

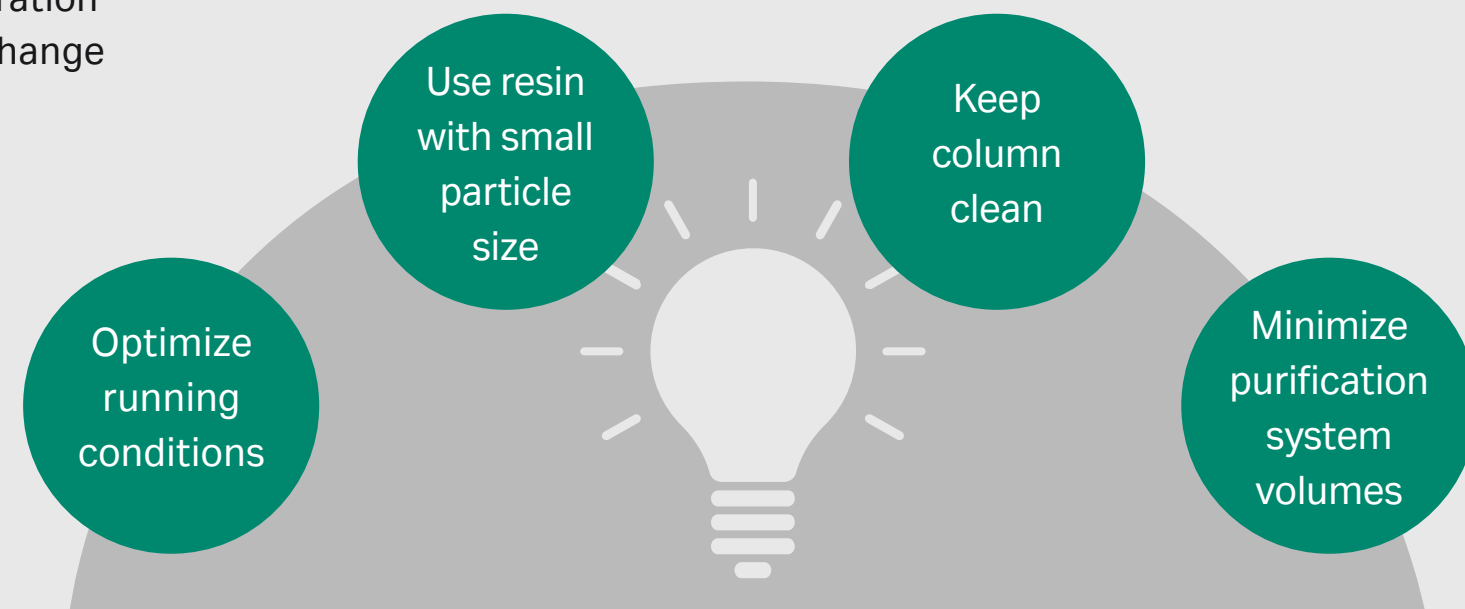
Ion exchange chromatography

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.

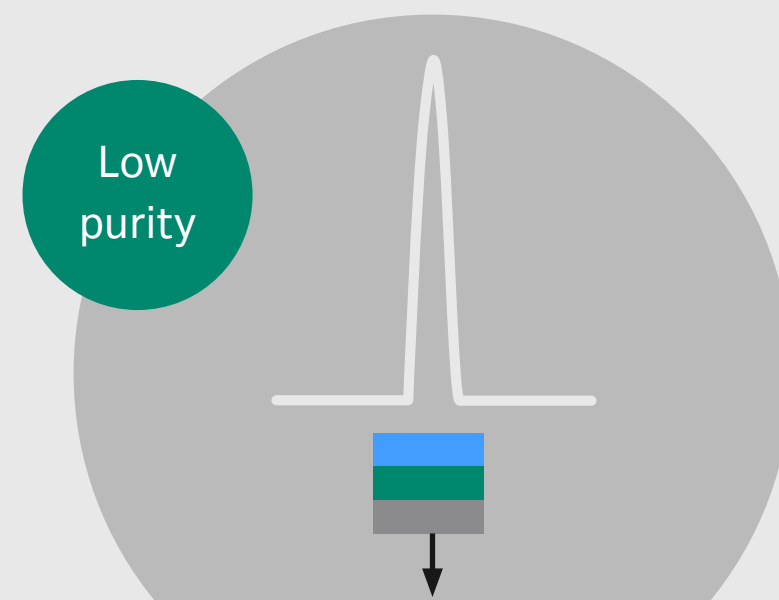


More than 60% of consulted scientists¹ say that high resolution or purity is their top priority or challenge in IEX.



Well-separated peaks = the route to high protein purity

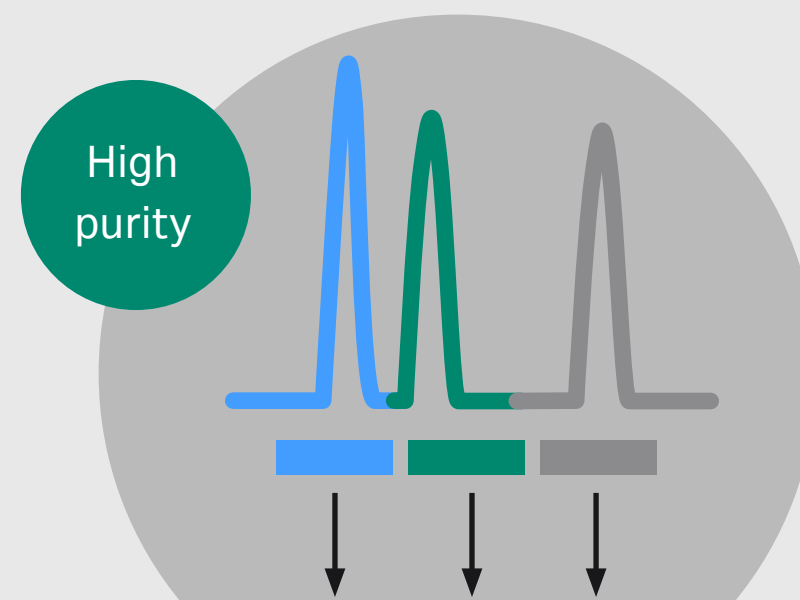
Peak separation = resolution



Low purity

No separation of peaks

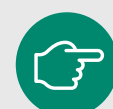
- Mixed fraction containing: Protein of interest + closely charged proteins + impurities



High purity

Well-separated peaks

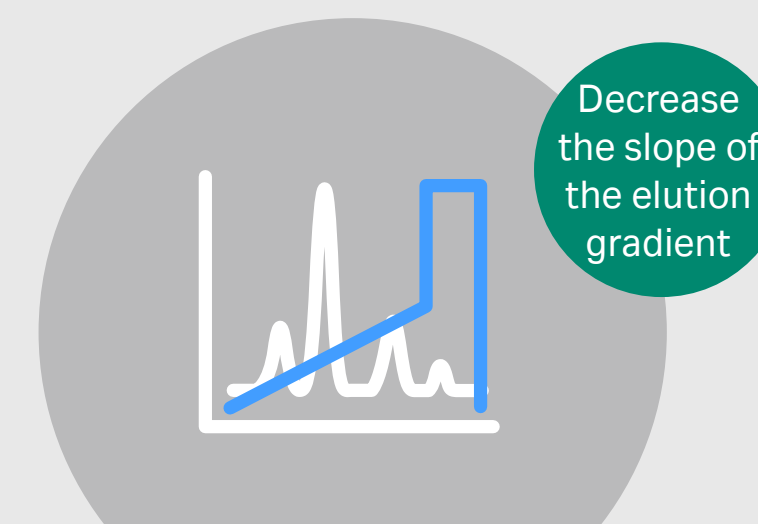
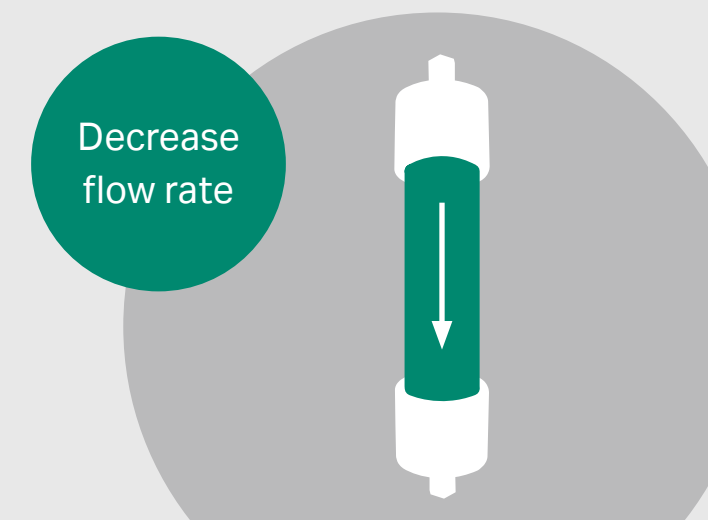
- Only contains the pure protein of interest
- Closely charged proteins
- Impurities



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!

Increasing resolution



Increasing back pressure

Examples

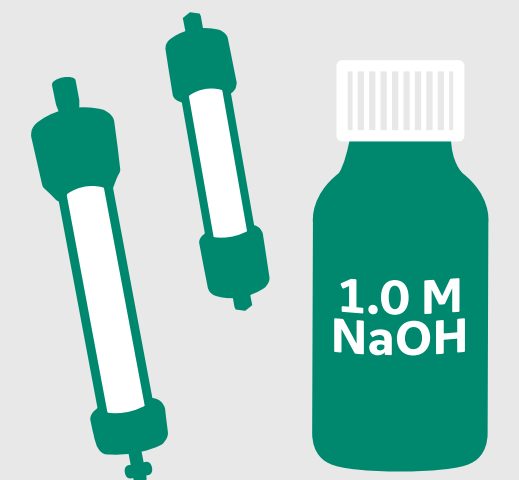
- High resolution needed (e.g., structural studies): < 10 μm
- For routine preparative purification: > 30 μm

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

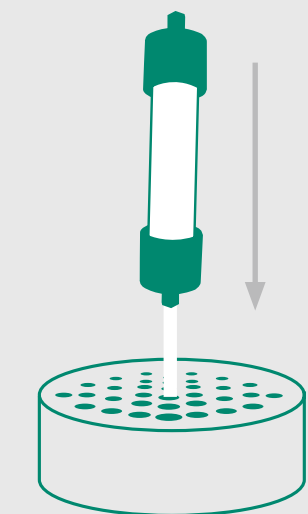


4 Minimize purification system volumes

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.

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