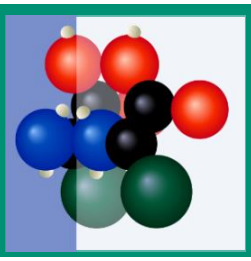


Research and Development Supported by HiPep® Technologies

Most of amino acid molecules are consisting of D and L enantiomers (mirror image) such as a right and a left hand. Biological actions often depend on the conformation of molecules. HiPep Laboratories has the potential to open the door of those mirror and to reveal molecular recognition. Thus, we are going to develop applications of those molecules. (Logo mark)

HiPep Laboratories

HiPep® Technology

High Throughput + High Quality + Highly Efficient Synthesis and Characterization of Peptides and their Related Materials

PepTenChip®

A sensor device, discriminates protein-structures, not only 1:1 recognition. Proteins can be mimicked by peptides. Designed Peptide Arrays have numerous advantages and high-quality industrial production are promised. Unlike proteins peptides as capture molecules can be used even after dried. Due to the original technologies, PepTenChip® can be repeatedly used, realization of SDGs, Sustainable Development Goals.

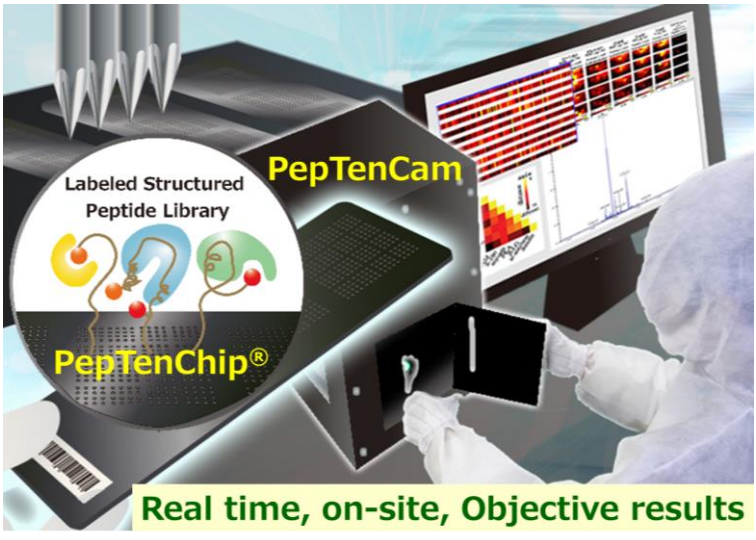
HiPep Laboratories was founded focusing on development of novel bio-detection systems, exploration and clinical applications of biologically active peptides and their derivatives such as synthetic vaccines, biomimetic materials, etc. HiPep provides consulting services for biomedical research, contract-based research involves syntheses, analyses, characterization. Additionally **HiPep Laboratories** performs production and sales of instruments, laboratory equipment, and reagents useful for construction of combinatorial chemical libraries. The core technology designated **"HiPep®"** is syntheses, characterization, and purification of peptides and their derivatives in a highly efficient manner. **HiPep Laboratories** has been focusing on molecular recognition in the context of peptides/proteins and their conformations/functions. The major R & D targets is production of bio-chip with the novel & original concepts.

Dr. Kiyoshi Nokihara
Founder & CEO/CSO, HiPep Laboratories

Corporate Philosophy

HiPep Laboratories was founded in 2002 for development of novel biochips with designed peptide arrays, research and clinical application of novel active peptide, application of peptide to biomaterial and development of drugs. Parallel performing own R and D, consultations and contract basis R and D. Additionally we provide compound library and related devices/ instruments & reagents.

Through our research and development in biomedical science, we would like to contribute to early detection, prevention and effective treatment of diseases which will help QOL improvement, including development of human resources. By taking advantages of founder's career and proficiency, we are proceeding interdisciplinary research and business collaboration and expanding world wide.



（株）ハイペップ研究所
https://hipep.com

Biographical Sketch of Kiyoshi NOKIHARA (Doctor of Pharmaceutical Sciences)

HiPep Laboratories Contributes to Society Growing to be a Leading Company for Bio-Medical Research

The CEO and CSO of the HiPep Laboratories, Dr. Nokihara, initially studied Organic Chemistry and Chemical Engineering in Tokyo, followed by many years of academic research in Germany. His involvement with bio-molecules commenced at Deutsches Wollforschungs Institute at the Technischen Hochschule Aachen (1973-1979). In 1980-1985, he was Assistant Professor of the Faculty of Pharmaceutical Sciences, Shizuoka Prefectural University, where he undertook extensive research on brain-gastrointestinal peptides, syntheses as well as immunochemical and endocrinology studies. He subsequently relocated to the Faculty of Medicine, University of Heidelberg in the role of visiting Professor, and later at the GBF (German Research Centre for Biotechnology). In 1990 he joined the Shimadzu Corporation, as a General Manager in R & D of the Biotechnology Instruments Department and Head of the Life Science Center. He has developed several novel instruments focusing on applications, including the simultaneous multiple automated peptide synthesizers, for which his team received a prize bestowed by the Japanese Agency of Science and Technology. His expertise was focused on the biochemical and medical fields, especially proteins and peptides, from isolation, synthesis and characterization to biochemical and medical applications. As well as his business commitments he received visiting Professor positions at the Tokyo University of Agriculture and Technology and the Tokyo Metropolitan Institute for Neuroscience Research to continue his academic research. In 1997 he founded Shimadzu Scientific Research Inc. and established a novel research business as the Chief Scientist. In 2002 he has independently founded a new venture company, the HiPep Laboratories, based on his knowledge and experiences in bioscience. Since 2002 he is a visiting Professor in Nanjing Medical University (China). In 2017 he was invited as a first foreign Professor at the School of 1st Clinical Medicine of Nanjing Medical University in addition to visiting Prof. of Faculty. Since 2019 he was invited additional the vising Professor position in Kangda Medical College, Lenyung Gan, China. He has around 300 publications and 130 patent applications.

Current Business

- Applications of on biological functions and molecular recognitions based on peptides and proteins
- Construction of peptide libraries, related devices, reagents and consumers: **Major commercial products were developed for in-house use (own research)**
- Application oriented consultation, contract research, and training
- Contract production for peptides and bioconjugates and characterization, discovery, structural optimization by design
- Amino acid analysis, chiral analysis, detection of contaminants, MS analyses for proteins and peptides

HiPep® Technology

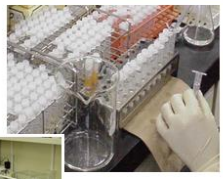
① Production of starting resin, by the in-house developed PetiSyzer®

② Automated Synthesizer PSSM-8, by Nokihara in 1991 (operated over night)

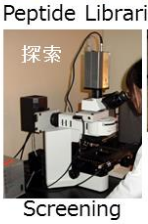
③ Manual syntheses for costly reagents, difficult sequences ④ Cleavage by PetiSyzer®

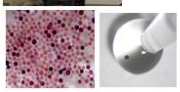
⑤ Large scle purification by in-house packed columns

⑥ Use of multiple LC-MS

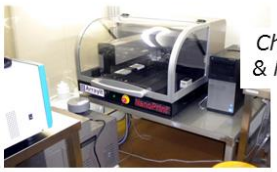


Peptide Libraries





Screening



Chip & MS

Analytes

Tailor made array and detection service

Biochip-fabrication in clean room

MALDI-TOF/TOF de novo seq.

Major Technologies and Products from Recent Research

Based on academic research in an interdisciplinary field as well as business experiences, HiPep Laboratories was founded in 2002 focusing on the biomedical applications of molecular recognition, which is one of the most important issues in the homeostasis of a living organism, for diagnostics and theranostics. Recently development of peptides-microarray systems for protein detection has been completed. Additionally a production system for polyamides consisting of pyrrole and imidazole residues which recognize double-stranded DNA has been also studied by which gene-related therapies and novel probes were developed.

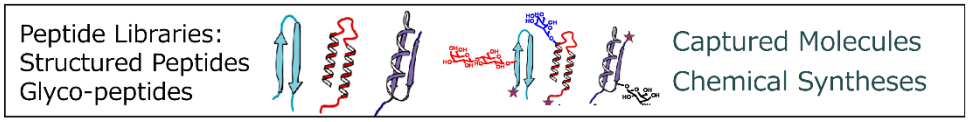
Major products were developed originally for in-house use

- Applications of biological functions based on peptides/proteins
- Design and construction of compound libraries, related devices & reagents, and consumers, which are originally for in-house use
- Novel separation columns with high resolution (HiPep-HPLC Columns)
- Consultation, Contract research, Contract characterization (Analyses), Training **: based on good facility with expertise**
- Designed compound libraries, on beads immobilized peptides
- PepTenChip®, novel bio-detection system for Protein detection/Profiling, Novel materials made from amorphous carbon for bio-chips, micro-reactors and micro-analyzers.

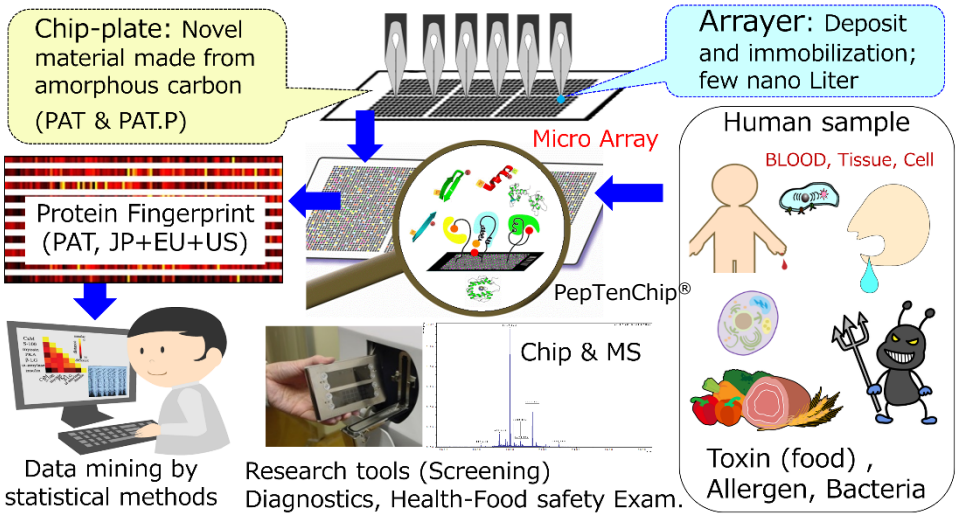
PepTenChip®

Protein-protein interaction mimicked by peptides: Biodetection, Diagnostics

Based on the principle that proteins can be mimicked by *de novo*-designed peptides, we are focusing on two major research areas; one is the novel bio-detection technology for diagnostics and another is development of functional molecules for theranostics. Characterization of proteins by high-throughput technologies are in great demand. During the last decade considerable effort has been devoted to the development of a novel bio-detection system using structured peptides with fluorescent dye as labels for capture molecules. These peptides are arrayed on the surface of novel chip material, amorphous carbon, with the novel surface chemistry. The novel concept and key technologies for bio-detection have been established [Reviewed: Solid-Phase Synthesis and Combinatorial Chemical Libraries 2004, ed. Epton, R. pp 836, **2004**]. The microarray system allowed minimization of amounts of both analytes (nano gram) and capture molecules (several femto moles).Designed peptides have been used as capture molecules in our bio-detection [FEBS Letters, 586, 325, **2012**, *ibid*. 587, 673, **2013**]. "The structural changes of the protein as an analyte are reflected in the florescence intensity changes of peptides as capture molecules in a dose dependent manner. The changes can be visualized as a pattern in a color bar-code which is designated a "Protein Fingerprint" [Chemistry and Biology, 10, 53, **2003**; Molecular BioSystems, 2**,** 113, **2006**, Patents of EU, Japan and USA; Bull. Chem. Soc. Jpn., 83, 799, **2010**]. In contrast to a conventional detection method, using a set of "antigen-antibodies", our detection system does not involve the detection of specific molecules themselves (so called "biomarkers") in a 1:1 manner, but the principle of detection is the differences in fluorescence intensity change caused by the analytes derived from normal *vs.* diseased subjects, for example. Recently the novel concept and key technologies for bio-detection have been completed.



- PepTenChip®, a sensor device, discriminates protein-structures.
- PepTenChip® is not only 1:1 recognition.
- **Not disposable, repeatedly used**



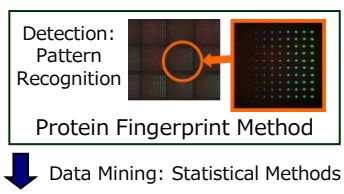
Advantages of amorphous carbon

① Mechanically stable
② Chemically inert
③ No self-fluorescence
④ Easily manufactured
⑤ Environmentally friendly (easier regeneration) , allowed to re-use.
⑥ Higher thermal & electro-conductivity (allowing ionization)
⑦ Extremely low back ground

Excellent Surface Technologies

- Lower non-specific protein absorption
- Uniformed distribution of functional groups
- Uniformed spot-formation on chips
- Easy handling
- Functional group-rich surface (easier immobilization)
- QC-methods for quantization of surface is established (PAT)

Peptide Design & Library Construction → High Density Peptide Arrays



Detector, PepTenCam (PAT), adaptable to PFP with easy handling, maintenance free, Low cost, portable, can be used in biosafety level 3 & 4 laboratories.

PepTenChip® is: powerful diagnostic tools/examination, diagnostics, prognostics and health management; provide the objective indicator not depending on doctor's skill, *i.e.* oral healthcare, neuronal diseases such as multiple sclerosis and early detection of pre-cancer status. allows avoiding to visit clinics/hospitals, because of examination requires long waiting time, accompanies with pain etc. Not compete conventional diagnostics, used by clinicians without hesitation because of the revenue.

Recent R&D: While PepTenChip® allows rapid differentiation of diseases to enable an objective evaluation by clinicians, on-going targets are neuronal diseases such as multiple sucraloses of which cause is unknown. Atrophic gastritis is considered as an early stage of cancer and the risk in the carcinogenic rate is higher. Instead of pathological observations, rapid and simple differentiation method is desired. PepTenChip® system with multivariate analyses are underway. Additionally the chip can directly be inserted into MALDI-TOF-MS after fluorescent detection and discovery of undefined materials caused diseases and/or surrogate markers can be possible. Since the system allows noninvasive diagnostics and on-site Point-Of-Care, the PepTenChip® has potential for application in diagnosis of incapacitated patients. Real time and on-site manner serve AI-assisted remote diagnostics and home care. Not compete conventional diagnostics, used by clinicians without hesitation because of the revenue.

Microarray printing services: Utilizing the most advanced microarray manufacturing facilities (Class 100 clean room) and expertise using patented concepts and novel substrate made from amorphous carbon. Detection service is also available.

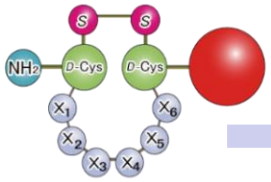
Alliance and Collaboration

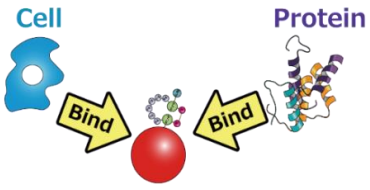
HiPep® (Core Technologies), PetiSyzer® (Personal Synthesizers)
LibraTube® (Multi purpose vessel) , PepTenChip® (Biodetection tool)

Peptide derivatives & Construction of Library; High-through put screening, Drug discovery; DDS-design = Peptide Vehicle;
Bio-detection tools (Peptide Microarray) ★ Devices, Reagents and HPLC-columns developed for in- house use.
★ Contract syntheses analyses and consultation

Bio-conjugates & Drug-discovery: Recognition of Proteins and DNAs

Discovery & Cell Therapy (DDS) Bioconjugate: Peptide-Vehicle





One cyclic peptide on One Bead Diversity 24^6 *ca.* 200 millions (X = 19 natural & 5 non-nat. AAs) Novel OPOB-Library, focusing on drug-likeness and expanded Chemical space.

Deconvolution service available.

Peptide-vehicle

Peptide-vehicle: Tetrahedron Lett. 55, 4091, **2014**

Peptide nucleic acids (PNAs) [Pipkorn. R., et al., Int. J. Med. Sci., 9, 1, **2012**] and used as chemical probes for FISH.

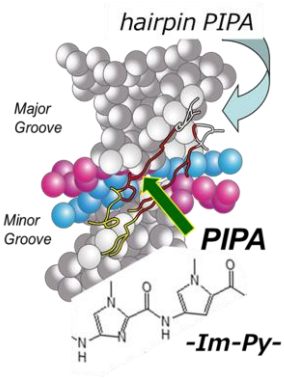
Novel aspects and advantages of the Peptide-vehicle

1. DDS; 2. Specificities; 3. Novel Patents; 4. Selectivity to cell lines; 5. Against drug-resistance; 5. Combinatorial functional modules; 6. The vehicles are expected to be more effective novel drug

PIPA-drugs as Gene Silencer: Novel therapeutic agents

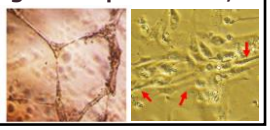
PIPAs, peptides consisting of non-proteinogenic amino acids, *N*-methyl-pyrrole and *N*-methyl-imidazole as major building blocks. PIPAs bind to specific nucleotide sequences in the minor groove of double helical DNA with high affinity and specificity [P. B. Dervan et. al., Nature, 391, 468, **1998**].

PIPAs are resistant to nucleases and do not require any particular delivery systems. PIPA blocks binding of transcription factors and inhibiting gene transcription. Thus PIPA can be applied as novel chemical probes or vehicle for delivery that is the use for diagnostics and/or therapeutic agents. PIPA is difficult to be synthesized, although we have constructed a industrial scale production system and quality controls for PIPA have been established.



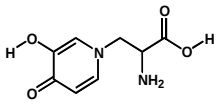
Regenerative Medicine: Angiogenic Peptide: AGP;

BBRC, 310, 153, 2003; BMC: DOI:10.1016/j.bmc.2020.115685 JPRTher, 28, 1-4, 2022. DOI 10.1007/s10989-022-10404-2.



Mimosine, non-proteinogenic amino acid & Mimosyl Peptides as ingredients for cosmeceuticals or pharmaceuticals

A large scale extraction, purification, physicochemical characterization of high quality Mimosine from plant *Leucaena leucocephala de Wit* has been established Nokihara, K, et.al., Amino Acids, 54, 27-36, **2012**. Nokihara, K, et.al., Peptide Science 2010, p 282, **2011.**



Company Profile

Corporate History

2002 March: Based on the successful results of the Millennium Project (MEXT, 2000-2002), HiPep Laboratories was founded by Dr. Nokihara focusing on development of novel biodetection systems. HiPep Laboratories also provide contract research in discovery and screening, syntheses, analyses & characterization of bio-molecules. Additionally, HiPep produces laboratory instruments and consumers fundamentally developed in house use. The core technology, designated HiPep® Technology, is the founder's life work. May: Wet laboratories were completed in Kyoto-city. November: Memorial conference on HiPep-opening was held and current collaborators and dealers were attended.

2004 September: Okinawa bio-project was started and a new additional laboratory was set up in the middle part of main island. December: Bio-Venture Project has been approved by the New Energy and Industrial Technology Development Organization and received a grant-in-aid.

2005 February: Opening conference of HiPep-Okinawa Laboratory was held. April: The prize in the Bio-Business Competition was bestowed. June: Laboratory space in Kyoto has been expanded and laboratory for protein characterization was newly opened.

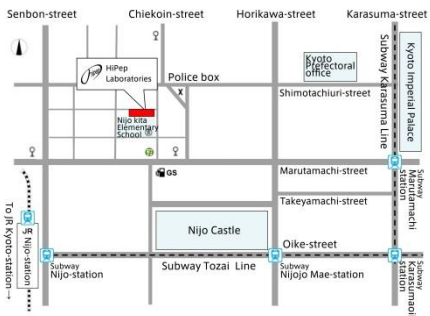
2006 November: Celebration 5th anniversary of HiPep and scientific conference was held with several invited speakers, mainly collaborators.

2007 March: Both office and laboratory spaces in Kyoto were further expanded up to 1.4 times lager. July: A five years national project, R&D Program for New Bio-industry Initiatives National Agriculture and Food Research Organization was started with grants(Prion Project).

2008 May: The Program for Creating Okinawa Innovation was adopted by the Gant-in-Aid for Cabinet Office, Government of Japan and Okinawa Prefecture, and laboratories for cell biology were completed.

2009 October: The Adaptable and Seamless Technology Transfer Program through Target-Driven R & D, Japan Science and Technology Agency, was bestowed.

2010 A novel application of PepTenChip® has been developed which can be used directly as a sample plate of MALDI-TOF mass spectrometry, thus the dual mode biodetection could be shown as a "hyphenated technologies".



Access to HiPep Labs from Kyoto Station **(1)** JR-**Saga-San-in**-line (track 33) getting off at **Nijo-eki**, the 2nd stop (5 min ride). From **Nijo-eki,** [a] *ca.* 15 min walk (north and then east), [b] Taxi (5 min), to show your driver **"下立売通千本東入：二条北小学校"**, [c] City bus, No. 201, 206, 6 or 46 get off at **Senbon-Marutamachi** and crossing **Senbon-Dori** 4 min. walk eastward.

(2) By Subway at **Karasuma-Oike** transfer to the **Tozai**-line and get off at **Nijo-Castle,** 10 min walk north & **Marutamachi-dori** west).

2011 April: Taking in account of economical influences caused by tsunami and earthquake in Tohoku, Okinawa lab was closed and all activities were focused in Kyoto. September: A clean room for biochip manufacturing was reconstructed.

2012 The conference of 10 years anniversary-celebration of HiPep was performed and several invited scientific collaborators gave their excellent lectures.

2013 R&D Prize of New Technology Foundation 2013.

2014 By the aid received in 2013 all fundamental technologies concerning novel biodetection system, PepTenChip®, consisting of libraries for captured molecules, chip-plate (novel material made from amorphous carbon, high through-put arrayer and fluorescent detector) have been completed. NEDO-Grant, Innovation Venture R&D Support, was began, the theme was development of industrial production of PIPA as API. Hence, not only proteins but also double strand DNA-specific recognition was proceeded.

2015 NEDO-project has been successfully terminated. Further, the system-design and improvements for practical production were carried out and larger scale syntheses have been performed for evaluation. The grant of Ikeda-Senshu Bank was bestowed for Oral-Health care hence PepTenChip® system ws applied.

2016 March: HiPep-Korea (HPK) was founded for Korean life science markets. May: HPK-Foundation Memorial Conference in Suwon Bio-Center. August: a spin off company, PIPLS Pharma (Peptides containing Imidazole and Pyrrole as building blocks-Large scale Syntheses) was founded based on R&D results during the last decade performed by HiPep Laboratories. PIPLS Pharma focuses on GMP production of high quality peptide derivatives, especially peptide conjugates and PIPA for clinical applications. PIPA is a novel drug candidates with novel action mechanisms such as gene controls. Industrial scale GMP-products is indispensable for clinical trial as well as FDA-approval.

2017 Two big exhibitions in March at the BioKorea, lecture was also given) and in April Korea-Pharm. June joined in Bio2017, San Diego, ca 70 business partnering were carried out. Two grants for Longer-Life, Kyoto-City and local collaboration project with academia (Kyoto-prefecture) have been received: former regenerative medicine and latter biodetection in neuronal diseases . Dec. Dr. Nokihara was invited as a first foreign Professor at School 1st Clinical Medicine, Nanjing Medical University, China. And a Visiting Professor in Nanjing Medical University (Faculty) is further continued.

2019 July, Grant "Project for Strategic Fundamental Technology Advancement Support" (METI) was received, which is focusing on applications of PepTenChip®.

Trade Name	HiPep Laboratories
Principal Office	Nakatsukasa-cho, 486-46, Kamigyo-ku, Kyoto, 602-8158, Japan
Contact	E-mail info@hipep.jp URL http://www.hipep.jp Tel: +81-75-813-2101 Fax:+81-75-801-0280
Founded	March 29, 2002
Capital	22.5 million Japanese Yen (as of April 1, 2022)
Main Banks	Mitsubishi-UFJ Bank, Kyoto Bank, Mizuho Bank, Kyoto-Shinyoukinko
Administration Board	CEO & CSO Dr. Kiyoshi NOKIHARA Dr. Yoshihiro OHTAKI , Tomoko NOKIHARA, Mitsuo FUJINAMI