Biophysics in Kanazawa University

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The history of Kanazawa University can be traced back to the Edo era, a period of ancient regime before the Meiji restoration in 1868. In 1862, the vaccination facility was founded by the Kaga (Maeda clan) governing the Hokuriku area including Kanazawa. This facility was the origin of the Kanazawa Medical College and the Pharmacy College founded in 1923. In 1949, several colleges in Kanazawa were unified into Kanazawa University in possession of six departments (Law and Literature, Education, Science, Medicine, Pharmacy, and Engineering) and an attached hospital. Kanazawa University is of medium size: ~10,000 students, ~1300 researchers, and ~2300 technical and administrative staffs.

The first biophysics laboratory in Kanazawa University was initiated by Ikuo Yamagata at the Physics Department. He studied the origin of life. In 1986, Toshio Ando returned to Japan after studying for six and half years at Manuel Morales's laboratory in UC San Francisco and took over the biophysics laboratory. He started biophysical studies on several topics: motor proteins (actin and myosin), measurements of local electrostatic potential of proteins by exploiting diffusion-enhanced fluorescence energy transfer, and the long-term potentiation (LTP) at synapses in hippocampal brain slices by electrophysiological methods. The technique of brain slicing was taught from the pioneer of this method, Chozaburo Yamamoto in the Medical School. But after 5 years, he abandoned the LTP study because of too much competition at that time. He started building an AFM system immediately after learning the advent of AFM (in 1986) from

Kazuhiro Oiwa at the summer school held in 1989 by Toshio Yanagida. The AFM system was built in 3 years and used to image myosin II. But the result was of course only static images, which prompted Ando to start developing high-speed AFM (HS-AFM) in 1993. However, it was initially very difficult due to a shortage of funds for this technical development. After a large fund was granted from the NEDO (New Energy and Industrial Technology Development Organization) in 1997, this study went into orbit. At the Biophysics Society Meeting in Boston in 2000, Ando presented a poster to show the first result of HS-AFM imaging. Shinya Inoué came to the poster and discussed. Next year, the first paper of this line of studies was published in PNAS (Ando et al. 2001), in which a movie showing Brownian motion of myosin V taken by a student, Noriyukin Kodera, was included. Soon after, Ando attempted to image myosin V walking on actin filaments, since his student, Takeshi Sakamoto, had already succeeded at that time in observing the processive movement of single molecules of myosin V using fluorescence microscopy (Sakamoto et al. 2000). But it was not successful because the HS-AFM system was not sophisticated enough, resulting in breakage of actin filaments during imaging. Then, various improvements were carried out with new ideas, and finally, the HS-AFM system was established in 2008 (Ando et al. 2008), soon followed by Kodera's success in filming walking myosin V (Kodera et al. 2010).

In 2010, a new institute, the Bio-AFM Frontier Research Center (BAFRC), was founded in Kanazawa University under the president's leadership, resulting in the addition of five faculty members to the previous three. In 2017, our application for the World Premier International Research Center Initiative (WPI) program was adopted by JSPS. Then, the BAFRC was dissolved and absorbed into a new institute, the Nano Life Science Institute (WPI-NanoLSI), virtually comprising the biophysics group and 5 other groups (cancer

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research, cell biology, nano-metrology, super-molecular chemistry, and computational science). The biophysics group is the largest one, comprising 13 faculty members (four are from overseas). Their backgrounds range from Physics to Applied Physics, Chemistry, Biochemistry, Cell Biology, and Nanotechnology. All are young (in middle 30s or early 40s), except for one.

The research activity in the biophysics group is largely classified into two sections: technical developments and application studies of HS-AFM. Some members are working on both. In the former, we are developing faster and noninvasive HS-AFM, HS-AFM combined with optical techniques including optical tweezers, and high-speed/high-resolution scanning ion conductance microscopy (SICM). In the latter, we are performing HS-AFM imaging of a variety of purified proteins during their functional activity and trying to find ways to visualize with HS-AFM or HS-SICM dynamic molecular processes occurring in the interior of de-roofed cells or on the surface of isolated intracellular organelles. In the technical developments, we are aiming to (i) increase the imaging rate of HS-AFM from the current 15 frames/s to 100 frames/s in order to expand the targets and phenomena that can be observed with HS-AFM; (ii) visualize protein molecules under the influence of external forces, such as unfolding and refolding; and (iii) establish noncontact imaging with HS-SICM to visualize proteins molecules on fragile membranes or in suspended structures such as myofibrils. It takes time to reach these goals, as was the case with the development of

HS-AFM. In contrast, the applications studies of HS-AFM have been progressing smoothly. Following the pioneering imaging studies on myosin V, F₁-ATPase (Uchihashi et al. 2011) and cellulases (Igarashi et al. 2011), various proteins have been successfully imaged with HS-AFM: To name a few, F-actin-cofilin interactions (Ngo et al. 2015), AAA+ molecular chaperone ClpB undergoing massive conformational changes (Uchihashi et al. 2018) (Fig. 1a), dynamin rings constricting a lipid tube (Takeda et al. 2018) (Fig. 1b), and Cas9 interacting with a guide RNA and DNA (Shibata et al. 2017). Using interactive HS-AFM, in which a controlled strong force can be applied to a specified locus of the sample during imaging, the role of inner lumen proteins (FAP45/FAP52) bound to doublet microtubules was elucidated (Owa et al. 2019) (Fig. 1c,d). Besides, the entity of high-molecular weight complexes formed by 2-Cys peroxiredoxin was disclosed to be a kind of exosome formed by incorporation of lipids and nucleotide binding or overoxidation (Haruyama et al. 2018).

Under the strong WPI funding support, the NanoLSI has been running programs to promote collaborations with external institutes and worldwide dissemination of the developed techniques. Every year, we invite ~30 PhD students and researchers from the interior and overseas. They can study freely using our facilities for more than a month (up to 3 months). Since 2012, we have been holding the hands-on style of Bio-AFM Summer School, where more than 20 attendees from the interior and overseas observe their own samples using HS-AFM, SICM, or frequency modulation AFM.

Fig. 1 HS-AFM and interactive HS-AFM images recently filmed for protein molecules. **a** Molecular chaperone ClpB undergoing massive conformational changes during ATPase reaction. **b** Cluster formation of lipid tube-surrounding dynamin/amphiphysin helical rings upon addition of GTP. Arrowheads indicate the positions of individual rings. **c** Strong

tip tapping-caused partial depolymerization of B-tubule in wild-type doublet microtubule. **d** Strong tip tapping-caused fast and extended depolymerization of B-tubule in FAP45/FAP52-deletion mutant of doublet microtubule. The arrowheads in **c** and **d** indicate portions to which a strong tip force was applied

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