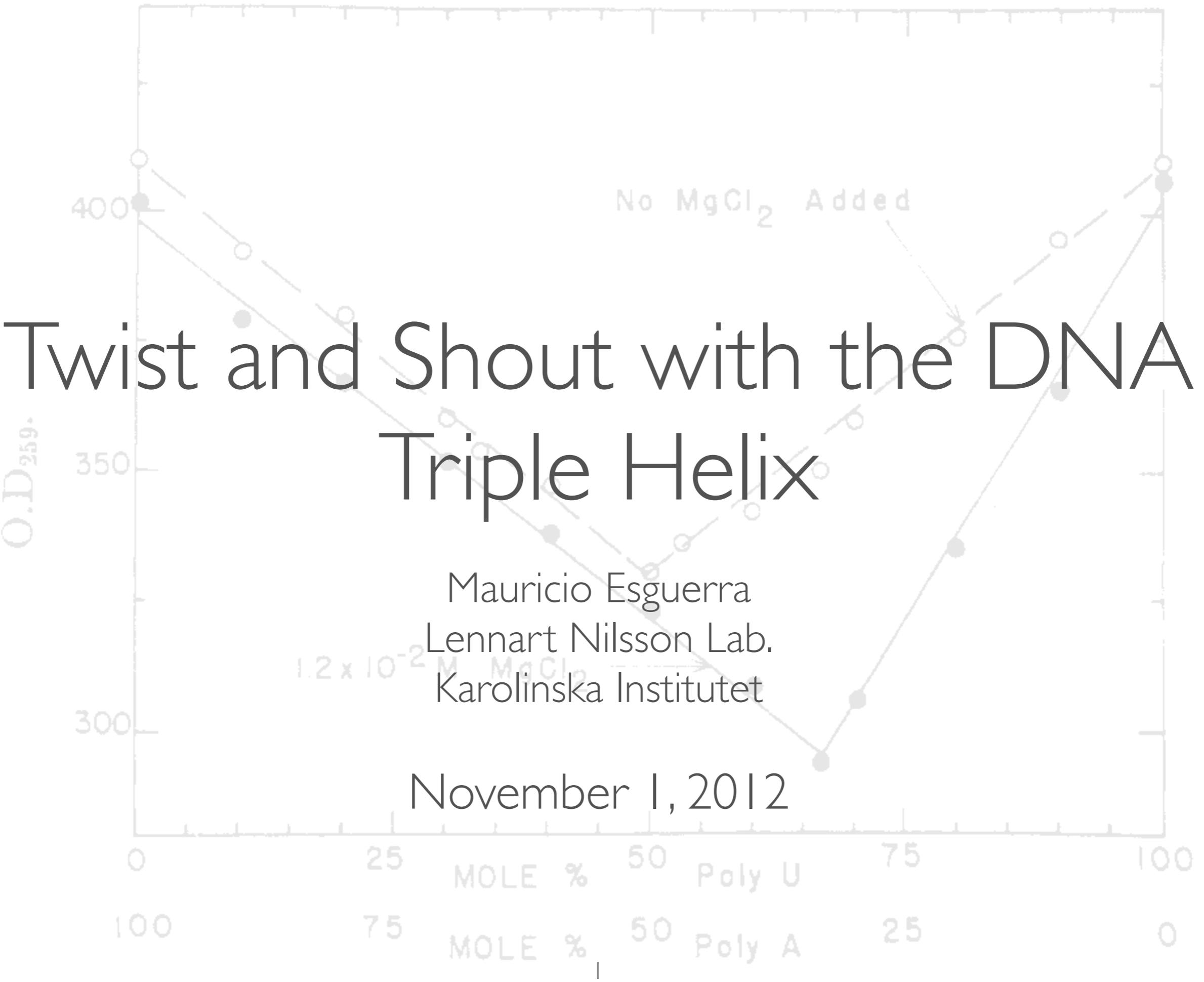


Twist and Shout with the DNA Triple Helix

Mauricio Esguerra
Lennart Nilsson Lab.
Karolinska Institutet

November 1, 2012



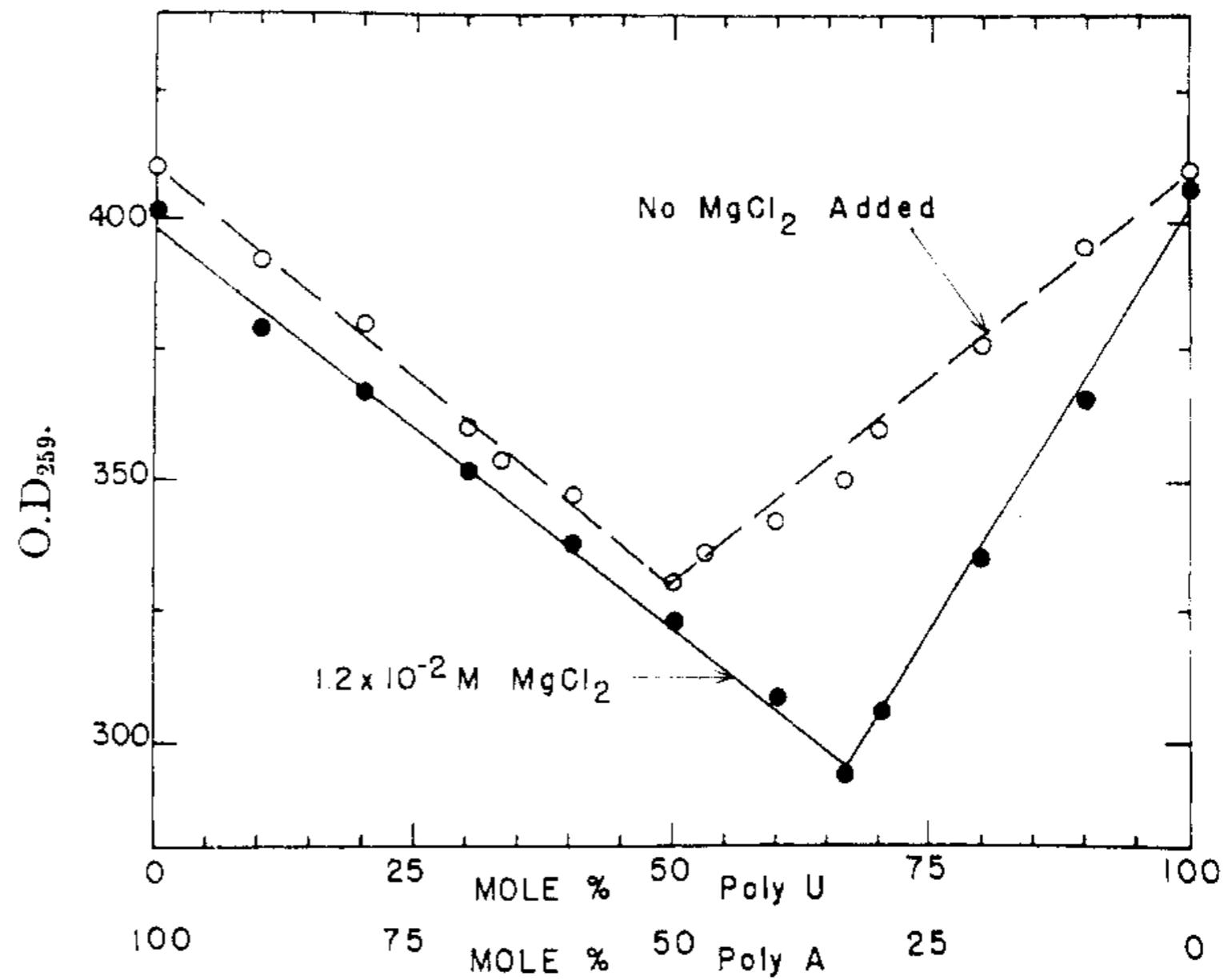
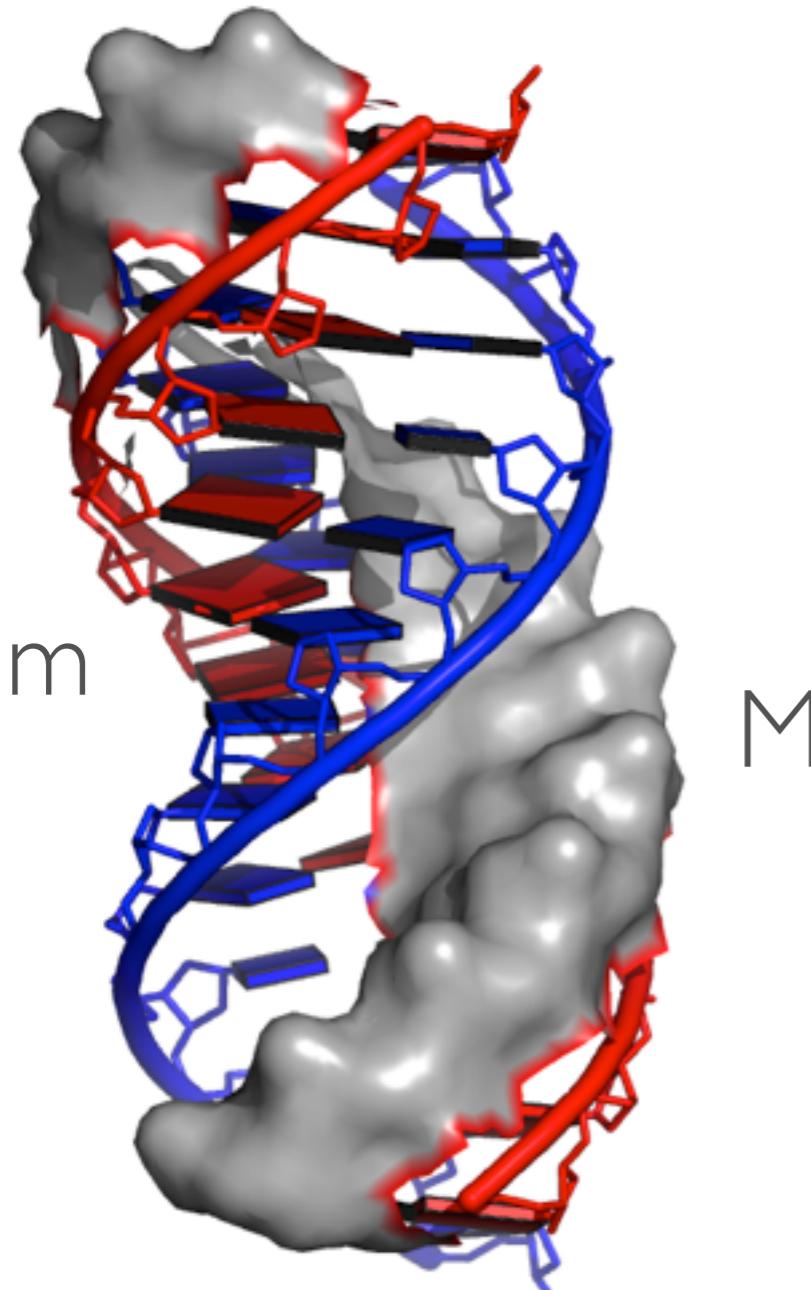
Twist and Slide with the DNA Triple Helix



November 1, 2012

Mauricio Esguerra
Lennart Nilsson Lab.
Karolinska Institutet

The RNA Triple Helix Discovered in 1957

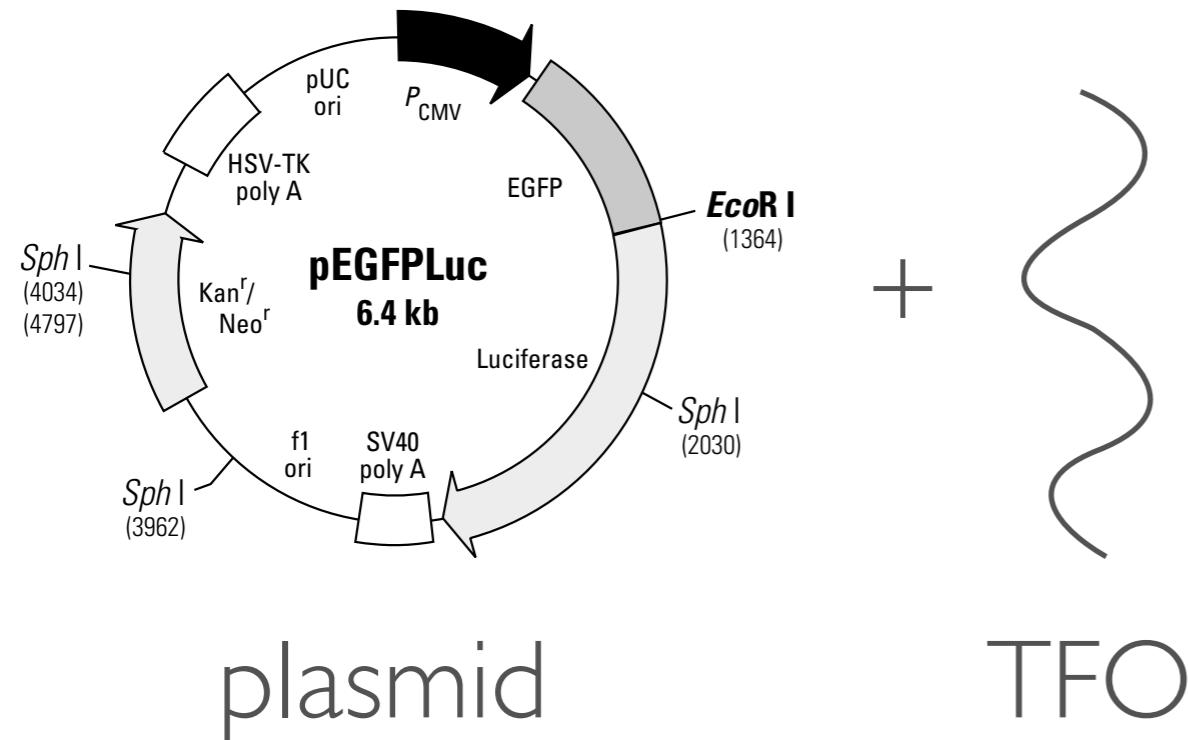


SECTION ON PHYSICAL CHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
BETHESDA 14, MARYLAND

G. FELSENFELD
DAVID R. DAVIES
ALEXANDER RICH
RECEIVED MARCH 21, 1957

Smith's Lab. Experiment with pEGFPLuc/G6 and Del-I Plasmids

The antigenic strategy



6 G sites with 2 bases in between

[pEGFPLuc/G6] = 100ng/ μ L

pH = 5.8

20mM NaPO₄ NaCl
37°C, 16-20h

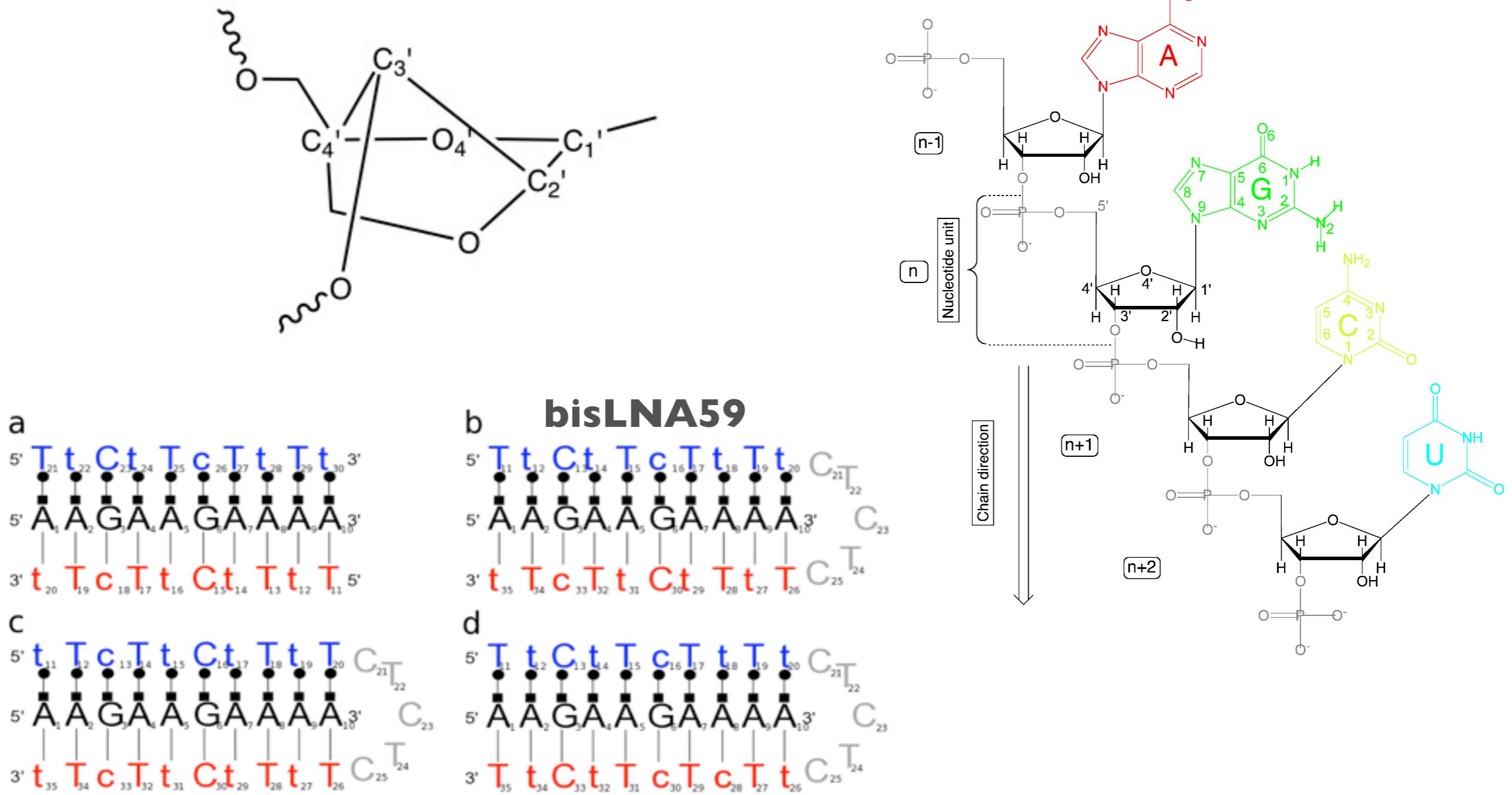
[Del-I] = 100ng/ μ L

pH = 7.3 - 7.4

50mM Tris-acetate, 120mM KCl,
5mM NaCl, 0.5mM Mg-acetate
37°C, 16-20h

GOAL:
Increase binding constants
via single-strand modification

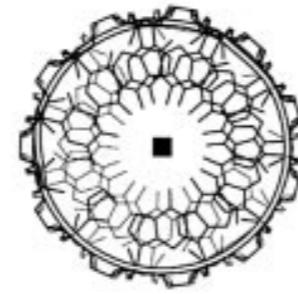
Modified Single Strands of DNA with LNA (Locked Nucleic Acids) or TFO's



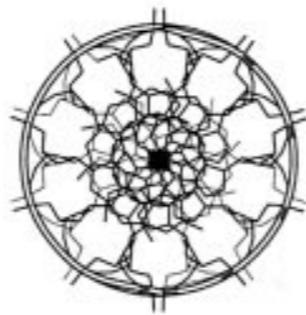
Adding Layers to DNA's Simple Complexity



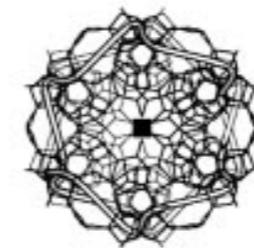
ADNA



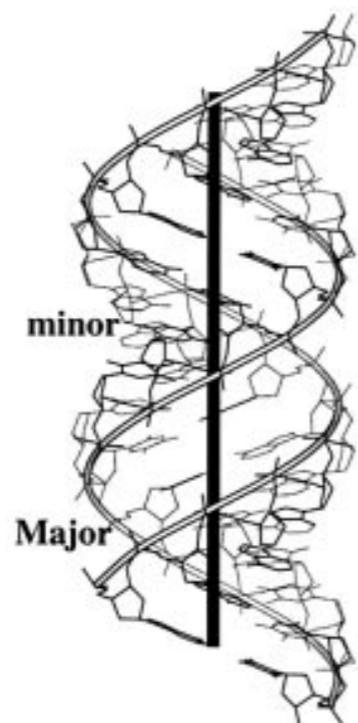
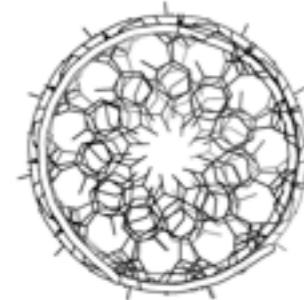
BDNA



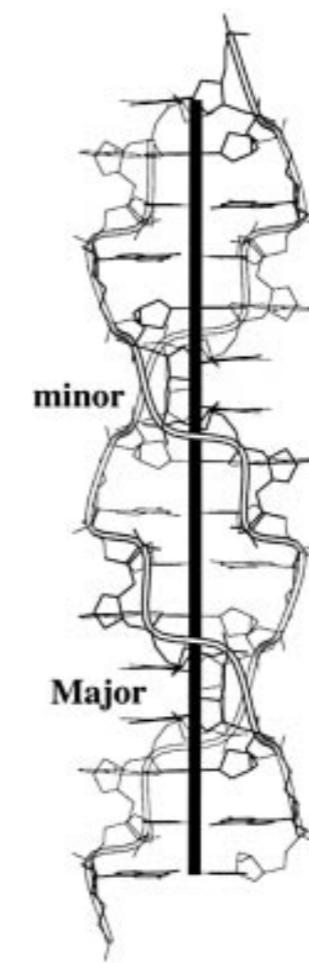
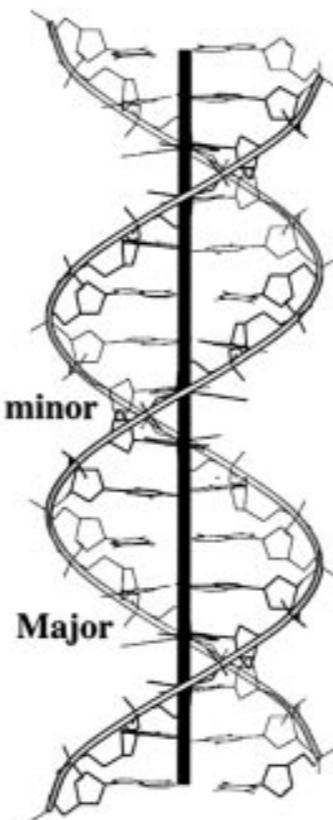
ZDNA



Triplex

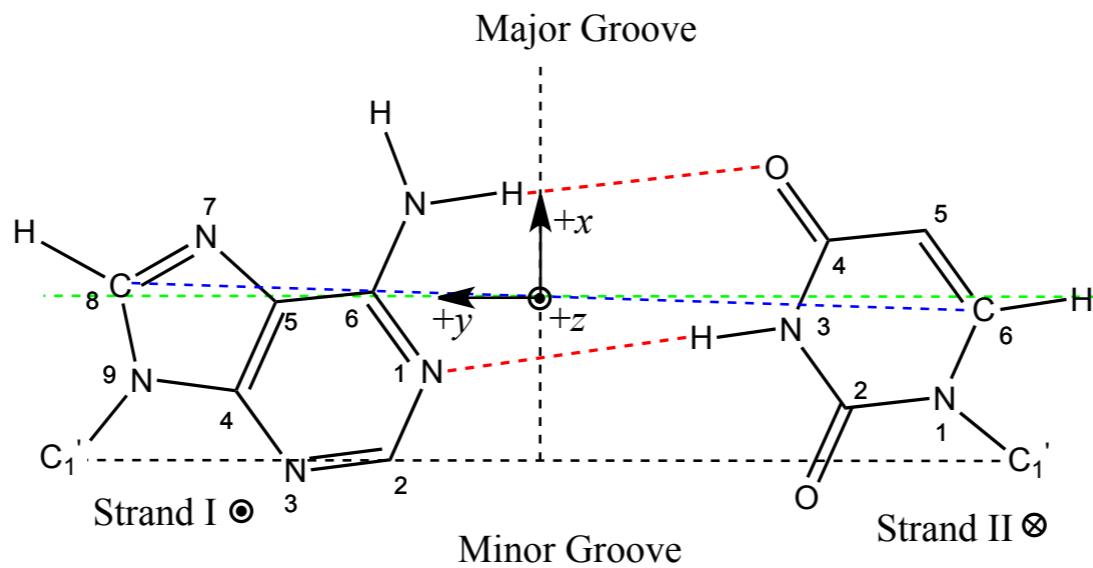


This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis



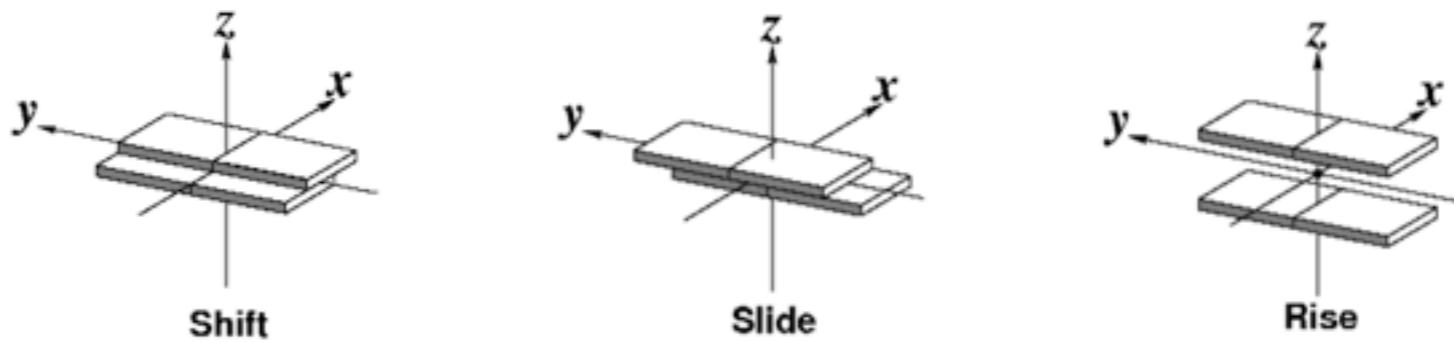
Lu-Olson NAR, 2003, 31, 5108-5121

The Rigid-Block Perspective. A Local, Base-Centered Approach

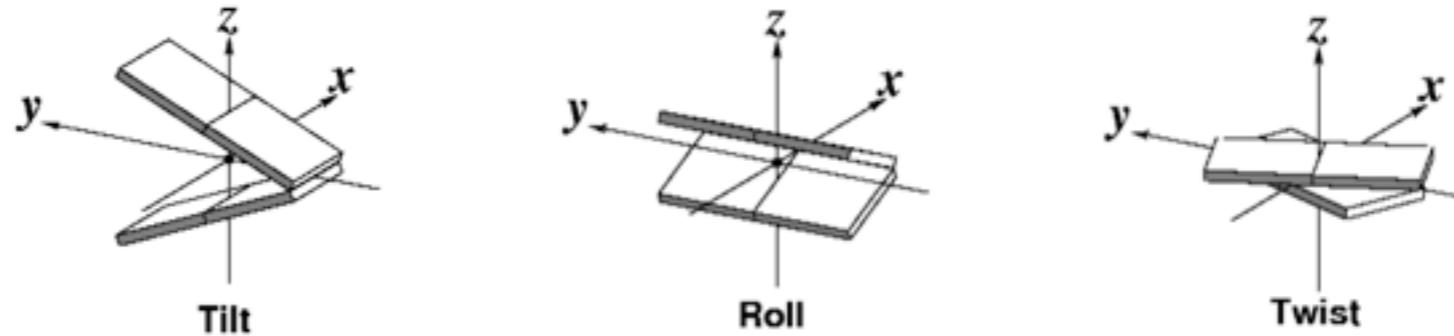


Base-Pair-Step Parameters

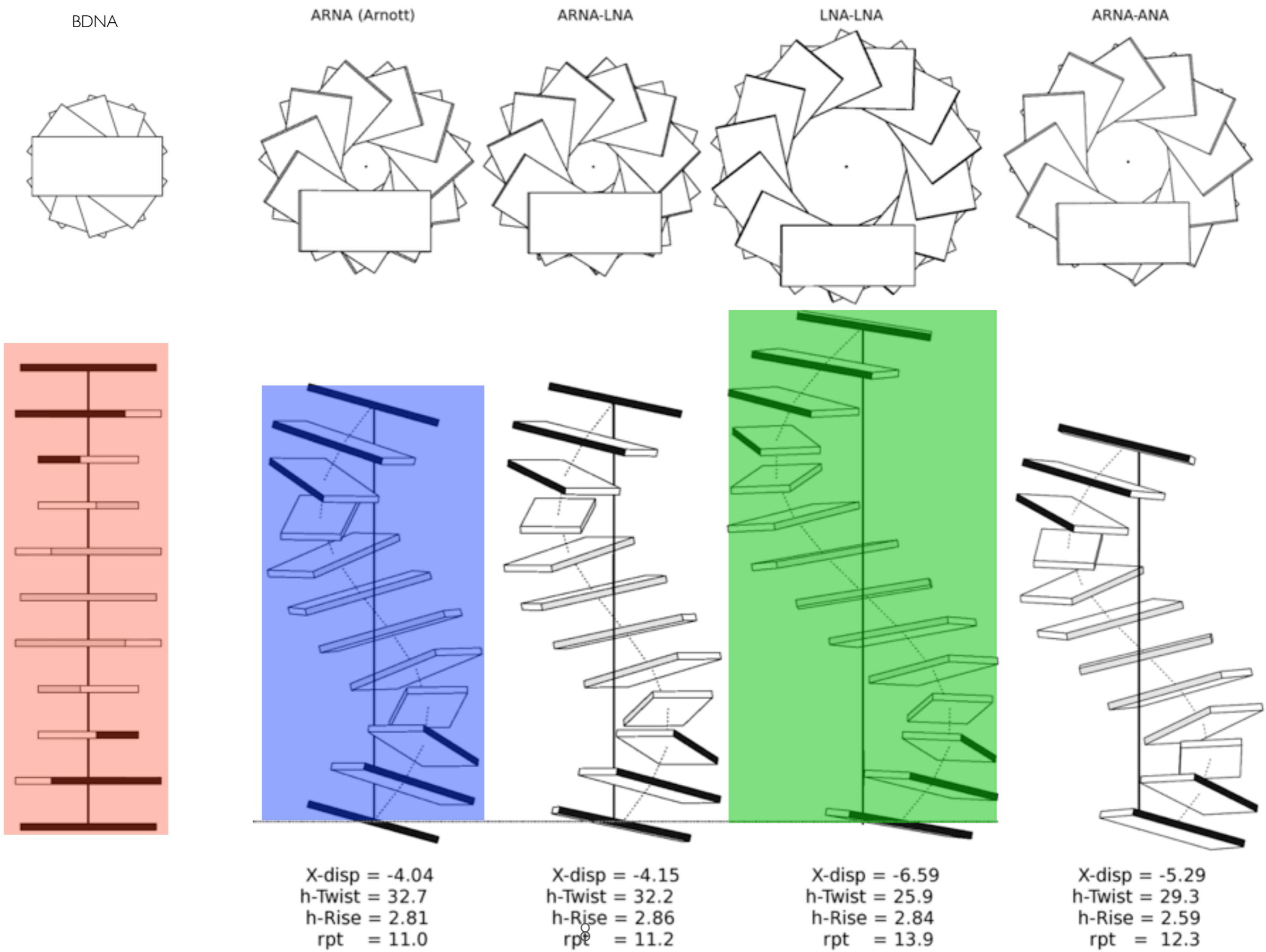
translation



rotation



A Mechanical Perspective of Helicoidal Polymers



How Do We Run Our Experiments? 19th Century Physics i.e. Molecular Dynamics

$$F_i = m_i a_i$$

$$F = -\nabla_i V(r^N)$$

$$s = v_0 \delta t + \frac{a \delta t^2}{2}$$

Verlet's algorithm:

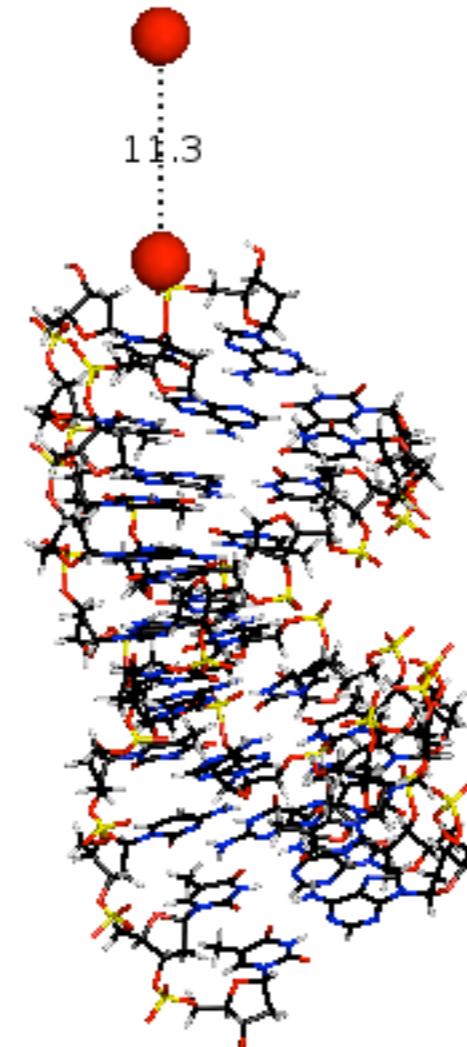
$$r(t + \delta t) = 2r(t) - r(t - \delta t) + a(t)\delta t^2$$

Leap-frog algorithm:

$$r(t + \delta t) = r(t) + v(t - \frac{1}{2}\delta t)\delta t$$

$$v(t + \frac{1}{2}\delta t) = v(t - \frac{1}{2}\delta t) + a(t)\delta t$$

The timestep has to be chosen around 1-2 fs



Borrowed from L. Nilsson

Add Cations to Neutralize Polyanion

$$F_i = m_i a_i$$

$$F = -\nabla_i V(r^N)$$

$$s = v_0 \delta t + \frac{a \delta t^2}{2}$$

Verlet's algorithm:

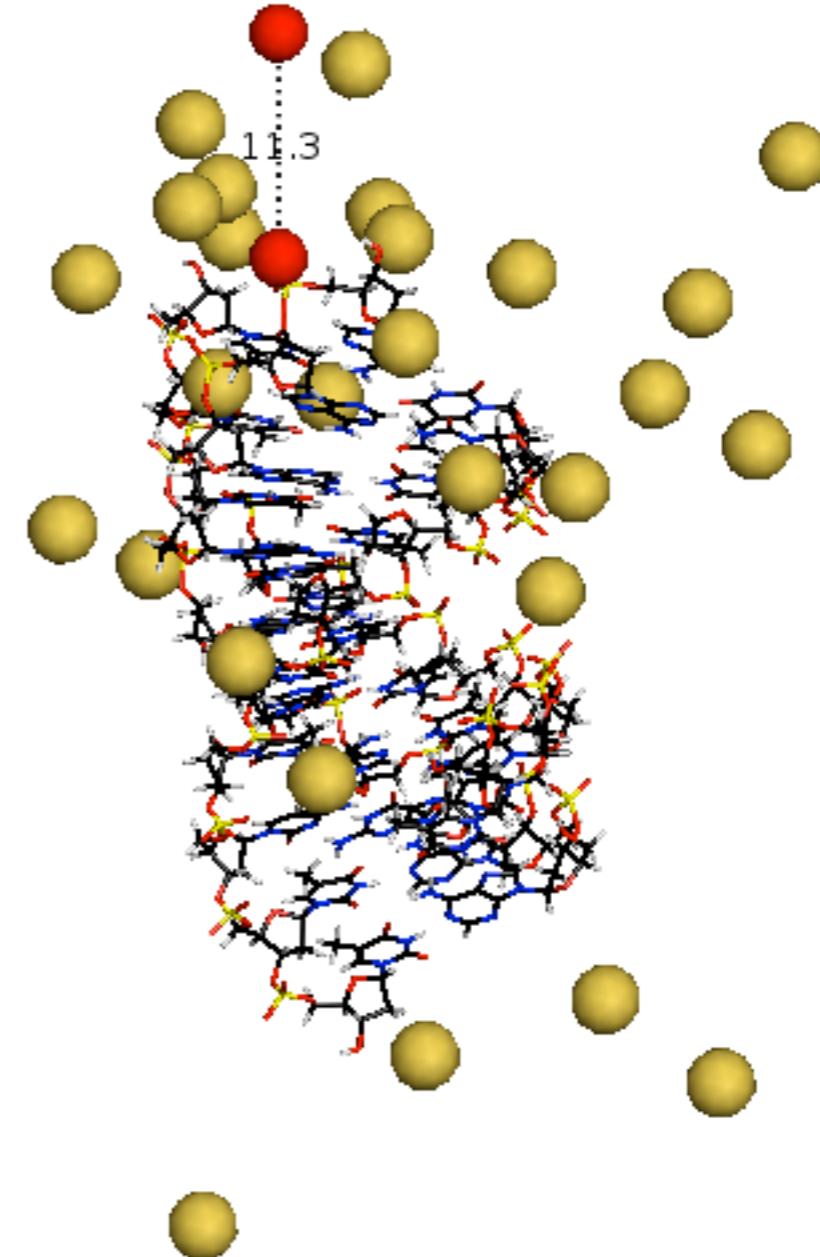
$$r(t + \delta t) = 2r(t) - r(t - \delta t) + a(t)\delta t^2$$

Leap-frog algorithm:

$$r(t + \delta t) = r(t) + v(t - \frac{1}{2} \delta t) \delta t$$

$$v(t + \frac{1}{2} \delta t) = v(t - \frac{1}{2} \delta t) + a(t) \delta t$$

The timestep has to be chosen around 1-2 fs



Borrowed from L. Nilsson

Add Some Waters (TIP3)

$$F_i = m_i a_i$$

$$F = -\nabla_i V(r^N)$$

$$s = v_0 \delta t + \frac{a \delta t^2}{2}$$

Verlet's algorithm:

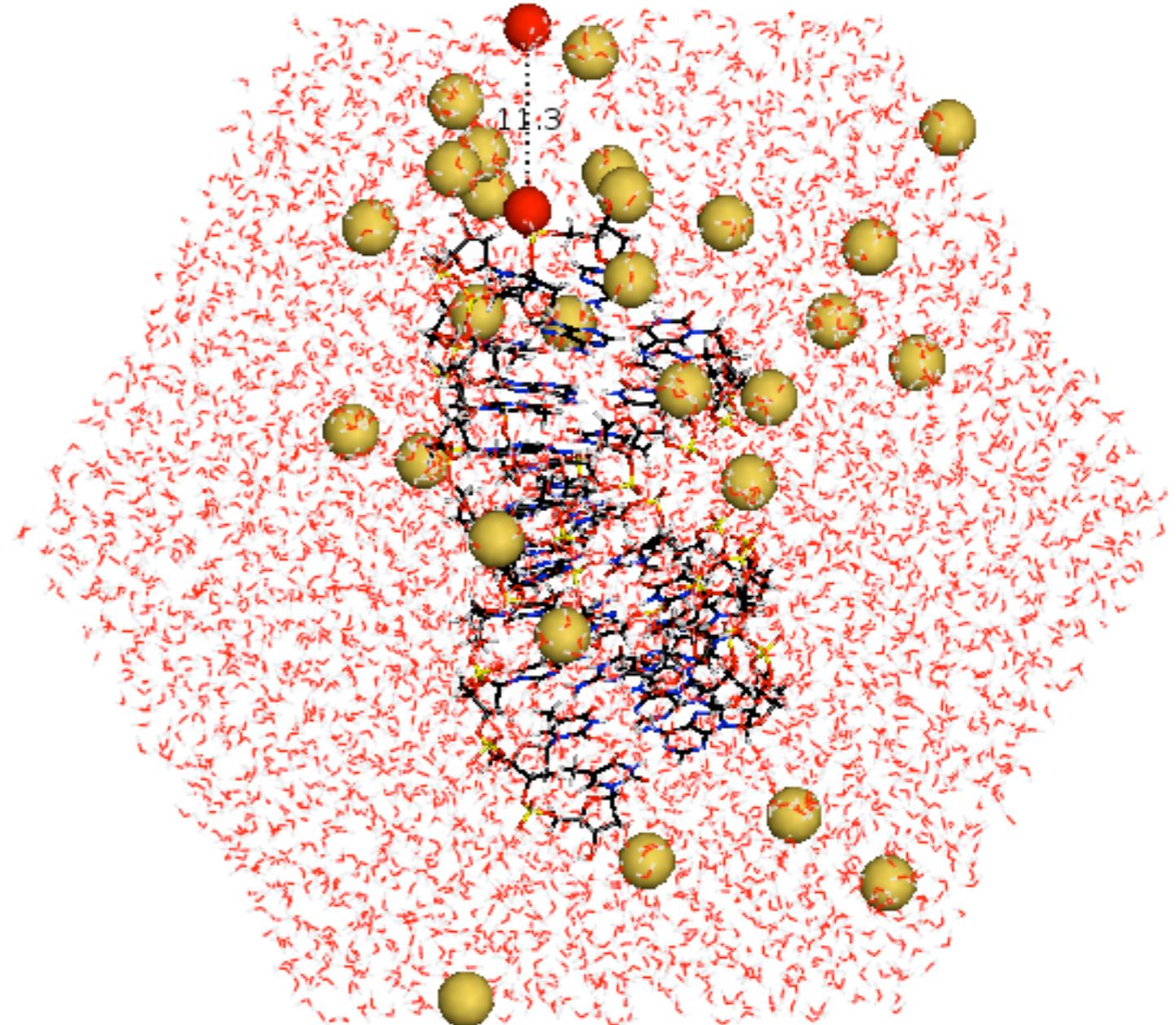
$$r(t + \delta t) = 2r(t) - r(t - \delta t) + a(t) \delta t$$

Leap-frog algorithm:

$$r(t + \delta t) = r(t) + v(t - \frac{1}{2} \delta t) \delta t$$

$$v(t + \frac{1}{2} \delta t) = v(t - \frac{1}{2} \delta t) + a(t) \delta t$$

The timestep has to be chosen around



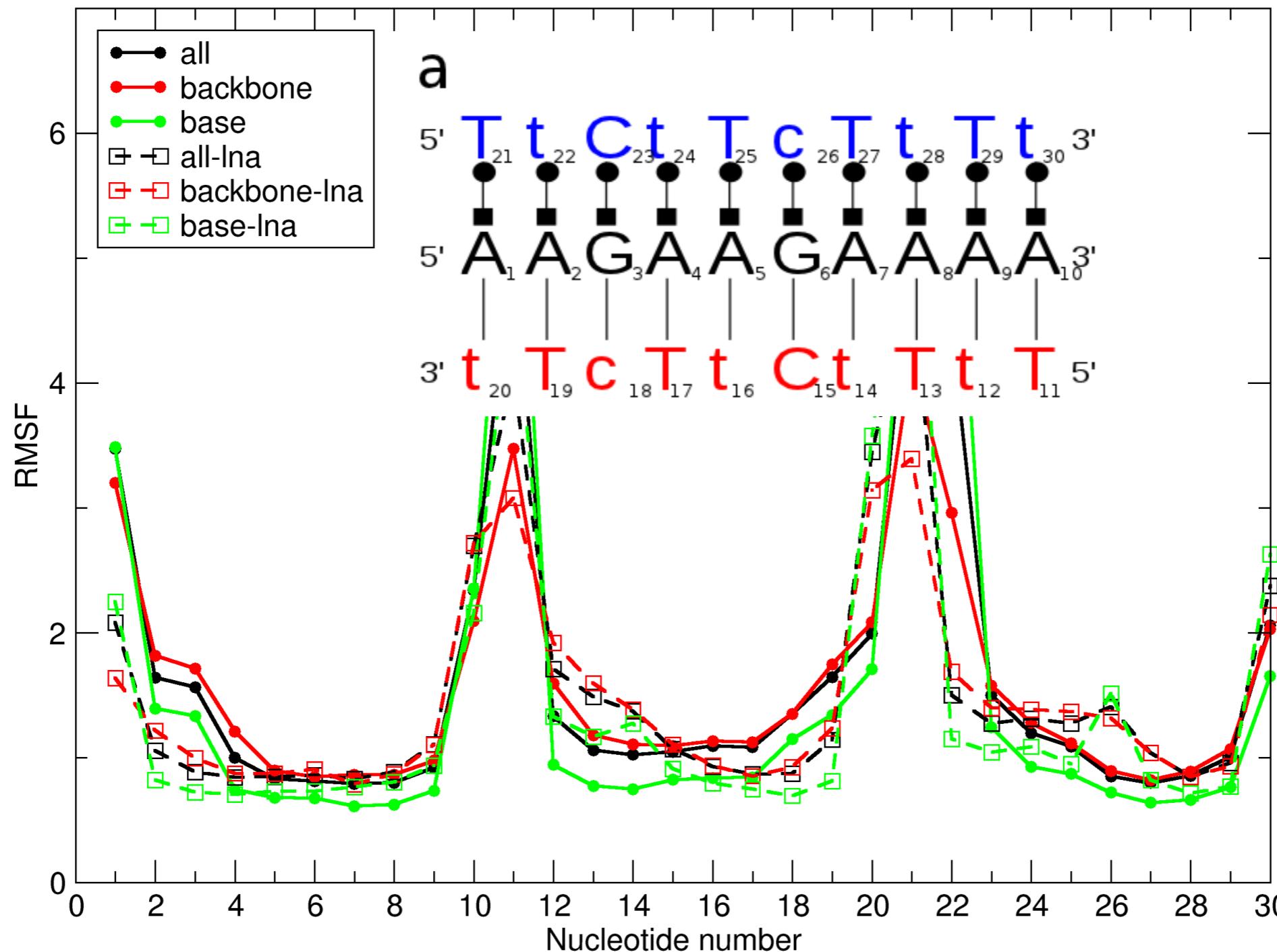
RHDO = Rhombic Dodecahedron
14997 TIP3 Water atoms
951 Triplex atoms
15975 Total atoms

Borrowed from L. Nilsson

SHOW ME THE RESULTS!
WILL YA!

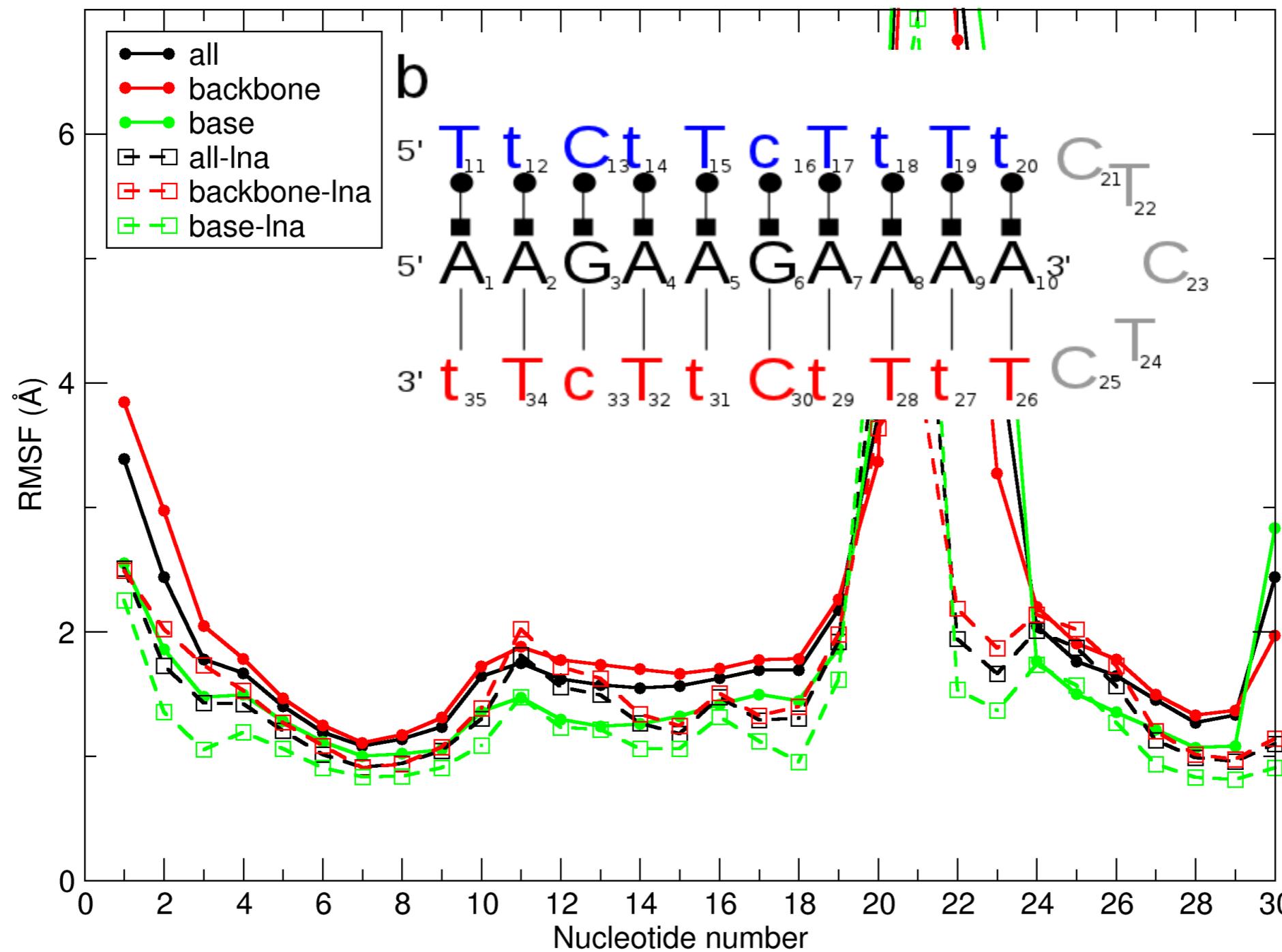
“Flexibility” On a 40ns Window

RMSF of nucleotides
triplex and triplex-Ina

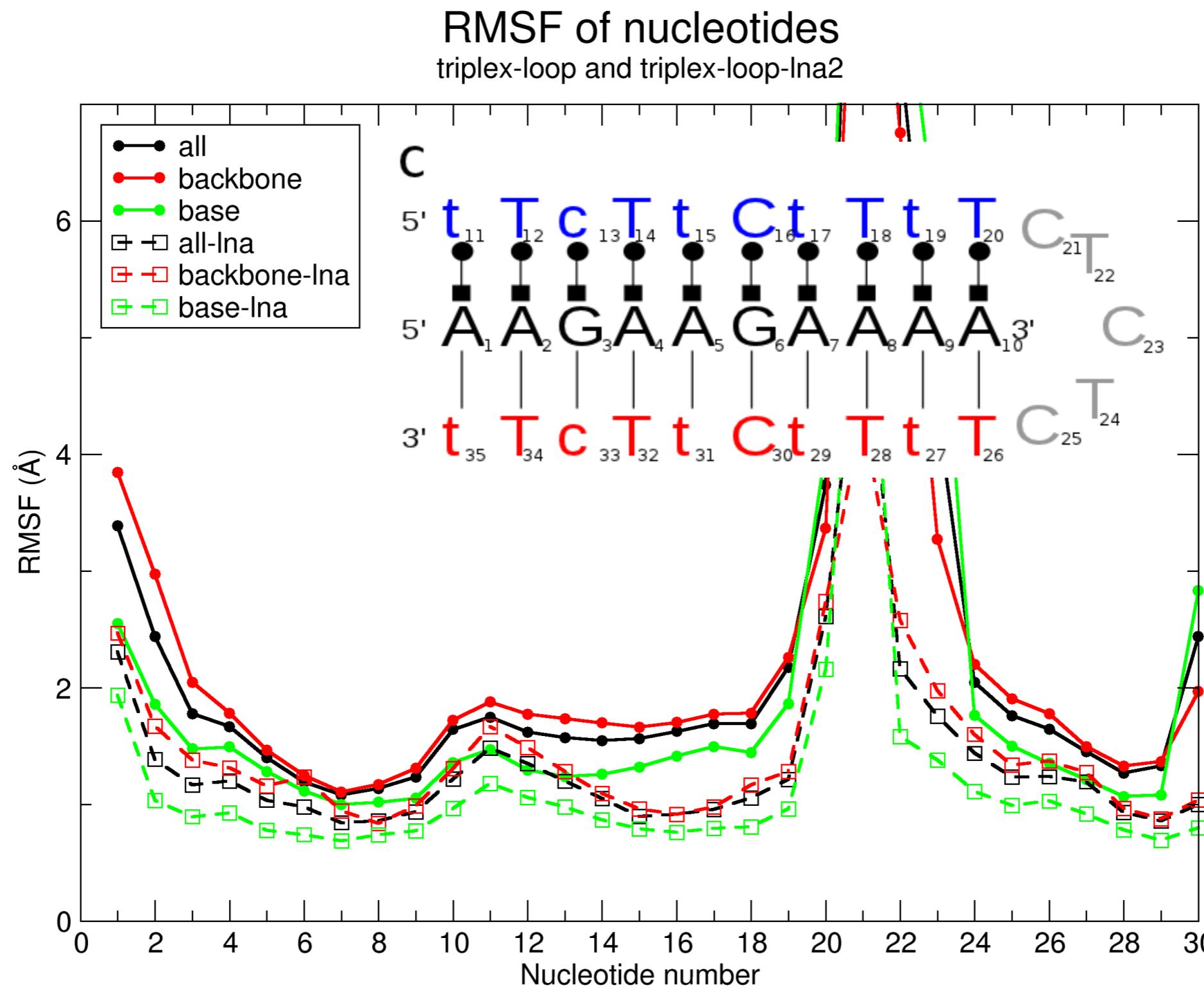


“Flexibility” On a 40ns Window

RMSF of nucleotides
triplex-loop and triplex-loop-Ina

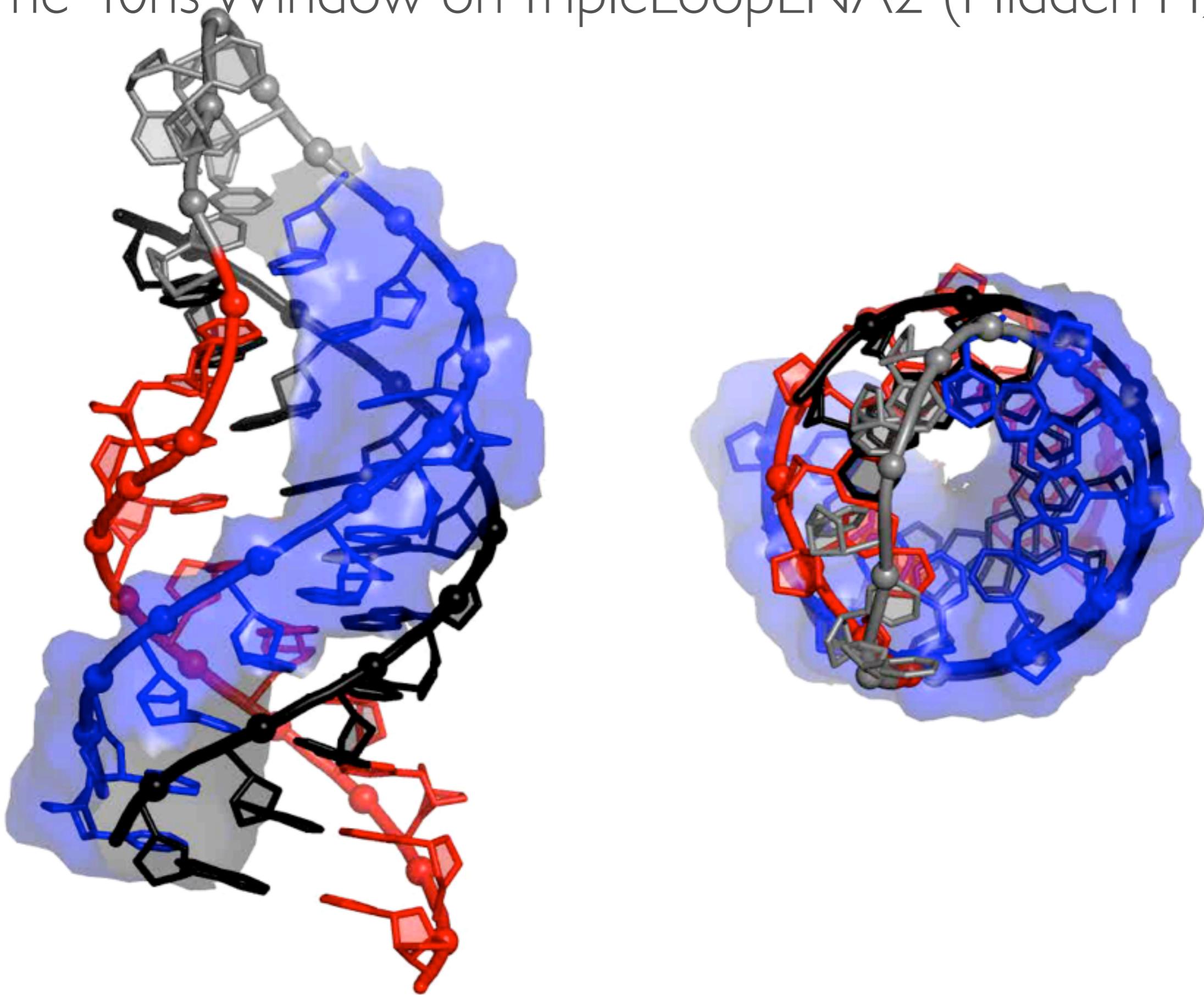


“Flexibility” On a 40ns Window

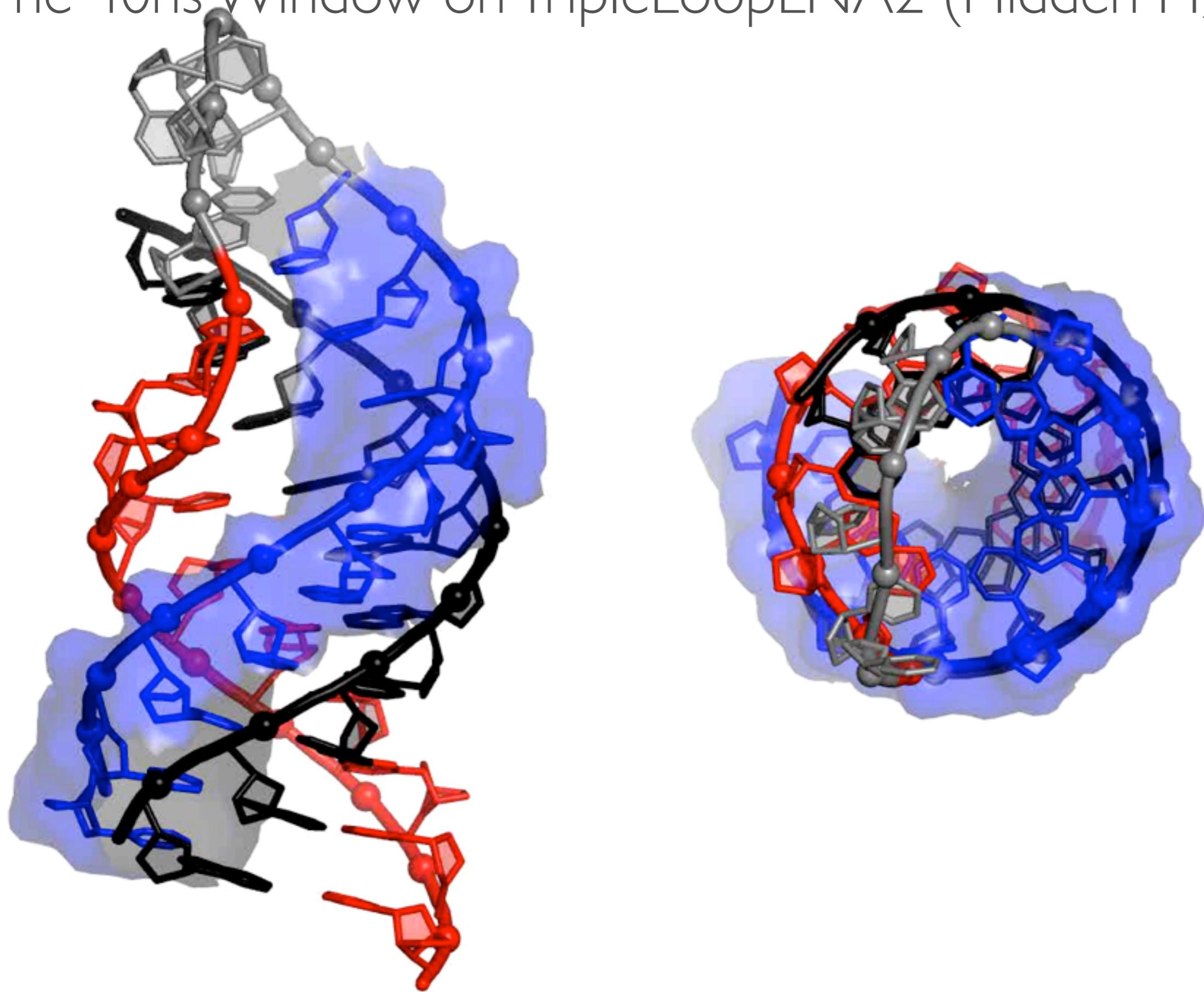


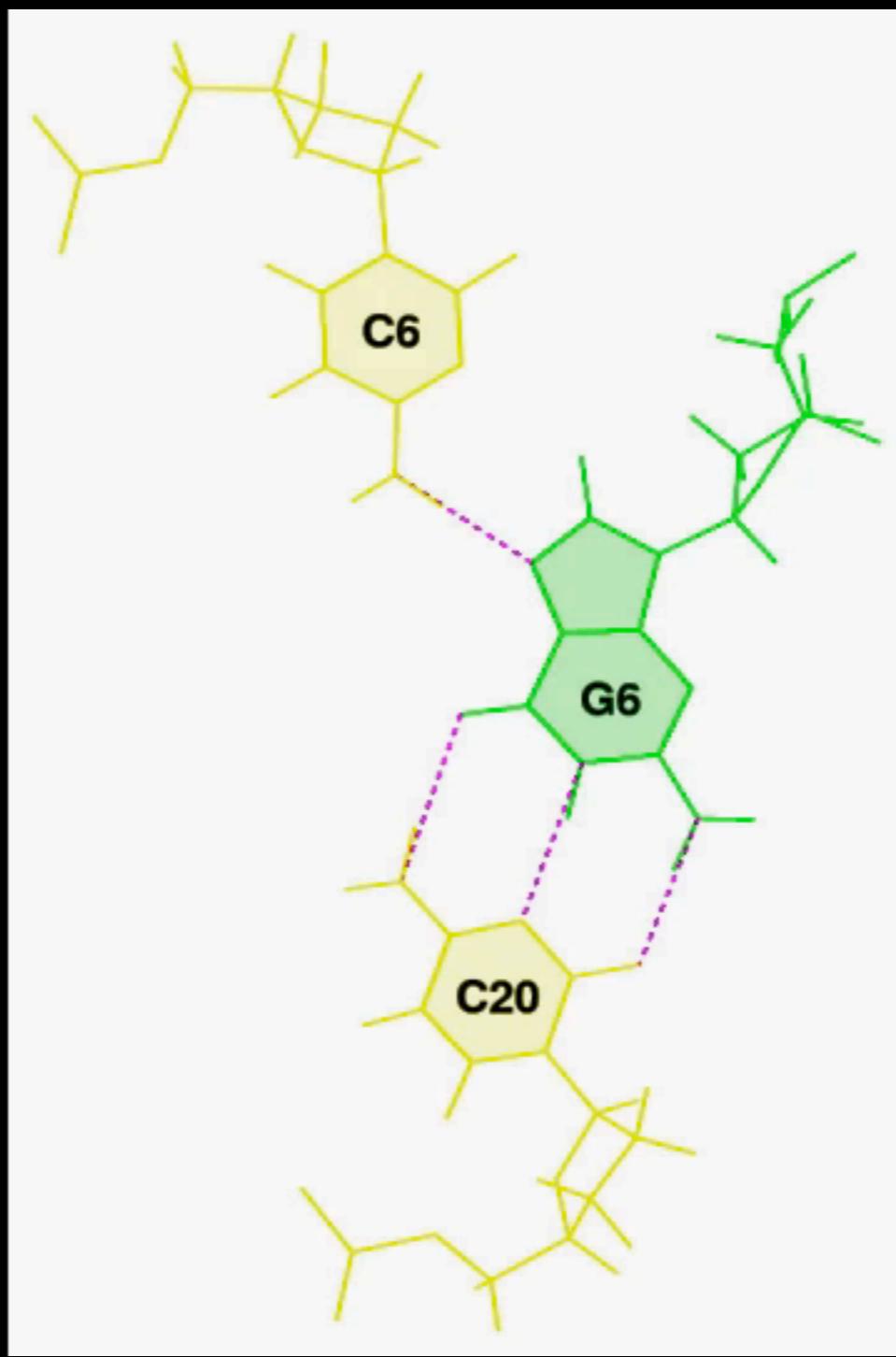
The 40ns Window on TripleLoopLNA2 (Hidden H₂O)

The 40ns Window on TripleLoopLNA2 (Hidden H₂O)

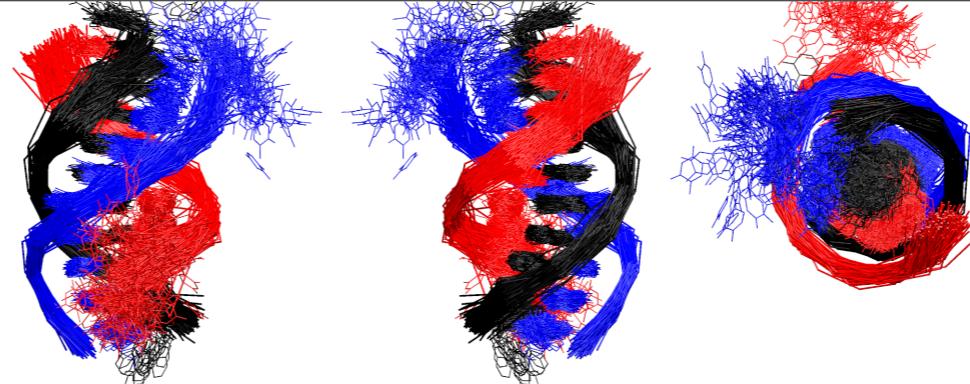


The 40ns Window on TripleLoopLNA2 (Hidden H₂O)

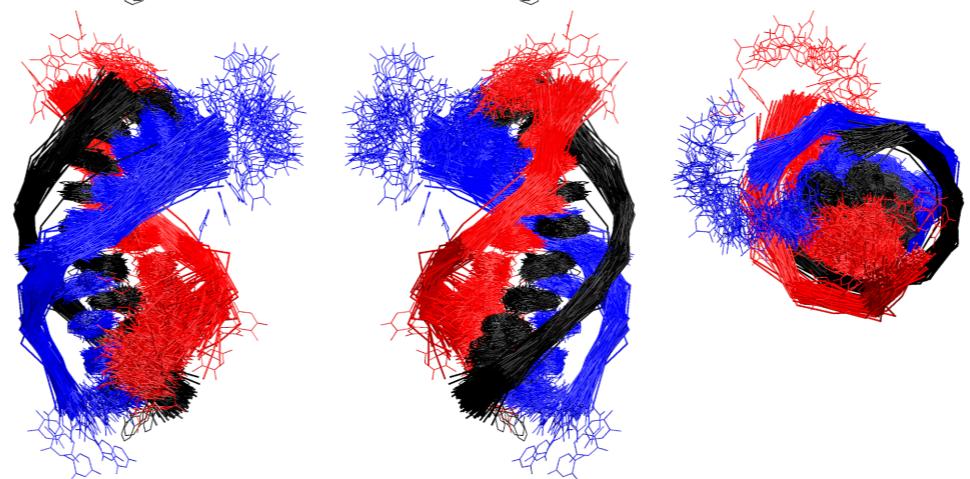




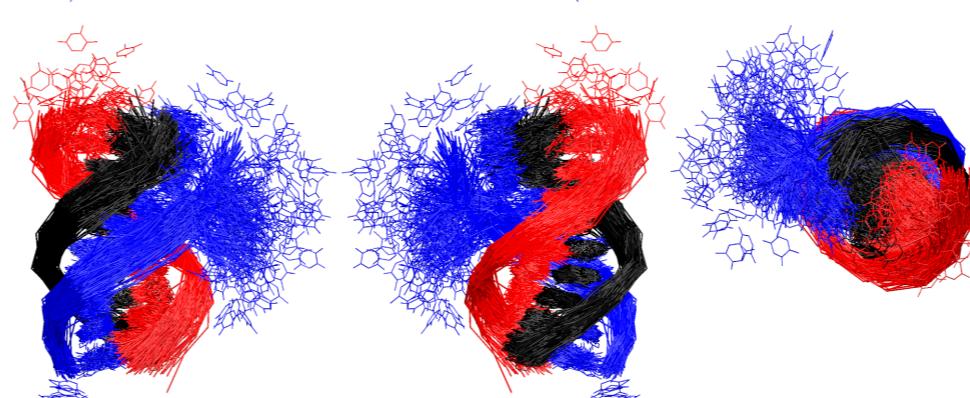
Triplex



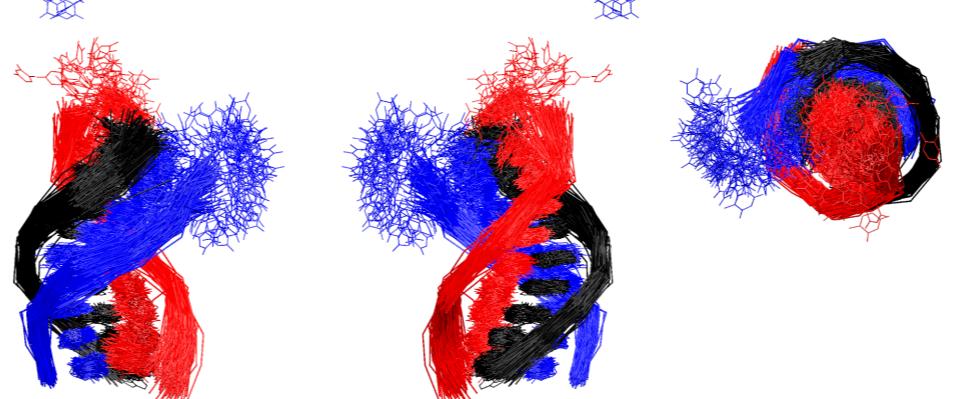
TriplexLNA



TriplexLoop



TriplexLoopLNA

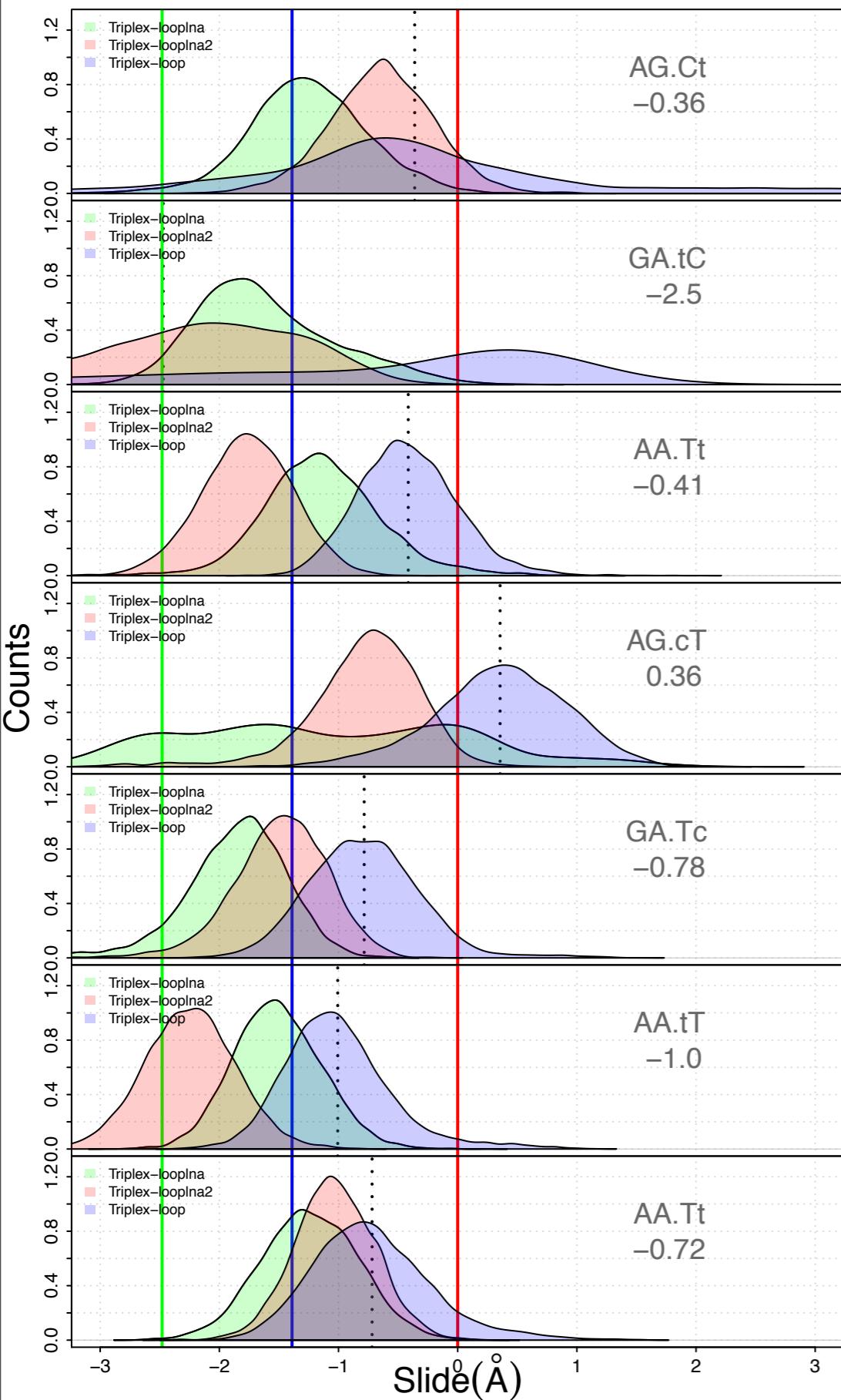


TriplexLoopLNA2



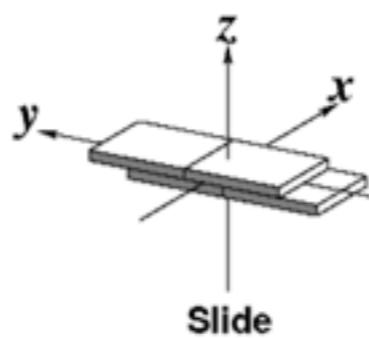
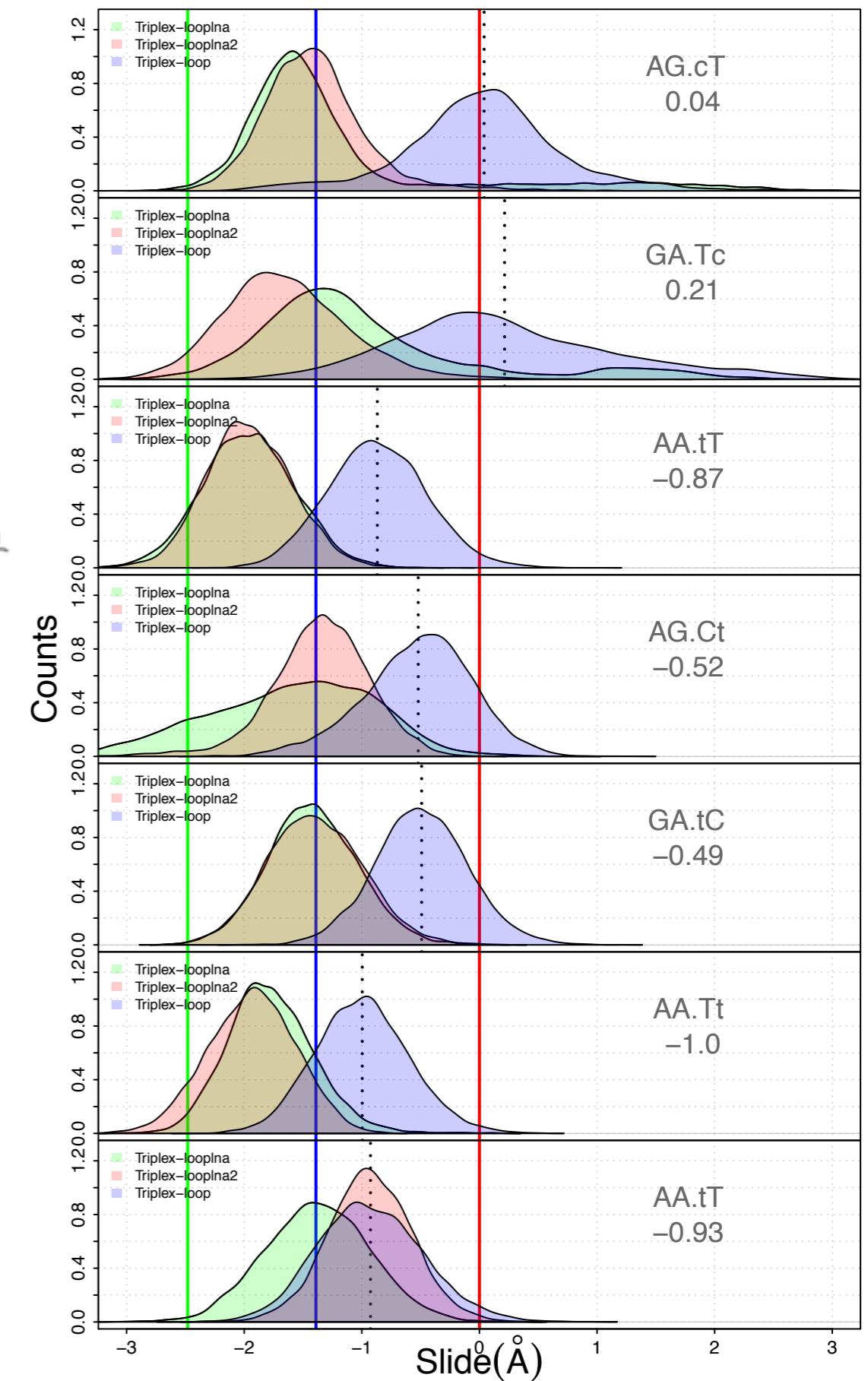
Watson-Crick Steps

Slide shifts to lower values with intercalated LNA's



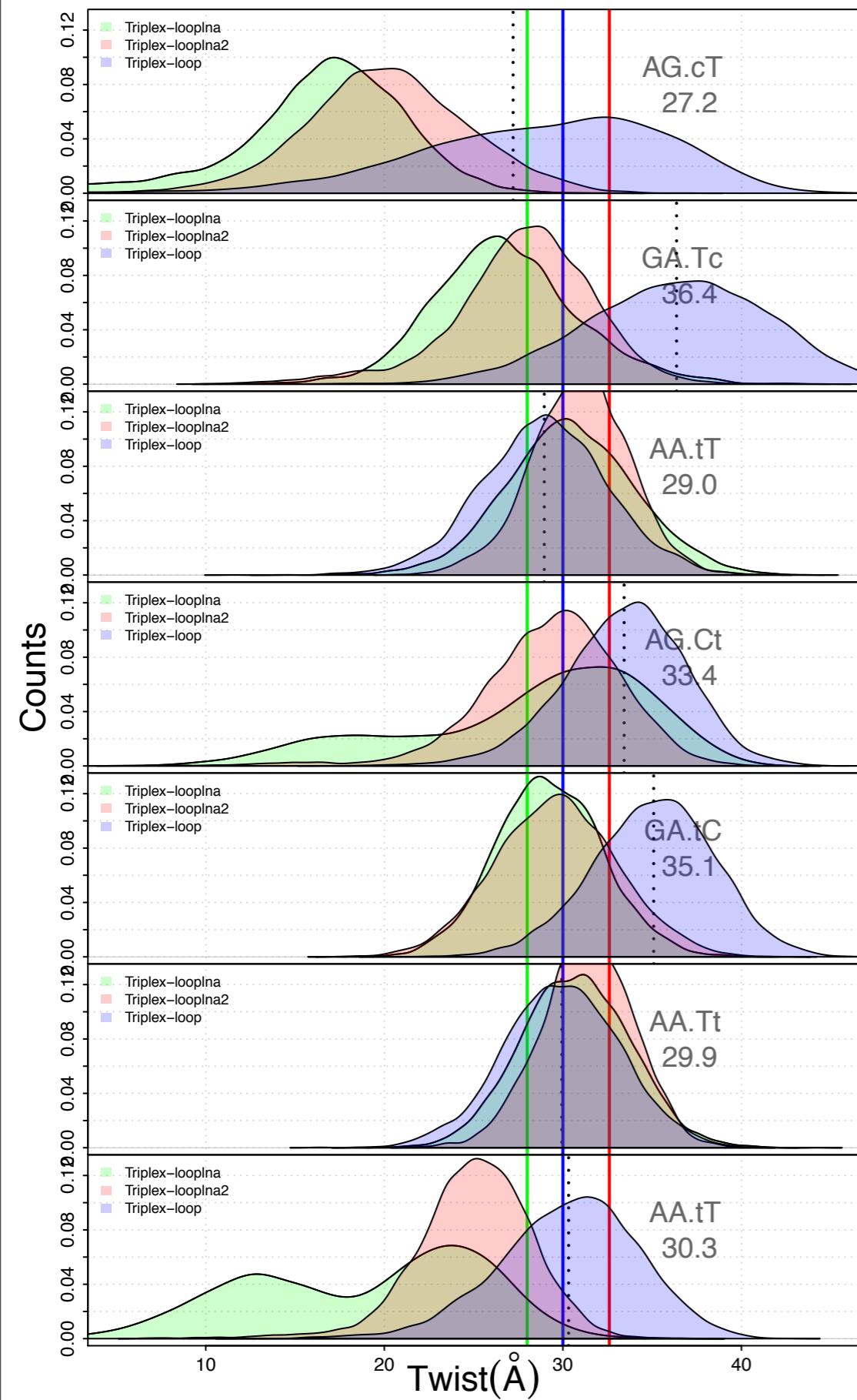
Hoogsteen Steps

Slide shifts to lower values with intercalated LNA's



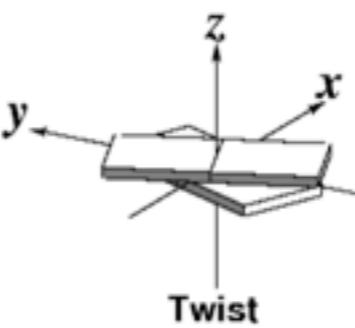
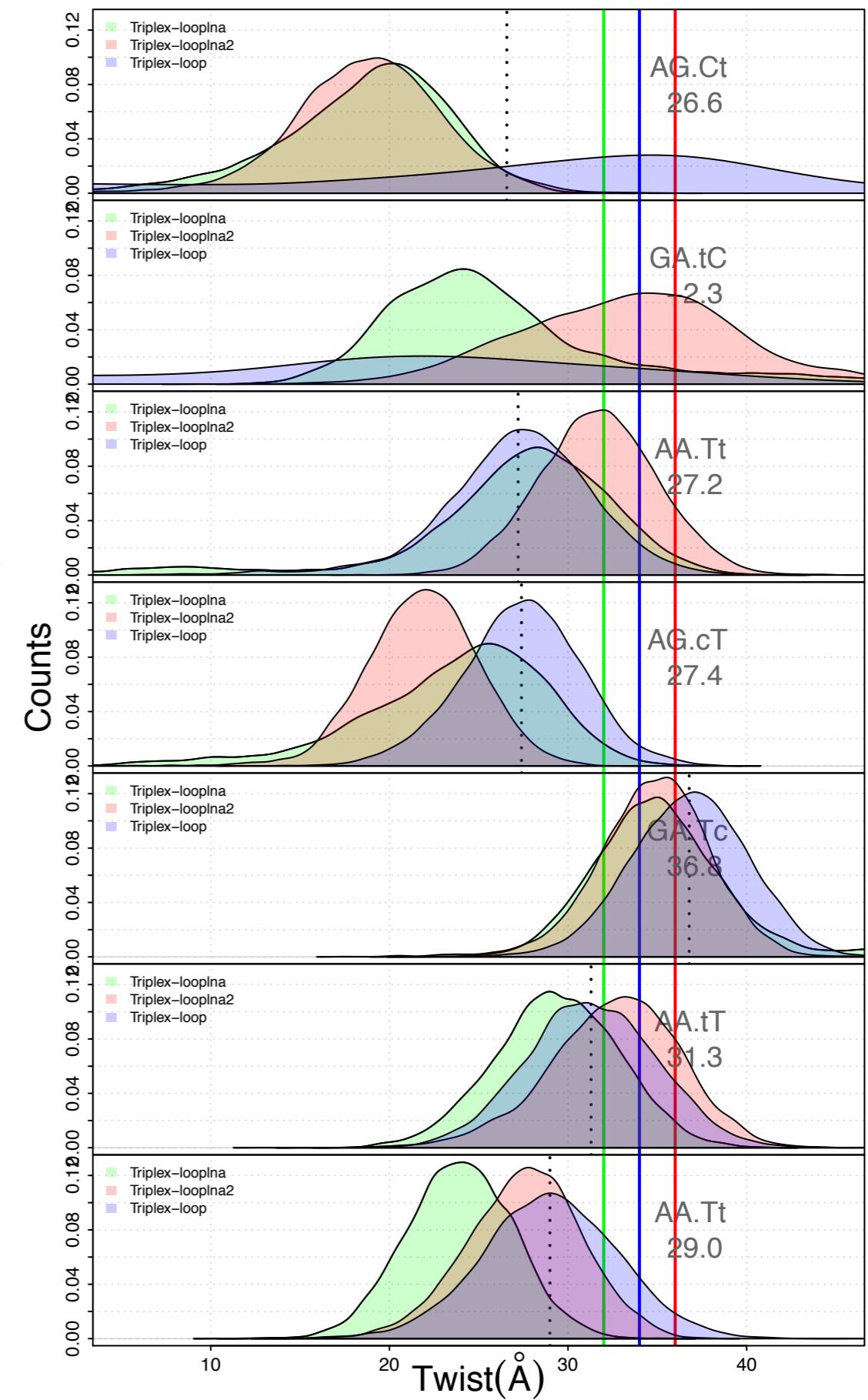
Watson-Crick Steps

Twist shifts to lower values with intercalated LNA's



Hoogsteen Steps

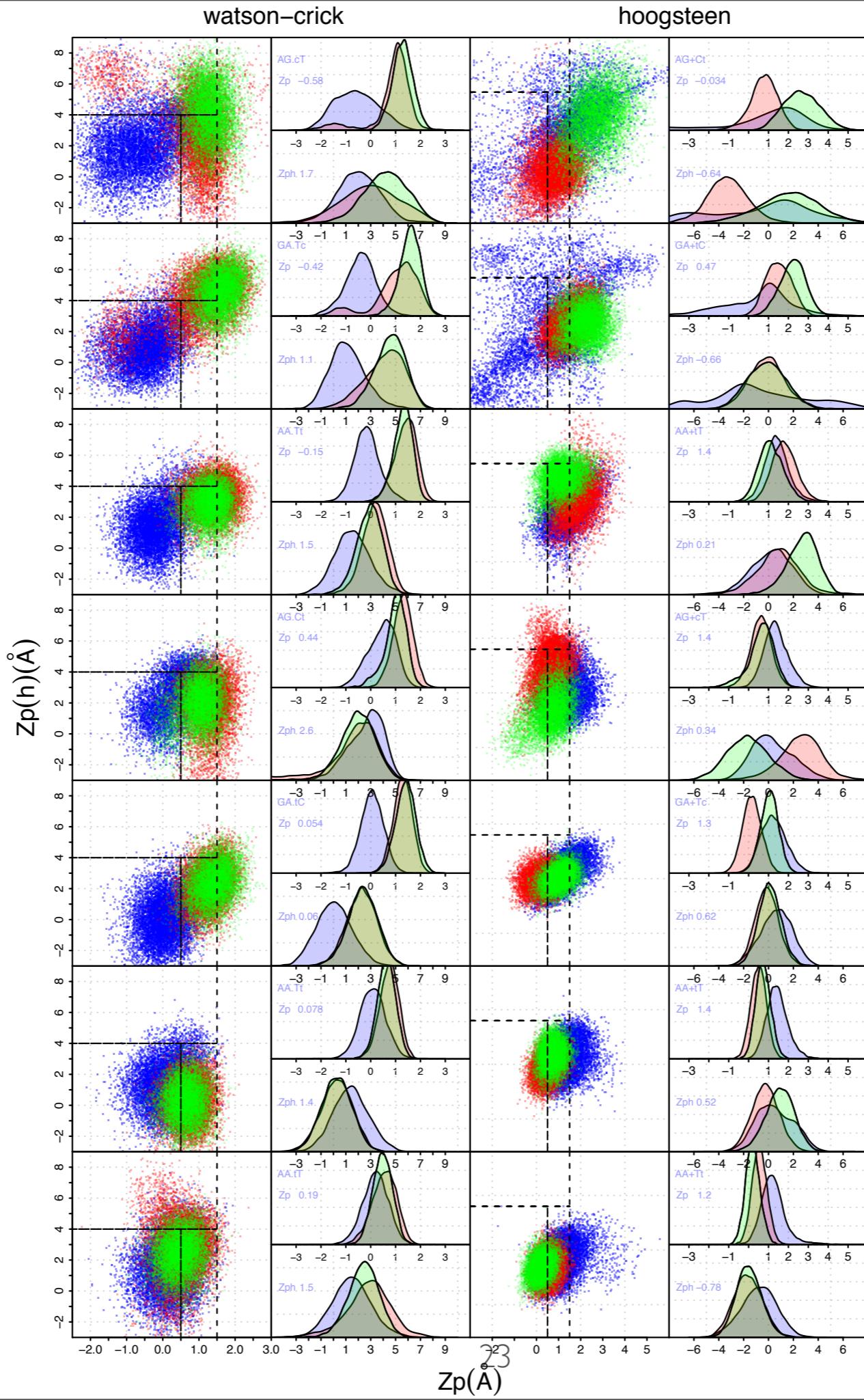
Twist shifts to lower values with intercalated LNA's

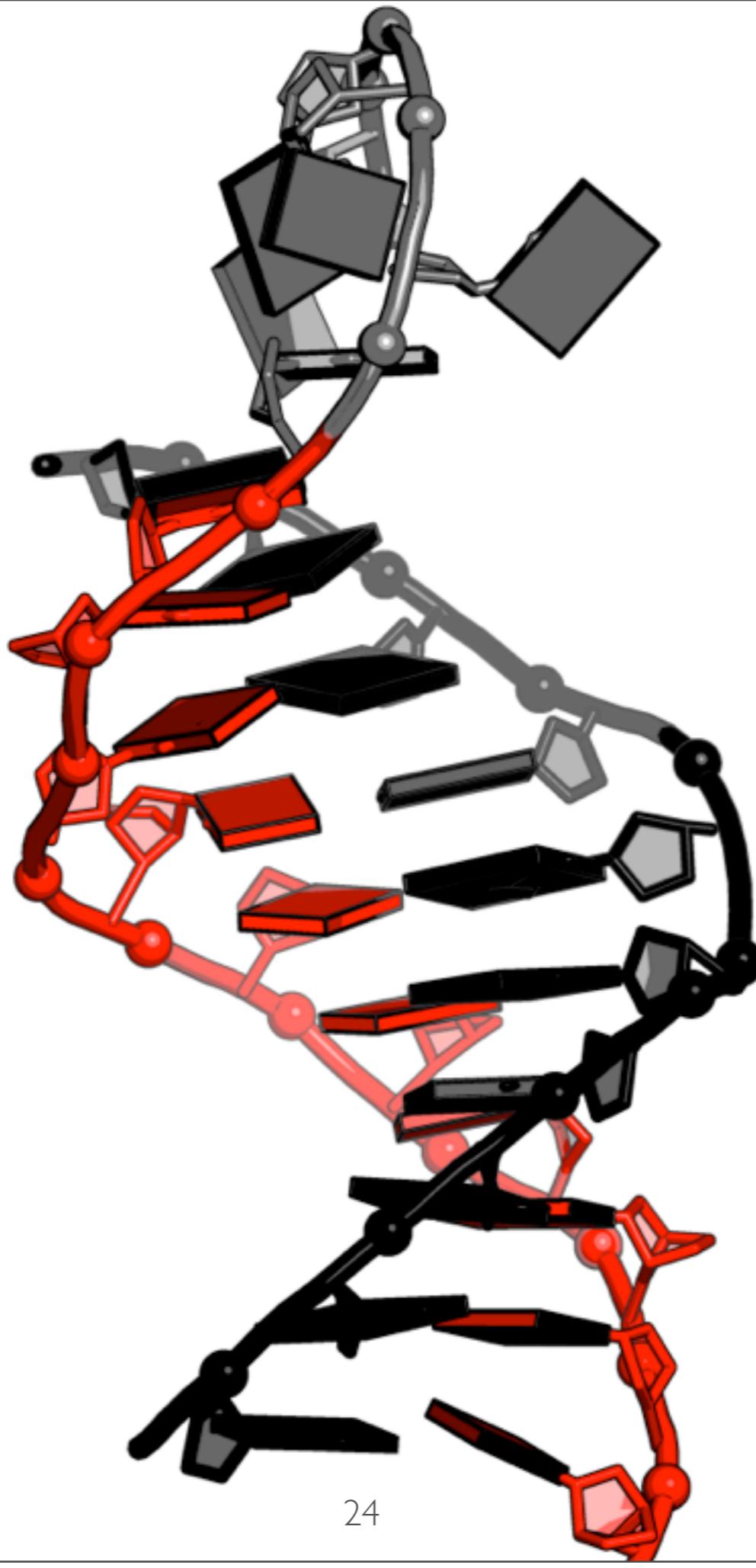


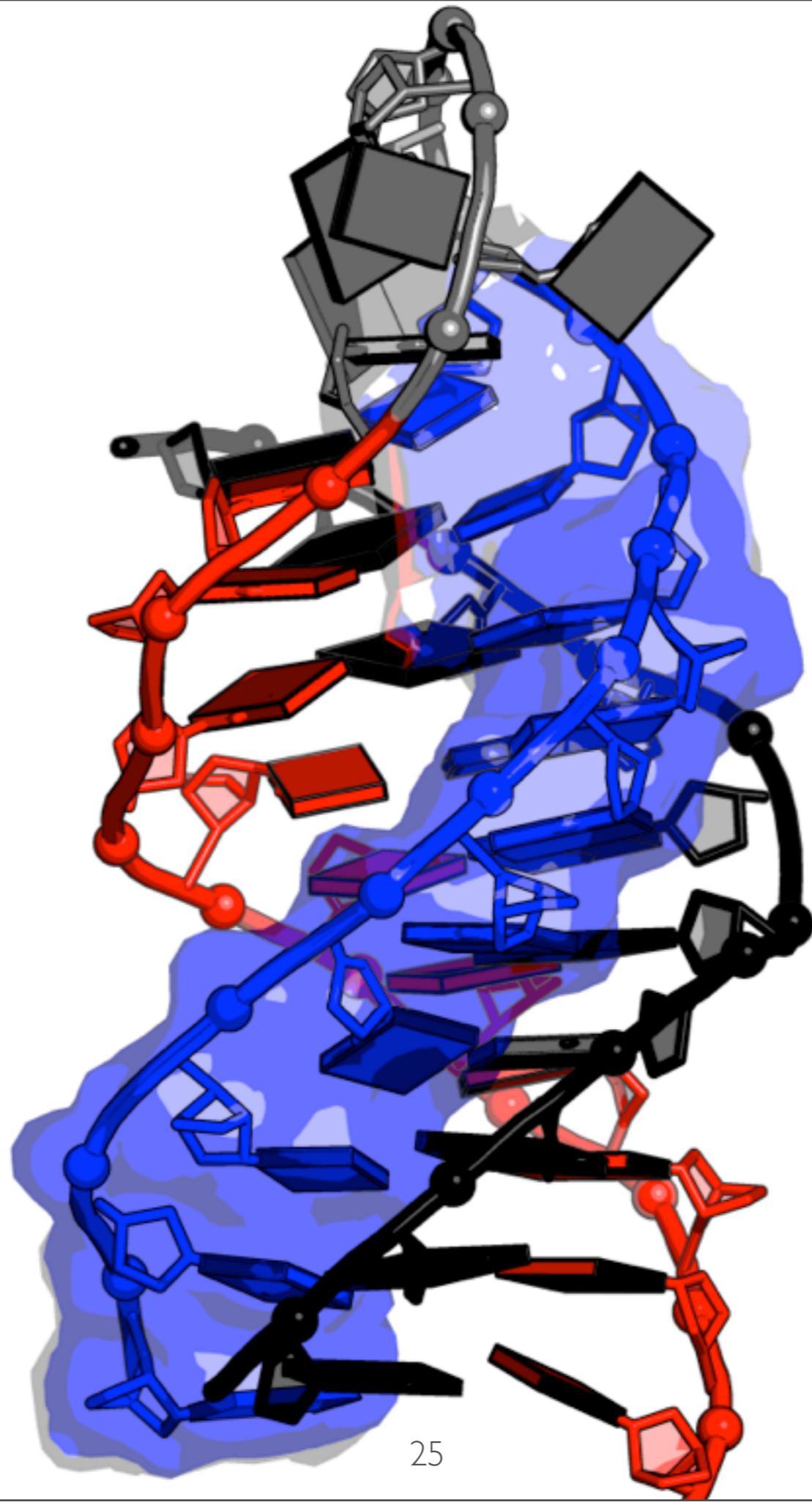
Further directions

- Ion and Water Distributions.
- ENA (Need ff-parameters?)
- ENA+LNA+normal
- Binding Constants via PMF (Potential of Mean Force)
- Explore Binding Mechanism Using Montecarlo Modeling
(Covariance Derived Force constants)

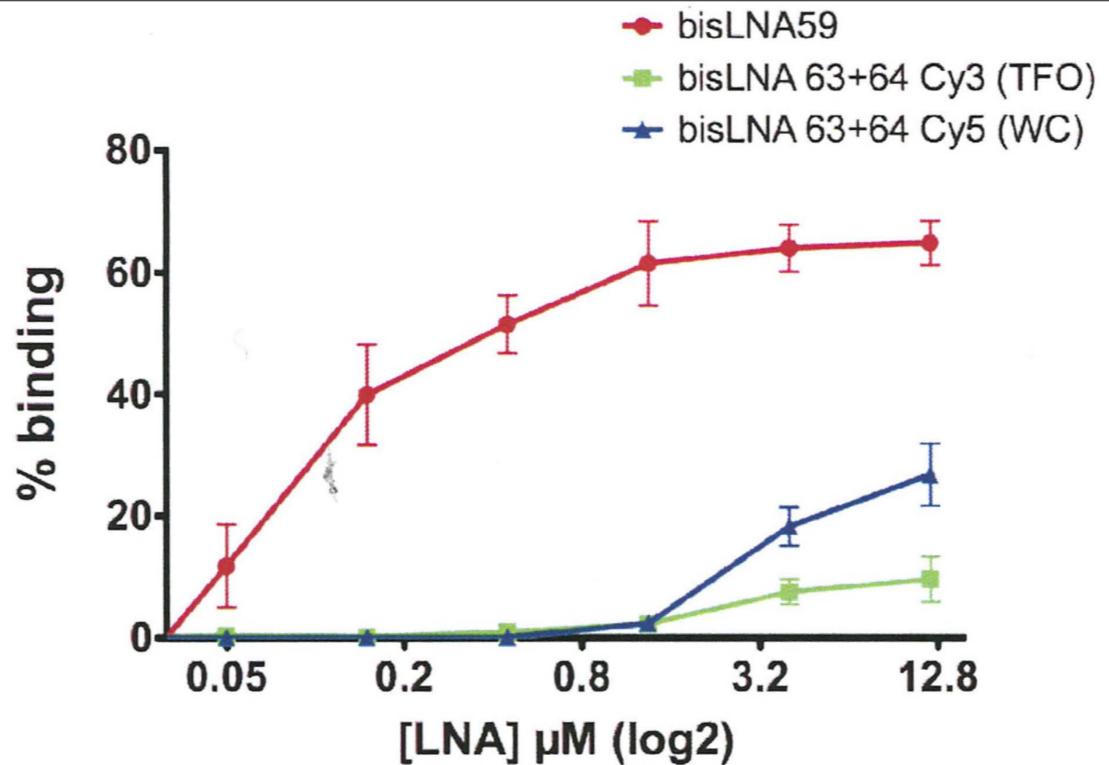
THANKS FOR YOUR ATTENTION







25



Taken from Pedro Moreno's Thesis

