

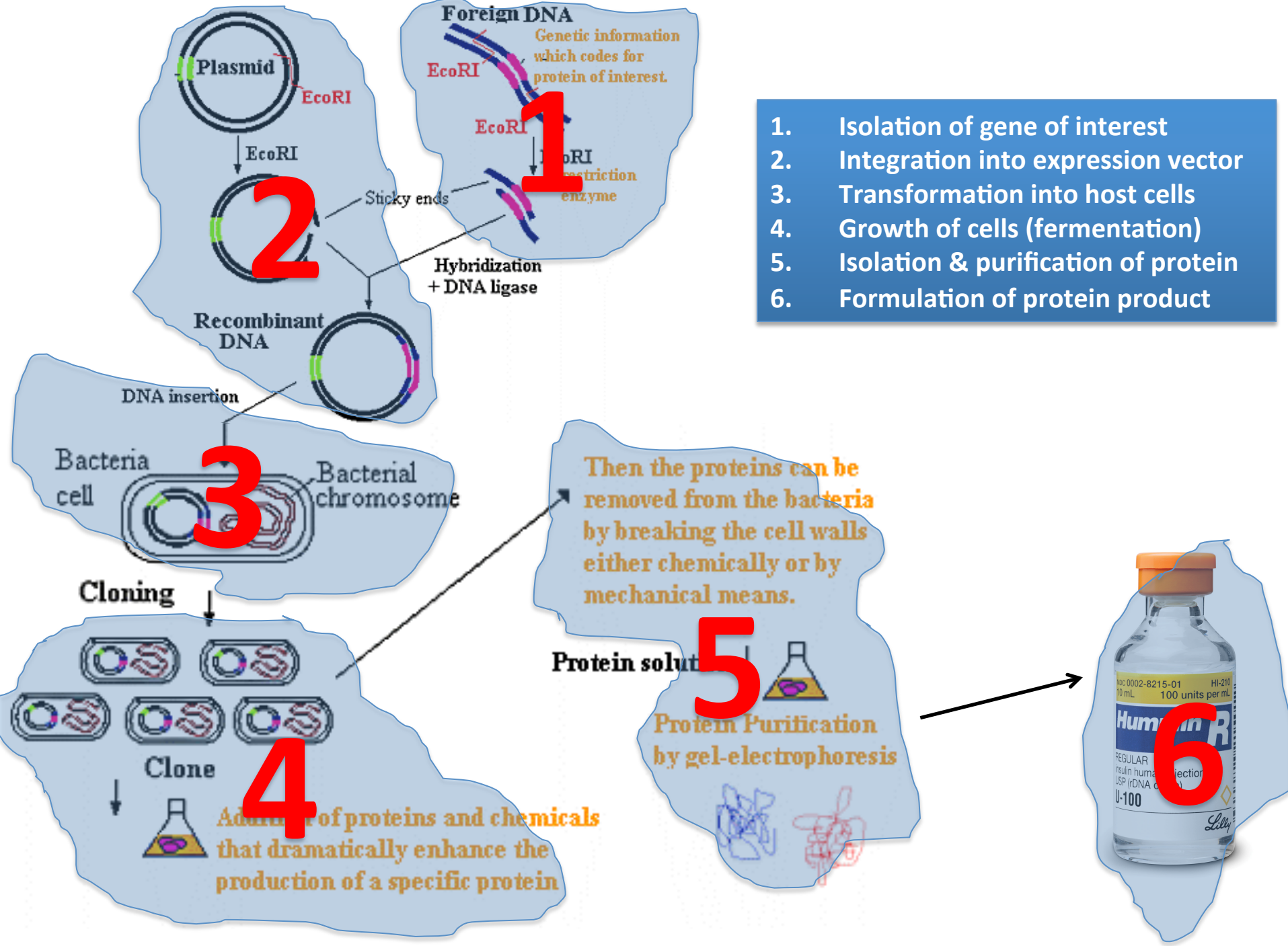
Pharmaceutical Protein Production (I)

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Objectives of this lecture

By the end of this lecture you will be able to:

1. Understand the role of proteins in health and disease
2. Recognize the limitations of traditional protein production methods
3. Recognize the advantages of recombinant over naturally-isolated proteins



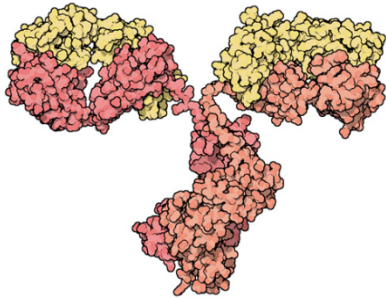
Functions of Proteins

Structural



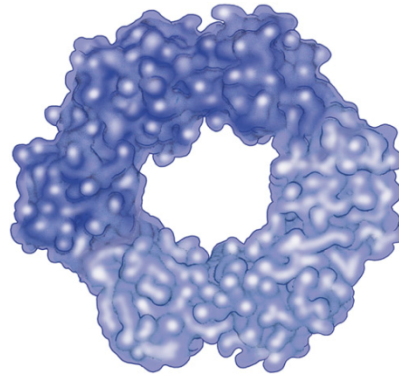
collagen

Immune



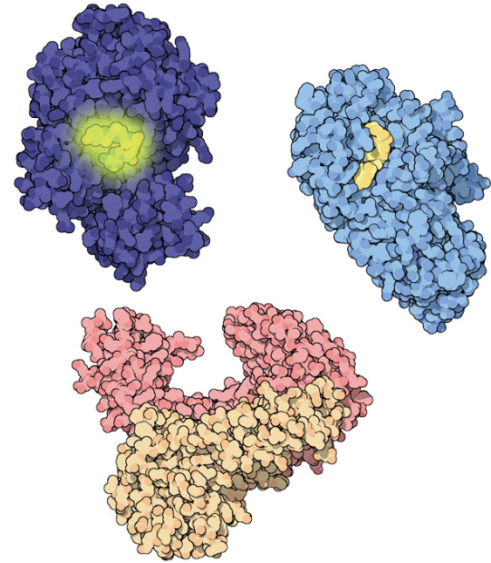
antibodies

DNA Regulation



DNA polymerase III

Enzymes



luciferase

amylase

reverse
transcriptase

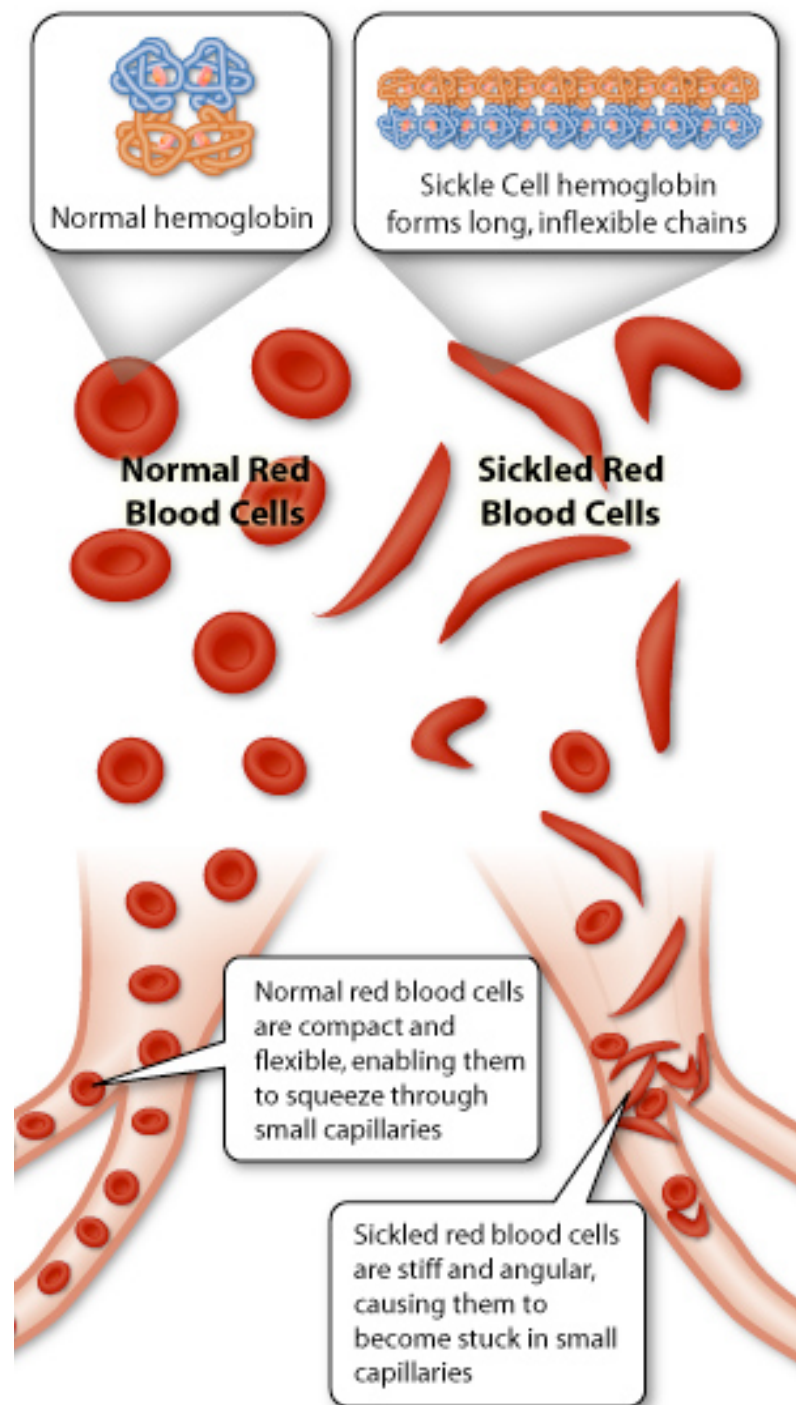
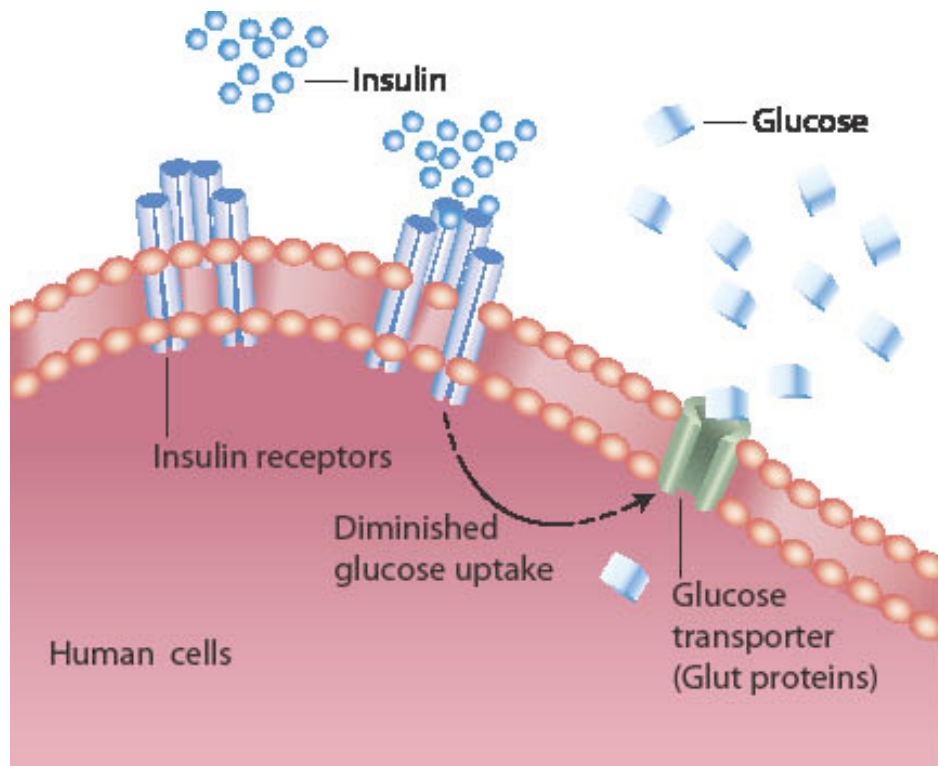
Proteins in diseases and therapy

DISEASE-CAUSING PROTEINS

Loss in protein production or functionality might lead to serious diseases

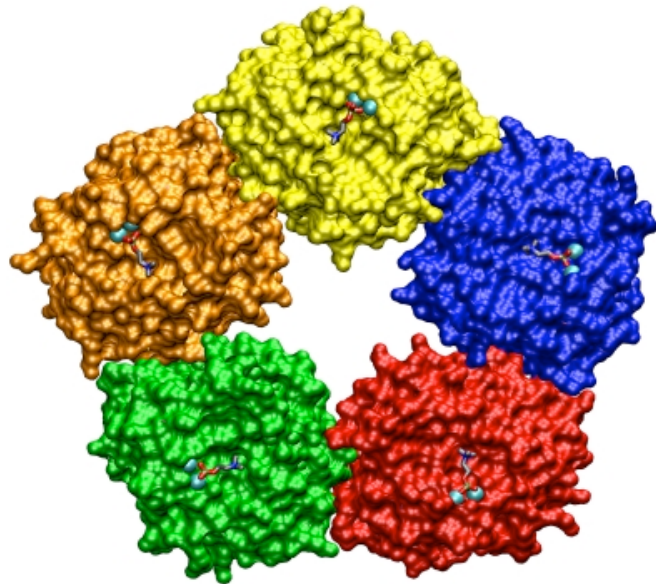
Diabetes Mellitus

Sickle cell anemia

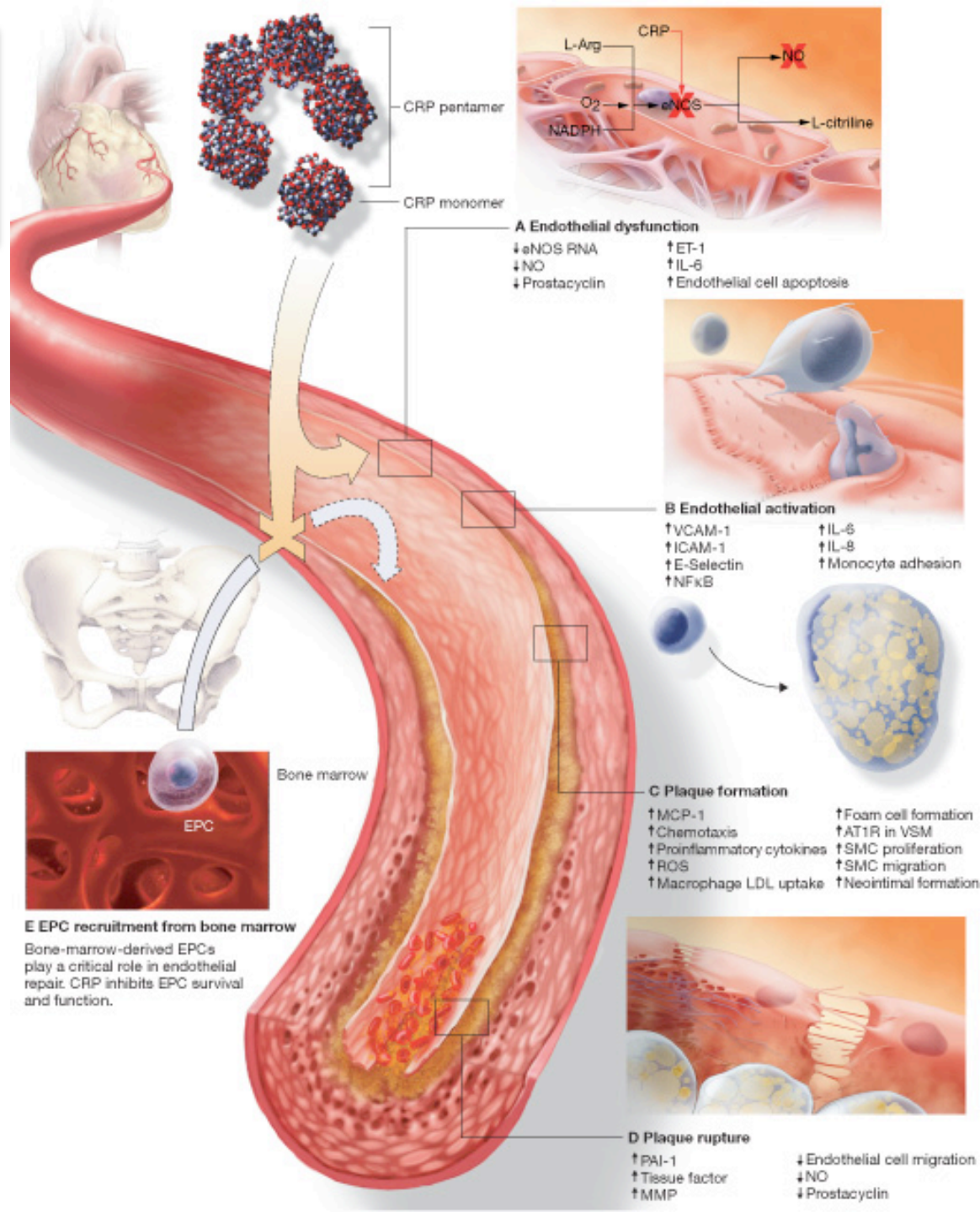


Proteins might mediate diseases as well

C-reactive protein mediates cardiovascular disorders

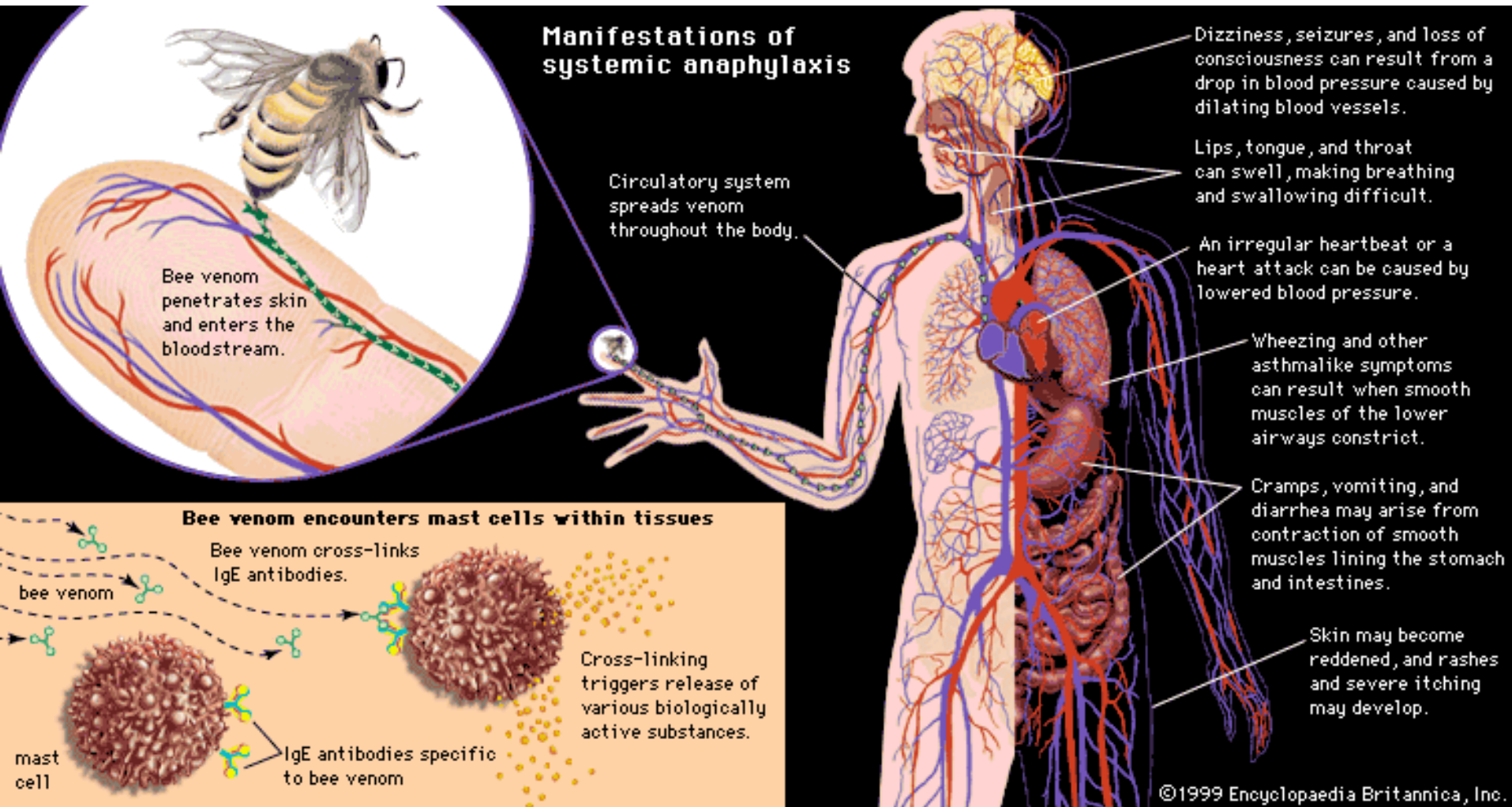


C-reactive protein



Exogenous proteins might mediate serious reactions

Hypersensitivity reactions



Proteins in diseases and therapy

THERAPEUTIC PROTEINS

Serving or mimicking an endogenous function

Hormonal therapy (Insulin, GH)

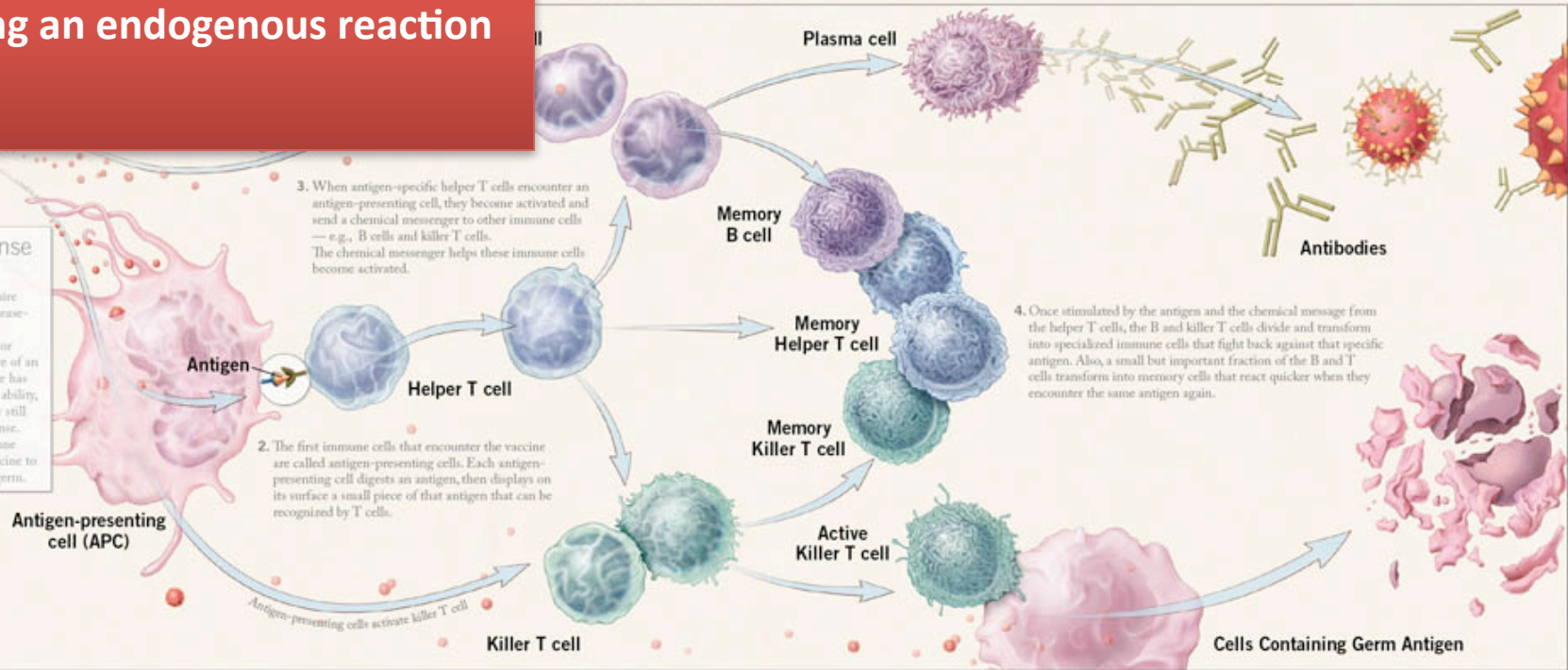


Exploiting an endogenous reaction

Vaccines

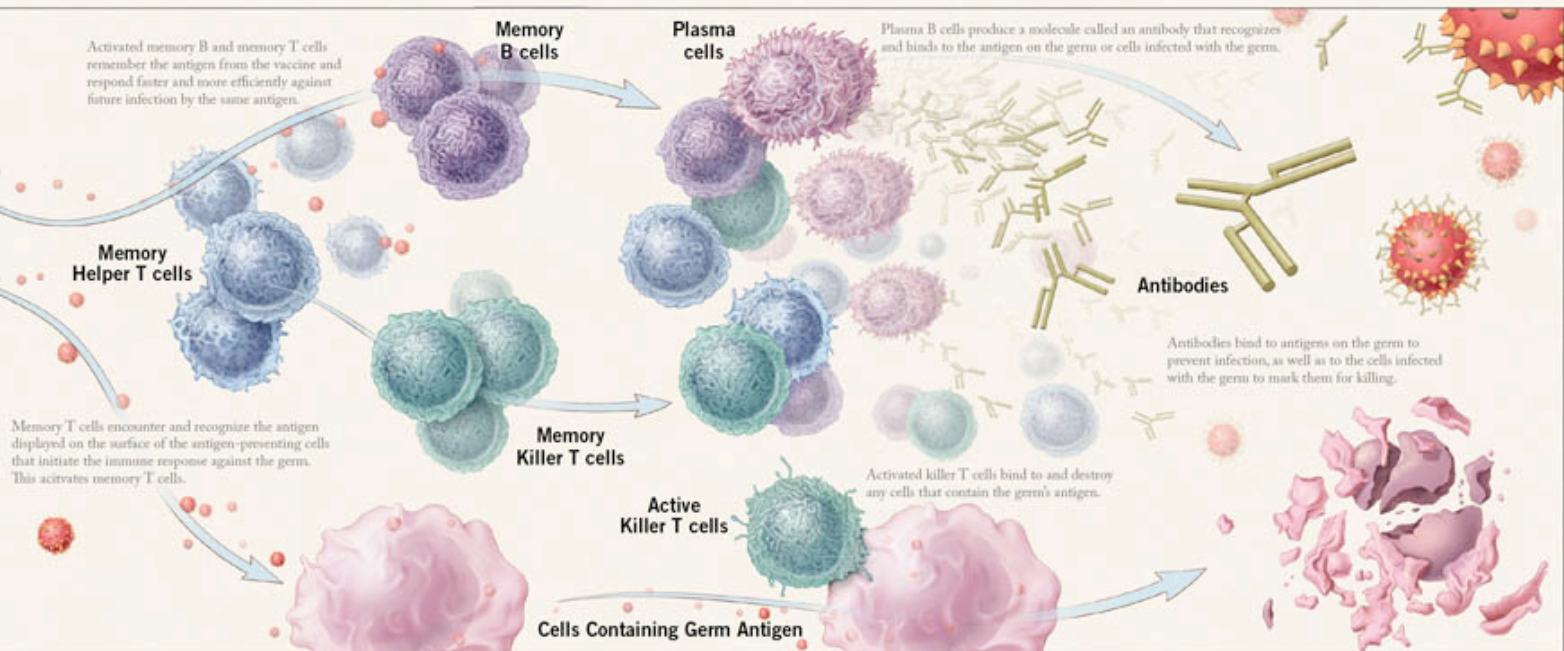
Immune Response to a Vaccine

Vaccines help the body acquire immunity against many disease-causing germs and cancers. A vaccine contains a killed or weakened form or derivative of an infectious germ. The vaccine has little or no disease-causing ability, but its presence in the body still provokes an immune response. This activates various immune cells that learn from the vaccine to recognize and destroy the germ.



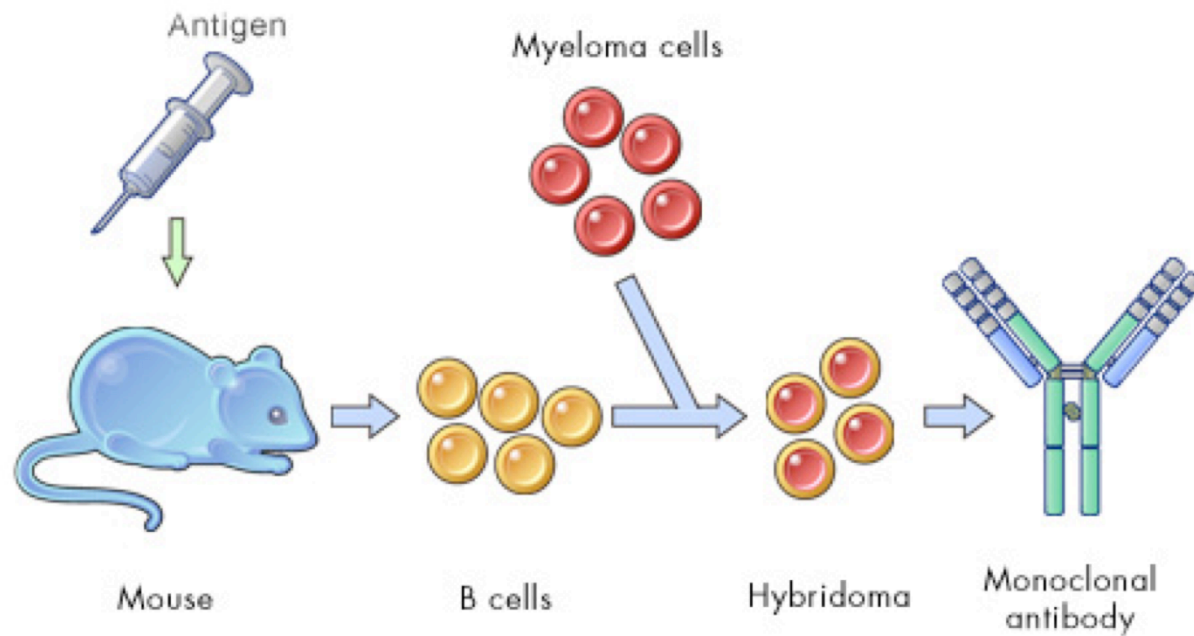
Immune Response after Vaccination

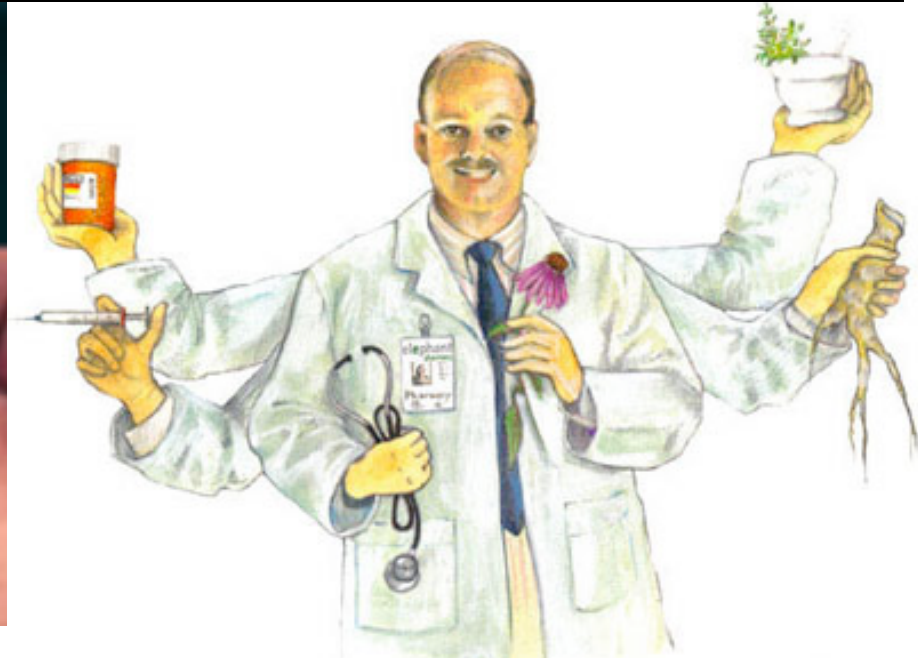
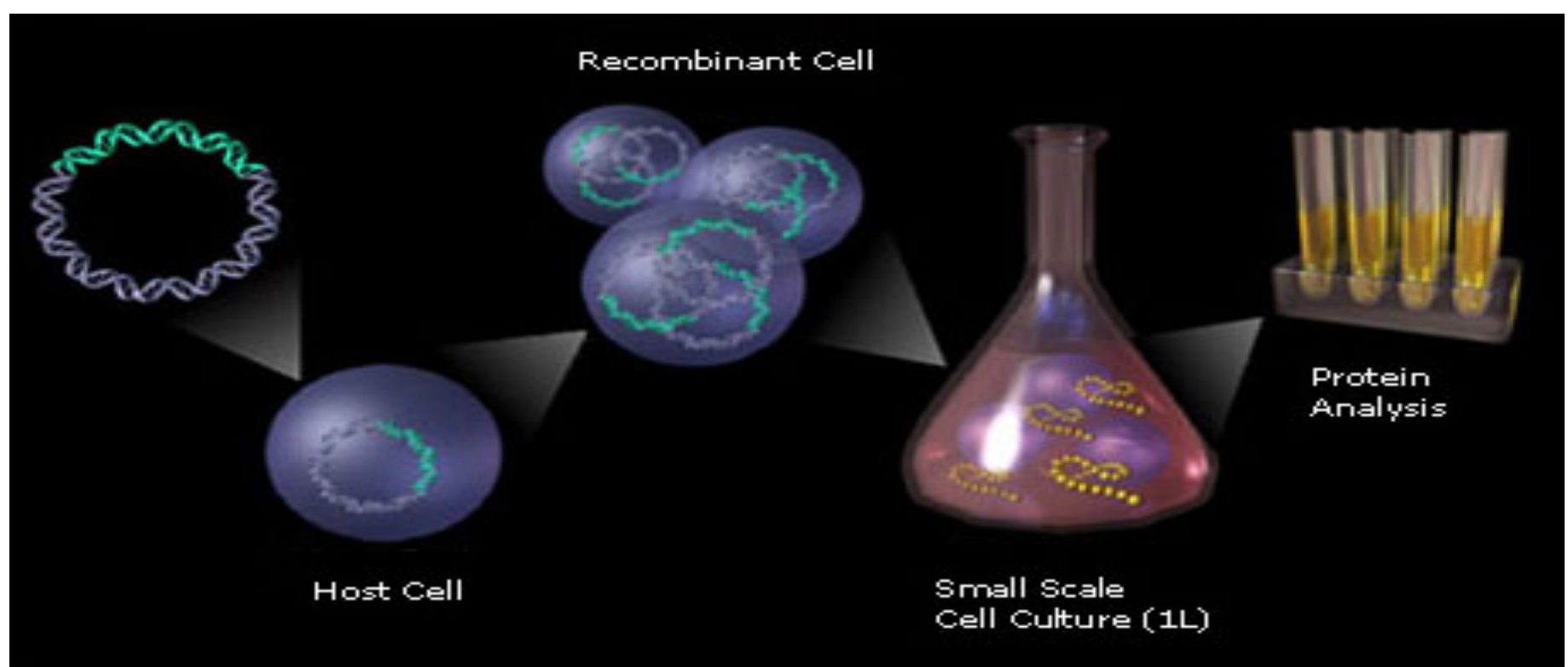
An exposure to a germ after vaccination stimulates the memory B and memory T cells, which recognize the antigen from the germ and respond quickly and effectively to prevent disease.

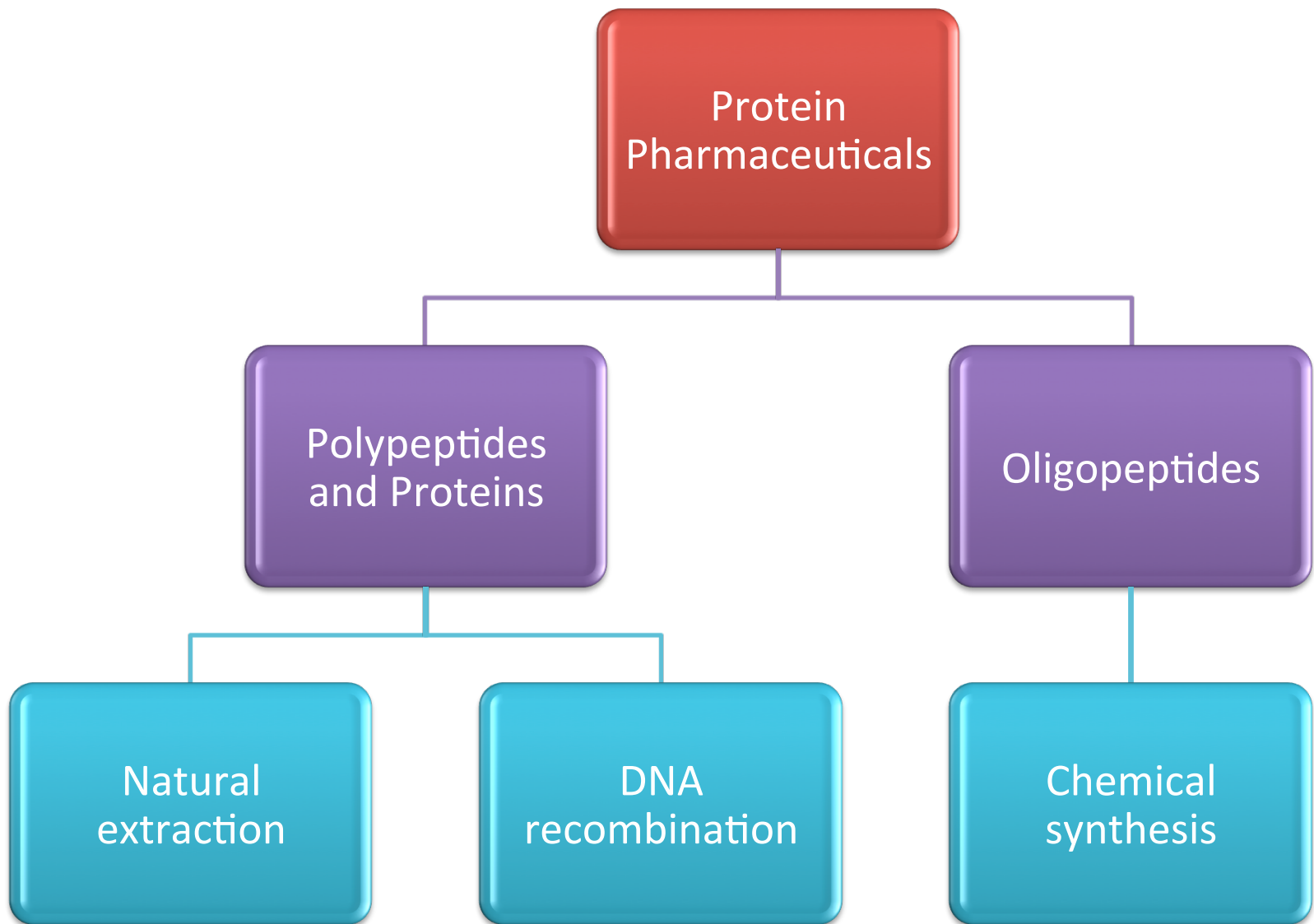


Antagonizing an endogenous function

mAb therapy (Herceptin)







Protein Pharmaceuticals

- **Peptide:** consists of 2 or more amino acids
- **Oligopeptide:** consists of 10 or fewer amino acids
- **Polypeptides:** chains of 10 to 50 amino acids
- **Proteins:** polypeptides of more than 50 amino acids

Naturally-Occurring Proteins

Hormone	Size (a.a.)	Location	Site of action includes
ACTH	39	Anterior pituitary	Adrenal cortex
ADH (VP)	9	Posterior pituitary	Kidney / CVS / CNS
Calcitonin	32	Thyroid	Bones
GH	191	Anterior pituitary	Bones / muscles / liver
Oxytocin	9	Posterior pituitary	Mammary gld. / Uterus / CNS
Prolactin	198	Anterior pituitary	Mammary gland
Insulin	51	Pancreas	Liver / Muscles / Adipocytes

Oligopeptides

- Many hormones in our body are actually oligopeptides such as Oxytocin and Vasopressin (ADH)

Oligopeptides

- Pharmaceutical production of oligopeptides can be achieved by:
 1. Extraction from natural sources (e.g. salmon calcitonin)
 2. Solid-phase peptide synthesis (SPPS)
 3. Recombinant DNA technology

Solid Phase Peptide Synthesis

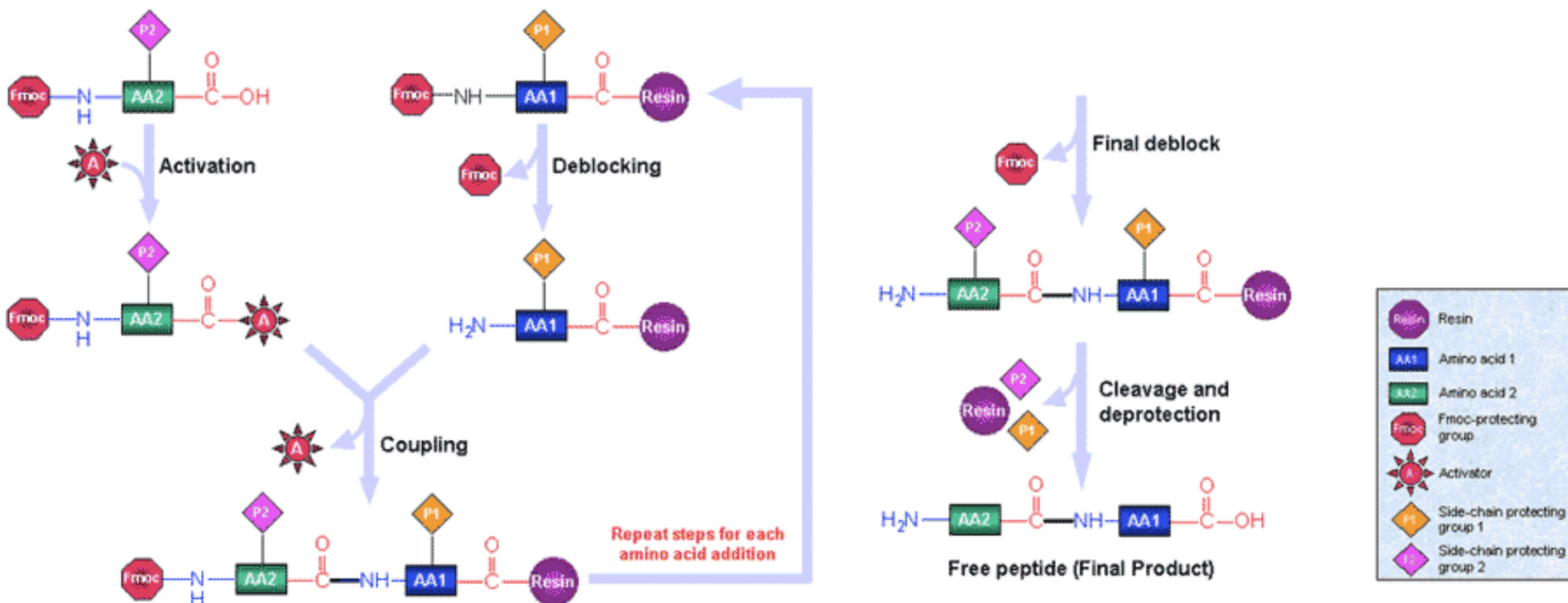


Bruce Merrifield 1921-2006

- Method developed by Bruce Merrifield and published in JACS in 1963
- He won the Nobel Prize in Chemistry in 1984

Solid Phase Peptide Synthesis

Solid Phase Peptide Synthesis Scheme



Solid Phase Peptide Synthesis

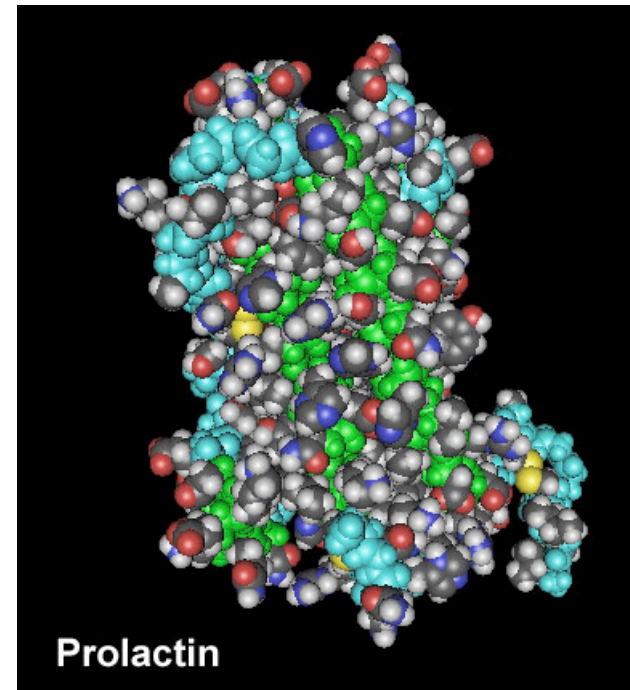
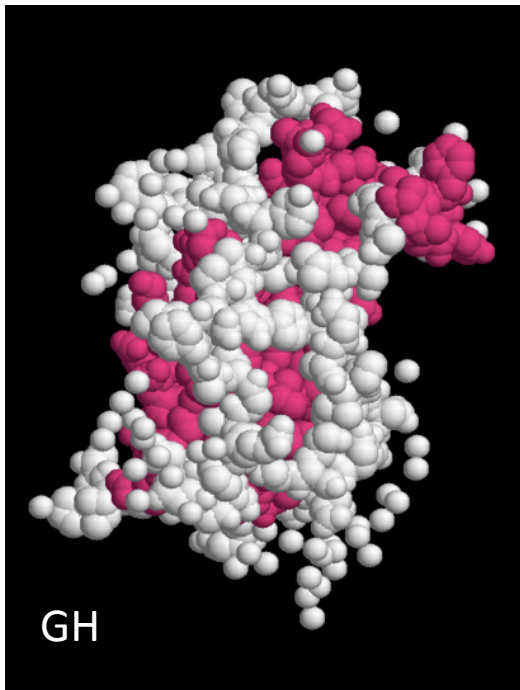


Solid Phase Peptide Synthesis

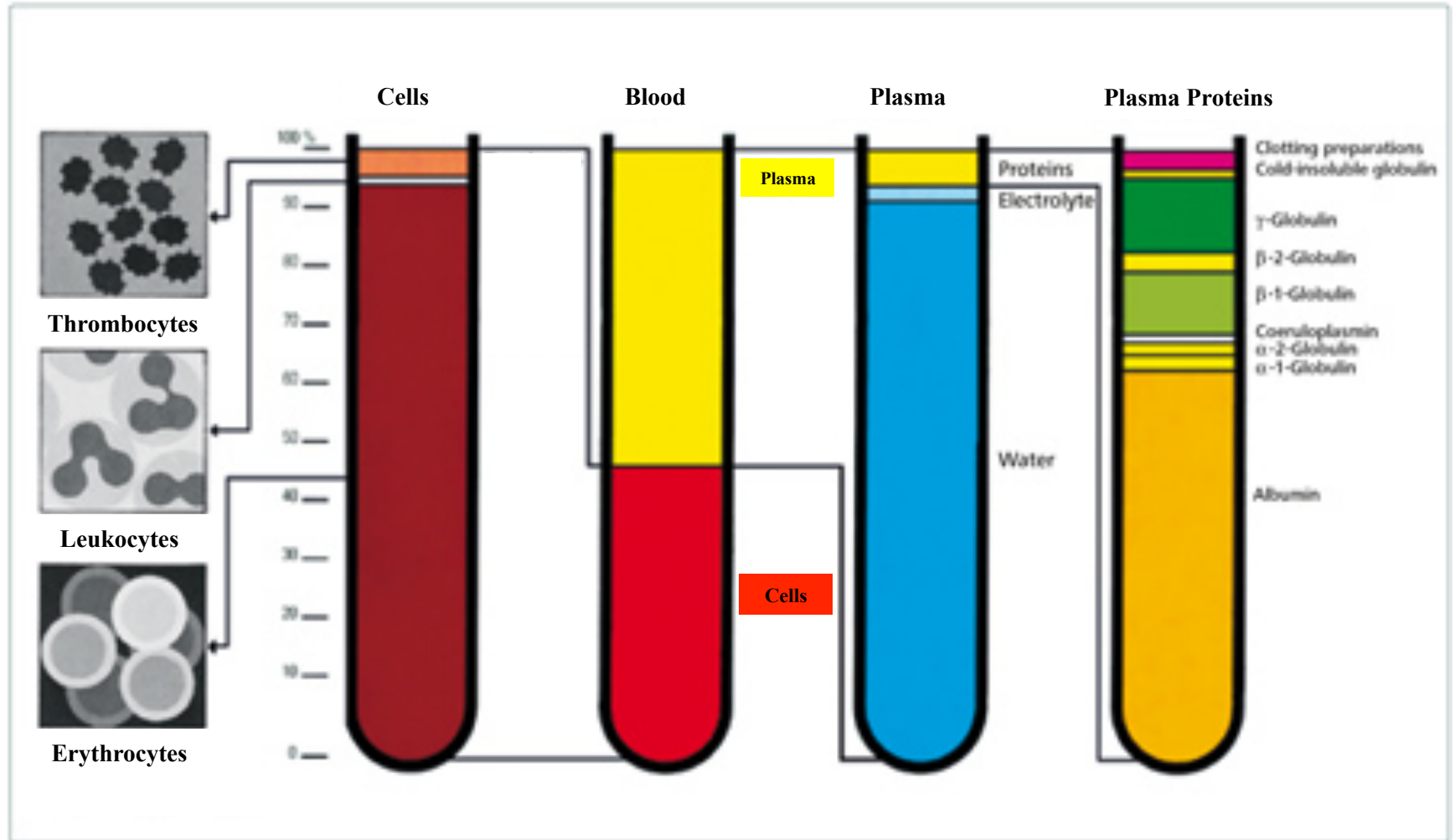
- Very efficient synthesis (>99% per couple)
 - 10 residue peptide, 99% coupling
 - Yield = $0.99^{10} = 90.4\%$
 - 50 residue peptide, 99% coupling
 - Yield = $0.99^{50} = 60.5\%$
- Limited to small peptides
- No glycosylation ability

Polypeptides and Proteins

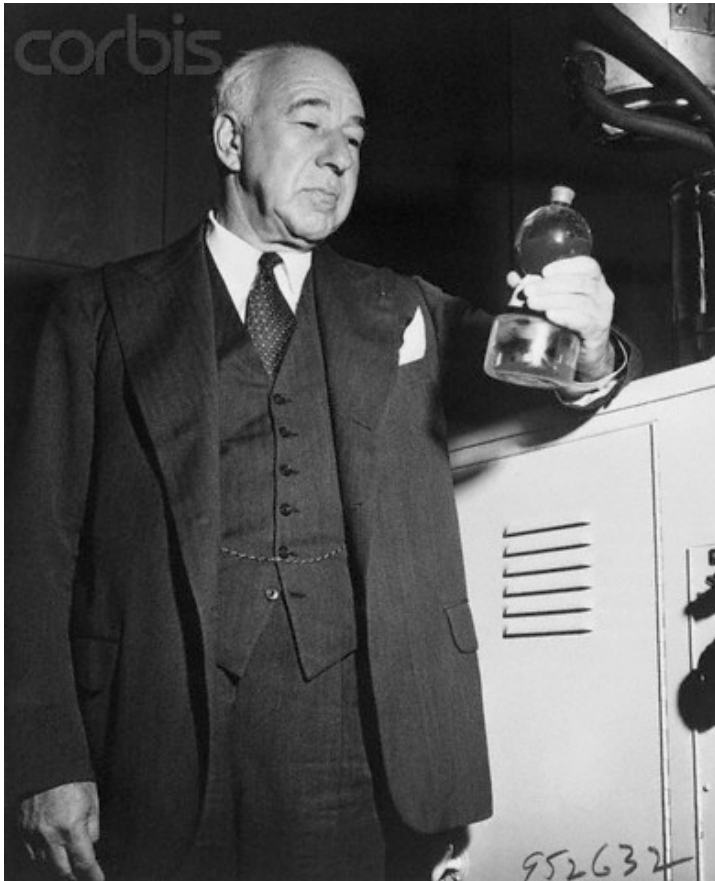
- Other hormones in our body consist of larger peptide chains or more complex proteins such as GH, ACTH, and Prolactin



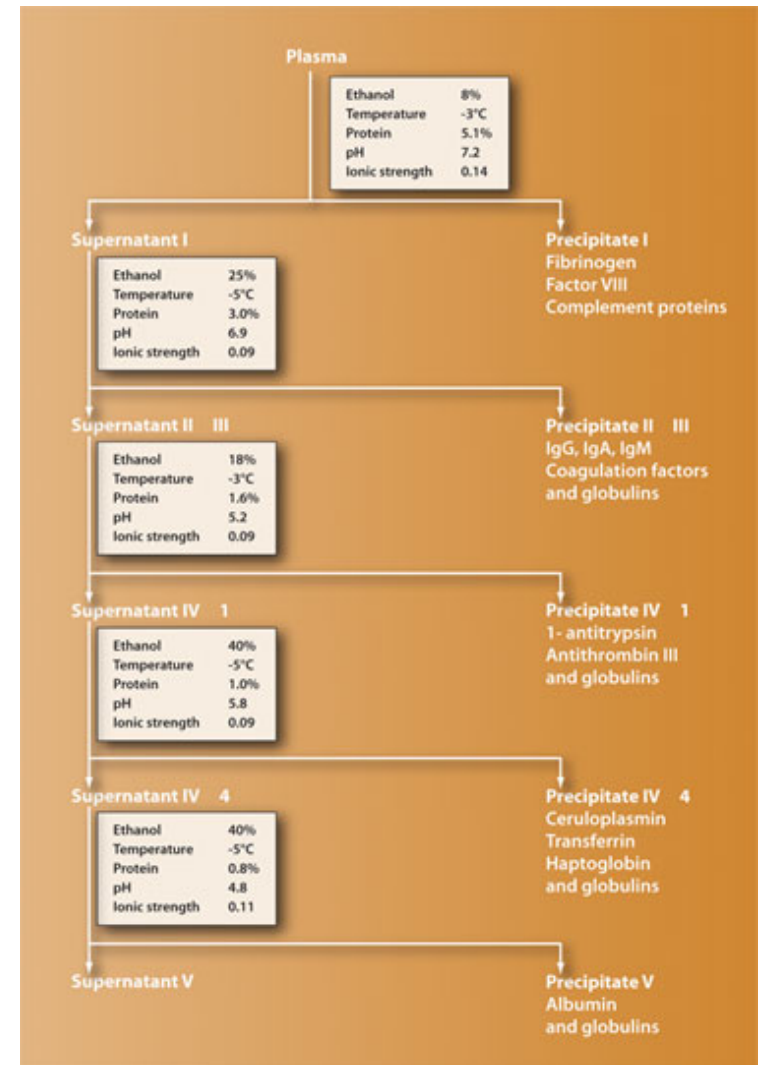
Blood Fractionation



Cohn Process



Edwin Cohn 1892-1953



Blood Fractionation

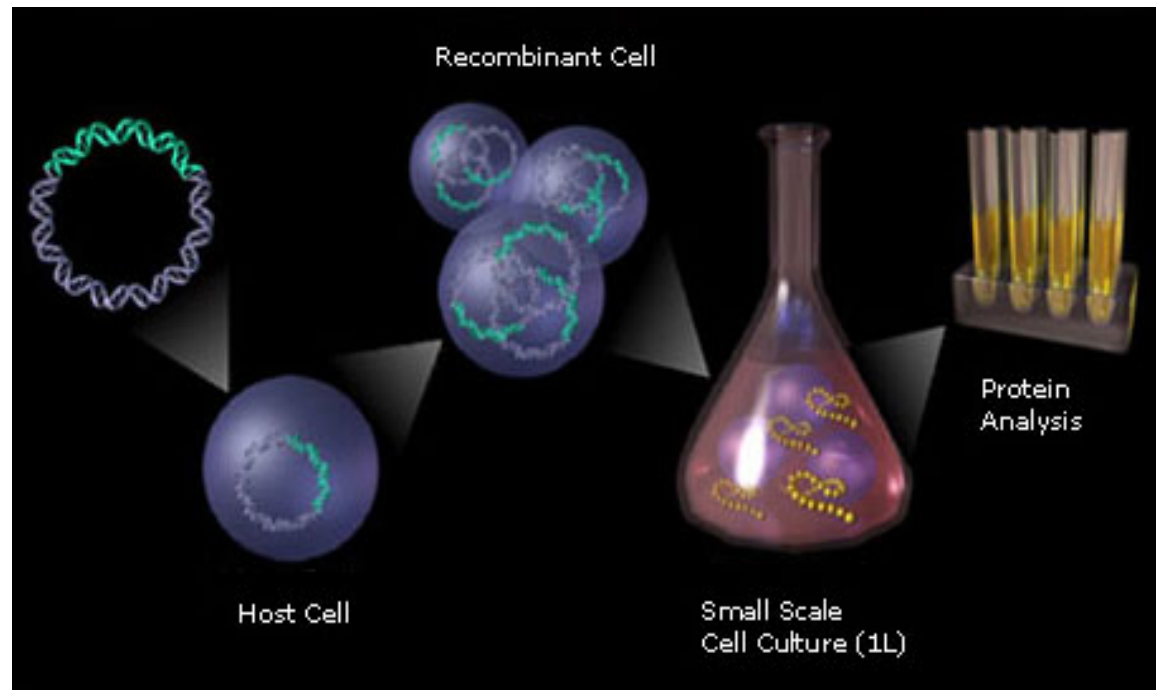


Polypeptides and Proteins

- Natural sources are often rare and expensive
 - Difficult to keep up with demand
 - Hard to isolate product
 - Lead to immune reactions (e.g. HAMA response)
 - Viral & pathogen contamination

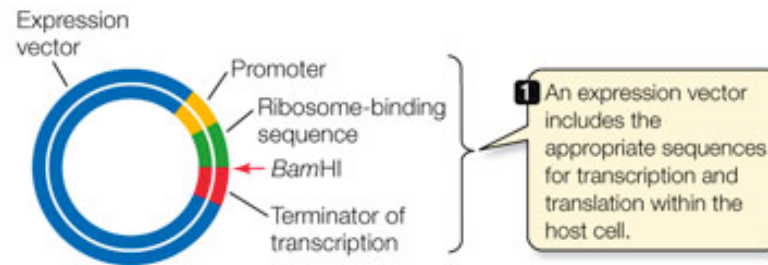
Polypeptides and Proteins

- Most protein pharmaceuticals today are recombinant products
 - Cheaper, safer, abundant supply



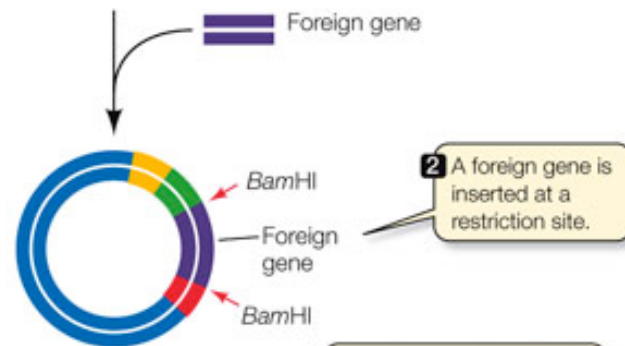
Requirements for recombinant protein production

Vector



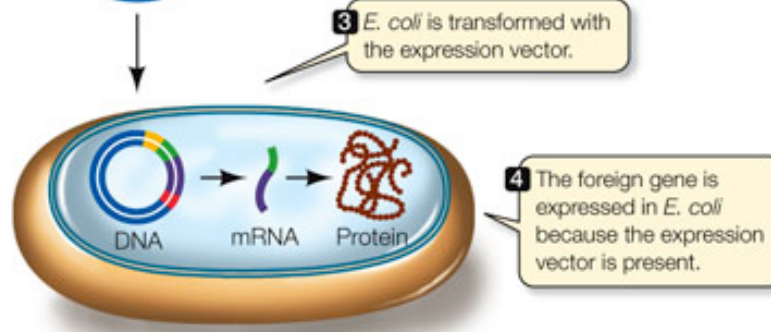
Plasmid

Gene



Insulin

Host



E. coli

Now you are able to:

- ✓ Understand the role of proteins in health and disease
- ✓ Recognize the limitations of traditional protein production methods
- ✓ Recognize the advantages of recombinant over naturally-isolated protein