# Temperature Dependency on Conformational Sampling of 12-Crown-4 by Simulated Annealing

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## Abstract

In this manuscript, we report a protocol to determine most of the lowest energy conformations from the ensemble of conformations. 12-crown-4 was taken as study compound to get the most of energy minima conformations. Molecular dynamic (MD) simulation for 1 nanosecond (ns) was performed at 300, 500, 700, 900 and 1100 K temperature. At particular interval conformations were sampled. Then Gaussian program was used to minimize compounds using PM6 energy levels. Duplicates were removed by checking energy as well as mirror image conformations, and only unique conformations were retained for the next 6-31+G* level minimization. It was observed that upto certain increment in temperature the number of unique conformations were increased, but afterword it decreased.

Keywords: Molecular Dynamics, Simulated Annealing, Conformational Sampling, PM6, 6-31*

## 1. Introduction

In the drug discovery process, getting a low energy conformation of the drug or protein molecule is always a big task. However, there are number of methods to get a lowest energy or global minimum conformation. It includes simulated annealing, replica exchange molecular dynamics and locally enhanced sampling[1-3]. The usual goal of a conformational analysis is the identification of all accessible minimum energy structures of a molecule. Even for the small and simple molecules there may be a large number of such structures. Lowest energy conformation is referred as the global minimum energy conformation. It is not necessary to be a global minimum energy conformation to a biologically active 3D structure.

Systematic search method is a conformational search method in which variable torsion angles are used to get different conformations. The idea to improve systematic search have been described by the Bruccoleri et al[4]. A random conformational search method have been devised, in which structure is selected from the previously generated iterative process then randomly modified and minimized[5-7]. If this process resulted in new structure, then it is added to the list, otherwise discarded.

The aim of this study is not to get all the possible conformations, but it is to get most of the lowest energy conformations for a molecule. Here, we developed a protocol to get access to the lowest energy conformations. 12-crown-4 is used as a case study compound (Fig. 1).
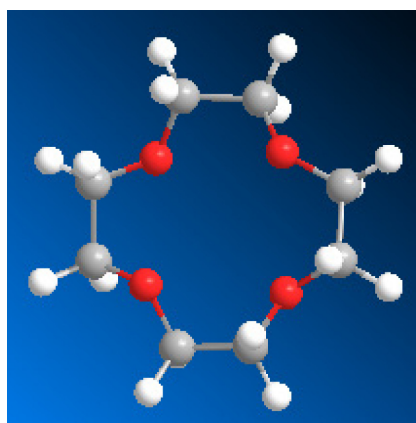


Fig. 1. Three dimensional (3D) structure of 12-crown-4 shown in ball-stick model. Oxygen represented in red, carbon in grey and hydrogen in white.

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## 2. Experimental Section

Developed protocol to access lowest energy conformations of molecule is shown in Fig. 2. First 12-crown-4 was sketched by the SYBYL8.1[8] software package and minimized by setting all the default parameters. Minimized structure was saved as protein database (pdb) format. This structure was imported in AMBER 12[9] suite of package. The energy minimization of the structure was performed using the AM1-BCC[10] charge and antechamber program with general amber force field[11]. Importance of calculation of partial charge and its application to atoms is reviewed elsewhere[12,13]. Amber parameter of the molecule was saved into topology and coordinate files. *In vacuo* steepest descent and conjugate gradient minimization for total 500 steps with 12 Å cutoff were performed, which include first 250 steps of steepest descent and remaining conjugate gradient minimization. After energy minimization the restart coordinates were generated which were used in the next MD simulation process. MD simulation was performed *in vacuo* with Langevin temperature controller coupled with collision frequency of 1.0 (gamma_ln). The output and trajectory coordinates were saved after every 100 steps. Total 500000 steps simulation with disintegration time of 0.002 picosecond (ps) was performed with 12 Å cutoff. Initial and final temperature was set each to 300, 500, 700, 900 and 1100 K, respectively. Sander program was used to run production simulation of 1 nanosecond. Five simulations with the simulated annealing temperature were performed and coordinate and trajectories were saved for further analyses.

All the simulations were post-processed using ptraj program and snapshots were generated at particular interval. We generated 100 snapshots at 10 ps interval for all the simulated annealing and saved in pdb format. Total 500 snapshots were generated. Internally developed script was used to process 500 structures and converted in Gaussian readable input format. We performed two steps optimization for all the 500 structures, because it is considered that the obtained structures at particular interval are very high in energy or crude. First we performed PM6 optimization and then the same structures were deleted using self consistent field (SCF) energy and mirror image conformation. Next, the obtained structures after SCF and mirror image detection were further used as an input in 6-31+G* optimization.
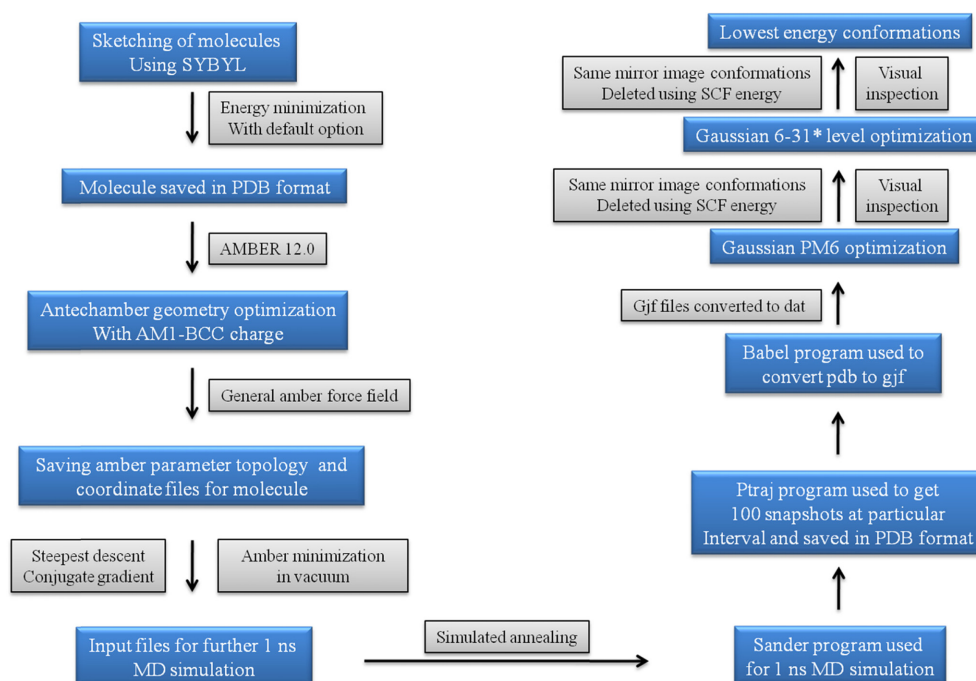


Fig. 2. Protocol used in this study to access lowest energy conformations. (Developed by Prof. Seung Joo Cho).

## 3. Results and Discussion

After simulated annealing and post-processing PM6 minimization, the number of structures retained is given in Table 1. The obtained lowest energy structure among all was considered to be relatively most stable and ranked as one, and other structures were assigned rank relative to first. The retained structure after PM6 minimization has been converted again for the next level of minimization using developed script. More expensive and time consuming 6-31+G* level energy optimization was performed to get the lowest energy minimum structures from first level. Analyses were performed again based on the mirror image detection and SCF energy. The number of lowest energy structures retained after this analyses were reported in Table 1 and plotted in Fig. 3. It is observed that the increased temperature leads to retention of more structures, but at 1100 K the numbers of retained structures are decreased.

## 4. Conclusion

We developed a protocol to access most of the lowest energy conformations for a molecule. Here, we used

SYBYL, AMBER and Gaussian suites of packages to get most of the lowest energy structures. Temperature dependencies were observed in the retention of structures upto certain level and later on reduction in number of structures was observed. Upto 900 K, the number of lowest energy structures were increased gradually, but at 1100 K it decreased with the higher level of optimization (6-31+G*). It concludes that the simulated annealing with quantum mechanical optimization could be an effective approach to get lowest energy conformations.

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Table 1. Temperatures used to simulate 12-crown-4 and number of structures retained after PM6 and 6-31+G* optimizations after deletion of mirror image structure.

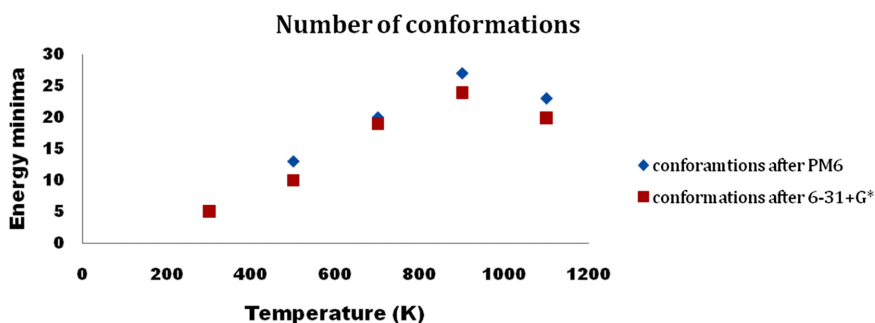| Temperature (K) | Unique conformation after PM6 minimization | Unique conformation after 6-31+G* minimization |
|---|---|---|
| 300 | 5 | 5 |
| 500 | 13 | 10 |
| 700 | 20 | 19 |
| 900 | 27 | 24 |
| 1100 | 23 | 20 |



Fig. 3. Plot of temperature dependent energy minima.

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