***Lec.7Bioseparation Technique* Affinity chromatography**

**Affinity chromatography is a technique for separate and purification of most biological molecules depends on the specific interaction between pairs of biological materials such an enzymes. Affinity chromatography was used initially in protein isolation and purification particularly enzymes. Since there many other applications have been developed for enzyme inhibitors, antibodies, nucleicacid and recombinant proteins.**

**Affinity chromatography is a method of separating**[**biochemical**](https://en.wikipedia.org/wiki/Biochemistry)**mixtures based on a highly specific interaction between**[**antigen**](https://en.wikipedia.org/wiki/Antigen)**and**[**antibody**](https://en.wikipedia.org/wiki/Antibody)**,**[**enzyme**](https://en.wikipedia.org/wiki/Enzyme)**and**[**substrate**](https://en.wikipedia.org/wiki/Substrate_%28biochemistry%29)**, or receptor and**[**ligand**](https://en.wikipedia.org/wiki/Ligand_%28biochemistry%29)**. Biological macromolecules, such as enzymes and other proteins, interact with other molecules with high specificity through several different types of bonds and interaction. The high selectivity of affinity chromatography is caused by allowing the desired molecule to interact with the stationary phase and be bound within the column in order to be separated from the undesired material which will not interact and elute first. The molecules no longer needed are first washed away with a buffer while the desired proteins are let go in the presence of the eluting solvent. This process creates a competitive interaction between the desired protein and the immobilized stationary molecules, which eventually lets the now highly purified proteins be released.**

***Affinity chromatography principle:***

**The stationary phase is typically a gel matrix, four polymers which are often used for most materials (agarose,sepharose- Cl-4B, dextrose, cellulose and polyacrylamide) to prevent steric interference during the binding process of the target molecule to the ligand, an inhibitor containing a hydrocarbon chain is first attached to the agarose bead which commonly used as a gel matrix, this inhibitor with a hydrocarbon chain is commonly known as the spacer arm between the agarose bead and the target molecule. Agarose activated with cyanogen bromide is one of the most commonly used supports for coupling of amino acids.**

***Column setups:***

**Usually the starting point is an undefined heterogeneous group of molecules in solution; the molecule of interest defined property, and can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on stationary phase, the other molecules in the mobile phase will not become trapped. The stationary phase can then be removed from the mixture, washed and the target molecule released from the entrapment in a process known as dialysis.**

**The initial mixture run through the column to allow setting, a wash buffer run through the column and the elution buffer subsequently applied to the column and collected.**

**The ligand binding substances which immobilized on an insoluble support (matrix), the ligand is attached to the matrix by physical adsorption or chemically by covalent bond, the ligands used in affinity chromatography are obtained from both organic and inorganic sources; examples of biological sources are serum proteins, lectins and antibodies, inorganic sources as metal chelates and triazinedyes. Affinity columns can be**[**eluted**](https://en.wikipedia.org/wiki/Elution)**by changing salt concentrations, pH, pI, charge and ionic strength directly or through a gradient to resolve the particles of interest.**

***Affinity chromatography uses:***

**Affinity chromatography can be used to purify and concentrate a substance from a mixture into a buffering solution, reduce the amount of undesired substances in a mixture, identify the biological compounds binding to a particular substance, purify and concentrate an enzyme solution.**

**Affinity chromatography can be used in a number of applications, including nucleic acid purification, protein purification from cell free extracts, and purification from blood. By using affinity chromatography, one can separate proteins that bind a certain fragment from proteins that do not bind that specific fragment,because this technique of purification relies on the biological properties of the protein needed, it is a useful technique and proteins can be purified many folds in one step.**

***Lectin:***

**Lectins are carbohydrate-binding proteins, macromolecules that are highly specific for sugar moieties. Lectin affinity chromatography is forms of affinity chromatography where**[**lectins**](https://en.wikipedia.org/wiki/Lectin)**are used to separate components within the sample,Lectins, such as**[**concanavalin A**](https://en.wikipedia.org/wiki/Concanavalin_A)**are proteins which can bind specific alpha-D-mannose and alpha-D-glucose carbohydrate molecules, the most common application is to separate**[**glycoproteins**](https://en.wikipedia.org/wiki/Glycoprotein)**from non-glycosylated proteins.**

**The principle of affinity chromatography is as follows:

1) Inject a sample into an initially equilibrated affinity chromatography column.
2) Only the substances with affinity for the ligand are retained in the column.
3) Other substances with no affinity for the ligand are eluted from the column.
4) The substances retained in the column can be eluted from the column by changing pH or salt concentration of the eluent.**

**Affinity chromatography separates proteins on the basis of a reversible interaction between a protein and a specific ligand coupled to a chromatography matrix, eluent is then passed throw the column to release the highly purified and concentrated molecule.**

 **The affinity chromatography technique offers:**

**1-High selectivity, hence high resolution.**

**2- High capacity for the protein(s) of interest.**

**3-Target protein(s) is collected in a purified, concentrated form.**

 **Biological interactions between ligand and target molecule can be a result of electrostatic or hydrophobic interactions, van der Waals’ forces and/or hydrogen bonding. To elute the target molecule from the affinity medium, the interaction can be reversed, either specifically using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity. Target molecules can be purified from complex biological mixtures, native forms can be separated from denatured forms of the same substance. For an even higher degree of purity, or when there is no suitable ligand for affinity purification, an efficient multi-step process must be developed using the purification strategy of Capture. When applying this strategy affinity chromatography offers an ideal capture or intermediate step in any purification protocol and can be used whenever a suitable ligand is available for the protein of interest. Successful affinity purification requires a biospecific ligand that can be covalently attached to a chromatography matrix. The coupled ligand must retain its specific binding affinity for the target molecules and, after washing away unbound material, the binding between the ligand and target molecule must be reversible to allow the target molecules to be removed in an active form. Any component can be used as a ligand to purify its respective binding partner.**

 **Some typical biological interactions, frequently used in affinity chromatography, are listed below:**

 **• Enzyme ⌠ substrate analogue, inhibitor, and cofactor.**

 **• Antibody ⌠ antigen, virus, cell.**

 **• Lectin⌠ polysaccharide, glycoprotein, cell surface receptor, cell.**

 **• Nucleic acid ⌠ complementary base sequence, histones, nucleic acid polymerase, nucleic acid binding protein.**

 **• Hormone, vitamin ⌠ receptor, carrier protein.**

 **• Glutathione ⌠ glutathione-S-transferase.**

 **• Metal ions ⌠ native proteins with histidine, cysteine and/or tryptophan residues on their surfaces.**



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