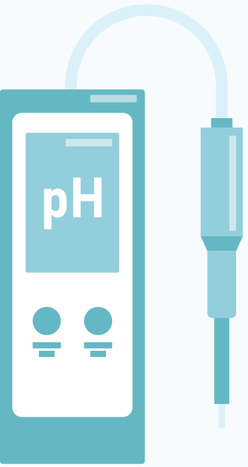
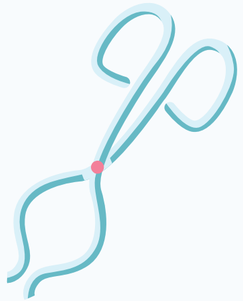
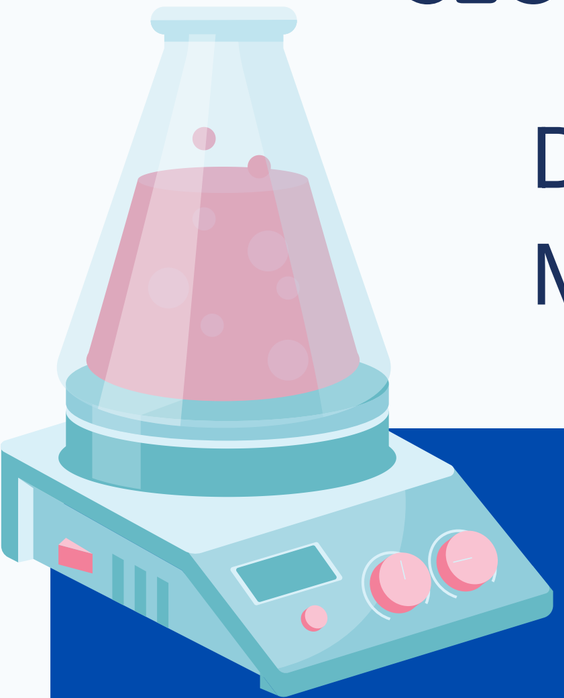


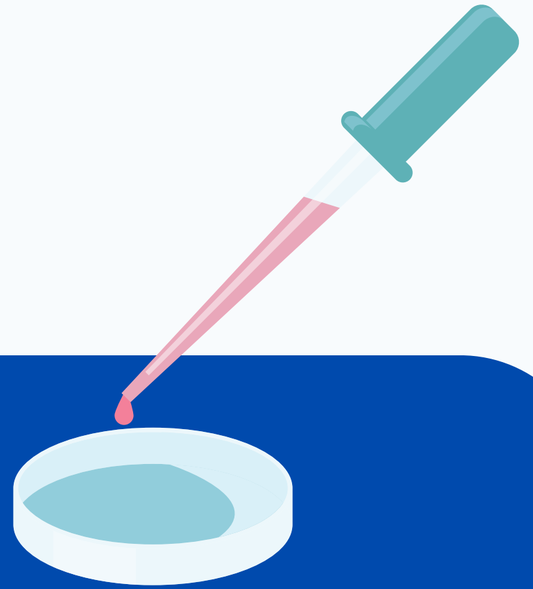


MECHANISM OF ENZYME ACTION

SECOND STAGE

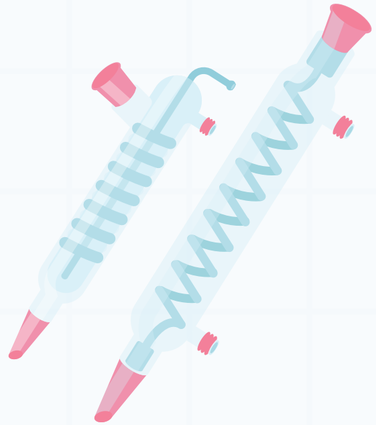
DR.RASHAD AL - TUUAMAH
MEDICAL BIOCHEMISTRY





MECHANISM OF ENZYME ACTION

ENZYME ACTION CAN BE UNDERSTOOD FROM TWO

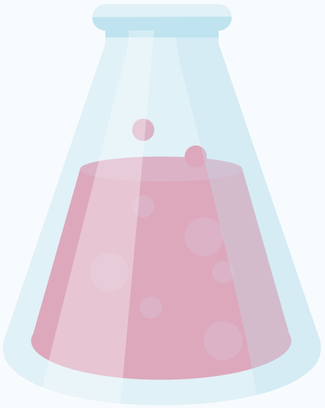


PERSPECTIVES: ONE HIGHLIGHTS THE ENERGY CHANGES THAT CREATE A MORE FAVORABLE REACTION

PATHWAY, WHILE THE OTHER FOCUSES ON HOW THE ACTIVE SITE CHEMICALLY FACILITATES CATALYSIS.

ENERGY CHANGES OCCURRING DURING THE REACTION:-

- ACTIVATION ENERGY
- RATE OF REACTION
- ALTERNATE REACTION PATHWAY

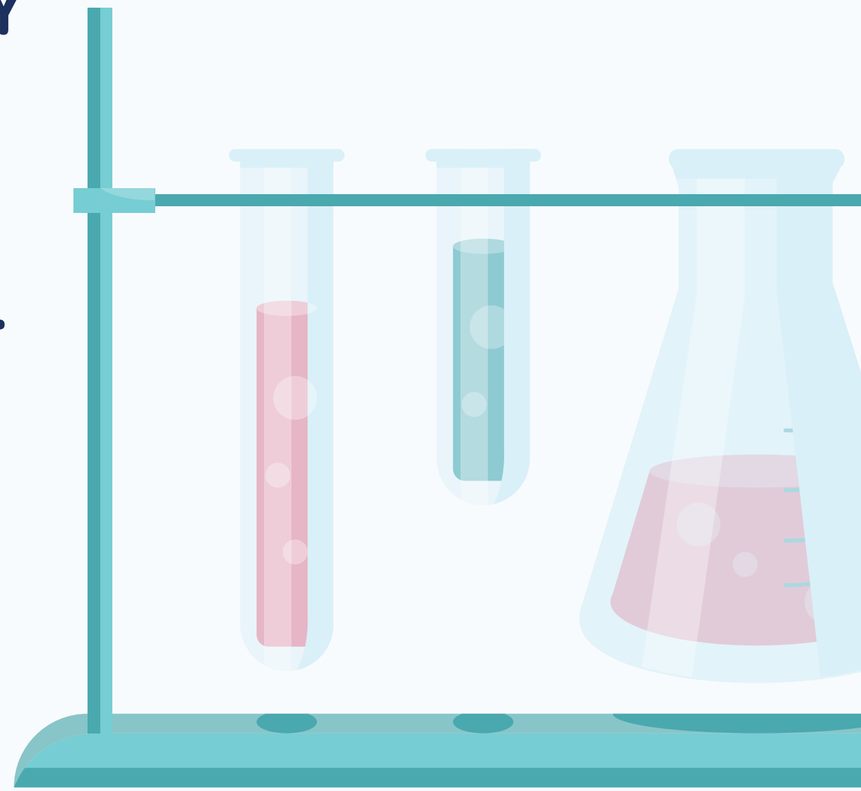


NEARLY ALL CHEMICAL REACTIONS ENCOUNTER AN ENERGY BARRIER KNOWN AS

THE ACTIVATION ENERGY (EA), WHICH REPRESENTS THE DIFFERENCE IN ENERGY

BETWEEN THE REACTANTS AND THE HIGH-ENERGY TRANSITION STATE (T*)

THIS TRANSITION STATE IS A CRUCIAL INTERMEDIATE IN THE CONVERSION OF REACTANT A TO PRODUCT B

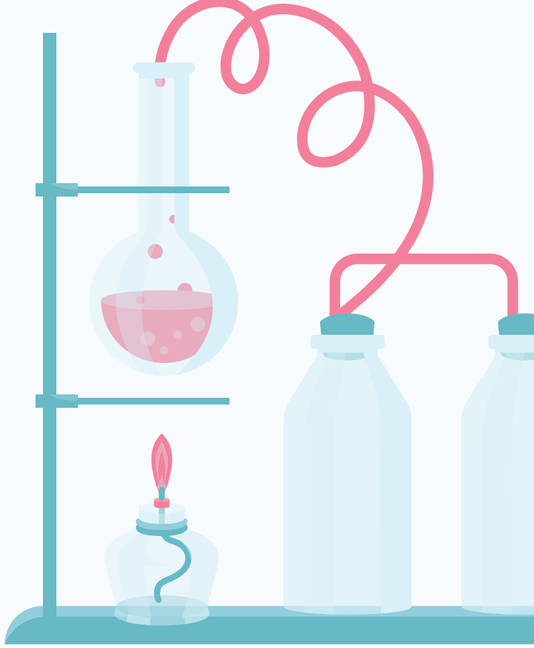


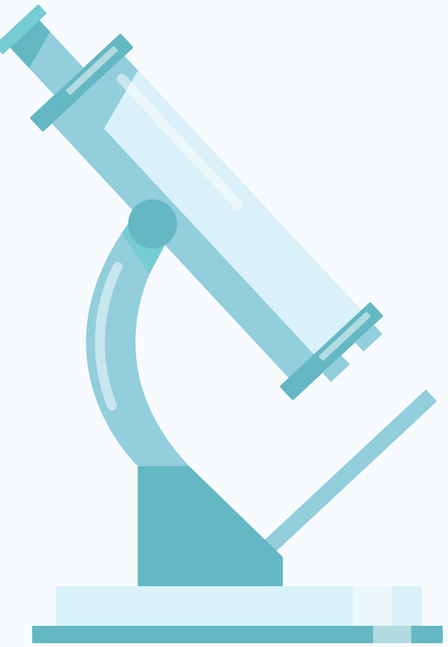
THE PEAK ENERGY IN THE TRANSITION STATE (T*) REPRESENTS THE FREE

ENERGY DIFFERENCE BETWEEN THE REACTANTS AND THIS HIGH-ENERGY

INTERMEDIATE, WHICH CONTRIBUTES TO THE SLOW RATES OF UNCATALYZED

CHEMICAL REACTIONS DUE TO THE HIGH ACTIVATION ENERGY (EA).



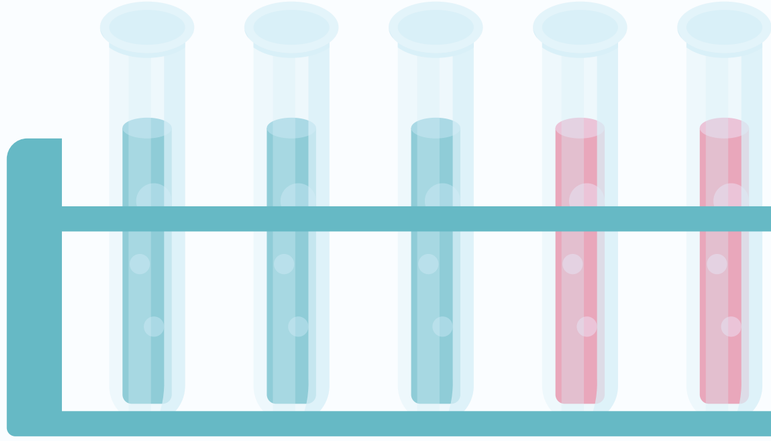


FOR MOLECULES TO REACT THEY MUST POSSESS ENOUGH ENERGY TO SURPASS THE TRANSITION STATE'S ENERGY BARRIER

WITHOUT AN ENZYME ONLY A SMALL FRACTION OF MOLECULES TYPICALLY HAVE

THIS ENERGY LIMITING THE REACTION RATE GENERALLY A LOWER ACTIVATION
ENERGY (EA) RESULTS IN

MORE ENERGIZED
MOLECULES THEREBY ACCELERATING THE REACTION RATE.

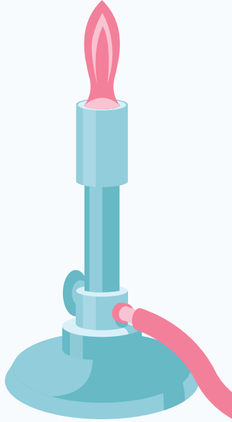


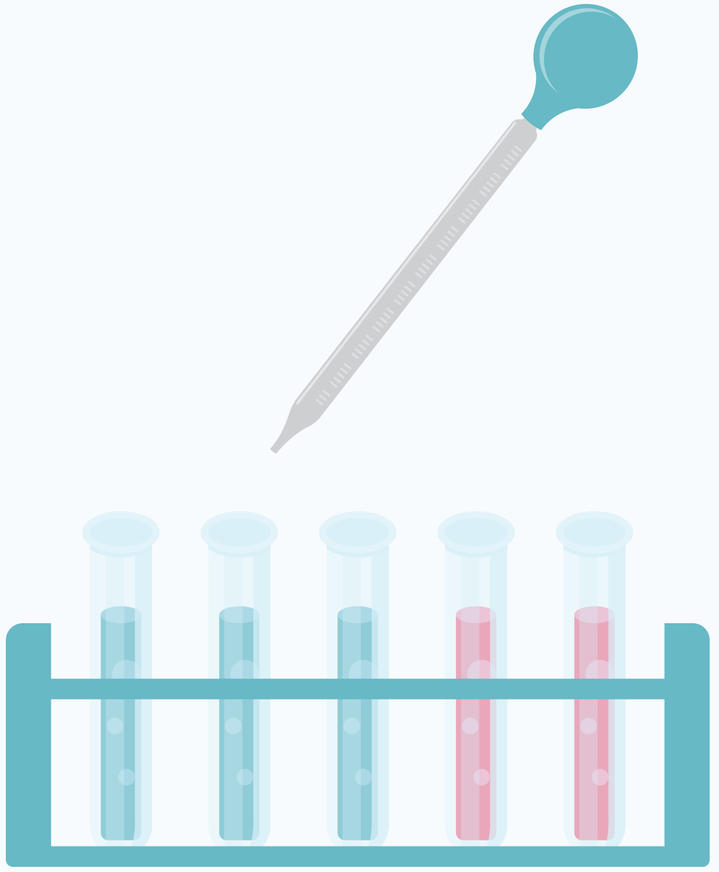
AN ENZYME FACILITATES A REACTION BY OFFERING AN ALTERNATIVE PATHWAY WITH A LOWER ACTIVATION ENERGY (EA)

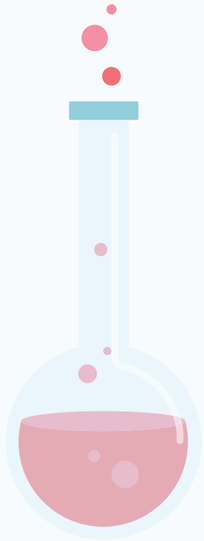
ENABLING THE REACTION TO PROCEED RAPIDLY UNDER CELLULAR CONDITIONS WHILE IT DOES NOT ALTER THE FREE

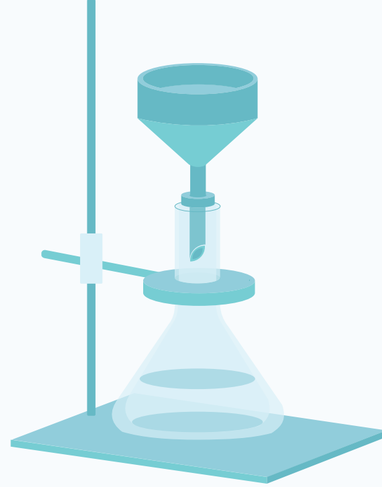
03

ENERGIES OF THE REACTANTS OR PRODUCTS, AND THUS THE EQUILIBRIUM, IT SIGNIFICANTLY ACCELERATES THE RATE AT WHICH EQUILIBRIUM IS ACHIEVED.





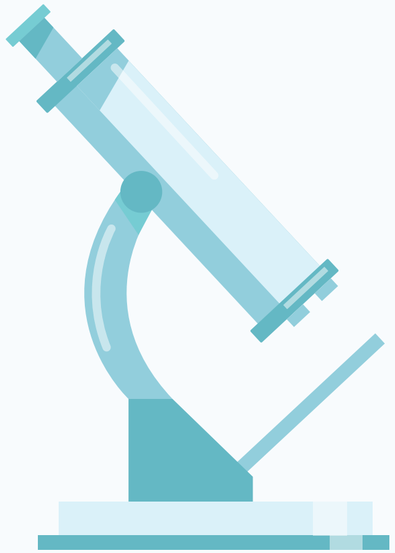


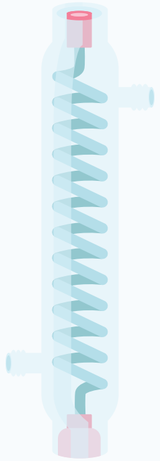


ACTIVE SITE CHEMISTRYTRANSITION-STATE STABILIZATION

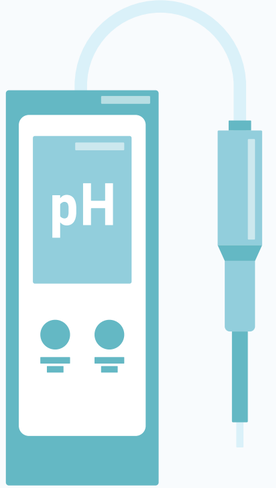
THE ACTIVE SITE FUNCTIONS AS AN INTRICATE MOLECULAR MACHINE, ACTIVELY ENGAGING IN VARIOUS CHEMICAL MECHANISMS TO

CONVERT SUBSTRATE INTO PRODUCT RATHER THAN MERELY SERVING AS A PASSIVE BINDING SITE

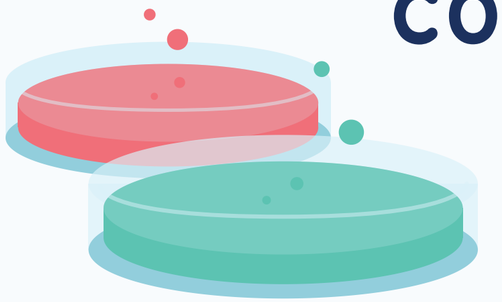


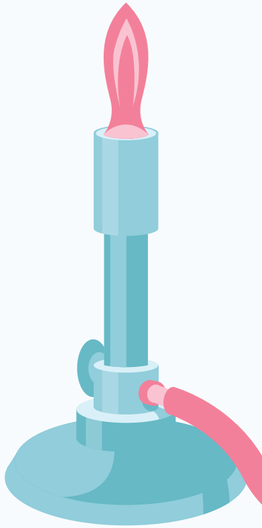


SEVERAL FACTORS CONTRIBUTE TO THE CATALYTIC EFFICIENCY OF ENZYMES, EXEMPLIFYING THIS DYNAMIC ROLE



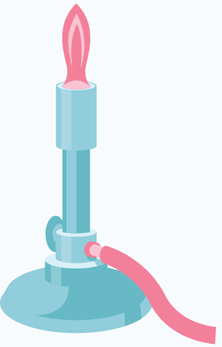
THE ACTIVE SITE STABILIZES THE TRANSITION STATE BY ACTING AS A FLEXIBLE MOLECULAR TEMPLATE THAT BINDS THE SUBSTRATE, FACILITATING ITS CONVERSION TO THIS HIGH-ENERGY STRUCTURE.



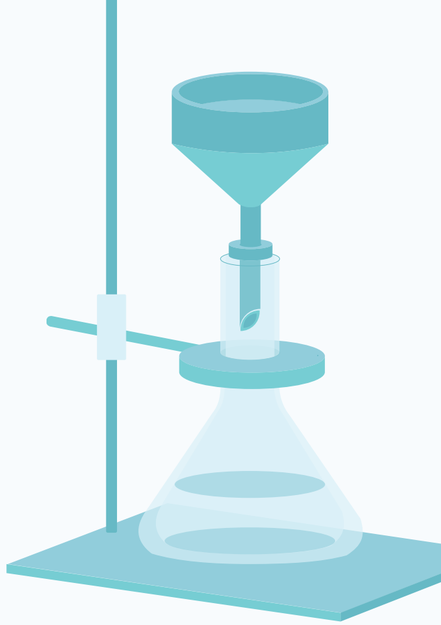


THIS STABILIZATION SIGNIFICANTLY INCREASES THE CONCENTRATION OF THE REACTIVE INTERMEDIATE, THEREBY ACCELERATING THE REACTION. NOTABLY, THE TRANSITION STATE ITSELF CANNOT BE ISOLATED.

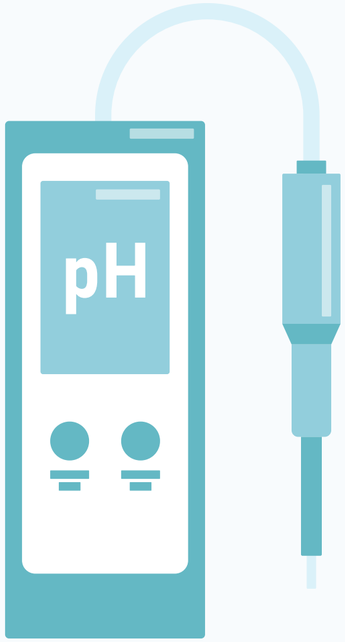
THE ACTIVE SITE OFFERS CATALYTIC GROUPS THAT INCREASE THE LIKELIHOOD OF FORMING THE TRANSITION STATE, WITH SOME ENZYMES UTILIZING GENERAL ACID-BASE CATALYSIS THROUGH PROTON TRANSFER BY AMINO ACID RESIDUES, WHILE OTHERS MAY FORM TRANSIENT COVALENT ENZYME-SUBSTRATE (ES) COMPLEXES.



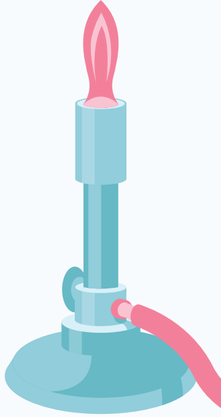
THE ENZYME-CATALYZED CONVERSION OF SUBSTRATE TO PRODUCT CAN BE LIKENED TO REMOVING A SWEATER



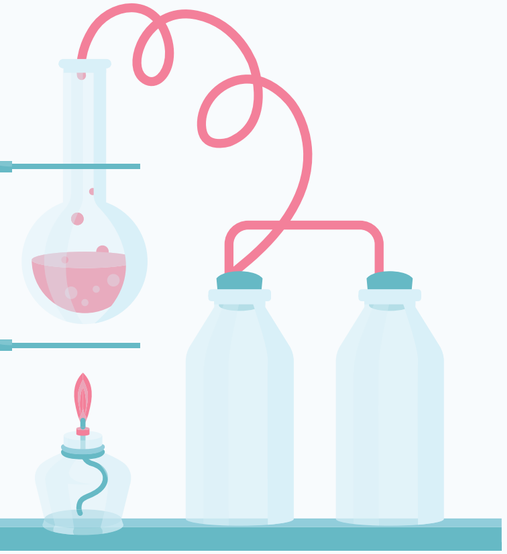
FROM AN UNCOOPERATIVE INFANT, WHERE THE HIGH ACTIVATION ENERGY (EA) REFLECTS THE DIFFICULTY OF ACHIEVING THE NECESSARY POSTURE WITHOUT A CATALYST.



HERE, A PARENT ACTS AS THE ENZYME, FIRST FORMING AN ENZYME-SUBSTRATE (ES) COMPLEX AND THEN GUIDING THE INFANT'S ARMS



INTO THE REQUIRED POSITION, ANALOGOUS TO THE TRANSITION STATE. THIS CONFORMATION FACILITATES THE SWEATER'S REMOVAL



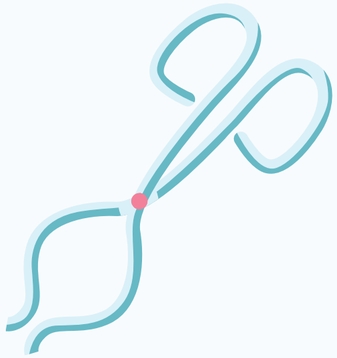
RESULTING IN THE DISROBED BABY AS THE PRODUCT. THE ES COMPLEX EXISTS AT A SLIGHTLY LOWER ENERGY THAN THE UNBOUND SUBSTRATE, WHICH ACCOUNTS FOR THE MINOR DIP IN THE ENERGY CURVE.

FACTORS AFFECTING REACTION VELOCITY

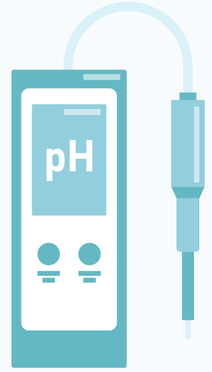
ENZYMES CAN BE ISOLATED FROM CELLS AND THEIR PROPERTIES STUDIED IN A TEST TUBE, THAT IS, IN VITRO. DIFFERENT ENZYMES SHOW DIFFERENT

RESPONSES TO CHANGES IN SUBSTRATE CONCENTRATION, TEMPERATURE, AND PH. THIS SECTION DESCRIBES FACTORS THAT INFLUENCE THE REACTION VELOCITY OF ENZYMES. ENZYMATIC RESPONSES

TO THESE FACTORS GIVE US VALUABLE CLUES AS TO HOW ENZYMES FUNCTION IN LIVING CELLS, THAT IS, IN VIVO.

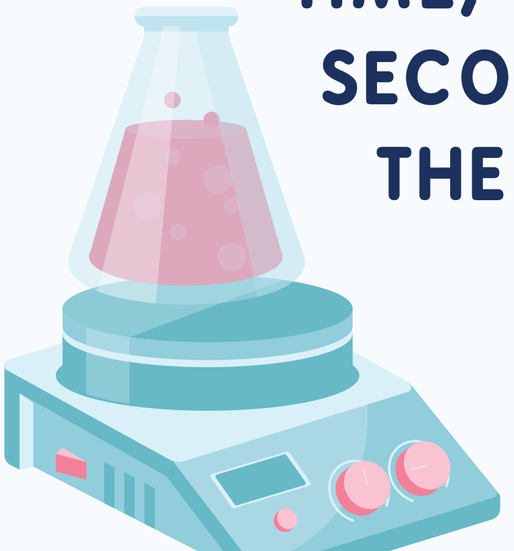


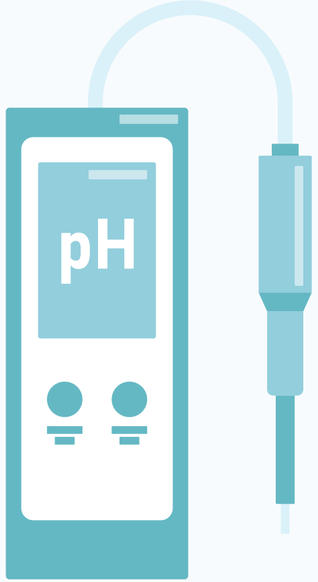
SUBSTRATE CONCENTRATIONM:-
MAXIMAL VELOCITY
SHAPE OF THE ENZYME KINETICS CURVE



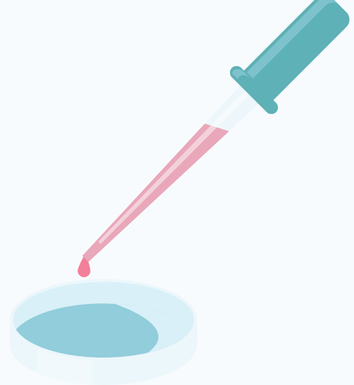
THE RATE OR VELOCITY OF A REACTION (V) QUANTIFIES THE NUMBER OF SUBSTRATE MOLECULES CONVERTED TO PRODUCT PER UNIT

TIME, TYPICALLY MEASURED IN MMOL OF PRODUCT PER SECOND. AS SUBSTRATE CONCENTRATION INCREASES, THE REACTION VELOCITY RISES UNTIL IT REACHES A MAXIMUM (VMAX),



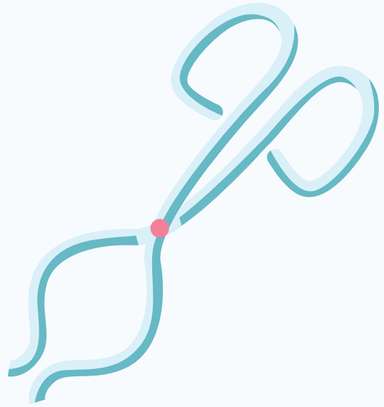


AT WHICH POINT ALL AVAILABLE ENZYME BINDING SITES ARE SATURATED, RESULTING IN A PLATEAU IN THE REACTION RATE.



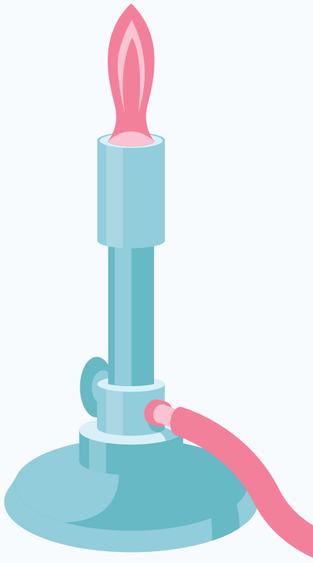
MOST ENZYMES EXHIBIT MICHAELIS-MENTEN KINETICS, CHARACTERIZED BY A HYPERBOLIC PLOT OF INITIAL REACTION VELOCITY (VO) AGAINST SUBSTRATE CONCENTRATION

IN CONTRAST, ALLOSTERIC ENZYMES DISPLAY A SIGMOIDAL CURVE, RESEMBLING THE OXYGEN-DISSOCIATION CURVE OF HEMOGLOBIN, RATHER THAN FOLLOWING MICHAELIS-MENTEN KINETICS

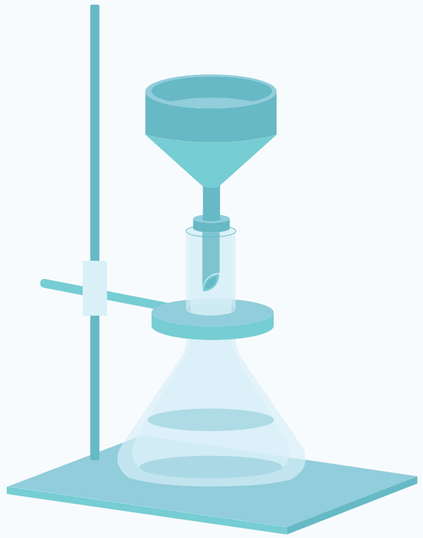


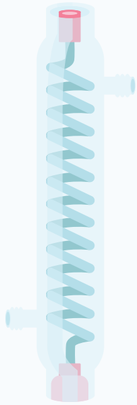
TEMPERATURE:-

VELOCITY INCREASE WITH TEMPERATURE: THE REACTION VELOCITY INCREASES WITH TEMPERATURE UNTIL A PEAK VELOCITY IS REACHED.



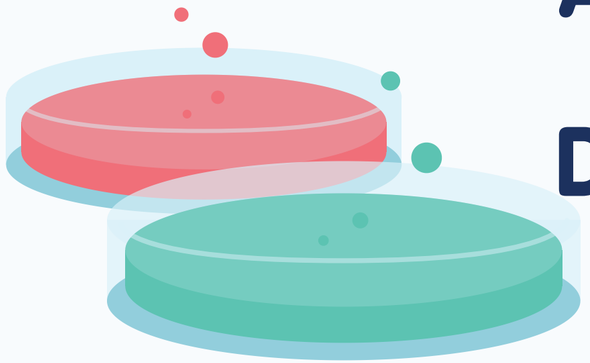
THIS INCREASE IS THE RESULT OF THE INCREASED NUMBER OF SUBSTRATE MOLECULES HAVING SUFFICIENT ENERGY TO PASS OVER THE ENERGY BARRIER AND FORM THE PRODUCTS OF THE REACTION

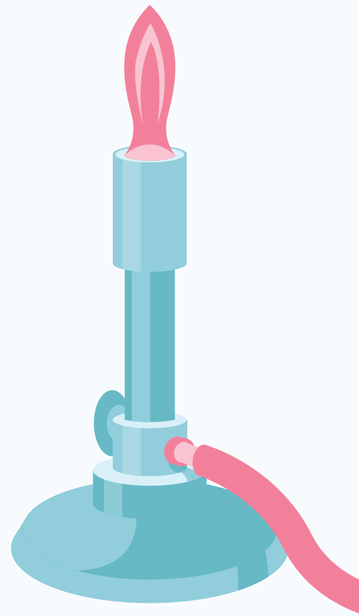




VELOCITY DECREASE WITH HIGHER TEMPERATURE: FURTHER ELEVATION OF THE TEMPERATURE CAUSES A

DECREASE IN REACTION VELOCITY AS A RESULT OF TEMPERATURE-INDUCED DENATURATION OF THE ENZYME.



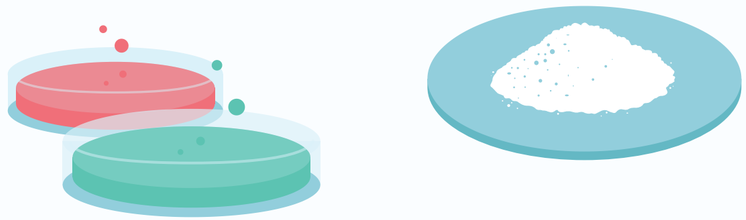


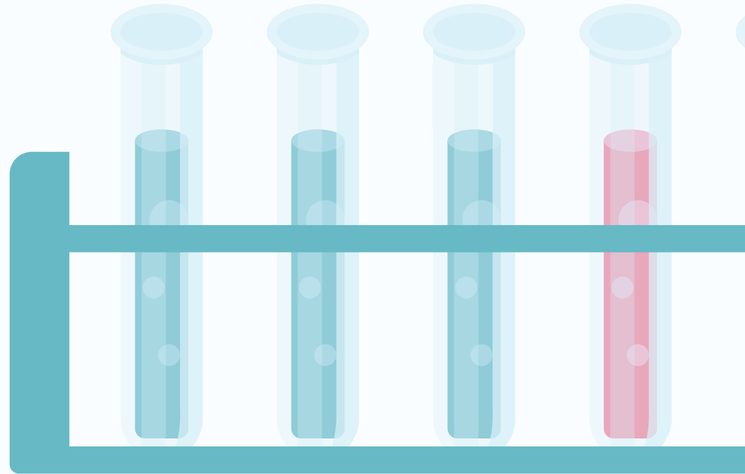
PH EFFECT ON ACTIVE SITE IONIZATION: THE CONCENTRATION OF PROTONS ([H+]) INFLUENCES REACTION VELOCITY BY

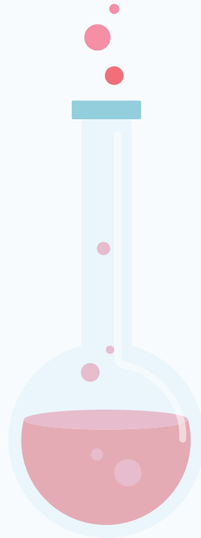
NECESSITATING THAT SPECIFIC CHEMICAL GROUPS OF THE ENZYME AND SUBSTRATE EXIST IN EITHER AN IONIZED OR UNIONIZED

STATE FOR EFFECTIVE INTERACTION. FOR INSTANCE, IF AN AMINO GROUP OF THE ENZYME MUST BE PROTONATED (-NH3+)

A DECLINE IN REACTION RATE OCCURS AT ALKALINE PH WHEN THIS GROUP BECOMES DEPROTONATED.



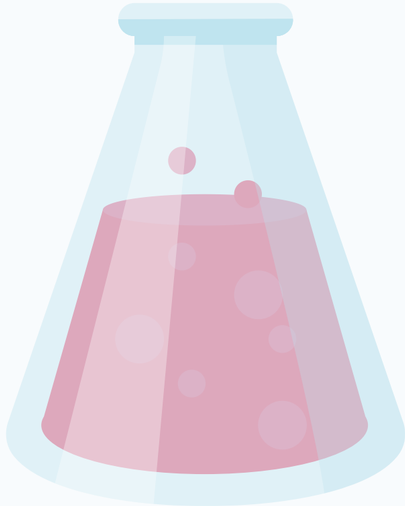




PH EFFECT ON ENZYME DENATURATION:

EXTREMES OF PH CAN ALSO LEAD TO DENATURATION OF THE ENZYME, BECAUSE THE STRUCTURE OF THE

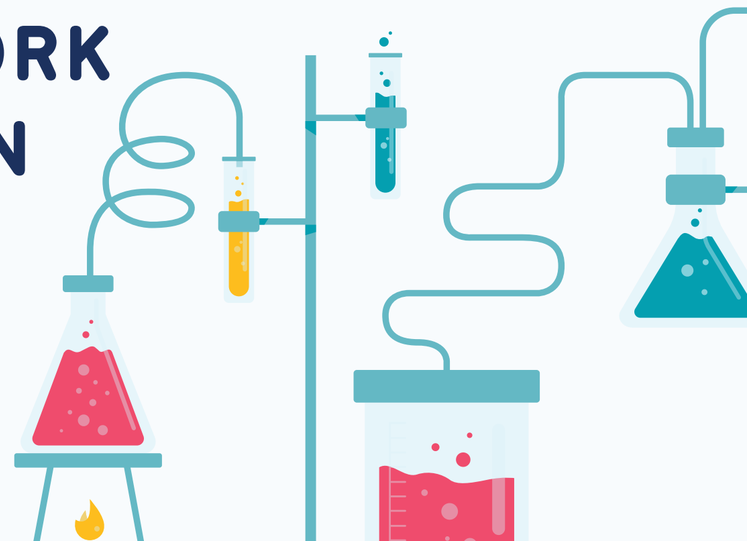
CATALYTICALLY ACTIVE PROTEIN MOLECULE DEPENDS ON THE IONIC CHARACTER OF THE AMINO ACID SIDE CHAINS.



VARIABLE PH OPTIMUM: THE PH AT WHICH MAXIMAL ENZYME ACTIVITY IS ACHIEVED IS DIFFERENT FOR DIFFERENT ENZYMES AND OFTEN REFLECTS THE [H+] AT

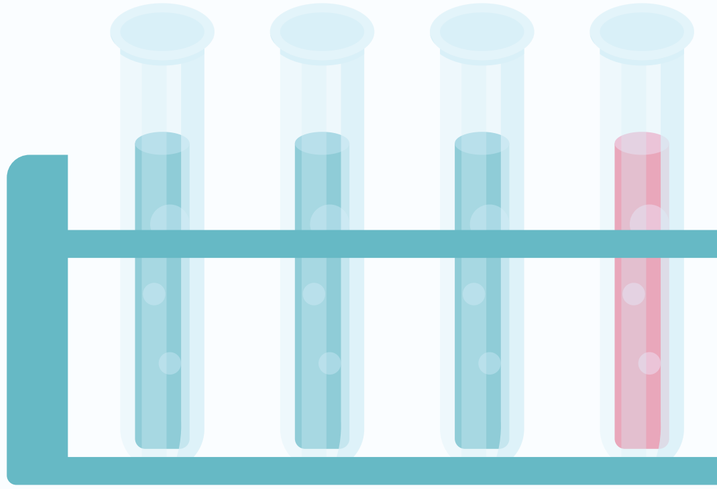
WHICH THE ENZYME FUNCTIONS IN THE BODY. FOR EXAMPLE, PEPSIN, A DIGESTIVE ENZYME IN THE STOMACH, IS MAXIMALLY ACTIVE AT PH 2

WHEREAS OTHER ENZYMES, DESIGNED TO WORK AT NEUTRAL PH, ARE DENATURED BY SUCH AN ACIDIC ENVIRONMENT.

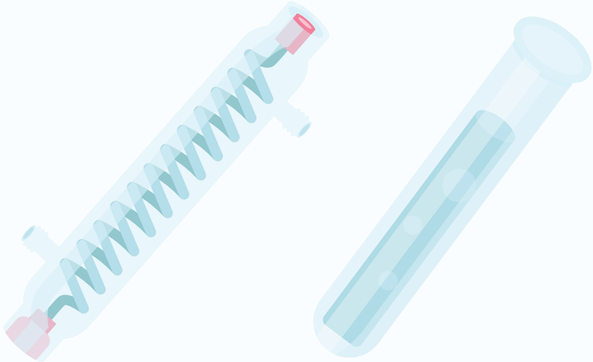


MICHAELIS-MENTEN KINETICS

IN 1913, LEONOR MICHAELIS AND MAUD MENTEN PROPOSED A MODEL WHERE AN ENZYME REVERSIBLY FORMS AN ENZYME-SUBSTRATE (ES) COMPLEX THAT GENERATES PRODUCT, THEREBY REGENERATING THE FREE ENZYME.



$$Vo = \frac{Vmax[S]}{Km + [S]}$$



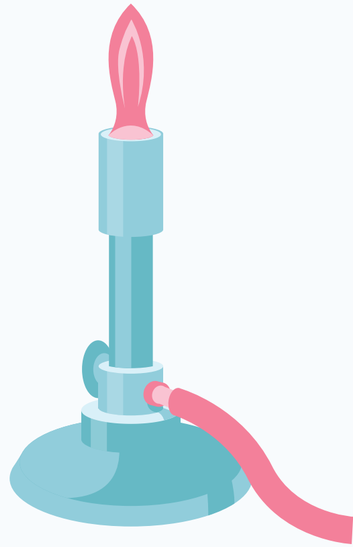
WHERE VO = INITIAL VELOCITY; VMAX = MAXIMAL VELOCITY = KCAT [E]TOTAL; KM = MICHAELIS CONSTANT = (K-1 + K2)/K1; [S] = SUBSTRATE CONCENTRATION.

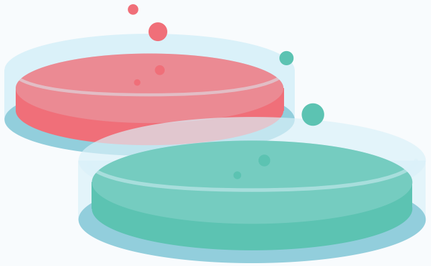
IMPORTANT CONCLUSIONS:

THE MICHAELIS CONSTANT (KM) REFLECTS THE AFFINITY OF AN ENZYME FOR ITS SUBSTRATE, BEING NUMERICALLY EQUAL

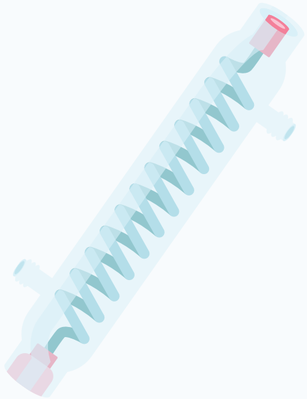
TO THE SUBSTRATE CONCENTRATION AT WHICH THE REACTION VELOCITY IS HALF OF VMAX, AND IT REMAINS UNCHANGED

REGARDLESS OF ENZYME CONCENTRATION.

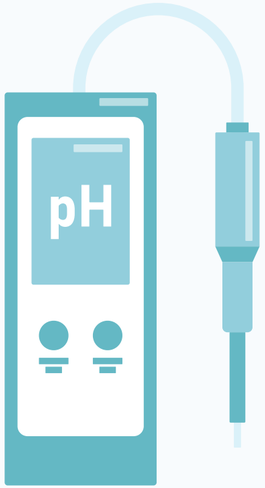


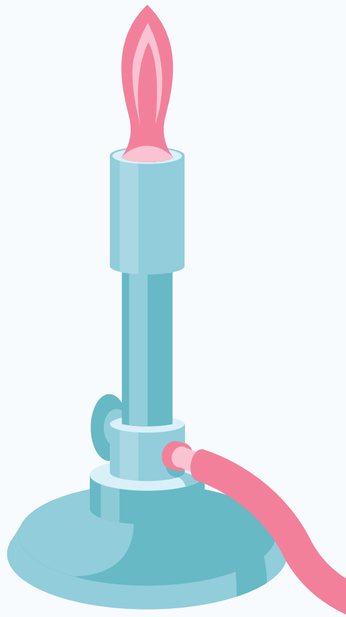


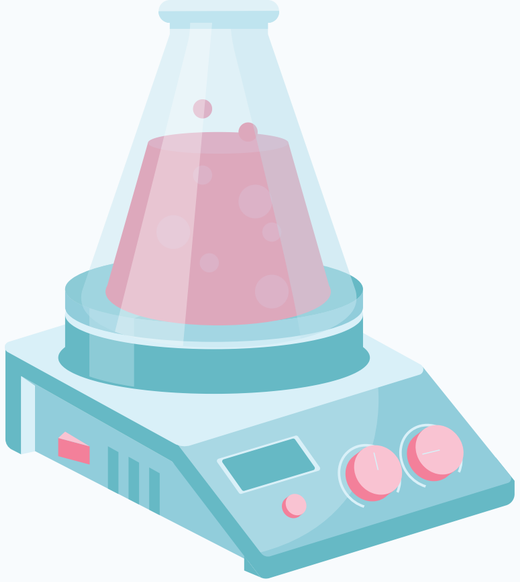
A SMALL KM INDICATES A HIGH AFFINITY OF THE ENZYME FOR ITS SUBSTRATE, AS A LOWER SUBSTRATE CONCENTRATION

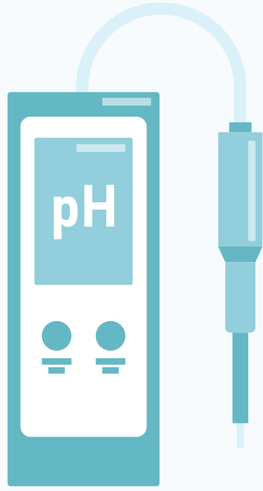


IS SUFFICIENT TO ACHIEVE HALF-SATURATION AND REACH A REACTION VELOCITY OF HALF VMAX.



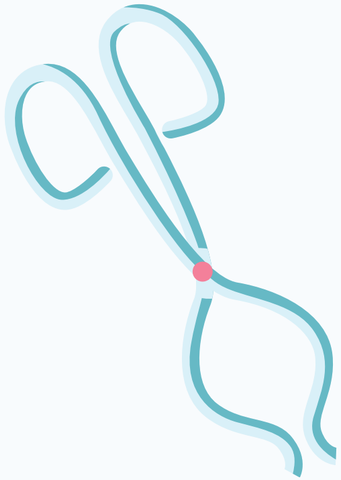


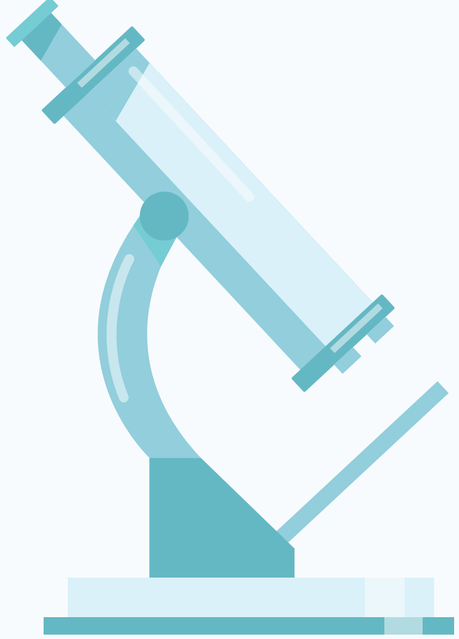




A LARGE KM SIGNIFIES A LOW AFFINITY OF THE ENZYME FOR ITS SUBSTRATE, AS A HIGHER

SUBSTRATE CONCENTRATION IS REQUIRED TO ACHIEVE HALF-SATURATION.

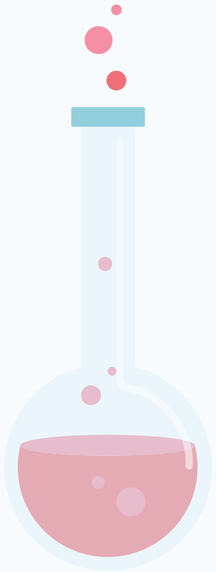


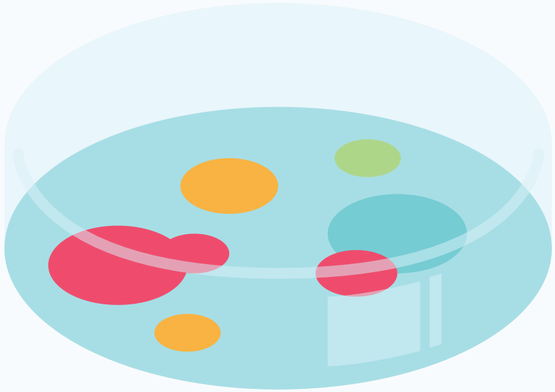


THE REACTION RATE IS DIRECTLY PROPORTIONAL TO ENZYME CONCENTRATION WHEN SUBSTRATE CONCENTRATION IS NOT LIMITING; THUS, HALVING THE

03

ENZYME CONCENTRATION REDUCES BOTH THE INITIAL REACTION RATE (VO) AND VMAX TO HALF OF THEIR ORIGINAL VALUES.

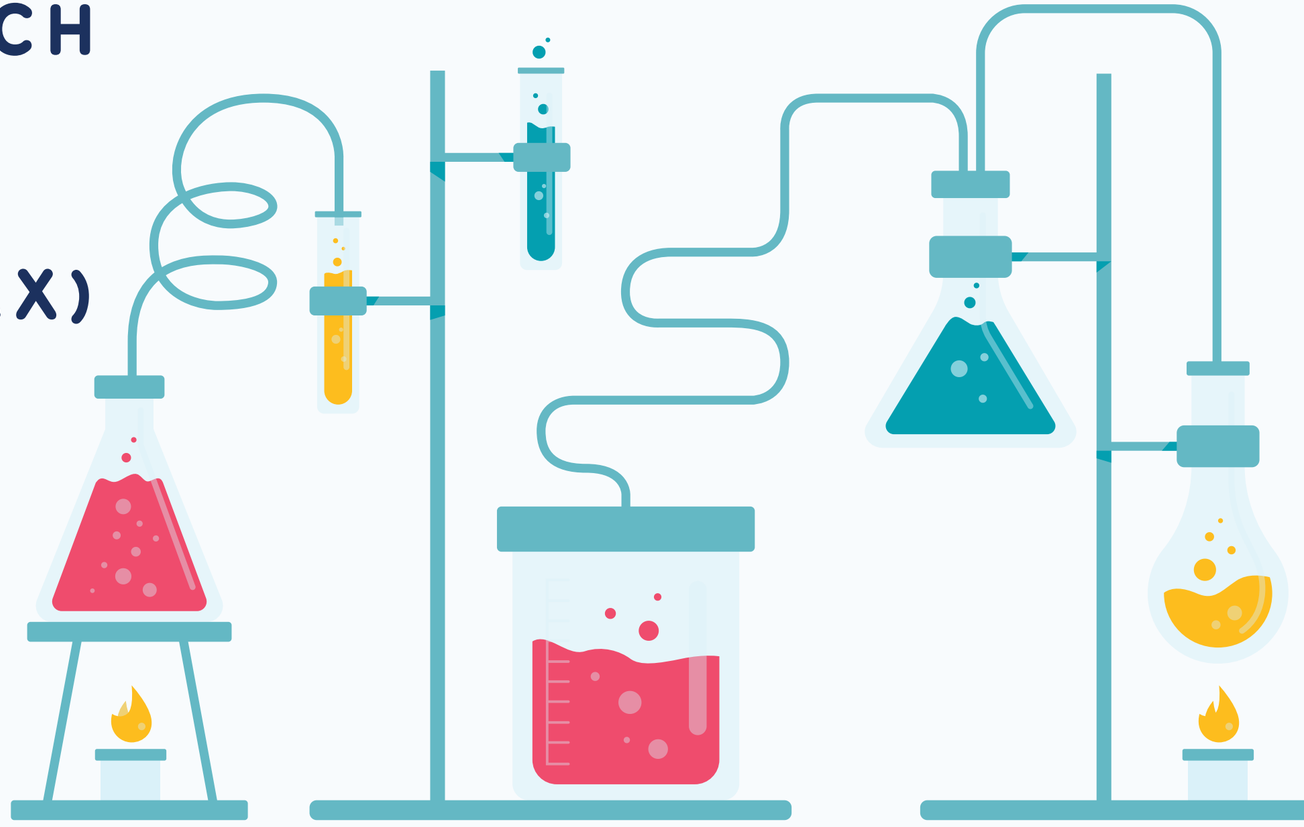




WHEN SUBSTRATE CONCENTRATION ([S]) IS MUCH LESS THAN KM, THE REACTION VELOCITY IS FIRST-ORDER

AND PROPORTIONAL TO [S]; CONVERSELY, WHEN [S] IS MUCH GREATER THAN KM, THE

VELOCITY IS CONSTANT (VMAX) AND INDEPENDENT OF SUBSTRATE CONCENTRATION, INDICATING ZERO-ORDER KINETICS.

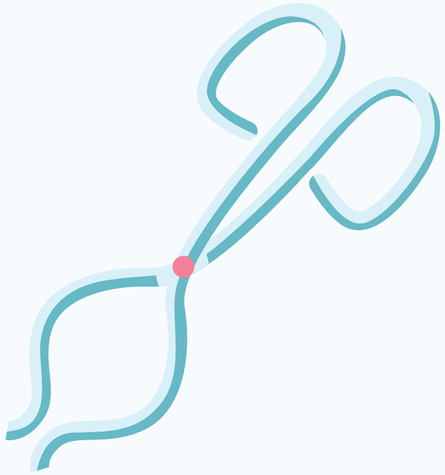


B. LINEWEAVER-BURK PLOT

THE LINEWEAVER-BURK PLOT, OR DOUBLE-RECIPROCAL PLOT, ALLOWS FOR THE

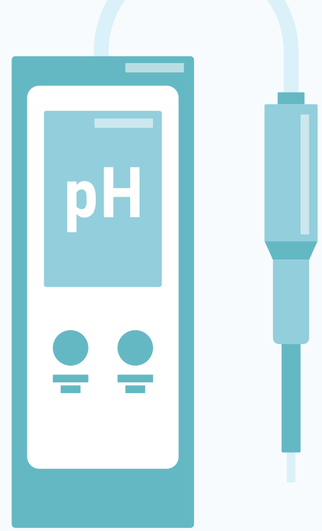
DETERMINATION OF KM AND VMAX BY PLOTTING 1/VO AGAINST 1/[S], PRODUCING A STRAIGHT LINE THAT FACILITATES THE

ANALYSIS OF ENZYME KINETICS AND INHIBITOR MECHANISMS.



ENZYME INHIBITION

INHIBITORS REDUCE THE VELOCITY OF ENZYME-CATALYZED REACTIONS AND CAN BE REVERSIBLE, FORMING NONCOVALENT ENZYME-INHIBITOR

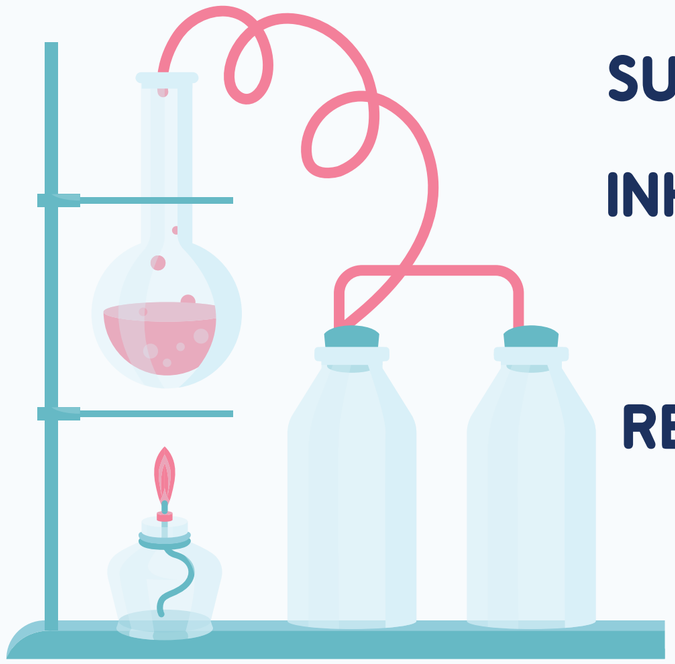


COMPLEXES, OR IRREVERSIBLE, BINDING COVALENTLY, AS SEEN WITH LEAD INHIBITING FERROCHELATASE; COMMON REVERSIBLE TYPES INCLUDE COMPETITIVE AND

NONCOMPETITIVE INHIBITION. REVERSIBLE INHIBITORS BIND NONCOVALENTLY, ALLOWING FOR RECOVERY OF ENZYME ACTIVITY UPON DILUTION.

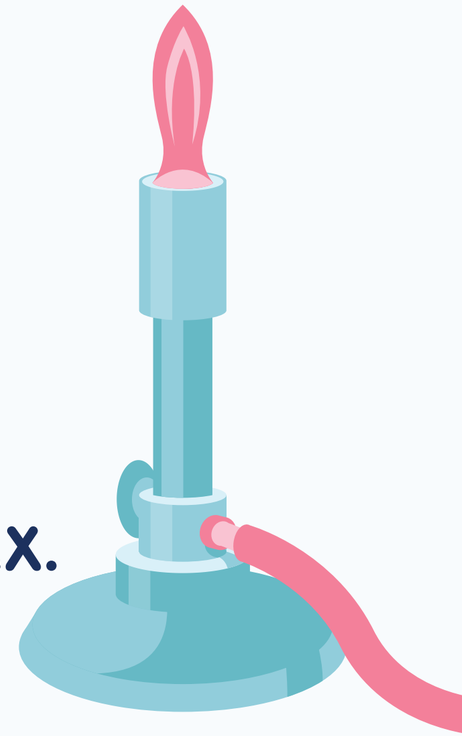
COMPETITIVE INHIBITION

COMPETITIVE INHIBITION OCCURS WHEN AN INHIBITOR BINDS REVERSIBLY TO THE ENZYME'S ACTIVE SITE, COMPETING WITH THE SUBSTRATE.

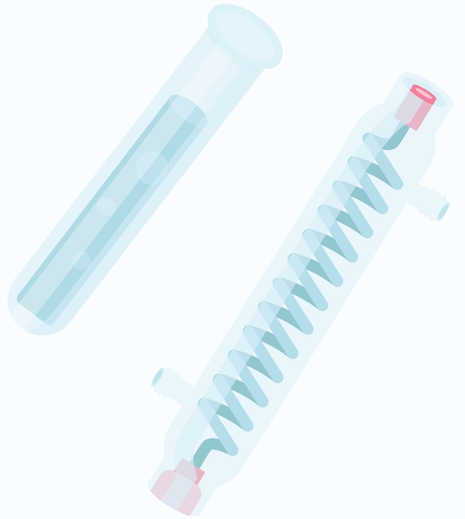


WHILE VMAX REMAINS UNCHANGED SINCE HIGH SUBSTRATE CONCENTRATIONS CAN OVERCOME THE INHIBITOR, THE APPARENT KM INCREASES,

REQUIRING MORE SUBSTRATE TO ACHIEVE HALF VMAX.

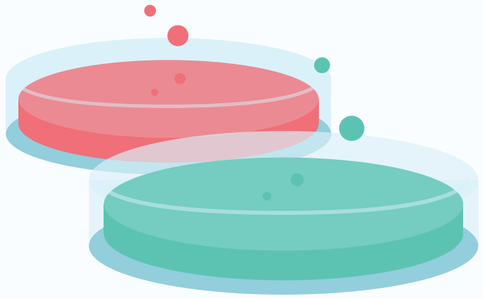


IN THE LINEWEAVER-BURK PLOT, THE LINES FOR INHIBITED AND UNINHIBITED REACTIONS INTERSECT ON THE Y-AXIS, REFLECTING THE UNCHANGED VMAX,



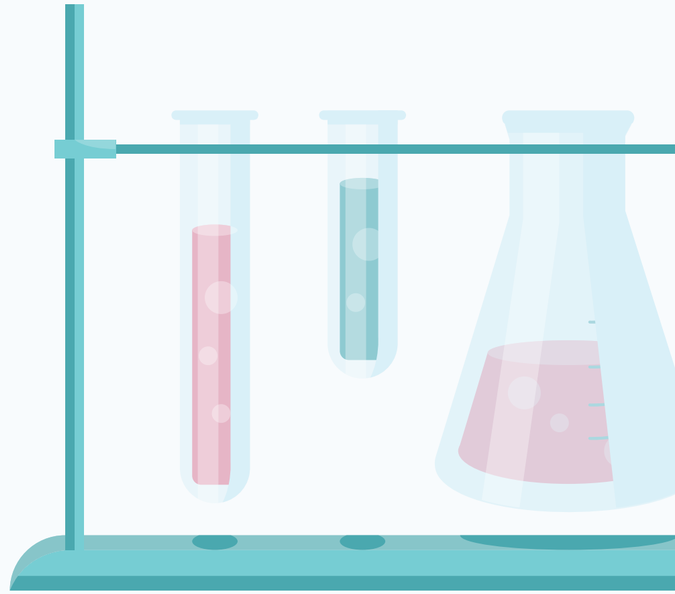
WHILE DIFFERING X-AXIS INTERCEPTS INDICATE AN INCREASED APPARENT KM. NOTABLY, TRANSITION STATE ANALOGS

ARE A SIGNIFICANT CLASS OF COMPETITIVE INHIBITORS, BINDING MORE TIGHTLY TO THE ENZYME THAN THE SUBSTRATE.



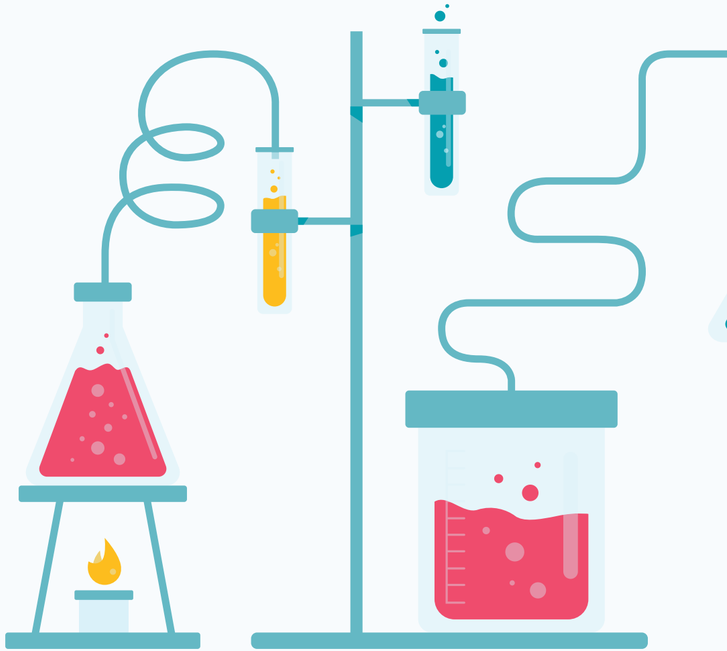
NONCOMPETITIVE INHIBITION

NONCOMPETITIVE INHIBITION IS MARKED BY A DECREASE IN VMAX, AS THE INHIBITOR



BINDS TO DIFFERENT SITES ON THE ENZYME, PREVENTING THE REACTION REGARDLESS OF SUBSTRATE CONCENTRATION. THIS TYPE O

F INHIBITION DOES NOT AFFECT KM, MEANING THE ENZYME'S AFFINITY FOR THE SUBSTRATE REMAINS UNCHANGED. IN THE

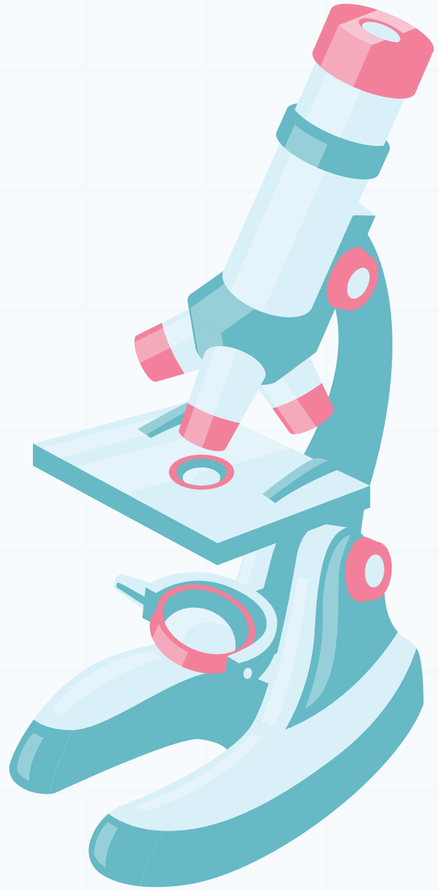


LINEWEAVER-BURK PLOT, NONCOMPETITIVE INHIBITION IS DISTINGUISHED BY A REDUCED VMAX WHILE

MAINTAINING THE SAME KM, CONTRASTING WITH COMPETITIVE INHIBITION.

ENZYME INHIBITORS AS DRUGS

MANY OF THE MOST PRESCRIBED DRUGS IN THE U.S. ARE ENZYME INHIBITORS, INCLUDING B-LACTAM ANTIBIOTICS

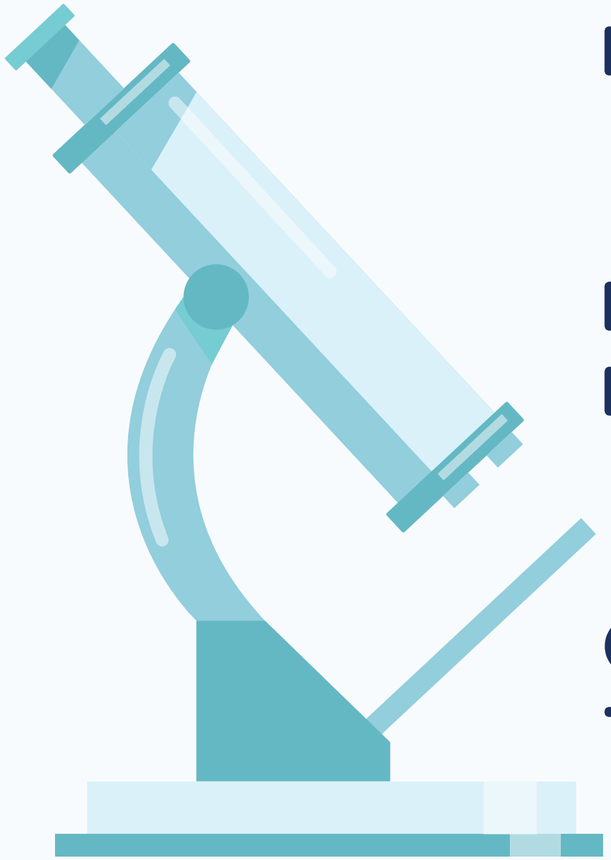


LIKE PENICILLIN AND AMOXICILLIN, WHICH INHIBIT BACTERIAL CELL WALL SYNTHESIS. ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS, SUCH AS

CAPTOPRIL AND LISINOPRIL, LOWER BLOOD PRESSURE BY BLOCKING THE CONVERSION OF ANGIOTENSIN I TO THE VASOCONSTRICTOR ANGIOTENSIN II.

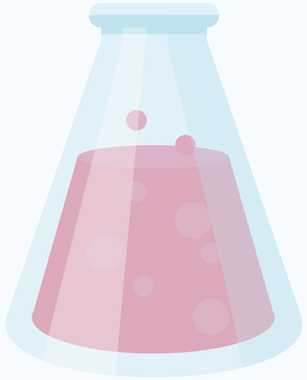
ADDITIONALLY, ASPIRIN IRREVERSIBLY INHIBITS CYCLOOXYGENASE, AFFECTING PROSTAGLANDIN AND

THROMBOXANE SYNTHESIS. STATIN DRUGS, SUCH AS ATORVASTATIN AND PRAVASTATIN, ARE COMPETITIVE



INHIBITORS OF HMG COA REDUCTASE, THE RATE-LIMITING ENZYME IN CHOLESTEROL BIOSYNTHESIS,

EFFECTIVELY REDUCING DE NOVO CHOLESTEROL SYNTHESIS BY MIMICKING THE NATURAL SUBSTRATE.

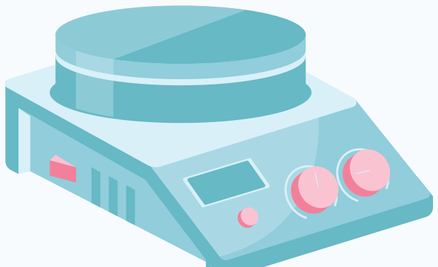


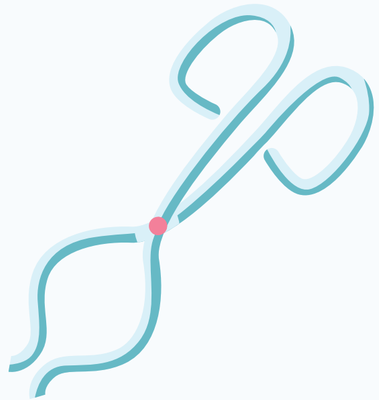
ENZYME REGULATION

THE REGULATION OF ENZYME REACTION VELOCITY IS CRUCIAL FOR COORDINATING METABOLIC PROCESSES

, AS MANY ENZYMES RESPOND TO CHANGES IN SUBSTRATE CONCENTRATION NEAR THEIR KM, LEADING TO INCREASED REACTION RATES THAT HELP NORMALIZE

SUBSTRATE LEVELS. ADDITIONALLY, SOME ENZYMES ARE REGULATED BY ALLOSTERIC EFFECTORS, COVALENT MODIFICATIONS, OR CHANGES IN THEIR SYNTHESIS AND



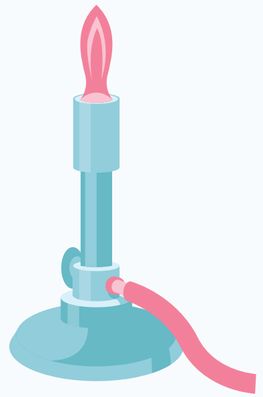


DEGRADATION IN RESPONSE TO PHYSIOLOGICAL CONDITIONS.

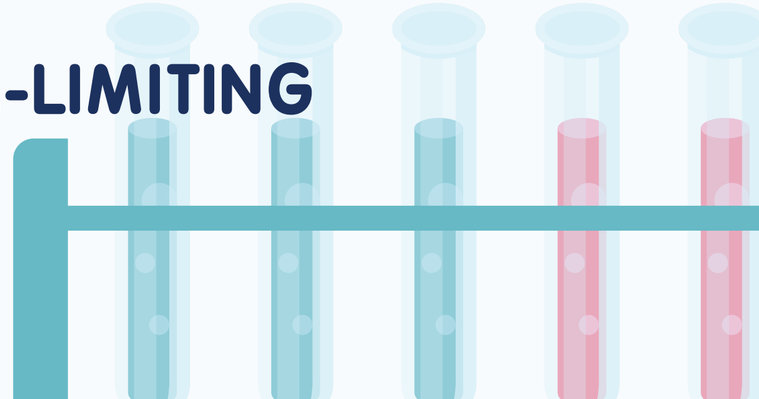
ALLOSTERIC ENZYMES

ALLOSTERIC ENZYMES, WHICH DO NOT FOLLOW MICHAELIS-MENTEN KINETICS, ARE REGULATED BY

NONCOVALENT EFFECTORS THAT BIND TO DISTINCT REGULATORY SITES, OFTEN ON NON-CATALYTIC SUBUNITS. POSITIVE EFFECTORS ENHANCE ENZYME



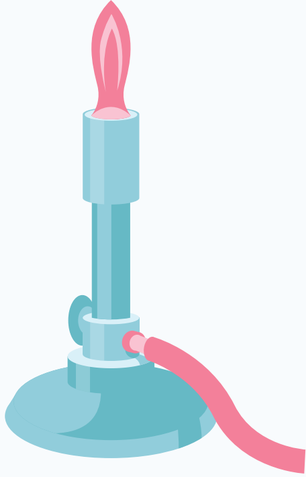
ACTIVITY, WHILE NEGATIVE EFFECTORS INHIBIT IT, INFLUENCING BOTH SUBSTRATE AFFINITY (K0.5) AND MAXIMAL CATALYTIC ACTIVITY (VMAX). TYPICALLY



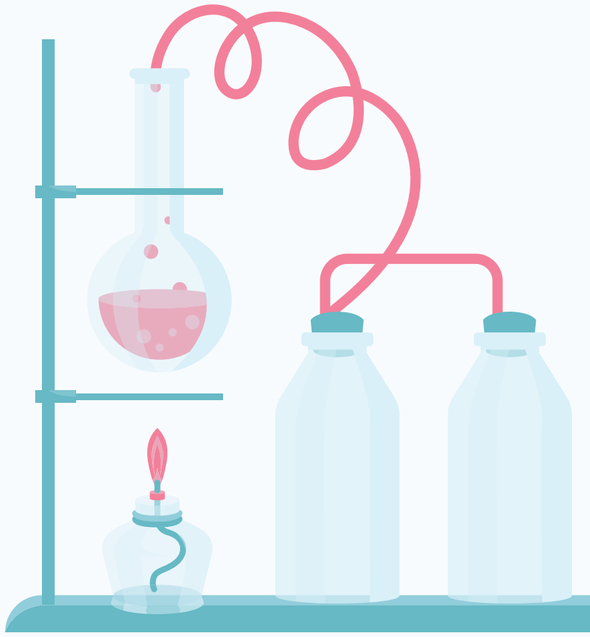
, THESE ENZYMES CATALYZE KEY COMMITTED OR RATE-LIMITING STEPS EARLY IN METABOLIC PATHWAYS.

HOMOTROPIC EFFECTORS

OCCUR WHEN THE SUBSTRATE ITSELF ACTS AS AN EFFECTOR, OFTEN ENHANCING THE CATALYTIC ACTIVITY OF OTHER



SUBSTRATE-BINDING SITES ON THE ENZYME, LEADING TO COOPERATIVE BEHAVIOR. THIS RESULTS IN A SIGMOIDAL CURVE OF REACTION VELOCITY (VO) VERSUS SUBSTRATE



CONCENTRATION, CONTRASTING WITH THE HYPERBOLIC CURVE TYPICAL OF MICHAELIS-MENTEN KINETICS, SIMILAR TO OXYGEN BINDING IN HEMOGLOBIN.

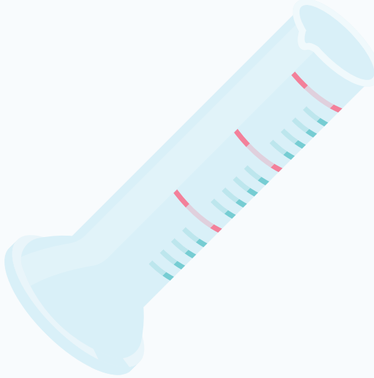
HETEROTROPIC EFFECTORS
INVOLVE DIFFERENT MOLECULES THAN THE SUBSTRATE, EXEMPLIFIED BY FEEDBACK INHIBITION WHERE AN END

PRODUCT, LIKE G, BINDS TO AN ALLOSTERIC SITE, REGULATING ENZYME ACTIVITY AND ENSURING BALANCED PRODUCT SYNTHESIS. A NOTABLE EXAMPLE

IS PHOSPHOFRUCTOKINASE-1, INHIBITED BY CITRATE, WHICH IS NOT A SUBSTRATE.

COVALENT MODIFICATION

ENZYMES ARE PRIMARILY REGULATED THROUGH COVALENT MODIFICATION



NOTABLY BY THE PHOSPHORYLATION OF SERINE, THREONINE, OR TYROSINE RESIDUES, A KEY MECHANISM IN

CELLULAR PROCESS REGULATION.

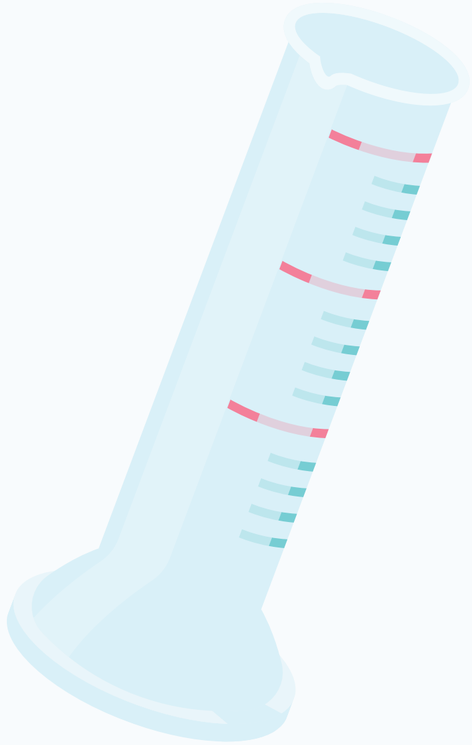
PHOSPHORYLATION AND DEPHOSPHORYLATION:

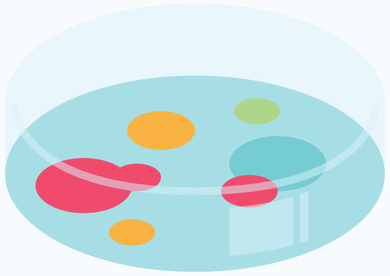
PHOSPHORYLATION, CATALYZED BY PROTEIN KINASES USING ATP, ADDS PHOSPHATE GROUPS TO PROTEINS, WHILE

PHOSPHOPROTEIN PHOSPHATASES REMOVE THESE GROUPS, REGULATING ENZYMATIC ACTIVITY.

ENZYME RESPONSE TO PHOSPHORYLATION:

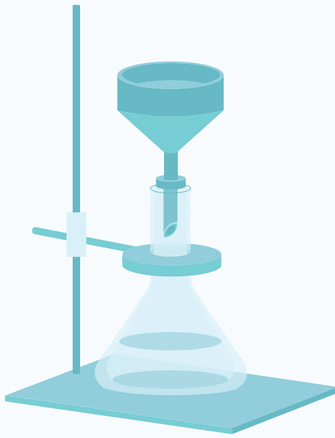
PHOSPHORYLATION: PHOSPHORYLATION CAN EITHER ENHANCE OR INHIBIT ENZYME ACTIVITY; FOR INSTANCE, IT ACTIVATES GLYCOGEN PHOSPHORYLASE WHILE DIMINISHING THE ACTIVITY OF GLYCOGEN SYNTHASE.



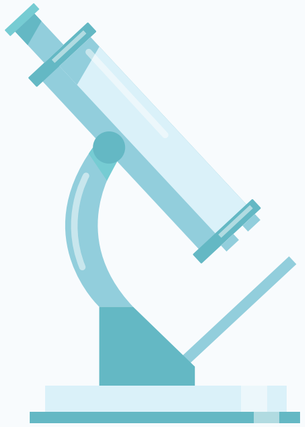


ENZYME SYNTHESIS

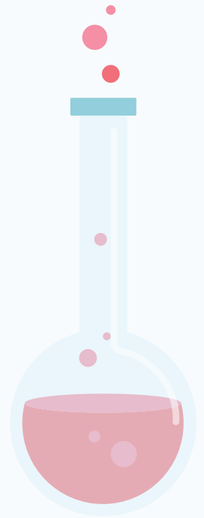
CELLS REGULATE ENZYME ACTIVITY NOT ONLY THROUGH MODIFICATION OF EXISTING ENZYMES BUT ALSO BY ALTERING ENZYME SYNTHESIS RATES, LEADING TO INDUCTION OR



REPRESSION BASED ON PHYSIOLOGICAL CONDITIONS. FOR INSTANCE, ELEVATED INSULIN LEVELS INCREASE THE SYNTHESIS OF ENZYMES FOR GLUCOSE METABOLISM, WHILE ENZYMES IN



CONSTANT USE REMAIN UNAFFECTED. THIS REGULATION OCCURS GRADUALLY, TAKING HOURS TO DAYS, UNLIKE RAPID ALLOSTERIC OR COVALENT MODIFICATIONS THAT HAPPEN WITHIN SECONDS TO MINUTES.



THANK YOU FOR YOUR ATTENTION