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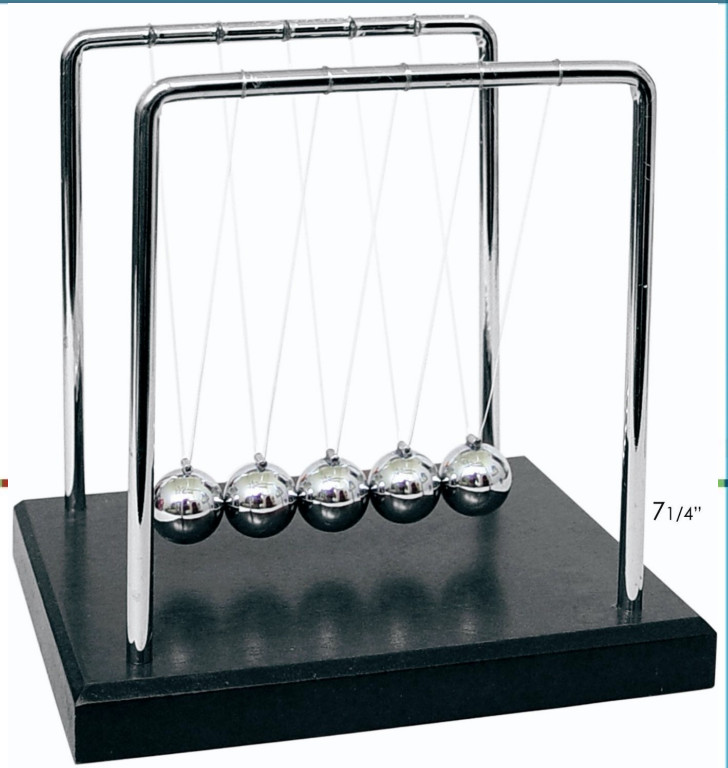
K Channel Transport: Hard Knocks, Soft Knocks, Other?

Susan Rempe

Sr. Scientist, Sandia National Labs



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NNSA National Nuclear Security Administration

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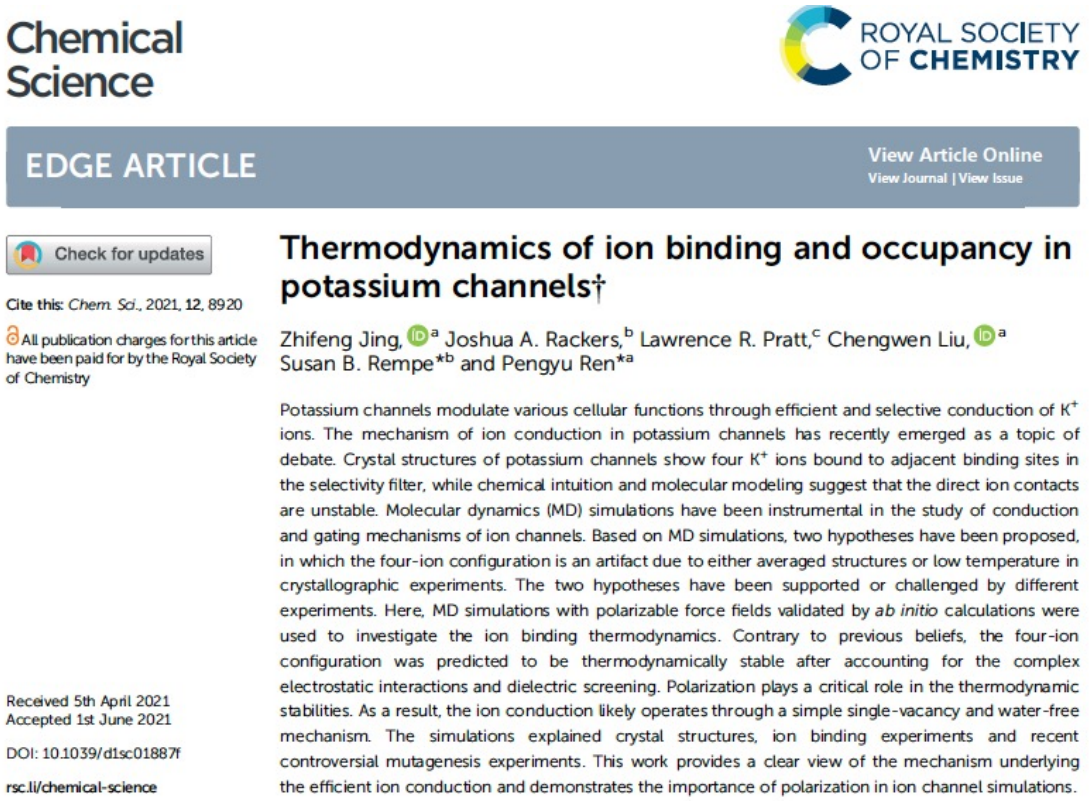
Zhifeng “Francis” Jing
UT Austin



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Thermodynamics of ion binding and occupancy in potassium channels†

Zhifeng Jing,[a] Joshua A. Rackers,[b] Lawrence R. Pratt,[c] Chengwen Liu,[a] Susan B. Rempe*[b] and Pengyu Ren*[a]

Potassium channels modulate various cellular functions through efficient and selective conduction of K^+ ions. The mechanism of ion conduction in potassium channels has recently emerged as a topic of debate. Crystal structures of potassium channels show four K^+ ions bound to adjacent binding sites in the selectivity filter, while chemical intuition and molecular modeling suggest that the direct ion contacts are unstable. Molecular dynamics (MD) simulations have been instrumental in the study of conduction and gating mechanisms of ion channels. Based on MD simulations, two hypotheses have been proposed, in which the four-ion configuration is an artifact due to either averaged structures or low temperature in crystallographic experiments. The two hypotheses have been supported or challenged by different experiments. Here, MD simulations with polarizable force fields validated by *ab initio* calculations were used to investigate the ion binding thermodynamics. Contrary to previous beliefs, the four-ion configuration was predicted to be thermodynamically stable after accounting for the complex electrostatic interactions and dielectric screening. Polarization plays a critical role in the thermodynamic stabilities. As a result, the ion conduction likely operates through a simple single-vacancy and water-free mechanism. The simulations explained crystal structures, ion binding experiments and recent controversial mutagenesis experiments. This work provides a clear view of the mechanism underlying the efficient ion conduction and demonstrates the importance of polarization in ion channel simulations.

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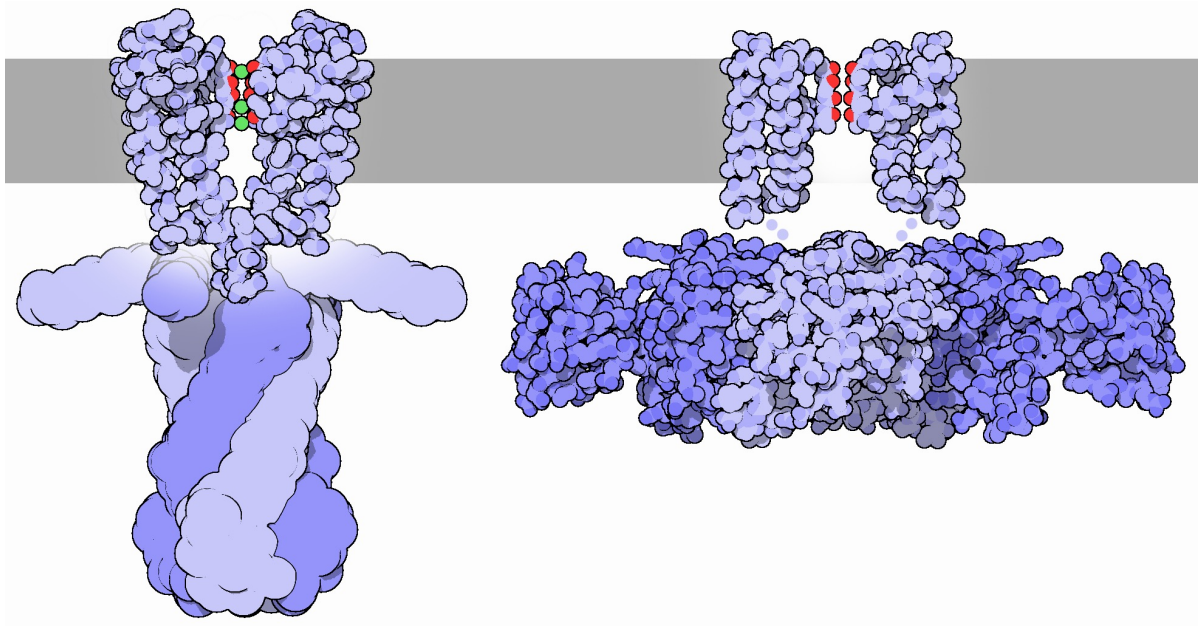
rsc.li/chemical-science

LDRD

CINT

The problem: Fast *and* selective?

K channels: Model channel

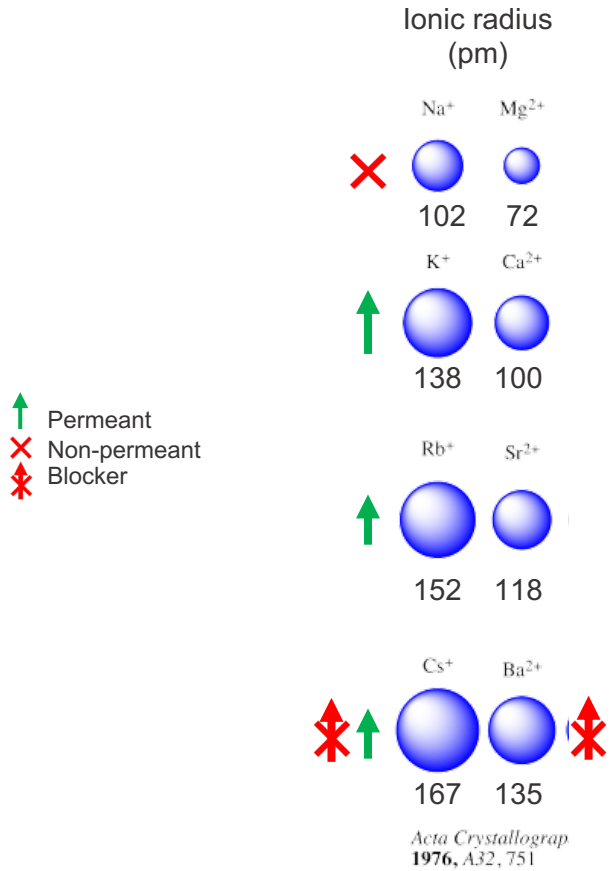


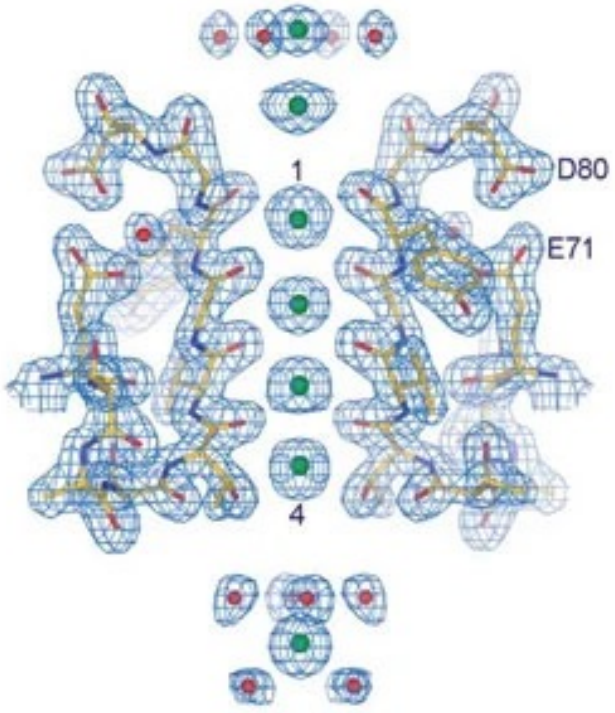
Resting/action potential homeostasis signaling

https://pdb101.rcsb.org/motm/38

- High selectivity: 1000x K^+/Na^+
- Rapid transport: diffusion limit

Selectivity: Architectural constraints

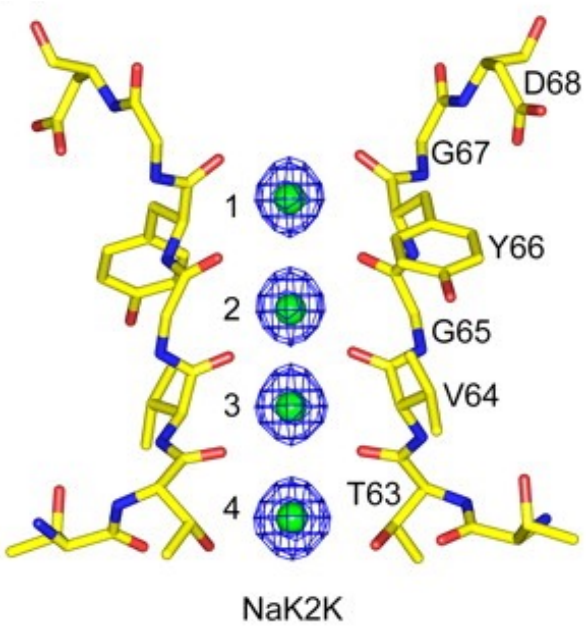




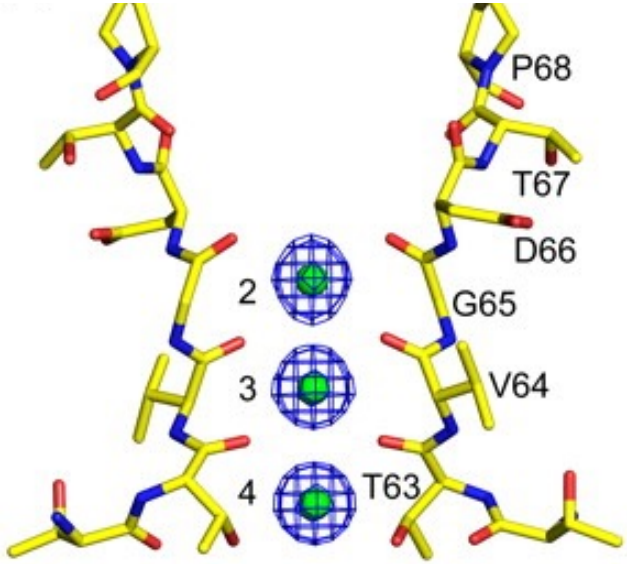
K permeates naked,
but not Na.

- Perspectives on Ion Selectivity *JGP* (2011) 137

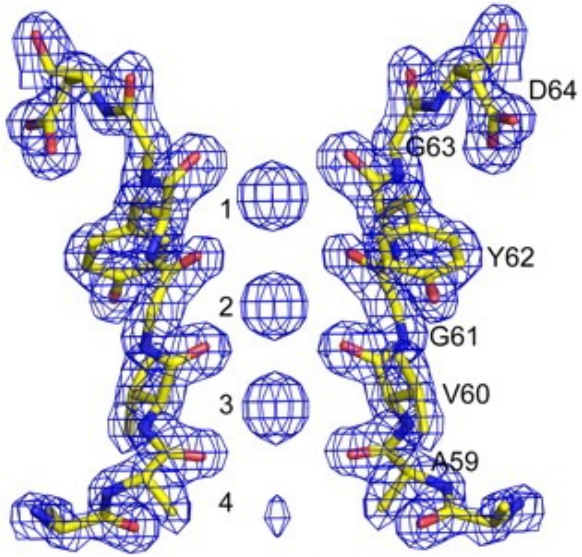
Experiments: 4 binding sites essential



NaK2K



NaK2CNG-D



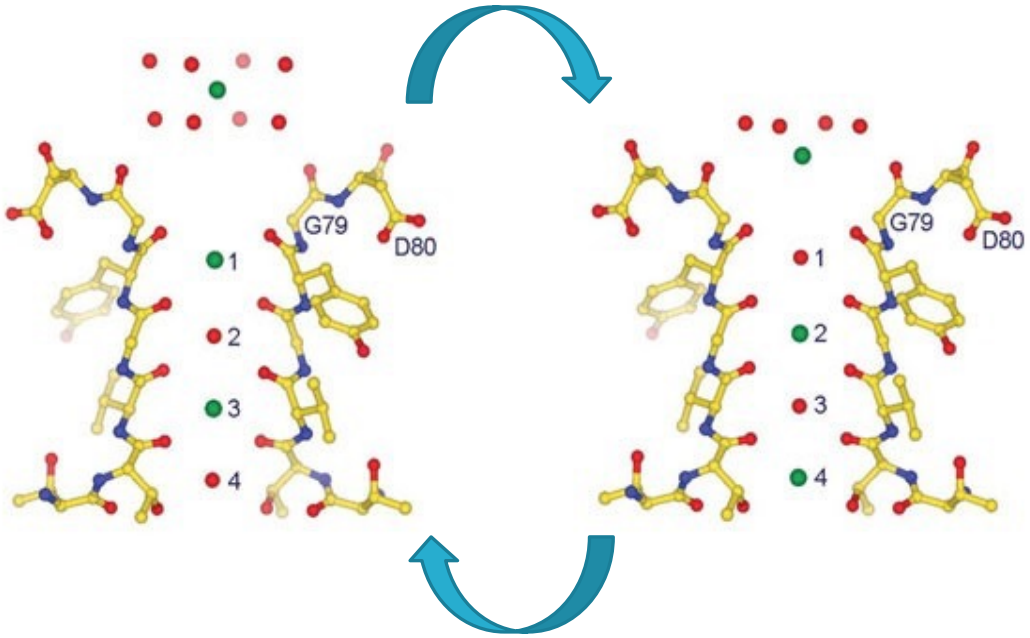
MthK pore (T59A)

Selective
High conductance

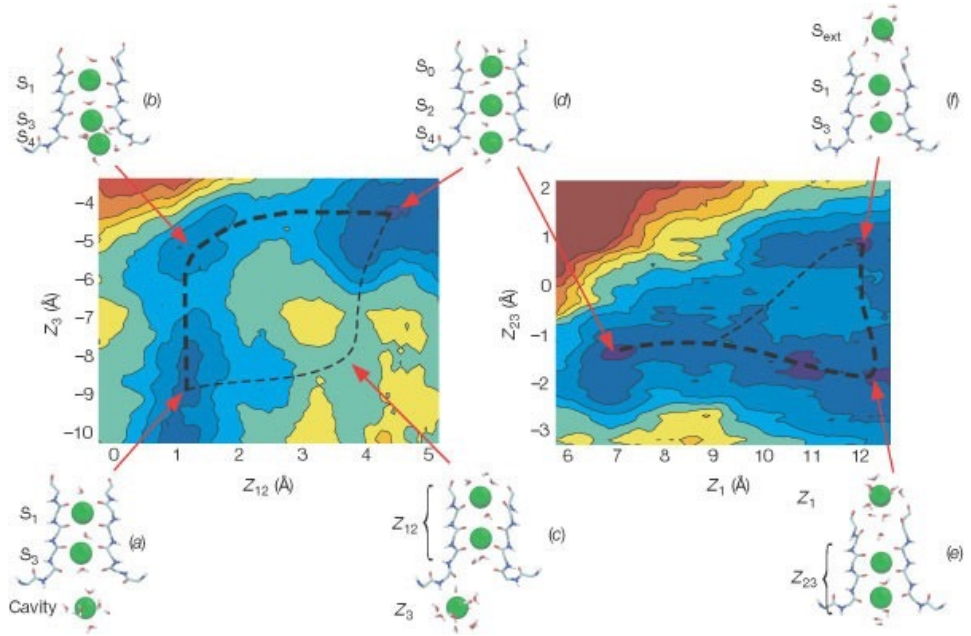
Nonselective
Low conductance

Y. Jiang & co, PNAS January 11, 2011 108 (2) 598-602

Interpretation: Transport by Soft Knock-On



Experiments
Zhou et al. Nature 414, 43–48 (2001)
Morais-Cabral et al. Nature 414, 37–42 (2001)



Fixed Charge Molecular Simulation (FC-MD)
Bernèche et al. Nature 414, 73–77 (2001)
Jensen *et al.* PNAS 2010, 107 (13) 5833-5838

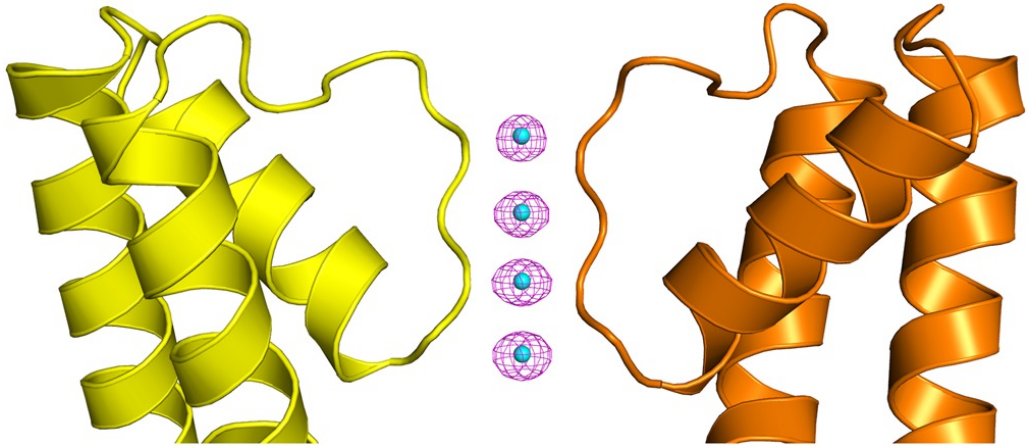
- 2/2 I-W-I-W + W-I-W-I

More Experiments: Occupancy ≥ 3

Hill coefficient from tetramer protection titration (±10%)

Cation	*n*
K^+	3.2
Rb^+	2.9
Cs^+	3.3
Ba^{2+}	0.9

$$\theta = \frac{[L]^n}{(K_{0.5})^n + [L]^n}$$

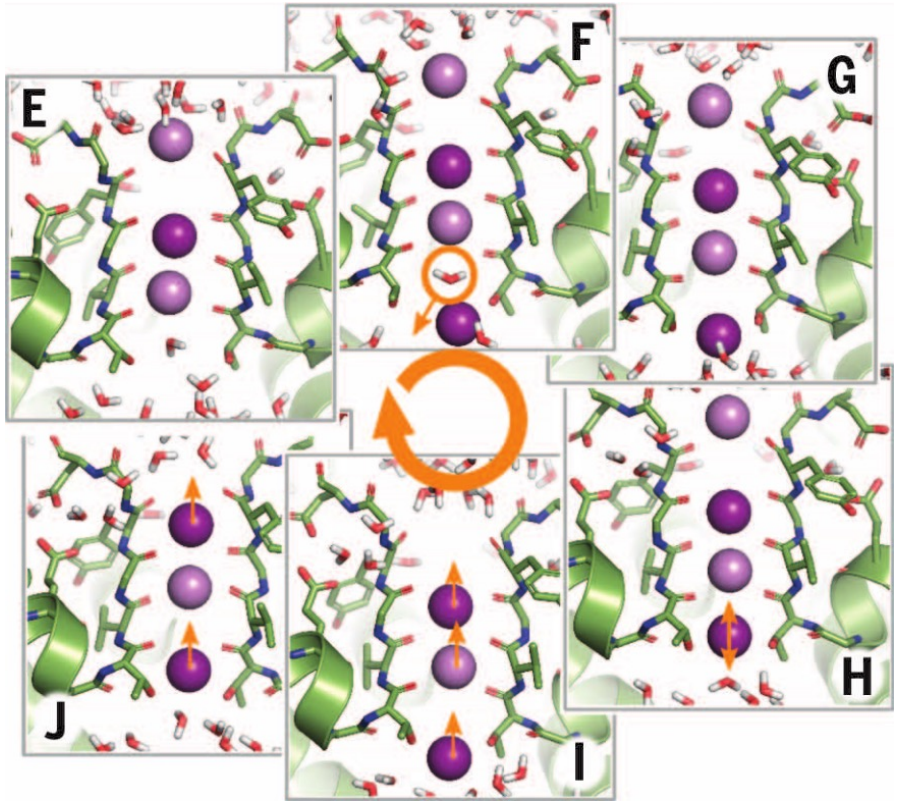


Anomalous dispersion (SAD) X-rays of K^+

Krishnan et al. J. Gen. Physiol. 126, 271–283 (2005)
Langan et al. Nature Commun. 9, 4540 (2018)

- Cooperative binding & full occupancy

More FC-MD: Transport by Hard Knock-On

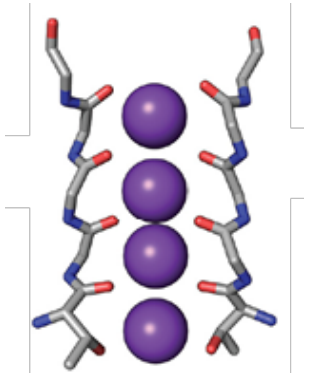


Kopec et al. Nature Chemistry 10, 813–820 (2018)
Kopfer, Zachariae, de Groot et al. Science 346, 352-355 (2014)

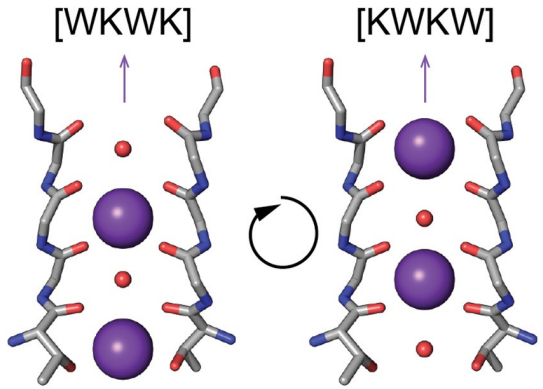
- 2-3 ion contacts dominate

Question: Occupancy During Transport?

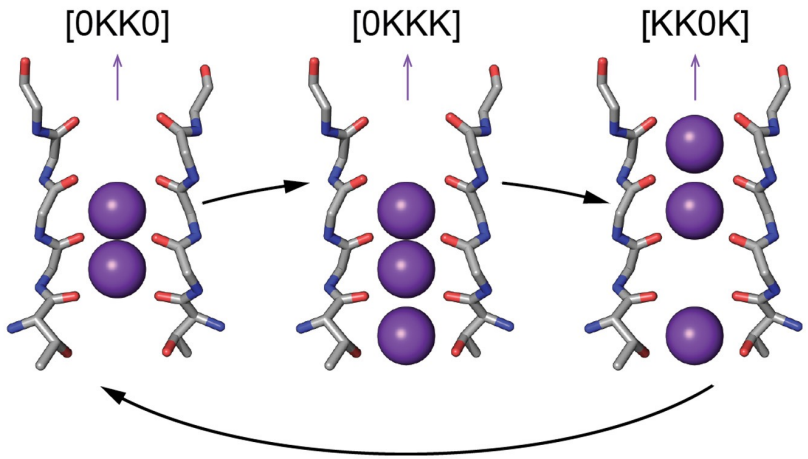
Crystal structure



Soft knock-on



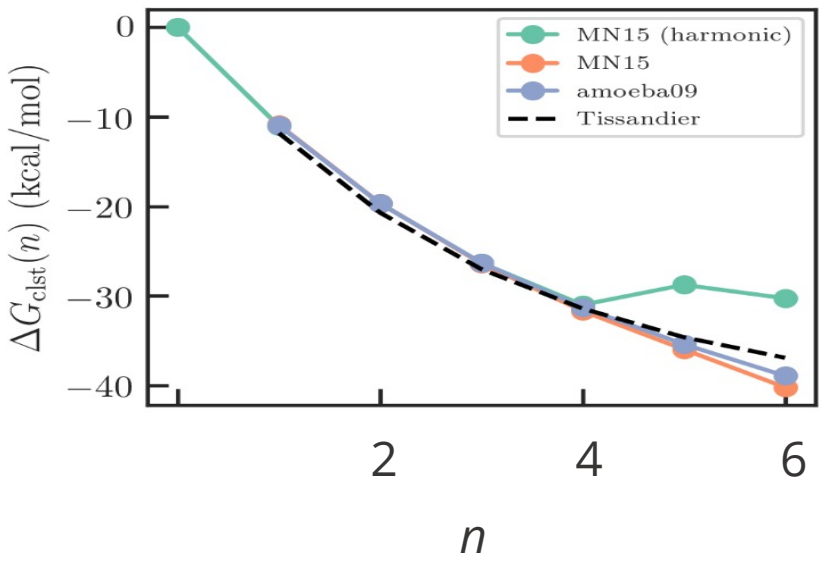
Hard knock-on



Oster et al. Science Advances 5, 7, eaaw6756 (2019)
Zhou et al. Nature 414, 43–48 (2001)

- How to reconcile crystal structure with mechanisms?

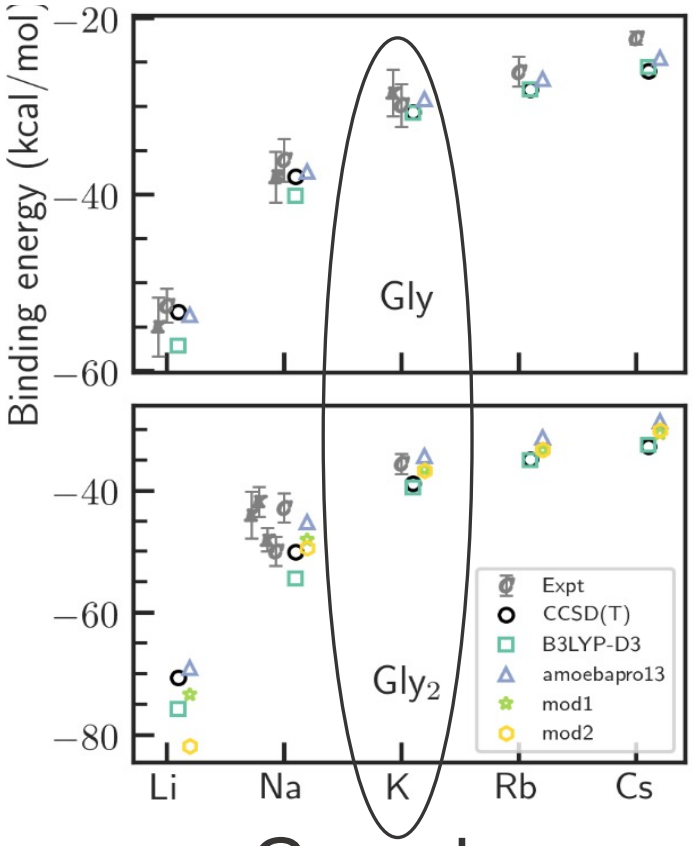
Validate Force Fields



DFT-harmonic

Pol-MD
DFT

$K^+(H_2O)_n$ free energy



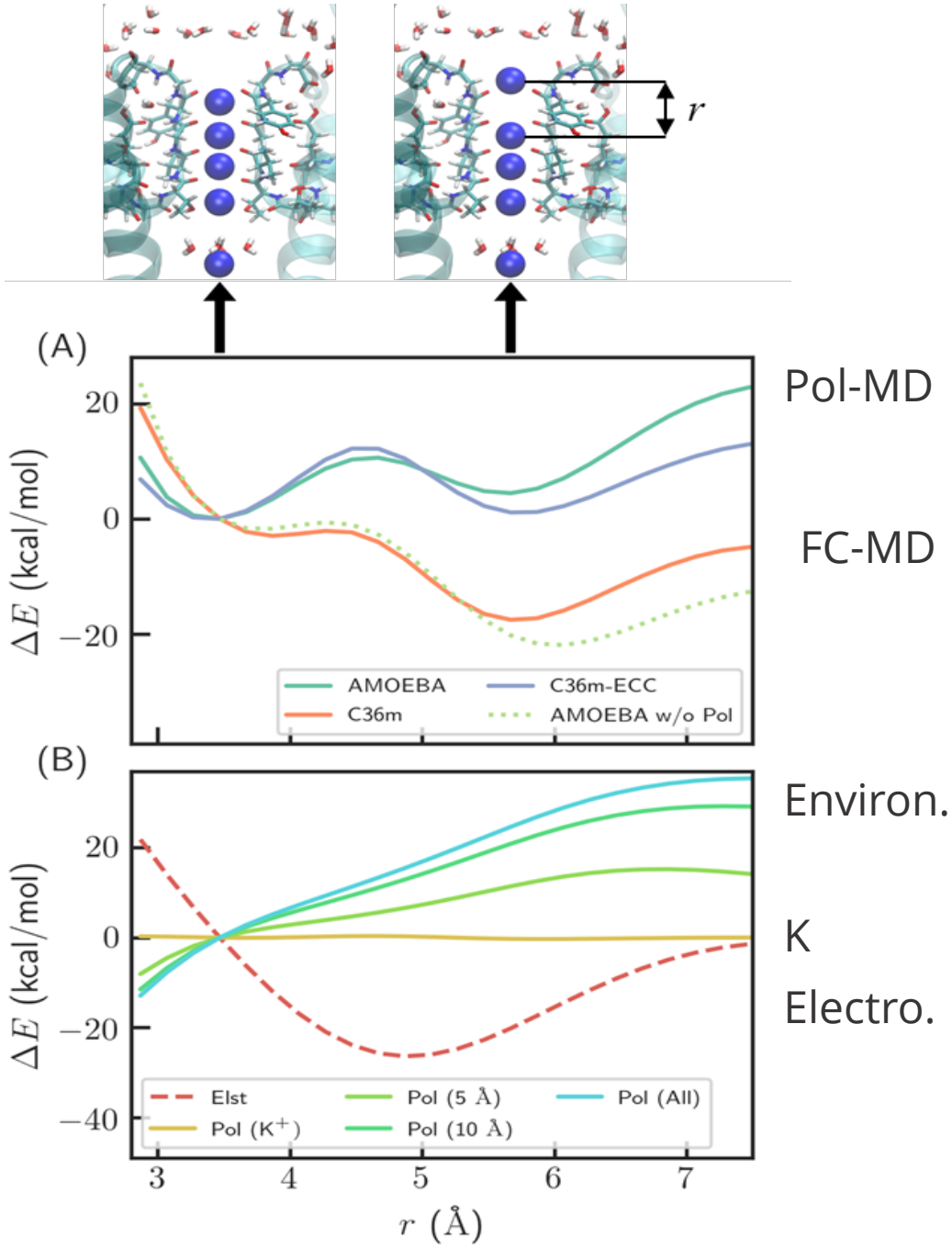
Gas-phase
binding energy

Jing et al. Chem Sci (2021)
Tissandier et al. J. Phys. Chem. A 1998, 102, 40, 7787–7794

- **Cluster experiments, QM/DFT, Polarizable MD agree**

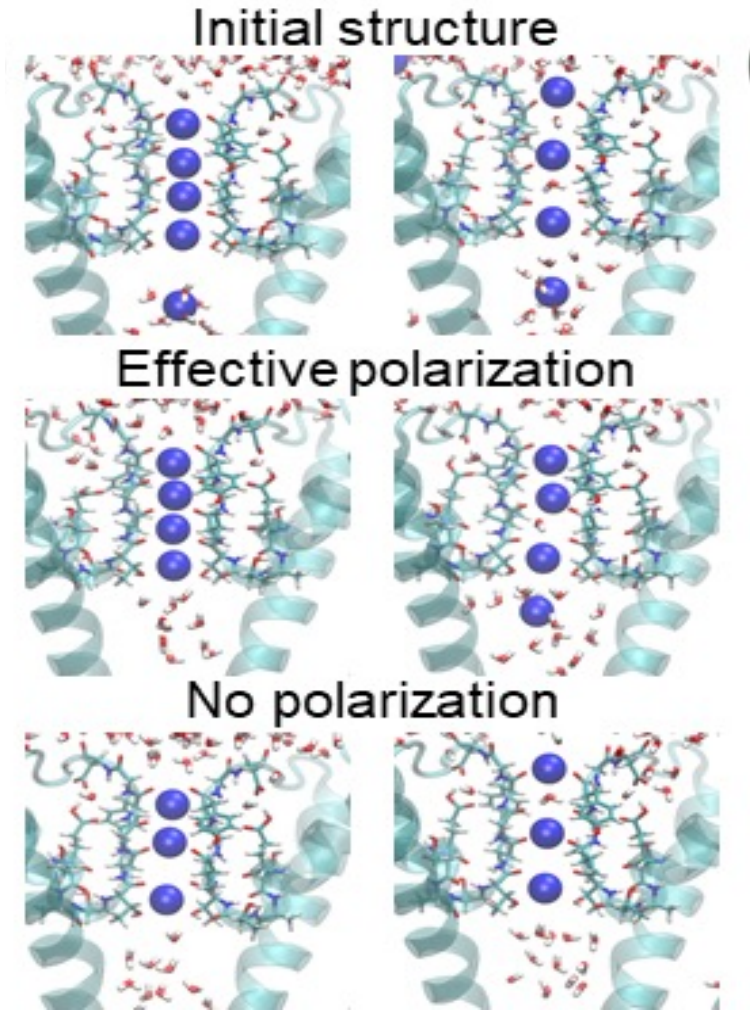
Polarization Effect

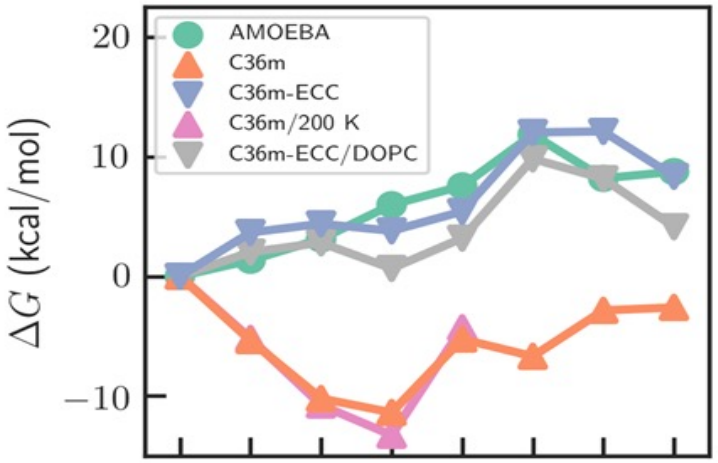
- **Weakens ion-ion repulsion**
- **Environment is polarized**



Jing et al. Chem Sci (2021)
Tomachev et al J. Chem. Theory Comput. 2020, 16, 1, 677–687

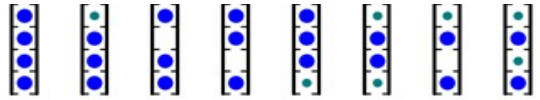
Ion Occupancy – Full is Stable (Pol-MD)





Pol-MD

FC-MD



Binding Free Energies Consistent w/ Experiments

		K+		Na+→K+	
Protein	Conformation	Pol-MD	ITC	Pol-MD	ITC
KcsA-WT	collapsed	-4.9 ± 0.7	-4.7	-1.6 ± 0.3	-2.1
KcsA-WT	conductive	-6.8 ± 0.6 -1.3 ± 0.6	-5.7	-2.0 ± 0.2	-2.9

1 K (S1/S4) → 2(S1 + S4)

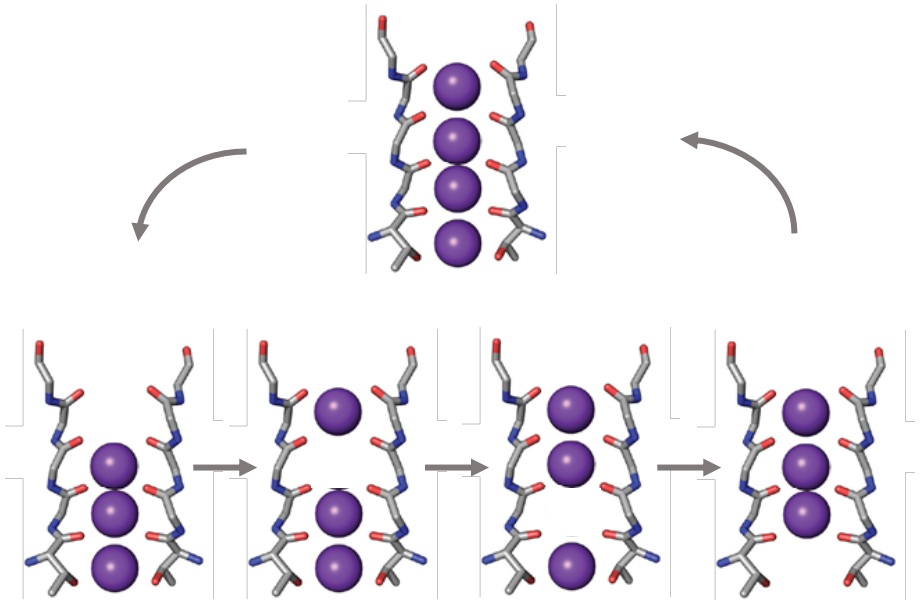
2 K → 3 K
(3K → 4K)

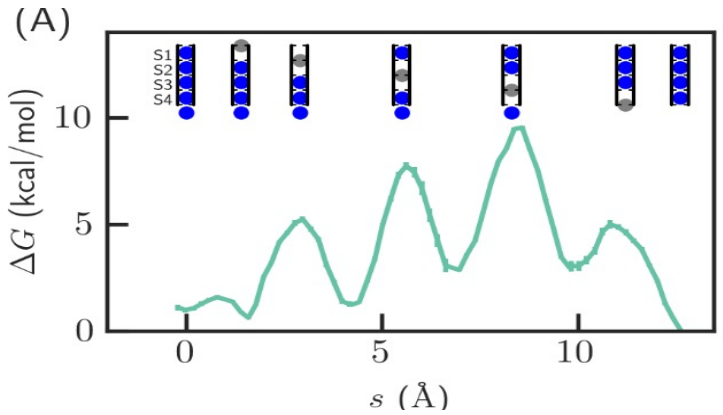
2 Na (S1+S4) → Na + K (S1+S4)

4K → 3K + 1Na (S1/S4)

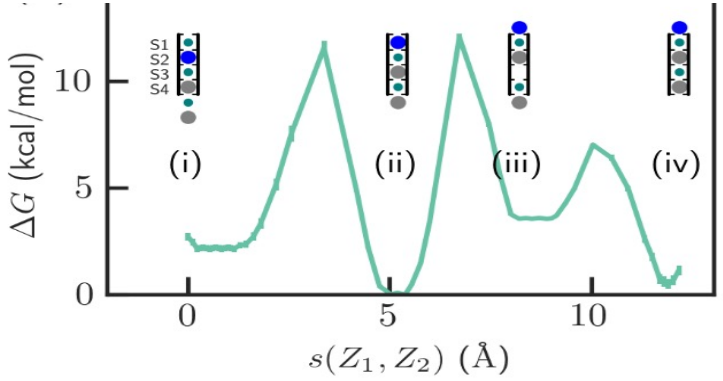
Liu et al. PNAS 112(49):15096-15100 (2015).
Jing et al. Chem Sci (2018)

Barriers Support 4 Occupancy + Single Vacancy





- 4 occupancy + single vacancy



- 2/2 K-W-K-W + W-K-W-K

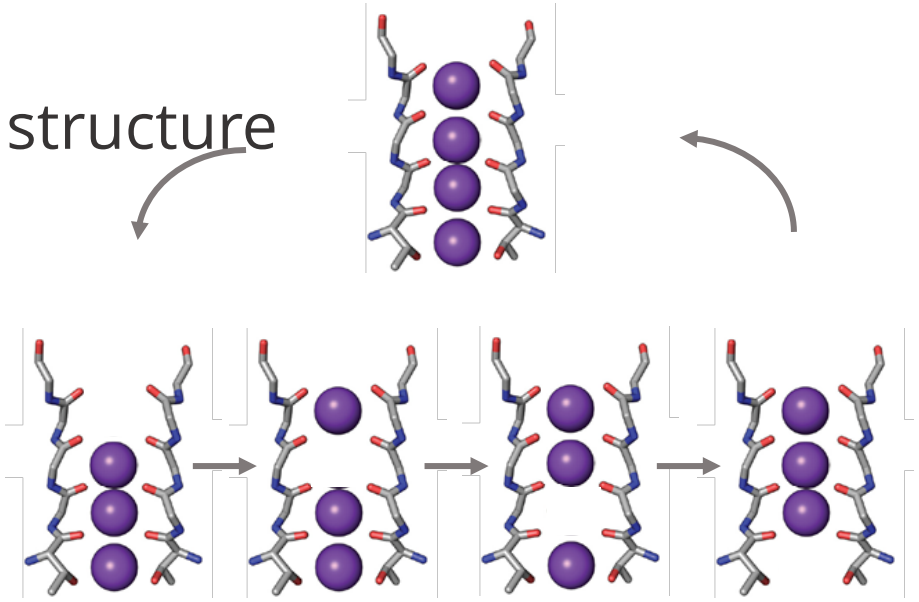
- Lower barriers for single-vacancy vs soft knock-on

Jing, et al Chem Sci (2018)

SUMMARY

- Polarization is important to ion occupancy

- Ion transport likely operates at near ion-saturated states, consistent with crystal structure



4 occupancy + single vacancy