HiPep Labs has been focused on ligand-based medium-sized molecules as a modality for discovery and diagnostics

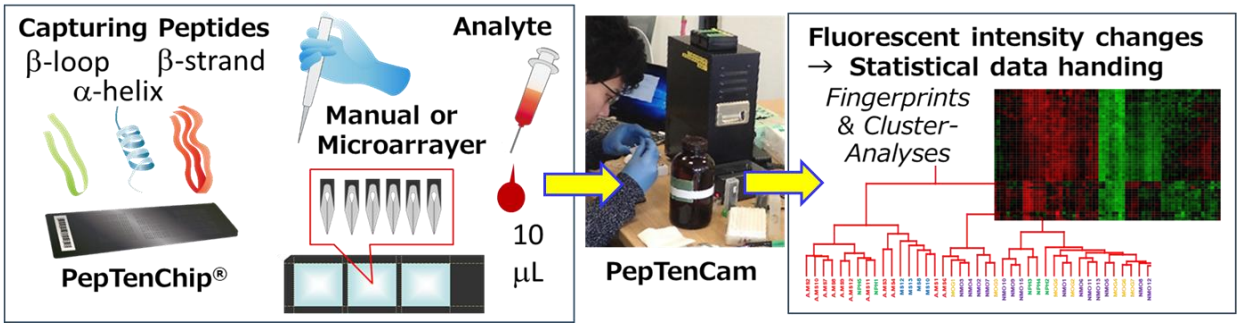
Bio-molecular recognition ➡ Innovative Diagnostics & Theranostics (Drug discovery)

[1] Analyses using microarrays with capturing molecules immobilized on a novel substrate made from amorphous carbon. [2] Discovery tools: libraries prepared by cyclic peptides immobilized on a gel-type supports instead of a chip substrate. [3] DNA-recognition by PNA-CPP-conjugates, subsequently shifted to PIPA. Immobilization is the key technology.

We are looking for collaborators having clinical specimens, investors, and distributors.

[1] Bio-detection and applied to diagnostics

HiPep Labs focuses on protein interactions based on molecular recognition, advancing R and D of biochips, designated PepTenChip®, consisting of *de novo* designed peptide derivatives as capture molecules (>2500) arrayed on novel substrate made from amorphous carbon. The novel detection principles was established. Fluorescent intensity changes before and after sample addition are dose-dependent and can be visualized as a protein fingerprint, recognition is not limited to a 1:1 correspondence. Data handling is performed by statistical methods, which allow (1) the disease-associated positive molecules (markers) are unknown, (2) objective assessment independent of the physician's personal skills, (3) rapid and simple non-invasive tests. PepTenChip® is not disposable but can be used repeatedly. One of key technology for this is materilas and immobilization. Approval as a Class 1 medical device is in preparation. Detector, PepTenCam, is easy setting up almost no maintenance, carry-on baggage size, no special reagents or pretreatment required and training free → Enables the use in the field and suitable for remote medical care (home healthcare). *Anal. Methods.* (RSC), **17**, 4590, 2025.



Clinical applications: useful even when disease markers are unknown/definitive diagnostic methods are unavailable

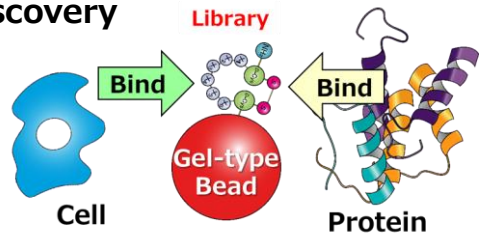
Pre-cancer diagnosis using gastric fluid, without tissue sampling. Classification of patients will develop stomach cancer of not (taking ca 30 min). The reproducibility and accuracy were confirmed by pathological examination of simultaneously collected tissues.

Multiple sclerosis: Both the typical form (MS) and atypical form (AMS) are types of central demyelinating disorders, arising from inflammation in the brain, spinal cord, and optic nerves. MS is a designated intractable disease with no known cause or cure, classified as Disease No. 13 in Japan.

MS/AMS differ in their treatments and their effectiveness, and practical diagnostic method is unknown and early diagnosis and treatment before progression are considered crucial. Using cerebrospinal fluid, we successfully classified MS and AMS and identified multiple peptide sequences contributing to classification. We fabricated a classification chip incorporating these sequences and confirmed its reproducibility and accuracy.

[2] Chemically synthesized OPOB library for drug discovery

- The key to successful discovery lies in uniform composition and high-quality libraries (equal fractionation, only intramolecular cross-linking, and D-Cys for endogenous enzyme resistance).
- OPOB contains a large number of molecules per molecular type and is also suitable for drug discovery oriented to activity.
- Parallel Simultaneous Solid-Phase Synthesis and improved methods for sequence analysis give high throughput
- Identify ligands and target molecules together
- Solving the limitations of nucleic acid-based molecular display methods, Phage/mRNA display relies on translation on ribosomes

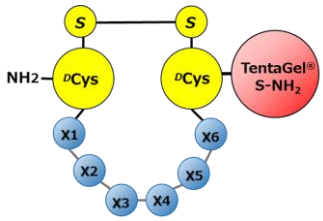


OPOB = One Peptide immobilized on One (single) Bead =OPOB

Two OPOB-libraries

OPOB	CP24OB	CP12FD
Building Blocks **(BB)**	24 BB (19 nat.+ 5 non-nat. **Cha, Hyp, Nle, Nva, Phg** *Amino Acids*, 48, 2491, 2016.	Selected 12 BB (10~11 non Nat.) does not contain the same mol. wt.) focusing on drug-likeness.
Diversity	**24^6 = 200 million**	**12^6 = 3 million**
Sequencing	① Edman-degradation ② Partial hydrolysis followed by MS/MS ③ **Direct liberation chemistry One pot reaction** Analytical Sciences, 40, 1219, 2024.	Site specific direct liberation ➡ MS/MS **without LC purification before MS/MS analyses** *Chem Biol & Drug Des.* 102, 1327, 2023
Size/Loading	90 μm, 0.27 mmol/g; 80~100 pmol/bead	

CP24OB



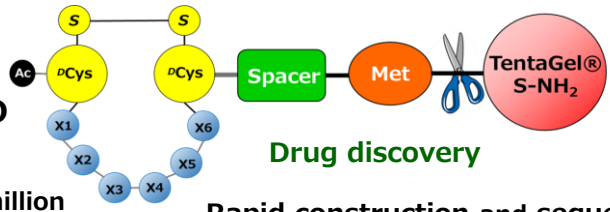
PPI-Elucidation

24 BBs 24^6 = ca 200 million 19 natural & 5 non-proteogenic AA, Cha, Hyp, Nle, Nva, Phg + *D*-Cys

Splitting resin requires effort and time. Seq. requires skill & time

➡ Novel chemistry was developed: cyanylation by NTCB

CP12FD



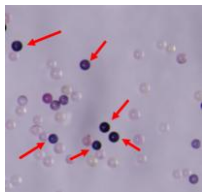
Drug discovery

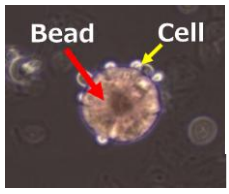
12^6 = 3 million

Rapid construction and sequencing

Selected 12 BBs involved 1 or 2natural mainly non-nat. in consideration that each compound is drug like-ness & Emphasizing on pharmacophore considered structure diversification of main chain. Met-residue of thio-ether structure ensured the liberation from support, without **LC-purification** to MS/MS characterization

Screening : OPOB captures proteins

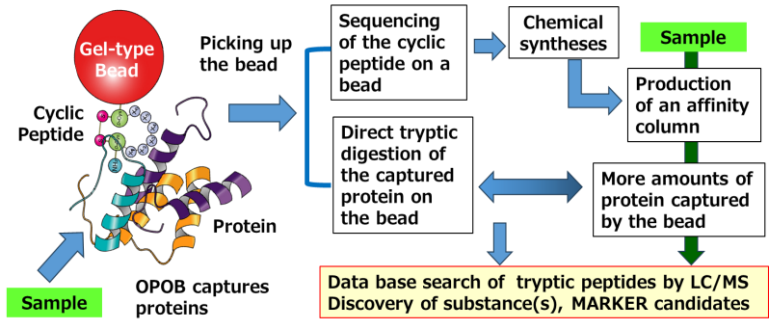




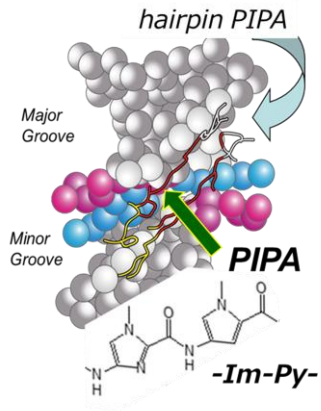
- Target protein tagged by alkaline phosphatase.
- Cell-targeting; Peptide-Vehicle: Obtained target can be used solely and also bio-conjugates for controlling molecules of functional proteins. *THL. 55, 4091, 2014*
- Affinity separation: fishing of recognized molecules
- Discovery of marker candidates from a complex of "Bead+Ligand"
- Characterization by MSpec offers Target molecules & +Ligand together
- OPOB allows discovery on "Multiple-to-Multiple"
- 1/5 bead is enough for seq. (one single bead carries 80-90 pico-mole)
- The skill on interpretation are the key issues.

- Novel PPI-inhibitors
- Novel Molecular Glue
- Discovery of dark-protein controlling molecules

Offering contract-research



[3] PIPA candidates as gene-control drugs



- PIPAs are middle sized chemically synthesized drug candidates, consisting of N−methylpyrrole (Py) and N−methylimidazole (Im) as major building blocks **reversibly bind to dsDNA** in a minor groove via hydrogen bonding in sequence specific manner with high affinity & selectivity: **P. Dervan,** *Science, 282, 111 (1998), Nature, 391, 468 (1998).*
- PIPA is an alternative gene silencer other than **siRNA** or **PNA** and designable to any gene.
- PIPA blocks binding of transcription factors inhibiting gene expression, controls expression of gene. Basic patents of PIPA were expired in 2017.
- **PIPA is Stable** in cells or bodies because of nuclease resistance and **No-toxicity** has been found in animal experiments. It takes 3-4 weeks to be excreted in urine (∼100%)
- PIPA can be delivered into cell-nucleus **without vectors**.
- PIPA with payload can be applied to DDS = Bio-conjugates: Peptide Vehicle (THL, 55, 4091, 2014), Alkylation (Würtz & Dervan, Chem & Bio, 7, 153, 2000) of target DNAs
- Specific **DNA visualization** by labelled PIPAs: ex. Human telomere visualization.
- Target diseases of PIPA: small molecules or antibodies can not be applied.
- PIPA suppresses only the increased gene transcription activity not gene expression knockdown (advantageous from a side effect perspective).
- Chemical syntheses as well as QC is more difficult than that of conventional peptides.
- Leading the world, HiPep Labs has established industrial manufacturing methods (design, chemical synthesis methods, manufacturing, purification, and quality control technologies).

Commercially available
PIPA reported
https://hipep.com/?p=283

Target DNA search
Target DNA seq.

Binding study
Pharmaco-actions
Candidate PIPA

Individual-level activity assessment
Safety tests

HiPep established a production for PIPA

PIPA design
Syntheses &purification
Characterization

Scaling up production of Candidate PIPA

Technology transfer of Industrial production system of PIPA together with SOP

Structural optimization cab be performed in a short period due to the reduced time required from target sequence selection to design.

Collaboration, Contract based research Technology transfer