アカデミックプログラム［A講演］ | 17. 生体機能関連化学・バイオテクノロジー：口頭A講演

2024年3月19日(火) 15:55 ~ 17:05 H932(9号館 [3階] 932)

[H932-2vn] 17. 生体機能関連化学・バイオテクノロジー

座長：高橋 俊太郎、寺 正行

英語
15:55 ~ 16:05
[H932-2vn-01]
核酸化学のNew Data Science (4): 擬似細胞システムと機械学習を用いたG-四重らせん構造を標的としたハイスループット・スクリーニング法の開発

○大山 達也[1]、建石 寿枝[1]、川内 敬子[2]、高橋 俊太郎[1]、田中 成典[3]、杉本 直己[1,2] (1. 甲南大学　先端生命工学研究所（FIBER)、2. 甲南大学　フロンティアサイエンス研究科(FIRST)、3. 神戸大学　システム情報学)

英語
16:05 ~ 16:15
[H932-2vn-02]
New Data Science in Nucleic Acids Chemistry (5): Effect of local environments on the stability of nucleic acids in mitochondria

○Saptarshi Ghosh[1], Lutan Liu[1], Shuntaro Takahashi[1], Tamaki Endoh[1], Naoto Yoshinaga[3], Keiji Numata[3,4], Naoki Sugimoto[1,2] (1. FIBER, Konan University, 2. FIRST, Konan University, 3. RIKEN, 4. Kyoto University)

英語
16:15 ~ 16:25
[H932-2vn-03]
New Data Science in Nucleic Acids Chemistry (6): Complementary effect of hydrogen bonding and base stacking on the stability of double-stranded nucleic acids

○Lutan Liu[1], Shuntaro Takahashi[1], Naoki Sugimoto[1,2] (1. FIBER, Konan University, 2. FIRST, Konan University)

英語
16:25 ~ 16:35
[H932-2vn-04]
New Data Science in Nucleic Acids Chemistry (7): Quantitative study of formation of DNA tetraplexes during cell cycle

○SINJAN DAS[1], SHUNTARO TAKAHASHI[1], NAOKI SUGIMOTO[1,2] (1. FIBER, Konan University, 2. FIRST, Konan University)

日本語
16:35 ~ 16:45
[H932-2vn-05]
四重鎖DNAを内包した人工ウイルスキャプシドの創製

○石井 楽乃[1]、稲葉 央[1]、遠藤 玉樹[2]、建石 寿枝[2]、杉本 直己[2]、松浦 和則[1] (1. 鳥取大院工、2. 甲南大学 先端生命工学研究所)

日本語
16:45 ~ 16:55
[H932-2vn-06]
ループ領域が決定するDNA四重らせん構造の構造形成速度

○中田 実紀[1]、小坂 直暉[1]、三好 大輔[1] (1. 甲南大学)

日本語

16:55 ~ 17:05

[H932-2vn-07]

チオフラビンT誘導体/グアニン四重鎖複合体の多価金属イオンに対する蛍光特性評価

○割石 智子[1]、片岡 由佳[1]、藤田 博仁[1]、笠原 勇矢[2,3]、小比賀 聡[2,3]、桒原 正靖[1] (1. 日大院総合基、2. 医薬基盤健栄研、3. 阪大院薬)

New Data Science in Nucleic Acids Chemistry (4): Development of high-throughput screening method targeting G-quadruplexes using the pseudo-cellular system and machine learning

([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] *Department of Computational Science, Graduate School of System Informatics, Kobe University*)
Tatsuya Ohyama,[1] ○ Hisae Tateishi-Karimata,[1] Keiko Kawauchi,[2] Shuntaro Takahashi,[1] Shigenori Tanaka,[3] Naoki Sugimoto,[1,2]
Keywords: Pseudo-cellular system; G-quadruplex; Transcription; High-throughput screening; Machine learning

Nucleic-acids-targeting compounds are being screened worldwide for inhibiting diseases-related biological reactions. However, compounds optimized *in vitro* often fail to function in cells, because biomacromolecules are under molecular crowding environments in cells. Here, we developed a new pseudo-cellular system (Figure 1) for highlighting the environments inside the cell and quantitatively investigated the environmental effects on DNA G-quadruplexes, which have recently attracted attention as targets for pharmaceuticals.[1] We screened compounds that bind to the G-quadruplexes and inhibit transcription *in vitro*, in the pseudo-cellular system, and in living cells. As results, the compounds showed similar trends of transcriptional inhibition both in the living cells and the pseudo-cellular system. In the presentation, we will discuss the structural features of the ligands which bound to the G-quadruplex and inhibited transcription using machine learning analysis.

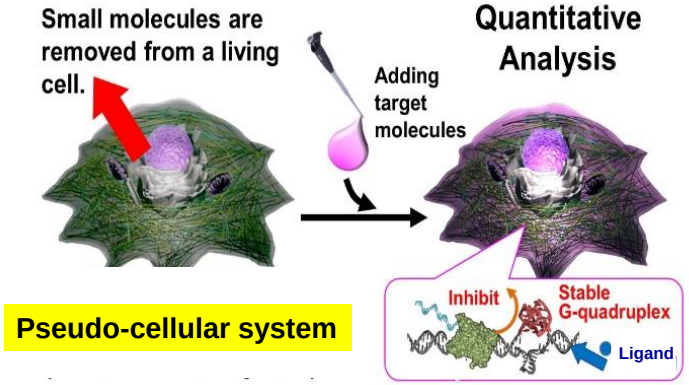

Figure 1. Schematic illustration of the construction of a pseudo-cellular system.

1) H. Tateishi-Karimata, K. Kawauchi, N. Sugimoto, *J. Am. Chem. Soc*. **2018**, *140*, 642.

New Data Science in Nucleic Acids Chemistry (5): Effect of local environments on the stability of nucleic acids in mitochondria

(*[1]FIBER, Konan Univ., [2]RIKEN., [3]Kyoto Univ., [4]FIRST, Konan Univ.*)
○Saptarshi Ghosh,[1] Lutan Liu,[1] Shuntaro Takahashi,[1] Tamaki Endoh,[1] Naoto Yoshinaga,[2] Keiji Numata,[2,3] Naoki Sugimoto[1,4]
Keywords: DNA stability, Mitochondria, Molecular crowding, G-quadruplex, Prediction

Molecular crowding affects the stability and conformation of nucleic acids (DNA and RNA),[1] leading to the formation of non-canonical DNA structures such as a guanine-quadruplex (G4) and i-motif that alter DNA replication and transcription and can cause diseases like cancer.[2] Although a link between molecular crowding and cellular function has been suggested, detailed information in localized region of the cells on this phenomenon is still lacking. For example, mitochondria, which are a key organelle for supplying the energy for the cell to exert all its functions, have own DNAs (mtDNAs). It has been suggested that G4s on mtDNAs have roles in regulation of replication and transcription of mtDNAs, which are vital for mitochondria functions. To clarify the roles of non-canonical structure in mitochondria, it is beneficial to predict the behavior of DNA structures depending on mitochondrial environment.

We have recently developed the stability prediction of DNA duplexes from their sequences in solutions containing different concentrations of cations and cosolutes.[3] As it has been suggested that the molecular crowding condition in mitochondria is more crowded with macromolecules than cytosol and nucleus,[4] we tested how the duplex formations could be predicted in the presence of polyethylene glycol (PEG) having large molecular weight like PEG8000. We found that the duplex formation having GC-rich sequence more destabilized in PEG8000 containing solution, compared with our prediction for in nucleus using PEG200 as a cosolute. Thus, the molecular environment in mitochondria may destabilize duplex and induce G4 formations more than that in nucleus. We are further investigating the effect on mitochondrial environment on G4 stability by using mitochondria-specific reporter assay and will discuss in the presentation.

1) S. Nakano, D. Miyoshi, N. Sugimoto, *Chem. Rev.,* **2014**, *114*, 2733. 2) a) S. Takahashi, J. A. Brazier, N. Sugimoto, *Proc. Natl. Acad. Sci. U. S. A.,* **2017**, *114*, 9605. b) H. Tateishi-Karimata, K. Kawauchi, N. Sugimoto, *J. Am. Chem. Soc.,* **2018**, *140*, 642. c) S. Takahashi, K. T. Kim, P. Podbevsek, J. Plavec, B. H. Kim, N. Sugimoto, *J. Am. Chem. Soc.,* **2018**, *140*, 5774. 3) S. Ghosh, S. Takahashi, T. Ohyama, T. Endoh, H. Tateishi-Karimata, and N. Sugimoto. *Proc. Natl. Acad. Sci. U. S. A.,* **2020**, *117*, 14194. 4) E. P. Bulthuis et al., *EMBO J.*, **2023**, *42*, e108533

New Data Science in Nucleic Acids Chemistry (6): Complementary effect of hydrogen bonding and base stacking on the stability of double-stranded nucleic acids

(*[1]FIBER, Konan Univ.,[2]FIRST, Konan Univ.*)
○ Lutan Liu[1]; Shuntaro Takahashi[1]; Naoki Sugimoto[1,2]
Keywords: Nucleic Acids, Watson-Crick base pairs, Hydrogen bonding, Stacking interaction, Conformational entropy

Hydrogen bonding, base stacking, and conformational entropy are widely accepted as the main structural determinants of duplex stability of nucleic acids. Conformational entropy has a significant effect on the free energy (ΔG^{o}_{37}) of hydrogen bonding and base stacking, since these interactions can be enhanced through translational and rotational movements of the nucleotides. However, conformational entropy is often neglected in the thermodynamic characterization of macromolecular folding. For instance, although the Nearest Neighbor (NN) parameters of various duplexes are well characterized [1-3], studies on the energetic constituents of the NN parameters rarely considers conformational entropy effect. Deconstructing the energetic contributions of NN parameters will aid the development of models that predict the stability of other secondary structures of nucleic acids.

In this study, we quantified and compared the ΔG^{o}_{37} contributions of base stacking and hydrogen bonding in DNA/DNA, RNA/RNA, and RNA/DNA duplexes. The ΔG^{o}_{37} of base stacking was obtained by comparing the ΔG^{o}_{37} of the core duplex and the duplexes with dangling ends. The ΔG^{o}_{37} of hydrogen bonding was obtained by subtracting ΔG^{o}_{37} contributions of base stacking from the ΔG^{o}_{37} of the terminal base pair, or by comparing the ΔG^{o}_{37} of two terminal base pairs with a single hydrogen bond difference. In line with the past studies [4], our results indicated that all the duplexes have a complementary relationship between ΔG^{o}_{37} of hydrogen bonding and base stacking (Figure 1). The duplexes showed varying degree of complementarity, indicating that there are distinct effects of conformational entropy on each duplex. Identifying the energetic contributions of structural factors to macromolecular folding is essential for elucidating the thermodynamic basis of biomolecular stability.

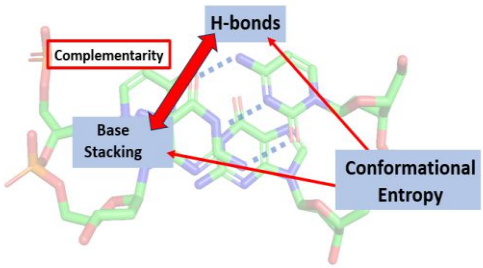

Figure 1. Schematic diagram of the relationship between hydrogen bonding, base stacking and conformational entropy

1) D. Banerjee, H. Tateishi-Karimata, T. Ohyama, S. Ghosh, T. Endoh, S. Takahashi, N. Sugimoto. *Nucleic Acids Res,* **2020,** 48, 12042. 2) S. Ghosh, S. Takahashi, T. Ohyama, T. Endoh, H. Tateishi-Karimata, N. Sugimoto. *Proc. Natl. Acad. Sc. U.S.A.,* **2020,** 117, 14194. 3) S. Ghosh, S. Takahashi, D. Banerjee, T. Ohyama, T. Endoh, H. Tateishi-Karimata, N. Sugimoto. *Nucleic Acids Res.*, **2023,** 51: 4101. 4) D. H. Turner, N. Sugimoto, R. Kierzek, S.D. Dreiker. *J. Am. Chem. Soc,* **1987**, 109, 3783.

New Data Science in Nucleic Acids Chemistry (7): Quantitative study of formation of DNA tetraplexes during cell cycle

([1]*FIBER, Konan University*, [2]*FIRST, Konan University*) ○Sinjan Das,[1] Shuntaro Takahashi,[1] Naoki Sugimoto[1,2]

Keywords: Cell cycle; Thermodynamics; Molecular crowding; i-motif; G-quadruplex

The cell cycle is the series of precisely controlled events in which a eukaryotic cell undergoes as it synthesizes proteins, replicates its DNA, and finally divides into two identical daughter cells (called G1, S, and G2/M phases, respectively, as shown in Fig. 1). During cell cycle progression, tetraplex DNAs such as G-quadruplexes (G4) and i-motifs (iM), which appear in the complementary sequences of each other, form along genomic DNAs interdependently in S and G1 phases of the cell cycle, respectively.[1,2] As thermodynamics of these tetraplex formations depend on molecular crowding in solution,[3] the phase specific cellular environments such as fluctuation of ATP concentration during the cell cycle[4] can regulate gene expressions according to the cell cycle progression through the interdependent formations of G4s and iMs.

To elucidate the mechanism, we investigated the G4 and iM formations on the genes related to the phase transition from G1 to S in the solution by tuning the concentration of ATP. We found a conserved motif of consecutive 3-5 G bases followed by GGAGG motif in the potential G4 forming sequences in the cyclin-dependent kinases 4 and 6 (CDK4 and CDK6) and their activating partners, D-type cyclins (CCND), which are the genes responsible for controlling core cell cycle machinery. Thermodynamic analysis indicated that the $-\Delta G°_{37}$ values of the these G4 formations were lower than those of the complementary iMs with increasing ATP concentrations. Molecular crowding conditions also facilitated the stabilization of iMs over G4s in the presence of high ATP condition. Our study suggests that spontaneous and dynamic changes of ATP concentration affect the specific regions of human genome to precisely drive the cell cycle progression through the formation of specific tetraplex structures. In the presentation, we will also discuss the formations of G4 and iM under different Mg^{2+} concentrations, which maintains a reciprocal relation with ATP concentration.

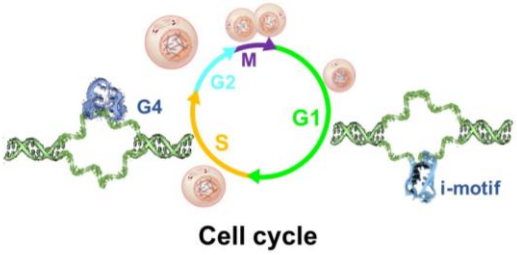

Fig. 1. Cell cycle dependent formation of tetraplex structures.

1) G. Biffi, D. Tannahill, J. McCafferty, S. Balasubramanian, *Nat. Chem*., **2013**, *5*, 182. 2) M. Zeraati, D. B. Langley, P. Schofield, A. L. Moye, R. Rouet, W. E. Hughes, T. M. Bryan, M. E. Dinger, D. Christ, *Nat. Chem*., **2018**, *10*, 631. 3) S. Takahashi, N. Sugimoto, *Chem. Soc. Rev*., **2020**, *49*, 8439. 4) K. Mitra, C. Wunder, B. Roysam, G. Lin, J. Lippincott-Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 11960.

四重鎖 DNA を内包した人工ウイルスキャプシドの創製

（鳥取大院工 [1]・甲南大 FIBER[2]）○石井 楽乃 [1]・稲葉 央 [1]・遠藤 玉樹 [2]・建石 寿枝 [2]・杉本 直己 [2]・松浦 和則 [1]

Creation of artificial viral capsid encapsulated quadruplex DNA ([1]*Graduate School of Engineering, Tottori University*, [2]*The Frontier Institute for Biomolecular Engineering Research, Konan University*) ○ Motono Ishii,[1] Hiroshi Inaba,[1] Tamaki Endoh,[2] Hisae Karimata Tateishi,[2] Naoki Sugimoto,[2] Kazunori Matsuura [1]

G-quadruplex DNA (G4) is known to be involved in regulating the gene expression of tumor-related genes, but the details of the structure and function of G4 in response to the intracellular environment have not been clarified. Therefore, there is a need for a technology for intracellular delivery of G4.[1)] We have previously created artificial viral capsids by self-assembly of *β*-annulus peptide derived from TBSV.[2)] In this study, we created artificial viral capsids encapsulated G4 DNA via disulfide bond as material for intracellular delivery of G4 (Fig. 1a). Co-assembly of G4 DNA-SS-*β*-Annulus and unmodified *β*-Annulus peptides (1:9 ratio) in 10 mM potassium phosphate buffer (pH7.5) afforded spherical assemblies of about 100 nm, suggesting the construction of G4-encapsulated artificial viral capsids (Fig. 1b,c).

Keywords : β-Annulus peptide; Artificial viral capsid; G-quadruplex DNA; Disulfide bond

グアニン四重鎖 DNA(G4)の形成が癌関連遺伝子のプロモーター領域に存在していることから、G4 は癌のバイオマーカーおよび治療の標的としての可能性を持っている [1)]。しかし、細胞内環境に応じた G4 の構造や機能の詳細は明らかになっていないため、G4 を細胞内にデリバリーする技術が求められている。一方我々は、TSBV 由来の 24 残基の*β*-Annulus peptide の自己集合により、30~50 nm の人工ウイルスキャプシドを構築することに成功しており、様々な分子を内包できることを示している [2)]。本研究では、ジスルフィド結合を介して*β*-Annulus ペプチドの N 末端に G4 を連結した G4 DNA-SS-*β*-Annulus を合成し、G4 DNA を内包した人工ウイルスキャプシドを創製した(Fig. 1a)。10 mM リン酸カリウム buffer(pH7.5)中で、G4 DNA-SS-*β*-Annulus と未修飾*β*-Annulus ペプチドを 1:9 で共集合させたところ、100 nm 程度の球状集合体を形成することを DLS 測定と TEM 観察により確認した (Fig. 1b,c)。

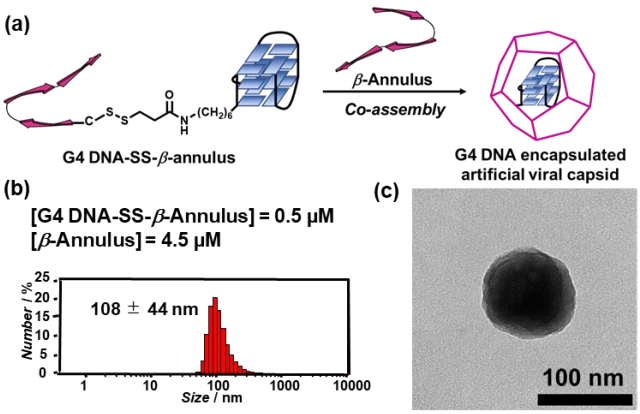

Fig. 1 (a) Construction of G4 DNA-encapsulated artificial viral capsid. DLS size distribution (b) and TEM image (c) of G4 DNA-encapsulated artificial viral capsids at 5 μM.

1) R. H. Hertsch, M. D. Antonio, S. Balasubramanian, *Nat. Rev. Mol. Cell Biol.*, **18**, 279 (2017).
2) K. Matsuura, *Chem. Commun*., **54**, 8944 (2018).

ループ領域が決定する DNA 四重らせん構造の構造形成速度

（甲南大 FIRST）○中田 実紀・小坂 直暉・三好 大輔

The Loop Region Dominating Folding Kinetics of DNA G-quadruplexes

(*Frontiers of Innovative Research in Science and Technology FIRST, Konan University*)
○Minori Nakata, Naoki Kosaka, Daisuke Miyoshi

Guanine-rich nucleic acid sequences have a propensity to form G-quadruplexes (G4s). The G4 folding mechanism is important topic to confirm the role of G4 in living cells and to design drugs which control biological events with targeting G4s. To date, it has revealed human telomere G4 folds through intermediates such as an antiparallel G4 and a guanine triple helix (G-triplex) after formation of guanine-guanine base pairs (G-hairpin). However, the G4 folding kinetics is totally depending on various factors such as nucleotide sequence and cation. Noteworthy, it was recently reported that a G4 with a loop region, in which a hairpin structure is formed (hairpin G4), folded faster than that did not contain the hairpin. In this study, we investigated the effects of hairpin loop structure on G4 folding kinetics. It was found that the hairpin at the loop region accelerated folding reactions as a nucleation site of a G-hairpin, which is former to the G4 formation. Furthermore, we quantitatively revealed for the first time that the relationship between G4 folding kinetics and RNA polymerase inhibitory function. These results suggest not only the general mechanism “nucleation-elongation” of G4 folding but also the importance of G4 folding kinetics for regulating biological events.

Keywords : G-quadruplex; kinetics; thermodynamics; folding, nucleation

グアニンに富む核酸鎖は、グアニン四重らせん構造(G4)を形成する。これまでに G4 の構造形成機構は、ヒトテロメア DNA G4 を対象に検討されてきた。現在では、ヒトテロメア G4 が、グアニン同士の塩基対からなる G ヘアピンが形成されたのち、アンチパラレル型 G4、グアニン三重らせん構造といった中間体を介して形成することが提唱されている [1]。しかし、G4 の構造形成機構や速度は条件によって様々であるため、G4 が関与している生物学的反応の分子機構解明や、それらを制御する方法を確立することは困難になっている。興味深いことに近年、ループ領域にヘアピン構造を形成する G4 がそうでない G4 と比較して、速く形成することが明らかになった [2](Fig. 1)。そこで本研究では、ループ領域のヘアピン構造における、G4 形成速度に対する効果を検討した。その結果、ヘアピン構造が核形成サイトとして働き、G4 形成速度を加速させることが明らかになった。これらの結果は、G4 の構造形成機構が、二重らせん構造やタンパク質にも見られる、一般的な機構であることが示唆された。さらに、G4 形成速度が G4 の酵素阻害能に大きく影響することも示された。

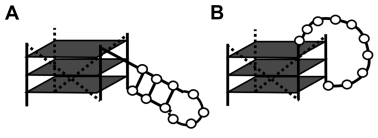

Fig. 1 Scheme of (A) structured loop G4 (hairpin G4) and (B) unstructured loop G4.

1) R. D. Gray, *et al., J. Mol. Biol.* **2014**, *426*, 1629.
2) T. Q. N. Nguyen, *et al., J. Phys. Chem. B.* **2020**, *124*, 5122.

チオフラビンT誘導体/グアニン四重鎖複合体の多価金属イオンに対する蛍光特性評価

（日大院総合基[1]・医薬基盤健栄研[2]・阪大院薬[3]）○割石 智子[1]・片岡 由佳[1]・藤田 博仁[1]・笠原 勇矢[2,3]・小比賀 聡[2,3]・桒原 正靖[1]
Fluorescence property of Thioflavin T derivatives/G-quadruplex complexes for multivalent metal ions ([1]*Graduate School of Integrated Basic Science, Nihon University,* [2]*National Institutes of Biomedical Innovation, Health and Nutrition*, [3]*Graduate School of Pharmaceutical Sciences, Osaka University*) ○Tomoko Wariishi,[1] Yuka Kataoka,[1] Hiroto Fujita,[1] Yuuya Kasahara,[2,3] Satoshi Obika,[2,3] Masayasu Kuwahara[1]

The fluorescence of thioflavin T (ThT) varies greatly depending on the dihedral angle between the benzothiazole ring and the dimethylaminobenzene ring in the excited state. It is known to emit strong fluorescence upon binding to amyloid fibrils and guanine quadruplex (G4) [1–5]. Taking advantage of this property of ThT, we devised fluorescent sensors for the detection of divalent metal ions by combining a chelate-conjugating ThT with G4. The fluorescence sensors revealed that the fluorescence increased or decreased depending on the kind of divalent metal ions. Furthermore, the effects of divalent metal ions on the formation of ThT derivative/G4 complexes and fluorescence emission were examined by circular dichroism (CD) analysis, surface plasmon resonance (SPR) measurement, and Job plot methods. We have also investigated the luminescence properties (phosphorescence) of complexes of lanthanide metals such as Eu^{3+} and Tb^{3+} with ThT derivatives in the presence of G4 with different topology.

Keywords : Thioflavin T, Chelator, Metal ion, Fluorescent sensor, G-quadruplex

チオフラビン T (ThT) は、励起状態におけるベンゾチアゾール環とジメチルアミノベンゼン環の二面角によって蛍光が大きく変化する。これにより、アミロイド線維やグアニン四重鎖 (G4) と結合することで強い蛍光を発することが知られている [1–5]。この ThT の性質を利用して、我々はキレート剤とコンジュゲート化した ThT 誘導体と G4 とを組み合わせた二価金属イオンを検出するための蛍光センサーを開発した。この蛍光センサーは、二価金属イオンの種類によって、蛍光が増大したり減少したりすることを明らかにした。さらに、円偏光二色性スペクトル (CD) 法や表面プラズモン共鳴 (SPR) 法、Job plot 法を用いて二価金属イオンが ThT 誘導体/G4 複合体の形成や蛍光発光に及ぼす影響について検証した。また、トポロジーの異なる G4 の存在下で、ランタノイド金属である Eu^{3+}や Tb^{3+}と ThT 誘導体の錯体の発光特性（りん光）について検討を行ったので合わせて報告する。

1) Mohanty J, Barooah N, Dhamodharan V, Harikrishna S, Pradeepkumar PI, Bhasikuttan AC. *J. Am. Chem. Soc*. **2013**, 135, 367–376.
2) Kataoka Y, Fujita H, Kasahara Y, Yoshihara T, Tobita S, Kuwahara M. *Anal. Chem*. **2014**, 86, 12078–12084.
3) Kataoka Y, Fujita H, Endoh T, Sugimito N, Kuwahara M. *Molecules* **2020**, 25, 4936.
4) Kataoka Y, Fujita H, Afanaseva A, Nagao C, Mizuguchi K, Kasahara Y, Obika S, Kuwahara M. *Biochemistry* **2019**, 58, 493–498.
5) Zhao L, Ahmed F, Zeng Y, Xu W, Xiong H. *ACS. Sens.* **2022**, 7, 2833–2856.