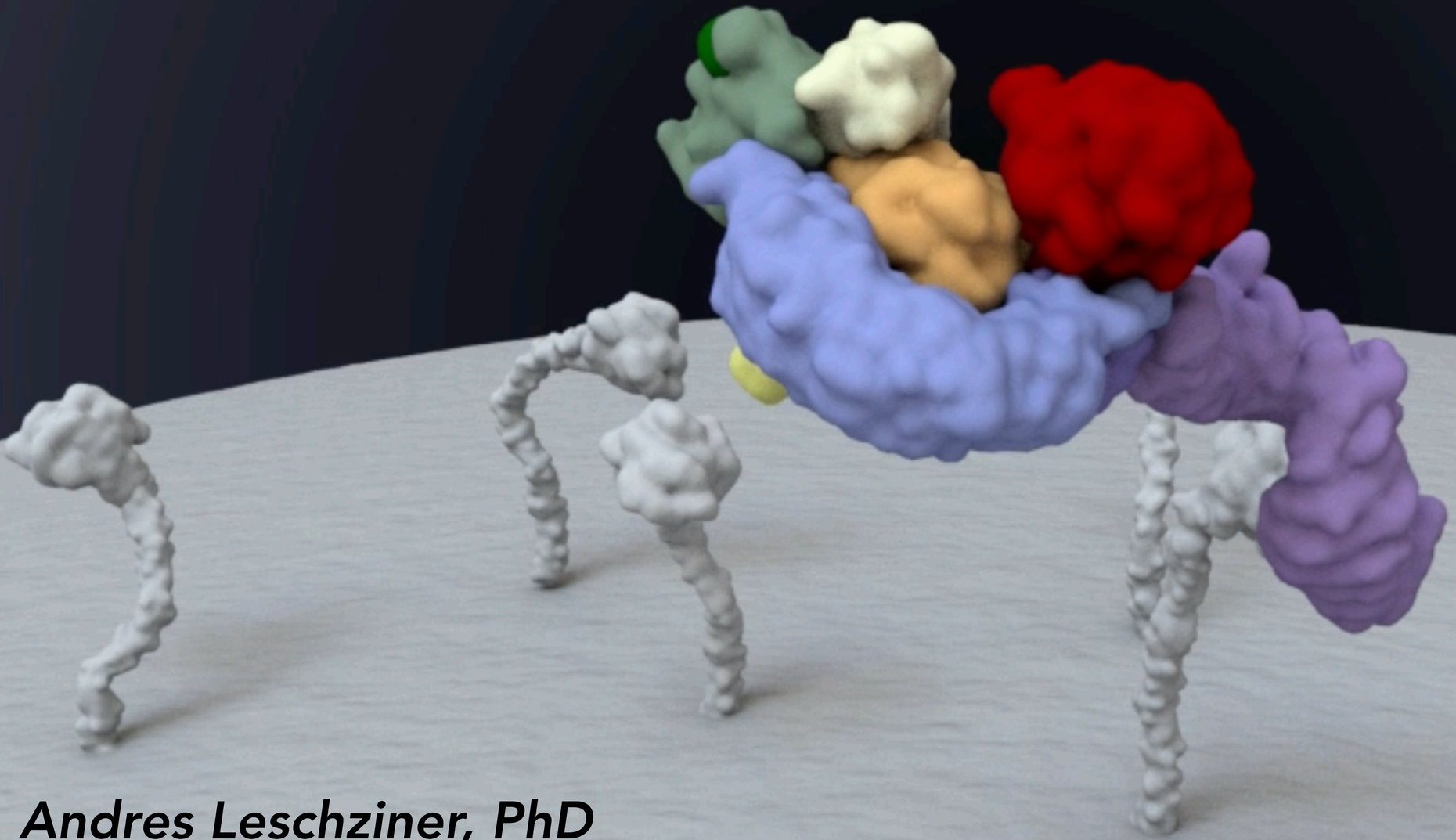
Applications of SPA to biological systems & data visualization



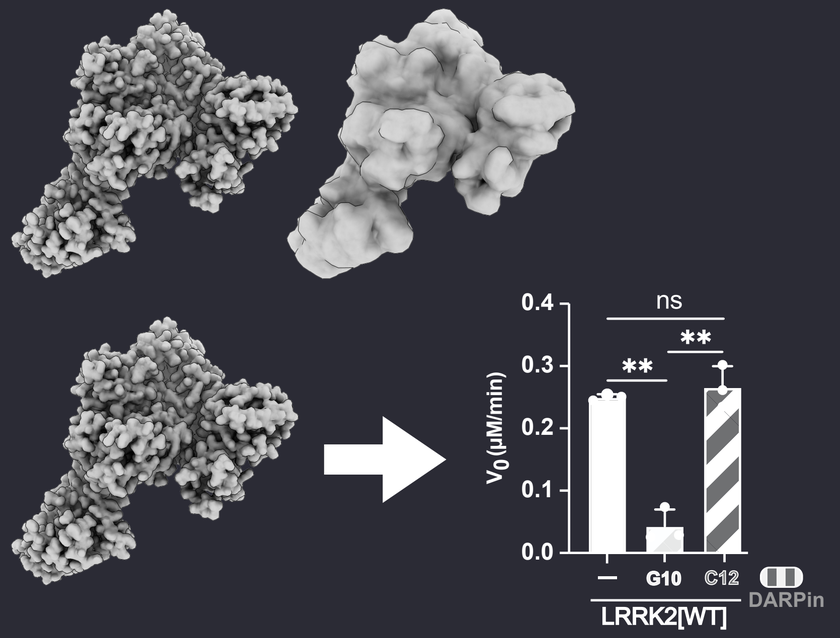
Andres Leschziner, PhD
Department of Biochemistry & Biophysics

Weill Cornell Medicine

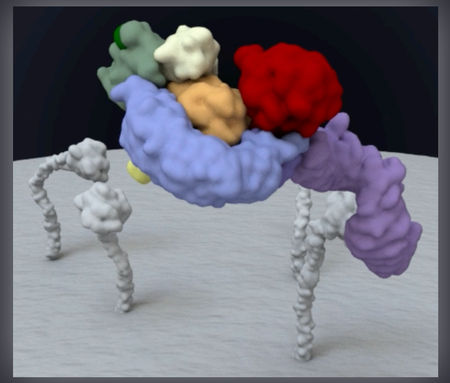
Applications of SPA to biological systems & data visualization

What resolution do I need to answer the question?

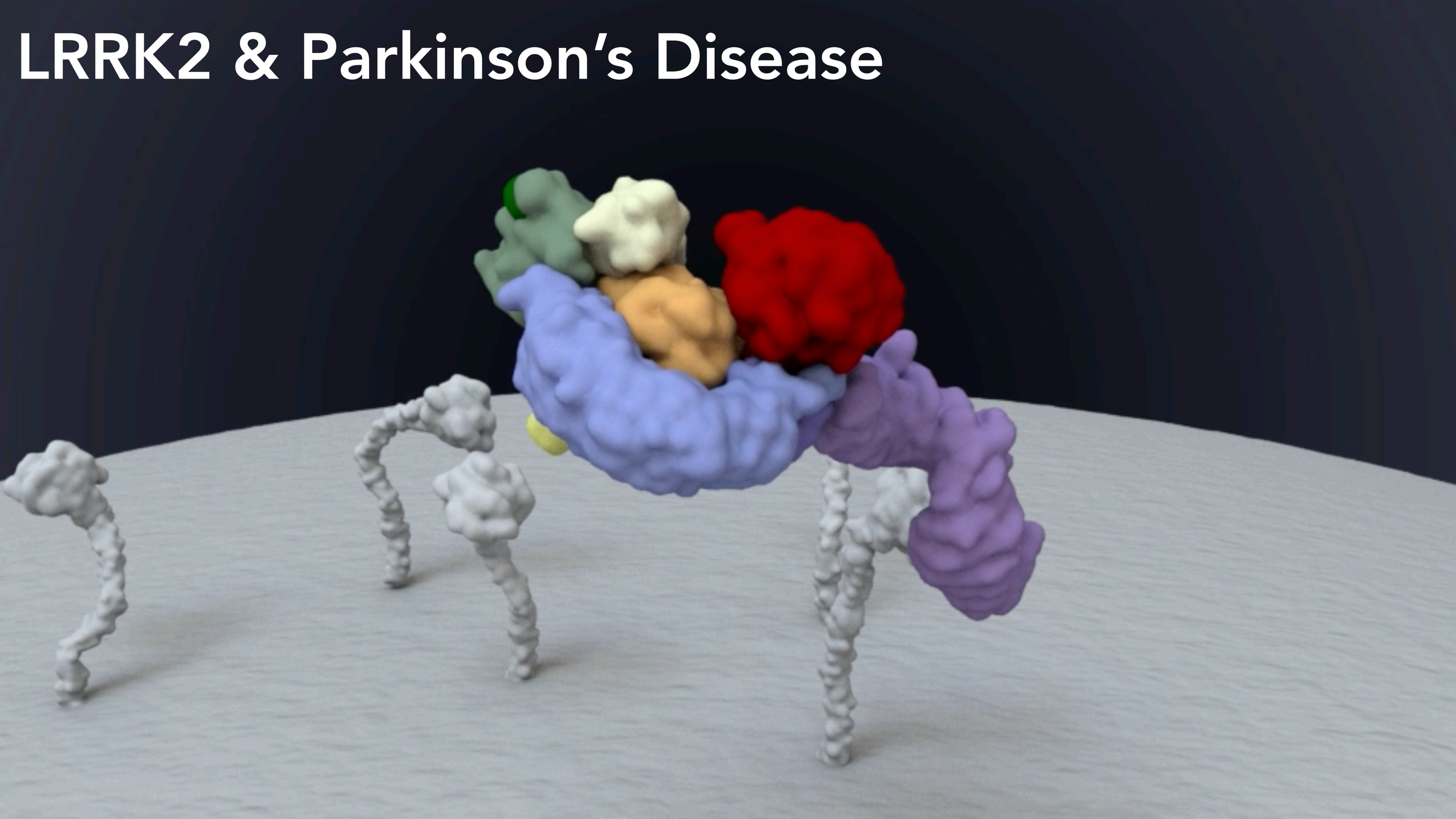
Structure = Function



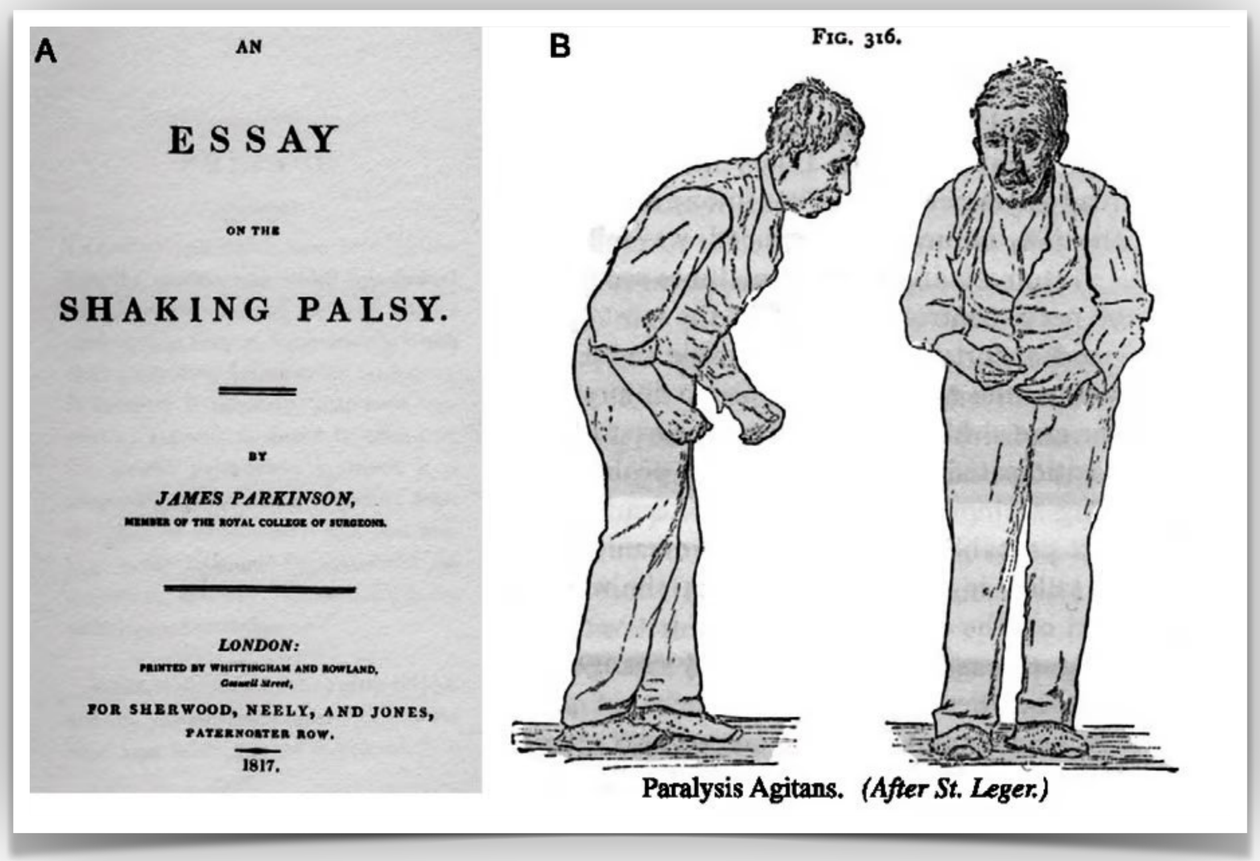
Data visualization = hypothesis generation



LRRK2 & Parkinson's Disease



Parkinson's Disease (PD)



- Second most common neurodegenerative disease
- ~10 million affected worldwide
- 10-15% of cases have a direct genetic component

Leucine **R**ich **R**epeat **K**inase **2** (LRRK2)

Leucine **R**ich **R**epeat **K**inase **2** (LRRK2) & Parkinson's Disease: a brief history

2002

A New Locus for Parkinson's Dis… Chron…

Manabu Funayama, M… Masaaki Saito,

Funayama …

…nd PD

2004

Neuron, Vol. 44, 595–600…

Cloning o… that Caus…

Coro Pa… E. Whitn…

Neuron, V…

Mut… Park…

Alexand… Petra L…

Pai… Zi…

2005/6

PNAS

Parkinso… leucine-r…

An… Val…

The … I2020…

Christian… Eric O'Ne…

Glo…

…ctivate its kinase

2018

SCIENCE TRAN…

PARKINSON'S DI…

LRRK2 activa…

Roberto Di Maio[1,2,3], Eric K. …, Laurie H. Sanders[1,2,4], Briana R. De Miranda[1,2], Alevtina Zharikov[1,2], Amber Van Laar[1,2], Antonia F. Stepan[5], Thomas A. Lanz[5], Julia K. Kofler[6], Edward A. Burton[1,2,7], Dario R. Alessi[8], Teresa G. Hastings[1,2], J. Timothy Greenamyre[1,2,7]*

…n idiopathic PD

Di Maio et al. (2018) *PMID: 30045977*

2026

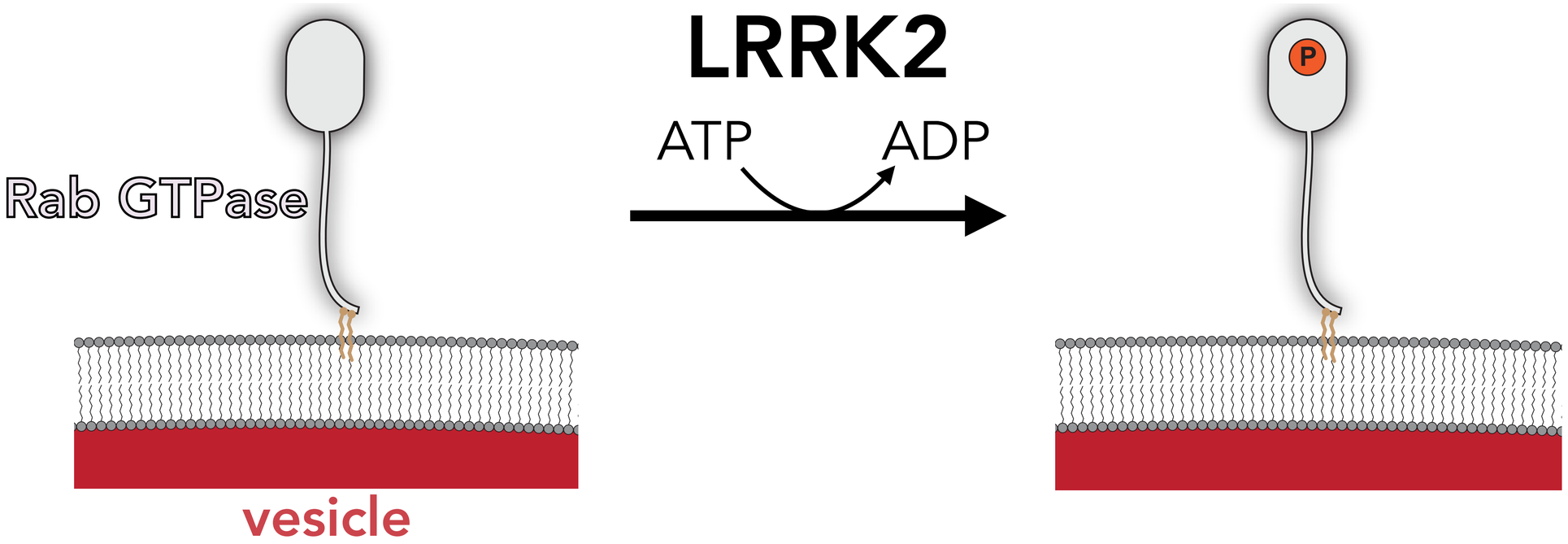
DENALI Biogen — Kinase inhibitor (Phase II)

Neuron 23 — Kinase inhibitor (Phase I)

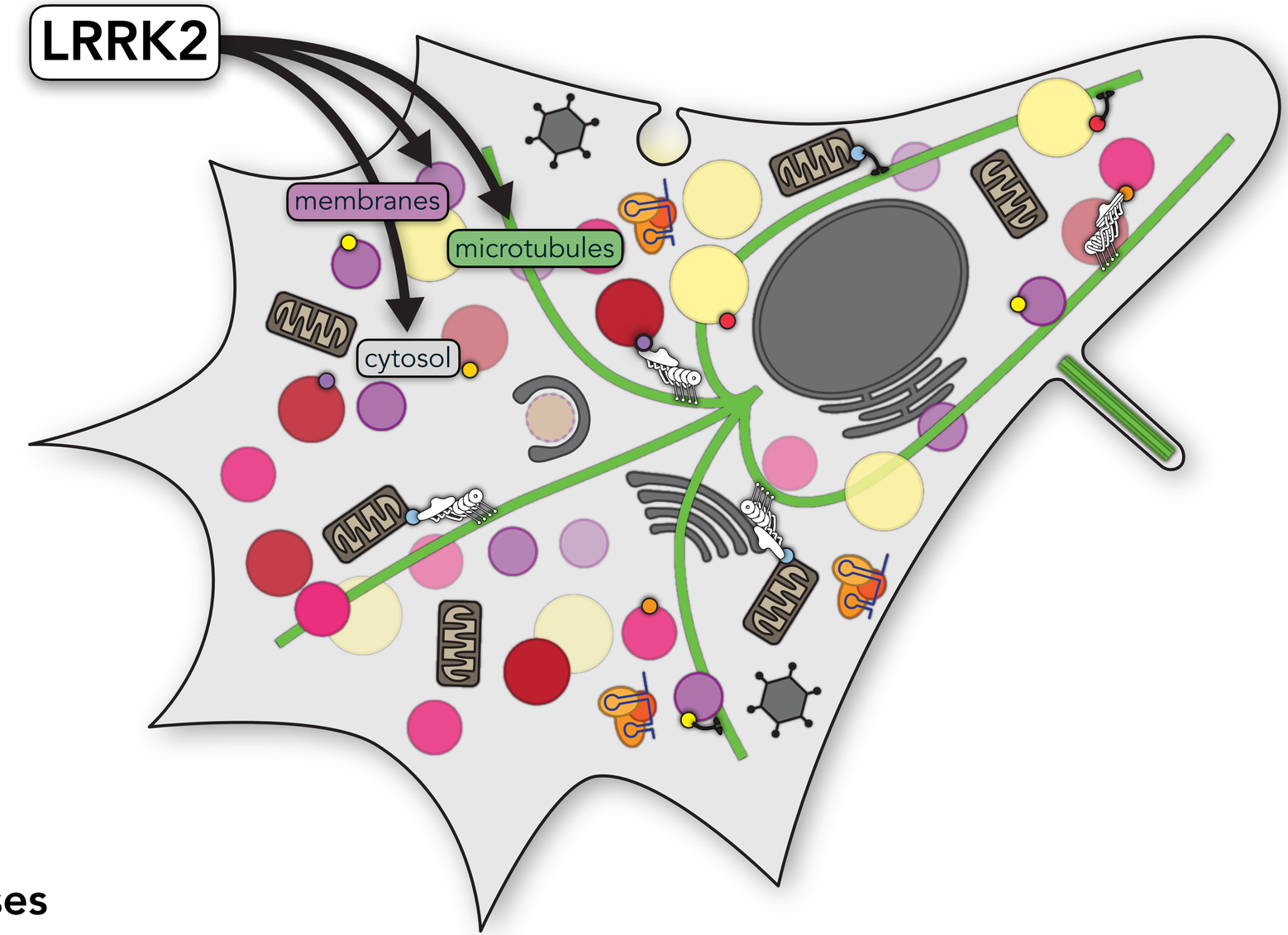
Biogen — Anti-Sense Oligo (Phase I)

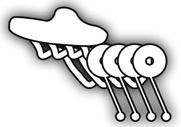
ARVINAS — PROTAC (Phase I)

LRRK2 is Rab kinase



LRRK2 is Rab kinase





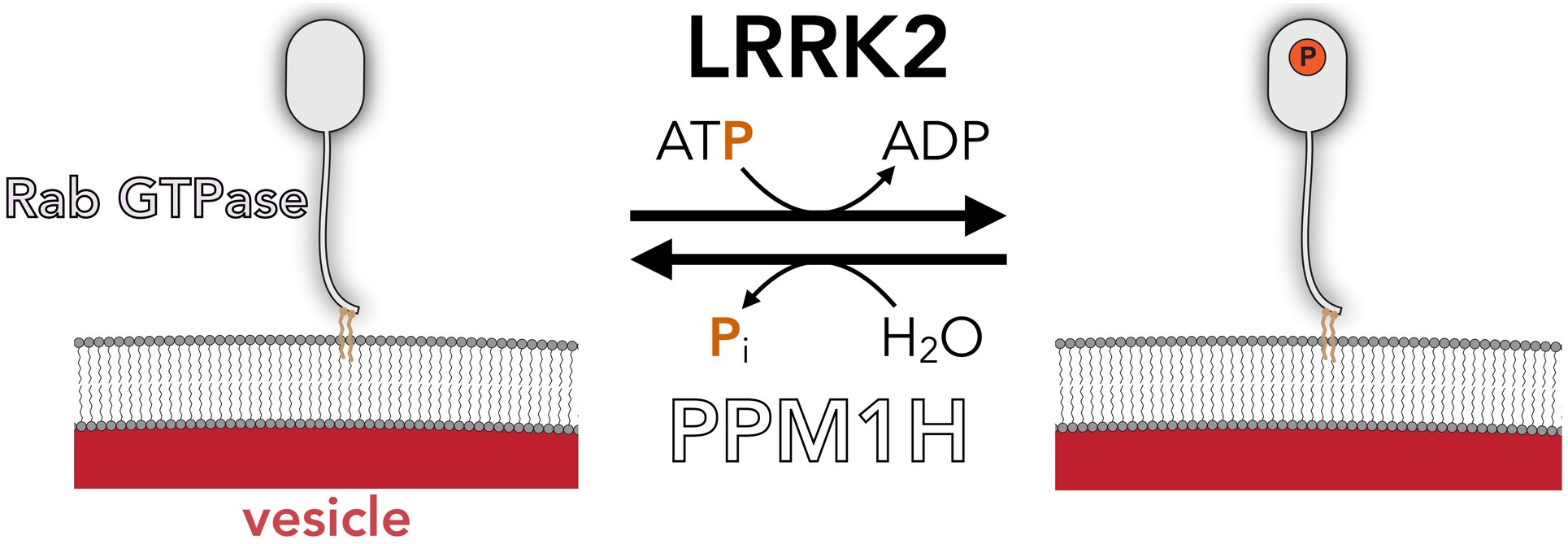
dynein

kinesin

Rab GTPases

Steger et al (2016) *PMID: 26824392*, and Steger et al (2017) *PMID: 29125462*
Reviewed in Usmani et al. (2021) *PMID: 33526455*

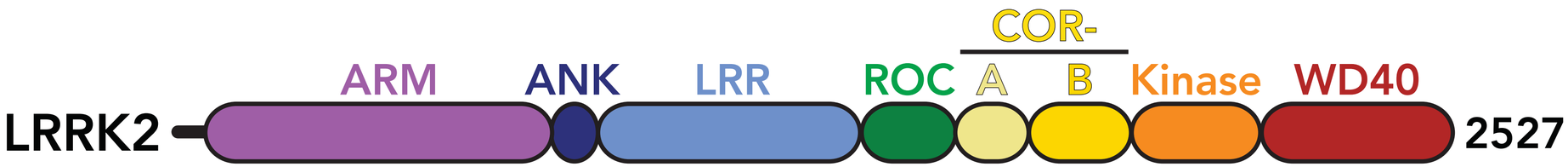
LRRK2 is Rab kinase



LRRK2

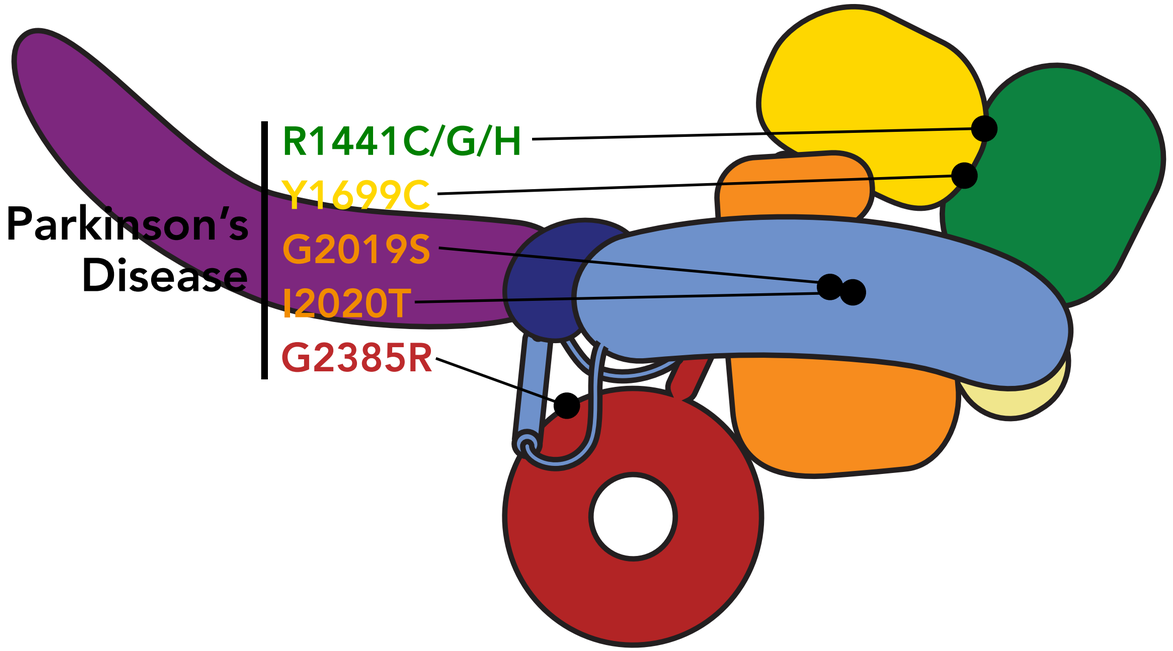
ARM: Armadillo repeats
ANK: Ankyrin repeats
LRR: Leucine Rich Repeats

ROC: Ras-Of-Complex
COR: C-terminal Of ROC



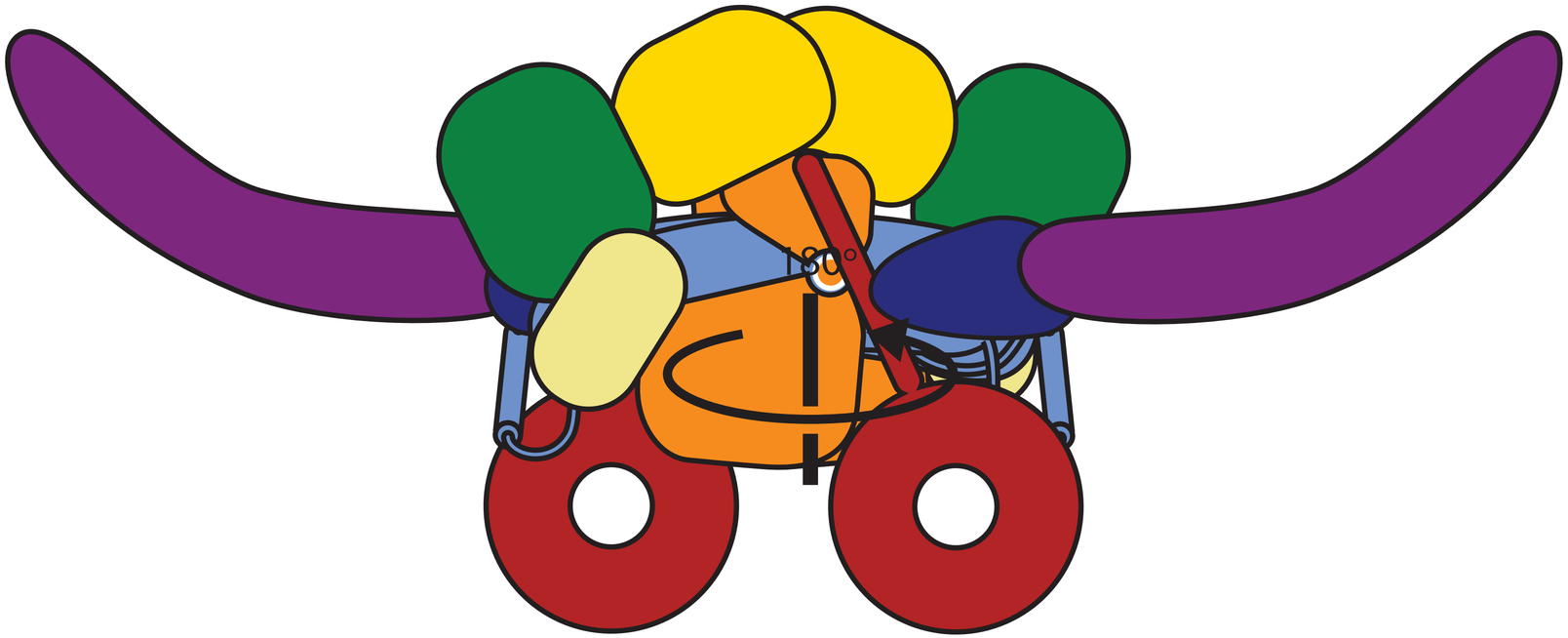
LRRK2

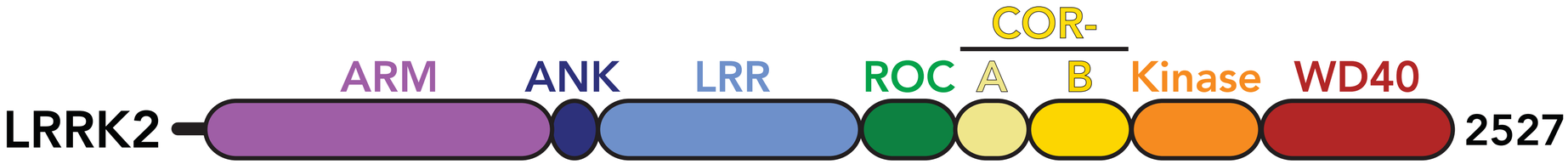
PD mutations



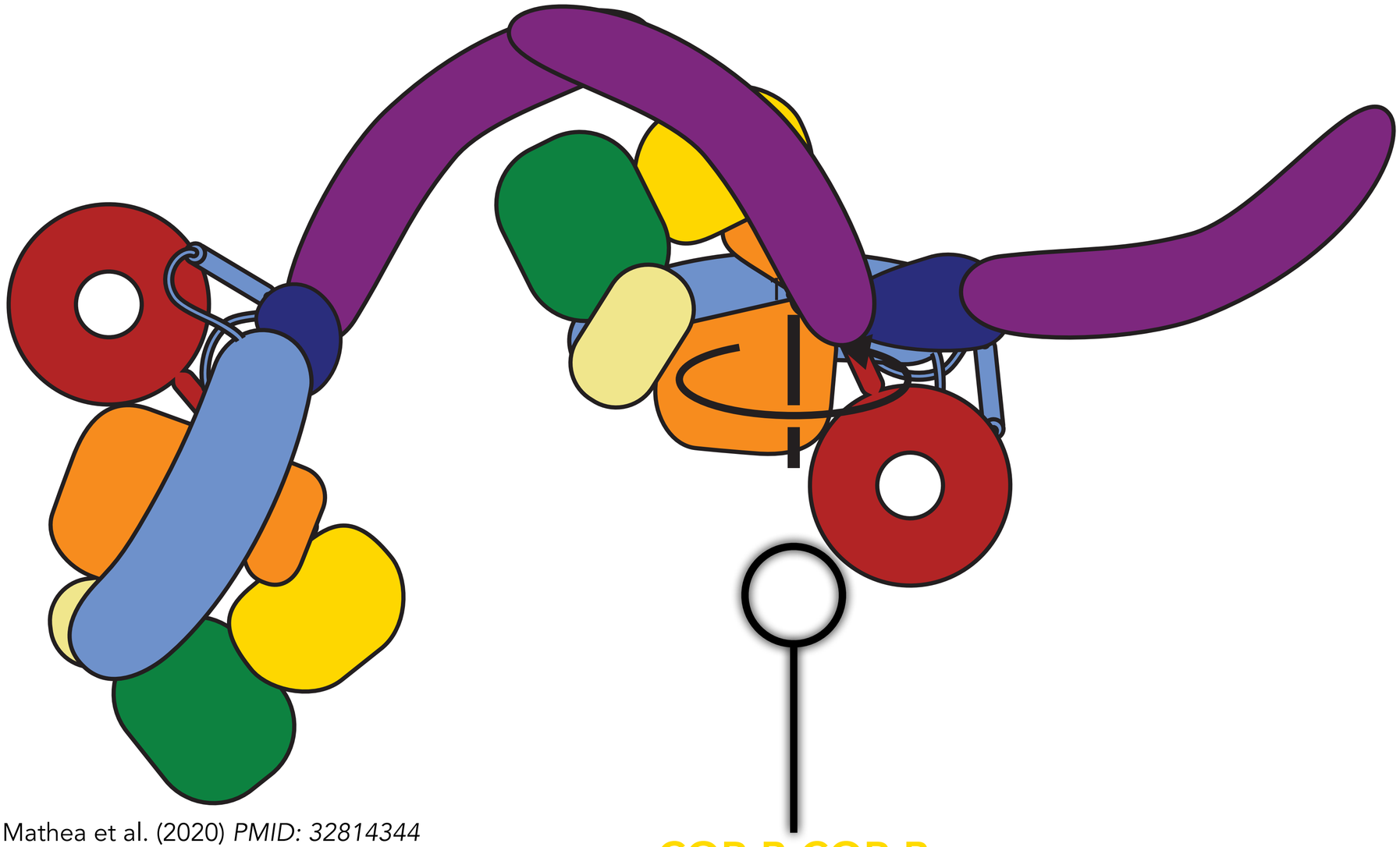
Deniston, Salogiannis, Mathea et al. (2020) *PMID: 32814344*

LRRK2



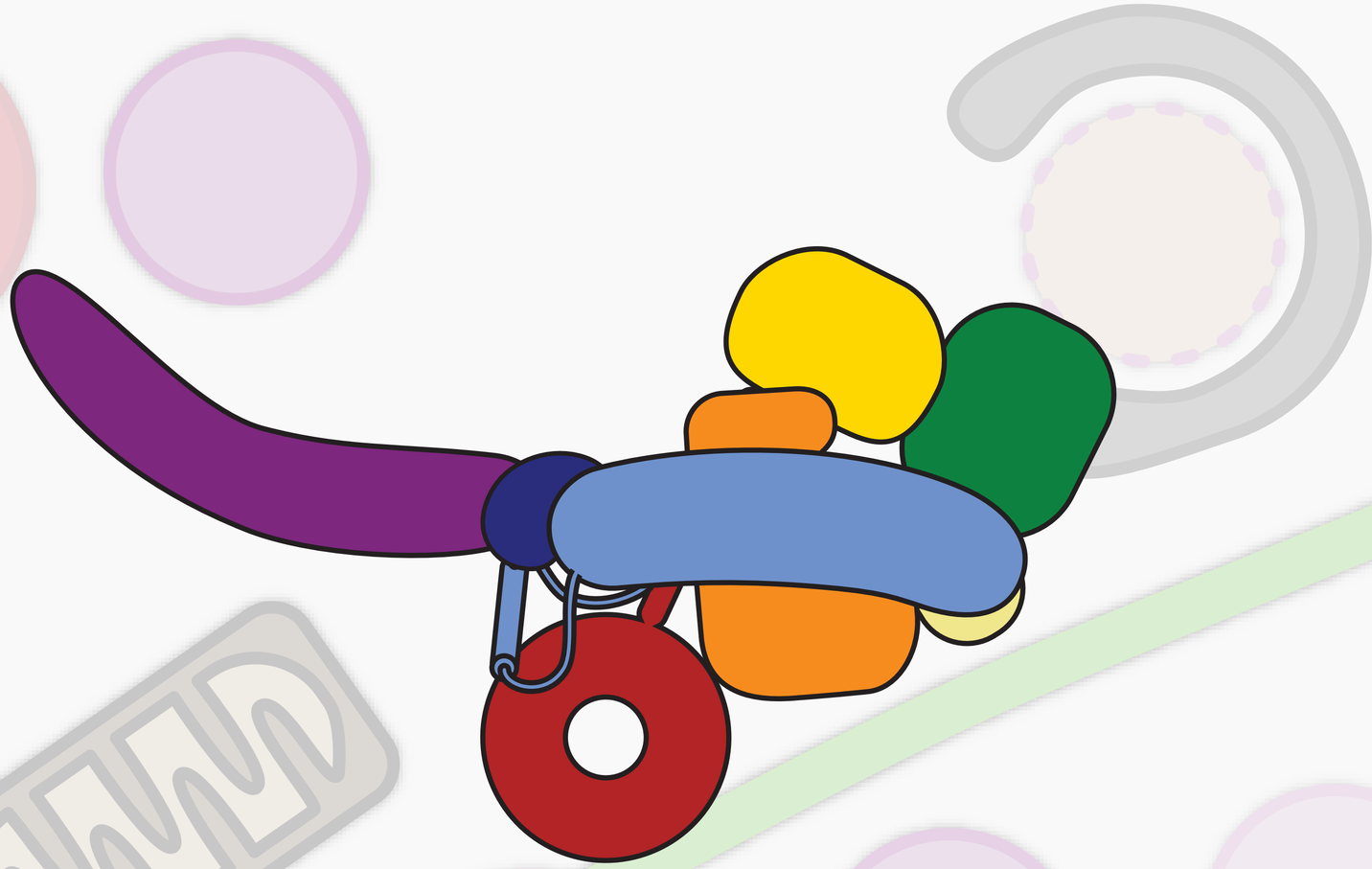


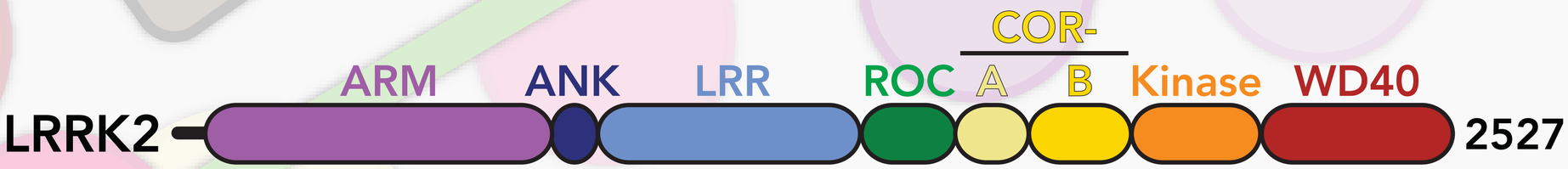
LRRK2
dimer



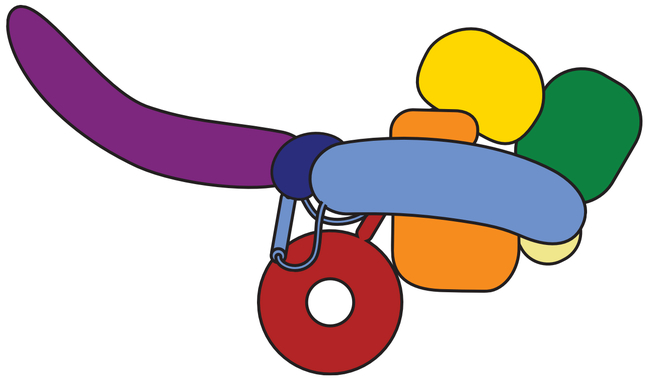
Deniston, Salogiannis, Mathea et al. (2020) *PMID: 32814344*
Myasnikov et al. (2021) *PMID: 34107286*

LRRK2





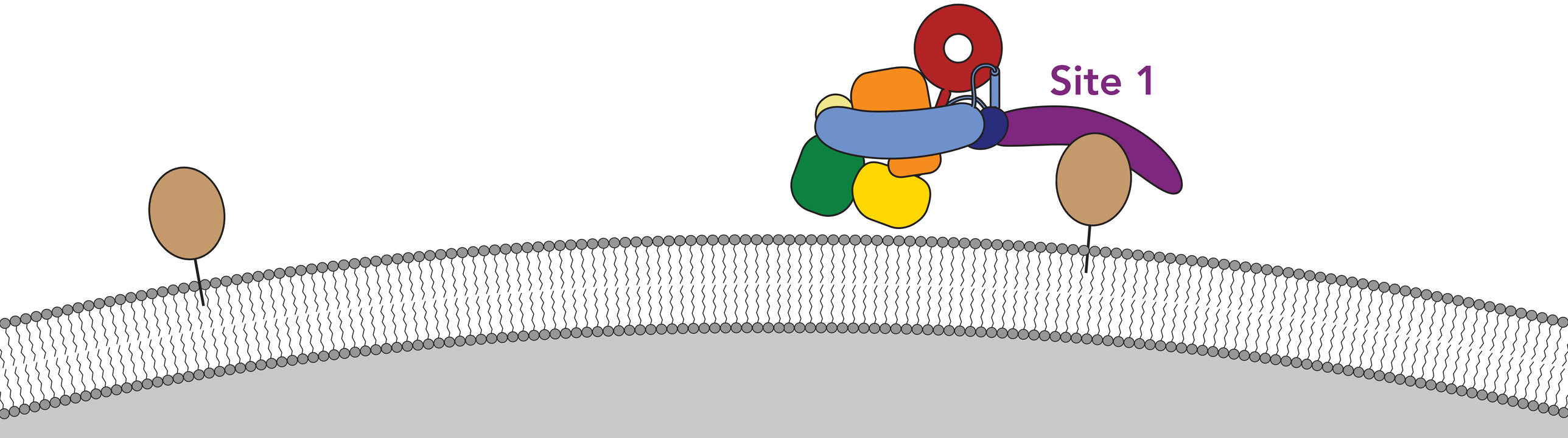
Recruitment



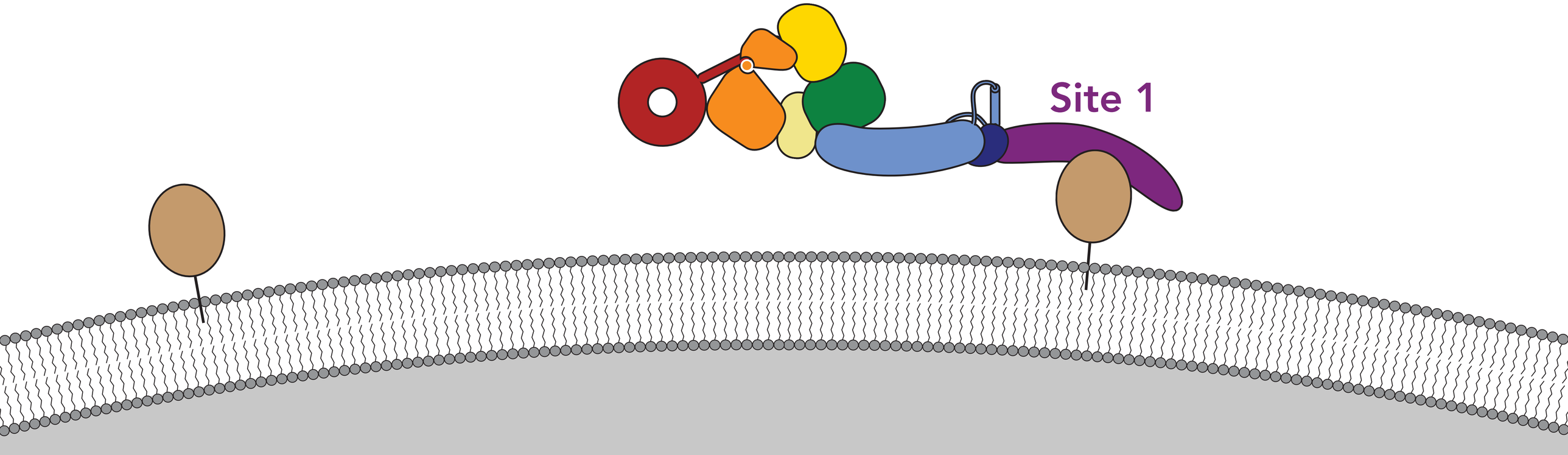
Site 1

Vides et al., (2022) *PMID: 36149401*

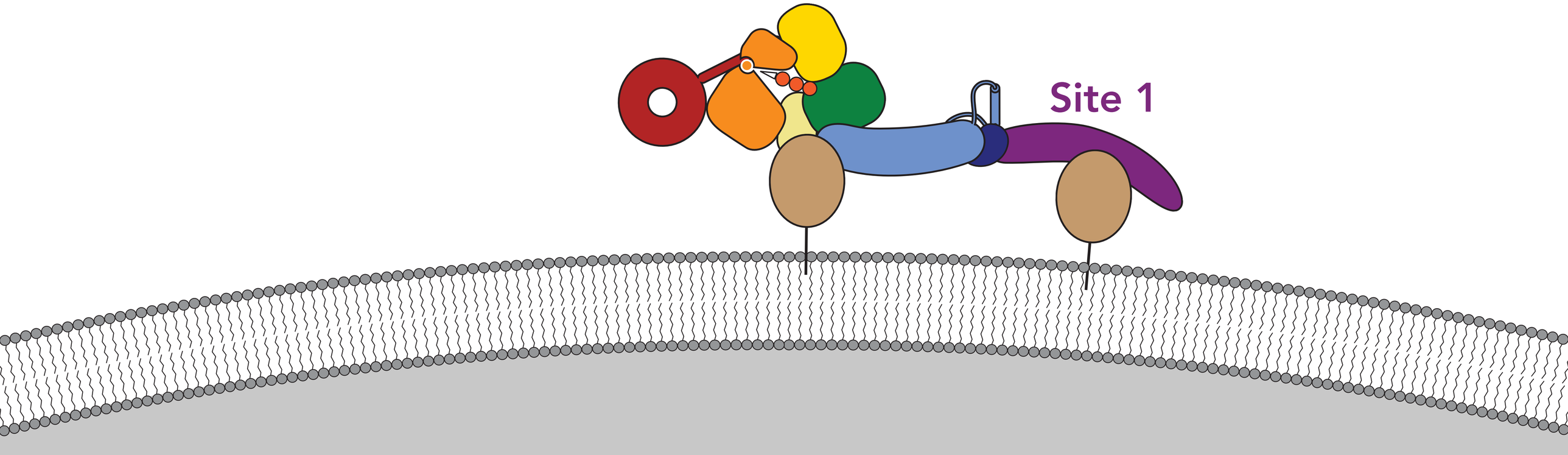
Release of Autoinhibition



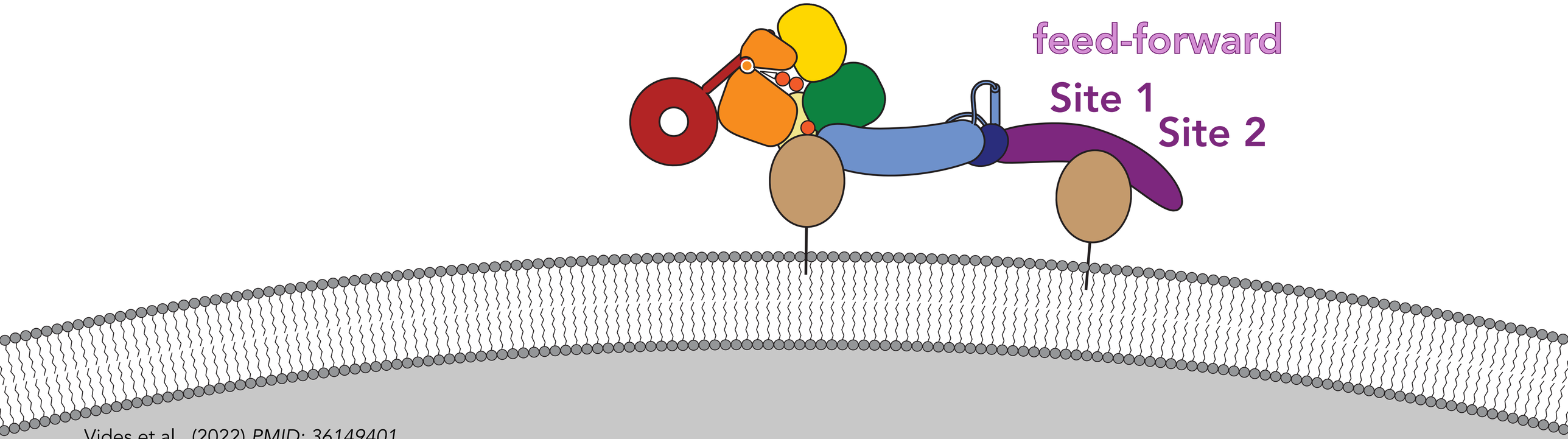
Substrate binding



Kinase
activity

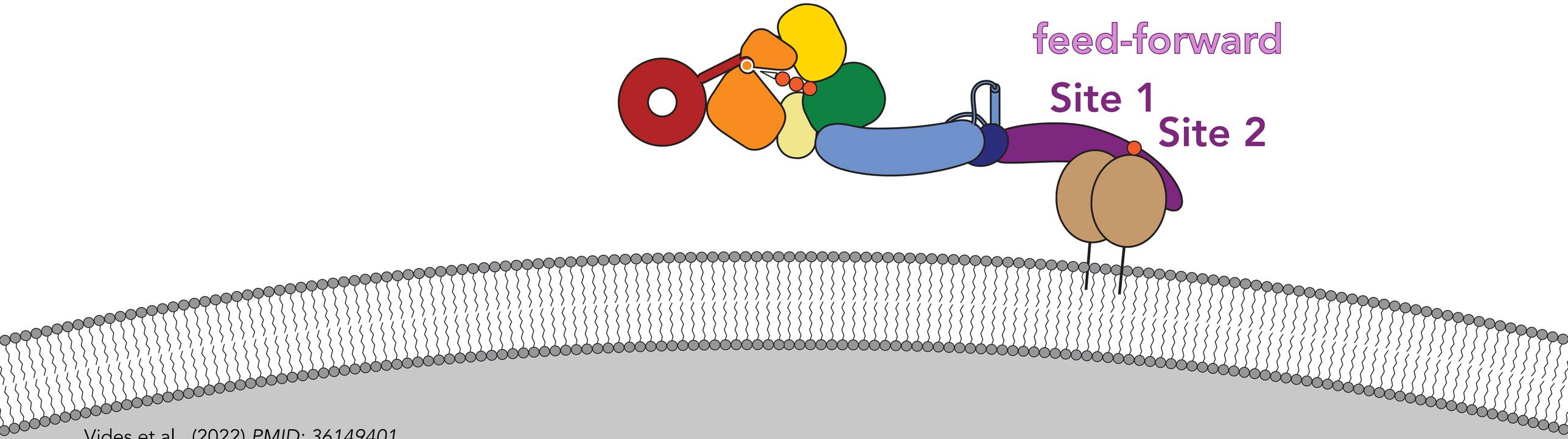


Strengthening of Recruitment

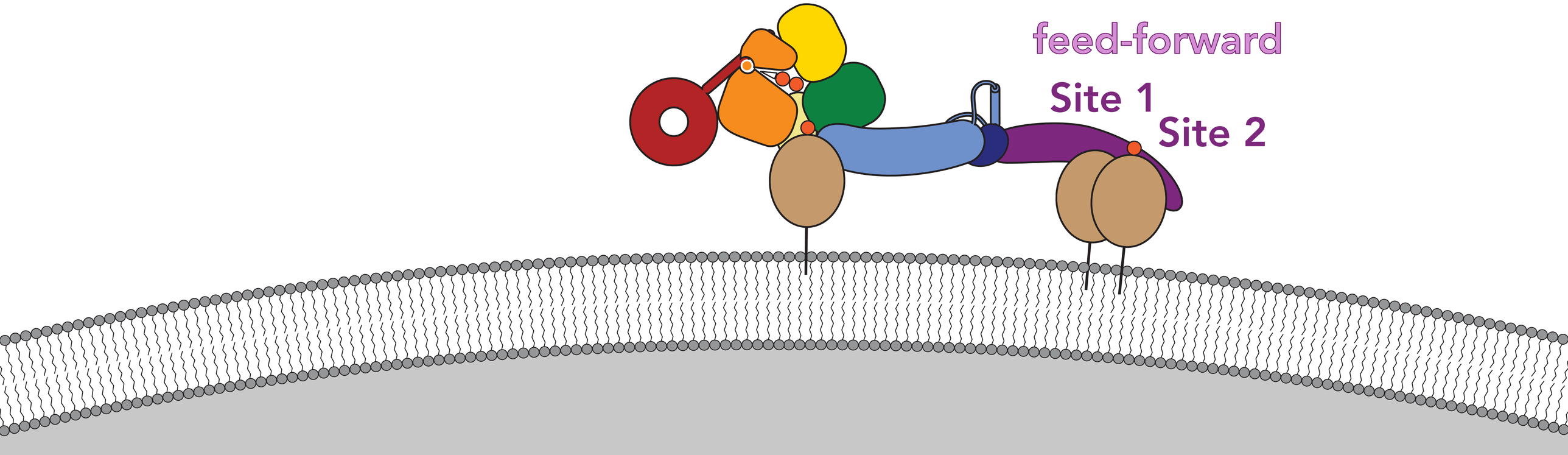


Vides et al., (2022) *PMID: 36149401*

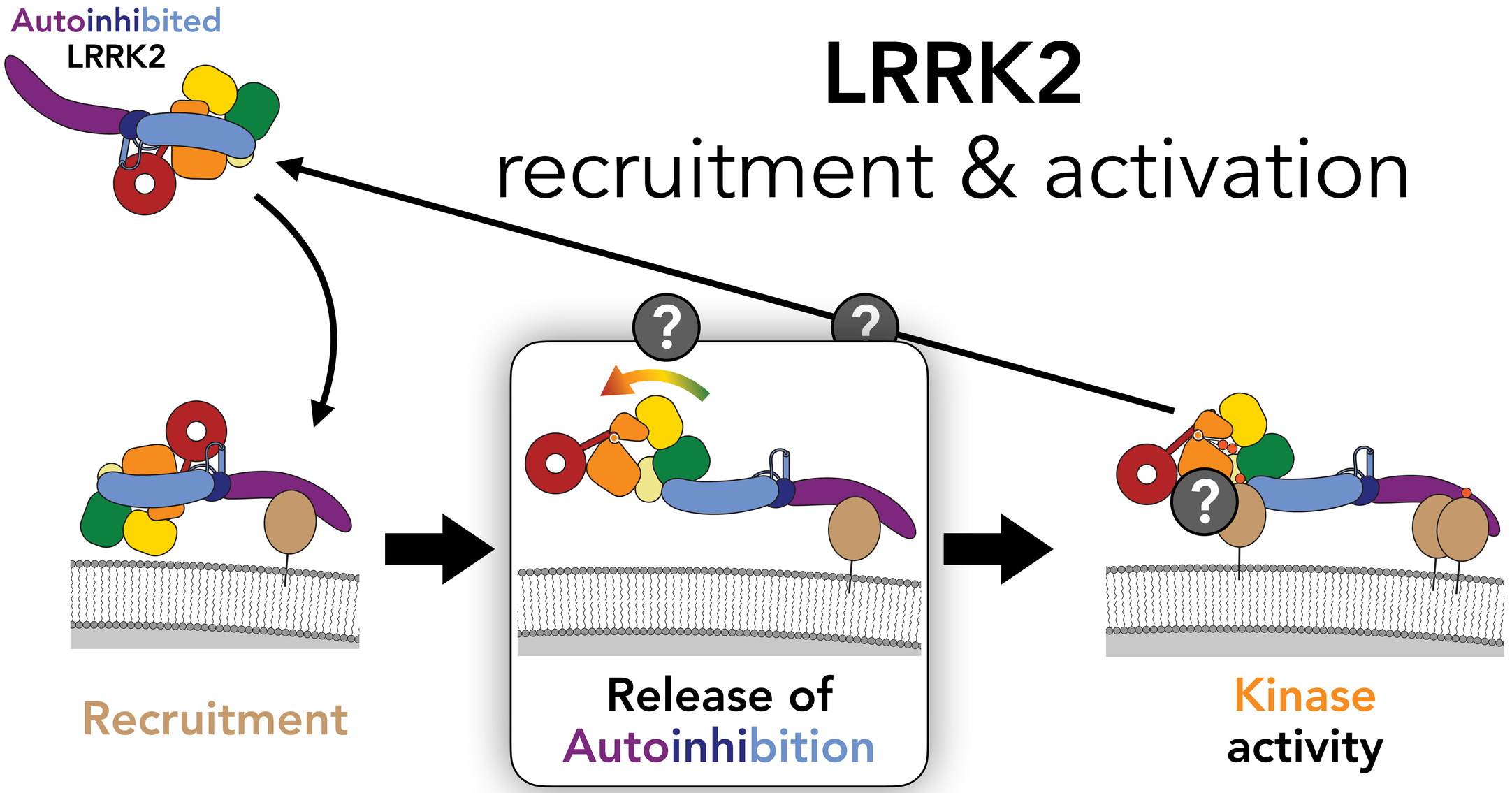
Kinase
activity



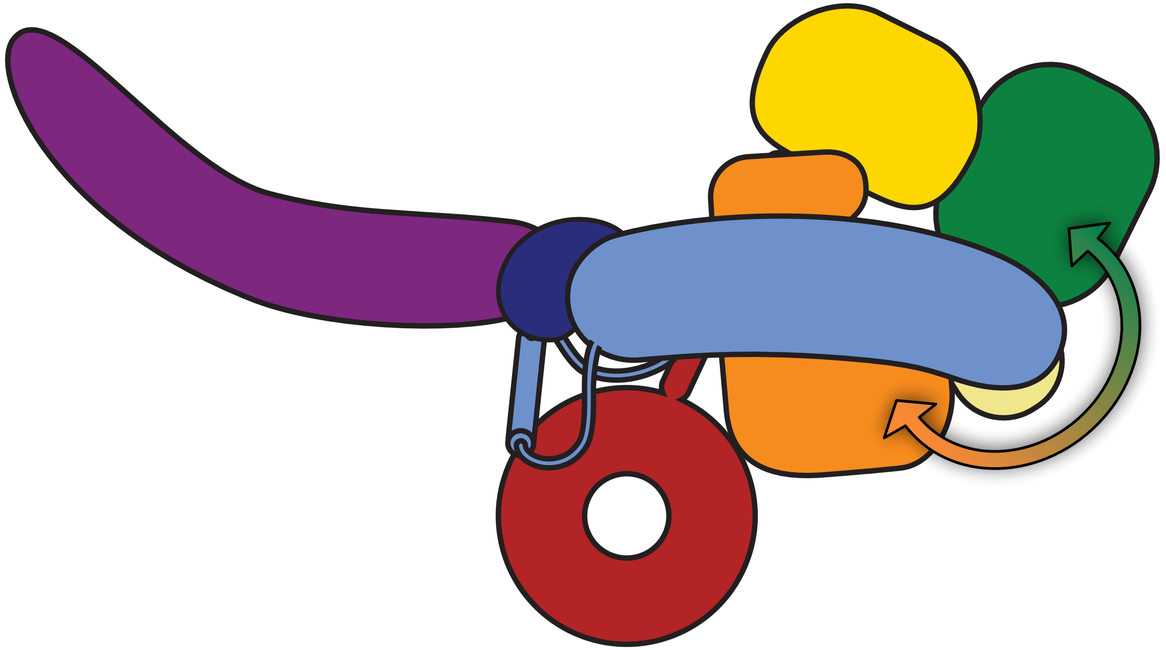
Vides et al., (2022) *PMID: 36149401*


feed-forward
Site 1
Site 2

LRRK2
recruitment & activation



LRRK2

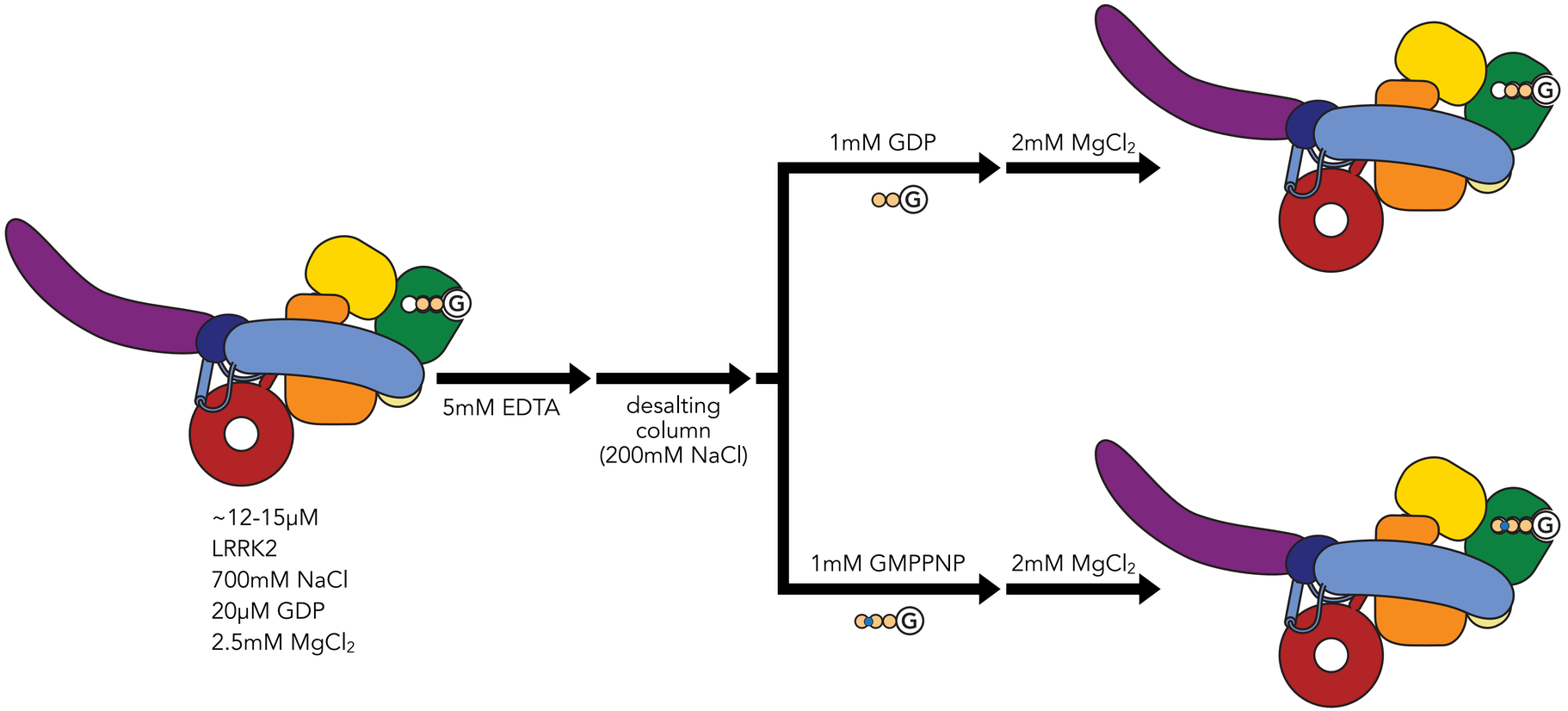


West et al. (2007) *PMID: 17200152*
Guo et al. (2007) *PMID: 17706965*
Ito et al. (2007) *PMID: 17260967*
Greggio et al. (2009) *PMID: 19733152*
Taymans et al. (2011) *PMID: 21858031*
Webber et al. (2011) *PMID: 21806997*
Biosa et al. (2013) *PMID: 23241358*
Blanca Ramirez et al. (2017) *PMID: 28453723*

How does the GTPase control LRRK2's activation?

An unbiased structural approach

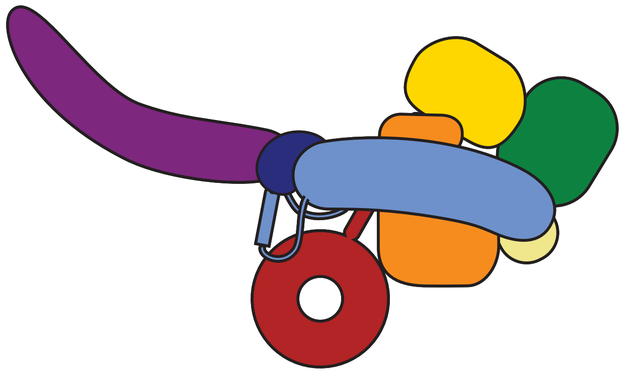
Nucleotide exchange @ROC



Lawrence Zhu
(Shokat lab, UCSF)

Amalia Villagran Suarez
Delisa Ramos

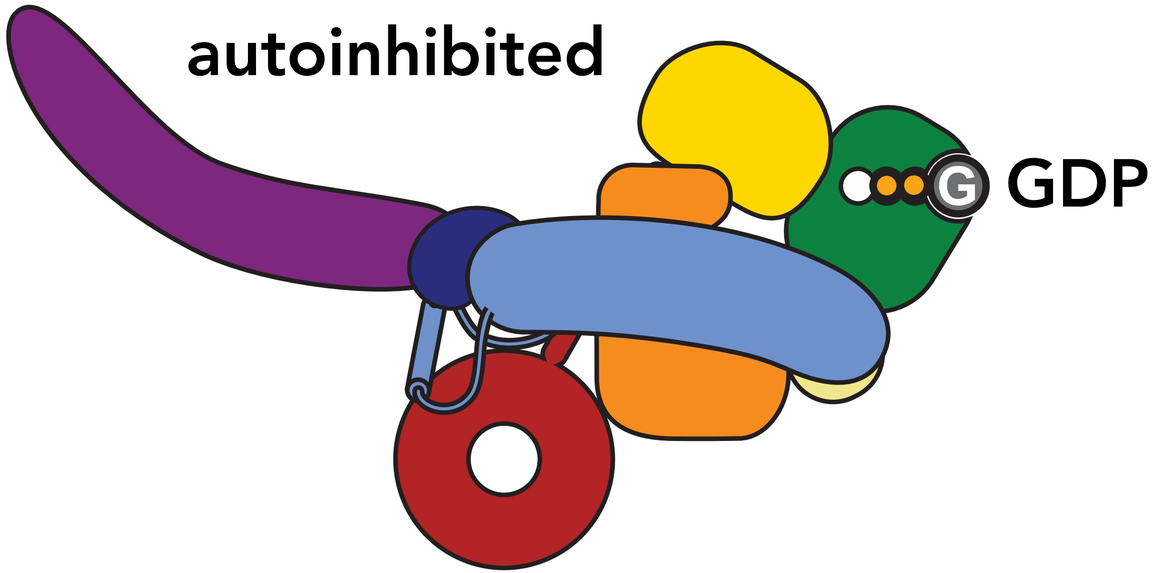
How to “score” activation



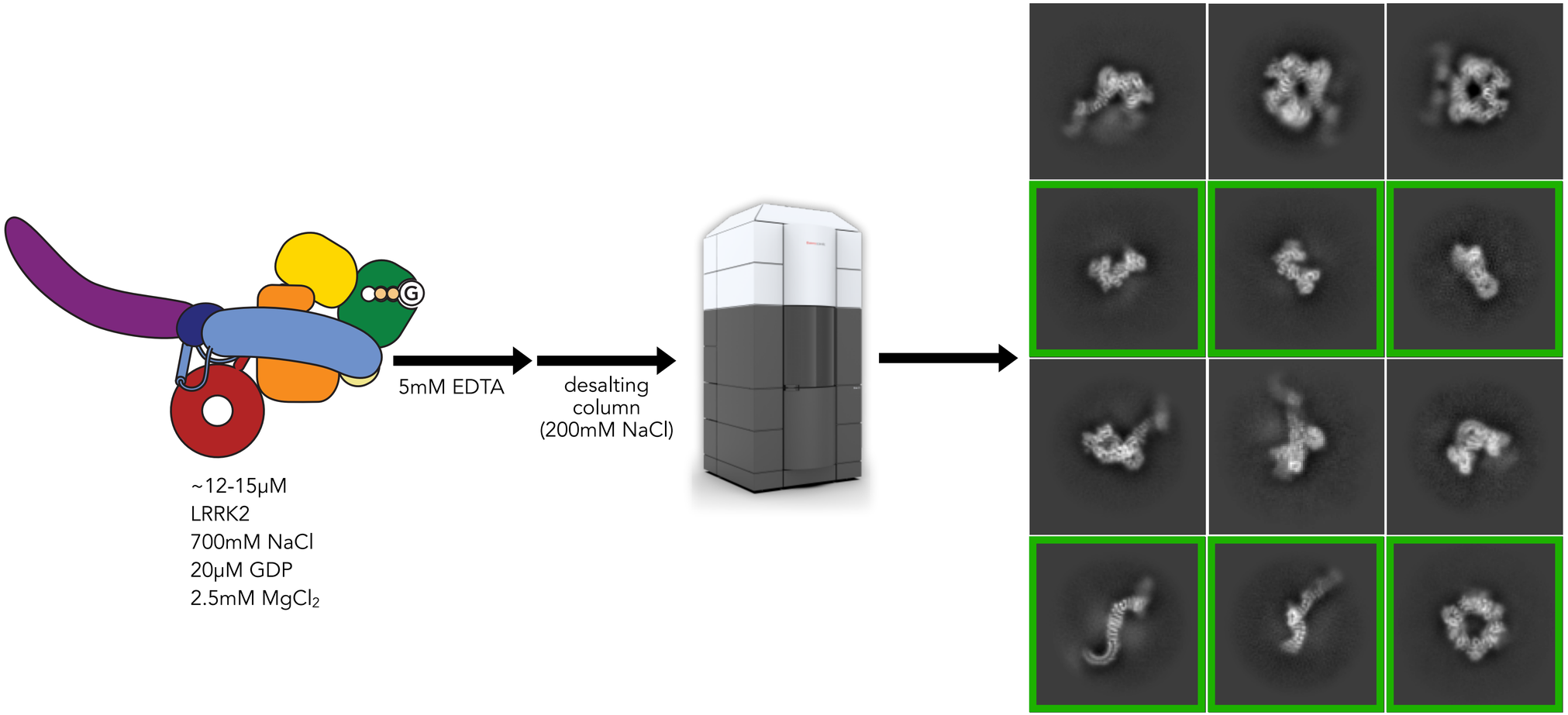
autoinhibited

activated

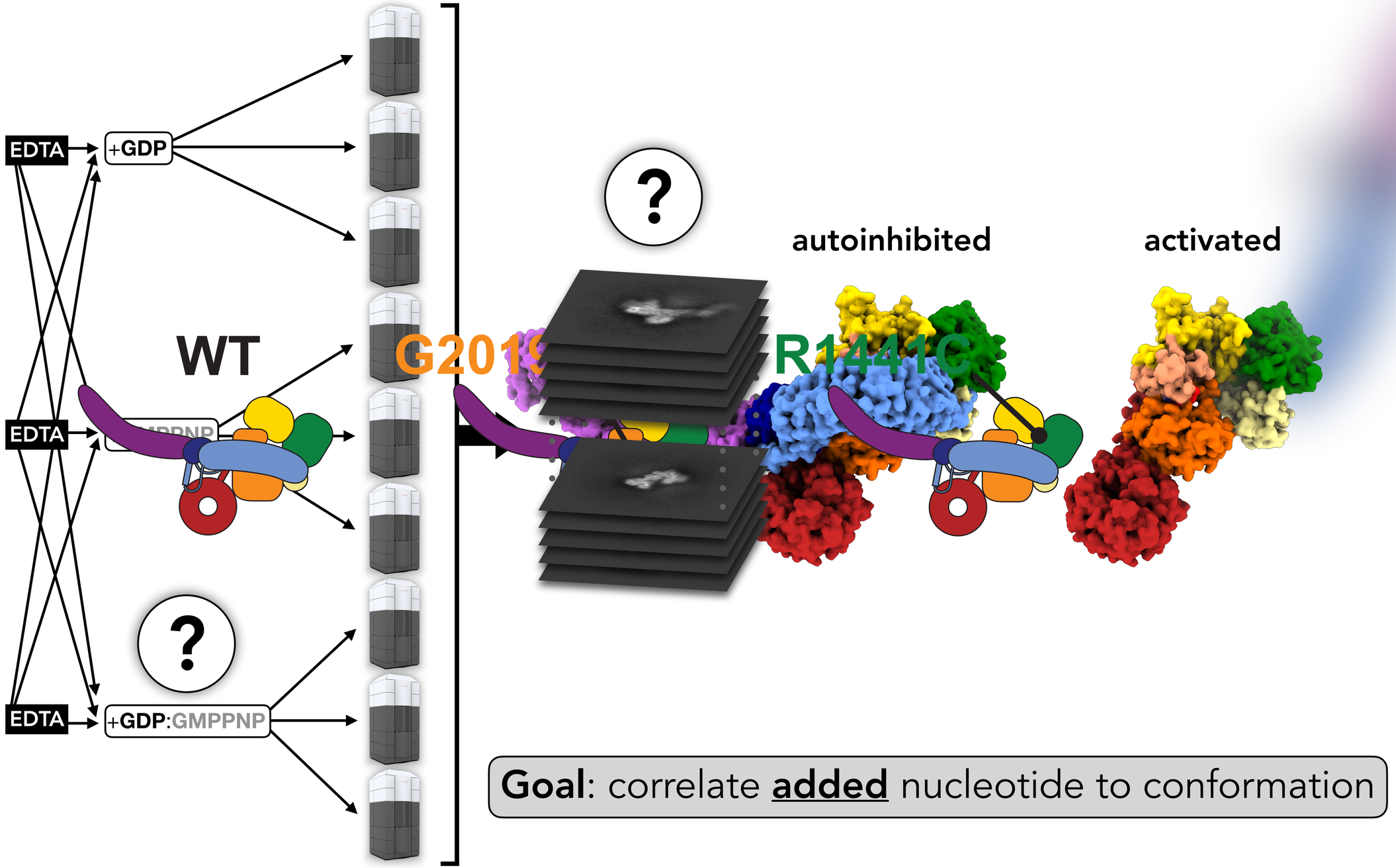
Important Fact #1:
Purified LRRK2 is mostly autoinhibited and GDP-bound



Important Fact #2:
Nucleotide stripping leads to activation

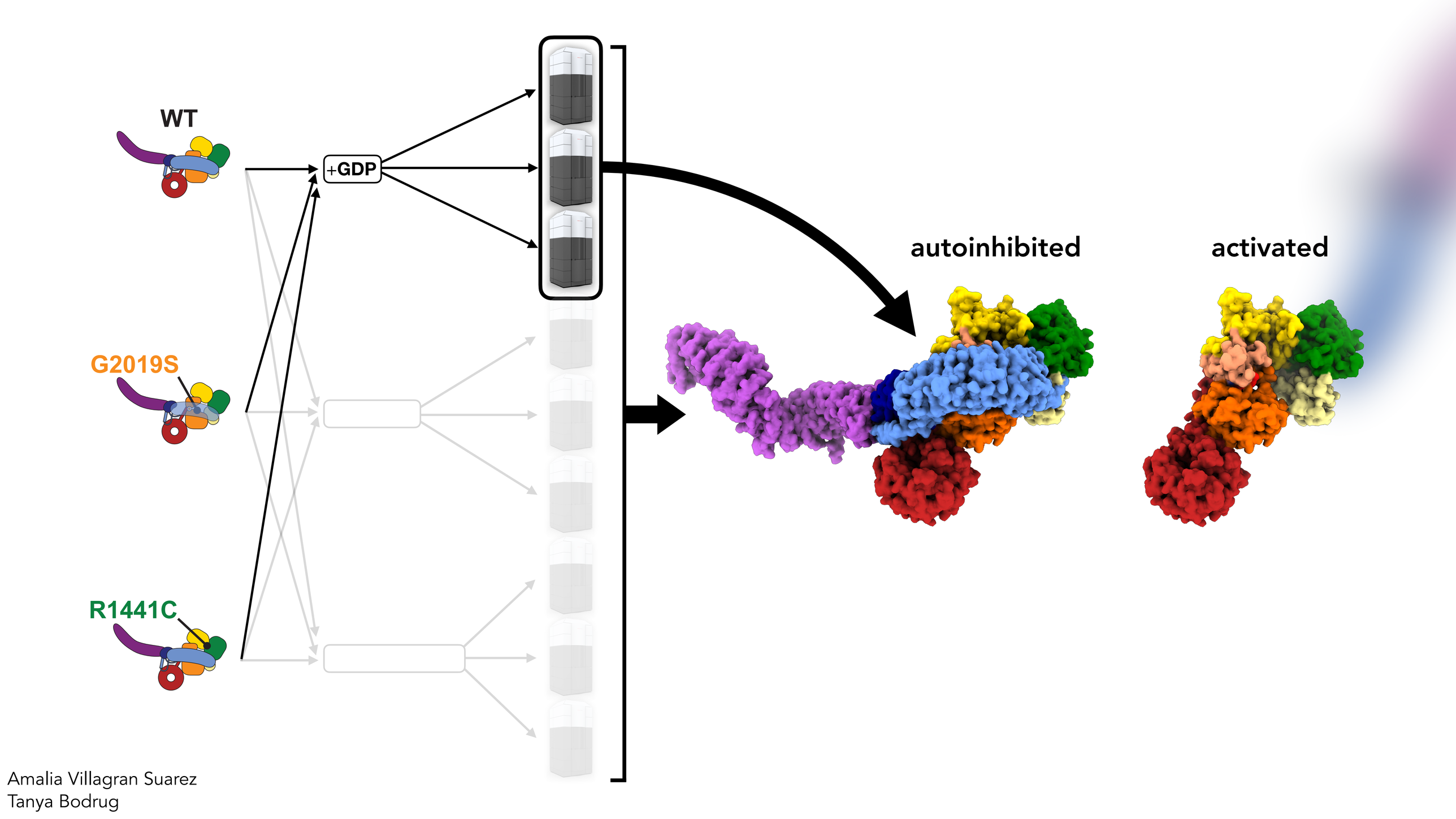


Amalia Villagran Suarez
Tanya Bodrug

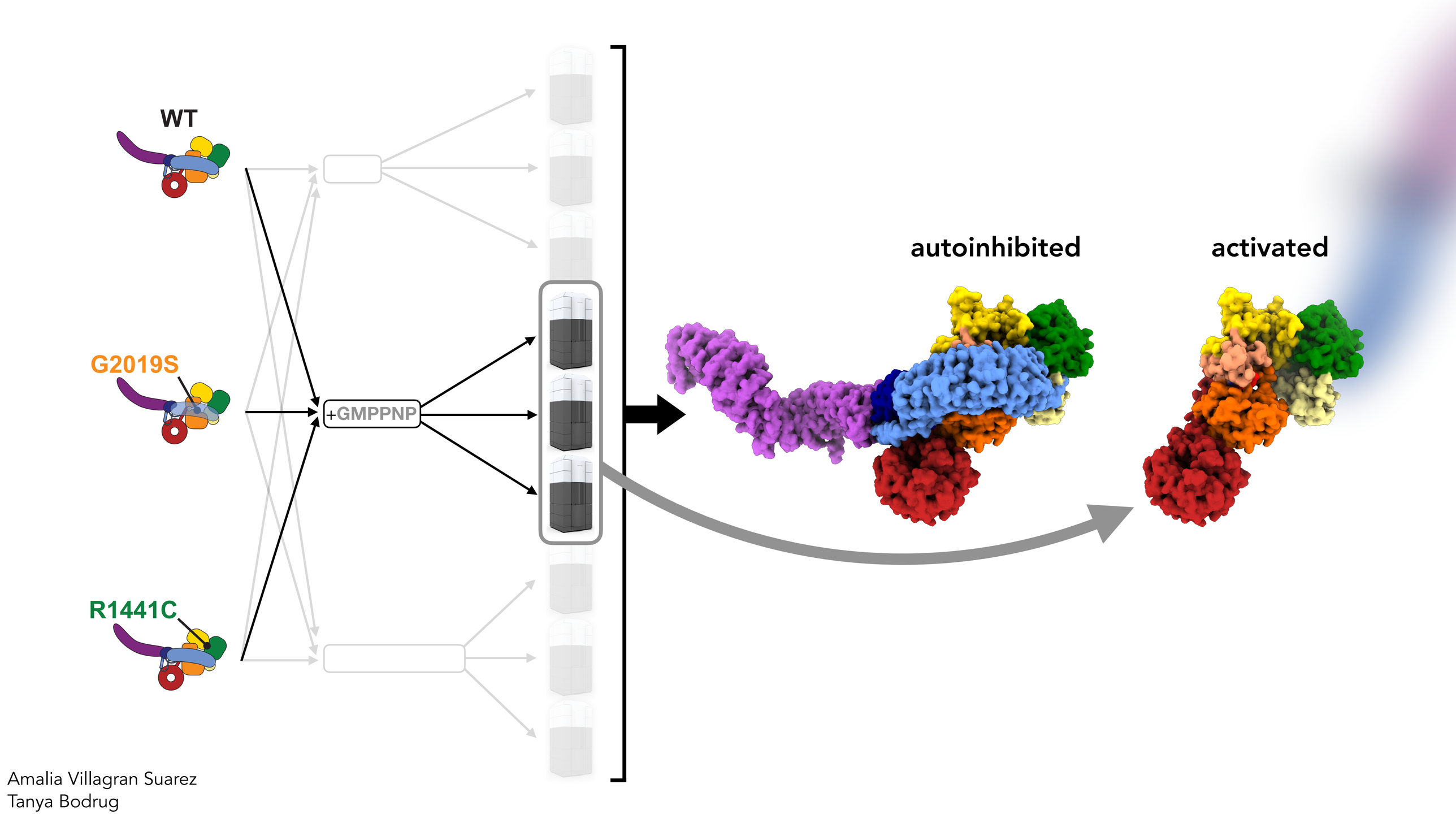


Goal: correlate **added** nucleotide to conformation

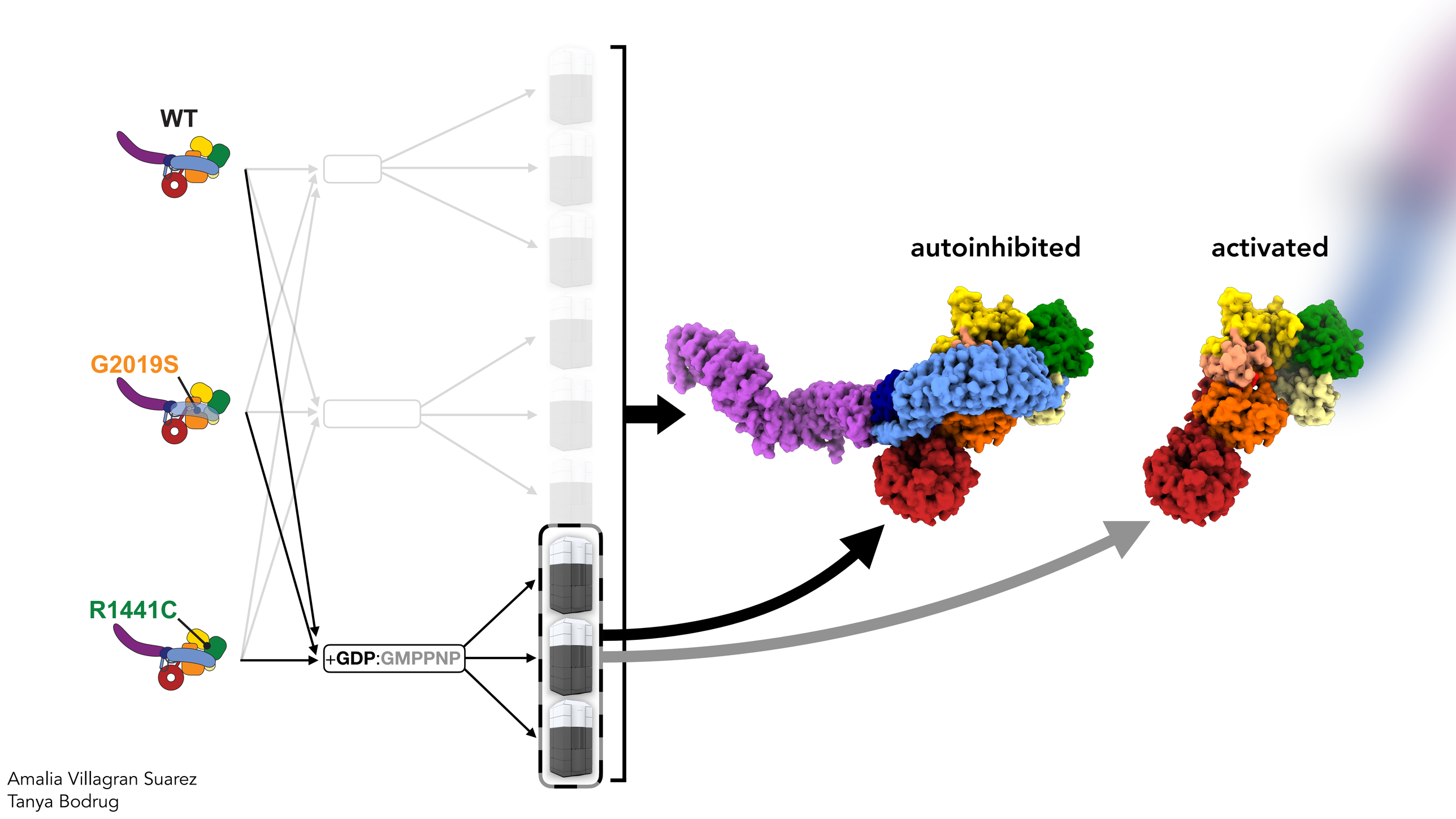
Amalia Villagran Suarez
Tanya Bodrug



Amalia Villagran Suarez
Tanya Bodrug



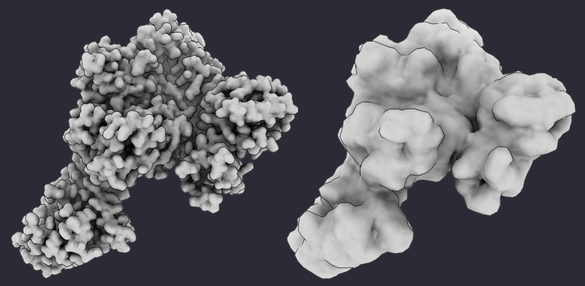
Amalia Villagran Suarez
Tanya Bodrug

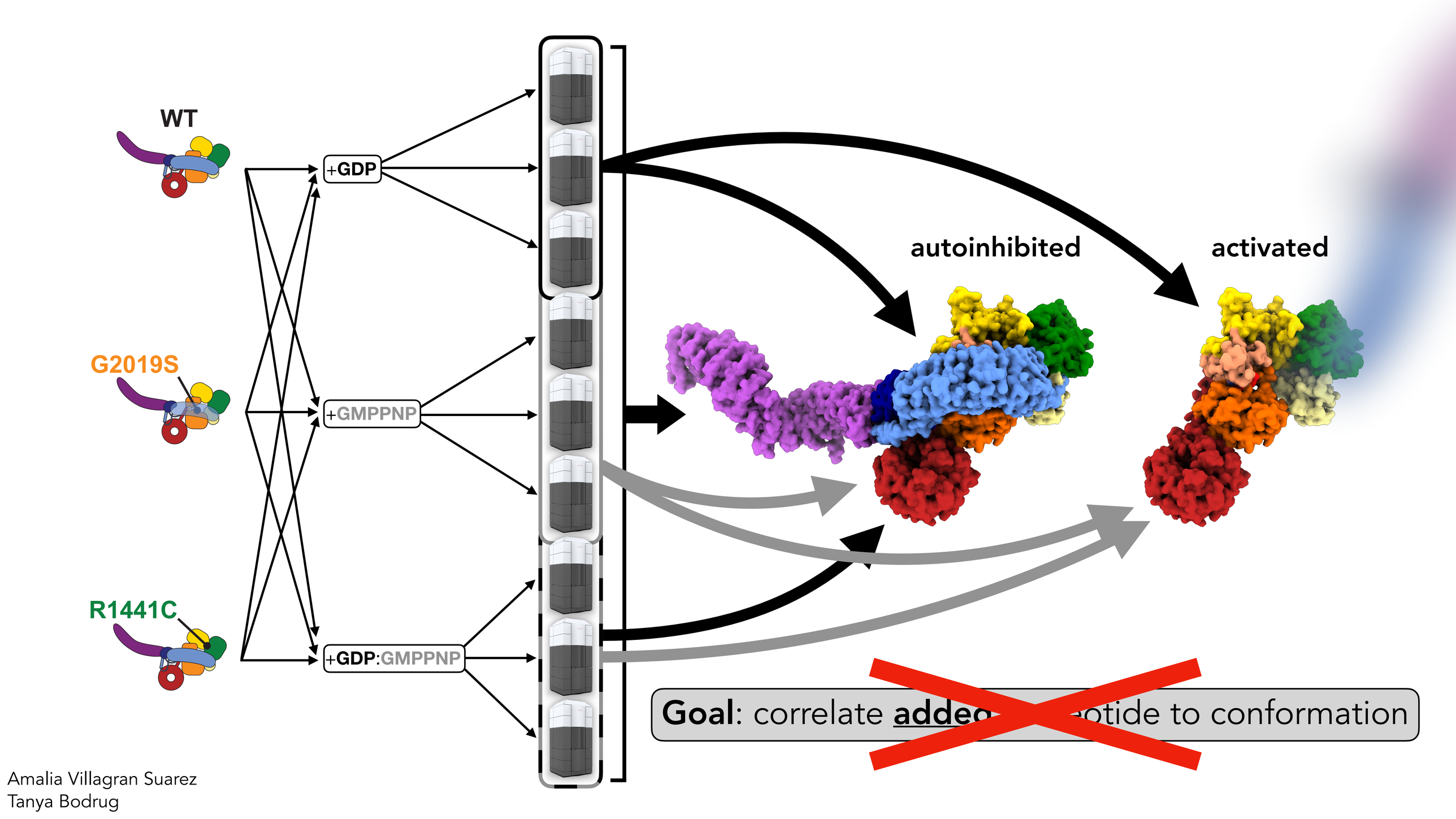


Amalia Villagran Suarez
Tanya Bodrug

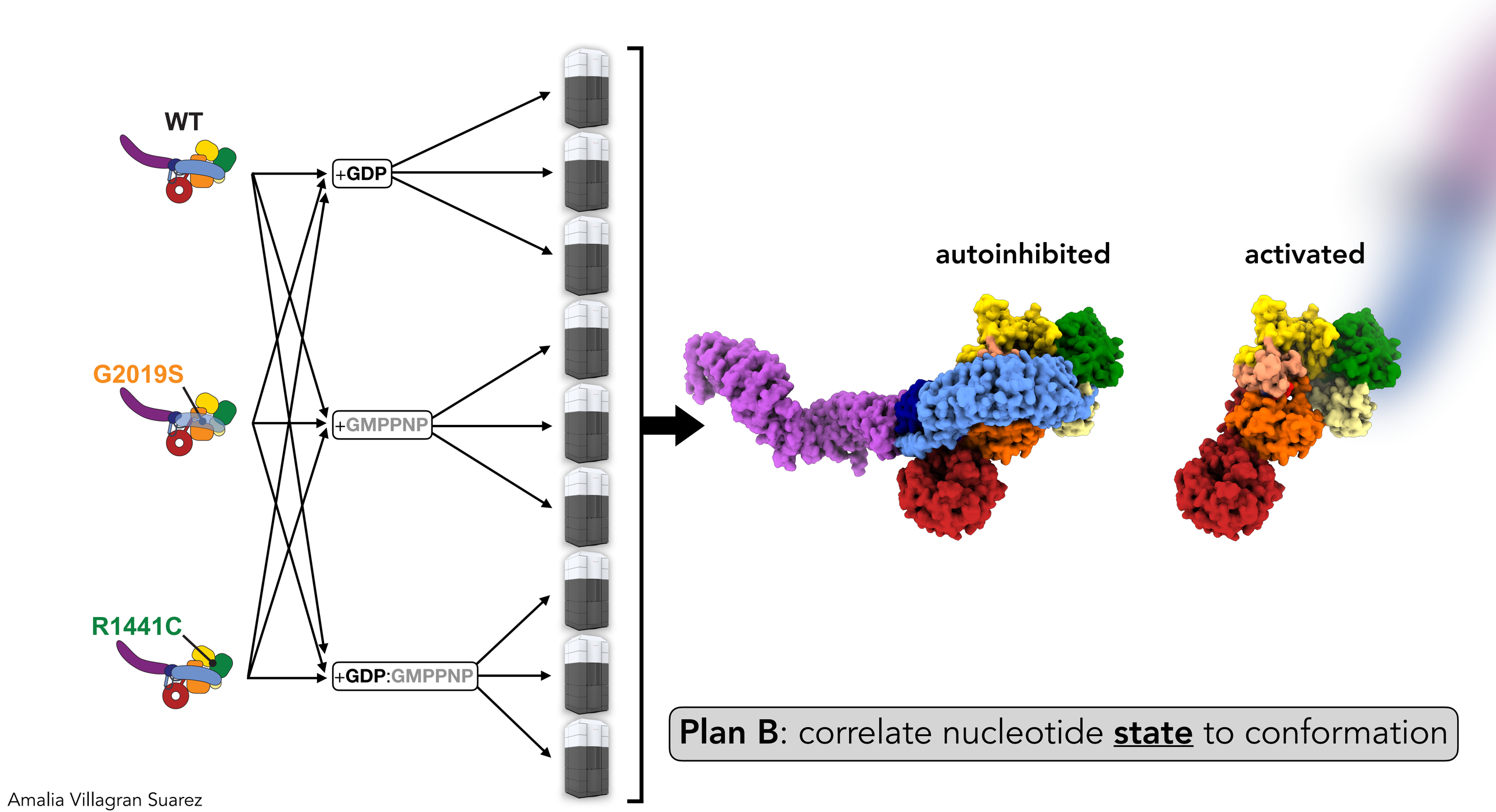
Applications of SPA to biological systems & data visualization

What resolution do I need to answer the question?





Amalia Villagran Suarez
Tanya Bodrug

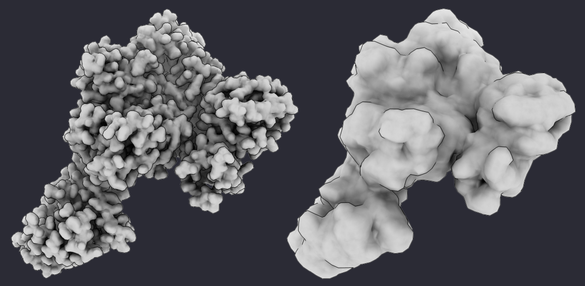


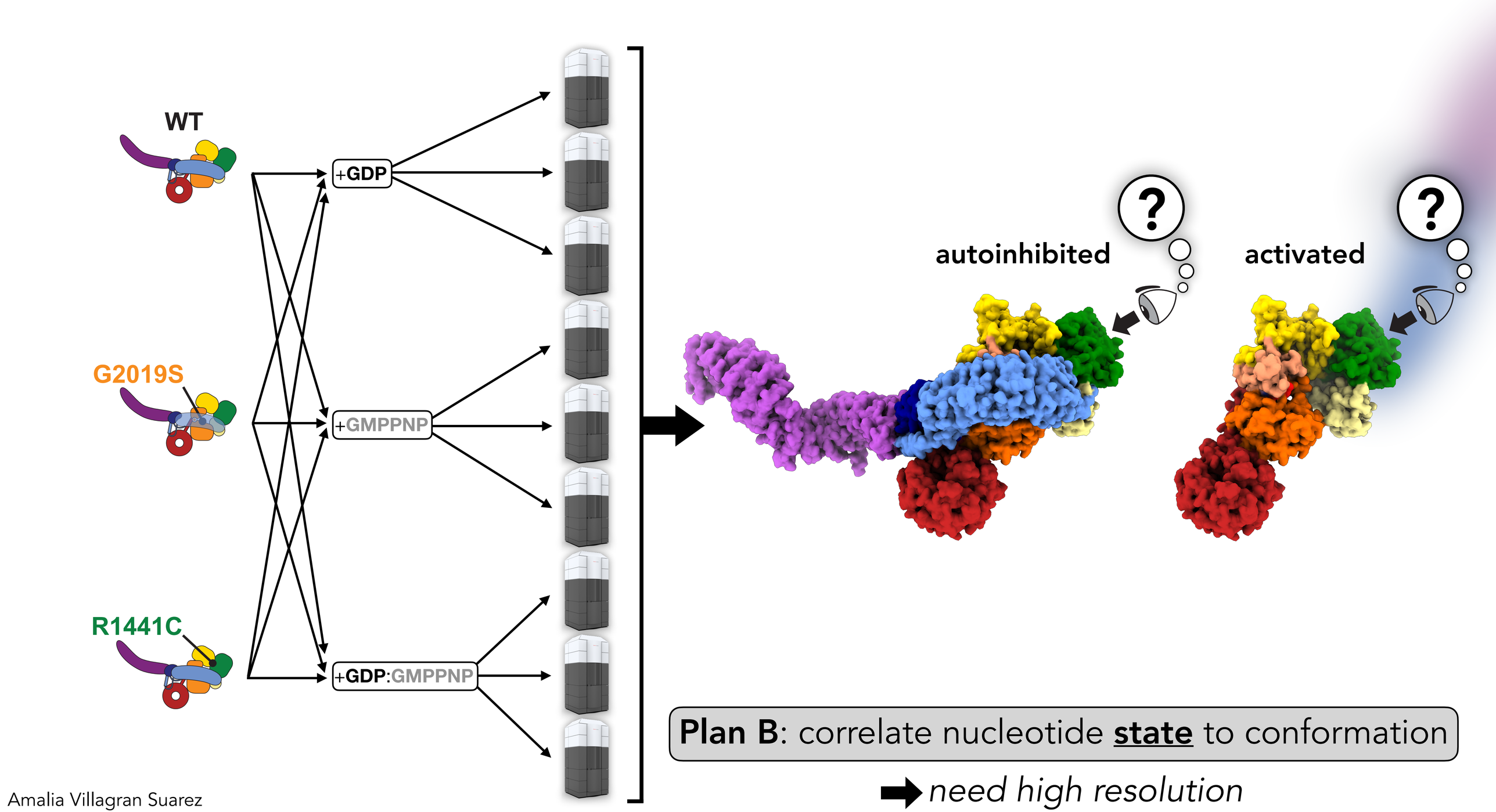
Plan B: correlate nucleotide **state** to conformation

Amalia Villagran Suarez
Tanya Bodrug

Applications of SPA to biological systems & data visualization

What resolution do I need to answer the question?





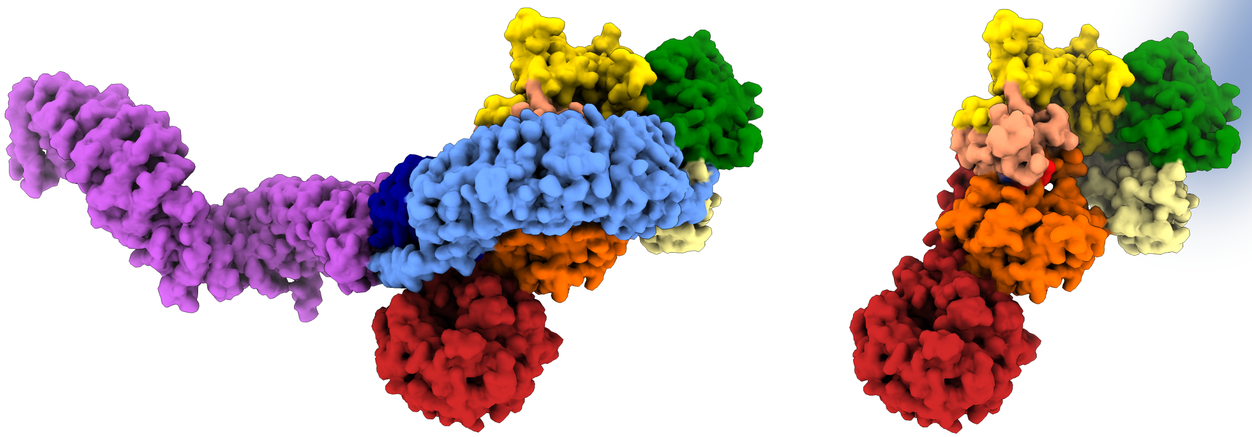
Plan B: correlate nucleotide **state** to conformation

➡ *need high resolution*

Amalia Villagran Suarez
Tanya Bodrug

autoinhibited

activated



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Tanya Bodrug

A

	GDP	GMPPNP	GDP/ GMPPNP
WT	3,505	8.234	4,723
R1441C	4,736	7,425	N/A
G2019S	13,968	26,151	8,219

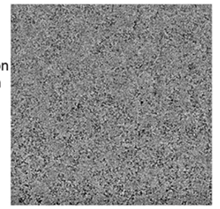
Collected movies

	GDP	GMPPNP	GDP/ GMPPNP
WT	3,499	8,056	3,065
R1441C	4,736	7,324	N/A
G2019S	13,153	13,068	8,219

Accepted movies

Patch motion correction
Patch CTF correction

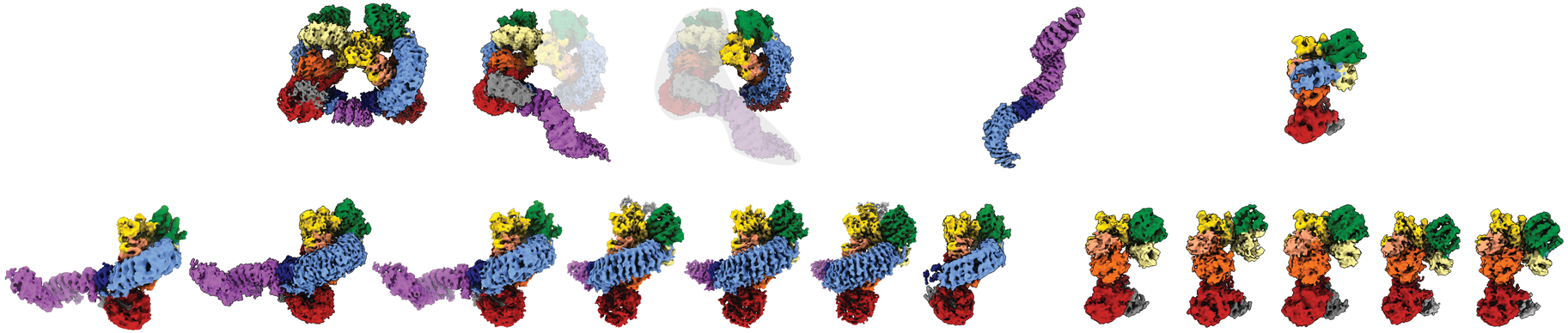
B



Blob picker
Extract
3.74 Å/px

31,248,339
particles

a structural bonanza…



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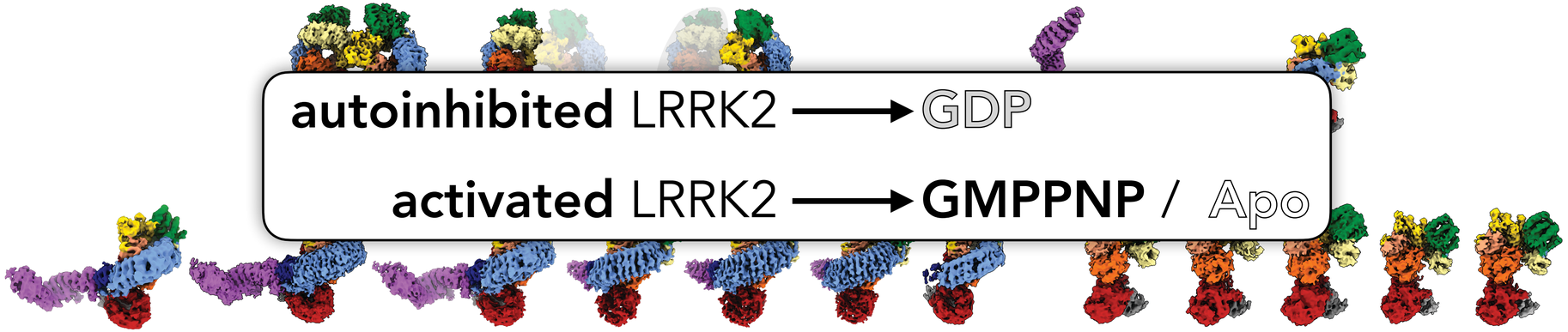
Tanya Bodrug



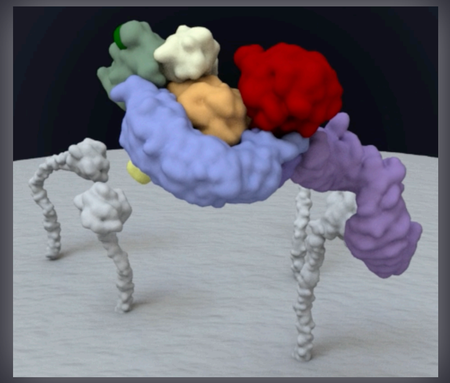
autoinhibited LRRK2 ⟶ GDP

activated LRRK2 ⟶ **GMPPNP** / Apo

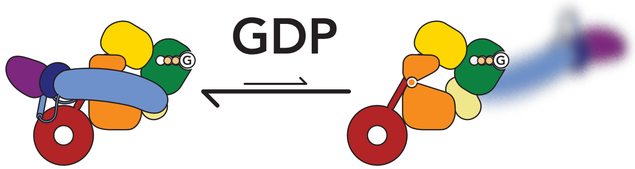
	homodimer	heterodimer		monomer			
	GDP	GDP	Apo	GDP	Apo	**GMPPNP**	unkn.
autoinhibited	* * *						
activated							

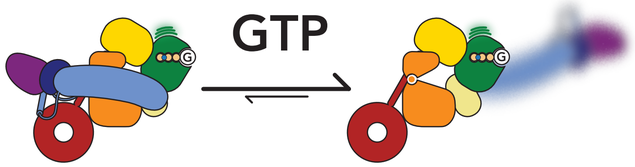
Applications of SPA to biological systems & data visualization

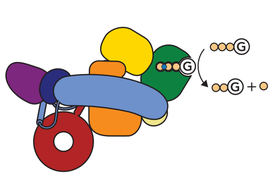
Data visualization = hypothesis generation



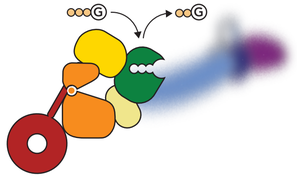
Main hypothesis underlying the model





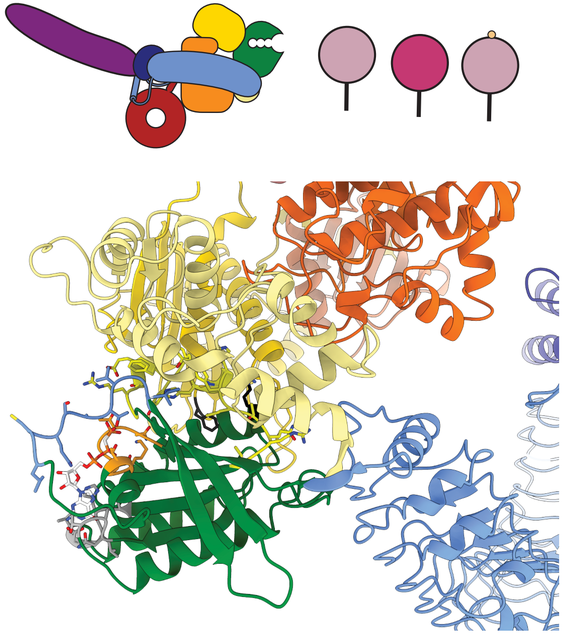


Repeats docked
= GAP

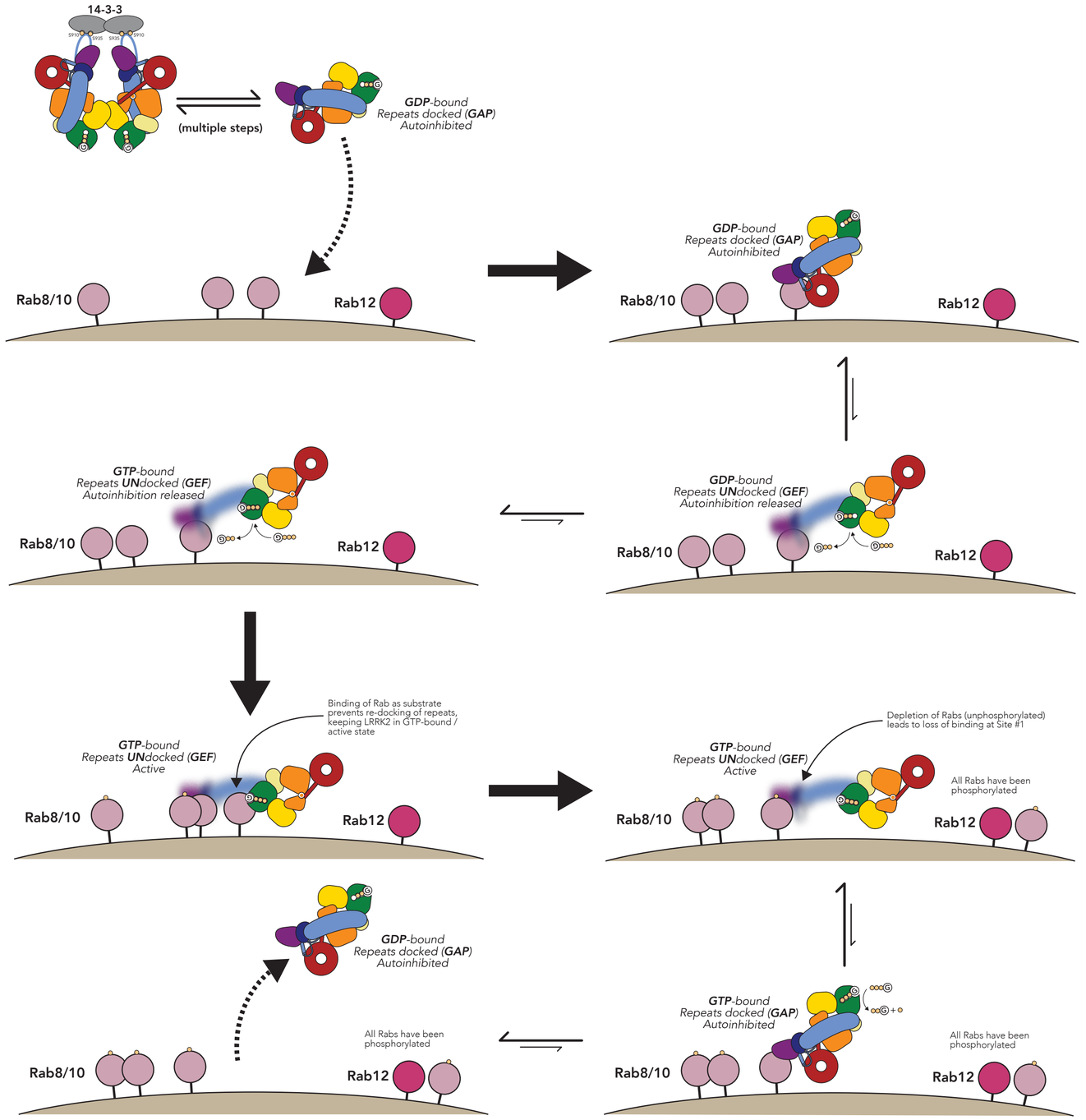


Repeats UNdocked
= GEF

The ROC domain is nestled against COR-A/B. Changes in Switch II (yellow) upon GTP binding force ROC away from COR-A/B.

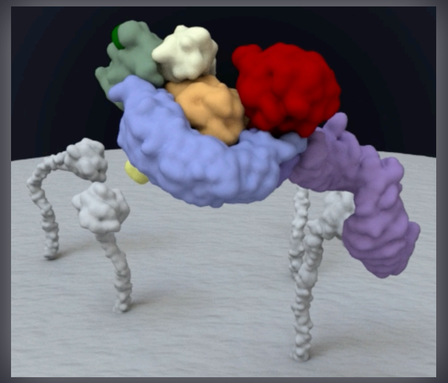


The LRR is anchored in place by the small b-sheet it forms with the C-ter of ROC. Movement of ROC relative to COR-A would disrupt this docking and release the N-terminal repeats.


14-3-3
S910
S935
S935
S910
(multiple steps)
GDP-bound
Repeats docked (GAP)
Autoinhibited
Rab8/10
Rab12
GDP-bound
Repeats docked (GAP)
Autoinhibited
Rab8/10
Rab12
GTP-bound
Repeats UNdocked (GEF)
Autoinhibition released
Rab8/10
Rab12
GDP-bound
Repeats UNdocked (GEF)
Autoinhibition released
Rab8/10
Rab12
Binding of Rab as substrate prevents re-docking of repeats, keeping LRRK2 in GTP-bound / active state
GTP-bound
Repeats UNdocked (GEF)
Active
Rab8/10
Rab12
Depletion of Rabs (unphosphorylated) leads to loss of binding at Site #1
GTP-bound
Repeats UNdocked (GEF)
Active
All Rabs have been phosphorylated
Rab8/10
Rab12
GDP-bound
Repeats docked (GAP)
Autoinhibited
All Rabs have been phosphorylated
Rab8/10
Rab12
GTP-bound
Repeats docked (GAP)
Autoinhibited
All Rabs have been phosphorylated
Rab8/10
Rab12

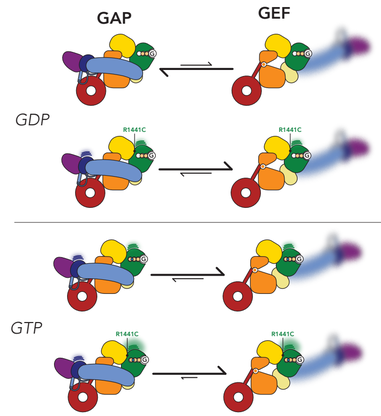
Applications of SPA to biological systems & data visualization

Data visualization = *hypothesis generation*



Predictions for nucleotide exchange assays

WT vs. R1441C



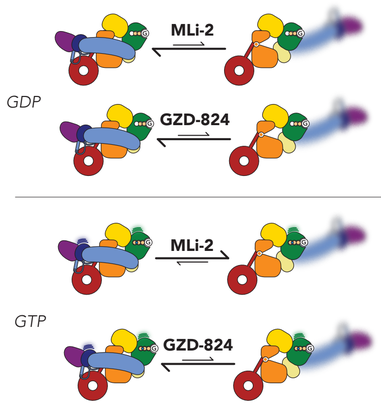
- Mostly GDP-bound, autoinhibited conformation
- GAP like
- Refractory to nucleotide exchange without stripping

- Mostly GDP-bound, NOT autoinhibited
- GEF like
- Should release GDP and take up GTP without stripping

- Mostly GTP-bound, NOT autoinhibited
- GEF like
- Should exchange its own GTP with GTP from solution without stripping

- Same as WT but more extreme

WT - MLi-2 vs. GZD-824



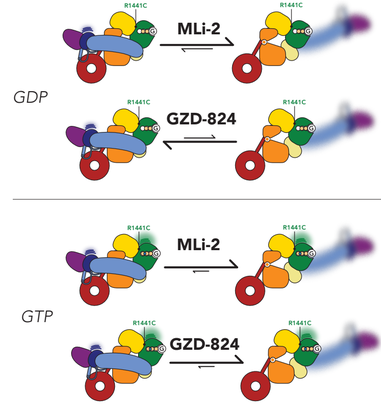
- Mostly GDP-bound, autoinhibited
- GAP like
- Refractory to nucleotide exchange without stripping

- Mostly GDP-bound, autoinhibited
- GAP like
- Refractory to nucleotide exchange without stripping

- Mostly GDP-bound, NOT autoinhibited
- GEF like
- Should exchange its own GTP with GTP from solution without stripping

- Mostly GDP-bound, more autoinhibited than MLi-2
- GAP like
- Should NOT exchange (easily) its own GTP with GTP from solution without stripping

R1441C- MLi-2 vs. GZD-824

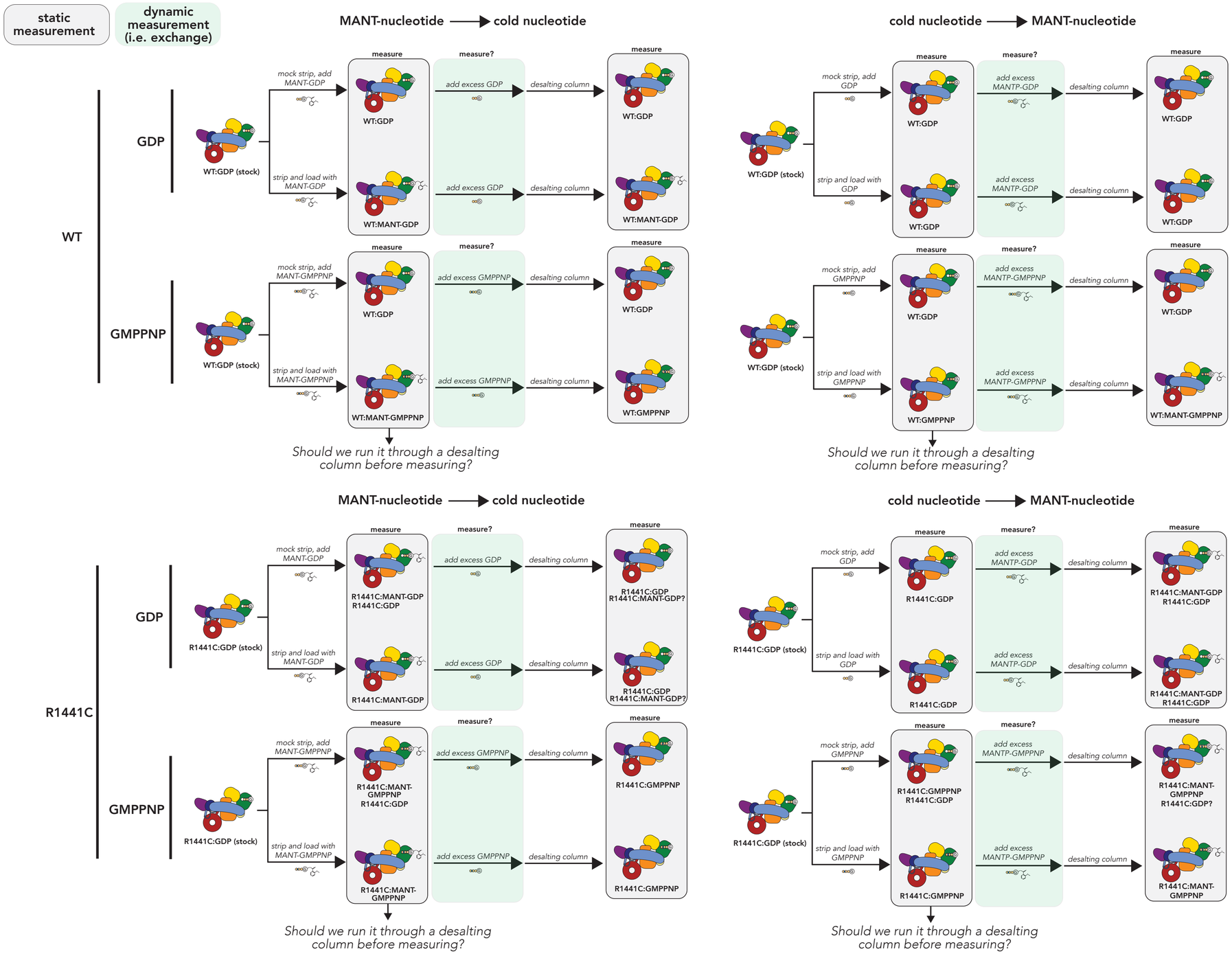


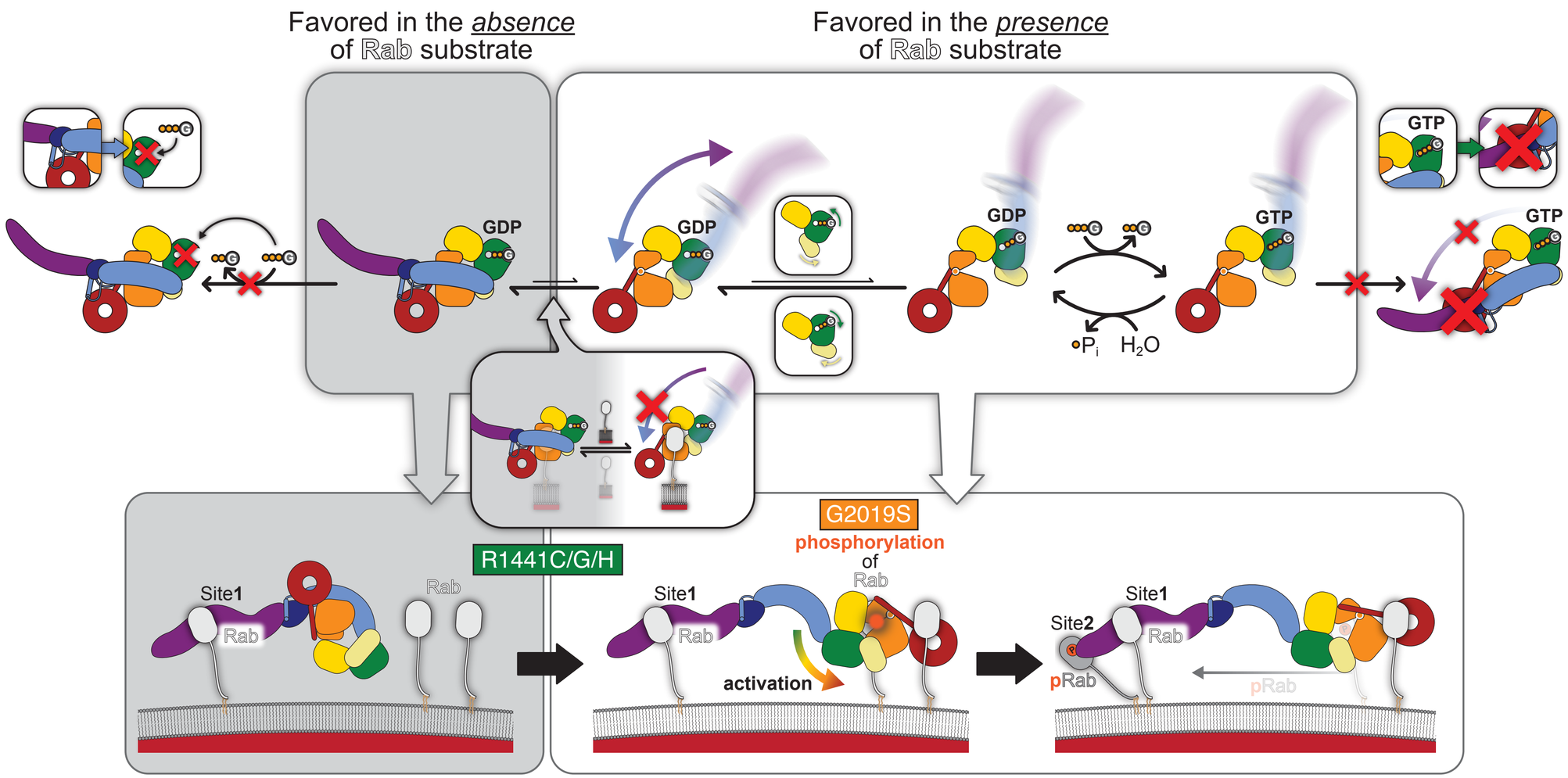
- Mostly GDP-bound, NOT autoinhibited
- GEF like
- Should exchange GDP with GTP from solution without stripping

- Mostly GDP-bound, more autoinhibited than MLi-2
- GAP like
- Should exchange GDP with GTP from solution less well than with MLi-2 without stripping

- Mostly GTP-bound, NOT autoinhibited
- GEF like
- Should exchange its own GTP with GTP from solution without stripping (this should be the sample that exchanges best)

- Mostly GTP-bound, more autoinhibited than MLi-2
- less GEF like
- Should exchange its own GTP with GTP from solution without stripping less well than with MLi-2


static measurement
dynamic measurement (i.e. exchange)
MANT-nucleotide → cold nucleotide
cold nucleotide → MANT-nucleotide
WT
R1441C
GDP
GMPPNP
WT:GDP (stock)
R1441C:GDP (stock)
mock strip, add MANT-GDP
strip and load with MANT-GDP
mock strip, add MANT-GMPPNP
strip and load with MANT-GMPPNP
mock strip, add GDP
strip and load with GDP
mock strip, add GMPPNP
strip and load with GMPPNP
measure
measure?
add excess GDP
add excess GMPPNP
add excess MANTP-GDP
add excess MANTP-GMPPNP
desalting column
WT:GDP
WT:MANT-GDP
WT:MANT-GMPPNP
WT:GMPPNP
R1441C:MANT-GDP
R1441C:GDP
R1441C:GDP R1441C:MANT-GDP?
R1441C:MANT-GDP R1441C:GDP
R1441C:MANT-GMPPNP
R1441C:GMPPNP
R1441C:GMPPNP R1441C:GDP
R1441C:MANT-GMPPNP R1441C:GDP?
Should we run it through a desalting column before measuring?

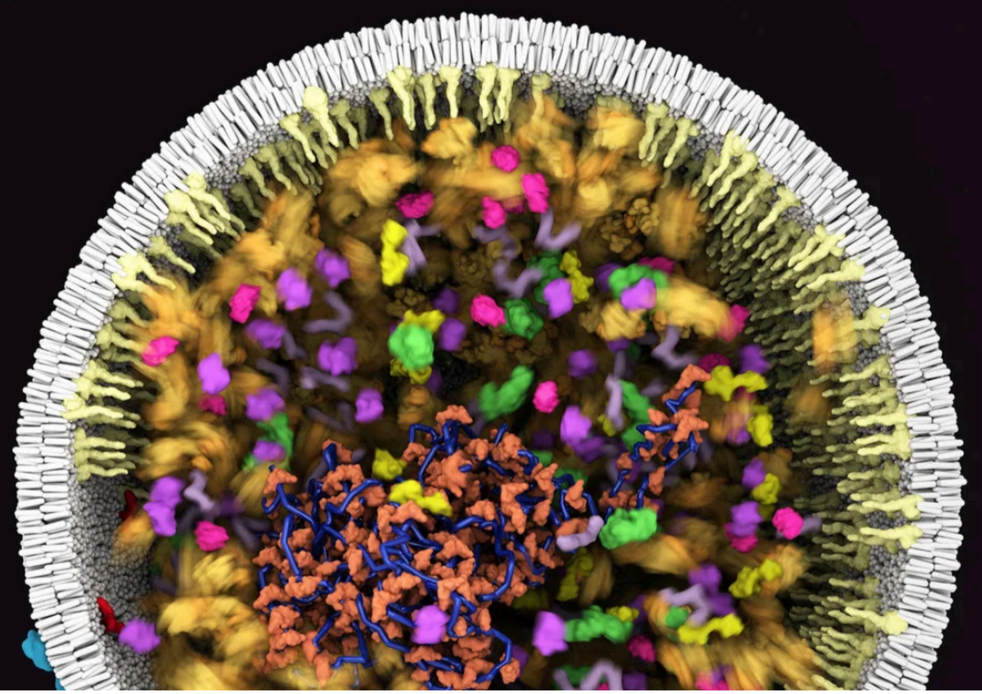

Favored in the absence of Rab substrate
Favored in the presence of Rab substrate
GDP
GDP
GDP
GTP
GTP
GTP
P_i
H_2O
R1441C/G/H
G2019S
phosphorylation of Rab
Site1
Rab
Rab
Site1
Rab
activation
Site2
pRab
Site1
Rab
pRab

3D visualization is a powerful hypothesis-generating/refining tool

THE ANIMATION LAB

VISUALIZING

BIOLOGY



Janet Iwasa, Ph.D.

Janet Iwasa is an Associate Professor of Biochemistry and the Director of the GSLC at the University of Utah. After receiving her Ph.D. from UCSF for work completed in Dyche Mullins' lab, she completed a postdoc with Jack Szostak (MGH/Harvard) and later worked on biological visualizations as a faculty member at Harvard Medical School.

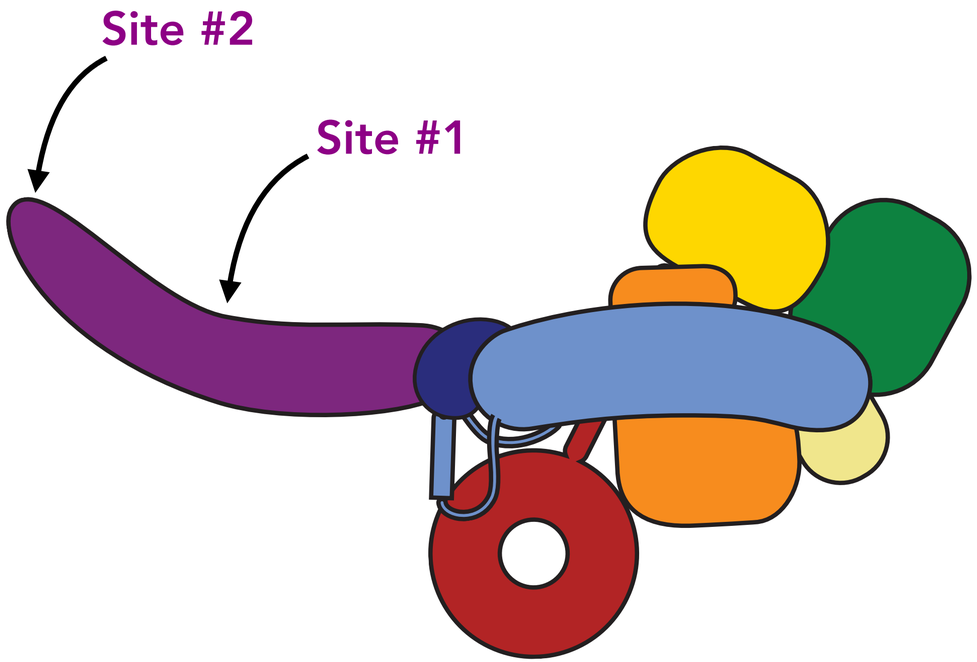
Building visual hypotheses

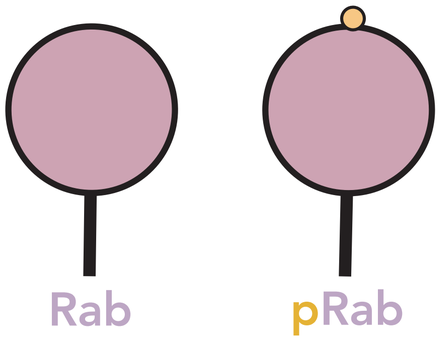
The Animation Lab creates information-rich and visually compelling animations that capture current hypotheses on diverse molecular and cellular processes. These visualizations have broad applications in scientific research, communication, education and outreach. We are also interested in creating new tools and workflows that will enable researchers to more readily create dynamic visualizations of the processes they study.

We welcome new collaborations!

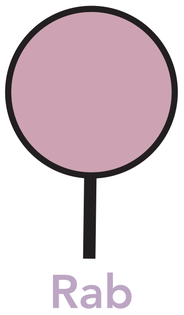
A model for LRRK2 activation and autoinhibition

LRRK2



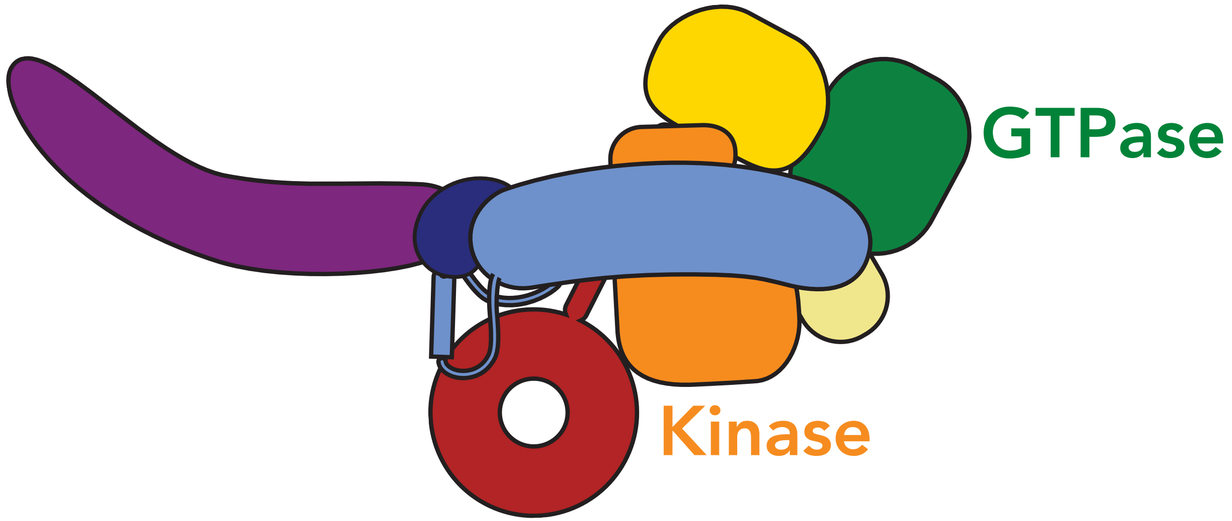


Substrate

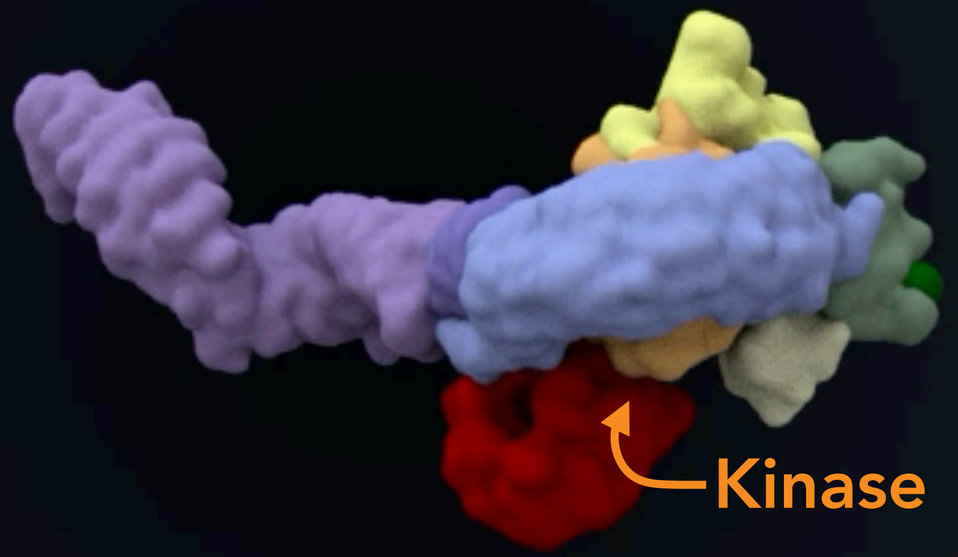


Vides et al., (2022) *PMID: 36149401*
Dhekne, Tonelli, Yeshaw, Chiang, et al., (2023) *PMID: 37874635*

LRRK2



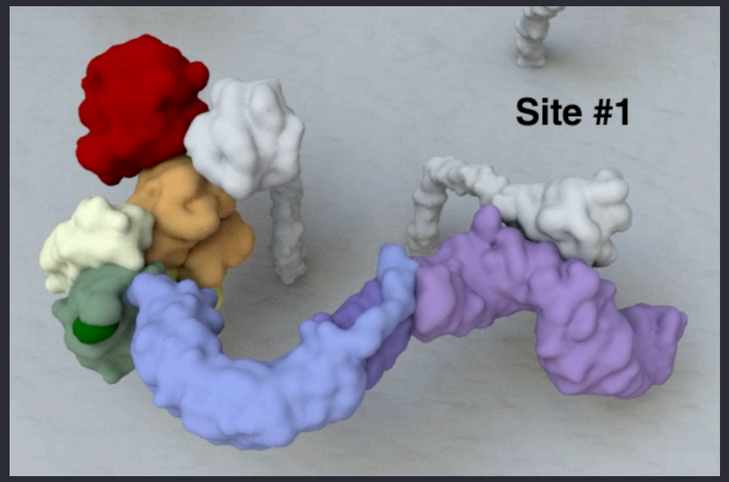
LRRK2



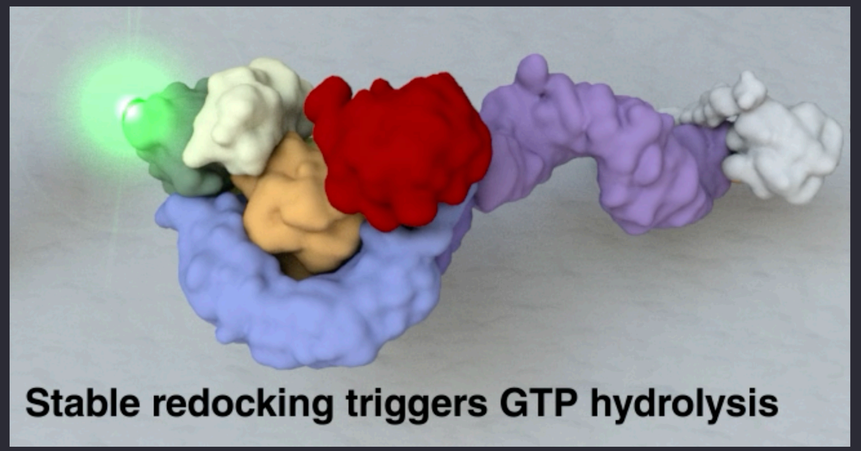
Animation by **Janet Iwasa**
w/Andres Leschziner & Samara Reck-Peterson

September 29, 2024

Some features of the animation that needed revision...

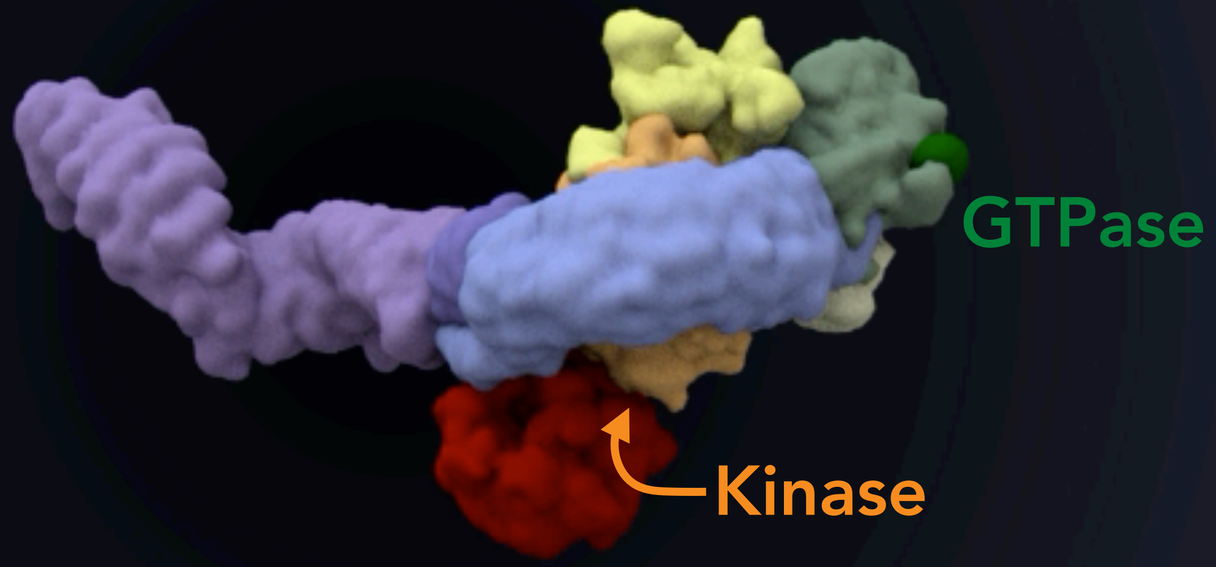


only conformational change is docking/ undocking of N-terminal repeats



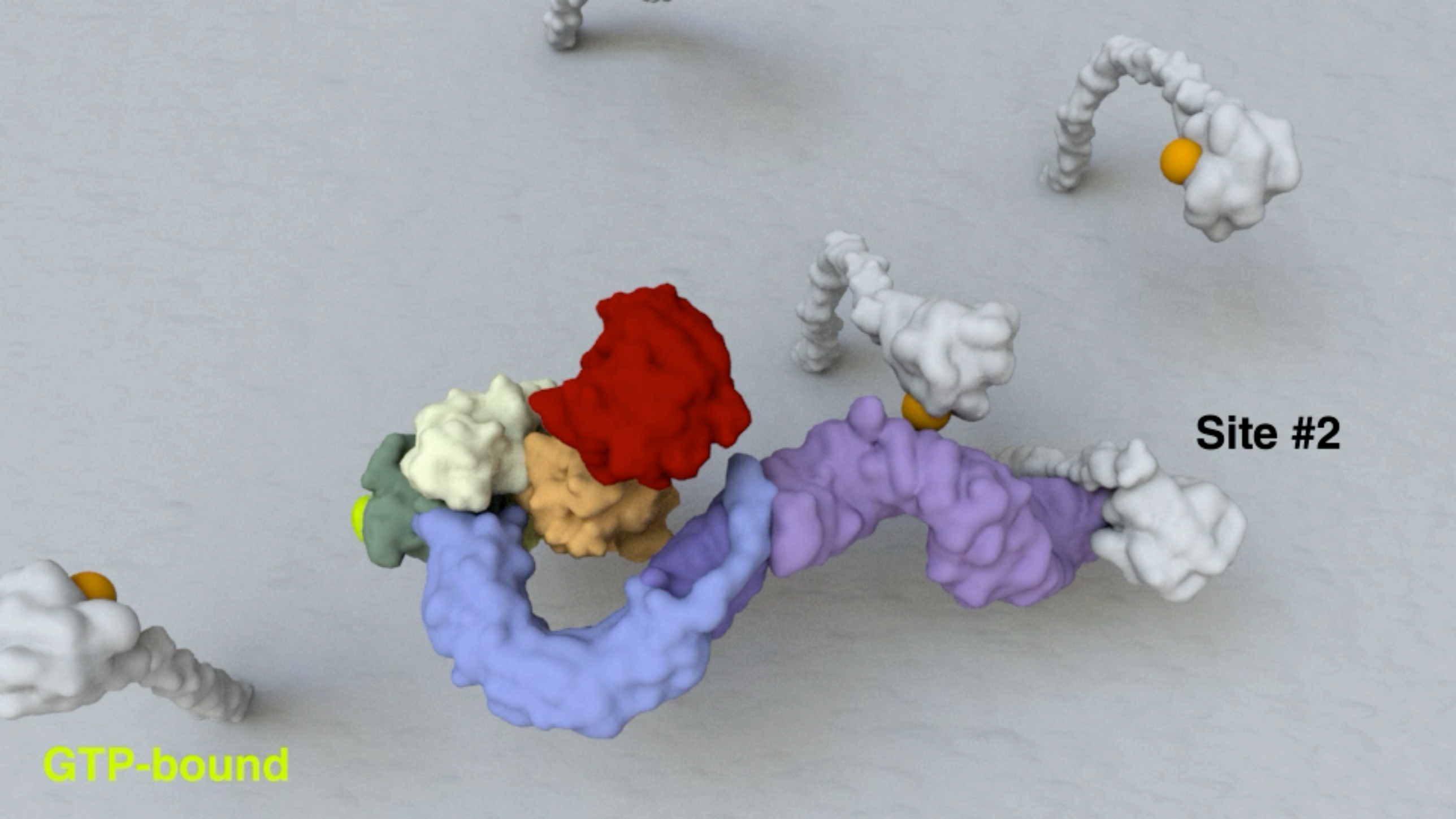
GTP hydrolysis triggered by re-docking

LRRK2



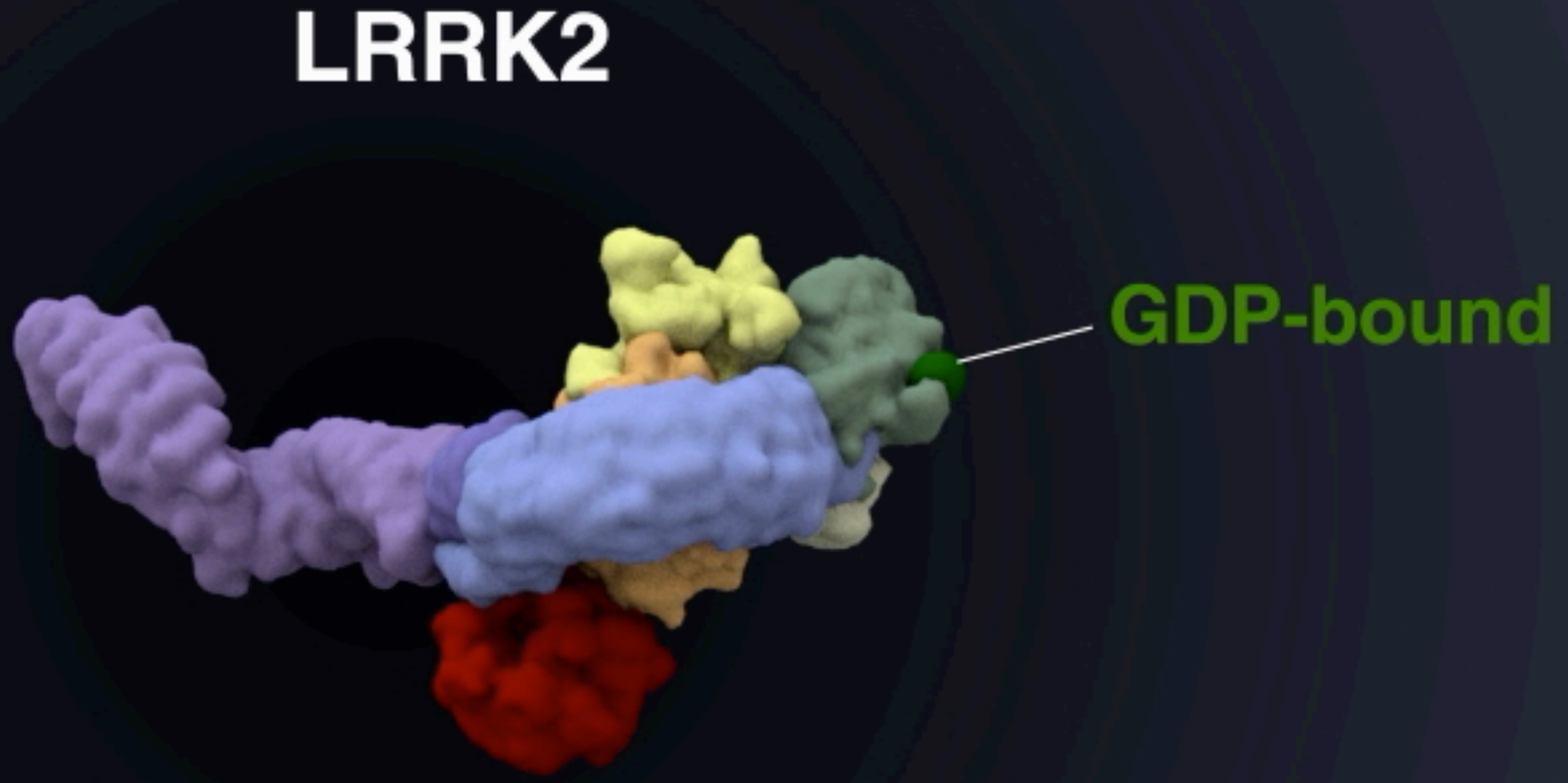
Animation by **Janet Iwasa**
w/Andres Leschziner & Samara Reck-Peterson

November 2, 2025

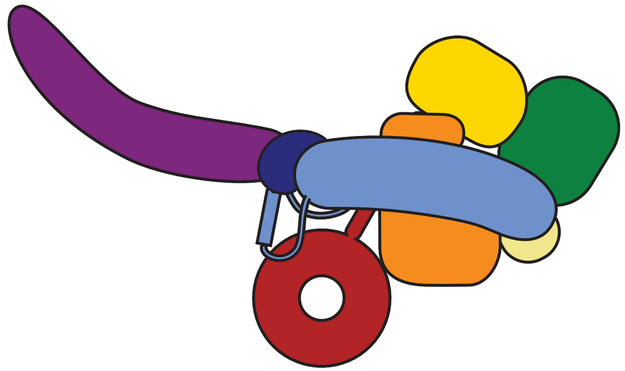

Site #2
GTP-bound

The data behind the model

Autoinhibited LRRK2

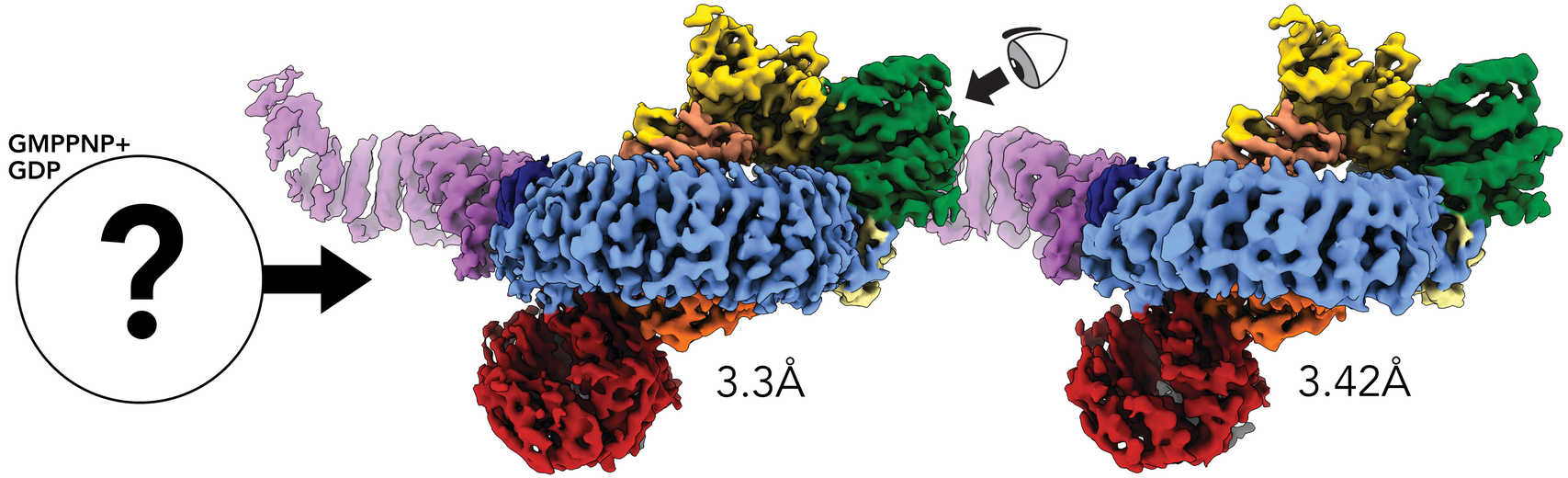


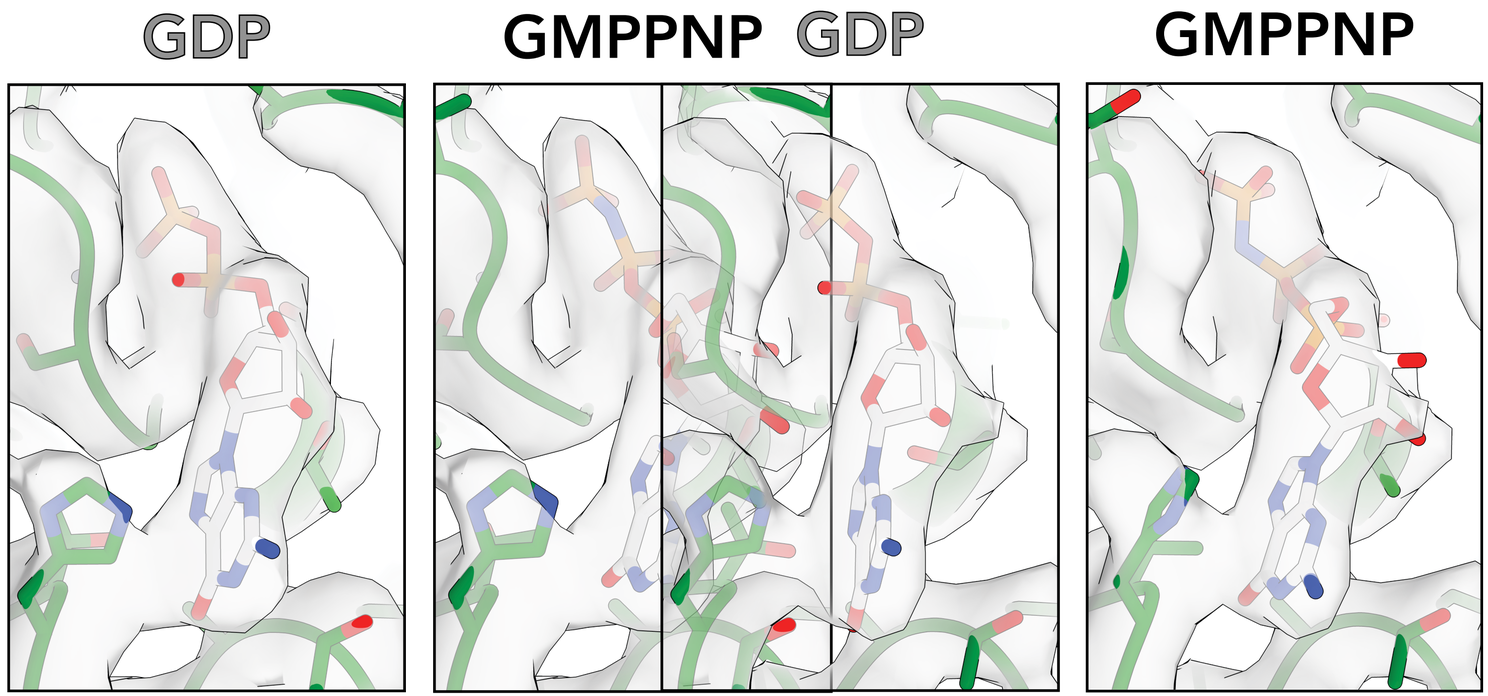
Autoinhibited LRRK2 is bound to GDP



autoinhibited
LRRK2

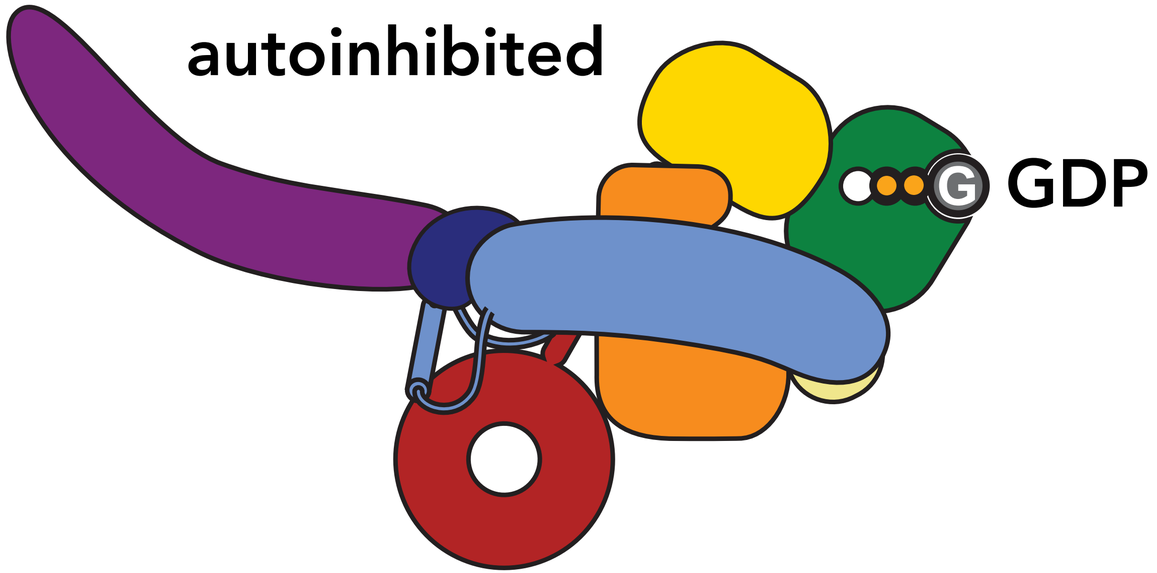
LRRK2 [WT / R1441C / G2019S] + GDP / GMPPNP / GDP+GMPPNP



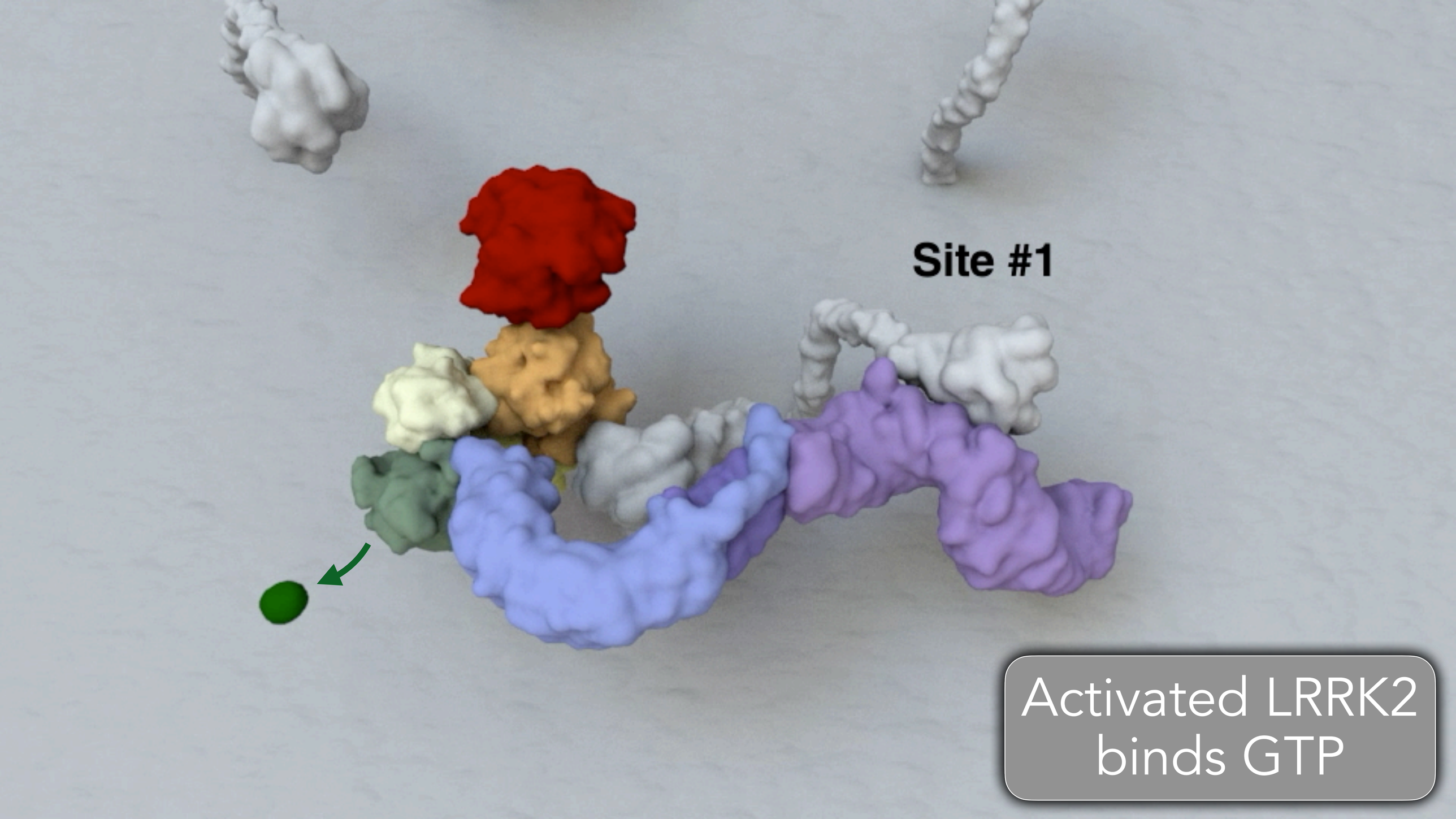


Amalia Villagran Suarez & Tanya Bodrug

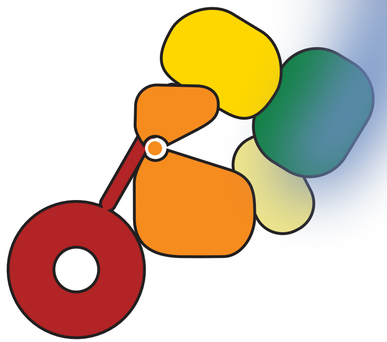
Important Fact #1:
Purified LRRK2 is mostly autoinhibited and GDP-bound



Autoinhibited LRRK2

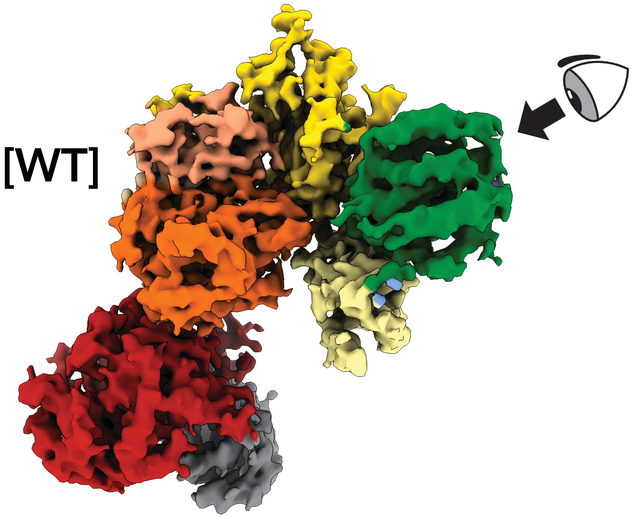


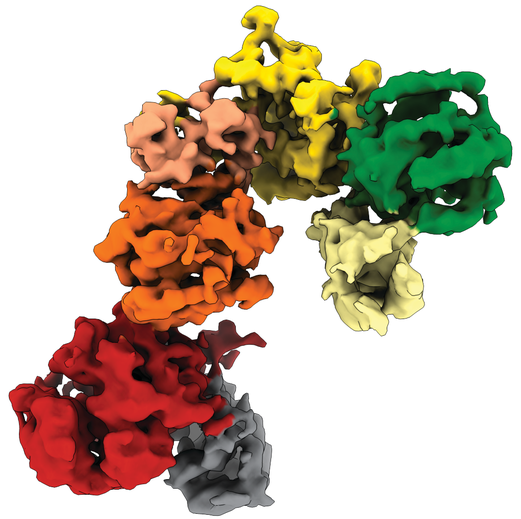
Activated LRRK2 binds GTP



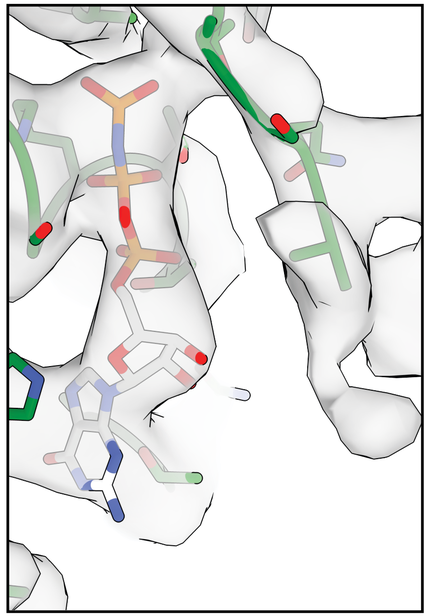
activated
LRRK2

Activated LRRK2 is bound to **GMPPNP** or Apo

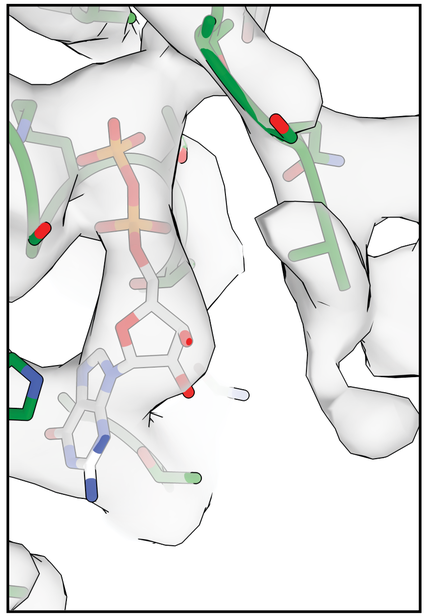




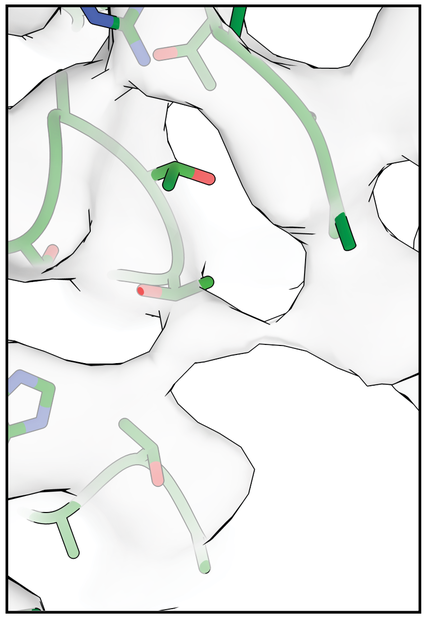
GMPPNP



GDP



Apo



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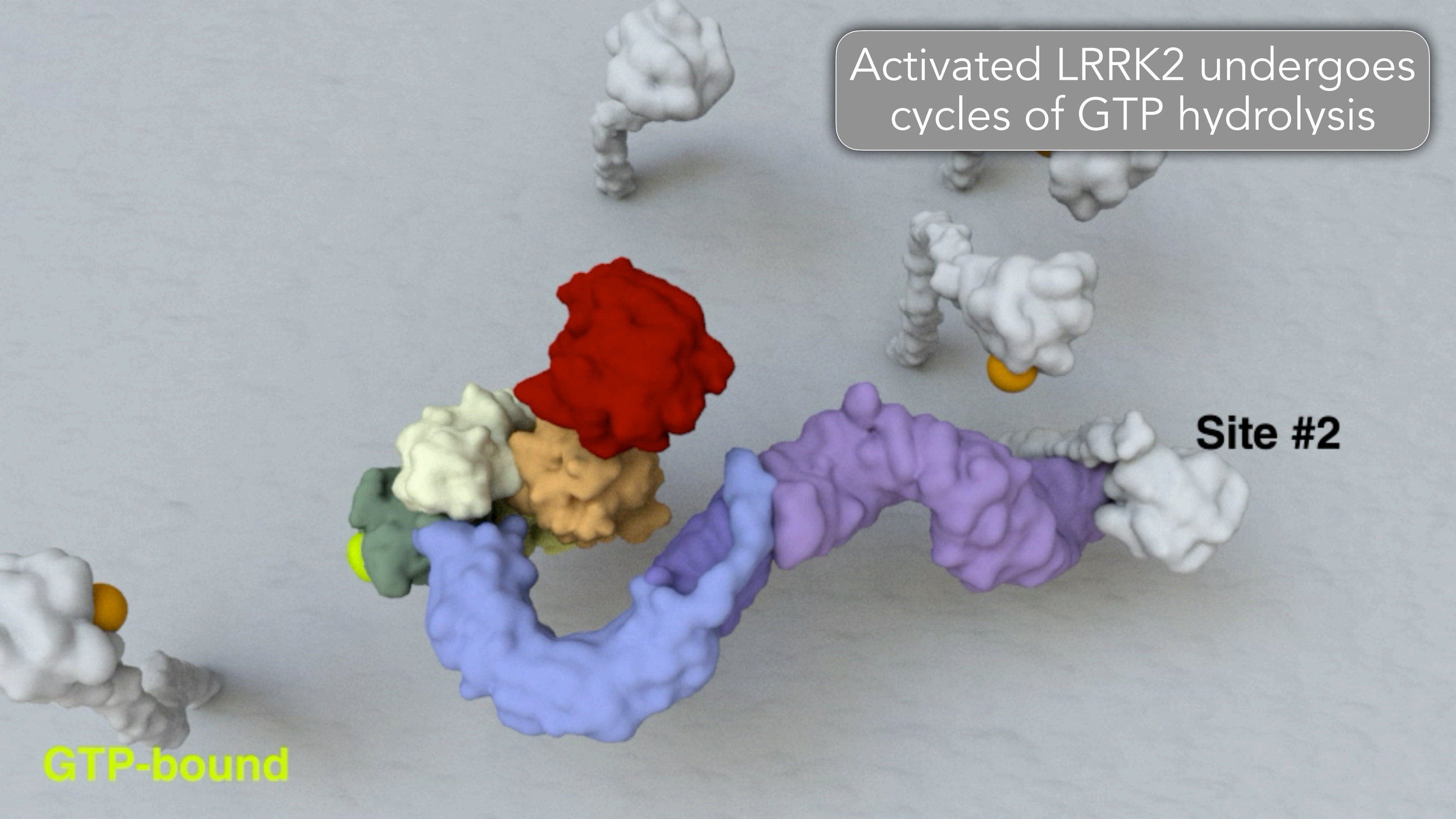
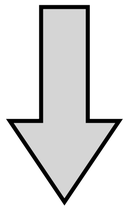
Activated
LRRK2

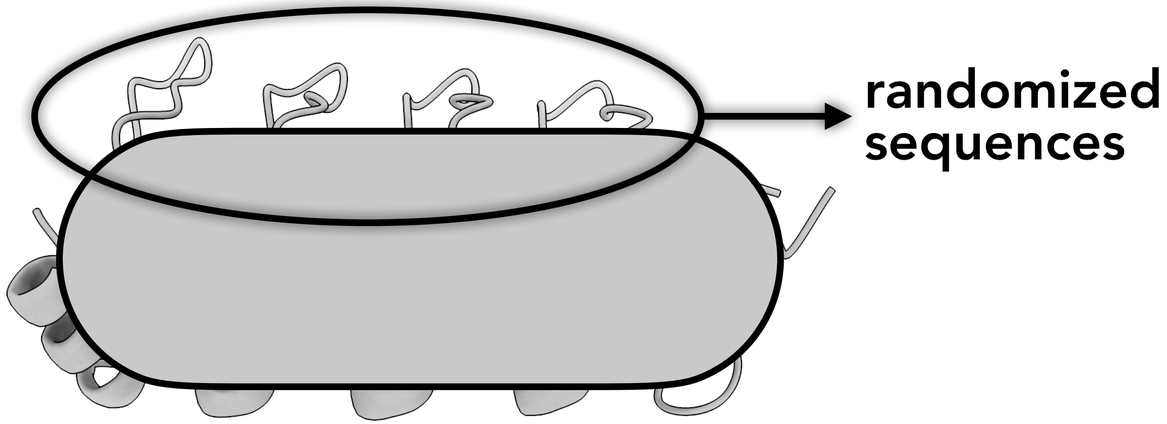

Activated LRRK2 undergoes cycles of GTP hydrolysis
Site #2
GTP-bound

Is the GTPase a timing mechanism for autoinhibition?

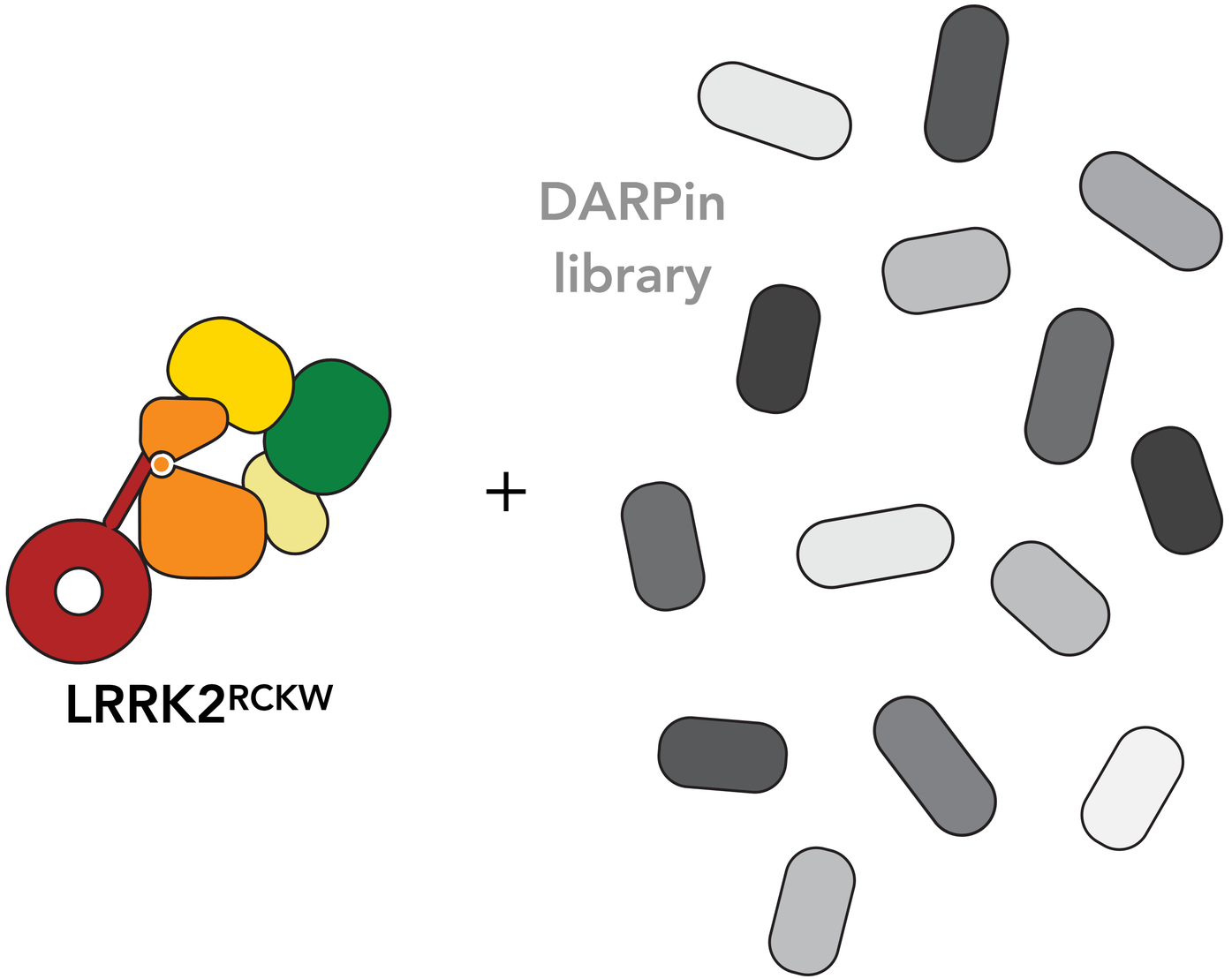


We need tools to control activation and autoinhibition

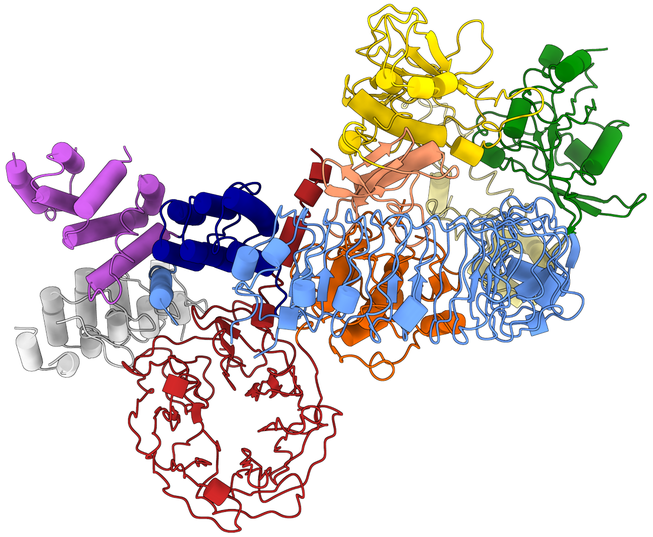
DARPins

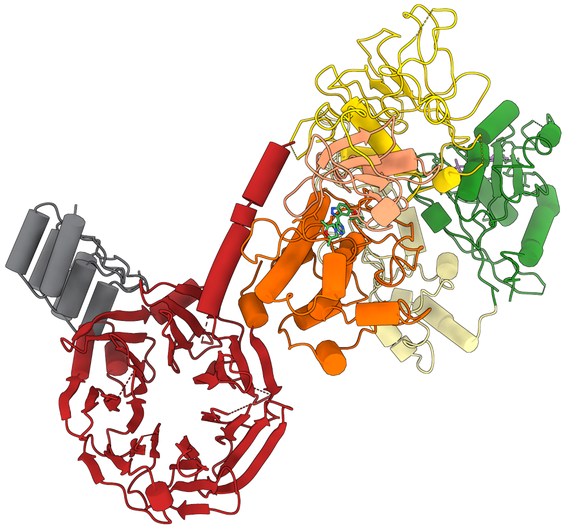


DARPin = **D**esigned **A**nkyrin **R**epeat **P**rote**in**

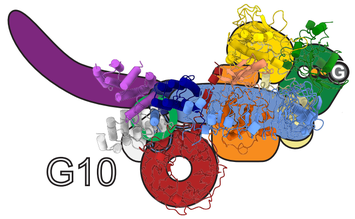


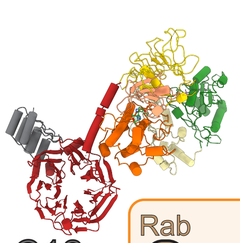
Dederer, Sanz Murillo, Karasmanis et al (2024) *PMID: 38876305*

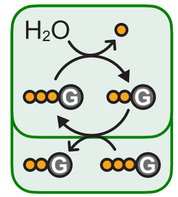


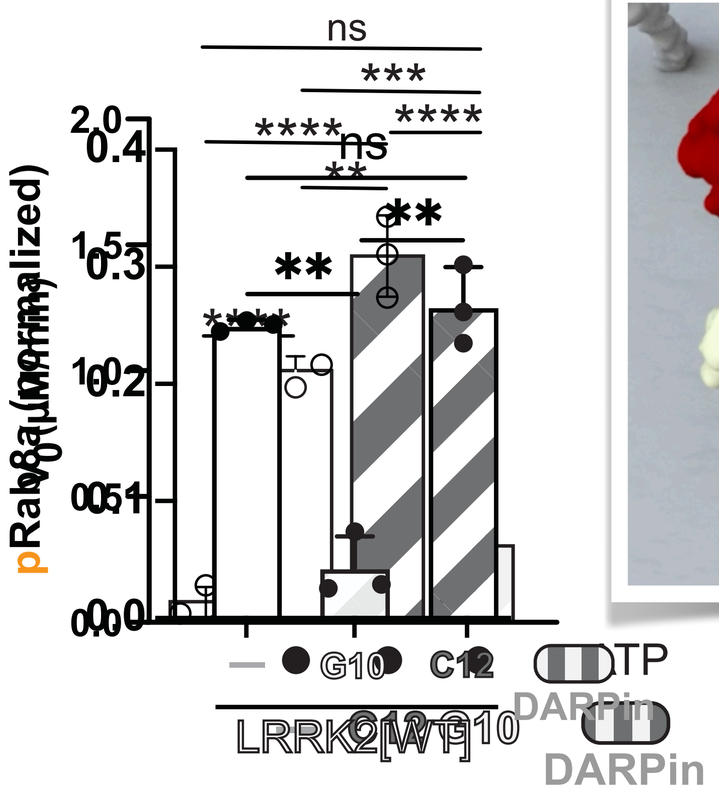


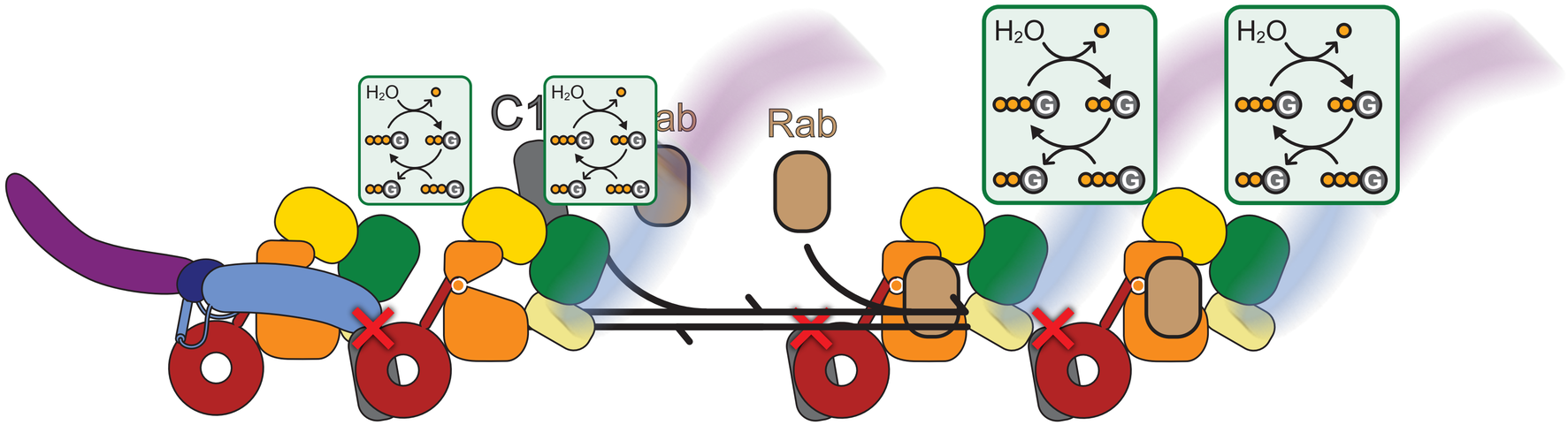
Is the GTPase a timing mechanism for autoinhibition?

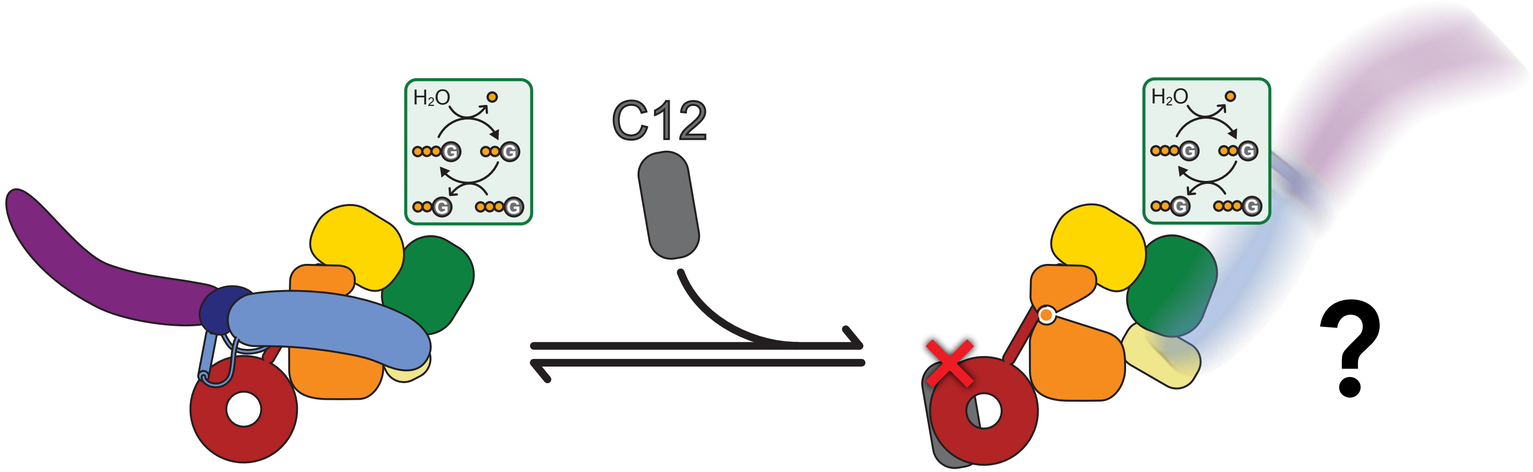


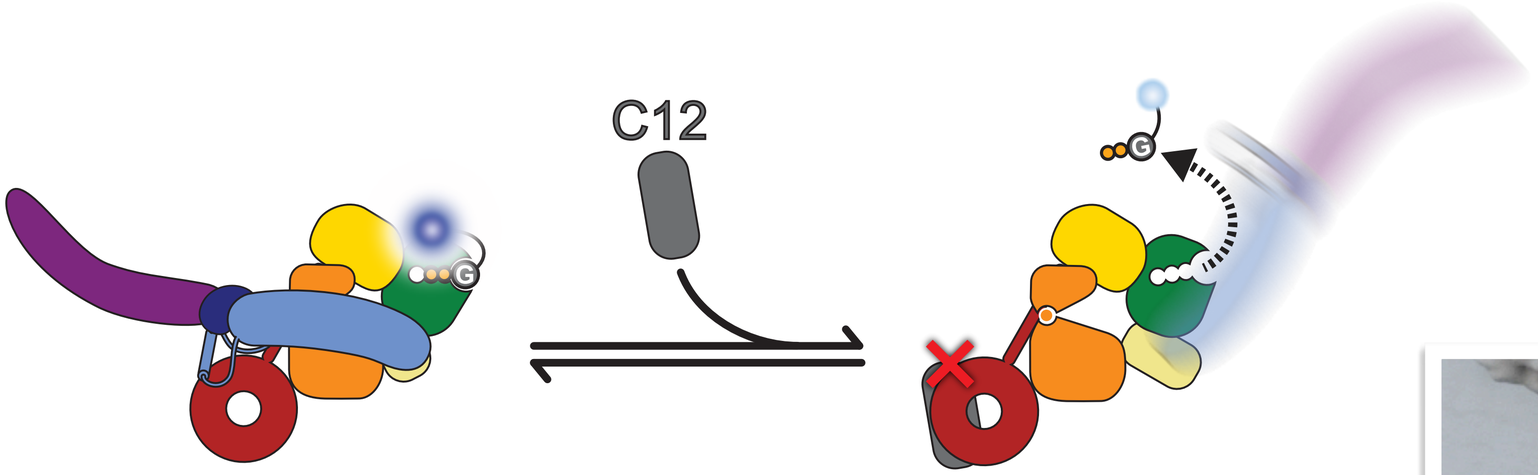


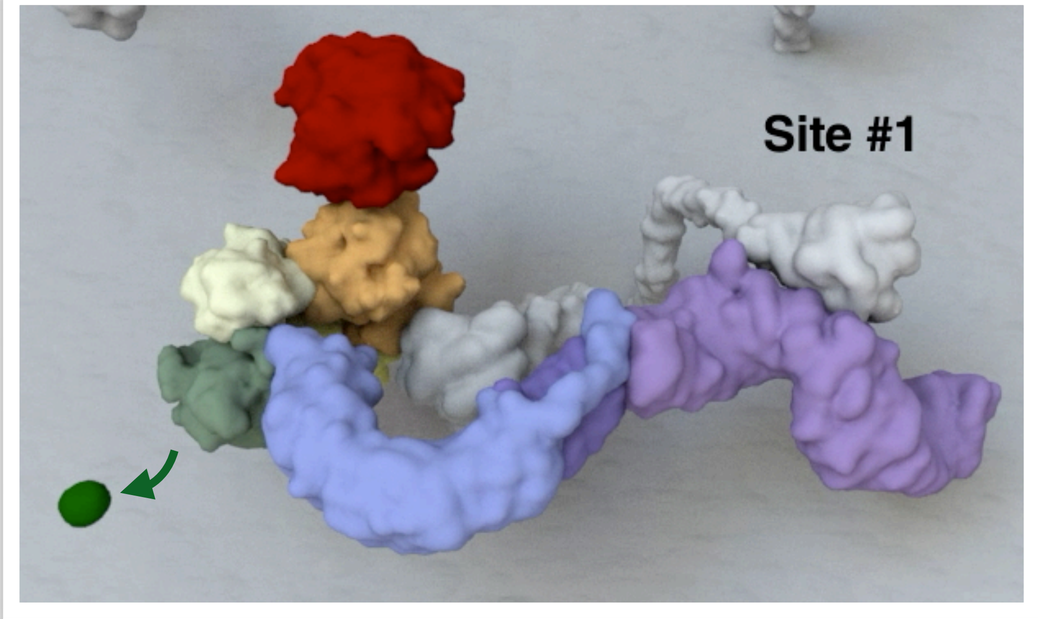


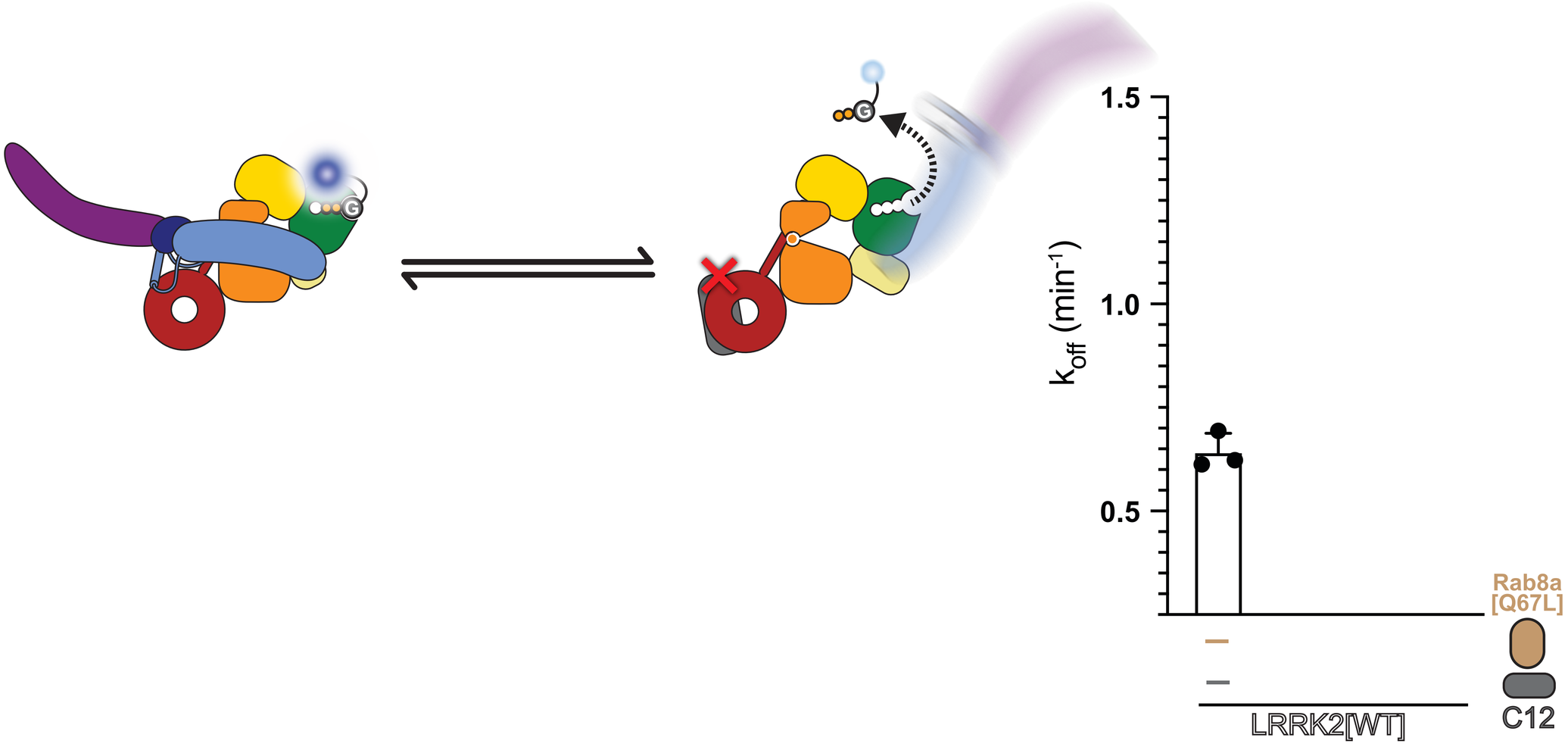


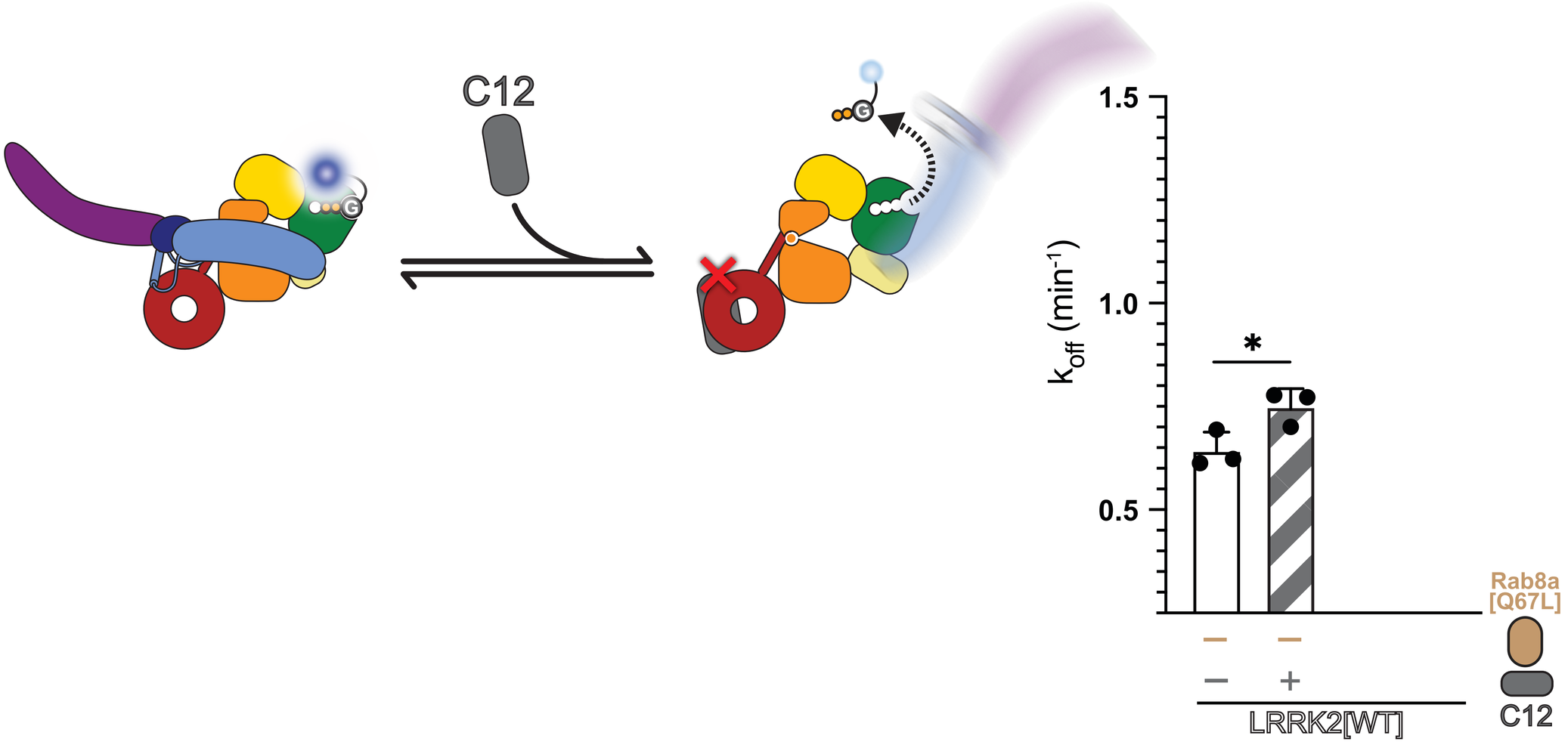

H2O
G
C1
ab
Rab
H2O
G
H2O
G

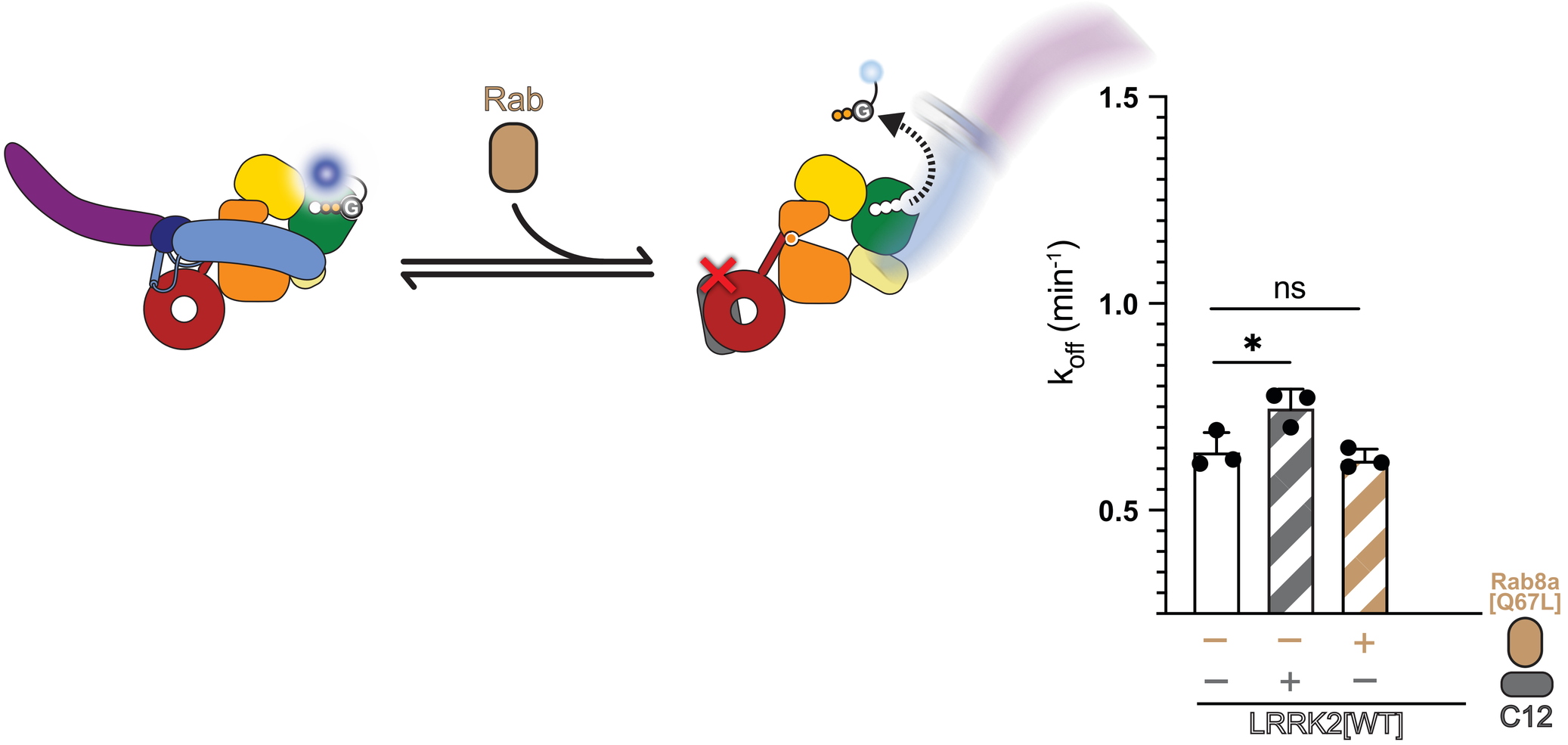

H2O
C12
H2O
?

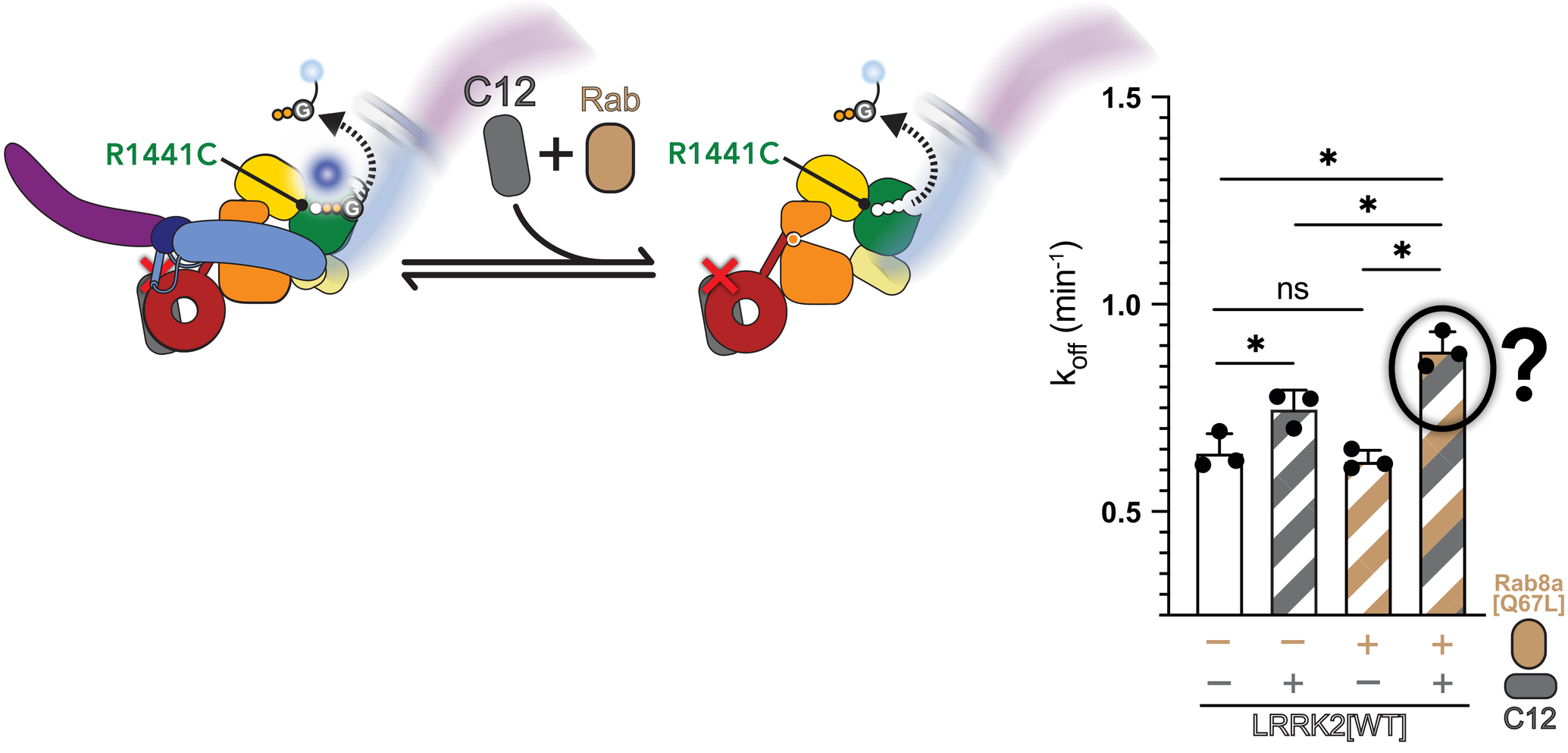

C12
G
G

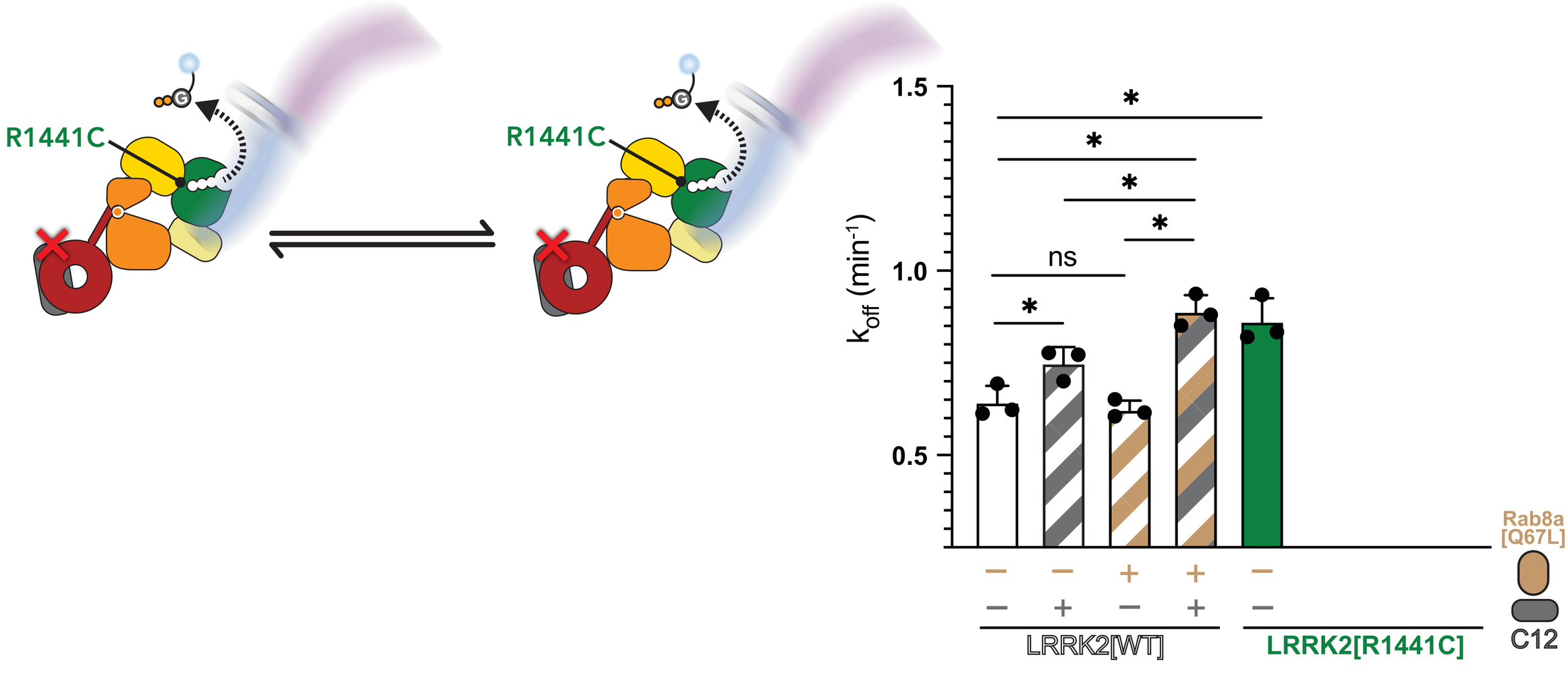

Site #1

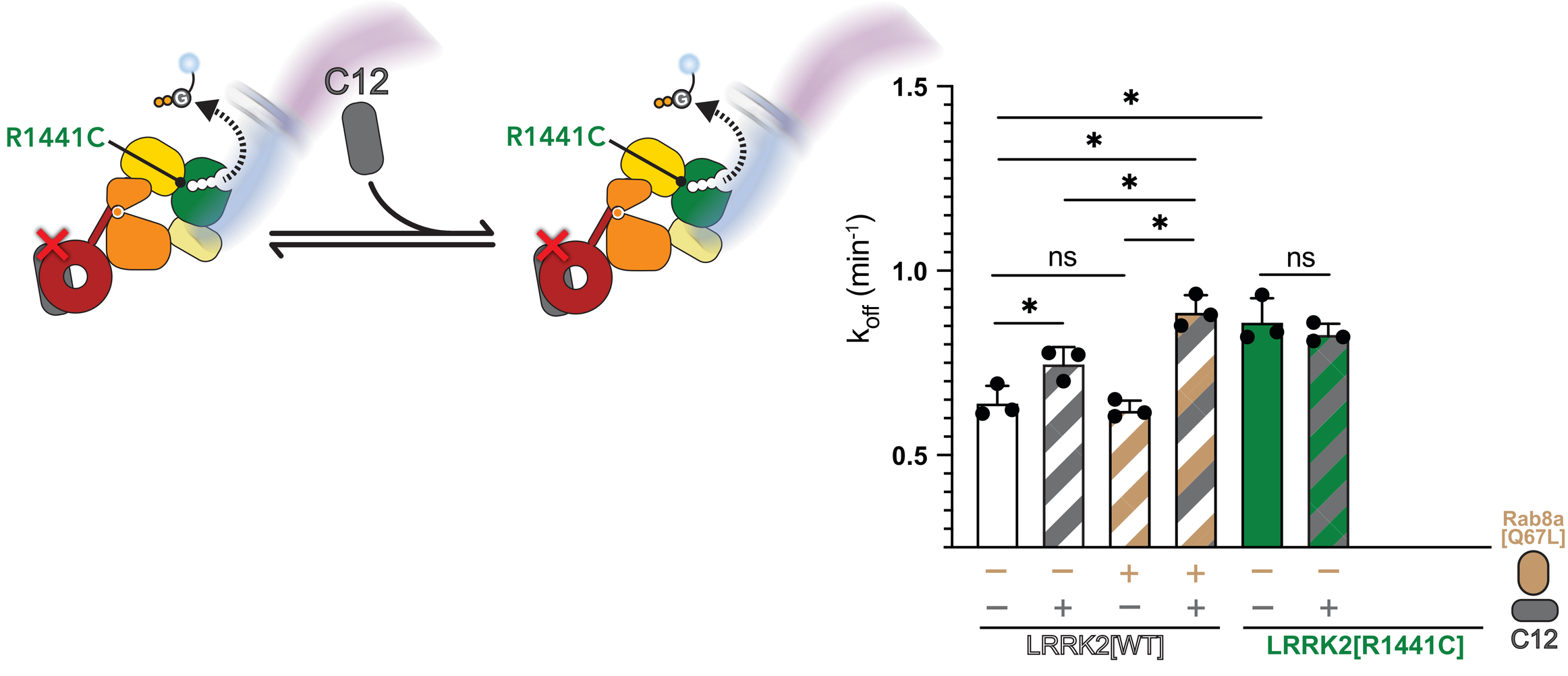

G
G
1.5
1.0
0.5
k_{off} (min^{-1})
Rab8a
[Q67L]
–
–
LRRK2[WT]
C12

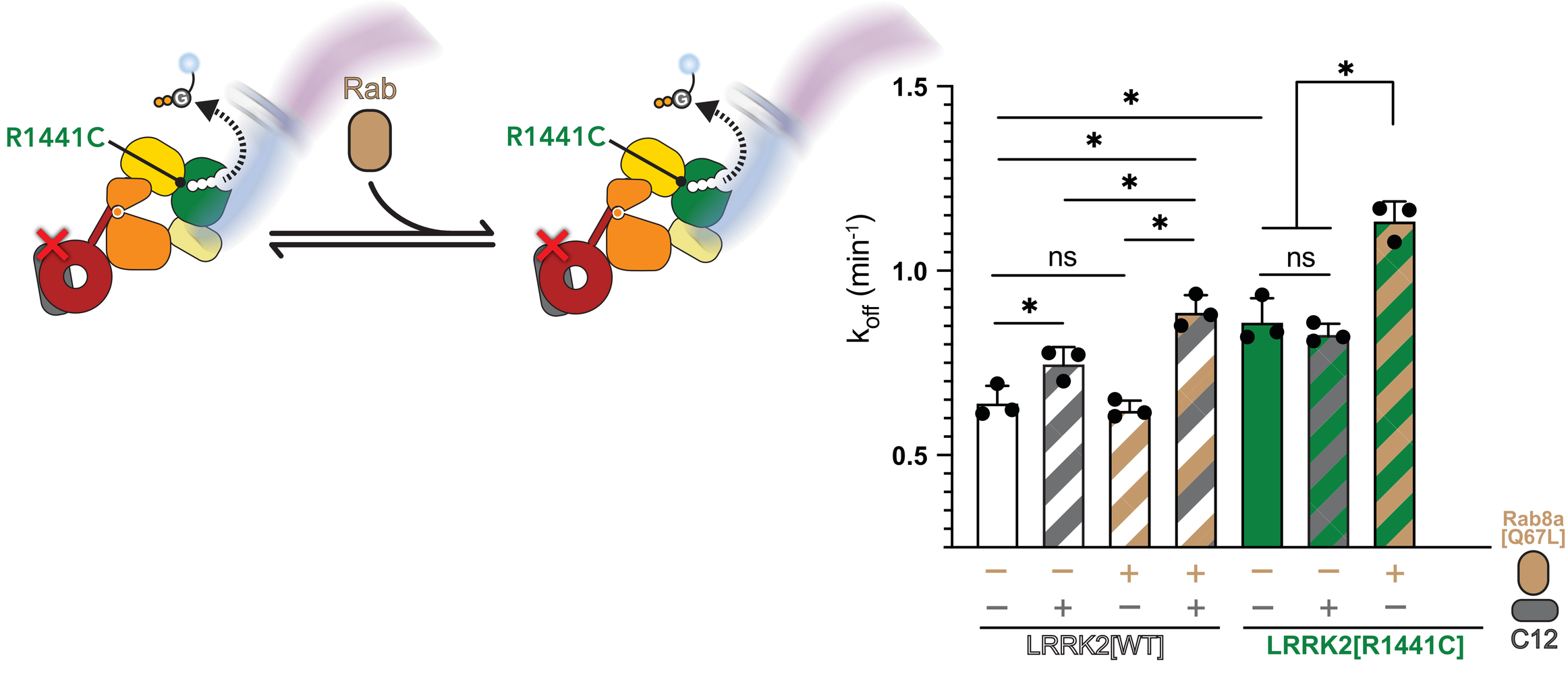

C12
G
G
1.5
1.0
0.5
k_{off} (min^{-1})
*
Rab8a
[Q67L]
–
–
–
+
LRRK2[WT]
C12

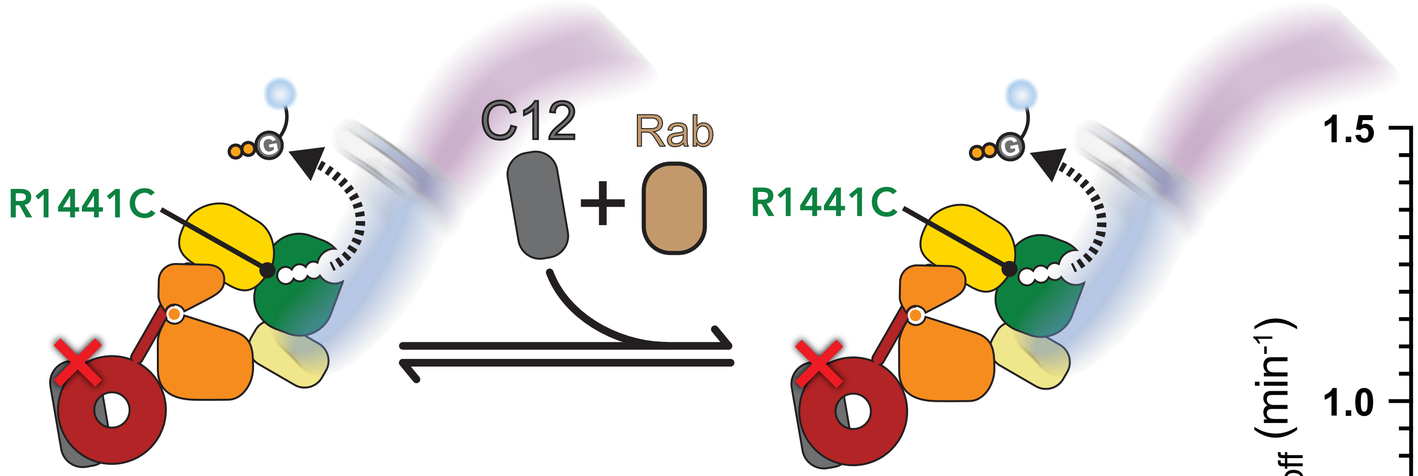

Rab
G
G
1.5
1.0
0.5
k_{off} (min^{-1})
ns
*
Rab8a
[Q67L]
−
−
+
−
+
−
LRRK2[WT]
C12

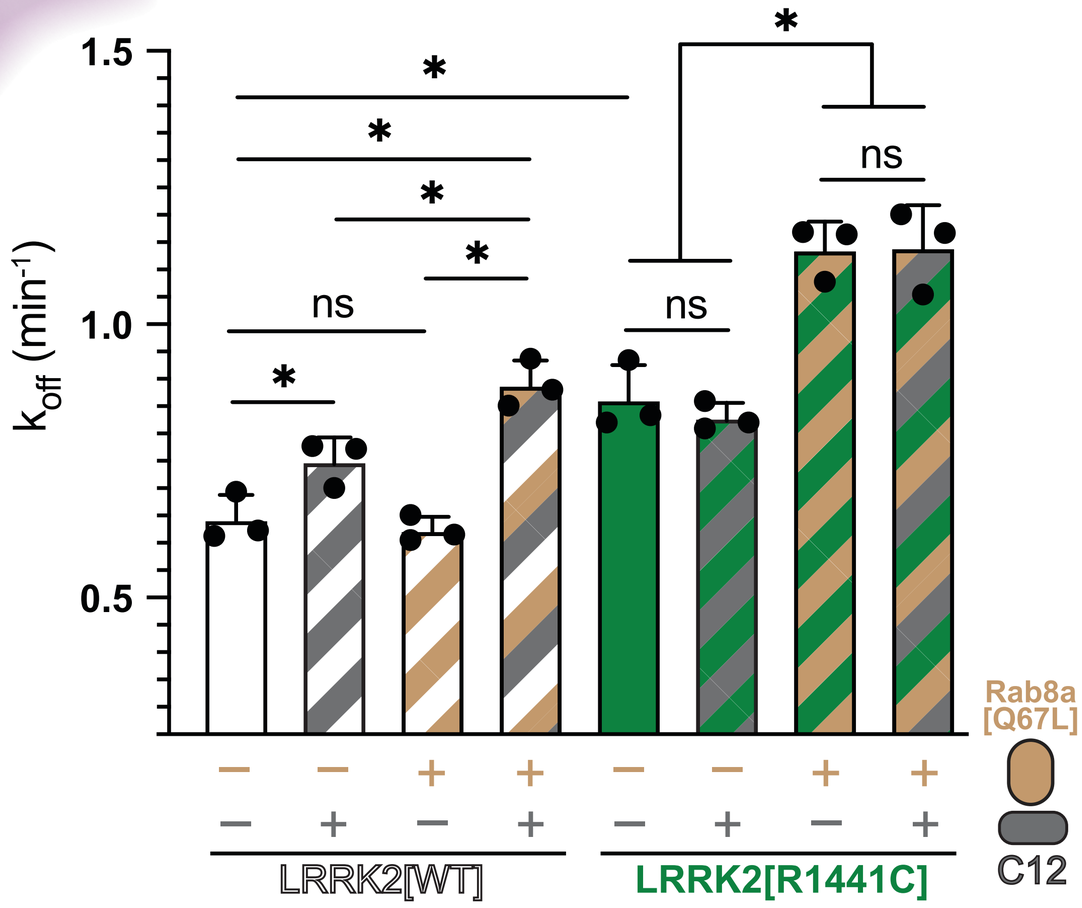

R1441C
C12
Rab
R1441C
1.5
1.0
0.5
k_{off} (min^{-1})
*
*
*
ns
*
?
Rab8a
[Q67L]
−
−
+
+
−
+
−
+
LRRK2[WT]
C12

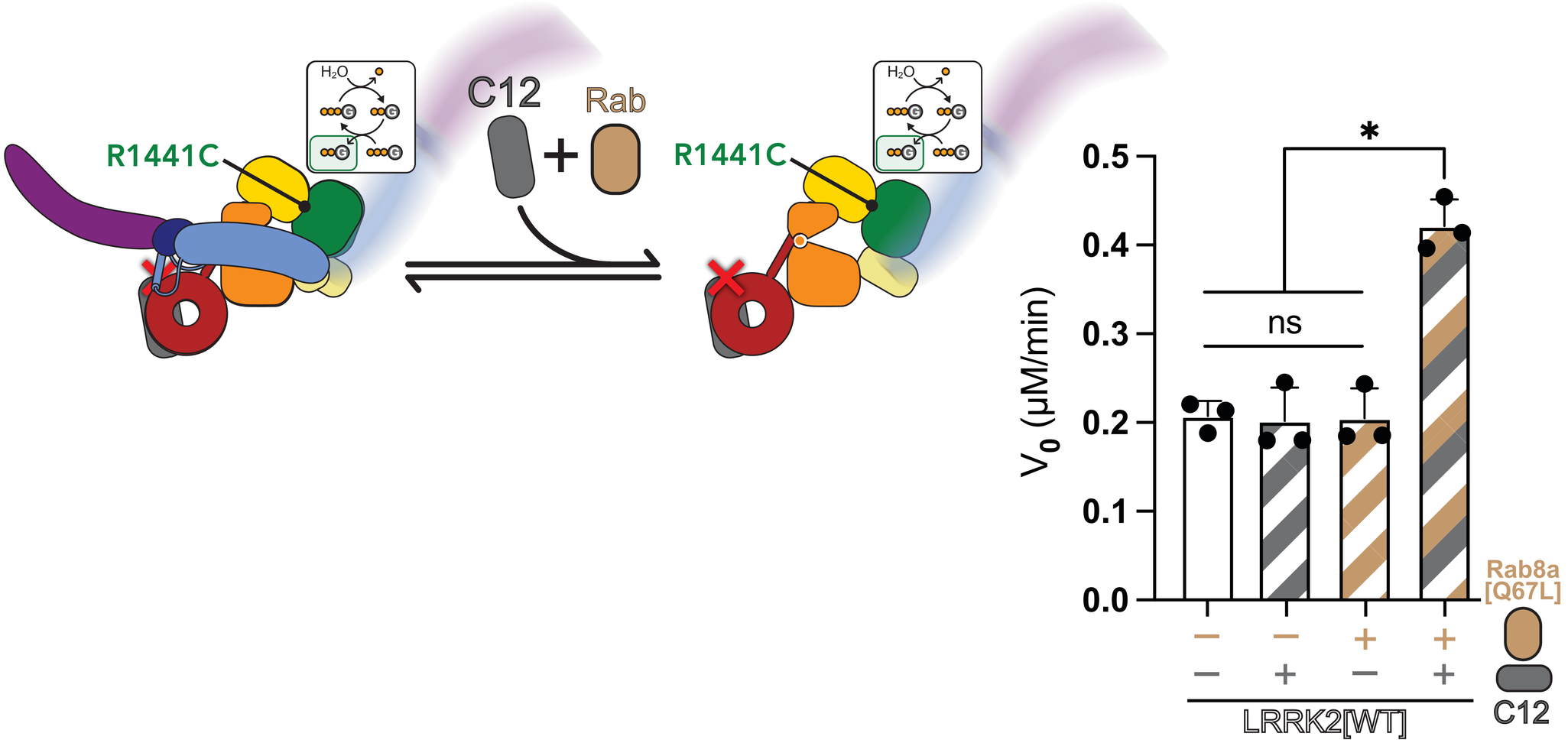

R1441C
G
R1441C
G
1.5
1.0
0.5
k_{off} (min^{-1})
*
*
*
*
*
ns
*
− − + + −
− + − + −
LRRK2[WT]
LRRK2[R1441C]
Rab8a
[Q67L]
C12

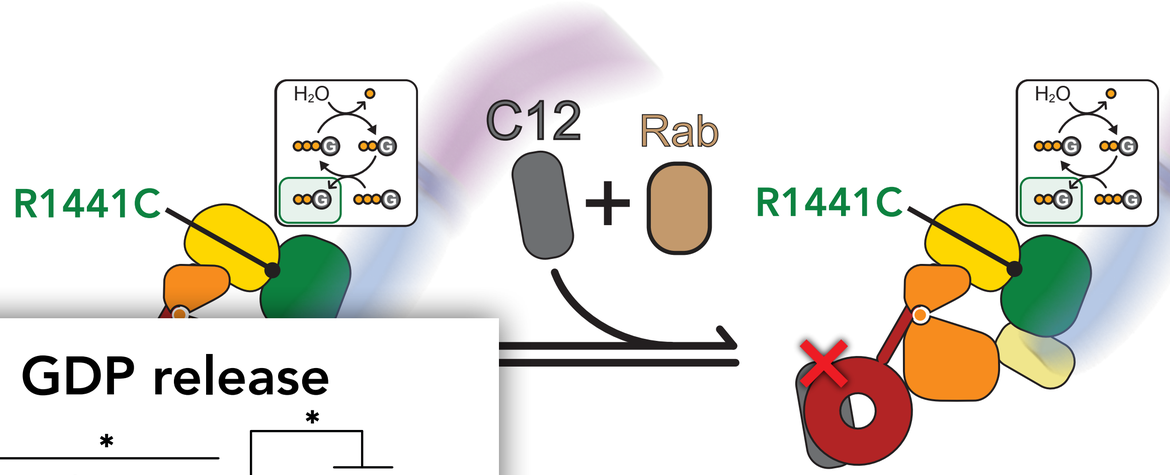

R1441C
C12
R1441C
1.5
1.0
0.5
k_{off} (min^{-1})
*
*
*
*
*
ns
ns
− − + + − −
− + − + − +
LRRK2[WT]
LRRK2[R1441C]
Rab8a
[Q67L]
C12

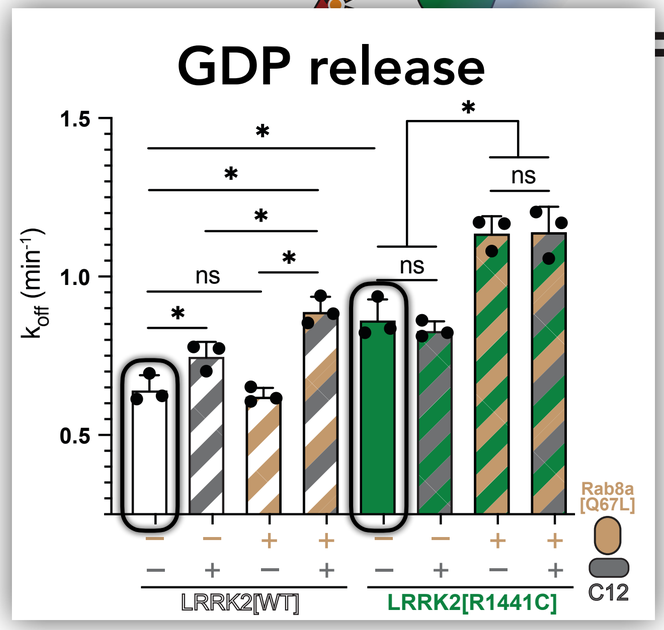

R1441C
Rab
R1441C
1.5
1.0
0.5
k_{off} (min^{-1})
*
*
*
*
*
*
ns
ns
− − + + − − +
− + − + − + −
LRRK2[WT]
LRRK2[R1441C]
Rab8a [Q67L]
C12

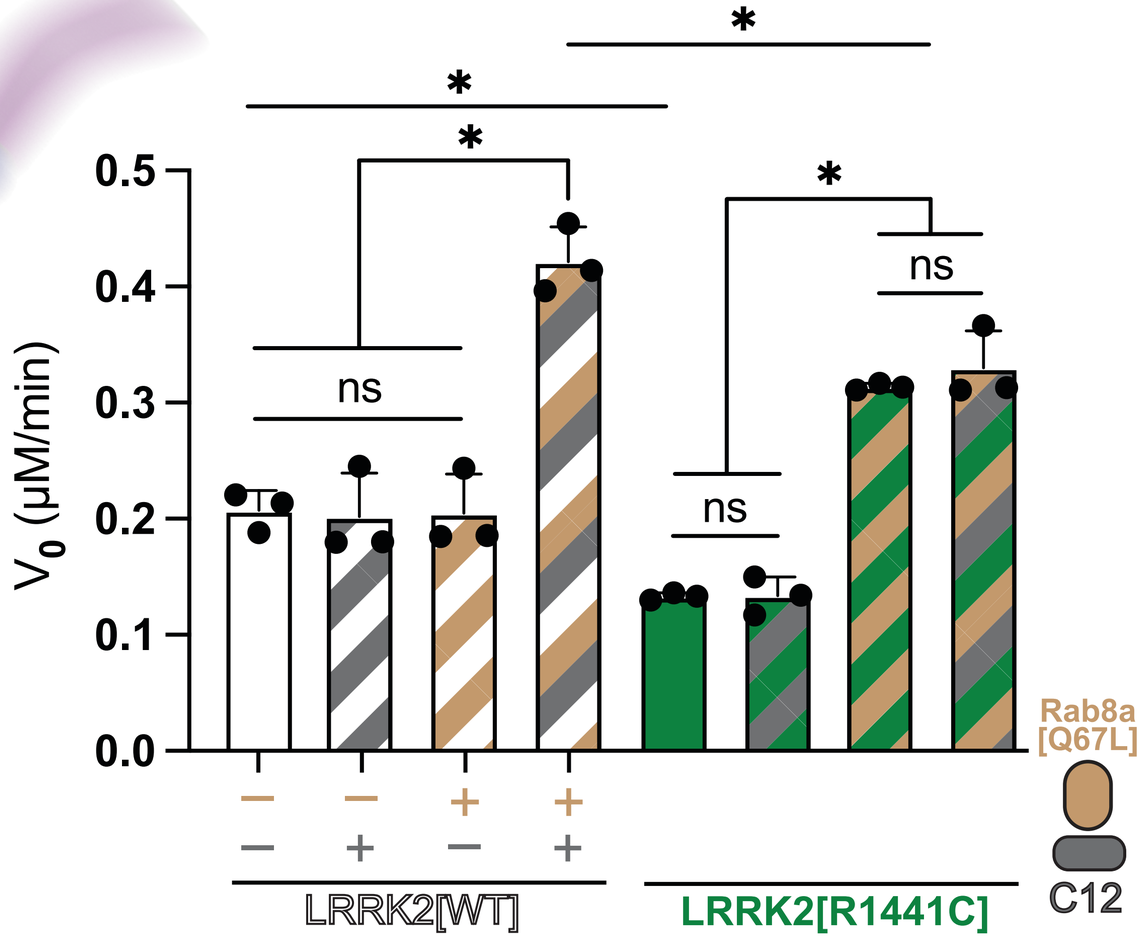

R1441C
C12
Rab
R1441C


1.5
1.0
0.5
k_{off} (min^{-1})
*
*
*
*
*
ns
ns
ns
− − + + − − + +
− + − + − + − +
LRRK2[WT]
LRRK2[R1441C]
Rab8a [Q67L]
C12

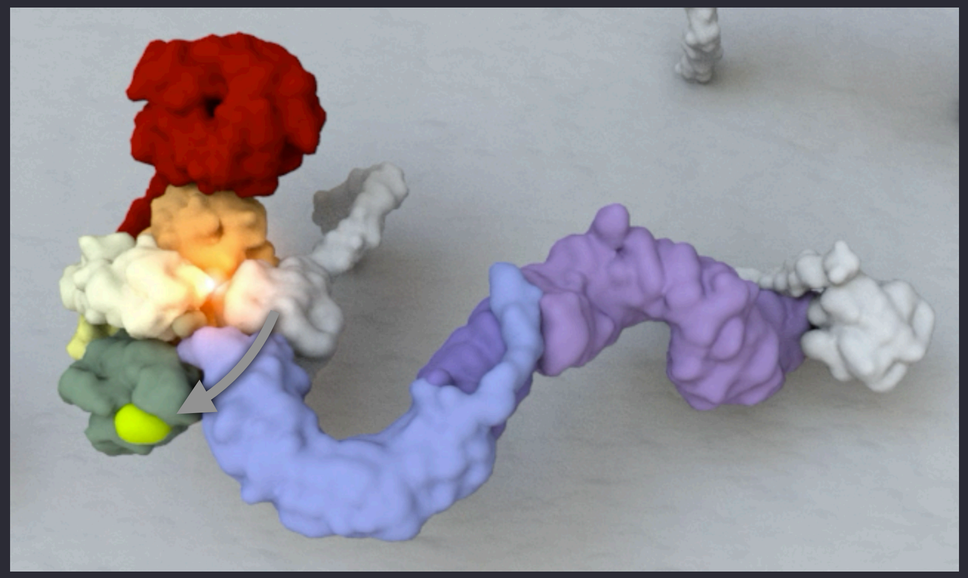

R1441C
C12
Rab
R1441C
H_2O
V_0 (µM/min)
0.5
0.4
0.3
0.2
0.1
0.0
*
ns
Rab8a [Q67L]
C12
LRRK2[WT]


H2O
R1441C
C12
Rab
R1441C
H2O


GDP release
koff (min-1)
1.5
1.0
0.5
*
*
*
*
ns
*
ns
ns
Rab8a [Q67L]
C12
LRRK2[WT]
LRRK2[R1441C]

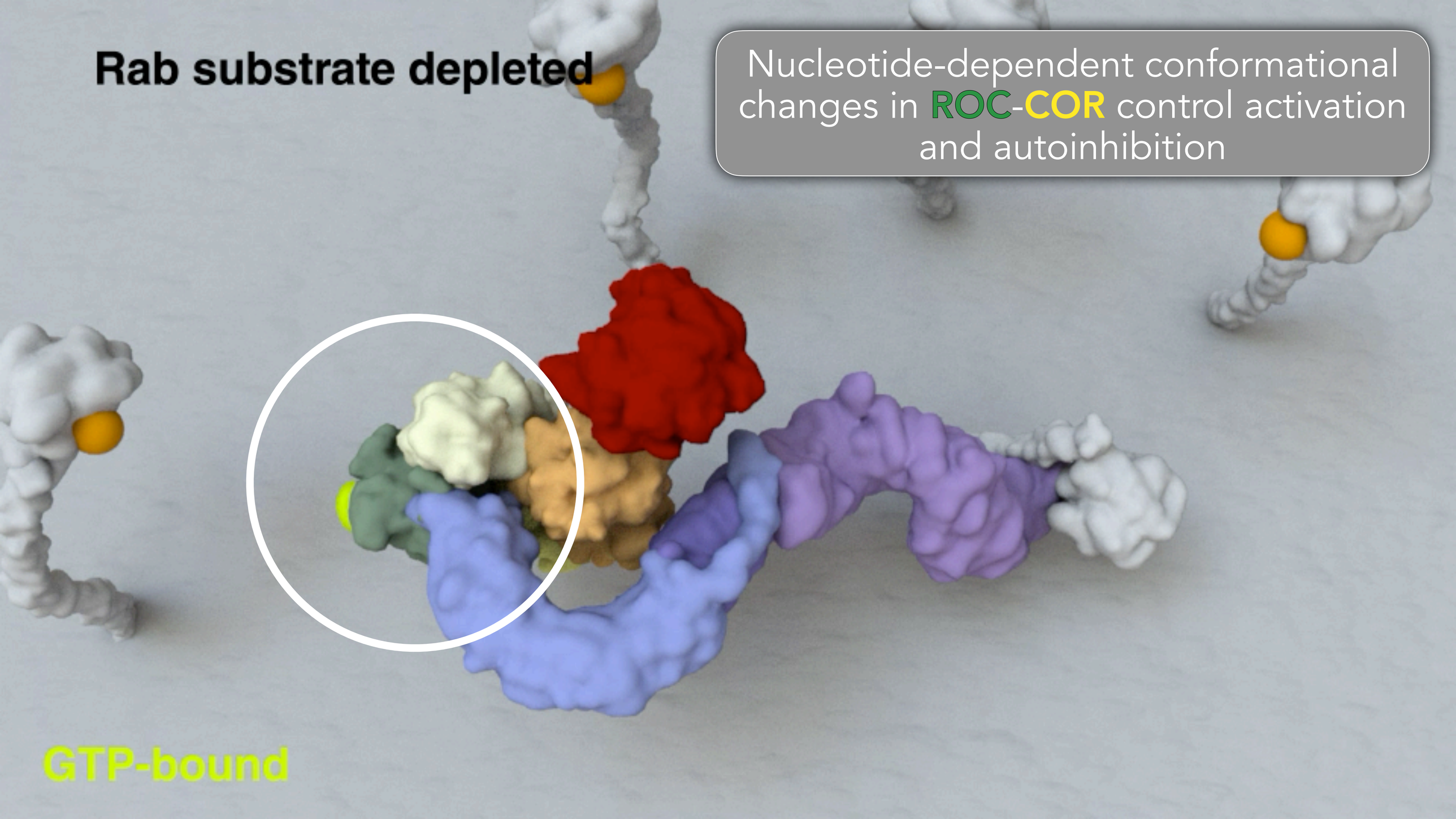

V0 (µM/min)
0.5
0.4
0.3
0.2
0.1
0.0
*
*
*
*
ns
ns
ns
Rab8a [Q67L]
C12
LRRK2[WT]
LRRK2[R1441C]

New data we must incorporate into the animation



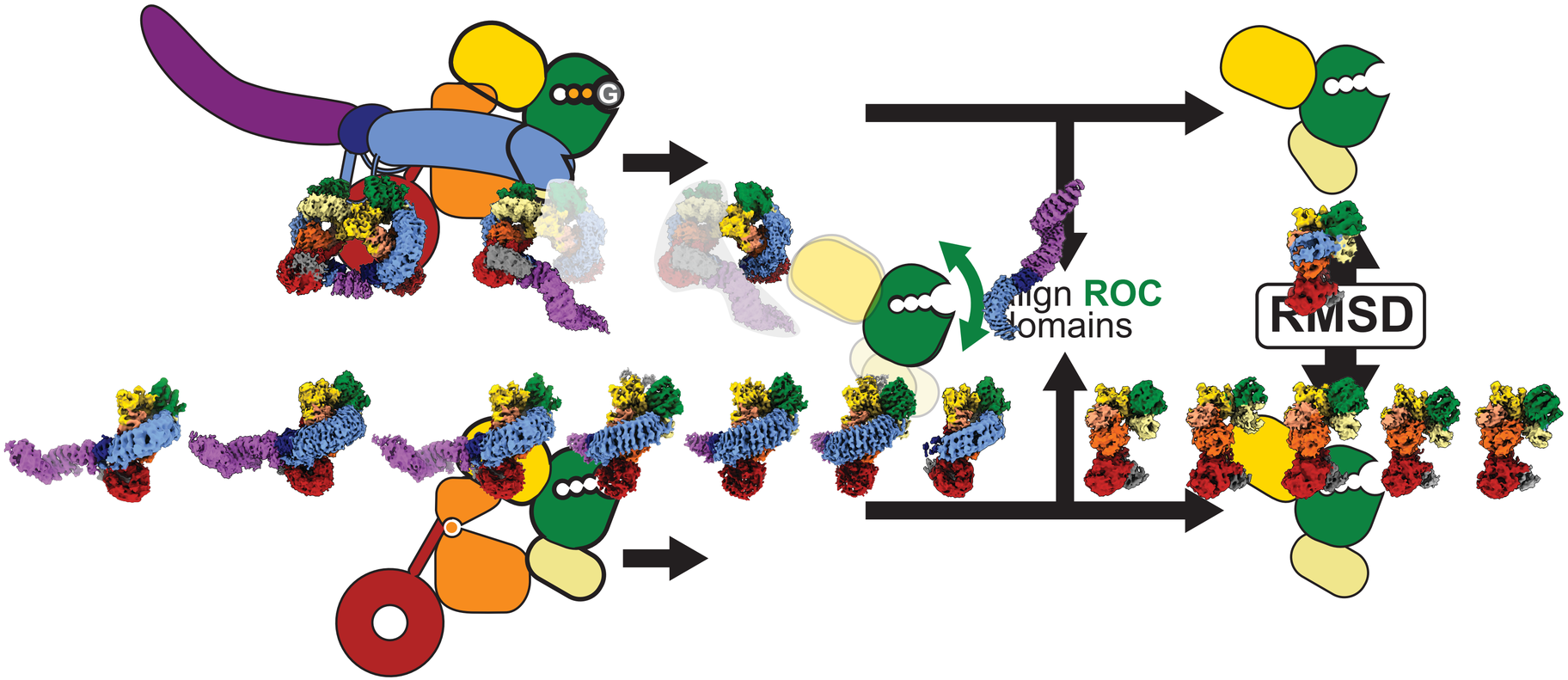
Binding of Rab substrate promotes GTP hydrolysis and GDP release

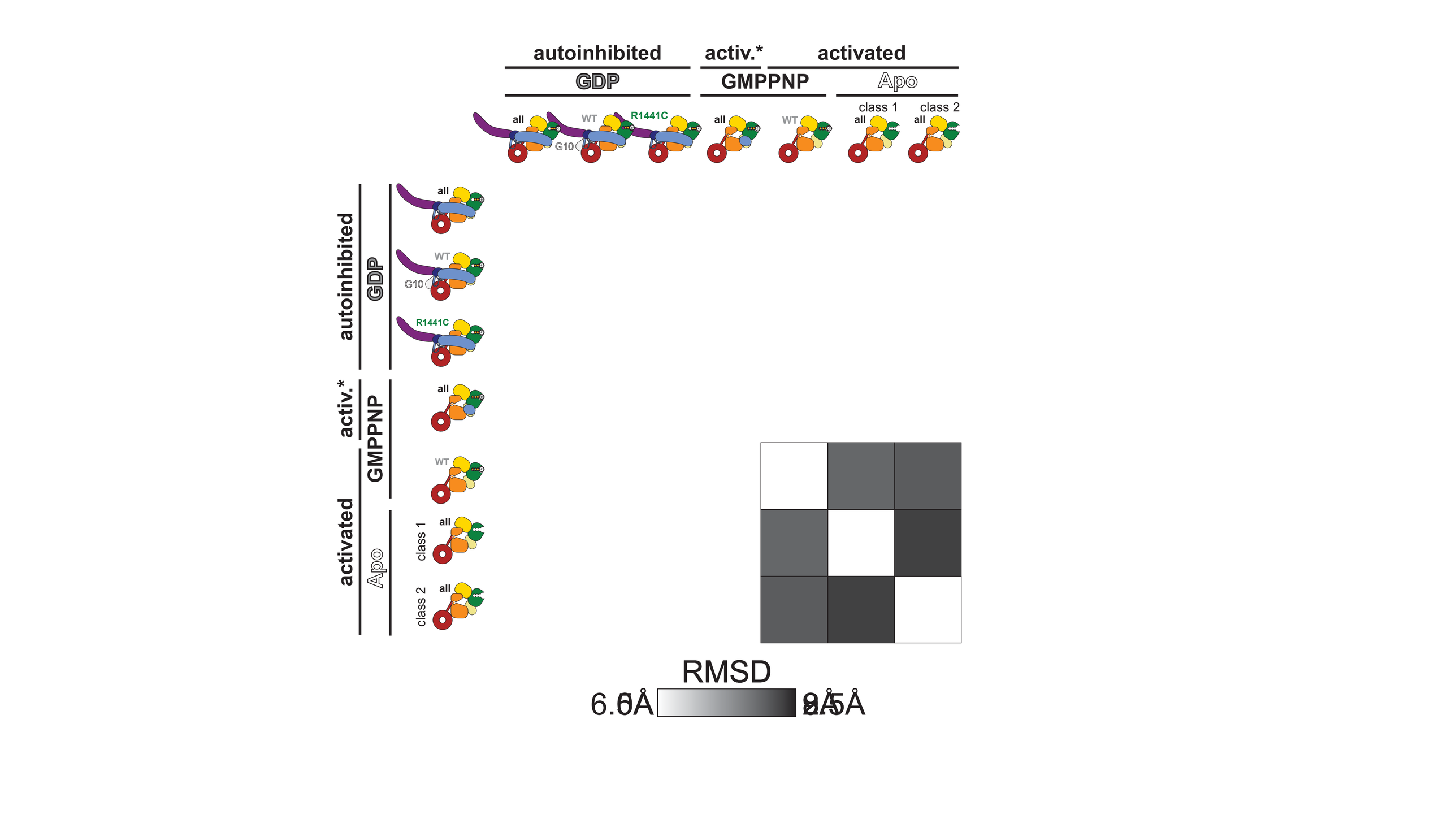
The GTPase cycle


Rab substrate depleted
Nucleotide-dependent conformational changes in ROC-COR control activation and autoinhibition
GTP-bound

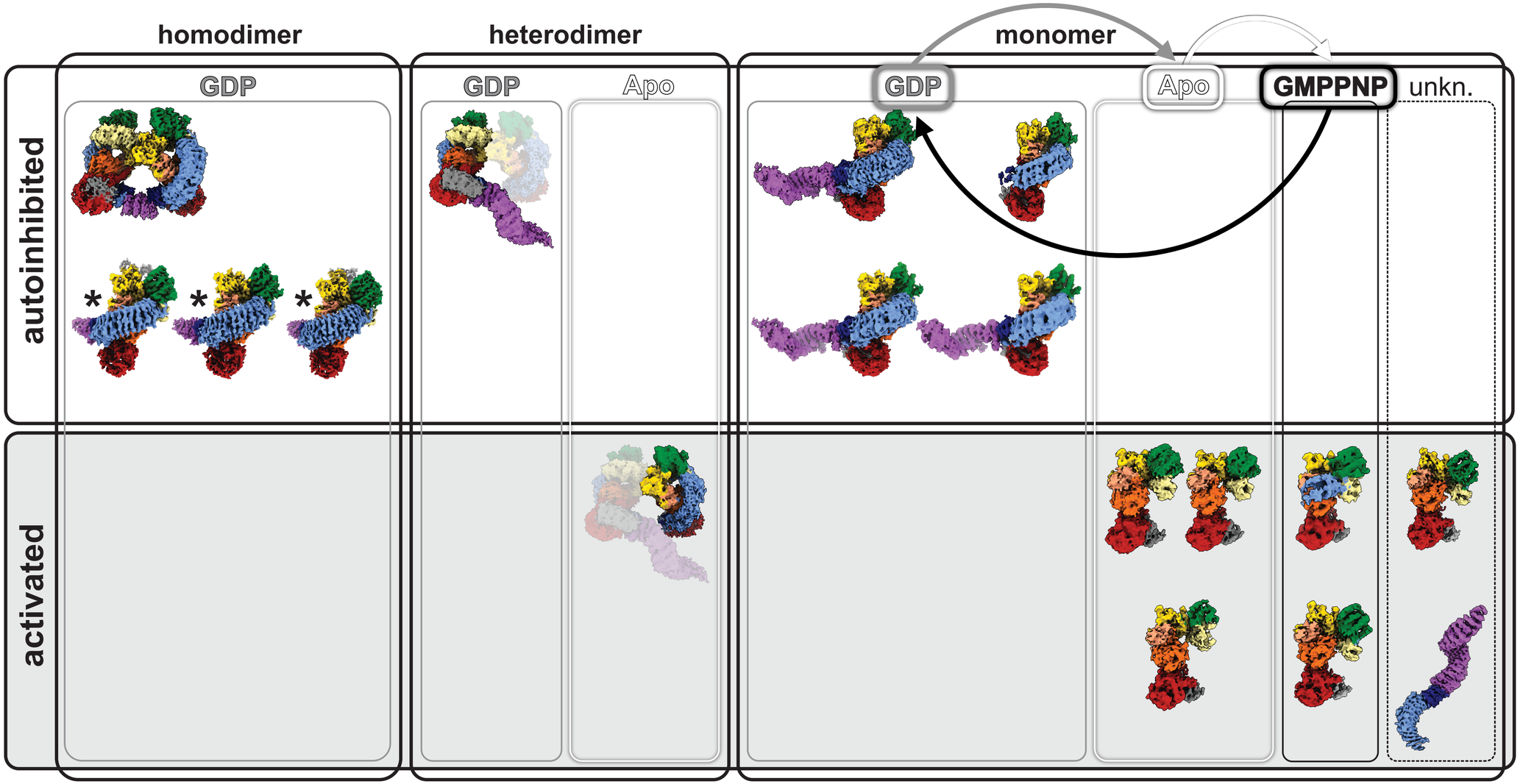
Conformational changes in LRRK2 activation

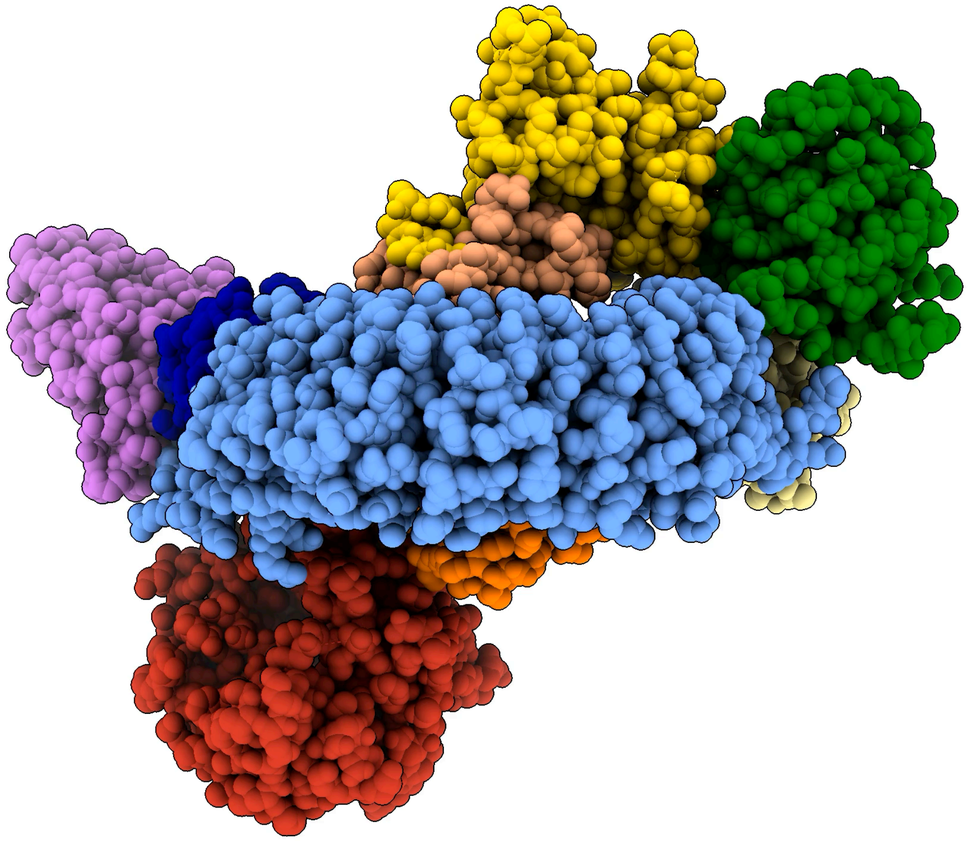
How do we quantify ?



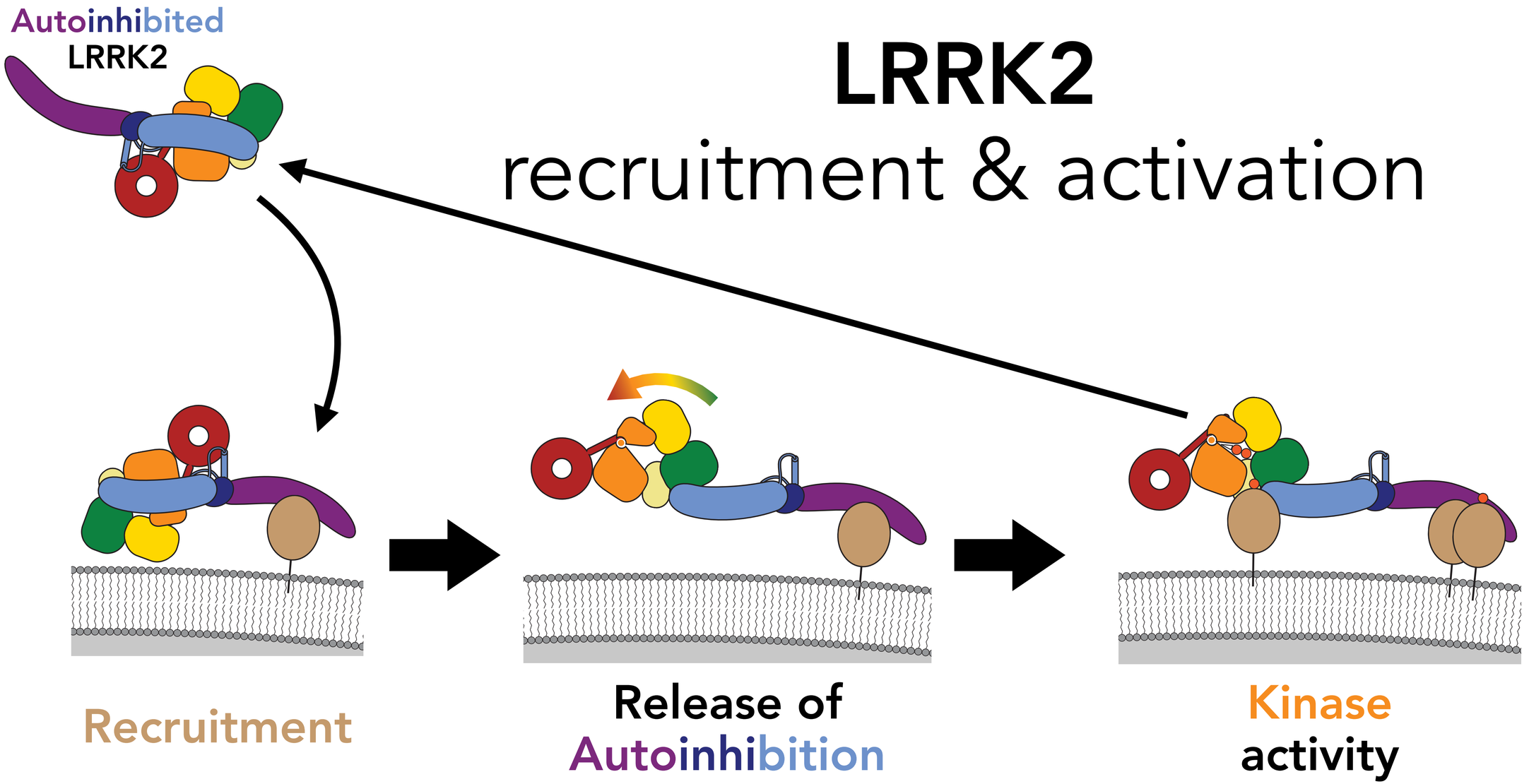

autoinhibited
activ.*
activated
GDP
GMPPNP
Apo
all
WT
R1441C
G10
class 1
class 2
RMSD
6.0Å
9.5Å

Conformational changes in LRRK2 activation

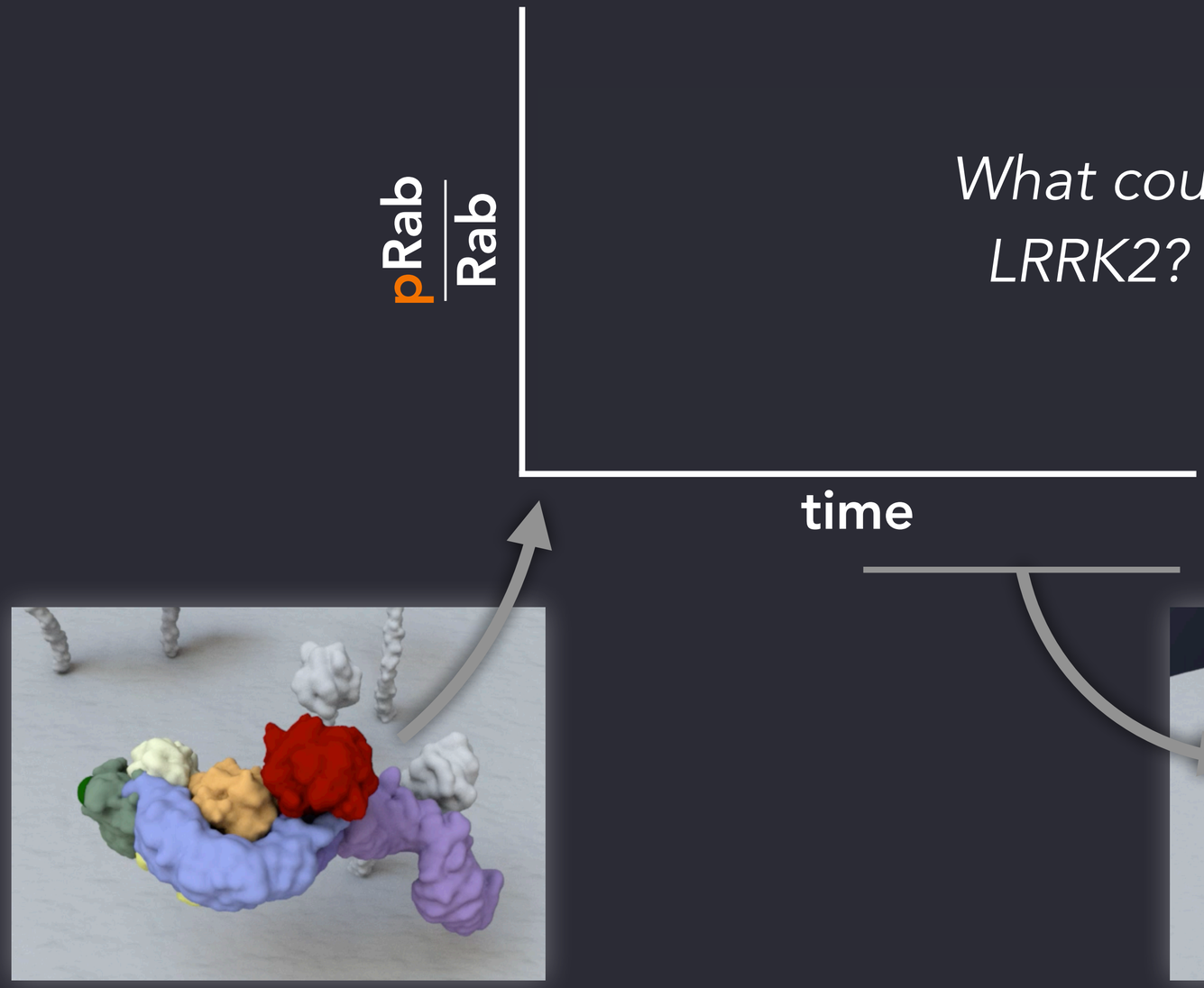




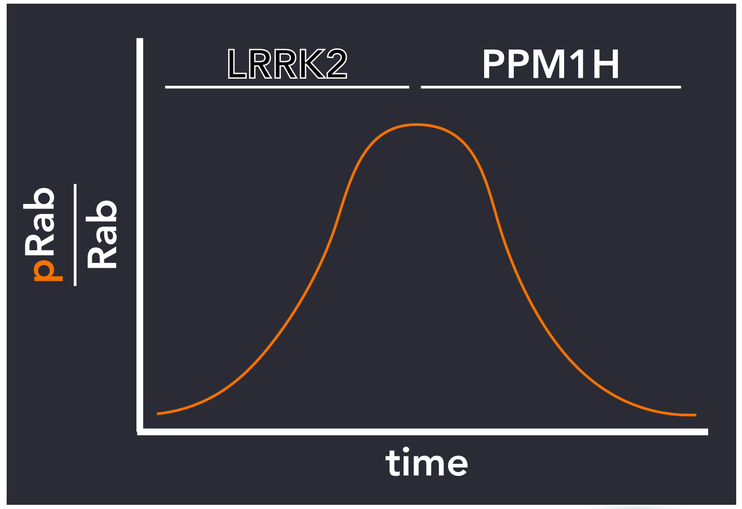
LRRK2
recruitment & activation

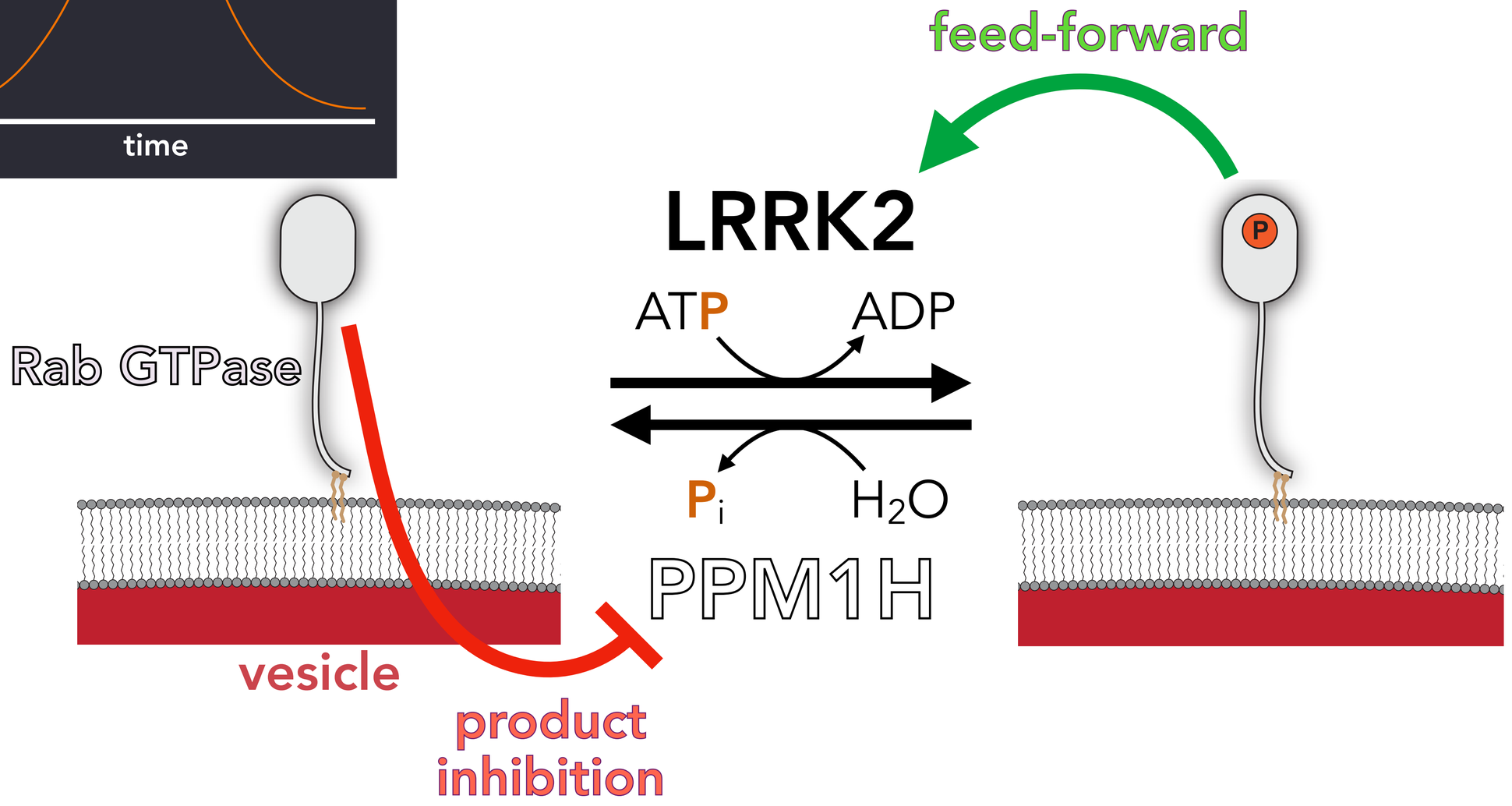


How does LRRK2 create a “burst” of pRab?

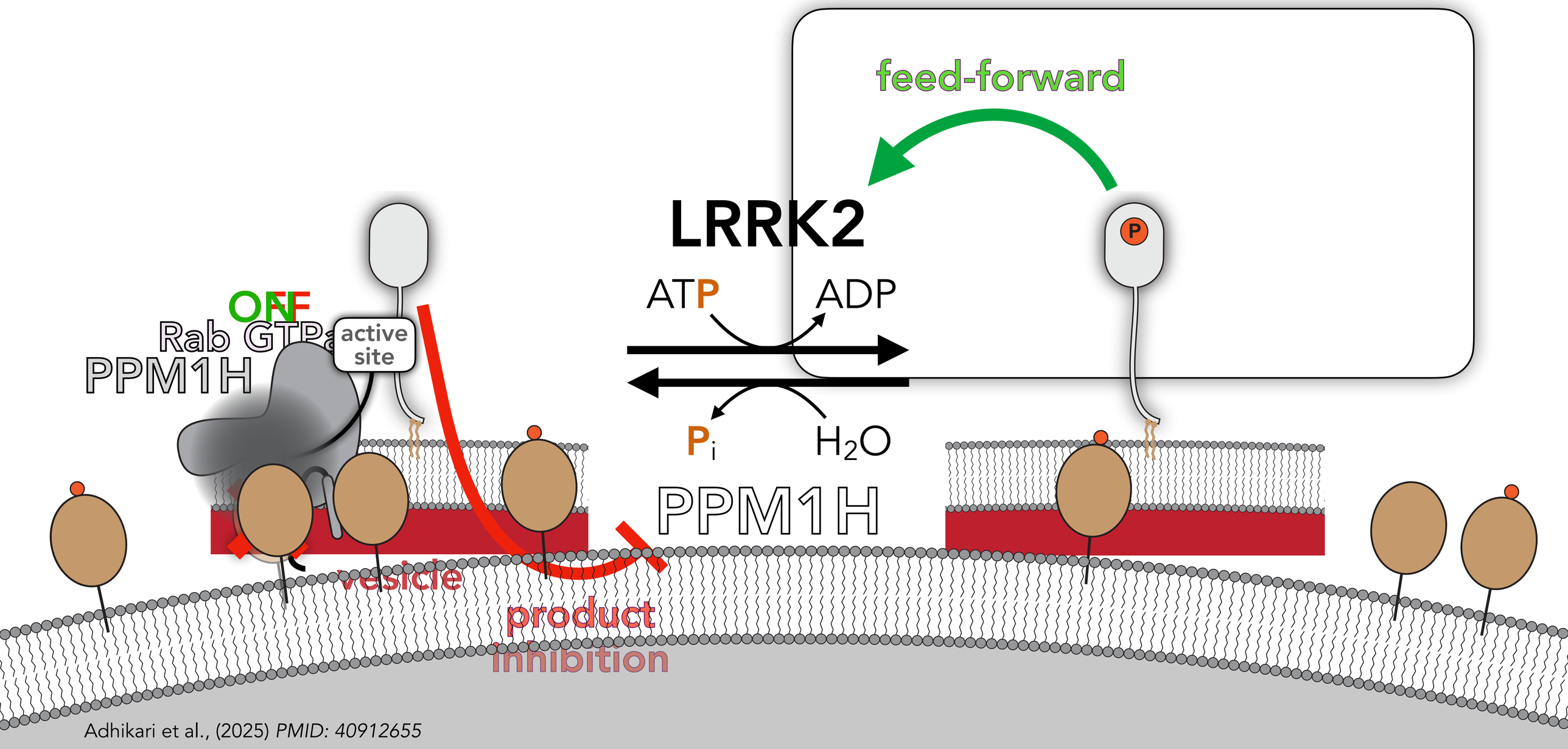


LRRK2 is Rab kinase

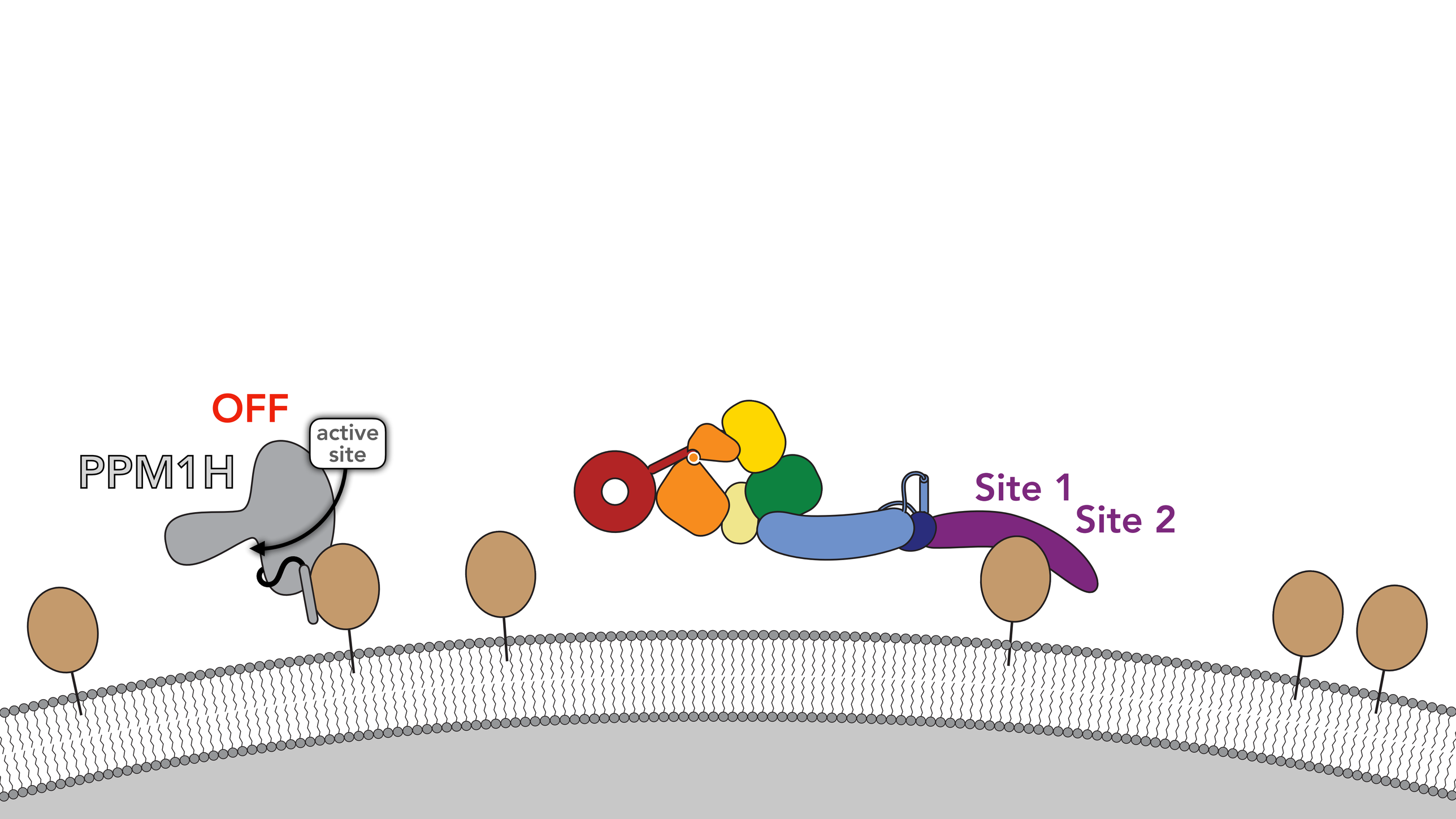


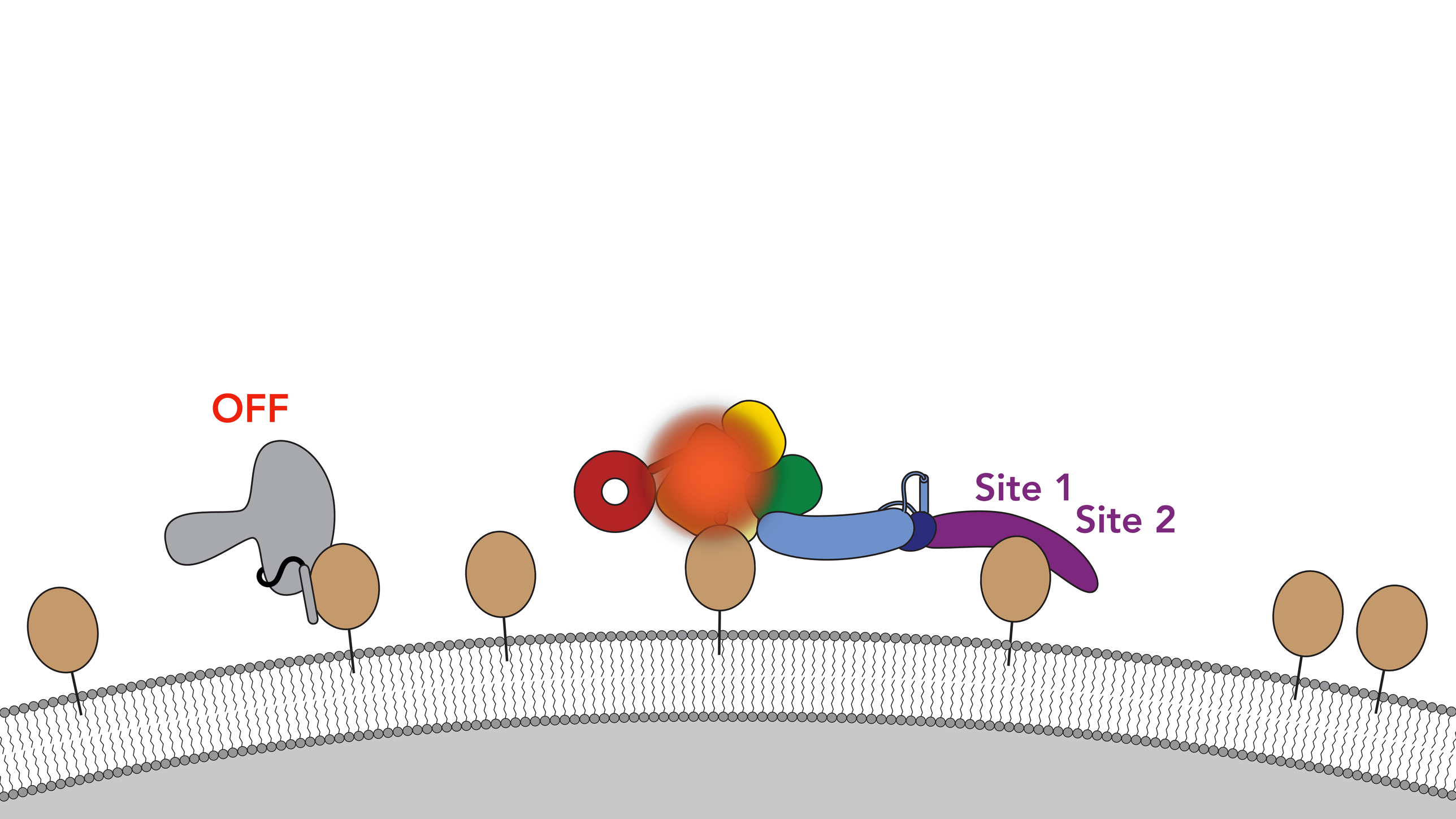


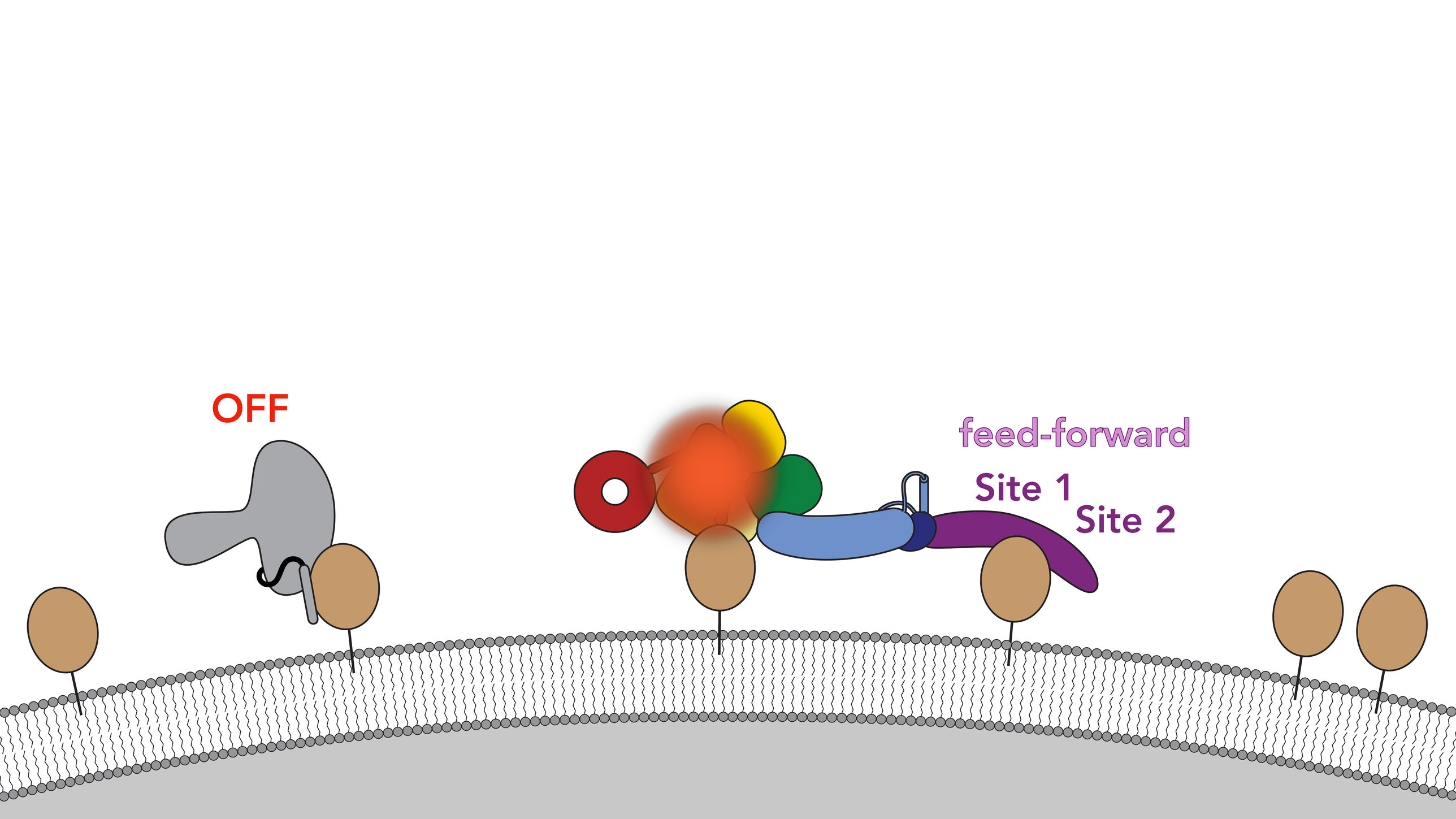
PPM1H is a pRab phosphatase

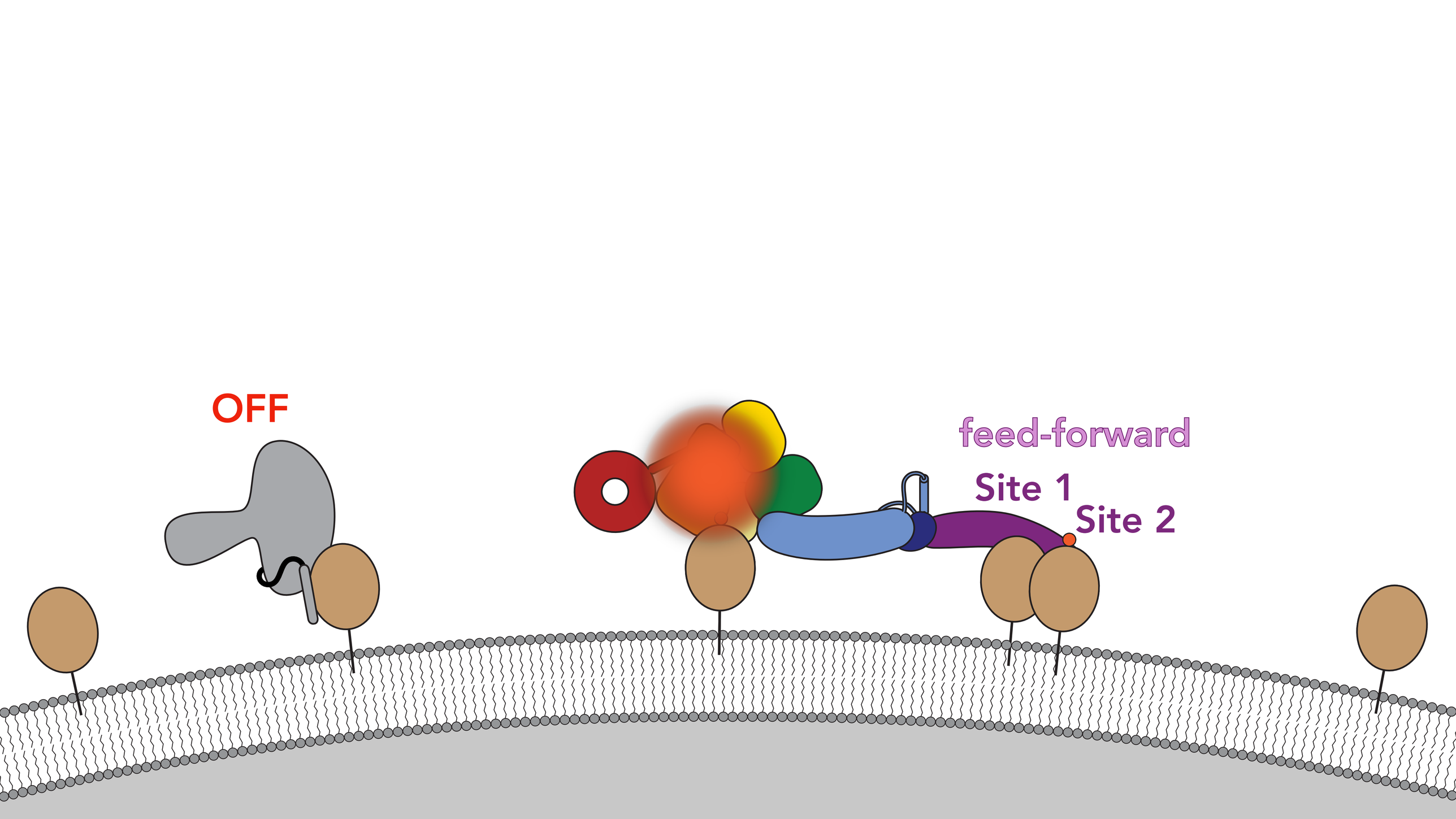


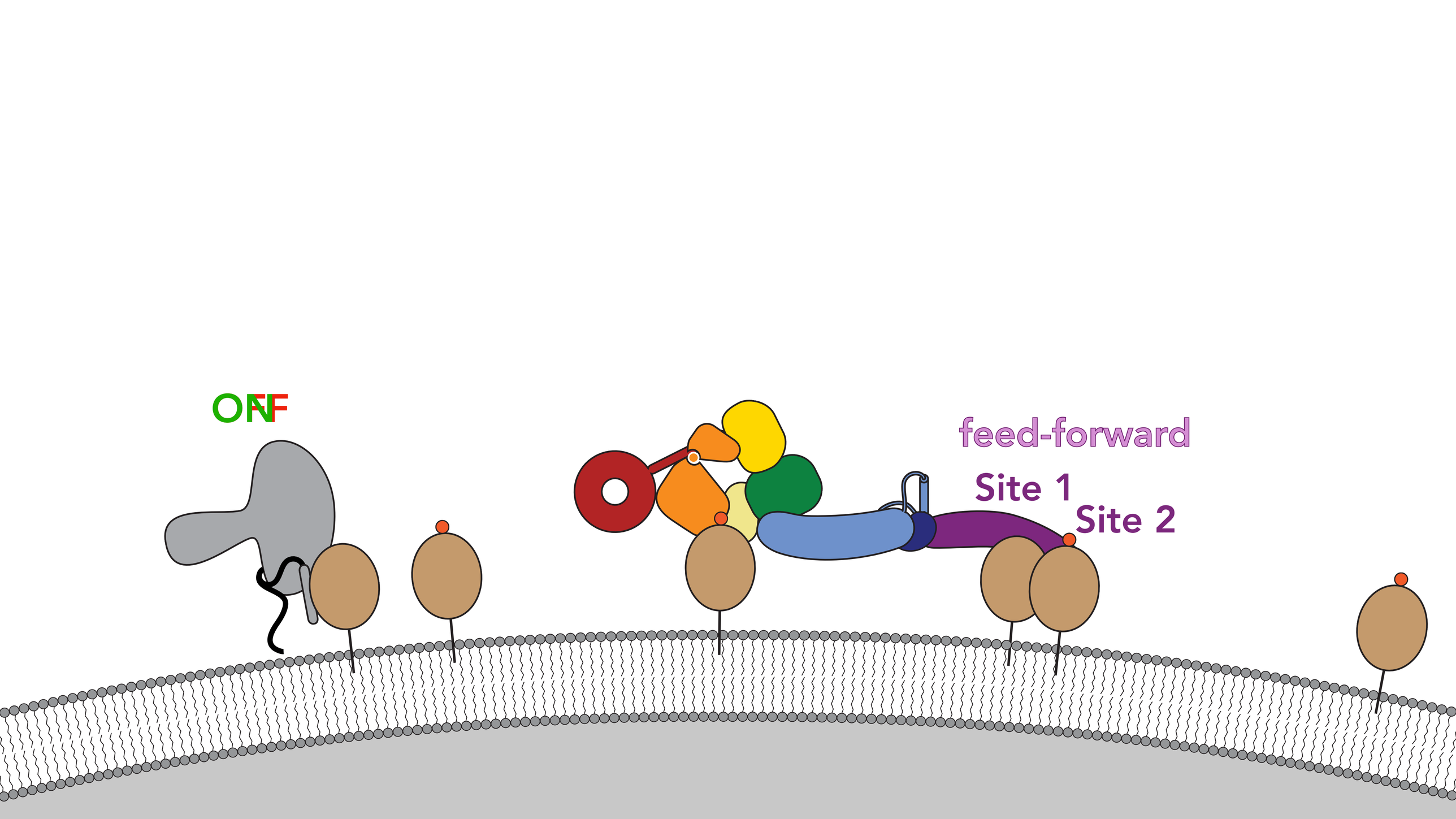
Adhikari et al., (2025) *PMID: 40912655*

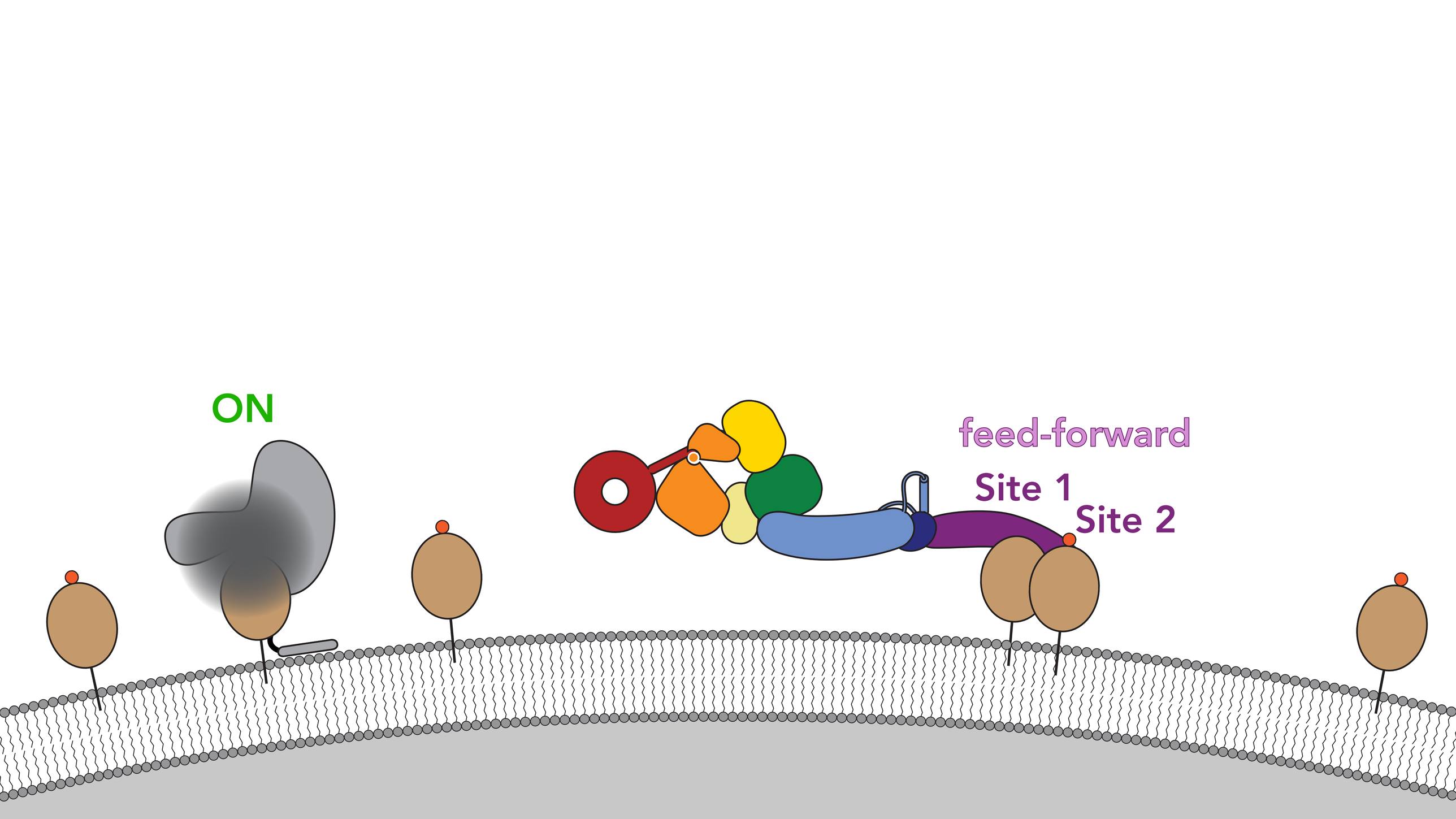

OFF
PPM1H
active site
Site 1
Site 2

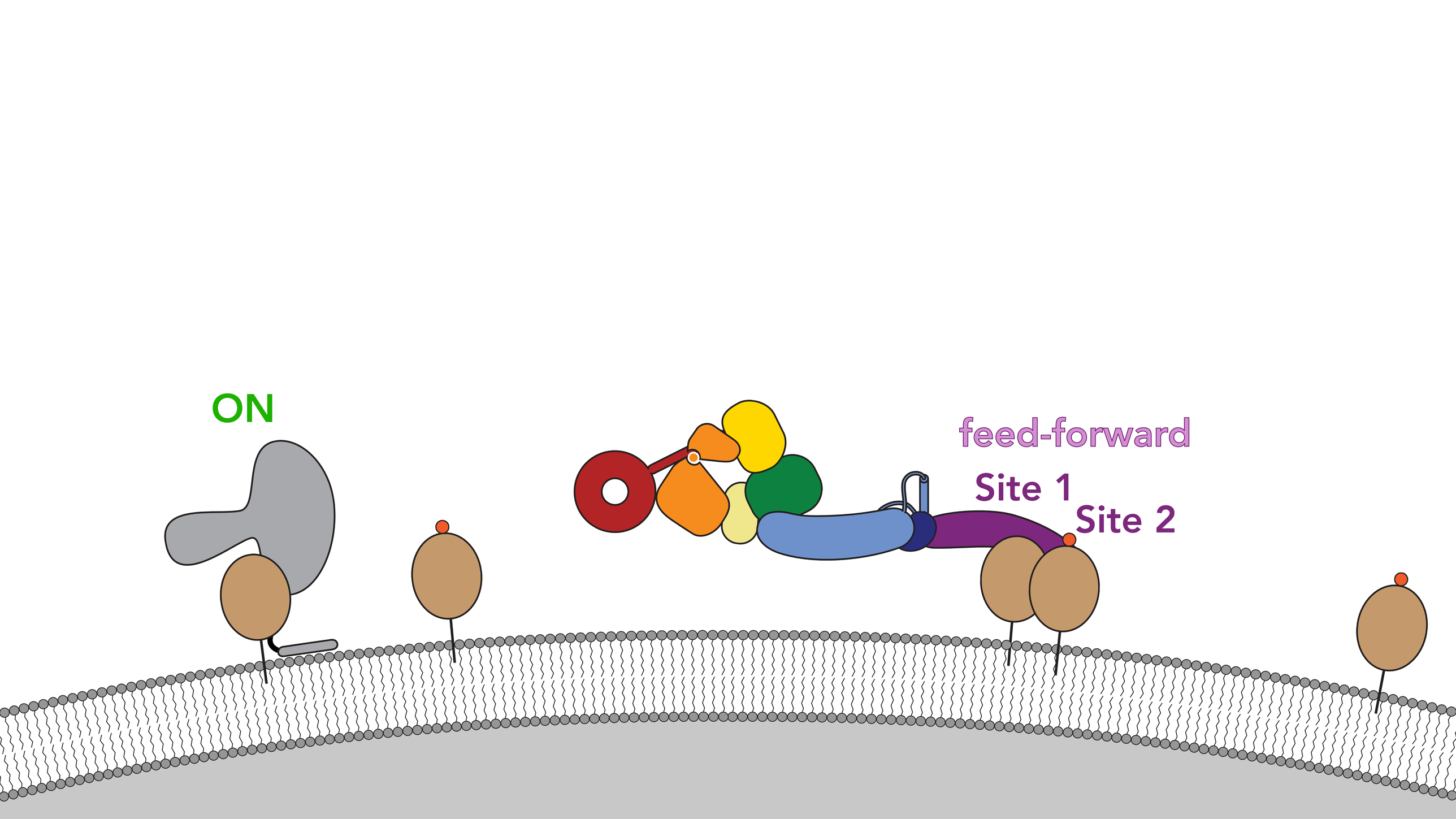

OFF
Site 1
Site 2

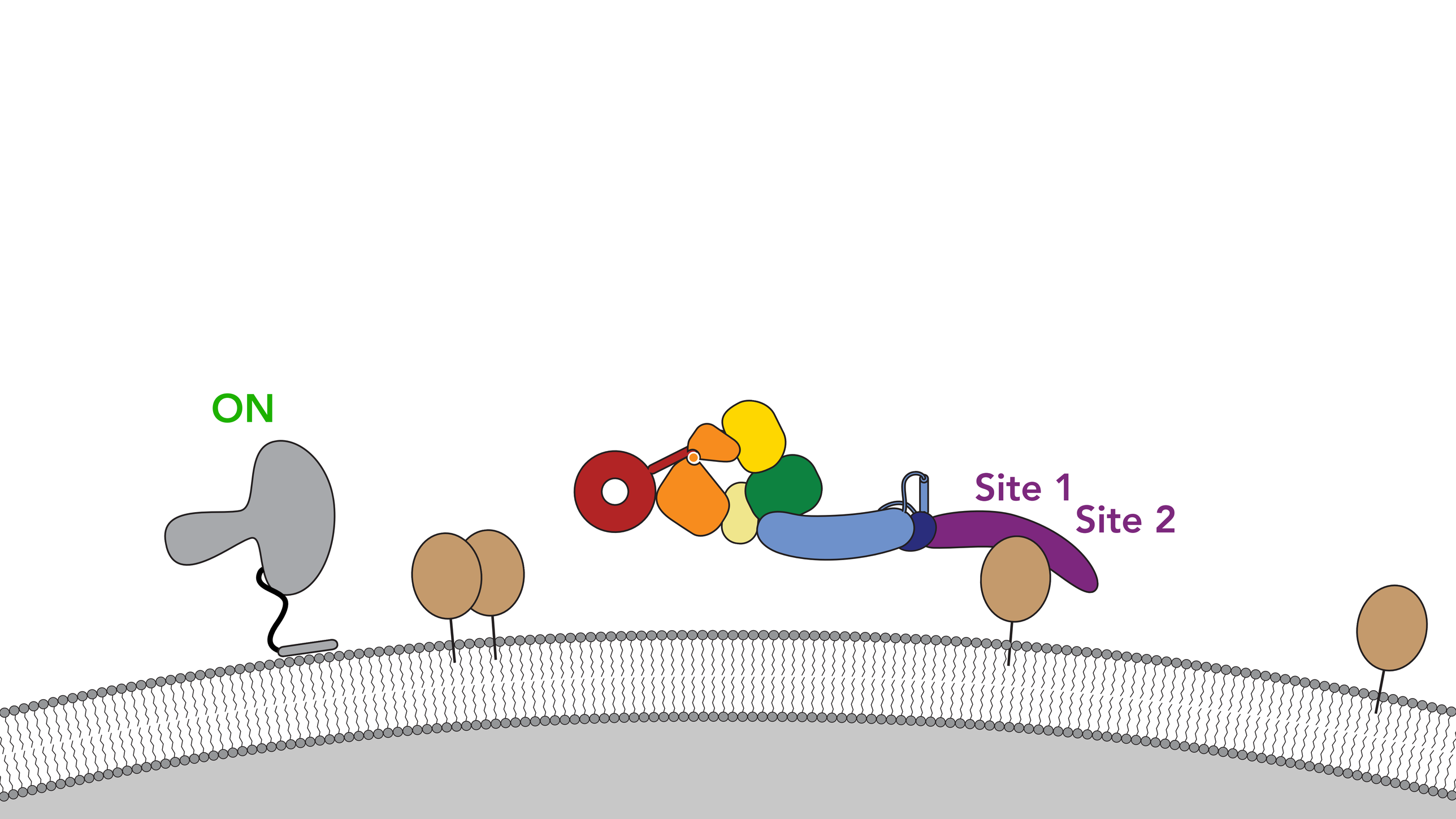

OFF
feed-forward
Site 1
Site 2

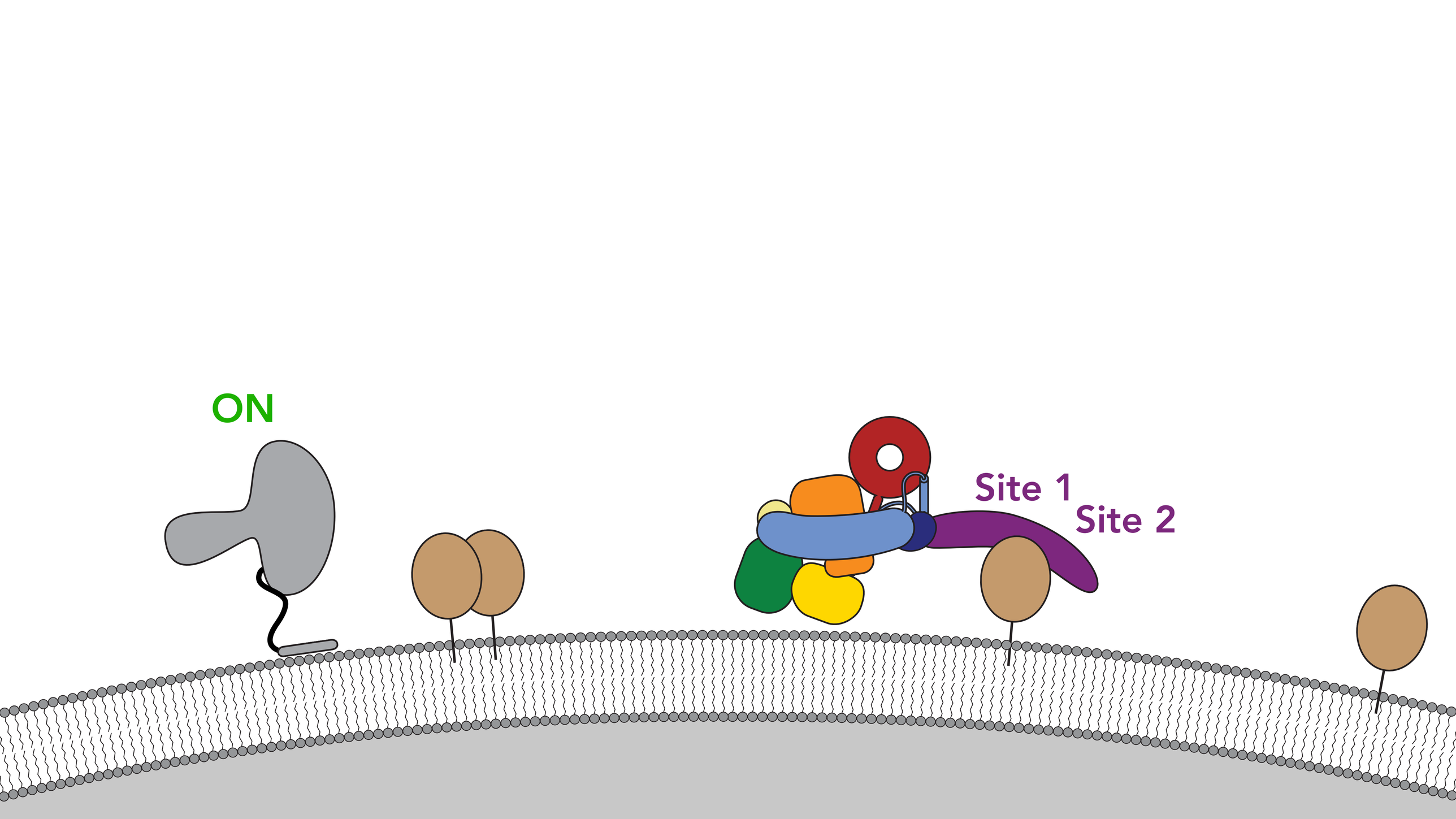

OFF
feed-forward
Site 1
Site 2

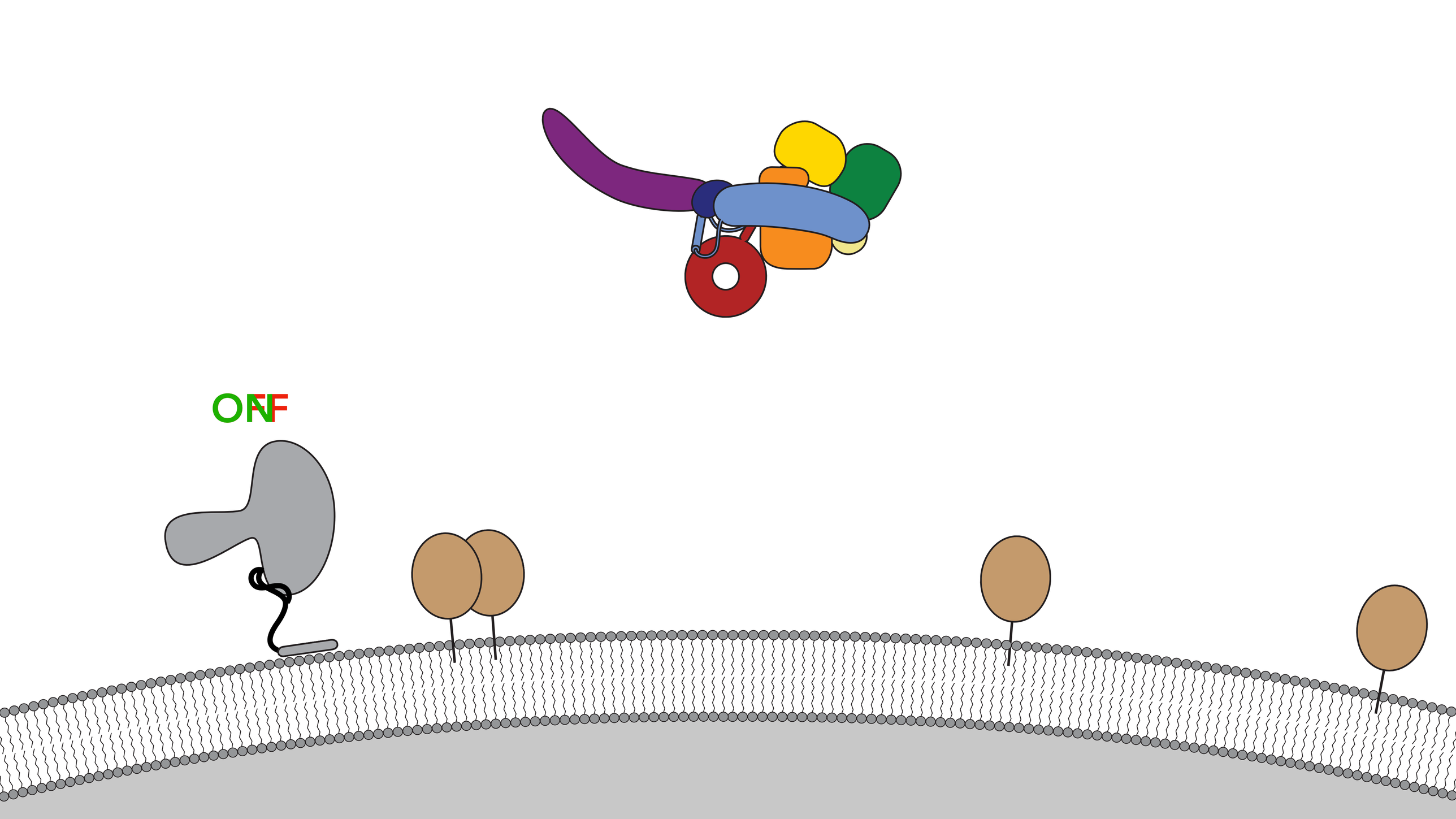

ON
OFF
feed-forward
Site 1
Site 2


ON
feed-forward
Site 1
Site 2

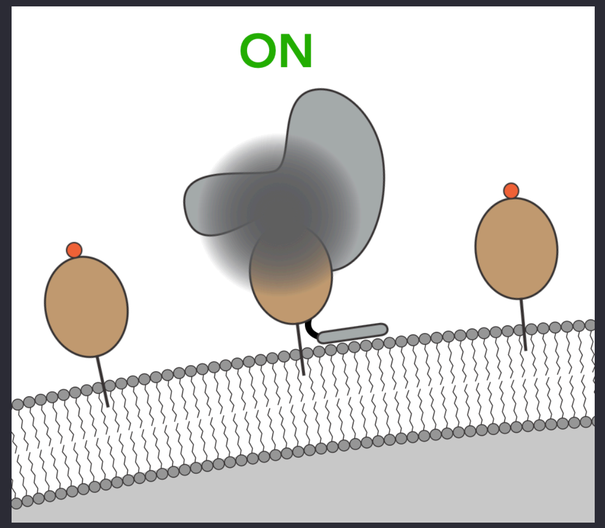

ON
feed-forward
Site 1
Site 2


ON
Site 1
Site 2


ON
Site 1
Site 2

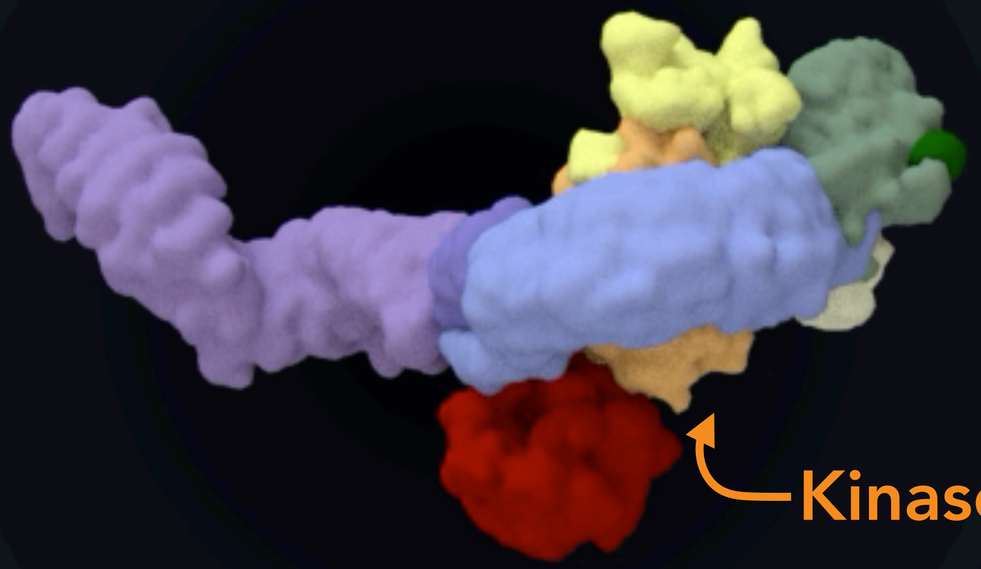

ON
OFF

Another element we need to add to the animation



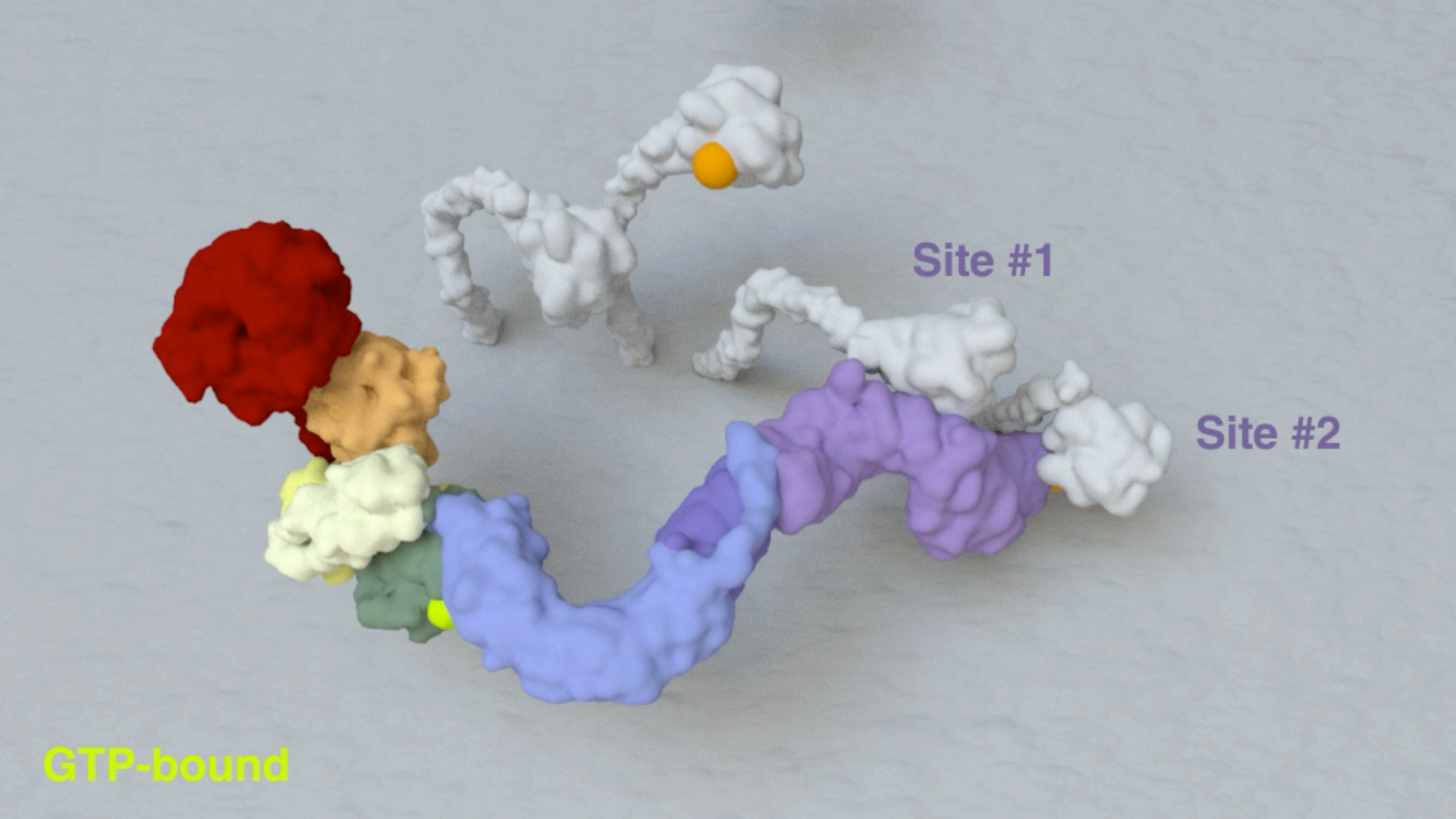
The role of PPM1H in resolving the pRab burst

LRRK2



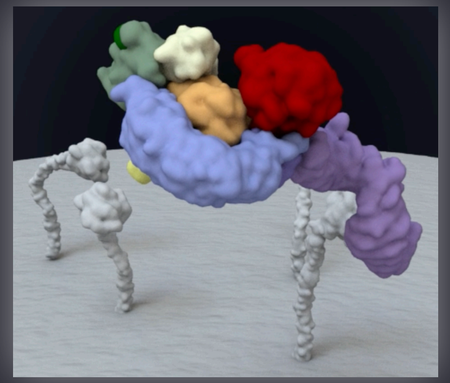
Animation by **Janet Iwasa**
w/Andres Leschziner & Samara Reck-Peterson

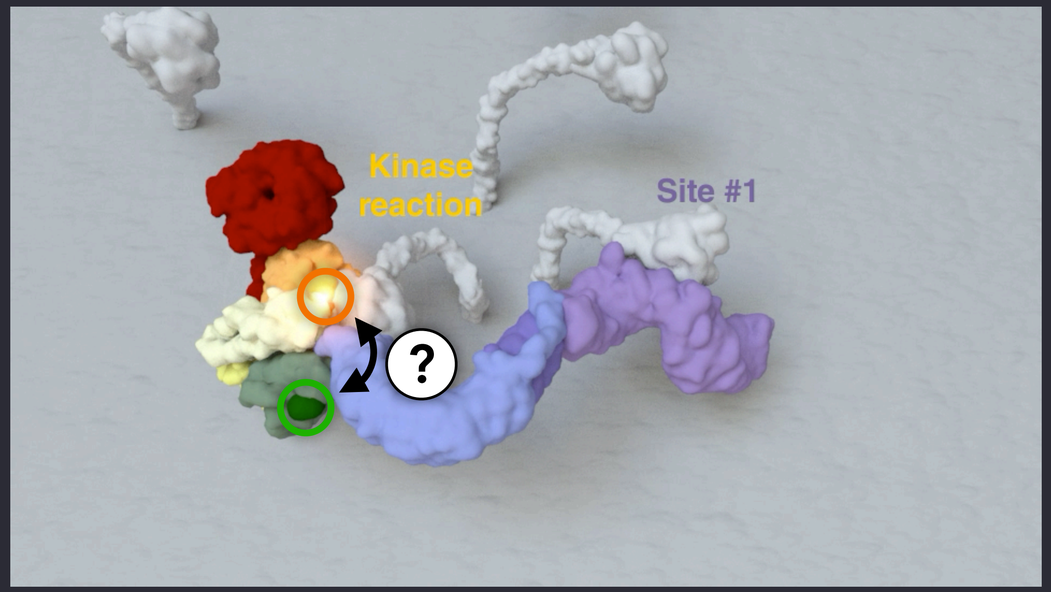
March 14, 2026

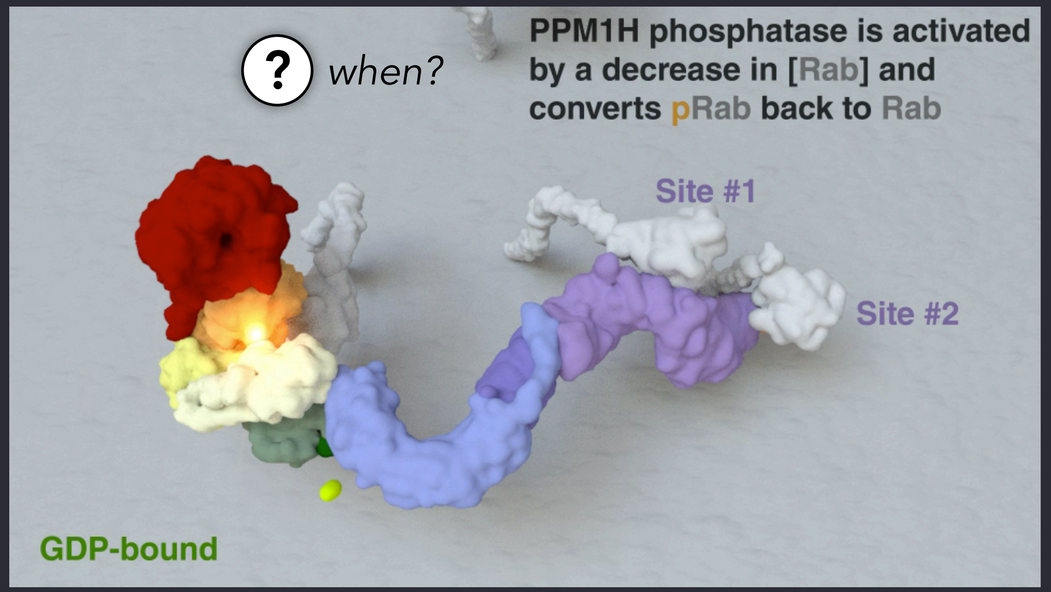

Site #1
Site #2
GTP-bound

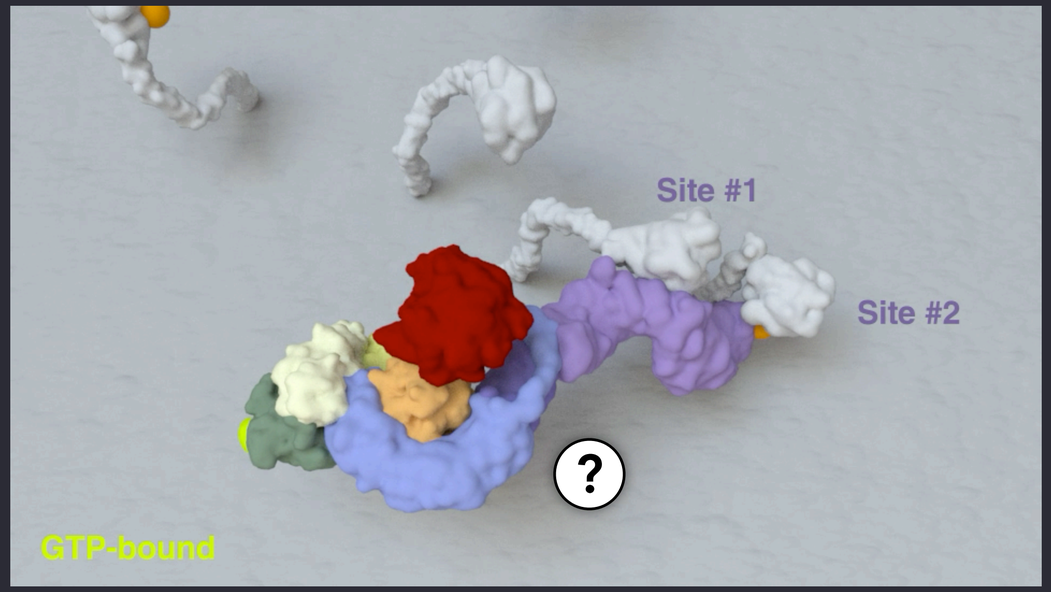
Applications of SPA to biological systems & data visualization

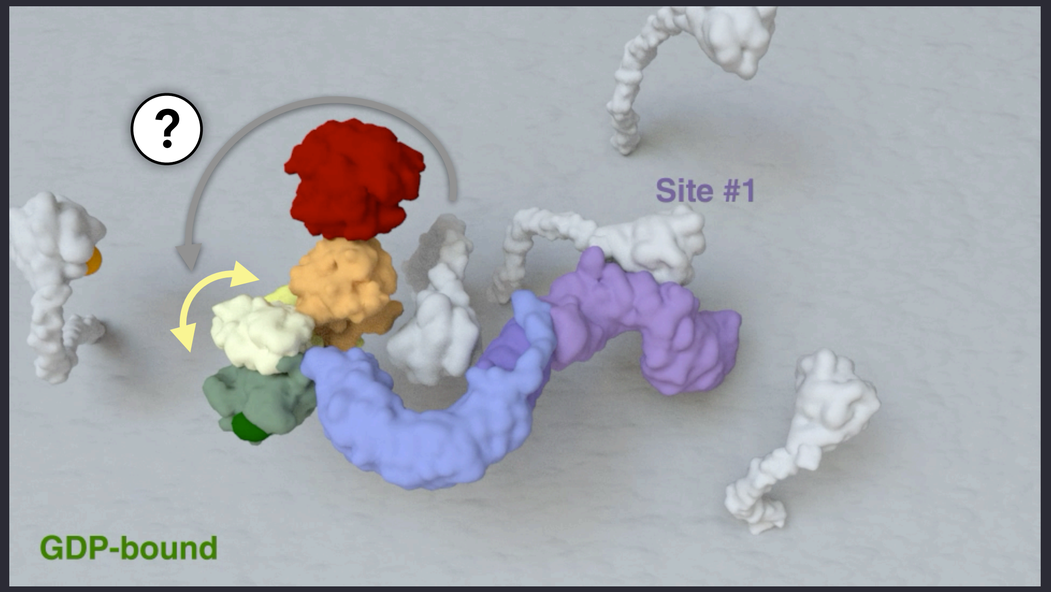
Data visualization = hypothesis generation



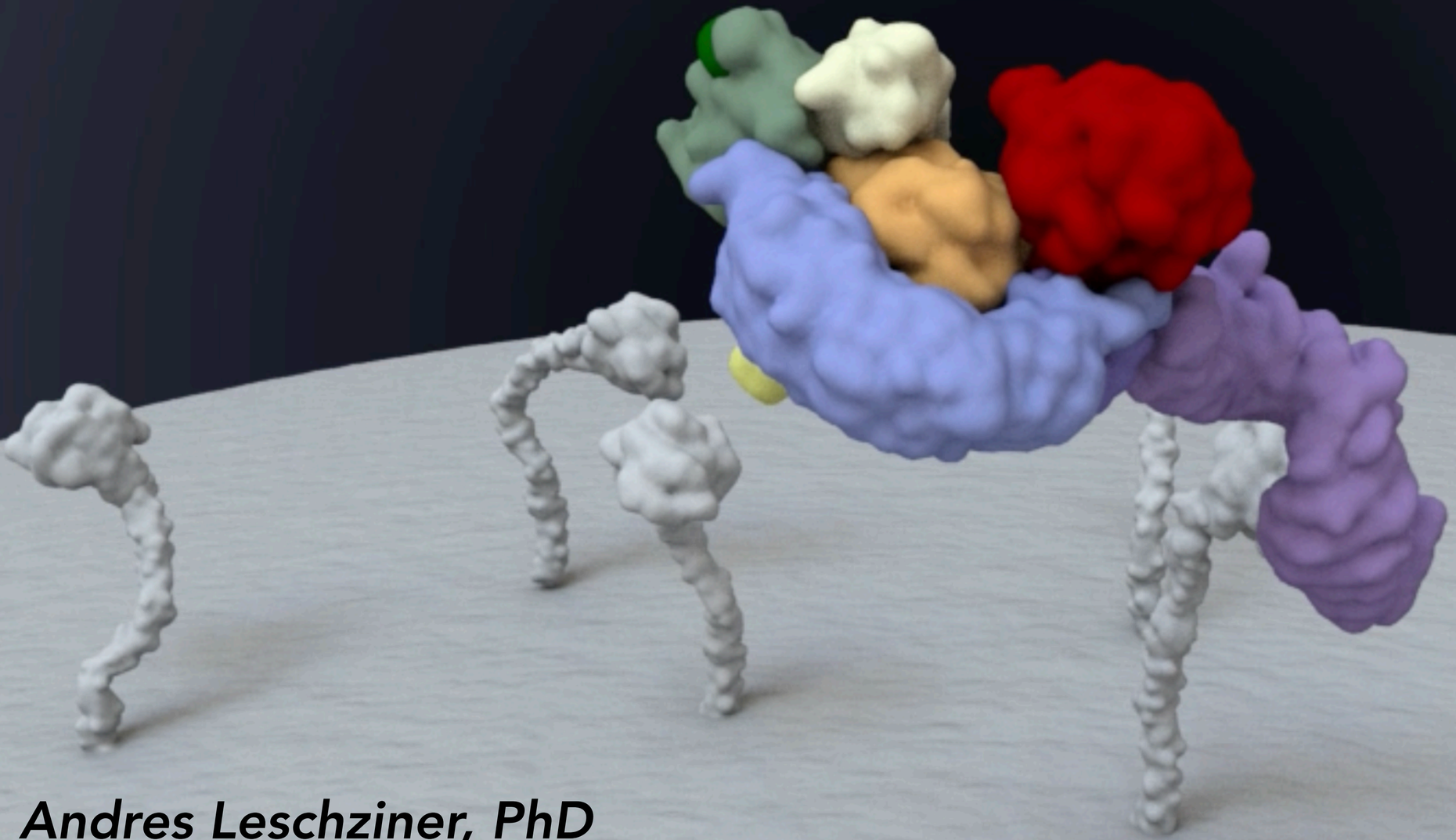

Kinase
reaction
Site #1
?


?
when?
PPM1H phosphatase is activated
by a decrease in [Rab] and
converts pRab back to Rab
Site #1
Site #2
GDP-bound


Site #1
Site #2
?
GTP-bound


?
Site #1
GDP-bound

Applications of SPA to biological systems & data visualization



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Department of Biochemistry & Biophysics

Weill Cornell Medicine