DX DNAs as Templates for Multiple Arrangement of Zinc Fingers

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ABSTRACT
The present paper reveals that double crossover-DNAs (DX) serve as scaffolds for the multiple arrangement of a $[\text{Ru(bpy)}_3]^{2+}$-bound zinc finger (ZF) protein, Ru-ZF. This series of results would lead to the realization of the two-dimensional arrangement of functional molecules and nanomaterials on DX-tiles.

INTRODUCTION
The two-dimensional arrangement of functional molecules and nanomaterials is of great importance in the realization of molecule-based devices. Among various solutions for this problem, the utilization of DNA is one of the predominating candidates because of the strong complementation of base pairs, and programmable and fixed compositions. As a practical matter, there are a number of works where the one-dimensional assemblies of functional molecules and nanoparticles onto dsDNAs are attained (1, 2). Recently, DNA superstructures, which were initiated by Fu and Seeman with the innovation of double crossover DNAs, DX (3), have begun to be exploited as scaffolds to realize higher-dimensional arrangements (4); however, this series of reports is limited to periodic assemblies, and the arrangement of functional molecules and nanomaterials on the desired positions of DXs has never been attained.

Zinc finger (ZF) is the most abundant transcription factor. Although the minimal units of ZFs consist of as few as about 30 amino residues, its selectivity and affinity for the specific triplets of dsDNAs are extremely high. It should be pointed out that ZFs include a peculiar $\beta\alpha\beta$ secondary structure, where one Zn(II) ion is indispensable for the folding, and the $\alpha$-helix plays a main role in the fine recognition ability. Choo and Klug clarified “recognition codes” for a zif268-type of ZFs, which affords a prediction on the specificity (5).

We have adopted ZFs as anchors, and DXs as scaffolds for functional molecules and nanomaterials, respectively. The virtues of the employment of ZFs are as follows: (1) the small bodies of ZFs afford proximity among the functional components; (2) both periodic and non-periodic arrangement of multiple and different components are possible with the proper designs of DNAs.

This paper reports quantitative binding assays between DXs and a $[\text{Ru(bpy)}_3]^{2+}$-bound ZF, Ru-F1F2 (Scheme 1 and Figure 1). In addition, the multiple arrangement of Ru-F1F2 is also discussed (Figure 3).

MATERIALS
ssDNAs were purchased from NIHON GENE RESEARCH LABORATORIES Inc. as lyophilized forms, and quantified with OD260 values in TE Buffer. Fluorescent and isotope labels, IRD700 and $^{32}$P, respectively, were appended onto the 5'-terminus of strand 4 (Figure 1). IRD-tagged strand 4 (1 $\mu$M in TE Buffer) was supplied by LI-COR and used as it was. Strand 4 was radiolabeled with T4 polynucleotide kinase and $[^{32}P]ATP$. F1F2 was synthesized by the solid-phase method. Ru-F1F2 was synthesized by chemical ligation between F1F2 and thioester-terminated $[\text{Ru(bpy)}_3]^{2+}$. DXs were fabricated from corresponding ssDNAs in TAE5m buffer with the annealing from 95°C to 4°C. The formation of DXs was confirmed by using Native-PAGE (Figure 3).

RESULTS AND DISCUSSION
F1F2 was programmed to show selective affinity for 5'-CGCGGGG-3'. Among the five classes of DXs (3), the DAE type was employed here considering its linearity and durability against EMSA. This class of superstructures, however, consists of two proximal double strands, which

![Figure 1 Schematic illustrations of DX1-DX4 and the “phase problem” with Ru-F1F2.](https://academic.oup.com/nass/article-abstract/52/1/695/1108904/DX-DNAs-as-Templates-for-Multiple-Arrangement-of)

F1F2: CRICGRNFSRSDTLRHIRHTGTGECPKYGCRCGRNFSRSDHLTRHIRHTHG
Ru-F1F2: Ru(bpy)$_3^{2+}$-GTGECPKYGCRCGRNFSRSDTLRHIRHTGTGECPKYGCRCGRNFSRSDHLTRHIRHTHG

Scheme 1 Amino acid sequences of F1F2 and Ru-F1F2.
could be a barrier for the binding of ZFs. More precisely, it is the “phase” of the recognition site that dominates this problem (Figure 1). The binding assay of F1F2 and Ru-F1F2 onto DX1-DX4 was firstly carried out in terms of this “phase” problem.

The quantification of the conjugation was performed by EMSA with the 32P-labeled DXs. Table 1 showed the dissociation constant, $K_d$, for F1F2 and Ru-F1F2 for each DX. Ru-F1F2 showed higher affinity for all the DXs than that of F1F2, which is attributable to the electrostatic attraction between the cationic Ru complex and negatively charged DXs. It is also to be noted that DX3, where the complexation had been expected to be suppressed due to the steric problem (Figure 1), showed the smallest $K_d$ among the DXs employed here. This behavior should come

Table 1  Dissociation Constants ($K_d$) for Ru-F1F2 and F1F2 Binding to DXs and dsDNA

<table>
<thead>
<tr>
<th>Template</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DX1</td>
<td>42</td>
</tr>
<tr>
<td>DX2</td>
<td>71</td>
</tr>
<tr>
<td>DX3</td>
<td>23</td>
</tr>
<tr>
<td>DX4</td>
<td>68</td>
</tr>
<tr>
<td>dsDNA (50 bp)</td>
<td>96</td>
</tr>
</tbody>
</table>

from the deceleration of the dissociation process by the Ru complex moiety serving as a “stopper”.

The two dimensional and multiple conjugation between Ru-F1F2 and DX4R4 (Figure 2), which bears four recognition sites, was also verified by EMSA with the IRD700-tagged DX. Due to the quenching of the fluorescence from IRD700 by the Ru complex, this analysis lost a quantitative performance, though, the quadruple conjugation was observed.

CONCLUSION

We have elucidated that a ZF motif, F1F2 has binding affinity for DXs. The modification of F1F2 with functional molecules, [Ru(bpy)$_3$]$,^2+$, does not spoil the binding affinity; in fact, this modification enhances the binding affinity, which is attributable to electrostatic attraction between the cationic charges of the Ru complex and anionic ones of the DXs. We have also attained the two-dimensional and quadruple binding of Ru-F1F2 onto a DX with four recognition sites. This series of results would open the door to the desired arrangement of functional molecules and nanoparticles on two-dimensionally expanded DX tiles composed of DXs.

REFERENCES


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