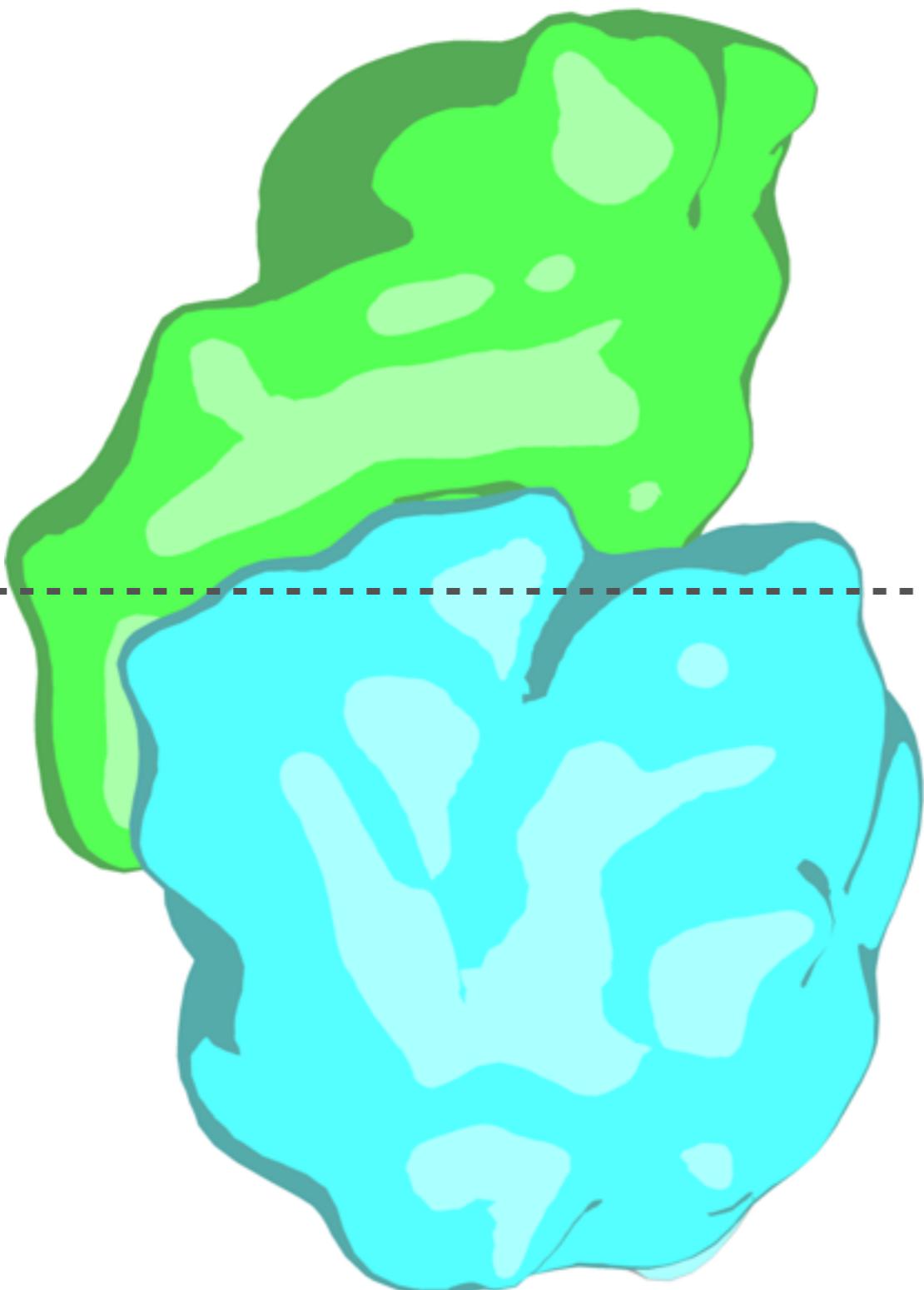


# Picking-up the slack on the bisLNA Triplex

Mauricio Esguerra  
Hugo Gutiérrez de Terán  
Uppsala Universitet  
[mesguerra.org](http://mesguerra.org)

October 15, 2014

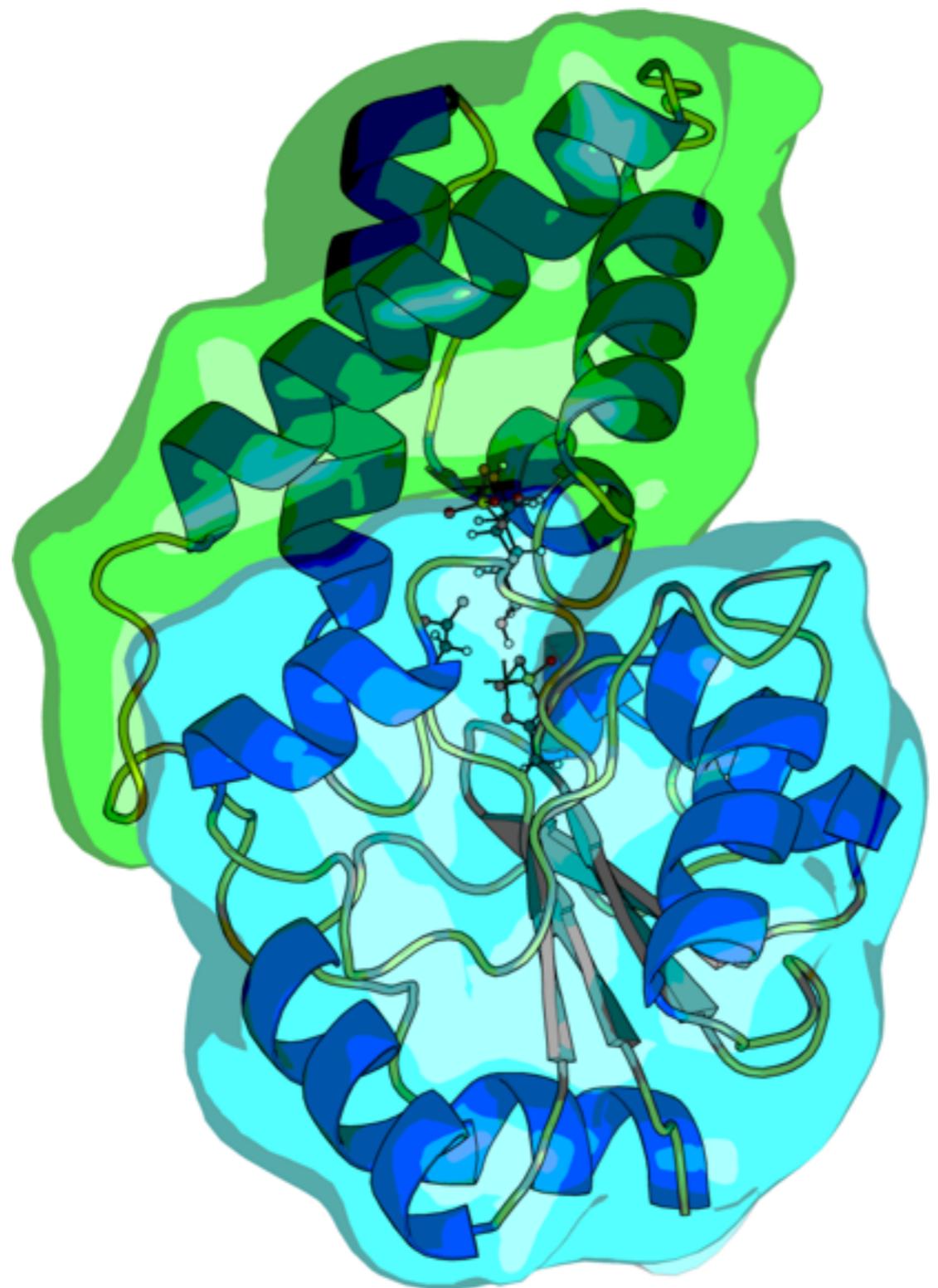
cap

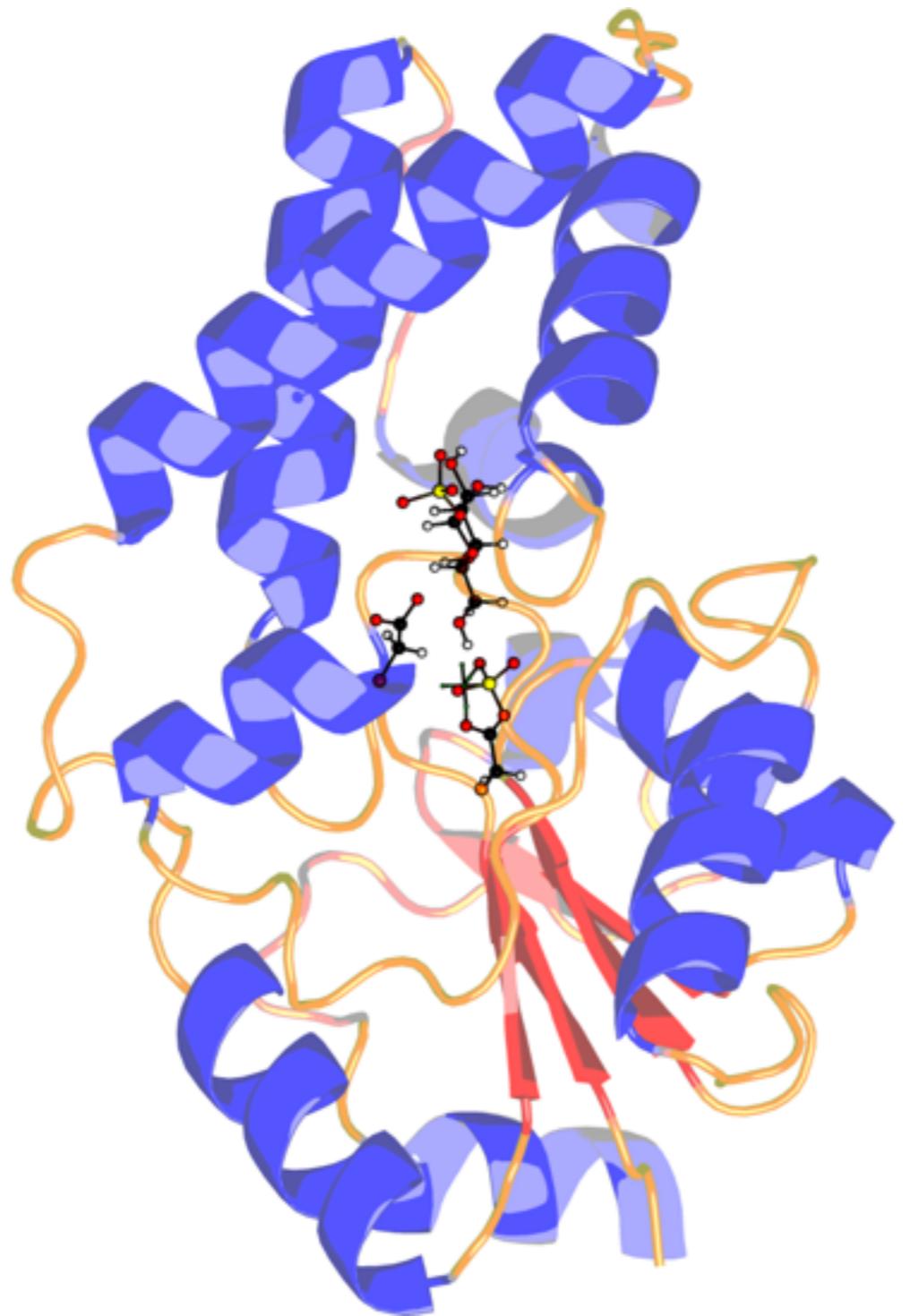


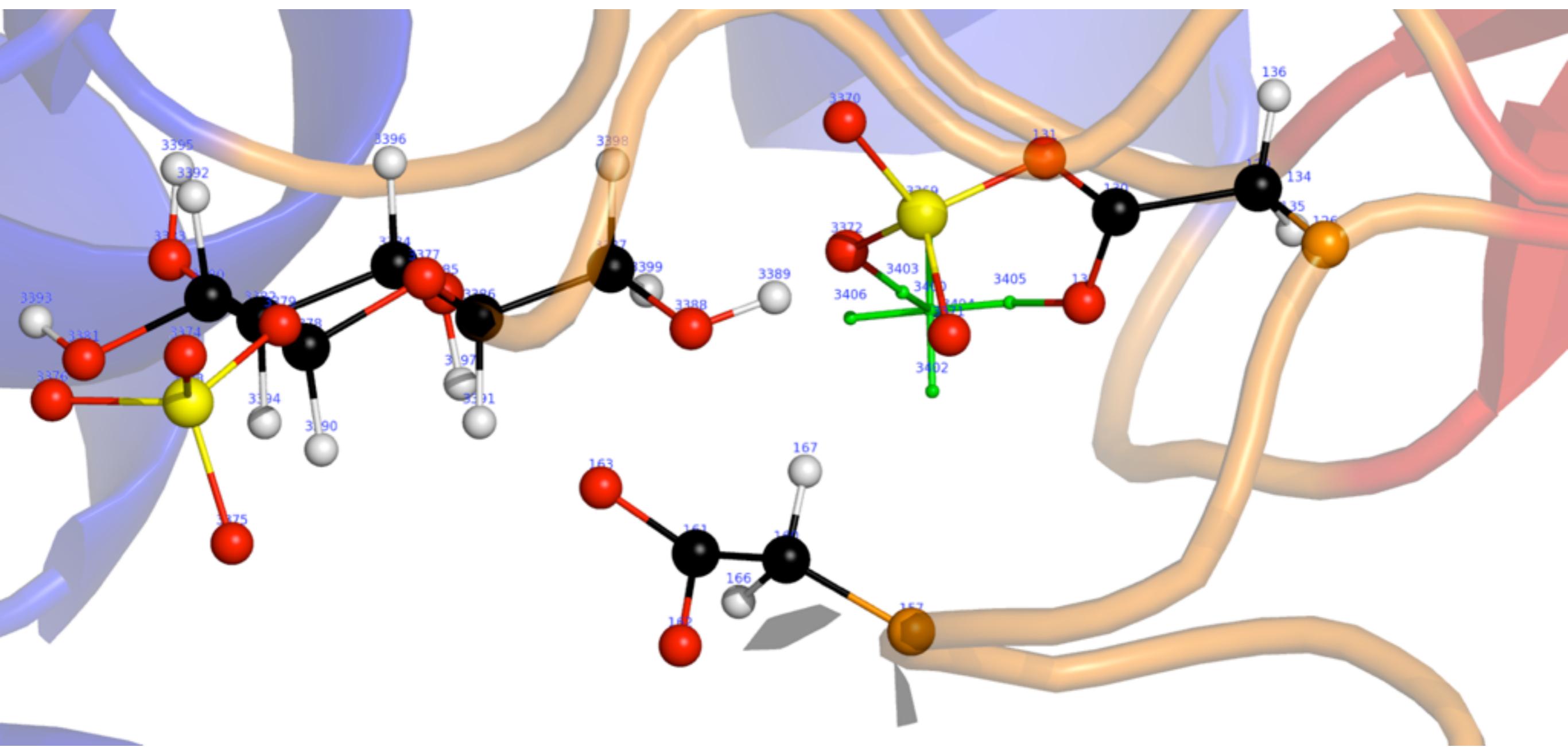
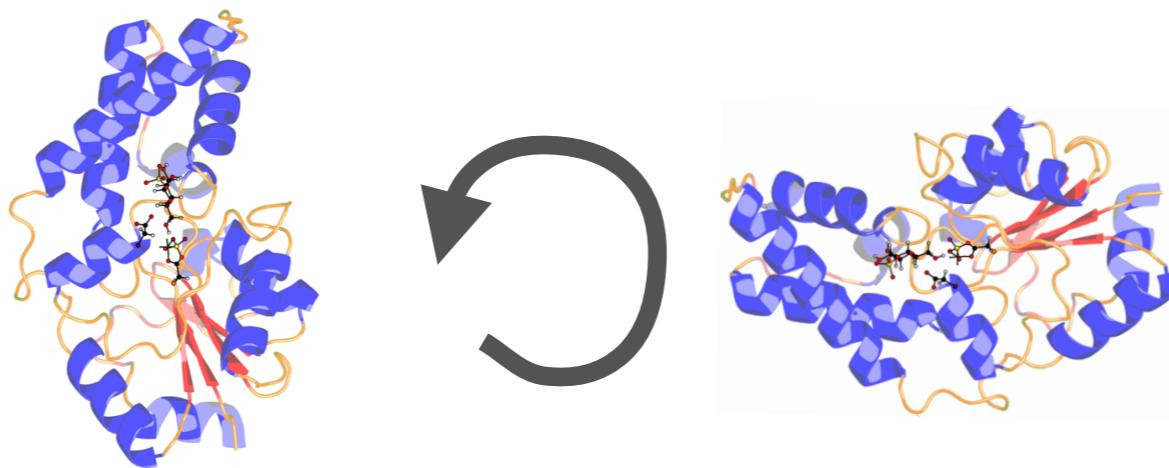
221 residues  
~1714 protein atoms

24.2 KDa  
4.4 - 61.2 KDa prot in ribosome  
2.6 MDa prokaryote ribosomes  
4.3MDa eukaryote ribosomes

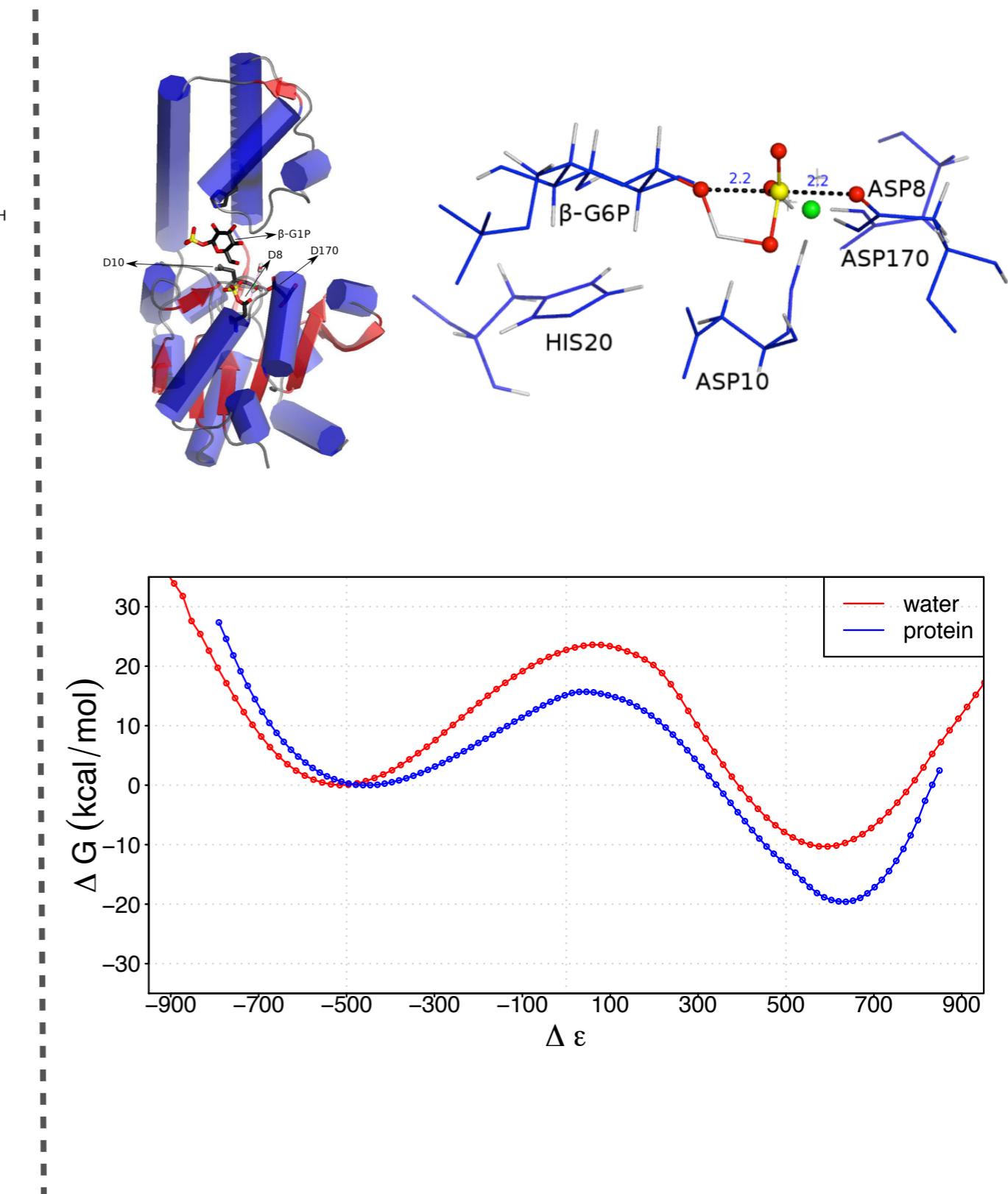
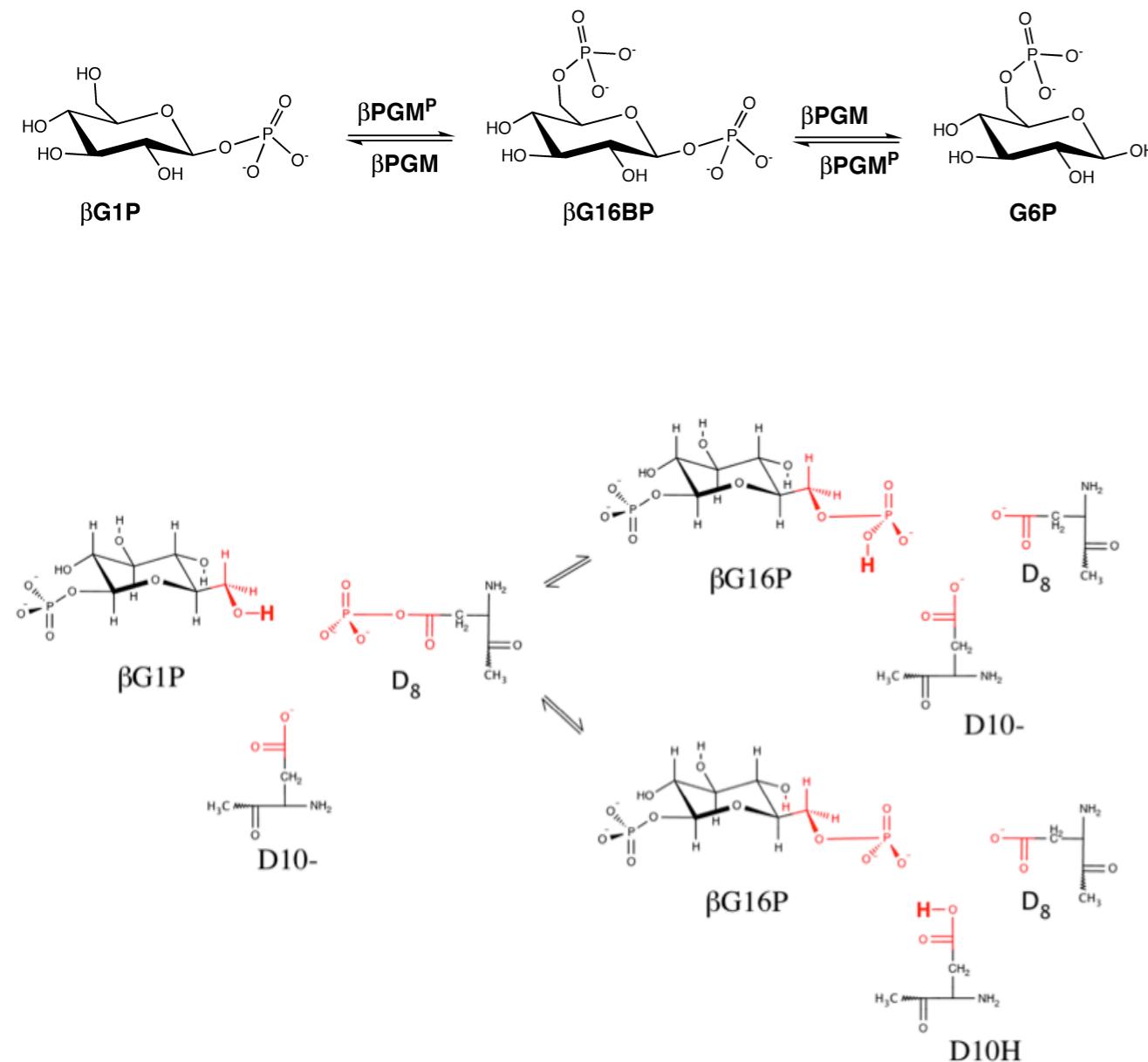
core

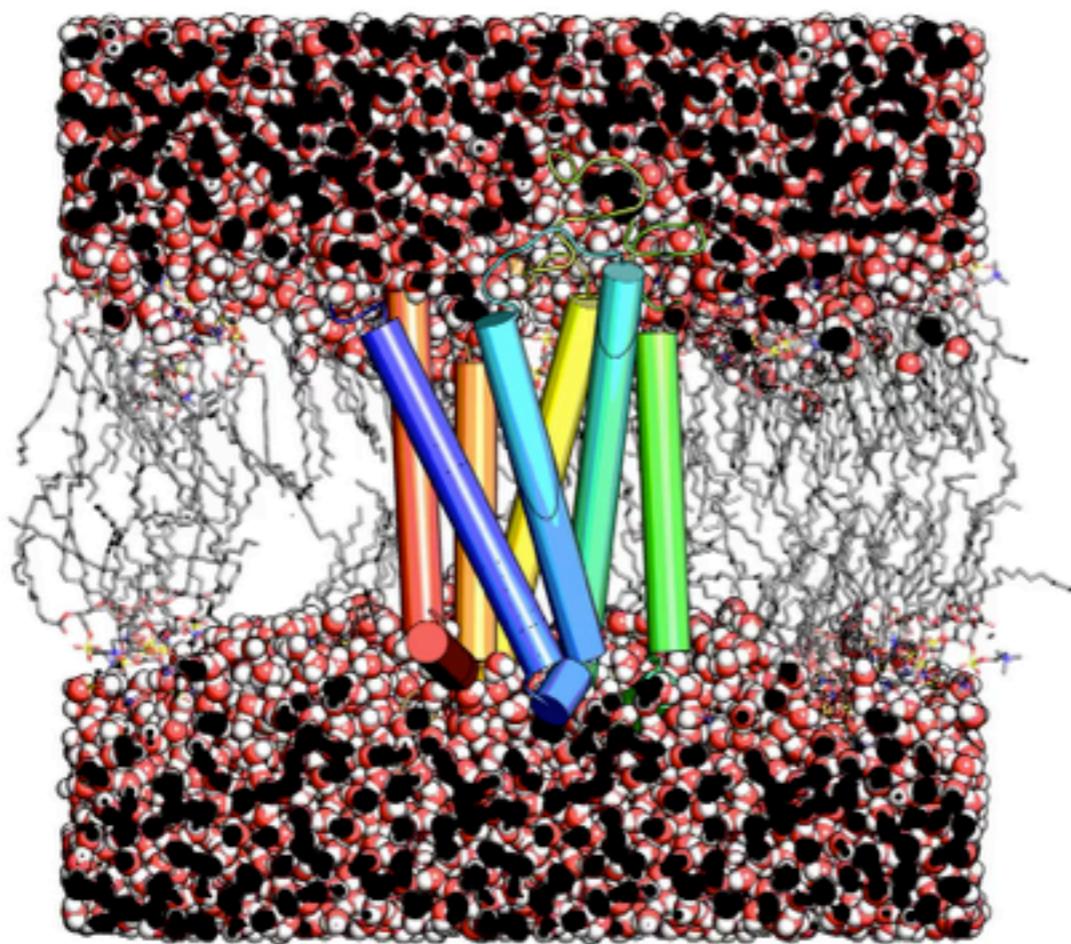






# A brave new world. Moving from Nucleic Acids to a tongue-twister Beta-phosphoglucomutase





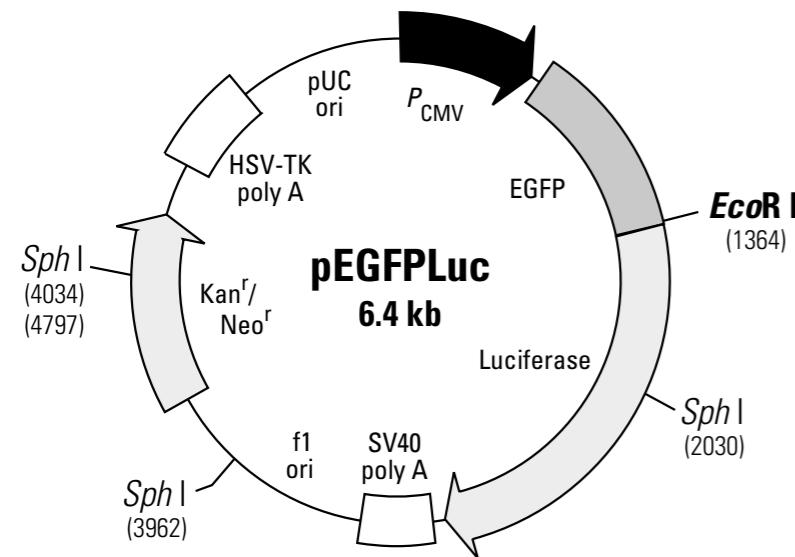
stiff calphas



more relaxed consensus  
network

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

The antigenic strategy



plasmid

6 G sites with 2 bases in between

[pEGFPLuc/G6] = 100ng/ $\mu$ L

**pH = 5.8**

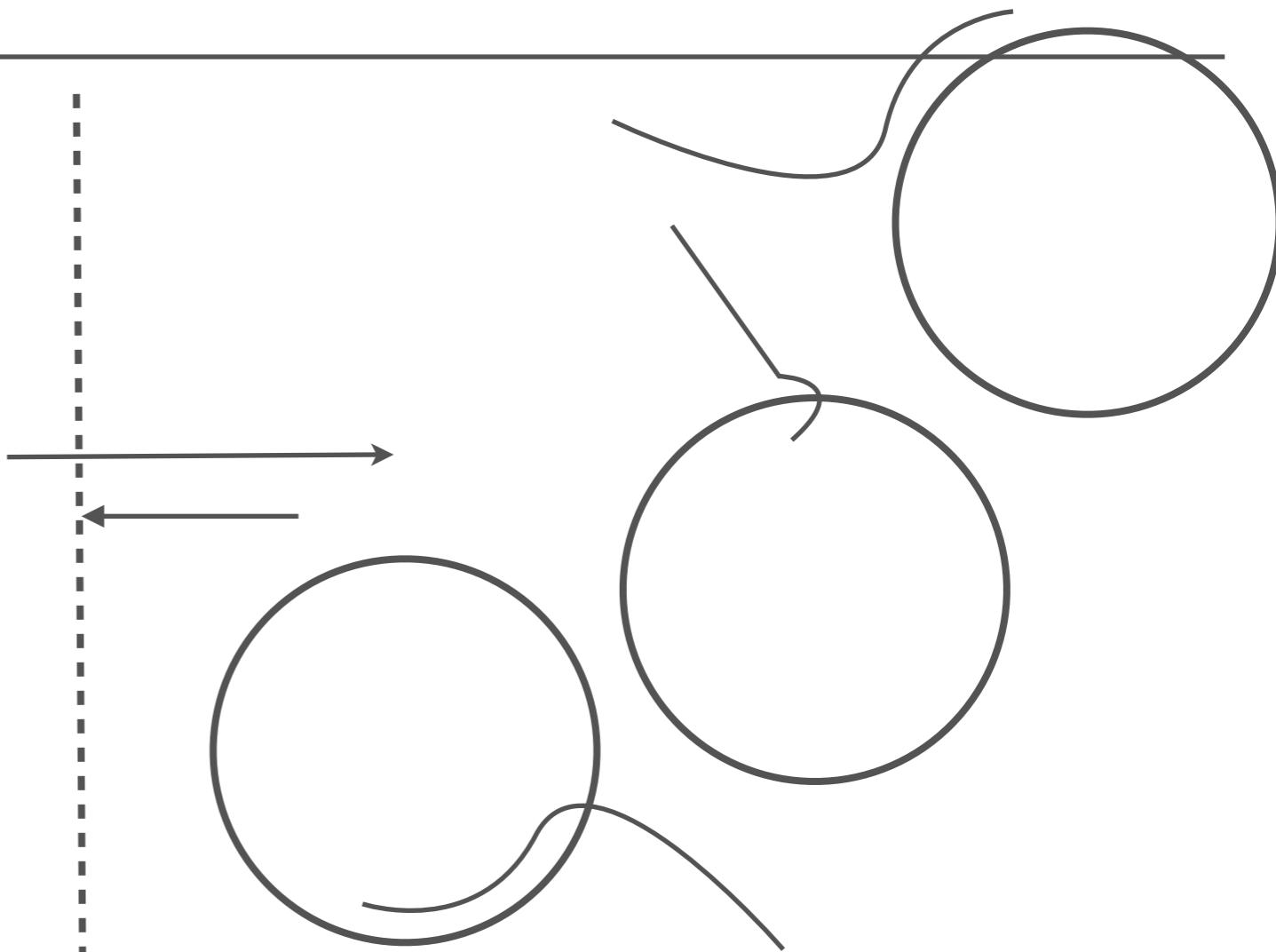
20mM NaPO<sub>4</sub> NaCl  
37°C, 16-20h

[Del-I] = 100ng/ $\mu$ L

**pH = 7.3 - 7.4**

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

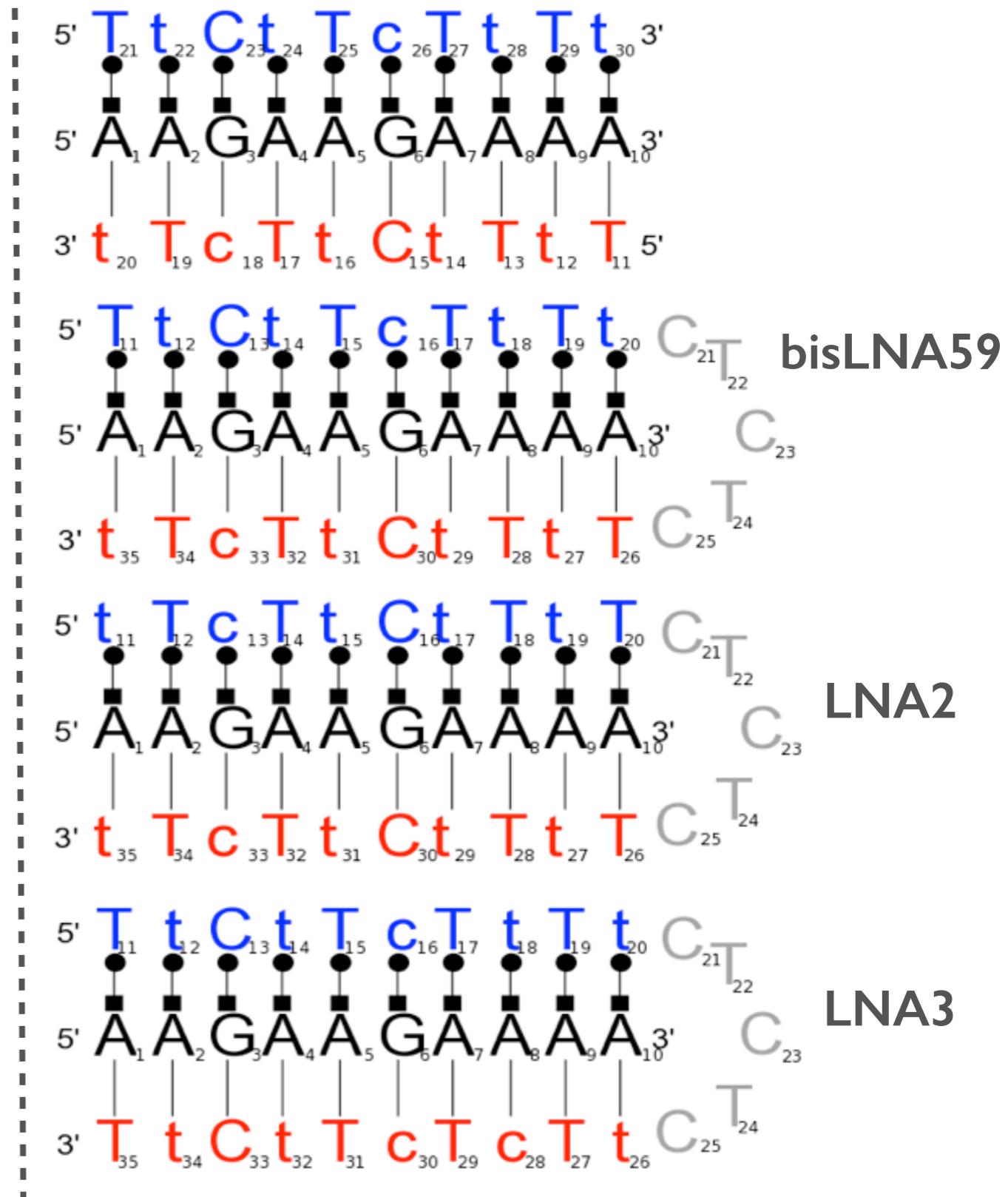
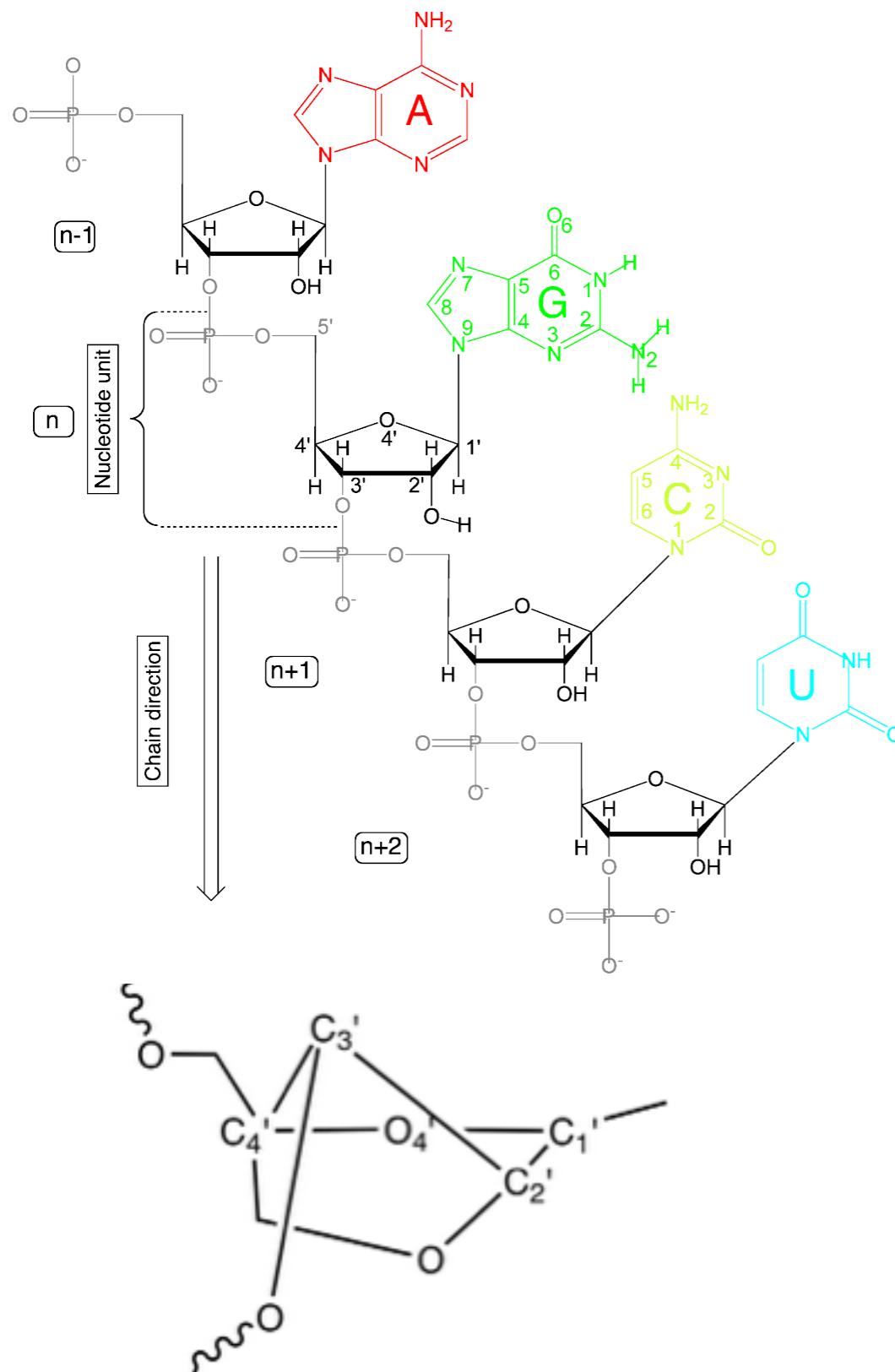
+ TFO



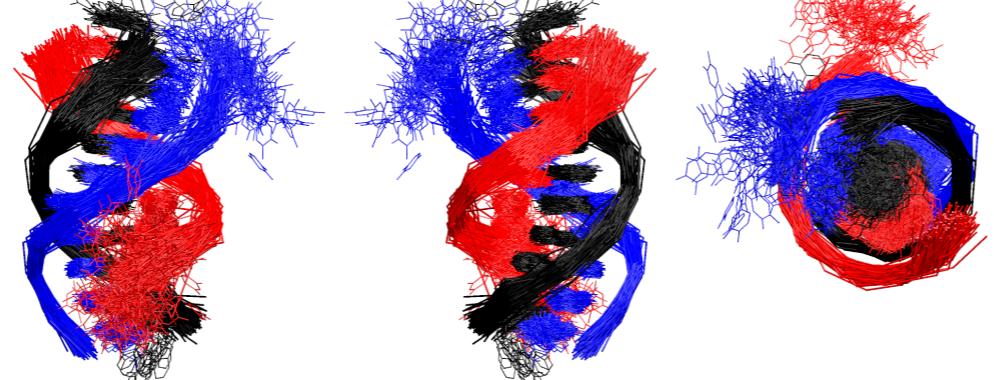
**GOAL:**  
Increase binding constants  
via single-strand modification

Hélène (1991) Anti-Cancer Drug Design, 6, 569-584.

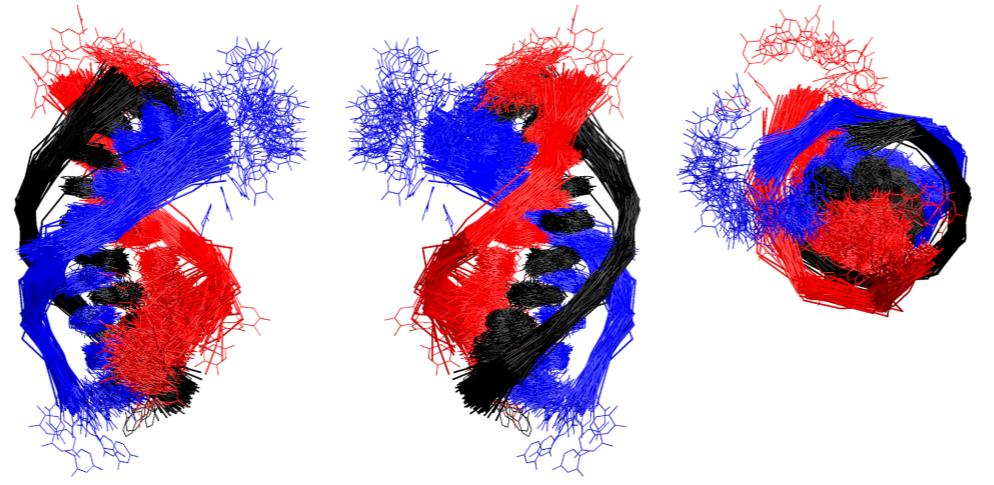
# Modified Single Strands of DNA with LNA (Locked Nucleic Acids) also called Triplex-Forming Oligonucleotides (TFO's)



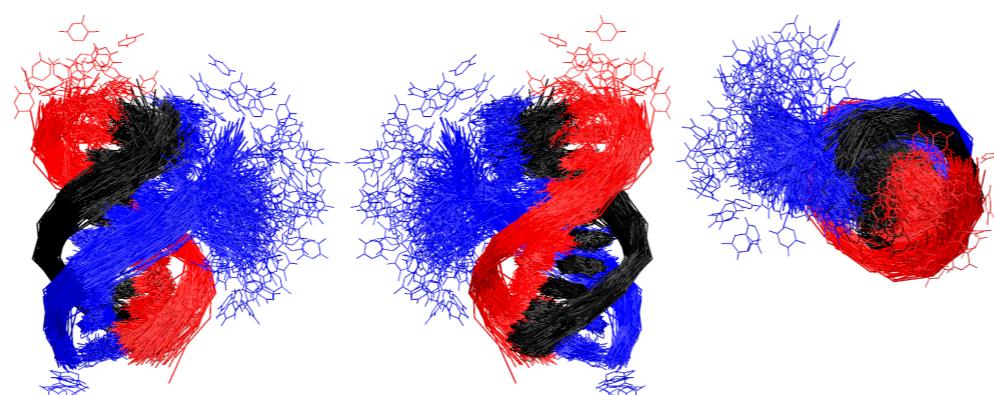
Triplex



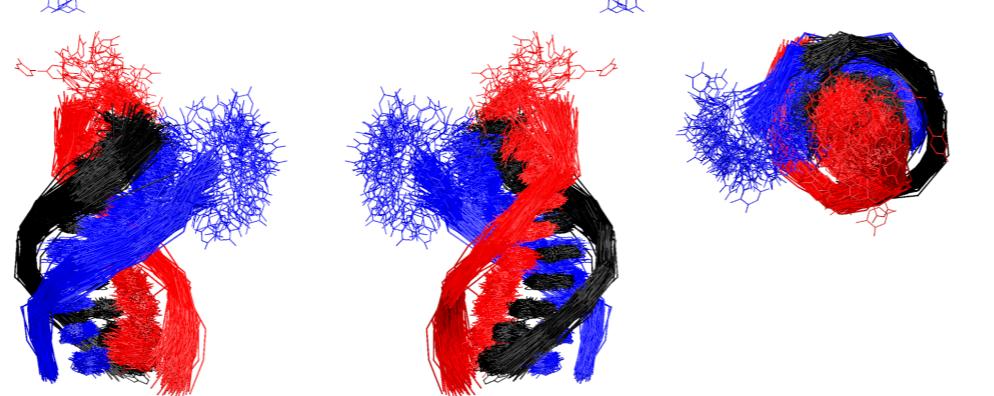
TriplexLNA



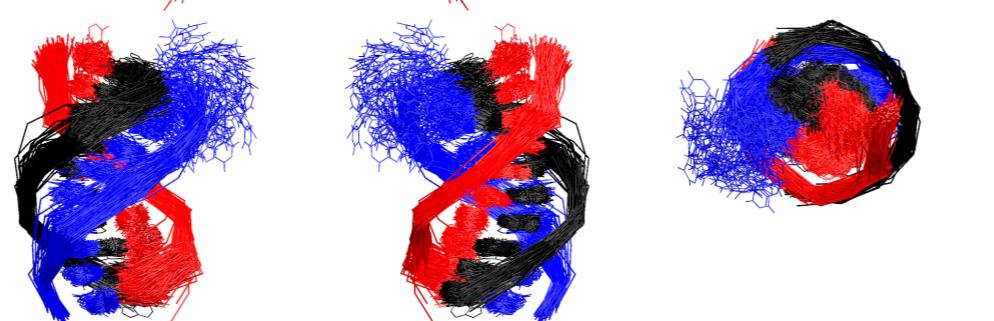
TriplexLoop



TriplexLoopLNA

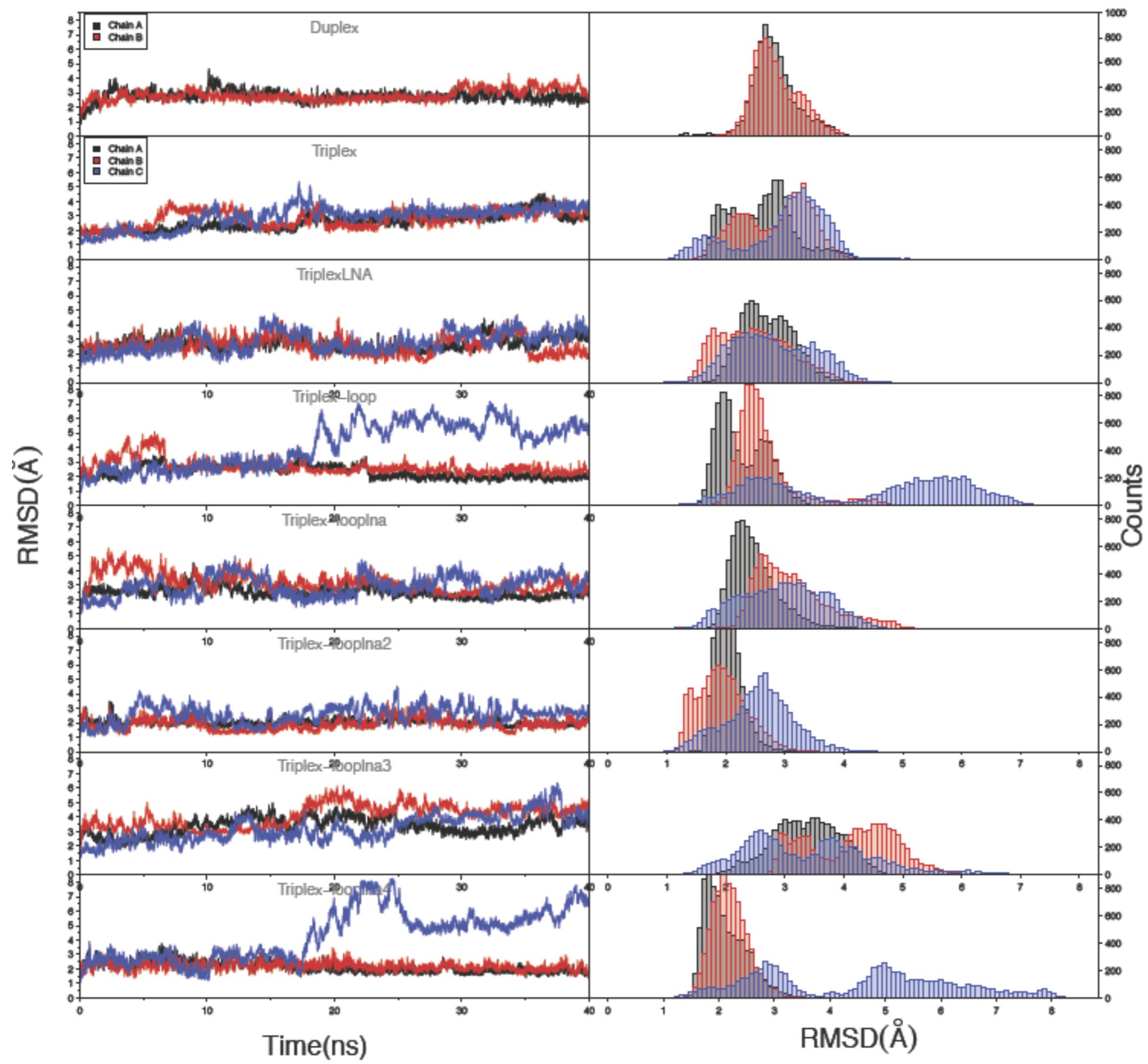


TriplexLoopLNA2

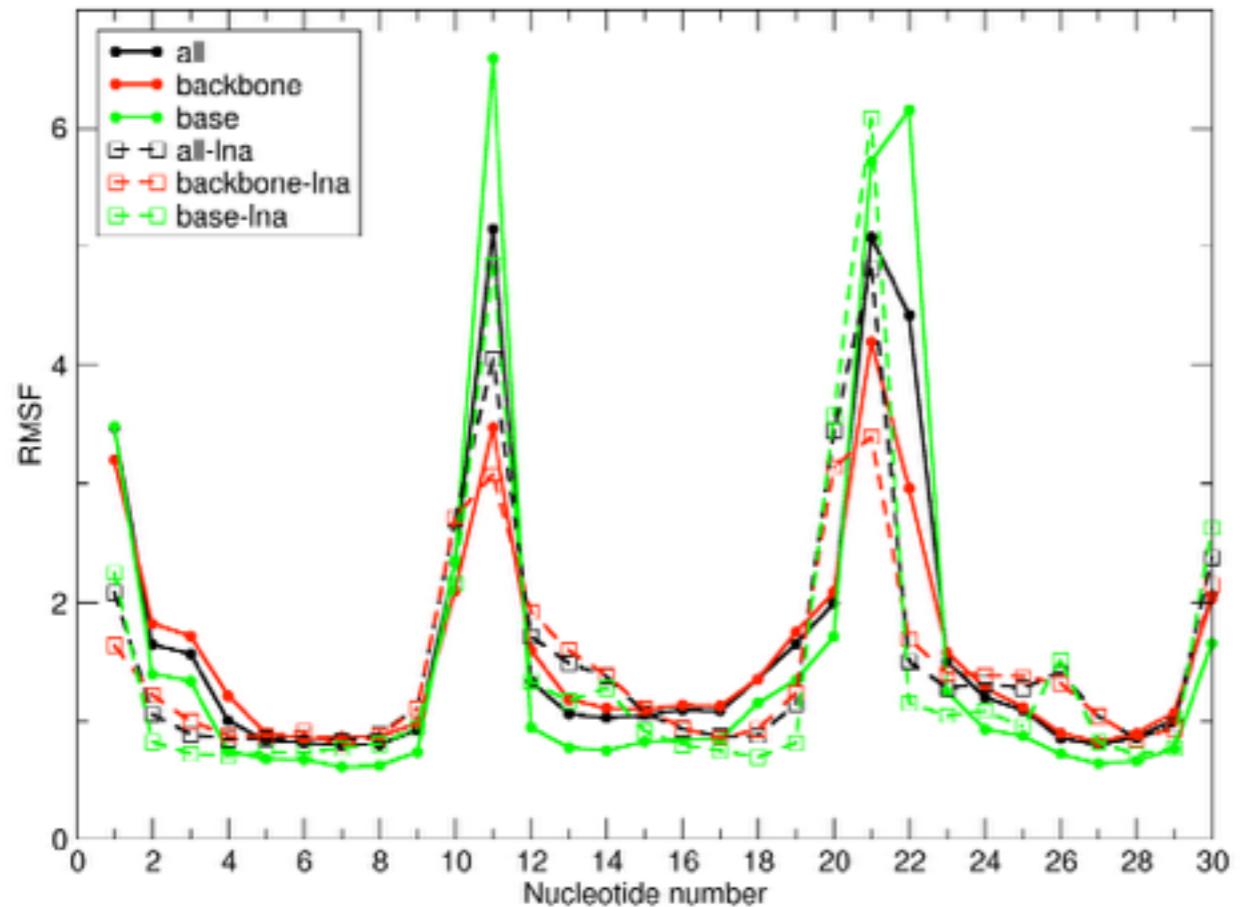


Snapshots of 40ns simulations. The **Watson** strand is colored **black**, the **Crick** strand is colored **red**, and the **Hoogsteen** strand is colored **blue**. In the left triplexes have been centered with the major-groove between base-pairs 5 and 6 facing the viewer. In the center panel the view has been rotated 180 degrees and now the minor groove of the middle base-pairs faces the viewer. In the right panel the view has been rotated 90 degrees and the no-loop end faces the viewer

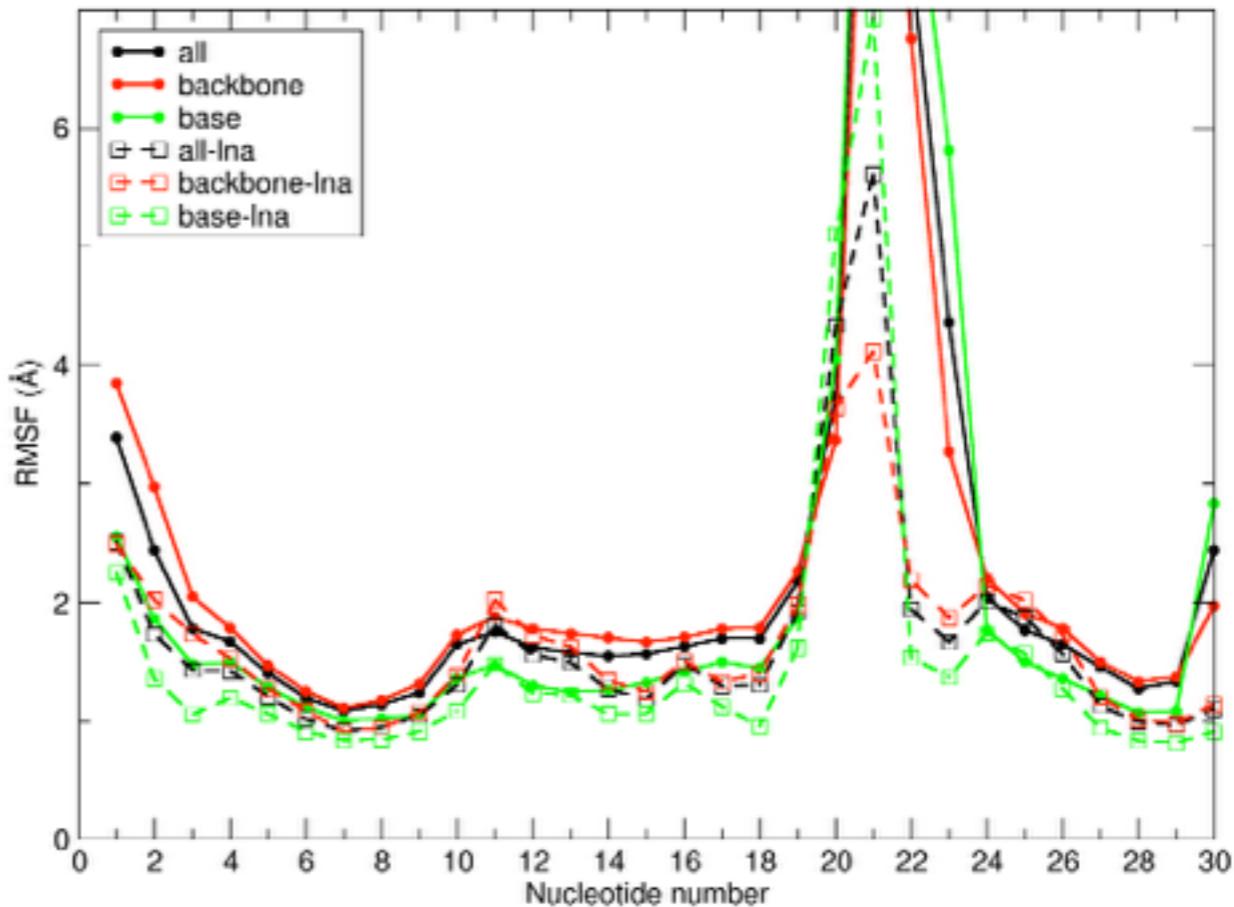
# RMSD of all atoms



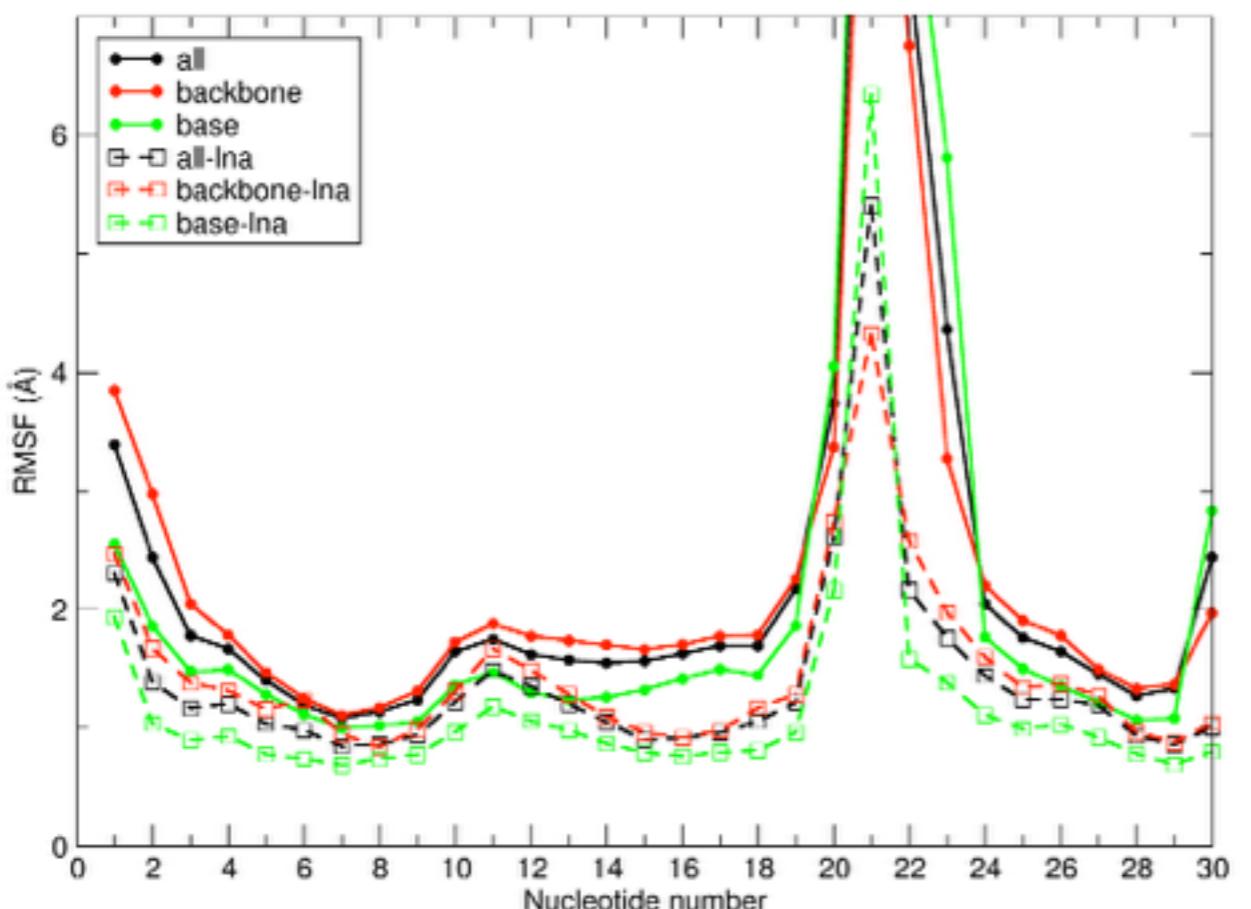
RMSF of nucleotides  
triplex and triplex-Ina



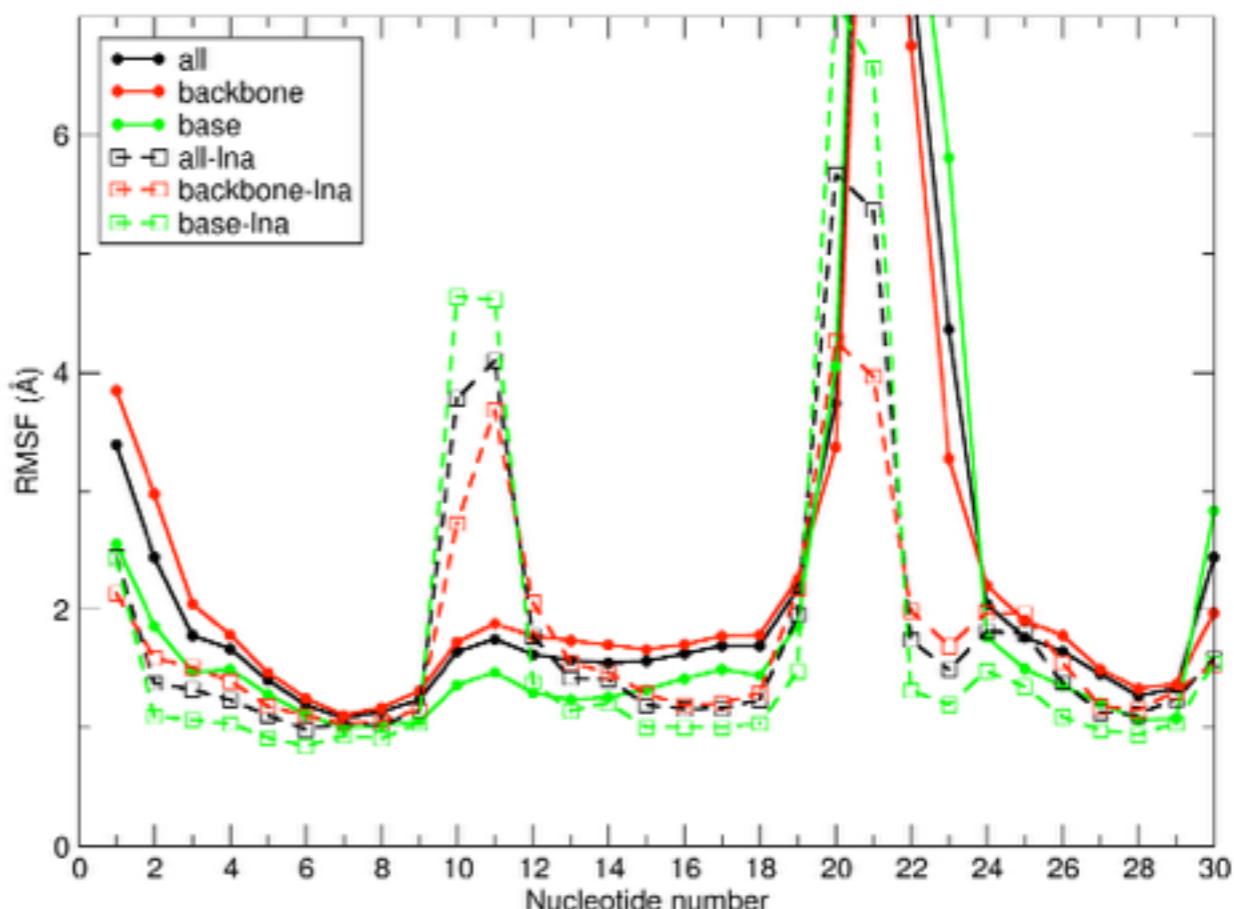
RMSF of nucleotides  
triplex-loop and triplex-loop-Ina



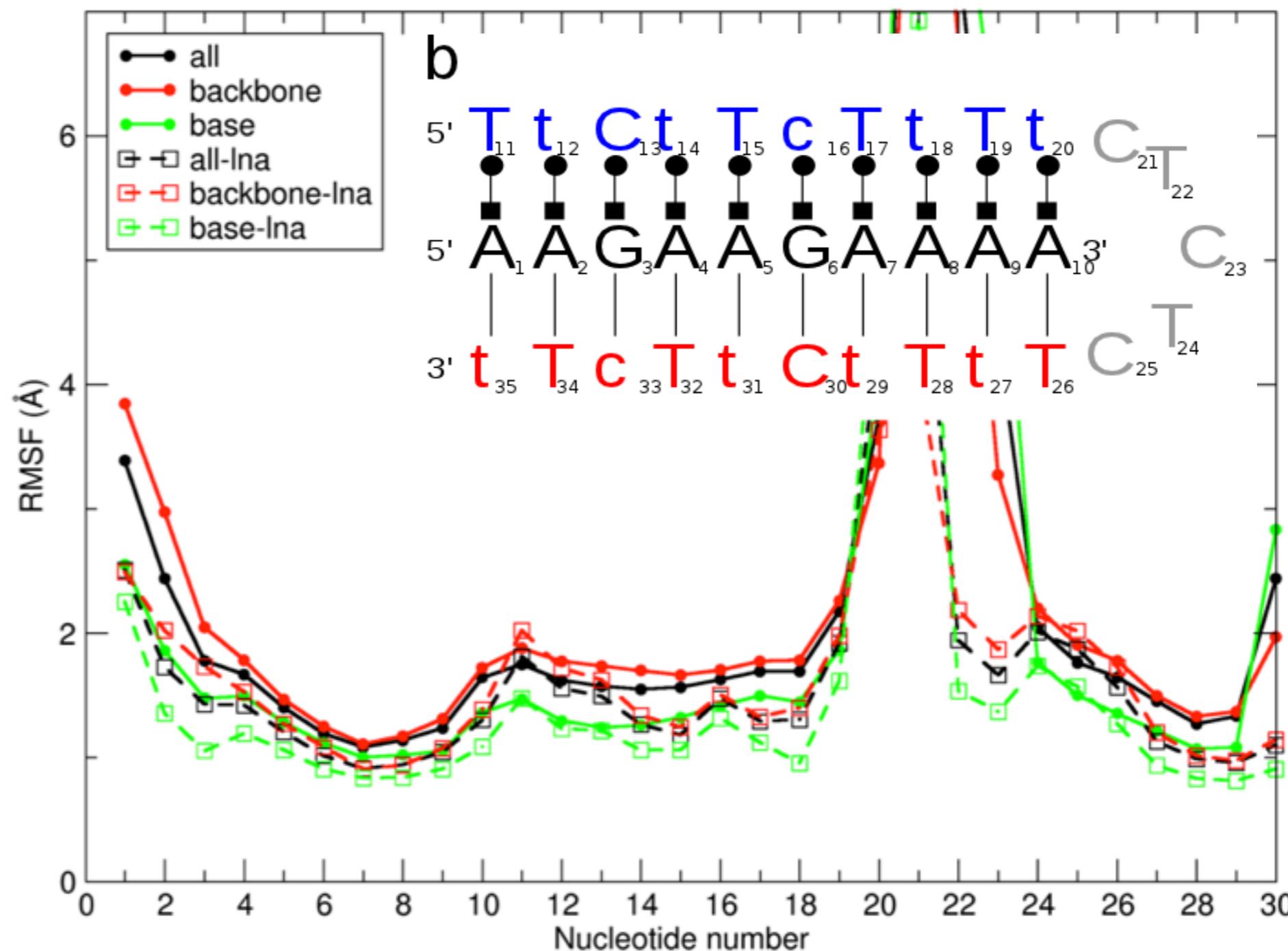
RMSF of nucleotides  
triplex-loop and triplex-loop-Ina2



RMSF of nucleotides  
triplex-loop and triplex-loop-Ina3

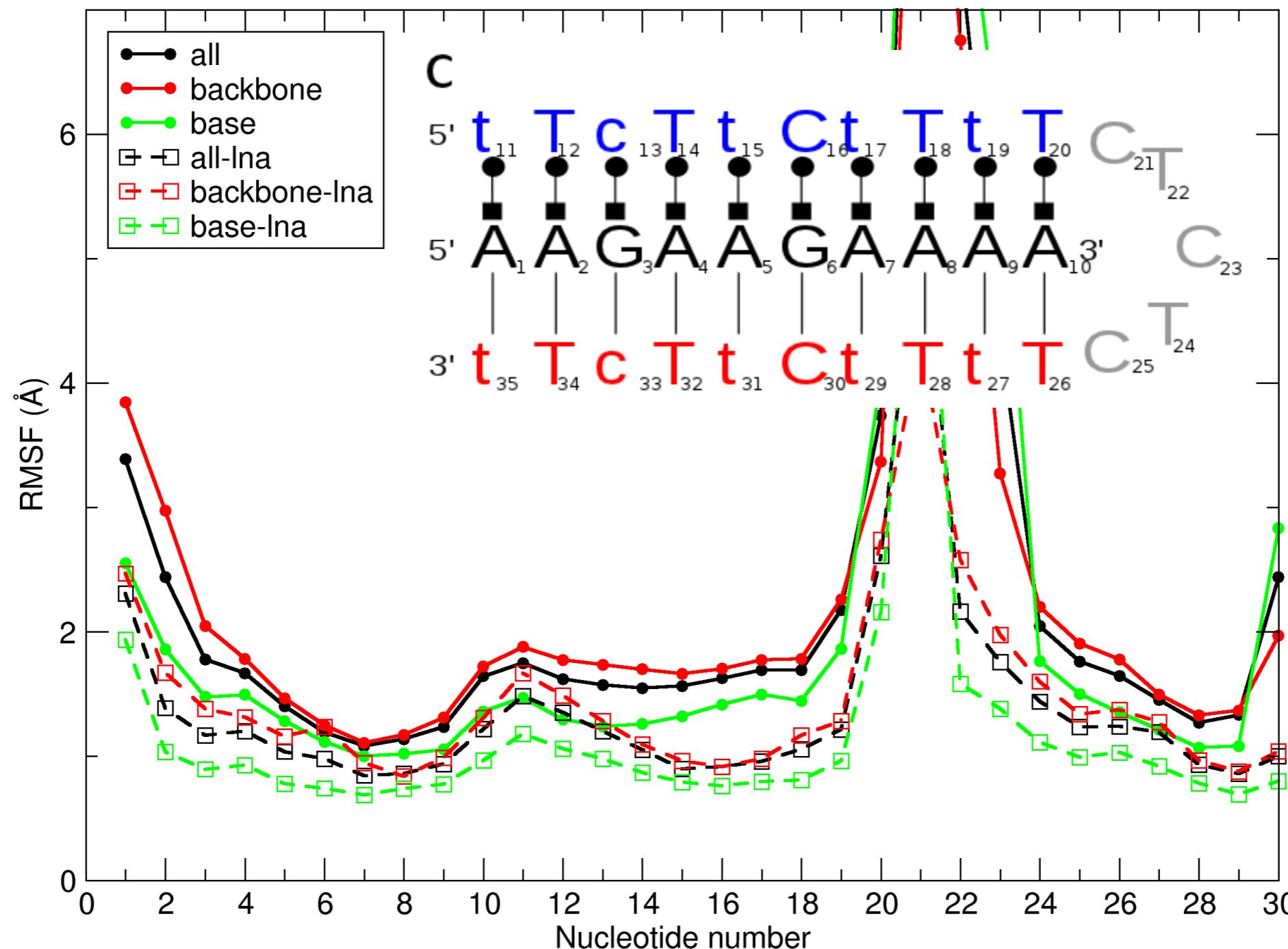


## RMSF of nucleotides triplex-loop and triplex-loop-Ina



# RMSF of nucleotides

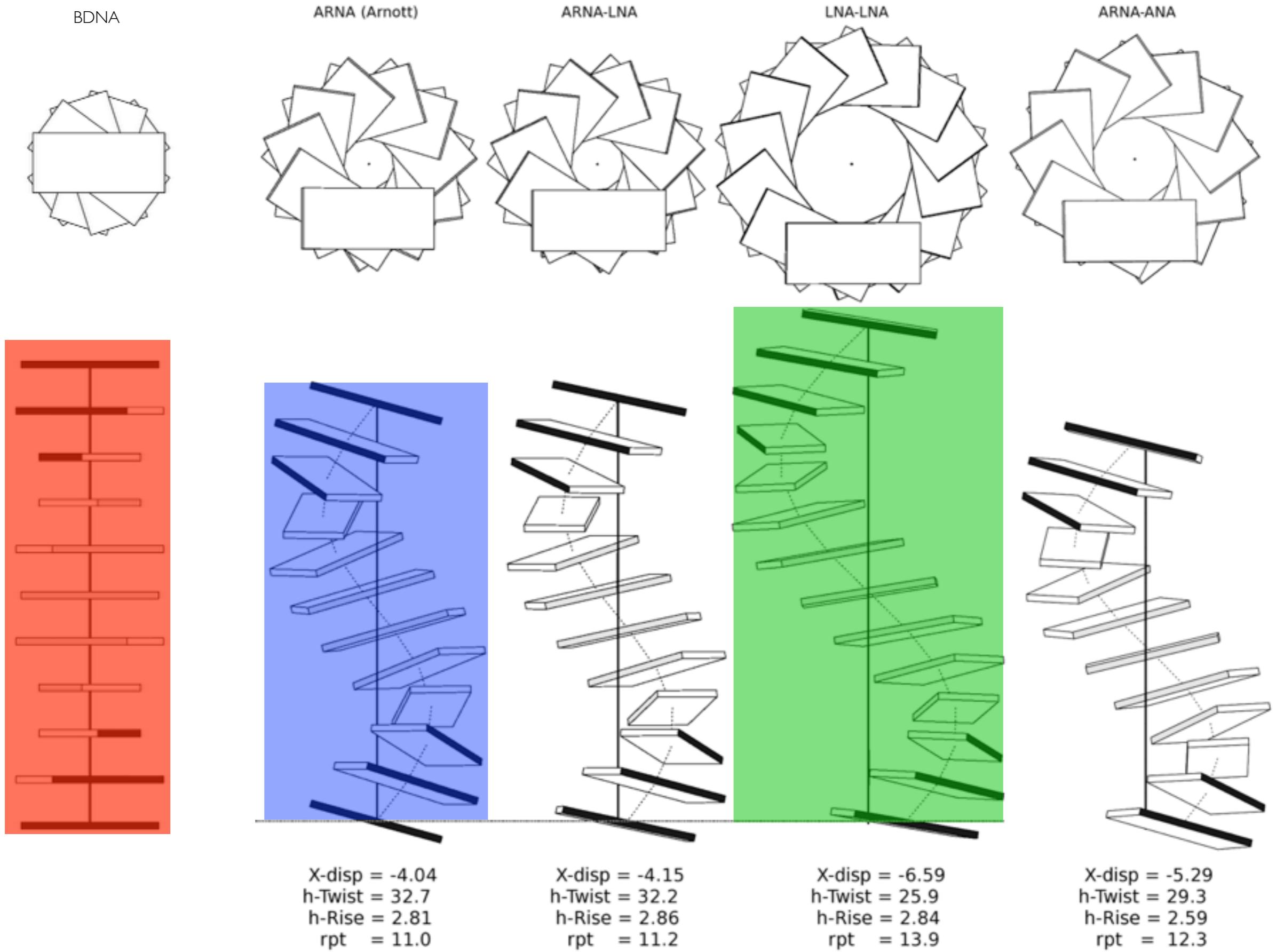
triplex-loop and triplex-loop-Ina2



# LESSON I

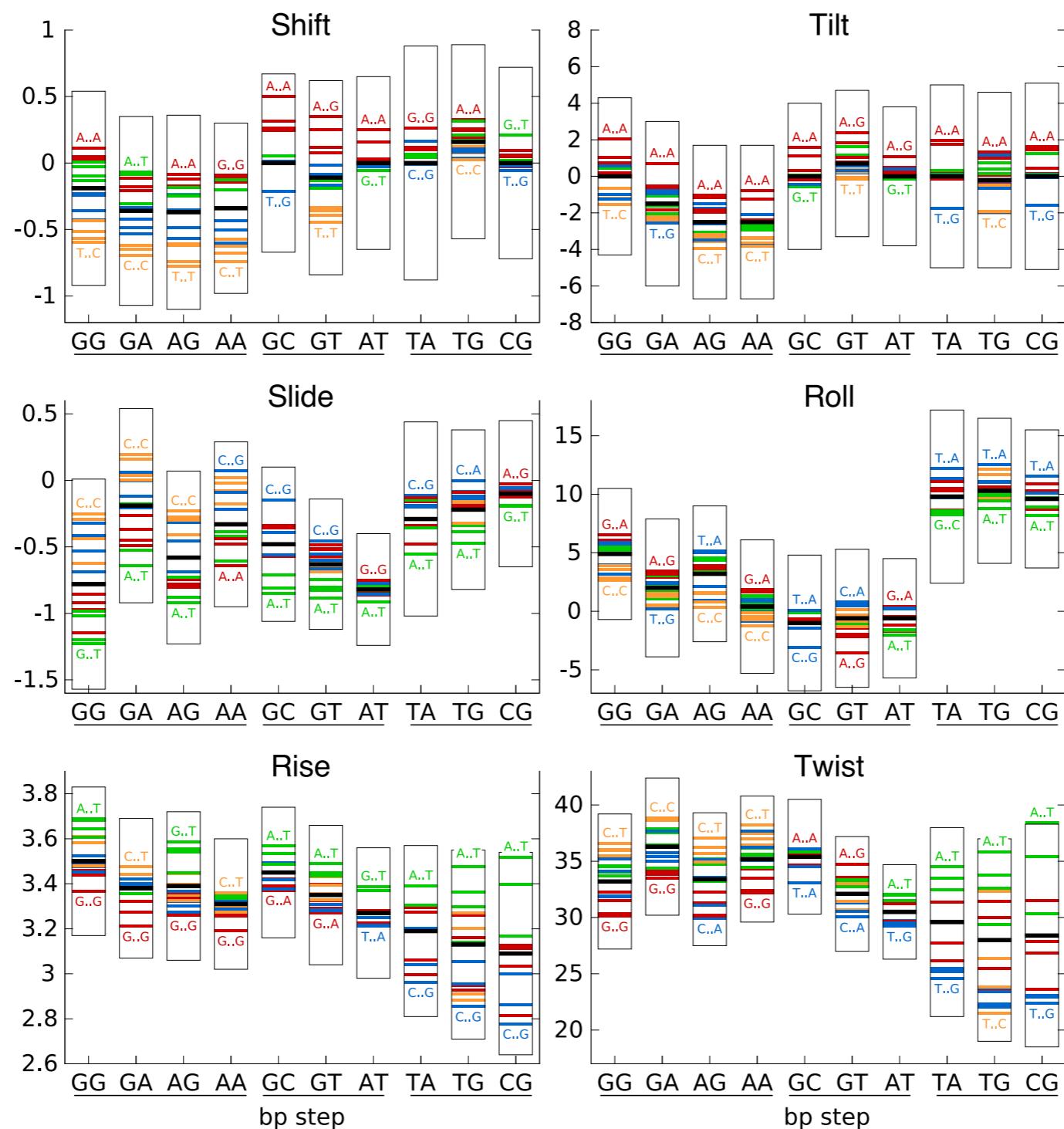
- LNA on both sides of the **triplet** results in a more rigid (perhaps more stable) triplex in contrast to one side of the triplet LNA-ing.

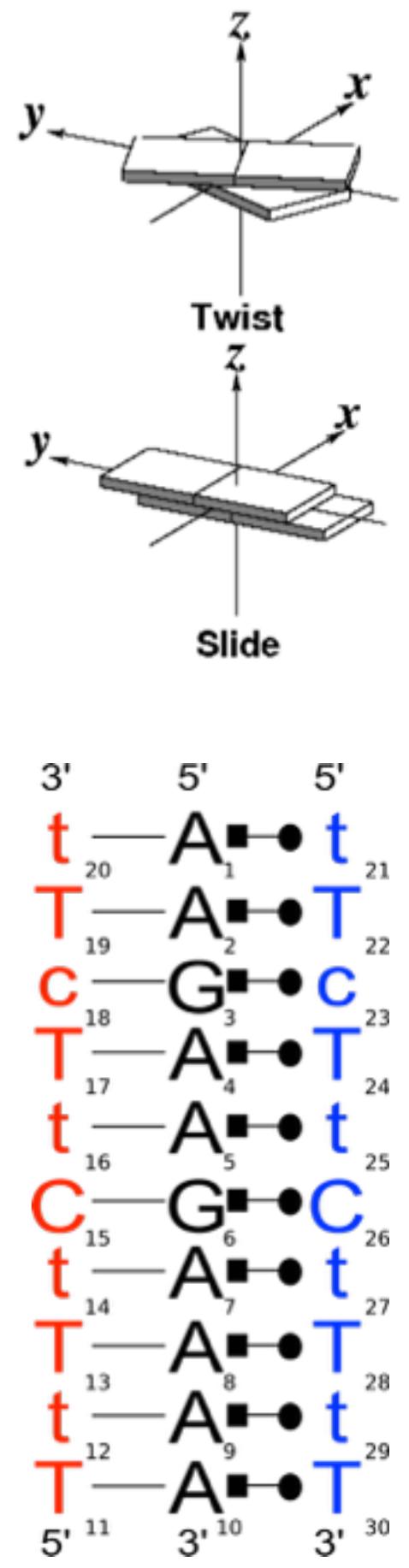
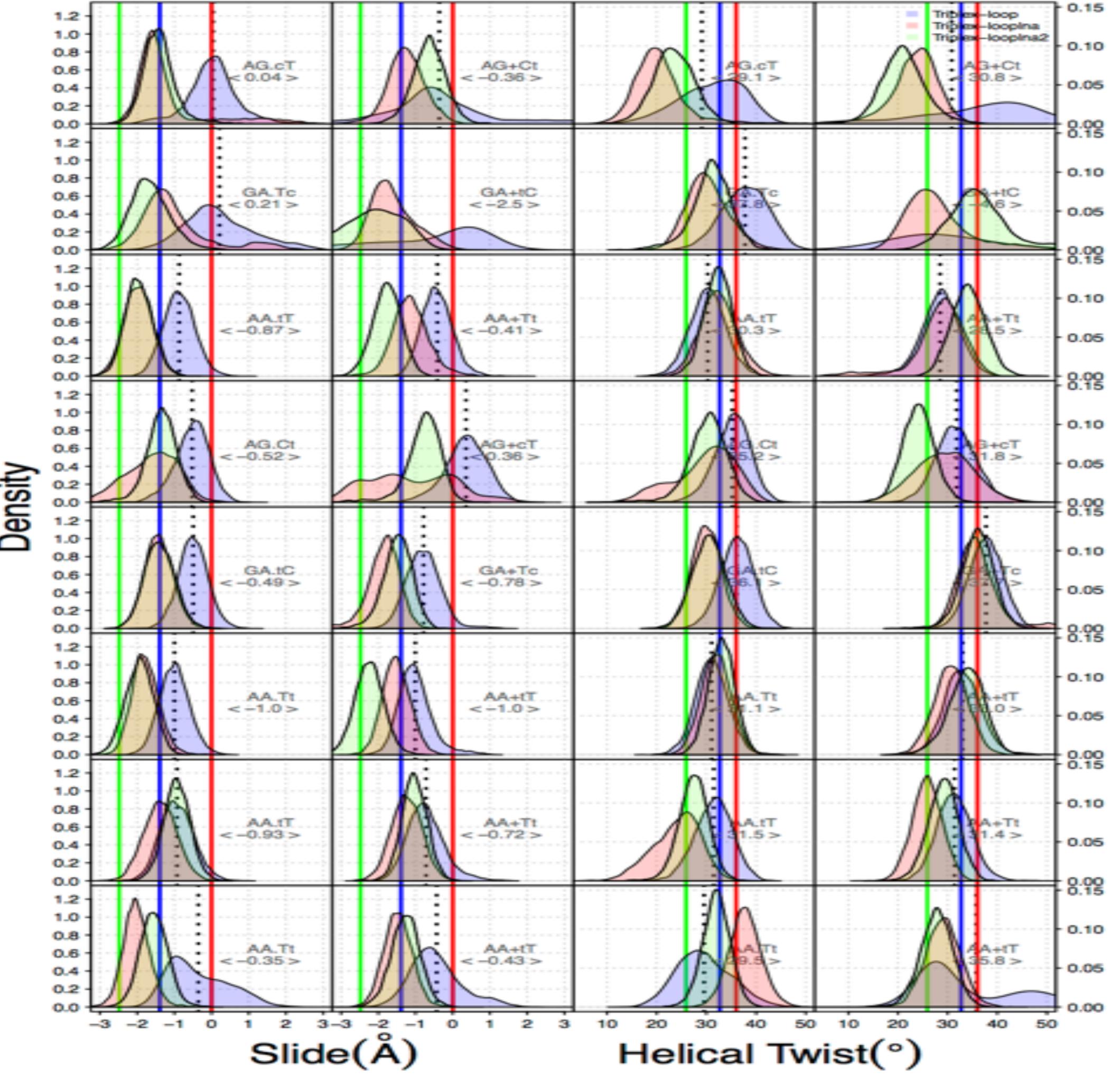
# red BDNA, blue ADNA, green all-LNA



# $\mu$ ABC: a systematic microsecond molecular dynamics study of tetranucleotide sequence effects in B-DNA

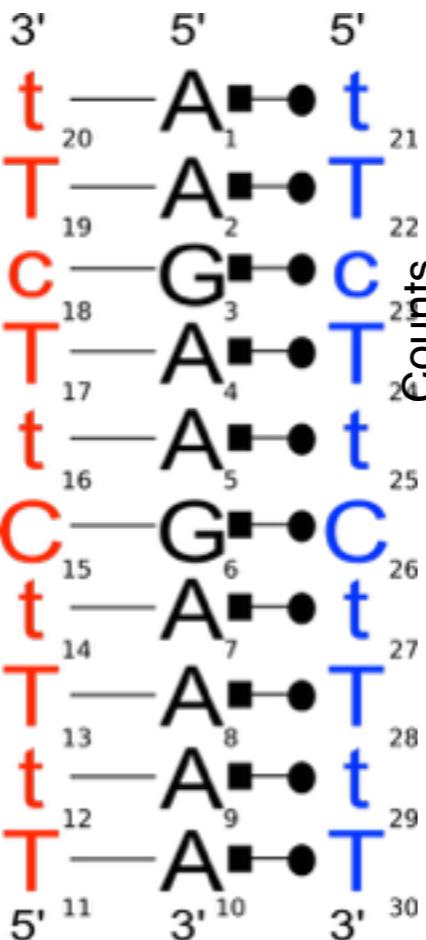
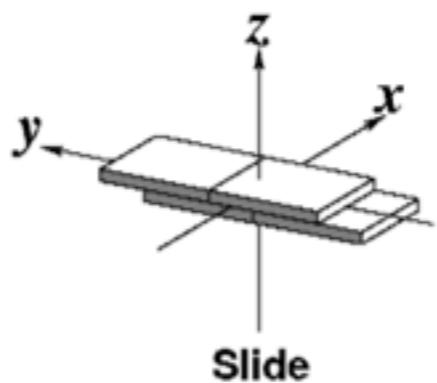
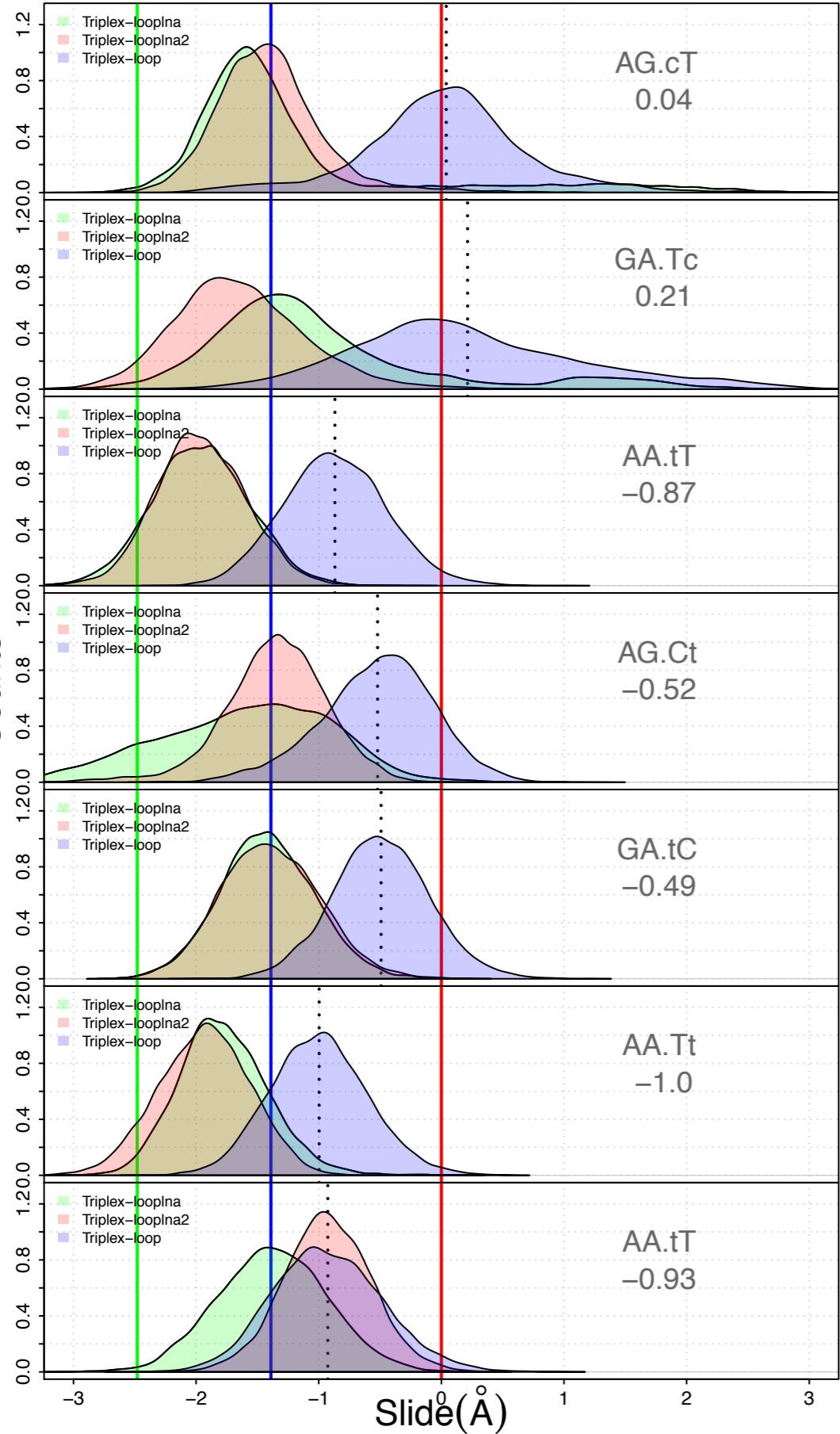
Marco Pasi<sup>1</sup>, John H. Maddocks<sup>1,\*</sup>, David Beveridge<sup>2</sup>, Thomas C. Bishop<sup>3</sup>, David A. Case<sup>4</sup>, Thomas Cheatham, III<sup>5</sup>, Pablo D. Dans<sup>6,7,8</sup>, B. Jayaram<sup>9</sup>, Filip Lankas<sup>10</sup>, Charles Laughton<sup>11</sup>, Jonathan Mitchell<sup>1</sup>, Roman Osman<sup>12</sup>, Modesto Orozco<sup>6,7,8</sup>, Alberto Pérez<sup>6,7,8</sup>, Daiva Petkevičiūtė<sup>1</sup>, Nada Spackova<sup>13</sup>, Jiri Sponer<sup>13,14</sup>, Krystyna Zakrzewska<sup>15</sup> and Richard Lavery<sup>15</sup>





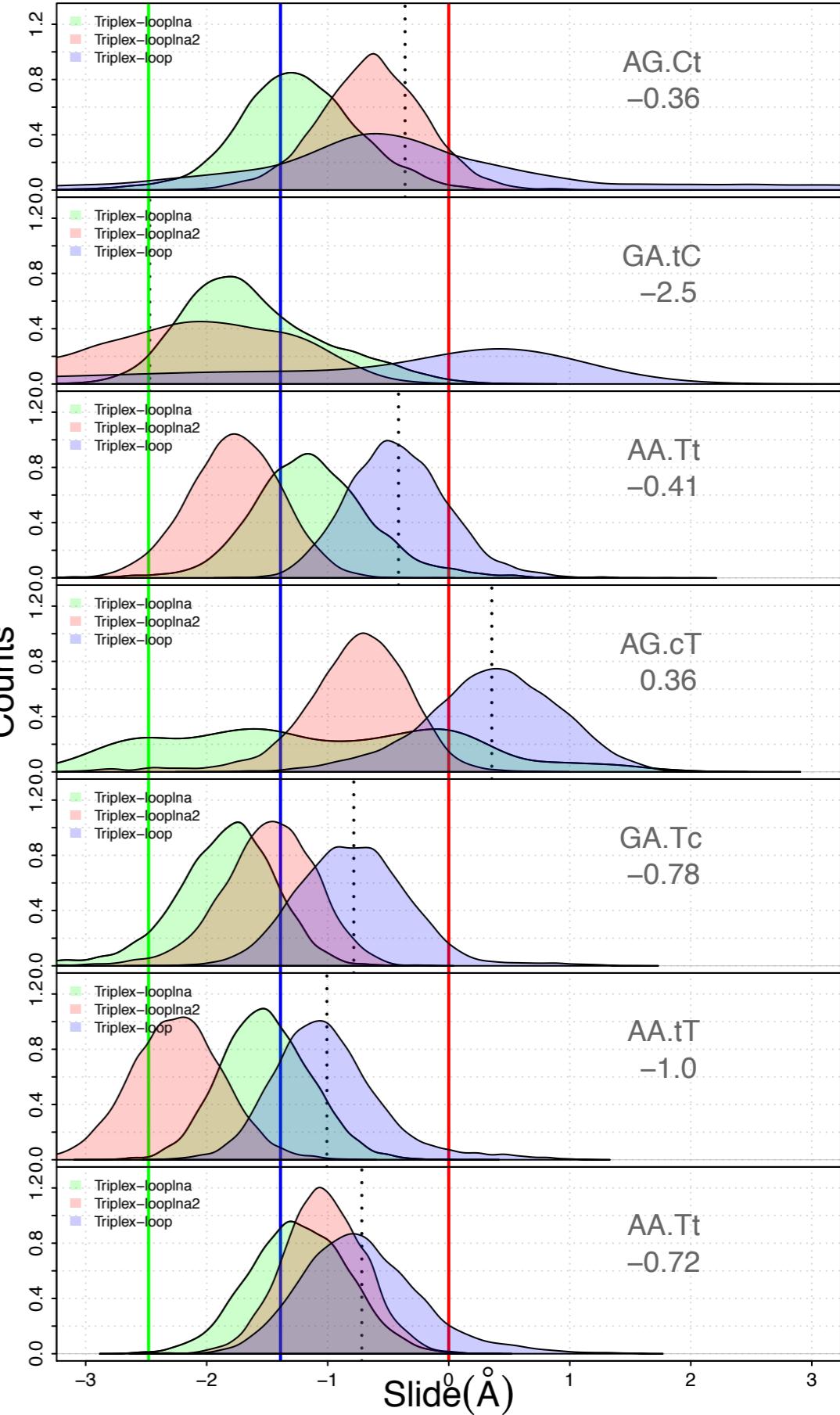
# Watson-Crick Steps

Slide shifts to lower values with intercalated LNA's



# Hoogsteen Steps

Slide shifts to lower values with intercalated LNA's



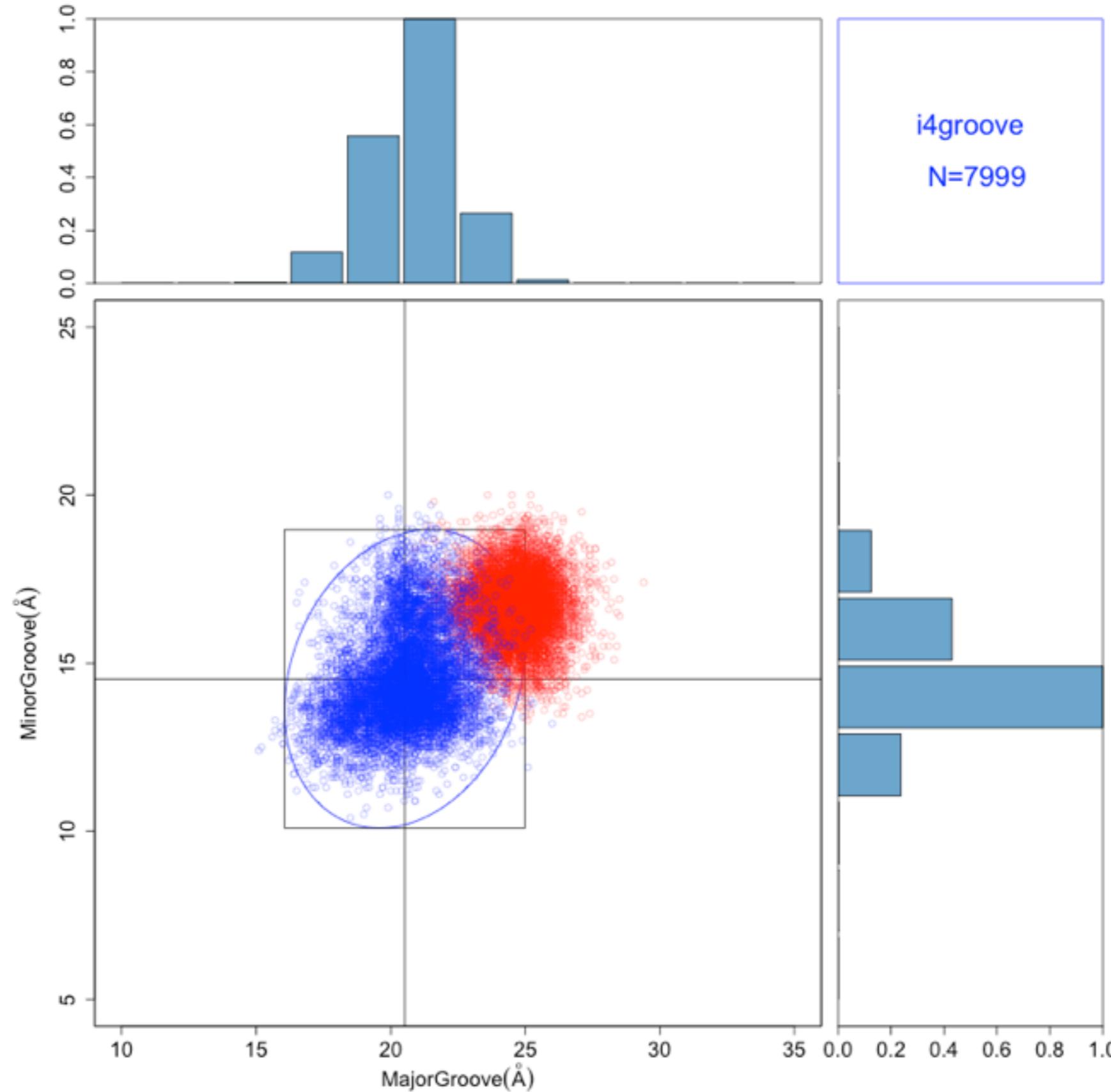
## LESSON I

- LNA on both sides of the triplet results in a more rigid (perhaps more stable) triplex in contrast to one side of the triplet LNA-ing.

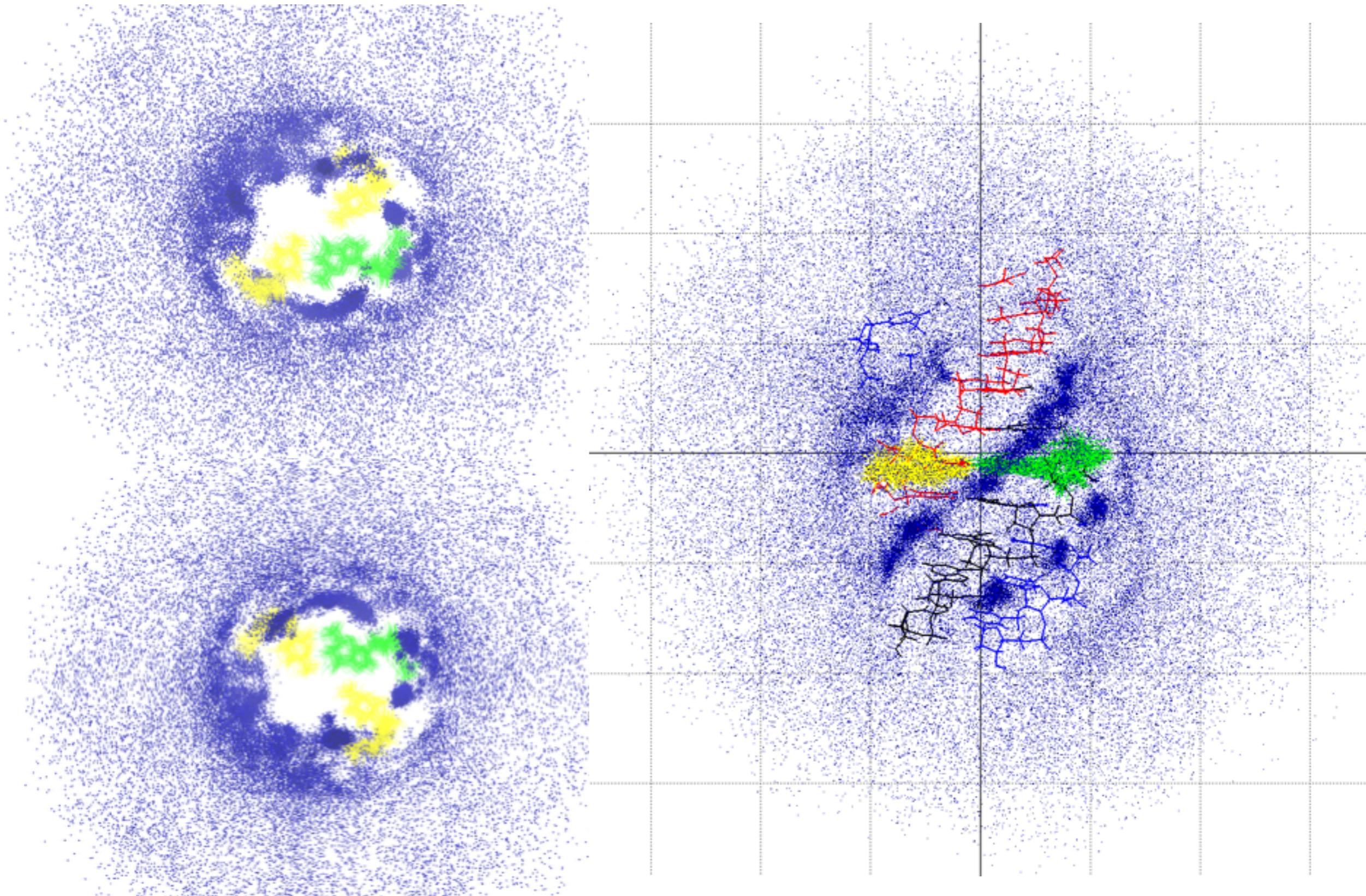
## LESSON II

- The modified triplex undertwists and overslides almost in every step and its sequence-dependent.  
Result is more repeats per turn and fixed at constant rise the helix becomes longer.  
Both major and minor groove widths are enlarged.

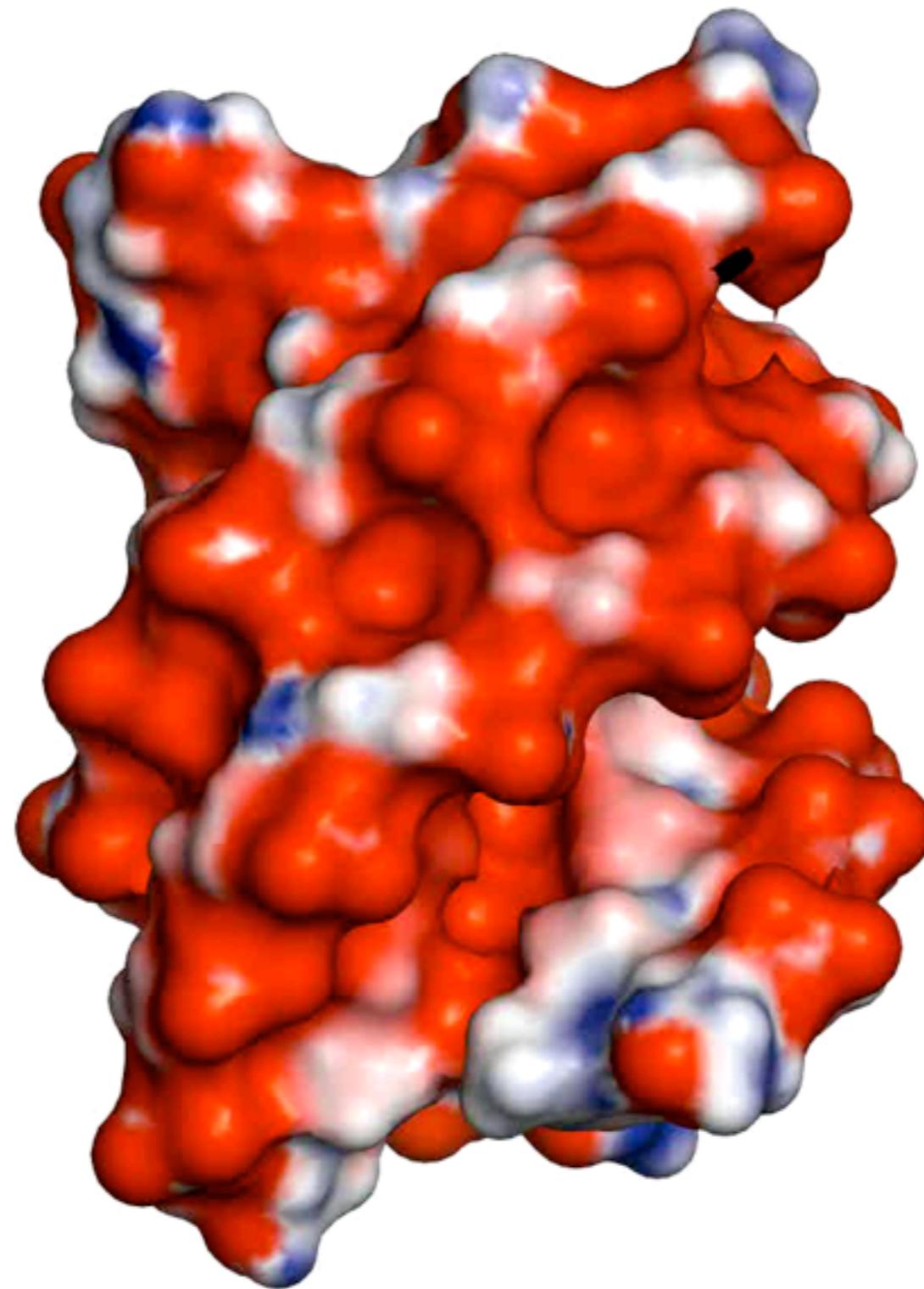
Major and minor groove widths are larger when LNAs are in.



# Ion Distribution



# Apbs Non-linear

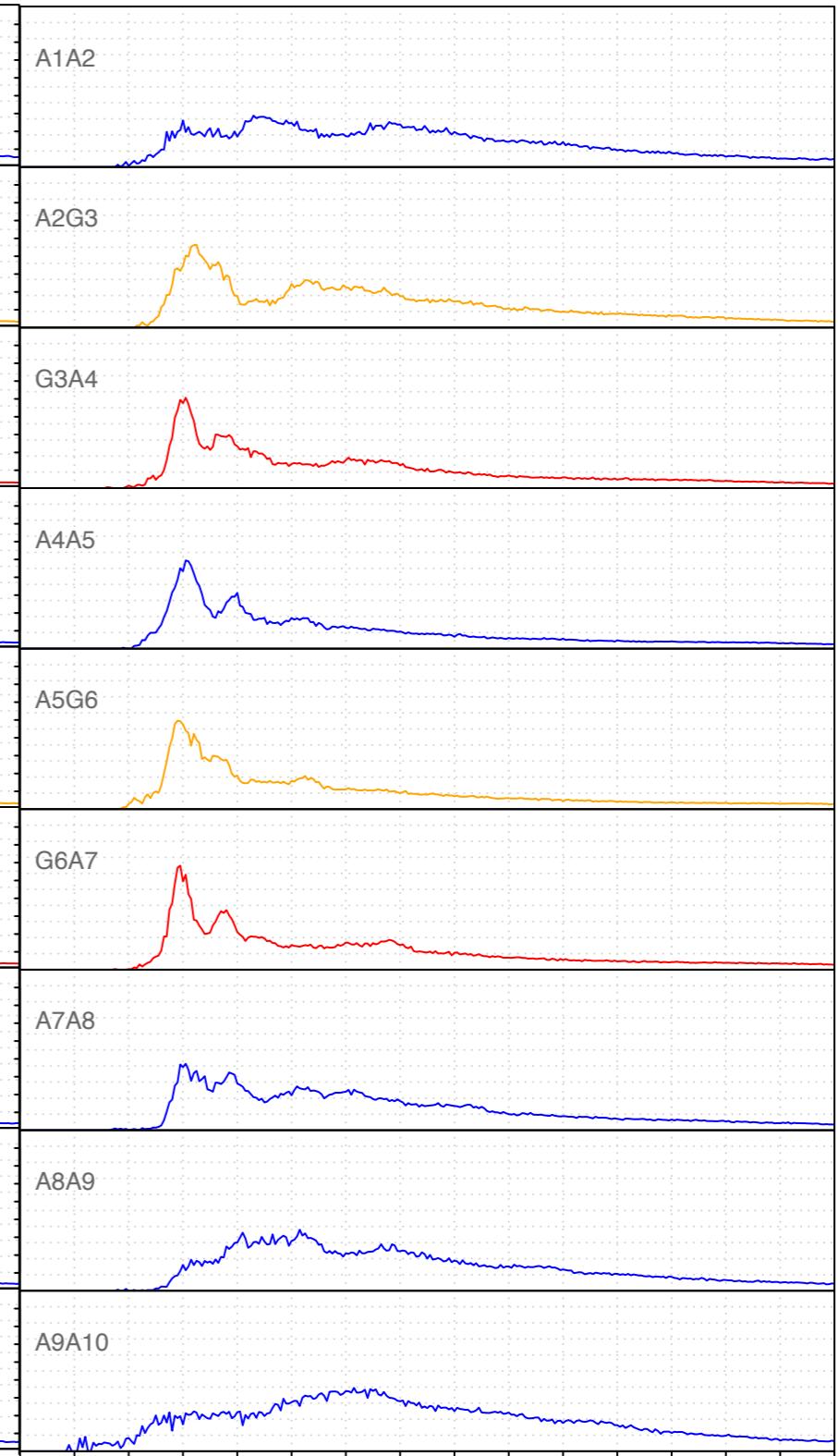
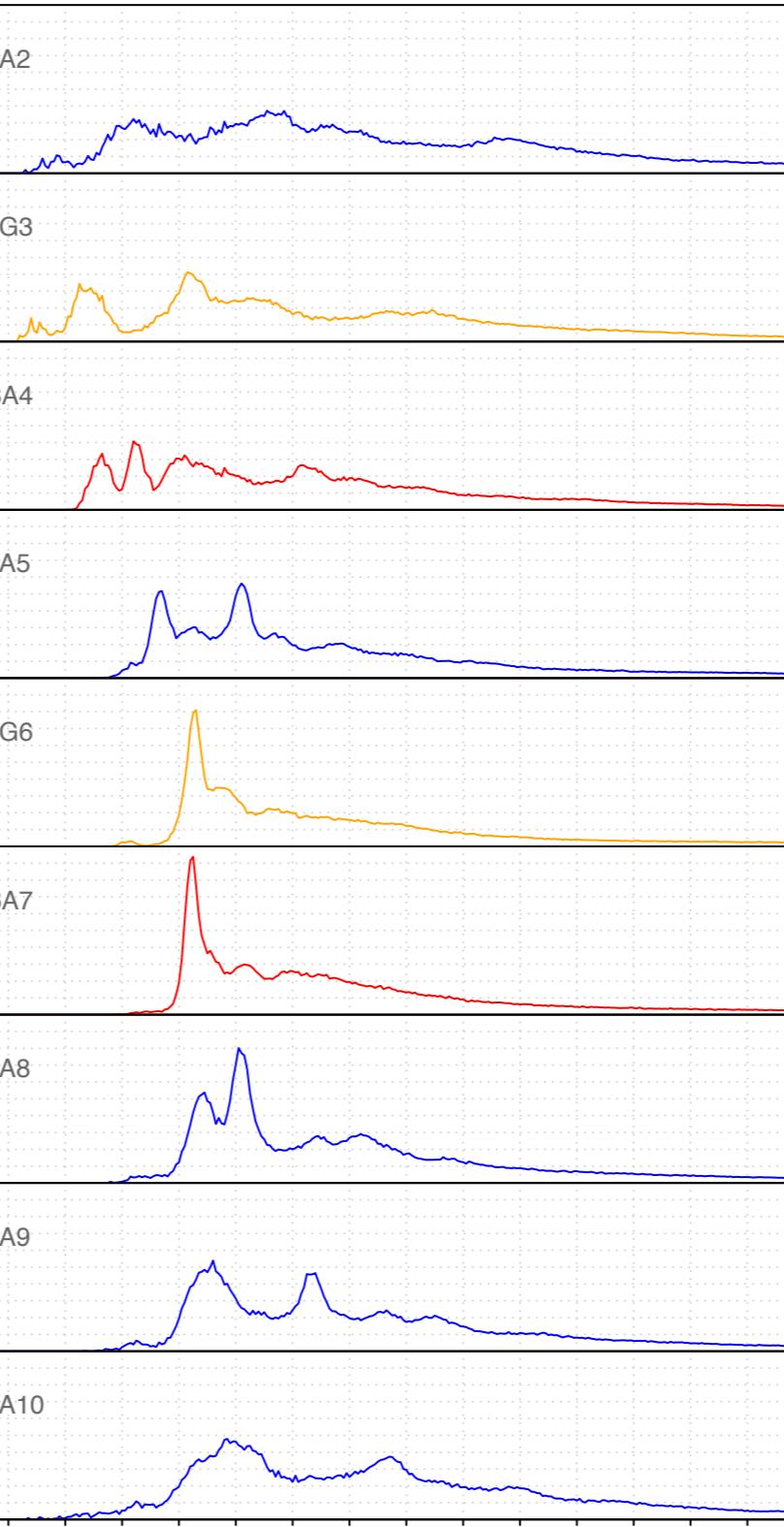
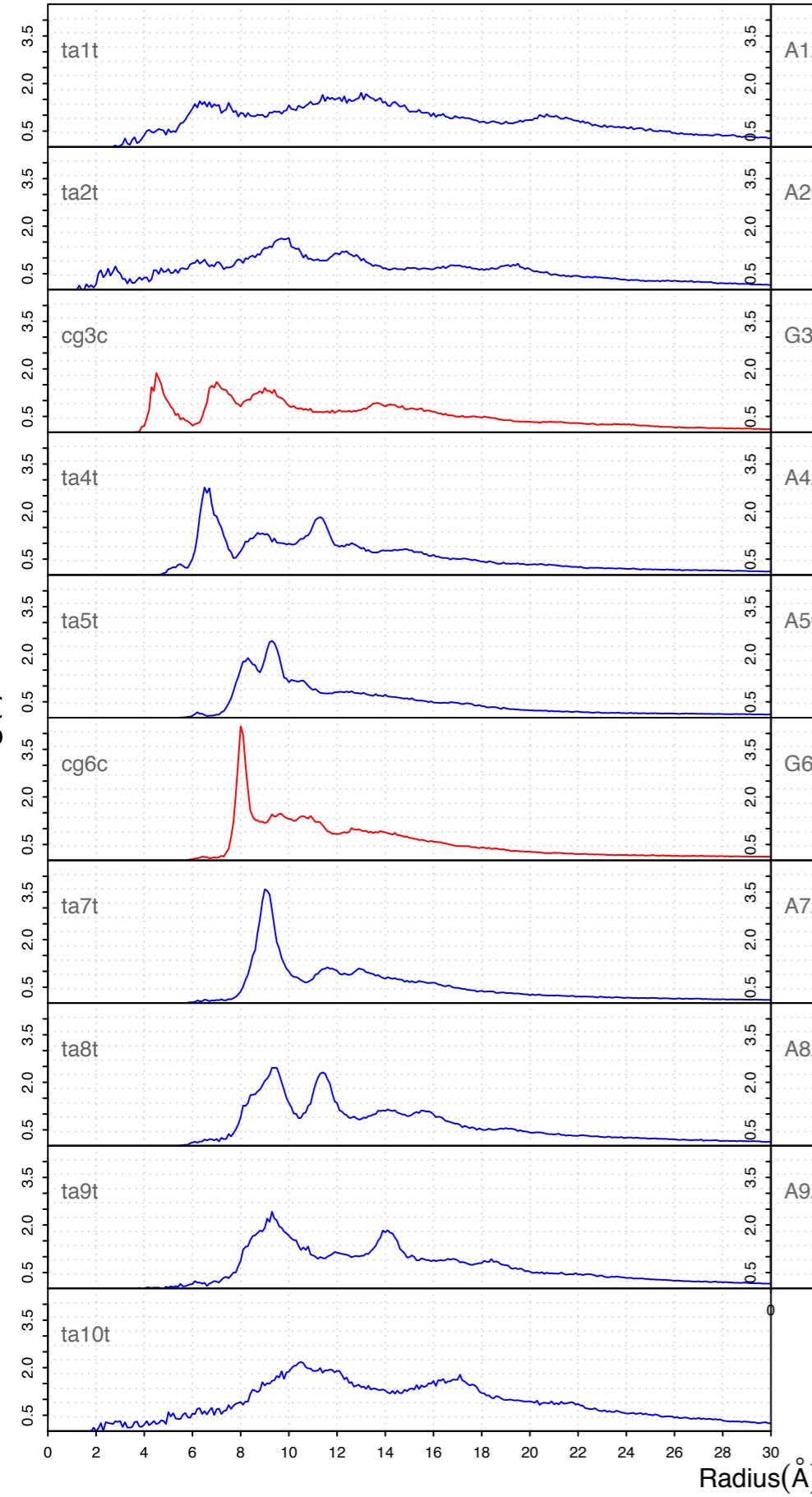


Pair

g(r) of Na to Triplets

g(r) of Na to Triplet-Steps

g(r) of Na to Triplet-Steps



Triplex

DNA