Topic 1: Protein-DNA Interaction

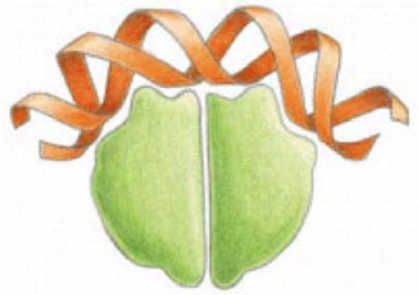
- Goals:
  - find DNA binding target seq for each transcription factor (TF)
  - find the affinity of a TF to its DNA target as a function of its cellular concentration *in vivo*
  - find how the TF-DNA affinity may be modulated by the target sequence
- Problems:
  - thousands of TFs each with distinct target sequence; only a few characterized in detail experimentally
  - *ab initio* molecular calculation difficult even when TF-DNA co-crystal structure available
  - need to deal with the entire genomic DNA seqs *in vivo*

Statistical physics:

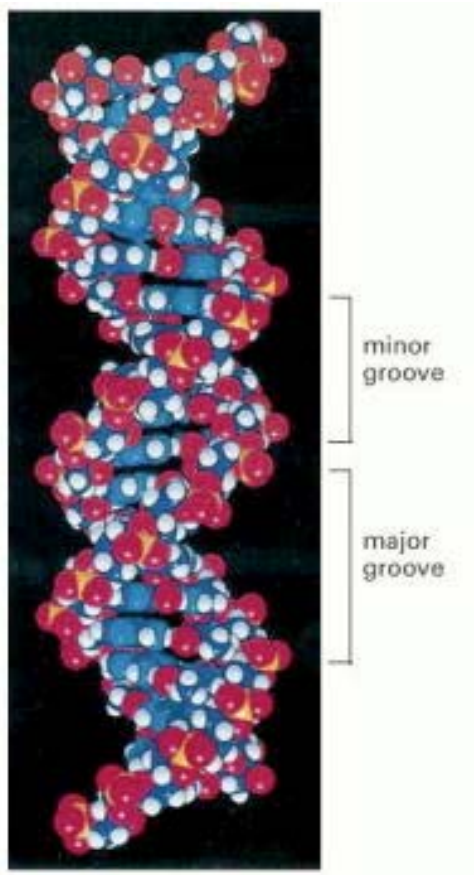
➔ ways to think quantitatively about TF-DNA interaction
in the absence of detailed microscopic information

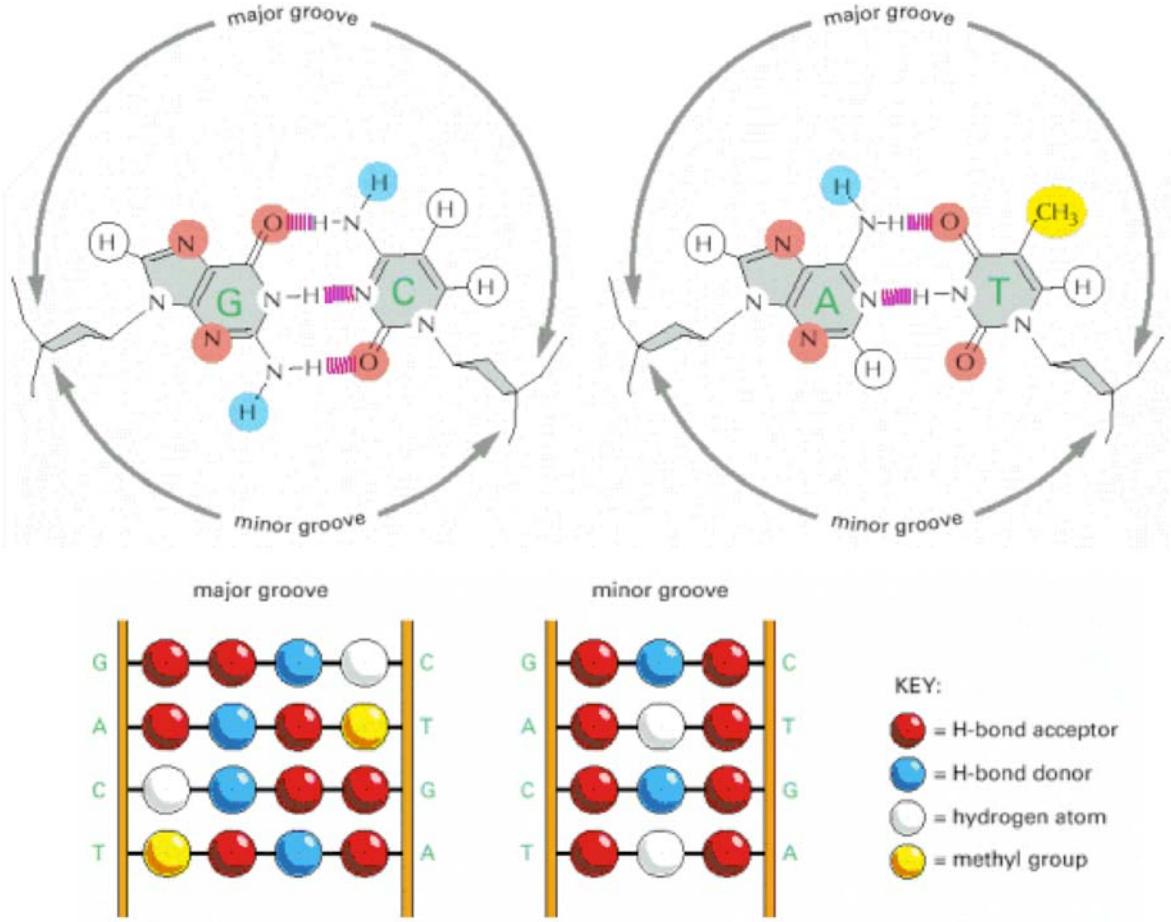
➔ link from molecule to function

A. Empirical facts

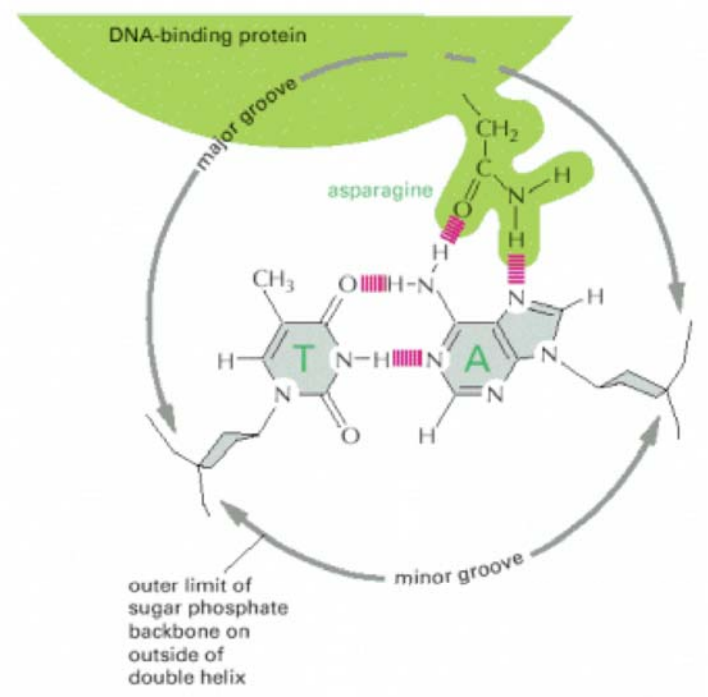


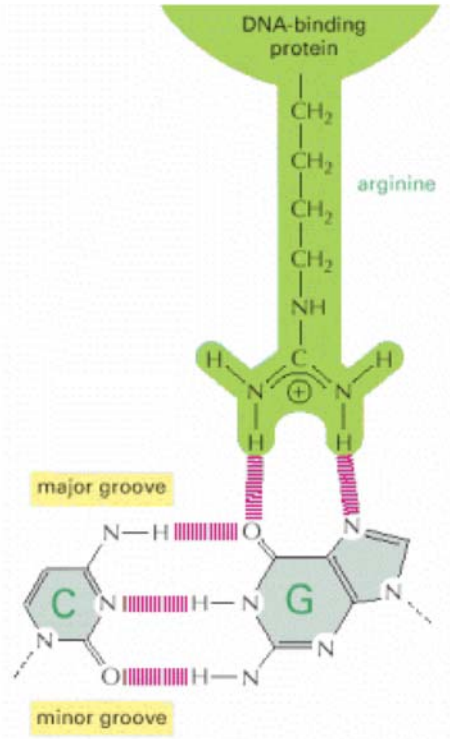
1. Transcription Factors
   - size: ~5nm (10-20 bp)
   - molecular basis of sequence recognition





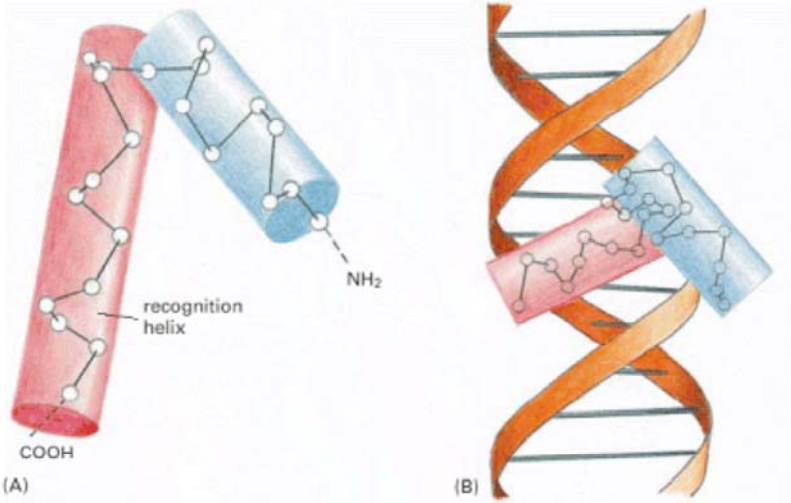
- contact between TF and DNA

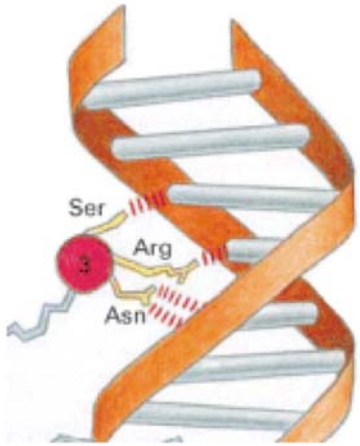




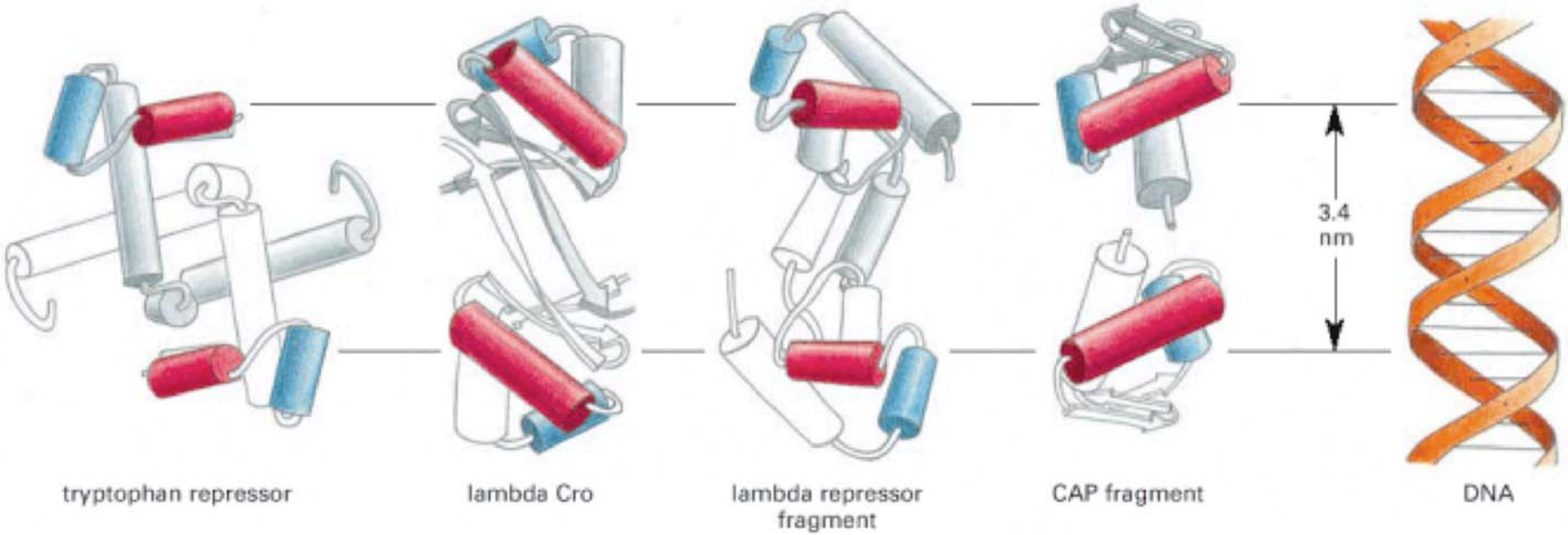
➔ structure of a TF must place the appropriate amino acids next to the base pairs they contact

- various molecular strategies
  - Helix-Turn-Helix

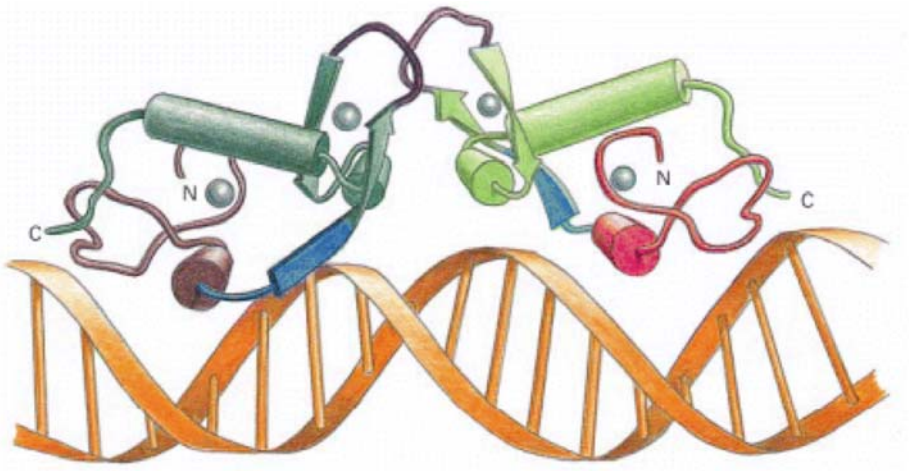




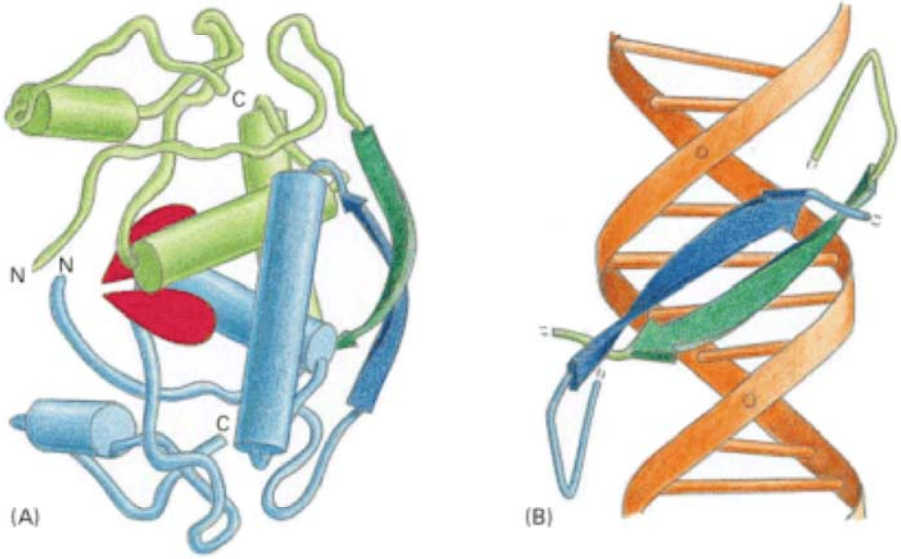
well-known examples in bacteria (note: homodimers)



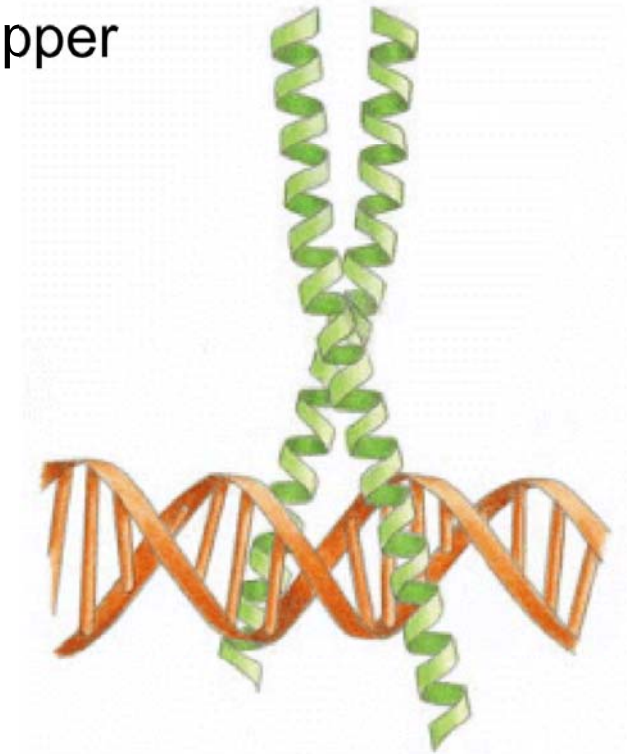
– zinc-finger domain



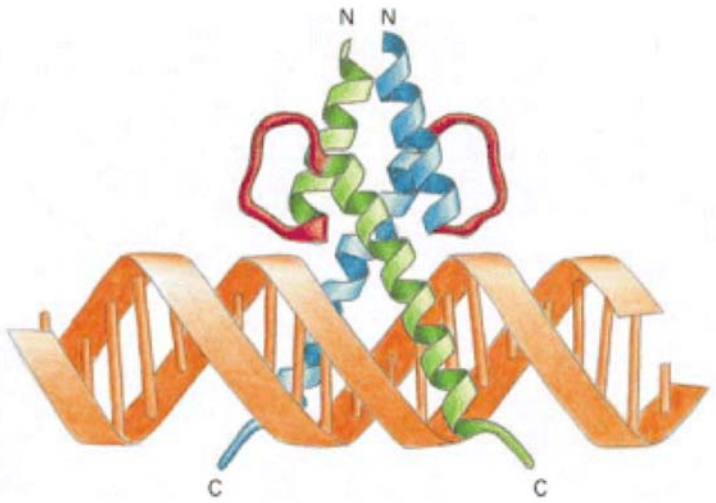
– beta-sheets



– leucine zipper



– helix-loop-helix



2. DNA binding sequences

- typically 10-20 bp in bacteria

protein	target sequence
lac repressor	5' AATTGTGAGCGGATAACAATT 3' TTAACACTCGCCTATTGTTAA
CRP	TGTGAGTTAGCTCACT ACACTCAATCGAGTGA
λ repressor	TATCACCGCCAGAGGTA ATAGTGGCGGTCTCCAT

- lots of sequence variants
- consensus sequence often palindromic
- common to have 2~3 mismatches from the core consensus sequence -- "fuzzy" binding motif

3. TF-DNA interaction

- passive (no energy consumption)
- strong electrostatic attraction indept of binding seq

  e.g., $[TF-DNA] > 10 \times [TF]_{free}$ for LacI in 0.1M salt

  ➔non-specific binding: $G_{ns} - G_{cyto} \simeq -15RT$

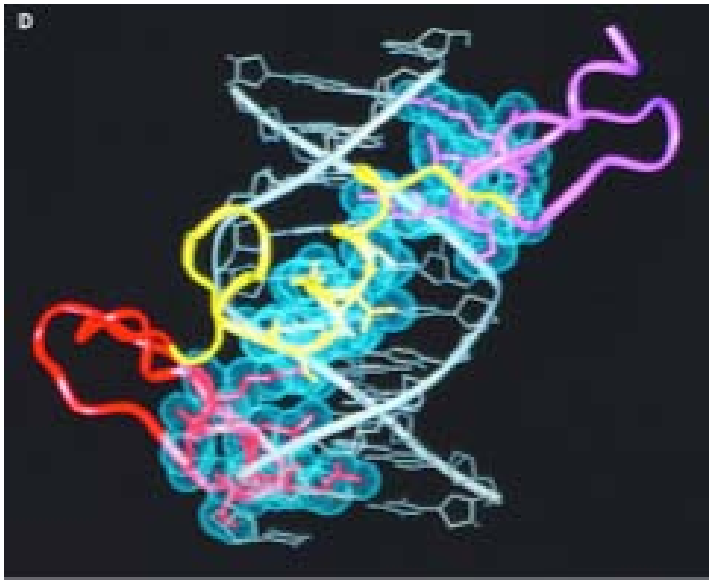
  ($RT \approx$ 0.62 kcal/mole at 37C)

- additional energy gained from hydrogen bonds to preferred sequences

  strongest binder: $G^{*} - G_{ns} \simeq -15RT$

- graded increase in binding energy for sequences with partial match to the preferred sequence

- relative binding affinity for Mnt



Binding energies for Mnt (in $k_B T \equiv 1$):

pos.	10	11	12	13	14	15	16	17
A	1.8	2.4	1.6	1.0	0	2.1	0.8	1.1
C	2.4	1.9	4.2	2.1	0.3	0	0	0
G	0	1.6	0	0	1.2	3.2	1.0	1.2
T	3.0	0	2.2	2.2	0.6	2.2	0.7	0.3

(D.S. Fields, Y. He, A.Y. Al-Uzri & G. Stormo, 1997)

(from competitive binding expts)

➔ weak energetic preference -- weak specificity

➔ similar results for other TFs studied (e.g., LacI, λ-CI, λ-Cro)

- double mutation: binding energy approx additive

- Issues to be addressed here:
  - range of TF-DNA affinity *in vivo*
  - dependence of this affinity on variation in target sequence
  - why weak specificity of TF-DNA interaction?
    ["design rule" for TF]
  - why fuzzy motifs
    [choice of DNA targets]
- Issues not addressed:
  - what is the target sequence of a given TF
    [can be probed experimentally]
  - fluctuations in TF-DNA binding