Recent development of high-speed atomic force microscopy in molecular biology

Ke Xu ✉, *Xin Huang, Yusheng Pan*

School of Information & Control Engineering, Shenyang Jianzhu University, Shenyang, 110000, People's Republic of China
✉ *E-mail: xksky1234@163.com*

Published in Micro & Nano Letters; Received on 11th May 2019; Revised on 15th November 2019; Accepted on 17th January 2020

In the aspect of biomacromolecule imaging, high-speed atomic force microscopy (HS-AFM) has many wonderful characteristics, such as rapid imaging and real-time visualisation of the structure and dynamic behaviour of biological macromolecules, which is unparalleled by other technologies. In this review, state-of-the-art achievements on HS-AFM in molecular biology are summarised. Firstly, biomacromolecule characteristics determination and nanostructure recombination using HS-AFM are introduced, which confirm that HS-AFM is such a mature technology that realises high-speed imaging. Then, some improvements including the cantilever of HS-AFM, mechanical scanner, position sensor, and closed-loop control algorithm are reviewed to obtain higher resolution images. Finally, future development directions that could enhance the performance of HS-AFM are discussed.

1. Introduction: Atomic force microscopy (AFM) is an excellent detection tool for high-resolution imaging without being affected by the operating environment and conductivity. AFM uses the force between tip and sample surface (mainly van der Waals force) for imaging, which causes the cantilever bend. By detecting the change of spot position on the position sensitive device, the surface topography of the sample can be obtained (as shown in Fig. 1). Owing to the impact of the mechanical devices (e.g. cantilever and scanner), the imaging rate of traditional AFM is slow, which always takes a few minutes to draw a picture [1]. For example, when observing the dynamic behaviour of macromolecules, the time variation is usually at the level of milliseconds [1, 2], which severely limits the wide application of AFM. Therefore, how to effectively improve the imaging rate of AFM has been plagued by researchers. In recent years, high-speed AFM (HS-AFM) has been successfully invented by the efforts of many research groups (especially, the Ando team at Kanazawa University in Japan and the Hansma team at the University of California, Santa Barbara in the United States) [2, 3].

To overcome the effect of tip force, HS-AFM generally uses small cantilevers with high-resonant frequency and small spring constant, fast scanners and fast electronics to increase the imaging rate [1]. HS-AFM not only possesses the high adaptability of AFM, which can work normally in the vacuum environment, atmospheric pressure environment and liquids but also has the advantages of high-resolution imaging of different samples, even the living biological samples [4–13]. Although the imaging rate depends on imaging conditions, HS-AFM is able to generally capture the image of protein molecules within <100 ms [14]. The acquired molecular images have provided new mechanistic insights, which cannot have been achieved in other ways [15]. HS-AFM solves problems that cannot be addressed by previous technology, such as studying the dynamic behaviour of highly dynamic and disordered protein molecules [16], determining the characteristics and structure of some specific proteins, and capturing the dynamic assembly process at the molecular level in real time. As a result, HS-AFM has been widely used in many fields of biology.

As the imaging rate of HS-AFM is greatly improved, the components of conventional AFM such as ordinary scanners or cantilever beams cannot be adapted to the high-speed working state of HS-AFM [17]. Therefore, it is necessary to improve the HS-AFM cantilever beam, a piezoelectric actuator, a piezoelectric scanner, and a tracking control algorithm, which are very vital to the improvement of the HS-AFM imaging rate and imaging resolution. This Letter summarises the improved methods of various HS-AFM components and many new control algorithms to achieve the best performance of HS-AFM. The novel cantilever using SU-8 polymer as new cantilever material, some improved components and many new control algorithms, are, respectively, presented.

Many reviews of HS-AFM have been reported. However, they usually focus on the structure and dynamic behaviour of biomacromolecule [18, 19]. The aim of this review is to overview the recent progress of HS-AFM in molecular biology research. Then, some recent biomacromolecule studies are summarised. Next, improvements that can help enhance imaging rates and resolutions are introduced. In the last part, opportunities in the future development of HS-AFM are proposed.

2. Characterisation of protein: Bacterial micro-compartments (BMCs) are protein organelles, which were widely found in bacteria phyla. They compartmentalise enzymes via a selectively permeable shell and play an important role in carbon fixation, microbial ecology, and pathogenesis. Sutter *et al.* [20] used HS-AFM combined with X-ray crystallography to find that the preformed hexamers were assembled into uniformly oriented shells, a single hexamer thick. They observed the shell facet assembly dynamic process and found that the shell hexamers can be dissociated from and incorporated into assembled sheets, indicating a flexible interaction between the molecules. Moreover, they also demonstrated that specific contacts at the shell protein interface are major factors, controlling the self-assembly and kinetics of shell proteins. This experimental result helps to further understand the self-assembly process and kinetics of the BMC shell. It is worth noticing that, this research contributes new insights into the formation, kinetics and interaction of BMC shell facets, and gives new directions for the design of self-assembled biological nanoreactors and scaffolds based on BMC structures.

Annexins are cytoplasmic proteins that act in calcium ion (Ca^{2+}) storage and membrane healing, which can bind to negatively charged phospholipids in a Ca^{2+}-dependent manner. Miyagi *et al.* [21] used HS-AFM to repeatedly induce and disrupt annexins, and study their structure, dynamics, and interactions. It was found that the same annexin V exhibited different Ca^{2+} and membrane affinities at different positions, and provided a wide Ca^{2+} buffering mechanism while maintaining membrane stability. This study, which is on the structure and properties of annexin V, deepens the understanding of annexin.

Metazoan fatty acid synthase (FAS) are molecular machines used in critical biosynthetic processes [1]. Benning *et al.* [22] used HS-AFM to perform real-time imaging of FAS with different

doi: 10.1049/mnl.2019.0313

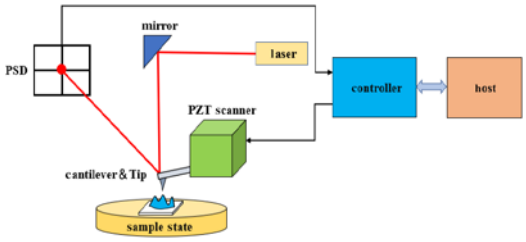

Fig. 1 *Flow of working process of AFM*

viewing angles and high spatiotemporal resolution. They identified the mobility of individual FAS regions in the video sequence, and the correlation of shape features via reference-free 2D class averaging to identify group conformational variety. Electron microscopy showed that the conformation of FAS varied greatly, but this traditional detection method can only analyse the molecular structure of FAS via static high-resolution crystallographic analysis, and the conformational dynamics of FAS could not be obtained. Unlike electron microscopy, HS-AFM is able to explore the kinetics of single FAS molecules, the correlation between catalysis and structural changes, and can be used to study the kinetics of FAS and other multi-enzymes in aqueous solution at the single molecule level.

The MinDE protein system was considered to be the smallest biological oscillator currently discovered. This protein system consists of only two MinD and MinE protein molecules that regulate the molecules in mechanisms that lead to protein patterns and oscillation is still known little. Accordingly, Miyagi *et al.* [23] analysed the mechanism of the MinDE membrane association/dissociation kinetics on isolating membrane patches. The experimental results demonstrated that the association and dissociation of MinD and membrane are highly cooperative with each other, but mechanistically different processes. They proposed that these processes represent the two directions of a single allosteric switch, resulting in the formation and depolymerisation of MinD filaments. They also analysed the association/dissociation kinetics of varying MinD and MinE concentrations and obtained the basic kinetic variables of the oscillation cycle of the MinDE protein system, which enables us to more clearly understand the working principle and the molecular mechanism of the biological oscillator.

The clustered regularly interspaced short palindromic repeats (CRISPR)-related endonuclease (Cas9) and RNA can be combined to form the Cas9–RNA system, Shibata *et al.* [24] used HS-AFM to perform real-time imaging of CRISPR-Cas9 to obtain real-time dynamics of CRISPR-Cas9 operation. They found in real-time images that apo-Cas9 has a flexible conformation and a stable bilobed structure after the formation of Cas9–RNA. Furthermore, they gained a DNA cleavage process involving Cas9 based on real-time images, and the Cas9 HNH nuclease domain formed an active conformation after fluctuates upon DNA binding. Notably, the HNH active site falls on the cleavage site of the target DNA. Through studying the real-time image of CRISPR-Cas9, the operation mechanism of CRISPR-Cas9 can be known better, which can benefit many fields, including genome editing.

HS-AFM also plays an important role in the characterisation of viral proteins. Lim *et al.* [25] used HS-AFM to visualise the conformational change of HA0, which is a trimeric precursor of haemagglutinin (HA) of influenza A. HA0 monomer consist of designated HA1 and HA2 domains. In the acidic endosomal environment, HA0 can be cleaved by proteases and produces a metastable HA in which HA2 undergoes conformational changes. This change can initiate membrane fusion, which is a vital process for viral infectivity. This is the first report on the conformational change of HA0 trimer of H5N1 in real time by using HS-AFM. It is worth noticing that this research contributes new insights into avian influenza H5N1 HA precursor, and gives new directions for the treatment of influenza A and the production of therapeutic drugs.

3. Visualisation of protein dynamic behaviour: As researchers explore the functions of HS-AFM more deeply, it is found that HS-AFM can successfully capture protein molecules in physiological solutions at sub-100 ms temporal resolution, without interfering with the biological functions of protein molecules [26, 27]. Fisher *et al.* [28] used a programmable DNA origami platform to create a DNA origami cylinder (as shown in Fig. 2). The cylinder simulated the size of the central transport channel and could accommodate a specific number of phenylalanine-glycine (FG)-domains at specific locations. The design parameters were adjusted by topology, graft density, and so on. The molecular dynamics simulation based on the coarse-grained polymer model was the same as the actual working process in the FG-domain. Moreover, compared with previous studies, HS-AFM was utilised to reveal the process of local and reversible FG-domain condensation transiently occludes the central channel. Although the underlying mechanism for establishing the permeability properties of nuclear pore complexes (NPCs) has not been fully elucidated, it provides a new idea for researching how NPCs establish their permeability properties.

In order to solve the selective barrier mechanism of NPCs, Sakiyama *et al.* [29] choose to use HS-AFM to observe FG nucleoporins (FG Nups). FG Nups is a disordered protein existing in the nuclear pore of NPCs, which interferes with the regulation of NPCs on the bidirectional transmission of macromolecules between cytoplasm and nucleus of eukaryotic cells. HS-AFM is used to visualise the dynamic behaviour of FG Nups inside Xenopus laevis oocyte NPCs. The results show that the cytoplasmic pores are limited by the highly flexible FG Nups. FG Nups can be rapidly elongated and retracted, intermingling FG Nups exhibited transient entanglements in the central channel, but do not coalesce into a tightly cross-linked meshwork. Therefore, they conclude that the selective barrier of NPCs is mainly composed of FG Nups. This Letter fully demonstrates that HS-AFM plays an important role in the research of highly dynamic and disordered protein molecules. Additionally, direct evidence that the selective barrier of NPCs is composed of FG Nups is obtained, which solves the long-standing doubts about the selective barrier mechanism of NPCs and deepens the understanding of the selective barrier mechanism of NPCs.

Zhang *et al.* [30] studied the structural flexibility of α-synuclein (α-syn, the first discovered Parkinson's disease pathogenic gene) in an aqueous solution environment. They used discrete molecular dynamics methods to simulate the molecular basis of structural transformation. This research assumed that the α-syn monomer is in a globular conformation, and can form tail-like protrusions over dozens of seconds. More importantly, the globular monomer can adopt a fully extended conformation. However, the *α*-syn dimer is less dynamic and has a dumbbell shape, and morphological

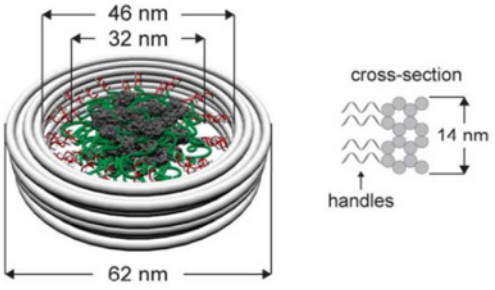

Fig. 2 *Design and assembly of nucleoporins organised on DNA. Cartoon and cross-section of DNA origami cylinder with protruding inner DNA 'handles' hybridised to DNA-labelled FG Nups (green with red 'anti-handles')*

changes occur over time. Parkinson's disease still has not found effective treatment methods. Research on the conformation and characterisation of α-syn can help researchers find the pathogenesis of Parkinson's disease and provide a direction for researchers to explore ways for curing Parkinson's disease.

Amyloid β-protein (Aβ) oligomers are one of the main pathogenic factors of Alzheimer's syndrome, and the development of oligomer-specific therapeutic agents requires detailed characterisation of the structure and dynamics of oligomers. Banerjee *et al.* [31] used a time-lapse HS-AFM to Letter the conformational changes and structural dynamics of various Aβ42 oligomers at nanometre resolution on a millisecond time scale. The experimental results show that the trimer maintains a single-globular shape, which elongates adopting an ellipsoidal shape, while the cross-linked pentamer and heptamer shape dynamically change between compact single-globular and multi-globular shapes. Oligomers exist transiently that complicates their structural analysis. At present, only photochemical crosslinking of native oligomers can be used. In this case, oligomers can be purified and isolated for physical and chemical research. This research obtained the conformational change between the oligomers of Aβ42 by HS-AFM and detailed the structural dynamics of the oligomers, which laid the foundation for the further development of oligomer-specific therapeutic agents.

Zhang *et al.* [32] used HS-AFM to image carbon nanotube porins (CNTPs) in real time. This porin has the characteristics of being able to move laterally in the supported lipid membrane and mimic the behaviour of biological proteins. The movement of the CNTP in the supported lipid bilayer membrane was captured by HS-AFM in real time to build the long-term trajectory of the CNTP (Fig. 3) and determine the diffusion coefficients associated with the motion.

They found that the diffusion coefficients of CNTPs in the supported lipid membrane are the same as those of conventional proteins. In addition, CNTPs often exhibit directed diffusion behaviour, which is similar to proteins in living cells. This research showed that CNTPs are similar to biological proteins and have wide application prospects.

4. Improvements of HS-AFM imaging function: The traditional method can only improve the imaging rate of the AFM by reducing the size of the cantilever beam, but blindly reducing the size of the cantilever beam will affect the detection and imaging of the microscope [33–36]. Hence, Adams *et al.* [37] achieved high-speed imaging in the atmosphere by changing the material of the cantilever. The Adams team used SU-8 polymer to fabricate cantilevers. The new cantilever can simulate the high-damping environment of liquids, and its imaging-in-air detection bandwidth is 19 times faster than traditional cantilevers with the same size, resonance frequency, and elastic constant. Different from other improvements, the Adams team attempted to improve the materials used in the cantilever and successfully solved the problem of relatively slow imaging of HS-AFM in the atmospheric environment, which is important for HS-AFM in atmospheric environments. This research also provides great help for the further development of HS-AFM research on biomacromolecules in an atmospheric environment.

HS-AFM brings high-speed and high-resolution imaging while also placing higher demands on position sensors. Hosseini *et al.* [38] used a new miniaturised micro-electromechanical system position sensor combined with a simple proportional–integral–derivative controller to achieve high-speed, high-precision nanopositioning. The new position sensor effectively reduces nonlinearity, improves control accuracy, and stabilises imaging at line speeds up to 300 Hz. Conventional AFM imaging accuracy depends on the piezoelectric actuator, but the non-linear characteristics of the piezoelectric actuator can affect the accuracy of the imaging. Therefore, HS-AFM requires a new type of position sensor with high bandwidth and low non-linearity. The new position sensor effectively solves the problem of distortion in the previous imaging using piezoelectric actuators, providing a new solution for HS-AFM to image faster and more accurately.

Different from the Hosseini team, Cai *et al.* [39] improved on the traditional piezoelectric scanner and got a new high-speed scanner. The high-speed scanner uses a piezoelectric bimorph, one of which acts to drive the bimorph beam at high speed and the other one monitors the bimorph vibration. The Cai team also proposed a phase-lag compensation and non-linear motion compensation correction method based on data shift and non-linear mapping relationship. This method can compensate and correct the image distortion caused by sinusoidal scanning and phase-lag. The improved HS-AFM is capable of operating at a maximum rate of ~30 frames/s and effectively eliminates image distortion caused by

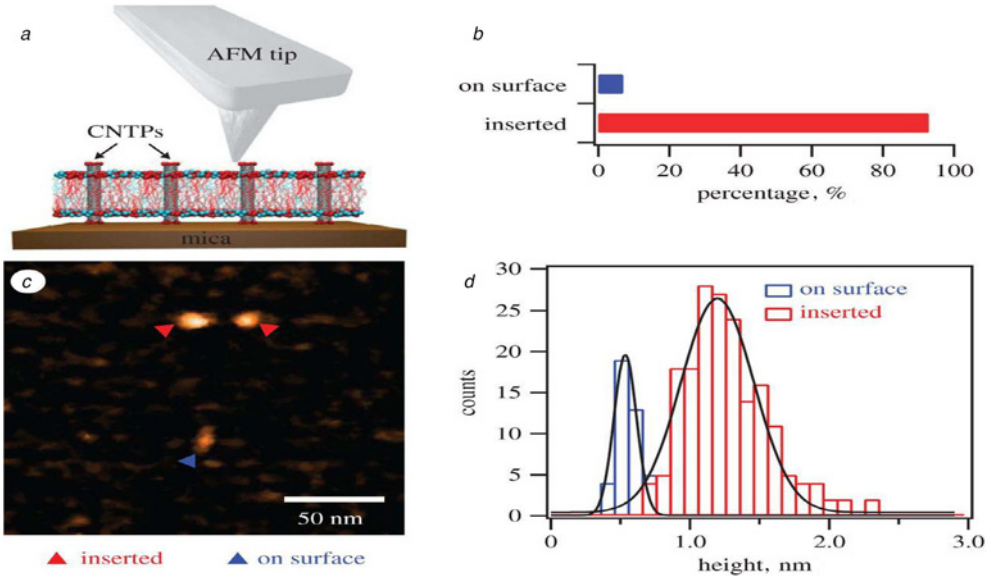

Fig. 3 *Analysis of the diffusion coefficients of CNTPs in supported lipid proteins*
a Schematics of HS-AFM measurement showing AFM tip scanning over CNTPs inserted into lipid bilayer matrix supported on mica surface
b Representative AFM image showing CNTPs inserted into lipid bilayer (red triangles) and adsorbed on bilayer surface (blue triangle)
c Histogram showing fractions of CNTPs inserted into bilayer ($n = 184$) and adsorbed on bilayer surface ($n = 14$)
d Histograms of height of CNTP features relative to bilayer surface level (solid lines represent Gaussian fits to data)

doi: 10.1049/mnl.2019.0313

sinusoidal scanning and phase-lag. This improvement has facilitated the further development of HS-AFM, providing a new approach to high-resolution imaging at the nanoscale.

Hartman *et al.* [40] designed a feedback-based feature tracking algorithm that reduces imaging time by focusing on the characteristics of the sample, thus reducing the total imaging area. The Hartman team used the same parameters to perform comparative tests on circular gratings, square gratings, silicon steps as well as DNA strands and found that the imaging time of raster scanning was reduced by 3–12 times. This design effectively solves the problem that the scanning range of the previous commercial microscope is too large, and the frame rate needs to be increased. Using this algorithm, local circular scan (LCS) can achieve an order of magnitude higher scans than traditional raster scans with the appropriate parameters selected, and can significantly increase the frame rate of imaging without significant hardware changes. The algorithm has many advantages such as flexibility, tracking ability strong and scanning rate fast, which makes it especially suitable for imaging of dynamic biological systems.

5. Components optimised for HS-AFM: One of the main reasons why the traditional AFM speed cannot be effectively improved is that the bandwidth during mechanical scanning cannot be continuously improved. As the first eigenfrequency of the system has been limited by the first eigenfrequency of the AFM head in terms of tip scanning and by the sample stage in case of sample scanning, the first eigenfrequency of the system has reached the limit [41, 42].

Braunsmann *et al.* [43] used optical knife-edge technique to design a simple, contact-free and high-speed displacement sensor, which can be used in HS-AFM scanners. The experiment results showed that after using the displacement sensor, a root-mean-square sensor noise of 0.8 nm can be achieved in a bandwidth of 1 Hz to 1.1 MHz, and a force curve of up to 5 kHz can be obtained by detecting and correcting the non-linear z-piezo displacement. This novel sensor can measure the true z-piezo displacement while recording the force, thus realising high-speed quantitative measurement of mechanical sample performance. The novel optical knife-edge displacement sensor breaks through the fundamental resolution limits of traditional sensors and improves their linearity, and can be applied to other areas of high-speed displacement sensing that require sub-nanometre resolution.

The most critical operation of the traditional AFM tapping mode is to detect the amplitude of the cantilever oscillation. As a matter of fact, the change in the amplitude of the cantilever oscillation drives the feedback loop. Therefore, the accuracy and speed of amplitude detection are critical to improving the performance of HS-AFM. Miyagi *et al.* [44] developed a new phase-shift-based amplitude detector for HS-AFM, which has a phase delay of ∼138° when the amplitude of the actual operation is abrupt, while the phase delay is ∼682° by using the Fourier analysis method. This research demonstrates that the novel amplitude detector can be used in the HS-AFM system to detect the change of the amplitude of the cantilever oscillation more quickly and accurately, thus achieving lower invasiveness and faster image acquisition.

The temperature-dependent state of the lipid bilayer is called the phase, and the main phase is the solid phase and liquid phase. Under certain conditions, certain lipid bilayer structures employ a so-called ripple phase, a structure in which the solid phase and the liquid phase alternate at a constant period. Takahashi *et al.* [45] developed a temperature control device for HS-AFM that is capable of observing the reversible phase transition of the ripple phase to the liquid phase in real time. The experiment results demonstrate that the phase transition hysteresis is obvious during the fast cooling and rapid heating process, while in the quasi-steady state, both melting and condensation occur at 24.15°C. HS-AFM combining with this temperature control device is a novel experimental system, which can be used to observe the kinetics of nanoscale temperature sensitive processes. This design helps people to understand the ripple phase and its transition dynamics and provides specific analysis and research on the reversible phase transition process from the ripple phase to the liquid phase.

6. Conclusion: This review summarises the latest applications and improvements of HS-AFM. HS-AFM has been successfully applied in the research of various proteins. Unlike other technologies, HS-AFM is capable of simultaneously assessing the structure and kinetics of proteins and is transforming static structural biology into dynamic structural biology.

Although the molecular images captured by HS-AFM directly provide evidence for the inference of many biomolecule dynamic processes and solve problems that were previously difficult or unresolved by using other methods, there are still many biomolecular phenomena that cannot be solved by HS-AFM in its current form. Therefore, HS-AFM is currently being combined with other powerful technologies to expand the application of HS-AFM in biological research [46]. After the combination of HS-AFM with a super-resolution optical microscope, optical tweezers, fluorescence microscope and other powerful technologies, a super hybrid microscope system can be formed, which can visualise the dynamic process of biomolecules better, and also have a great impact on the single molecule biophysics. Also, with the development of microfabrication technology, we can use emerging elastomer-based microfabrication technologies to improve the HS-AFM cantilever beam fabrication [47, 48]. The application of HS-AFM will be further extended in the near future.

7. Acknowledgments: This work was supported by the National Natural Science Foundation of China (grant no. 11704263).

8 References

[1] Takayuki U., Noriyuki K., Toshio A.: 'Guide to video recording of structure dynamics and dynamic processes of proteins by high-speed atomic force microscopy', *Nat. Protoc.*, 2012, **7**, (6), pp. 1193–1206
[2] Ando T, Uchihashi T, Fukuma T: 'High-speed atomic force microscopy for nano-visualization of dynamic biomolecular processes', *Prog. Surf. Sci.*, 2008, **83**, (7–9), pp. 337–437
[3] Ando T.: 'High-speed atomic force microscopy and its future prospects', *Biophys. Rev.*, 2017, **10**, (2), pp. 285–292
[4] Uchihashi T., Watanabe H., Fukuda S., *ET AL.*: 'Functional extension of high-speed AFM for wider biological applications', *Ultramicroscopy*, 2016, **160**, pp. 182–196
[5] Ando T.: 'Directly watching biomolecules in action by high-speed atomic force microscopy', *Biophys. Rev.*, 2017, **9**, (4), pp. 1–9
[6] Colom A., Casuso I., Rico F., *ET AL.*: 'A hybrid high-speed atomic force-optical microscope for visualizing single membrane proteins on eukaryotic cells', *Nat. Commun.*, 2013, **4**, pp. 2155–2162
[7] Shibata M., Watanabe H., Uchihashi T., *ET AL.*: 'High-speed atomic force microscopy imaging of live mammalian cells', *Biophys. Physicobiol.*, 2017, **14**, pp. 127–135
[8] Kodera N., Yamamoto D., Ishikawa R., *ET AL.*: 'Video imaging of walking myosin V by high-speed atomic force microscopy', *Nature*, 2010, **468**, pp. 72–76
[9] Rangl M., Rima L., Klement J., *ET AL.*: 'Real-time visualization of phospholipid degradation by outer membrane phospholipase a using high-speed atomic force microscopy', *J. Mol. Biol.*, 2017, **429**, (7), pp. 977–986
[10] Rahman M., Day S., Neff D., *ET AL.*: 'Origami arrays as substrates for the determination of reaction kinetics using high-speed atomic force microscopy', *Langmuir*, 2017, **33**, (30), pp. 7389–7392
[11] Imamura M., Uchihashi T., Ando T., *ET AL.*: 'Probing structural dynamics of an artificial protein cage using high-speed atomic force microscopy', *Nano Lett.*, 2015, **15**, (2), pp. 1331–1335
[12] Sugiyasu K., Fukui T., Uchihashi T., *ET AL.*: 'Direct observation and manipulation of supramolecular polymerization by using high-speed atomic force microscopy', *Angew. Chem. Int. Ed.*, 2018, **130**
[13] Rangl M., Miyagi A., Kowal J., *ET AL.*: 'Real-time visualization of conformational changes within single MloK1 cyclic nucleotide-modulated channels', *Nat. Commun.*, 2016, **7**, p. 12789
[14] Ando T., Uchihashi T., Scheuring S.: 'Filming biomoleculear processes by high-speed atomic force microscopy', *Chem. Rev.*, 2014, **114**, pp. 3120–3188

[15] Shibata M., Uchihashi T., Ando T., *ET AL.*: 'Long-tip high-speed atomic force microscopy for nanometer-scale imaging in live cells', *Sci. Rep.*, 2015, **5**, p. 8724
[16] Yamashita H., Inoue K., Shibata M., *ET AL.*: 'Role of trimer–trimer interaction of bacteriorhodopsin studied by optical spectroscopy and high-speed atomic force microscopy', *J. Struct. Biol.*, 2013, **184**, (1), pp. 2–11
[17] Ando T.: 'High-speed atomic force microscopy (AFM)', *Encycl. Biophys.*, 2018, **1**, pp. 1–7
[18] Fukuda S., Uchihashi T., Iino R., *ET AL.*: 'High-speed atomic force microscope combined with single-molecule fluorescence microscope', *Rev. Sci. Instrum.*, 2013, **84**, (7), p. 073706
[19] Oestreicher Z., Taoka A., Fukumori Y.: 'A comparison of the surface nanostructure from two different types of gram-negative cells: *Escherichia coli* and *Rhodobacter sphaeroides*', *Micron*, 2015, **72**, pp. 8–14
[20] Sutter M., Faulkner M., Aussignargues C., *ET AL.*: 'Visualization of bacterial microcompartment facet assembly using high-speed atomic force microscopy', *Nano Lett.*, 2015, **16**, (3), pp. 1590–1595
[21] Miyagi A., Chipot C., Rangl M., *ET AL.*: 'High-speed atomic force microscopy shows that annexin V stabilizes membranes on the second timescale', *Nat. Nanotechnol.*, 2016, **11**, (9), pp. 783–790
[22] Benning C., Sakiyama Y., Mazur A., *ET AL.*: 'High-speed atomic force microscopy visualization of the dynamics of the multienzyme fatty acid synthase', *ACS Nano*, 2017, **11**, (11), pp. 10852–10859
[23] Miyagi A., Ramm B., Schwille P., *ET AL.*: 'High-speed atomic force microscopy reveals the inner workings of the MinDE protein oscillator', *Nano Lett.*, 2017, **18**, (1), pp. 288–296
[24] Shibata M., Nishimasu H., Kodera N., *ET AL.*: 'Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy', *Nat. Commun.*, 2017, **8**, (1), pp. 1262
[25] Lim K.S., Mohamed M.S., Wang H., *ET AL.*: 'Direct visualization of avian influenza H5N1 hemagglutinin precursor and its conformational change by high-speed atomic force microscopy', *Biochim. Biophys. Acta, Gen. Subj.*, 2020, **1864**, pp. 10–16, doi: 10.1016/j.bbagen.2019.02.015
[26] Mohamed M., Kobayashi A., Taoka A., *ET AL.*: 'High-speed atomic force microscopy reveals loss of nuclear pore resilience as a dying code in colorectal cancer cells', *ACS Nano*, 2017, **11**, (6), pp. 5567–5578
[27] Aybeke N., Belliot G., Lemaire-Ewing S., *ET AL.*: 'HS-AFM and SERS analysis of murine norovirus infection: involvement of the lipid rafts', *Small*, 2017, **13**, (1), p. 1600918
[28] Fisher P., Shen Q., Akpinar B., *ET AL.*: 'A programmable DNA origami platform for organizing intrinsically disordered nucleoporins within nanopore confinement', *ACS Nano*, 2018, **12**, (2), pp. 1508–1518
[29] Sakiyama Y., Mazur A., Kapinos L., *ET AL.*: 'Spatiotemporal dynamics of the nuclear pore complex transport barrier resolved by high-speed atomic force microscopy', *Nat. Nanotechnol.*, 2016, **11**, (8), pp. 719–723
[30] Zhang Y., Hashemi M., Lv Z., *ET AL.*: 'High-speed atomic force microscopy reveals structural dynamics of α-synuclein monomers and dimers', *J. Chem. Phys.*, 2018, **148**, (12), p. 123322
[31] Banerjee S., Sun Z., Hayden Y., *ET AL.*: 'Nanoscale dynamics of amyloid β-42 oligomers as revealed by high-speed atomic force microscopy', *ACS Nano*, 2017, **11**, (12), pp. 12202–12209
[32] Zhang Y., Tunuguntla H., Choi O., *ET AL.*: 'Real-time dynamics of carbon nanotube porins in supported lipid membranes visualized by high-speed atomic force microscopy', *Philos. Trans. R. Soc. B, Biol. Sci.*, 2017, **372**, p. 20160226
[33] Noshiro D., Ando T.: 'Substrate protein dependence of GroEL–GroES interaction cycle revealed by high-speed atomic force microscopy imaging', *Philos. Trans. R. Soc. B, Biol. Sci.*, 2018, **373**, p. 20170180
[34] Wang Y., Wan J., Hu X., *ET AL.*: 'A rate adaptive control method for improving the imaging speed of atomic force microscopy', *Ultramicroscopy*, 2015, **155**, pp. 49–54
[35] Schächtele M., Hänel E., Schäffer E.: 'Resonance compensating chirp mode for mapping the rheology of live cells by high-speed atomic force microscopy', *Appl. Phys. Lett.*, 2018, **113**, (9), p. 093701
[36] Herfst R., Dekker B., Witvoet G., *ET AL.*: 'A miniaturized, high frequency mechanical scanner for high speed atomic force microscope using suspension on dynamically determined points', *Rev. Sci. Instrum.*, 2015, **86**, (11), p. 113703
[37] Adams D., Erickson W., Grossenbacher J., *ET AL.*: 'Harnessing the damping properties of materials for high-speed atomic force microscopy', *Nat. Nanotechnol.*, 2015, **11**, (2), pp. 147–151
[38] Hosseini N., Nievergelt P., Adams D., *ET AL.*: 'A monolithic MEMS position sensor for closed-loop high-speed atomic force microscopy', *Nanotechnology*, 2016, **27**, (13), p. 135705
[39] Cai W., Zhao J., Gong W., *ET AL.*: 'Resonance-type bimorph-based high-speed atomic force microscopy: real-time imaging and distortion correction', *Meas. Sci. Technol.*, 2014, **25**, (12), p. 125404
[40] Hartman B., Andersson S.: 'Feature tracking for high speed AFM imaging of biopolymers', *Int. J. Mol. Sci.*, 2018, **19**, (4), p. 1044
[41] Liao S., Yang W., Ko C., *ET AL.*: 'Imaging initial formation processes of nanobubbles at the graphite–water interface through high-speed atomic force microscopy', *Appl. Surf. Sci.*, 2018, **434**, pp. 913–917
[42] Wadikhaye P., Yong K., Reza O.: 'A serial-kinematic nanopositioner for high-speed atomic force microscopy', *Rev. Sci. Instrum.*, 2014, **85**, (10), p. 105104
[43] Braunsmann C., Prucker V., Schäffer E.: 'Optical knife-edge displacement sensor for high-speed atomic force microscopy', *Appl. Phys. Lett.*, 2014, **104**, (10), p. 103101
[44] Miyagi A., Scheuring S.: 'A novel phase-shift-based amplitude detector for a high-speed atomic force microscope', *Rev. Sci. Instrum.*, 2018, **89**, (8), p. 083704
[45] Takahashi H., Miyagi A., Redondo-Morata L., *ET AL.*: 'Temperature-controlled high-speed AFM: real-time observation of ripple phase transitions', *Small*, 2016, **12**, (44), pp. 6106–6113
[46] Umakoshi T., Fukuda S., Iino R., *ET AL.*: 'High-speed near-field fluorescence microscopy combined with high-speed atomic force microscopy for biological studies', *Biochim. Biophys. Acta, Gen. Subj.*, 2020, **1864**, pp. 35–47, doi: 10.1016/j.bbagen.2019.03.011
[47] Kung Y.C., Huang K.W., Fan Y.J., *ET AL.*: 'Fabrication of 3D high aspect ratio PDMS microfluidic networks with a hybrid stamp', *Lab Chip*, 2015, **15**, (8), pp. 1861–1868
[48] Liu T.L., Wen X, Kung Y.C., *ET AL.*: 'Fabrication strategy for micro soft robotics with semiconductor devices integration'. Proc. 30th IEEE Int. Conf. on Micro Electro Mechanical Systems, Las Vegas, NV, USA, 2017, pp. 663–666

doi: 10.1049/mnl.2019.0313