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Dynamic Observation of 2686 bp DNA–BAL 31 Nuclease Interaction with Single Molecule Level Using High-Speed Atomic Force Microscopy

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Conventional atomic force microscopy (AFM) can visualize not only the specific binding between a DNA molecule and an enzyme directly, but also the resulting conformation of DNA that reacted with the enzyme. However, it is difficult to visualize the dynamic behavior of biomolecules by conventional AFM, which need a few minutes to image a sample, because the conformation of a biomolecule changes within seconds. In the present study, we successfully observed the dynamic behavior of the interaction between a DNA and an enzyme in a reaction buffer by high-speed AFM. Moreover, we successfully visualized the digestion of the DNA by the enzyme and the motions of the enzyme on a DNA strand. The dynamic observation by high-speed AFM enabled us the quantitative evaluation of what. The movement speed of the enzyme along the DNA strand was about 23 nm/100 ms. [DOI: 10.1143/JJAP.47.6168]

KEYWORDS: AFM, high-speed AFM, DNA, enzyme, BAL 31 nuclease, real-time imaging, dynamics, interactions, digestion

1. Introduction

Atomic force microscopy (AFM) is used widely to observe a sample surface at a spatial resolution of sub nanometer.[1,2] In particular, intermittent-contact-mode measurement in conventional AFM is a helpful technique for observing soft materials, such as biomolecules, without destroying the sample by the cantilever used.[3]

Electrophoresis and transmission electron microscopy are convenient techniques for the structural analysis of DNA. Although it can not be used to evaluate the structure of DNA molecules, electrophoresis enables us to analyze the mass of DNAs of any length. On the other hand, although transmission electron microscopy enables us to observe the precise structure of a DNA molecule, is technique leads to the loss of the physiological activity of a DNA molecule because of the staining treatment and the use of vacuum atmosphere. However, conventional AFM enables us to observe the precise structure of a DNA molecule while maintaining its physiological activity. For example, various types of interactions between DNAs and proteins have been observed by conventional AFM.[4–9]

Recently, "high-speed AFM" has been developed, which enables us to observe the dynamical behavior of relating to proteins.[10–13] Previously, we studied the interaction between DNA and DNase I in a reaction buffer by high-speed AFM, which enables us to perform a real-time observation of the dynamical behavior of biomolecules.[14] It is known that BAL 31 nuclease, which degrades double-stranded nucleic acids from both the 3′- and 5′-ends and single-stranded nucleic acids. In this study, we studied the dynamical behavior of the interaction between DNA and BAL 31 nuclease in a reaction buffer by high-speed AFM.

2. Materials and Methods

2.1 DNA and enzyme preparation

A 2686-bp circular DNA (PUC-18, Toyobo), which was also a supercoil structure, was excised by *Eco*RI (ECO-111, Toyobo) and purified with a DNA purification kit (27-9602-01, GE Healthcare). BAL 31 nuclease (BAL-301, Toyobo) was used for the experiment on 2686 bp DNA digestion. The buffer solution [20 mM tris(hydroxymethyl)aminomethane–HCl, 12.5 mM $MgCl_2$, 12.5 mM $CaCl_2$ (pH 8.1)], which was used as the reaction buffer of BAL 31 nuclease, was prepared and used for all the experiments.

2.2 High-speed AFM

The sample of the 2686 bp linear DNA was diluted with the reaction buffer to a final concentration of 0.4 ng/μl. For high-speed AFM, a 1.5 μl droplet of the sample was applied onto a freshly cleaved mica (1 mm^2) and incubated 5 min at room temperature to electrostatically immobilize the linear DNA onto the surface. After gently rinsing the substrate surface with 90 μl of Milli Q water, 70 μl of reaction buffer was placed onto the substrate surface. Then a small amount of BAL 31 nuclease (0.007 units/μl) was added to the reaction buffer placed on the substrate, and the sample was immediately observed at room temperature by high-speed AFM (NanoLiveVision). Here, the observation was performed in the intermittent-contact-mode using a special cantilever made of silicon nitride using standard micromachining techniques and whose dimensions were 7 μm long, 2 μm wide, and 0.1 μm thick, with an electron beam deposition tip that is 600 nm long.[15] The resonance frequencies of the cantilever in water were about 636–705 kHz, and the spring constants measured in water by the thermal vibration method were about 0.1 N/m.[16] For wide-area imaging, 192 point × 144 line images were obtained at a scan speed of 144 lines/s (i.e., 1 s/frame). For narrow area imaging, 80 point × 60 line images were obtained at scan speeds ranging from 600 to 900 lines/s (i.e., 0.067–0.100 s/frame).

3. Results and Discussion

3.1 Dynamic observation of 2686 bp DNA by high-speed AFM

The 2686 bp DNA treated with the restriction enzyme, i.e., *Eco*RI, was observed in the reaction buffer of BAL 31 nuclease by high-speed AFM (Fig. 1). We could observe many DNA strands with a linear shape within each frame and only a few DNAs with a circular shape. The observation results show that *Eco*RI treatment of the circular DNA (PUC-18) was successful in the preparation of a double-stranded linear DNA, with a few circular DNAs remaining. We found that the linear and circular DNAs adsorbed onto

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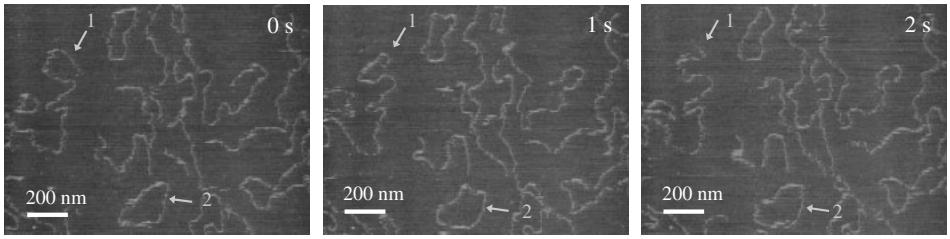


Fig. 1. Dynamic observation images of 2686 bp DNA in reaction buffer. The images from (0 s) to (2 s) are consecutive images extracted from dynamic observation. Arrow 1 shows the same single molecule of linear DNA. Arrow 2 shows the same single molecule of circular DNA.

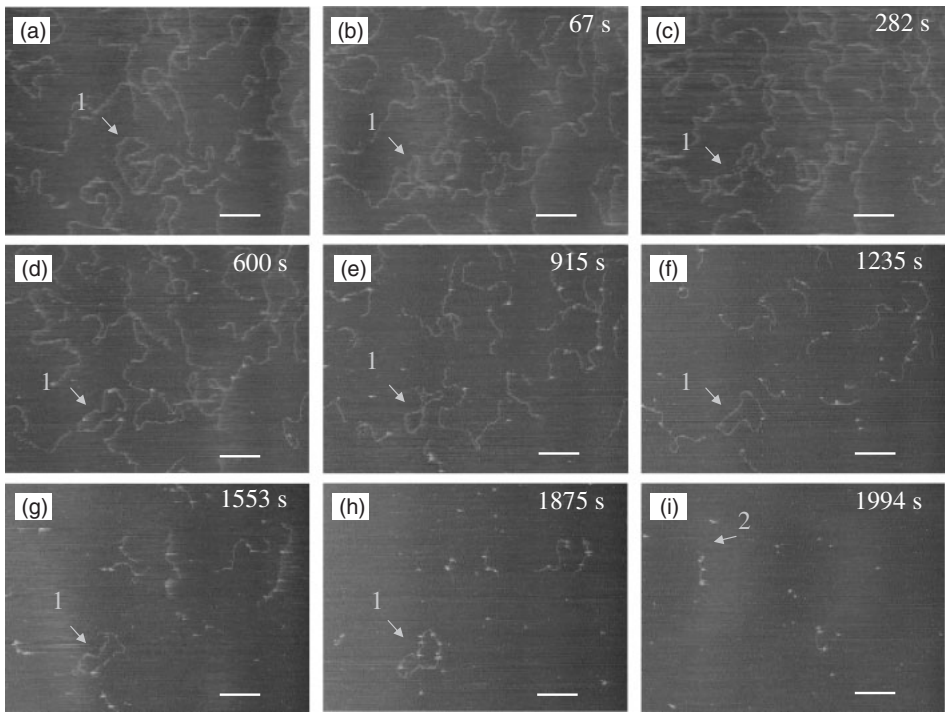


Fig. 2. Dynamic observation images of 2686 bp linear DNA–BAL 31 nuclease interaction and 2686 bp circular DNA–BAL 31 nuclease interaction. (a) Image of DNA before adding BAL 31. (b-i) Dynamic images of DNA after adding BAL 31. The images from (b) to (h) were extracted from the dynamic observation. Arrow 1 from (a) to (h) shows the same single molecule of circular DNA. (i) Image in another field of view. Arrow 2 of (i) shows the DNA digested by BAL 31 nuclease. The scale bars represent 200 nm.

the mica surface moved, and were differed in these molecular structure in every frame. It was thought that DNA molecules on the mica surface changed to these structures, because of their very loose binding onto the mica surface. In addition, the linear and circular DNAs were observed dynamically for a few minutes. Hence, we could not determine whether the AFM cantilever can mechanically cut any DNA strand adsorbed on the mica substrate during dynamic observation.

3.2 Dynamic observation of 2686 bp linear DNA–BAL 31 nuclease interaction

The 2686 bp linear and circular DNAs were observed dynamically in the reaction buffer including BAL 31 nuclease by high-speed AFM (Fig. 2). In the first step, the linear and circular DNAs were observed simultaneously in the frame [Fig. 2(a)]. In the second step, BAL 31 nuclease was added in the reaction buffer and the dynamic observation was started immediately [Figs. 2(b)–2(h)]. After adding BAL 31, the linear DNA was digested through its reaction with BAL 31 nuclease. However, the linear and circular DNAs maintained each structural type 67 s after the addition of BAL 31 [Figs. 2(a) and 2(b)]. The circular DNA maintained a circular structure during the dynamic observation, although a cut of the linear DNA was observed [Figs. 2(b)–2(h)]. Here, we note that BAL 31 degrades double-stranded nucleic acids from both the 3′- and 5′-ends when no single-stranded nucleic acids are present.[17] In Fig. 2(i), we could not find the 2686 bp linear DNA 1994 s after the addition of BAL 31. The result suggests that the digestion of the 2686 bp linear DNA on the mica surface had already been completed by BAL 31 nuclease at this time.

Figure 3 shows the direct observation results of the enzymatic reaction in which BAL 31 nuclease digests the linear DNA. We succeeded in directly observing that, immediately after the binding of BAL 31 to the linear DNA in both 3′- and 5′-ends, the digestion of the DNA by BAL 31

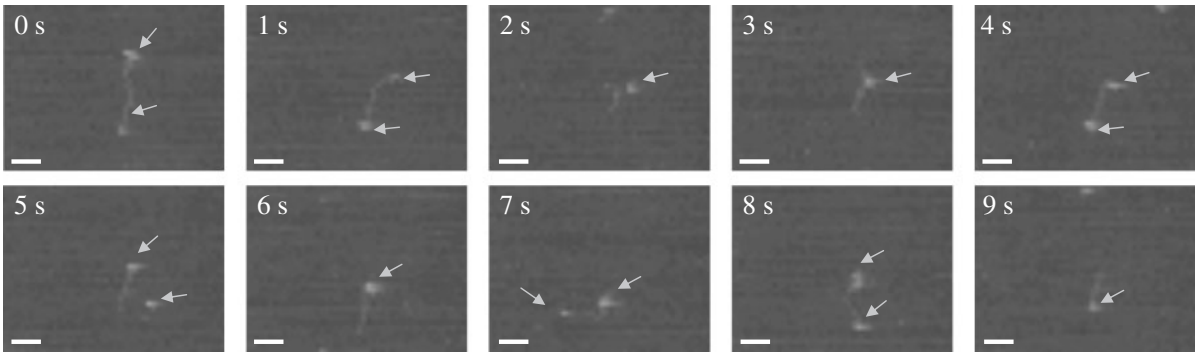
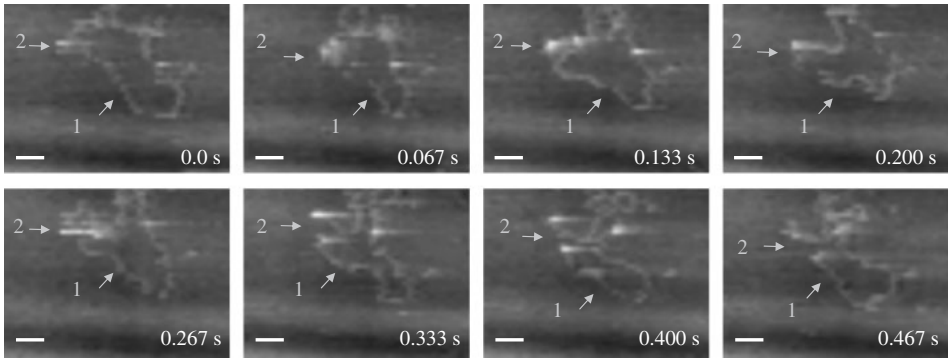
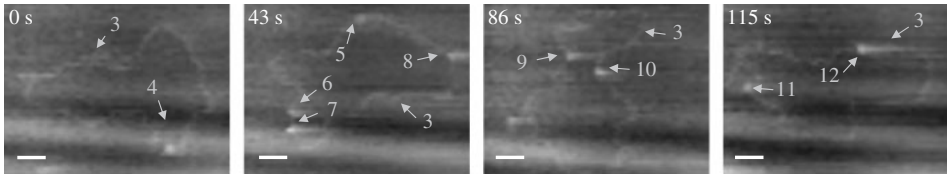


Fig. 3. Dynamic observation images of linear DNA digestion. The images from (0 s) to (9 s) are consecutive images extracted from dynamic observation. The arrows from (0 s) to (9 s) show a single molecule of BAL 31 nuclease binding to the DNA specifically. A DNA of 89 nm length was digested by BAL 31 within nine seconds. The scale bars represent 50 nm.



(a)



(b)

Fig. 4. Dynamic images of BAL 31 nuclease movement onto circular DNA. (a) Images of circular DNA cut off using BAL 31 specifically. Arrow 1 from (0.0 s) to (0.467 s) shows the same DNA. The circular DNA was cut off using BAL 31 as shown by arrow 2 specifically. (b) Images of circular DNA–BAL 31 interaction extracted from dynamic observation. Arrow 3 from (0 s) to (115 s) shows the same DNA. Arrows 4–12 from (0 s) to (115 s) show BAL 31 moving on the DNA strand. The scale bars represent 50 nm.

began, and finally the linear DNA was digested to a length of about 90 nm within 9 s. Although the digestion of the linear DNA strand by the enzyme is a well-known phenomenon, we succeeded in the direct observation of the process for the first time, as shown in Fig. 3.

3.3 Dynamic observation of circular DNA–BAL 31 nuclease interaction

The interactions between the 2686 bp circular DNA and BAL 31 nuclease were observed dynamically (Fig. 4). We successfully observed that BAL 31 moves on the circular DNA or on the mica surface.

Although BAL 31 generally digests only linear DNA that has both the 3′- and 5′-ends, we observed that only a few circular DNAs were excised and digested by BAL 31 [Fig. 4(a)]. In other words, it could be considered that the circular DNA degraded had active strand sequences for BAL 31 nuclease. Here, "the active strand sequences" in the present study could not be clarified. Most of the circular DNAs were not cut off, even when BAL 31 bound to the circular DNA [Fig. 4(b)]. In Fig. 4(b), BAL 31 molecules moved only along the circular DNA strand.

3.4 Dynamic observation of BAL 31 nuclease movement on linear DNA

The behavior of BAL 31 nuclease on the linear DNA was observed directly to quantitatively evaluate the movement speed of BAL 31 along the linear DNA strand (Fig. 5). We observed that almost all BAL 31 on the DNA strand moved at random speed along the helical structure of DNA [Figs. 5(a) and 5(b)]. We thought that BAL 31 slide on the DNA strand was to recognized DNA sequences. We also observed that

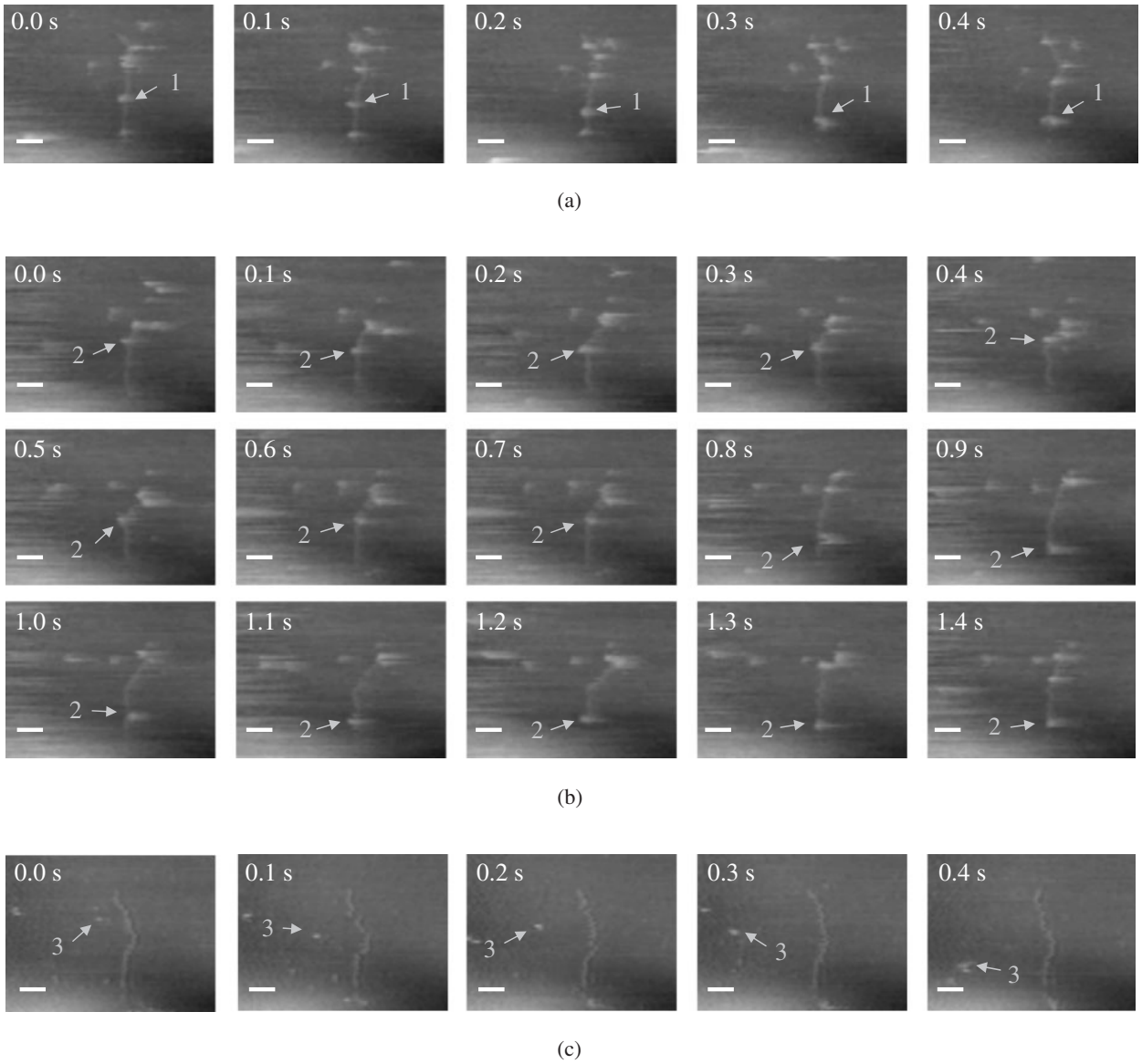


Fig. 5. Dynamic images of BAL 31 nuclease movement. Images a–c are consecutive images extracted from dynamic observation. (a, b) Movement of BAL 31 on linear DNA. (c) Movement of BAL 31 on mica surface. The BAL 31 indicated by arrows 1–3 in the consecutive images show the same single molecule of BAL 31. The scale bars represent 50 nm.

BAL 31 released from DNA was moving on the mica surface at a random speed like the Brownian motion of particles [Fig. 5(c)].

The movement distance of BAL 31 in 100 ms was measured from two consecutive images extracted from the dynamic image. Figure 6 shows the histogram of movement distance in 100 ms of BAL 31 on the DNA strand, and that of BAL 31 on the mica surface. In the case of BAL 31 on the DNA strand, we calculated the variance of movement distance while assuming a one-dimensional system, and obtained a value of about 23 nm. We found that the movement speed of BAL 31 on the DNA strand was about 23 nm/100 ms or about 78 bp/s. It could considered that the obtained speed of about 78 bp/s was the reaction speed of DNA sequences for BAL 31. In the case of BAL 31 on the mica surface, we also calculated the variance of movement distance while assuming a two-dimensional system, and obtained a value of about 32 nm. Here, we found that movement speed of BAL 31 on the mica surface was about 32 nm/100 ms, which was slower than that on the DNA strand. The result suggests that the interaction between BAL 31 and the DNA strand limited the movement of BAL 31 on the DNA strand. Also, note that the present reaction speed of DNA sequences caused by BAL 31 was derived from the observation that was limited on the mica surface. On the other hand, BAL 31 nuclease moved around freely in the buffer solution at random speed. Here, we considered that the movement behavior of BAL 31 limited on the mica surface differed from that of BAL 31 in bulk solution, because of the binding interaction between BAL 31 and the substrate.

4. Conclusions

In the present study, we studied the dynamic behavior of the interaction between a 2686 bp linear DNA and BAL 31 nuclease in a reaction buffer by high-speed AFM. We successfully observed such a phenomenon in which a single molecule of the 2686 bp linear DNA on a mica surface was digested gradually from both ends of the DNA strand by BAL

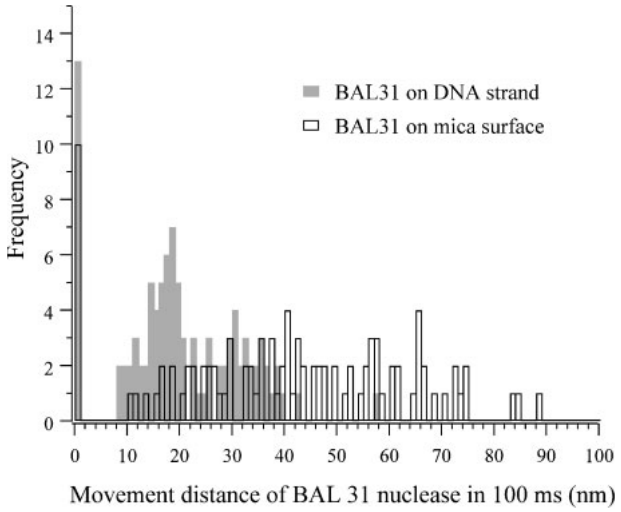


Fig. 6. Analysis result of movement distances of BAL 31 in 100 ms on mica surface (transparent bar) and DNA strand (gray bar). The movement distances of BAL 31 in 100 ms were measured from two consecutive images. The average speed of BAL 31 on the DNA was 199 ± 12 nm/s. The average speed of BAL 31 on the mica surface was 401 ± 22 nm/s.

31 nuclease. We also succeeded in directly observing a process in which a single molecule of BAL 31 nuclease slid along the DNA strand. We found that the movement speed of BAL 31 nuclease along the DNA strand differed from that on the mica surface. Moreover, the direct observation by high-speed AFM clarified that BAL 31 nuclease moves along DNA strands at a speed of about 23 nm/100 ms, and enable the observation of the actual behavior of the interaction processes between a DNA strand and BAL 31 nuclease.

Consequently, we conclude that interesting results of the present study were obtained by high-speed AFM. We think that this AFM is a powerful tool for elucidating the biological mechanism of various types of biomolecules.

Acknowledgments

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