Activity Report: MERIT Self-Directed Joint Research

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Research Topic

Structural studies on Cu^{II} assemblies inside DNA duplexes having ethenoadenosine bases as metal binding sites.

Background

Arranging metal ions in a nanoscale is imperative towards the miniaturization of present-day devices. Bottom-up development of Metal-Organic Frameworks (MOFs) remains a powerful strategy to develop metallo-architectures that offer a wide range of applications in fields ranging from electronics to bionanotechnology. The arrangement of metal ions in nanoscale can be aided by organic frameworks, which provides flexibility in design as well as synthetic tactics. The development of suitable organic frameworks to capture metal ions remains a challenge due to the cumbersome synthetic steps.

DNA-templated metal assembly is a simple, yet powerful strategy to construct metal nanowires in a highly selective and programmable manner. Using an automated DNA synthesizer, it is possible to incorporate ligand-type unnatural nucleobases at predetermined sites inside the DNA. The ligand-type nucleobases can coordinate to metal ions resulting in the formation of "metal-mediated base pairs" inside DNA duplexes (**Figure 1**). Shionoya Group has made a great deal of progress in this area by developing several artificial nucleobases suited for the metal-mediated base pairing and constructted one-dimensional assemblies of Cu^{II}, Hg^{II}, Gd^{III}, etc. inside DNA duplexes.[1] We have also utilized these metal-mediated base-pairs in constructing highly sophisticated DNA architectures such as DNA-three way junctions[2] and allosteric DNAzymes.[3]

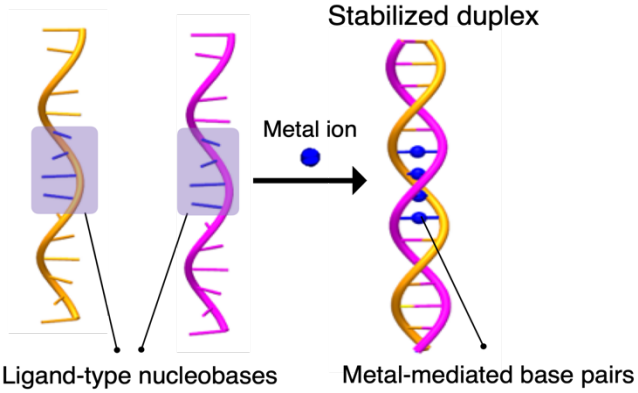


Figure 1. Schematic representation of formation of metal-mediated base pairs inside DNA duplexes

Early research on the development of metal-mediated base pairs widely made use of fully artificial nucleobases such *O,O*-bidentate hydroxypyridone (**H**) to capture metal ions.[4] Yet their multi-step, low yielding synthesis limited their utility in further application in nanotechnology. In contrast to artificial nucleobases, modified nucleobases are excellent candidates because they are well-studied and can be easily derived from their natural analogues. Therefore, I have been making use of modified nucleobases for the development of metallo-DNA architectures. Using a well-known damaged nucleobase 1,N^6-ethenoadenosine (**εA**) as metal-binding sites, I was able to capture and arrange Cu^{II} ions inside a DNA duplex. The duplexes containing **εA**–**εA** pair were stabilized by adding Cu^{II} ions through the formation of **εA**–Cu^{II}–**εA** pairs. By constructing DNA duplexes bearing varying number of **εA**–**εA** mismatch pairs, I synthesized and characterized programmable Cu^{II} self-assembly (**Figure 2**).[5–7]

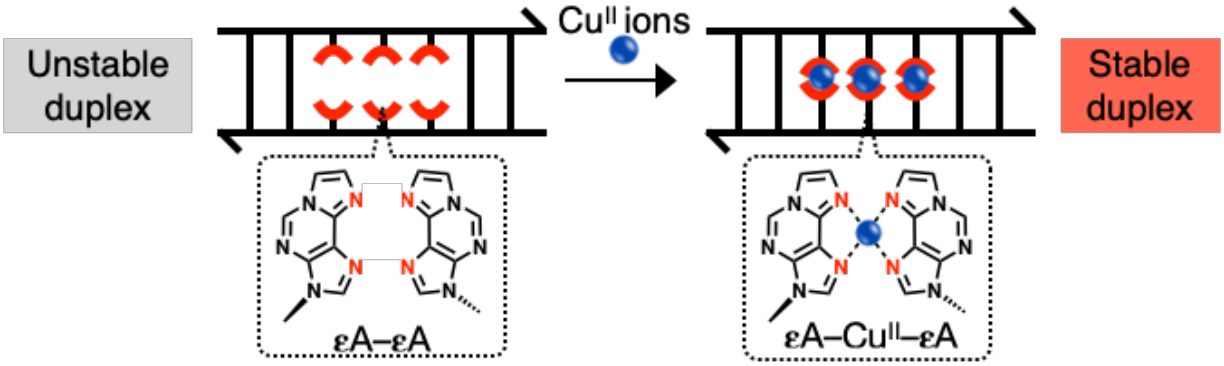


Figure. 2. Self-assembly of Cu^{II} ions within DNA duplexes bearing 1,N^6-ethenoadenosine (**ε**A) modified nucleobases as metal-binding sites.

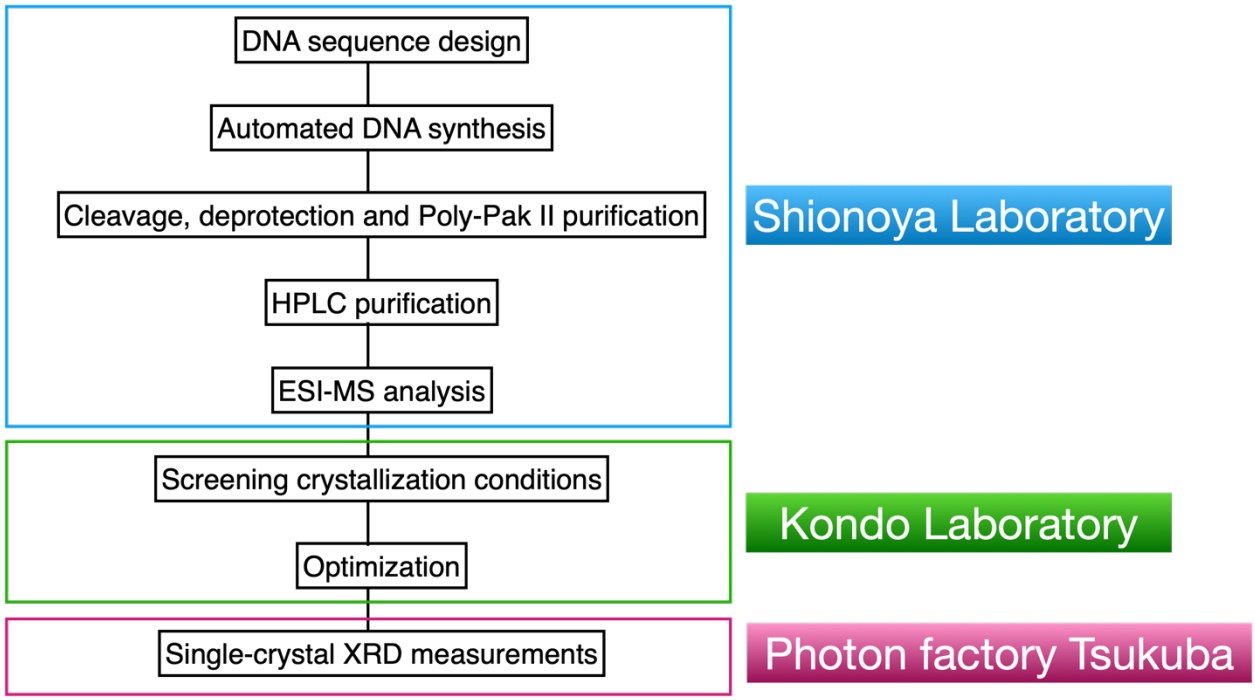
Problem Statements

Elucidating the structural information of these metal-mediated base pairs has been one of the bottlenecks of the previous researches. Structural studies of these DNA-templated metal assemblies will unravel vital information regarding plausible structural distortions, helicity, and metal–metal interactions arising from the incorporation of **εA**–Cu^{II}–**εA** base pairs inside the duplexes. Understanding such factors would enable us to develop molecular devices based on metallo-DNAs and to design metal-responsive higher-order DNA architectures. This study would open a new research area of solid-state chemistry of metallo-DNAs.

Experimental flow

Towards decoding the structural parameters of DNA duplexes bearing **εA**–Cu^{II}–**εA** base pairs, I initiated a collaboration project with Ms. Natsumi Eguchi (M2 student), belonging to the Kondo laboratory at Sophia university. Prof. Jiro Kondo has previously reported the crystal structures of several metallo-DNA duplexes having Ag^{I}- and Hg^{II}-mediated base pairs.[8,9] This study began with the DNA sequence design apt for the formation of crystal structure, followed by the DNA synthesis and purification, using previously established protocols in our laboratory. Following this, various conditions (buffer, counter cations, etc.) were examined to find the optimal

conditions for the crystal formation at Kondo laboratory. Single crystal X-ray crystallography was carried out at the synchrotron radiation facilities at the Photon factory in Tsukuba. (**Scheme. 1**)



Scheme 1. Research flow of this self-directed joint-research project

Results and Discussions

This section has been abstracted for confidential reasons.

(1) DNA sequence design

The crystallization of DNA molecules is generally very difficult due to its polyanionic feature. The first and arguably the most important step in this study is the DNA sequence design apt for crystallization experiments. We have designed five DNA sequences in total, and I will detail the rationale behind two of the sequences in this report. The first two DNA strands synthesized were designed following the famous Dickerson-Drew dodecamer sequences. Dickerson-Drew DNA (DDD) sequence is an ideal model system that offers us the following advantages:

a) DDD has biologically relevant sequences that adopt the canonical B-DNA structure.
b) Buffer conditions appropriate for the crystallization of DDD have been already known.
c) DDD sequences have been extensively investigated, providing us with several structural references in the PDB databases. In fact, the first ever report on the single-crystal XRD analysis on DNA made use of DDD sequence design.
d) MD simulation studies on DDD sequences are widely reported making the analysis easier.

We incorporated the unnatural **εA** nucleobases into the DDD sequences by replacing the natural nucleotides in the original sequences with **εA** nucleotides. The incorporation of **εA**

nucleobases in the middle of the duplex stabilizes the overall structure via preorganization due to the formation of canonical base pairs on both ends. The sequences of the DDD DNA strands used in this study are shown below:
1. DD-2eA: 5′–CGC GA**εA εATC** GCG–3′
2. DD-4eA: 5′–CGC G**εAεA εAεA**C GCG–3′
We later redesigned the sequences to additionally incorporate 5-bromouridine (^{Br}**U**) to resolve the phase problem by the anomalous dispersion method.

(2) DNA synthesis and purification

The DNA synthesis was carried out using standard solid-phase phosphoramidite chemistry. DNA strands containing 1,N^6-ethenoadenosine (**εA**) and 5-bromouridine (^{Br}**U**) nucleotides were synthesized on an NTS M-2-MX DNA/RNA synthesizer. The synthesis was carried out on a 1-μmol scale in a DMTr-on mode with ultramild deprotection phosphoramidites and reagents (Glen Research). All DNA strands were obtained in good yields around 99%.

The cleavage from the solid supports and the deprotection were performed according to previously optimized protocols. The products were cleaved and deprotected using 25% NH_3 aqueous solution at room temperature for 2.5 hours. The oligonucleotides were firstly purified and detritylated using a Poly-Pak™ II cartridge (Glen Research) using the protocol suggested by the manufacturer. Further purification was done by reverse-phase HPLC (Waters XBridge C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, flow rate: 1 mL min^{-1}, temperature: 60 °C).

(3) Characterization

All the DNA strands were identified using ESI-TOF mass spectrometry. The characterized oligomers were freeze-dried under vacuum, packed, and sent to Sophia University for crystallization experiments.

(4) Screening of crystallization conditions

Crystallization experiments were conducted at Kondo's laboratory in Sophia University. The DNA crystal formation was performed using the Hanging-drop vapor diffusion method. A drop containing unsaturated precipitant and DNA solution was placed in a well containing a reservoir with precipitant in higher concentration. The well was hermetically sealed to prevent droplet evaporation and to allow vapor equilibrium of the droplet and the reservoir. Equilibration of the water vapor from the protein containing droplet to the reservoir solution causes the DNA solution to reach supersaturation level, instigating nucleation and initial growth, culminating in crystal formation.

Crystallization was performed by the hanging-drop vapour diffusion method at 293 K in the presence of Cu^{II} and/or Ag^{I} ions. The DNA solution (4 mM) was mixed with the same volume of 8 mM $CuSO_4$ or $AgNO_3$. Crystallization droplets were prepared by mixing 1 μL of the sample solutions and 1 μL of crystallization solutions containing 50 mM 3-morpholinopropanesulfonic acid (MOPS) (pH 7.0), 10 mM spermine, 10% (v/v) 2-methyl-2,4-pentanediol and 10–500 mM

cation nitrates. The droplets were equilibrated against reservoir solutions containing 40% (v/v) 2-methyl-2,4-pentanediol. Dozens of the crystallization conditions were examined for each DNA sample.

(5) Analysis of crystals using single crystal XRD measurements

Two of the five synthesized DNA strands yielded good quality crystals. The conditions for crystallization and sequence information are not disclosed due to confidentiality. Fresh crystals were mounted in nylon cryoloops (Hampton Research) with the crystallization solution that contained 40% (v/v) 2-methyl-2,4-pentanediol as a cryoprotectant and stored in liquid nitrogen prior to the X-ray experiments. The yielded crystals were then sent to Photon factory in Tsukuba for single crystal XRD measurements. Upon analysis the crystals were revealed to have different space groups. The structure solved so far did not contain desired **εA**–Cu^{II}–**εA** base pairs. Currently the optimization of crystallization conditions as well as the analysis of XRD measurements are ongoing.

References

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