



Development of the Integrated Computer Simulation Model of the Intracellular, Transmembrane, and Extracellular Domain of Platelet Integrin $\alpha_{IIb}\beta_3$ (Platelet Membrane Glycoprotein: GPIIb–IIIa)

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TH Open 2024;8:e96–e105.

Abstract

Background The structure and functions of the extracellular domain of platelet integrin $\alpha_{IIb}\beta_3$ (platelet membrane glycoprotein: GPIIb–IIIa) change substantially upon platelet activation. However, the stability of the integrated model of extracellular/transmembrane/intracellular domains of integrin $\alpha_{IIb}\beta_3$ with the inactive state of the extracellular domain has not been clarified.

Methods The integrated model of integrin $\alpha_{IIb}\beta_3$ was developed by combining the extracellular domain adopted from the crystal structure and the transmembrane and intracellular domain obtained by Nuclear Magnetic Resonance (NMR). The transmembrane domain was settled into the phosphatidylcholine (2-oleoyl-1-palmitoyl-sn-glycerol-3-phosphocholine (POPC)) lipid bilayer model. The position coordinates and velocity vectors of all atoms and water molecules around them were calculated by molecular dynamic (MD) simulation with the use of Chemistry at Harvard Macromolecular Mechanics force field in every 2×10^{-15} seconds.

Results The root-mean-square deviations (RMSDs) of atoms constructing the integrated $\alpha_{IIb}\beta_3$ model apparently stabilized at approximately 23 Å after 200 ns of calculation. However, minor fluctuation persisted during the entire calculation period of 650 ns. The RMSDs of both α_{IIb} and β_3 showed similar trends before 200 ns. The RMSD of β_3 apparently stabilized approximately at 15 Å at 400 ns with persisting minor fluctuation afterward, while the structural fluctuation in α_{IIb} persisted throughout the 650 ns calculation period.

Conclusion In conclusion, the integrated model of the intracellular, transmembrane, and extracellular domain of integrin $\alpha_{IIb}\beta_3$ suggested persisting fluctuation even after convergence of MD calculation.

Keywords

- platelet
- integrin $\alpha_{IIb}\beta_3$
- molecular dynamic simulation
- GPIIb/IIIa

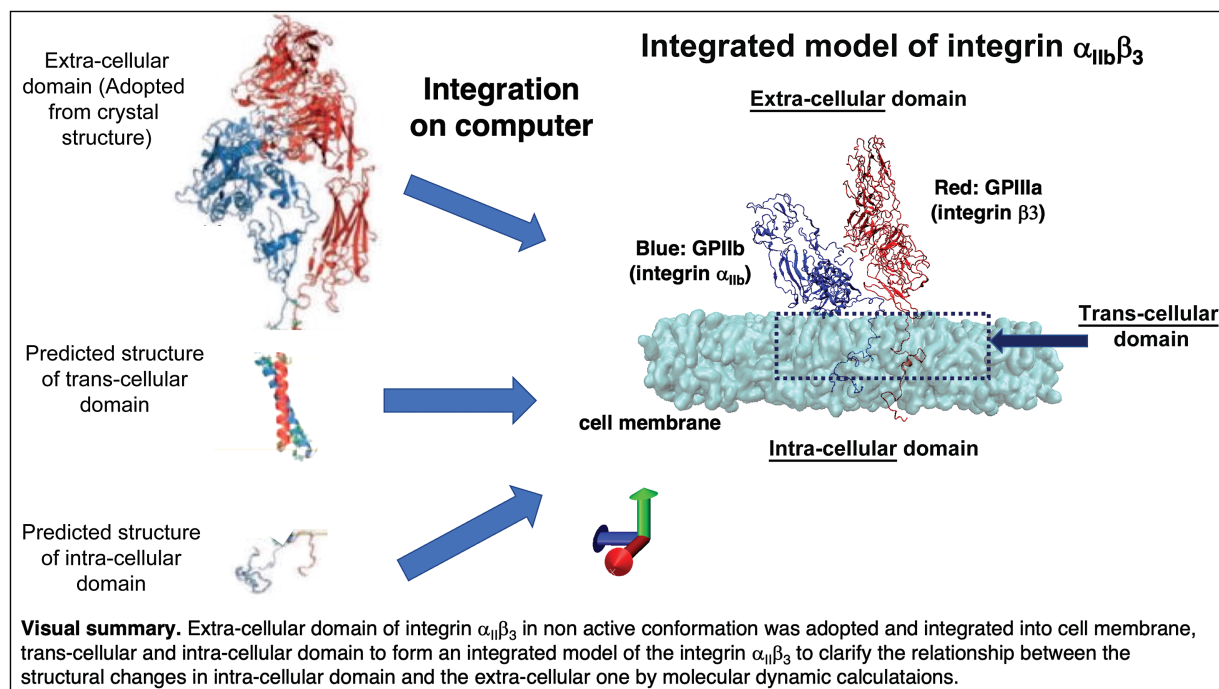
* The contribution of Shinichi Goto on this paper is equal to Masamitsu Nakayama.

received
May 11, 2023
accepted after revision
January 4, 2024
accepted manuscript online
January 17, 2024

DOI <https://doi.org/10.1055/a-2247-9438>.
ISSN 2512-9465.

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Introduction

The integrin $\alpha_{IIb}\beta_3$ molecules known as platelet glycoprotein (GP) IIb/IIIa change their affinity to various plasma ligand proteins such as fibrinogen and von Willebrand factor (VWF) upon platelet activation.¹ Serious bleeding phenotype appears in patients deficient in the functions of $\alpha_{IIb}\beta_3$, namely Glanzmann thrombasthenia² or fetal/neonatal alloimmune thrombocytopenia.³ The functional blockage of integrin $\alpha_{IIb}\beta_3$ reduces the risk of thrombosis such as myocardial infarction but increases the risk of bleeding.⁴ Thus, the function of integrin $\alpha_{IIb}\beta_3$ is essentially important for hemostasis and thrombus formation. A large body of studies have revealed that the mechanism of platelet activation depends on the functional changes in integrin $\alpha_{IIb}\beta_3$.¹ The structural characteristics of both the extracellular domain that mediates biological function^{5–7} and the intracellular domain that induce functional changes^{8–11} were deeply investigated. Recently, Tong et al revealed the importance of an intermediate structure between active and nonactive conformation for platelet adhesion by the use of molecular dynamic (MD) calculations.¹² However, the stability of the structure of the integrin $\alpha_{IIb}\beta_3$ incorporated into the lipid membrane with the nonactive state of extracellular domain still needs to be elucidated.

Recent progress in computer technology and the evolutions of the force field incorporating quantum mechanics coarse-grained into molecular mechanics such as the CHARMM (Chemistry at Harvard Macromolecular Mechanics) enabled the construction of the biological functions of various proteins from the accumulations of simple physical movements of the atoms.^{13–15} Various biological functions

such as transmembrane water transportation were constructed by structural fluctuations of specific proteins such as aquaporin.^{16,17} Specific biological functions of platelets such as adhesion on VWF under high shear stress conditions^{18,19} were also simulated from dynamic movements of atoms.^{20,21} The MD simulation calculation has also been applied in parts of integrin $\alpha_{IIb}\beta_3$ previously²² and, recently, for whole molecules incorporated into lipid membrane by Tong et al.¹² Several previous studies revealed the important regions within the extracellular domain of $\alpha_{IIb}\beta_3$ to achieve its biological functions.^{23,24} Moreover, the MD simulation was also applied to the intracellular domain of $\alpha_{IIb}\beta_3$.²² The integrated model of integrin $\alpha_{IIb}\beta_3$ constructed from intracellular, transmembrane, and extracellular domain was published recently.¹² Here, we have attempted to confirm the structural fluctuation of integrin $\alpha_{IIb}\beta_3$ incorporated into the lipid membrane.

We are proposing the hypothesis here that the structure of integrin $\alpha_{IIb}\beta_3$ is unstable as compared to other platelet glycoproteins such as GPIIb even in the inactive conformation of extracellular domain.

Material and Methods

Molecular Dynamic Simulation

Initial Structure of GPIIb/IIIa

The initial structure of the extracellular domain of integrin $\alpha_{IIb}\beta_3$ was obtained from the previously published crystal structure representing the nonactivated conformation.^{7,25} While the platelet membrane is known to contain phosphatidyl serine,²⁶ the cell membrane model composed from lipid

bilayer 2-oleoyl-1-palmitoyl-sn-glycerol-3-phosphocholine (POPC)²⁷ was used in this study because the distributions of POPS were shown to be influenced substantially after platelet activation and the precise distributions of POPS before and after platelet activation have only been partly quantified.²⁸ The structure of the transmembrane and the intracellular domain was adopted from the previously published model predicted from NMR, electron cryo-microscopy, and single particle image reconstruction.^{29,30} The POPC membrane model was settled at the transcellular domain of the integrated $\alpha_{IIb}\beta_3$ model. The integrated model of whole integrin $\alpha_{IIb}\beta_3$ was constructed according to the previously published conceptional model.³⁰

Molecular Dynamic Simulation Calculation

The water molecules were modeled as CHARMM transferable intermolecular potential with three interaction sites and were arranged around the atoms constructing the integrated model of $\alpha_{IIb}\beta_3$ according to the previous publication.³¹ Newton's second law of $F(\text{force}) = M(\text{mass}) \times A(\text{acceleration})$ was solved for all atoms constructing the integrated model of $\alpha_{IIb}\beta_3$, lipid membrane, and water molecules. The calculation was conducted using NANOscale Molecular Dynamics software^{20,21} on a computer equipped with four NVIDIA Tesla V100 GPUs (HPC5000-XSLGPU4TS, HPC systems Inc., Tokyo,

Japan). Since biological events occur in stable temperature and pressure, neither constant-temperature, constant-pressure ensemble (NPT) nor constant-temperature, constant-volume ensemble (NVP) ensembles were skipped. The position coordinates and velocity vectors of atoms and water molecules were calculated in each 2.0 femtosecond (10^{-15} s) using the CHARMM-36 force field.^{32,33} The calculation started immediately from the initial structure. Visual molecular dynamics version 1.9.3 was used for the visualization of the results.^{20,21}

Root Mean Square Deviations

In each calculated structure, the average distances between various atoms excluding lipid bilayer and water molecules were calculated as the root mean square deviations (RMSDs) for all atoms constructing the integrated model of $\alpha_{IIb}\beta_3$. To identify the specifically unstable regions within this calculation, the RMSDs were also calculated separately for α_{IIb} , in β_3 , in the intracellular domain, in the transmembrane, and in the extracellular domains. The RMSDs were calculated every 10 picoseconds from the beginning to the end of the calculation.

The validity of calculation results was intuitively assessed by comparing the structure of extracellular domain of the integrated model of $\alpha_{IIb}\beta_3$ before and after MD calculation. The stability of RMSDs of atoms constructing the

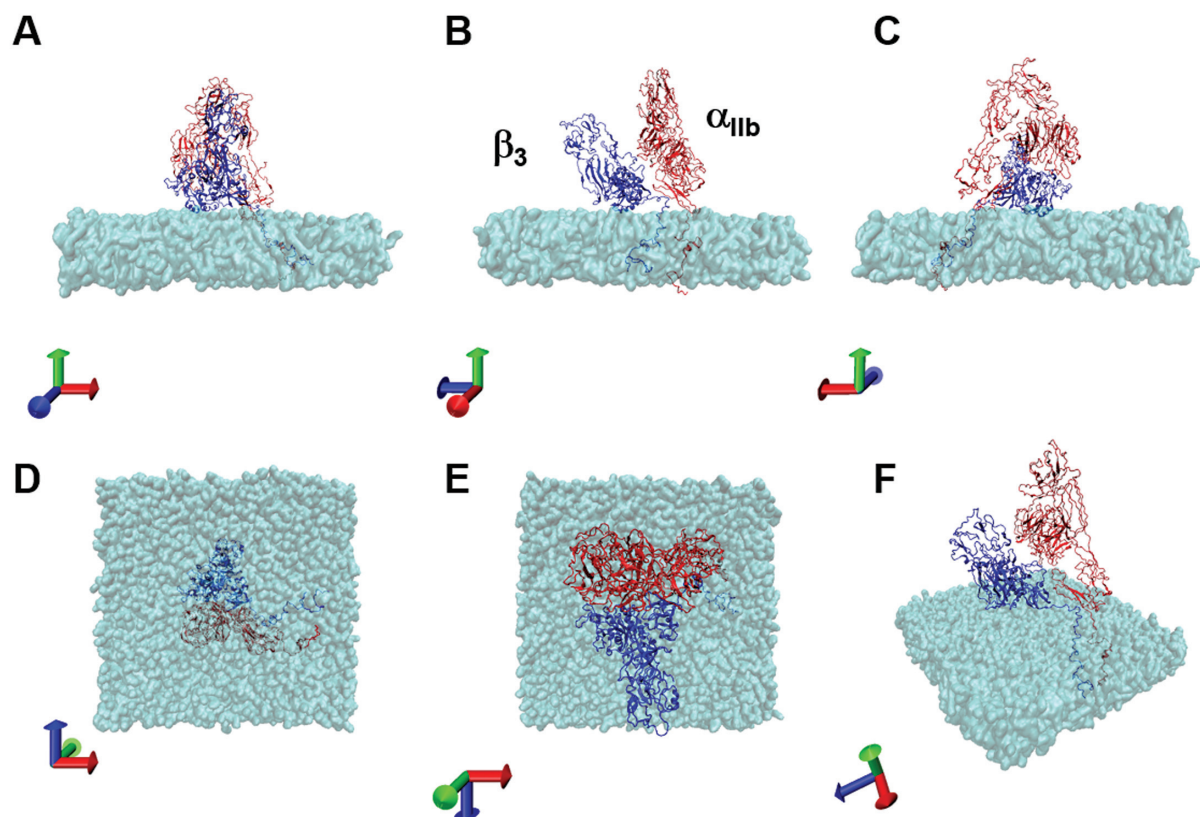


Fig. 1 Initial structure of integrated model of integrin $\alpha_{IIb}\beta_3$. The composed initial structure of integrin $\alpha_{IIb}\beta_3$ along with membrane bilayer are shown in each panel. The molecules constructing α_{IIb} and β_3 are shown in red and blue, respectively. The membrane constructed from the bilayer of 2-oleoyl-1-palmitoyl-sn-glycerol-3-phosphocholine is shown in light blue. The panel A to F show the view of the initial structure of the integrated model from the direction shown at left bottom of each panel.

extracellular domain of the integrated model of $\alpha_{IIb}\beta_3$ within 20 \AA objectively confirms that the calculated structure is not extremely different from the crystal structure.

Results

Initial Structure

► **Fig. 1** shows that the initial structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ composed of the extracellular, transmembrane, and intracellular domain arranged within the POPC lipid bilayer. The protein structures are also provided as a pdb file as attached (**Supplemental pdb files 1**, available in the online version). The transmembrane domain is shown through a lipid bilayer.

Structure after 700 ns of Molecular Dynamic Calculation

► **Fig. 2** shows the structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ after 700 ns (3.5×10^8 step) of MD calculation. The protein structures provided as pdb files (**Supplemental pdb files 2**, available in the online version). There were apparent changes as compared to the initial structures. To further clarify the changes from the initial structure, each panels of ► **Fig. 1** and ► **Fig. 2** was overlaid to make ► **Fig. 3**. The structural fluctuations of the integrated $\alpha_{IIb}\beta_3$ model from the beginning to the end of the calculation is summarized in two movies (**Supplemental movie A** and **Supplemental movie B**, available

in the online version). Apparently, the structural fluctuation was larger in α_{IIb} than in β_3 . As compared to the heavy chain, the light chain of α_{IIb} appeared most unstable.

Supplemental Movie A

Time-dependent changes in the structure of integrin $\alpha_{IIb}\beta_3$ from the frontal view. Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-2247-9438>.

Supplemental Movie B

Time-dependent changes in the structure of integrin $\alpha_{IIb}\beta_3$ from the diagonal view. Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-2247-9438>.

► **Fig. 4** shows the detailed structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ focusing on the transmembrane domain after 700 ns of calculation. The amino acid from

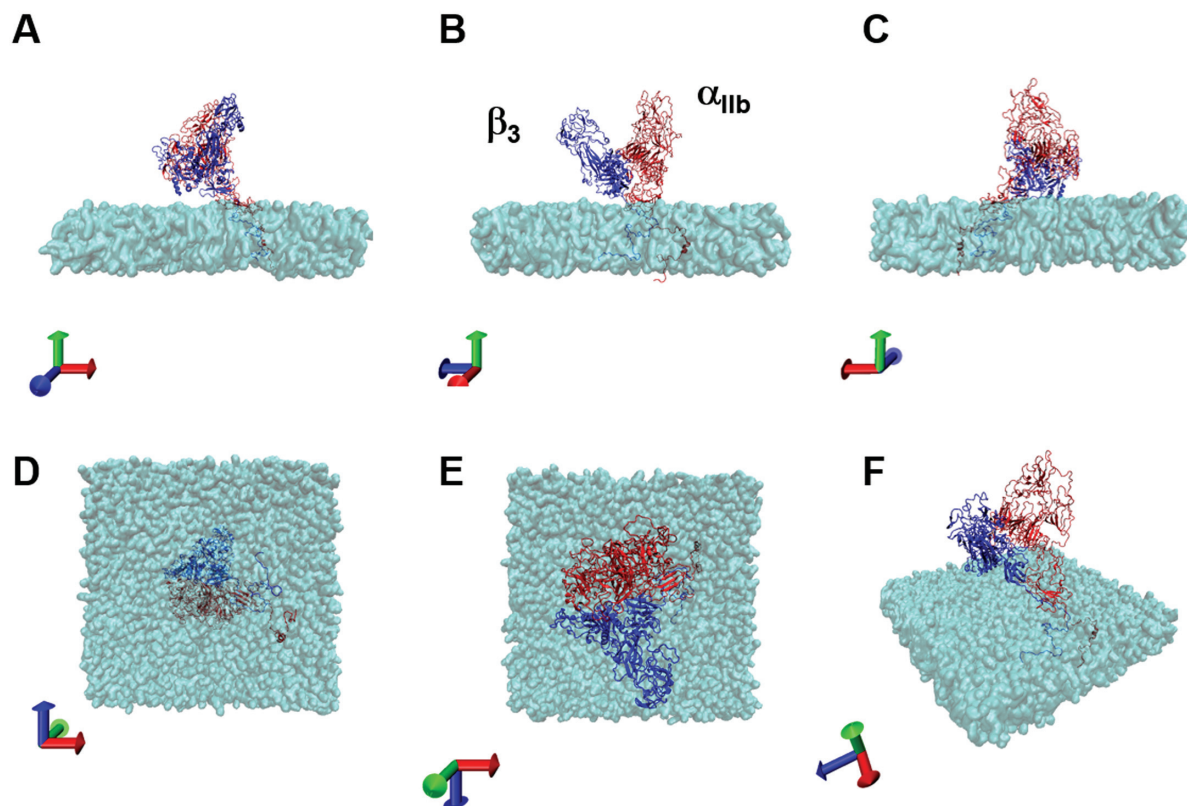


Fig. 2 The structure of the integrated model of integrin $\alpha_{IIb}\beta_3$. After 700 ns of calculation. The composed structure of integrin $\alpha_{IIb}\beta_3$ along with the membrane bilayer after 700 ns of MD calculation is shown. The panel A to F show the view of the initial structure of the integrated model from the direction shown at left bottom of each panel.

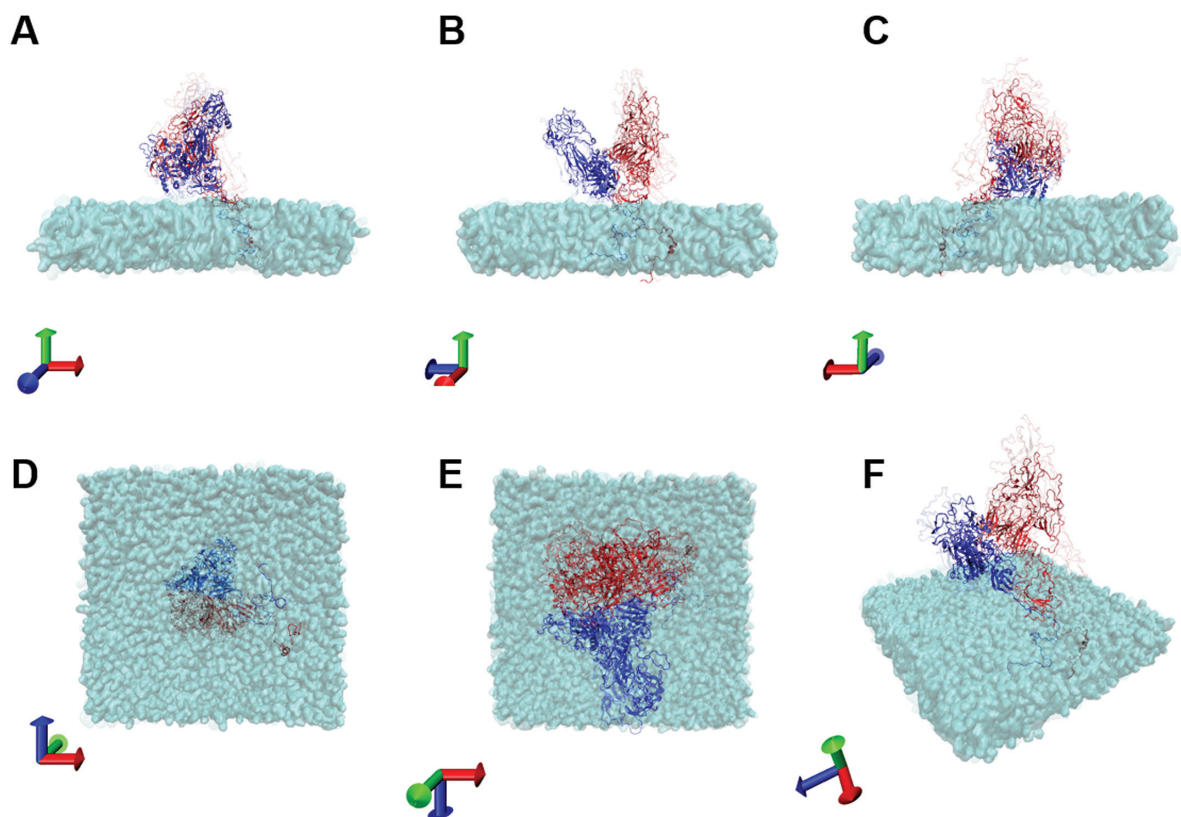


Fig. 3 Overlaid images of initial structure and the one after 700 ns of calculations. The panel A to F are constructed by overlaying the images in the initial structure and the one after 700 ns of calculation.

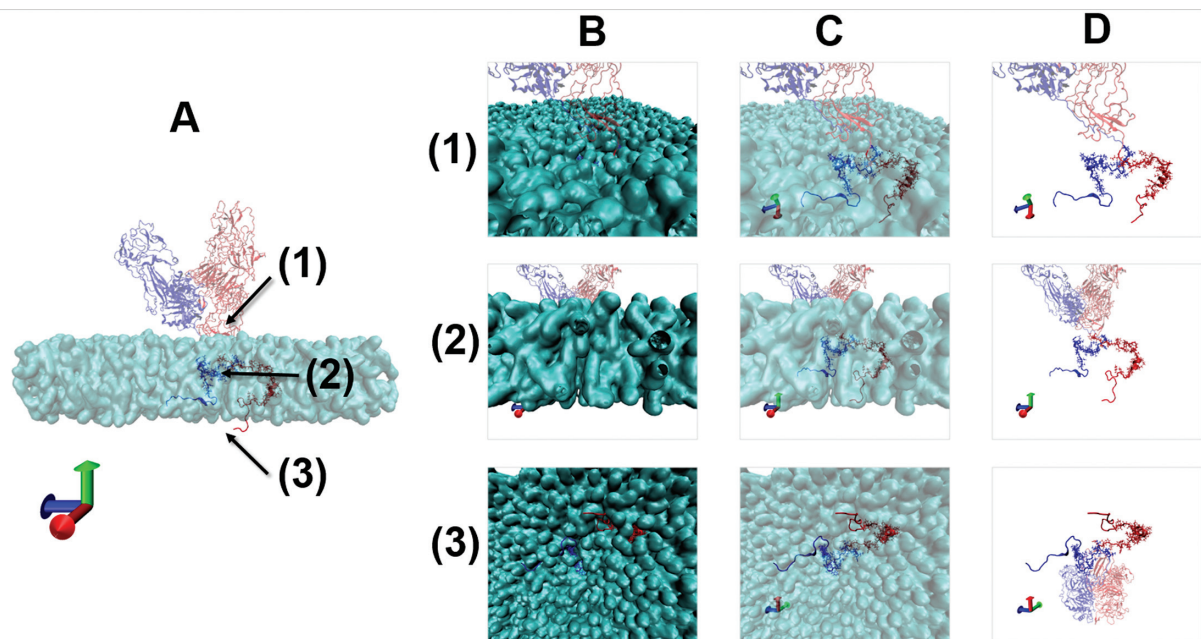


Fig. 4 The detailed structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ focusing transmembrane domain after 700 ns of calculation. Panel A shows the overview of the calculation results at 700 ns. The arrow (1) indicates the base of the extracellular domain of integrin $\alpha_{IIb}\beta_3$. The arrow (2) and (3) indicated the transmembrane and extracellular domain of integrin $\alpha_{IIb}\beta_3$. Both the extracellular and intracellular domains are shown as ribbon diagram. The transmembrane domain is shown as ribbon diagram/ball and stick. Panel B, C, and D show the detailed structure of integrin $\alpha_{IIb}\beta_3$ around platelet membrane. (1), (2), and (3) correspond to the views shown in panel A. The lipid membrane was shown thick, transparent, and clear in panel B, C, and D. The red line indicated the structure of integrin α_{IIb} and the blue line indicates the structure of β_3 .

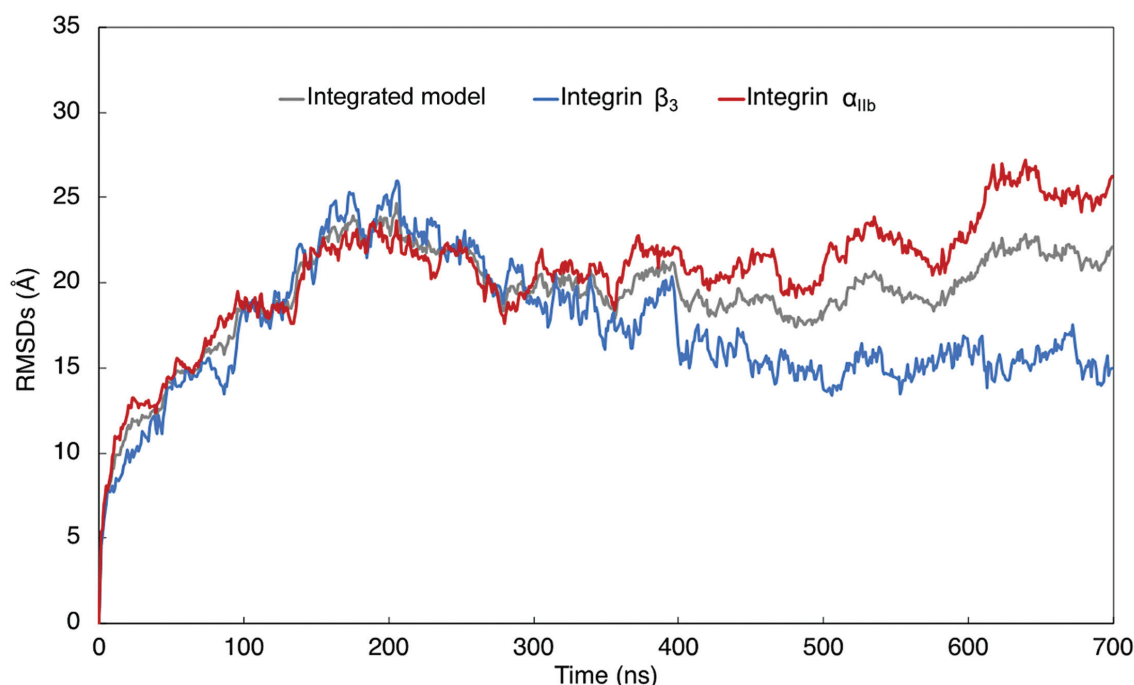


Fig. 5 The root mean square deviations of atoms constructing the integrated model of integrin $\alpha_{IIb}\beta_3$. The time-dependent changes in the root mean square deviations (RMSDs) of atoms constructing the whole integrated model of $\alpha_{IIb}\beta_3$, excluding the water and the lipid, are shown as a gray line. The blue line represents the time-dependent changes in RMSDs in atoms constructing the β_3 subunit in the integrin $\alpha_{IIb}\beta_3$, while the red line represents that in α_{IIb} domain.

961 to 933 in α_{IIb} and 715 to 742 in β_3 integrins are located within the lipid membrane.

Root Mean Square Deviations

The calculated results of RMSDs in the integrated $\alpha_{IIb}\beta_3$ model and in the individual structure of α_{IIb} and β_3 are shown in ►Fig. 5. The RMSD of integrated $\alpha_{IIb}\beta_3$ model apparently stabilized at approximately 23 Å after 200 ns of calculation. However, minor fluctuation persisted until 650 ns to approximately 24 Å. The RMSD of both α_{IIb} (red line in ►Fig. 5) and β_3 (blue line in ►Fig. 5) showed similar trends before 200 ns. The RMSD of β_3 stabilized approximately at 15 Å at 400 ns, while it persisted to fluctuate until 650 ns in α_{IIb} .

►Fig. 6 shows the RMSDs in β_3 within the integrated $\alpha_{IIb}\beta_3$ model. Overall, the RMSDs of β_3 in the integrated $\alpha_{IIb}\beta_3$ model apparently stabilized at 15 Å after 600 ns of calculations. As compared to the extracellular domain, both intracellular and transmembrane domains were more unstable even after 500 ns of calculation.

►Fig. 7 shows the RMSDs in α_{IIb} within the integrated $\alpha_{IIb}\beta_3$ model. The RMSDs of α_{IIb} in the integrated $\alpha_{IIb}\beta_3$ model did not stabilize even after 600 ns of calculation. The RMSD in α_{II} and extracellular domain were larger than that in their intracellular and transmembrane domains.

Discussion

Integrin $\alpha_{IIb}\beta_3$ is one of the most commonly expressed platelet membrane GP. Unlike other commonly expressed pairs of protein complexes such as GPIb/IX, the biological functions of integrin $\alpha_{IIb}\beta_3$ change dramatically after platelet activation.

The functional changes in integrin $\alpha_{IIb}\beta_3$ upon platelet activation are mediated mostly by the conformational changes in its extracellular domain.³⁴ Various ions including cations such as calcium and magnesium play important roles in keeping both inactive and active conformation of the extracellular domain of integrin $\alpha_{IIb}\beta_3$.^{35–37} The activated form of integrin $\alpha_{IIb}\beta_3$ can bind with ligand proteins such as fibrinogen and VWF although it could not bind them in its inactive form. The mechanisms of intracellular signaling pathways to achieve active conformation of integrin $\alpha_{IIb}\beta_3$ have deeply been investigated so far. Recently, the logical link between the structural changes in intracellular domain of $\alpha_{IIb}\beta_3$ on the substantial conformational changes in its extra-cellular domain was suggested by combining all-atom simulations, principal component analysis, and mesoscale modeling by Tong et al.¹² Here, the MD simulation of the integrated model of the extracellular, transmembrane, and intracellular domain of the integrin $\alpha_{IIb}\beta_3$ incorporated into the lipid bilayer membrane was conducted in all atoms constructing them. The structure of the integrated model continuously fluctuated even when the calculation was started from the inactive conformation of extracellular domain suggesting the structural instability of integrin $\alpha_{IIb}\beta_3$ even at the resting state.

As compared to other platelet membrane GPs such as GPIb α , the integrin $\alpha_{IIb}\beta_3$ model was structurally unstable even with a similar extent of calculation length. Indeed, the RMSD became apparently stable after the initial 200 ns of calculation but continued to fluctuate until 650 ns. The time-dependent fluctuation is clearer in the α_{IIb} domain than β_3 . Within α_{IIb} , time-dependent fluctuation was clearer in the extracellular domain. For the future, we aim to apply this

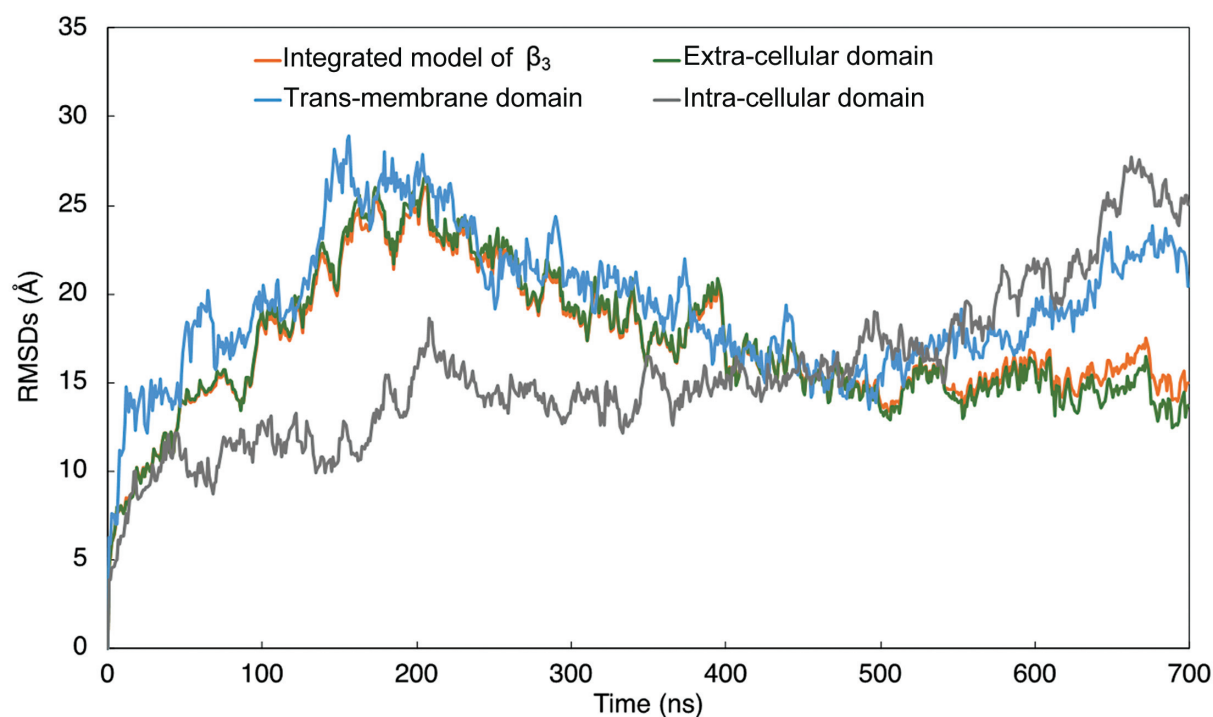


Fig. 6 The root mean square deviations of atoms constructing β_3 in the integrated model of integrin $\alpha_{IIb}\beta_3$. The time-dependent changes in the root mean square deviations (RMSDs) of atoms constructing the β_3 subunit in the integrated model of $\alpha_{IIb}\beta_3$, excluding the water and the lipids, are shown as an orange line. The blue line represents the time-dependent changes in RMSDs in transmembrane domain of β_3 molecule in the integrin $\alpha_{IIb}\beta_3$. The time-dependent changes in RMSDs in the extracellular and the intracellular domains are shown in green and gray lines, respectively.

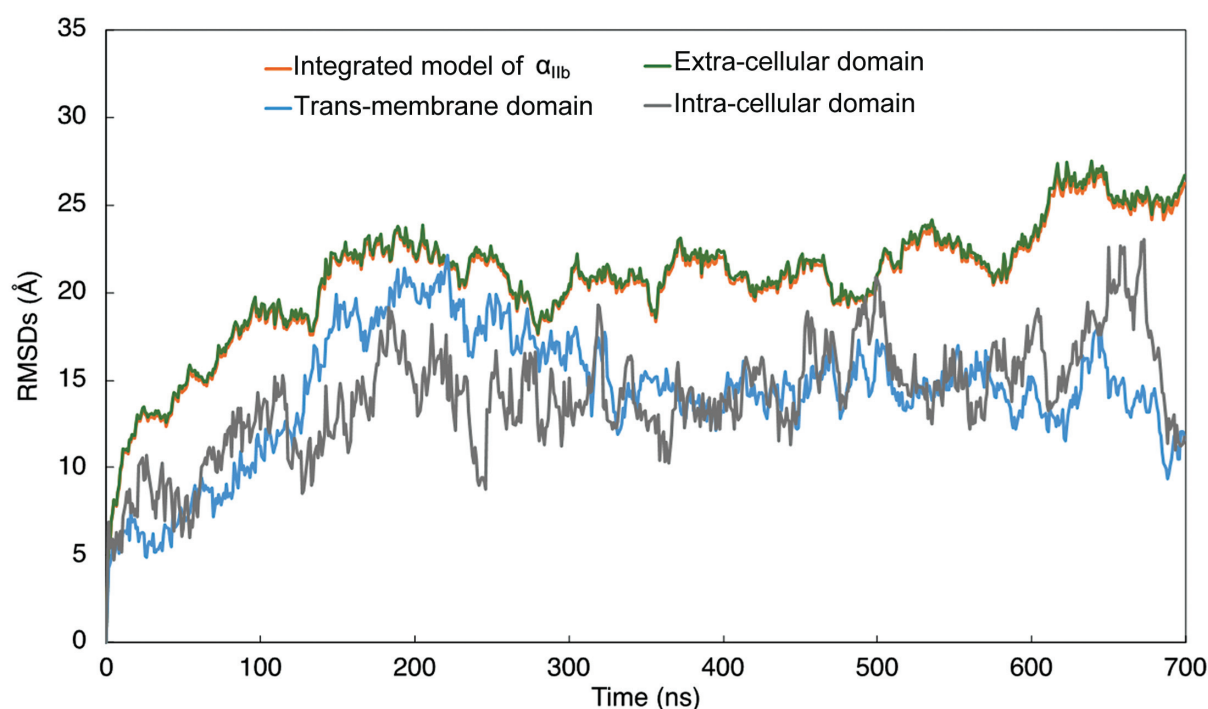


Fig. 7 The root mean square deviations of atoms constructing α_{IIb} in the integrated model of integrin $\alpha_{IIb}\beta_3$. The time-dependent changes in the root mean square deviations (RMSDs) of atoms constructing α_{IIb} in the integrated model of $\alpha_{IIb}\beta_3$, excluding the water and the lipids, is shown as an orange line. The blue line represents the time-dependent changes in RMSDs in the transmembrane domain of β_3 molecule in the integrin $\alpha_{IIb}\beta_3$. The time-dependent changes in RMSDs in the extracellular and the intracellular domains are shown in green and gray lines, respectively.

model to understand the logical link between the conformational changes in the intracellular domain in integrin $\alpha_{IIb}\beta_3$ induced by increased intracellular calcium ion concentration upon the activation of platelets on the conformational changes in its extracellular domain.³⁷

Molecular dynamic simulation is not a novel technic.³⁸ But, recent advances in high-performance computers enabled clarification of the specific biological functions by large-scale and long-time simulation calculation³⁹ such as water transportation by dynamic structural changes in specific proteins.^{40,41} For the platelet membrane protein, the structural fluctuation and biological functions of platelet GPIIb α binding with the A1 domain of VWF were extensively investigated.^{20,21,42} Unlike the integrated $\alpha_{IIb}\beta_3$ model, RMSD of GPIIb α binding with VWF converged to approximately 2 Å and stabilized after several hundred nanoseconds of calculation. As compared to GPIIb α , the structure of integrin $\alpha_{IIb}\beta_3$ was apparently unstable as shown by the attached movies. Our MD calculation results are in agreement with the previous publication.¹² The substantial difference in the stability of the structure in commonly present platelet membrane GPIIb α and GPIIb/IIIa of integrin $\alpha_{IIb}\beta_3$ is suggested.

Platelet activation initiated by various receptor stimulations rapidly increases the intracellular calcium ion concentration ($[Ca^{2+}]_i$). The activation-dependent changes in the structure of the extracellular domain of the integrin $\alpha_{IIb}\beta_3$ occur subsequently to this. It is of note that active conformation of the extracellular domain of integrin $\alpha_{IIb}\beta_3$ rapidly reversed to the inactive state without continuous stimulation of the P2Y₁₂ ADP receptor that is necessary for the cyclic increase in $[Ca^{2+}]_i$.^{37,43} These experimental findings suggest that the changes in the structure of the extracellular domain of integrin $\alpha_{IIb}\beta_3$ are reversible events. Yet, the precise mechanism is still to be elucidated. Our computer simulation calculation findings that the structure of integrin $\alpha_{IIb}\beta_3$ in nature is not as stable as other membranous proteins such as GPIIb α do not contradict with these previous findings. Various intracellular proteins such as talin^{8,44,45} and kindlin¹¹ play a role in achieving and maintaining the active conformation of the extracellular domain of $\alpha_{IIb}\beta_3$. The dynamic structural regulation process should be controlled by a cyclic increase in $[Ca^{2+}]_i$. Most likely, these intracellular proteins cause structural change in intracellular domain of integrin $\alpha_{IIb}\beta_3$. Our integrated model of integrin $\alpha_{IIb}\beta_3$ started from the inactive conformation of extra-cellular domain. We have shown here the structural fluctuation of our model even within the inactive conformation of extracellular domain. These structural fluctuations may explain the redundant biological function of integrin $\alpha_{IIb}\beta_3$ including its binding capacity to bind to fibrinogen with RGD (arginine-glycine-aspartate) and NGD (asparagine-glycine-arginine) peptides.⁴⁶ In the future, we are aiming to test the hypothesis whether the conformation of extra-cellular domain becomes an active form by modifying the structure of intracellular domain mimicking platelet activation.

There are several clear limitations in our study. We have adopted the previously published crystal structure of the inactive form of integrin $\alpha_{IIb}\beta_3$ ⁴⁷ as the extracellular domain of our integrated model of integrin $\alpha_{IIb}\beta_3$. However, the crystal structure may not be identical to the functional structure of integrin $\alpha_{IIb}\beta_3$ in the human body. Moreover, the structure of

transmembrane and intracellular domain was adopted from the prediction from NMR, electron cryo-microscopy, and single particle image reconstruction.^{29,30} The precise structure of the transmembrane domain, especially α_{IIb} integrin was hard to be determined in a biochemical manner.⁴⁸ MD simulation revealed positional fluctuations of amino acids in integrin $\alpha_{IIb}\beta_3$ as shown in the attached **Supplemental movie 1** and **movie 2** (available in the online version). The results shown in the figures in this paper only reflect the snapshot of the fluctuating structure. Accordingly, we are not aiming to provide a new structural model as compared to the previously established ones.^{49,50} Our goal in this paper is to show the persisting structural fluctuation of integrin $\alpha_{IIb}\beta_3$ even after convergence of MD calculation starting from inactive extracellular conformation. The use of the lipid membrane composed only from POPC without POPS may also influence the experimental results. Biological experiments revealed that the position of POPS changed from the inside to the outside of the platelet membrane.⁵¹ However, the precise location of POPS in the membrane is still to be elucidated. While the initial structure did not contradict with previously published findings,^{48,52,53} one may argue that our model was artificially developed even though we followed the previous publication to construct the integrated model.^{29,30} To quantify the structural fluctuations of atoms constructing $\alpha_{IIb}\beta_3$, the RMSDs were calculated in our study. However, the RMSD values may be influenced by errors such as inappropriate selection of initial structures.⁵⁴ The highest value of RMSD shown in extracellular domain of α_{IIb} may reflect the largest structural difference between the initial and calculated structure in the extracellular domain of α_{IIb} . Despite these limitations, our major findings showing fluctuations even after the convergence of the integrated model is not influenced.

The Supplemental Movie 1

Time-dependent change in the structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ in frontal view excluding the water and lipid molecules. The results are expressed as the sequential snap-shot images obtained every 10 ns from the initial structure to the end of 700 ns. Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-2247-9438>.

The Supplemental Movie 2

Time-dependent change in the structure of the integrated model of integrin $\alpha_{IIb}\beta_3$ in a diagonal view excluding the water and lipid molecules. The results are expressed as the sequential snap-shot images obtained every 10 ns from the initial structure to the end of 700 ns. Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-2247-9438>.

In conclusion, an integrated model of intracellular, trans-membrane, and extracellular domain of integrin $\alpha_{IIb}\beta_3$ was developed on a computer. Molecular dynamic simulation calculation on our model suggests persisting structural fluctuation of integrin $\alpha_{IIb}\beta_3$ with inactive extracellular conformation incorporated into lipid membrane even after the convergence of MDs calculations.

What is Known on this Topic?

- The structure and functions of the extracellular domain of platelet integrin $\alpha_{IIb}\beta_3$ (platelet membrane glycoprotein: GPIIb-IIIa) change substantially upon platelet activation.
- The talins and kindlins binding to the intracellular domain of integrin β_3 are necessary to transform the extracellular domain of integrin $\alpha_{IIb}\beta_3$ into active forms.
- Molecular dynamic simulation can provide a clue to understand the biological functions of integrin $\alpha_{IIb}\beta_3$.

What Does this Paper Add?

- The integrated model of platelet integrin $\alpha_{IIb}\beta_3$ (platelet membrane glycoprotein: GPIIb-IIIa) constructed from intracellular, transmembrane, and extracellular domain suggested structural instability of integrin $\alpha_{IIb}\beta_3$ even in the inactive conformation of extracellular domain.
- Our calculation results are in agreement with previous publication but still provide hypothesis to be tested in the future.

Funding

This work was funded by the Ministry of Education, Culture, Sports, Science and Technology, Japan Society for the Promotion of Science, Kakenhi19H03661.

Conflict of Interests

The authors S.G. declare that he is the Associate Editor for Circulation by the American Heart Association. He also declares that he is the President of the Japanese Society of Biorheology, Vice President of the Japanese College of Angiology, and Vice President of the Japanese Organization of Clinical Research Evaluation and Review. He also declares that he is a member of the executive and steering committee for several clinical trials (details could be provided with CA). The authors M.N. and Shinichi G. have nothing to disclose.

Acknowledgement

This research was funded by grant-in-aid for MEXT/JSPS KAKENHI 19H03661. The authors acknowledge the funding support by the Vehicle Racing Commemorative Foundation (6236), the AMED grant number A368TS, A447TR,

Fukuda Foundation for Medical Technology, and the 16th Nakatani Grand Prix Award. The authors also acknowledge the grant support by the next-generation supercomputer Research and Development program supported by RIKEN.

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