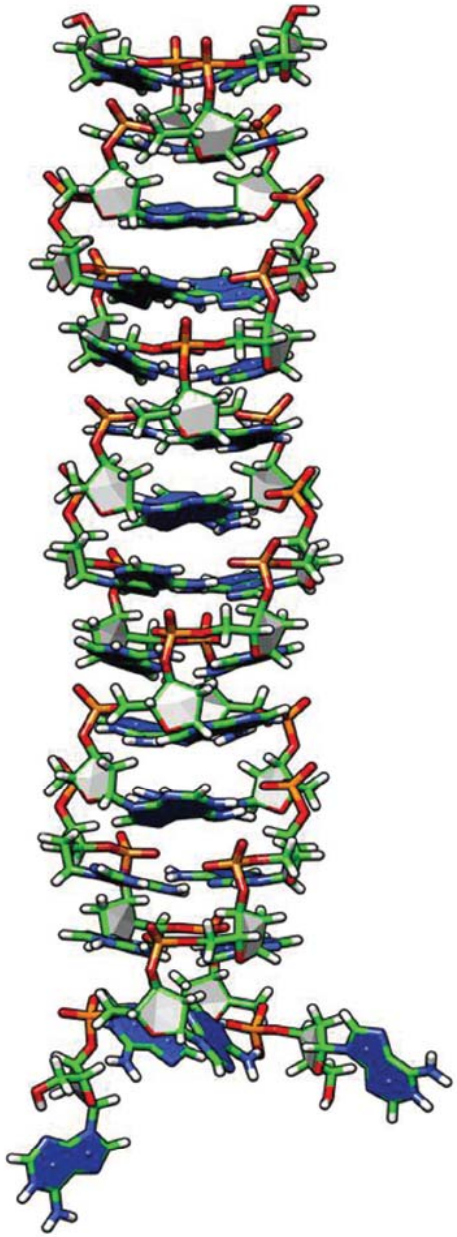

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The poly dA helix: a new structural motif for high performance DNA-based molecular switches

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Received December 16, 2008; Revised February 16, 2009; Accepted February 17, 2009

ABSTRACT

We report a pH-dependent conformational transition in short, defined homopolymeric deoxyadenosines (dA_{15}) from a single helical structure with stacked nucleobases at neutral pH to a double-helical, parallel-stranded duplex held together by AH^+-H^+A base pairs at acidic pH. Using native PAGE, 2D NMR, circular dichroism (CD) and fluorescence spectroscopy, we have characterized the two different pH dependent forms of dA_{15}. The pH-triggered transition between the two defined helical forms of dA_{15} is characterized by CD and fluorescence. The kinetics of this conformational switch is found to occur on a millisecond time scale. This robust, highly reversible, pH-induced transition between the two well-defined structured states of dA_{15} represents a new molecular building block for the construction of quick-response, pH-switchable architectures in structural DNA nanotechnology.

INTRODUCTION

Structural DNA nanotechnology is an emerging field that uses DNA to create either rigid architectures or dynamic switches (1–4). Dynamic, DNA-based nanodevices may also be described as molecular switches. They are based on structural transitions between two well-defined conformations of DNA upon the application of a stimulus. Several devices have been developed based on B-DNA assemblies employing differential hybridization of complementary strands, metal ions and indeed protons (5–11). Here we describe the poly dA helix as a new structural motif that functions as a molecular switch, which at low pH forms a parallel-stranded double helix and at neutral pH exists as a structured, single helix.

Early studies on understanding the structure, base-pairing scheme and base stacking properties of DNA and RNA duplexes used synthetic homopolymeric DNA and RNA as they were considered simplified model systems. Eventually it was found that these synthetic homopolymers actually formed different unusual conformations involving non-Watson-Crick base pairing. Poly rC and poly dC formed i-tetraplexes (12,13), while poly rG and poly dG formed G-quadruplexes (14–16). Interestingly, poly rA formed a parallel-stranded double helix, called pi-helix at acidic pH due to N1 protonation of the adenines at pH <5 (17–19). At neutral pH poly rA was found to exist as a single, right-handed helix with nine nucleotides per pitch of 25.4 Å (20,21). In fact, characteristic of the distinct nature of this helix, there are proteins called poly rA binding proteins (PABPs) that specifically bind poly rA over any random ssRNA (22,23). At neutral pH poly dA is known to exist as a structured single helix, similar to poly rA (24–27), except that the nucleobases in poly dA are more strongly stacked than in poly rA and are in the C2′-endo configuration. However, the behavior of poly dA at acidic pH is still unknown. We were encouraged by the fact that poly rA could form these structures, with no indication of any special role for the 2′OH. Further we also found a sprinkling of short DNA sequences that had an over-representation of adenines that formed parallel duplexes at acidic pH (28–30), all of which contained A–A base pairs. But, these sequences would be expected to exist as unstructured single strands at neutral pH.

We have been interested in developing alternative, non-B-DNA building blocks that rely on non-Watson-Crick base pairing, for applications in structural DNA nanotechnology (31–35). Given that poly rA exists as a right-handed, parallel-stranded, double helix at acidic pH (17) and a structured right-handed single helix at neutral pH we reasoned that poly dA may have potential as a new building block for DNA based pH-switches if is able to recapitulate poly rA behavior. In order to see whether poly dA alone could form a duplex at acidic pH and if so, could it switch reversibly between its structured single helical state at pH 7 to a structured duplex at acidic pH, we investigated a segment of poly dA. We chose a segment of poly dA 15 nucleotides long, because this is within the limits of the observed persistence length of the poly dA single helix (36). Using gel electrophoresis, circular dichroism (CD) spectroscopy and concentration dependent

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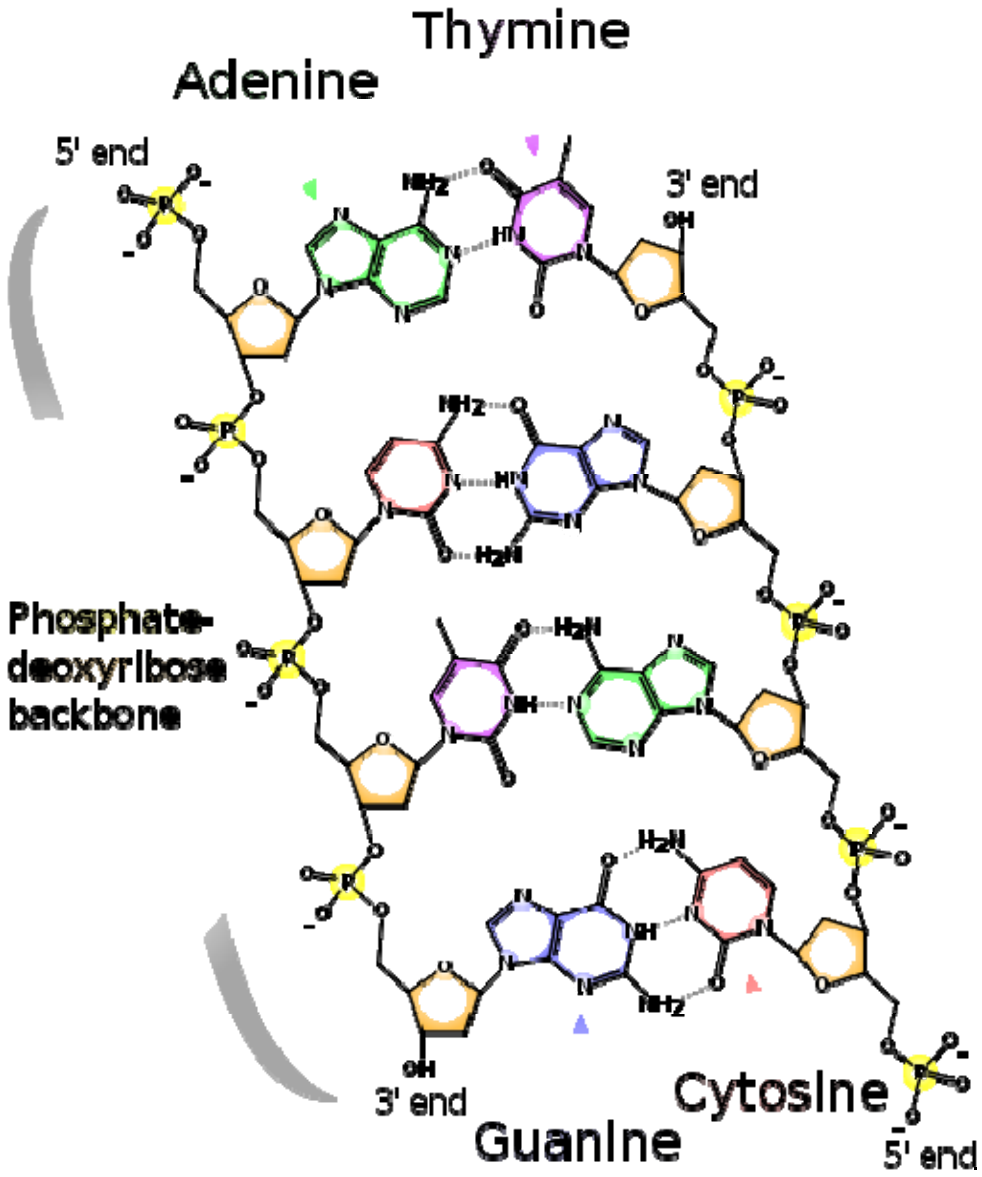
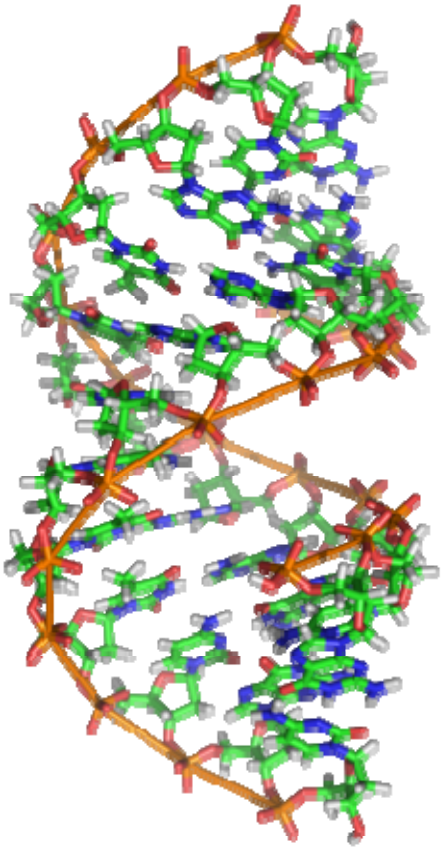
DNA



http://en.wikipedia.org/wiki/DNA

DNA helical structure



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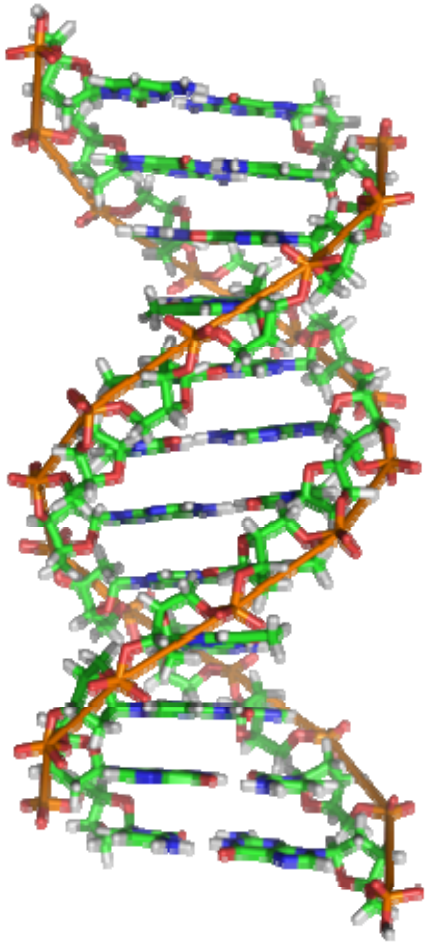

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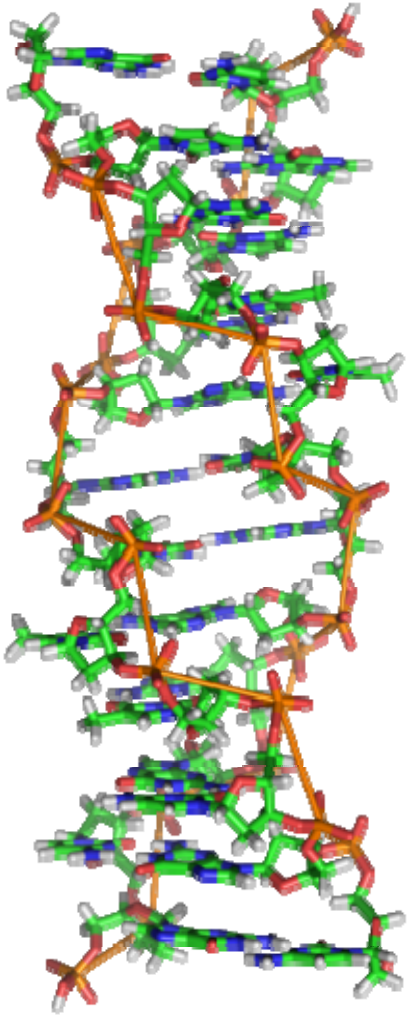
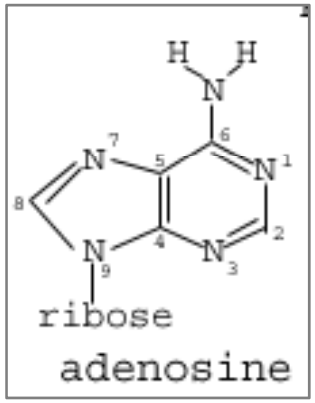
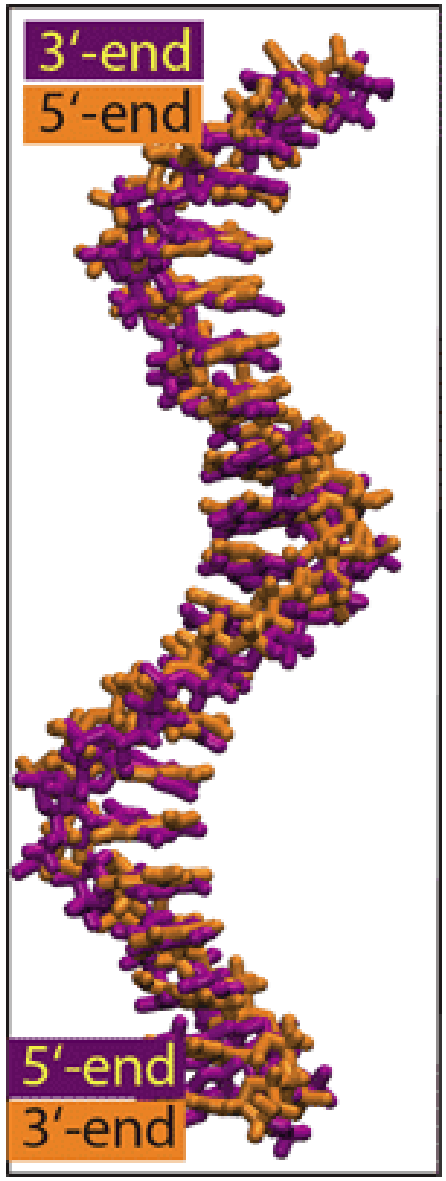


Z

http://en.wikipedia.org/wiki/DNA

PolydA





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The poly dA helix: a new structural motif for high performance DNA-based molecular switches

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Poly r**C** and poly d**C** formed i-tetraplexes (12,13),
poly r**G** and poly d**G** formed G-quadruplexes (1
Interestingly, poly r**A** formed a parallel-stranded
helix, called pi-helix at acidic pH due to N1 protonat
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pH poly d**A** is known to exist as a structured single
similar to poly r**A** (24–27), except that the nucleob
poly d**A** are more strongly stacked than in poly r**A** a
in the C2′-endo configuration. However, the behav
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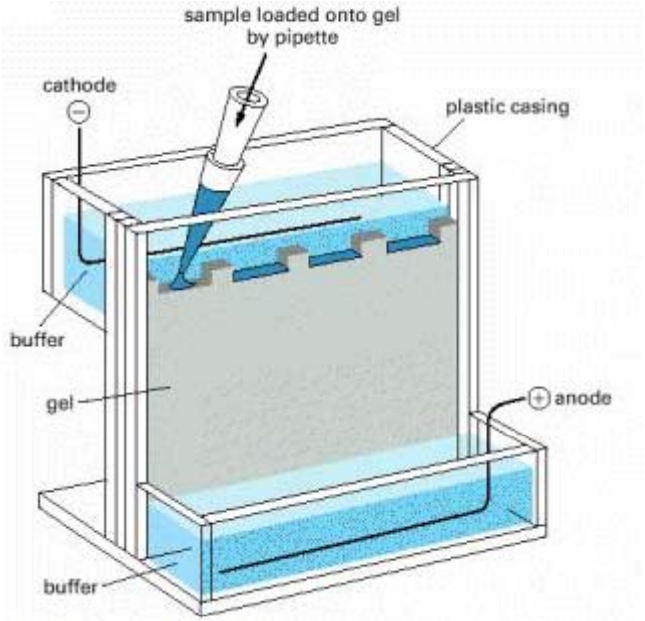
aged by the fact that poly r**A** could form these stru
with no indication of any special role for the
Further we also found a sprinkling of short
sequences that had an over-representation of ad

DNA

Table 1. Poly d**A** sequences used in this study

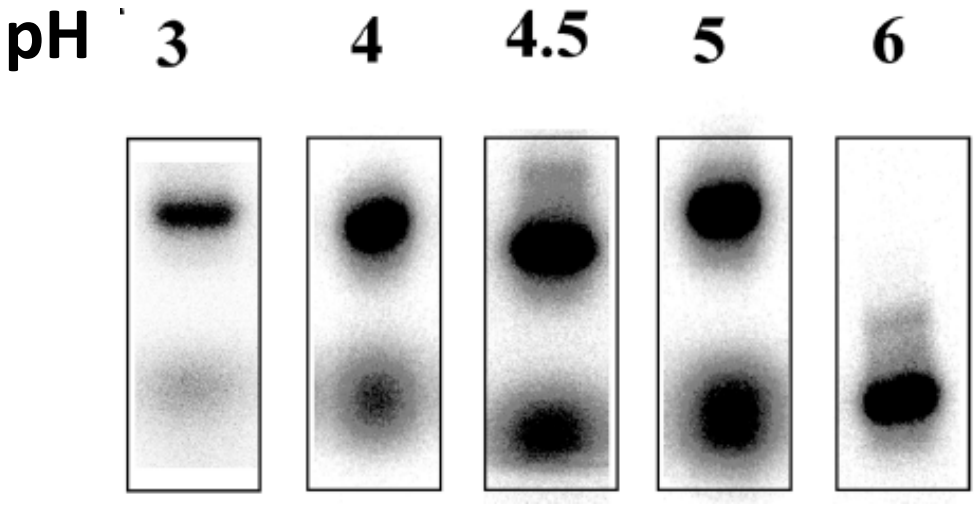
Name	Sequence
Poly d$\mathbf{A}_{15}$	5′-d(AAAAAAAAAAAAAAA)-3′
dTA6	5′-d(TAAAAAA)-3′
3′-Dabcyl-d$\mathbf{A}_{15}$	5′-d(AAAAAAAAAAAAAAA)-Dabcyl-3′
3′-TMR-d$\mathbf{A}_{15}$	5′-d(AAAAAAAAAAAAAAA)-TMR-3′
5′-TAMRA-dA_{15}	5′-TAMRA-d(AAAAAAAAAAAAAAA)-3′

Gel-electrophoresis

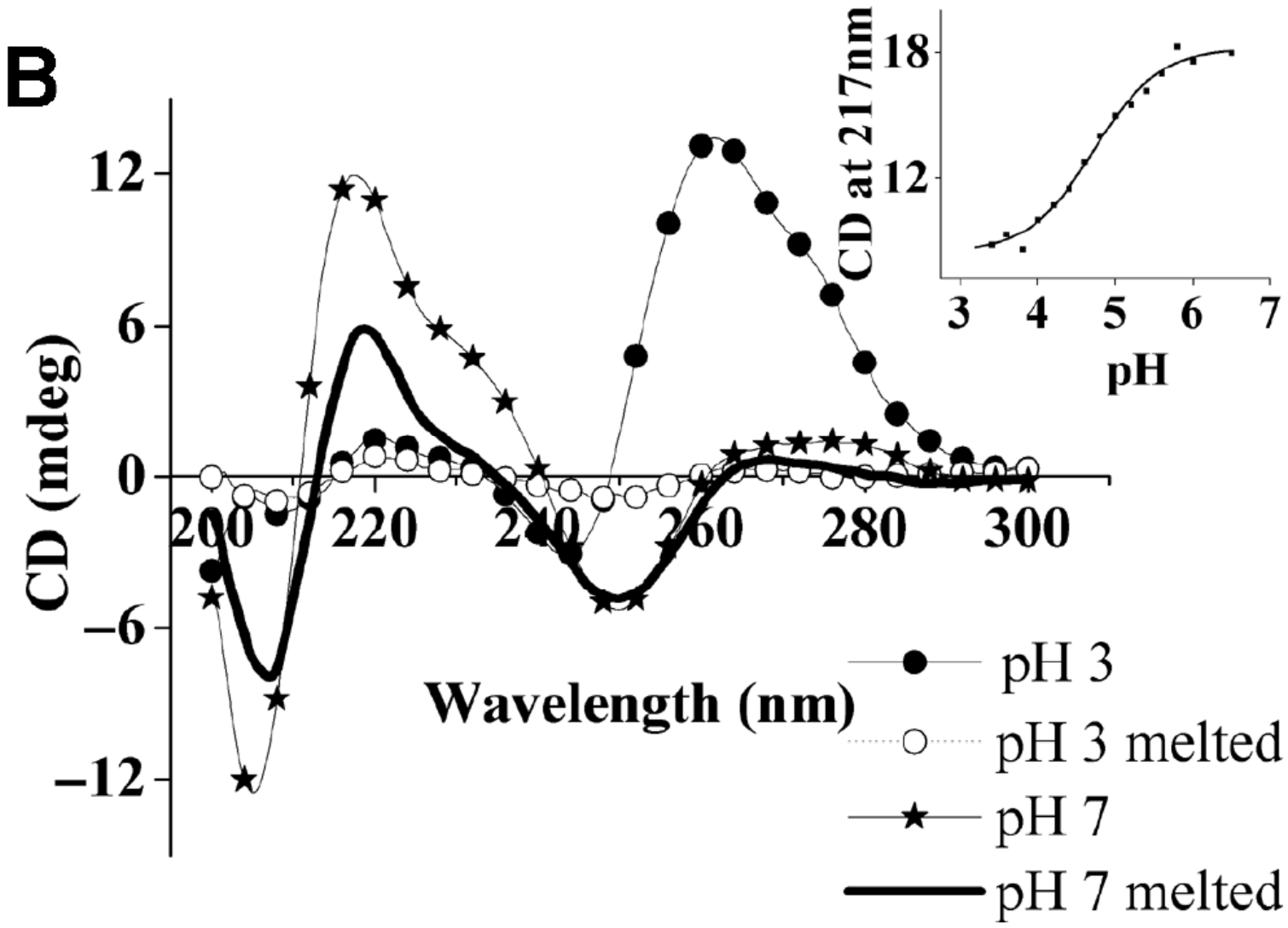


An Electrophoresis Apparatus.
A solution of proteins is applied to indentations made in a thin gel that is held between two clear plates of plastic. An electric current is applied, and over time the proteins separate.

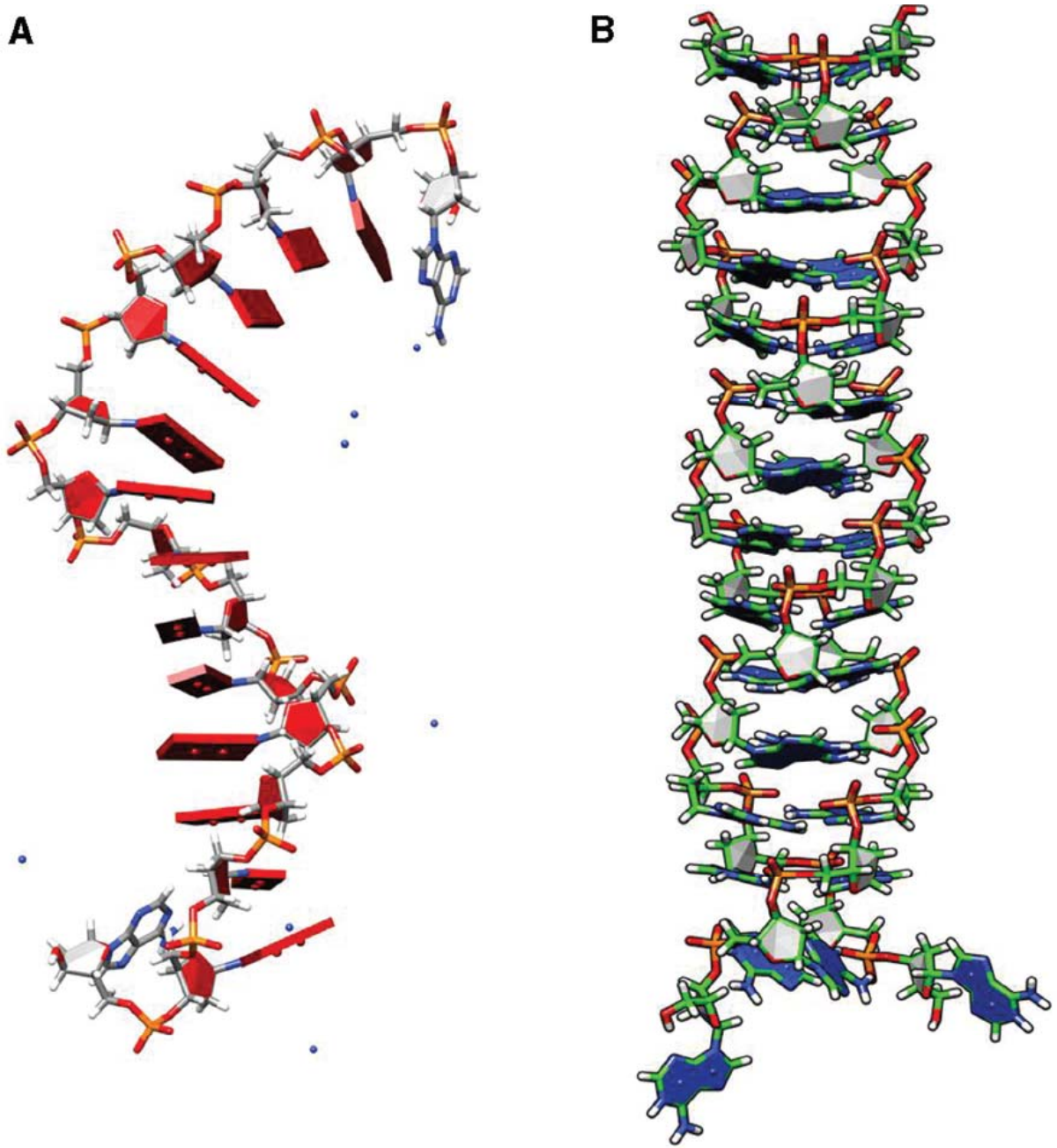
Poly dA_{15}



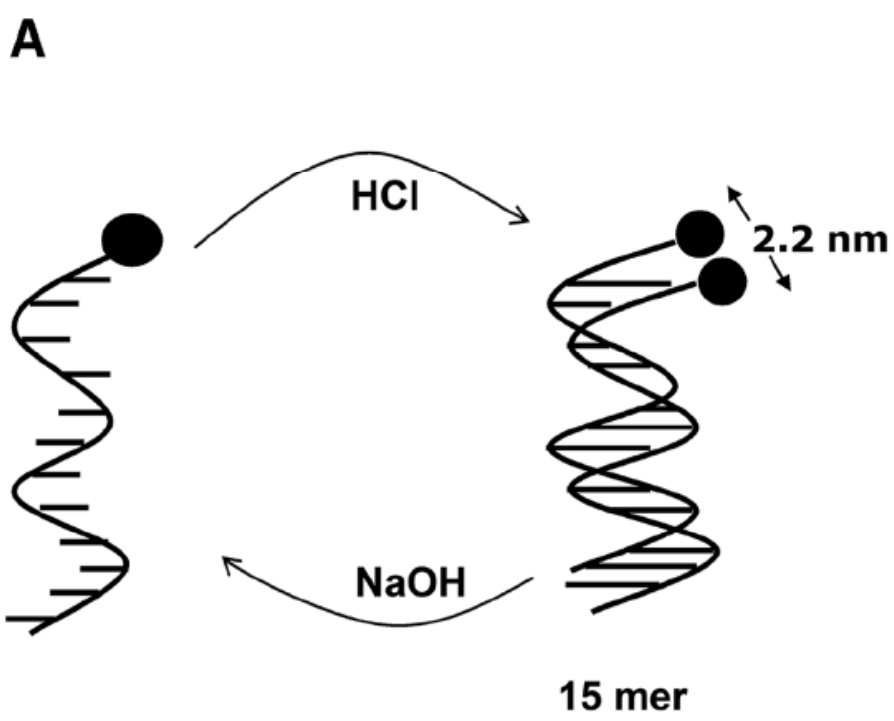
CD spectrum

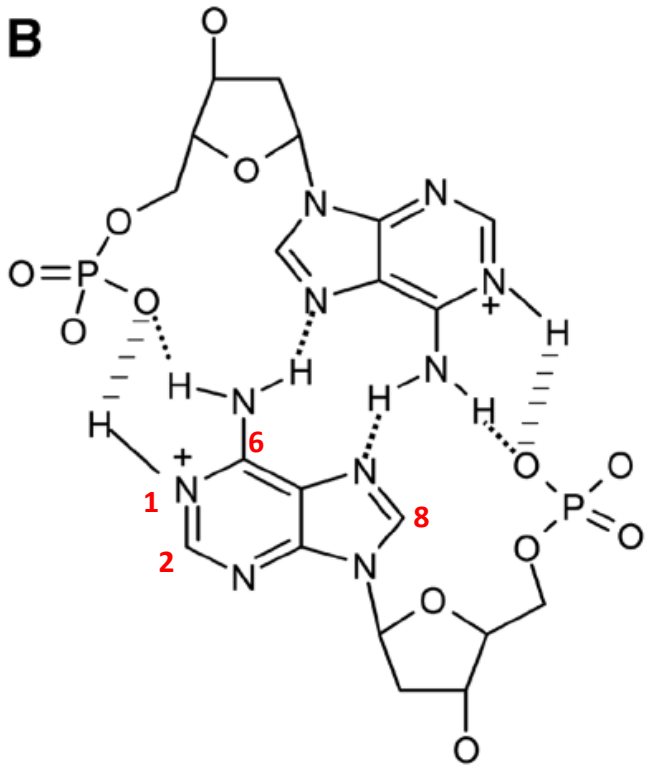

B
12
6
0
−6
−12
CD (mdeg)
200
220
240
260
280
300
Wavelength (nm)
CD at 217nm
18
12
3
4
5
6
7
pH
pH 3
pH 3 melted
pH 7
pH 7 melted

MD

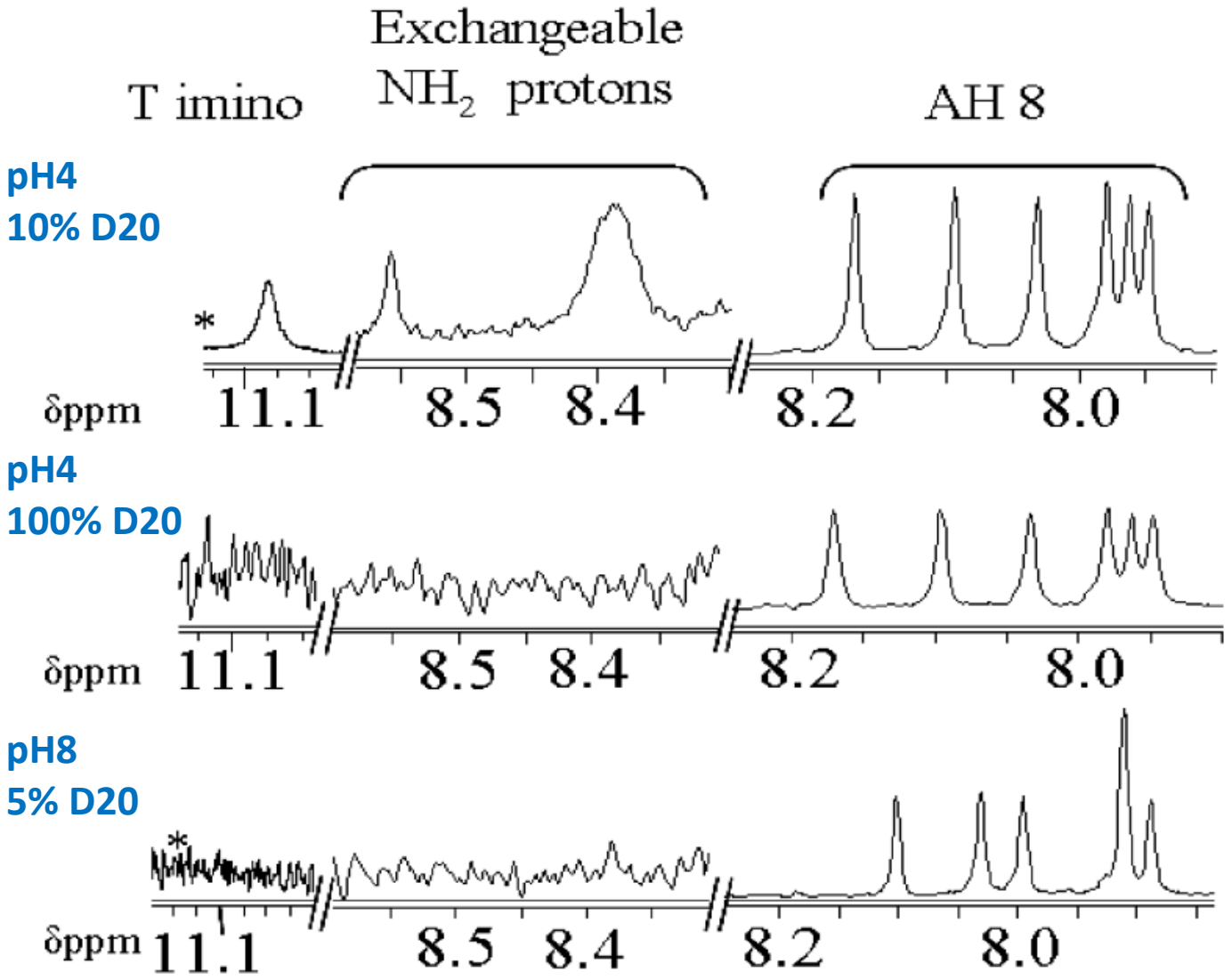


The scheme



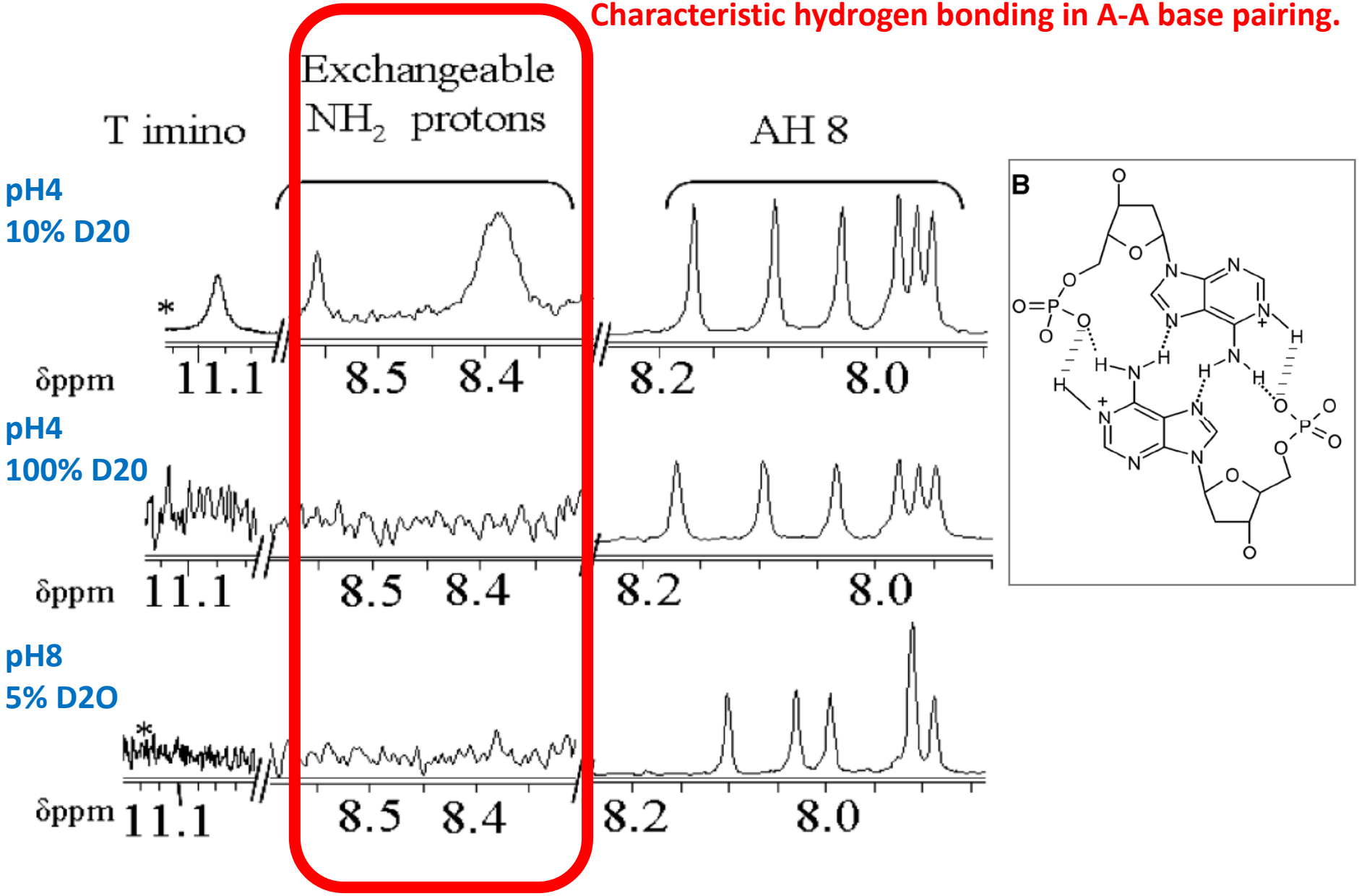


1D-NMR


T imino
Exchangeable
NH2 protons
AH 8
pH4
10% D20
*
δppm
11.1
8.5
8.4
8.2
8.0
pH4
100% D20
δppm
11.1
8.5
8.4
8.2
8.0
pH8
5% D20
*
δppm
11.1
8.5
8.4
8.2
8.0

1D-NMR

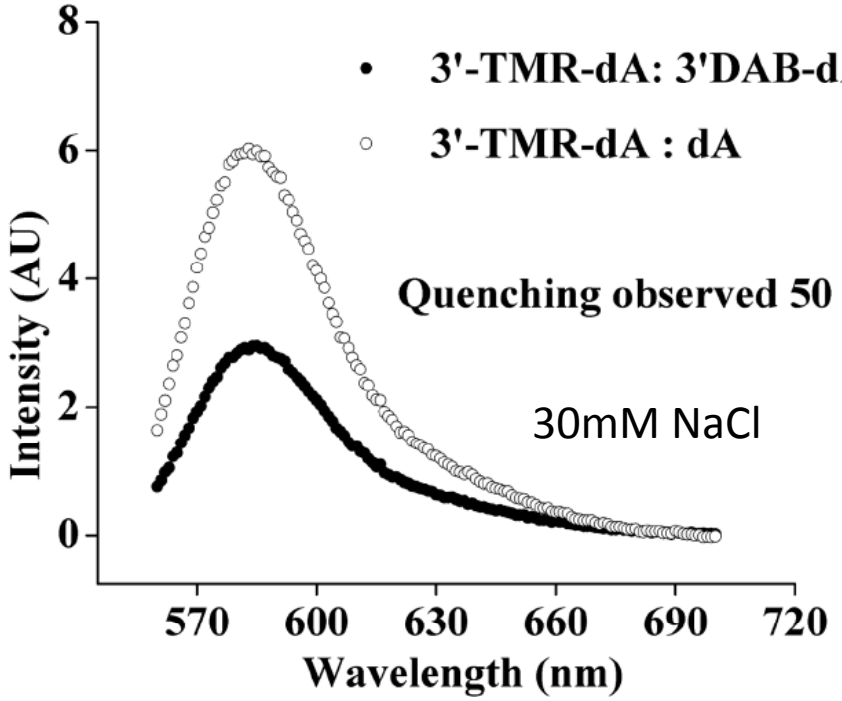
This NH2 protons usually showed up at 6-7 δppm.
Characteristic hydrogen bonding in A-A base pairing.

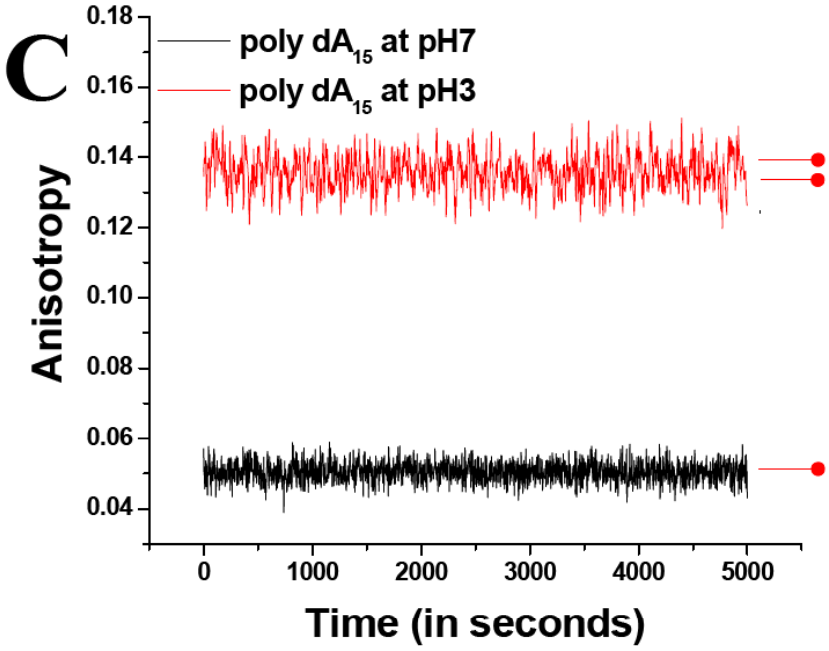


Fluorescence Quenching

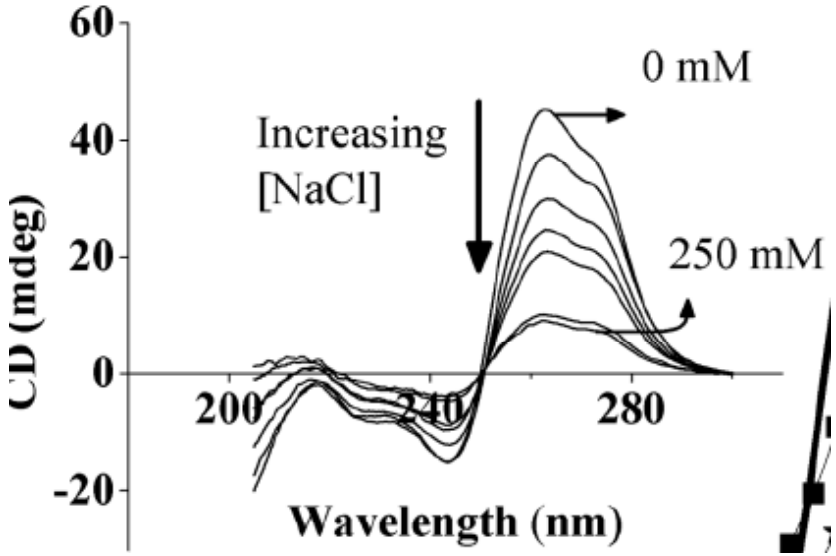
3′-Dabcyl-dA$_{15}$ 5′-d(AAAAAAAAAAAAAAA)-Dabcyl-3′

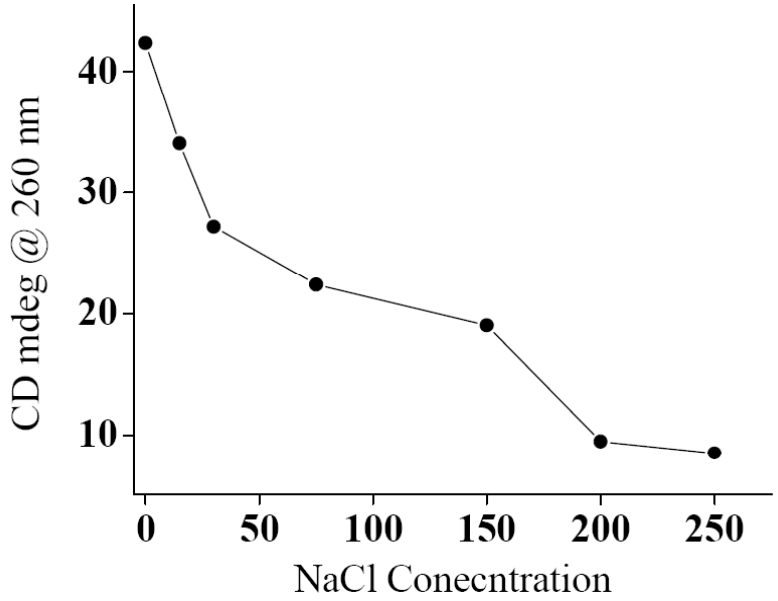
3′-TMR-dA$_{15}$ 5′-d(AAAAAAAAAAAAAAA)-TMR-3′

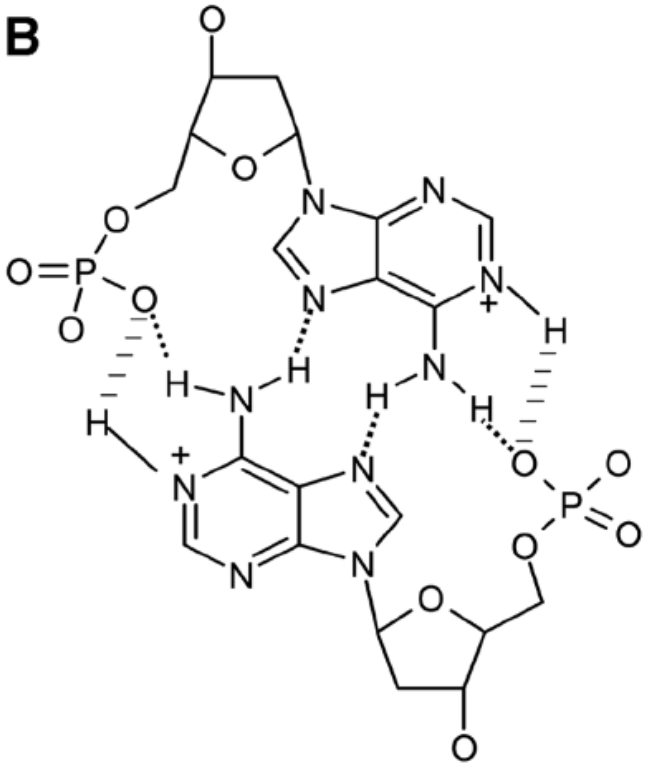




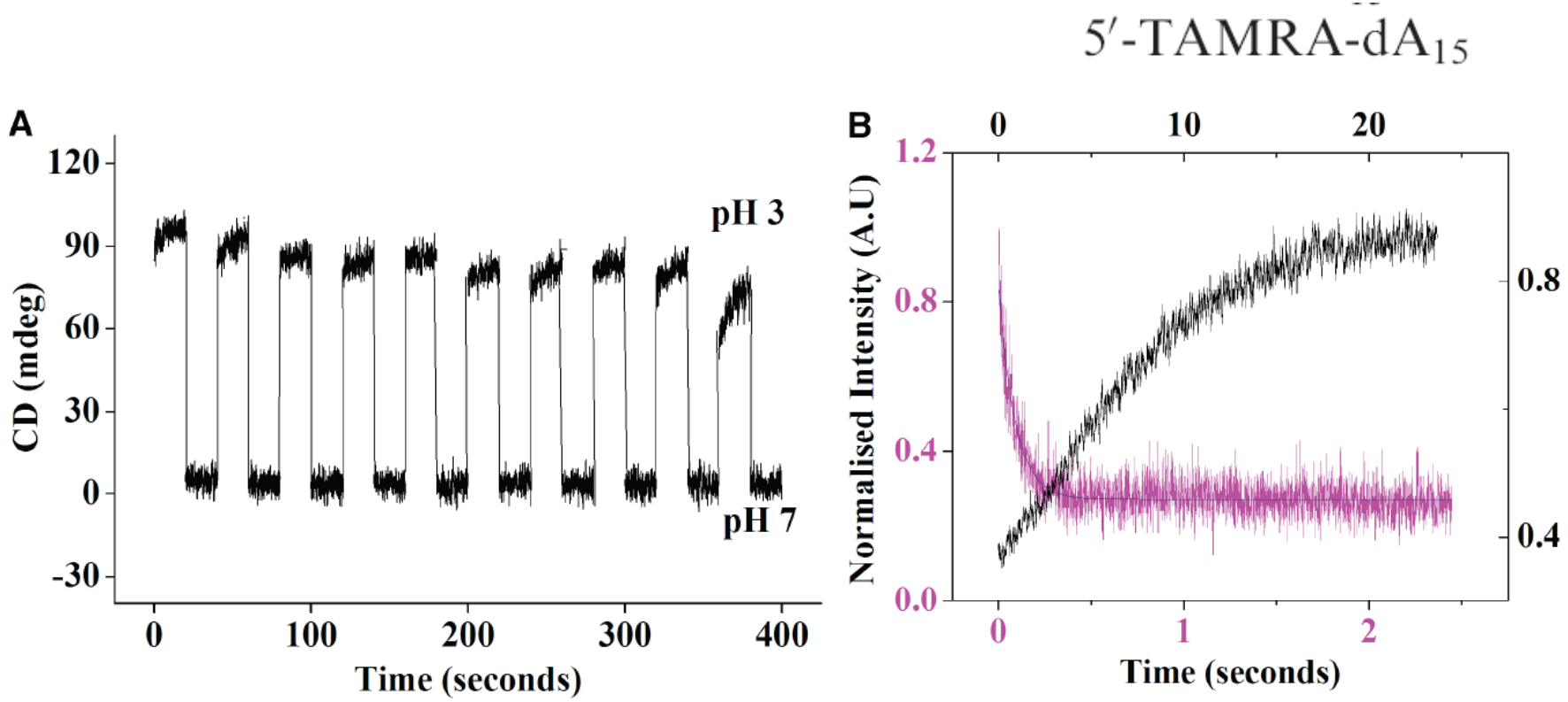
Salt dependence - CD







Structural transition is reversible



CONCLUSIONS

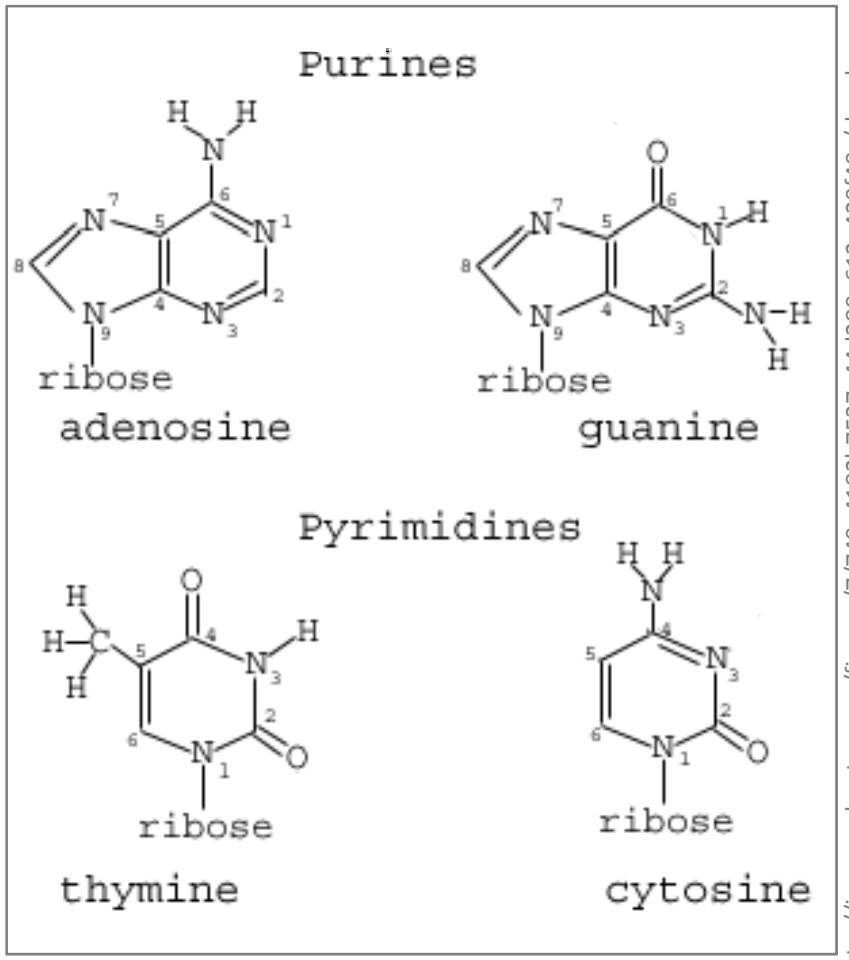
1)Poly dA exist as a structured single helix at neutral pH

2)Poly dA forms a right-handed parallel-stranded double helix at acidic pH

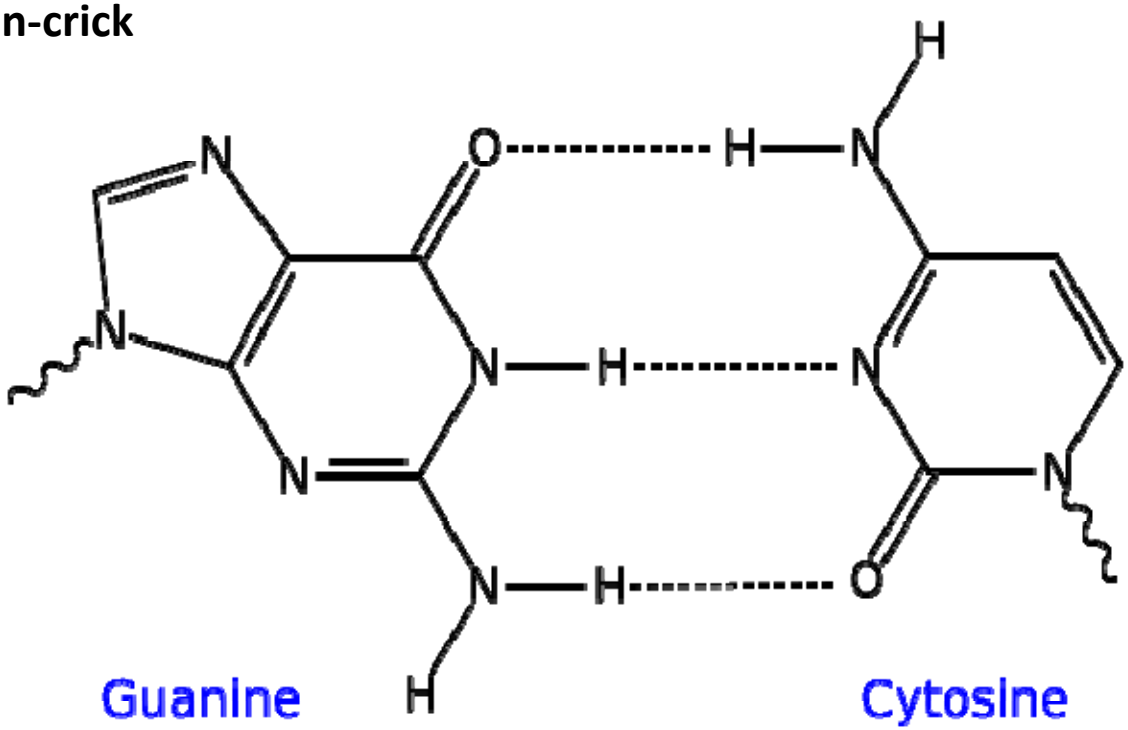
3) the duplex at low pH are held by Hoogsteen pairing bet protonated dA

4)This can be used as a proton driven molecular switch

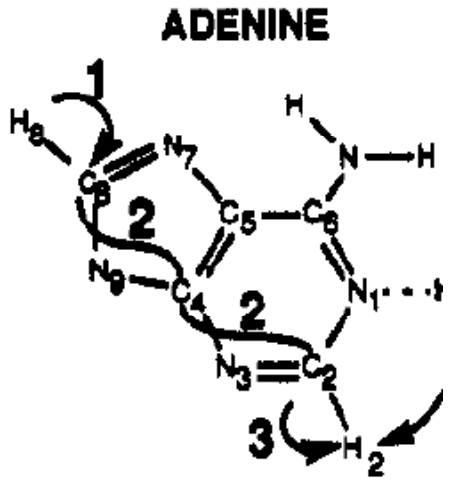
Supp.

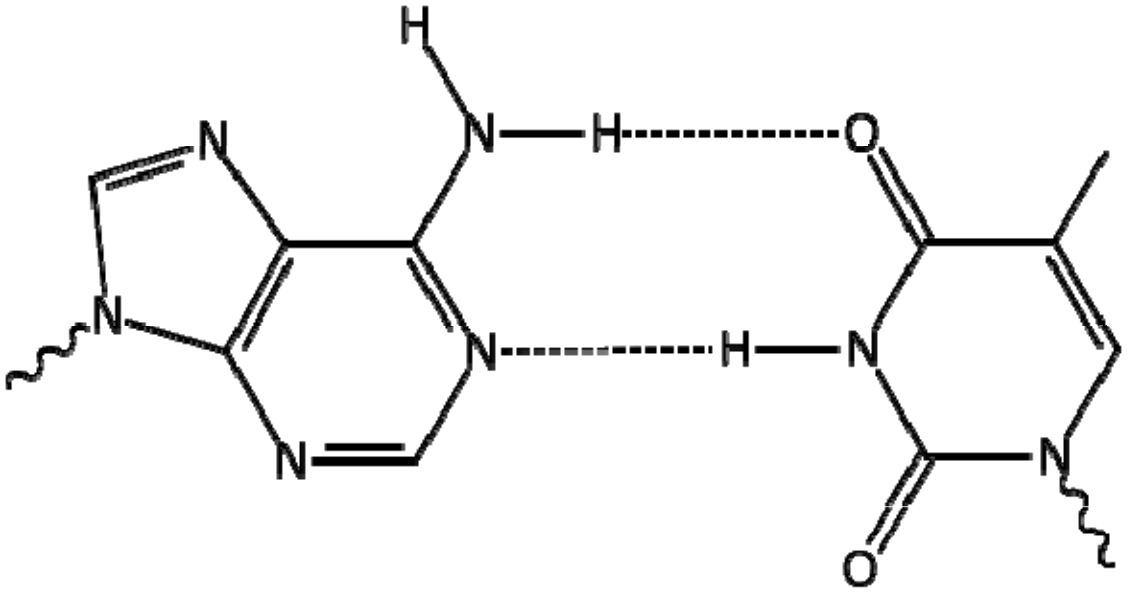


Watson-crick



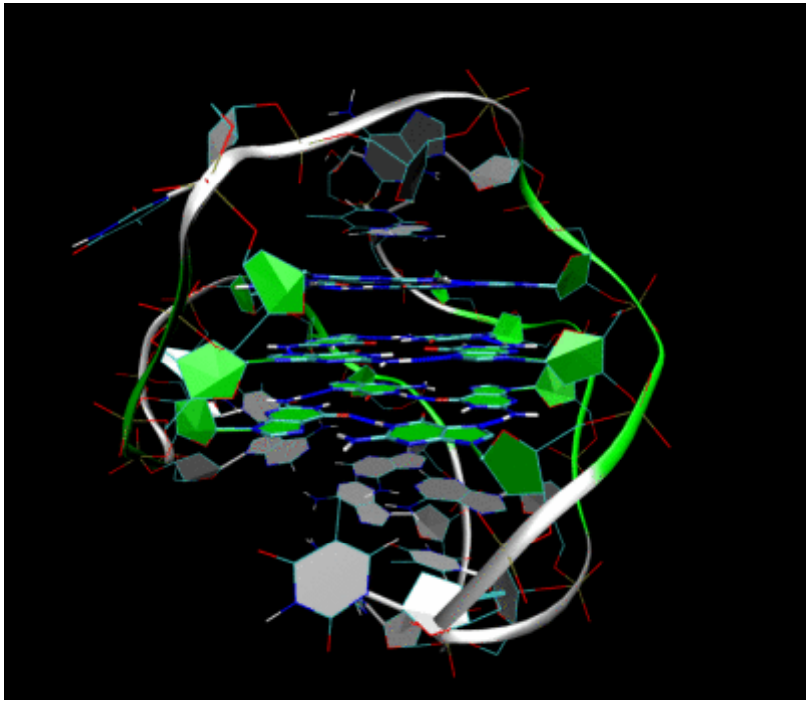
J. Am. Chem. Soc. 1994,116, 2205-2206

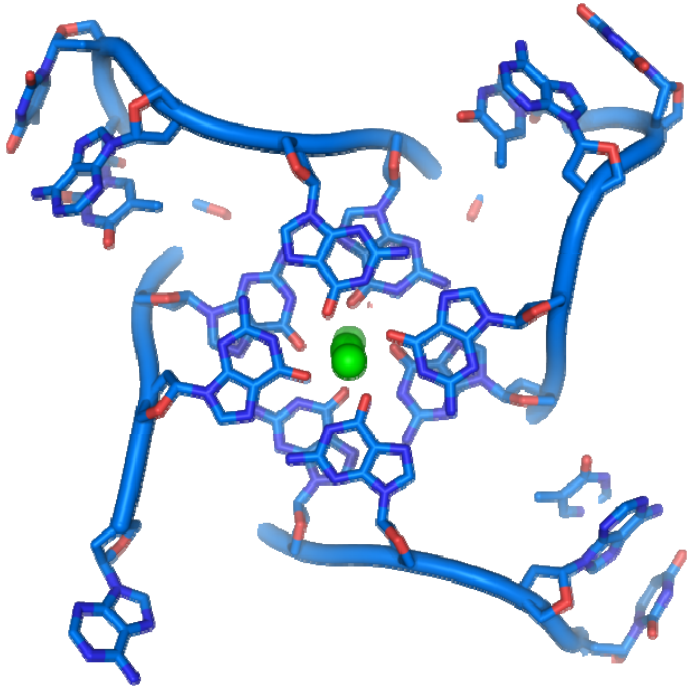


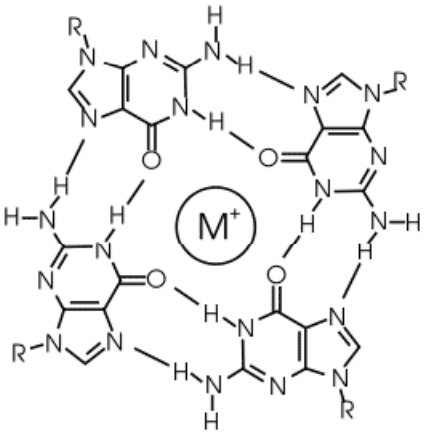


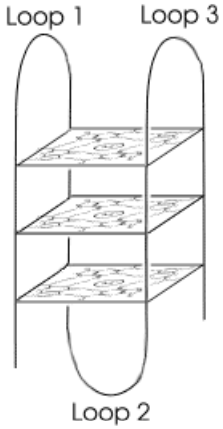
http://en.wikipedia.org/wiki/DNA

G-quadruplex



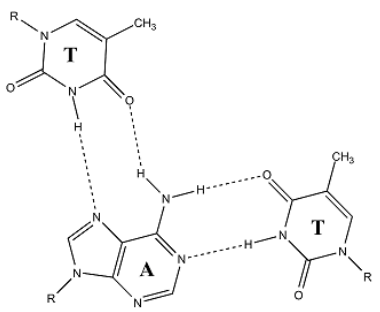




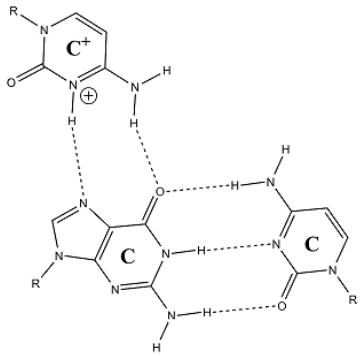


http://en.wikipedia.org/wiki/G-quadruplex

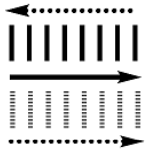
Hoogsteen base pair



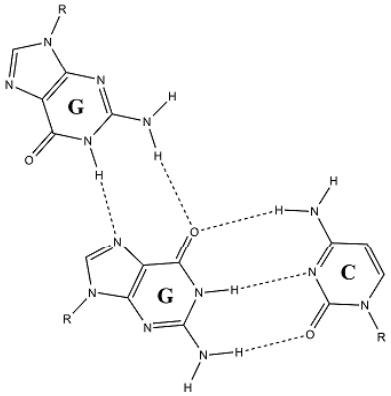
TA*T



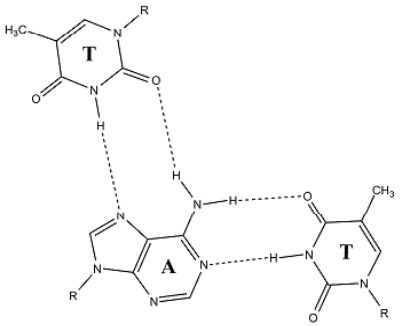
CG*C+



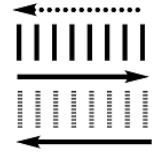
YR*Y



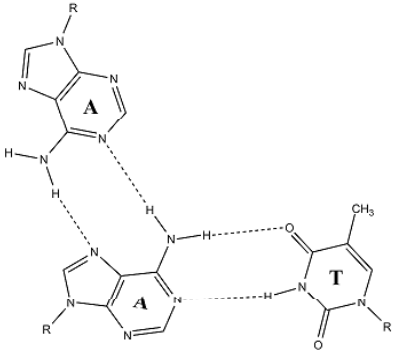
CG*G



TA*A



YR*R



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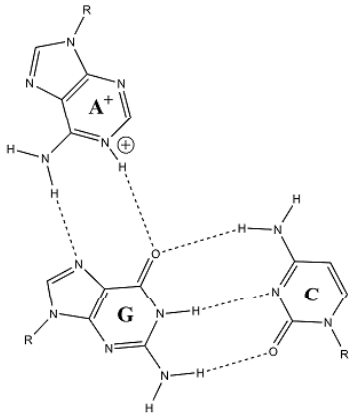
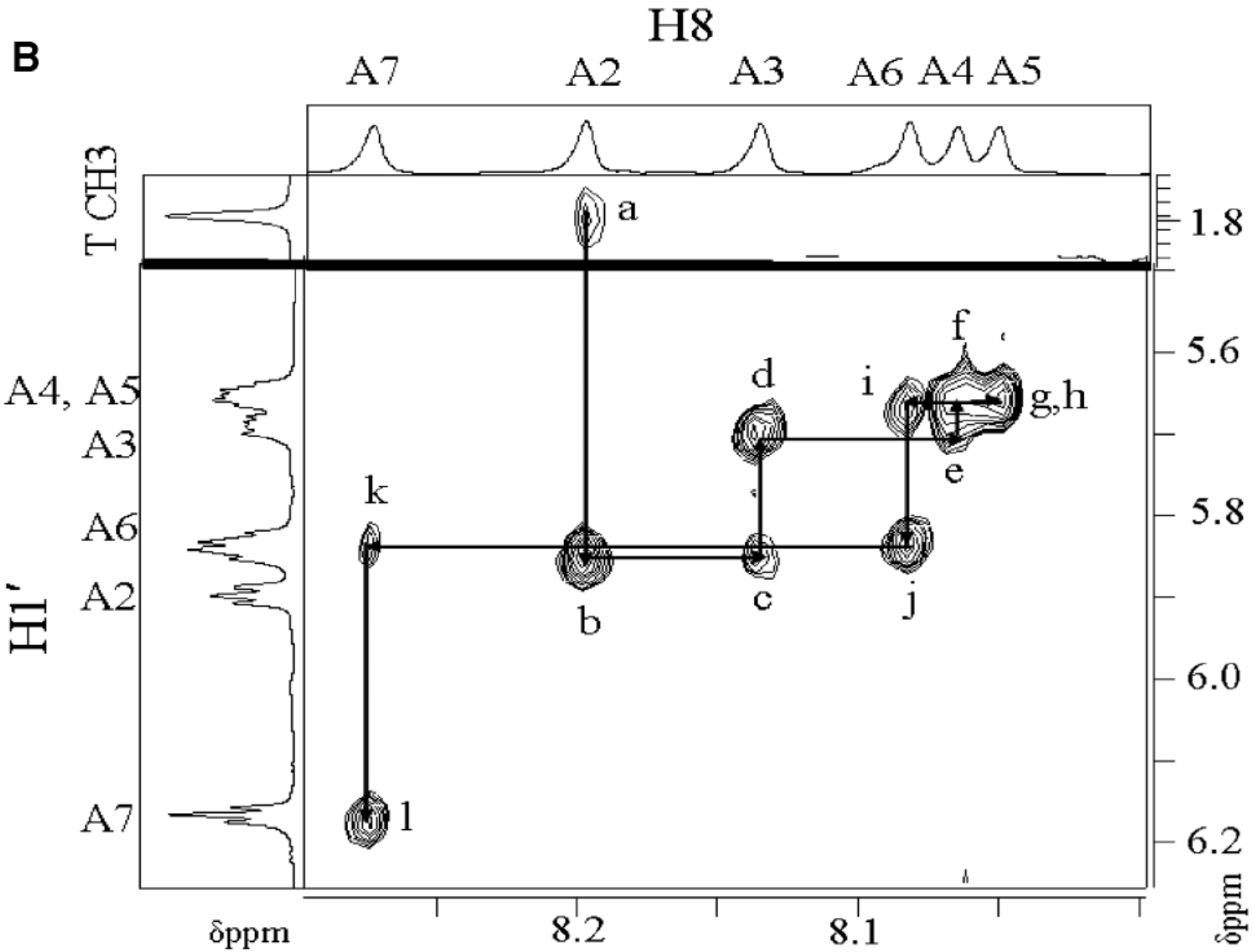


CG*A+

2D-NMR


B
H8
A7
A2
A3
A6 A4 A5
T CH3
a
1.8
f
d
i
g,h
5.6
A4, A5
A3
e
k
A6
5.8
A2
H1'
b
c
j
6.0
A7
l
6.2
δppm
8.2
8.1
δppm

Thermal stability