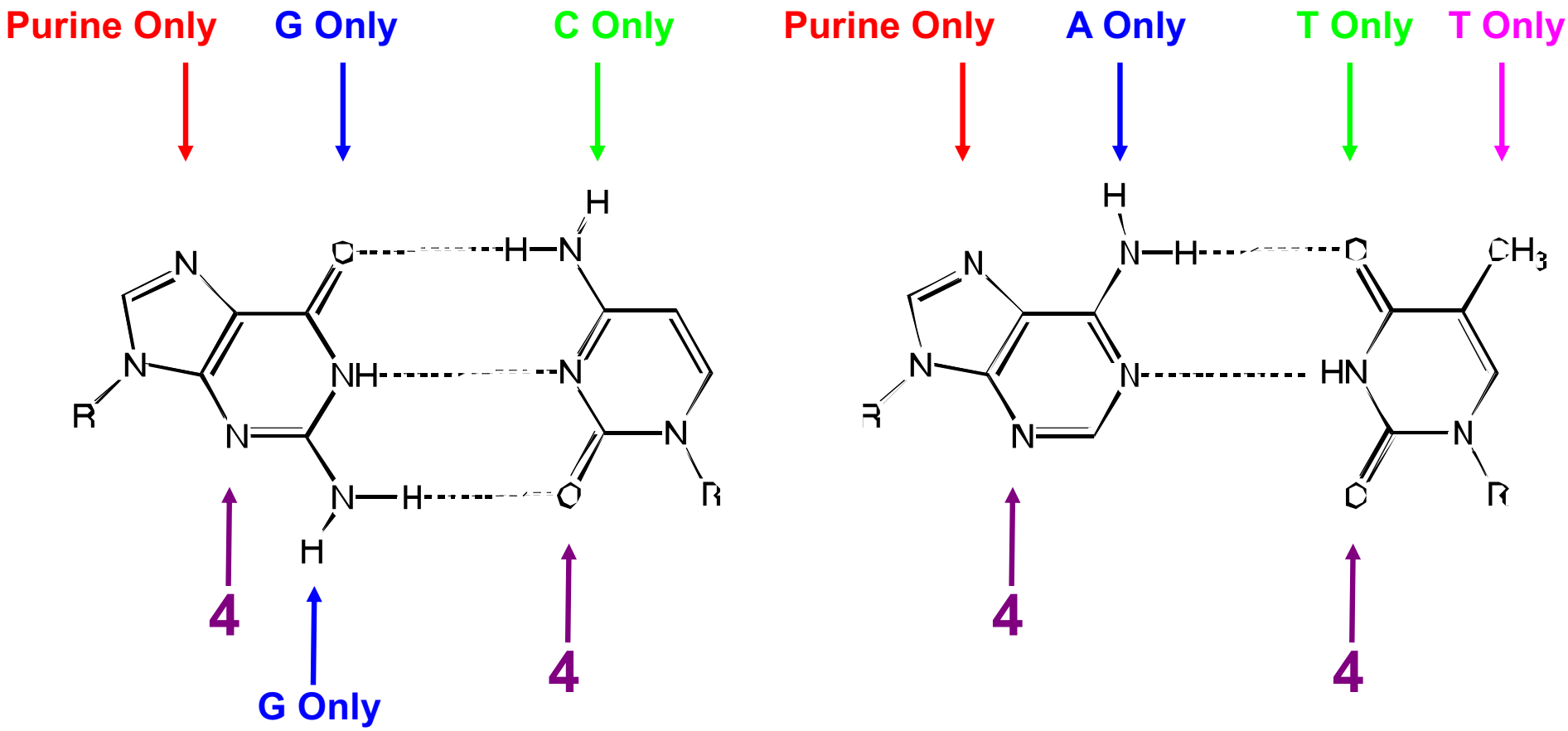
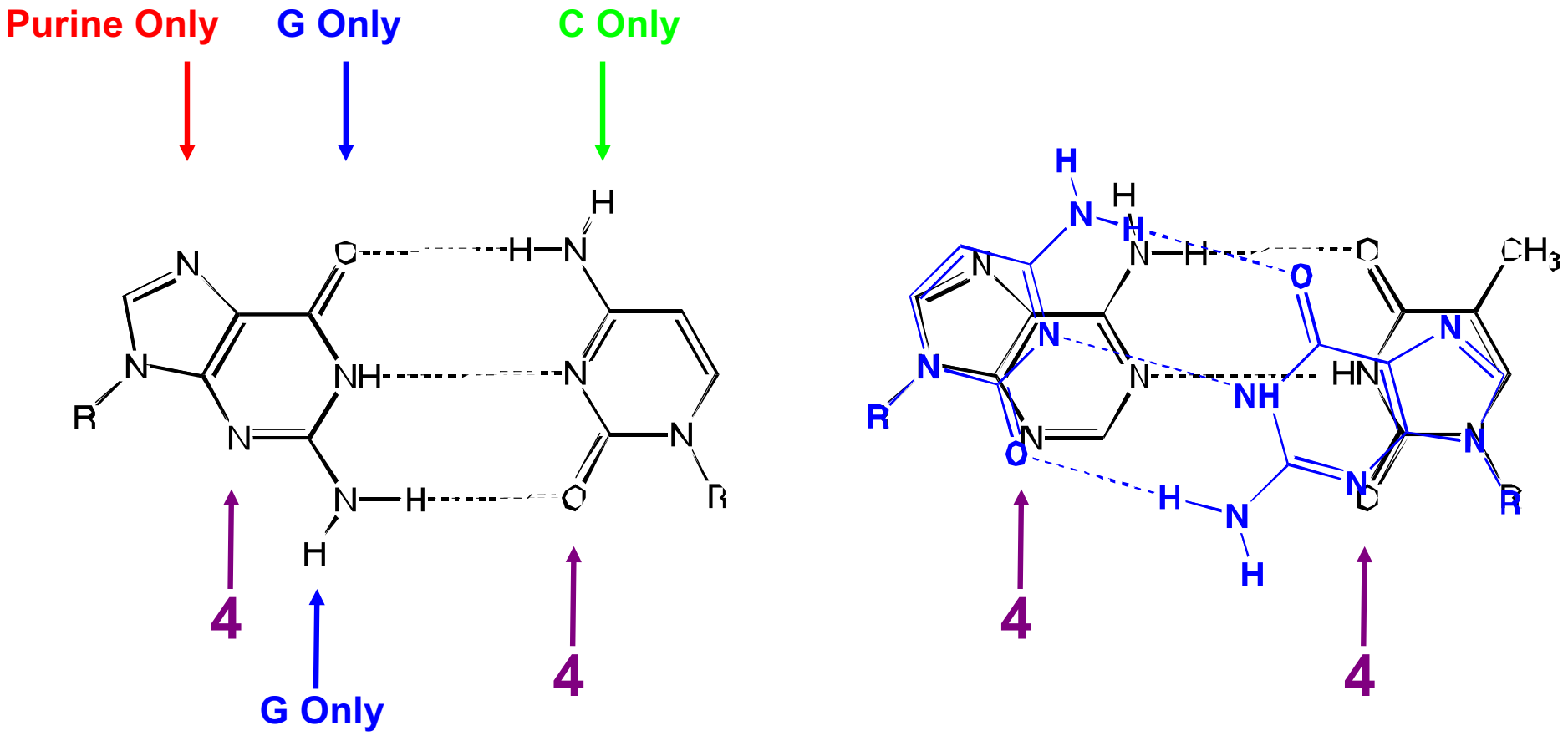
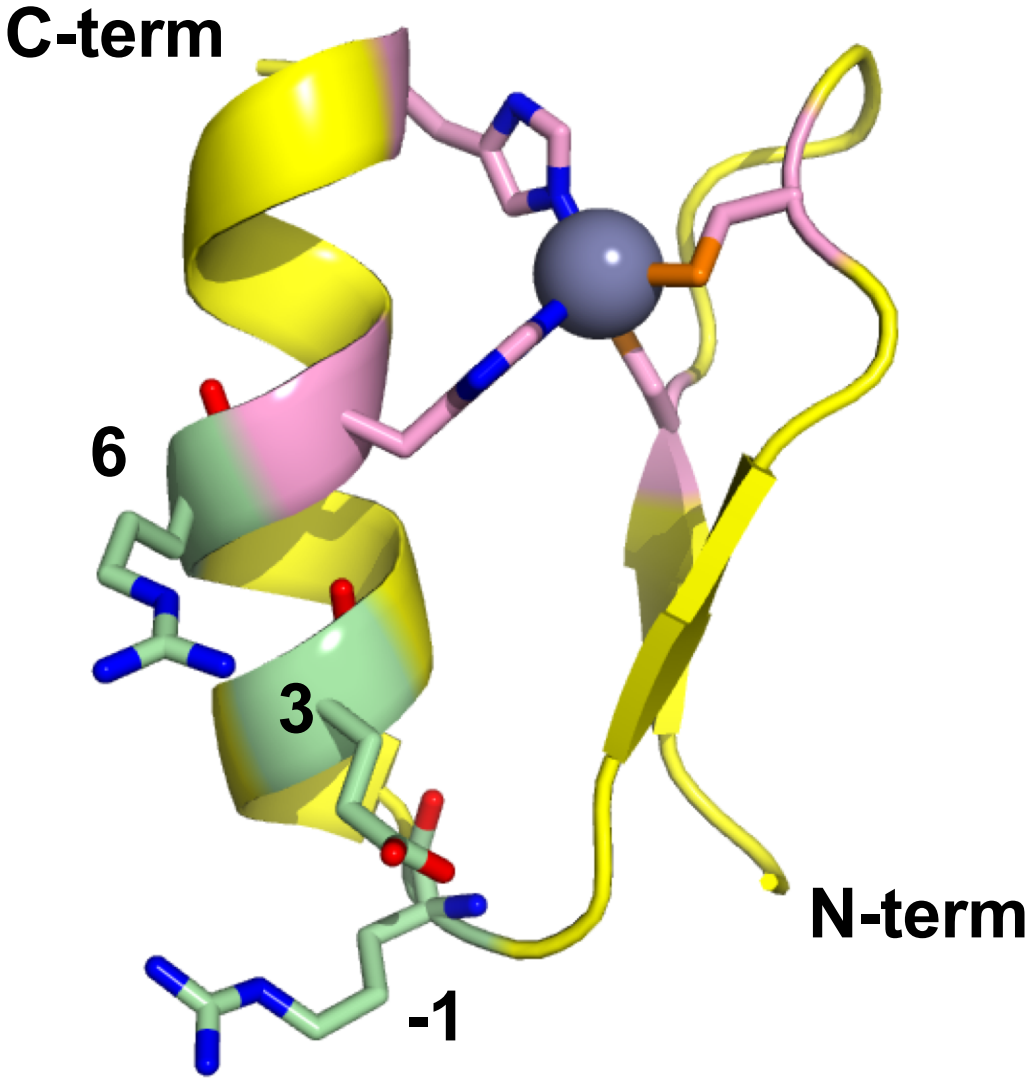
Opportunities for Direct Readout



Opportunities for Direct Readout

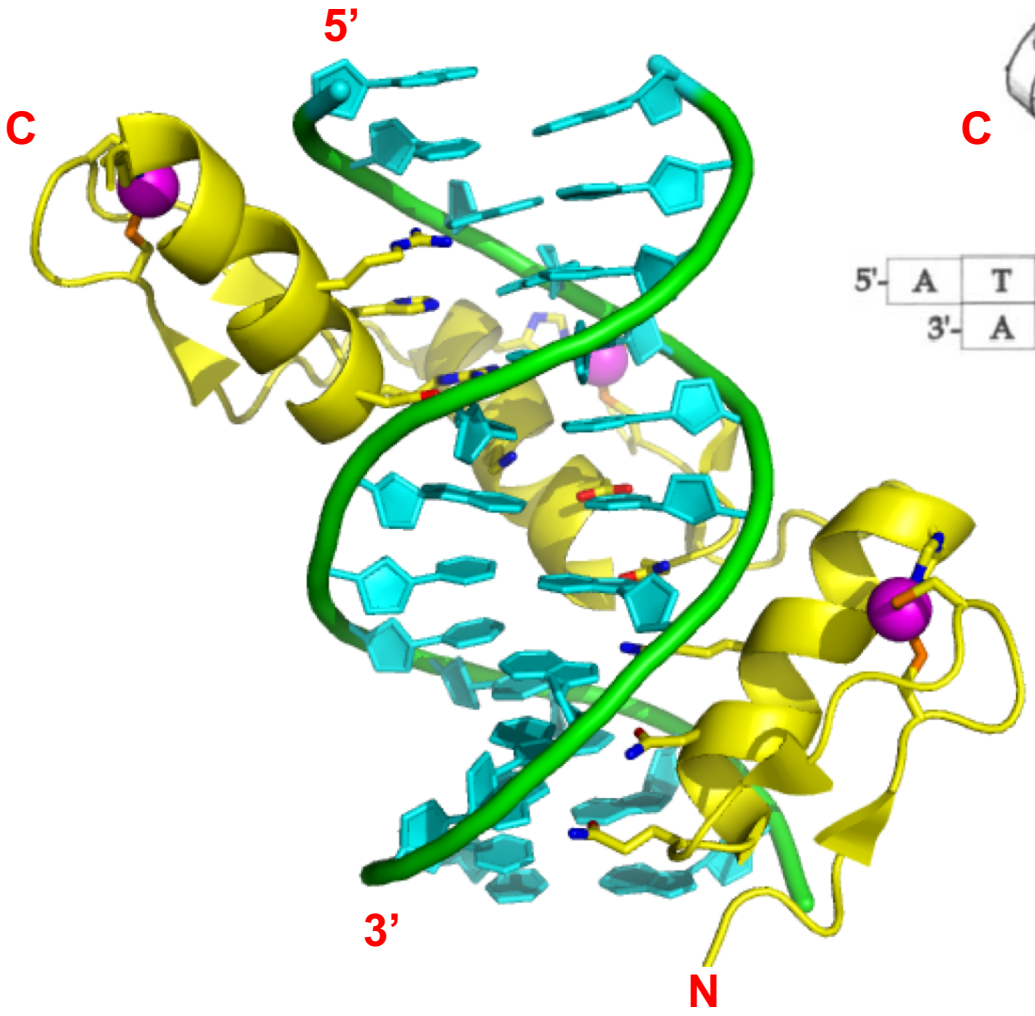


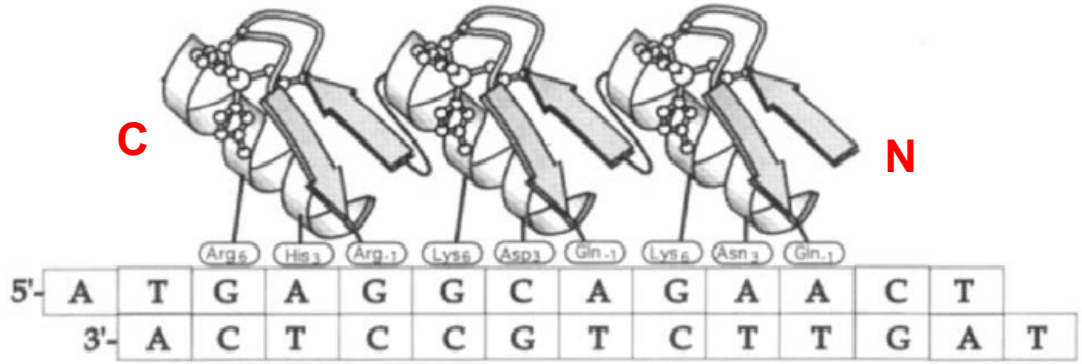
The Zn Finger Domain



Zif268 RPYA**C**PVES**C**DRRFS**R**SD**E**LT**R****H**IRI**H**T

Zinc Finger - DNA Complex





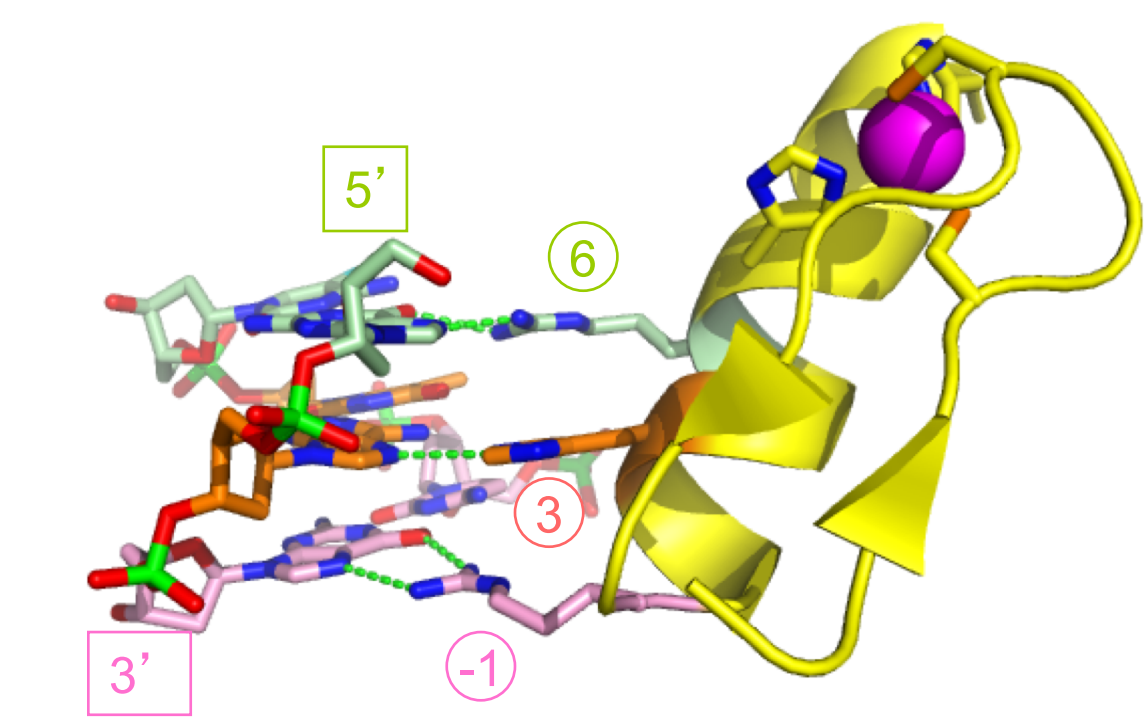
Resolution (Å)	2.74
Measured reflections	43976
Unique reflections	12537
Data coverage (last shell)	92
R_{merge}[1] (%)	5.90
R-factor[3]	0.224
free *R*-factor (10%)	0.319

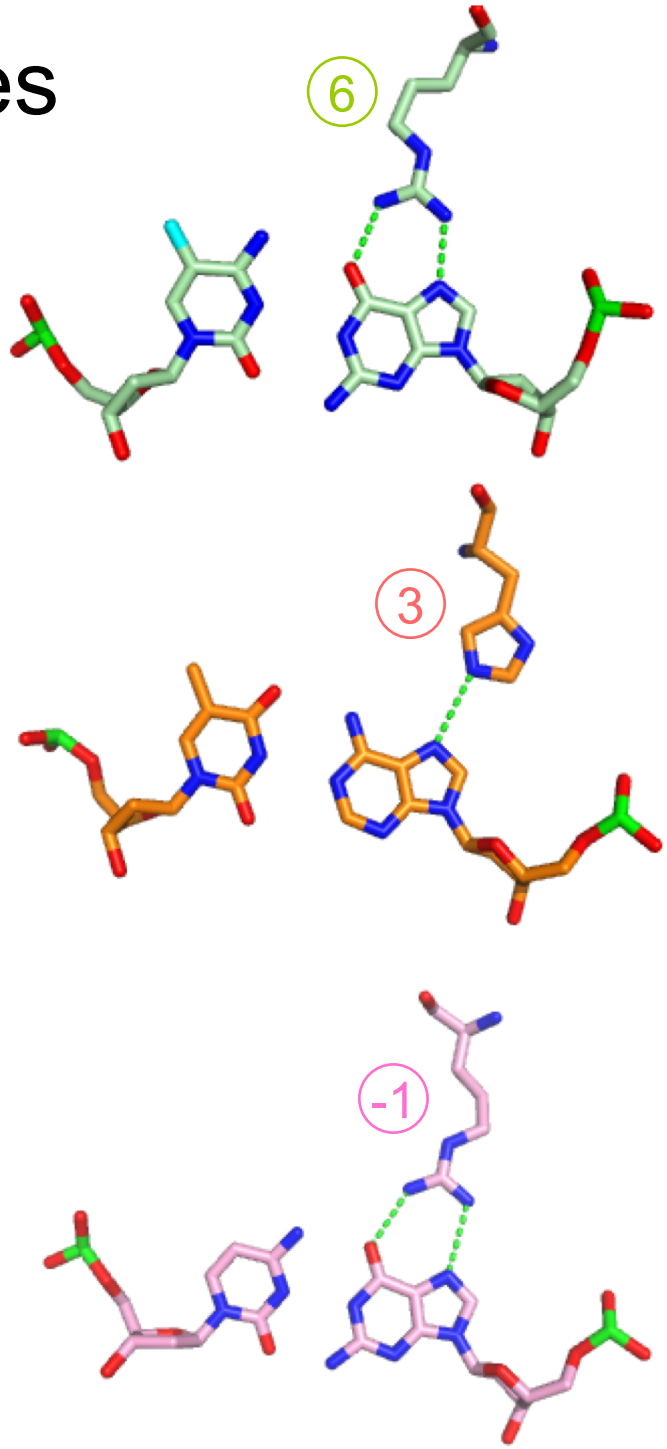
	DNA	protein
R.m.s. in bond lengths (Å):	0.014	0.019
R.m.s. in bond angles (degrees):	1.947	2.030

Kim & Berg (1996) *Nat. Struct. Biol.* **3**, 940

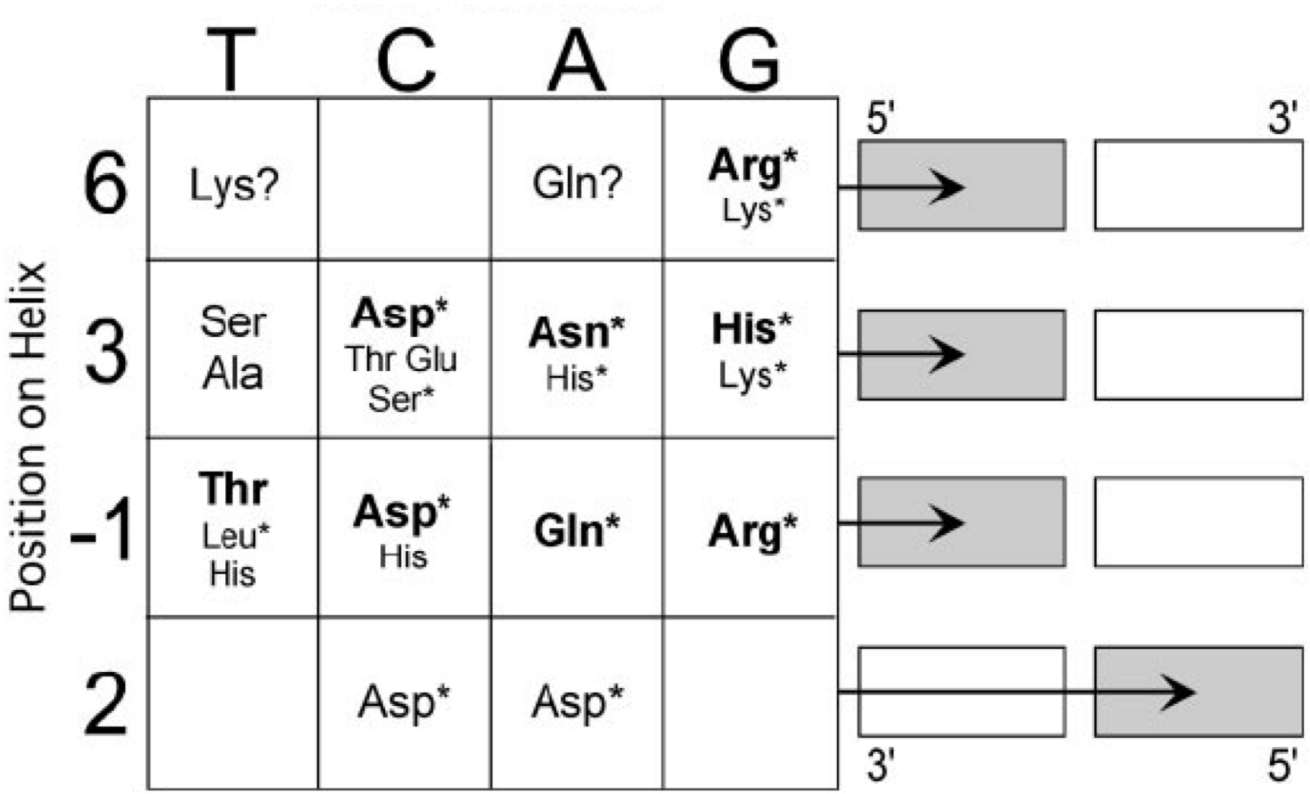
Direct Readout of Base Edges

$^{N}\underline{R}HR^{C}$ - $^{5'}GA\underline{G}^{3'}$





Recipe for Recognition?



Bold = most common
* = Xray structure
? = unsure
Blank = really unsure

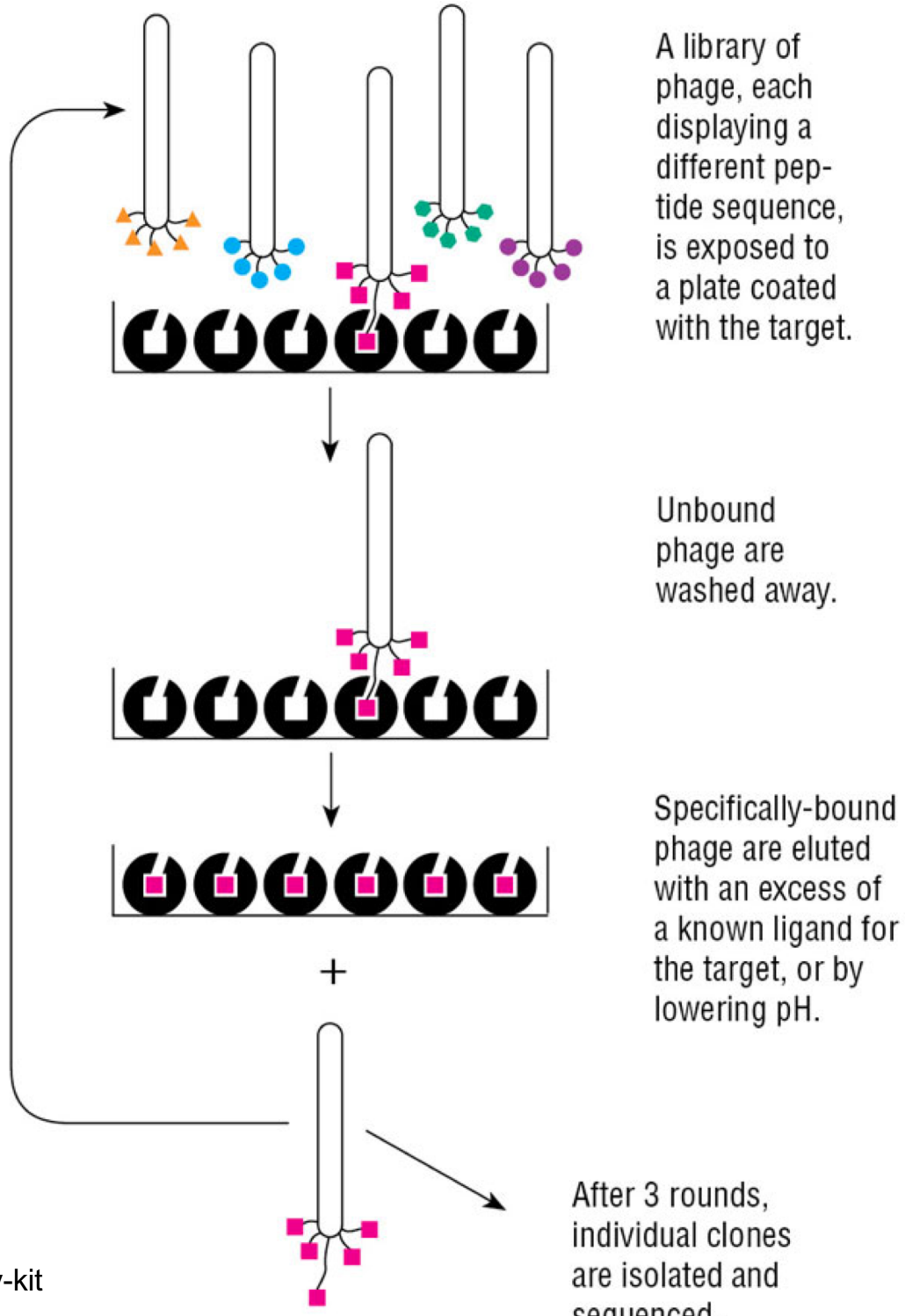
Pabo (1999) Ann Rev Biochem Biophys

Mix and Match Fingers

Protein			DNA			
Finger 1	**Finger 2**	**Finger 3**	**Triplet 1**	**Triplet 2**	**Triplet 3**	**K_d (μM)**
RER	**RER**	**RER**	**GCG**	**GCG**	**GCG**	**11***
RER	RER	RER	GGG	GCG	GGG	nd
QDR	**RER**	**RHR**	**GCT**	**GCG**	**GGG**	**.002***
QDR	RER	RHR	GGG	GCG	GGG	.015
QDR	RER	RHR	GCG	GCG	GCG	1.0
RER	**QDR**	**RHR**	**GCG**	**GCT**	**GGG**	**0.010***
RER	QDR	RHR	GCT	GCG	GGG	0.066
QDR	RER	RHR	GCG	GCT	GGG	0.55
QDR	**RER**	**RHR**	**GCT**	**GCG**	**GGG**	**0.003***

*=cognate: RER/GCG, QDR/GCT, RHR/GGG

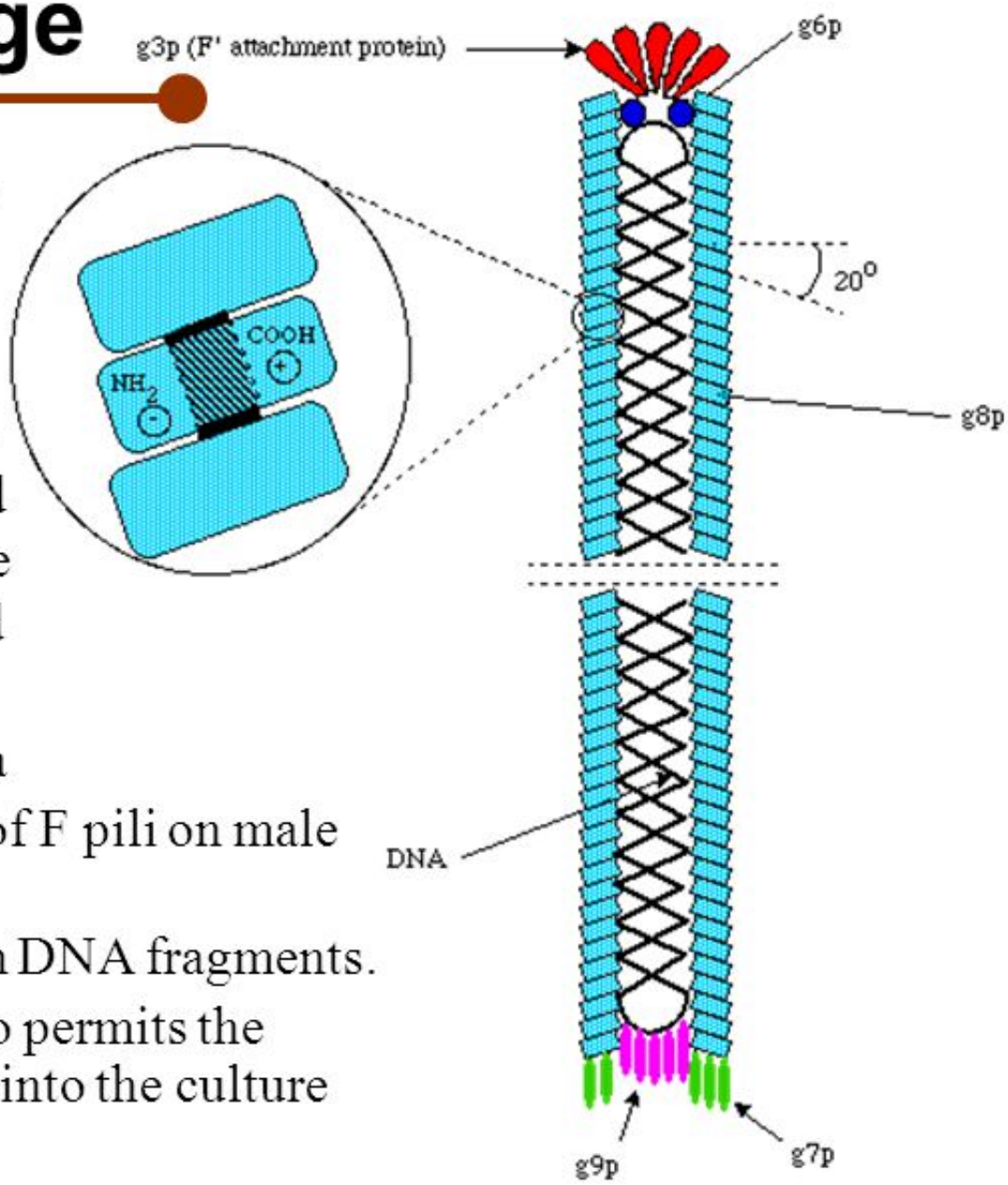
Phage Display Courtesy of New England Biolabs



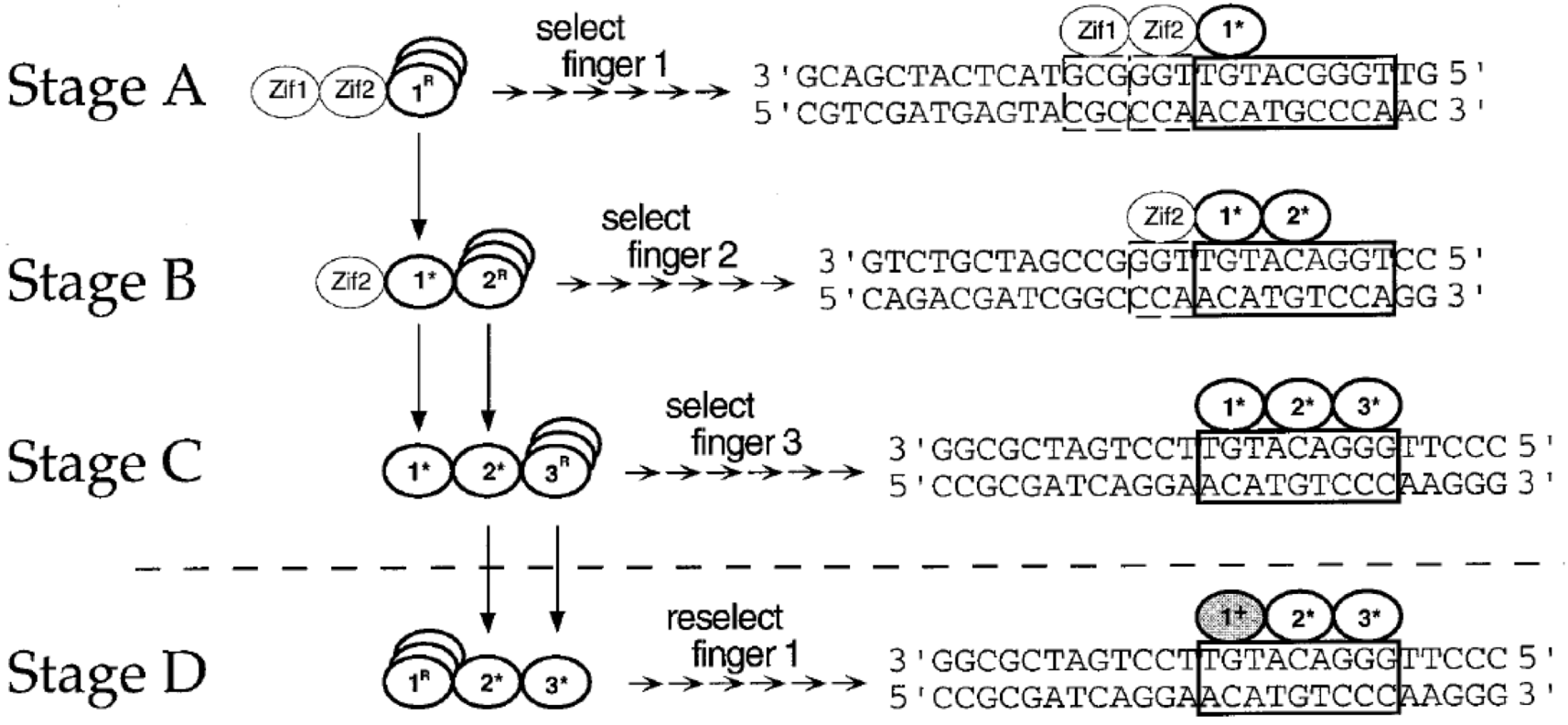
https://www.neb.com/products/e8120-phd-c7c-phage-display-peptide-library-kit

Filamentous Phage

- long, thin, and flexible particles that contain a closed circular single-strained DNA molecule, such as fd, f1, and M13.
- The major coat protein is pVIII. The minor coat proteins pIII and pVI are located at one end of the phage; pVII and pIX are located at the other end of the phage.
- to infect Gram-negative bacteria
- to adsorb specifically to the tip of F pili on male cells.
- Be able to accommodate foreign DNA fragments.
- its nonlysogenic characteristic to permits the extrusion of recombinant phage into the culture supernatant.



Strategy For Finding High Affinity Triple Finger

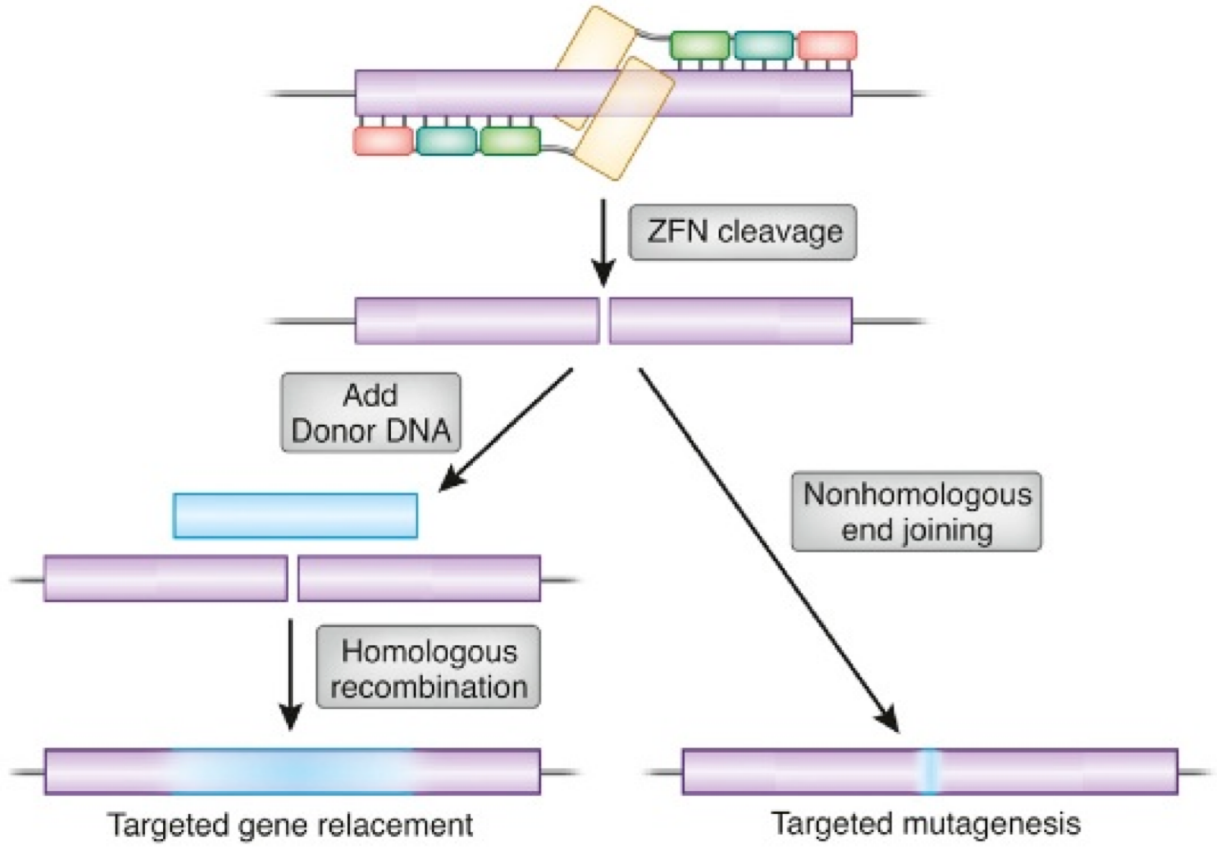


Target Sites: Zif268 3'- G G C G G G T G C G T -5'

p53 3'- T T G T A C A G G G T -5'

J. Mol. Biol. (1999) 285, 1917

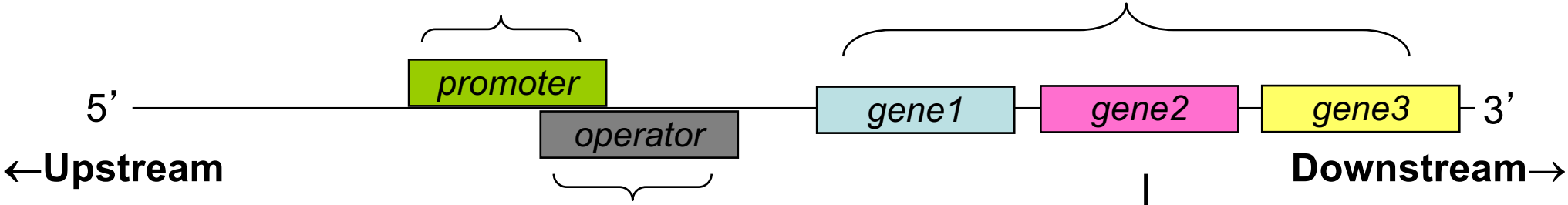
Zinc Finger Nucleases



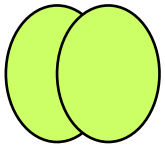
An Illustrated Glossary of Terms

DNA Sequence
Bound by RNA Polymerase
To Initiate Transcription

Operon - Cluster of Genes
Under control of 1 promoter



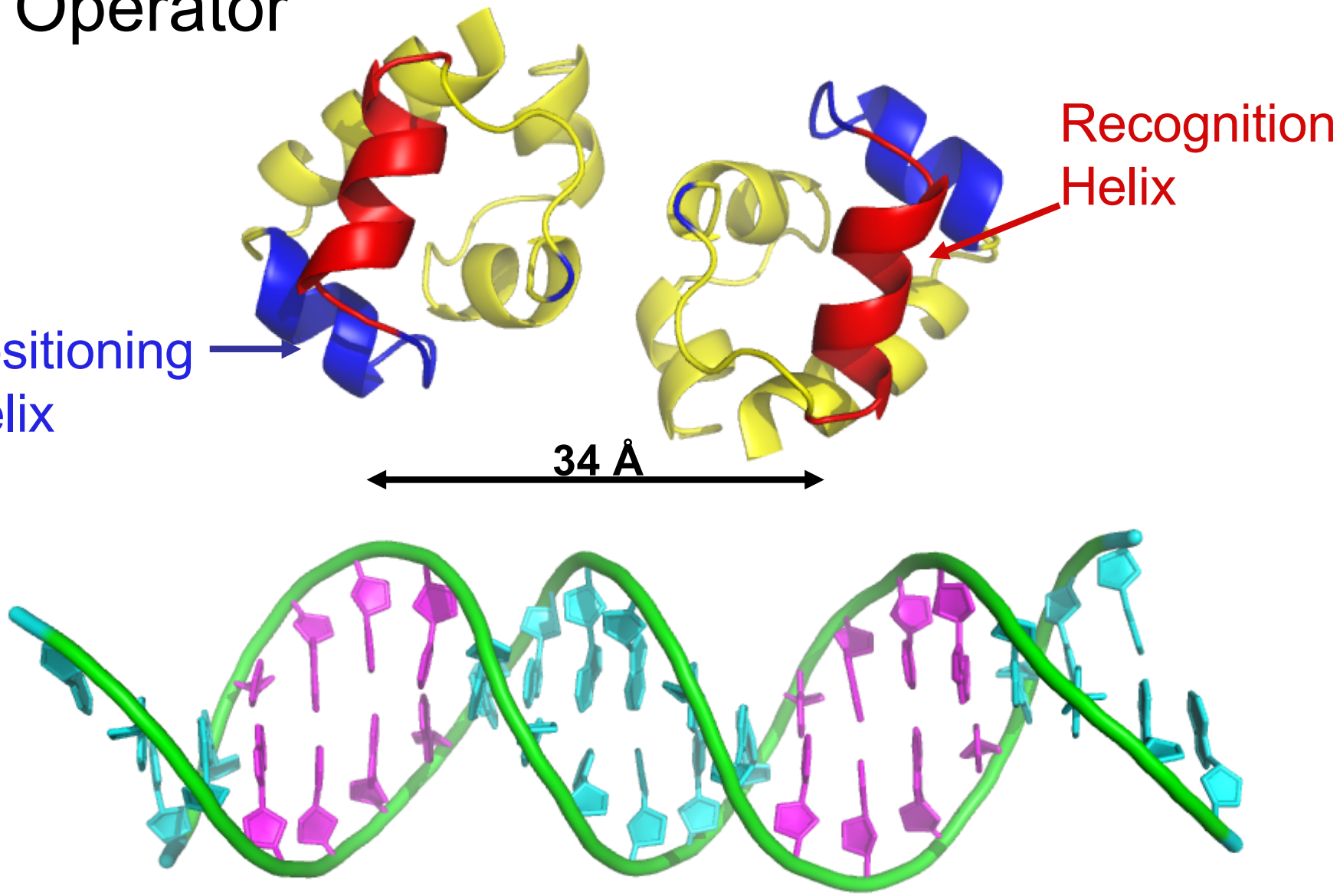
DNA Sequence
Bound by Regulatory
Protein



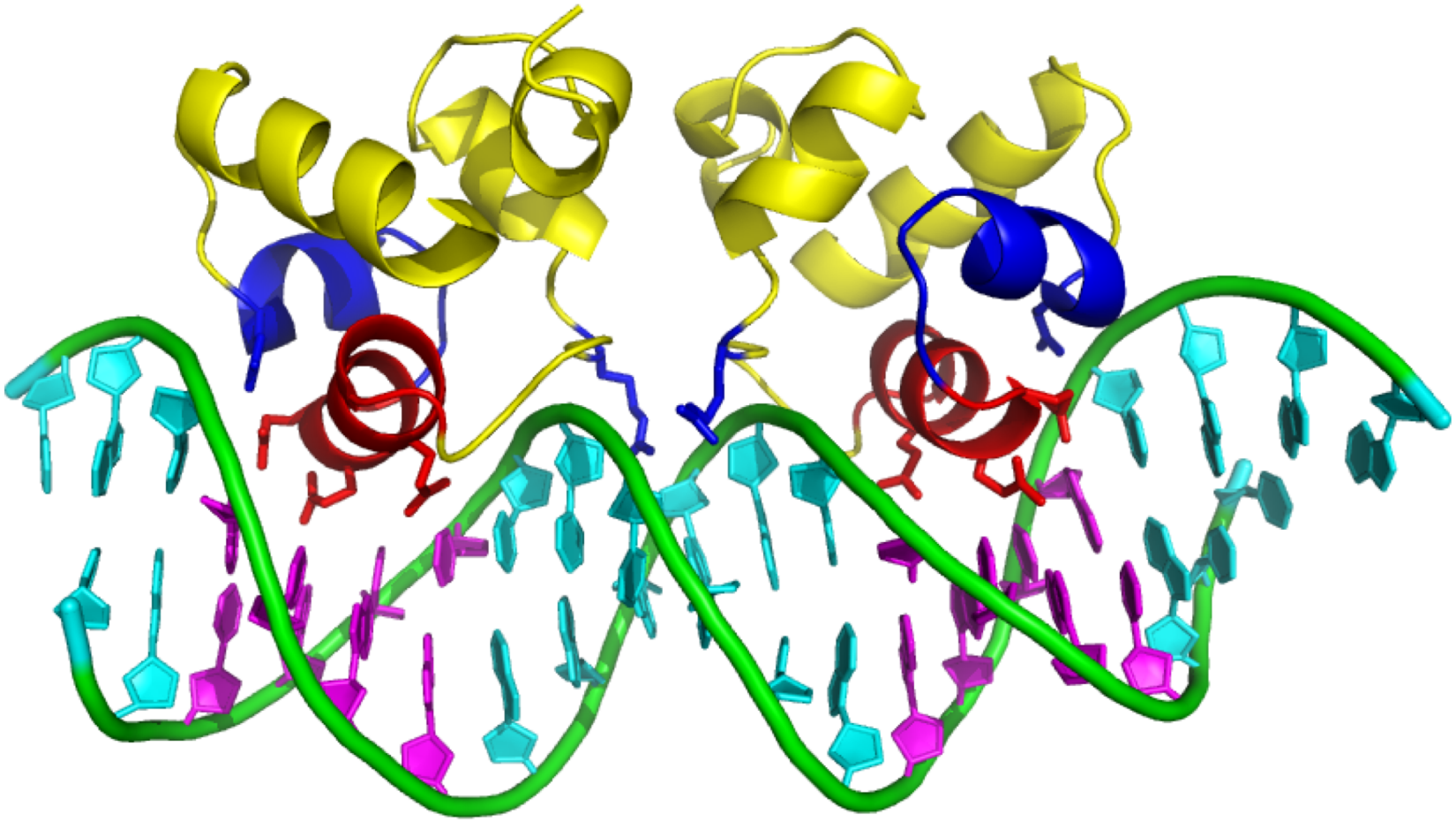
Regulatory Protein
May bind an allosteric effector. Binding
the effector leads to a change in conformation
That changes DNA affinity/specificity

Each gene encodes one
protein. A gene named *lacZ*
encodes protein named LacZ

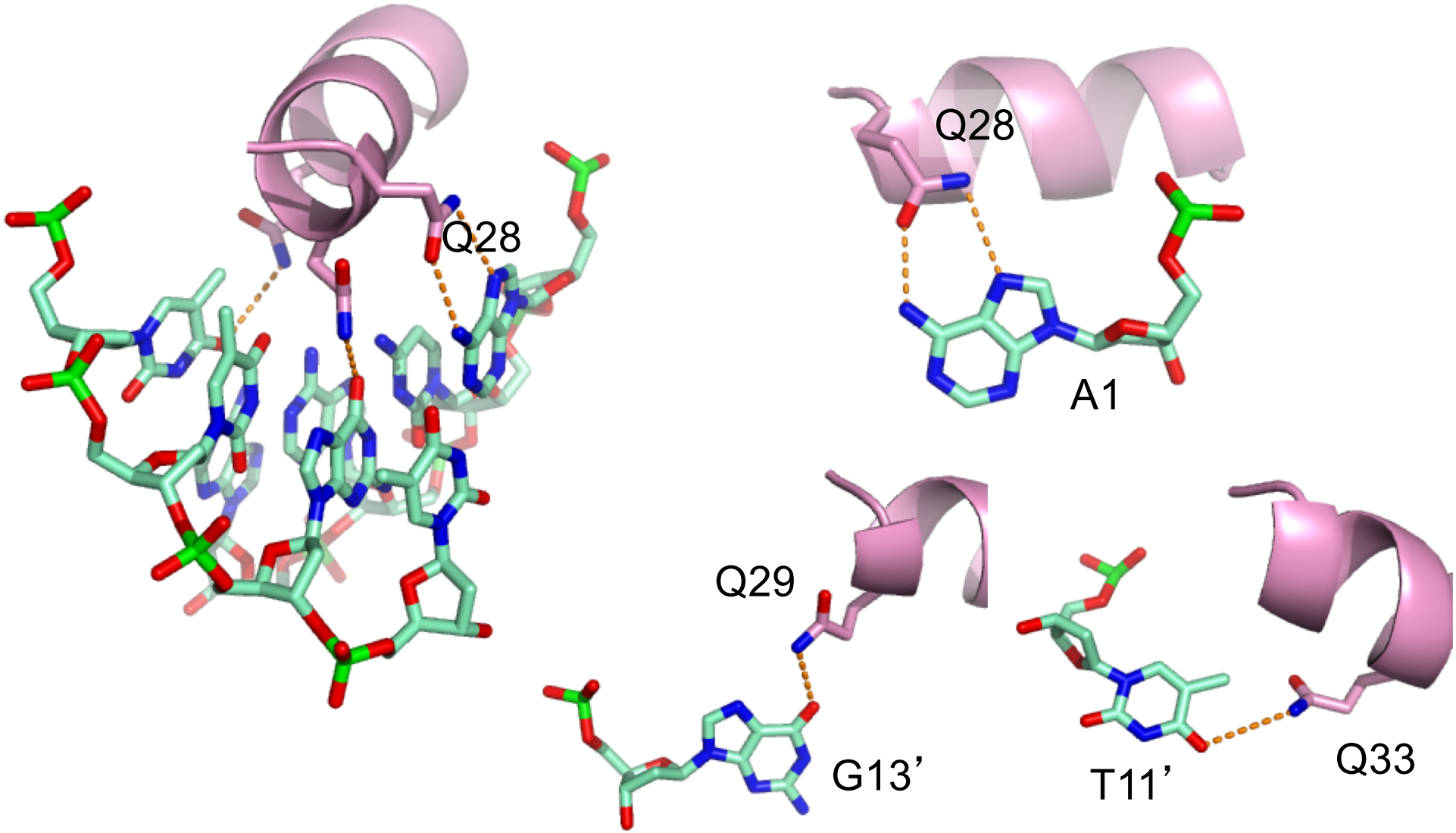
434 repressor and its Operator



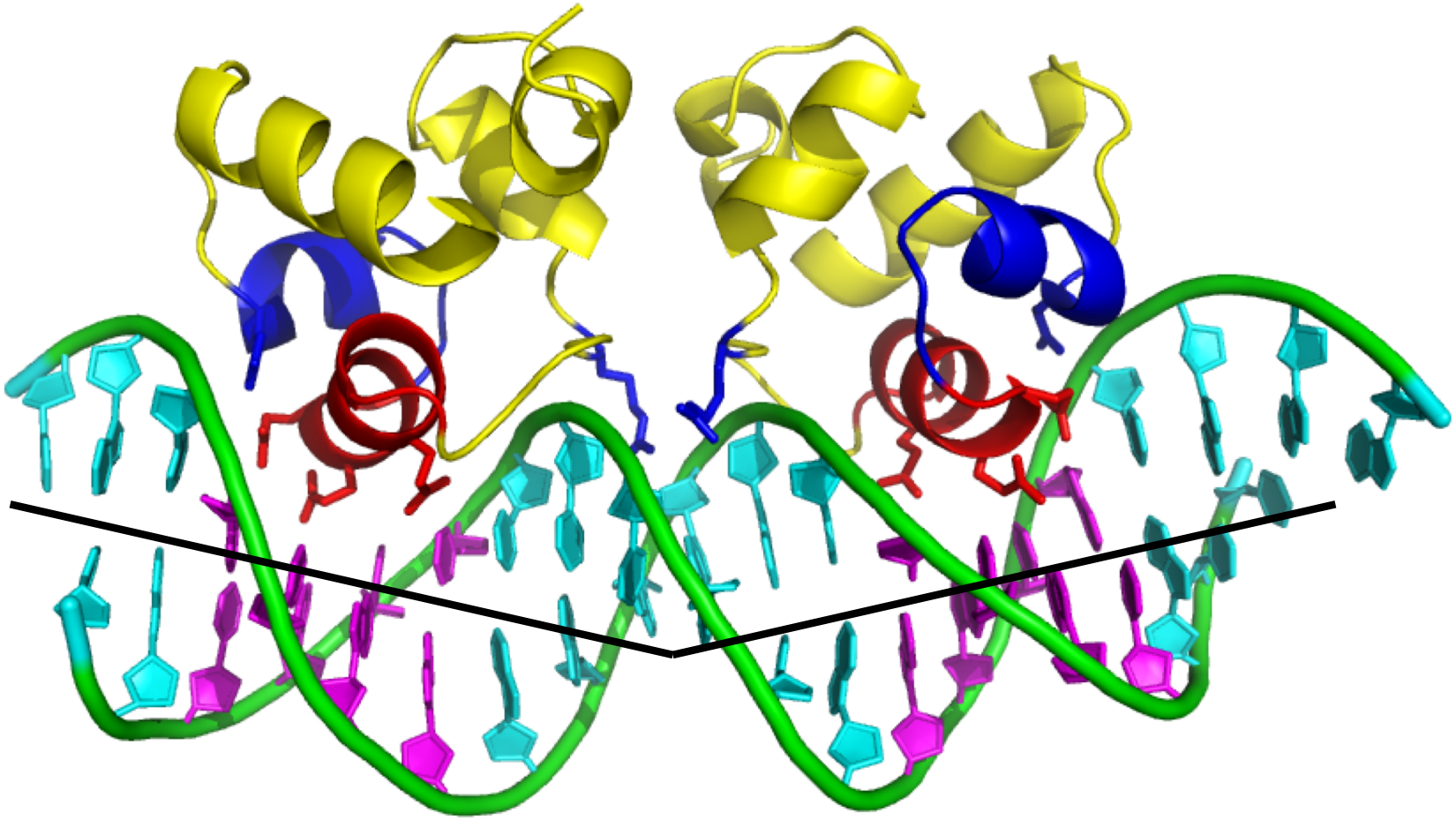
The 434 Repressor-Operator Complex



Direct Readout of Major Groove

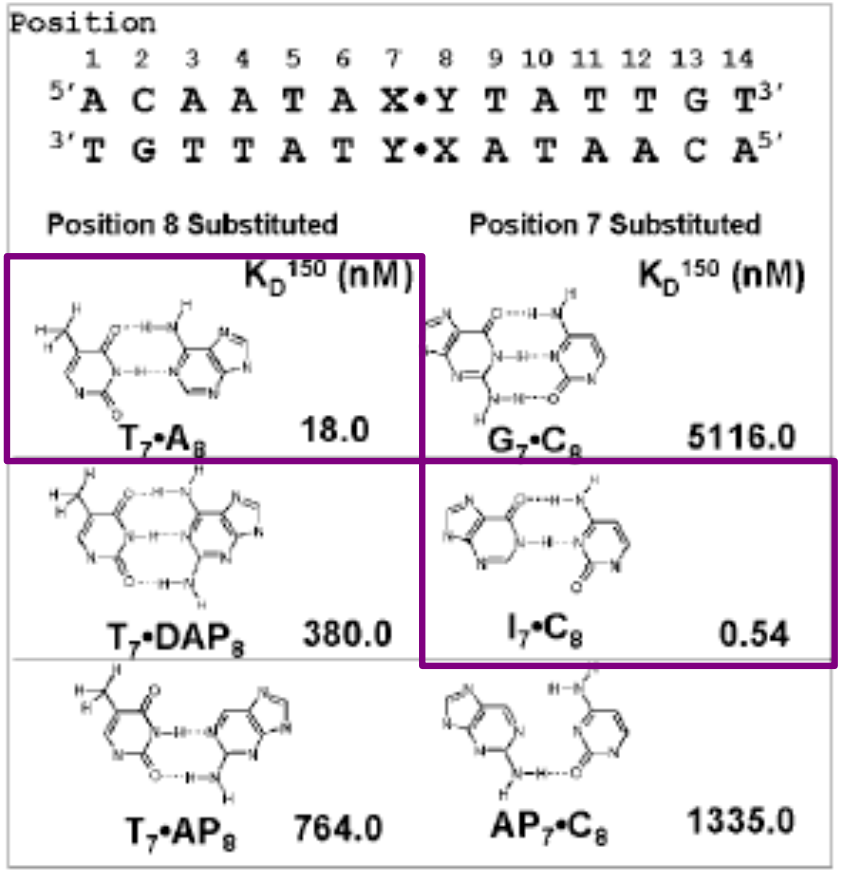


Indirect Readout of Minor Groove?



24˚ bend arises
from minor groove
Compression

Indirect Readout of DNA Conformation



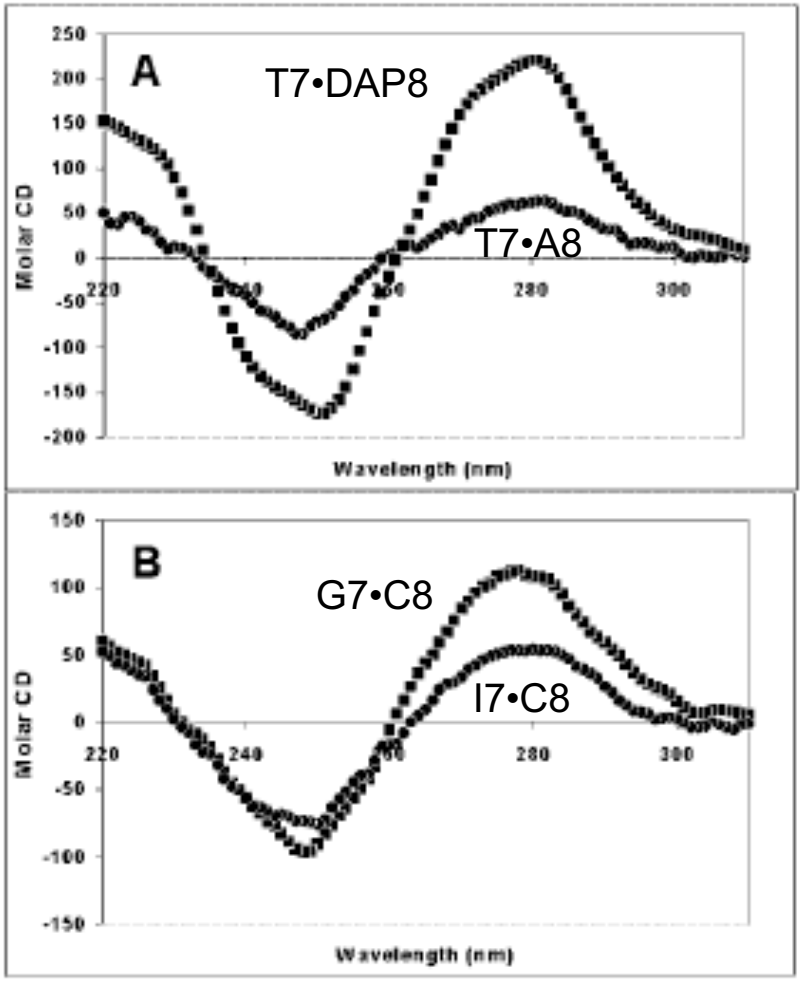
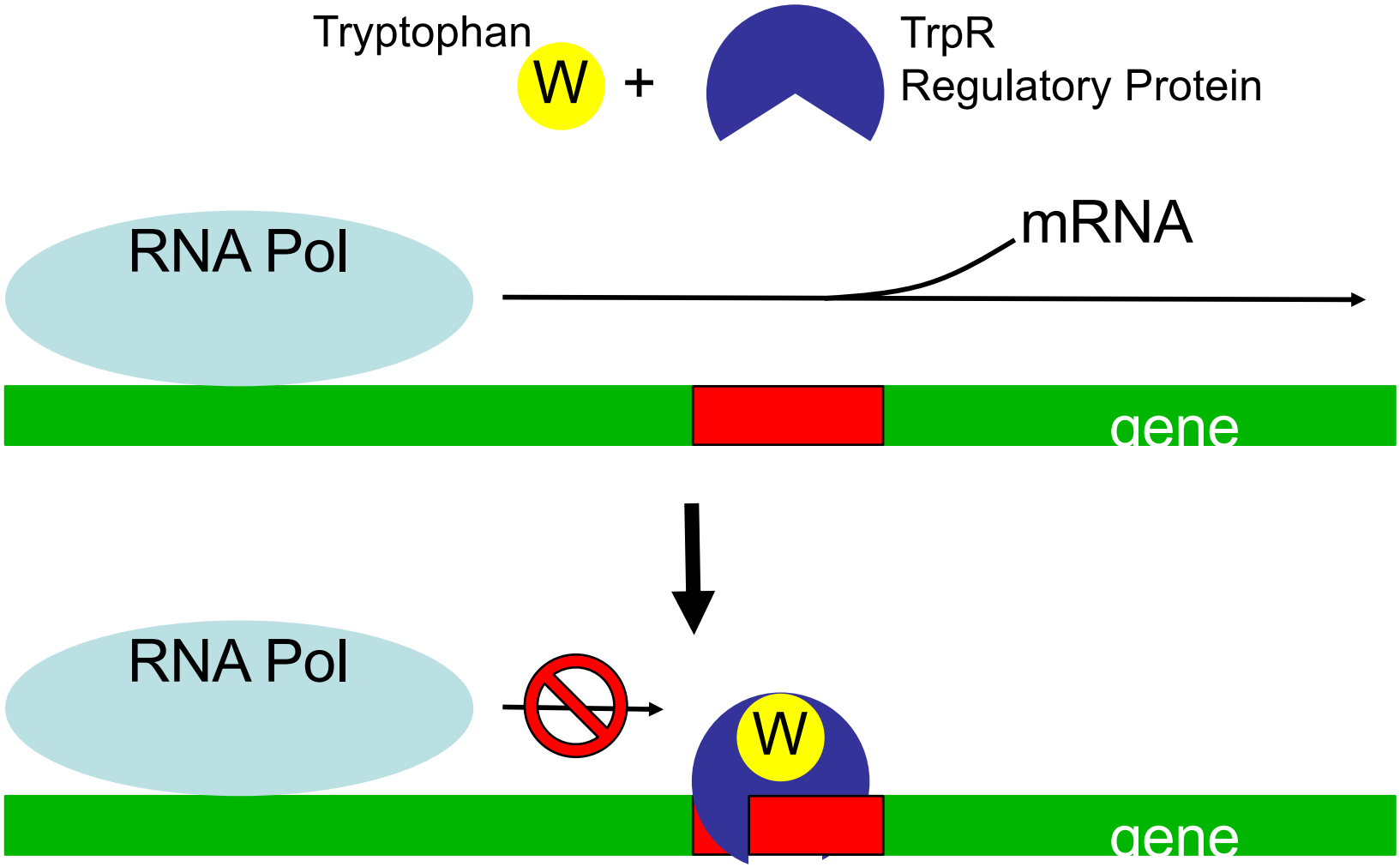


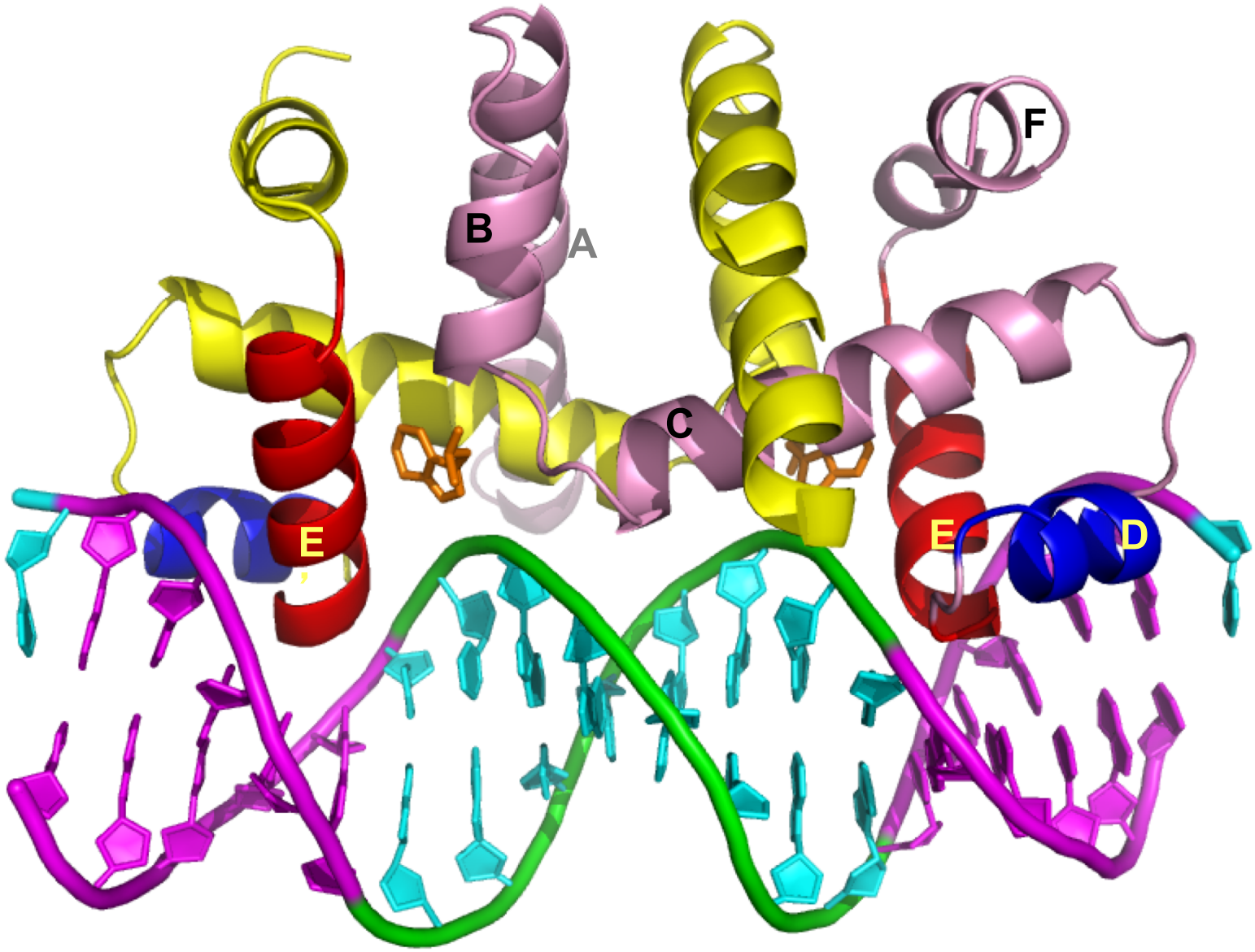
FIG. 4. **Circular dichroism spectra of 2 mM of DNAs bearing modified bases at their central positions.** Spectra were acquired at 50 mM KCl, 25 °C (see also "Experimental Procedures"). The spectra of T7·A8 (●) and T7·DAP8 (■) are shown in *A*. *B* displays the spectra of I7·C8 (●) and G7·C8 (■).

Mauro et al. (2003) *J. Biol. Chem.* **278**, 12995

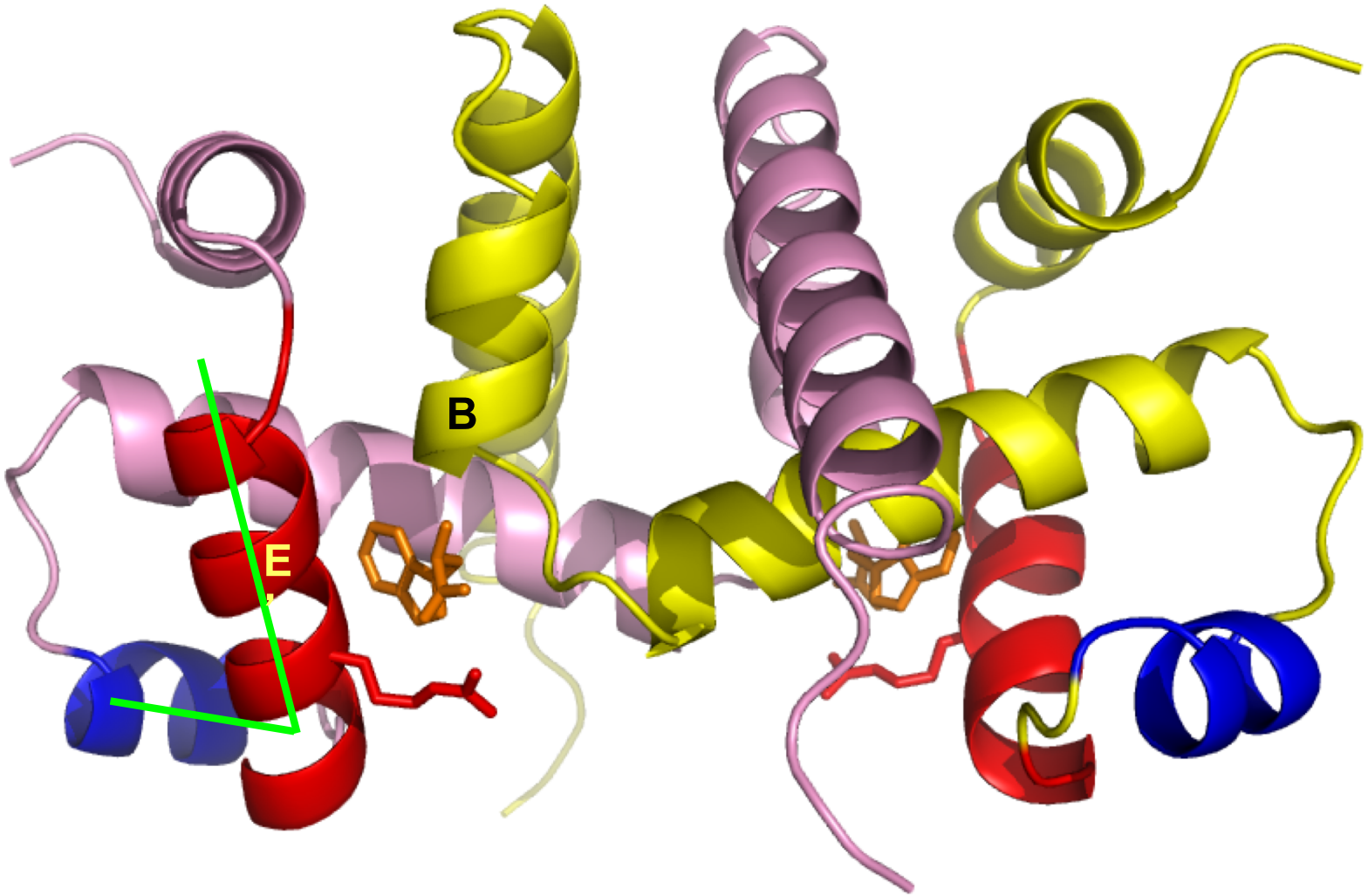
TrpR Binds Tryptophan and Blocks mRNA Synthesis by Binding DNA



Trp Repressor Bound to Operator

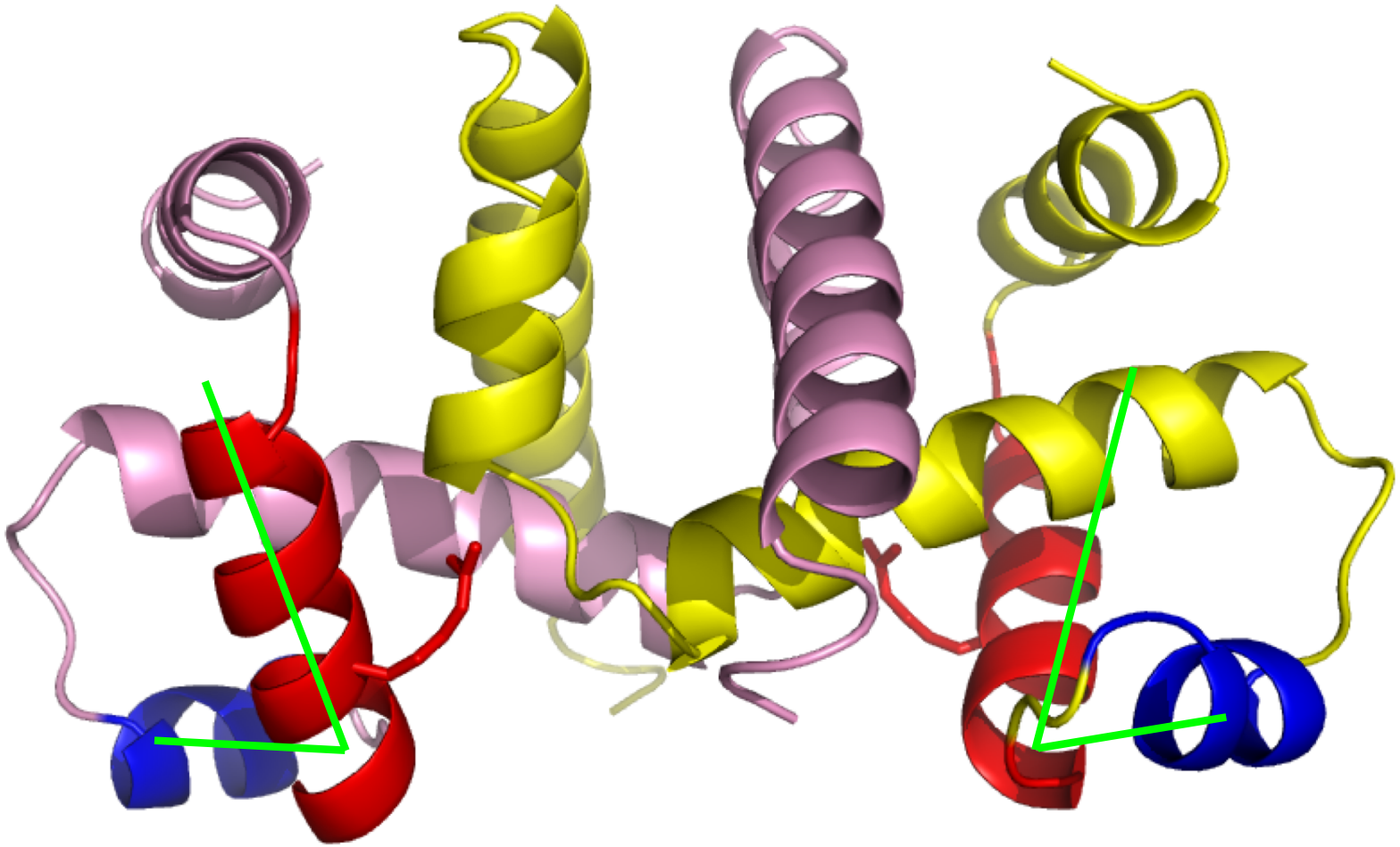


Tryptophan is an Allosteric Effector



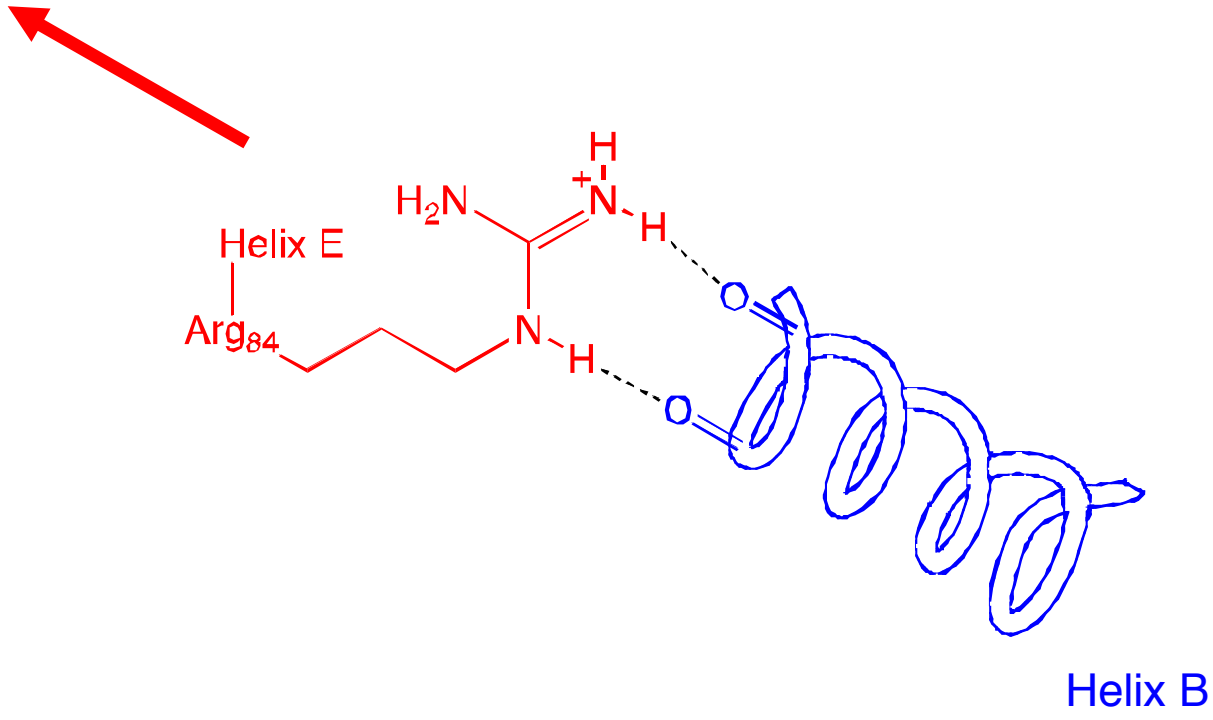
In the presence of bound tryptophan, Arg84 H-bonds to α-carboxylate of the co-repressor

Tryptophan is an Allosteric Effector

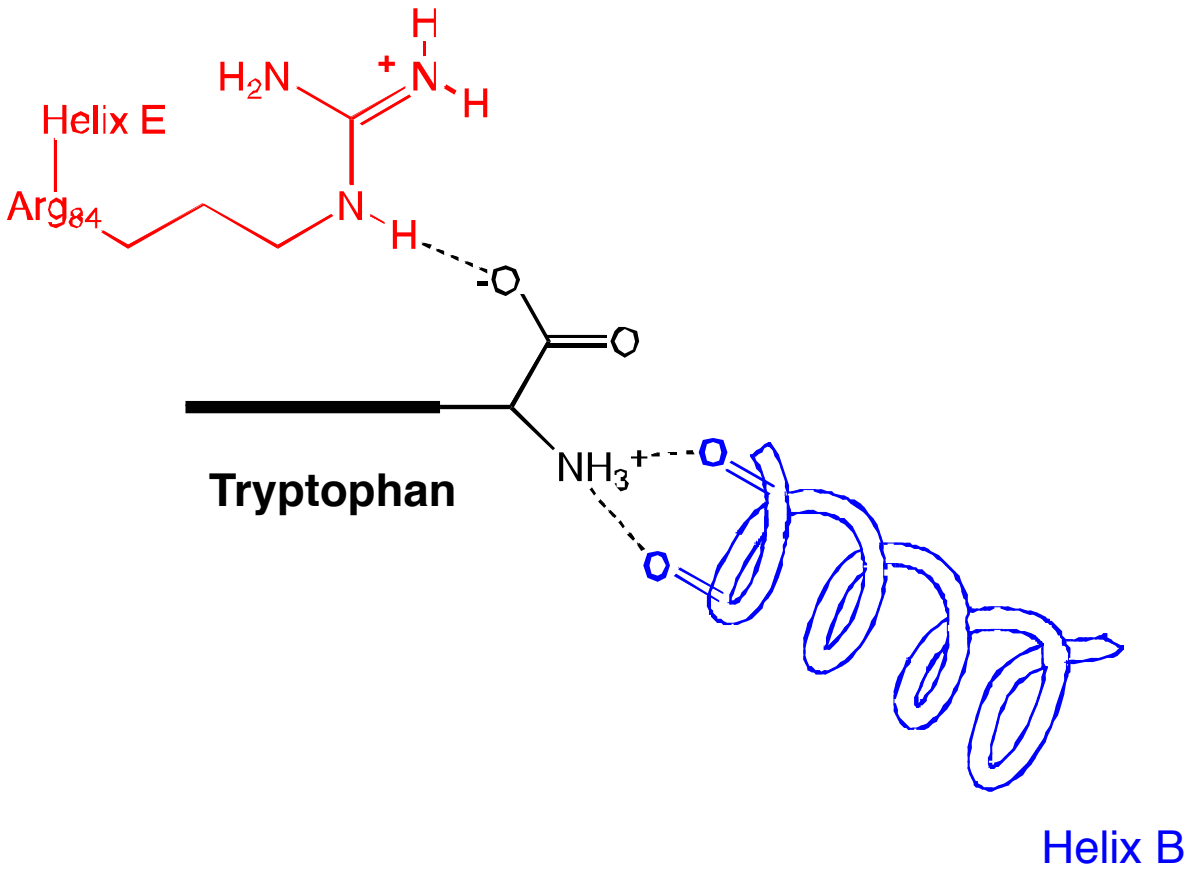


Without tryptophan co-repressor, Arg84 reaches to H-bond with carbonyls at C-term of Helix B of 2nd subunit

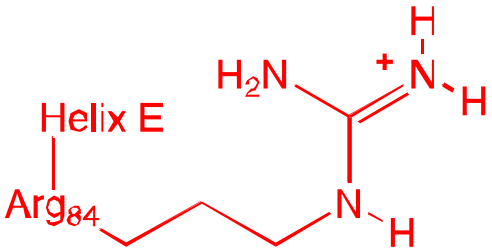
Tryptophan Binding Site

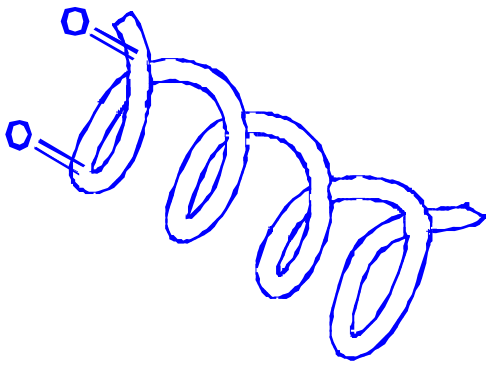


Tryptophan Binding Site



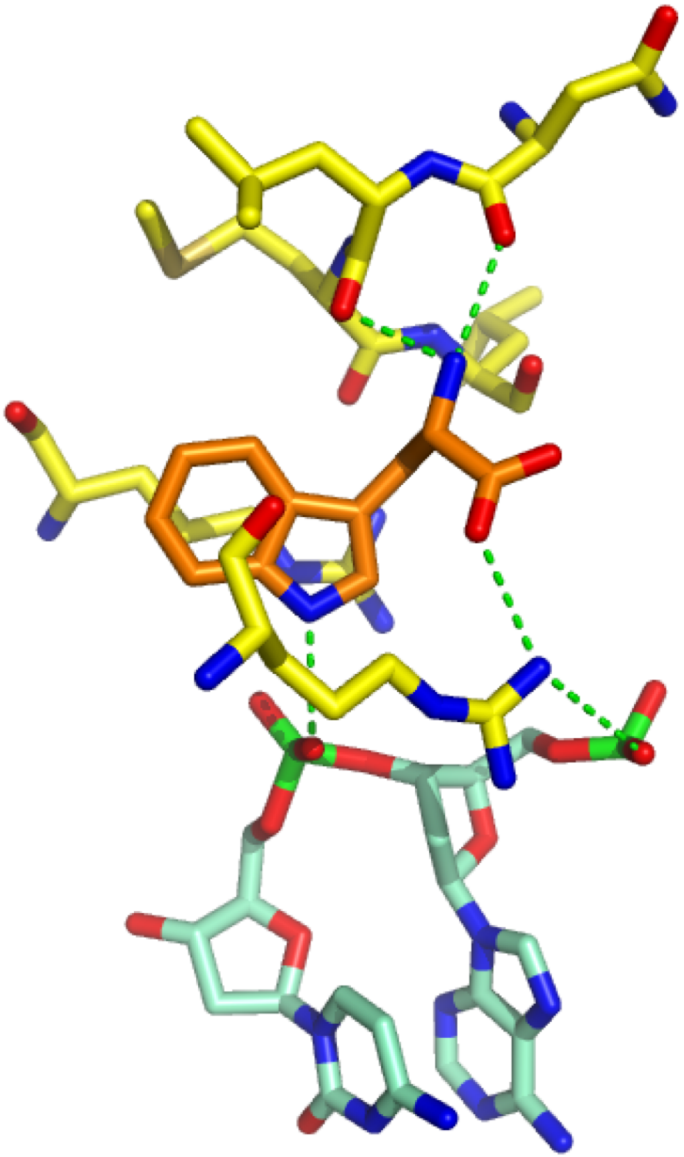
Tryptophan Binding Site



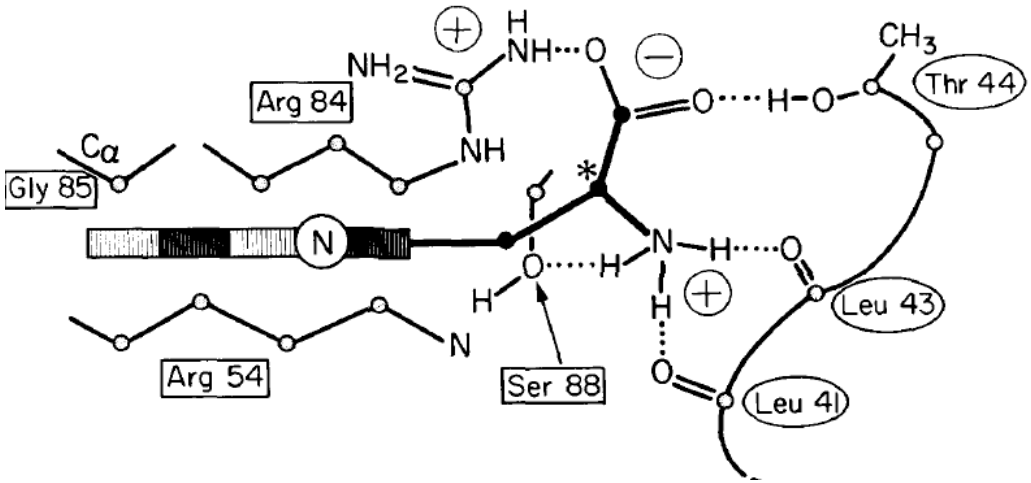


Helix B

Trp Interacts with DNA



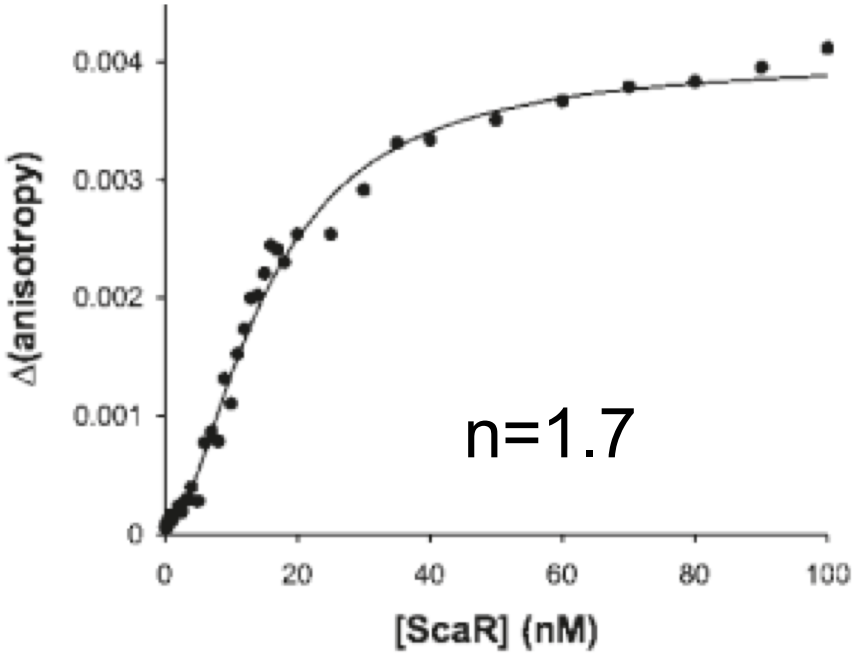
Interaction of indole NH with phosphate indicates why Phe and Tyr wouldn't be able to activate TrpR even if they bound, which they don't.



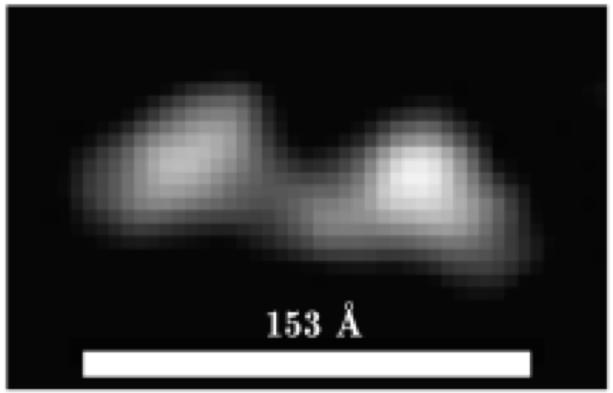
DNA's eye view

Cooperativity in DNA-Binding

Two adjacent binding sites lead to Protein-Protein Interactions



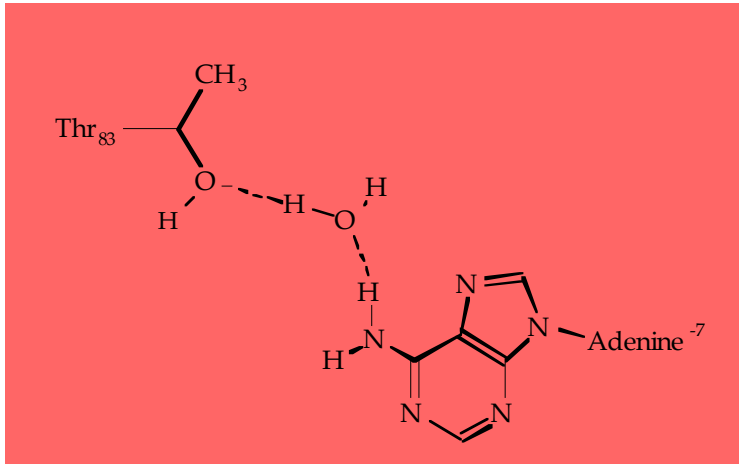
Biochemistry **2009,** *48*, 10308–10320

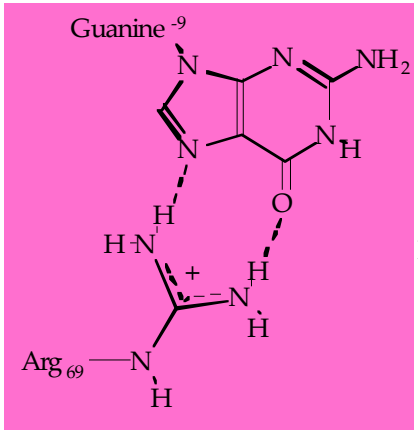


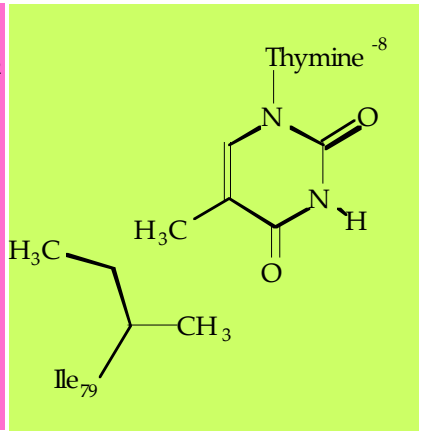
Weisman, Reed Thesis, 2016

TrpR-Operator Specificity

```
-9-8-7-6-5-4-3-2-1   1 2 3 4 5 6 7 8 9
 G T A C T A G T T   A A C T A G T A C
                 •
 C A T G A T C A A   T T G A T C A T G
```

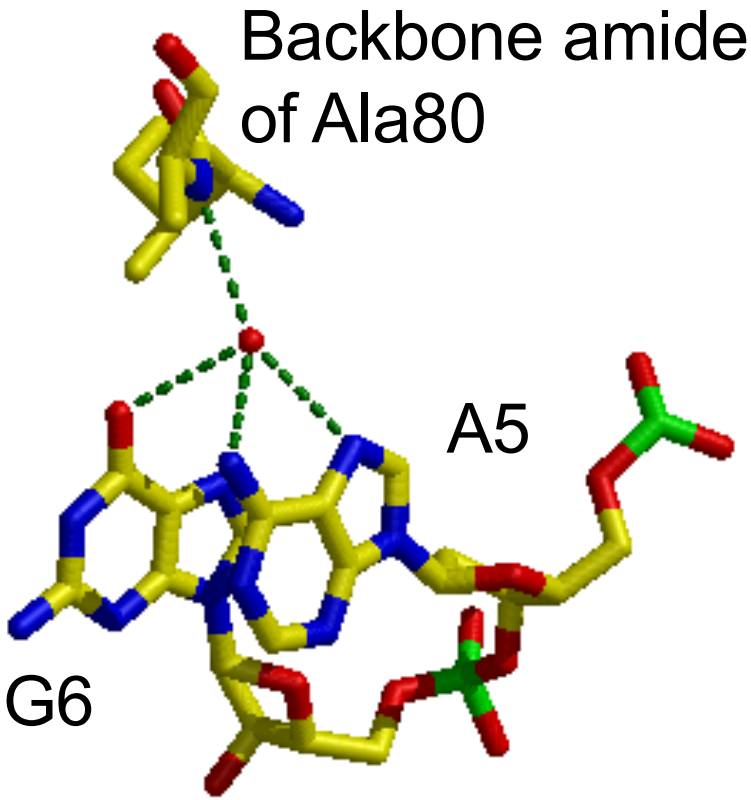




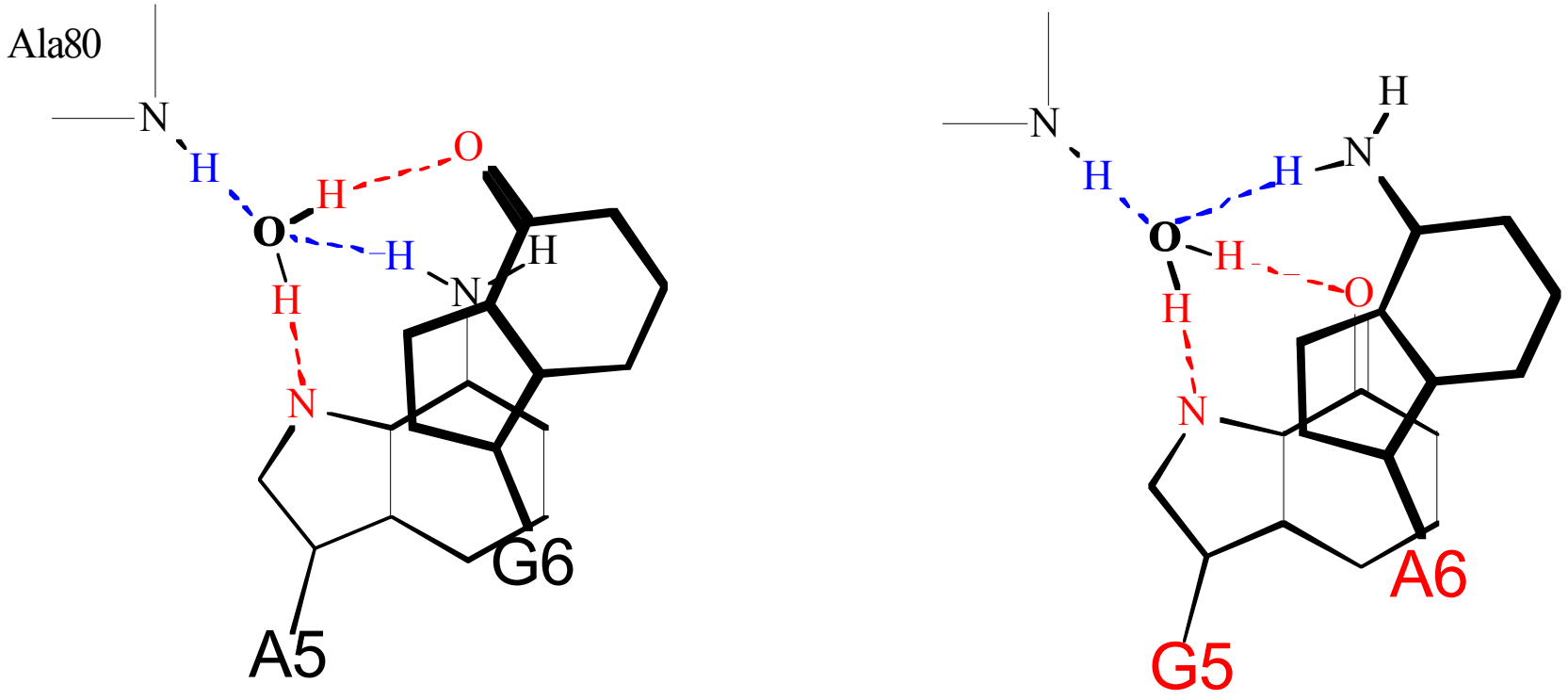


Water-Mediated Specificity

```
-9-8-7-6-5-4-3-2-1   1 2 3 4 5 6 7 8 9
 G T A C T A G T T   A A C T A G T A C
 C A T G A T C A A   T T G A T C A T G
```



Water-Mediated Specificity



Sigler (1994) Nature, 368, 469: “These water molecules can thus be regarded as non-covalent extensions of the DNA bases which may be used as stereospecific recognition elements of the DNA target sequence.”