

Mechanisms of Ion Recognition by Biomolecules

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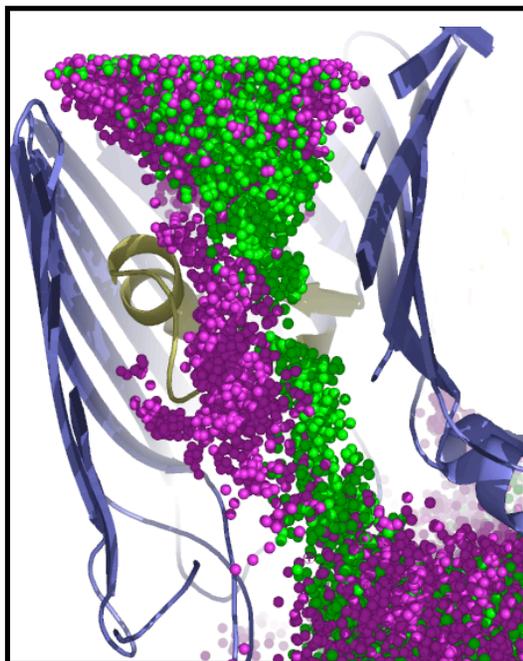
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AAAS Regional Meeting, 12th April 2008

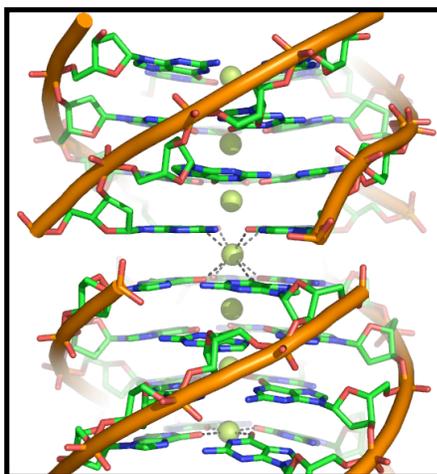
Examples of ion selective biomolecules

Weakly Cation Selective
Bacterial ion Channel:
OmpF



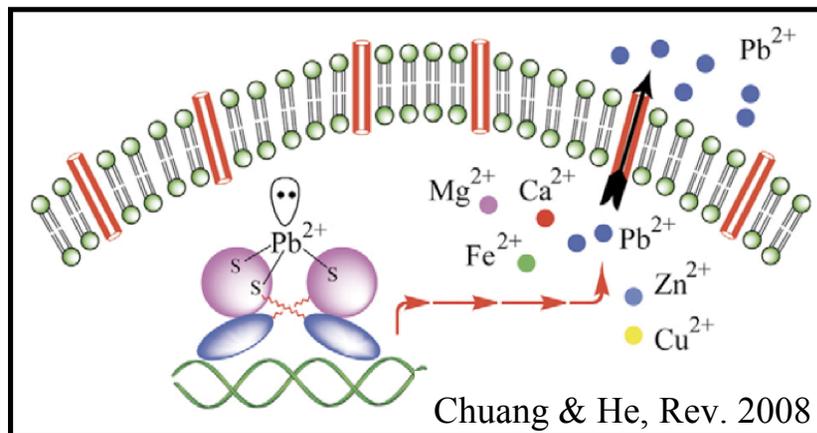
Varma & Jakobsson., BJ, 2006

Strongly Na⁺ selective
DNA quartet



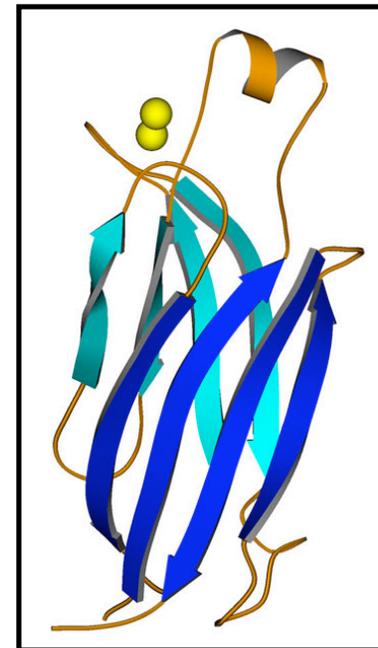
Laughlan *et al.*, Nature. 1994

**Transition metal ion selective
biomolecules**



Chuang & He, Rev. 2008

Strongly Divalent ion
selective:
C2 domains:



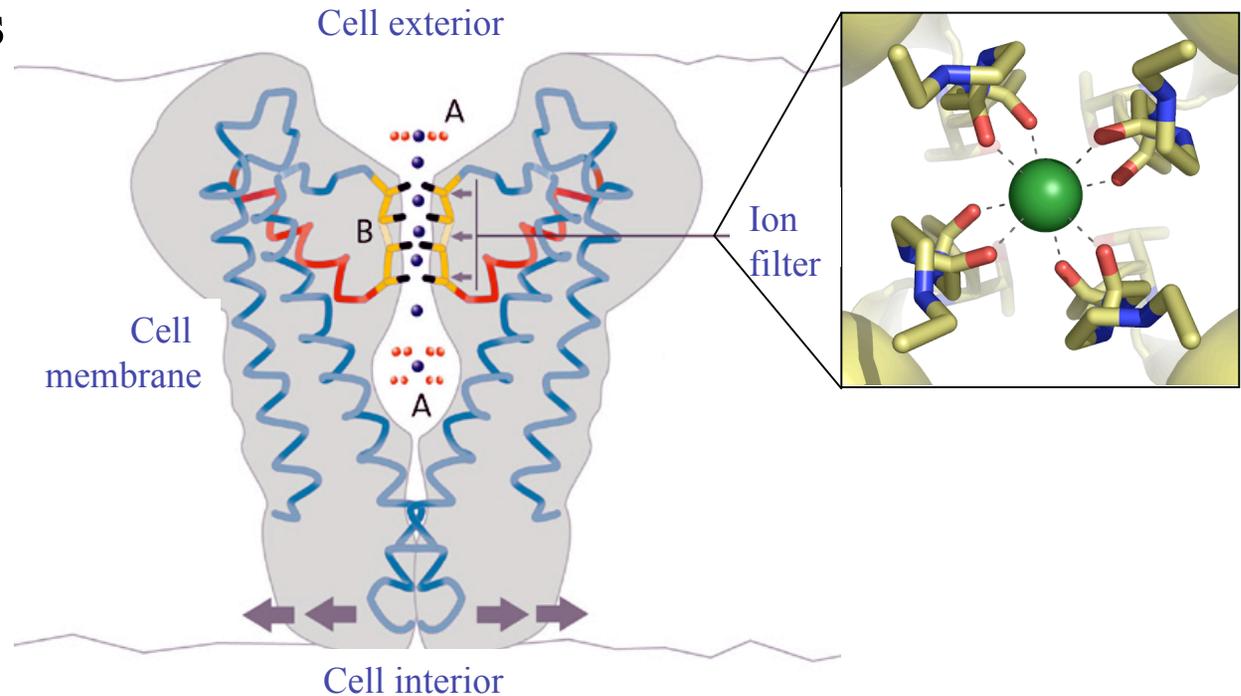
Varma & Jakobsson., BJ, 2007

K^+/Na^+ selective biomolecules

The radius of K^+ is larger than that of Na^+ by 0.4 \AA

Potassium Ion Channels

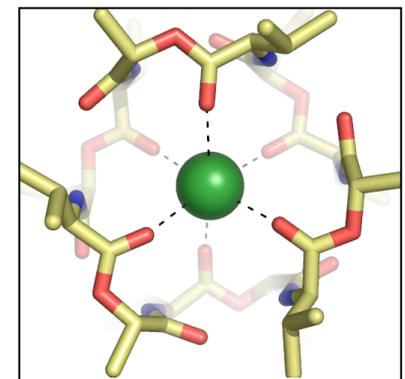
Regulates K^+ ion gradients across cell membranes to enable numerous physiological tasks including nerve conduction and muscle contraction



Valinomycin

Bacterial toxin that disrupts K^+ ion gradients across cell membranes

169 atoms



Driving force for selectivity

Each ion has a characteristic solvation free energy



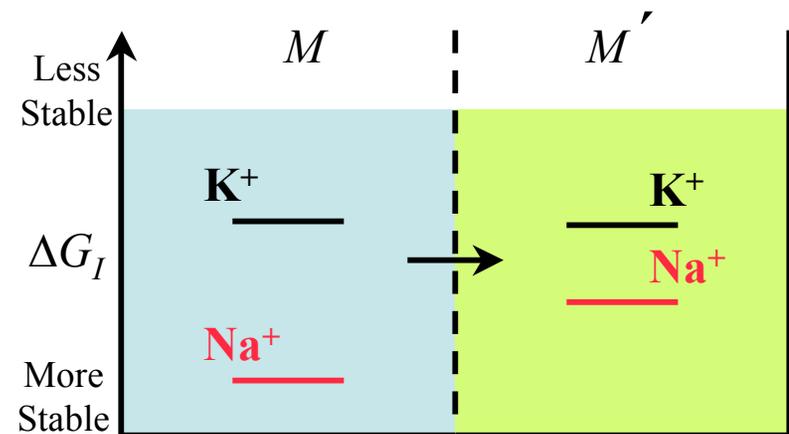
In liquid media, Na^+ ions have larger solvation free energies than K^+ ions

$$\Delta\Delta G_{\text{Na}^+ \rightarrow \text{K}^+}(M) = \Delta G_{\text{K}^+}(M) - \Delta G_{\text{Na}^+}(M)$$

Thermodynamics dictates that K^+/Na^+ selective partitioning will occur when

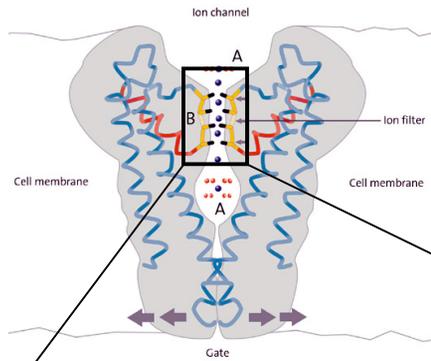
$$\Delta\Delta\Delta G_{\text{Na}^+ \rightarrow \text{K}^+}(M \rightarrow M') < 0$$

Solvent (M)	Dipole moment (Debye)	Dielectric constant (at 25° C)	$\Delta\Delta G_{\text{Na}^+ \rightarrow \text{K}^+}(M)$ (kcal/mol)
Liquid Formamide	3.7	109	18 (Cox & Schmid)
Liquid Water	1.9	78	17 (Schmid & Tissandier)
Liquid Methanol	1.7	33	18 (Burgess)

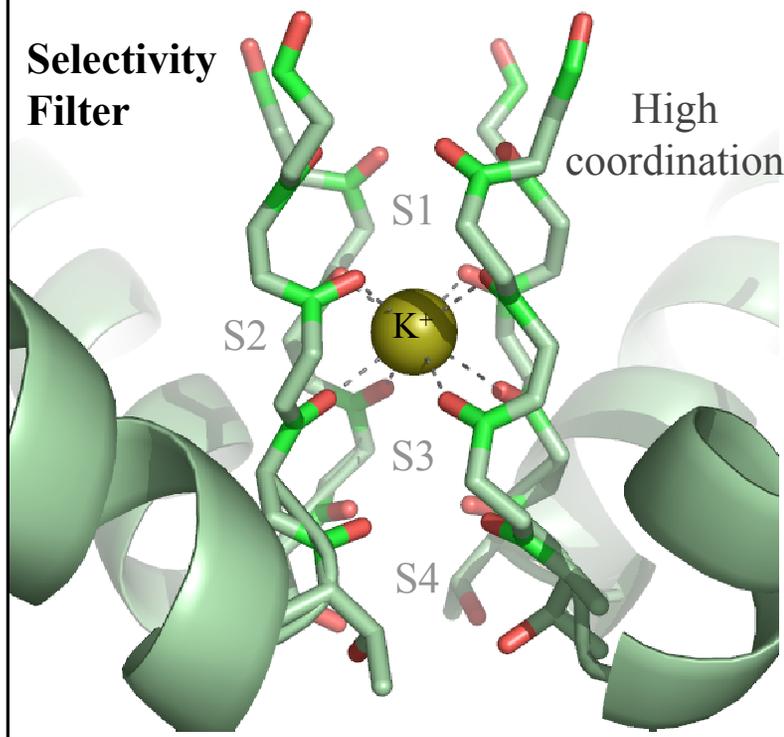


$$\Delta\Delta G_{\text{Na}^+ \rightarrow \text{K}^+}(M) > \Delta\Delta G_{\text{Na}^+ \rightarrow \text{K}^+}(M')$$

K-channel selectivity - The conventional picture



Selectivity Filter

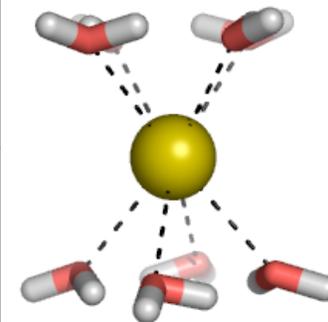


Central Ideas

- (1) K^+ ions readily partition into K-channel binding sites as they **mimic** K^+ ion hydration structures.
- (2) **Selectivity** occurs because of **specific cavity sizes** that match the larger K^+ ion

<http://nobelprize.org>

K^+ in Bulk Water



High coordination

Theoretical Approach

Molecular Association Reaction



Utilize molecular association statistical theory developed by Pratt and Co. to determine individual contributions of each K_n to net chemical potential μ_I

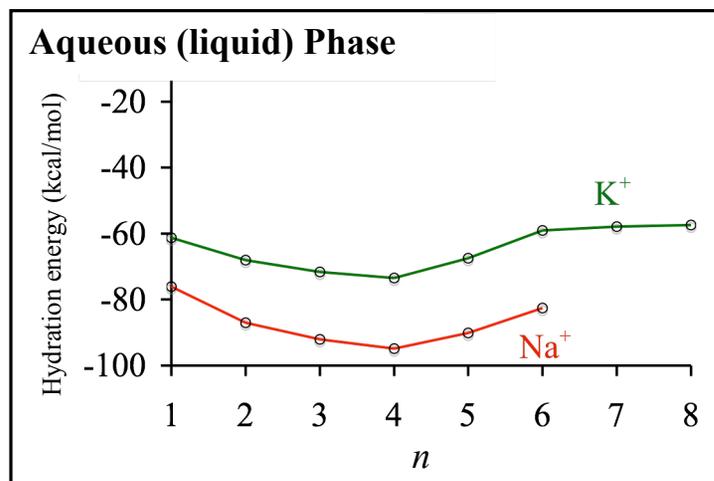
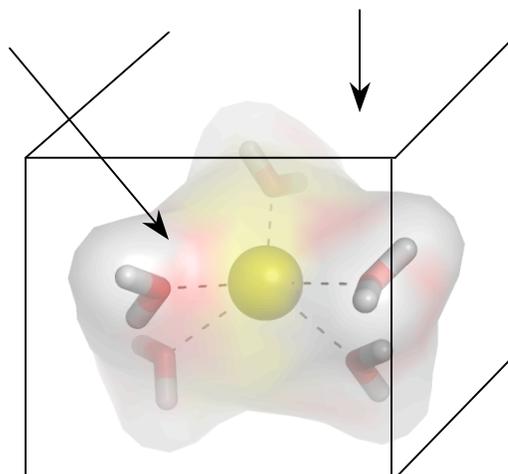
$$\beta\mu_I = \ln\left[\frac{\rho_I V}{q_I}\right] - \ln\left[\left\langle e^{-\beta\Delta U} \prod_j (1 + f_{Ij}) \right\rangle_0\right] - \ln\left[\sum_{n \geq 0} K_n (\rho_X)^n\right]$$

$$f_{Ij} = \left. \begin{array}{l} 0 \quad ; \text{ solvent } j \text{ inside cluster} \\ -1 \quad ; \text{ solvent } j \text{ outside cluster} \end{array} \right\}$$

This method has reproduced simultaneously and accurately both the structural and energetic hydration properties of several ions, including Li^+ , Na^+ , K^+ , Be^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Zn^{2+} , OH^- and H_3O^+

Inner Domain
treated quantum
chemically
(DFT/B3LYP)

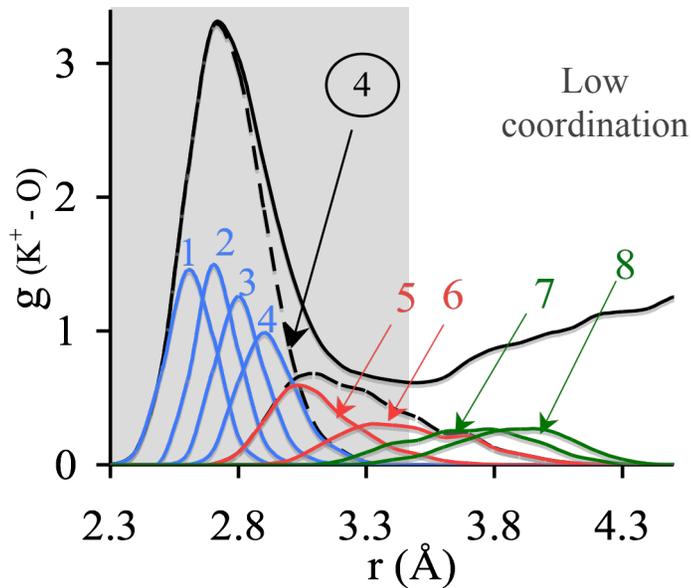
Outer Domain
treated classically
(Elec./MD)



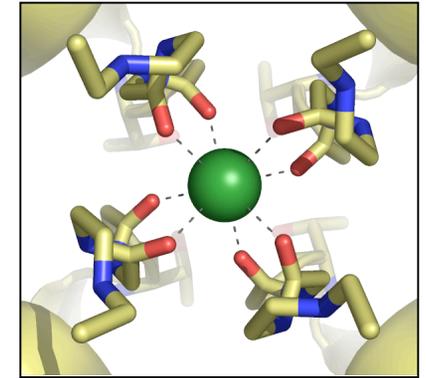
Determinants of selectivity in K-channels

- 1a) New picture of K⁺ in water:**
The probability to find an 8-fold coordination is very low in liquid phases including water, formamide & methanol.

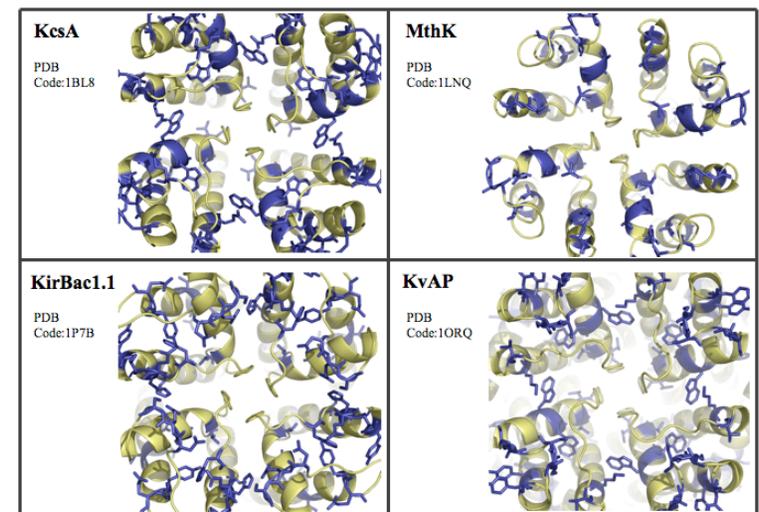
40 ps AIMD (PW91) trajectory of K⁺ in water



- 1b) The energetic barrier** associated with transferring K⁺ from low coordination in water to high coordination in the filter can be **substantially reduced** by **eliminating direct favorable interactions** of the carbonyl oxygens with everything outside their binding sites.



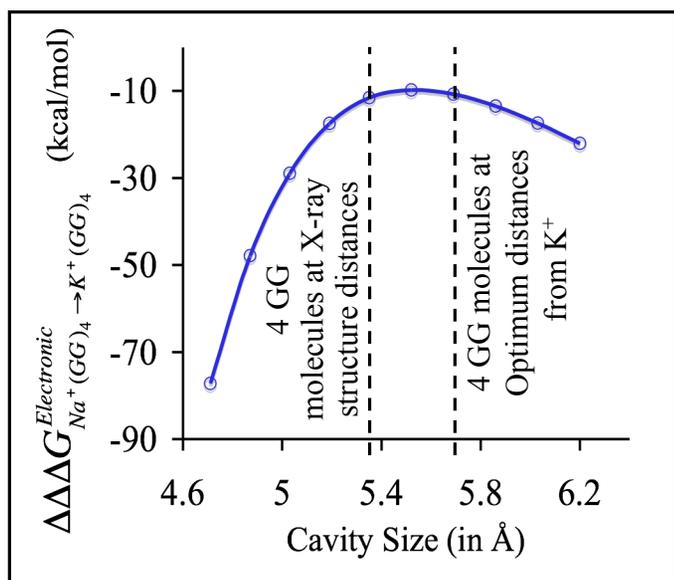
- 1c) No H-bond donor groups** within 6 \AA from carbonyl oxygens.



Determinants of selectivity in K-channels

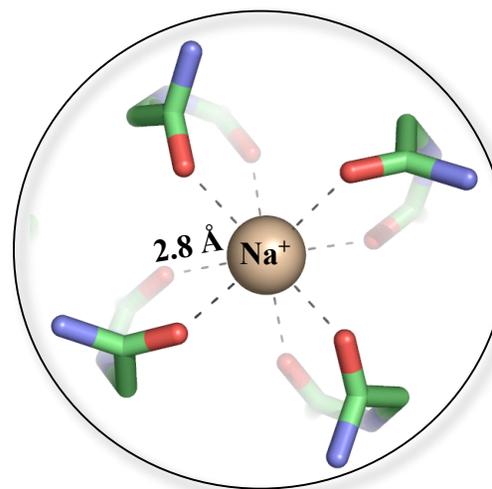
The specific combination with Carbonyl Ligands

- 2a) Rigidity in terms of a specific **cavity size is not important**
- 2b) However, in such a scenario, **constraints on a > 6 coordination are a must**. Some form of rigidity is therefore necessary.



Maintain an 8-fold coordination -
Retain K⁺/Na⁺ selectivity

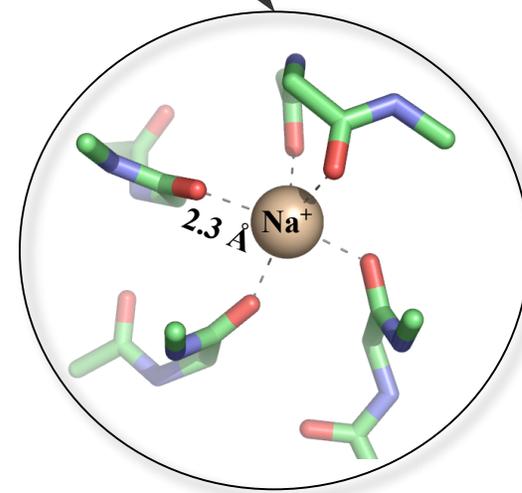
X-ray Coordinates



Allow binding site full flexibility to wrap around a Na⁺

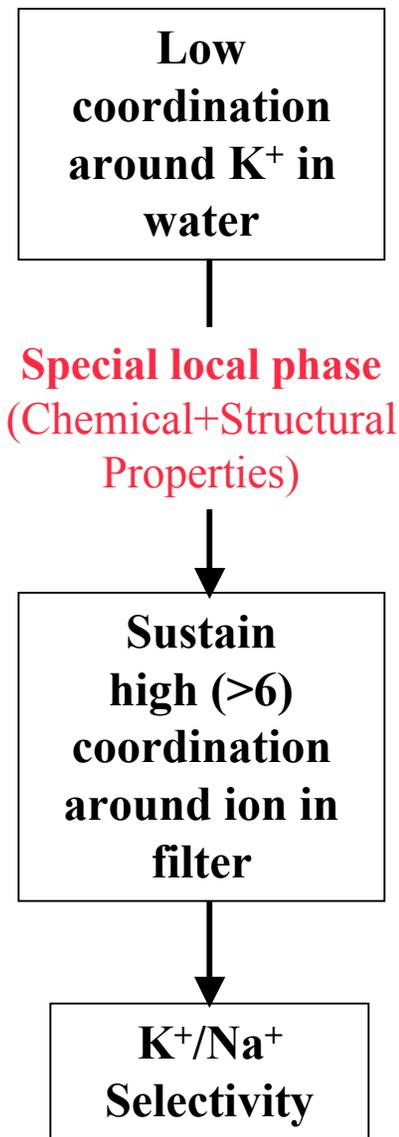
↓
Coordination around Na⁺ drops to 5 or 6

↓
Eliminate K⁺/Na⁺ selectivity



RMS Deviation = **1.8 Å**

Over-coordination mechanism - Experimental support



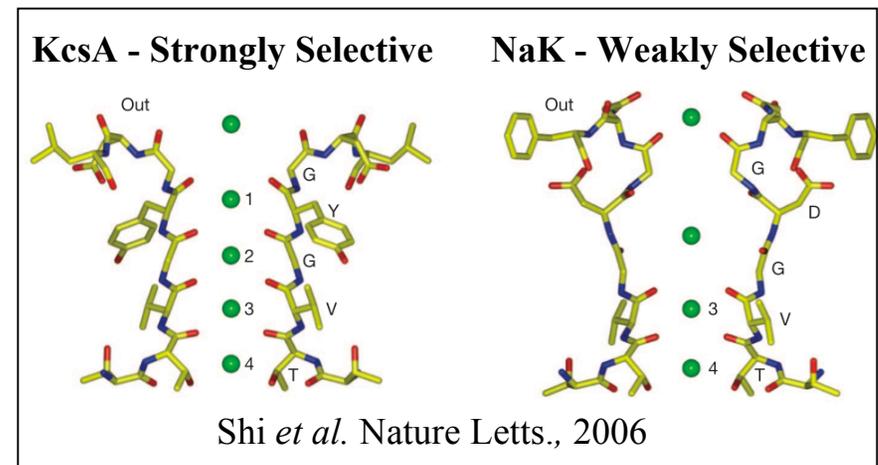
Varma & Rempe, Submitted., 2008
 Varma & Rempe, Biophys. J, 2007
 Varma & Rempe, Biophys. Chem., 2006

- local phase
- Disturb
- 1. “Mutations** that disturb the local phase via introduction of hydrogen bond donors.

Bichet *et al.* PNAS, 2006
 - 2. Sequence alignment of Weakly selective K-channels**

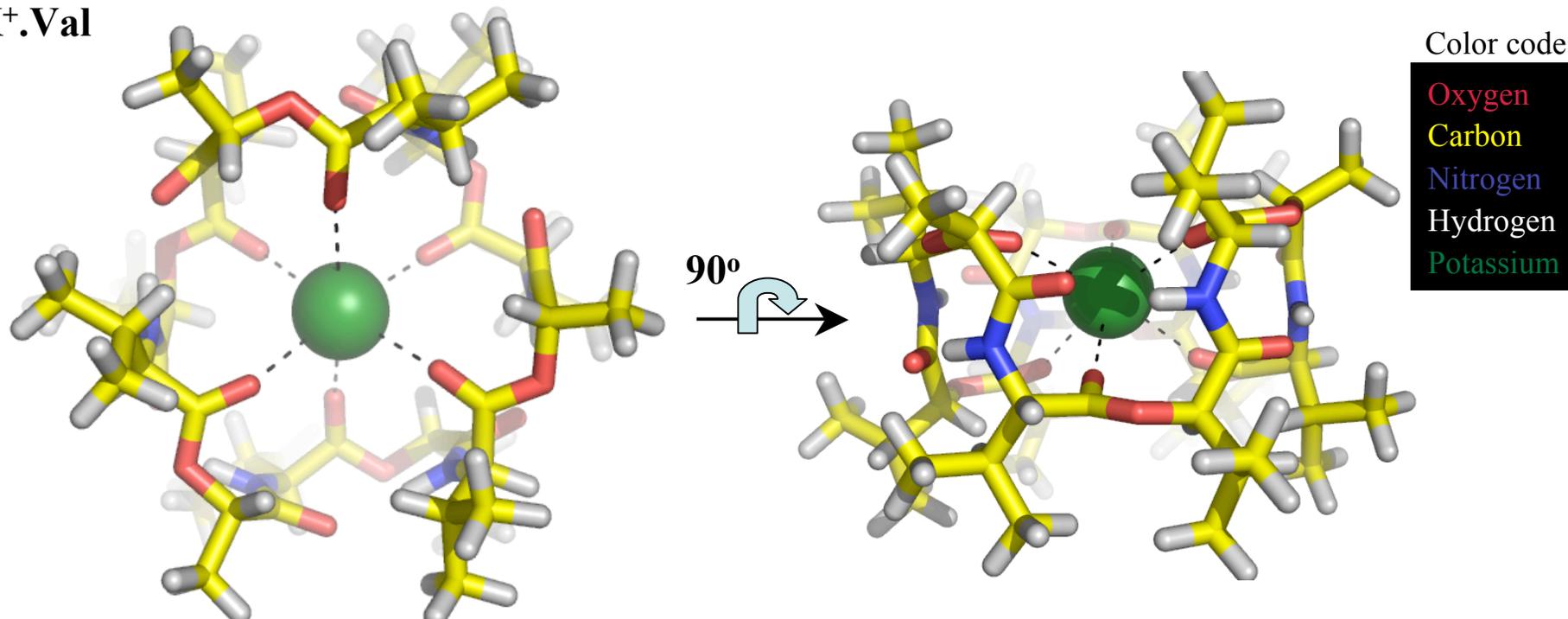
(Shealy *et al.* Biophys. J, 2003)

Weakly selective K-channels =
 Strongly selective K-channels + Arginines in local phase
 - 3. Structure**
 Introduce H-bond donors in the form of water & increase conformational freedom.



Valinomycin *appears* to utilize 6 carbonyl ligands for selectivity

$K^+ \cdot Val$



L.K. Steinrauf; J.A.Hamilton; M.N. Sabesan, JACS, 1982. 101:4085.

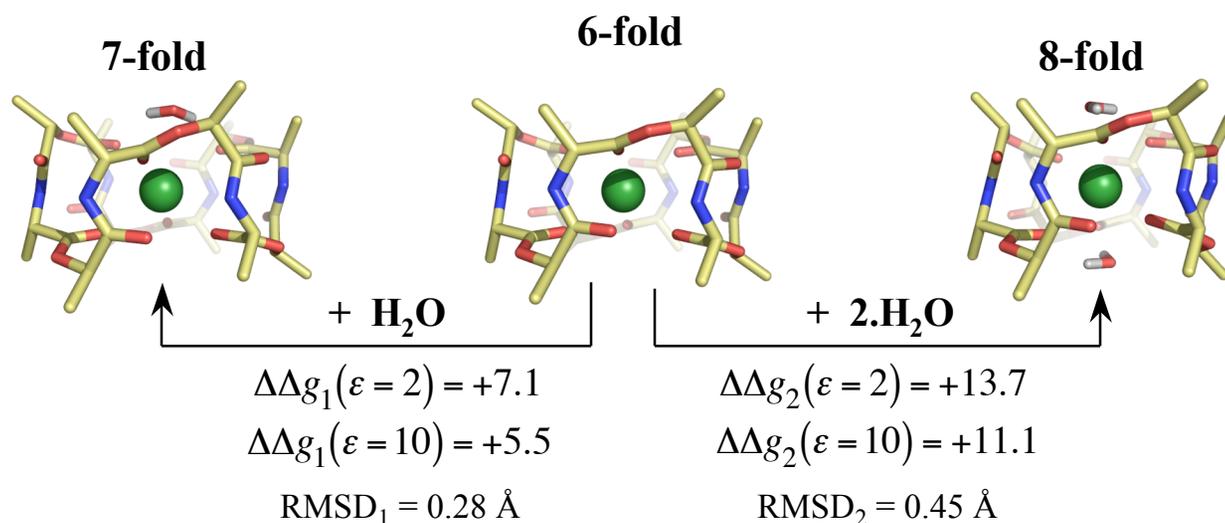
Its structure in the absence of bound cations is solvent dependent,
but
when bound to K^+ , it adopts a configuration wherein its amino- and hydroxy-acid side-chains
always point outward creating a hydrophobic exterior, while the 6 carbonyl oxygens from the
hydroxy-acid residues point inward

Dobler, Ivanov, *et al.*

Does valinomycin utilize additional ligands from solvent ?

We choose water as a representative additional ligand as it simulates the physiologically most relevant scenario where ion complexation to valinomycin occurs in lipid membranes.

Molecular Association Reaction



Reaction free energies $\Delta\Delta g$ determined using quasi-chemical approach developed by Pratt and Co.

Additional ligation by water significantly destabilizes the complex. This implies that their contribution to the free energy difference between the K⁺- and Na⁺-complexed states will be negligible.

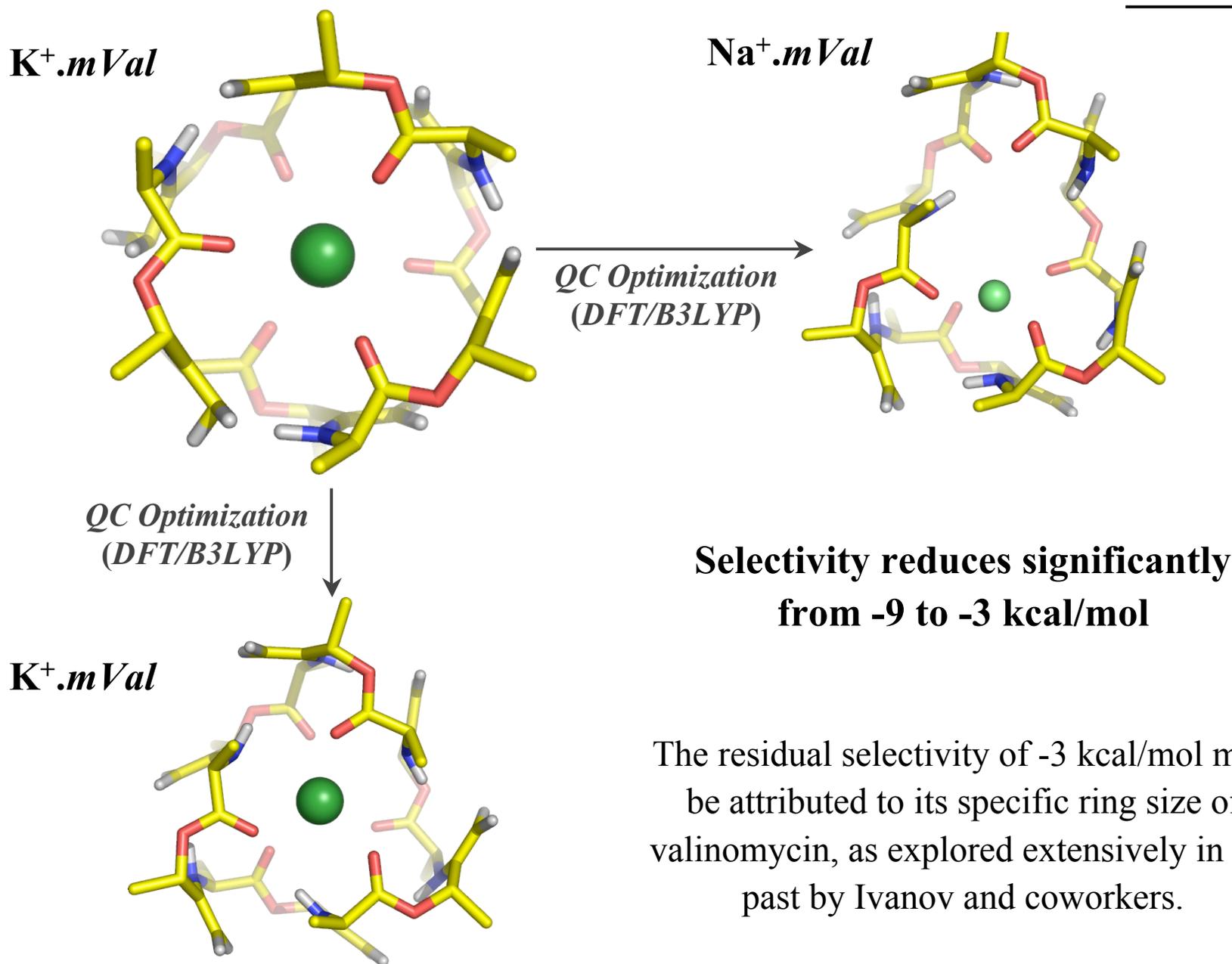
Comparing with other 6-fold coordinations

Computed (DFT/B3LYP) structural and thermo-chemical properties of 6-fold ion-oxygen clusters in gas phase.

Ligand	Chemistry	$\Delta\Delta\Delta G_{Na^+ \rightarrow K^+} (p \rightarrow Val)$ (kcal/mol)	Na⁺-O	K⁺-O
			(in Å)	
Valinomycin	carbonyl	-8.7	2.6	2.8
Formamide	carbonyl	-0.4	2.4	2.8
Glycine Dipeptide	carbonyl	-0.2	2.4	2.8
Water	hydroxyl	-2.2	2.4	2.8
Methanol	hydroxyl	-2.5	2.4	2.8

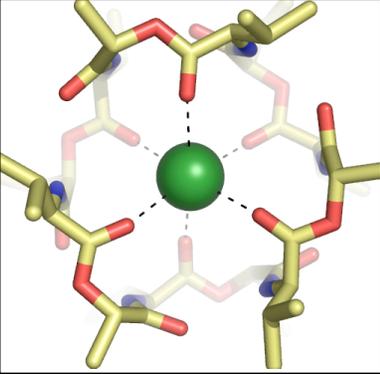
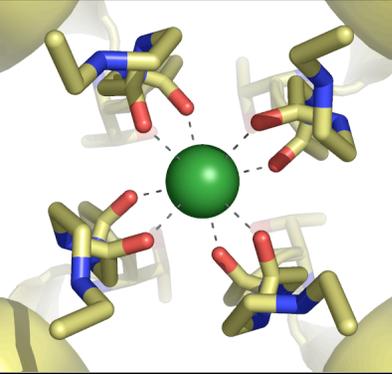
What constrains valinomycin from collapsing onto the smaller Na⁺ ion?

Turn off a source of constraints



Conclusions and Summary

- 1) We **introduced the individual roles and interplay** of two major determinants of ion selectivity and partitioning: (a) **local phase** and (b) **coordination number**.
- 2) Nature is famous for her parsimony in recycling functional motifs. These investigations present an anomaly:

Valinomycin	Strongly selective K-Channels
<p>Uses 6 carbonyl ligands</p> <p>Utilizes constraints on specific cavity sizes for selectivity</p>  <p>Primary advantage of this design: Selectivity is not dependent on the local environment.</p>	<p>Use > 6 carbonyl ligands</p> <p>Utilize constraints on over-coordination (> 6) for selectivity</p>  <p>Primary advantage of this design: Selectivity can be easily tuned via specific mutations in the local neighborhood.</p>

Varma, Sabo & Rempe, J Mol. Bio. 2008

Varma & Rempe, 2008, Submitted

Varma & Rempe, Biophys. J, 2007

Varma & Rempe, Biophys. Chem., 2006

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2. Sandia Supercomputing Center

- **Collaborators**

Desalination Team at Sandia

PI: Susan Rempe

- Jeff Brinker
- Kevin Leung
- Steve Plimpton
- **Dubravko Sabo**
- Seema Singh

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(<http://www.nanoconductor.org>)

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- Steve Plimpton (SNL)
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