

論文の内容の要旨

Stochastic Dynamic Behaviors of Single Molecules Revealed by Sub-Millisecond Electron Microscopic Imaging

(サブミリ秒電子顕微鏡動画撮影による確率論的な単分子動的挙動の解析)

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1. Introduction

In situ observation of nanoscale events such as chemical reactions and molecular dynamics have remained to be a big challenge for scientists for a long time. Time-resolved imaging of the molecular events is of great importance to unveil the behavior of an individual molecule in the nanoscale system and thus to break into an unexplored field of science. To understand these events in detail, both spatial and temporal resolutions are essential. Recently, the speed of molecular imaging with a high-speed atomic force microscope has reached a practical limit of 16 frames/second (fps), yet with its spatial resolution far from atomic.¹ Pump-probe microscopy has achieved high spatial and temporal resolution,² but it requires the events observed to be easily reproducible and repeatable, and not suitable for the stochastic and dynamic phenomena of single molecules, which need to be imaged continuously. This limitation would be lifted by fast electron microscope (EM) imaging. However, the fast EM imaging against mobile molecules at a molecular level far lagged behind reported to be 12 fps, limited by the speed of the camera.³

Under these circumstances, our group has developed a single-molecule atomic-resolution real-time electron microscopy (SMART-EM) imaging method to observe and analyze the dynamic behavior of a single molecule in situ.^{4,5} In this work, by using a transmission electron microscope (TEM), a fast shutter speed camera, and the optimized denoising algorithm for EM imaging, I succeeded in recording the world's fastest video of stochastic dynamic behaviors of a molecule at a sub-millisecond level. By analyzing the video statistically over time, I could obtain the whole picture of the molecular events. As a target molecule, [60]fullerene (C_{60}) encapsulated in carbon nanotubes (CNTs) were used. First, I analyzed the fusion reaction of C_{60} dimers with the time resolution of a millisecond to capture the structures of the short-life intermediates. Second, I succeeded in recording a video of a single molecular shuttle at the time resolution of sub-millisecond and analyzed these mechanical motions to reveal the rich molecular dynamics of a single C_{60} molecule shuttling, rotating, and interacting with a vibrating CNT.

2. Sub-Millisecond Molecular Imaging Enabled by Denoising

The low frame rate imaging (e.g. 1 fps) usually causes blurring because the molecules move around during observation. To solve this problem, I used a fast shutter speed camera for molecular imaging. However, a high frame rate makes the raw image (Figure 1a) noisy due to a low electron dose for a single frame. In this work, I solved this problem with a collaborator, Dr. Joshua Stuckner, by investigating various noise reduction methods. I concluded that the most appropriate denoising method for EM molecular imaging is Chambolle total variation denoising algorithm.⁶ While the Chambolle denoising preserves molecular edges, which are indispensable for precise size and distance measurements, noises are reduced to give a good signal to noise ratio, improving the image contrast. As shown in Figure 1b, I identified C_{60} molecules in the Chambolle denoised single-frame image. After simultaneous three-frame superimposition and Chambolle denoising, I obtained a clear molecular image (1.875 ms/frame, Figure 1c) with keeping a time resolution of a millisecond. With this denoising method, I have successfully achieved a temporal precision of 0.9 ms and a localization precision of 0.01 nm for imaging of non-periodic molecular structures. Millisecond-level time-resolution EM images described here are the fastest EM time resolution so far for imaging of dynamic molecular events.

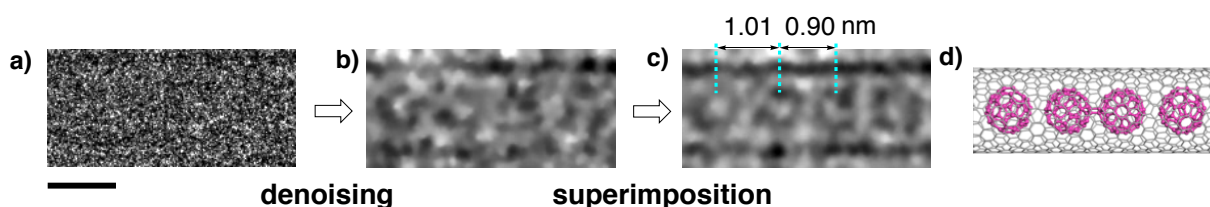


Figure 1. Fast video imaging of C_{60} molecules with Chambolle denoising and superimposition.

- a) A single-frame image of C_{60} molecules in a CNT at 0.625 ms/frame (1600 fps) without denoising and b) with denoising. c) A three-frame superimposed image (1.875 ms/frame) with Chambolle denoising. d) Corresponding molecular model of C_{60} molecules in a CNT. Scale bar: 1 nm.

3. Millisecond-Order Analysis of Chemical Reactions of C_{60} molecules

Chemical reactions are usually discussed and analyzed in the averaged numbers based on Avogadro's constant, for example in nuclear magnetic resonance (NMR) spectroscopy. However, in reality, each reaction occurs independently in stochastic manners. By using the SMART-EM technique, we are now able to observe chemical reactions focusing on each molecule.

I report here on the in situ imaging of reacting C_{60} molecules in a CNT up to a time resolution of a millisecond. It is known that under electron beam irradiation, C_{60} dimerization occurs in a CNT.⁷ From low fps EM imaging analysis, only several types of intermediates are seen, such as van der Waals (vdW) complex, cycloadduct, and unidentified dimer and oligomers. In this work, I monitored C_{60} dimerization at 1600 fps imaging and measured the center to center distance between two C_{60} cores with sub-angstrom level as an indicator of the progress of the dimerization reaction. (Figure 2a) I have achieved recording the decisive moment of cycloaddition reaction with <1.9 ms time resolution. Furthermore, by using proposed isomer models (OT isomer models) from the computational calculation,⁸ I conducted simulated distance analysis (Figure 2b), which showed similarity to experimental distance data. The denoised TEM image of each intermediates (Figure 2c) and TEM simulation images of OT isomer models from various angles were automatically compared to obtain the best matched TEM simulation images (Figure 2d), determining plausible isomer models (Figure 2e). These results confirm that the fusion reaction of C_{60} dimers occurs in stepwise, and I succeeded in capturing the structures of the short-life intermediates.

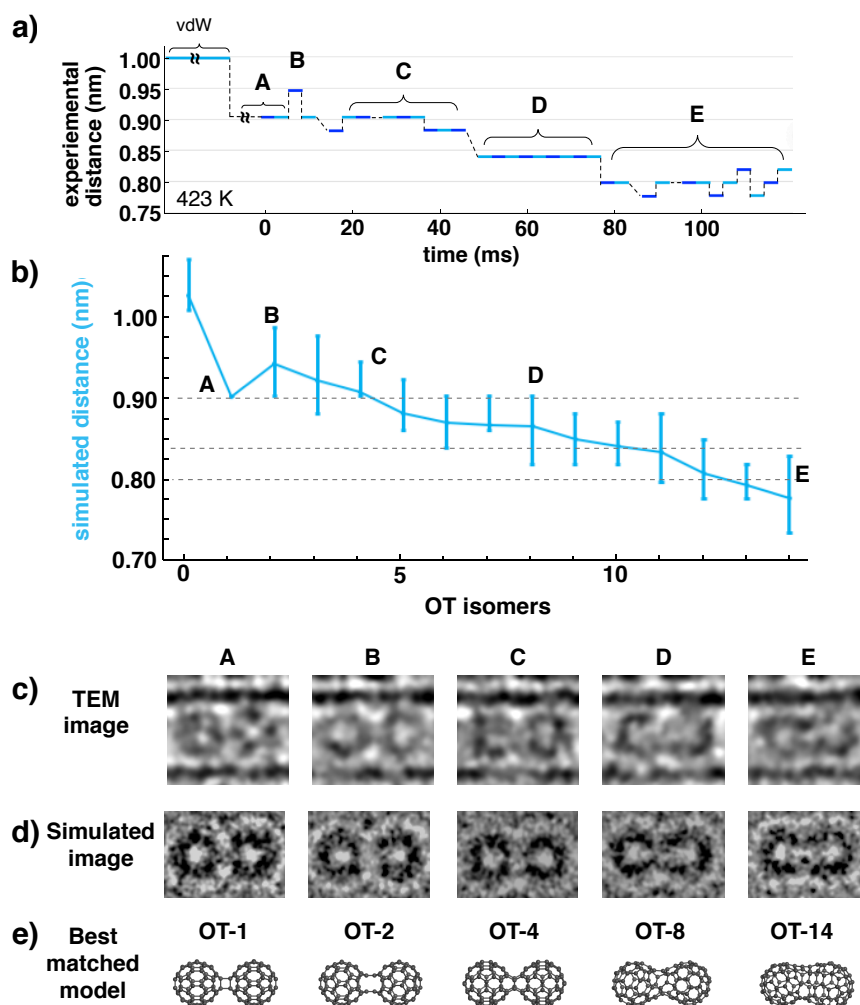


Figure 2. Multistep conversion of cycloadduct **A** to fused dimer **E** based on TEM videos. a) Time evolution of center to center distance measured every 3.125 ms at 423 K. b) The simulated distance of intermediates proposed by Osawa and Tomanek, who used the numbering shown (OT-#). c) Experimental TEM images of **A**–**E** (3.125 ms/frame, with denoising). d) Simulated TEM images obtained from automated cross-correlation image matching. e) Best matched OT isomer models.

4. Real-Time Video Imaging of Mechanical Motions of a Single Molecular Shuttle

A molecular shuttle is a typical molecular machine studied for a long time. However, in situ observation of the shuttling motion has not been achieved, and more importantly, the mechanism of the molecular shuttling in nanoscale from a perspective of molecular dynamics has been unknown because this kind of mechanics is stochastic and non-repeatable. In addition, conventional dynamic imaging for nanoscale mechanical events has achieved time resolution only down to tens of milliseconds. Here I elucidated the mechanism of a nanomechanical behavior of a molecular shuttle by recording of the 1600 fps video of a single molecular shuttle. C_{60} encapsulated in a CNT reacted to each other under an electron beam irradiation to form C_{60} dimers or oligomers, as I mentioned above. Due to the reaction, the distance between each C_{60} molecule got shorter and some spaces were made in the CNT. Then, I found a C_{60} dimer or oligomer moving back and forth in the CNT during TEM observation. I analyzed these mechanical motions in detail with sub-millisecond sub-angstrom precision in situ video imaging. In Figure 3a, I showed a series of TEM images of an oligomer moving back and forth for three times and the results of the analysis for the location of an encapsulated

molecule and CNT container were summarized in Figures 3b and 3c. I found that the translation of the molecule occurred when the direction of the CNT vibration changes, suggesting that the motion was induced by receiving an energy from the vibrating CNT. In other words, the molecule and the CNT container as a whole behave as a mechanically coupled oscillator, where the molecular motion is coupled with the mechanical motion of CNT.

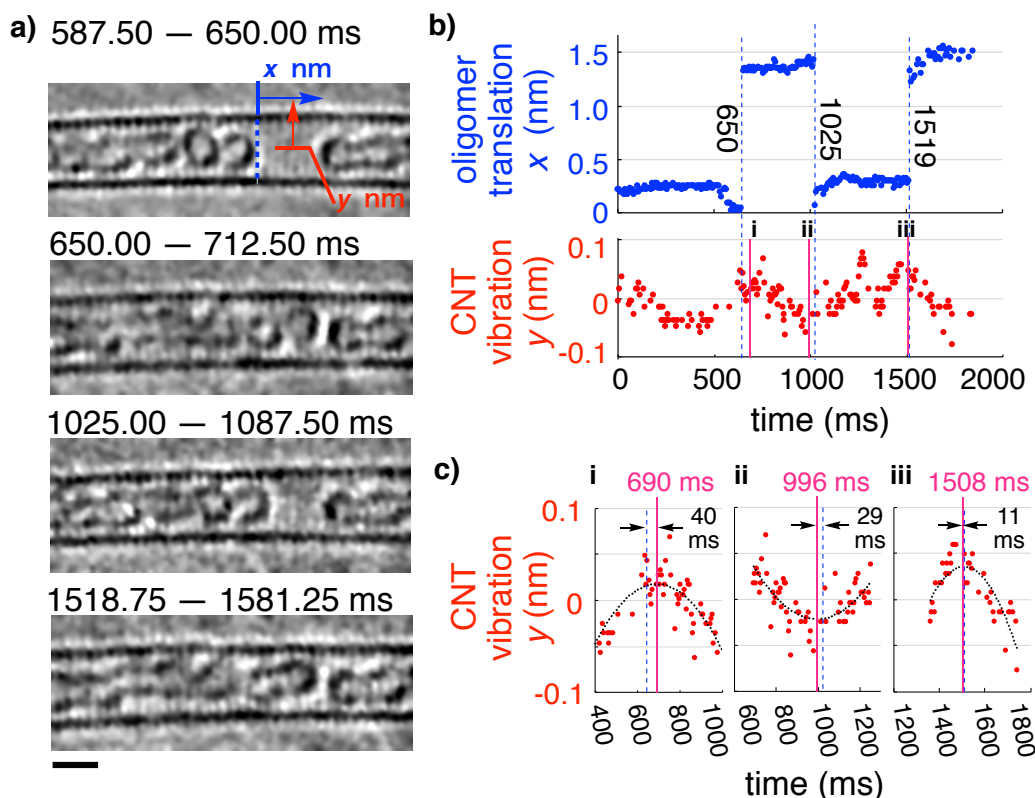


Figure 3. SMART-EM video frames showing the motions of a C_{60} oligomer in a vibrating CNT. a) TEM imaging and b) the analysis of the shuttling C_{60} oligomer in a CNT. c) Expansion of 690, 996, and 1508 ms areas of CNT vibration. Scale bar: 1 nm.

5. Conclusion

During my doctoral course study, I have succeeded in observing and analyzing in situ individual nanomechanical events coupled with mechanical vibration of the CNT container, as well as chemical reactions of individual molecules at maximum sub-millisecond and sub-angstrom precision by combining an atomic-resolution electron microscope, a fast shutter speed camera, and the optimized denoising algorithm for EM imaging. This sub-millisecond electron microscopic imaging techniques will open up a new field of stochastic dynamics of single molecules, elucidating the mechanism of molecular conformational changes and the equilibrium state of chemical reactions, which are more fundamental molecular behaviors. It is expected to be applied for the study of various scientific phenomena that could only be conducted in theoretical calculations.

6. References

- [1] T. Ando, *Biophys. Rev.* **2017**, *9*, 421. [2] A. H. Zewail, *Science* **2010**, *328*, 187. [3] J. H. Warner et al., *ACS Nano* **2009**, *3*, 3037. [4] M. Koshino et al., *Science* **2007**, *316*, 853. [5] E. Nakamura, *Acc. Chem. Res.* **2017**, *50*, 1281. [6] A. Chambolle, *J. Math. Imaging Vis.* **2004**, *20*, 89. [7] S. Okada et al., *J. Am. Chem. Soc.* **2017**, *139*, 18281. [8] S. Han et al. *Phys. Rev. B*, **2004**, *70*, 113402.