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

令和　５ 年　 ６ 月 ２１ 日現在

機関番号：１３３０１
研究種目：研究活動スタート支援
研究期間：2021～2022
課題番号：２１Ｋ２０６３３

研究課題名（和文）Biophysical modelling of virus-cell entry

研究課題名（英文）Biophysical modelling of virus-cell entry

研究代表者
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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）：計算科学研究では、2つの計算モデルを開発しました。微分方程式に基づく1つの単純なモデルと、ブラウン力学に基づくもう1つの複雑なモデル。実験では、化学コーティングされた金ガラス表面への金属コロイド吸着を光学顕微鏡で可視化したVLP/MLSアッセイシステムを確立しました。これらの結果は現在論文にまとめられています。スタートアップファンドは他に2つの論文を発表しています。近い将来、この分野の研究をまとめた総説を書く予定です。

研究成果の学術的意義や社会的意義
This work potentially serves society by, 1. It provides greater knowledge about the colloidal forces experienced by a virus at the cell membrane. 2. It brings ideas ideas from two scientific disciplines together (virology and colloidal science). 3. It highlights new avenues for pharmacotherapy.

研究成果の概要（英文）：On the computational side two computer models were developed, and on the experimental side a VLP/MLS assay was established based on optical microscope visualization of metal colloid adsorption to a chemically coated gold glass surface. These results are currently being written up. The funds from this start-up project were acknowledged in the following published or submitted manuscript manuscripts using the following appropriation, ‘This work was supported, in part, by KAKENHI Start-Up grant 21K20633 awarded to D.H.’ (i) Hall, D. and Foster, A.S. (2022) Practical considerations for feature assignment in high-speed AFM of live cell membranes. Biophysics and Physicobiology, 19, p.e190016. https://doi.org/10.2142/biophysico.bppb-v19.0016 (ii) Hall, D. (2023) MIL Cell: A tool for multi-scale simulation of yeast replication and prion transmission. BioRxiv, pp.2023-03. https://doi.org/10.1101/2023.03.21.533288

研究分野：biophysics/biochemistry

キーワード：virus　cell membrane　virus entry　colloidal adsorption　computer modelling

１．研究開始当初の背景(Introduction)

Despite their diversity in terms of structure and core genetic material, all viruses are by definition, obligate parasites meaning that for a virus to reproduce it requires access to the genetic machinery of a host cell. To obtain such access all viruses have to first enter the cell by passing through the cell membrane. Viruses accomplish cell-entry in two steps (i) adsorption to the cell membrane, and (ii) internalization into the cell through virus/cell membrane fusion (for viruses having a membrane envelope) or through employing virus/cell directed endocytotic (membrane encapsulation) mechanisms. Virus directed admission to the cell typically requires either specific recognition events between the virus and cell surface receptors /ligands, or alternatively, non-specific recognition events between the virus membrane coat and local patches of cell membrane rich in a particular type of lipid. However irrespective of the virus or cell type, all virus infection cycles begin with some form of intimate approach and attachment event to the cell membrane as a prerequisite first step. Up until the present time, the biophysical study of this virus/cell encounter/attachment stage has predominantly focused on structural and computational analysis of the individual specific virus protein and cell membrane receptor components involved in the virus/host interaction, with less attention devoted to the adsorption process as a whole. However, as virus capsid sizes typically range from ~50 to 500 nm virus attachment to the cell membrane shares many of the characteristics of the well-studied phenomena of colloidal adsorption to a partially 'clean' two-dimensional matrix of binding sites. This research sought to create experimental and computational models of the virus adsorption/attachment process described in terms of such colloidal adsorption theory with the goal of provide insight into the process of virus infection potentially highlighting new avenues for drug development.

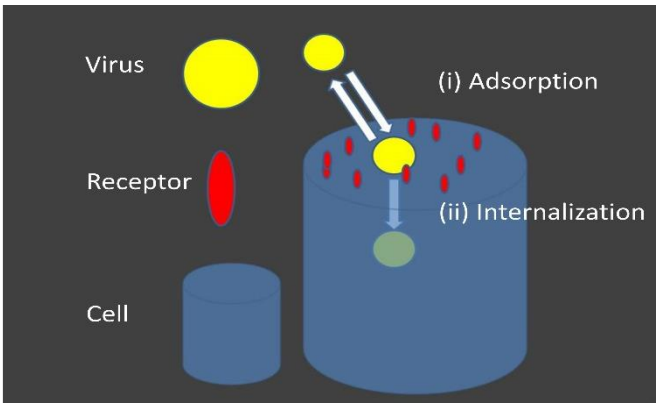

Fig.1. To infect a cell all viruses must typically (i) adsorb to the surface and then (ii) internalize into the cell by playing a biochemical trick (such as binding to a receptor, fusing with the membrane or utilizing pinocytosis). **The aim of this proposal was to experimentally investigate and computationally model the virus adsorption step**. By biophysically characterizing this universal feature of virus cell infection this project hoped to uncover features that are particularly susceptible to drug intervention strategies.

２．研究の目的(Aim of research)

This project aimed to experimentally and computationally model the virus cell entry step. It sought to do this in a novel way by framing the virus-host interaction event as a problem of colloid adsorption to a complex surface. It introduced concepts familiar to colloidal chemists, but less familiar to virologists (such as displacement series, sieving effects, conditional sequential adsorption effects), to highlight aspects of the viral entry process that are susceptible to single or combined-dose pharmaceutical intervention.

３．研究の方法(Experimental methods)

This study involved both experimental development of *in vitro* model systems of virus-like-particles (VLP) binding to a membrane-like-surface (MLS), and computational development of simulation models of VLP/MLS interactions. This joint approach allowed for exploration of the effects of various virus properties, membrane properties and supporting solution characteristics. Predominantly 'single particle tracking' (SPT) approaches (high speed atomic force microscopy (HS-AFM)/ light microscopy (LM)) were used to record the kinetic adsorption isotherms of VLPs to an MLS and examine how a number of non-typical biological factors affected the VLP/MLS adsorption profiles. Computationally, two kinetic models of VLP adsorption were constructed (one microscopic and one macroscopic) to examine the effects of VLP

properties (such as size, shape, lifetime), MLS properties (such as density, mobility, heterogeneity) and interfacial solution characteristics.

４．研究成果(Results)

On the computational side two computer models were developed, and on the experimental side a VLP/MLS assay was established that was based on optical microscope visualization of metal colloid adsorption to a chemically coated gold glass surface. These results are currently being written up.

The funds from this start-up project were acknowledged in the following published or submitted manuscript manuscripts using the following appropriation

'This work supported, in part, by KAKENHI Start-Up grant 21K20633 awarded to D.H.'

(i) Hall, D. and Foster, A.S. (2022) Practical considerations for feature assignment in high-speed AFM of live cell membranes. Biophysics and Physicobiology, 19, p.e190016. https://doi.org/10.2142/biophysico.bppb-v19.0016

(ii) Hall, D. (2023) MIL Cell: A tool for multi-scale simulation of yeast replication and prion transmission. BioRxiv, pp.2023-03. https://doi.org/10.1101/2023.03.21.533288

(iii) Hall D. (2023) HSAFM-MIREBA – Methodology for Inferring REsolution in Biological Applications. (submitted)

(iv) Hall D. (2023) Equations describing semi-confluent cell growth (I) Analytical approximations. (submitted)

The following manuscripts are currently in advanced stages preparation and will carry an acknowledgement of the funds ssociated with this startup grant.

(v) Hall D. (2023) Time over target: A novel viewpoint for development of antiviral drugs.

(vi) Hall D. (2023) A optical microscope-based assay for investigating virus-like-particle adsorption to a membrane-like-surface.

5．主な発表論文等

〔雑誌論文〕　計2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　2件）

1．著者名 Damien Hall	4．巻 19
2．論文標題 Practical considerations for feature assignment in high-speed AFM of live cell membranes	5．発行年 2022年
3．雑誌名 Biophysics and Physicobiology	6．最初と最後の頁 1-21
掲載論文のDOI（デジタルオブジェクト識別子） 10.2142/biophysico.bppb-v19.0016	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Damien Hall	4．巻 2023
2．論文標題 MIL Cell: A tool for multi-scale simulation of yeast replication and prion transmission.	5．発行年 2023年
3．雑誌名 Biorxiv	6．最初と最後の頁 1-27
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2023.03.21.533288	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計0件

〔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