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研究課題名（和文）Influence of microtubule defects on single motor protein motility studied by HS-AFM

研究課題名（英文）nfluence of microtubule defects on single motor protein motility studied by HS-AFM

研究代表者

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研究者番号：５０８４６０９５

交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：(Google 翻訳による翻訳。)
このプロジェクトでは、高速原子間力顕微鏡（HS-AFM）と全反射蛍光顕微鏡（TIRFM）を組み合わせた既存のシステム、および従来の HS-AFM システムを使用して、微小管上のキネシンの運動性を研究しました。 キネシンは、従来の HS-AFM を使用してさまざまな濃度のアデノシン三リン酸（ATP）で観察でき、文献から予想される動作を示しました。 これは、HS-AFM が最小限の侵襲でキネシン運動を画像化するのに適していることを確認しました。

研究成果の学術的意義や社会的意義
HS-AFMとTIRFMを組み合わせてモータータンパク質を研究する新たなアプローチを導入しました。
このアプローチは、HS-AFMの空間分解能とTIRFMの広域観測能および時間分解能を組み合わせたマルチスケール観測によるものです。
これは、モータータンパク質の研究に大きなインパクトを与え、モータータンパク質の理解を深めるのに役立つものとなります。

研究成果の概要（英文）：In this project, an existing system then combined high-speed atomic force microscopy (HS-AFM) with total internal reflection fluorescence microscopy (TIRFM) as well as a conventional HS-AFM system were used to study the motility of kinesin on microtubules. Kinesin could be observed at different concentrations of adenosine triphosphate (ATP) with conventional HS-AFM and showed a behavior expected from literature. This confirmed that HS-AFM is suitable to image kinesin motility with minimum invasiveness. Further, the combined HS-AFM/TIRFM system was improved significantly and allowed to observe movement of kinesin with HS-AFM and TIRFM simultaneously. It was found that small defects can actually be overcome by kinesin. However, the detailed investigation of how defects in the microtubule lattice will impact kinesin motility is still ongoing. This proces requires extensive measurements with the combined system at different ATP concentrations and with various defect sizes.

研究分野：Material Science

キーワード：HS-AFM　kinesin　microtubules　TIRFM

１．研究開始当初の背景

Kinesins are motor proteins walking on microtubules. They are responsible for a variety of functions and have been associated with neurodegenerative diseases. While their motility is often studied with fluorescence microscopy, the details of the motion are often obscured due to the lack of spatial resolution. Single protein defects could hinder the movement of kinesin and could therefore play a role in neurodegenerative diseases. However, such defects cannot be observed reliably with optical microscopy alone and the interaction of kinesin with such defects remains unclear. High-speed atomic force microscopy, would allow to resolve all the details on the single protein scale but lacks the low invasiveness of optical microscopy and has only a very limited field of view.

２．研究の目的

In this project, a combined high-speed atomic force microscopy (HS-AFM) and total internal reflection fluorescence microscopy (TIRFM) system was used to overcome the limitations of both systems. Using this system, it is possible to image the details of the kinesin motility with HS-AFM while observe the large-scale motion using TIRFM. Furthermore, HS-AFM allows to locally manipulate the microtubule and induce defects. The main purpose of this project was to study the interaction of kinesin with such defects and to what extent the microtubule must be damaged to severely impact motor protein motility.

３．研究の方法

First, the combined HS-AFM/TIRFM had to be improved to allow a low invasiveness comparable to conventional HS-AFM. Also, the scan area was limited and synchronized measurement of both parts was not fully achieved initially. Further, while larger scale samples such as cells could be located in with the combined system, a single-protein measurement had not been achieved previously. In order to progress with the research plan, these issues needed to be fixed.

The combined system was fully re-calibrated and mechanical problems such as unwanted resonances in the scanning piezos were eliminated successfully. Also, optical problems such as bleed-through of the HS-AFM readout laser into the TIRF signal were improved. Finally, both systems were synchronized and work truly as a combined machine. A proof of concept of a successful combined single-protein observation is presented in Figure 1.

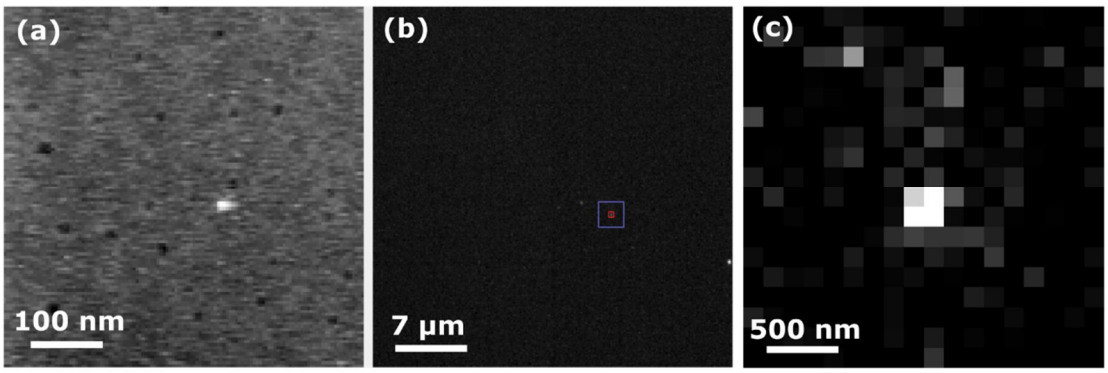

Figure 1: Proof of concept for single-protein observation with the combined HS-AFM/TIRFM with GFP. (a) HS-AFM image. (b) Full TIRFM image. The red square marks the HS-AFM observation area and the blue square marks the zoomed-in in (c). Please note the vastly different scales of (a) and (b).

４．研究成果

First, in order to confirm that HS-AFM can observe kinesin motility with relatively low invasiveness, conventional HS-AFM was used to measure the kinesin velocity at different concentration of adenosine triphosphate (ATP), which should result in an increasing velocity with increasing ATP concentration, according to previous findings. The results are summarized in Figure 2 and clearly show that the HS-AFM data is in accordance with the literature. Figure 2 also shows that with HS-AFM we have an observational windo w of ATP concentration ranging from about 2 µM to less than 20 µM. The lower boundary comes from a limited ATP supply, while the upper boundary is caused by kinesin moving too fast for HS-AFM to reliably image it.

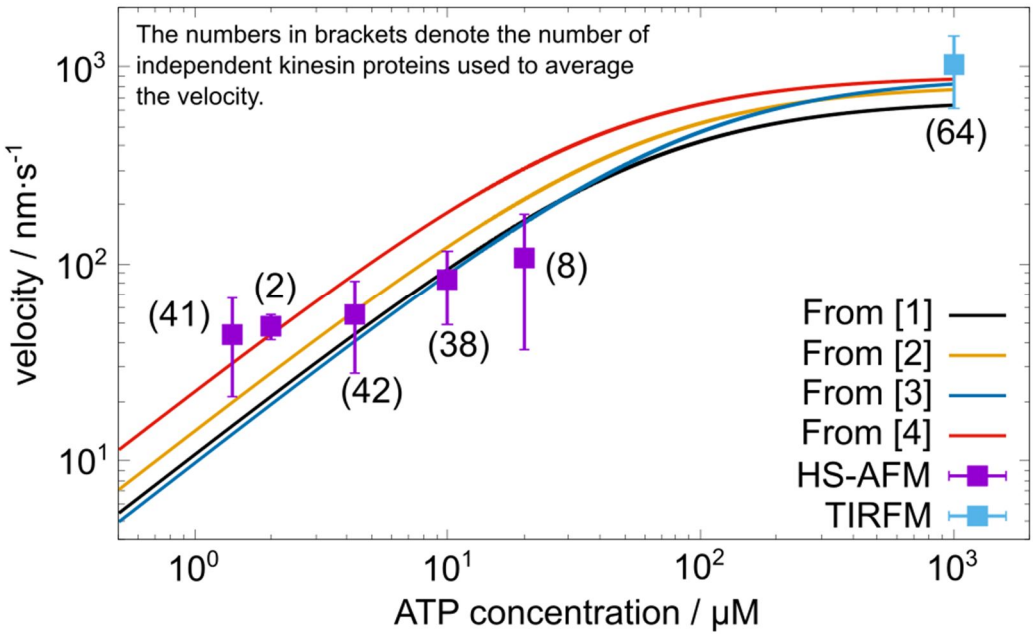

Figure 2: Kinesin velocity dependence on ATP concentration. The solid lines are models based on experimental observations (see [1 – 4] for details).

Also, using conventional HS-AFM, it was possible to record the interaction of kinesin with a small defect. It was found that it was possible for kinesin to overcome such a single tubulin defect, as shown in Figure 3.

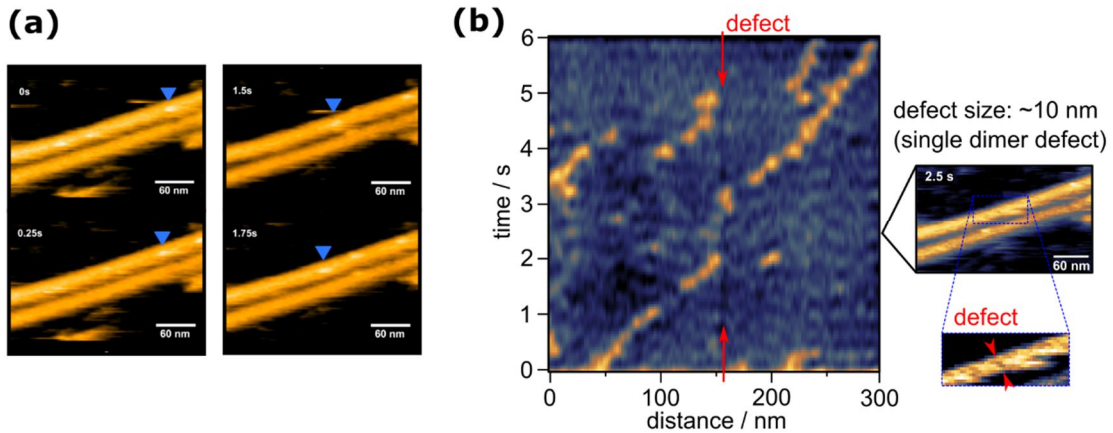

Figure 3: Kinesin moving on a microtubule and encountering a single tubulin defect. (a) Time lapse HS-AFM image of kinesin moving along a microtubule (indicated by the blue arrow). (b) Kymograph of kinesin encountering a defect. The red arrows indicate the defect. Notice that the kinesin continues its trajectory after stopping for about 1 s at the defect.

Finally, after improving the combined HS-AFM/TIRFM as described above, a combined observation was conduced and it was possible to confirm the movement of kinesin with HS-AFM and TIRFM simultaneously, as demonstrated in Figure 4.

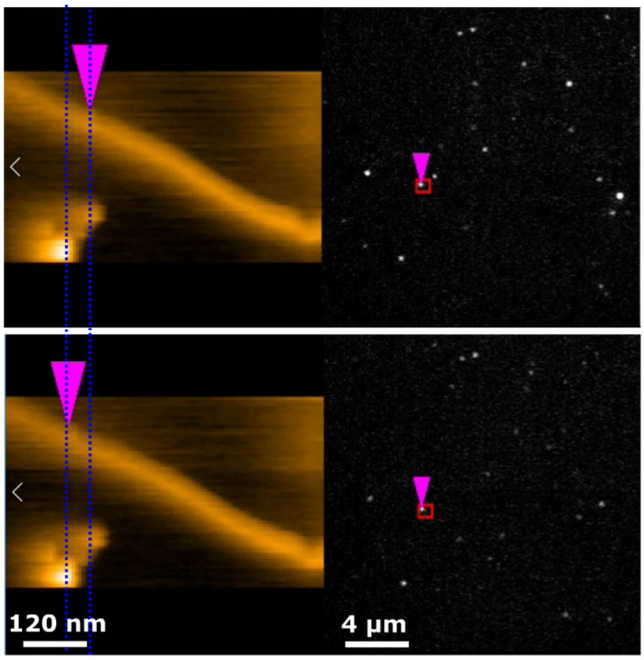

Figure 4: Combined HS-AFM/TIRFM observation of kinesin (pink arrow) on a microtubule. The top and bottom images are recorded consecutively and are 500 ms apart.

The movement of kinesin in Figure 4 shows, that it is easily possible to see a kinesin movement of a few tens of nanometers, which is not reliably observable with TIRF imaging. However, finding the kinesin at dilute concentrations is basically impossible with a small scan area as with HS-AFM, so, combining the two methods offers a unique combination of their advantages.
From now, data collection will continue and defect generation will be performed to study how defects impact kinesin motility. This has become only possible due to establishing such a multiscale observation system.

References

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５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Uchihashi Takayuki、Ganser Christian	４．巻 12
２．論文標題 Recent advances in bioimaging with high-speed atomic force microscopy	５．発行年 2020年
３．雑誌名 Biophysical Reviews	６．最初と最後の頁 363～369
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s12551-020-00670-z	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Christian Ganser, Syeda Rubaiya Nasrin, Akira Kakugo, Ryota Iino, Takayuki Uchihashi
２．発表標題 Kinesin transport on microtubules studied by high-speed AFM
３．学会等名 Meeting of the Biophysical Society of Japan（国際学会）
４．発表年 2019年

１．発表者名 Christian Ganser, Kimitoshi Takeda, Ryota Iino, Koichi Kato, Takayuki Uchihashi
２．発表標題 Defect creation in microtubules and kinesin motility studied by HS-AFM
３．学会等名 ICSPM27（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関