Crystallography and Molecular Dynamics:

Water–Free K^+ Transport through Membrane Channels

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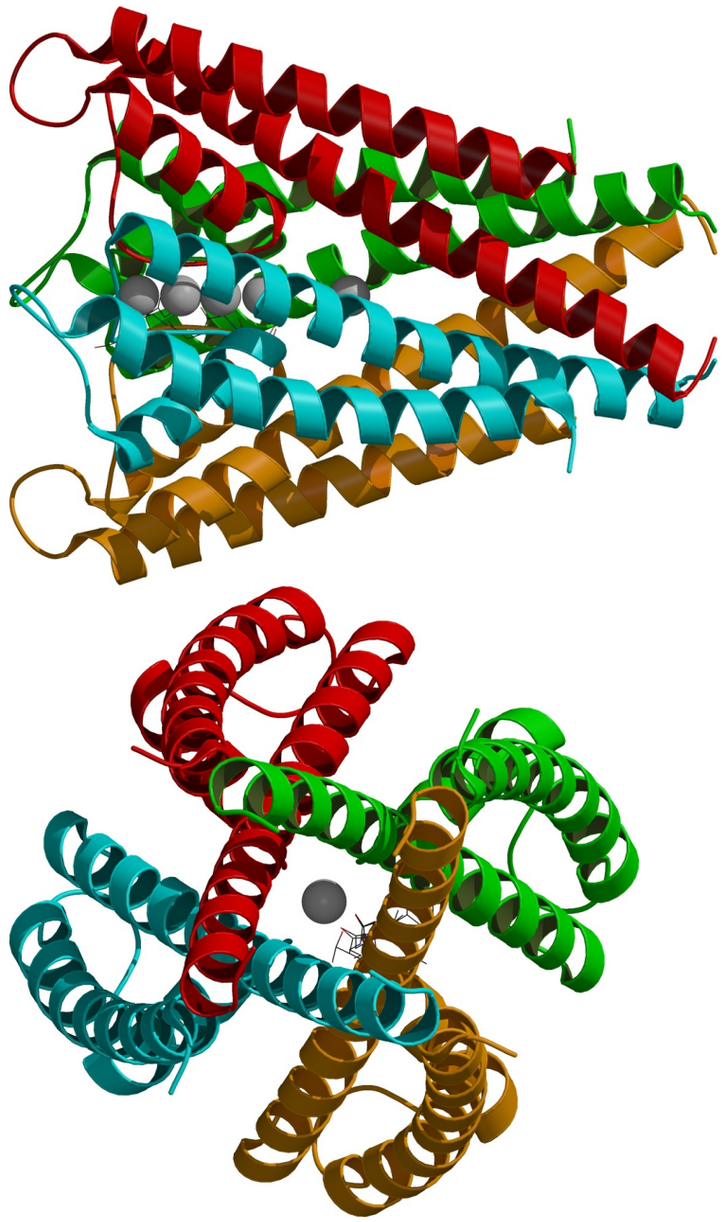
Potassium Channels — Transport Properties

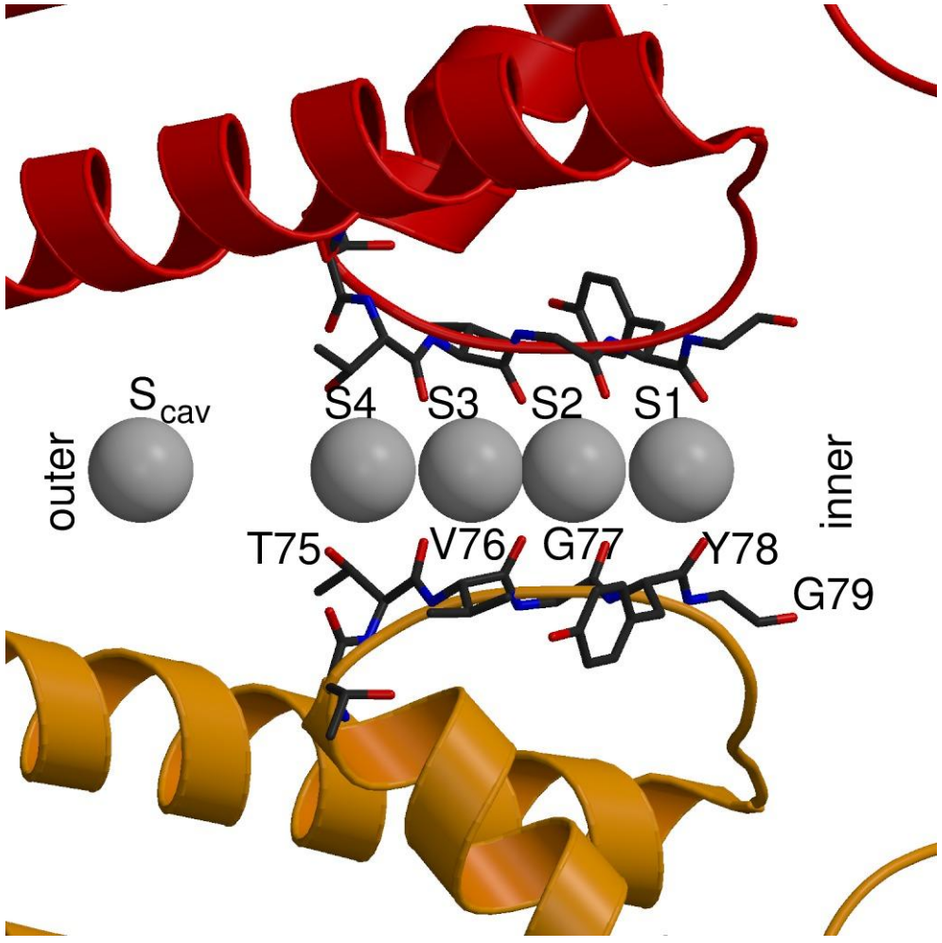
- Transport of Potassium ions across cell membranes
- Abundant in pro– and eukaryotes (≥ 78 genes in humans)
- Nervous signal transduction, muscle contraction
- Highly K^+ specific selectivity
- Passive transport, *i.e.* gradient driven
- Transport rate nearly diffusion limited



(PDB-ID 1R3J)

The Selectivity Filter

1998 First Crystal Structures of the K^+ channel KcsA by Roderick MacKinnon.



- Core of channel: 12Å tube
- $S_{\text{cav}} \rightarrow S_4 \rightarrow S_1$: transport into membrane compartment
- Conserved sequence TVGYG
- K^+–$O \approx$ 2.8Å
- (Backbone–) oxygen atoms mimic 1^{st} hydration shell

$\Rightarrow$ Blocks other ions, *e.g.* Na^+, Ca^{2+}: X–$O \approx$ 2.4Å

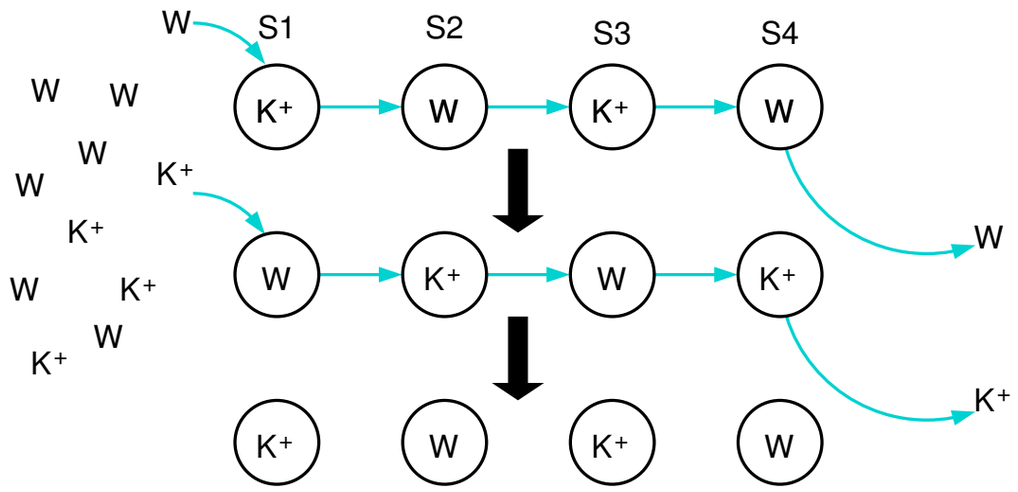
The "$KWKW$" Conduction Mechanism

- A. L. Hodgkin, R. D. Keynes (1955): electrostatic repulsion prohibits $K^+K^+K^+K^+$. Shielding by water molecules
- Morais–Cabral, Zhou, MacKinnon, Nature (2001), 414, 37–42: determination of electron density with K^+ and Rb^+
- Zhou & MacKinnon, JMB (2003), 333, 965–975: Crystallographic occupancy by means of the anomalous signal of Tl^+ and $K+$

1. Tl^+–$O \approx K^+$–$O \approx$ 2.8Å: interchangeable
2. Occupancy refinement based on the anomalous signal of Tl^+
3. Comparison with K^+–density

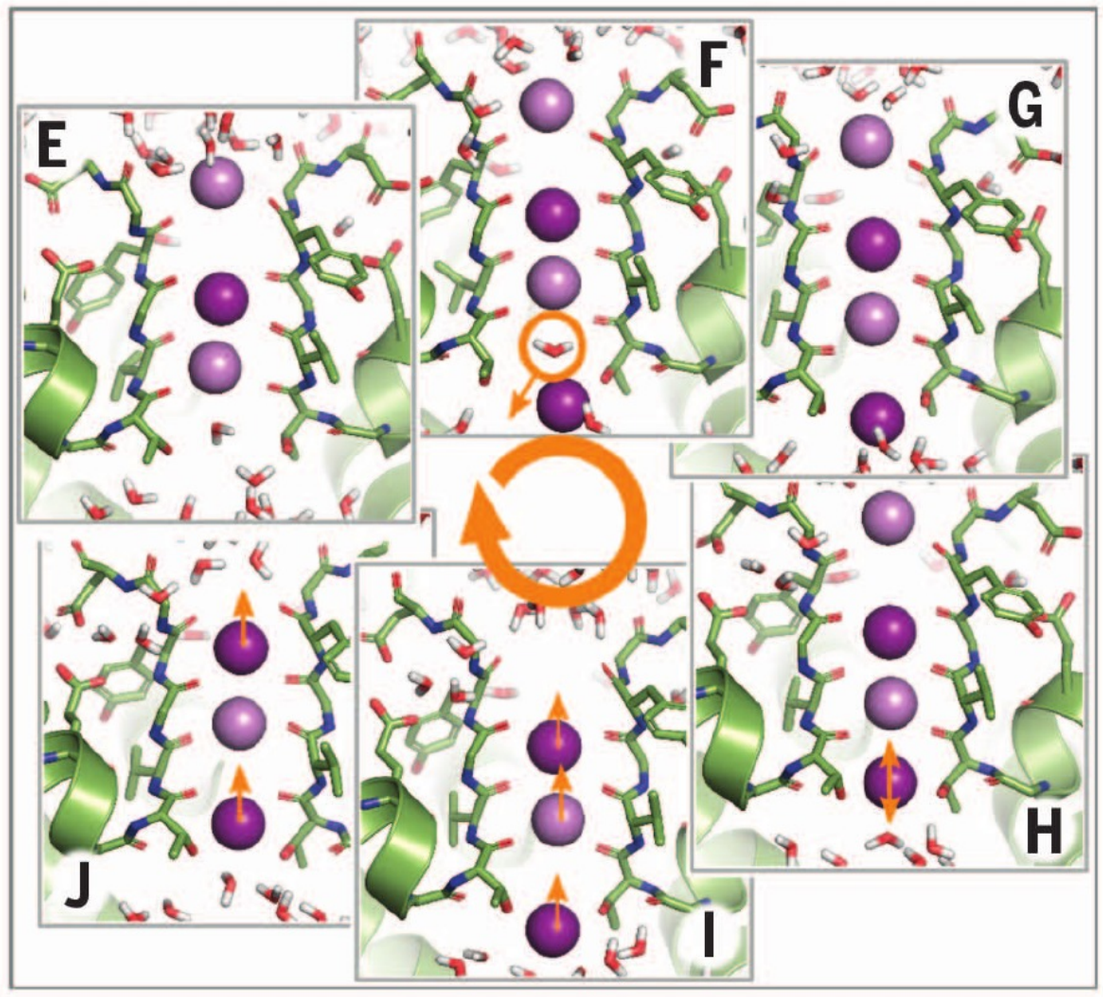
$\Rightarrow$ on average 2 K^+ inside filter

$\Rightarrow$ Crystal structure = mixture between "$KWKW$" and "$WKWK$"



Established view: co–transport of water through Selectivity Filter

The de Groot/Köpfer Mechanism



Based on Molecular Dynamics: **no water** inside Selectivity Filter

Köpfer, Song, Gruene, Sheldrick, Zachariae, de Groot: *Science* (2014), 346, pp. 352–355

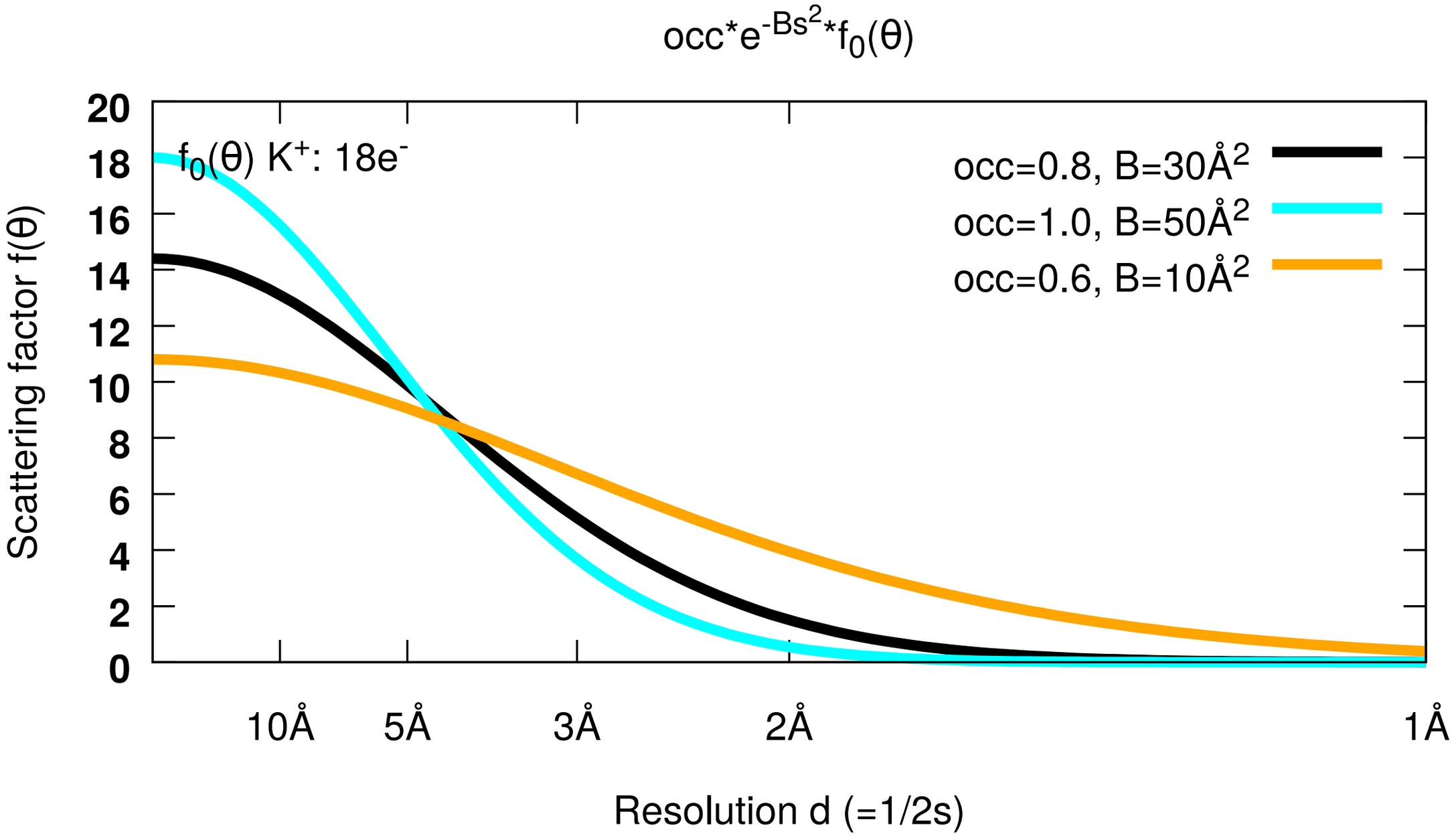
Molecular Dynamics: Transport Model

Crystallography: Experimental Data

MD simulation **contradicts** established model. Crystallogrpahy:

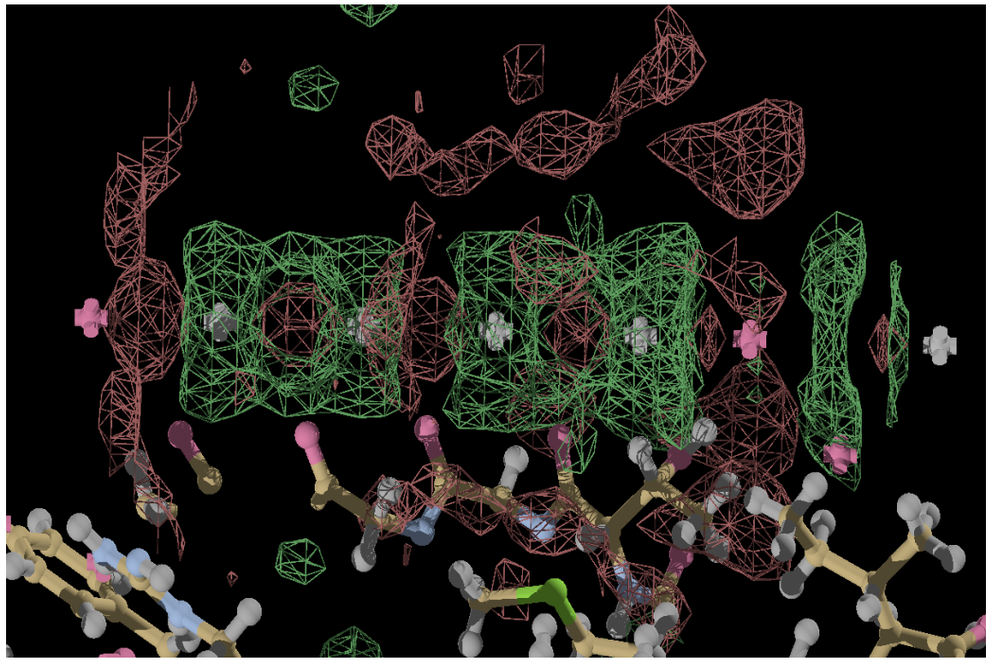
Can K^+ ions occupy adjacent sites within the selectivity filter?

Crystallography: Occupancy *vs.* thermal vibration



Refinement “sees” the **area** underneath each curve

Atomic Resolution does not Resolve all Problems

- Correlation between atomic displacement parameter and occupancy > 90%
- Restraints: bind B–values to chemical environment?
- K^+ ions on 4–fold symmetry axis: artefacts  (1R3J (Tl^+))

Step 1: Anomalous Signal in Reciprocal Space

Relative Occupancy of Tl^+ from SAD Phasing

Advantages

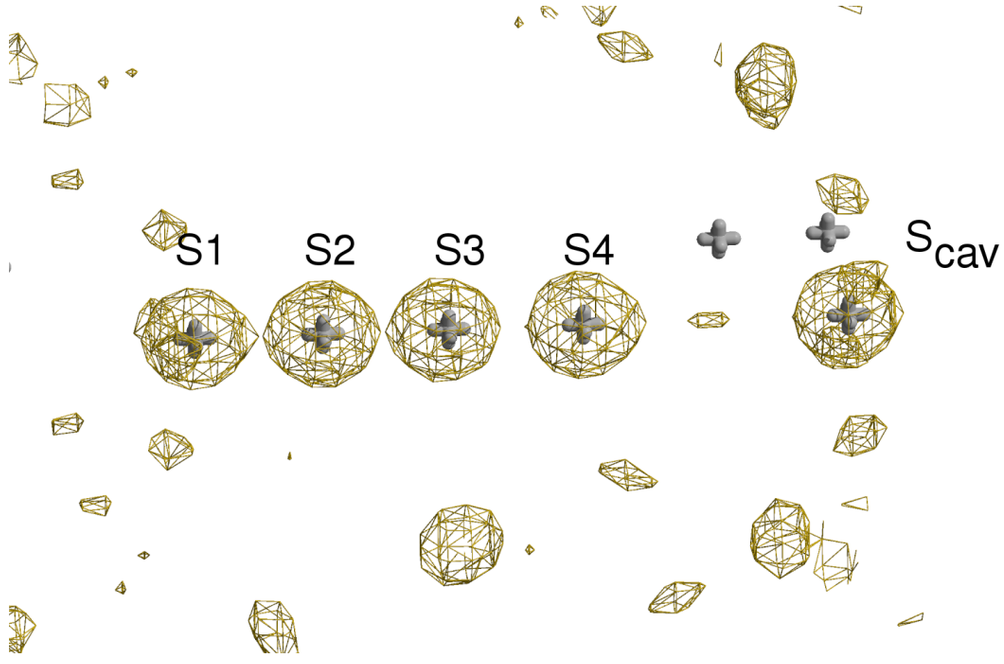
- **Only** anomalous signal: no contribution from water molecules

- "Normalized structure factors": point like atoms at $0\ K$, *i.e.* independence from B–values

Drawback: occupancies only *relative* to strongest peak

Results Step 1: Ions **equally distributed** inside Selectivity Filter

1r3j_fa.res	Tl^+					
	S1	S2	S3	S4	S_{cav}	#6
relative occ.	1.0	0.9	0.9	1.0	0.6	0.12



experimental anomalous electron density

Drawback: occupancies only *relative* to strongest peak

Consequence from Equal Distribution for Occupancy

SAD Phasing: occ(S1) = occ(S2) = occ(S3) = occ(S4), unknown absolute.

Possible Scenarios:

average SF content	Distribution				occupancy
$\langle 1\ Tl^+ \rangle$	Tl	W	W	W	
	W	Tl	W	W	
	W	W	Tl	W	
	W	W	W	Tl	$\langle occ \rangle \leq 0.25$
$\langle 2\ Tl^+ \rangle$	Tl	W	Tl	W	
	W	Tl	W	Tl	$\langle occ \rangle \leq 0.5$
$\langle 3\ Tl^+ \rangle$	Tl	Tl	Tl	W	
	Tl	W	Tl	Tl	
	W	Tl	Tl	Tl	
	Tl	Tl	W	Tl	$\langle occ \rangle \leq 0.75$
$\langle 4\ Tl^+ \rangle$	Tl	Tl	Tl	Tl	$\langle occ \rangle \leq 1.00$

Ion separation by water requires all four occupancy at least < 0.75.

Step 2: **Absolute** Occupancy of Tl^+ from Structure Refinement

- Tl^+: unrestrained B–values
- Tl^+: anisotropic B–values
- including anomalous signal

Result Step 2: Ions in Selectivity Filter **fully occupied**

1R3J (Tl^+)					
	S1	S2	S3	S4	S_{cav}
absolute occupancy (SHELXL)	1.02 (4)	0.93 (3)	0.92 (4)	0.99 (4)	0.47 (3)
B[Å^2]	28(1)	26(1)	25(1)	27(1)	20(2)
relative occupancy (SHELXD)	1.0	0.9	0.9	1.0	0.6

- No occupancy $< 75\%$

$\Rightarrow$ $Tl^+Tl^+Tl^+Tl^+$ **predominant in crystal**

Why are the Results Different from the Original Work?

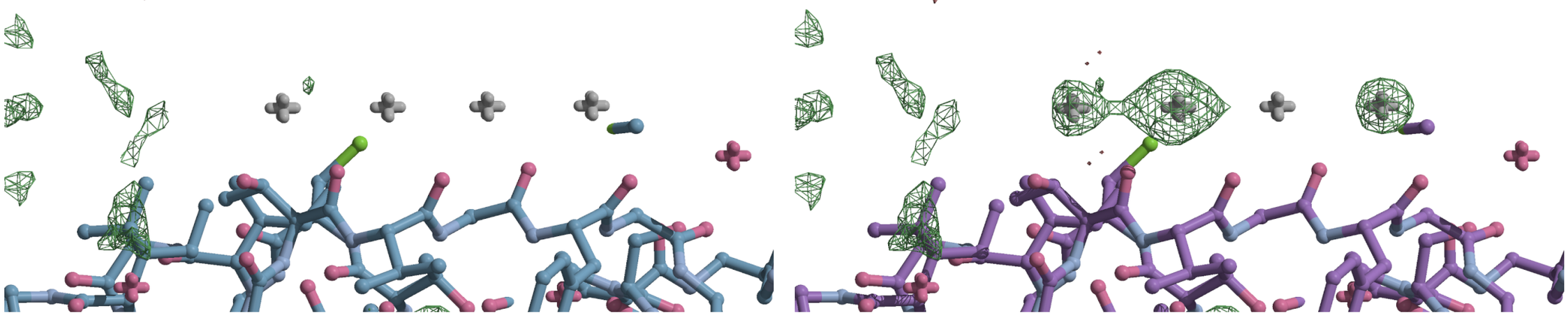
3LDC: MthK pore with 100mM K^+, $d_{\text{min}} = 1.45$Å

Refined occupancies

	S4	S3	S2	S1
occupancy:	1.00	1.00	0.80	0.92
B-value:	20(1)	18(1)	17(1)	18(1)

Fixed occupancies: 50% K^+, 50% H_2O

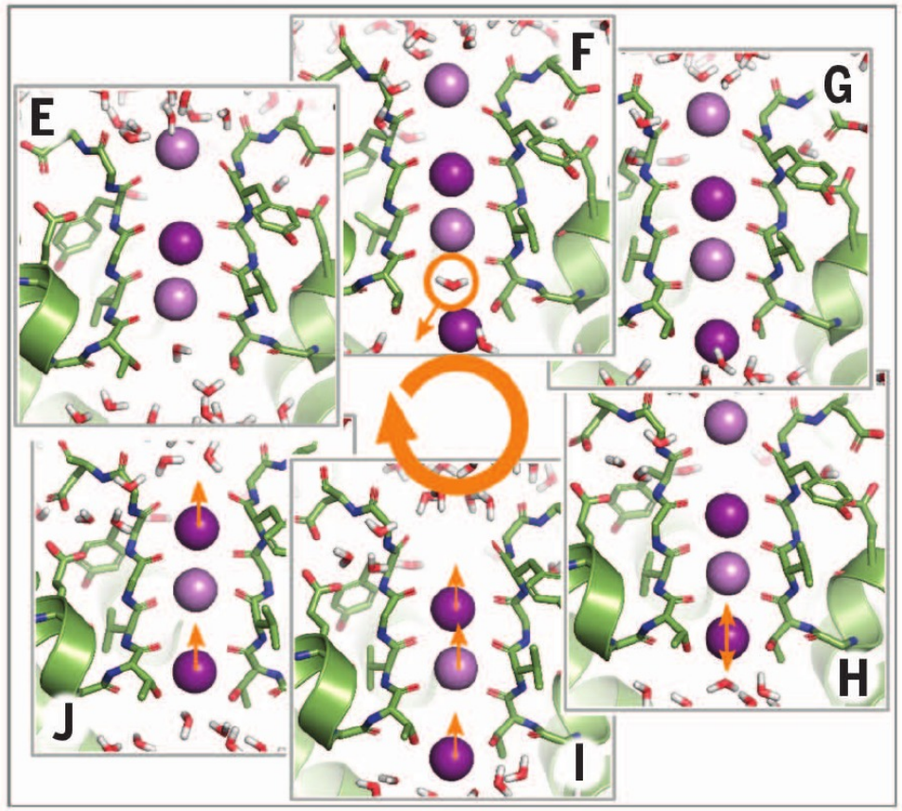
	S4	S3	S2	S1
occupancy:	0.50	0.50	0.50	0.50
B-value:	12	12	11	12



(Especially) S2: Fluctuations in map due to $B \leftrightarrow occ$

Conclusions

- Molecular dynamics simulations correct “text book” mechanism
- Crystallographic experimental data confirm molecular dynamics simulations
- K^+–transport through membrane channels without shielding water



Acknowledgements

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Reference: Köpfer, Song, Gruene, Sheldrick, Zachariae, de Groot: *Science* (2014), 346, pp. 352–355