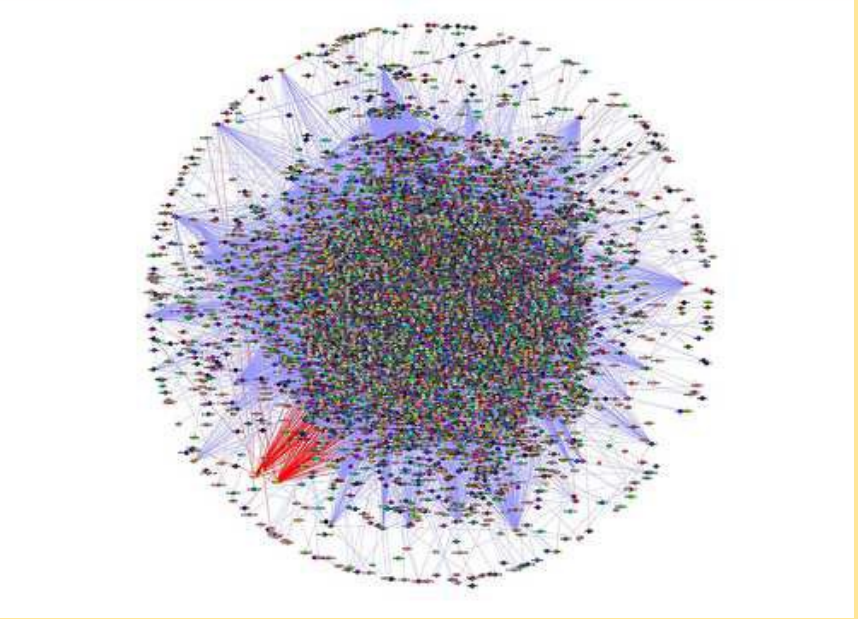
TEST

Interaktom

- soubor všech interakcí v dané buňce nebo organismu
- meziproteinových – protein.protein
- protein–DNA interaktom
- ……



- Interactions between molecules are central to how cells detect and respond to signals and affect:
  - Gene expression (transcription & translation)
  - DNA replication, repair and recombination
  - Signalling
  - And many other processes....
- Interactions are (mainly) mediated by many weak chemical bonds (van der Waals forces, hydrogen bonds, hydrophobic interactions)
- Accumulation of many bonds influences affinity and specificity of interactions

Protein-protien

- Dvouhybridní systém
- Ko-imunoprecipitace
- Knihovna „Phage display“
- „Pull-down assay“
- „Far-western blotting“
- Proteinová „microarray“

Protein-Nucleic Acid Interactions

- A wide range of Biophysical Chemistry methods have been used to study interactions between proteins and nucleic acids
- Particularly good for determining the strength (**affinity**) of the interactions
  - ➢ High affinity, µM – nM: tend to involve sequence-specific interactions, e.g. restriction enzymes
  - ➢ Low affinity, mM – µM: proteins tend to recognise aspects of "overall" structure i.e. not sequence-dependent

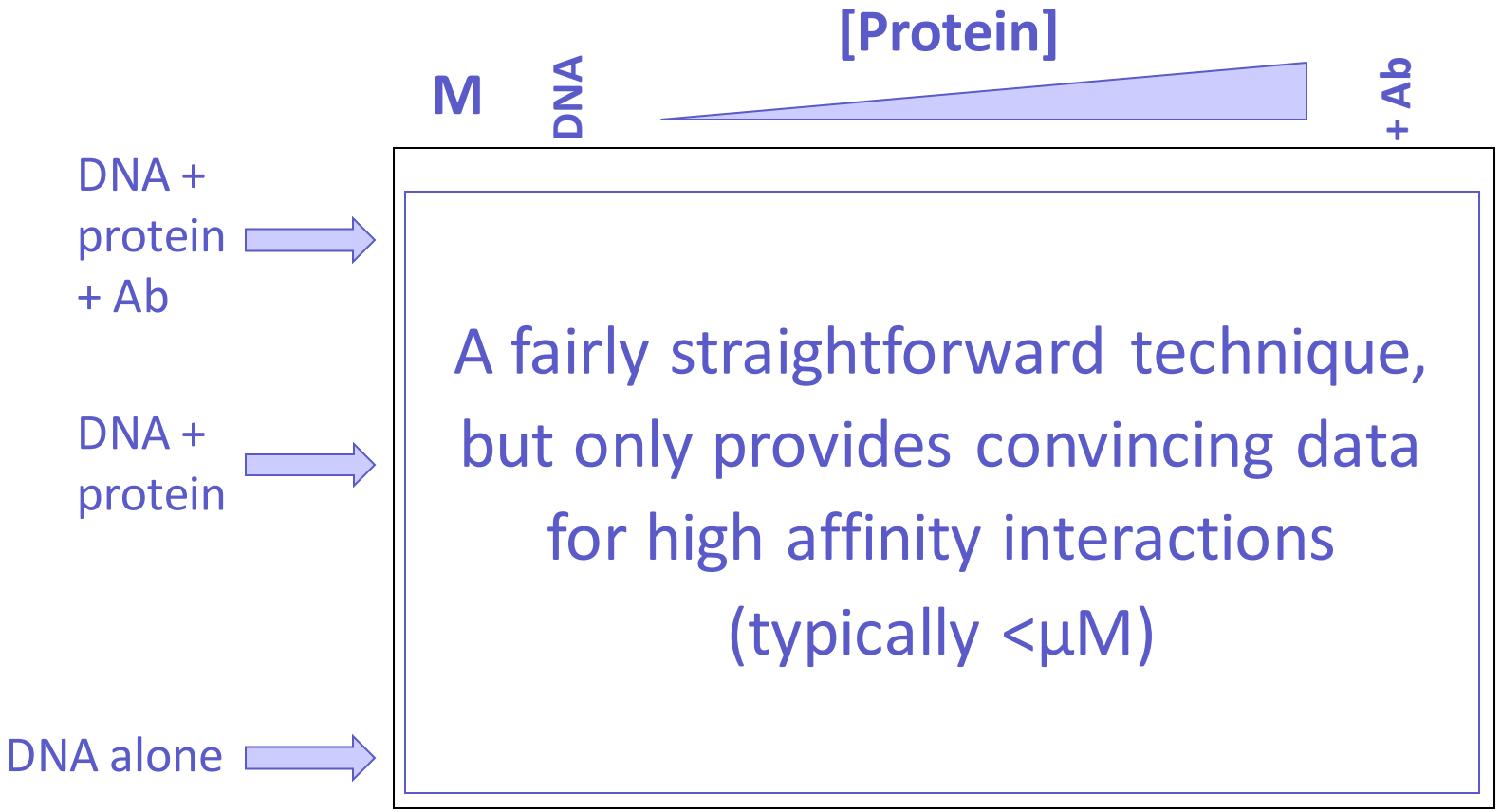
Biophysical Chemistry Approaches for Studies of Molecular Interactions

- Wide range of Biophysical Chemistry approaches are useful for studying molecular interactions:
  - NMR
  - X-ray crystallography
  - SPR (Surface plasmon resonance)
  - ITC (Isothermal titration calorimetry)
  - CD spectroscopy (Circular dichroism)
  - Gel electrophoresis
  - EPR (Electron paramagnetic resonance spectroscopy)
  - Mass spectrometry
  - Fluorescence
  - **Footprinting**

Many of these techniques are particularly useful for determining the strength (affinity) of interactions

EMSA ("Gel Shift" Assay)

- Electrophoretic Mobility Shift Assay (EMSA) or "gel shift" can provide information about protein-NA interactions

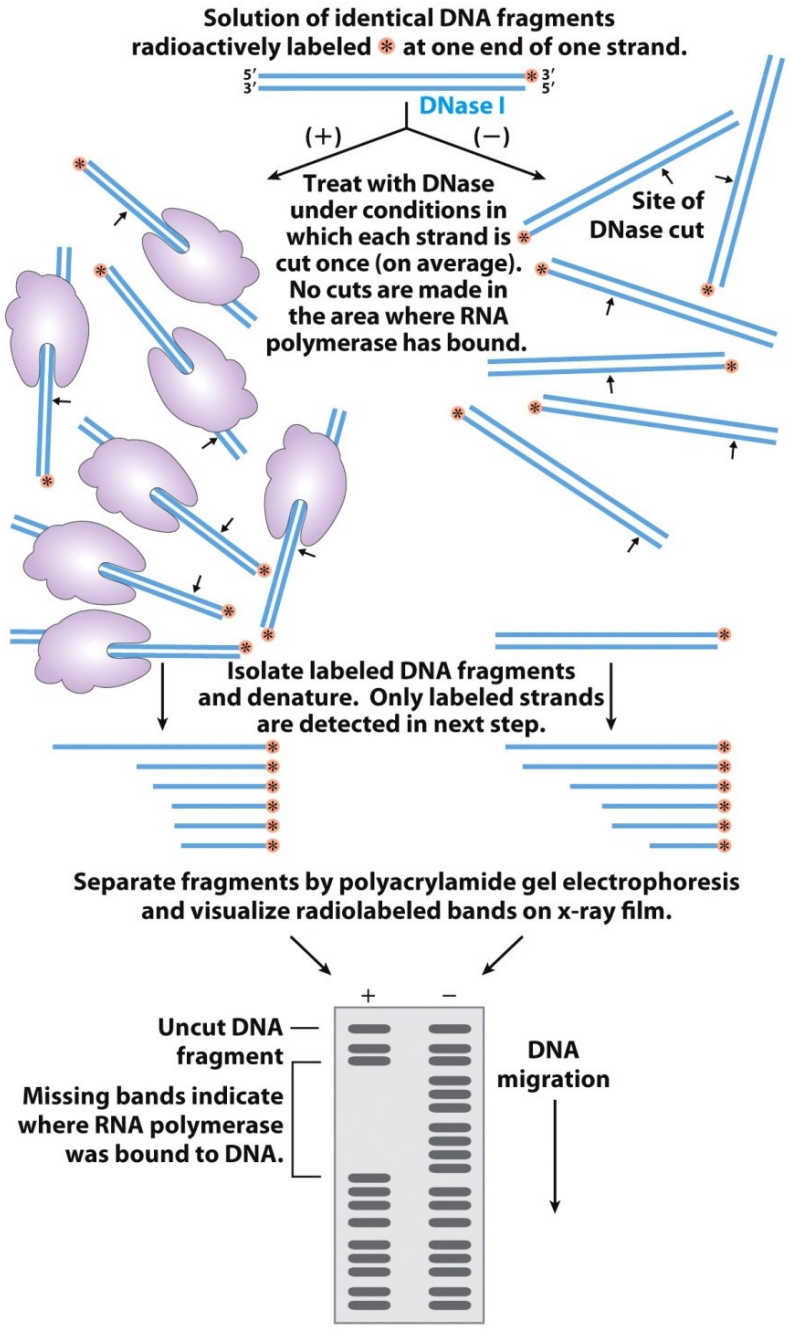


“Footprinting” is a Technique to Identify a DNA-binding site

Premise: DNA bound by protein will be protected from chemical cleavage at its binding site

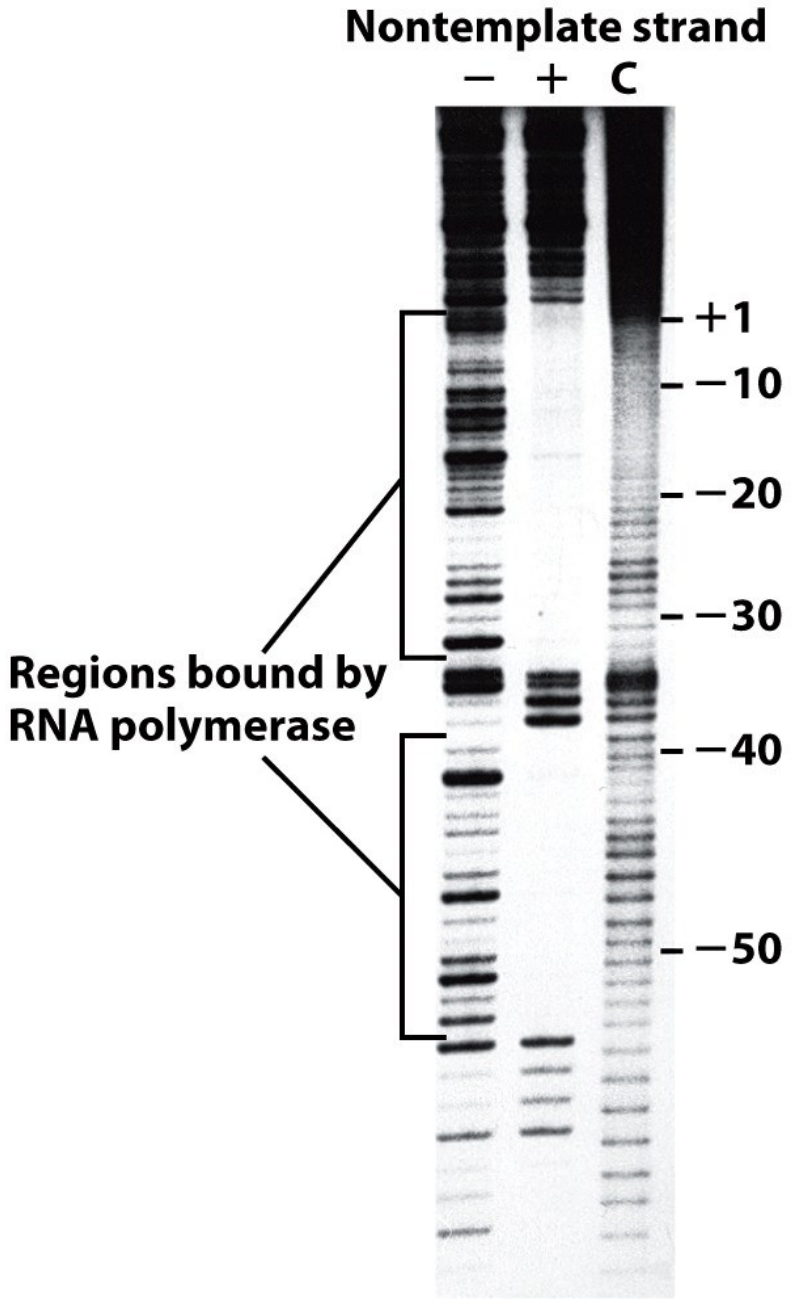
1) Isolate a DNA fragment thought to contain a binding site and “label” it
2) Bind protein to DNA in one tube; keep another as a “naked DNA” control
3) Treat both samples with chemical or enzymatic agent to cleave the DNA
4) Separate the fragments by gel electrophoresis and visualize bands on X-ray film or imager plate

Protein-DNA Footprinting



Box 26-1 Figure 1
Lehninger Principles of Biochemistry, Sixth Edition

Footprinting Results of RNA Polymerase Bound to Promoter



Box 26-1 Figure 2
Lehninger Principles of Biochemistry, Sixth Edition

Binding of Proteins to DNA Often Involves Hydrogen Bonding

- **Gln/Asn** can form specific H-bond with Adenine's N-6 and H-7 H's
- **Arg** can form specific H-bonds with Cytosine-Guanine base pair

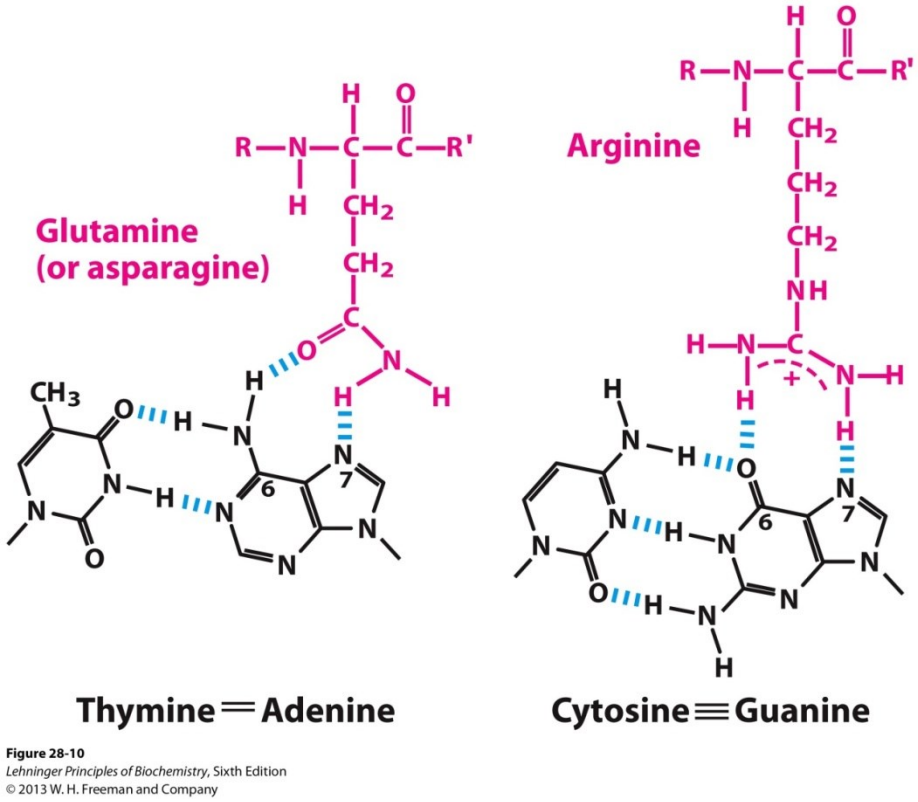


Figure 28-10
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- **Major groove** is right size for α-helix and has exposed H-bonding groups

DNA-binding domains

- Proteins generally recognise aspects of nucleic acid sequence, or variations in structure and/or flexibility
- High-resolution structures of many protein-DNA complexes have now been solved
- Similar **structural domains** occur in different proteins:
  - Helix-turn-helix
  - Zinc-finger
  - Zinc-binding domain
  - Basic region-leucine zipper (bZIP)
  - β-sheet recognition

The Helix-turn-helix Motif is Common in DNA-binding Proteins

- Each "helix-turn-helix" covers ~ 20 aa
  - One α-helix for DNA recognition, then β-turn, then another α-helix
  - Sequence-specific binding due to contacts between the recognition helix and the major groove

- Four DNA-binding helix-turn-helix motifs in the **Lac repressor**

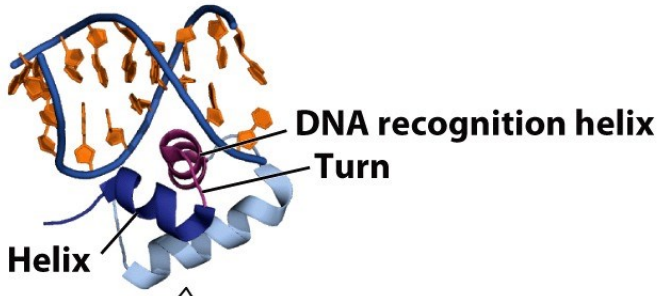

Figure 28-11
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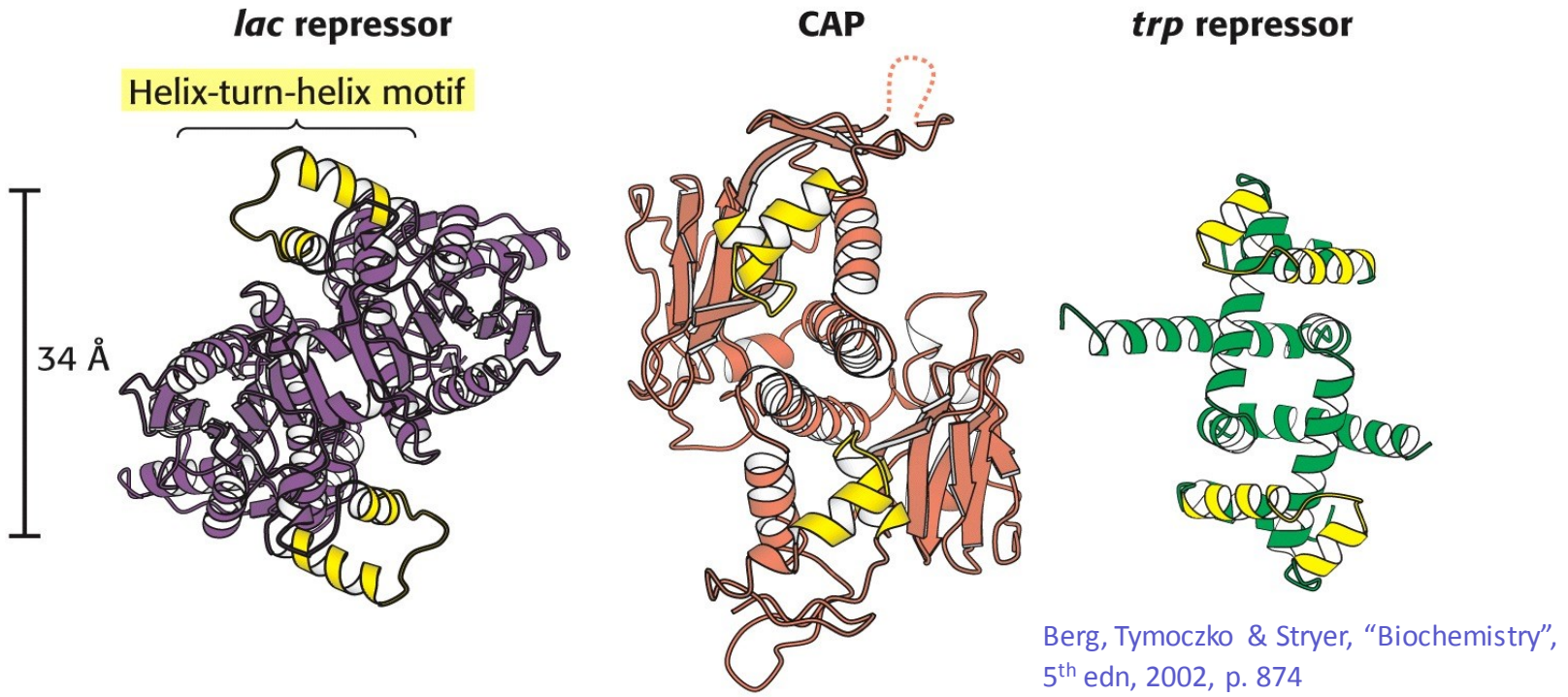
Helix-turn-helix

- Helix-turn-helix is most common observed DNA-binding unit in prokaryotes

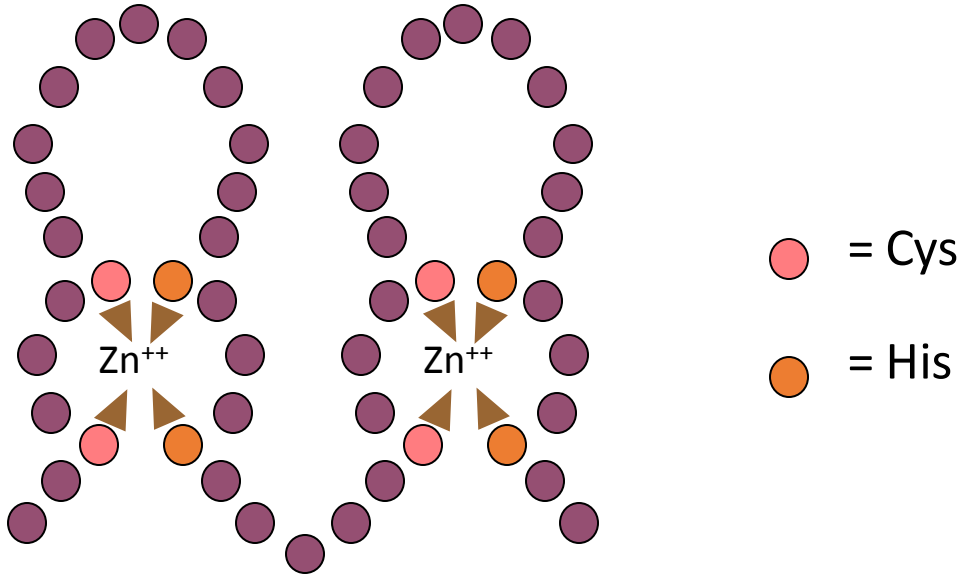


Berg, Tymoczko & Stryer, "Biochemistry", 5th edn, 2002, p. 874

- Note that 34 Å corresponds to 1 turn of DNA

Zinc-finger

- One of best-studied examples of DNA binding domain, but also binds RNA
- Each covers ~30 aa
- Binding is relatively weak, so typically there are a series of zinc fingers



Zinc Finger Motif is Common in Eukaryotic Transcription Factors

- Regulatory protein Zif268, complexed with DNA

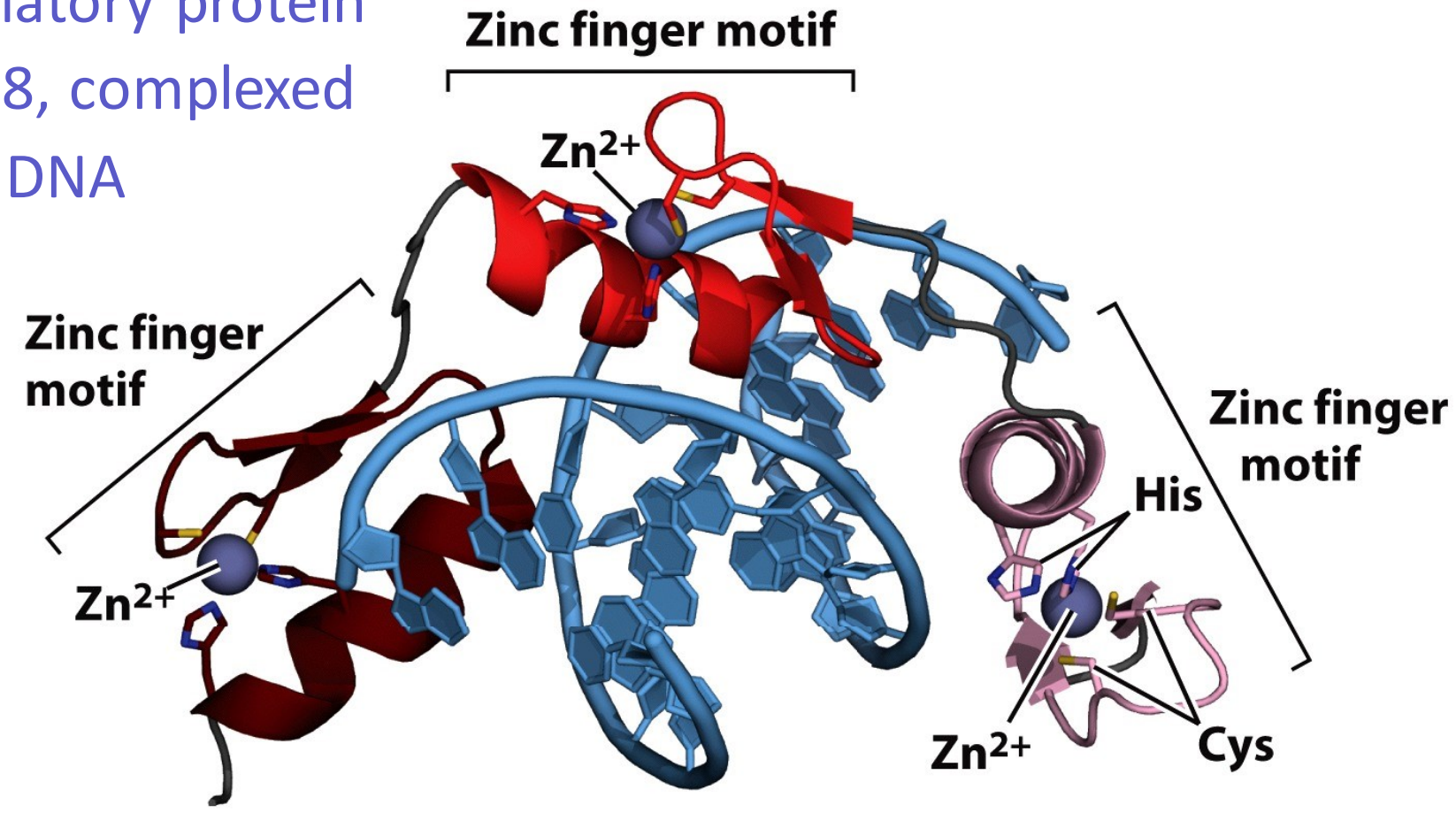


Figure 28-12
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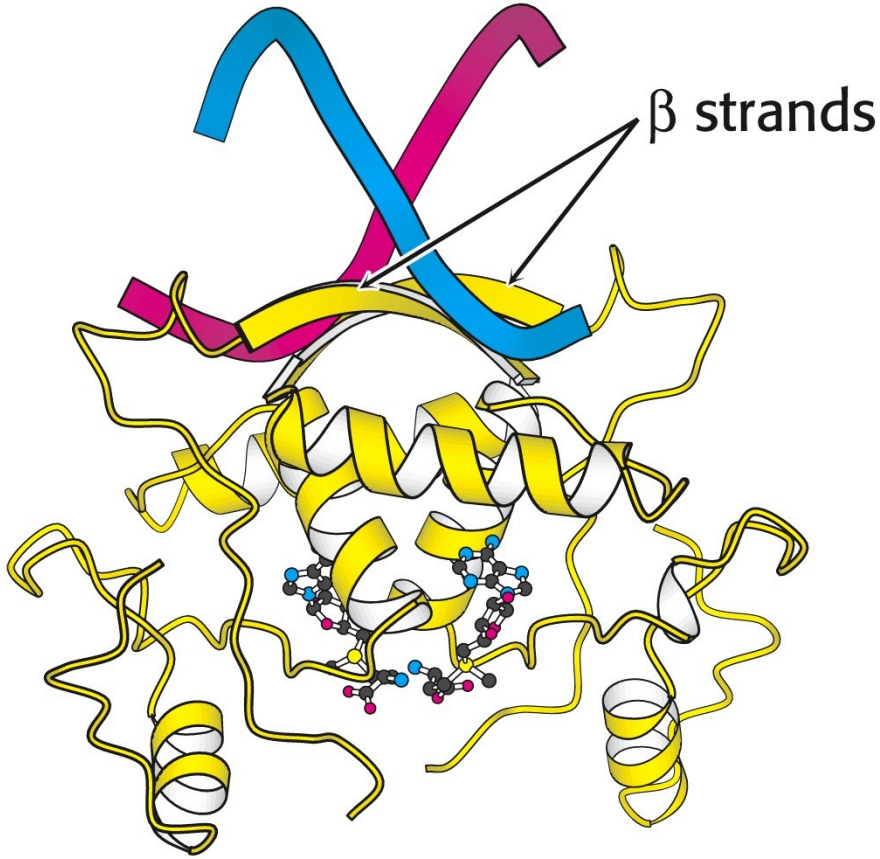
β-recognition motif

- In some prokaryotic regulatory proteins, this is an alternative DNA-binding motif

- *E. coli* methionine repressor binds DNA through insertion of pair of β-strands into major groove



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Studium interakcí protein-protein

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