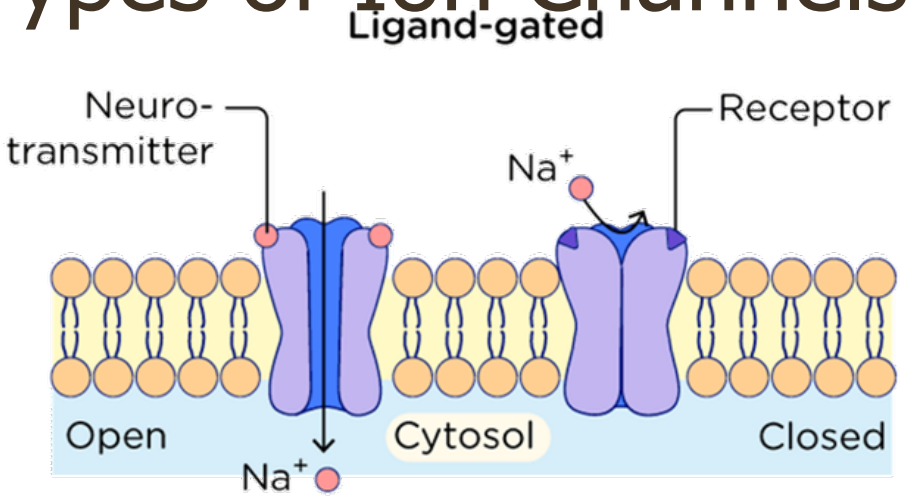
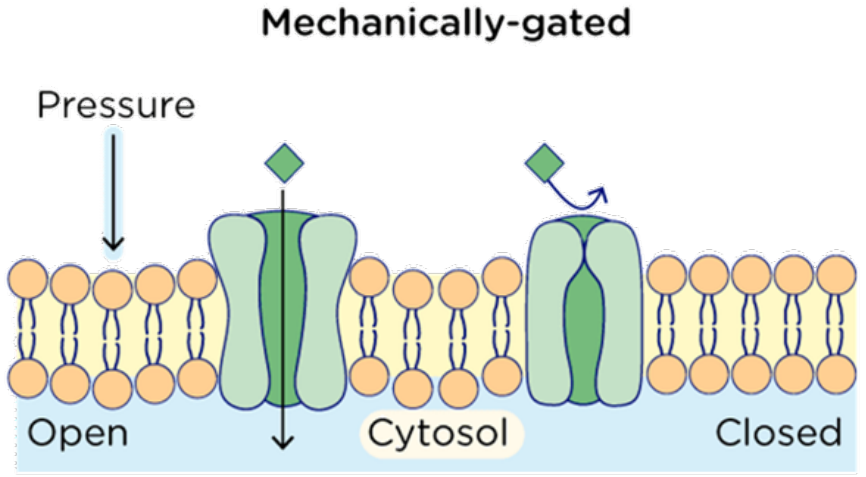
Birth of an idea (2007). Julian Voss-Andreae

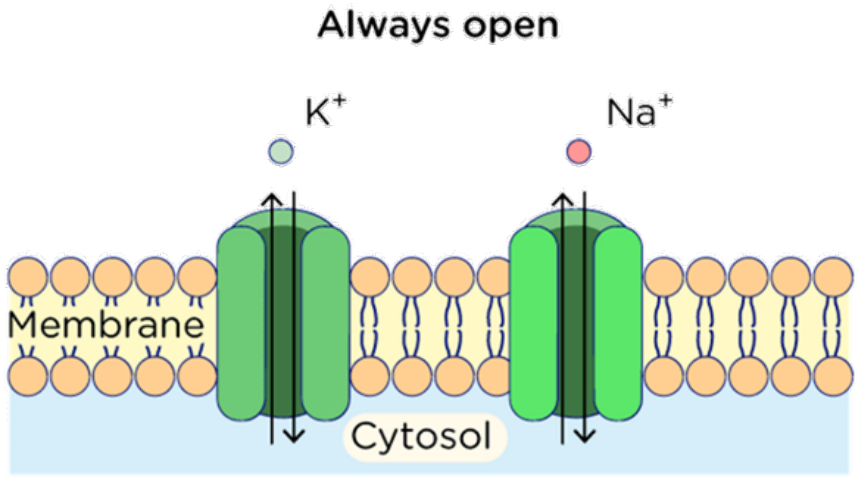
Square Antiprism is a Key Determinant for Potassium Ion Selectivity

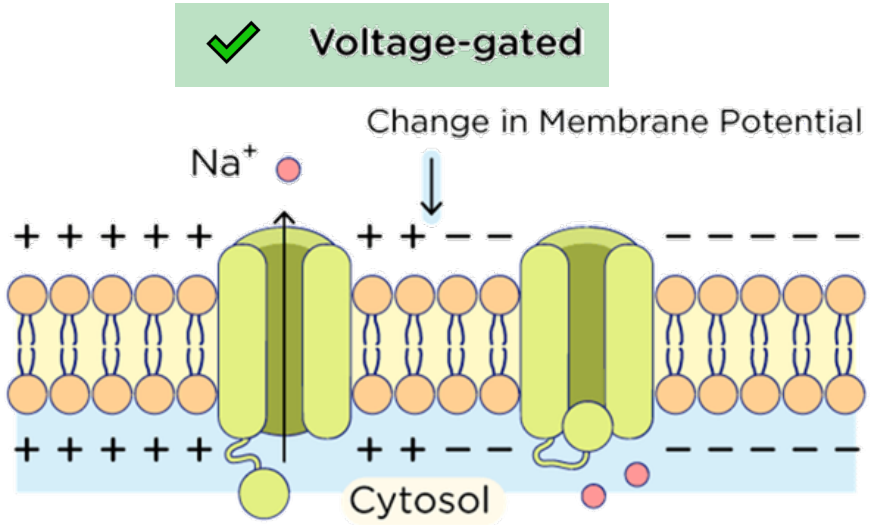
Kirill Scherbakov, Alexander Vassilevski, Anton Chugunov

Types of Ion Channels





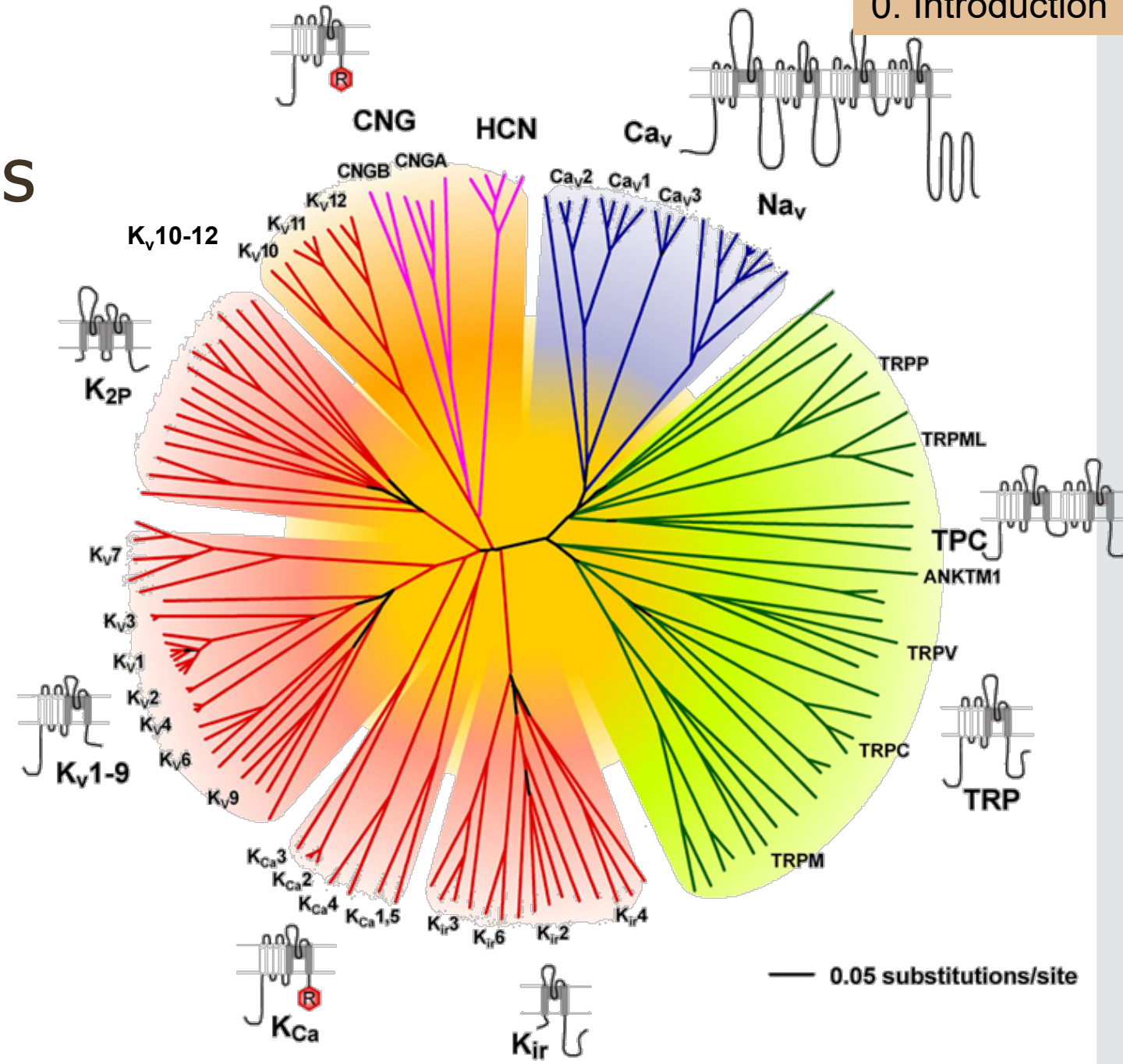




Jack Westin

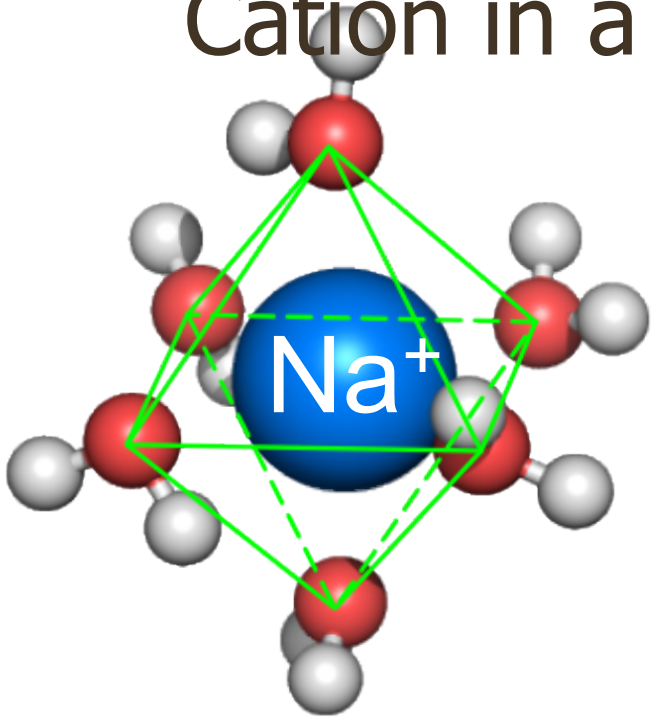
Ion Channels Families

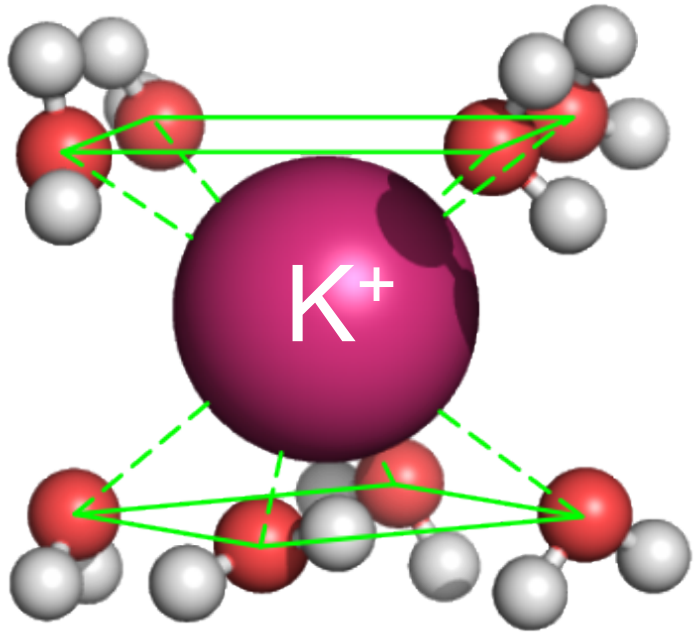
- **Ligand-gated**
  - Serotonin (5-HT_3R)
  - Acid-sensing (ASIC)
  - Epithelial sodium channel (ENaC)
  - $GABA_A$ receptors
  - Glycine receptors
  - Glutamate receptors
  - Nicotinic acetylcholine receptors
  - ATP receptors (P2X)
- **Voltage-gated**
- **Other**
  - Aquaporins
  - Chloride

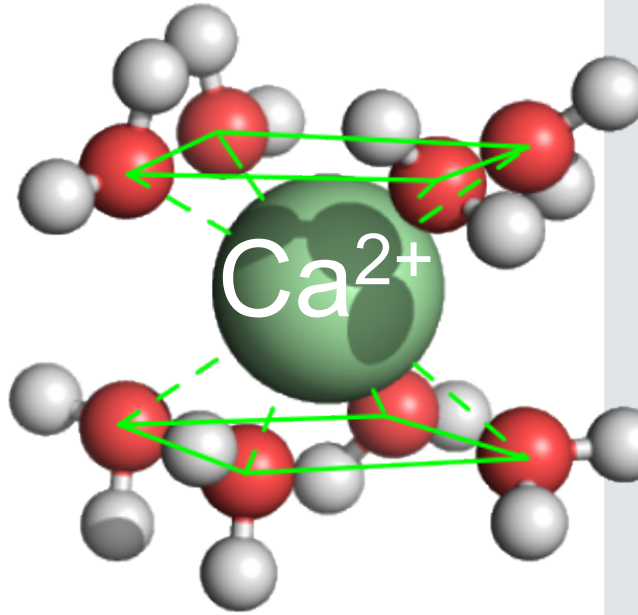


IUPHAR Guide to Pharmacology

Cation in a coat

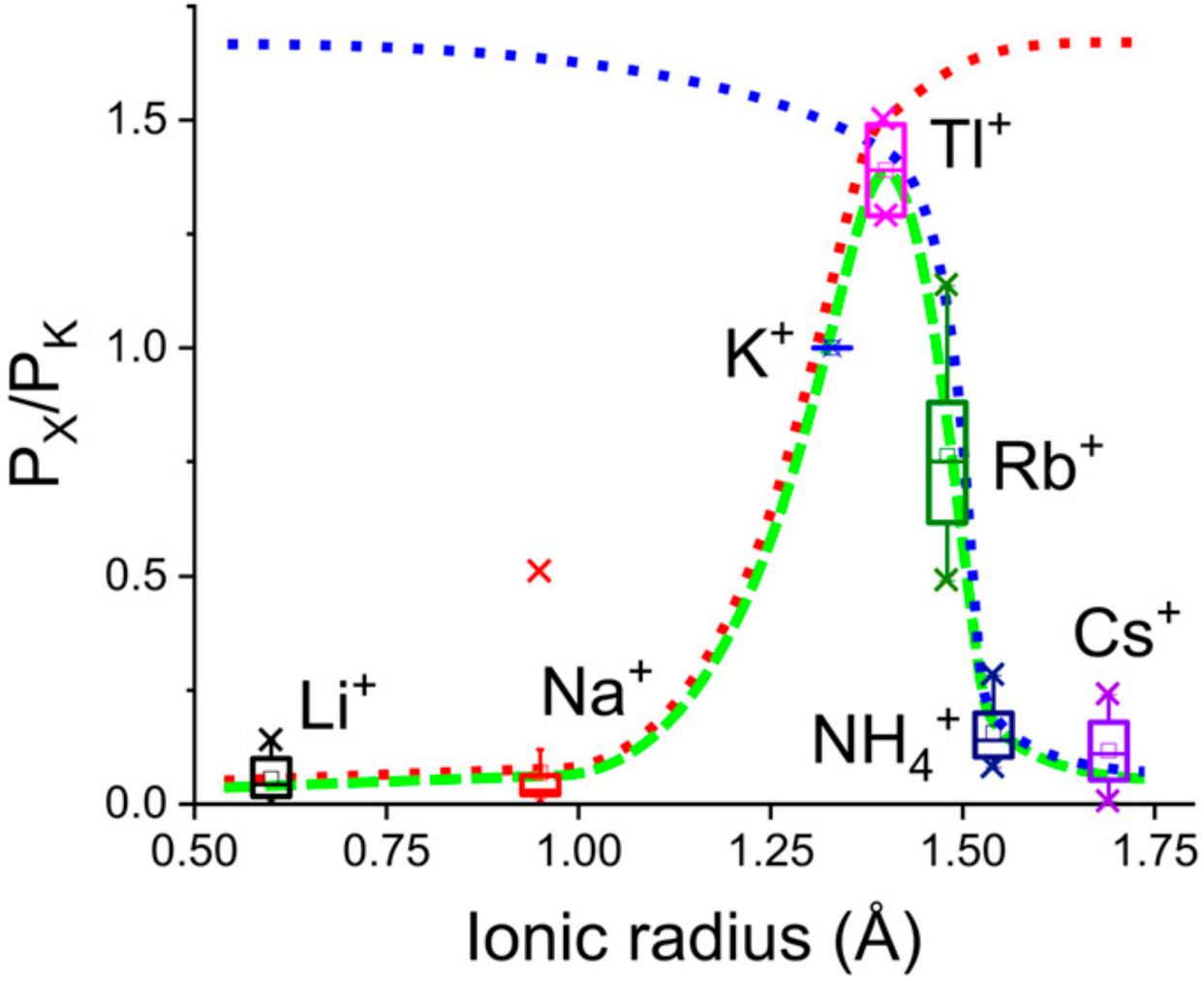






R_{ion}, Å	1.02	1.51	1.12
R_{Me-O}, Å	2.43	2.84	2.46
Coord. number	6	8	8
Geometry	Octahedron (bipyramid)	Square antiprism	Square antiprism

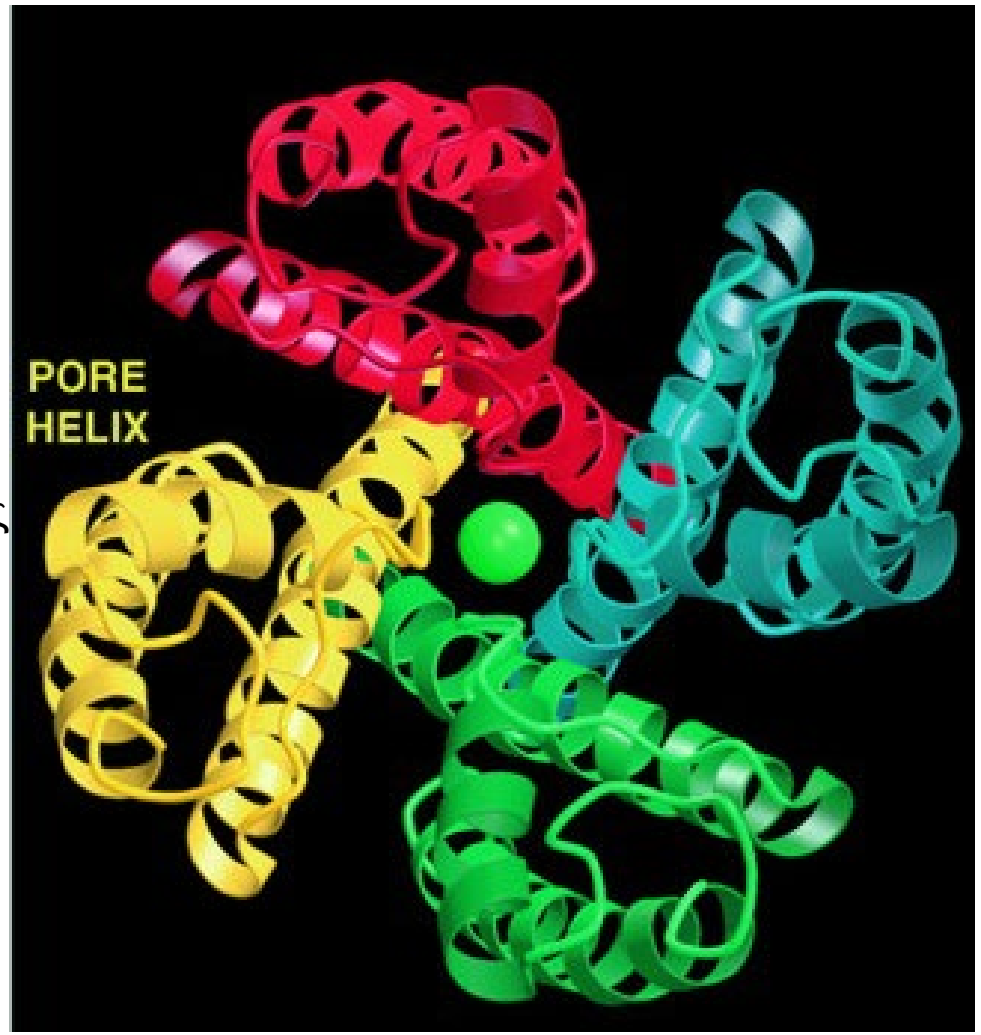
A Unique Potassium Channel

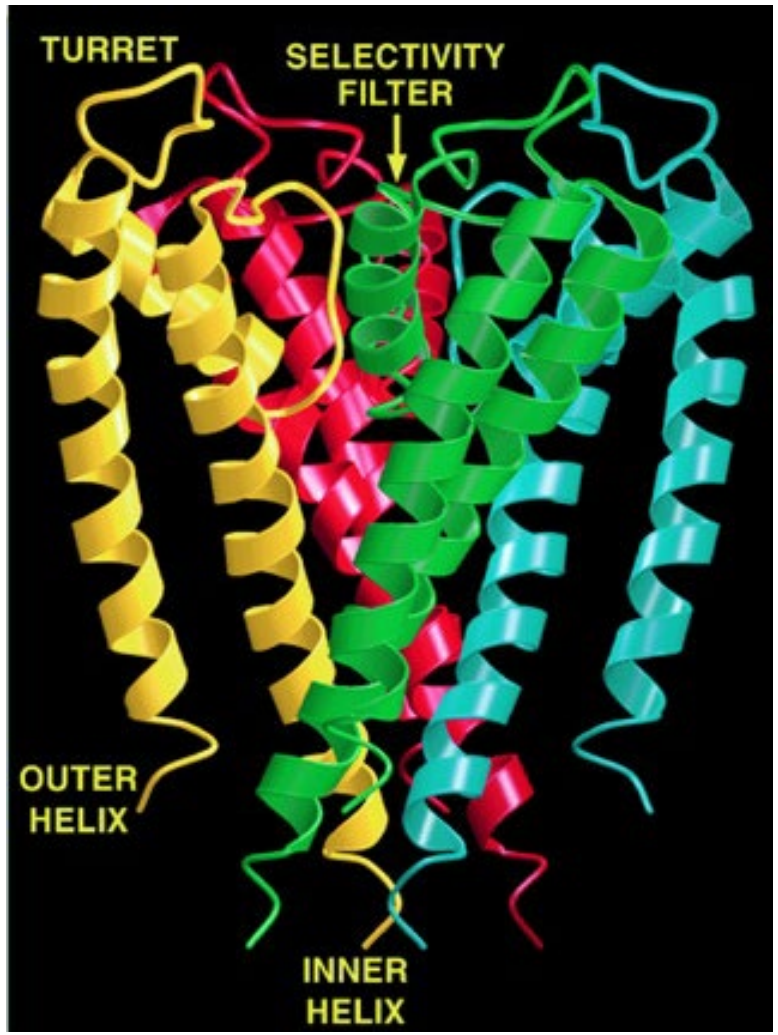


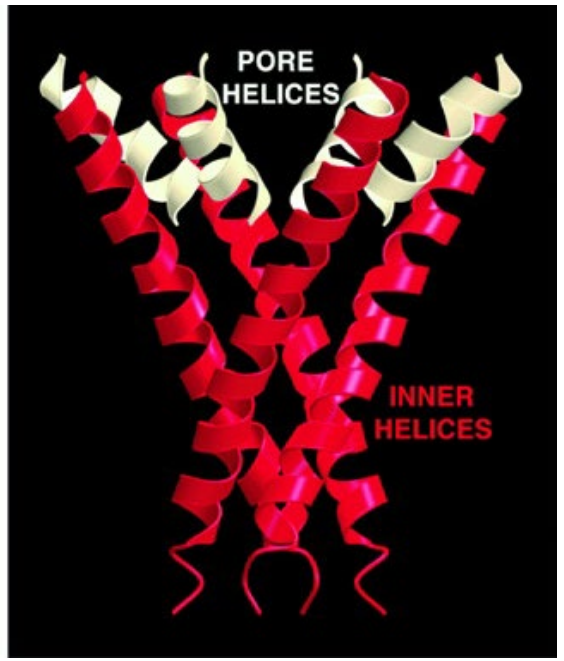
Mita *et al., PNAS 2021*

- Selectivity up to 10^4
- Rapid conductance (10^8 K^+/s), close to a diffusion limit
- Electrostatic knock-on mechanism of conduction: K^+ ions come one after the other, interspersed by H_2O molecules
- Time of ion passage is <1 µs, thus MD modeling may be applied

Potassium Channel Structure (KcsA)



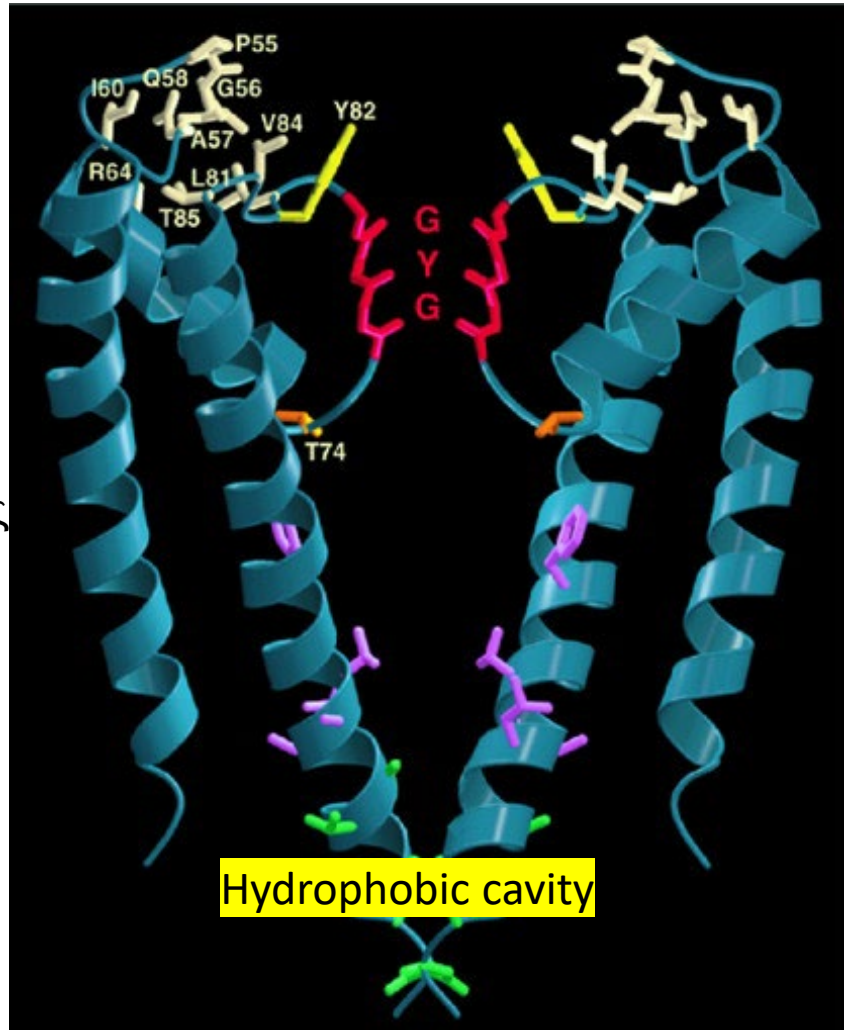


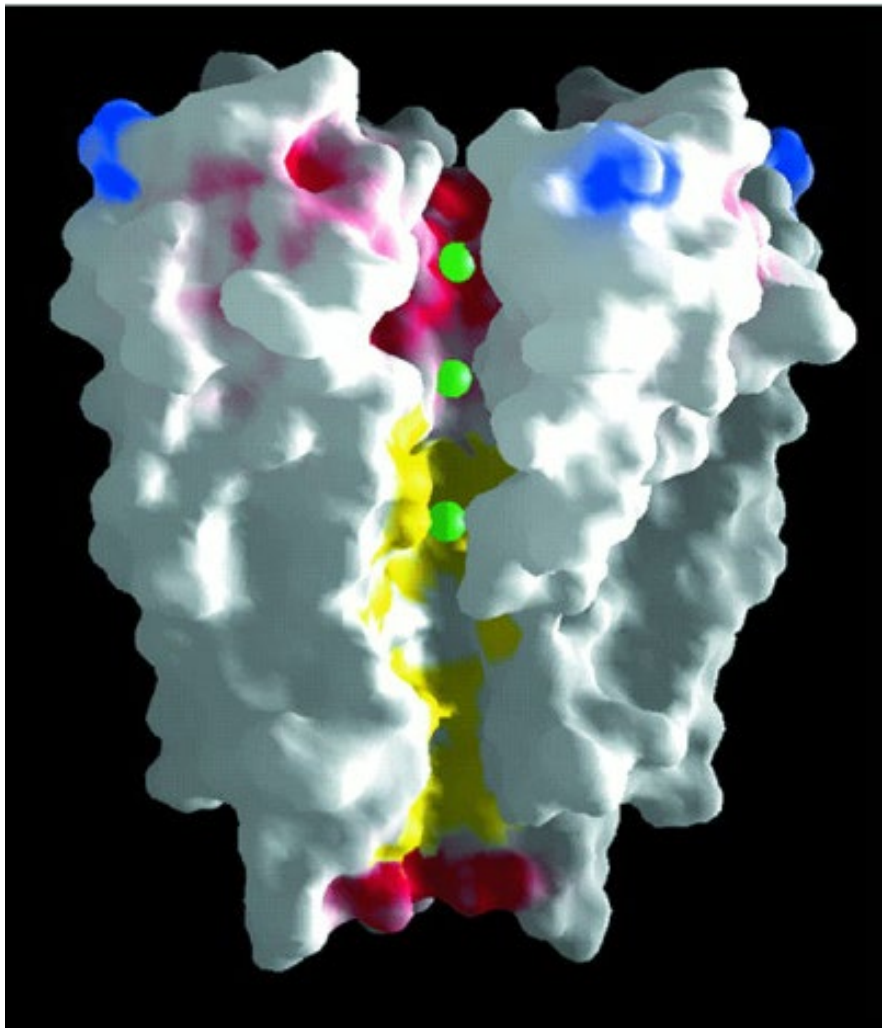


Doyle, ..., MacKinnon, *Science* 1998

Nobel Prize in Chemistry, 2003

Selective Filter is a "Quasi-Water"



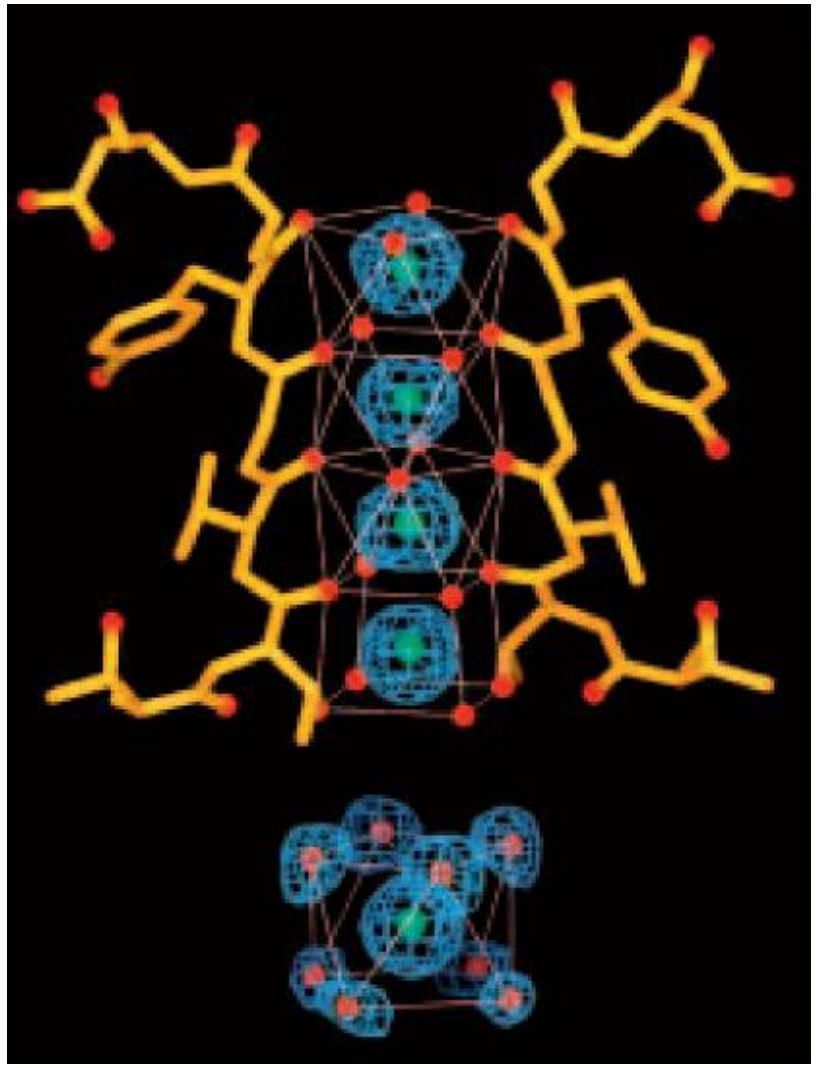


Doyle, ..., MacKinnon, *Science* 1998

Nobel Prize in Chemistry, 2003

- SF: just 12 Å for potential to drop
- There are four SF sites, which are (probably) occupied by K^+ over one (others contain H_2O)
- K^+ come one after the other (two ions in SF at a time)
- 7.5 Å → 4 M KCl (!)
- Hydrophobic gating

Origin of Selectivity



- K^+-Channels conduct potassium at that high rate and selectivity because carbonyl oxygens layers in the SF form a rotated quads that perfectly copy a square antiprism of the solvated K^+ ion
- K^+ desolvates **completely** without any energy penalty
- There are several K^+-binding sites in the SF (4+2), but just two are occupied at the same time
- Upcoming K^+ electrostatically pushes another ion from the SF — a “knock-on” mechanism (“soft”, “hard”)
- Sodium (Na^+), apart being smaller:
  - does not come into SF
  - Cannot desolvate without a penalty (since the channel is optimized for K^+)

MacKinnon’s Nobel Lecture

In this talk:

0. Introduction

1. An algorithm to seek K^+-binding sites in proteins

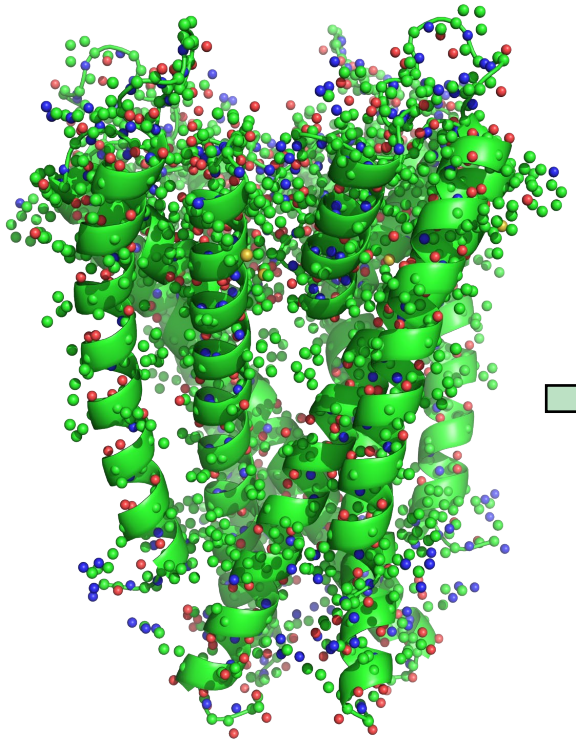
2. K^+ Selectivity filter: unique and conserved for all K^+-channels

3. Yes we can distinguish active and inactivated SF in K^+-channels by antiprismatic match

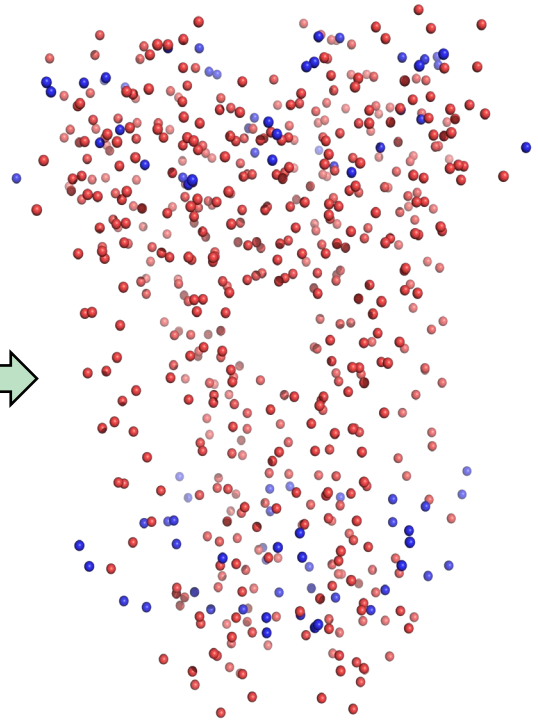
4. K^+-binding sites in other (membrane) proteins

5. Small differences for other K^+-binding proteins explain lack of selectivity and other features

A quest for K^+-binding sites using template



All KcsA heavy atoms: 3060
$N_{var} = 10^{23}$ 🤦🤦🤦

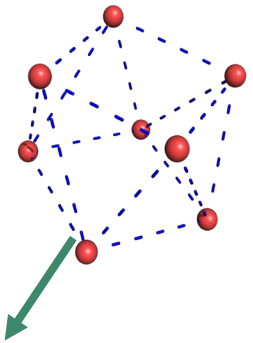


Potential cation-chelating atoms (N_{sc}, O): 612
$N_{var} = 10^{17}$ 🤦

Distance matrices $[A_{prot}]$ and $[A_{ion}]$

For $[A_{ion}]$: R_{min}, R_{max}

For i^{th} protein atom: the search vicinity $[R_{min} - \Delta; R_{max} + \Delta]$



Enumeration of octets of atoms, superposition and RMSD calculation

RMSD < cut-off?

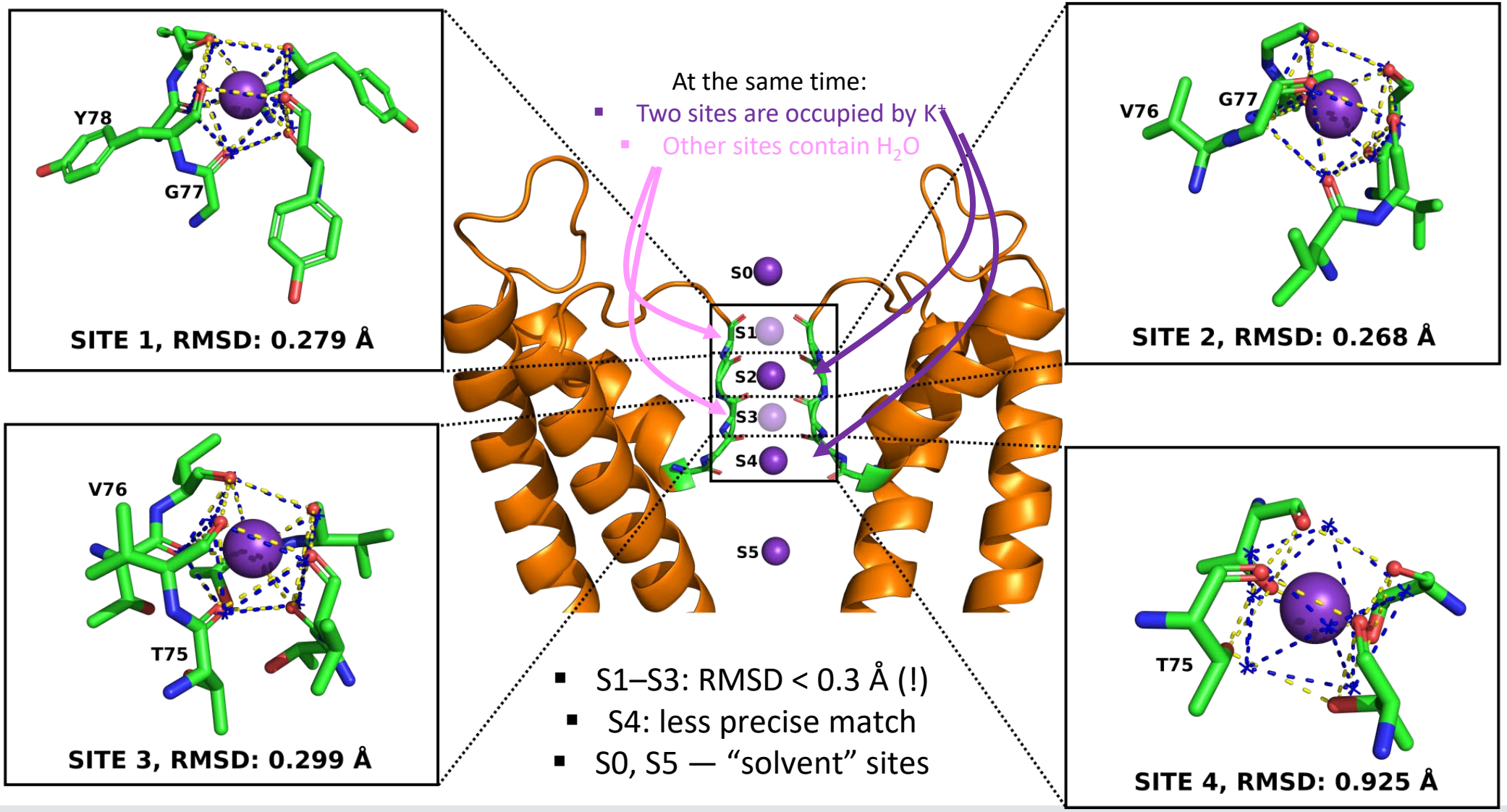
Yes

Site stored

No

Site rejected

Four KcsA Sites Found at Their Best👌

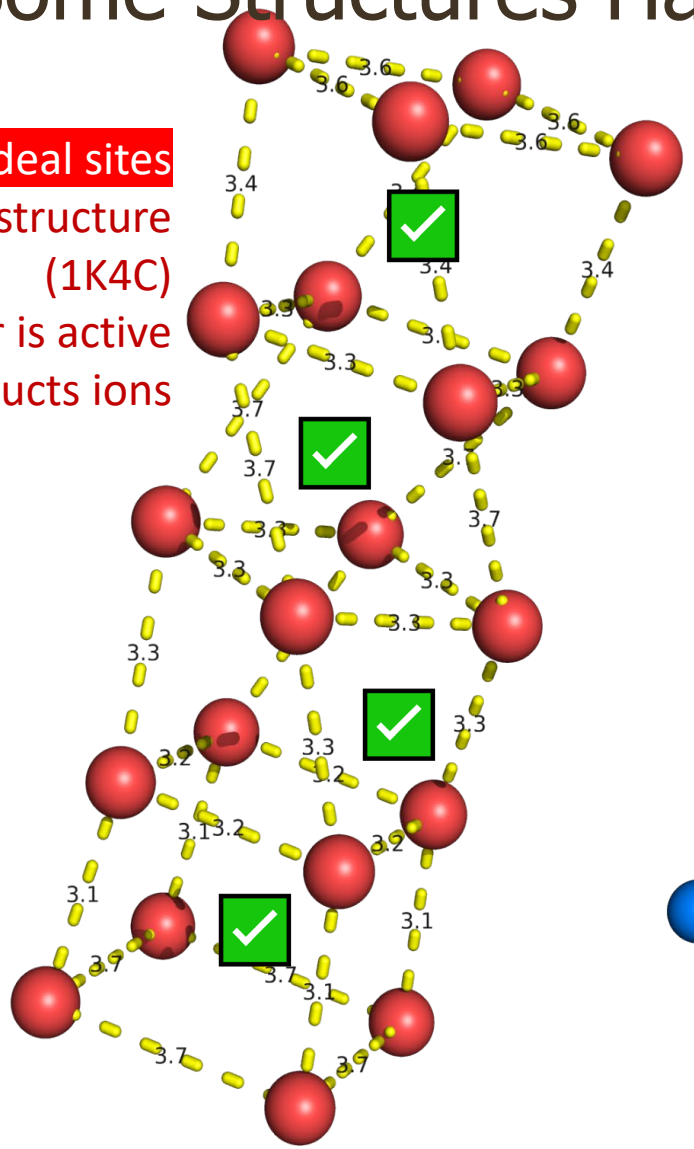


But Some Structures Haven't K^+-Binding Sites... 🤷

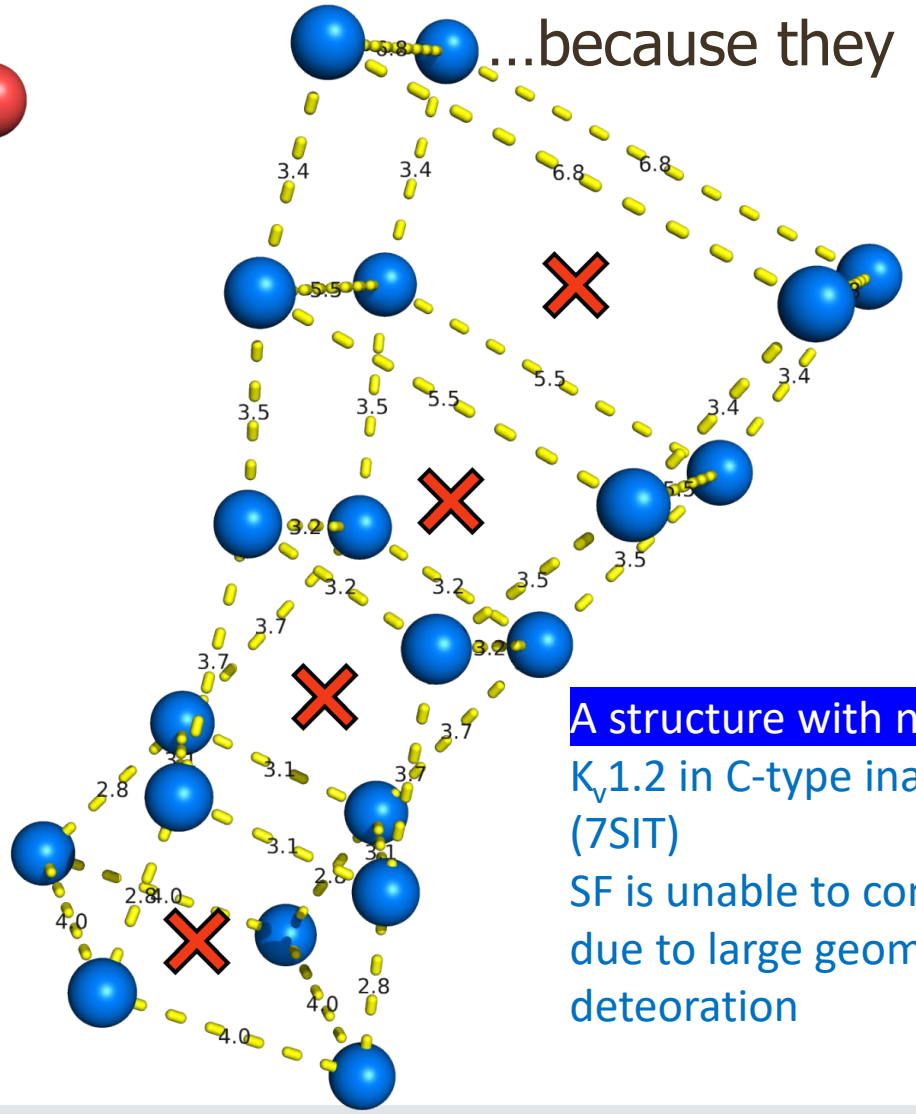
Ideal sites

"Classic" KcsA structure (1K4C)

Selective filter is active and conducts ions



...because they don't have to! 👌

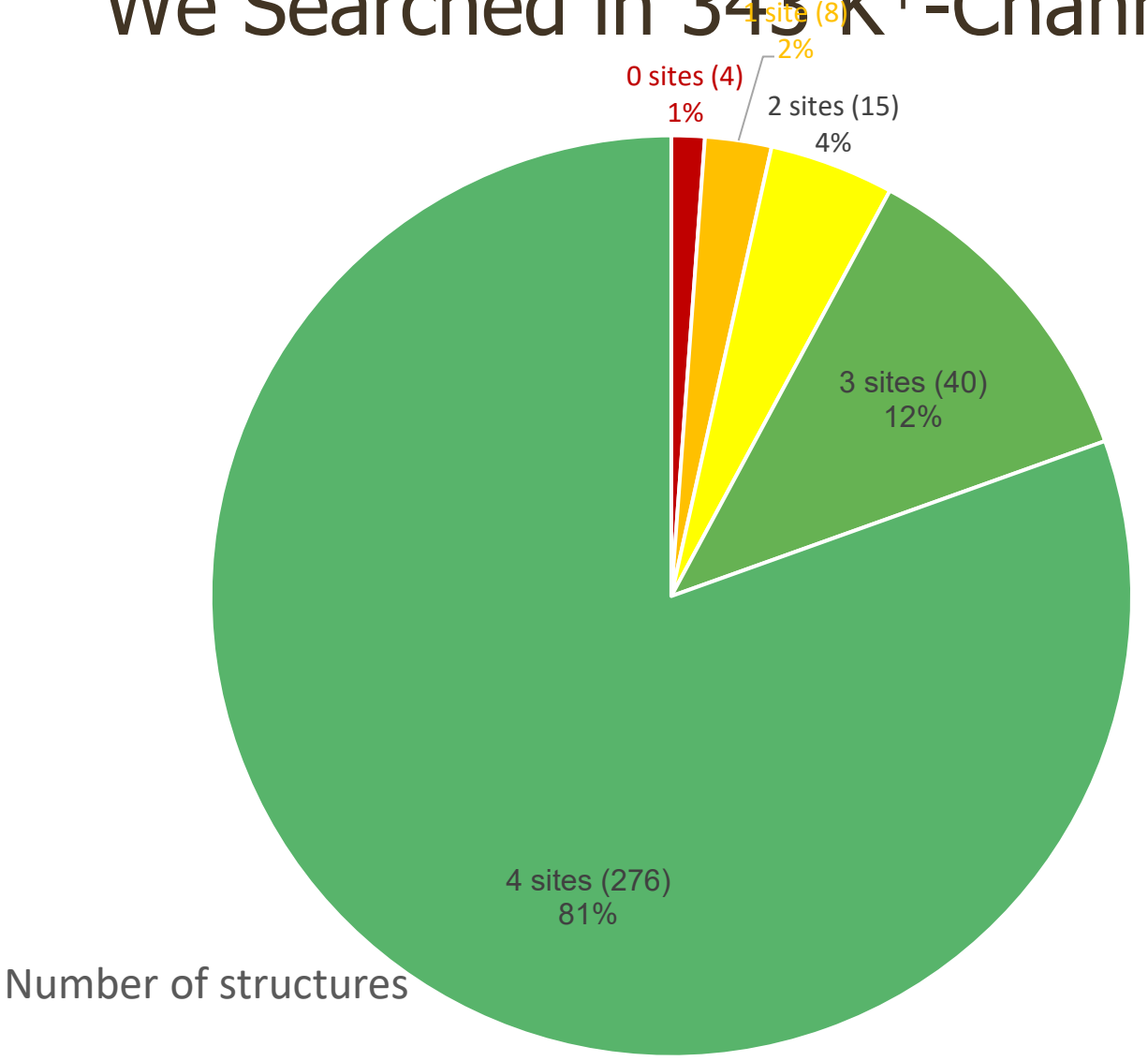


A structure with no sites

$K_v1.2$ in C-type inactivated state (7SIT)

SF is unable to conduct ions due to large geometry deteoration

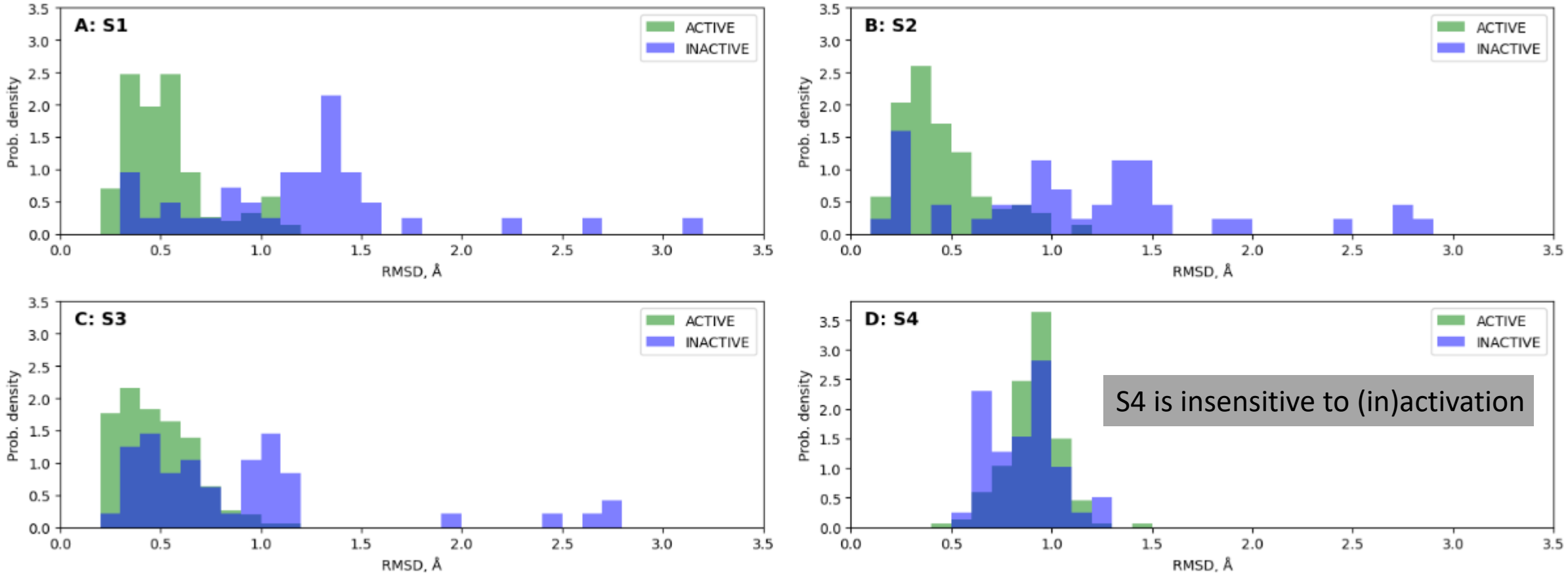
We Searched in 343 K^+-Channels Structures…



…and found that structures described as active (conductive) mostly exhibit 3–4 sites, while (deeply) inactivated may contain 0–2

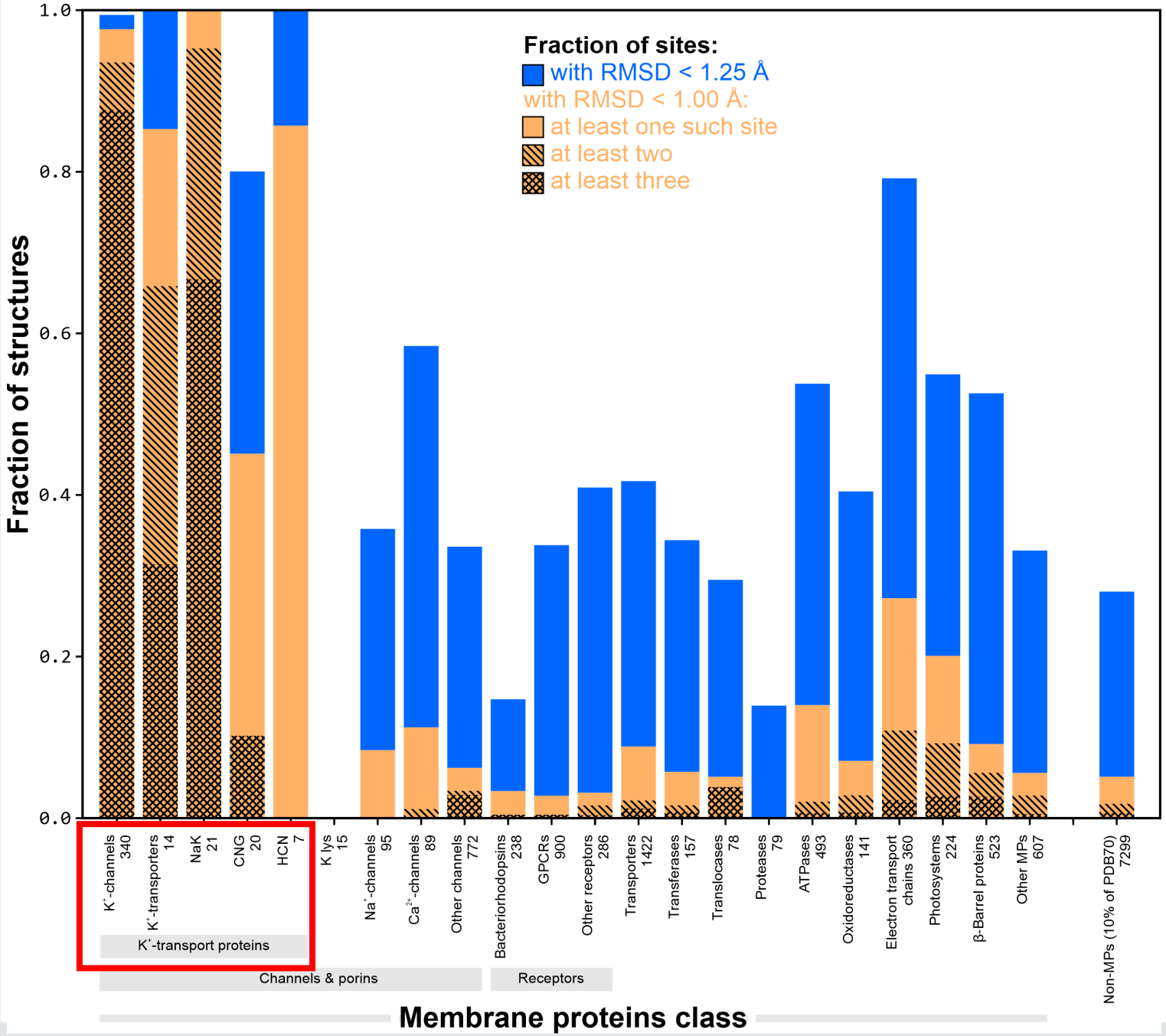
- RMSD cut-off for antiprismatic match was chosen 1.25 Å
- For structure to be active, it must feature at least three (S1–S3) K^+-binding sites
- S4 has higher RMSD and is not affected by (in)activation
- This prediction method is rather precise:
  - TPR = 0.99
  - TNR = 0.80

RMSD Cut-off of 1.25 Å at S1–S3 Sites Clearly Distinguish Active and Inactive SFs



All three S1–S3 have to match to square antiprismatic template for SF to be active

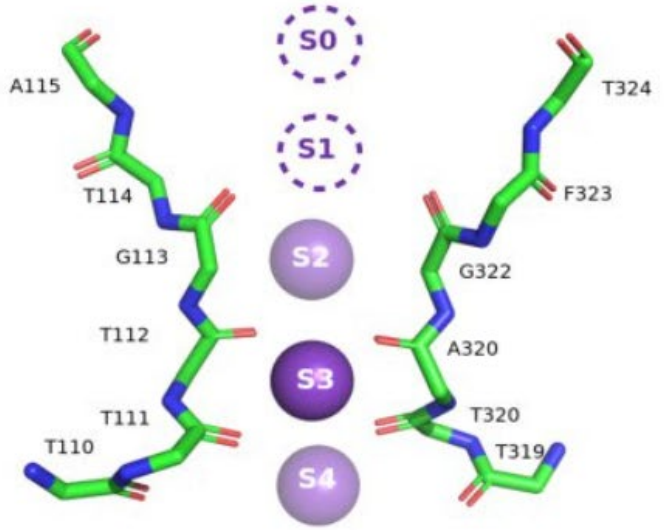
Only K⁺-transport Proteins Have Many Precise K+-Binding Sites



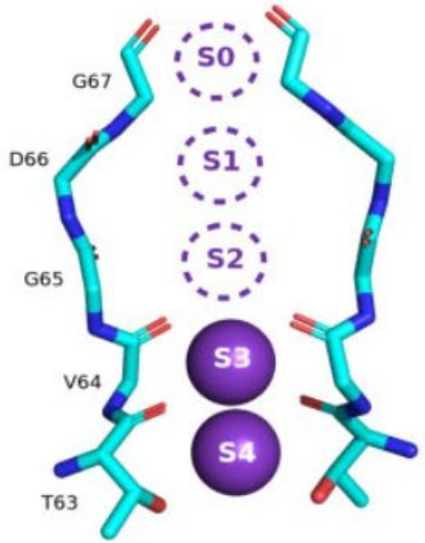
These proteins include:

- K⁺-channels (discussed earlier)
- K⁺-transporters
- NaK channels
- CNG channels
- HCN channels

A: K+-transporter



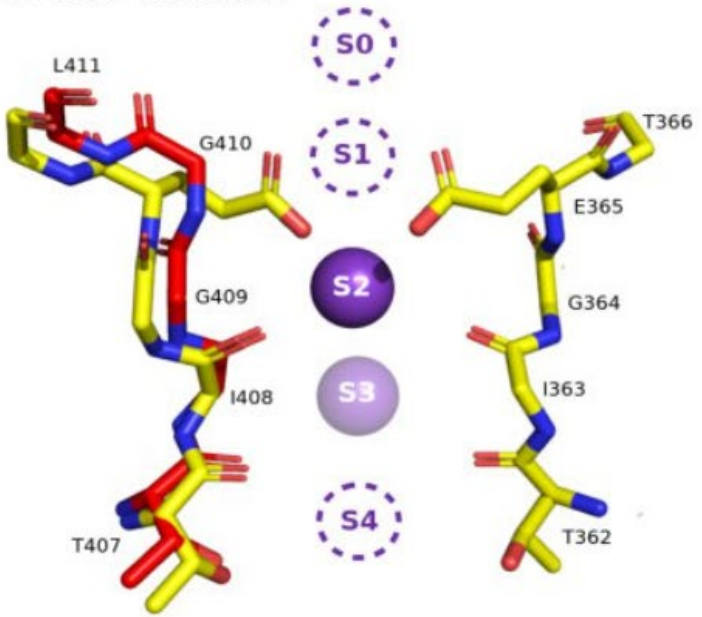
B: NaK channel



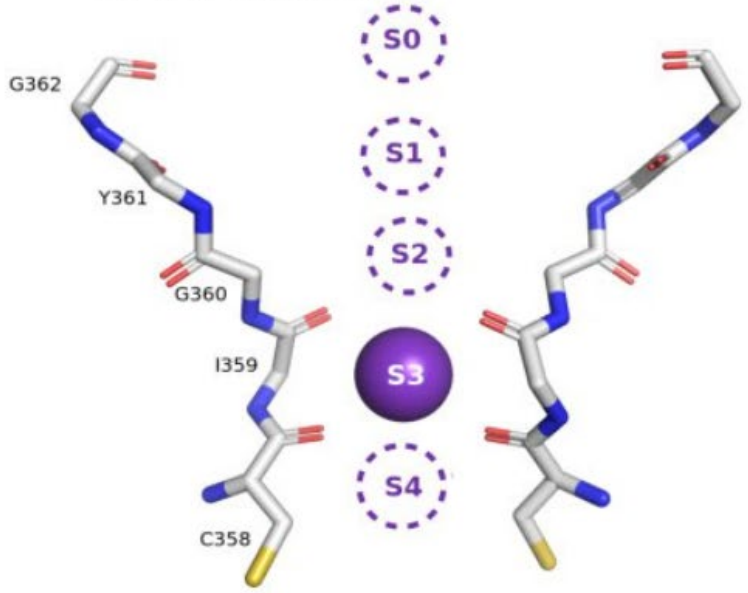
Other K+-Transport Proteins: Similar yet Different

In contrast to K^+-channels, these proteins possess only one or two K^+-binding sites, leading to significantly lower K^+ selectivity

C: CNG channels



D: HCN channels



Conclusions

1. The SF is a unique structure persisting in K^+-channels and some related proteins (K^+-transporters and NaK, CNG, and HCN channels) in a highly conserved form, but it is absent from other proteins.

2. Conductive and non-conductive SFs may be clearly delineated by a 1.25 Å RMSD threshold at sites S1–S3 — the way for geometric assessment of the functional state of SFs.

3. Antiprismatic sites are found in different channel domains and other membrane as well as non-membrane proteins, where they may be of functional significance.

Thanks for the attention!