# DNA nanostructure with non-canonical base pairs responding to enviromental condition

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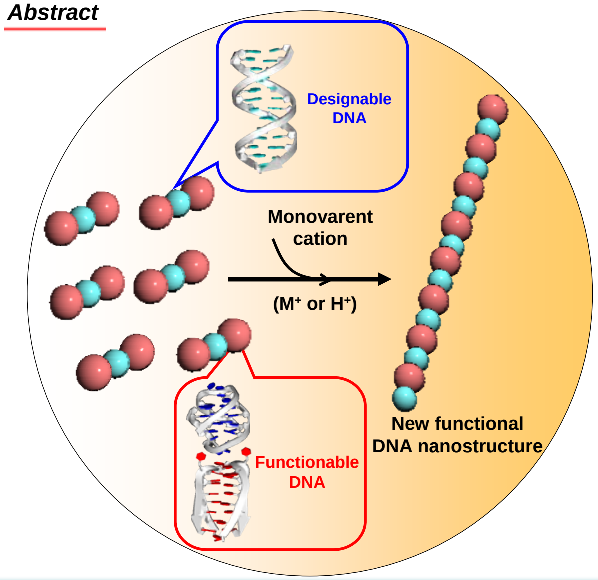
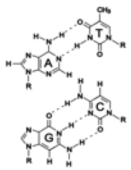
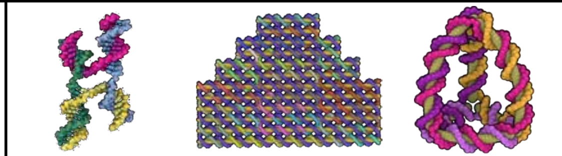
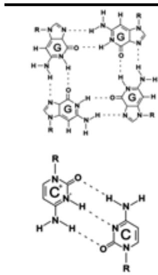
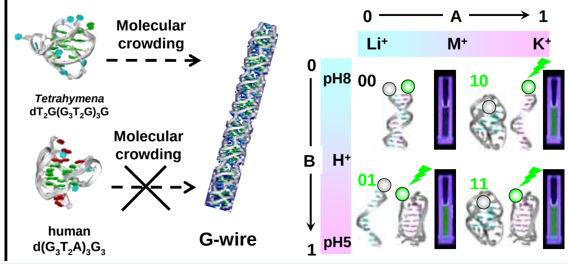
## Abstract




In order to develop new functional DNA scaffolds with advantage of both canonical and non-canonical structures, we have here attempted to combine the sequence specificity of canonical base pairs and environmental condition-dependent assembly of non-canonical base pairs. Structural analysis demonstrated that rational design of DNA G-quadruplex or i-motif arrays connected by duplex were successfully constructed. Moreover, we showed evidence that the formation of the designed nanostructure can be regulated by monovarent cations ($M^+$ or $H^+$).

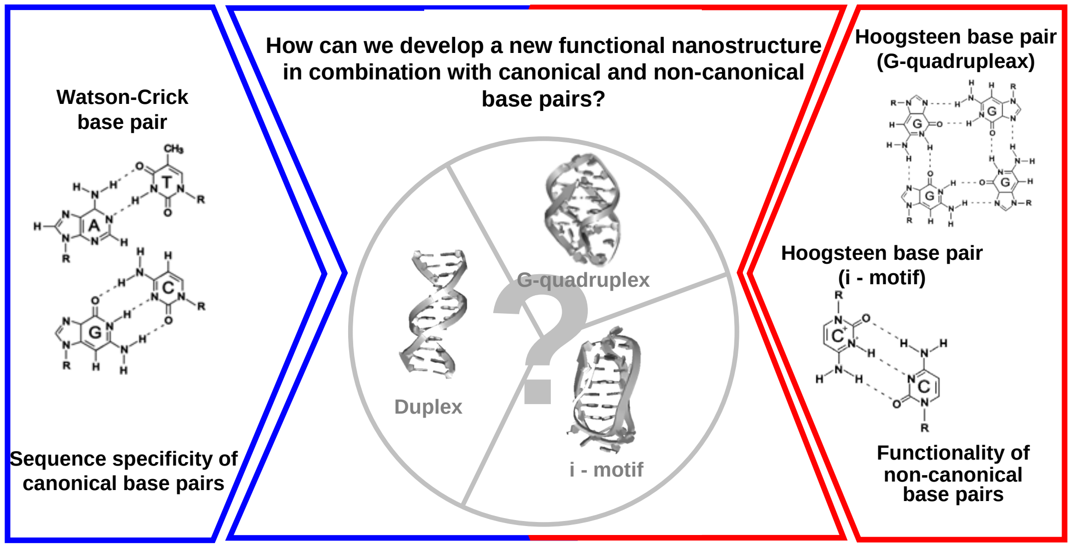
## Introduction

### Advantages of DNA for nanostructures with various base pairs

| Base pair type | | Advantages | Regulation |
|---|---|---|---|
|  | Canonical base pair | Addressable<br>Designable |  |
|  | Non-canonical base pair | Switchable<br>Functionable |  |



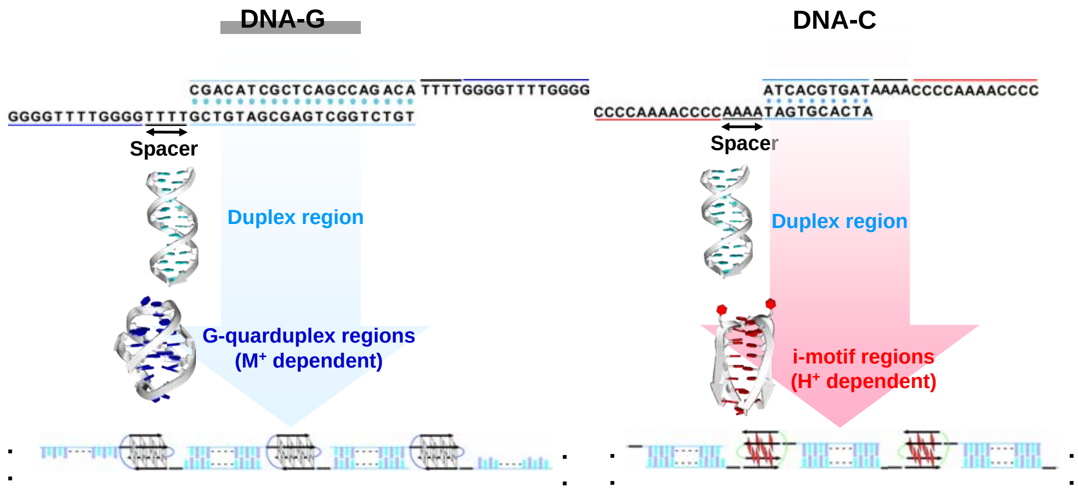
## Problem




## Results and Discussion

### Molecular design

**Strategy: Connecting G-quadruplex or i - motif units by duplex**



## Structure of DNA-G and DNA-C

・DNA-G

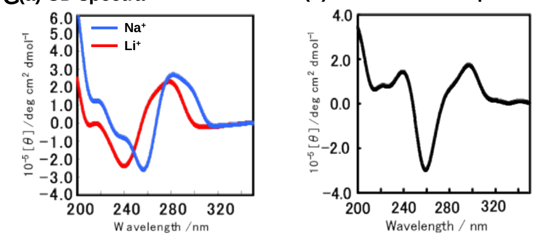
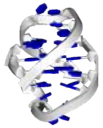



Figure 1. (a) CD spectra for 20 μM DNA-G in buffers containing 100 mM NaCl (blue) or LiCl (red) and 50 mM Tris-HCl (pH 7.0) at 260 nm (b) Differential CD spectrum between CD spectra obtained with NaCl and LiCl.

duplex

265 nm : +
240 nm : –



G-quadruplex

295 nm: +
260 nm: –

・DNA-C

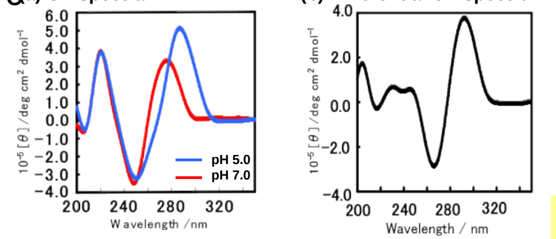



Figure 2. (a) CD spectra for 20 μM DNA-C in buffers containing 100 mM NaCl 50 mM MES (pH 5.0) (blue) or 50 mM Tris-HCl (pH 7.0) (red) at 260 nm (b) Differential CD spectrum between CD spectra obtained with pH 5.0 and 7.0.

i - motif

285 nm : +
260 nm : –

**Structure formation depending on cations and pH**

## Stability of DNA-G and DNA-C

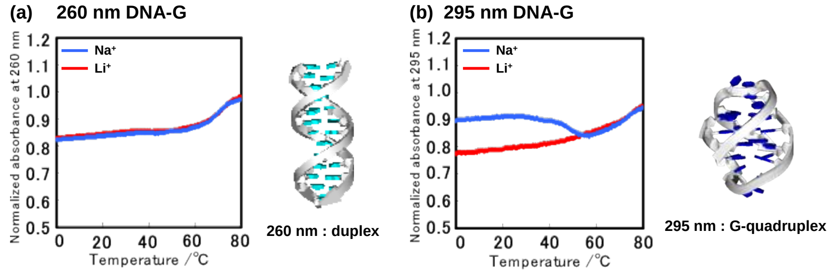
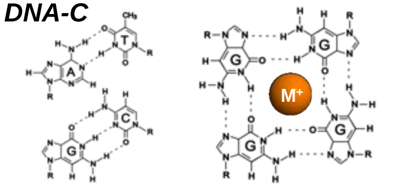



Figure 3. Normalized melting curves 20 μM DNA-G in buffers containing 100 mM NaCl (blue) or LiCl (red) and 50 mM Tris-HCl ( pH 7.0) at 260 nm (a) and 295 nm (b).



| | Duplex (260 nm) | G-Quadruplex (295 nm) |
|---|---|---|
| $Na^+$ | 70.3℃ | 47.4℃ |
| $Li^+$ | 72.6℃ | < 0℃ |
| | Cation independent | Cation dependent |

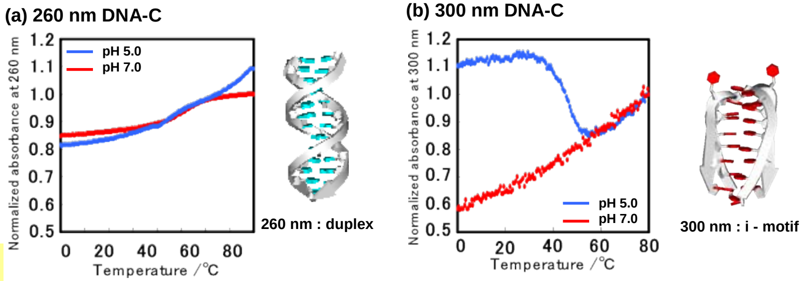
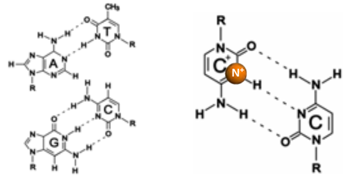



Figure 4. Normalized melting curves 20 μM DNA-C in buffers containing 100 mM NaCl (blue), or 50 mM MES (pH 5.0) (blue), 50 mM Tris-HCl ( pH 7.0) (red) at 260 nm (a) and 295 nm (b).



| | Duplex (260 nm) | i - motif (300 nm) |
|---|---|---|
| pH 5.0 | 46.8℃ | 45.7℃ |
| pH 7.0 | 46.8℃ | < 0℃ |
| | pH independent | pH dependent |

## Regulating DNA nanostructures by cation and pH

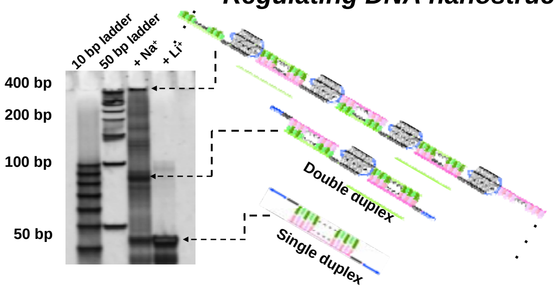



Figure 5. 10% Native PAGE of 10 μM DNA-G in buffers of 100 mM NaCl or 100 mM LiCl and 50 mM Tris-HCl (pH 7.0) at 4 ℃. Lane 1: 10 bp step ladder. Lane 2: 50 bp step ladder. Lane 3: DNA with NaCl. Lane 4: DNA with LiCl.

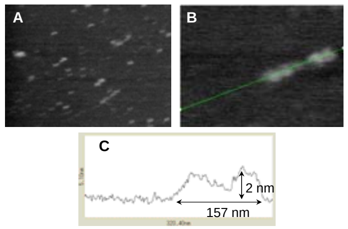
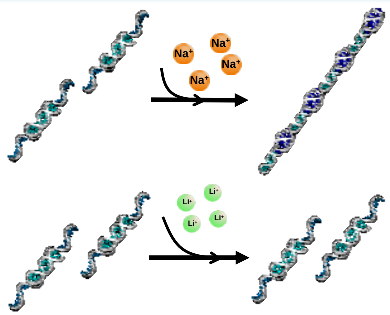



Figure 6. (A) AFM image of DNA-G with NaCl. (Dimensions: 300 × 225 nm, Z 5.1 nm). (B) AFM image (Dimensions: 100 × 75 nm, Z 5.1nm). (C) The section analysis of a typical fiber along the green line in panel B.



Cation dependent nanostructures consisting of both DNA duplex and G-quadruplex were developed.

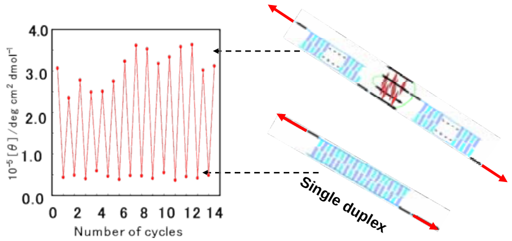



Figure 7. Cycling of switch as observed by measurement of the CD intensity at 300 nm at 4 ℃. The first cycle was produced by addition of HCl followed by addition of NaOH. The second and later cycle were produced by alternating addition of HCl and NaOH.

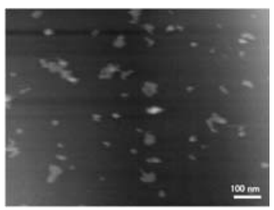
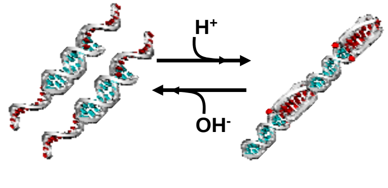


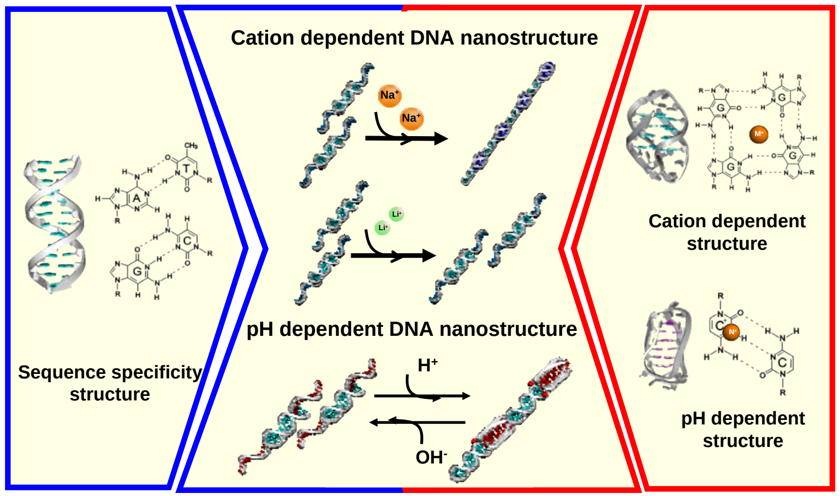
Figure 8. AFM image of DNA-C at pH 5.0 liquid (Z 5 nm).




pH dependent nanostructures consisting of both DNA duplex and i-motif were developed.

## Conclusion

**We developed new functional nanostructures by designing the array that could form duplex and G-quadruplex or i-motif.**




## Related papers from our group


1) D. Miyoshi, H. Karimata, Z. –M. Wang , K. Koumoto, and N. Sugimoto, *J. Am. Chem. Soc.*, 129, 5919-5925 (2007).
2) D. Miyoshi, M. Inoue, N. Sugimoto, *Angew. Chem. Int. Ed.* 42, 7716- 7719 (2006).
3) D. Miyoshi, H. Karimata, and N. Sugimoto, *Angew. Chem. Int. Ed.*, 44, 3740-3744 (2005).