Spontaneous Formation of a Molecular Net Assembly by Using Nucleotide Complementarity

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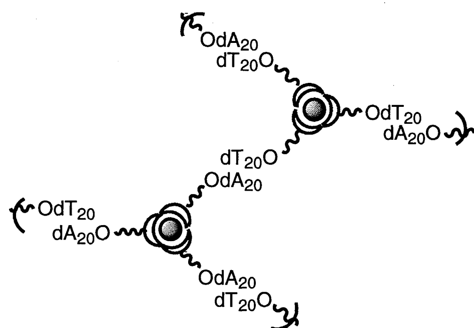
A mixture of ferrous-bipyridine complexes carrying an adenosine or thymidine 20-mer on the ligand forms a two-dimensional molecular network through hybridization of the complementary oligonucleotides.

DNA recognizes its complementary sequence with a very high specificity. DNA hybridization techniques take advantage of this character.[1] This special feature of DNA may also be exploited for the construction of a two- or three-dimensional assembly of functional molecules. If such molecules are furnished with an oligonucleotide of a certain sequence, they may be assembled in any desired manner by choosing a proper nucleotide sequence. This idea was substantiated only by a pioneering work of Chen and Seeman, who built a molecular cube.[2]

In this paper, we report a novel molecular assembly system. We exploit ferrous-bipyridine complexes as a core molecule. The bipyridine ligand is furnished with either an adenosine or thymidine 20-mer (dA_{20} or dT_{20}) at its terminus. The two ligands were allowed to react with a ferrous ion to form respective metal complexes. Upon mixing, these two complexes form a supra-molecular assembly through hybridization of the complementary oligonucleotides. Scheme 1 depicts an expected structure of the assembly to be a two-dimensional one like a molecular net. Since the ferrous-bipyridine complex and oligonucleotide unit are separated by a six-methylene chain, the molecular assemblies obtained here are regarded as a two-dimensional net in spite of the configuration of the metal complex. However, other forms of an assembly such as spheres are also possible.



Scheme 1.

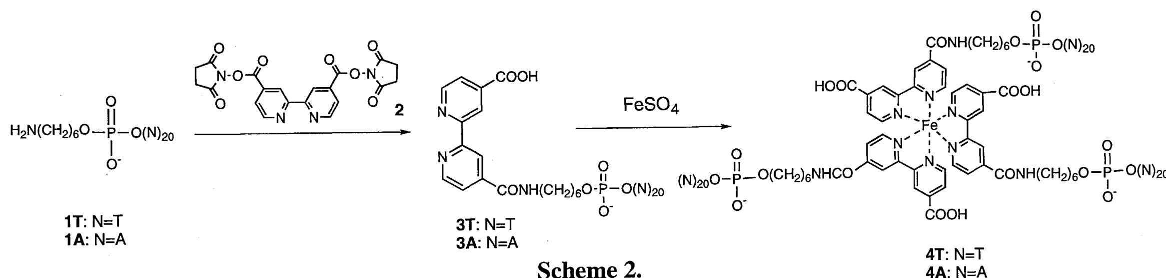
We first synthesized bipyridine derivatives **3A** and **3T** carrying an adenosine or thymidine 20-mer, respectively (Scheme 2). 4,4'-Dicarboxy-2,2'-bipyridine was activated by converting it to N-hydroxysuccinimide ester **2**[3] and a 100-fold excess of **2** was allowed to react with amino-linked oligonucleotides **1A** and **1T** to yield **3A** and **3T**, respectively.[4] Compounds **3A** and **3T** reacted with 1/3 equivalent of ferrous ion to form their ferrous complexes **4A** and **4T**, respectively, as a red substance.[5] Compounds **4A** and **4T** showed an absorption maximum at 540 nm due to the iron (II) tris-bipyridine complex,[6] proving the formation of the desired metal complexes. Comparison of the absorption at 260 nm for the oligonucleotide[7] with that at 540 nm for the iron (II) tris-bipyridine complex[6] revealed that the complexes have three oligonucleotide tails per metal complex. When the complexes **4A** and **4T** were mixed in a 1:1 molar ratio and then two volumes of ethanol added, the assembly precipitated. This suggested that the assembly has a high molecular weight.

We measured the melting temperature (T_m) of the double helix of the network from **4A** and **4T** in 1 mM tris-(hydroxymethyl)aminomethane·HCl buffer (pH 7.5) and 0.25 M NaCl. The duplexes from $dA_{20}\cdot dT_{20}$ and **3A+3T** also were measured for comparison. The T_m of the complex of **4A** and **4T** was slightly higher than that of both duplexes from $dA_{20}\cdot dT_{20}$ and **3A+3T** (Table 1). This result shows that double helix formation was enhanced in the assembly of **4A** and **4T** to some extent. The effect of the metal complex core on the duplex formation was minimal, presumably because the six-methylene chain separates the ferrous-bipyridine complex from the oligonucleotide unit. Cyclic voltammetry for **4A**, **4T** and their

Table 1. DNA thermal melting in the complex of **4A** and **4T** and their precursors[a]

Oligonucleotides	T_m (°C)
$dA_{20}\cdot dT_{20}$	48.0
3A + 3T	49.2
4A + 4T	50.8

[a]Experiments were conducted in 1 mM tris(hydroxymethyl)-aminomethane·HCl buffer (pH 7.5) and 0.25M NaCl. Scan rate, 0.33 °C/min. [oligonucleotide] = 1.0 μM-ds.



Scheme 2.

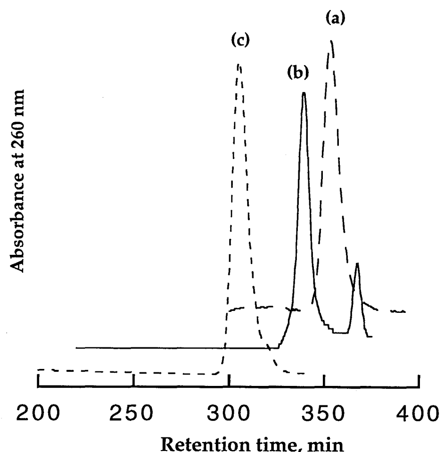


Figure 1. Gel permeation HPLC analysis of the molecular net of **4T** and **4A**. About 10 nmol of a sample (based on bipyridine) were applied. (a) **4T** (352 min), (b) **3T+3A** (339 min), **3T** (368 min), and (c) **4T+4A** (303 min). Column, Tosoh G-DNA-PW x 4; eluent, 0.1 M Tris·HCl buffer (pH 7.5) and 0.3 M NaCl; flow rate, 0.15 ml/min; detection, 260 nm; temperature, 25 °C.

mixture was also measured in 0.25 M sodium phosphate buffer (pH 6.9) and 0.3 M KCl at 100 mV/s of scan rate. The current for **4A** or **4T** alone at 700 mV decreased by 10% in the mixture (data not shown). This behavior is explicable in terms of the formation of a high molecular assembly, because its diffusion toward the electrode should be slower than that of the monomers.

We estimated the mean molecular weight of these assemblies by gel filtration chromatography (Figure 1).[8] The assembly from **4A** and **4T** gave a single peak with a retention time of 303 min (Figure 1 (c)). When compared with the retention times of linear DNA fragments of various length, this value corresponds to that of 4.4 kb DNA. In other words, the assembly consists of nearly 100 molecules each of **4A** and **4T**. The same retention time was observed on gel filtration chromatography upon mixing the double helix of **3A+3T** with ferrous ion. Although this estimate is an approximate one because of a lack of proper size-standardizing samples, the data do suggest that the assembly is considerably large. Since the T_m profile of the assembly was reversible and the same chromatogram was obtained after repeated denaturation and re-naturation, the morphology of this assembly seems to be stable under broad conditions.

In summary, a novel type of supra-molecular assembly has been constructed successfully. The size and structure of the assembly may be altered by a proper choice of the sequence and length of the oligonucleotides as well as the ligand and metal ion. Hence, this system is versatile and will find various extensions and applications in the future.

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References and Notes

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3 2,2'-Bipyridine-4,4'-dicarboxylic acid[9] was dissolved in DMF with triethylamine and allowed to react with N-hydroxysuccinimide to yield **2** in 95% yield. Ester **2** was purified by silica gel chromatography using chloroform as an eluent.
4 A 100-fold molar excess of **2** was allowed to react with amino-linked oligonucleotide **1A** or **1T** in sodium bicarbonate buffer and DMSO. Mono-substituted products **3A** and **3T** and di-substituted products were obtained in a 2:1 ratio with 60% yield. Compounds **3A** and **3T** were purified by HPLC (Tosoh TSK-gel ODS-80Ts, 4.6 mm ID x 15 cm) to homogeneity: the retention times for **3A** and **3T** were 18.0 and 22.0 min, respectively, in a linear gradient of 10-30% of acetonitrile in 0.1 M triethylammonium acetate buffer (pH 7.0).
5 A titration curve of **3T** in water saturated at 1/3 molar equivalent of ferrous ion.
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8 We failed to estimate the mean molecular weight of the assembly by agarose gel electrophoresis because the metal complex of the assembly broke in this condition.
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