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**Ribosomal Synthesis and Selection of macrocyclic peptides for bacterial metallo-β-lactamase inhibition**

**Introduction**

The naturally generated proteins, or polypeptides, are derived from the transcription of DNA into messenger RNA codons and translation into peptide chains. However, the central dogma and thus codon table for peptide translation are shared for all known living systems and restricted within the arrangement of twenty kinds of amino acids (Figure 1).

Synthesis of non-canonical peptides - those that cannot be naturally synthesized in human body - has been a challenge, especially in the pharmaceutical field. With their increased target-binding affinity, resistance to proteases and biological membrane permeability to the peptides, macrocyclic peptides can become favorable drug candidates\(^1\), such as cyclosporine A (Figure 2). The majority of macrocyclic peptides, are synthesized by nonribosomal peptide synthases (NRPSs), and often contain noncanonical structural features, such as atypical amino acid side chains or stereochemistries, in addition to their unusual macrocyclic structures\(^2\).
Figure 1. Each mRNA codon and its correspondent amino acid

(Openstax College 2017)

Flexible in vitro translation (FIT) was introduced to enable the synthesis of macrocyclic peptides. The method utilizes the specificity of aminoacyl-tRNA synthase and reconstitute a translation system that contains flexizyme-mediated AA-tRNA synthesis. Flexizymes, ribozymes purified from E. coli ribosomes with certain recombinant cofactors and proteins, are discovered to catalyze the aminoacylation in tRNA with 3'-CCA terminus. The removal of the initiator tRNA's cognate
aminoacyl-tRNA synthase from the translation reaction led to the “vacated” methionine codon. The addition of flexizyme would then catalyze the misacylation of initiator tRNA with a non-canonical amino acid with unique side chain groups. Finally, the intramolecular reaction between side chains would generate the macrocyclic structure (Figure 3).

Figure 3. The scheme of cyclic peptide formation

After the construction of macrocyclic peptide library, RaPID (Random Nonstandard Peptide Integrated Display) was uniquely developed in Suga Lab as a screening method against target proteins. The screening starts with ligation of oligo by puromycin, an antibiotic that is a protein synthesis inhibitor by causing premature chain termination. Puromycin is structurally similar to aminoacyl-tRNA but its amine group is more stabilized by the adjacent amide group (Figure 4). Thus, the translation
would generate an incompletely hydrolyzed mRNA-peptide fusion and subsequent reverse transcription gives the cDNA-peptide fusion. The fusion molecules are treated with the target protein for selection of binders. The cognate mRNA of selected peptides could be enhanced by PCR and transcription for more rounds of RaPID selection.

![Figure 4. The structures of puromycin and aminoacyl-tRNA](image)

**Research Project**

Bacterial metallo-β-lactamases (MBL) are the carbapenemases belonging to the class B β-lactamases on a molecular level. MBL can hydrolyze rather resistant antibiotics as carbapenem and thus its inhibition would advance the medical effectiveness of certain antibiotics. Two types of the metallo-β-lactamase, IMP-1 and VIM-1, which have been detected mainly from Pseudomonas aeruginosa in Japan and in Europe, are especially important because of the broad substrate
specificity\textsuperscript{4}. In this research project, three types of MBL are set as target proteins: IMP-1, NDM-1 and VIM-2. The selected macrocyclic peptides should ideally inhibit all three at the same time.

**Results**

16 macrocyclic peptides were synthesized and pre-selected as the mRNA codon library by Dr. Passioura (Figure 5). Among them, MBL-L-02, MBL-L-06 and MBL-L-07 were chosen to reproduce a smaller mRNA codon library and peptide library. Both L-CiAc-Tyr and D-CiAc-Tyr were used to replace Met in the codon table by flexizyme. Due to time limit, two rounds of selections were performed. After each round of selection, qPCR was used to monitor and obtain the recovered amount.

![Figure 5. Pre-selected macrocyclic peptides for MBL inhibition](image)

<table>
<thead>
<tr>
<th></th>
<th>Percent Recovery (%)</th>
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</thead>
<tbody>
<tr>
<td>L - Positive selection</td>
<td>MBL-L-07 YWTLCG---RRPCPCG</td>
</tr>
<tr>
<td>L - Negative selection</td>
<td>MBL-L-01 YQLGCYSLIRKYPCG</td>
</tr>
<tr>
<td>D - Positive selection</td>
<td>MBL-D-01 YGCQITWTECGPRM-YPG</td>
</tr>
<tr>
<td>D - Negative selection</td>
<td>MBL-D-02 Y-----ECGPCKPRPCG</td>
</tr>
<tr>
<td></td>
<td>MBL-D-05 Y-----WTECGPSR---NGC</td>
</tr>
<tr>
<td></td>
<td>MBL-D-09 YCWRDWM---VHSCG</td>
</tr>
<tr>
<td></td>
<td>MBL-D-03 YTSI----RCRTYETRACG</td>
</tr>
</tbody>
</table>
Table 1. Percent recovery of selections for library of each stereochemistry

<table>
<thead>
<tr>
<th></th>
<th>Round 1</th>
<th>Round 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.01</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>0.009</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

If the isolation of peptide of interest was successful, an increase in % recovery of positive selection while a decrease in that of negative selection should be observed. From the table, negative selections seemed to prove the success, with both L and D peptides showing % recovery decrease from 0.003 to 0.001. However, the positive selection did not demonstrate an increase in % recovery. This may result from the loss during transfer of products, given the long procedure and steps involved. With more time, more rounds of RaPID selection should be performed to substantiate the success of isolation and purify the desired peptides.

**Reference**


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