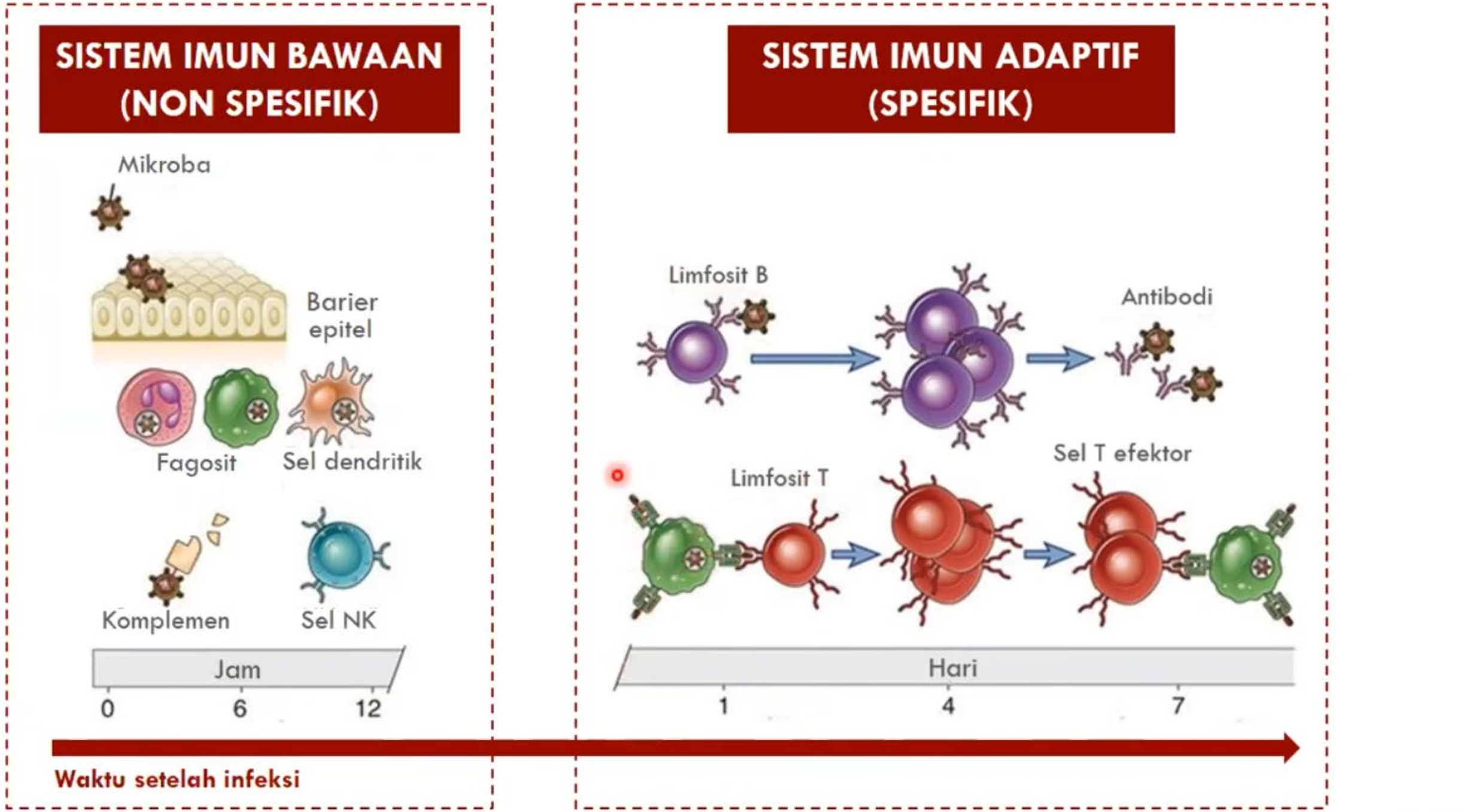
Advanced Pharma Industry
Industri Bioteknologi

More Slide take from Dosen Apt. Octavia Rahayu. M. Biomed - Brawijaya

Bioteknologi Bidang Medis (*Medical Biotechnology*)

Suatu tindakan manipulasi (rekayasa genetika) dari organisme hidup atau komponen dari organisme tersebut untuk meningkatkan kualitas hidup manusia di bidang kesehatan

- Pharmaceutical products discovery and production (therapeutic protein, mAb, vaccine, gene therapy)



(Abbas *et al.*, 2010)

APLIKASI BIOTEKNOLOGI DALAM BIDANG MEDIS: PHARMACEUTICAL PRODUCTS DISCOVERY & PRODUCTION

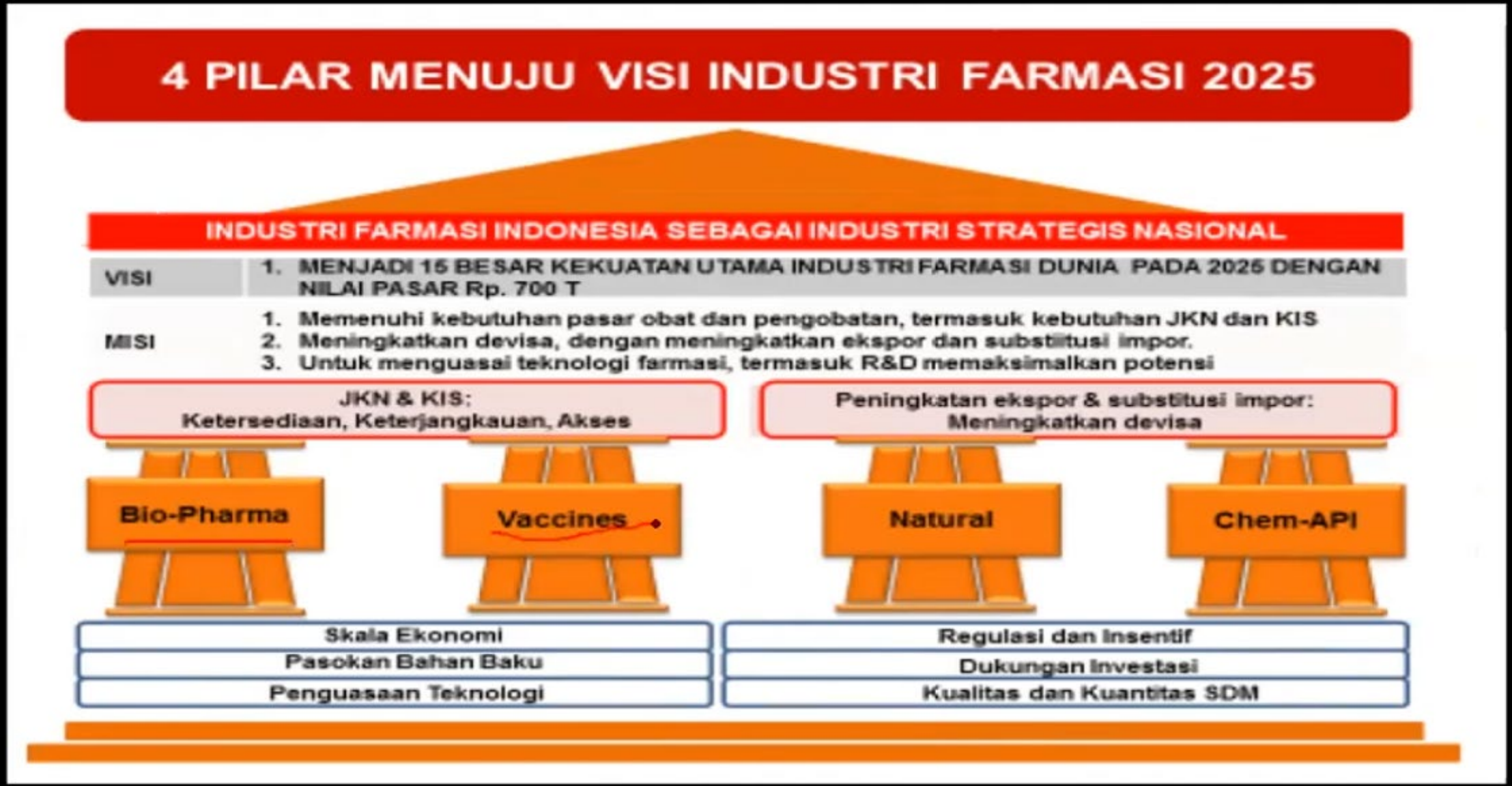
Protein terapetik
(*therapeutic protein*)

Antibodi monoklonal
(*monoclonal antibody*)

Terapi gen
(*gene therapy*)

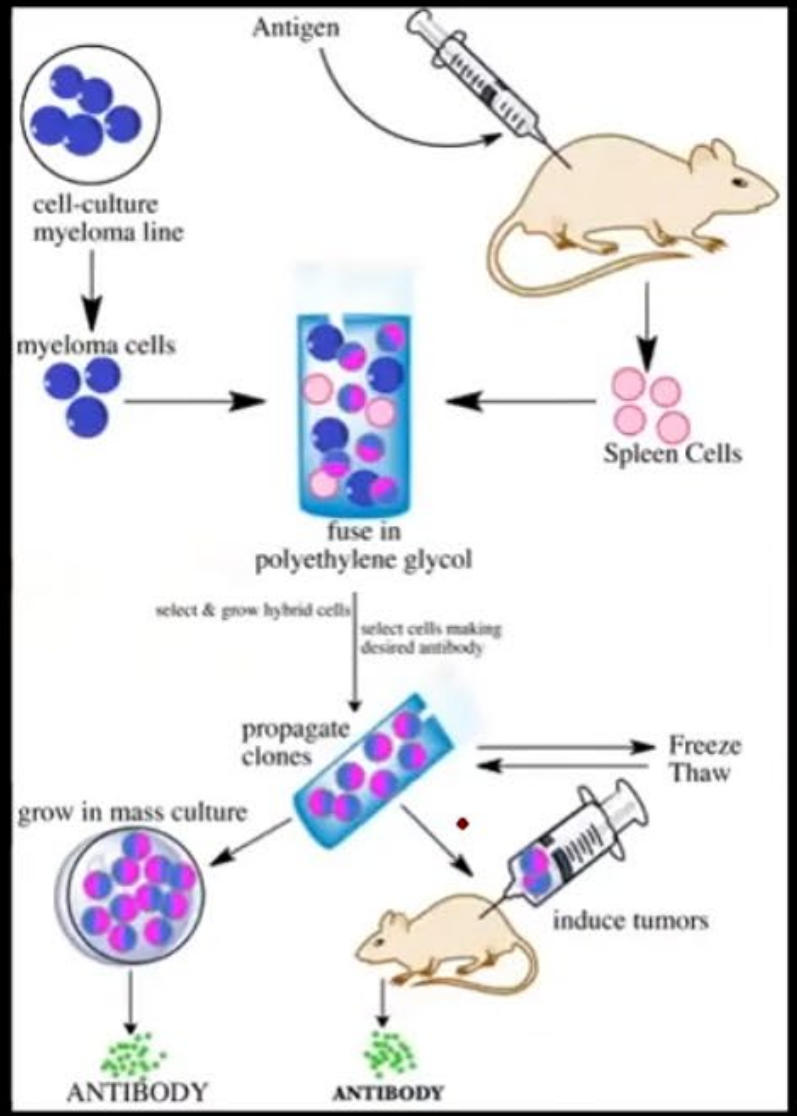
Vaksin
(*vaccine*)

PENGEMBANGAN INDUSTRI FARMASI



SKENARIO PENGEMBANGAN PRODUK BIOTEKNOLOGI

Industri Farmasi Produk bioteknologi		
2016-2018	2019-2021	2022-2025
1. *EPO (Erythropoetin)* 2. *GCSF (Granulocyte Colony Stimulating Factor)* 3. *Probiotic* 4. Insulin 5. *Stem cell protein (Wound care and cosmetics)* 6. Somatropin 7. *EGF (Epidermal Growth Factor)* 8. Enoxaparin 9. *Plasma Fractionation* (albumin, Immunogbulin)	1. *Blood Fractionation* 2. *Growth Hormone* 3. Interferon 4. Trastuzumab 5. Insulin 6. MAB (oncology) Rituximab, Bevacizumab	1. MAB (Monoclonal Anti Body) 2. Insulin *analogue*



PMK Republik Indonesia Nomor 17 Tahun 2017 tentang rencana aksi pengembangan industri farmasi dan alat kesehatan

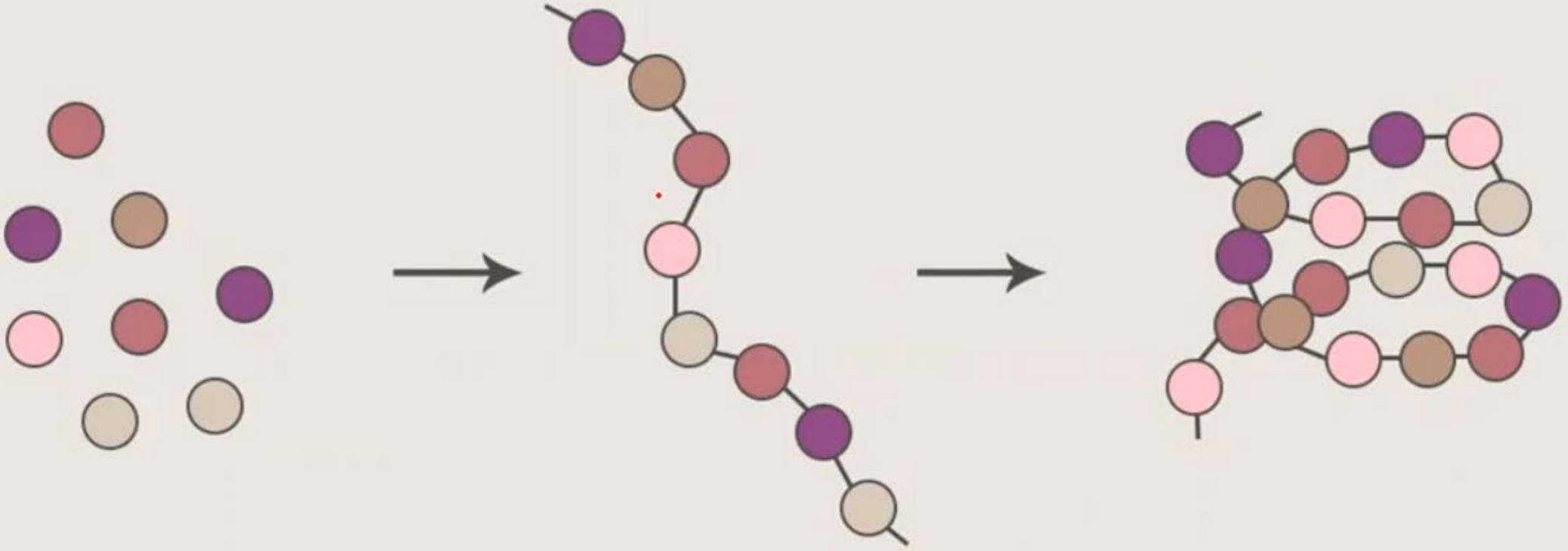
SKENARIO PENGEMBANGAN PRODUK VAKSIN

Industri Farmasi Produk Vaksin		
2016-2018	2019-2021	2022-2025
1. Dengue (Demam Berdarah) 2. MR (Measles Rubella) 3. HB (Hepatitis-B) 4. Hexavalent 5. Sabin IPV (*Inactivated Polio Vaccine*)	1. DTaP (*Diphteri, Tetanus, acellular Pertussis*) 2. Hexavalent 3. MenACWY 4. New OPV type 2 5. Pneumococcal 6. Rotavirus 7. Rabies	1. HPV (Human Papiloma Virus) 2. New TB Recombinant

PMK Republik Indonesia Nomor 17 Tahun 2017 tentang rencana aksi pengembangan industri farmasi dan alat kesehatan


<1> PROTEIN TERAPETIK

PROTEIN vs PEPTIDE?



Amino Acids

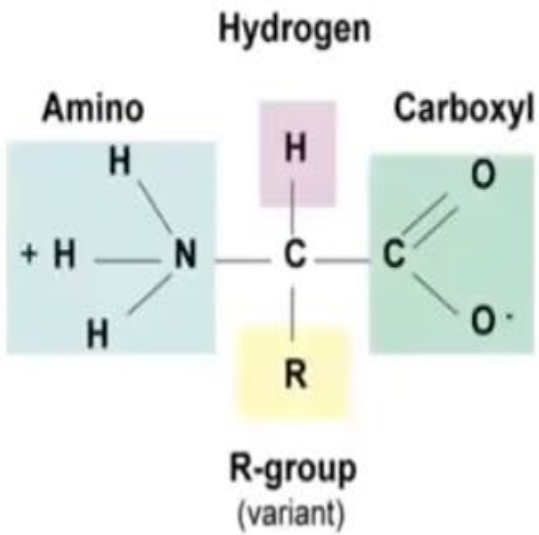
Peptide

short chain of amino acid residues with a defined sequence (e.g. leuprolide)

Protein

polypeptides which occur naturally and have a defined sequence of amino acids and a three-dimensional structure (e.g insulin)

Amino Acid Structure

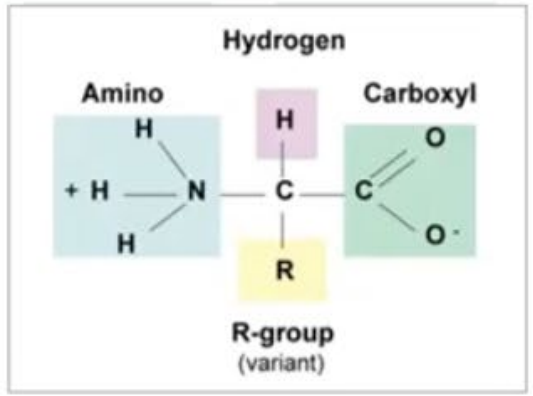


Amino acids have a range of physical properties, each having a greater or lesser degree of hydrophilic or hydrophobic nature

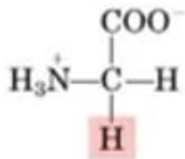
ABBREVIATIONS FOR AMINO ACIDS

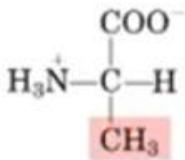
Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z

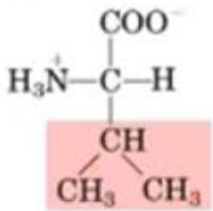
Classification of amino acids on the basis of R-group

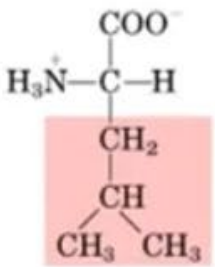


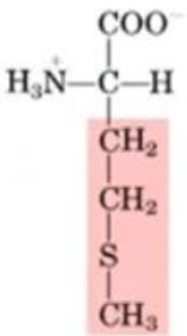
Nonpolar, aliphatic R groups

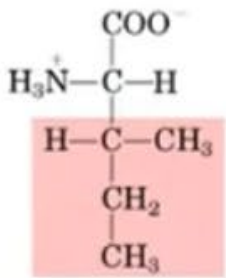

Glycine


Alanine

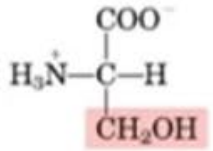

Valine

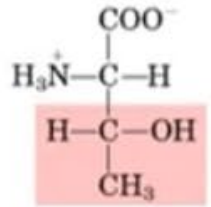

Leucine

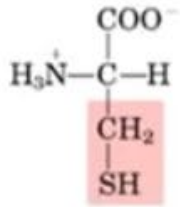

Methionine

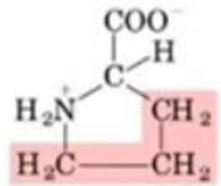

Isoleucine

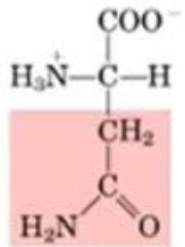
Polar, uncharged R groups

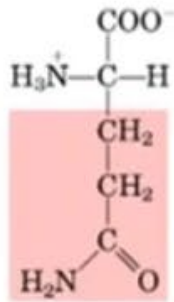

Serine


Threonine

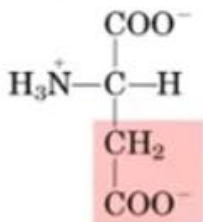

Cysteine

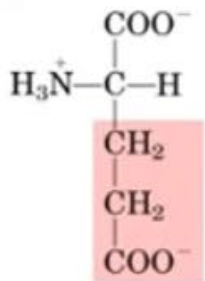

Proline


Asparagine

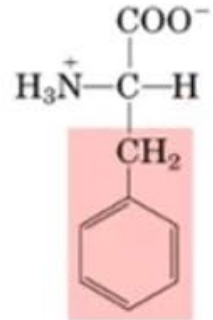

Glutamine

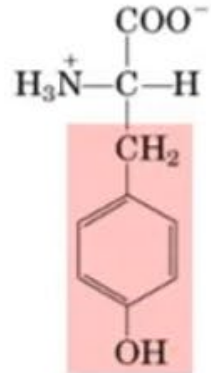
Negatively charged R groups

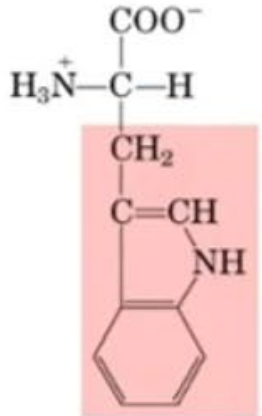

Aspartate


Glutamate

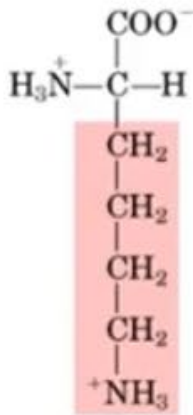
Aromatic R groups

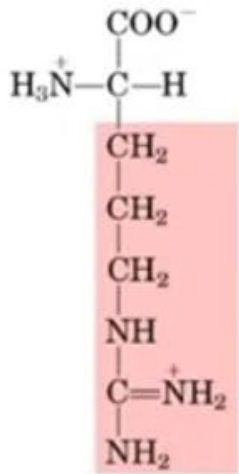

Phenylalanine

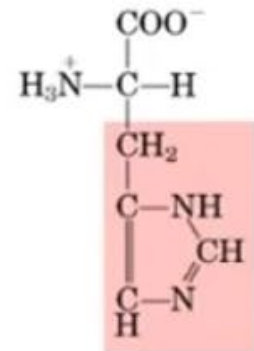

Tyrosine


Tryptophan

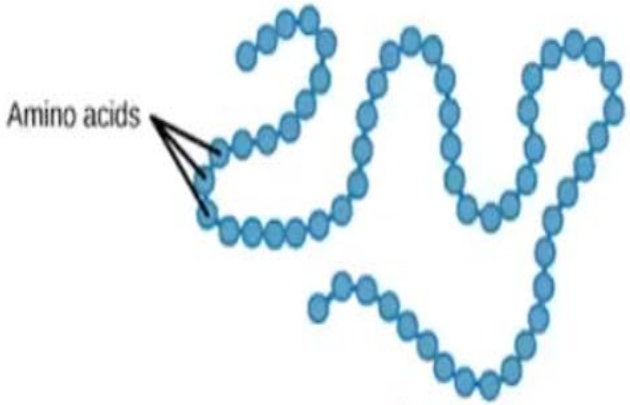
Positively charged R groups


Lysine

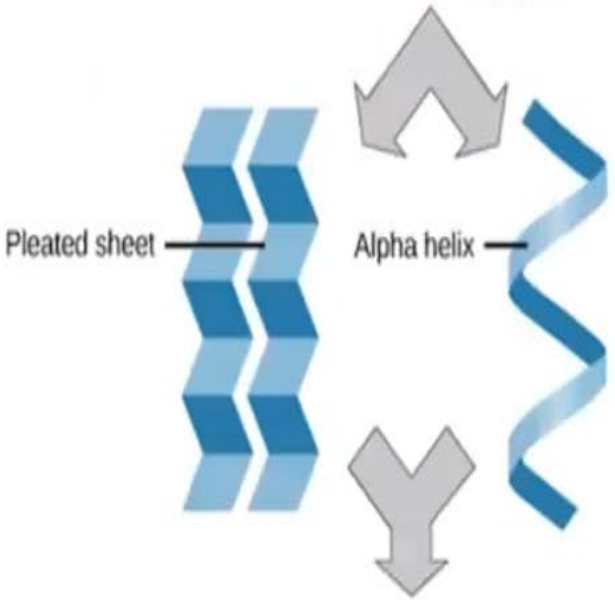

Arginine


Histidine

PROTEIN STRUCTURE



Primary protein structure
sequence of a chain of animo acids

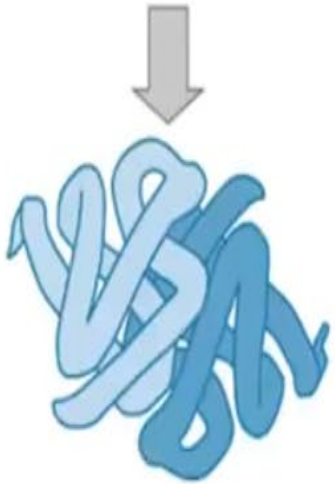


Secondary protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

including coiled α-helix and pleated sheets

Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions

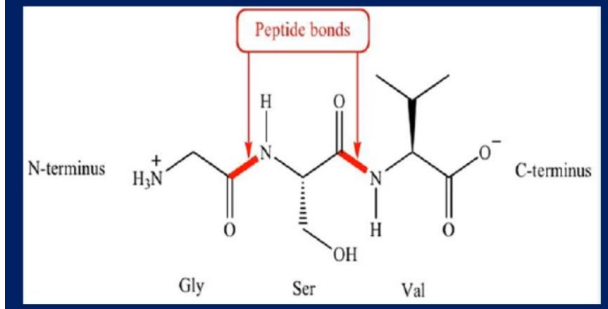
3D arrangement of helices and coils



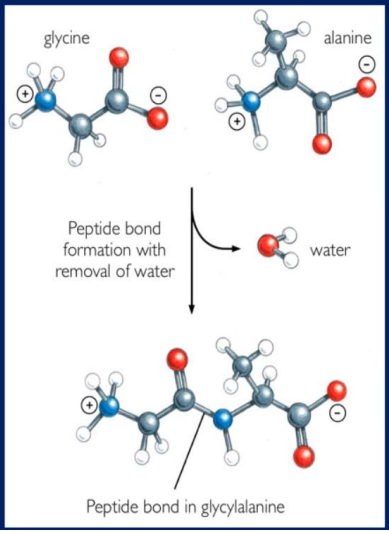
Quaternary protein structure
protein consisting of more than one amino acid chain

(e.g. the hexameric form of insulin)

STRUKTUR IKATAN PEPTIDA

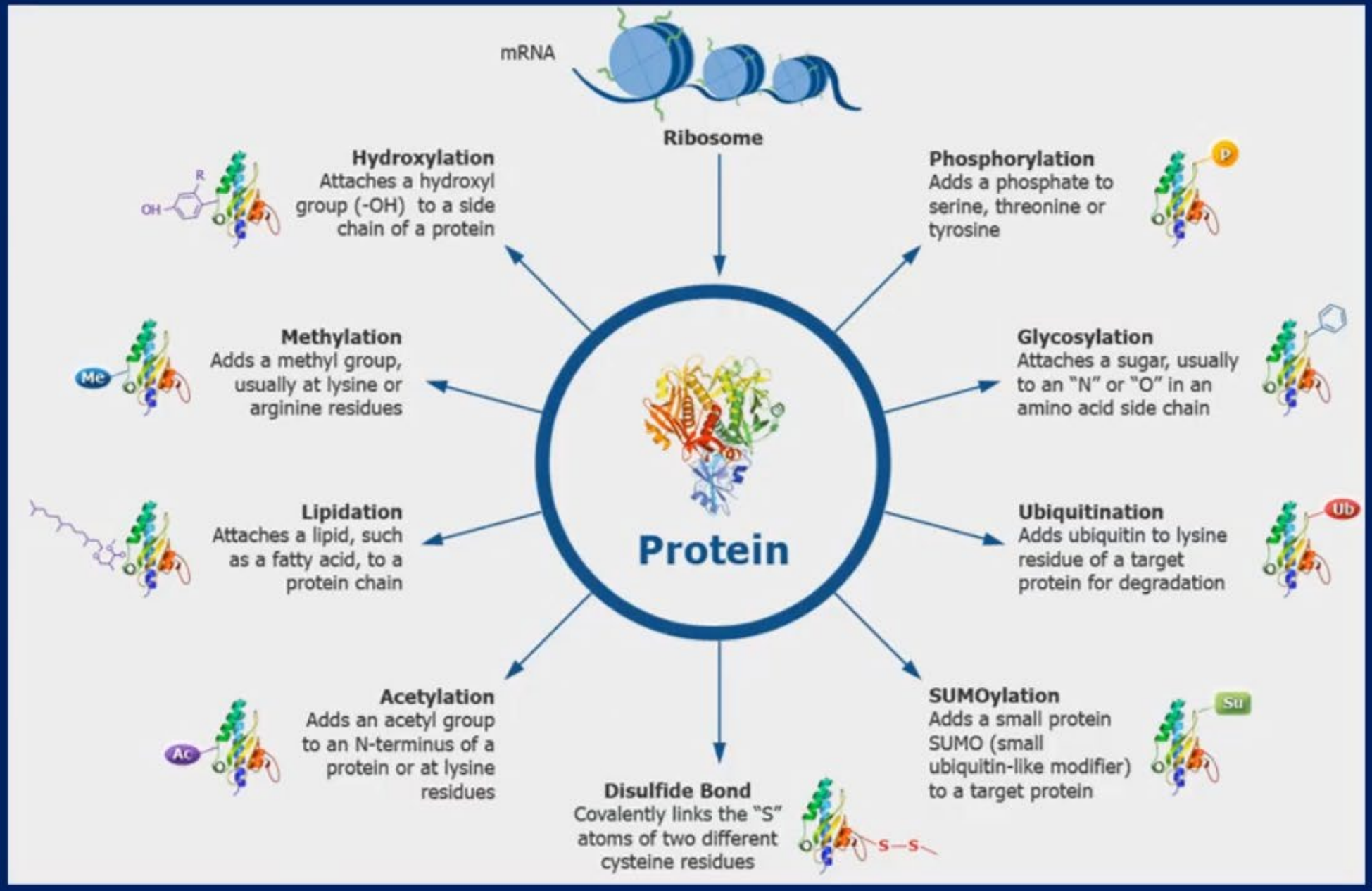


Asam amino penyusun protein dihubungkan melalui ikatan peptida (ikatan amida)

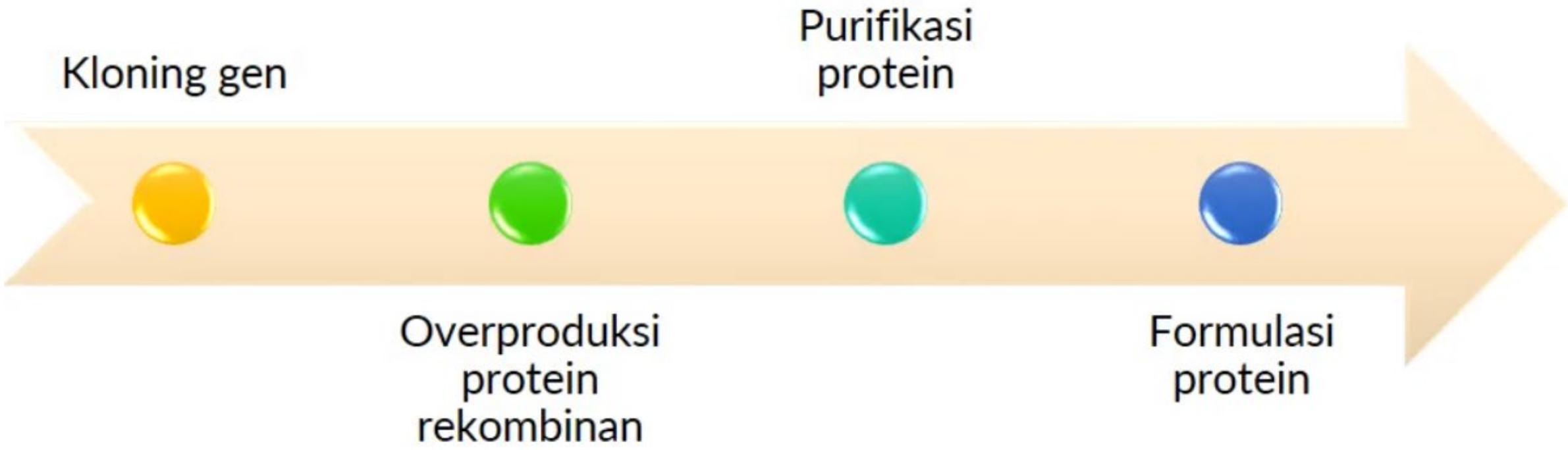


POST TRANSLATIONAL MODIFICATION (PTM)

Proses modifikasi protein setelah ditranslasi supaya dapat memberikan fungsi, seperti folding, glikosilasi pada N dan O terminal atau penambahan asam sialat, ubikuitinasi, dsb

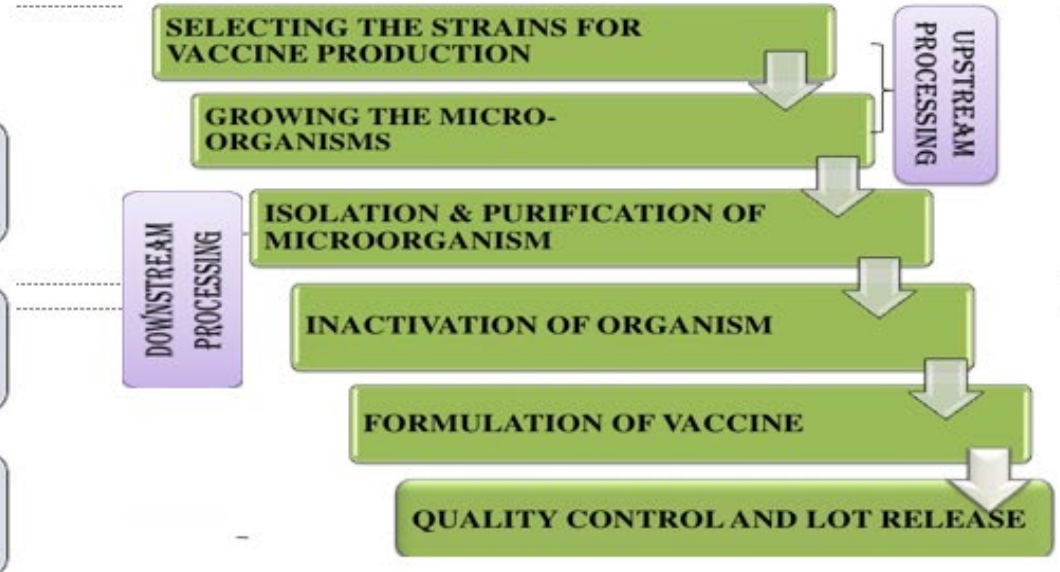


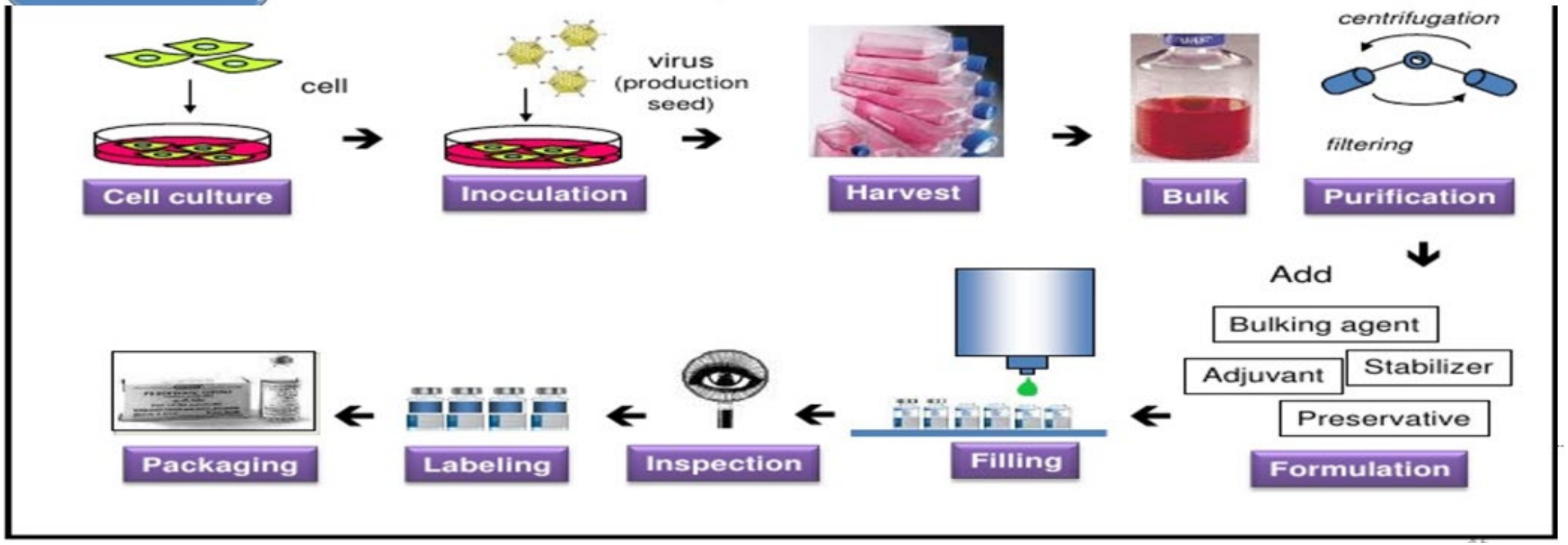
PRODUKSI PROTEIN TERAPETIK



PRODUCTION

Single harvest	• Media preparation • Fermentation
Bulk	• Inactivation • Concentration • Purification
Finished product	• Formulation • Filling • Packaging

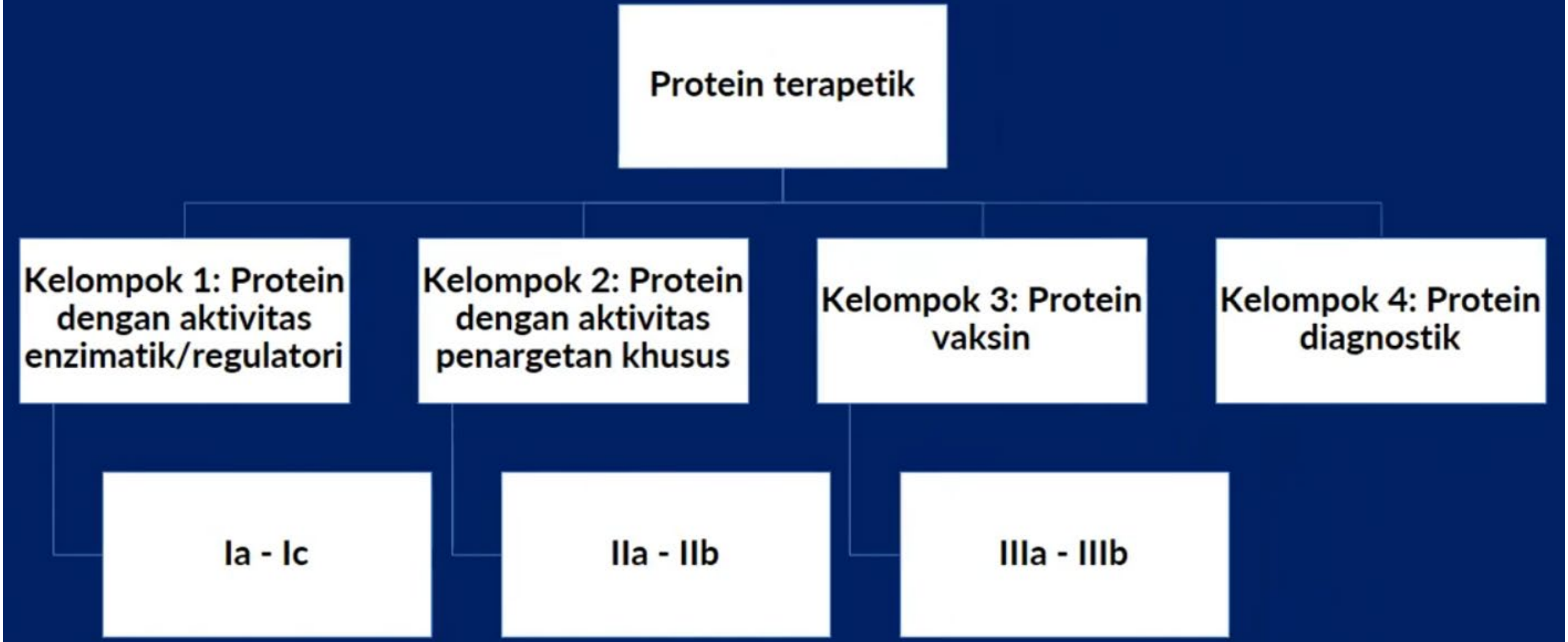




Contoh Protein Terapetik

No	Generic drug name	Size	Indication	Route
1	Etanercept	150 kDa	RA, psoriatic arthritis, plaque psoriasis, ankylosing spondylitis	sc
2	Insulin gargline	53 AA, 6.1 kDa	Type 1 and 2 DM	sc
3	Pegfilgastrim	39 kDa	Neutropenia	sc
4	Salmon calcitonin	32 AA	Osteoporosis	im, sc, intranasal
5	Cyclosporine	Cyclic, 11 AA	Phrophylaxis, solid organ, rejection	po, iv
6	Ocreotide	8 AA, somatostatin analog	Acromegaly, gigantism, symptomatic relief of carcinoid syndrome	iv, sc, im
7	Liraglutide	31 AA, 3.8 kDa	Type 2 DM (GLP-1 agonist)	sc
8	Bivalirudin	2.2 kDa	Anticoagulant	iv
9	Desmopressin	9 AA (8 D-AA)	Nocturnal enuresis	iv, im, sc, intranasal

KLASIFIKASI PROTEIN TERAPETIK BERDASARKAN AKTIVITAS FARMAKOLOGI

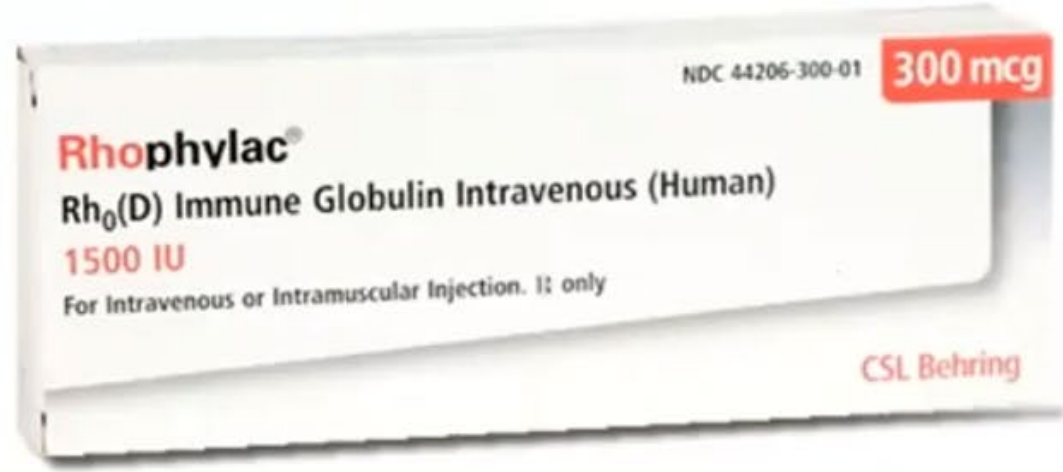


Kelompok 2: Protein dengan aktivitas penargetan khusus

- IIa: Menginterfensi molekul atau organisme. Contoh: Avastin
- IIb: Menghantarkan komponen atau protein lain (seperti radionuklei, obat sitostatik, atau protein efektor). Contoh: Ontak

Kelompok 3: Protein vaksin

- IIIIa: Melindungi terhadap senyawa asing. Contoh: Engerix
- IIIb: Mengobati penyakit autoimun. Contoh: Rhophylac

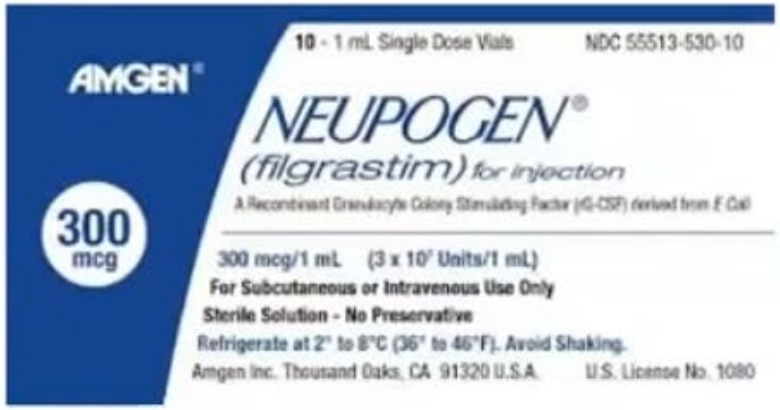


Kelompok 4: Protein diagnostik

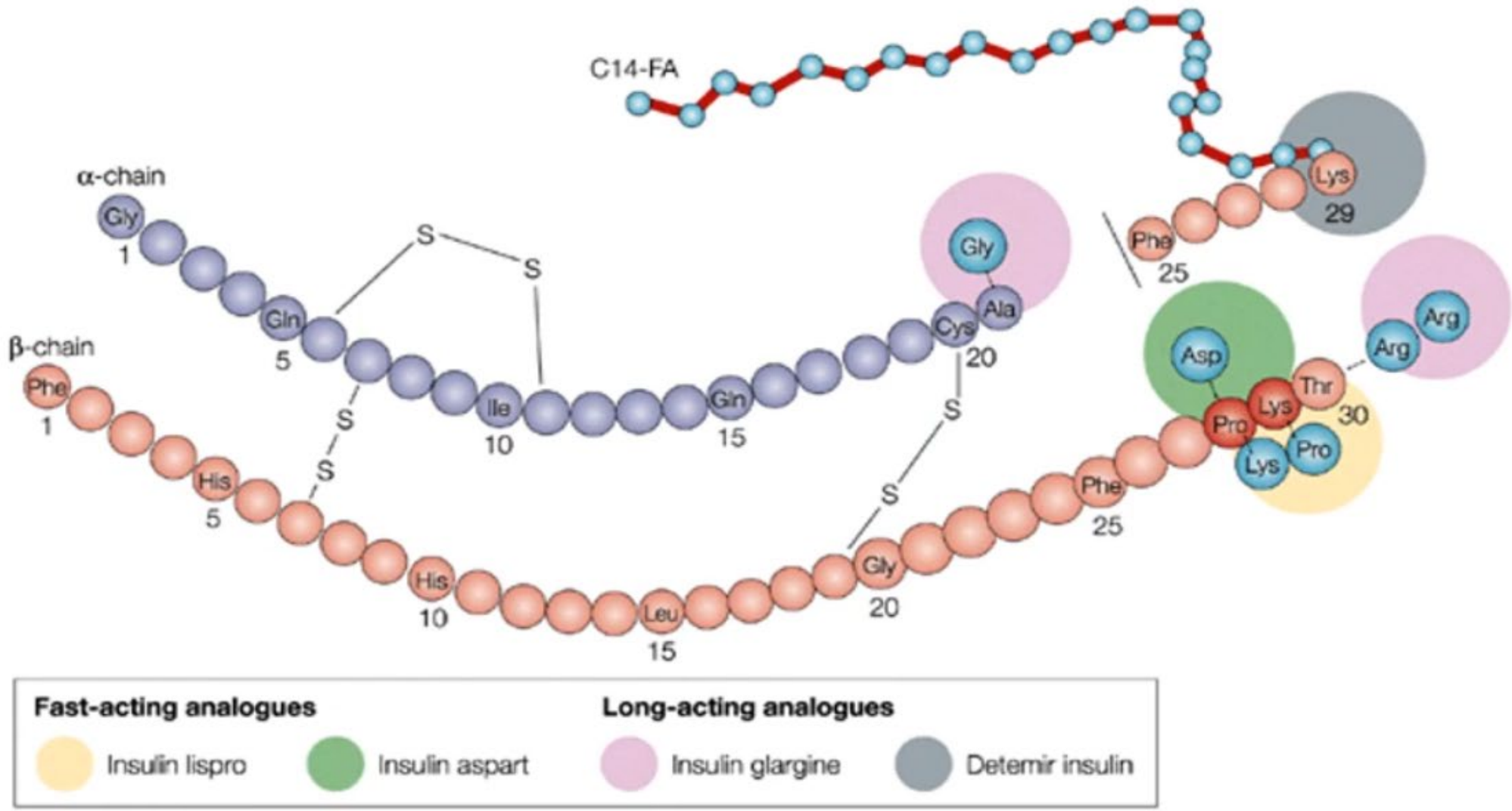
- Contoh: Geref

Kelompok 1: Protein dengan aktivitas enzimatik/regulatori

- Ia: Mengganti protein yang kurang/ abnormal. Contoh: Exubera, Increlex
- Ib: Augmentasi jalur yang telah ada. Contoh: Ovidrel, Neupogen
- Ic: Memberikan aktivitas/fungsi baru. Contoh: Myoblock



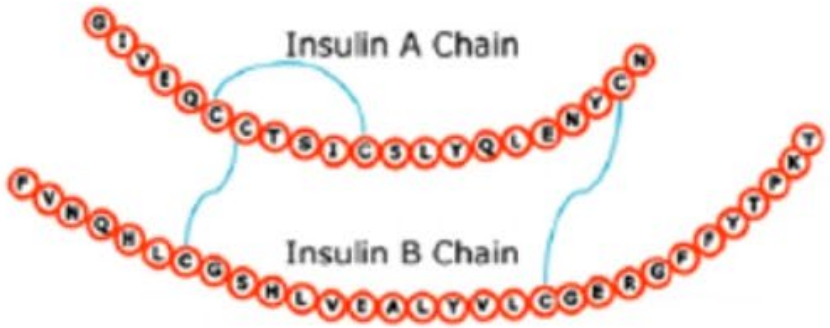
INSULIN ANALOG



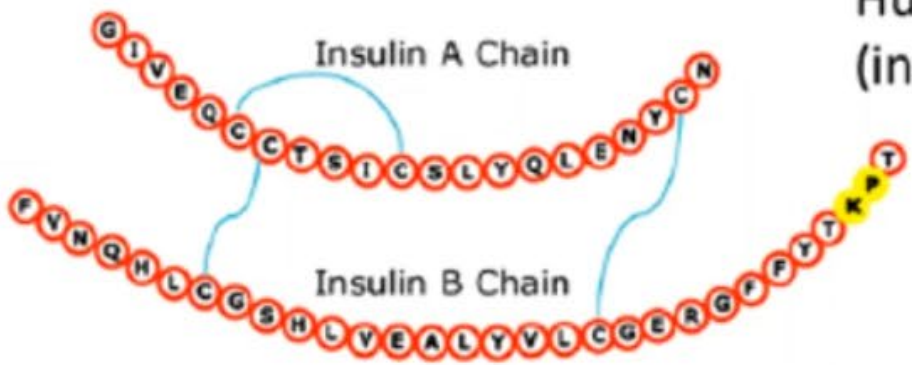
Nature Reviews | Drug Discovery

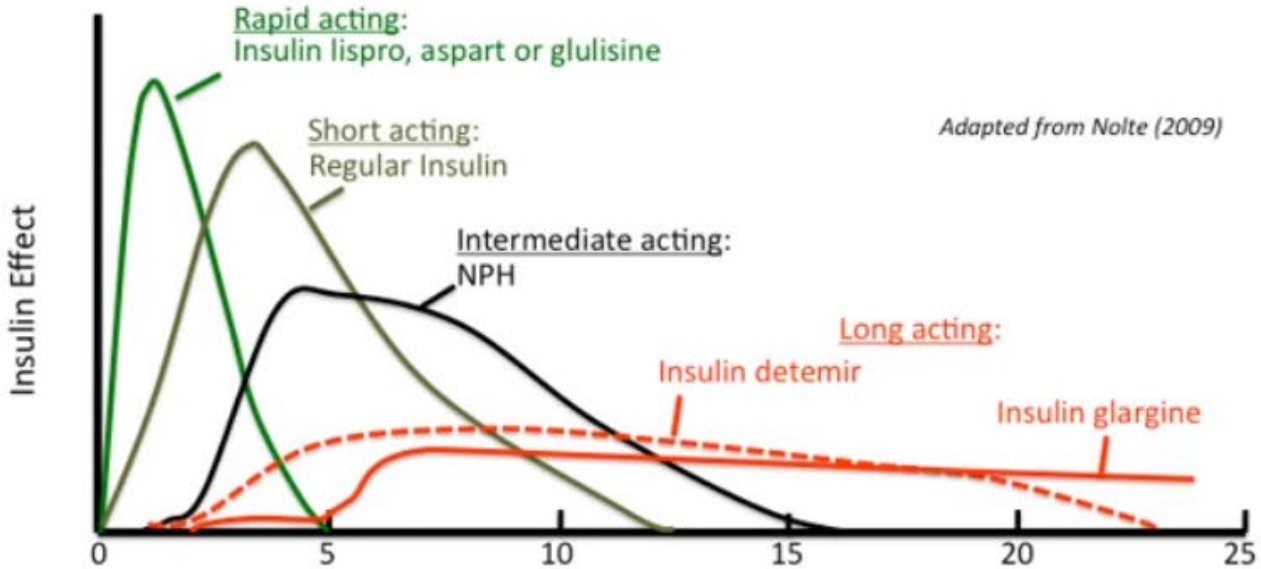
RAPID ACTING INSULIN

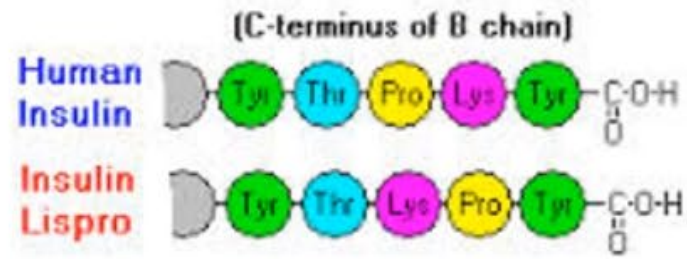
Human Insulin
MW 5808



Humalog
(insulin lispro)



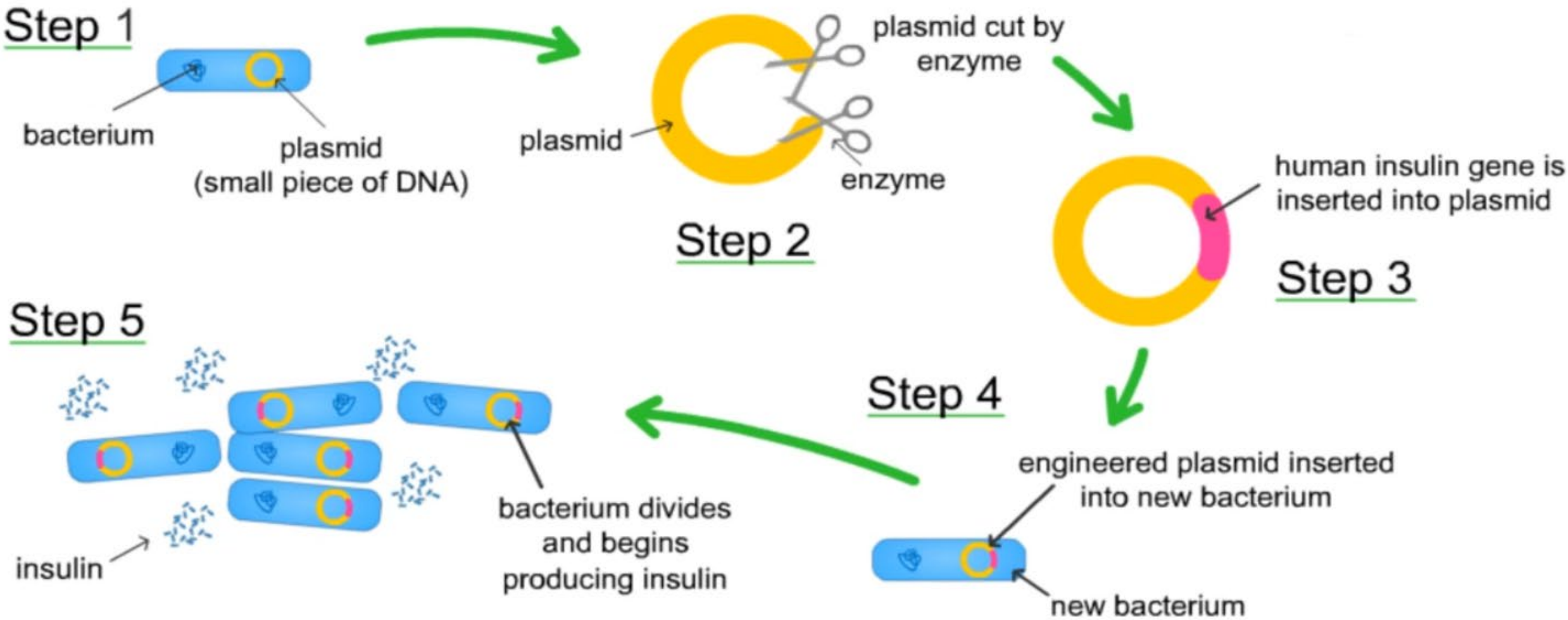




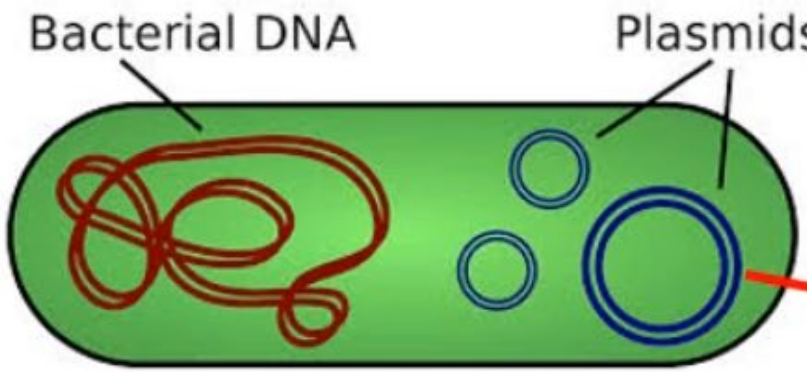
*Perubahan asam amino **prolin-lisin** pada human insulin menjadi **lisin-prolin** pada insulin lispro menyebabkan insulin lispro bekerja lebih cepat karena tidak membentuk dimer dan heksamer, tetapi berbentuk monomer sehingga absorpsi lebih cepat*

<1> Kloning

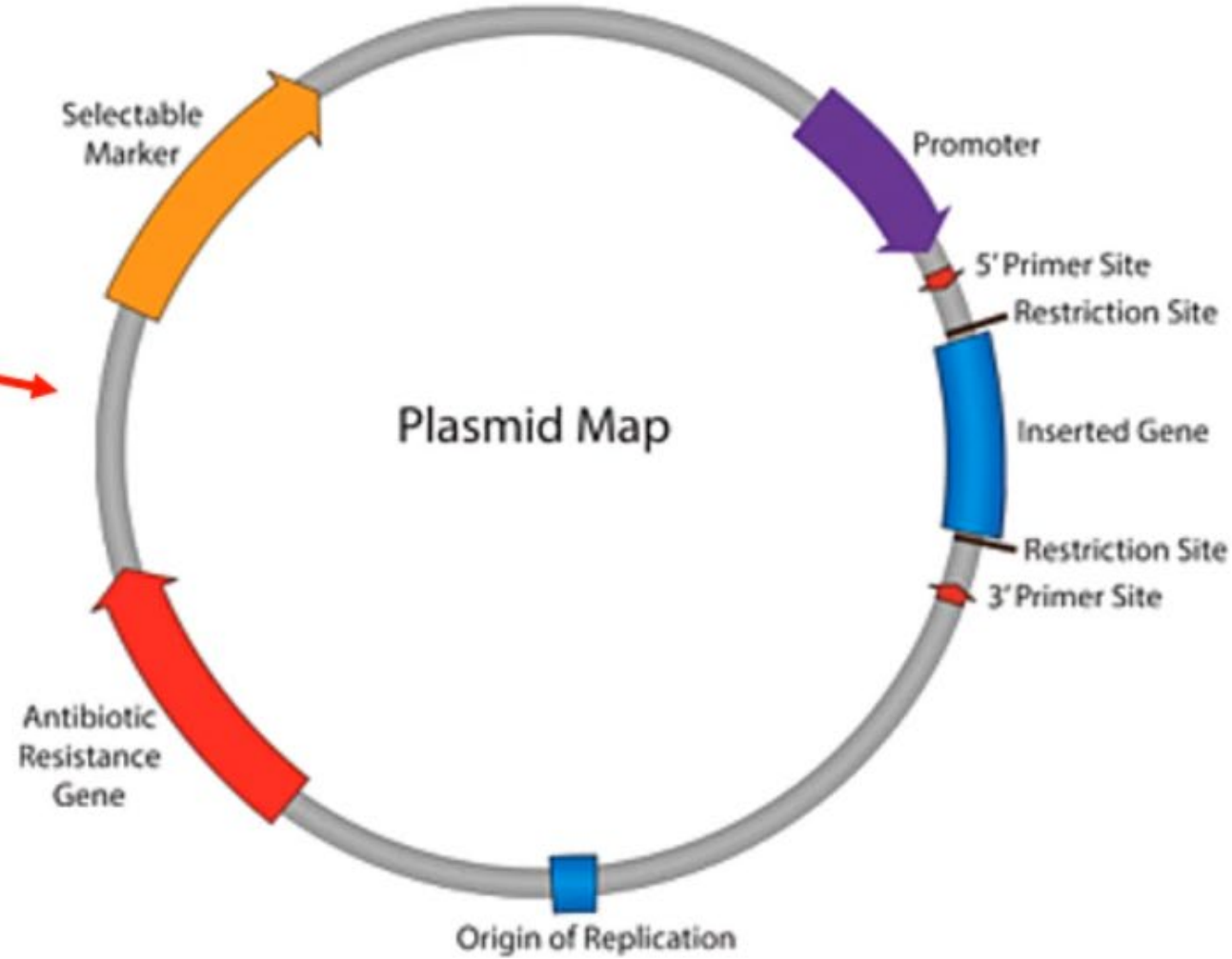
Pemotongan DNA dengan enzim restriksi → Ligasi DNA → Transformasi



PLASMID

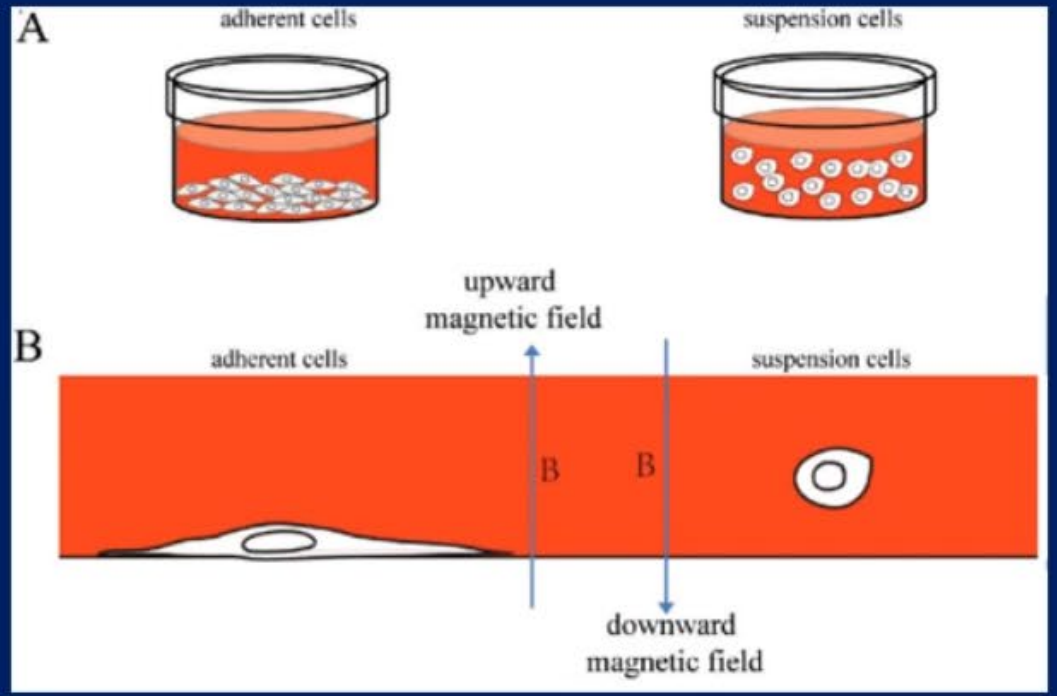


- dsDNA
- Berbentuk lingkaran
- Dapat bereplikasi sendiri di dalam sel
- Dapat disisipi DNA asing



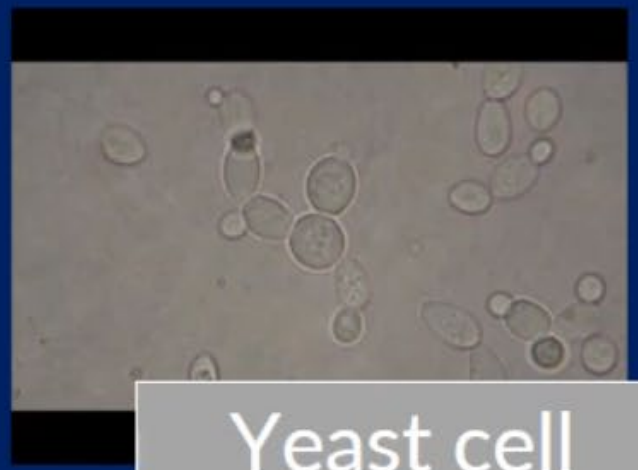
<2> Overproduksi Protein

SEL INANG (HOST)



Insect cells

Bacterial cell



Yeast cell

Mamalian cell

GROWING THE MICROORGANISMS

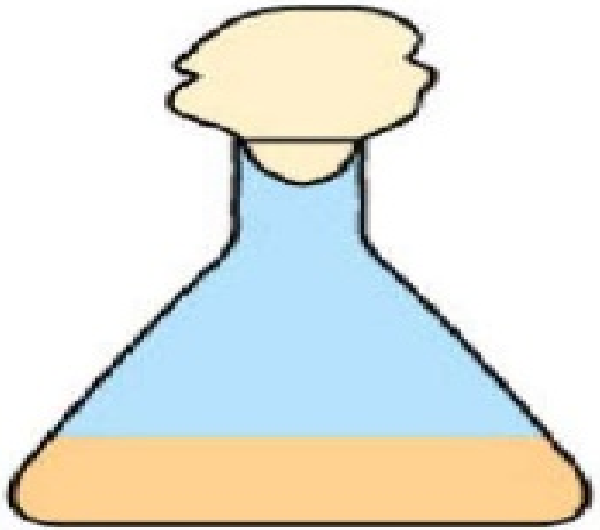
GROWING BACTERIA

Methods used are :

- **BATCH CULTURE**
  - the microbe is grown in a closed vessel
  - typically in a test tube or flask
- **CONTINUOUS CULTURE**
  - the microbe is grown in vessel which has medium constantly added and spent medium constantly removed.
  - It is performed in a **chemostat.**

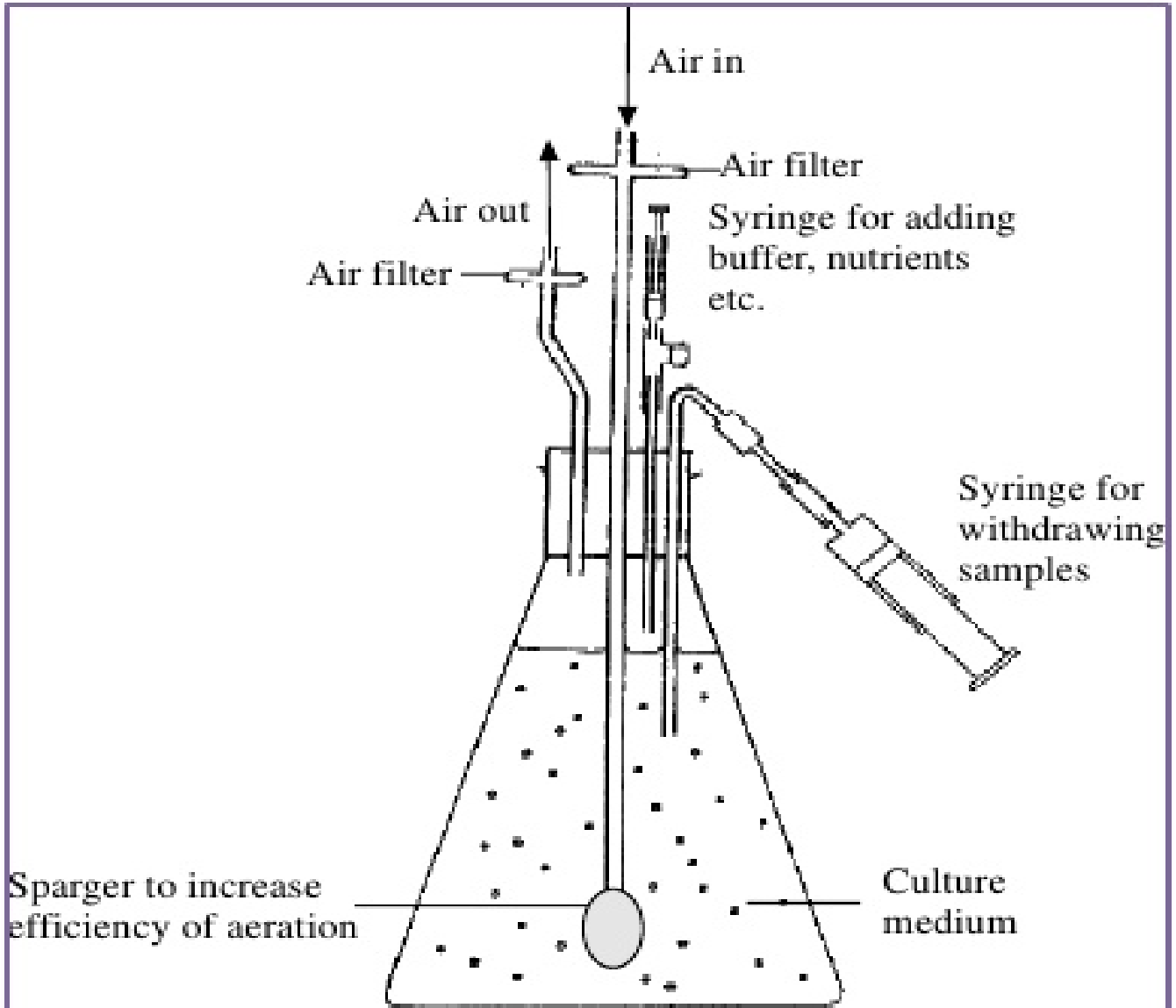
Batch culture

- Culture incubated in a closed vessel with a single batch of medium.
- The fermenter shown here is set up for a **batch culture.**



Flask culture

A batch culture



- Batch processing is a way of providing the best conditions for a micro-organism.
- All the raw materials are put in the fermenter at the start and then the micro-organism is added.
- The system is then left for a long time – possibly a week – until all the raw materials have been used up and there is loads of the product.
- The fermenter is then emptied and other processes are used to separate the product from the micro-organism.
- The fermenter is then cleaned out and the whole process begins again.

Continuous culture

- Growth in an open system
  - continual provision of nutrients
  - continual removal of wastes
- **Continuous culture** aims to keep a culture growing indefinitely. This can be done if:
  - fresh nutrients are continually supplied
  - Accumulated cells and waste products are removed at the same rate
  - Conditions such as temperature and pH are kept at their optimum values.

METODE KULTUR

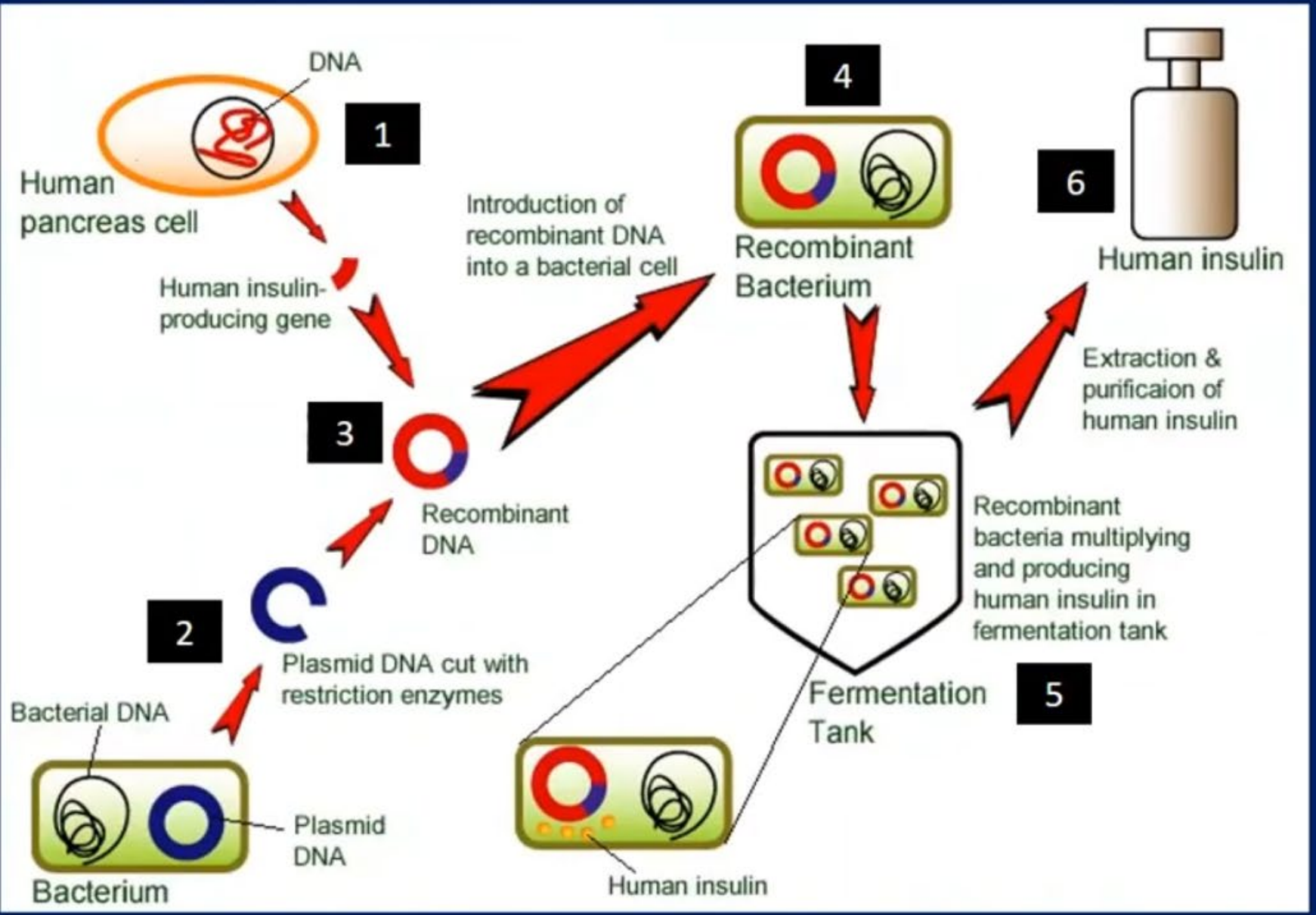
MANUAL

T flask

Roller bottle

·BIOREAKTOR

Produksi Human Insulin



1. Ekstraksi insulin dari sel pankreas → isolasi gen yang memproduksi insulin
2. Ekstraksi plasmid DNA dari bakteri dan potong dengan enzim restriksi → membentuk vektor plasmid
3. Insersi gen yang memproduksi insulin ke vector plasmid bakteri untuk membentuk DNA rekombinan
4. Transfeksi DNA rekombinan ke sel bakteri untuk memproduksi bakteri rekombinan
5. Bakteri rekombinan memperbanyak diri pada tank fermentasi dan memproduksi insulin
6. Ekstraksi insulin, purifikasi dan kemas → siap diinjeksikan

<3> Purifikasi Protein

Protein ekstrasel

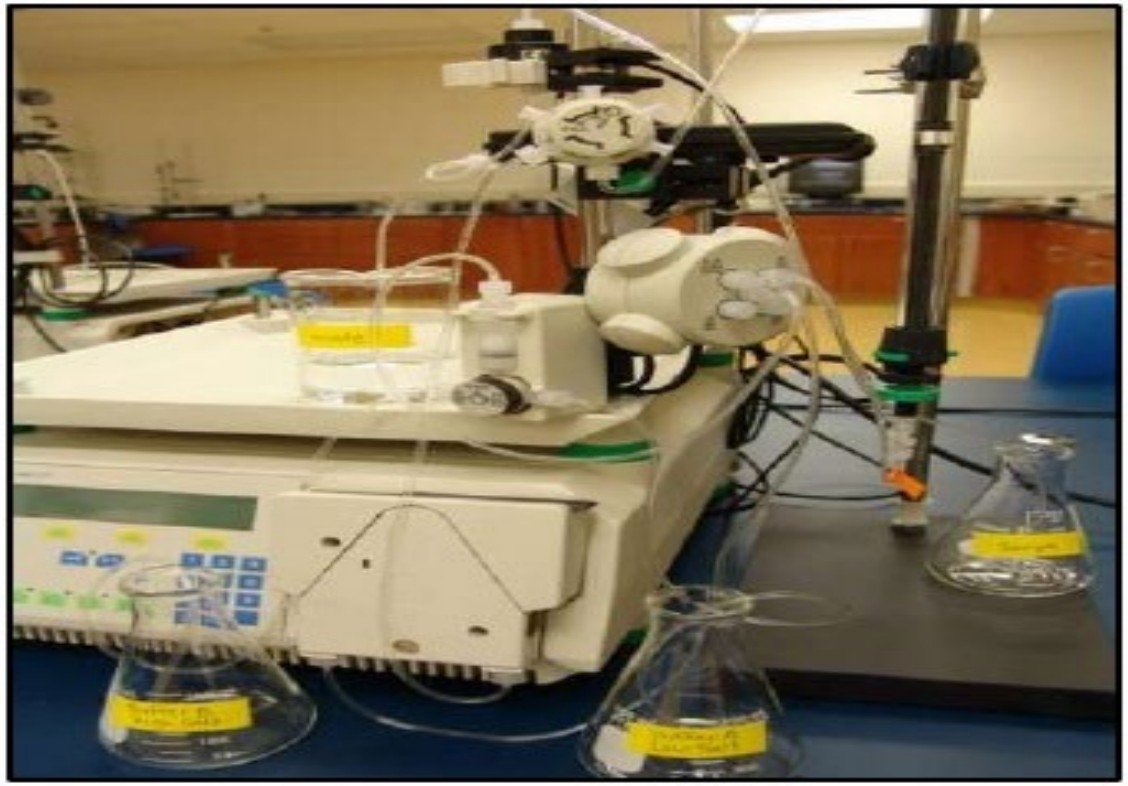
Pemisahan sel → Pemekatan protein → Purifikasi protein (kromatografi)

Protein intrasel

Pemecahan sel → Pemisahan serpihan sel → Pemekatan protein → Purifikasi protein (kromatografi)

Purification Process

Lab Scale Chromatography System



Large Scale Chromatography System

CONTOH TAHAP PURIFIKASI PROTEIN (PROTEIN EKSTRASELULER)

Pemanenan media kultur (harvest)

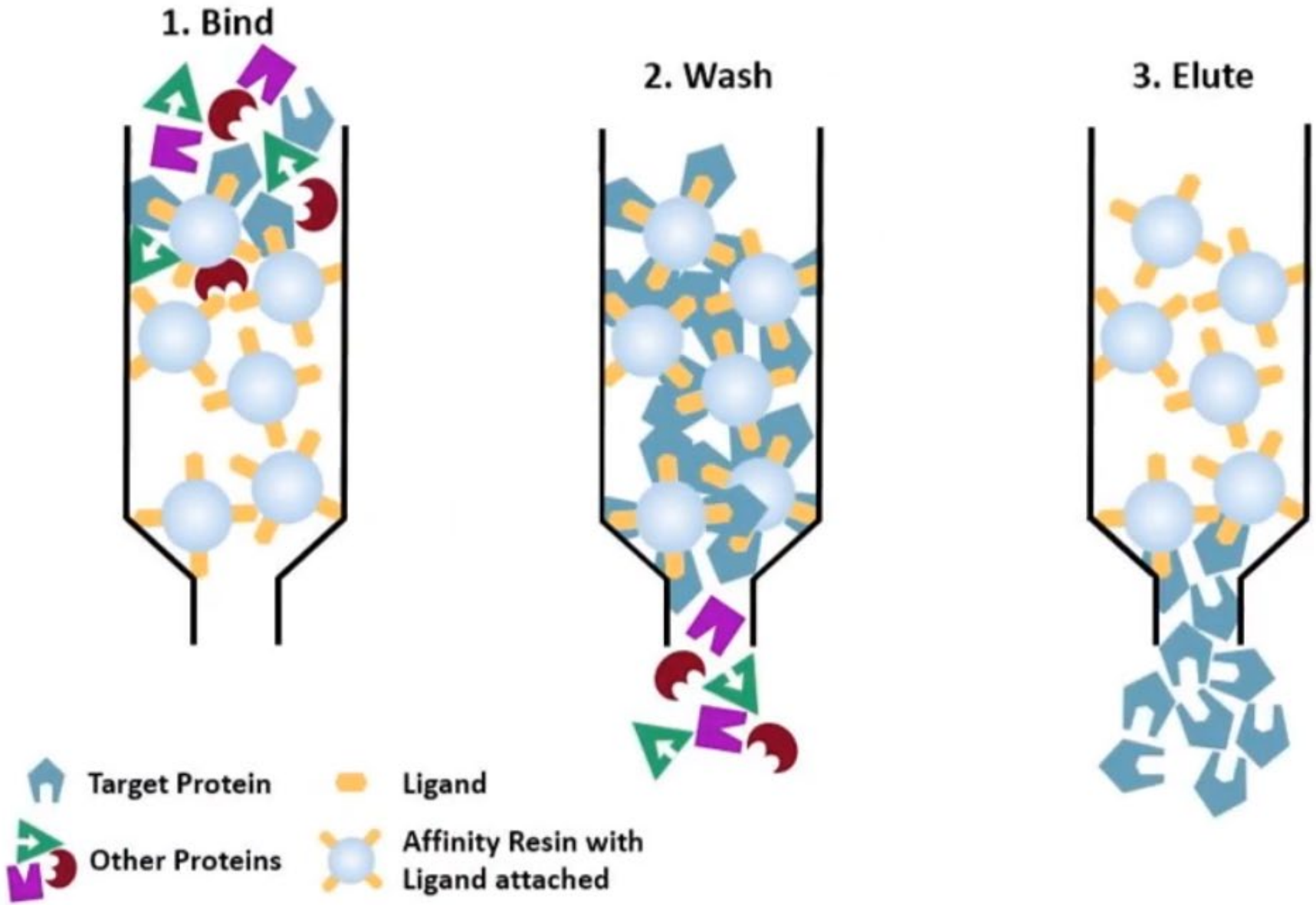
↓

Penyaringan (filtrasi)

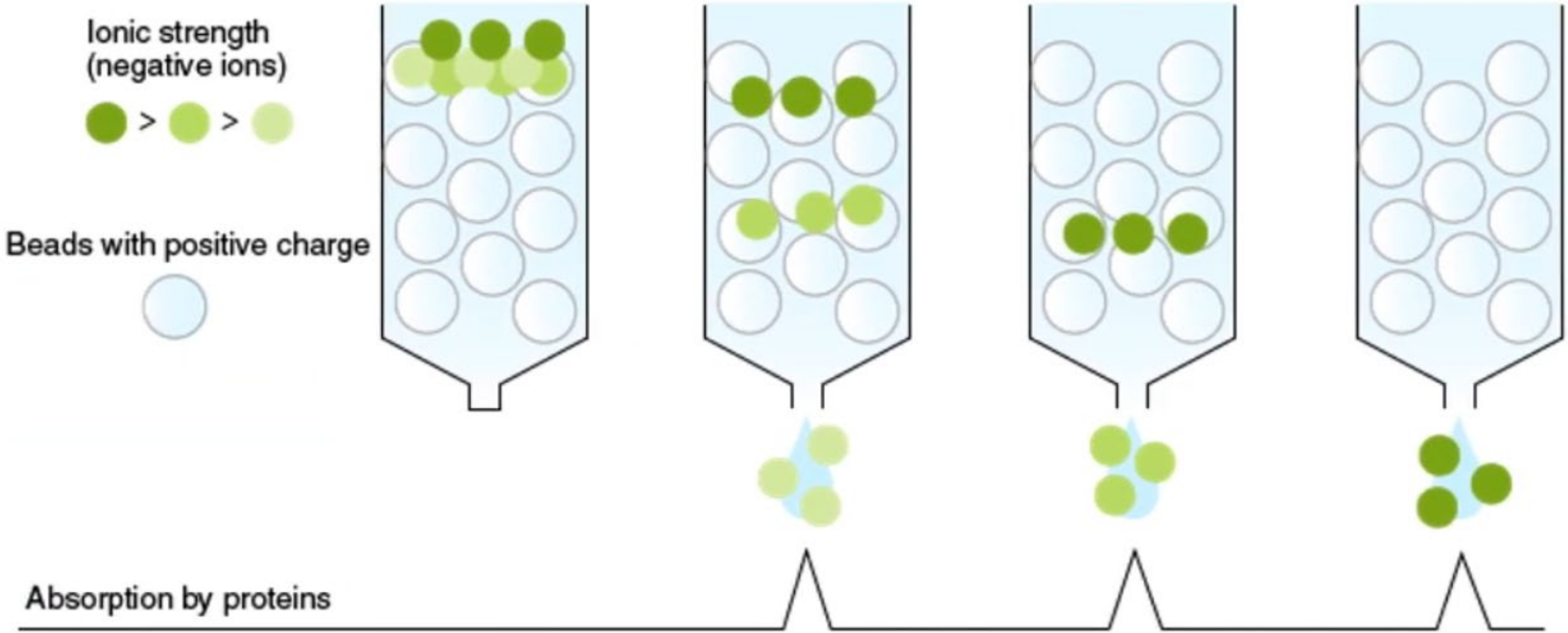
↓

Pemurnian (purifikasi)

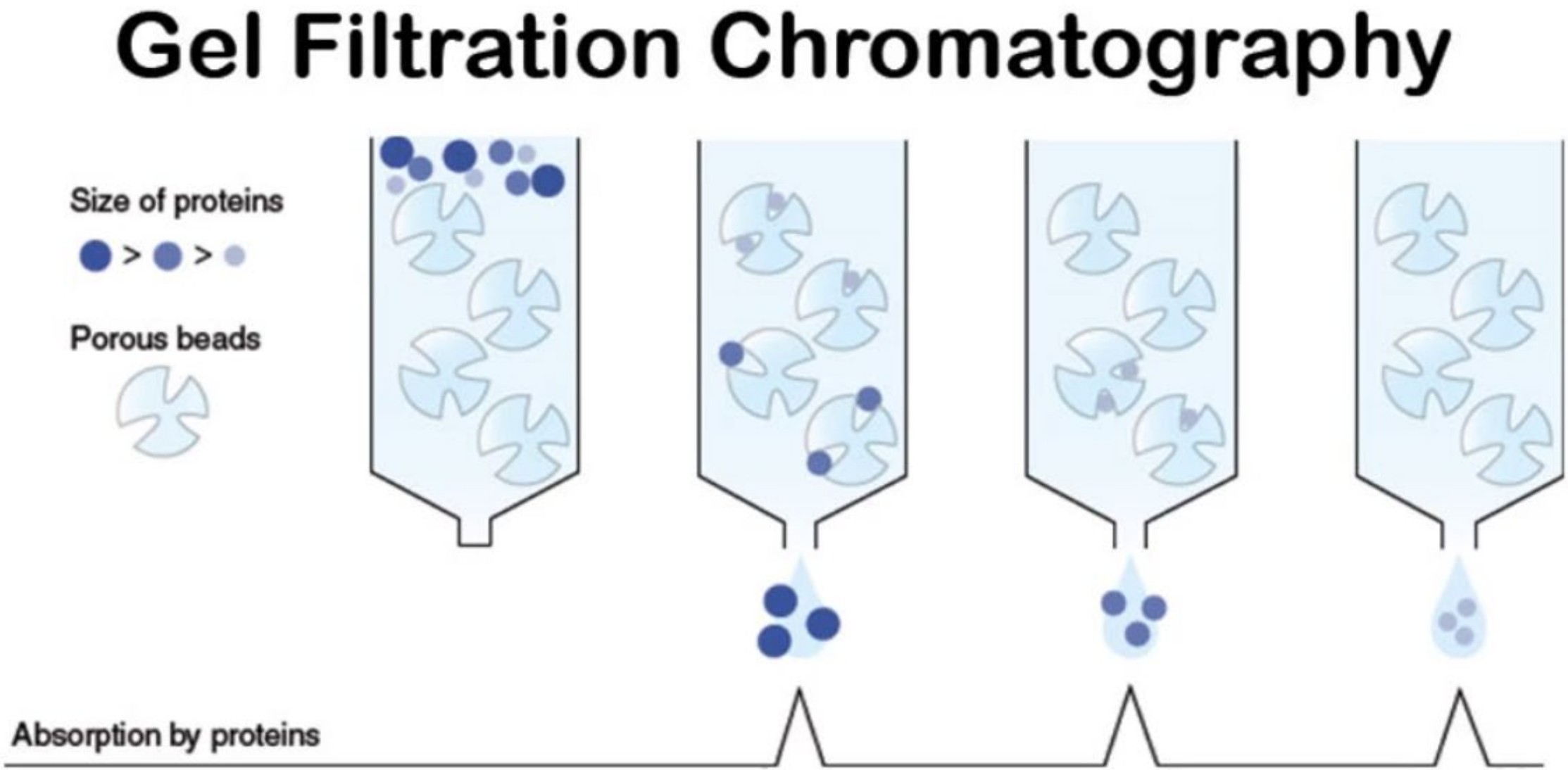
Affinity chromatography
(pemisahan berdasarkan afinitas terhadap ligan)



Ion exchange chromatography
(pemisahan berdasarkan muatan)



Gel filtration chromatography
(pemisahan berdasarkan ukuran)



TAHAP PURIFIKASI PROTEIN

<1> Dye affinity chromatography → IPC: ELISA

(menghilangkan media kultur & menangkap protein yg diinginkan)

<2> Diafiltration → IPC: pH, konduktivitas, protein content

(ganti buffer & pemekatan)

<4> Ion exchange chromatography → IPC: elektroforesis, isoelectric focusing

(menghilangkan protein dengan pI tinggi)

<6> Diafiltration → IPC: pH, konduktivitas, protein content, elektroforesis, isoelectric focusing

(ganti buffer & pemekatan)

<7>Nanofiltration → IPC: protein content

(menghilangkan virus)

<5> Multi mode chromatography → IPC: endotoxin, protein content

(menghilangkan endotoksin & host cell protein)

<3> Absorption chromatography → IPC: elektroforesis, protein content

(menghilangkan host cell protein)

Active Pharmaceutical Ingredients (API) → QC: Uji aktivitas biologi

Specifications for Purified Bulk Drug Concentrate

Test Methods
1. *Physical Evaluation*
Appearance
pH
2. *Identity*
Bioassay
Peptide mapping
Amino acid analysis
3. *Protein Potency*
Nitrogen content
HPLC
4. *Biological Potency*
Specific activity
5. *Purity*
HPLC
SDS-PAGE
(CZE)
IEF
DNA
Endotoxins
Sterility
Other specifications can be included, depending on the specific requirements of the individual protein.

<4> Formulasi Sediaan Protein Terapetik

Sediaan Likuid

- Stabilitas rendah
- Membutuhkan kondisi penyimpanan Khusus (suhu rendah)

Sediaan Solid

- Liofilisasi → ↑ stabilitas
- Cara kerja: pembekuan protein & pengeringan dalam kondisi vakum
- Liofilisasi dapat menyebabkan perubahan struktur protein → perlu ditambahkan *cyroprotector* (albumin, senyawa poliol)

KEMASAN UNTUK SEDIAAN STERIL PROTEIN TERAPETIK (INJEKSI)

<1> Ampul (gelas)

<2> Vial
(gelas & plastik)

<3> *Prefilled syringes*
(gelas & plastik)

<4> *Cartridges* (gelas)

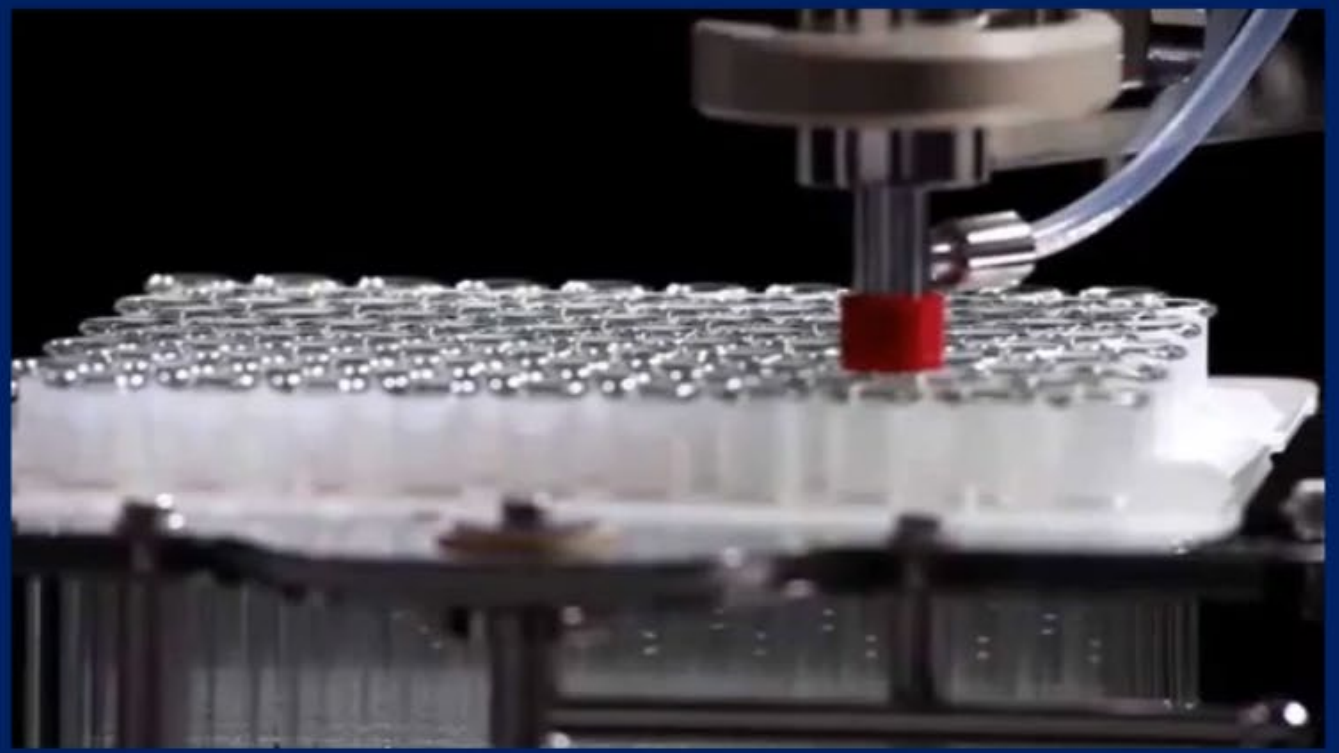
<4> Formulasi Sediaan Protein Terapetik

Formulasi sediaan steril

Pengisian (filling)

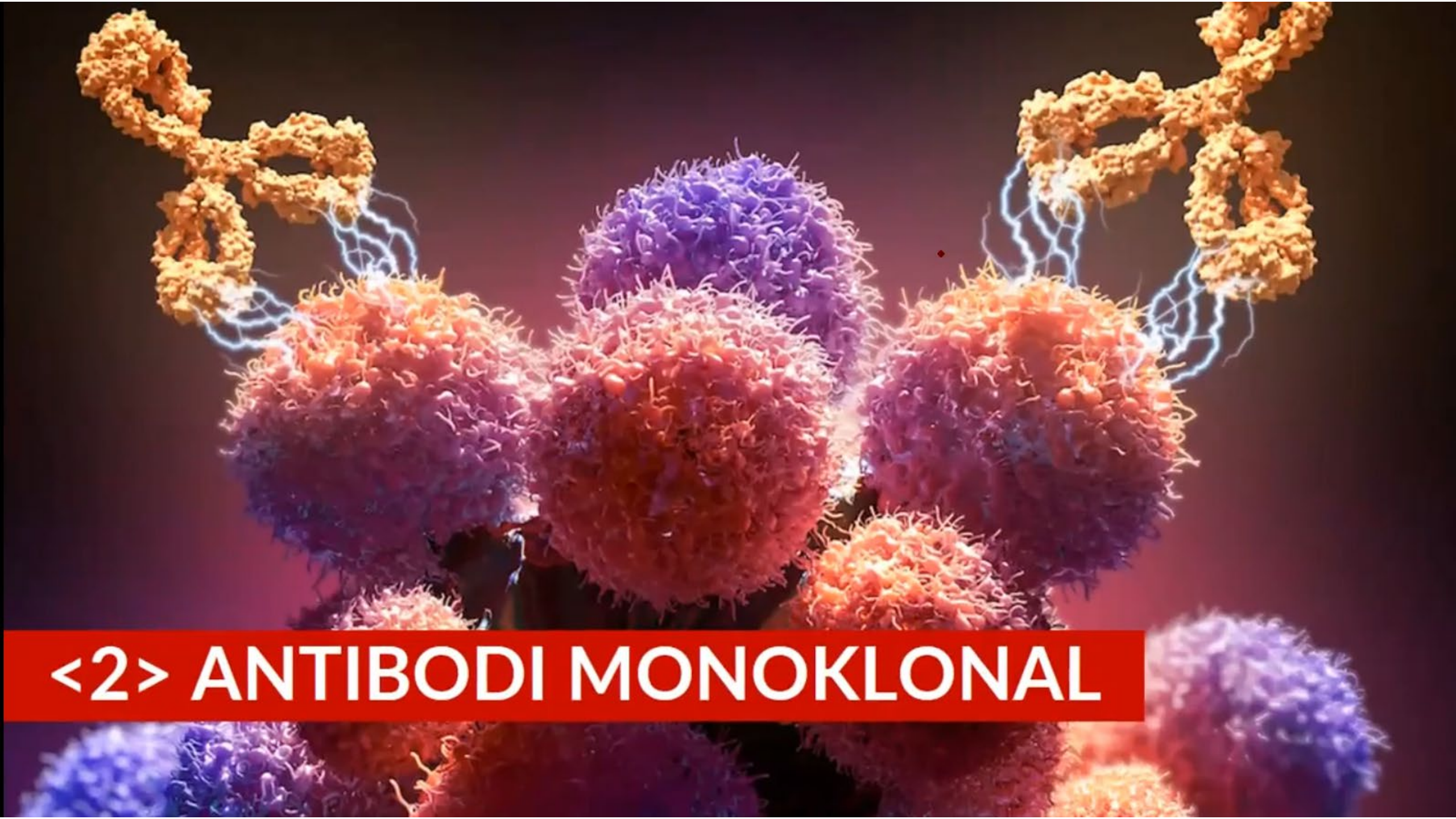
Visual inspection

Pengemasan

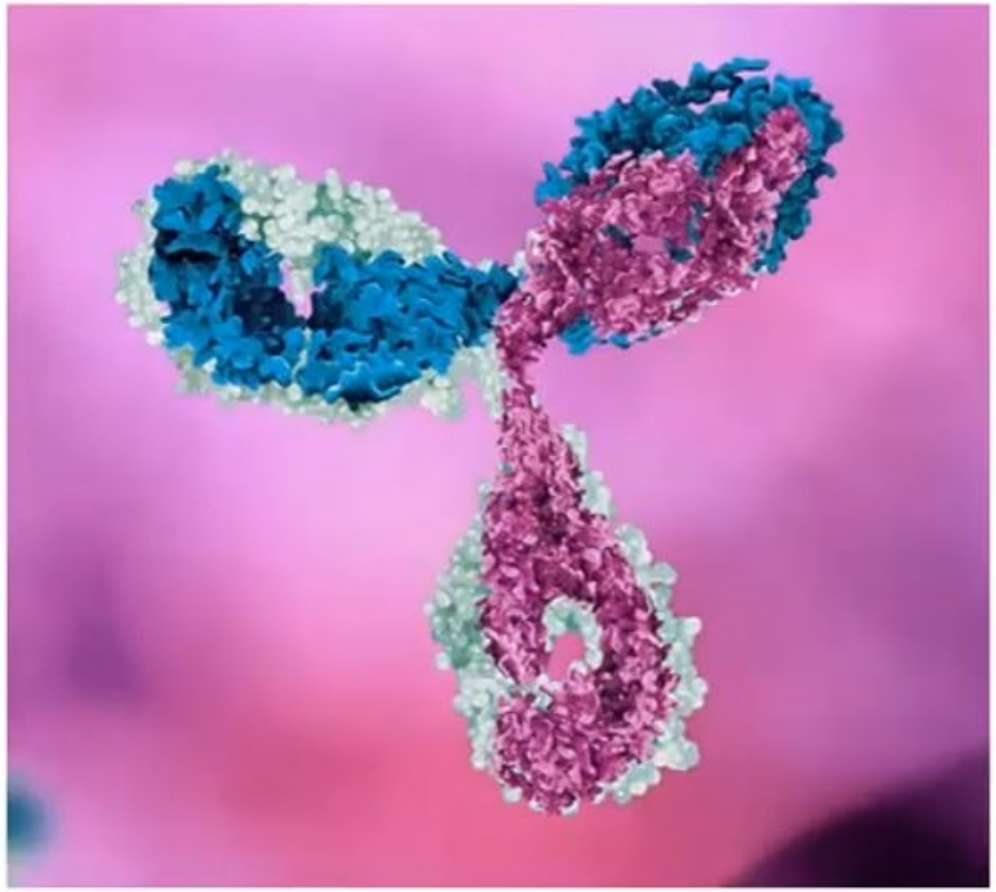


Specifications for Purified Protein/Peptide Drug in Finished Dosage Form

1. *Physical Evaluation*
   - Appearance
   - pH
   - Volume/container
   - Moisture (lyophilized product)
   - Total protein
   - Particulates (for both liquid and lyophilized formulations)
2. *Potency Tests*
   - *In vitro* assays
   - Radioimmunoassays
   - Enzyme immunoassays
   - Chromatographic methods
   - Bioassays (animal model or cell-line derived)
   - Protein content
3. *Identity*
   - Peptide mapping
   - NH_2 terminal analysis
   - Western blot
   - Isoelectric focusing
   - SDS-PAGE
   - Coomassie stain (reduced and unreduced)
   - Biological activity
4. *Purity*
   - SDS-PAGE
   - Coomassie stain
   - HPLC-RP
   - HPLC-SEC
   - HPLC-gel filtration
   - DNA contamination
   - Other specifications can be included, depending on the specific requirement of the protein
5. *Microbiological Tests*
   - Sterility
   - Pyrogens
   - Mycoplasma
6. *Safety*
7. *Degradation Assays*
   - SDS-PAGE
   - Enzyme-linked immunosorbent assay (ELISA)
   - HPLC
   - Electrophoresis


<2> ANTIBODI MONOKLONAL

KONSEP ANTIBODI MONOKLONAL

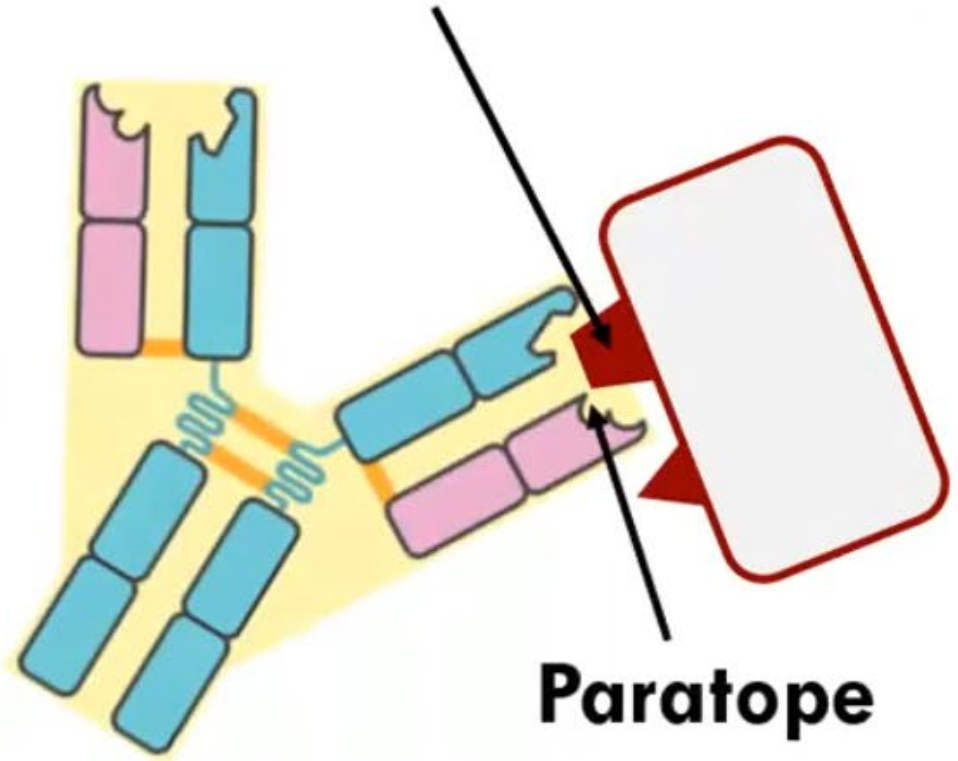


ANTIBODI

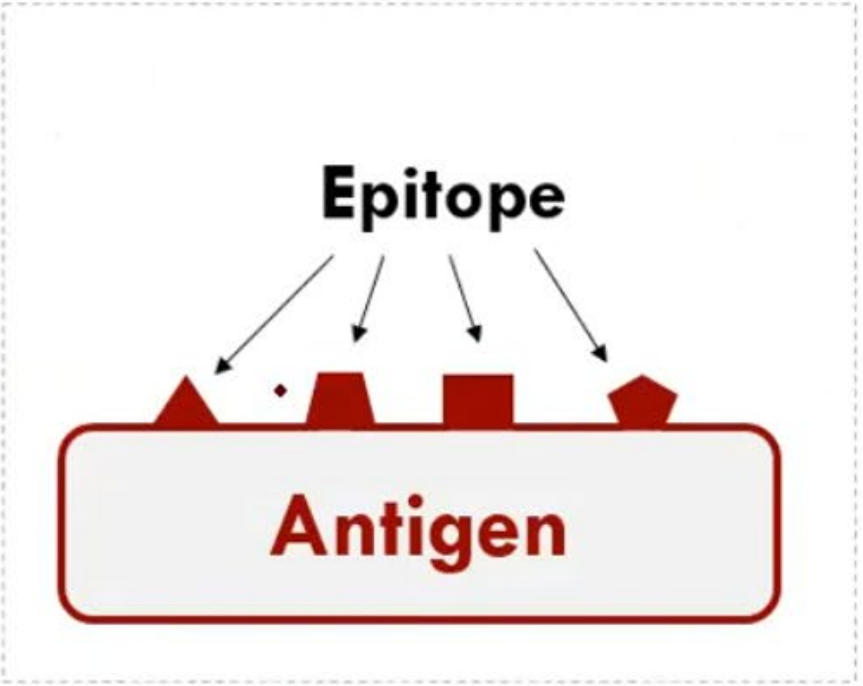
- Merupakan glikoprotein yang diproduksi oleh **sel B** sebagai respons dari adanya patogen asing (**imunitas humoral**)
- Dikenal sebagai immunoglobulin (Ig)/ *Y-shaped protein*
- Sangat spesifik karena hanya mengenali satu jenis antigen → Ab monoklonal
- Bervariasi tergantung dari ukuran, muatan, afinitas dan spesifisitas → berpengaruh ketika antibodi digunakan dalam SPO → beberapa jenis memiliki waktu paruh yang lebih lama dibandingkan yang lain

EPITOPE & PARATOPE

Epitope
(sisi antigen yang berikatan dengan antibodi)



Paratope
(sisi antibodi yang berikatan dengan epitope)



Suatu antigen dapat memiliki beberapa epitop sehingga dapat menginisiasi respon dari beberapa tipe sel B

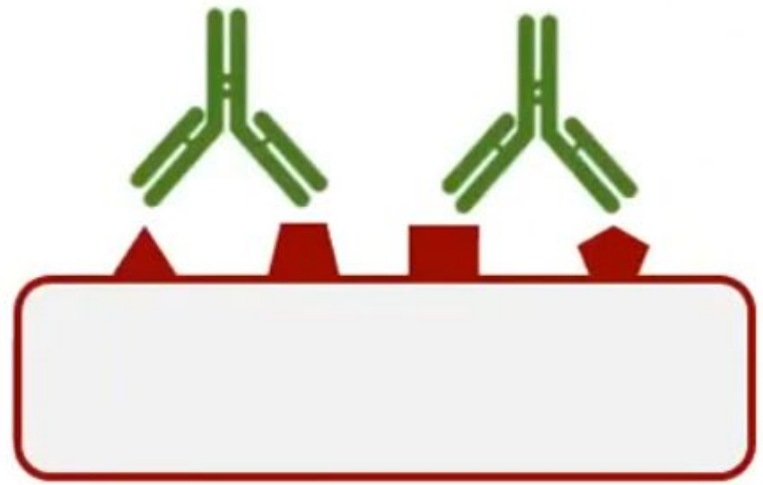
ANTIBODI MONOKLONAL & POLIKLONAL

ANTIBODI MONOKLONAL

(bereaksi dengan **epitope yang spesifik** pada antigen)

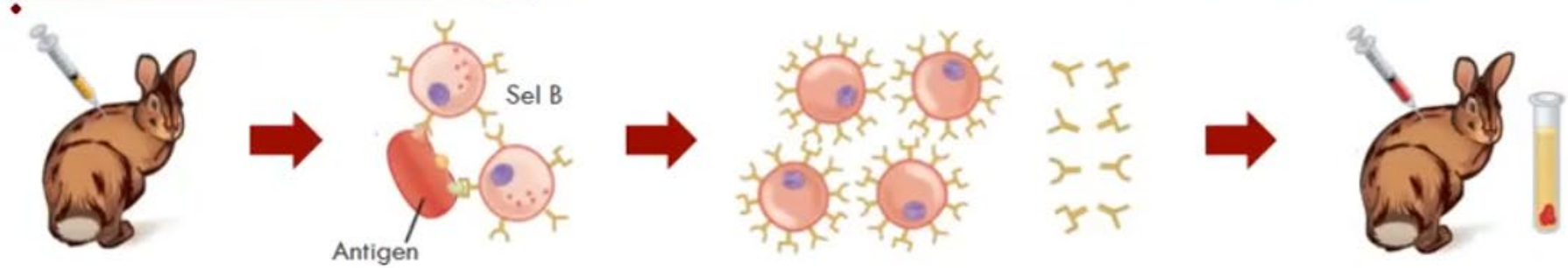
ANTIBODI POLIKLONAL

(bereaksi dengan **berbagai macam epitope** pada antigen)



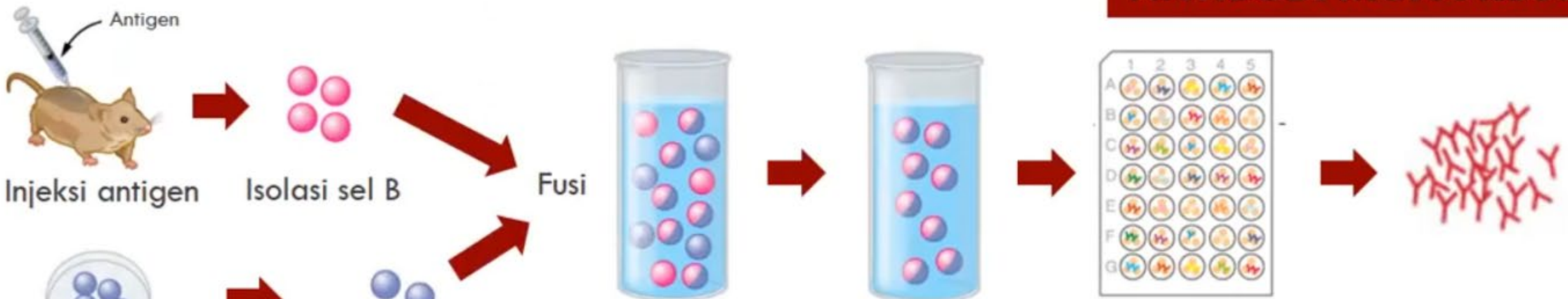
PRODUKSI ANTIBODI SECARA UMUM

ANTIBODI POLIKLONAL



Injeksi antigen → Aktivasi sel B → Pembentukan antibodi → Ekstraksi serum berisi antibodi

ANTIBODI MONOKLONAL



Injeksi antigen → Isolasi sel B

Kultur sel myeloma → Isolasi sel myeloma

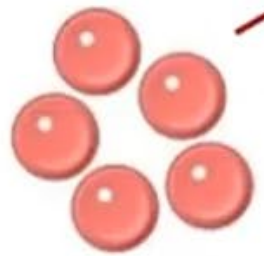
Fusi → Pembentukan hibridoma → Seleksi hibdridoma → Seleksi & ekspansi klon → Purifikasi antibodi monoklonal

(Greenfield, 2014)

PRODUKSI ANTIBODI MONOKLONAL

mAb → kumpulan antibodi yang identik karena dihasilkan oleh satu jenis sel B dan hanya mengenali satu epitop saja

B cells diisolasi dari limfa hewan yang telah diinjeksi dengan suatu antigen

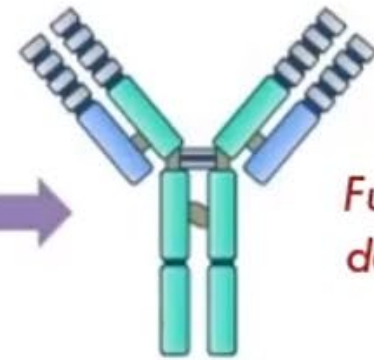


Myeloma cells memiliki kemampuan untuk replikasi tak terbatas & clonogenic - dapat membentuk klon saat ditumbuhkan in vitro

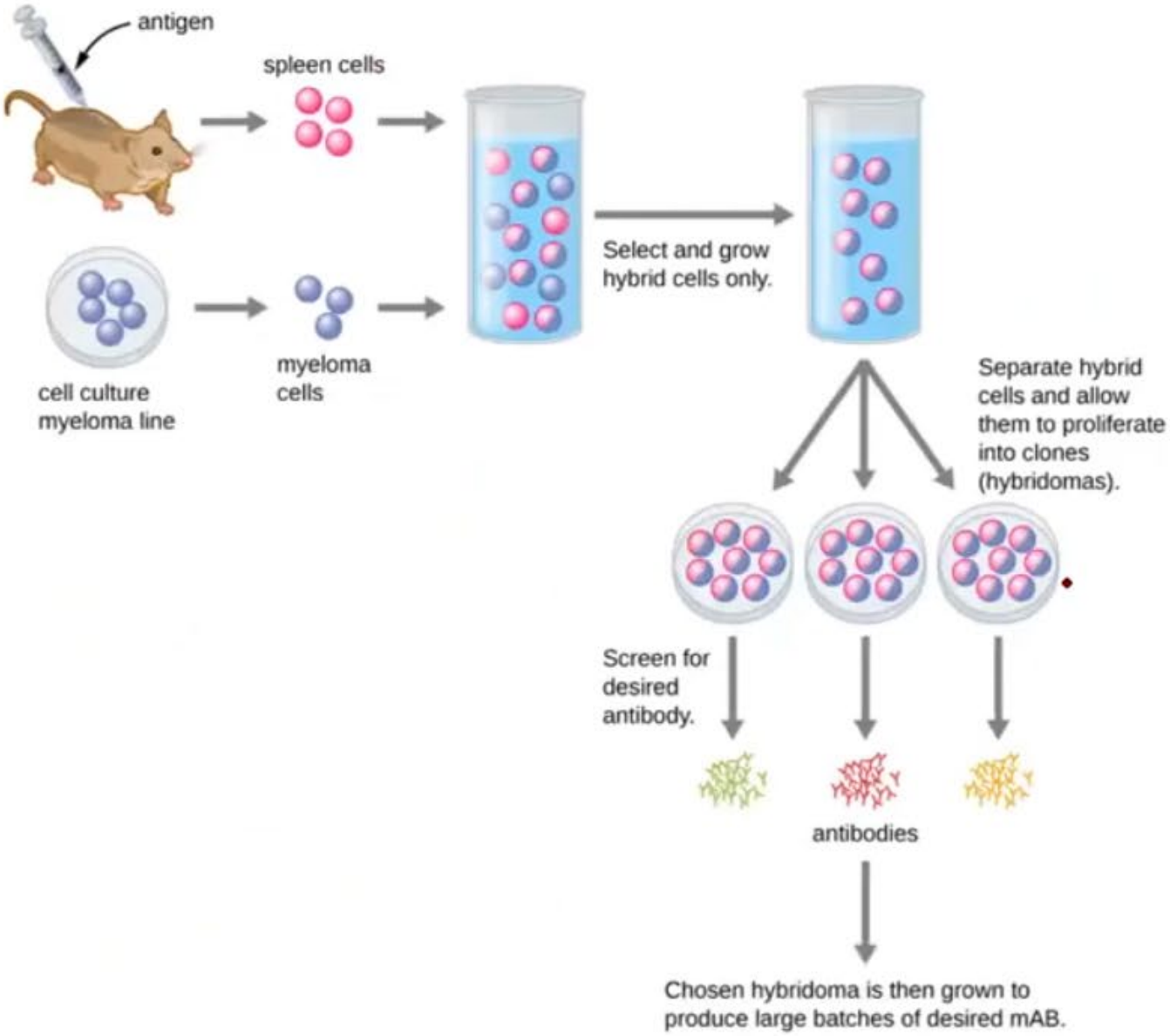
IMMORTAL **(malignant myeloma cells)**

PEG

*Hybridoma (sel hibrid yang memiliki sifat gabungan dari kedua parent cells) → dihasilkan dengan memfusikan **malignant myeloma cells** dan **antibody-producing B cells***



Fusi sel myeloma dan B cell → sel imortal yang menghasilkan antibodi spesifik



Tahap 1: Imunisasi hewan uji

Tahap 2a: Isolasi sel B dari limpa hewan uji

Tahap 2b: Kultur sel myeloma

Tahap 3: Fusi antara sel B dan sel myeloma

Tahap 4: Seleksi sel hibridoma

Tahap 5: Seleksi klon

Tahap 6: Ekspansi klon

Tahap 7: Purifikasi

EKSPANSI KLON

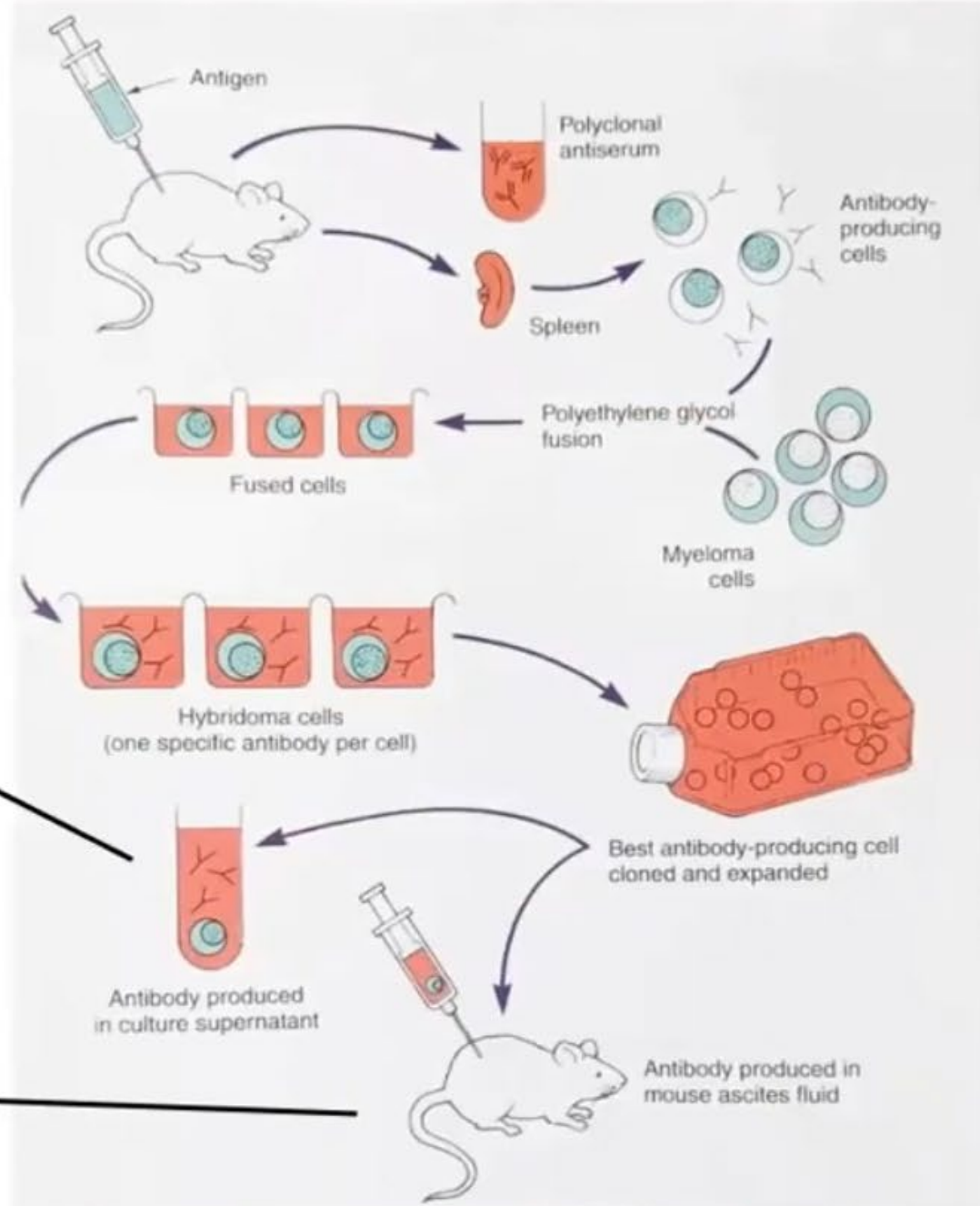
Untuk memperbanyak antibodi monoklonal

1> Teknik kultur

menggunakan **chamber fermentasi** untuk memproduksi antibodi dalam skala besar → produksi menggunakan kultur sel lebih dikembangkan karena teknik ascites sangat menyakitkan bagi hewan uji

2> Teknik ascites

Ketika sel hibridoma diinjeksikan ke tikus (pada ruang peritoneal, perut) maka akan diproduksi tumors yang mengangandung cairan yang kaya antibodi → disebut dengan **ascites fluid**



mAb dapat diperbanyak pada kultur sel atau hewan

TIPE ANTIBODI MONOKLONAL

Imunogenesitas

Murine Ab
(-omab)

100% tikus

Chimeric Ab
(-ximab)

20% tikus
80% manusia

Humanized Ab
(-zumab)

10% tikus
90% manusia

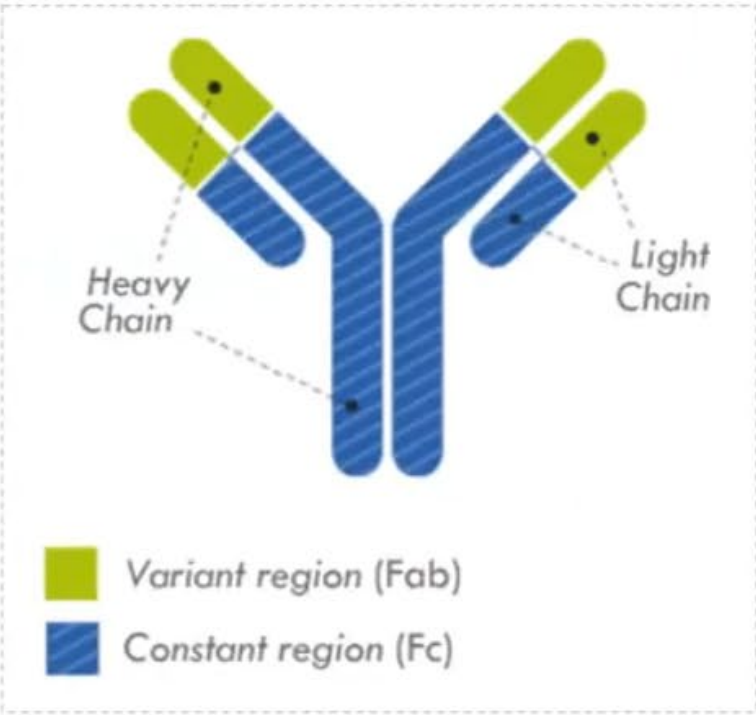
Human Ab
(-umab)

100% manusia

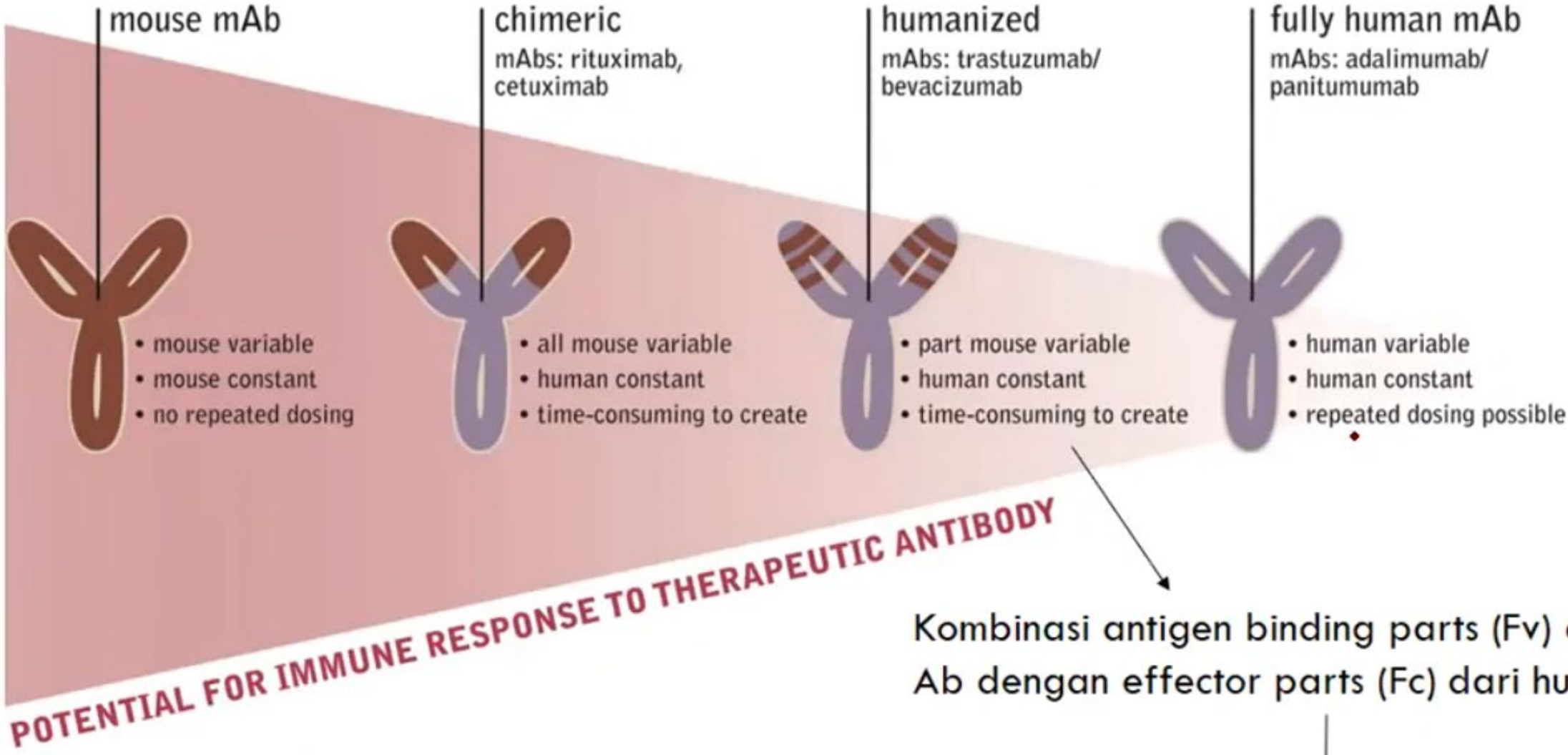
Contoh: ibritum**omab** infli**ximab** transtu**zumab** adalim**umab**

Tikus
Manusia

Adanya komponen dari tikus memicu pembentukan
human anti-mouse antibody (HAMA)



Muncul respons **human anti murine antibody (HAMA)** → alergi / hipersensitivitas kompleks imun



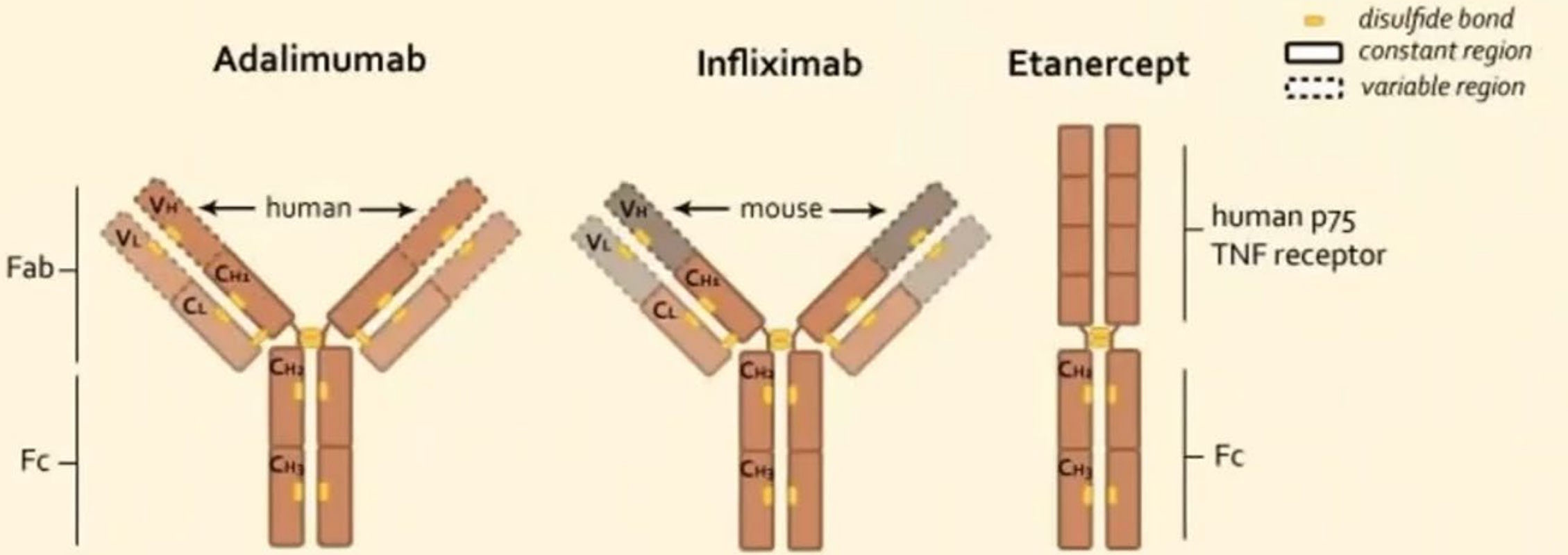
Kombinasi antigen binding parts (Fv) dari mouse Ab dengan effector parts (Fc) dari human Ab

↓

Menurunkan risiko respons **HAMA**

Contoh: Anti-TNFα Antibody (Adalimumab vs Infliximab)

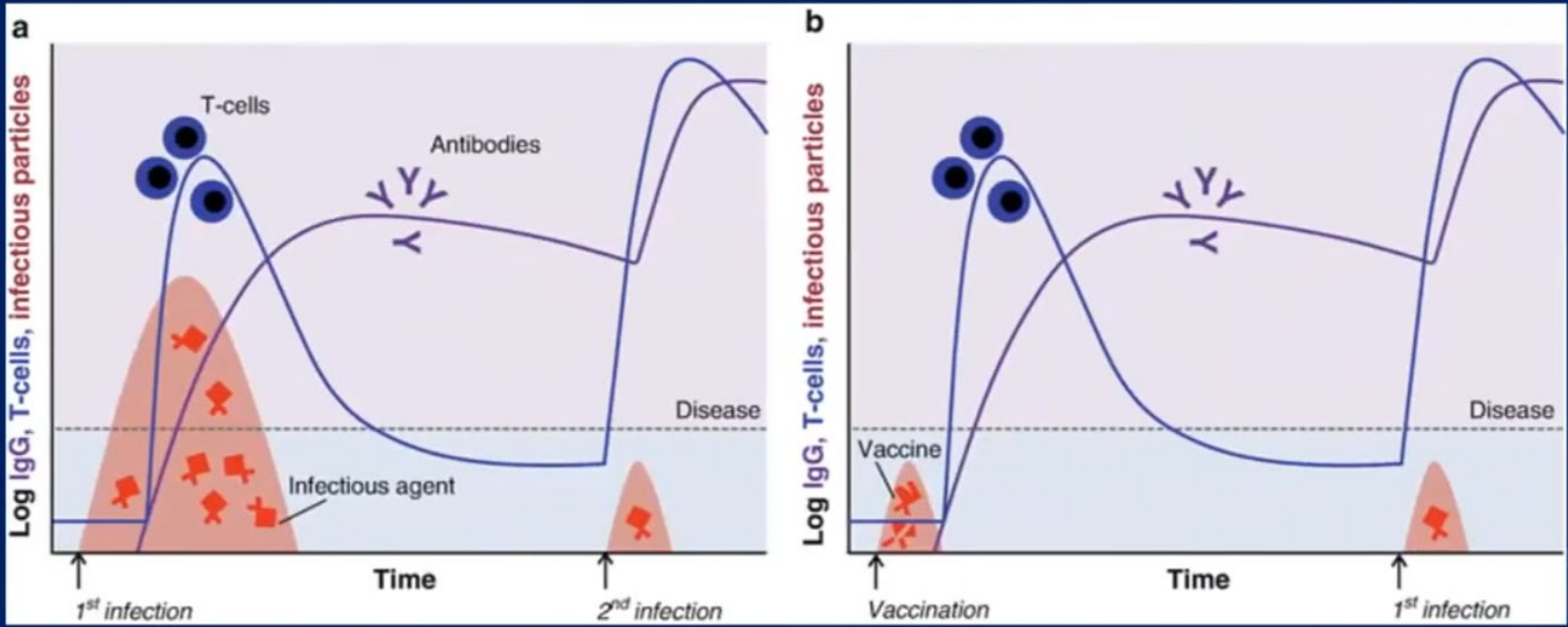
TNF-α Inhibitors



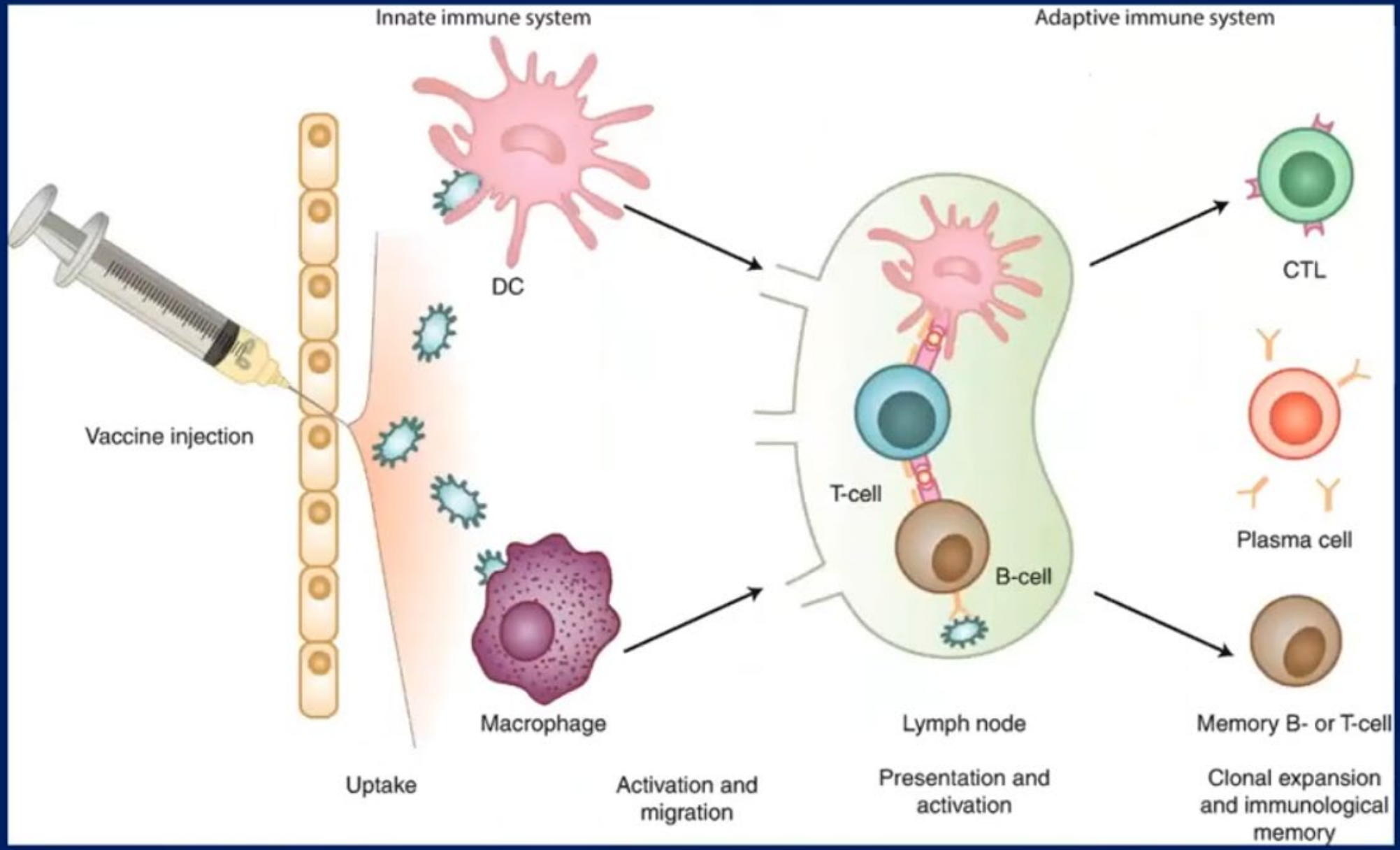
Indikasi: rheumatoid arthritis

<3> VAKSIN

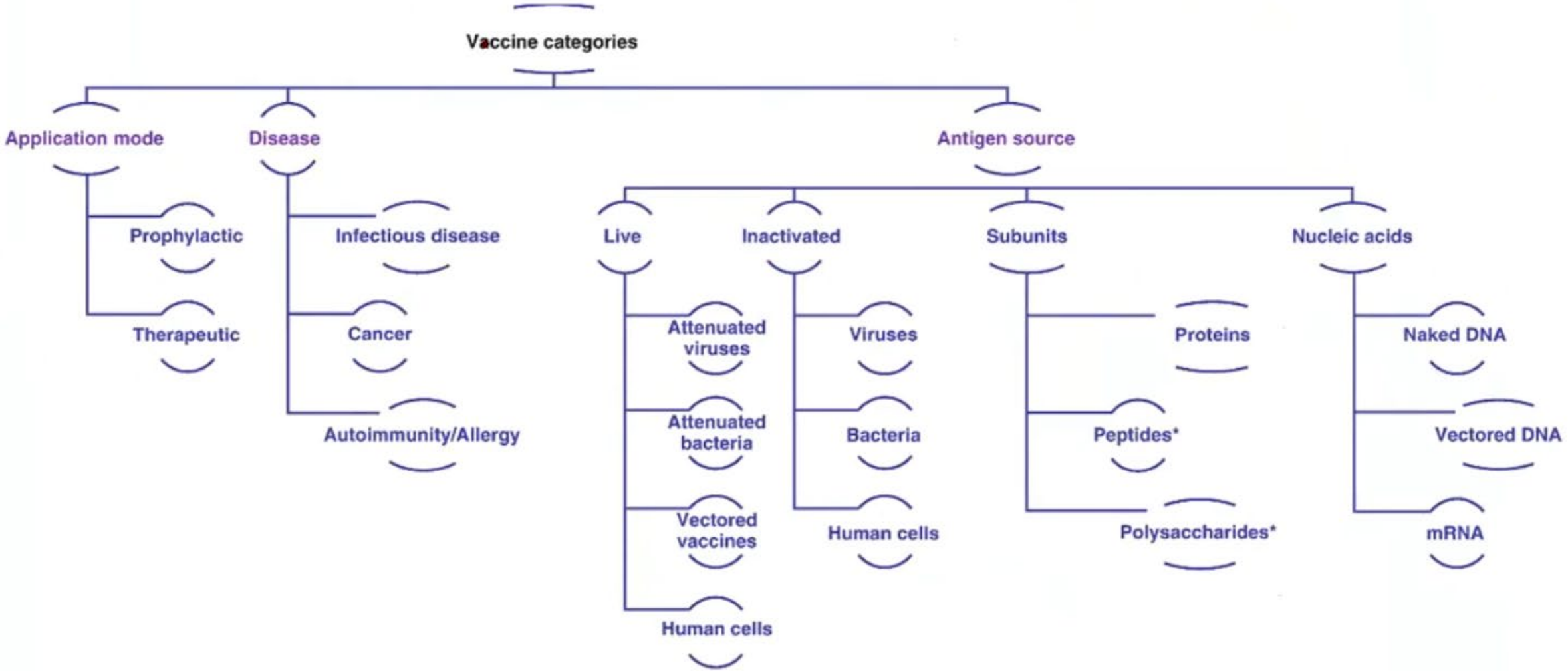
PRINSIP RESPONS IMUN ADAPTIF TERHADAP INFEKSI DAN VAKSINASI



RESPONS IMUN TERHADAP VAKSINASI



KLASIFIKASI VAKSIN



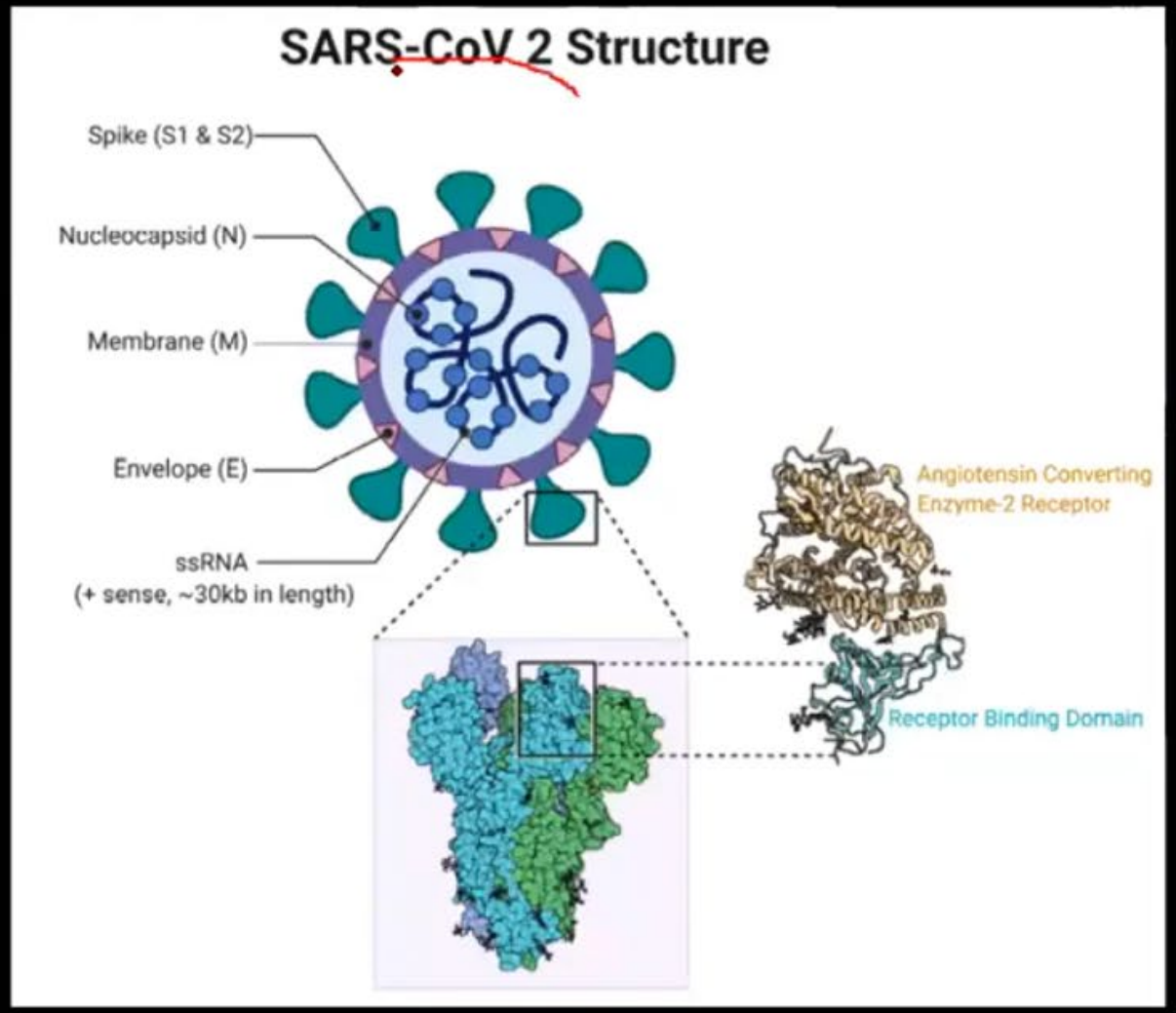
Vaksin BCG (Beku Kering)

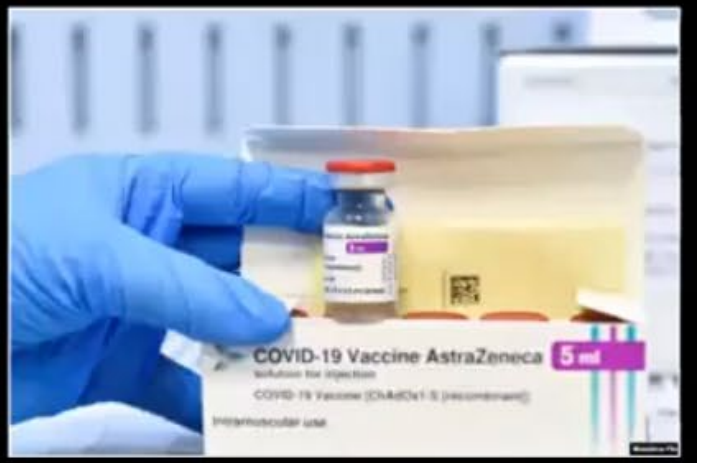
Vaksin BCG merupakan vaksin beku kering yang mengandung Mycobacterium bovis hidup yang dilemahkan (*Bacillus Calmette Guerin*), strain Paris

Bagikan:

Contact Us

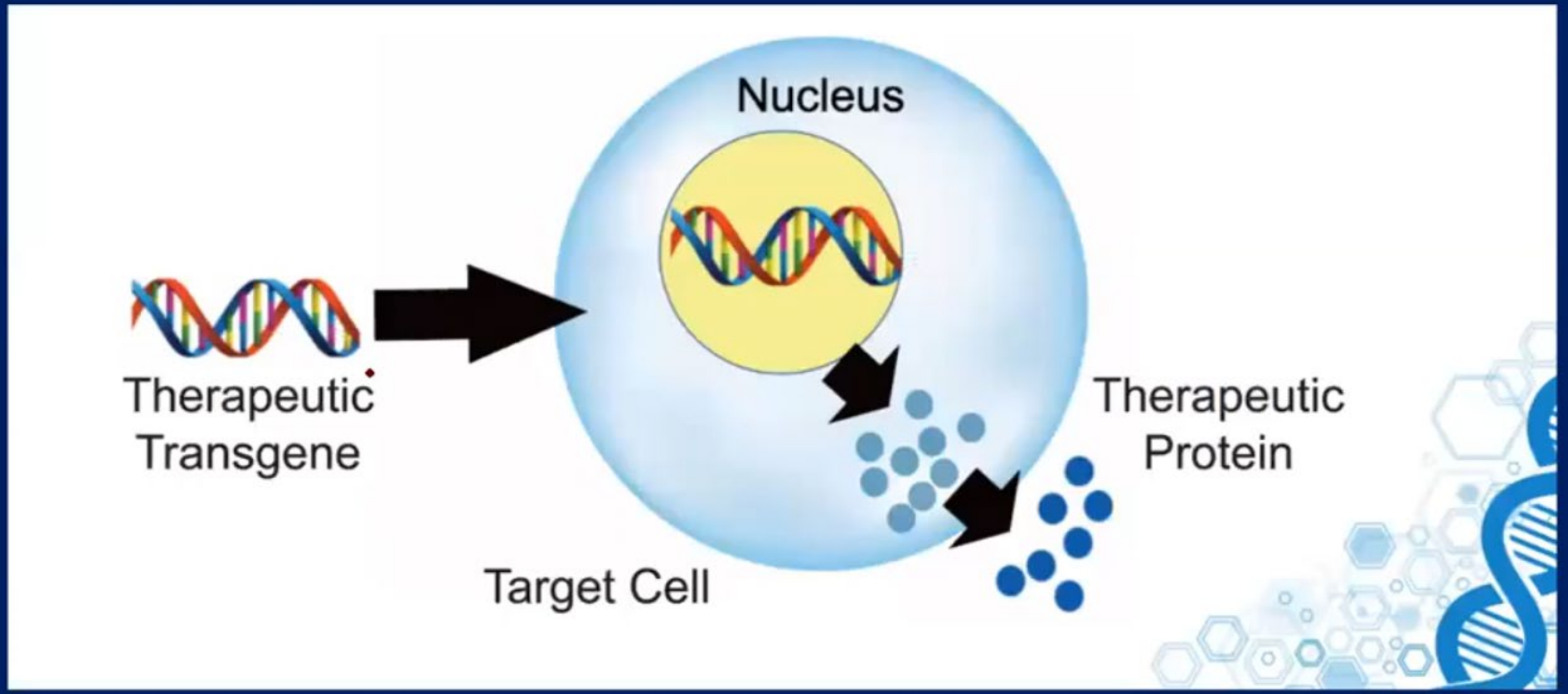
Vaksin COVID-19?





Terapi Gen

Prinsip dari Terapi Gen



Terapi Gen *In Vivo* dan *Ex Vivo*

Ex vivo | Gene delivery to cells outside the body

Delivery vector, e.g.

Lentivirus

Electroporation

Cargo / Payload

Nucleic Acid

Genome editing tool

Collect
(from **patient** (autologous) or **donor** (allogenic))

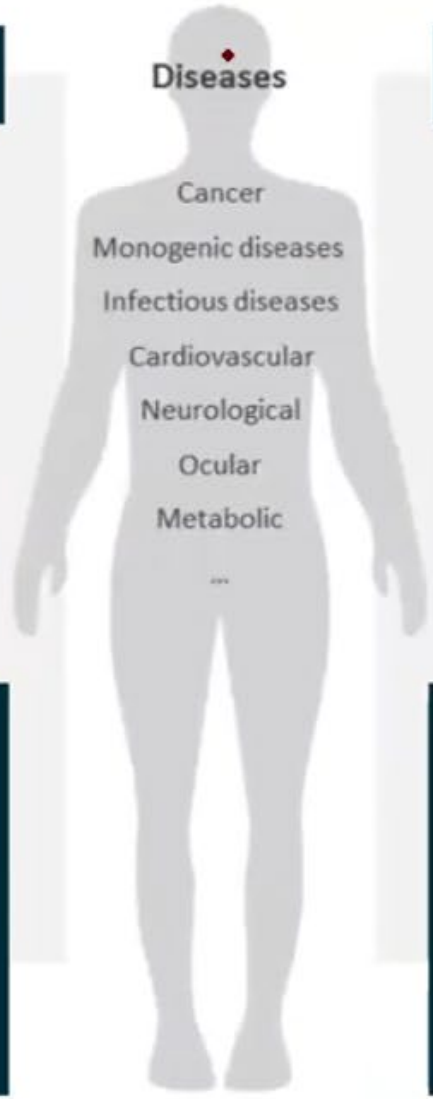
Select

Modify

Amplify

Deliver

Sel diambil dari pasien/donor → seleksi → dimodifikasi secara genetik → amplifikasi (diperbanyak) → diberikan ke pasien



In vivo | Gene delivery to patient cells

Local injection

Systemic injection

Delivery vector, e.g.

AAV LNP

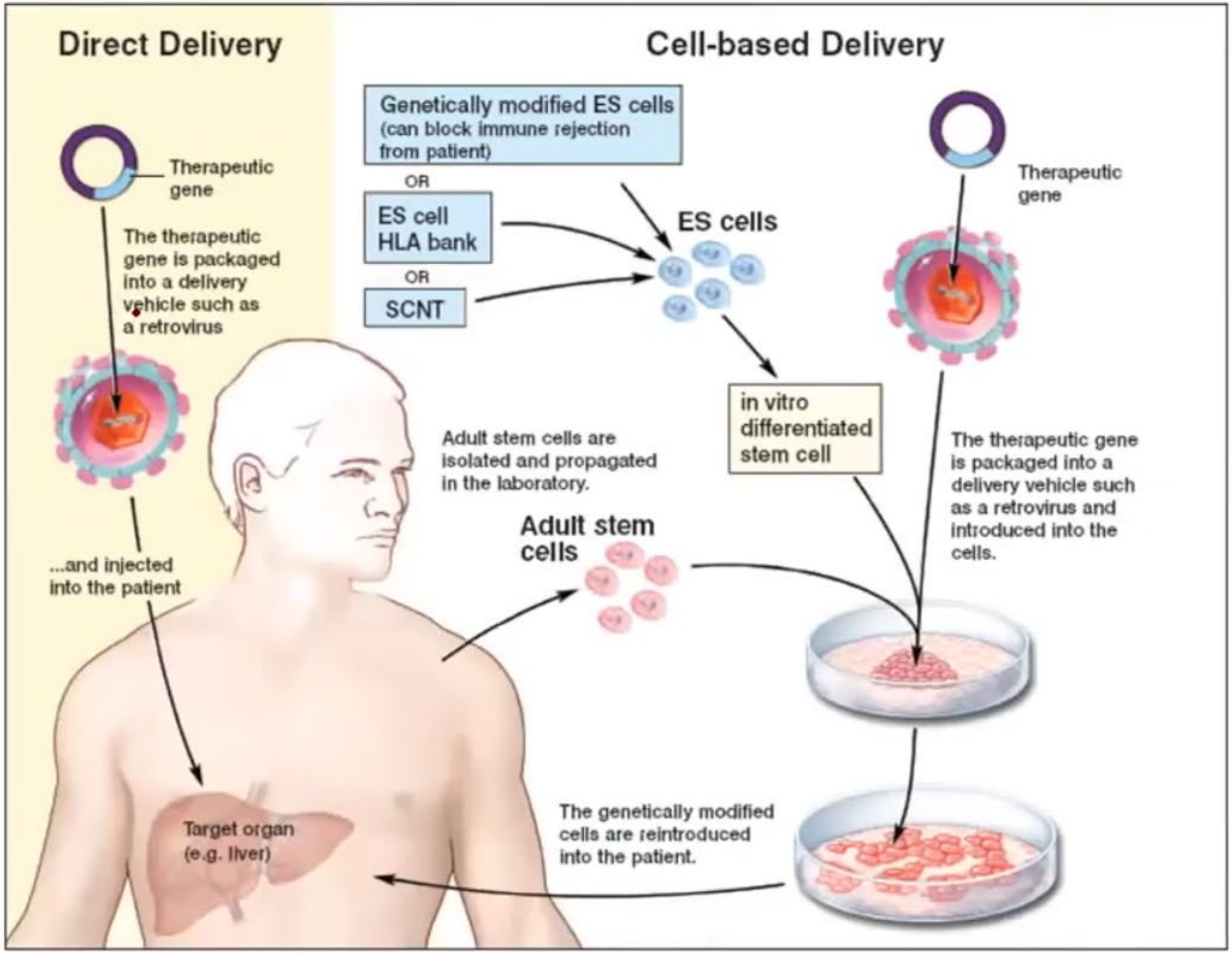
Cargo / Payload

Nucleic Acid

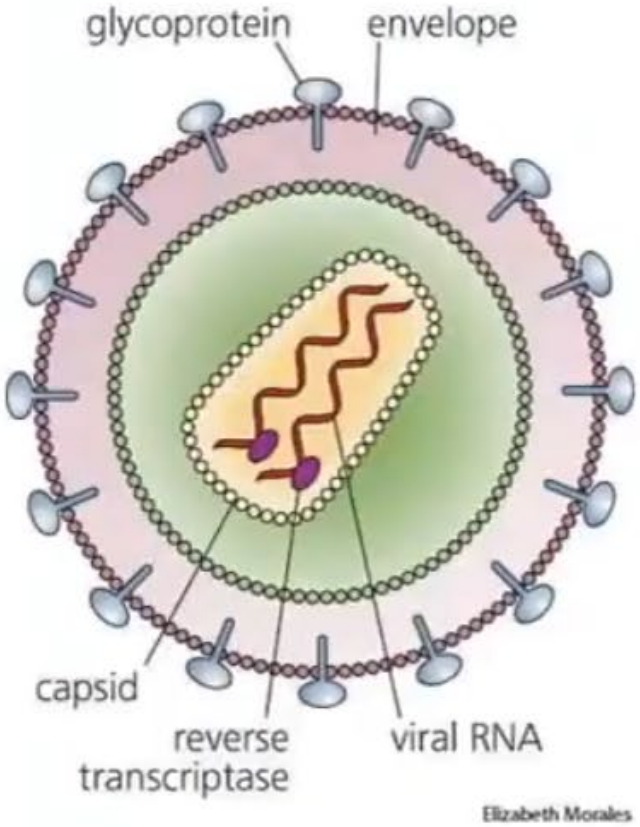
Genome editing tool

Gen diberikan ke pasien secara langsung, baik menggunakan vector (virus atau non virus) maupun tidak

in vivo



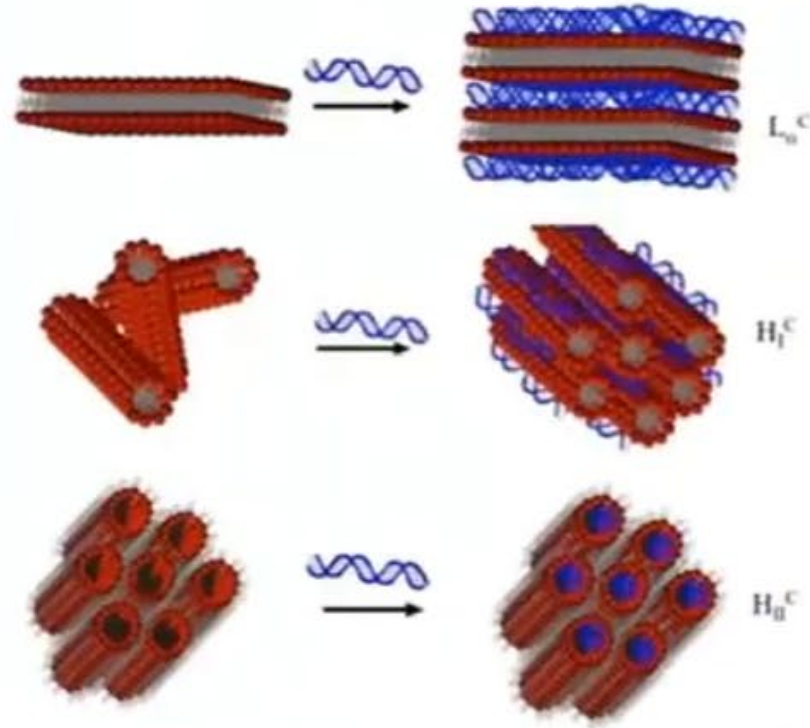
ex vivo



Vektor Virus

\+ : Efisiensi tinggi

\- : Memicu respon imun saat diberikan berulang



Vektor Non Virus

\+ : Tidak memicu respon imun dalam jumlah signifikan

\- : Efisiensi rendah

Q&A

THANK YOU