

Doctoral Dissertation (Censored)

博士論文（要約）

Discovery of thioether macrocyclic peptide inhibitors
targeting the intrinsically disordered protein on Hepatitis B virus, preS1,
and
development of an in vitro screening system for backbone macrocyclic peptides

(B型肝炎ウイルスの天然変性タンパク質 preS1 を標的とした
チオエーテル環状ペプチド阻害剤
および
主鎖環状ペプチドの in vitro スクリーニングシステムの開発)

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Abstract

Macrocyclic peptides (MPs) have attractive pharmacological properties such as strong binding affinity against targets, remarkable *in vivo* stability, and cell membrane permeability thanks to their structural rigidity. To discover such bioactive MPs, screening against a target with a library composed of diverse MP members is an efficient approach, with methods such as one-bead-one-compound (OBOC) method, phage display, and split-intein circular ligation of peptides and proteins (SICROPs) allowing for screening of up to 10^7 , 10^8 , and 10^9 MP members, respectively. However, the diversity of MP libraries can be increased for more efficient discovery, leading to development of random non-standard peptide integrated discovery (RaPID system) in our laboratory, enabling screening of 10^{12} MP members. In this technology, a cognate mRNA connected to the corresponding MP via a C-terminal peptide enables identification of screened peptides. Discovery of bioactive MPs has been achieved by RaPID system against target proteins with ordered structures. However, there has been no reported application to intrinsically disordered proteins (IDPs), which are relevant to diverse diseases but difficult to design binders due to their flexible structures. This research shows RaPID system against such the IDP, preS1, which is a target for inhibition of HBV infection. As post-screening approaches, modification, dimerization, and grafting of an identified MP inhibitor into Fc scaffold were tried to improve inhibitory activity.

MPs are classified into three categories by their cyclization modes: Cyclization by sidechain-to-sidechain, head-to-sidechain, and head-to-tail (i.e. backbone). Among them, backbone macrocyclic peptides (BMPs) have the most rigid structure due to their closed structure by nonrotatable peptide bonds. In addition, BMP exhibits an inherent resistance to exopeptidases because of its loss of N- and C- terminus. In fact, BMPs comprise diverse natural bioactive peptides such as Cyclosporin A. However, such BMPs had not been screened by RaPID system because BMPs do not have C-terminal peptide, which is intrinsically connected to the cognate mRNA. To overcome this stumbling block, an alternative way has been developed by Dr. Takatsuji in our laboratory to connect the cognate mRNA to a sidechain of BMP via a thioether covalent linkage. This research shows an application of this method for construction of a BMP library and screening against targets of interest.

In chapter 1, the general introduction in this study is described. Advantages of MPs, their screening system to date, and IDP were introduced.

In chapter 2, RaPID system was carried out to identify thioether macrocyclic peptides (TMPs) inhibiting the infection of HBV. Modifications, dimerization, and lasso-grafting of the TMP inhibitors were performed as post-screening approaches to improve the

inhibition activity.

In chapter 3, a BMP library where BMPs were conjugated with the respective mRNAs was constructed, and screenings were performed against several targets such as cMet, Akt2, and Ankyrin repeats (AR) protein. The characteristics of the screened each BMP binder against cMet and Akt2 were compared to the TMP mutant with the same sequence and the same number of atoms in the cyclic ring. Finally, a comparison of the screening with a BMP library and a TMP library is discussed.

In chapter 4, the general conclusion of this study is described. The achievements of this research were summarized, and the perspectives were discussed.

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Abbreviations

AA: Amino Acid	IDP: Intrinsically disordered protein
Ala: Alanine	LC-MS: Liquid Chromatograph Mass Spectrometry
Alloc: Allyloxycarbonyl	MP: Macrocyclic peptide
BMP: Backbone Macrocyclic Peptide	Met: Methionine
CIAC: Chloroacetyl	Myr-B: Myrcludex-B
Cys: Cysteine	NCL: Native chemical ligation
Dap: L-2,3-Diaminopropionic acid	NHS: N-Hydroxysuccinimide
ELISA: Enzyme-Linked Immuno Sorbent Assay	NMR: Nuclear Magnetic Resonance
Gly: Glycine	NPAA: Nonproteinogenic amino acid
HBV: Hepatitis B virus	NRPs: Non-ribosomal peptides
HCl: Hydrogen chloride	NTCP: Sodium taurocholate cotransporting polypeptide
HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid	OBOC: One-bead-one-compound method
HPLC: High-Performance Liquid Chromatography	Orn: Ornithine
IAA: Iodoacetamide	PAA: Proteinogenic amino acid
PDB: Protein Data Bank	PAC: Proanthocyanidin
PEG: Polyethylene glycol	
PGA: Penicillin G amidase	
PPI: Protein-Protein Interaction	
PROTAC: Proteolysis targeting chimera	
Phacm: Phenylacetylaminomethyl	
Pro: Proline	
RiPPs: Ribosomally synthesized and post-translationally modified peptides	
SFTI-1: Sunflower trypsin inhibitor-1	
SPPS: Solid phase peptide synthesis	
SPR: Surface plasmon resonance	
TMP: Thioether macrocyclic peptide	
Tyr: Tyrosine	
UTR: Untranslated region	

Chapter 1. General introduction

1.1. Macrocyclic peptides in drug discovery

1.1.1. Peptide drugs

The number of approved peptide drugs has been increasing over the years and a total of 83 peptide drugs have been approved by 2019¹ (Figure 1-1). This is attributed to excellent properties of peptides for therapeutic agents. For example, a peptide can bind against an activity pocket or relatively flat surface of a target protein with high binding affinity and specificity comparable to an antibody². In addition, it can be synthesized at low cost as well as a small drug, which leads to easy optimization of a peptide according to a target.

However, normal linear peptides especially composed of proteinogenic amino acids (PAAs) have challenges before becoming effective therapeutic agents. For example, such linear peptides are decomposed by proteolysis in vivo because proteases can easily recognize and cleave them. In addition, they have poor cell permeability caused by their larger size and aquaphobic property due to multiple hydrogen bonding donors/acceptors in the peptide backbones.

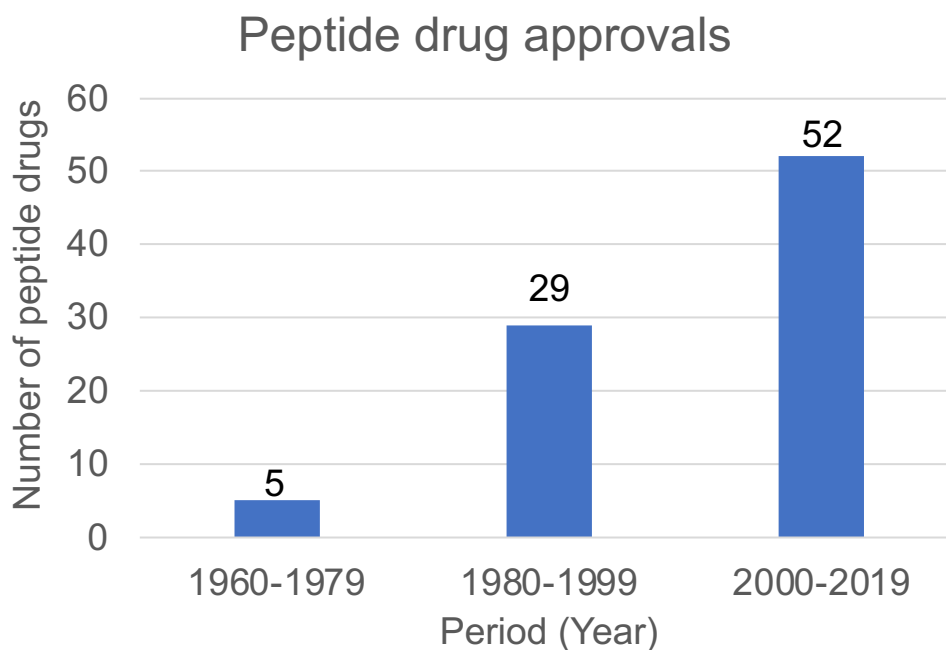


Figure 1-1. The number of approved peptide drugs. The top of the graph shows the number of approved drugs for each 20-year period.

1.1.2. Macrocyclic peptides (MPs)

Macrocyclization can overcome the challenges of easy decomposition *in vivo* and poor cell membrane permeability by imparting rigidity to the linear peptides. The rigidity prevents the recognition by protease, and thus the stability *in vivo* increases³. Besides, the rigidity contributes to a closed conformation, wherein hydrophobic regions are exposed to the surface, but hydrophilic regions are hidden inside of the cyclic structure, leading to the increase in their cell-membrane permeability⁴. Furthermore, the rigidity decreases the entropy on binding mode against a target, and thus binding affinity improves.

Macrocyclic peptides (MPs) are classified into three categories by cyclization modes: macrocycles closed by head-to-tail (backbone), sidechain-to-sidechain, and sidechain-to-backbone (Figure 1-2). Many peptides belonging to the first two of the three are often clinically used. For instance, Cyclosporin A and Pasireotide are categorized for the backbone macrocycle and Lanreotide and Vasopressin for the sidechain-to-sidechain macrocycle⁵. In contrast, fewer peptides belonging to the side-to-backbone cycle, such as Bremelanotide, are reported⁵.

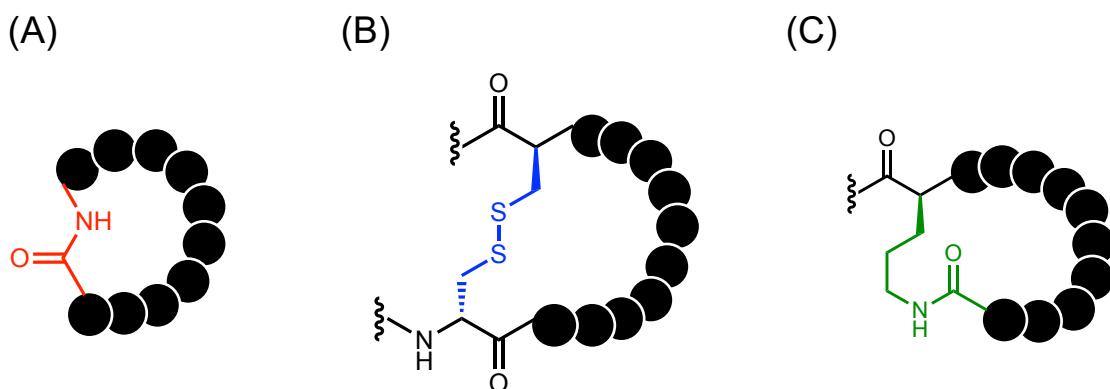


Figure 1-2. MPs classified by cyclization mode. (A) Backbone cyclization, (B) sidechain-to-sidechain cyclization (e.g. disulfide cyclization), and (C) side-to-backbone cyclization (e.g. sidechain of Orn and C-terminus cyclization)

1.2. Screening technologies of MPs

Bioactive MPs have been isolated from nature, but their discovery is often fortuitous, and the isolated MPs do not always exhibit binding to desired targets^{6,7}. Furthermore, bioactive MPs have also been engineered from a small molecule binder and a protein-protein interaction (PPI) motif, yet, these designs necessitate the availability of template molecules and their previously known interactions with specific targets^{8,9}. Thus, these designs are limited in application to de novo targets of interest. To discover bioactive MPs against a specific target, screening technologies with an MP library are a viable strategy. An optimal screening methodology should encompass a high diversity and a broad chemical space of the MP library and be adaptable to any target. In this section, recent advancements in MP screening systems that address these considerations will be discussed.

1.2.1. In silico screening of MPs

Novel MP candidates inhibiting PPI interaction can be discovered by in silico screening, such as cyclic Peptide Matching (cPEPmatch) approach¹⁰. The cPEPmatch characterizes distance of atoms in a PPI interface of a template protein and then searches for MPs satisfying the characteristics from a small MP database (~ 30), which is sourced from the Protein Data Bank (PDB) and thus comprises a variety of MP types. This software demonstrated its utility by identifying a BMP in agreement with the structure of Sunflower trypsin inhibitor-1 (SFTI-1) from the PPI of bovine trypsin inhibitor (BPTI) and trypsin protease, even though SFTI-1 was not present in the library. In addition, it was applied for 154 PPI interactions, resulting in successful identification of MPs with a 71% probability. The chemical space of this library is large because an MP in the PDB can be included in the library, but the reported diversity is quite small. In addition, elucidation of a target structure is necessary.

1.2.2. In vitro screening of MPs

Library sizes of in vitro screening techniques are much larger than the library generated in silico. However, the techniques require identification of screened peptide sequences. The identification of linear peptides can be accomplished by edman degradation and MSMS analysis, which produce and compile information on peptide fragments. However, they are hardly applicable to MPs because of difficulty of producing peptide fragments of the MP. To identify the MP sequence, each MP should coexist with the corresponding tag, such as a linear peptide or cognate DNA. In this section, recent in vitro screening technologies of MP, such as the one-bead-one-compound (OBOC) method and phage display, will be introduced.

OBOC method utilizes a linear peptide tag with the same sequences of the MP. The MP sequences are identified by the deconvolution of the linear peptides using partial edman degradation/mass spectrometry¹¹. The linear peptide with the MP sequence is connected to an inner surface of a microbead, while the MP is connected to an outer surface of the bead^{12,13}. The MP library is constructed by split-and-pool method and the following backbone cyclization. While this library has the potential to theoretically comprise all types of homogeneous MPs, many BMP libraries appear to be the primary focus^{12,14–17}. This method is as follows (Figure 1-3)¹²: First of all, beads immobilized with a linker peptide are soaked in water, and then organic solvent, which makes a bilayer with a water layer inside and an organic phase outside. The linker peptide in the outer layer is derivatized by N^α-Fmoc-Glu(δ-NHS)-O-CH₂CH=CH₂ to carry out backbone cyclization after constructing BMP precursors. Construction of BMP is performed by the split-and-pool method (Figure 1-3A). The beads coupled with an AA are split into X fractions, followed by coupling X number of AAs onto each fraction separately. The beads are combined and then split again into X fractions. This process of coupling, combining, and splitting is repeated for n cycles to produce a library containing n number of length and Xⁿ diversity of BMP precursors. Finally, backbone cyclization is carried out to produce a BMP library in the outer layer where linear peptides in the inner layer are far, so the following screening does not interfere (Figure 1-3B, the case N = 7). Screening with the BMP library against a target of interest has been carried out by fluorescence-based assay^{12,14,15} or the colored beads sorting¹², etc to discover the bioactive BMPs such as human prolactin receptor (hPRLr) antagonist¹², calcineurin (Cn)/nuclear factor of activated T cell (NFAT) interaction inhibitor¹⁸, HIV-1 capsid (CA)-human lysyl-tRNA synthetase (hLysRS) interaction inhibitor¹⁴, and Ras-effector interaction inhibitor^{15,19}. A beneficial point of the OBOC is to deal with a BMP library containing

nonproteinogenic amino acids (NPAAs), which often improves the in vivo stability of peptides. In addition, chemical space of the library may expand as the number of used NPAAs increases. However, diversity of the library is limited to less than 10^7 .

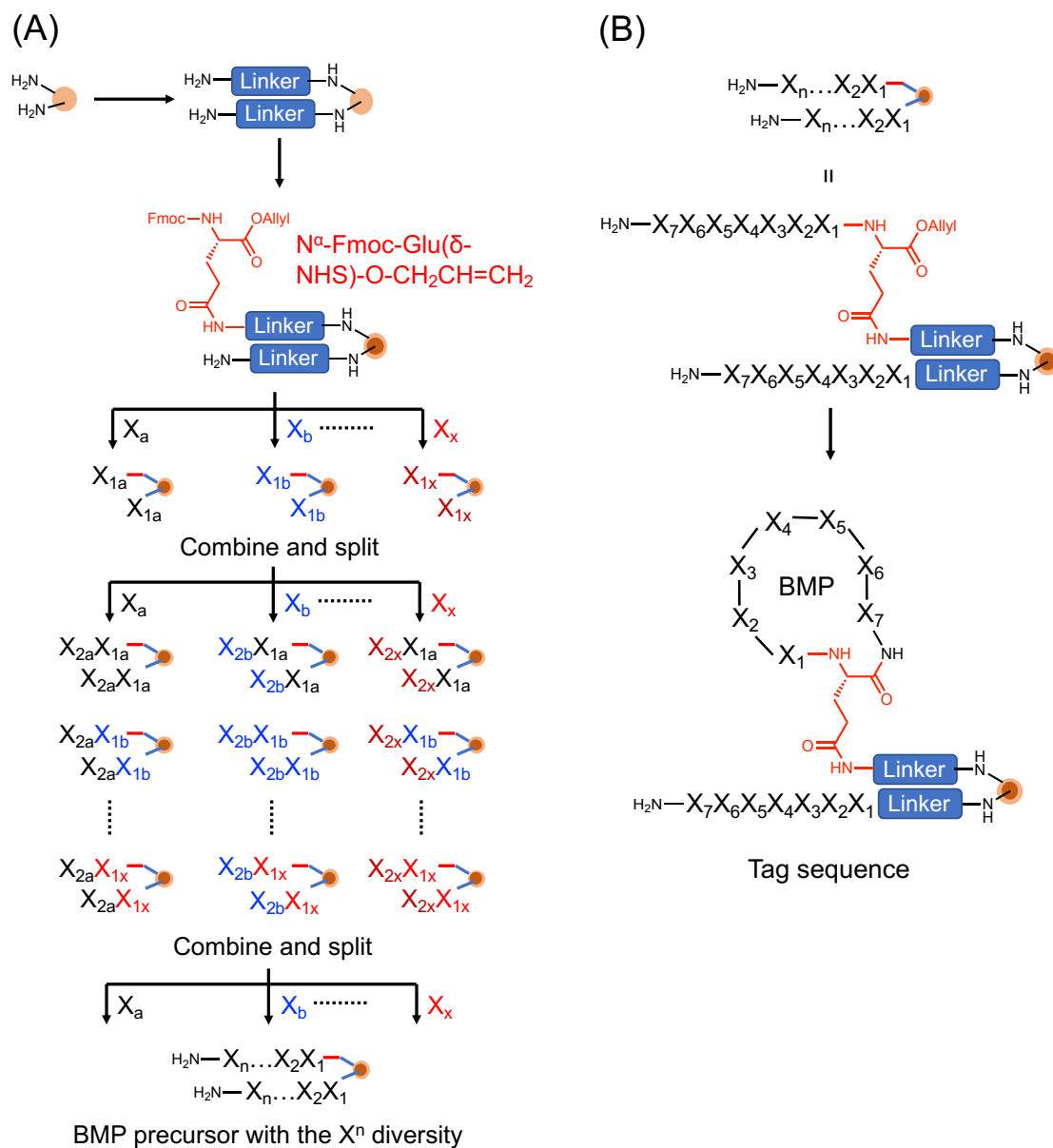


Figure 1-3. OBOC method. (A) Construction of BMP precursors by the split-and-pool method. Before the split-and-pool, the linker peptide in the outer layer is derivatized by N^α -Fmoc-Glu(δ -NHS)-O-CH₂CH=CH₂ (shown in red). (B) Construction of a BMP library (e.g. N=7). Backbone cyclization of the linear peptide in the outer layer is carried out. The tag sequence will be used for the identification of BMP.

Phage display utilizes a cognate plasmid DNA on an inside of a bacteriophage for identification of the corresponding peptide on the exterior of the phage. To produce an MP, a genetic code reprogramming, amber suppression, can incorporate an NPAA bearing an electrophile into a peptide, and macrocyclization is carried out between the electrophile and a downstream Cys (Figure 1-4)²⁰. In addition, crosslink reagent is also used for reaction with two thiols of Cys to produce a macrocyclic peptide^{21,22}. Therefore, the library comprises a homogeneous sidechain-to-backbone or sidechain-to-sidechain MP library. A peptide library is constructed based on an inside DNA library and then used for affinity-based screening, discovering a carbonic anhydrase binder with K_D value of 40.8 ± 4.9 nM, a Kelch-like ECH-associated protein 1 (Keap1) binder with K_D value of 110 nM, and a sonic hedgehog binder with K_D value of 550 nM, etc^{20,21}. However, only few NPAAAs can be incorporated into the peptide. In addition, the library size is limited to 10^8 because the transformation efficiency is low.

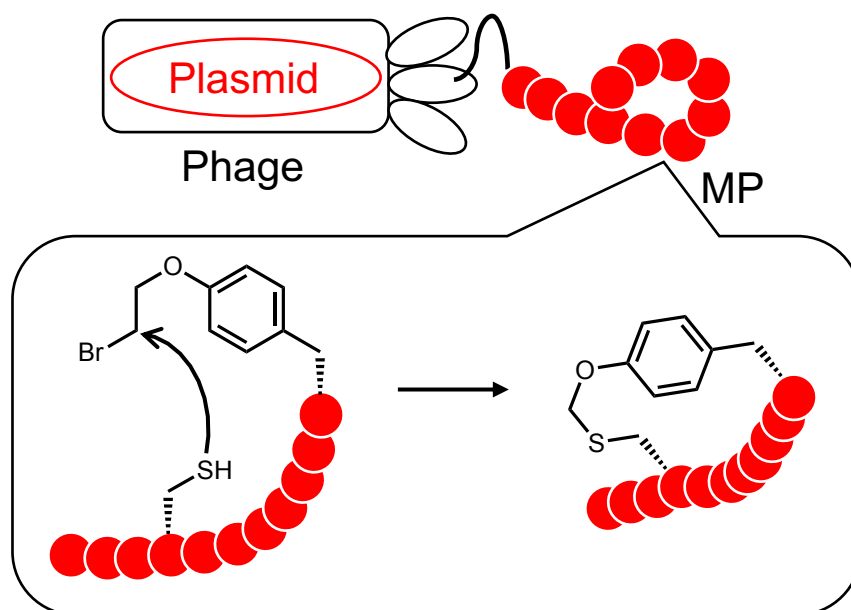


Figure 1-4. Phage display for MPs. A peptide initiated with NPAA bearing a nucleophile is expressed on the exterior of the phage. The nucleophile is reacted with the thiol of Cys to produce an MP.

1.2.3. In vivo screening of MPs

Split-intein circular ligation of peptides and proteins (SICROPPTS) utilizes a *in vivo* natural process of protein intein splicing to generate a BMP after protein expression from plasmid DNA. Thus, the BMP is identified by the plasmid DNA. A homogeneous BMP library is produced by placing a randomized core between a C-terminal and N-terminal intein domain, followed by an intein splicing process, which allows for intramolecular rearrangements such as *N*-to-*S* acyl shift to produce a thioester and asparagine succinimide formation to produce an N-terminal Cys, providing opportunities for backbone cyclization via trans-thioesterification between the Cys and the thioester and *S*-to-*N* acyl shift (Figure 1-5). The BMP library has been screened using a phenotype screening, reverse two-hybrid screening, which discovered the inhibitor of PPI such as the interaction between *Bacillus anthracis* protective antigen and the human CMG2 receptor²³, the interaction between the HIV Gag protein and human TSG101 protein²⁴, and homo dimerization of BCL6²⁵. A beneficial point of SICROPPTS is the compatibility of the phenotype screening, improving efficiency of finding bioactive hits. However, a BMP library containing many NPAAAs cannot be used, and diversity of the library is limited to 10^9 because of low transformation efficiency as is the case with phage display.

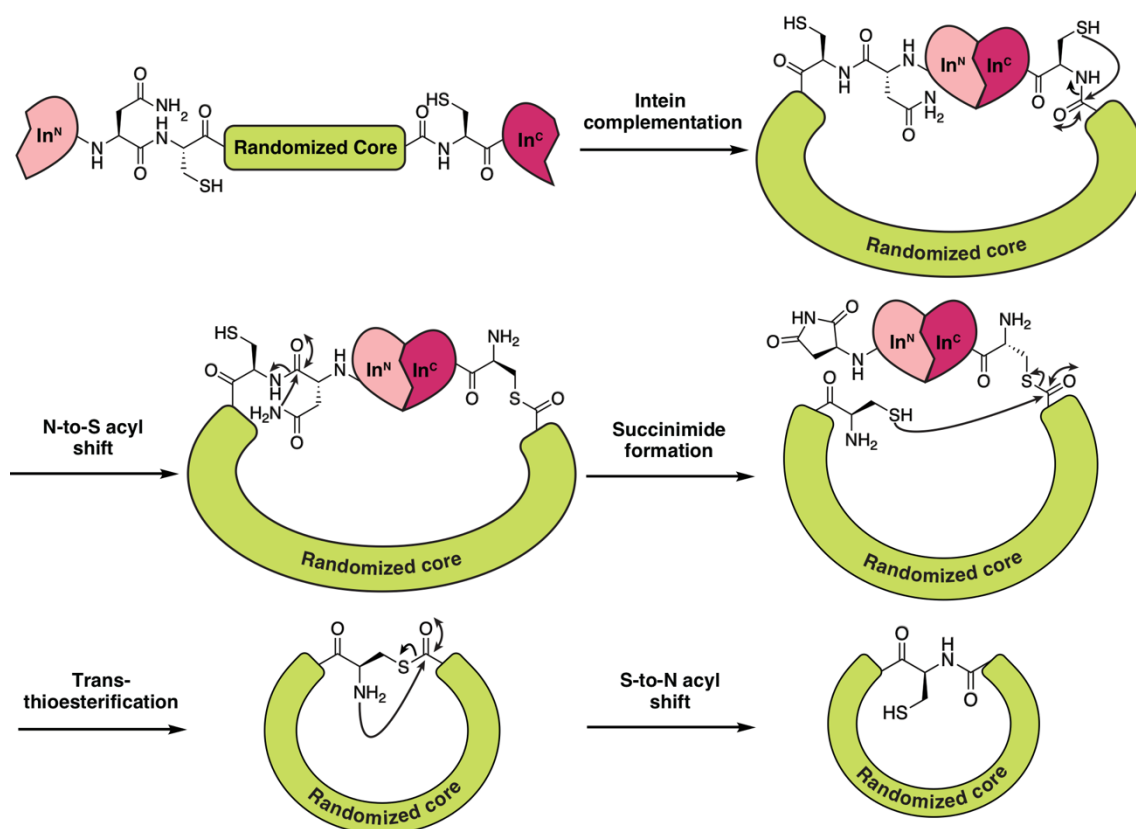


Figure 1-5. SICROPPS. Figure from our review (Shinbara. K. *et. al.*, *Front. Chem.* 8:447). After protein expression, split inteins are interacted with each other. It provokes *N*-to-*S* acylshift to produce a thioester. On the other hand, the side chain of Asn attacked its carbonyl residue to produce a succinimide and a terminal Cys, the thiol of which in turn attacks the thioester. After *S*-to-*N* acylshift, the backbone cyclization is completed.

1.2.4. Random non-standard peptide integrated discovery (RaPID) system

mRNA display screens peptides from a PAA library, and disulfide formation and crosslink reagents enable production of a homogeneous side-to-side chain MP library^{26,27}. mRNA display is also compatible with a genetic code reprogramming system such as amber suppression and flexible in vitro translation (FIT) system to produce peptides containing multiple NAAs and a sidechain-to-backbone MPs. The integrated technology is called random non-standard peptide integrated discovery (RaPID system). A C-terminus of an MP is connected to 3' terminus of the respective mRNA, which enables mRNA sequencing to identify the MP. Construction of a sidechain-to-backbone MP library and following screening are as follows (Figure 1-6): An mRNA library transcribed from the corresponding DNA library is translated into a peptide library, where respective mRNAs are connected via puromycin to the respective peptides. An NPAA bearing electrophile such as an N-chloroacetyl residue is usually incorporated as an initiator by means of FIT system. A random region is placed between the initiator NPAA and Cys, by which the attack of the thiol of Cys to the chloroacetyl residue produces a thioether macrocyclic peptide (TMP) library. Then, reverse transcription is performed not to obtain an aptamer. Next, the TMP-mRNA/cDNA complex library is mixed with a target of interest to perform affinity-based screening. Finally, the cDNAs coupled with the peptides binding to the target are collected and transcribed into an mRNA library, which is used for the next round of screening. This screening cycle is repeated several times until peptide sequences converge. An advantage of RaPID system is the compatibility with incorporation of various NPAAs into a peptide chain, such as N-methyl amino acids (N-methyl AAs), D-AAs, and cyclic β -AAs, by using the FIT system and discovered the bioactive TMPs against targets such as ubiquitin ligase²⁸, epidermal growth factor receptor (EGFR)²⁹, hFXIIa and interferon-gamma (IFN- γ) receptor 1 (IFNGR1)³⁰, respectively. In addition, diversity of the MP library, less than 10^{12} , is superior to that of other screening systems due to capacity of in vitro translation system.

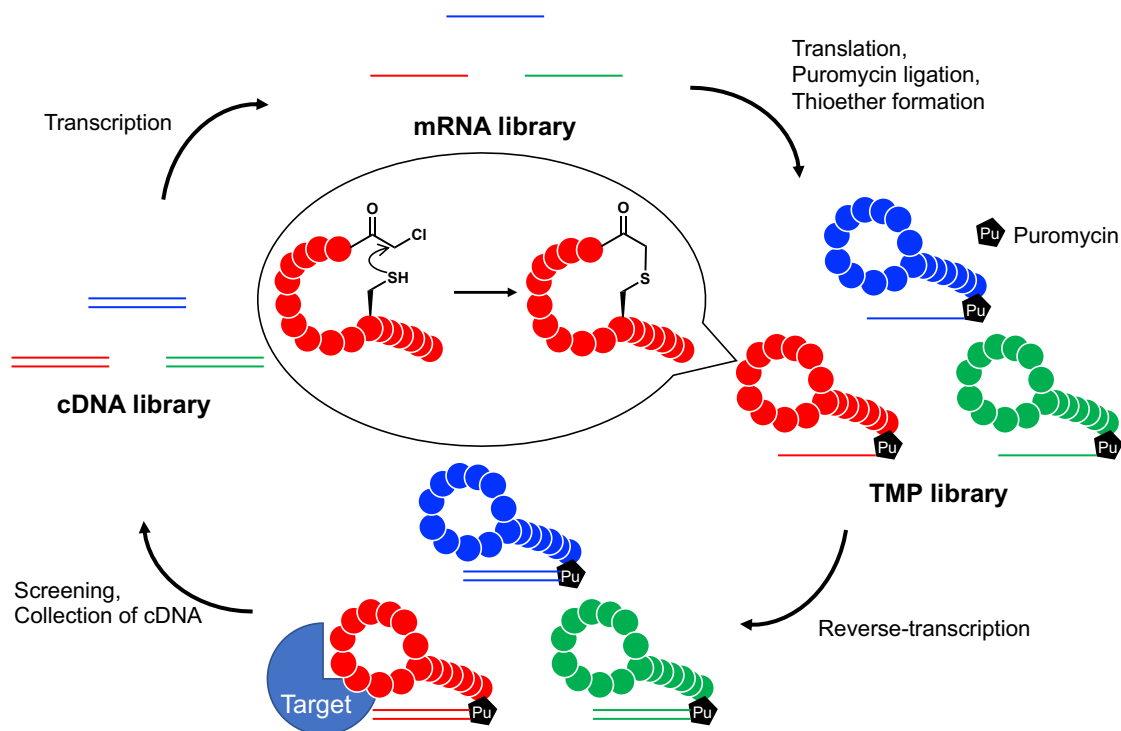


Figure 1-6. RaPID system with a TMP library. A TMP library is constructed from the corresponding mRNA library using incorporation of NPAA bearing a nucleophile such as chloroacetyl residue and thioether cyclization between the chloroacetyl residue and a thiol of Cys. Screening against a target of interest is performed and cDNAs connected to TMP binders are amplified. The cDNA library is transcribed to produce an mRNA library again, which is used for the next round of screening.

1.3. Intrinsically disordered proteins (IDPs)

A target protein of screening generally has an orderly structure, and it exhibits activity by binding to a structured partner molecule. However, in recent years, proteins and regions with little or only partial structure under physiological conditions have been discovered. They are referred to as intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs), respectively³¹. Their flexible structures attribute strong electrostatic repulsions due to many hydrophilic residues and lack of compaction because of a small number of hydrophobic residue³². However, IDPs/IDRs can bind to many partner molecules with high specificity and low binding affinity through induced folding by the partners. This binding can sometimes involve the same region of the IDP for recognition of different partner molecules and sometimes involve different regions of the IDP for the same site³³. Due to the high flexibility of this binding ability, one molecule can bind to various molecules, so it becomes chaperon proteins or hubs of signal pathways such as p53³¹. Also, 33% of eukaryotic proteins compose IDPs/IDRs³⁴.

However, they are also related to various diseases related to signaling, cancer, and neurodegenerative, etc. this state is called "disorder in disorders"³⁵. Well-studied examples of IDPs related to human disorders are p53, c-Myc, α -synuclein, etc. In many cases, IDPs are overexpressed, and their concentration in the body increases, which leads to side effects³⁶. In addition, virus proteins are often IDPs and have IDRs, which imparts themselves the capacity for quick adaption against changing surroundings³⁷. Therefore, development of drugs targeting these IDPs is desired. However, due to the structural characteristics of IDPs, applications of existing evaluation assays and computer-based methods is often limited³¹. In addition, rational designs of inhibitors from structural data of X-ray crystallography and NMR are also challenging³⁸. On the other hand, discovery of an IDP inhibitor using screening systems with a small molecule library is expected. However, obtained small molecules often suffer from their low binding affinities and specificities³⁹. In addition, the number of reports about IDPs/IDRs binders was few, and most binders are small molecule⁴⁰.

Peptides have higher binding affinities and specificities against targets, and their screening systems have also been applied to IDPs. Regarding the peptide screening system described in section 1.2., phage display and SICROPPS have been applied to IDP^{24,41–43}. Note that phage display could obtain linear binders, but the system with MP libraries has not yet applied to IDP. On the other hand, cPEP match, OBOC method, and RaPID system have not yet been applied to IDP. mRNA display, the basis of RaPID system, was applied to NS1 protein of influenza A virus, whose C-terminus is IDP, but a binding site of an NS1 protein binder remained elusive^{44,45}.

1.4. Research objectives

Table 1-1 summarizes the properties of current MP screening technologies. Although the diversity of the MP library of RaPID system is the highest and various NPAAAs can be incorporated, the application to IDPs and BMP library has not yet been achieved. To break out of this status quo, in this research, RaPID system was applied to the IDP, preS1, which is a target of Hepatitis B virus infection (Chapter 2) and a BMP library (Chapter 3).

Novel TMP inhibitors against preS1 could be identified, and then a TMP inhibitor's sequence was optimized through single substitution scanning with PAAs, D-amino acid (AA), and N-methyl AA. Dimerization and lasso-grafting of the TMP into the Fc scaffold were also tried to improve the inhibition of HBV infection (Chapter 2).

Cognate mRNA is usually connected to the peptide C-terminus on RaPID system. Thus, RaPID system is not intrinsically applicable to BMPs (i.e. N-terminus and C-terminus cyclized peptides) because of their loss of the C-terminus. To solve this problem, the cognate mRNA was connected to the corresponding BMP via its sidechain. Construction of a BMP library and screenings against cMet (HGF receptor), Akt2, and an ankyrin repeat (AR) protein were carried out to demonstrate the RaPID system with a BMP library. In addition, BMP binders from this screening were compared to the TMP mutants with the same sequence and the same number of atoms in the cyclic ring to show the unique character of BMP. Finally, a comparison of RaPID system with BMP and TMP libraries was discussed (Chapter 3).

	cPEPmatch	OBOC	Phage display	SICROPSS	RaPID system (mRNA display)
Location of use	In silico	In vitro	In vitro	In vivo	In vitro
Diversity	~30	~10 ⁷	~10 ⁸	~10 ⁹	~10 ¹²
Style of a MP library	Backbone, Sidechain-to-sidechain, Sidechain-to backbone (Mixture)	Backbone, Sidechain-to-backbone (Homogeneous)	Sidechain-to-Sidechain, Sidechain-to-backbone (Homogeneous)	Backbone (Homogeneous)	Sidechain-to-Sidechain, Sidechain-to-backbone (Homogeneous) Not backbone
NPAA	can be incorporated	can be incorporated	A few NPAAs can be incorporated	A few NPAAs can be incorporated	can be incorporated
Application to IDP	No report	No report	Yes (Linear peptide)	Yes	No report

Table 1-1. Current screening technologies for discovery of MP candidates and its application to IDPs. Red letters show the problem solved in this study.

Chapter 2. Discovery of thioether macrocyclic peptide inhibitors targeting the intrinsically disordered protein on Hepatitis B virus, preS1

本章に関しては特許申請のため、非公開

Chapter 3. Development of an in vitro screening system for backbone macrocyclic peptides

本章に関しては特許申請のため、非公開

Chapter 4. General conclusion

IDPs play important roles in cellular activities and diseases. The study in chapter 2 demonstrated identification of MP inhibitors against the IDP, preS1, using RaPID system. RaPID system has strength compared to other screening systems applied for targeting IDPs. Although small compound binders screened from their library often suffer from their low binding affinity and low specificity against a target, MP binders obtained by RaPID system have strong binding affinity and specificity. Compared to the other MP screening such as SICROPPS, library diversity of RaPID system is extremely higher (SICROPPS: less than 10^9 , RaPID system: less than 10^{12}), which enables more efficient screening. In addition, RaPID system enables discovery of binders within one month. This rapid discovery also allows for application of RaPID system to mutants caused by prone viral mutation. In fact, preS1 is very prone to mutation, which has been confirmed in different liver diseases. MPs targeting the mutants will also be identified by RaPID system and contributes to treatment of the diseases. Chapter 2 also showed post-screening approaches to improve the activity of an MP binder against preS1. Substitution of AAs in an MP with other PAAs showed improvement of the inhibition activity, whereas substitution with D-AA and N-methyl AA and dimerization did not improve the inhibitory activity. These results of post-screening approaches will help to choose suitable approaches in development of IDP inhibitors. Further modification of sidechains of the AAs in the MP will improve the inhibitory activity.

The research described in chapter 3 also demonstrated a novel in vitro screening system for BMPs using RaPID system. This screening system deals with a BMP library with the largest members to date ($\sim 10^{12}$). Because BMPs have more resistance against a protease than the TMP mutants, this screening system with a BMP library would be more useful for discovering MPs with high stability against proteases than screening with a TMP library. Although BMPs with lower activity could be obtained from this BMP screening than TMPs obtained from the TMP screening due to lower diversity of a BMP library, the BMPs could bind the same site where the TMPs bind. Further screening with a focused library will enable identification of BMPs with equal to or greater activity than TMPs. In addition, disulfide formation of cysteines and incorporation of NPAAAs into BMPs will enable more RiPPs-like and NRPs-like BMP library, respectively. These contribute to discovery of more natural-like and bioactive peptide drugs.

These studies contributed enlargement of RaPID system's applicability to an IDP and expansion of chemical space to BMPs. Further utilization and modification of this system will enable discovery of peptide therapeutics against targets of interest.

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List of accomplishments

【Publications】

1. Takatsuji Ryo, **Shinbara Koki**, Katoh Takayuki, Goto Yuki, Passioura Toby, Yajima Ryo, Komatsu Yamato, Suga Hiroaki., Ribosomal synthesis of backbone-cyclic peptides compatible with in vitro display, J. Am. Chem. Soc., 141, pp 2279-2287, 2019
2. **Koki Shinbara**, Wenyu Liu, Renier Herman Pieter van Neer, Takayuki Katoh, Hiroaki Suga., Methodologies for backbone macrocyclic peptide synthesis compatible with screening technologies, Front. Chem., 8, 447, 2020
3. 西村仁孝、**新原光貴**、加藤敬行、菅裕明 ペプチドの環化手法と特殊ペプチドライブラリー構築への応用、ペプチド医薬の最前線、シーエムシー出版、第4章、2019年
4. **新原光貴** 東京大学科学技術インタープリター養成講座 2021 年 修了論文「血糖値の継続的可視化による糖尿病当事者への心理的影響とその対応」 http://science-interpreter.c.u-tokyo.ac.jp/wp-content/uploads/2021/03/2020_08_SHINBARA.pdf
5. **Koki Shinbara**, Takayuki Katoh, and Hiroaki Suga., The RaPID System Applied for Selection of Backbone Macrocyclic Peptides, Peptide Science 2022

【Presentation】

1. **新原光貴**、高辻諒、加藤敬行、菅裕明 mRNA ディスプレイに適用可能な主鎖環状ペプチド合成法の確立、日本、ケミカルバイオロジー学会第14回年会、2019年6月
2. **Shinbara Koki**, Takatsuji Ryo, Katoh Takayuki, Suga Hiroaki., Development of A Methodology for Displaying Head-to-tail Macrocyclic Peptides by Means of mRNA Display, 10th International Peptide Symposium, 2018年12月
3. **Shinbara Koki**, Takatsuji Ryo, Katoh Takayuki, Suga Hiroaki. Development of A Methodology for Displaying Head- to-tail Macrocyclic Peptides by Means of mRNA Display, 3rd ETH Zürich-UTokyo Strategic Partnership Symposium, 2019年1月
4. **新原光貴**、高辻諒、加藤敬行、菅裕明 mRNA ディスプレイを用いた主鎖環状ペプチドのディスプレイ法の開発、第51回若手ペプチド夏の勉強会 2019年8月
5. **新原光貴**、高辻諒、加藤敬行、菅裕明 Development of a novel in vitro screening system for backbone macrocyclic peptides, The 58th Japanese Peptide Symposium, 2021年10月

6. Nakajima Seiru, **Shinabra Koki**, Katoh Takayuki, Suga Hiroaki. In vitro ribosomal synthesis of Cyclosporin A, PacificChem 2021, Honolulu, Hawaii, USA, 2021, 12
7. **新原光貴**、高辻諒、加藤敬行、菅裕明 RaPID system for backbone macrocyclic peptides, The 59th Japanese Peptide Symposium, 2022 年 10 月

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