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# APPLICATION OF DYE-LIGAND CHROMATOGRAPHY IN PROTEIN PURIFICATION

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## DESCRIPTION

The separation of compounds from a mixture is referred to as chromatography. Specific proteins can be separated from a mixture, and constantly affinity chromatography is used for this purpose. Then, ligands that interact with the targeted proteins are used to capture these proteins as the sample is passed through the chromatography column.

Still, the ligands used in these columns, similar as antibodies, lectins and nucleotide co-factors, are unstable, precious and have a low capacity. It may cause problem for the use in chromatography as they are not readily soluble.

Synthetic dyes can also act as ligands for proteins. The dyes used are grounded on a chromophore, generally either azo, anthraquinone or phthalocyanine, and also have a reactive part, generally a chlorotriazine ring. The chromophore gives the color to the dye. It should have sulfonic groups to allow it to be soluble in water. The reactive part allows the dye to bind to the matrix. Synthetic dyes are affordable, can fluently be immobilized onto the matrix of the chromatography, are resistant to chemical or enzymatic degradation, and have a capacity far higher than other ligands. Therefore, this technique is good for large scale protein purification.

Still, indeed with those advantages, dyes aren't as specific as other types of ligands, potentially limiting their usage. Alternately, this low specificity could be an advantage as it could reduce the need for having to use multiple ligands. Increased understanding of how these dyes interact with proteins has led to an improvement in the design of the dyes. Synthetic dyes (also known as “biomimetic dye-ligands”) are fairly new and are designed to mimic natural ligands, either structurally or by mimicking ligand-protein commerce. It's believed that these biomimetic color-ligands have increased perceptivity over the older dyes.

Several studies have shown that dyes similar as Cibacron Blue 3-GA bind readily to nucleotide binding regions of certain enzymes although, there is always the possibility that the dye will bind to other regions of the proteins. For non-enzymatic proteins, Cibacron Blue 3-GA can bind using other mechanisms, including ionic forces, hydrophobic relations, or exclusion- diffusion. The chromatography process depends on a variety of factors, including dye concentration, buffer conditions, flow rate, and column figure. The following steps are involved

1. Purification of the dye
2. Immobilizing the dye onto the chromatography matrix
3. Running the chromatogram

First, the dyes need to be purified as they're generally stored in buffers with added stabilizers, and could also have other pollutants. In the coming stage, the dye is immobilized onto the matrix, and this can be either through the chlorotriazine ring or by a spacer molecule. Using a spacer molecule, similar as hexamethyldiamine, may increase the dye selectivity due to the reduction of steric interference from the matrix. After the color is immobilized, the matrix is washed to remove the excess color.

The matrix with the color is de-gassed, and also packed into a chromatography column. The de-gassing is to help air bubbles from forming. The column is washed with buffer, previous to the loading of the protein samples.

Once the protein sample has passed through the column, the column is washed with the buffer again to remove any proteins that didn't bind to the color, and this fraction is collected as well. An alternate buffer is also used to elute the bound proteins from the column and the performing alternate bit is also collected. Both fractions are then tested for the absence/ presence of the protein of interest.