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研究課題名（和文）The Effect of Active Zone Protein Dynamics on Synaptic Vesicle Release Probability

研究課題名（英文）The Effect of Active Zone Protein Dynamics on Synaptic Vesicle Release Probability

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）：細胞内小胞による生体分子の能動輸送は、例えば神経細胞の化学的情報伝達に不可欠である。小胞動態を捉えるシミュレータの開発より、今まで以上に空間的、分子化学的に詳細な海馬シナプスのシミュレーションに成功した。
このシミュレーションより小胞リサイクリングでは、電極刺激の周波数に応じて活用される小胞プールが変化し、tomosyn-1やRab3-GTPが神経伝達物質の放出に重要であることを明らかにした。

研究成果の学術的意義や社会的意義
神経情報伝達物質の放出は脳の情報伝達の根幹にあり、これらの異変はアルツハイマー病、パーキンソン病、そして統合失調症に関連すると考えられている。よって、神経伝達の理解を深める本研究の成果はこれらの病理学に大きく貢献し、治療法確立の礎となる。本研究で開発されたシミュレータは今後細胞内小胞のシミュレーションにも役立つ。

研究成果の概要（英文）：We developed new technology for simulating vesicles in cells and related processes such as exocytosis, clustering, active transport and endocytosis. Such phenomena play crucial roles in biology, for example by controlling neurotransmitter release in nerve terminals, which is the fundamental process behind information transfer in the brain. Being able to simulate these systems computationally allows us to explore them in ways not previously possible, and uncover new insights into their function.

We used this new technology to develop a model of the synaptic vesicle cycle at a hippocampal en passant synapse to unprecedented spatial and molecular detail. In doing so, we uncovered many new insights into this system, such as the use of the distinct vesicle pools at different stimulation frequencies and the role of crucial molecules such as tomosyn-1 and Rab3-GTP in regulating neurotransmitter release. The tools developed will allow further discoveries to be made in the future.

研究分野：Computational Neuroscience

キーワード：Synapse　Plasticity　Hippocampus　Computational biology　Systems biology

１．研究開始当初の背景(Background)

Information transfer in the brain at chemical synapses is underpinned by neurotransmitter release in response to a nerve cell action potential, but much remains to be elucidated about the molecular behavior involved in this fundamental component of brain function. We apply computational methods to further our understanding of this and other systems.

Synaptic vesicles form distinct pools in the presynaptic neuron, which may broadly be distinguished into a large, clustered pool known as the reserve pool, a much smaller number of docked vesicles ready to undergo exocytosis upon receiving the right signal, known as the readily releasable pool, and the recycling pool, which is dynamic in size, mobile, and available for replenishment of the readily releasable pool. Vesicles contain proteins embedded in their membrane that can interact with their environment, enabling processes such as neurotransmitter filling, active transport, docking and fusion. The synaptic vesicle cycle consists of exocytosis of docked vesicles upon sensing a calcium signal, upon which neurotransmitter is released into the synaptic cleft, recycling of the proteins from the exocytosed vesicle in the cell membrane by endocytosis, and replenishment of the readily releasable pool ready for the next stimulus. Under sustained high-frequency stimulation, reserve pool vesicles can be released and can become available for docking and fusion.

It is our hypothesis that the mobility and availability of proteins in the presynaptic neuron strongly influences neurotransmitter release probability, but the practical difficulty of measuring protein dynamics in living cells makes experiments infeasible, and we take a computational approach. However, the tools to simulate these processes have simply been absent until now, and so first the computational tools must be developed, and they must be reliable and powerful enough to be applied to our research question, specifically spatial, detailed molecular modeling of the full presynaptic vesicle cycle.

Computational techniques for application to biological systems have advanced significantly in recent years. Specifically in our STochastic Engine for Pathway Simulation (STEPS) project, spatial reaction-diffusion systems were supported in 2012 with realistic morphology represented by tetrahedral meshes [1]. Then came voltage calculations, which are crucially important to neuronal process, in the 2013 release of finite volume membrane potential calculations [2], coupled with the reaction-diffusion systems. Parallelization followed in 2017 [3], opening up much more complex models that could be run on a supercomputer in a workable wall-clock time. However, despite these advances, some fundamental processes related to chemical synapse neurotransmitter release were not supported and required further development and novel advances in the field of computational molecular biology.

By 2019 we had developed a prototype vesicle modeling technology and applied it to post-synaptic AMPAR vesicle trafficking [4], but much remained to be done to develop mature, reliable and powerful software with the functionality required to model the phenomena of interest in the synaptic vesicle cycle. Specifically required was the need to simulate processes such as endocytosis, vesicle transport, docking, fusion and release, and to couple these processes with the reaction-diffusion techniques already in place. The software needed to be tested rigorously and be parallelized in order to achieve workable runtimes.

[1] Hepburn I, Chen W, Wils S, and De Schutter E (2012) STEPS: efficient simulation of stochastic reaction-diffusion models in realistic morphologies. *BMC Systems Biology* 6, 19
[2] Hepburn I, Cannon R and De Schutter E (2013) Efficient calculation of the quasi-static electrical potential on a tetrahedral mesh and its implementation in STEPS. *Front Comp Neuro* 7:129.
[3] Chen W and De Schutter E (2017) Parallel STEPS: Large Scale Stochastic Spatial Reaction-Diffusion Simulation with High Performance Computers. *Front Neuroinf* 11, 15
[4] De Schutter E, Nagasawa S, Hepburn I and Gallimore A (2018) Spatial Modeling of AMPA Receptor Trafficking and Sorting at the Endosome. in CNS2018 (Computational Neuroscience Society, Seattle)

２．研究の目的(Purpose)

The objective of this project is to design the tools to enable better understanding of neurotransmitter release at chemical synapses, which could also potentially be applied to further understanding of other

biological systems. Better understanding of the process of neurotransmitter release would have many benefits, such as, eventually, allowing for breakthroughs to be made in treating certain neurological disorders. This is because there is increasing evidence that synaptic disfunction is involved in numerous neurological disorders such as Alzheimer's disease, Parkinson's disease and schizophrenia and so understanding the regulation of synaptic transmission may, eventually, have clinical significance, leading to new treatments, and this project could be an important step towards these goals. Better understanding of these systems may have other benefits, such as perhaps enabling more advanced and efficient AI systems. For our specific purpose in this research project, we want to design the tools that can simulate vesicle dynamics realistically and to demonstrate the power of these tools by application to the synaptic vesicle cycle. If successfully achieved, this both advances our understanding of neurotransmitter release at chemical synapses and further opens up new avenues of research in computational biology in the future that simply haven't been possible until now.

Overall, our goal was to develop a computational model of the complete vesicular cycle at an unprecedented level of spatial and biochemical detail in a relatively short timeframe. This ambitious goal required concurrent work in two different aspects of the project, allowing researchers of different expertise to collaborate towards the final goal.

３．研究の方法(Research method)

The development of the software, STEPS, to enable modeling of the phenomena of interest, namely synaptic vesicles and their interactions with their environment, broadly encompasses the research methods of this project.

Many components of vesicle-related function were developed and tested with rigorous validation models. The functionality is summarized in Figure 1 and the validation models can be viewed, downloaded and run from:
https://github.com/CNS-OIST/STEPS_Validation/tree/main/vesicles.

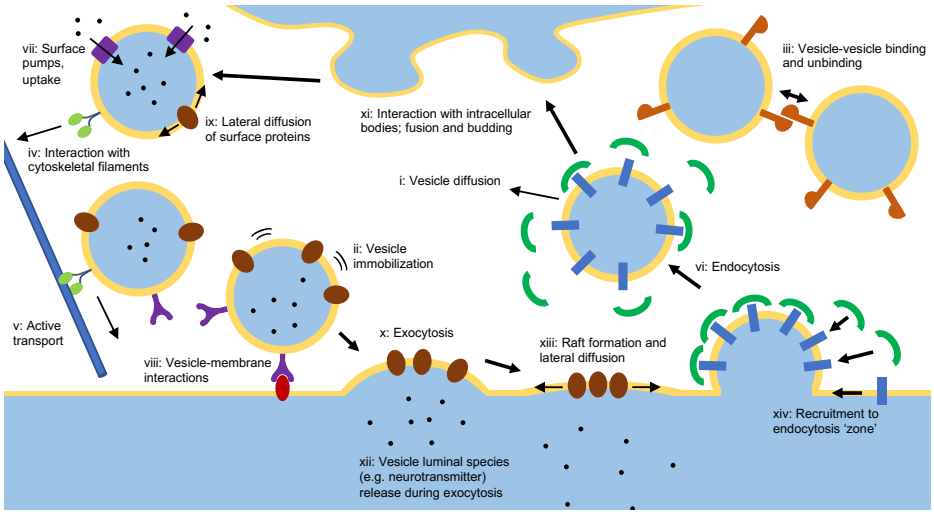

Figure 1 The vesicle modeling functionality in STEPS developed for this project

The novel functionality that was developed are summarized in Figure 1. Firstly, vesicle mobility was captured with free Brownian-motion diffusion (Fig. 1i), immobilization when docked to membrane (Fig. 1ii), partial mobility when clustered with other vesicles (Fig. 1iii) and active transport on cytoskeletal filaments (Fig. 1v). Vesicle membrane proteins were added, enabling processes such as neurotransmitter filling (Fig. 1vii), docking to the cell membrane (Fig. 1viii) and binding to other vesicles (Fig. 1iii). Diffusion of proteins on the vesicle surface was added (Fig. 1ix) which amounted to solving diffusion on the surface of a sphere. The important processes of endocytosis (Fig. 1vi) and exocytosis (Fig. 1x) were added enabling the creation of vesicles and neurotransmitter release (Fig. 1xii), respectively. Support for lipid rafts was added, along with related processes such as raft surface diffusion on cell membranes (Fig. 1xiii) and raft endocytosis (Fig. 1vi).

To test all of this functionally, we developed a validation model for each component, available from the above public Github repository. A manuscript detailing these methods and their corresponding validation models was accepted by Communications Biology in the Nature series by the time of this project's conclusion, and was published on May 15th 2024 [1].

The prototype version of STEPS in 2019 that included some of this functionality did so entirely with serial computation, severely restricting what was achievable with this software in acceptable runtimes. During this project, the major development of the software was to completely design parallel routines for these methods from scratch, which resulted in a major overhaul of the codebase, and to couple these methods with our parallel reaction-diffusion methods previously developed [2,3]. The parallel routines are shown in Figure 2 a,b, and described in detail in our publication [1]. For our realistic synaptic vesicle cycle model, we achieved a good speedup of 50x (Fig. 2c) bringing acceptable runtimes, which was vital for the modeling component of this project. In addition, we developed a Blender extension module, demonstrated in Figure 2d and described in more detail in our publication [1].

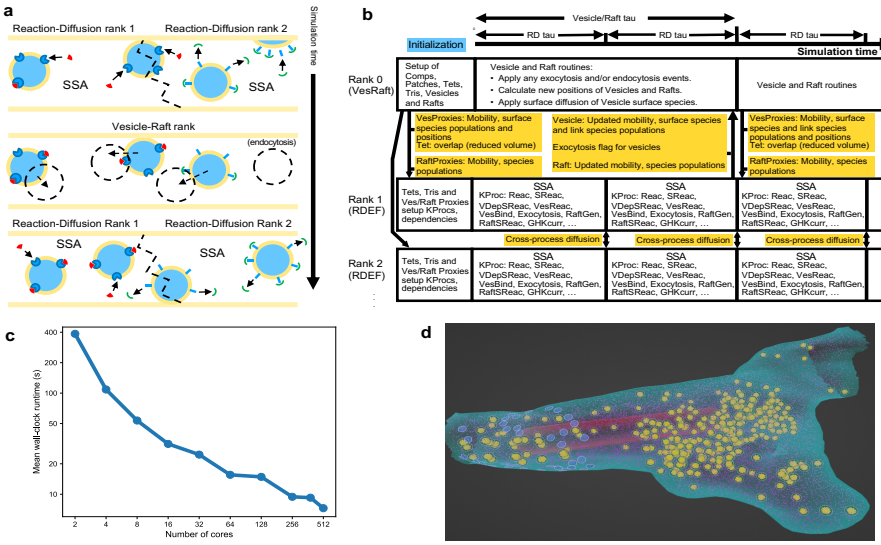

Figure 2 The MPI implementation in STEPS 5 and the Blender extension module

STEPS 5, the first public release to include all the described functionality, was released in March 2024 (https://github.com/CNS-OIST/STEPS/releases/tag/5.0.1).

[1] Hepburn I, Lallouette J, Chen W, Gallimore AR, Nagasawa-Soeda S and De Schutter E (2024) Vesicle and reaction-diffusion hybrid modeling with STEPS. *Communications Biology* **7**, 573
[2] Hepburn I, Chen W, De Schutter E (2016) Accurate reaction-diffusion operator splitting on tetrahedral meshes for parallel stochastic molecular simulations. *J Chem Phys* 145, 054118
[3] Chen W and De Schutter E (2017) Parallel STEPS: Large Scale Stochastic Spatial Reaction-Diffusion Simulation with High Performance Computers. *Front Neuroinf* 11, 15

4．研究成果(Outcomes)

Enabled by the development of our novel vesicle simulation technology, we built a model of the synaptic vesicle cycle at a hippocampal *en passant* synapse to unprecedented molecular and spatial detail [1]. The model is summarized in Figure 3. It includes vesicle clustering and pool formation (Fig. 3A), the recruitment of proteins to the vesicle surface (Fig. 3C), vesicle surface protein to vesicle surface protein interactions (enabling clustering, Fig. 3C,D), docking and priming interactions involving proteins on the vesicle surface and proteins in the cell membrane (Fig. 3B,C), exocytosis (Fig. 3D), along with endocytosis and recycling (Fig. 3D). A snapshot of the complete computational model can be visualized in Figure 2d.

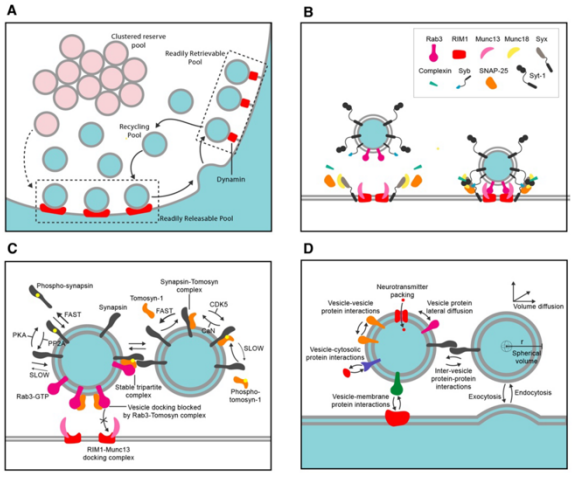

Figure 3 Structure of our model

We prepared a manuscript describing all of our modeling outcomes, which we published in preprint [1], and an updated version, which includes supportive experimental data, is currently under review at a major journal. In the model, we typically simulated neural firing at ranges from 5 Hz to 50 Hz, allowing 5s for docking, cluster formation and equilibration of the model, then simulating for 45 seconds of stimulation. In summary, our modeling outcomes (described in more detail in [1]) were as follows:

- Our model captured physiological release probabilities (0.5), recycling times (between 2.5 and 18.6 seconds) and increasing accumulation of membrane vesicle material at higher frequencies.
- At all neural firing frequencies studied, endocytosis closely followed exocytosis with a delay of approximately 2 seconds. This suggests the recycling system can keep pace with fusion even at non-physiologically high firing rates.
- Dynamin-mediated scission is the final step in vesicle endocytosis. We observed that, while the rate of accumulation of bound dynamin increased with frequency, it never reached saturation, indicating that the number of dynamin complexes is not rate-limiting in maintaining endocytosis.
- On the timeframe observed, vesicle recycling was largely responsible for maintaining vesicle release over time. Reserve vesicle usage increased with frequency, from 9% at 5 Hz to 45% at 50 Hz (Figure 4C red line shows reserve vesicle usage over time at 10Hz).

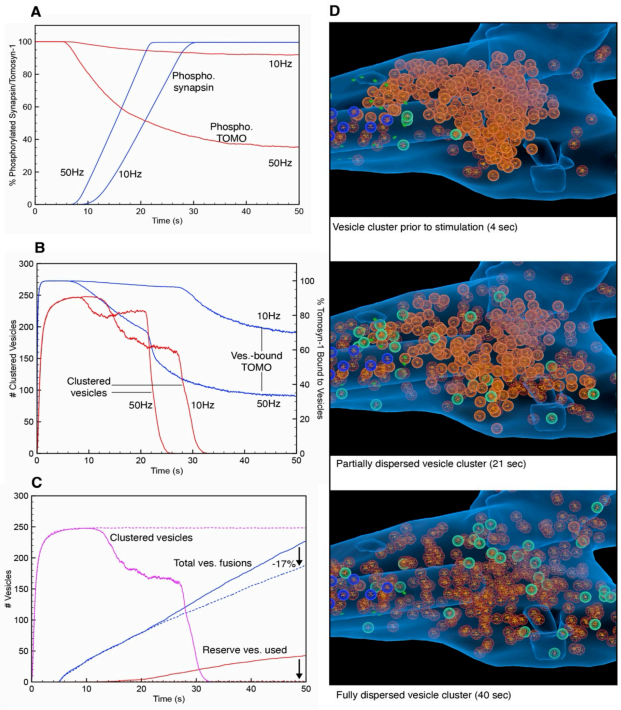

Figure 4 Regulation of vesicle clustering and dispersion

- We observed vesicle clustering and dispersion at two different frequencies of stimulation, 10 Hz and 50 Hz (Figure 4). We observed that the dispersion of the vesicle cluster occurs rapidly in both cases but is quicker at the higher frequency (Fig. 4B) driven by a faster rate of synapsin phosphorylation (Fig. 4A,D). Simulating without phosphorylation of synapsin blocked vesicle usage completely from the reserve pool (Fig 4C).
- We found that tomosyn-1 not only inhibits docking but, by forming a stable tripartite complex (Fig. 3C), ensures that the vesicle cycle is maintained by endocytosed vesicles, avoiding depletion of the reserve pool.
- We found that selective tethering of vesicles with free Rab3-GTP in our model both enhances the rate of vesicle release over time whilst also reducing the number of vesicles from the reserve pool to maintain synaptic release. Selective tethering allows a smaller number of recycling vesicles to maintain the vesicle cycle with minimal reserve pool recruitment.

Our novel simulation technology and synaptic vesicle cycle model at unprecedented spatial and molecular detailed has brought many new insights into the molecular behavior involved in neurotransmitter release, furthering our understanding of this crucial process in brain function. The tools developed will allow further discoveries to be made in the future.

[1] Gallimore AR, Hepburn I, Rizzoli S and De Schutter E (2023) Dynamic Regulation of Vesicle Pools in a Detailed Spatial Model of the Complete Synaptic Vesicle Cycle. bioRxiv 2023.08.03.551909

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　2件／うちオープンアクセス　2件）

１．著者名 Hepburn Iain、Lallouette Jules、Chen Weiliang、Gallimore Andrew R.、Nagasawa-Soeda Sarah Y.、De Schutter Erik	４．巻 7
２．論文標題 Vesicle and reaction-diffusion hybrid modeling with STEPS	５．発行年 2024年
３．雑誌名 Communications Biology	６．最初と最後の頁 573
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s42003-024-06276-5	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 Gallimore Andrew R.、Hepburn Iain、Rizzoli Silvio、Schutter Erik De	４．巻 -
２．論文標題 Dynamic Regulation of Vesicle Pools in a Detailed Spatial Model of the Complete Synaptic Vesicle Cycle	５．発行年 2023年
３．雑誌名 bioRxiv	６．最初と最後の頁 551909
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2023.08.03.551909	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 該当する

〔学会発表〕　計1件（うち招待講演　1件／うち国際学会　0件）

１．発表者名 Erik De Schutter
２．発表標題 Modeling the tripartite synapse.
３．学会等名 Quantitative Synaptology talk, University of Goettingen, Germany.（招待講演）
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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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