様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Real-time observation of native conformations and molecular behaviors of viral fusion proteins using high-speed atomic force microscopy (HS-AFM).

研究課題名（英文）Real-time observation of native conformations and molecular behaviors of viral fusion proteins using high-speed atomic force microscopy (HS-AFM)

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：応募者は、HS-AFMを用いて2つのウイルス融合タンパク質、インフルエンザAヘマグルチニン（HA）およびSARS-CoV-2スパイクタンパク質（Sタンパク質）の分子特性を調査しました。HS-AFMを使用して、HAのネイティブコンフォメーションおよび動的挙動、特に融合遷移、およびHAと脂質層の相互作用が記録されました。同様に、Sタンパク質の構造動態、SタンパクとACE2タンパク質の相互作用、Sタンパク質とエクソソームの相互作用、およびSタンパク質の中和相互作用もHS-AFMを使用して可視化および分析されました。その結果は、高インパクトのジャーナルに報告されています

研究成果の学術的意義や社会的意義
HS-AFM imaging allows the applicant to study structural dynamics of viral proteins and dynamic viral protein-antibody interactions, in which it is difficult to do using conventional methods such as cryo-EM. The applicant's studies have been reported in newspapers and on news television channels.

研究成果の概要（英文）：The applicant has investigated molecular properties of two viral fusion proteins: influenza A hemagglutinin (HA) and SARS-CoV-2 spike protein (S protein) using HS-AFM. Native conformation and dynamic behavior of HA, particularly the fusogenic transition, and the interaction between lipid layer and HA have been recorded using HS-AFM. Likewise, structural dynamics of S protein, S protein-ACE2 protein interaction, S protein-exosome interaction, and S protein-neutralizing interaction, have been visualized and analyzed using HS-AFM. The results have been reported in high impact journals.

研究分野：ウイルス学関連

キーワード：Viral fusion proteins　HS-AFM　Hemagglutinin　Spike protein　SARS-CoV-2　Influenza A

１．研究開始当初の背景

Viral-cell fusion is a critical event for viral infection. This event is mediated by one or more viral glycoproteins including one in general called fusion protein. Viral fusion proteins are highly dynamic. These proteins share a unified conformer known as a-trimer-of-hairpin. This conformer executes the fusion between viral membrane and host membrane to release viral genomes into host cytoplasm. Conformational changes of viral fusion proteins are triggered by several mechanisms such as low pH and receptor binding. Current conventional techniques including X-ray crystallography, NMR, Cryo-EM, and FRET, are incapable of capturing transient conformations of viral fusion proteins after triggered. In addition, the molecular behavior of viral fusion proteins when interacting with their target membranes remains a mystery. High-speed atomic force microscopy (HS-AFM) allows scientists to direct visualize the conformational changes of biomolecules and the interaction between biomolecules at nanoscopic range with high spatiotemporal resolution. The key scientific question in this proposal is- “Can we use HS-AFM to visualize the native conformations of viral fusion proteins and their conformational dynamics after triggered by low pH with or without the presence of target membrane?”

２．研究の目的

The purpose of this project was to investigate the native conformation and conformational changes of viral fusion protein triggered by low pH either with or without the presence of membrane (exosomes). Furthermore, this project determined the interaction between neutralizing antibodies with native or triggered form of viral fusion proteins. This project provides significant findings in molecular behavior of viral fusion proteins after triggered by acidic environment, particularly the conformational dynamic of the viral fusion proteins while interacting with exosome at acidic environment.　These findings are essential for developing neutralizing antibodies and inhibitors to prevent membrane fusion.

３．研究の方法

Prior to HS-AFM imaging, the applicant performed in silico analysis to obtain HS-AFM simulated images of viral fusion proteins and their surface charges. The simulated images were used as references by the applicant to identify target proteins during HS-AFM scanning. Protein surface charges information is important for the applicant to select suitable substrates for sample adsorption.

Recombinant viral fusion proteins, respective receptors, and neutralizing antibodies were purchased from companies. Exosomes were isolated by collaborators. Viral fusion proteins were scanned under a near-physiological buffer to obtain their native structures and dynamic behaviors. Manipulations of scanning condition were performed to investigate several aims planned in this research including: fusogenic transition of viral fusion proteins, viral fusion protein-receptors interactions, viral fusion proteins-exosomes interactions, and viral fusion proteins-neutralizing antibodies interaction.

HS-AFM videos were processed and analyzed using ImageJ software. Quantitative analyses and statistical comparisons were performed using SPSS. Graphs were plotted using GraphPad prisms. Adobe Illustrator was used to prepare figures with publication qualities. Movies were edited and compiled using Adobe Premiere Pro software.

４．研究成果

First, the applicant elucidated the conformational dynamics of hemagglutinin (HA) of Influenza A virus. He managed to capture fusogenic transition of HA at low pH. Additionally, real-time docking of HA on lipid membrane of exosome was successfully filmed. Distinct interaction patterns between HA and exosomes at neutral pH or acidic pH were found, which HA destabilized exosomal layer only at low pH. At low pH, HA underwent conformational change and released fusion peptide. The fusion peptide inserted into exosomal layer and disrupted its integrity. The results are published in Nano Letters (PMID: 32787163).

Next, during the COVID outbreak, the applicant performed HS-AFM imaging to study molecular properties of SARS-CoV-2 spike protein (S protein). The results revealed rigid trimeric head of S protein (S1 subunit) together with a flexible tail (S2 subunit). Furthermore, HS-AFM captured conformational changes of S protein after interacting with its receptor, ACE2. Exosomes isolated from ACE2-expressing cells were found interacting with S protein. This observation was not found in exosomes isolated from ACE2-deficient cells, suggesting that the S protein-exosome interaction required ACE2. Lastly, the applicant filmed the insertion of S2 subunit (containing fusion peptide) into exosomes in a real-time manner. Like fusion peptide of HA, fusion peptide of S protein also disrupted exosomal layer stability. The results are published in the Journal of Extracellular Vesicles (PMID: 34874124).

Lastly, the applicant conducted HS-AFM imaging to study the interaction between anti-S protein neutralizing antibody (S NAbs) and S protein or S protein-expressing exosomes. This study demonstrates that HS-AFM could function as a nanoscopic platform for assessment of S NAbs against S proteins. The results are reported in Nano Letters (PMID: 36641798).

５．主な発表論文等

〔雑誌論文〕　計9件（うち査読付論文　9件／うち国際共著　9件／うちオープンアクセス　9件）

１．著者名 Lim Keesiang、Hazawa Masaharu、Wong Richard W.	４．巻 32
２．論文標題 Crafty mimicry grants nuclear pore entry to HIV	５．発行年 2024年
３．雑誌名 Cell Host & Microbe	６．最初と最後の頁 441～442
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.chom.2024.03.004	査読の有無 有
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１．著者名 Lostao Anabel、Lim Keesiang、Carmen Maria Pallares、Ptak Arkadiusz、Marcuello Carlos	４．巻 238
２．論文標題 Recent advances in sensing the inter-biomolecular interactions at the nanoscale - A comprehensive review of AFM-based force spectroscopy	５．発行年 2023年
３．雑誌名 International Journal of Biological Macromolecules	６．最初と最後の頁 124089～124089
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２．論文標題 Nanoscopic Assessment of Anti-SARS-CoV-2 Spike Neutralizing Antibody Using High-Speed AFM	５．発行年 2023年
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１．著者名 Sajidah Elma Sakinatus、Lim Keesiang、Yamano Tomoyoshi、Nishide Goro、Qiu Yujia、Yoshida Takeshi、Wang Hanbo、Kobayashi Akiko、Hazawa Masaharu、Dewi Firli R. P.、Hanayama Rikinari、Ando Toshio、Wong Richard W.	４．巻 11
２．論文標題 Spatiotemporal tracking of small extracellular vesicle nanotopology in response to physicochemical stresses revealed by HS‐AFM	５．発行年 2022年
３．雑誌名 Journal of Extracellular Vesicles	６．最初と最後の頁 -
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1．著者名 Makiyama Kei、Hazawa Masaharu、Kobayashi Akiko、Lim Keesiang、Voon Dominic C.、Wong Richard W.	4．巻 586
2．論文標題 NSP9 of SARS-CoV-2 attenuates nuclear transport by hampering nucleoporin 62 dynamics and functions in host cells	5．発行年 2022年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bbrc.2021.11.046	査読の有無 有
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2．論文標題 Millisecond dynamic of SARS‐CoV‐2 spike and its interaction with ACE2 receptor and small extracellular vesicles	5．発行年 2021年
3．雑誌名 Journal of Extracellular Vesicles	6．最初と最後の頁 -
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1．著者名 Sajidah Elma Sakinatus、Lim Keesiang、Wong Richard W.	4．巻 10
2．論文標題 How SARS-CoV-2 and Other Viruses Build an Invasion Route to Hijack the Host Nucleocytoplasmic Trafficking System	5．発行年 2021年
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2．論文標題 High-Speed Atomic Force Microscopy Reveals Spatiotemporal Dynamics of Histone Protein H2A Involution by DNA Inchworming	5．発行年 2021年
3．雑誌名 The Journal of Physical Chemistry Letters	6．最初と最後の頁 3837～3846
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2．論文標題 High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes	5．発行年 2020年
3．雑誌名 Nano Letters	6．最初と最後の頁 6320～6328
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.nanolett.0c01755	査読の有無 有
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〔学会発表〕　計8件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 Keesiang Lim
2．発表標題 Structural dynamics of SARS-CoV-2 spike (S) protein and its interaction with ACE2 receptor and small extracellular vesicles
3．学会等名 Cross-Scale Biology 1st Wakate meeting
4．発表年 2022年

1．発表者名 Keesiang Lim
2．発表標題 Structural dynamics of SARS-CoV-2 spike (S) protein and its interaction with ACE2 receptor and small extracellular vesicle
3．学会等名 The 69th Annual Meeting of the Japanese Society for Virology
4．発表年 2022年

1．発表者名 Keesiang Lim
2．発表標題 Millisecond tracking of structural dynamics of SARS-CoV-2 spike protein and its interaction with ACE2 receptor and small extracellular vesicles.
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4．発表年 2022年

１．発表者名
Keesiang Lim

２．発表標題
Spatiotemporal tracking of SARS-CoV-2 spike (S) protein and its interaction with ACE2 receptor and small extracellular vesicles

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４．発表年
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１．発表者名
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２．発表標題
Conformational dynamics of SARS-CoV-2 spike (S) protein and its interaction with ACE2 receptor and small extracellular vesicles.

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１．発表者名
Keesiang Lim

２．発表標題
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The Molecular Biology Society of Japan

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2021年

１．発表者名
Lim Kee Siang

２．発表標題
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MBSJ (The Molecular Biology Society of Japan) 2020

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Lim Kee Siang

２．発表標題
High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes

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〔図書〕　計0件

〔産業財産権〕

〔その他〕
世界初！高速AFMで新型コロナウイルススパイクタンパク質の分子ナノ動態を可視化！
https://nanolsi.kanazawa-u.ac.jp/achievements/achievements-18490/

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