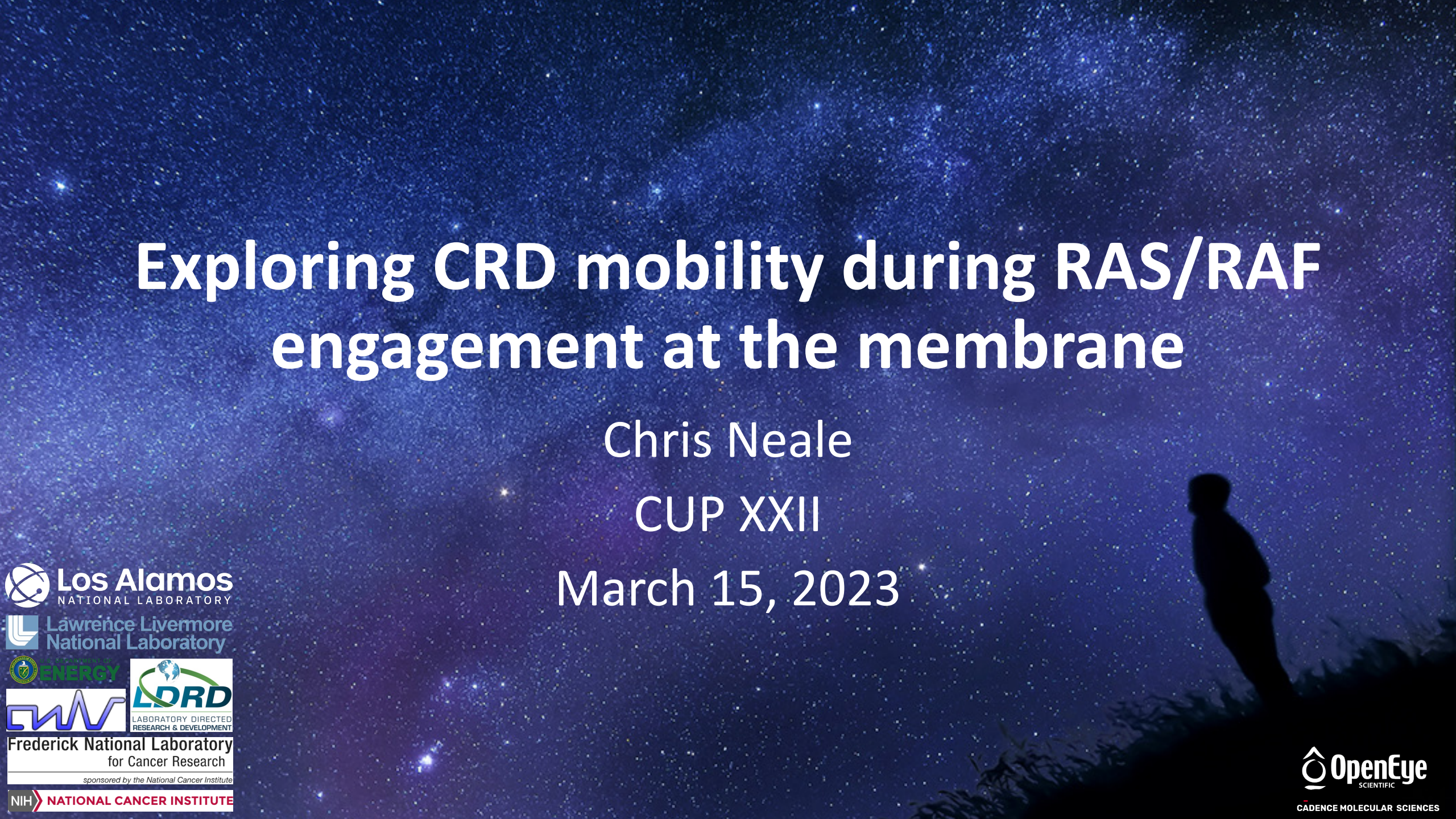
Exploring CRD mobility during RAS/RAF engagement at the membrane

Chris Neale

CUP XXII

March 15, 2023



Los Alamos NATIONAL LABORATORY
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Frederick National Laboratory for Cancer Research
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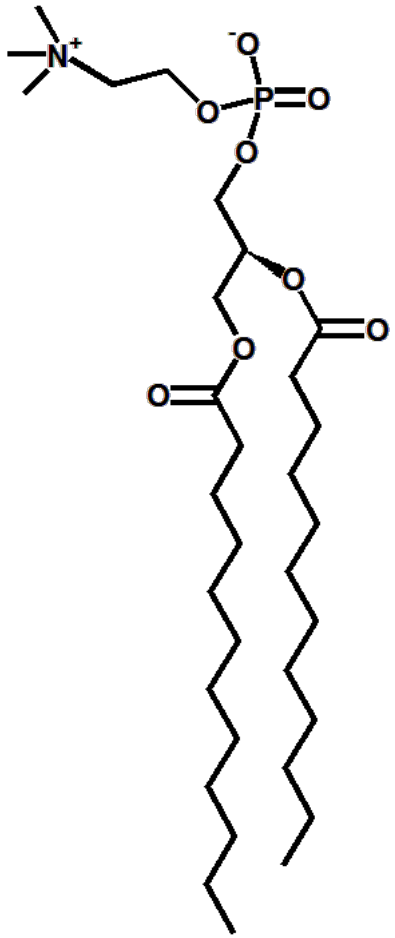
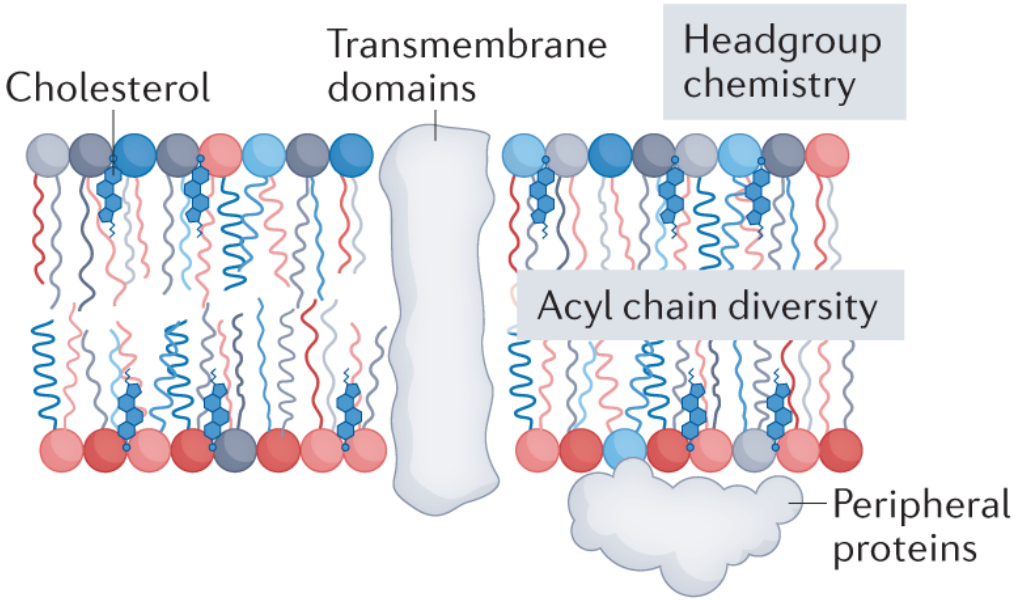
Embryonic stem cells divide quickly. Why don't all cells?

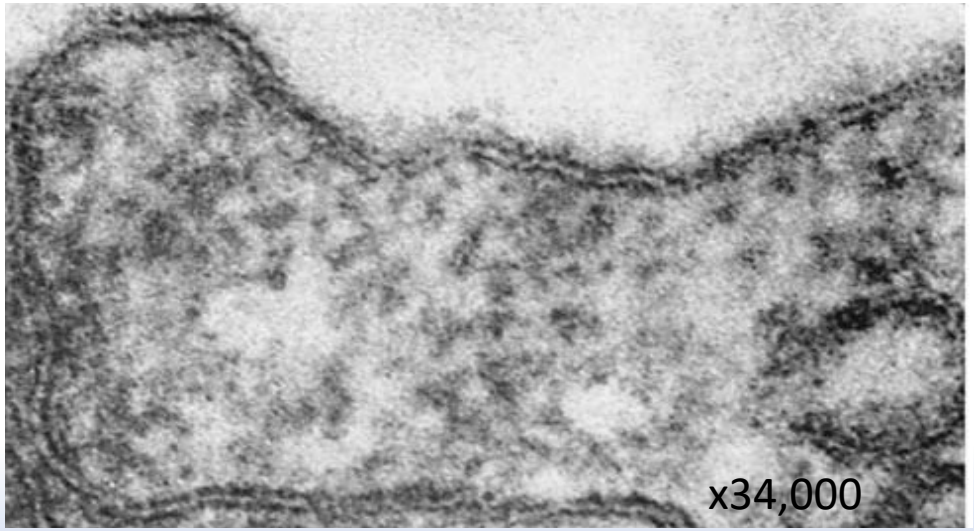
Mio and Maeda, *Am. J. Obstet. Gynecol.*, **2008**, 199, 660.e1-660.e5

Cell membranes



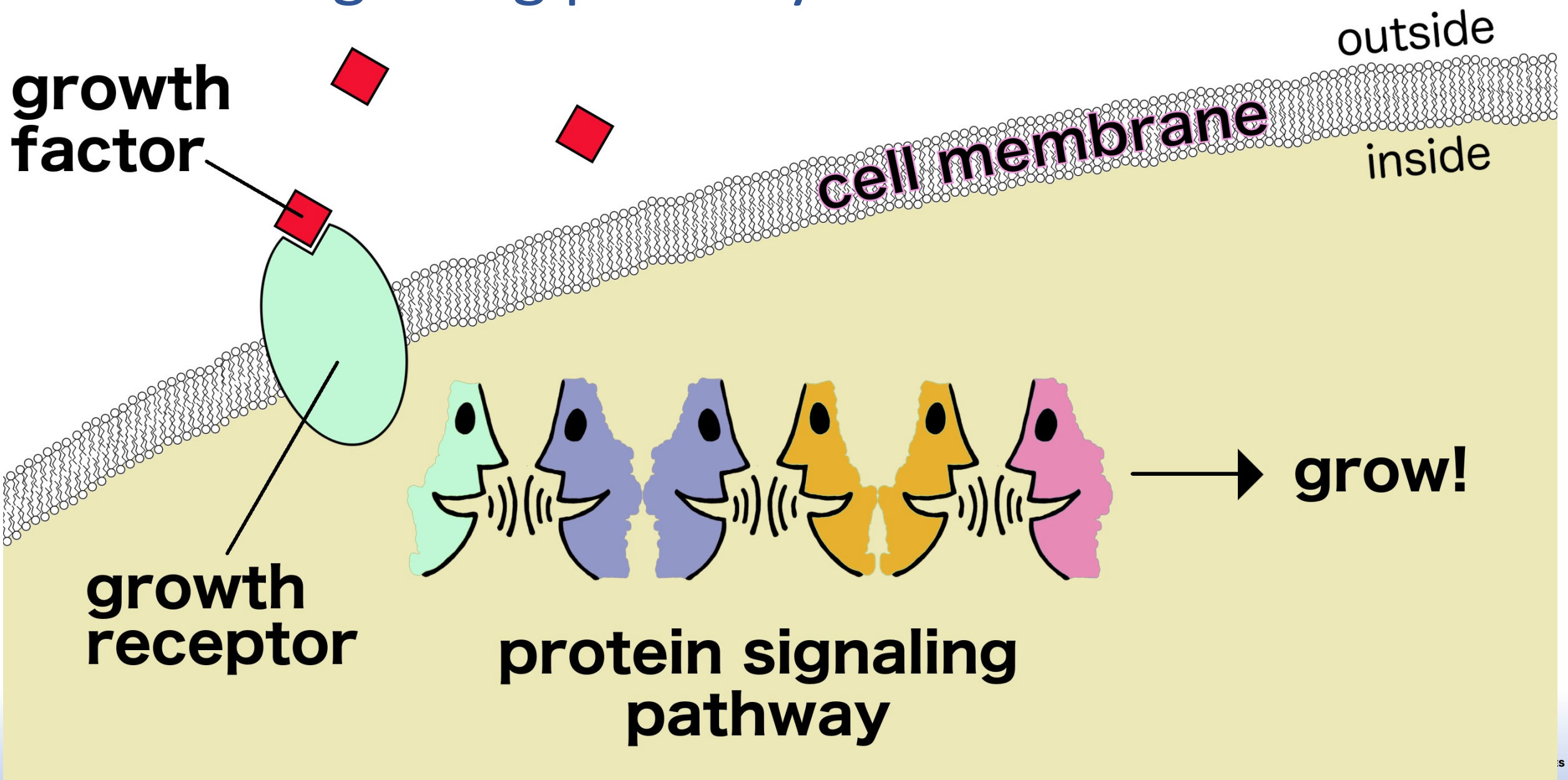


Levental and Lyman, **2023**, *Nat. Rev. Mol. Cell Biol.* 24:107-122

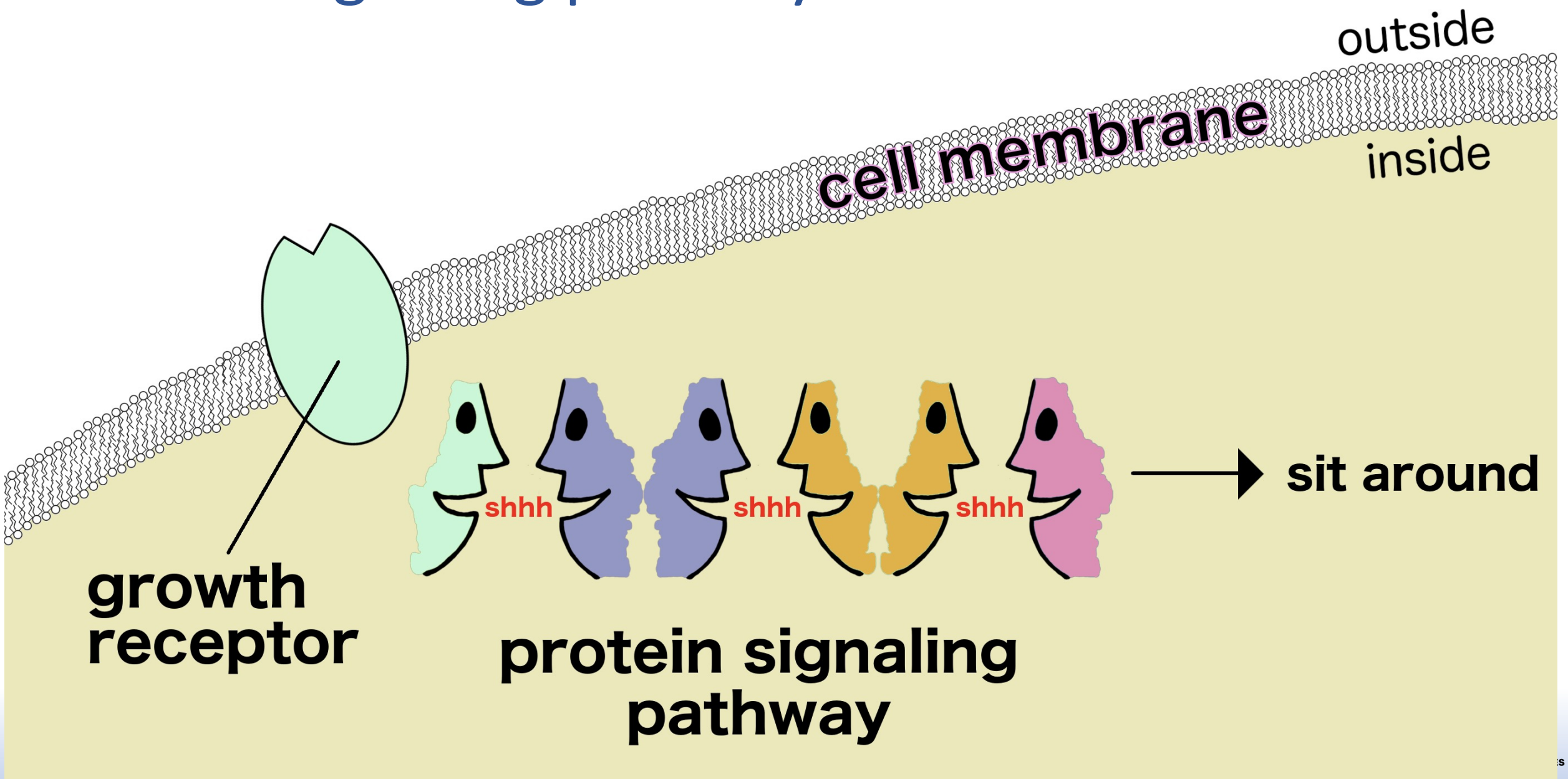


M.W. Brightman and T.S. Reese. **1969**, *J. Cell. Biol.*, 40(3):648-77

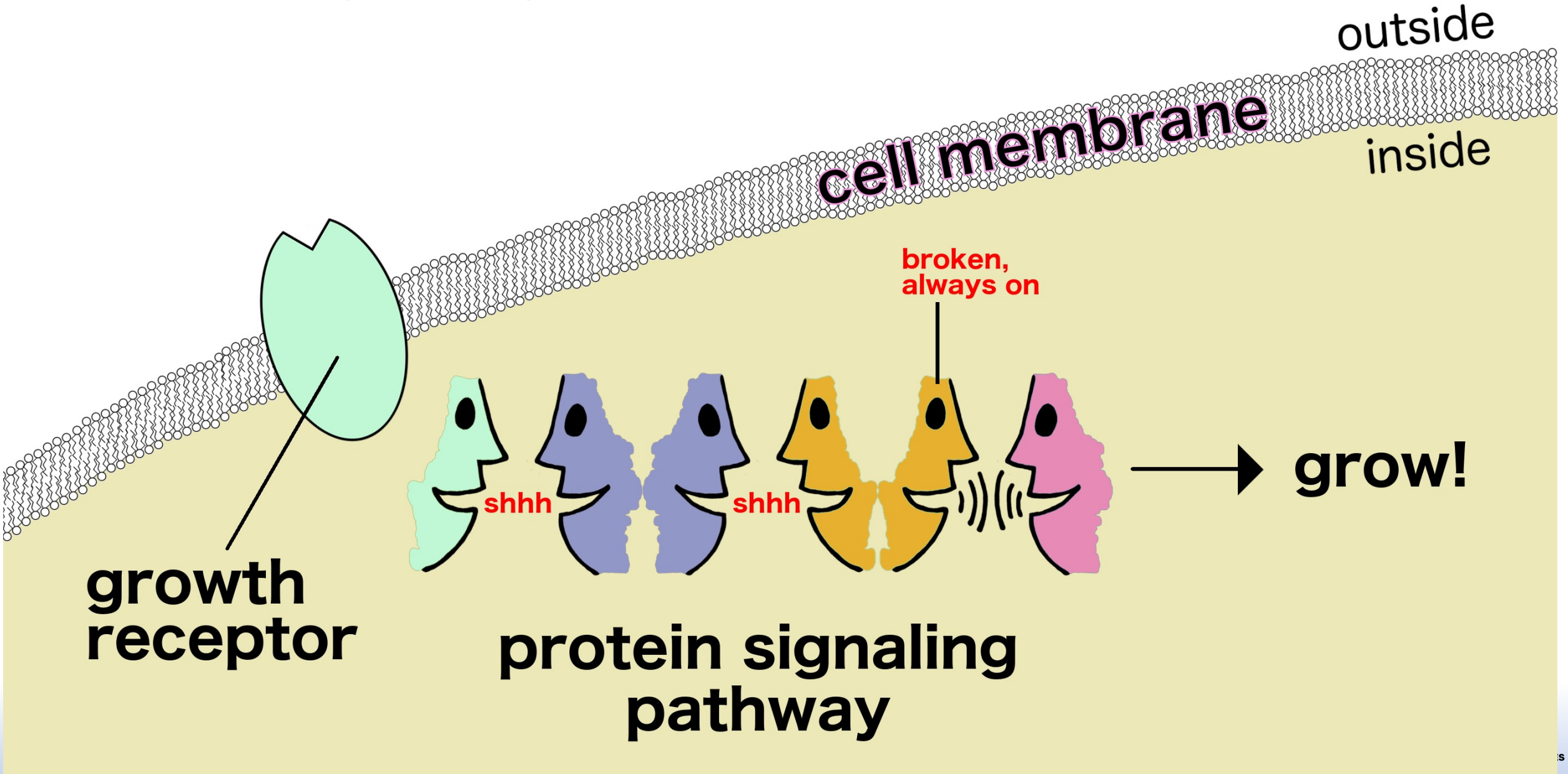
Cellular signaling pathways



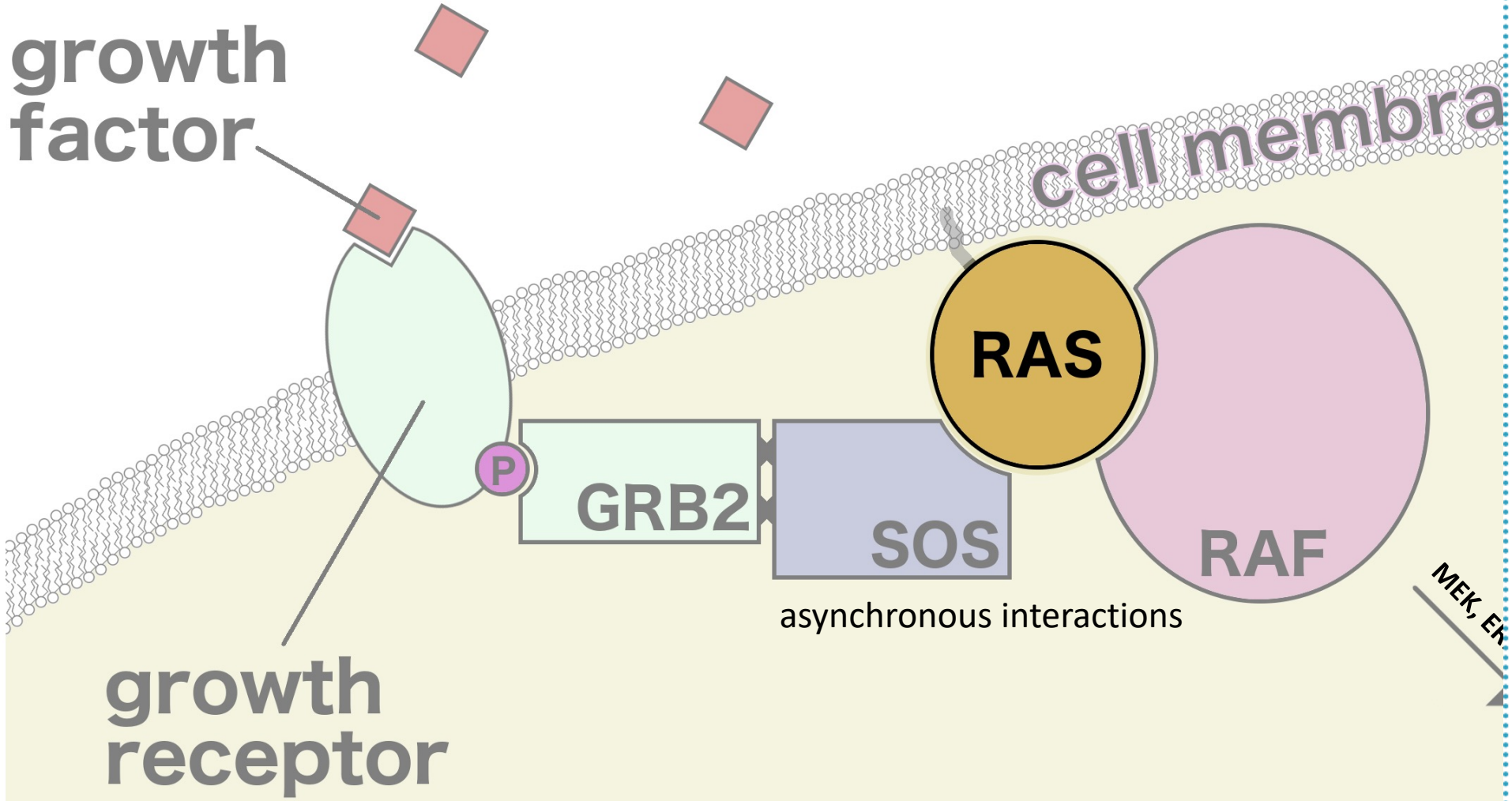
Cellular signaling pathways



Cellular signaling pathways



Ras is a signaling switch



MORE THAN
30%
OF ALL HUMAN CANCERS
ARE DRIVEN BY MUTATIONS OF

RAS GENES

RAS MUTATIONS
IN HUMAN CANCERS

PANCREAS – KRAS	95%
COLORECTAL – KRAS	45%
LUNG – KRAS	35%
AML – NRAS	15%
MELANOMA – NRAS	15%
BLADDER CANCER – HRAS	10%

"RAS ONCOGENES ARE THE **WORST** ONCOGENES."
– Dr. Frank McCormick,
RAS National Program Advisor

Ras is a challenging target

Nras in melanoma: Targeting the undruggable target

Mario Mandalà[a,*], Barbara Merelli[a], Daniela Massi[b]

CANCER

Drug for an 'undruggable' protein

Scientists have long aimed to develop drugs against the cancer-associated protein KRAS, but without success. An approach that targets the oncoprotein's cellular localization reignites lost enthusiasm. SEE LETTER P.638

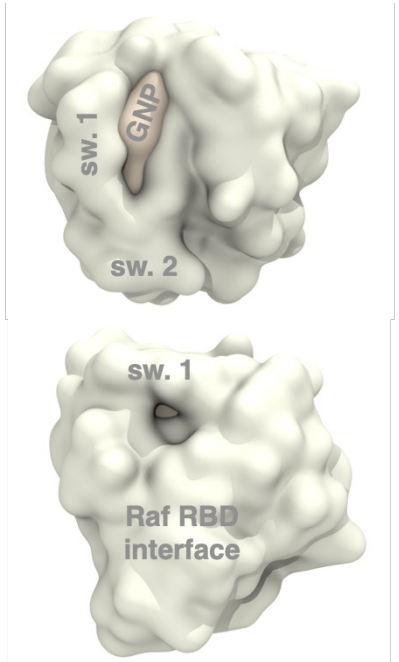
RAS-targeted therapies: is the undruggable drugged?

Amanda R. Moore[1], Scott C. Rosenberg[1], Frank McCormick[2] and Shiva Malek[1]

Drugging the undruggable RAS: Mission Possible?

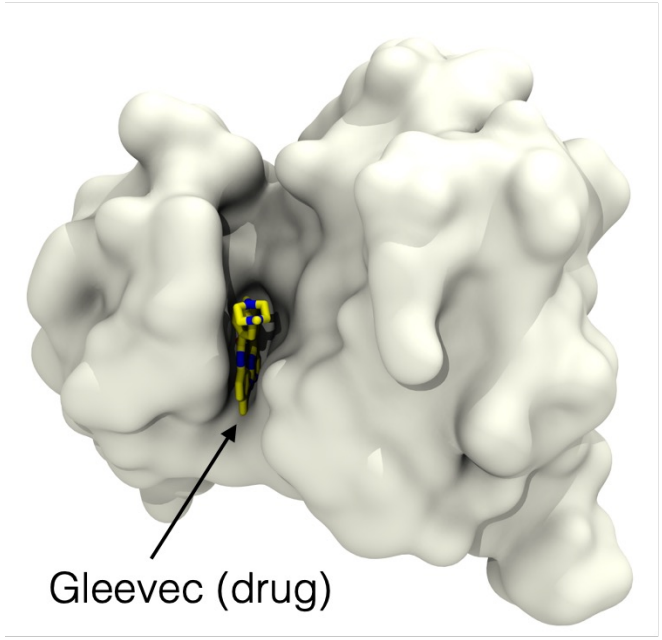
Adrienne D. Cox[1], Stephen W. Fesik[2], Alec C. Kimmelman[3], Ji Luo[4] and Channing J. Der[1]

K-Ras G domain



Welsch *et al.*, *Cell*, **2017**, 168:878-889

Syk kinase domain



Atwell *et al.*, *J. Biol. Chem.*, **2004**, 279:55827-32

Drugging an undruggable pocket on KRAS

Dirk Kessler[a,1], Michael Gmachl[a,1], Andreas Mantoulidis[a,1], Laetitia J. Martin[a,1], Andreas Zoephel[a], Moriz Mayer[a], Andreas Gollner[a], David Covini[a], Silke Fischer[a], Thomas Gerstberger[a], Teresa Gmaschitz[a], Craig Goodwin[b], Peter Greb[a], Daniela Häring[a], Wolfgang Hela[a], Johann Hoffmann[a], Jale Karolyi-Oezguer[a], Petr Knesl[a], Stefan Kornigg[a], Manfred Koegl[a], Roland Kousek[a], Lyne Lamarre[a], Franziska Moser[c], Silvia Munico-Martinez[a], Christoph Peinsipp[a], Jason Phan[b], Jörg Rinnenthal[a], Jiqing Sai[b], Christian Salamon[a], Yvonne Scherbantin[a], Katharina Schipany[a], Renate Schnitzer[a], Andreas Schrenk[a], Bernadette Sharps[a], Gabriella Siszler[a], Qi Sun[b], Alex Waterson[d,e], Bernhard Wolkerstorfer[a], Markus Zeeb[c], Mark Pearson[a], Stephen W. Fesik[b,d,e], and Darryl B. McConnell[a,2]

[a]Discovery Research, Boehringer Ingelheim Regional Center Vienna GmbH & Co KG, 1120 Vienna, Austria; [b]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37235; [c]Discovery Research, Boehringer Ingelheim Pharma GmbH & Co KG, D-88397 Biberach an der Riss, Germany; [d]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37235; and [e]Department of Chemistry, Vanderbilt University, Nashville, TN 37235

Ras is druggable

Sotorasib (AMG510) FDA approved May 2021

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use LUMAKRAS safely and effectively. See full prescribing information for LUMAKRAS.

LUMAKRAS™ (sotorasib) tablets, for oral use
Initial U.S. Approval: 2021

----------INDICATIONS AND USAGE----------
LUMAKRAS is an inhibitor of the RAS GTPase family indicated for the treatment of adult patients with *KRAS G12C*-mutated locally advanced or metastatic non-small cell lung cancer (NSCLC), as determined by an FDA-approved test, who have received at least one prior systemic therapy. (1)
This indication is approved under accelerated approval based on overall response rate (ORR) and duration of response (DOR). Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial(s). (1)

----------DOSAGE AND ADMINISTRATION----------
- Recommended dosage: 960 mg orally once daily. (2.2)
- Swallow tablets whole with or without food. (2.2)

Adagrasib (MRTX849) FDA approved Dec 2022

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use KRAZATI safely and effectively. See full prescribing information for KRAZATI.

KRAZATI™ (adagrasib) tablets, for oral use
Initial U.S. Approval: 2022

INDICATIONS AND USAGE
KRAZATI is an inhibitor of the RAS GTPase family indicated for the treatment of adult patients with *KRAS* G12C-mutated locally advanced or metastatic non-small cell lung cancer (NSCLC), as determined by an FDA approved test, who have received at least one prior systemic therapy. (1)

This indication is approved under accelerated approval based on objective response rate (ORR) and duration of response (DOR). Continued approval for this indication may be contingent upon verification and description of a clinical benefit in a confirmatory trial(s). (1)

DOSAGE AND ADMINISTRATION
- Recommended dosage: 600 mg orally twice daily. (2.2)
- Swallow tablets whole with or without food. (2.2)

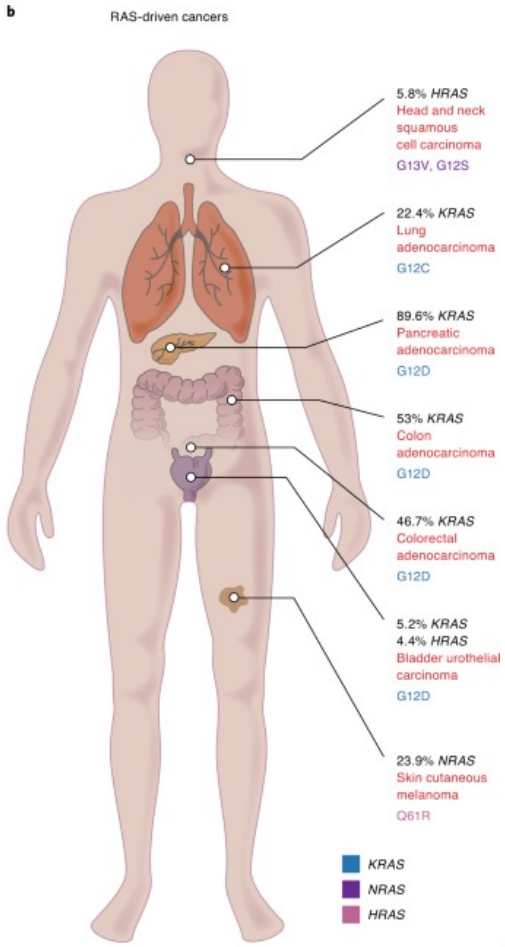
Many Ras driver mutants remain undrugged

prevalence of mutated Ras

a

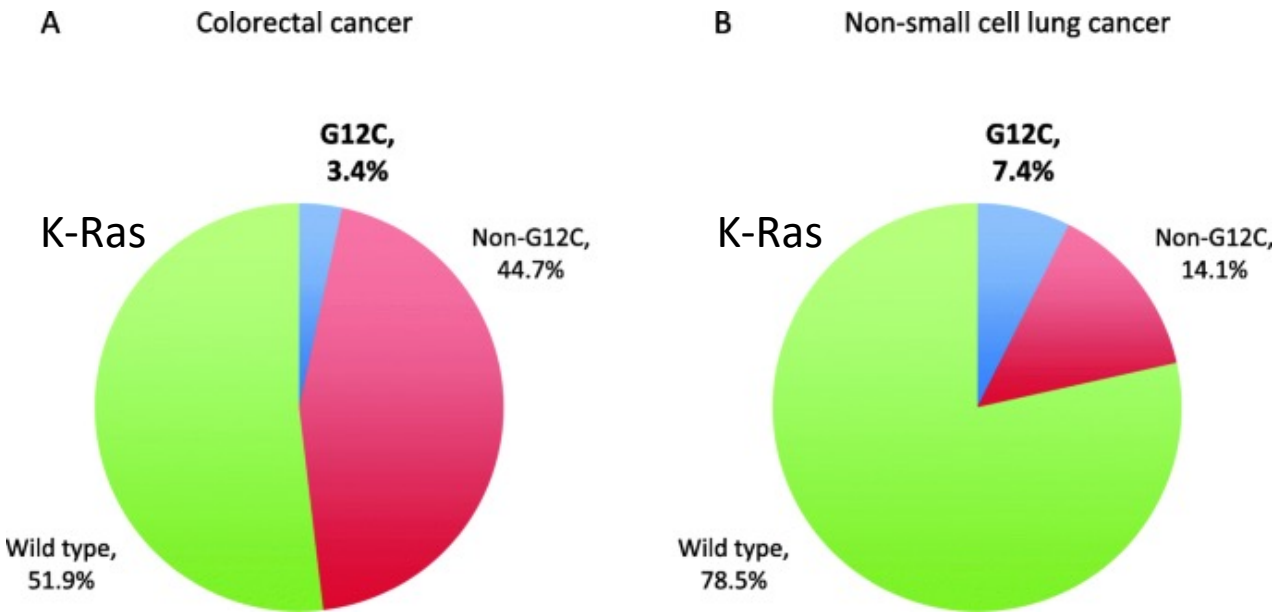
Cancer tissue	Frequency of mutation (%)		Death rate per year
Biliary tract	23.3	*KRAS*: 20	6.6
		NRAS: 1.9	
		HRAS: 1.4	
Bowel	51.5	*KRAS*: 49.7	13.7
		NRAS: 1.6	
		HRAS: 0.2	
Cervix	17.1	*KRAS*: 13.8	2.2
		NRAS: 0.9	
		HRAS: 2.4	
Lung	20.9	*KRAS*: 20.1	38.5
		NRAS: 0.6	
		HRAS: 0.2	
Myeloid	21.4	*KRAS*: 5.5	3.2
		NRAS: 11.1	
		HRAS: 4.8	
Ovary/ Fallopian tube	15	*KRAS*: 13.9	6.7
		NRAS: 1	
		HRAS: 0.1	
Pancreas	79.2	*KRAS*: 78.7	11
		NRAS: 0.4	
		HRAS: 0.1	
Skin	18.9	*KRAS*: 1.3	2.3
		NRAS: 14.2	
		HRAS: 3.4	
Testis	19.5	*KRAS*: 14.4	0.3
		NRAS: 4.4	
		HRAS: 0.7	
Uterus	19.4	*KRAS*: 16.7	4.9
		NRAS: 2	
		HRAS: 0.7	
Vulva/vagina	16.4	*KRAS*: 8.6	0.6
		NRAS: 2.7	
		HRAS: 5.1	

b



Mukhopadhyay, Heiden, McCormick, **2021**, *Nature Cancer*, 2:271–283

prevalence of K-Ras G12C



Araujo *et al.*, **2021**, *BMC Cancer*, 21:193

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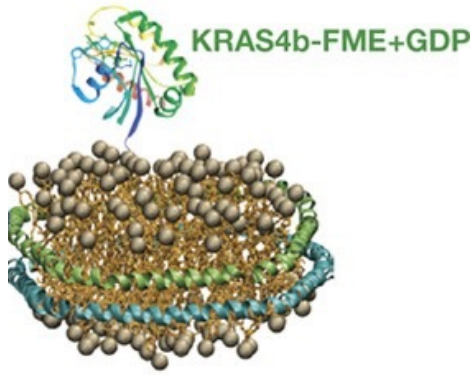
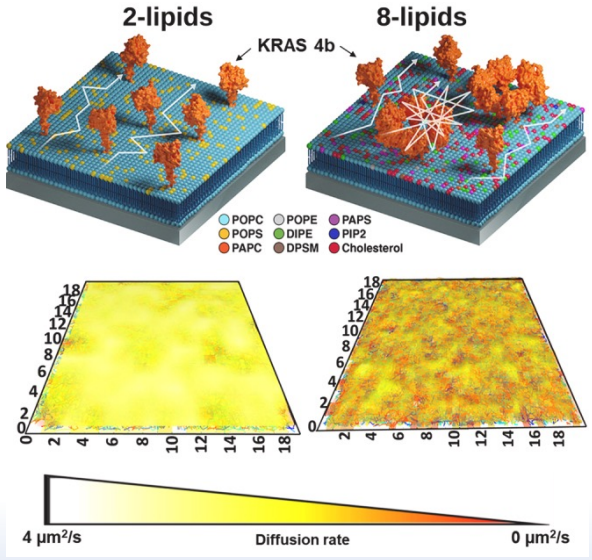
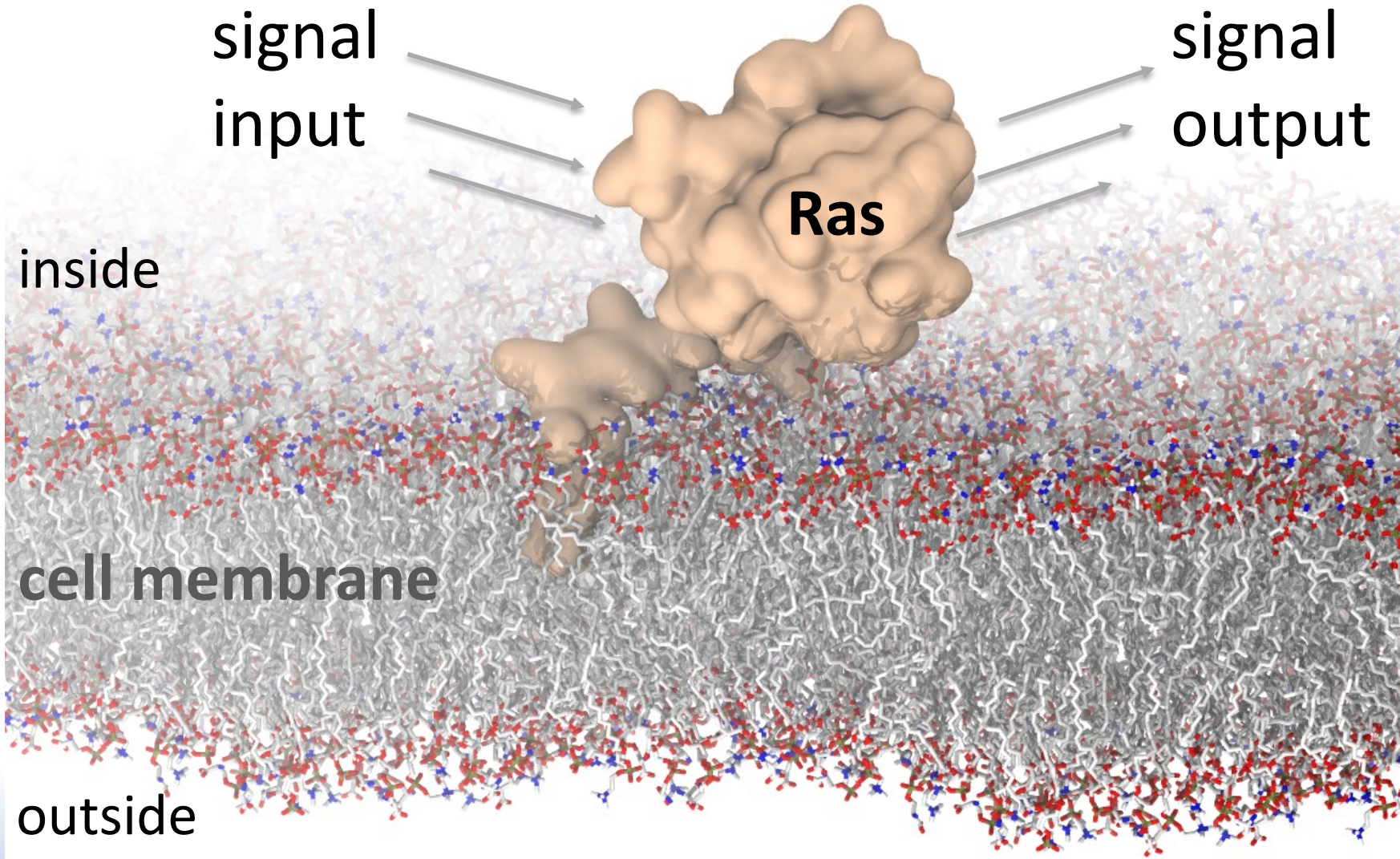
Can we target Ras-membrane systems?

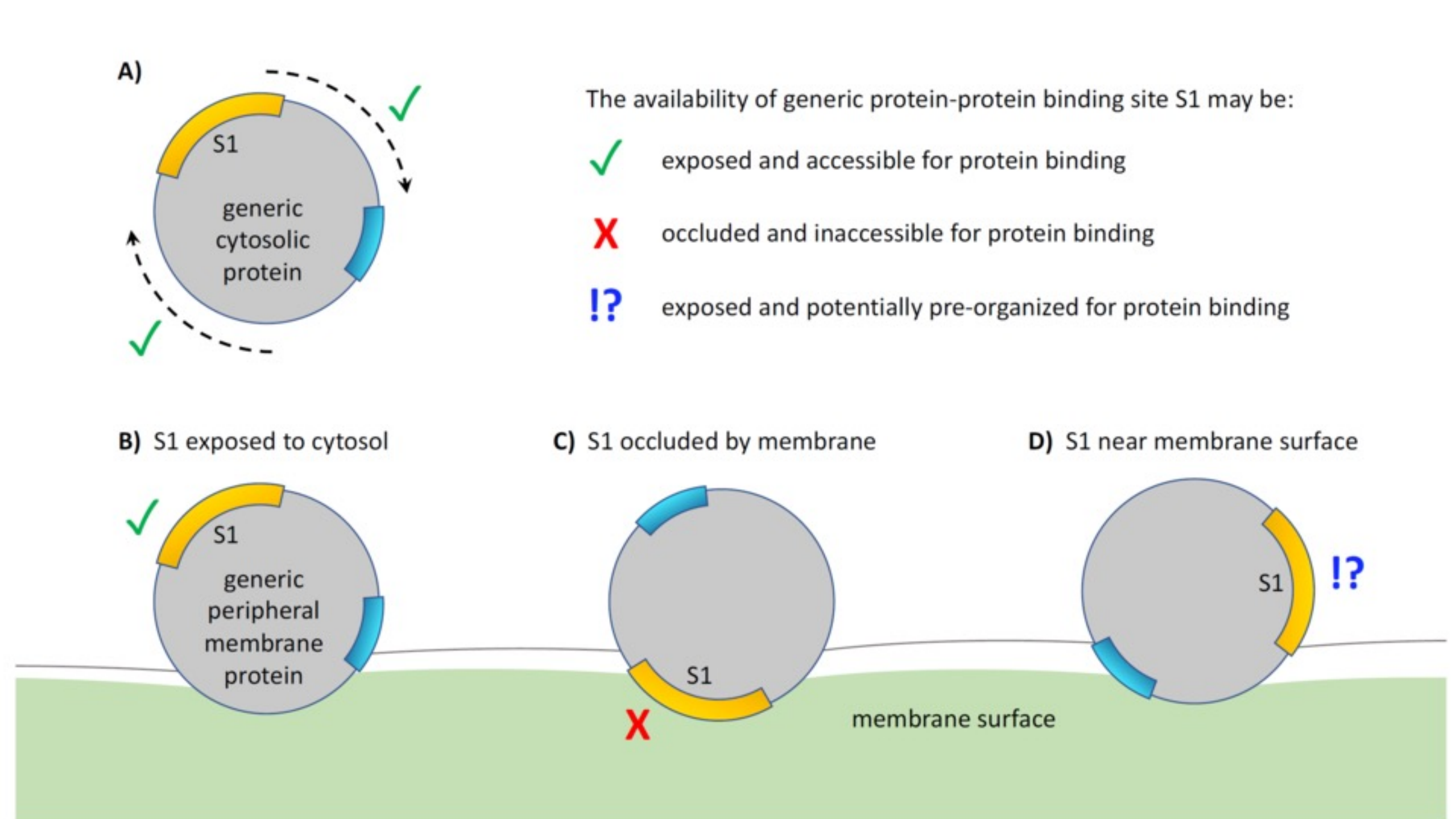


Gillette *et al.*, **2015**, *Sci. Rep.*, 5:15916

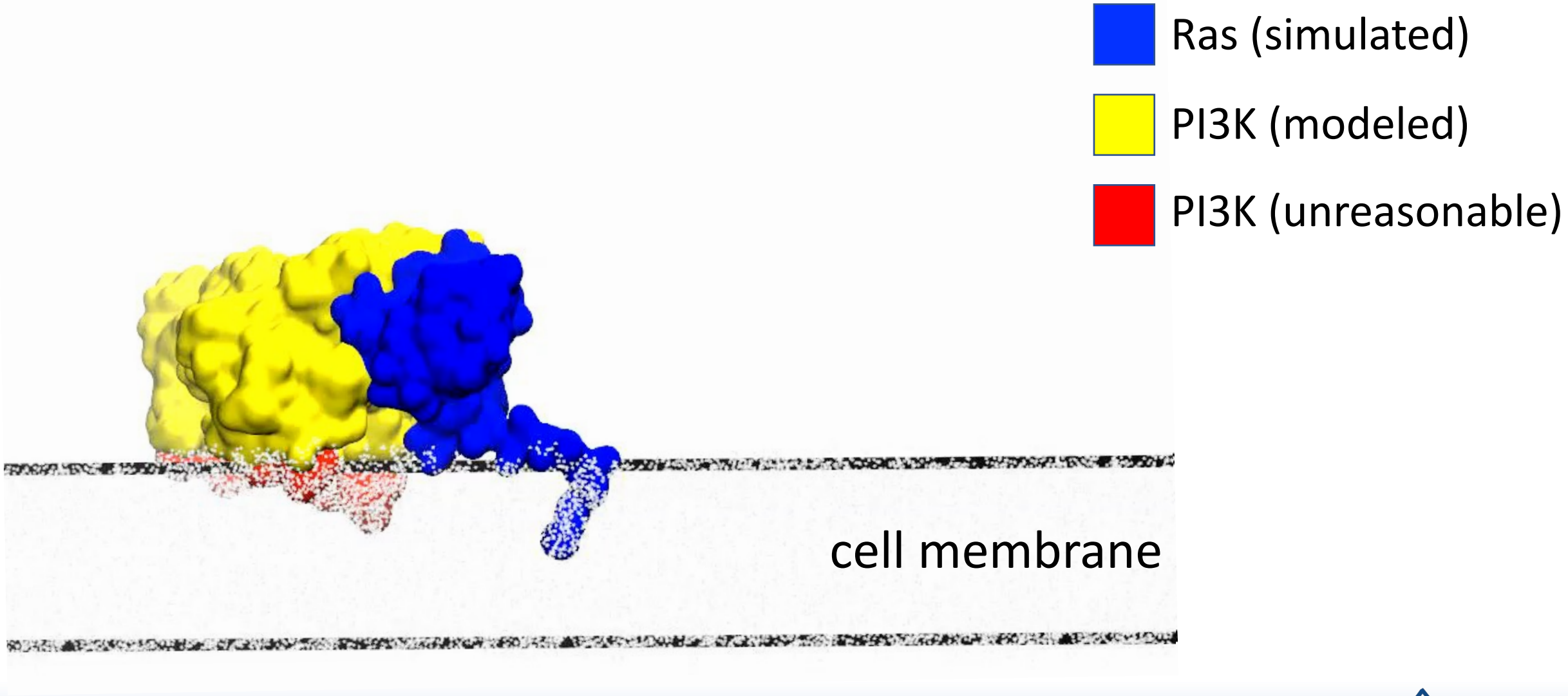


Shrestha *et al.*, **2021**, *iScience*, 25(1):103608



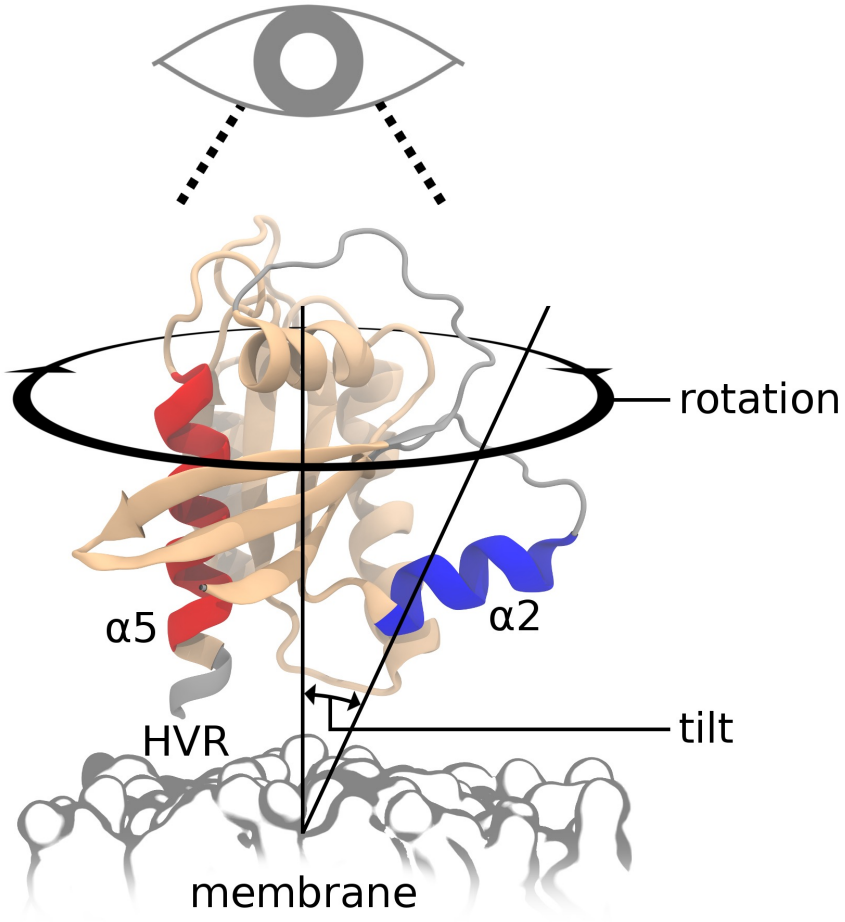

A)
S1
generic
cytosolic
protein
The availability of generic protein-protein binding site S1 may be:
exposed and accessible for protein binding
occluded and inaccessible for protein binding
!?
exposed and potentially pre-organized for protein binding
B) S1 exposed to cytosol
S1
generic
peripheral
membrane
protein
C) S1 occluded by membrane
S1
D) S1 near membrane surface
S1
!?
membrane surface

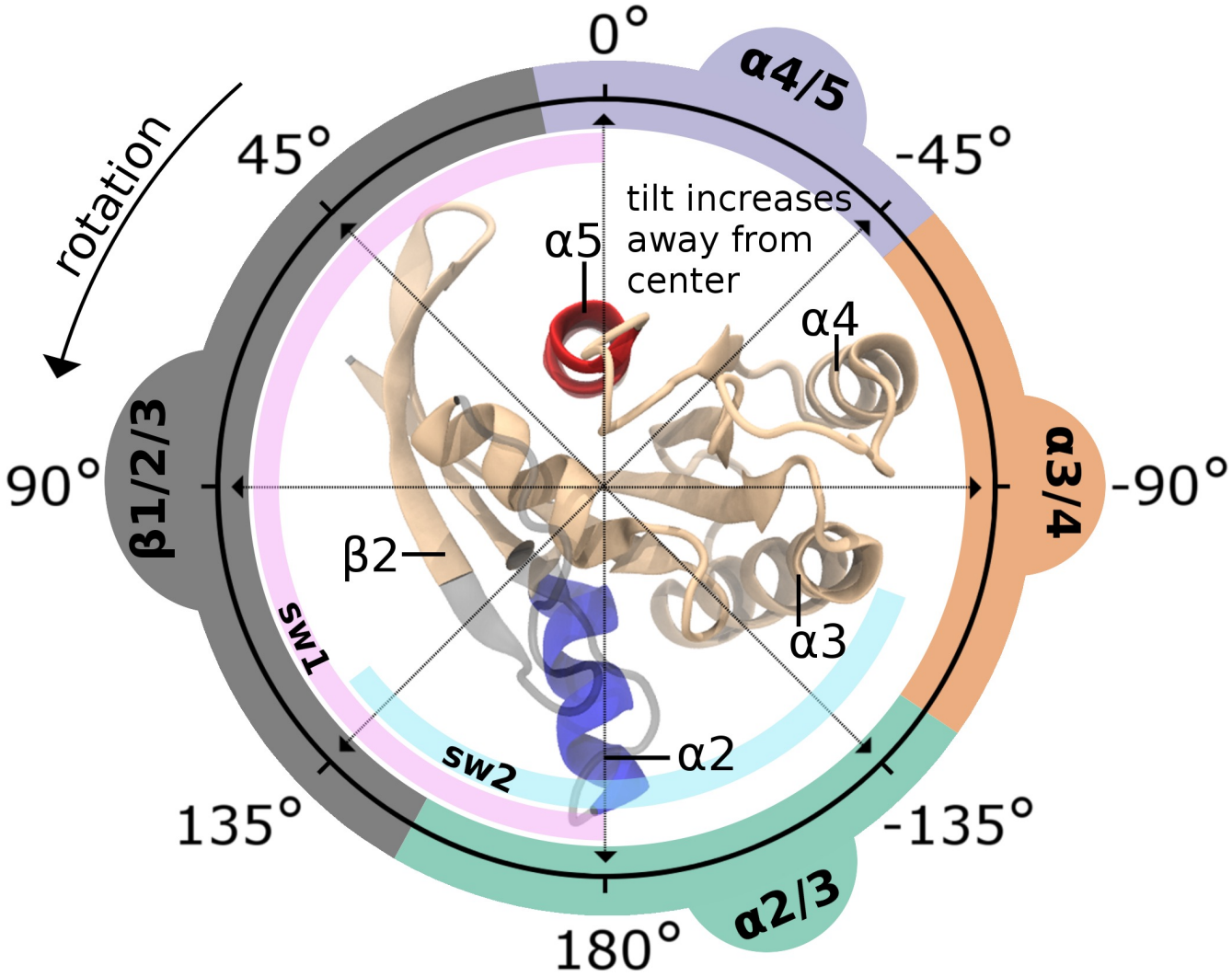
Ras orientation regulates binding site accessibility



Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

Ras orientation is regulated by lipids





Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

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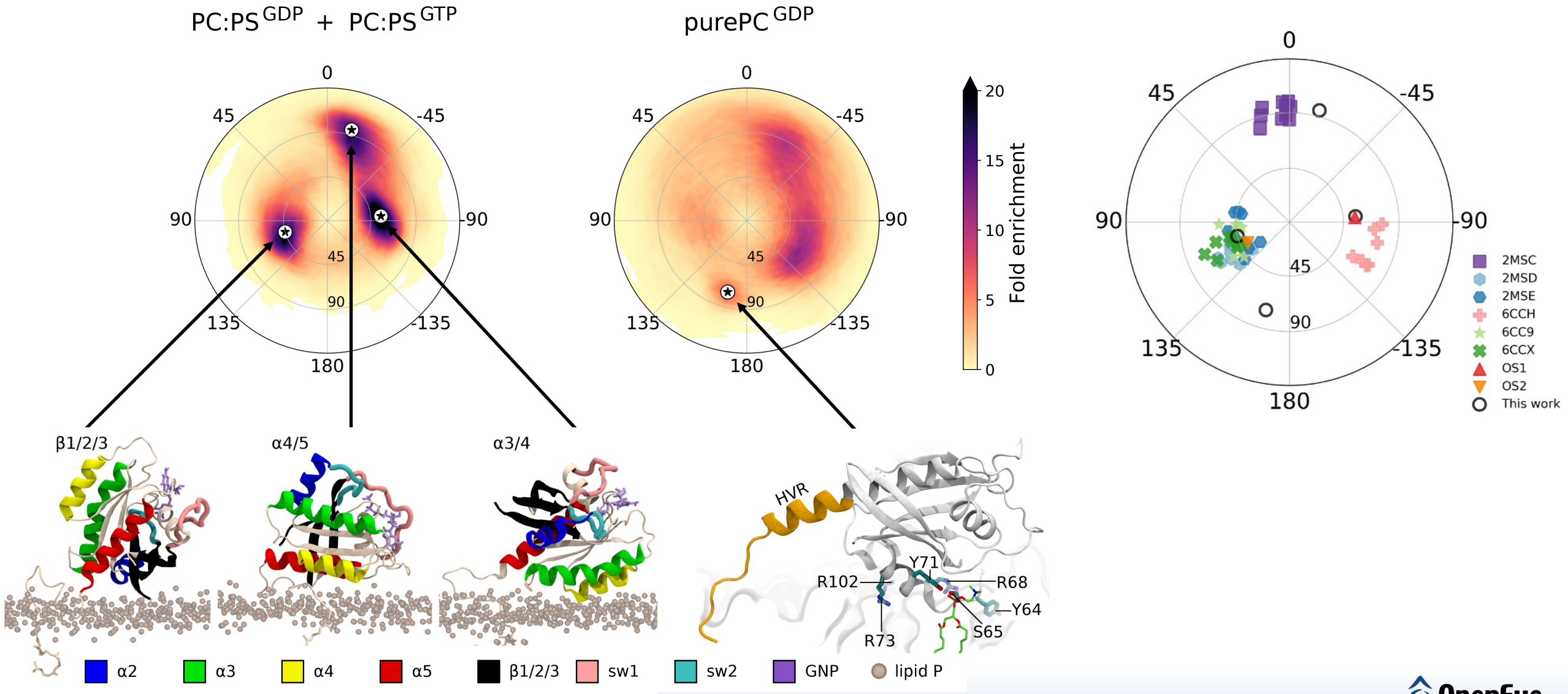
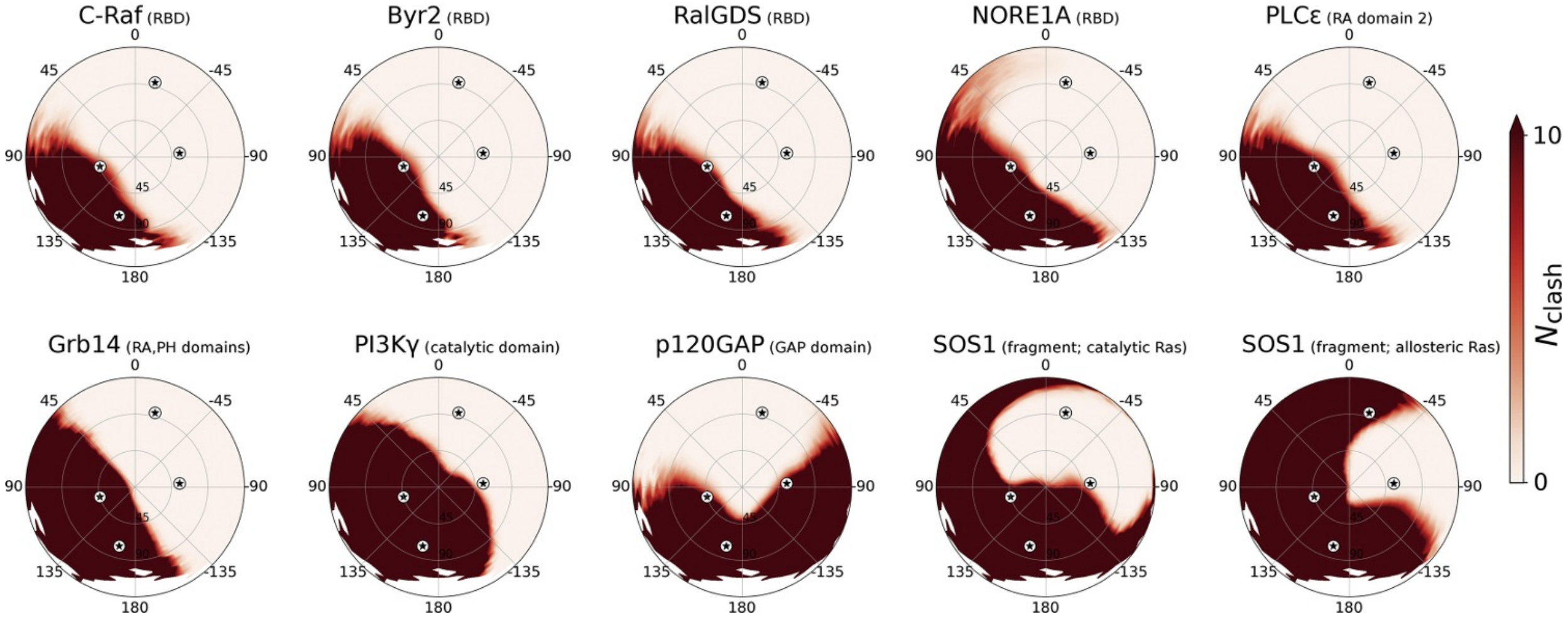
Ras orientation is regulated by lipids



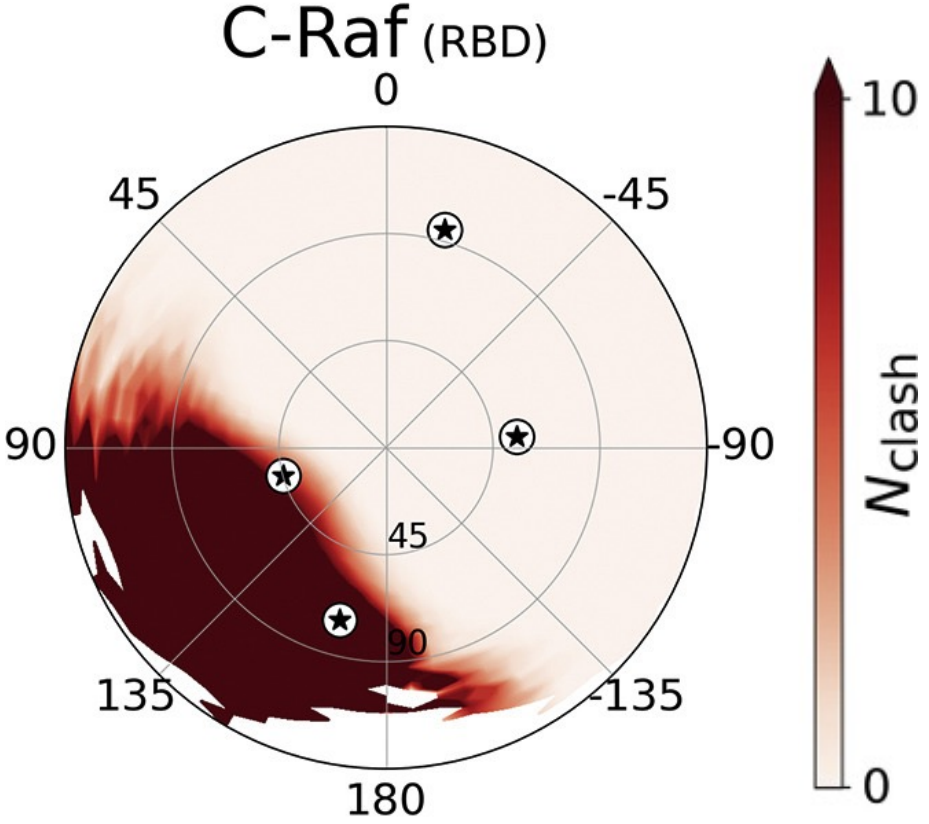
Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

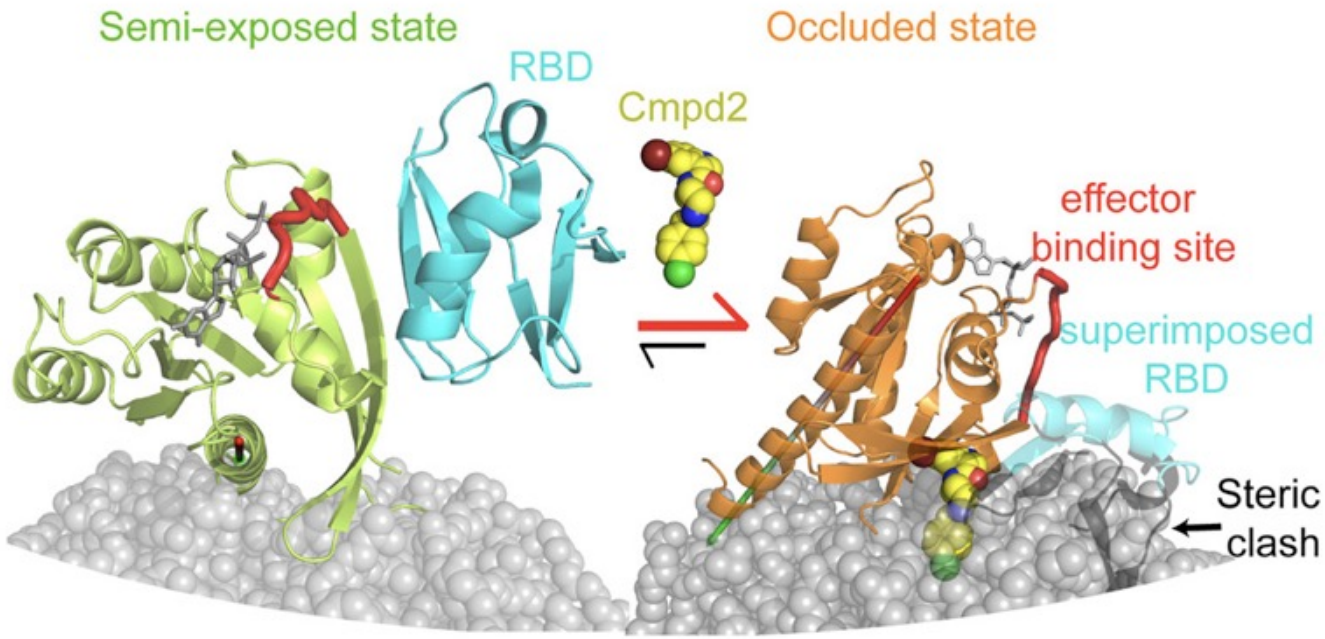
Ras orientation regulates binding site accessibility



Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

Ras orientation regulates binding site accessibility



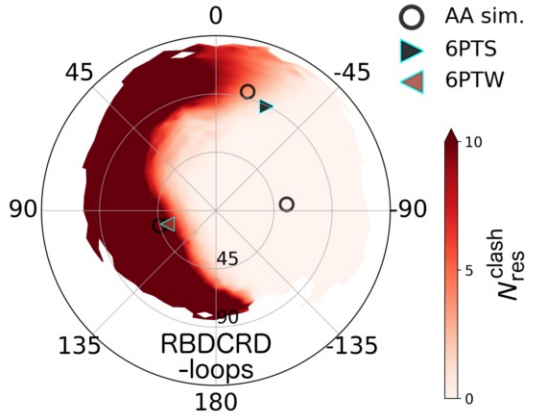


Cmpd2 leads to occlusion of effector binding site of KRAS

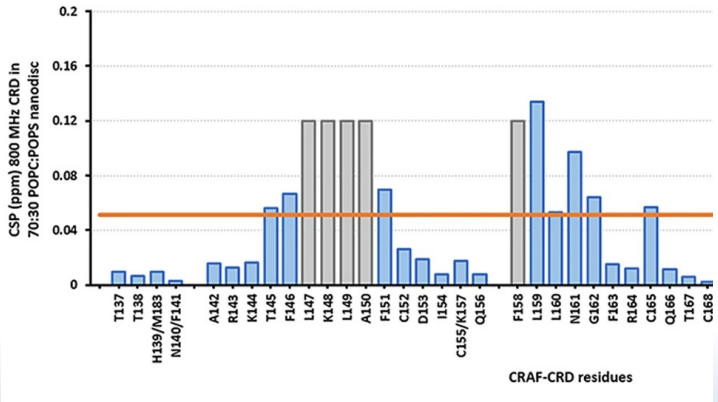
Fang, *et al.*, **2018**, *Cell Chem. Biol.*, 25, 1327-1336

Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

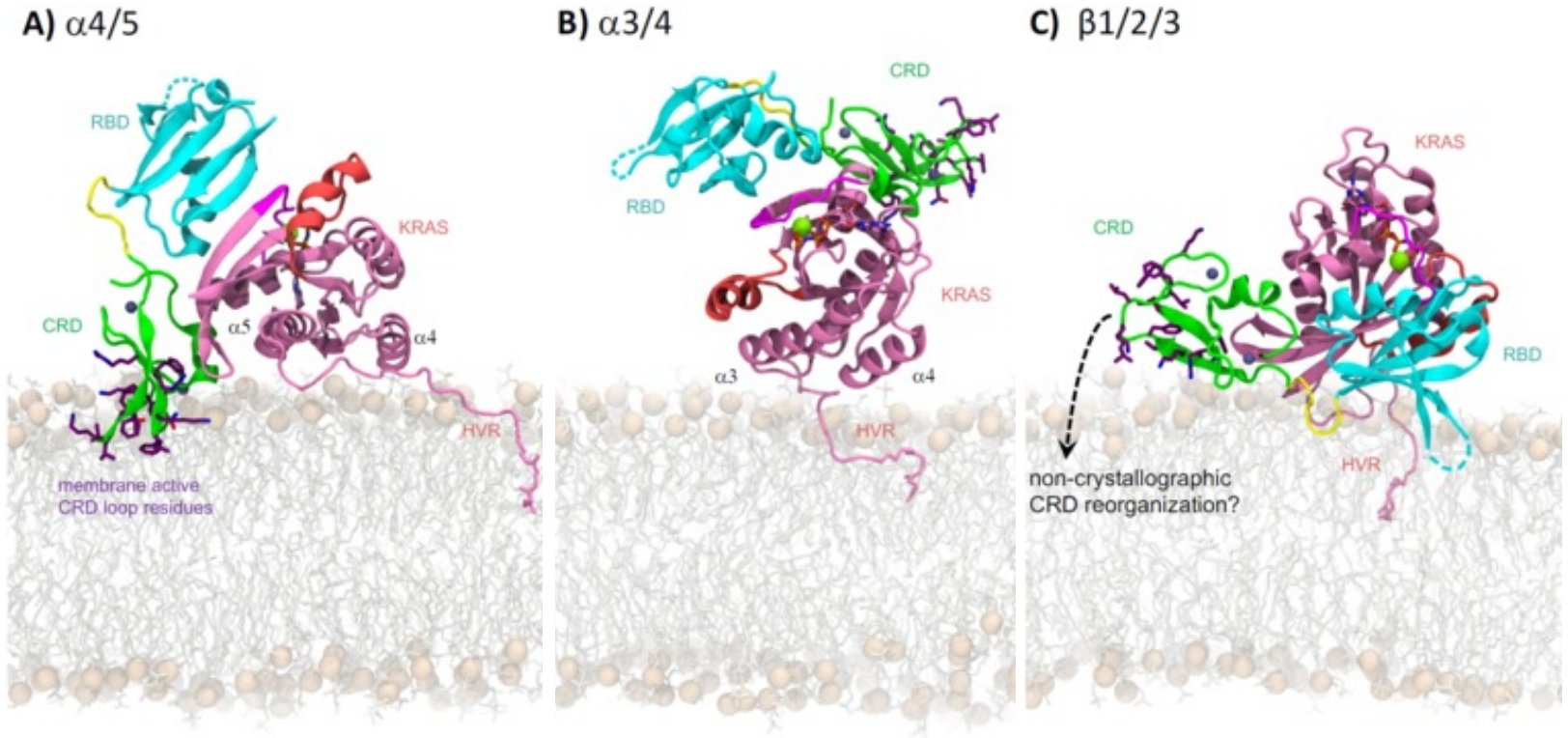
MD simulations of K-Ras4B populate a Raf binding pose consistent with data from NMR and crystallography



Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141



Travers *et al.*, **2018**, *Sci. Rep.*, 8:8461



Tran *et al.*, **2021**, *Nat. Commun.*, 12:1176

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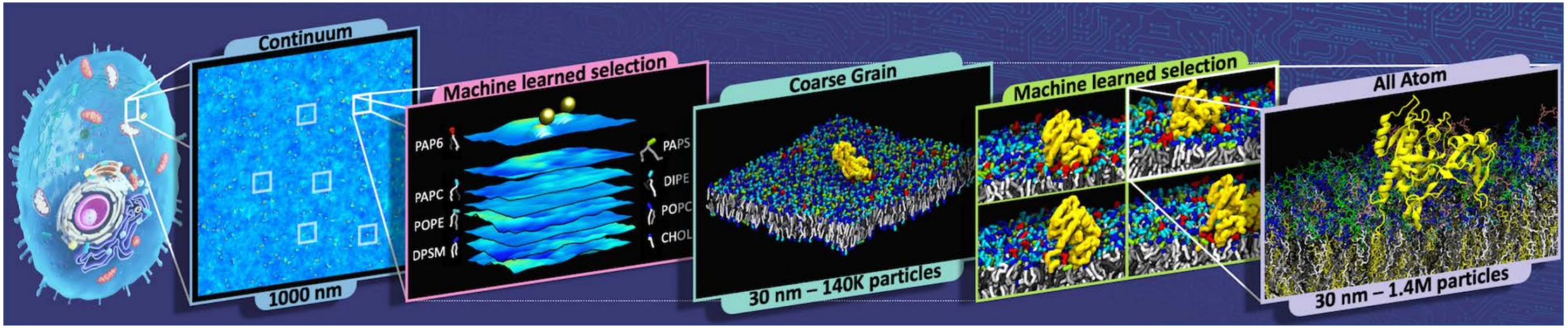
Multiscale simulations



Di Natale *et al*., **2019**, *SC '19*, 57:1-16

Bhatia *et al*., **2021**, *SC '21*, 10:1-16

Ingólfsson *et al*., **2022**, *PNAS*, 119:e2113297119

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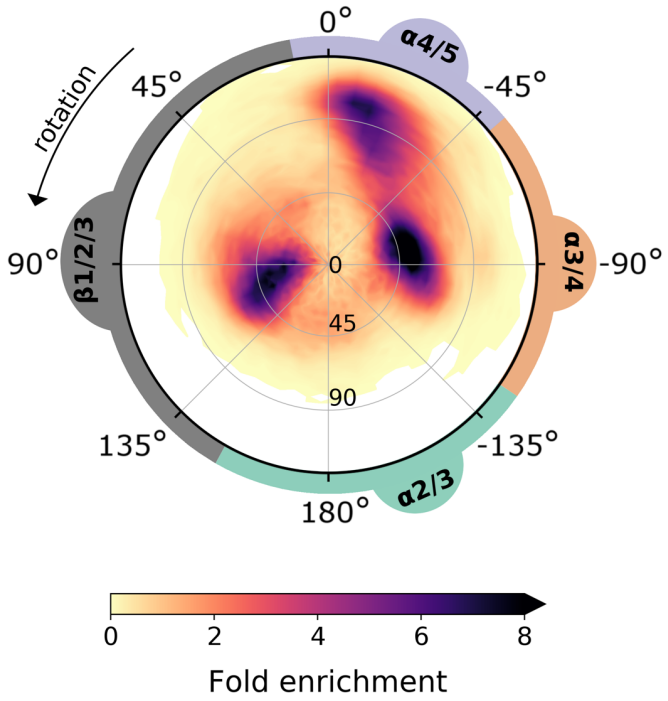
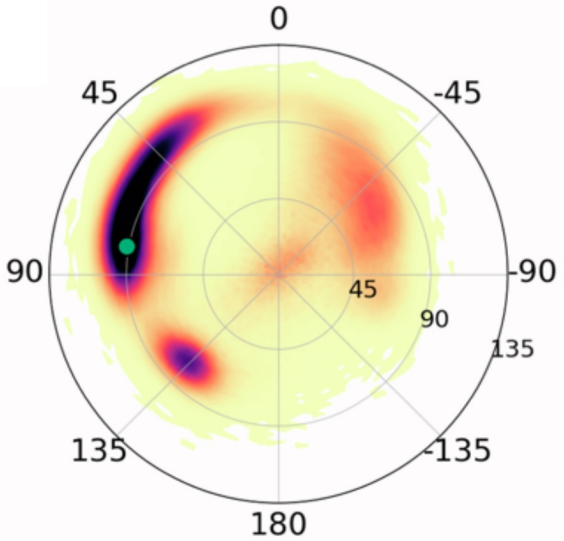
Multiscale simulations reveal sensitivity & uncertainty

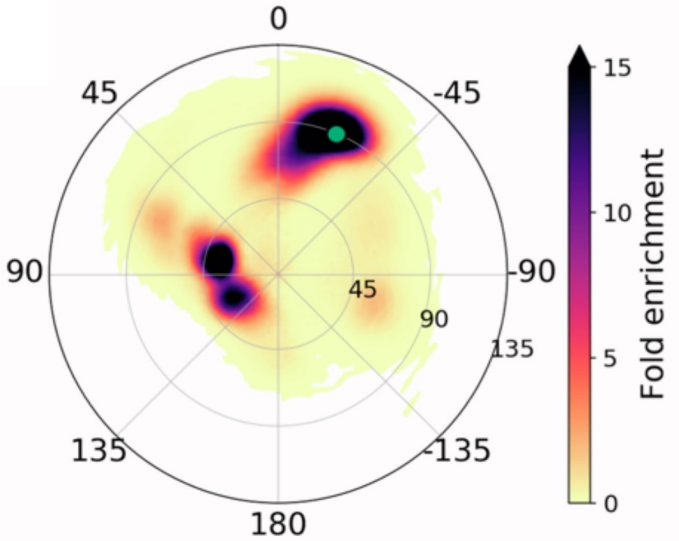
(AA) Ras + 2-lipids

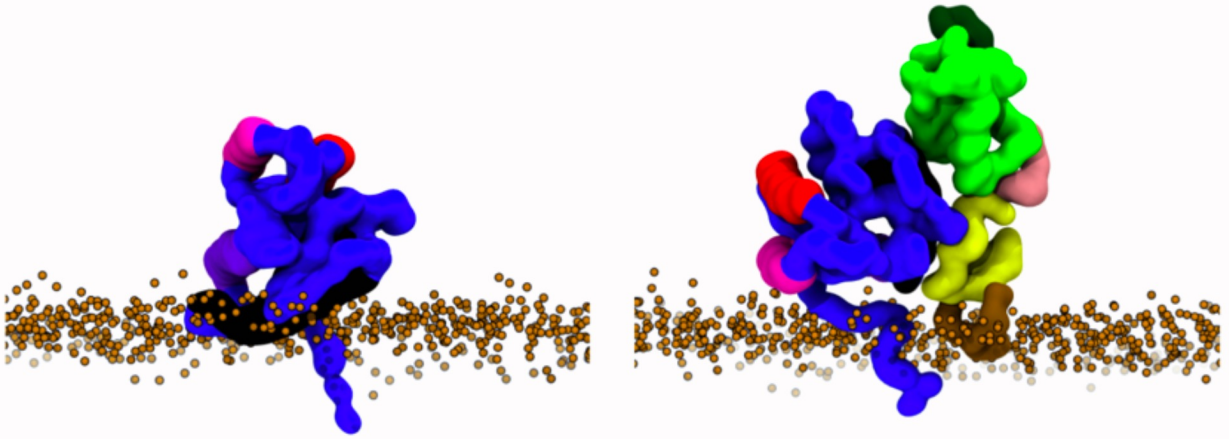


(CG) Ras + 8-lipids



(CG) Ras + RBD/CRD + 8-lipids

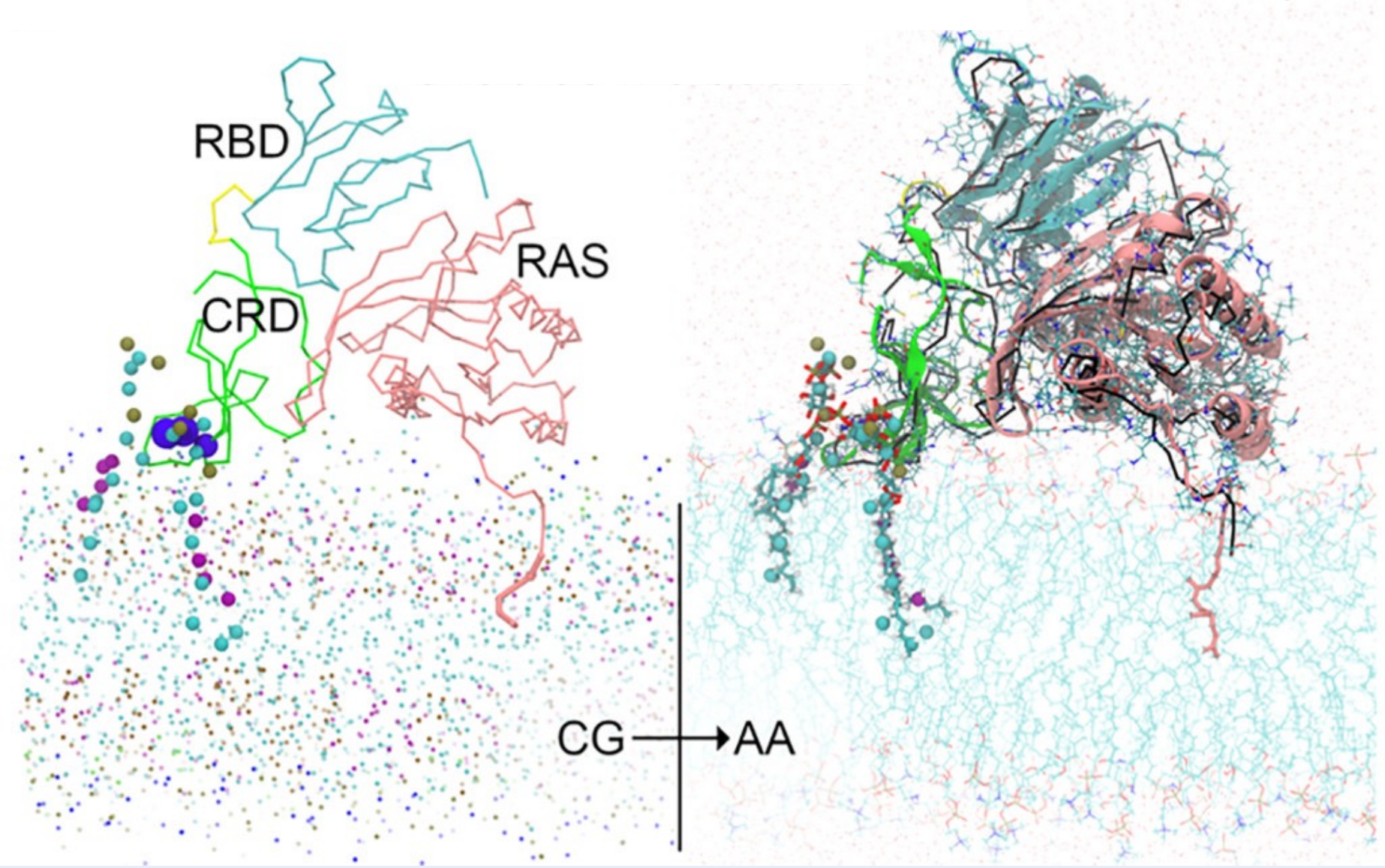




Neale and García, *Biophys. J.*, **2020**, 118(5):1129-1141

Nguyen *et al.*, **2022**, *Biophys. J.*, 121(19):3630-3650

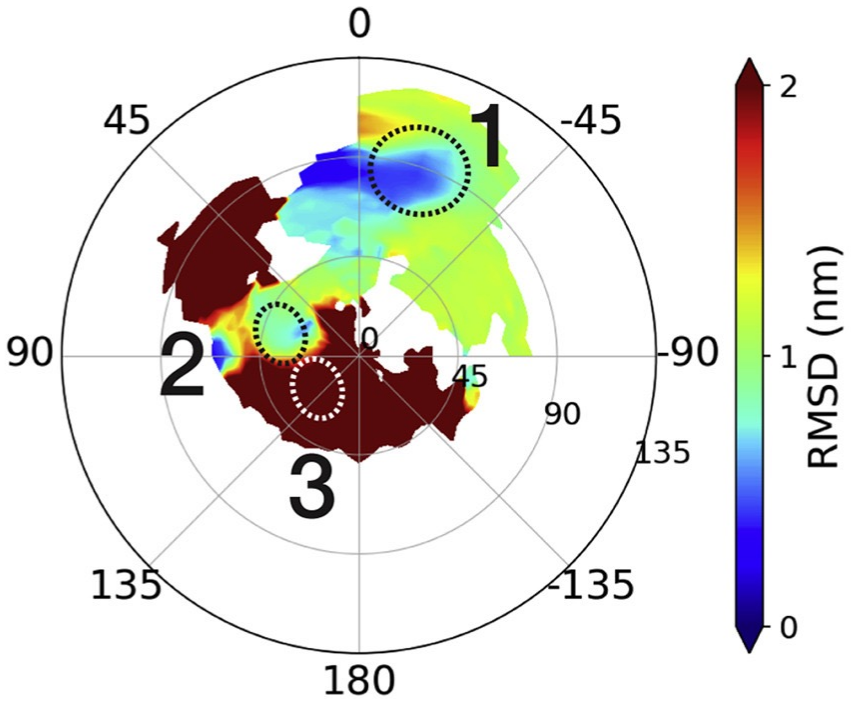
CG-to-AA reenables conformational changes

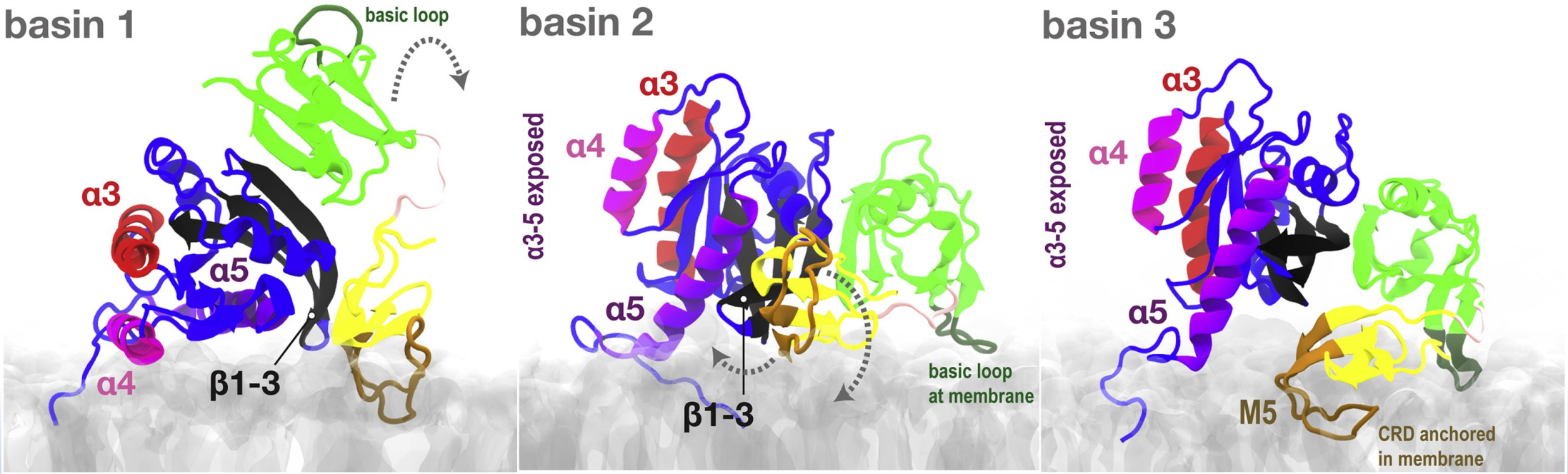


Lopez *et al.*, **2022**, *J. Chem. Theory Comput.*, 18(8):5025-5045

CRD Reorganization vs. Ras orientation

Nguyen *et al*., **2022**, *Biophys. J*., 121(19):3630-3650





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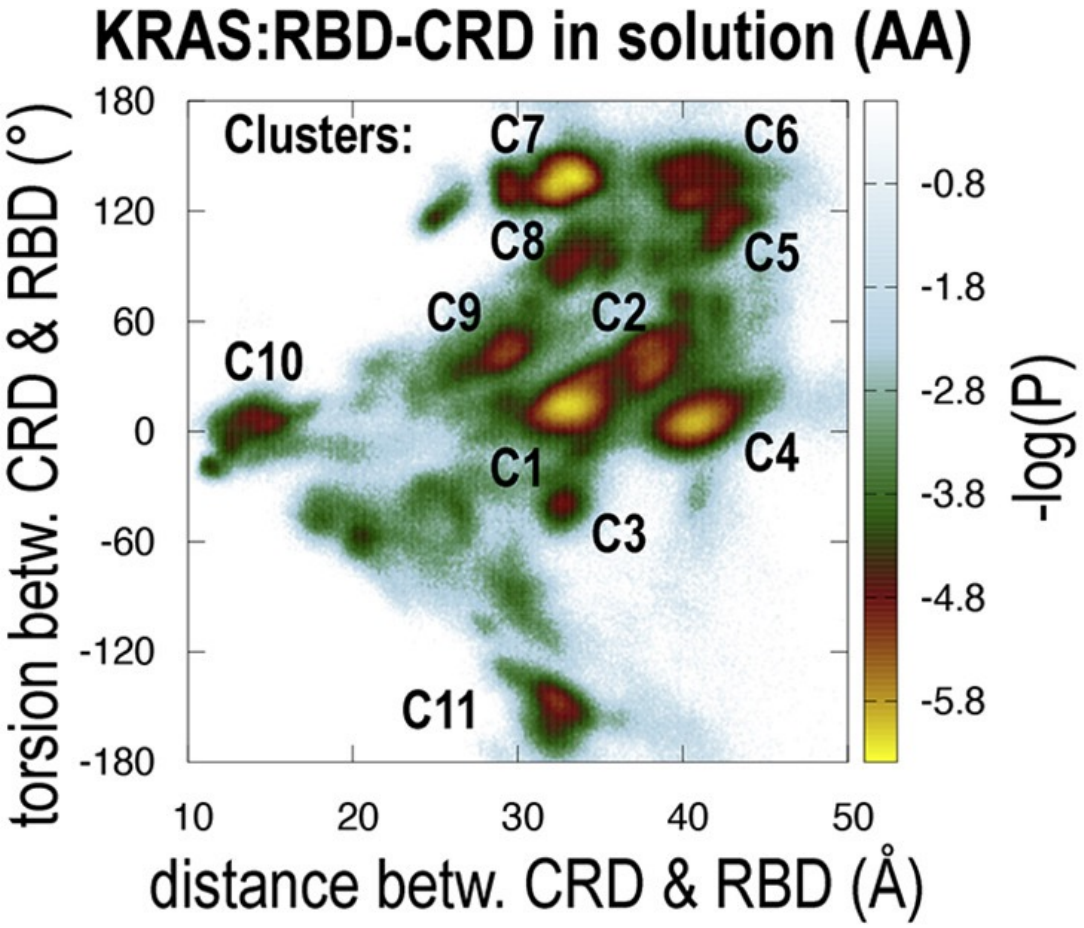
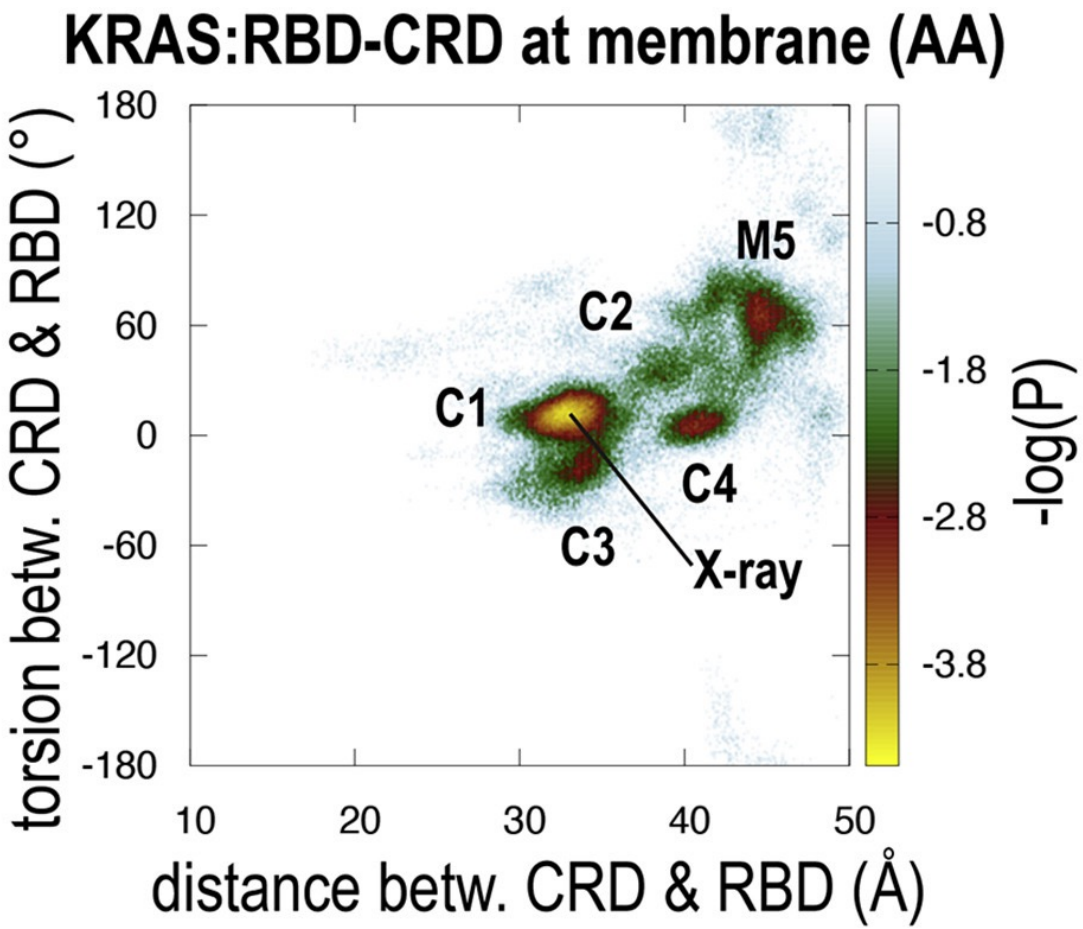
CRD reorganization is larger in solution





Nguyen *et al*., **2022**, *Biophys. J*., 121(19):3630-3650

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Summary

- Disposition
  - Lipids (not GNP) define K-Ras4B orientation
  - membrane as a competitive inhibitor or PAM
  - orientation may be druggable
- Conformation
  - CG/AA multiscale yields benefits of each scale
  - membrane protein surfaces may be more (or differently) druggable than is apparent in crystal structures

We are interested in enabling a membrane protein focus in our floes

Thank You

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