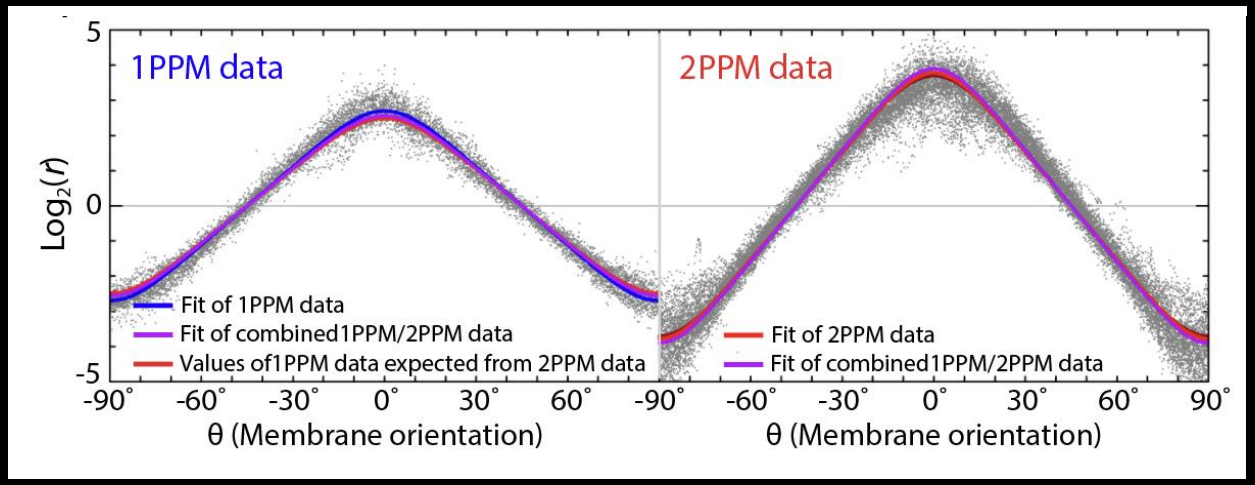
Bondar A. & al., Communications Biology 2021

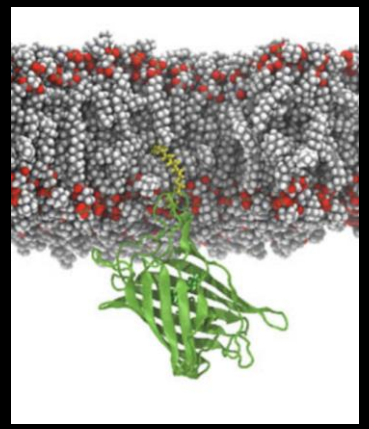
ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS



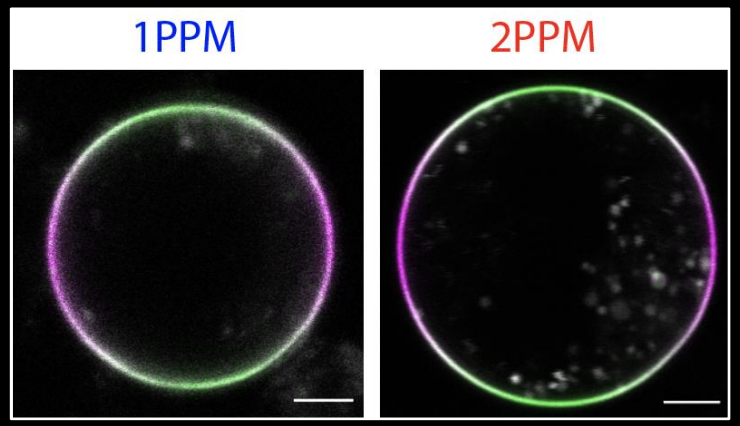
Doubly lipidated eGFP

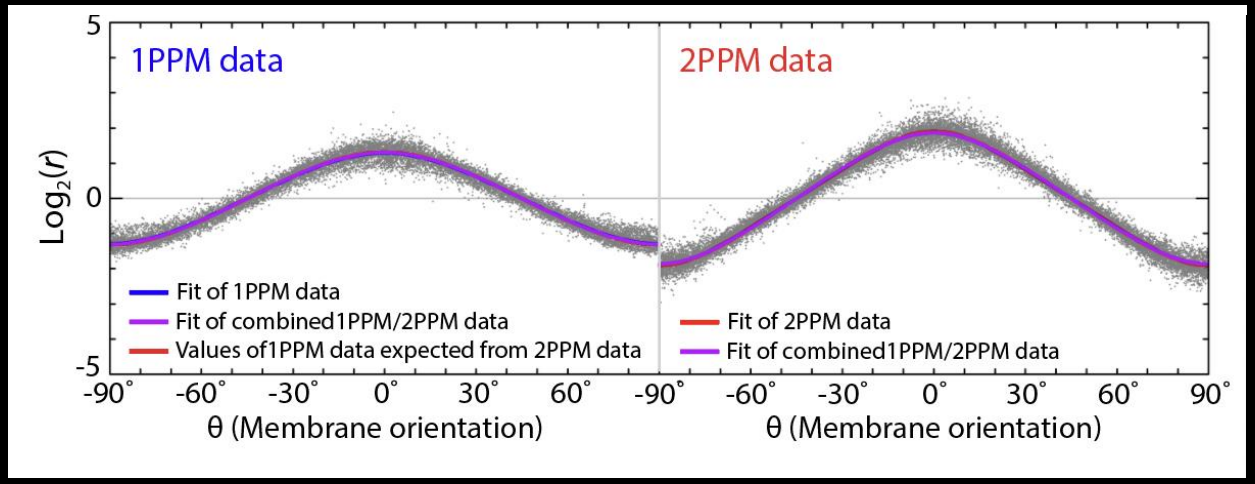






C-terminally lipidated eGFP





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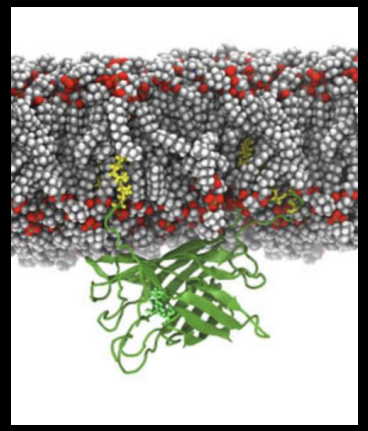
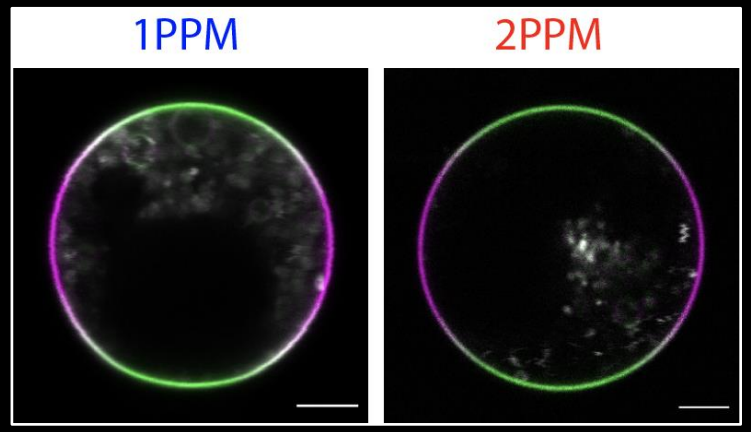
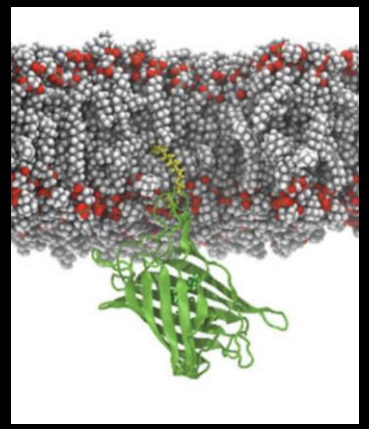
ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS

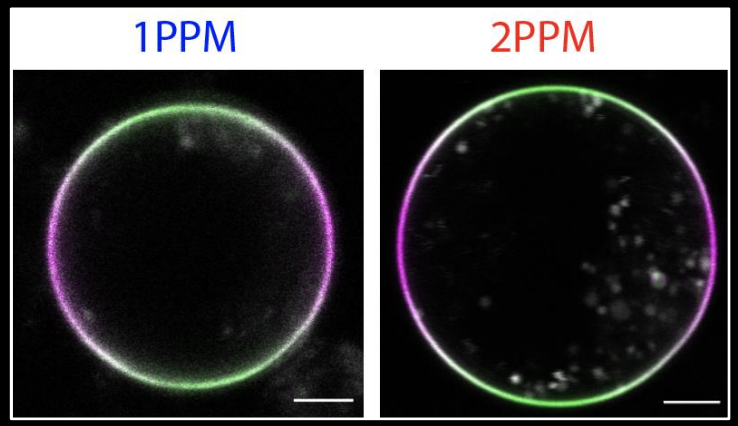


Doubly lipidated eGFP

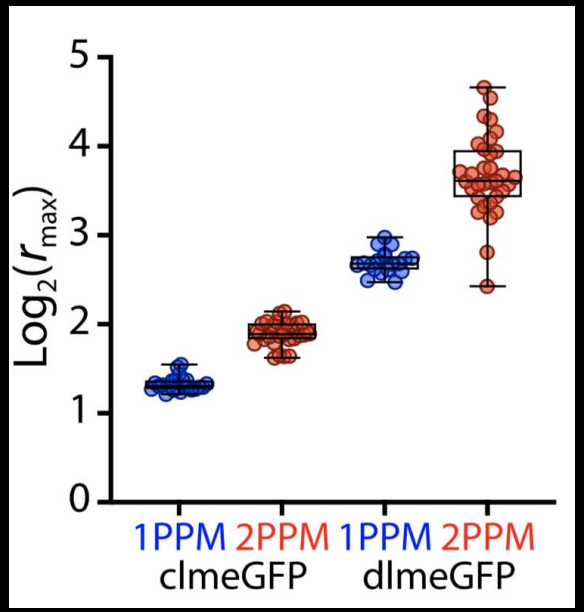




C-terminally lipidated eGFP



Linear dichroism quantitation:



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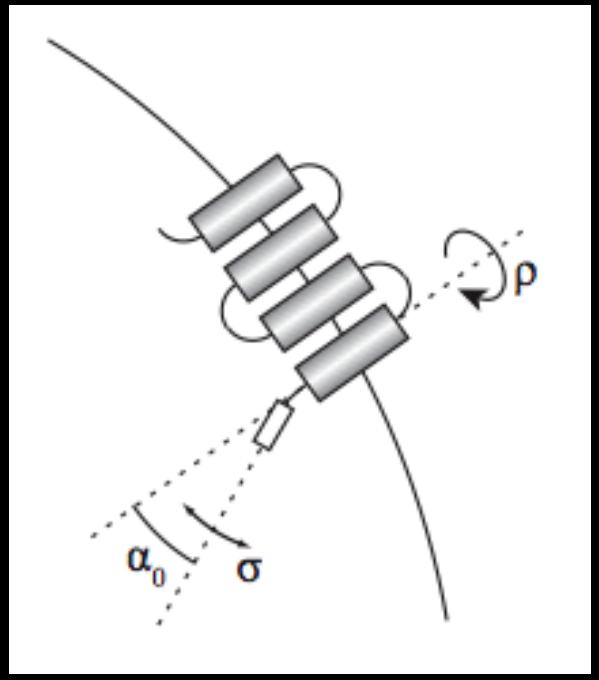
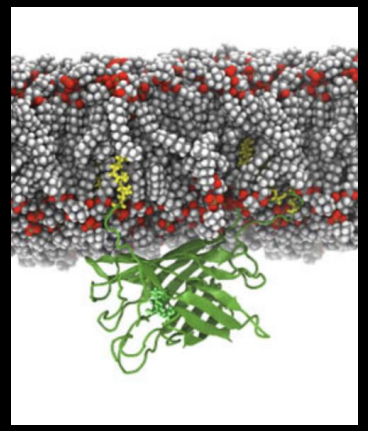
INSIGHTS INTO PROTEIN STRUCTURE

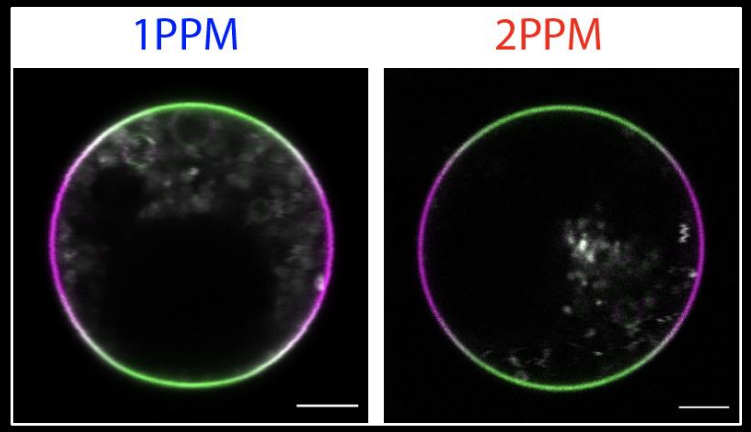
INSIGHTS INTO PROTEIN STRUCTURE



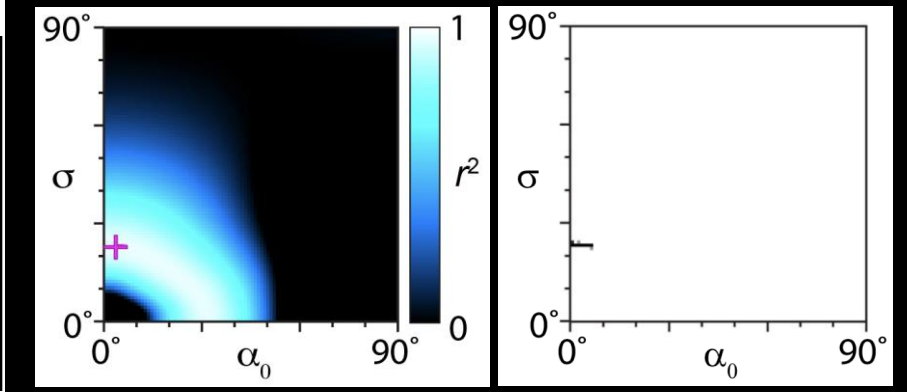
ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS



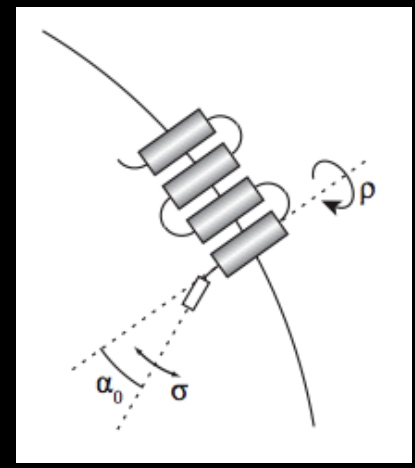
Doubly lipidated eGFP

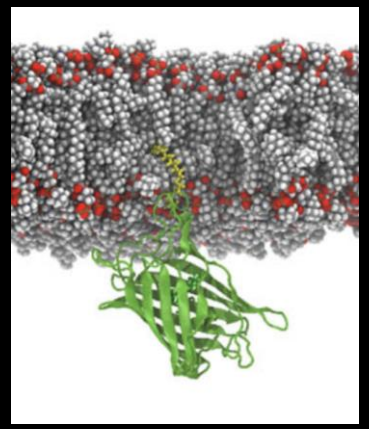


Linear dichroism structural interpretation:

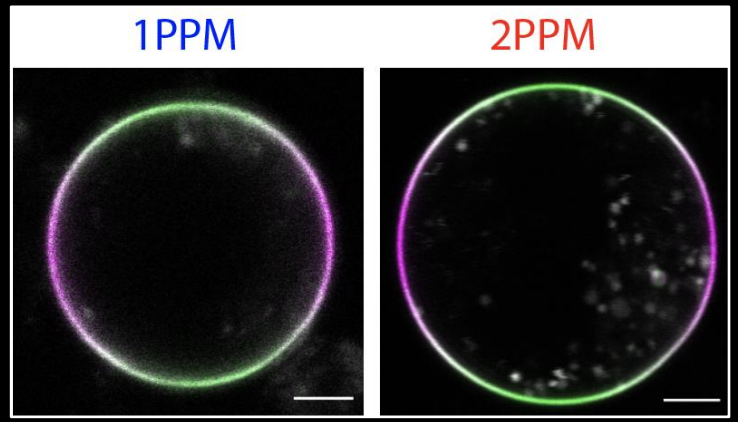


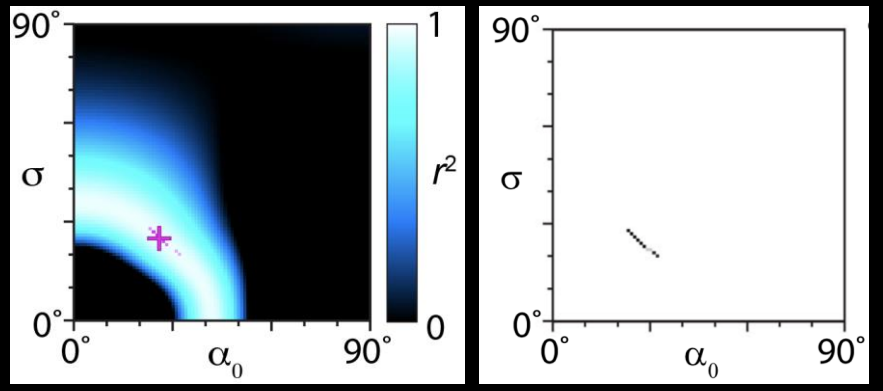
$\alpha_0 = 3°$, $\sigma = 23°$





C-terminally lipidated eGFP





$\alpha_0 = 26°$, $\sigma = 25°$

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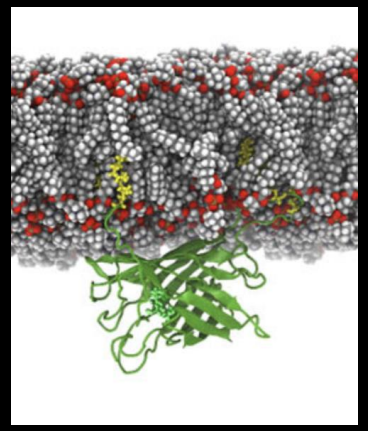
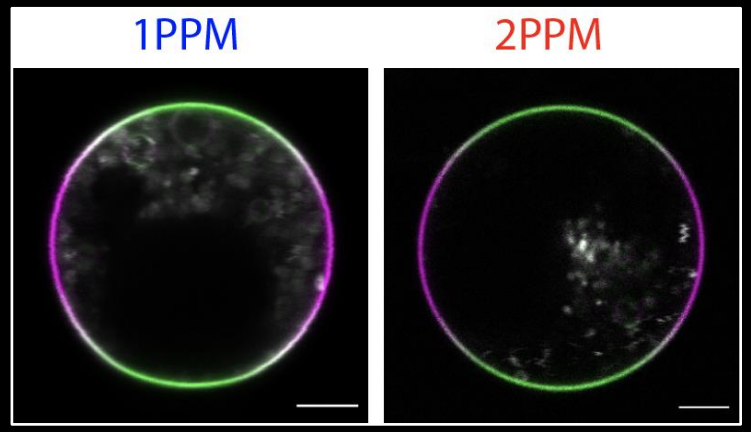
ROUND CELLS + MODEL FLUORESCENT PROTEIN CONSTRUCTS



Doubly lipidated eGFP



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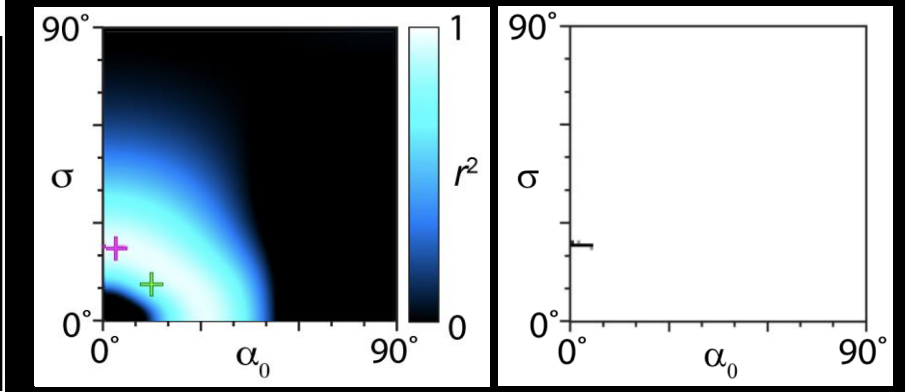
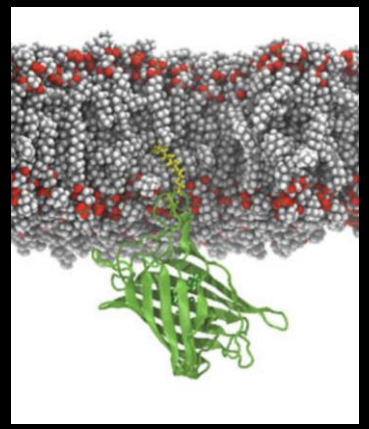
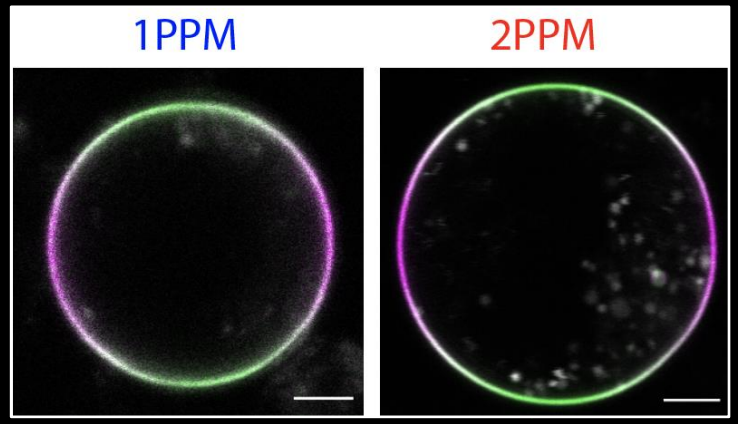
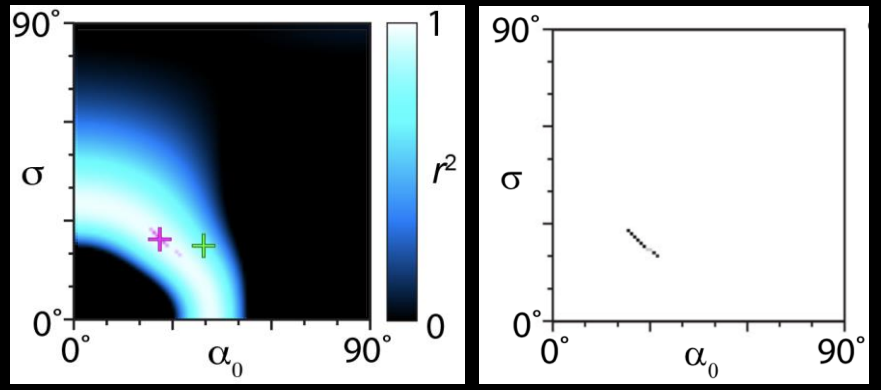


$\alpha_0 = 3°$, $\sigma = 23°$

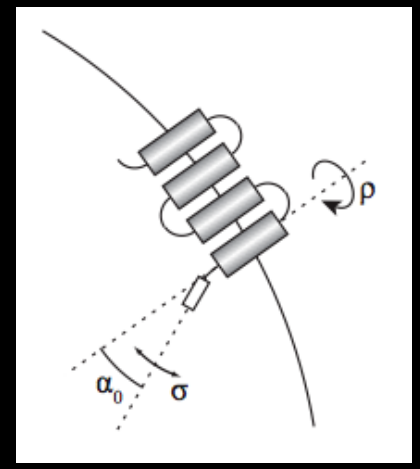


C-terminally lipidated eGFP





$\alpha_0 = 26°$, $\sigma = 25°$



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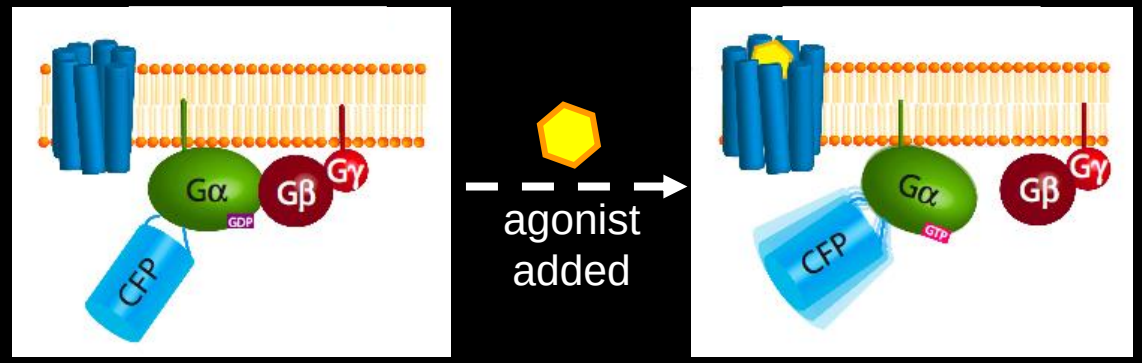
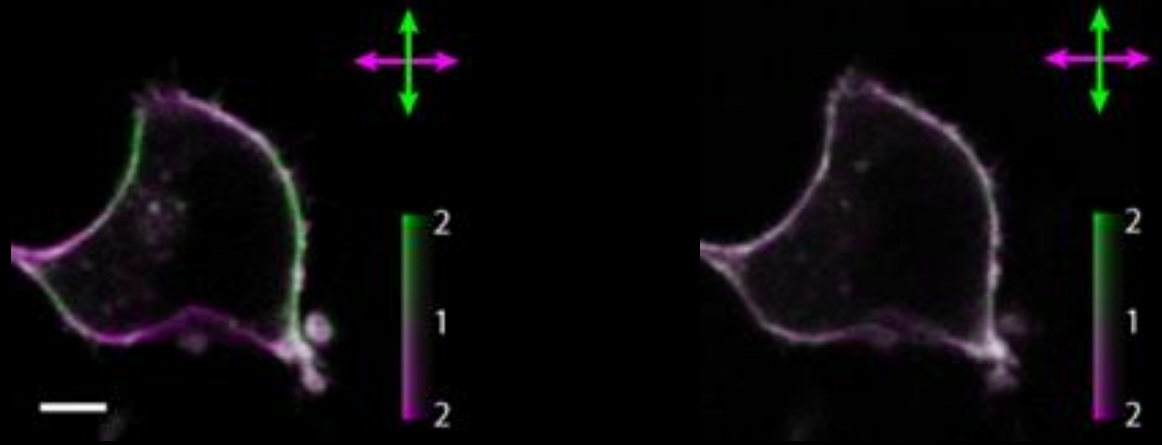
IMAGING PROTEIN-PROTEIN INTERACTIONS

Activation of heterotrimeric G-proteins:



resting state activated state



Gαi2 (GAP43-CFP-Gαi2, Gβ1, Gγ2, α2-AR-YFP)

IMAGING PROTEIN-PROTEIN INTERACTIONS

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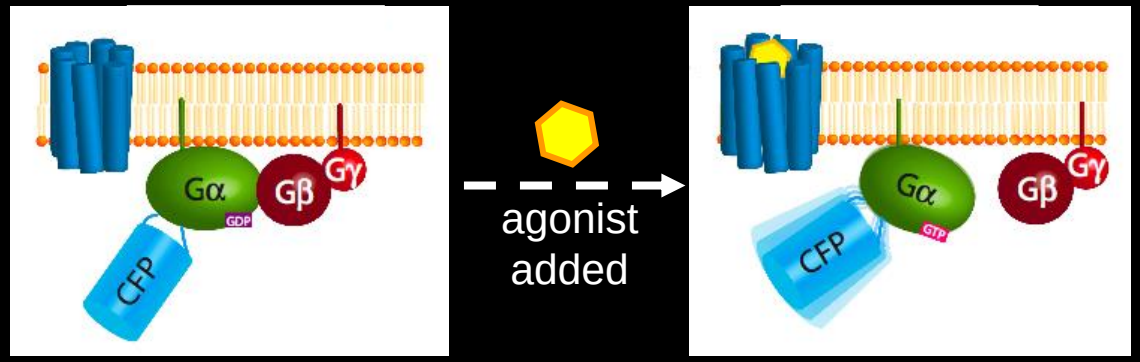
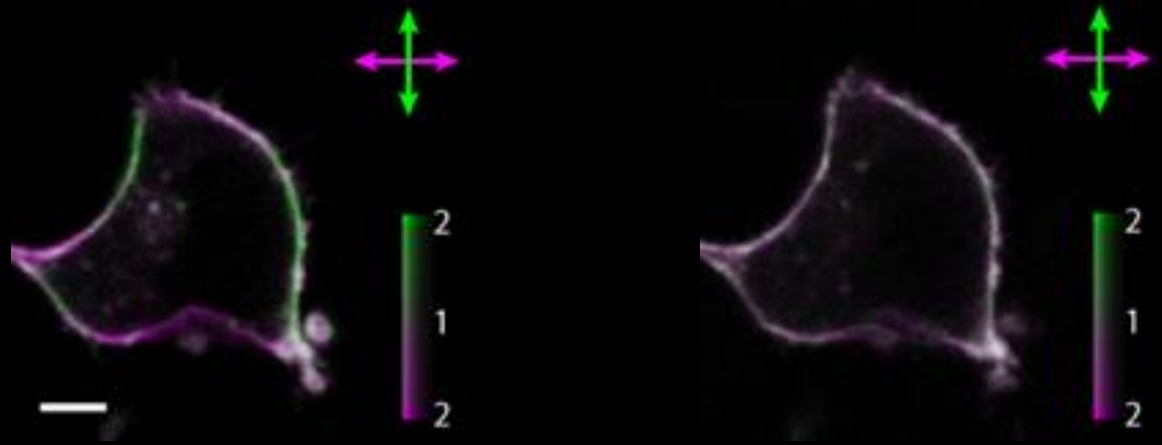


resting state activated state



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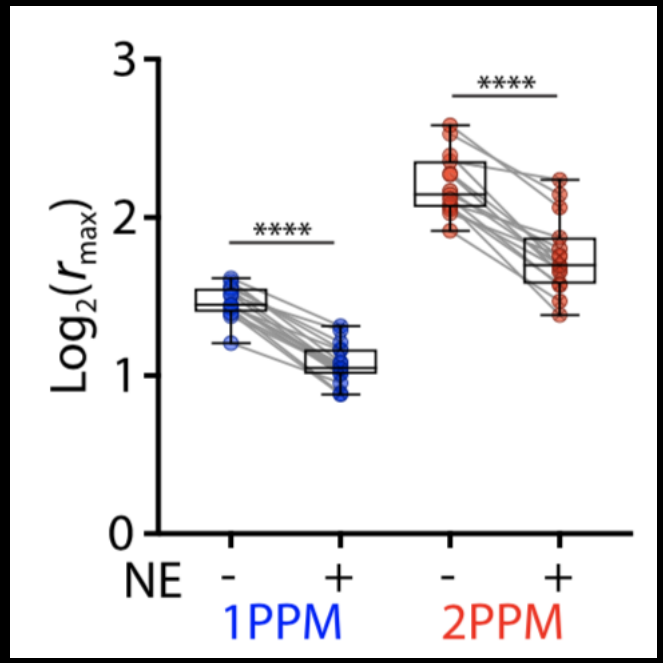
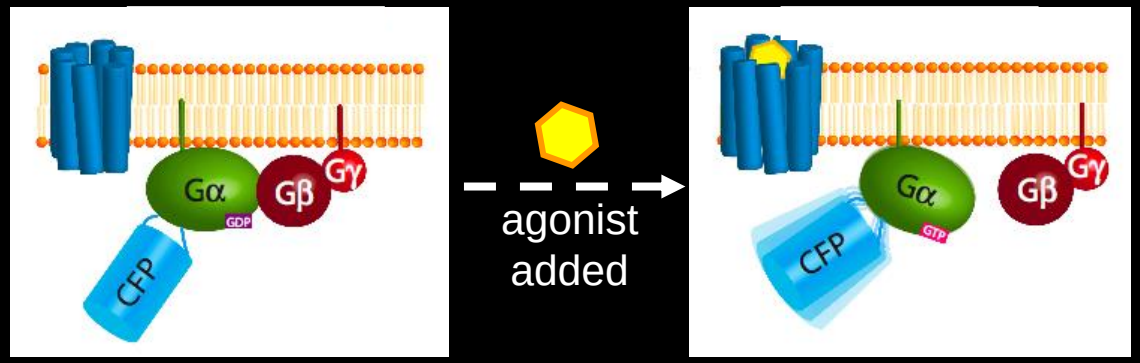
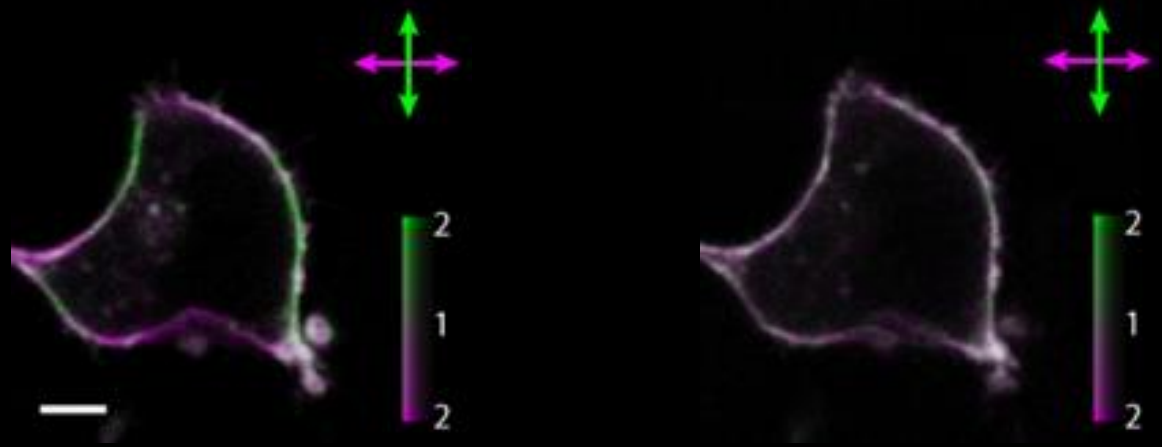


INSIGHTS INTO PROTEIN STRUCTURE

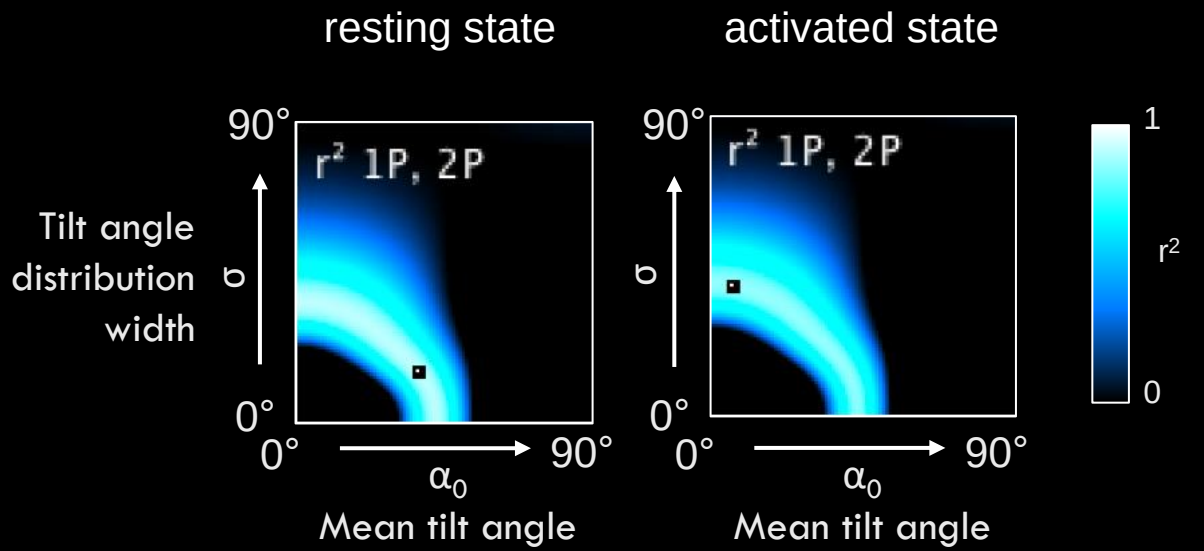
Activation of heterotrimeric G-proteins:



resting state activated state

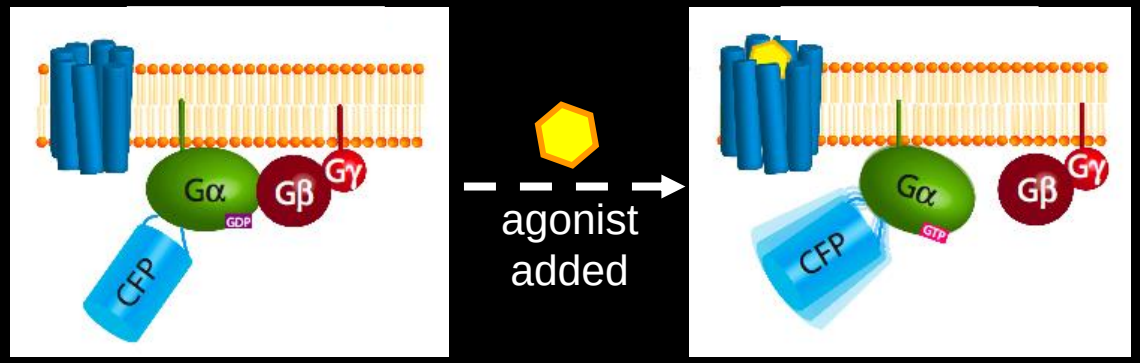


Gαi2 (GAP43-CFP-Gαi2, Gβ1, Gγ2, α2-AR-YFP)

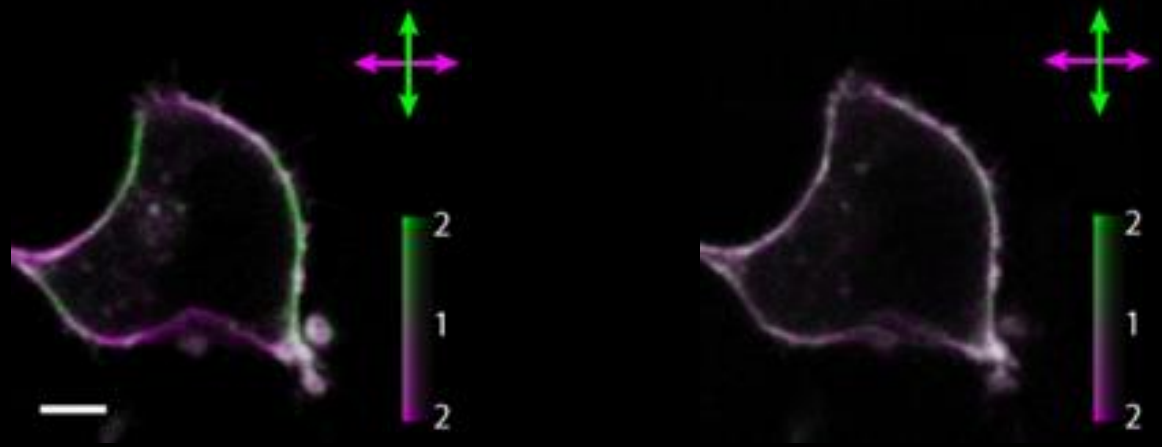


INSIGHTS INTO PROTEIN STRUCTURE

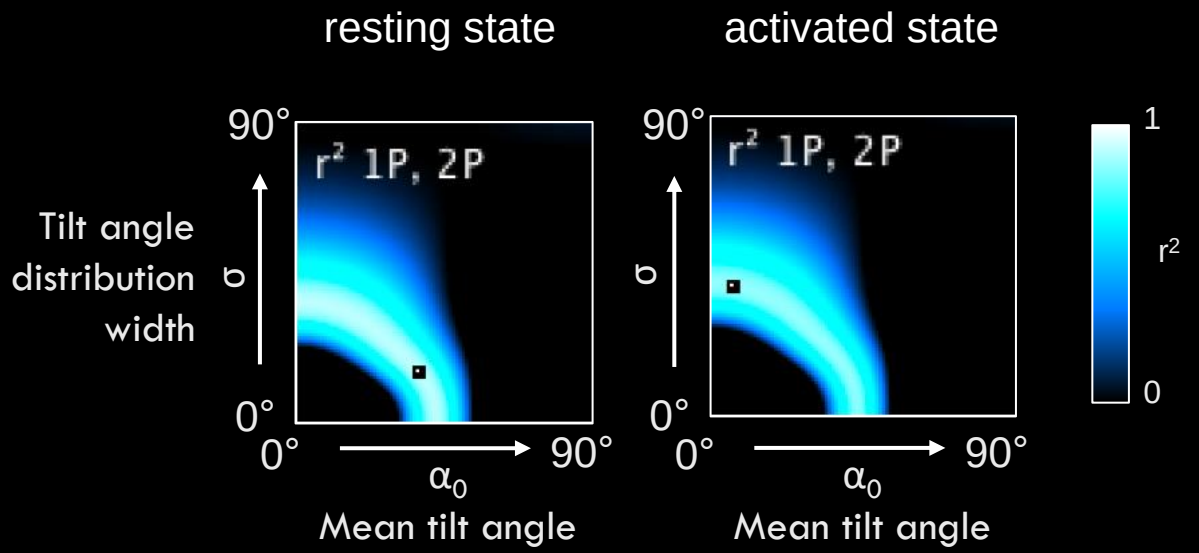
Activation of heterotrimeric G-proteins:



resting state activated state



Gαi2 (GAP43-CFP-Gαi2, Gβ1, Gγ2, α2-AR-YFP)

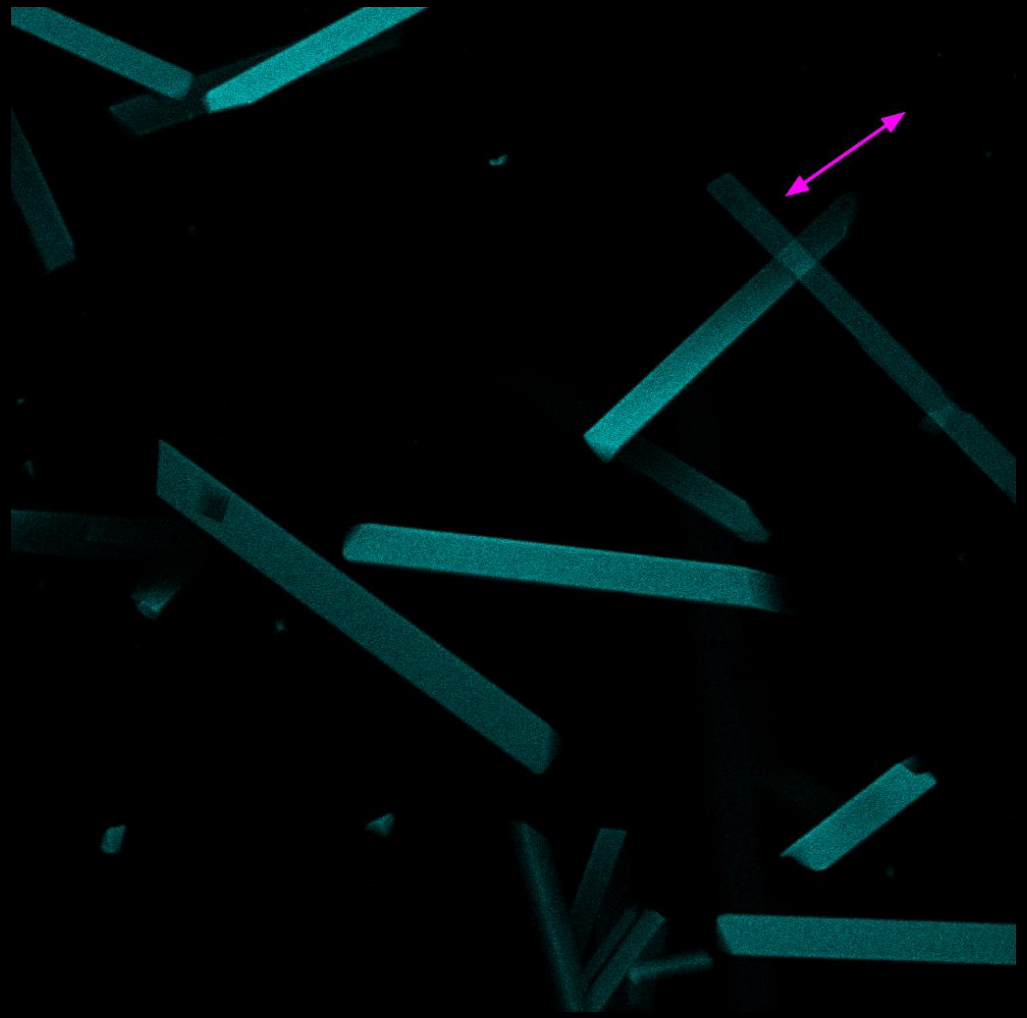


Rational genetically encoded probe development

DIRECTIONALITY OF FP ABSORPTION

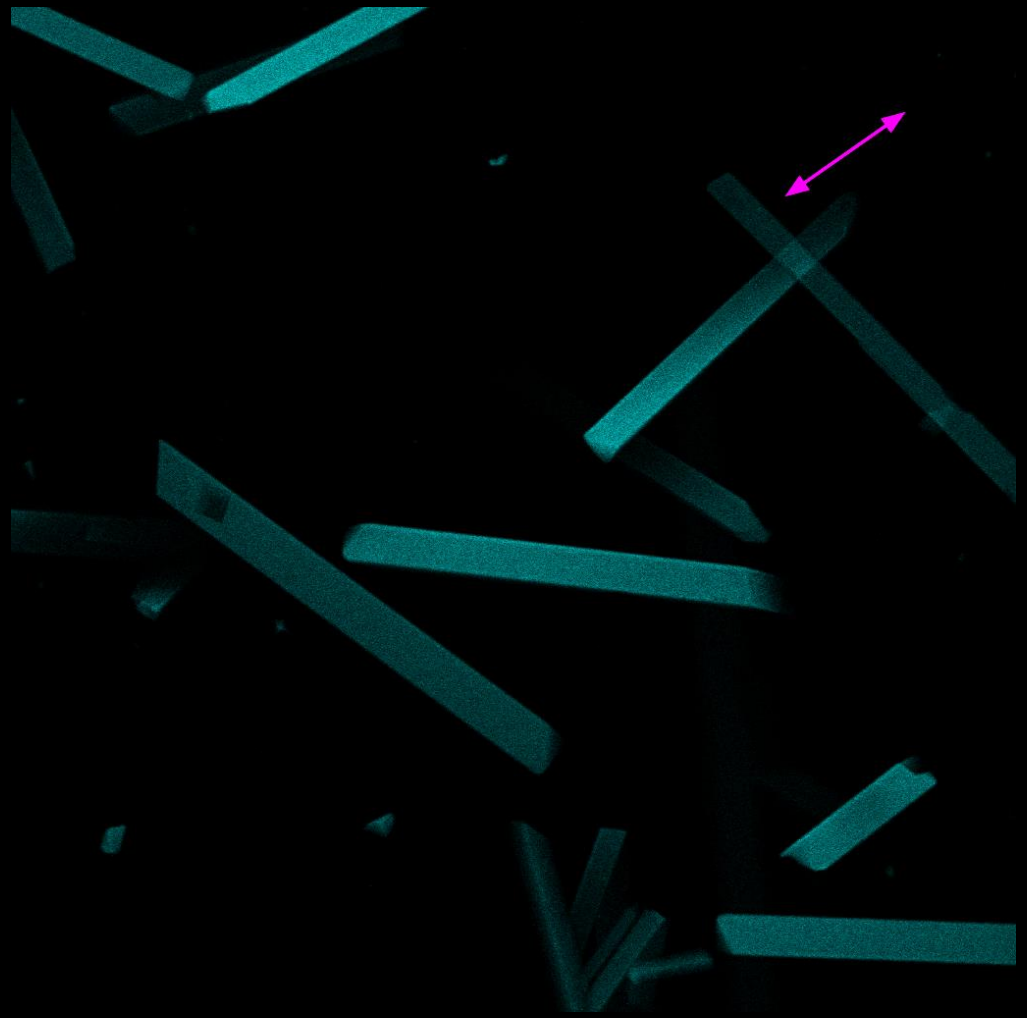
Crystals of mTurquoise2

DIRECTIONALITY OF FP ABSORPTION



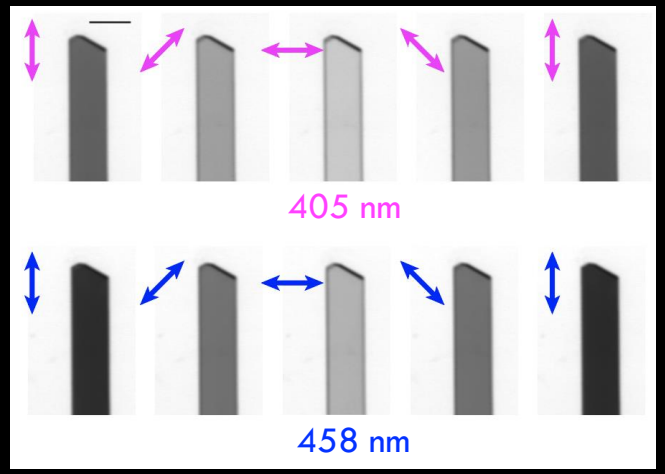
Crystals of mTurquoise2

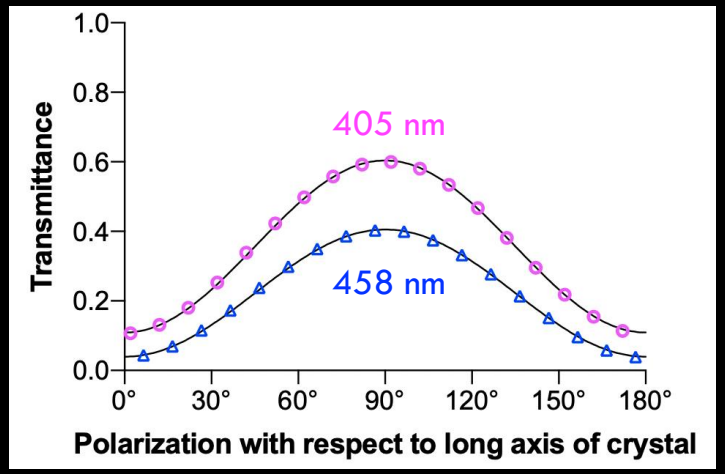
DIRECTIONALITY OF FP ABSORPTION



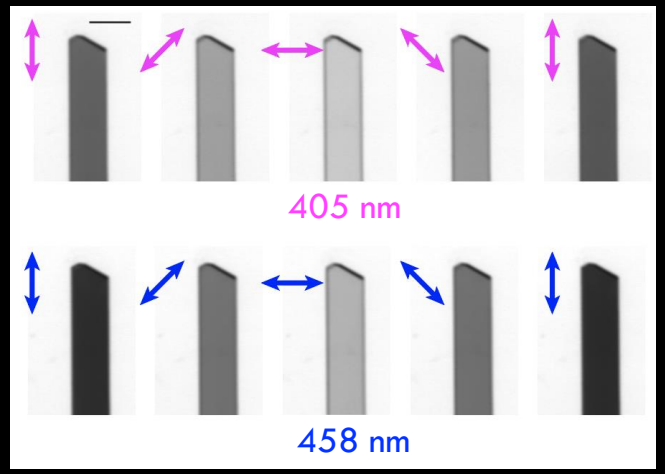
Crystals of mTurquoise2

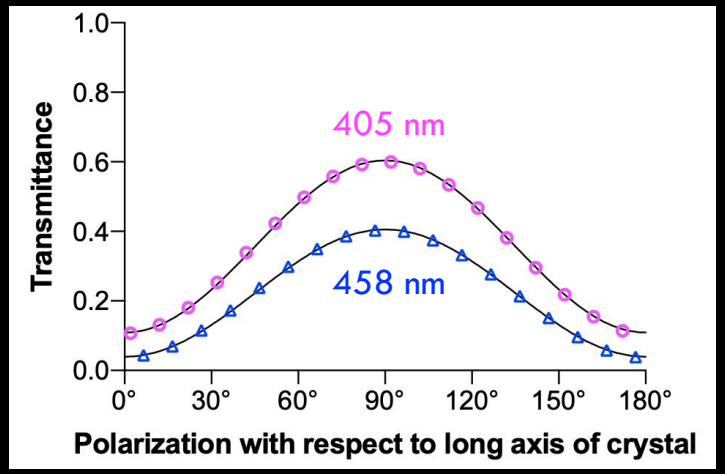
DIRECTIONALITY OF FP ABSORPTION: MTURQUOISE2

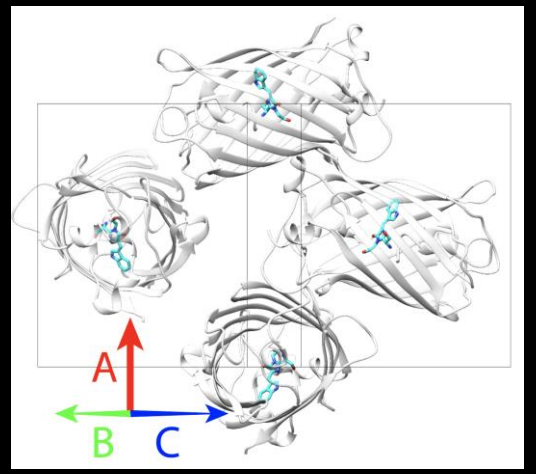




DIRECTIONALITY OF FP ABSORPTION: MTURQUOISE2

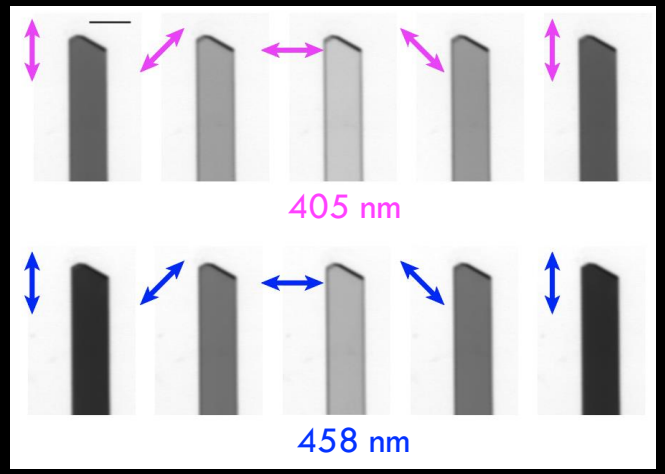


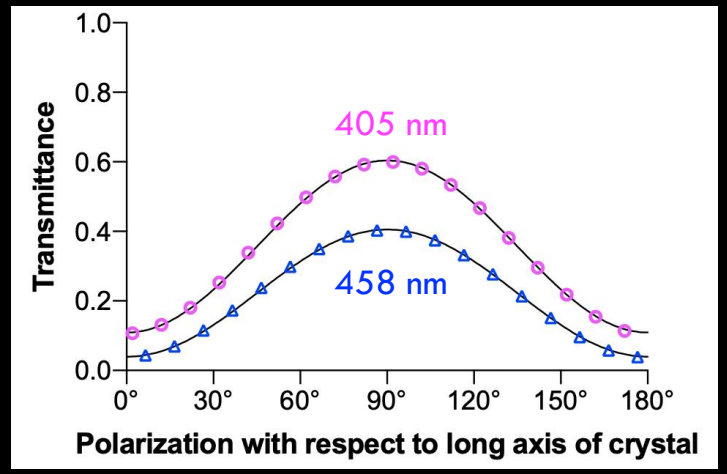


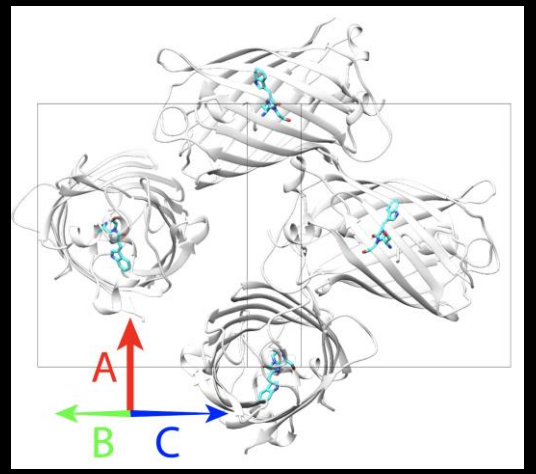


mTurquoise2: P212121

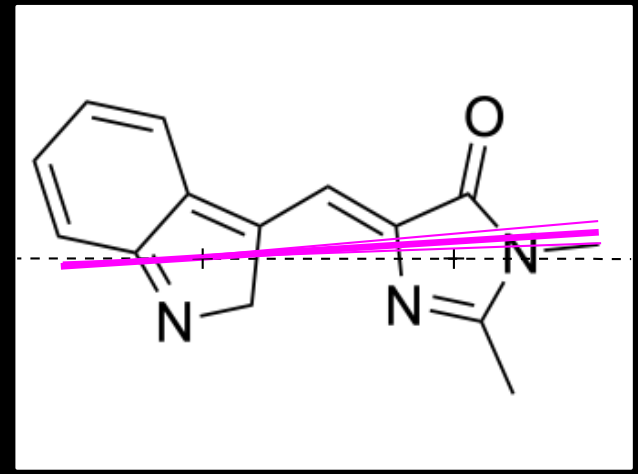
DIRECTIONALITY OF FP ABSORPTION: MTURQUOISE2



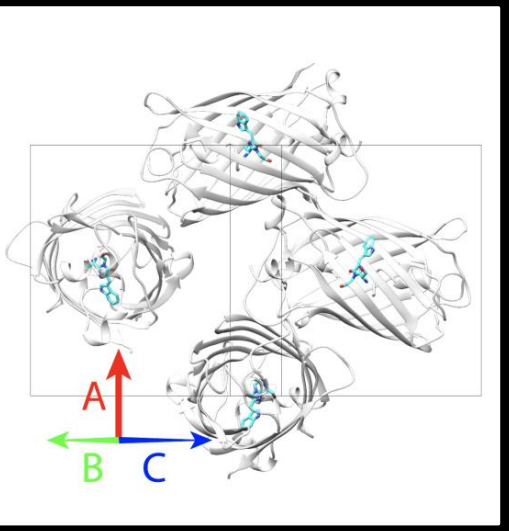




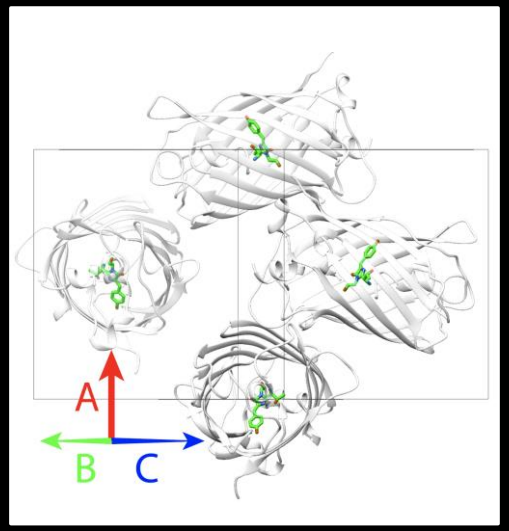
mTurquoise2: P212121



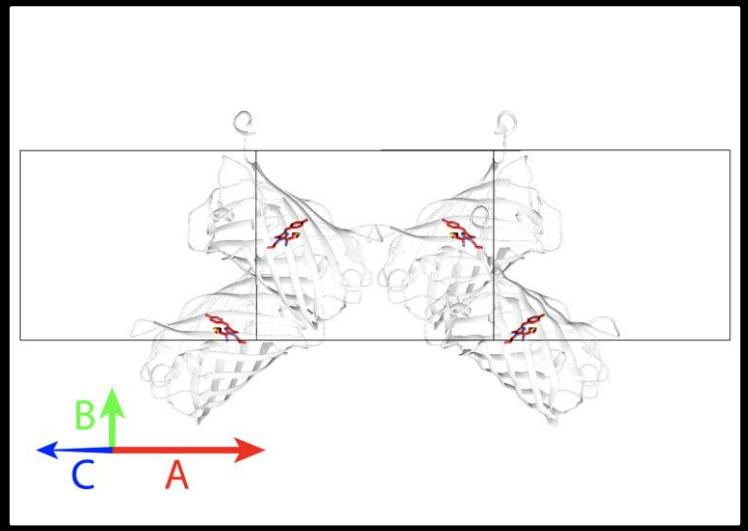
DIRECTIONALITY OF FP ABSORPTION AND EMISSION



mTurquoise2
(P212121)



eGFP
(P212121)



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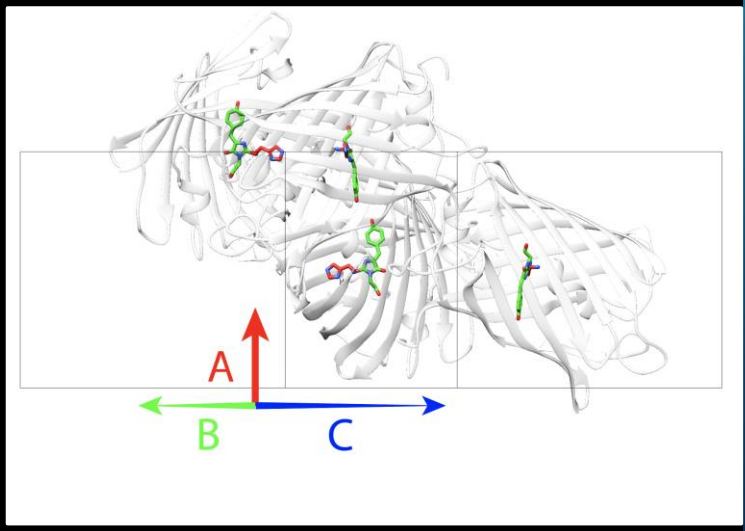
(C121)



mEos4b
(P212121)

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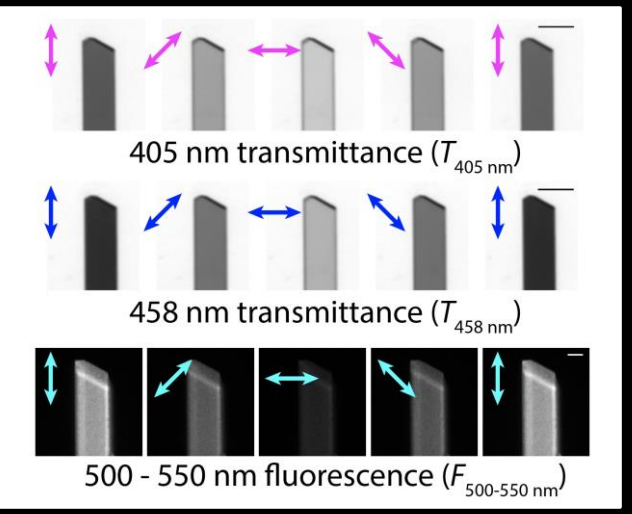
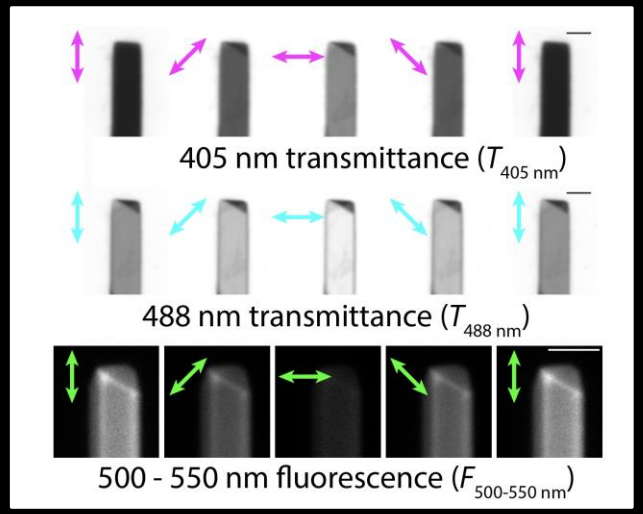
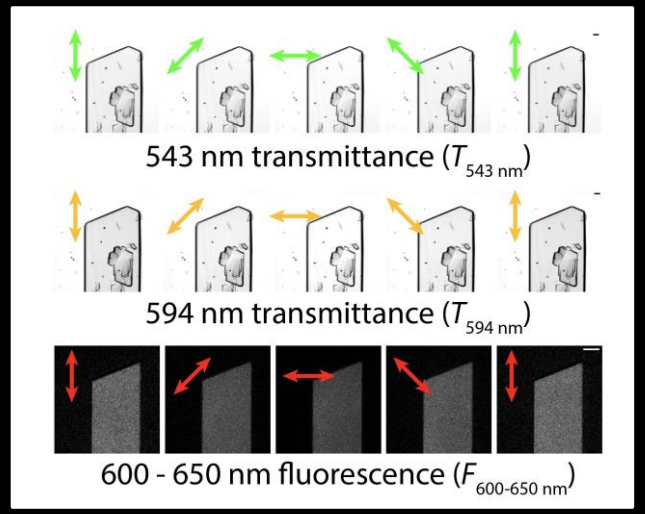
DIRECTIONALITY OF FP ABSORPTION AND EMISSION



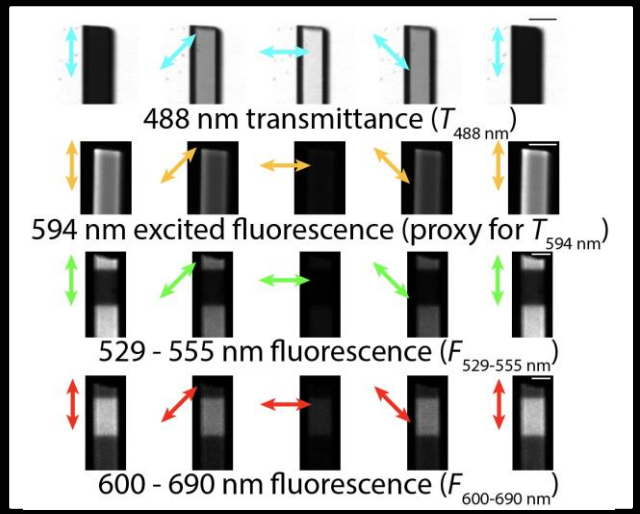
mTurquoise2
(P212121)



eGFP
(P212121)



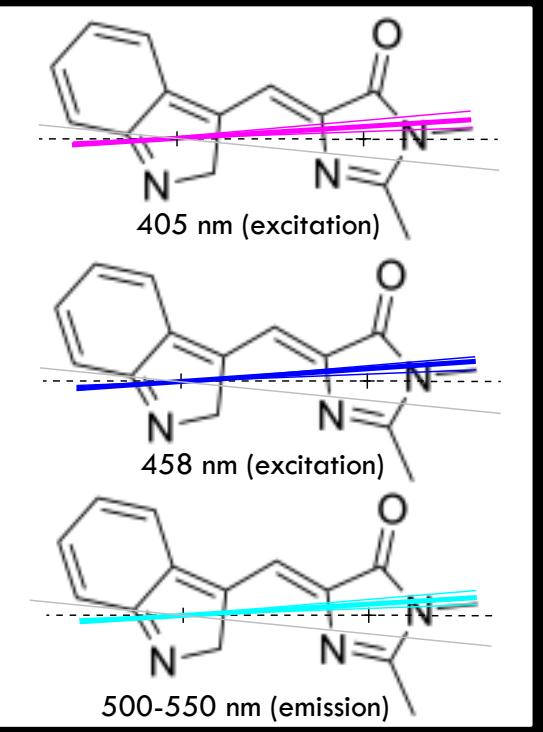
mCherry
(C121)



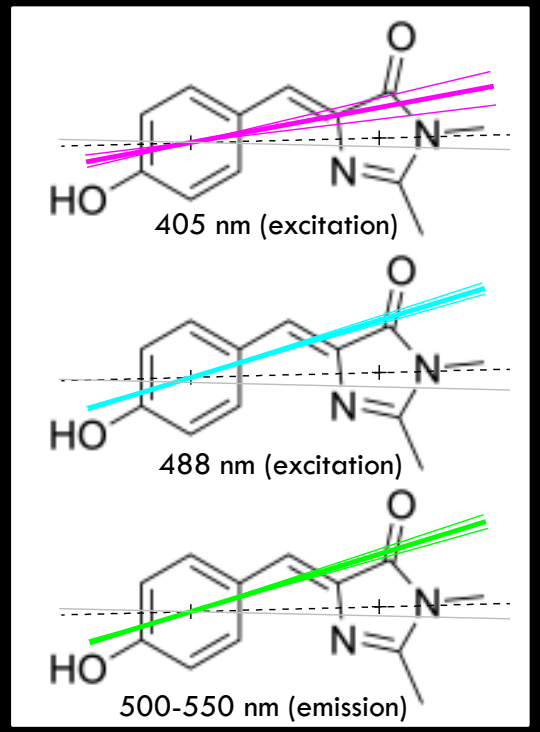
mEos4b
(P212121)

Myšková J. & al., PNAS 2020

DIRECTIONALITY OF FP ABSORPTION AND EMISSION

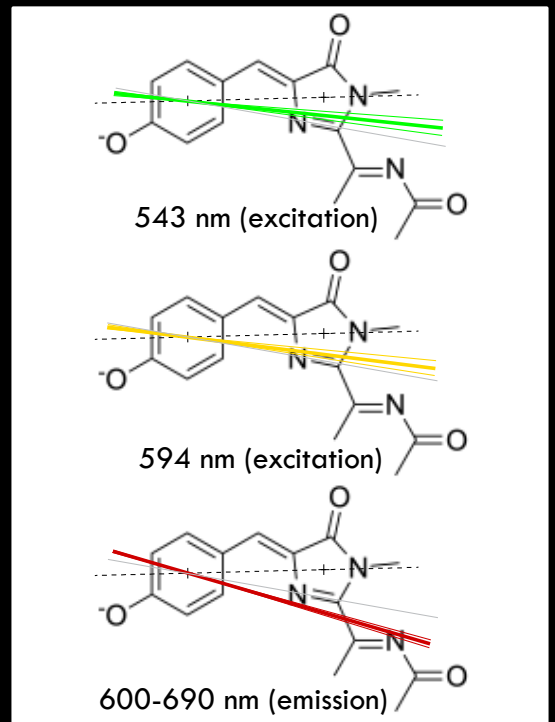


mTurquoise2



eGFP

- in agreement with published experimental determination



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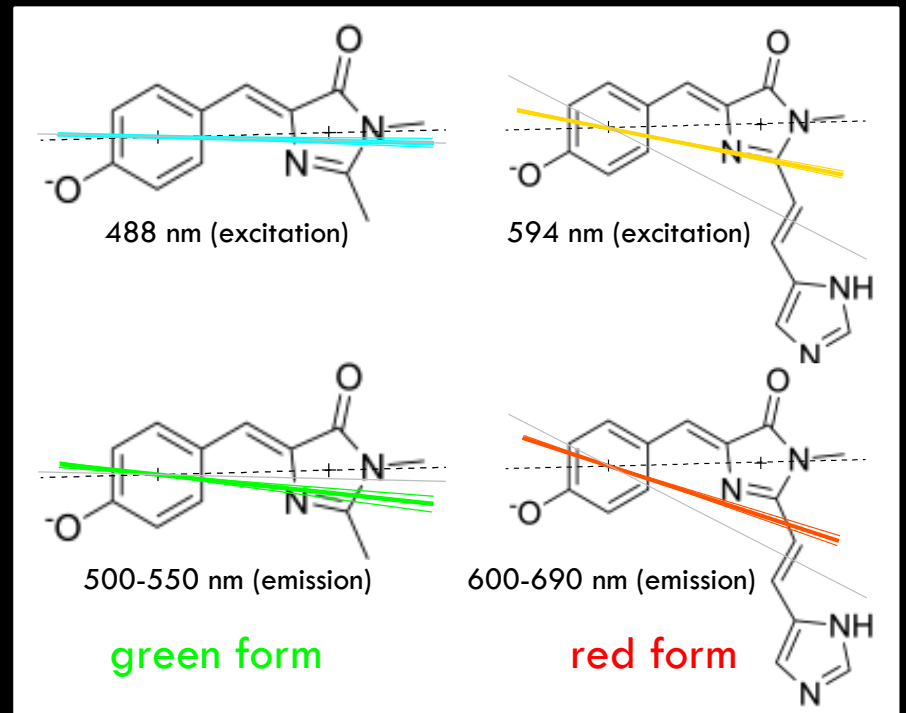
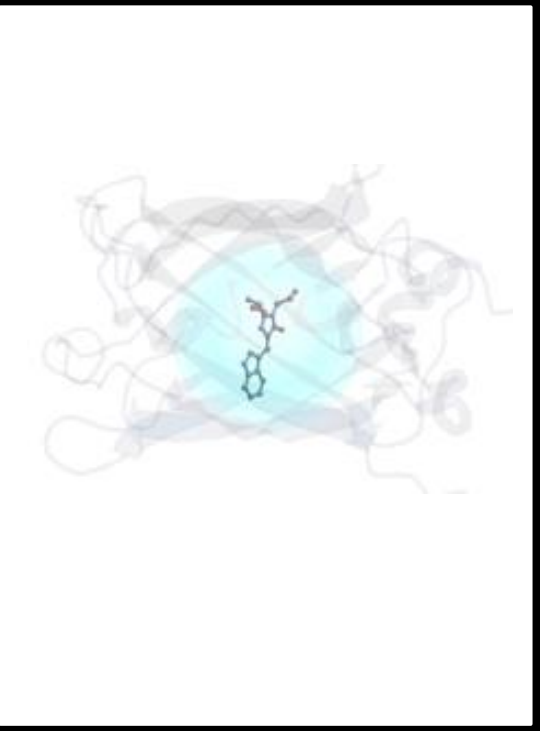


mEos4b

Myšková J. & al., PNAS 2020

DIRECTIONALITY OF FP ABSORPTION AND EMISSION



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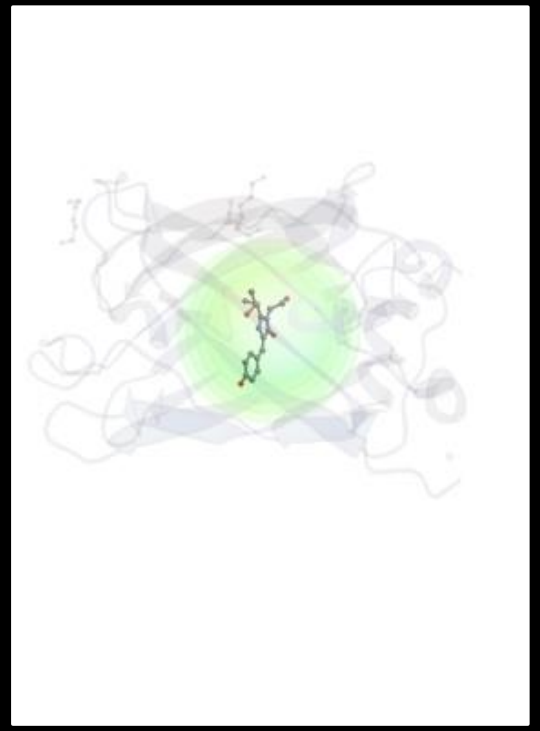
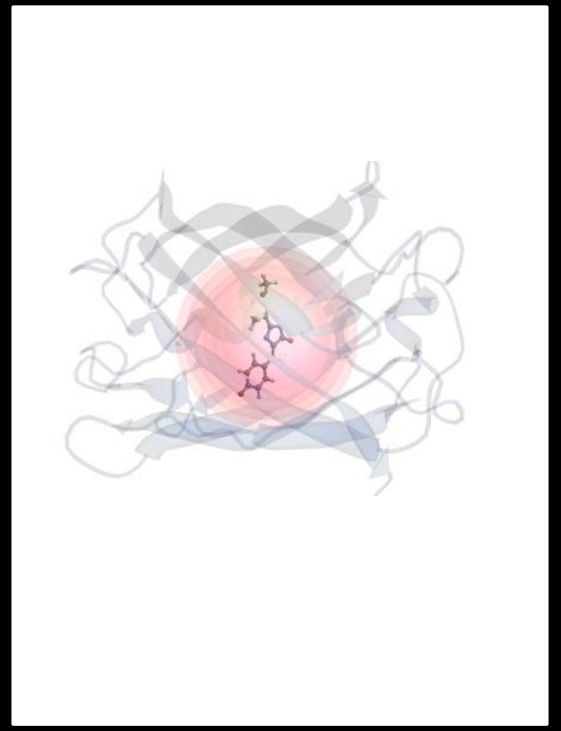


eGFP



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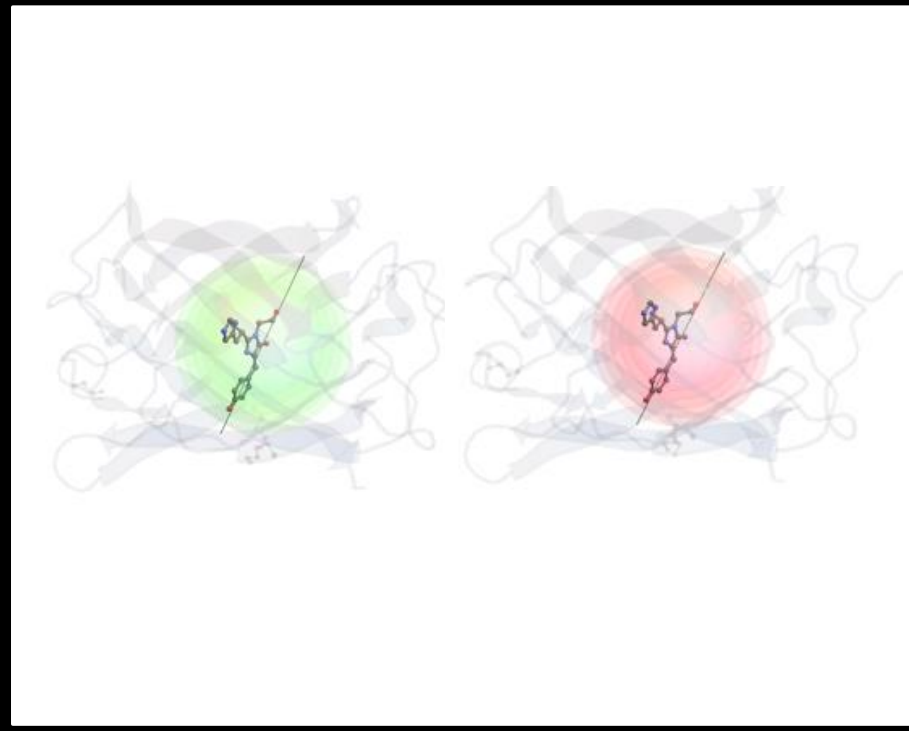
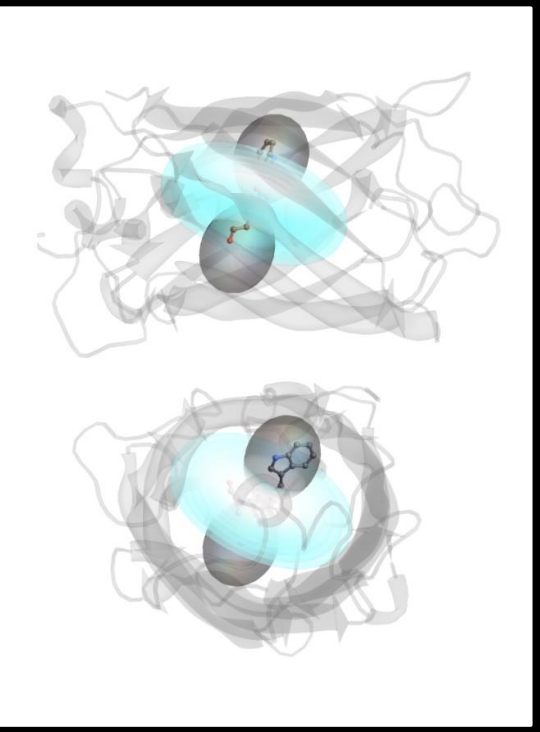


mEos4b

DIRECTIONALITY OF FP ABSORPTION AND EMISSION



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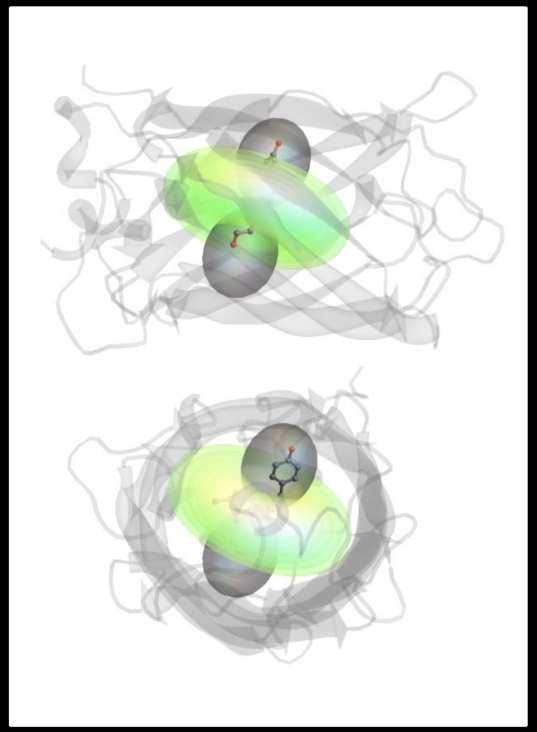
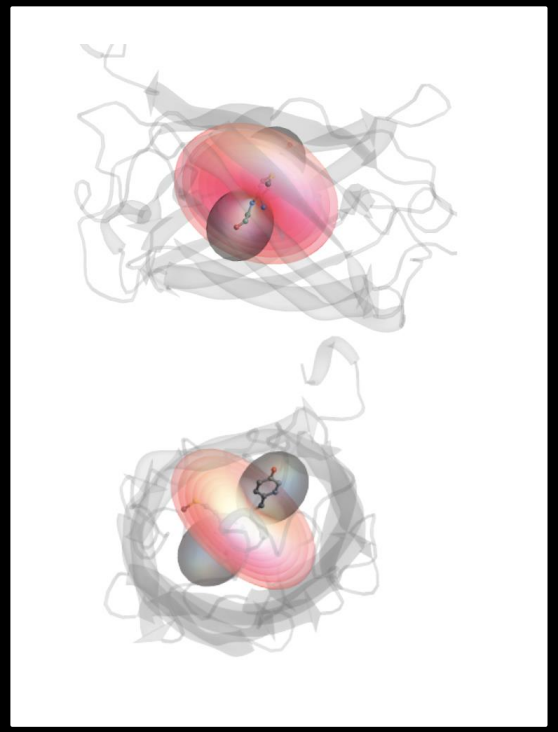


eGFP



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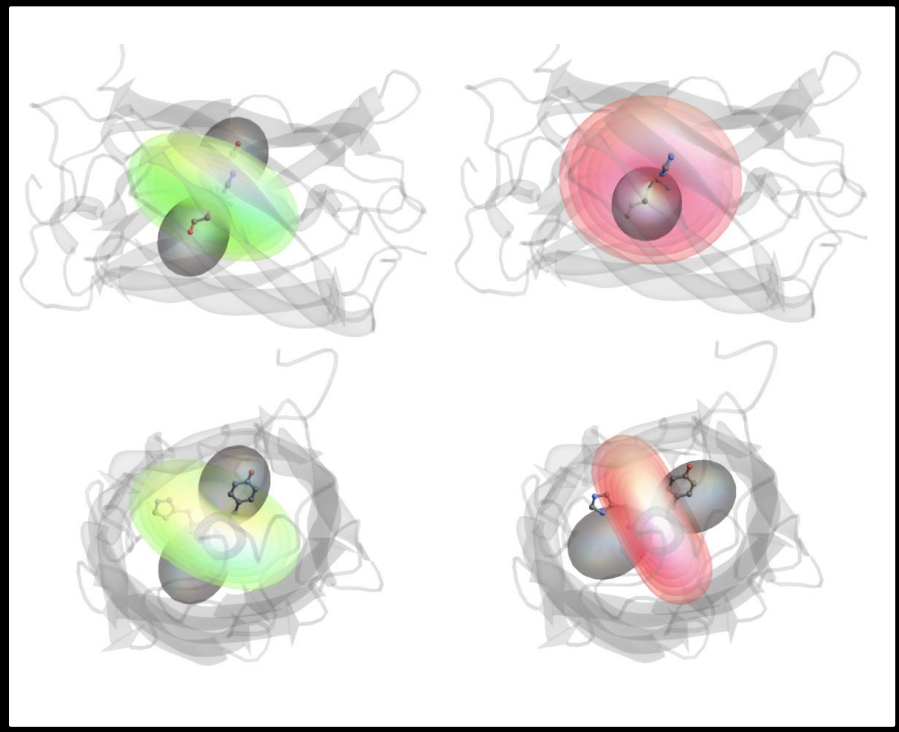

mEos4b

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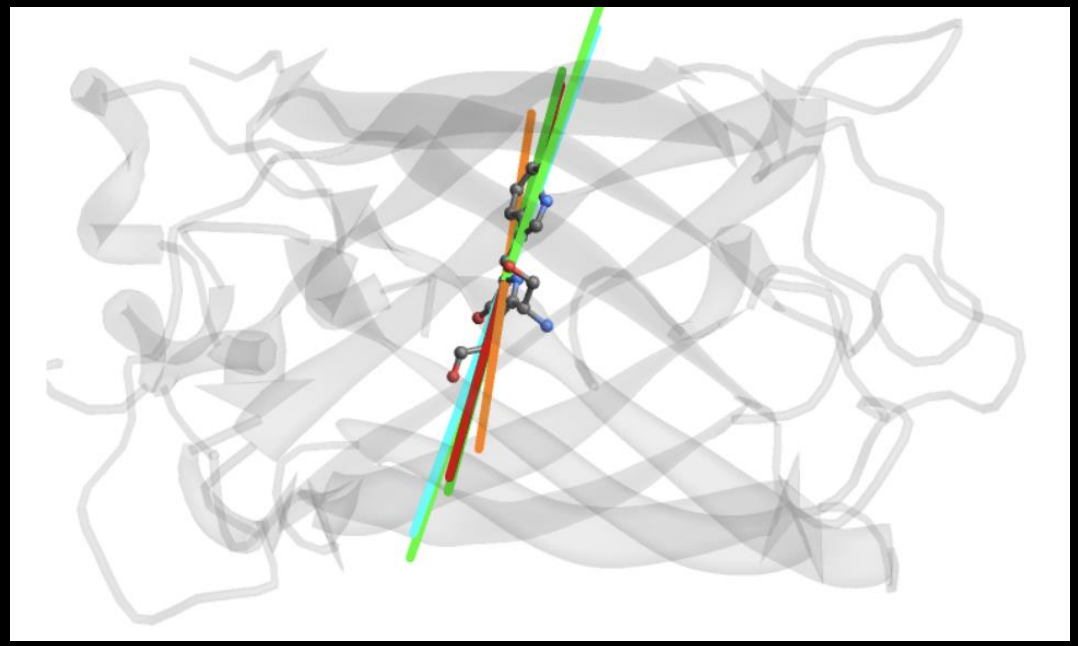
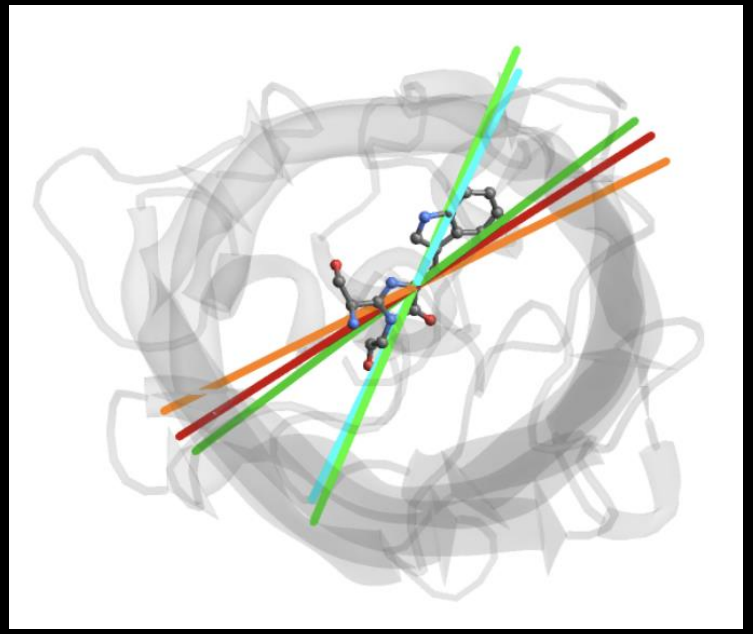
DIRECTIONALITY OF FP ABSORPTION AND EMISSION



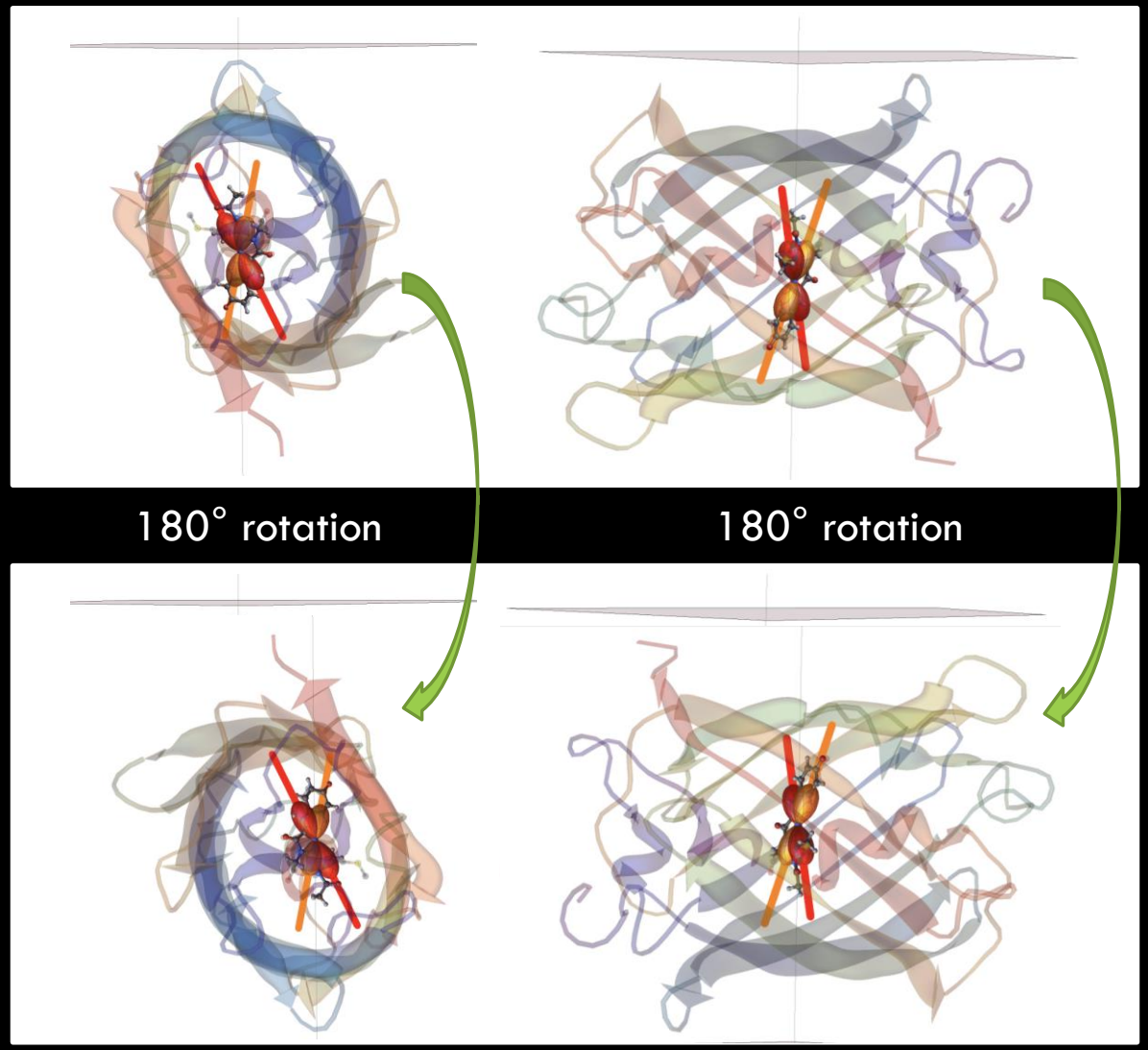


mTurquoise2 eGFP mCherry mEos4b

Myšková J. & al., PNAS 2020

FP ORIENTATION WITH RESPECT TO THE CELL MEMBRANE

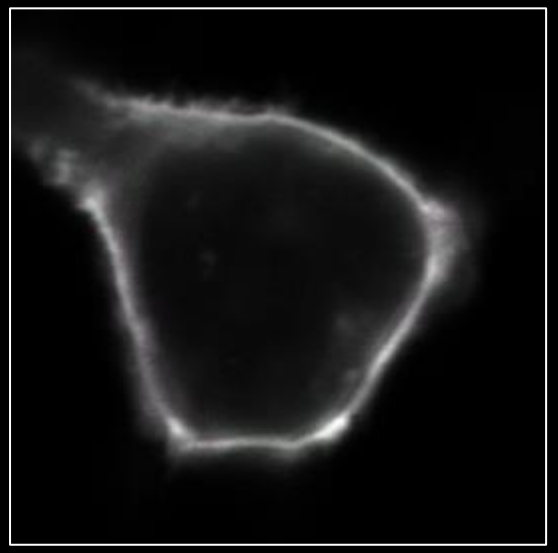
Work in progress:
Two indistinguishable possible orientations



DEVELOPMENT OF PROBES – IN PROGRESS

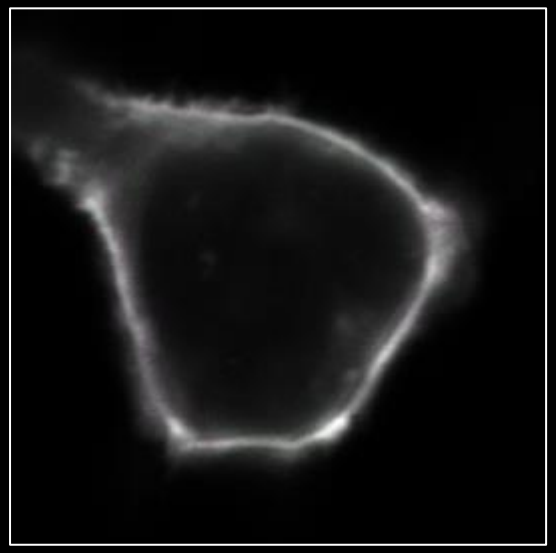
DEVELOPMENT OF PROBES – IN PROGRESS

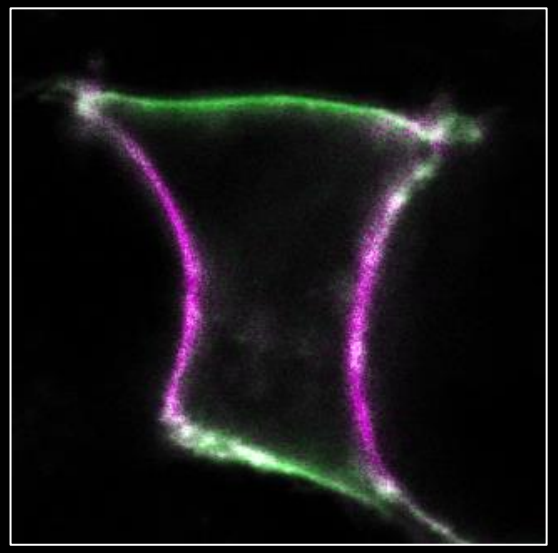
Probe K1:



DEVELOPMENT OF PROBES – IN PROGRESS

Probe K1:

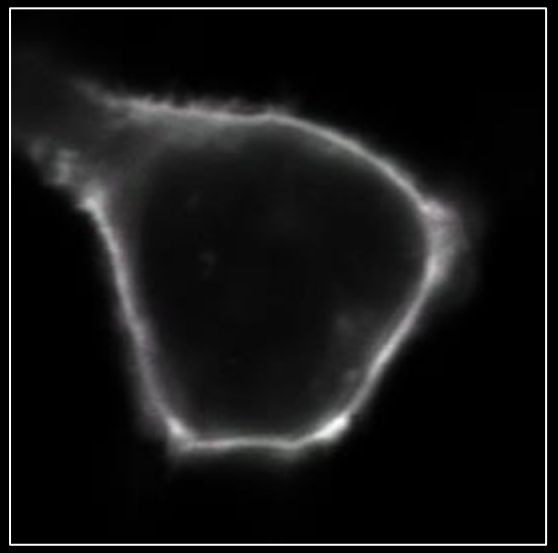


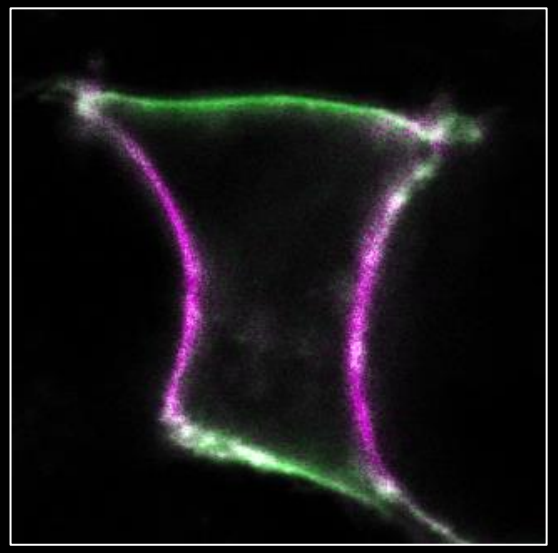


In presence of
activated Gαi1

DEVELOPMENT OF PROBES – IN PROGRESS

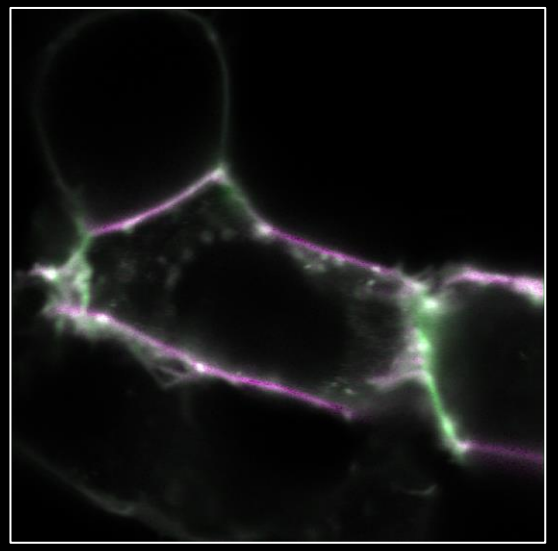
Probe K1:





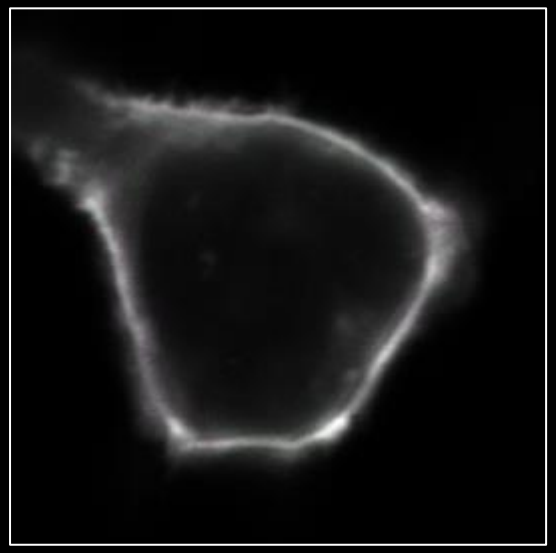
In presence of
activated Gαi1

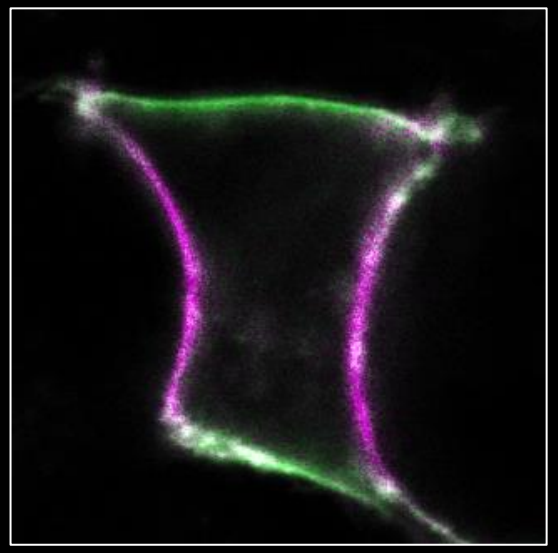
Probe K2:



DEVELOPMENT OF PROBES – IN PROGRESS

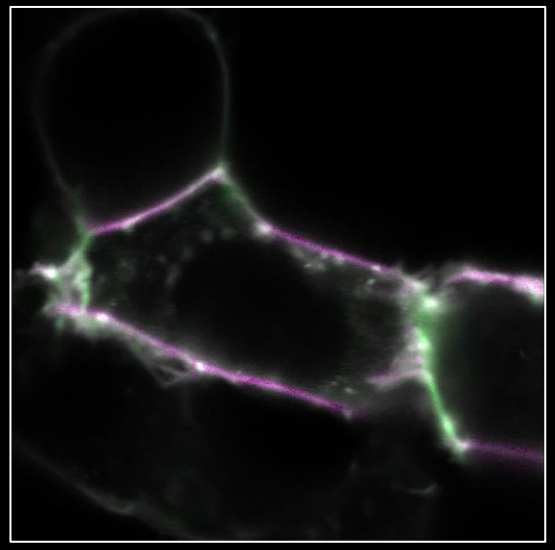
Probe K1:

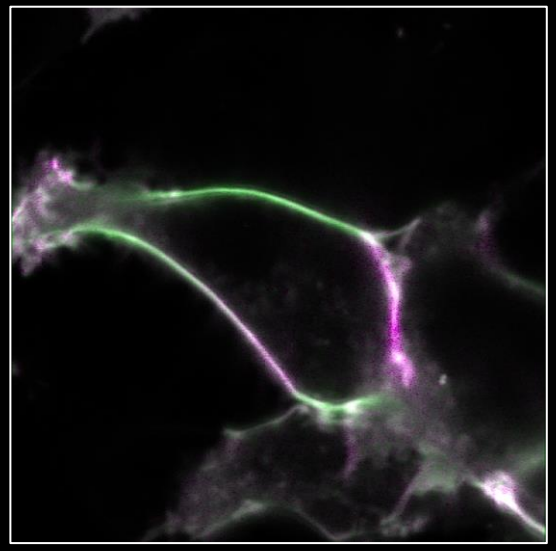




In presence of activated Gαi1

Probe K2:

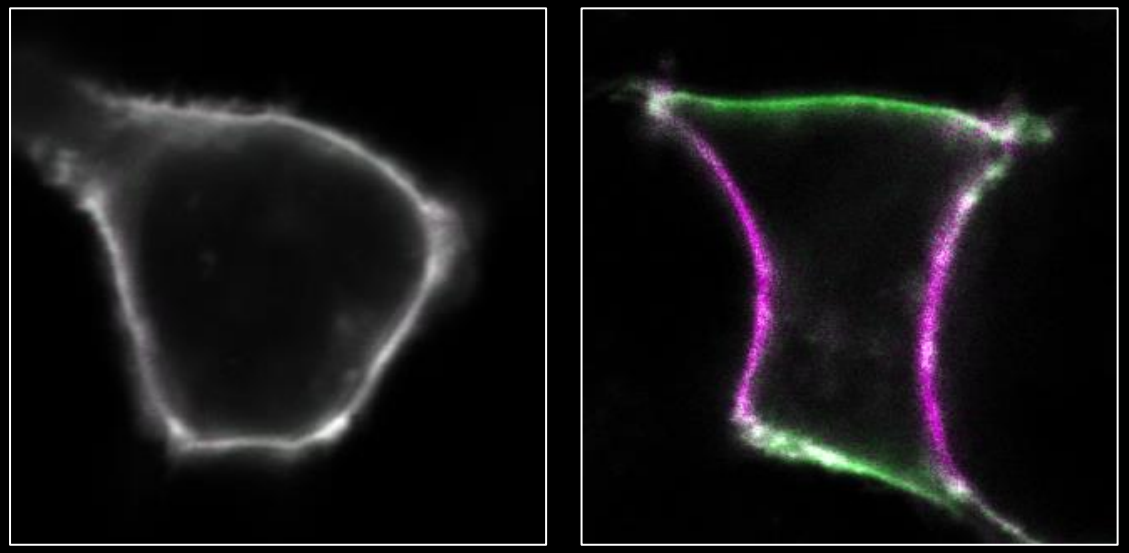




In presence of activated Gαi1

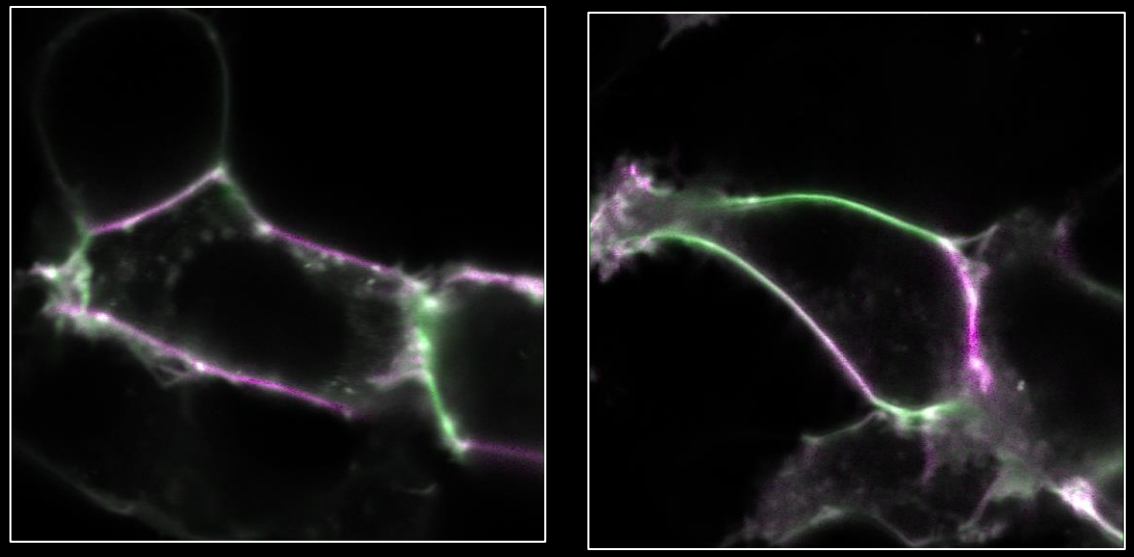
DEVELOPMENT OF PROBES – IN PROGRESS

Probe K1:



In presence of
activated Gαi1

Probe K2:

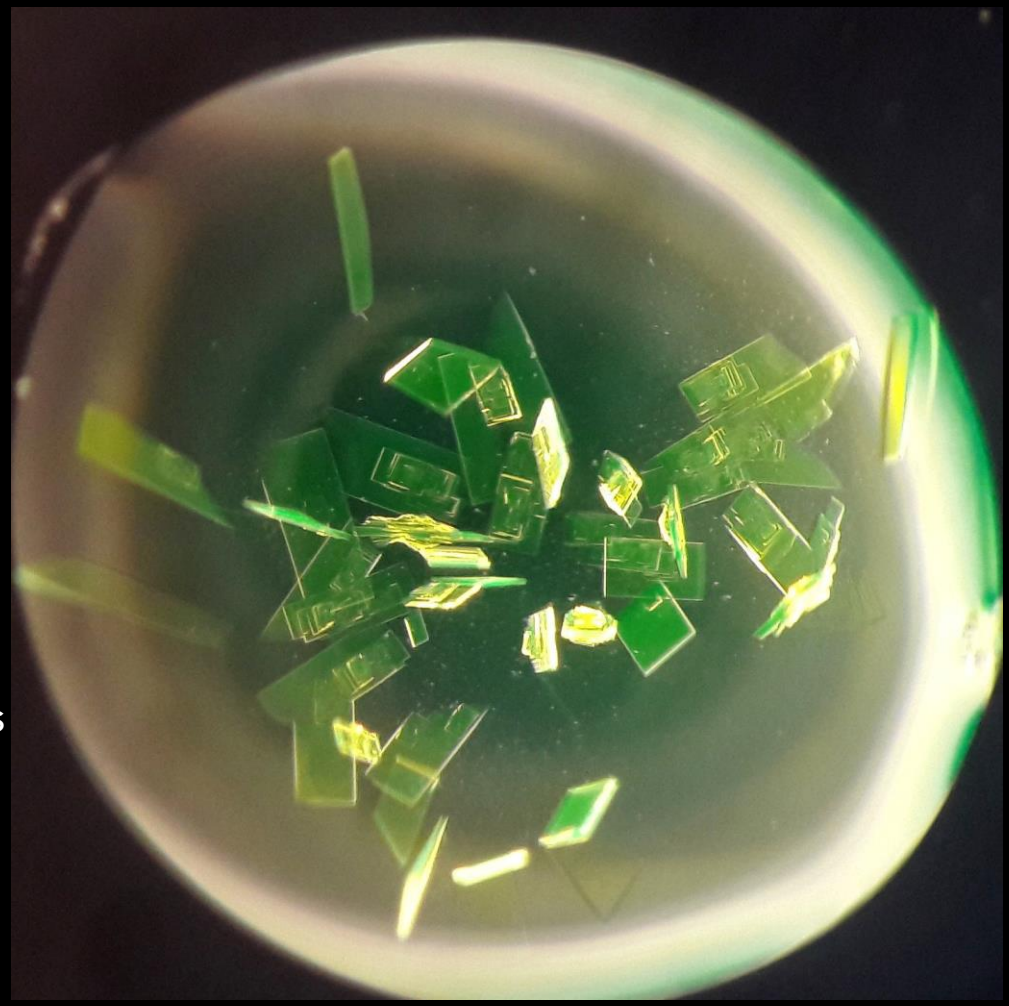


In presence of
activated Gαi1

Nowadays:
sensitive probes of activation of Gαi1, Gαs, Gα12, Gβγ , RhoA, Rac1, β2-adrenergic receptor, μ-opioid receptor, others

POLARIZATION MICROSCOPY

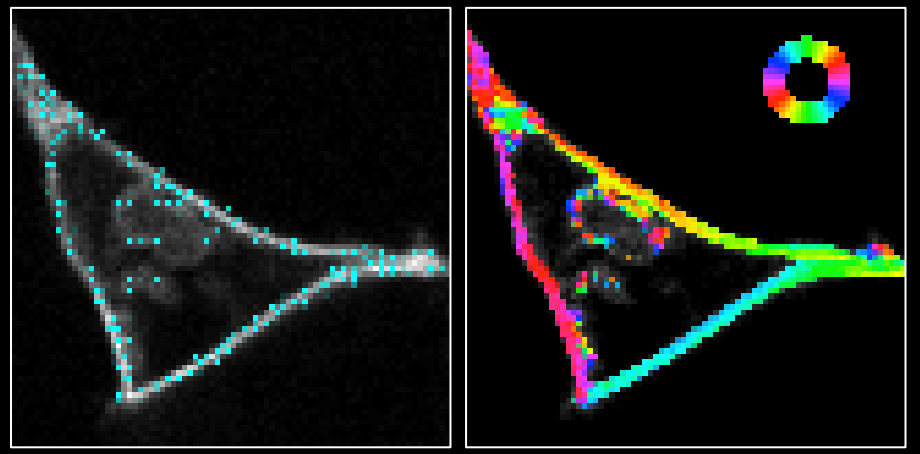
- Molecules behave like antennas
- Needs a single fluorescent label
- Allows multiplexing
- Ratiometric
- Can be quantitated
- Direction of TDM with respect to the cell membrane can be determined
- Orientation of an FP with respect to the cell membrane should soon be determinable (with some ambiguity?)
- Compatible with confocal/2P biological imaging
- Detecting conformational changes in proteins
- Detecting protein-protein interactions
- Can use existing constructs
- Allows easy, rapid, rational development of genetically encoded probes
- Allows observation of many important molecular processes
- Ample room for growth: HALO-, SNAP-, CLIP-tags, …



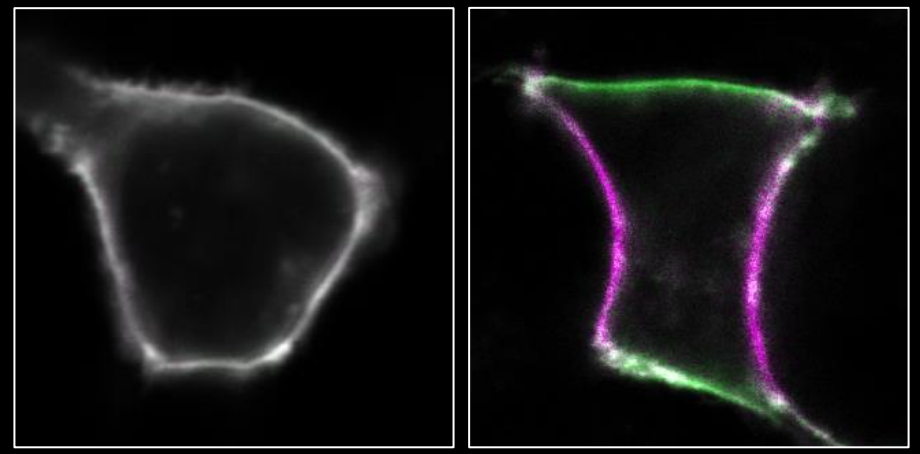
INNOVATIVE BIOIMAGING

Innovative Bioimaging

Hardware



Software



Probes

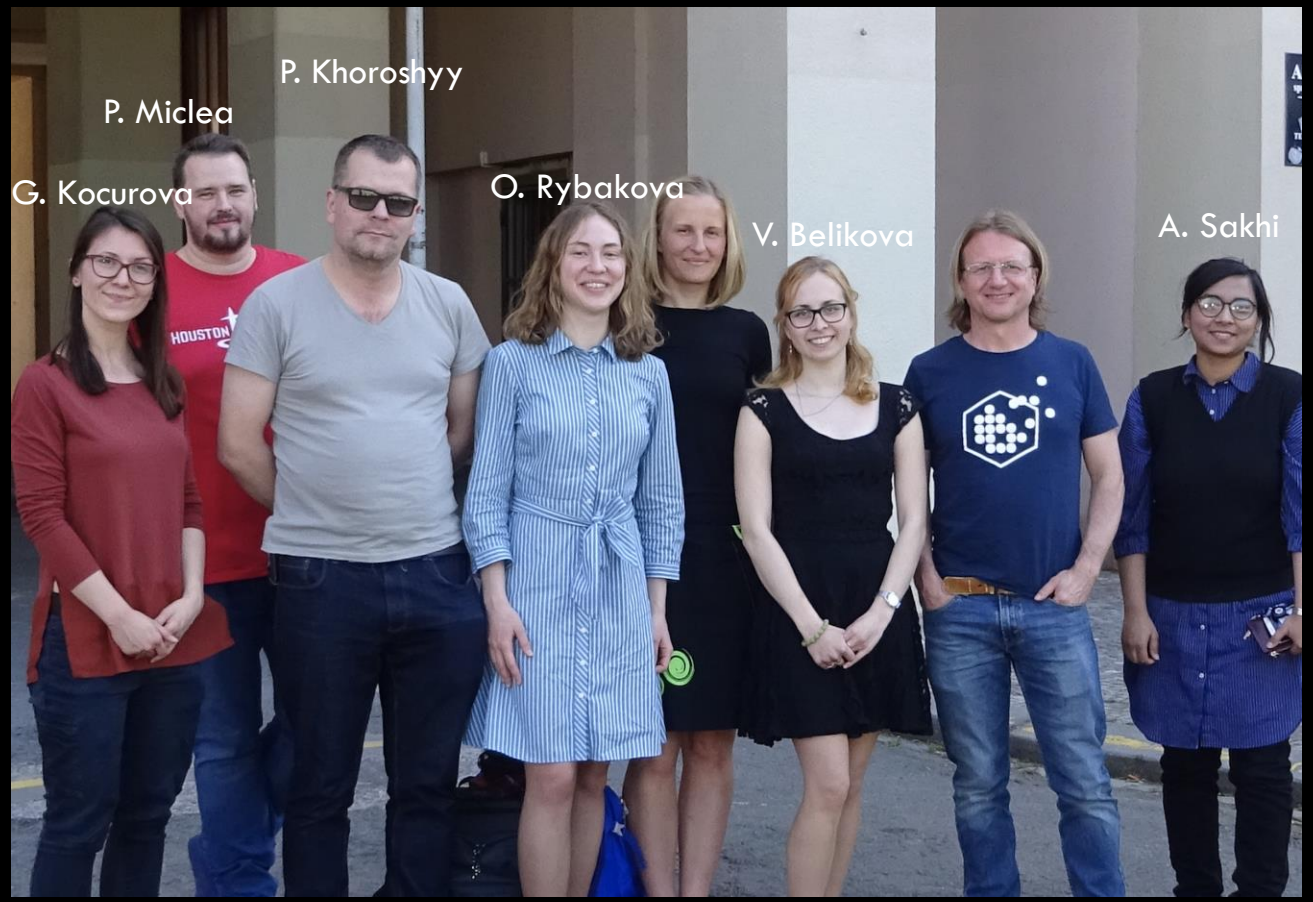
INNOVATIVE BIOIMAGING

Innovative Bioimaging

Partners:
- technical development
- marketing/sales

Funding

THANK YOU



A. Bondar

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MINISTRY OF EDUCATION, YOUTH AND SPORTS