

# Artificial Zinc Finger Proteins with Modified Metal Moiety: Toward the Nano-Biodevices and Artificial Transcriptional Regulation.

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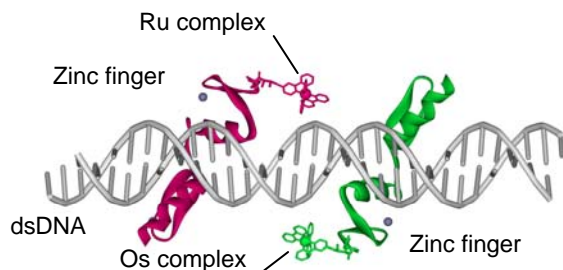
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## 1. Ru, Os-Modified Zinc Finger Proteins on DNA Template Directing Multiple Metal Array

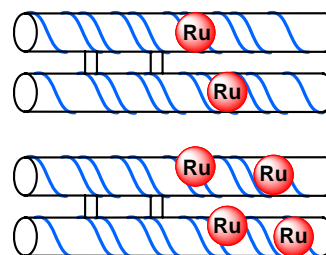
DNA-templated molecular assembly is one of promising methods to construct sophisticated nano device at will. Modification of ssDNA with various functional molecules of organic, inorganic compounds or colloids enables desired alignment of multiple target molecules on dsDNA scaffold. For example, sequential array of chromophores at regulated distances on DNA successfully leads to efficient multistep fluorescence energy transfer, which act as single molecular photonic wire. Here we report DNA-templated metal array system via DNA-binding protein anchor. We focused on zinc finger proteins as a reader molecule to utilize sequence information of DNA.(Figure 1)

To achieve metal array on DNA, we have selected tris(bipyridine)ruthenium(II) and osmium(II) complex as the luminescence and energy transfer probes. Ru-tagged single finger, **Ru-F1**, and two finger, **Ru-F1F2**, were synthesized. The coordination environment of Cys2His2 moiety was investigated by d-d transition band in Co-loaded protein. CD spectroscopy also indicates that the **Ru-F1** gives typical zinc finger folding. Each finger domain, F1 and F2, was designed to bind to GGG and GCG, respectively, according to reported recognition code. DNA binding ability of **Ru-F1F2** to target DNA subsite was confirmed by electrophoretic mobility shift assay (EMSA).

We have demonstrated that selective Ru array on DNA duplex using our Ru-tagged zinc finger anchor. The DNA duplex containing two target subsite was prepared and complexation with **Ru-F1F2** was investigated by EMSA analyses. Increasing the concentration of **Ru-F1F2**, we have observed the band of two **Ru-F1F2** on DNA. We have also successfully array five Ru complex on 50 bp DNA. We have prepared Os-tagged zinc



**Figure 1.** Positioning of Ru and Os complexes on dsDNA via zinc finger



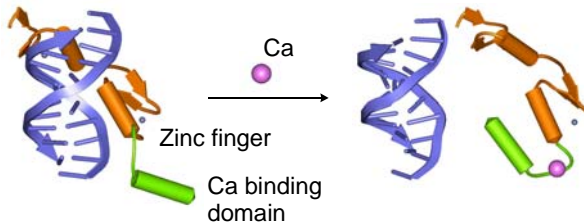
**Figure 2.** Various Ru array on DX DNA templates via zinc finger anchor.

finger proteins, **F1F1-Os**, binding to GGGGCG subsite and demonstrated the selective positioning of Ru and Os complex. The successful positioning of Ru and Os complexes can be expanded to 2D array using Double Crossover DNA templates.(Figure 2) Detailed investigation of photophysical properties Ru and Os array on DNA will be also discussed.

## 2. Artificial Ca Ion Responsive Zinc Finger Transcriptional Factor<sup>1</sup>

The use of zinc fingers is an attractive strategy for the design for artificial transcriptional factors. Controlling the binding availability by further improvement on zinc fingers is a promising method for the regulation of gene expression. Zinc finger domains are frequently found as a part of various kinds of transcriptional factors, and the DNA binding ability of the zinc finger region can be regulated by the other part of the protein. Thus, artificial transcriptional factors with switching functions have recently been reported with zinc finger fusion proteins or their mutants. We were motivated to create artificial zinc finger fusion proteins in order to develop the metal responsive regulation of DNA binding. Thus, we have designed a Ca-responsive finger that is a fusion protein with a representative EF-hand motif of a Ca-binding protein in order to control DNA binding.

In this study, single finger with Troponin C domain, F2-Tn and double finger with Troponin C domain, F1F2-Tn, were synthesized by the combination of solid phase and chemical ligation methods. Cys2His2 coordination and folding structure of F2-Tn were investigated by UV-vis. and CD spectroscopy indicating that our fusion protein keeps native zinc finger folding. Detailed solution structure of F2-Tn was determined by conventional NMR analyses. The results clarify that our fusion protein gives native zinc finger folding with the artificial Ca binding helix domain. Ca responsive DNA-binding affinity of troponin-fused protein, F1F2-Tn/Zn, was investigated using EMSA. F1F2-Tn/Zn has  $K_d$  value of 5.8 nM in the absence of Ca ion and 13 nM in 100 equiv. of Ca ion. The  $K_d$  values are gradually up to roughly twice higher value. While F1F2/Zn without Tn Ca-binding site does not exhibit such increase of  $K_d$ . Therefore, the results clearly indicate that the fusion zinc finger has potential for Ca-responsive DNA binding properties. Our fusion protein with two finger unit has DNA binding affinity, which is controlled by coordination of Ca ion.(Figure 3)



**Figure 3.** Strategy of Ca sensing zinc finger fusion protein with an additional Ca-binding site from troponin C.

### Reference

- (1) Onoda, A. and Yamamura, T. *et al.*, *J. Am. Chem. Soc.* **2005**, 127, 16535-16540.