SAND2008-0593C

Sandia National Laboratories

Classical and quantum chemical studies of Rb^+ & Cs^+ to understand mechanisms in K-channels

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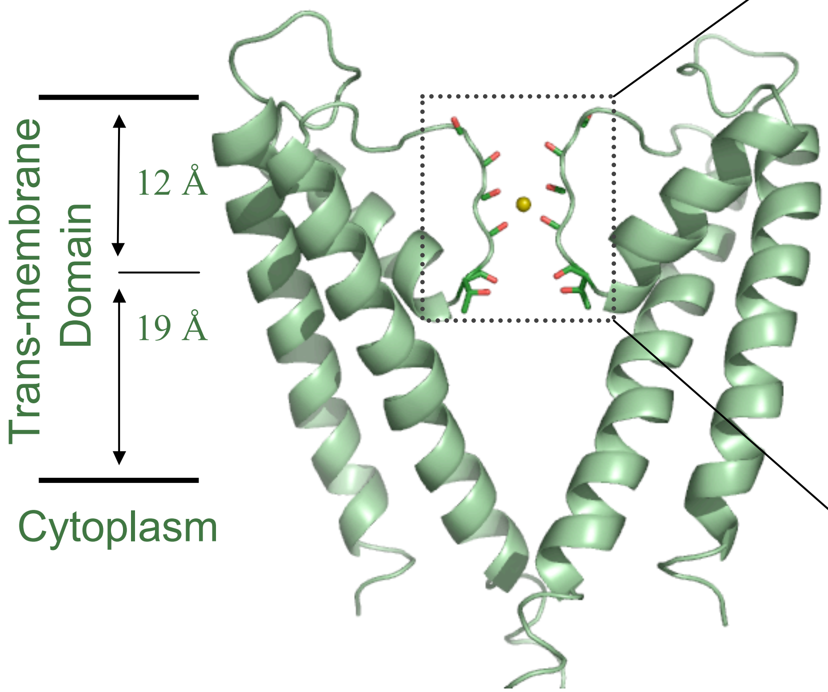
ABSTRACT

Cs^+ interferes with the permeation of K^+ ions through potassium (K-) channels and serves as a channel blocker. Presumably, the difference between its binding free energy to the selectivity filter and its hydration free energy must be more negative than that of K^+, or even the permeable Rb^+ ions. Nonetheless, what still remains unclear regarding the mechanism by which it interacts with K-channels includes the following. In our previous quantum chemical studies, we found that highly selective K-channels maintain a special local environment around their binding sites devoid of competing hydrogen bond donor groups, which enables spontaneous transfer of K^+ from states of low coordinations in water into states of over-coordination by 8 carbonyl ligands in the channel filter. This over-coordination is physiologically important to achieve K^+ over Na^+ selectivity. Does the binding of Rb^+ or Cs^+ to the 8-fold sites in the channel also require the presence of this special local phase? In addition to the properties of the solvation phase beyond the individual binding sites, are there any structural differences between the inner coordination shells of the Cs^+, Rb^+ and K^+ ions that designate the former as a blocker and the latter two as permeable ions? To resolve these issues, we carry out a series of classical and quantum chemical simulations and probe the effects of such determinants as coordination number, ligand chemistry and local phase on the structural and thermodynamic solvation properties of Rb^+ and Cs^+ ions. We then compare these results to our previous results on Na^+ and K^+ ions to understand why K-channels appear to be selective for ion size (not by size).

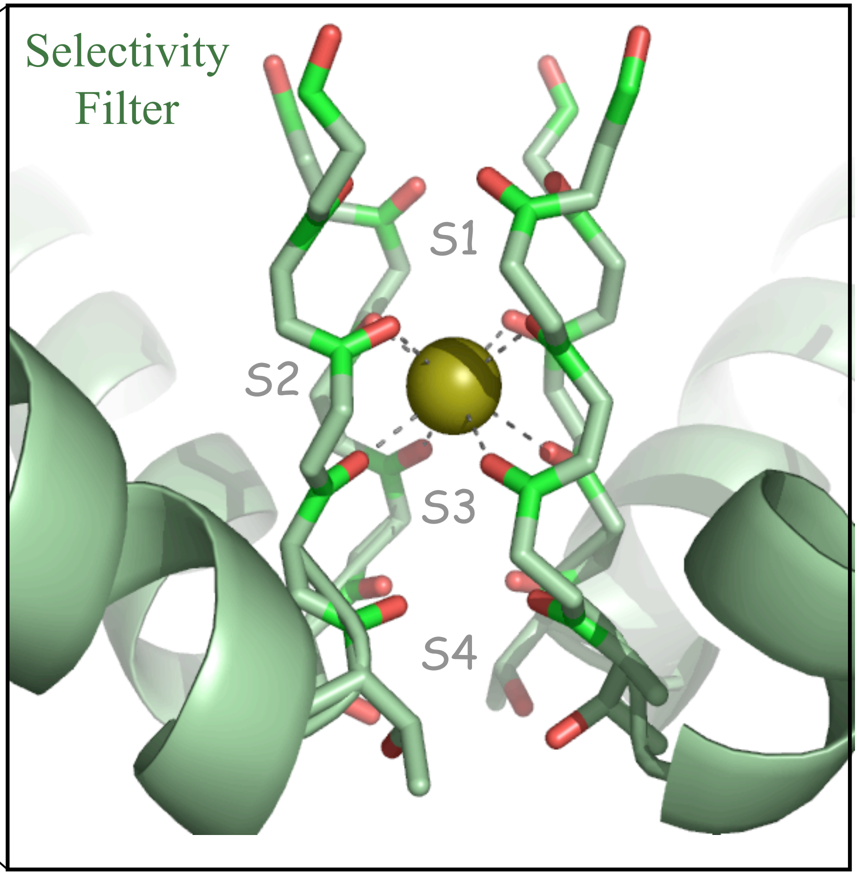
Representative Structure of K-channels

X-ray Structure of KcsA

PDB ID: 1K4C, MacKinnon and Co.



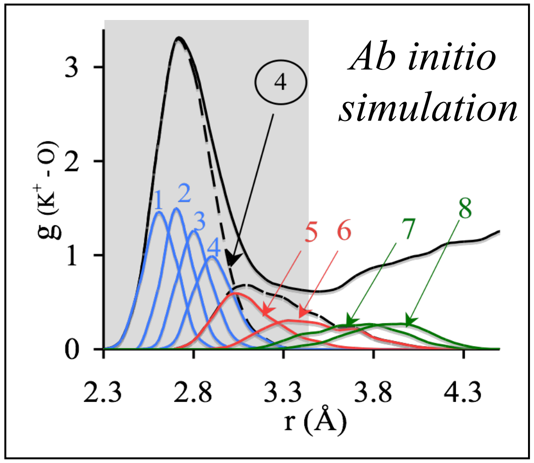
Narrowest portion of the permeation pathway
in the channel's conductive state



Summary of previous quantum chemical studies of K^+ over Na^+ selectivity

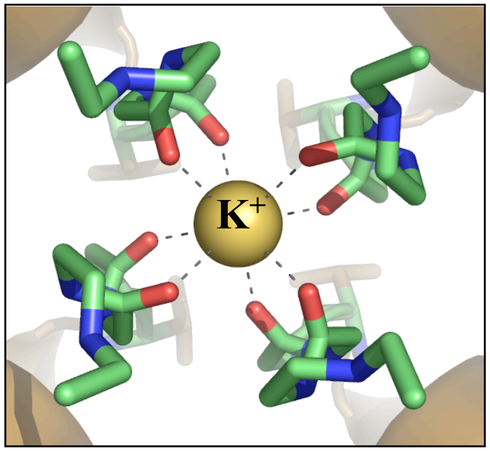
1. Selectivity in K-channels is due to constraints on over-coordination

K^+ in Bulk Water



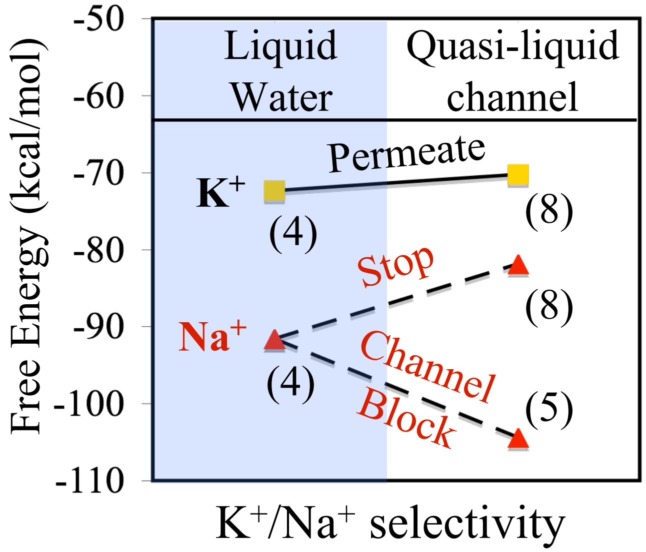
Low coordination preferred

Ion selectivity filter



High 8-fold coordination in equilibrium

Phase



Introduce Special Quasi-Liquid Phase

Introduce constraint on over-coordination

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

Tuning ion selectivity in K-channels

Low coordination around K⁺ in water

Special local phase (Chemical+Structural Properties of phase)

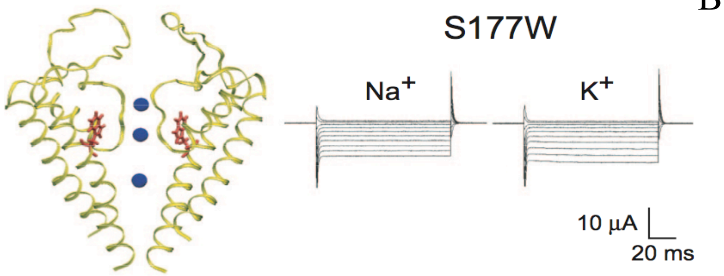
Sustain high (>6) coordination around ion in filter

K⁺/Na⁺ Selectivity

Disturb phase

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)

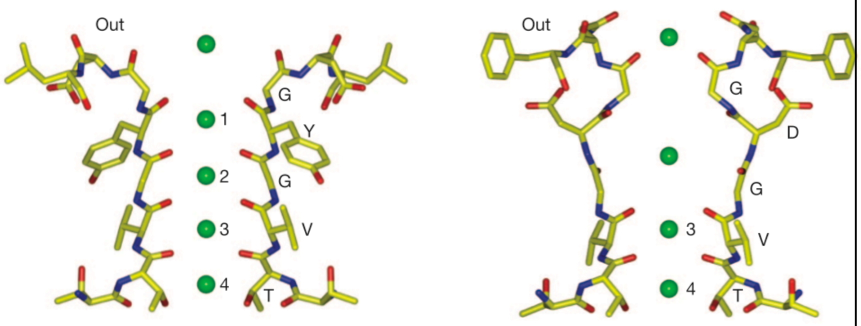
Weakly selective K-channels =

Strongly selective K-channels + Arginines in local phase

3. **Structure**

Introduce H-bond donors in the form of water & increase conformational freedom.

KcsA - Strongly Selective **NaK - Weakly Selective**



Shi *et al.* Nature Letts., 2006

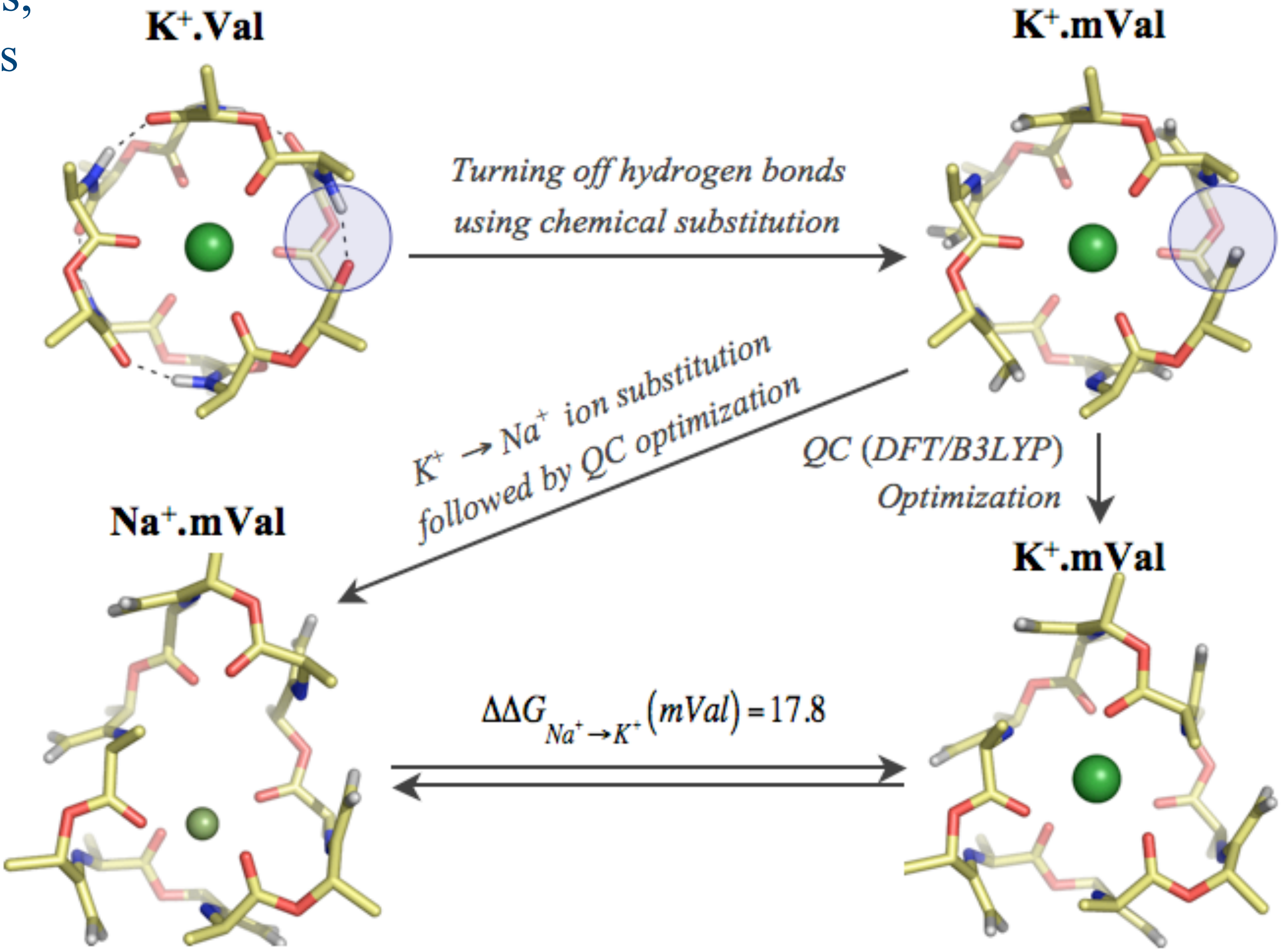
2. Selectivity in Valinomycin is due to constraints on cavity size

In contrast to K-channels, **Valinomycin** molecules achieve K^+/Na^+ selectivity by using:

6 carbonyl ligands

+

constraints on cavity size



Intra-molecular Hydrogen bonds play a vital role in enforcing cavity size constraints.

Issues with respect to understanding Rb^+ and Cs^+ conduction through K-channels

1) Does the binding of Rb^+ or Cs^+ to the 8-fold sites in the channel also require the presence of a special local quasi-liquid phase?

2) In contrast to K^+ and Rb^+, Cs^+ moves sluggishly through the channel. Why?
    a) Is it because it is too large to fit through the selectivity filter?
    b) Or does it bind more tightly to the selectivity filter as compared to K^+ and Rb^+?

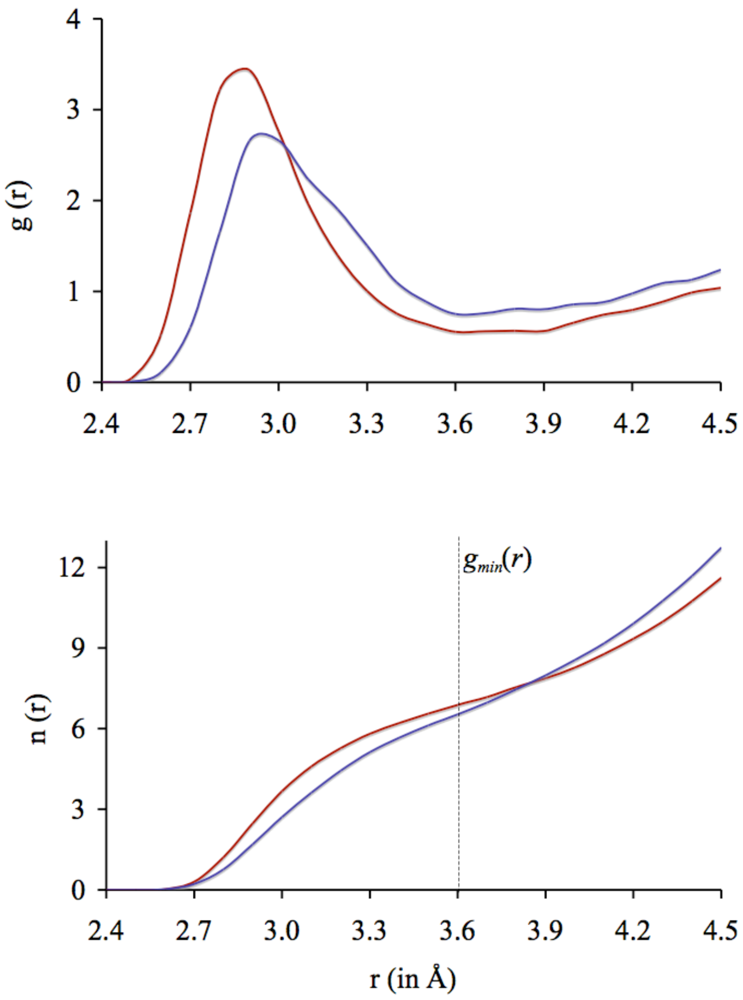
        a) If so, then is it because of specific differences between the intrinsic coordination properties of the ions?
        b) And/or is it because specific chemical + structural properties of the binding sites make them bind more tightly to Cs^+?
        c) And/or, is it because specific chemical + structural properties of the region beyond the binding sites make the binding sites bind more tightly to Cs^+?

Structural properties of Rb$^+$ and Cs$^+$ ions in water: Classical Versus *Ab initio* simulations

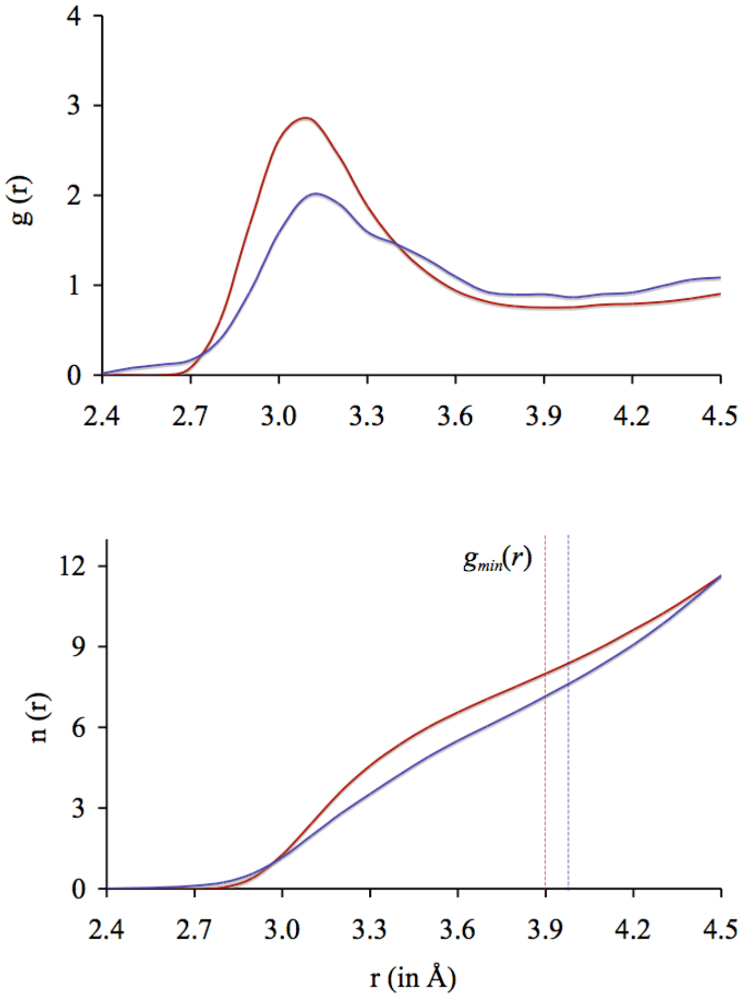
Classical - Åqvist Ion + SPC/E Water

***Ab initio* - PW91 Functional**

Rb$^+$



Cs$^+$

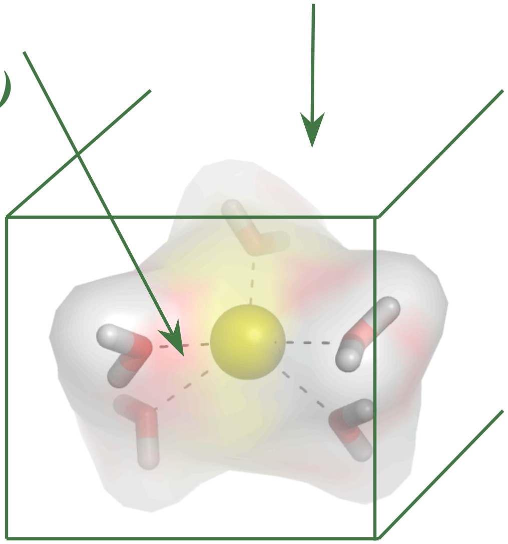


Hydration free energy difference between Rb^+ & Cs^+

Computed Value = $\Delta\Delta G(aq.) =$

$\Delta G_{Rb^+}(aq.) - \Delta G_{Cs^+}(aq.) =$ -7 kcal/mol

Experimental Value = -6 kcal/mol



Outer Domain treated classically (Elec./MD)

Inner Domain treated quantum chemically (DFT/B3LYP)

Computed using the molecular association theory of liquids:
(Widom, 1982; Pratt & Co.)

$$\beta\Delta G_A = -\ln\left\langle \prod_{j\neq\{1,\ldots,n\}} \left(1+f_{\sigma j}\right)\right\rangle_0 - \ln\left[\sum_n K_n^{(g)} \left[\frac{\left\langle\left\langle e^{-\beta\Delta U_\sigma}\right\rangle_0\right\rangle_{n,\Gamma}}{\left\{\left\langle e^{-\beta\Delta U_{H_2O}}\right\rangle_0\right\}^n}\right]\rho_{H_2O}^n\right]$$

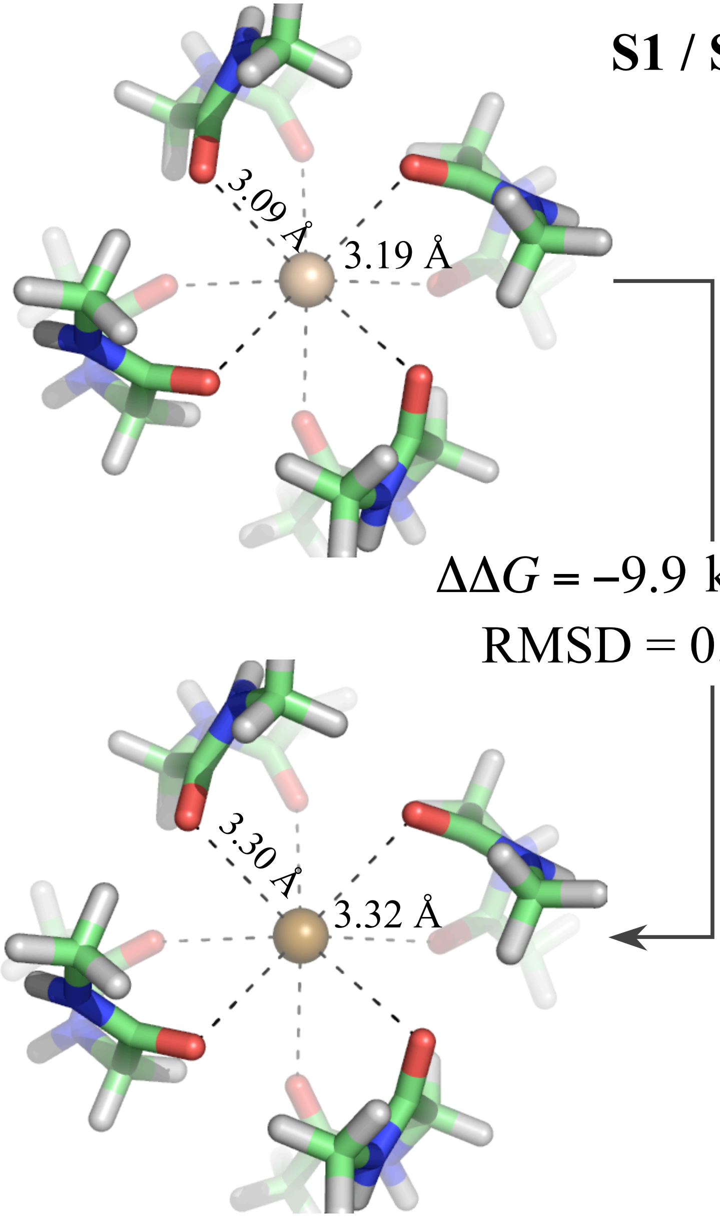
where, $K_n^{(g)}$ is the equilibrium constant of the following gas phase reaction

$$A + nH_2O \xrightarrow{K_n^{(g)}} \left[A\left(H_2O\right)_n\right]$$

Interaction of Rb$^+$ & Cs$^+$ with Individual binding sites

Individual binding sites are more selective for Rb$^+$

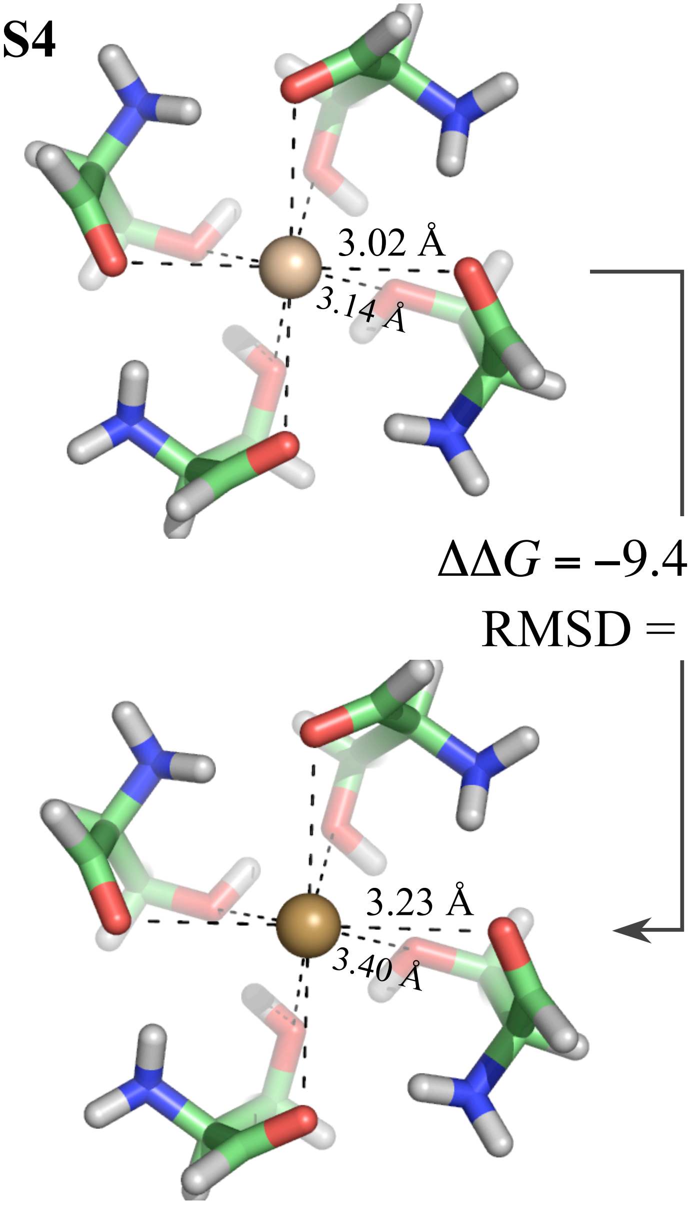
S1 / S2 / S3



$\Delta\Delta G = -9.9$ kcal/mol

RMSD = 0.64 Å

S4



$\Delta\Delta G = -9.4$ kcal/mol

RMSD = 0.13 Å

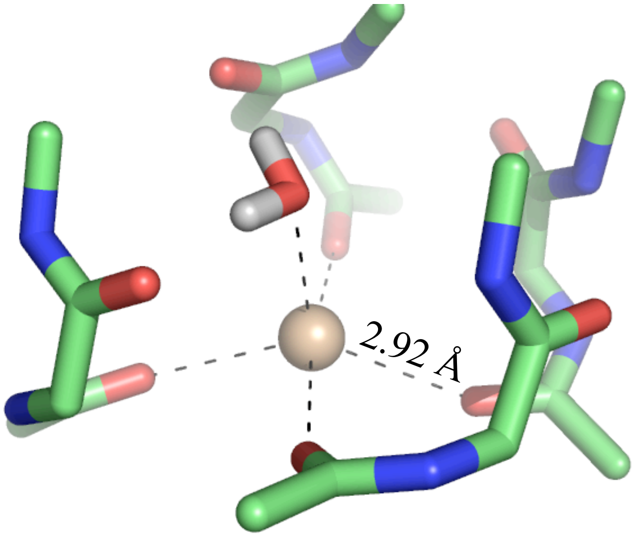
Interaction of Rb^+ & Cs^+ with Individual binding sites & water

S1 with One Extra-cellular Water

Optimized S1 with Rb^+

+ Water

RMSD = 1.2 Å



$Rb^+ \rightarrow Cs^+$

Large Distortion resulting in 5-fold coordination

$\Delta\Delta G = -10.3$ kcal/mol

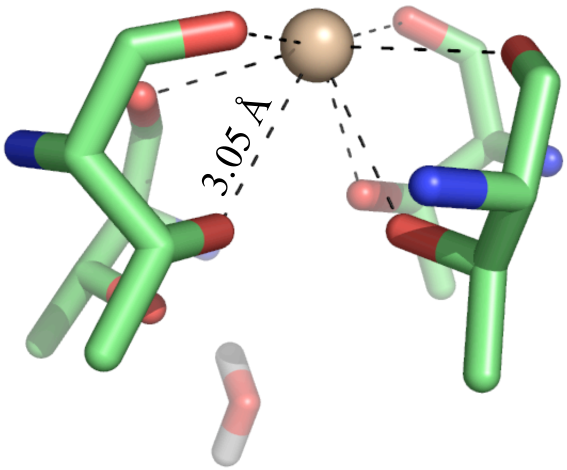
RMSD = 0.25 Å

S4 with One Intra-cellular Water

Optimized S4 with Rb^+

+ Water

RMSD = 0.7 Å



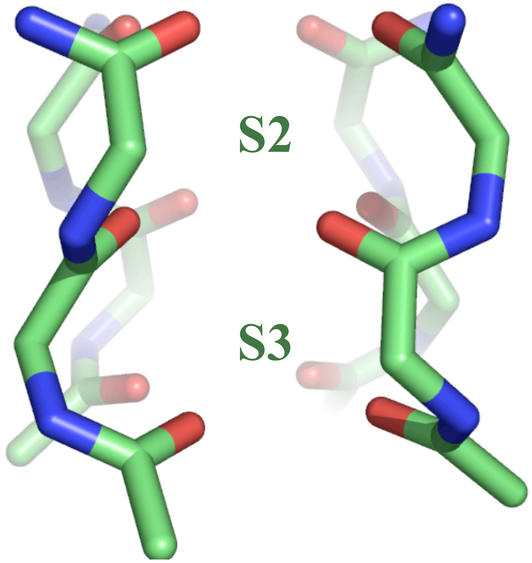
$Rb^+ \rightarrow Cs^+$

7-fold coordination, but not with water

$\Delta\Delta G = -9.4$ kcal/mol

RMSD = 0.12 Å

Interaction of Rb^+ & Cs^+ with Multiple binding sites

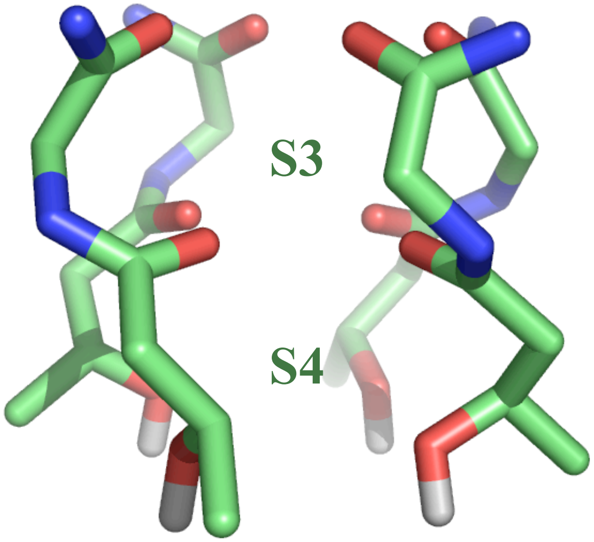


+ H_2O

$Rb^+ \rightarrow Cs^+$

$\rightarrow \Delta\Delta E = -7.9$ kcal/mol

With a water molecule in site S3, site S2 is still more selective for Rb^+



+ H_2O

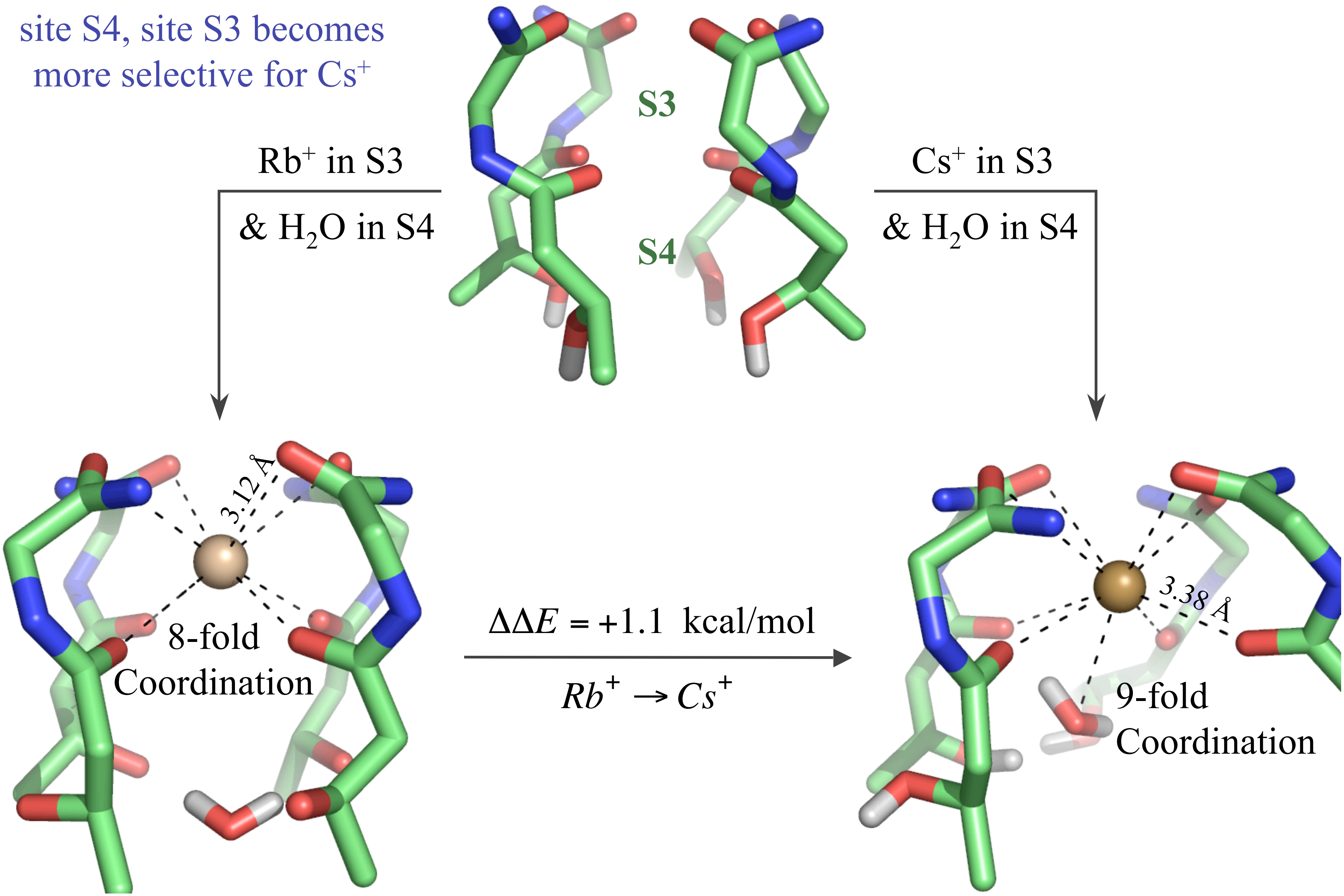
$Rb^+ \rightarrow Cs^+$

$\rightarrow \Delta\Delta E = -8.2$ kcal/mol

With a water molecule in site S3, site S4 is also more selective for Rb^+

Interaction of Rb^+ & Cs^+ with Multiple binding sites

With a water molecule in site S4, site S3 becomes more selective for Cs^+



Rb^+ in S3 & H_2O in S4

Cs^+ in S3 & H_2O in S4

$\Delta\Delta E = +1.1$ kcal/mol

$Rb^+ \rightarrow Cs^+$

Summary & Conclusions

1) Binding of Cs^+ to the 8-fold sites in the channel does not require the presence of a special local quasi-liquid phase, as it is also 8-fold coordinated in liquid water.

2) This is in contrast to the requirements for the binding of both K^+ and Rb^+ ions, which are over-coordinated in the selectivity filter.

3) In contrast to K^+ and Rb^+, Cs^+ moves sluggishly through the channel because
    a) Cs^+ may be too large to fit through the selectivity filter (Add Citations)
    b) In addition, it also binds more tightly to site S3, provided

        a) site S4 is occupied by a water molecule, and
        b) site S3 is surrounded by a quasi-liquid pocket to allow Cs^+ to over-coordinate with 9 ligands

Acknowledgements

Software

(1) Classical Molecular Dynamics simulations - GROMACS v3.1
(Lindahl *et al*., J Mol. Mod. 2001)
(2) *Ab initio* Molecular Dynamics simulations - VASP vX.X
(3) Quantum Chemical calculations - Gaussian03 (Gaussian Inc.)
(4) Implicit Solvent Simulations - APBS (Baker *et al*., PNAS 2001)
(5) Figures - PyMol (DeLano Scientific)
(6) Analysis Codes - Home grown at UIUC under the auspices of the Ion Channel Portal, and at Sandia National Labs.

Computer Time (150,000 hours)

(1) The National Center for Supercomputing Applications at UIUC.
(2) Sandia Supercomputing Center.

Funding

(1) DOE Grants to SV and SBR
(2) NIH grant to SBR through the NIH roadmap for Medical Research (www.nanoconductor.org).