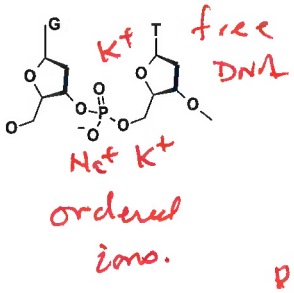
Lecture 36: Protein-DNA Interactions

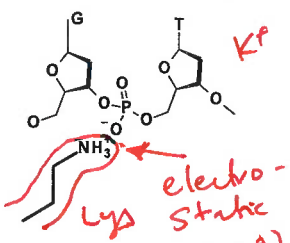
DNA-Protein Interactions - Forces and functional groups involved in recognition.

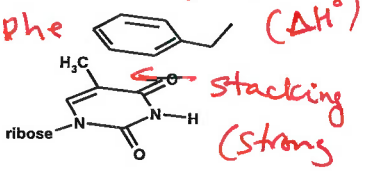
A. Non-sequence specific (no sequence recognized)

1. Electrostatic bonding to the backbone.
   a) side chains of Lys and Arg to phosphates.
   b) release of metal ions (e.g. K^+) favors binding (large increase in ΔS of ions).
   c) Binding affected by NaCl concentration.



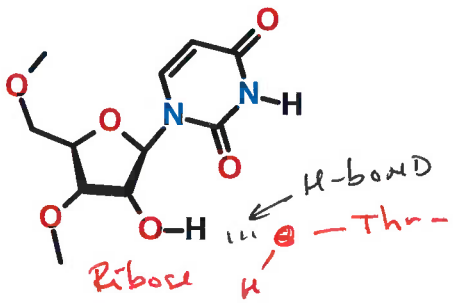
disordered (favor ΔS°) COMPLEX

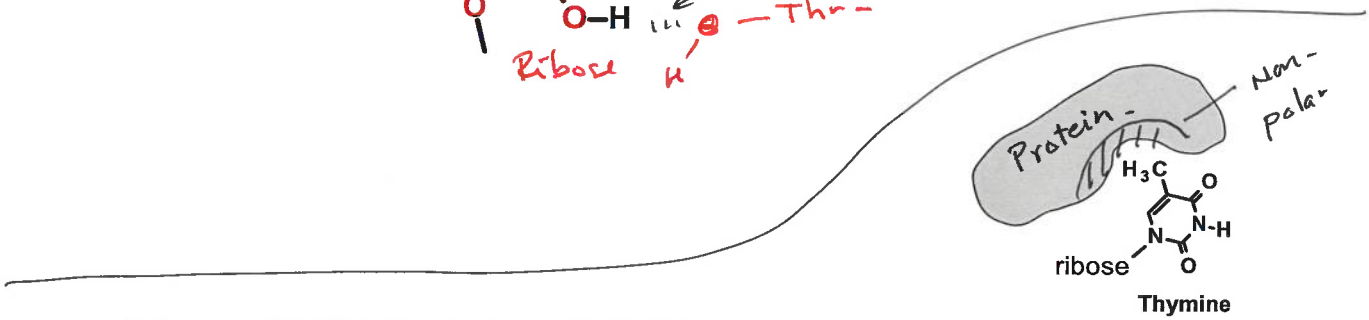




2. Van der Waals: Stacking (and intercalation) of Phe, Trp, and Tyr side chains. More prevalent in single stranded (ss) nucleic acid. (e.g. RNA)

3. H-bonding to ribose (may distinguish between DNA and RNA).





Thymine

B. Sequence Specific: *Changing Base affects binding*

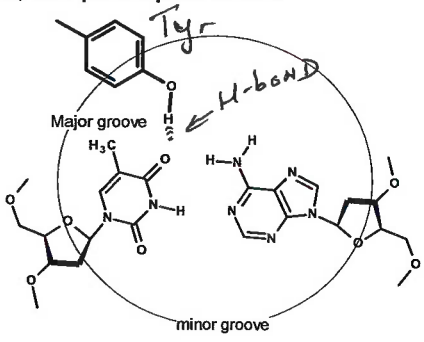
3. Hydrophobic interaction with the 5-methyl of T.

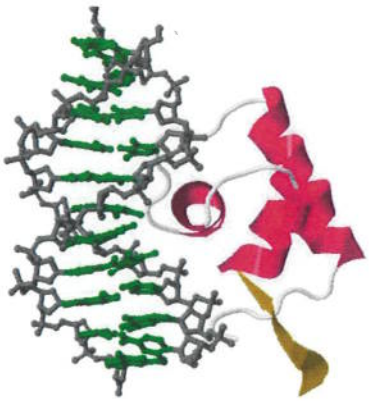
4. Non- Watson-Crick Hydrogen bonding to the polar edges of the bases, **usually in the major groove**. *(Major groove is large arc of a circle drawn through the basepair, minor groove is small arc).*

   a) Side chains of Arg, Asn, Gln, Tyr...

   b) Protein mainchain, C=O, NH.

   Note: WC H-bond acceptor, C=O, can participate in one additional non-WC H-bonds.



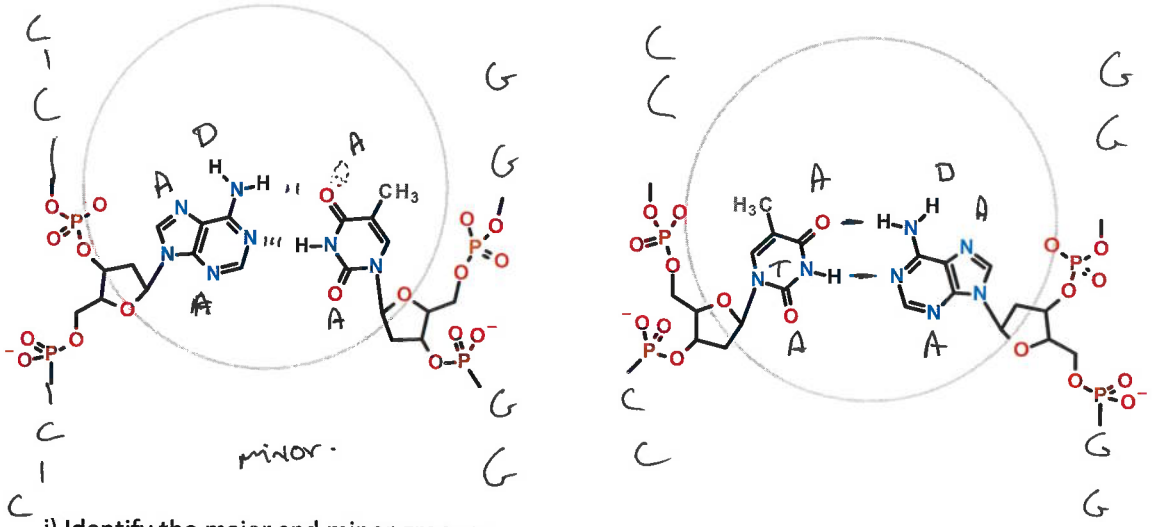


$\Delta G^\circ_{BIND}{}^{TOTAL} = \Delta G^\circ_{NON\text{-}SPEC} + \Delta G^\circ_{SPECIFIC}$

Reflection: How do proteins recognize DNA sequences without using Watson-Crick hydrogen bonds? The following represent two different sequences:

--CCACC--
--GGTGG--

--CCTCC--
--GGACC--

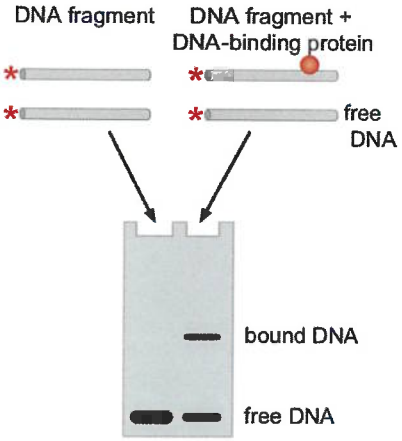


i) Identify the major and minor grooves.
ii) Mark the hydrogen bond donors and acceptors in the major and minor groove.
iii) Compare the pattern for each basepair in each groove, are they the same or different.

Measuring DNA-Protein Affinity: Y = [DNA-P]/([P] + [DNA-P])

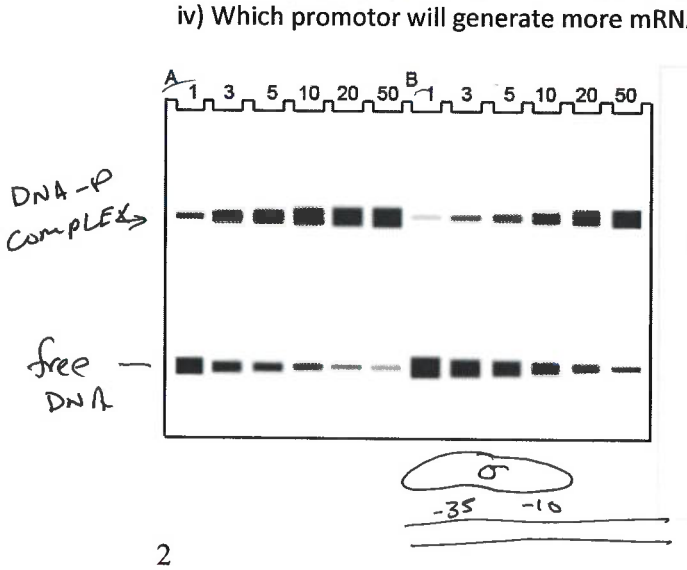
1. Gel Shift (EMSA-electrophoretic mobility shift assay):

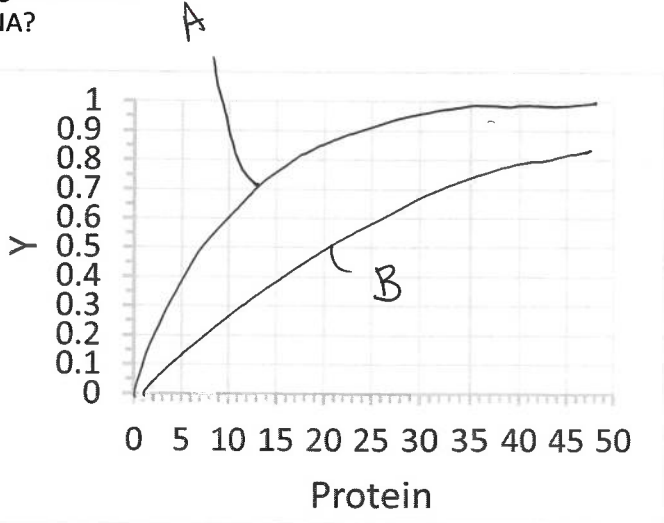
- Native agarose or acrylamide gels, DNA is labeled (fluorescent, radioactive)
- Protein-DNA complex has different electrophoretic mobility than free DNA due to size and charge differences.
- Band intensities give amount of free and bound DNA.



Example:
The binding of sigma factor to two different promotors (A, B) is measured using EMSA, DNA was constant, protein was varied.

i) Which band is the free DNA and which is the sigma-DNA complex?
ii) Sketch the approximate binding curves from these data.
iii) Which promotor has higher affinity for sigma factor?
iv) Which promotor will generate more mRNA?



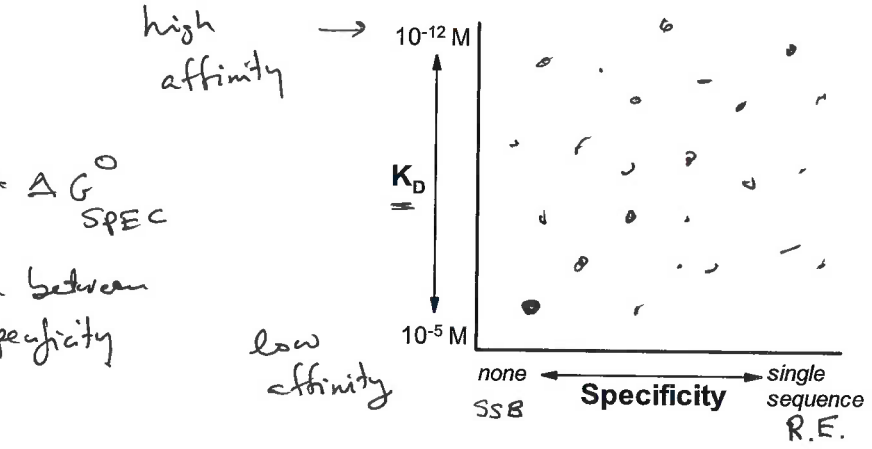


Reflection: Predict the relationship between binding specificity and affinity?

high affinity →

$\Delta G^\circ = \Delta G^\circ_{NON} + \Delta G^\circ_{SPEC}$

No correlation between affinity & specificity



Restriction Endonuclease: [*endo* - cut within, *nuclease* - cleave nucleic acid].

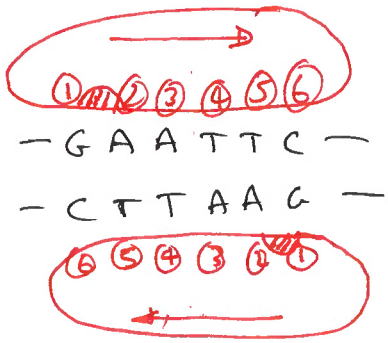
- **Provide a tool to cleave DNA at specific DNA sequences.**

Enzyme	Recognition Sequence		Products	
EcoR1: (G^AATTC)	-G-A-A-T-T-C- -C-T-T-A-A-G-	-G -C-T-T-A-A	A-A-T-T-C- G-	5′ overhang
PstI: (CTGCA^G)	-C-T-G-C-A-G- -G-A-C-G-T-C-	-C-T-G-C-A -G	G- A-C-G-T-C-	3′ overhang
EcoRV: (GAT^ATC)	-G-A-T-A-T-C- -C-T-A-T-A-G-	-G-A-T -C-T-A	A-T-C- T-A-G-	blunt end

Isolated from bacteria, normal biological function is to degrade invading viral DNA. Named after bacterial species the enzyme was isolated from. There are hundreds of these enzymes, and several profitable biotech companies who make and sell them.

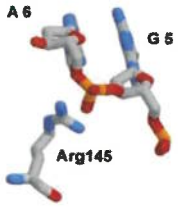
A. DNA Recognition – "Restriction Site"

1. Restriction enzymes binds to specific **recognition sequences** with near absolute specificity and high affinity ($K_D = 10^{-10}$ M).
2. Enzymes usually bind in *major* groove, forming *both* specific and non-specific interactions.
3. These homodimeric enzymes have 180-degree rotational symmetry. Because of the symmetry in the enzyme, the DNA sequence also symmetrical. The sequence is the same on the top and bottom strands (referred to as palindromic sequences). The cut site is also the same on both strands.



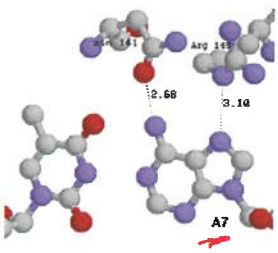
Example: EcoR1: GAATTC

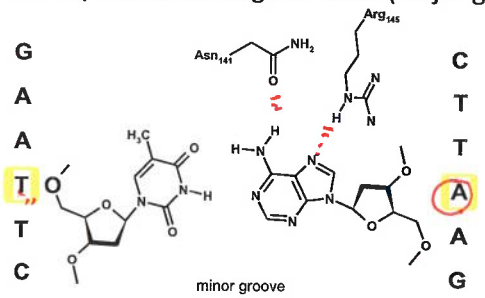
a) Non-specific interactions with DNA phosphates.



---G-A-A-T-T-C---
---C-T-T-A-A-G---

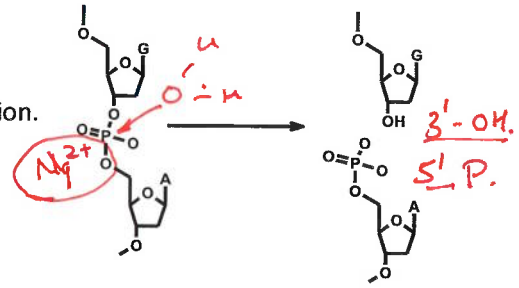
b) Specific hydrogen bonds with donor and acceptors at the edge of bases (major groove):





B. DNA Cleavage

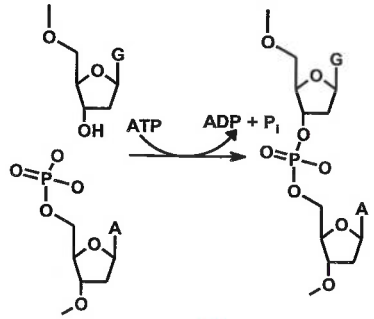
1. Require Mg^{2+} for cleavage. Generate a 3'OH and 5' phosphate.
2. Cleave both strands at the same location.
3. Generate different types of ends, dep. on cleavage location.



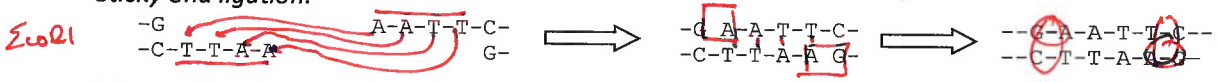
DNA Ligase:

- **Provides a tool to join DNA fragments together.**

1. Uses ATP to join 5'phosphate to 3'-OH
2. Two ends of the DNA have to be held in close proximity to allow reformation of the phosphodiester bond.



Sticky end ligation:



Blunt end ligation: