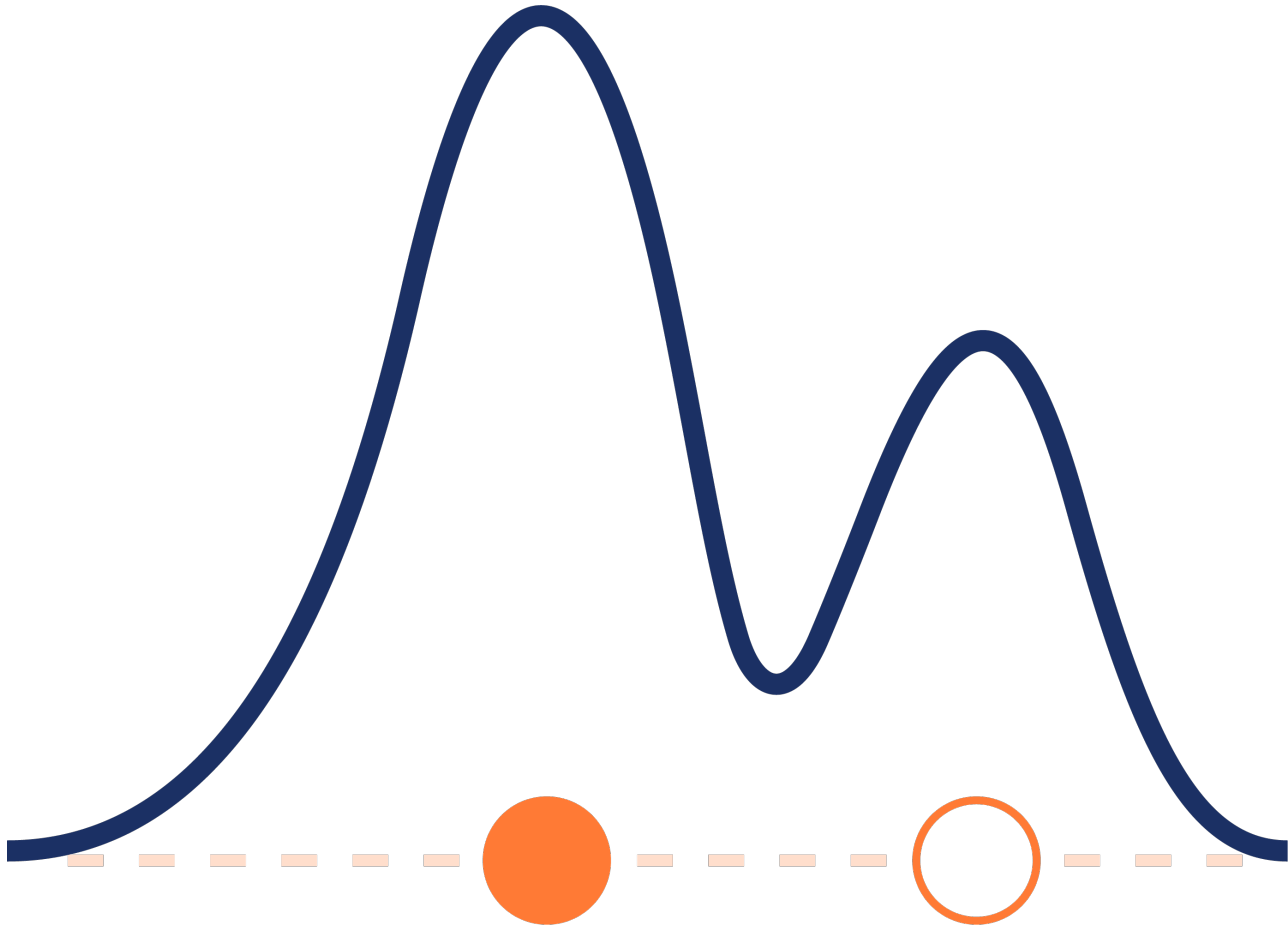
cytiva

Ion exchange chromatography columns for protein purification in research



Outline

Fundamentals about ion exchange chromatography (IEX)

When is IEX relevant to use?

Important considerations when preparing IEX runs

Cytiva IEX columns for basic research applications

Useful tools

IEX fundamentals

What are the main chromatography techniques used for protein purification?

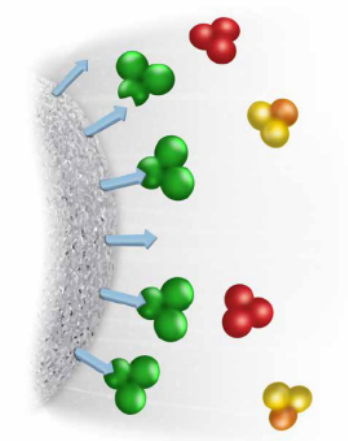
Size exclusion chromatography

(SEC)

Size

Affinity chromatography

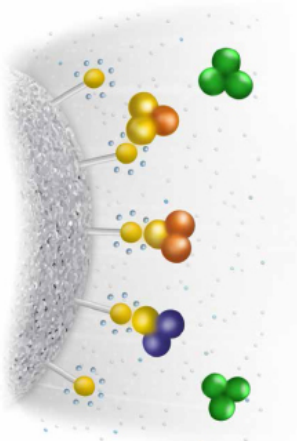
(AC)



Biorecognition

Hydrophobic interaction chromatography

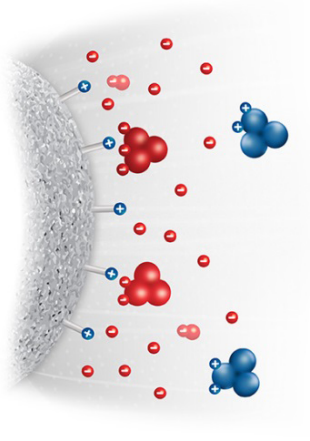
(HIC)



Hydrophobicity

Ion exchange chromatography

(IEX)



Charge

Chromatography techniques enable separation of proteins based on differences in specific properties.

IEX separates proteins with differences in net surface charge

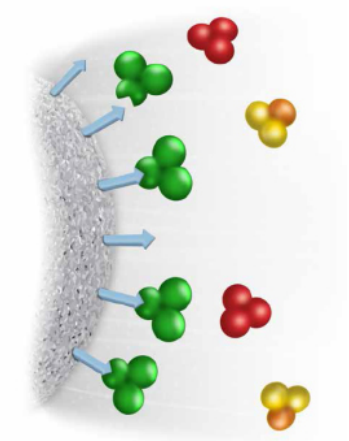
Size exclusion chromatography

(SEC)

Size

Affinity chromatography

(AC)



Biorecognition

Hydrophobic interaction chromatography

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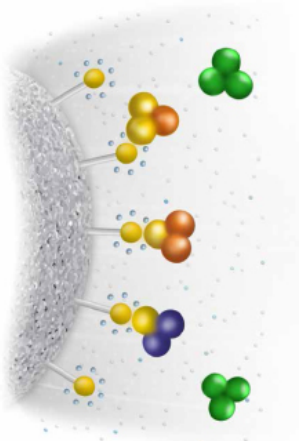
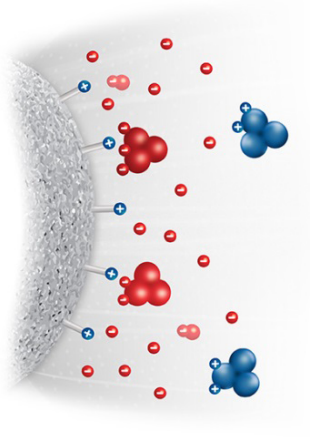


Hydrophobicity

Ion exchange chromatography

(IEX)



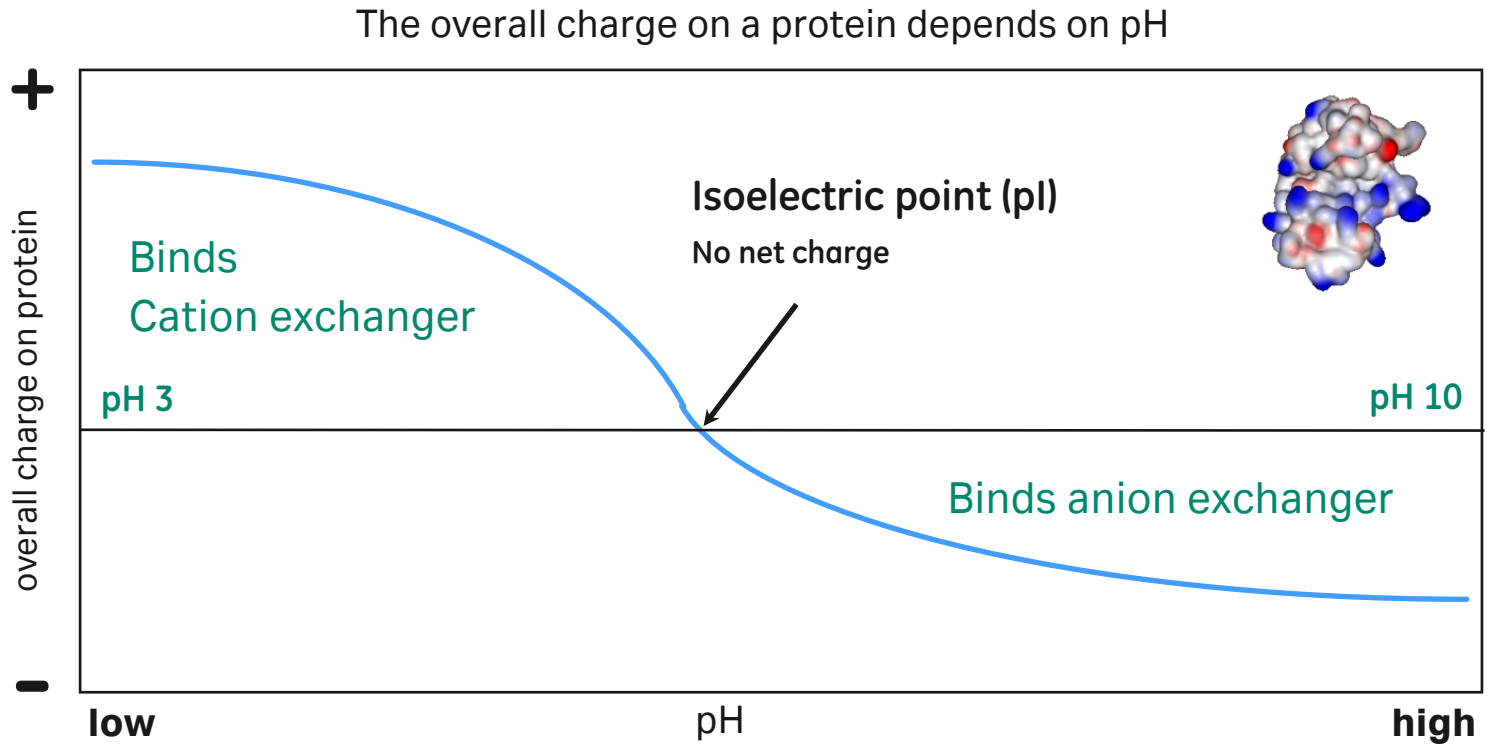
Charge

Chromatography techniques enable separation of proteins based on differences in specific properties.

What makes IEX so versatile?

- Isoelectric point (pI) = pH at which a protein has no net charge
- Every protein has its own pI

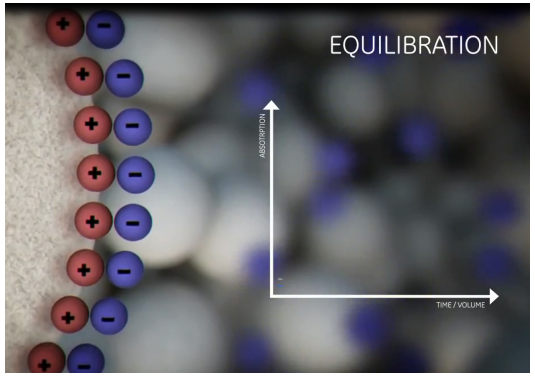
This property allows proteins to interact to different degrees with an IEX resin.



A protein's net surface charge is highly pH dependent; surface charge can be utilized to separate proteins from each other.

How does IEX work?

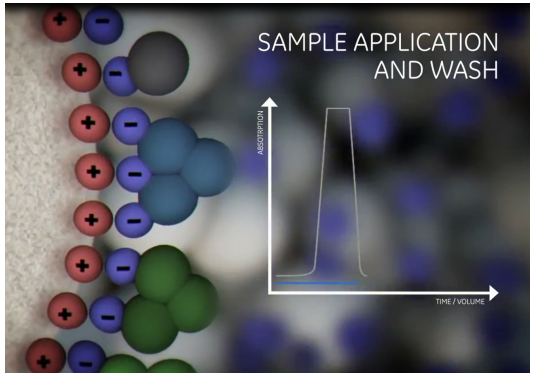
Equilibration



The first step is the equilibration of the stationary phase to the desired start conditions.

When equilibration is reached, all stationary phase charged groups are associated with exchangeable counter-ions such as chloride or sodium.

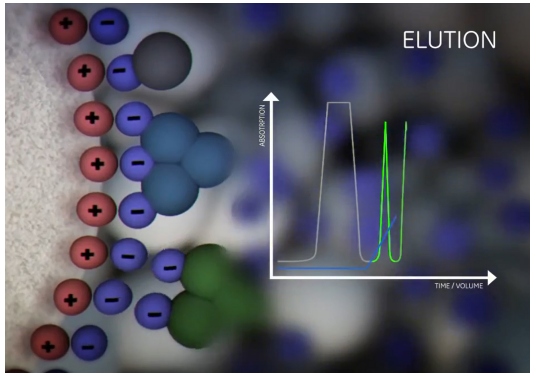
Sample application and wash



The goal in this step is to bind the target molecules and wash out all unbound material.

The sample buffer should have the same pH and ionic strength as the starting buffer in order to bind all appropriately charged proteins.

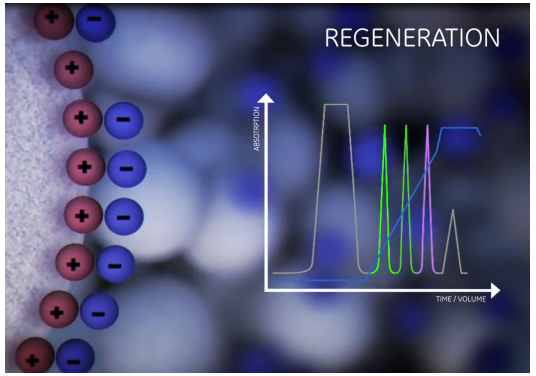
Elution



Biomolecules are released from the ionic exchanger by a change in the buffer composition.

A common elution method is to increase the ionic strength with sodium chloride or another simple salt in order to desorb the bound proteins. Proteins are desorbed relative to their number of charged groups on their surface.

Regeneration

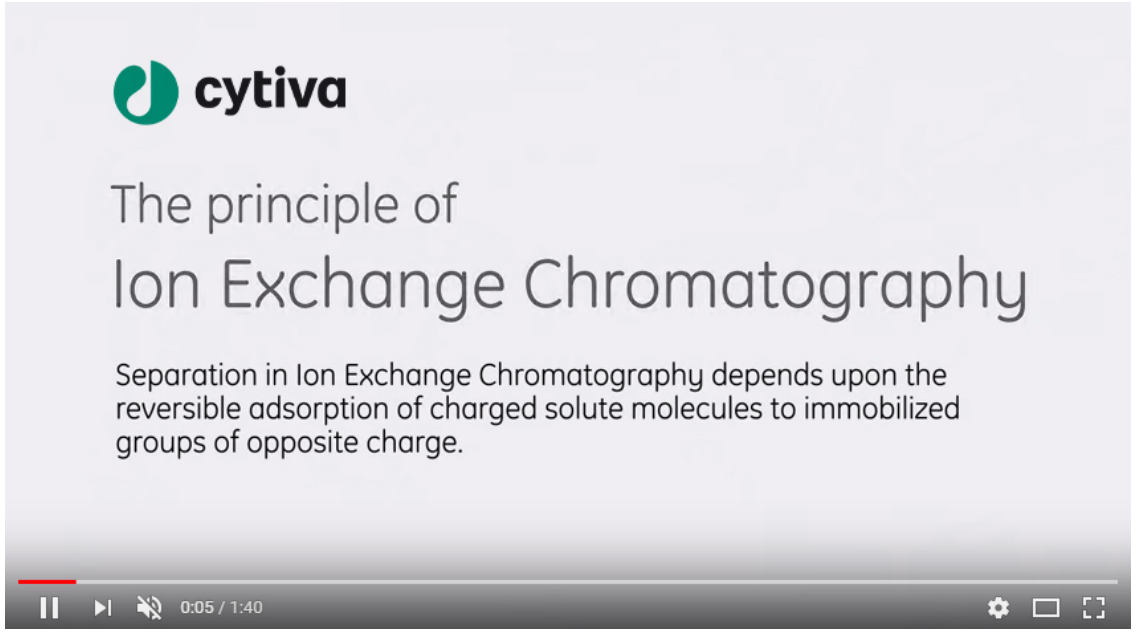


The final step, regeneration, removes all molecules still bound.

This ensures that the full capacity of the stationary phase is available for the next run.

More on IEX animation and Cytiva handbook

Watch the video on YouTube >>
The principle of ion exchange chromatography



Download the handbook >>
Ion exchange chromatography — Principles and methods

When to use IEX

What are the applications?

Purification or analysis of:

- Native/untagged proteins
- Closely related proteins

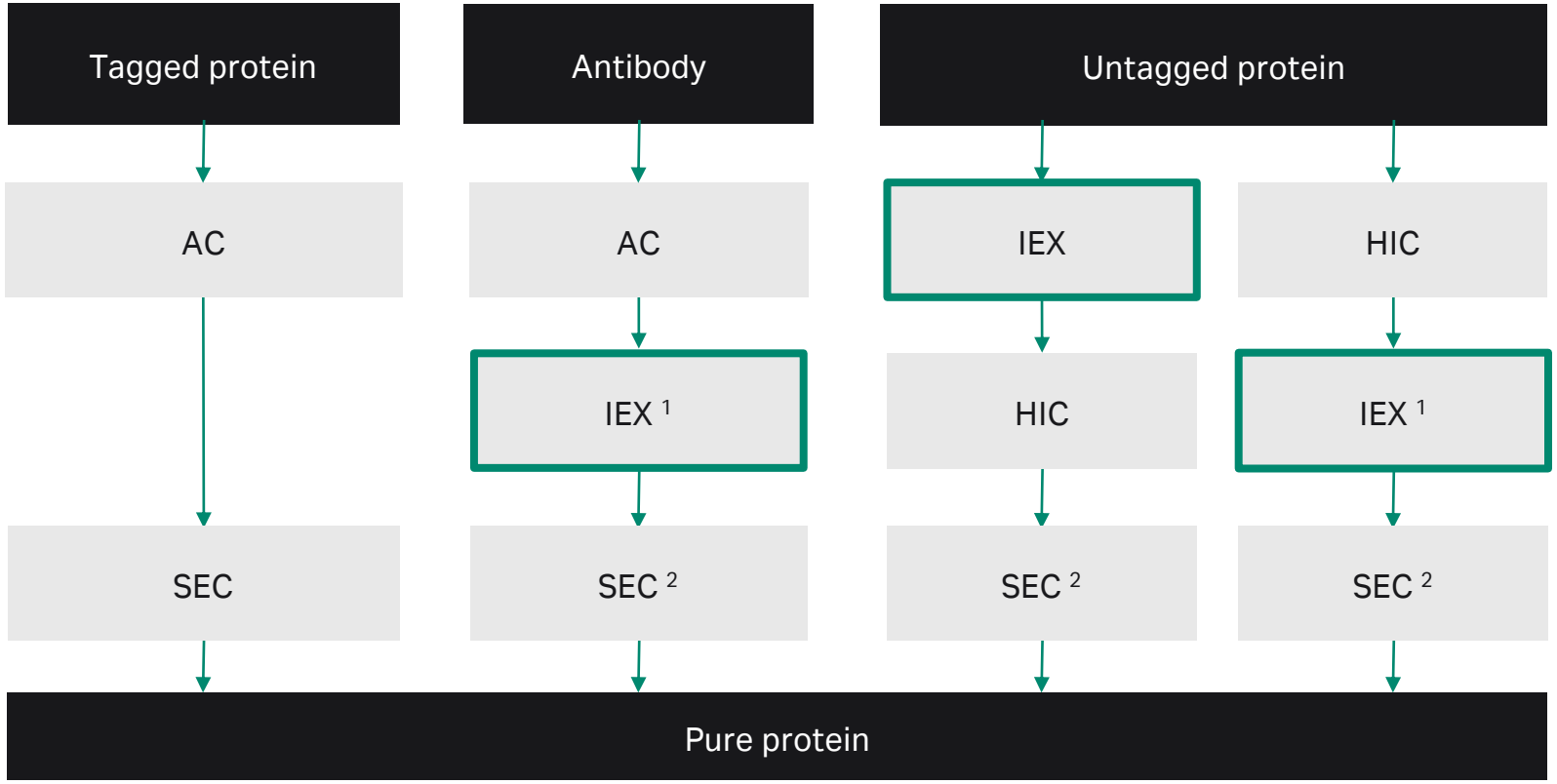
IEX can also be used for purification of peptides, amino acids, and nucleotides.

IEX can be used in various stages of the protein purification protocol

Capture:
Isolate, concentrate, stabilize

Intermediate purification:
Remove bulk impurities

Polishing:
Remove final contaminants

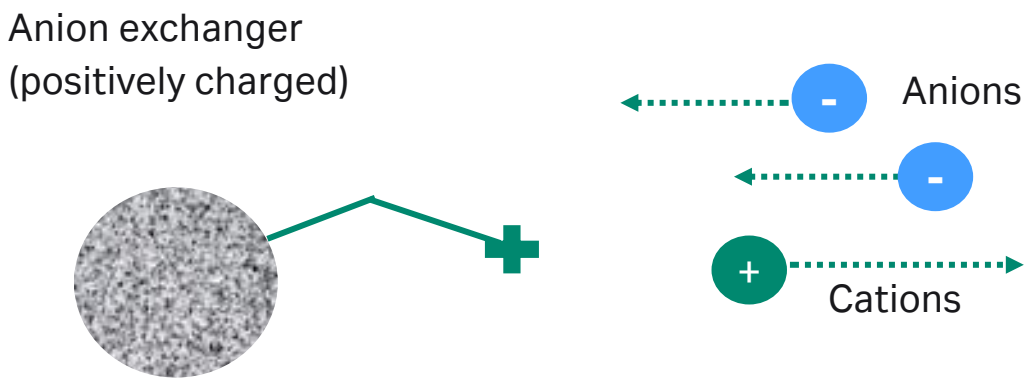


[1] Use of IEX as an intermediate step is not always used and will depend on the level of purity needed.
[2] SEC is not typically used as a polishing step in industrial applications because scale-up is particularly challenging.

Important considerations when preparing IEX runs

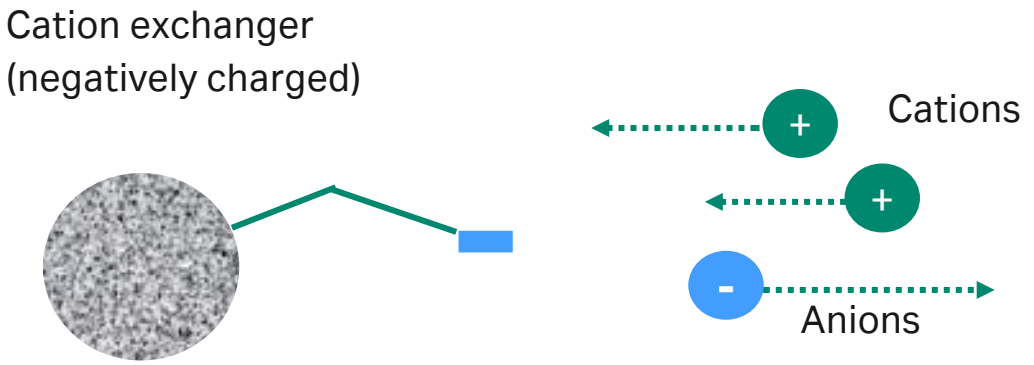
Choose a resin that has the most appropriate ion exchanger for your protein to ensure protein binding

Anion exchangers = bind anions



Most common ligands: Q (strong), DEAE (weak), ANX (weak)

Cation exchangers = bind cations



Most common ligands: S (strong), SP (strong), CM (weak)

What does "strong" and "weak" mean?

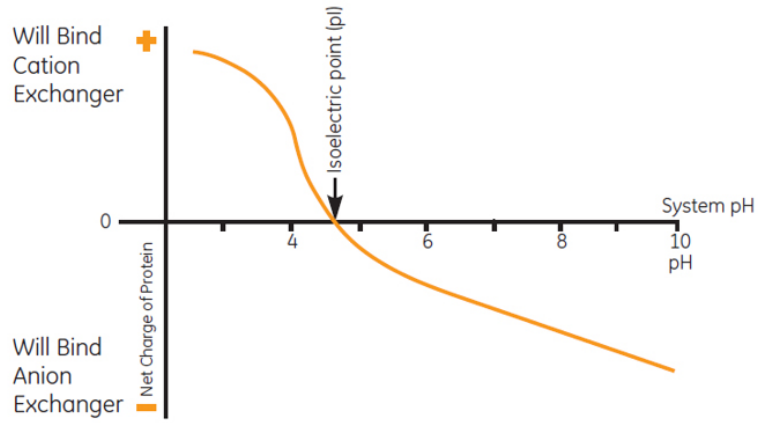
- Strong ion exchangers → the ion exchanger is fully charged over a broad pH range
- Weak ion exchangers → the charge of the ion exchanger varies with pH

Tip!

Start with a strong ion exchanger. If the selectivity is not good enough (peaks on the chromatogram are not sufficiently separated), try a weak ion exchanger.

How to select the most appropriate ion exchanger?

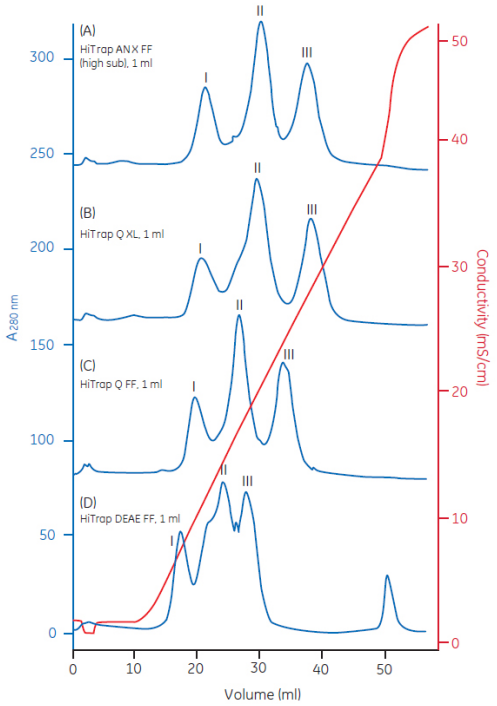
If pI of your protein is known

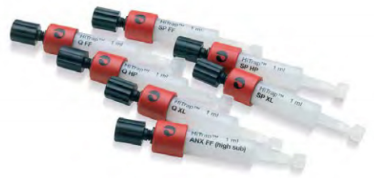


- Select an anion exchanger (Q, DEAE, ANX) with a buffer pH above pI
- Select a cation exchanger (S, SP, CM) with a buffer pH below pI

If pI of your protein is unknown

- Start by using a strong anion exchanger (Q)
- Use IEX selection kits for screening of the most appropriate ion exchanger



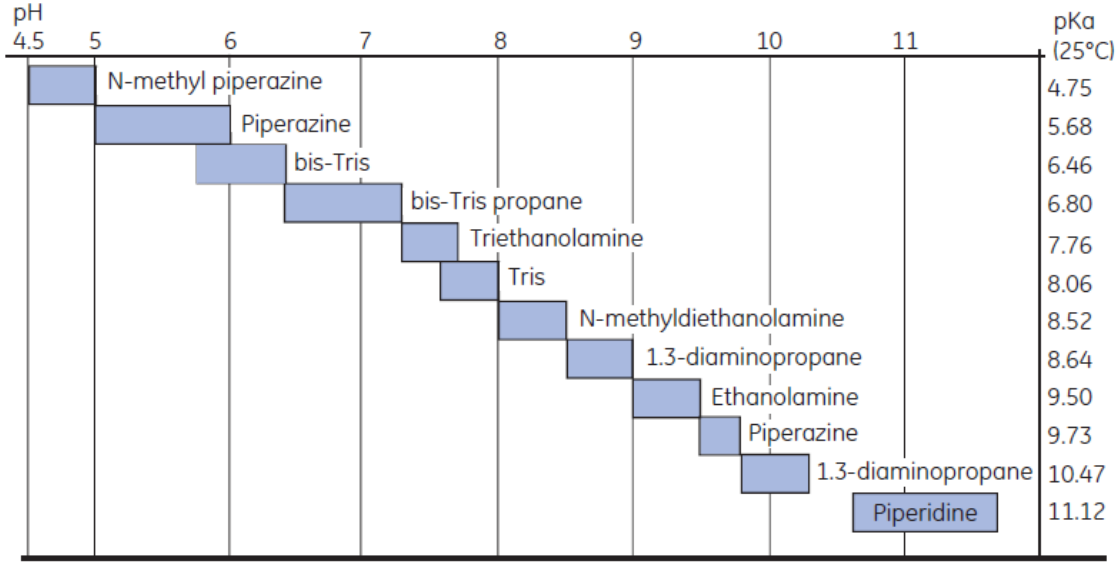


HiTrap™ IEX Selection kit

Choose the appropriate buffer substances to ensure protein binding

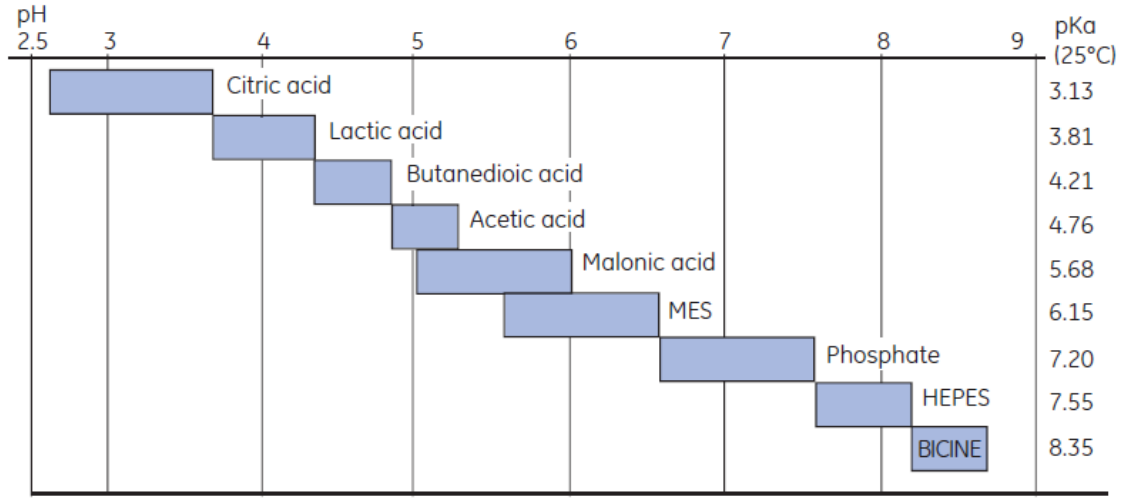
Buffer choice depends on protein pI and type of IEX

Buffers for anion exchange chromatography



- Choose buffer 0.5 to 1.0 pH units <u>above</u> pI

Buffers for cation exchange chromatography



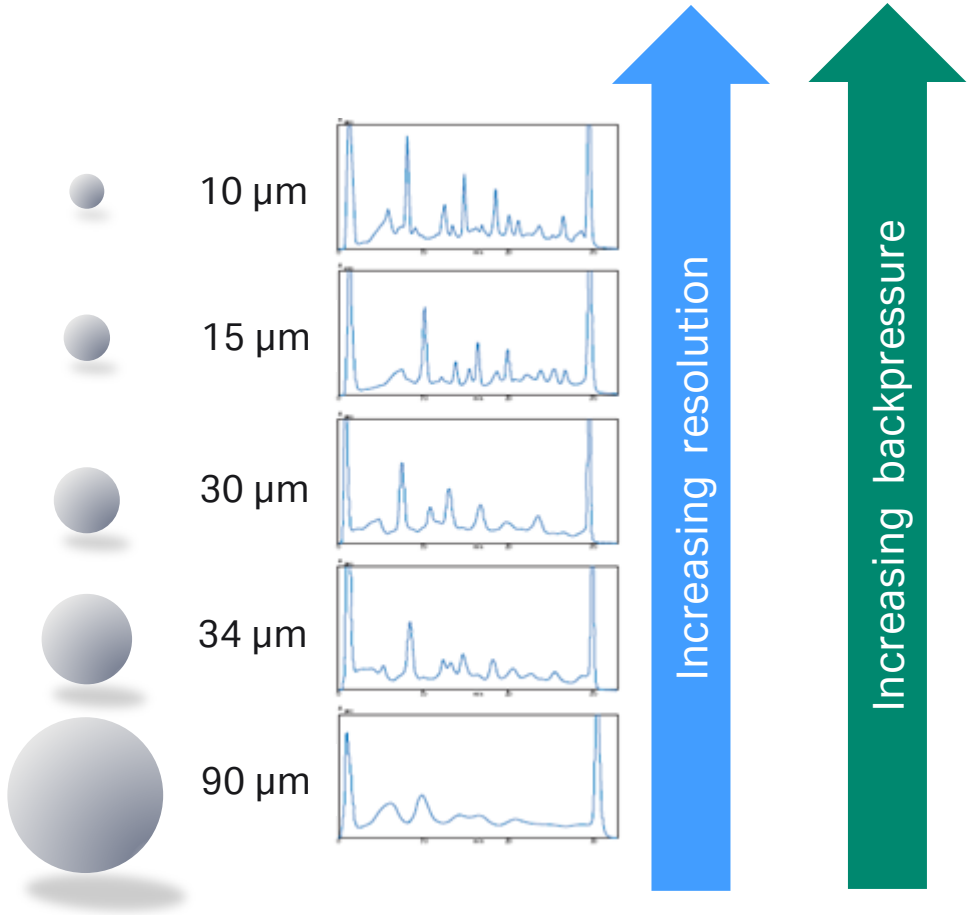
- Choose buffer 0.5 to 1.0 pH units <u>below</u> pI

✓ **Consider stability window of protein (often unstable around where pH ≈ pI)**

✓ **Ensure that your column is sufficiently equilibrated in buffer**

✓ **Choose a buffering ion with same charge as the resin to prevent it from taking part in the ion exchange process**

Smaller resin bead size delivers increased resolution but higher back pressure



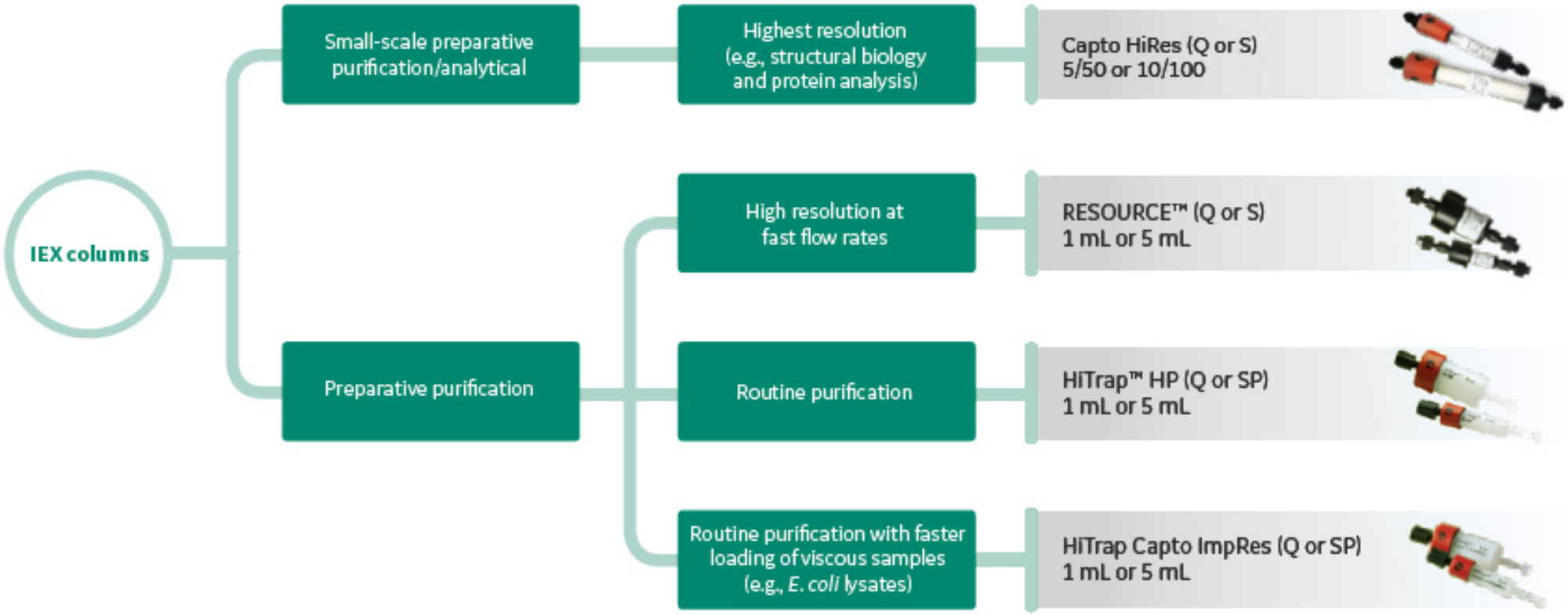
Choose an ion exchanger that has the right balance between resolution and backpressure for your purification needs.

High resolution gives high purity.

Too high back pressure can cause column bed compression, column leakage, and breakage of system components.

IEX columns for basic research applications

IEX columns recommended by Cytiva for research use[1]



HiTrap Capto™ ImpRes (Q/SP) compared with HiTrap HP (Q/SP) delivers similar protein purity AND shorter loading time with viscous samples (such as *E. coli* lysates).

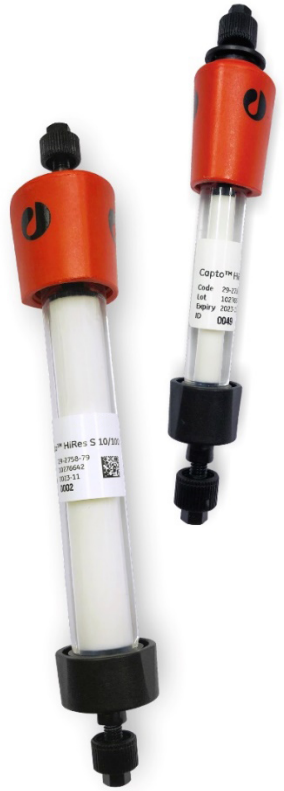
[1] For larger sample loads or scale-up needs, other formats are available. (e.g., HiScreen™, HiPrep™).

Highest resolution — small-scale preparative and analytical IEX

Capto HiRes 5/50 and 10/100 ion exchange chromatography columns

Capto™ HiRes Q and Capto HiRes S columns are IEX columns for protein analysis or small-scale, high-resolution polishing of proteins.

They replace MonoBeads™ columns.



Learn more about Capto HiRes columns

Capto HiRes S (strong cation) >>

Capto Hires Q (strong anion) >>

Preparative purification — high resolution at high flow rates

RESOURCE 1 mL and 6 mL IEX columns

RESOURCE™ columns are prepacked with SOURCE™ 15 IEX resin exchanger for high-resolution polishing purification of proteins, at high flow rates.

Learn more about RESOURCE columns

RESOURCE S (strong cation) >>

RESOURCE Q (strong anion) >>

Preparative routine purification
HiTrap HP (1 mL and 5 mL) IEX columns

HiTrap™ SP HP and HiTrap Q HP columns are for routine high-resolution, small-scale preparative protein purification.

Learn more about HiTrap HP columns
HiTrap SP HP (strong cation) >>
HiTrap Q HP (strong anion) >>

Preparative routine purification of viscous samples

HiTrap Capto ImpRes (1 mL and 5 mL) IEX columns

HiTrap™ Capto™ ImpRes chromatography columns are packed with ion exchange modern resins for routine high-resolution, small-scale protein purification.

Learn more about HiTrap HP columns

HiTrap Capto SP ImpRes (strong cation) >>

HiTrap Capto Q ImpRes (strong anion) >>

Check next slide to learn about modern resins

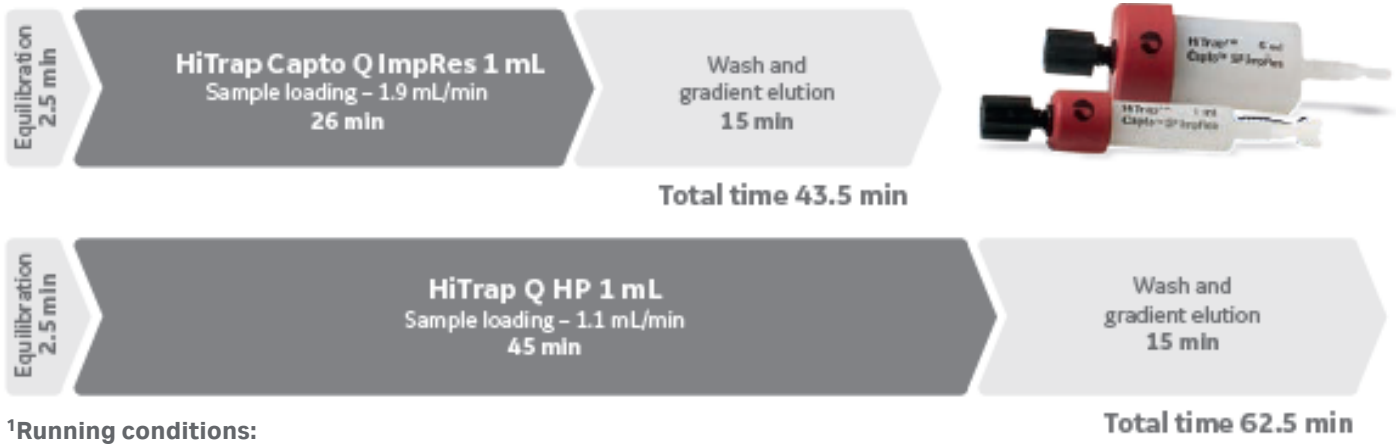
HiTrap Capto ImpRes is packed with modern IEX resin

Time saving vs HiTrap HP, when working with viscous samples such as *E. coli* lysates

Combine high resolution with shorter total run time at comparable price

HiTrap™ Capto™ ImpRes (SP or Q) = **Similar resolution as HiTrap HP (SP or Q)** + **Similar price as HiTrap HP (SP or Q)** + **Faster sample load than HiTrap HP** when working with viscous samples such as *E. coli* lysates

Example: time saving with 50 mL *E. coli* lysate run in cold room[1]



[1]**Running conditions:**
Equilibration: 2 mL/min, 5 CV - Wash: 2 mL/min, 10 CV, Gradient elution: 1 mL/min, 10 CV

For larger sample loads or scale-up needs, other formats are available (e.g., HiScreen, HiPrep)

HiSreen™ columns for method and process development

Learn more about HiScreen columns
HiScreen Q HP (strong anion) >>
HiScreen SP HP (strong cation) >>
HiScreen Capto™ Q ImpRes (strong anion) >>
HiScreen Capto SP ImpRes (strong cation) >>

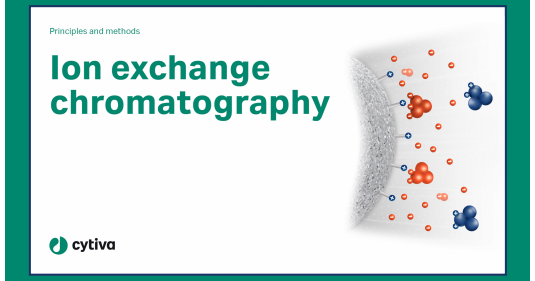
HiPrep™ columns - convenient for scale-up

Learn more about HiPrep columns
HiPrep SP HP (strong cation) >>
HiPrep Q HP (strong anion)) >>

Useful tools to ensure successful IEX runs

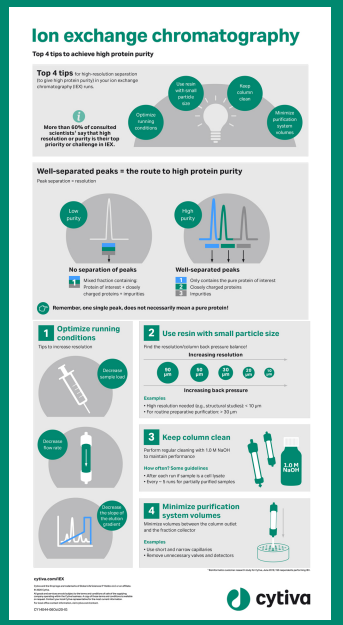
Cytiva expertise made available for you on cytiva.com/IEX

Principles and methods Cytiva handbook >>



IEX resins and columns selection guide>>

IEX infographic>>



Thank you

For local office contact information, visit cytiva.com/contact