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RESEARCH ARTICLE

PROTEIN SEQUENCING AND DRUG DISCOVERY BY USING A HIGH THROUGHPUT SCREENING ASSAY

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Abstract

This topic explains to us the technique of drug discovery. In this we take a protein sample which under-go sequencing forcers by using either with chemical and chromatography process or by mass spectroscopy which gives us the detailed information of monomeric amino acid groping or sequencing. After this the protein sample undergoes crystallization for the X-ray crystallography this helps to know about the 3D structure of the protein. By getting the information of protein we can now perform the high throughput screening which is an interrogation of more than 10,000 compounds that have a drug life property. High throughput screening is a fully automatic process in which with the help of advanced technology and robotics helps to test many compounds from compounds library at the same time. In this, we learn about protein sequencing, mass spectroscopy, HPLC, TLC, X-Ray crystallography, high throughput screening (HTS), and the phenomena of drug discovery.

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Introduction:-

This is a technique of drug discovery by using protein sequencing and high throughput screening. In this we extracted the protein from the deceased person's cells sample and sequence it, this helps to know detailed about the monomeric sequence. This sequencing process is either performed by chemical method or by spectroscopy then we perform X-Ray crystallography for its 3D structure. With this all the possible detail we have and can-do high throughput screening which is an interrogation of more than 10,000 compounds with the respected protein. This is a fully automatic process in which advanced robotic arms and computers do the hit and try the method on large scale.

Protein

Protein, as we all know that in cells every action every performance whether it is a replication of DNA, formation of RNA, production of protein, sending or receiving the message all the task is performed by a protein. The protein is the polymeric chain of amino acids, these amino acids are connected with the bond called the peptide bond. The amino acids consist of one acidic, one basic terminal, and the R-group attached to the α-carbon.

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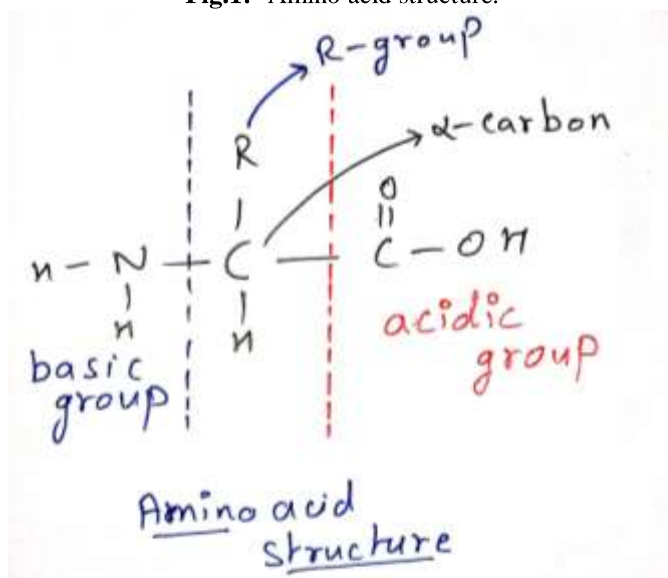
Fig.1:- Amino acid structure.



The R-group of the amino acids is different organic compounds which are specific. There are around 20 amino acids all have different R-group. These 20 amino acids are classified in 5 parts based of their structure and nature such as Nonpolar aliphatic R-group, Polar uncharged R-group, Aromatic R-group positively charged R-group, negatively charged R-group. As shown in fig.

Fig.2:- R-group of amino acid.

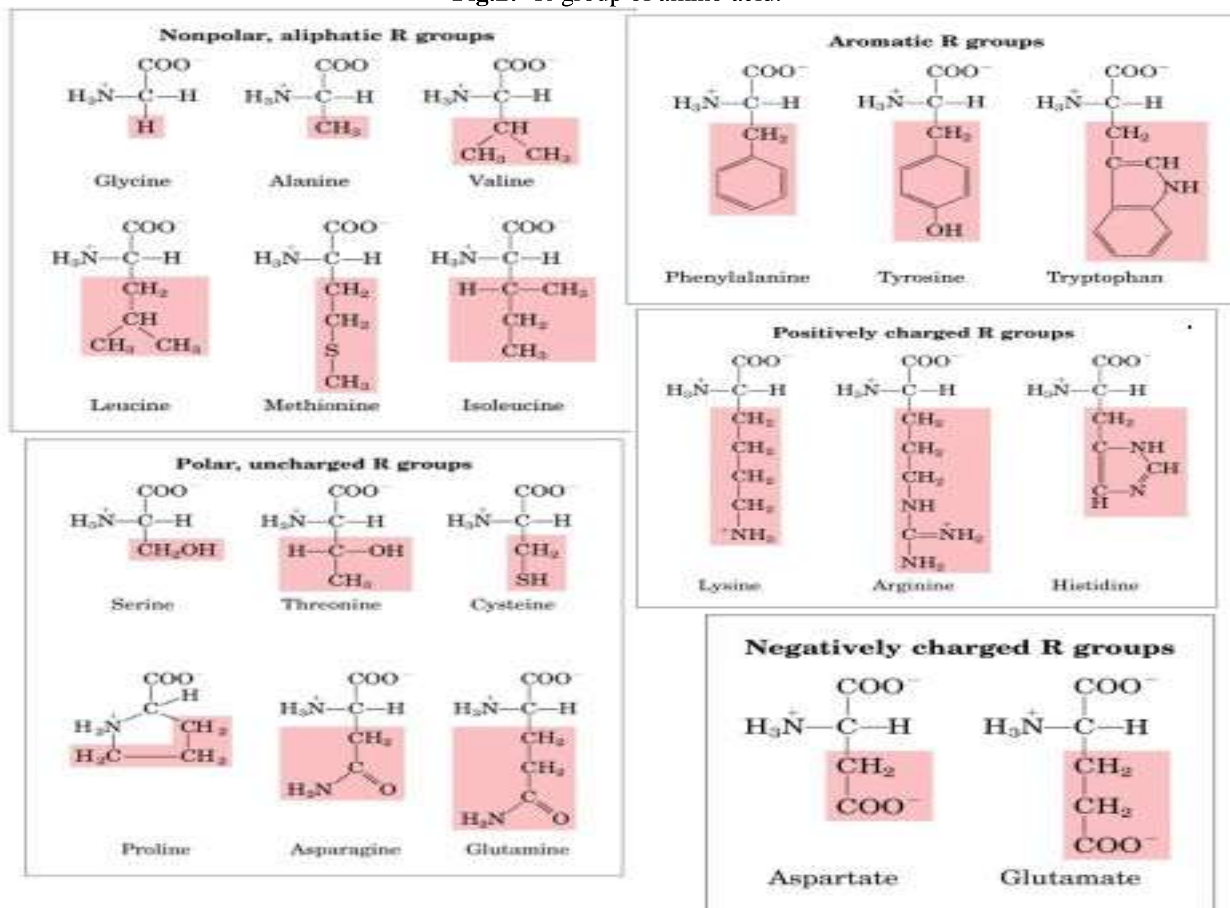
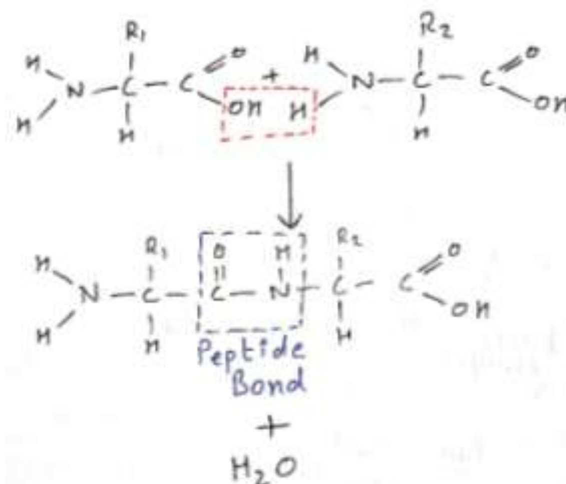


Fig.3:- Formation of peptide bond.



The amino acids have both acidic and basic terminal as they called the zwitterion. This amino acid under-go neutralization reaction with another amino acid and relies on water. This creates a bond called peptide bond, one protein consists of such type of several bonds hence they also called polypeptides.

In many diseases any specific protein is responsible and sometime just by deactivating the protein the disease get cure. This can be done by change the shape of protein binding site. As the protein is highly sensitive towards the binding site structure.

So, it is important to understand the protein monomer composition, this can help to know about the shape and structure of the protein to make a suitable drug, or in other words, the study of the sequence of amino acids is important in the drug discovery process. The finding of the protein composition process is called protein sequencing.

Protein sequencing

This is a process to find out the sequence or grouping of amino acids of protein or peptide and analysis the monomeric compositions. This is important because by this we can understand the structure, as the structure of the protein is highly important for its activeness. Proteins are only got active in a specific shape. Each protein has its specific shape and this is different because of its monomeric amino acid as each amino acid applies different forces and attrition to another amino acid in the chain these forces are weak forces and disulfide bonds.

Let’s have an example if there is a disease in which any specific protein is causing the disease to the body so deactivating the protein, we can cure the disease. To do so we can introduce any organic or inorganic compound which is structurally similar to the shape of the active site so that the protein's original shape gets changed and it not recognizable by the ligand or receptors. By this, we can avoid the disease. This is the general idea to understand the importance of protein sequencing in drug discovery.

In the general idea of protein sequencing, we have to take the protein that extracted from the cells, as we know that protein is a complex compound one side it has a peptide bond between each amino acid, and on the other side, we have disulfide bonds, this is a strong bond between 2 Sulphur containing R group and this play important role in the protein 3D structure. To start the sequencing preparation, we have to first destroy the disulfide bonds, this can be done either by reduction and oxidation process.

In oxidation we have to treat the protein sample with performic acid by this the protein disulfide bonds get cleaved and become cysteic acid this is a stable compound and prevents the re-forming of disulfide bonds. On the other hand, we treat the protein sample with a reducing agent like Dithiothreitol (DTT) or β-mercaptoethanol (β-me) this reduces the disulfide bonds and this becomes -SH and -SH. But still, after this it can able to re-form the disulfide bonds to stabilize, we have to add iodoacetate to form carboxymethyl-cystic. As shown in the reaction shown.

Fig.4:- Destroying reaction of disulfide bond.

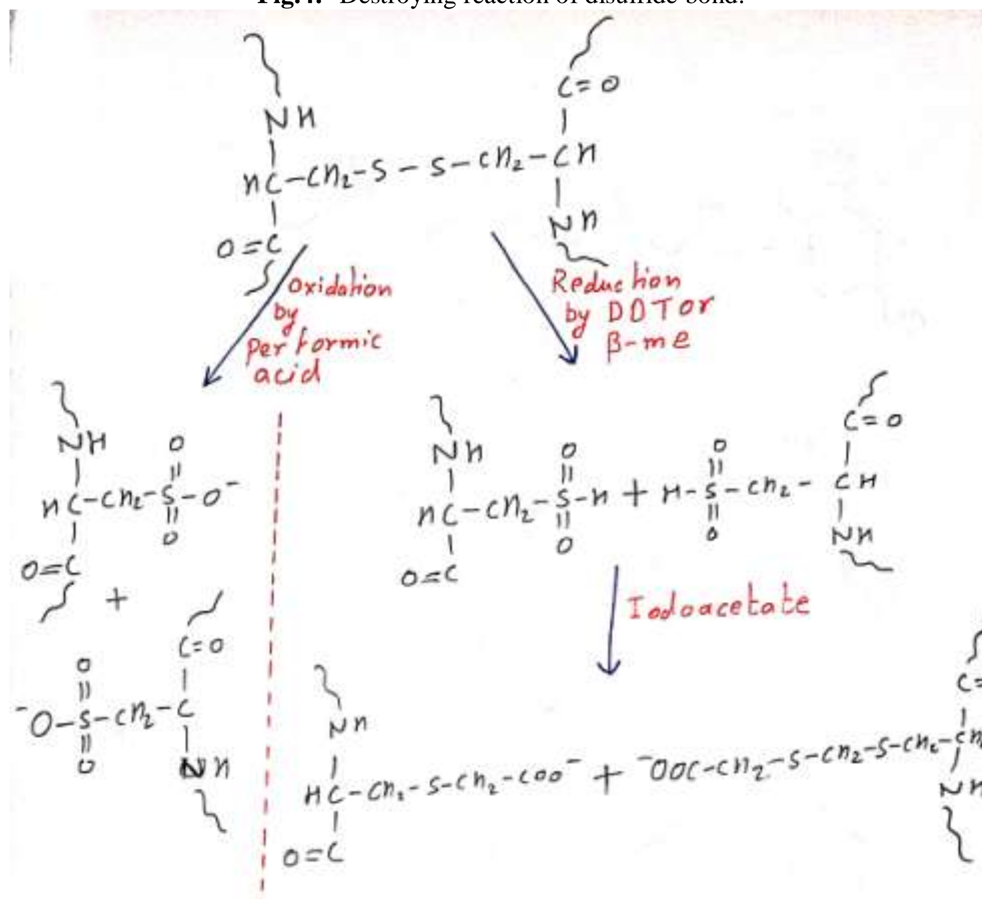


Table 1:- Specific site protein cleavage reagents.

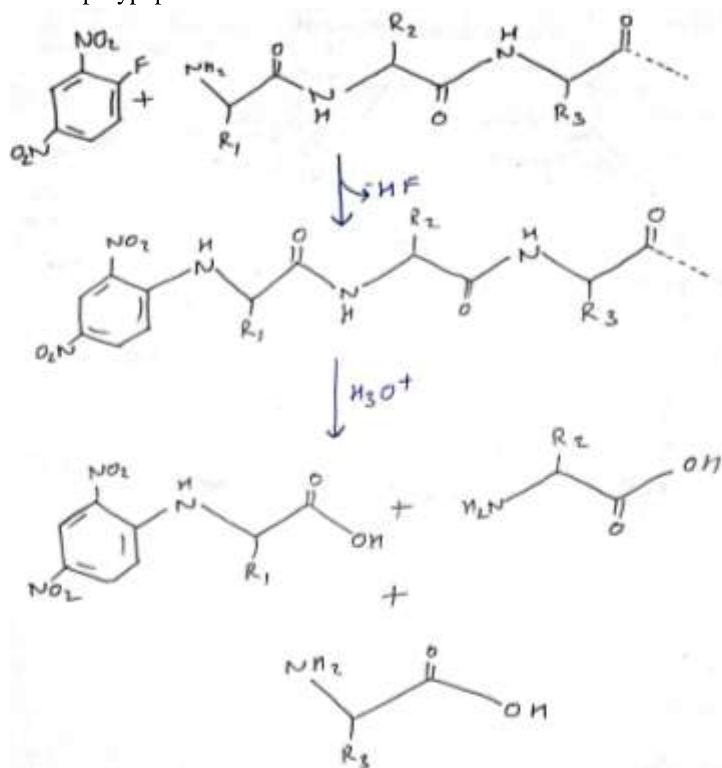
S.NO	Reagents	Cleavage site
1	Trypsin	After Lys, Arg
2	chymotrypsin	After Phe, Trp, Tyr
3	pepsin	After Leu, Phe, Trp, Tyr
4	Cynogen bromide	After met

After destroying the disulfide bonds the chain is free from any strong bond that can change the shape of the protein sequence by this, we get a linear polypeptide chain but it is not possible to deal with such a large molecule for sequencing, for this, we have to cleaved the protein chain to get small fragment that can go for the sequencing process. To do so we have both biological and chemical reagents for example we have Trypsin, chymotrypsin, pepsin as a biological reagent, and cyanogen bromide as a chemical reagent. Each reagent has a specific cleavage site as shown in the table.

By applying these reagents to the sample, we get a small fragment of the polypeptide for the sequencing process.

Methods:-

Fig.5:- Reaction of DFNB with polypeptide chain.



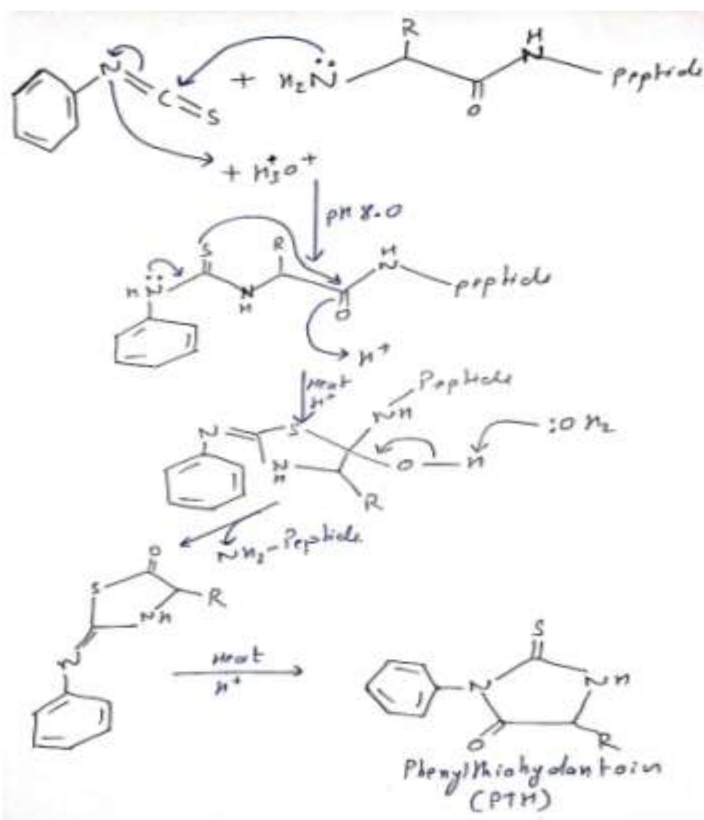
There are mainly 3 methods by which we can sequence the polypeptide chain these processesare Identifying N-terminal by using sanger reagent, Edman degradation sequencing, and C-terminal residues. In the first method, we are going to identifying the nitrogen end terminal of a small fragment polypeptide chain. In this, the sample is treated with a sanger reagent which is 1-fluoro-2,4-dinitrobenzene (DFNB) this reagent combined the amino-terminal of the polypeptide chain and release HF. DFNB can also react with a free amino group to form a Dinitrophenyl-amino acid complex. After this step, we have to hydrolysis the chain, under the acidic condition the is braking of a peptide bond between two R-group and as a result, we get free amino acids that can be combined with other DFNB present in the solution. Now the product can be analyzed by the HPLC or TLC separation and compare with the standard amino acid data (reaction as shown).

Fig.6:- Edman degradation reaction.

The 2nd method is called Edman degradation sequencing in which we use phenylisothiocyanate reagent this reacts with a terminal amino group and forms cyclic Phenylthiocarbomoyl derivatives. Under acidic conditions, the terminal amino acid is cleaved from the main chain as a thiazolinone derivative. This compound can be extracted into the organic solvent and it forms phenyl-thiohydantoin-amino acid (PTH-amino acid) in the presence of acid it can be separated by TLC or HPLC and then by comparing standard amino acid data (Reaction as follow).

The 3rd method is C-terminal Residue in this carboxylic terminal will attach with reagent and help to identify the amino acid. There is not too much method are developed for this but the most common method is the polypeptide sample will be treated with carboxypeptidase this attached with carboxylic terminal and relies C-terminal and then we can go for the Edman or N-terminal sequencing method for the amino acid identity.

After all the above steps we can identify the R-groups of the amino acid but not the actual sequence as initially we have a whole protein chain, this is because now it is in the fragmented form. But to identify the actual sequence we have a technique that initiallywe use different protein cleavage reagents it

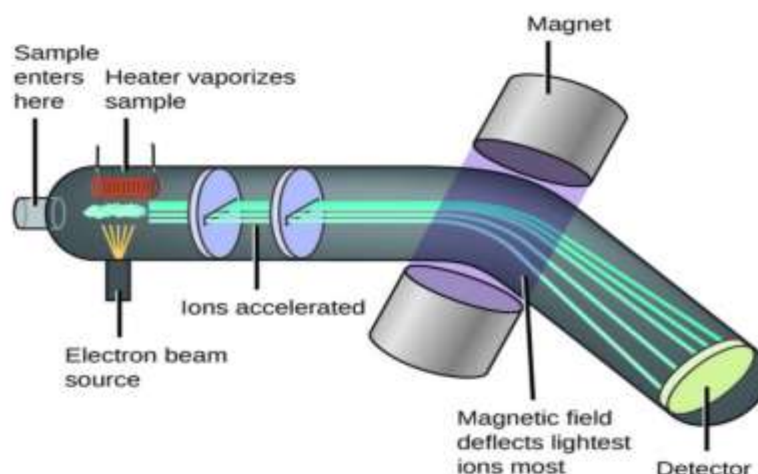


produces different overlapping amino acid stretches and these stretches can be used to put the whole sequence. For example, we have the protein chain and we cleave the chain into 4 fragments, so we have a fragment like fragment-1, fragment-2, fragment-3, fragment-4 but apart from this we also get fragment which not get cleaved and become fragment-1 and 2 or fragment-2 and 3 or fragment-3 and 4 respectively, by analyses this mega fragment we can compare the micro fragment and orientated the fragment as it is in the initial stage before cleaving.

To understand the original shape of protein it is also important to locate the Disulfide bond this can help to get a clear image of the sequence of the protein. To do so we can do the same protein sequence process but without destroying the Disulfide bond and by comparing both the result (with and without destroying Disulfide bond) we can get an idea that in which point of the chain does the Disulfide bond is present. In normal condition if the chain brock between Disulfide bond we get 2 independent chains but without breaking Disulfide bond it appears as one long chain of protein we can use this for comparing. For the last step we or protein sequencing we can go for the Mass spectrometry method or UV-vis spectroscopy method for the identification of the monomeric unit of a polypeptide chain.

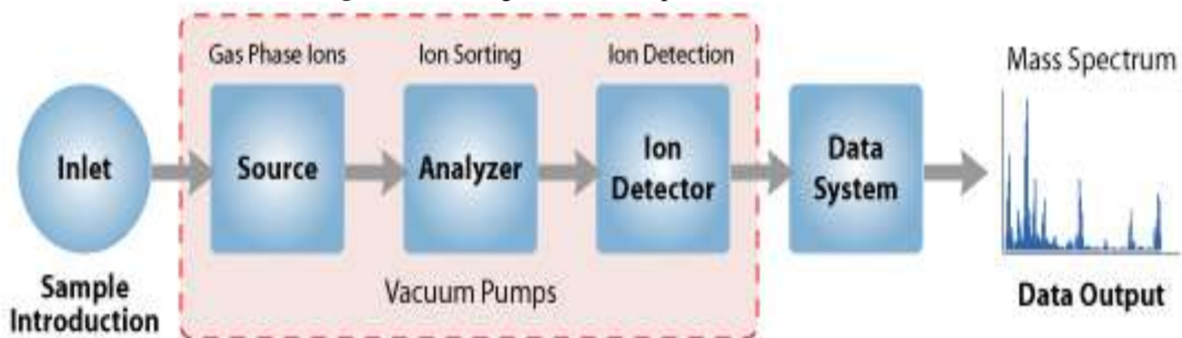
The equipment uses in the protein sequence process: -
Mass spectrometry
Above is the chemical method but the new and more reliable method is Mass spectrometry. This is a technique in which the biomolecules such as protein are analyzed or identify the unknown protein. This is a process in which we get to know the structure and chemical properties of the protein. In this, the sample is converted into gas phase ions by applying arc or high temperature. In this process, we test the sample with or without the fragmentation steps. The sample is characterized by its mass-to-charge ratio and compares with standard molecular data.

Fig.7:- Mass spectrometer.



Basicprinciple: -
The principle of this technique is generating multiple ions from the sample that has to be identified. After making samples as gas ions, it separates them according to their mass-to-charge ratio, and then it records the relative abundance of each ion type.
The first step of this process is to convert the sample into the gas phase ion this is done just by electron ionization. Due to this molecule undergo fragmentation in this it converts to the primary ions which derived from molecular ions and then it separated according to their mass-to-charge ratio then detected in proportion to their abundance. By this process, the mass spectrum of the sample molecules is produced. This result is displayed as a plot of ion abundance versus mass-to-charge ratio.

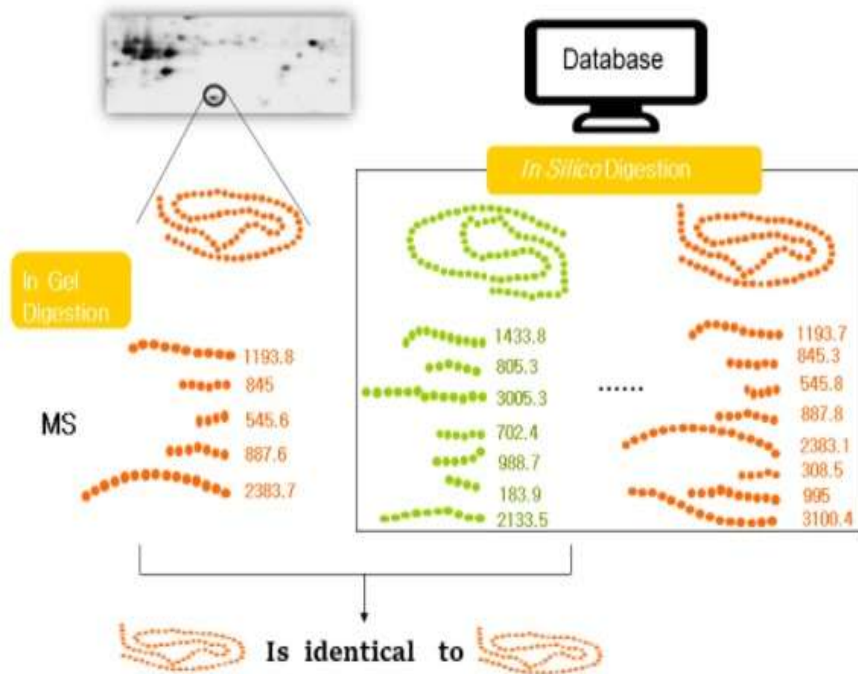
Fig.8:- Block diagram of mass spectrometer.



Components:-
1. Ion source
2. Analyzer
3. Detector system
4. Operating system

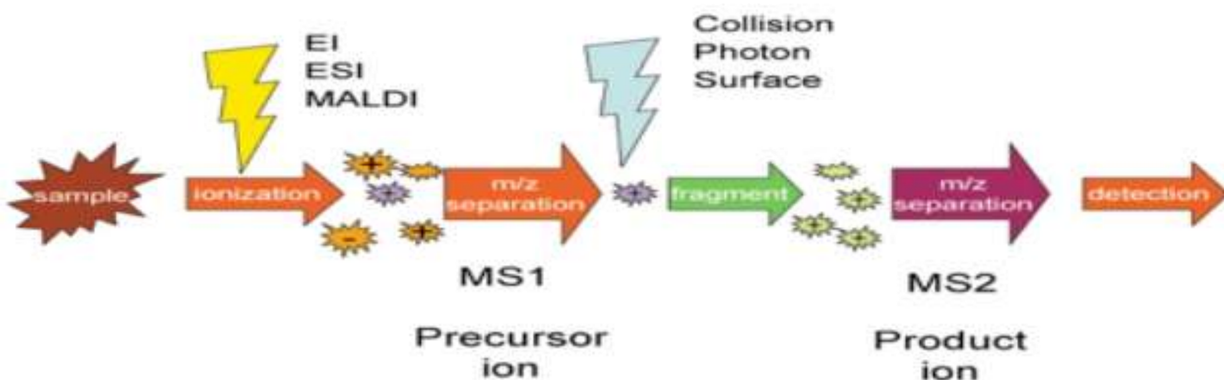
Peptide mass fingerprinting: -
After the mass spectrometry, the related data has to be analyzed with the database to find out the actual protein that is present in the sample for that we use peptide mass fingerprinting. This is a high throughput process in which the sample data is compared to all the protein sequence MS data present in the protein database.Peptide mass fingerprinting is always performed withMatrix-assisted laser/desorption ionization-time of flight (MALDI-TOF) mass spectrometry.In this technique, the protein is treated with proteolytic enzyme and separate with the help of SDS-PAGE electrophoresis or HPLC technique and gather the sample protein data by mass spectrometry and on other hand, the protein database is used which have nearly all the protein sequencing mass spectrometry data that is known by the mankind. And compare it and show the best possible matches.

Fig.9:- Layout of peptide mass fingerprinting.



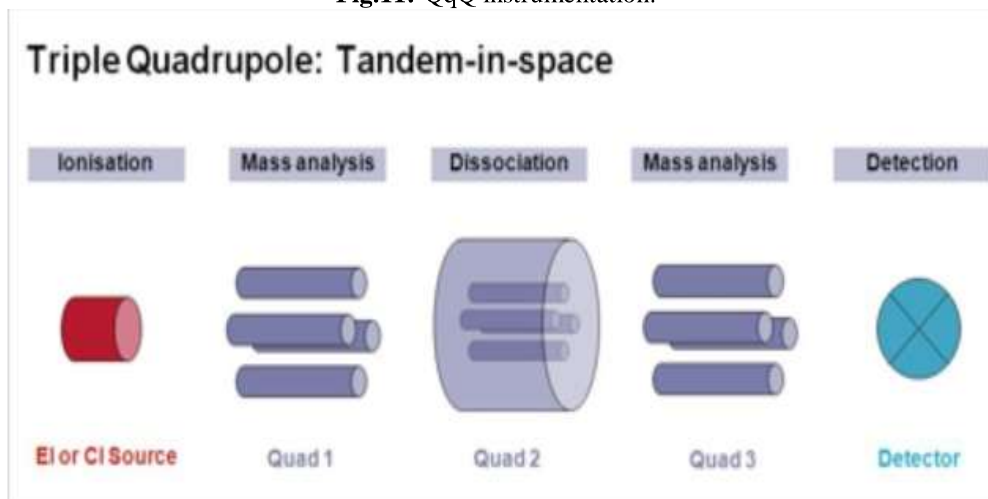
Tandem spectrometry:-
Tandem mass spectrometry is a technique in which samples undergo two separate ionization techniques. In this, the loaded sample in our case its protein is first to undergo product ionization in which molecule ionization step occurs, then it separates as per their mass-to-charge ratio this hole step is called MS1. After this step, it undergoes fragmentation in which the components become ions and go for the detection this is called MS2.

Fig.10:- Layout of tandem spectrometer.



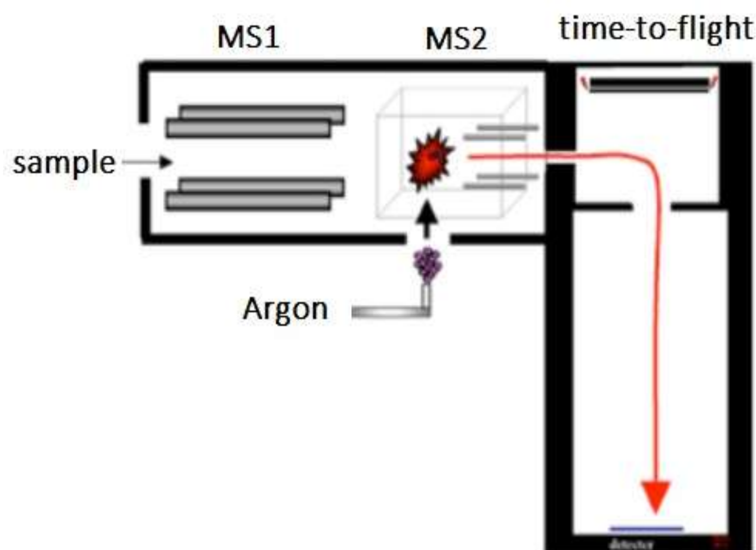
QqQ (triple quadrupole): -this is a 3-part ionizer quad 1, quad 2, quad 3 in this precursor ion are selected, fragmentation (dissociation), the final generation of product-ions respectively. After this, it goes for detection.

Fig.11:-QqQ instrumentation.



QTOF (quadrupole time-of-flight): -in this, the sample is converted into ions and selected then it goes to the collision cell for fragmentation. The productsions are separate as per the time-of-flight mass spectrometry.

Fig.12:- QTOF instrumentation.



High-performance liquid chromatography or high presser liquid chromatography (HPLC): -
This is a type of chromatography specifically used for the analysis of biochemical with the help of this we can separate the biomolecules and analysis by comparing standard data. This consists of a column filled with packing material (stationary phase), a pump that helps to move the mobile phase through the column, and a detector that show the retention time of molecules. This retention time depends on the interaction of the stationary phase, the molecule being analyzed and the solvent used. In this, a small sample is introduced to the stream of mobile phase, in this sample experience retarded force by the specific chemical or physical interaction with a substance present as stationary phase. This retardation is depending on the nature of analyte, solvent, and stationary phase components. The solvent can be any organic solvent, water, or combination of both water and organic liquid. The retardation phenomena help to separate the components present in the sample, this is done to analyze the compound present in the sample and this analysis is performed by spectroscopy method.

Fig.13:- HTLC devise.

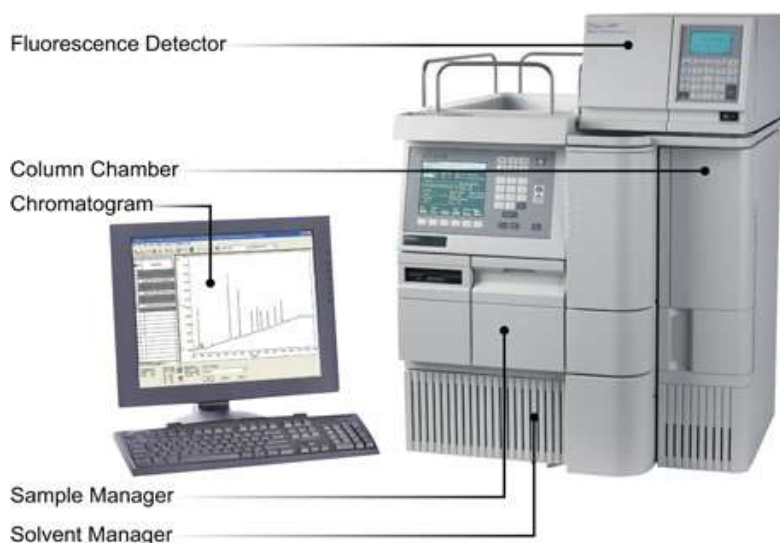
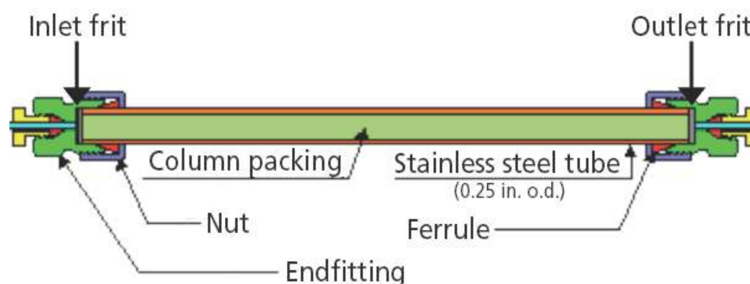


Fig.14:- HTLC filament for resting and mobile phase.



Types of HPLC: -

1. Normal phase chromatography
2. Reversed-phase chromatography
3. Size exclusion chromatography
4. Ion exchange chromatography
5. Bio-affinity chromatography

Instrumentation: -

1. Injection of sample: -

Septum injectors are used to inject the sample solution in the mobile phase where it gets to mix with the solvent of the mobile phase.

2. The detector: -

There can be many detectors attached with the device, the most common is a UV-visible spectroscope, many organic compounds observe the wavelength of this range energy by calculating the observed energy we can analyze the components of the sample.

3. Interpreting the output from the detector: -

the output is recorded as series of peaks, each peak represents the components present in the sample pass through the detector. This can be possible by calculating the absorbed energy, this process is automatically done by a computer connected with this device.

Thin-layer chromatography (TLC): -

This is a technique in which we separate the non-volatile mixture, mainly this technique is used to separate the biomolecules sample such as protein fragments, carbohydrates, etc. this experiment is performed on the aluminum foil, plastic, or glass which coated with a thin layer of absorbent material. For example, aluminum oxide, cellulose, silica gel, etc. In this the sample and sometimes control sample also loaded at a sport few centimeters above the bottom of a plate and put inside the gas jar and add any volatile organic compound such as ethanol, methanol, acetone, etc. this helps to raise the sample and the control at a point, from that point we can calculate the distance traveled by the sample and this help to find the R_f value (retention factor).

$$R_f = \frac{\text{distance traveled by sample}}{\text{distance traveled by the solvent}}$$

Each compound has its specific value concerning the solvent by comparing it we can find out the component present in the sample. Many times, the sample did not have any pigment so to spot the growth we put the plate under UV

light after completing the experiment this help to find the spot of the growth of the sample for each component as shown in fig.

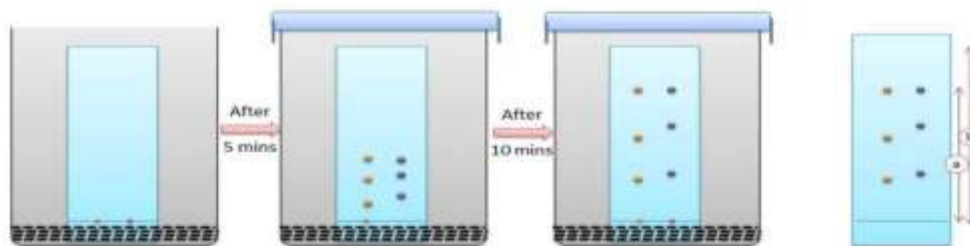


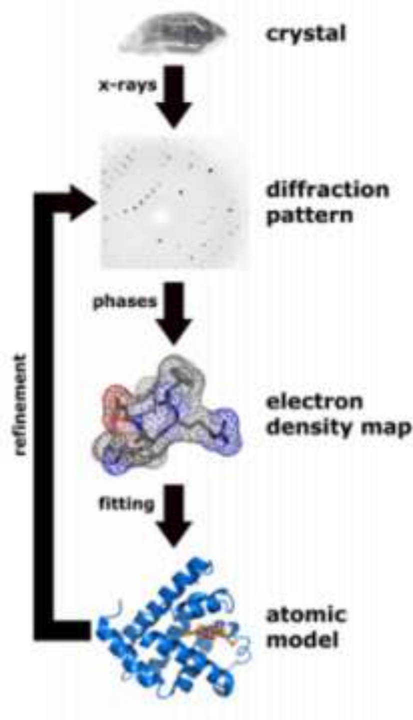
Fig 15:- TLC process.

After all the abovetechnique we get to know about the sequence of protein monomer by which we can get to know about the nature and biochemical property of the protein but in the case of protein, this is not sufficient because protein activeness is dependon its structure so it is important to know about its 3D structure by which we can make the road map for the drug discovery. By knowing the 3D structure, we can make the idea that which compound is most suitable for binding with the desirable protein and go for the high throughput process. To know about the 3D structure the best way is X-ray protein crystallography.

X-ray protein crystallography: -

This is a process in which the protein converts into the crystal and then is placed between detector and X-ray emitter module which supply X-Ray this X-ray is pass through the crystal. The crystal changes the path of the ray with an angle these rays fall in the detector plat which connected to the computer which shows the 3D structure of the protein crystal by this we can analyze the 3D structure of the signal protein structure.

Fig.16:- X-Ray protein crystallography layout



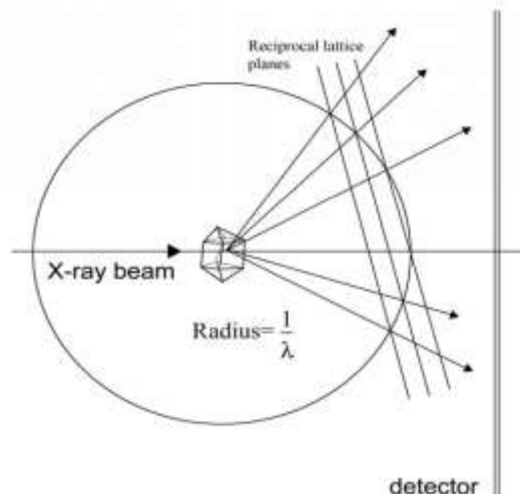


Fig.17:- X-Ray deflection on detector due toprotein crystal.

The most important step of this technique is to make a pure protein crystal that is without any impurity. For this step sample is treated for its purity should be 90-95% then adjust the PH and temperature.

It is typically important to complete an incredible number of analyses to decide the best crystallization conditions while utilizing a base measure of protein per analysis. The protein fixation ought to be about 10mg/ml, in this manner 1mg of cleaned protein is adequate to perform around 100 crystallization tests. Crystallization can be completed by utilizing various strategies, the most reliable are fluid dissemination techniques, crystallization under dialysis, and fume dispersion procedure. since it is not difficult to set up and permits the bio crystallographer to use a base protein sum. The fume dispersion process is the "hanging drop" and the "sitting drop" techniques.

The "sitting drop" technique is ideal when the protein arrangement has low surface pressure and the equilibration rate between drop arrangement and repository arrangement should be eased back down. A schematic outline of a sitting drop vessel is displayed in the boundaries that can be fluctuated incorporate nature and centralization of the encouraging specialist; supports to investigate the whole pH range; extra salts and cleansers, etc.

Crystal cryoprotection: -

The most broadly utilized cryo-mounting technique comprises the suspension of the gem in a film of a "liquid catalyst" arrangement, held by surface pressure across a little measurement circle of fiber, and the fast addition of a vaporous nitrogen stream. The cryo-preserved arrangement is acquired by adding, cryo-protectant specialists like glycerol, ethylene glycol, MPD (2-Methyl-2,4-pentandiol), or low atomic weight Stake (polyethylene glycol) to the precipitant arrangement. The precious stone is immersed in this sample for a couple of moments preceding being streak frozen. The technique puts minimal mechanical weight on the precious stone, so it is astounded for delicate examples. Circles are produced using exceptionally fine (~10 µm breadth) strands of nylon. As certain precious stones debase in development and reap arrangements, fluid nitrogen stockpiling is a phenomenal method to settle gems for significant stretches. This framework is especially valuable when planning tests for information assortment at synchrotron radiation sources, in that, by limiting the time needed by test arrangement, it give the time to gather the information.

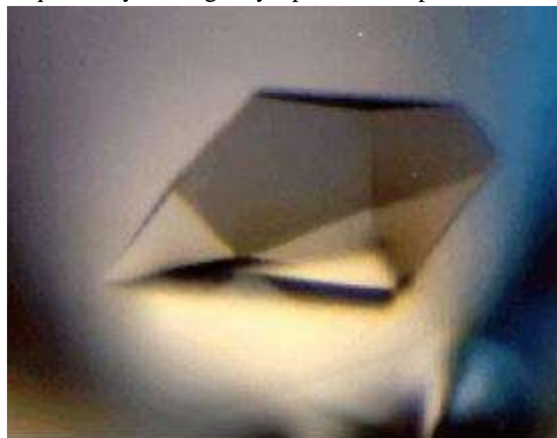


Fig.18:- protein crystal.

By the above all the techniques, steps, and efforts we get all the possible details about the spotted protein such as monomeric sequence, the location of bonds, its shape, and most important its 3D structure. But this is just a half battle that we won because we have to discover the drug that can treat the disease by de-activating the protein that causing the disease. This is the longest and time-consuming step as we have more than 100,000 compounds and only one or two can de-activating the protein. But luckily, we discover the technique that helps to discover the drug compound this technique is called high throughput screening. Due to this technique, the pain taking and the long process becomes much easier, and also it is more effective.

High throughput screening (HTS) of drug discovery: -

High throughput screening is a technique in which a large number of compounds (compound library) are tested against the selected protein sample. On early days of this technique each test of a compound is performed manually this is a painful task and also create many errors but nowadays thanks to advance technology all the process is automatic with the help of advance robotic, better liquid handling devises, and advance computer which can interrogate more than 10,000 compounds at a time this highly efficient technique is called high throughput screening. In this process we take more than 10,000 same samples such as a protein that has to be the target for the drug discovery this sample is placed in a plate which consist of many micro-wells this help to separate the sample in a different chamber, this well is small in volume so that the sample is in small quantity, this pouring of sample is nowadays done by robotic arms which have many pipettes this help to pouring the sample to the well of the microtiter plate after this from the compound library we introduce more than 100,000 compounds as per the capacity (one compound in one well) with some indicator such as fluorescence or luminescence this help to show the drug action into the protein or sample. By doing these steps we can generate hits which is a basic starting point for this process. This whole process is performed by an integrated system, robotics, instrument platform, computer management, bioinformatics, and specialized peoples.

Fig.19 high throughput screening robotic arm

Steps of high throughput screening: -

Assay development and optimization: -

This the step the function instruments are checks for the process and one duplicate runs in perform to check that all the components are working up to the mark or not. This test run was performed with 10,000 compounds with the sample.

primary screen:-

This is just the starting points of the assay; in this, we test all the possible compound from the compound library to find the hit molecules which can interact with the selected protein of component present in a given sample. In this process, we interrogate a large number of drug nature compounds so that a few of them who can take any action with the sample is taken out and go for secondary assay such compounds are called hit compounds.

Secondary assay: -

In this, by using hit compound we again run the same process in which this selected compound again interrogates with the sample for conformation and can go for pre-medical trials and be the drug for the suitable drug for the disease.

Pre-medical trials

Informatics: -

This is a group or cluster of computer software that acts as back support to store, compare, save data or receive data from the server from all over the world. All the information, notes, result, conclusion, errors are inserted in a database from where the information can retrieve in future or during the research. In this there are severalsoftware's are present in which the result of an experiment is plotted as a graph or peaks which we can use for the comparing with another data to get the result of getting any conclusion.

This process has many complex stages and required respected planning to start this technique.

Components: -

1. **Compound collection: -**this is a library on all types of different compounds such as biochemical, organic chemical, and inorganic chemical which can be used as a drug for any disease.
   This chemical must have drug-like properties, compound cluster, or grouping of common structure motets this can help in the arrangement of chemicals or reduce the complexity.
   Each cluster must have its representatives so that it's much easy to select the respected cluster of compounds. For example, if we have 1 million compounds in the library there must be at least 50,000 clusters and each cluster at least 20 representatives must present.
2. **Assay amenable to HTS: -** as this topic name suggests high throughput screening which means higher speed in this process the systems must design like they can support a large number of compound interrogations in a short period. It is a fully automatic process and is mainly categorized into two parts (homogenous and heterogeneous).

the homogeneous assay is a process in which we observe the interaction between analyte and surroundings it has a simple step, the reagent can be introduced to the sample in one step or more than one step according to their requirements. It includes only simple principal steps such as the addition of components or reagents, incubation, and reading or observation also it consists of different detection techniques such as fluorescence, radiometric, etc. its main advantage is its simplicity in the HTS technique which contribute to reducing cost and complexity. During detection, the interference can come because of the presence of different assay components due to this the signal-to-noise ratio is less than 10.

On the other hand, the heterogeneous essay is composed of additional setups such as centrifugation, filtration, etc. also for this separate computer setup, this increases components, increases complexity but on another hand, this increases the accuracy and signal-to-noise ratio. A heterogeneous assay is required when a homogenous assay fails to give qualitative results.

Types of high throughput assay: -

Biochemical assay: -

these assays are for the receptors, proteins, enzymes. In such assays, drug target is known and this technique uses scintillation proximity assay (SPA), radiometric, colorimetric fluorescence detector for the detection of the assay actions.

Scintillation proximity assay (SPA) was a technique in which reaction was performed as per homogenous types which means in this washing or filtration steps are ignored, radioactive which emit electron at about 10μm to the surrounding solvent especially in water for the assay. This technique was performed for the cell surface receptor due to its low receptor requirements or high binding tendency. There are many biochemical assays types are there such as: -

Fluorescence resonance energy transfer (FRET): -

FRET is a non-radioactive quantum mechanical process. In this transfer of energy from the excited donor to the suitable acceptor fluorophore, in this process, the donor molecules take energy from incident light and transfer it to the nearby acceptor molecules. The most common pair of such molecules are cyan fluorescent protein (CFP) – yellow fluorescent protein (YEP), green fluorescent protein (GFP). For this reaction the pair molecules must be in a nearby or short distance, there ought to be critical contrast in degree of the extinguishing of the beginning material and component, Progress dipole directions of benefactor and acceptor should be roughly equal.

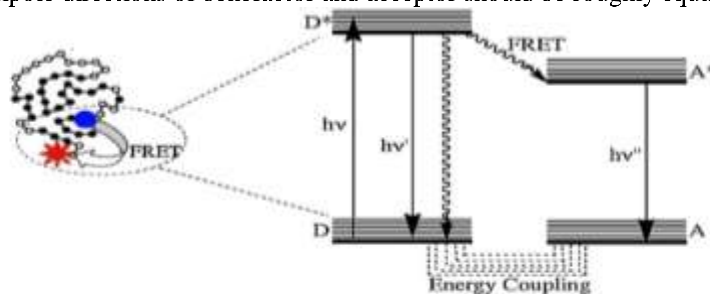


Fig.21:- Principle of fluorescence resonance energy transfer.

Fluorescence polarization (FP): -

When the fluorophore is illuminated with light, it gets excited and it stays consistent all through the excitation state, it emits light energy in a polarizing plate, but if it rotates it give light in another phase (depolarized). Large molecules show less rotation hence they stay more in the polarized state. hence give more light but when molecules are small or it does not bind with another molecule they can rotate and give less light as now it is in the depolarized state. By comparing this we can analyze the action of the drug with protein or substrate. This has been used to measure biochemical properties like protein denaturation, and connection of proteins to nucleic corrosive, and so on, used to consider receptor/ligand contemplates, Tyrosine Kinase Tests, and so on.

$$\text{Polarization (mP)} = 1000\left(\frac{S - G(P)}{S + G(P)}\right)$$

S = intensity with polarizer parallel
P = intensity with polarizer perpendicular
G = intensity and assay dependent grating factor

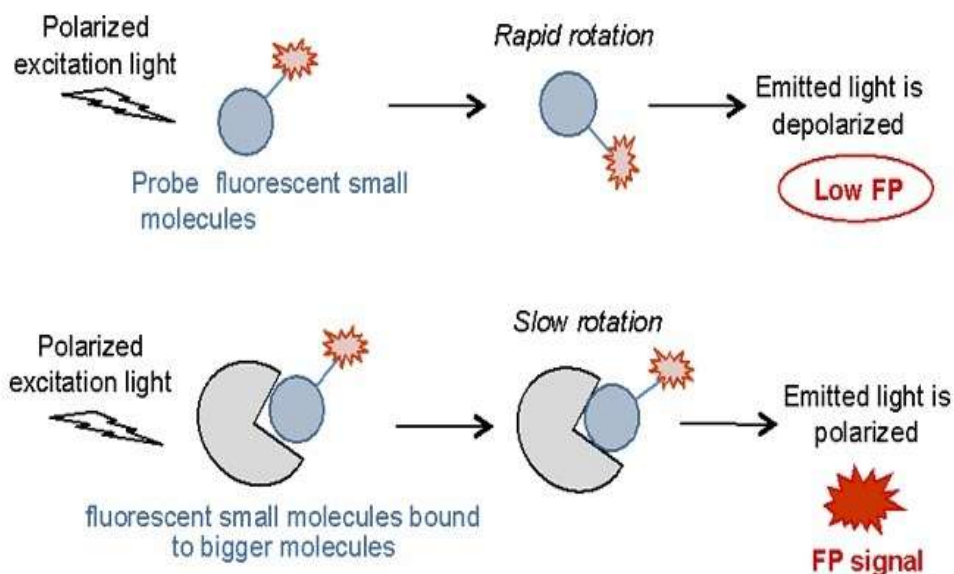


Fig.22:- Principal of fluorescence polarization.

Homogeneous time-resolved fluorescence: -

This is a combination of standard FRET technology and time-resolved measurement (TR) of fluorescence**.** This allows ignoring most of the short-lived background fluorescence which happens due to interfering material present in the sample or solvent. This result in a delay of approximately 50 to 150µsec between the initial excitation and the fluorescence measurement. europium cryptate (Eu^{3+} cryptate) is used as an energy donor, this is a rare-earth complex consisting of macrocycle within which Eu^{3+} ion is tightly embedded. The presence of receptor looks like cage it acts as an antenna that collects and transfers energy to Eu3+ which is the energy with the long-lived fluorescent pattern which is specific. this is used as a fluorescent donor with allophycocyanin or XL665 as a cross-linked. This is a psychobilly protein pigment extracted from red algae. This worked as an acceptor Donor. This is a form of hybrid technique that has a long fluorescence life period of europium cryptate. This doner gated from a short period this initial activeness of fluorescence not measured.

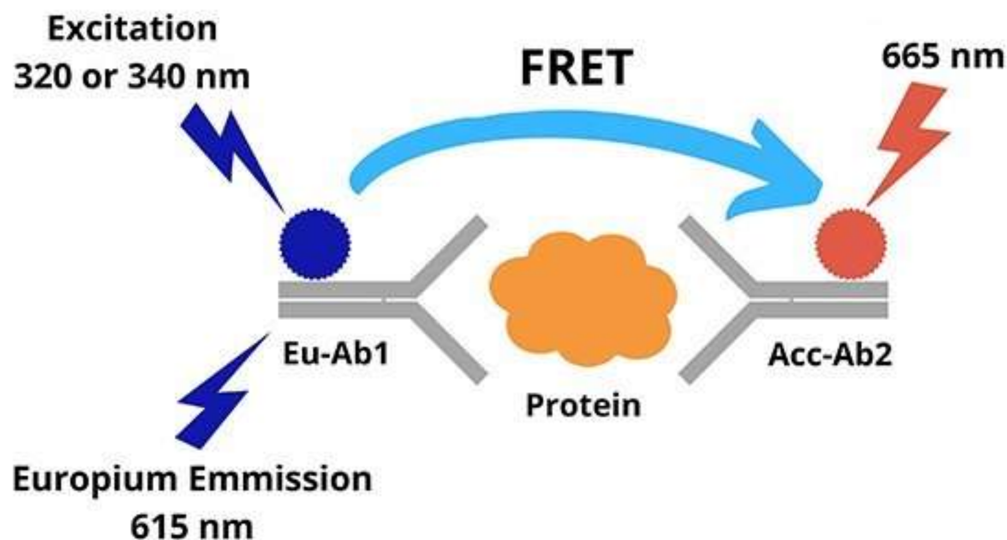


Fig.23:- Principal of homogeneous time-resolved fluorescence.

Advantage of the biochemical assay: -
1. Simple
2. Consistency
3. Direct measuring of target engagement
4. Increase specificity of compounds
5. Can measure compound characteristics

Disadvantage biochemical assay: -
1. Maybe non-physiological
2. Not sensitive to compound properties such as membrane permeability, toxicity, or off-target effects.

Cell-based assays: -
This is a most unique assay because of its complexity in this the target is unknown in this we specially have to deal with hole cell components such as reporter protein, receptor protein, ion canal gates, etc. but the target is unknown and we have to work just as hit and trial method, the cell-based assay is classified.

Second messenger assays: -
It screens signal transduction from actuated cell-surface receptors. Second courier tests normally measure quick, transient fluorescent signals that takes less than seconds or milliseconds. many fluorescent particles are known to react to changes in intracellular Calcium particle focus, film potential, and different boundaries, consequently, they are utilized being developed second courier tests for receptor incitement and particle channel activity. The improvement of hydrophobic voltage-sensitive probes and FRET-viable microplate instrumentation has helped the headway of the evaluating strategy for particle station drug discovery.

Cell proliferation assays: -
It screens the general development or no development of the cell to outer improvements. These are fast and simple to be utilized for robotization.

Advantage of the cell-based assay: -
1. more physiological, amenable to a systems approach
2. may sensitive to compound properties (membrane permeability, toxicity, or off-target effects)

A disadvantage of the cell-based assay: -
1. Phenotypic assay dos not directly measure target engagement
2. Complex

3. Variable
4. Exclusion of less soluble permeable compound

Fig.24:- Barcoded microtiter plate for HTS.

High throughput assay is performed in barcoded microtiter plates, this is the high-density plates consisting of many wells in which the separate experiments are performed with different compounds and for specific targets in these few columns are dedicated for the control runs and others are for test runs as per the protocol, by using these plates we can perform and take around 30,000 to 100,000 data points per day thanks to the advance robotics and the multi well plates. The volumes of these plates are very plate to plate for example for 96 well there may be 100-200µl will be the volume for 384 well plate 25-50µl, 1536 well plate 4-10µl, 1456 well plates 1-2µl can be the internal volume and so on this plate can be transparent, black or white. The reagents can be used with some stabilizer, water, organic solvents such as DMSO, 0.1% trypsin, etc. as per the requirements. The moto of this assay performing in HTS is to test and observe the activeness of the compound with the given analyte.
For this, we have to calculate the signals, background, noise, visibility, and their ratios such as: -

$$\text{signal : background} = \frac{\text{mean of the sample signal}}{\text{mean of the control signal}}$$

$$\text{signal to noise ratio} = \frac{\text{mean of sample} - \text{mean of control}}{\text{standard deviation control}}$$

$$\text{low visibility (cv) to \%cv ratio} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Z-factor:-
Z-factor is the quality of the assay itself without the intervention of test compounds, the Z will be between 0 to 1 after calculation as per the formula.

$$Z = 1 - \frac{3\ (\text{standerd deviation sample}) +\ 3\ (\text{standerd deviation of control})}{|\text{mean of sample} - \text{mean of control}|}$$

This is done to check the compounds which we test is either need to consider or not with the help of this we can eliminate the batch of compounds and reduce the compounds and able to focus on our desirable compound. By this, we get the results that which of the all-drug compound is working with the protein and do any effects on the protein as our desirable the sensors receive the florescent comes from the experimental wells and interpreted with the data by calculating this, we can get to know the Z-factor.

Conclusion And Discussion:-

Form this technique we get to know about how the new drug is discovered for disease. From this, we get to know about how protein is used as a reference for discovery also how much the protein is important for drug discovery. This shows that how a complex long-chain biomolecule (protein) is taken and sequence them to get full details of the protein how spectroscopy and chemical method help for sequencing, how to advance chromatography technique help in identification and finally one fully automatic technique which has advance robotic arms many advance

fluorescence technique reduces the time and cost of drug discovery and find one best drug out of more than 10,000 compounds which have a drug nature this is a complete technique of protein sequencing and drug discovery by using high throughput screening assay.

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