Assiut University

Workshop
Protein electrophoresis and immunoblot (Western blot)

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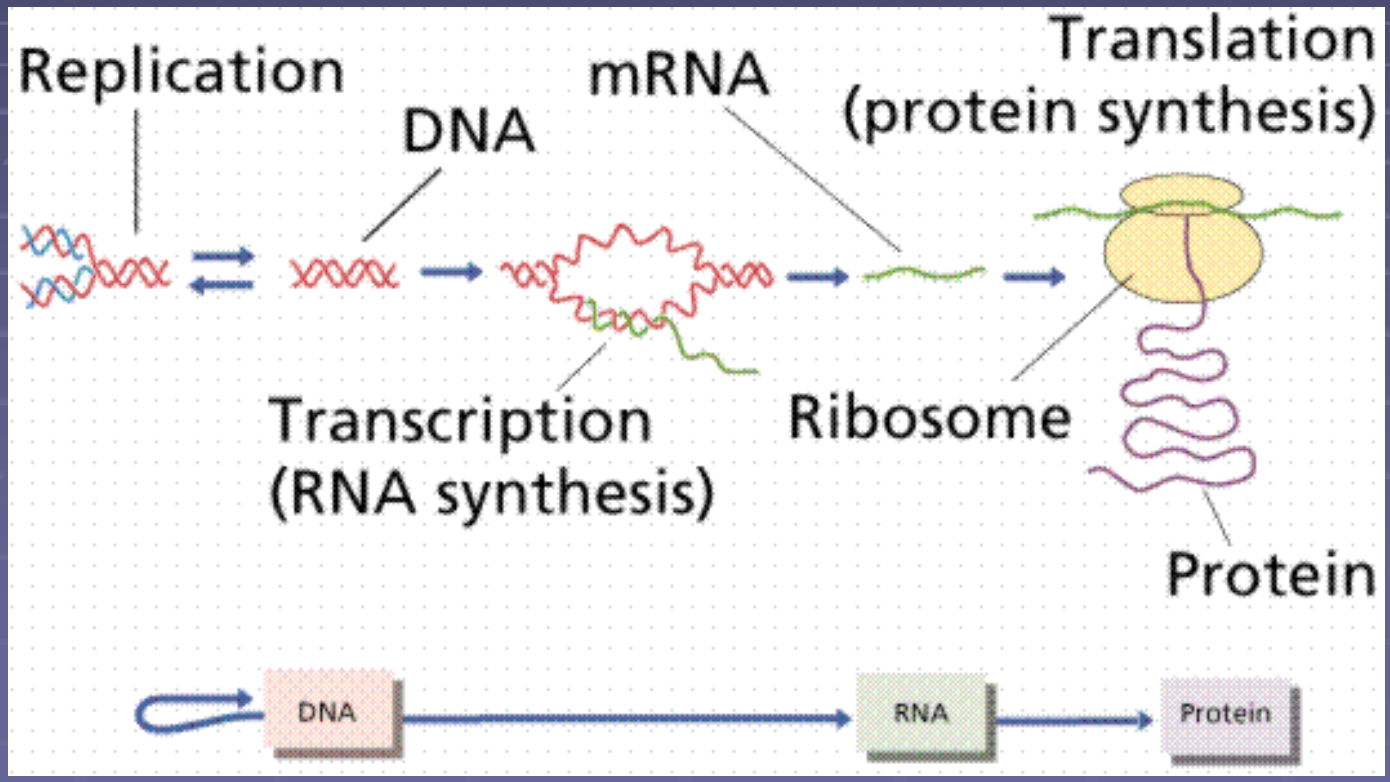
Introduction of protein structure and isolation

Protein Function

- **Enzymes** - proteases, synthetases, polymerases, kinases
- **Structural** tubulin collagen, elastin a-keratin
- **Transport** - serum albumin, hemoglobin, transferrin
- **Motor** - myosin, kinesin, dynein
- **Storage** - ferritin, ovalbumin, calmodulin
- **Signaling** - insulin, nerve growth factor, integrins
- **Receptor** - acetylcholine receptor, insulin receptor, EG recept
- **Gene regulatory** - lactose repressor, homeodomain proteins
- **Special purpose** - green fluorescent protein, glue proteins

Protein synthesized by ribosomal machinery which translate the nucleotide sequence in mRNA into amino acid sequence of protein



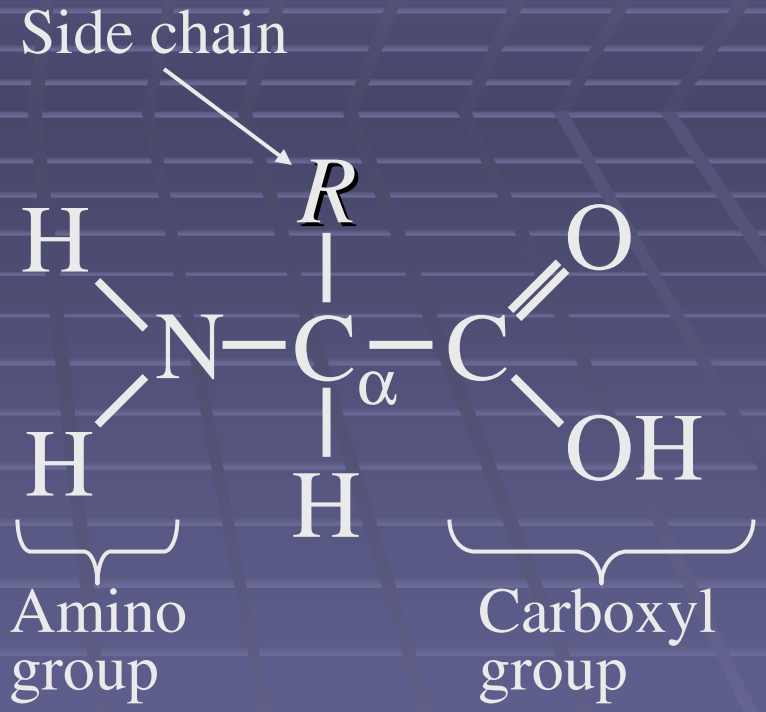
Codon Usage Table

	AGA									UUA
	AGG									UUG
GCA	CGA						GGA			CUA
GCC	CGC						GGC		AUA	CUC
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC	CUG
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU	CUU
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu
A	R	D	N	C	E	Q	G	H	I	L

				AGC					
				AGU					
			CCA	UCA	ACA			GUA	
			CCC	UCC	ACC			GUC	UAA
AAA		UUC	CCG	UCG	ACG		UAC	GUG	UAG
AAG	AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA
Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
K	M	F	P	S	T	W	Y	V	

Amino acid composition

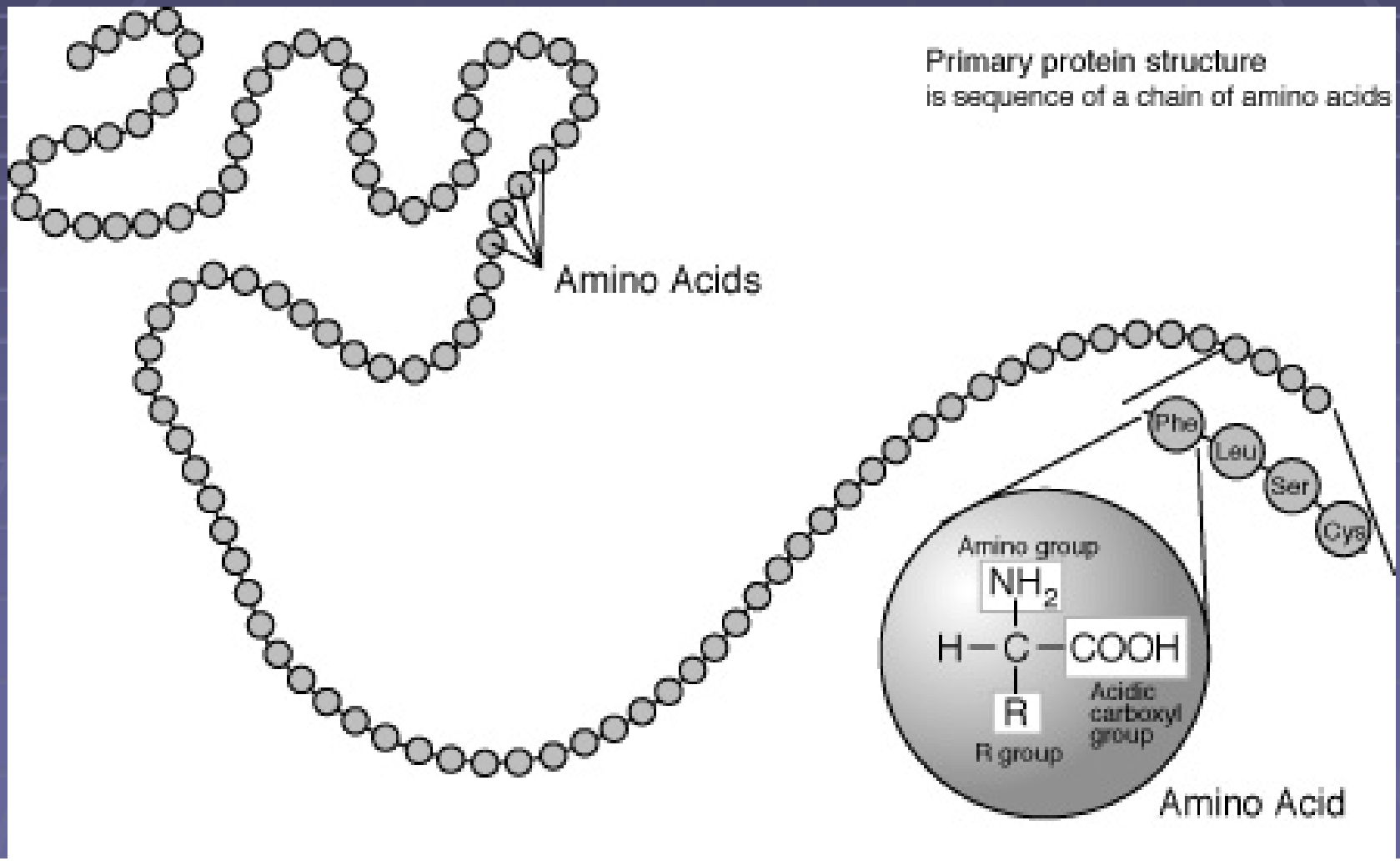
- Basic Amino Acid Structure:
  - The side chain, R, varies for each of the 20 amino acids



We group the amino acids into three general groups:
Hydrophobic
Charged (positive/basic & negative/acidic)
Polar

Proteins are chains of amino acids

- *Polymer* – a molecule composed of repeating units



Amino Acids

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Theonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	P	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

Peptidyl polymers

- A few amino acids in a chain are called a *polypeptide*. A *protein* is usually composed of 50 to 400+ amino acids.
- Since part of the amino acid is lost during dehydration synthesis, we call the units of a protein *amino acid* ***residues***.

Protein Structure and Function

Protein Structure

Primary structure - amino acid sequence.

Secondary structure - formation of a helices and b sheets.

Tertiary structure - the three-dimensional conformation of polypeptide chain.

Quaternary structure - formation of a protein molecule as complex of more than one polypeptide chain.

Biology/Chemistry of Protein Structure

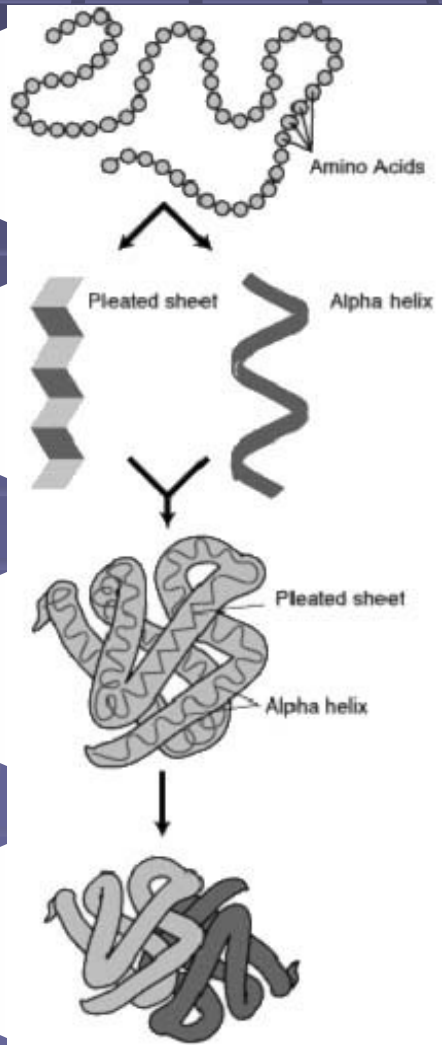
STRUCTURE

Primary

Secondary

Tertiary

Quaternary



Assembly

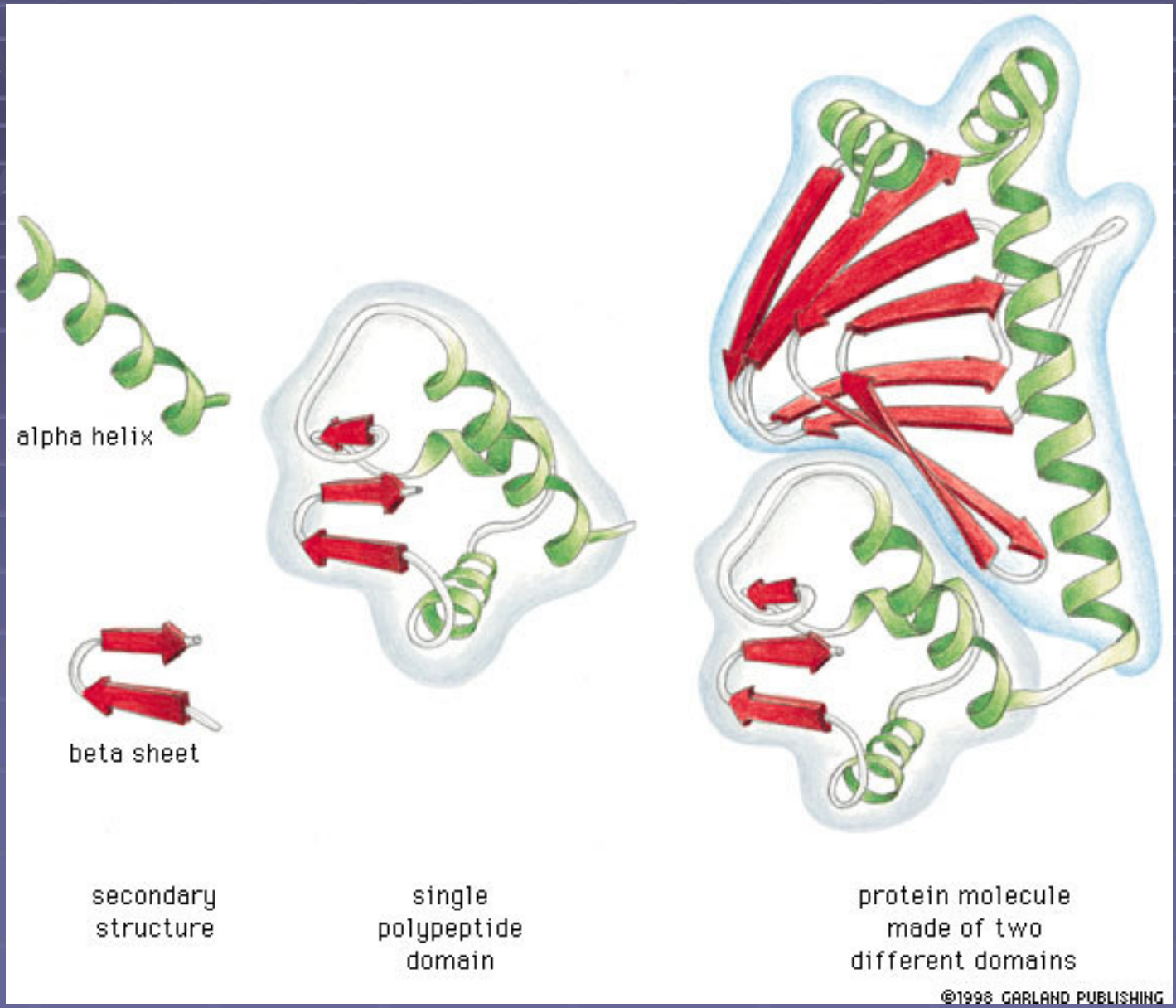
Folding

Packing

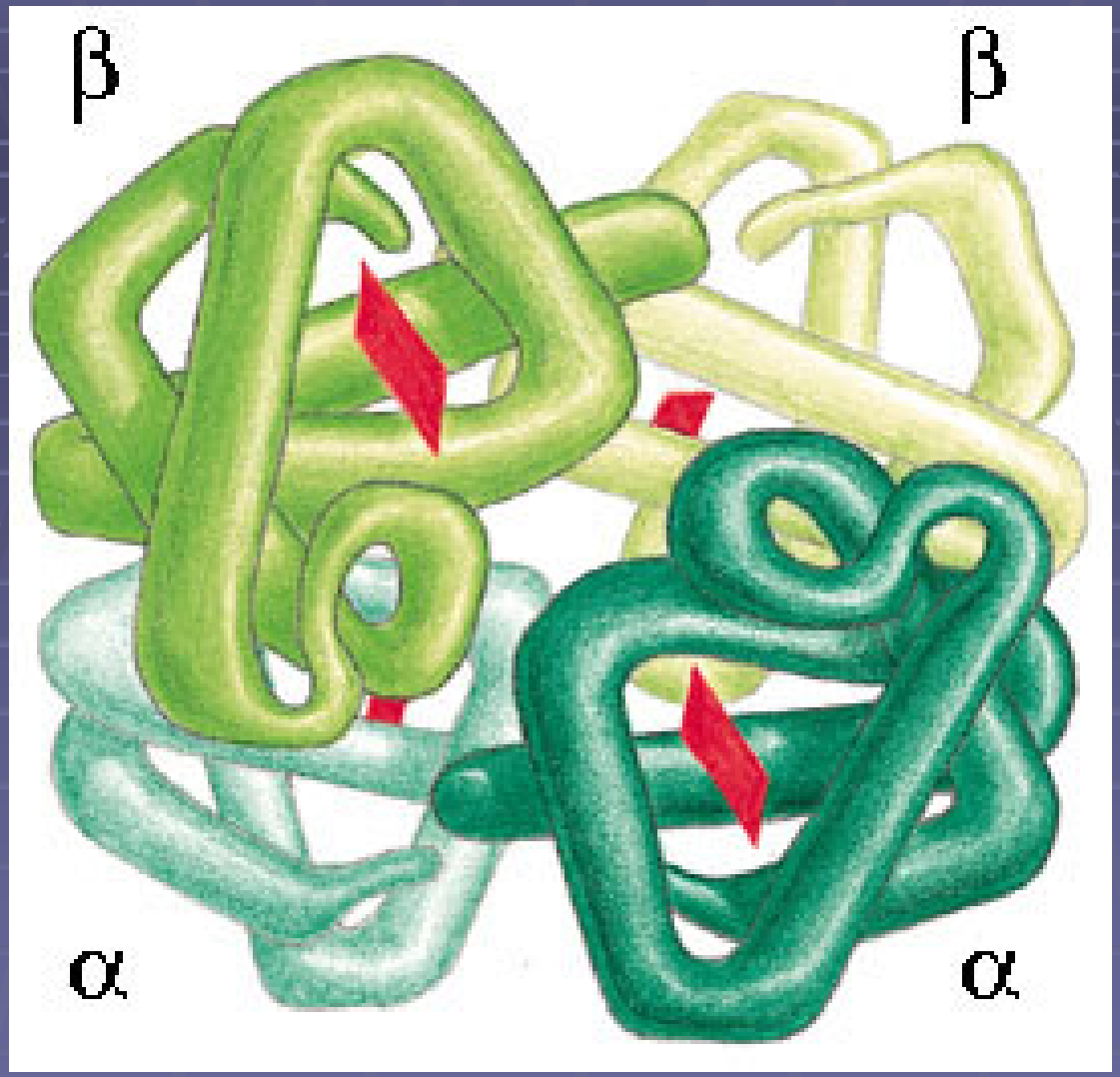
Interaction

PROCESS

Tertiary Structure



Quaternary Structure

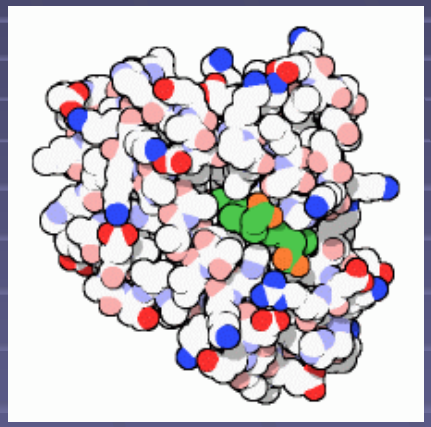


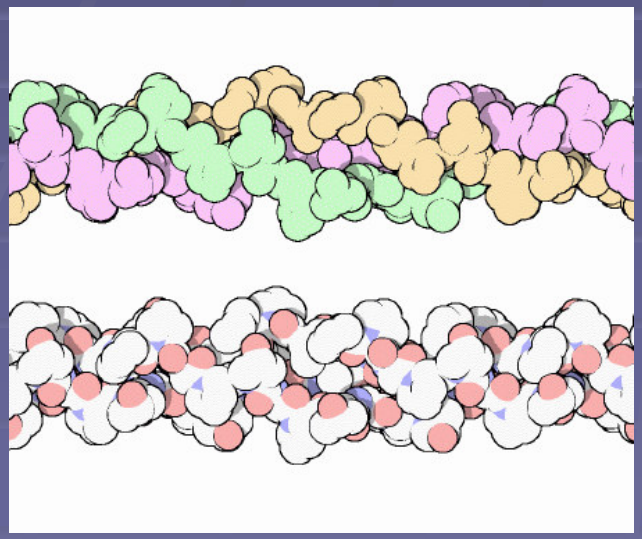
Hemoglobin

There are Different Forms of Classification apart from Structural

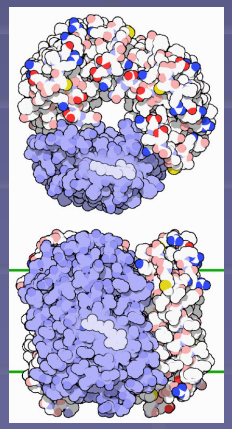
- Biochemical
- Globular
- Membrane
- Fibrous

myoglobin



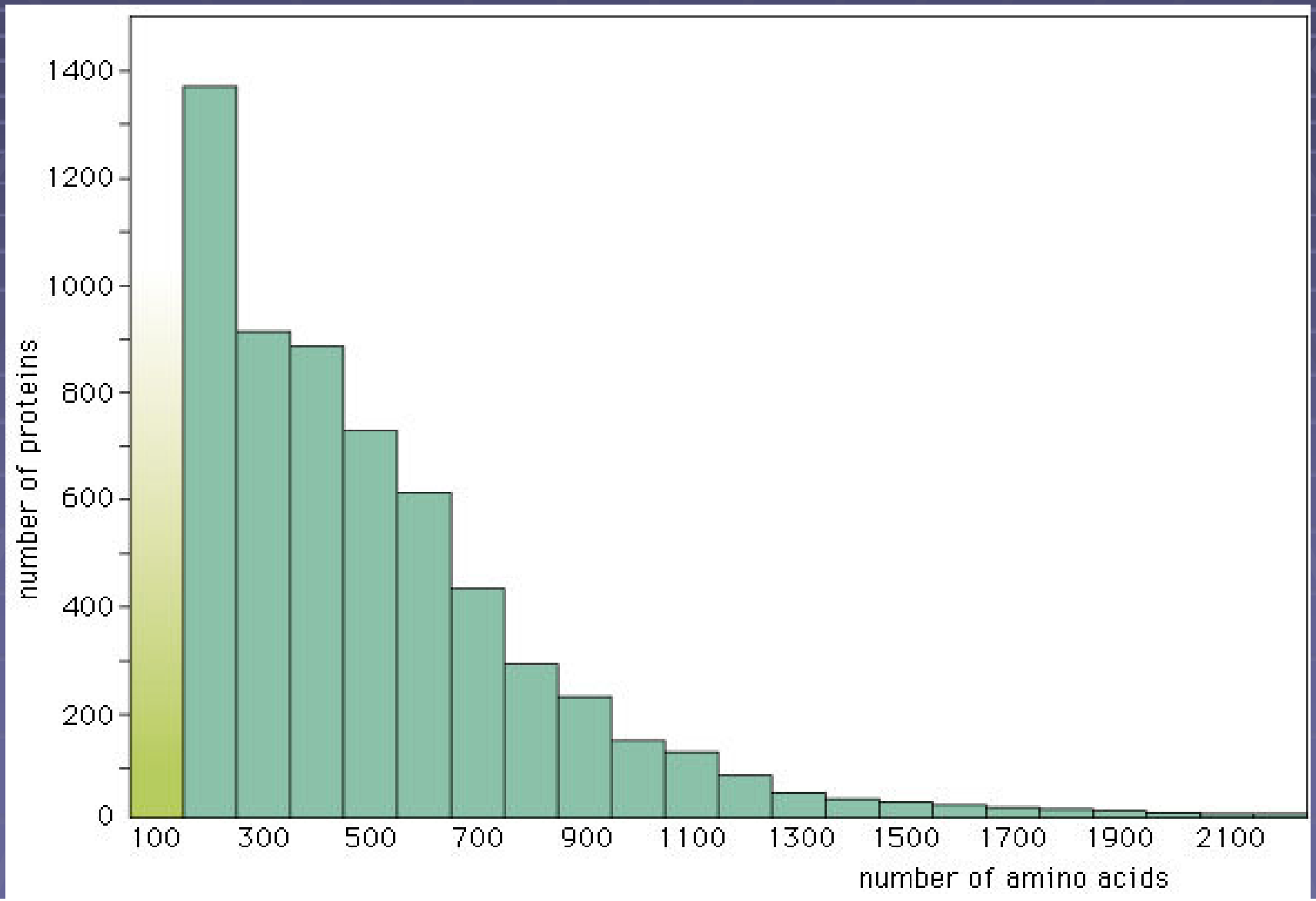


Collagen

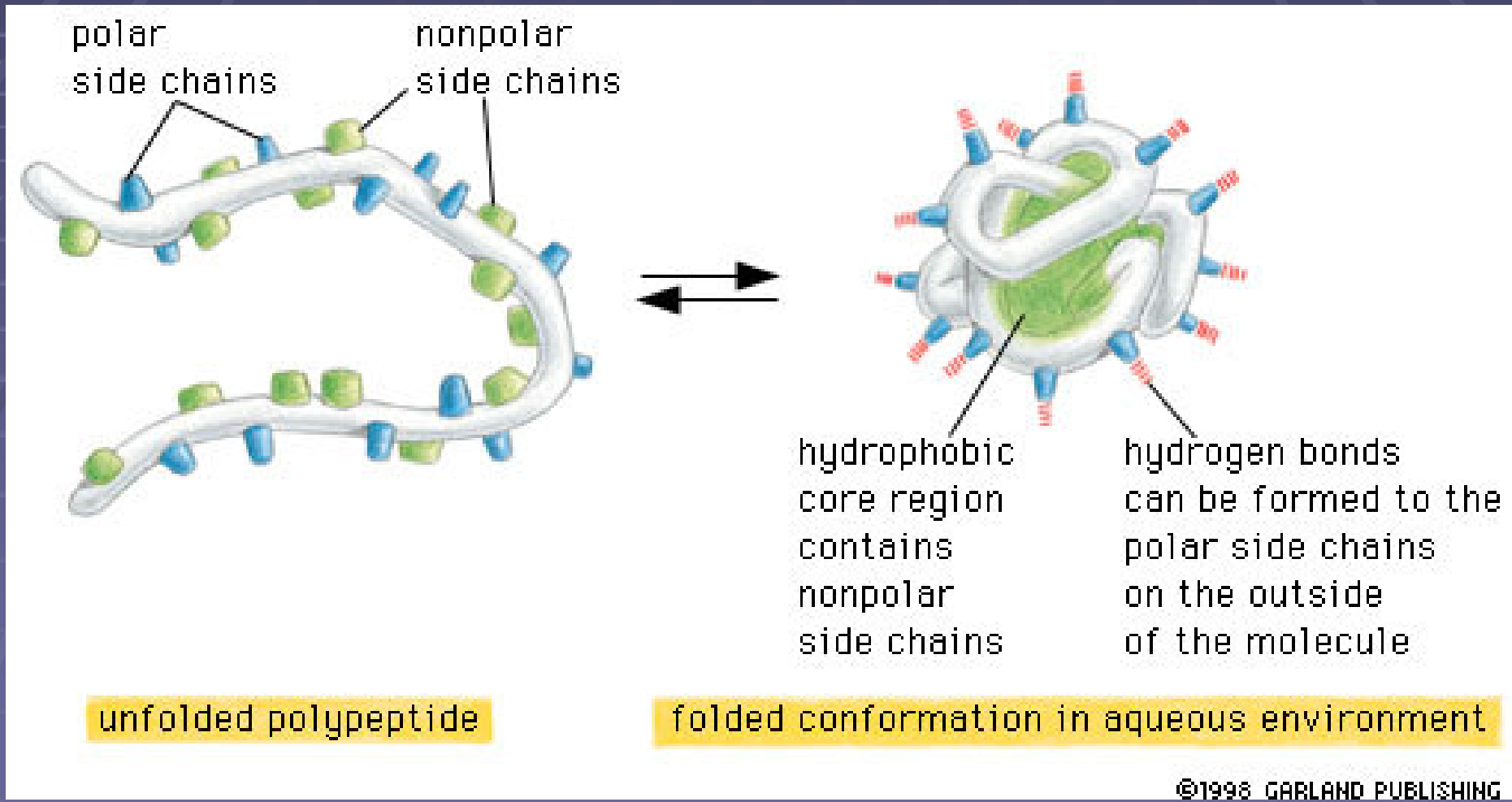


Bacteriorhodopsin

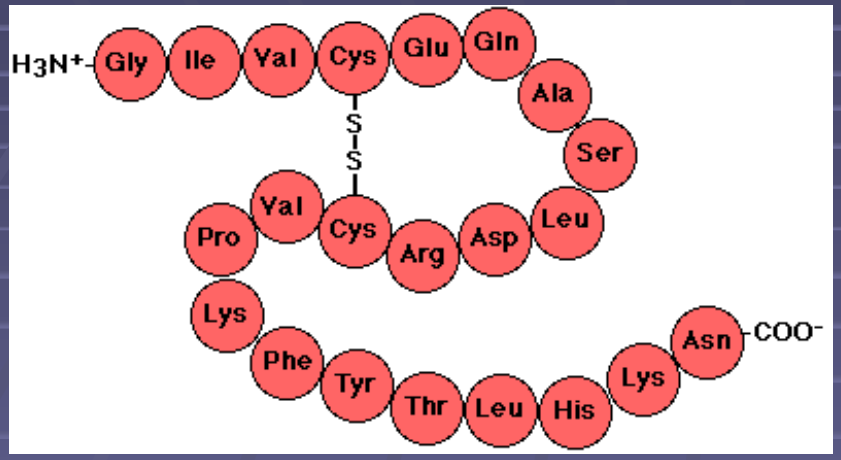
Number & Size Distribution of Cellular Proteins



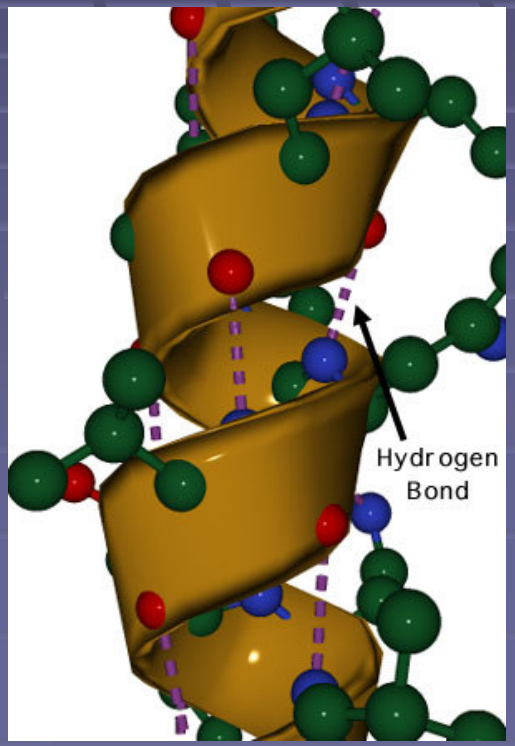
Protein Folding



Disulfide Bridge – Linking Distant Amino Acids



Hydrogen Bonding And Secondary Structure



Why purify a protein?

- Characterize function, activity, structure
- Use in assays
- Raise antibodies
- many other reasons ...

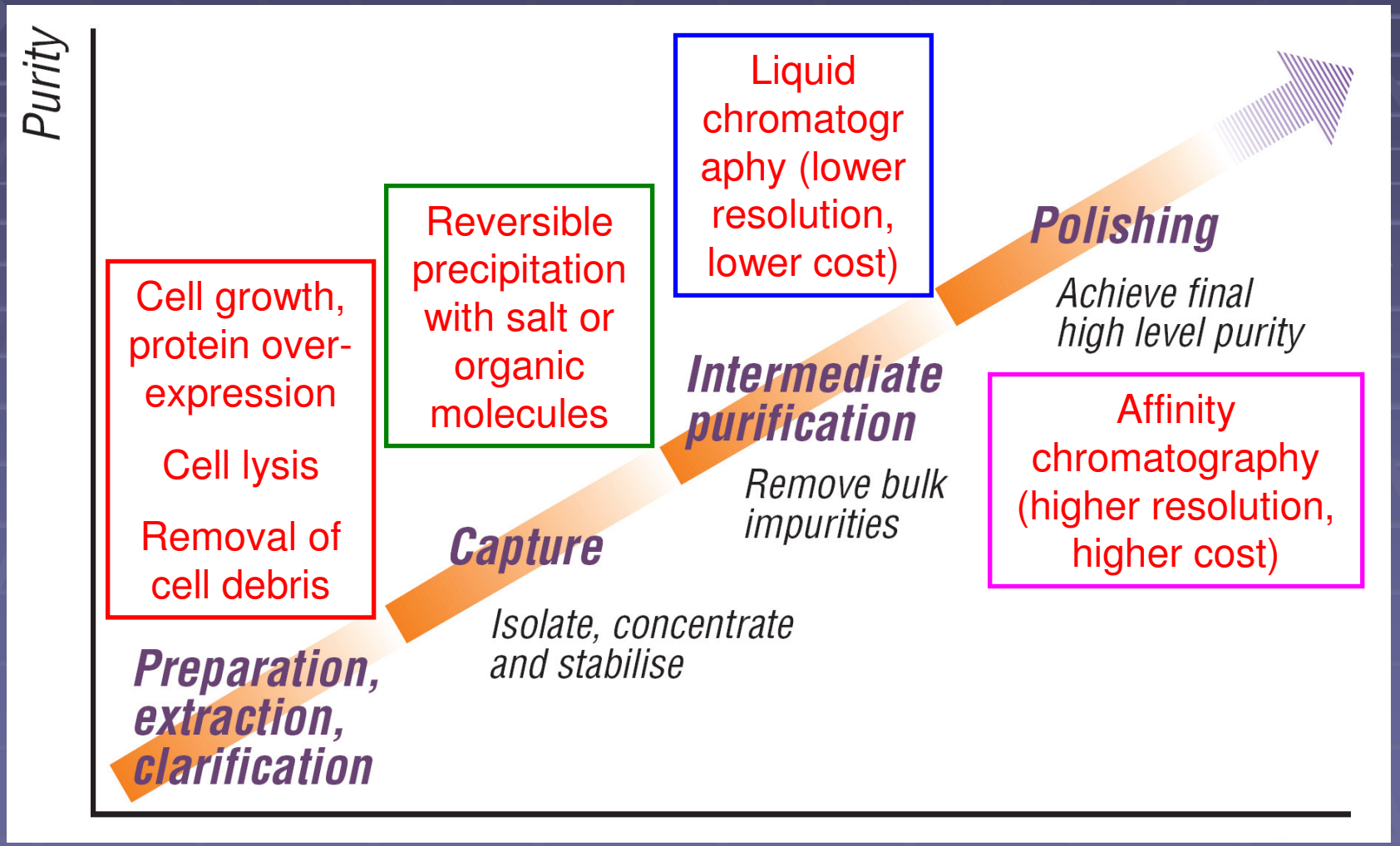
How pure should my protein be?

Application	Required Purity
Therapeutic use, *in vivo* studies	Extremely high > 99%
Biochemical assays, X-ray crystallography	High 95-99%
N-terminal sequencing, antigen for antibody production, NMR	Moderately high < 95%

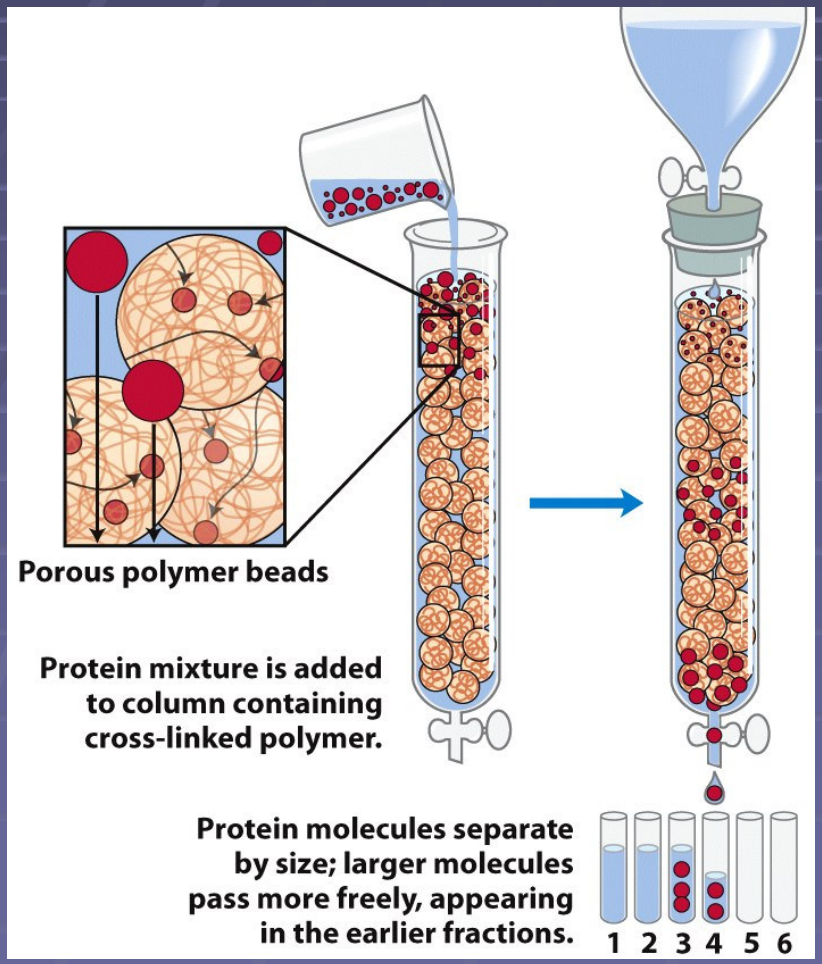
Separation of proteins based on physical and chemical properties

- Solubility
- Binding interactions
- Surface-exposed hydrophobic residues
- Charged surface residues

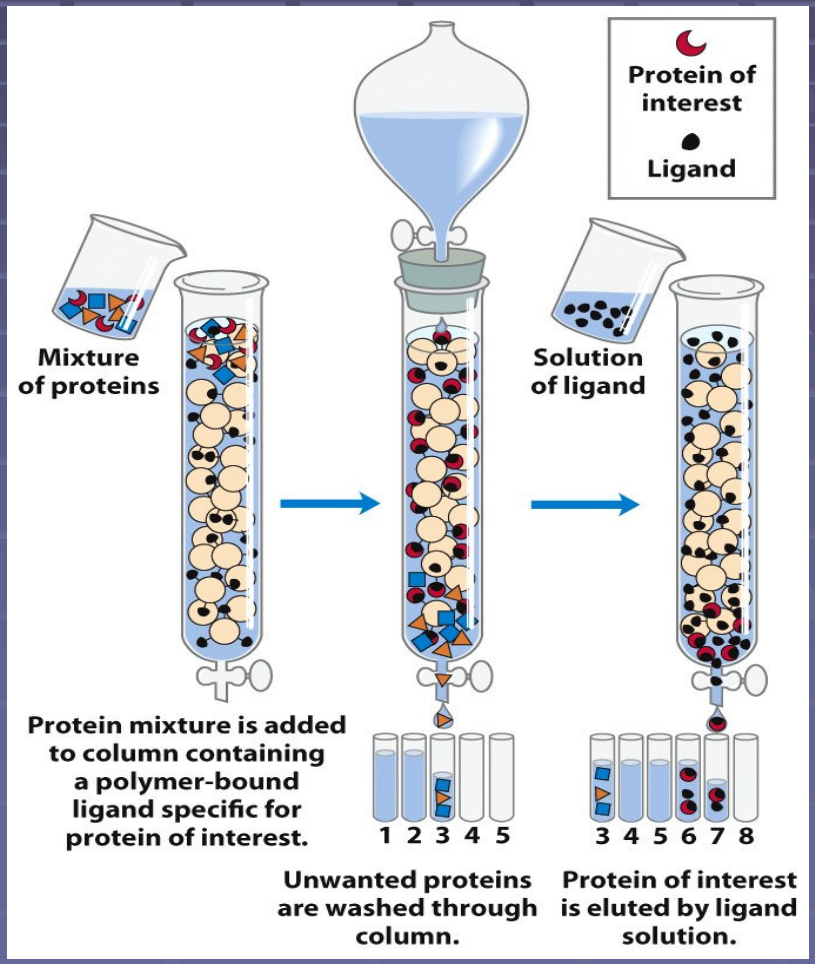
Protein isolation, concentration, and stabilization



Size exclusion chromatography



Affinity Chromatography



Protein detection methods

- SDS-PAGE
  - Visual confirmation
- UV Spectrophotometry
  - Absorbance @ 280 nm
- Colorimetric Techniques
  - Color change proportional to [protein]
  - Bradford, Lowry, BCA

References and additional Reading

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  An excellent introduction

- Richardson (1981) The Anatomy and Taxonomy of Protein Structure Adv. Protein Chem. 34: 167-339

  Good historical perspective

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