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**\(^{1,2,3}\)
  - Requires inorganic ions for electroneutrality

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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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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
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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
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


- LiI 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


- 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

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

\[ \mu_{\text{blue}}^{\text{ex}}, \mu_{\text{red}}^{\text{ex}} \text{ account for energetic interactions of the particles} \]
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
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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

\[ \mu^{ex} = k_B T \ln \int e^{\varepsilon / k_B T} P(\varepsilon) d\varepsilon \]
\[ = k_B T \ln \langle e^{\beta \varepsilon} \rangle \]

- \( \varepsilon \) is the interaction energy of the particle
- \( P(\varepsilon) \) is the probability distribution of interaction energy \( \varepsilon \)
- Focus on the local, chemically relevant interactions
Quasichemical theory of solution

\[ \mu_{\text{ex}}(0) = k_B T \ln p_0 \]

\[ \mu_{\text{ex}}^X = -k_B T \ln p_0 + \mu_{\text{ex}}(0) + k_B T \ln x_0 \]

- Decomposes hydration into physically motivated pieces
- \( \mu_{\text{ex}}(0) \) is the regularized problem, easier to estimate than \( \mu_{\text{ex}} \)
- Regularization helps to bring out local chemical interactions clearly
Multistate organization
Regularization and sorting based on occupation

- 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

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

$$P(\varepsilon) = \sum x_n P(\varepsilon|n)$$
Multistate organization of $\mu^{\text{ex}}$

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

$\mu^{\text{ex}}$ is given as a partition sum over local coordination states

$\mu^{\text{ex}}(n)$ is the excess chemical potential in $n$-coordinate state

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

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

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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 quasichemical approach
Molecular \textit{aufbau} description of local chemistry

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

\[ \mu_{\text{ex}} = -k_B T \ln \sum_n p_n e^{-\beta \mu_{\text{ex}}(n)} \]
Molecular *aufbau* description of local chemistry

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

\[ \Rightarrow \ln x_0 = -\ln \sum_{n=0}^{\infty} e^{-\beta (\mu^{\text{ex}}(n) - \mu^{\text{ex}}(0))} \frac{p_n}{p_0} \]

\[ \mu^{\text{ex}} = -k_B T \ln \left( \sum_n p_n e^{-\beta \mu^{\text{ex}}(n)} \right) \]

\[ p_{3}^{P(0)}(\varepsilon|n = 3) \]

\[ p_{4}^{P(0)}(\varepsilon|n = 4) \]
Energetic reweighing of pure solvent states

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

\[
\ln x_{0,i} = - \ln \sum_{n=0}^{i} e^{-\beta(\mu_{ex}(n) - \mu_{ex}(0))} \frac{p_n}{p_0}
\]
Energetic reweighing of pure solvent states

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

- Occupancy variations at the size and scale of observation volume
  $$\frac{p_n}{p_0}$$

$$\ln x_{0,i} = -\ln \sum_{n=0}^{i} e^{-\beta(\mu_{\text{ex}}(n) - \mu_{\text{ex}}(0))} \frac{p_n}{p_0}$$
Hydration of Na$^+$ (aq)

$g(r)$ is obtained from neutron scattering or MD simulations.$^5$

Typically one would quote a mean coordination number: for Na$^+$ (aq) mean coordination number varies from 4 to 8.

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Hydration of Na\(^+\)(aq)

- \(g(r)\) is obtained from neutron scattering or MD simulations\(^5\)
- Typically one would quote a mean coordination number: for Na\(^+\)(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

$\mu_{\text{Na}^+}^\text{ex}(n) - \mu_{\text{Na}^+}^\text{ex}(0)$ (kcal/mol)

- 2.54 Å
- 2.70 Å
- 3.10 Å
- 3.45 Å
- 3.70 Å

- 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 Na\(^+\)(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

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

If only a small number of states are chemically important why sample others?

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Intrinsic occupancy variation for $K^+$

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

- High coordination states are a result of occupancy variations of the pure solvent

Using $\{p_n\}$ and calculated values of $\mu^{ex}(n)$ we reconstruct $\{x_n\}$
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 $\approx 1000$

Studies suggest $Na^+$ does not bind to the channel\(^7\)

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\(^7\) S. W. Lockless et al., PLoS Biology, 2007
The selectivity filter is conserved
Defining equilibrium selectivity

Selectivity is defined by:

\[ \Delta \mu_{\text{ex}} \approx -20 \text{ kcal/mol} \]

\[ \Delta \mu_{\text{ex}}(\text{aq}) = \mu_{\text{Na}^+}(\text{aq}) - \mu_{\text{K}^+}(\text{aq}) \]
Thermodynamics of K$^+$ selectivity

Conventional wisdom

- 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 studies\(^8\)

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

Thermodynamics of K$^+$ selectivity
Energetic considerations

Compared to K$^+$, Na$^+$ interacts more favorably with the channel

The spread of the \( P_X(\varepsilon) \) distribution is larger for Na$^+$\(^9\)

\(^9\) Asthagiri et al., JCP, 2006, Dixit et al., Biophys. J. 2009
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 contrasting observations
Thermodynamics of $K^+$ selectivity

Theory\textsuperscript{10}

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

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

$\langle \varepsilon \rangle_X$ is the mean binding energy of the ion

$\mu_{X,\text{fluc}}^{\text{ex}}$ reflects the spread of the binding energy distribution $P_X(\varepsilon)$

Let $\Delta(\cdot) = (\cdot)_{\text{Na}^+} - (\cdot)_{K^+}$,

$$
\Rightarrow \Delta \mu_X^{\text{ex}}(S) = \Delta \langle \varepsilon \rangle + \Delta \mu_{\text{fluc}}^{\text{ex}}
\Delta \mu^{\text{ex}} = \Delta \mu^{\text{ex}}(S) - \Delta \mu^{\text{ex}}(\text{aq})
$$

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

\textsuperscript{10} Dixit and Asthagiri, Biophys. J., 2009
Thermodynamics of $K^+$ selectivity

Results

$$\Delta \mu^{\text{ex}} = \Delta \langle \varepsilon \rangle + \Delta \mu^{\text{ex}}_{\text{fluc}} - \Delta \mu^{\text{ex}}(\text{aq})$$

$$= -21.0 - (-20.7)$$

▶ The binding energy difference alone cannot explain the exclusion of $Na^+$ from the $S_2$ site
Thermodynamics of $K^+$ selectivity

Results

\[ \Delta \mu_{\text{ex}} = \Delta \langle \varepsilon \rangle + \Delta \mu_{\text{fluc}}^{\text{ex}} - \Delta \mu_{\text{ex(aq)}} \]

\[ 4.8 = -21.0 + 5.3 - (-20.7) \]

- 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 $P_{Na^+}(\varepsilon)$ 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 interaction\textsuperscript{11}

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

\[ u^{\text{ex}} \approx \langle \varepsilon \rangle + \langle U_{\text{CO-CO}} \rangle \]

\[ \Delta \langle \varepsilon \rangle + \Delta \mu^{\text{ex}}_{\text{fluc}} = \Delta \langle \varepsilon \rangle + \Delta \langle U_{\text{CO-CO}} \rangle - T \Delta s^{\text{ex}} \]

\[ \Rightarrow \Delta \mu^{\text{ex}}_{\text{fluc}}(S) = \Delta \langle U_{\text{CO-CO}} \rangle - T \Delta s^{\text{ex}} \]

\textsuperscript{11} Dixit and Asthagiri, Biophys. J., 2011
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\[ \Delta \langle \varepsilon \rangle + \Delta \mu^{\text{ex}}_{\text{fluc}} = \Delta \langle \varepsilon \rangle + \Delta \langle U_{\text{CO-CO}} \rangle - T \Delta s^{\text{ex}} \]
\[ \Rightarrow \Delta \mu^{\text{ex}}_{\text{fluc}}(S) = \Delta \langle U_{\text{CO-CO}} \rangle - T \Delta s^{\text{ex}} \]

\[ \Delta \langle U_{\text{CO-CO}} \rangle = 7.3, \]
\[ T \Delta s^{\text{ex}}(S) = 2.2, \text{ and} \]
\[ \Delta \mu^{\text{ex}}_{\text{fluc}}(S) = 5.1 \]

- 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\textsuperscript{+}

\textsuperscript{11} Dixit and Asthagiri, Biophys. J., 2011
Summary

- Potential distribution theorem allows a physically motivated decomposition of the hydration free energy of solutes:
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  - 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.
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
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