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Zoom ブレイクアウトルーム

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# Free energy profile of tRNA dissociation from ribosome studied by coarse-grained molecular dynamics simulations

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**Keywords:** Protein synthesis, Molecular simulation, Free energy calculation

Ribosomes are large protein-RNA complexes that are responsible for protein synthesis in cells. The process of protein synthesis in ribosomes mainly consists of three steps: initiation, elongation, and termination. The elongation process is essential for protein synthesis, while the initiation and termination processes are also essential for maintaining the high quality of protein synthesis. In this study, we focused on the initiation process of translation in ribosomes. During the translation initiation phase, a pre-translational complex consisting of ribosomes and initiation-related proteins is formed to ensure the initiation of protein synthesis. These initiation-specific proteins are called initiation factors (IFs), and in the case of bacteria, there are three initiation factor proteins; IF1, IF2, and IF3. In bacteria, protein synthesis is initiated by methionine bound to an initiator tRNA. One of the roles of initiation factors is to enable the selective binding of such tRNAs. This study aims to elucidate the roles of these factors and ribosomal proteins using computational methods.

Because the pre-translation complex consists of more than 20 protein and RNA molecules, it is difficult to perform an all-atom molecular dynamics simulation. In this study, we performed molecular dynamics simulations of the protein-RNA complex using a coarse-grained model to elucidate the roles of initiation factors and ribosomal proteins in the complex. The complex consists of a small ribosomal subunit, a messenger RNA (mRNA), an initiator transfer RNA (tRNA), and initiation factors IF1, IF2, and IF3. The used structures of the complexes were obtained by cryo-electron microscopy, and these structures were treated with the Martini coarse-grained model. The pre-initiation complex is further classified into various states, and in this study, we focused on the incomplete (state *a*) and nearly complete (state *b*) states of the complex. The coarse-grained pre-translational complex using the Martini model consisted of about 17,000 particles, and the total number of particles, including solvent, was about 173,000. Molecular dynamics calculations were performed by GROMACS.

In order to understand how the presence of the initiation factors and the ribosomal proteins contribute to the stability of the initiator tRNA, coarse-grained molecular dynamics simulations using umbrella sampling were performed for each of the treated states *a* and *b*. The free energy profile of tRNA dissociation from the complex was calculated. Furthermore, by analyzing the structure of the ribosome-RNA complex, we revealed how the IFs and ribosomal proteins are related to the accurate recognition of the initiator tRNA by the ribosome. Comparing the free energy profile of the complex in the incomplete state with that in the nearly complete state (state *b*), the tRNA-bound state is stable and the activation barrier for dissociation is high in the nearly complete state. One of the reasons for these different free energy profiles is that the interactions between ribosomal proteins and tRNAs are different. The interactions between the C-terminal region of the ribosomal proteins and tRNA may stabilize the ribosome and tRNA. This interaction could be necessary for the accurate recognition of tRNA by the ribosome. The structure of ribosomal proteins and their interactions with tRNA will be discussed in the presentation.

# Dynamic residue interaction network analysis of the neuraminidase H274Y mutant conferring drug resistance in influenza virus

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**Keywords:** Residue interaction network, Molecular dynamics simulation, Drug resistance, Influenza virus neuraminidase

Influenza is a highly contagious respiratory disease caused by influenza virus, resulting in mild to severe illness and sometimes even death. Influenza virus glycoproteins, hemagglutinin (HA) and neuraminidase (NA), play essential roles in its replication process. HA mediates virus entry into the host cell by binding to the terminal sialic acid (SA) of sugar chains on the host cell surface. NA plays a role in the virus release, by cleaving the bond between SA and the sugar chain in the last stage of infection, which allows the progeny viruses to liberate to begin infecting the surrounding host cells.

Oseltamivir (OTV) is a leading NA inhibitor used in the treatment and prevention of influenza. However, the OTV resistant influenza virus strains are emerging rapidly. One of the major OTV resistant influenza virus strain exhibits His-to-Tyr mutation at residue 274 in N1 NA. However, the molecular mechanism of the reduction in H274Y mutant N1 NA binding affinity to OTV have not been fully elucidated. Hence, in this study, we theoretically investigated the changes in residue-residue and residue-ligand interactions associated with the H274Y mutation in N1 NA bound to OTV using dynamic residue interaction network (dRIN) analysis based on molecular dynamics (MD) simulation.<sup>1</sup>

dRIN analysis revealed that the OTV binding site of N1 NA and its H274Y mutation site interact via the three interface residues, S246, E276, and R292, connecting them. Due to H274Y mutation in N1 NA, the interaction between residue 274 and the three interface residues, S246, E276, and R292, significantly increased, thereby significantly decreasing the interaction between OTV and its surrounding 150-loop residues, D151, and R152. Such changes in residue interactions could reduce the binding affinity of N1 NA to OTV, resulting in OTV resistance in influenza viruses. In conclusion, using dRIN analysis, we succeeded in understanding the characteristic changes in residue interactions due to H274Y mutation in N1 NA, which can elucidate the molecular mechanism of reduction in OTV binding affinity to N1 NA. Finally, the dRIN analysis used in this study can be widely applied to various systems such as individual proteins, protein-protein complexes, and protein-ligand complexes to characterize the dynamic aspects of the interactions.

[1] Yadav, M.; Igarashi, M.; Yamamoto, N. Dynamic residue interaction network analysis of the oseltamivir binding site of N1 neuraminidase and its H274Y mutation site conferring drug resistance in influenza A virus, *PeerJ*, **2021**, e11552.

# Interaction analysis of AKR1C3-inhibitor complexes using fragment molecular orbital method and molecular dynamics simulation

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**Keywords:** AKR1C3, Fragment Molecular Orbital Method, Molecular Dynamics Simulation

AKR1C3 belongs to the aldehyde-reductase (AKRs) family and has high-homology with AKR1C1, C2, and C4 [1]. Therefore, there is concern that side effects due to cross-inhibition may occur. In AKR1C3, it is expected that a more potent and selective inhibitor can be obtained by binding the inhibitor to the SP1 region consisting of Ser118, Asn167, Phe306, Phe311, and Tyr319 [2].

In this study, we performed interaction analysis of AKR1C3 inhibitor complexes and AKR1C2 inhibitor complexes by fragment molecular orbital (FMO) method and molecular dynamics (MDs) simulations to develop inhibitors with high selectivity for AKR1C3.

The crystal structures of AKR1C3 and AKR1C2 inhibitor complex which IC<sub>50</sub> have been known in the literature [3-5] were downloaded from Protein Data Bank (PDB). Structural modifications and optimizations were performed using MOE. We also prepared the complexes that was not registered in the PDB by MOE Dock. 100 ns MD-simulations were performed using GROMACS for these structures, and a total of 10 structures were acquired in each complex, one for every 10 ns. FMO calculations were performed for the acquired structures and the inter-fragment interaction energies (IFIE) and PIEDA between each residue and inhibitor were calculated. ABINIT-MP was used as the FMO calculation, and MP2/6-31G\* was used as the calculation level.

MD-simulations indicated that the interaction between SP1 in the ligand binding pocket and the inhibitor was weak in AKR1C2, and the inhibitor tended to approach Lys84 in many cases, so the interaction between the SP1 residue and the inhibitor is important. PIEDA also suggest that the dispersion energy (DI) of Phe306, Phe311 of SP1 contribute to the selectivity.

This research was done in activities of the FMO drug design consortium (FMOOD). For FMO calculations, RIKEN R-CCS Fugaku and Oakforest-PACS at JCAHPC were used (hp210130 quota).

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# FMO-based Intermolecular Interaction Energy Analysis between SARS-CoV-2 Spike Protein and Its Drug Candidate Molecules

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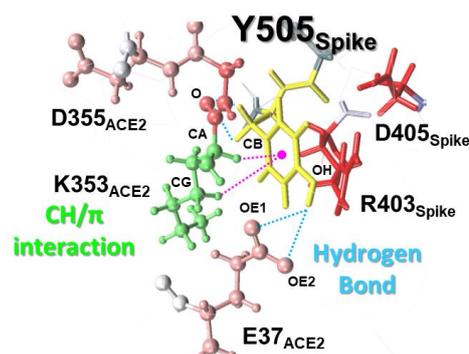
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**Keywords:** SARS-CoV-2, PPI, antibody, FMO DB

Due to the COVID-19 pandemic, researchers have attempted to identify complex structures of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoprotein (S-protein) with angiotensin-converting enzyme 2 (ACE2), a blocking antibody, or a drug candidate molecule. We have performed fragment molecular orbital (FMO) calculations on the complexes of S-protein with ACE2 and antibodies to reveal key residues for molecular recognition mechanism [1]. To quantitatively evaluate hydrogen bonds, XH/ $\pi$  interactions (X = N, O, and C), and salt bridges, we performed FMO-based epitope analysis with inter-fragment interaction energy (IFIE) and its energy decomposition analysis (PIEDA). In addition, molecular recognition between S-protein and another drug candidate molecule will be also reported by IFIE/PIEDA analysis. These FMO calculation data will be registered in FMO DB (<https://drugdesign.riken.jp/FMO DB/>).



**Fig. 1. PIEDA between S-protein and ACE2**

## Acknowledgement

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# Cryo-EM structure of the MT<sub>1</sub>-G<sub>i</sub> signaling complex

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**Keywords:** Cryo electron microscopy, G-protein coupled receptor, Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) activates melatonin receptors (MT<sub>1</sub> and MT<sub>2</sub>), which are one of the G<sub>i</sub>-coupled class A GPCRs and transduce inhibitory signaling by inhibiting the adenylyl cyclase (AC). Melatonin thus induces our sleep and modulates our circadian rhythm, and melatonin receptors have long been regarded as an important therapeutic target for an insomnia. Although melatonin itself serves as a sleep-inducing supplement, its property is not enough to use clinically, because it is rapidly cleared from our body. Therefore, a lot of melatonin analogs with prolonged release properties have been developed so far, such as ramelteon, agomelatine, tasimelteon.

Recently reported crystal structures of ligand-bound MT<sub>1</sub> and MT<sub>2</sub> elucidated the structural basis of ligand entry and recognition, but the molecular mechanism of the ligand-induced MT<sub>1</sub> structural change that would lead to G<sub>i</sub>-coupling remains unclear.

Here we report the cryo-EM structure of the MT<sub>1</sub>-G<sub>i</sub> signaling complex at 3.3 Å resolution. The structure reveals the receptor activation mechanism, in which the ligand-induced conformational changes are propagated to the G-protein coupling interface. As compared to other G<sub>i</sub>-coupled receptors, MT<sub>1</sub> exhibits a large outward movement of TM6, which is considered to be a specific feature of G<sub>s</sub>-coupled receptors. The structural comparison among the G<sub>i</sub>- and G<sub>s</sub>-complexes demonstrated the conformational diversity of the C-terminal entry of the G<sub>i</sub> protein, suggesting the loose and variable interactions at the helix end. These notions, together with our biochemical and computational analyses, highlight the different binding modes of Gα<sub>i</sub> and provide the basis for the selectivity of G-protein signaling.

# SynergyUltra opens a new era with digits of different performances and capabilities in protein crystallography; 4s-4m-40m

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**Keywords:** SynergyUltra, 4 seconds whole measurement, 4s-4m-40m

The accurate protein structures and their complex targets compounds are necessary and critical for designing and developing effective medicines and potent substances. To date, massive numbers of these structures have been determined by single-crystal X-ray diffraction (SC-XRD) analysis. Other new technologies like single-particle analysis using a Cryo-electron microscope or, more recently, utilization of three-dimensional electron diffraction (3D-ED) are emerging to overcome the difficulty of crystallization and hence to be thought the enhancement in the speed.

The geometry of the atoms defines interactions among the molecules, and some of those are highly sensitive to the directions and/or distances; thus, the accurate and precise structural information of the molecule is an indispensable firm base for successful drug design processes. In this context, SC-XRD is still the best approach if it could be performed as its necessity.

Meanwhile, it has still been widely believed that SC-XRD requires considerable time and effort to crystalize the target molecules and other efforts to collect the datasets and phasing.

This presentation introduces our next-generation instrument that opens a new era of protein SC-XRD with laboratory instruments formats. Ultra-fast and accurate measurements of protein crystals using our latest system SynergyUltra will be presented. The following numbers characterize the power of SynergyUltra.

- 4 seconds, the whole data collection for the molecular replacement phasing:  
The 4 seconds in total data collection fulfilling the 1.8Å resolution range. The obtained results were accurate enough for molecular replacement phasing employing Alpha Fold 2 deduced structure as a searching model. As a result, detailed electron densities were obtained, in which disorder of the sidechain can be modeled and refined for their population. Utilizing the SynergyFlow robotics system enables to obtain the unique crystal structures every several minutes that enable curtain bombing (carpet bombing) structure analyses of mutant proteins and/or its complexes with various candidates.
- 4 minutes, the whole data collection good for SAD phasing:  
The 4 minutes in total data collection fulfilling the 1.75Å resolution range. This measurement allows obtaining highly even and redundant data sets that are accurate enough for successful S-SAD phasing. 97% of residues were automatically modeled and refined to yield the detailed electron densities. 4 minutes measurements would be useful unless above 4 seconds MR experiments did not work well.
- 40 minutes, the whole data collection for ultra-high resolution data collection.  
The 40 minutes in total data collection fulfilling the 1.40Å resolution range. This measurement allows obtaining further precise and highly accurate electron density that shows the spherical shapes for each atom similar to those obtained in small-molecule crystallography in that atom types can be identified by their density.

# Conserved allostery in heme-copper oxidases

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**Keywords:** Ligand-based virtual screening, X-ray crystallography, Cryo-EM, molecular dynamics, Raman spectroscopy

Heme-copper oxidases (HCOs) are terminal components of the respiratory electron transport chain. Recent studies have shown the enzymes in the respiratory chain containing HCOs as a target of antibiotics.

Recently we identified novel inhibitors of mitochondrial cytochrome c oxidase (mtCcO), which is a mammalian HCOs, and determined mtCcO structure in complex with the inhibitors. These structures showed two inhibitors bound to the same site of mtCcO. The inhibitor binding pocket is distant from the ligand-binding sites, suggesting that the pocket is an allosteric site of mtCcO.

The allosteric site of mtCcO forms a narrow tunnel with four helices. Three of them are conserved in bacterial HCOs, but the other isn't. These led us to hypothesize that the allosteric inhibitory mechanism is conserved in bacterial HCOs and we could reasonably screen novel antibiotics from derivatives of mtCcO inhibitor.

To this end, we established a custom library by using the ligand-based virtual screening system, LAILAPS, with the mtCcO inhibitors as queries from commercially available compounds. The custom library has a high probability of binding to not only the allosteric site of mtCcO but also the homologous position in bacterial HCOs: *E. coli* ubiquinol oxidase (ecoUqO). As results of the enzymatic analysis with the library, we identified both improved mtCcO inhibitors and importantly ecoUqO specific inhibitors. Cryogenic electron microscopy shows the ecoUqO specific inhibitor binds to the corresponding allosteric site in ecoUqO. Finally, antibacterial activity analysis showed the inhibitor prevented the growth of UqO-dependent *E. coli* strain, whereas the effect was abrogated in UqO-independent strain, thus the growth inhibition is not a nonspecific effect.

From these results, we conclude that the allosteric site is conserved among HCOs and can lead to the development of specific antibiotics for bacterial HCOs. In this presentation, we will also mention the mechanism of conserved allostery which was unveiled by molecular dynamics analysis and resonance Raman spectroscopy.

# Large-scale membrane permeability prediction of cyclic peptides crossing a lipid bilayer based on enhanced sampling molecular dynamics simulations

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**Keywords:** Cyclic peptides, Membrane permeability, Molecular dynamics

Membrane permeability is a significant obstacle facing the development of cyclic peptide drugs. However, the membrane permeation mechanism of cyclic peptides with high membrane permeability is not yet understood. To investigate common features of permeable peptides, it is necessary to reproduce the membrane permeation process of cyclic peptides through the lipid bilayer for a large number of peptides. In this study, we first simulated the membrane permeation process of 100 six-residue cyclic peptides across the lipid bilayer based on steered molecular dynamics (MD) and replica-exchange umbrella sampling (REUS) simulations, and predicted membrane permeability using the inhomogeneous solubility-diffusion model (ISDM) and a modified version of it.[1] Furthermore, we confirmed the effectiveness of this protocol by predicting the membrane permeability of 56 eight-residue cyclic peptides with diverse chemical structures, including some confidential designs from a pharmaceutical company. As a result, a reasonable correlation between experimentally assessed and calculated membrane permeability was observed for the peptides, except for strongly hydrophobic peptides. Our analysis of the MD trajectory demonstrated that most peptides were stabilized in the boundary region between bulk water and membrane, and that for most peptides the process of crossing the center of the membrane is the main obstacle to membrane permeation. The height of this barrier is well correlated with the electrostatic interaction between the peptide and the surrounding media. This feature of the result emphasizes the importance of the smooth desolvation during penetration into the membrane.

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# Prediction of the binding mechanism for DNA methyltransferase 3A selective inhibitor using molecular simulation approach

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**Keywords:** DNA Methyltransferase, Molecular Dynamics, Drug Selectivity

DNA methyltransferase(DNMT) catalyzes the methylation reaction in the DNA cytosine, and several isoforms have been reported, such as DNMT1 and DNMT3A. Although the overall structure of DNMT1 and DNMT3A are different, the active sites of both proteins are similar to each other [1]. DNA methylation is involved in the inhibition of gene expression, and DNMT hypermethylation is related to the formation and progression of cancer cells since it causes inhibition of the tumor suppressor gene [2]. To prevent hypermethylation, many DNMT inhibitors have been developed. Especially, inhibitors which bind selectively to specific a DNMT isoform are attractive because selective drugs are expected to have few side effects [3].

Halby L *et al.* [4] have reported a selective inhibitor can bind to DNMT3A with about 100 times more activity than DNMT1. However, the molecular mechanism of selectivity is still unknown because the complex structure of DNMT3A and any selective inhibitor has not been clarified.

In this study, we predict the complex structure and the residues responsible for binding selectivity by molecular simulation. Docking simulations were first carried to generate initial structures of several candidate complexes, and MD simulations were performed to relax the structures and analyze the stability of complexes. The most stable complex structure was determined by RMSD analyze and the binding free energy using MM/PBSA method. Finally, we analyzed the energetic contribution of each residue of the protein, interactions, and the structural difference with DNMT1. Binding free energy and interaction analysis are suggested that VAL665 and ARG688 of DNMT3A form an interaction with selective inhibitor. Because these residues are replaced by MET1169 and ASN1192 in DNMT1, compounds that interact with VAL665 and ARG688 may selectively inhibit DNMT3A. In conclusion, we have predicted the complex structure of a DNMT3A-selective inhibitor and the cause of its binding selectivity.

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# Omics-based Generation of Drug Candidate Molecules with Desired Phenotypes by Machine Learning

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**Keywords:** Structure generator, Deep generative model, Machine learning, Omics data

The efficient identification of small molecules with desired phenotypes such as bioactivities is a challenging task in the drug-discovery process. In recent years, machine learning techniques have been used for *de novo* drug design. In particular, molecular generative models are able to learn the characteristics of specific real-world data and generate the chemical structures of new molecules with similar properties<sup>[1]</sup>. However, the biological phenomena have not been taken into account in molecular generation.

In this study, we present a novel machine learning method for *de novo* drug design using omics data such as gene expression profiles. We investigated the correlation between chemically induced gene expression profiles (reflecting cellular responses to compound treatment) and target perturbation gene expression profiles (reflecting cellular responses to gene knock-down or gene over-expression of target proteins) in terms of interactions between compounds and target proteins. Then, we proposed novel machine learning methods to generate the chemical structures of new molecules with desired gene expression profiles in the framework of variational auto-encoder (VAE)<sup>[2]</sup> using a new chemical representation, which does not depend on the SMILES strings. The novelty of the proposed method comes from the bridge between the chemical space and the biological space, the high chemical validity of the newly generated structures, the generation of drug candidate compounds from omics data with high accuracy, and the applicability to target proteins without information on ligands. We confirmed that the molecules that were newly generated using our structure generator were valid from the viewpoint of medicinal chemistry, comparing the structures between the newly generated molecules and known ligands. It was observed that the newly generated molecules were more similar to known ligands than those generated by the previous method with similar goals<sup>[3]</sup>. Our proposed method for omics-based molecular generation is expected to be useful for *de novo* drug design in practice.

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## Visualized and quantitative structural analysis of peptidomimetics for PPI drug discovery

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**Keywords:** Peptidomimetics, Conformational analysis, PepMetrics<sup>TM</sup> Library, PPIs

Protein–protein interactions (PPIs) are significant drug targets and many peptidomimetic molecules have been developed for modulating PPIs [1]. Nevertheless, no analytical method has been established to evaluate how precisely these molecules can mimic target peptide structures.

We developed new methods which enable visual, comprehensive, and quantitative analysis of three-dimensional structures of peptidomimetics: peptide conformation distribution (PCD) plot and peptidomimetic analysis (PMA) map [2]. These methods are based on the conformational analysis of multiple side-chain C<sub>α</sub>-C<sub>β</sub> bonds of a peptide fragment and their corresponding bonds (pseudo-C<sub>α</sub>-C<sub>β</sub> bonds) in a peptidomimetic molecule, instead of the Ramachandran plot [3] using φ and ψ angles of a single amino acid. PCD-plot is an alignment-free method and visually clarifies conformation distribution of peptidomimetic molecules in the chemical space and similarity with its target peptide secondary structures. PMA-map is an alignment-based method and can quantitatively evaluate similarity between various types of peptidomimetic molecules and their target peptide structures.

Results obtained from analysis using these two methods indicated that our PepMetrics<sup>TM</sup> scaffold, multi-facial α-helix mimetics, is an excellent peptidomimetics that can mimic spatial positioning of the side-chains of α-helix precisely. We have developed a peptidomimetic compound library (PepMetrics<sup>TM</sup> Library) using our PepMetrics<sup>TM</sup> scaffolds and have found PPI hit compounds for the interactions of KIX and c-Myb/MLL proteins known as targets for cancer and leukemia.

In summary, our methods and strategies are useful for development of new peptidomimetic molecules and rational PPI drug discovery.

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# Redesigning the low-molecular weight SHP2 inhibitors with Computational Chemistry.

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**Keywords:** *de novo* drug design, SH2-domain containing protein tyrosine phosphatase 2, in silico FBDD, in silico screening, molecular dynamics (MD) simulation, binding free energy calculation

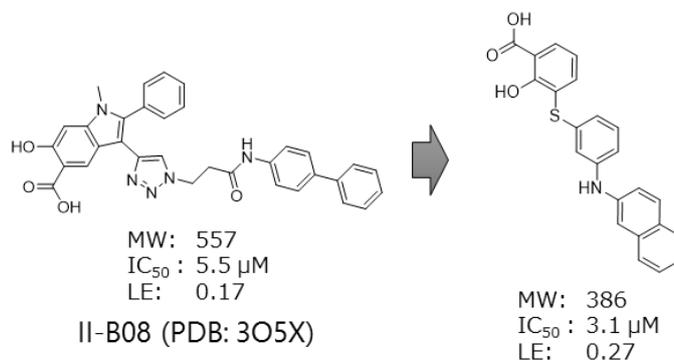
In recent years, thanks to the epoch-making improvement of structural analysis technology supported AI, various protein-ligand complexes have been revealed and have been available for structure-based drug design. In this study, we obtained novel active compounds based on the co-crystallized X-ray structures of anti-cancer target protein SHP2 which is related to the immune checkpoint pathway associated with PD-1 [1].

Method is as follows:

- Analyze the SHP2 protein-ligand interactions of PDB structures [2-4] and design novel compounds with the same interactions using OPMF<sup>TM</sup> [5], our in silico FBDD technology.
- Check the synthetic accessibility and the charged state of the experiment and calculation conditions.
- Evaluate and select the compounds.
  - The binding stabilities calculated by MD simulation
  - The differences of the conformation energies between the complex form and free form calculated with molecular mechanics (MM) and quantum mechanics (QM)
  - The binding free energy calculated by MAPLECAFEE<sup>TM</sup> V2
- Synthesize the compounds and their analogues and evaluate SHP2 inhibitory activities.

We synthesized 10 of 18 selected compounds and 3 of 10 compounds showed  $IC_{50} < \text{approximately } 3 \mu\text{M}$ . The molecular weight, activity value and ligand efficiency (LE) of the three compounds were improved than the values of the PDB structure which we used for the drug design.

These results indicate that combination of the crystal structure of existing active compounds and our design technology could accelerate the development of novel low-molecular drugs.



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# Interpretable Deep Learning Using Multimodal Graph Convolutional Network for Predicting Compound-Protein Interactions

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**Keywords:** in silico Screening, Compound-Protein Interaction, Graph Convolutional Network

Understanding compound-protein interactions (CPI) is important for identifying hit compounds in the early stages of drug discovery. Recent advances in deep learning technology have enabled rapid and accurate calculations[1], but it remains difficult to interpret the physicochemical and biological aspects of the prediction results. For example, to visualize the prediction results, mapping the weight of Attention mechanism to the 3D structure of the protein or using Grad-CAM has been attempted[2,3]. However, this is still not enough to evaluate the results objectively, and no discussion has been obtained to validate the model. In our study, we propose a multimodal and interpretable CPI prediction model by adapting Graph Convolutional Networks (GCN) for small molecules and Convolutional Neural Networks (CNN) for proteins, and by using Integrated Gradients (IG) to visualize the contribution of each atom in small molecules and each residue in proteins to the prediction results. As a result of visualization with our model, it is suggested that the prediction results are obtained by capturing the features of chemical structures of small molecules and protein sequences. This indicates that when screening hit compounds using Deep Learning-based CPI prediction models, biological reliability of the prediction results needs to be carefully considered.

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# Drug Target Gene-based Analyses of Drug Repositionability in Rare and Intractable Diseases

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**Keywords:** Drug repositioning, Clinical trials, Inter-disease analyses, Drug target genes

Drug development for rare and intractable diseases has been challenging for decades due to the low prevalence and insufficient information on these diseases. Drug repositioning is increasingly being used as a promising option in drug development. We aimed to analyze the trend of drug repositioning and inter-disease drug repositionability among rare and intractable diseases. We created a list of rare and intractable diseases based on the designated diseases in Japan. Drug information extracted from clinical trial data were integrated with information of drug target genes, which represent the mechanism of drug action. We obtained 753 drugs and 551 drug target genes from 8,307 clinical trials for 189 diseases or disease groups. Trend analysis of drug sharing between a disease pair revealed that 1,676 drug repositioning events occurred in 4,401 disease pairs. A score, Rgene, was invented to investigate the proportion of drug target genes shared between a disease pair. Annual changes of Rgene corresponded to the trend of drug repositioning and predicted drug repositioning events occurring within a year or two. Drug target gene-based analyses well visualized the drug repositioning landscape. This approach facilitates drug development for rare and intractable diseases.

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