Communication

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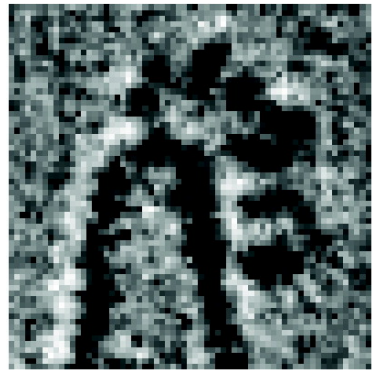

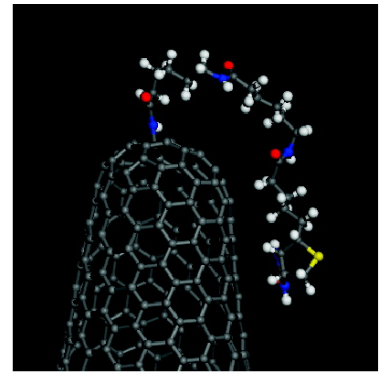

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Imaging of Conformational Changes of Biotinylated Triamide Molecules Covalently Bonded to a Carbon Nanotube Surface

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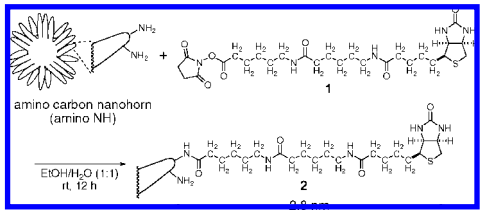
Complex organic molecules exist in numerous conformations in equilibrium with each other, under kinetically or thermodynamically controlled conditions. The conformation exerts decisive effects on the molecular interactions, and therefore, on the activity of the molecules. Chemists and biologists routinely discuss the conformational changes through theoretical and manual analysis of a single model molecule, yet there have been no experimental methods to study conformational changes of complex organic molecules on a single molecule basis in a time-resolved manner with near atomic precision. We recently reported that transmission electron microscopy (TEM) provides near-atomic resolution movie images of the conformational change for an alkyl carborane[1] and an aromatic amide molecule,[2] both of which were loosely encapsulated in the interior of a carbon nanotube (CNT).[3] However, the CNT confinement limited not only the size of the specimen molecules but also the conformational possibilities, and hence reduced the generality of the new methodology. While attachment of the specimen molecules on the exterior of a CNT is an obvious alternative to alleviate these limitations,[4] it poses new problems; how the molecules are to be fixed on the CNT exterior, how stable the molecules can be if they are directly exposed to the electron beam, and how fast the conformational change will be without the constraint of encapsulation. We probed these issues for single molecules of a biotinylated diamide **1** (Scheme 1) bonded to aminated carbon nanohorn (NH) through an amide linkage.[5] We report herein that the amide linkage is a viable tool to link a complex molecule to NH under mild conditions, the triamide molecule **2** is qualitatively as stable as the molecules in nanotubes (i.e., at least for a few minutes of observation time and a total electron dose of ca. 10^7 e·nm^{-2}),[6] and the conformational change is on the same time scale of seconds as those reported previously for confined molecules.[1−4]

Scheme 1. Attachment of **1** to Amino NH To Form Conjugate



In this study, we used aminated NHs of molecular formula approximating $C_{70000000}N_{200000}H_{500000}$∼$C_{170000000}N_{400000}H_{1300000}$, on the surface of which there exists one amine residue per ca. 90 carbon atoms. NHs possess more of the functionalizable cap regions than the less reactive side walls, which is an attractive feature of the NHs among other CNT variants.[5] We have chosen to study the structure of biotinylated diamide **1** because of its importance in biological research.[7] Despite the moderate complexity of **2**, the number of possible conformers is formidable, $>10^8$. We treated the amino NH with the *N*-hydroxysuccinimide ester of **1**. The yield of the acylation of the amino NH under these conditions is several %,[5b] and hence we expect one molecule of **1** per 1000 to 2000 surface carbon atoms, that is, a few molecules per single NH composed of several thousand carbon atoms.

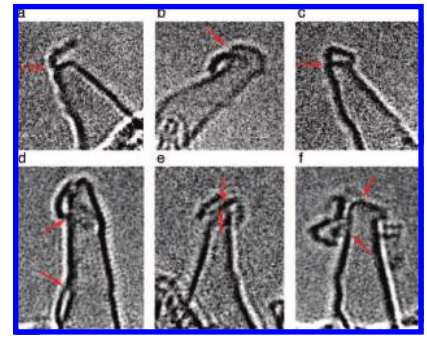


Figure 1. TEM images of the triamide−NH conjugate **2**: (a−c) a single triamide molecule on an NH; (d−f) multiple attachment. Red arrows point to the positions of the amide bonding to the NH surface. Identification of such positions can be made more easily on movie images than on still images. The scale bar represents 1 nm.

We examined the specimen with a TEM instrument (120 kV, Cs = 0.45 mm, 2.3 Å resolution) operating at 293 K and 1×10^{-5} Pa. A TEM-microgrid was soaked in a suspension of **2** in H_2O, and the triamide molecules on the NHs were imaged with a 0.5 s imaging time (i.e., electron irradiation of 0.5 C/cm^2) followed by a 1.6 s charge-coupled device data readout time (no irradiation) for a total observation time of up to a few minutes.

As illustrated by the six representative images in Figure 1, we could detect one to a few triamide molecules in various conformations attached frequently in the curved regions. Thus, the images provide experimental support for the accepted wisdom that such regions are the predominant reactive sites of pristine CNTs.[8] The conformers did not significantly change in structure during the observation time, hence each must represent a kinetically separated local minimum that undergoes small conformational changes in its local potential well (vide infra). All images in Figure 1 except image a appear to have a contact of the terminal biotin moiety to the π-surface of NH (vide infra).

We take a series of TEM images of the time evolution of the conformational changes of **2** shown in Figure 2a as an example and discuss the structural information that we can draw from such images. The images were extracted from a movie recorded for 101 s (video 1). The movie indicates that the molecule is bonded to the cap of the

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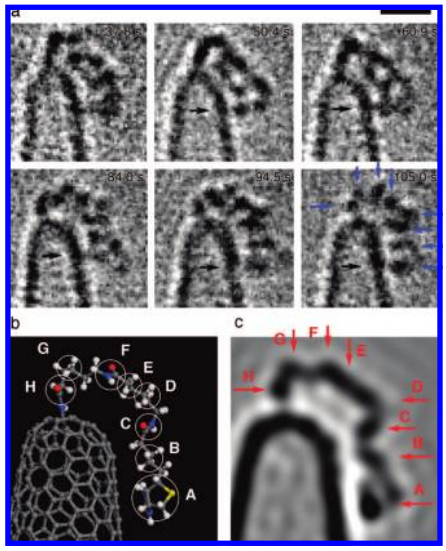

Figure 2. (a) TEM images of triamide−NH conjugate **2** in a series of bent conformations. The figure captions refer to the time (seconds) after initiation of the observation, i.e., initiation of electron irradiation. (b,c) Molecular structure of **2** and its simulated image at 105.0 s in Figure 2a. Scale bar = 1 nm.

Ⓦ A TEM movie, video 1, of tiramide–NH conjugate **2**.

Ⓦ A TEM movie, video 2, of a second molecule showing larger structural changes.

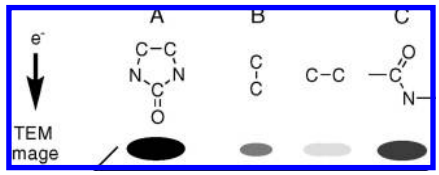

Figure 3. Schematic representations of TEM images generated by overapping atoms. The spot becomes darker as more atoms overlap along the direction of the electron beam with some modulation of the image contrast by electron interference. A−C correspond to those points in Figure 2b,c.

NH and stretches to the right, keeping contact with the NH surface (cf. the molecular model, Figure 2b), that the structural change occurs smoothly as illustrated by the appearance of images, which are mutually related one after another, and that the molecular motion is quite slow relative to the exposure time of 0.5 s (i.e., the images generally showed well-defined dark spots).

The dark spots reflect structural characteristics of the conformers, and their time-dependent change reflects the conformational change. As seen by the positions of the biotin terminus indicated by black arrows (vide infra), the molecule largely retained its structure during the observation time (the movie image of another molecule showing much larger structural changes is shown in video 2).

As illustrated schematically in Figure 3, alignment of more than one carbon, oxygen, or nitrogen atoms parallel to the electron beam creates characteristic dark contrasts in their TEM image, which may be further enhanced or attenuated by electron interference, as illustrated by the white/black fringes in Figure 2c. As shown in the cartoon and simulations (structure A in Figures 2b,c, 3, and 4), biotin generates complex patterns as viewed from various directions (Figure 4). Similarly, a C−C bond generates either a moderately dark spot (B) or a very weak contrast depending on the orientation of the bond, and an amide (structure C in Figures 2b,c and 3) may give a rather dark spot. The blurring of the dark spots suggests that the atoms moved during the 0.5 s exposure time.

We therefore considered that structure assignment at a certain confidence level can be made from the experimental TEM image by

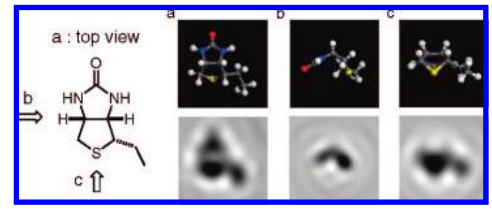

Figure 4. TEM simulation of the bicyclic moiety of biotin as viewed from three directions.

carrying out a series of iterative model constructions/TEM simulations and comparing the simulated and real images. One plausible structure of the conformer responsible for the 105.0 s image thus obtained is shown in Figure 2b. This model was constructed on the basis of the standard conformational analysis[9] with appropriate help of theoretical calculations,[10,11] and then the simulation in Figure 2c was created. The special structural features are highlighted by eight circles A−H, which generated eight dark spots in the simulation. The locations of these spots agree quite well with those in the actual 105.0 s image (and the 94.5 s image, too) that are indicated by blue arrows. The agreement is good enough to convince us that this 3-D model structure is one probable structure of the conformer out of the $>10^8$ possibilities. Figure 1 suggests contact between the biotin and the π surface of the NH. Figure 2b and the TEM images reinforce this conjecture, because the molecule maintained contact with the NH at least in region A throughout the observation. Lone pair/π, π/π, and/or CH/π interactions may be responsible for this contact.

In summary, in contrast to the encapsulation protocol in which a carbon chain of the specimen molecule is placed perpendicular to the electron beam and viewed largely in its stretched conformation,[1] this exterior protocol allows the chain to have orientations that are more diverse and hence give more diverse contrast images of more diverse conformers, thereby giving richer structural information. The confidence level of the assignment will become higher in the future through improvement in the time and spatial resolution of the microscopes, and the method will then become an ultimate microanalytical method for studies of molecular structures and their conformational behavior.

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Supporting Information Available: Experimental procedures and additional TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The capacity of the current theoretical methods to deal with weak interactions precluded full structural optimization of the whole system.
(11) We assumed that all TEM images are due to conformers in local minima of potential surface.

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