Weill Cornell Medicine
Graduate School of Medical Sciences
A partnership with the Sloan Kettering Institute

TMEM16 scramblases thin the membrane to enable lipid scrambling

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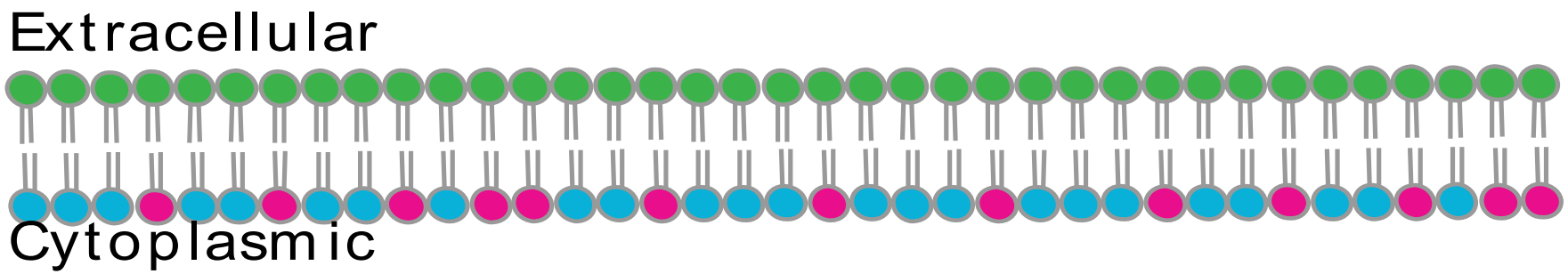
The eukaryotic cell membrane is asymmetric at rest

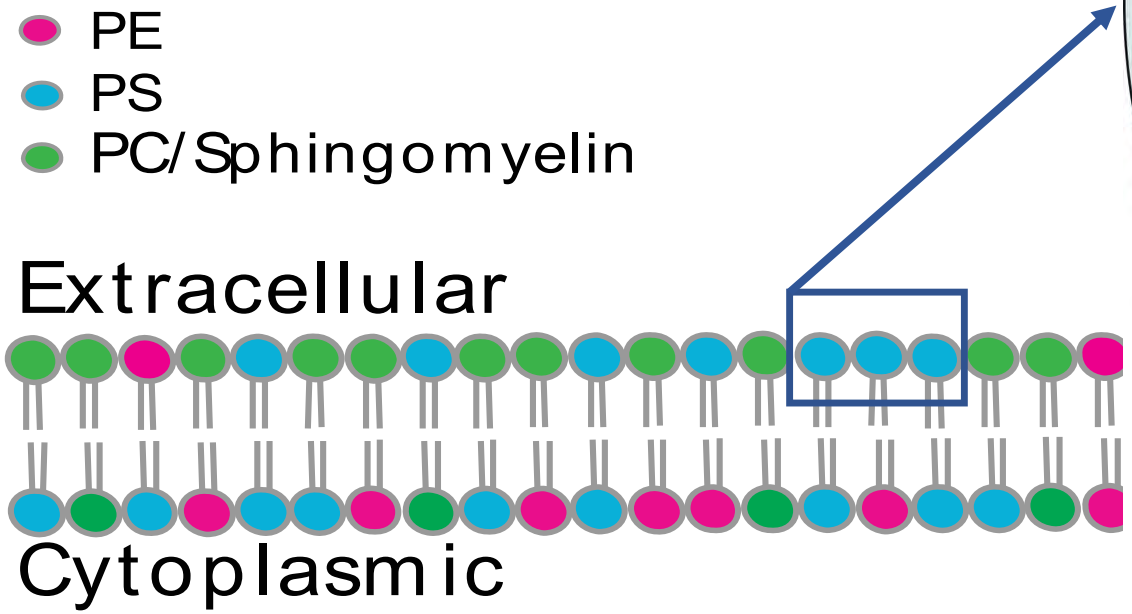
- Phosphatidylethanolamine (PE)
- Phosphatidylserine (PS)
- Phosphatidylcholine (PC)/Sphingomyelin

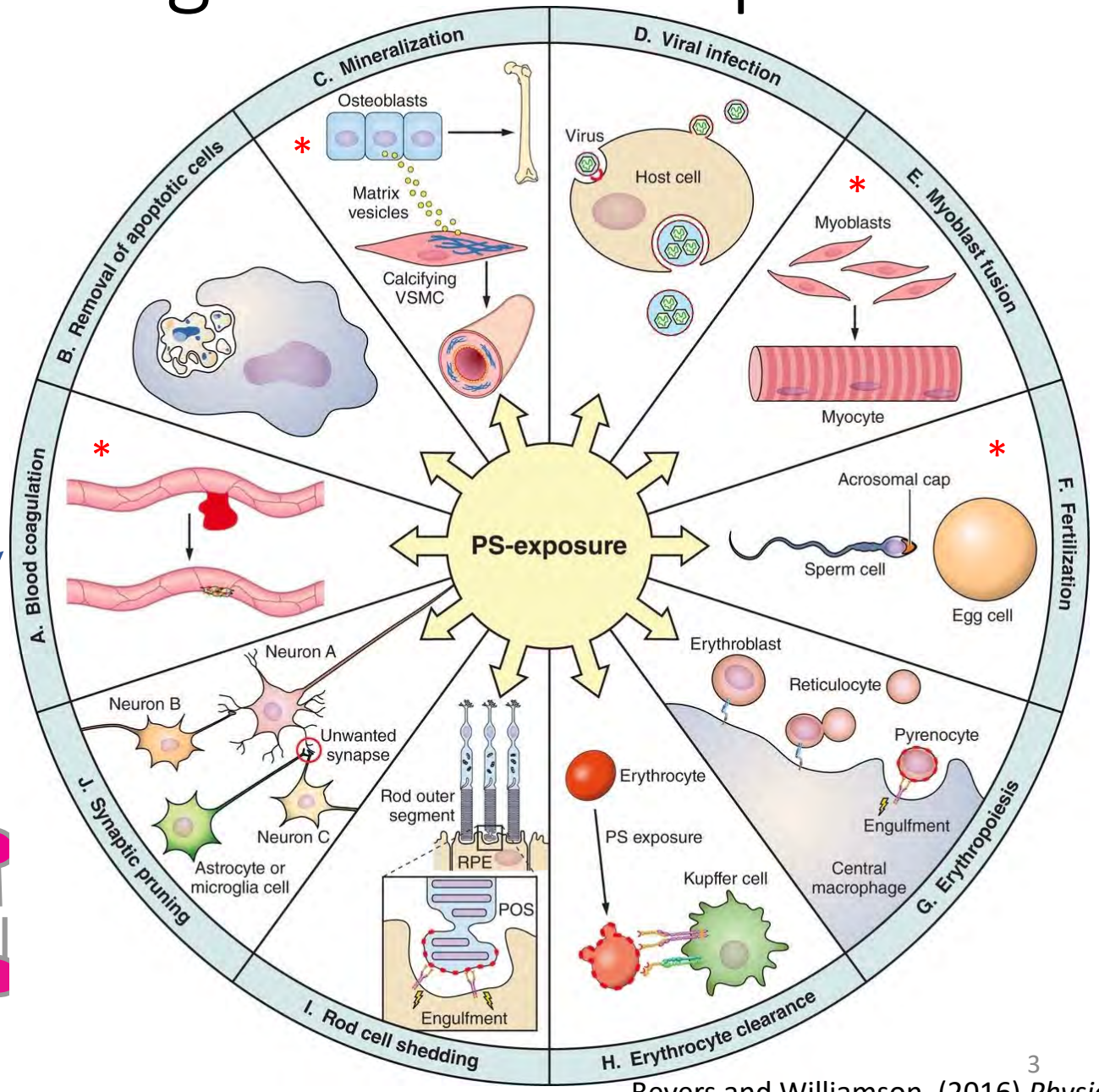


Phospholipid scrambling causes PS exposure

PS exposure is essential for many cellular signaling processes including:

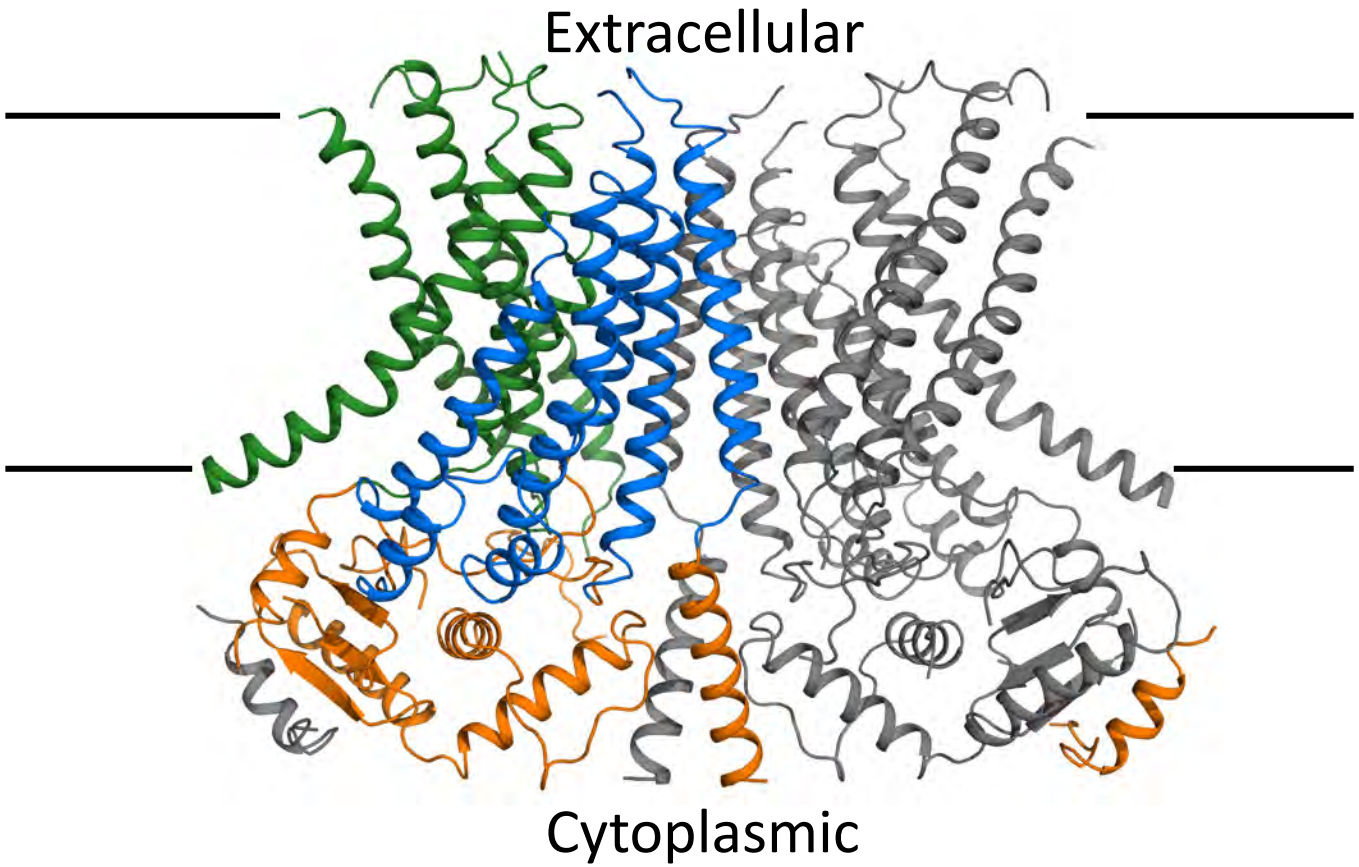
- PE
- PS
- PC/Sphingomyelin





Bevers and Williamson, (2016) *Physiol Rev.*

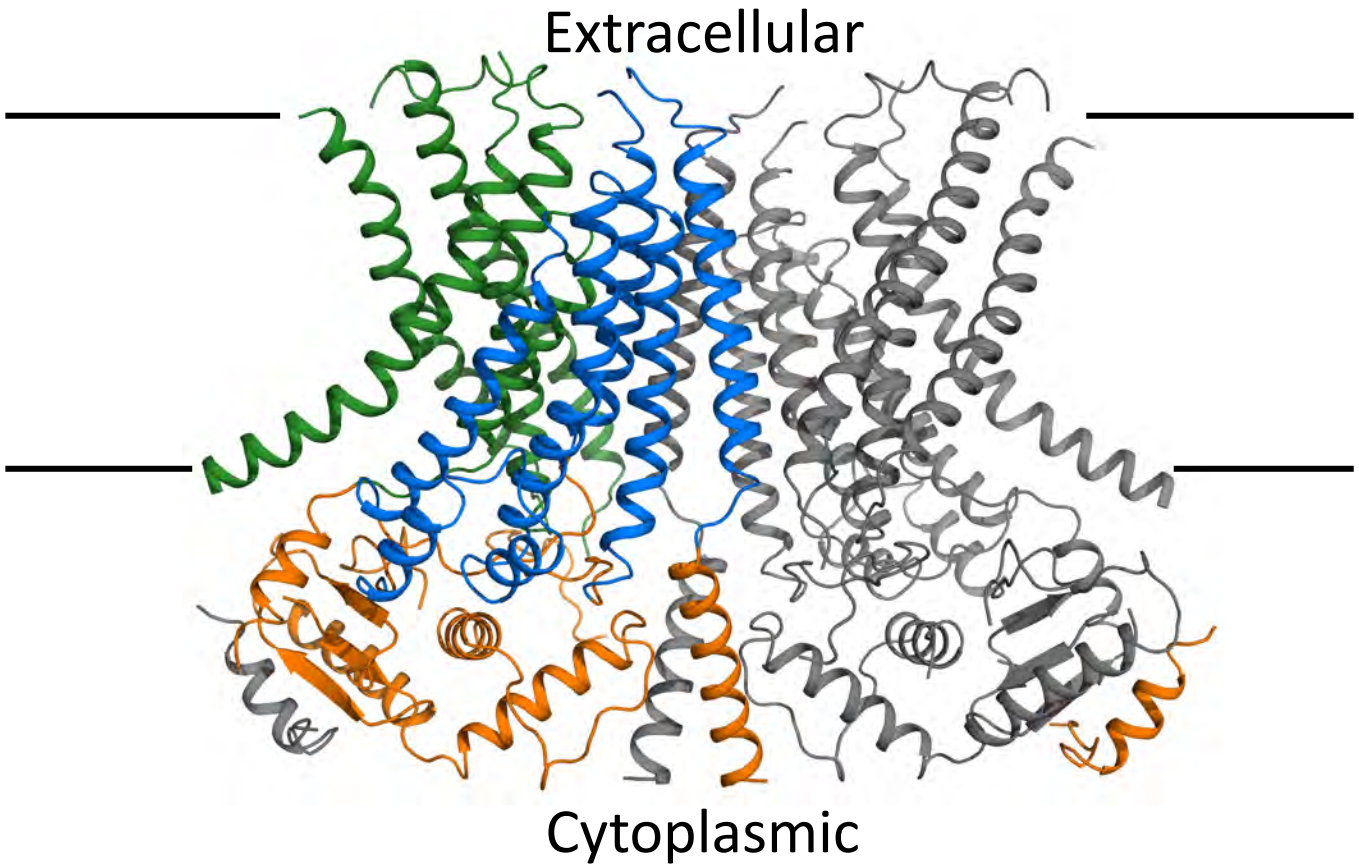
TMEM16s are Ca^{2+}-activated scramblases



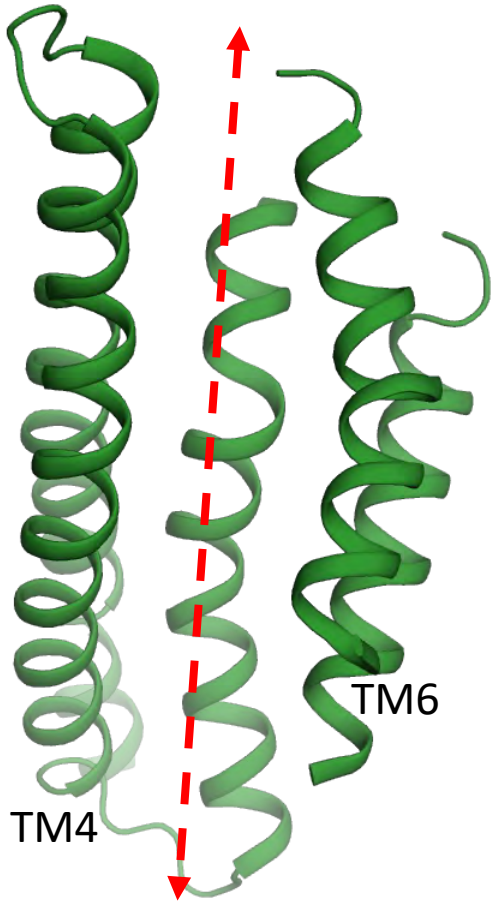
- Several TMEM16s function as Ca^{2+}-activated scramblases
- Fungal homologues afTMEM16 is used as a model system

Falzone et al., (2019). *Elife*.

Scrambling is mediated by a hydrophilic groove



Rotate 90°

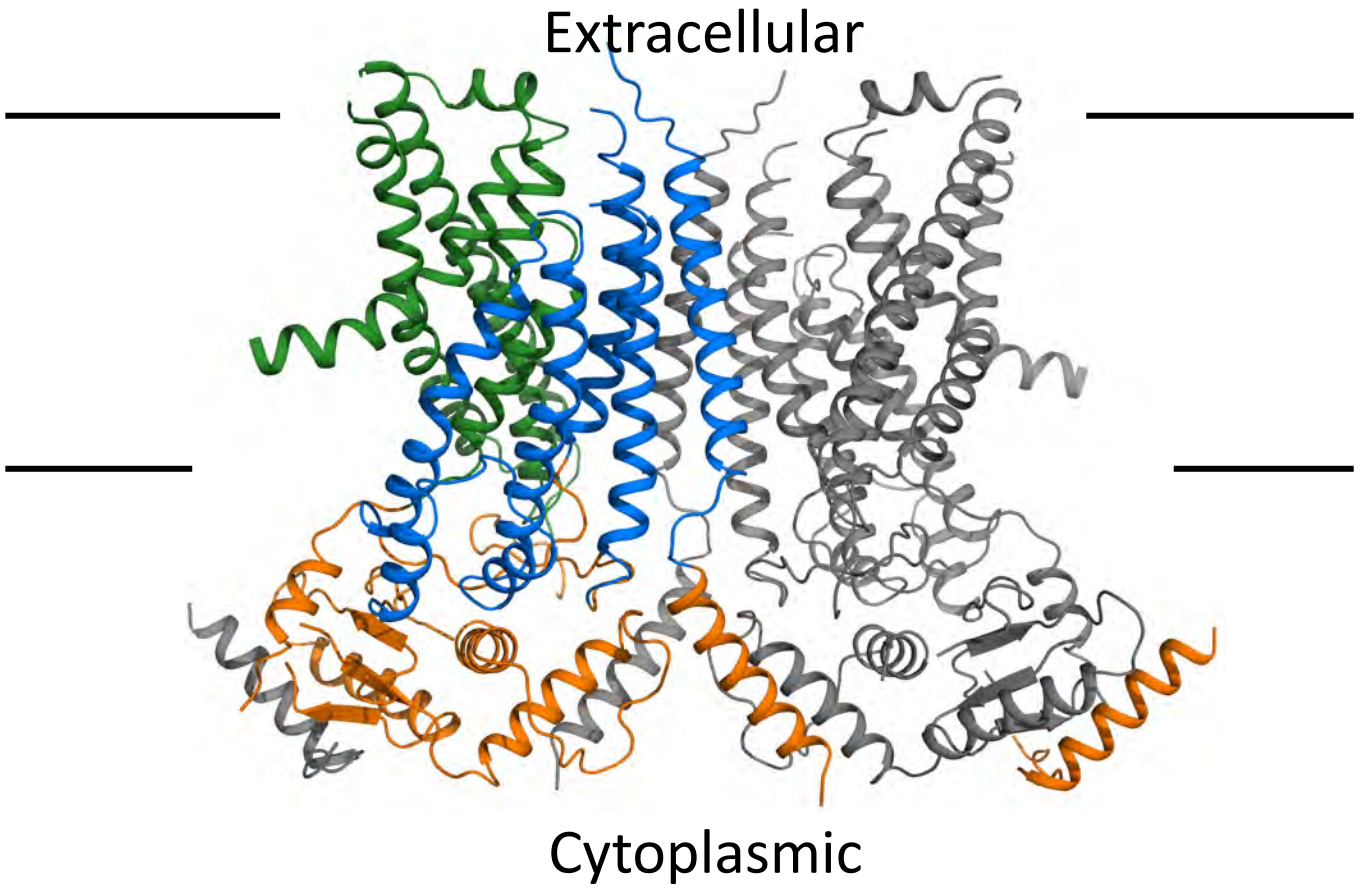


formed by TM 3-7

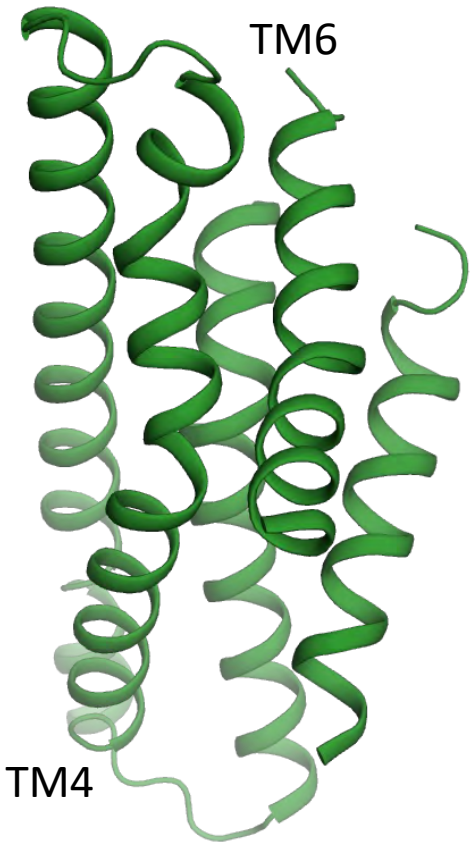
- Homodimers
- Mediate scrambling via a membrane exposed, hydrophilic groove on the side of the protein

Falzone et al., (2019). *Elife*.

The lipid permeation pathway is Ca^{2+}-gated



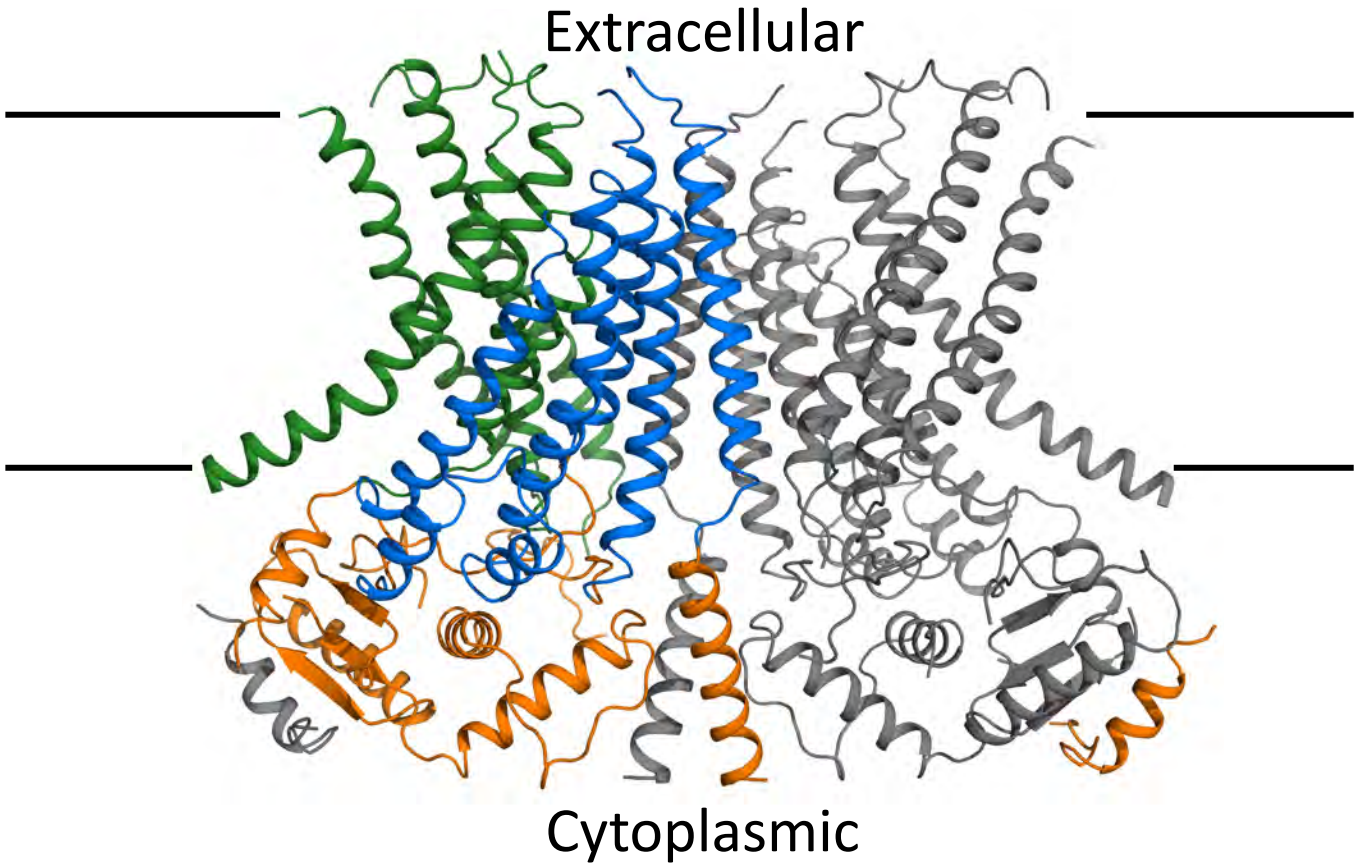
Rotate 90°

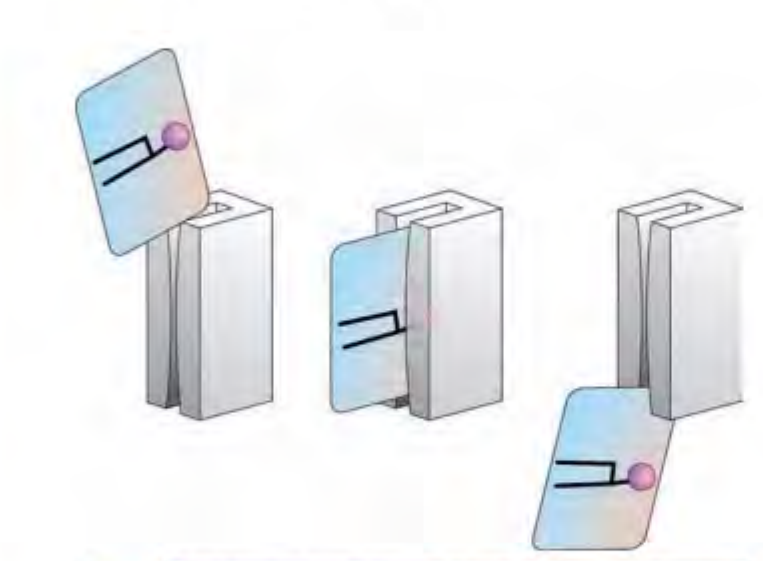


- In the absence of Ca^{2+}, the groove closes which reduces scrambling

Falzone et al., (2019). *Elife*.

Scrambling is proposed to occur via a credit-card mechanism



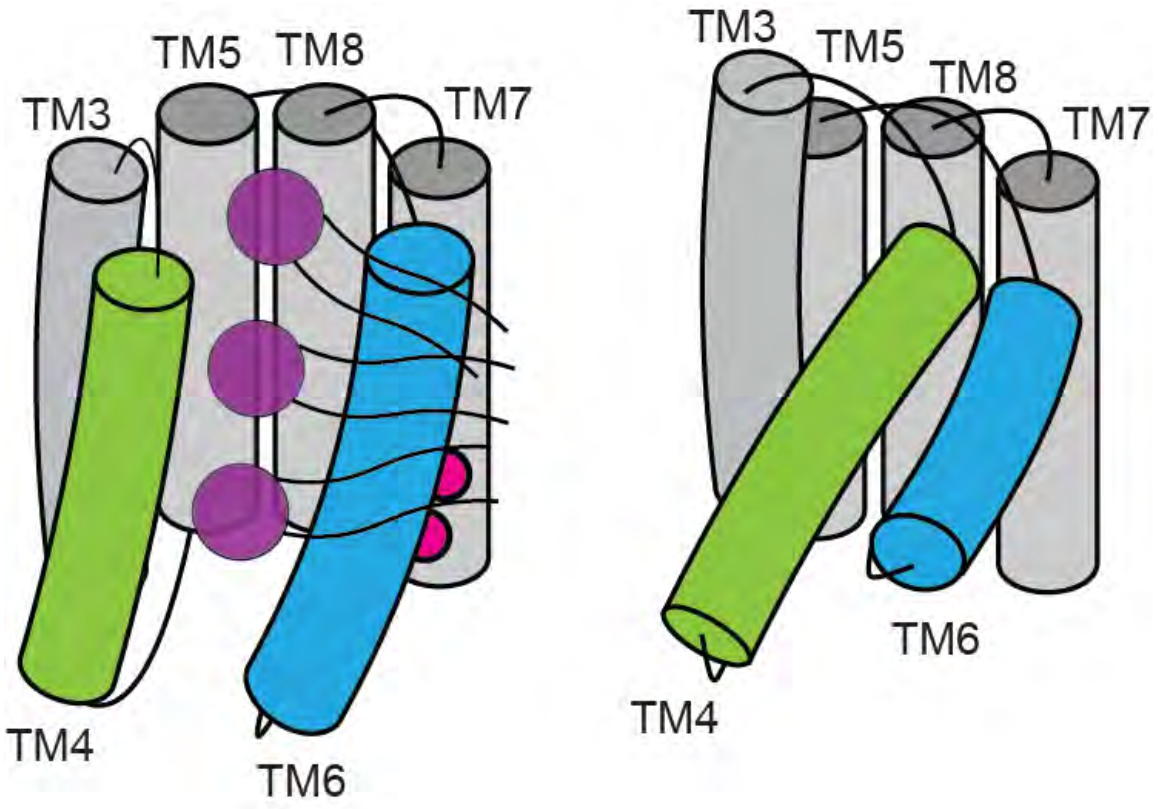


- The protein functions as a card reader which stabilizes the charged lipid headgroups as they move through the membrane

Falzone et al., (2019). *Elife*. Pomorski, and Menon, (2006). *Cellular and Molecular Life Sciences.*

Scrambling is proposed to occur via a credit-card mechanism

Ca^{2+} is bond
Pathway is open
Scrambling is fast

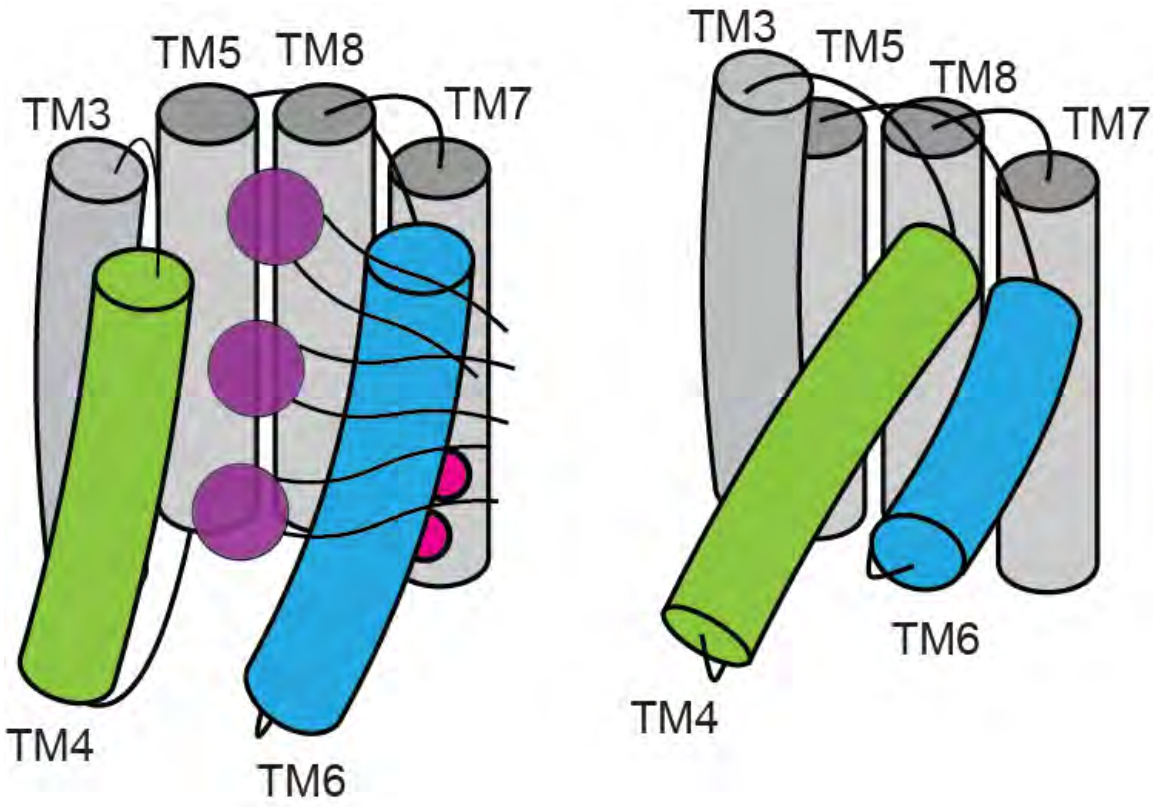


No Ca^{2+} is bond
Pathway is closed
No Scrambling

- Fits well with the observed permeation pathway and Ca^{2+}-dependent gating

But the credit-card mechanism contradicts some functional properties of TMEM16s

Ca^{2+} is bond
Pathway is open
Scrambling is fast

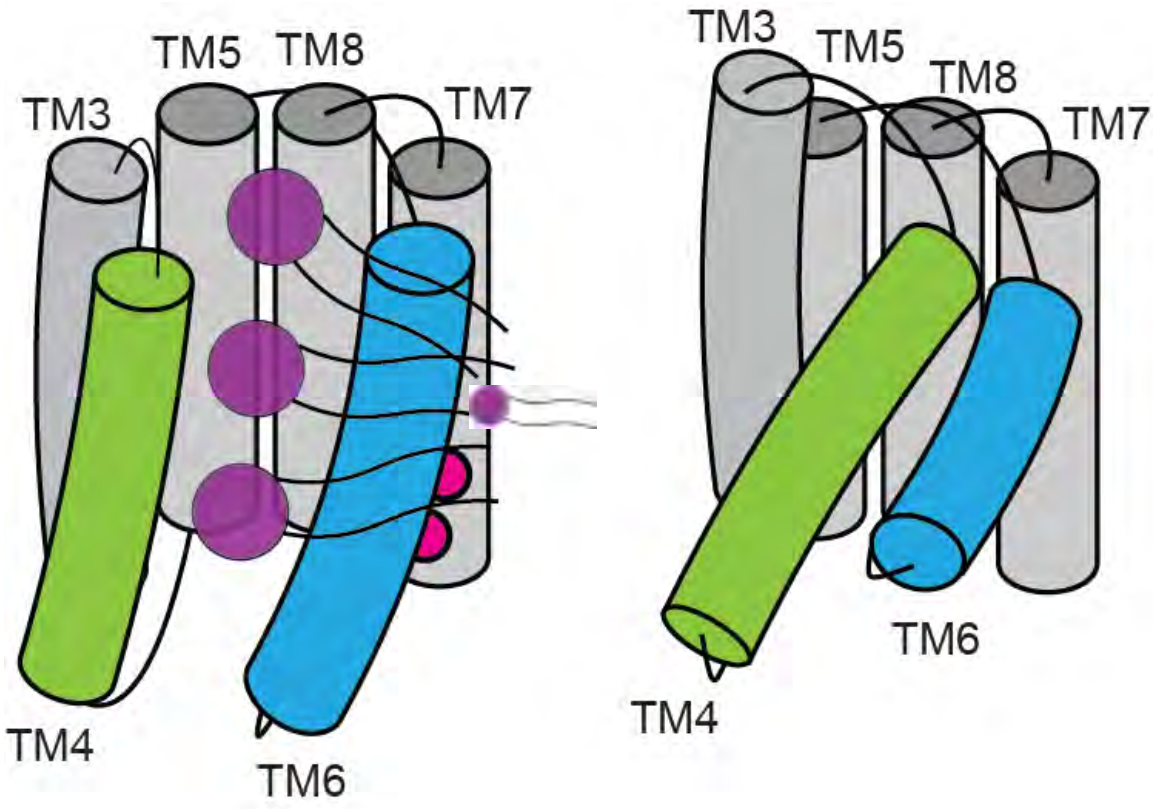


No Ca^{2+} is bond
Pathway is closed
No Scrambling

- The credit card mechanism predicts specific lipid-protein interactions within the permeation pathway, but TMEM16s show no substrate specificity for headgroup charge or size.
- Scrambling is also very fast (~10^4 lipids/s), further challenging the idea of specific protein-lipid interaction

But the credit-card mechanism contradicts some functional properties of TMEM16s

Ca^{2+} is bond
Pathway is open
Scrambling is fast

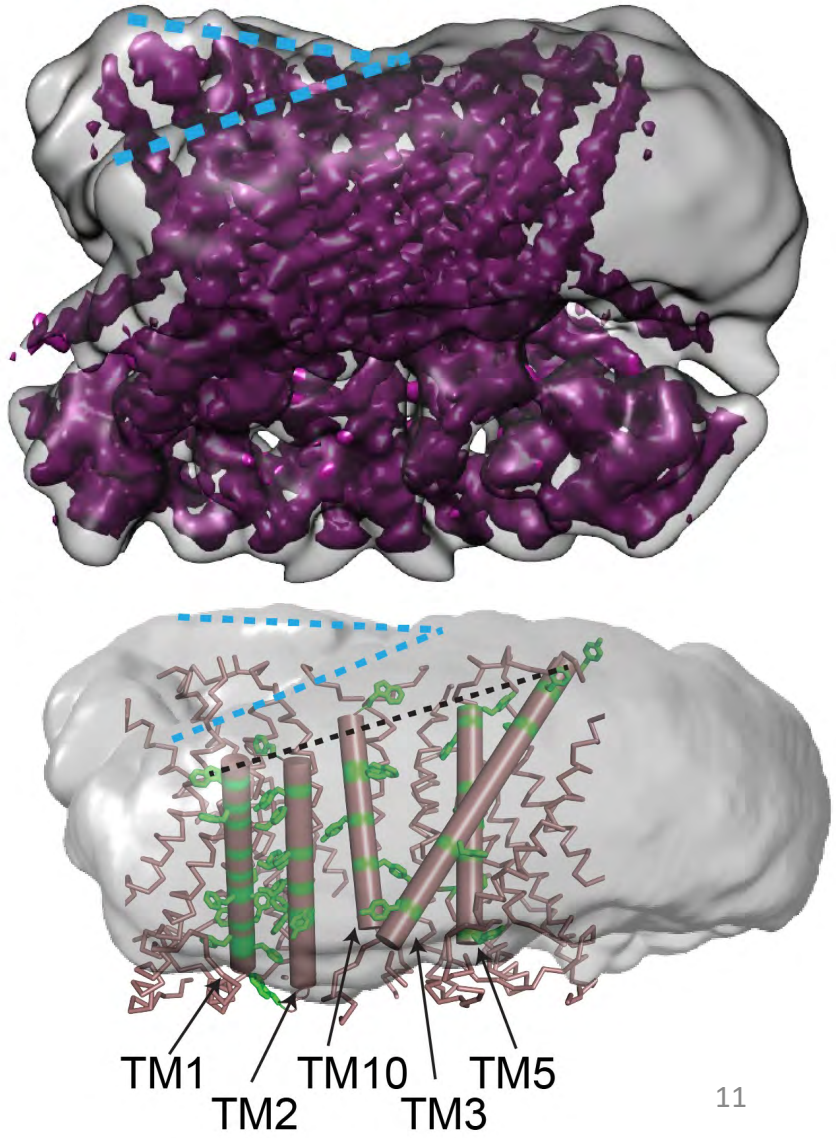


No Ca^{2+} is bond
Pathway is closed
No Scrambling??

- And some TMEM16s do not clearly open in response to Ca^{2+} and are proposed to scramble through the closed groove conformation

TMEM16 scramblases alter the organization of their surrounding membrane

- The membrane surrounding afTMEM16 is bent and thinned at the open permeation pathway
- We proposed that these changes are due to the architecture of the protein and are important for scrambling
- Supported by the observation that scrambling function is regulated by membrane properties like thickness and rigidity
- But these are lower resolution structures and don’t capture specific protein-lipid interactions



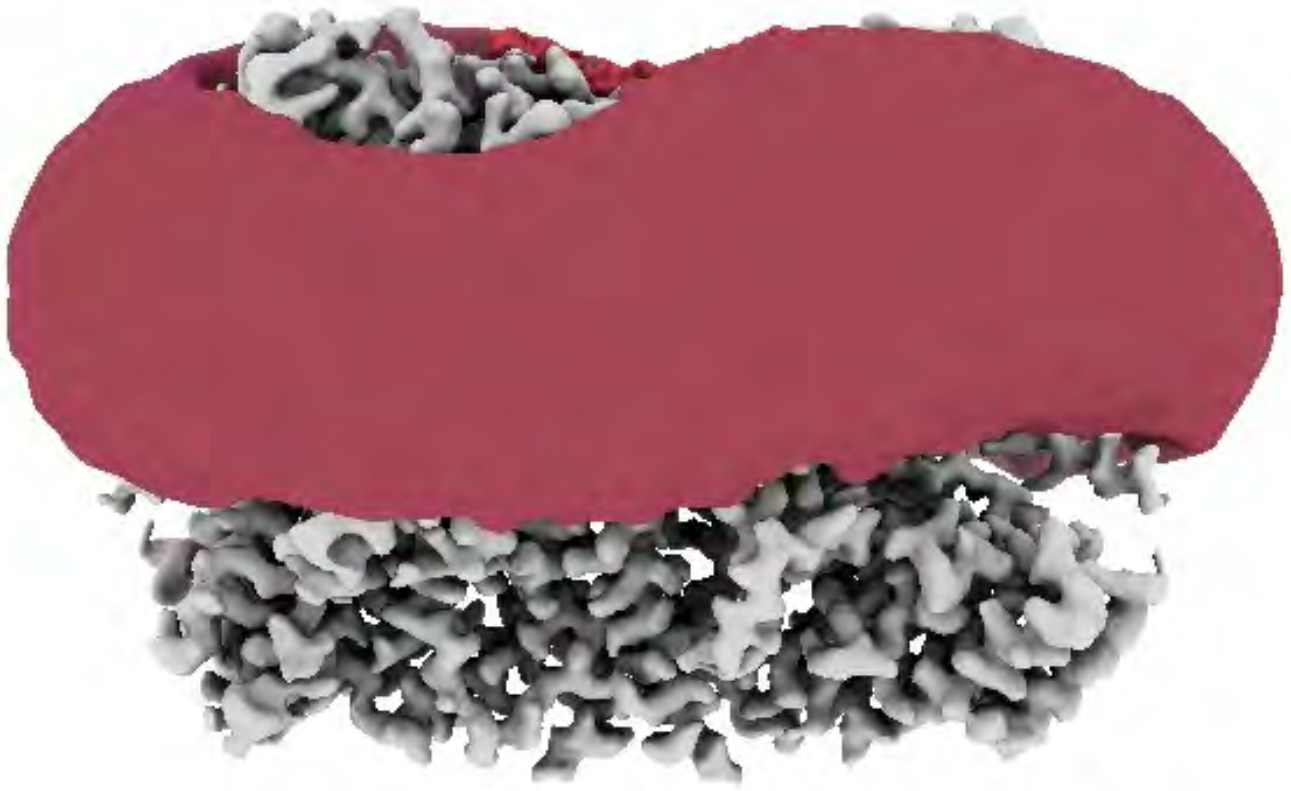
Falzone et al., (2019) *eLife*.

How do lipids interact with TMEM16 scramblases? What is the role of membrane thinning in scrambling?

Determined higher resolution structures to visualize the protein-lipid interface

High resolution structure of afTMEM16 reveals surrounding lipids

- Determined the structure of afTMEM16 +Ca^{2+} in nanodiscs to 2.3 Å
- Can resolve a continuous layer of lipids from both leaflets, including lipids across the opening of the permeation pathway
- Individual lipids align with the bulk membrane density surrounding the protein
- Consistent with our previous observation of altered membrane organization



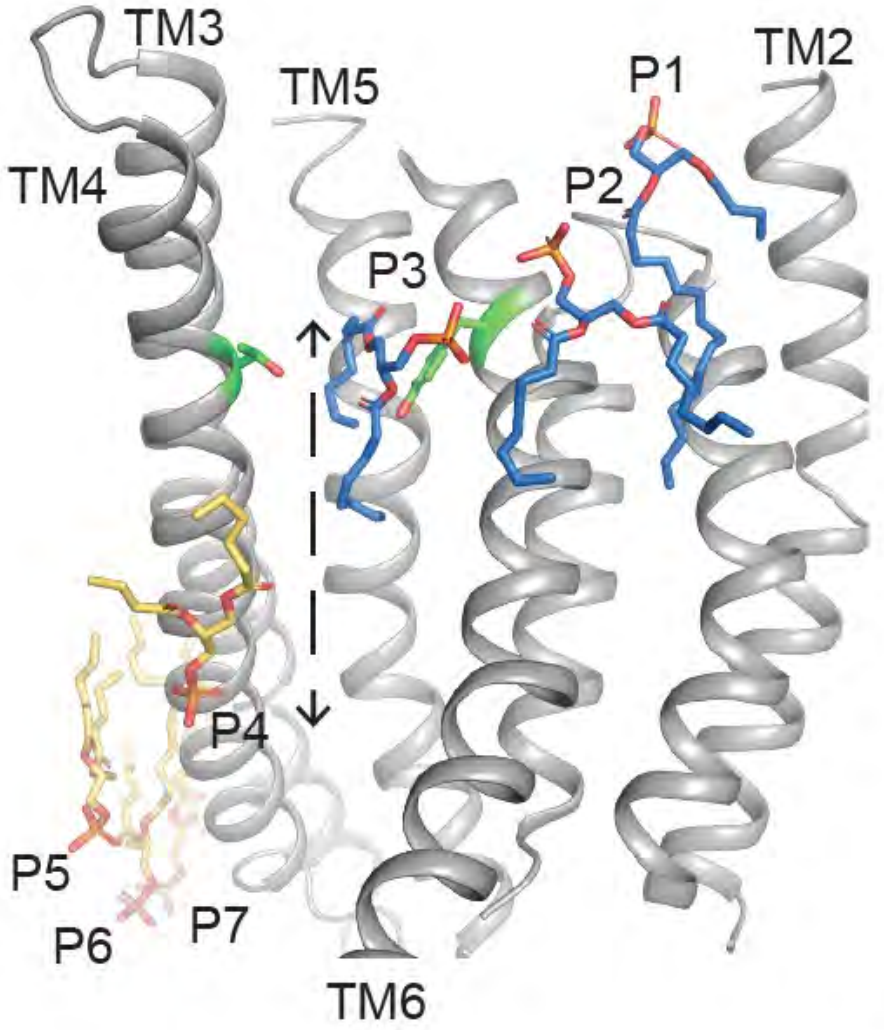
Falzone, Feng et al., (2022) *Nature Communications*.

Lipids interact with the periphery of the pathway and rotate as they approach is

- Lipids adopt rotated positions as they approach the permeation pathway from both leaflets
- All lipids except P3 are associated with the periphery of the pathway rather than deep inside of the opening as proposed by the credit card mechanism

Are the observed lipids substrates?

Or do they reflect the membrane footprint imposed by the protein?

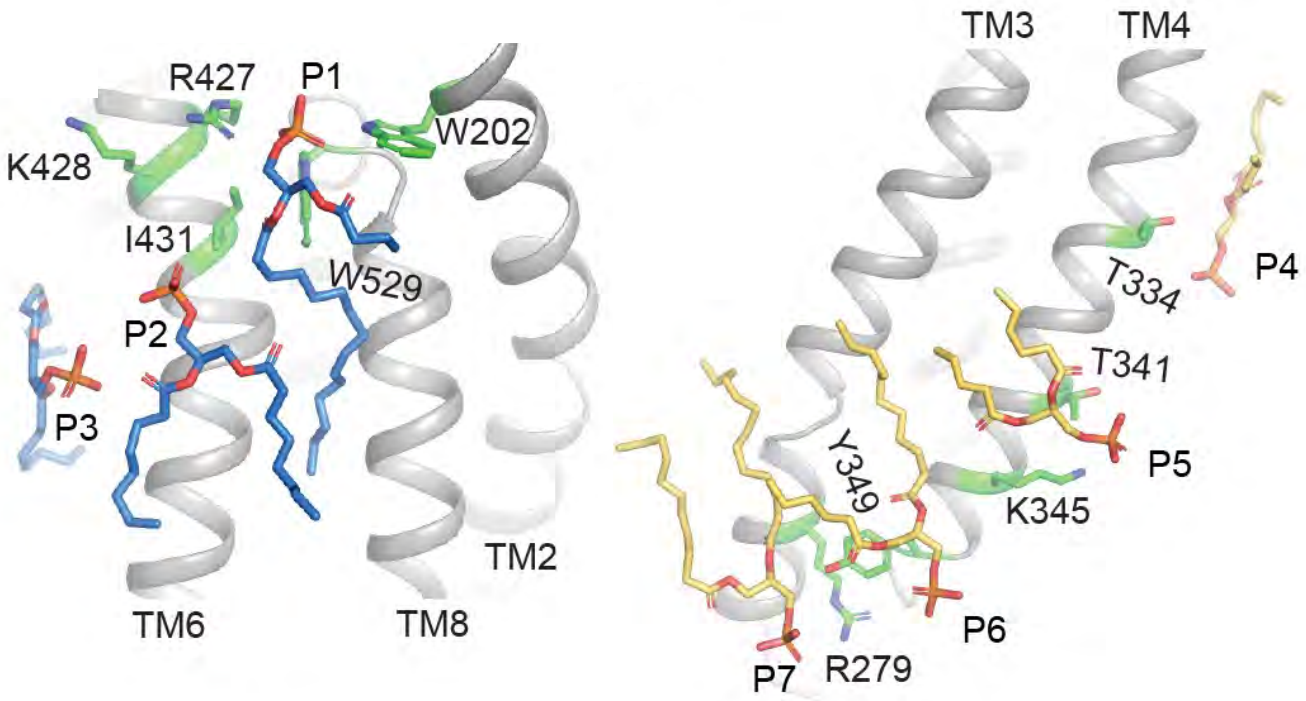


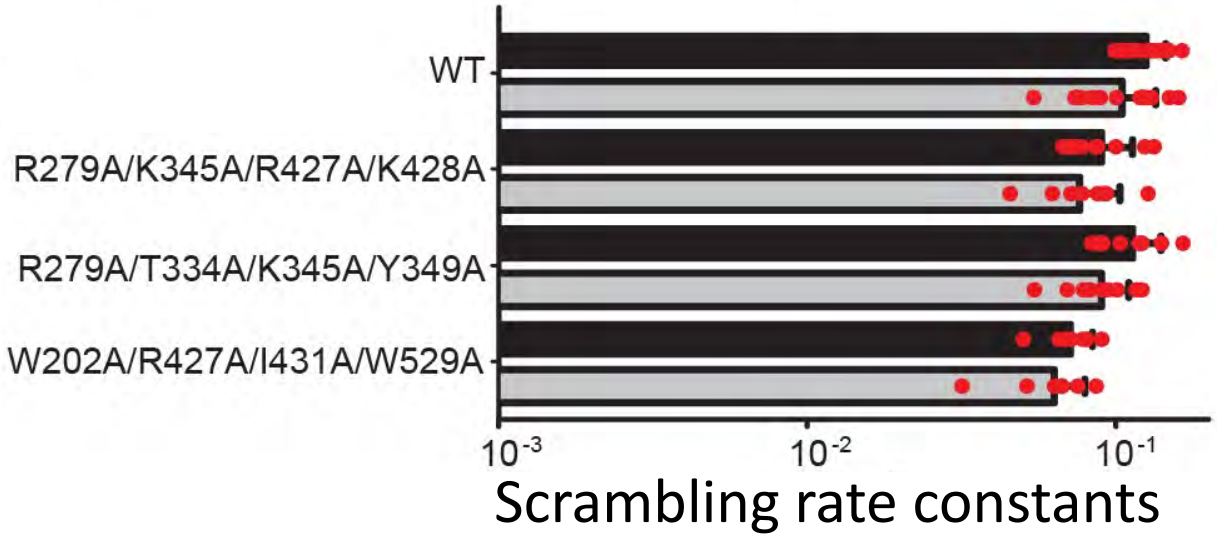
Falzone, Feng et al., (2022) *Nature Communications*.

Scrambling in unaffected by mutating lipid-interacting residues

Outer leaflet

Inner leaflet





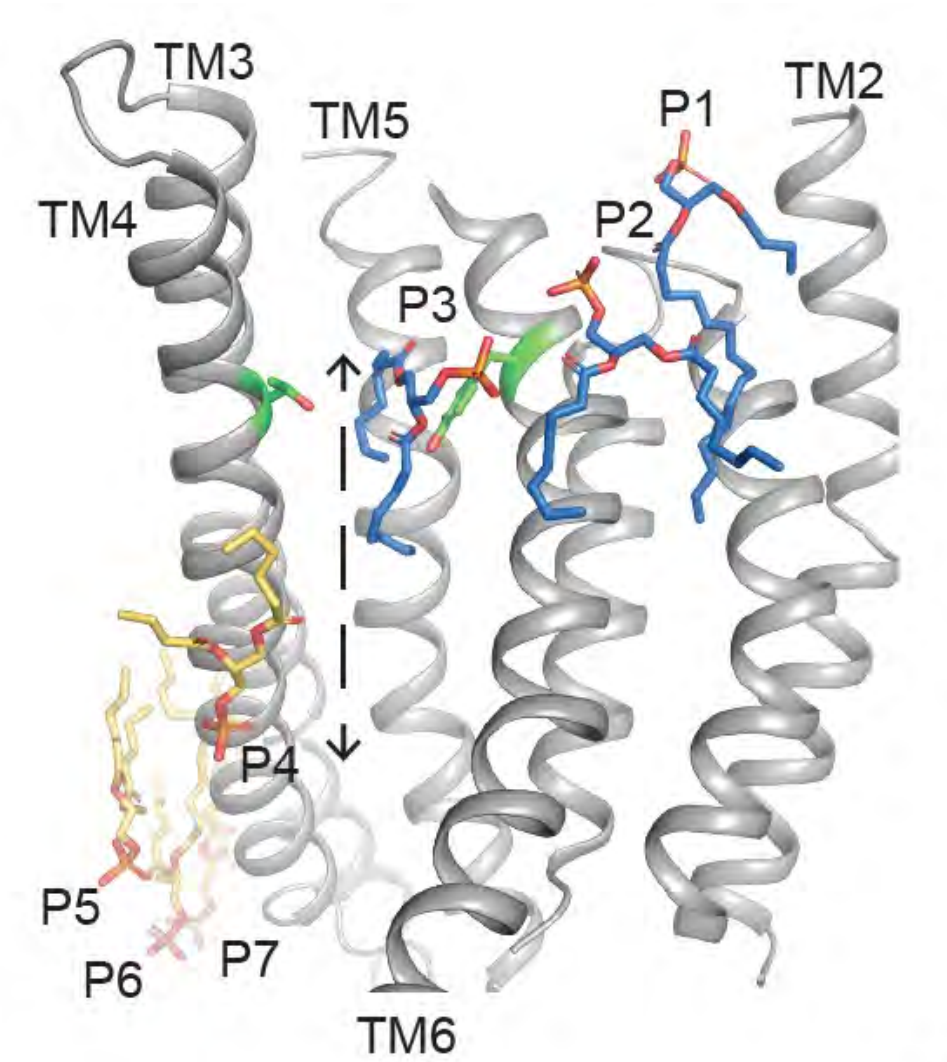
- Mutated all the residues that interact with lipids and none disrupt scrambling function

Suggests that these are not substrates but rather part of the re-organization imposed by the protein

Falzone, Feng et al., (2022) *Nature Communications*.

The membrane is significantly thinner at the permeation pathway

- The membrane is 50% thinner at the pathway compared to other regions of the protein

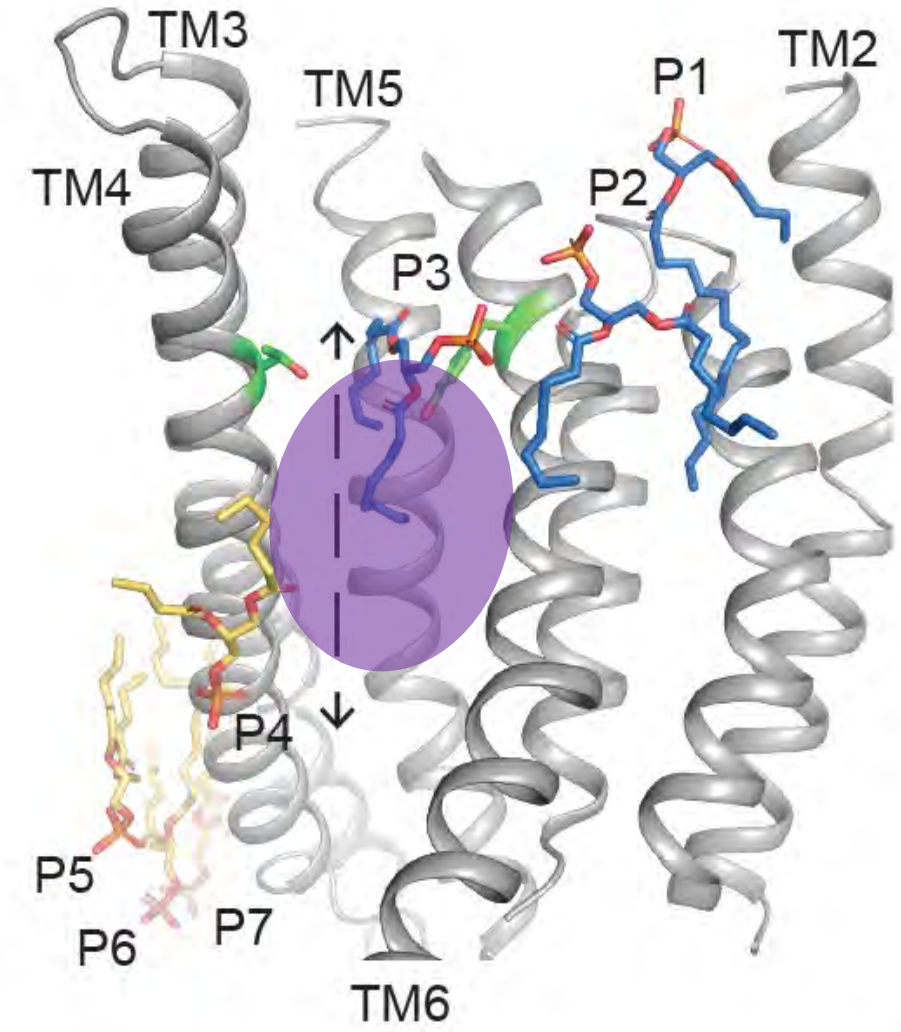


Falzone, Feng et al., (2022) *Nature Communications*.

Observed lipid positions define the region where flipping must occur

- P4 is the last observed lipid in the inner leaflet and P3 is the last observed lipid from the outer leaflet
- Suggests that the flip of substrate lipids must occur between these two

If this is true, then lipids wouldn't need to enter the narrow upper part of the pathway



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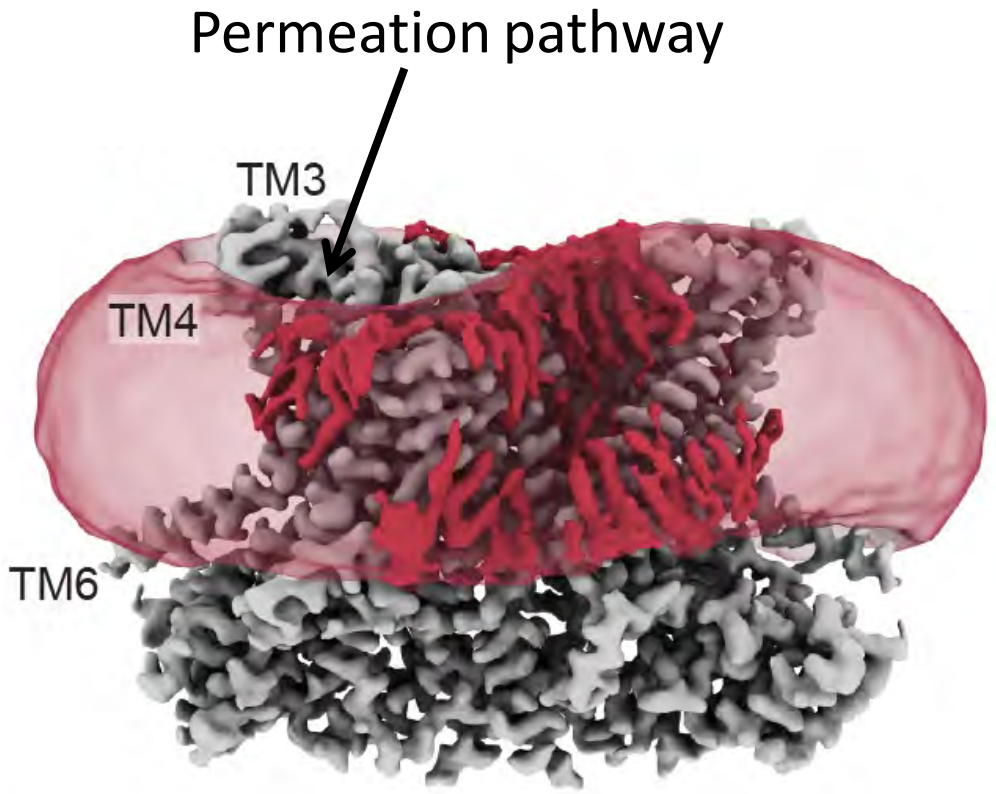
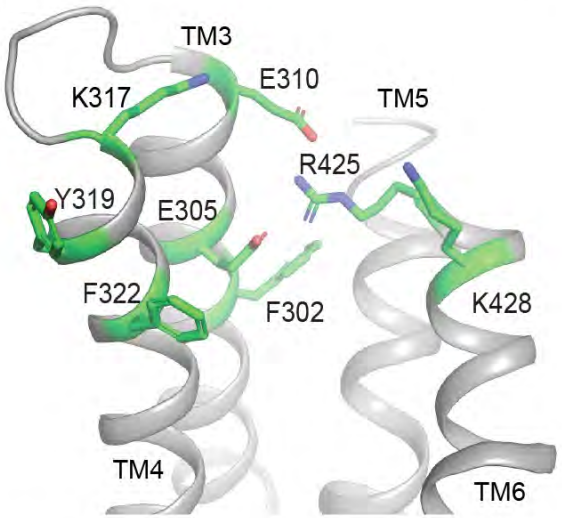


Can see that the membrane is actually excluded from this region of the protein in our structure

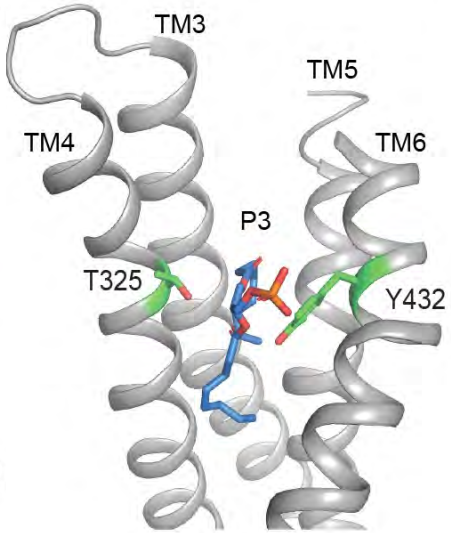
Falzone, Feng et al., (2022) *Nature Communications*.

Observed lipid positions define the region where flipping must occur

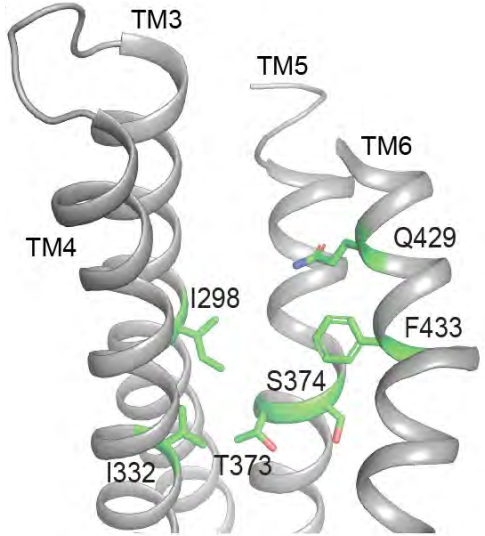
Extracellular vestibule

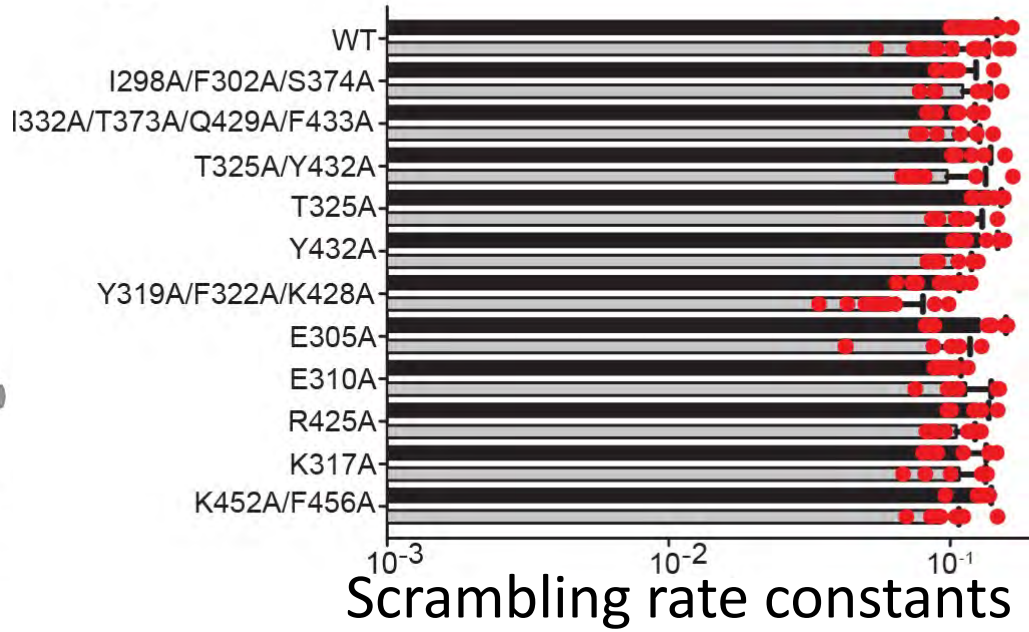


P3 binding site



Central constriction



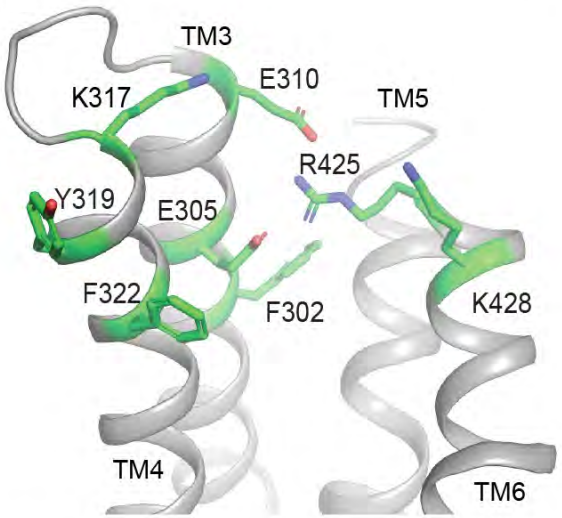


- Mutated residues lining the extracellular vestibule and facing the interior of the pathway near the central constriction
  - None impair scrambling

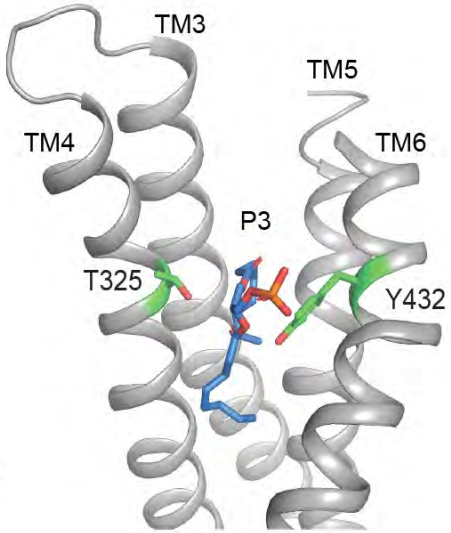
Falzone, Feng et al., (2022) *Nature Communications*.

Observed lipid positions define the region where flipping must occur

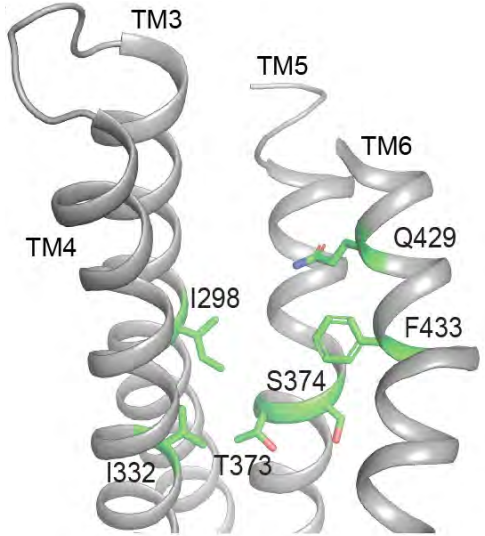
Extracellular vestibule

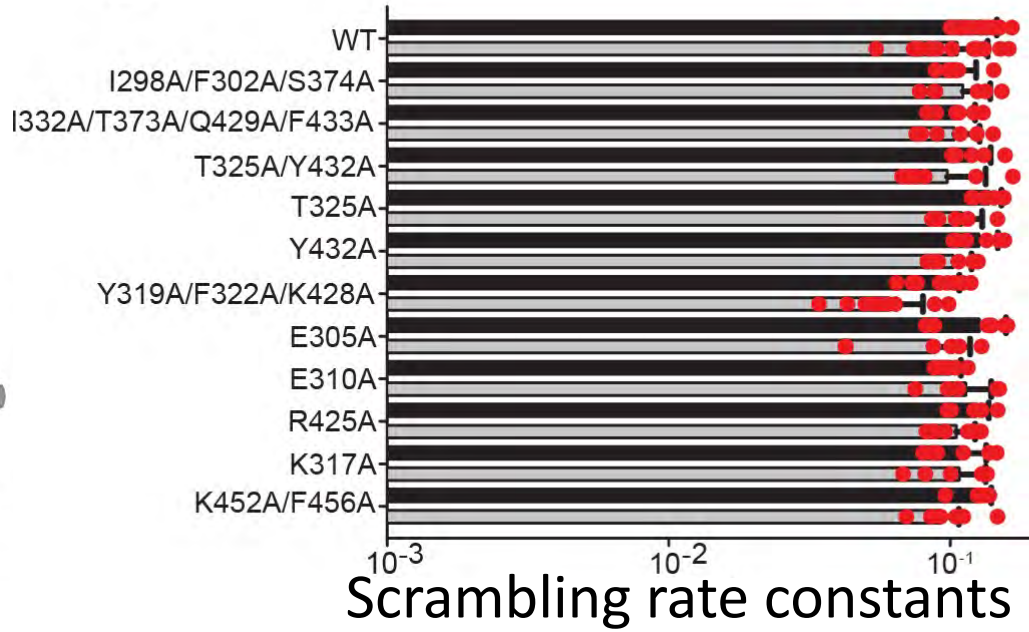


P3 binding site



Central constriction



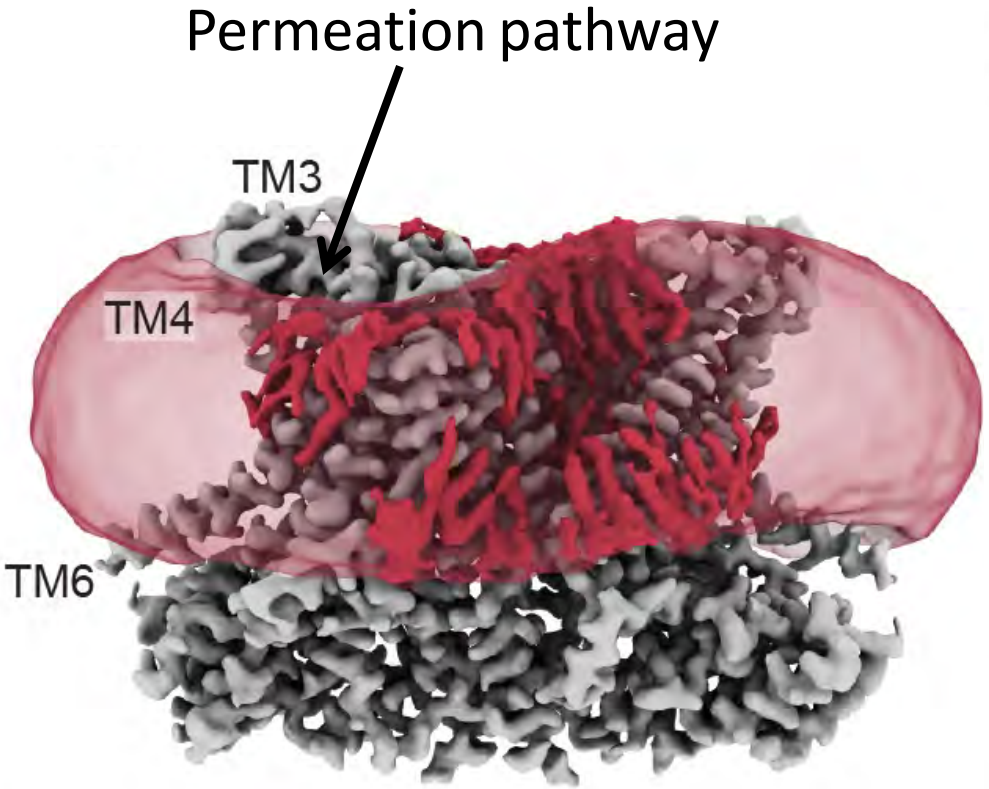


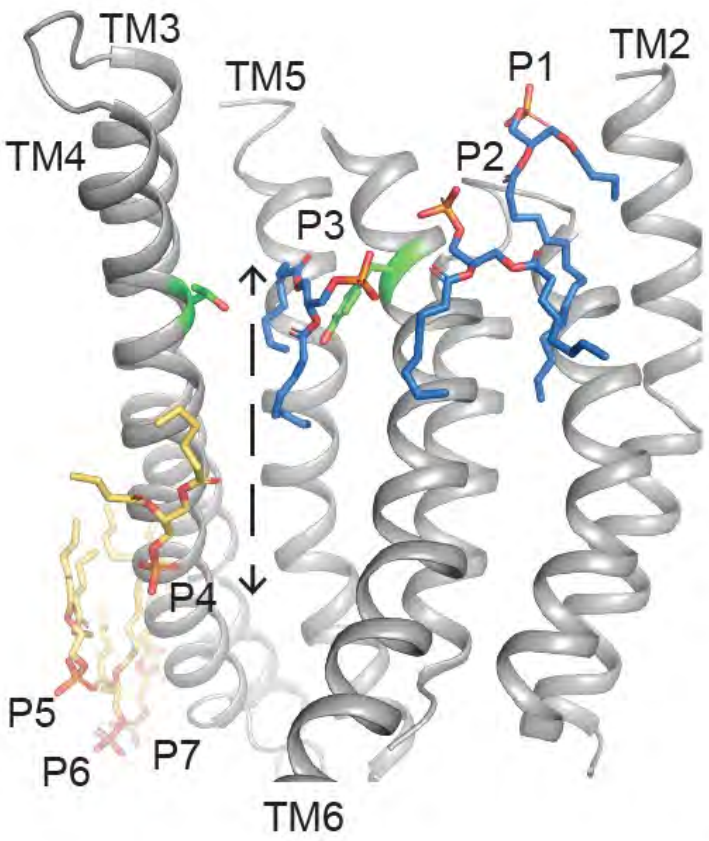
Supports our hypothesis that flipping occurs between P3 and P4

Suggests that scrambled lipids do not need to enter the pathway as proposed by the credit card model

Falzone, Feng et al., (2022) *Nature Communications*.

A role for membrane thinning in scrambling?

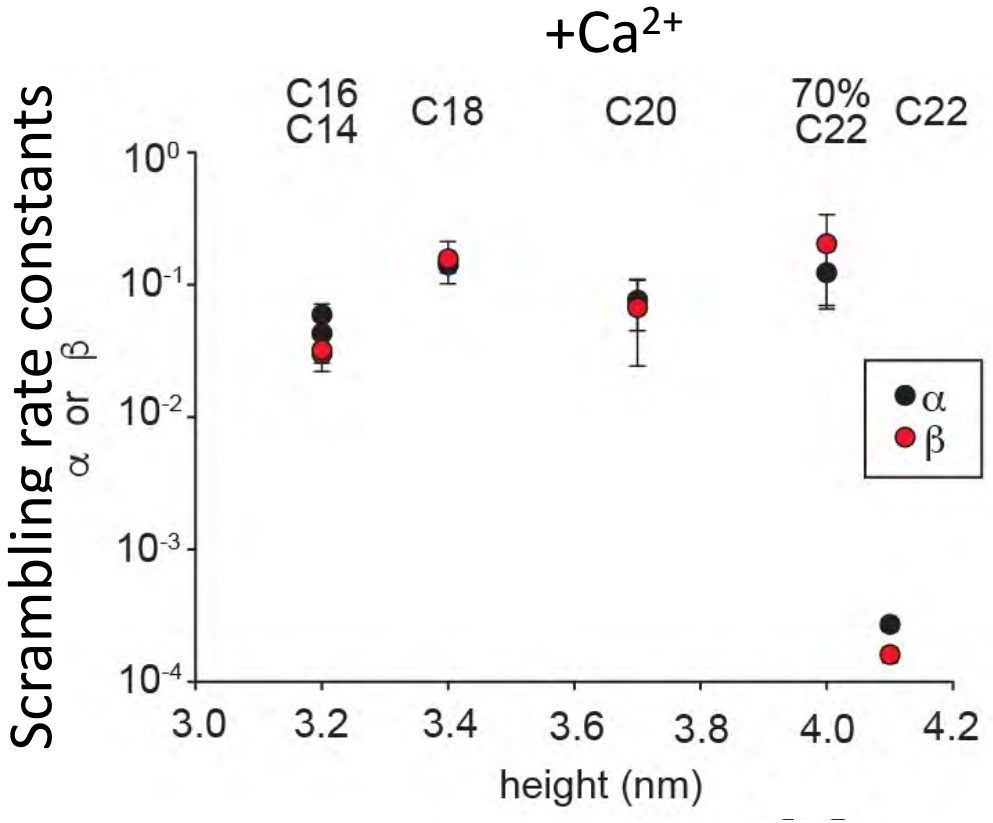




- Hypothesis: membrane thinning is the main mediator of scrambling
  - Consistent with previous results showing that afTMEM16 and hTMEM16K are modulated by membrane thickness
  - *Investigated the relationship between membrane thickness and scrambling function*

Falzone, Feng et al., (2022) *Nature Communications*.

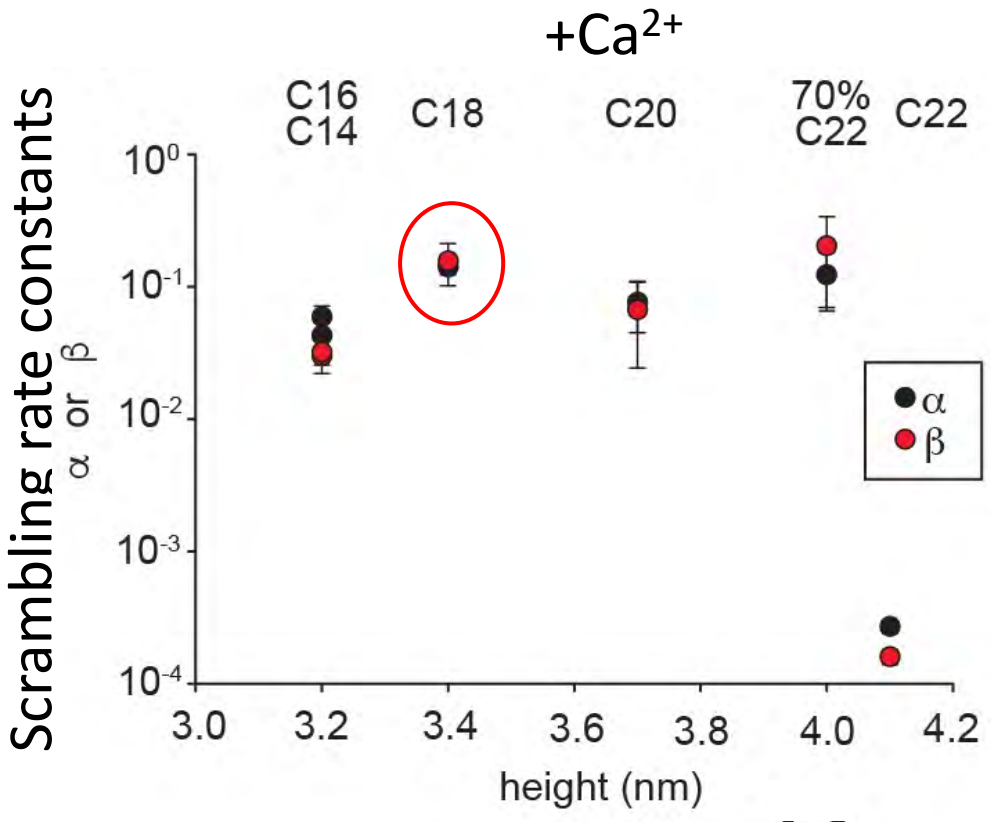
afTMEM16 is inhibited by thicker membranes



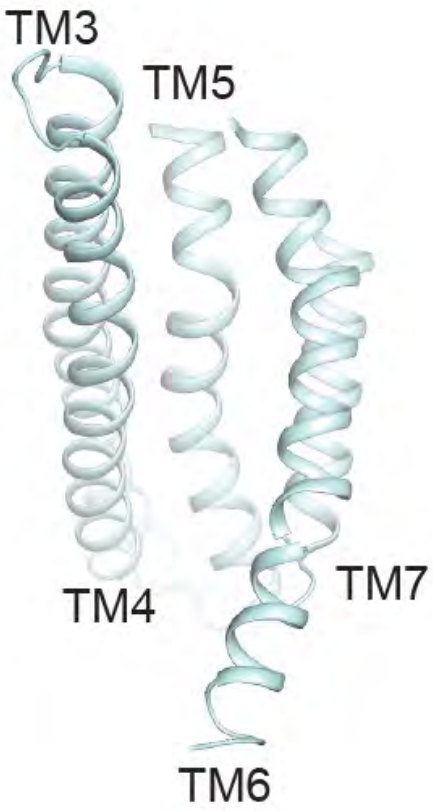
- In the presence of Ca^{2+}, scrambling is unaffected by thickness below 4 nm
  - Above 4 nm (C22 lipids), scrambling is completely inhibited

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes



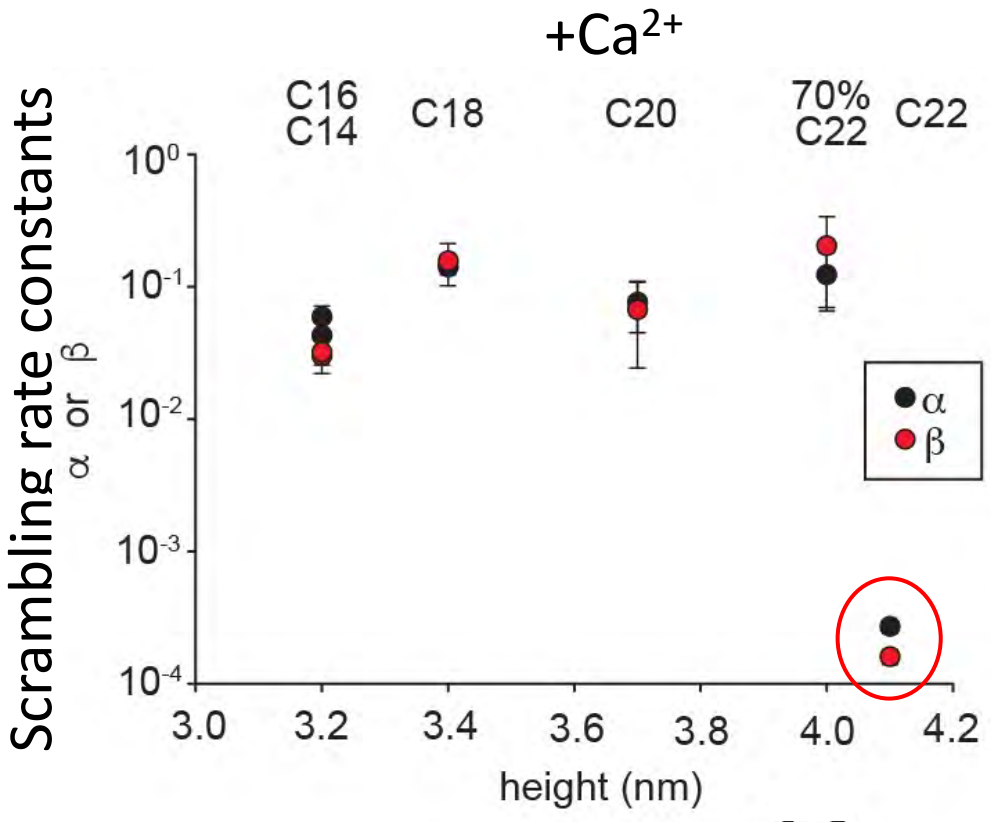
C18 +Ca^{2+}, active, open



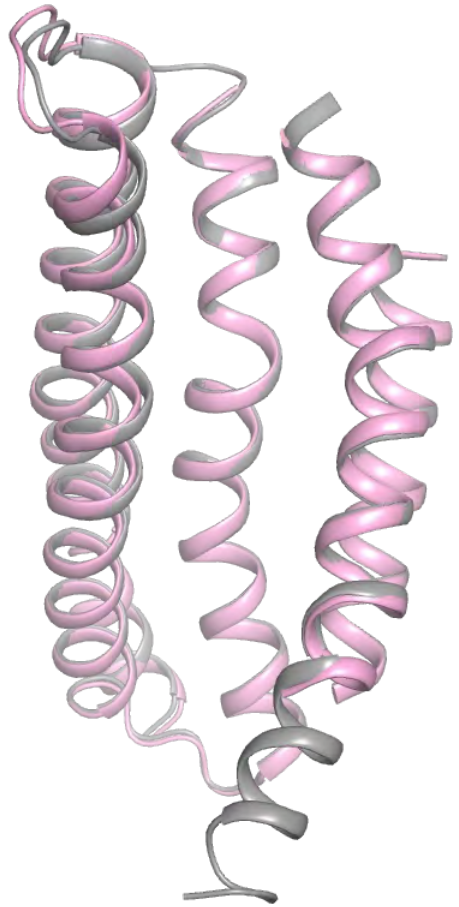
- What is the mechanism of inhibition?
- C18 is our control condition with full activity
  - Previous structure in the presence of Ca^{2+} was determined in C18 lipids and has an open pathway

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes



C22 +Ca^{2+} (pink), inactive, open

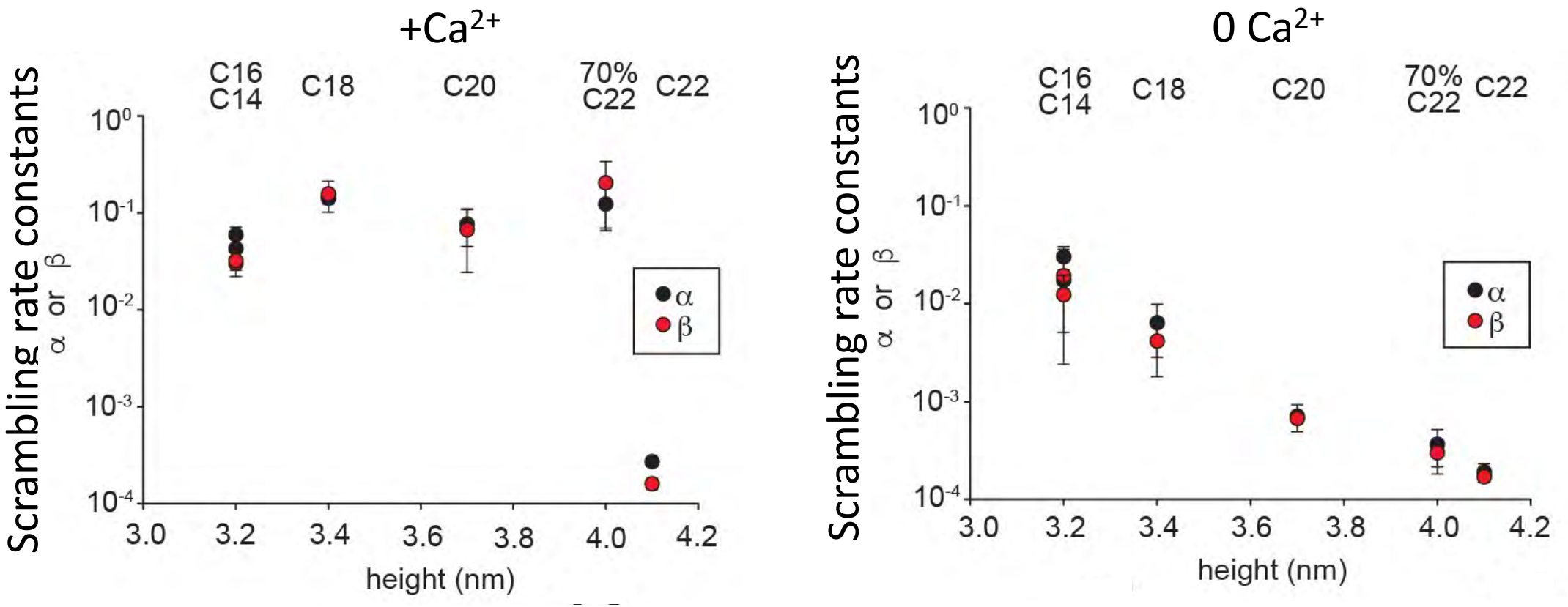


- C22 has ~500-fold less activity
  - But the permeation pathway is still open

Thicker membranes do not alter gating of the permeation pathway

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes

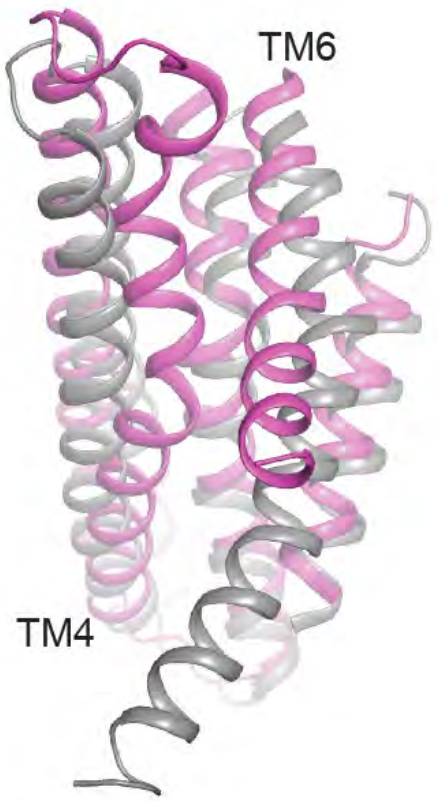


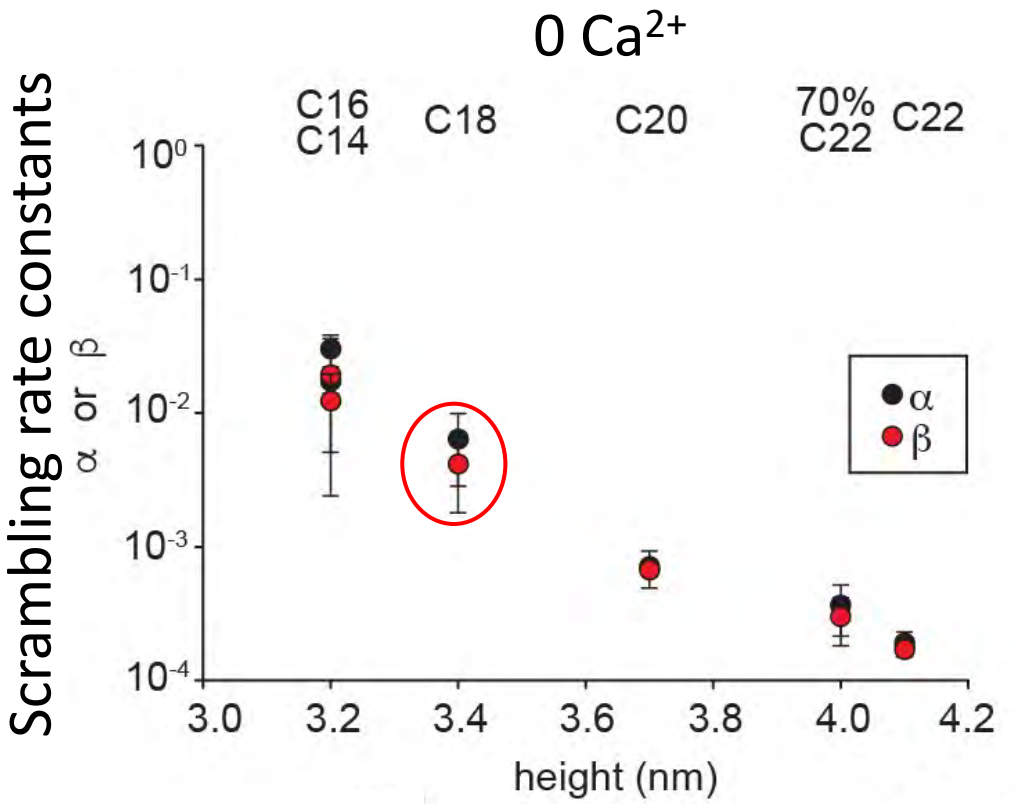
- In the absence of Ca^{2+}, scrambling shows a near exponential relationship with membrane thickness

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes

C18 0Ca^{2+} (pink), slow, closed

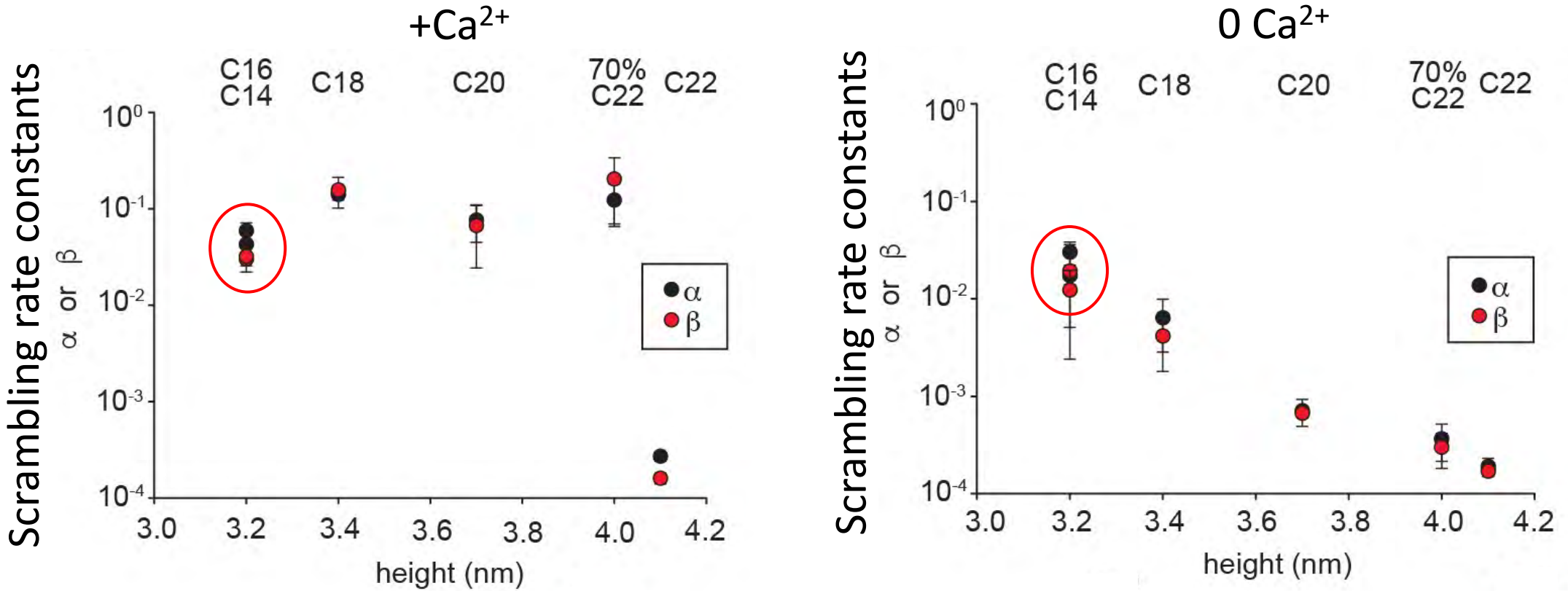




- In the absence of Ca^{2+} where scrambling is slow the pathway is closed

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes

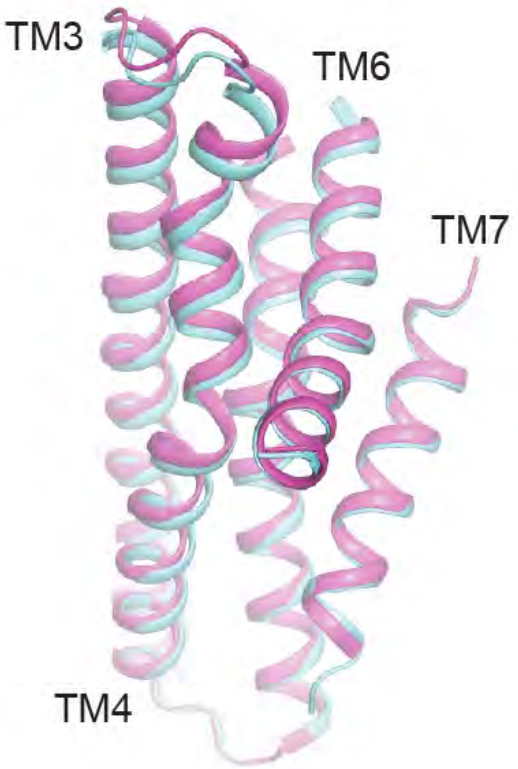


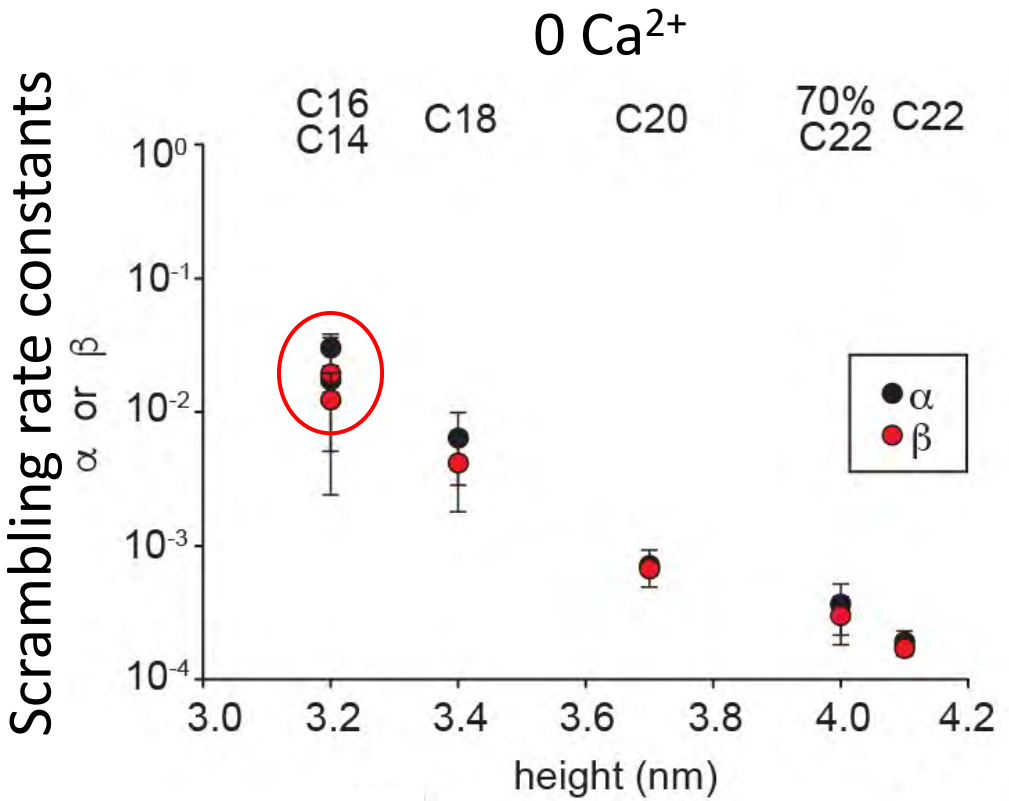
- In C14 lipids, scrambling is almost Ca^{2+}-independent

Falzone, Feng et al., (2022) *Nature Communications*.

afTMEM16 is inhibited by thicker membranes

C14 0Ca^{2+} (blue), active, closed



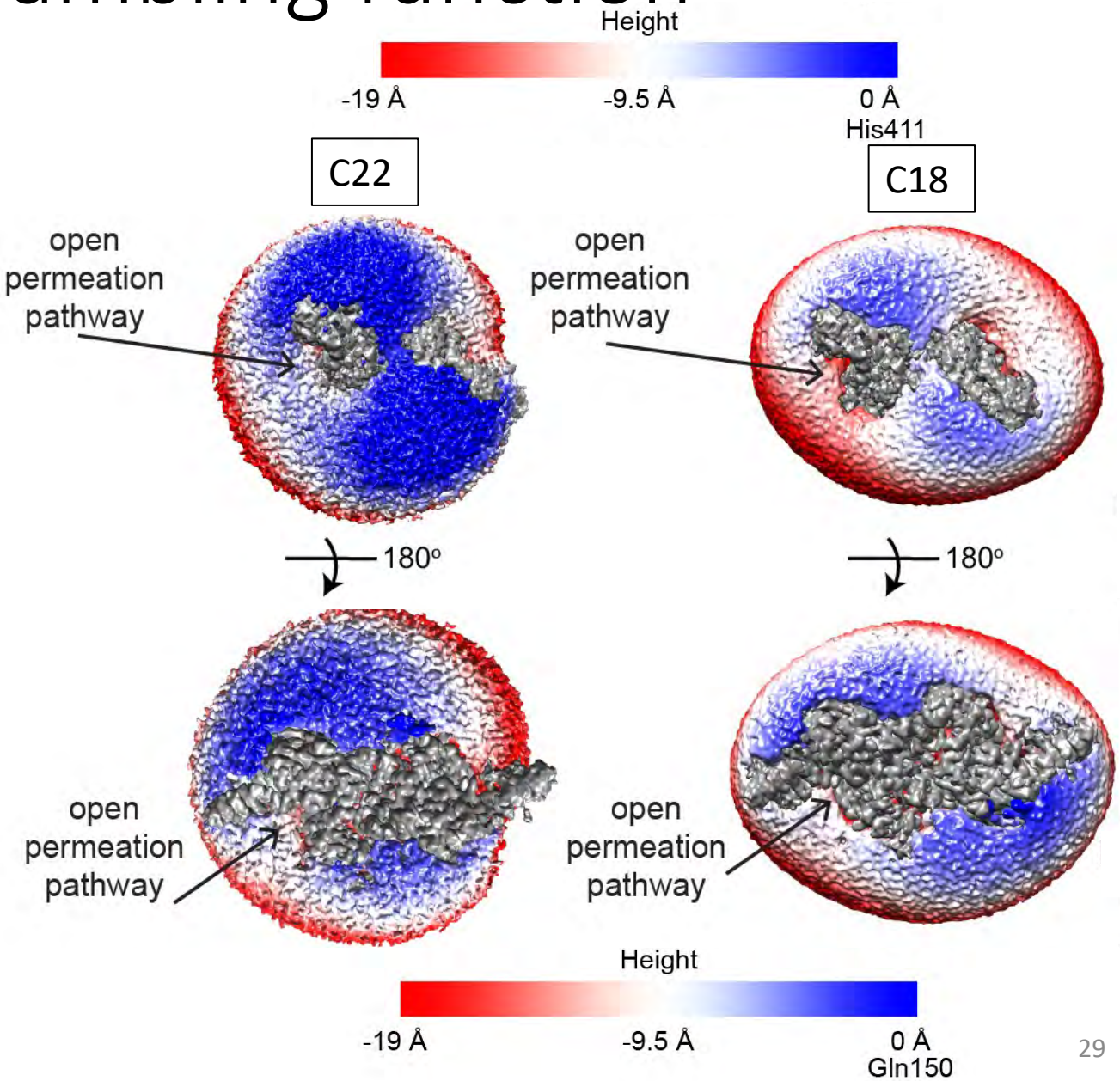


- In C14 lipids, scrambling is almost Ca^{2+}-independent
  - But the permeation pathway is still closed without Ca^{2+}

Falzone, Feng et al., (2022) *Nature Communications*.

Membrane thickness at the permeation pathway correlates to scrambling function

- Colored the density from the nanodisc membranes by height relative to a fixed point on the protein near the outer leaflet (His411) or the inner leaflet (Q150)
- Highlights overall difference in thickness between the C18 and C22 membranes
- Shows that the membrane is also thicker at the pathway in C22 lipids, suggesting that a lack of thinning might explain the lack of function



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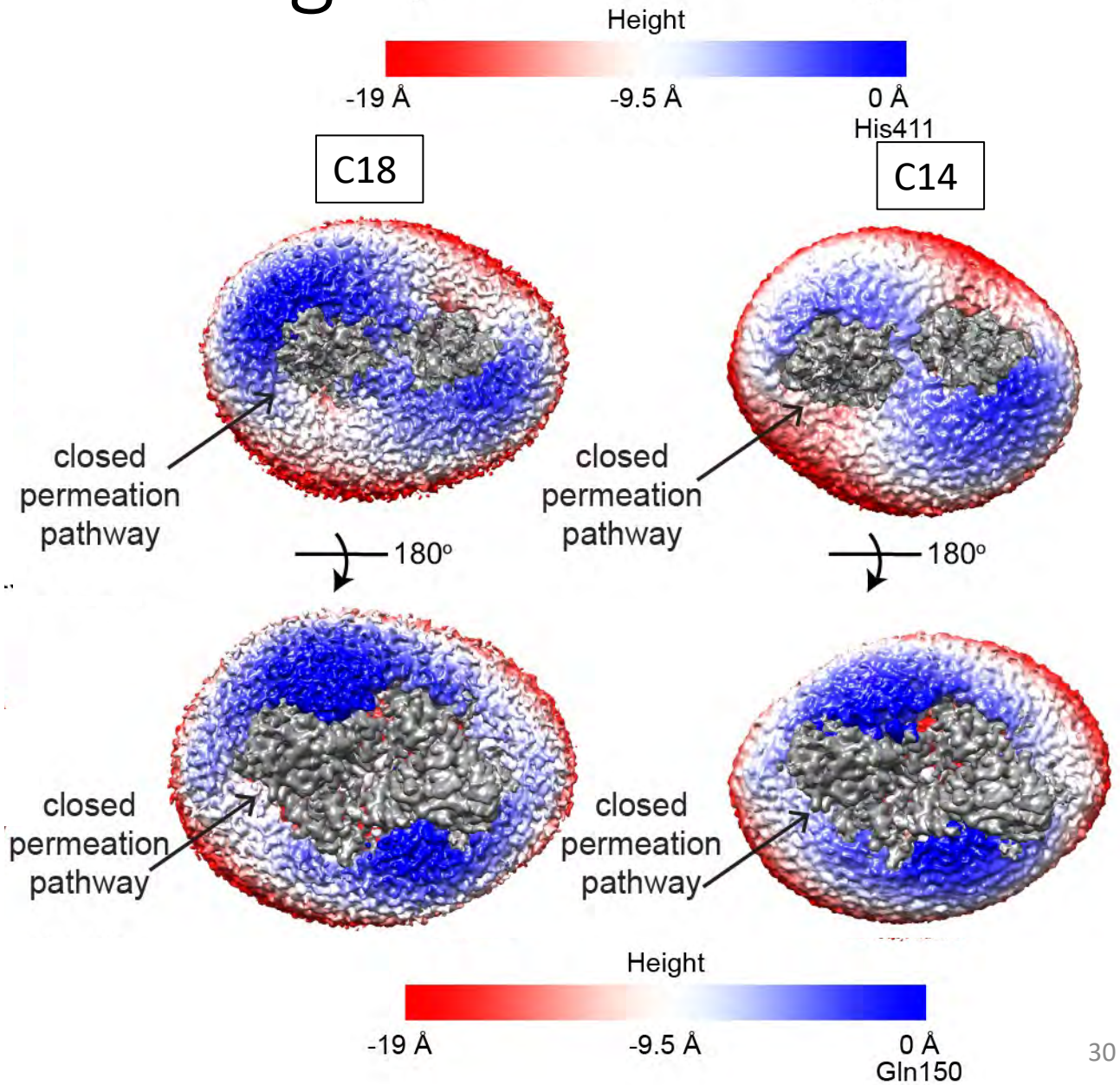
- Highlights overall difference in thickness between the C18 and C14 membranes
- Shows that the membrane is also thinner at the pathway in C14 lipids, suggesting that this could explain the enhanced function



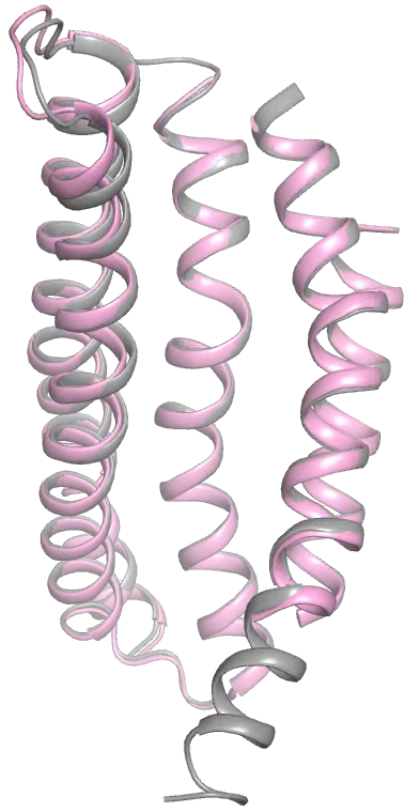
Falzone, Feng et al., (2022) *Nature Communications*.

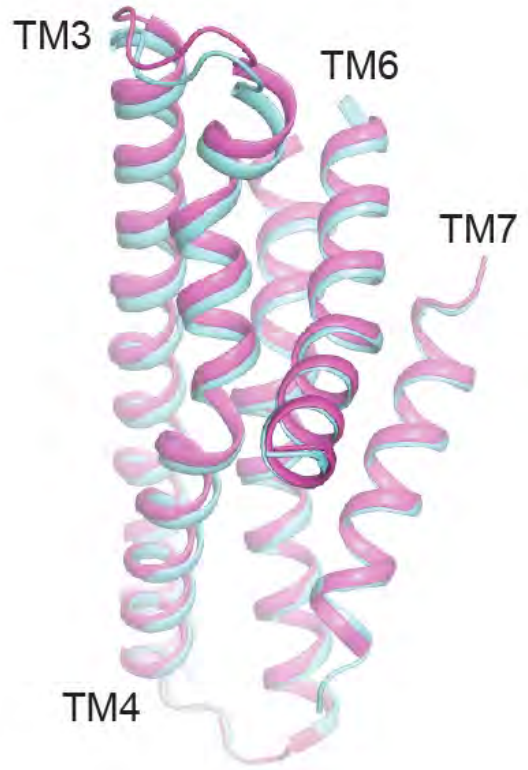
Ca^{2+} gates the permeation pathway but membrane thinning controls scrambling

- Scrambling is inhibited in thicker membranes despite an open permeation pathway
- Scrambling in the absence of Ca^{2+} is enhanced in thinner membranes despite a closed permeation pathway

C22 +Ca^{2+} (pink), inactive, open

C14 0Ca^{2+} (blue), active, closed





Suggests that Ca^{2+} binding gates the conformation of the pathway but protein-imposed membrane thinning determines if scrambling occurs, regardless of the pathway conformation

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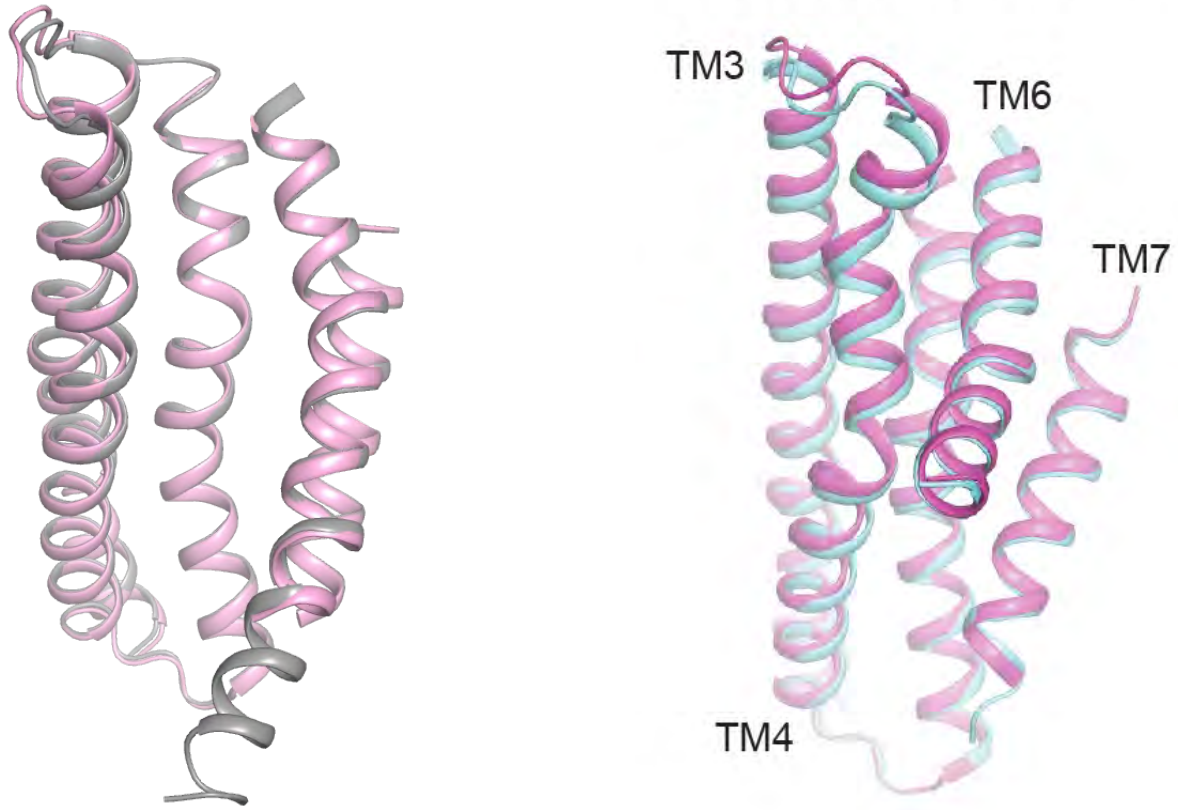
Ca^{2+} gates the permeation pathway but membrane thinning controls scrambling

- We hypothesize that in cells membrane thickness is used to impose an additional layer of regulation on TMEM16s
  - For example, scramblases could be localized to a thicker membrane region to reduce scrambling despite the presence of intracellular Ca^{2+}.
- Thinning-mediated scrambling can be applied to other scramblases, which lack an explicit wide hydrophilic groove such at XKR's.

C22 +Ca^{2+} (pink), inactive, open

C14 0Ca^{2+} (blue), active, closed



Suggests that Ca^{2+} binding gates the conformation of the pathway but protein-imposed membrane thinning determines if scrambling occurs, regardless of the pathway conformation

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