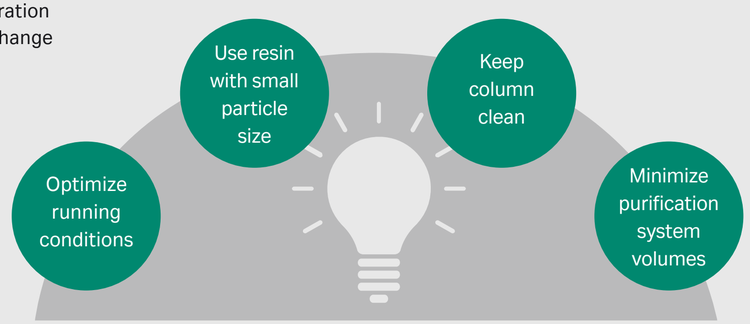
Ion exchange chromatography

Top 4 tips to achieve high protein purity

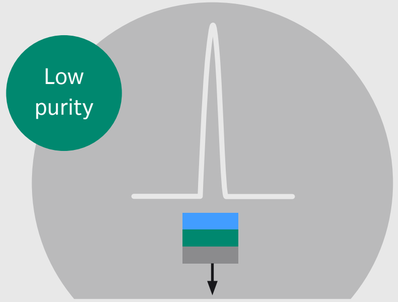
Top 4 tips for high-resolution separation (to give high protein purity) in your ion exchange chromatography (IEX) runs.



More than 60% of consulted scientists[1] say that high resolution or purity is their top priority or challenge in IEX.

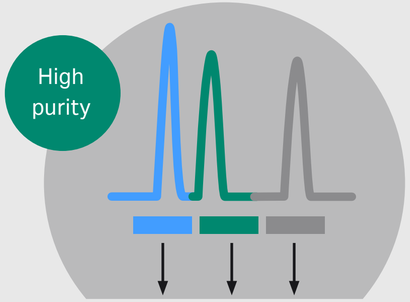
Well-separated peaks = the route to high protein purity

Peak separation = resolution



No separation of peaks

1 Mixed fraction containing: Protein of interest + closely charged proteins + impurities



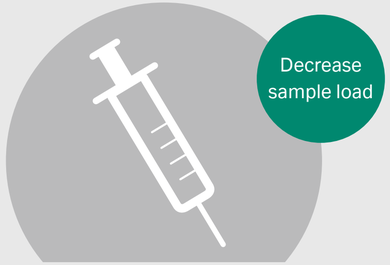
Well-separated peaks

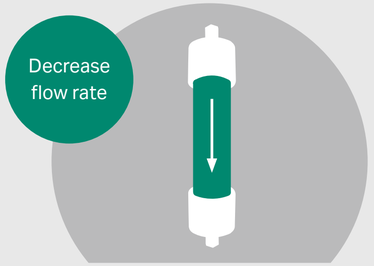
1 Only contains the pure protein of interest
2 Closely charged proteins
3 Impurities

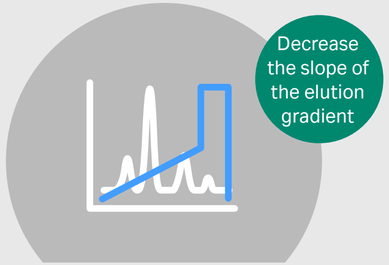
Remember, one single peak, does not necessarily mean a pure protein!

1 Optimize running conditions

Tips to increase resolution

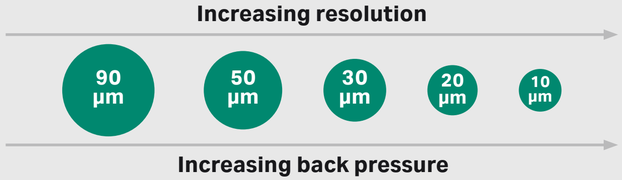






2 Use resin with small particle size

Find the resolution/column back pressure balance!

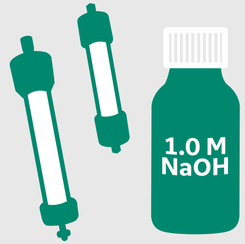


Examples

- High resolution needed (e.g., structural studies): < 10 µm
- For routine preparative purification: > 30 µm

3 Keep column clean

Perform regular cleaning with 1.0 M NaOH to maintain performance

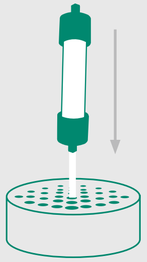


How often? Some guidelines

- After each run if sample is a cell lysate
- Every ~ 5 runs for partially purified samples

4 Minimize purification system volumes

Minimize volumes between the column outlet and the fraction collector



Examples

- Use short and narrow capillaries
- Remove unnecessary valves and detectors

[1] BioInformatics customer research study for Cytiva, June 2016; 190 respondents performing IEX.

cytiva.com/IEX

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