"Enzymes"

I. Overview

- Virtually all body reactions are mediated by enzymes.
- Enzymes are protein catalysts, usually intracellular.
- They increase reaction rates without being changed in the overall process.
- Among many energetically possible biologic reactions, enzymes selectively channel substrates into useful pathways.
- Enzymes direct all metabolic events.
- This chapter examines the nature and mechanisms of enzymes.

II. Nomenclature

A. Enzyme Names

- Each enzyme has two names:

    a. Recommended name (short, everyday use)
    b. Systematic name (complete, unambiguous identification)

B. Recommended Name

- Most enzyme names end with suffix "-ase", attached to the substrate, e.g.,
    - Glucosidase
    - Urease
- Some names describe the action performed, e.g.,
    - Lactate dehydrogenase (LDH)
    - Adenylyl cyclase
- Some enzymes retain trivial names without indication of reaction, e.g.,
    - Trypsin
    - Pepsin

C. Systematic Name

- Systematic names reflect the chemical reaction catalyzed, including all substrate names.

- The suffix "-ase" is attached to a full description, e.g.,
    - Lactate:nicotinamide adenine dinucleotide (NAD^+) oxidoreductase
- Each enzyme is assigned a classification number, e.g.,
    - Lactate:NAD^+ oxidoreductase = 1.1.1.27
- Systematic names are unambiguous and informative but often cumbersome for general use.
- Enzymes are divided into six major classes, each with numerous subgroups.

II. Potentially Confusing Enzyme Nomenclature

- Enzymes with similar names but different functions/mechanisms:
    - Synthetases
        - Require ATP for their action.
    - Synthases
        - Do not require ATP.
    - Phosphatases
        - Use water to remove a phosphate group.

- Phosphorylases
  - Use inorganic phosphate to break a bond and generate a phosphorylated product.
- Dehydrogenases
  - Use NAD^+ or flavin adenine dinucleotide (FAD) as electron acceptors in redox reactions.
- Oxidases
  - Use oxygen as electron acceptor without incorporating oxygen atoms into the substrate.
- Oxygenases
  - Incorporate oxygen atoms into their substrates.

III. Properties

- An enzyme is an efficient, specific protein catalyst.
- It combines with a substrate at the enzyme active site.
- Performs chemistry on the substrate to convert it to product.

- Without enzymes, most biochemical reactions would be too slow to have physiologic importance in the human body.

- Enzymes increase the velocity of chemical reactions but are not consumed during the reaction.

- Some RNAs can catalyze reactions affecting phosphodiesterase and peptide bonds.

    - RNAs with catalytic activity are called ribozymes.
    - Ribozymes are much less common than protein catalysts.

A. Active Site

- Enzyme molecules contain a special pocket or cleft called the active site.

- The active site is formed by folding of the protein.

- Contains amino acid residues whose side chains participate in:

    - Substrate binding
    - Catalysis

- The substrate binds the enzyme forming an enzyme-substrate (ES) complex.

- Binding causes a conformational change in the enzyme (induced fit model).

- This allows rapid conversion of ES to enzyme-product (EP) complex.

- EP complex subsequently dissociates into free enzyme + product.

B. Efficiency

- Enzyme-catalyzed reactions proceed 103 to 108 times faster than uncatalyzed reactions.

- The number of substrate molecules converted to product per enzyme molecule per second is the turnover number (kcat).

- Typical kcat values are 10^2 to 10^4 s^{-1}.

- Note: kcat is the rate constant for conversion of ES to E + P.

C. Specificity

- Enzymes are highly specific.
- Capable of interacting with one or very few substrates.
- Can catalyze only one type of chemical reaction.
- The set of enzymes synthesized within a cell determines which reactions occur in that cell.

D. Holoenzymes, Apoenzymes, Cofactors, and Coenzymes

- Some enzymes require nonprotein components for enzymatic activity.
- Holoenzyme = protein component + nonprotein component (active enzyme).
- Apoenzyme = enzyme without its nonprotein moiety (inactive enzyme).
- Nonprotein components must be present for catalytic function.

- Types of nonprotein components:
    - Cofactor: metal ion (e.g., zinc Zn^{2+}, iron Fe^{2+}).
    - Coenzyme: small organic molecule.
- Coenzymes (cosubstrates):
    - Bind transiently to the enzyme.
    - Dissociate in an altered state (example: NAD^+).
- Prosthetic group:
    - Coenzyme permanently bound to enzyme.
    - Returned to original form after reaction (example: FAD).
- Coenzymes are often vitamin-derived:
    - NAD^+ contains niacin.
    - FAD contains riboflavin.

E. Regulation

- Enzyme activity can be increased or decreased.
- Allows rate of product formation to match cellular needs.

F. Location within the Cell

- Most enzymes act inside cells, confined by plasma membranes.
- Many enzymes are localized to specific organelles
- Compartmentalization benefits:
    - Isolates substrate/product from competing reactions.
    - Creates a favorable environment for reactions.
    - Organizes enzymes into specific metabolic pathways.

IV. Mechanism of Enzyme Action

- Enzyme action can be understood from two perspectives:
    - Energy changes during the reaction.
    - Chemical facilitation by the active site.

A. Energy Changes Occurring During the Reaction

- All chemical reactions have an energy barrier between reactants and products called the activation energy (Ea).

- Activation energy (Ea):

    - Energy difference between reactants and a high-energy intermediate called the transition state (T*).
    - Transition state is a short-lived, high-energy intermediate formed during conversion from reactant to product.

- Reaction pathway:

$$A \leftrightarrow T* \leftrightarrow B$$

where A = reactant, T* = transition state, B = product.

- Activation energy significance:

    - Ea represents the peak of energy on the reaction coordinate.
    - High Ea causes slow rates for uncatalyzed reactions.

- Rate of reaction:
    - Molecules must have sufficient energy to overcome Ea to react.
    - Without enzymes, only a small fraction of molecules reach the transition state.
    - The rate depends on the number of molecules with enough energy to surpass Ea.
    - Lower Ea → more molecules can cross transition state → faster reaction rate.

3. Alternate Reaction Pathway

- Enzymes provide an alternate reaction pathway with a lower activation energy (Ea).
- This allows reactions to proceed rapidly under cellular conditions.
- Enzymes do not change the free energy of reactants or products.
- Therefore, enzymes do not alter the equilibrium of the reaction.
- Enzymes accelerate the rate at which equilibrium is reached.

B. Active Site Chemistry

- The active site is a complex molecular machine, not just a substrate binding pocket.
- It uses diverse chemical mechanisms to facilitate substrate → product conversion.
- Factors contributing to catalytic efficiency include:

1. Transition-State Stabilization

- Active site acts as a flexible molecular template binding substrate and initiating conversion to the transition state (T*).
- Transition state has bond structures different from substrate or product.
- By stabilizing the transition state, the enzyme increases the concentration of the reactive intermediate.
- This stabilization accelerates the reaction.
- Note: The transition state cannot be isolated experimentally.

2. Catalysis

- The active site provides catalytic groups that increase the probability of transition state formation.
- Some enzymes use general acid–base catalysis: amino acid residues donate or accept protons.
- Other enzymes catalyze via transient covalent enzyme–substrate (ES) complexes.
- Example: Chymotrypsin (protein digestion enzyme in intestine) uses:
    - General base catalysis: Histidine gains a proton.
    - General acid catalysis: Histidine loses a proton.
    - Covalent catalysis: Serine forms a transient covalent bond with substrate.
- Histidine's pK near physiologic pH allows it to switch between protonated and deprotonated states.

3. Transition-State Visualization

- Enzyme-catalyzed substrate → product conversion likened to removing a sweater from an uncooperative infant.

- High activation energy because both arms must be fully extended over the head, an unlikely substrate conformation without enzyme.

- Enzyme acts like a parent:

    - Binds substrate (forming ES complex).
    - Guides substrate to adopt extended arm posture (transition state).

- This conformational change facilitates reaction to product (disrobed baby).

- Note: ES complex is at a slightly lower energy than free substrate (explains small dip in energy curve at ES).

V. Factors Affecting Reaction Velocity

Enzymes show different responses to substrate concentration, temperature, and pH—both in vitro and in vivo.

A. Substrate Concentration

1. Maximal Velocity (Vmax)

- Reaction velocity (v) = number of substrate molecules converted to product per unit time.
    - Expressed as: µmol of product/second.
- As substrate concentration increases, the reaction rate increases — up to a point.
- At high [S], all enzyme active sites are occupied, so the enzyme becomes saturated.
- Once saturation occurs, further increases in [S] do not increase the rate → velocity reaches Vmax.
- Vmax reflects the point where all enzyme molecules are bound to substrate.

2. Shape of the Enzyme Kinetics Curve

- Most enzymes follow Michaelis–Menten kinetics:
    - Plot of initial velocity (v_0) vs. substrate concentration = hyperbolic curve (like myoglobin's O_2 curve).
- Allosteric enzymes show a sigmoidal curve (like hemoglobin's O_2 curve):
    - Indicates cooperative binding and regulation.

B. Temperature

1. Velocity Increases with Temperature

- As temperature increases, more substrate molecules have sufficient energy to overcome activation energy (Ea).
- Velocity increases until it reaches a peak.

2. Velocity Decreases at High Temperature

- Higher temperatures denature enzymes, decreasing activity.
- Optimum for human enzymes: ~35°C–40°C.
    - Start to denature >40°C.
- Thermophilic bacterial enzymes: optimum ≈ 70°C.

C. pH

1. Effect on Active Site Ionization

- Proper ionization of functional groups (e.g. $-NH_3^+$) is critical.
- pH affects binding and catalysis.
    - E.g. deprotonation at high pH → loss of activity.

2. Effect on Enzyme Denaturation

- Extremely low or high pH disrupts ionic interactions → denatures protein structure.

3. Variable pH Optimum

- Each enzyme has a specific pH for optimal activity, depending on its location:
    - Pepsin (stomach) → optimal at pH 2.
    - Neutral pH enzymes → inactive in acidic pH.

VI. Michaelis-Menten Kinetics:

- Proposed in 1913 by Leonor Michaelis and Maud Menten.
- Explains most enzyme-catalyzed reactions involving a single substrate.

Reaction Model

$E + S \rightleftharpoons ES \rightarrow E + P$

- E = Enzyme
- S = Substrate
- ES = Enzyme-substrate complex
- P = Product
- k_1 = rate constant for $E + S \rightarrow ES$
- k_{-1} = rate constant for $ES \rightarrow E + S$
- k_2 (or kcat) = rate constant for $ES \rightarrow E + P$

A. Michaelis-Menten Equation

$v_0 = (Vmax \times [S]) / (Km + [S])$

- v_0 = Initial velocity of the reaction
- Vmax = Maximum velocity = kcat × [E]total
- Km = Michaelis constant = $(k_{-1} + k_2) / k_1$
- [S] = Substrate concentration

Assumptions of Michaelis-Menten Model

1. Enzyme and Substrate Relative Concentrations

- [S] ≫ [E], so only a small fraction of substrate is enzyme-bound.

2. Steady-State Assumption

- Concentration of ES complex remains constant during the reaction.
- Rate of ES formation = rate of ES breakdown.
- Applies to intermediates in any pathway where synthesis rate = degradation rate.

3. Initial Velocity (v_0)

- v_0 is measured immediately after mixing enzyme and substrate.
- At this early stage, product concentration is negligible, so reverse reaction (P → S) can be ignored.
- Initial velocity allows clearer analysis of forward reaction kinetics.

B. Important Conclusions from Michaelis-Menten Kinetics

1. Km Characteristics (Michaelis constant)

- Km is unique for each enzyme-substrate pair.
- Reflects enzyme's affinity for the substrate.
- Defined as the substrate concentration at which v = ½ Vmax.
- Km is independent of enzyme concentration.

a. Low Km

- Indicates high affinity → enzyme binds substrate easily.
- Low [S] is sufficient to reach half Vmax.

b. High Km

- Indicates low affinity → more [S] needed for half-saturation.

2. Velocity Relation to Enzyme Concentration

- When [S] is not limiting, $v_0 \propto [E]$.
- Halving [E] → both v_0 and Vmax are halved.

3. Reaction Order

a. First-Order Kinetics

- When $[S] \ll Km$, velocity $\propto [S]$.
- Reaction is first-order with respect to substrate.

b. Zero-Order Kinetics

- When [S] ≫ Km, velocity = Vmax.
- Reaction is zero-order → independent of [S] due to enzyme saturation.

C. Lineweaver-Burk Plot (Double-Reciprocal Plot)

- Developed in 1934 by Hans Lineweaver and Dean Burk.
- Overcomes limitations of hyperbolic curve by using reciprocals.

Lineweaver-Burk Equation:

$1/v_0 = (Km/Vmax) \times (1/[S]) + 1/Vmax$

- Plot: $1/v_0$ vs $1/[S]$ → yields a straight line.
- x-intercept = $-1/Km$
- y-intercept = $1/Vmax$
- Slope = $Km/Vmax$
- Useful for:
    - Calculating Km and Vmax
    - Identifying types of enzyme inhibition

VII. Enzyme Inhibition

Definition

- Any substance that decreases the velocity of an enzyme-catalyzed reaction.

- Two major types:

➤ Irreversible inhibition
➤ Reversible inhibition

A. Irreversible Inhibition

- Inhibitor forms covalent bonds with the enzyme.

- Permanent inactivation of enzyme function.

- Example: Lead (Pb^{2+})

    - Covalently binds to the sulfhydryl (-SH) group of cysteine residues.
    - Ferrochelatase, an enzyme in heme synthesis, is irreversibly inhibited by lead.

B. Reversible Inhibition

- Inhibitor binds via noncovalent interactions (e.g., hydrogen bonds, ionic bonds).
- Can form enzyme–inhibitor complex (EI).
- Enzyme activity can be restored by dilution or removal of inhibitor.
- Two major types:

➤ Competitive inhibition
➤ Noncompetitive inhibition

A. Competitive Inhibition

- Inhibitor competes with the substrate for the active site.
- Binds reversibly to the same site as the substrate.

I. Effect on Vmax

- Vmax remains unchanged.
- Because high [S] can outcompete the inhibitor and restore full enzyme activity.

2. Effect on Km

- Apparent Km increases.
- More substrate is required to reach ½ Vmax, indicating a decrease in enzyme affinity for the substrate in the presence of inhibitor.

3. Effect on Lineweaver-Burk Plot

- Inhibited and uninhibited plots intersect on the Y-axis at 1/Vmax → Vmax is unchanged.
- The X-intercept changes:
    - Inhibited plot shifts leftward (-1/Km moves closer to zero).
    - Indicates an increase in apparent Km.

- Competitive inhibitors increase slope of Lineweaver-Burk plot:

➤ Slope = Km/Vmax ↑

- Transition state analogs:
    - Stable molecules that mimic the transition state.
    - Bind more tightly than the actual substrate.
    - Act as potent competitive inhibitors.

4. Example: Statin Drugs

- Statins (e.g., atorvastatin, pravastatin) are competitive inhibitors.
- Inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis.
- They are structural analogs of the natural substrate.
- Reduce de novo cholesterol synthesis → lower blood cholesterol levels.

B. Noncompetitive Inhibition

Mechanism

- Inhibitor binds to a site different from the substrate-binding site.

- Can bind:

➤ To free enzyme (E)
➤ Or to the enzyme-substrate complex (ES)

- Binding prevents the catalytic activity, regardless of whether the substrate is bound.

Effect on Vmax and Km

- Vmax decreases → enzyme's overall capacity is reduced.

- Km remains unchanged → substrate binding affinity is not affected.

- Lineweaver-Burk plot:

➤ Same X-intercept (-1/Km)
➤ Higher Y-intercept (1/Vmax)

1. Effect on Vmax

- Vmax decreases.
- Effect cannot be reversed by increasing substrate concentration.
- Indicates reduced overall enzyme catalytic capacity.

2. Effect on Km

- Km remains unchanged.
- Substrate binding is not affected, as inhibitor binds elsewhere (not at active site).
- Enzyme shows same affinity for substrate in presence of inhibitor.

3. Effect on Lineweaver-Burk Plot

- Plots show:

➤ Same X-intercept (-1/Km) → Km unchanged
➤ Higher Y-intercept (1/Vmax) → Vmax decreased

- Useful to distinguish noncompetitive inhibition from competitive inhibition.

C. Enzyme Inhibitors as Drugs

1. β-Lactam Antibiotics

- Include penicillin, amoxicillin.
- Inhibit bacterial enzymes involved in cell wall synthesis.

2. ACE Inhibitors

- Drugs like captopril, enalapril, lisinopril.
- Inhibit angiotensin-converting enzyme (ACE).
- Prevent conversion of angiotensin I → angiotensin II.
- Result: Vasodilation and lower blood pressure.

3. Aspirin

- A nonprescription drug.
- Irreversibly inhibits cyclooxygenase (COX enzyme).
- Blocks prostaglandin and thromboxane synthesis.
- Results in anti-inflammatory and antiplatelet effects.

VIII. Enzyme Regulation

Importance of Regulation

- Enzyme regulation is essential for coordinating metabolic processes.
- Most enzymes respond to changes in substrate concentration.
    - Substrate levels often near Km.
    - ↑ [Substrate] → ↑ reaction rate → helps normalize substrate levels.

Specialized Regulatory Mechanisms

- Some enzymes respond to:
    - Allosteric effectors
    - Covalent modification
    - Altered synthesis/degradation rates in response to physiological changes.

A. Allosteric Enzymes

Basic Characteristics

- Do not follow Michaelis–Menten kinetics.
- Regulated by effectors (bind noncovalently at sites other than active site).
- Usually multimeric (multiple subunits).
- Effector–binding site ≠ catalytic site.

Types of Effectors

- Positive effectors: Increase enzyme activity.
- Negative effectors: Decrease enzyme activity.

Effects of Effectors

- Modify:
    - Affinity for substrate ($K_{0.5}$)
    - Catalytic activity (Vmax)
    - Or both

Role in Metabolic Pathways

- Often catalyze:
    - Committed step in a pathway.
    - Rate-limiting step.

1. Homotropic Effectors

- Substrate itself acts as the effector.
- Example: Cooperative binding seen in some enzymes (like hemoglobin* – although not an enzyme, it shows similar behavior).
- Term "homotropic" = same as substrate.

Cooperativity in Allosteric Enzymes

- Substrate often acts as a positive effector.
- Binding of substrate to one site enhances catalytic activity at other sites.
- This interaction between sites is called cooperativity.
- Leads to a sigmoidal (S-shaped) vo vs. [S] curve.
    - In contrast to hyperbolic curve seen with Michaelis-Menten enzymes.
- Analogy: Similar to O_2 binding to hemoglobin.

2. Heterotropic Effectors

- Effector ≠ substrate → called heterotropic.

- Example: Feedback inhibition:
    - End product G binds to an allosteric site of an earlier enzyme (e.g., enzyme converting D → E).
    - High [G] → inhibits the first irreversible step unique to the pathway.
    - Regulates product levels by controlling pathway flow.

- Common Example:
    - Phosphofructokinase-1 (PFK-1) in glycolysis:
        - Inhibited by citrate (not a substrate of PFK-1).
        - Citrate acts as a heterotropic inhibitor.

B. Covalent Modification

- Many enzymes are regulated by addition/removal of phosphate groups.

- Modification usually occurs on serine, threonine, or tyrosine residues.

- Protein phosphorylation is a key method of cellular regulation.

1. Phosphorylation and Dephosphorylation

- Phosphorylation catalyzed by protein kinases:
    - Add phosphate group to enzyme/protein.
    - Use ATP as phosphate donor.
- Dephosphorylation catalyzed by phosphoprotein phosphatases:
    - Remove phosphate groups.

2. Enzyme Response to Phosphorylation

- Effect of phosphorylation varies by enzyme:
    - Some become more active, others less active.
- Examples:
    - Glycogen phosphorylase (breaks down glycogen):
        - Activity increased by phosphorylation.
    - Glycogen synthase (synthesizes glycogen):
        - Activity decreased by phosphorylation.

C. Enzyme Synthesis

- Regulation can occur by changing enzyme quantity, not just activity.
- Achieved by altering the rate of enzyme synthesis or degradation.

Key Concepts:

- Induction: Increased enzyme synthesis.
- Repression: Decreased enzyme synthesis.
- Alters total number of active sites.

Example:

- High blood glucose → High insulin:
  - ↑ Synthesis of key glucose metabolism enzymes.

Additional Notes:

- Enzymes regulated by synthesis are typically:
  - Required only during certain stages or specific conditions.

- Housekeeping enzymes:
    - Used constantly → not typically regulated this way.
- Synthesis-based regulation is slow:
    - Takes hours to days.
    - In contrast, allosteric and covalent regulation acts in seconds to minutes.

IX. Enzymes In Human Blood

Overview:

- Most enzymes function intracellularly, but some are found in extracellular fluids like blood plasma.
- Plasma contains enzymes from two main sources:

A. Types of Enzymes Found in Blood Plasma

1. Actively Secreted Enzymes:

- A small group secreted into blood by specific cells.

- Example: Liver secretes zymogens (inactive precursors) of protease enzymes for blood coagulation.
    - These become activated in blood to perform enzymatic functions.

2. Enzymes Released from Cells During Normal Turnover:

- Not secreted purposefully — result of routine cell death.
- Are normally intracellular and nonfunctional in plasma.
- Their levels remain constant in healthy individuals:
    - Steady state: Rate of release = Rate of clearance.
- Elevated plasma enzyme levels suggest:
    - Tissue damage or increased cell death beyond normal turnover.

Additional Definitions:

- Blood plasma: Fluid, noncellular portion of blood.
- Serum:
    - Obtained after coagulation and centrifugation of whole blood.
    - Used in most laboratory enzyme activity assays.
- Plasma vs. Serum:
    - Plasma = Physiologic body fluid.
    - Serum = Laboratory-prepared fluid (no clotting factors).

A. Blood Plasma Enzyme Levels in Disease States

- Tissue damage → Cell lysis → Release of intracellular enzymes into plasma.
- These enzymes do not function outside the cell but are used for diagnosis.

- Clinical importance:
    - Enzyme levels correlate with:
        - Extent of tissue damage.
        - Therapy effectiveness.
        - Prognosis.

B. Plasma Enzymes as Diagnostic Tools

- Some enzymes are tissue-specific → Useful for localizing tissue damage.

Example:

- Alanine aminotransferase (ALT):
    - High in liver.
    - Elevated plasma ALT = Possible liver damage.
    - Included in liver function tests.

- Enzymes with wide tissue distribution:
    - Less specific for diagnosis.
    - Only indicate general cell injury, not the tissue source.

C. Isoenzymes

Definition:

- Isoenzymes (isozymes) = Variant forms of the same enzyme.
- Same catalytic activity, but differ in:
    - Amino acid sequence (genetically determined).
    - Physical properties (e.g., charge, structure).
- Due to differences in charged amino acids, they can be separated via:
    - Electrophoresis – movement in an electric field.

I. Clinical Importance of Isoenzymes:

- Different tissues have characteristic proportions of isoenzymes.
- Pattern of isoenzymes in blood plasma helps identify tissue damage.

Examples:

- LDH (Lactate Dehydrogenase):
    - Found in many tissues.
    - Has five isoenzymes: LD1–LD5
        - LD1: Myocardial muscle
        - LD2: Red blood cells
        - LD5: Liver, skeletal muscle
- CK (Creatine Kinase):
    - Isoenzymes: CK1 (BB), CK2 (MB), CK3 (MM)
    - Distribution:
        - CK1 (BB): Brain
        - CK2 (MB): Myocardium (cardiac-specific!)
        - CK3 (MM): Skeletal muscle

2. Isoenzyme Quaternary Structure

LDH Isoenzymes:

- All are tetramers (4 subunits).
- Two types of subunits: H (heart) and M (muscle).

- Combinations:
    - LD1 = HHHH
    - LD2 = HHHM
    - LD3 = HHMM
    - LD4 = HMMM
    - LD5 = MMMM

CK Isoenzymes:

- All are dimers (2 subunits).
- Two types of subunits: B (brain) and M (muscle).
- Combinations:
    - CK1 = BB
    - CK2 = MB (unique to cardiac muscle)
    - CK3 = MM

Each isoenzyme has a characteristic electrophoretic mobility.

3. Historical Use in Diagnosis of Myocardial Infarction (MI)

- Before troponins, CK MB (CK2) was used as a biomarker for MI.
- CK MB is specific to myocardium:
    - Only tissue with >5% total CK activity as CK MB.
    - Its presence in blood = Myocardial damage.

CK MB Timeline Post-MI:

- Appears in plasma: 4–8 hours after chest pain.
- Peaks: ~24 hours.
- Used in early MI detection before troponin testing became standard.

Clinical Application: Diagnostic Use of Troponins

Troponins:

- Regulatory proteins in muscle contractility.
- Cardiac-specific isoforms: cTnT (Troponin T) and cTnI (Troponin I).

cTn Characteristics:

- Released into plasma after cardiac muscle damage.
- Highly sensitive and specific for myocardial injury.

Timeline of cTn Post-MI:

- Appear: 4–6 hours after onset.
- Peak: 24–36 hours.
- Remain elevated: 3–10 days.

Clinical Use:

- Considered the "gold standard" for MI diagnosis.
- Interpretation includes:
    - Elevated cTn
    - Clinical symptoms
    - ECG changes

Note: Though similar in timing to CK MB, cTn shows a greater change from baseline, making it more reliable.