

BioChem 330 - Course Outline

September 29, 2011

- **Bio-molecular Structure/Function (I cont'd)**

- NUCLEIC ACID

- DNA sequence and structure
 - Protein/nucleic acid interactions

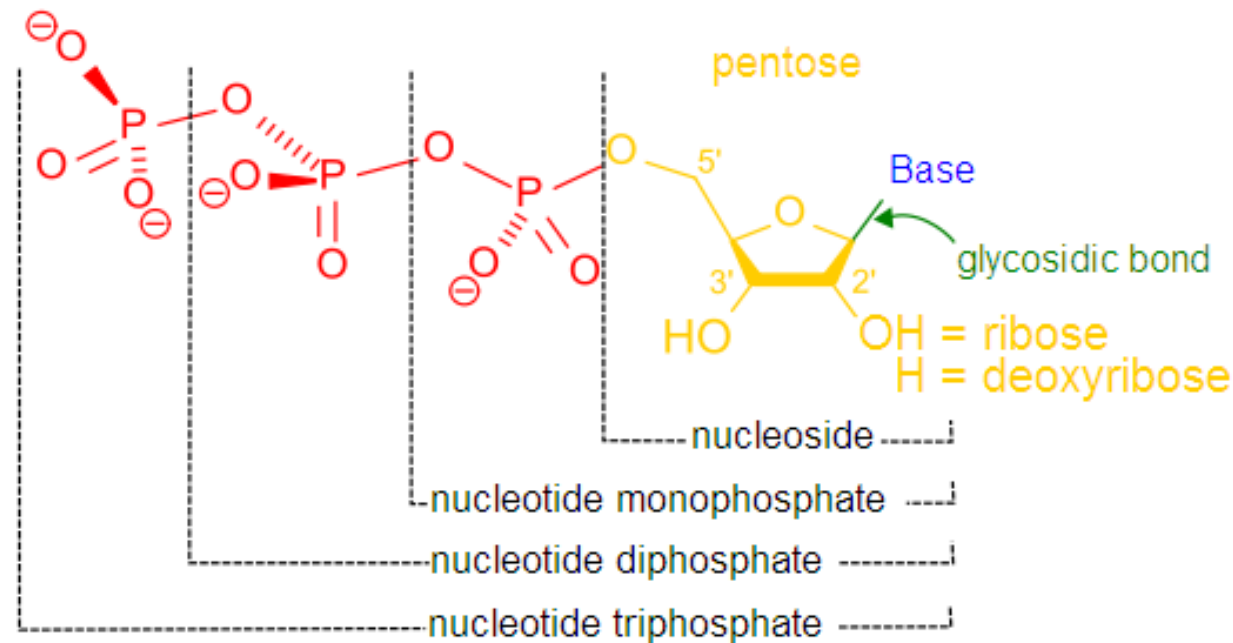
- CARBOHYDRATES

- Sugars - mono and disaccharides
 - Polysaccharides
 - Glycerides and glycerol

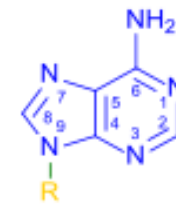
- FATS AND LIPIDS

- Chemistry and nomenclature for fatty acids
 - Saturated and unsaturated fatty acids
 - Fluid mosaic model of membrane structure

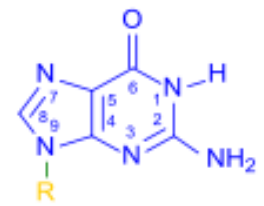
Fundamental Pieces of a Nucleotide



Purines

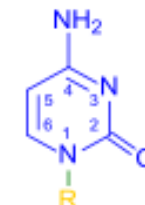


Adenine

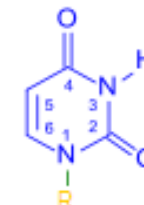


Guanine

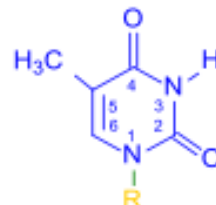
Pyrimidines



Cytosine

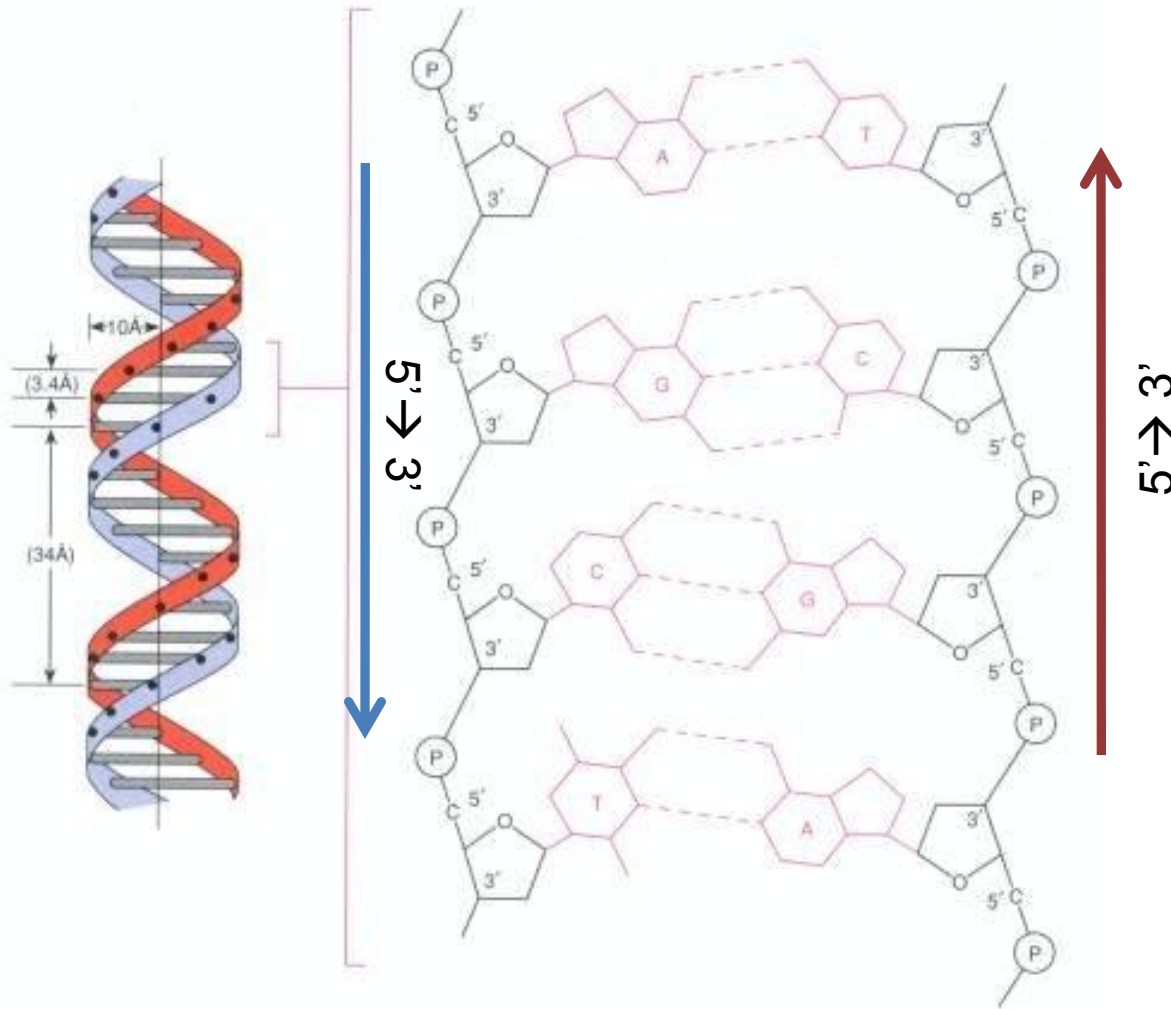


Uracil

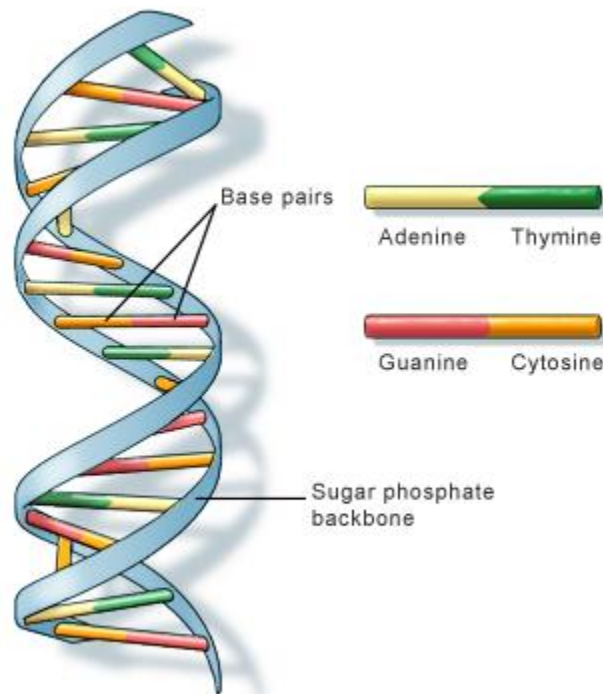


Thymine

How do you put together a DNA duplex?



Double stranded DNA helix (ds DNA)

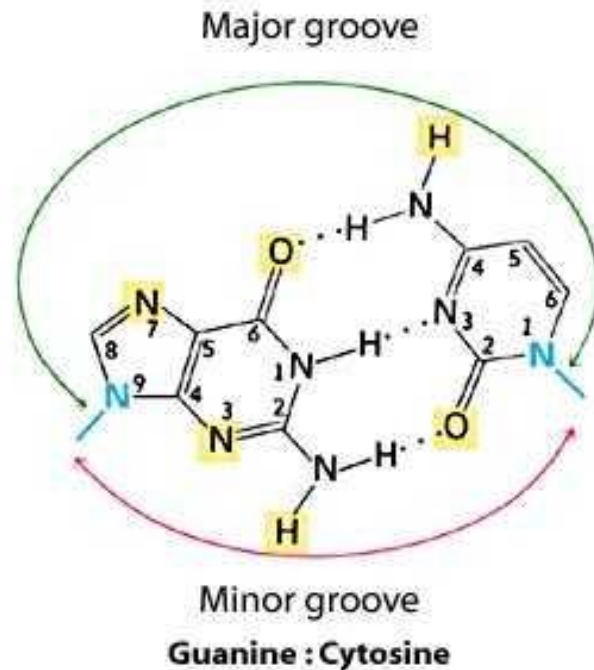
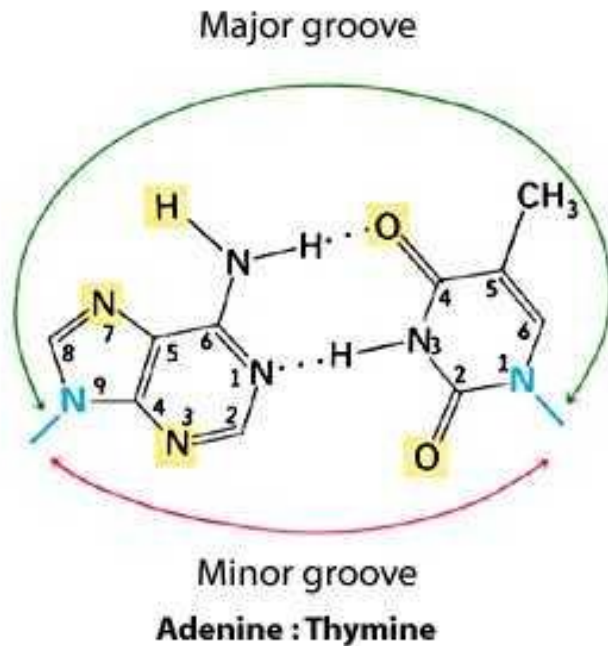


U.S. National Library of Medicine

- The strands are made up of ribose sugars that run in anti parallel fashion
- The nucleic bases point towards the center
 - A on one strand H-bonds with T on the other
 - G on one strand H-bonds with C on the other
- The phosphate groups are on the outside

What holds DNA strands together?

Nucleic acid bases are internally hydrogen bonded **G:C** (forming 3 H bonds) and **A:T** (forming two H bonds) in classic **Watson Crick** base pairs.



Major Structural Determinants in DNA

(as seen by a protein)

- B form DNA is a double stranded, antiparallel, right handed
 - Anionic polymer with a width of 20 Å , length??
 - Rise/base pair of 3.4 Å
 - 10 base pairs/turn
 - 34 Å/turn
 - 36°/step
 - Polyanionic phosphate backbone is on the outside of the helix and phosphate groups are associated with either positively charged groups on proteins (i.e. histones) or metal ions (i.e. Mg(II)) in vivo.

Major Structural Determinants in DNA

(as seen by a protein)

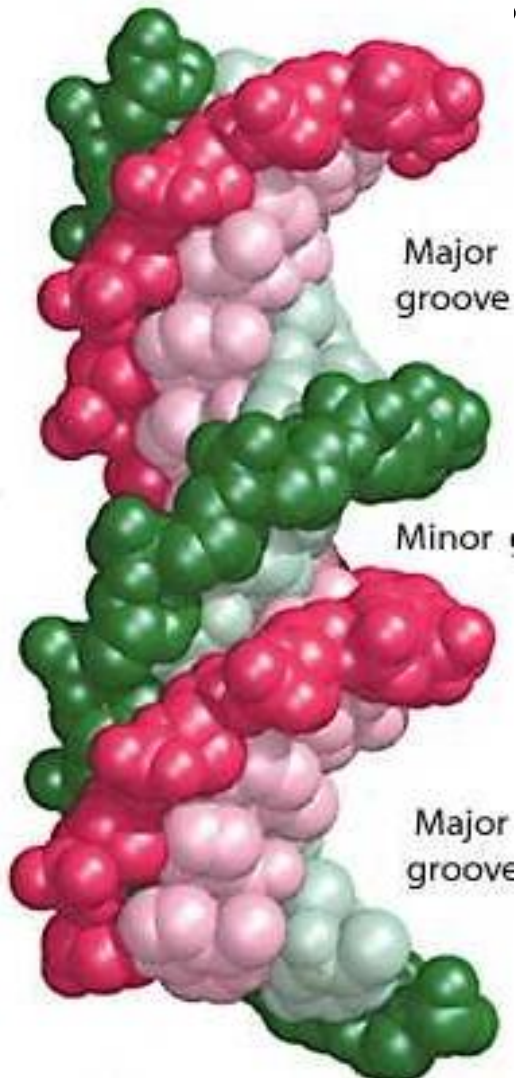
- GC and AT base pairs are normally perpendicular to helical axis but the DNA is structurally dynamic and can be distorted by:
 - **rolling** (in plane rotation along long axis of base pair plane)
 - **tilting** (in plane rotation along short axis of base pair plane)
 - **twisting** (rotation about axis perpendicular to base pair plane)
 - **propellar twisting** (rotation of planes of base pairs relative to one another)

What Does a Protein “see” in DS DNA?

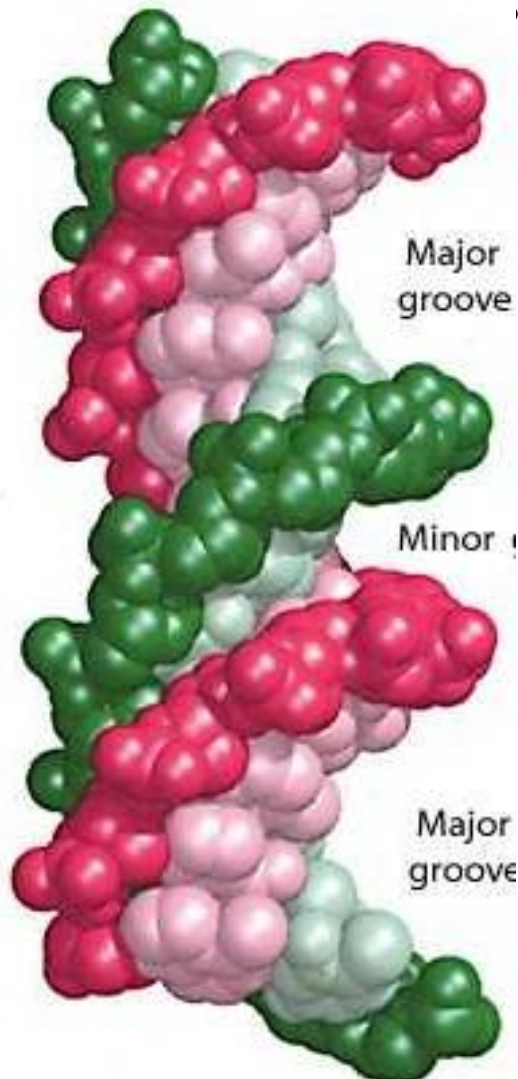
“Reading” the major and minor groove

– Grooves are lined by sequence specific H-bonding groups

- **Major Groove** 8.5 \AA deep & 11.5 \AA wide
- **Minor Groove** 7.5 \AA deep & 4.5 \AA narrow



What Does a Protein “See” in DS DNA?



“Reading” the major and minor groove

- Both Grooves Are Lined by Sequence Specific H-bonding Groups

- B DNA major groove

- GC

- CG

- AT

- TA

- B DNA minor groove

- GC

- CG

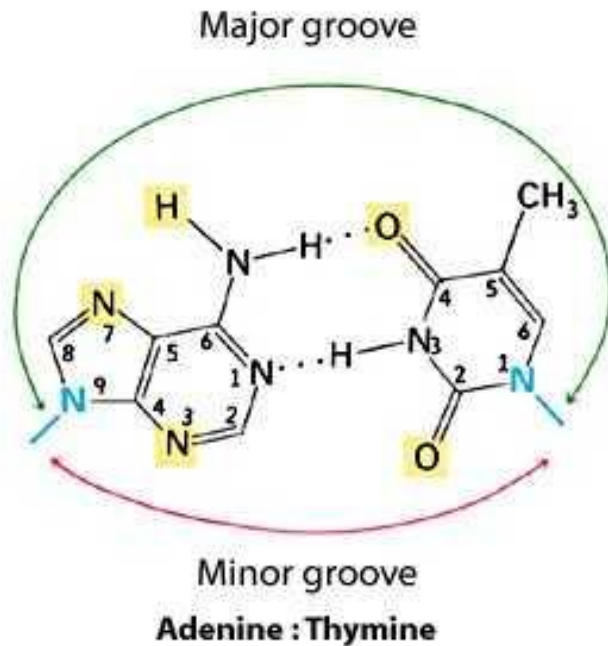
- AT

- TA

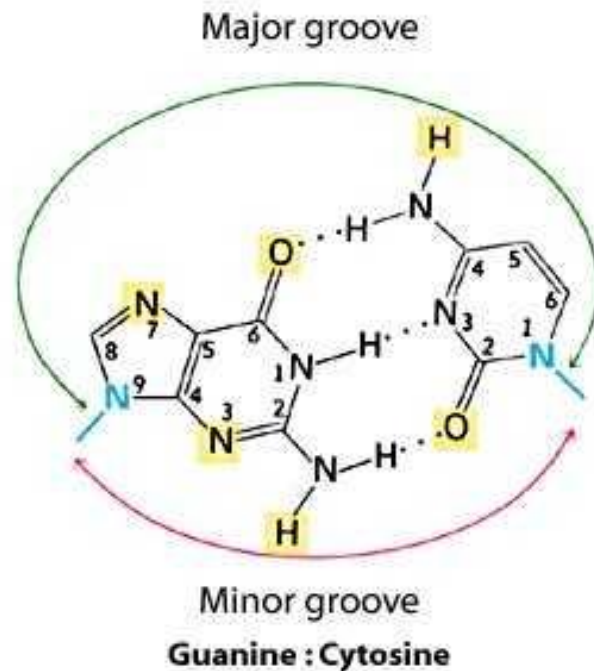
How does a binding protein “read” a 5’->3’ sequence such as GCAT on the Watson strand and “ATGC” on the Crick stand

What Does a Protein “See” in DS DNA?

AT_{major}: N^hO^{me}



GC_{major}: N^hO^h



AT_{minor}: N^hO

GC_{minor}: N^hO

What Does a Protein “See” in DS DNA?

“Reading” the major and minor groove

- Both Grooves Are Lined by Sequence Specific H-bonding Groups

- **B DNA** *major groove*

- GC *N O h*

- CG *h O N*

- AT *N h O*

- TA *O h N*

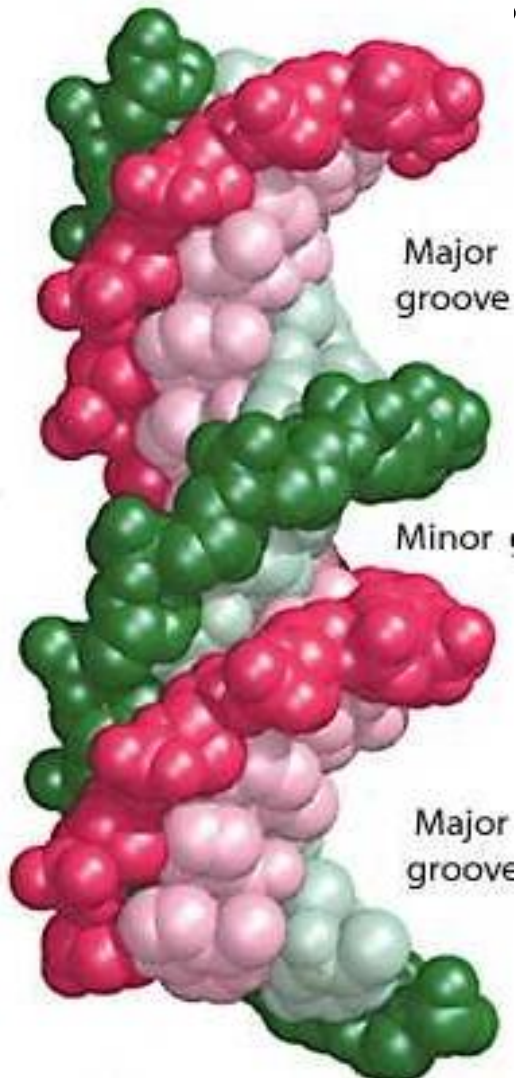
- **B DNA** *minor groove.*

- GC *N h O*

- CG *O h N*

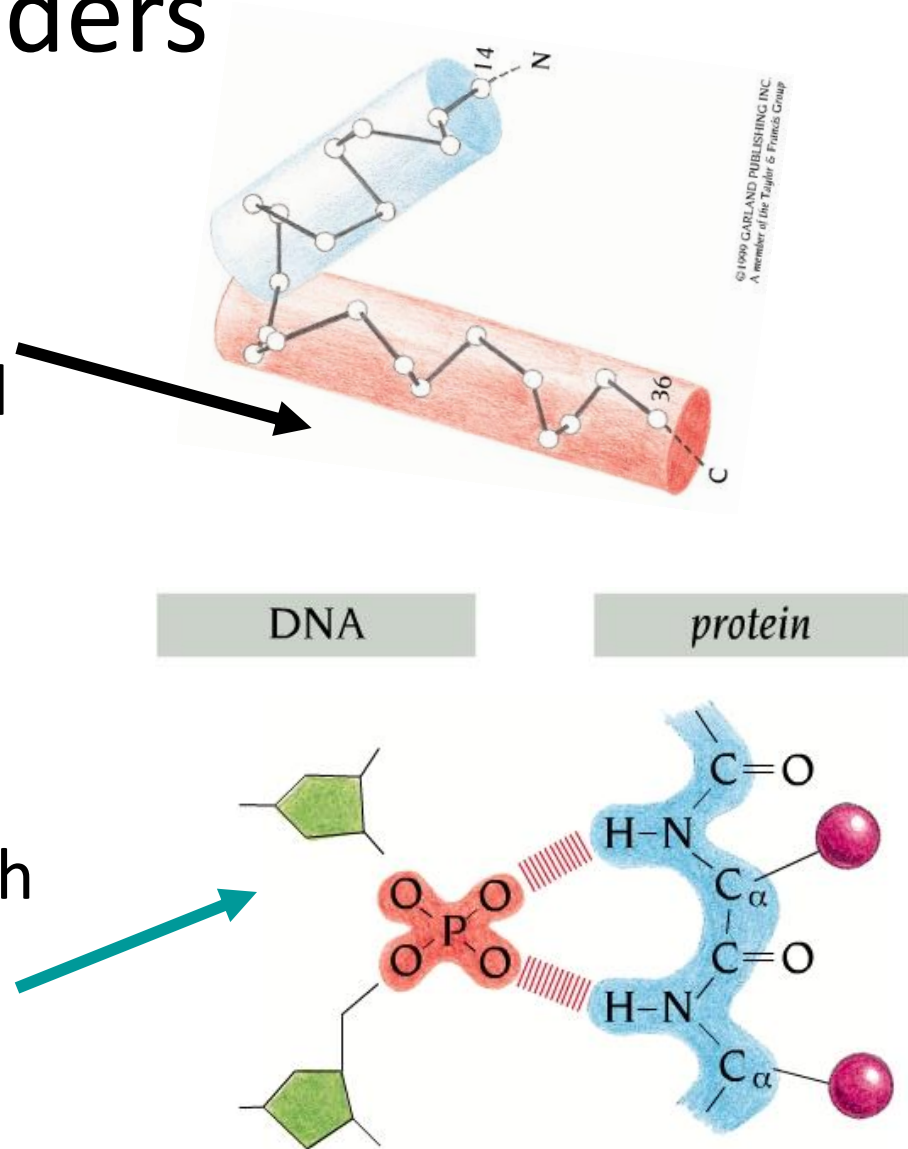
- AT *N O*

- TA *O N*



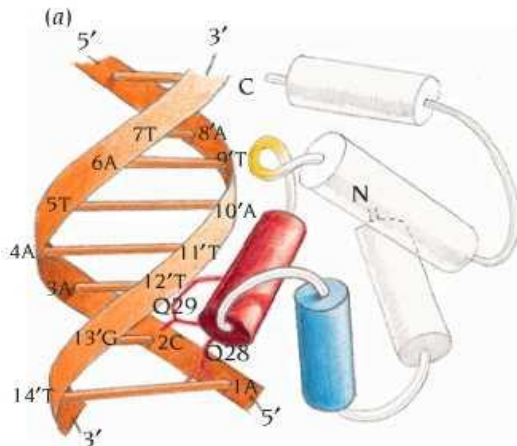
I. Major Groove Binders

- Specific Interactions:
Recognition helices use side group interactions to H-bond to sequence specific nucleic acids displayed in the major groove.
- Non-specific interactions with phosphate backbone (here with amide backbone)

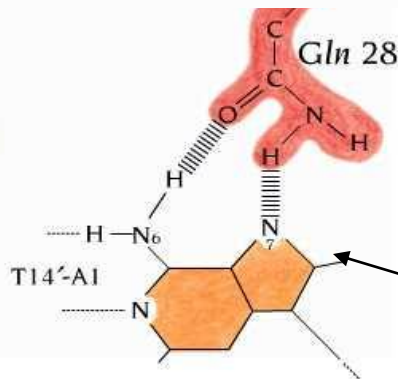


I. Major Groove Binders

- helix-loop-helix
 - recognition helix
 - Red aa 25-36
 - orientation helix
 - Blue aa 14-23

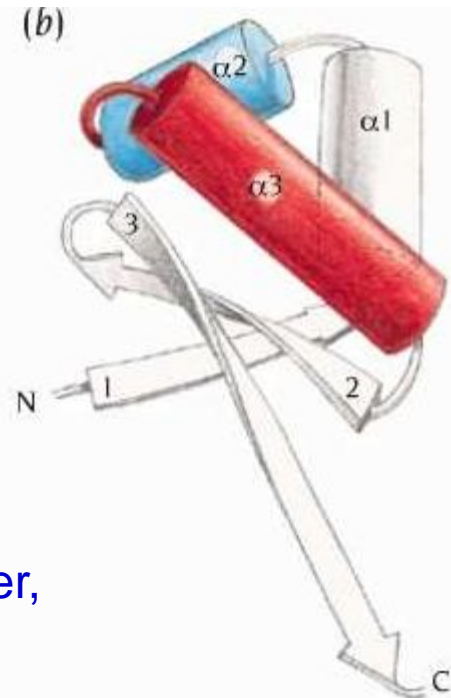
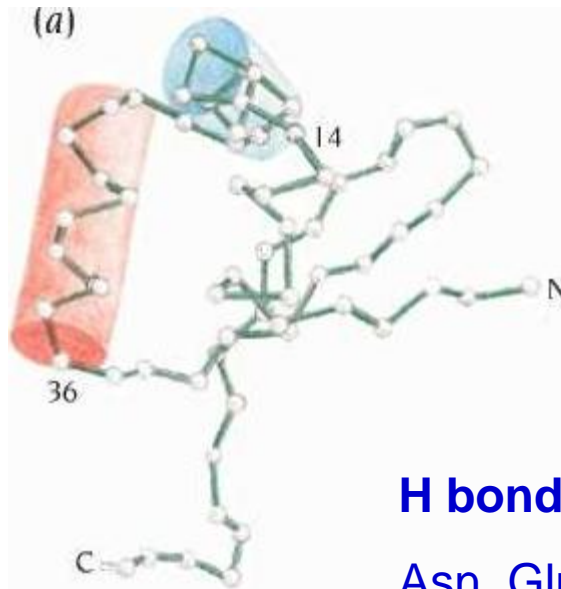


(b)



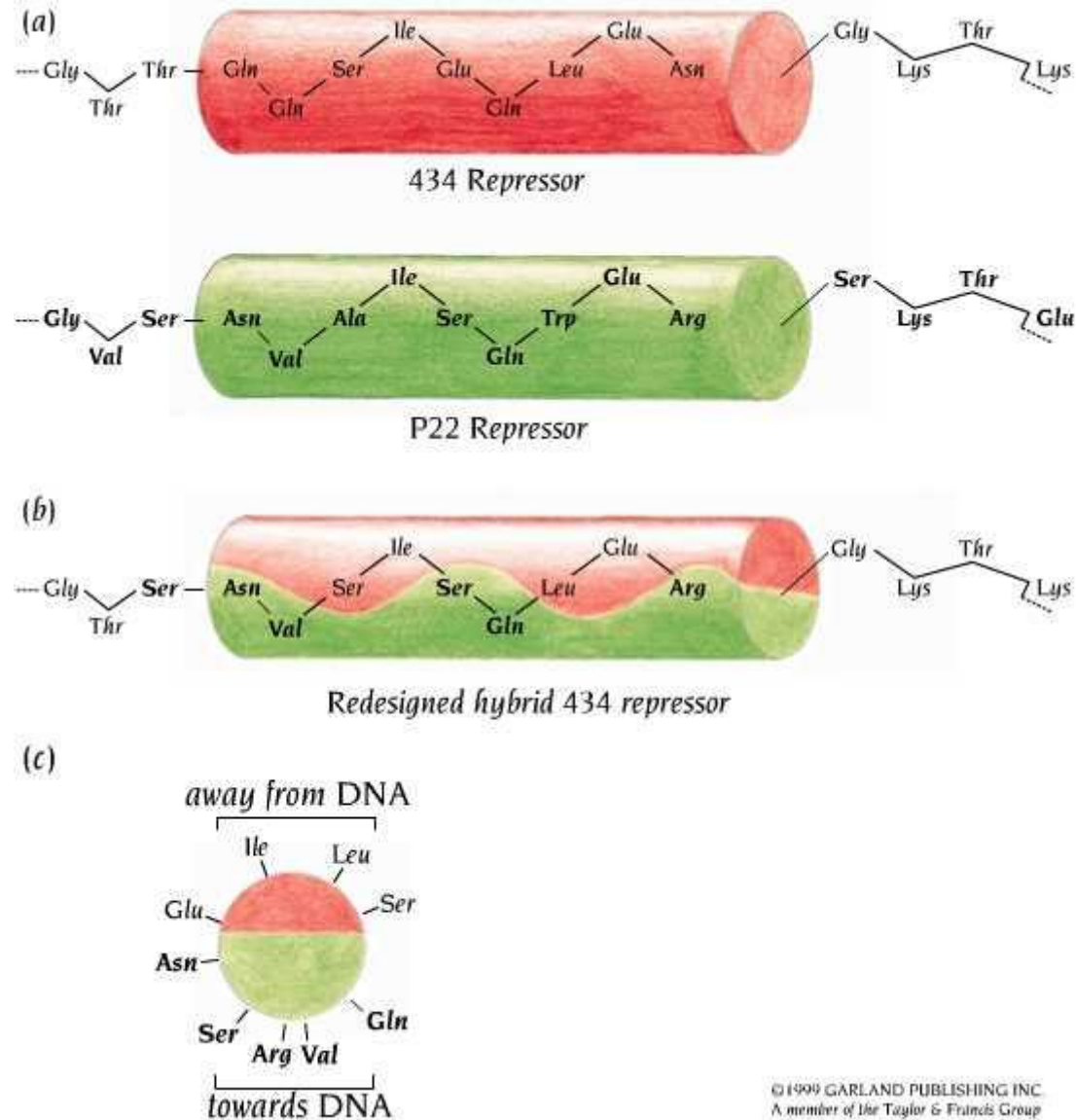
H bond aa

Asn, Gln, Ser,
His, Tyr, Trp

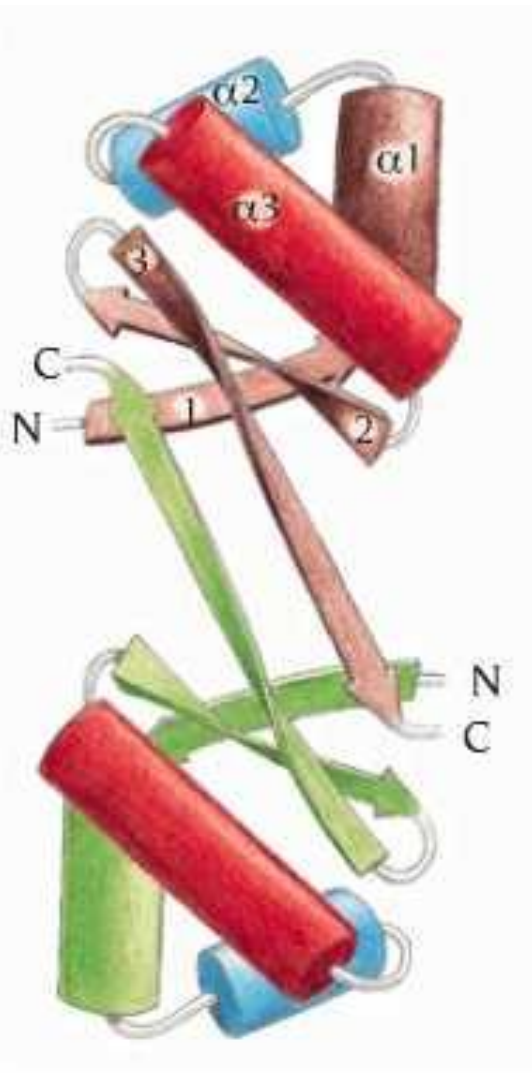


Mutagenesis proves that recognition of specific DNA is moderated by recognition helix

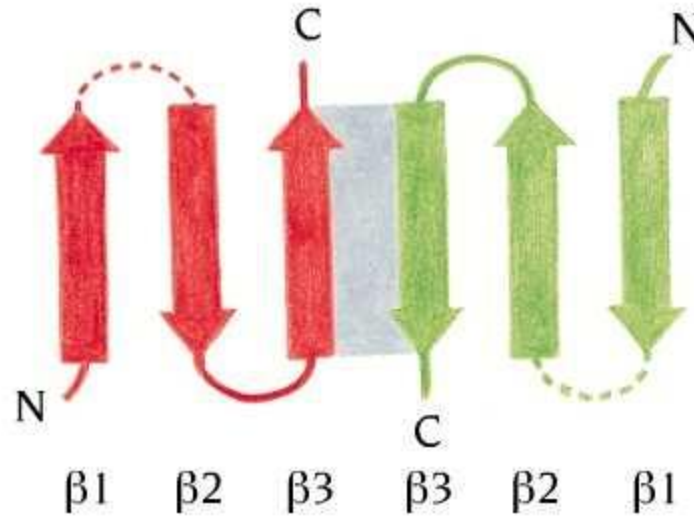
- 434 repressor is from one organism, recognizes specific DNA and represses its transcription
- p22 repressor is of similar type but from a different organism, binds to a different DNA and represses its transcription
- change of six amino acids changed 434 repressor into a p22 repressor



Dimer formation through B strands

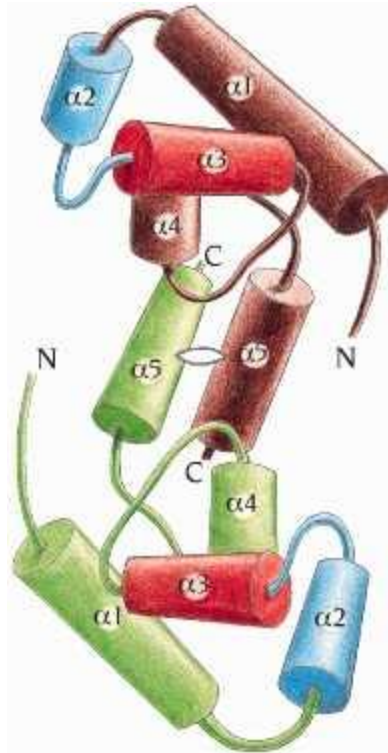
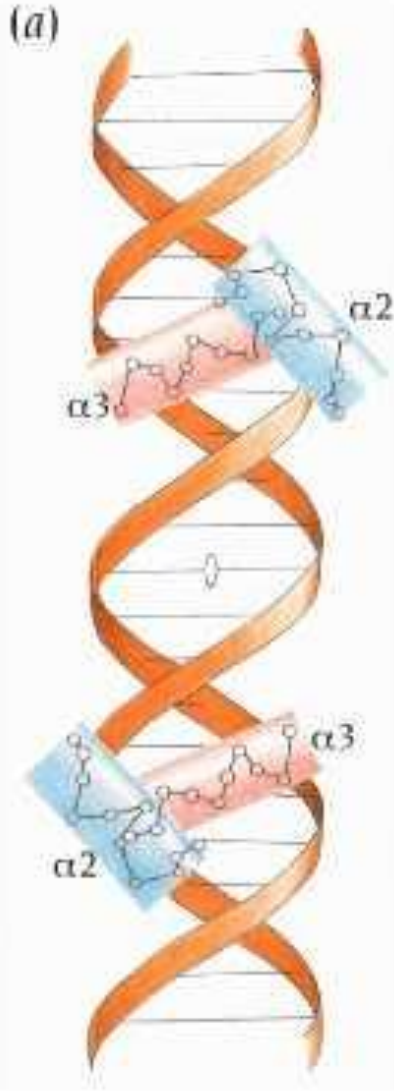


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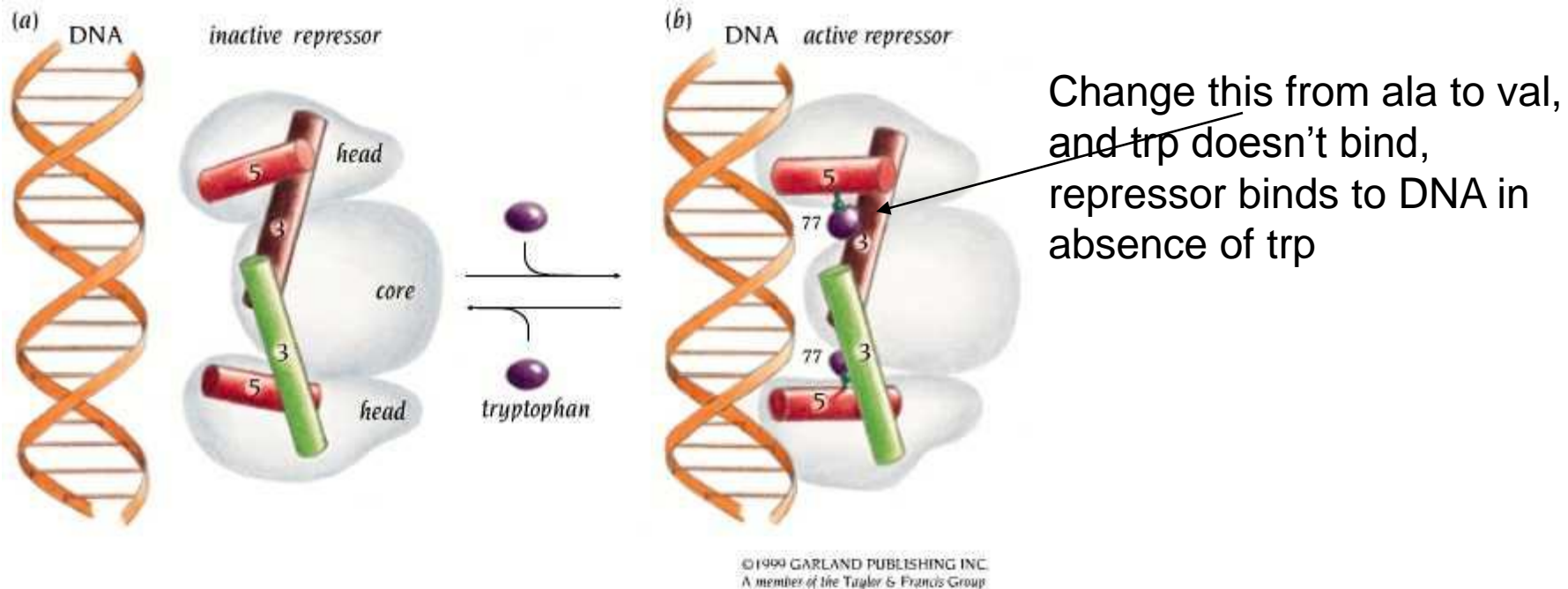
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Dimerization through helices



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H-L-H Protein: Trp Repressor

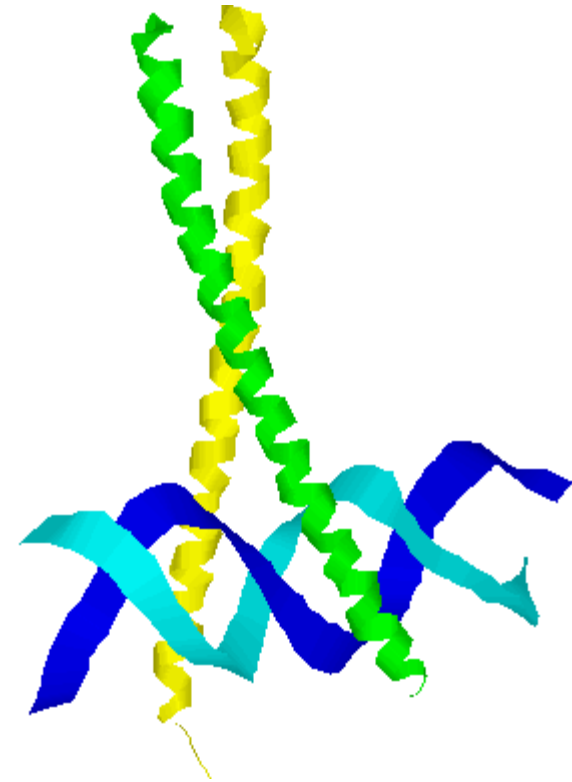
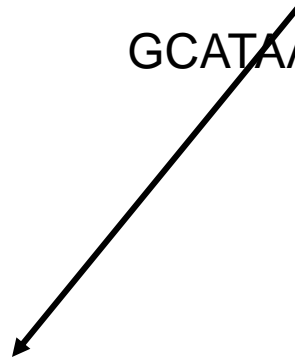


- Allosteric Effector: trp, the corepressor
 - trp binding in a crevice between helices 3 and 5 changes the tilt of helices and facilitates interaction of trp repressor protein with regulatory region of DNA which codes for synthesis of trp synthase

Leucine Zipper Proteins bind to DNA Major Groove

- Two long parallel α helices grip the DNA like the legs of a cowboy on a bucking bronco
 - ◆ Side groups on protein interact with palindromic sequences in major groove
 - ◆ one α helix interacts with the other through leu side groups.

Madam I'm Adam
GCATAATACG

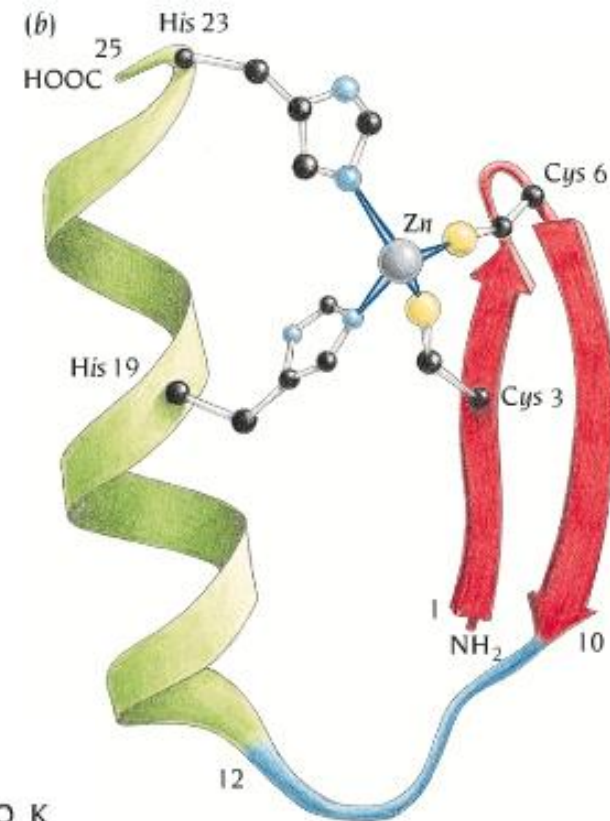
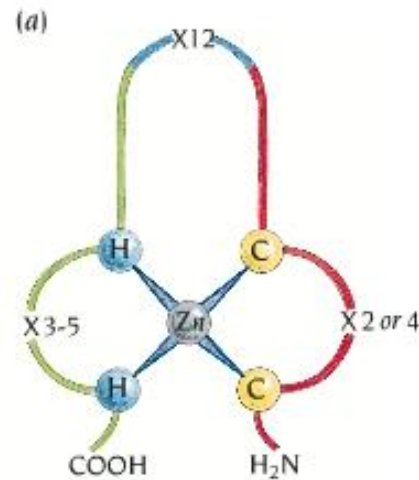


Shown in ribbon format,
here the regulatory protein
GCN4 binds to DNA using a
leucine zipper motif

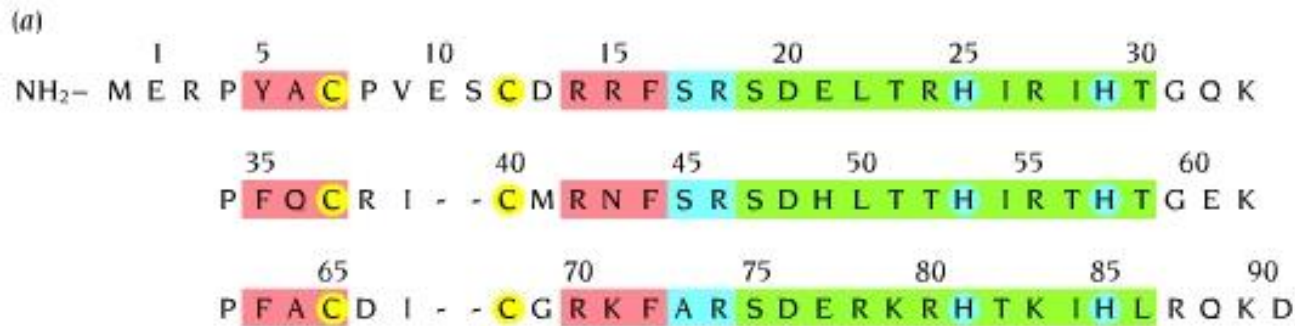


leucine zipper.pdb

Zn Finger Protein also bind to DNA major grooves



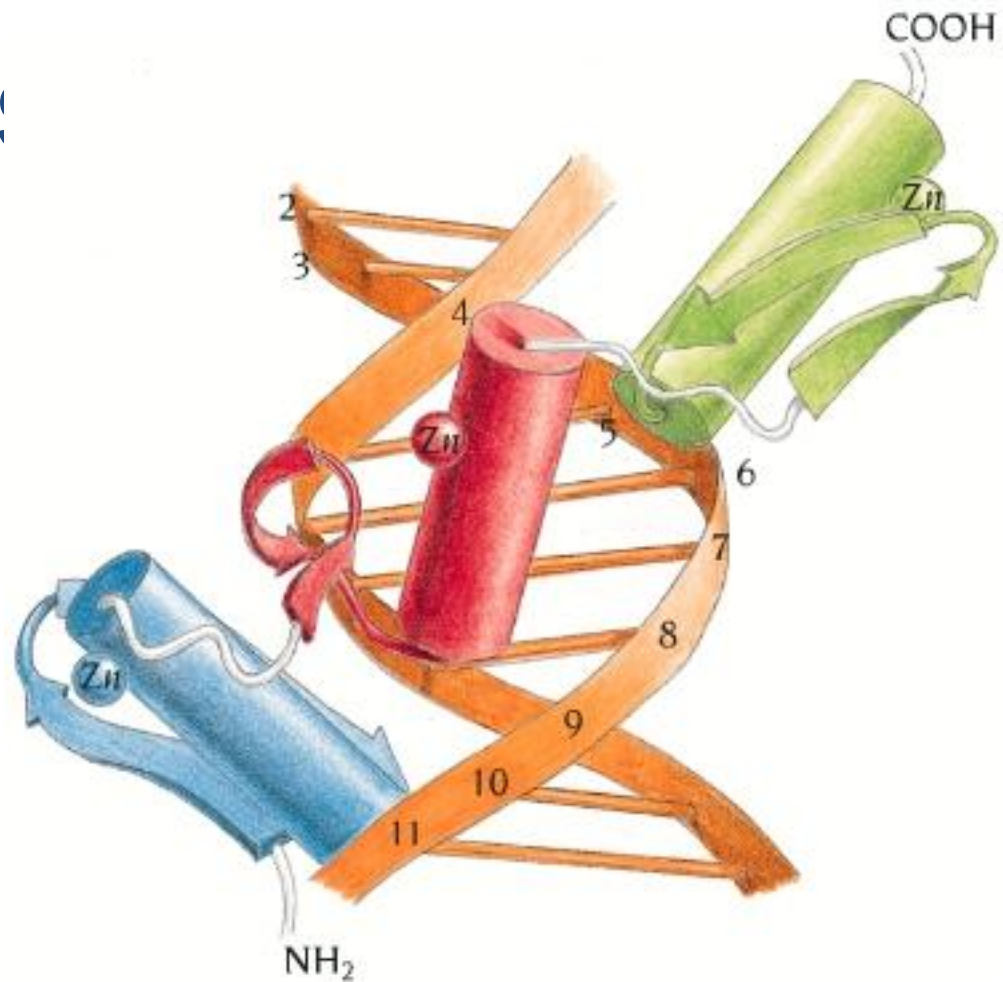
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Zn Finger Proteins

- * The 3 Zn fingers of Zif 268 bind in tandem to the major groove of DNA
- * The zinc fingers bind in a similar way with the N-terminus of the α helix pointing into the major groove, here oriented by beta strands
- * N.P. Pavletich et al., Science 261: 1701-1707, 1993.)



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