HiPep Laboratories

Gene control reagents: PIPA

HiPep Laboratories focused on the research of double strand DNAs recognition in addition to proteins. Recently industrial production of PIPA (peptides consisting of pyrrole and imidazole as building units) has been established. By using this technology several PIPAs for research works on gene expression control are launched. Price: inquire. Abbreviations and related information see also URL http://hipep.jp/?p=1032

P/N	Structure (C terminal amide)	Moe. weight	Target Gene	Ref.
HPTB01	Ac-Im-Py-Py-βAla-Im-Py-Py-γAbu-PyPy-Py-βAla-Py-Py-βAla-Dp	1787.90	Human TGF-β1	1
HPTB02	Ac-Im-Im-βAla-Im-Py-Im-γAbu-Py-Py-Py-βAla-Py-Py-βAla-Dp	1667.75	Human TGF-β1	1
HPTB03	Ac-Im-Py-Im-βAla-Py-Py-Py-γAbu-Im-Py-Im-βAla-Py-Py-Py-βAla-Dp	1912.00	Human TGF-β1	1
HPTB04	Ac-Py-Py-βAla-Py-Im-Py-γAbu-Py-Py-Py-βAla-Im-Py-βAla-Dp	1665.78	Rat TGF-β1	2
HPLH05	Ac-Im-Py-Im-βAla-Py-Py-Im-γAbu-Py-Py-Py-βAla-Py-Py-Py-βAla-Dp	1911.01	Human LOX-1	3
HPLR06	Ac-Py-Py-βAla-Py-Py-Im-γAbu-Py-Im-Im-βAla-Py-Im-βAla-Dp	1667.75	Rat LOX-1	4
HPCT07	Ac-Im-Py-Py-βAla-Py-Py-Py-γAbu-Im-Py-Im-βAla-Im-Py-Py-βAla-Dp	1912.00	Human CTGF	5
HPTE08	Ac-Py-Im-βAla-Im-Im-Im-γAbu-Py-Py-Py-βAla-Py-Py-βAla-Dp	1667.75	TMPRSS2-ERG	6
HPOC09	Ac-Py-Py-βAla-Im-Py-Py-γAbu-Py-Im-Py-βAla-Im-Py-βAla-Dp	1666.76	Oct1	7
HPOC10	Ac-Im-Py-βAla-Py-Py-Py-γAbu-Py-Py-Py-βAla-Im-Py-βAla-Dp	1665.78	Oct1	7
HPRU11	Chlorambucil-βAla-Im-Py-Im-Im-γAbu-Py-Py-Py-Py-Dp	1523.41	RUNX	8

Freeze dried, No additives, 100 μg/vial ～ 1 mg (upon requests larger scale can also be supplied)

＊Labeling, Derivatizing, Design, Modifications can be performed upon requests (see also contract syntheses)

References upon request pdf can be send personally

1. Igarashi, J. et. al. Preclinical Study of Novel Gene Silencer Pyrrole-Imidazole Polyamide Targeting Human TGF-β1 Promoter for Hypertrophic Scars in a Common Marmoset Primate Model, PLoS One. 10, e0125295, 2015.
2. Matsuda, H. et al. Transcriptional inhibition of progressive renal disease by gene silencing pyrrole imidazole polyamide targeting of the transforming growth factor-β1 promoter, Kidney Int. 79, 46-56, 2011.
3. Ueno, T. et al. A novel gene silencer, pyrrole–imidazole polyamide targeting human lectin-like oxidized low-density lipoprotein receptor-1 gene improves endothelial cell function, J. Hypertens 27, 508-516, 2009.
4. Yao, E. H. et al. Novel gene silencer pyrrole-imidazole polyamide targeting lectin-like oxidized low-density lipoprotein receptor-1 attenuates restenosis of the artery after injury, Hypertension, 52, 86-92, 2008.
5. Wan, J. X. et al. Development of a novel gene silencer pyrrole-imidazole polyamide targeting human connective tissue growth factor, Biol. Pharm. Bull. 34, 1572-1577, 2011.
6. Obinata, D. et al. Pyrrole-imidazole polyamide targeted to break fusion sites in TMPRSS2 and ERG gene fusion represses prostate tumor growth, Cancer Sci. 105, 1272-1278, 2014.
7. Obinata, D. et al. Targeting Oct1 genomic function inhibits androgen receptor signaling and castration-resistant prostate cancer growth, Oncogene, 1-6, 2016.
8. Morita, K. et al. Genetic requlation of the RUNX transcription factor family has antitumor effects, J. Clin. Invest. 127, 2815-2828, 2017.

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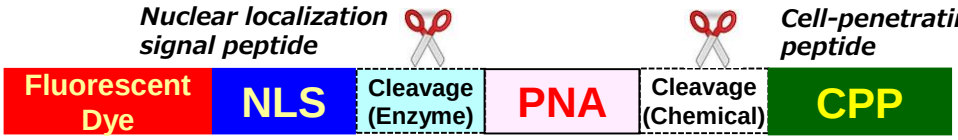
HiPep Laboratories

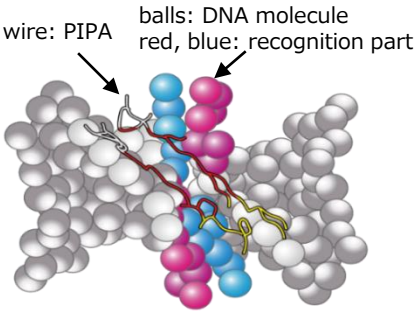
Drug discovery (Gene Therapy by DNA specific recognition) & Biodetection

Recently, gene therapy with genome editing is expected as the novel medical treatment.
HiPep Laboratory has been performed **Peptide Nucleic Acid (PNA)** and **Pyrrole-Imidazole Polyamide (PIPA)**.
Both can be performed by the contract syntheses. Moreover the facility of GMP-production is under construction.

PNA is a kind of peptide consisting of base units with amide bonds. PNA can bind complementary with DNA, such as A-T; C-G, and recognize to specific nucleotide sequence. DNA/PNA double-stranded structure is more stable than dsDNA. DNA expression can be blocked by hybridization with PNA and DNA. However, the limitation of PNA is poor membrane permeability. So devised to delivery to pass through the cell membrane and nuclear membrane is required. Especially efficient introduction technology into the nucleus of the target cell is indispensable. In addition, molecules recognition can be applied for biodetection (e.g. FISH etc.). In order to deliver PNA into the cell, we propose a conjugate of module type molecule (designated "**Peptide-vehicle**" ; "**Bio-shuttle**").

Peptide Vehicle; Bio-shuttle module type molecule (Middle Molecular)





Advantages of bioconjugate

1. Improvement of drug delivery: Introduced only in target (cancer) cells
2. Cell specific; reduction of side effects: Target cells rather than molecular targets
3. New patent: Drugs out of patent secured new intellectual property right
4. Improve selectivity for cell lines
5. Activity even in cancer-resistant cancer cells (peptide vehicle with anticancer drug)

PIPA is a peptide containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) as major building units. PIPA can bind to specific nucleotide sequences in the minor groove of double-helical DNA with high affinity and specificity, suggesting that PIPA blocks binding of transcription factors inhibiting gene expression, thus PIPA can be used for gene-control. PIPA is stable in cells or bodies because of nuclease resistance and enters into cell nucleus without any DDS.

Comparison of PNA and PIPA

	PNA	PIPA
Form of recognition DNA	single-stranded DNA	double-stranded DNA
Bonding mechanism	Bind complementary such as A-T ; C-G	Bind in the minor groove of dsDNA: Py/Py with A-T & T-A; Py/Im with C-G; Im/Py with G-C
Stability of binding state with DNA	more stable than dsDNA	Binding to the DNA double helix structure containing the target DNA sequence shows high affinity as nano M order (Kd value)
Average molecular weight / unite	300/base	120/unite (smaller molecules than PNA)
Optional modification	・Conjugation with modules to pass into membranes (e.g. CPP, NLS) ・N-terminus acetylation; C-terminus amidation	・N-terminus acetylation; C-terminus amidation or diamine derivative (Improve recognition)
Optional modification Common	・Fluorescent label for imaging (selectable fluorescent dye suitable for wavelength to be observed) ・Biotinylation ・PEG modification ・Introduction of non-natural amino acid	

[Literatures in our activities] *upon requests reprints are available*

Pipkorn, R. et al., *Int. J. Med. Sci.*, **9**, 1-10, 2012. Improved Synthesis Strategy for Peptide Nucleic Acids (PNA) appropriate for Cell-specific Fluorescence Imaging

Braun, K. et al. *Int. J. Med. Sci.* **9**, 339-352, 2012. BioShuttle Mobility in Living Cells Studied with High-Resolution FCS & CLSM Methodologies

Nokihara, K. et al. *Peptide Science 2012*: K. Sugimura (Ed) The Japanese Peptide Society, pp 167-170, 2013. Improved Coupling Methods for the Difficult Amide-Bond Formation in Solid-Phase Assembly

Hirata, A. et al. *Peptide Science 2011*: K. Sakaguchi (Ed), The Japanese Peptide Society , pp 377-380, 2012. Synthesis and Mass-Spectrometric Characterization of the Peptide-Vehicle, Peptide that Carry Molecules into Cells

Nokihara, K. et al. *Peptide Science 2011*: K. Sakaguchi (Ed), The Japanese Peptide Society pp 73-76, 2012. Studies of Bioconjugates Focusing on Drug Delivery; Peptide-Vehicles Consisting of Recognition and Cell Penetrating Peptide-Modules with Anticancer Drugs

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Hirata,A., Nokihara, K et al. *J. Am. Chem. Soc.,* **136**, 11546–11554, 2014. Structural Evaluation of Tandem Hairpin Pyrrole–Imidazole Polyamides Recognizing Human Telomeres

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HiPep Laboratories

PIPA: a Novel Gene Silencer

Why don't consider the use of PIPAs over knockout by RNAi or knockdown by genome editing

Characteristics of PIPA

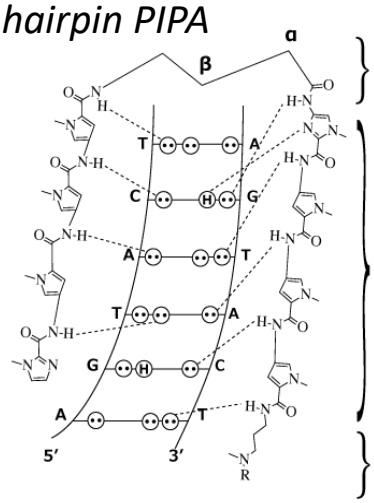
- PIPA is a kind of peptide, masks with an minor groove on the outside of the double stand DNA.
- Recognizing the target double stand DNA in dormant state, the mechanism differs from that of single stranded RNAi.
- PIPA is consisting of all non-proteinogenic amino acids. Stable in cells or bodies because of nuclease resistance.
- Enter into cell nucleus without any DDS.
- Flexible Design against any gene.
- As with RNAi, it is a temporary reduction in expression, its effect is also weakened and restored by passaging.

PIPAs are expected as a novel drugs for gene therapy with a novel mechanism; interfering the transcription of disease-related genes, suppressing expression of diseased proteins, stopping onset and progression of diseases.

[References]
P. B. Dervan et. al., Nature 391 (1998) 468-471; P. B. Dervan and R. W. Burli, Current Opinion in Chemical Biology 3 (1999) 688-693; P. B. Dervan, Bioorganic & Medicinal Chemistry 9 (2001) 2215-2235.

Principle: double strand DNA recognition by PIPA

hairpin PIPA

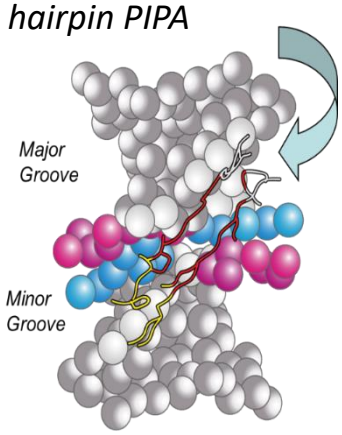


Central gamma aminobutyric acid linker makes the molecule folds into a hairpin type, selectively binds to a specific base sequence.

Adjust the length of the base sequence recognized by introducing beta-alanine at an appropriate position.

At the C terminus, special functional groups are placed to increase affinity for DNA.

hairpin PIPA

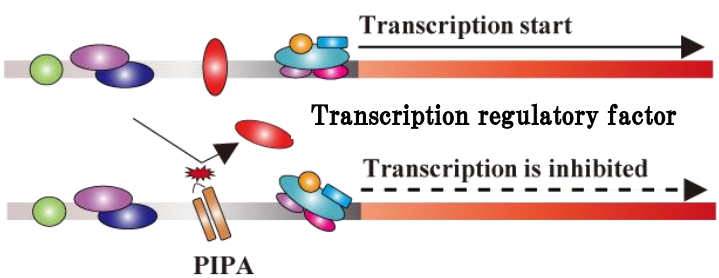


PIPA binds reversibly to specific nucleotide sequences in the minor groove of double-helical DNA by hydrogen bonding.

HiPep Laboratory has been performed R&D on industrial application of molecular recognition of living organisms. In addition to biodetection using PepTenChip®, a novel microarrays, drug development are also carried out.

Hence, double-stranded DNA binding using PIPAs, peptides consisting of pyrrole/imidazole rings, control **transcription factors and inhibiting gene transcription**. Academic research on PIPA has been carried out in the past two decades and now clinical applications are reported.

Method	Target	Cleavage Activity	Gene expression suppression type	Genetic code change	Change in expression level	Necessity of clone isolation
PIPA	Genomic dsDNA		Knock down gene transcriptional activity	No	✓	
RNAi	mRNA	✓	Knock down gene expression	No	✓	
CRISPR	Genomic dsDNA	✓	Knock out	Yes	✓	✓



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