BIOSCIENCE RESEARCH FOUNDATION

Ion Discrimination by Nanoscale Design

water tower: www.pbase.com/mescaleroman/images/57217408

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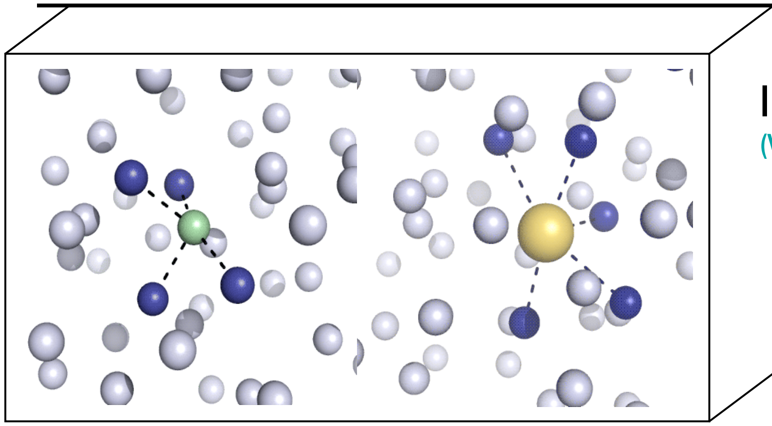
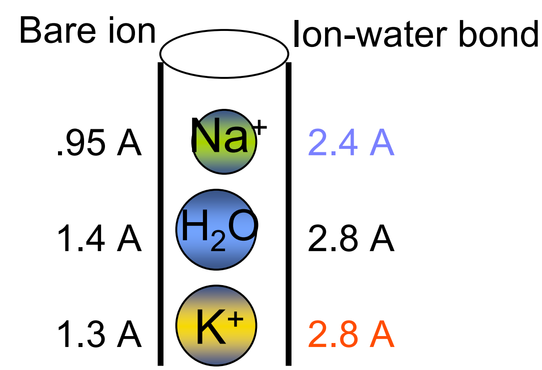
Sandia National Laboratories

Natural protein channels: Not just simple holes!



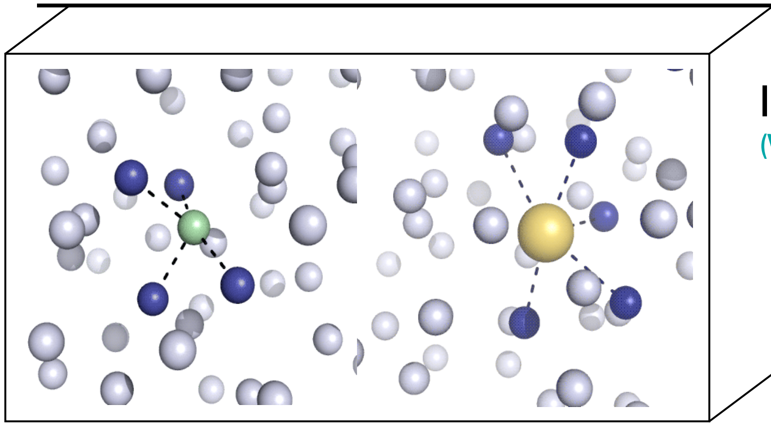
Ions in water
(Varma & Rempe, 2007)

- Ions are 'happy' **coordinated** with water **ligands** in liquid water



- K^+/Na^+ exquisite discrimination:
  - same charge
  - same size at kT
  - larger ion transported, fast!

Natural protein channels: Not just simple holes!



Ions in water
(Varma & Rempe, 2007)

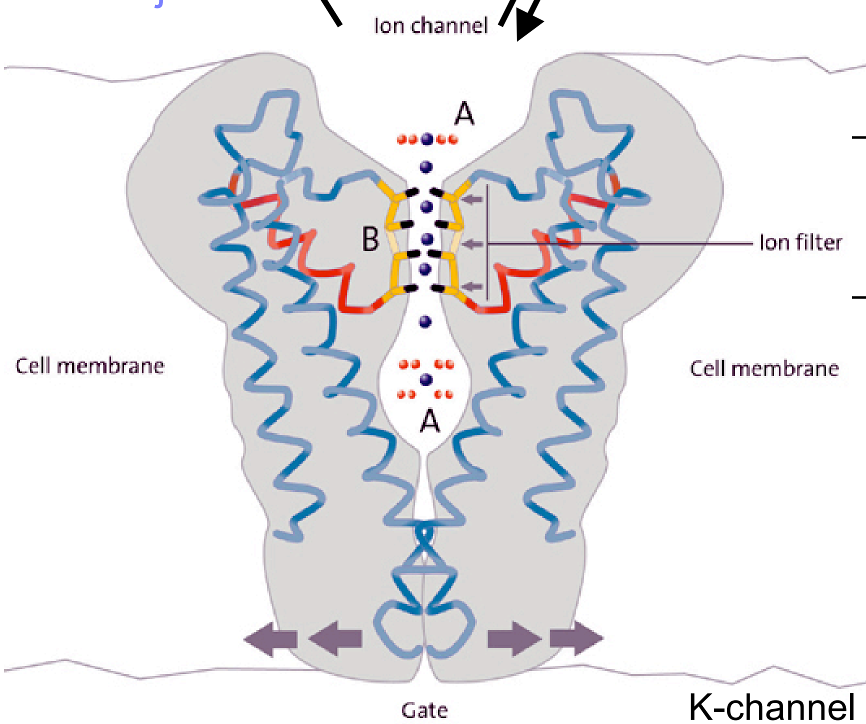
- Ions are 'happy' **coordinated** with water **ligands** in liquid water

Unresolved Questions:

- How do channels work?

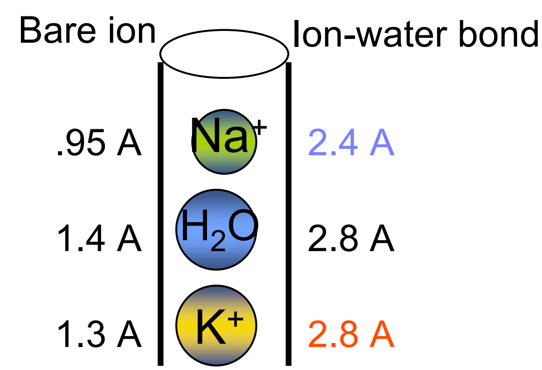
Smaller Na^+ ions rejected

Larger K^+ ions permeate fast



4 nm x
2.8 A radius

K-channel molecule
(www.nobelprize.org)



- K^+/Na^+ exquisite discrimination:
  - same charge
  - same size at kT
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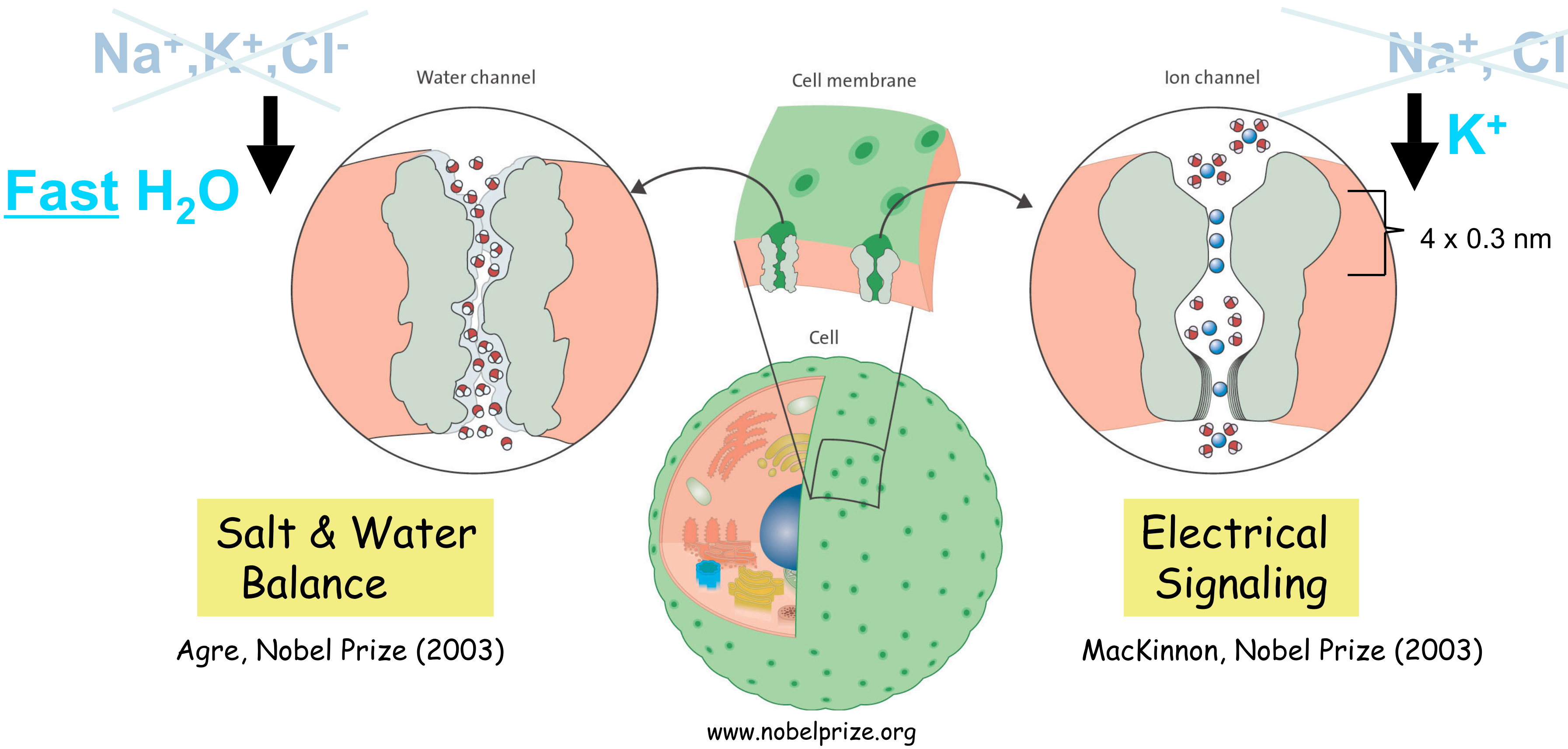
Natural channel proteins:
Small dimensions, large biological impact



Channels are fundamental to life: severe Health consequences if disrupted.

Nobel Prizes underscore Health significance of Channels….

Time and Nobel Prize Field	Laureates	Specific Research
1963 Nobel Prize in Medicine	Alan L. Hodgkin, Andrew F. Huxley	Ion mechanisms of nerve impulse
1985 Nobel Prize in Medicine	Michael S. Brown, Joseph L. Goldstein	Regulation of cholesterol metabolism
1988 Nobel Prize in Chemistry	Johann Deisenhofer, Robert Huber, Hartmut Michel	Structure of photosynthetic reaction center
1991 Nobel Prize in Medicine	Erwin Neher, Bert Sakmann	Function of single ion channels
1997 Nobel Prize in Chemistry	Jens C. Skou	Membrane-bound turnover of ATP
1999 Nobel Prize in Medicine	Günter Blobel	Principles of protein compartmentalization
2003 Nobel Prize in Chemistry	Roderick MacKinnon, Peter Agre	Molecular structural analysis of membrane channels, existence of water channels
2004 Nobel Prize in Medicine	Richard Axel, Linda B. Buck	Odorant receptors

Biederer, *course: biological membranes*, 2005

Global Problem: Desalination

Clean water: a global precious commodity

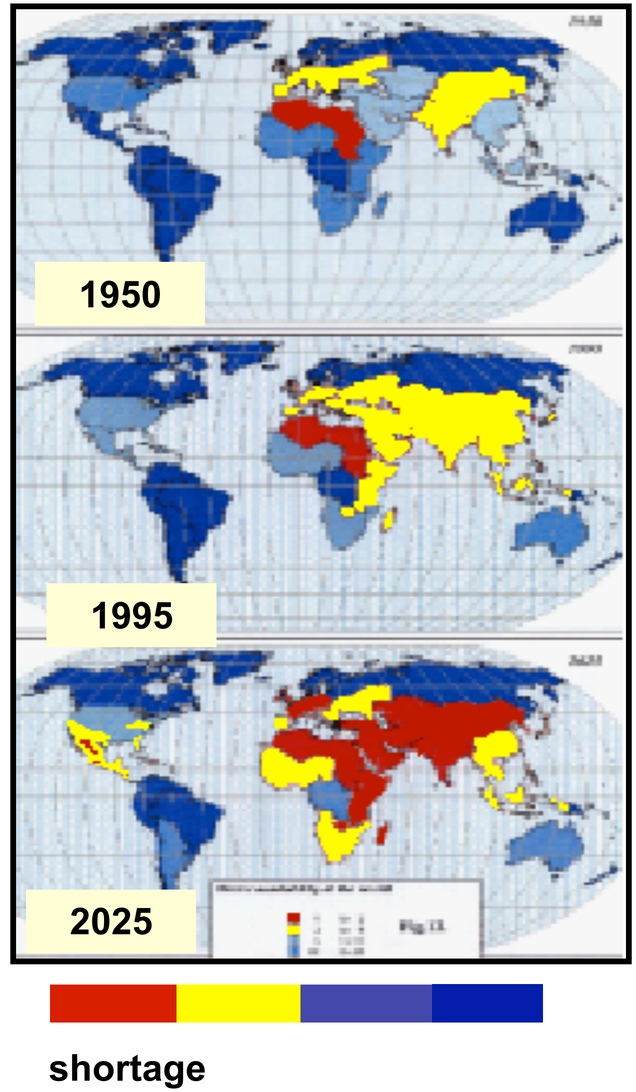
- water is recyclable
- but RO is expensive, produces sterile water

Salinity Levels:		
Seawater:	~35 g/l	(0.6 M)
Brackish:	~1-5 g/l	(0.08 M)
Potable:	<0.5 g/l	(0.008 M)

Efficient membranes: critical challenge

- fast (barrierless) water transport
- select ion exclusion (mineral water)

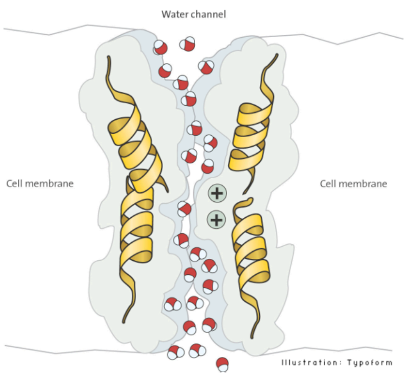
"Water promises to be to the 21st century what oil was to the 20th century: the precious commodity that determines the wealth of nations."
Fortune Magazine, May 15, 2000



Efficient Membranes:

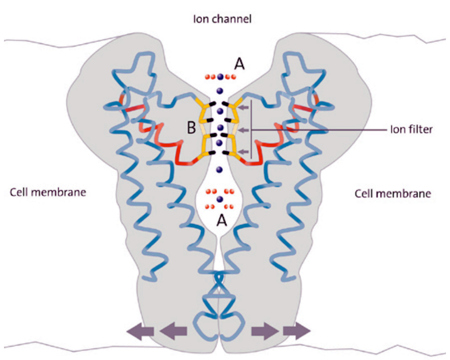
Understand, design, engineer nano-channels for desalination

Water channels

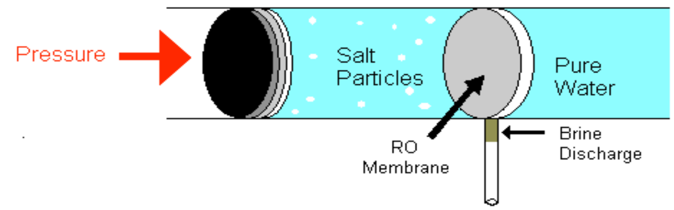


- transport H_2O fast

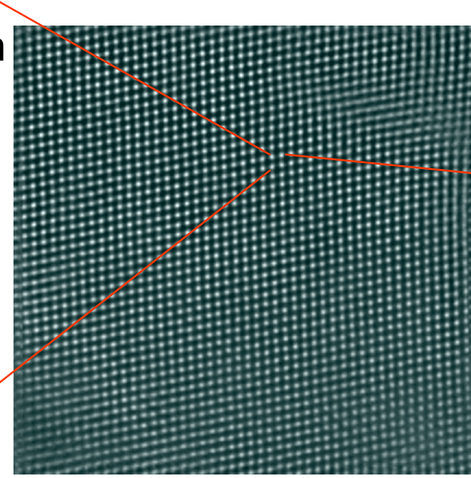
Ion channels



- transport select minerals



- Bio-inspired design
- Theory & modeling



Brinker Lab

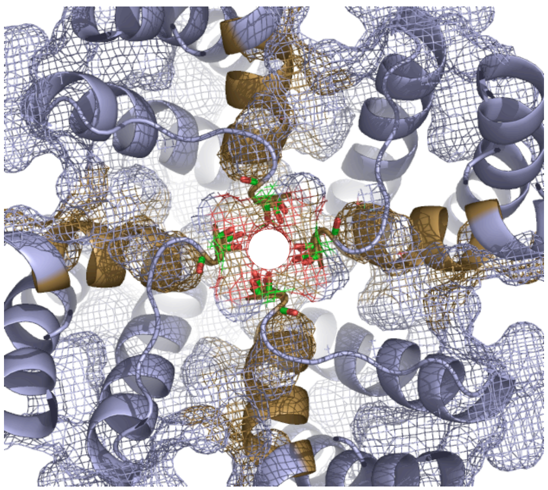
- Inorganic membrane synthesis

Solution: Harness molecular biomechanisms.
Gain 10x in water flux + minerals.

What parameters do we give our engineers?

Natural Channels

4 x 0.3 nm
Active site

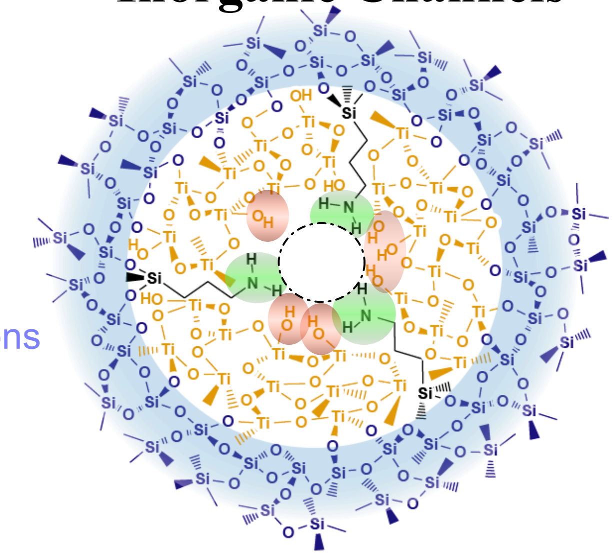


Bio mechanisms

→

Engineering solutions

Inorganic Channels



Critical Channel Design Issues:

What's significant about **(1)** Mouth?

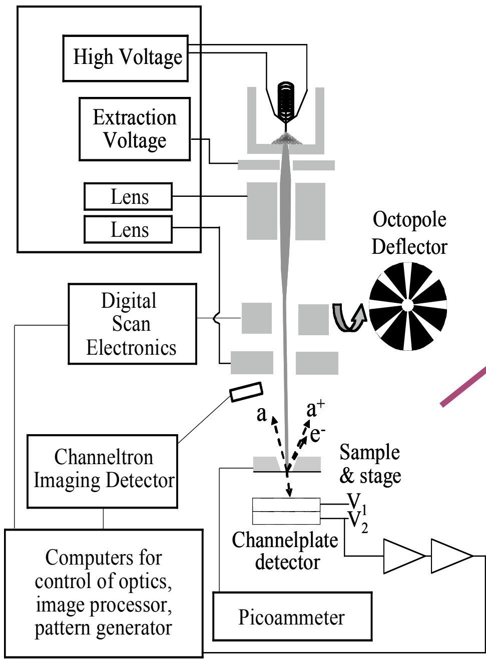
(2) Narrow filter?

(3) Chemistry, architecture?

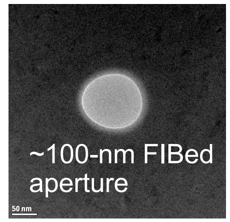
Subtle, challenging questions demand molecular precision:

→ **Molecular Modeling** + Molecular Synthesis

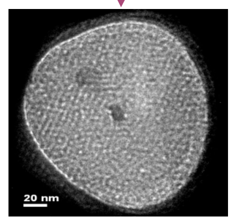
Platforms for Experiments & Modeling



FIB experimental set-up



EISA



PA-ALD

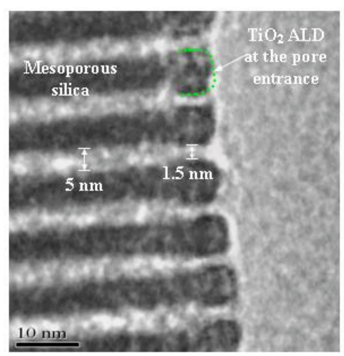
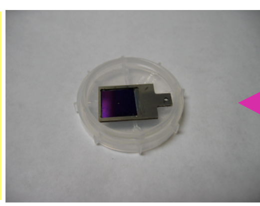
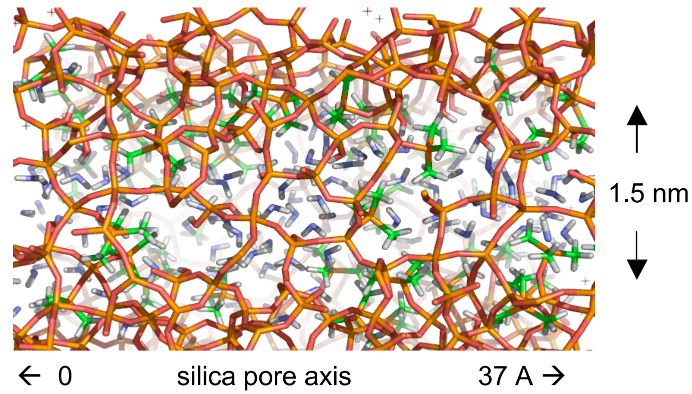


Fig. 1a

Special holder device
•TEM observation
•Patch Clamp
•PA-ALD
•Iterate



• Multiscale modeling essential to understand combined effects of pore size, structure, chemistry and charge.



- Classical (large, long times)
- Quantum (accuracy)
  - Thermodynamics (work)
  - Dynamics (transport)

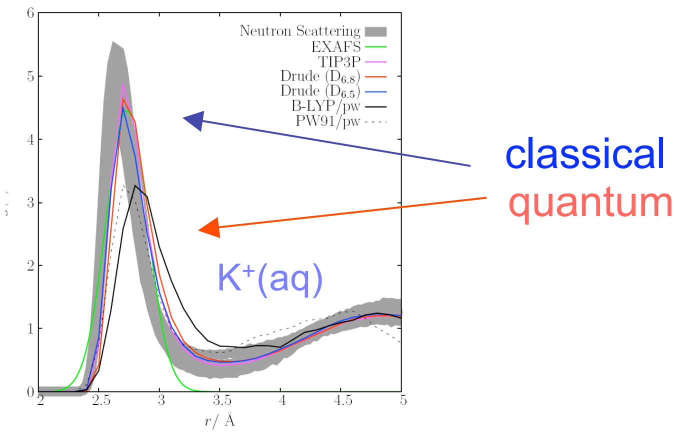
• Experimental platform allows successive modification (pore size, and surface chemistry), imaging and transport measurements on identical sample.

Modeling Approach:
Molecular + quantum accuracy

- Quantum (ab initio) interactions:
  - expensive; describe complex interactions (ex. ion-ligand chemical bonds)
- Classical molecular interactions:
  - inexpensive; simplified, parameterized

• Example 1

More ligands bind classical ions??

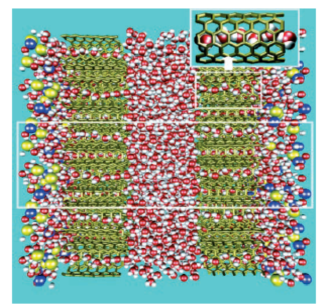


Whitfield, Rempe, *et al. JCTC (2007)*

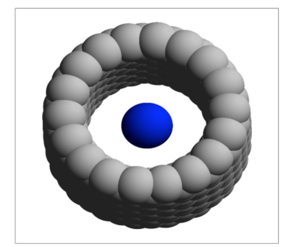
• Example 2

Classical nanotubes Exclude ions??

Quantum tubes Admit ions!!



Hummer & co *PNAS* (2003)

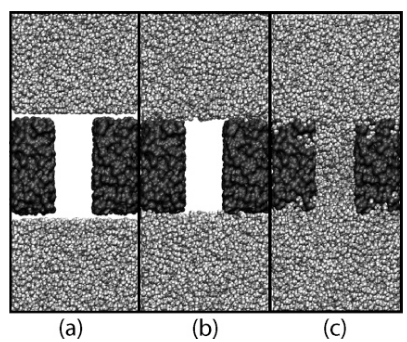


Na+ : -210 kJ/mol

Leung, Marsman *JCP* (2007))

• Example 3

<10% change in classical parameters: Water fills/empties?



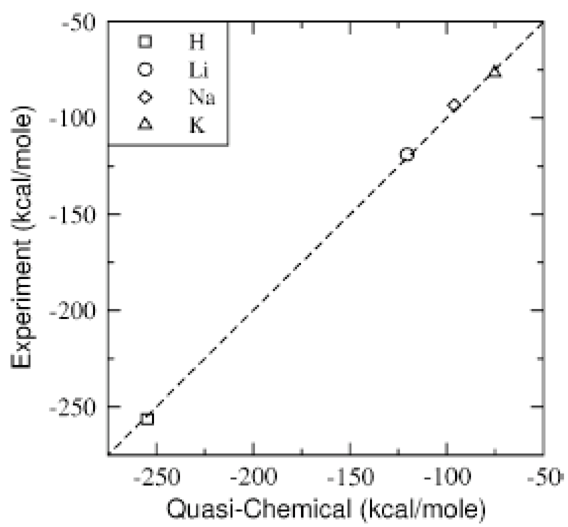
Leung, Rempe, Lorenz *PRL* (2006) channel construction

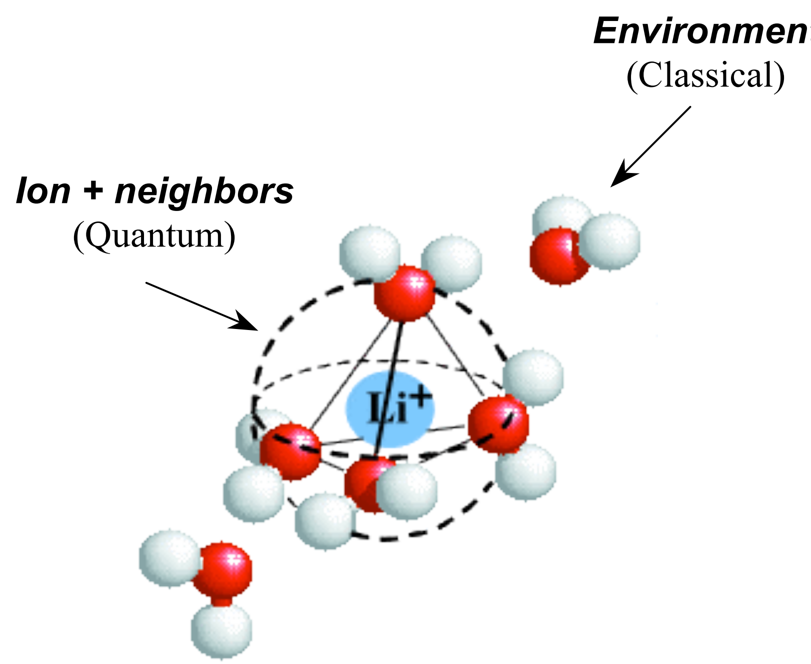
Cruz-Chu *et al JPCB* (2006)

Predictions:
Ion transfer thermodynamics using liquid state theory

- Calculate work to transfer ions (water → channels), efficiently & accurately
  - ’quasi-chemical’ theory[1]

- Theory well-tested for ion hydration:[2]



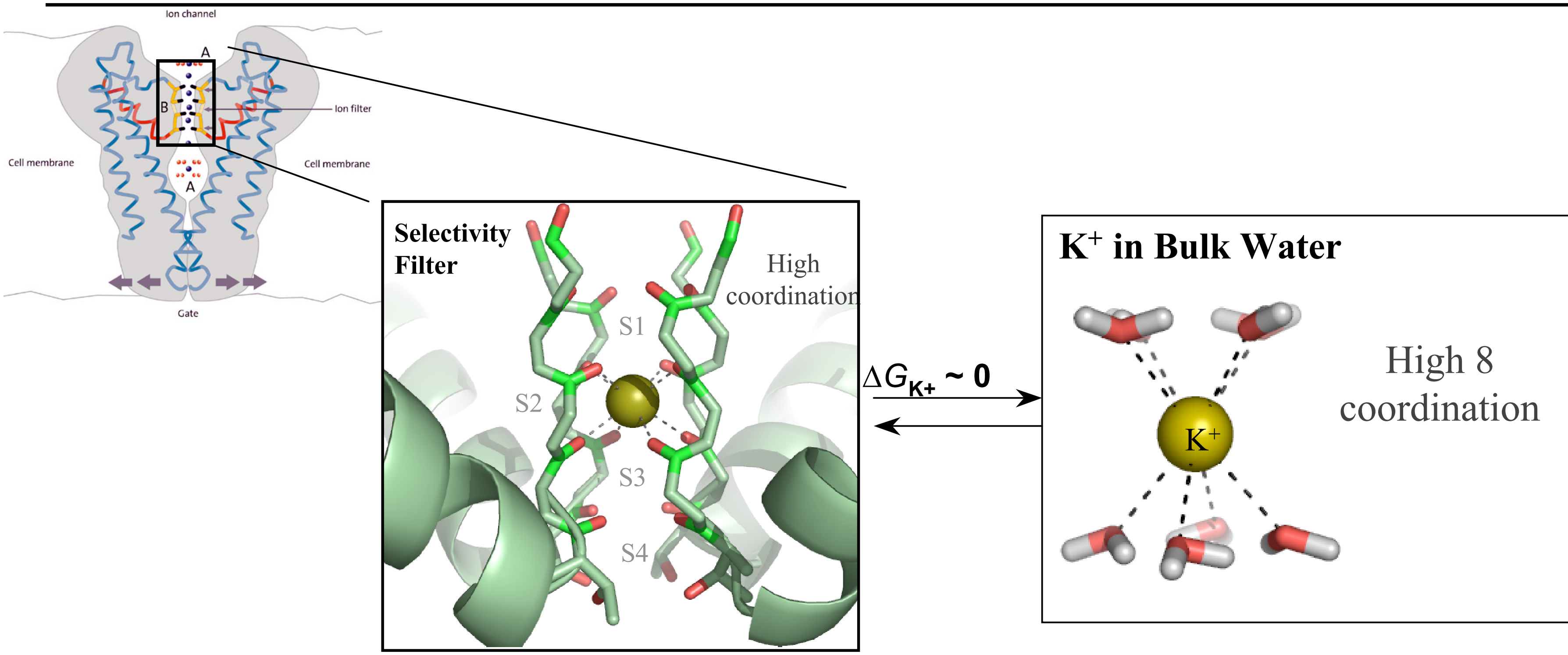


- Apply to Bio/Inorganic channels for Nanoscale Design Parameters[3,4,5]

- [1]Pratt & Rempe (1999); Sabo, Rempe, *et al JPCB* (2008)
- [2]*JACS* (2004), *PCCP* (2004), *FPE*(2001), *JACS* (2000)
- [3]Varma & Rempe *Biophys J* (2007)
- [4]Varma, Sabo, Rempe *J Molec Bio* (2008)
- [5]Leung, Rempe, Lorenz *PRL* (2006)

K^+/Na^+ Ion Discrimination Problem:

How do K-selective channels work? Prevailing Views

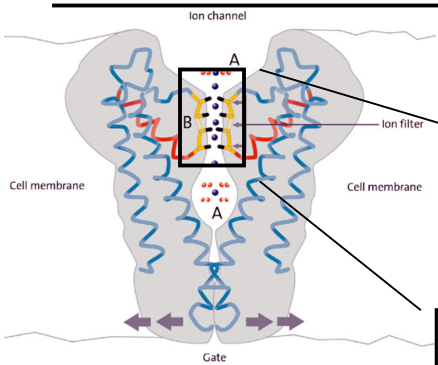


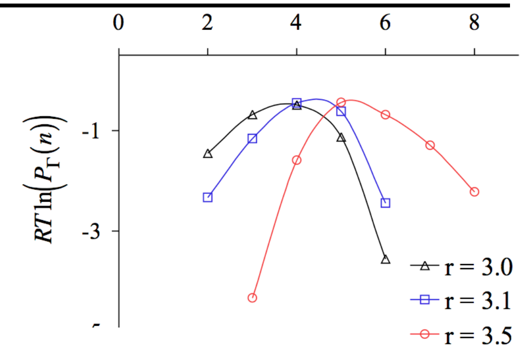
1. Mimic K^+ ion hydration structures for fast K^+ transfer[1,2,3]
2. Specific cavity size fits K^+ [1,2] vs Liquid-like flexibility[3]

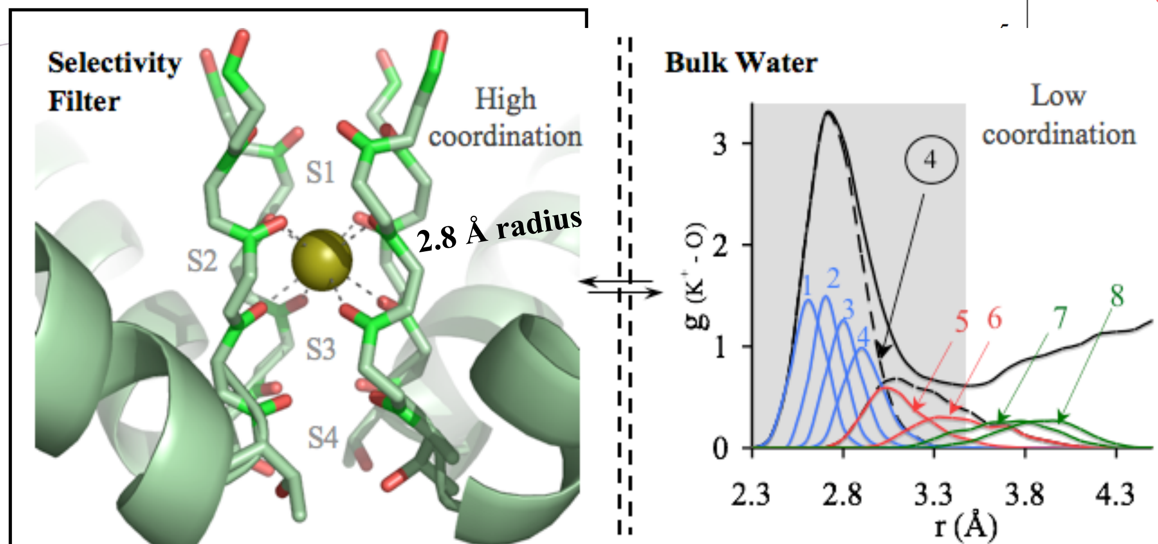
[1]Bezanilla & Armstrong (1972), [2]Zhou *et al.* (2001), [3]Noskov *et al* (2004)

K+/Na+ Ion Discrimination Problem:

How do K-selective channels work? New View #1







1. **Not** Mimic of K^+ ion hydration structures for fast K^+ transfer[1,2]

==> Work avoided by special channel environment (no H-bonders)[3]

[1]Rempe *et al PCCP* (2004), [2]Varma & Rempe *Biophys Chem* (2006), [3]Varma & Rempe *J Molec Bio* (2008)

Special Channel Environment:

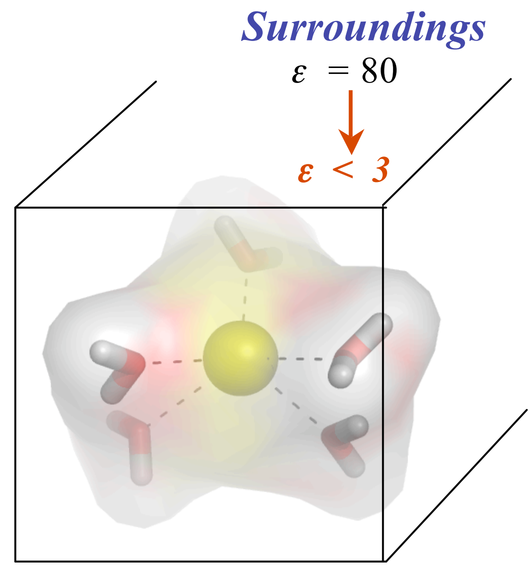
No local competitors for O-ligands stabilizes high ion coordinations

- **Coordination preferences controlled by ligand desolvation penalty**

$$I^+ + n\,H_2O\ (\varepsilon<3) \rightleftharpoons I^+H_2O_n\ (\varepsilon<3)$$

In this **"quasi-liquid"** environment, preferred ion coordination is higher

$K^+ = 8$ $Na^+ = 6$



Special Channel Environment:

No local competitors for O-ligands stabilizes high ion coordinations

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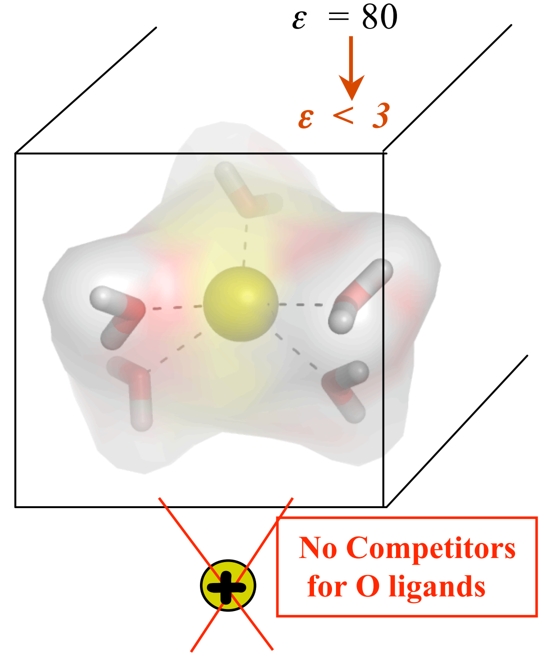
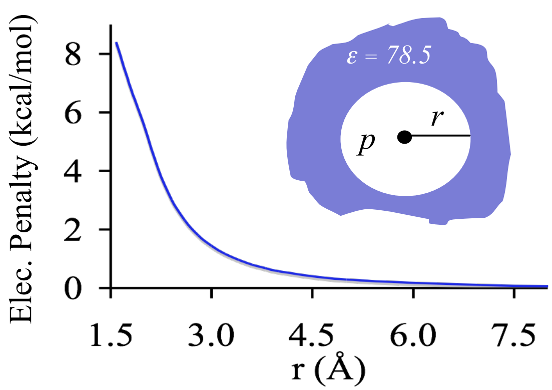
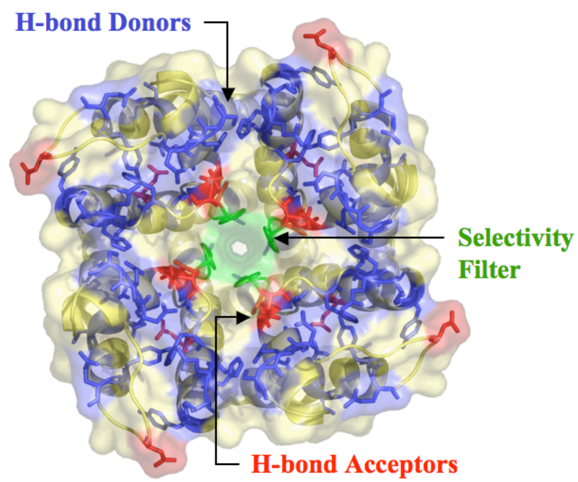
$I^+ + n\,H_2O$ (ε<3) ⇌ $I^+H_2O_n$ (ε<3)



- **Ligand desolvation penalty eliminated locally (6 A)**





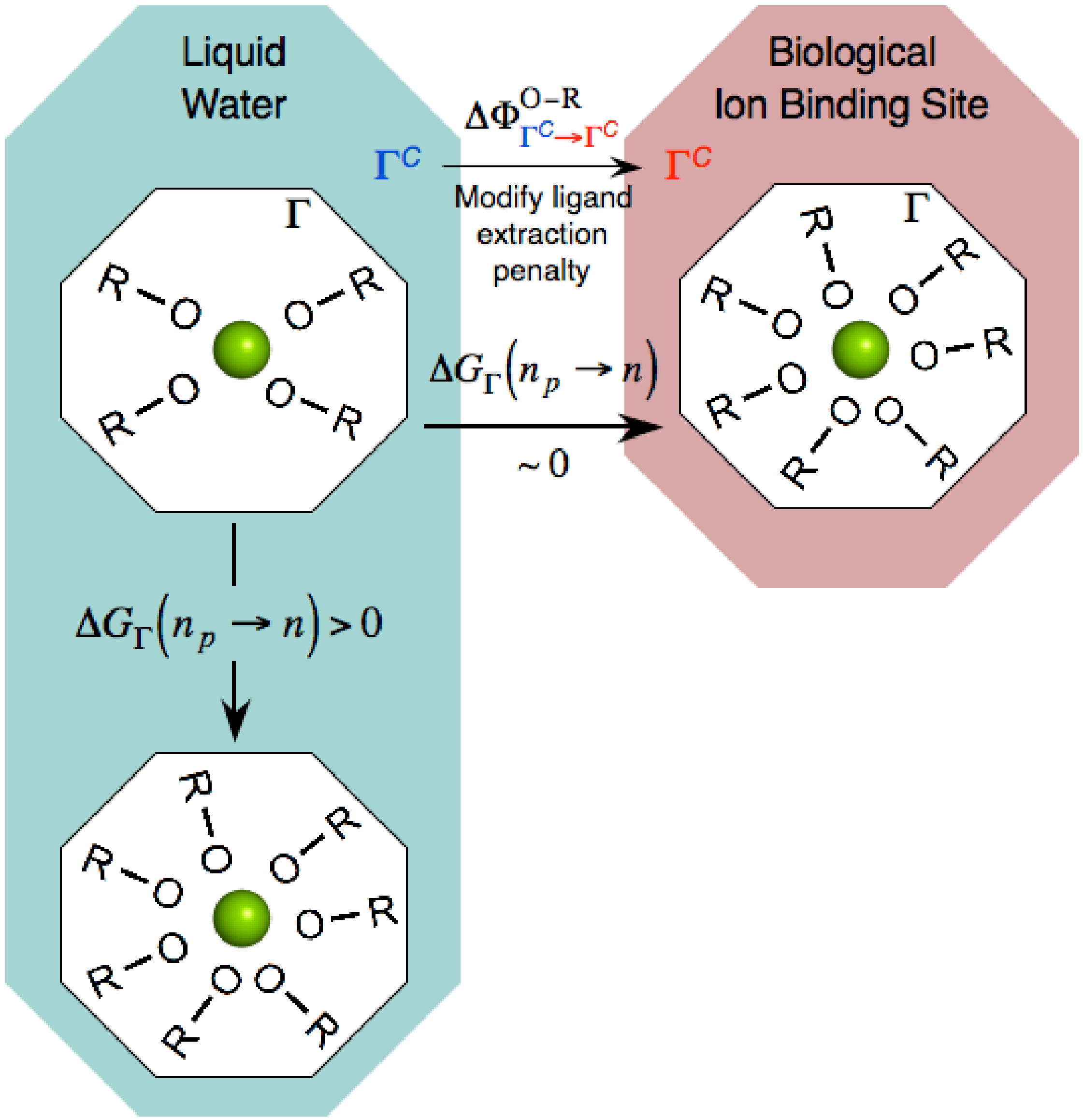
KirBac1.1

(His, Arg, Lys, Gln, Asn, Trp, Tyr, Cys > 6.8 A away from binding site ligands)

- **Highly selective K-channels <u>lack H-bond donors</u>** locally
  - explains how 8 ligands bind K^+ with no work

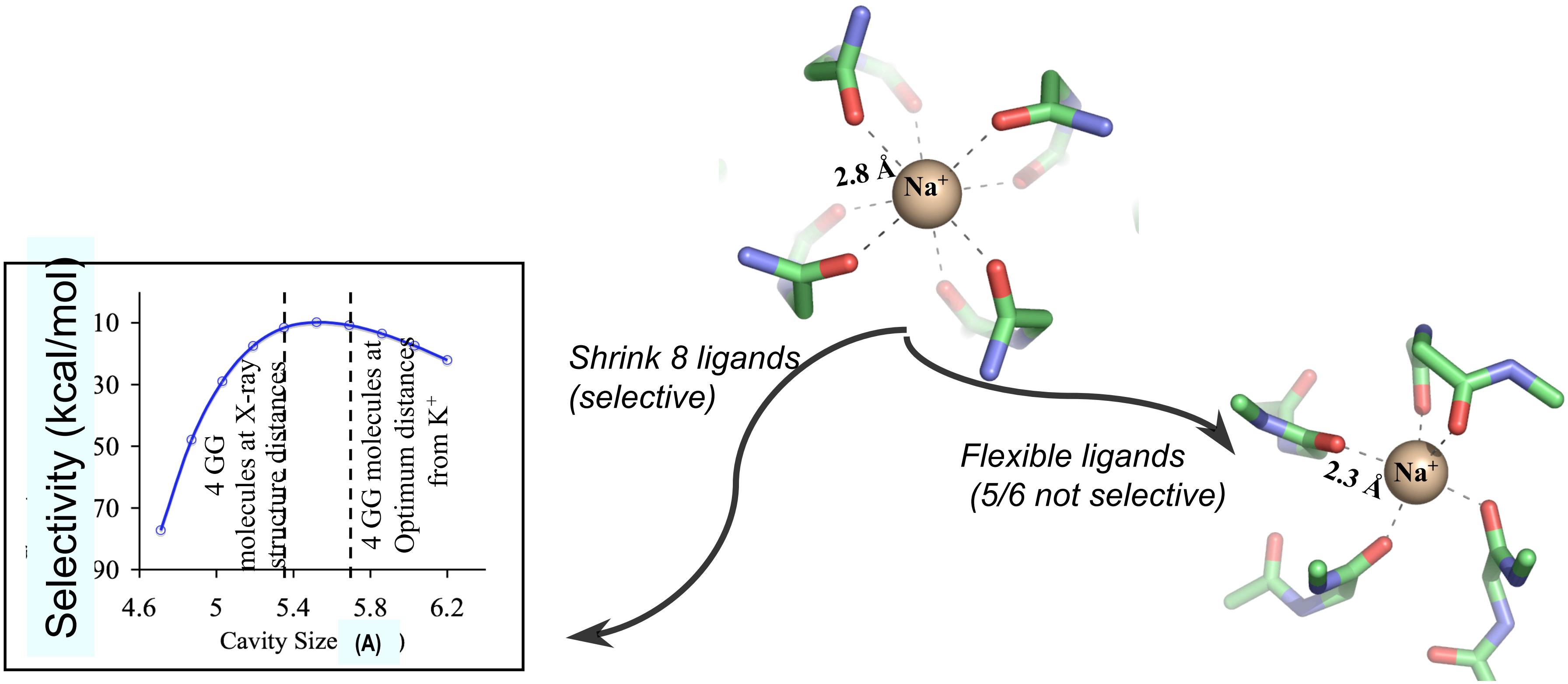
Special Channel Environment:

Structural transitions in ion coordination driven by changes in competition for ligand binding



K⁺/Na⁺ Ion Discrimination Problem:

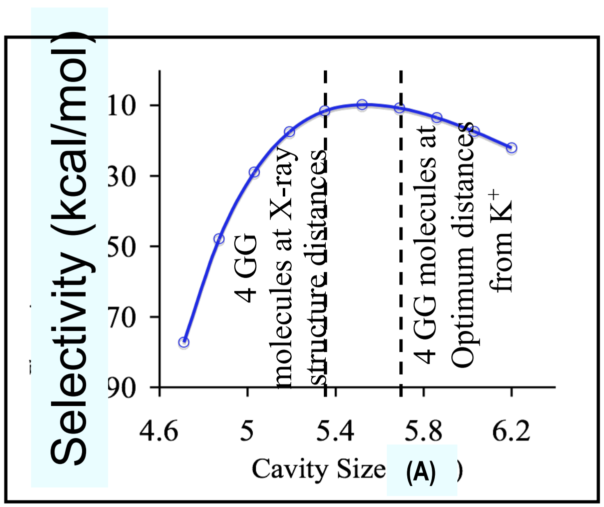
How do K-selective channels work? New View #2

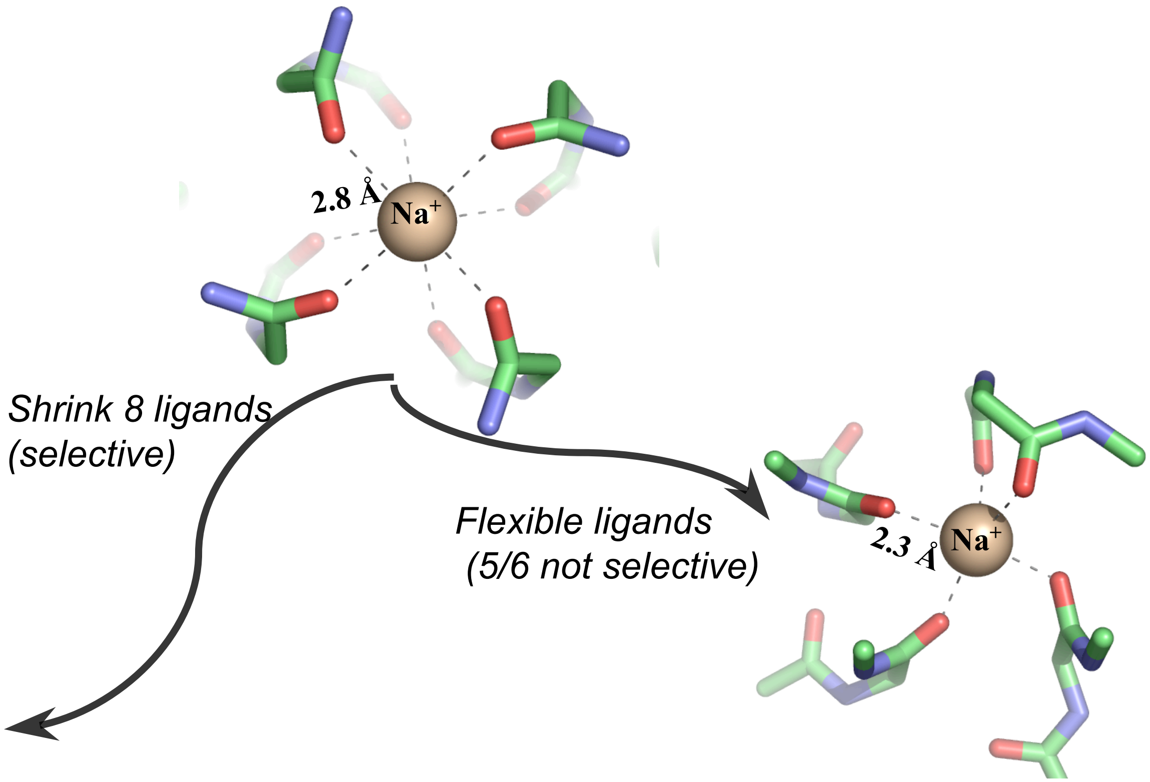


1. Cavity size important
2. Flexibility important

K+/Na+ Ion Discrimination Problem:

How do K-selective channels work? New View #2





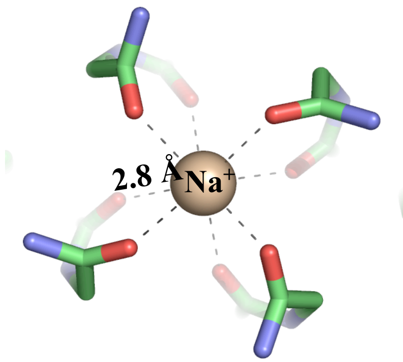
1. Cavity size important, but specific size not necessary
2. Flexibility important, but can reduce ligands & eliminate selectivity

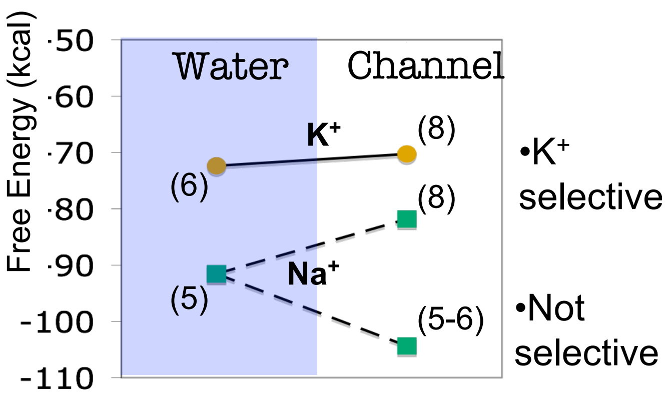
==> Rigidity[1-5] important to maintain Over-Coordinated[1-4] ions (>6 ligands)

[1]Varma & Rempe *Biophys J* (2007); [2]Varma, Sabo, Rempe *J Molec Bio* (2008);
[3]Bostick & Brooks *PNAS* (2007); [4]Thomas *et al. Biophys J* (2007); [5]Asthagiri *et al. JCP* (2006)

Fast K^+/Na^+ discrimination:
Mechanism & Translation Strategy #1

Natural Channels[1]





Nanoscale design parameters (natural channel):

"The caress of the surroundings,[2] the crowding of the ligands"

[1]Varma & Rempe *Biophys J* (2007); [2]Jordan *Biophys J* (2007); [3]Jiang, Brinker, *et al. JACS* (2006)

Fast K+/Na+ discrimination:

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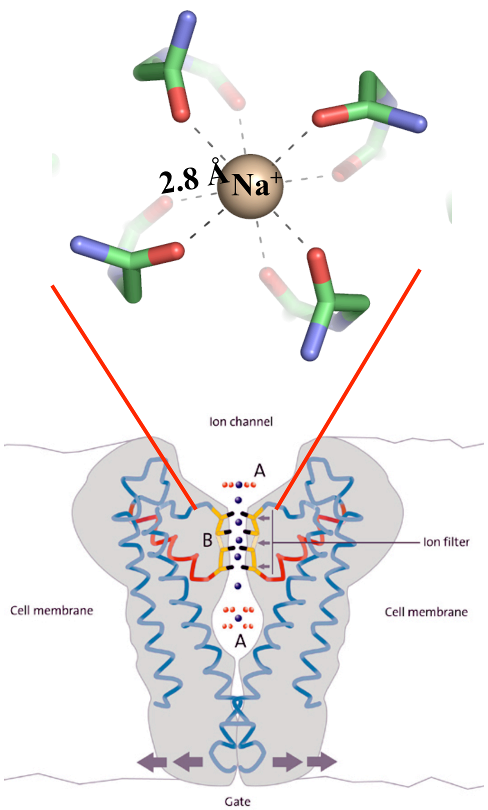
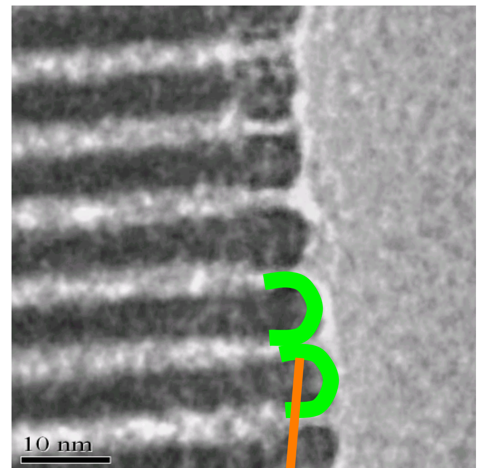


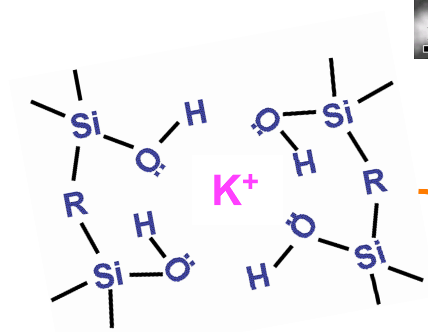
Bio mechanisms

Engineered Mimicry

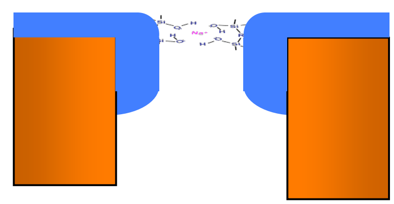
Inorganic Channels



4 nm x 1.5 nm



•[3]New ALD + molecular imprinting?



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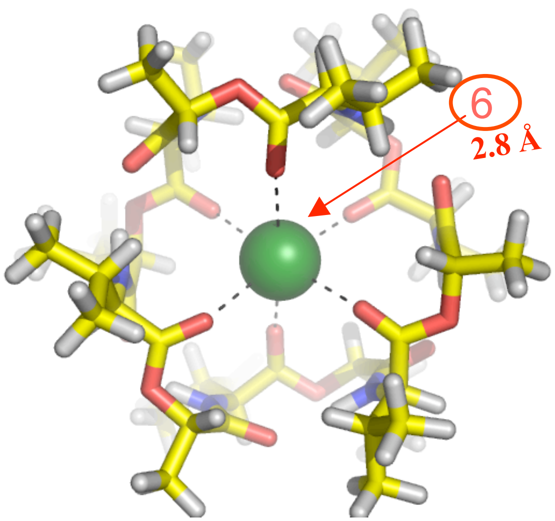
"The caress of the surroundings,[2] the crowding of the ligands"

[1]Varma & Rempe *Biophys J* (2007); [2]Jordan *Biophys J* (2007); [3]Jiang, Brinker, *et al. JACS* (2006,2007)

Fast K^+/Na^+ discrimination:

Mechanism #2

Natural K-selective Molecule

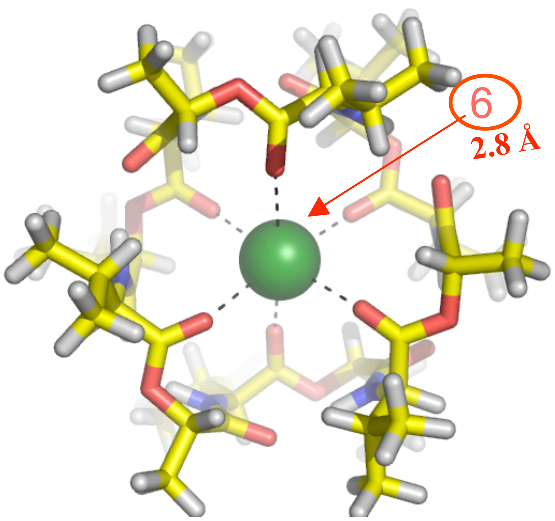


- **6 C=O ligands, not >6?**

[1]Varma, Sabo, Rempe *J Molec Biol* (2008)

Fast K^+/Na^+ discrimination:
Mechanism #2

Natural K-selective Molecule



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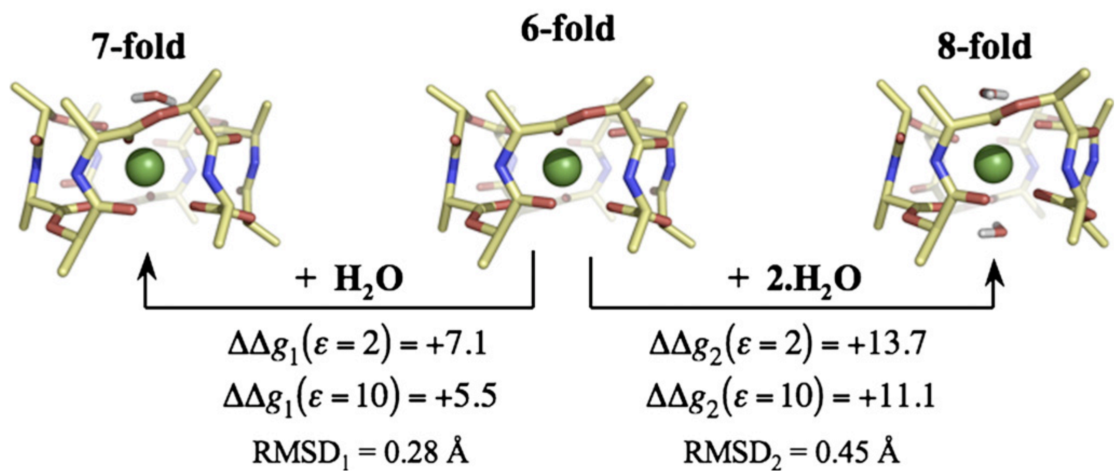


$$[K^+.Val](\varepsilon) + n.H_2O(aq.) \xrightarrow{\Delta\Delta g_n(\varepsilon)} [K^+.Val.(H_2O)_n](\varepsilon)$$

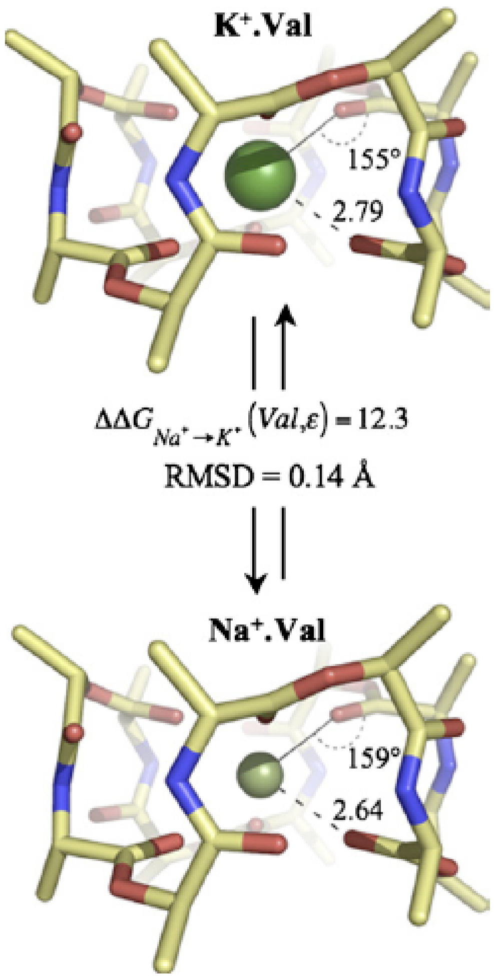
ΔΔgn(ε): change in complexation energy for 2 values of ε, which represent dielectric constant lipid membranes. 6-fold coordination is also more stable for higher values of ε, such as ε=80 . $RMSD_n$ reflects change in backbone structure of K^+-bound valinomycin due to complexation by *n* water molecules.

[1]Varma, Sabo, Rempe *J Molec Biol* (2008)

Fast K^+/Na^+ discrimination:

Mechanism #2

Natural K-selective Molecule



Optimized structures (DFT/B3LP).

- **Special C=O chemistry?**

Table 1. Computed (DFT/B3LYP) structural and thermochemical properties of 6-fold ion-oxygen clusters in gas phase

Ligand	Chemistry	$\Delta\Delta G_{Na+ \to K+}$ ($\varepsilon = 1$) (kcal/mol)	Na^+-O	K^+-O
Valinomycin	Carbonyl	12.3	2.64	2.79
Formamide[20]	Carbonyl	20.6	2.42	2.80
Glycine Dipeptide[20]	Carbonyl	20.8	2.43	2.76
Water[20]	Hydroxyl	18.8	2.42	2.84
Methanol	Hydroxyl	18.5	2.44	2.84

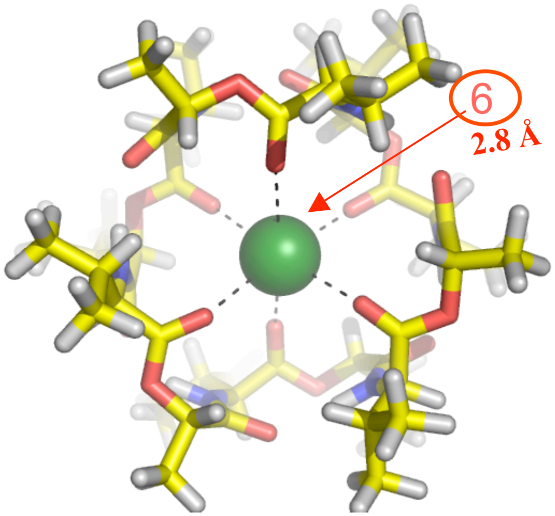
Ion–oxygen distances Na^+-O and K^+-O are in ångström units and $\Delta\Delta G_{Na^+ \to K^+}$ ($\varepsilon = 1$) are the free energy differences between the Na^+ and K^+ complexes in gas phase.

[1]Varma, Sabo, Rempe *J Molec Biol* (2008)

Fast K+/Na+ discrimination:

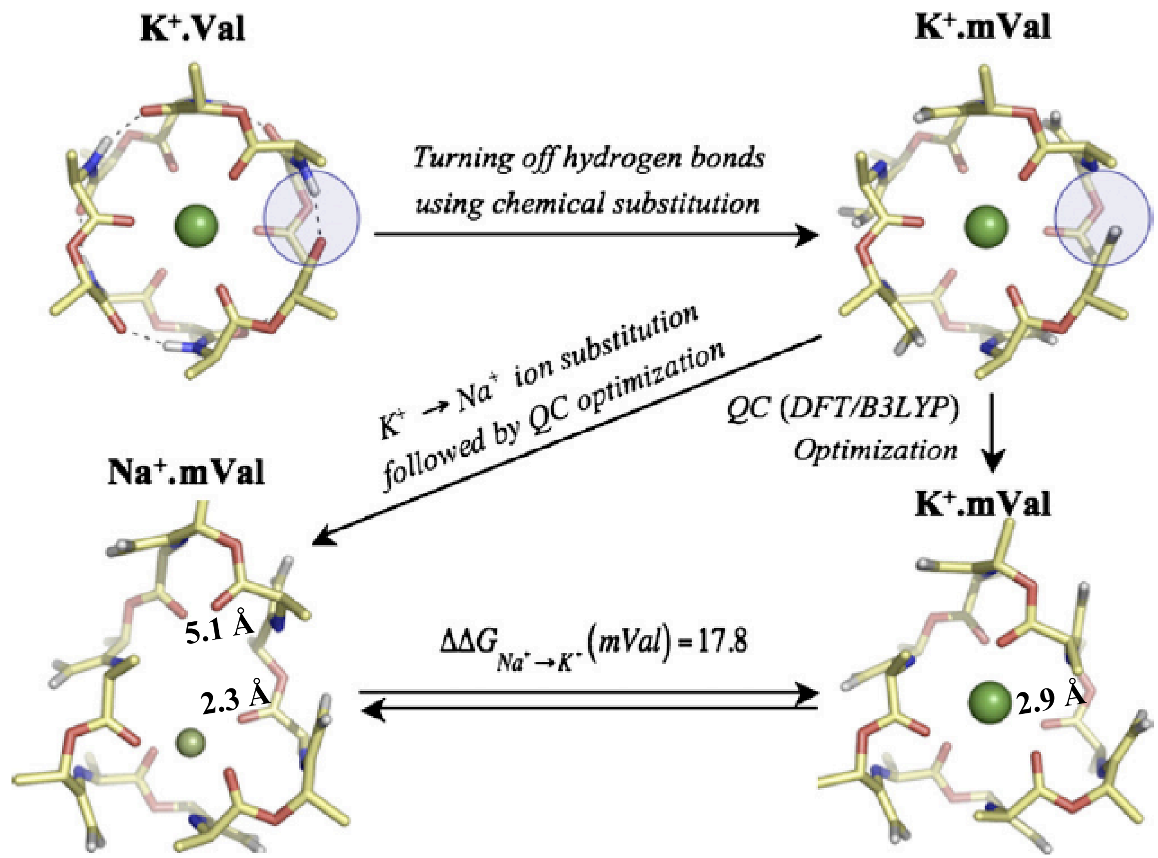
Mechanism #2

Natural K-selective Molecule



[1]Varma, Sabo, Rempe *J Molec Biol* (2008)

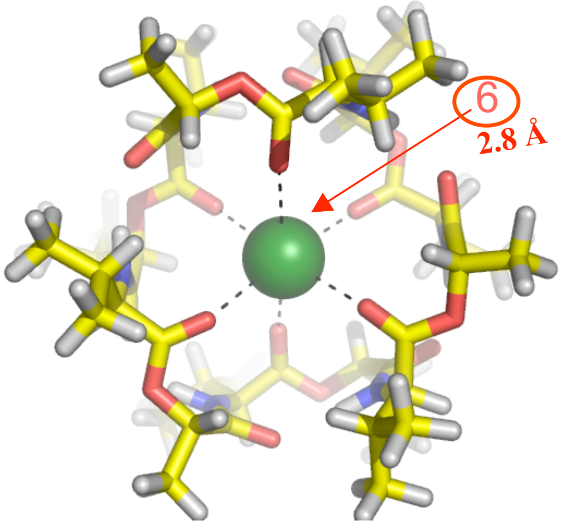
- **H-bonds maintain cavity size**



H-bonds turned off by replacing proton acceptor atoms =O with $=CH_2$ groups. QC optimization results in small K+ complex changes, but large changes in Na+ complex. Absence of H-bonds also increases free energy difference between Na^+ & K^+ complexes from 12.3 to 17.8 kcal/mol, thus reducing K^+/Na^+ selectivity relative to liquid water.

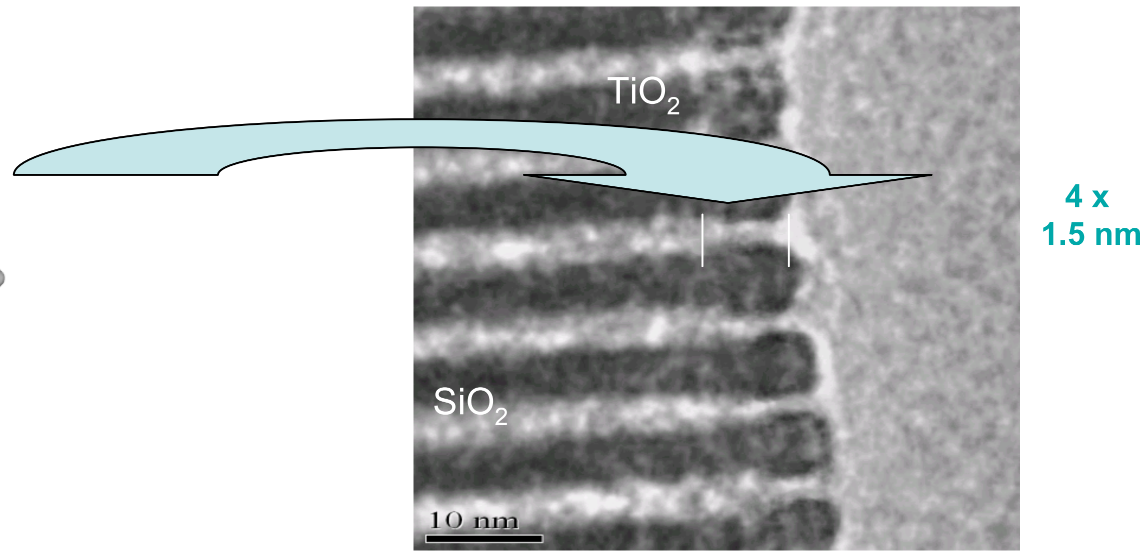
Fast K^+/Na^+ discrimination:
Mechanism & Translation Strategy #2

Natural K-selective Molecule



- **Specific cavity** fits K^+, not Na^+
- Less selective in water

Inorganic Channels



- **Ion-selective aperture?**

- **Outstanding questions:**
  - Queue of ion binding sites?
  - Avoid block?
  - Fast water transport + ion selectivity?

[1]Varma, Sabo, Rempe *J Molec Biol* (2008)

Fast size discrimination:

Translated (Fabricated) by Atomic Layer Deposition

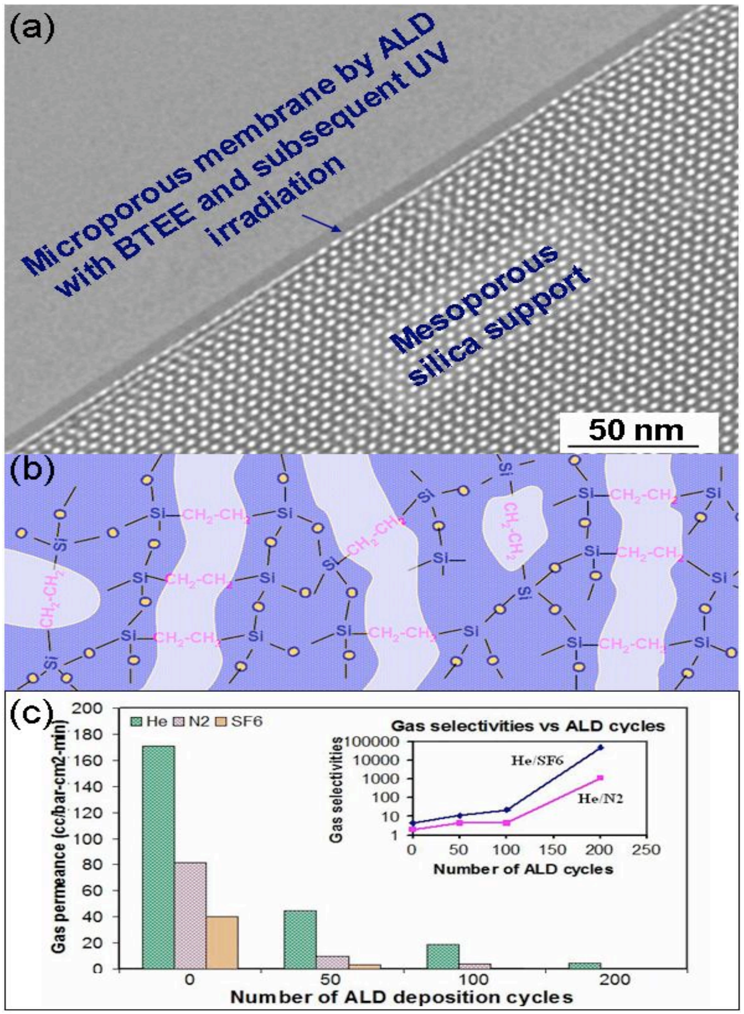


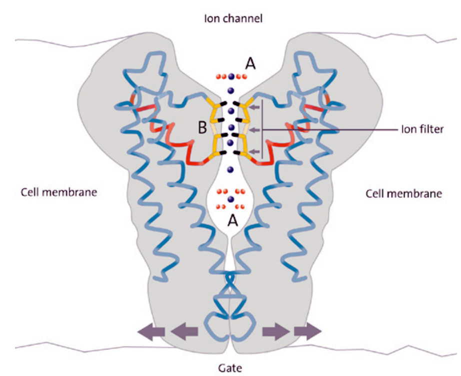
Fig. 1 a) TEM: micropore membrane self-assembled on mesopore support;
b) UV/ozone removes C_2 bridging ligands (pore templates), from dense hybrid film;
c)gas permeance vs ALD cycle; after 200 cycles, N_2 excluded/He transports;
thus pore size ~0.3 nm.

- [1]biomimetic pores (**0.3 nm diameter**) via new ALD & molecular templating

  - high flux + high size-selectivity (He/N_2)

- template-based approach for uniform molecular-sized pores established

- mimic biological K^+/Na^+ selectivity?

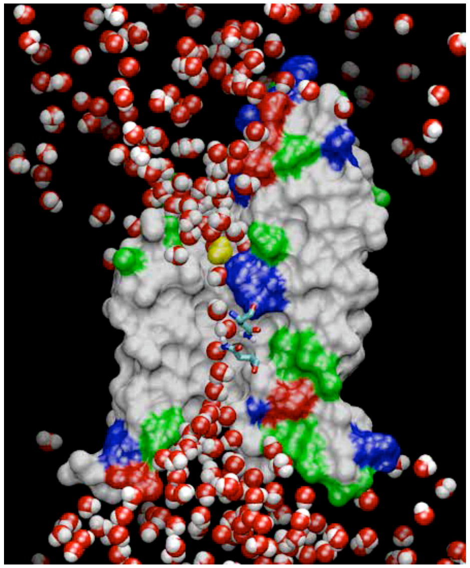


4 x 0.3 nm
Active site

[1]Jiang, Brinker, *et al JACS*, 2007

Summary:
Nanoscale Channel Structures for Big Problems

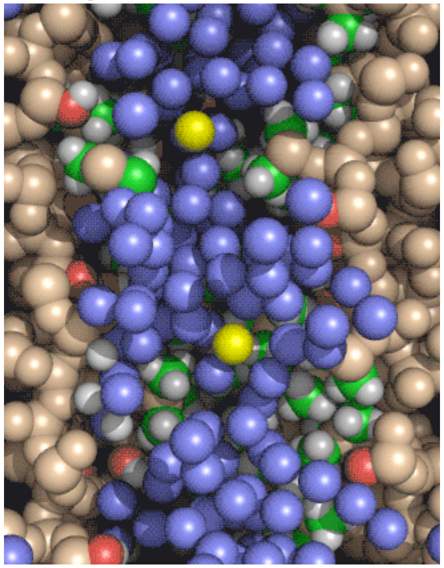
Natural Water Channel



(Tajkhorshid & co)

Bio mechanisms → Engineering solutions

Inorganic Water Channel



(Desal Team, Sandia)

- Channels: molecular structures & subtle, important functions
- Solve problems:
  - **Health** (medicine + biodefense)
  - **Nanomedicine** (smart dialysis)
  - **Water-Energy** (mineral water, efficiently)

Biology ↔ inorganic nanostructures
Quantum Modeling ↔ experiments

Acknowledgements

- **Funding**
  1. DOE: Sandia's Water Desalination Program
  2. DOE: Sandia's LDRD program (BST, ERN)
  3. NIH: National Nanomedicine Center
- **Compute time (~ 300,000 hours cpu)**
  1. Sandia Computing: Thunderbird
  2. National Center of Supercomputing Applications (NCSA), UIUC
- **Collaborative Science, Engineering, Technology Teams**

Desalination Team at Sandia

PI: Susan Rempe

- Jeff Brinker
- Kevin Leung
- Steve Plimpton
- Dubravko Sabo (postdoc)
- Seema Singh
- Sameer Varma (postdoc)
- Ying-Bing Jiang (postdoc)
- Tom Mayer (project manager)

NIH Center for Design of Biomimetic Nanoconductors

(http://www.nanoconductor.org)

PI: Eric Jakobsson, UIUC

Senior Scientists

- Narayan Aluru (UIUC)
- Hagan Baylay (U Oxford)
- Jeff Brinker (SNL)
- Millicent Firestone (ANL)
- David LaVan (Yale)
- Kevin Leung (SNL)
- Steve Plimpton (SNL)
- Atul Parikh (UC Davis)
- Umberto Ravioli (UIUC)
- Susan Rempe (SNL PI)
- Benoit Roux (U Chicago)
- Marco Saraniti (IIT)
- Larry Scott (IIT)

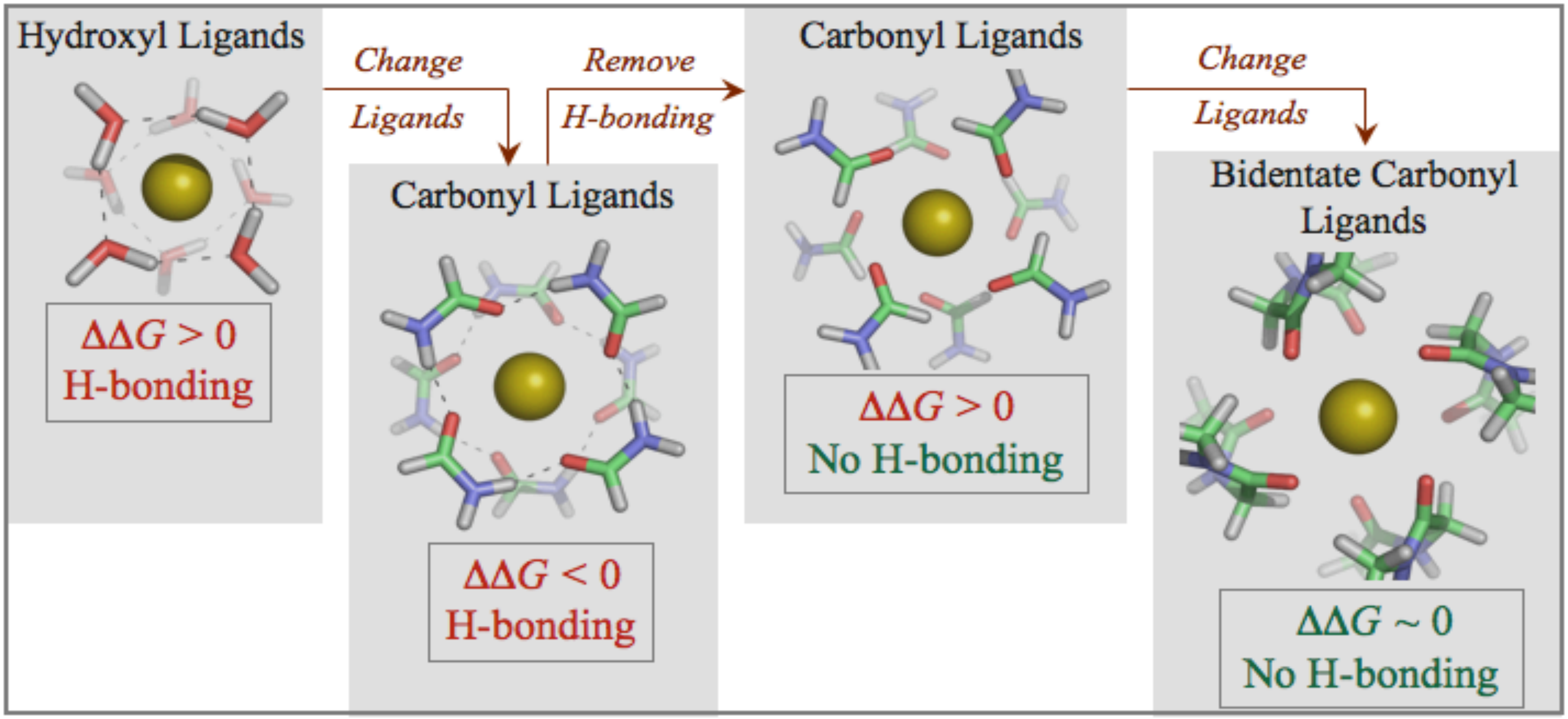
BIOSCIENCE RESEARCH FOUNDATION

Ion Discrimination by Nanoscale Design

Susan Rempe, Sameer Varma, Dubravko Sabo, Kevin Leung, Ying-bing Jiang, Jeff Brinker

Sandia National Labs

K+ transfer from Water into fully flexible K-channel binding sites



- Build molecular models and predict:
  - Optimized complexes; overlay X-ray structure
  - K^+ transfer thermodynamics
  - Ion selectivity

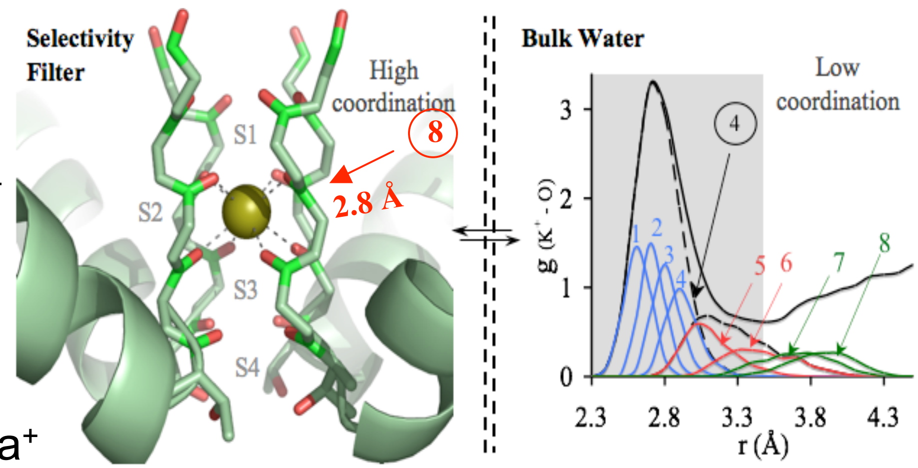
- **Achieve a model that represents known data:**
  - reproduces measured ion channel properties
  - reveals new determinants of selectivity: environment & coordination

K^+/Na^+ Ion Discrimination Problem:

How do K-selective channels work?

Traditional Mechanism

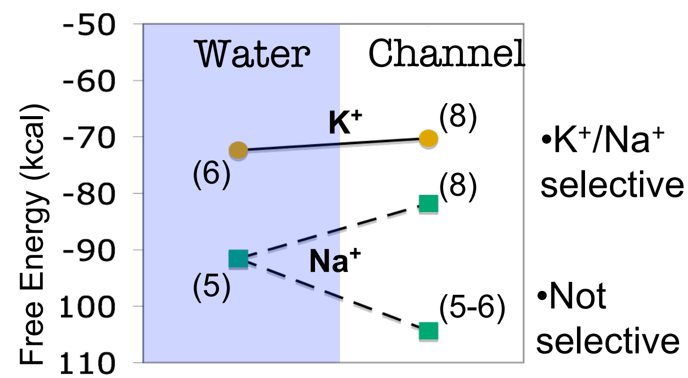
- Ion coordination assumed **fixed**, **mimicked**
- **Specific cavity size** fits permeant K^+, not Na^+



Over-coordination Mechanism

- Ion **coordination linked to environment**
- **Specific** (C=O) **ligand number '**fits' K^+, not Na^+

- "The caress of the surroundings, the crowding of the ligands." Jordan (2007), New & Notable *BJ*



Impact

- **New explanation** of K-channel selectivity
- **Engineering parameters**
- **Health**, **Water**, **Nanoengineering**

Varma & Rempe (2007). *Biophys J*
Varma & Rempe (2006), *Biophys Chem*
Varma, Sabo, Rempe (2008), *J Molec. Bio*