Introduction

✓ What is Biotechnology?

- Purposeful design and modification/assembly of bio-oriented materials (e.g., proteins/enzymes, microorganisms, plant/animal cells, tissues, stem cells etc..) and unit processes to benefit humans or make a profit.

- Use and applications of biological system (cells, tissues etc..) or biomolecules (enzymes/proteins, antibodies, DNA/RNA) and key technologies to produce valuable products at commercial scale and to treat diseases:

→ **Cost-effectiveness → economically feasible**

✓ **Basic Biology / Medical sciences**

- To discover and understand the underlying mechanisms of behaviors and disorders in living organisms

➢ **Definition of Biotechnology based on the use of techniques and methods**

✓ **Traditional Biotechnology** (Before 1970)

- Broad definition of Biotech : Using a biological system to make products

- **Food processing** : Fermented foods, Brewery, Dairy products, etc.
  The process of brewing beer : conversion of starch to sugar followed by addition of specific yeast

- **Agriculture** : Modifications of living plants for improved yield of food crops via artificial selection and hybridization
  ex) Crops with reduced vulnerability to frost, draught, and the cold

✓ **Simple process**

- Direct use of or isolation from original biological sources
- Fermentation

✓ Modern Biotechnology (After 1970s)

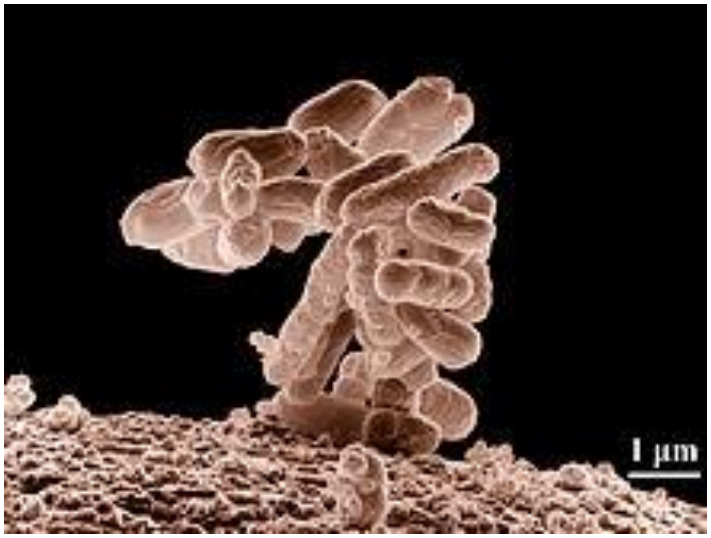
- Use of recombinant DNA technology since 1973
  - Stanley Cohen and Herbert Boyer perfect techniques to cut and paste DNA (using restriction enzymes and ligases) and reproduce the new DNA in bacteria.
- Combined use of different disciplines:

-Biology-based knowledge : Cell biology, genetics, molecular biology, etc

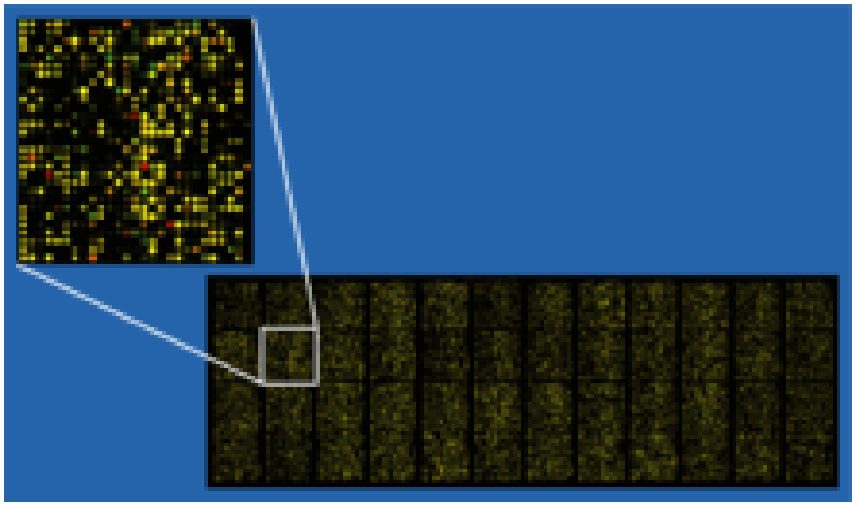
-Knowledge linked with practical applications :Biochemical Eng, Bioinformatics, computational design, etc.

- Use of genetically altered organisms
  - Enabling the production of existing medicines or products easily and cheaply (ex: insulin ; price between recombinant human insulin and synthetic pig insulin ?)

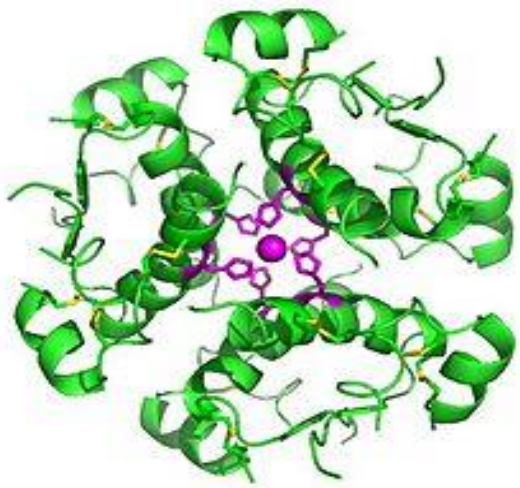
- **Traditional Biotechnology industries** : adopts new approaches and modern techniques to improve the quality and productivity of their products



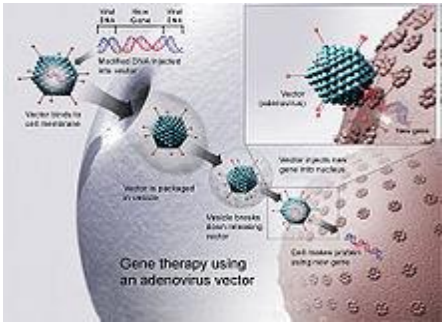
Recombinant *E. coli*



DNA microarray



Computer-generated insulin structure



Gene therapy using adenovirus

Impact of recombinant DNA technology on the production of proteins

- Overcomes the problem of source availability : allows the manufacture of any protein in whatever quantity it is required

- Overcomes the problem of product safety:
  Transmission of blood-born pathogens such as hepatitis B, C, and HIV via infected blood products

- Provides an alternative to direct extraction from inappropriate or dangerous source materials : The fertility hormones(FSH and hCG) from the urine of pregnant women; Urokinase from urines

- Facilitates the generation of newly designed proteins:
  Therapeutic proteins or enzymes with desired property

✓ **Biotechnology focuses on :**

- Development of therapeutics based on underlying mechanisms of diseases
- Development of new methods to cure diseases : Gene and cell (stem cells) therapies
- Production of valuable products at commercial scale

  Organic acids, Antibiotics, Amino acids, Proteins(enzymes), Biofuels, Vitamins, Hormones, Alcohols, Fermented foods, Fine chemicals, etc..

- Development of tools and methodology

  Expression systems, Gene synthesis/Sequencing, Purification process, Formulation, Bioassays, Diagnosis, Delivery

✓ **Integration of biological sciences with Engineering principles**

→ **cost-effectiveness**

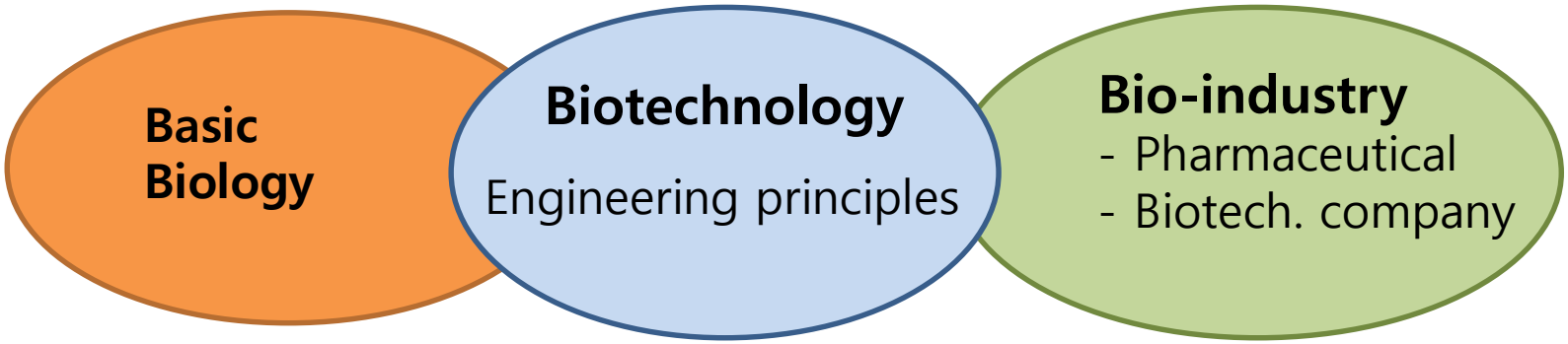
- **Process development/design/optimization**

✓ **Required disciplines**

- Basic biology, - Mass/Energy balance, - Thermodynamics

- Physical, organic chemistry / Pharmacology

- Biochemical engineering : Extension of chemical engineering principles to biological system → Bioreaction engineering, plane design, process control, optimization, and separations



✓ Major application areas

- **Health care / Diagnostics** :
  - Development of therapeutics: efficacy, toxicity
  - Diagnosis : early detection and prevention of diseases

- **Agriculture** : Crop production with high yield and quality

- **Bio-based process:** Pollution, CO_2 emission, global warming

- **Alternative energy (Bio-energy)** :
  - Depletion of fossil fuels
  - Use of renewable sources :Corn, sugar cane, cellulose
  - Cost (?)

Key technologies and fields

- **Protein engineering** : Design of proteins/enzymes based on structural and mechanistic knowledge, molecular evolution, computational design
- **Metabolic pathway engineering:** Design of more efficient metabolic pathways: high yield of target product, low by-product

- **Computational modeling and optimization:** Systems Biology, Genome-wide analysis
- **Nano-biotechnology** : Use of NPs for diagnosis and imaging

- **Cell culture engineering :** Microorganisms and mammalian cells

  - **Hybridoma technology :** A technology of forming hybrid cell lines (called **hybridomas**) by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell that is selected for its ability to grow in tissue culture

- **Separation technology :** Recovery and purification of a target product

- **Synthetic biology**：Creation of new biosystems (**Cells and biomolecules**): Systematic, hierarchical design of artificial, bio-inspired system using robust, standardized and well-characterized building block

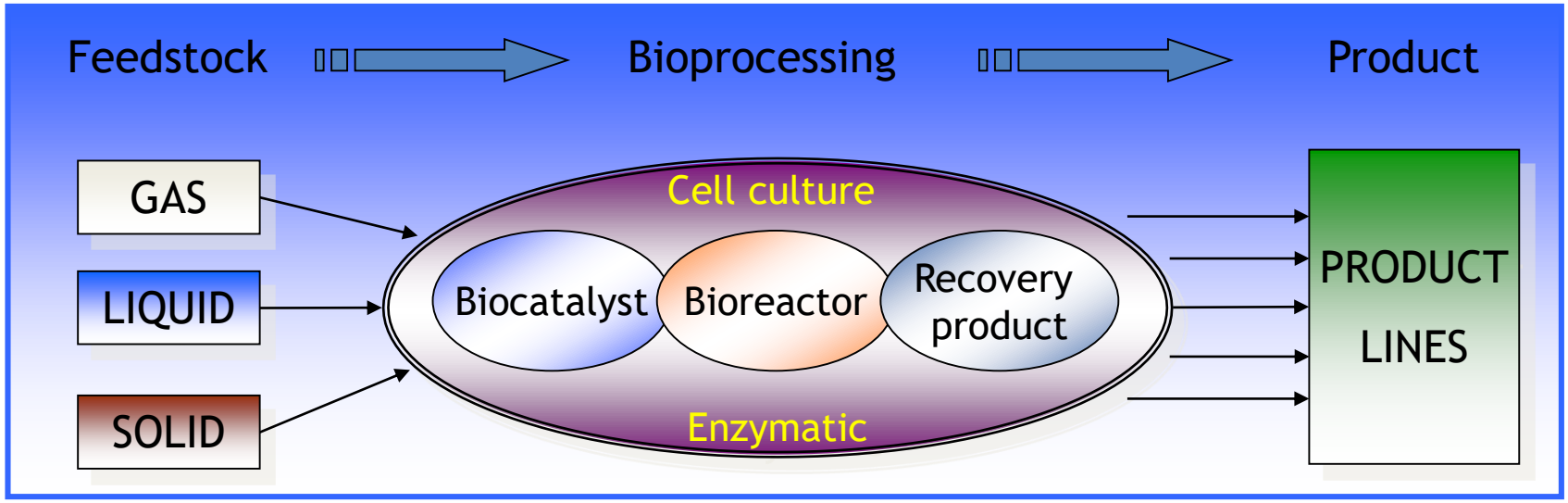
Branches of Biotechnology

- **Blue biotechnology**：Marine and aquatic applications of biotechnology
- **Green biotechnology**：Agricultural applications
- **Red biotechnology**：Medical applications
- **White biotechnology**：Industrial applications

Typical examples of Bio-Products

Company	Procucts
BASF	✓ Vitamin B-2 ✓ Methoxy isopropyl amine (chiral intermediate) ✓ Styrene oxide ✓Amino acids
Eastman Chemical / Genencor	✓ Ascorbic acid
Degussa	✓ Acrylamide ✓ Fatty acid – derived esters ✓ Polyglycerine ester ✓ Organo modified silicones and oleochemicals
Celanese / Diversa	✓ Acetic acid ✓ Polyunsaturated fatty acids ✓ Non-digestible starch ✓ Polylactic acid (PLA)
Cargill	✓ Polylactic acid (PLA) (140,000 MT/yr)
DuPont / Genencor	✓ 1,3-Propanediol ✓ Terephthalic acid ✓ Adipic acid
Chevron / Maxygen	✓ Methanol

General Bioprocess



Feedstock

- Gas
  - Syn. Gas
  - CO_2
  - Organic vapor
- Liquid
  - Organic
  - Sugar solution
- Solid
  - Biomass
  - Consumer Waste

Bioprocessing

- Immobilized Enzymes
  - Ambient to Extreme
- Fermentation
  - Immobilized
  - Free cell
  - Ambient to Extreme
- Bioreactors
  - Continuous Systems
  - Membrane
  - Batch or Fed-batch
- Separation
  - In situ
  - Secondary
- Media
  - Gaseous
  - Aqueous
  - Organic

Product

- Pharmaceuticals
- Fine chemicals
- Specialty Chemicals
- Feedstock
- Bulk chemicals

New paradigms in Biotechnology

- ✓ **Advent of Bio-based Economy**
- ✓ **Genome and proteom wide analyses: Global analysis**
- ✓ **Integration of high throughput analysis system**

- **Massive and high speed analysis system**
  - Genome and proteom-wide approach : Systemic approach
  - High amounts of relevant knowledge

- **Genomics** (Gene chips) : 1 million genes / chip
  - Gene (mRNA) expression profiling in high throughput way
  - Single nucleotide polymorphism (SNP)

- **Proteomics** (2-D gel, LC/MS, protein microarray)
  - Functional genomics
  - Bio-molecular interactions (Interactom)

- **Development and commercialization of target products**
  - Bioinformatics
  - Drug target identification via high throughput screening

Bio-based economy: Impact on global economy

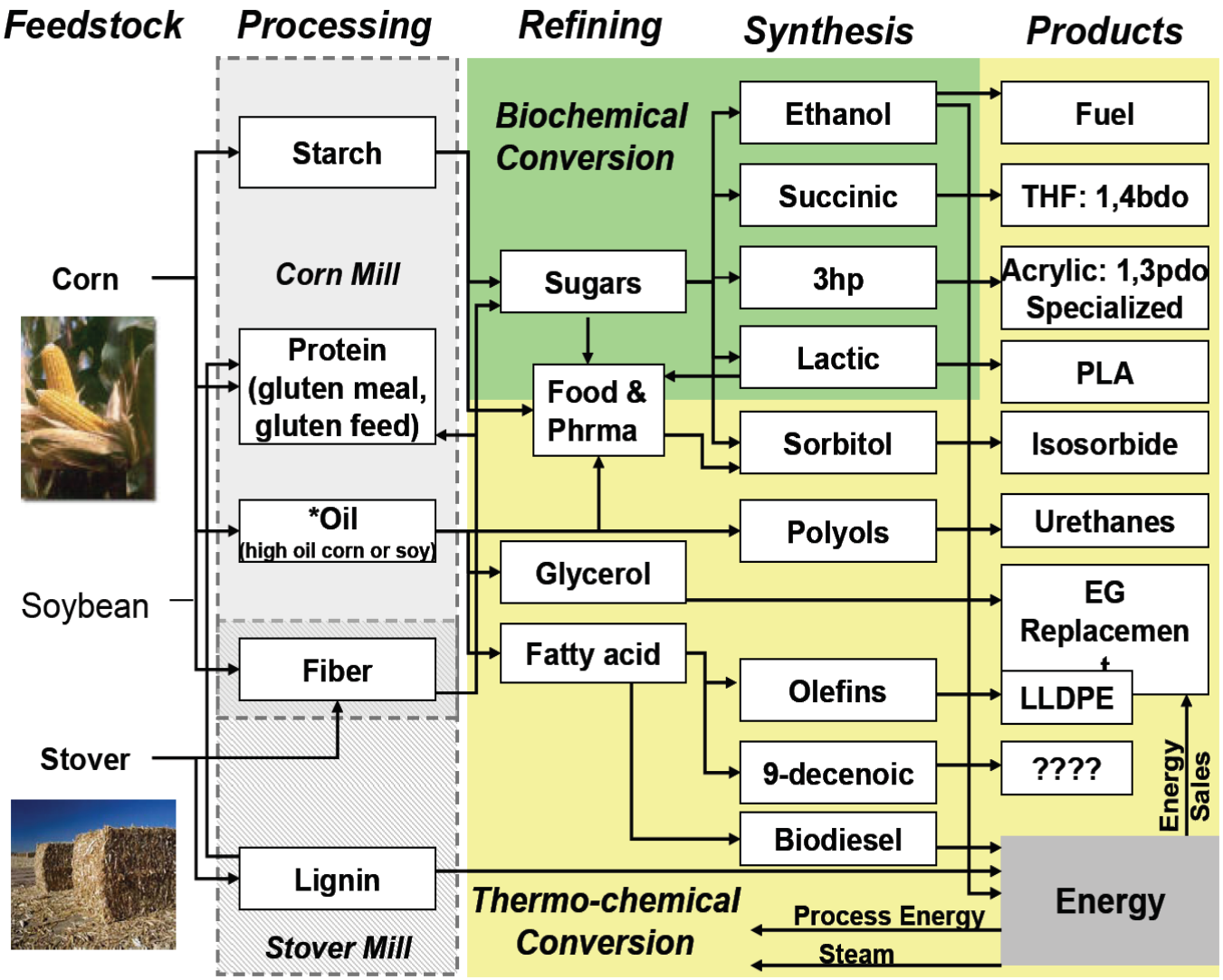
✓ **Shift from petroleum-based economy**

- Exhaustion and soaring price of petroleum (> $ 100 /gallon)
- Environmental issue
  Global warming (greenhouse gas, CO_2 , emission)
  Pollution

- **Development of renewable source-based Bioprocess**
- **Replacement of chemical processes with Bio-based ones**

White Biotechnology

Value chains from renewable sources



Alternative energy sources

✓ **Production of biofuels from natural resources**

- Increase in the yield and alcohol tolerance
  - Redesign of pathway for the ethanol production in yeast to use raw materials : corn starch, cellulose, soybean, sugar cane
  - Elucidation of enzyme mechanisms
  - Redesign of pathway to increase the yield and to reduce by-products
  - Design of critical enzymes in the pathway
- Process development : Fermentation process
- Separation and concentration

✓ **Role of Agricultural Biotech in the production of biofuels ?**
✓ **Adverse effects due to the production of biofuels from corn ?**

Key role of enzymes in Bio-based economy

Energy and Environmental issues
- **Depletion of fossil fuels**
- **Limitation to CO_2 emission (Kyoto protocol)**

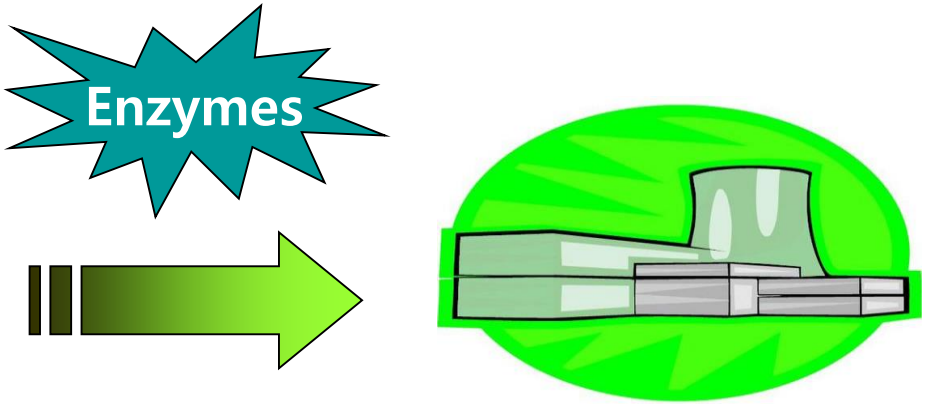
Petrochemical-based economy

Chemical process

→

Renewable source-based economy

Bio-based process



Use of enzymes in Biofuel production from renewable biomass such as starch and cellulose → amylase, cellulase etc.

Enzymes

- Most proficient catalysts with high specificity
- Competitive and cost-effective processes

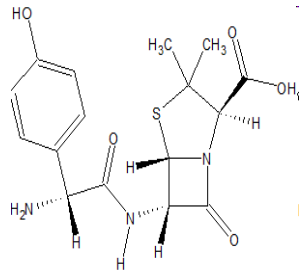
- Cleaning (Detergents)
- Textiles
- Starch Processing
- Brewing
- Leather
- Baking
- Pulp and Paper
- Food and Specialties
- Animal feeds
- Cosmetics

Use for biosciences

- DNA polymerase: Thermostability, fidelity
- Restriction enzymes: Specificity
- Alkaline phosphatase
- Peroxidase

Industrial use for specialty chemicals

- Chiral drugs
- Chiral intermediates
- Semi-synthetic antibiotics
- Organic acids



Therapeutics

- Treatment of Gaucher's disease

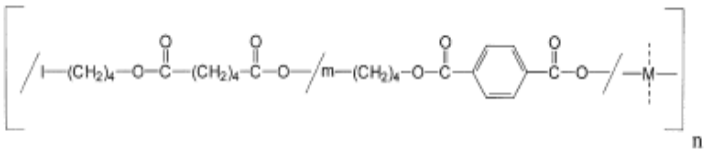
Chemical company devoting to Biotechnology : BASF

Emphasis on Bio-products mainly using Enzymes

BASF
The Chemical Company

Agrochemicals	• Fungicides • Herbicides • Insecticides
Fine Chemicals	• Vitamins • Carotenoids • Amino acids • Enzymes • Nutraceuticals • Pharmaceutical Active Ingredients • Chipros™

Ecoflex®



Therapeutic proteins

- ✓ **High specificity and less toxicity → high safety and efficacy**
- ✓ **Therapeutic proteins**
  - **Antibodies, proteins, enzymes, peptides etc.**

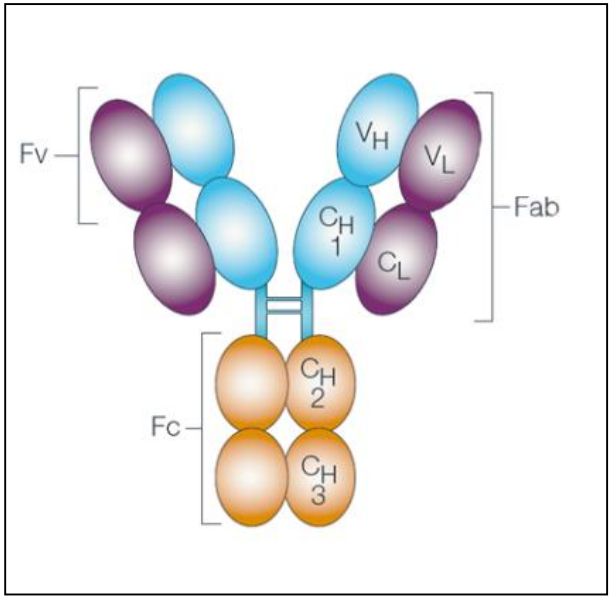
    ex) EPO, Interferon, Insulin, Avastin, Enbrel, Remicade, Herceptin,

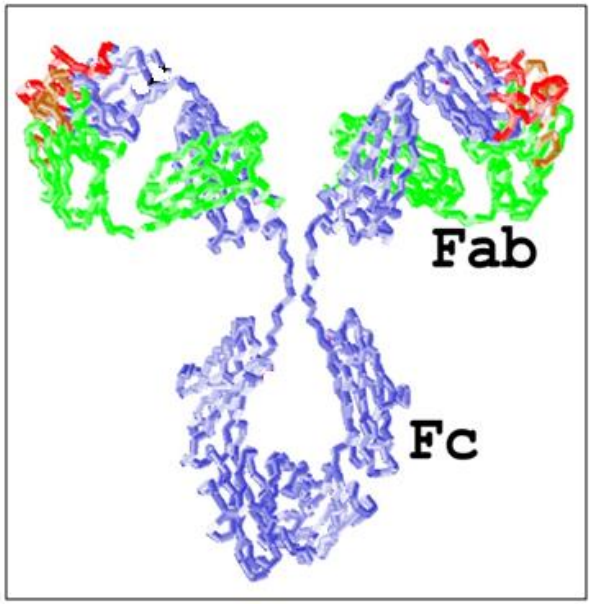
    EPO (Erythropoietin) : Stimulating the proliferation of red blood cells
    Herceptin : Mab against EGFR2(Epidermal growth factor receptor 2)
    Avastin : Mab against VEGF (Vascular endothelial growth factor)
    Remicade: Mab against TNF-α (**Tumor necrosis factor- α)**

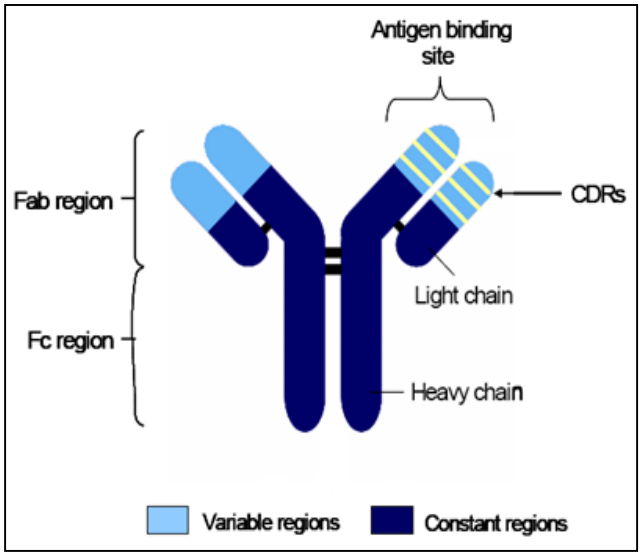
- ✓ **World market**
  - **EPO alone : ~ $ 11 Billion per year**
  - **$ 50 Billion (2007)→ $ 190 Billion (2015)**
  - **Antibodies > 50 %**
  - **Intensive investment in monoclonal antibodies: Biosimilar**

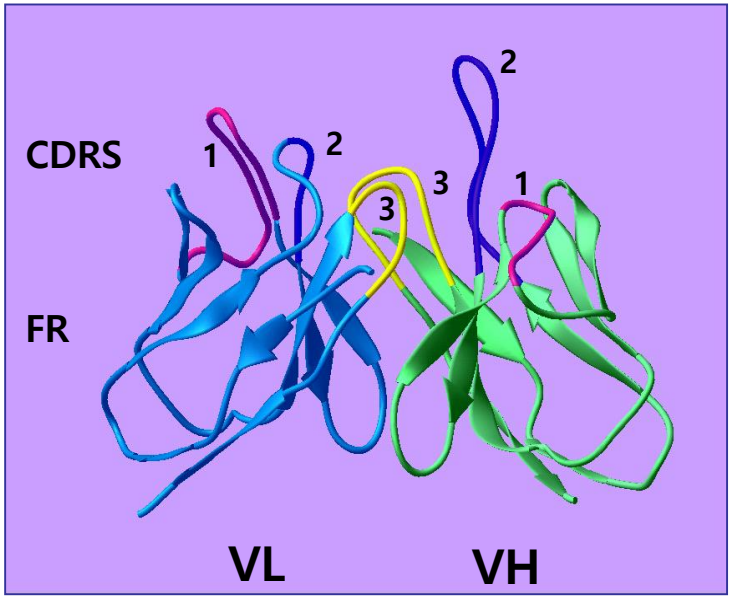
Therapeutic proteins will form the back-born of future medicinal therapy

Structural and functional features of antibodies









Blockbuster Therapeutic Antibodies

Approved Year	Product	Target	Indication	Company	Market size(07)*	Antibody Type
1997	Rituxan	CD20	Non-Hodgkin's lymphoma	Genentech	4,603	Chimeric
1998	Herceptin	Her2/neu	Breast cancer	Genentech	4,047	Humanized
1998	Synagis	RSV	RSV prophylaxis	MedImmune	1,100	Humanized
1998	Remicade	TNF- α	RA, Chron's disease	J&J	5,234	Chiemric
2002	Humira	TNF-α	RA	Abbott	3,064	Human
2003	Raptiva	CD11a	Psoriasis	Genentech Xoma	211	Humanized
2004	Erbitux	EGFR	Colorectal cancer	Imclone Bristol-Myers	1,336	Chiemric
2004	Avastin	VEGF	Colorectal cancer	Genentech	3,335	Humanized
2006	Vectibix	EGFR	Colorectal cancer	Amgen	170	Human

* Million $ (Data Monitor 'Monoclonal 2008)

Drawbacks of immunoglobulin antibodies

- Complicated process for selecting cell lines and the production using mammalian cells → very expensive
- Intellectual property barriers
- Tend to aggregate due to large size (~ 150 KDa)
- Difficult to penetrate inside the cells
- Limited binding affinity due to confined binding surface

Ideal scaffold for alternative therapeutics

- High-level soluble expression in bacteria
- High stability (thermodynamic, pH)
- Easy design of binders with high affinity for a target
- Low immunogenicity and cytotoxicity

Therapeutics based on non-antibody scaffold

New paradigm in therapeutic proteins

- Development of new therapeutics with high efficacy and low side effect from non-antibody protein scaffold
- Designer therapeutic proteins
- IP issue and cost-effectiveness

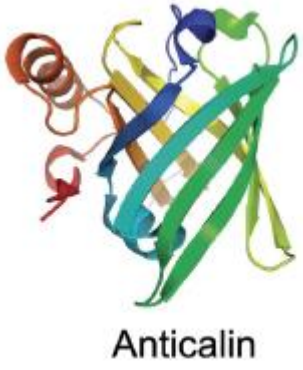
GlaxoSmithKline,
Amgen
Bristol-Myers-Squibb,
Boehringer Ingelheim
Eli Lilly,
Roche,
Avidia, Ammunex. Affibody,
Ablynex, Adnexus Therapeutics
…….

Strategic alliance or merger between big pharma and biotech companies

Non-antibody scaffolds

Human fibronectin

Z domain of Staphylococcal protein A



Human lipocalin

Ankyrin

Therapeutic Enzymes

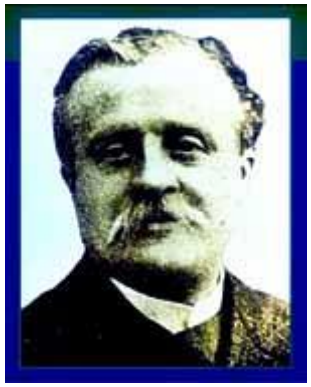
Disease	Product	Developer	Sales (US$Millions)		Features
			2004	2007	
Gaucher's	Ceredase®	Genzyme	443	N/A	▪ Glucocerebrosidase ▪ Purified from human placenta
	Cerezyme®	Genzyme	932 (2005)	1,048	▪ Produced in CHO cells ▪ 3 Exoglycosidases process for Terminal Mannose
Fabry's	Fabrazyme®	Genzyme	209	397	▪ alpha-galactosidase ▪ Mannose-6-phosphate for Glycotargeting
	Replagal	TKT	57	168	
MPS-1	Aldurazyme®	Genzyme	12	204	▪ alpha –L-iduronidase
Pompe	Myozyme®	Genzyme	Approved (2006)		▪ alfa-glucosidase

Treatment of Gaucher's disease by Cerezyme costs up to $550,000 annually

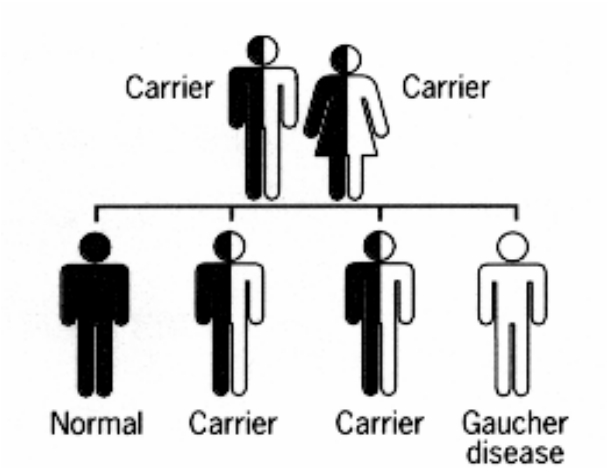
Most of therapeutic enzymes : glycoproteins

Gaucher's Disease : Lysosomal Storage Disease

Caused by a recessive mutation in a gene located on chromosome 1, affecting both males and females.

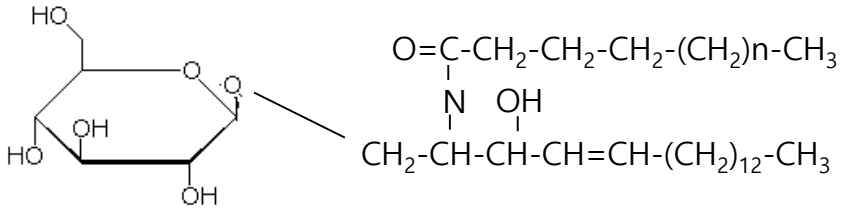


- **Found by Phillipe Gaucher in 1882**
- **Biochemical basis for the disease in 1965 by Brady *et al*..**

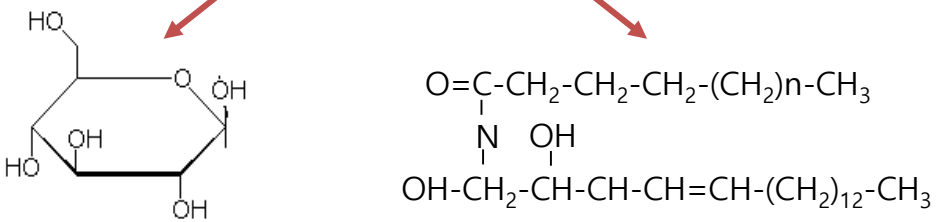


Autosomal recessive inheritance

Glucosyl **Ceramide**

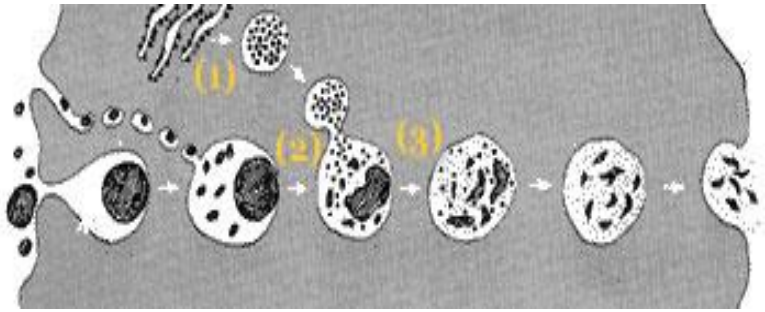


Glucocerebrosidase (b-glucosidase)



Glucose **Ceramide**

Lysosomal storage diseases (LSDs): Lysosomal Enzymes



(1) The ER and Golgi apparatus make a lysosome

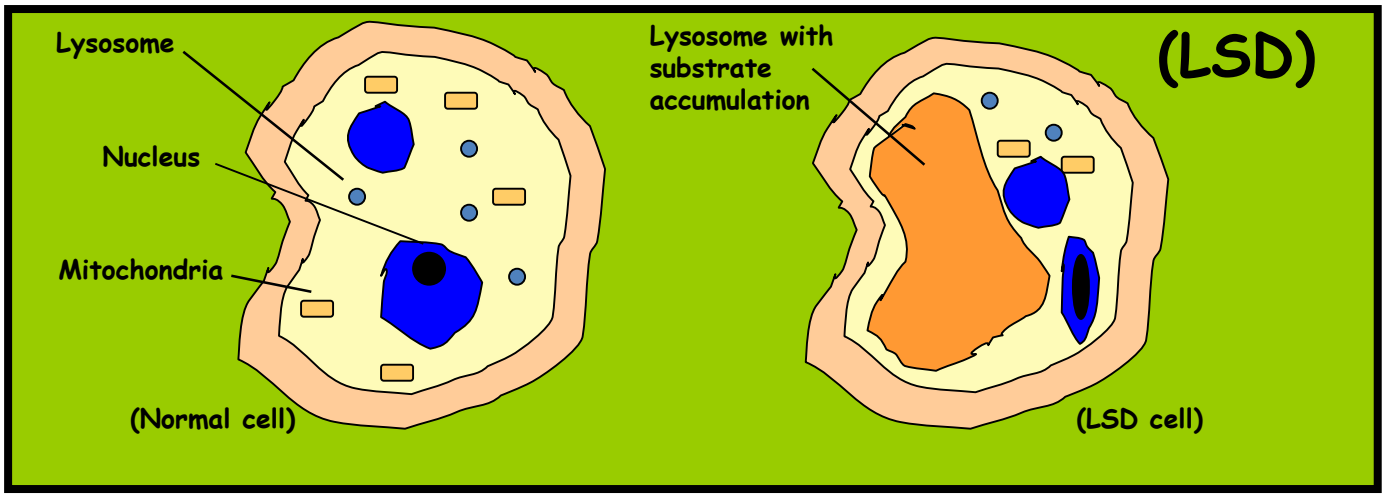
(2) The lysosome fuses with a digestive vacuole

(3) Activated acid hydrolases digest the contents

- **Lysosomes:** Cellular organelles containing acid hydrolase enzymes to break down waste materials and cellular debris

- **Cells' garbage disposal system**

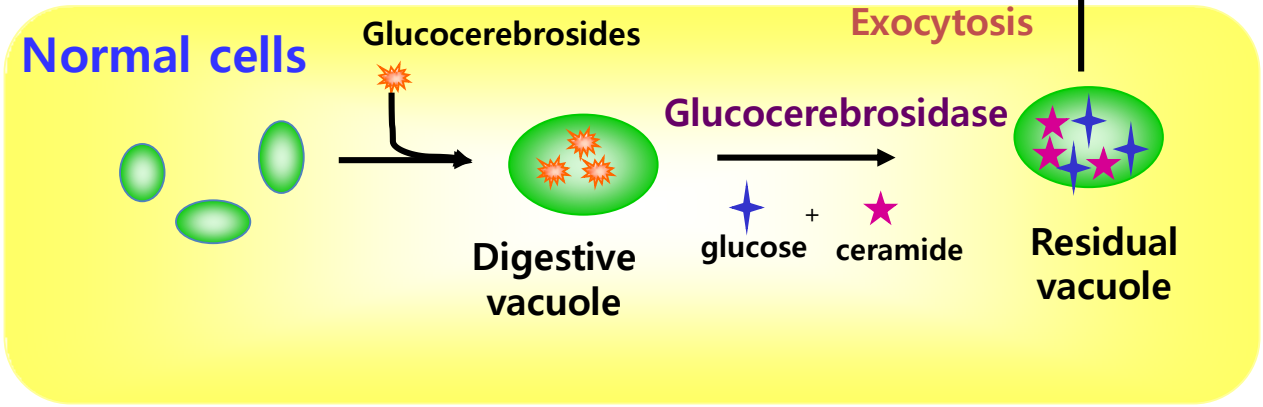
- Digestive organelle in the cell
- Contains ~40 hydrolytic enzyme
- Acidic pH (about pH4.8) within the lysosome is required for lysosomal enzymes to be active

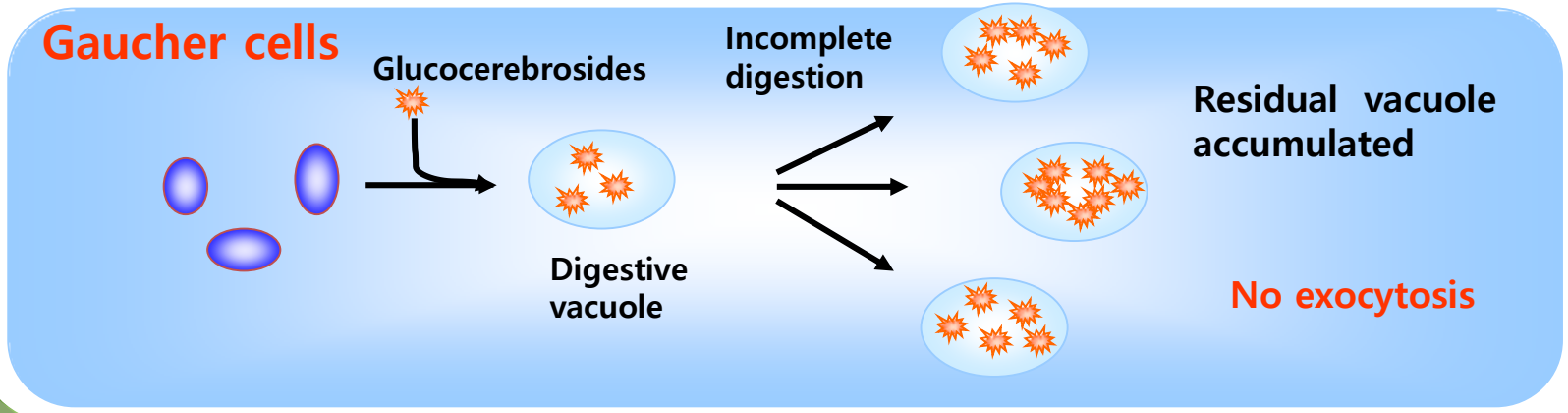


Gaucher's disease : Occurrence and symptoms

- 1/ 40,000~60,000 (Jew 1/~500)
- Swollen vacuoles → Gaucher cells
- Accumulation in spleen, liver, kidney, brain
- Enlarged spleen and liver, liver malfunction, neurological complications etc..

Distended abdomen





Diagnostics

- Diagnosis of disease as early as possible :
  Best solution compared to treatments

- Prediction and treatment of diseases based on individual genomes
  - personalized medicine
  - treatment with appropriate therapeutic agents

- Analysis / Detection of disease biomarkers:
  - Invasive or non-invasive analysis

Perspectives

- Biotechnology will have the greatest impact on humans in the future in terms of health, life-style, and economy.
  - Therapeutic proteins
  - Bio-based economy : Bioprocess and Bio-Energy
  - Diagnostics

- Modern Biotechnology constitutes a variety of diverse areas and technologies, requiring interdisciplinary collaborations.