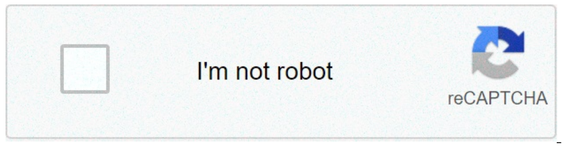

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Ion exchange chromatography is used for separation of

Highly pure proteins are vital for successful experiments; they play roles in research as assay reagents (especially for SPR applications), therapeutic candidates, and of course, as the subjects of structural and biochemical studies. Chromatography is the science of separation and we utilize it to isolate and purify proteins based on their unique physiochemical properties. One of the most fundamental and important skill sets a budding life scientist can master is protein chromatography. This is the first in a series of articles written to introduce the various separation methods and provide some practical tips. We will begin with ion-exchange chromatography. A Brief Overview of Ion-Exchange Chromatography Ion-exchange chromatography (IEX) separates biomolecules based on differences in their net charge at a particular pH. Protein charge depends on the number and type of ionizable amino acid side chain groups. Each protein has an isoelectric point (pI), a pH at which the overall number of negative and positive charges is zero. In a buffered solution below the protein's pI, the protein is positively charged and will bind to the negatively charged functional groups of a cation exchange resin. In a buffered solution above the protein's pI, the protein is negatively charged and will bind to the positively charged functional groups of an anion exchange resin. In principle, a protein could bind to either a cation or anion exchange resin, but in practice, proteins are only stable within a narrow pH range and the choice of resin depends on the pH stability of the protein. Resins for Ion-Exchange Chromatography Ion-exchange chromatography resins have charged functional groups bound to resin beads which attract biomolecules of the opposite charge. Cation exchange resins are negatively charged, and anion exchange resins are positively charged. Ion-exchange resins are also categorized as "weak" or "strong" exchangers. These terms aren't related to the strength of ion binding, but instead, refer to the extent that the ionization state of the functional groups varies with pH. A "weak" exchanger is ionized over only a limited pH range, while a "strong" exchanger shows no variation in ion exchange capacity with changes in pH. Weak exchange resins can take up or lose protons with changes in buffer pH, and that added variation in charge offers an additional dimension of selectivity for binding and elution. Strong exchangers do not vary and remain fully charged over a broad pH range, which can make optimization of separation simpler than with weak exchangers. The table below summarizes the most common IEX resins. In addition to type of functional group, you need to consider the physical properties of the resin. The size, material and porosity of the resin beads dictate the maximum operating pressures and flow-rates (affects the speed of purification). More importantly, bead size and porosity affect the resolution of the separation. Larger beads are generally conducive to fast flow rates and provide resolution appropriate for early and intermediate stages of purification. Smaller beads provide the best resolution and are ideal for later purification stages. Table 1: Summary of ion-exchange chromatography resins & their functional groups. Easy as pI The first step in designing an IEX purification scheme could be the in silico determination of the pI of your protein of interest. The pI of a protein is determined by the aggregate charge of every amino acid in the protein chain. The Henderson-Hasselbach equation is used to iteratively compute protein charge at certain pH's until one is found in which the net charge of the protein is zero. The math can get complicated for proteins, but luckily, there are several online tools you can use to painlessly estimate your protein's pI and guide your experiments. ProtParam, hosted by ExPASy. This is the "classic" tool that most life scientists know of. You can calculate the theoretical molecular weight, isoelectric point, extinction coefficient and other physiochemical properties based on protein sequence. The algorithm is based off the work of Bjellqvist et al. in the early 1990's1–2. Isoeletric Point Calculator (IPC). The IPC is a "new school" online tool. It computes a series of pI predictions using several published algorithms and pK datasets. In addition to displaying the range of computed isoelectric points, it also provides an average pI based for all methods. I find the output from IPC to be more useful and comprehensive as compared to the value determined by ProtParam. The ProMoST web tool. Post-translational modifications (PTM) can have a significant effect on a protein's pI. The majority of PTMs occur on ionizable sidechains, and some PTMs, such as phosphorylation or acetylation, introduce new ionizable chemical groups. If you are purifying a eukaryotic protein that is likely post-translationally modified, use the ProMoST tool to calculate and compares the effect of post-translational modifications on pI, molecular mass, and other physiochemical properties. Remember that the theoretical pI is likely to be different from the true isoelectric point, and may not reflect the actual charge distribution on the protein surface. Charge distribution is not uniform and a protein is capable of having both positively and negatively charged patches on a surface. When working with a new protein, I always screen a range of cation and anion exchangers, both weak and strong, to optimize the purification step. Buffer Conditions The choice of buffer system, pH, additives and salt concentration all have a direct effect on the success of your IEX experiment. Buffer scouting is frequently required to find the optimal pH for solubility and adsorption to the IEX resin. When screening resins and buffer conditions, keep the following in mind: Keep the pH of any protein purification or storage buffer 0.5 to 1 pH units above or below its pI to promote solubility. A protein's pI is the point at which it has no net charge; it is likely to be unstable, less reactive, and least soluble at that pH. It's a simple concept, but may not be the first thing that comes to mind if your protein begins crashing out of solution during buffer exchange. Use a buffer concentration sufficient to maintain buffering capacity, typically 25 to 100 mM. Pay attention to the ionic strength of the starting material and wash buffers, as the affinity of the protein for the column decreases as ionic strength increases due to salt concentration. There are more modern salt-tolerant ion exchangers that can help overcome this issue if you have to work with buffers at higher ionic strength (these will be highlighted in a later article). If loading a small volume of protein onto an IEX column, dilute the protein solution with the starting buffer, which will assure that conditions are ideal for binding. Slower flow-rates during column loading and elution increases the interaction time between the protein and the exchange resin, promoting specific binding interactions during loading. Elution & Selectivity Proteins are most often eluted from IEX columns by increasing the concentration of counterions (salts) in the buffer solution. You may also consider using pH shifts as well. They can be helpful in specific cases when using a weak ion exchange resin, or if adjusting the salt concentration cannot achieve sufficient resolution. Choice of elution method, either via linear gradient or a step elution, affects selectivity. Also, keep in mind down stream techniques, such as surface plasmon resonance. Linear gradients gradually raise the ionic strength and are ideal when starting with an unknown sample or if peak resolution is important. Peak resolution is also improved by reducing flow-rates, eluting over a greater volume, or eluting with a shallower gradient. Step elution speeds purification time and minimizes the final protein elution volume. However, it should be used once the IEX separation has been optimized. Some elution strategies use a step gradient like a high-stringency wash, and then start a linear gradient at the higher concentration of salt. Differential column chromatography, sometimes referred to as group elution or flow-through mode, is used to remove contaminants by choosing conditions that maximize binding of the contaminants and allow target proteins to pass through the column. This is especially helpful in reducing contaminants and improving column specificity in a later purification step. It's a popular and very effective strategy for removing nucleic acid contaminants. DNA and RNA have strong negative charges at a neutral to basic pH, so they adsorb tightly to anion exchange resins, but not cation exchangers. Ion-exchange chromatography is an incredibly versatile method for protein purification, which is vital for certain analyses (such as in a SPR technique). The method can be used at any stage of purification, and the diversity of available resins provide a broad spectrum of selectivity that can be fine-tuned for your protein of interest. Later articles will go into the nitty-gritty of troubleshooting and optimizing IEX, so please comment below to share what kind of snafus you have run into in the past. References Further Reading Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.; Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). 571-607. Halligan, B. D. et al. ProMoST (Protein Modification Screening Tool): a web-based tool for mapping protein modifications on two-dimensional gels. Nucleic Acids Research 32, W638- 644, doi:10.1093/nar/gkh356 (2004). Kozlowski LP (2016) IPC – Isoelectric Point Calculator CSHL Press. doi: Facebook Twitter LinkedIn More Image Credit: Ricardo's Photography

ion exchange chromatography is used for separation of mcq. ion exchange chromatography is used for separation of polar or nonpolar molecule. ion exchange chromatography is used for separation of sterols. ion exchange chromatography is used for separation of sugars. ion exchange chromatography is used for separation of fatty acid. ion exchange chromatography is used for the separation of which molecules. how does ion exchange chromatography work. how to use ion exchange chromatography

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