Structural Changes in the Selectivity Filter of the Open-State KcsA channel

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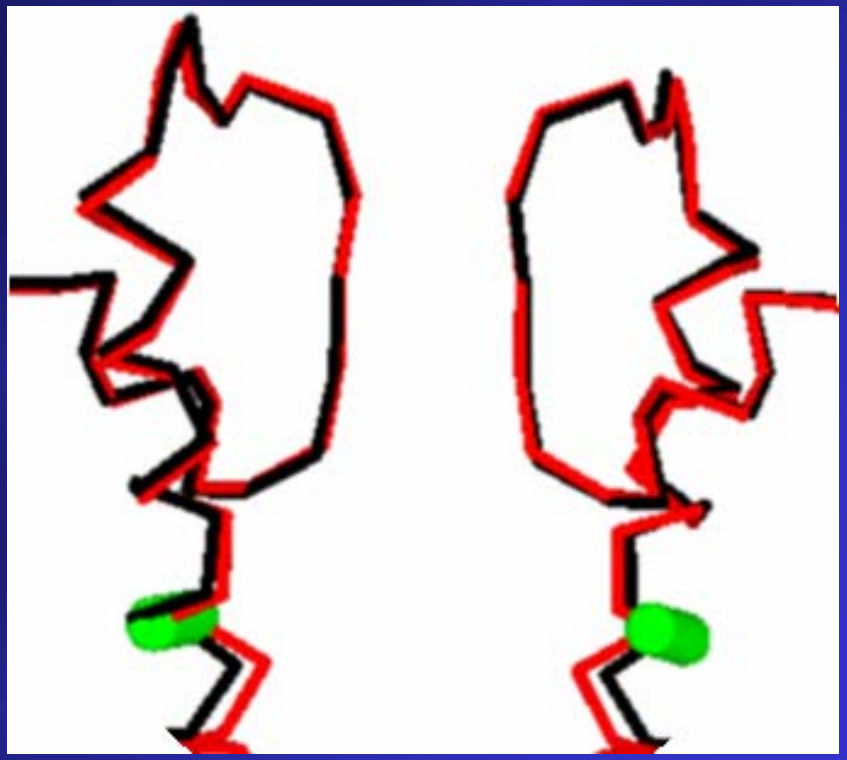
Outline

1. **Key Concepts & Model**
2. **Conformations of the SF**
3. **Ionic Selectivity in KcsA**

Telluride, CO

July 31, 2007

Conformation of the SF P-loops



Superposed C_α traces of the SF P-loops. Closed structure shown in red, open structure shown in black. Green marks show the location of a gating hinge, G99.

- SF P-loops are structurally unaltered in the open-state KcsA channel

Therefore

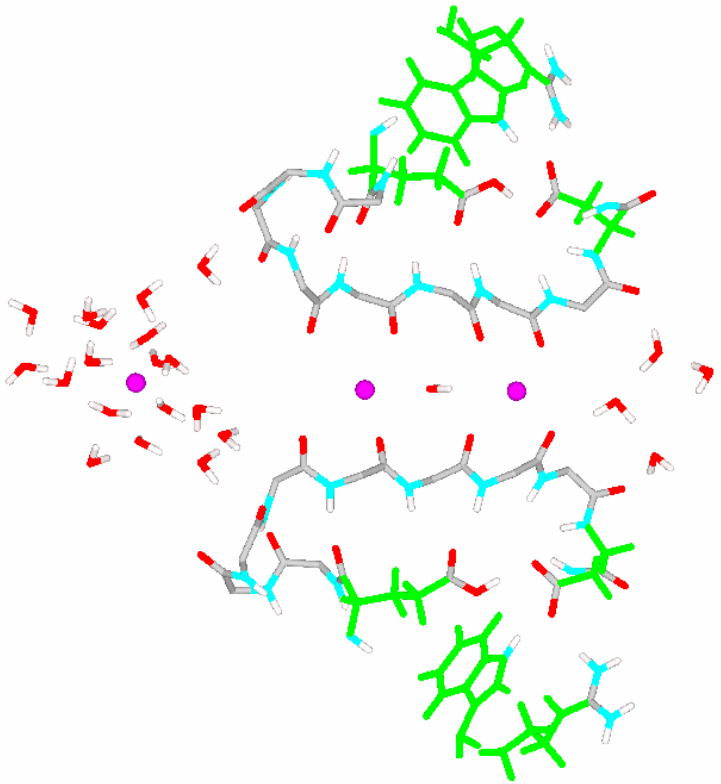
➢ closed-state crystal structure (1K4C) of the SF can be used as a reference state :

to determine structural changes in response to mutations or to $K^+ \Rightarrow Na^+$ substitution in the SF of the open-state KcsA

Conformation of the SF P-loops

- primary gate – the IC bundle; secondary gate – the selectivity filter
- are the two gates independent or they somehow coupled?

Cordero-Morales et al. *Nat. Struct. Mol. Biol.* **13**, 311-318 (2006); Blunck et al. *J. Gen. Physiol.* **128**, 569-581 (2006)



The movie shows conformations of the SF P-loops and residues during the closed-open gating transition

- SF P-loops and residues fluctuate near the initial conformation
- side chains of R89 and W67 undergo deviations during gating, but they stay close to the initial conformation in the open state
- no crucial structural changes are observed that could inactivate the SF

Ultimate goal: *use a computational approach to predict:*

mutant protein structures and local structural changes due to substitutions, insertions or deletions of residues, waters and ions in the SF of the open-state KcsA

MD simulations?

- MD yields trajectories that provide a comprehensive description of molecular motion and appear quite complicated resembling "random noise“
- trajectories do not go to a final well-tuned conformation, but represent the fluctuating protein with small-scale conformational transitions
- thermal fluctuations cause the protein structure to wander away from the crystal structure making comparisons difficult
- MD simulations are not well suitable for our goal

Why should energy minimization work?

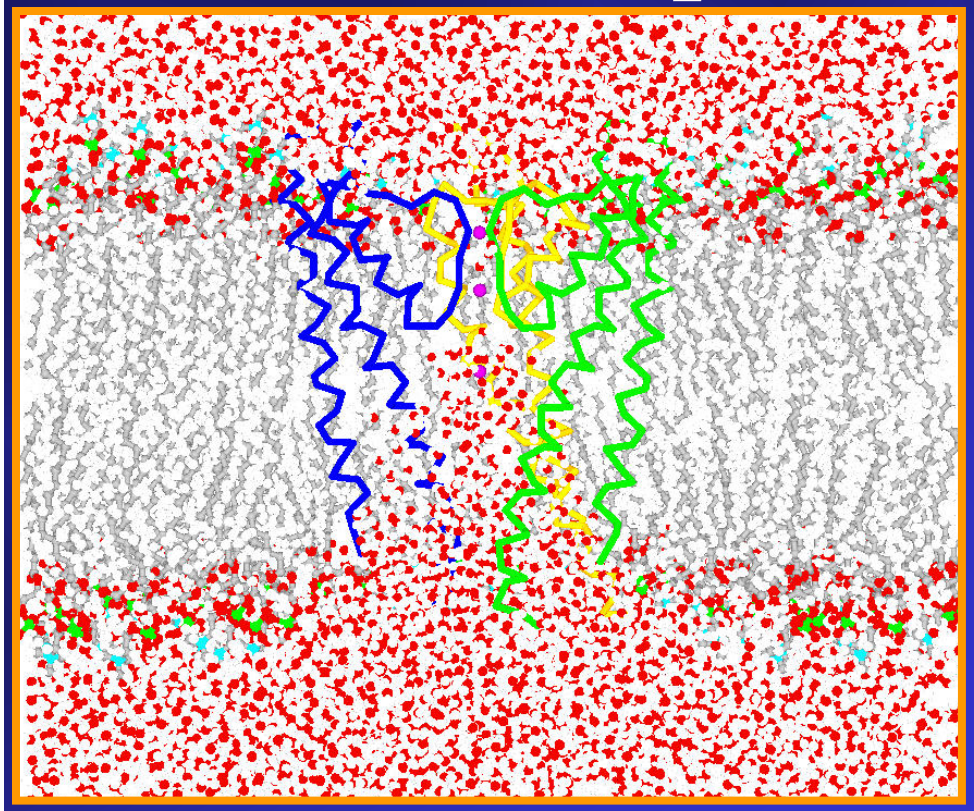
Because ...

- a protein's native-fold undergoes only ***local*** structural adjustments at mutation sites in the SF during minimization, and deviates somewhat ***locally*** from the experimental structure; no major repacking of the rest of the KcsA protein occurs

- the final conformation of the energy-minimized mutant is also a low-temperature structure, similar to the X-ray crystallographic structure; thus, structural refinement of the mutant is performed relative to low-temperature data with ***local*** adjustments at mutation sites

- energy minimization fine tunes (refines) the location, the optimal geometry and the energy of the mutant protein's preferred conformation that can be used for comparisons with the available experimental structures

Computational Model



Side view of the central simulation cell showing open-state KcsA, 3 ions, 123 DPPC lipids and ~7,000 waters (~400 in the IC vestibule). Front lipids and one KcsA subunit are not displayed.

- the open-state KcsA structure with the cavity as an integral part of the cytoplasm is used

- periodic boundary conditions applied in all dimensions; the Coulomb energy calculated by a parallelized FMM; minimizations were performed using the steepest descent method and a new conjugate gradient method with guaranteed descent

- the large solvent box: **1**) stabilizes polar and charged residues on the surface of KcsA; **2**) accounts for surroundings' effect on SF conformations

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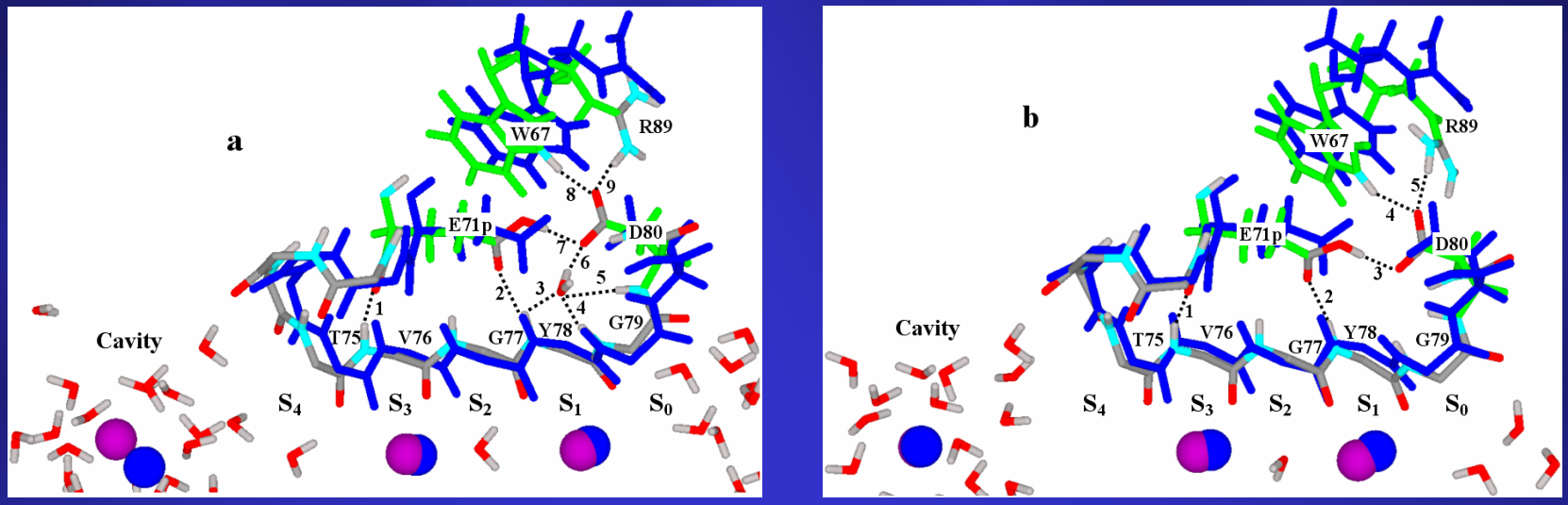
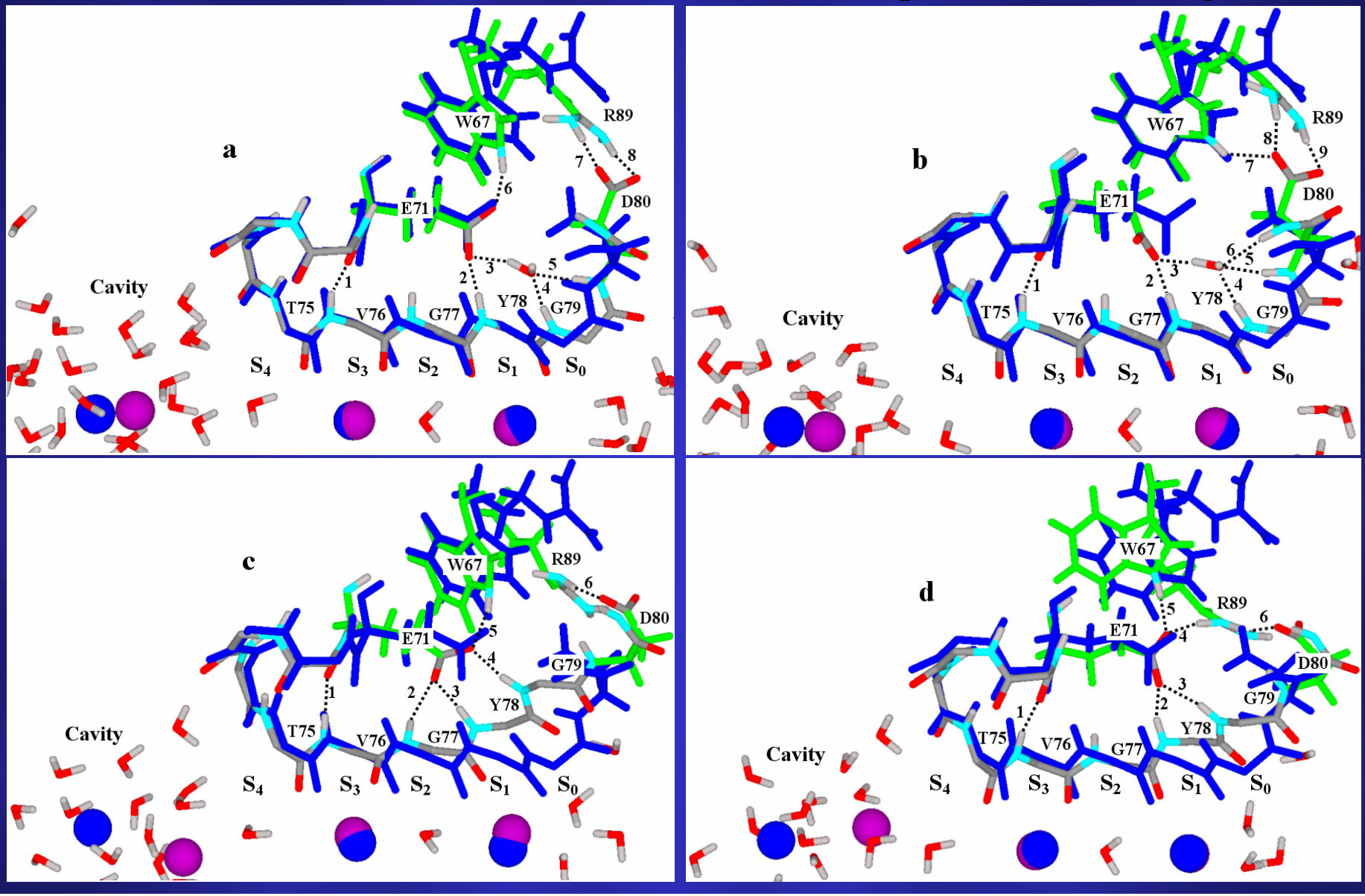
Validating E71p KcsA against 1K4C

Superposing SF of closed (high [K+] structure, 1K4C in blue) and open state (energy-minimized E71p KcsA, native colors) structures. With (a) and without (b) water behind the P-loop

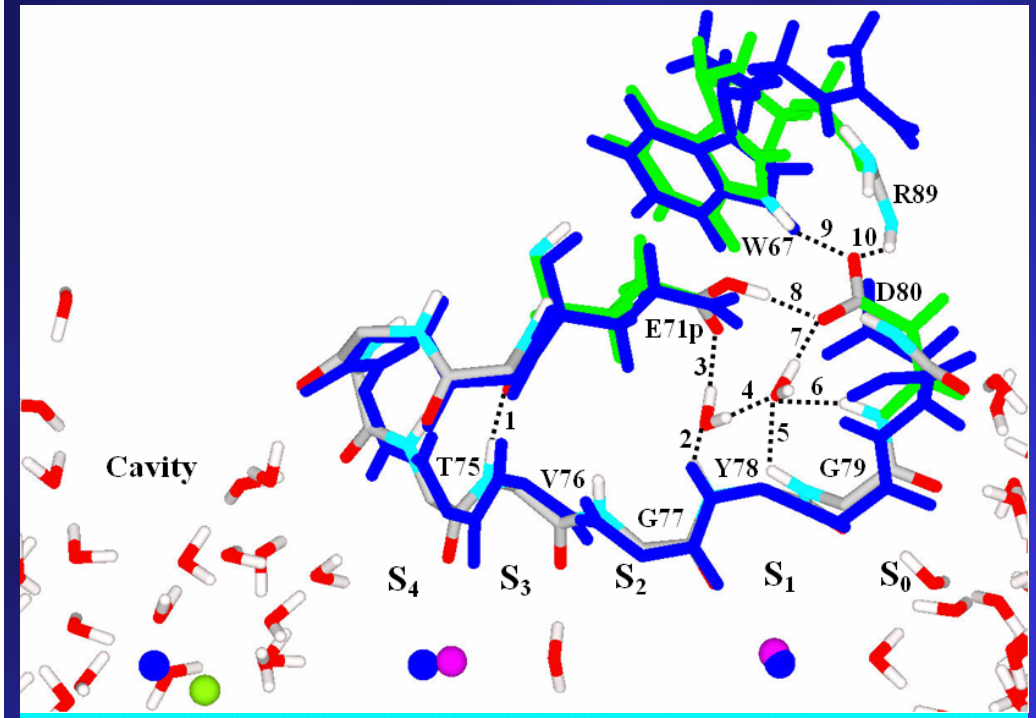


- in both (**a**) and (**b**) E71p and D80 stay close to the 1K4C locations, thus strongly supporting the idea that they share a proton
- R89 from the neighboring subunit reorganizes forming a salt bridge with D80
- the amide Hs of both G77 and G79 are not H-bonded in variant (**b**), but the amide H of G79 is well stabilized by water in variant (**a**)

Superposing SF of closed (crystalline 1K4C) and open state (energy-minimized) KcsA structures – E71 and D80 both ionized. With (a,b) and without (c,d) a water molecule behind the P-loop. Note rearrangement.



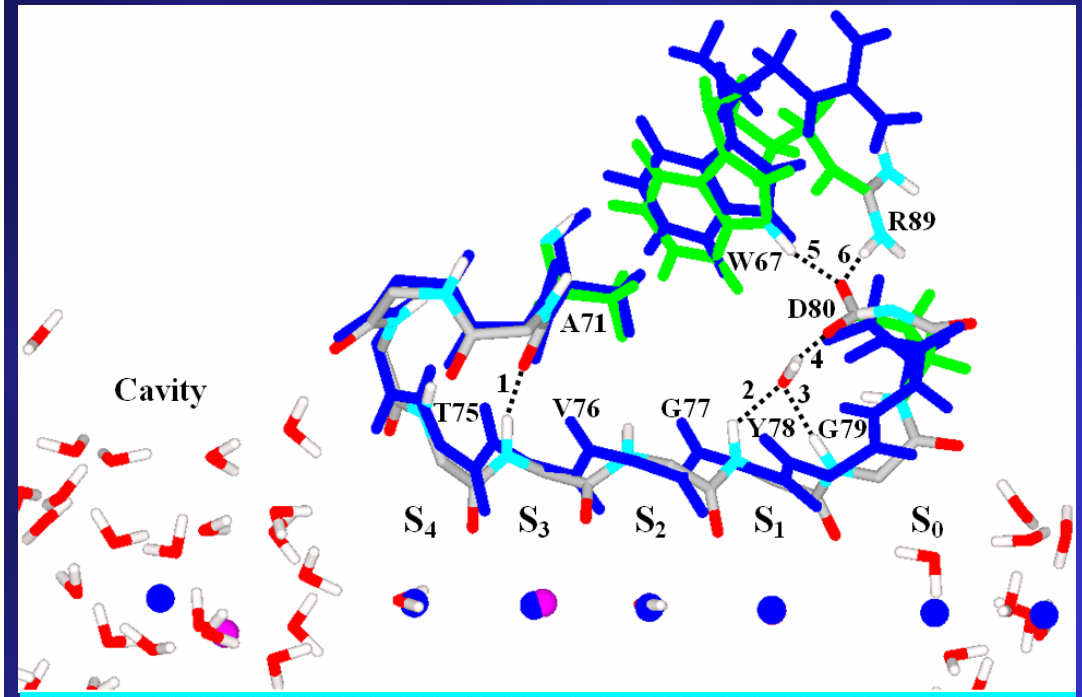
Validating E71p KcsA against 1K4D



Superposing SF of closed (low [K+] structure, 1K4D in blue) and open state (energy-minimized E71p KcsA, native colors) structure with a vacant S_2 site and two water molecules behind the P-loop

- E71p and D80 remain close to 1K4D locations
- R89 side chain moves toward D80 forming a salt bridge
- two water molecules behind the P-loop form H-bonded network with amide Hs of G79 and Y78 and carboxyl Os of E71 and D80
- P-loop conformation highly distorted in low [K^+] structure relative to high [K^+] structure

Validating E71A KcsA against 1ZWI

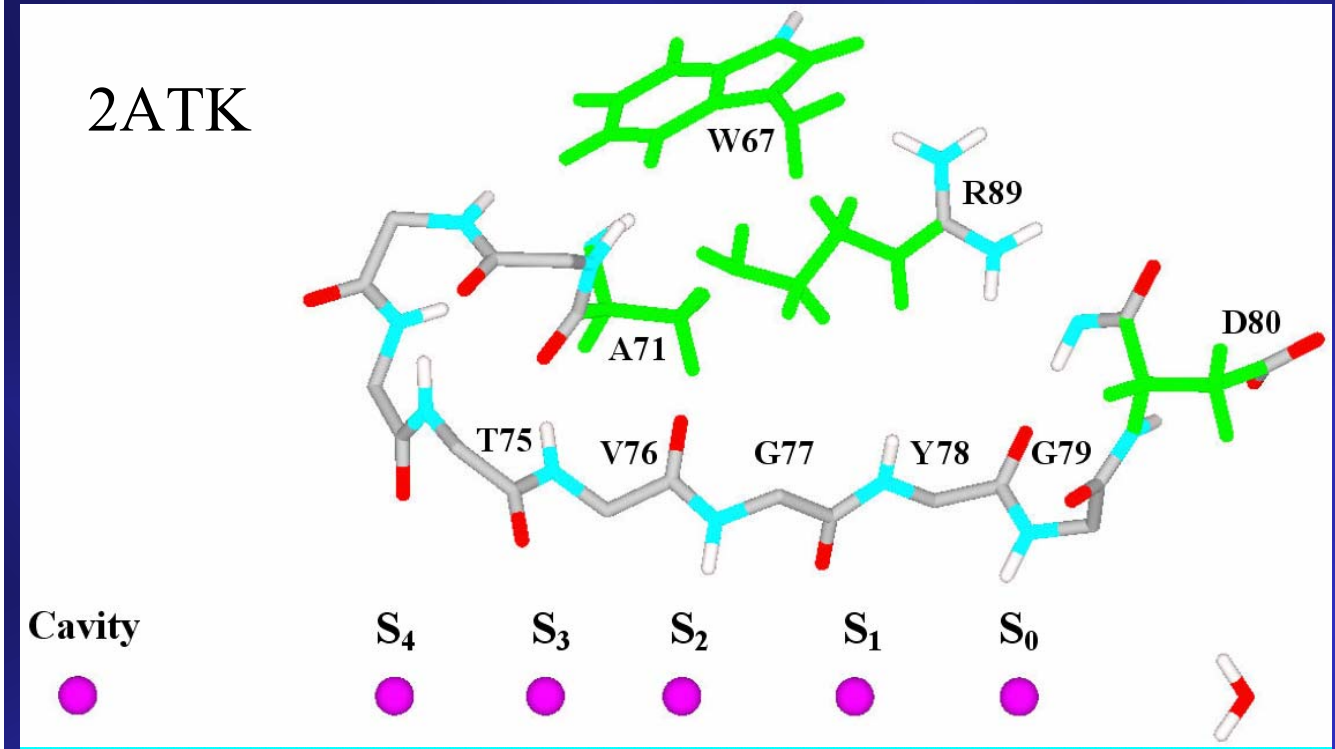


Superposing SF of closed (non-flipped E71A KcsA, 1ZWI in blue) and open state (energy-minimized E71A KcsA, in native colors) structures.

Cordero-Morales et al. Nat. Struct. Mol. Biol. **13**, 311-318 (2006)

- good overall agreement for P-loops and location of A71, D80 and W67 side chains; R89 side chain moves toward D80 forming a salt bridge
- conformation of D80 is close to that in the E71p mutant and differs from that with both E71 and D80 ionized
- central part of the P-loop behind the SF is not well stabilized

"Flipped" E71A KcsA Crystal Structure



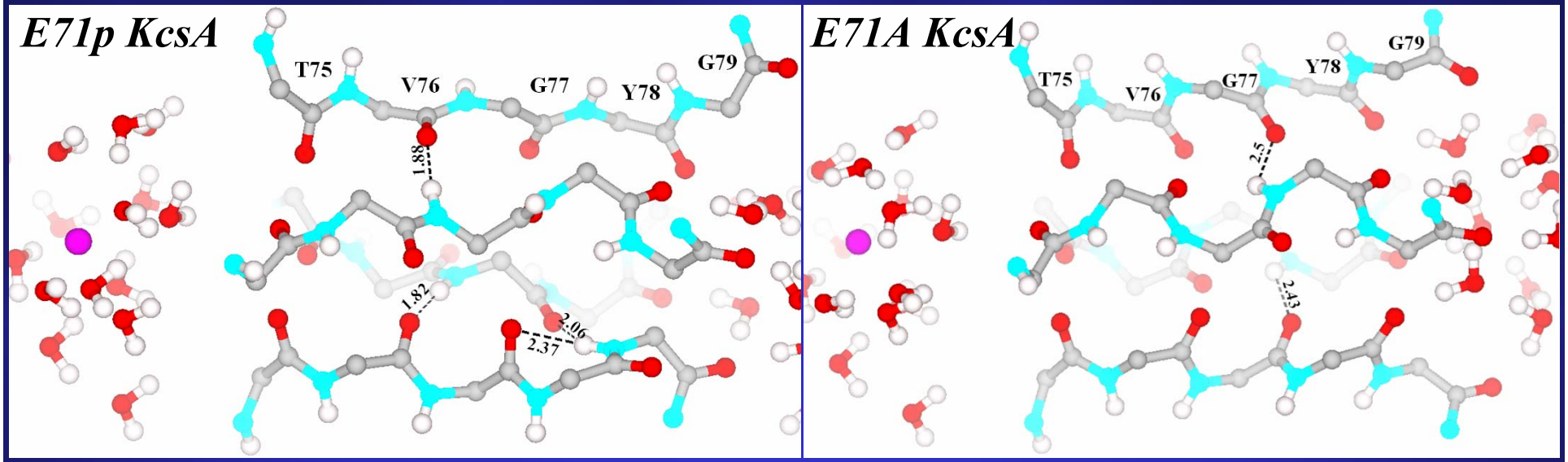
Conformation of the SF P-loop of the flipped E71A KcsA crystal structure (in native colors), pdb code 2ATK.

- D80 is displaced toward the extra-cellular side; W67 observed in two rotameric states
- V76 & Y78 COs flipped away from the pore
- positions of the binding sites are displaced

energy-minimization runs failed to yield conformations similar to this flipped E71A mutant

Cordero-Morales et al. *Nat. Struct. Mol. Biol.* **13**, 311-318 (2006)

Conformation of the empty SF



- KcsA remains assembled, but peptide planes of the P-loops undergo strong distortions and flipping; C_α of residues G77, in the center of the SF, twist inward, occluding the pore
- the distorted SF is well stabilized by a network of HBs between Hs and Os from the adjacent P-loops
- peptide planes **G79-Y78** undergo deviation of ~90^0; **Y78-G77** & **G77-V76** planes flip ~90^0-180^0; **V76-T75** plane never strongly distorted

Summary

- energy-minimized open state conformations of E71 mutants agree well with available crystal data accept the “flipped” E71A structure
- H-bonding network stabilizing amide Hs behind the P-loops is sensitive to E71 mutations and to the presence of water molecule(s)
- peptide plane distortion and flipping was observed in a SF partially or entirely depleted of ions and waters; the SF void of ions and waters undergoes a conformational change involving all four P-loops and the new conformation is well stabilized by H-bonds between amide Hs and COs from the adjacent P-loops
- our data suggest that inactivated state of the SF corresponds to conformations with partially unoccupied or entirely empty SF
- the selectivity filter is very flexible, and can easily rearrange depending on the supply of ions or the configuration of hydrogen-bonding network stabilizing the P-loops

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Current Perspectives on Ion Selectivity

MD Simulations (Sansom's group):

- K^+ ions sit in a cage of eight COs, Na^+ ions interact tightly with a single ring of four COs plus two waters
- the mean Na^+-O distance is ~2.36 Å; K^+-O distance is ~2.85 Å
- SF is blocked by collapse (local constriction of a CO ring) around Na^+ ions

Biggin et al. *BBA* **1510**, 1-9 (2001); Shrivastava et al. *BJ* **83**, 633-645 (2002)

Reduced Models:

- discrimination against Na^+ due to strong binding, filter constriction, and enhanced energetic and positional fluctuations

Asthagiri & Pratt, *JCP* **125**, 024701 (2006)

- selectivity arises from "external" or "topological" constraints/forces imposed on an ion-coordinated complex by the channel protein

Bostick & Brooks, *PNAS* **104**, 9260-9265 (2007)

Current Perspectives on Ion Selectivity

Quantum Chemical Calculations:

- both *environmental* effects and the protein's ability to provide a proper *coordination number* are determinants of selectivity

- both Na^+ and K^+ prefer higher coordination numbers in a low dielectric environment, while lower coordination numbers are found in high dielectric surroundings (bulk water)

Varma & Rempe, *BJ* **93**, 1093-1099 (2007)

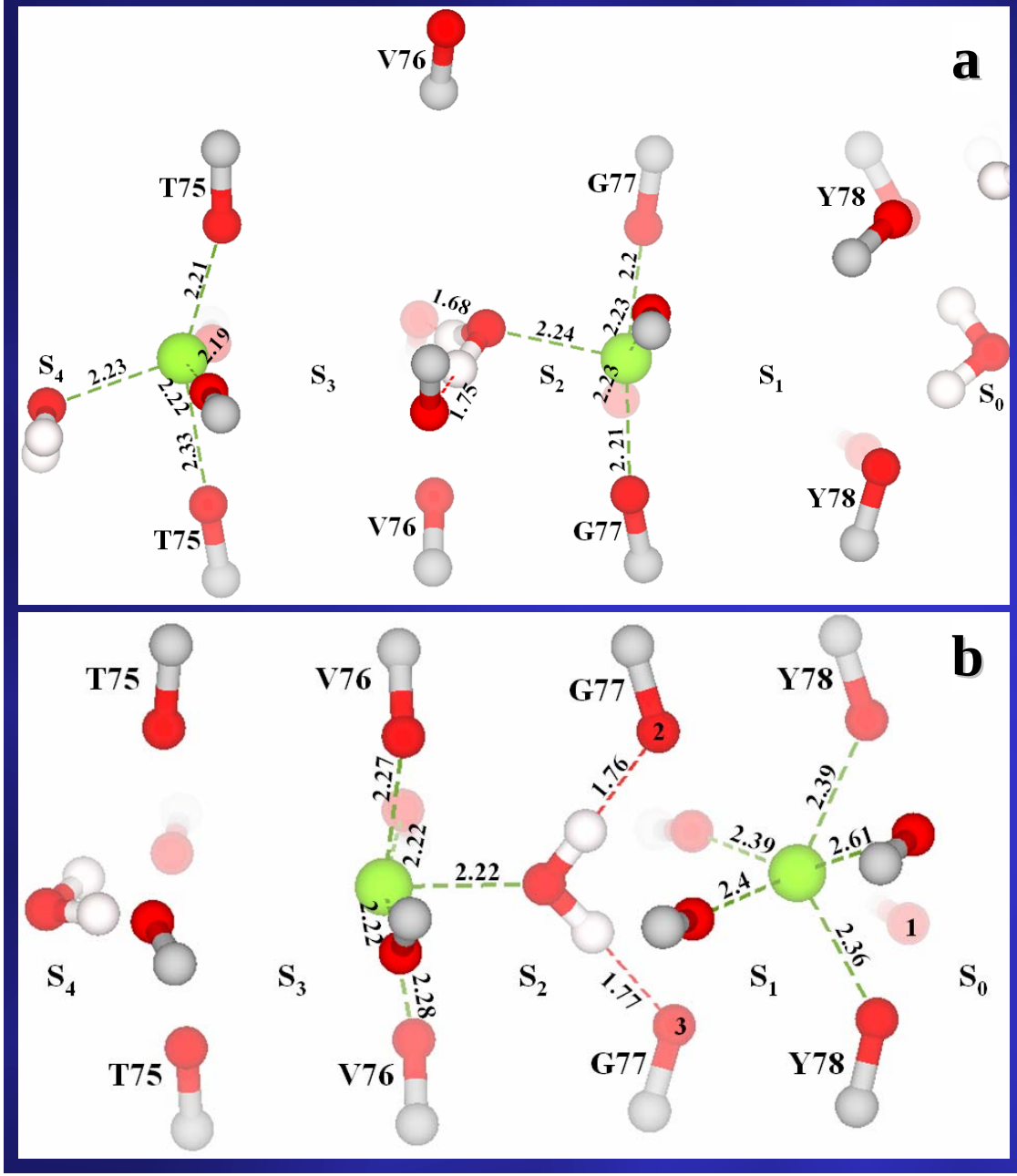
MD Simulations (Roux's group):

- the key to determining selectivity is the strength of the electric field arising from the ligands coordinating the cation

- the physical origin of the strain energy (~6 kcal/mol) in favoring K^+ over Na^+ was solely attributed to "through-space" electrostatic repulsions between COs, not to structural deformations of the protein

Noskov et al, *Nature* **431**, 830-834 (2004); Noskov & Roux, *JGP* **129**, 135-143 (2007)

Conformation of the SF loaded with Na^+



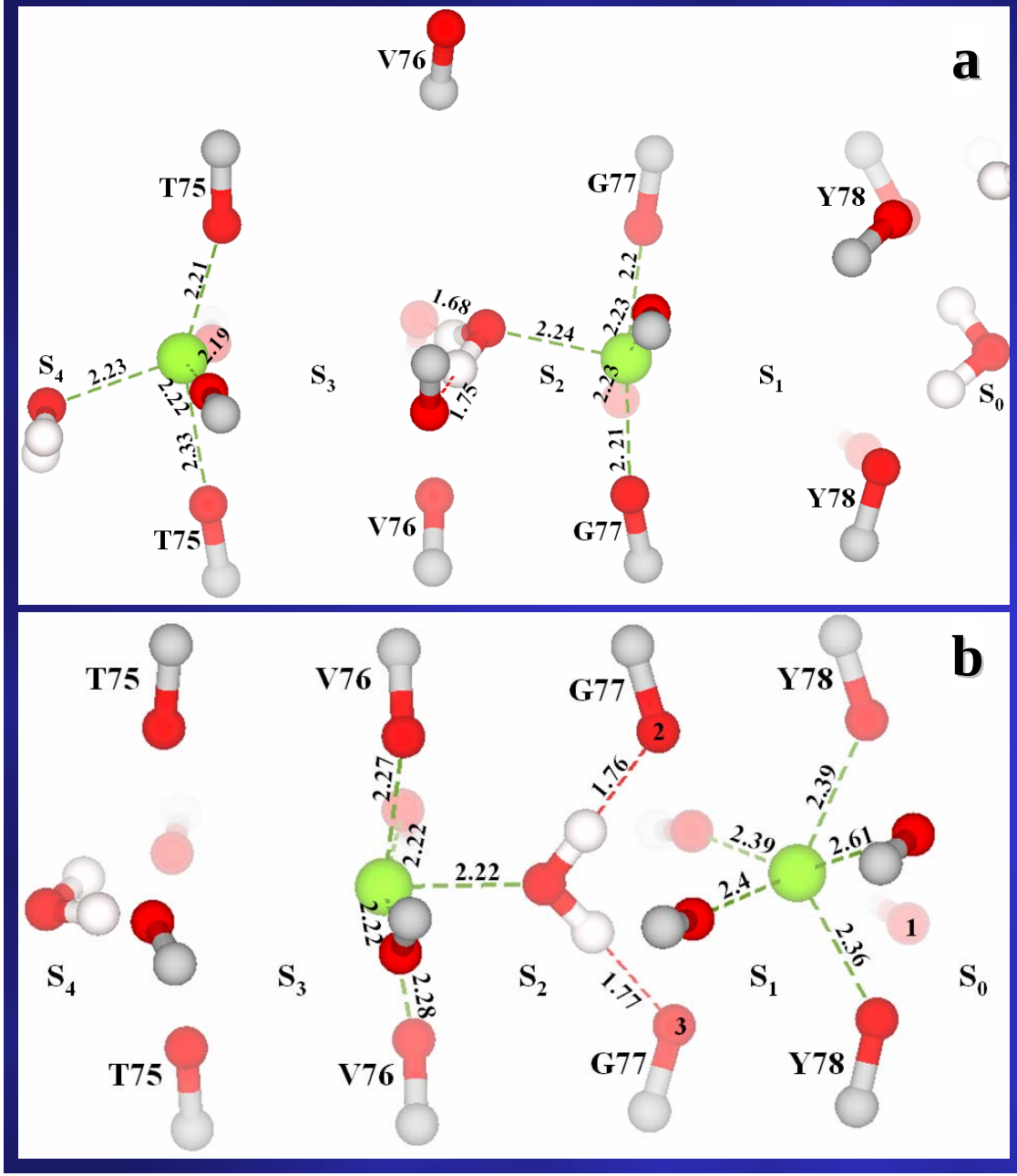
The energy-minimized SF loaded with Na^+ for E71p (**a**) and ionized E71&D80 (**b**) variants. Waters, Na^+ ions and COs from the four subunits are shown. Na^+-O bonds are shown as dashed lines and distances are labeled. In (**a**) one CO of V76 flips. In (**b**), distance from Na^+ to Os 1, 2 and 3 > 2.8 Å.

The change of the strain energy

Energy component	Δ_{tot} *kcal/mol*	Δ_{ion} *kcal/mol*
Electrostatic	-65.55	-21.85
van der Waals	6.41	2.14
Total non-bonded	**-59.1**	**-19.7**
Bond	1.19	0.397
Angle	4.89	1.63
Urey-Bradley Angle	0.0945	0.0315
Dihedral	3.11	1.037
Improper	0.419	0.1397
Total bonded	**9.7**	**3.23**
Total	**-49.44**	**-16.48**

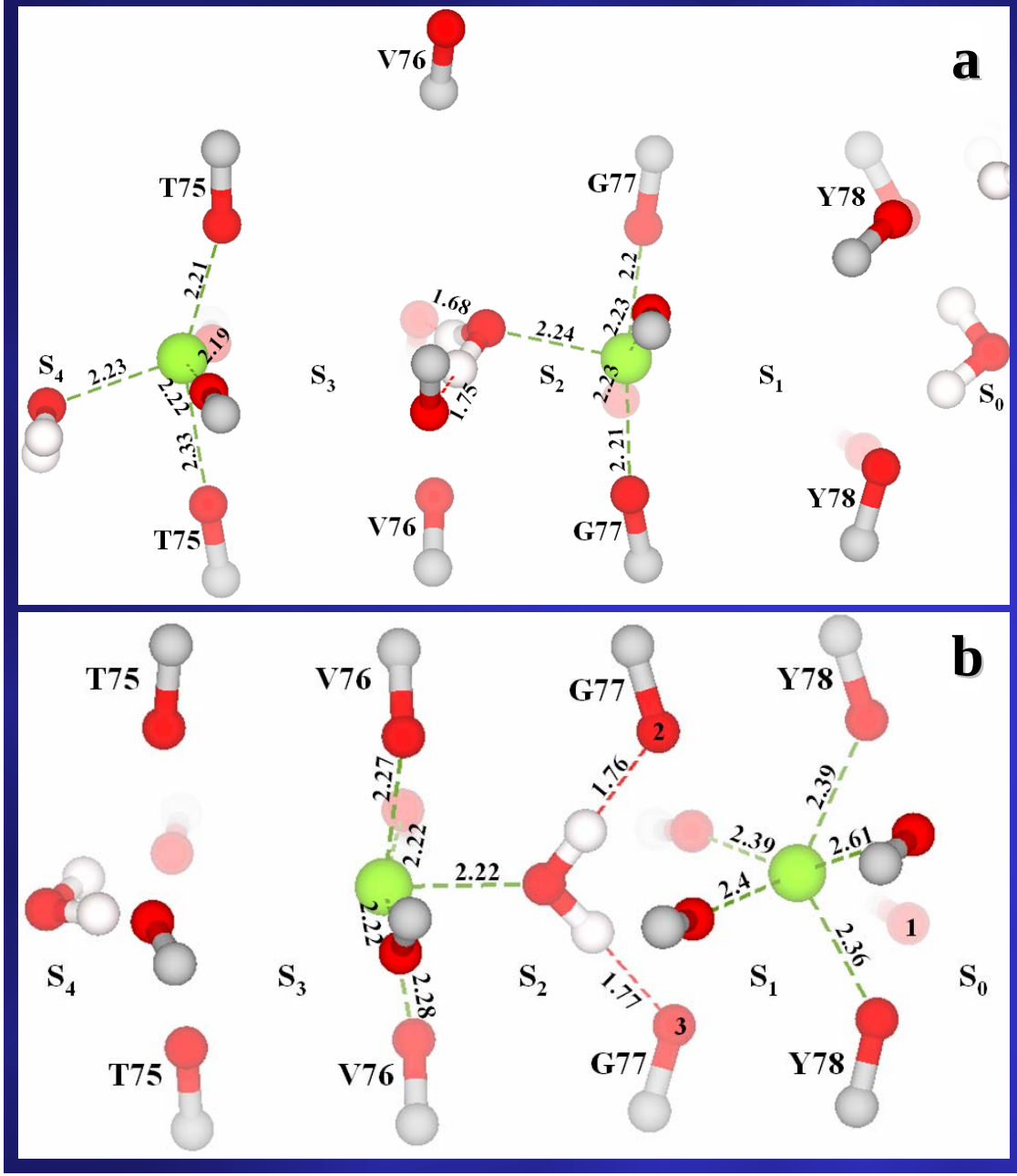
the difference in hydration energy between Na^+ and K^+ in bulk solution ~ **-18** kcal/mol

Conformation of the SF loaded with Na^+



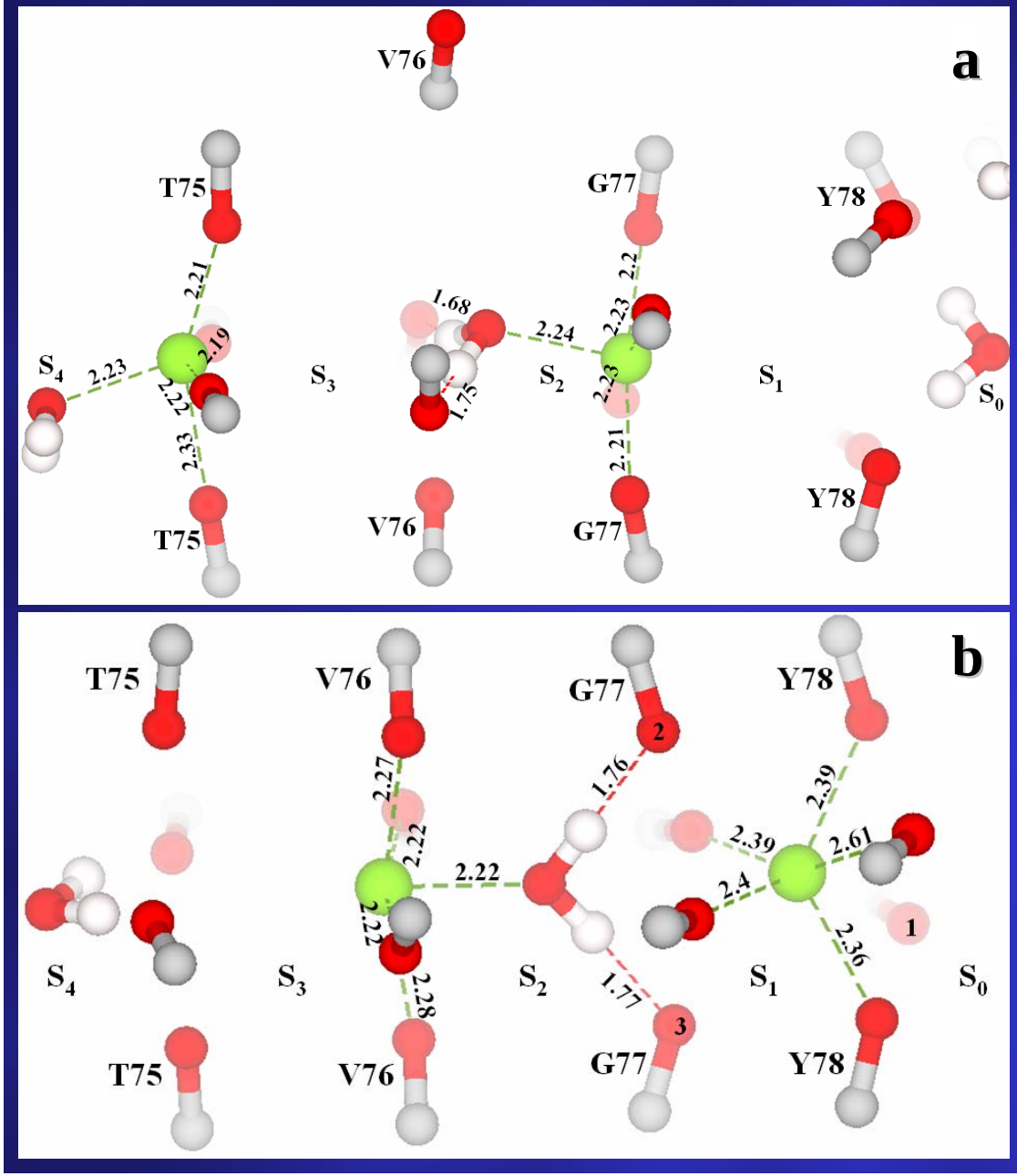
➢ ***Conformation change:*** the SF rearranges to bind the smaller Na^+. The ions either move from the center of S_1 and S_3 toward a ring of COs and are solvated by four COs and a water molecule (**a,b**) or the original eight-CO cage deforms and five Os collapse around Na^+ (**b**). Three COs (labeled 1, 2 and 3 in (**b**)) do not directly coordinate Na^+.

Conformation of the SF loaded with Na^+



➢ ***Coordination number change:*** the SF rearranges and five Os properly coordinate Na^+; the SF structure is deformed and presumably nonconductive. Both COs and waters coordinate Na^+.

Conformation of the SF loaded with Na^+



➢ ***Size selectivity:*** ionic size matters. K^+ ions fit comfortably in their binding sites without deforming the SF. The Na^+ binding cavity is smaller; the SF deforms providing five coordinating ligands to favorably cradle Na^+. SF collapse around Na^+ locally occludes the conduction pathway.

Summary

- **Conformation and binding sites differ with Na^+ in the SF:**

➢The native binding sites (S1-S3) are a good fit for K^+, but when Na^+ replaces K^+, these sites are no longer favorable

➢The SF rearranges to form new binding sites that comfortably accommodate Na^+, with direct coordination to five Os

➢Binding is preferably with a ring of four COs and one water molecule, but binding in a deformed eight-fold CO cavity is also seen

- **The SF narrows in the vicinity of Na^+ ions:**

➢The cylindrical geometry of the SF, nearly ideal for K^+, is distorted with local contraction around Na^+, releasing ligands not involved in Na^+ coordination and leading to occlusion of the permeation pathway

➢Strong binding to Na^+ induces local collapse of the filter

Acknowledgements

Prof. Peter Jordan

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