Exercise 4

You're about to purify a protein called BtcA, which is linked to a GST tag. Following is the amino acid sequence:

```
>BtcA-GST
MVIRNLRHLLGLPVGADEPVTSLAIDEQWAVHIGCEDDMVTVLLPLGPAPDPLPGAALV
NSLAQWPPVLLDLSEQGEAILWAREHVGRLTAEQLHALLVRVAARAAALMAPAAAPPAP
QDTAEVKLAAALEGSSLVPRGSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDE
GDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFML
YDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGD
HPPKSDLVPRGSP
```

For this purification you have in your disposal a modern FPLC system with the following array of columns:

- Superdex 75 26-60 pg
- Superdex 200 26-60 pg
- HiTrap™ SP HP 5ml column
- HiTrap™ Q HP 5ml column
- His Trap HP column 5ml column
- HiTrap HIC 5ml column
- GSTrap HP 1ml column

Note that the column sheet are attached at the end of this homework

1. Plan a purification scheme from the wet bacterial pellet to pure protein, assuming you will need at least two chromatography steps. Please fill in the details in the tables below and give a **brief** description, in steps, to the purification process. Detail the lysis method, centrifugation and buffer composition (including concentration details).

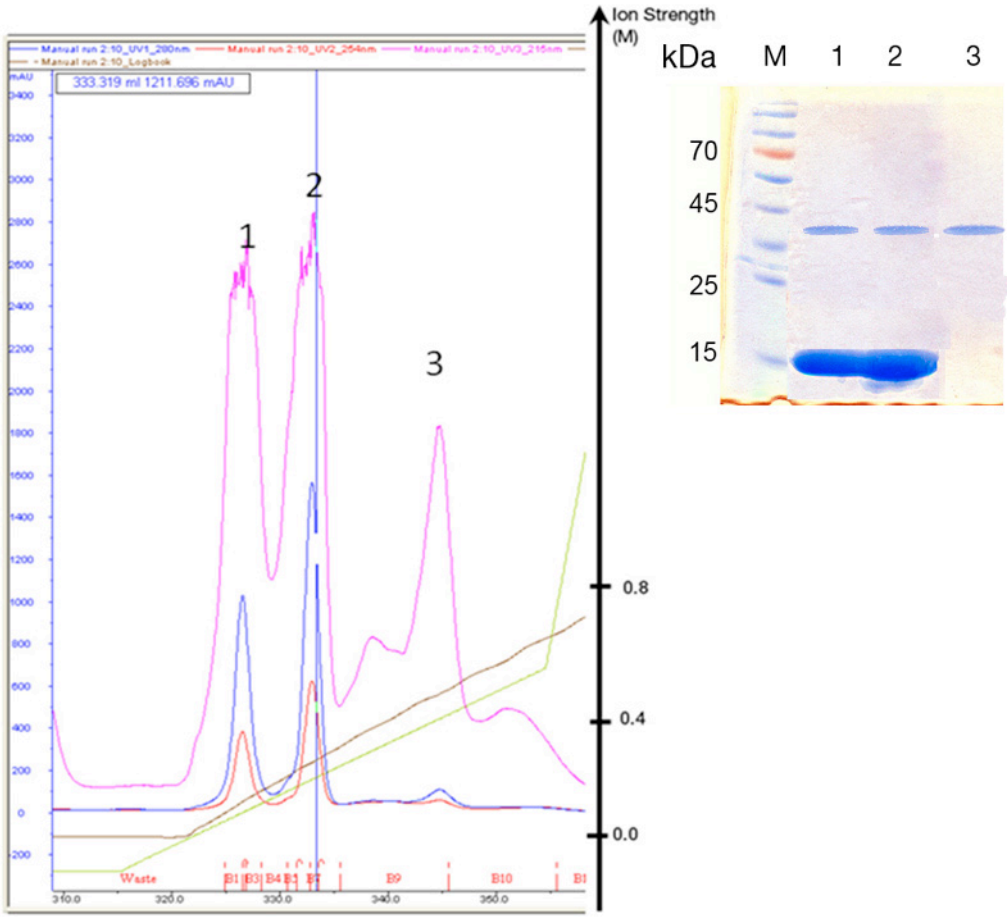
Step #	Buffer #	Buffer composition	pH

Step #	Speed	Duration

Step#	Column	Buffer # used

2. Following one of the purification steps, you obtain the following gel and chromatogram:

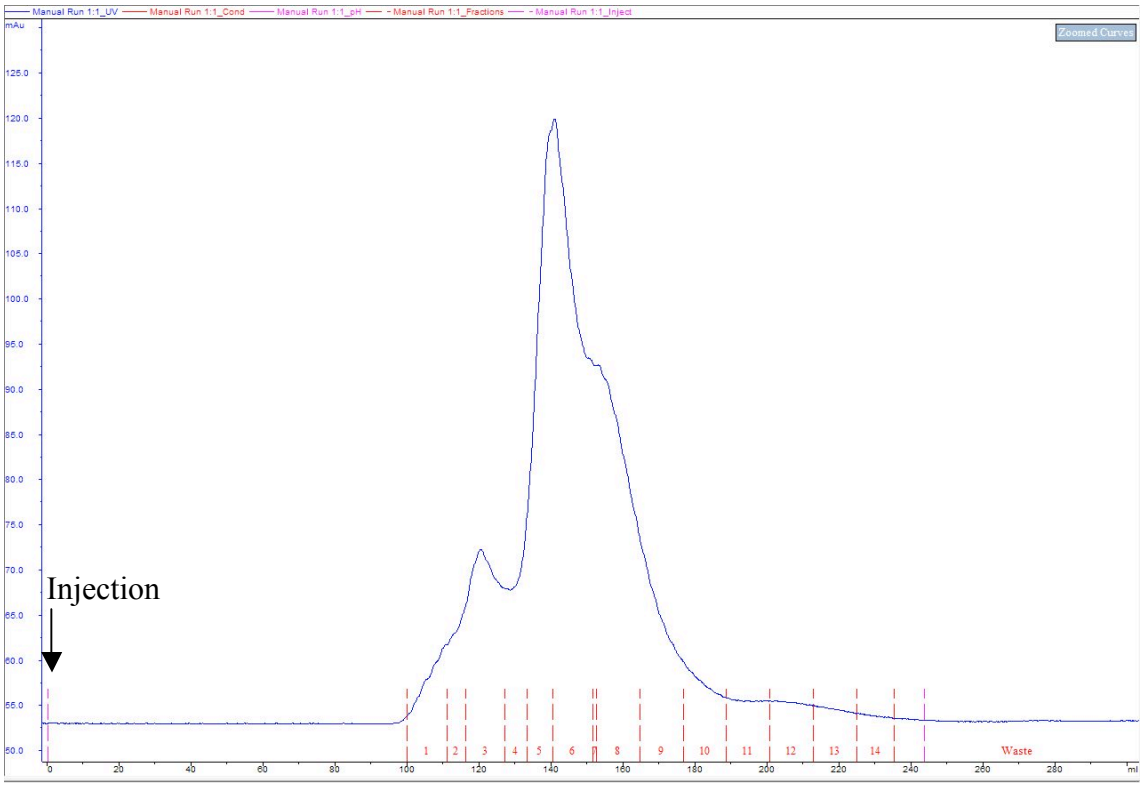
Blue curve ⇒ 280nm
Red curve ⇒ 254nm
Pink curve ⇒ 215nm
Green curve ⇒ %B buffer
Brown curve ⇒ conductance (mS)



Numbers denote peaks and their respective samples run on SDS-PAGE

a. Which chromatography technique has been used? Explain how you reached this conclusion.
b. Please explain how the gel results fit with the acquired chromatogram.
c. Suggest further purification steps (based on your answer in b).

3. You conduct one more chromatography step:



a. What type of purification is being presented in the above chromatogram?
b. How many protein populations can you detect? Please estimate the size of each population in kDa.
c. After running a representative sample of each population, you see that there are only two bands after staining with Coomassie. Please explain the results.
d. Your objective is to crystallize the protein – is this sample homogenous enough for crystallization?

HisTrap™ HP Columns - Easy, High-performance Purification

Technical Information

** For licensing information, see Legal Info*

HisTrap HP 1-ml and 5-ml columns are designed for simple, one-step purification of histidine-tagged proteins. The columns are prepacked with Ni Sepharose High Performance, which has high binding capacity and low nickel ion leakage that ensures reliable capture of target protein in repeated IMAC purifications.

HisTrap HP columns can also be used for the purification of tagged proteins containing shorter or longer polyhistidine tags, such as $(histidine)_4$ or $(histidine)_{10}$. The shorter $(histidine)_4$ will bind more weakly and the longer $(histidine)_{10}$ will bind more strongly compared with $(histidine)_6$. This difference in binding strength can be used during purification; since $(histidine)_{10}$ binds more strongly, a higher concentration of imidazole can be added to the lysed cells. This can facilitate the removal of contaminants that can otherwise be co-purified with the tagged target protein.

The high stability and broad compatibility of Ni Sepharose™ High Performance maintains biological activity and increases product yield, at the same time as it greatly expands the range of suitable operating conditions.

For convenient scaling up of histidine-tagged protein purification, use 20-ml HisPrep™ FF 16/10 columns. Ni Sepharose™ 6 Fast Flow, the medium prepacked in HisTrap™ FF and HisPrep™ FF 16/10 columns, allows high flow rates, which facilitates scale-up of histidine-tagged protein purification.

HiTrap™ IMAC HP, see IMAC Sepharose High Performance Media/HiTrap IMAC HP Columns, is the product of choice when charging the medium with different metal ions for optimization of purification protocols.

For information on the complete range of products for histidine-tagged protein purification, see Introduction to Tagged Protein Purification.

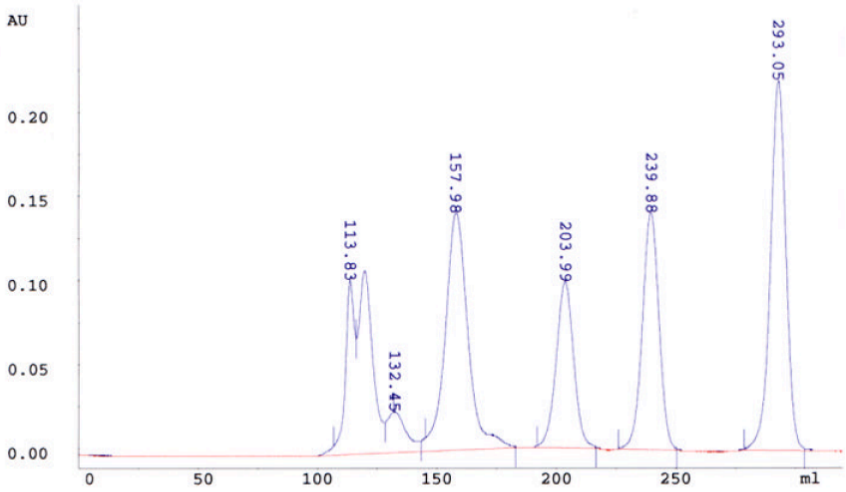
TECHNICAL SPECIFICATIONS	
Medium	Ni Sepharose™ High Performance
Column volume	1 ml and 5 ml
Dynamic binding capacity*	At least 40 mg histidine-tagged protein/ml medium
Column dimensions	0.7 × 2.5 cm (1 ml); 1.6 × 2.5 cm (5 ml)
Recommended flow rate	1 ml/min (1 ml); 5 ml/min (5 ml)
Max flow rate†	4 ml/min (1 ml); 20 ml/min (5 ml)
Max. pressure†	0.3 MPa, 3 bar
pH stability ‡	2–14 (short term), 3–12 (long term)
Compatibility	Stable in all commonly used buffers, reducing agents, denaturants and detergents (see Ni Sepharose™ High Performance - High-performance Purification) for more information.
Chemical stability	For more information see Introduction to Tagged Protein Purification.
Storage	20% ethanol
Storage temperature	4°C to 30°C
** Protein binding capacity is protein-to protein dependent.* *† H_2O at room temperature* *‡ Ni^{2+}-stripped medium*	

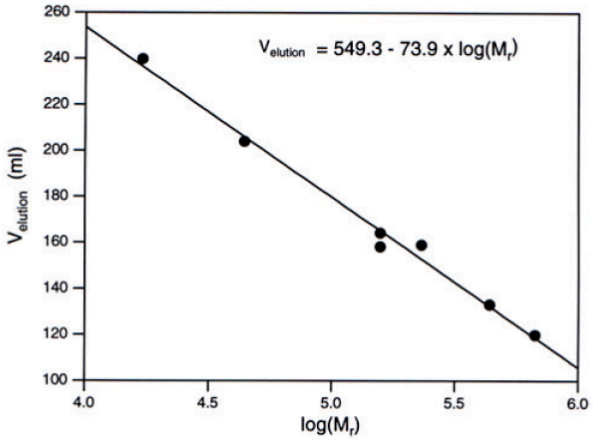
Superdex 75/200 26 60 pg (XK 26/60)

Column data

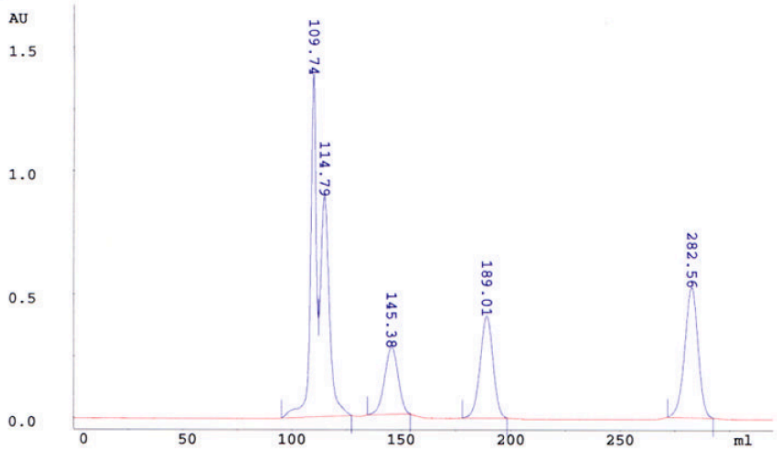
Matrix	Dextran covalently bound to highly cross-linked agarose
Mean particle size	34 µm
Separation range (M_r)	
globular proteins	<10 000 (Superdex 30 pg) 3×10^3–7×10^4 (Superdex 75 pg) 1×10^4–6×10^5 (Superdex 200 pg)
dextrans	5×10^2–3×10^4 (Superdex 75 pg) 1×10^3–1×10^5 (Superdex 200 pg)
Column volume[1]	120–124 ml (XK 16/60) 319–330 ml (XK 26/60)
Bed volume	220 ml
Sample volume[2]	Up to 5 ml (XK 16/60) Up to 13 ml (XK 26/60)
Recommended flow rate	10–50 cm/h at room temperature (0.3–1.6 ml/min for XK 16/60 or 0.9–4.4 ml/min for XK 26/60)
Theoretical plates	>13 000 m^{-1}
Maximum pressure over the packed bed during operation[3]	0.3 MPa, 3 bar, 42 psi
HiLoad column hardware pressure limit[3]	0.5 MPa, 5 bar, 73 psi
pH stability	
long term and working range	3–12
short term	1–14
Storage	20% ethanol

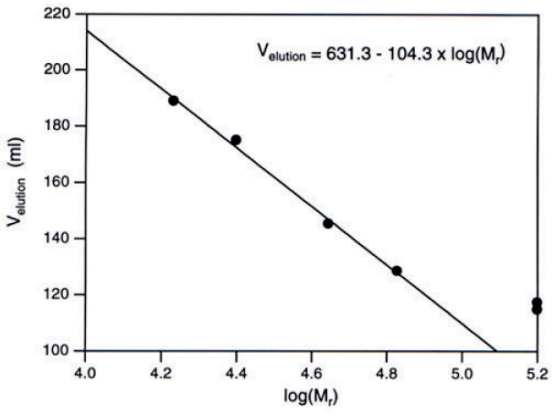
Calibration of HiLoad 26/60 Superdex 200 prep grade





Calibration of 26/60 HiLoad Superdex 75 prep grade





HiTrap™ SP HP and HiTrap™ Q HP ion exchange columns are fast and convenient to use.

The ion exchange media packed in HiTrap™ ion exchange columns are based on Sepharose™ High Performance. The small particle size (34µm) allow s fast adsorption and desorption even at high sample loadings and flow rates. SP Sepharose™ High Performance is a strong cation exchange medium and Q Sepharose™ High Performance is a strong anion exchange medium. Both remain charged and have high loading capacities over broad pH ranges.

TECHNICAL SPECIFICATIONS

HiTrap™ SP HP and HiTrap™ Q HP

Media	SP Sepharose™ High Performance Q Sepharose™ High Performance
Column volume	1 ml or 5 ml
Max. flow rate	
1-ml column	4 ml/min
5-ml column	20 ml/min
Recommended flow rate	
1-ml column	1 ml/min
5-ml column	5 ml/min
Max. back pressure	3 bar (43 psi, 0.3 MPa)
Storage	20% ethanol (Q), 0.2 M sodium acetate in 20% ethanol (SP)
Storage temperature	4°C to 30°C

HiTrap™ HIC HP and FF Columns

Technical Information

For more information about these columns, please see HiTrap™ HIC Selection Kit.

** See licensing information at back of catalog.*

TECHNICAL SPECIFICATIONS	
Media	Phenyl Sepharose™ 6 Fast Flow (high sub)
	Phenyl Sepharose™ 6 Fast Flow (low sub)
	Phenyl Sepharose™ High Performance
	Butyl Sepharose™ High Performance
	Butyl Sepharose™ 4 Fast Flow
	Butyl-S Sepharose™ 6 Fast Flow
	Octyl Sepharose™ 4 Fast Flow
	Butyl Sepharose™ High Performance
Column volume	1-ml or 5-ml
Max. flow rate*	
1-ml column	4 ml/min
5-ml column	20 ml/min
Recommended flow rate	
1-ml column	1 ml/min
5-ml column	5 ml/min
Max. pressure	3 bar (0.3 MPa, 43 psi)
Storage	20% ethanol
Storage temperature	4°C to 30°C
** H_2O at 25°C*	

GSTrap HP — Technical Specifications

Bed Volume	1 ml
Bed Dimensions	7 x 25 mm
Flow rate	<4 ml/min[1)]
Storage Conditions	4 to 30°C, 20% Ethanol
Pressure Max. [Over the Packed Bed During Operation]	3 bar [0.3 MPa] (42 psi[2)])

[1)]The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

[2)]H_2O at room temperature.

Media

Ligand	Glutathione
Average Particle Size	34 µm
Matrix	Highly crossed-linked agarose, 6%
Binding Capacity/ml Chromatography Medium	>7 mg/ml medium[1)]
pH stability Working Range	3-12
pH stability Cleaning	3-12
Flow Velocity	<600 cm/h
Storage Conditions	4 to 30°C, 20% Ethanol
Chemical Stability	All commonly used aqueous buffers

[1)]Binding capacity will vary depending of the type of cell lysate, target protein, flow rate, temperature, pH, etc. This is an important consideration, especially during sample loading and elution.

Column

Complete Packsize	1 ml
Column i.d.	7 mm
Material [Column Hardware]	Polypropylene (PP)