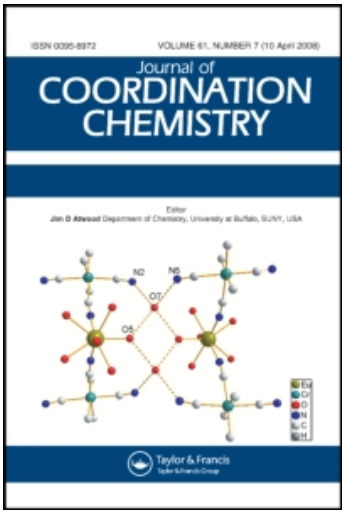

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Molecular Modeling Studies on Molecular Recognition: Crown Ethers, Cryptands and Cryptates. From Static Models *in vacuo* to Dynamical Models in Solution

Georges Wipff[a]
[a] Institut de Chimie, Strasbourg, France

To cite this Article Wipff, Georges(1992) 'Molecular Modeling Studies on Molecular Recognition: Crown Ethers, Cryptands and Cryptates. From Static Models *in vacuo* to Dynamical Models in Solution', Journal of Coordination Chemistry, 27: 1, 7 — 37
To link to this Article: DOI: 10.1080/00958979209407942
URL: http://dx.doi.org/10.1080/00958979209407942

J. Coord. Chem. 1992, Vol. 27, pp. 7-37
Reprints available directly from the publisher
Photocopying permitted by license only

MOLECULAR MODELING STUDIES ON MOLECULAR RECOGNITION: CROWN ETHERS, CRYPTANDS AND CRYPTATES. FROM STATIC MODELS *IN VACUO* TO DYNAMICAL MODELS IN SOLUTION

GEORGES WIPFF

Institut de Chimie, 1, rue Blaise Pascal, 67000 Strasbourg, France

Molecular Mechanics, Monte Carlo and Molecular Dynamics simulations on free and complexed crown ethers, on bicyclic cryptands and cryptates provide deeper insights into their conformational and recognition properties and allow to address the questions of preorganisation, complementarity, and binding selectivity. Alternatively, references to experimental data allow to outline present theoretical and computational limitations. Of particular interest are the microscopic pictures obtained in solution, which demonstrate *the importance of solvent and environment effects on the precise structure of free and complexed receptors, and on their dynamics.* Quantitative insights into relative free energies in solution represents a most promising breakthrough for computational studies in molecular recognition.

Keywords: Molecular modeling; molecular mechanics; molecular dynamics; molecular regognition; crown ethers; cryptands and cryptates; solvation; supramolecular chemistry

INTRODUCTION

Host-guest chemistry, and molecules such as crown ethers, cryptands, spherands, podands and derivatives represent a field of particular interest for molecular modelling. These are small enough to be built and manipulated with classical CPK models and in the chemist's hands: conformations and architecture have been designed in such a way that they could act as receptors representing a concave molecular region to bind strongly with marked specificities small species such as cations, anions, neutral molecules.[1-6] Cram quoted "From the beginning, we used Corey-Pauling-Koltun molecular models, which served as a compass on an otherwise uncharted sea of synthesizable target complexes. We have spent hundreds of hours building CPK models of potential complexes and grading them for desirability as research targets".[6] Lehn and Pedersen who shared with Cram the Nobel prize in 1987,[4] and many of the chemists active in the field of supramolecular chemistry performed also similar molecular modelling studies using CPK type models.

With the advent of high speed computers and computer graphics systems, and with the development of applied theoretical methods, synthetic macro(poly)cyclic compounds can be studied by molecular modelling techniques. Their remarkable recognition properties are inherently similar to those observed in large and complex biological systems, extending beyond simple non covalent associations to processes following complexation, such as transport, or catalysis with chemical transformations. However, in contrast to large biological systems, detailed information is available

on their constitution and structure(s) in the solid state, concerning their spectroscopic, thermodynamic and kinetic properties in various solutions and environments. Their small size allows us also to perform computer experiments in a reasonable time. Since our first molecular mechanics study on 18-crown-6 and its alkali complexes in 1980[7] this system has become to molecular modelling what the hydrogen molecule has been for quantum chemistry: subjected to various molecular mechanics,[8] Monte Carlo calculations,[9] conformational searches,[10] molecular dynamics simulations *in vacuo*,[11] in water[12] as well as in the solid state.[13] Complexation of alkali cations in water,[14] and in non-aqueous solvents[15] as well as complexation of neutral species[16] has also been modelled.

In the following, applications of computer simulations on quantitative aspects which could not be tackled with hand-made traditional models are presented. From the cartesian coordinates of the system, the energy can be computed rapidly by using empirical representations, and optimized close to the nearest minimum by molecular mechanics. Minimization can be followed by a stochastic hunt of the conformational space (Monte Carlo), or by the computation of trajectories as a function of time by molecular dynamics (MD) simulations. Computer modelling and graphical representation involve also very attractive aesthetic aspects, which can hardly be reproduced in such a publication[130].

CONFORMATIONAL ANALYSIS AND ORGANIZATION

One of the guidelines to build models in the field of molecular recognition by macro(poly)cyclic receptors is the "lock and key" concept, as introduced by E. Fischer, and later on essentially used for biological systems. Translation of this phenomenological scheme[17–20] into structural features implies that the conformation of the macrocycle is such that a cavity, or at least a concave region is present with a suitable orientation of the putative binding sites. Some of the receptors, like the Cram's anisole spherands[3] are topologically and conformationally preorganized in the course of their synthesis. Most often, however the receptor and the substrate are flexible, and their complementarity may be achieved only in very restricted parts of their conformational space. Cavity may not be present in the free receptor (in the "gas phase"), but may be induced by the substrate, or by a given solvent or environment. For instance, X-ray structures of 18-crown-6 (**18C6**) or of the bicyclic **222** cryptand in their free state and in the absence of molecular environment have no preformed cavity suitable for complexation.[21,22] X-ray structures of the receptor of cadaverine similarly shows that removal of this molecular substrate, leads to a more compact structure, with no cavity.[23] In such systems one would like to estimate computationally the energy needed to organize cavities, i.e. the "conformational organization energy". In fact, this energy is not simply an intrinsic feature of the host, since the solute/solvent interaction energies depend also markedly on the conformation of the solute, as shown with crown ethers[9] and cryptands[67]. Molecular mechanics affords relative energies of static structures "in the gas phase". In solution, molecular dynamics or Monte Carlo simulations are required to provide statistical representations of the solvent around the solute.

Classical conformational analysis describes primary intramolecular processes such as inversion at atomic centres or rotation around bonds in molecules taking into account steric and electrostatic intramolecular interactions. For host/guest or

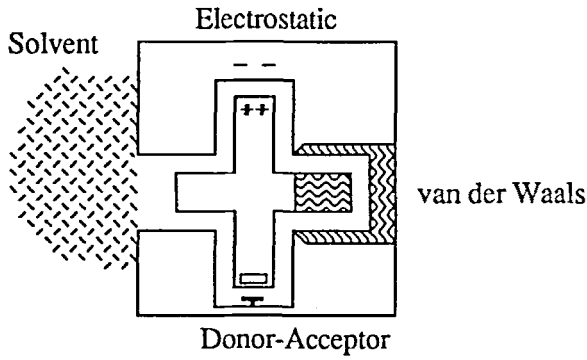

FIGURE 1 The lock and Key Scheme.

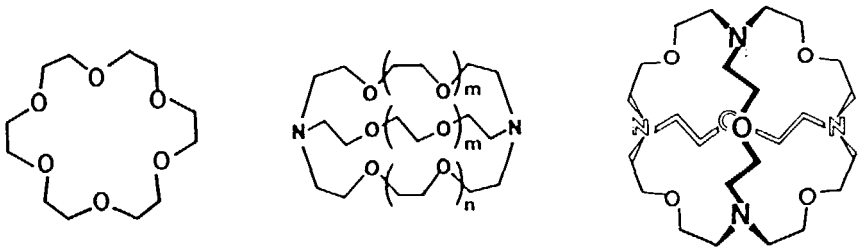

FIGURE 2 18-crown-6, the bicyclic and tricyclic cryptands.

receptor/substrate (*R/S*) complexes and for their recognition process non-covalent interactions are of primary importance and a "supermolecule" type approach involving the internal energy of *R* and *S*, their mutual interaction and the effect of the environment (*e.g.* counter ions, solvent molecules, *etc.*) should be used.

The basic computational procedures require to find "representative coordinates" of the system, *i.e.* an adequate sampling of the conformational space of *R*, of *R/S* isolated or in their environment, and to calculate rapidly the energy for each set of coordinates. One favourable case is when coordinates are available from X-ray diffraction studies on crystals, but they may not be representative of the structures in solution where molecular recognition takes place. We will see examples and limitations of molecular mechanics calculations on typical macrocycles in vacuo: monocyclic **18C6**,[7] bicyclic **222**[24] and tricyclic **SC24**[25] (Figure 2). To characterize in short the conformers, we refer either to their symmetry (*e.g.* **18C6-D_{3d}**), or to the structure of the complex from which it has been extracted. For instance, **SC24-N** and **222-K** are conformers of **SC24** and **222** cryptands, extracted from the NH_4^+ and K^+ cryptates, respectively.

We will then try to get dynamics pictures of these systems first *in vacuo* from the analysis of their low frequency normal modes of vibration and from MD simulations at 300K.[11] Statistical analysis of structures generated by high temperature annealed MD simulations provides alternative pictures of the flexibility and conformational preferences.[26] Reaction paths for ion inclusion into compact cages can also be

modelled and illustrate the importance of flexibility/rigidity of their cage, and of the size of the ion on intrinsic barriers.[27]

Finally, we shall illustrate how MD simulations on crown ethers, cryptands and cryptates in water provide microscopic details of hydration, and emphasize the *importance of solvent effects on molecular conformation and on the recognition process.* Of particular interest in supramolecular chemistry are applications of free energy pertubation calculations, which provide the thermodynamic measure of relative interaction and binding free energies *in solution.*

ENERGY REPRESENTATION AND COMPUTATIONAL TECHNIQUES

With present computer means, molecules of the size of crown ethers or host-guest complexes cannot be handled accurately with quantum–mechanical (QM) techniques, especially for energy minimization, or conformational sampling purposes. The few examples reported so far deal either with semi empirical QM such as CNDO[97], or with *ab initio* QM using small basis sets[99]. With QM techniques, the solvent can be at best represented via continuum models[100]. This is why a *force field* (FF) representation of the potential energy is used for MM, MC and MD simulations for supramolecular systems. It is conceptually easier to understand that the QM approach, because it attempts to quantify the ball-and-stick model used for years by chemists. The "balls" centered on atoms are connected by "sticks" (for covalent bonds). Problems arise when the covalent/non covalent character of the bonds is not clear-cut, e.g. for transition metal complexes[101,102], multicentered bonds, strong hydrogen bonds[103], etc...

We used the AMBER software[28] for MM and MD simulations, with the following representation of the potential energy:

$$E_T = \sum_{\text{bonds}} K_r(r-r_{eq})^2 + \sum_{\text{angles}} K_\theta(\theta-\theta_{eq})^2 + \sum_{\text{dihedrals}} (1+\cos(n\phi-\gamma)) + \sum_{i<j}[B_{ij}/R_{ij}^{12} - A_{ij}/R_{ij}^{6} + q_iq_j/\varepsilon R_{ij}]$$

The bonds and bond angles are treated as harmonic springs, and a torsional term is associated to the dihedral angles. The interactions between atoms separated by at least three bonds are described within a pairwise additive scheme by a 1-6-12 potential.

The parameters are derived from the AMBER force field[30] and can be found in references 7, 24, 25 and 67. Most critical is the electrostatic representation of the system, which was as much as possible calibrated from experimental gas phase data. The lack of an explicit polarization term in this force field led us to use and test different sets of charges depending of the presence and nature of host ion. For the uncomplexed macrocycles, the charges ($q_O = -0.3$, $q_N = -0.24$) account for the dipole moment of the OMe_2 and NMe_3 building units. For the alkali cation complexes, larger values ($q_O = -0.6$, $q_N = -0.6$) were required to reproduce experimental $M^+ \ldots OMe_2$ interaction enthalpies in the gas phase. A similar procedure was used to derive charges involving ammonium sites or the NH_4^+ substrate. Explicit account of distance and orientation dependent polarization energy and of many body effects would be more satisfactory,[30] but this is not incorporated in current modelling software performing MD simulations with reliable parameters, and requires extensive computer time. The

CH_2 groups were represented in the united atom approximation for the calculations *in vacuo*. For simulations in aqueous solution, the C—H hydrogen atoms were represented explicitely, and the charges on **222** and **222**,nH^+ were fitted on electrostatic potentials from *ab initio* calculations on adequate fragments.[67]. An interesting alternative procedure for force field derivation for crown ether complexes has been proposed by Pretsch *et al.* who combine *ab initio* derived pair potentials for the intermolecular energies and MM2 for internal energy of the crown[104].

Molecular mechanics optimizations relaxing the starting structure to the nearest energy minimum have to be performed consistently in terms of force field and procedure: we used conjugate gradient minimization followed in most cases by a Newton Raphson optimization to check for the second derivatives of the energy, and to obtain the normal modes of vibration.

Unless otherwise stated, MD simulations *in vacuo* were run for 100 ps at 300K starting with random velocities and using the Verlet algorithm with a time step of 1 fs.[29] MD simulations in water were performed for 50 ps with TIP3P water, constraining the water molecules and C—H bonds by SHAKE, and using a time step of 2 fs.

METHODOLOGIES FOR CONFORMATIONAL SEARCHING

In practice, one faces two (related) problems before running MM, or MD calculations. First, obtain the cartesian coordinates of "reasonable" 3D structures, as fashioned for instance in the hands of the chemist by manipulation of CPK models. Secondly, because the calculated energies have no absolute meaning, and have to be compared with "the" most stable conformers, a search for "representative" minima on a complex hypersurface of high dimensionality is required, although rigorously unfeasible. An excellent review of these questions can be found in reference 40. In supramolecular chemistry, specific problems arise because of the (poly)cyclic nature of the hosts, and of the way the structural requirements are formulated. For instance, one wants to build the complex of **18C6** with ammonium derivatives (Figure 3a) such that the crown anchors the NH_3^+ moiety, that the lateral substituents are in axial position, and that the ester carbonyl of the peptidic substrate may be attacked by one sulfur atom of the lateral chains of the receptor. Another challenging problem was to build conformers of the Barrel,$6H^+$ cage (Figure 3b) with conformations of three $-CH_2-NH_2^+-(CH_2)_3-NH_2^+-CH_2-$chains suitable for anchoring the encapsulated NO_3-anion[39].

Classical methods based on incremental building in terms of internal coordinates cannot be used because dihedral angles are unknown. Manipulations of 3D structures done easily in the hands of the chemist with CPK models,[31] involving concerted rotations around several bonds in such a way that a cavity forms, keeping the connectivity, and such that the binding sites are properly oriented cannot be done with current modelling software, especially for polycyclic systems. On the other hand, building methods such as symmetry repetition of given fragments[32] or drawing structures within a diamond lattice[33] are specific and cannot be used for general purposes. An elegant method based on the Fourier component analysis of the shape of cyclic systems has also been proposed, but seems hardly applicable to polycyclic systems with topological constraints[89]. The "corner flapping" method proposed for

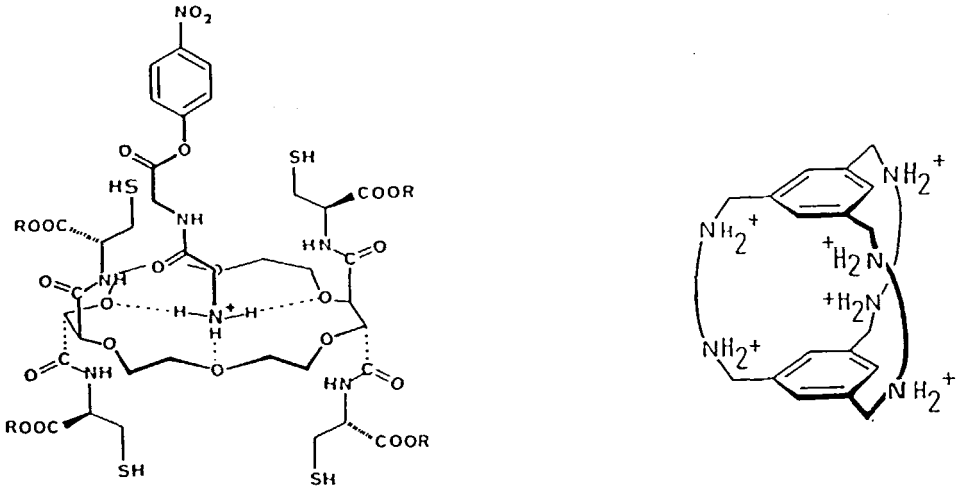

FIGURE 3 (3a left) "supermolecule" formed between a lateral derivative of 18C6 and an ammonium substrate (ref. 5). (3b right) the "Barrel,$6H^+$" receptor of NO_3^- (from reference 64).

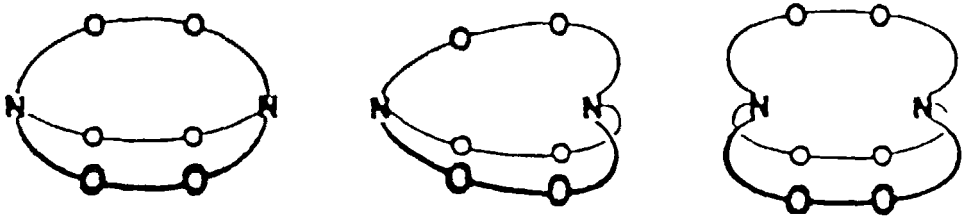

FIGURE 4 "Out-out", "out-in" and "in-in" topomers of the bicyclic 222 cryptand.

ring systems[105] seems hardly applicable to (poly)cyclic supermolecules meeting constraints such as cavity size and topology, convergence of binding sites, etc....

The Crippen's approach[34] based on the partially known matrix of interatomic distances revealed to be successful in translating constraints such as range of distances between non bonded centres or of dihedral angles into distances. From the upper and lower bounds of that matrix, several sets of coordinates could be calculated.[35] For instance, "in-out" forms of **222** (Figure 4) or of SC24[25] were generated by imposing Cc-N-LP angles of 0° and 180°, respectively (Cc is the Centre of the cavity, and LP is a point along the nitrogen lone pair).[24] One limitation of this approach is the small number of different structures produced and the bias introduced by the constraints. In addition, in order to find solutions a balance between short range and long range constraints is required.

The ELLIPSE technique of Billeter *et al.*[37,10] like DISMAN[38] uses the torsional angles as variables. Purely geometrical techniques such as ELLIPSE or distance geometry may not lead to low energy forms, nor to the absolute minimum. They have therefore to be coupled with energy optimizations and structure relaxations.[40] P. A. Kollman reported such a combined use of ELLIPSE and MD simulations for

18C6.[10] Houk *et al.* modelled phenanthrene macrocycles using ELLIPSE and AMBER or MM2 optimisations.[41] We performed a conformational analysis of **18C6**, **222**, **SC24** neutral and protonated combining distance geometry searches with AMBER.[7,24,25]

Monte Carlo searches taking the dihedral angles as variables[42] can hardly be used for polycyclic systems, especially when additional structural criteria have to be met. The Saunders stochastic method[43] combines random atomic displacements with energy refinements, sampling largely the conformational space of (poly)cyclic hydrocarbons and "hunting" for the global minimum. The latter method might be used for macrocyclic receptors as well. Annealing procedures used in conjunction with Monte Carlo simulations[44] have been reported for open or monocyclic molecules, but have not been tested to our knowledge on polycyclic systems.

Finally, quenched or annealed MD simulations at high temperature incorporating constraints should also provide suitable structures, as used for modelling structures from NMR data.[45] We will illustrate such an application for the **222** cryptand.[26]

A comparison of current techniques for conformational searching has been made recently, using consistently a MM2 energy representation for cycloheptadecane.[46] In this study, the focus was on low energy structures rather than on *structural organization*, and it is stressed that adding constraints such as size and organization of the cavity would make some of the methods (like Monte Carlo or systematic searches) significantly less performant.

ENERGY COMPARISON OF EXPERIMENTAL STRUCTURES: MOLECULAR MECHANICS "*IN VACUO*".

As a first, but non trivial exercise, one can calculate relative energies of hosts as fragments of solid state "supermolecules". Given the approximation of pairwise additivity of energy components, the receptor/substrate interaction energies can also be compared for various substrates. These gas phase investigations, although insufficient for solution studies, contribute to "supramolecular conformational analysis". Many results on the modeling of conformationally restricted hosts has been reviewed by Toner[106].

Energy comparison of selected forms, although being in principle a straightforward procedure suffers limitations related to the force field and to the optimisation procedures. Comparison of molecular mechanics and of *ab initio* calculations on **18C6** in its C_i, D_{3d} and C_1 conformations (Figure 5) illustrate that point.[7,8,10] There is agreement on the fact that the distorted C_1 form is the least stable, but not on the

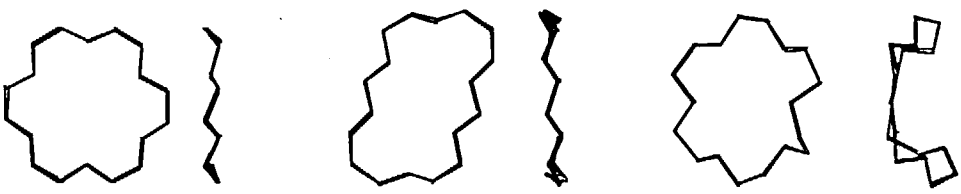

FIGURE 5 The D_{3d}, C_i and C_1 forms of **18C6**.

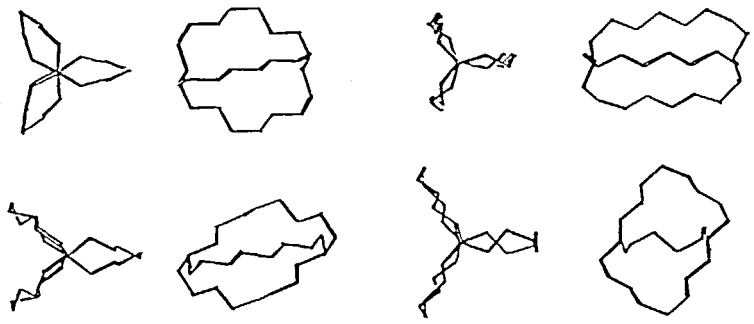

FIGURE 6 The K, II (first line), OO and SS (second line) forms of 222.

relative C_i/D_{3d} stabilities. The discrepancy is mostly related to the electrostatic effects: the repulsive 1-4 O—C—C—O interactions in the gauche arrangement disfavour D_{3d} relative to C_i. Similarly, relative conformational energies of **222** obtained by AMBER[24] and MM2[47] differ. Force field effects on relative stabilities of cone, partial cone and alternate conformers of calixarenes have recently been assessed.[86] It is therefore important to run several sets of calculations varying critical parameters such as the dielectric constant or the atomic charges in order to assess the origin and range of conformational preferences. The dependence of the relative stabilities on the FF and parameters, may be considered to result from the inadequacy of the MM approach. The FF needs to be consistent and makes implicit hypothesis of transferability of bond and fragment properties (e.g. chemical, electronic, spectroscopic, structural features), which represents a compromise between computational efficiency and accuracy of results. The QM results, although a priori more satisfactory, depend on the size of basis set, and on the level of approximation[111].

Relative stabilities and optimized structures depend also on the minimization procedure. For instance using the conjugate gradients algorithm gave increased stability for the **Ca**, **Na**, and **K** conformers of the **222** cryptand, in qualitative agreement with the expected strain induced in the Ca^{2+}, Na^+ and K^+ cryptates, respectively. However, Newton-Raphson optimizations converged to a same minimum of D_3 symmetry.[24]

Constrained minimizations may be used in principle to relax partially experimental structures and calculate the deformation energies induced by complexation. We found with the **222** cryptand that no conclusive results could be obtained by constraining the coordinates to their initial values because the results depend on the choice of the restraining force constant. On the other hand, experimental structures cannot be compared simply without being relaxed because of inhomogeneities in bond lengths due to crystal disorder, or to thermal effects in the crystal (for instance, the C—O bonds of **18C6** are on the average 1.411 Å at 300K, and 1.423 Å at 100K[48]). For model-built or randomly generated structures, which, contrary to experimental ones, may be far away from energy minima, more extensive relaxation is required combining for instance molecular mechanics and molecular dynamics optimizations.

Conformational preorganization. Ring closure and the (poly)cyclic nature of the receptors leads to high stability complexes, as compared to their open chain analogs. This is the *macrocyclic effect*, dominated by the enthalpic component of the complexation energy[2,95]. Comparison of the MM optimized K^+ complexes of

18-crown-6 and of pentaglyme supports qualitatively this view[7]. When the free receptor adopts a conformation suitable for binding a guest, it is said to be *preorganized* for this process, which contributes to enhanced stabilities. This is the case for anisole spherands [3,131] but not for most crown ethers and cryptands. For **18C6** and **222** uncomplexed, MM optimisations confirmed that their free forms found in the solid state (respectively **222-II** and C_i) are more stable than those extracted from the complexes, which indicates that these structures do not result from significant packing forces. However, the energy difference is weak (of a few kcal/mole) compared to the interaction energy with an ion (respectively about 95 and 115 kcal/mole, for the **18C6** and **222/K$^+$** complexes). In other words, *complexation induces a structural reorganization, but with no significant strain*, especially for the best "recognized" cation in solution (K^+ for **18C6** and **222**). Unlike crown ethers and cryptands, anisole spherands[49] display little conformational flexibility, and become preorganized for complexation during their chemical synthesis.[3,6]

Optimisations of the alkali cation complexes of **18C6** in various conformations were able to account for their structures: Na^+ prefers the C_1 form, K^+ the D_{3d} form in a nested position, and Cs^+ a perched position over the D_{3d} ring, as found in the crystal.[7] Similarly, optimisations of the **222/K$^+$** cryptates starting from structures with no cavity converged to the **K** form because of the electrostatic and steric strain induced by K^+.

The calculated binding energies. In these cation complexes, we calculate a decreasing complexation energy from Na^+ to Cs^+, as observed in the gas phase for binding of these ions to ether or amine binding sites. In aqueous solution, however, the selectivity peaks experimentally at K^+ for **18C6** and **222**, at Rb^+ for **SC24**, and at Na^+ for the anisole spherand.[2,5,6] Similar peaks are calculated when one substracts simply the experimental hydration enthalpies of these ions from the calculated complexation energies. However, such a crude procedure is unable to account for the binding selectivity of one given ion to different receptors[11,27] and could not be used safely for prediction purposes. In this context, it was remarkable that this simple approach used on spherands derivatives led to a correct prediction concerning their Li^+ binding selectivity, over Na^+ or K^+[49].

To model ammonium complexes of **18C6** and of its lateral amide derivatives (Figure 3a) we used a step by step building procedure, from fragments of experimental structures.[50] Here, molecular mechanics optimisations account for the binding selectivity observed in the gas phase (primary > secondary > tertiary ammonium) and in solution, as well as for the different modes of binding. However, when the structural complexity of the system increases, e.g. with longer ammonium substrates or lateral

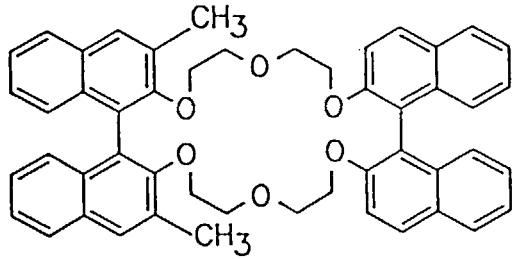

FIGURE 7 The Cram's chiral macrocycle.

arms, no firm conclusion could be drawn because of the multiple minima problem.[50] Particularly, the ability of that system to display chiral recognition could not be determined.

Chiral recognition of ammonium substrates. In the MM study of the chiral Cram's receptor based on binaphtyl units (Figure 7) and its L/D complexes with (Phenyl)-$^{+}$Gly(OMe) a weak energy difference (2.0 kcal/mole) involved in the chiral recognition displayed by this macrocycle was found, in agreement with experiment[50].

Similar molecular mechanics studies on chiral recognition for a series of ammonium substrates have been reported, and led to a satisfactory agreement between calculated relative L/D binding energies and experimental activation energies for decomplexation.[90]

MOLECULAR MOTIONS DESCRIBED WITH THE HARMONIC MODEL: LOW FREQUENCIES OF VIBRATION.

The molecules are not rigid, and undergo fluctuations around their equilibrium structure, with possible conformational interconversions. In the solid state, atomic motions which are temperature-dependent, are pictured by the ellipsoïds centered on the average positions. It has been shown by solid state NMR that complexes of **18C6** undergoes a "merry-go-round" process[108]. From a theoretical point of view, it is easy to calculate the 3N-6 normal modes of vibration of molecules of a few hundreds of atoms. Schematically, as observed in vibrational spectroscopy, the highest frequencies correspond to bond stretching motions localized on strong bonds, and lower values are observed for angle bending processes. We found of interest to analyze the lowest frequency modes, because they are related to torsional motions around bonds, leading to global molecular deformations. In the first frequencies (below 100 cm^{-1}), harmonic displacements of the atoms of about 3 Å take place at low energy

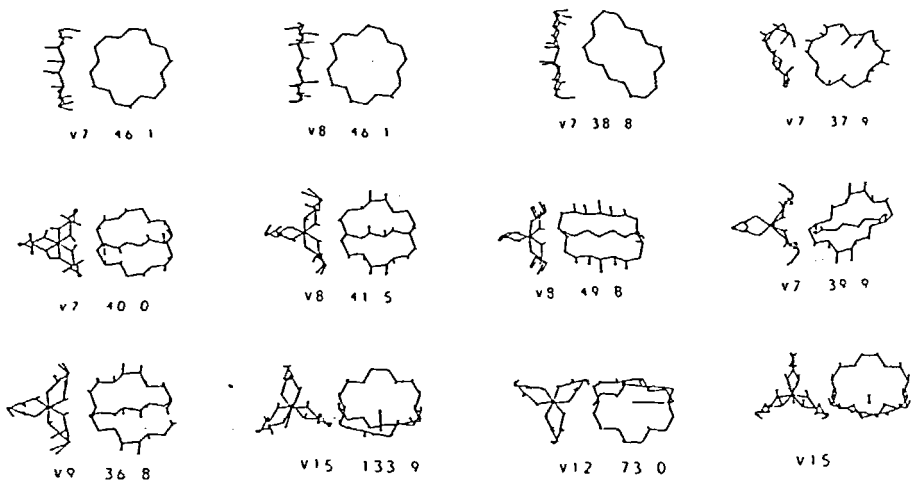

FIGURE 8 Typical low frequency normal modes of vibration (orthogonal views) of **18C6** free (line 1) in its D_{3d}, C_i and C_1 forms, of **222-K, 222-II, 222-OOO** free (line 2), of **222/M^{+}** (respectively K^{+}, Na^{+}, Cs^{+}) cryptates (line 3).

cost (less than 5 kcal/mole). The graphics display of these vibrations either with static vectors of elongation, or as dynamic pictures,[51] revealed interesting qualitative features concerning the type of motions and deformations, which may be important for the recognition properties of these macrocyclic receptors.[11]

First, for *the free receptors, there are normal modes of vibration which provide a path for substrate inclusion.* For instance, among the three first normal modes of vibration of the monocyclic **18C6**, the bicyclic **222**, and the tricyclic **SC24** receptors, some lead to an opening of the cavity and make its binding sites accessible to the solvent or to an approaching substrate (Figure 8). This feature is not restricted to the conformers extracted from cation complexes. For instance for **18C6**, ν_7 and ν_8 of the D_{3d} form, as well as ν_7 and ν_{10} of the C_i form, or ν_7 and ν_9 of the C_1 form correspond to a same type of ring folding. For the bicyclic **222** cryptand, ν_8 and ν_9 of the **222-K** form, ν_7 and ν_8 of the **222-II** form, or ν_7 of the **222-OO** form are such that two bridges open/close and make the cavity less shielded. This contrasts with ν_7 of **222-K** which is a symmetrical breathing of the cage (Figure 8). In **SC24** neutral, or tetraprotonated in the "in-in-in-in" form, one face of the tetrahedron formed by the nitrogens opens while the opposed nitrogen approaches, making a kind of breathing motion of C_3 symmetry.[11]

In a barrel type tricyclic topology, the "**Barrel,6H$^+$**" (Figure 3b) receptor of NO_3^- [64,132] displays opening motions of the ammonium chains among the first vibrations. Such molecular motions are probably a general feature of receptors, and have been characterized in larger biological systems like lysozyme or trypsin.[52]

In the complexed macrocycles, some low frequency vibrations provide paths for cation extrusion and solvation. For the **222**/Na^+, K^+ and Cs^+ cryptates, ν_8 is the characteristic modes, degenerated with ν_9 (Figure 8). Similarly, ν_8 of the **SC24**/K^+ or **SC24**/NH_4^+ cryptates is a "decomplexation mode".[11,27]

Vibrations of the ion inside the cages reflects also the ion/cage complementarity. Indeed, when the cation fits into the cage like K^+ in **18C6** or in **222**, it is immobile in the lowest frequency modes. Otherwise its moves in a characteristic way. For instance in ν_7 of the **18C6-D_{3d}** complex, Na^+, which is slightly too small undergoes in plane librations of large amplitude; Cs^+ which is too large oscillates along the C_3 axis above the ring. In the **222** cryptates, the first motion for Na^+ is a large oscillation along the N...N axis inside the cage (in ν_{12}, 73 cm^{-1}), whereas K^+ is expelled through one face at higher frequency (ν_{15}, 134 cm^{-1}) and Cs^+, squeezed in the cage, is prevented from moving (Figure 8).

Although these motions are pictured in the harmonic approximation, they should be of significance for the recognition processes and express more generally the receptor-substrate complementarity. From a quantitative point of view, the lowest frequency modes contribute significantly to the vibrational entropy, and therefore to the relative free energies[109]. Interestingly, the gas phase conformers of **18C6** generated by a MD simulation of 6 ns have been shown to approach a Boltzmann ensemble when, and only when the vibrational energy contribution were included[110].

MOLECULAR MOTIONS *IN VACUO* AT 300 K, OBTAINED FROM MOLECULAR DYNAMICS SIMULATIONS

During "real" molecular dynamics ("MD") simulations, the particles follow trajectories determined by the classical Newtonian equations of motion. If the kinetic

energy of the N atoms (related to the temperature T by $\sum m_i v_i^2 = 3N\,kT$) is sufficient, the system can move over energy barriers from one potential well to the other, i.e. undergo conformational transitions. At 300 K, the time scale of 100 ps used here, is too short to allow for significant conformational interconversions and sampling. The system may relax and escape from structural instabilities, or fluctuate around an energy minimum. We calculated the root mean square atomic displacements (RMS) from the average position during the MD simulation to size the amplitude of the motions[11].

The uncomplexed macrocycles: conformers with cavities undergo the largest motions.

Based on topological criteria alone, one would expect a decrease in atomic fluctuations from monocyclic, to bicyclic and tricyclic receptors. The RMS values reported Figure 9 for several conformers of **18C6, 222** and **SC24** show that this is not the case. For a given receptor, there is no general relation either between stability and amplitude of motion. One could anticipate that the size of the systems relates to its mobility. However, within a series of bicyclic cryptands (**111, 211, 221, 222** as defined Figure 2), no such relationship is found. The *largest RMS values correspond in fact to conformers which display a large cavity;* conversely, the forms without cavity are more rigid[11]: in the free state of the receptor "in the gas phase", the atoms move in such a way as to fill this cavity[41] (compare for instance the **K** and **II** forms of **222**, Figures

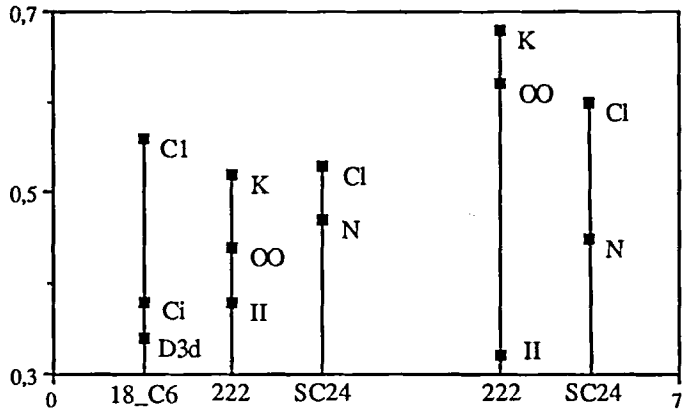

FIGURE 9 RMS fluctuations (Å) for 18-crown-6, the 222 and SC24 cryptands in various conformations, calculated with two different charge representations, *Left:* with charges $q_O = -0.30$, $q_N = -0.24$. *Right:* with $q_O = -0.40$, $q_N = -0.54$.

FIGURE 10 Cumulative views of the **222** cryptand and **222** cryptates from 50 ps of MD in vacuo (orthogonal representations).

9 and 10). It is thus expected that the solvent will play a major role on the dynamics, because of the possibility to fill the cavities, and of specific interactions with the host.

The cation complexes: complementarity and dynamics

The substrate encapsulated inside the cavity of the receptor reduces its conformational freedom, due to steric and electrostatic interactions. Indeed, in the cation complexes of **18C6**, **222** and **SC24** one observes a *rigidification* compared to the free receptor (compare **222-K** with **222/K$^+$**, Figure 10). For the K^+ complexes of **18C6**, **222-K** and **SC24-N**, the RMS's drop respectively by 0.21, 0.40 and 0.12 Å.

The relative fluctuations of the cation, as compared to the cage, depend also clearly on their complementarity. For instance, from Na^+ to Cs^+, the fluctuations of the cage decreases in the three macrocycles: from 0.33 to 0.28 Å in **18C6**, from 0.40 to 0.23 Å in **222** and from 0.33 to 0.29 Å in **SC24**, as a result of the increased size of the ion. Although Na^+ interacts more strongly with the cages than K^+ or Cs^+, it does not rigidify them as much.

Dynamic coupling between a flexible cage and the complexed cation. It is interesting to compare the relative mobility of the cations within the **222** and the **SC24** cryptates (Table I). It decreases as the size of the ion increases and Cs^+, which is somewhat compressed in **222** as in **SC24**, has the weakest fluctuation. A dramatic difference is observed for the smallest ion. Na^+ fluctuates in **SC24** more than twice as in **222**! We believe that this is a result of the *difference in flexibilities* of these cages: **222** is flexible not only in its ability to adopt various conformations, but also in being able, in a given conformation, to adjust its cavity size to that of the substrate. The flexibility of **222** reaches however a limit in the Ca^{2+} cryptate, in which the Ca^{2+} ... O distances are larger than the sum of van der Waals radii.[21] On the other hand, the highly connected **SC24** cryptand is quite rigid and keeps a fairly constant cavity size in its free state as in its inclusion complexes, but that cavity is too large for Na^+.

The cations complexes of **18C6-D$_{3d}$**, in contrast with those of **222** and **SC24**, display an interesting non regular behaviour from Na^+ to Cs^+. This is because Na^+ and K^+ can fit into the ring, whereas Cs^+ too big for **18C6** sits and fluctuates over the ring. As a result, Cs^+ is less anchored than K^+ and fluctuates more, respectively by 0.28 and 0.24 Å. For **18C6**, the RMS's peak at Rb^+, whose size allows fluctuations between "inclusive" and "perched" type positions.

A similar dynamic coupling between the motions of the host and of the guest has been calculated also when linear diammonium substrates $^+H_3N\text{-}(CH_2)_n\text{-}NH_3^+$ of increasing length (n = 4 to 7) are complexed by a tricyclic barrel type receptor[23] (see figure 27 of ref. 5). Interestingly, the complexation energies calculated *in vacuo* do

TABLE I
Fluctuations of the cations (Å) in **18C6-D$_{3d}$**, **222-K** and **SC24-N**.

Host	Na^+	K^+	Rb^+	Cs^+
18C6	0.33	0.24	0.30	0.28
222	0.25	0.16	0.15	0.14
SC24	0.55	0.29	0.20	0.16

not peak at n = 5, as observed experimentally in chloroform solution. However, the correlation coefficient between the deformations of the cage and positioning of the substrate peaks at n = 5, because of *optimal dynamic complementarity*[112].

Importance of mobility for catalytic processes. MD simulations on ammonium complexes of **18C6** unsubstituted and substituted by lateral amide arms,[50] modelled as anchoring site in an "artificial enzyme" (Figure 3a) shed light on the *possible importance of the mobility for efficient catalysis* by such systems. Indeed, we calculate that amidic fragments increase both the stability and the mobility of R-NH_3^+ complexes, compared to **18C6** unsubstituted. It might have been anticipated that increased stability induces reduced mobility. Above, for the alkali cation complexes of **222** and **SC24**, we have seen that it was not so. In the ammonium complexes of lateral amide derivatives of **18C6**, the electric field of the amide carbonyles facilitates indeed both the binding of the substrate and its oscillations away from the crown ether oxygens. Catalytic behaviour requires the supermolecule to be flexible enough to move from stable non covalent "Michaelis complexes" to stabilized transition states involving covalent binding between reactive centres of the receptor and of the substrate.[53–55] Our MD results confirm that such an effect operates in "artificial enzymes" based on macrocyclic units in which enhanced stability is accompanied by increased mobility.[50] Biological catalytic systems might take advantage of that dynamic effect as well.

SAMPLING OF THE CONFORMATIONAL SPACE USING HIGH TEMPERATURE ANNEALED MD SIMULATIONS

Solid state structures of host-guest complexes are essential as proofs of their inclusive nature, and for understanding the related non-covalent interactions leading to their stability[21,22]. The crystal itself can be considered of supramolecular nature[113], and the conformers found in the solid state complexes may not be representative of the intrinsic preferences. In terms of "receptor design", it would be desirable to build computationally conformers suitable for selective complexation of a given substrate, i.e. with a cavity of appropriate size and orientation of binding sites.

In order to gain insight into these questions, we consider the **222** cryptand and sample its conformational space *in vacuo* by using several annealing MD simulations[26,56]. The structures are compared with solid state conformers, and analyzed from the point of view of their binding properties. Briefly, the procedure used for "High Temperature Annealing" simulations is the following. First the molecule is severely shaken by MD at high temperature (1000K) for 100 ps, then each of the 500 structures saved every 0.2 ps is energy minimized by MM, reshaken at 300K during 20 ps of MD in order to allow for enough relaxation, and finally reoptimized.

Starting from several structures, it was gratifying to find in the final collection of 500 annealed structures the experimental **222-II** one which was calculated as being the most stable among X-ray conformers, as well as new "in-in" slightly more stable ones. Thus, this procedure is able to produce "the" global minimum and confirms that several forms similar in shape (quite elongated and without cavity) are in equilibrium.

One remarkable result is the *absence of any form of cryptate* in that set, and particularly of the **K** conformer which displays the highest complementarity for K^+. Several simulations were repeated including a gradual representation of the complexed

cation, either as a pure electrostatic driver (+1 dimensionless charge) or with an electrostatic+steric representation which simulates K^+. We found that the **K** form was generated only in the presence of K^+, although being only a few kcal/mole higher in energy than **222-II**. This shows that particular forms of **222** acting as receptors and ionophores, like those which bind Ca^{2+}, Ag^+, or Pb^{2+} have a low probability to be present in the absence of their substrate, and that *222 is topologically, but not conformationally preorganized for cation complexation.* More generally, in terms of drug design, this suggests that conformations of a flexible drug able to bind to a receptor have very low probability to be found computationally *in vacuo* if the supermolecule formed with the receptor is not considered explicitly.

During the MD run at 1000K several "in"/"out" conversions take place, and the final set of annealed structures contains populations of "in-in", "in-out" and "out-out" topomers. The energy distribution of these classes (Figure 11) shows a clear preference for converging orientations of the bridgeheads. The populations increase in the order "out-out" < "in-out" < "in-in" (respectively 7%, 33% and 61%) and the peaks of these classes (respectively at 10.1, 8.7 and 6.2 kcal/mole from **222-II**), as well as the lowest energy forms (respectively at 4.4, 3.4, −0.8 kcal/mole from **222-II**) confirm the preference for "in" forms. New minima of each topology are also found (Figure 12).

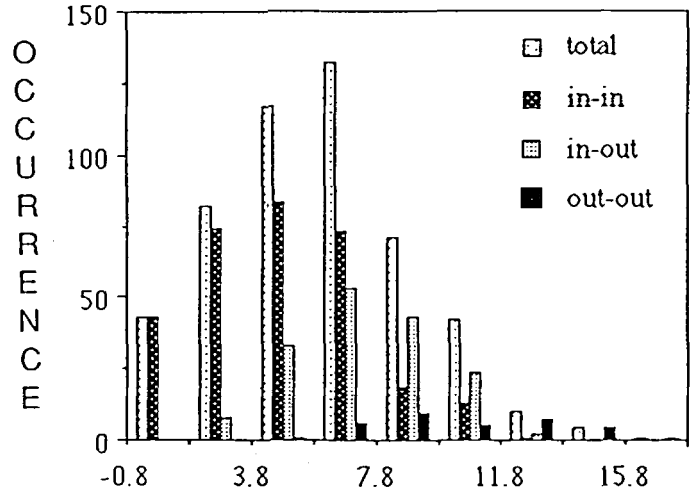

FIGURE 11 Occurrence of "in-in", "in-out" and "out-out" forms of **222** after the High Temperature Annealed MD simulations.

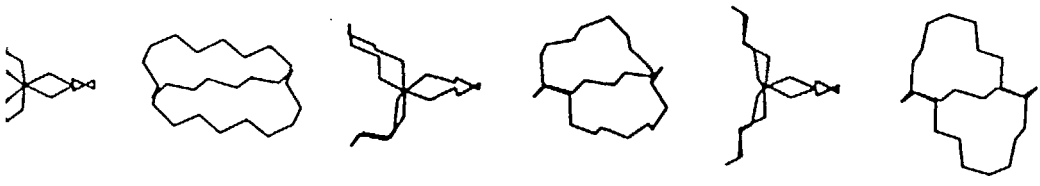

FIGURE 12 Lowest energy conformers of **222** "in-in", "in-out" and "out-out" generated by the High Temperature Annealed MD simulations (orthogonal views).

Interestingly, most of low energy forms have non converging orientations of the oxygen binding sites in the gas phase. Several have indeed a large "solvent accessible surface".[57] Such conformers, not characterized in the solid state for **222** itself, are expected to be better hydrated than the hydrophobic ones extracted from cation inclusion complexes, and to play a particular role for ion capture, as well as for the ionophoric behaviour. The energy difference between stable "in" and "out" forms is small enough to be compensated by hydrogen bonds involving a protic solvent, and therefore these forms are expected to be in equilibrium in solution.

This study pinpoints the difficulty of sampling the conformational space of macro(poly)cyclic receptors, and of finding "blindly" by computer the conformations adequate for complexation, because of sampling procedure and of force field effects. The conformational search of Billeter *et al.* on **18C6** failed also to produce *in vacuo* the D_{3d} conformer, which is the most relevant for K^+ complexation.[10] This apparent failure may be related to insufficient sampling and to the too small number of conformers generated. We feel however that it tells something more fundamental about these flexible systems: (i) in the solid state, the conformational space is very restricted, and the collection of structures of the host is not representative of conformational minima in other phases; (ii) in the gas phase, the relative population of structures of "receptor type" is low. Most of the conformers are "unactive" for complexation purposes. In terms of complexation, there is therefore an *entropy cost for conformational organization of the receptor*, which is largely compensated by the enthalpic components of receptor-substrate interactions. (iii) Gas phase sampling of structures, even with very precise energy calculations, may be irrelevant for exploration of conformational minima in solution (see next the examples of **16C6** and of **222** in water).

From a methodological point of view, we did not analyze the effect of temperature, and of the length of simulation on the results. These effects have been discussed by Sun and Kollman in their 6 ns MD simulation of **18C6** *in vacuo*[110]. The annealing procedure tested above for **222** could in principle be used more generally for receptor-substrate "supermolecules", taking into account intra- and inter-molecular energy components, whereas the calculations reported so far with MM2 type calculations are restricted, to our knowledge, to the receptors alone.[88]

REACTION PATHS FOR INCLUSION OF THE SUBSTRATE INTO THE RECEPTOR

Conformational analysis of macrocyclic supermolecules should not only consider the extreme states (free *vs* complexed macrocycle), but also the process of ion capture. The energy and structure of transition states for ion inclusion as a function of the substrate is of particular interest. Experimental data for complexation of alkali cation by cryptands in solution show that complexation is fast compared to decomplexation, and driven by the stability of the inclusion complex formed.[59]

In the gas phase, reaction path and kinetics for complexation of Na^+ by **18C6** *in vacuo* has been calculated using canonical variational transition-state theory.[61] We modelled the inclusion of alkali cations and of NH_4^+ into **222** and **SC24**, and the inclusion of Cl^- and Br^- halides into **SC24,4H**$^+$ in the gas phase,[25,27] using step by step molecular mechanics optimisations and molecular dynamics simulations.

Modelling the complexation process in solution is a difficult task due to the

conformational changes of the receptor, and to desolvation/solvation effects. Pohorille *et al.* modelled the capture of Cl^- by **SC24,4H**$^+$ in water using MD simulations and found that desolvation of Cl^- and deformation of the receptor contribute mostly to the barrier.[60] Recently, the free energy profile ("potential of mean force") for the complexation of K^+ by 18-crown-6 has been calculated to be a downhill process[14].

More generally, understanding and prediction of the *nature* of the complexes as a function the partners and environment is a very challenging computational problem. Whereas X-ray data confirm that the Cs^+ complexes of **222** are of inclusion type[21], NMR data in solution show that, depending on the solvent and on the counterion, the complexes may be either "inclusive" or "exclusive".[63] A more dramatic case is the "**Barrel,6H**$^+$"/NO_3^- complex (Figure 3b) where NMR data in aqueous solution provide convincing evidence for formation of an inclusive cryptate of 1:1 stoechiometry, whereas X-ray analysis of the crystal shows that the NO_3^- anions have *external* coordination with the ammonium sites of the cage![64] We are presently investigating these problems using MD simulations.[132]

MODELLING CONFORMATIONS IN SOLUTION AND SOLVATION PATTERN

The prediction of structures in solution can be in principle achieved through long MD simulations taking into account explicitely the solvent. Simulation times of a few hundreds of ps are too short to allow for significant conformational interconversions but allow for reorientation of water molecules around the solute. The longest MD simulation reported so far for a macrocyclic receptor has been of 1.5 ns for **18C6** in water.[12] Significant structural interconversions took place, leading to more than a thousand of different conformers, but no convergence was found.

A less computer time consuming task is the analysis of solvation pattern for given conformers of the solute, a problem of particular interest given the ionophoric properties of many synthetic macrocycles, analogous than those of natural antibiotics.[21] In solid state structures, solvent molecules may be strongly coordinated

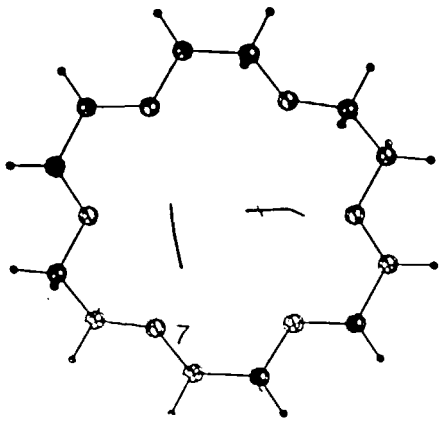

FIGURE 13 Hydration pattern simulated by Monte Carlo for **18C6** in the D_{3d} conformation[9]. The water molecules are represented on the top face only. On the bottom face of the crown, they are related by an inversion center.

to the macrocycle or to its complexed ion[21,22] and have to be considered as part of the "supermolecule". Modelling solvation pattern can be in principle achieved through MC or MD techniques, the latter allowing for the dynamics of the solute and of the solvent. Monte Carlo simulations reported so far in supramolecular chemistry deal either with macrocyclic receptors restricted as rigid solutes[66], or with flexible molecular clefts with limited degrees of freedom[82].

Hydration pattern of 18-crown-6 from Monte Carlo simulations

We performed Monte Carlo simulations on **18C6** hydrated in its D_{3d}, C_i and C_1 conformations.[9] From these simulations, it was suggested that dissolution of the crystalline C_i form would lead to a conformational change, and that the D_{3d} conformer (Figure 13) might be populated in solution. In addition, the water structure around **18C6** was predicted for the three conformers of the crown, and confirmed later by a X-ray study of a **18C6/PO_4H_3/6H_2O** complex.[65] This prediction has been supported by subsequent MD simulations of **18C6** in water.[12] Similar Monte Carlo simulations have been performed on monocyclic polyamines uncomplexed and complexed by Mg^{2+}.[66]

Hydration of the 222 cryptand from MD simulations

How an "hydrophobic" conformer can be particularly well hydrated! We simulated by MD the hydration of the 222 cryptand in water for 50 ps.[67] Four typical conformers were compared: **II, K, OO** (see above) and **SS**, taken from a dithia analogue of **222**,[58] and the average solute-water interaction energies E_{SW} calculated (Table II). We expected the **K** form, with converging N, O binding sites and diverging CH_2 groups to have the poorest hydration, compared to **OO** or **SS** in which the hydrophilic N or O binding are diverging and can make hydrogen bonds with the water molecules. The results turned out to be surprisingly not so. Among these forms, **II, OO, SS** have comparable E_{SW} energies, and the **K** form appears to have a significantly better hydration! A more detailed analysis showed that the main difference comes from the hydration of the oxygen atoms of **222**. Radial distribution functions, dynamical hydrogen bond analysis and graphical display confirmed that the **K** form is stabilized *via* a very specific water structure, as shown in Figure 14. Typically, there are three water molecules, each of them sitting between two bridges of **222** and being doubly hydrogen bonded to the oxygens atoms of two bridges. Without imposing any symmetry constraint during the simulation, an arrangement of approximate D_3 symmetry, involving three such water molecules appeared. These latter bring about 3 times 13 kcal/mole of interaction with **222** and contribute mostly to the more

TABLE II
The **222** cryptand in water. Energy component analysis (kcal/mole).

Conformer	II	OO	SS	K
$\langle E_{SW} \rangle$	−49(5)	−48(5)	−42(4)	−79(5)
$\langle E_{222} \rangle$	+27(4)	+29(4)	+25(4)	+39(4)
ΔE_{opt}	0	+1	−1	+9

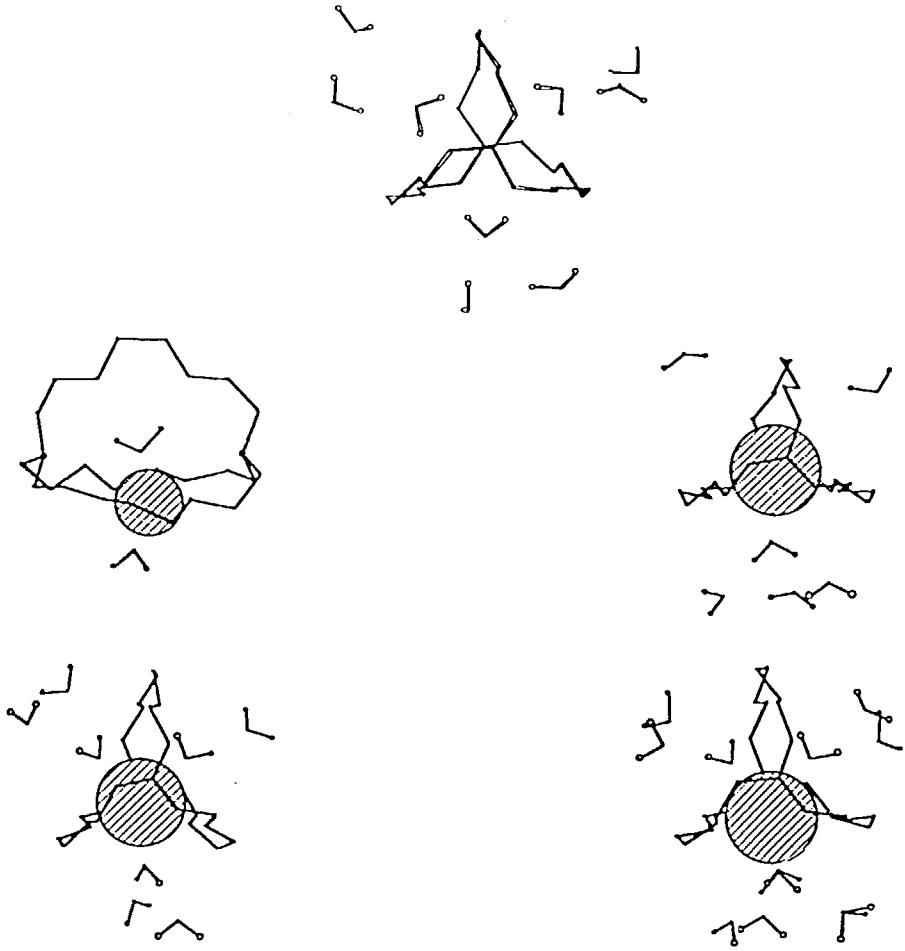

FIGURE 14 Selected water molecules around the K form of the **222** cryptand (first line), and the Li^+, Na^+ (second line), Ca^{2+} and Eu^{3+} (third line) **222**cryptates.

negative E_{sw} for the **K** form, compared to **II**, **OO**, or **SS**. Accordingly, we would predict the **K** form to have a significantly higher population in water.

Does a water molecule take the place of the cation in the uncomplexed form of the cage? We explored the possible role of water in filling the cavity of such ionophores and initiated MD simulations on the **K** form in water, starting with one water molecule inside the cavity. After a few ps, this water molecule moved to a facial position as displayed in Figure 14. Thus, although the cavity of **222** is of hydrophilic type and large enough to accommodate water, we find no water inside, and that an alternative scheme of facial hydration is preferred.

Water coordination to the free host: importance of bridging water molecules. As for **18C6**, this result emphasizes the *importance of bridging structures of water in stabilizing specific conformations of crown ethers, cryptands, and related compounds.*

It appears clearly also that stabilization by water cannot be simply predicted from the surface of the heteroatoms of the solute accessible to the solvent. The solvent granularity is at least as important as its macroscopic properties in determining the conformations in solution. Some of the strongly coordinated solvent molecules have to be considered as part of a "**222**-water supermolecule".

Analogous bridging structures of water surrounding polyethers have been found by a systematic search in the Crystallographic Cambridge Data Base[112].

It appears, like for **18C6**, that *water stabilizes the conformation of 222 conformationally preorganized* for complexing K^+! On the other hand, it is stressed that the complexation of cations into the particularly well hydrated form will require removal or reorientation of these water molecules, which might contribute to the abnormally low rates of complexation by cryptands.[68] Hydration of the other conformers is less favoured because topological features prevent formation of strong and/or cooperative hydrogen bonds with water.

Hydration of the 222/M^{n+} cryptates. Does the cage shield the cation from the solvent?

Similar MD simulations on cryptates of **222** in water have been performed for the series of alkali cations (Li^+ to Cs^+) and for Ca^{2+} and Eu^{3+} cryptates to study the effect of size and of cationic charge on the mobility of the complex and on its hydration pattern.[67] It was found that for all cryptates, there are significant interactions between the cation and water, in the first hydration shell and at larger distances (see Figure 14).

In the first coordination shell of the cations, the water molecules are coordinated *via* their oxygen atom, and their dipole is reversed compared to the hydration of the **K** form uncomplexed. Typical structures are displayed in Figure 14 and supported by statistical analysis of the results, and by the radial distribution functions. In the Na^+, K^+, Rb^+ and Cs^+ cryptates, the cation sits near the centre of the cavity, and has a coordination number of 0.9, 1.4, 1.3 and 1.3, respectively. The Li^+ cation, is on the average at the centre of the cavity during the dynamics *in vacuo*, but moves to one face of **222** in water. It is bound to two water molecules, one of them being held inside the cavity of **222** by additional hydrogen bonds. This pattern of cooperative binding of Li^+,H_2O is strikingly similar to that found in the solid state structure of the 18-crown-6/Li^+/H_2O complex. From Li^+ to Cs^+, dynamical coordination also differs. Indeed, the smallest cations have stronger interactions with water, which is less labile. Accordingly, the number of water molecules involved, and the relative residence time is 2.0 for Li^+ (100%, 100%), 1 for Na^+ (90%), 2 for K^+ (80% and 20%), 4 for Rb^+ (19%, 16%, 18%, 43%) and 3 for Cs^+ (65%, 16%, 24%). The Na^+, Ca^{2+} and Eu^{3+} cations have been modelled with a same radius, and a charge going from 1.0 to 3.0. During the dynamics in water, they move slightly to one face of **222**, being on the average at 0.5, 0.8 and 1.3 ± 0.1 Å from the centre of mass of the cage. This is because these ions are somewhat too small for **222**, and additional coordination to the solvent is required when the charge increases. Indeed, we find a coordination number of 3.0 for Ca^{2+} (3 distinct water molecules, bound for 100% of the time) and 3.9 for Eu^{3+} (4 distinct water molecules bound for 94, 100, 100 and 100% of the time).

Water is also structured in the second shell of the cations, with an orientation reversed compared to the uncomplexed **K** form. This is due to water first shell/second shell interactions, and to the electrostatic field of the cation, particularly in the Na^+, Ca^{2+} and Eu^{3+} series (Figure 14). Finally, in the third shell of the cation, *i.e.* around

the CH_2 "hydrophobic" region, water in oriented like in the uncomplexed **222** for all cations, except for highly charged Eu^{3+} cryptate.

These results are consistent with several thermodynamic and spectroscopic observations which show that the encapsulated cation is not completely shielded from the solvent, and that there may be significant interactions between the solvent and the uncomplexed ligand as well.[69–72]

The 222/M^{n+},n Cl- cryptates in water: are the ion pairs separated?

Cation complexation by cryptates is used experimentally to separate the anion from the cation, and to enhance its chemical reactivity[2]. In the solid state structures of alkali cation cryptates, the anion is not in contact with the inclusive cation. Divalent or trivalent cations however, are coordinated to solvent molecules and/or to anions[21]. We investigated the question of ion pairing by MD simulations of 50 ps for **222/M^{n+}** cryptates in the presence of *n* Cl- anions, first in the gas phase, then in aqueous solution (M = Li, Na, K, Cs, Ca, Eu). The starting structures correspond to intimate ion pairs, optimized by molecular mechanics. There are interesting new features, as compared to those obtained previously[114].

First, in the gas phase, M^+ does not remain, on the average, at the centre of the cage. Because of the Cl- attraction, the cations move to one face of **222**, i.e. evolve from one inclusion type complex to a *facial complex*. In particular, the K^+ structure (Figure 15 Left) resembles the "exclusive complex" proposed by Popov *et al.* in non-aqueous solutions[69]. Cs^+ is nearly outside the cage.

Second, in water, *the dynamics of the complexes depends on the size and on the charge of cation.* The **222/Na^+** cryptate remains for 50 ps in contact with Cl-, whereas Cl- and the K^+ and Cs^+ cryptates dissociate in the first ps of the simulation. The monovalent cations remain now close to centr of the cavity. As the charge of M^{n+} increases, the Cl- anions are more attracted by M^{n+}. Depending on the protocol used for the simulation, 1 or 2 Cl- anions remain in direct contact with the Ca^{2+}, and 2 or 3 Cl- in contact with **222/Eu^{3+}** cryptate in water. Figure 15 shows an interesting configuration of the **Ca^{2+}/222** cryptate in water after 50 ps: one water molecule is anchored inside the cage by Ca^{2+} and by hydrogen bonds to ether oxygens. Clearly, the substrate is not Ca^{2+}, but Ca^{2+},1H_2O, similar to the Li^+,1H_2O substrate calculated in water[67].

These microscopic results are in qualitative agreement with experimental data, and demonstrate *the importance of solvent and environment effects on the precise structure and dynamics and the complexes*, and therefore on their stability. Similar studies on

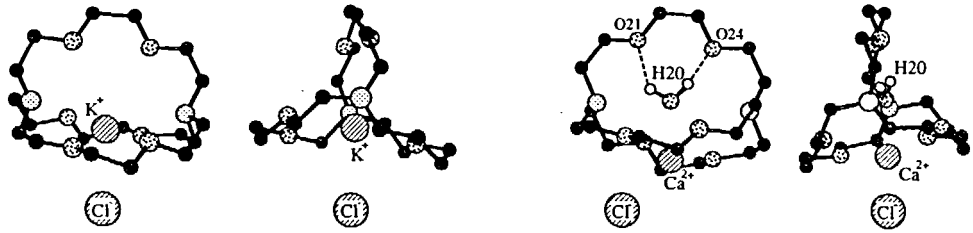

FIGURE 15 *Left*: The **222/K^+,Cl^-** cryptate *in vacuo*. *Right*: The **222/Ca^{2+},2Cl^-** cryptate in water. (Orthogonal views).

calixarene/M^{n+} complexes showed that the structures in solution cannot be simply inferred from one extracted from the solid state[115]. This structural flexibility is important for (de)complexation and ionophoric behaviour. It is stressed that traces of water present in non-aqueous solvents may play a particular role for complexation processes.

The protonated 222,H^+ and 222,$2H^+$ cryptands in water: "in vs *"out" topomers*

We modelled monoprotonated and diprotonated forms of **222** in water, in order to have insight into their "in"/"out" conformations. There has been indeed much debate about this question, without clear conclusion.[72,77] Classical MD cannot simulate in-out proton exchange involving bond breaking and bond making processes, *via* solvent molecules. We therefore restricted the study to a comparison of the average E_{SW} energies and of the internal energies for two typical conformers: **K** and **OO** (Figure 15). The results show that intrinsically, internal protonation is favoured over external protonation (for **222,1H**$^+$ and **222,2H**$^+$, respectively, $\Delta E = 9$ and 20 kcal/mole for molecular mechanics optimized energies; $\Delta E = 4 \pm 4$ and 20 ± 12 kcal/mole for the average MD energies). This may be related to internal N-H$^+$ ... O hydrogen bonding in the **K** form (Figure 15), as found previously in the **SC24,4H**$^+$ cryptand.[25] On the other hand, solvation favours "out" protonated forms, due to their larger steric accessibility ($\Delta E_{SW} = 7 \pm 8$ kcal/mole for **222,1H**$^+$ and 40 ± 12 kcal/mole for **222,2H**$^+$).

It is difficult to conclude on "in"/"out" preferences from these simulations, due to statistical uncertainties, and to possible force field dependence on the calculated energies. The relative free energies in water depend also on solvent-solvent interactions, and on entropy effects, not accessible to those MD simulations. The **K** and **OO** forms may also not be the most stable ones for "in" and "out" topomers, respectively. The above results however illustrate at least qualitatively how intrinsic stabilities may oppose to solvation effects, and suggest that water has an important levelling effect on the various conformers which should be therefore in equilibrium.

TABLE III

The "in-in" and "out-out" forms of **222,H**$^+$ and **222,2H**$^+$ in water. Average energies (kcal/mole).

	222,H$^+$		**222,2H**$^+$	
Conformer	in-in	out-out	in-in	out-out
$\langle E_{solute} \rangle$	+41(4)	+45(4)	+90(4)	+110(4)
$\langle E_{SW} \rangle$	−127(9)	−134(8)	−239(12)	−279(12)
E_{opt}	+4	+13	+58	+79

FIGURE 16 The "out-out" (**OO**) and "in-in" (**K**) conformers of diprotonated **222,2H**$^+$.

CALCULATING FREE ENERGIES IN SOLUTION: RELATIVE SOLVATION ENERGIES, BINDING SELECTIVITIES AND STABILITY CONSTANTS.

In the last years, procedures have emerged for calculating free energy differences *in solution* with a good precision[78]. They are based on the slow pertubation of one state into another, coupled with Monte Carlo or MD generation of statistical ensembles of each state. The ΔG between states i and j can be calculated as an average of a function of their potential energy difference:

$$G_i - G_j = -k_B T \, \mathrm{Log} < \exp(E_j - E_i)/k_B T >_i$$

Gradual mutation of state i into state j can be achieved via intermediate "hybrid" states. Examples of small pertubations in van der Waals parameters are Na^+ to K^+, or Cl^- to Br^- mutations in solution, either uncomplexed, or complexed by macrocyclic hosts. More severe mutations involve for instance C-H to C-Alkyl, or C-Aryl transformations, or annihilation processes[78]. Because of the neglect of long range electrostatic interactions, and of polarization effects, charge mutations are presently more problematic (e.g. NH_4^+ to Ca^{2+}).

The quantitative measure of the *binding selectivity* of the receptor **R** for the substrates S_1 and S_2, given by the difference of free energies of complexation $\Delta\Delta G = \Delta G_1 - \Delta G_2$ is obtained computationally by $\Delta\Delta G = \Delta G_3 - \Delta G_4$, by using the thermodynamic cycle:

$$\begin{array}{lll} R + S_1 \longrightarrow R/S_1 & & \Delta G_1 \\ \Delta G_3 \downarrow & \downarrow \Delta G_4 & \\ R + S_2 \longrightarrow R/S_2 & & \Delta G_2 \end{array}$$

ΔG_3 and ΔG_4 correspond respectively to the mutations of the free and complexed substrates *in solution*, and are calculated with small statistical fluctuations, as compared to the direct calculation of ΔG_1 and ΔG_2 for the complexation processes. Comparative binding of a substrate S to similar receptors R_1 and R_2 can be computed similarly[78].

This procedure accounts quantitatively for the Cl^-/Br^- binding selectivity of **SC24,4H**$^+$.[79] It has been used recently in the macrocyclic area to calculate the relative binding free energies of Na^+/K^+ by **18C6** in water[80] and in methanol,[15] of nitromethane/malononitrile/acetonitrile by **18C6**,[16] or of Na^+/K^+ by dibenzo-crown ethers[81] as well as the binding of pyridine/pyrazine by one of Rebek's acridine diacid receptors in chloroform.[82] We also compared the $Na^+/K^+/Rb^+$ selectivity of **222** in water and in methanol[93]. In both solvents, the peak of selectivity calculated for K^+ was in qualitative agreement with experiment. Selective complexation of alkali cations by calixspherands[116], or by naturally occuring antibiotics like valinomycine[117] was similarly accounted for. From identical mutations performed in different solvents **A** and **B**, the relative free energies of transfer ΔG_t from **A** to **B** can be similarly computed with good accuracy, by using an appropriate thermodynamic cycle[93,118]. By annihilation of Rb^+ free, and in its calixspherand complex, the *absolute* free energy of complexation in water was calculated, in good agreement with experiment[116]. The choice of starting structure may be crucial for the results, especially if the sampling is restricted to about 100 ps[93]. Most of the mutations reported so far in chemistry

or biology were initiated with an experiment structure, and the role of the starting configuration remains to be further investigated.

For species **R** and **S**, the change of free energy ΔG as a function of their separation corresponds to the *"potential of mean force"*, or "pmf". The "pmf" for decomplexation of K^+ from its **18C6** complex in water, obtained by step by step removal of K^+ from the centre of mass of the crown led to a fascinating result[14]: the free energy minimum does not correspond to an "inclusion" complex (with K^+ at the centre of a crown of approximate D_{3d} symmetry), as found in the solid state or calculated *in vacuo*. Instead, K^+ is perched over a tennis ball seam type conformer of the crown, in order to achieve better coordination to water. Again, these microscopic pictures in water differ dramatically from the ones obtained in other phases. Such free energy simulations, still in their infancy, need intensive calculations, but become feasible with present workstations in a few days. Very promising results have been obtained so far, despite the force field limitations (e.g. no polarization terms) and small time scales (about 0.1 ns). One particularly interesting issue will be to investigate non-aqueous solutions of these complexes[120].

COMPUTER DETERMINATION OF THE RULES FOR SUPRAMOLECULAR MODELING IN SOLUTION.

Supramolecular structures result from the delicate balance between intramolecular conformational preferences (torsional and inversion barriers), and non-covalent interactions involving the receptor, the substrate, and their molecular environment. In solution, it is therefore important to assess the *effectiveness* of van der Waals, hydrogen bonding and ionic interactions[133,135], and of rotational and inversion barriers, as compared to the gas phase. Important insights have been obtained computationally by using the free energy perturbation technique[78]. For instance, acetamide was calculated to dimerize in chloroform via $C{=}O\cdots H\text{-}N$ hydrogen bonding, but not in water solution[121]. Stacking versus hydrogen bonding of nucleic bases is dependent of the simulation phases[83]. In water, the "effective attraction" between M^+ and X^- is much weaker then in the gas phase, of a few kcal/mole only[123]. The CC-CC rotation barrier of butane has been calculated to be reduced in water, as compared to the gas phase[124], and the hydration effect on the cis-trans energy difference for the peptide bond assessed *in vacuo* and in water[125].

Of particular interest in supramolecular chemistry are computational results unexpected from common concepts. For instance, despite of coulombic repulsions, some like ion pairs of $X^-\cdots X^-$ type, or of $M^+\cdots M^+$ type have been found to be "locally attractive" in water, because of specific hydration pattern! For instance, approaching 2 Cl^- anions[122,126–128], or 2 Guanidinium$^+$ cations[129] from 7 Å to about 4 Å in water leads to a *decrease* in free energy! A microscopic analysis of such interactions is important for understanding molecular interactions in general and for designing host molecules for such ions.

Another interesting result concerns the *complexation of anionic substrates*, which has been much less modelled[25,60,91,98,132], as compared to cationic substrates. It is generally achieved via protonated amino binding sites of the receptor, or via strong hydrogen bonds, which present an accumulation of like-charges or dipoles, and requires explicit treatment of the solvent. In polyammonium macrocycles which bind anions like Cl^-, ATP^{4-} the $1\cdots4$ interactions between NH_2^+ groups are expected

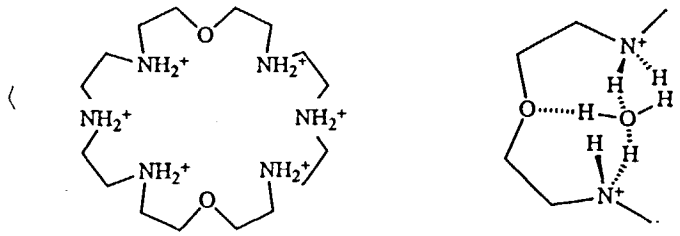

FIGURE 17 *Left*: The $[24]N_6O_2$, $6H^+$ macrocyle. *Right*: Typical representation of briding water coordination obtained from a MD simulation of the free macrocyle in water.

to prevent the $^+NC\text{-}CN^+$ *gauche* arrangements required for convergent structures suitable for binding X^- (Figure 17). Calculation of the change in free energy upon C-C rotation in ethane diammonium $^+H_3N\text{-}CH_2\text{-}CH_2\text{-}NH_3{}^+$[92] confirms that in the gas phase, the only stable form is *trans*, as expected. In aqueous solution however, a better hydration of the *cis* and *gauche* forms, compared to the *trans* form, brings the *gauche* conformer as a local minimum, at 3.5 kcal/mole only above the *trans* conformer. In other words, *water may stabilize preferentially conformers of the polyammonium hosts with convergent binding sites, and suitable for anion binding.*

This *role of water in preorganizing the protonated macrocycle* for complexation of anions has been shown subsequently by MD simulations[91,119]. Whereas in the gas phase, the macrocycle $[24]N_60_2,6H^+$ (Figure 17) adopts conformations with diverging ammonium binding sites and *trans* $^+NC\text{-}CN^+$ dihedrals, in solution it retains converging $NH_2{}^+$ binding sites, because of specific hydration pattern. As found for crown ethers and cryptands, *bridging water* molecules, (see Figures 13, 14 and 17), play a major role in the conformation dependent solvation and on the accumulated of like-charges. How other solvents would interact with such macrocycles and to which extent water is "unique" remains to be investigated.

CONCLUSION

Computer modelling of macro(poly)cyclic molecules brings new or deeper insights into their conformations and recognition properties. The different techniques used to built selected conformers of "supermolecules" with constraints and to sample their conformational space *in vacuo*, provide dynamic views of the free receptors and of their "lock and key" complementarity with various substrates. Similar modelling studies have been performed for instance in the field of cyclodextrins[85], calixarenes[86], siderophores[87], etc.... They generally involve combination of graphics software, of Molecular Mechanics or Molecular Dynamics software, and analysis programs[84,107] From a practical point of view, it would certainly be useful to have dedicated modelling tools, similar to those available for proteins, or for small organic molecules. Particularly, linking fragments of known cyclic structures with modelbuilt pieces, combined with energy refinements and searches in the structural databases would improve significantly the chances of producing reasonable models.

Attempts are made to evaluate conformational and complexation energies, but care must be taken about the significance of these numbers because of the theoretical approximations used to simulate the "gas phase" situation. In addition the relevance of gas phase energy data and solid state structures for recognition properties in solution has to be questioned. In particular, the nature of the complex (inclusive or exclusive) and whether complexation in a given solvent will take place or not, is presently hardly predictable, especially when the complex is of entropic, rather than of enthalpic origin.[95] It is clear that molecular dynamics at longer time scales, which will become feasible with high speed computers in the next future will provide higher prediction capability concerning structures and energies in solution.[96]

Compared to traditional tools of theoretical chemistry, free energy simulations represent a real breakthrough[78], allowing for new quantitative insights into fundamental features of molecular structure and recognition in chemistry and biology, i.e. the thermodynamic characterization of complex dynamical supramolecular frameworks.

Computer graphics, coupled with a powerful workstation is indispensable for visualizing experimental complex structures, and the calculated trajectories of the solutes, solvent shells, etc..... The fleeting character of these illuminating pictures raises however a problem of communication. We filmed graphics pictures of receptor/substrate type complexes referred to in this paper (crown ethers, cryptands, cryptates, calixarenes) and of supramolecular assemblies (helicates, catenates, model mesophases and channels)[130]. The movie gives some feeling of the dynamics, although less than the interactive manipulation of stereo pictures on the screen of the graphics display. Like for computer models of complex biological systems, it would be desirable to make such results available and easily transferable to other users and workstations, e.g. by using high flow computer communication networks. A second problem may arise: pictures and models versus "reality": can we trust pictures? While computer simulations are revolutionizing our understanding of chemical processes, by considering more realistic situations and coding hypothetical rules governing chemical structures, there are but improved *models*[134], and permanent feedback with experiment is of crucial importance.

As far as model manipulations are concerned, we feel that despite the spectacular development of computer graphics techniques and of supercomputers, computer modelling is presently not performant enough to replace completely classical CPK type models in the chemist's hands. Technical difficulties such as synchronous bond rotations keeping the (poly)cycles closed will certainly be solved, but most important is that the inspired way the structure is made and manipulated is hardly codable. Further efforts will have to be made to develop artificial, but real intelligence and knowledge!

ACKNOWLEDGEMENTS

G.W. thanks his collaborators P. Auffinger, J. M. Wurtz and S. Boudon for their contributions to the manuscript, and a NATO exchange grant 0478/82 with P. A. Kollman for supporting much of this work. Generous allocation of computer time on the IBM-3090 VF computers at Strasbourg-Cronenbourg and at the CIRCE through the CNRS-IBM Scientific Group is also greatly acknowledged. The author also thanks the "Region Alsace" and the BIOSTRUCTURE company for supporting a Computer graphics system.

REFERENCES

1. C.J. Pedersen, *J. Am. Chem. Soc.*, **89**, 2495, 7017 (1967), *ibid. Pure Appl. Chem.*, **60**, 450 (1988).
2. B. Dietrich, J.-M. Lehn and J.-P. Sauvage, *Tetrahedron Lett.*, 2886, 2889 (1969); J.-M. Lehn, *Struct. Bond.*, **16**, 1 (1973); *Acc. Chem. Res.*, **11**, 49 (1978).
3. D. J. Cram and J. M. Cram, *Science*, **183**, 803 (1974).
4. Nobel Prize Citation; Nobel Festival, December 10 (1987).
5. J.-M. Lehn, *Angew. Chem. Int. Ed.*, **27**, 89 (1988).
6. D.J. Cram, *Science*, **240**, 760 (1988).
7. G. Wipff and P. Kollman, *J. Am. Chem. Soc.*, **104**, 3249 (1982).
8. R. Perrin, C. Decoret, G. Bertholon and R. Lamartine, *Nouv. J. Chim.*, **7**, 263 (1983); M.J. Bovill, D.J. Chadwick and I.O. Sutherland, *J. Chem. Soc. Perk. Trans.*,**2**, 1529 (1980); M. Badetscher, M. Welti, P. Portmann and E. Pretsch, *Topics Curr. Chem.*, **136**, 17 (1986).
9. G. Ranghino, S. Romano, J.-M. Lehn and G. Wipff, *J. Am. Chem. Soc.*, **107**, 7873 (1985).
10. M. Billeter, A.E. Howard, I.D. Kuntz and P.A. Kollman, *J. Am. Chem. Soc.*, **110**, 8385 (1988). See also reference 33.
11. J.-M. Wurtz and G. Wipff, in "Transport through Membranes: Carriers, Channels, Pumps", A. Pullman *et al.*, Ed. Kluwer Academic Publishers, 1 (1988).
12. T.P. Straatsma and J.A. McCammon, *J. Chem. Phys.*, **91**, 3631 (1989).
13. J. van Eerden, S. Harkema and D. Feil, *Acta Cryst.*, **B34**, 222 (1990).
14. J. van Eerden, W.J. Briels, S. Harkema and D. Feil, *Chem, Phys. Lett.*, **164**, 370 (1989); L. Dang and P.A. Kollman, *J. Am. Chem. Soc.*, **112**, 5176 (1990).
15. M.H. Mazor, J.A. McCammon and T.P. Lybrand, *J. Am. Chem. Soc.*, **111**, 55 (1989); M.H. Mazor, J.A. McCammon and T.P. Lybrand, *J. Am. Chem. Soc.*, **112**, 4411 (1990).
16. P.D.J. Grootenhuis and P.A. Kollman, *J. Am. Chem. Soc.*, **111**, 4046 (1989).
17. E. Fischer, *Chem. Ber.*, **27**, 2985 (1894).
18. L. Pauling, *Nature*, **161**, 707 (1948).
19. D.E. Koschland, *Adv. Enzym.*, **22**, 45 (1960). *ibid. Ann. Rev. Biochim.* **37**, 672 (1968).
20. W.P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw Hill, New York (1969).
21. M. Dobler, "Ionophores and their Structures", Wiley Interscience, New York (1981).
22. F. Vögtle, W.M. Müller and W.H. Watson, *Topics Curr. Chem.*, **125**, 131 (1984).
23. C. Pascard, C. Riche, M. Cesario, F. Kotzyba-Hibert and J.-M. Lehn, *J. Chem. Soc. Chem. Commun*, 557 (1982).
24. G. Wipff and P. Kollman, *Nouv. J. Chim.*, **9**, 457 (1985).
25. G. Wipff and J.-M. Wurtz, *Nouv. J. Chim.*, **13**, 807 (1989).
26. P. Auffinger and G. Wipff, *J. Comput. Chem.*, **11**, 19 (1990).
27. J.-M. Wurtz, "Logiciels Infographiques de Modélisation Moléculaire. Application à l'étude d'un récepteur macrocyclique". Thèse de doctorat, Université Louis Pasteur, Strasbourg (1988).
28. U.C. Singh, P. Weiner, J. Caldwell and P. Kollman, AMBER-3.0, U.C. San Francisco (1987).
29. S.J. Weiner, P.A. Kollman, D.A. Case, U.C.Singh, C. Ghio, G. Alagona, S. Profeta, Jr and P. Weiner, *J. Am. Chem. Soc.*, **106**, 765 (1984).
30. N. Gresh, P. Claverie and A. Pullman, *Theoret. Chim. Acta*, **66**, 1 (1984); A.E. Howard, U.C. Singh, M. Billeter and P.A. Kollman, *J. Am. Chem. Soc.*, **110**, 6984 (1988); A. Warshel and M. Levitt, *J. Mol. Biol.*, **103**, 227 (1976); T. P. Lybrand and P.A. Kollman, *J. Chem. Phys.*, **83**, 2923 (1985).
31. W.L. Koltun, *Biopolymers*, **3**, 665 (1965).
32. S. Lifson, C.E. Felder and A. Shanzer, *J. Am. Chem. Soc.*, **105**, 3866 (1983); S. Lifson, C.E. Felder and A. Shanzer, *Biochemistry*, **23**, 2577 (1984).
33. W.H.M. Uiterwijk, S. Harkema, B.W. van de Waal, F. Göbel and H.T.M. Nibbeling, *J. Chem. Soc. Perk. Trans. 2*, 1843 (1983).
34. G.M. Crippen, "Distance geometry and conformational calculations", Research Studies Press Wiley, New York (1981).
35. P.K. Weiner, S. Profeta, G. Wipff, T. Havel, I.D. Kuntz, R. Langridge and P. Kollman, *Tetrahedron*, **39**, 1113 (1983).
36. "2D3D: a program to build 3D structures on the VAX+PS300 graphics system, based on the distance geometry approach.", J.-M. Wurtz and G. Wipff (1988).

37. M. Billeter, T.F. Havel and K. Wüthrig, *J. Comput. Chem.*, **8**, 132 (1987).
38. W. Braun, *Quarter. Rev. Biophys.*, **19**, 115 (1987).
39. M. Billeter and G. Wipff, unpublished results.
40. A.E. Howard and P.A. Kollman, *J. Med. Chem.*, **32**, 1669 (1988).
41. R.J. Loncharich, E. Seward, S.B. Ferguson, F.K. Brown, F. Diederich and K.N. Houk, *J. Org. Chem.*, **53**, 3479 (1988).
42. G. Chang, W.C. Guida and W. C. Still, *J. Am. Chem. Soc.*, **111**, 4379 (1989).
43. M. Saunders, *J. Comput. Chem.*, **10**, 203 (1989). ibid. *J. Am. Chem. Soc.*, **109**, 3150 (1987).
44. S.R. Wilson, W. Cui, J.W. Mosckowitz and K.E. Schmidt, *Tetrahedron Lett.*, **29**, 4373 (1988).
45. M. Nilges, A.M. Gronenborn, A.T. Brünger and G.M. Clore, *Prot. Eng.*, **2**, 27 (1988); A.T. Brünger, G.M. Clore, A.M. Gronenborn and M. Karplus, *Prot. Eng.*, **1**, 399 (1987).
46. M. Saunders, K.N. Houk, Y.-D. Wu, W.C. Still, M. Lipton, G. Chang and W.C. Guida, *J. Am. Chem. Soc.*, **112**, 1419 (1990).
47. R. Geue, S.H. Jacobson and R. Pizer, *J. Am. Chem. Soc.*, **108**, 1150 (1986).
48. J.D. Dunitz and P. Seiler, *Acta Cryst.*, **B30**, 2739 (1974); E. Maverick, P. Seiler, B. Schweizer and J.D. Dunitz, *Acta Cryst.*, **B36**, 615 (1980).
49. P.A. Kollman, G. Wipff and U.C. Singh, *J. Am. Chem. Soc.*, **107**, 2212 (1985).
50. G. Wipff, D. Gehin and P. Kollman, *J. Am. Chem. Soc.*, **111**, 3011 (1989).
51. J.-M. Wurtz and G. Wipff, "MDNM, an interactive program to display Molecular Dynamics and Normal Modes of Vibration on a Evans and Sutherland PS300 picture system." (1988).
52. M. Levitt, C. Sander and P.S. Stern, *J. Mol. Biol.*, **181**, 423 (1985); B. Brooks and M. Karplus, *Proc. Nat. Acad. Sc. USA*, **82**, 4995 (1985).
53. C. Sirlin, *Bull. Soc. Chim. Fr.*, **2**, 25 (1984); J.-M. Lehn and C. Sirlin, *J. Chem. Soc. Chem. Comm.*, 949 (1978). ibid. *Nouv. J. Chim.*, **11**, 693 (1987).
54. J.F. Stoddart, *Annu. Rep. Prog. Chem. Sect. B*, **80**, 353 (1983); I. Tabushi, *Tetrahedron*, **40**, 269 (1984); R. Breslow, *Adv. Enzymol.*,**58**, 1 (1986); I.O. Sutherland, *Chem. Soc. Rev.*, **15**, 63 (1986); F.M. Menger, *Topics Curr. Chem.*, **136**, 1 (1986); V.T. D'Souza and M.L. Bender, *Acc. Chem. Res.*, **20**, 146 (1987); J. Rebek, Jr, *Science*, **235**, 1478 (1987).
55. C.A. Venanzi, *Int. J. Quant. Chem., Quant. Biol. Symp.*, **12**, 69 (1986).
56. D.H.J. McKay, A.J. Cross and A.T. Hagler, "The role of energy minimisation in simulation strategies of biomolecular systems", Fassman Ed. (1988).
57. M.L. Connolly, *J. Am. Chem. Soc.*, **107**, 1118 (1985); B. Lee and F.M. Richards, *J. Mol. Biol.*, **55**, 379 (1971).
58. M.J. Heeg, *Acta Cryst.*, **C44**, 2219 (1988).
59. E. Graf, J.-P. Kintzinger, J.-M. Lehn and J. Lemoigne, *J. Am. Chem. Soc.*, **104**, 1672 (1982); E. Graf and J.-M. Lehn, *Helv. Chim. Acta*, **64**, 1040 (1981).
60. B. Owenson, R.D. McElroy and A. Pohorille, *J. Am. Chem. Soc.*, **110**, 6992 (1988); B. Owenson, R.D. McElroy and A. Pohorille, *J. Mol. Struct. (Theochem)*, **179**, 467 (1988).
61. W.L. Hase, M.-C. Richou and S.L. Mondro, *J. Phys. Chem.*, **93**, 539 (1989).
62. E. Graf, J.-M. Lehn, *J. Am. Chem. Soc.*, **98**, 6403 (1976).
63. E. Kauffmann, J.L. Dye, J.-M. Lehn and A.I. Popov, *J. Am. Chem. Soc.*, **102**, 2274 (1980).
64. D. Heyer, J.-M. Lehn, *Tetrahedron, Lett.*, **27**, 5869 (1986); D. Heyer, postdoctoral report (1986).
65. E.H. Nordlander and J.H. Burns, *Inorg. Chim. Acta*, **115**, 31 (1986).
66. S.V. Hannongbua and B. Rode, *J. Chem. Soc. Farad. Trans. 2*, **82**, 1021 (1986); V.W. Ruangpornvisuti, M. M. Probst and B.M. Rode, *Inorg. Chim. Acta*, **134**, 297 (1987).
67. P. Auffinger and G. Wipff, *J. Am. Chem. Soc.*, **113**,5976 (1991).
68. V.M. Loyola, R. Pizer and R.G. Wilkins, *J. Am. Chem., Soc.*, **99**, 7185 (1977); J.M. Bemtgen, M.E. Springer, V.M. Loyola, R.G. Wilkins and R.W. Taylor, *J. Am. Chem. Soc.*, **23**, 3348 (1984).
69. Y.M. Cahen and A.I. Popov, *J. Sol. Chem.*, **4**, 599 (1975); E. Kauffmann, J.L. Dye, J.-M. Lehn, J. M. and A.I. Popov, *J. Am. Chem. Soc.*, **102**, 2274 (1980); P.A. Mosier-Boss and A.I. Popov, *J. Am. Chem. Soc.*, **107**, 6168 (1985); E. Schmidt, J.M. Tremillon, J.P. Kintzinger and A.I. Popov, *J. Am. Chem. Soc.*, **105**, 7563 (1983).
70. M.H. Abraham, A.F. Danil de Namor and R.A. Schulz, *J. Chem. Soc. Faraday Trans. I*, **76**, 869 (1980); A.F. Danil de Namor, *J. Chem. Soc. Faraday Trans. I*, **84**, 2441 (1988).

71. N. Morel-Desrosiers and J.P. Morel, *J. Am. Chem. Soc.* **103**, 4743 (1981); N. Morel-Desrosiers and J.P. Morel, *Nouv. J. Chim.*, **3**, 539 (1979).
72. N. Sabbatini, M. Ciano, S. Dellonte, A. Bonazzi, F. Bolleta and V. Balzani, *J. Phys. Chem.*, **88**, 1534 (1984); N. Sabbatini, S. Dellonte, M. Ciano, A. Bonazzi and V. Balzani, *J. Am. Chem. Soc.*, **107**, 212 (1984).
73. B.G. Cox and H. Schneider, **99**, 2809 (1977).
74. B. Dietrich, J.-M. Lehn and J.P. Sauvage, *Tetrahedron*, **29**, 1647 (1973); B. Dietrich, J.-M. Lehn, J.P. Sauvage and J. Blanzat, *Tetrahedron*, **29**, 1629 (1973).
75. R. Pizer, *J. Am. Chem. Soc.*, **100**, 4239 (1978).
76. H. Schneider, S. Rauh and S. Petrucci, *J. Am. Chem. Soc.*, **85**, 2287 (1981).
77. H.E. Simmons and C.H. Park, *J. Am. Chem. Soc.*, **90**, 2428 (1968).
78. W.L. Jorgensen, *Acc. Chem. Res.*, **22**, 184 (1989); O.A. Bash, U.C. Singh, R. Langridge and P.A. Kollman, *Science*, **236**, 564 (1987); P.A. Bash, U.C. Singh, F.K. Brown, R. Langridge and P.A. Kollman, *Science*, **235**, 574 (1987); J.A. McCammon, *Science*, **238**, 486 (1987); M. Mezei and D.L. Beveridge, *Ann. New York Acad. Sc.*, **482**, 1 (1980); W.F. van Gunsteren, *Prot. Eng.*, **2**, 5 (1988); M. Migliore, G. Corongiu and E. Clementi, *J. Chem. Phys.*, **88**, 7766 (1988).
79. T.P. Lybrand, J.A. McCammon and G. Wipff, *Proc. Nat. Acad. Sc. USA*, **83**, 833 (1986); J.A. McCammon, T.P. Lybrand, S.A. Allison and S.H. Northrup, in "Biomolecular Stereodynamics III", Sarma Ed., Adenine, New York, p. 227 (1986).
80. J. van Eerden, S. Harkema and D. Feil, *J. Phys. Chem.*, **92**, 5076 (1988).
81. P.D.J. Grootenhuis and P.A. Kollman, *J. Am. Chem. Soc.*, **111**, 2152 (1989).
82. W.L. Jorgensen, S. Boudon and T.B. Nguyen, *J. Am. Chem. Soc.*, **111**, 755 (1989).
83. L.X. Dang and P.A. Kollman, *J. Am. Chem. Soc.*, **112**, 503 (1990).
84. P.A. Kollman, P.D.J. Grootenhuis and M.A. Lopez, *Pure Appl. Chem.*, **61**, 593 (1989).
85. F.M. Menger and M.J. Sherrod, *J. Am. Chem. Soc.*, **110**, 8606 (1988); H.-J. Thiem, M. Brandl and R. Breslow, *J. Am. Chem. Soc.*, **110**, 8612 (1988); M. Ohashi, K. Kasatani, H. Shinohara and H. Sato, *J. Am. Chem. Soc.*, **112**, 5824 (1990); J.E.H. Koehler, W. Saenger and W.F. van Gunsteren, *Eur. Biophys. J.*, **15**, 197 (1987). ibid., **15**, 211 (1987); C.A. Venanzi, P.M. Canzius, Z. Zhang and J.D. Bunce, *J. Comput. Chem.*, **10**, 1038 (1989); D.W. Armstrong, T.J. Ward, R.D. Armstrong, T.E. Beesley, *Science*, **232**, 1132 (1986); I. Tabushi, T. Mizutani, *Tetrahedron*, **7**, 1439 (1987); D.A. Wertz, C. Shi and C. Venanzi, *J. Comput. Chem.* **13**, 41 (1992).
86. P.D.J. Grootenhuis, P.A. Kollman, L.C. Groenen, D.N. Reinhoudt, G.J. van Hummel, F. Ugozzoli and G.D. Andreetti, *J. Am. Chem. Soc.* **112**, 4165 (1990).
87. A. Shanzer, J. Libman, S. Lifson and C.E. Felder, *J. Am. Chem. Soc.* **108**, 7609 (1986); A. Bouraoui, M. Fathallah, B. Blaive and R. Gallo, *Chem. Soc. Perk. Trans.* **2**, 1211 (1990).
88. R. Dharanipragada, S. Ferguson and F. Diederich, *J. Am. Chem. Soc.*, **110**, 1679 (1988). For other MM2 calculations on macrocyclic compounds, see for example S.P. Miller and H.W. Whitlock, Jr, *J. Am. Chem. Soc.*, **106**, 1492 (1984); B.M. Masek, B.D. Santarsiero and D.A. Dougherty, *J. Am. Chem. Soc.*, **109**, 4373 (1987); K.B. Lipkovitz and R. Zegarra, *J. Comput. Chem.* **10**, 595 (1989); Y. Murakami *et al.*, *J. Am. Chem Soc.* **113**, 8229 (1991).
89. P.R. Gerber, K. Gubernator and K. Müller, *Helv. Chim. Acta*, **71**, 1429 (1988).
90. J.S. Bradshaw, P. Huszthy, C.W. McDaniel, C.Y. Zhu, N.K. Dalley, R.M. Izatt and S. Lifson, *J. Coord. Chem.*, **55**, 3129 (1990). For theoretical studies on eniantioselectivity in chiral chromatography, see K.B. Lipkowitz, B. Baker and R. Zegarra, *J. Comput. Chem.* **10**, 718 (1989) and references cited therein.
91. S. Boudon, A. Decian, J. Fischer, M.W. Hosseini, J.-M. Lehn and G. Wipff, *J. Coord. Chem. B*, **23**, 113 (1991).
92. S. Boudon and G. Wipff, **228**, 61, *J. Mol. Struct. (Theochem)*, **23**, 113 (1991)
93. P. Auffinger and G. Wipff, *J. Chimie Physique*, **88**, 2525 (1991).
94. P. Auffinger and G. Wipff, *J. Inclusion Phenom.*, **11**, 71 (1991).
95. E. Kauffmann, J.-M. Lehn and J.-P. Sauvage, *Helv. Chim. Acta*, **59** 1099 (1976); R.M. Izatt, J.S. Bradshaw, S.A. Nielsen, J.D. Lamb, J.J. Christensen and D. Sen, *Chem. Rev.*, **85**, 271 (1985); J. Cullinane, R.I. Gelb, T.N. Margulis and L.J. Zompa, *J. Am. Chem. Soc.*, **104**, 3048 (1982).
96. W.F. van Gunsteren and H.J.C. Berendsen, *Angew. Chem. Int. Ed.*, **29**, 992 (1990).

97. T. Yamabe, K. Hori, K. Akagi and K. Fukui, *Tetrahedron*, **35**, 1065 (1979); K. Hori, H. Yamada and T. Yamabe, *Tetrahedron*, **39**, 67 (1983); E.D. Lovrinenko-Ometsinskaya, V.B. Volkov, *Teor. Eksp. Khim.*, **20**, 722 (1984).
98. M.A. Santos, M.G.B. Drew, *J. Chem. Soc. Farad. Trans.* **87**, 1321 (1991).
99. M. Billeter, A.E. Howard, I.D. Kuntz and P.A. Kollman, *J. Am. Chem. Soc.*, **110**, 8385 (1988); A. Pullman, C. Giessner-Prette and Yu. V. Kruglyak, *Chem. Phys. Lett.* **12**, 382 (1976).
100. R. Bonaccorsi, R. Cammi, J. Tomasi, *J. Comput. Chem.* **12**, 301 (1991), and references cited therein
101. V.S. Allured, C.M. Kelly and C.R. Landis, *J. Am. Chem. Soc.* **113**, 1513 (1991).
102. R.D. Hancock, *Acc. Chem. Res.*, **23**, 253 (1990); *Prog. Inorg. Chem.* **37**, 187 (1989); G.R. Brubaker and D.W. Johnson, *Coord. Chem. Rev.*, **53**, 36 (1984); S.C. Hoops, K.W. Anderson, K.M. Merz, Jr., *J. Am. Chem. Soc.*, **113**, 8262 (1991); Y. Yoshikawa, *J. Comput. Chem.*, **11**, 326 (1990); K.R. Adam, M.A. Antolovitch, L.G. Brigden and L.F. Lindoy, *J. Am. Chem. Soc.*, **113**, 3346 (1991).
103. B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, **4**, 187 (1983).
104. M. Badertscher, S. Musso, M. Welti, E. Pretsch, T. Maruizumi and T.-K. Ha, *J. Comput. Chem.*, **11**, 819 (1990).
105. H. Goto and E. Osawa, *J. Amer. Chem. Soc.*, **111**, 8950 (1989).
106. J.-L. Toner in *"Modern Aspects of Host-Guest Chemistry: Molecular Modeling and Conformationally restricted Hosts."*, Pataï (Eds), J. Wiley & Sons; Interscience Publ., pp. 77 (1989).
107. P.A. Kollman, *Acc. Chem. Res.*, **23**, 246 (1990).
108. C.I. Rarcliffe, J.A. Ripmeister, G.W. Buchanan and J.K. Denike, *J. Amer. Chem. Soc.*, **114**, 3294 (1992).
109. A.T. Hagler in *"Theoretical Simulation of Conformation, Energetics, and Dynamics of Peptides"*., Academic Press, 1985, p. 213.
110. Y. Sun and P.A. Kollman, *J. Comput. Chem.*, **12**, 33 (1992).
111. J. Hehre, L. Radom, P. Schleyer, J. Pople, *"Ab Initio Molecular Orbital Theory"*, Wiley, New York (1986).
112. P. Auffinger and G. Wipff, *Unpublished results;* P. Auffinger, "Simulations par Dynamique Moléculaire de Cryptates de Cations: de la Phase Gazeuse à la Solution", *Thèse de Doctorat, Université Louis-Pasteur, Strasbourg. (1991).*
113. J. Dunitz in *"Host-Guest Molecular Interactions: from Chemistry to Biology"* CIBA Foundation Symp. 158, J. Wiley Ed. (1991), p. 92.
114. L. Troxler and G. Wipff, *Unpublished results*
115. A. Varnek, G. Wipff and P. Guilbaud, "MD Simulations of Cation Complexes of Calix [4]arene Amides in Water", *to be published.*
116. S. Miyamoto and P.A. Kollman, *J. Am. Chem. Soc.*, **114**, 3668 (1992).
117. G. Eisenmann, O. Alvarez and J. Aqvist, *J. Inclus. Phenom. Mol. Recogn. Chem.*, **12**, 23 (1992).
118. W.L. Jorgensen, J.M. Briggs and M.L. Contreras, *J. Phys. Chem.*, **94**, 1683 (1990).
119. S. Boudon, G. Wipff, *J. Chimie Physique*, **88**, 2443 (1991).
120. T.J. Marrone and J. Merz K.M., *J. Amer. Chem. Soc.*, **114**, 7542 (1992).
121. W.L. Jorgensen, *J. Am. Chem. Soc.*, **111**, 3770 (1989).
122. L.X. Dang and B.M. Pettitt, *J. Phys. Chem.*, **94**, 4303 (1990).
123. M. Berkowitz, O.A. Karim, J.A. McCammon and P.J. Rossky, *Chemical Physics Letters*, **105**, 577 (1984); M. Berkowitz and W. Wan, *J. Chem. Phys.*, **86**, 376 (1987).
124. W.L. Jorgensen and J.K. Buckner, *J. PHys. Chem.*, **91**, 6083 (1987); W.L. Jorgensen, *J. Phys. Chem.*, **87**, 5304 (1983).
125. W.L. Jorgensen and J. Gao, *J. Am. Chem. Soc.*, **110**, 4212 (1988).
126. J. Gao, S. Boudon and G. Wipff, *J. Am. Chem. Soc.*, **113**, 9610 (1991).
127. L.X. Dang and B.M. Pettitt, *J. Am. Chem. Soc.*, **109**, 5531 (1987).
128. J.K. Buckner and W.L. Jorgensen, *J. Am. Chem. Soc.*, **111**, 2507 (1989).
129. S. Boudon and G. Wipff, *J. Phys. Chem.*, **94**, 6056 (1990).
130. J.-M. Lehn, J.-P. Sauvage and G. Wipff, *"Molecular Modeling in Supramolecular Chemistry"*, *Image- Media CNRS*, (1992).

131. P.V. Maye and C. Venanzi, *J. Comput. Chem.*, **12**, 994 (1991).
132. C. Rubin-Carrez, *"Influence de l'Environnement sur les Interactions Récepteur Substrat: Modélisation et Dynamique Moléculaire d'un Récepteur Synthétique d'Anions"*, Thèse de Pharmacie, Nancy (1991).
133. For a discussion of experimental host-guest energies, see H.-J. Schneider, *Agnew. Chem. Int. Ed.*, 30, 1417 (1991).
134. The definition of "structuralist activity", given in the context of semiology, language and human sciences applies particularly well to molecular modeling: "L'attitude structuraliste consiste...à reconstituer un objet, de façon à manifester, dans cette reconstitution, les *règles de fonctionnement* de cet objet. La structure est en fait un *simulacre* de l'objet, mais un simulacre dirigé, intéressé, puisque l'objet imité fait apparaître quelque-chose qui restait....inintelligible dans l'état naturel." (Roland Barthes, *Essais Critiques, Seuil*, 1964, p. 213).
135. H.J. Schneider, T. Schiestel, P. Zimmermann, *J. Am. Chem. Soc.* **114**, 7698 (1992).