Understanding RNA sequence specificity of inhibitors of translation initiation factor by dynamical FMO analysis

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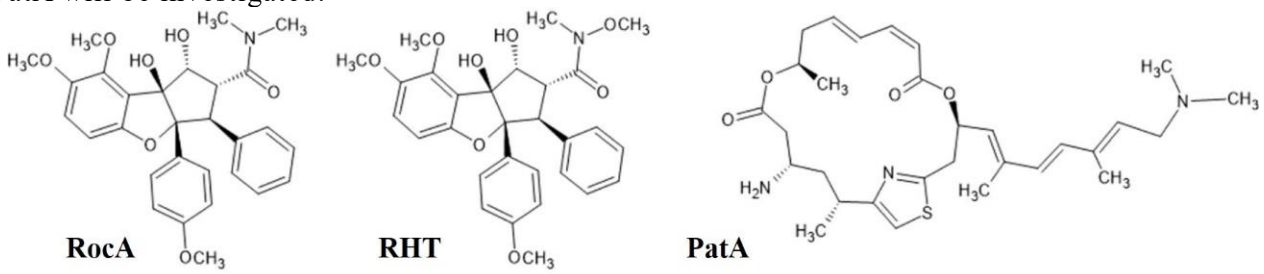
Keywords: eIF4A, RNA, Fragment molecular orbital method, Molecular Dynamics, Rocaglamide A, Pateamine A, RHT

Rocaglamide A (RocA) is a natural compound found in *Aglaia odorata*, which has potent anticancer activity and acts as a translation inhibitor targeting to translation initiation factor eIF4A. This compound is known to function in a unique mechanism; clamping eIF4A onto purine sequence specificity [1] for steric hindrance for scanning ribosomes. Our group recently identified that another eIF4A-targeting small molecule Pateamine A (PatA), which was isolated from a sponge, works as a translation inhibitor in a similar manner, but with a different RNA specificity; GNG motifs. However, the detailed molecular mechanism for such base selectivity provided by the compounds remains unclear. To tackle this issue, here we employed dynamical fragment molecular orbital (FMO) calculations combining classical molecular dynamics (MD) and FMO methods.

For RocA, four structures were created, each with one of the G6, A7, G8, and A9 bases around ligand mutated to U in addition to the wild-type ^{6}GAGA^{9} RNA. In addition, we analyzed structure with the RocA analog RHT. For PatA, we created ^{6}AGAG^{9}, polyA, and polyG sequences by mutating the ^{6}GAGA^{9} sequence around ligand in addition to the wild type. For all model structures, MD simulations were performed for 50 ns each. From the MD results, we extracted 10 structures every 3 ns, and performed FMO calculations at the MP2/6-31G* level. The MD and FMO calculations were performed on the supercomputer Fugaku (hp220143) and TSUBAME 3.0 (AMED-BINDS, JP21am0101113).

In the RocA complex, π/π interaction with U7 was lost in the A7U mutant and the hydrogen bond and π/π interaction with U8 were lost in the G8U mutant. These results suggest that the interaction with these two bases is important for purine sequence-specific recognition of RocA. RHT showed no significant change in major interactions compared to RocA. PatA showed loss of hydrogen bonding at G8 and weakened π/π interactions at A7 and G8 compared to RocA. The mutant form of PatA will be investigated.



[1] Iwasaki S. *et al.,* The Translation Inhibitor Rocaglamide Targets a Bimolecular Cavity between eIF4A and Polypurine RNA, *Mol Cell. 2019;73(4):738-748.e9*

Development of protein-ligand binding affinity evaluation method by combining classical MD and FMO calculations

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Keywords: Protein-ligand interaction, Fragment molecular orbital method (FMO), Molecular dynamic simulation

Molecular simulations to evaluate protein-ligand interactions are useful for identifying drug candidates from a huge number of compounds. In SBDD-based computational drug discovery, protein-ligand interactions are usually evaluated using classical force field-based methods. On the other hand, quantum mechanical calculations are desirable in terms of accuracy but are difficult due to the enormous computational cost. The fragment molecular orbital (FMO) method has been proposed as a unique method that can calculate the entire protein system quantum-chemically.

Interaction evaluation by the FMO method is usually performed by single-point calculations in vacuum using X-ray crystal structures. However, recent studies have suggested that including thermal fluctuations gives a more reliable interaction, which is not evaluated by single-point calculations in vacuum [1]. Therefore, we focused on the possibility of further improving the accuracy by taking into account not only thermal fluctuations but also desolvation effects and ligand deformation during binding.

In this research, evaluations were performed on three proteins with the different total numbers of residues, ER, CDK2, and SARS-CoV-2 Mpro ligand complexes.

For each complex, two 10 ns molecular dynamics (MD) simulations were performed using GROMACS. From the results of each MD simulation, 20 structures were extracted every 1 ns. The interaction energies (ΔE_{FMO}) were obtained by FMO calculations at the FMO2-MP2/6-31G* level using ABINIT-MP. Then MM-PB/SA calculations were performed to evaluate each desolvation energy (ΔG_{solv}) classically. Ligand deformation energies (ΔE_{lig}) were calculated as the energy difference between conformations of the ligand in the complex structure and in the water. Finally, we added up their effects and averaged them over 20 samples to obtain a binding affinity value ($\Delta G_{\mathrm{bind}} = \overline{\Delta E_{\mathrm{FMO}} + \Delta G_{\mathrm{solv}} + \Delta E_{\mathrm{lig}}}$). The results of FMO calculations were obtained using the supercomputer "Fugaku" (hp220143).

For ER complexes, 5 compounds were used. The enhancement of correlation between calculation values and experimental values (pIC_{50}) was confirmed by considering thermal fluctuation, ligand deformation energy, and desolvation energy. The R^2 between simple single-point FMO calculation energies and pIC_{50} was about 0.11 (Fig. 1 (a)), but it was improved to about 0.44 (Fig. 1 (b)) by taking all these effects into account. The results for other complexes will be presented in detail.

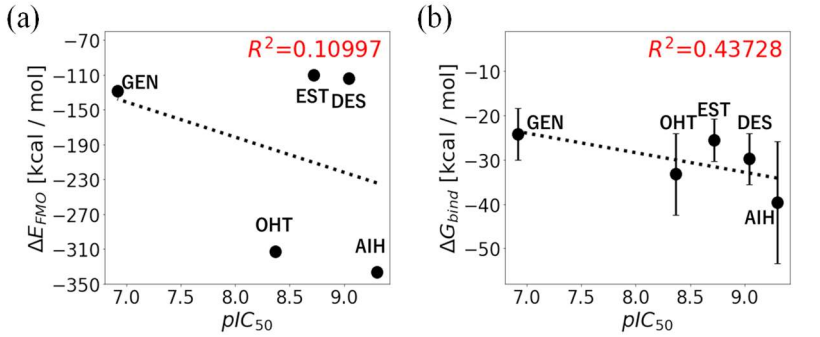

Fig. 1 pIC50 vs (a) single-point $\boldsymbol{\Delta E_{\mathrm{FMO}}}$ and (b) $\boldsymbol{\Delta G_{\mathrm{bind}}}$ based on sampling of ER-complex

[1] Hatada, R.; *et al.*, *Appl. Phys. Express*, **2021**, *14*, 027003

Structure-Activity Relationship Study of PROTACs against Hematopoietic Prostaglandin D_2 Synthase

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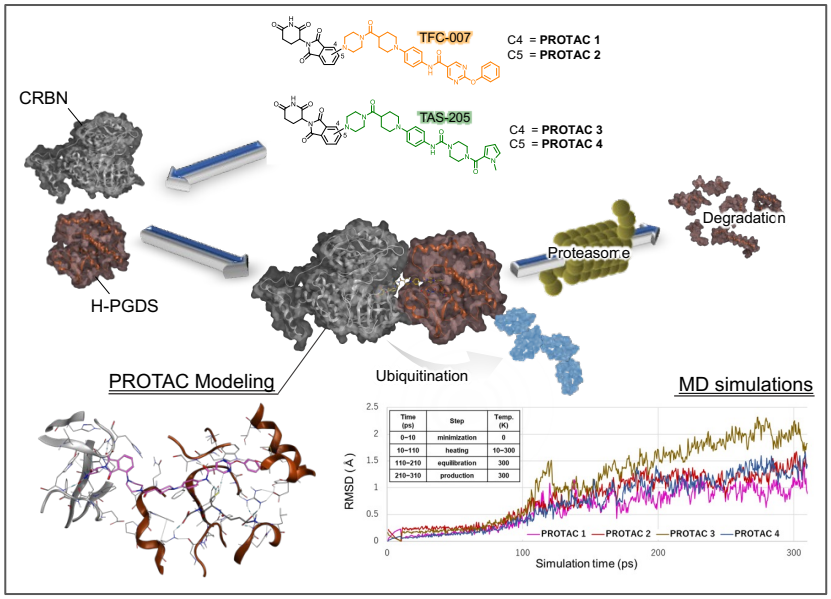
Keywords: PROTAC, H-PGDS, PROTAC Modeling, MD simulation

Degradation of hematopoietic prostaglandin D_2 synthase (H-PGDS) by proteolysis-targeting chimeras (PROTACs) is expected to be important in the treatment of allergic diseases and Duchenne's muscular dystrophy. We recently reported that **PROTAC 1**, which is composed of H-PGDS inhibitor (TFC-007) and cereblon (CRBN) E3 ligase ligand (pomalidomide), showed potent H-PGDS degradation activity[1].

In this study, we investigated the structure–activity relationships of **PROTAC 1**, focusing on the conjugation site between the H-PGDS ligand and the E3 ligand, and the effect of changing the H-PGDS ligand from TFC-007 to TAS-205. Three new **PROTACs 2-4** were evaluated for H-PGDS affinity, H-PGDS reducing activity, and inhibition of prostaglandin D_2 production. All compounds showed high H-PGDS reducing activities, but **PROTAC 3** was slightly less active than the other compounds.



To investigate these differences, the PROTAC Modeling Tools and molecular dynamics (MD) simulations were used to evaluate the stability of each PROTAC ternary complex. The results showed that the ternary complex including **PROTAC 3** had the highest RMSD value, suggesting that the reduced activity of PROTAC 3 is due to the lower stability of the H-PGDS–PROTAC–CRBN ternary complex.

[1] H. Yokoo. et al., *J. Med. Chem.* **2021**, *64*, 15868–15882.

Investigation of hydrogen bond network in the active center of catechol *O*-methyltransferase by X-ray crystallography and FMO method.

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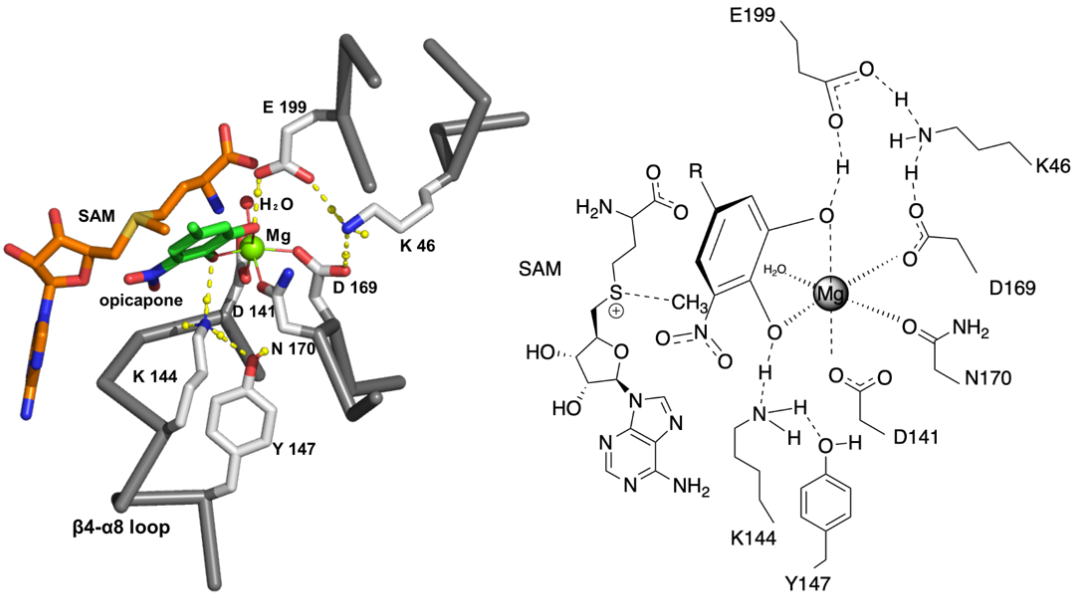
Keywords: X-ray crystallography, FMO method, hydrogen network

Catechol *O*-methyltransferase (COMT) is a target enzyme for the drug development for Parkinson's disease. COMT uses *S*-adenosylmethionine (SAM) as a substrate for L-Dopa methylation to produce *S*-Adenosyl-L-homocysteine (SAH).

We determined the structures of COMT/SAM/Mg/opicpaone and COMT/SAH/Mg/opicapone complexes by X-ray crystallography. In both structures, a complex hydrogen bond network between COMT and opicapone was observed. Determineation of precise position of hydrogen atoms involved in the network is important to design new inhibitors. However, it is difficult to discuss the hydrogen position based only on X-ray crystallography. We evaluated the energy of the hydrogen network for possible models by computing with the FMO method. Finally multiple network models were sucessfully obtained.



(Figure) Structure of active center determined by X-ray diffraction COMT/SAM/Mg/opicapone

PoSSuMds Update : Addition Of Clinical Candidates And COVID-19 Clinical Trials Information

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Keywords: PDB, Pocket similarity, Clinical candidates, COVID-19

PoSSuM is a database of comprehensive pocket similarity pairs in proteins registered in the PDB [1]. PoSSuMds (PoSSuM Drug Search) is a subset for investigating the diversity of ligands and receptors among a set of pockets that can bind to a drug compound [2]. We provide here the new PoSSuMds, updated with the latest approved drugs and candidates, resulting in an increase in the number of drug compounds that can be queried. A total of 4,142 compounds, selected from the ChEMBL [3] (version 29), were matched with PDB ligand structures, provided at Ligand Expo, and determined presence in PoSSuM with HET code. When matching ligand structures, the structure standardization protocol was refined from the previous version for precise structure matching. As a result, 599 unique compounds had at least one protein-ligand complex structure in the PDB. Of these, 580 compounds had pocket similarity data in the PoSSuM, indicating an increase of 386 compounds compared to the previous version (194 compounds). From this increase, the number of binding pockets (query pockets) has increased 8-fold, previous 2,595 pockets to current 20,866, and comparable pockets (target pockets) has increased 5-fold, previous 26,509 pockets to current 151,482, and the number of pockets pair similarities has increased 7-fold, previous 530,898 pairs to current 3,858,525. In addition, a survey using clinical candidate data from the ClinicalTrials.gov reveals that 68 out of 580 compounds in PoSSuMds were tested in clinical trials as potential treatments for COVID-19. With this update, a similar pocket search on a larger scale has become possible, increasing the effectiveness of investigations such as drug reuse, including for infectious diseases.

[1] https://possum.cbrc.pj.aist.go.jp/PoSSuM/
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Databases of similar epitopes, PoSSuMAg and putative ligand-binding pockets on AlphaFold models, PoSSuMAF

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Keywords: similarity search of binding sites, epitopes, ligand-binding pockets, AlphaFold

PoSSuM is a database of similar ligand-binding and putative pockets on proteins [1]. Based on the PoSSuM scheme for detecting putative pockets and searching similar binding sites [2-5], we have developed two new databases. One is a database of similar epitopes, antibody-binding sites on (putative) antigen proteins [6]. The current version contains the data of about 75,000 similar pairs between known and known, and over two million similar pairs between known and putative epitopes, which includes the information of known and putative epitopes on SARS-CoV-2 proteins. We found several putative epitopes that are similar to known epitopes on the antigen proteins in complex with antibody drugs. The other is a database of putative ligand-binding pockets on AlphaFold models of 20,891 human proteins in AlphaFold database [7], which are similar to the known ligand-binding sites in the PoSSuM database [1]. Our method detected about 290,000 putative pockets on the AlphaFold models, and about 56 million similar pairs between known and putative pockets [8]. It is expected that these databases will be helpful for drug discovery and development.

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[8] https://possum.cbrc.pj.aist.go.jp/PoSSuMAF/