Academic Program [Oral B] | 17. Biofunctional Chemistry, Biotechnology：Oral B

Tue. Mar 19, 2024 9:00 AM - 11:30 AM JST | Tue. Mar 19, 2024 12:00 AM - 2:30 AM UTC H932(932, Bldg. 9 [3F])

[H932-2am] 17. Biofunctional Chemistry, Biotechnology

Chair: Hidenori Okamura, Yuichiro Aiba

Japanese
9:00 AM - 9:20 AM JST | 12:00 AM - 12:20 AM UTC
[H932-2am-01]
Recognition of Double-Stranded DNA with Multiple Pairs of Shifted Parallel-Stranded PNAs

○Naoya Mochizuki[1], Masanari Shibata[1], Yuichiro Aiba[1], Kota Ito[1], Shinya Ariyasu[1], Osami Shoji[1] (1. Nagoya University)

Japanese
9:20 AM - 9:40 AM JST | 12:20 AM - 12:40 AM UTC
[H932-2am-02]
Development of gene expression controlling system driven by host-guest interaction

○Takeyuki Yao[1,2], Hidenori Okamura[1,2], Fumi Nagatsugi[1,2] (1. IMRAM, Tohoku Univ., 2. Grad. Sch. Sci., Tohoku Univ.)

English
9:40 AM - 10:00 AM JST | 12:40 AM - 1:00 AM UTC
[H932-2am-03]
Pseudorotaxane formation by cyclized oligo DNAs and approach to accelerating its formation

○Kazuki Kuwahara[1,2], Kazumitsu Onizuka[1,2], Sayaka Yajima[1,2], Yuuhei Yamano[1], Fumi Nagatsugi[1,2] (1. Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2. Graduate School of Science, Tohoku University)

10:00 AM - 10:10 AM JST | 1:00 AM - 1:10 AM UTC
Break

English
10:10 AM - 10:30 AM JST | 1:10 AM - 1:30 AM UTC
[H932-2am-04]
Development of the Convergent Synthesis of Boranophosphate DNA by an *H*-boranophosphonate Method

○Yuhei Takahashi[1], Itsuki Kato[1], Kazuki Sato[1], Takeshi Wada[1] (1. Tokyo University of Scienece)

English
10:30 AM - 10:50 AM JST | 1:30 AM - 1:50 AM UTC
[H932-2am-05]
Reaction mechanism analysis of chemical ligation for long-chain elongation of L-*α*TNA

○Hikari Okita[1], Keiji Murayama[1], Hiroyuki Asanuma[1] (1. Nagoya Univ.)

English
10:50 AM - 11:10 AM JST | 1:50 AM - 2:10 AM UTC
[H932-2am-06]
New Data Science in Nucleic Acids Chemistry (10): Quantitative analysis for factors affecting i-motif formation in living cells estimated by the pseudo-cellular system

○Kun Chen[1], Hisae Tateishi-Karimata[1], Naoki Sugimoto[1,2] (1. Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 2. Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University)

English

11:10 AM - 11:30 AM JST | 2:10 AM - 2:30 AM UTC

[H932-2am-07]

New Data Science in Nucleic Acids Chemistry (11): Transcriptional regulation in cancer cells induced by formation of G-quadruplexes and i-motifs

○Hisae Tateishi-Karimata[1], Keiko Kawauchi[2], Yiwei LING[3], Shujiro Okuda[3,4], Naoki Sugimoto[1,2] (1. Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 2. Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 3. Medical AI Center, Niigata University School of Medicine, 4. Graduate School of Medical and Dental Sciences, Niigata University)

認識領域をずらした parallel 型 PNA による 2 本鎖 DNA へのインベージョン

(名大院理 [1]) ○望月 直哉 [1]・柴田 将成 [1]・愛場 雄一郎 [1]・伊藤 公太 [1]・有安 真也 [1]・荘司 長三 [1]

Recognition of Double-Stranded DNA with Multiple Pairs of Shifted Parallel PNAs

([1]*Graduate School of Science, Nagoya University*, [2]) ○Naoya Mochizuki,[1] Masanari Shibata,[1] Yuichiro Aiba,[1] Kota Ito,[1] Shinya Ariyasu,[1] Osami Shoji[1]

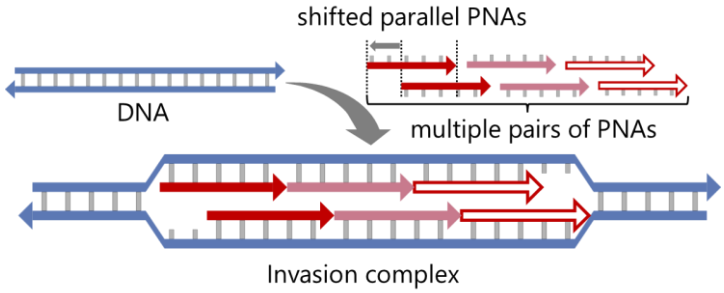
Peptide nucleic acid (PNA) [1] is a type of artificial nucleic acid in which the sugar-phosphate backbone of DNA is replaced by an *N*-(2-aminoethyl)glycine backbone. Since PNA has no negative charge on its backbone, there is no electrostatic repulsion between PNA and DNA, and PNA exhibits high DNA binding affinity. Furthermore, PNA can directly recognize the sequences in double-stranded DNA through a unique recognition called "invasion".[2]

In our laboratory, we have developed a novel invasion of parallel-stranded PNAs that does not require the modified nucleobases of PNA.[3] In this study, we aimed to make the parallel-stranded PNA invasion adoptable to a variety of applications. We confirmed that DNA recognition is possible when using multiple pairs of PNAs even in different sequence combinations. In addition, we succeeded in improving DNA recognition by shifted parallel-stranded PNAs.

Keywords : PNA; Invasion; DNA; artificial nucleic acids; genetic engineering

ペプチド核酸（PNA）[1]は、DNA の糖-リン酸骨格を *N*-(2-aminoethyl)glycine 骨格に置き換えた人工核酸である。PNA は骨格に負電荷を持たないことから、DNA と静電反発を生じず、DNA に対する高い結合力を示す。その結果、PNA が 2 本鎖中に潜り込みながら DNA を認識する「インベージョン」という結合様式が可能である。[2]

当研究室では、2 本の PNA を parallel 型で設計することで、これまでインベージョンに必要であった修飾核酸塩基を必要としない新規インベージョンの開発に成功している。[3]本研究では、この parallel 型 PNA インベージョンによる DNA 認識の適応範囲拡大を目指した。複数組の PNA を用いることで、様々な配列において DNA 認識が可能であることを明らかにした。さらに、各 PNA の認識領域を互い違いに設計することで、インベージョン効率の向上に成功した。



1) P. E. Nielsen, *et al., Science*, **1991**, 254, 1497. 2) Y. Aiba, *et al., Appl. Sci.*, **2022**, 12, 3677.
3) M. Shibata, *et al., ChemRxiv*, **2022**, doi:10.26434/chemrxiv-2022-wq3dm.

ホスト–ゲスト相互作用を利用した遺伝子発現制御法の開発

（東北大多元研[1]・東北大院理[2]）○矢尾 健行[1,2]・岡村 秀紀[1,2]・永次 史[1,2]
Development of gene expression controlling system driven by host-guest interaction (*[1]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University., [2]Graduate School of Science, Tohoku University*) ○Takeyuki Yao,[1,2] Hidenori Okamura,[1,2] Fumi Nagatsugi[1,2]

Artificial genes provide numerous applications ranging from elucidation of biological functions to gene expression-based therapeutics such as DNA vaccines. Lack of a methodology to control gene expression at arbitrary locations and timing is a challenge. To this end, we designed adenosine derivatives modified with cucurbit[7]uril (CB[7])-specific guest moieties; these nucleoside derivatives were expected to form a stable duplex by base-pairing with thymidine whereas in the presence of CB[7] forms a bulky host-guest complex to dissociate the duplex structure. Furthermore, we anticipated that addition of a high-affinity guest would induce guest exchange reaction to reassociate the duplex structure. By utilizing these nucleosides, we successfully demonstrated the reversible control of base pair formation and artificial gene expression control *in vitro*. In the presentation, we will report the details of molecular design and reversible control of gene expression.

Keywords : Functional nucleic acid; Host-guest interaction; Gene expression control; Supramolecular chemistry

人工遺伝子の発現を任意の場所とタイミングで制御できる方法論は、生命現象の解明のほか、合成生物学や創薬研究に有用である。本研究では、二重鎖の形成と解離を基盤とする遺伝子発現制御法の開発を目的として、ホスト–ゲスト相互作用によって塩基対形成を可逆的に制御できるヌクレオシド誘導体を設計した（**Fig. 1**）。本誘導体は、CB[7]の非存在下ではチミジンと塩基対を形成し安定な二重鎖を形成するが、CB[7]の存在下では、強固かつかさ高い CB[7]-ゲスト複合体形成により、二重鎖構造を解離すると考えられる。さらに、CB[7]に対してより強く結合するゲスト分子を添加することでゲスト交換が生じ、二重鎖構造の再形成を誘起できると期待した。本設計概念を検証したところ、二重鎖 DNA の解離と形成を可逆制御できることを確認した。さらに、本誘導体を用いることで、無細胞系における人工遺伝子発現の制御にも成功した。発表では、分子設計と遺伝子発現の可逆制御の詳細について議論する。

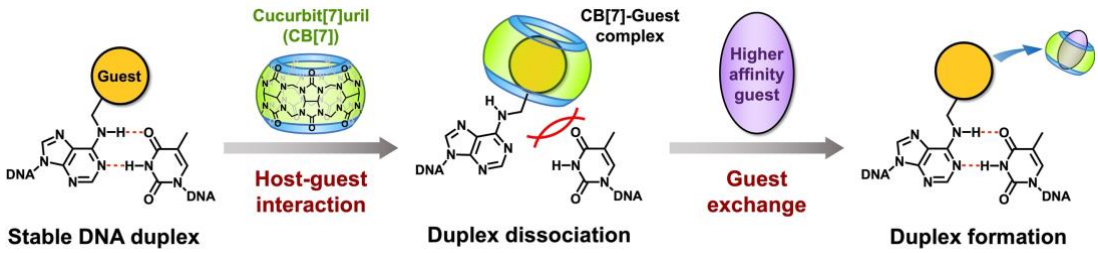


Fig. 1 ホスト–ゲスト相互作用による塩基対形成の可逆制御

Pseudorotaxane formation by cyclized oligo DNAs and approach to accelerating its formation

([1]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*, [2] *Department of Chemistry, Graduate School of Science, Tohoku University*) ○ Kazuki Kuwahara[1,2], Kazumitsu Onizuka[1,2], Sayaka Yajima[1,2], Yuuhei Yamano[1], Fumi Nagatsugi[1,2]
Keywords: Interlocked molecule; pseudorotaxane; cyclized nucleic acids

Since interlocked molecules such as rotaxane and catenane have unique properties that are not found in ordinary covalent molecules, they have been actively applied to artificial molecular machines driven by external stimuli. In nucleic acid chemistry, interlocked and threaded molecular structures have been studied for DNA nanotechnology, topological labeling, and complex stabilization. In our laboratory, we have discovered that a pseudorotaxane structure is spontaneously formed upon mixing cyclized DNA with a complementary target nucleic acid.[1)] However, complexity of the synthesis of cyclized nucleic acids made further investigation difficult. In this study, we successfully simplified the synthesis of pseudorotaxane-forming oligo DNAs (rfODN) and investigated the effects of linker position and length on the formation of the pseudorotaxane structures. These rfODNs formed pseudorotaxane structure at 37 °C with high efficiency (~90%, 2 h) simply by mixing with the complementary target RNA and rfODNs with longer linker showed the more efficient formation. Interestingly, rfODN with a ring at the 5'-side was found to be highly efficient in the formation of pseudorotaxane with RNA having a complementary region at the 3'-side. In contrast, rfODN with a ring at the 3'-side forms a highly efficient pseudorotaxane with RNA having a complementary region at the 5'-side. This result suggests that the threading direction can be controlled by changing the position of the ring (Figure 1). In addition, we found that the pseudorotaxane formation was accelerated by extending the duplex from the complementary sequence. We will report on them in detail.

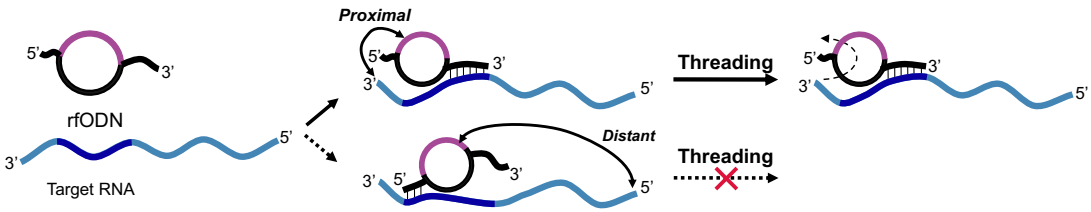


Figure 1. Schematic illustration of threading direction control.

1) K. Onizuka, T. Chikuni, T. Amemiya, T. Miyashita, K. Onizuka, H. Abe and F. Nagatsugi, *Nucleic Acids Res.* **2017**, *45*, 5036−5047.
2) K. Kuwahara, S. Yajima, Y. Yamano, F. Nagatsugi, K. Onizuka, *Bioconjugate Chem.* **2023**, *34*, 696−706.

ボラノホスフェート DNA のブロック合成法の確立

○高橋 裕平[1]、加藤 樹[1]、佐藤 一樹[1]、和田 猛[1] (1. 東京理科大学)

Development of the Convergent Synthesis of Boranophosphate DNA by an *H*-boranophosphonate Method ([1]Tokyo University of Scienece) ○Yuhei Takahashi[1], Itsuki Kato[1], Kazuki Sato[1], Takeshi Wada[1]

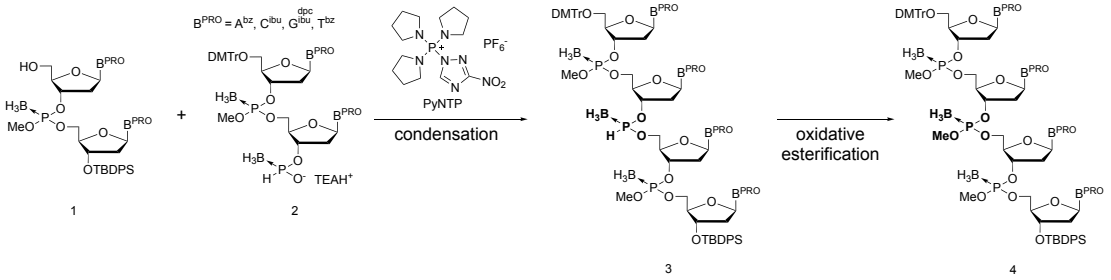
Boranophosphate DNAs (PB-DNAs), which have been suggested to be useful as antisense oligonucleotides, are difficult to synthesize by the conventional phosphoramidite method. In our laboratory, we developed the *H*-boranophosphonate method for the synthesis of boranophosphate DNAs. However, the synthesis of oligomer was conducted only by solid-phase synthesis, and large scale synthesis of PB-DNAs is remaining issue.

In this study, we apply this method to the block synthesis of boranophosphate oligonucleotides in solution by converting *H*-boranophosphonate diester **3** to the more stable boranophosphotriester **4** after condensation of *H*-boranophosphonate monoester **1** using a phosphonium type condensing reagent (PyNTP). By using this synthetic strategy, we have synthesized a boranophosphate DNA tetramer containing four nucleobases by condensation of the dimer building blocks followed by oxidative esterification of the internucleotidic bond.

Keywords : boranophosphate, H-boranophosphonate method, convergent synthesis

siRNA やアンチセンス核酸として有用であると示唆されているボラノホスフェート核酸は、一般的な核酸合成法であるホスホロアミダイト法では合成が困難である。当研究室は、*H*-ボラノホスホネート法を開発し、ボラノホスフェート核酸の合成を達成した。しかし、本手法でのオリゴマー合成は固相法に限られており、反応のスケールアップが困難であるという課題が残っている。

そこで本研究では、ホスホニウム型縮合剤を用いて化合物 **1** と *H*-ボラノホスホネートモノエステル **2** を縮合後、得られた *H*-ボラノホスホネートジエステル **3** をより安定なボラノホスホトリエステル **4** へと変換することで、本手法をブロック合成に応用し、液相にて長鎖のボラノホスフェート核酸のブロック合成を検討した。本合成戦略を用いて 2 量体のビルディングブロック同士の縮合・酸化を行うことで 4 種核酸塩基を有するボラノホスフェート核酸 4 量体の合成を達成したので、その詳細を報告する。



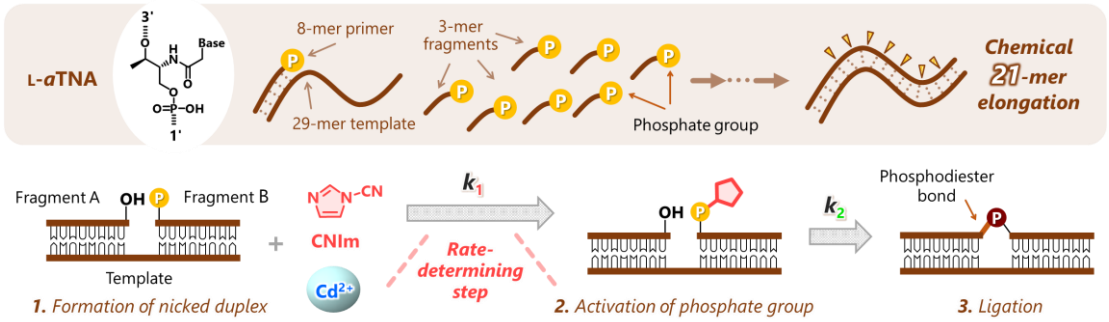
Reaction mechanism analysis of chemical ligation for long-chain elongation of L-*a*TNA

(*Graduate School of Engineering, Nagoya University*) ○Hikari Okita, Keiji Murayama, Hiroyuki Asanuma

Keywords: Chemical ligation; Artificial nucleic acid; Template-directed synthesis; DNA

DNA functions as a versatile biological tool due to its sequence specificity, but it is vulnerable to enzymatic degradations. Many artificial nucleic acids have been developed by chemical modification of DNA scaffold to provide enzyme resistivity. We have designed *acyclic* L-threoninol nucleic acids (L-*a*TNA) by changing D-ribose scaffold to acyclic scaffold. L-*a*TNA can form highly stable homo-duplex compared with DNA and it can hybridize with complementary strands of DNA and RNA.[1] However, since natural enzyme does not recognize L-*a*TNA, it cannot be a substrate of useful enzymes such as polymerase and ligase.

We have recently developed nonenzymatic pseudo-primer extension reactions by using *N*-cyanoimidazole (CNIm) and Mn^{2+} instead of enzymes, which enabled template-directed elongation of 9-mer L-*a*TNA from random trimer fragments.[2] If we achieve much efficient template-directed synthesis, design of L-*a*TNA aptamer, creation of artificial life, and nanotechnology based on L-*a*TNA will be possible. For this purpose, we focused on CNIm/M^{2+} system and ligation mechanisms were analyzed in detail to improve the efficiency of L-*a*TNA replication. It was revealed that Cd^{2+}, Ni^{2+}, and Co^{2+} dramatically increased the ligation rate more than Mn^{2+} for not only L-*a*TNA but also DNA ligations.[3] Furthermore, we performed kinetic analysis of chemical ligation of L-*a*TNA. The ligation proceeds mainly via three steps: (i) duplex formation between fragments and a template, (ii) activation by CNIm binding to phosphate group, and (iii) ligation of two fragments by generating a phosphodiester bond. We found that the activation was rate-determining step and stabilization of 3'-phopahte group of L-*a*TNA at nick site accelerated the ligation rate. Based on these results, we finally achieved elongation of 21-mer L-*a*TNA with Cd^{2+} and random trimers by reversing the elongation direction suitable for stabilizing phosphate group.[3]



1) K. Murayama *et al.*, *Chem. Commun.*, **2015**, *51*, 6500. 2) K. Murayama, H. Okita *et al.*, *Nat. Commun.*, **2021**, *12*, 804. 3) H. Okita *et al.*, *J. Am. Chem. Soc.*, **2023**, *145*, 17872.

New Data Science in Nucleic Acids Chemistry (10): Quantitative analysis for factors affecting i-motif formation in living cells estimated by the pseudo-cellular system

([1]*Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University,* [2]*Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University*) ○Kun Chen,[1] Hisae Tateishi-Karimata,[1] Naoki Sugimoto[1,2]

Keywords: Cancer; i-motif; Molecular crowding; Pseudo-cellular system; Living cell

The structural versatility of DNA is profoundly influenced by its surrounding environments, with the canonical duplex structure being just one facet of its dynamic conformational landscape. Beyond the conventional duplex, DNA exhibits the ability to adopt alternative structures such as triplexes, G-quadruplexes and i-motifs. The intricacies of DNA folding are further modulated by environmental factors, including the presence of cosolutes such as polyethylene glycol (PEG) and Ficoll, as well as cations like K^+ and Mg^{2+}.[1] These factors have been demonstrated to either stabilize noncanonical DNA structures or induce destabilization of short duplexes, thus adding an additional layer of complexity to the regulation of DNA stability.[2] However, molecular environment within living cells influencing i-motif structures are unknown. Intracellular environments are densely populated with an array of macromolecules, creating a highly crowding conditions and ionic strength setting for DNA structures like i-motif (Figure 1).

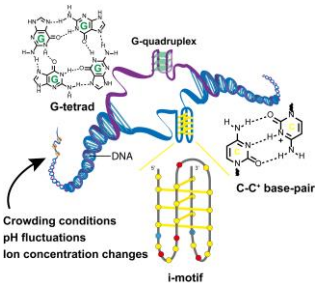


Figure 1. Scheme of intracellular environments providing conditions for i-motif formation.

The *CDH1* gene encodes E-cadherin, which is a protein responsible for cell adhesion. *CDH1* is a tumor suppressor gene, which contains many C-rich sequences that can form i-motif. In this study, as a typical example of an oncogene, we selected some C-rich sequences from *CDH1* to conduct a systematic study of pH dependence examining the relationship between intracellular conditions and i-motif dynamics in the context of cancer. Firstly, we employed biophysical techniques such as CD and UV spectroscopy to examine the stability of *CDH1*-derived C-rich sequences under varying pH and ion concentrations with cosolute mimicking cancer cell environments. Typically, we use PEG as a common cosolute. For example, i-motifs with PEG8000 is stable than in dilute solution. Our results demonstrated that i-motif stability was markedly altered in cosolute conditions, suggesting crowding conditions influences largely i-motif formation. In this presentation, we will show quantitative analysis of the determinants impacting i- motif formation within living cells, utilizing a novel pseudo-cellular system.

1) S. Takahashi, N. Sugimoto, *Chem. Soc. Rev.* **2020**, *49*, 8439. 2) H. Tateishi-Karimata, K. Kawauchi, N. Sugimoto, *J. Am. Chem. Soc.* **2018**, *140*, 642.

New Data Science in Nucleic Acids Chemistry (11): Transcriptional regulation in cancer cells induced by formation of G-quadruplexes and i-motifs

([1]*Frontier Institute for Biomolecular Engineering Research (FIBER) Konan University,* [2]*Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University,* [3]*Graduate School of Medical and Dental Sciences, Niigata University*)
○ Hisae Tateishi-Karimata,[1] Keiko Kawauchi,[2] Yiwei Ling,[3] Shujiro Okuda,[3] Naoki Sugimoto,[1,2]
Keywords: Molecular crowding; Cancer cell; G-quadruplex; i-motif; Transcript mutation

Formation of non-canonical DNA structures in response to the environment can regulate the expression of disease-related genes. It is known that the cell shape changes during with malignant transformation (Figure 1).[1] In addition, because of the increased expression of the cytoskeleton (F-actin), the environments in malignant cancer cells are more crowded than those in normal cells (Figure 1).[1] The changes should alter the intracellular molecular environments, affecting biological reactions responses to the structure and stability of nucleic acids. We have reported that G-quadruplexes are stabilized in normal and mild cancer cells and destabilized in a malignant cancer cells,[2] and that these changes regulate transcription although the detailed mechanism is still unknown. In this study, we investigated the effects of the intracellular environments on nucleic acids associated with malignant transformation of cancer. As results, the malignancy of the cancer cells is higher, the expression level of the cytoskeleton, which creates an intracellular crowding condition, was markedly increased. Moreover, we found that G-quadruplexes formed in normal cells, while, i-motifs were shown to form in malignant cancer cells. In the presentation, we will explain the effects of formation for G-quadruplexes and i-motifs on the transcriptional mutation.

1) S. Yamauchi, Y. Hou, A. Kunyao Guo, H. Hirata, W. Nakajima, A. Kia Yip, C. Yu, I. Harada, K. Chiam, Y. Sawada, N. Tanaka, K. Kawauchi, *J Cell Biol,* **2014**, *204*, 1191.
2) H. Tateishi-Karimata, K. Kawauchi, N. Sugimoto, *J. Am. Chem. Soc.* **2018**, *140*, 642.

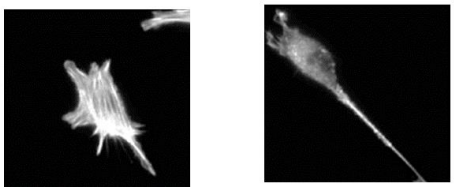


Figure 1. Comparison of cell shape between (a) normal cells and (b) malignant cancer cells. Images were taken with a confocal microscope. The cytoskeleton, which is F-actin, is shown in white.