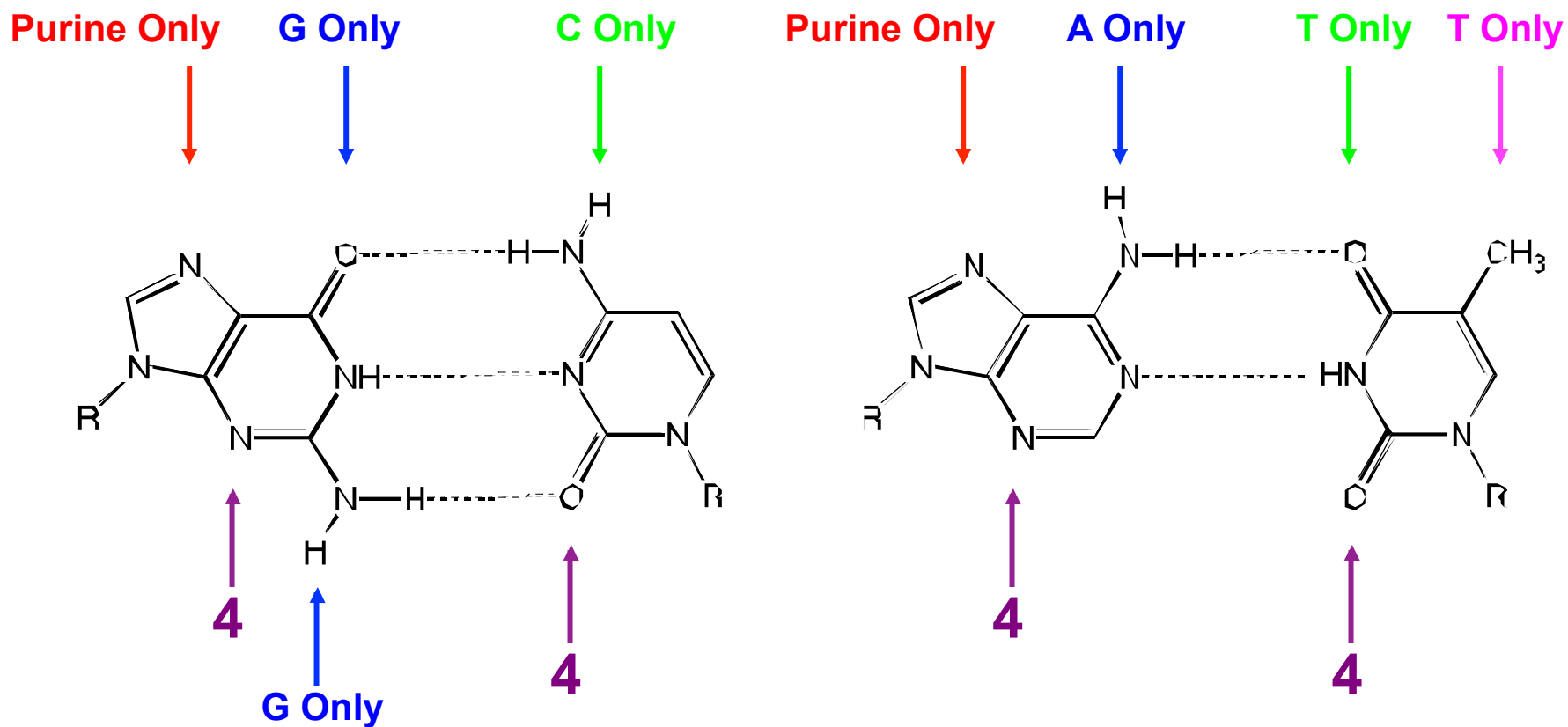
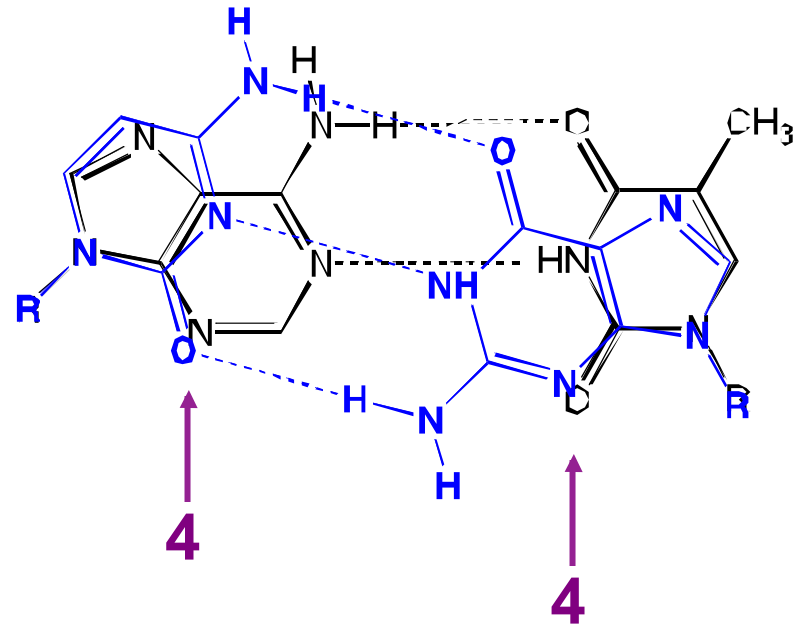
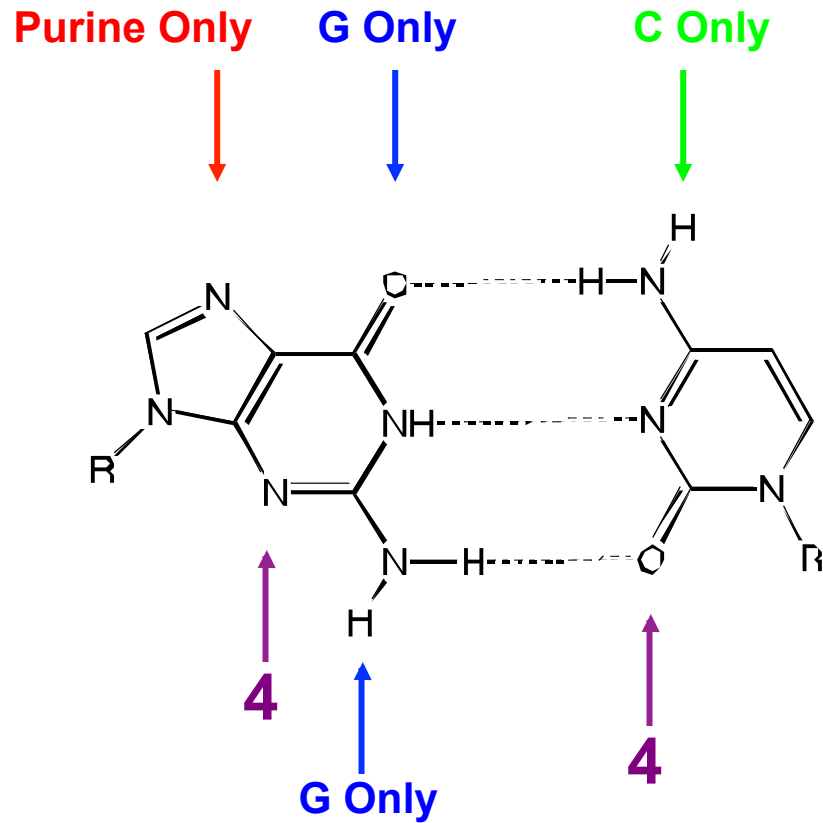


Opportunities for Direct Readout



Opportunities for Direct Readout



The Zinc Finger

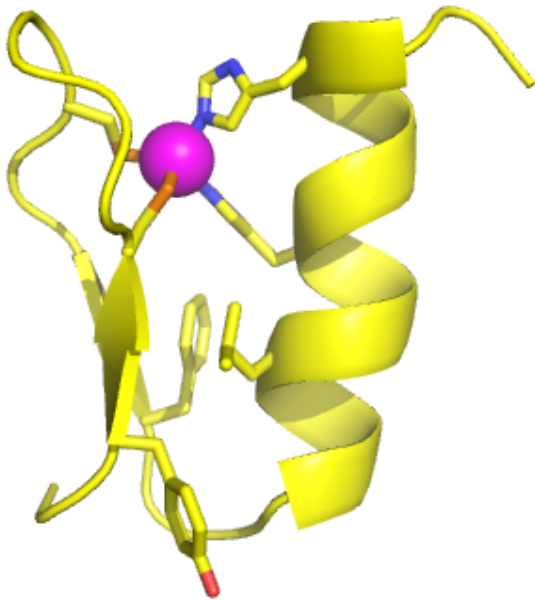
30 residue structural unit readily identified by sequence pattern: $\text{Cys}_{X_{2-5}}\text{Cys}_{X_{12-15}}\text{His}_{X_{3-5}}\text{His}$

RYICSF	ADCGAAYNKNWKLQAHLC	KHTG
PFPCKE	EGCEKGF T SLHHLTRHSL	THTG
NFTCDS	DGCDLRF T TKANM K KHFN	RFHNI
VYVCHF	ENCGKAF K KHNQLKVH Q F	SHTQ
PYECPH	EGCDKRFSLPSRLKRHEK	VHAG
-YPCKKDDSCSFVGKTWTLYLKHVA	ECHQD	
-AVCDV	CNRKFRHKDYLRDHQK	THEK
VYLCPR	DGCDRSYT T AFNLRSHIQ	SFHEE
PFVCEH	AGCGKCFAMKKSLE R HSV	VHDP

Single protein may contain many zinc finger **domains**

Metal Binding Necessary, but not Sufficient for Folding

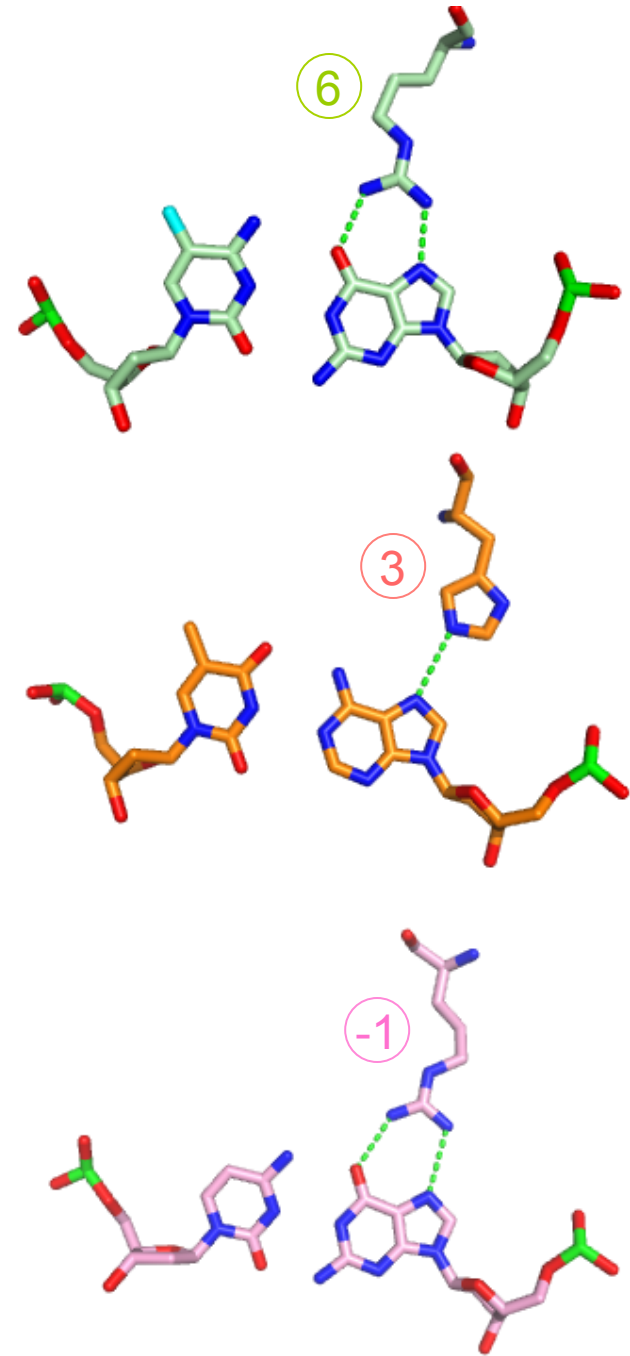
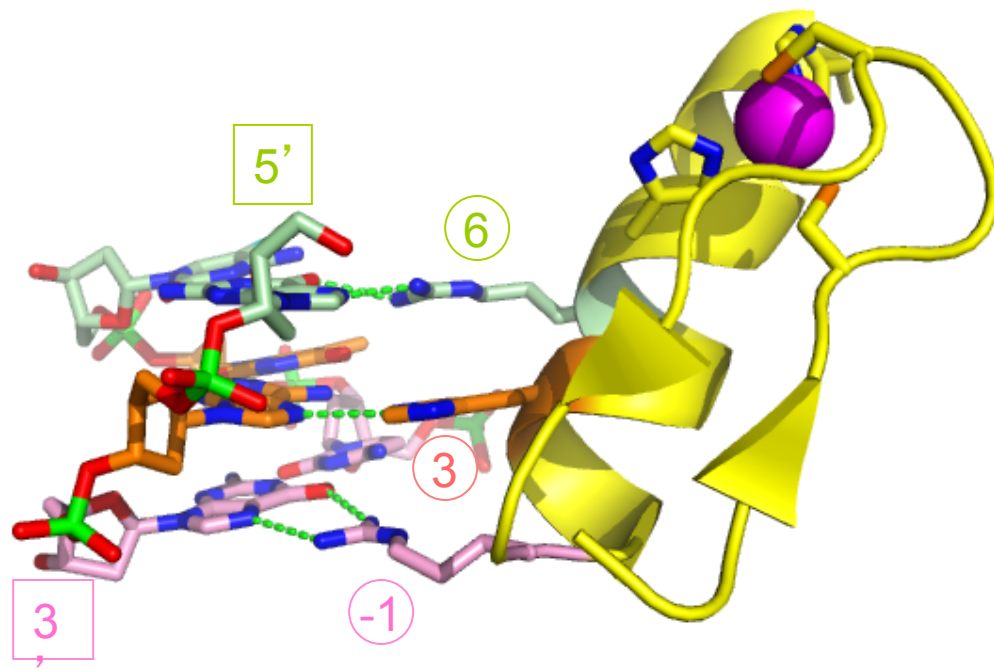
Cons. Peptide 1	P	Y	K	C	P	E	C	G	K	S	F	S	Q	K	S	D	L	V	K	H	Q	R	T	H	T	G
Min Zn Finger	K	Y	A	C	A	A	C	A	A	A	F	A	A	K	A	A	L	A	A	H	A	A	A	H	A	K
MZF-Coreless	K	A	A	C	A	A	C	A	A	A	A	A	A	K	A	A	A	A	A	H	A	A	A	H	A	K



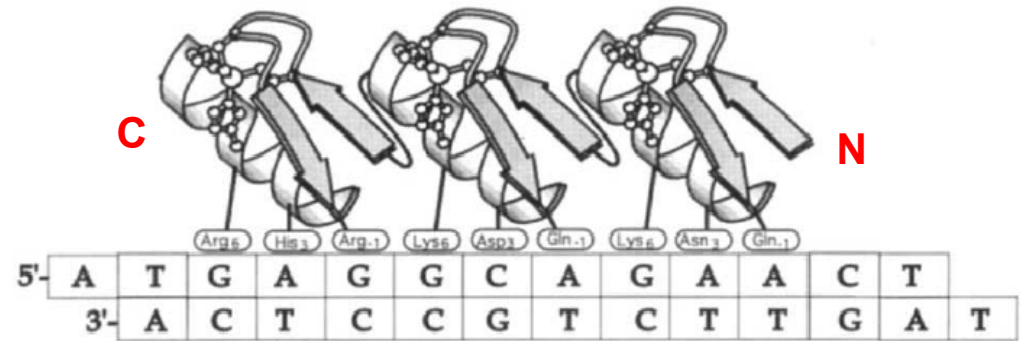
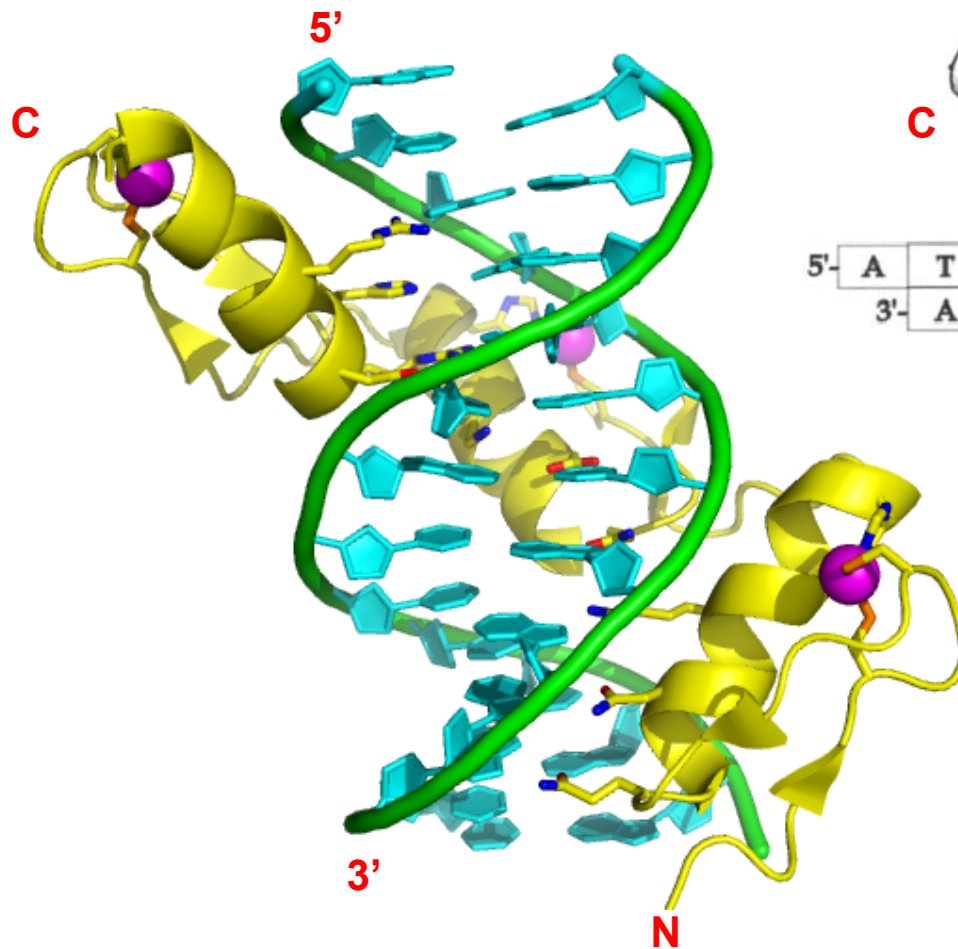
MZF retain native conformation.

Coreless fails to adopt stable fold.

NRHR^C - 5' GAG3'



Zinc Finger - DNA Complex

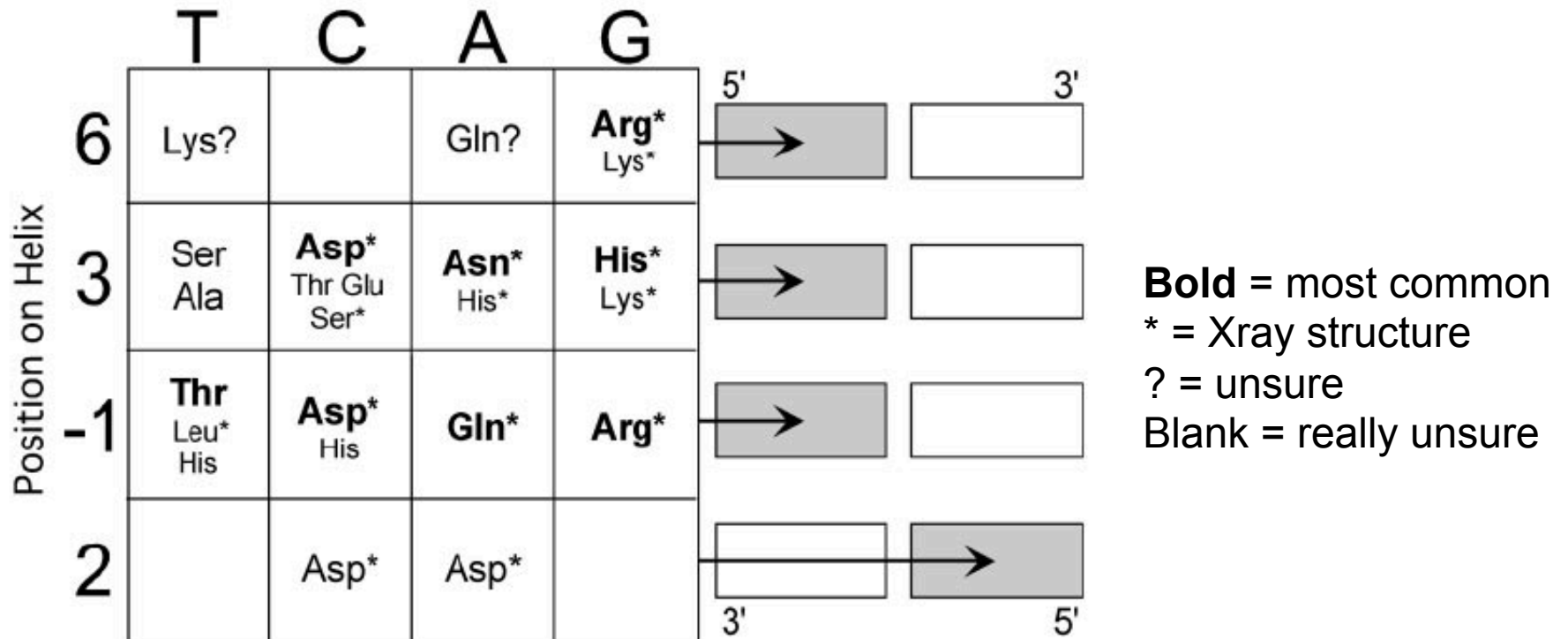


Resolution (Å)	2.74
Measured reflections	43976
Unique reflections	12537
Data coverage (last shell)	92
R_{merge}^1 (%)	5.90

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

Recipe for Recognition?



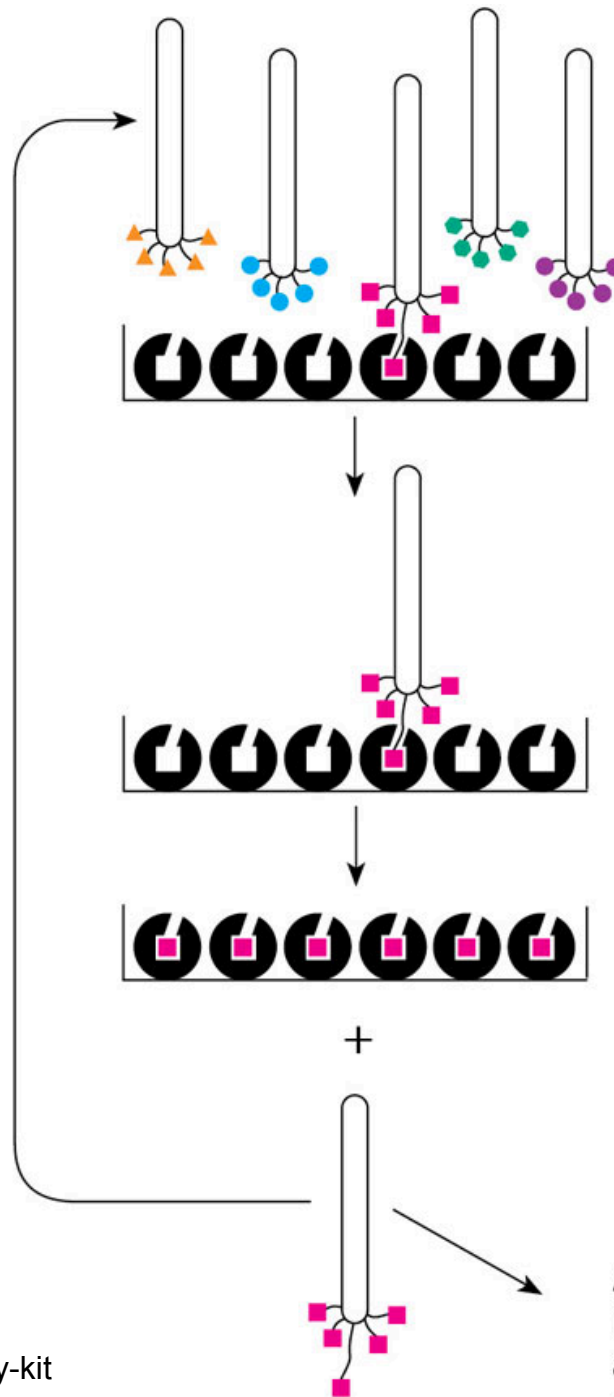
Pabo (1999) Ann Rev Biochem Biophys

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	<u>GGG</u>	GCG	<u>GGG</u>	nd
QDR	RER	<u>RHR</u>	GCT	GCG	<u>GGG</u>	.002*
QDR	RER	<u>RHR</u>	<u>GGG</u>	GCG	<u>GGG</u>	.015
QDR	RER	<u>RHR</u>	GCG	GCG	<u>GCG</u>	1.0
RER	QDR	<u>RHR</u>	GCG	GCT	<u>GGG</u>	0.010*
RER	QDR	<u>RHR</u>	GCT	GCG	<u>GGG</u>	0.066
QDR	RER	<u>RHR</u>	GCG	GCT	<u>GGG</u>	0.55
QDR	RER	<u>RHR</u>	GCT	GCG	<u>GGG</u>	0.003*

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

Phage Display Courtesy of New England Biolabs



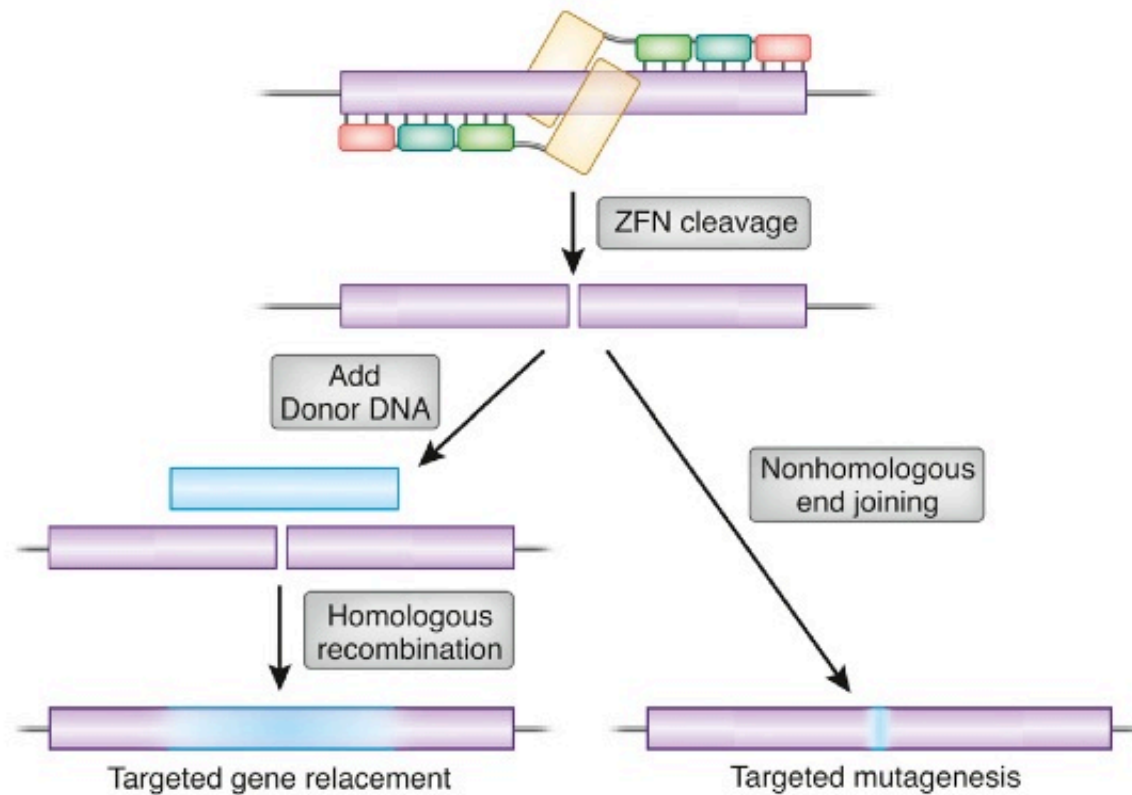
A library of phage, each displaying a different peptide sequence, is exposed to a plate coated with the target.

Unbound phage are washed away.

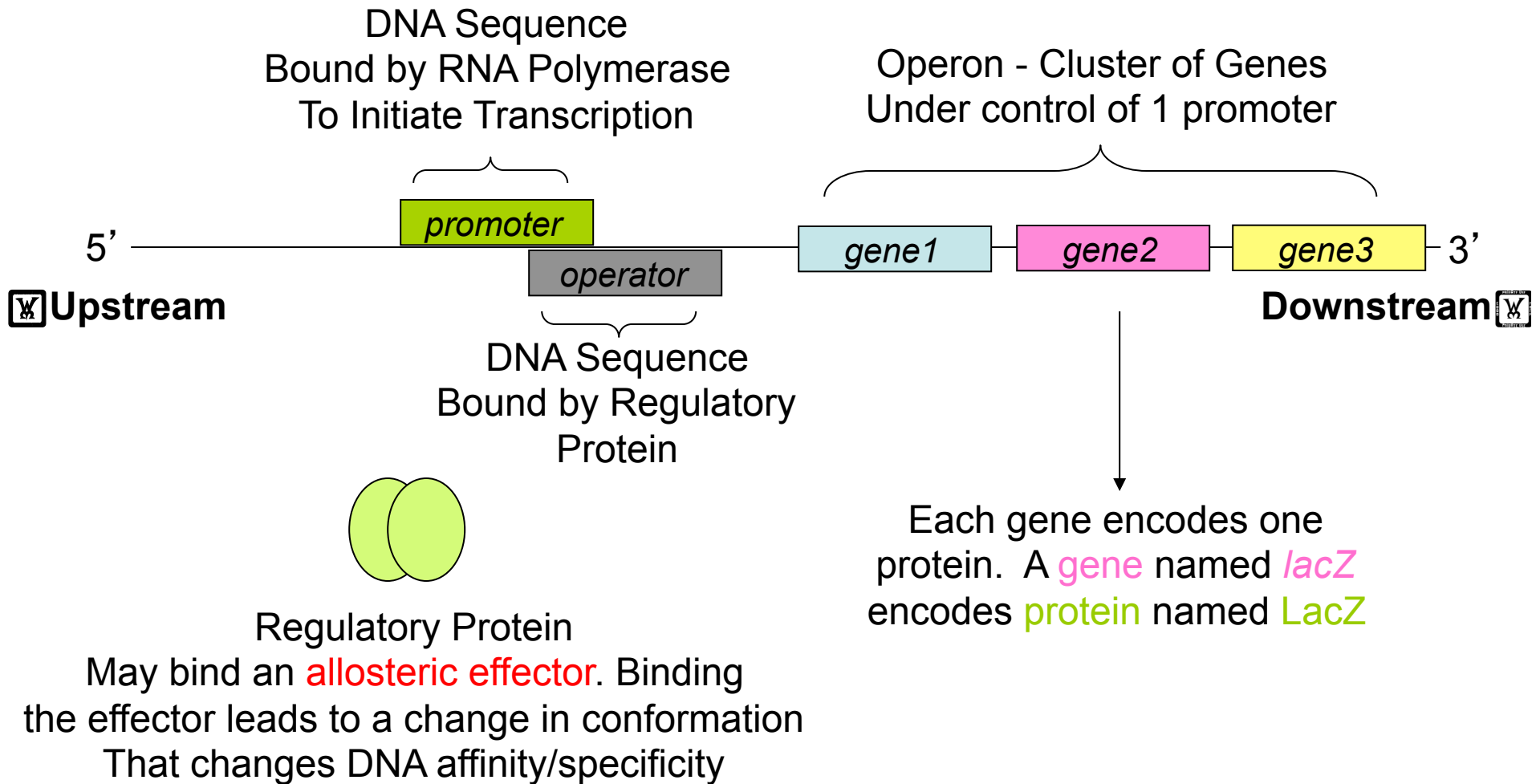
Specifically-bound phage are eluted with an excess of a known ligand for the target, or by lowering pH.

After 3 rounds, individual clones are isolated and sequenced.

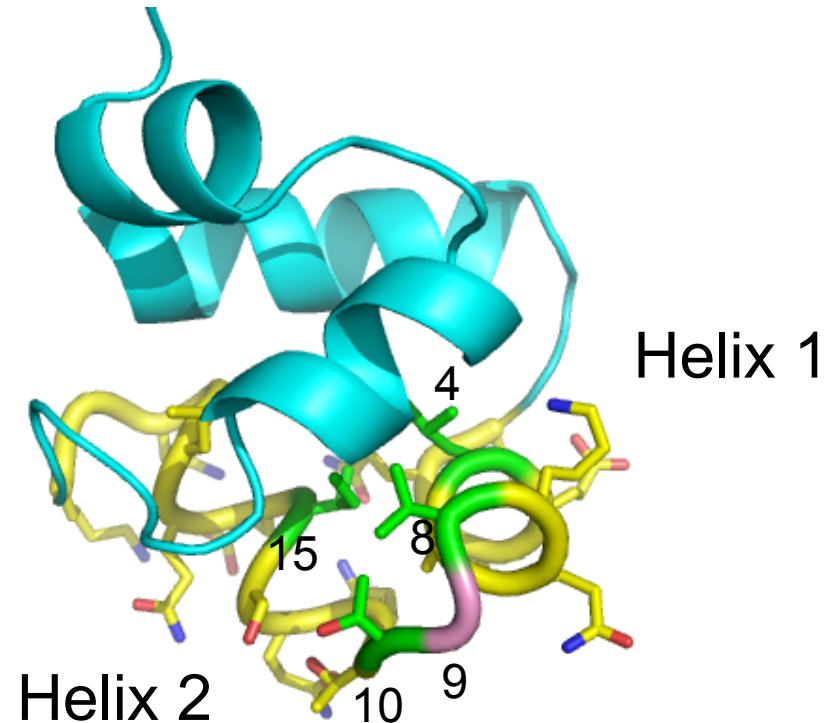
Zinc Finger Nucleases



An Illustrated Glossary of Terms



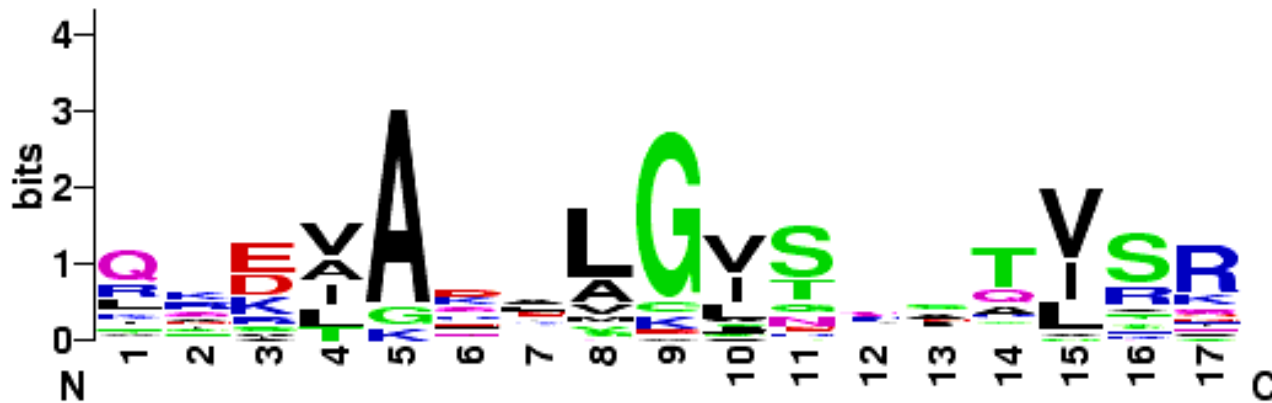
The HTH Motif



434 QAE**LAQK****VGT**TQQS**IEQ**LENGKKR
4 89 15

The HTH-motif is traditionally defined as a 20-amino-acid segment of two almost perpendicular helices connected by a four residue β turn.

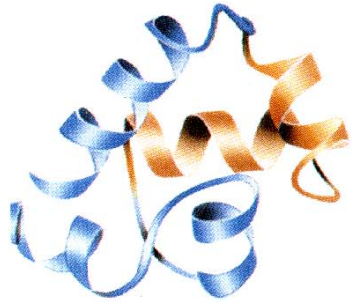
The HTH Motif



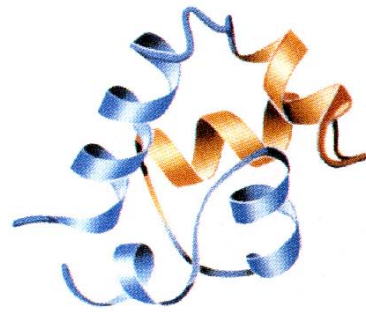
s70 LEE**V**GAY**F****G**VTRER**I**RQIENKALR
 Trp QRE**L**KNE**L****G**AGIAT**I**TRGSNSLKA
 Lac LYD**V**AEY**A****G**VSYQT**V**SRVVNQASH
 CAP RQE**I**GE**I****V****G**CSRET**V**GRILKMLED
 434 QAE**L**AQ**K****V****G**TTQQS**I**EQLENGKKR

4 89 15

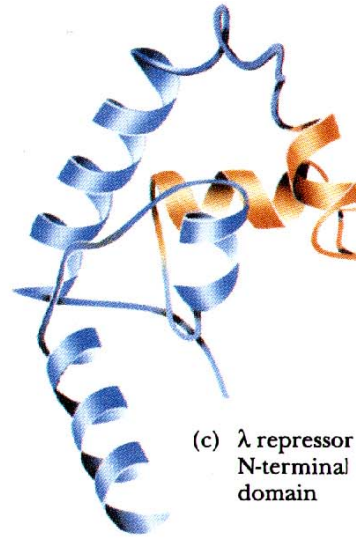
The HTH-motif is traditionally defined as a 20-amino-acid segment of two almost perpendicular α helices connected by a four residue β turn.



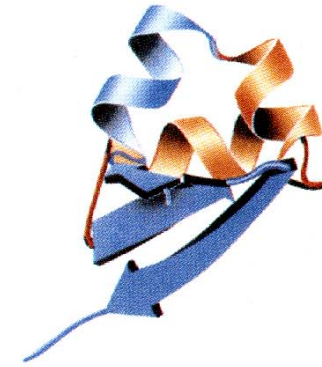
(a) 434 repressor
N-terminal
domain



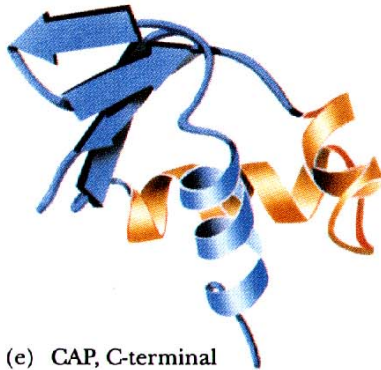
(b) 434 Cro



(c) λ repressor
N-terminal
domain



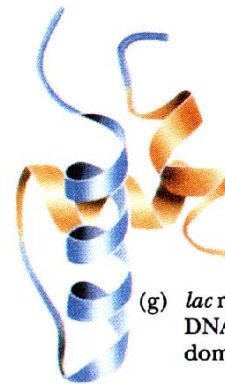
(d) λ Cro



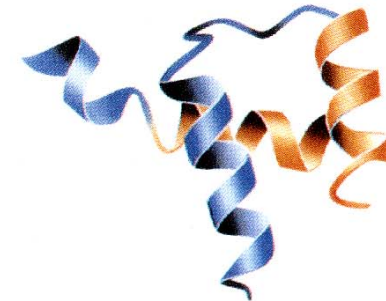
(e) CAP, C-terminal
domain



(f) *trp* repressor
subunit

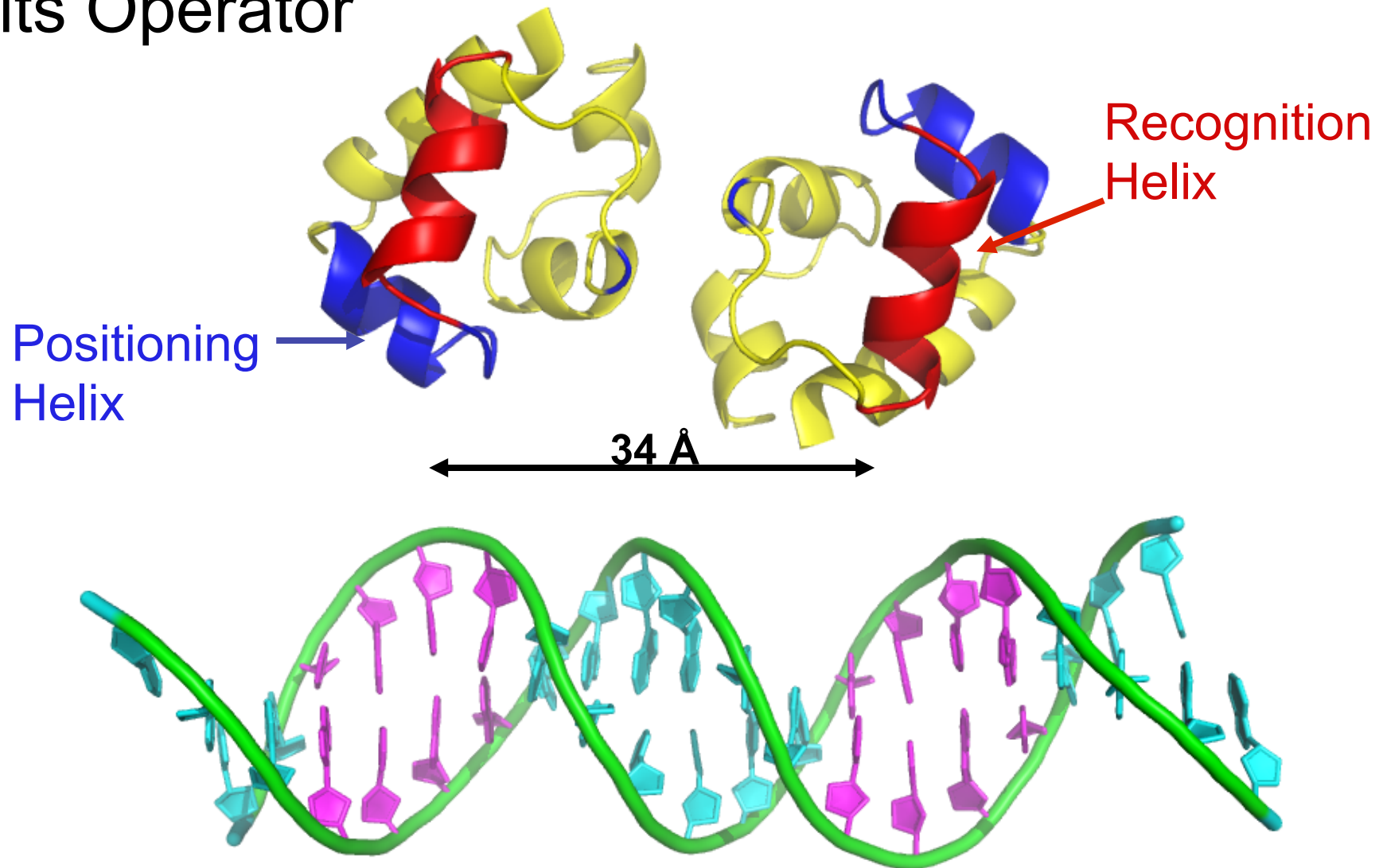


(g) *lac* repressor
DNA-binding
domain

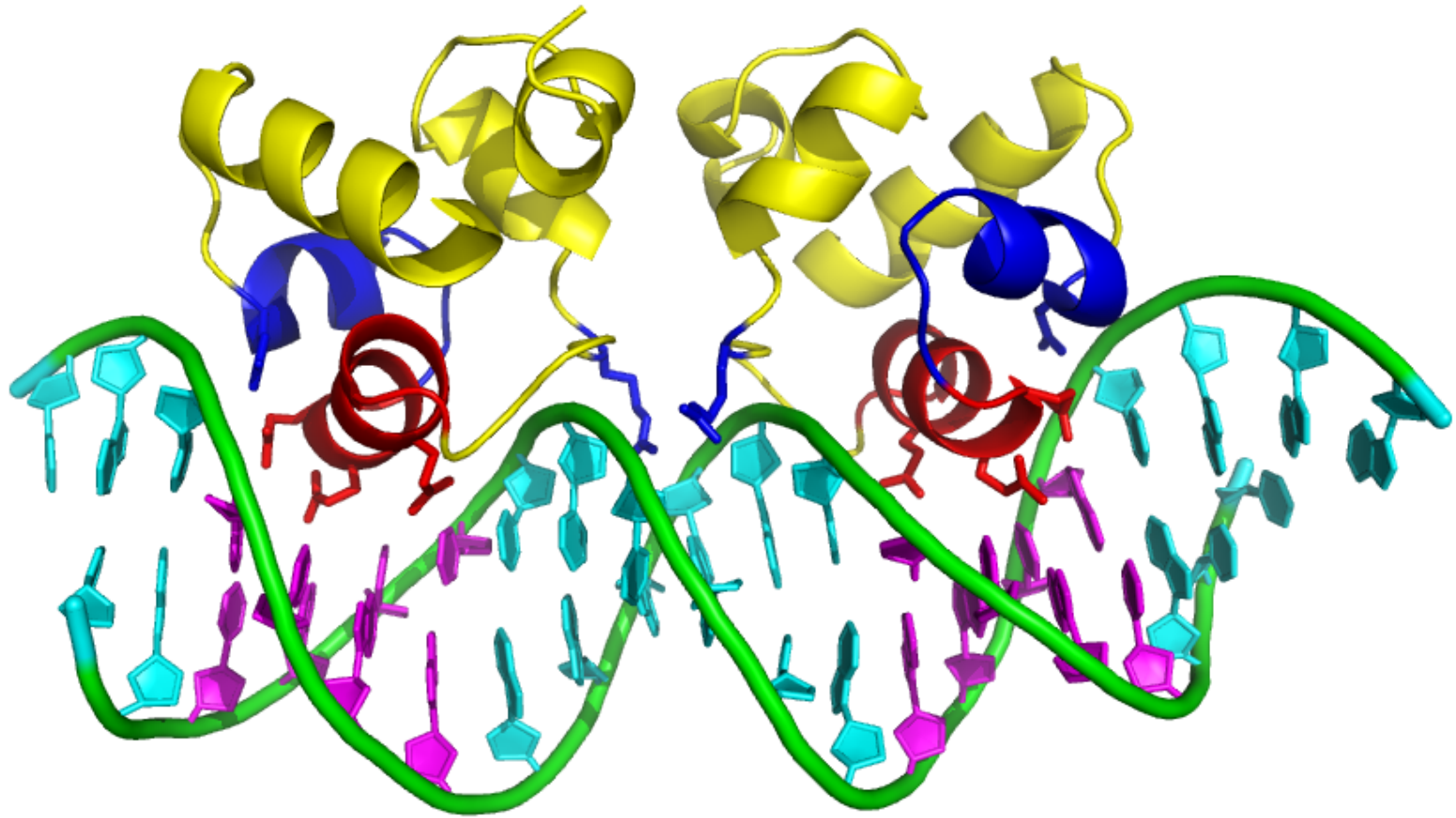


(h) *Antp* homeodomain

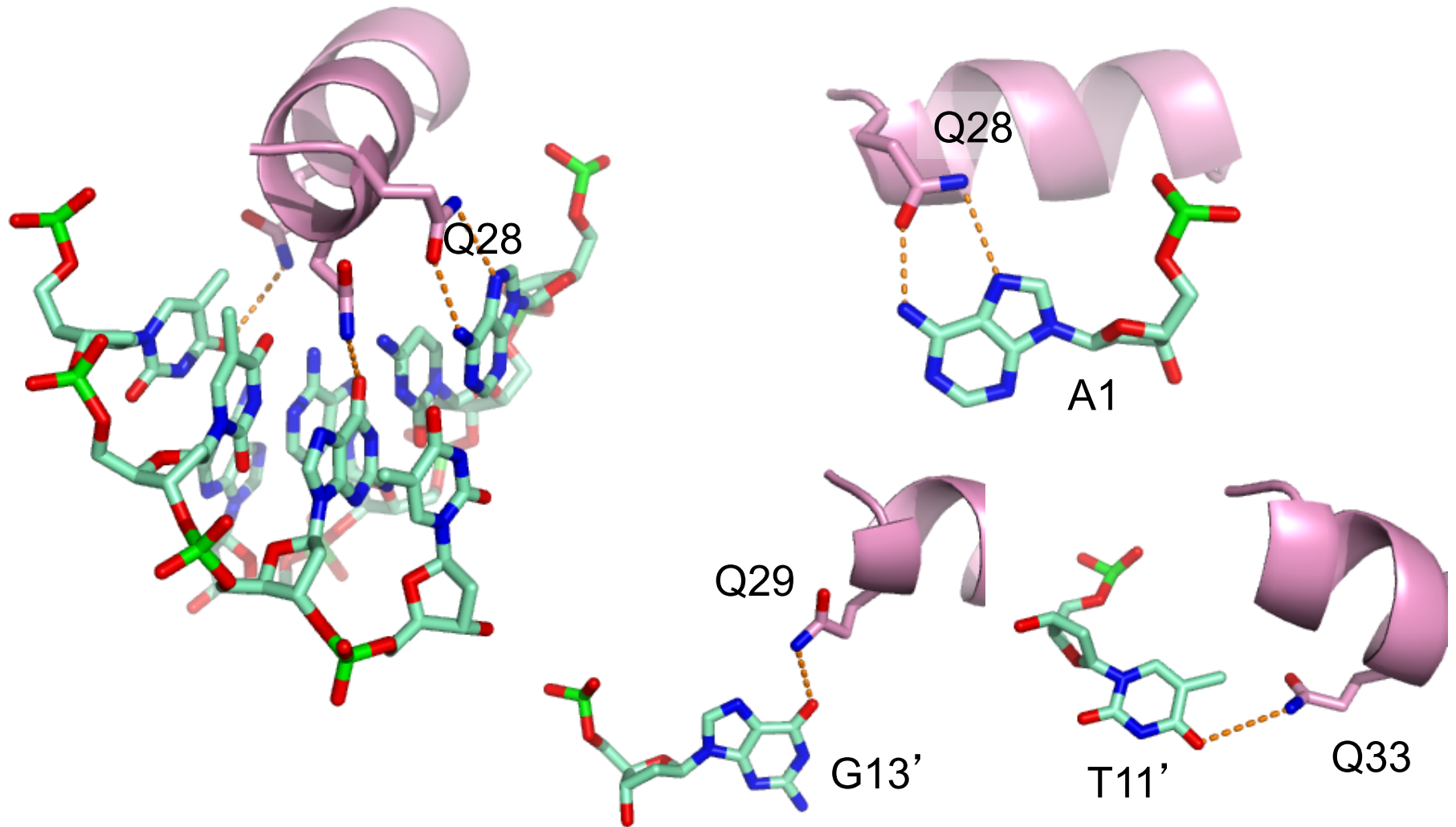
434 repressor and its Operator



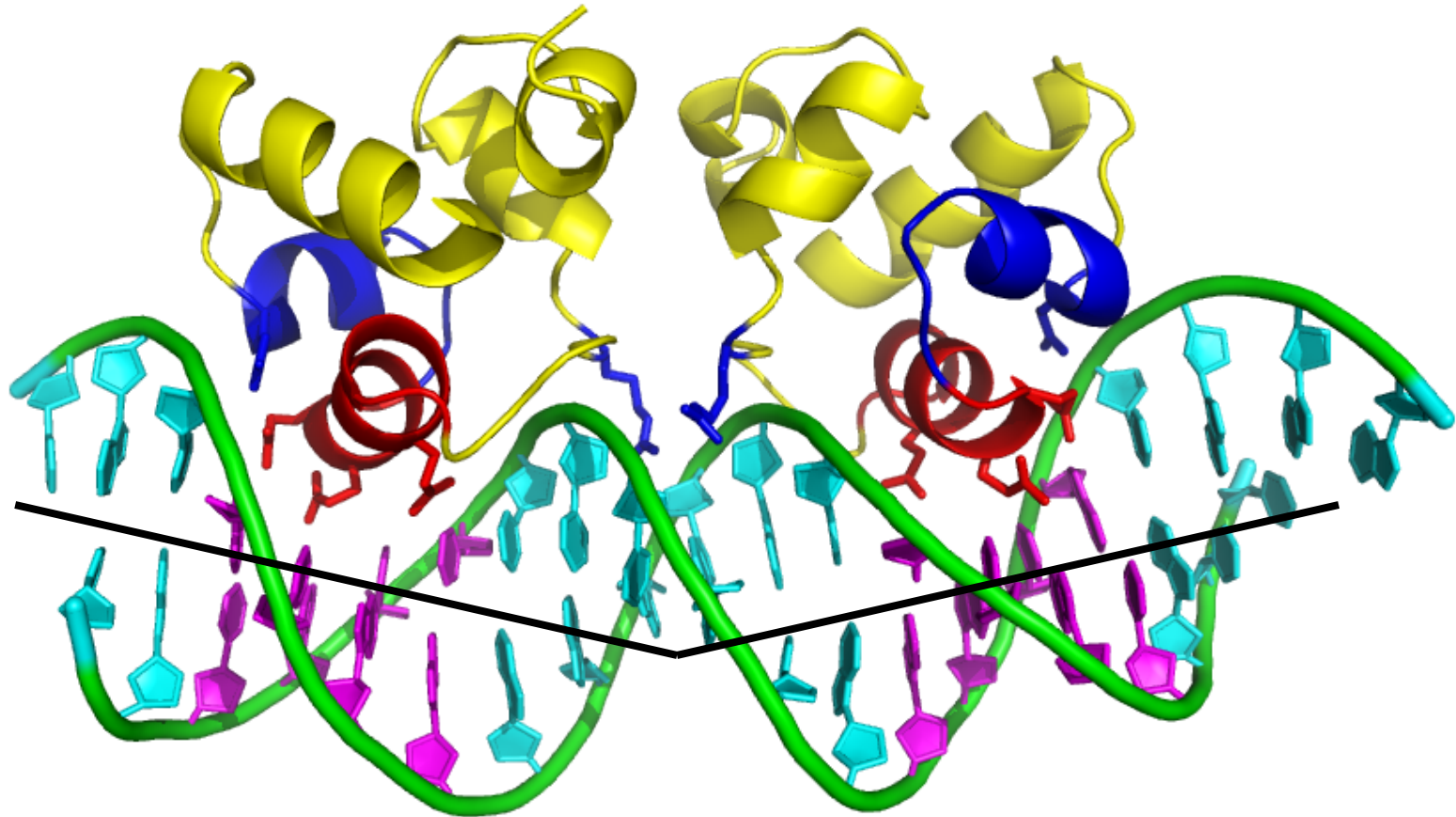
The 434 Repressor-Operator Complex



Direct Readout of Major Groove

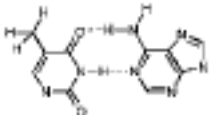
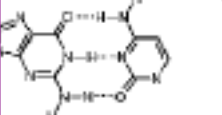
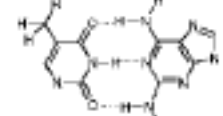
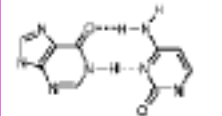
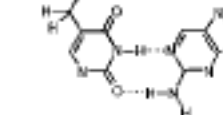
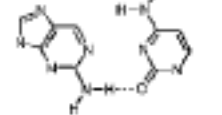


Indirect Readout of Minor Groove?



24° bend arises
from minor groove
Compression

Indirect Readout of DNA Conformation

Position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	
5'	A	C	A	A	T	A	X•Y	T	A	T	T	G	T	3'
3'	T	G	T	T	A	T	Y•X	A	T	A	A	C	A	5'
Position 8 Substituted							Position 7 Substituted							
K_D^{150} (nM)							K_D^{150} (nM)							
 T₇•A₈							 G₇•C₈							
18.0							5116.0							
 T₇•DAP₈							 I₇•C₈							
380.0							0.54							
 T₇•AP₈							 AP₇•C₈							
764.0							1335.0							

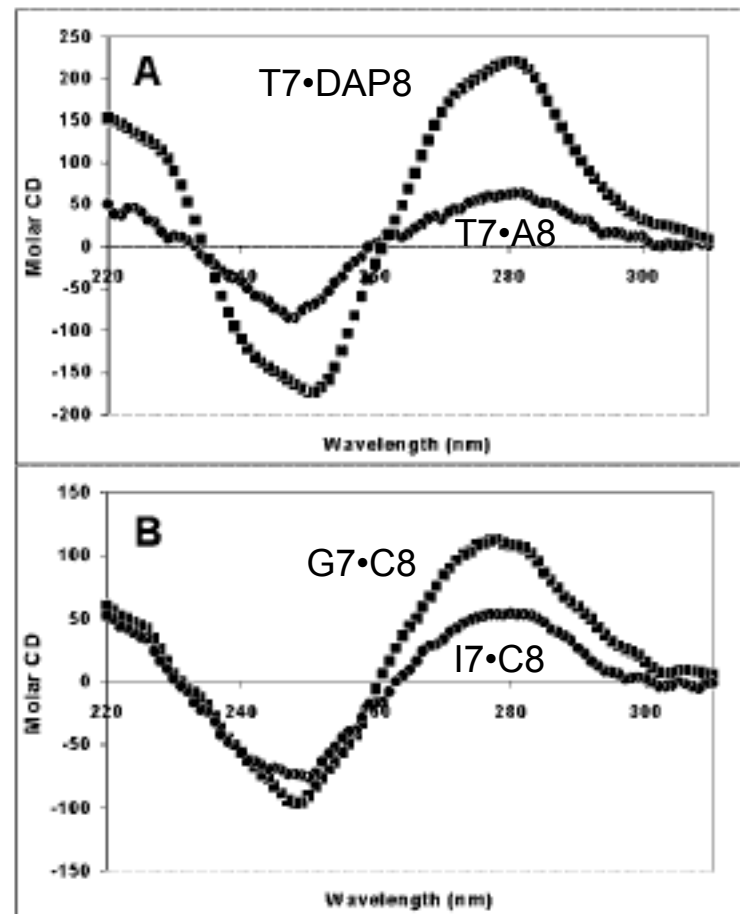
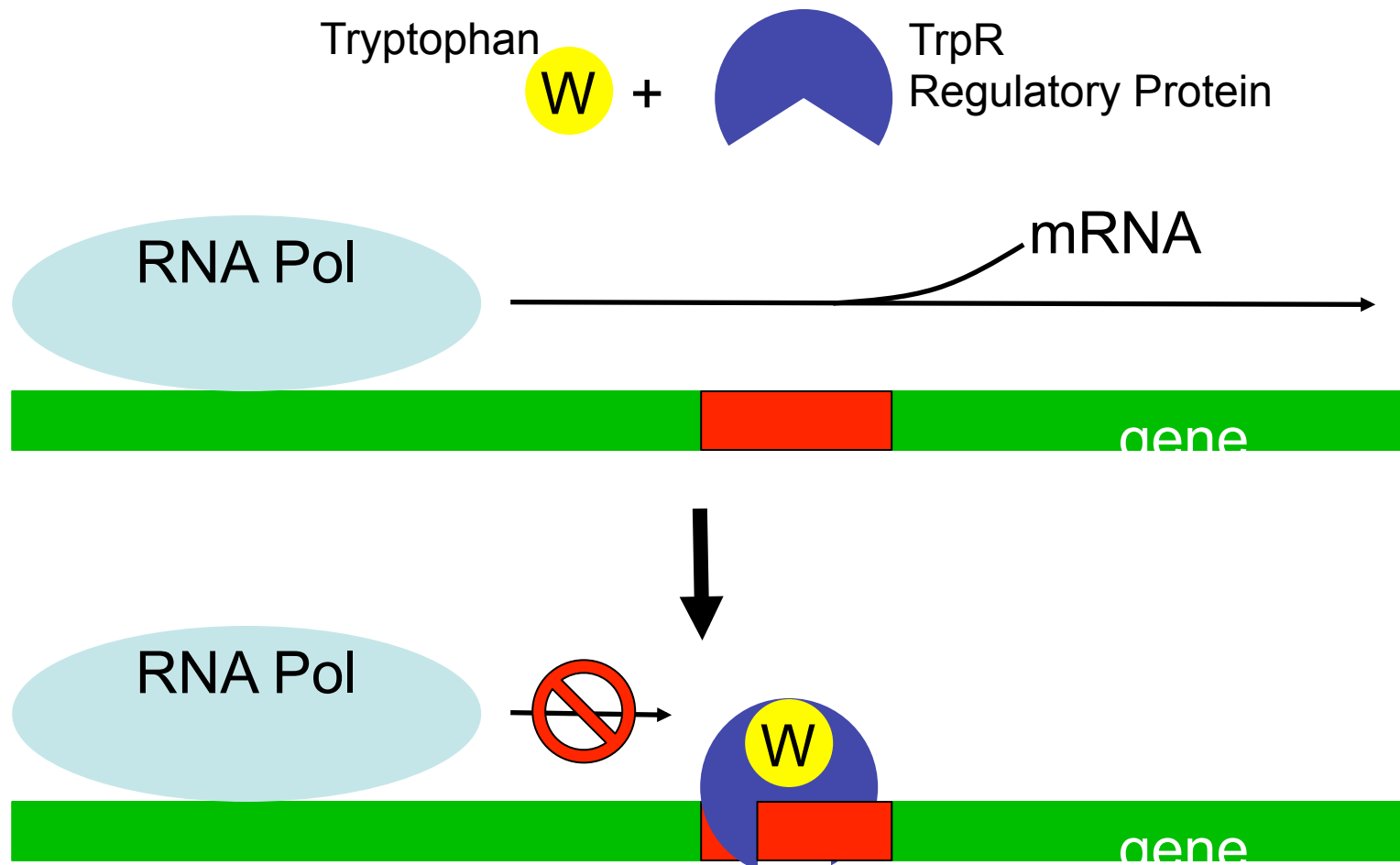
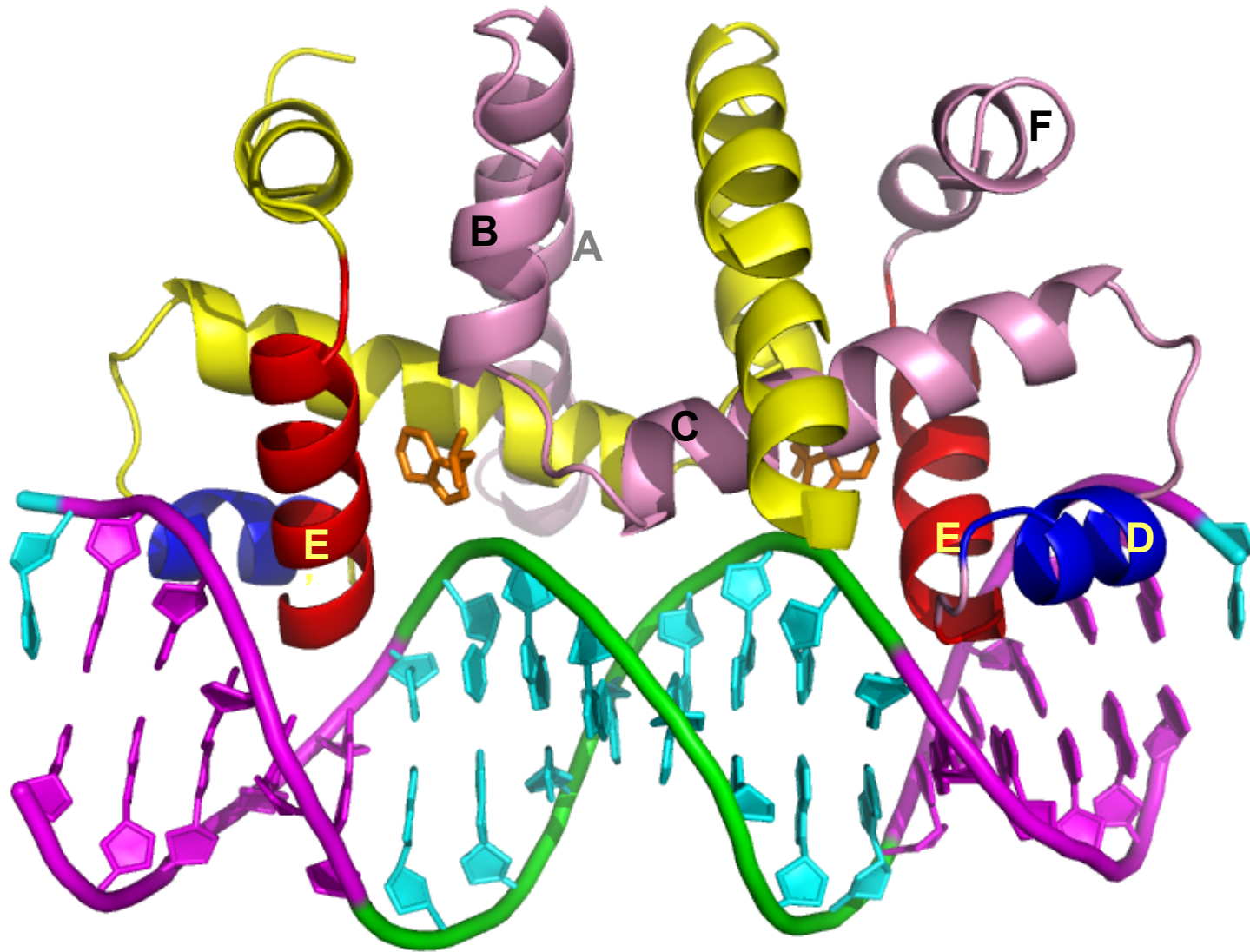


FIG. 4. Circular dichroism spectra of 2 mM of DNAs bearing modified bases at their central positions. Spectra were acquired at 50 mM KCl, 25 °C (see also "Experimental Procedures"). The spectra of T7•A8 (●) and T7•DAP8 (■) are shown in A. B displays the spectra of I7•C8 (●) and G7•C8 (■).

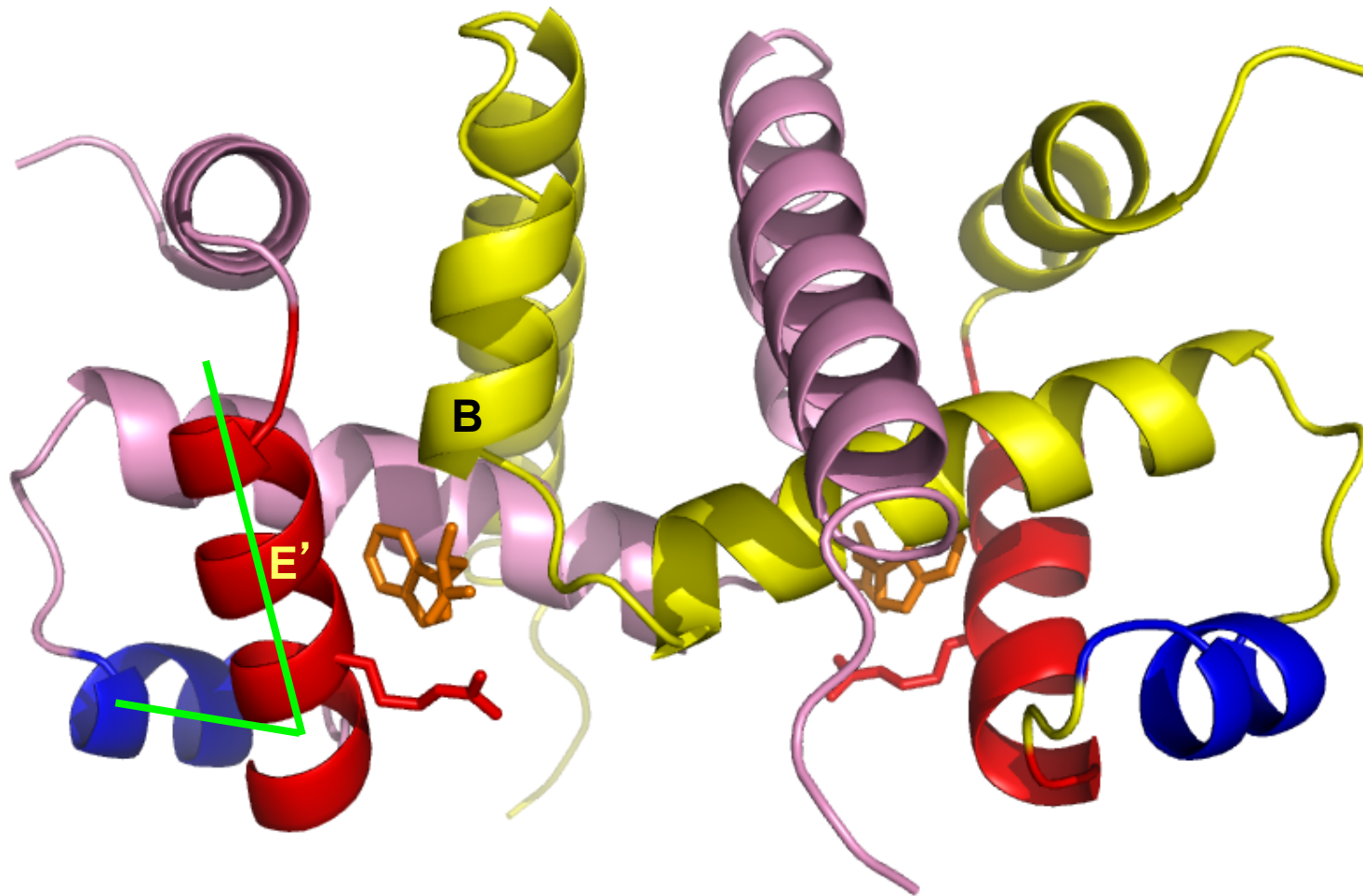
TrpR Binds Tryptophan and Blocks mRNA Synthesis by Binding DNA



Trp Repressor Bound to Operator

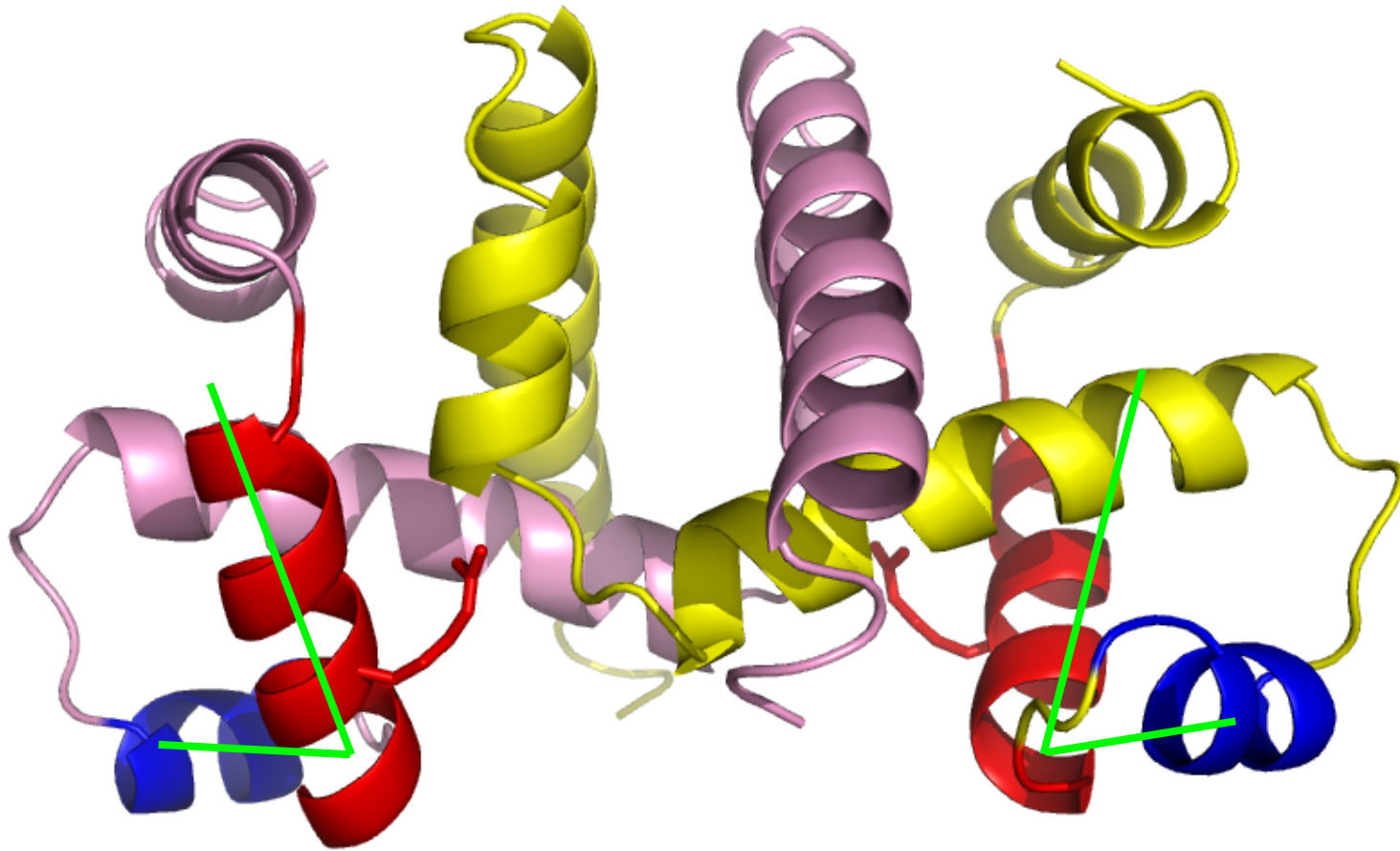


Tryptophan is an Allosteric Effector



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

