***Lec.13: Bioseparation Technique***

**Affinity chromatography composition**

***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.**

**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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