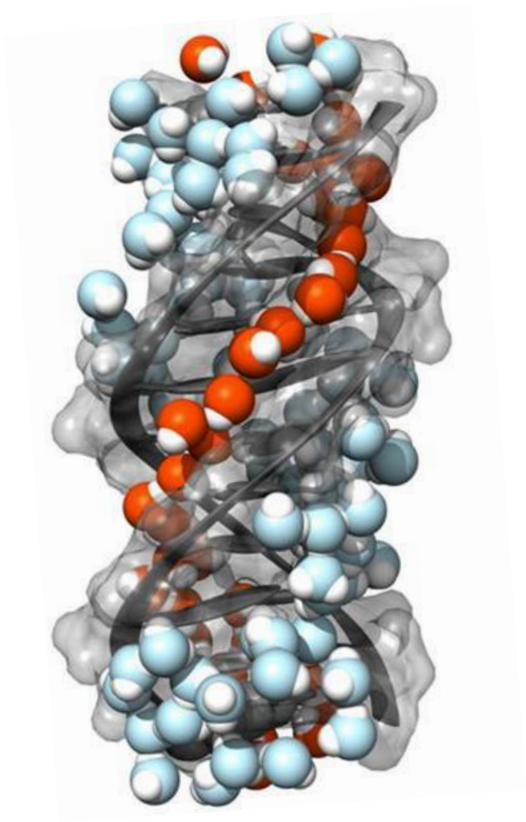
What drives the binding of a protein to DNA ?

Non-covalent interactions!

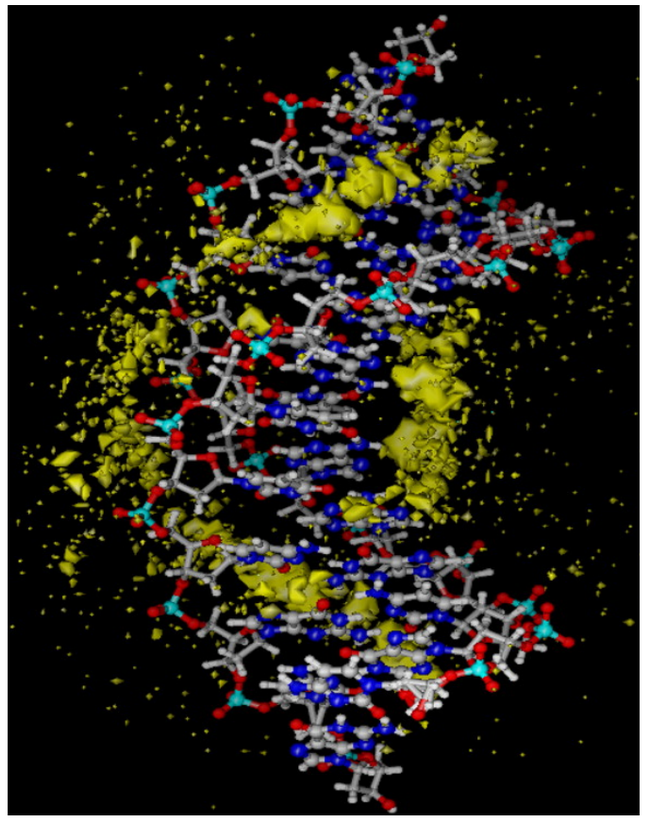
But: Non-covalent interactions can occur between macromolecule and water/ions just as well.

Water molecules (red) at DNA



McDermott ACS Centr. Sci., 2017
DOI: 10.1021/acscentsci.7b00100

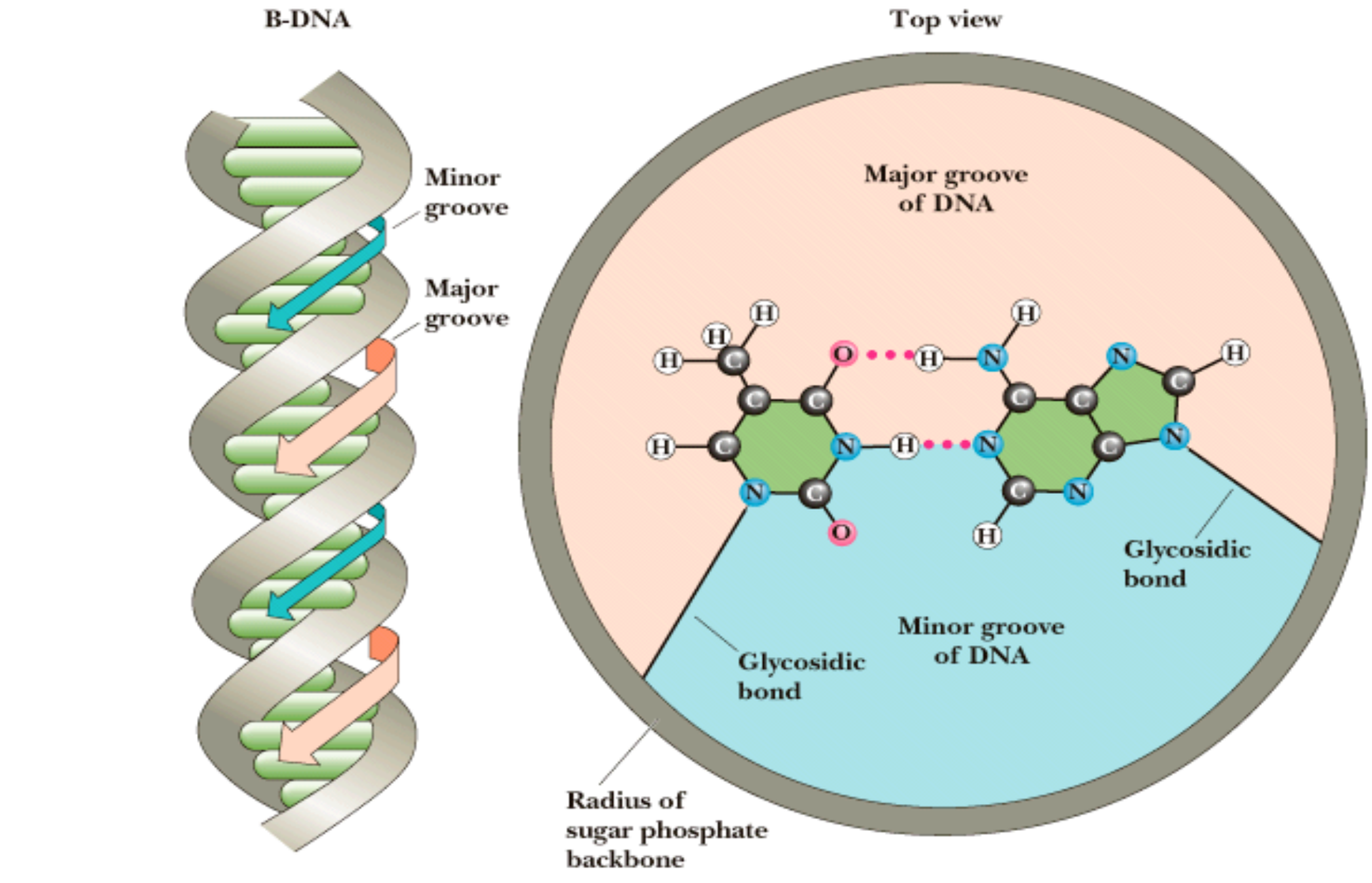
Regions of high Na^+ (yellow) density



Ponomarev, PNAS 2004,
DOI: 10.1073/pnas.0406435101

B-DNA – major & minor grooves

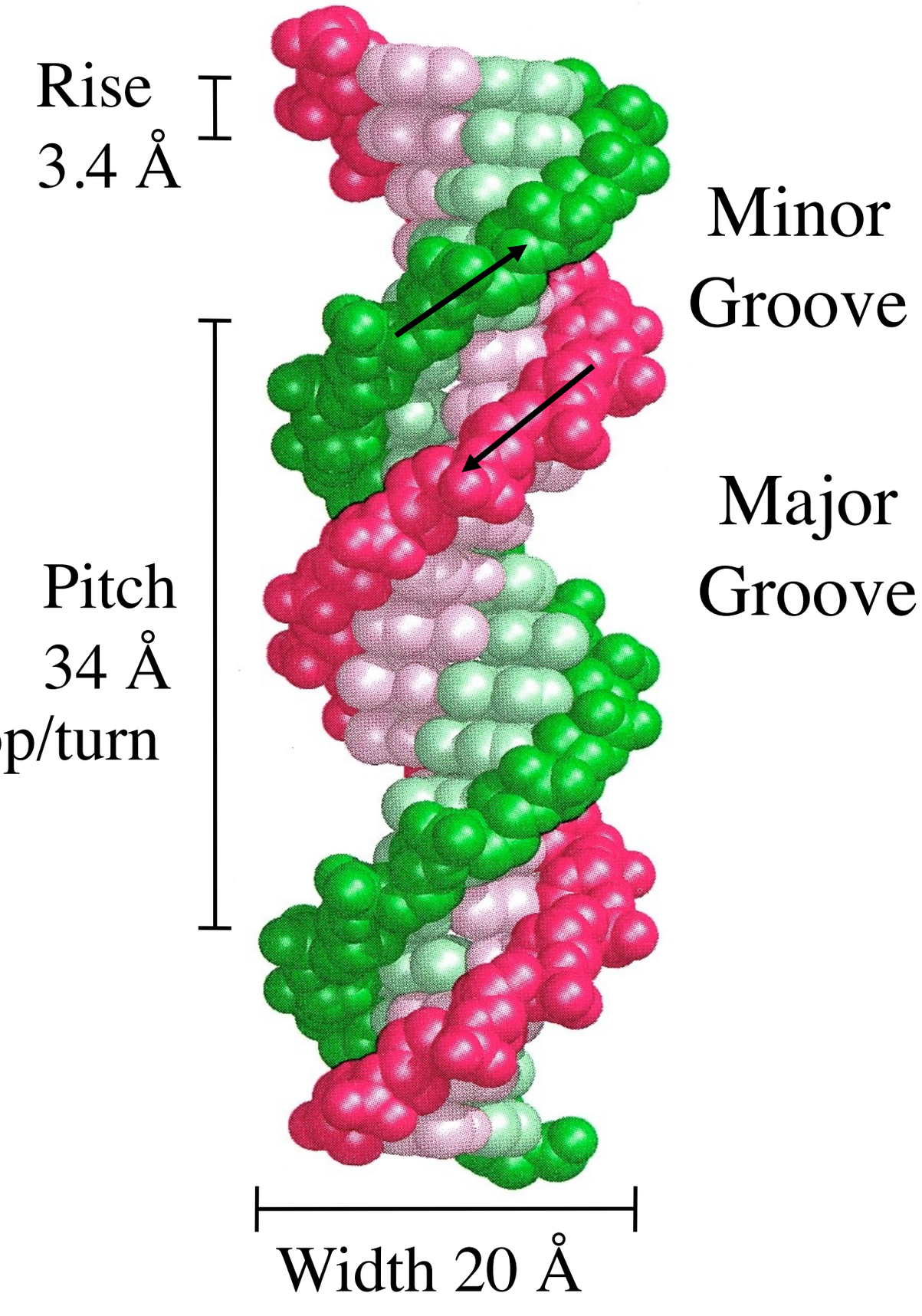
Garrett & Grisham: Biochemistry, 2/e
Figure 12.11

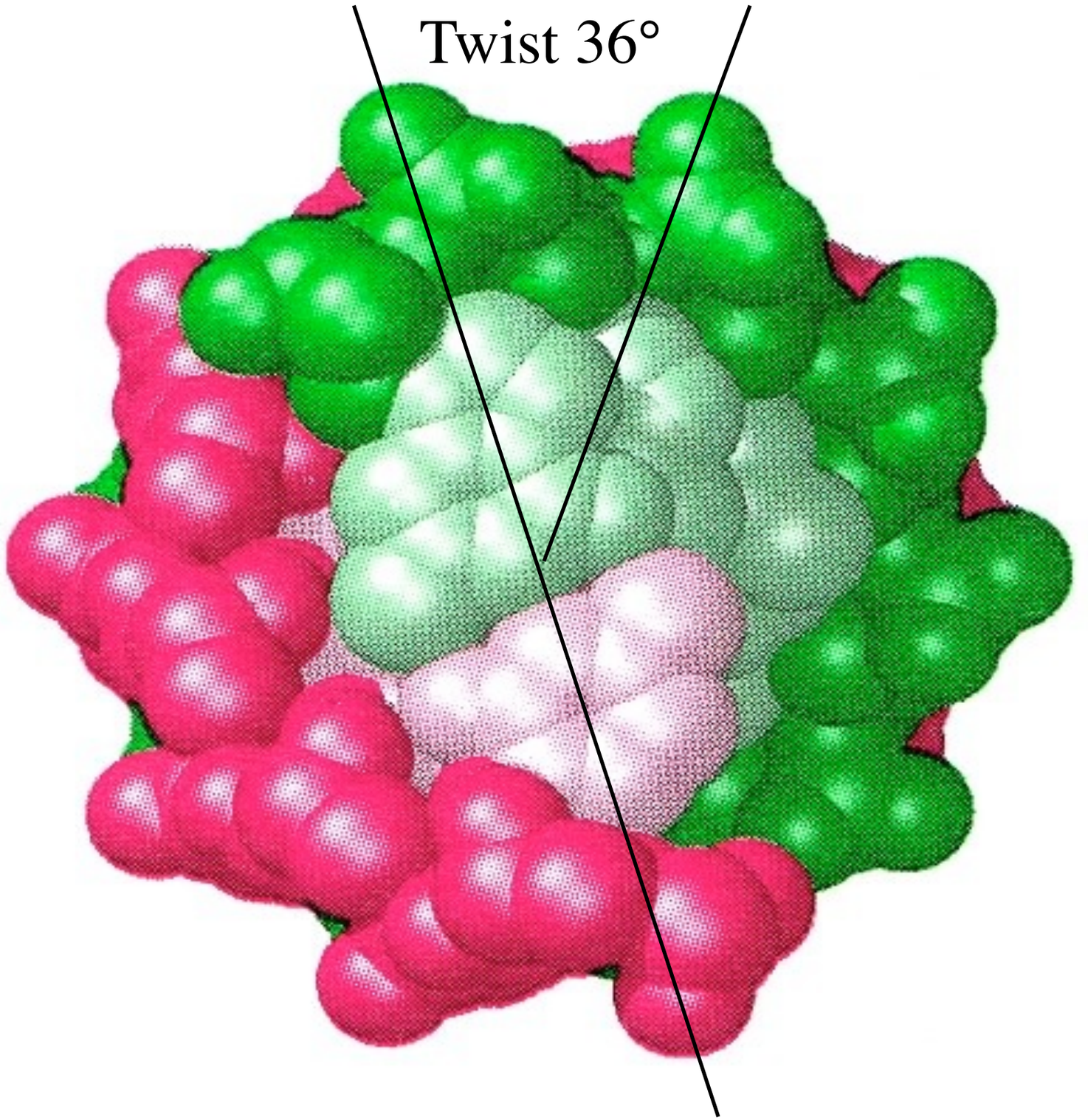


Saunders College Publishing

G & G 12.11

B-DNA: A right Handed double helix



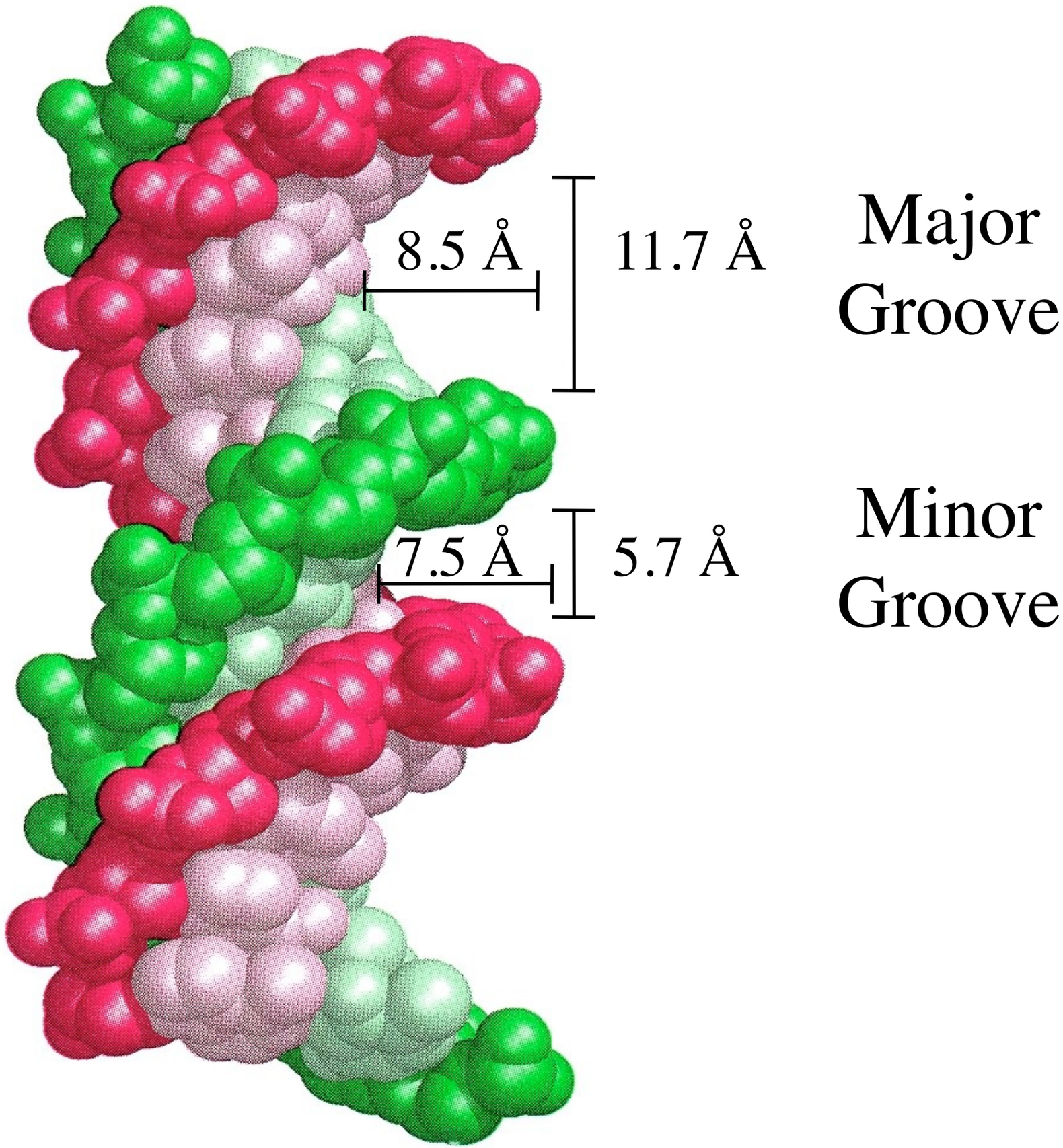

Twist 36°

α Helices and DNA - a perfect fit

- DNA-binding proteins often have an α-helical segment that fit directly into the major groove of B-form DNA.

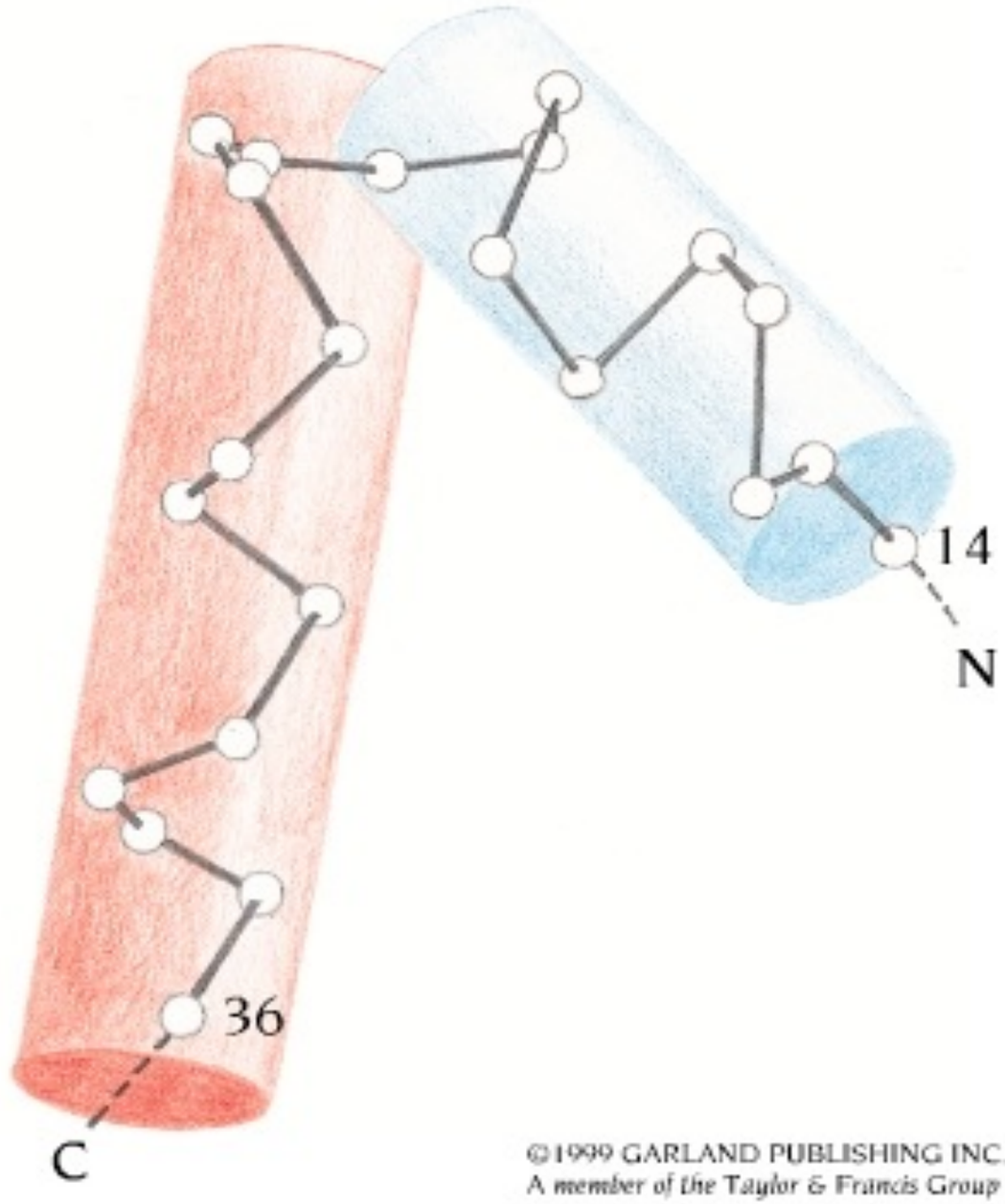
- The diameter of protein α-helix is 1.2 nm (12 Angstroms), and the major groove of DNA is about 1.2 nm wide and 0.6 to 0.8 nm deep.

- Proteins can recognize specific sites (sequences) via functional groups of base pairs in the major groove of the DNA.

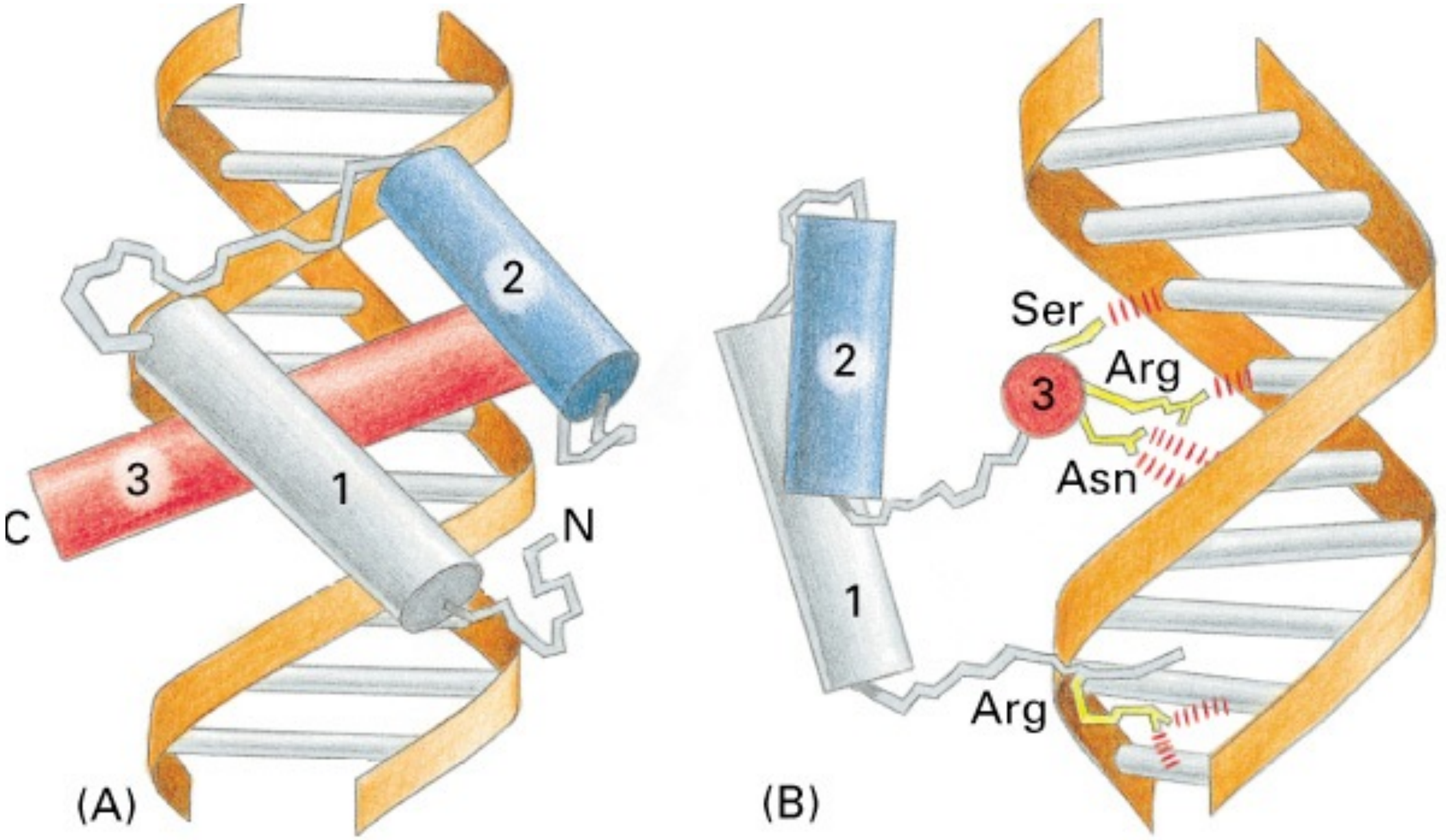

8.5 Å
11.7 Å
Major
Groove
7.5 Å
5.7 Å
Minor
Groove

The helix-turn-helix motif

- Generally bind as dimers to dyad-symmetric sites on DNA
- All contain two alpha helices separated by a loop with a beta turn
- The C-terminal helix fits in major groove of DNA
- N-terminal helix stabilised by hydrophobic interactions with C-terminal helix

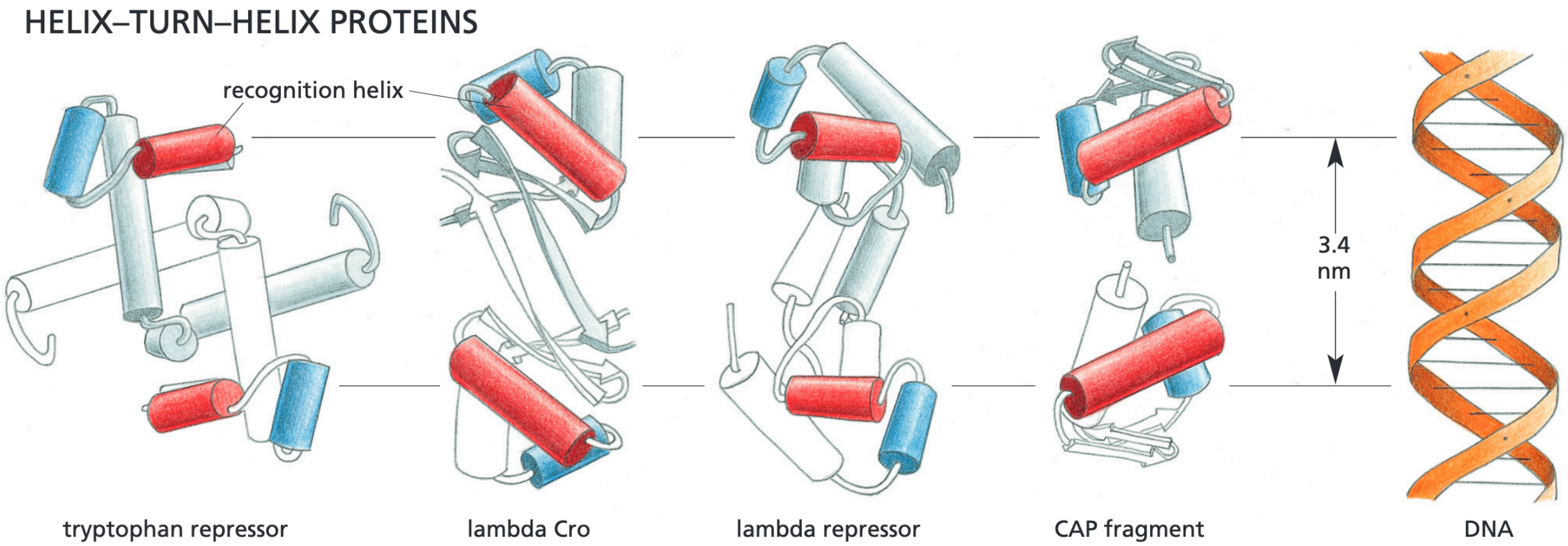


The helix-turn-helix motif: homeodomain transcription factor



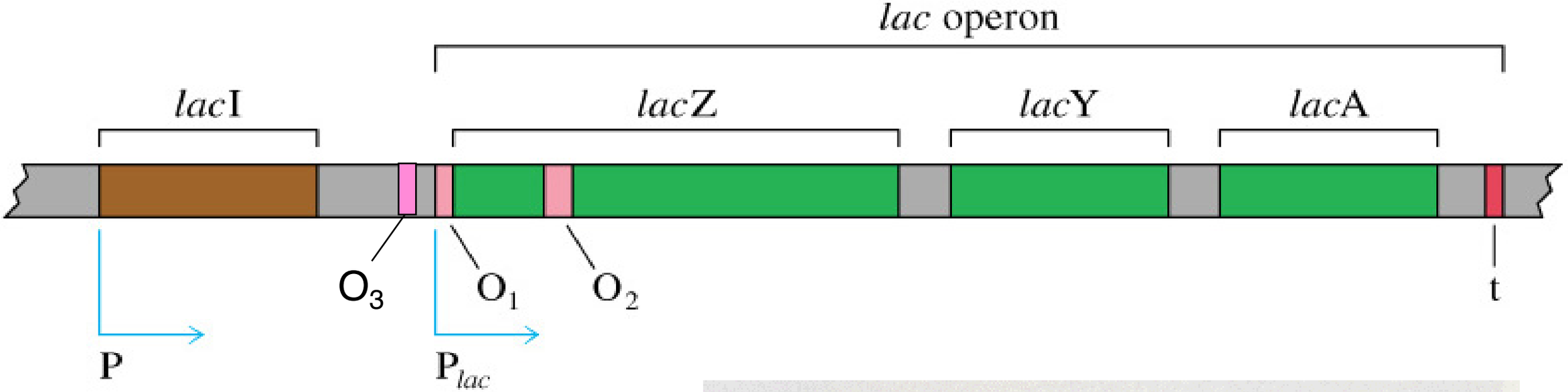
Control of gene expression, Chapter 7, MBC Alberts et al. 2022

Helix-turn-helix proteins

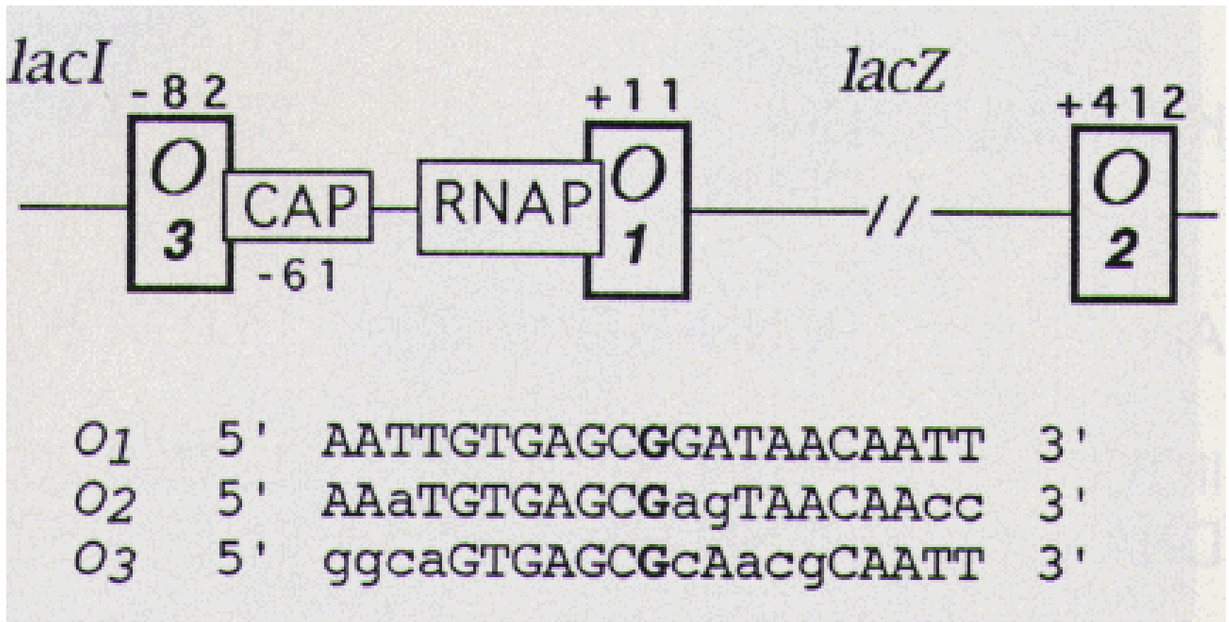


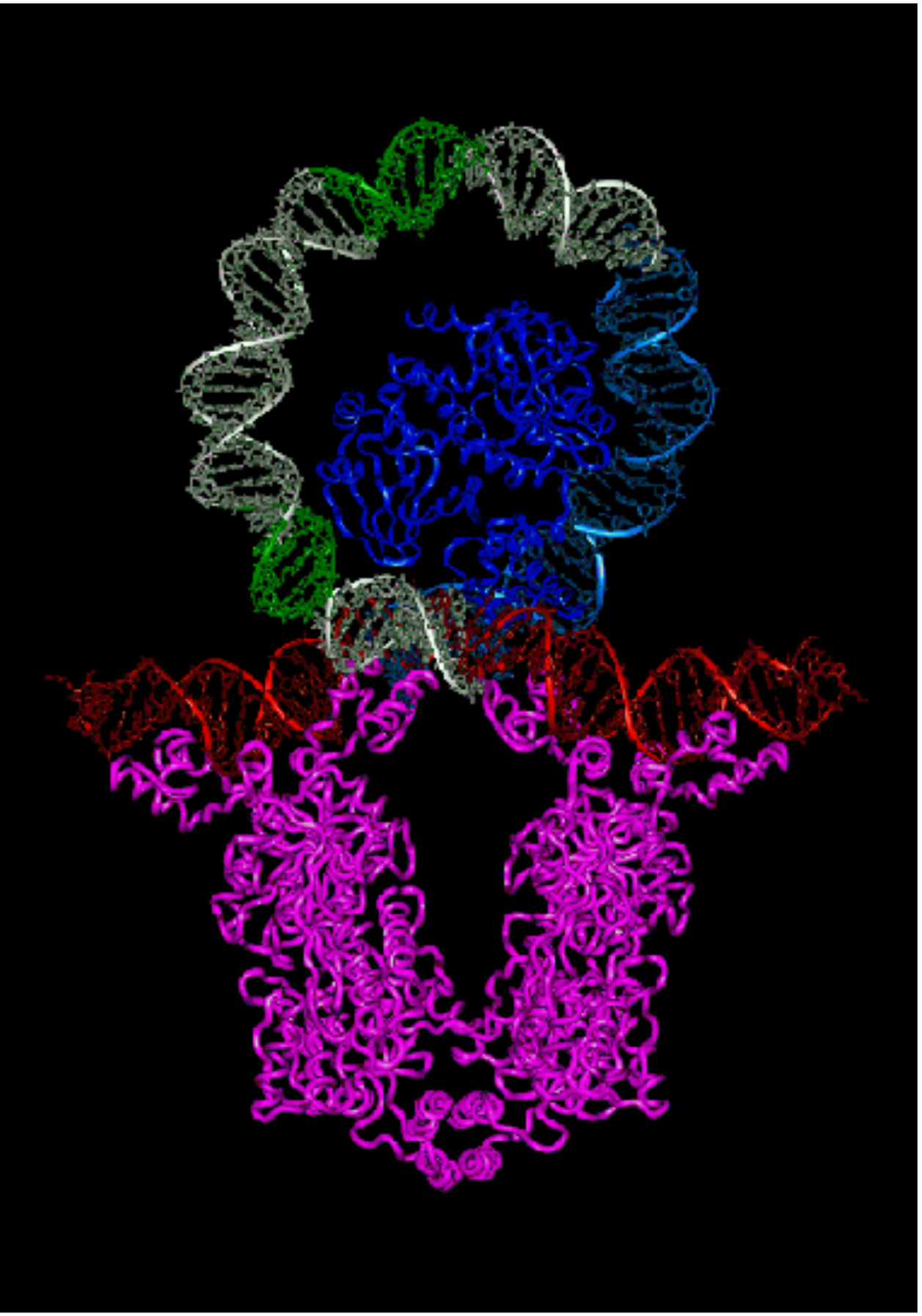
Control of gene expression, Chapter 7, MBC Alberts et al. 2022

Organization of the genes regulated by Lac repressor, a transcription repressor protein in the bacterium E. coli



Lac repressor binds to the operators O_1, O_2 and O_3





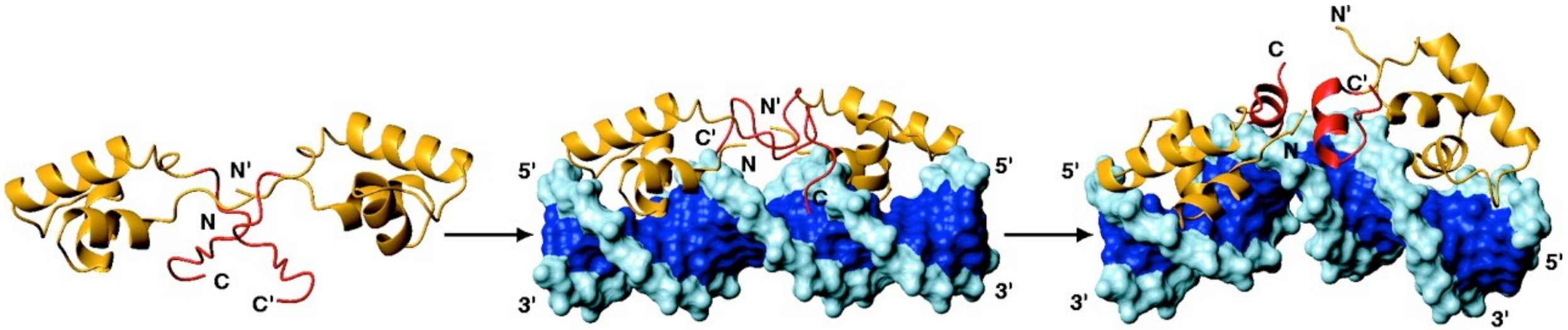
Model for the complex of CAP and LacI at the lac operator

← CAP

low glucose and low lactose
=> both CAP and LacI bound
=> repression

← Lac repressor bound to operator sites O1 and O3

The hinge region (50-62 in red) of Lac-DBD is folded only in the specific complex with DNA



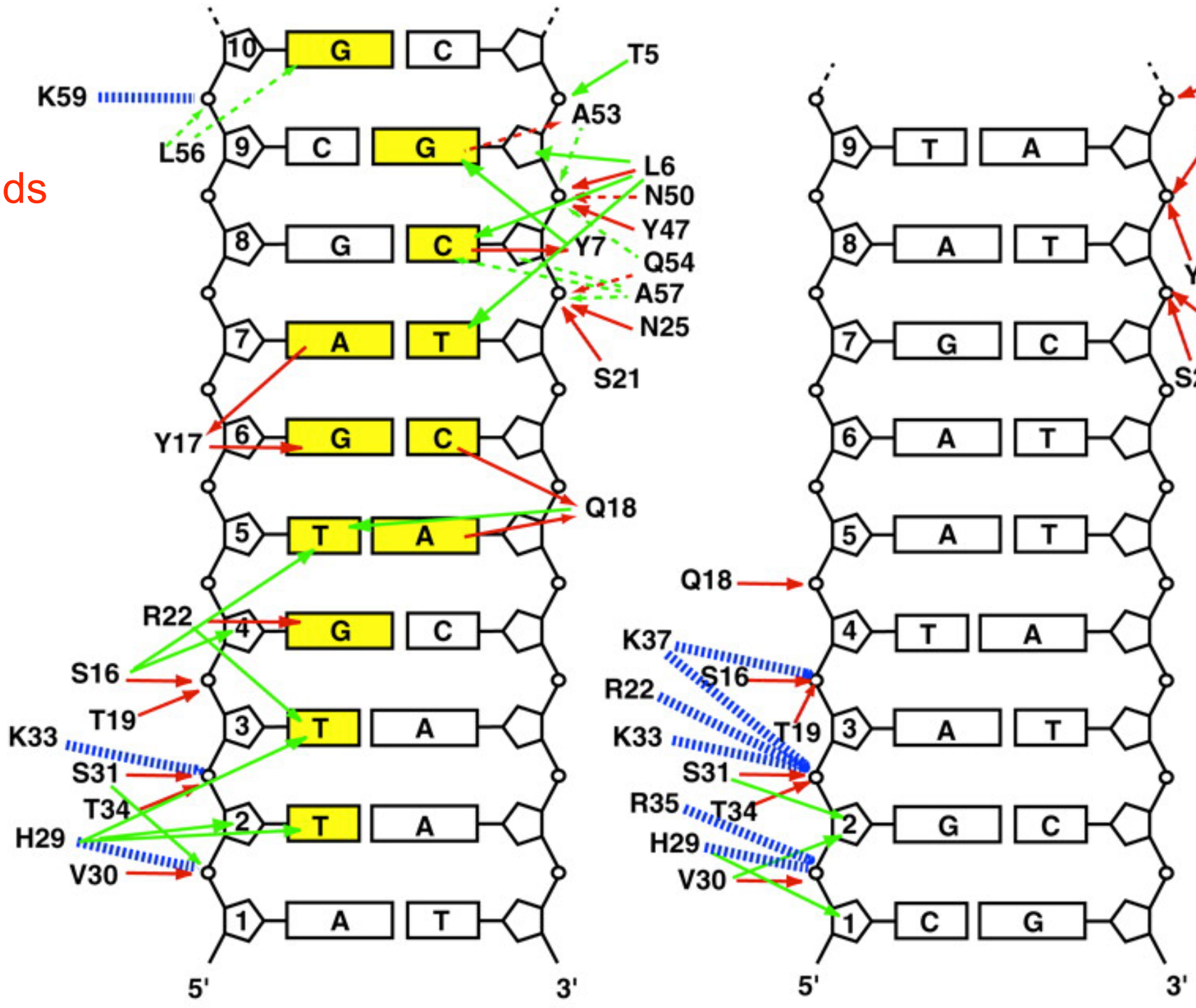
- folding of hinge region with specific contacts in minor groove
- specific interactions major groove
- less electrostatic interactions
- curvature of DNA

Specific (left) and nonspecific (right) protein-DNA contacts of Lac-DBD repressor with DNA

red:
hydrogen bonds

green:
hydrophobic
contacts

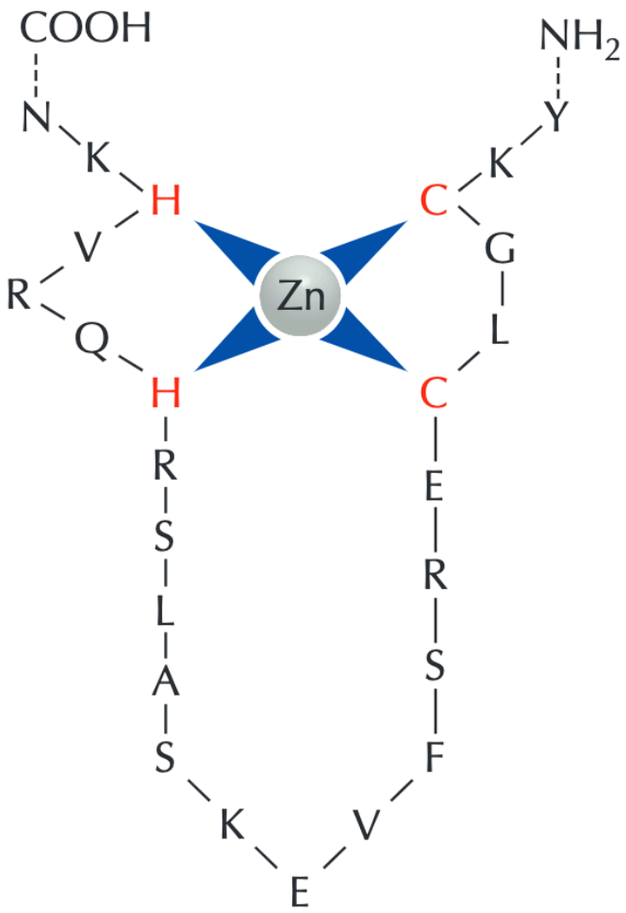
dashed blue:
electrostatic
contacts

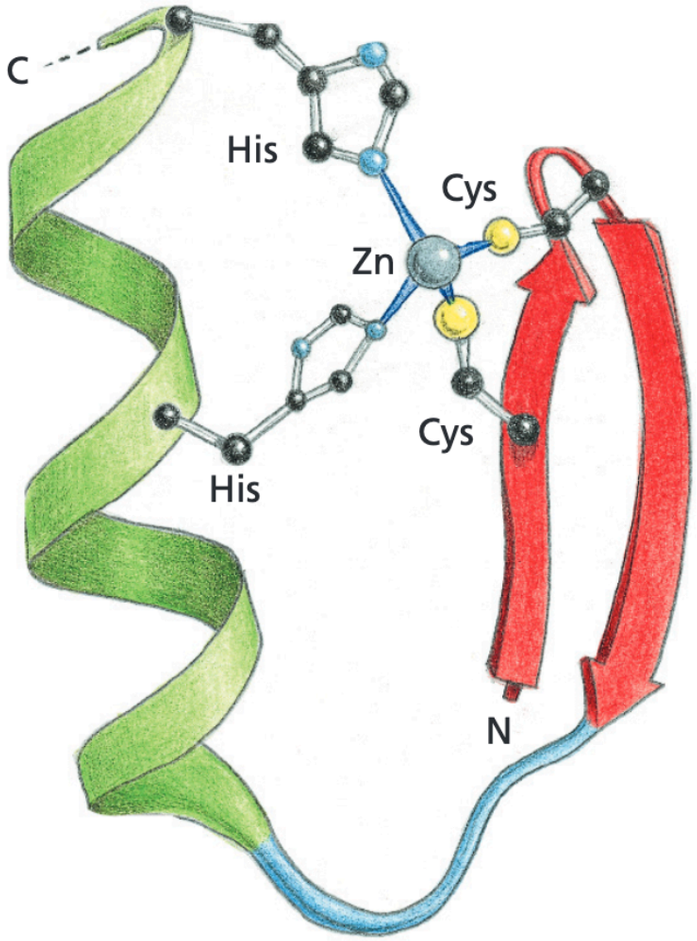


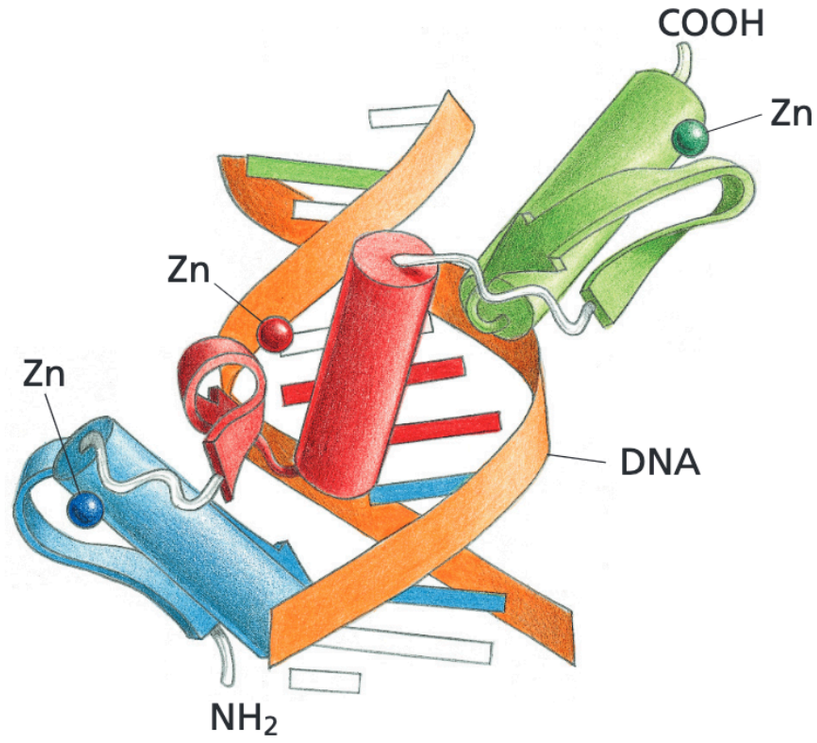
Specific complex

Non-specific complex

Zinc finger protein







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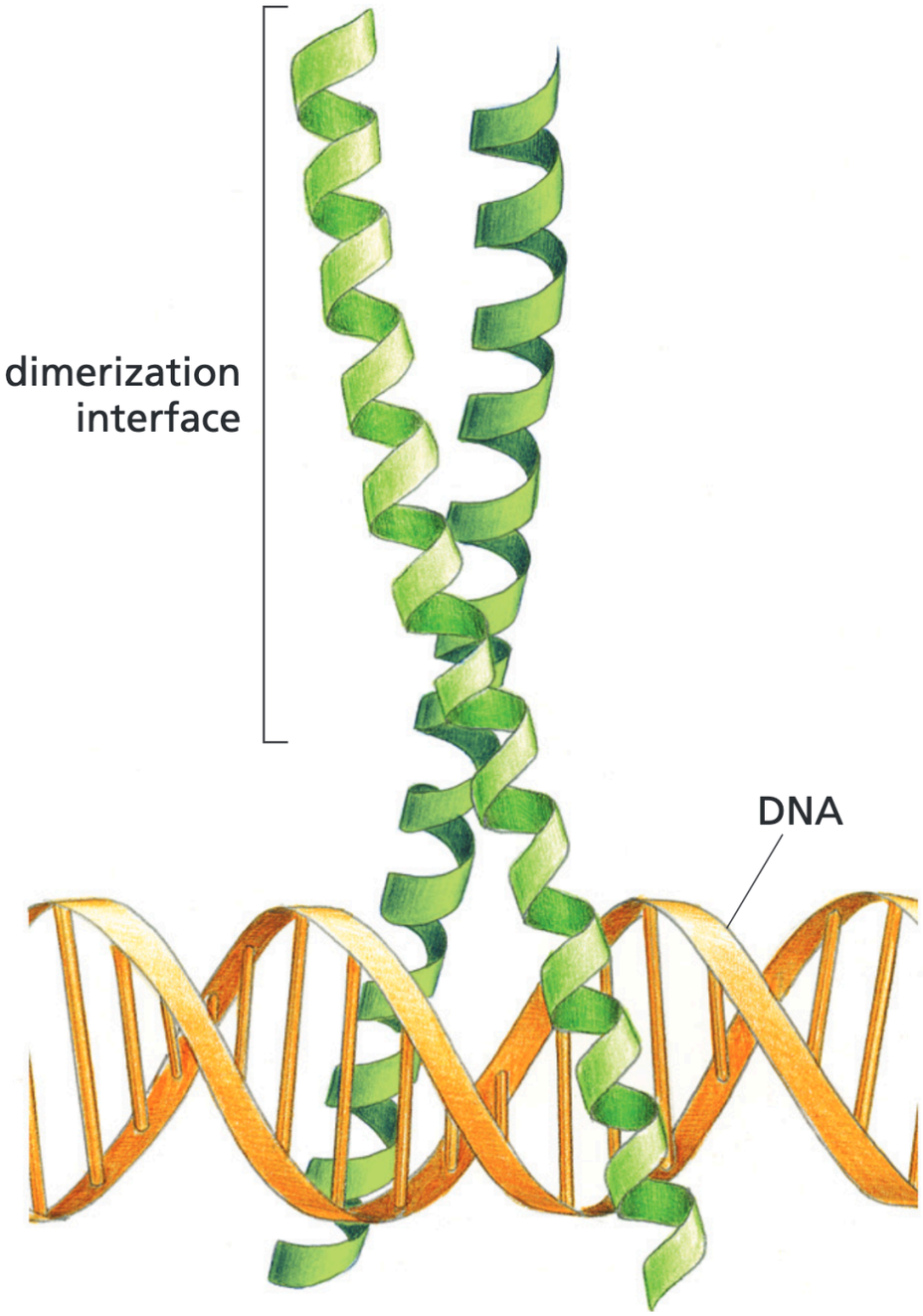
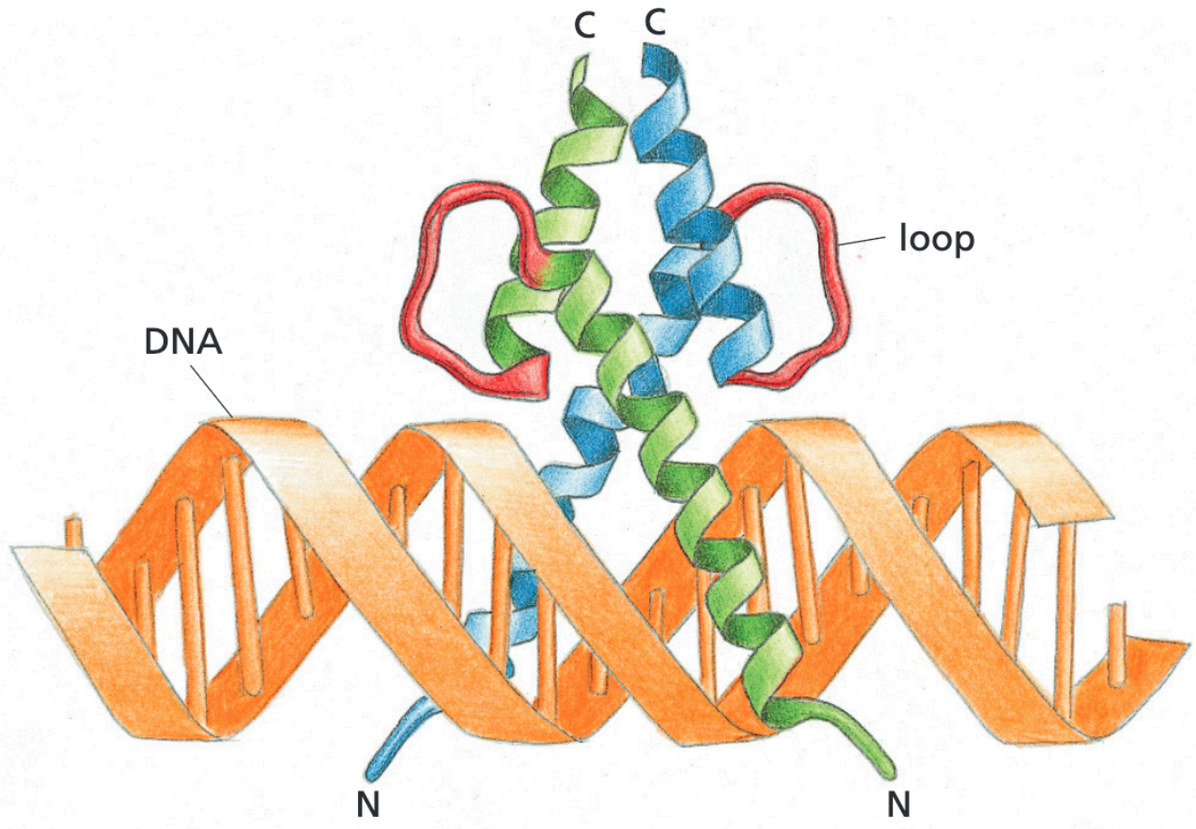
Leucine zipper proteins



Helix-loop-helix proteins



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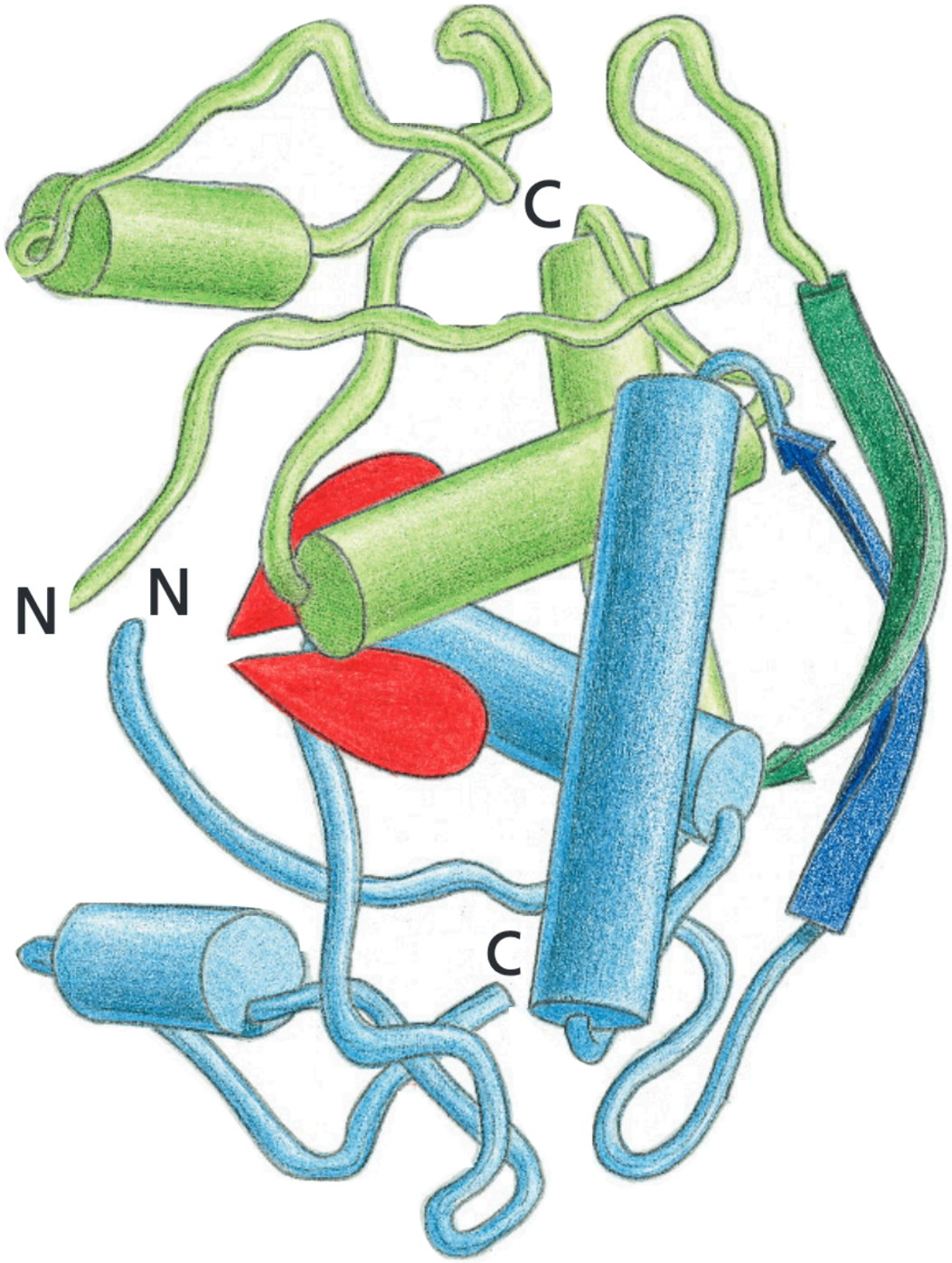
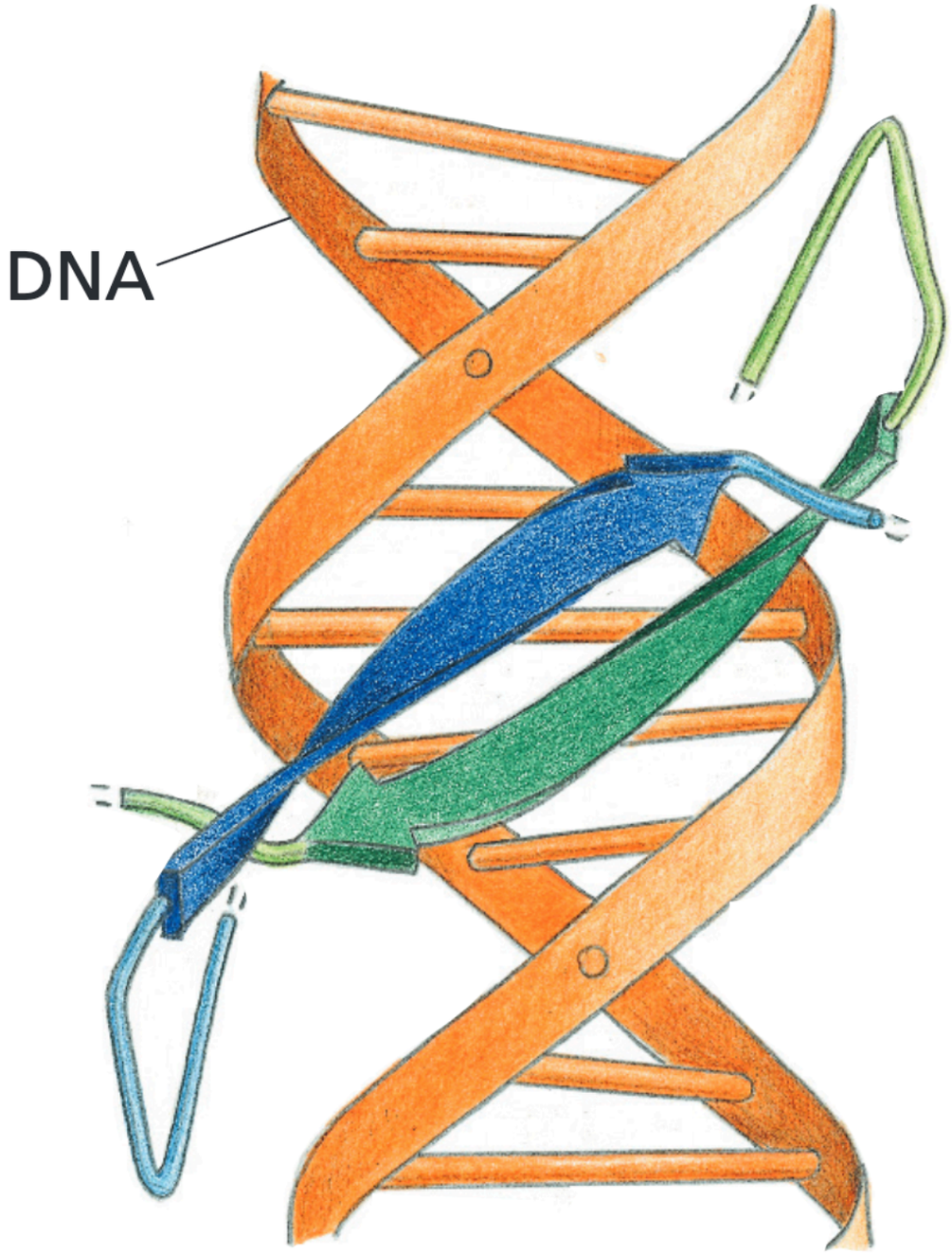
β-sheet DNA recognition proteins



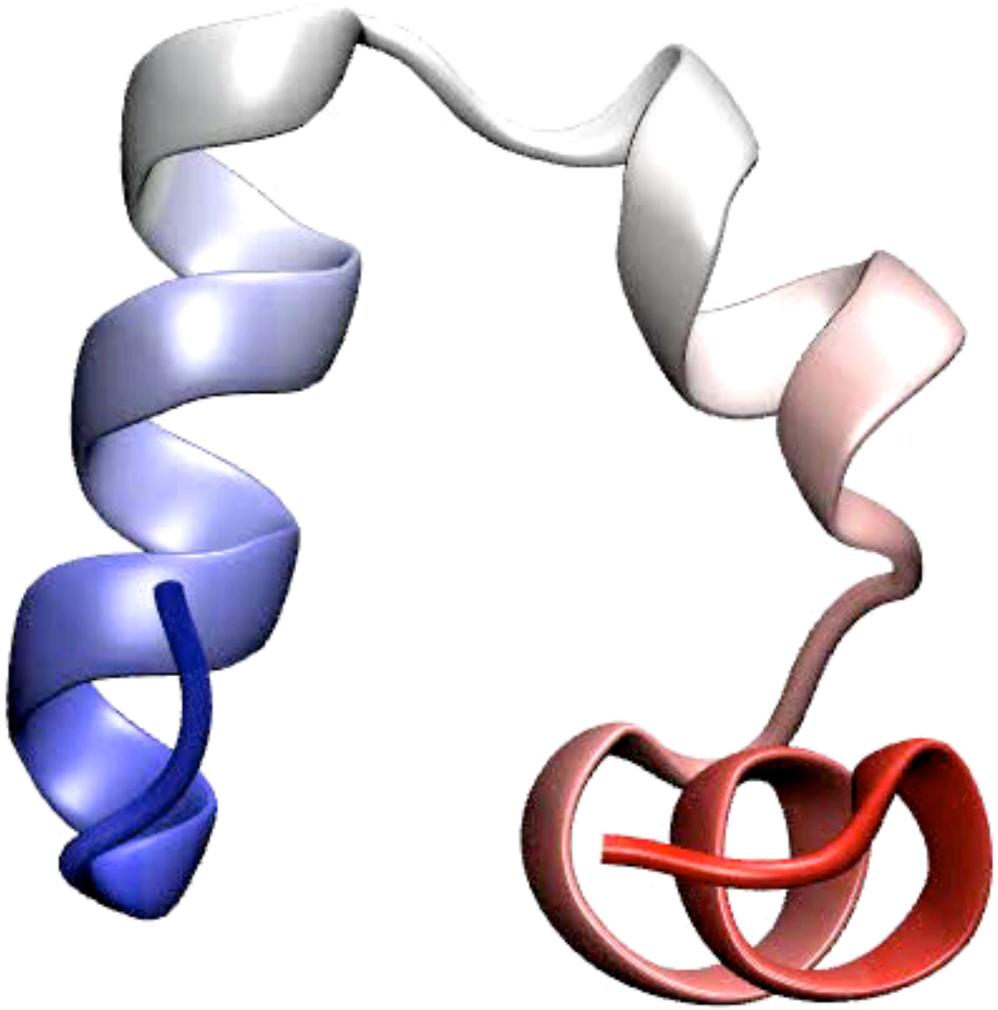


Control of gene expression, Chapter 7, MBC Alberts et al. 2022

Features of protein DNA interactions

- Unspecific electrostatic interactions of positively charge Arg and Lys with negatively charged sugar-phosphate DNA backbone
- Sequence recognition by non-covalent contacts between protein residues and bases
- Most contacts are in the major groove of DNA
- No simple recognition code between amino acid and base pair
- Frequently dimers that recognize palindromic sequence motifs
- 80% of regulatory proteins can be assigned to one of three classes:
  - helix-turn-helix (HTH)
  - zinc finger (Zn-finger)
  - leucine zipper (bZIP)
  - helix-loop-helix (HLH)
- In addition to DNA-binding domains, these proteins often possess other domains that interact with other proteins (“activation domain”)

What drives the folding of a protein?

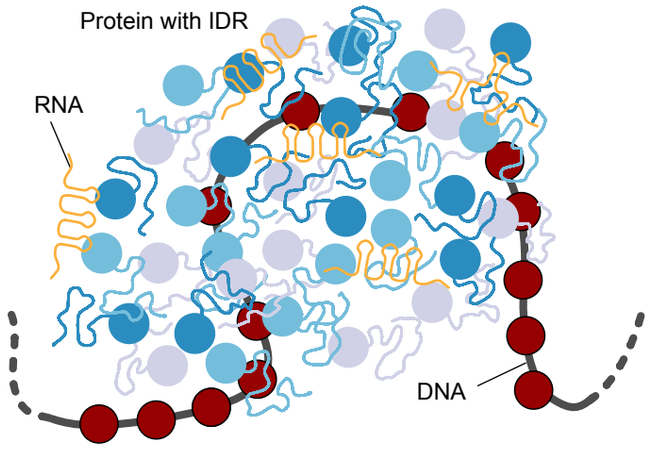


Protein folding as seen in molecular dynamics simulations of the villin protein headpiece (~10 μs time scale)

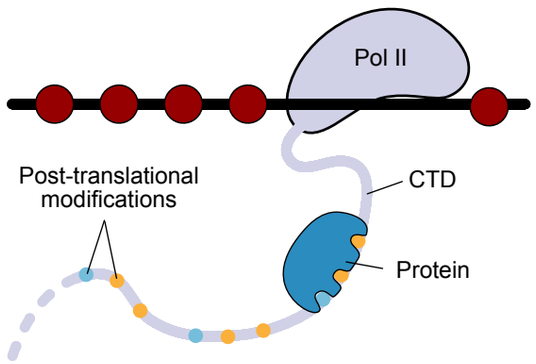
Peter L. Freddolino and Klaus Schulten. *Biophysical Journal*, 97:2338-2347, 2009

Intrinsically disordered protein regions (IDRs) can mediate macromolecular interactions

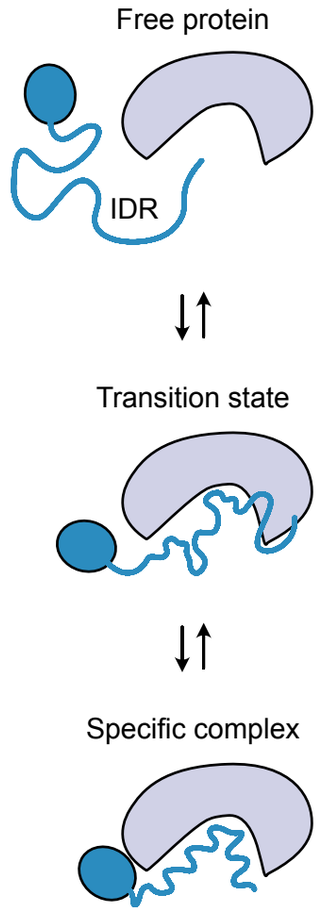
LLPS



Specific interactions with IDR

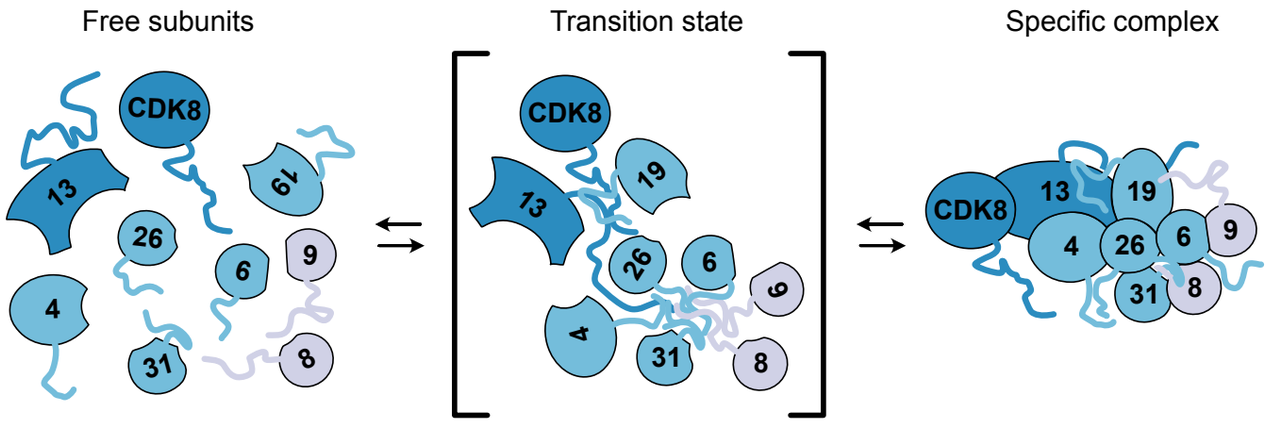


Induced folding interactions



Rippe & Papantonis 2022
Curr Opin Cell Biol

Increasing the kinetic rate for forming a multi-subunit complex



- 25–30% of eukaryotic proteins are predicted to be mostly disordered
- Half of all eukaryotic proteins have long regions (<50 aa) of disorder

Uversky 2021
Ann Rev Biophys

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY, MEDICAL CENTER, DURHAM, NORTH CAROLINA]

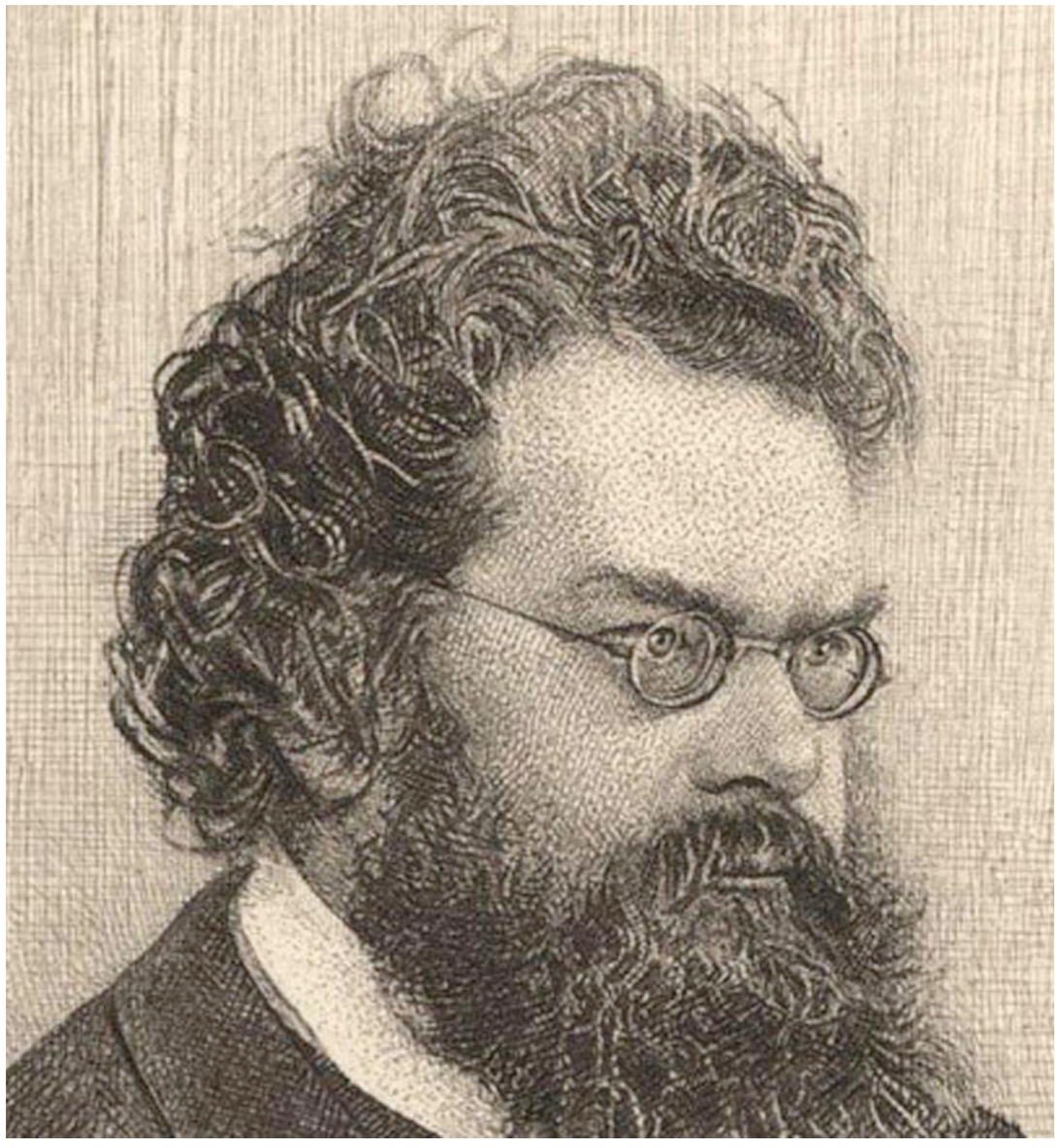
Contribution of Hydrophobic Interactions to the Stability of the Globular Conformation of Proteins

BY CHARLES TANFORD

RECEIVED APRIL 9, 1962

Tanford, C. *J Am Chem Soc* **84,** 4240–4247 (1962).

The Boltzmann constant



Ludwig Boltzmann

Definition of entropy as a measure of statistical disorder of a system

$$S \equiv k_B \ln W$$

- S: Molecular Entropy
- k_B: Boltzmann Constant
- W: Number of microscopic ways in which a particular outcome (macroscopic state) can be attained

Calculating entropy: how probable or disordered is the final state?

Entropy provides that measure (Boltzmann)…

$$S \equiv k_B \ln W$$

- S: Molecular Entropy
- k_B: Boltzmann Constant
- W: Number of microscopic ways in which a particular outcome (macroscopic state) can be attained

For Avogadro number's of molecules…

$$S = \underbrace{(N_{Avogadro} k_B)}_{R \text{ (gas constant)}} \ln W$$

Therefore: the most probable outcome maximizes entropy of <u>isolated systems</u>

Criterion for Spontaneity:

$\Delta S > 0$ (spontaneous)

$\Delta S < 0$ (non-spontaneous)

Unfavorable conformation entropy for protein folding

for the folded state: ~ 1 conformation

$$S_{\text{folded}} = R\ln(1) = 0$$

for the unfolded state: x is the number of flexible points per residue and z is the number or possible orientations of equal energy at each point.

$$S_{\text{unfolded}} = R\ln\left(z^{x}\right)$$

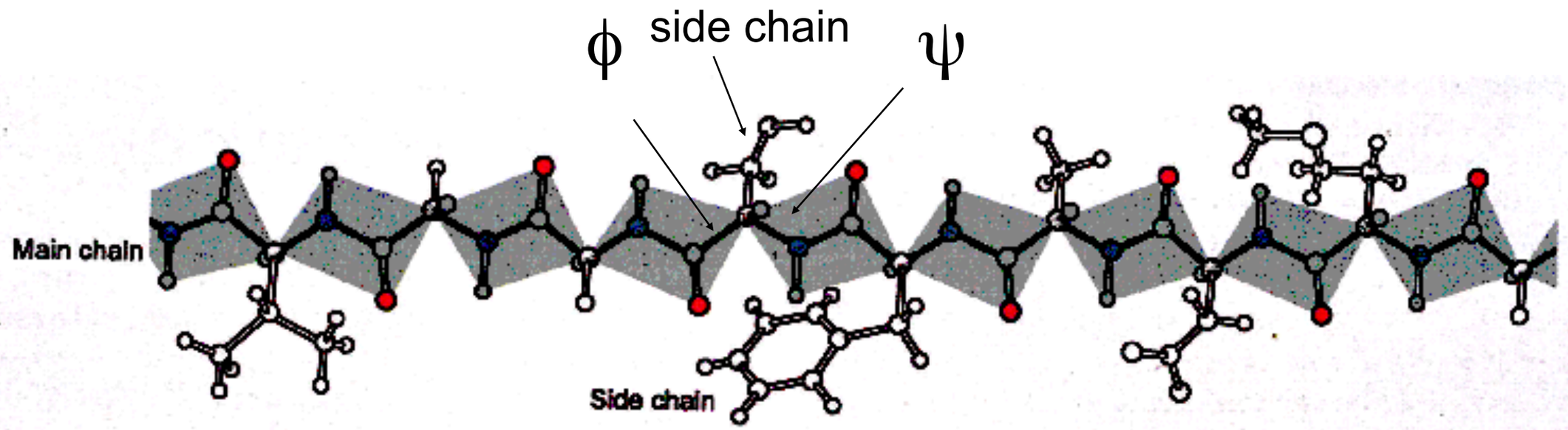
Estimating the unfavorable conformational entropy for protein folding

$$S_{\text{conf}} = R\ln\left(z^{x}\right) \qquad \Delta S_{\text{fold}} = R\ln\left(\frac{W_{\text{unfolded}}}{W_{\text{folded}}}\right)$$

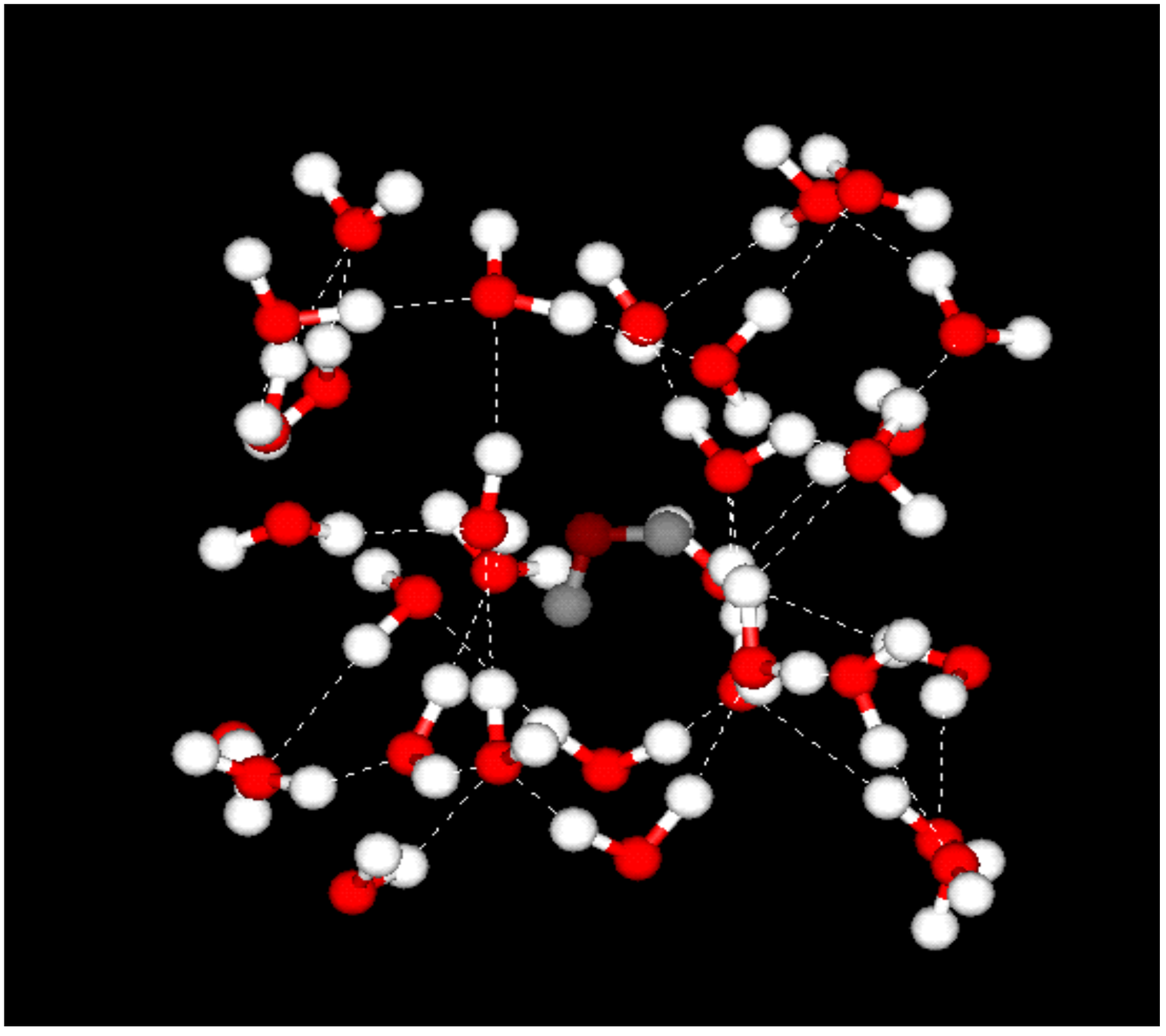
Tanford 1962: For three flexible positions (ϕ , ψ, side chain) with two possible orientations each we have 2^3 conformations per residue:

ΔS_{fold} = 8.31 J·mol^{-1}·K^{-1}·ln (⅛) = 17.3 J·mol^{-1}·K^{-1} or “entropy units” (e.u.).

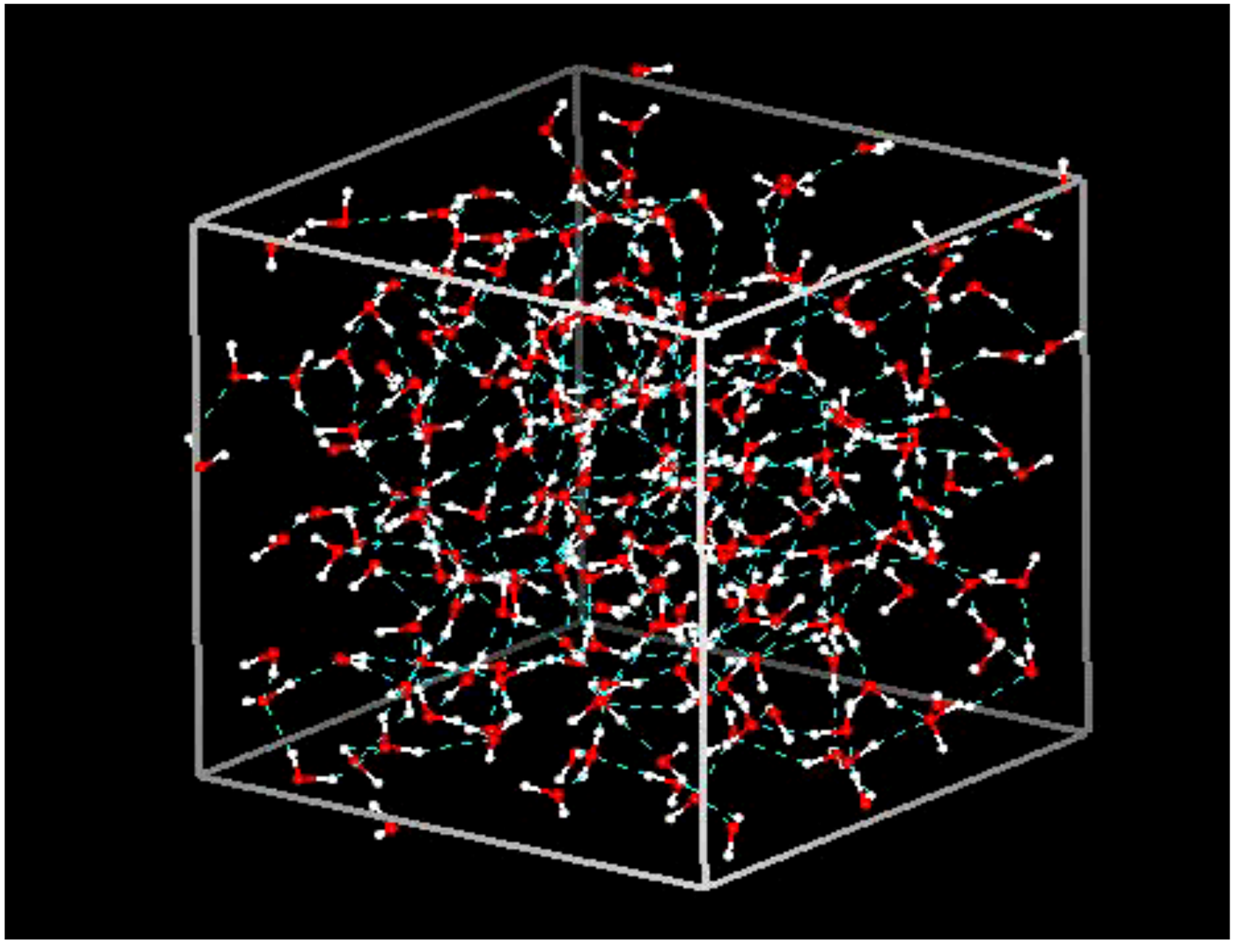
at 25 C: -TΔS =1.2 kcal/mol or 5.2 kJ/mol



Hydrogen bonding of liquid water

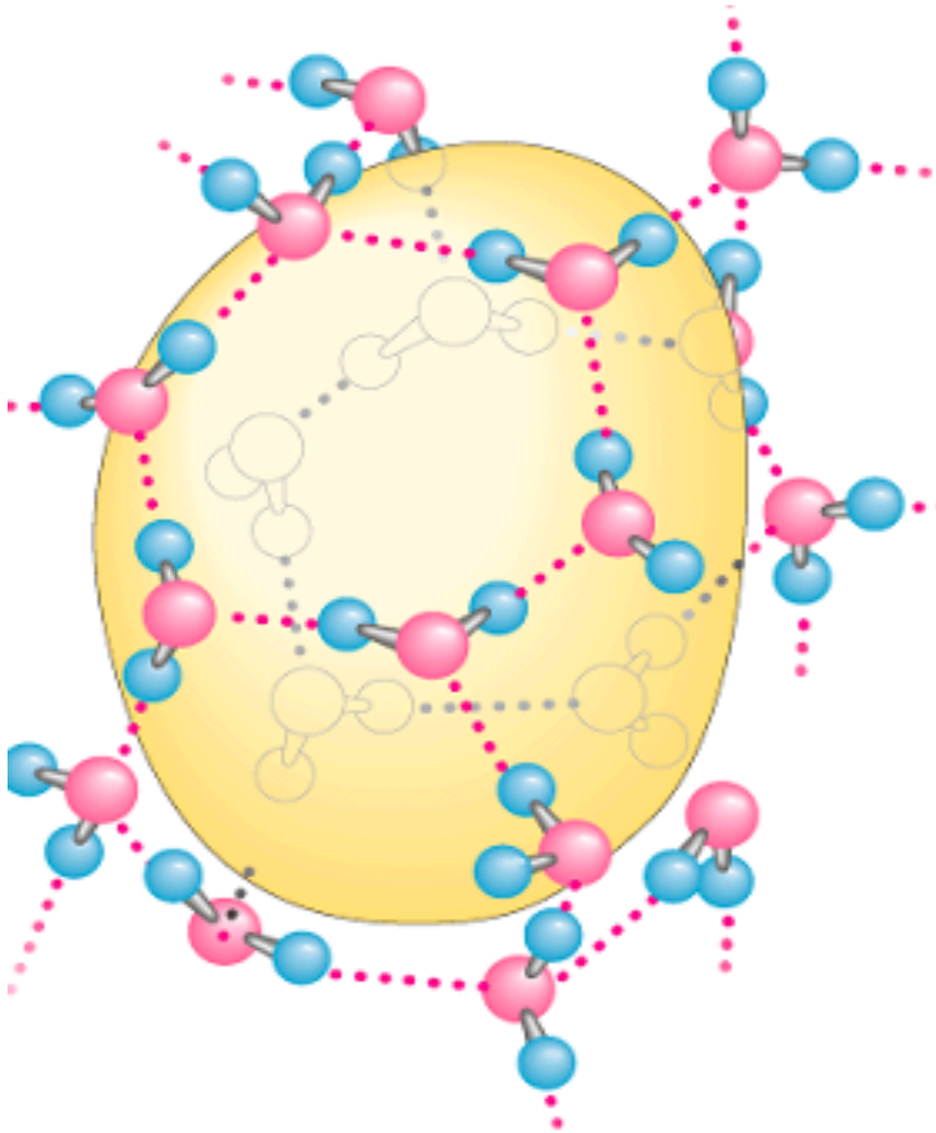


Hydrogen bonding of liquid water



The hydrophobic effect drives protein-protein and protein-DNA/RNA interactions in water

- Minimization of non-polar/water surface area leads to stability
- Complex mixture of physical properties
- Entropic contribution most significant
- Water must form a “cage” structure around non-polar surfaces

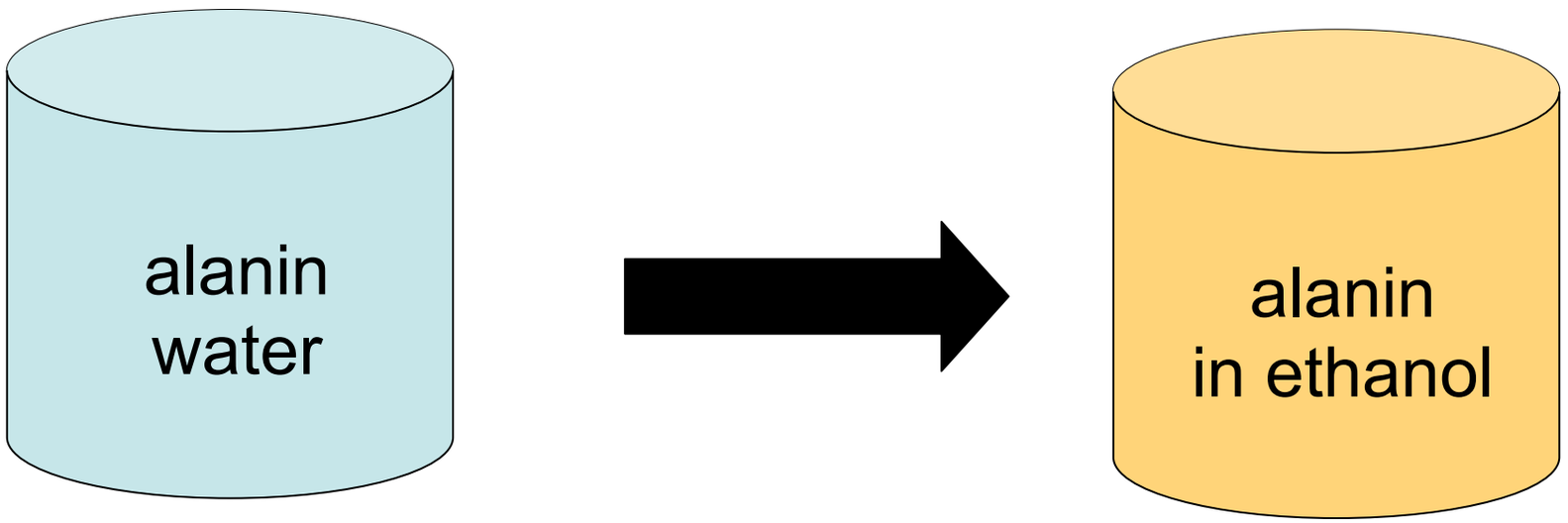


Measure solubility of amino acid in ethanol (= inside the folded protein) and in water (= unfolded state or at the protein surface)

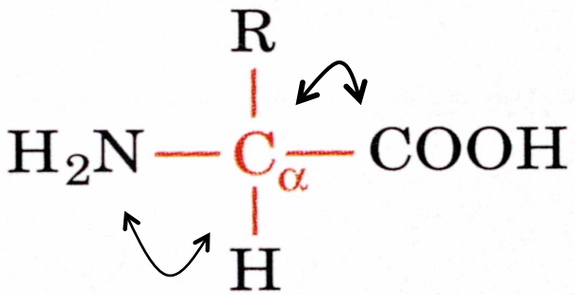
$$\text{partition coefficient } K_{\mathrm{D}} = \frac{\text{solubility } (Alanin_{\mathrm{EtOH}})}{\text{solubility } (Alanin_{\mathrm{H_2O}})}$$

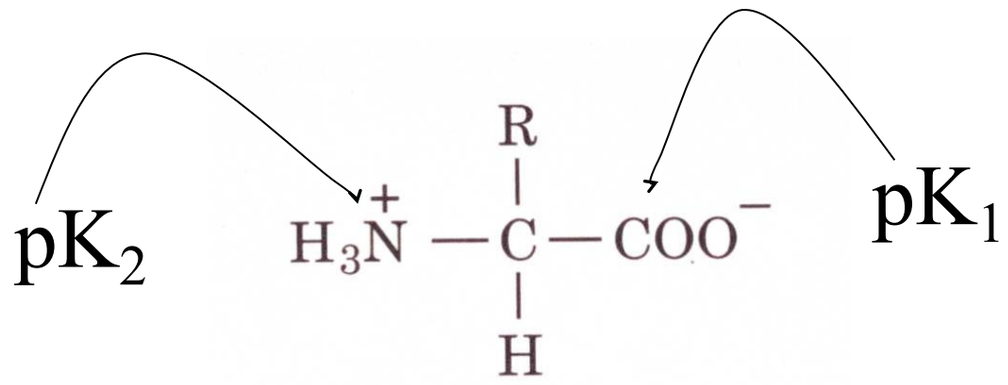
Calculate the free energy from transferring an amino acid from water to ethanol

$$\Delta G_{\mathrm{tr}} = -RT\ln\left(\frac{N_{\mathrm{EtOH}}}{N_{\mathrm{H_2O}}}\right) = -RT\ln\left(K_{\mathrm{D}}\right)$$



Free amino acids carry a positive and a negative charge that is not present in the peptide chain





α amino acids because of the α carboxylic and α amino groups pK_1 and pK_2 respectively pK_R is for R group pK's

$pK_1 \approx 2.2$ while $pK_2 \approx 9.4$

In the physiological pH range, both carboxylic and amino groups are completely ionized

TABLE I[a]

FREE ENERGY CHANGE IN CALORIES PER MOLE FOR TRANSFER FROM ETHANOL TO WATER AT 25°

Tanford 1962

	ΔF_t, whole molecule	Δf_t, side chain contribution	
Non-polar side chains			
Glycine	−4630	0	
Alanine	−3900	+ 730	
Valine	−2940	+1690	
Leucine	−2210	+2420	
Isoleucine	−1690[b]	+2970[b]	
Phenylalanine	−1980	+2650	
Proline	−2060[c]	+2600[c]	
Other side chains			
Methionine	−3330	+1300	
Tyrosine	− 930[d]	+2870[d]	
Threonine	−4190	+ 440	
Serine	−4590	+ 40	
Asparagine	−4640	− 10	
Glutamine	−4730	− 100	
Aspartic acid[e]	−4090	+ 540	uncharged
Glutamic acid[e]	−4080	+ 550	uncharged

Burying a charged amino acid in the interior (Born expression)

$$W_B = \frac{q^2}{4\pi\varepsilon_0 r}\left(\frac{1}{\varepsilon_1} - \frac{1}{\varepsilon_2}\right)$$

W_B is the free energy of transfer in moving a charged body from a region with a relative dielectric constant ε_2 to a medium with a with a relative dielectric constant ε_1. The parameter r is the radius of the charge.

q (charge of an electron) = 1.60 x 10^{-19} C
dielectric constant in vacuum ε_0 = 8.85 x 10^{-12} C^2 J^{-1} m^{-1}
r is ionic radius, with is typically 1-2 Å

For ε_1 = 2-8 and ε_2 = 80 (H_2O) => ΔG_{tr} = +30 to 50 kcal/mol

Sharp, K.A. and Honig, B. (1990) Electrostatic interactions in macromolecules: theory and applications. Annu Rev Biophys Biophys Chem, 19, 301-332.

Tanford 1962

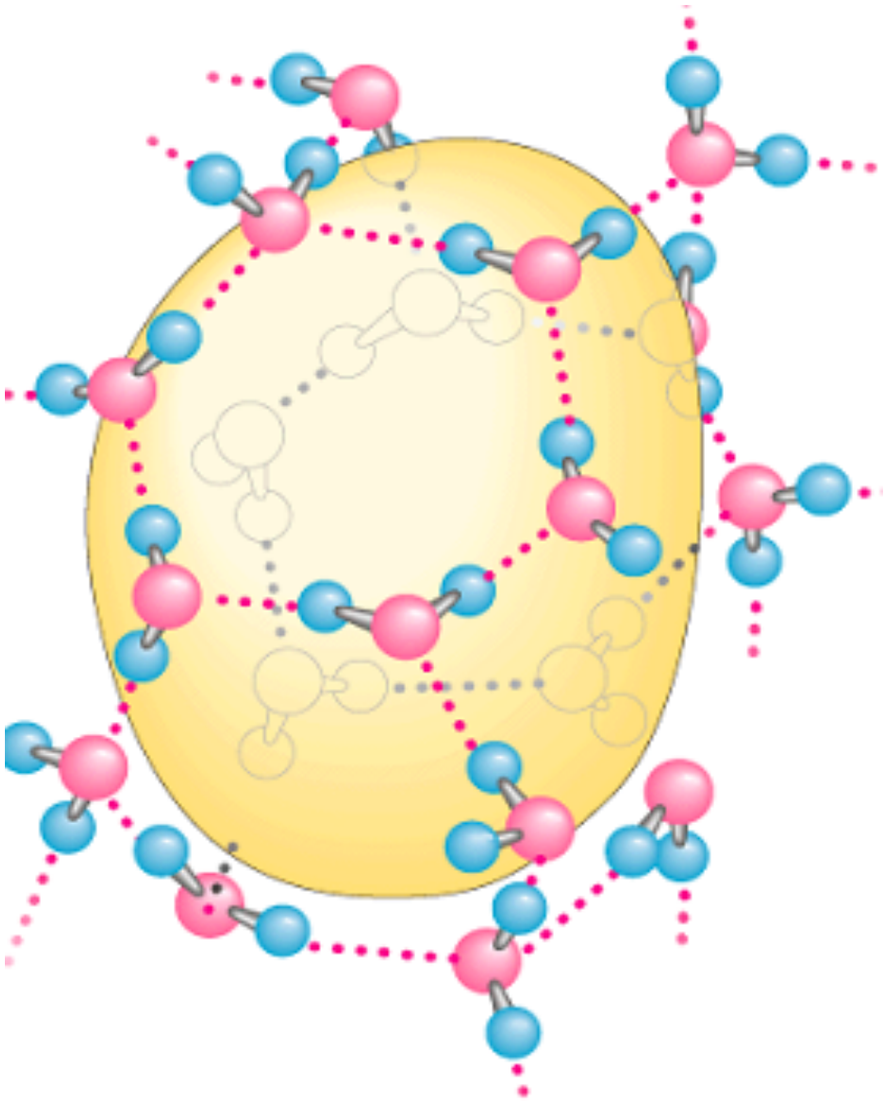
TABLE III

CONTRIBUTION OF THE MOST IMPORTANT HYDROPHOBIC INTERACTIONS TO THE FREE ENERGY OF UNFOLDING AT 25°

Side chain	Δf_u per side chain, cal./mole	Number present in myoglobin[a]	Number present in β-lactoglobulin[b]	Number present in ribonuclease[c]
Tryptophan	3000	2	2	0
Isoleucine	2970	9	10	3
Tyrosine	2870	3	4	6
Phenylalanine	2650	6	4	3
Proline	2600	4	8	4
Leucine	2420	18	22	2
Valine	1690	8	10	9
Lysine	1500	19	15	10
Methionine	1300	2	4	4
Alanine	730	17	14	11
Arginine	730	4	3	4
Threonine	440	5	8	10
Total number of residues		153	162	124
$-T\Delta S_{conf}$, kcal./mole		−184	−194	−149
$\Sigma\Delta f_u$, kcal./mole		+173	+192	+100

conformation entropy
hydrophobic effect

The hydrophobic effect drives protein-protein and protein-DNA interactions in water

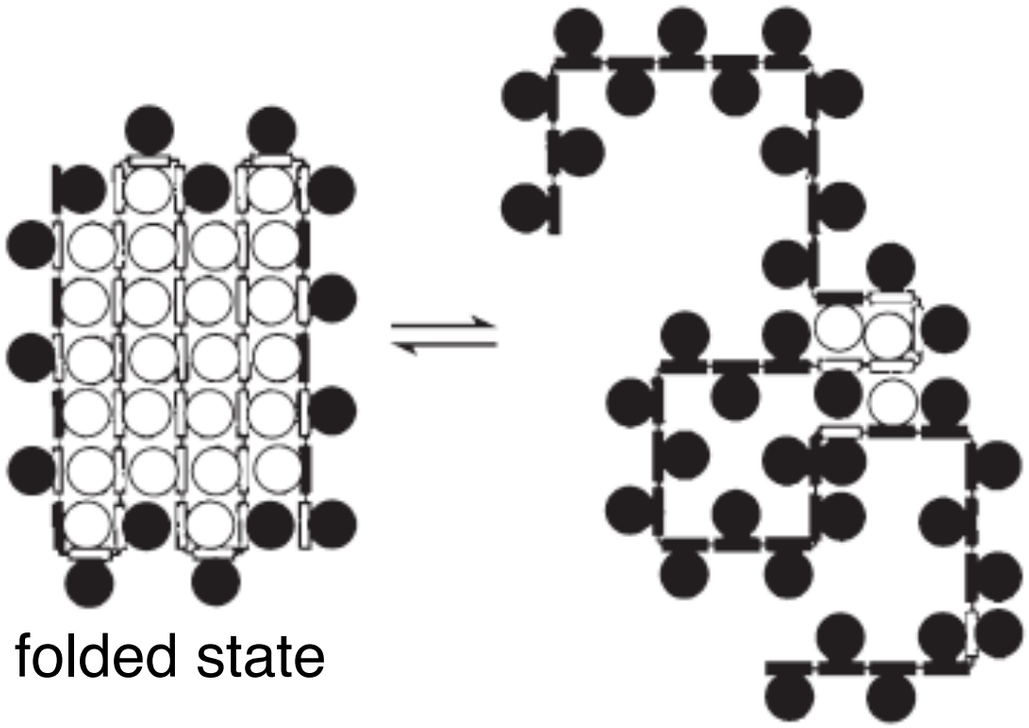


- Minimization of non-polar/water surface area
- Entropic contribution most significant
- Water“cage” around non-polar surfaces

Side-chain of amino acid residue	A^{np} (Å^2)	A^{pol} (Å^2)
Asp	48	58
Gln	53	91
Glu	61	77
Lys	119	48
Ala	67	—
Val	117	—
Leu	137	—
Ile	140	—

J. Mol. Biol. (1990) **213**, 375–384

Protein folding minimizes water accessible unpolar surfaces



folded state

random coil

Entropy change from
the number of states

$$\Delta S_{\text{fold}} = R \ln\left(\frac{W_{\text{unfolded}}}{W_{\text{folded}}}\right)$$

Folded: ~1 conformation
Unfolded: ~2^3 states (per aa)

ΔS_{fold} (2^3 states) = 8.31 J·mol^{-1}·K^{-1}·ln (⅛)
= 17.3 J·mol^{-1}·K^{-1} or “entropy units” (e.u.).

-T(25 ºC) x ΔS =1.2 kcal/mol or 5.2 kJ/mol

“Burying” an unpolar side chain: Leu = -2.4 kcal/mol, Val = -1.7 kcal/mol

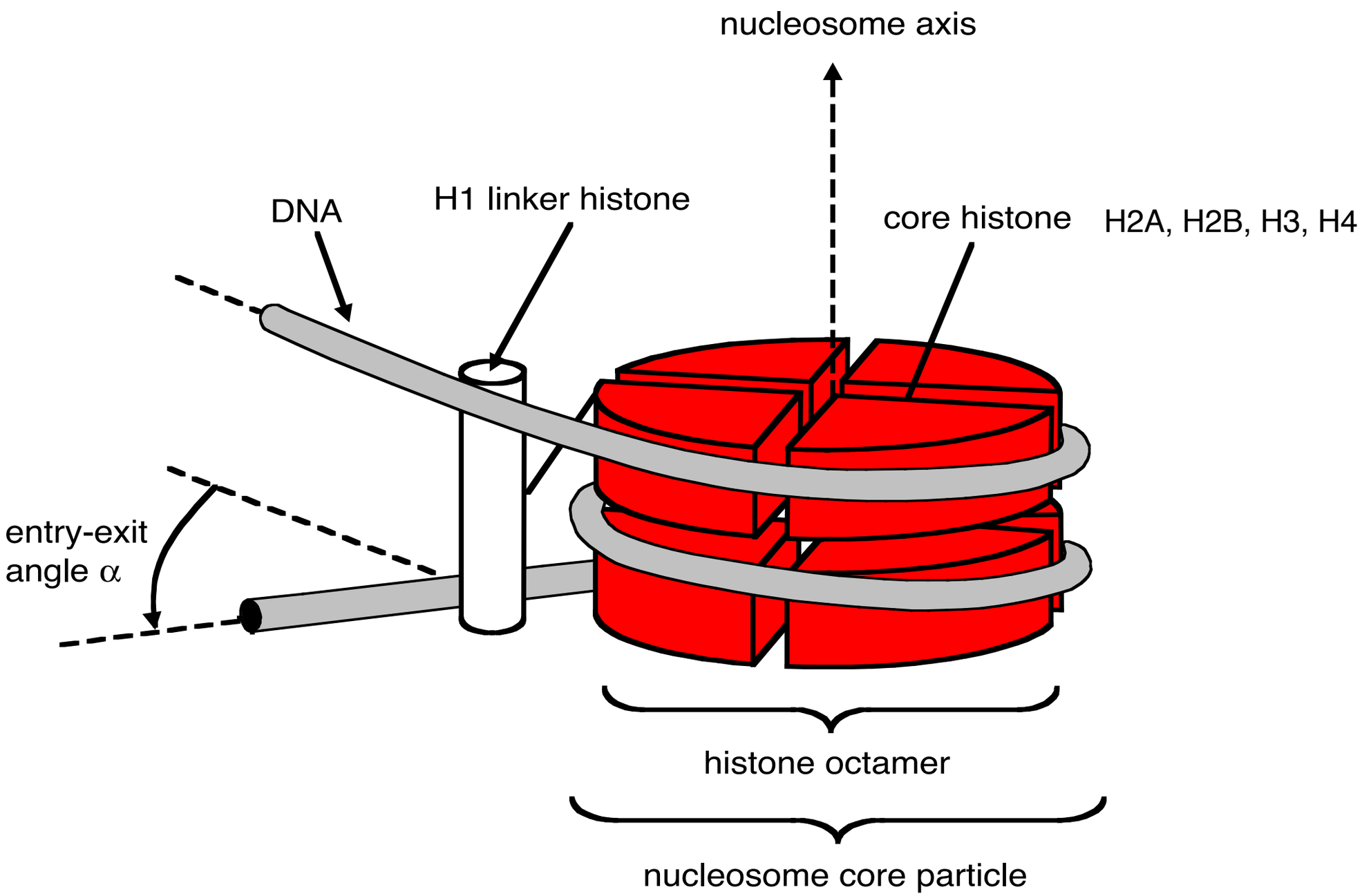
“Burying” a negative/positive charge: ΔG_{tr} = +30 to 50 kcal/mol

Making interactions between proteins and double-stranded DNA

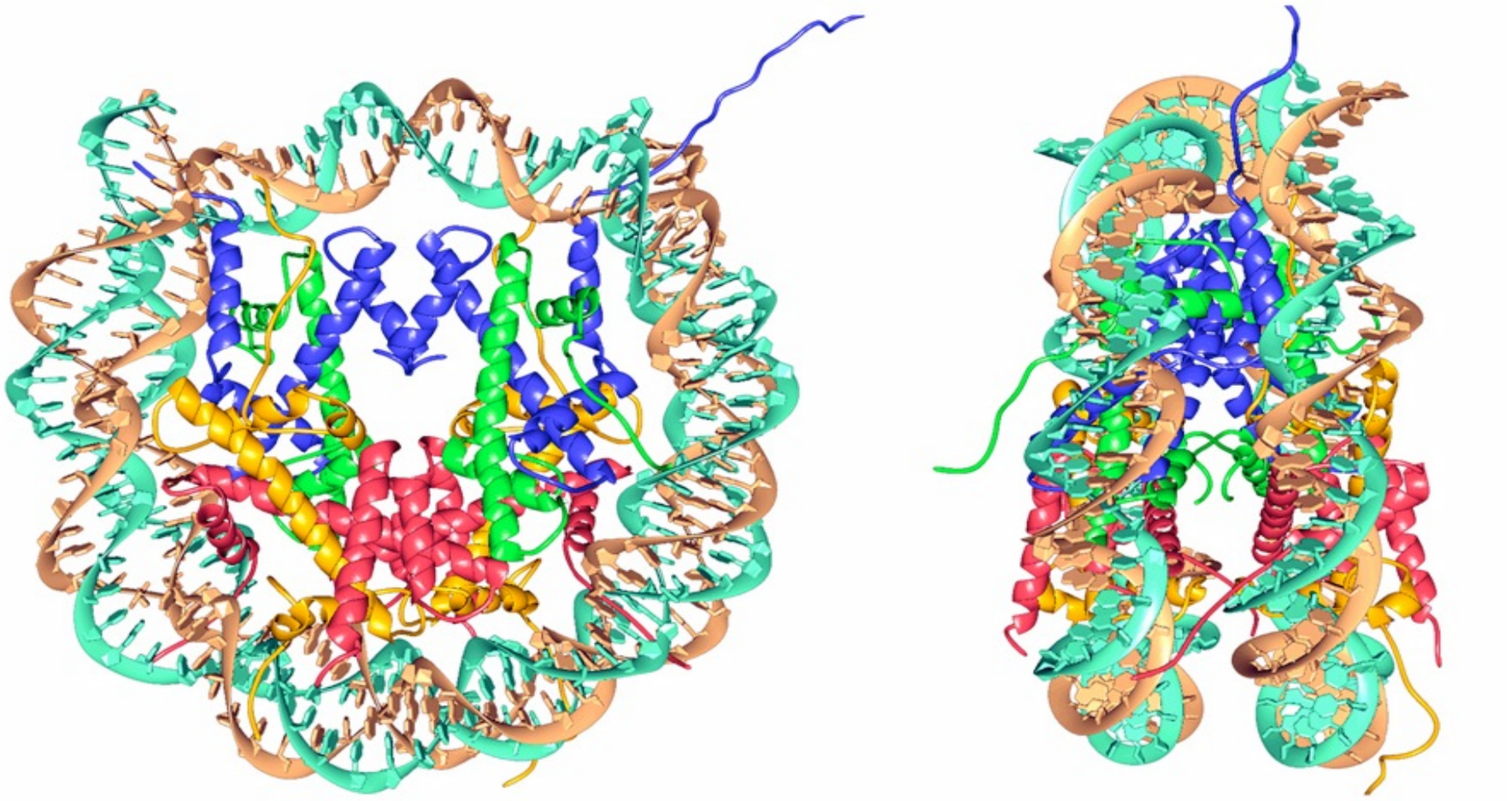
Protein-DNA interaction

- Sequence independent
  – may interact with the negatively charged sugar-phosphate backbone
- Sequence dependent
  – need to recognize the bases *in the double-helical structure* (don't have access to the atoms involved in base pair H-bonds)

Histone octamer - Nucleosome



Nucleosome crystal structure



Histones in the nucleosome

- Histone proteins are Lys & Arg rich and highly positiviely charge (“basic”)
- 2 copies of H2A-H2B dimer and (H3-4)2 tetramer form octamers
- Basic histones interact with the negatively charged DNA phosphates
- The DNA is wrapped around protein core in ~1.7 turns