# Structure and dynamics of membrane transporters - the hybrid approach

SYNEW 2015

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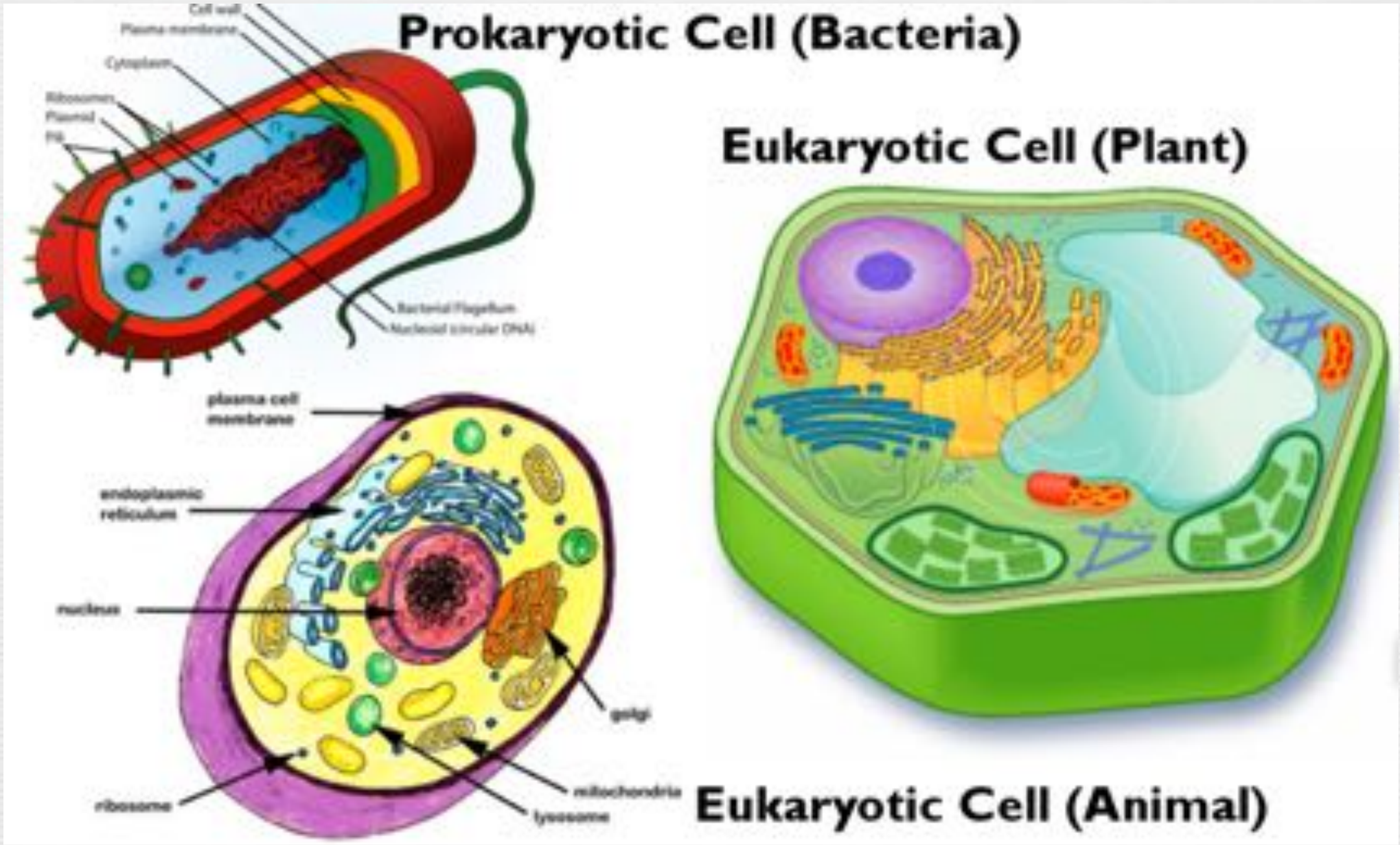
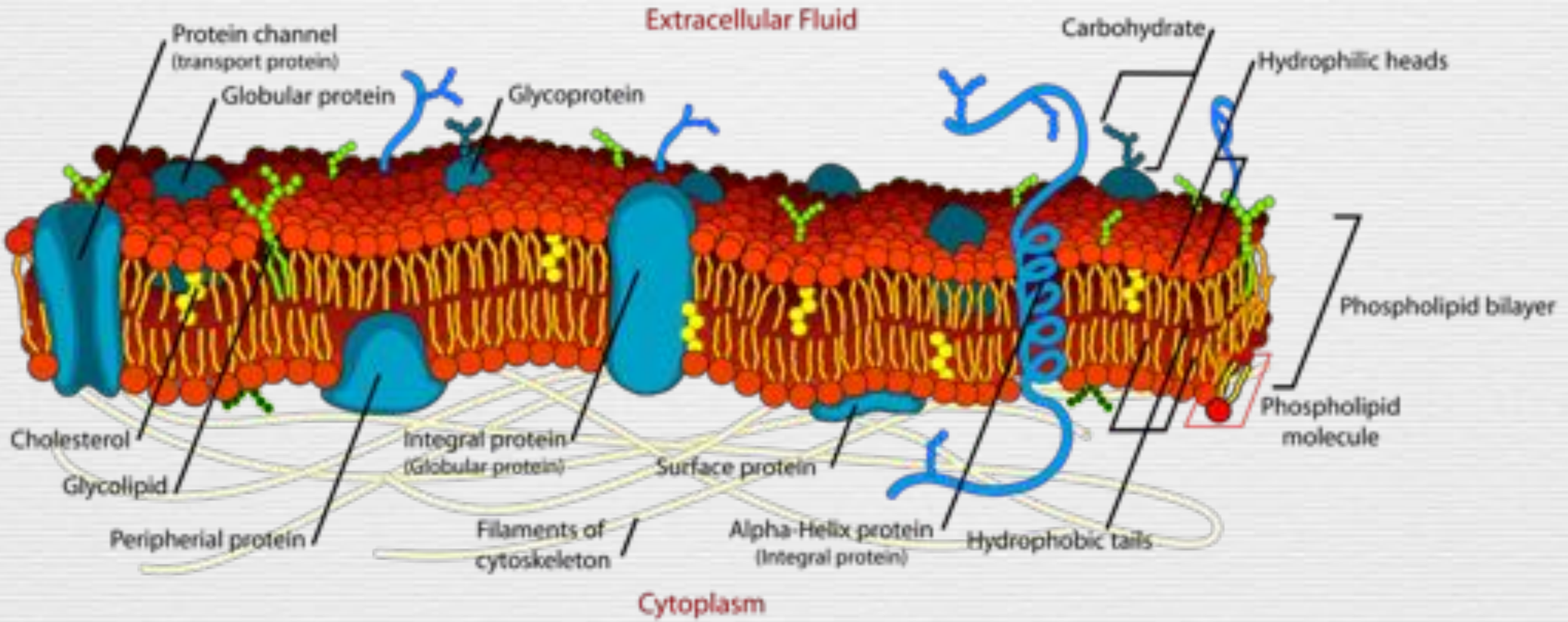
# Outline

- Why membrane proteins?
- Different types of membrane transporters
- Different mechanisms of transport
- Bright future

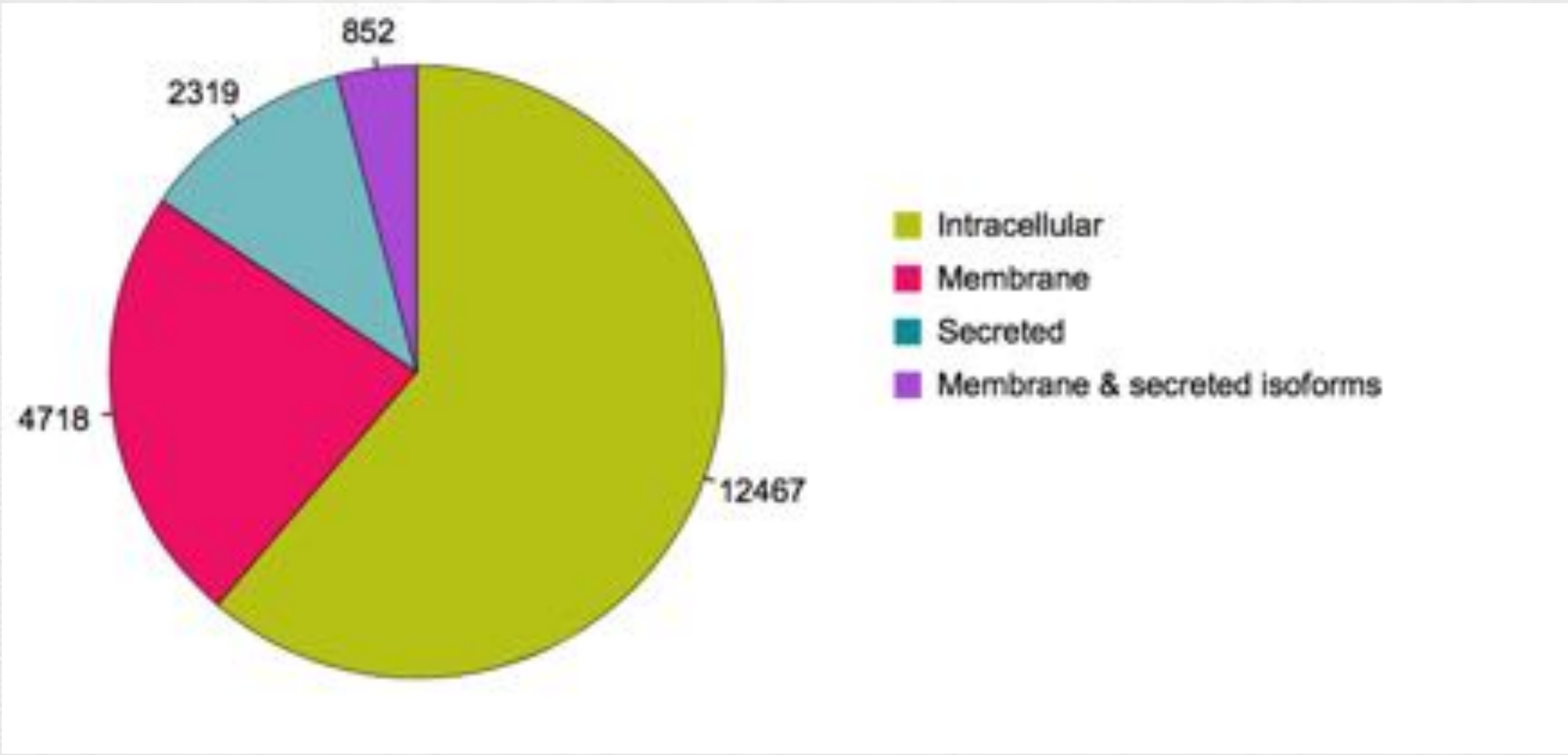
# Pro- and eukaryotic cells



# Membrane structure

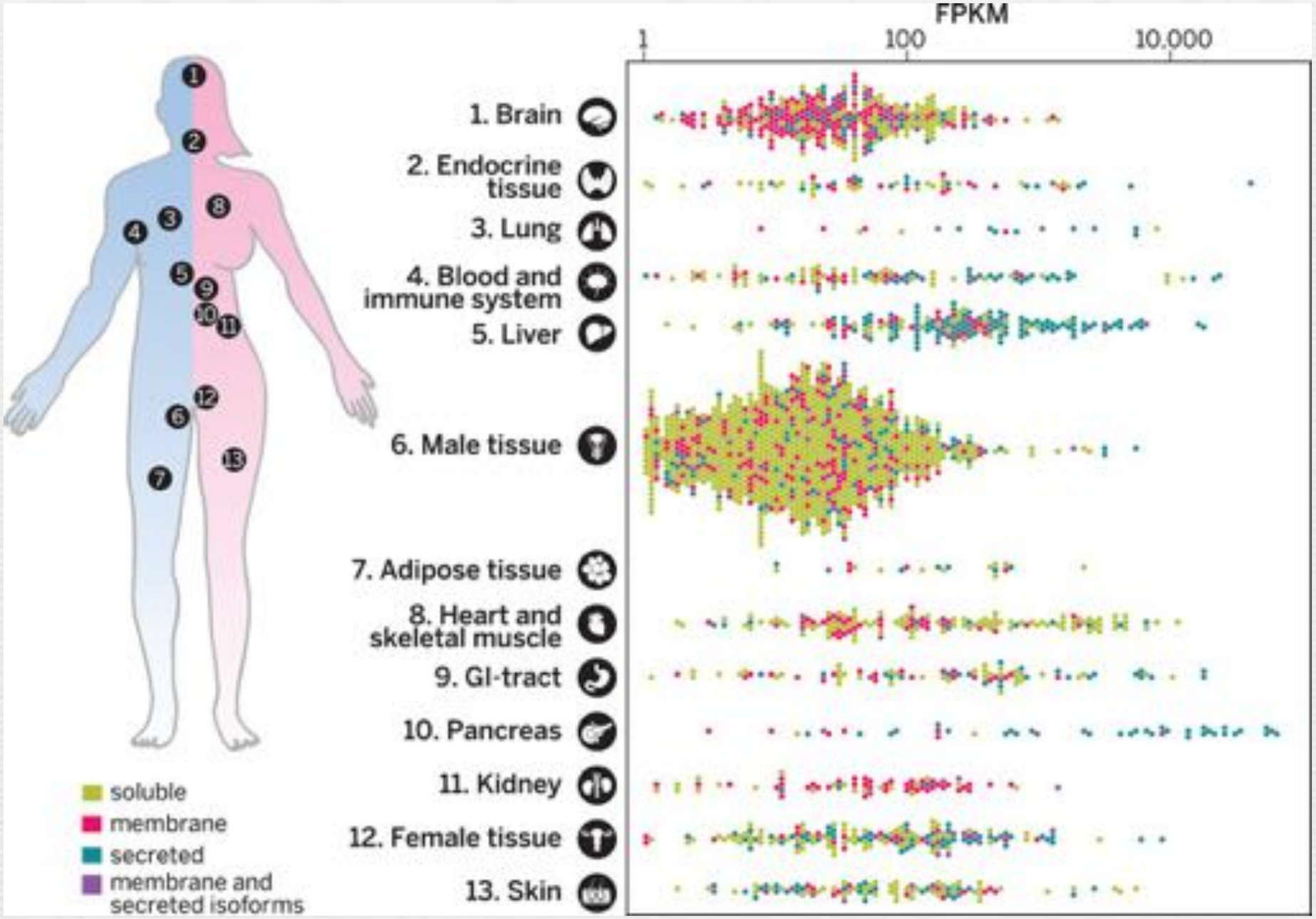


# Membrane proteins in human genome



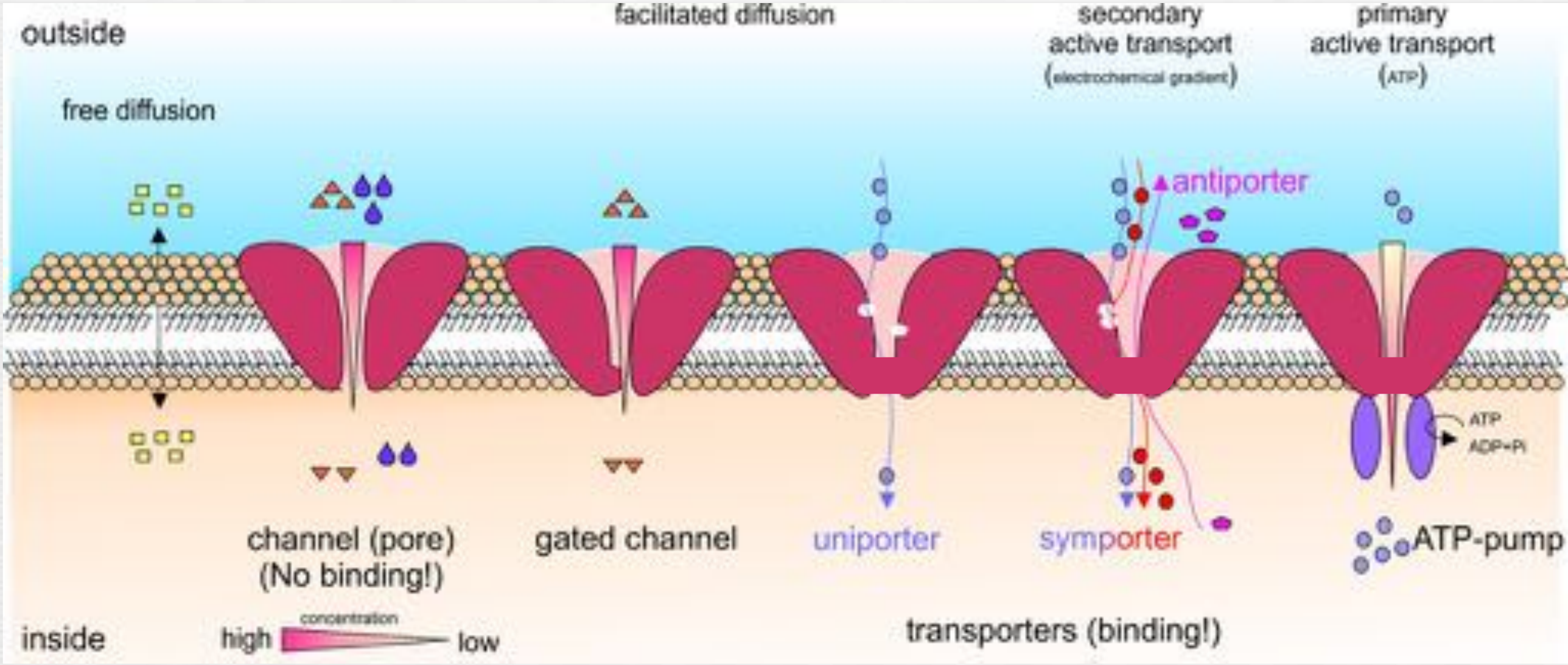

**~25% of genome!**

The human tissue–enriched proteins.All tissue-enriched proteins are shown for 13 representative tissues or groups of tissues, stratified according to their predicted subcellular localization.



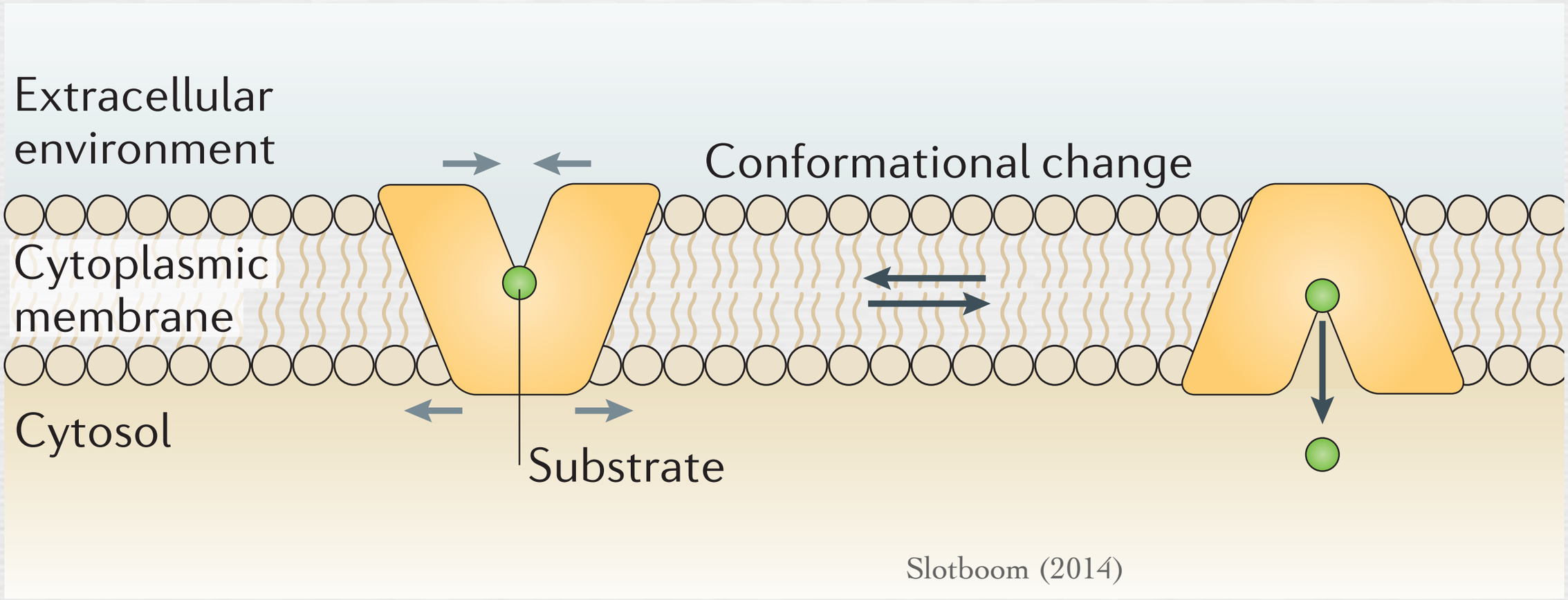

Mathias Uhlén et al. Science 2015;347:1260419

# Types of transporters



# Types of transport mechanisms

## Rocker-switch mechanism

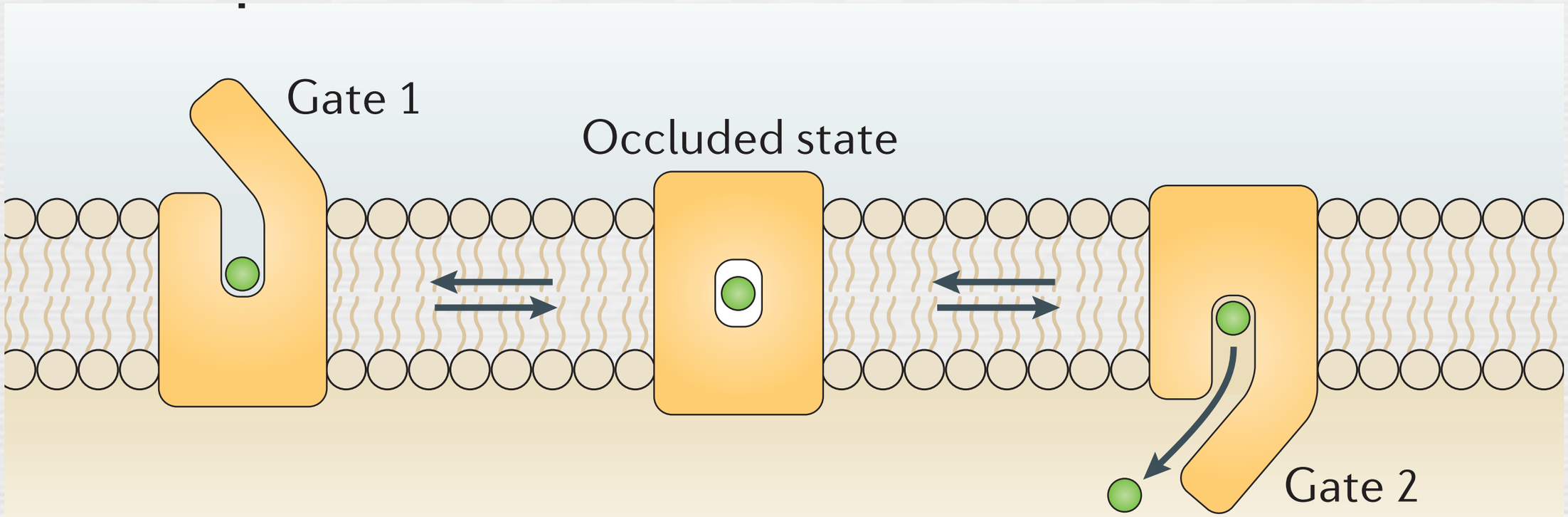



Slotboom (2014)

## Examples

# Types of transport mechanisms

## Gated-pore

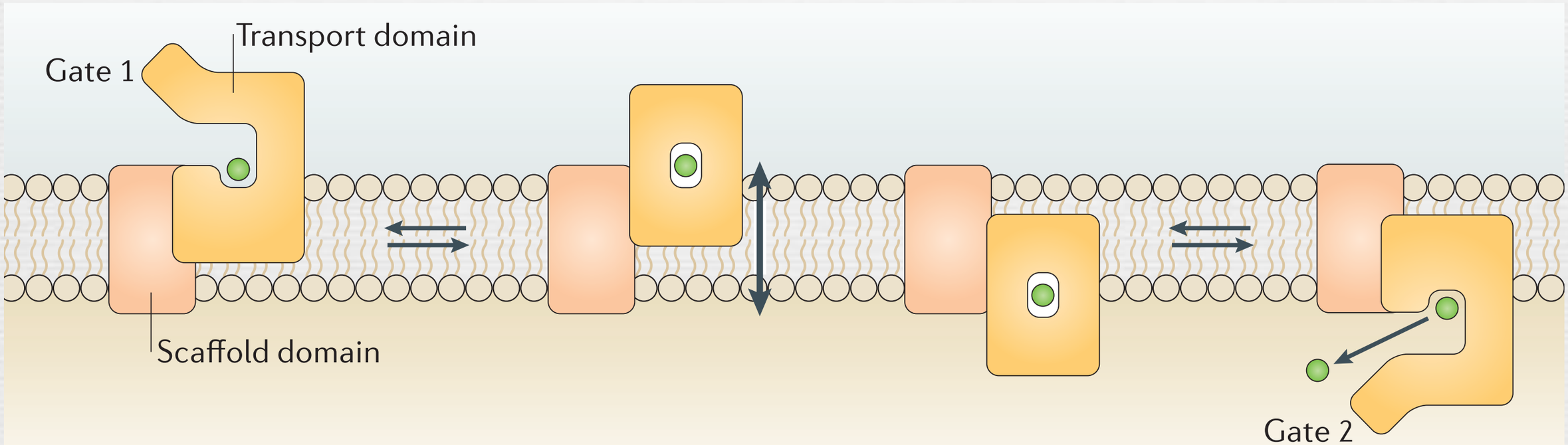



Slotboom (2014)

## Examples

# Types of transport mechanisms

## Elevator

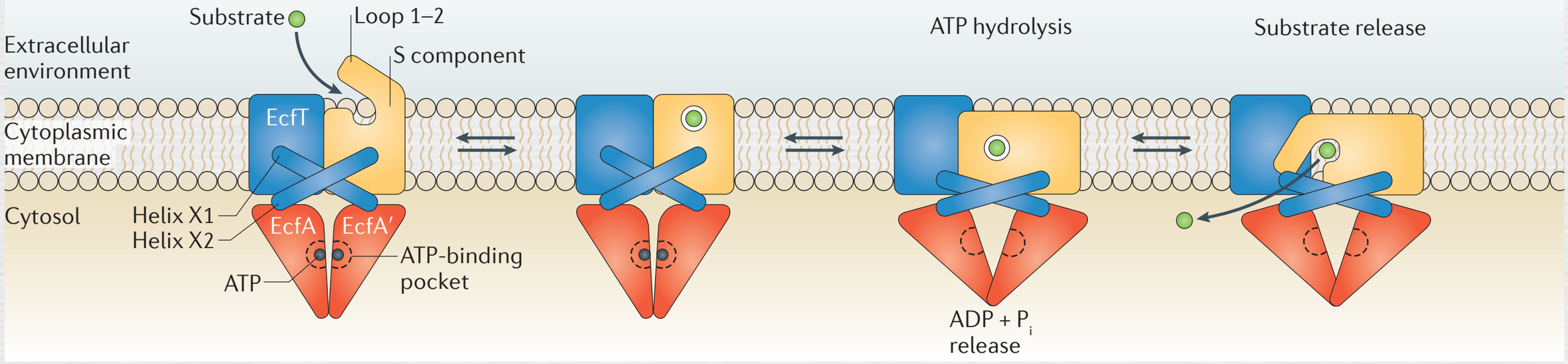



Slotboom (2014)

## Examples

# Types of transport mechanisms

## Toppling




Slotboom (2014)

## Examples

# Need for structures!

- What is the molecular mechanism of transport?
- Can we interfere with (block, modulate, enhance) this transport?
- Can we fix malfunctioning transporters?
- Can we copy this transport for biotechnology?
- How can we get reliable structures, and are the structures enough to answer all these questions?

# Protein Crystallography

>100.000 structures!


Some

1934 - Diffraction from a pepsine crystal (Bernal & Hodgkin)

1954 - Phase problem solved by MIR technique (Perutz et al., Proc. Royal Soc. Lond. A 225, 287–307

1958 - Myoglobin structure (Kendrew et al., Nature 185, 422–427

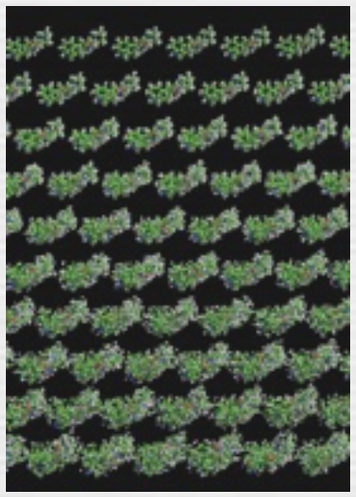
1978 - Tomato bushy stunt virus structure (Harrison et al., Nature 276, 368–373

1985 - First membrane protein structure (photosynthetic center) (Michel et al., Nature, 318 618–624

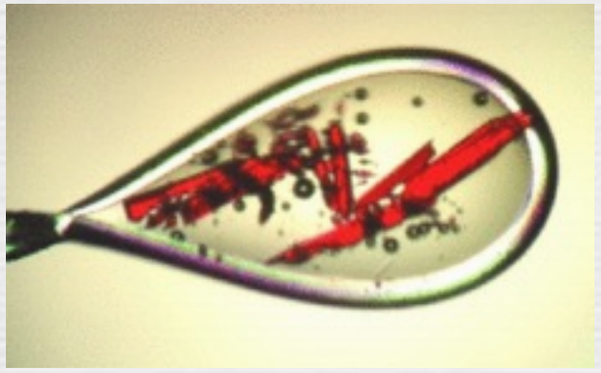
2000 - high-resolution Ribosome structures (Yonath, Steiz, Ramakrishnan)

2007 - First mammalian GPCR structure (Kobilka et al., Nature 450, 383-387

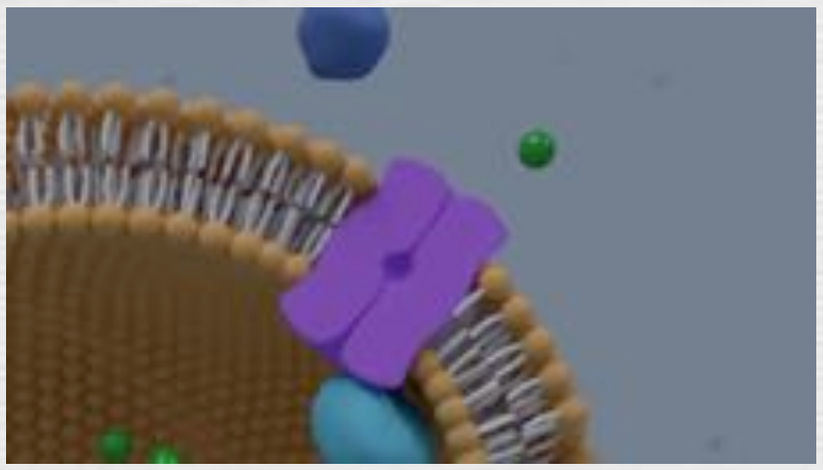
# Limitations of crystallography



Proteins in crystal is not in native conformation

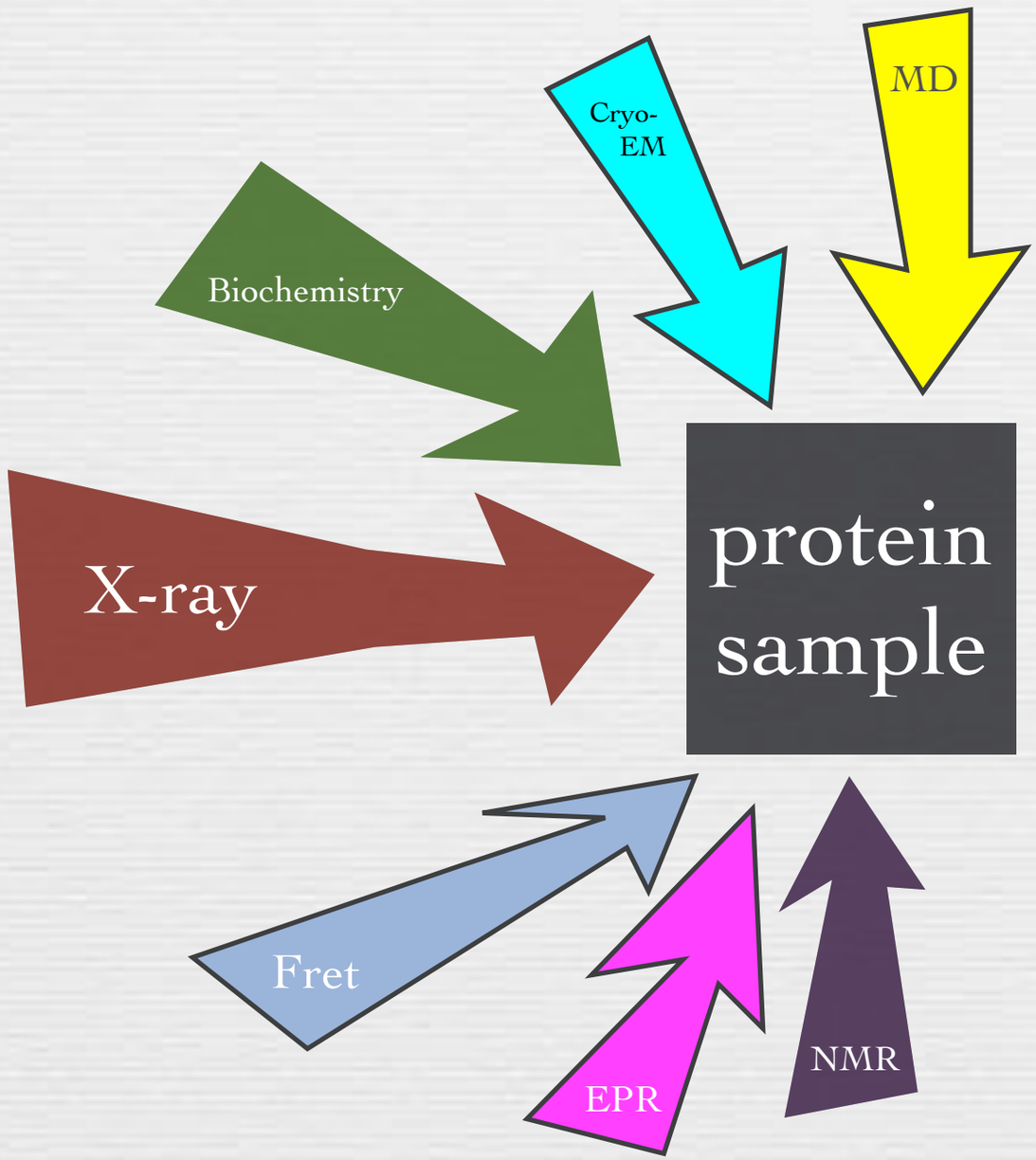


Radiation damage



Static picture!

# Synergetic approach



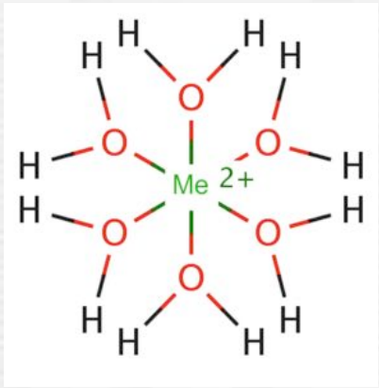

:

+ dynamics

# Examples of different transport mechanisms:

## 1. CorA Gated-channel

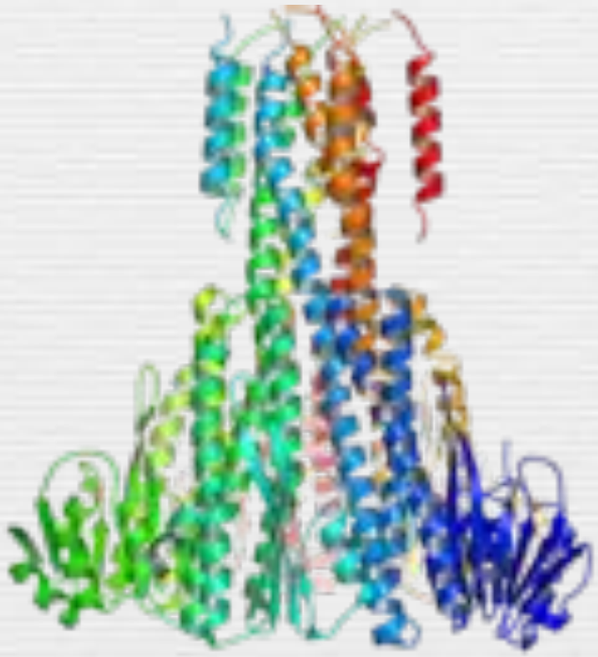
# The mystery of Mg



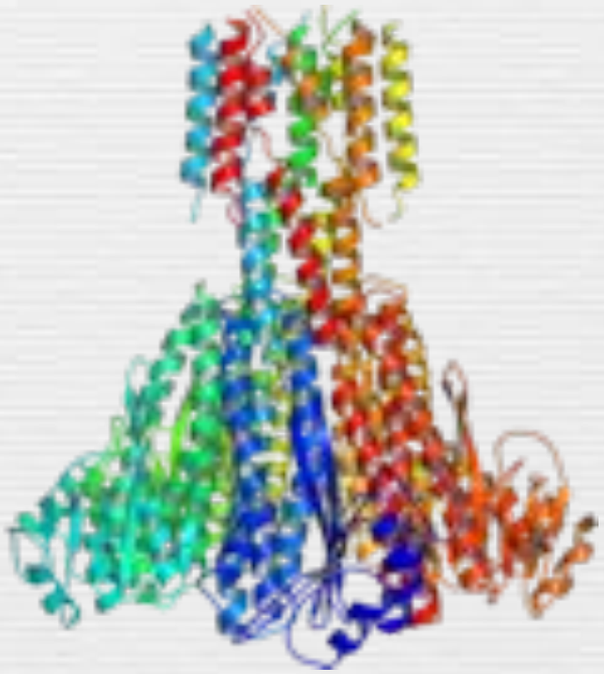

- The most abundant divalent cation in living cells
- Versatility – from structural roles to signalling (photosynthesis, Calvin cycle, ATP, DNA, RNA, enzymes, membrane walls…)
- The biggest difference between hydrated and ionic volume ~400 times!
- And very strong binding of water molecules due to the high charge density

# 2006: three structures of CorA

2.9Å Eshaghi et al



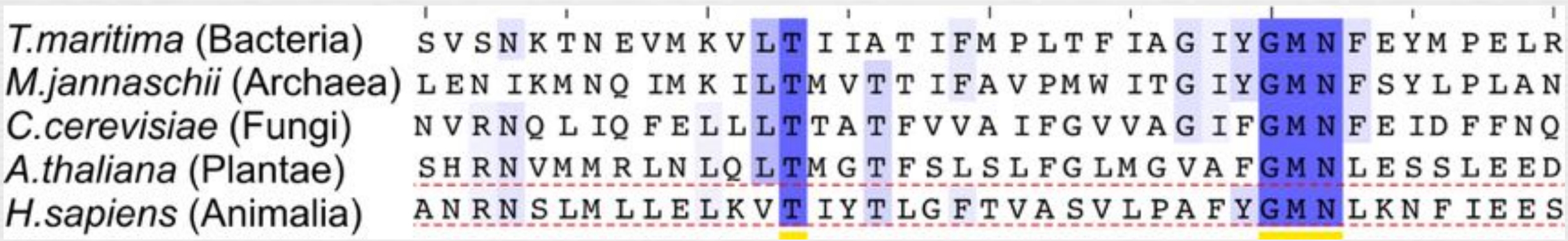
3.9Å Lunin et al



3.7Å Payadeh & Pai

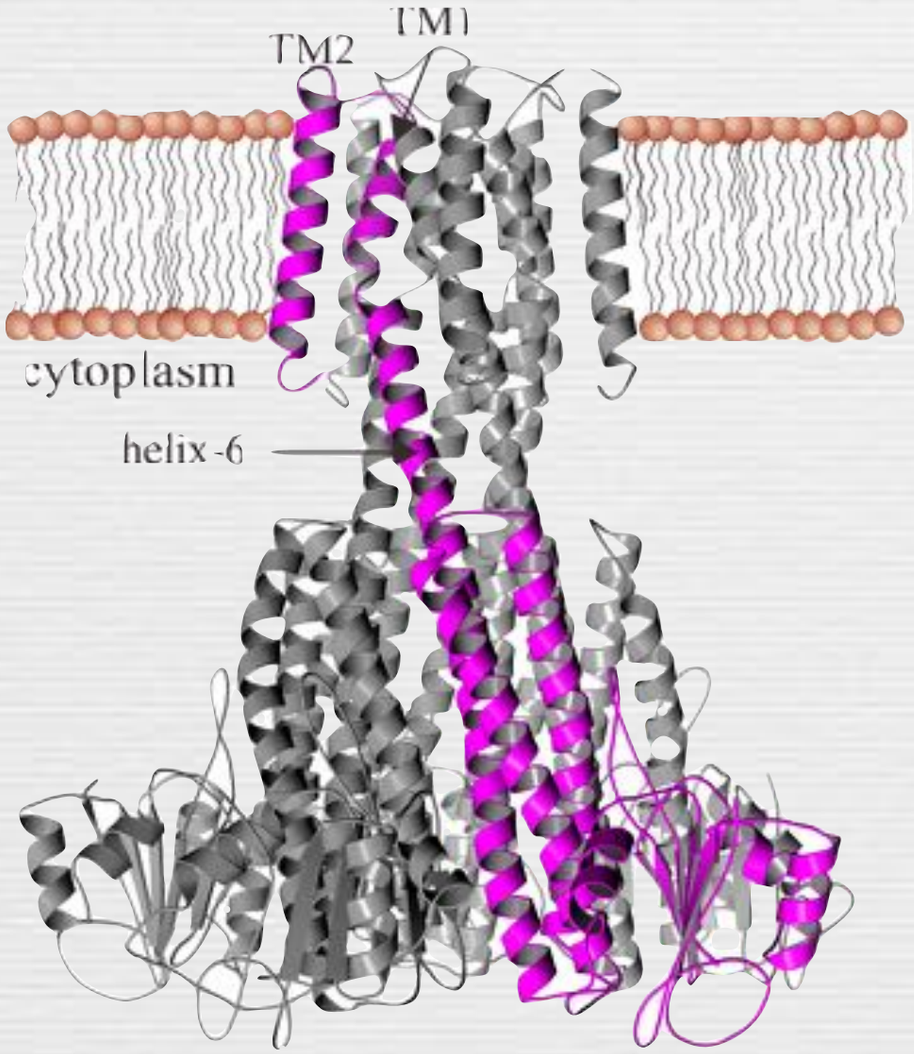

- Are all CorAs pentamers or some are tetramers?
- Only Mg
- What is the structure of the periplasmic loop (and thus selectivity filter)?
- How does the transport occur ( through the long hydrophobic “neck”)?
- What is the actual gating mechanism?

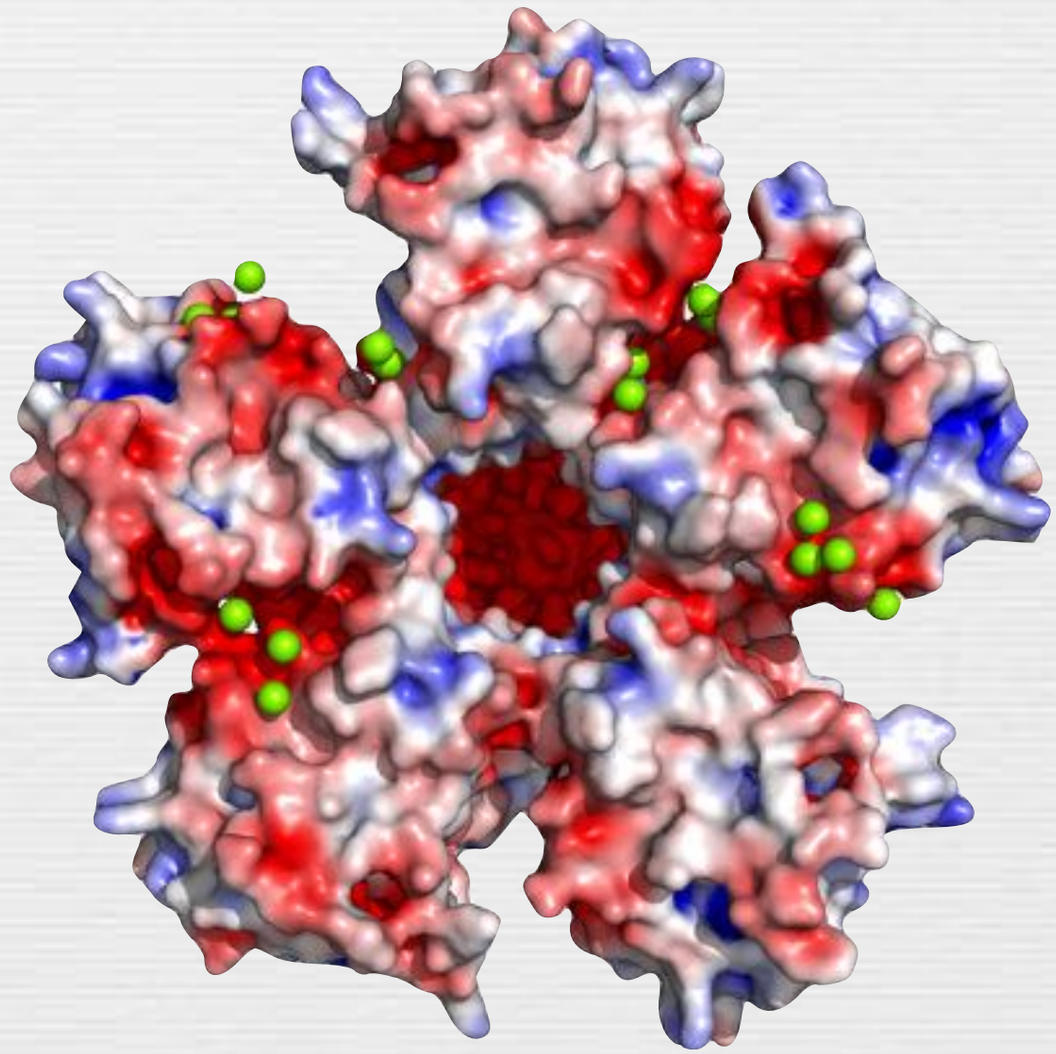
# The sequence diversity of the superfamily




Almost no identity among Kingdoms,
Very low identity even within Kingdoms (17-25%)

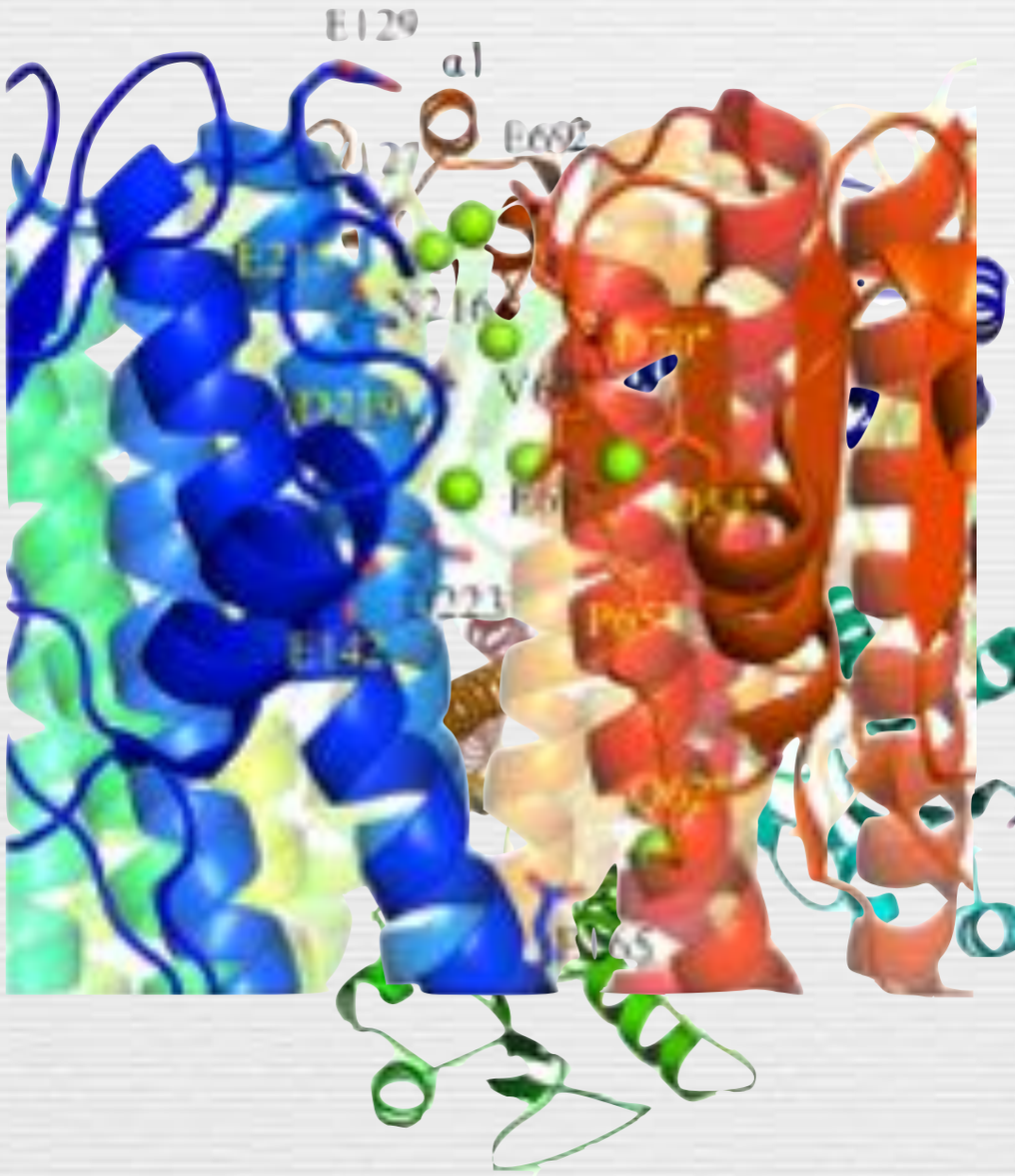
# The general architecture

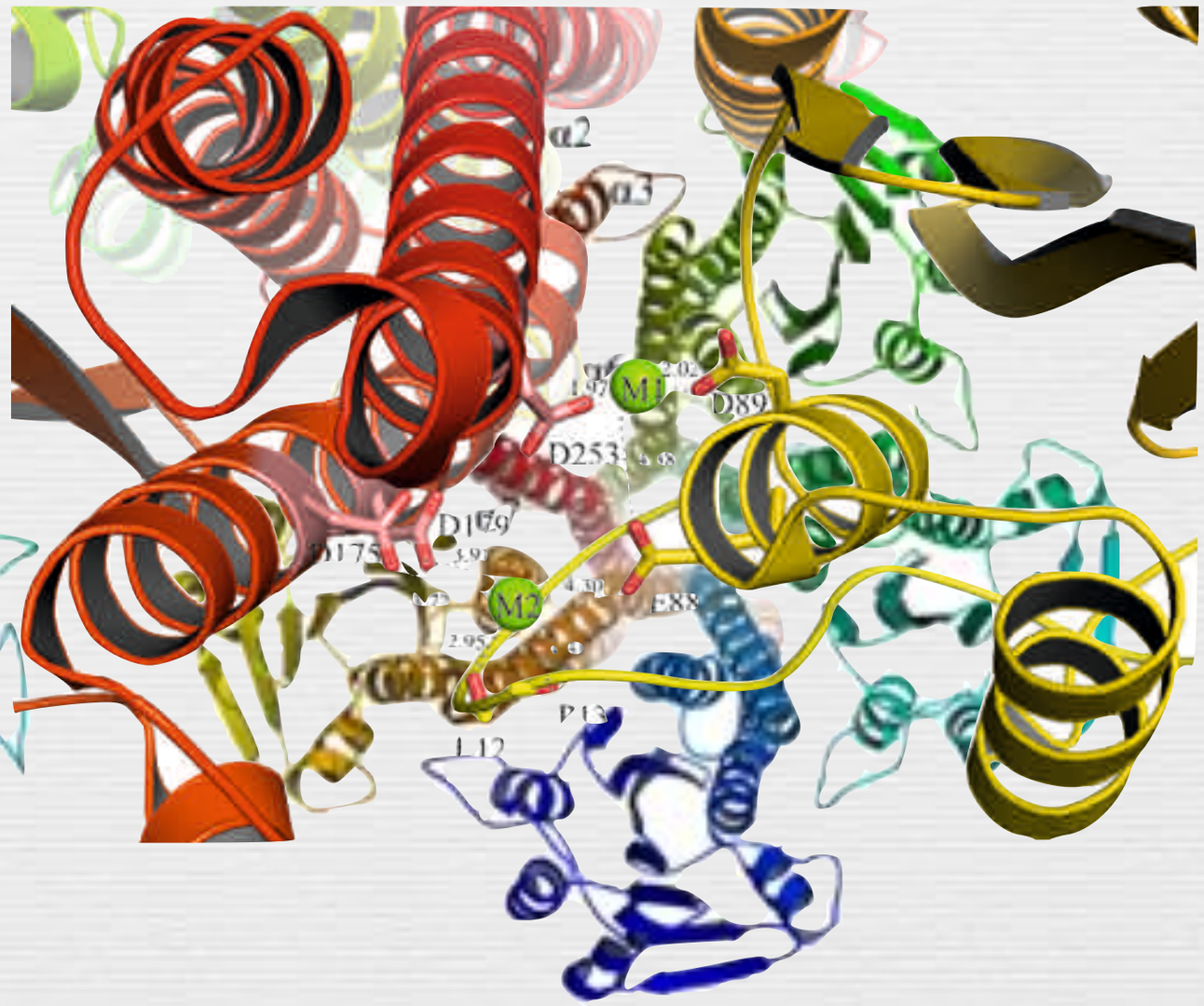


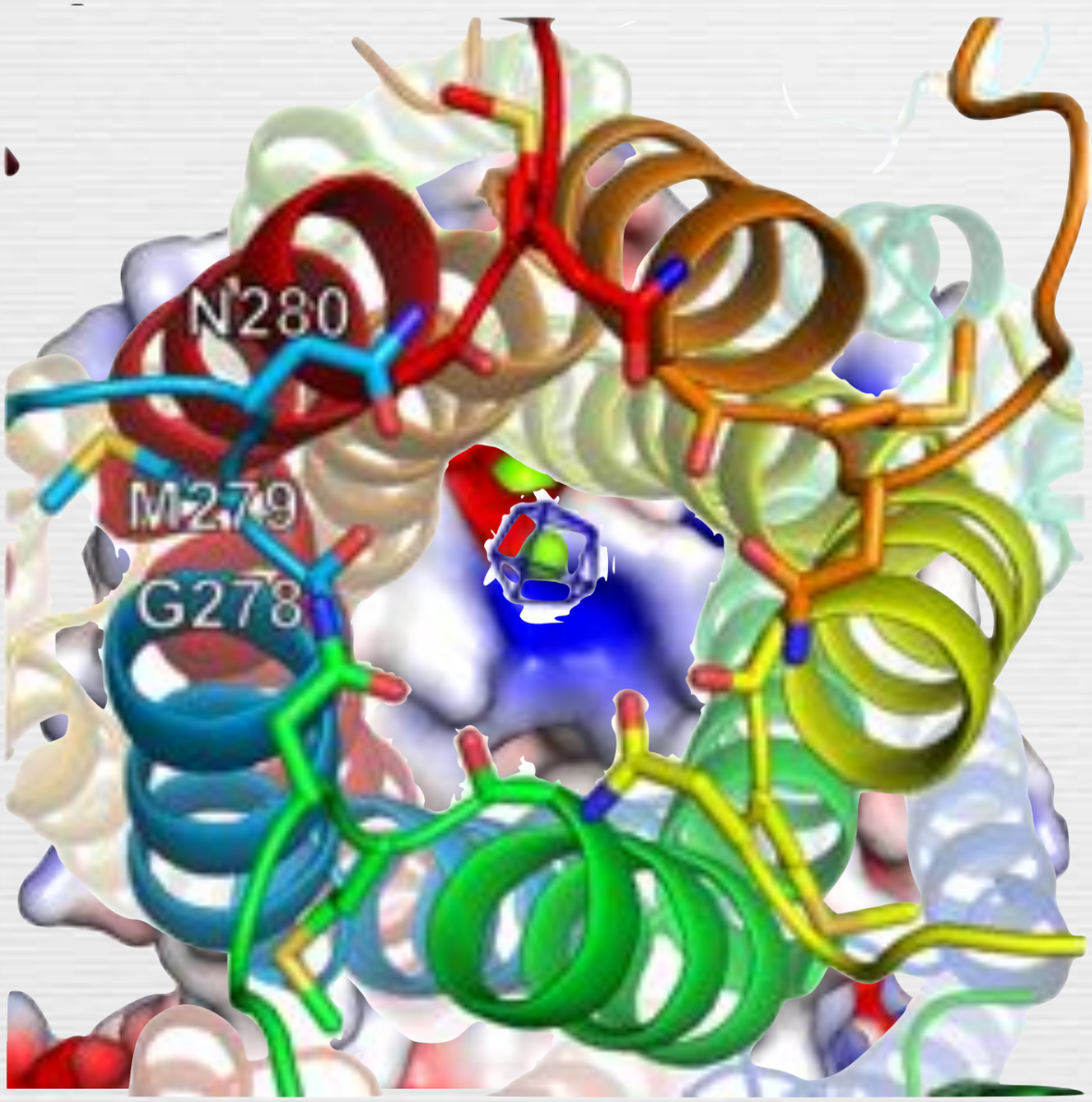
Guskov et al., (2012) PNAS,

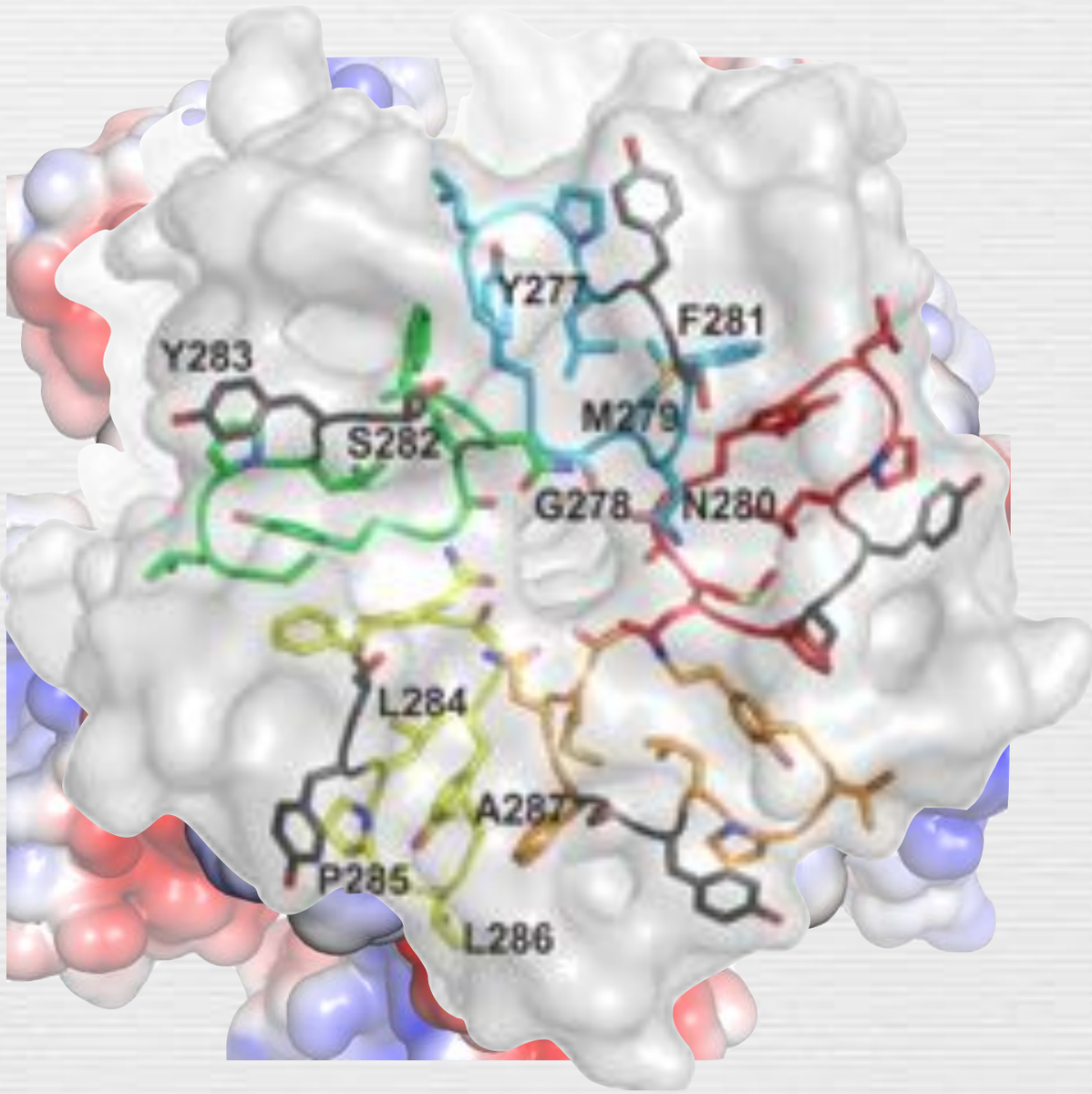
# Comparison with TmCorA



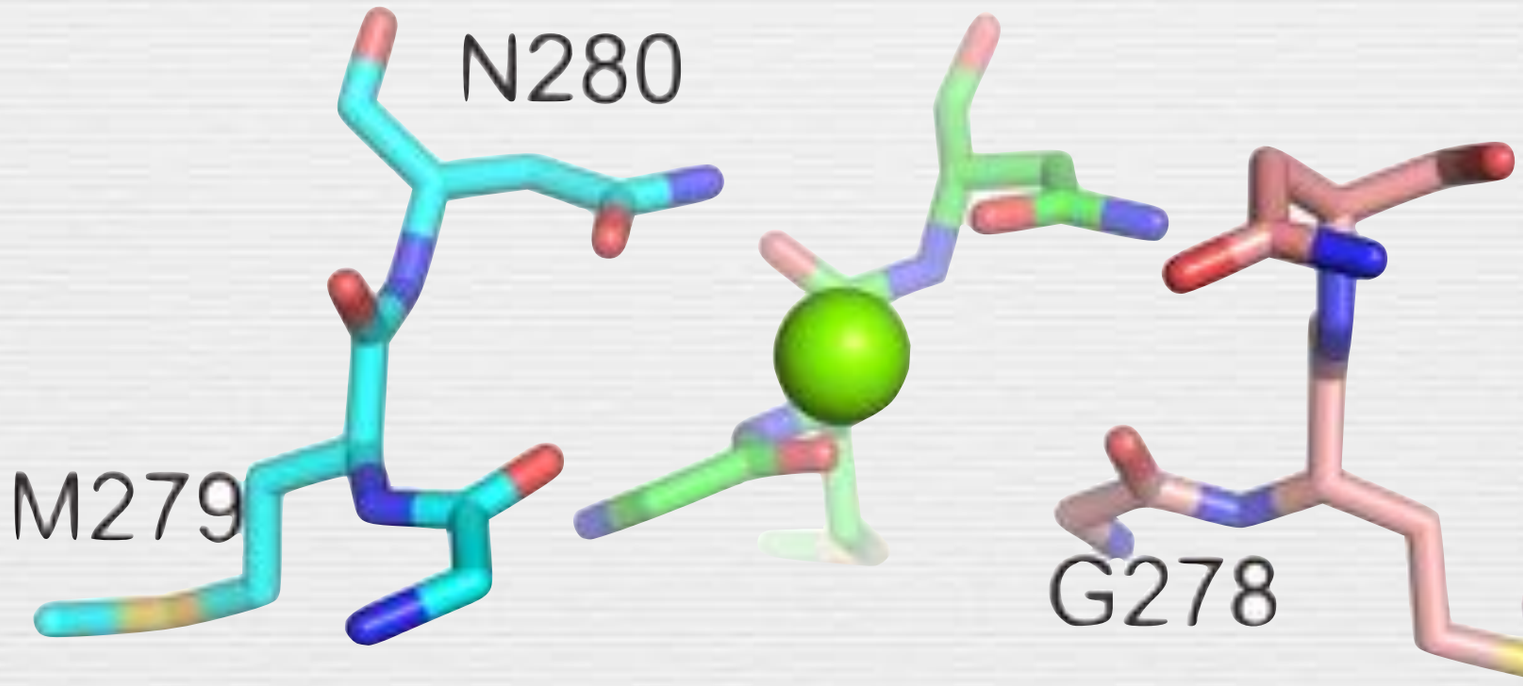



# Concavity and selectivity filter



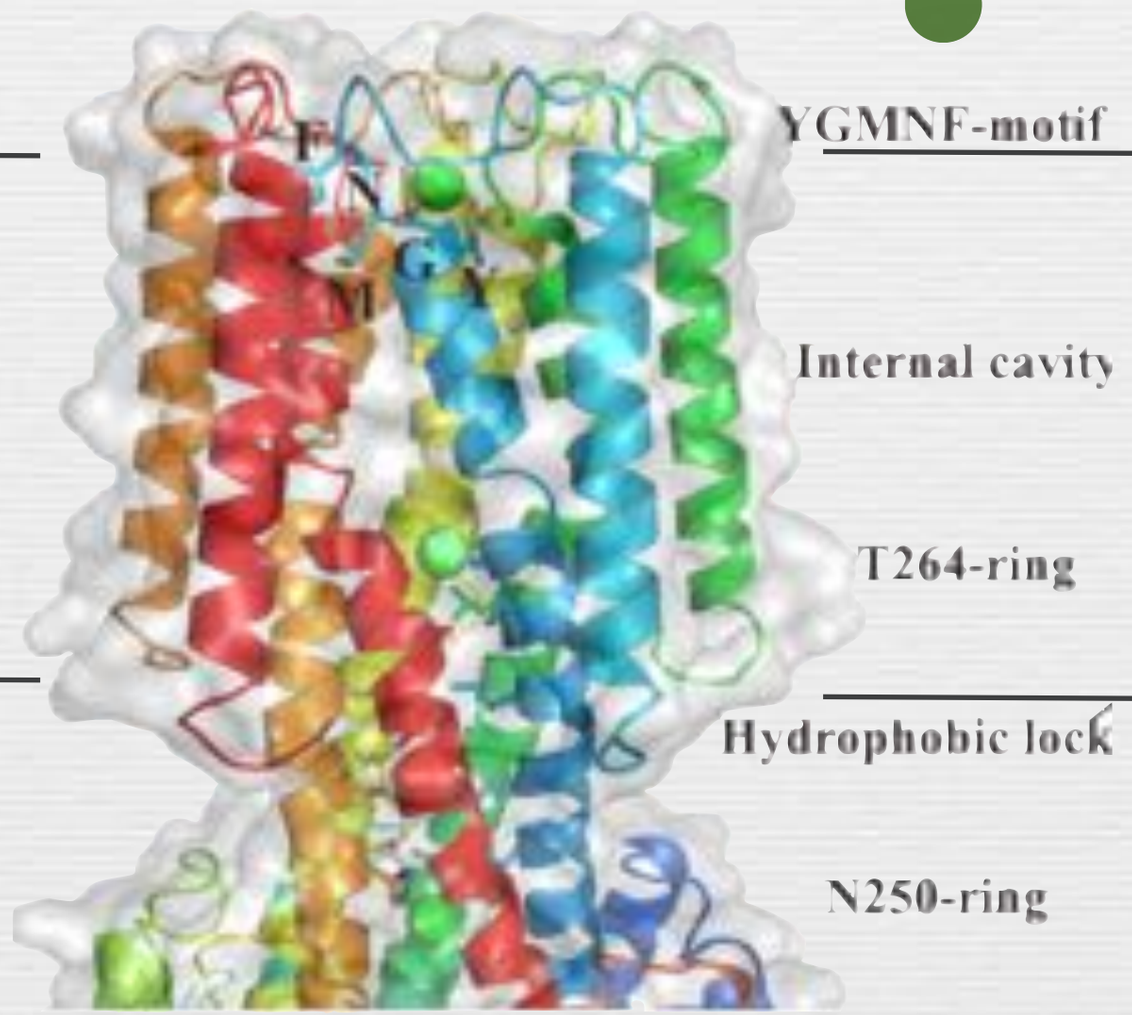
# GMN motif



# The entry point for magnesium ions

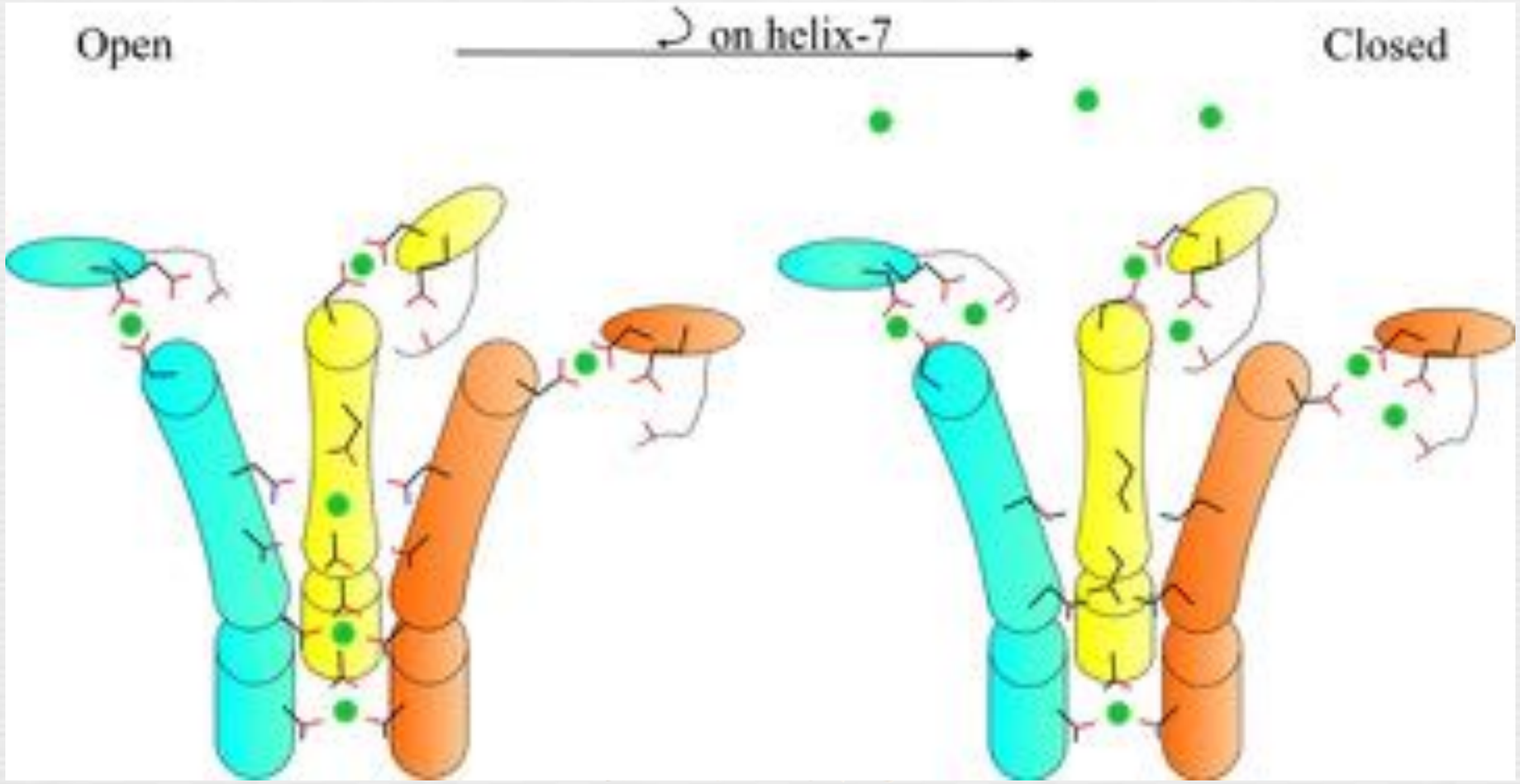
extracellular Mg pool

Outside

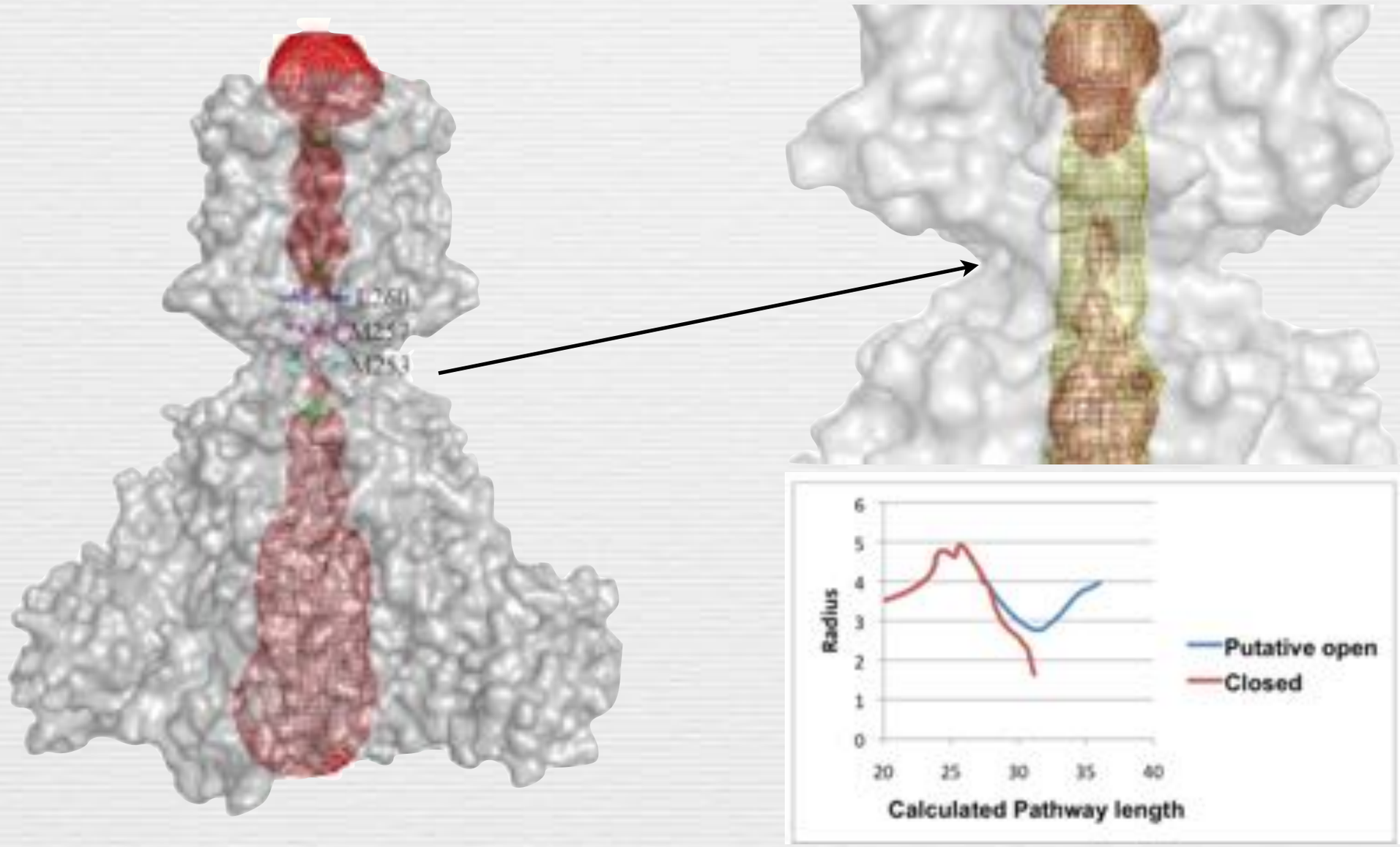



Inside

# Putative mechanism

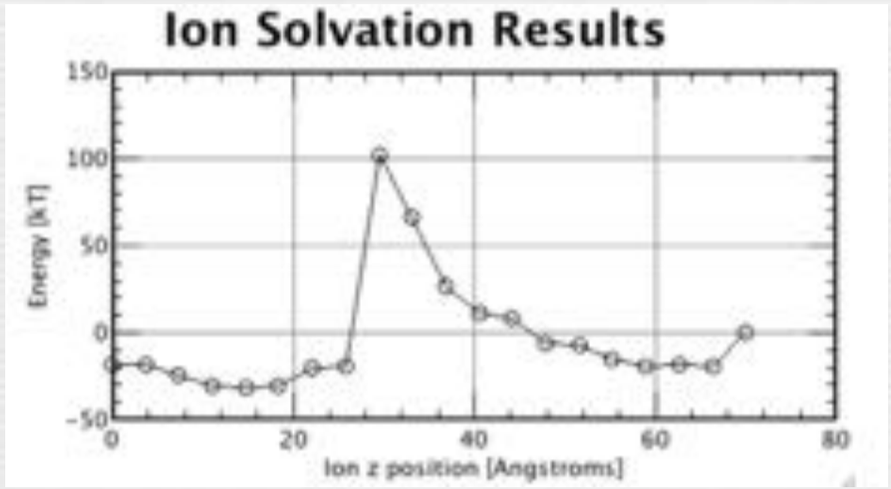


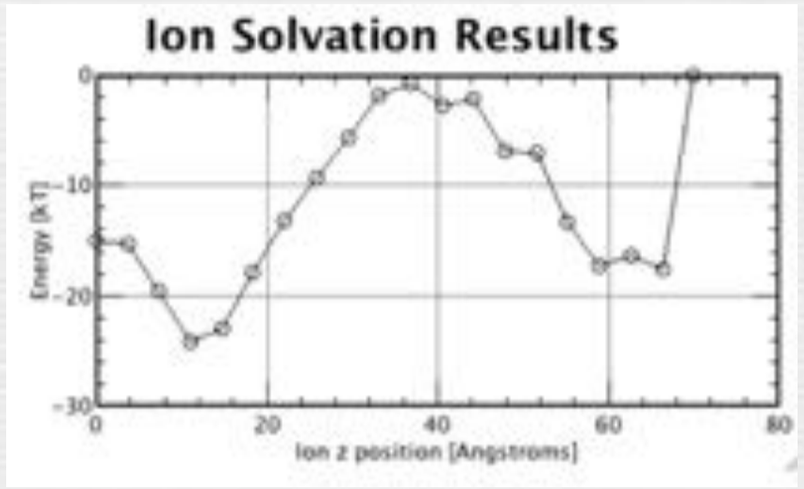
# Tunnel profile




Calculated with MOLEonline 2.0: interactive web-based analysis of biomacromolecular channels
K Berka et al Nucleic Acids Research, 2012, 1–6 doi:10.1093/nar/gks363

# Ion permeation energy profile




Closed conformation  Putative open conformation

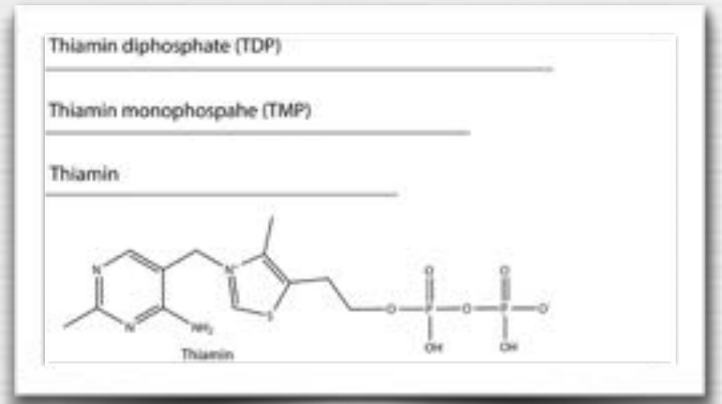
Calculated with APBSmem: A graphical interface for electrostatics calculations at the membrane.
KM Callenberg et al (2010) PLoS ONE 5(9): e12722

# Examples of different transport mechanisms:

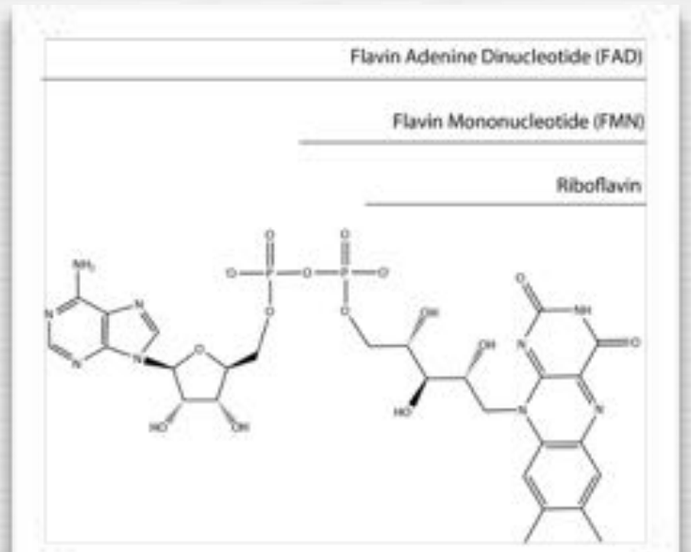
## 2. Facilitated diffusion (Gated-pore) by Pnu vitamin transporters

# B-Vitamins

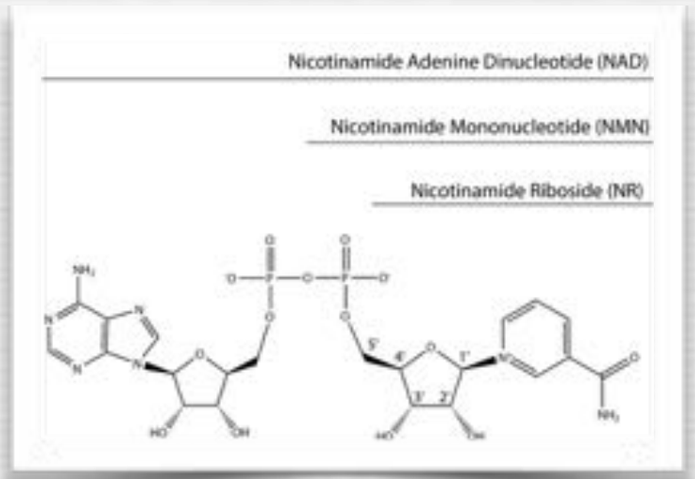
## Pnu proteins are poorly characterised membrane transporters involved in vitamin uptake in bacteria




PnuT

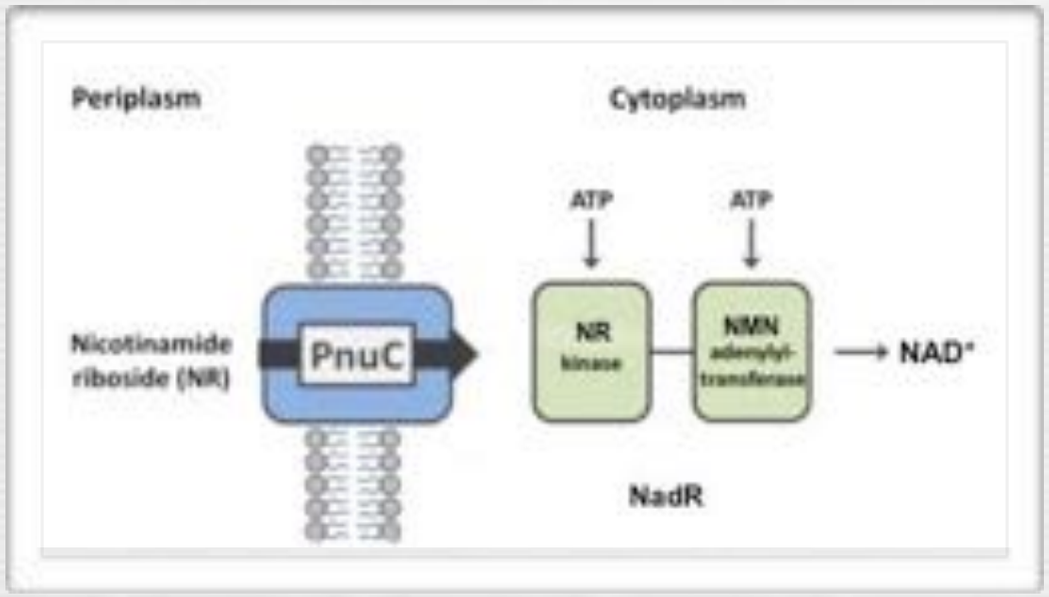



PnuX




PnuC

# Putative mechanism

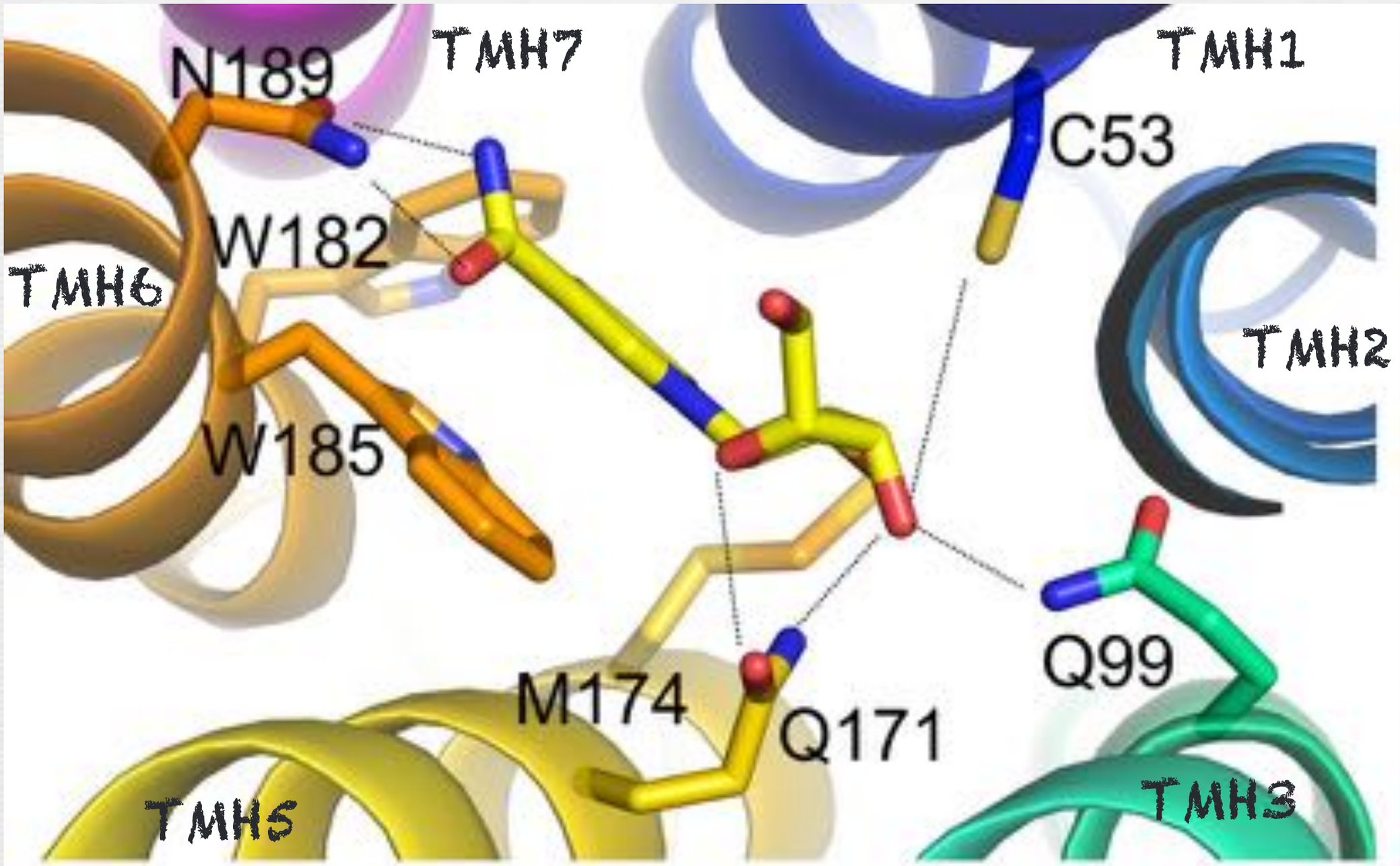



Facilitated diffusion coupled to the metabolic trapping

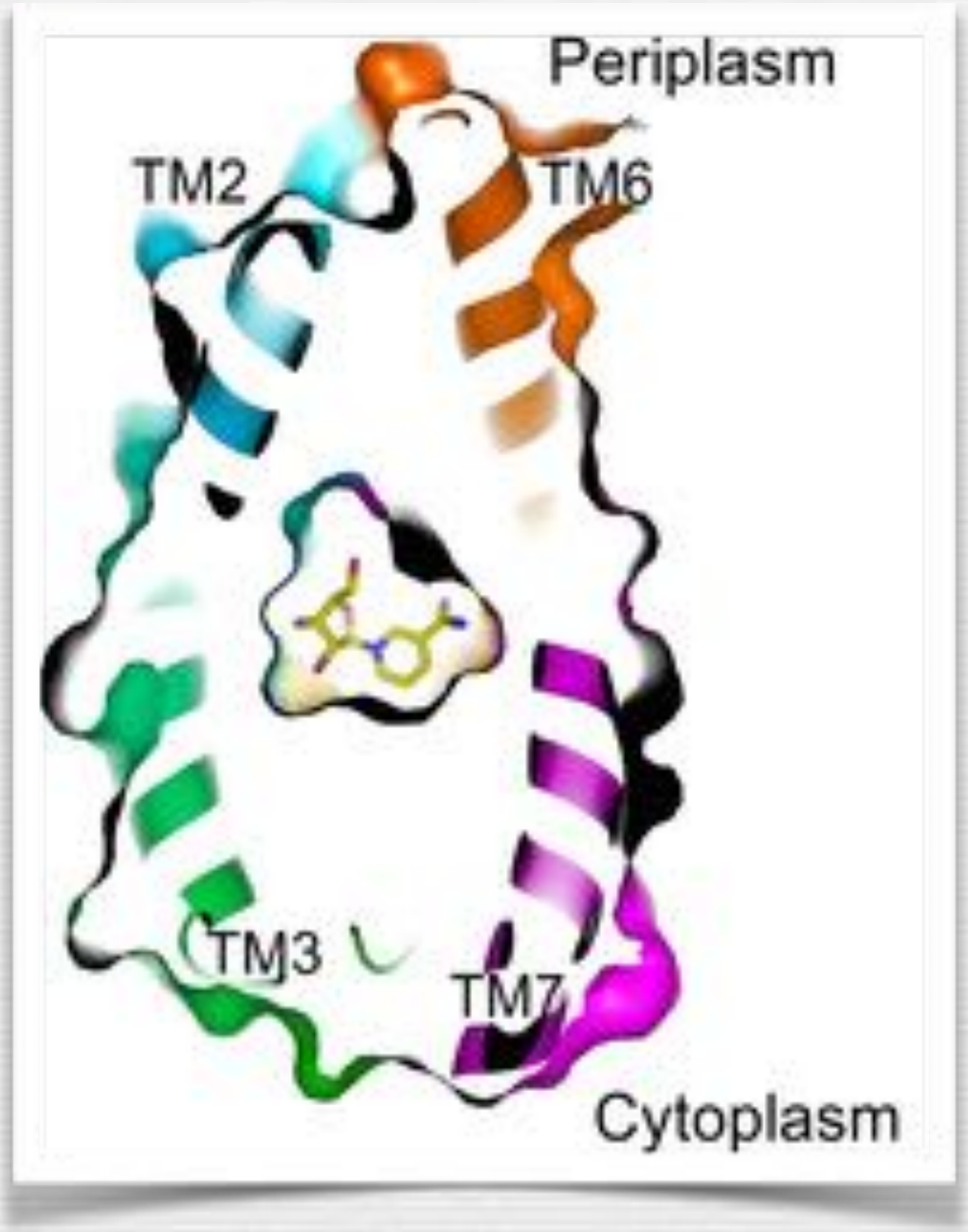
# Crystal structure of PnuC

Jaehme, Guskov, Slotboom (2014) Nat Struct Mol Biol,

# Ligand coordination



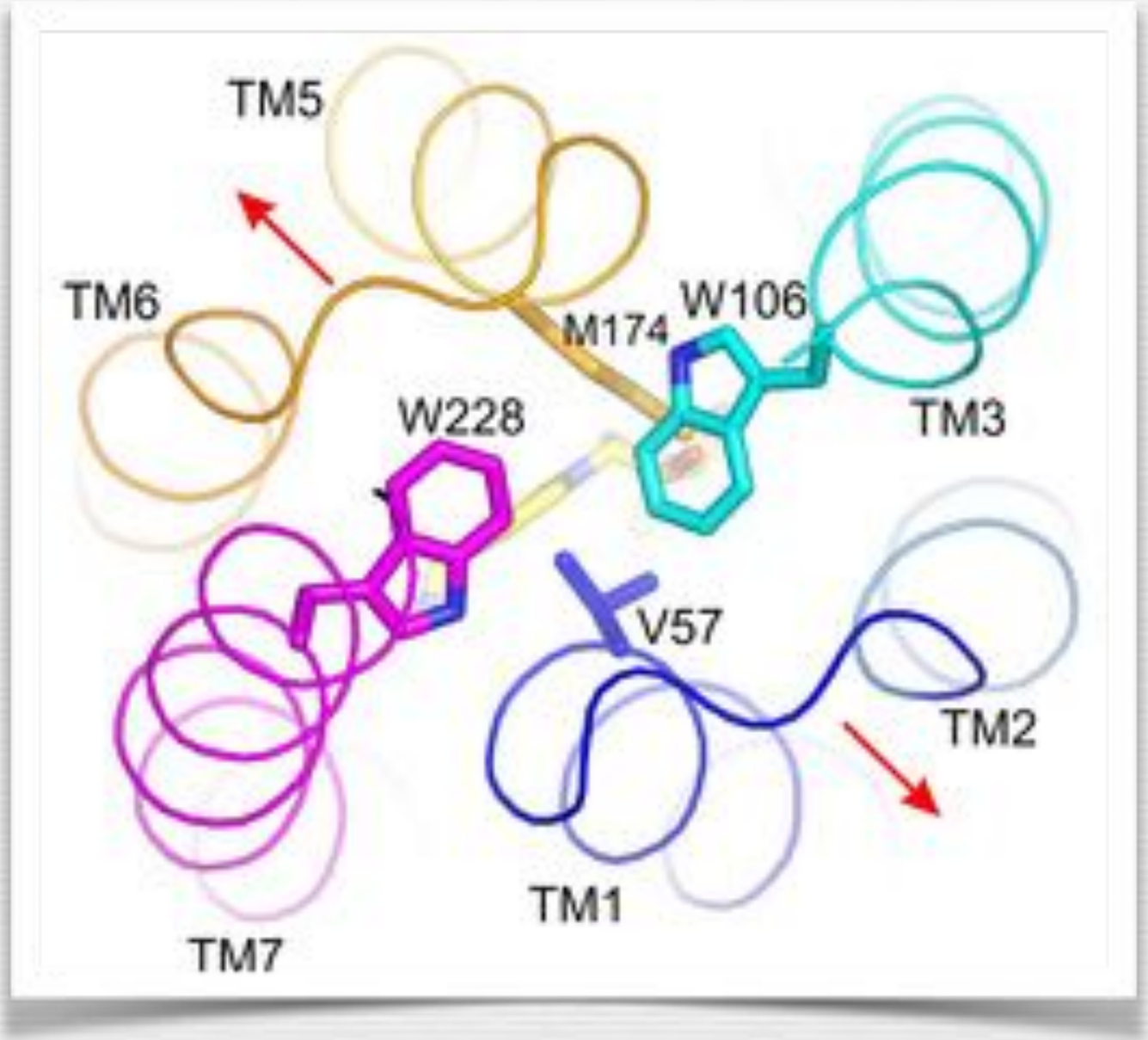
# The binding site




- The cavity is sealed from both sides - fully-occluded ligand-bound state

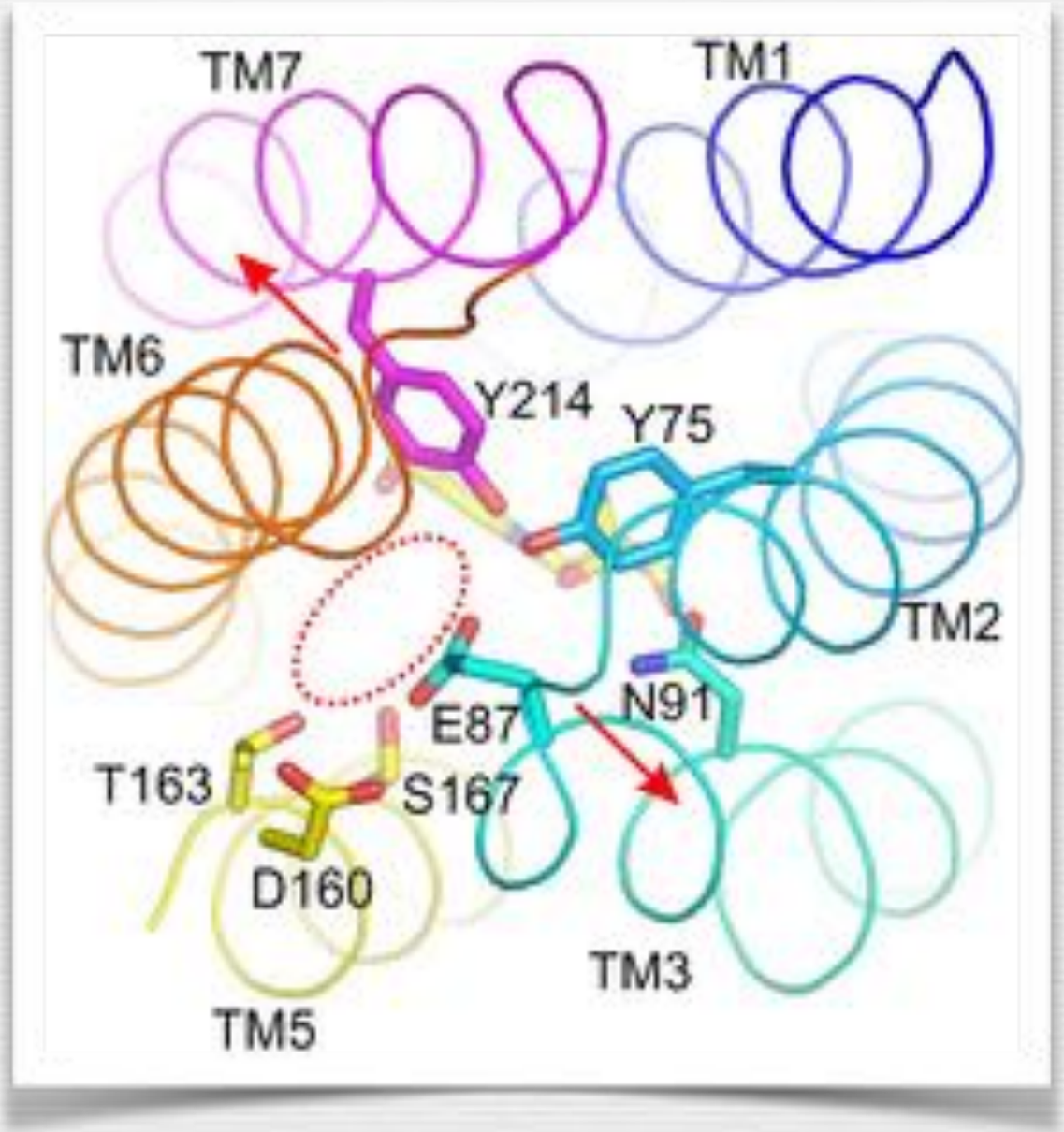
# Intracellular (cytoplasmic) gate

- two layers of symmetry related residues

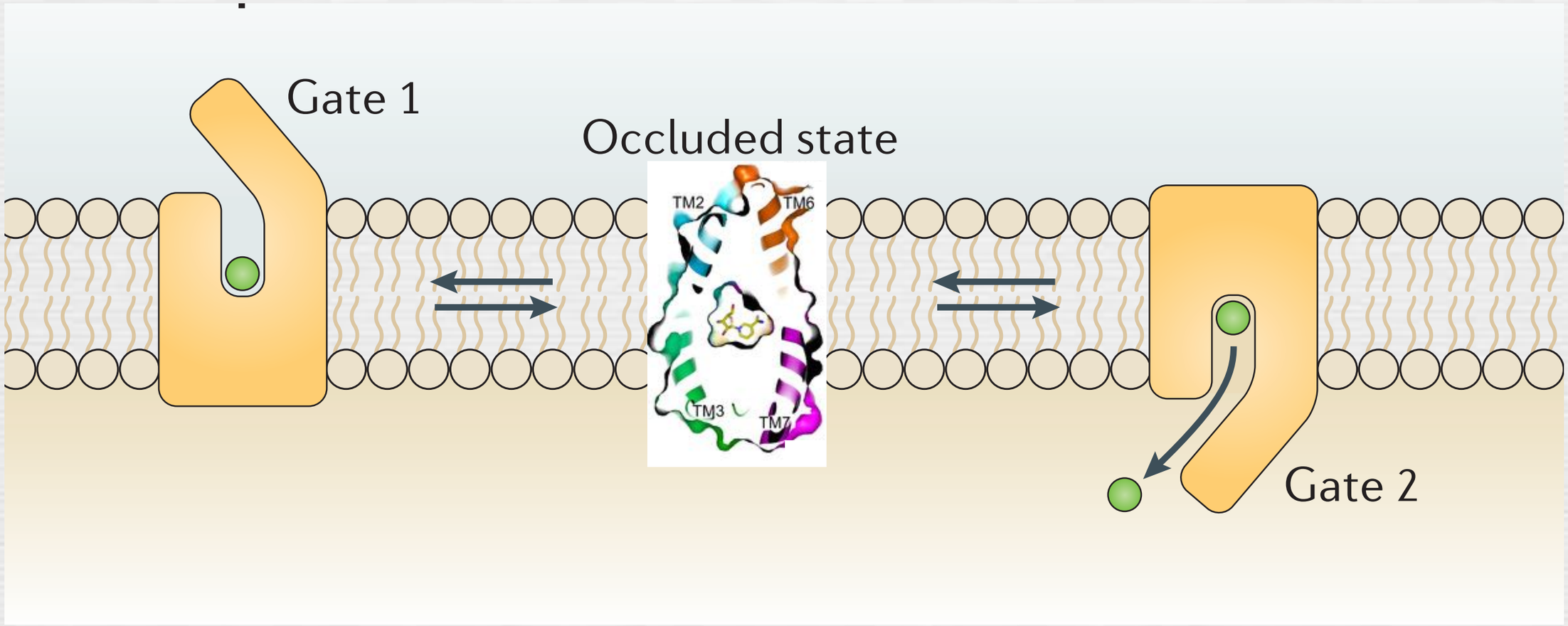


# Periplasmic gate

- The thick hydrophilic layer
- possible access path shown with the red dashed oval



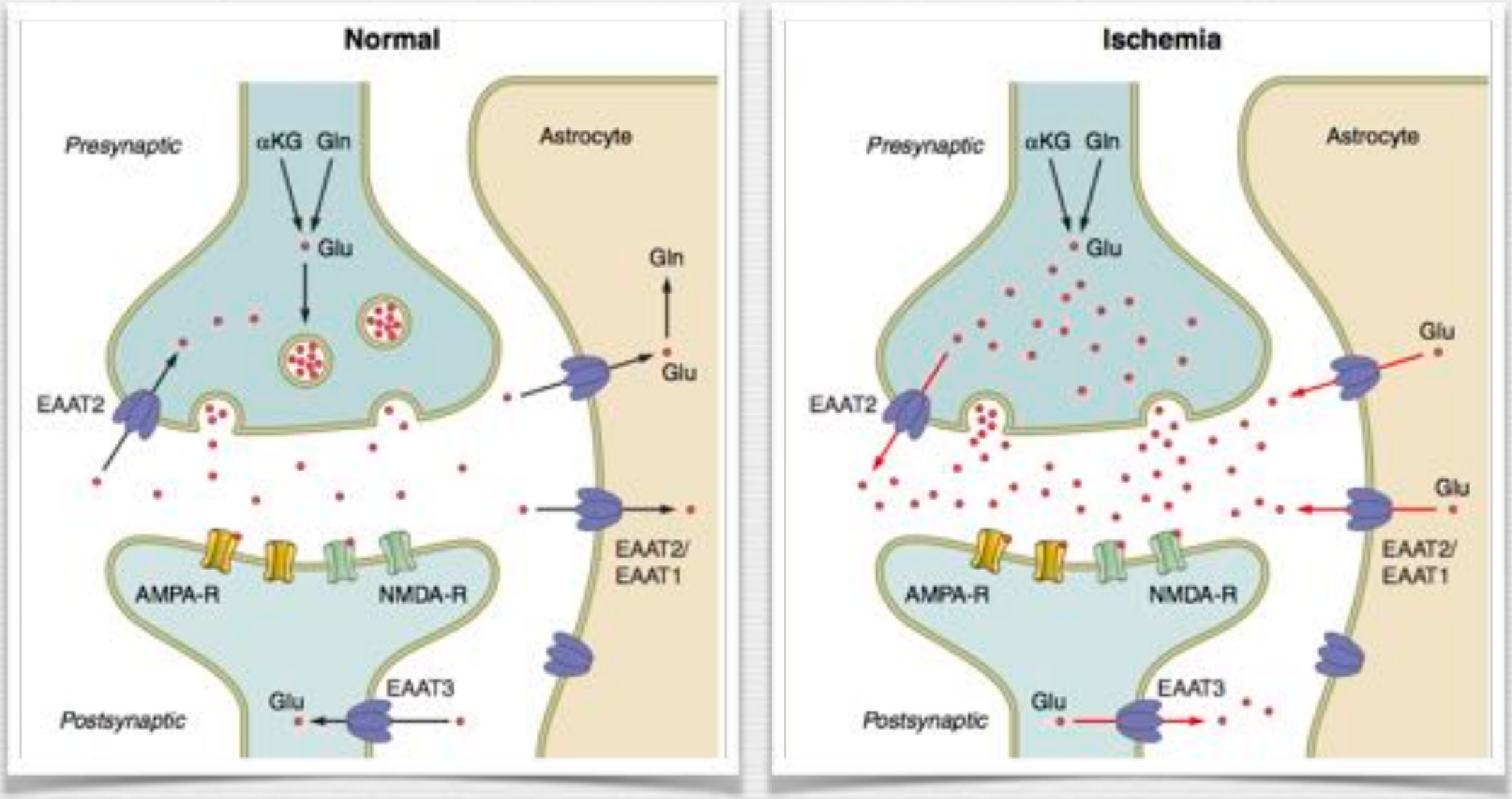
# Mechanism



Examples of different transport mechanisms:

3. Secondary active transport of glutamate/aspartate via an elevator mechanism

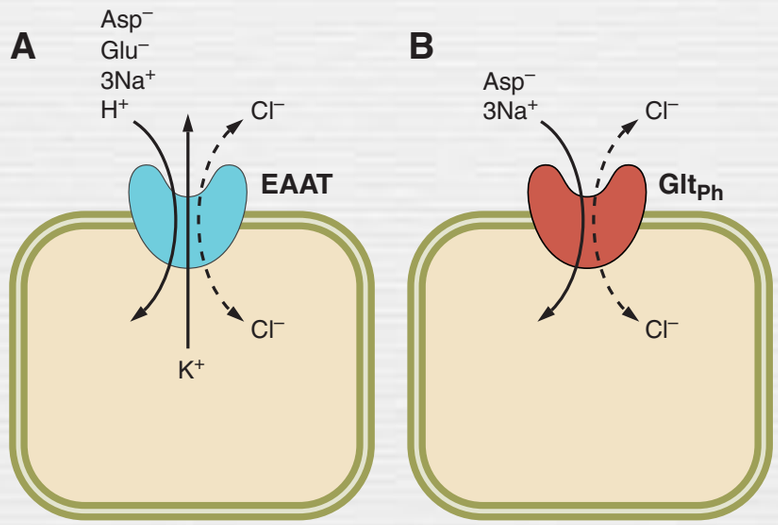
# Mammalian glutamate transporters take up the excitatory neurotransmitter glutamate




Vandenberg & Ryan (2013) Physiol Rev 93: 1621–1657

# Glutamate (aspartate) transporters in eu- and prokaryotes

- *Regulation of $[Glu]_{extra}$ to maintain synaptic signalling processes*
- *Glu is the predominant excitatory neurotransmitter*
- *Faster transport (ms)*




Vandenberg & Ryan (2013) Physiol Rev 93: 1621–1657

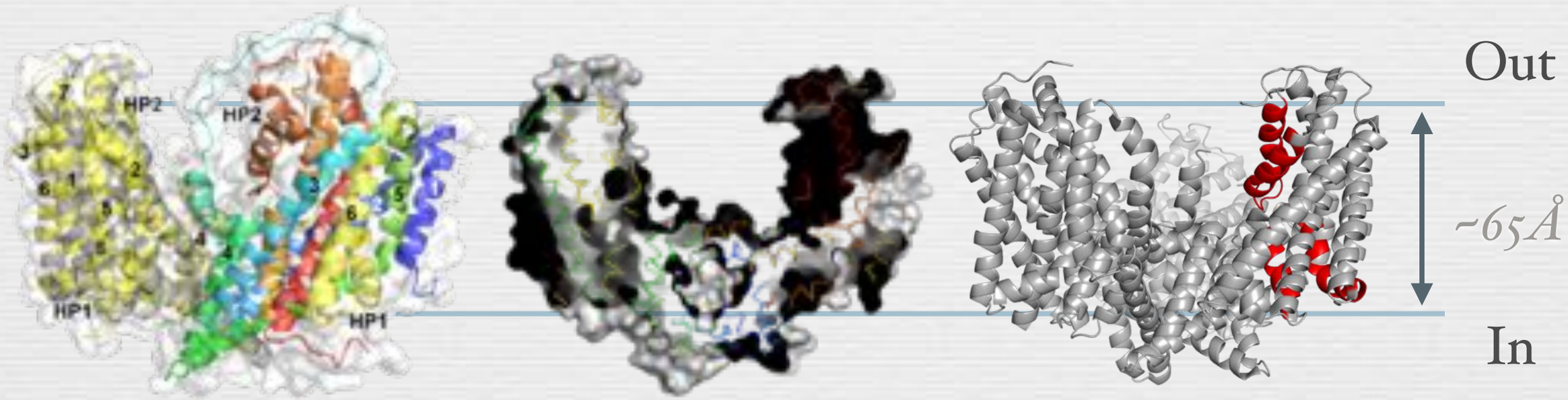
- *Prefers Asp over Glu*
- *Asp is a nutrient*
- *Slower transport (min)*

## Sequence identity

- 30-35% between EAAT(1-5) (Human) and GltPh
- 45-55% among EAAT

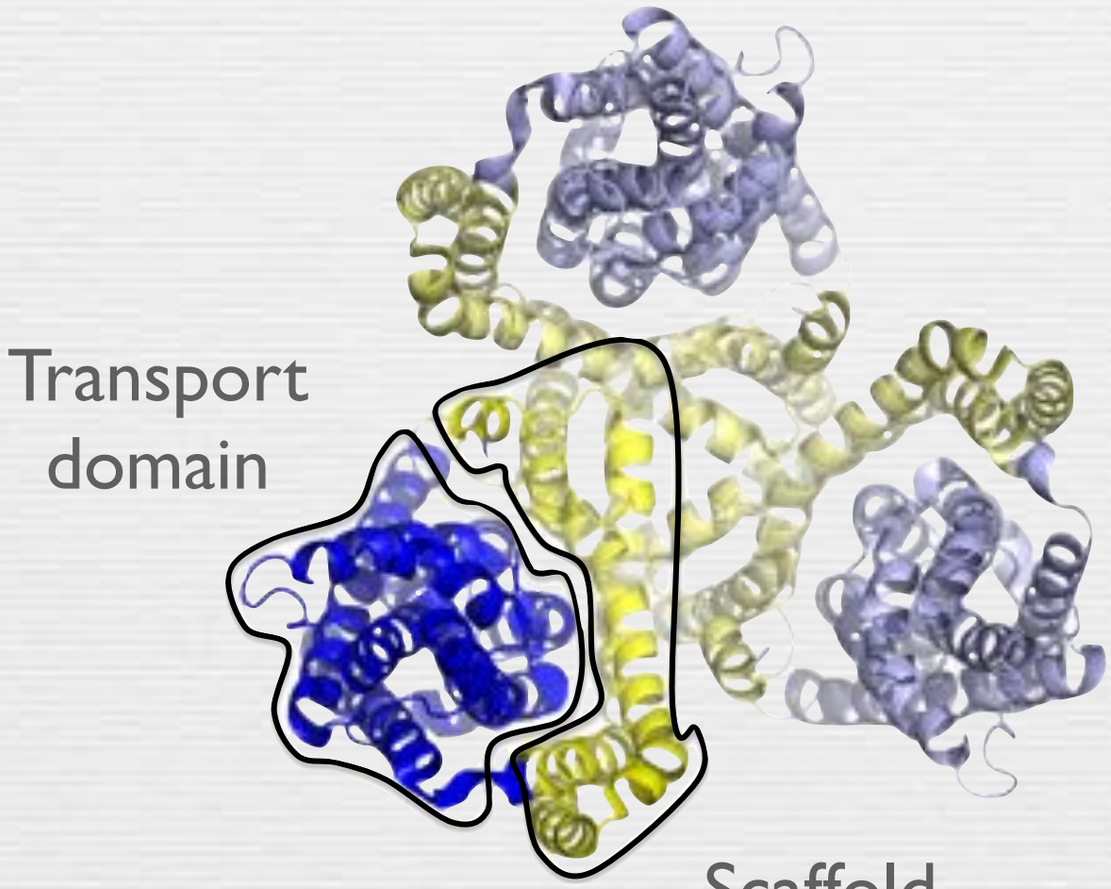
# Crystal structures of archaeal aspartate transporters

# General architecture



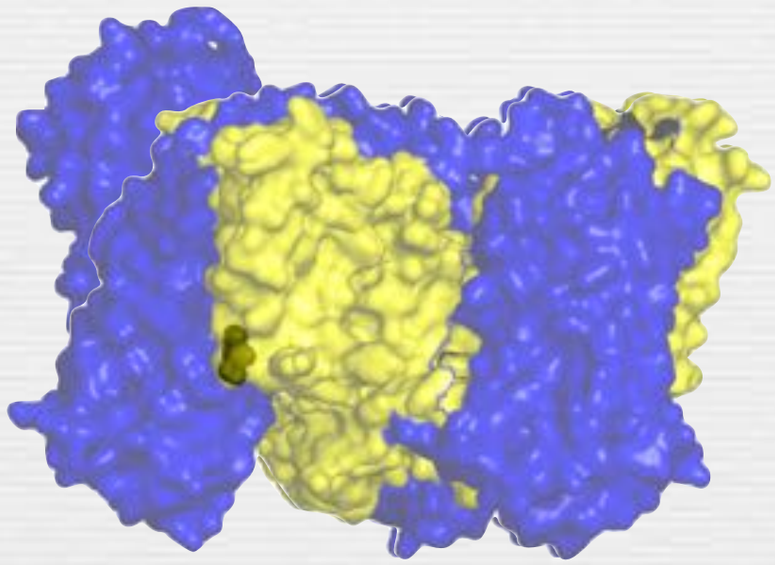

- E.Gouaux, O. Boudker and D.J. Slotboom labs

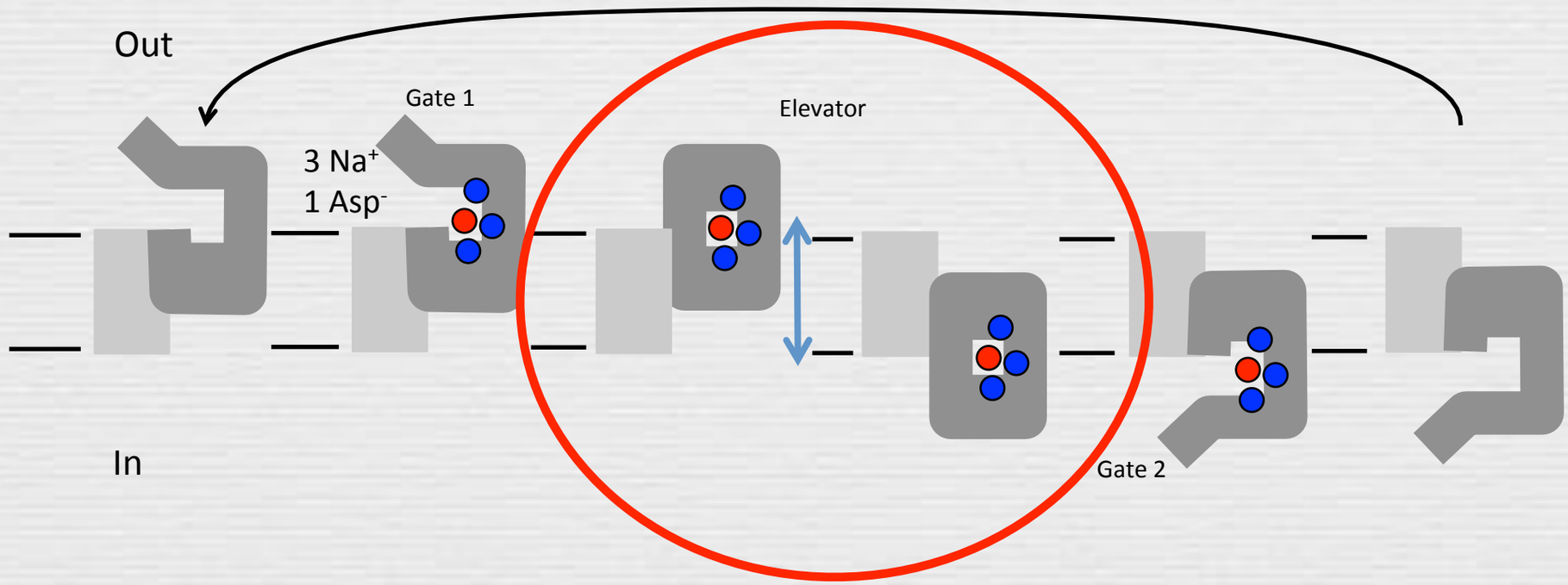
# Two rigid



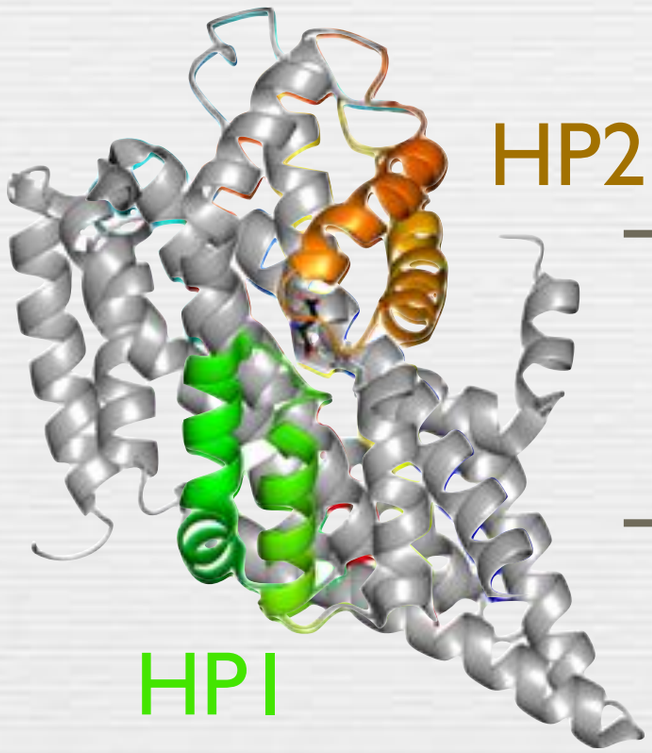
The trimerization domains form a scaffold in the membrane,

move





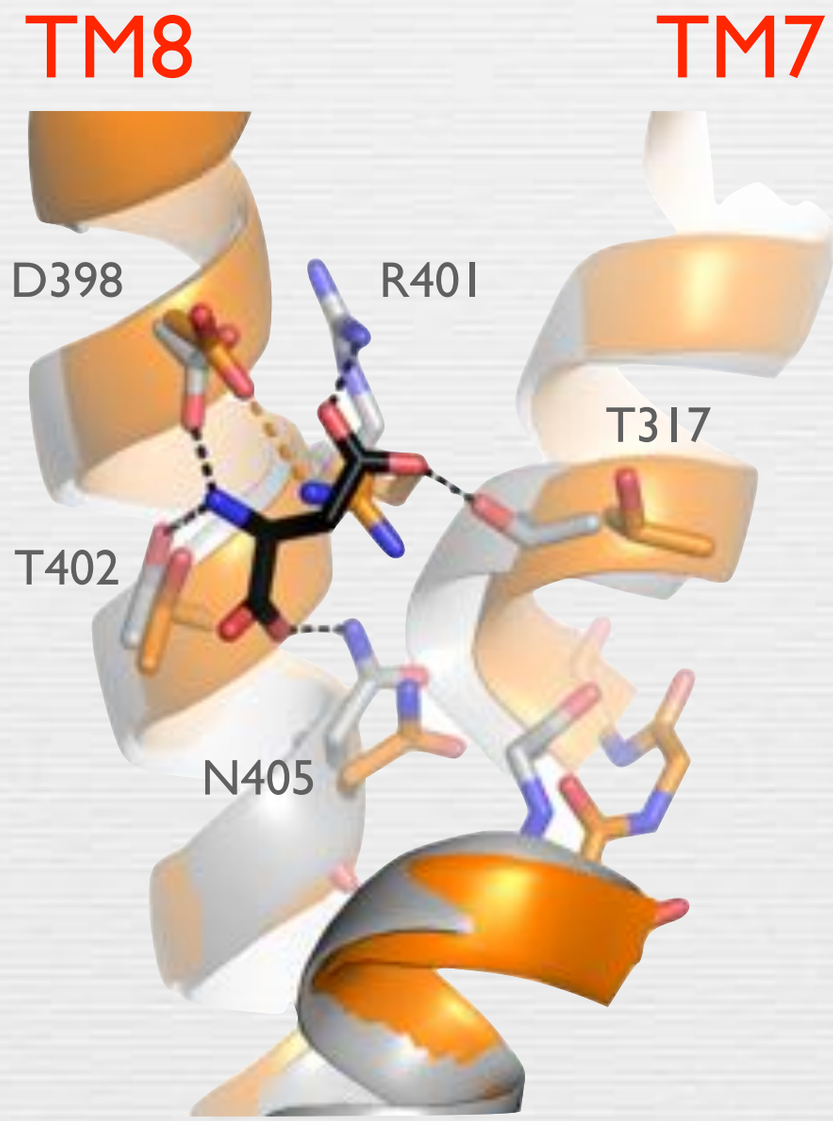
# Closer look at the elevator




Two helical hairpins (HP1 and HP2) are the **possible** lids ("doors") that can open

# The crystal structures explain the strong cooperativity between Na+ and Aspartate binding

# Conserved position of the substrate




with substrate

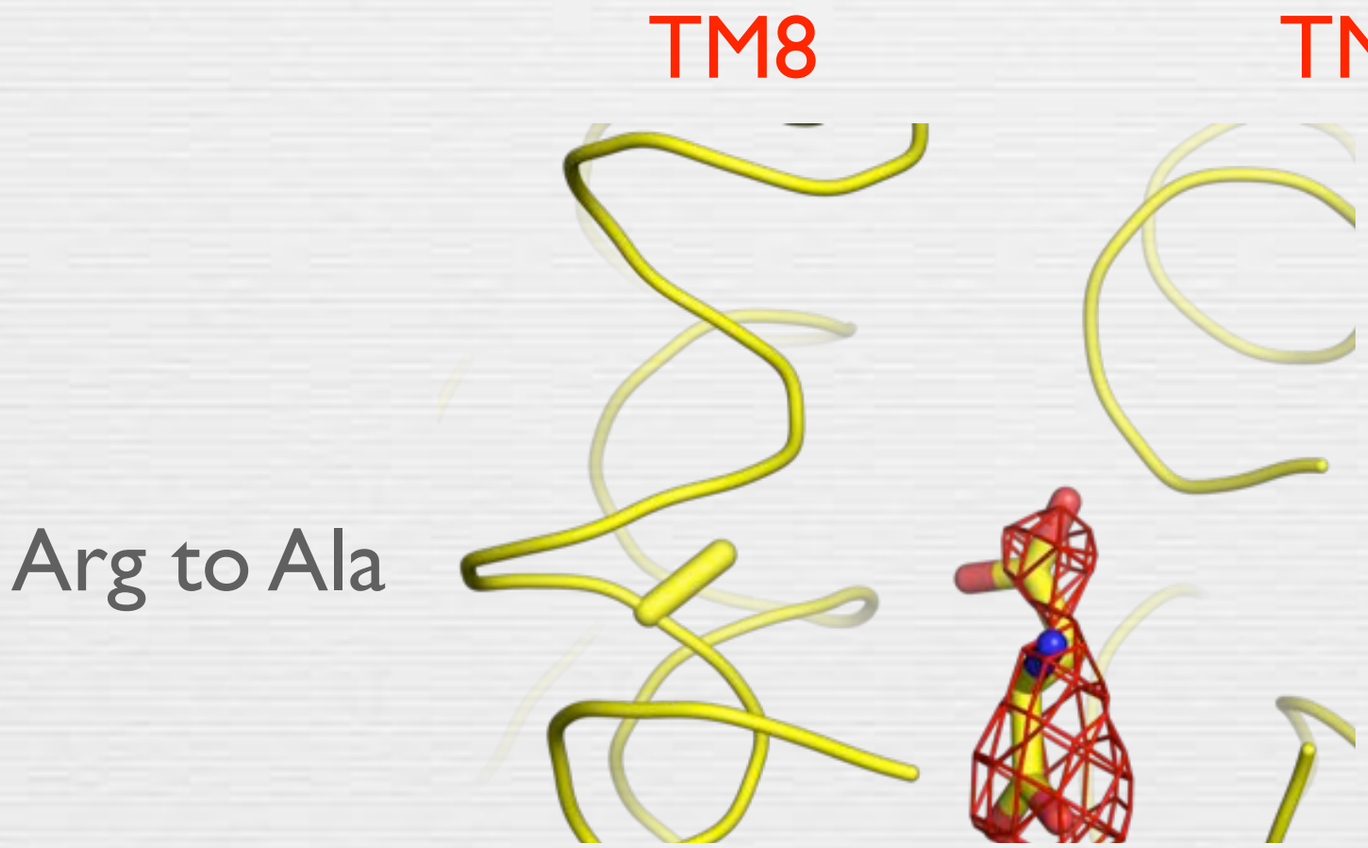
substrate-free

Arg401:

- Strictly conserved
- Essential for transport
- But also for re-orientation

Jensen et al., (2013) Nat Struct Mol Biol. 20(10):1224

# Mutations of Arg401 prevent substrate binding

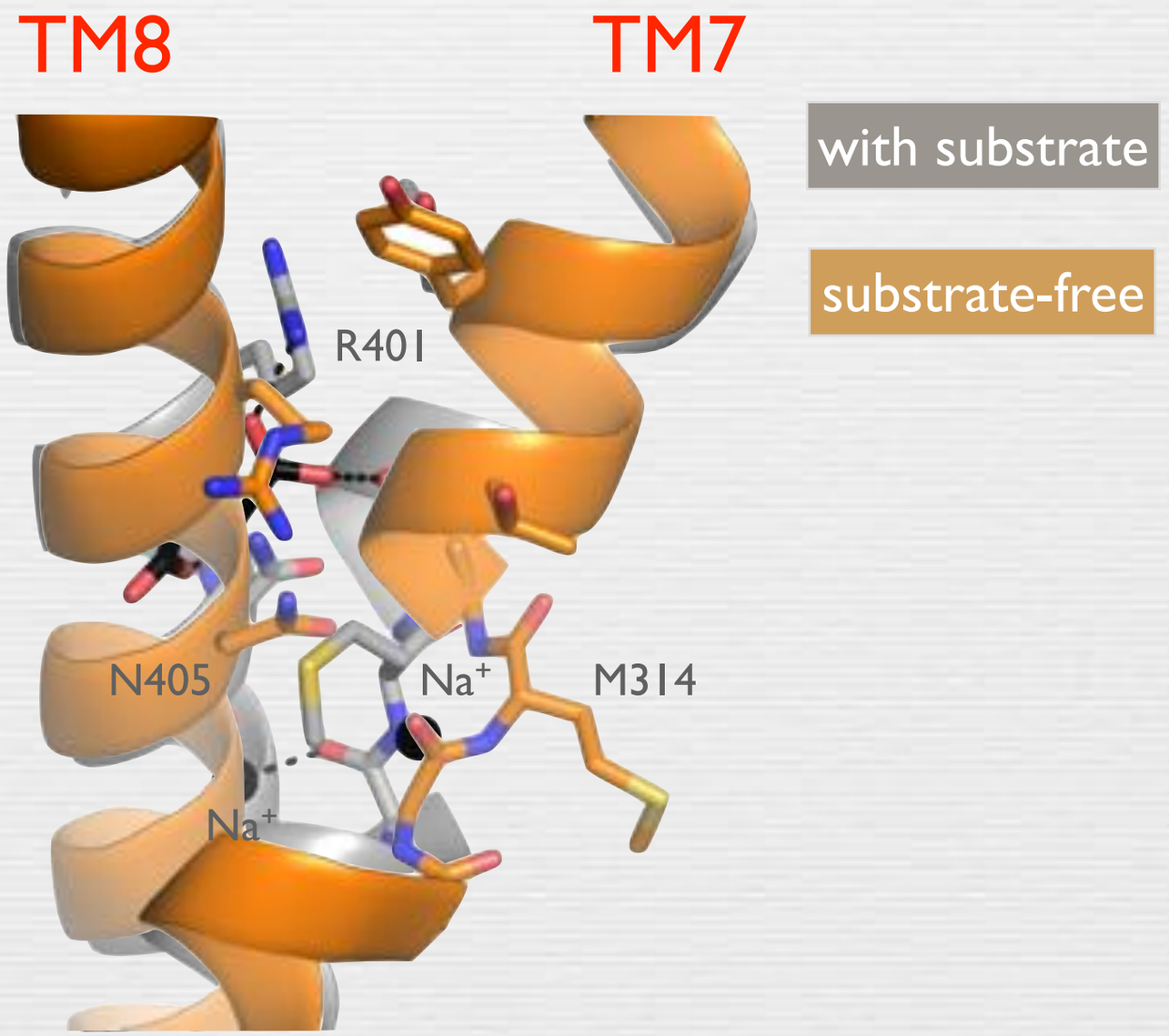



Arg to Ala

Fo-fc electron density for the substrate at -3σ

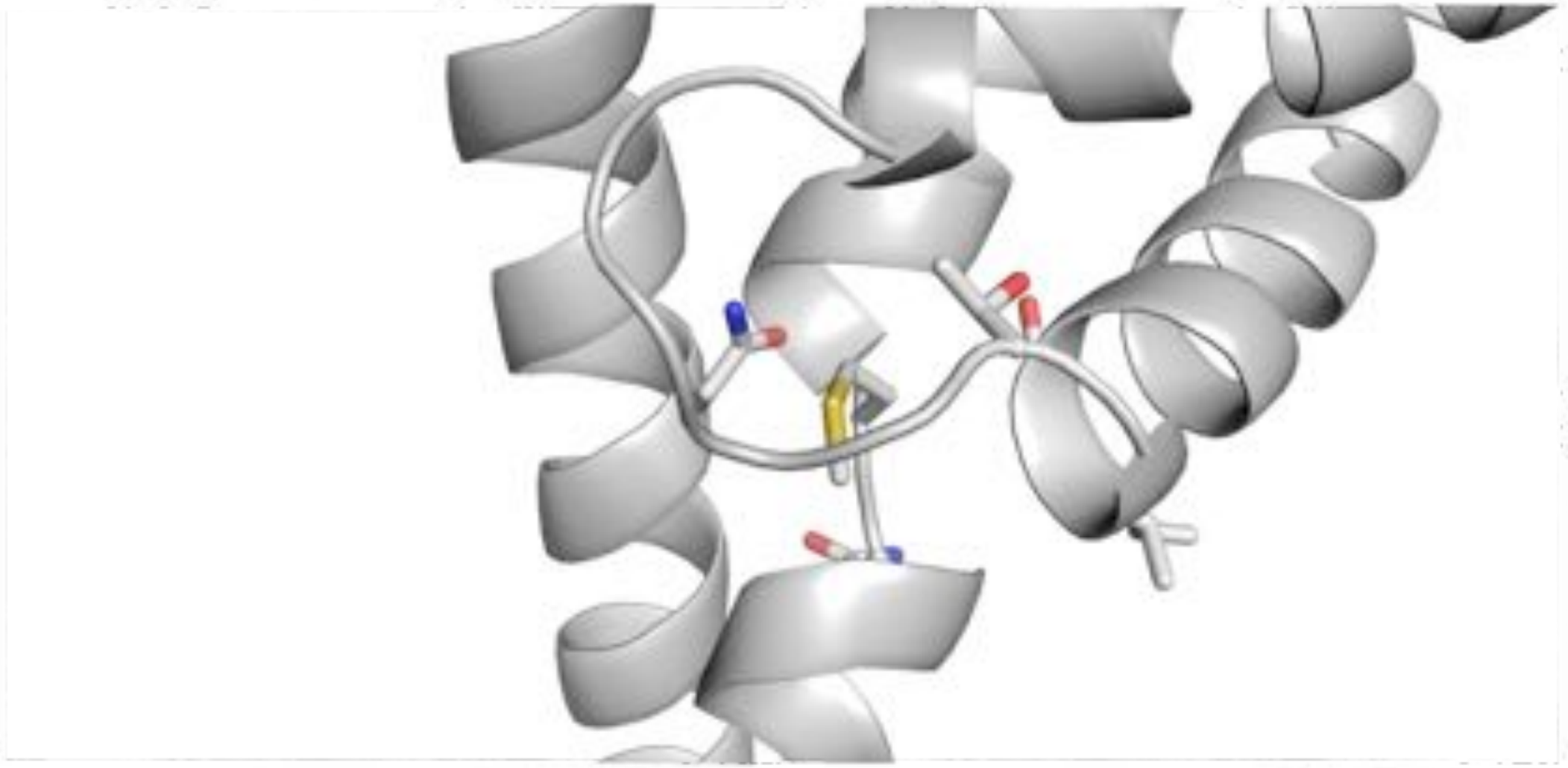
Verdon et al. (2014) eLife, 3:e02283

# Sodium binding sites




Jensen et al., (2013) Nat Struct Mol Biol. 20(10):1224

# Geometry of binding sites is destroyed




$1Million
Does the elevator-like movement really occur in membrane?
A: No
C: Perhaps
B: Yes
D: Only God knows

# Single-Molecule Studies

Outward-outward 72 Å

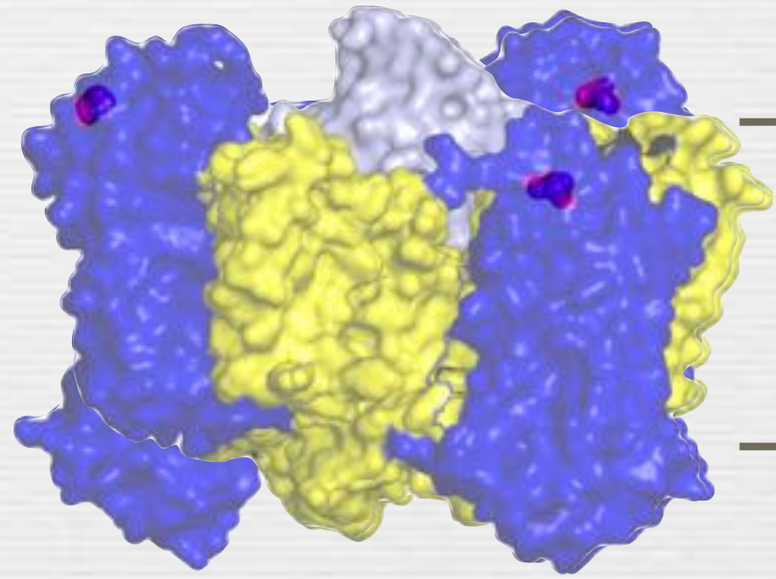
Outward-inward 62 Å

Intermediate-Intermediate 60 Å

Intermediate-inward 58 Å

Inward-inward 49 Å

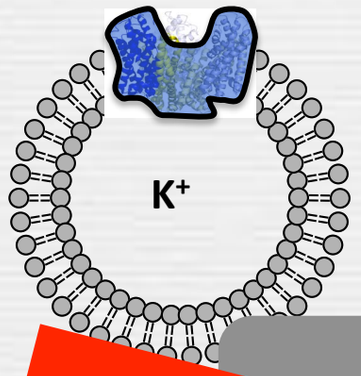
Low FRET

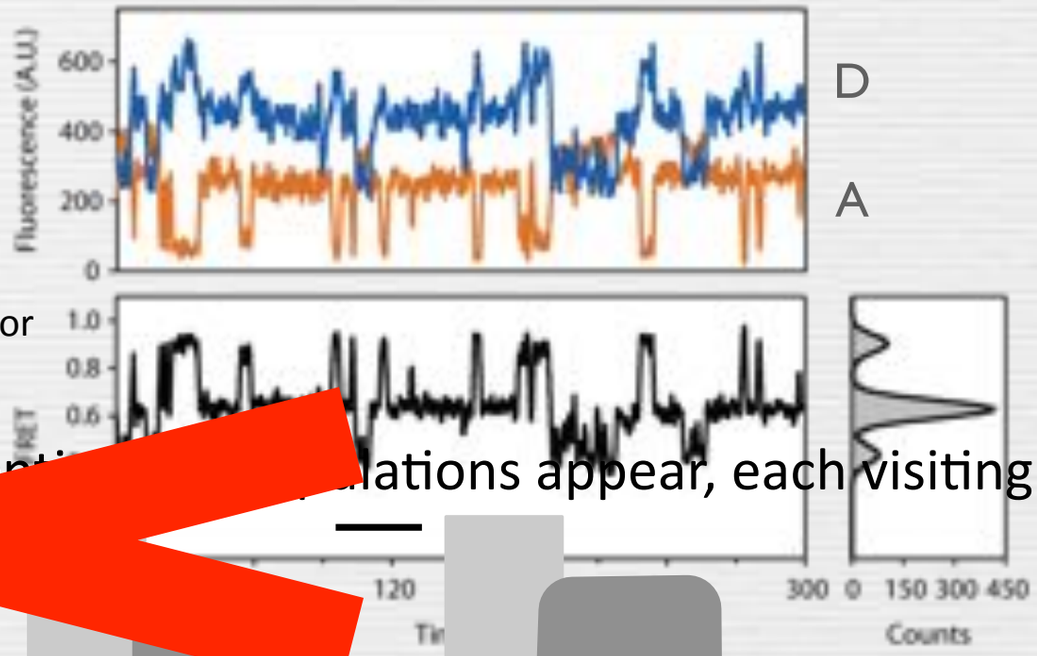


High FRET

# FRET

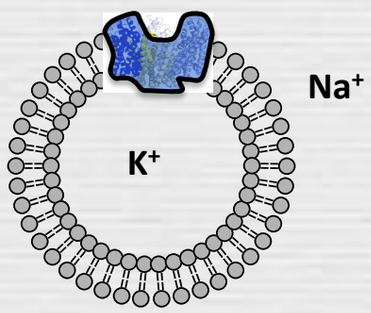
In the presence of a sodium, aspartate, and a membrane potential three FRET states are visited (low, intermediate, high)

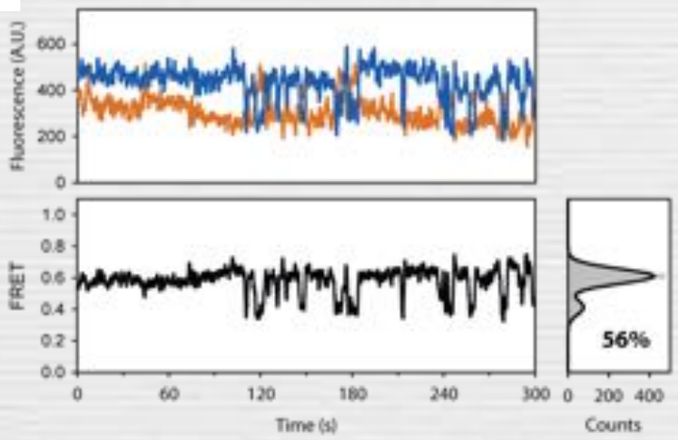


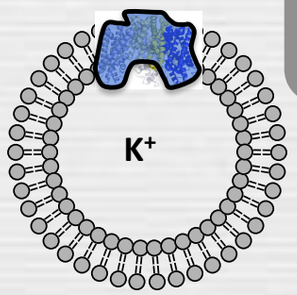




Gate 1

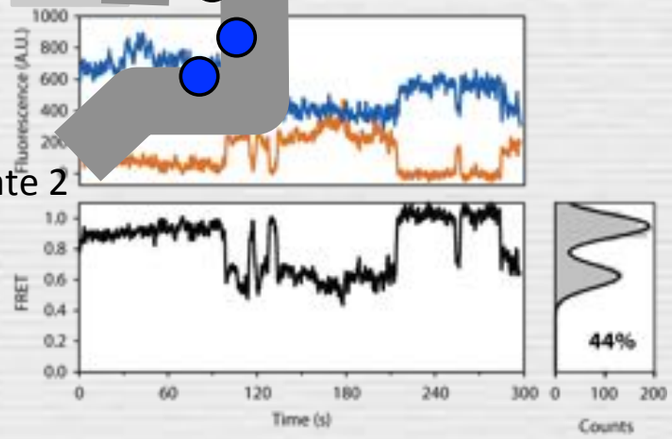
In the presence of a sodium gradient and potential three populations appear, each visiting




Gate 2

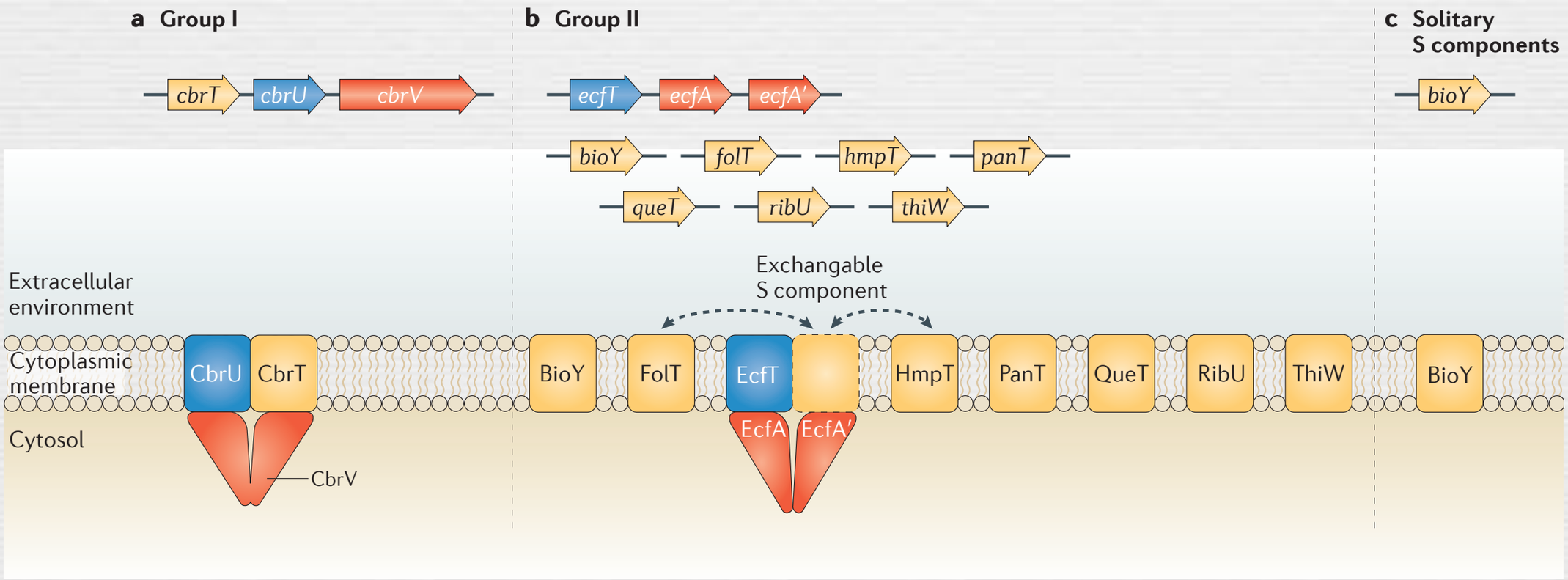



Erkens et al., (2013) Nature, 502(7469):119

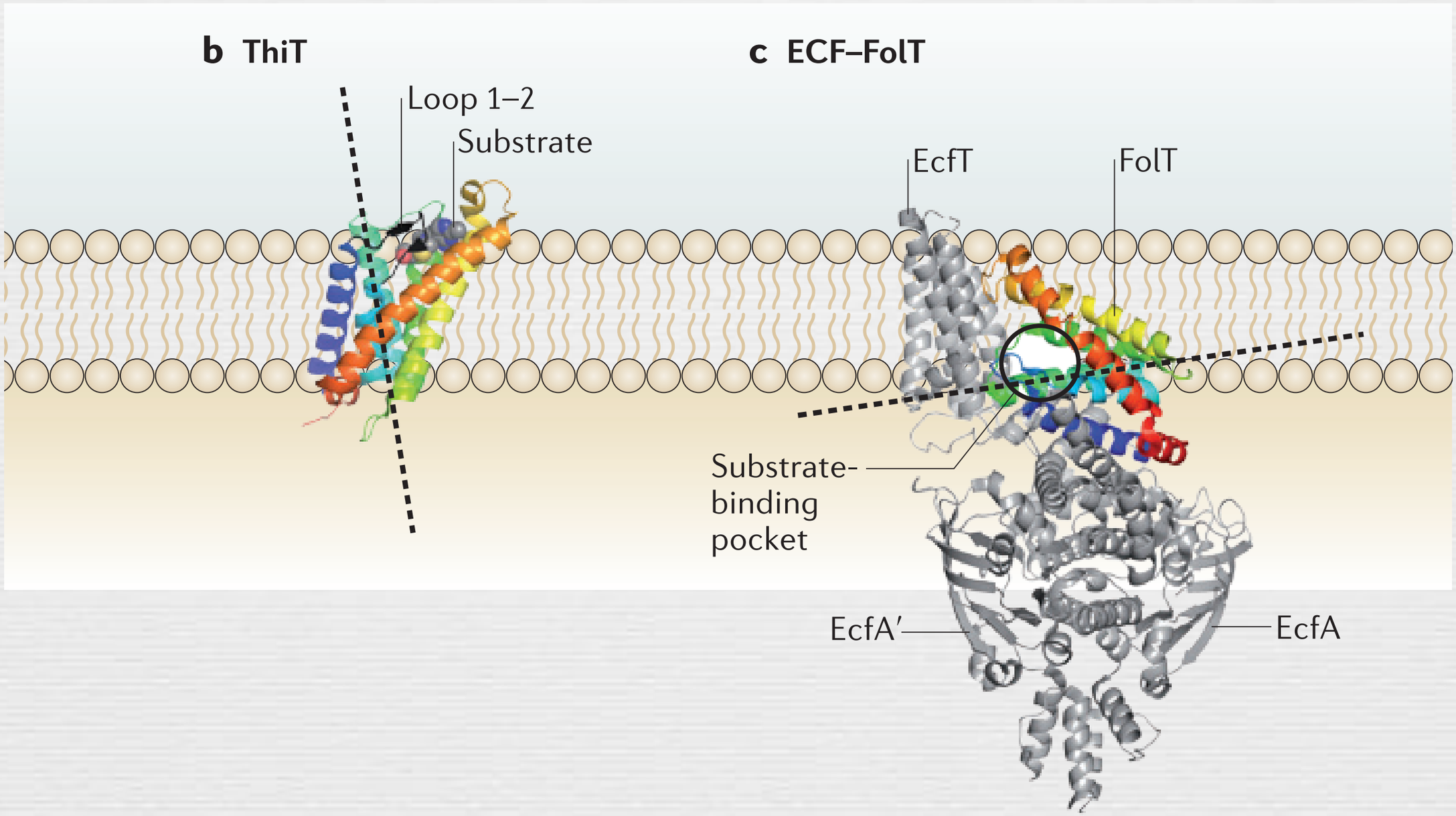
# Examples of different transport mechanisms:

## 4. Primary active transport via an toppled elevator mechanism?

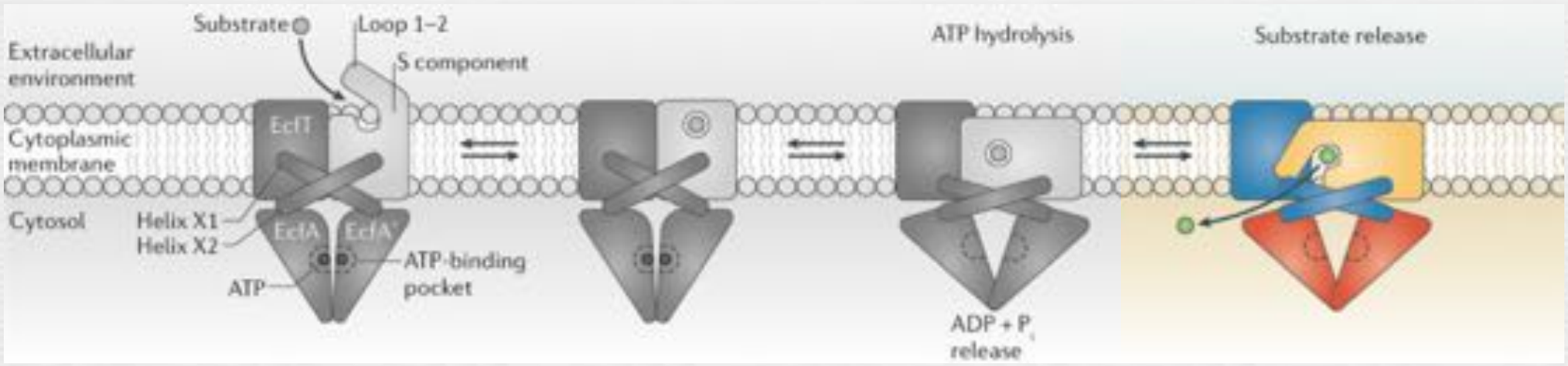
# General architecture



# Toppling

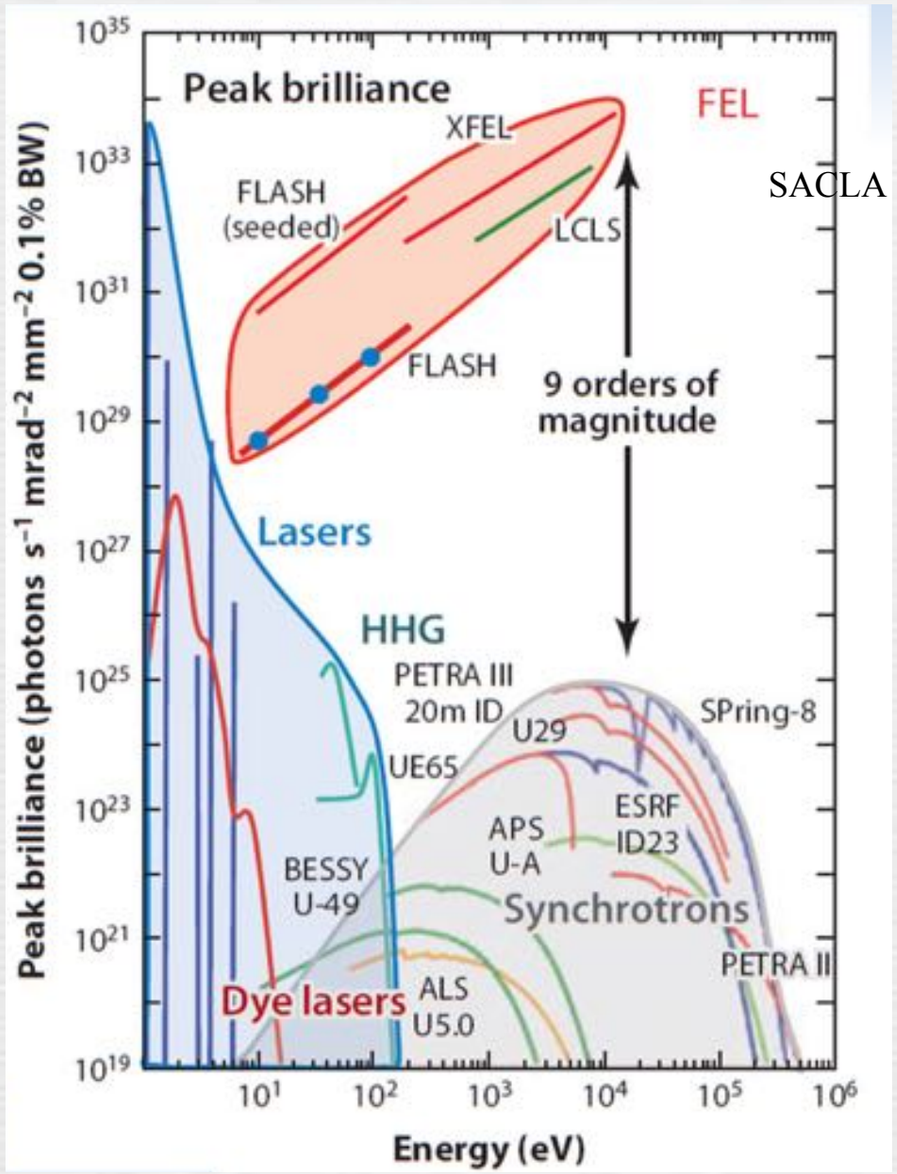

b ThiT
Loop 1–2
Substrate
c ECF–FolT
EcfT
FolT
Substrate-
binding
pocket
EcfA′
EcfA

# Possible mechanism of transport



# Future is here?

# X

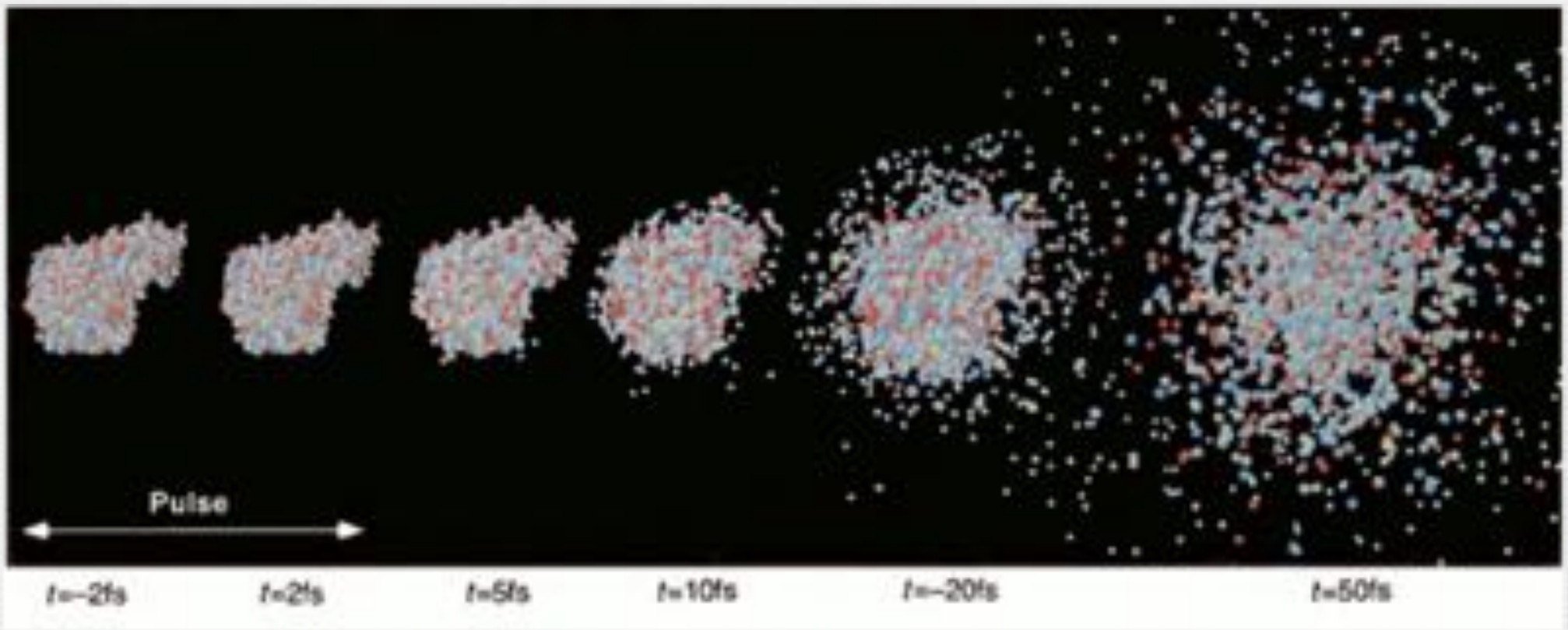



SACLA

- It is extremely **brilliant**
- it generates pulses on the **femto** ($10^{-15}$) second scale!

From J. Ullrich, A. Rudenko, R. Moshammer (2012) Ann. Rev. Phys. Chem. 63:635

# Diffraction principle

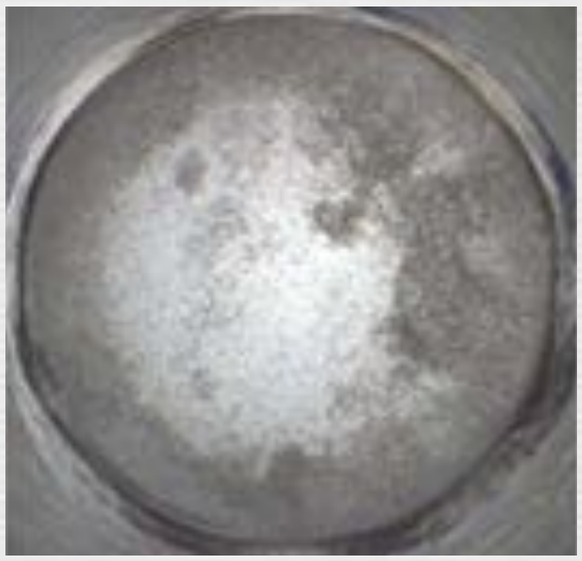



R. Neutze et al., (2000) Nature, 406(6797):752

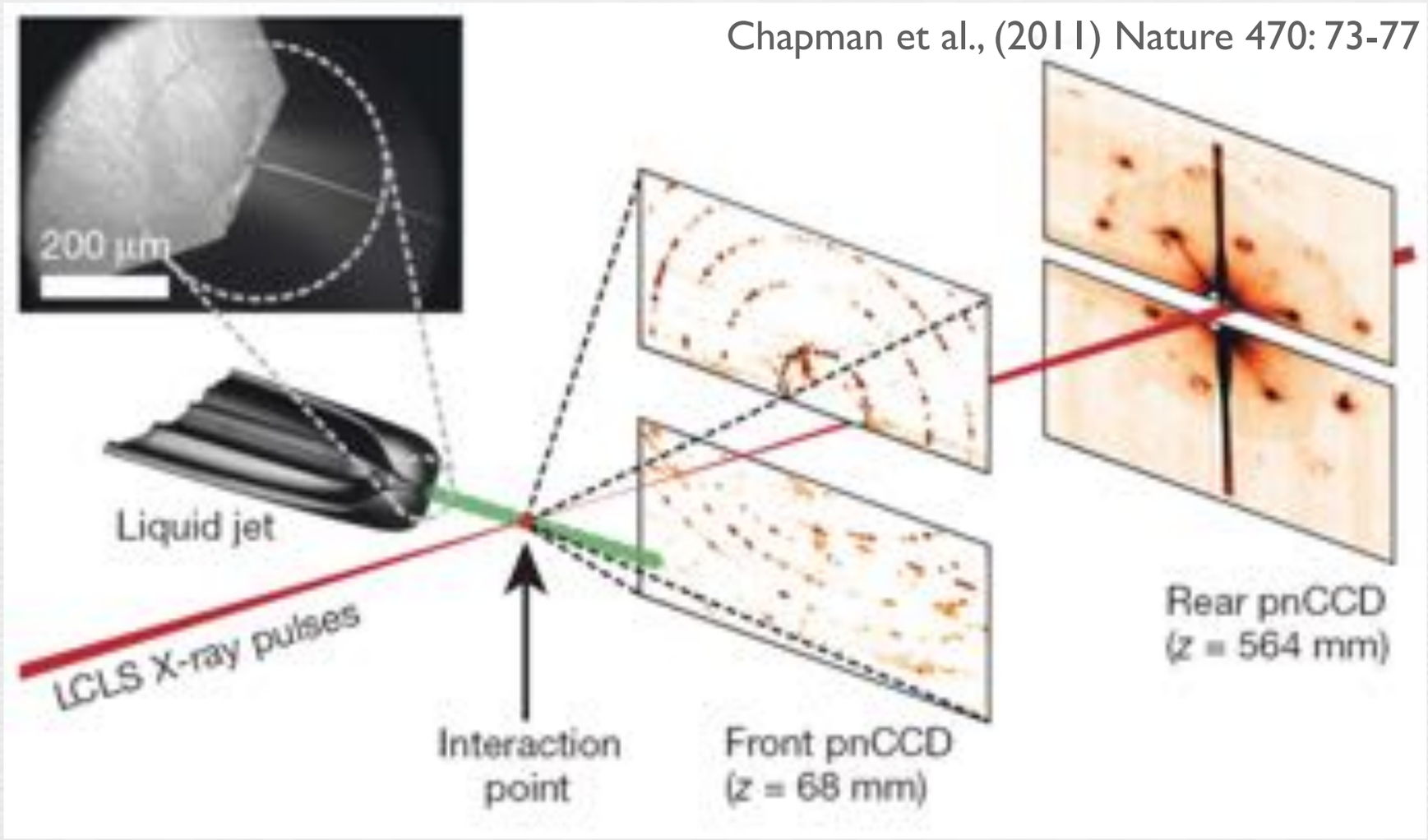
# Unprecedented possibilities for structural biology with XFELs

No need for time- and resource-consuming optimisation to get bigger crystals

Collecting (**room-temperature**) data **without radiation damage** and from **very tiny crystals!**



S

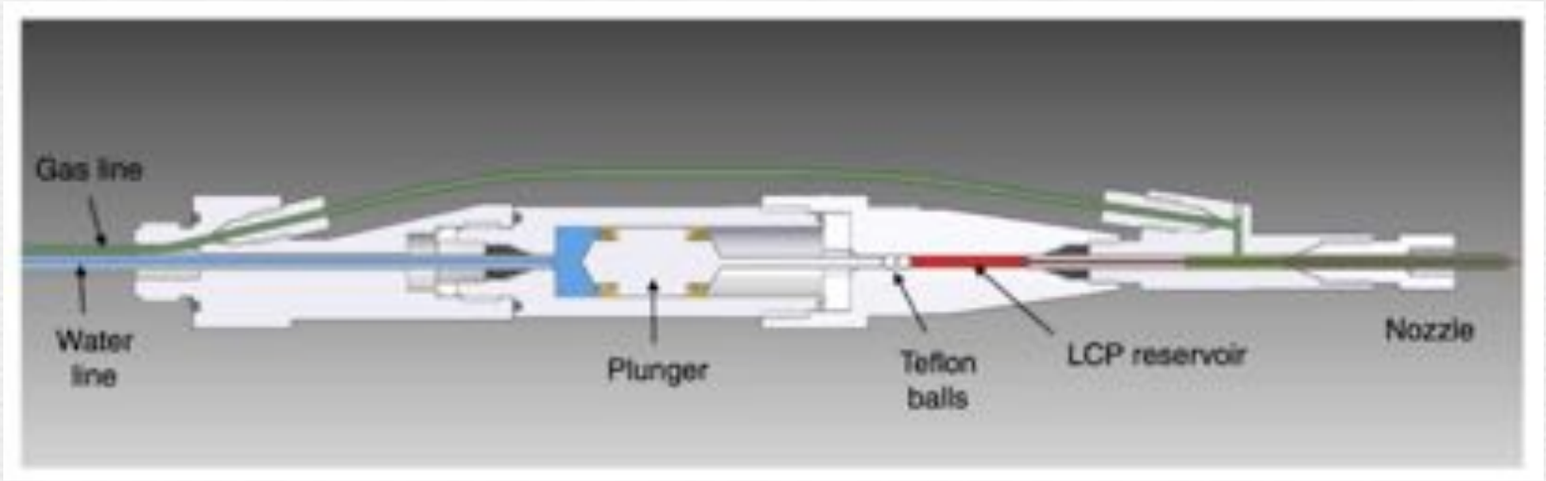



Chapman et al., (2011) Nature 470: 73-77

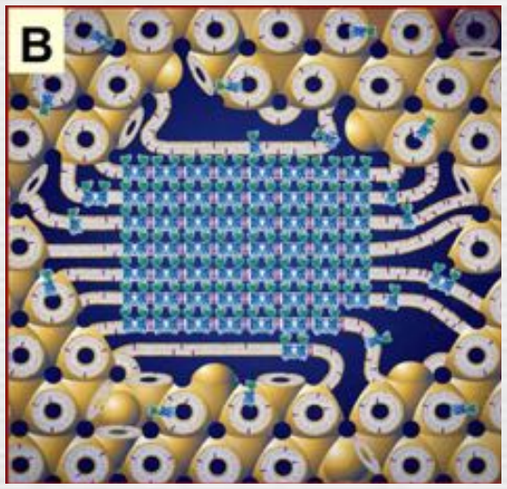
But there are more problems: low hit rate, non-trivial indexing (thousands of images are required),
and you need **ml** of highly concentrated suspension of microcrystals…

# L

Flow-rate down to **3-300nl/min** that equals to **~0.3mg** of purified protein!



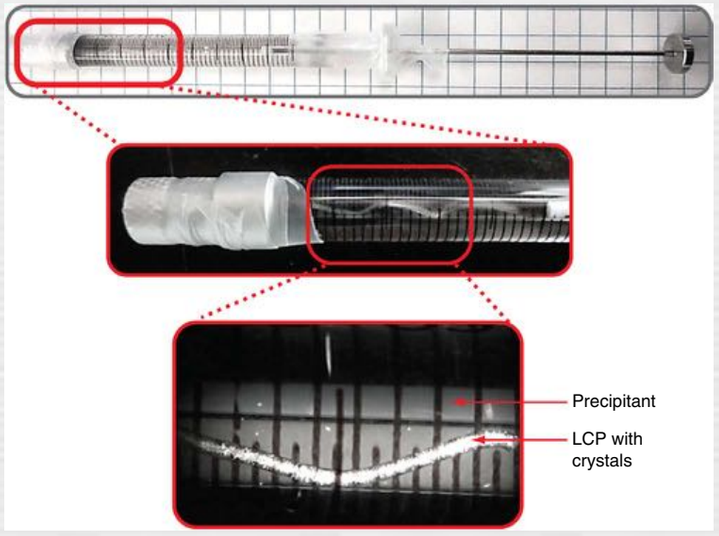

Weierstall, U. et al. , (2014) Nature Communications 5: 3309




Caffrey et al., (2012) Biochemistry 51(32):6266

Benefits:

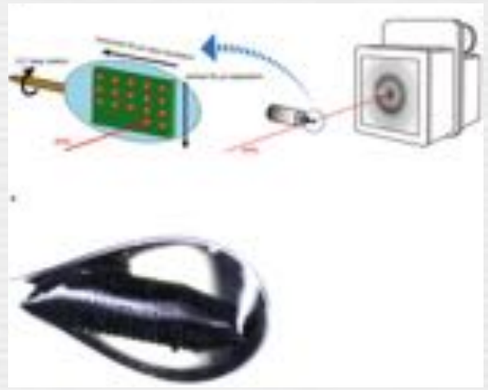
- native-like environment
- Type-I crystals
- No cryo-protection necessary

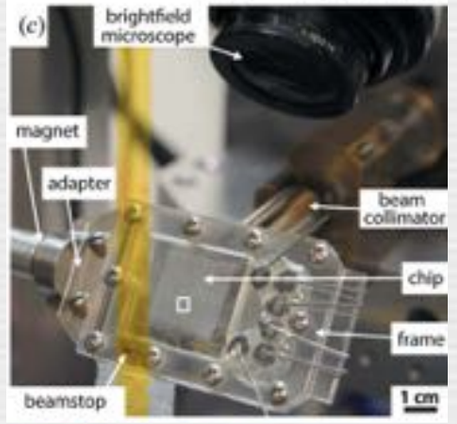



Liu et al., (2014) Nature Protocols 9:2123

# Alternative delivery methods

- Just use a big crystal if you have it – Suga et al., (2015) Nature 517: 99 Photosystem II structure at 1.95Å resolution
- fixed-target chips (theoretically 100% hit-rate)





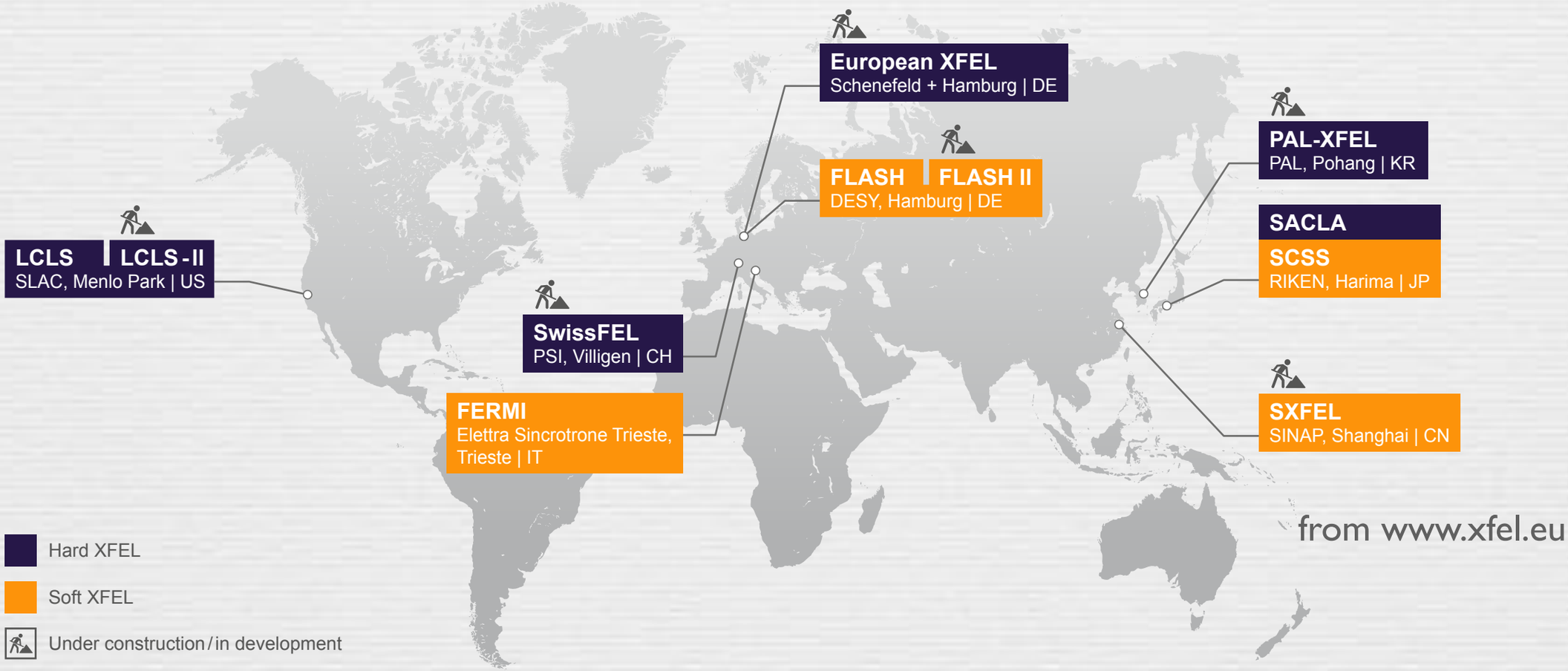

Heymann et al., (2014) IUCRJ 1:349

# Data Collection and processing issues

- Crystals are not always homogenous (different sizes)
- Crystals intersect the beam in random orientation
- Crystals stand still during exposure (no rotation)
- Only partial reflections are recorded
- Fringes rather than neat spots
- Per shot variation in photon energy and intensity of the pulse
- Enormous amount of data per experiment

Most issues can be resolved with Monte Carlo algorithms (CrystFel, cctbx.xfel) or by other software (nXDS)

# The main issue - Facility and Beam time availability

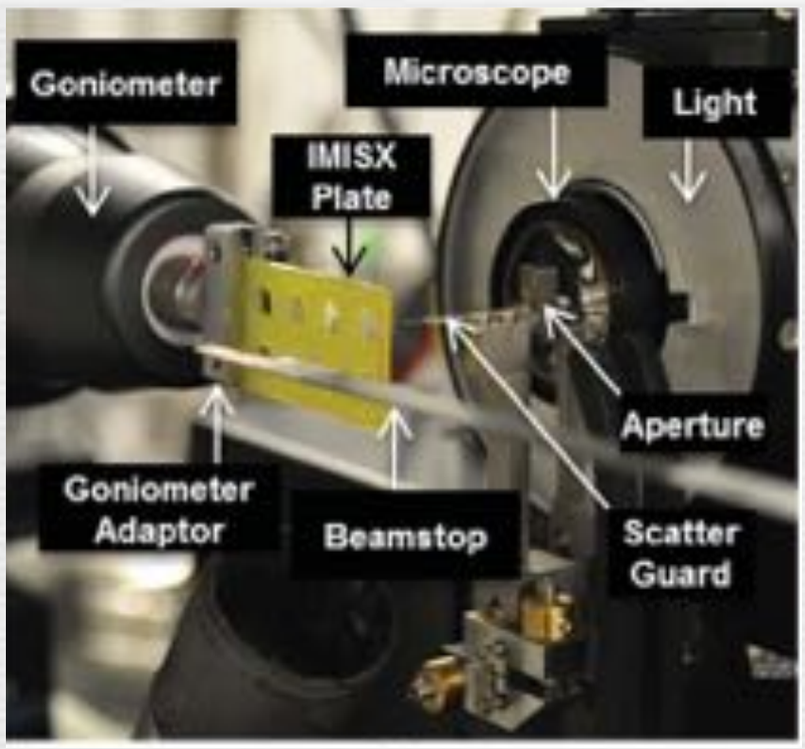



But SFX can be also performed at the most of modern synchrotrons!

# abbreviations competition

*in meso in situ*

MX
SFX
IMISX



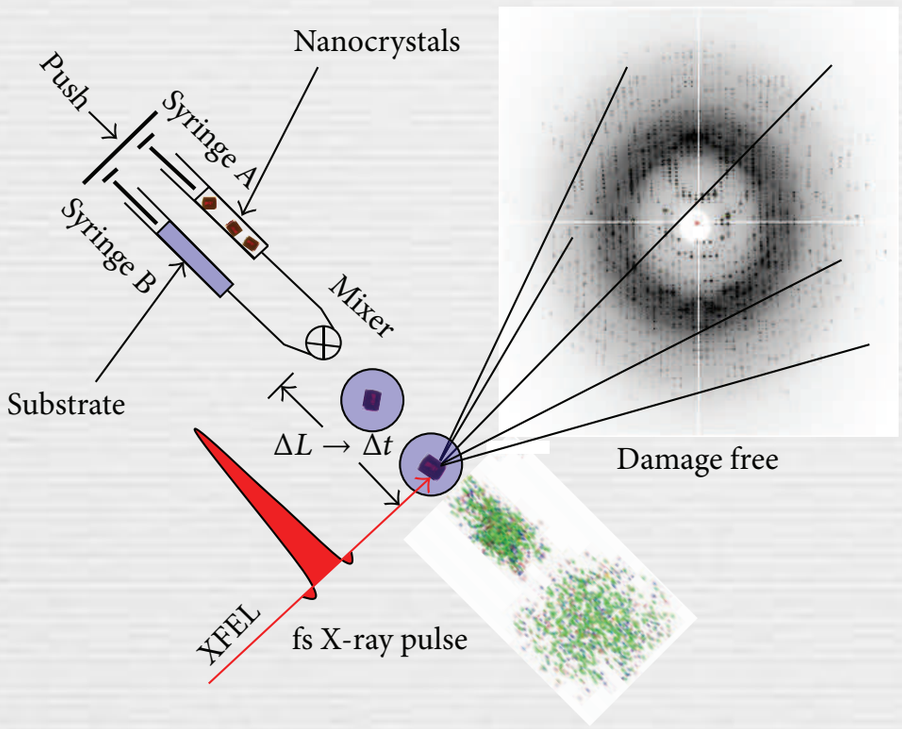

Huang et al., (2015) Acta Cryst D71

- +:No harvesting!
- RT
- No injectors
- Reduced amounts of samples
- Easy setup
- less data needed than with XFEL
- –: only RT, sensitive to t variations
- (data collection, delivery),
- short shelf lifetime

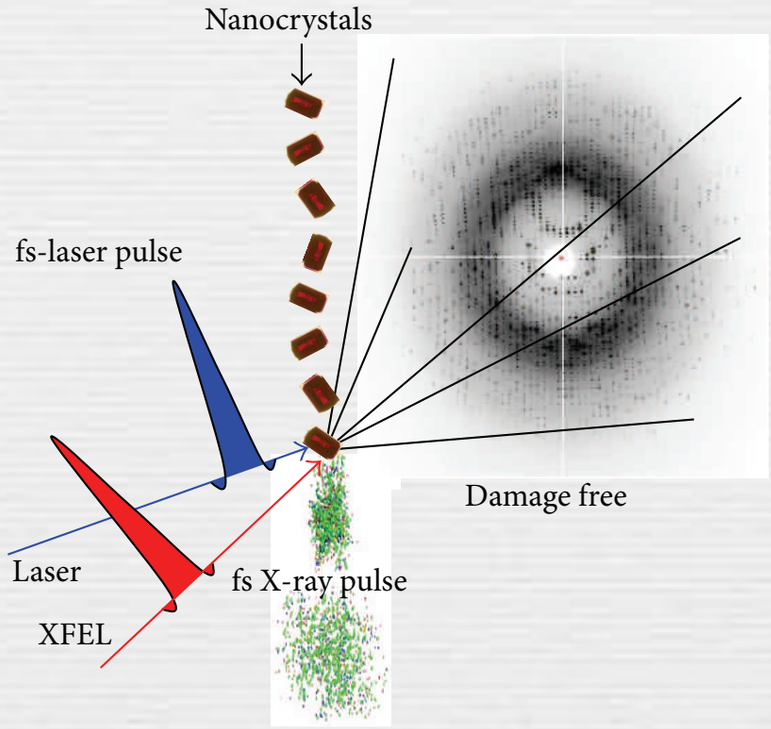
# benefits of using XFEL for time-resolved studies

- Small micro crystals allow  homogenous excitation
- Data is free from radiation damage caused by X-Rays and Laser
- Any sort of chemical reactions (including irreversible) can be studied
- No problem of “cryo-trapping”

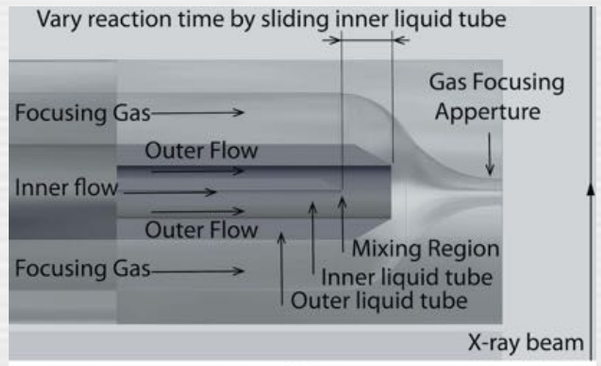
# Time-resolved studies




(a)



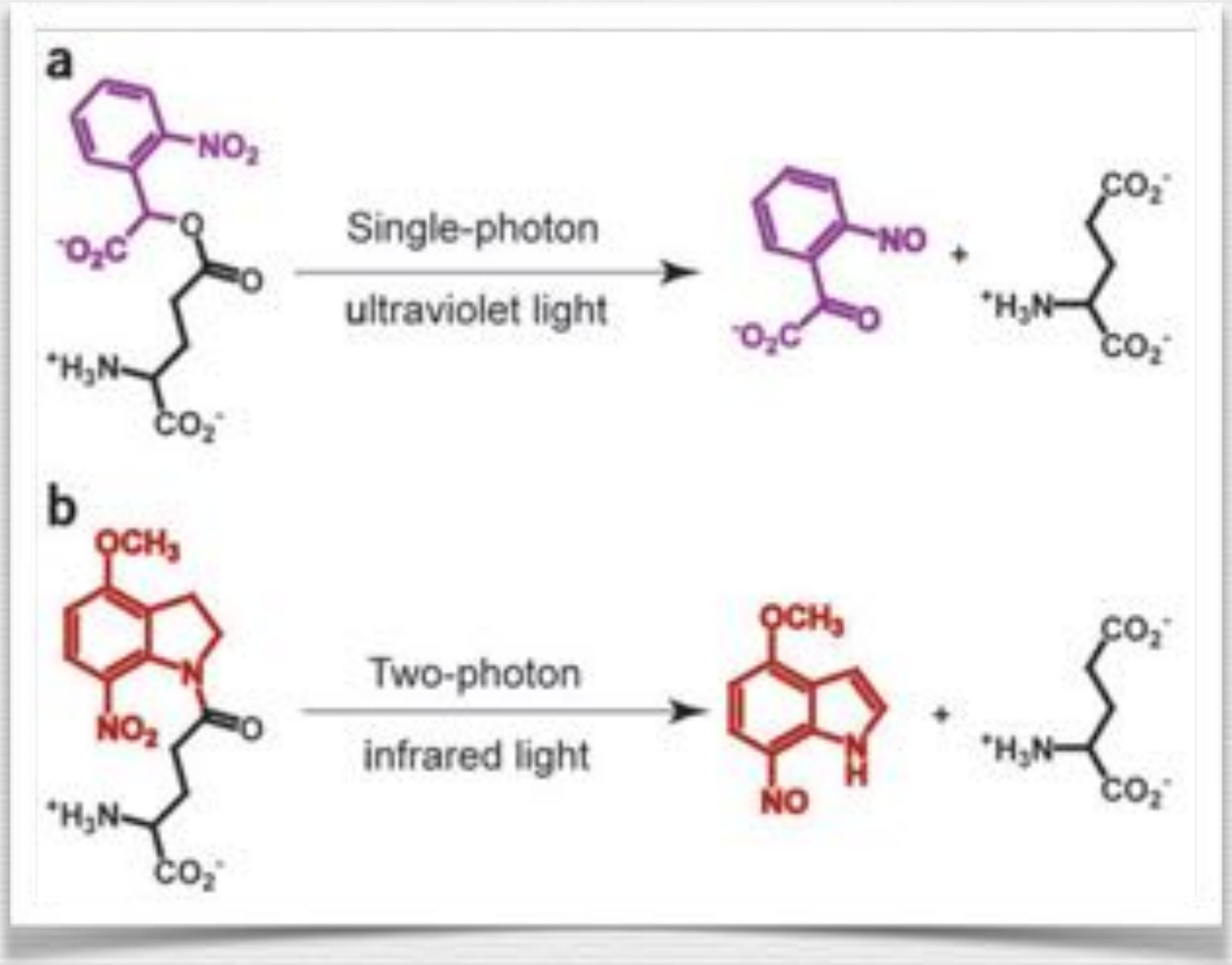

(b)




Schmidt M (2013), Adv in Con Mat Phys, doi: 10.1155/2013/167276

Wang et al., (2014) J of Synchr Rad. 21:1364

# Caged Glutamate (Aspartate)



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Dirk J Slotboom

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Andrii Ishchenko,
Thomas Barends,
Anastasya Shilova,
Manfred Burghammer

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Barbora Waclawikova,
Ria Duurkens,
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Maria Dosz-Majsnerowska
Dirk J Slotboom

## FRET

Antoine van Oijen

## TR

Arwen Pearson