Quantitative studies on molecular recognition:
free energy perturbation simulations on M+⊂222
cryptates in water and in methanol

P Auffinger, G Wipff*

Faculté de Chimie, 4, rue Blaise Pascal, 67000 Strasbourg, France

RESUME
Les différences d’énergie libre de complexation de Na+, K+ et Rb+ par le cryptand 222 ont été calculées dans l’eau et le méthanol, ainsi que les énergies libres relatives de transfert des ions libres et complexes entre ces deux solvants. Le 222 préfère intrinsèquement Na+ en phase gazeuse et en solution, mais la prise en compte des différences d’énergie libre de solvatisation des ions conduit à un pic de sélectivité pour K+ dans les deux solvants, en accord avec l’expérience. Des calculs préliminaires mettent en évidence l’importance de la conformation initiale du cryptate au cours de ces différentes mutations.

SUMMARY
Relative free energies of complexation of Na+, K+ and Rb+ cations by the 222 cryptand have been calculated in water and in methanol. This was achieved by "slow growth MD mutations" using the perturbation thermodynamic cycle. Starting with the experimental structure of the K⁺⊂222 cryptate we calculate that, in gas phase and in solution, 222 prefers intrinsically Na+. In solution, taking into account the relative solvation free energies, we find a peak of binding selectivity for K+, in qualitative agreement with experiment. Calculated relative free energies of transfer from water to methanol of the free cations and of their cryptates compare also reasonably well with experiment. However, calculations starting from another cryptate type structure give rather different energies, which questions the predictive power of FEP calculations when restricted to small simulation times, and when the experimental structures are not available!

INTRODUCTION
The 222 cryptand was the first molecule of macrobicyclic topology to complex with high stability and selectivity spherical cations inside its cavity forming M⁺⊂222 cryptate.¹,² The mathematical symbol ◻ stands for "inclusion of" (Figure 1). Control of the cavity size, the nature and number of binding sites, and of lipophilic substituents allowed to monitor the cation selectivity. Among M⁺ alkali cations, 222 is most selective
for K⁺ in water and in methanol, leading to complexes more stable than those made by naturally occurring ionophores.\textsuperscript{3,4,5}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1}
\caption{Inclusion of a cation into the 222 cryptand forming a M⁺\textsubscript{222} cryptate.}
\end{figure}

In this paper, we report preliminary calculations on these binding selectivities in solution, by using free energy perturbation (FEP\textsuperscript{+}) calculations. Mutations are performed in the Na⁺, K⁺, Rb⁺ series of cations in their free state, and complexed by 222, first in water, then in methanol. Following the first theoretical study of ion binding selectivity by the synthetic SC24,4H⁺ ionophore,\textsuperscript{6} there have been related free energy calculations of Na⁺, K⁺, Rb⁺ complexation by 18-crown-6 in water\textsuperscript{7} and in methanol for Na⁺ and K⁺,\textsuperscript{8} by dibenzo-crown ethers in methanol\textsuperscript{9} as well as calculations of the potential of mean force for inclusion of K⁺ into 18-crown-6 in water.\textsuperscript{10,11} Compared to 18-crown-6, the bicyclic 222 cryptand leads to complexes of higher stability since it provides six oxygen and two nitrogen binding sites to "solvate" the encapsulated cation. Its bicyclic topology prevents cation/solvent interactions, as compared to the more open 18-crown-6. Finally, in terms of ionophoric behaviour, 222 transports alkali cations through hydrophobic membranes with higher efficiency than monocyclic polyethers.\textsuperscript{12} It is therefore an excellent candidate for testing molecular mechanics, molecular dynamics studies in vacuo and in solution,\textsuperscript{13,14,15} as well as FEP calculations.

**COMPUTATIONAL PROCEDURE**

The AMBER 3.0 package\textsuperscript{16} has been used to perform the FEP calculations, based on molecular dynamics sampling of the conformational space. Mutation of one cation M\textsubscript{1}⁺ into M\textsubscript{2}⁺ was performed using the "slow growth" technique by changing gradually the coupling parameter \( \lambda \) from 0 to 1.\textsuperscript{17} This was achieved in water and in methanol for the uncomplexed cations, and for the cation cryptates. According to the thermodynamic cycle, the binding selectivity in solution \( \Delta \Delta G \) for M\textsubscript{1}⁺ versus M\textsubscript{2}⁺, measured experimentally by \( \Delta G_2 - \Delta G_1 \) should be equal to the calculated difference \( \Delta G_4 - \Delta G_3 \).
We used the AMBER energy representation, with a 1-6-12 potential for solute-solvent and solvent-solvent interactions. The parameters are the same as those used previously to simulate the dynamics of 222 cryptand and cryptates in water. Most are derived from the AMBER force field. The partial charges of the cryptand have been fitted from ab initio electrostatic potentials on isolated fragments of 222 and used throughout for the cryptates. For the solvent, we used TIP3P water and OPLS methanol with the methyl group represented in the united atom approximation. These solvent molecules were constrained to rigid bond lengths with SHAKE.

The solute was surrounded by water molecules put in a rectangular box with at least 10 Å of solvent thickness for water and 12 Å for methanol. All simulations were performed at constant temperature (300K) and pressure (1 atm) using periodic boundary conditions. For cryptate-water interactions a residue based cut-off of 8 Å in water and 10 Å in methanol was used. The solutions were first equilibrated for 51 ps for the K⁺C₂₂₂ cryptates and for 15 ps for K⁺ uncomplexed, using a time step of 2 fs. Then "slow growth" mutations were undertaken for 10, 20 and 40 ps in water, and for 40 ps in methanol. Checks for hysteresis were performed by comparing "forwards" (K⁺ to Na⁺ and K⁺ to Rb⁺) and "backwards" (Na⁺ to K⁺ and Rb⁺ to K⁺) mutations. As a matter of reference, the mutations starting from K⁺C₂₂₂ cryptates in vacuo were undertaken, (also for 400 ps) in order to assess the effect of the cage itself and of solvation on calculated selectivities.

All forwards mutations on the M⁺C₂₂₂ cryptates (K⁺ to Na⁺ and K⁺ to Rb⁺), were initiated with the structure of the K⁺C₂₂₂ cryptate, noted 222-K. The last structure of the MD mutation was then used to start the backwards mutation without additional equilibration. For the Na⁺/K⁺ cryptates in water, two additional simulations were performed starting from another structure, corresponding to the Ag⁺C₂₂₂ cryptate (noted 222-Ag). For simulation 1, the same procedure as above was used. For simulation 2, we equilibrated Na⁺C₂₂₂ for 12 ps in water, i.e. just before the first conformational transitions occurs. This was followed by the Na⁺C₂₂₂ -> K⁺C₂₂₂ mutation (forwards and backwards).
RESULTS

The ΔG values for mutation of Na⁺ into K⁺ and K⁺ into Rb⁺ and their cryptates in the gas phase, in water and in methanol are reported Table 1.

Table 1  Relative free energies of mutation (kcal/mol) of Na⁺, K⁺ and Rb⁺ uncomplexed and complexed by 222 (222-K starting structure) for different simulation times. When two values are given, they correspond to the forward and backward simulations.

<table>
<thead>
<tr>
<th>MUTATIONS</th>
<th>Na⁺ → K⁺</th>
<th>K⁺ → Rb⁺</th>
<th>Na⁺&lt;sub&gt;C222&lt;/sub&gt; → K⁺&lt;sub&gt;C222&lt;/sub&gt;</th>
<th>K⁺&lt;sub&gt;C222&lt;/sub&gt; → Rb⁺&lt;sub&gt;C222&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IN THE GAS PHASE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ps</td>
<td></td>
<td>13.0</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>20 ps</td>
<td></td>
<td>13.6</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>40 ps</td>
<td></td>
<td>12.6 / 10.0</td>
<td>11.7 / 9.3</td>
<td></td>
</tr>
<tr>
<td>400 ps</td>
<td></td>
<td>14.2 / 13.7</td>
<td>11.5 / 12.0</td>
<td></td>
</tr>
<tr>
<td>IN WATER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ps</td>
<td>18.4</td>
<td>7.3</td>
<td>12.9</td>
<td>10.9</td>
</tr>
<tr>
<td>20 ps</td>
<td>17.7</td>
<td>6.9</td>
<td>13.0</td>
<td>10.8</td>
</tr>
<tr>
<td>40 ps</td>
<td>18.7 / 18.8</td>
<td>7.2 / 7.2</td>
<td>13.3 / 13.5</td>
<td>10.8 / 10.9</td>
</tr>
<tr>
<td>40 ps&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3 / 15.2</td>
<td>11.3 / 11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>17.5&lt;sup&gt;b&lt;/sup&gt;; 17.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>20.1&lt;sup&gt;d&lt;/sup&gt;; 21.5&lt;sup&gt;e&lt;/sup&gt;; 6.1&lt;sup&gt;f&lt;/sup&gt;; 6.7&lt;sup&gt;j&lt;/sup&gt;</td>
<td>34.7&lt;sup&gt;h&lt;/sup&gt;; 13.8&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN METHANOL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ps</td>
<td>18.8 / 18.9</td>
<td>7.3 / 7.2</td>
<td>12.0 / 11.9</td>
<td>10.7 / 10.6</td>
</tr>
<tr>
<td>Experimental</td>
<td>17.9&lt;sup&gt;h&lt;/sup&gt;; 17.3&lt;sup&gt;l&lt;/sup&gt;; 17.0&lt;sup&gt;l&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>20.1&lt;sup&gt;i&lt;/sup&gt;; 19.8&lt;sup&gt;j&lt;/sup&gt;; 19.1&lt;sup&gt;j&lt;/sup&gt;; 5.9&lt;sup;k&lt;/sup&gt;; 7.6&lt;sup&gt;l&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;h&lt;/sup&gt;; 21.7&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Starting the simulations with the 222-Ag structure (see text).<sup>b</sup>Reference 23, <sup>c</sup>Reference 24, <sup>d</sup>Reference 25, <sup>e</sup>Reference 26, <sup>f</sup>Reference 7, <sup>g</sup>Reference 26, <sup>h</sup>Reference 27, for a cluster of 6 water molecules using non-additive potentials. <sup>i</sup>Reference 28, <sup>j</sup>Reference 6, <sup>l</sup>Reference 7, values calculated for clusters of 6 and 137 methanol molecules.

Gas phase selectivity. The gas phase simulations show, as found previously by simple molecular mechanics optimizations, that the order of intrinsic binding affinities is Na⁺<sub>C</sub> K⁺<sub>C</sub> Rb⁺<sub>C</sub>. The smallest cation has intrinsically the highest affinity, and no peak of selectivity is observed, although K⁺ shifts better in the cavity of 222 than Na⁺. This is consistent with the order of gas phase interaction energies between alkali cations and ethers or amines. At a quantitative level, taking the average of forwards and backwards simulations for the 400 ps simulation, the ΔG<sub>3</sub> of complexation would decrease by 13.9 and 11.7 kcal/mol respectively, from Na⁺<sub>C</sub> K⁺<sub>C</sub>, and from K⁺<sub>C</sub> to Rb⁺<sub>C</sub> (Table 1).
Relative free energies of solvation for Na+, K+ and Rb+ uncomplexed. We calculate that the smallest cation Na+ is better solvated than K+ or Rb+. The calculated \( \Delta G_{4} \text{H}_2O \) (18.8 kcal/mol for Na+/K+ and 7.2 kcal/mol for K+/Rb+) compare reasonably well with experimental values (respectively 17.5 and 5.5 kcal/mol), and with previously calculated values using slightly different force fields and procedures\(^7\) (Table I). The calculated energies \( \Delta G_{4} \text{MeOH} \) are also satisfactory, and comparable to the results obtained by McCammon et al.\(^8\) In agreement with experiment, the relative Na+/K+ solvation energies are about the same in water and in methanol, as calculated by Rao et al.\(^25\) This is also true for the K+/Rb+ relative solvation energies.

![Figure 2](image-url)  
**Figure 2.** Left, calculated \( \Delta G_3 \) and \( \Delta G_4 \) from mutating Na+, K+ and Rb+ cations uncomplexed and cryptated by 222 in water and in methanol. Right, calculated and experimental binding selectivity (\( \Delta \Delta G_C \)) for Na+, K+ and Rb+ by 222 in water and in methanol.

<table>
<thead>
<tr>
<th></th>
<th>Na+ C222 / K+ C222</th>
<th>K+ C222 / Rb+ C222</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IN WATER</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta \Delta G_{calc} )</td>
<td>5.4</td>
<td>-3.7</td>
</tr>
<tr>
<td>( \Delta \Delta G_{exp} )</td>
<td>2.0(^a)</td>
<td>-1.4(^b), -1.5(^c), -2.1(^d), -1.7(^b)</td>
</tr>
<tr>
<td><strong>IN METHANOL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta \Delta G_{calc} )</td>
<td>6.8</td>
<td>-3.6</td>
</tr>
<tr>
<td>( \Delta \Delta G_{exp} )</td>
<td>4.0(^a), 3.3(^b), 3.6(^c)</td>
<td>-2.0(^b), -2.2(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Oda by Izatt et al.; \(^b\)Reference 30; \(^c\)Reference 31

Binding selectivity in solution. The Na+ C222 to K+ C222 and K+ C222 to Rb+ C222 changes in free energy \( \Delta G_3 \) in solution are close to these calculated in the gas
phase (Table I) which suggests that interactions between the encapsulated cation and the solvent do not change significantly from Na+ to Rb+. This is first consistent with MD simulations of K+<222 cryptates in water\textsuperscript{15} and in methanol\textsuperscript{32} which indicated that about one solvent molecule remains directly coordinated to the cation complexed by 222 (Figure 3). Second, this suggests computationally that the binding selectivity results from desolvation of the complexed cation, as well as from receptor-cation complementarity.

![Figure 3](image)

Figure 3 The K+<222 in water and in methanol (from MD simulations\textsuperscript{15,32}) showing one solvent molecule coordinated to the encapsulated cation.

The relative complexation energies in water and in methanol, calculated as $\Delta \Delta G = \Delta G_A - \Delta G_B$ are given Table II and pictured Figure 2. There is now qualitative agreement with complexation experiments, since a peak of selectivity is found for K$^+$ in water and in methanol. Quantitatively, the selectivity is somewhat exaggerated, by about 3.5 kcal/mol for Na$^+$/$K^+$ and by about 1.2 to 2.0 kcal/mol for K$^+$/$Rb^+$ in water and in methanol respectively.

Relative free energies $\Delta \Delta G$ for transfer of cations and cryptates from water to methanol. According to the following scheme, these $\Delta \Delta G$ can be estimated and compared to experiment.\textsuperscript{33,34}

![Diagram](image)

The relative energy of transfer from water to methanol of A, compared to B is:

$$\Delta \Delta G(A,B) = \Delta G_B(B) - \Delta G_B(A) = \Delta G_m(A,B) - \Delta G_w(A,B)$$
The results (Table III) show that these $\Delta G_1$ are weak and in good agreement for Na$^+/K^+$, K$^+/Rb^+$ and for Na$^+/C_{222}/K^+/C_{222}$. For K$^+/C_{222}/Rb^+/C_{222}$, the calculated value (-0.20 kcal/mole) is of opposite sign compared to the experimental one (0.64 kcal/mole). Experiment shows that in fact $\Delta G_1$ results from enthalpic an entropic components, which tend to compensate each other (Table III). These relative free energies of transfer can also be related to relative partition coefficients.33,34

Table III. Experimental31 free energies, enthalpies and entropies of transfer from water to methanol for the free cations, for the cryptates and for the cryptand. Experimental and calculated relative free energies of transfer.

<table>
<thead>
<tr>
<th></th>
<th>Free cations</th>
<th>Cryptates</th>
<th>2 2 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na$^+$</td>
<td>K$^+$</td>
<td>Rb$^+$</td>
</tr>
<tr>
<td>$\Delta G_a^a$</td>
<td>2.05</td>
<td>2.35</td>
<td>2.45</td>
</tr>
<tr>
<td>$\Delta H_a^a$</td>
<td>-4.92</td>
<td>-4.52</td>
<td>-3.71</td>
</tr>
<tr>
<td>$\Delta S_a^b$</td>
<td>-23.4</td>
<td>-23.0</td>
<td>-20.7</td>
</tr>
<tr>
<td>$\Delta Q_b^b$</td>
<td>0.30</td>
<td>0.10</td>
<td>-1.39</td>
</tr>
<tr>
<td>$\Delta Q_c^c$</td>
<td>0.10</td>
<td>0.10</td>
<td>-1.30</td>
</tr>
</tbody>
</table>

*Experimental (kcal/mol), †Experimental (cal / K mol), ‡Calculated (kcal/mol)

DISCUSSION

It is known from experiment that stability constants of Na$^+$, K$^+$ and Rb$^+$ cryptates are higher in methanol and in other solvents than in water,5 but such a comparison cannot be made presently by FEP calculations. Transfer energies from water to methanol are also very different for free cations, the free cryptand and the cryptate and cannot be compared here. We compare relative binding affinities in solution, and we find a peak for K$^+$ in water and in methanol in agreement with experiment. However the calculated preference is somewhat exaggerated (by 2-3 kcal/mol in water and in methanol). This may be due to several approximations. First, no cage polarization by the cation was taken into account. Although polarization should be included35 it is not clear in which direction it would shift the present results! The sampling time might be indeed more critical and is too short to allow conformational interconversions. During the gas phase mutations, numerous dihedral transitions occur, especially with the smallest ion (about one every ps for Na$^+/K^+$, and one every 2 ps for K$^+/Rb^+$). The 10, 20, 40 and 400 ps simulations give sensible different relative free energies. This leads to an hysteresis of about 2 kcal/mol (Table I). In solution, no dihedral transitions are observed during 40 ps, and the hysteresis is smaller, but this may be an artefact related to the (too short)
simulation time. It is noticeable that in the solid state structures found in the Cambridge Data Base,36 all K+<C222 cryptates are similar, whereas the Na+ cryptate adopts diverse conformations, including that of K+<C222. We are presently investigating force field effects by mutating one set of charges on 222 into another one, as well as the question of sampling.

Does the selectivity calculated for K+ depend on the starting structure? All the above results have been obtained by starting with the 222-K structure,22 of adequate size to accommodate K+ rather than Na+ which prefers a smaller cavity, and this might be a source of selectivity for K+. We repeated therefore the mutations Na+<C222 -> K+<C222 and K+<C222 -> Rb+<C222 in water starting with the more compact 222-Ag structure22 which we calculated in the gas phase to have a higher affinity for Na+ than for K+.13 Two tests were performed. In simulation 1, the protocol is similar to that used above: 51 ps of MD on 222<|K+>, followed by mutations into Na+<C222 and Rb+<C222. We noticed that numerous dihedral transitions occurred during the *equilibration* stage. Although the hysteresis is weak (0.2 kcal/mol) and comparable to that obtained with the 222-K starting structure. The ΔG₄ for K+<C222 -> Na+<C222 is more negative by 2 kcal/mol. This computer experiment demonstrate thus that a weak hysteresis does not guarantee for thermodynamic equilibrium along the simulations! For K+<C222 mutated in Rb+<C222, both 222-Ag and 222-K starting structure gives a similar ΔG₄. This is because the size of these cations allows for less conformational freedom for 222 as Na+. In simulation 2, we start with 222<|Na+> which is *equilibrated* in water for 12 ps only (after, it undergoes dihedral transitions) and then mutated for 40 ps into K+<C222 forwards and backwards. For simulation 2 the hysteresis is much higher than that obtained with the 222-K form: ΔG₄ = 21.0 (forwards) and 17.6 kcal/mol (backwards). For the Na+/K+ cryptates, the average ΔG₄ (19.3 kcal/mol) is larger than that calculated with the 222-K conformer (13.4 kcal/mol) (Table I). As a result, this would lead to predict incorrectly a binding selectivity of 19.3 - 18.7 = 0.6 kcal/mol, i.e. a preference for complexing Na+ over K+ in water!

It is presently difficult to conclude on the problem of the starting geometry, which clearly influences the ΔΔG₂ by an amount similar to the binding selectivity itself, and may lead to wrong predictions if too small simulation times are used. It is not clear either what should be a good criteria for thermodynamic equilibrium. After 1.5 ns of MD simulations on 18-crown-6 in water, new conformers are still appearing.37 The conformational changes observed when the 222-Ag structure was used initially may result from thermodynamic instability or/and contribute to real conformational entropy. On the other hand, remaining in the same conformational region during the mutation, as observed with the 222-K starting structure is not a guarantee either. In the 18-crown-6 selectivity for
K+ over Na+ or Rb+, this question of knowing the "right structure" to start with appeared also to be of importance. From these results, one may question the ability to predict relative binding affinities of flexible receptors in solution, when experimental structures are not available. In the case of 22238 and of 18-crown-6,39 it is noticeable that conformational sampling in the gas phase did not provide the conformer adequate to complex K+, the cation which is best "recognized".

ACKNOWLEDGEMENTS

The authors thanks the CNRS-IBM Scientific Group for Molecular Modelling, and the CNRS Computer Center of Strasbourg Cronenbourg which have provided the computer means for this study. P.A. is also grateful to the French ministry of Research for providing a grant. G. W. thanks the "Région Alsace" and the Biostructure company for funding a computer graphics system.

REFERENCES