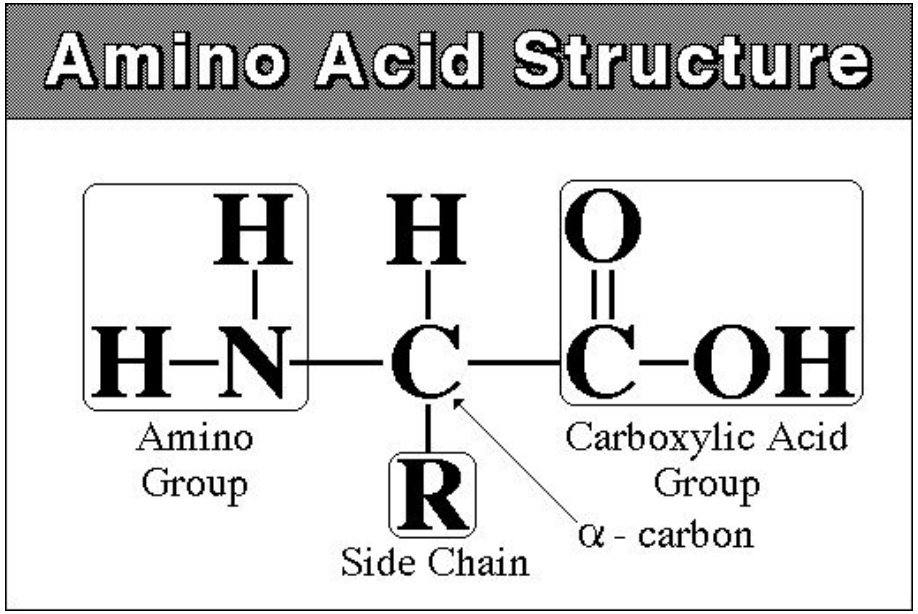
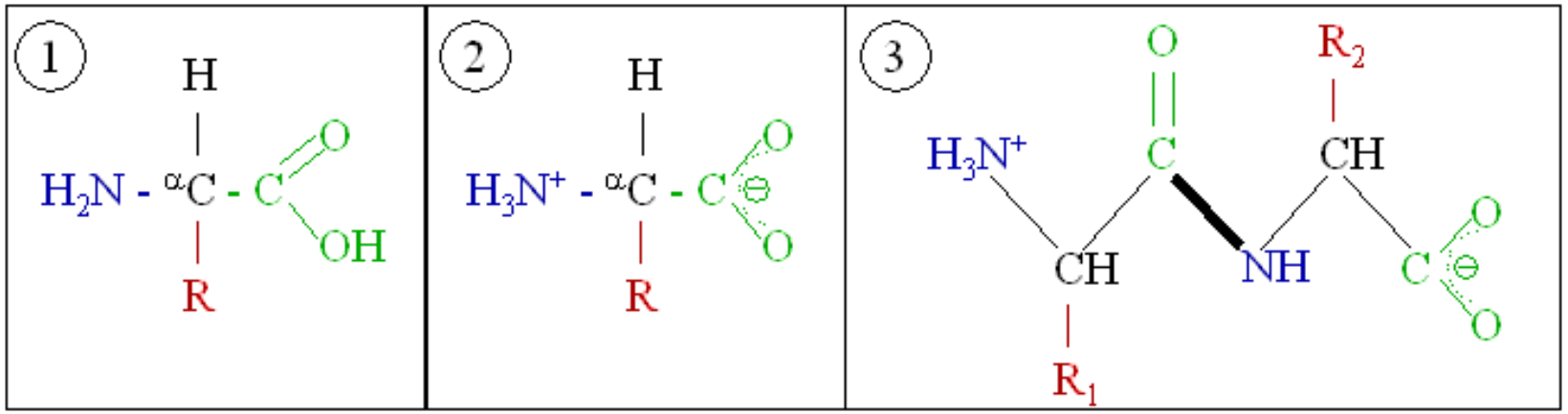
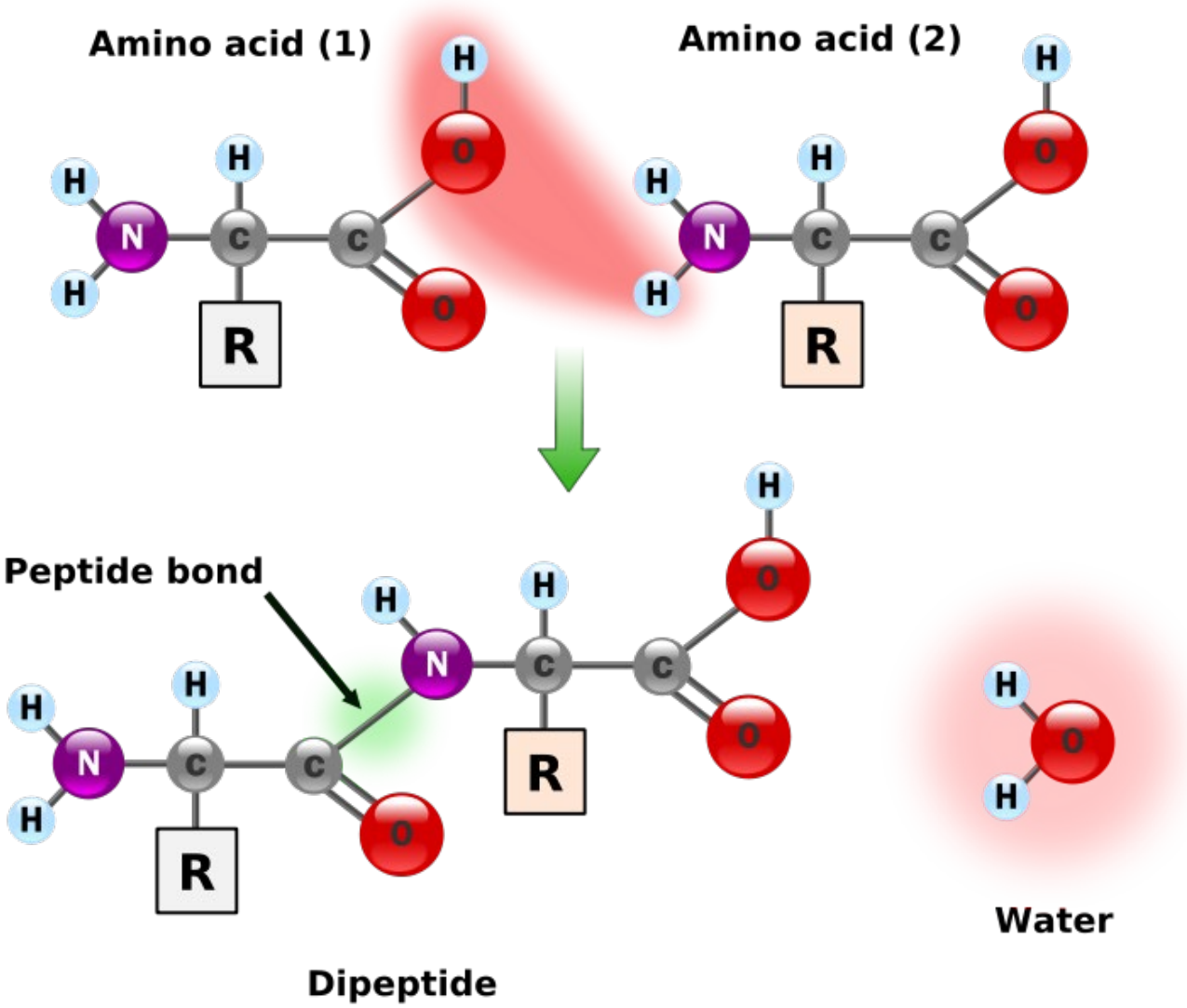
Amino Acid


Amino Acid Structure
H
H
O
H–N
C
C–OH
Amino Group
Carboxylic Acid Group
R
α - carbon
Side Chain

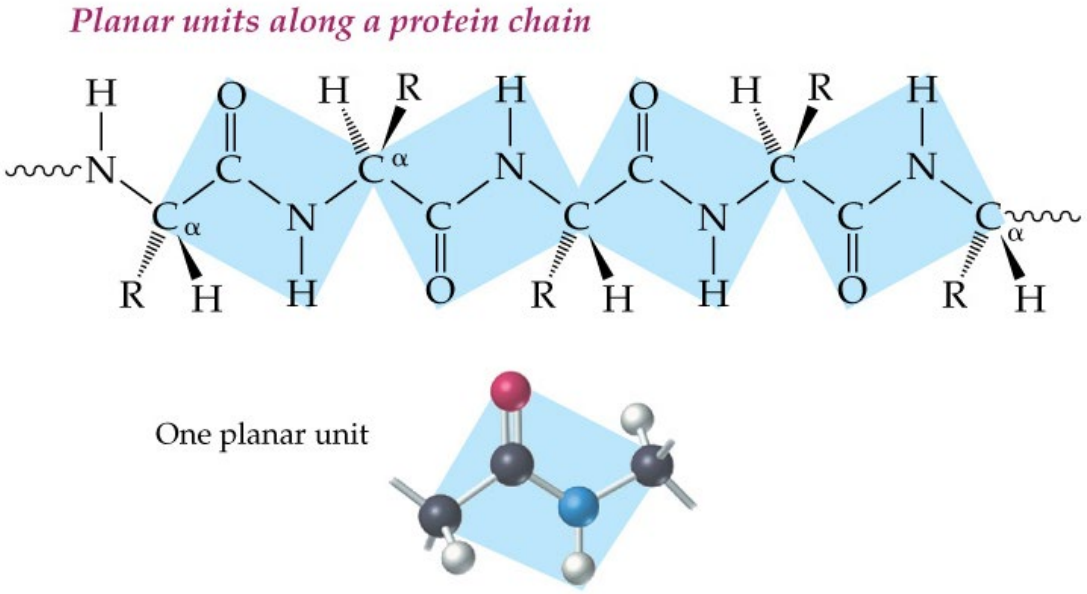

1
H
H_2N - $^{\alpha}C$ - C
O
OH
R
2
H
H_3N^+ - $^{\alpha}C$ - C
O
O
R
3
O
R_2
H_3N^+
C
CH
CH
NH
C
O
O
R_1

Peptide bond



Primary Protein Structure

- Primary structure of a proteins is the sequence of amino acids connected by peptide bonds. Along the backbone of the proteins is a chain of alternating peptide bonds and α-carbons and the amino acid side chains are connected to these

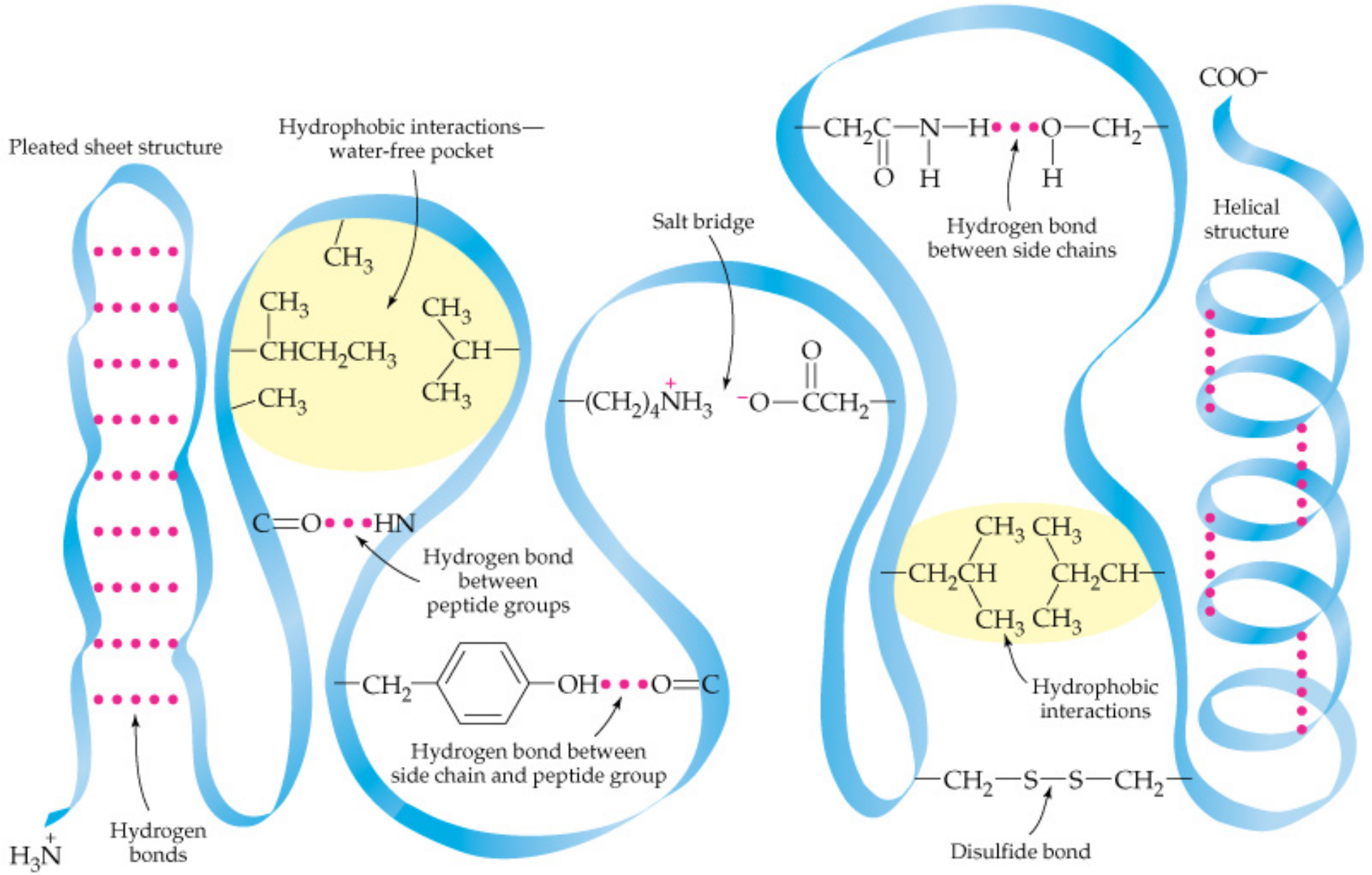


Secondary Protein Structure

- Secondary structure of a protein is the arrangement of polypeptide backbone of the protein in space. The secondary structure includes two kinds of repeating pattern known as the *α-helix* and *β-sheet*.
- Hydrogen bonding between backbone atoms are responsible for both of these secondary structures.

Tertiary Protein Structure

- ***Tertiary Structure of a proteins*** The overall three dimensional shape that results from the folding of a protein chain. Tertiary structure depends mainly on attractions of amino acid side chains that are far apart along the same backbone. Non-covalent interactions and disulfide covalent bonds govern tertiary structure.
- A protein with the shape in which it exist naturally in living organisms is known as a *native protein.*

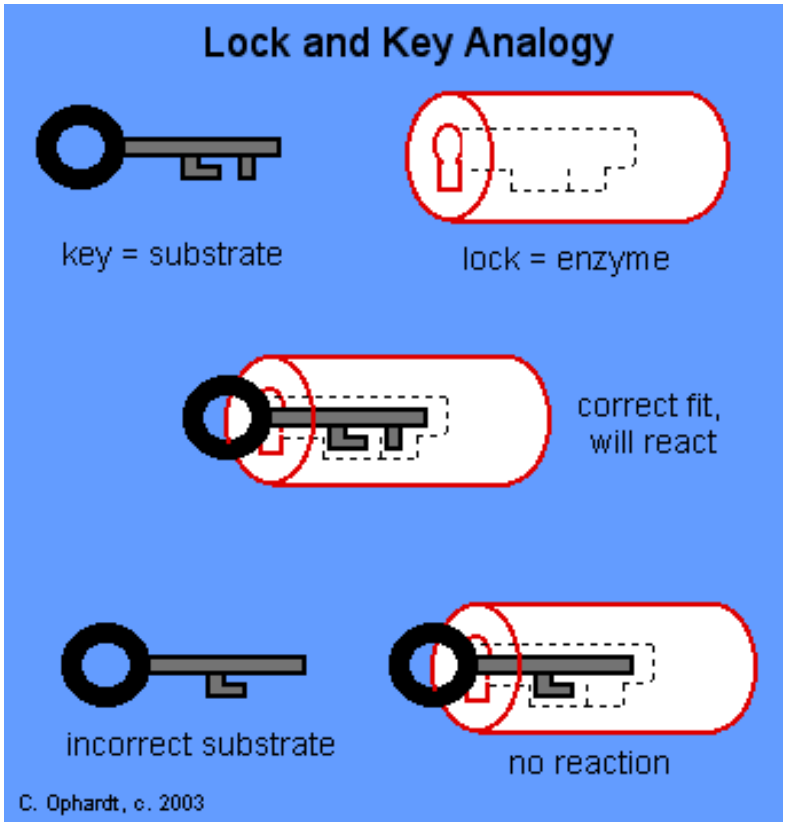

Pleated sheet structure
Hydrophobic interactions—
water-free pocket
Salt bridge
Hydrogen bond
between side chains
Helical
structure
Hydrogen bond
between
peptide groups
Hydrogen bond between
side chain and peptide group
Hydrophobic
interactions
Hydrogen
bonds
Disulfide bond

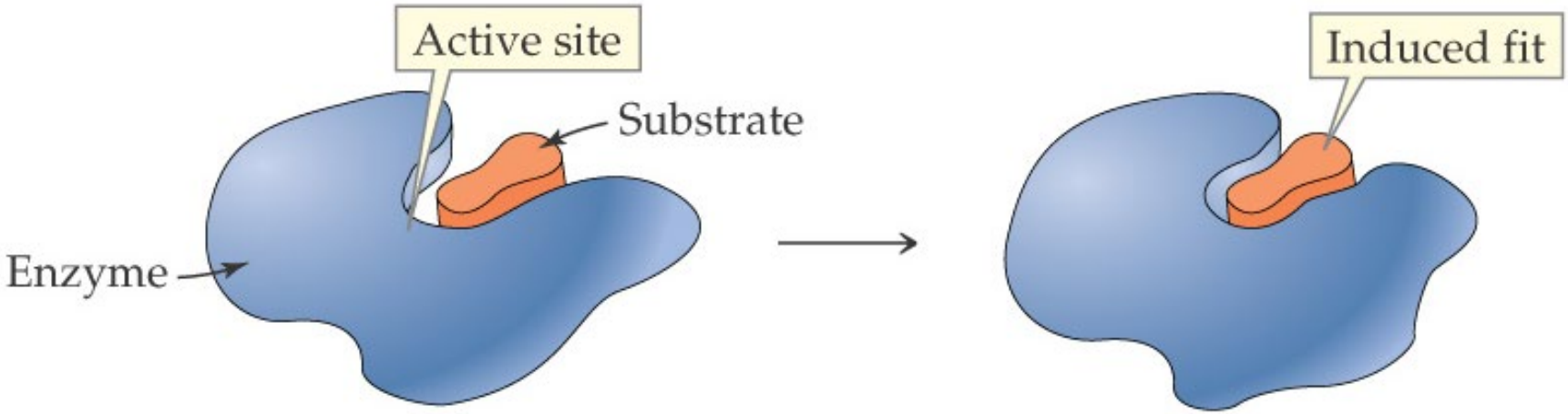
Quaternary Protein Structure

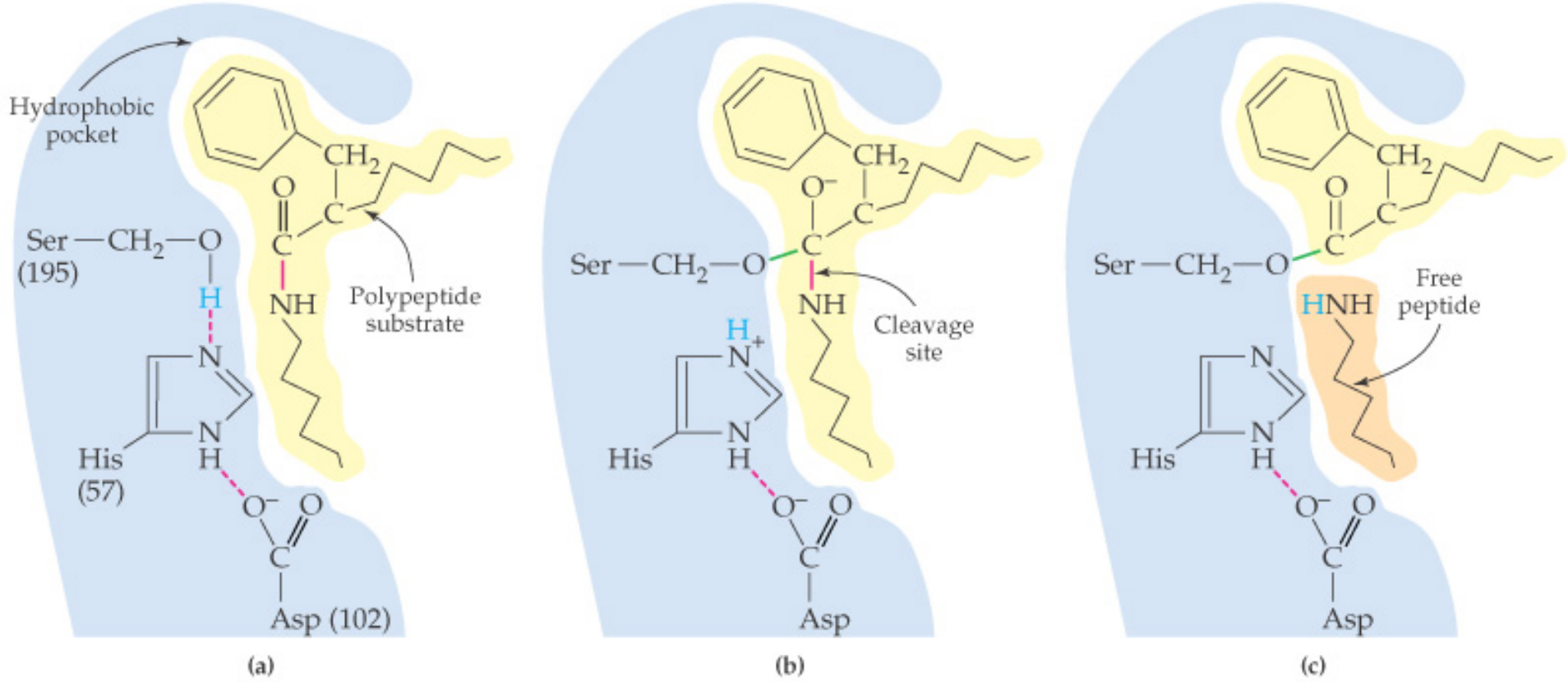
- ***Quaternary protein structure***: The way in which two or more polypeptide sub-units associate to form a single three-dimensional protein unit. Non-covalent forces are responsible for quaternary structure essential to the function of proteins.

How Enzyme Work

- Two modes are invoked to represent the interaction between substrate and enzymes. These are:
- ***Lock-and-key model***: The substrate is described as fitting into the active site as a key fit into a lock.
- ***Induced-fit-model***: The enzyme has a flexible active site that changes shape to accommodate the substrate and facilitate the reaction.


Lock and Key Analogy
key = substrate
lock = enzyme
correct fit,
will react
incorrect substrate
no reaction
C. Ophardt, c. 2003


Active site
Substrate
Enzyme
Induced fit


Hydrophobic pocket
Ser —CH2—O
(195)
H
N
N
H
His
(57)
O⁻ O
C
Asp (102)
CH2
O
C
C
NH
Polypeptide substrate
(a)
O⁻
C
Ser —CH2—O
NH
Cleavage site
H+
N
N
H
His
O⁻ O
C
Asp
(b)
HNH
Free peptide
(c)

19.5 Effect of Concentration on Enzyme Activity

- Variation in concentration of enzyme or substrate alters the rate of enzyme catalyzed reactions.
- *Substrate concentration*: At low substrate concentration, the reaction rate is directly proportional to the substrate concentration. With increasing substrate concentration, the rate drops off as more of the active sites are occupied.

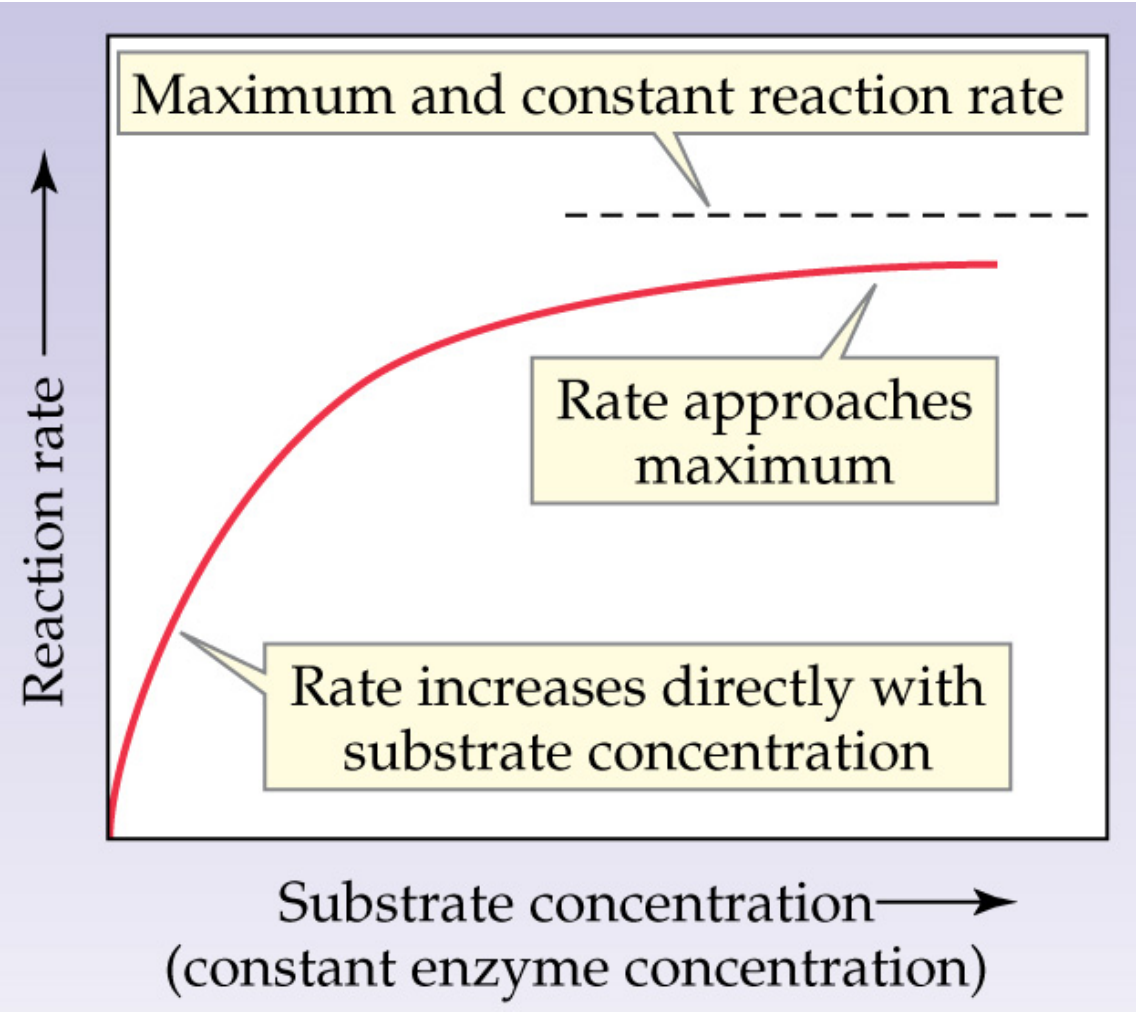
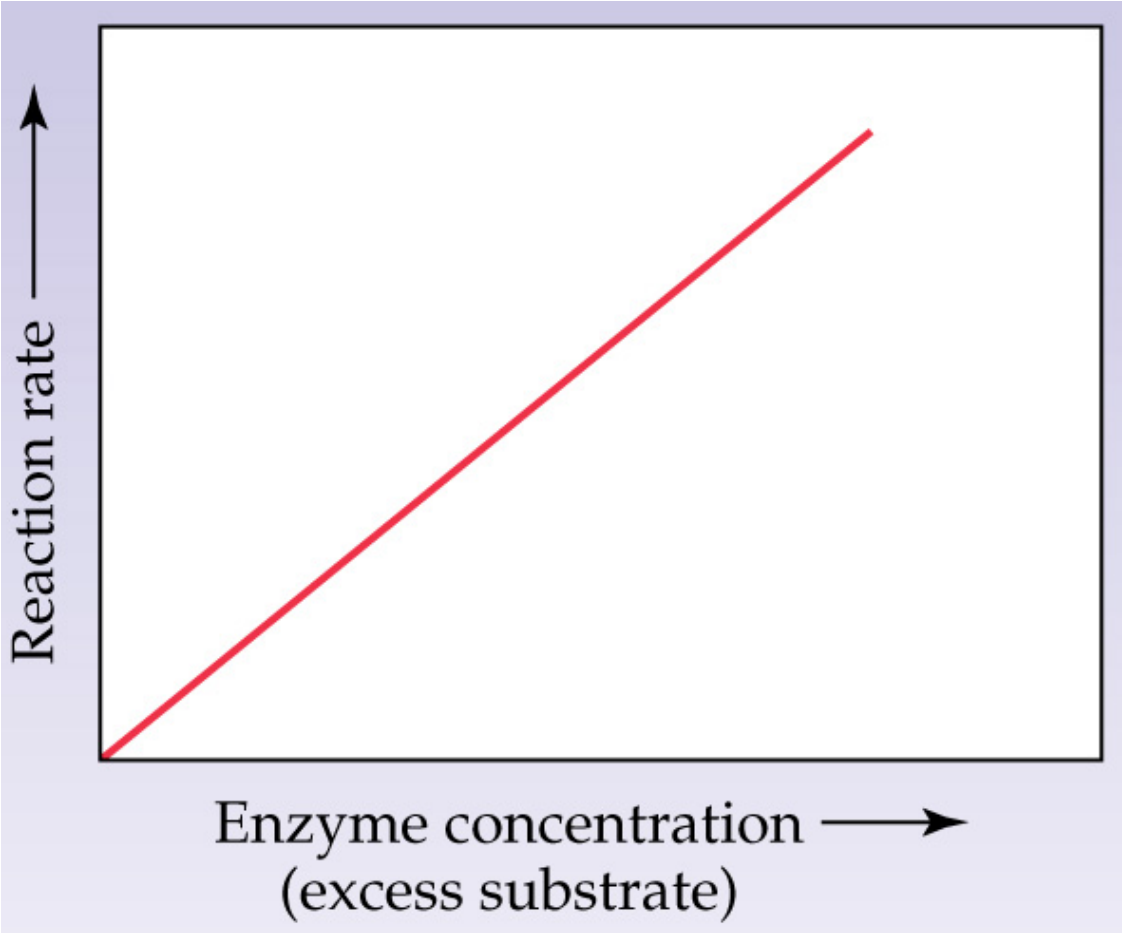


Fig 19.5 Change of reaction rate with substrate concentration when enzyme concentration is constant.

- *Enzyme concentration*: The reaction rate varies directly with the enzyme concentration as long as the substrate concentration does not become a limitation, Fig 19.6 below.



19.6 Effect of Temperature and pH on Enzyme Activity

- Enzymes maximum catalytic activity is highly dependent on temperature and pH.
- Increase in temperature increases the rate of enzyme catalyzed reactions. The rates reach a maximum and then begins to decrease. The decrease in rate at higher temperature is due to denaturation of enzymes.

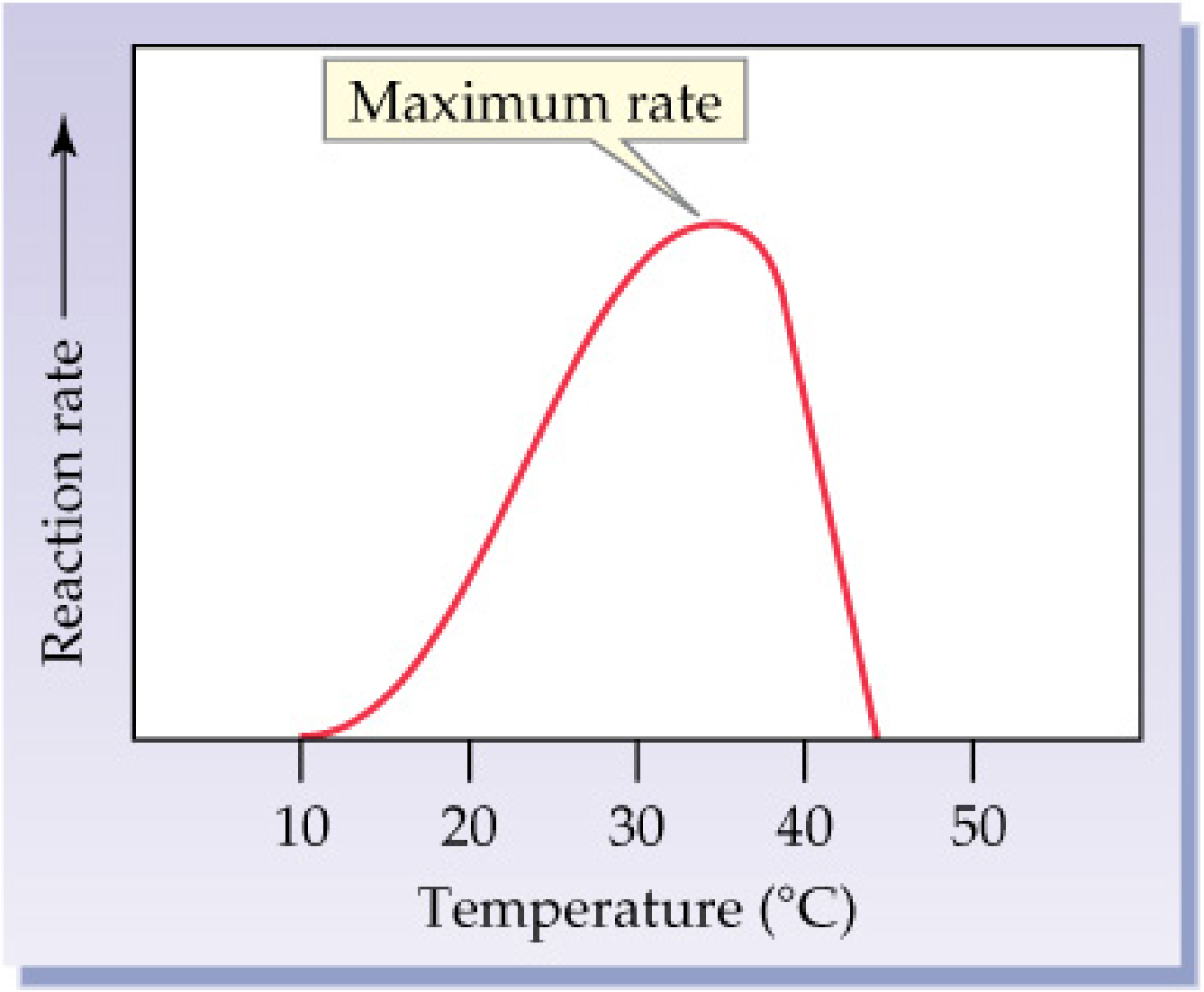
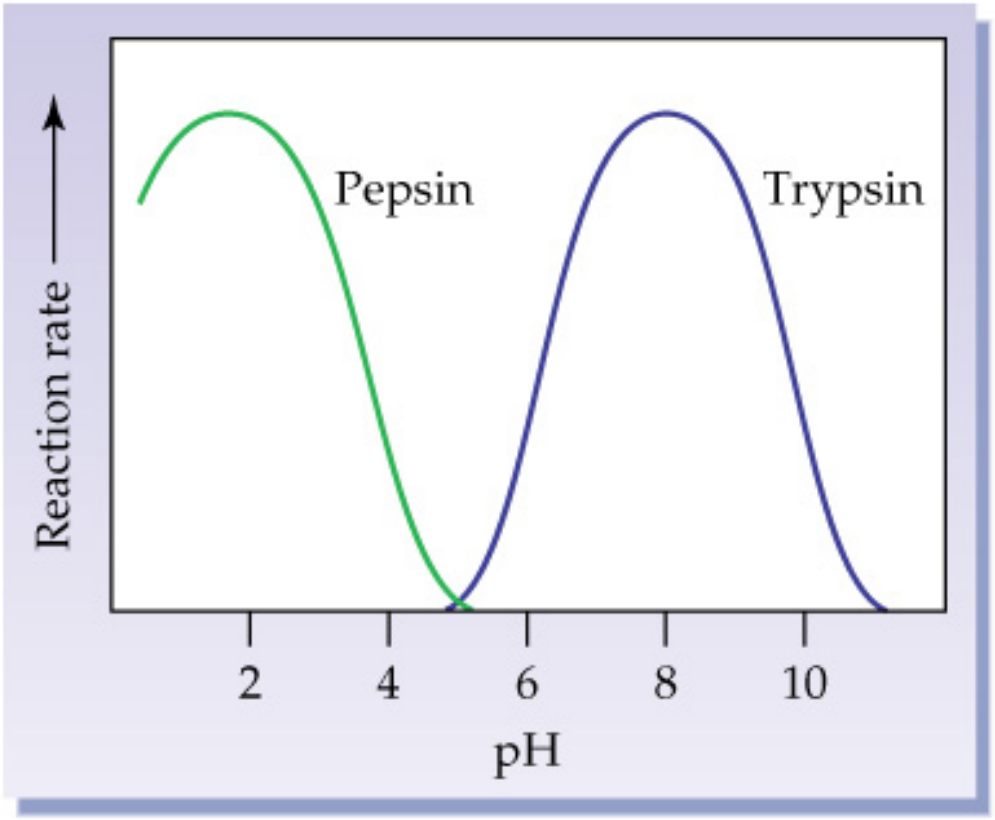


Fig 19.7 (a) Effect of temperature on reaction rate

- Effect of pH on Enzyme activity: The catalytic activity of enzymes depends on pH and usually has a well defined optimum point for maximum catalytic activity Fig 19.7 (b) below.

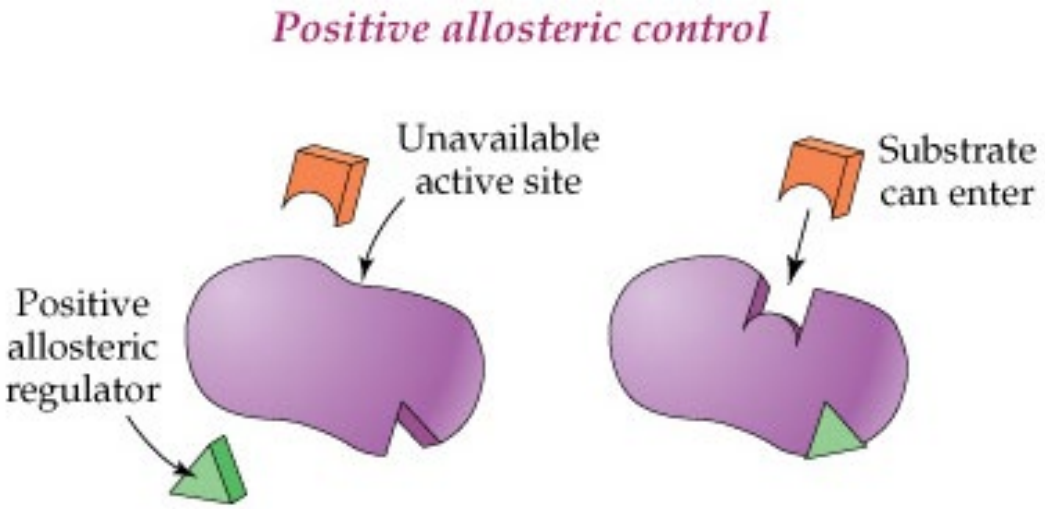


19.7 Enzyme Regulation: Feedback and Allosteric Control

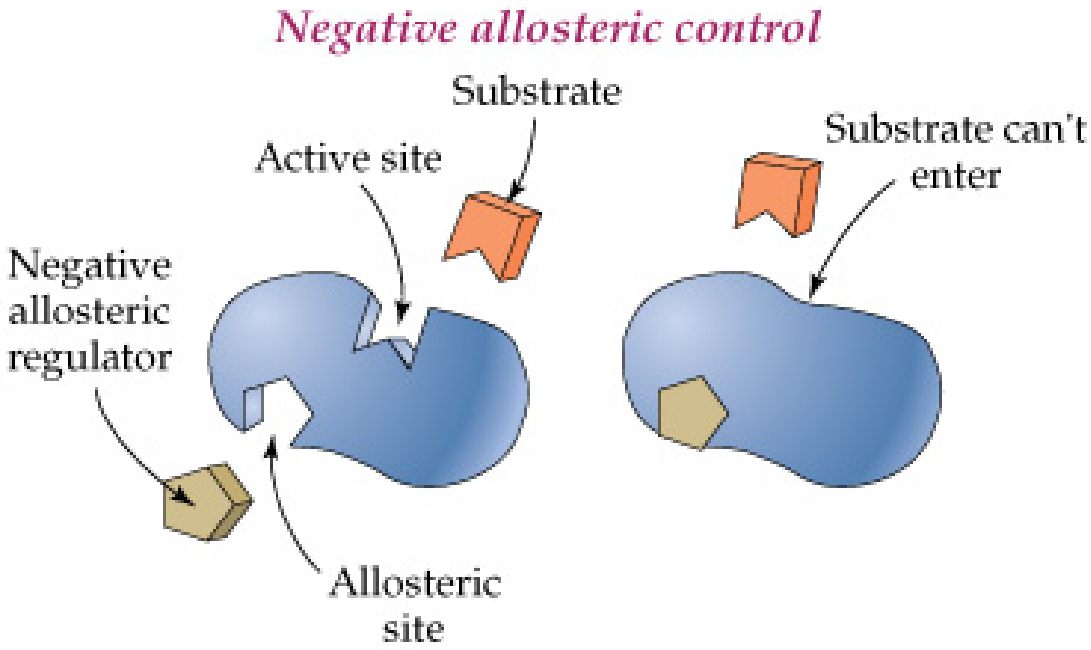
- Concentration of thousands of different chemicals vary continuously in living organisms which requires regulation of enzyme activity.
- Any process that starts or increase the activity of an enzyme is ***activation.***
- Any process that stops or slows the activity of an enzyme is ***inhibition.***

Two of the mechanism

- ***Feedback control***: Regulation of an enzyme's activity by the product of a reaction later in a pathway.
- ***Allosteric control***: Activity of an enzyme is controlled by the binding of an activator or inhibitor at a location other than the active site. Allosteric controls are further classified as positive or negative.
  - A positive regulator changes the activity site so that the enzyme becomes a better catalyst and rate accelerates.
  - A negative regulator changes the activity site so that the enzyme becomes less effective catalyst and rate slows down.



A positive regulator changes the activity site so that the enzyme becomes a better catalyst and rate accelerates.

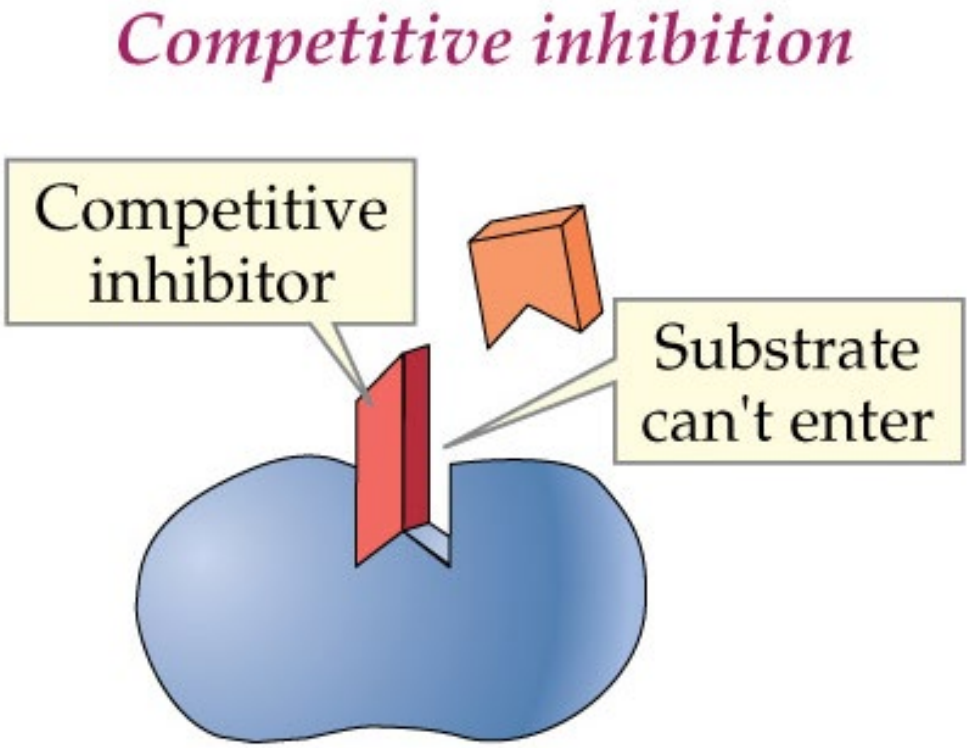


A negative regulator changes the activity site so that the enzyme becomes less effective catalyst and rate slows down.

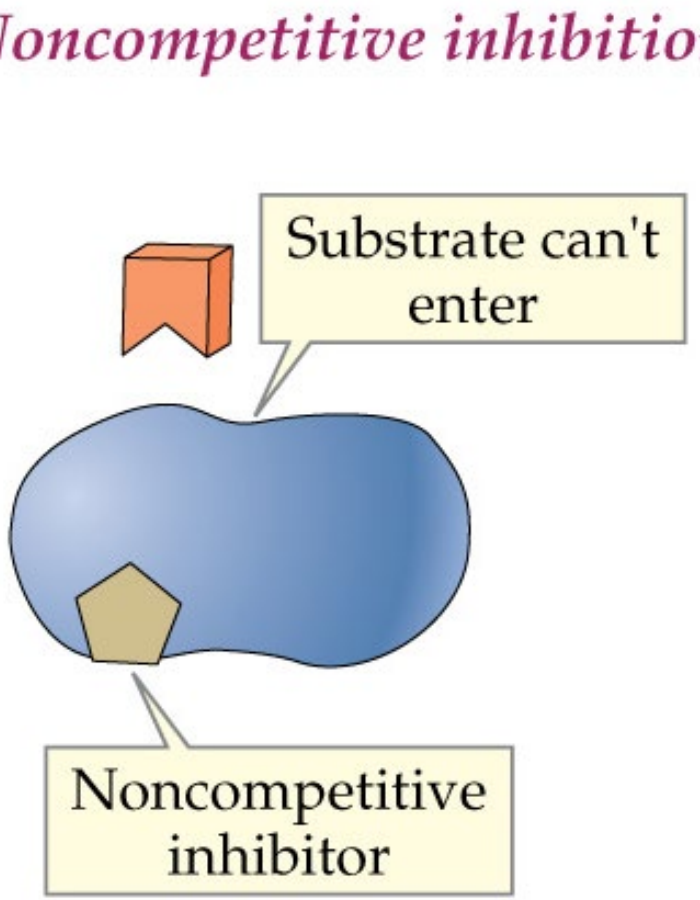
19.8 Enzyme Regulation: Inhibition

- The inhibition of an enzyme can be *reversible* or *irreversible.*
- In *reversible inhibition,* the inhibitor can leave, restoring the enzyme to its uninhibited level of activity.
- In *irreversible inhibition,* the inhibitor remains permanently bound to the enzyme and the enzyme is permanently inhibited.

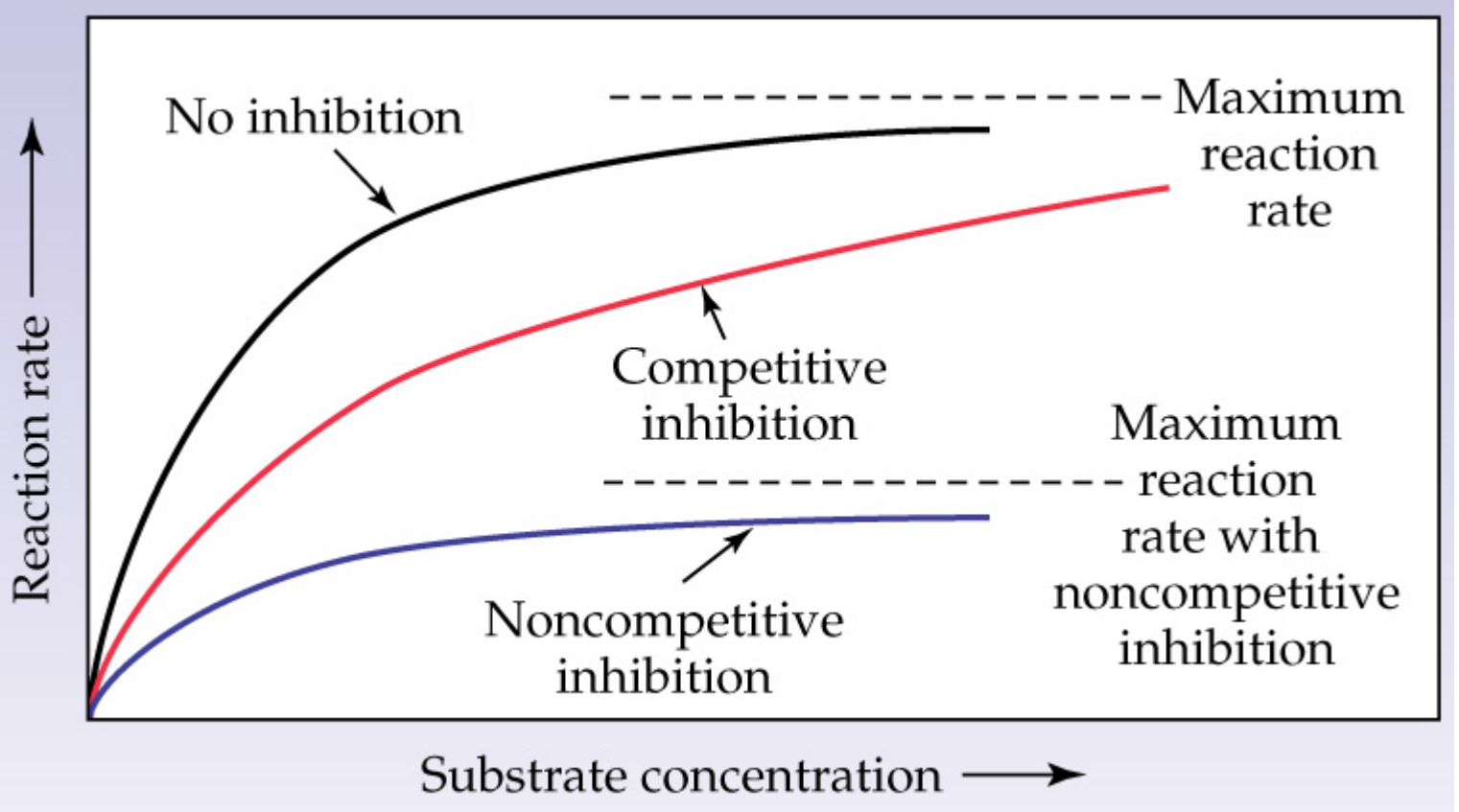
- Inhibitions are further classified as:
- *Competitive inhibition if* the inhibitor binds to the active site.



- *Noncompetitive inhibition,* if the inhibitor binds elsewhere and not to the active site.

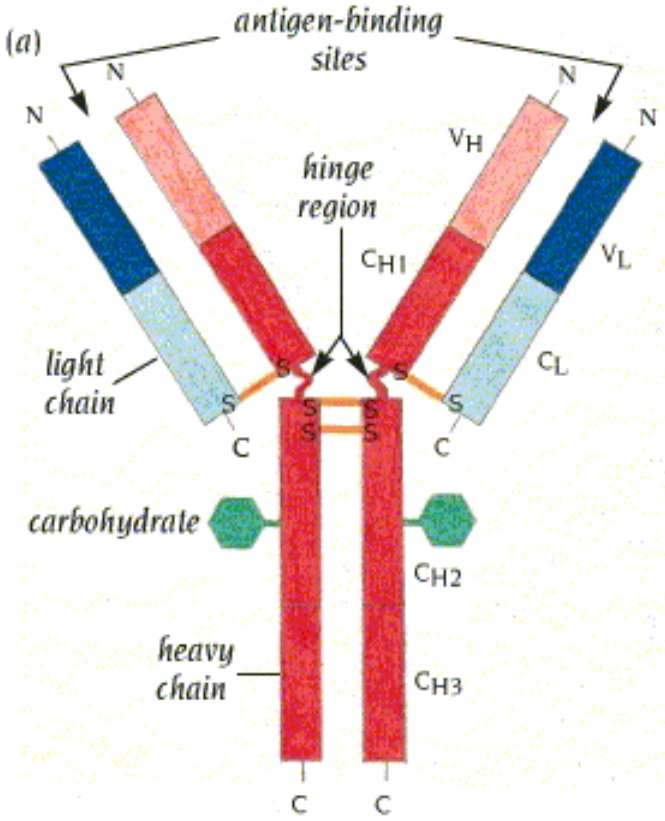


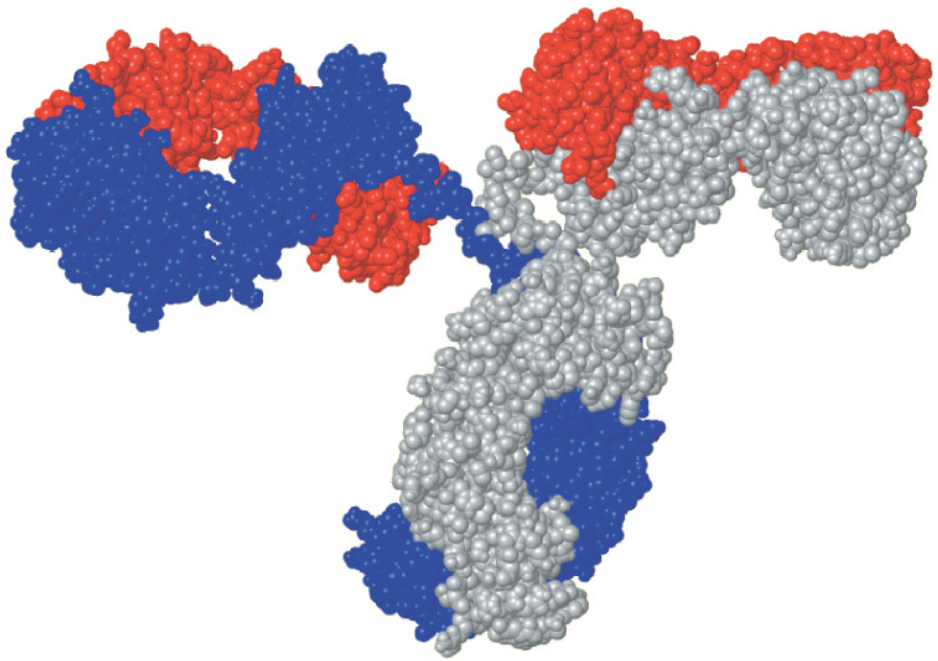
•The rates of enzyme catalyzed reactions with or without a competitive inhibitor are shown in the Fig 19.9 below.



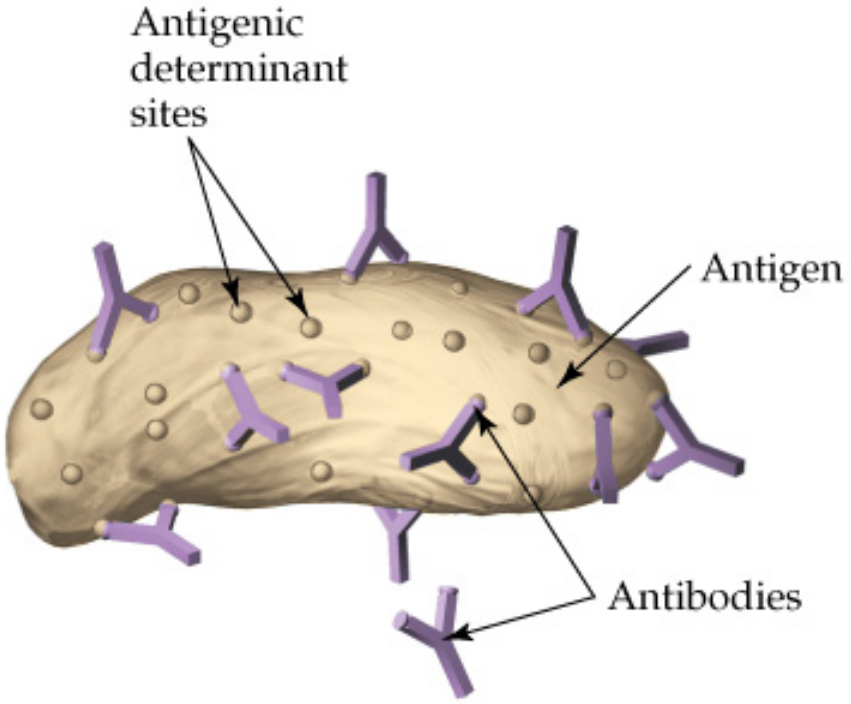
Shape-Determining Interactions in Proteins

- The essential structure-function relationship for each protein depends on the polypeptide chain being held in its necessary shape by the interactions of atoms in the side chains.

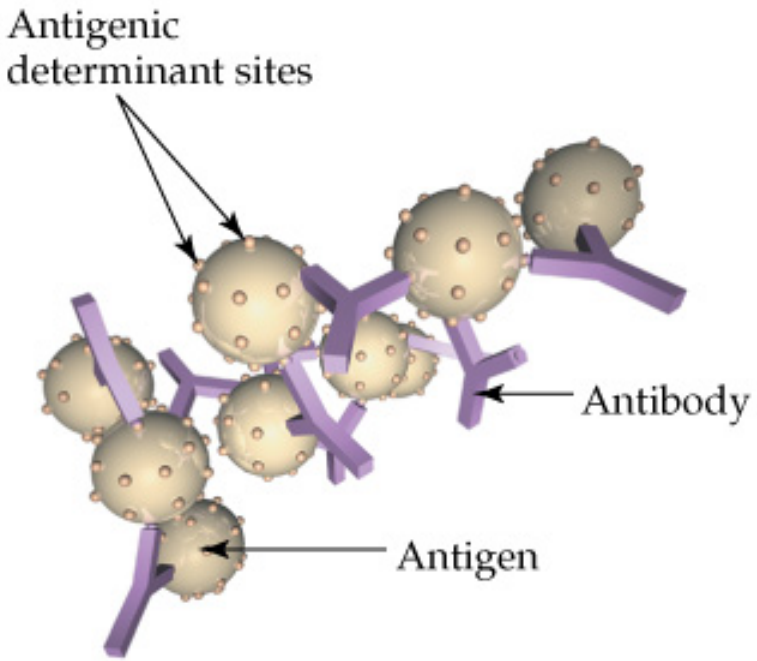

(a)
antigen-binding sites
N
N
N
N
VH
VL
hinge region
CH1
CL
light chain
S
S
S
S
S
S
S
S
C
C
carbohydrate
CH2
heavy chain
CH3
C
C



(b)

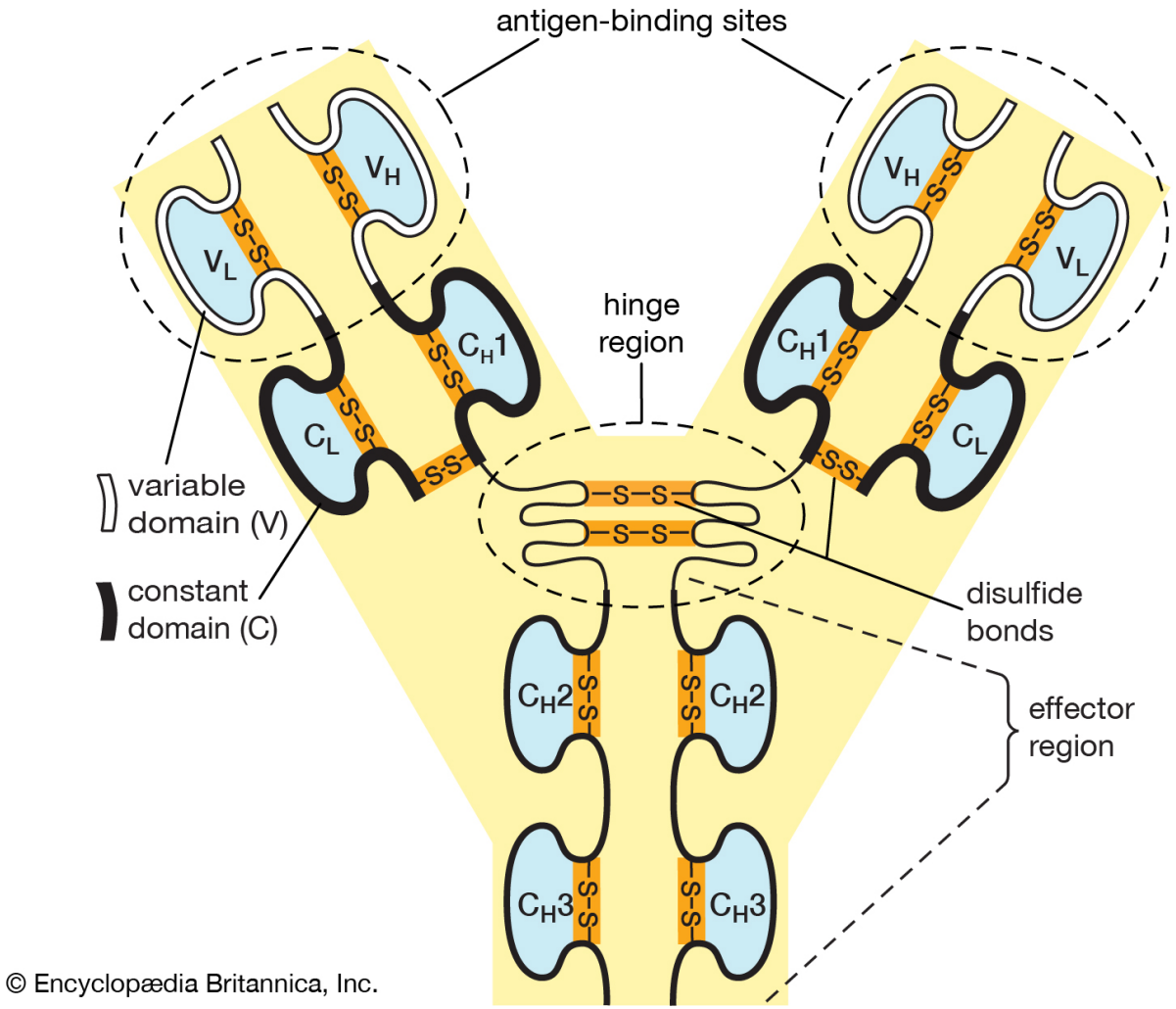

Antigenic determinant sites
Antigen
Antibodies

(a)

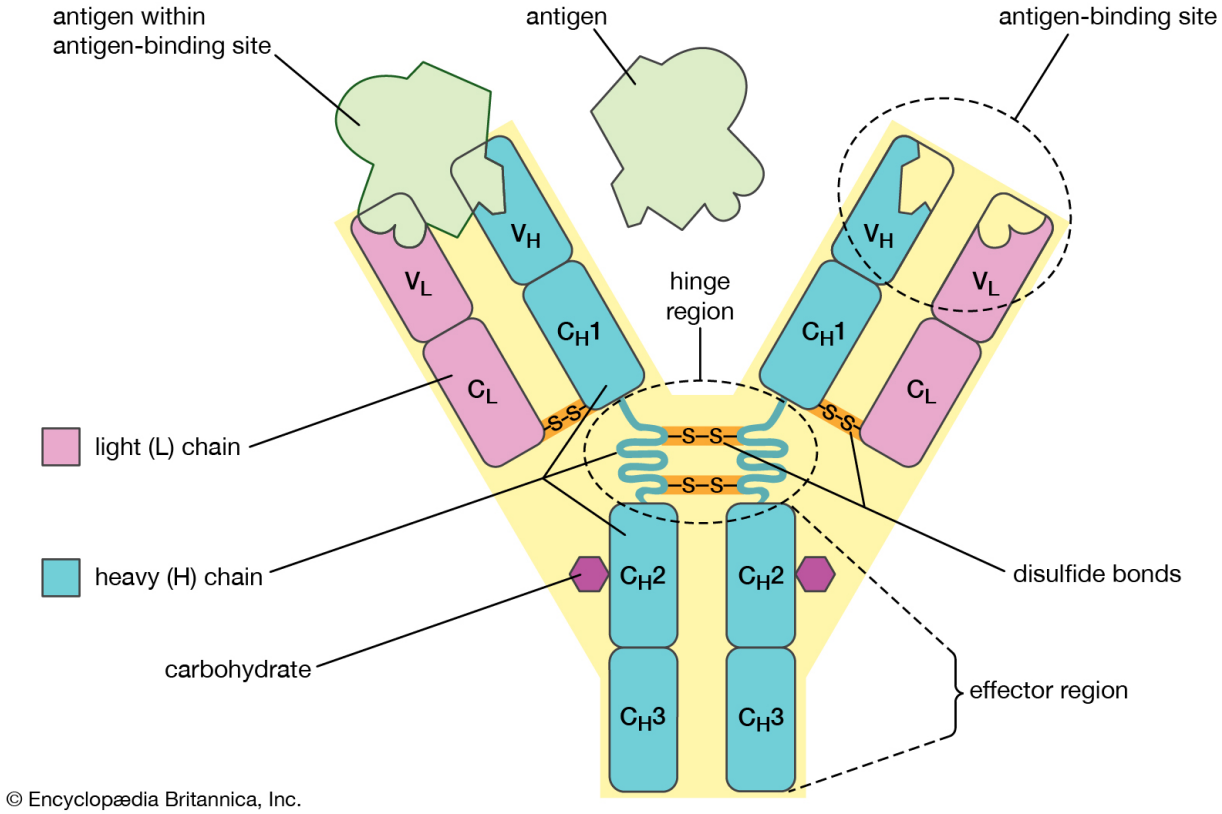

Antigenic determinant sites
Antibody
Antigen

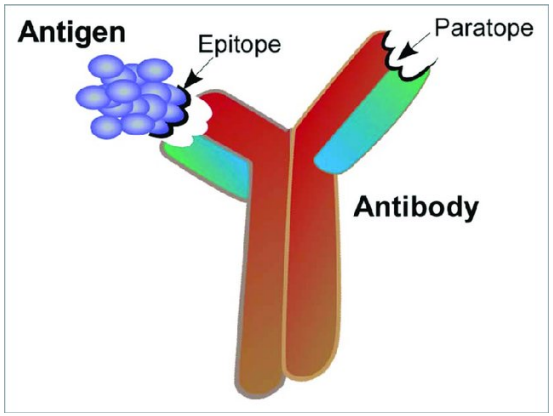
(b)

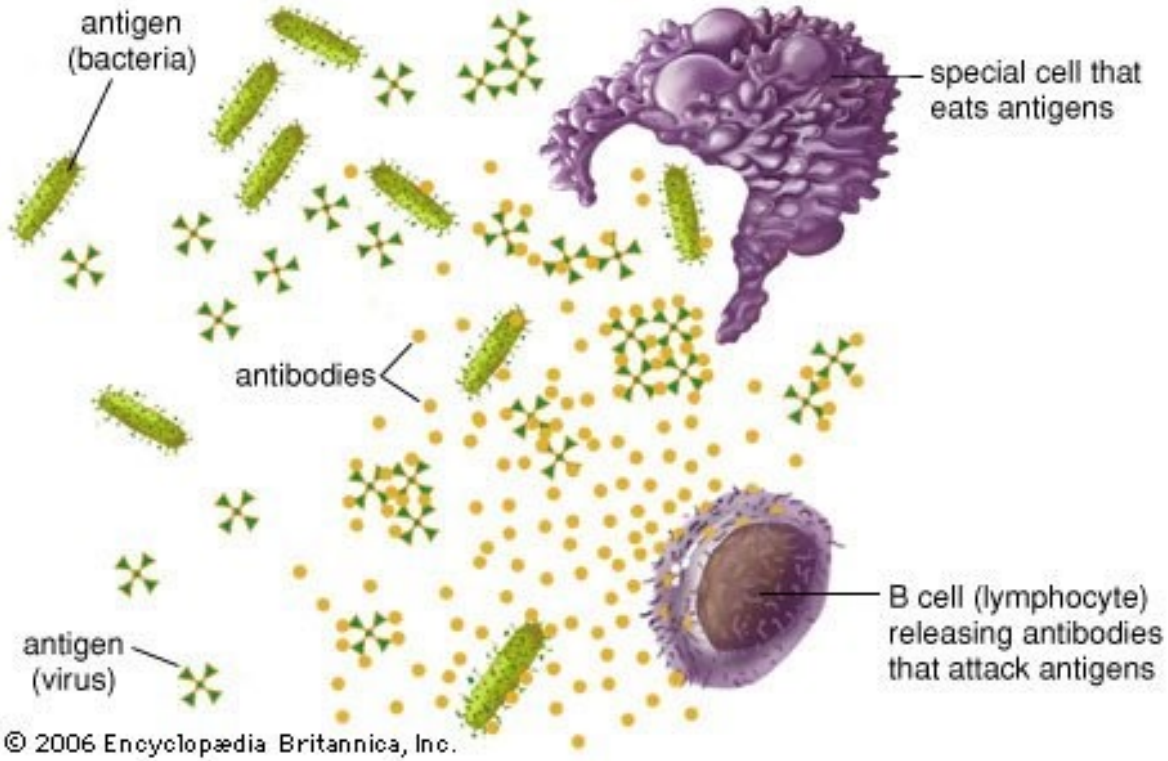
Antibody


antigen-binding sites
V_H
V_L
C_H1
C_L
hinge region
variable domain (V)
constant domain (C)
disulfide bonds
C_H2
C_H3
effector region
© Encyclopædia Britannica, Inc.

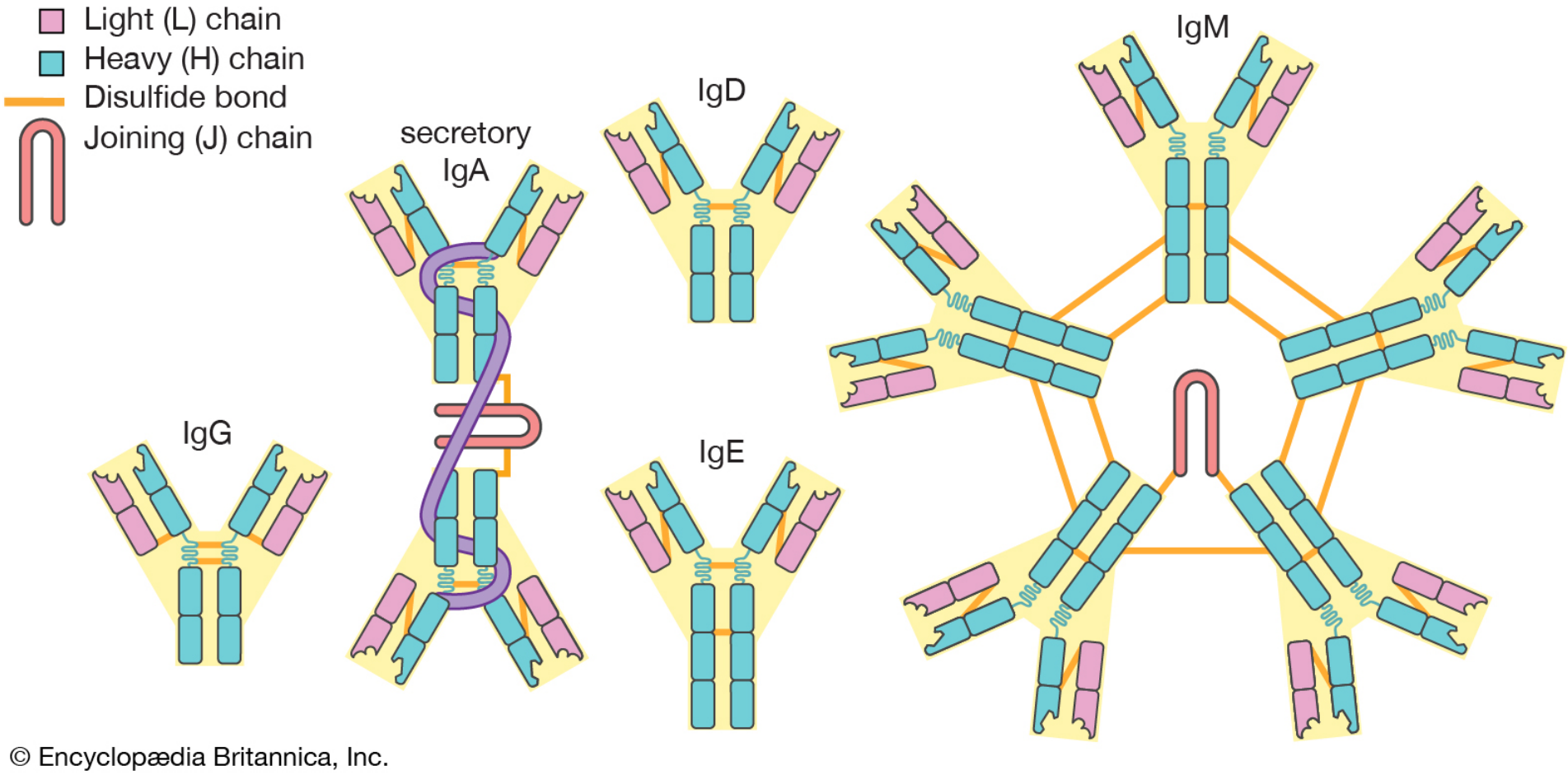
Antibody Binding Sites



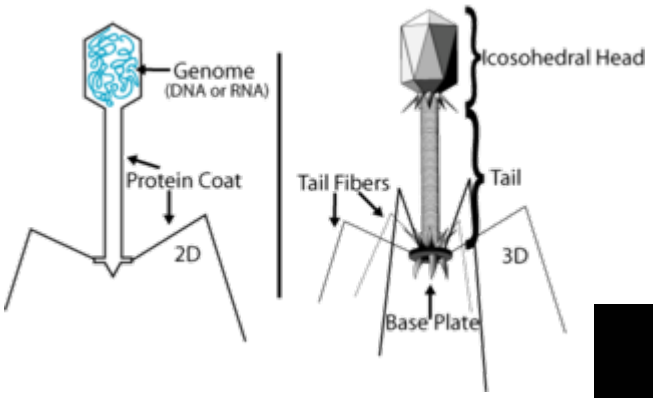


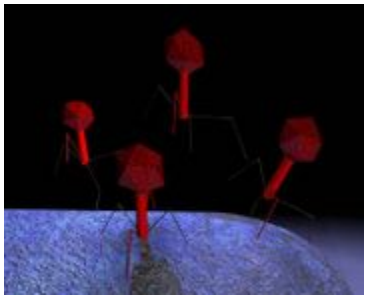

antigen
(bacteria)
special cell that
eats antigens
antibodies
B cell (lymphocyte)
releasing antibodies
that attack antigens
antigen
(virus)
© 2006 Encyclopædia Britannica, Inc.

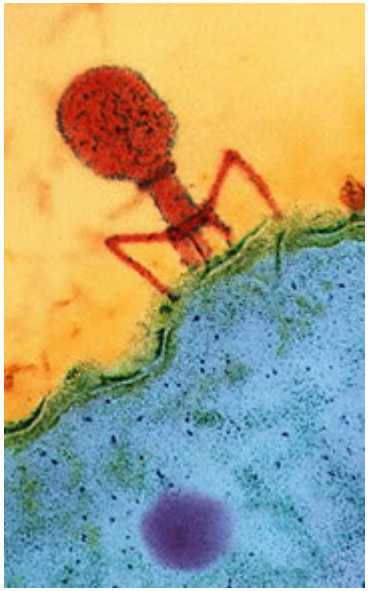
Different Types of Antibodies

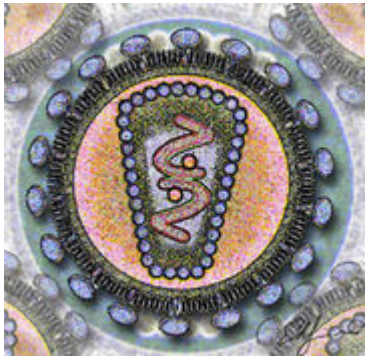


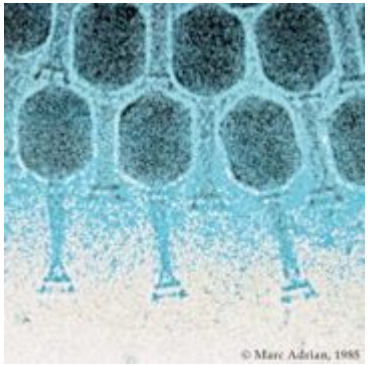
Virus


Genome
(DNA or RNA)
Protein Coat
2D
Icosohedral Head
Tail Fibers
Tail
3D
Base Plate

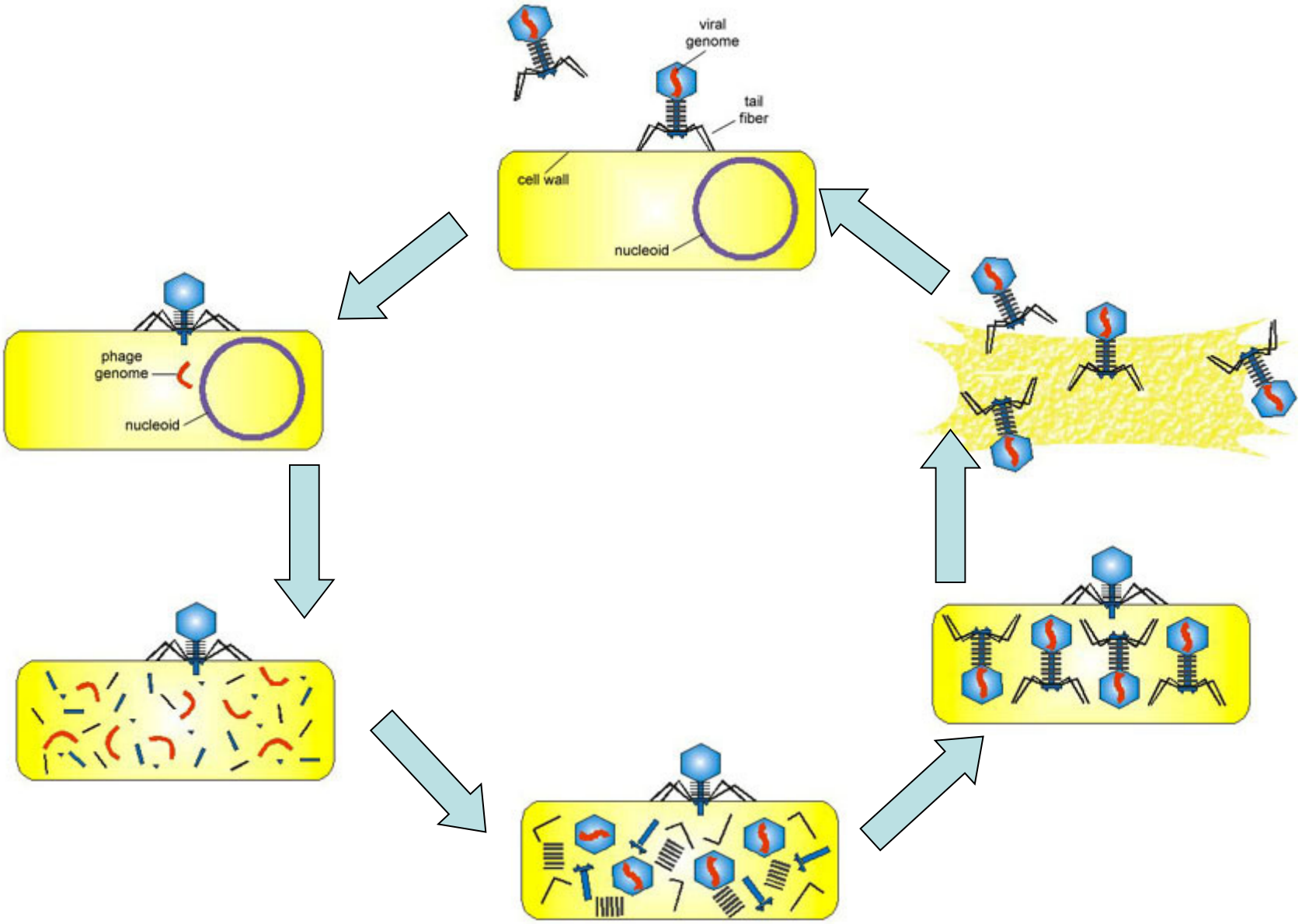




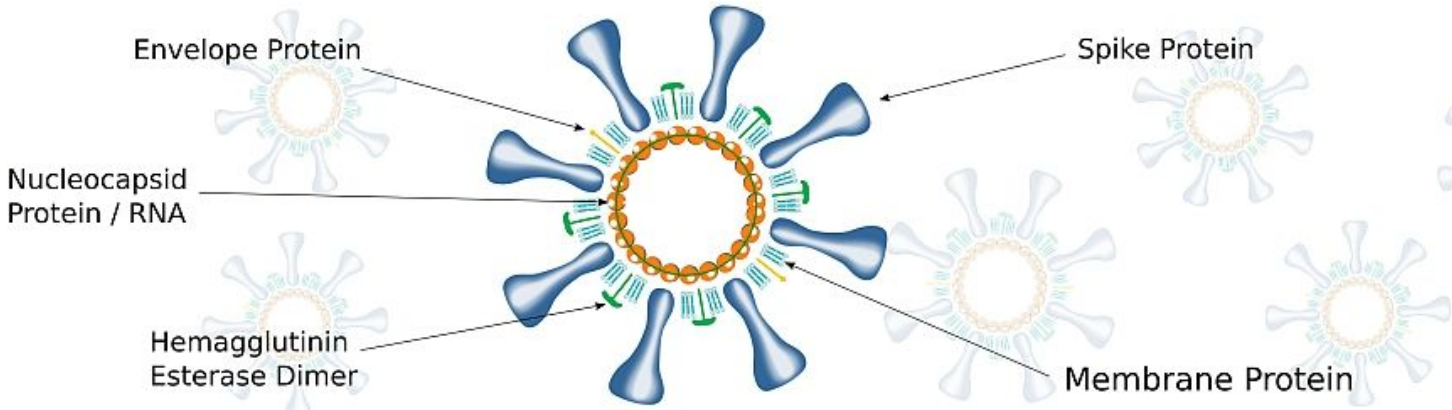


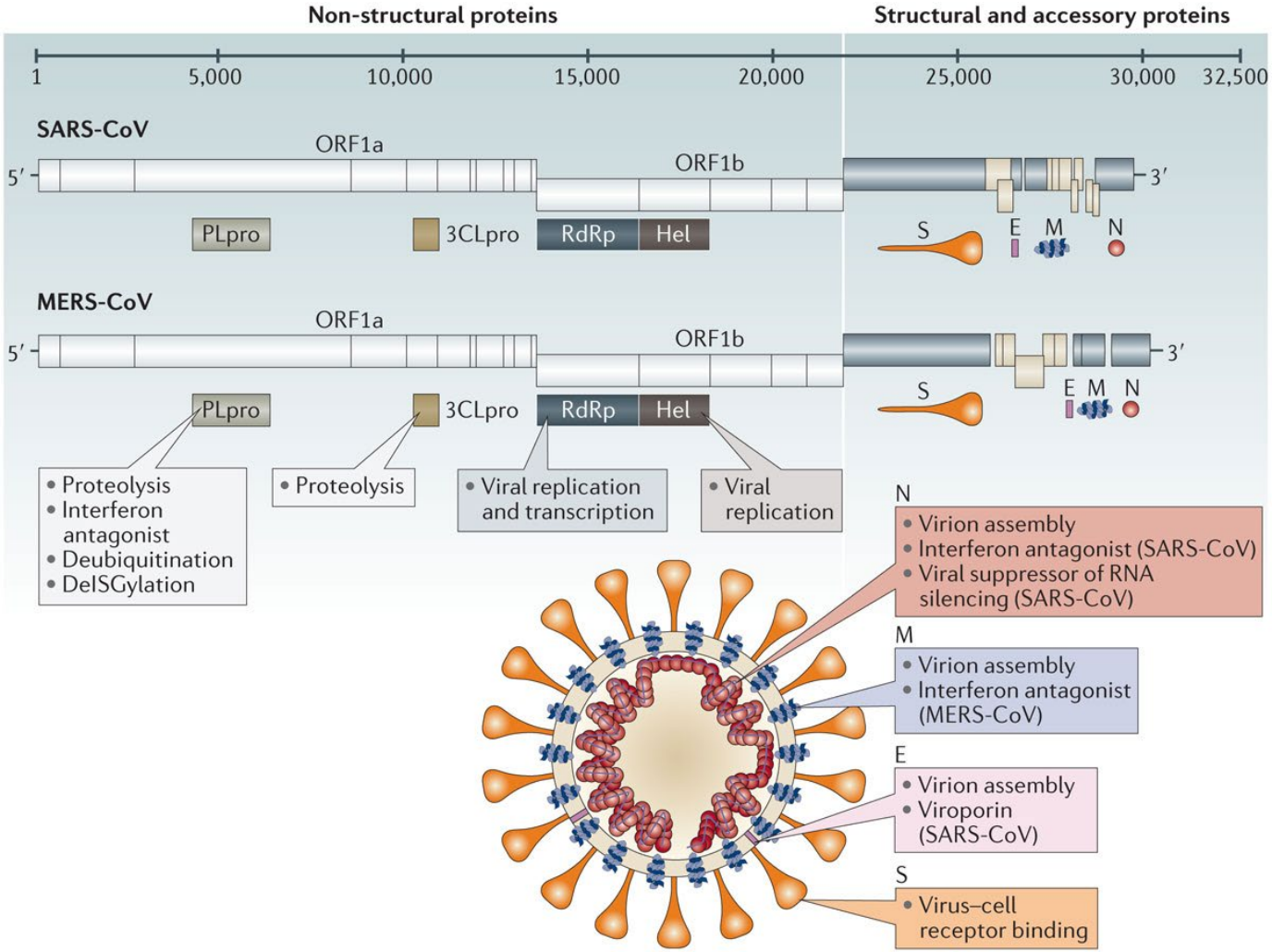

© Marc Adelan, 1985

Virus Reproduction



SARS-CoV-2

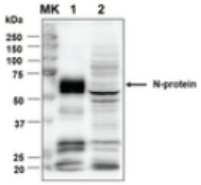

Envelope Protein
Spike Protein
Nucleocapsid
Protein / RNA
Hemagglutinin
Esterase Dimer
Membrane Protein


Non-structural proteins
Structural and accessory proteins
1
5,000
10,000
15,000
20,000
25,000
30,000
32,500
SARS-CoV
ORF1a
ORF1b
5′
3′
PLpro
3CLpro
RdRp
Hel
S
E
M
N
MERS-CoV
ORF1a
ORF1b
5′
3′
PLpro
3CLpro
RdRp
Hel
S
E
M
N
• Proteolysis
• Interferon antagonist
• Deubiquitination
• DelSGylation
• Proteolysis
• Viral replication and transcription
• Viral replication
N
• Virion assembly
• Interferon antagonist (SARS-CoV)
• Viral suppressor of RNA silencing (SARS-CoV)
M
• Virion assembly
• Interferon antagonist (MERS-CoV)
E
• Virion assembly
• Viroporin (SARS-CoV)
S
• Virus–cell receptor binding

COVID-19 Antibodies

Monoclonal & Polyclonal Antibodies to SARS-CoV-2

The antibodies available below have been validated to bind to proteins from SARS-CoV-2 (COVID-19), but were developed originally to target proteins from SARS-CoV-1, the virus responsible for the 2003 outbreak. We are currently developing monoclonal mouse and polyclonal rabbit antibodies specific to SARS-CoV-2 spike and nucleocapsid proteins. The polyclonal antibodies will be available in May. The monoclonal antibodies will be available sometime between July - August.



Rabbit Anti-SARS-CoV-2 Nucleocapsid Protein

Rabbit Anti-SARS-CoV-2 Coronavirus Nucleocapsid Protein

CODE: 128-10165-1

$1,450.00

SELECT SIZE ADD TO COMPARISON LIST

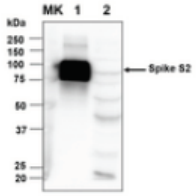
Mouse Anti-SARS-CoV-2 Nucleocapsid Protein

Mouse Anti-SARS-CoV-2 Coronavirus Nucleocapsid protein

CODE: 128-10166-1

$1,450.00

SELECT SIZE ADD TO COMPARISON LIST



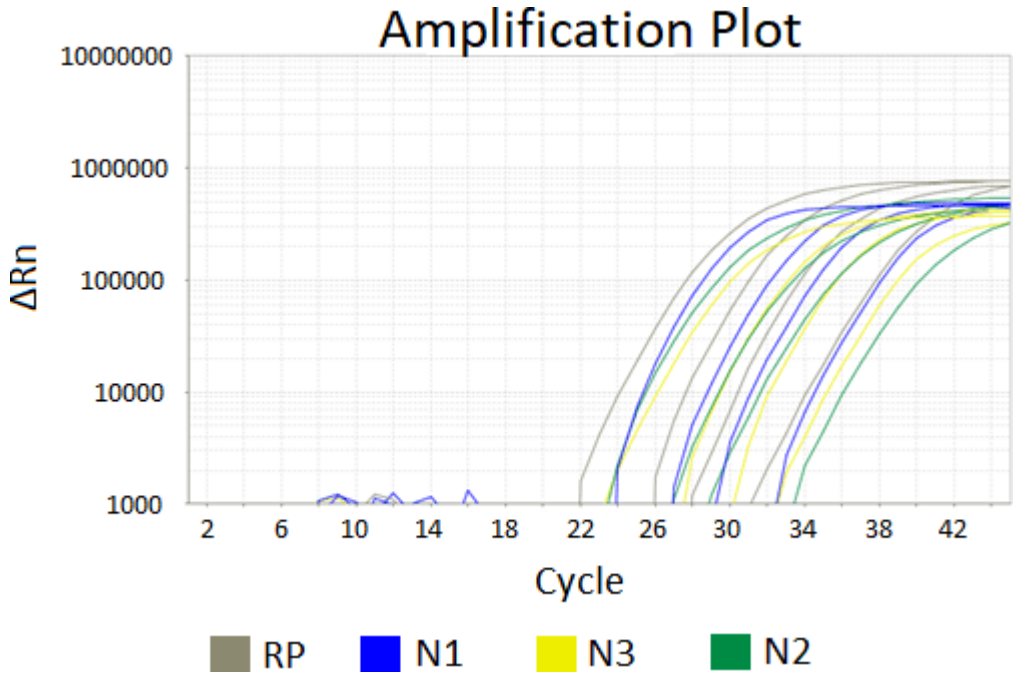
Rabbit Anti-SARS-CoV-2 Spike Protein

Rabbit Anti-SARS-Associated Coronavirus (COVID-19) Spike Protein

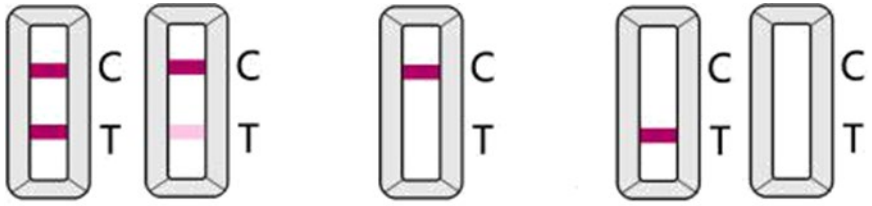
CODE: 128-10168-1

$1,450.00

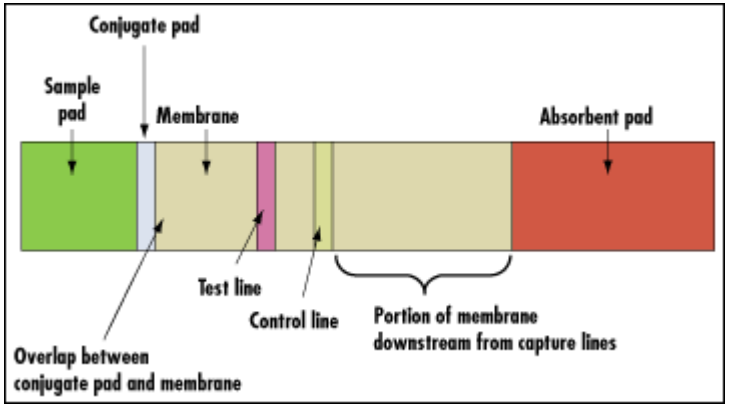
Real-time RT PCR



Fast Screening Kit

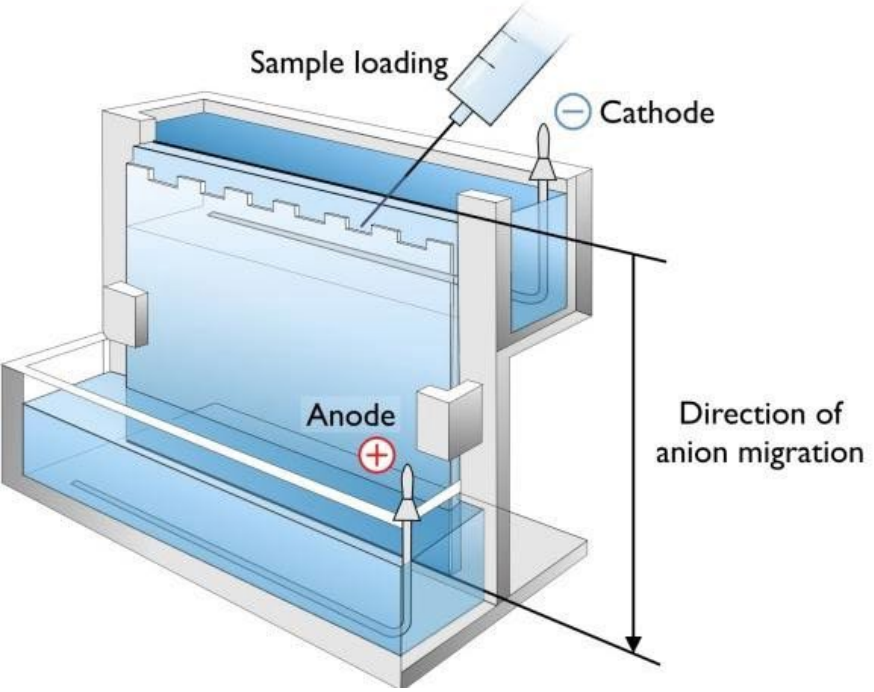


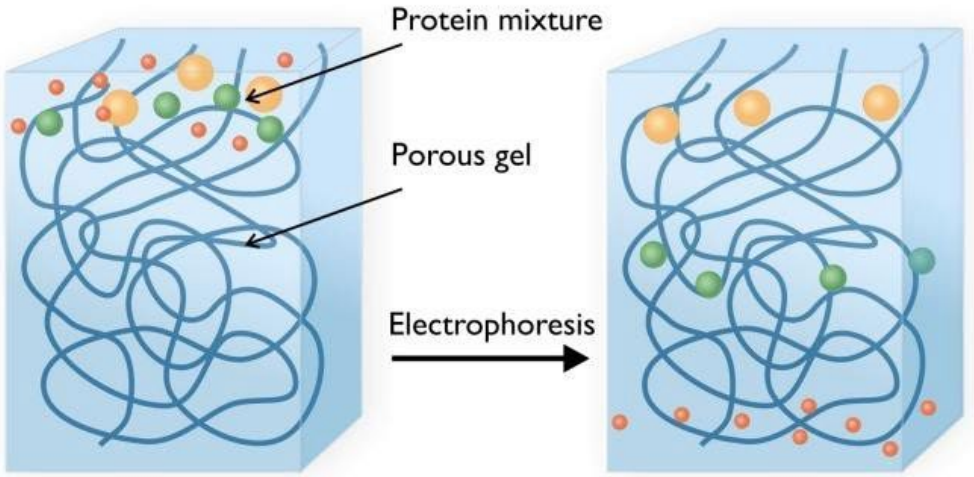
hCG immunoassay



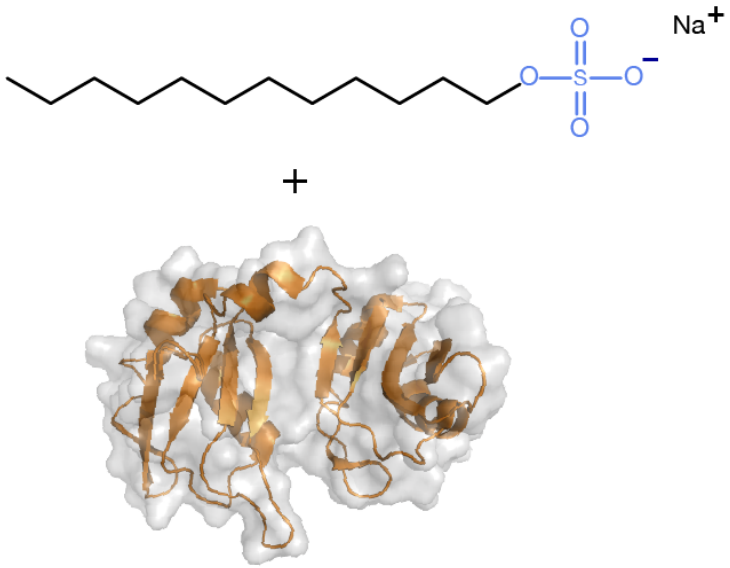
human chorionic gonadotropin (hCG)

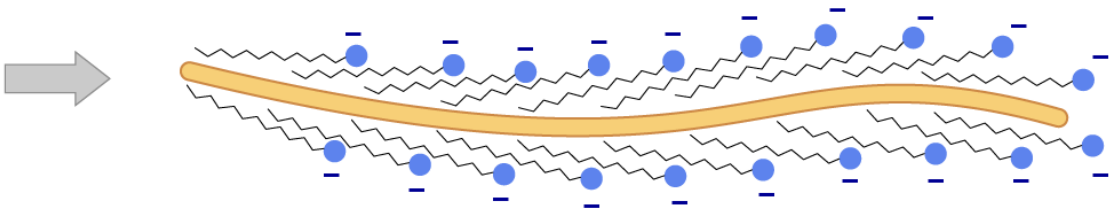
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



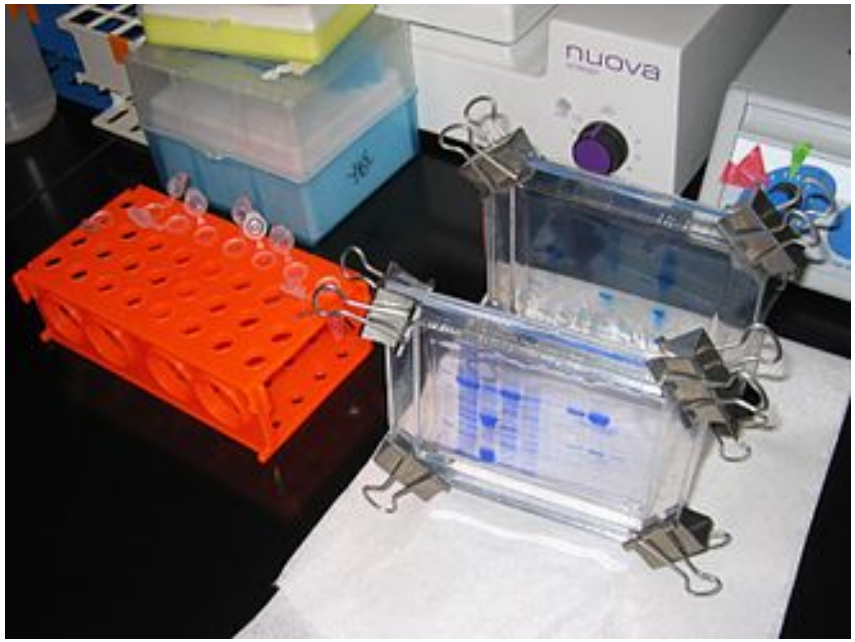


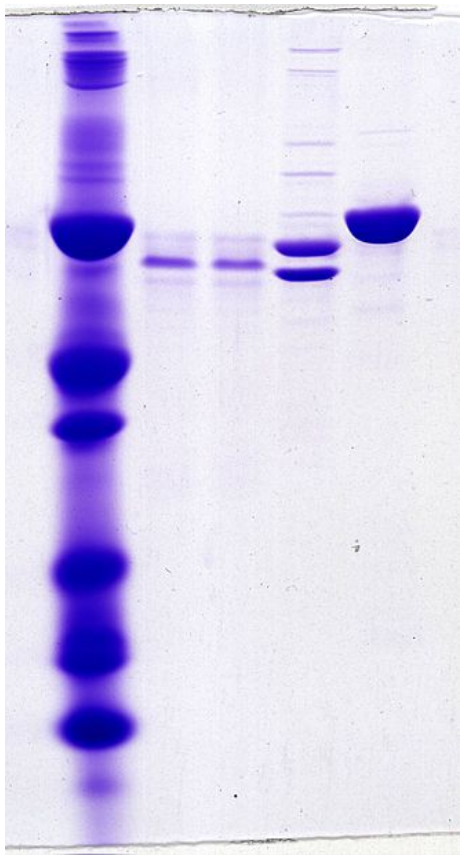
Protein Denature



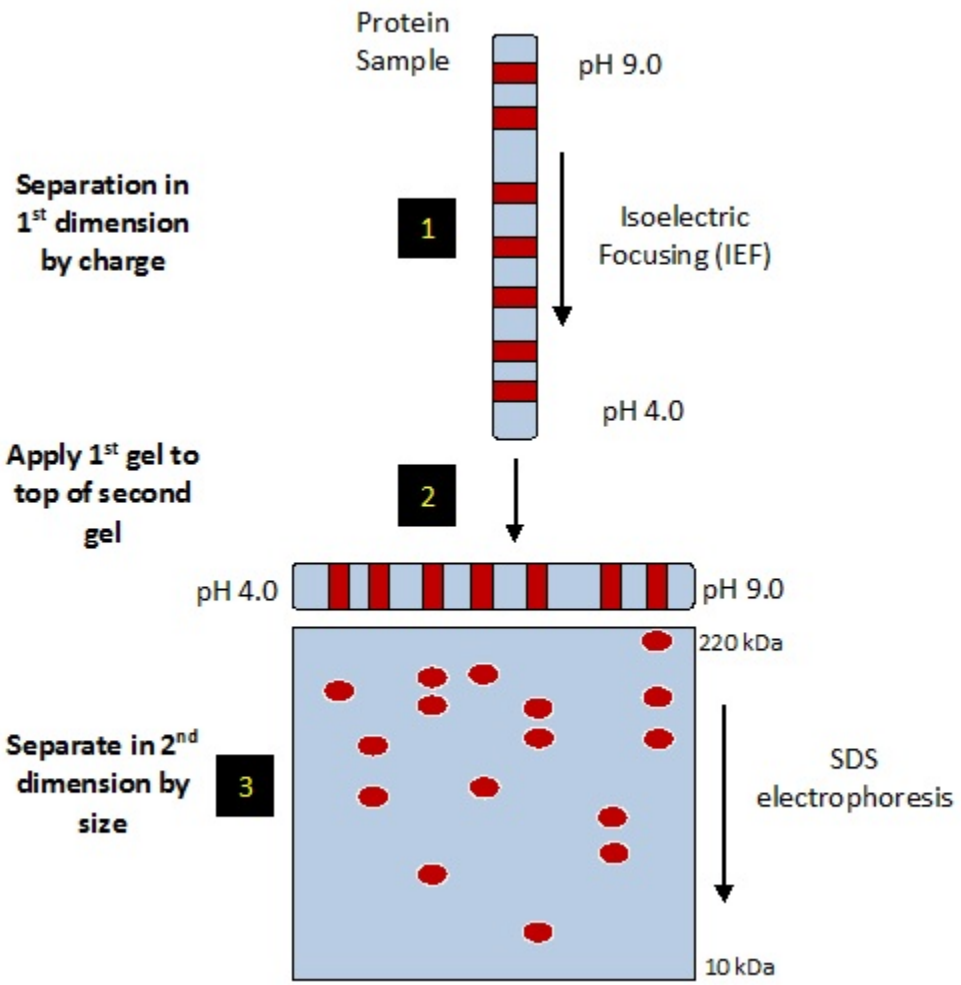


SDS-PAGE

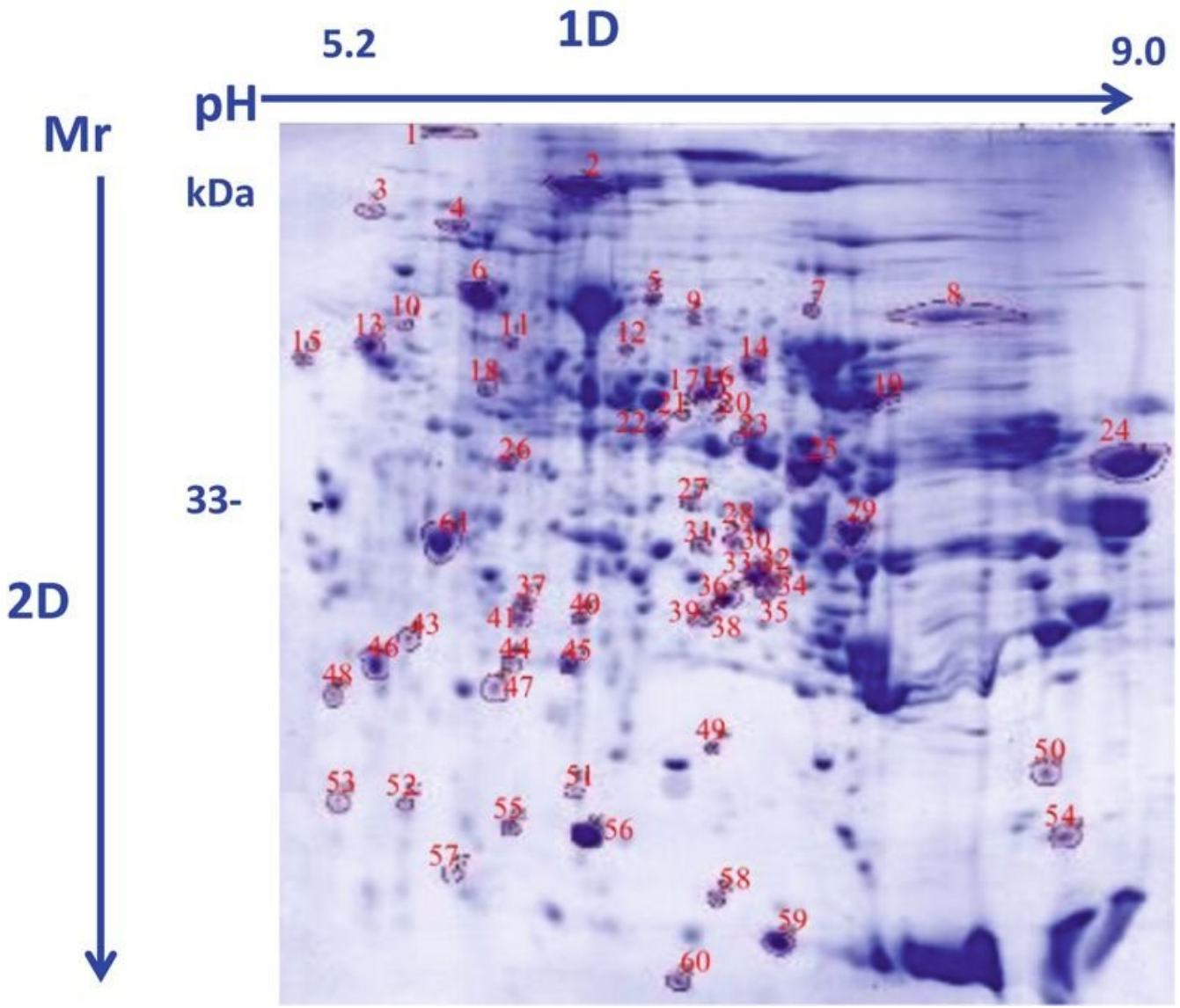


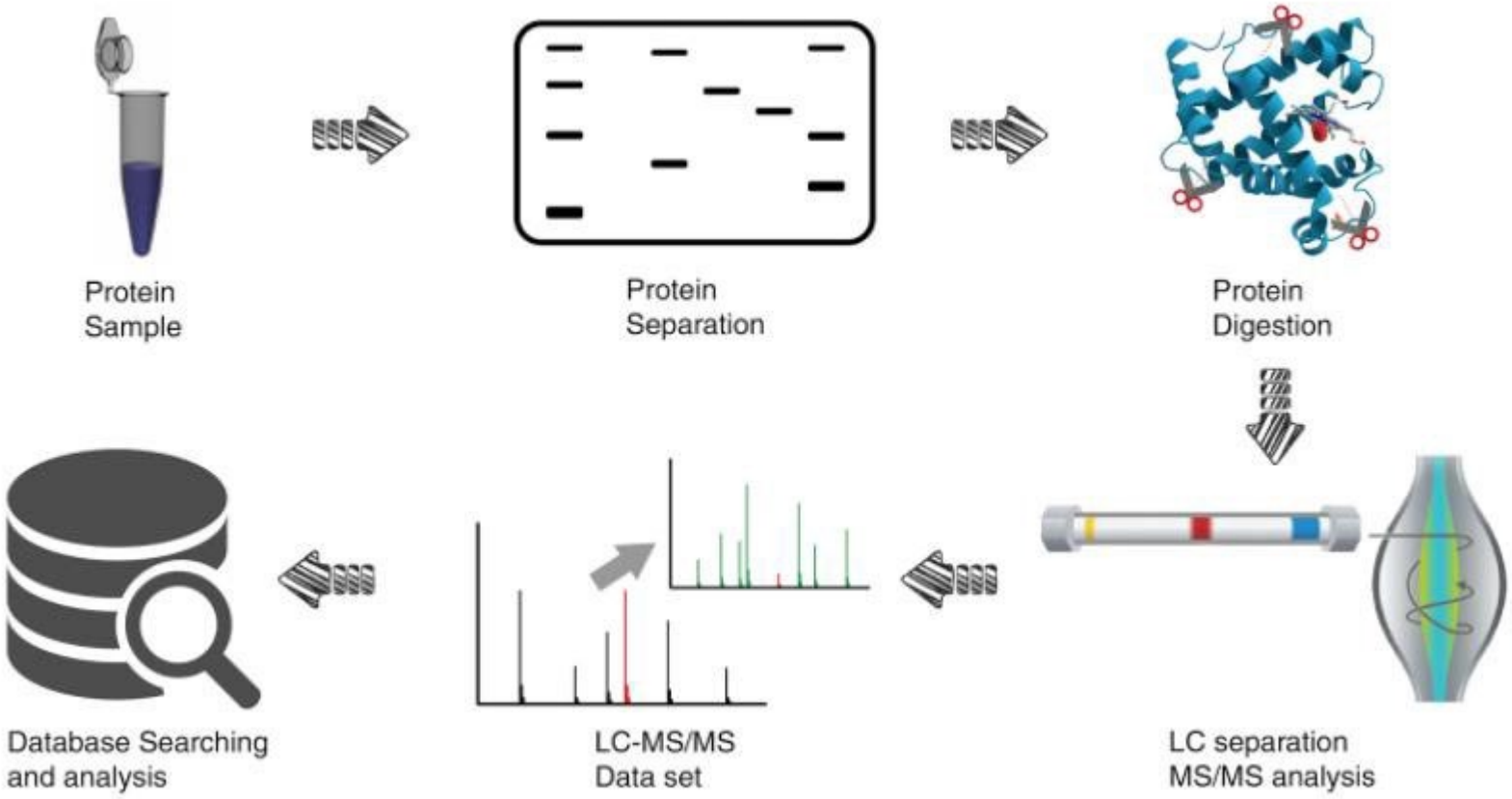


2D PAGE

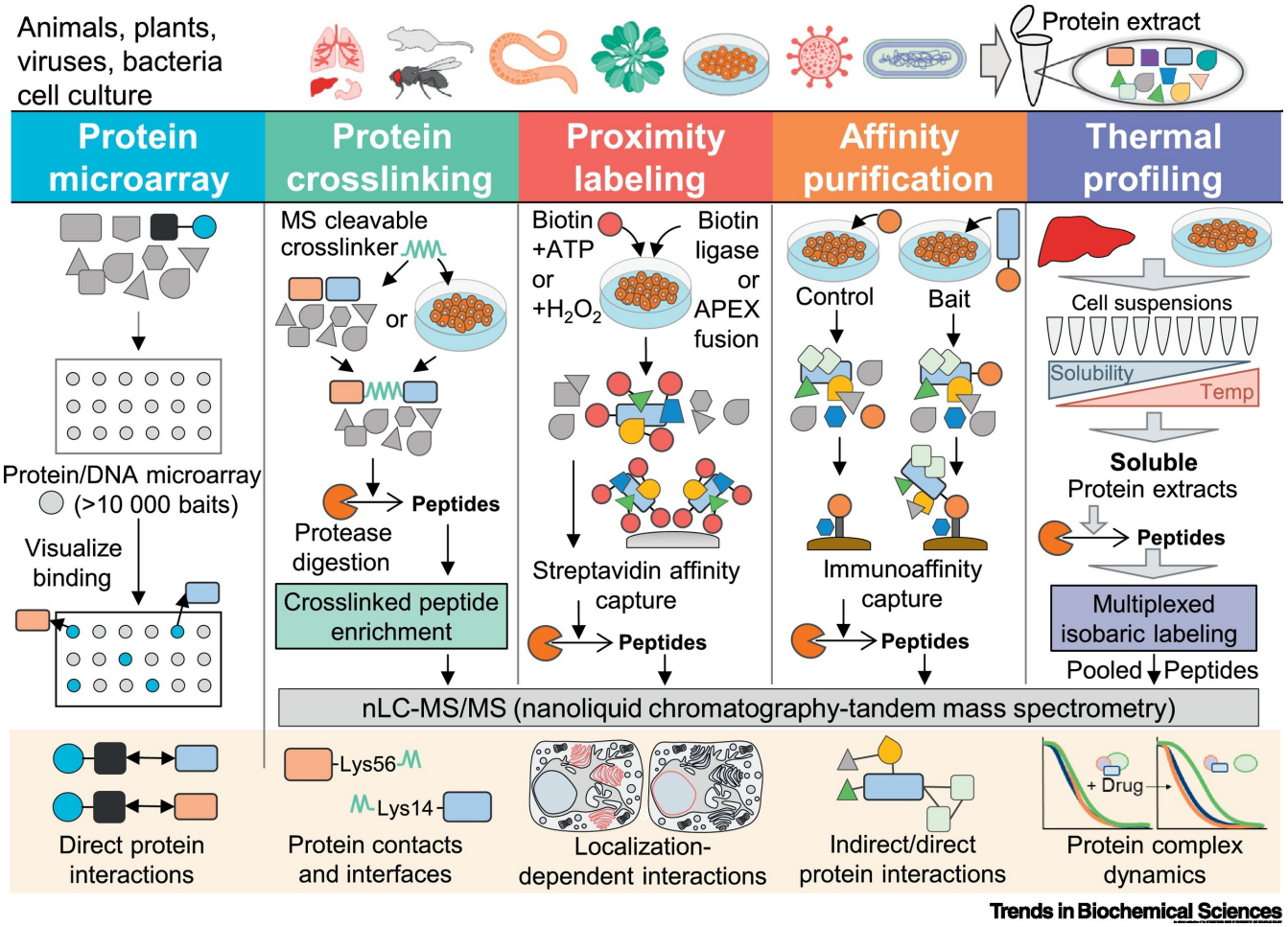

Protein Sample
pH 9.0
Separation in 1st dimension by charge
1
Isoelectric Focusing (IEF)
pH 4.0
Apply 1st gel to top of second gel
2
pH 4.0
pH 9.0
220 kDa
Separate in 2nd dimension by size
3
SDS electrophoresis
10 kDa

2D PAGE




Protein
Sample
Protein
Separation
Protein
Digestion
Database Searching
and analysis
LC-MS/MS
Data set
LC separation
MS/MS analysis

Proteomic



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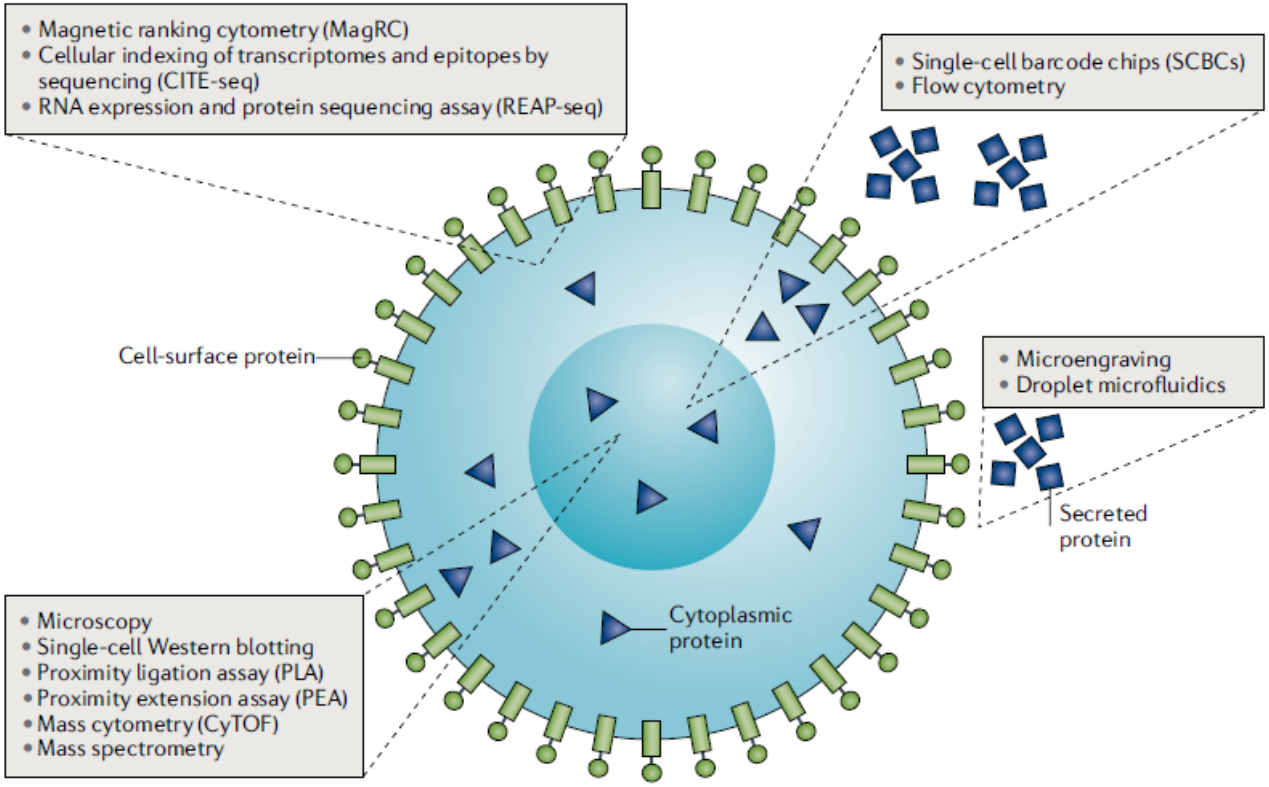
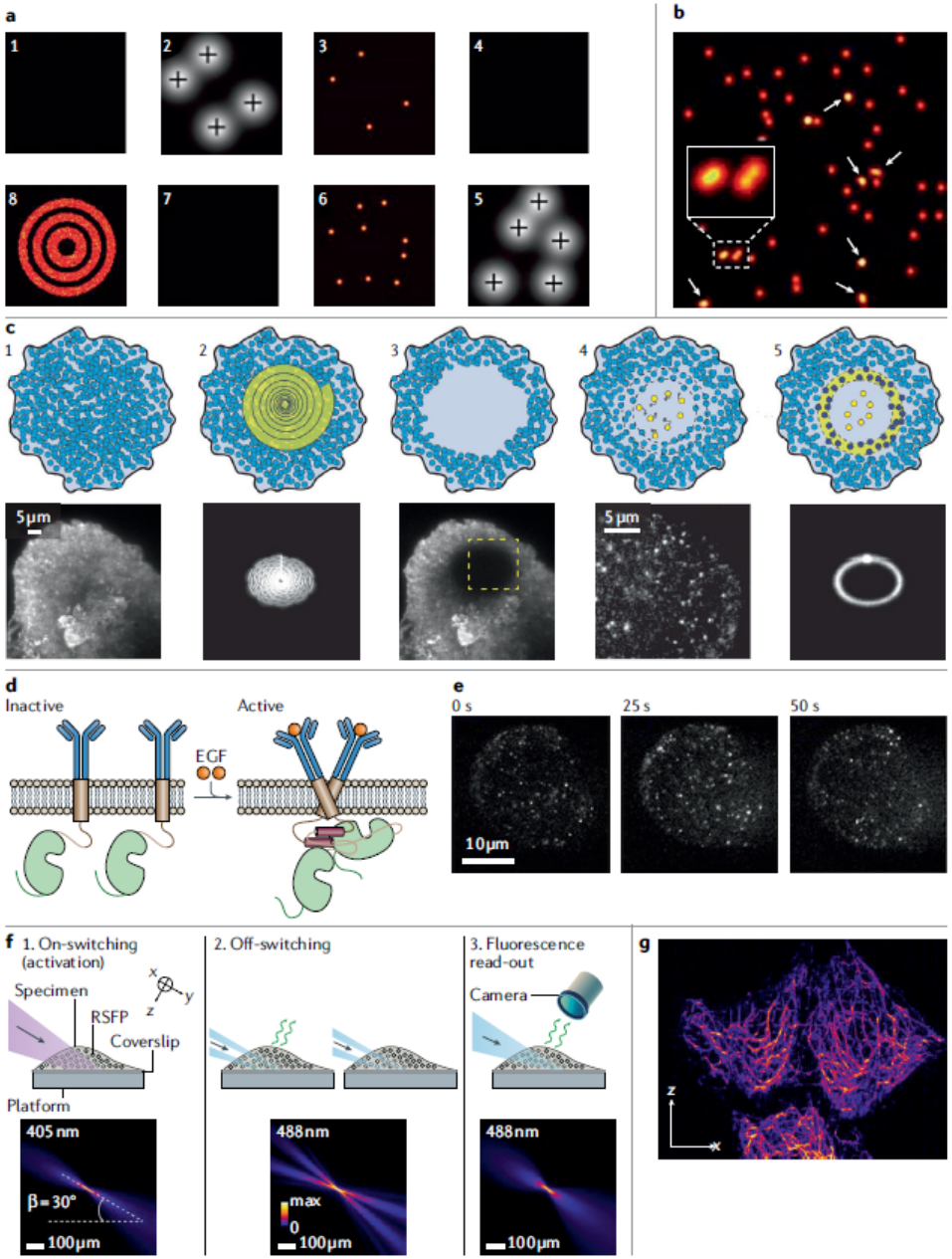
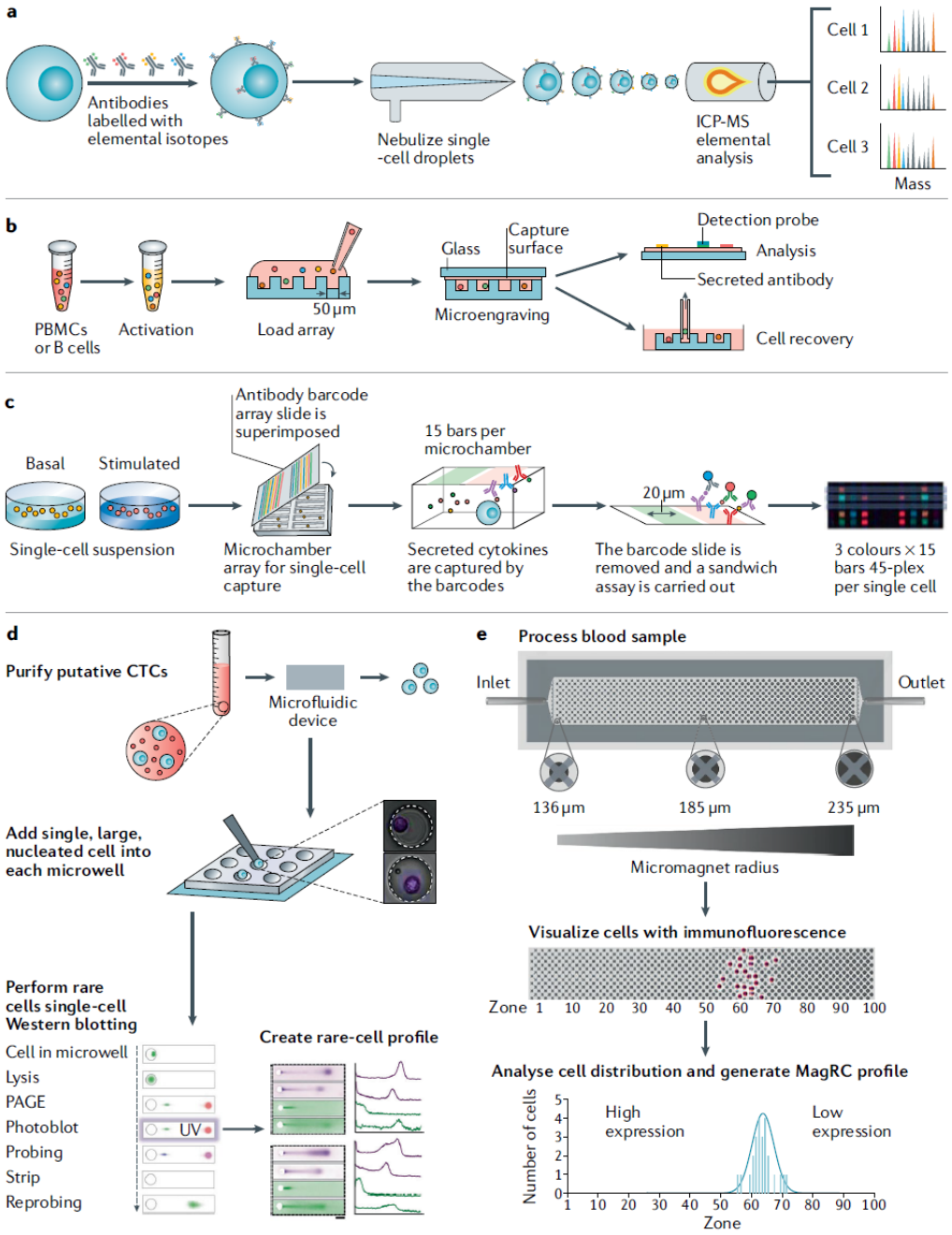
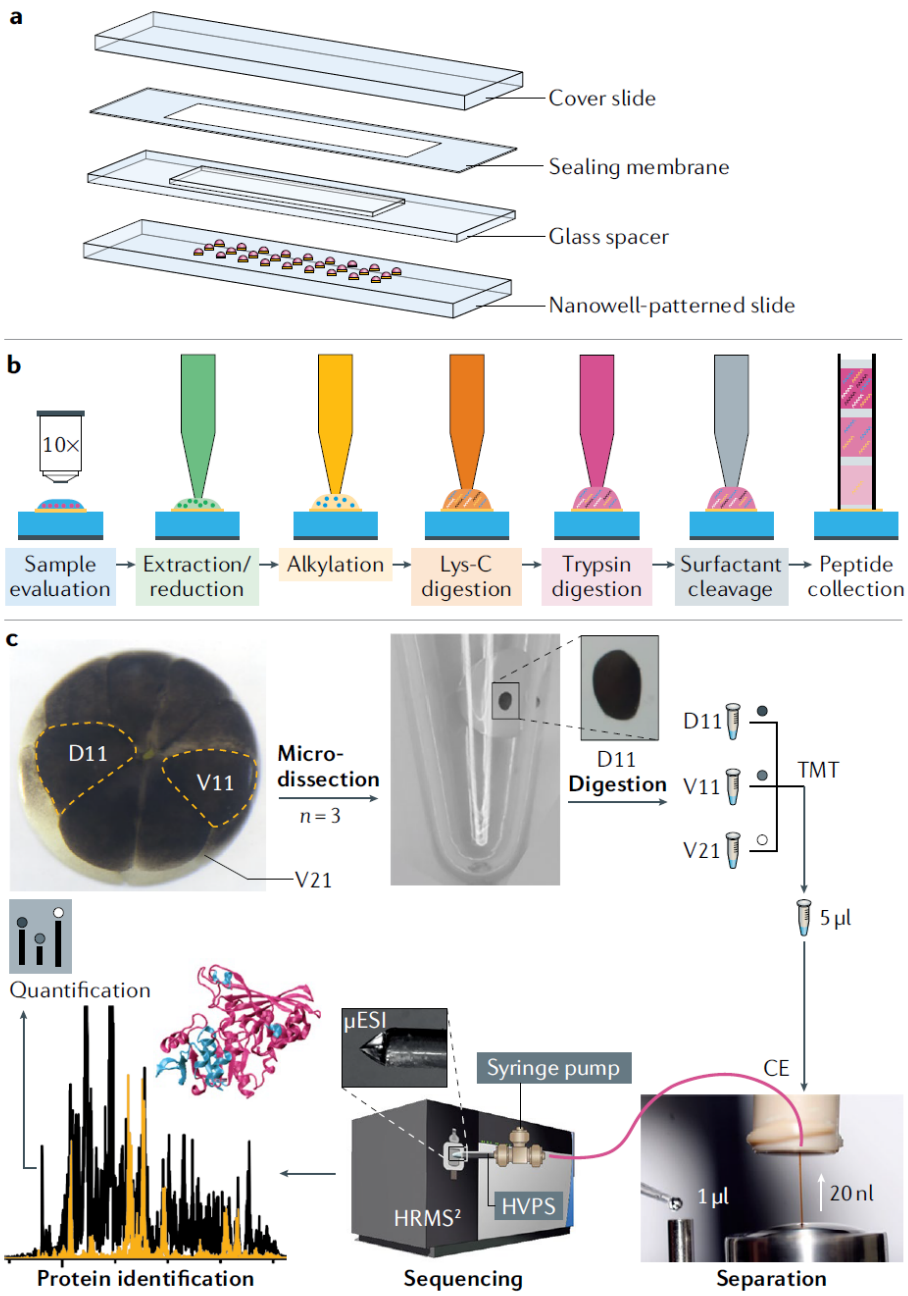
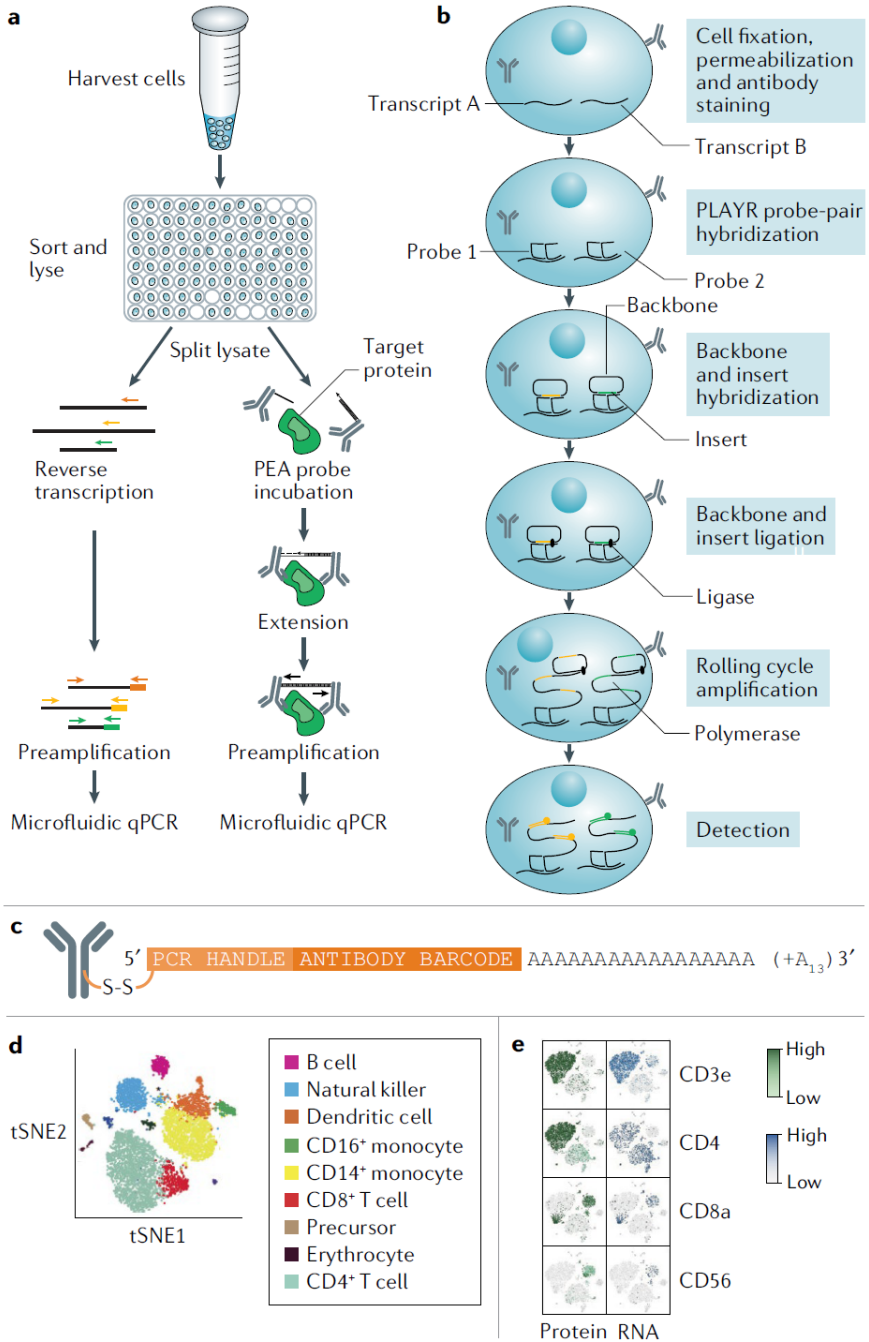


Fig. 1 | **Classification of single-cell protein analysis methods based on the location of target protein.** Cell-surface-protein analysis methods include magnetic ranking cytometry (MagRC), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), and RNA expression and protein sequencing assay (REAP-seq). Methods that can be used for the analysis of cell-surface and cytoplasmic proteins include microscopy, single-cell Western blotting, proximity ligation assay (PLA), proximity extension assay (PEA), mass cytometry (cytometry by time of flight; CyTOF) and mass spectrometry. Methods utilized for secreted-protein analysis include droplet microfluidics and microengraving techniques. Methods used for comprehensive analysis of the three proteins include flow cytometry and single-cell barcode chips (SCBCs).


a
1
2
3
4
8
7
6
5
b
c
1
2
3
4
5
5μm
5 μm
d
Inactive
Active
EGF
e
0 s
25 s
50 s
10μm
f
1. On-switching (activation)
Specimen
RSFP
Coverslip
Platform
x
y
z
2. Off-switching
3. Fluorescence read-out
Camera
405 nm
β=30°
100μm
488nm
max
0
100μm
488nm
100μm
g
z
x


a
Antibodies labelled with elemental isotopes
Nebulize single -cell droplets
ICP-MS elemental analysis
Cell 1
Cell 2
Cell 3
Mass
b
PBMCs or B cells
Activation
50 µm
Load array
Glass
Capture surface
Microengraving
Detection probe
Analysis
Secreted antibody
Cell recovery
c
Basal
Stimulated
Single-cell suspension
Antibody barcode array slide is superimposed
Microchamber array for single-cell capture
15 bars per microchamber
Secreted cytokines are captured by the barcodes
20 µm
The barcode slide is removed and a sandwich assay is carried out
3 colours × 15 bars 45-plex per single cell
d
Purify putative CTCs
Microfluidic device
Add single, large, nucleated cell into each microwell
Perform rare cells single-cell Western blotting
Cell in microwell
Lysis
PAGE
Photoblot
UV
Probing
Strip
Reprobing
Create rare-cell profile
e
Process blood sample
Inlet
Outlet
136 µm
185 µm
235 µm
Micromagnet radius
Visualize cells with immunofluorescence
Zone 1 10 20 30 40 50 60 70 80 90 100
Analyse cell distribution and generate MagRC profile
Number of cells
High expression
Low expression
Zone


a
Cover slide
Sealing membrane
Glass spacer
Nanowell-patterned slide
b
10×
Sample evaluation
Extraction/ reduction
Alkylation
Lys-C digestion
Trypsin digestion
Surfactant cleavage
Peptide collection
c
D11
V11
V21
Micro-dissection
n = 3
D11 Digestion
D11
V11
V21
TMT
5 µl
Quantification
µESI
Syringe pump
CE
HRMS²
HVPS
1 µl
20 nl
Protein identification
Sequencing
Separation


a
Harvest cells
Sort and lyse
Split lysate
Target protein
Reverse transcription
PEA probe incubation
Extension
Preamplification
Preamplification
Microfluidic qPCR
Microfluidic qPCR
b
Transcript A
Transcript B
Cell fixation, permeabilization and antibody staining
Probe 1
Probe 2
PLAYR probe-pair hybridization
Backbone
Backbone and insert hybridization
Insert
Backbone and insert ligation
Ligase
Rolling cycle amplification
Polymerase
Detection
c
5′
PCR HANDLE
ANTIBODY BARCODE
AAAAAAAAAAAAAAAAA (+A13) 3′
S-S
d
tSNE2
tSNE1
B cell
Natural killer
Dendritic cell
CD16+ monocyte
CD14+ monocyte
CD8+ T cell
Precursor
Erythrocyte
CD4+ T cell
e
CD3e
CD4
CD8a
CD56
High
Low
High
Low
Protein
RNA

ARTICLE

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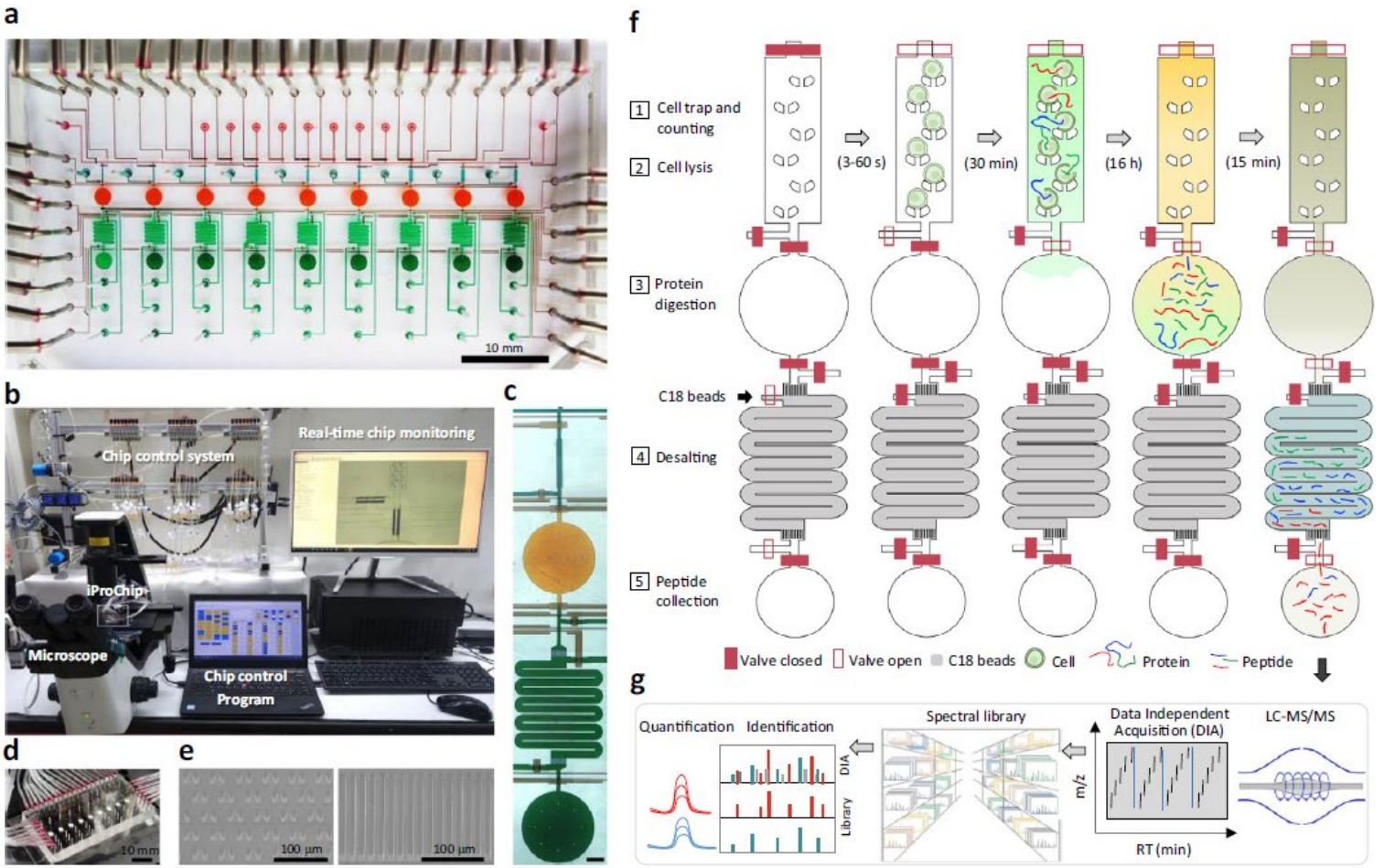
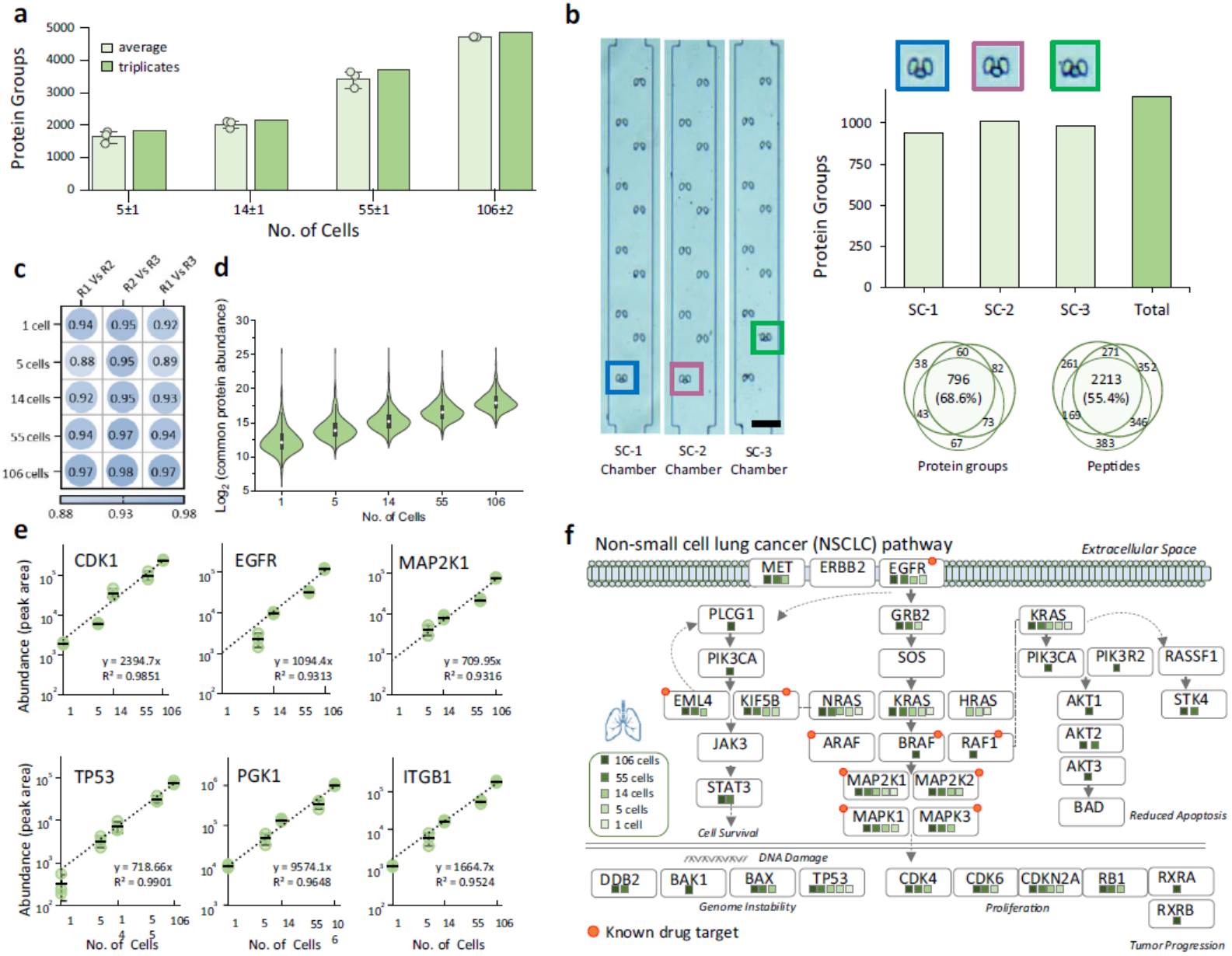
Streamlined single-cell proteomics by an integrated microfluidic chip and data-independent acquisition mass spectrometry

Sofani Tafesse Gebreyesus[1,2,3,9], Asad Ali Siyal[1,4,5,9], Reta Birhanu Kitata[1], Eric Sheng-Wen Chen[1], Bayarmaa Enkhbayar[4,6], Takashi Angata[6], Kuo-I Lin[7], Yu-Ju Chen[1,3,4,8✉] & Hsiung-Lin Tu[1,2,4,8✉]




a
Protein Groups
average
triplicates
No. of Cells
5±1
14±1
55±1
106±2
b
SC-1 Chamber
SC-2 Chamber
SC-3 Chamber
Protein Groups
SC-1
SC-2
SC-3
Total
796 (68.6%)
2213 (55.4%)
Protein groups
Peptides
c
R1 Vs R2
R2 Vs R3
R1 Vs R3
1 cell
5 cells
14 cells
55 cells
106 cells
d
Log2 (common protein abundance)
No. of Cells
e
CDK1
EGFR
MAP2K1
TP53
PGK1
ITGB1
Abundance (peak area)
No. of Cells
f
Non-small cell lung cancer (NSCLC) pathway
Extracellular Space
MET
ERBB2
EGFR
PLCG1
GRB2
KRAS
PIK3CA
SOS
PIK3CA
PIK3R2
RASSF1
EML4
KIF5B
NRAS
KRAS
HRAS
AKT1
STK4
JAK3
ARAF
BRAF
RAF1
AKT2
AKT3
STAT3
MAP2K1
MAP2K2
BAD
MAPK1
MAPK3
Reduced Apoptosis
Cell Survival
DNA Damage
DDB2
BAK1
BAX
TP53
CDK4
CDK6
CDKN2A
RB1
RXRA
RXRB
Genome Instability
Proliferation
Tumor Progression
Known drug target