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

Wed. Mar 26, 2025 9:00 AM - 11:40 AM JST | Wed. Mar 26, 2025 12:00 AM - 2:40 AM UTC
[A]D401(D401, Bldg. 4, Area 3 [4F])

[[A]D401-1am] 17. Biofunctional Chemistry, Biotechnology

Chair: Shuntaro Takahashi, Yousuke Katsuda

English
9:00 AM - 9:20 AM JST | 12:00 AM - 12:20 AM UTC
[[A]D401-1am-01]

Expanding the versatility of RNA hacking technology by G supply Staple oligomers

○Kida Kida Tomoki[1], Yousuke Katsuda[1,2], Yua Hasegawa[1], Miko Kato[1], Mahiro Ohtani[1], Shin-ichi Sato[1,2], Yusuke Kitamura[1], Toshihiro Ihara[1] (1. Faculty of Advance Science and Technology, Kumamoto University, 2. StapleBio inc.)

English
9:20 AM - 9:40 AM JST | 12:20 AM - 12:40 AM UTC
[[A]D401-1am-02]

Application of RNA hacking to enable enhanced gene expression

○Yua Hasegawa[1], Yousuke Katsuda[1,2], Sato Shin-ich[1,2], Yusuke Kitamura[1], Ihara Toshihiro[1] (1. Kumamoto University, 2. StapleBio Inc.)

English
9:40 AM - 10:00 AM JST | 12:40 AM - 1:00 AM UTC
[[A]D401-1am-03]

New Data Science in Nucleic Acids Chemistry (12): Role of groove hydration on stability and functions of biased DNA duplexes

Sarptarshi Ghosh[1], Shuntaro Takahashi[1,2], Tatsuya Ohyama[1], Lutan Liu[1], ○Naoki Sugimoto[1] (1. Konan Univ., FIBER, 2. Konan Univ., FIRST)

English
10:00 AM - 10:20 AM JST | 1:00 AM - 1:20 AM UTC
[[A]D401-1am-04]

New Data Science in Nucleic Acids Chemistry (13): Global and local molecular crowding effects depending on the size of crowding cosolute on stability of pseudoknot RNA

○Tamaki Endoh[1,2], Sagar Satpathi[1], Naoki Sugimoto[1] (1. FIBER, Konan University, 2. FIRST, Konan University)

10:20 AM - 10:40 AM JST | 1:20 AM - 1:40 AM UTC

Break

English
10:40 AM - 11:00 AM JST | 1:40 AM - 2:00 AM UTC
[[A]D401-1am-05]

New Data Science in Nucleic Acids Chemistry (14): Development of methods to predict RNA secondary structures in cells

○Hisae Tateishi-Karimata[1,2], Dipanwita Banerjee [1], Shuntaro Takahashi [1,2], Tomohiro Nishimura [3], Tsukasa Fukunaga[4], Michiaki Hamada[3], Maria Orehova[5], Janez Plavec [5], Naoki Sugimoto[1] (1. FIBER, Konan University, 2. FIRST, Konan University, 3. Department of Electrical

Engineering and Bioscience, Graduate School of Advanced Science and Engineering, Waseda University, 4. Waseda Institute for Advanced Study , 5. Slovenian NMR Centre)

English

11:00 AM - 11:20 AM JST | 2:00 AM - 2:20 AM UTC

[[A]D401-1am-06]

New Data Science in Nucleic Acids Chemistry (15): Universal prediction of DNAzyme activity using new nearest neighbor parameters and AI

○Shuntaro Takahashi[1,2], Tomohiro Nishimura[3], Saptarshi Ghosh[1], Hisae Tateishi-Karimata[1,2], Tsukasa Fukunaga[4], Michiaki Hamada[3], Naoki Sugimoto[1] (1. Konan Univ., FIBER, 2. Konan Univ., FIRST, 3. Waseda Univ., 4. Waseda Univ., WIAS)

English

11:20 AM - 11:40 AM JST | 2:20 AM - 2:40 AM UTC

[[A]D401-1am-07]

Chemical property and positioning of the nucleosome altered by platinum-based antineoplastics

○Xuanyu Liao[1], Takafumi Furuhata[1], Akimitsu Okamoto[1] (1. The University of Tokyo)

Expanding the versatility of RNA hacking technology by G supply Staple oligomer

([1] *Faculty of Advance Science and Technology, Kumamoto University,* [2]*StapleBio Inc.*) ○Tomoki Kida,[1] Yousuke Katsuda,[1,2] Yua Hasegawa,[1] Miko Kato,[1] Mahiro Ohtani,[1] Shin-ichi Sato, [1,2] Yusuke Kitamura,[1] Toshihiro Ihara,[1]

Keywords: *Nucleic acids medicine; RNA G-quadruplex; RNA hacking*

Nucleic acid medicines facilitate the expeditious design of seed molecules and hold promise for the development of medicines to treat rare diseases. Among these, small interfering RNA (siRNA), which leverages RNA interference (RNAi), forms a complex with Argonaute proteins to degrade target mRNAs, thereby providing a powerful gene-silencing tool. Despite its versatility in targeting a wide range of genes, siRNA suffers from reduced silencing efficacy when non-natural nucleic acids are incorporated and raises concerns about off-target effects due to unintended interactions with non-target mRNAs.

We previously described the development of RNA hacking (RNAh), a new technology that induces the formation of RNA G-quadruplexes (rG4) on target mRNAs using short nucleic acids named Staple oligomer. The rG4 structures induced by Staple oligomers inhibit the ribosomal translation process. Staple oligomer, in repressing protein expression level, differ from existing nucleic acids medicine mechanisms like siRNA in that they do not rely on endogenous enzyme such as Argonaute. This independent mechanism allows them to maintain their gene-silencing efficacy even in cases where non-natural nucleic acids are incorporated as components of the Staple oligomer. While this provides a significant advantage in terms of efficacy, there remains a challenge regarding the number of genes RNAh can target effectively. Bioinformatics analysis indicated that the previously reported Staple oligomers are applicable to only about 65% of all genes, indicating a significant limitation. To expand the applicability of RNAh, it is necessary to develop methods for targeting a broader range of mRNAs.

In this presentation, we are going to report G-tracts supply Staple oligomer (Gs Staple oligomer), which have been designed with the objective of enhancing the versatility of RNAh. Gs Staple oligomer incorporate G-tract sequences that are essential components of rG4, thereby reducing the sequence constraints of target mRNAs (**Fig. 1a**). Furthermore, we demonstrated that Gs Staple oligomer, designed for *m*TRPC6 mRNA, exhibit gene silencing effects *in vivo* (**Fig. 1b**). In addition to these findings, we will report how modifying the number of linkers between the two G-tracts in the Gs Staple oligomer allows for the fine-tuning of the gene silencing effect.

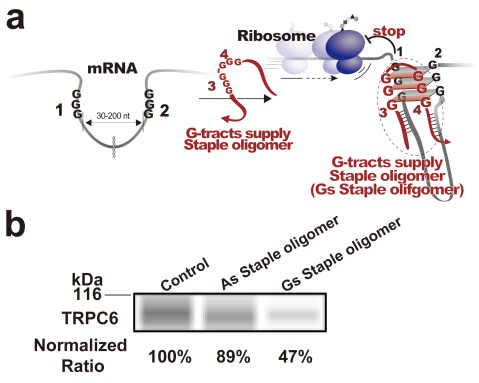


Fig. 1 a. Mechanism of action G-tracts supply Staple oligomer. **b.** Evaluation of protein expression levels of *m*TRPC6 *in vivo* by western blotting. As Staple oligomers are sequences in which G-tracts are replaced with adenine.

Application of RNA hacking to enable enhanced gene expression

([1]*Faculty of Advanced Science and Technology, Kumamoto University*, [2]*StapleBio Inc.*) ○Yua Hasegawa,[1] Yousuke Katsuda,[1,2] Shin-ichi Sato,[1,2] Yusuke Kitamura,[1] Toshihiro Ihara [1]
Keywords: *Nucleic acid medicine; RNA G-quadruplex; RNA hacking*

Insufficient expression of certain genes due to mutations or deletions is the cause of many serious diseases. Haploinsufficiency is a typical example, caused by a mutation in one allele that results in the lack of protein expression from that allele, leading to an overall deficiency in protein levels. Effective treatments for these diseases are still under development. While gene silencing technologies such as siRNA are well-established, technologies for increasing target gene expression have not yet been fully developed. There is a strong need for the development of universally applicable gene enhancement technologies that can be applied to a wide range of genes. We recently introduced an RNA hacking (RNAh) technology utilizing a short oligonucleotide known as the Staple oligomer, which promotes the formation of RNA G-quadruplexes (rG4) by bringing guanine-rich sequences into proximity. RNAh has been demonstrated to target the 5' UTR or ORF of mRNA, where it inhibits the ribosomal amino acid synthesis reaction, thereby reducing protein expression levels. In this study, we focus on Staple oligomer targeting the 3' UTR region. Staple oligomer designed to induce rG4 formation in the 3' UTR have the potential to enhance mRNA stability by preventing degradation pathways, such as exonuclease activity, ultimately leading to increased gene expression (Fig. 1).
In addition, RNAh can be applied to Xeno nucleic acid (XNA) based Staple oligomer that is suitable for pharmaceutical efficacy. Therefore, we conducted a proof-of-concept study to enhance gene expression using XNA Staple oligomer. In this presentation, we will share the latest findings demonstrating enhanced gene expression achieved through RNAh, highlighting its potential as a novel tool for precise regulation of gene expression.

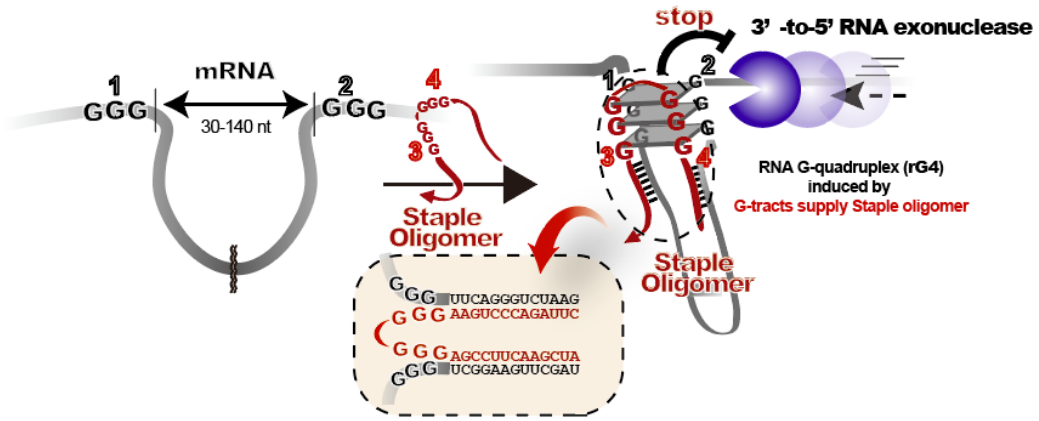


Fig. 1 Mechanism of mRNA degradation resistance enhancement by Staple oligomer. rG4 formation in the 3'UTR is thought to increase stability by inhibiting the reaction progression of nuclease.

New Data Science in Nucleic Acids Chemistry (12): Role of groove hydration on stability and functions of biased DNA duplexes

([1]*FIBER, Konan University,* [2]*FIRST, Konan University*) Saptarshi Ghosh,[1] Shuntaro Takahashi,[1,2] Tatsuya Ohyama,[1] Lutan Liu,[1] ○Naoki Sugimoto[1]
Keywords: DNA; Hydration; Stability; Function; Cell

We have determined new nearest neighbor parameters to predict stability of the canonical duplex structures of nucleic acids[1] and elucidated roles of their non-canonical structures.[2] DNA sequences with a biased composition of bases, such as repeated GC- or AT-tracts, are often found in regulatory regions of the genome, such as telomere and promoter regions, exhibiting dynamic duplex unfolding for gene replication and transcription. More importantly, under certain solution conditions, depending on pH and the presence of cations and cosolutes, these unfolded duplex regions with biased sequences can form non-canonical structures in each strand. For example, G-rich and C-rich sequences can fold into G-quadruplex and i-motif structures, respectively. The AT-biased regions can also form triplex structures upon binding of the third strand in the major groove of the duplex. These non-canonical DNA structures originating from the unfolded biased sequence of bases often regulate the expression of genes related to several diseases, including cancer and neurodegenerative disorders Thus, the intracellular stability of biased duplexes determines the relative population between the canonical duplex and non-canonical structures, and large deviations in this relative population could have pathological consequences due to the aberrant regulation of genes. Therefore, DNA duplex stability prediction is very important for understanding and designing functions of non-canonical structures in cell.

In this study, we systematically studied the thermodynamics of duplex formation for biased DNAs in a cell-mimicking solution and quantify the effects of groove hydration on their thermodynamics. The interaction of crowders with water molecules in the grooves was found to provide excess stabilization to biased DNAs than to unbiased DNAs, as estimated from the nearest-neighbor prediction model.

1) a) *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117*, 14194–14201. b) *Nucleic Acids Res.* **2023**, *51*, 4101-4111. c) *J. Am. Chem. Soc.,* **2023**, *145*, 23503-23518. d) *J. Am. Chem. Soc.,* **2024**, *146*, inpress.
2) a) N. Sugimoto, ed., “Handbook of Chemical Biology of Nucleic Acids”, SPRINGER NATURE, **2023**, Vols. 1, 2, and 3. b) “Chemistry and Biology of Non-Canonical Nucleic Acids”, WILEY, **2021**, 1-276. c) *Acc. Chem.Res.* **2021**, *54*, 2110-2120. d) *Chem. Soc. Rev.,* **2020**, *49*, 8439–8468. e) *J. Am. Chem. Soc.,* **2024**, *146*, 8005-8015.

New Data Science in Nucleic Acids Chemistry (13): Global and local molecular crowding effects depending on the size of crowding cosolute on stability of pseudoknot RNA

(*[1]FIBER, Konan Univ., [2]FIRST, Konan Univ.*)
○Tamaki Endoh,[1,2] Sagar Satpathi,[1] Naoki Sugimoto[1]
Keywords: High-order structure, RNA, Pseudoknot, Stability, Crowding

RNA pseudoknot (PK) is a common motif in functional RNAs. Intracellular functions of RNAs are expected to depend on the stability of their high-order structures, in which stabilities differ depending on molecular environments. We have demonstrated that crowding cosolutes, composed of diverse small and large molecular sizes, significantly alter the thermodynamic properties of RNA structures.[1] Understanding how these crowding cosolutes influence PK stability is essential for deciphering contribution of PK structures involved in RNA functions.

In this study, we evaluated the impact of the crowding cosolutes on PKs stability, focusing on their S2 stem region, which formation differentiates PK structure from simple hairpin conformer. Polyethylene glycol (PEG) with different average molecular weights of 200 (PEG200) and 8000 (PEG8000) were employed to mimic the crowding environments. Studies using PK variants containing abasic sites on a loop, revealed distinct stabilization mechanisms depending on the size of the crowding cosolutes. The nucleobases in the loop region destabilized the S2 stem under diluted conditions by disturbing hydration required to be stabilized.[2] Local stabilization of S2 stem region was observed in the presence of PEG200 through diminished destabilizing effects caused by the nucleobases in the loop region. On the other hand, PEG8000 exerted a global stabilizing effect by promoting the compact PK conformation via excluded volume effects (Fig. 1).[3] The results in this study provide critical insight and serve as a bridge between the diverse structural characteristics of PKs and mechanisms of their functionalization in varying cellular contexts.

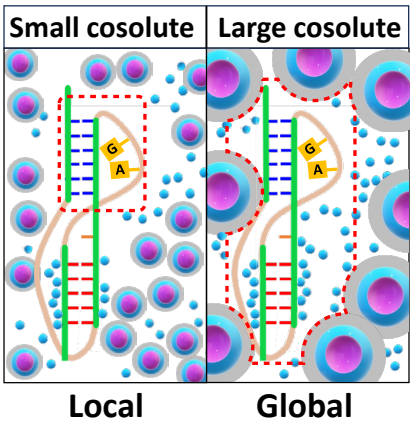


Fig. 1 Distinct stabilization mechanisms caused by small and large crowding cosolutes.

1) T. Endoh, H. Tateishi-Karimata, and N. Sugimoto, in ***Handbook of Chemical Biology of Nucleic Acids***, ed. N. Sugimoto, Springer Nature Singapore, Singapore, (2022), DOI: 10.1007/978-981-16-1313-5_40-1, pp. 1-45. 2) S. Satpathi, T, Endoh, N. Sugimoto, *Chem*. Commun., **2022**, *58*, 5952. 3) S. Satpathi, T, Endoh, N. Sugimoto, *Med. Chem. Res.*, **2024**, *33*, 2079.

New Data Science in Nucleic Acids Chemistry (14): Development of methods to predict RNA secondary structures in cells

(*[1]FIBER, Konan Univ., [2]FIRST, Konan Univ., [3]Waseda Univ., Faculty of Science and Engineering, [4]Waseda Univ. WIAS*)
○ Hisae Tateishi-Karimata,[1,2] Banerjee Dipanwita,[1] Shuntaro Takahashi,[1] Tomohiro Nishimura,[3] Tsukasa Fukunaga,[4] Michiaki Hamada,[3] Naoki Sugimoto[1]
Keywords: RNA/RNA duplex; Molecular crowding; Stability prediction; Nearest neighbor parameters; AI

The stability of RNA structures in cells is important for predicting RNA functions. Intracellular crowded environments affect significantly structure and stability of nucleic acids.[1,2] Living cells contain various organelles, cytoskeletons, and soluble and insoluble biomolecules. In order to predict the RNA structures, we have developed nearest neighbor parameters for RNA/RNA duplexes consisting with Watson-Crick base pairs under the molecular crowding contditions.[3] However, it is essential to predict accurately the stability of not only Watson-Crick base pairs, but also Non-Watson-Crick base pairs base pair because functional RNAs contain mismatches (Figure 1a). Here, we evaluated the thermodynamic parameters of duplexes with single mismatched and fully matched base pairs in dilute and crowded conditions (Figure 1b). Mismatched duplexes were destabilized with the increasing order of pyrimidine•pyrimidine < purine•pyrimidine < purine•purine under the crowding condition, with the exception of A•G mismatch. We proposed a method for predicting individual contribution of the 12 mismatches to the stability of RNA that provided a basis for an increasingly accurate prediction of intracellular RNA secondary structure. In the presentation we will discuss the results of using pseudo-cellular systems and AI to predict RNA structure in the intracellular environments.

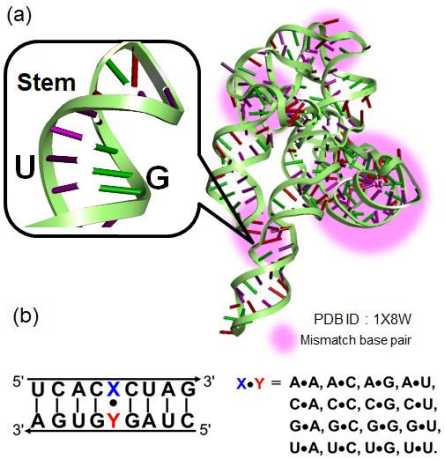


Figure 1. (a) Schematic image of the mismatch in functional RNA (Group I intron). (b) Schematic representation of RNA duplexes formed by 5′ -rUCACXCUAG-3′ and 5′ -rCUAGYGUGA-3′ sed in this study. "X" and "Y" denote positions of base pairs and mismatches shown on the right.

1) D. Banerjee, H. Tateishi-Karimata, M. Toplishek, T. Ohyama, S. Ghosh, S. Takahashi, M. Trajkovski, J. Plavec, N. Sugimoto, *J. Am. Chem. Soc*. **2023**, *145*, 23503. 2) H. Tateishi-Karimata, K. Kawauchi, S. Takahashi N. Sugimoto, *J. Am. Chem. Soc*. **2024**, *146,* 8005. 3) S. Ghosh, S. Takahashi, D. Banerjee, T. Ohyama, T. Endoh, H. Tateishi-Karimata, N. Sugimoto, *Nucleic Acids Res*. **2023**, *51*, 4101.

New Data Science in Nucleic Acids Chemistry (15): Universal prediction of DNAzyme activity using new nearest neighbor parameters and AI

(*[1]FIBER, Konan Univ. [2]FIRST, Konan Univ., [3]Waseda Univ., Faculty of Science and Engineering, [4]Waseda Univ. WIAS*)
○Shuntaro Takahashi,[1,2] Saptarshi Ghosh,[1] Hisae Tateishi-Karimata,[1,2] Tomohiro Nishimura.[3] Tsukasa Fukunaga,[4] Michiaki Hamada,[3] Naoki Sugimoto[1]
Keywords: Nearest neighbor (NN) model, Duplex, Stability prediction, AI, Functional prediction

For the functions of DNA and RNA based on base pairing such as Watson-Crick base pairs, the stability of forming base pairing is fundamental. Nearest neighbor (NN) model is the most successful method to predict the Gibbs free energy (ΔG°_{37}) of the formation of duplexes. We have recently reported NN parameters for all duplexes (DNA, RNA, and RNA/DNA hybrid) for the stability prediction universally available diverse crowding conditions.[1-4] As the catalytic functions of nucleic acids require the suitable solution conditions with especially Mg^{2+} and molecular crowding, such versatile prediction of the duplex stability is of interest to utilize the nucleic acids functions. Here, we demonstrated the prediction of the activity of the RNA-cleaving 8-17 DNAzyme by using ΔG°_{37} values for DNA duplexes predicted by our newly developed nearest neighbor (NN) parameters. As the enzymatic activity depends on Mg^{2+} concentrations and molecular crowding conditions, we improved the NN model to segmentate the thermodynamic parameters of each NN base pair into those affected by cation and cosolulte concentrations. By using the updated NN parameters and enzymatic activity in different Mg^{2+} concentrations and various molecular crowdings as a database, we characterized the relationship between the sequence information and enzymatic activity with machine learning techniques (Figure 1). Our results suggest that the NN parameters supported to efficiently and accurately predict the enzymatic reaction, which highlights the advantage of the NN parameters for predicting the functions of nucleic acids from the sequence information.

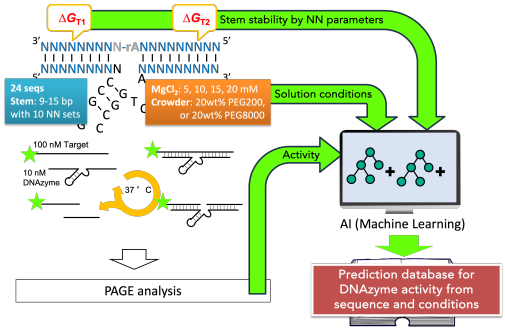


Figure 1. Schematic illustration of development of prediction database for DNAzyme activity from sequence and conditions.

1) S. Ghosh, S. Takahashi, T. Ohyama, T. Endoh, H. Tateishi-Karimata, and N. Sugimoto. *Proc. Natl. Acad. Sci. U. S. A.,* **2020,** *117*, 14194. 2) S. Ghosh, S. Takahashi, D. Banerjee, T. Ohyama, T. Endoh, H. Tateishi-Karimata and N. Sugimoto. *Nucleic Acids Res.*, **2023,** *51*, 4101. 3) D. Banerjee, H. Tateishi-Karimata, M. Toplishek, T. Ohyama, S. Ghosh, S. Takahashi, M. Trajkovski, J. Plavec, and N. Sugimoto. *J. Am. Chem. Soc.,* **2023,** *145*, 23503. 4) S. Ghosh, S. Takahashi, T. Ohyama, L. Liu, N. Sugimoto, *J. Am. Chem. Soc.,* **2024**, 146, 32479.

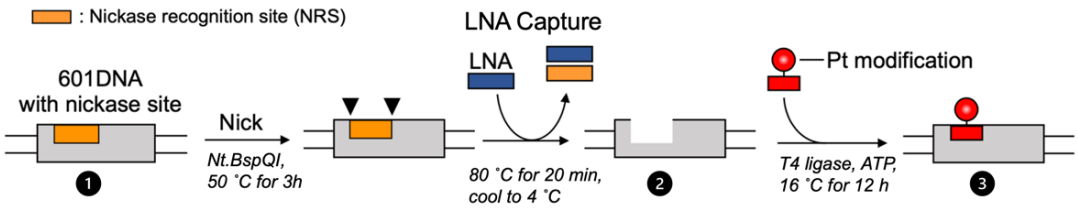
Chemical property and positioning of the nucleosome altered by platinum-based antineoplastics

([1]*Graduate School of Engineering, The University of Tokyo*,) ○Xuanyu Liao,[1] Takafumi Furuhata,[1] Akimitsu Okamoto[1]

Keywords: Platinum-Based Antineoplastic; Nucleosome; Nucleosome Sliding; Nucleosome Positioning

The platinum-based antineoplastic agent represented by cisplatin and its platinum analogs, carboplatin and dichloro (1,2-diaminocyclohexane) platinum (DACH-Pt), is one of the major classes of anti-cancer drugs currently. Their mode of action involves covalent binding to purine DNA bases to cause DNA lesion, which finally leads to cellular apoptosis.[1] Previous report shows that intracellular pathways that induce cellular apoptosis are different depending on their chemical structures. However, the molecular mechanisms underlying the influence of Pt-based drugs on nucleosome dynamics, which regulate biochemical reactions on DNA, still remains elusive.[2] Here, we want to further investigate the nucleosome dynamics of structure and localization caused by platinum-based drugs.

It is reported that DNA at SHL 1.5 (1.5 helical turns from the NCP dyad axis) is bent to be the preferred binding site for DNA damaging agents and it is close to the positively charged region of core histones and N-terminal tail of histones[3]. Therefore, I designed nucleosome site-specifically modified with platinum drugs, which is also charged positively, at the SHL1.5 to evaluate its effect on the nucleosome properties. To this aim, we took advantage of Plug-and-play method[4] to reconstitute model nucleosomes modified with two site-specifical types of platinum-based drugs, cisplatin (CisPt) and DACH-Pt which were suggested to have different mechanism actions depending on their chemical structures. Then, we inspected the physiochemical and biochemical characteristics of the constructed nucleosomes altered by platinum modification. Moreover, the changes of nucleosome positioning depending on the chemical structures of the platinum adducts were evaluated to get insights into their intrinsic effects on the nucleosome properties. The basic understanding of the relationships between nucleosome property dynamics and the mode of actions of drugs would expand the scope of molecular design for DNA targeting antineoplastics.



1) L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573-584. 2) E. C. Sutton *et al*., *J. Am. Chem. Soc*. **2019**, *141*, 18411-18415. 3) M. Ren *et al*., *Acc. Chem. Res.* **2022**, *55*, 1059–1073. 4) D. R. Banerjee *et al*. *J. Am. Chem. Soc*. **2018**, *140*, 8260-8267.