

## Host–Guest Interactions Influence Stability of the Rebek “Tennis Ball” Dimer Complex

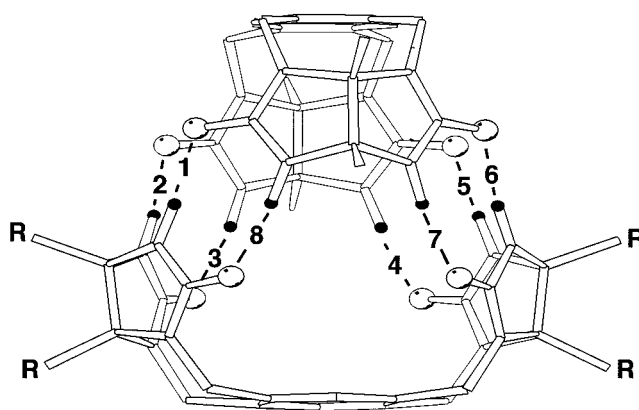
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Self-assembly and molecular recognition are governed by the formation of noncovalent interactions (hydrogen bonding, electrostatics, and weak van der Waals) between molecules in the liquid state.<sup>1,2</sup> Recently, Rebek and co-workers<sup>3</sup> described a self-assembling dimer (referred to as the “tennis ball”, Figure 1). The dimer assembles from two identical monomeric units, and the resulting complex is stabilized by eight hydrogen bonds. What is intriguing is that the dimer encapsulates solvent molecules. Hence, the “tennis ball” dimer system offers a unique opportunity to study not only self-assembly, but also host–guest complexation and related interactions. As shown by NMR spectroscopy, there are differences in the relative stabilities of empty dimer versus dimer–guest complexes.<sup>4</sup> It has been speculated that the empty dimer form may not be truly empty; rather, it may encapsulate dissolved gases. However, gas encapsulation and its effects on dimer stability have not yet been demonstrated by experiment.

To understand what forces influence the stability of the “tennis ball” dimer, theoretical simulations,<sup>5</sup> such as molecular dynamics, can provide a time-dependent window for observing pathways of formation and dissociation. Recently, free energy perturbation studies were conducted in an attempt to explain dimer–guest stabilities on the basis of energetics.<sup>6</sup> However, these simulations did not investigate the time-dependent variations in dimer structure in the presence or absence of guest molecules, including dissolved gases. Constant NVT (i.e., the number of particles, system volume, and system temperature remain constant during the simulation) molecular dynamics simulations may offer useful insights into the nature of dimer stabilities and provide a plausible mechanism for host–guest complexation and release. To examine these issues, we initiated constant NVT molecular dynamics simulations of the empty (“e”, Figure 2A) nitrogen-guest (“n”), and chloroform-guest (“c”) “tennis ball” dimers in the presence of explicit chloroform solvent. The nitrogen guest was chosen to represent a common gas molecule which would be present in solution. As shown herein, our studies indicate that



**Figure 1.** Empty “tennis ball” dimer (VFF energy-minimized structure). Hydrogen bonds (visualized as lines, numbered 1–8) are represented between carbonyl oxygen (large white spheres) and amide hydrogen (small black spheres) atoms; all other hydrogen atoms have been removed for clarity. R = Ph. Graphics were created using the MOLSCRIPT program.<sup>13</sup>

the “n-dimer” form is highly stable during the entire simulation. In contrast, the “e” dimer form is highly unstable, and the “c-dimer” complex experiences a number of conformational transitions. In both the “c” and “e” dimer systems, the most important transitions are centered on the hydrogen bonds, which are critical for both dimer assembly and retention of the “guest” solvent molecule within the framework of the organic superstructure.

**Methodology.** The “e”, “n”, and “c” dimer systems<sup>7</sup> were constructed using the INSIGHT II<sup>8</sup> molecular modeling suite. The Valence Force Field (VFF)<sup>9</sup> was used to perform the molecular dynamics simulations<sup>10</sup> for a

(7) The “e”, “c”, and “n” dimer systems were constructed using the INSIGHT II<sup>8</sup> molecular modeling suite and minimized until the derivatives were below  $10^{-4}$  kcal/mol. Point charges were assigned using the standard methodology for the CVFF force field. This results in the chloroform molecule having an appropriate dipole moment, and negatively charged chlorine atoms and positively charged carbon and hydrogen atoms as described elsewhere (Jorgensen, W. L.; Briggs, J. M.; Contreras, M. L. *J. Phys. Chem.* **1990**, *94*, 1683; Kovacs, H.; Kowalewski, J.; Laarksonen, A. J. *J. Phys. Chem.* **1990**, *94*, 7378; Wiberg, K. B.; Rablen, P. R. *J. Am. Chem. Soc.* **1993**, *115*, 614). This approach is in contrast to the RESP model used by Fox et al.<sup>6</sup> where the chlorine atoms are positively charged. Solvation was performed by placing the dimer complex in the center of a pre-equilibrated box of chloroform molecules (box dimensions:  $X = Y = Z = 40$  Å,  $\alpha = \beta = \gamma = 90^\circ$ ), and then removing any contacts closer than 3 Å.

(8) BIOSYM/Molecular Simulations Inc, San Diego, CA, 1995.

(9) Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. *Proteins: Structure Function and Genetics* **1988**, *4*, 31.

(10) Molecular dynamics simulations were performed for a period of 500 ps, with a time step of 1 fs, using periodic boundary conditions. The coupling constant for the temperature bath was 200 fs. System information, coordinates, velocities, and energetic properties were recorded every 20 fs. A spherical cutoff of 15 Å was applied to the nonbond forces with a 17 Å cutoff used to calculate the neighbor list. The dimer cavity diameter is within these cutoffs (i.e., 12.5 Å). Equilibration runs were performed on each system prior to the final simulations as follows. “E-dimer” system (100 ps total equilibration time): for the first 50 ps interval, the dimer was constrained and the solvent was allowed to move. In the second 50 ps interval, all constraints were removed. “C-dimer” and “n-dimer” systems (200 ps total equilibration time): for the first 50 ps interval, the host–guest complex was constrained and the solvent was permitted to move. For the second 50 ps interval, the guest was permitted to move, but the solvent and host were constrained. For the third 50 ps interval, the host–guest complex was allowed to move, but the solvent was constrained. For the final 50 ps interval, all constraints were removed.

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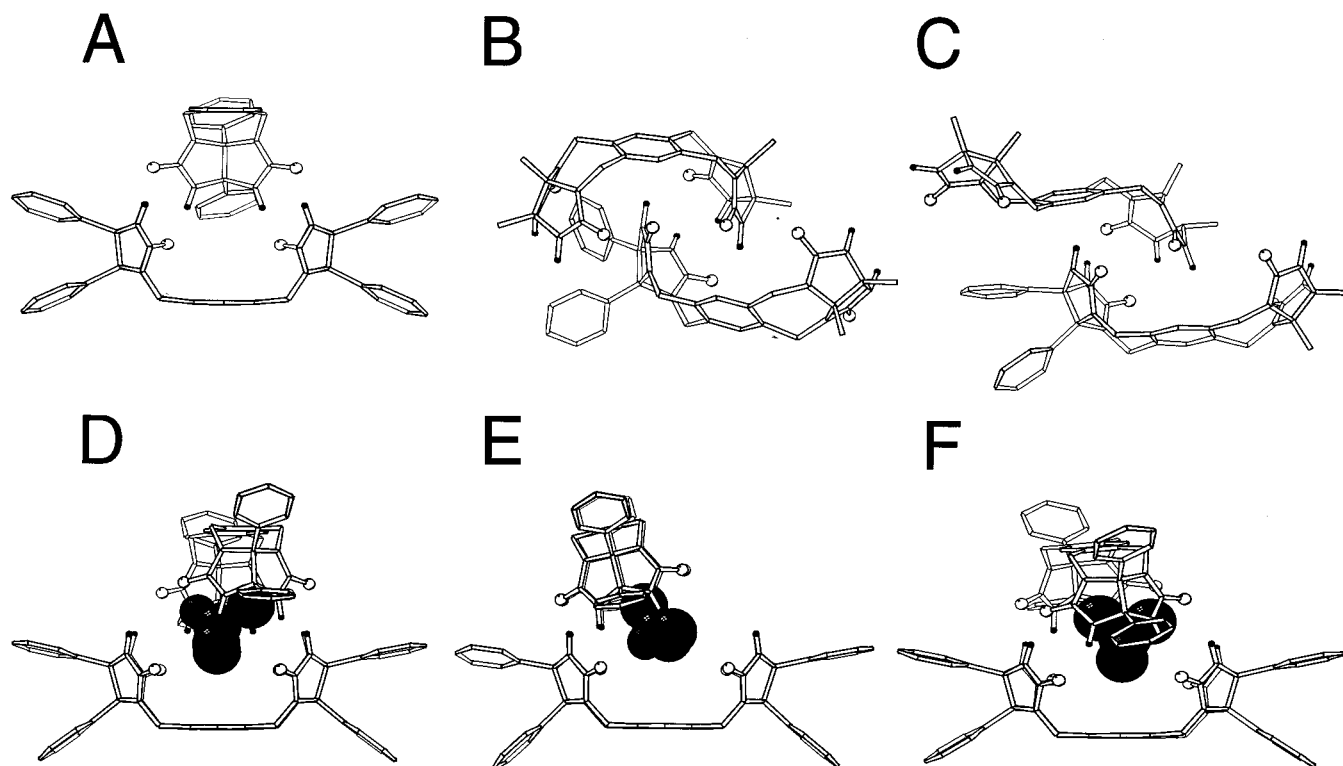
(2) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.

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(5) Allen, M. P.; Tildesley, D. J. In *Computer Simulation of Liquids*; Oxford Science Publications: Oxford, 1987; p 71.

(6) Fox, T.; Thomas, B. E., IV; McCarrick, M.; Kollman, P. A. *J. Phys. Chem.* **1996**, *100*, 10779.



**Figure 2.** “E-dimer” (A–C) and “c-dimer” (D–F) “snapshot” structures obtained from 500 ps molecular dynamics simulations. For clarity, all hydrogen atoms and solvent molecules have been deleted; furthermore, in B and C, we show only those phenyl groups which exhibit interactions with the glycouril rings. In all figures, the white spheres represent the carbonyl oxygens, and the black spheres represent the hydrogen atoms of the donor–acceptor pairs. In D–F, the chloroform molecule is presented space-filling spheres. Snapshots: (A) 0 ps; (B) 102 ps; (C) 216 ps; (D) 93 ps; (E) 120 ps; (F) 215 ps.

time period of 500 ps, with a coupled temperature bath<sup>11</sup> at 300 K. Using FOCUS,<sup>12</sup> the systems have been analyzed to examine time-dependent dimer stability and perturbations in the intra- and intermolecular forces and the hydrogen bonding distances.

### Results and Discussion

At  $t = 0$ , we find that all three equilibrated dimers exhibit the characteristic “tennis ball” configuration. Each dimer is stabilized by a total of eight identical hydrogen bonds (i.e., eight amide carbonyl oxygen to hydrogen donor–acceptor pairs, denoted as 1 through 8 in Figure 1). A comparison of each equilibrated dimer reveals that the average “c-dimer” hydrogen bonding distances (2.3 Å) are greater than those of the “n-dimer” (2.0 Å) and the “e-dimer” (1.9 Å). Note that the interior volume of the “e-dimer” has been estimated<sup>3</sup> at 50–53 Å<sup>3</sup>, whereas the volumes of a chloroform and nitrogen molecules are 73 Å<sup>3</sup> and 19 Å<sup>3</sup>, respectively. Hence, during the equilibration phase, both the “n” and “c” dimers undergo hydrogen bond expansion in order to accommodate their respective guest molecules.

Over the course of 500 ps, we observe differences in the relative stabilities of each dimer system. The “n-dimer” retains the “tennis-ball” configuration for the duration of the simulation. Over the 500 ps interval, the average O···H distances for sites 1–8 are 2.13, 2.10, 2.09,

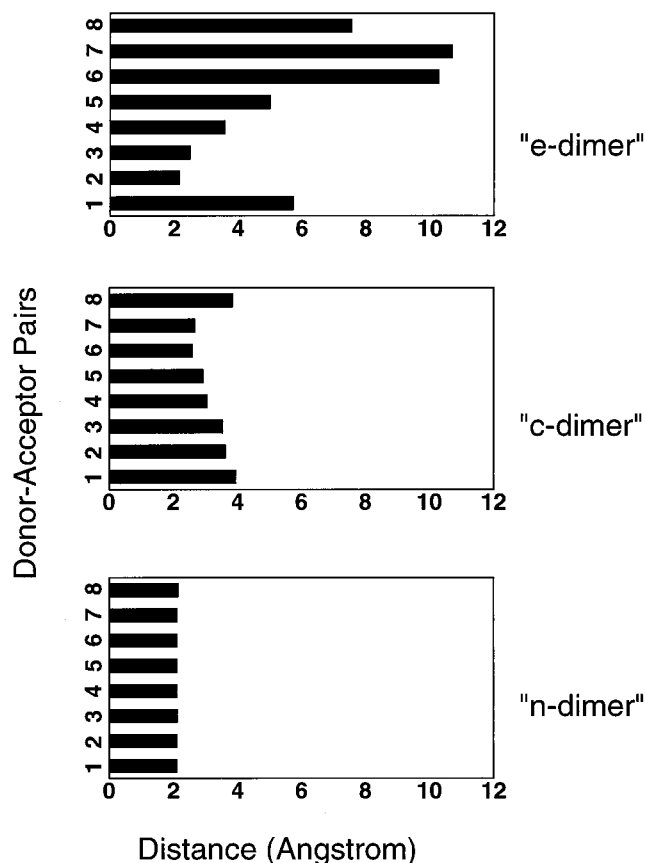
2.09, 2.08, 2.11, 2.09, and 2.09 Å, respectively (Figure 3). Each hydrogen bond undergoes small periods when the O···H distances are greater than 3 Å. However, these events are short-lived (<1 ps) and are followed by a return to the “average” value. Thus, there is monomer motion occurring, but the hydrogen bonding framework is retained and the complex remains stable.

In contrast, the “e-dimer” undergoes two significant structural changes. First, partial dimer dissociation is observed at  $t = 80$  ps. Only two of the original eight donor–acceptor pairs (i.e., 6, 7) are retained after this point in the simulation. Over the 500 ps interval, the average O···H distances for sites 1–8 are 7.54, 10.68, 10.26, 4.99, 3.57, 2.49, 2.15, and 5.71 Å, respectively (Figure 3). Interestingly, once the “e-dimer” undergoes partial dissociation, we observe a 90° rotation of one monomer relative to the other (Figure 2B). This rotation leads to a significant van der Waals repulsion energy between the two phenyl R groups of one monomer and the glycouril ring of the other. The second event occurs at  $t > 180$  ps, wherein the “collapsed” intermediate structure undergoes further rearrangement. Here, we observe a rotation about the two methylene torsion angles which join the glycouril ring to the central benzene ring. This rotation leads to the formation of a third type of dimer complex (Figure 2C). We did not observe any further rearrangements in this third complex up to 500 ps. Our analysis indicates that this torsional transition results from the buildup of van der Waals repulsion forces, and not electrostatics, in the collapsed intermediate. Collectively, our observations indicate that the “e-dimer” is unstable over the course of the 500 ps simulation and

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**Figure 3.** Donor–acceptor hydrogen bond distances in the “tennis ball” dimer complexes. For each donor–acceptor pair (identified on the *y*-axis), the O···H distances were calculated from system coordinate information in each completed trajectory file.<sup>10</sup> For each dimer, averaged distances were then obtained over the entire 500 ps trajectory.

is prone to internal rearrangements which are detrimental to the integrity of the complex. This observed instability is in contrast to experimental evidence<sup>3</sup> which suggests that the “e-dimer” is stable on the NMR time scale. However, it has been noted that the empty dimer may be encapsulating dissolved gases (such as  $N_2$ ).<sup>4</sup> If so, then the presence of the  $N_2$  guest would stabilize the dimer in chloroform solvent, in agreement with our observations.

The “c-dimer” does not exhibit full or partial monomer dissociation over the course of 500 ps simulation. Instead, the “c-dimer” complex experiences more limited motions that involve the movement of one monomer relative to the other. These motions are accompanied by increases in the hydrogen bonding distances and transient hydrogen bond dissociation–reassociation at certain donor–acceptor sites (Figure 3). Over the 500 ps interval, the average O···H distances for sites 1–8 are 3.83, 2.65, 2.57, 2.91, 3.03, 3.52, 3.61, and 3.92 Å, respectively. At  $t > 100$  ps, we find that all eight donor–acceptor pairs experienced some degree of positive hydrogen bond displacement. As shown in Figure 2E, this dimer expansion permits one monomer to “pivot” relative to the other monomer, resulting in an enlargement in the dimer cavity entrance. However, at no point in the 500 ps simulation do we observe any loss of the chloroform guest molecule from the “c-dimer” cavity. In fact, examination of the van der Waals surface of the dimer in Figure 2E indicates that the opening is insufficient in dimension to allow guest release. At  $t > 200$  ps, we observe that the “c-dimer” reverts to the initial “tennis ball” configu-

ration, with closure of the cavity entrance (Figure 2F). This opening and closing process is repeated at  $t > 300$  ps and  $t > 400$  ps, indicating that the expansion process is cyclic. Thus, unlike the “e-dimer”, the “c-dimer” experiences transient hydrogen bond dissociation and reassociation.

Clearly, our studies indicate that there are differences in the relative stabilities of the two-dimer systems that can only be accounted for by the presence of the encapsulated guest molecule. What is not clear is the nature of the stabilizing forces that occur between the encapsulated guest molecules and the self-assembled dimer complex. However, in recent free energy perturbation studies of the “tennis ball” dimer complexed with  $CH_4$  or  $CHCl_3$ , Fox et al.<sup>6</sup> showed that the  $CH_4$  guest binds more strongly to the dimer because of a reduction in the van der Waals interactions as compared to the  $CHCl_3$  guest. Branda et al.<sup>4</sup> also explained the more favorable binding of  $CH_4$  over  $CHCl_3$  in terms of the relative volume of the two guests compared to the interior volume of the dimer. It is estimated that optimal host–guest binding requires that the guest be ~20% smaller than the volume of the host, thus minimizing the van der Waals interactions.<sup>4</sup> This is in agreement with our observations of greater monomer–monomer motion in the “c-dimer” ( $CHCl_3$  volume = 73 Å<sup>3</sup>) as compared to the “n-dimer” ( $N_2$  volume = 19 Å<sup>3</sup>). Collectively, these observations suggest that van der Waals interactions, along with an optimal guest volume: host interior volume ratio, are the keys to host–guest complex stabilization.

It is known that  $CHCl_3$  is weakly bound to the dimer, with an inclusion constant ( $K_{inc}$ ) of 0.04 M<sup>-1</sup>.<sup>6</sup> Hence,  $CHCl_3$  molecules do enter and leave the dimer cavity with some frequency. On the basis of our observations of “c-dimer” monomer–monomer motions and “e-dimer” instability, we postulate that  $CHCl_3$  exchange with the “tennis ball” dimer complex may involve monomer–monomer “pivoting” or other motions which lead to expansion of the dimer and exposure of the dimer cavity to the solvent. Furthermore, it is reasonable to suggest that the empty dimer could then experience complete dissociation, or possibly encapsulate a new guest (e.g., another  $CHCl_3$  or a gas molecule). Obviously, these present studies represent a preliminary examination of the “tennis ball” dimer system and the possible mechanisms for host–guest capture and release.

As shown herein, the “tennis ball” self-assembly complex is dynamic and experiences periods of relative instability. Moreover, transient reversible or nonreversible rearrangements can affect the integrity of the dimer complex. We believe that mapping the occurrence and the net effects of time-dependent motions in supramolecular assemblies may enhance our ability to “engineer” a proper combination of noncovalent interactions and internal geometries that will prolong the lifetime of a given complex.

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**Supporting Information Available:** Plots of the eight hydrogen bond (proton – oxygen) distances versus simulation

time (Figure 1S), and, the average hydrogen bond distances (including standard deviation, maximum, and minimum distances)(Table 1S) for each dimer system (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and

can be ordered from the ACS; see any current masthead page for ordering information.

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