Innovation Center of NanoMedicine

Center of Healthy longevity And Nursing innovation with Global Ecosystem

Press Release

Main Title:

A novel technology to explore peptides as drug targets with high precision without using cells

Sub-title:

Overcomes the shortcomings of conventional methods, significantly enhancing quantifiability, flexibility, and speed

Summary

- Developed a technology (PL-Display method) that enables high-precision, high-speed drug target discovery without using cells
- By firmly immobilizing candidate peptide and their coding DNA onto a magnetic bead, highly accurate screening is possible
- A peptide found in only one out of 10,000 can be detected in a single screening
- Efficiently detect target peptides from a library of approximately 1.7 million random peptides
- Efficiently concentrate the target by amplifying the DNA encoding the detected peptide via PCR

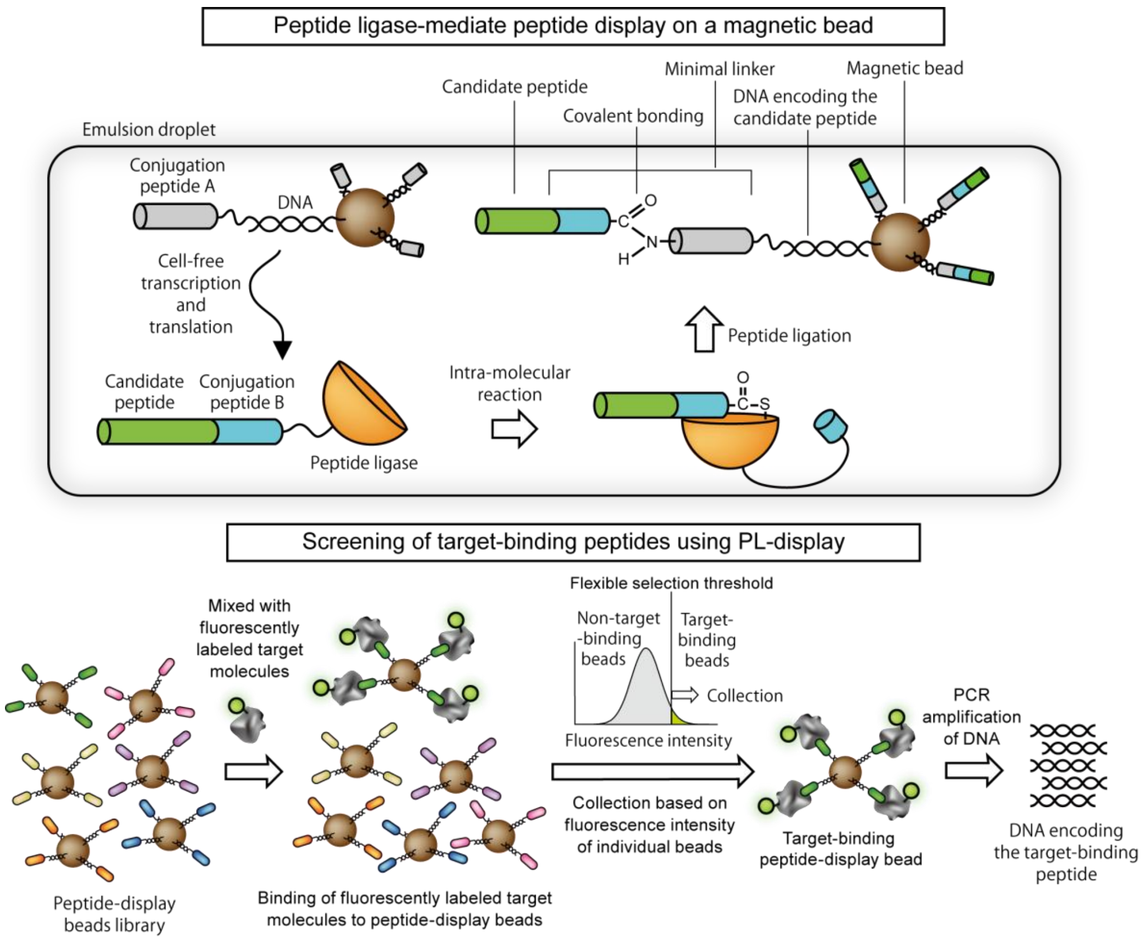
The Innovation Center of NanoMedicine (iCONM, Center Director: Prof. Kazunori Kataoka) is pleased to announce that research conducted by a group led by Shingo Ueno, Deputy Principal Research Scientist at the Ichiki Lab, has been published as an academic paper in PNAS Nexus (Note 1) and appeared online on February 13.

“Peptide ligase–mediated display: A cell-free platform for tunable selection of affinity peptides”

Authors : Shingo Ueno, Fumi Toshioka and Takanori Ichiki

DOI : https://doi.org/10.1093/pnasnexus/pgag031

Many biological functions are regulated by the switching on and off of mechanisms triggered by the matching of a keyhole (receptor) formed by a protein's three-dimensional structure and a molecule (ligand) that fits perfectly into it. If this keyhole deforms (protein mutation) or if a false key is created, biological functions become disrupted, leading to disease. Drug discovery research involves a process called screening to find compounds that fit into these receptor lock-and-key sites. The content published in this paper offers significant advantages, dramatically increasing the efficiency of this process and enabling the handling of highly toxic proteins that were previously difficult to work with. The underlying technology involves manufacturing and utilizing magnetic beads, each carrying a single peptide (a short-chain protein), without the need for cells. Traditional methods using living cells to produce peptides had drawbacks, such as inconsistent peptide quantities leading to unstable data. Compared to conventional approaches, this new method achieves over tenfold efficiency gains. Furthermore, its ability to perform screening under high-temperature and high-salt conditions significantly reduces the time required for lead compound discovery in drug development. Details are provided below.



➢ Research Background

The technology for finding proteins and peptides that bind precisely to specific targets is a crucial foundation for developing new drugs and diagnosing diseases. To date, various methods called “display technologies” have been developed to search for proteins that bind to the desired target (Note 2). Recently, “cell-free protein synthesis,” a method that produces proteins in test tubes without using living cells like E. coli or yeast, has become widely used. This method allows for more flexible and efficient searches for target proteins since cell cultivation is unnecessary. The iCONM research team developed a novel method to immobilize proteins produced via this cell-free synthesis onto the surface of small particles that stick to magnets (magnetic microbeads: Note 3). This technology enables the precise measurement of how strongly each individual bead binds to its target, allowing only the best-performing proteins to be selected.

➢ Research Results

In this study, we utilized the properties of an enzyme called “peptide ligase,” which connects peptides together. Using this enzyme, we developed a new technique to firmly anchor peptides (short protein fragments) and their template DNA onto the surface of small beads via a short connecting segment consisting of just nine amino acids. We named this method “PL-display.” The key feature of this technology is its ability to display one type of peptide and its corresponding DNA on a single bead without using any living cells. Furthermore, using an instrument called FACS (Note 4), beads can be examined one by one, allowing precise selection based on numerical values of only those beads possessing the desired properties. To verify that this technology functions correctly, we conducted the following experiment.

First, we mixed equal amounts of DNA for two types of marker peptides, HA-tag and His-tag, and displayed each on beads. We then added fluorescently labeled antibodies that bind only to each specific peptide. Beads bound to their target fluoresced more intensely, allowing FACS to select only the brightest beads. The DNA attached to these selected beads was then amplified using PCR (Note 5) to determine which genes they contained. The results confirmed that only the genes for the targeted peptides were accurately extracted. Furthermore, even from a mixture containing only 0.01% (one in ten thousand) of the HA-tag gene, we succeeded in completely isolating the HA-tag gene with just one round of selection. Additionally, from approximately 1.7 million peptides with random sequences, we efficiently collected peptides binding to the anti-HA-tag antibody in just two rounds of selection.

By determining the strictness of recovery criteria based on the fluorescence intensity of each individual bead, we achieved the high efficiency of "10,000-fold concentration in a single sorting step," which was difficult with conventional methods. This demonstrated that even from diverse peptide populations resembling those in actual drug discovery research, target peptides can be identified quickly.

- **Novelty**

This technology employs a method called "cell-free protein synthesis." This is a method for producing proteins in test tubes without using living cells. Consequently, it is not constrained by the conditions required to grow cells and is unaffected by variations in how proteins are produced by individual cells. Furthermore, it allows for the investigation of proteins that are harmful to cells or under special conditions different from the normal internal environment without issue. Additionally, in this technology, the protein and its template, DNA, are firmly connected by a strong bond that is difficult to break. Furthermore, each bead displays only one type of protein. This ensures stability even under harsh conditions like vigorous washing or high salt concentrations, allowing precise evaluation of "how strongly each bead binds to its target." Using FACS equipment, which sorts based on bead brightness (fluorescence intensity), allows researchers to freely and finely set criteria like "recovering only beads above a certain brightness threshold."

This mechanism theoretically enables the selection not only of proteins with very strong binding, but also of proteins possessing "just the right binding strength"—neither too strong nor too weak.

- **Future Potential and Contribution to Society**

Ultra-High-Throughput Screening of target-binding proteins using PL-display is expected to find applications across a wide range of fields, including drug discovery, diagnostics, and biomaterial development. These include the search for therapeutic and diagnostic peptides and low-molecular-weight antibodies, the development of industrial proteins for use in non-physiological environments, and high-speed screening through automation and robotics utilizing magnetic bead platforms.

Note 1

PNAS Nexus is an academic journal launched by the National Academy of

Sciences in 2022. It aims to disseminate high-impact research that breaks down disciplinary boundaries, such as in emerging fields and interdisciplinary studies. Positioned as an extension of the long-established Proceedings of the National Academy of Sciences (PNAS), it has garnered significant attention both domestically and internationally.

Note 2

Protein display technology is a method that physically links proteins or peptides with their corresponding DNA or RNA sequences to identify the genetic sequence for a selected protein. A representative example is the phage display method, which was awarded the Nobel Prize in Chemistry in 2018.

Note 3

Magnetic microbeads are tiny particles containing a magnetic core. They can be rapidly and selectively separated and recovered from a solution by applying an external magnetic field. By conjugating antibodies or nucleic acids to their surface, they are used for capturing, purifying, and concentrating specific molecules. Their ease of use, requiring no centrifugation, makes them widely employed in molecular biology and diagnostics.

Note 4

FACS (Fluorescence-Activated Cell Sorting) is a technology that reads fluorescently labeled cells or microparticles one by one using lasers, rapidly sorting and recovering them based on fluorescence intensity. Because it can select only particles with specific fluorescent signals, it is widely used for precise analysis and fractionation in research and medical fields.

Note 5

PCR (Polymerase Chain Reaction) is a technique that combines heating and cooling temperature cycles with enzymatic reactions to exponentially amplify specific DNA sequences. It is characterized by its ability to rapidly replicate large quantities of the target sequence and efficiently amplify even minute amounts of DNA. The amplified DNA is widely used for sequence determination, various analyses, and detection of specific sequences.