

Ions in biology: water and proteins

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Importance of ions in biology

- ▶ Biological processes need an aqueous medium and are built around chemistry involving **charged macromolecules**¹²³
 - ▶ Requires inorganic ions for electroneutrality

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³ Brenner et al., Curr Opin Chem Biol, 2004

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 - ▶ **Ion channels in excitable cells are a notable example**

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Importance of ions in biology

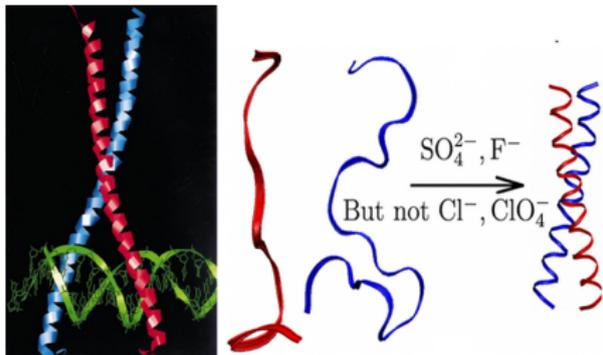
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- ▶ Approximately half of the human genome encodes for proteins that interact with metals
 - ▶ **Metal protein interactions are strong on a thermal energy scale and metal association confers stability to proteins**
- ▶ A general framework to elucidate the role of ion chemistry and charge is lacking

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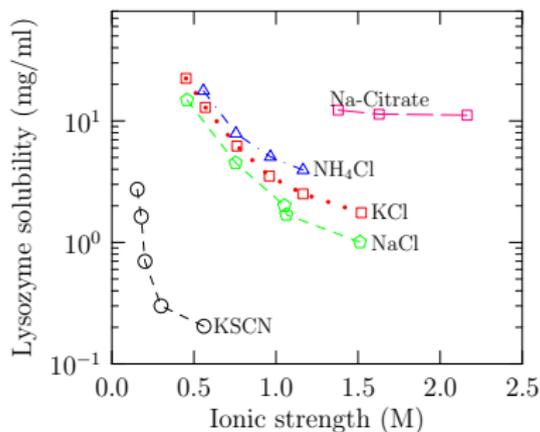
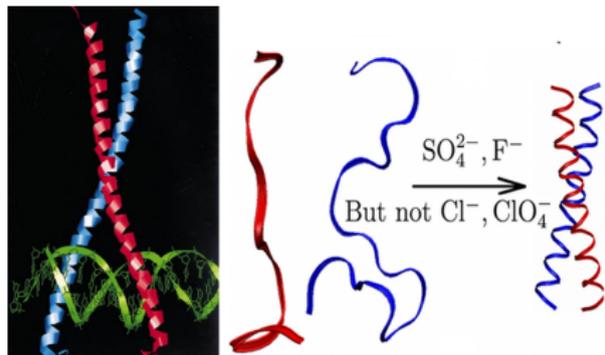
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Ions effects on solubility and structure of proteins



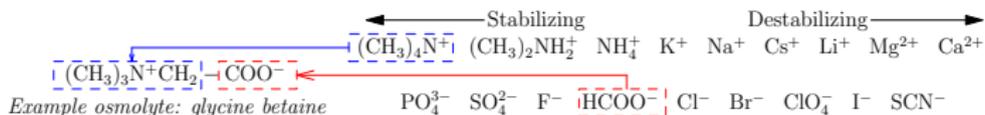
- ▶ These trends cannot be described by only an electrostatic description of ions
- ▶ They reflect the chemical nature of the dissolved ions

Ions effects on solubility and structure of proteins



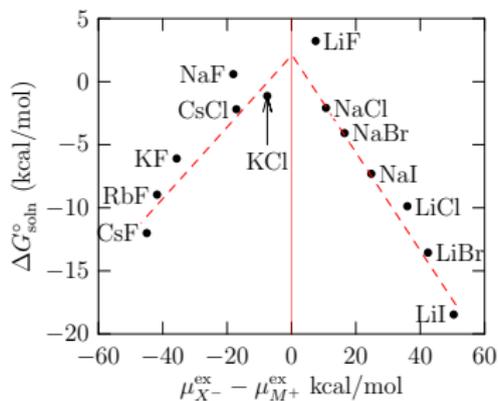
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Hofmeister series



- ▶ Weakly hydrated soft cations and strongly hydrated hard anions are stabilizing
- ▶ Strongly hydrated hard cations and weakly hydrated, soft anions are destabilizing
- ▶ Naturally occurring protein stabilizing osmolytes share motifs with stabilizing anions and cations
 - ▶ Molecular basis for this series is not understood
 - ▶ Potential application is designing buffers to solubilize/crystallize proteins

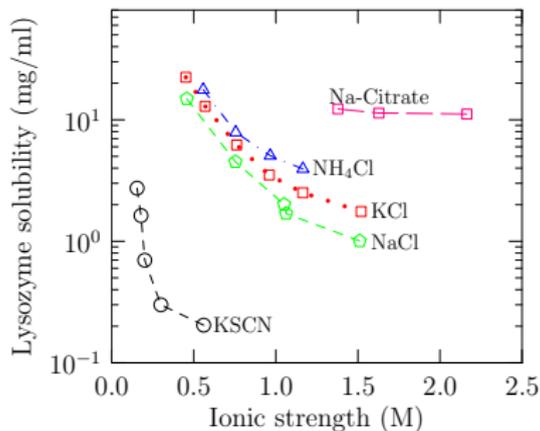
Like likes like



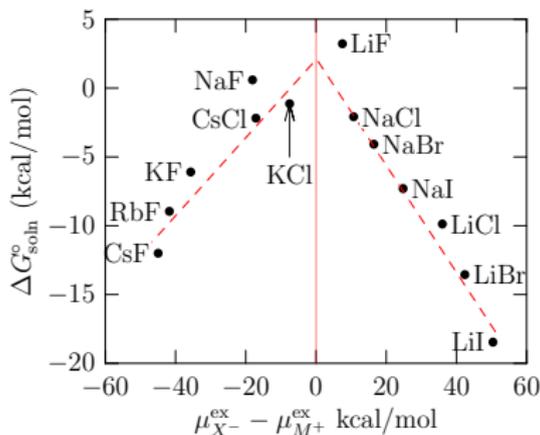
K. Collins, Biophys. J., 72, 65, (1997)

- ▶ Lil more soluble than LiF
- ▶ Na^+ pairs more readily with carboxylate group as compared to K^+
- ▶ Ion pairing is accompanied by local changes in hydration
- ▶ What is the thermodynamics associated with changing an ion's local environment ?

Ion-specific effects



M.M. Ries-Kaut and A.F. Ducruix, *JBC*, **264**, 7539, (1989)

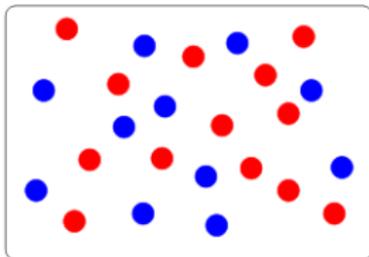


K. Collins, *Biophys. J.*, **72**, 65, (1997)

- ▶ Arise from ions modulating properties of the solvent in chemically specific ways
- ▶ What solute-solvent interactions are chemically specific ?
- ▶ How do these interactions contribute to hydration thermodynamics of the solute ?

Excess chemical potential

Thermodynamic descriptor of hydration

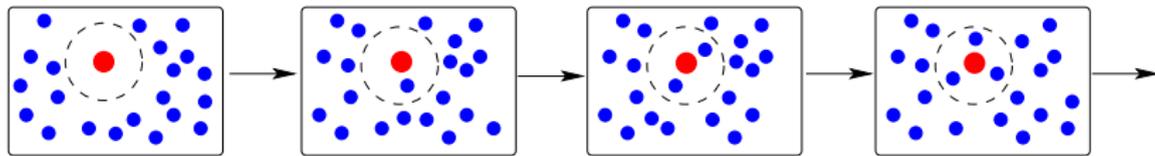


$$G(T, p, N_{\text{blue}}, N_{\text{red}}) = G_{\text{ideal}}(T, p, N_{\text{blue}}, N_{\text{red}}) + N_{\text{blue}} \cdot \mu_{\text{blue}}^{\text{ex}} + N_{\text{red}} \cdot \mu_{\text{red}}^{\text{ex}}$$

- ▶ $\mu_{\text{blue}}^{\text{ex}}, \mu_{\text{red}}^{\text{ex}}$ account for energetic interactions of the particles

Molecular aufbau approach

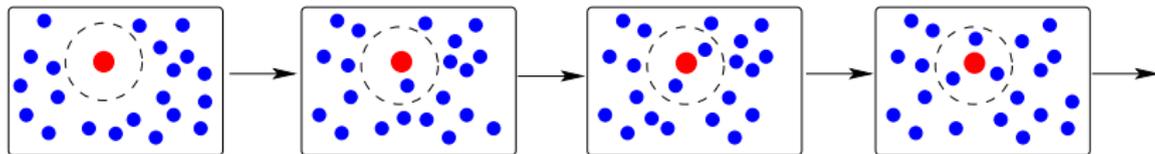
Merchant and Asthagiri, JCP, **130**, 195102, (2009)



- ▶ Define an inner shell around the ion
 - ▶ Imposing a spatial separation helps separate energies
 - ▶ Inner shell interactions will be chemically involved
- ▶ Populate the inner shell one solvent molecule at a time and assess its contribution to the inner-shell chemistry

Molecular aufbau approach

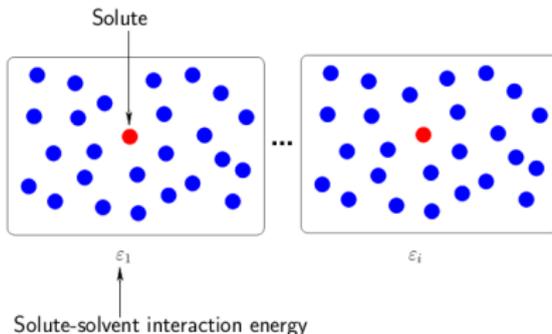
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- ▶ Define an inner shell around the ion
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- ▶ Populate the inner shell one solvent molecule at a time and assess its contribution to the inner-shell chemistry
- ▶ Mathematical formulation is obtained using a multi-state generalization of the potential distribution theorem

Potential distribution theorem

Estimating the excess chemical potential of a solute

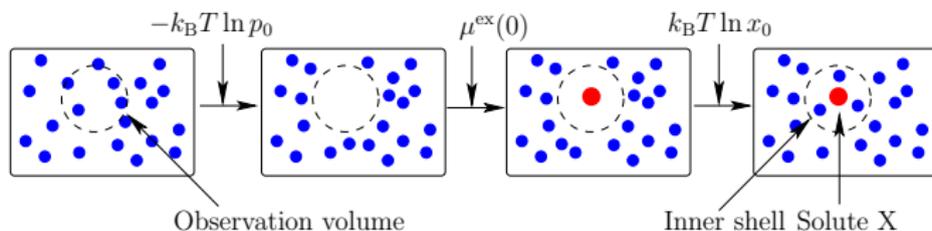


$$\begin{aligned}\mu^{\text{ex}} &= k_B T \ln \int e^{\varepsilon/k_B T} P(\varepsilon) d\varepsilon \\ &= k_B T \ln \langle e^{\beta \varepsilon} \rangle\end{aligned}$$

- ▶ ε is the interaction energy of the particle
- ▶ $P(\varepsilon)$ is the probability distribution of interaction energy ε
- ▶ Focus on the local, chemically relevant interactions

Quasichemical theory of solution

Beck, Paulaitis, Pratt, *The potential distribution theorem ...*, CUP, (2006)

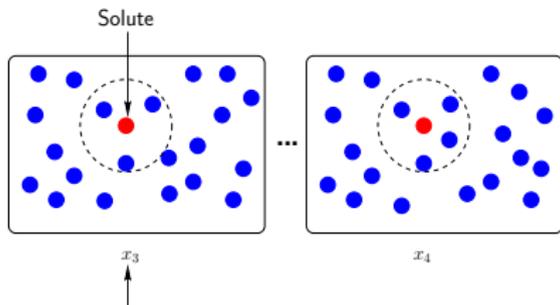


$$\begin{aligned}\mu^{\text{ex}}(0) &= k_B T \ln \int e^{\beta \varepsilon} P_X(\varepsilon | n = 0) d\varepsilon \\ \mu_X^{\text{ex}} &= \underbrace{-k_B T \ln p_0}_{\text{packing}} + \underbrace{\mu^{\text{ex}}(0)}_{\text{long range}} + \underbrace{k_B T \ln x_0}_{\text{chemistry}}\end{aligned}$$

- ▶ Decomposes hydration into physically motivated pieces
- ▶ $\mu^{\text{ex}}(0)$ is the **regularized problem**, easier to estimate than μ^{ex}
- ▶ Regularization helps to bring out local chemical interactions clearly

Multistate organization

Regularization and sorting based on occupation

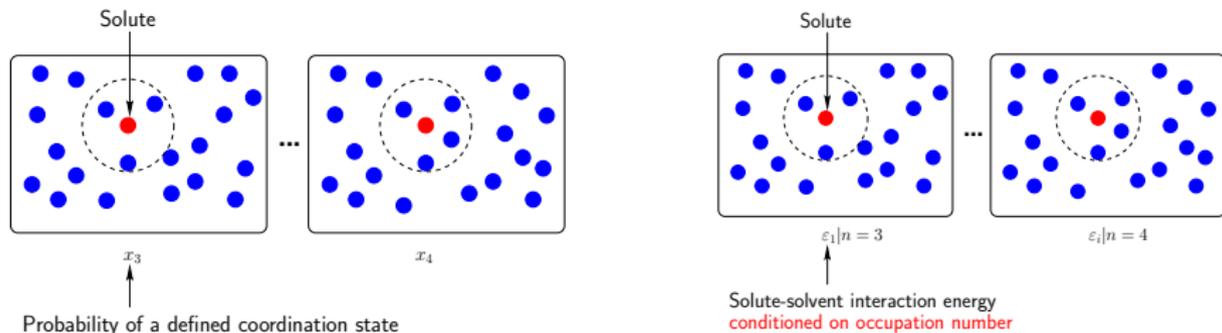


Probability of a defined coordination state

- ▶ Define a local region around the ion
- ▶ x_n is the probability of observing an n -coordinate state of the solute

Multistate organization

Regularization and sorting based on occupation

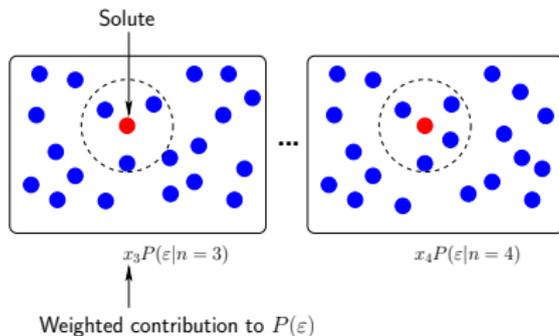


$$P(\varepsilon) = \sum x_n P(\varepsilon | n)$$

- ▶ Define a local region around the ion
- ▶ x_n is the probability of observing an n -coordinate state of the solute
- ▶ $P(\varepsilon | n)$ is the probability distribution of interaction energy **conditioned on there being n solvent molecules in the inner shell**

Multistate organization of μ^{ex}

Merchant and Asthagiri, JCP, **130**, 195102, (2009)

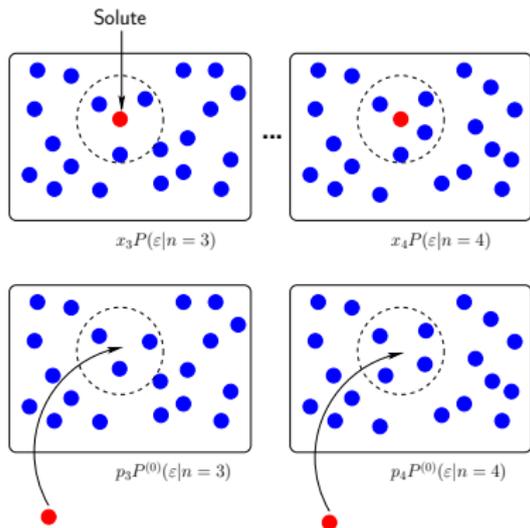


$$P(\epsilon) = \sum x_n P(\epsilon|n)$$
$$\mu^{\text{ex}} = k_B T \ln \sum x_n e^{\beta \mu^{\text{ex}}(n)}$$

- ▶ μ^{ex} is given as a partition sum over local coordination states
- ▶ $\mu^{\text{ex}}(n)$ is the excess chemical potential in n -coordinate state
- ▶ x_n serves as a weighing factor

Multistate organization leads to a fundamental identity

Merchant and Asthagiri, JCP, **130**, 195102, (2009)



$$\mu^{\text{ex}} = k_B T \log \sum x_n e^{\mu^{\text{ex}}(n)/k_B T}$$

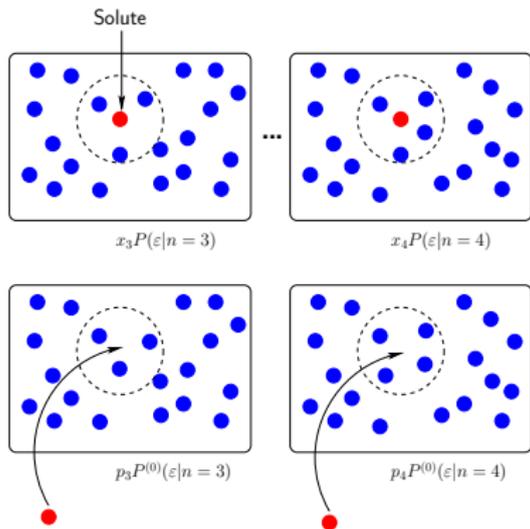
$$\mu^{\text{ex}} = -k_B T \log \sum p_n e^{-\beta \mu^{\text{ex}}(n)}$$

- ▶ State-wise contribution can also be obtained via insertions of solute ⁴
- ▶ $P^{(0)}(\varepsilon|n)$ is the uncoupled probability distribution of interaction energies

⁴ Hummer et al., JACS, **119**, 8523, (1997)

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$$\mu^{\text{ex}} = k_B T \log \sum x_n e^{\mu^{\text{ex}}(n)/k_B T}$$

$$\mu^{\text{ex}} = -k_B T \log \sum p_n e^{-\beta \mu^{\text{ex}}(n)}$$

$$\Rightarrow x_n = p_n e^{-[\mu^{\text{ex}}(n) - \mu^{\text{ex}}]/k_B T}$$

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Multistate organization leads to a fundamental identity

Separation into solvent-solvent and solute-solvent contributions

$$x_n = p_n e^{-\beta(\mu^{\text{ex}}(n) - \mu^{\text{ex}})}$$

- ▶ x_n is a property of the solute in the aqueous phase
- ▶ p_n is a **neat water property**
- ▶ $\mu^{\text{ex}}(n) - \mu^{\text{ex}}$ couples these distributions

Multistate organization leads to a fundamental identity

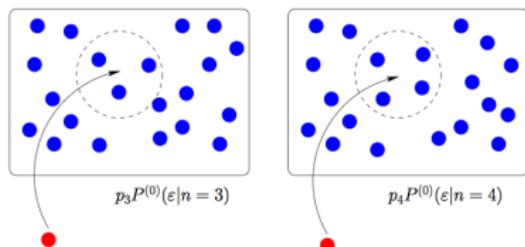
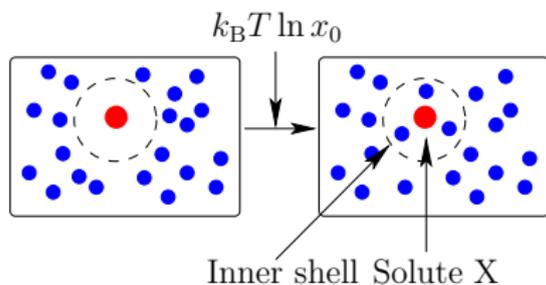
Separation into solvent-solvent and solute-solvent contributions

$$x_n = p_n e^{-\beta(\mu^{\text{ex}}(n) - \mu^{\text{ex}})}$$

$$\mu^{\text{ex}} = -k_B T \ln p_0 + \mu^{\text{ex}}(0) + k_B T \ln x_0$$

- ▶ x_n is a property of the solute in the aqueous phase
- ▶ p_n is a **neat water property**
- ▶ $\mu^{\text{ex}}(n) - \mu^{\text{ex}}$ couples these distributions
- ▶ $n = 0$ is the identity obtained in quasicheical approach

Molecular *aufbau* description of local chemistry

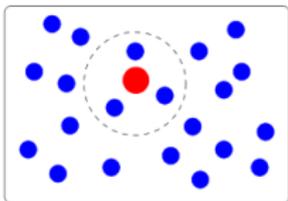


$$\mu^{\text{ex}} = -k_B T \ln \left[\frac{p_0}{x_0} e^{-\beta \mu^{\text{ex}}(0)} \right]$$

$$\mu^{\text{ex}} = -k_B T \ln \sum_n p_n e^{-\beta \mu^{\text{ex}}(n)}$$

Energetic reweighing of pure solvent states

- ▶ Free energy of assembling n -solvent molecules in the inner shell of the solute
- ▶ $e^{-\beta(\mu^{\text{ex}}(n) - \mu^{\text{ex}}(0))}$

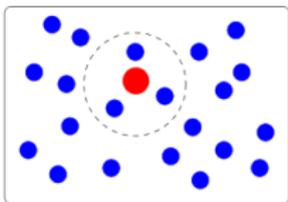


$$\ln x_{0,i} = -\ln \sum_{n=0}^i e^{-\beta(\mu^{\text{ex}}(n) - \mu^{\text{ex}}(0))} \frac{p_n}{p_0}$$

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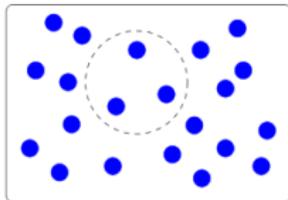
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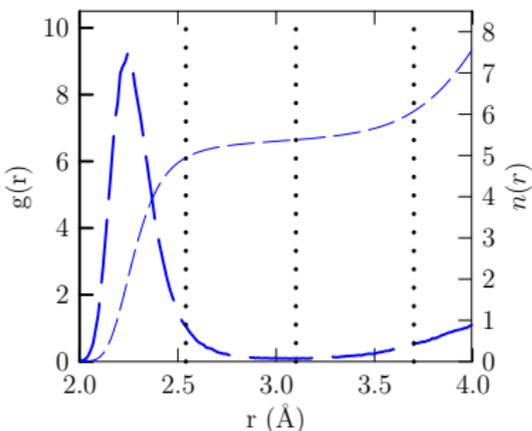
- ▶ Occupancy variations at the size and scale of observation volume

- ▶ $\frac{p_n}{p_0}$



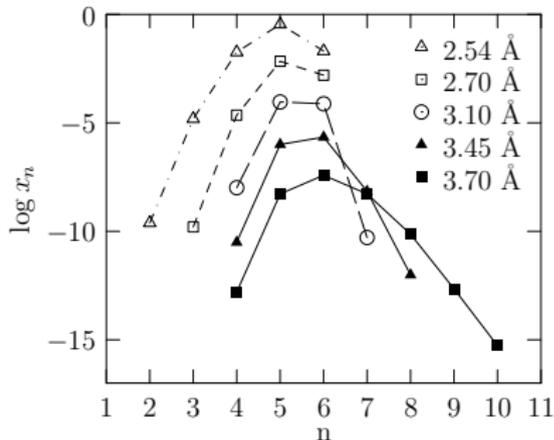
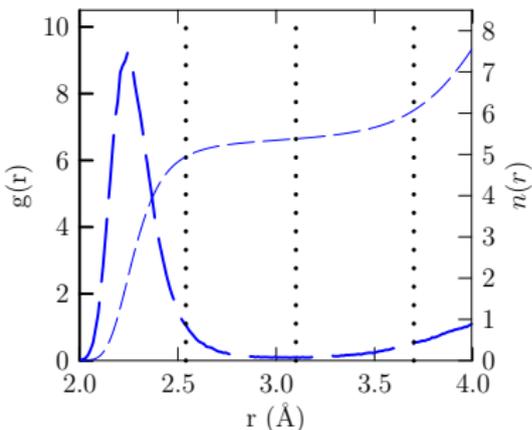
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Hydration of $\text{Na}^+(\text{aq})$



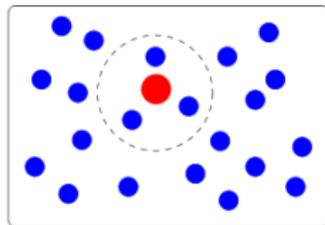
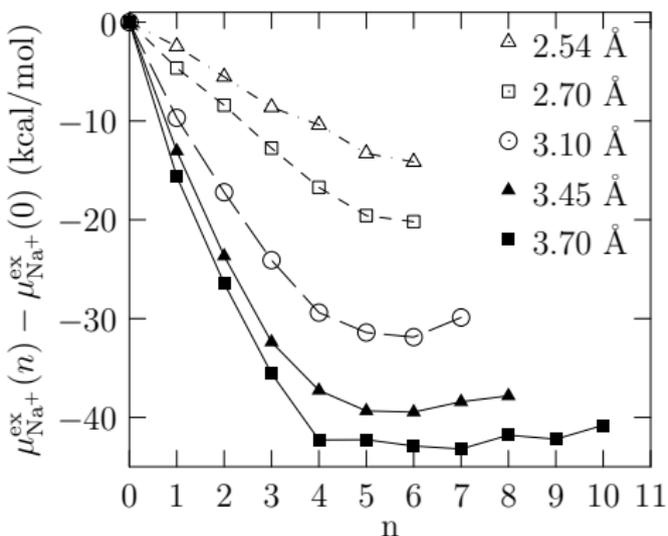
- ▶ $g(r)$ is obtained from neutron scattering or MD simulations ⁵
- ▶ Typically one would quote a mean coordination number: for $\text{Na}^+(\text{aq})$ mean coordination number varies from 4 to 8

Hydration of $\text{Na}^+(\text{aq})$



- ▶ $g(r)$ is obtained from neutron scattering or MD simulations⁵
- ▶ Typically one would quote a mean coordination number: for $\text{Na}^+(\text{aq})$ mean coordination number varies from 4 to 8
- ▶ Also the ion samples a broad distribution of states
- ▶ We need to understand implications of this broad sampling better

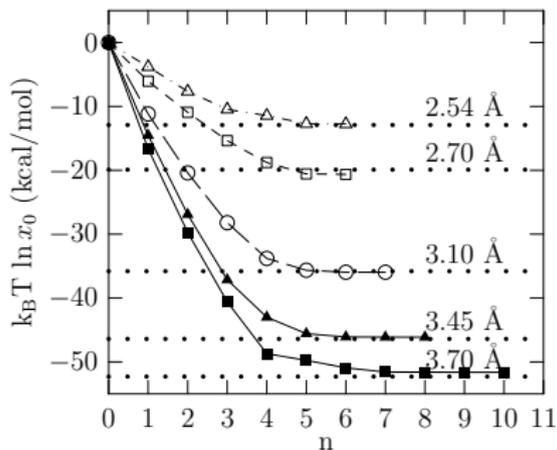
Free energy of forming $\text{Na}^+ + n$ -water clusters in water



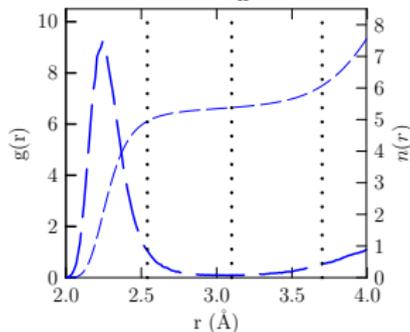
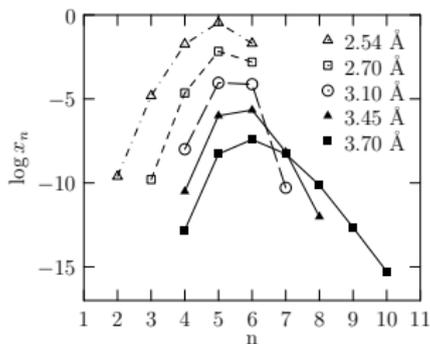
- ▶ A core set of water, $n \leq 4$, molecules bind the ion strongly
- ▶ These core water molecules screen the interaction of the ion with the rest of the medium

Coordination states and thermodynamics for $\text{Na}^+(\text{aq})$

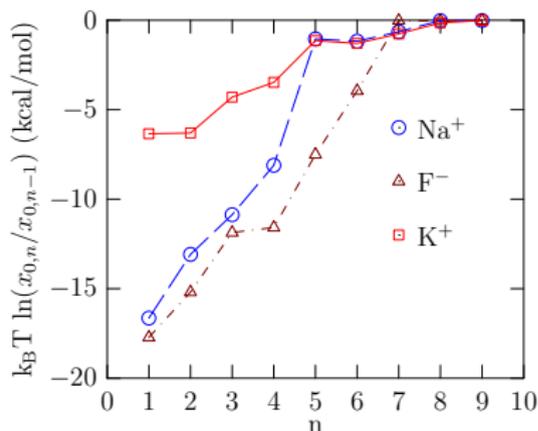
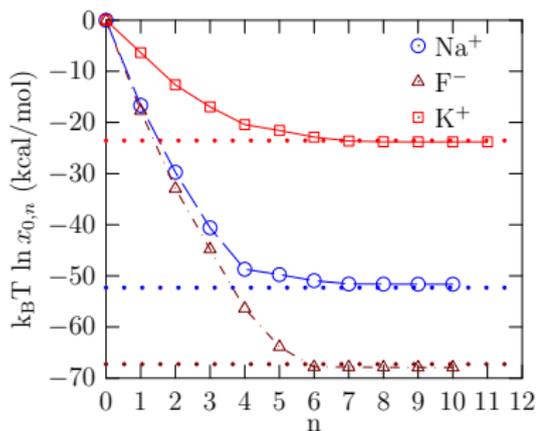
$n = 4$ is the dominant coordination state



- Most of the chemical term recovered by $n = 4$

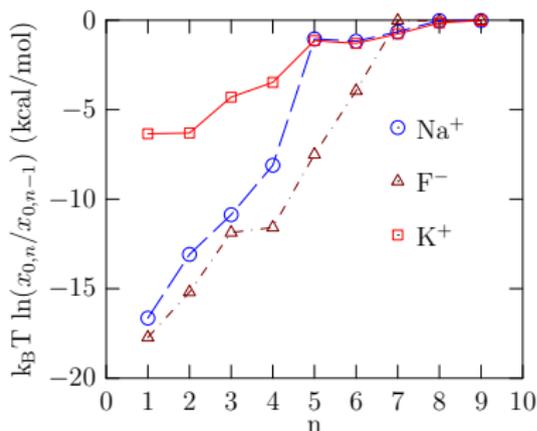
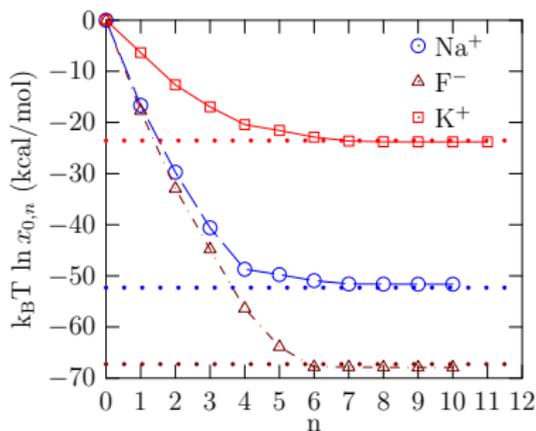


Contributions to chemistry for various ions



- ▶ Ion-specific contributions come from a small subset of water molecules
- ▶ Agrees with absence of long-range effect of ions on hydrogen bonding in water ⁶

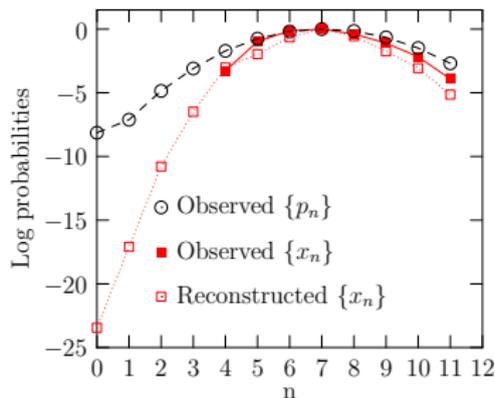
Contributions to chemistry for various ions



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- ▶ Agrees with absence of long-range effect of ions on hydrogen bonding in water ⁶
- ▶ If only a small number of states are chemically important why sample others ?

Intrinsic occupancy variation for K^+

If only a small subset of states are important then why sample others ?

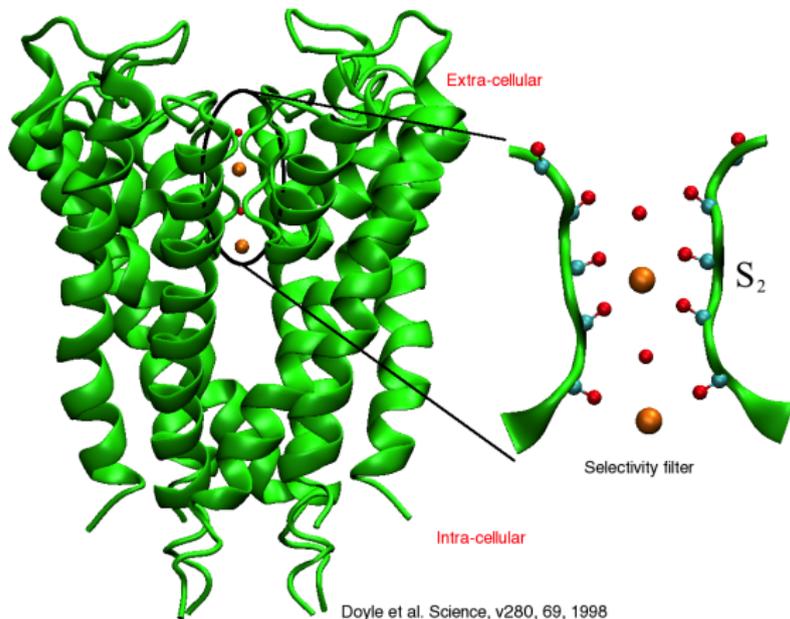


- ▶ Using $\{p_n\}$ and calculated values of $\mu^{\text{ex}}(n)$ we reconstruct $\{x_n\}$
- ▶ High coordination states are a result of occupancy variations of the pure solvent

Summary

- ▶ Probabilities $\{p_n\}$ of forming n -water clusters in an observation volume reflect occupancy number variations
 - ▶ The ion modifies the bare-probabilities $\{p_n\}$ to give the observed distribution $\{x_n\}$
- ▶ Ion interacts strongly only with a small number of core water molecules
 - ▶ The ion-specific contribution to hydration is entirely contained in these coordination states
 - ▶ Core waters screen the ion interaction with the rest of the medium leading to weak n -dependence beyond the most probable state

KcsA K⁺ channel and the selectivity filter

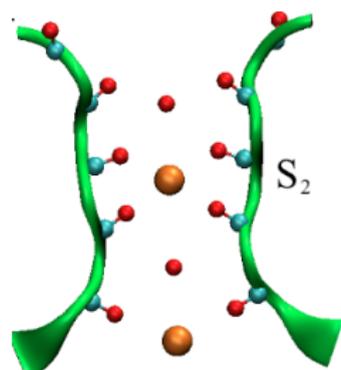


- ▶ K⁺-over-Na⁺ permeability is ≈ 1000
 - ▶ Studies suggest Na⁺ does not bind to the channel⁷

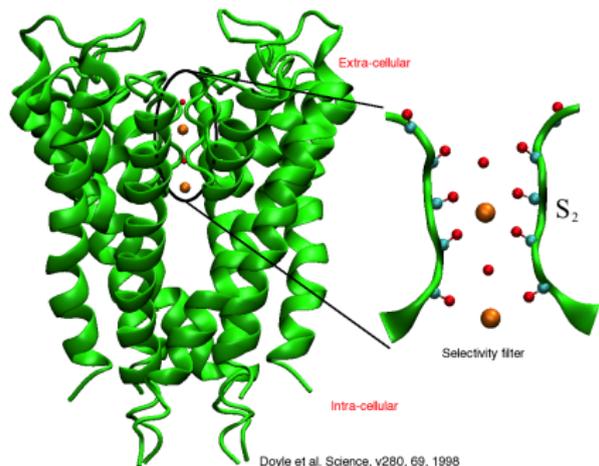
⁷S. W. Lockless et al., PLoS Biology, 2007

The selectivity filter is conserved

	-----	1	2	3	4	5	6	7	8	-----			
Sh	DAFWWAVVT	M	T	T	V	G	Y	G	D	M	T		
Kv1.1	DAFWWAVV	S	M	T	T	V	G	Y	G	D	M	Y	
Shab	EAFWWAGI	T	M	T	T	V	G	Y	G	D	I	C	
Kv2.1	ASFWWATI	T	M	T	T	V	G	Y	G	D	I	Y	
Shaw	LGLWWALV	T	M	T	T	V	G	Y	G	D	M	A	
Kv3.1	IGFWWAVV	T	M	T	T	L	G	Y	G	D	M	Y	
Sha1	AAFWYTI	V	T	M	T	T	L	G	Y	G	D	M	V
Kv4.1	AAFWYTI	V	T	M	T	T	L	G	Y	G	D	M	V
mSlo	ECVYLLM	V	T	N	S	T	V	G	Y	G	D	V	Y
fSlo	TCVYFLI	V	T	M	S	T	V	G	Y	G	D	V	Y
eag	TALYFTM	T	C	M	T	S	V	G	F	G	N	V	A
AKT1	TSMYWSI	T	T	L	T	T	V	G	Y	G	D	L	H
KAT1	TALYWSI	T	T	L	T	T	T	G	Y	G	D	F	H
ROMK1	SAFLFSLE	T	Q	V	T	I	G	Y	G	F	R	F	
IRK1	AAFLFSI	E	T	Q	T	T	I	G	Y	G	F	R	C
GIRK1	SAFLFFI	E	T	E	A	T	I	G	Y	G	Y	R	



Defining equilibrium selectivity



Doyle et al. Science, v280, 69, 1998

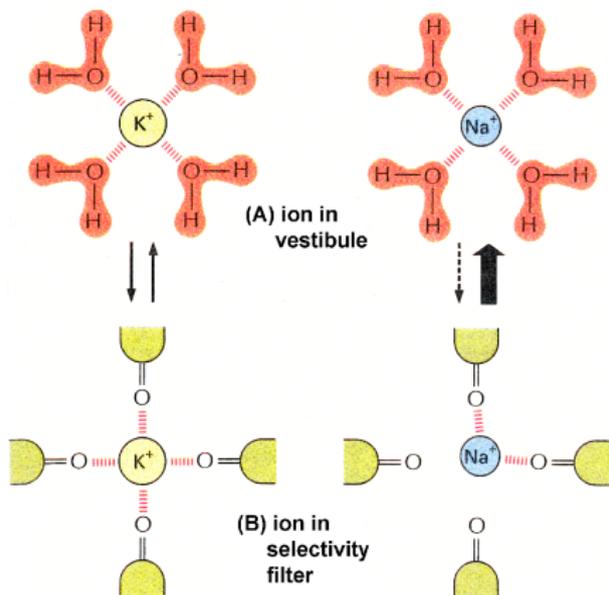
$$\begin{aligned}\Delta\mu^{\text{ex}}(\text{aq}) &= \mu_{\text{Na}^+}^{\text{ex}}(\text{aq}) - \mu_{\text{K}^+}^{\text{ex}}(\text{aq}) \\ &\approx -20 \text{ kcal/mol}\end{aligned}$$

- ▶ Selectivity is defined by:

$$\Delta\mu^{\text{ex}} = \Delta\mu^{\text{ex}}(\text{S}) - \Delta\mu^{\text{ex}}(\text{aq})$$

Thermodynamics of K^+ selectivity

Conventional wisdom



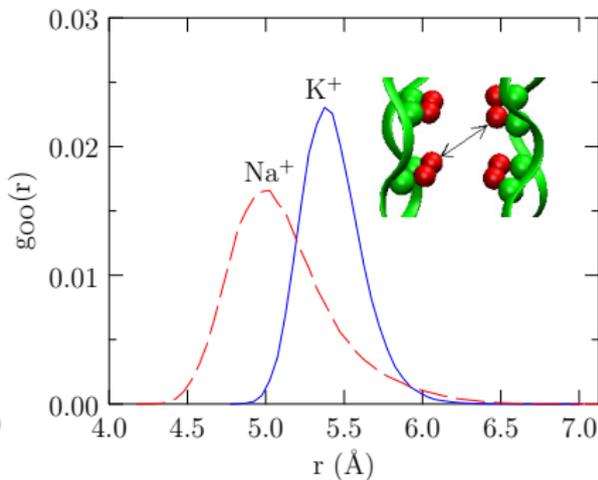
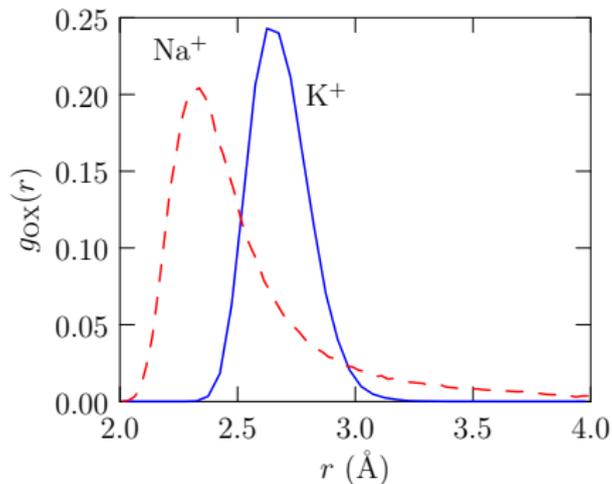
Alberts et al. Molecular Biology of the Cell, 2002

- ▶ Na^+ is too small to interact with all the carbonyls
- ▶ Dehydration remains uncompensated

" K^+ ion fits in the filter precisely so that the energetic costs and gains are well balanced. The structure of the selectivity filter with its molecular springs holding it open prevents the carbonyl oxygen atoms from approaching close enough to compensate for the cost of dehydration of a Na^+ ion." Doyle et al., Science 1998

Thermodynamics of K^+ selectivity

Simulation studies⁸

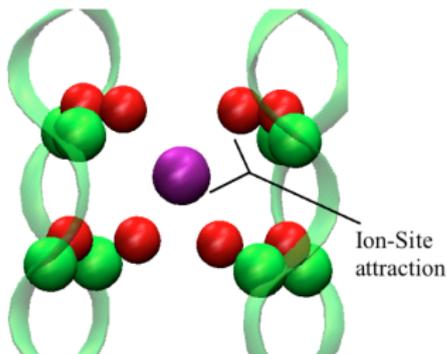
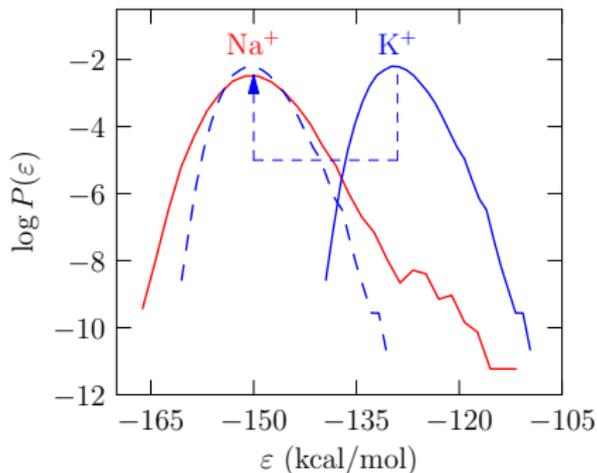


- ▶ The S_2 binding site can collapse onto the smaller Na^+
- ▶ The site is **more flexible in the presence of Na^+** compared to K^+

⁸Dixit et al. Biophys. J. 2009, Dixit and Asthagiri, Biophys. J. 2011, Dixit and Asthagiri, J. Gen. Physiol.

Thermodynamics of K^+ selectivity

Energetic considerations



- ▶ Compared to K^+ , Na^+ interacts more favorably with the channel
 - ▶ The spread of the $P_X(\epsilon)$ distribution is larger for Na^+ ⁹

Thermodynamics of K^+ selectivity

Computational findings

- ▶ The smaller Na^+ can pull the carbonyl ligands of the binding site towards itself
 - ▶ Compared to K^+ , the binding site is more flexible in the presence of Na^+
- ▶ Na^+ interacts with the channel protein **more favorably** than K^+
 - ▶ But the distribution of energies $P_X(\varepsilon)$ for Na^+ has a higher spread compared to K^+
- ▶ If the S_2 can accommodate and favorably interact with Na^+ , why does it select for K^+ ?

We need a theoretical framework to understand these constrasting observations

Thermodynamics of K^+ selectivity

Theory¹⁰

For $X = K^+$ or Na^+ , we can show,

$$\begin{aligned}\mu_X^{\text{ex}}(S) &= k_B T \log \int e^{\beta \epsilon} d\epsilon \\ &= \langle \epsilon \rangle_X + \mu_{X,\text{fluc}}^{\text{ex}}\end{aligned}$$

- ▶ $\langle \epsilon \rangle_X$ is the mean binding energy of the ion
- ▶ $\mu_{X,\text{fluc}}^{\text{ex}}$ reflects the spread of the binding energy distribution
 $P_X(\epsilon)$
- ▶ Let $\Delta(\cdot) = (\cdot)_{Na^+} - (\cdot)_{K^+}$,

$$\begin{aligned}\Rightarrow \Delta\mu^{\text{ex}}(S) &= \Delta\langle \epsilon \rangle + \Delta\mu_{\text{fluc}}^{\text{ex}} \\ \Delta\mu^{\text{ex}} &= \Delta\mu^{\text{ex}}(S) - \Delta\mu^{\text{ex}}(\text{aq})\end{aligned}$$

$$\Delta\mu^{\text{ex}}(\text{aq}) = -20.7 \text{ kcal/mole}$$

Thermodynamics of K^+ selectivity

Results

$$\begin{aligned}\Delta\mu^{\text{ex}} &= \Delta\langle\varepsilon\rangle + \Delta\mu_{\text{fluc}}^{\text{ex}} - \Delta\mu^{\text{ex}}(\text{aq}) \\ &= -21.0 \quad - \quad (-20.7)\end{aligned}$$

- ▶ The binding energy difference alone cannot explain the exclusion of Na^+ from the S_2 site

Thermodynamics of K^+ selectivity

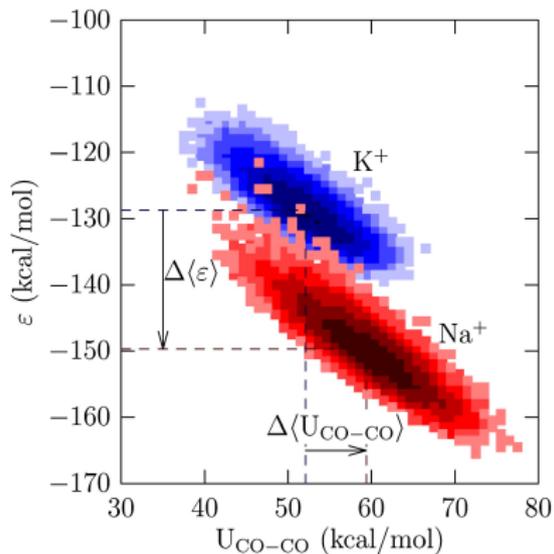
Results

$$\begin{aligned}\Delta\mu^{\text{ex}} &= \Delta\langle\varepsilon\rangle + \Delta\mu_{\text{fluc}}^{\text{ex}} - \Delta\mu^{\text{ex}}(\text{aq}) \\ 4.8 &= -21.0 + 5.3 - (-20.7)\end{aligned}$$

- ▶ The binding energy difference alone cannot explain the exclusion of Na^+ from the S_2 site
 - ▶ The larger spread of the Na^+ binding energy distribution $P_{\text{Na}^+}(\varepsilon)$ i.e. the higher fluctuation contribution is responsible for K^+ selectivity
- ▶ What are the molecular determinants of the positive fluctuation contribution $\Delta\mu_{\text{fluc}}^{\text{ex}}$?

Understanding selectivity

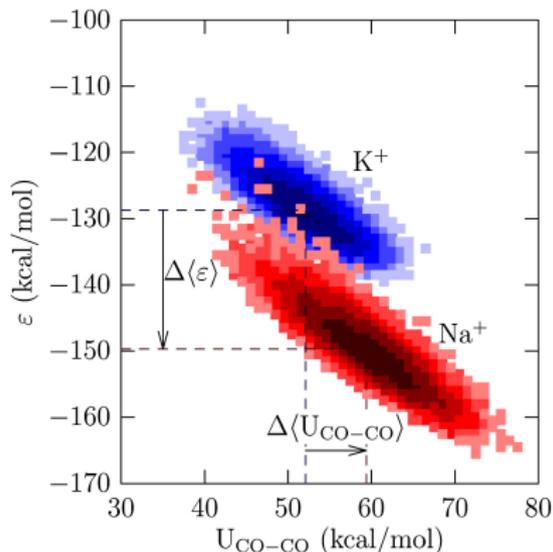
Fluctuation contribution depends on ligand-ligand interaction¹¹



$$\begin{aligned} T s^{\text{ex}}(S) &\approx u^{\text{ex}} - \mu^{\text{ex}}(S) \\ u^{\text{ex}} &\approx \langle\epsilon\rangle + \langle U_{\text{CO-CO}}\rangle \\ \Delta\langle\epsilon\rangle + \Delta\mu_{\text{fluc}}^{\text{ex}} &= \Delta\langle\epsilon\rangle + \Delta\langle U_{\text{CO-CO}}\rangle \\ &\quad - T\Delta s^{\text{ex}} \\ \Rightarrow \Delta\mu_{\text{fluc}}^{\text{ex}}(S) &= \Delta\langle U_{\text{CO-CO}}\rangle - T\Delta s^{\text{ex}} \end{aligned}$$

Understanding selectivity

Fluctuation contribution depends on ligand-ligand interaction¹¹



$$Ts^{\text{ex}}(S) \approx u^{\text{ex}} - \mu^{\text{ex}}(S)$$

$$u^{\text{ex}} \approx \langle\epsilon\rangle + \langle U_{CO-CO}\rangle$$

$$\Delta\langle\epsilon\rangle + \Delta\mu_{\text{fluc}}^{\text{ex}} = \Delta\langle\epsilon\rangle + \Delta\langle U_{CO-CO}\rangle - T\Delta s^{\text{ex}}$$

$$\Rightarrow \Delta\mu_{\text{fluc}}^{\text{ex}}(S) = \Delta\langle U_{CO-CO}\rangle - T\Delta s^{\text{ex}}$$

► $\Delta\langle U_{CO-CO}\rangle = 7.3$,
 $T\Delta s^{\text{ex}}(S) = 2.2$, and
 $\Delta\mu_{\text{fluc}}^{\text{ex}}(S) = 5.1$

- Configurations of the binding site favorable for the ion are exactly those with high energetic strain in the site and vice-versa
 - Binding site experiences a higher energetic strain in the presence of Na^+

Summary

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Summary

- ▶ Potential distribution theorem allows a physically motivated decomposition of the hydration free energy of solutes:
 - ▶ Highlights the role of specific solute-solvent interactions and
 - ▶ intrinsic solvent contribution
- ▶ Developments in the liquid state theory can help us understand ion-selectivity in proteins and suggests
 - ▶ design principles for artificial channels with desired conduction and selectivity profiles

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