ÄKTA™ and Biacore™: Enabling protein purification and characterization at microscale range

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cytiva

ÄKTA™ pure micro

Suitable for small sample volumes and micro-preparative columns

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Introduction

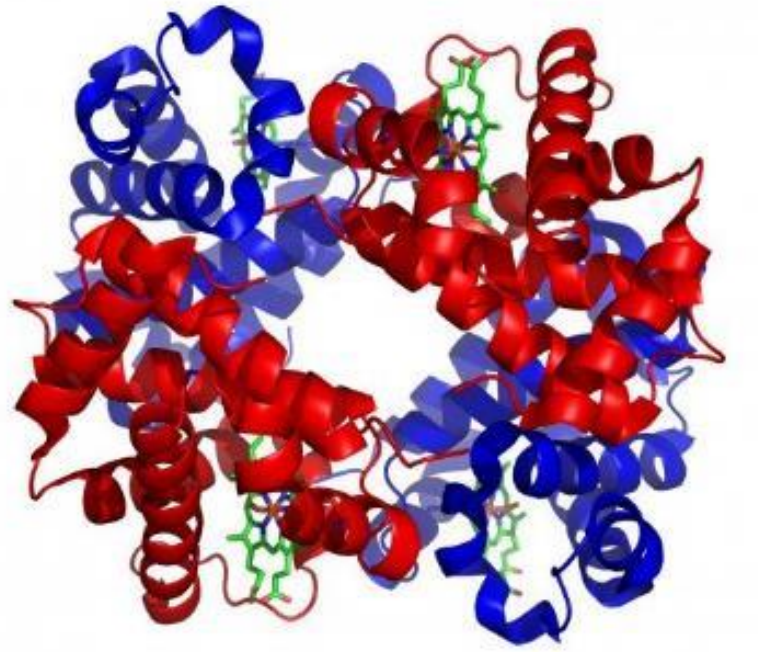
Knowing the 3D structure is essential to understand the function of a protein

In recent years, technical development has led to cryo-EM becoming the preferred technique in structural biology.

Cryo-EM enables structural determination of difficult targets and captures the native state of the molecules.

Top challenges when purifying proteins for cryo-EM experiments include:

- Limited sample volume in microliter (µL) scale.
- The inability of the chromatography system to handle limited sample volumes.

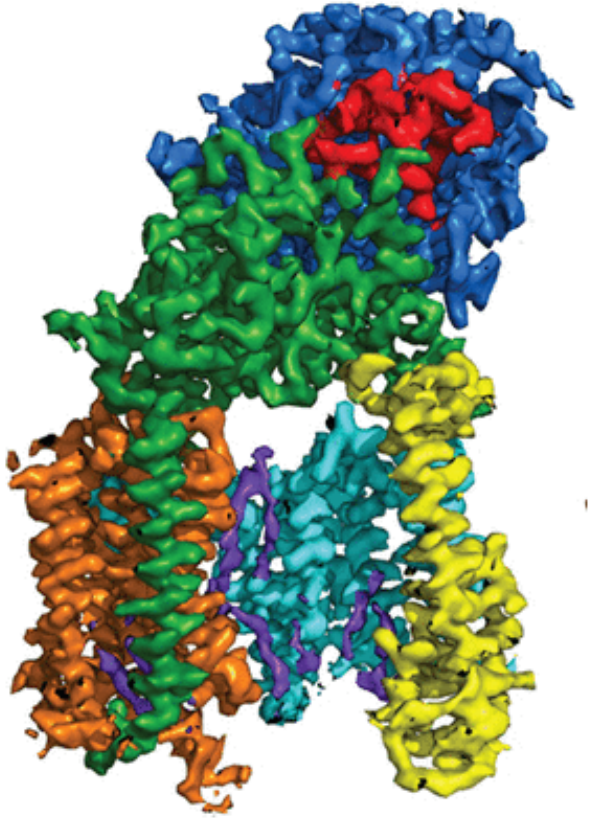


Hemoglobin

What is required for a succesful cryo-EM experiment?

- Only a few microliters (µL) of sample required
- Sample concentration in the range of 0.1 to 5 mg/mL
- Compositional and conformational homogeneity
- Modification/ treatment for increased stability (complexes, membrane proteins)

Ensuring sample quality puts high demand on protein characterization and purification.



Single particle reconstruction of the intramembrane protease γ-secretase at 3.4 Å resolution.

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Introducing ÄKTA™ pure micro

ÄKTA™ lab-scale systems: added functionality to ÄKTA™ pure supporting microscale purification

ÄKTA™ start

- Transition from manual to automated purification
- Educational tool
- Affordable and easy-to-use

ÄKTA™ go

- Achieve desired purity with ease
- Routine purifications
- Make the most of valuable bench/cold room space
- Quick method creation

ÄKTA™ pure

- Flexible to match current and future purification challenges
- Advanced automation setups
- Automated multistep
- Microscale purification

ÄKTA™ avant

- Productivity in PD
- Secure
- Scale-up

Cytiva

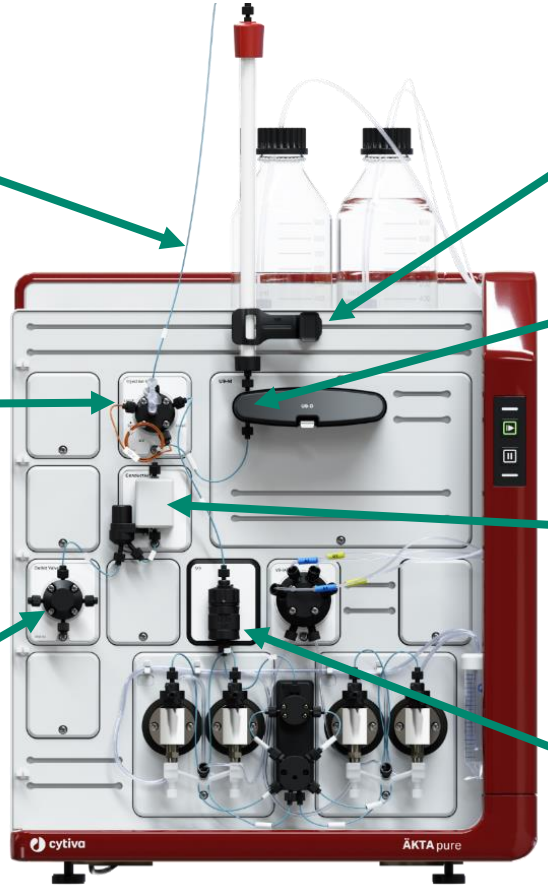
ÄKTA™ pure micro with optimized flow path for high performance microscale purification

Tubing kit
Minimize sample dilution and maintain peak resolution with low-volume tubing kit adapted for column size used.

Micro injection valve
Minimize sample dilution between injection valve and column inlet to ensures SEC[1] resolution.

Micro outlet valve
Minimize sample dilution after column for less peak broadening

Maintain peak resolution between outlet and fraction collector.



Column clamp
Hold SEC[1] column mounted directly on UV for minimized system volume

UV microflow cell
Minimize sample dilution after column and gives less peak broadening.

Micro conductivity monitor
Minimize sample dilution after column and gives less peak broadening.

Keep track of salt peak in SEC[1] and gradient formation for IEX[2]

Mixer chamber
Optimizes gradient for IEX[2]

[1] SEC = size exclusion chromatography. [2]IEX = ion exchange chromatography

Fraction collector F9-T for micro-preparative purification

- Recommended fraction collector for ÄKTA™ pure micro
- Dual plate faction collector
  - Microtiter plates
  - Microplate holder
- Micro nozzle creates small drops
- Drop sync for spillage free fractionation
- UNICORN™ 7.6 (or later version) required

Columns, autosampler, software and service for microscale purification

Cytiva columns for cryo-EM applications:

Technique	Prepacked columns	Dimensions
SEC	Superdex™ Increase	10/300, 5/150, 3.2/300
IEX	Capto™ HiRes	5/50

Resins with bead size down to 5 µm[1] can be used.

ALIAS™ Bio autosampler, to handle multiple samples, down to 1 µL.

UNICORN™ 7 software, designed to quickly get started using pre-defined methods for microscale purification.

Service offering available for installation.

[1] For long columns, back pressure may cause problems and require decreased flow rate.

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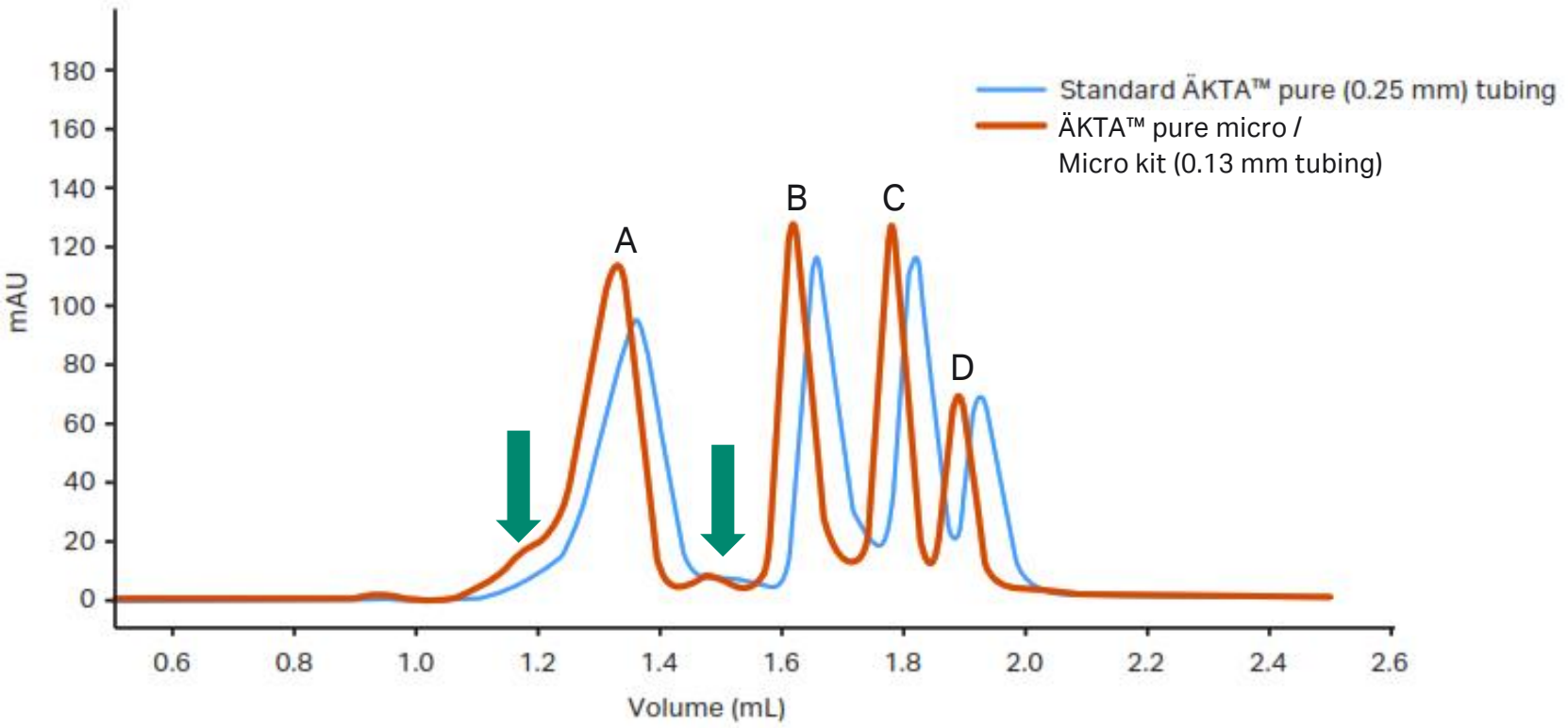
Performance

ÄKTA™ pure micro for improved resolution and sharper peaks



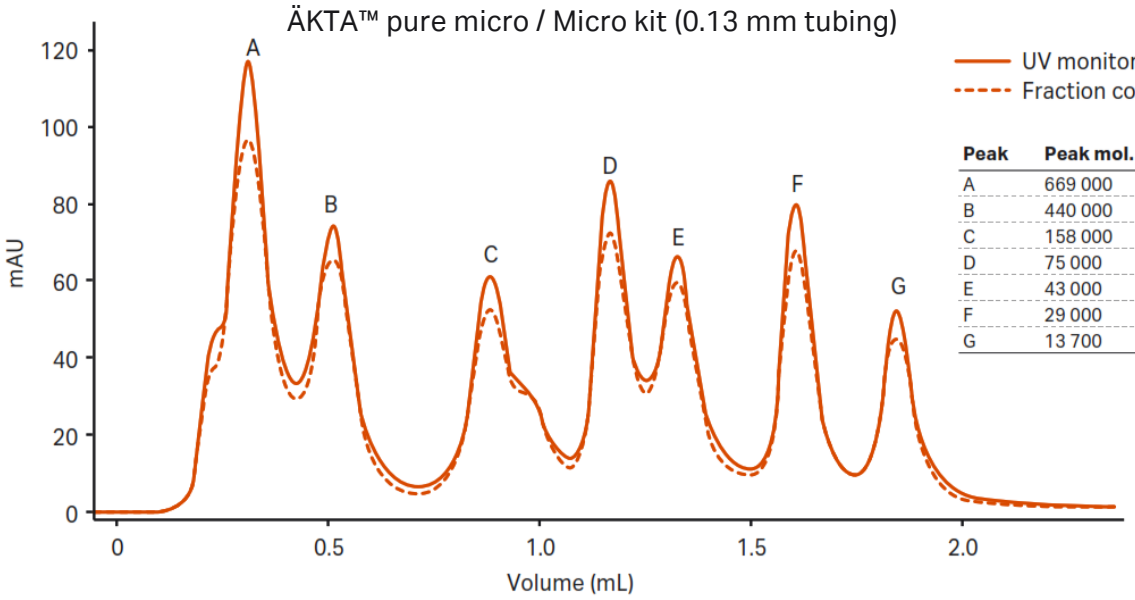
Superdex™ 200 Increase 3.2/300 column

Peak*	Peak volume at half height (mL)	
	Standard ÄKTA™ pure	ÄKTA™ pure micro/ Micro kit
Peak A	0.113	0.098
Peak B	0.063	0.054
Peak C	0.059	0.047
Peak D	0.054	0.049

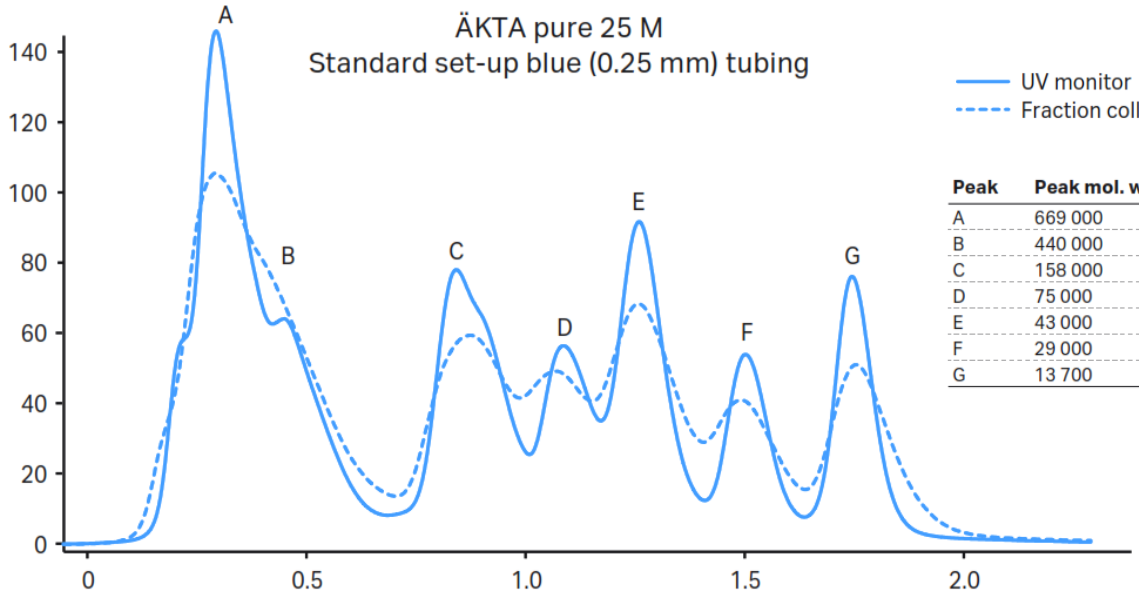
Peak*	Resolution	
	Standard ÄKTA™ pure	ÄKTA™ pure micro/ Micro kit
Peak A		
Peak B	1.97	2.23
Peak C	1.55	1.87
Peak D	1.13	1.35

*Peak A: Ferritin; Peak B: Conalbumin; Peak C: Carbonic anhydrase; Peak D: RNase

Comparing peak resolution at column outlet and fraction collector shows maintained resolution



Peak	Peak mol. weights (M_r)
A	669 000
B	440 000
C	158 000
D	75 000
E	43 000
F	29 000
G	13 700



Peak	Peak mol. weights (M_r)
A	669 000
B	440 000
C	158 000
D	75 000
E	43 000
F	29 000
G	13 700

Overlay Mix A[1] and Mix B[2] on Superdex™ 200 increase 3.2/300

[1] Mix A:
0.3 mg/mL ferritin
3 mg/mL conalbumin
3 mg/mL carbonic anhydrase

[2] Mix B:
5 mg/mL thyroglobulin
4 mg/mL aldolase
4 mg/mL ovalbumin
3 mg/mL ribonuclease

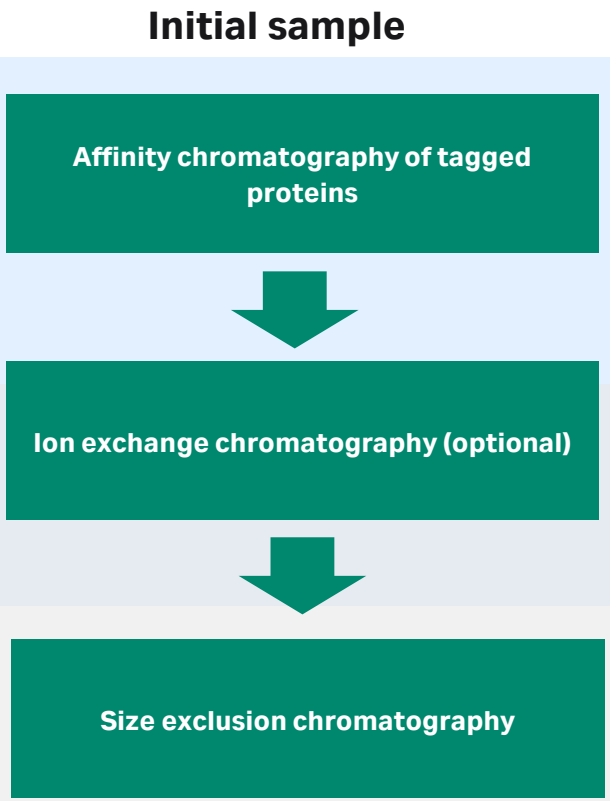
ÄKTA™ pure micro supports cryo-EM purifications

Standard ÄKTA™ pure:

For larger samples, revert to standard ÄKTA™ pure 25.

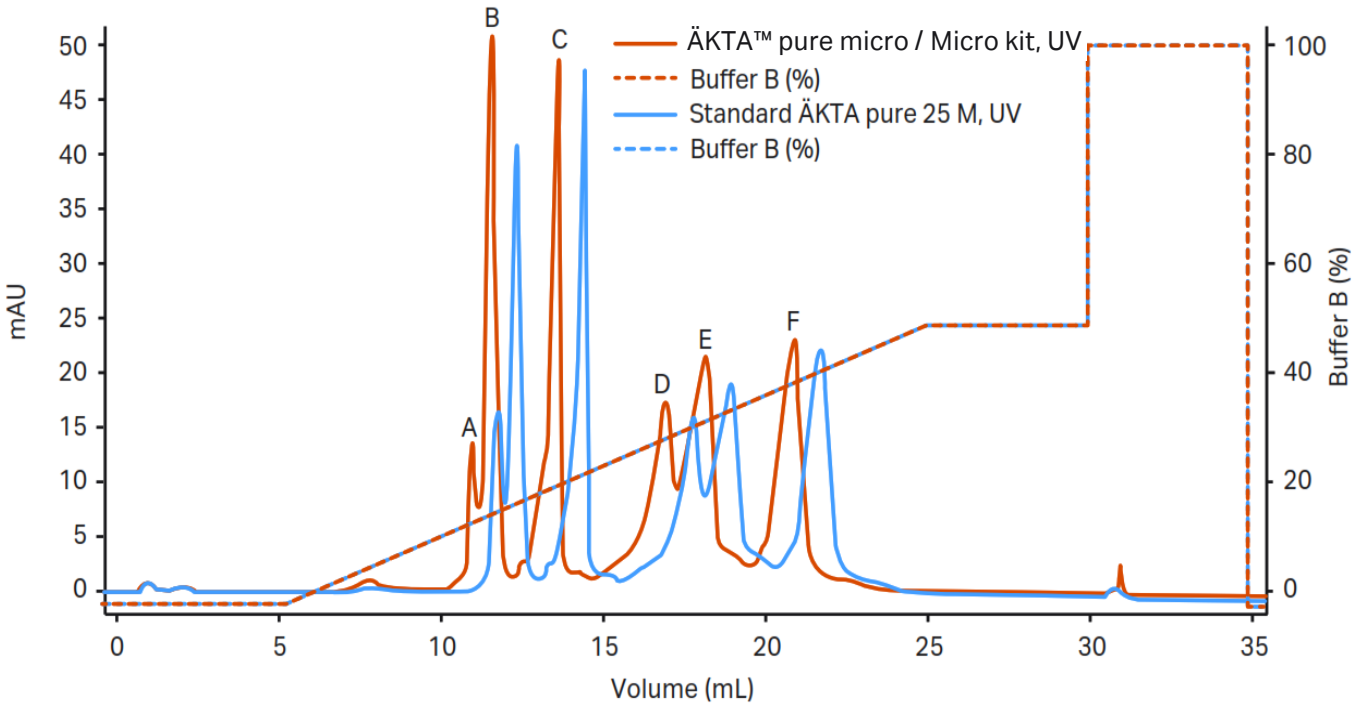
ÄKTA™ pure micro:

- Supports IEX runs on Capto™ HiRes 1 mL columns at microliter (µL) scale.
- SEC on Superdex™ Increase 3.2/300 columns at microliter scale.
- Simply change tubing after column valve outlet to fraction collector and run on the same system configuration.



Peak resolution – AIEX (Capto™ HiRes Q 5/50)

- ÄKTA™ pure micro system works with 1 mL Capto™ HiRes columns.
- Use the blue tubing kit to minimize system back pressure.



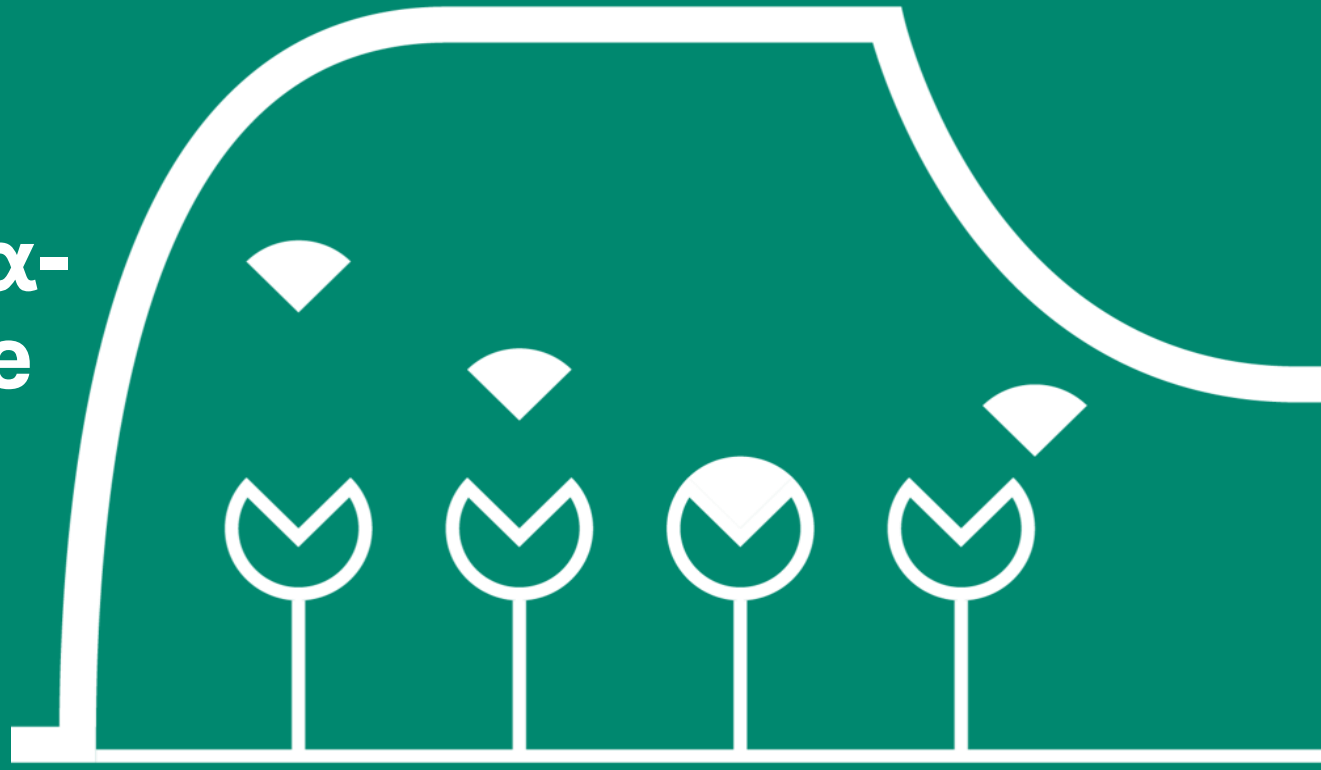
A. apo-Transferrin
B. apo-Transferrin
C. α–Lactalbumin
D. β-Lactoglobulin
E. β-Lactoglobulin
F. Amyloglucosidase

Peak	Peak volume at half height (mL)	
	Standard ÄKTA™ pure with blue (0.25 mm) tubing	ÄKTA™ pure micro/ Micro kit
Peak A	0.221	0.177
Peak B	0.278	0.242
Peak C	0.222	0.221
Peak D	0.559	0.537
Peak E	1.015	0.929
Peak F	0.837	0.831

ÄKTA™ pure micro for small sample volumes and micro-preparative columns

- Addresses protein purification challenges for cryo-EM samples
- High performance for increased resolution with minimized internal volumes
- Combine with fraction collector F9-T for collection in microtiter plates
- Equip your ÄKTA™ pure 25 M for micro-preparative runs using our Micro kit

Accurate concentration assessment of interferon α-2a (IFN α-2a) using Biacore systems



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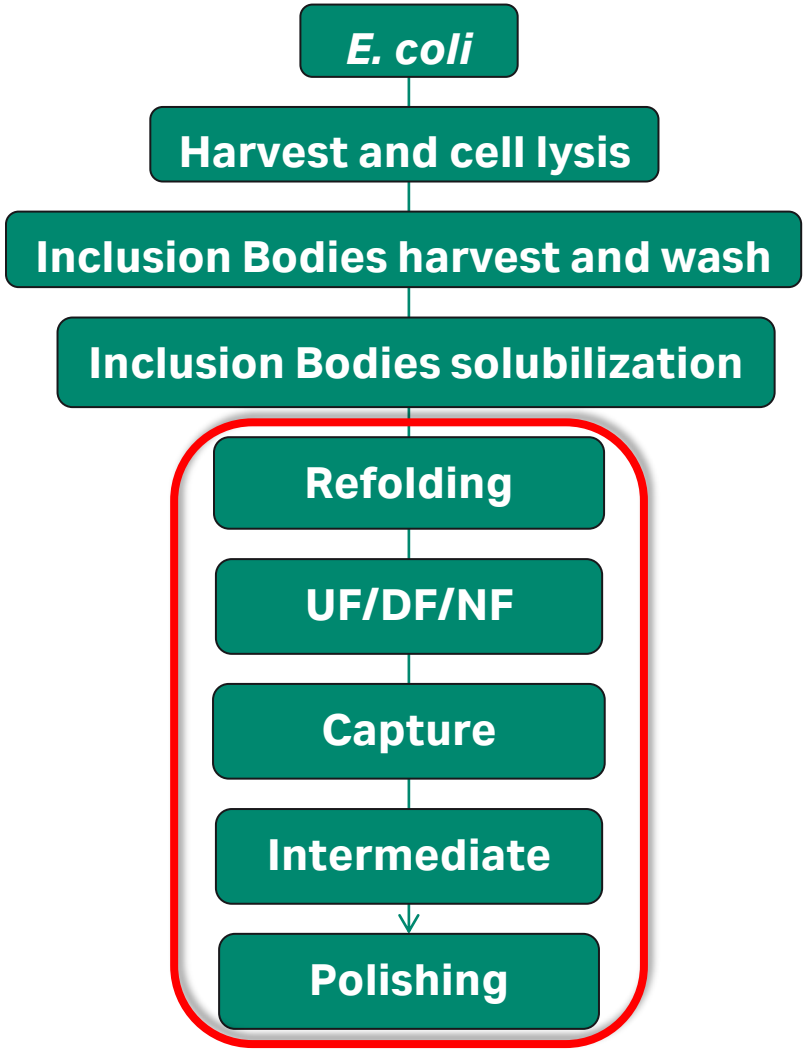
Purpose of project

We want to demonstrate the benefits of using Biacore™ systems in interferon α-2a (IFN α-2a) purification.

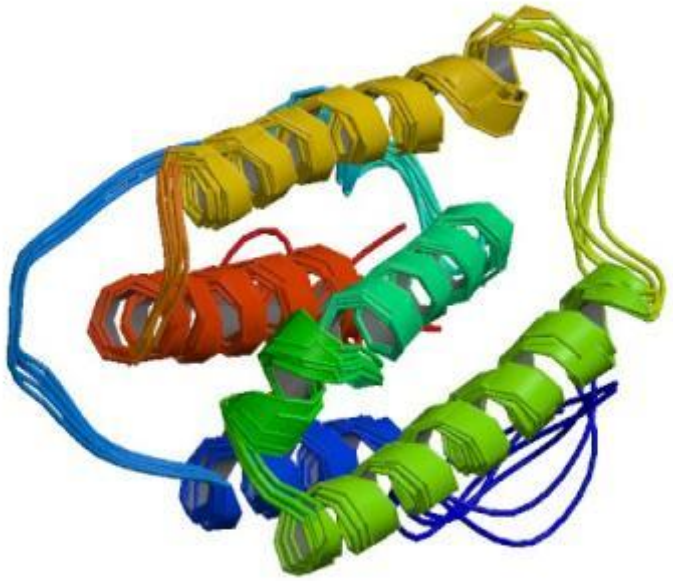


Interferon α-2a

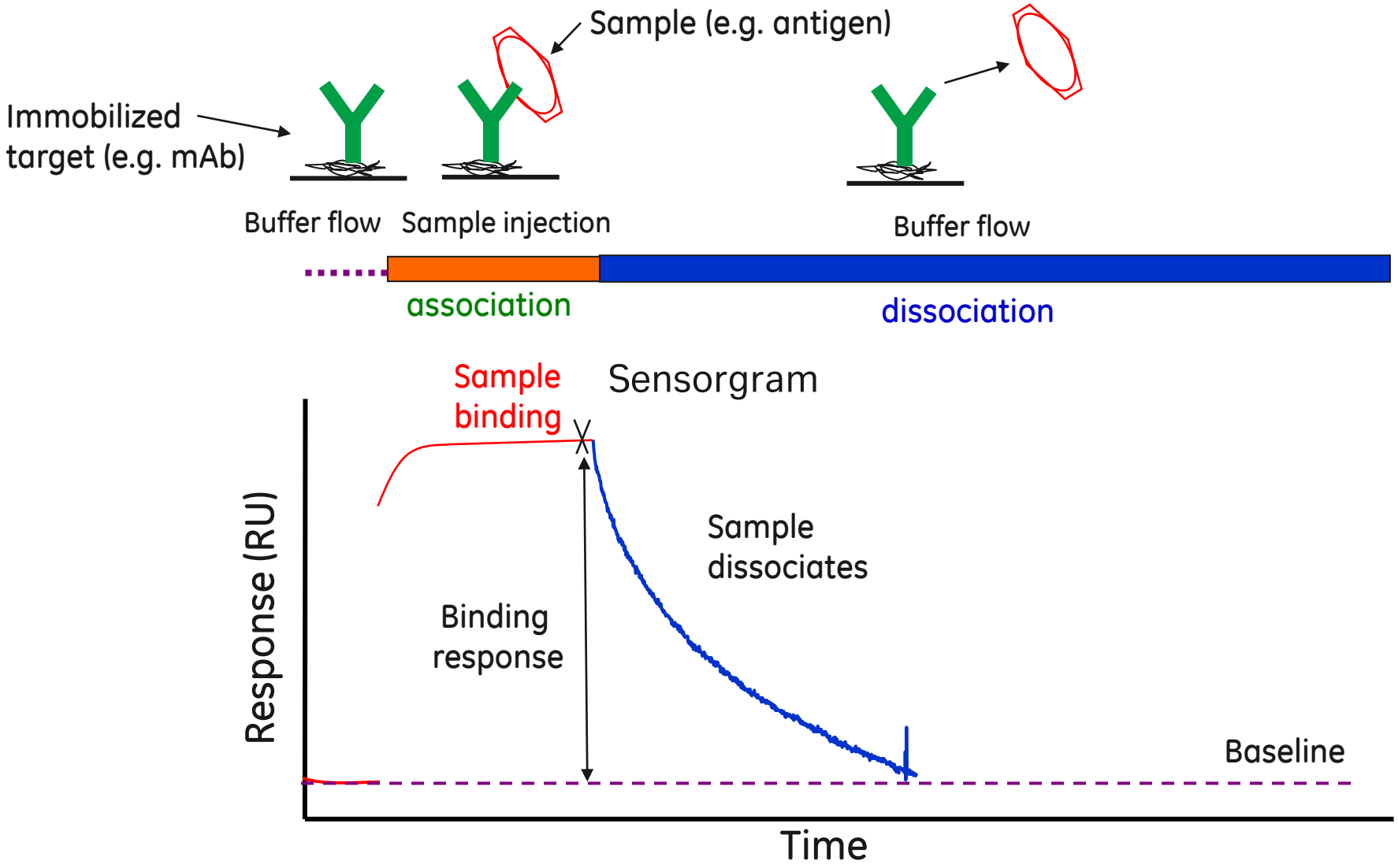
- α-interferon have potent antiviral activity
- Treatment of patients with chronic hepatitis, including hepatitis B, C, and D
- Inhibition of tumor growth

Molecular weight = 19241 Da

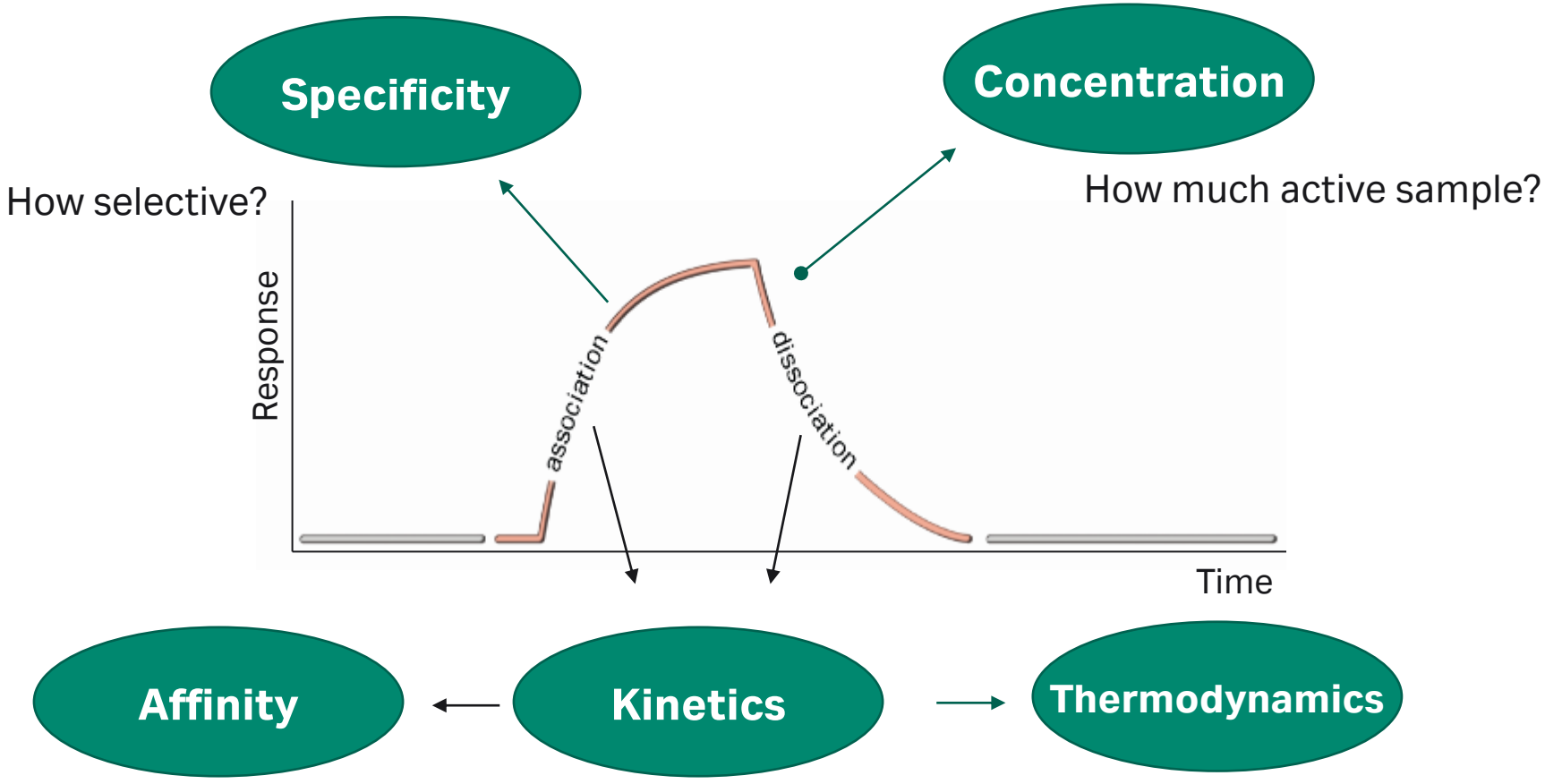
- Isoelectric point= 5.99
- Expressed in *Escherichia coli*
- 165 amino acids, 2 disulphite bonds



What do Biacore™ systems measure?

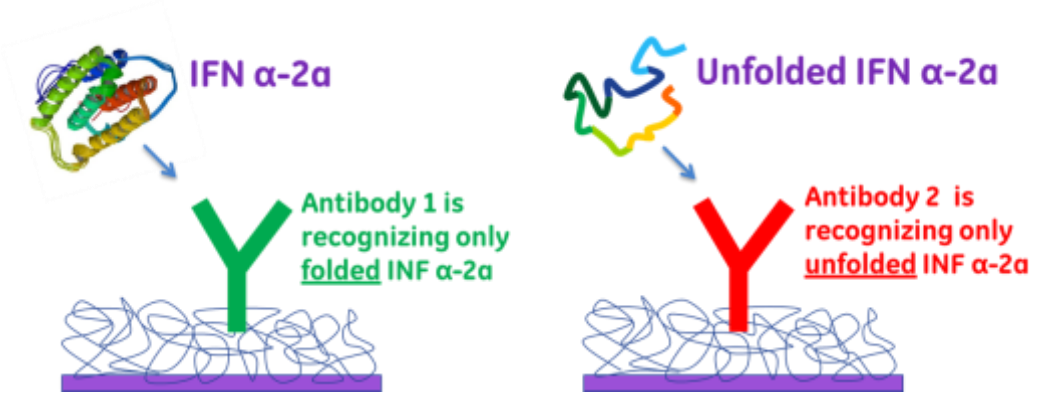


Insight into molecular interactions – The Sensorgram

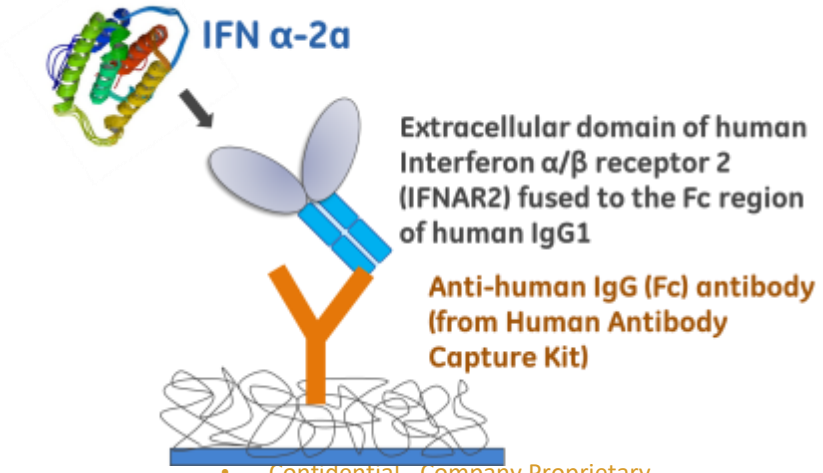


Concentration and kinetics analysis provides critical information in various steps

1. Concentration assay by Calibration-free concentration analysis (CFCA)



2. Kinetic analysis



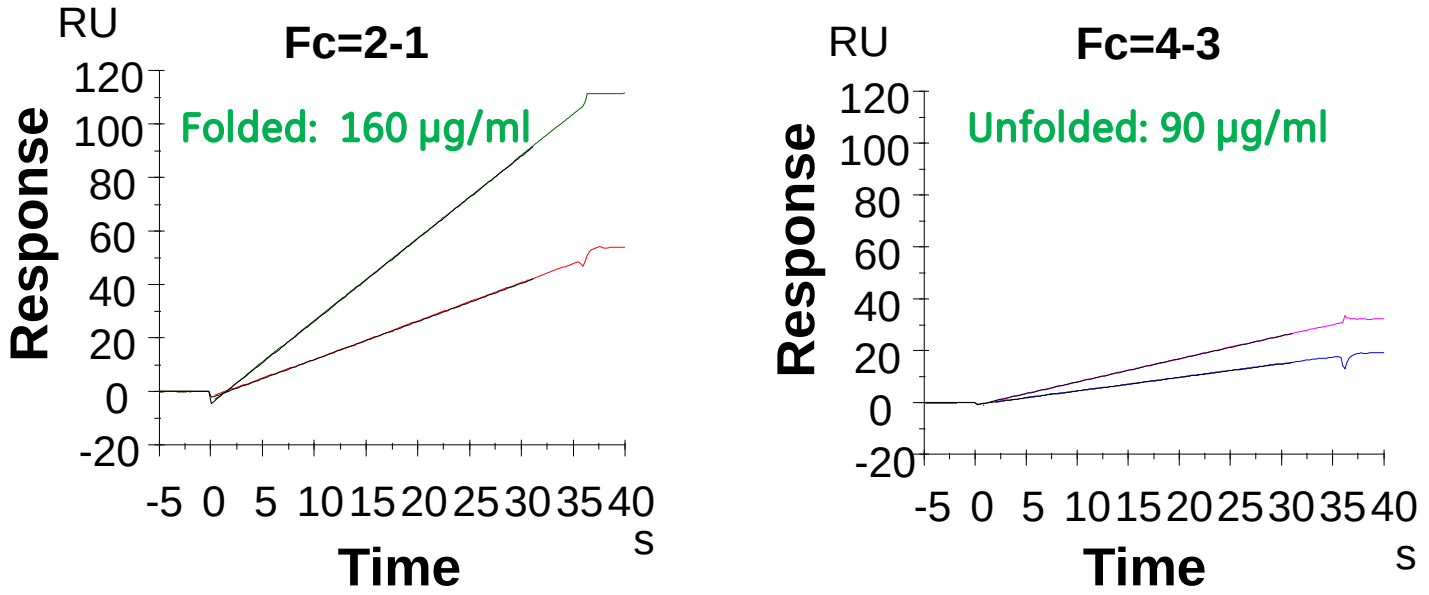
CFCA quickly finds the best refolding conditions with parallel measurement of folded and unfolded protein

Refolding buffers tested:

50 mM Tris/HCl pH 8.0 ± 0.5 M L-Arginine, or ± 10 mM Gluthathione

50 mM Tris/HCl pH 10.0 ± 0.5 M L-Arginine, or ± 10 mM Gluthathione

Refolding in 50 mM Tris/HCl pH 10.0 containing
0.5 M L-Arginin gave the highest fraction of folded protein.

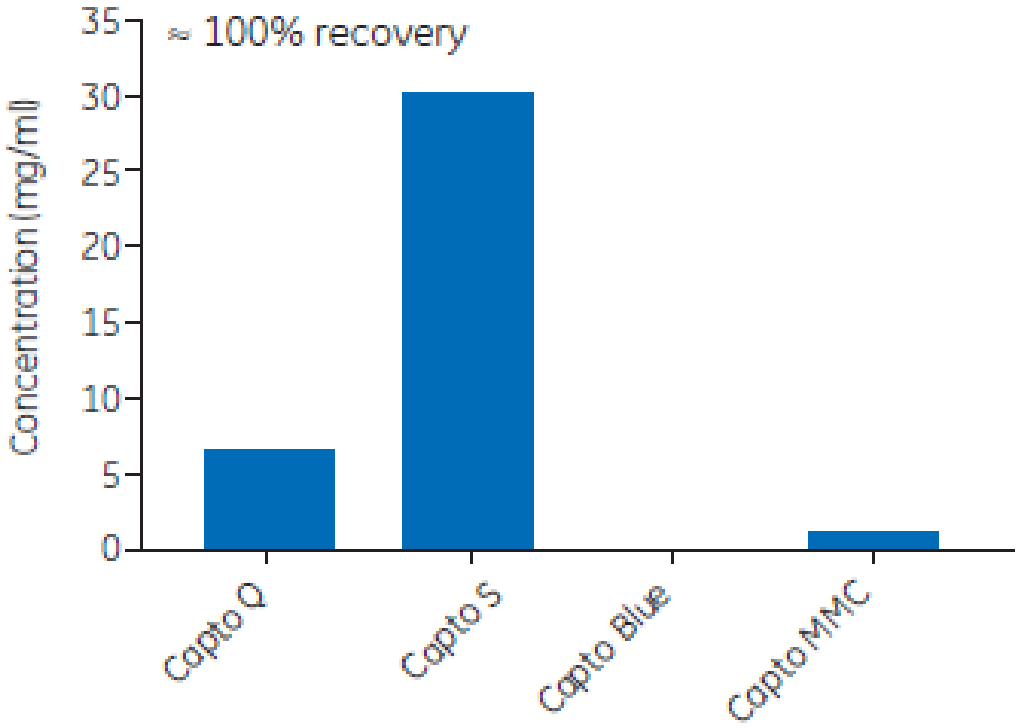


Refolding yield: 64%

CFCA guides the selection of the most appropriate chromatographic resin for the capture step

Fractions eluted from several types of columns were analyzed by CFCA, and recovery was calculated with respect to amount of protein applied to the column.

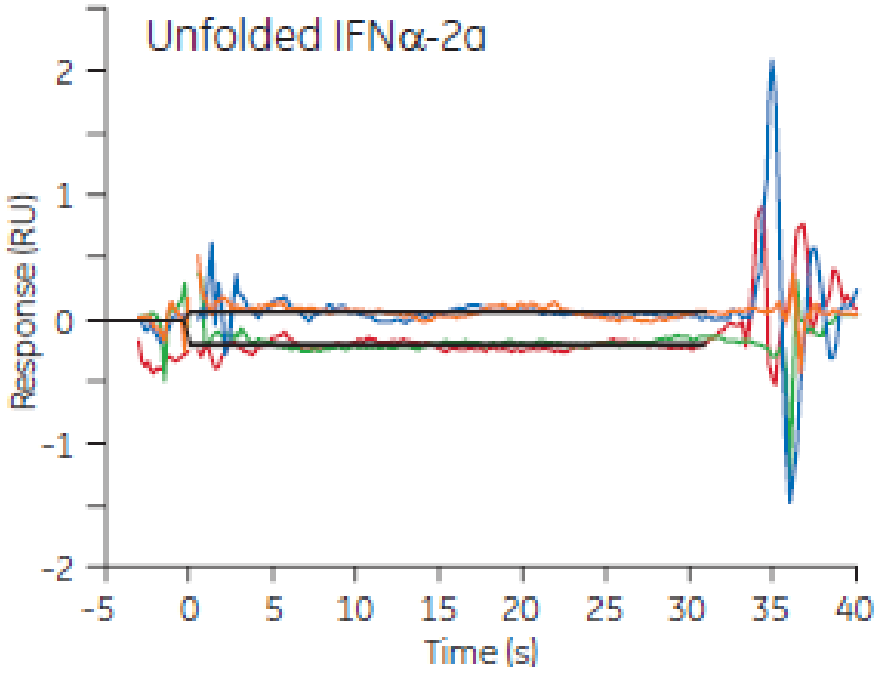
The highest eluted concentration and recovery was obtained with Capto™ S.

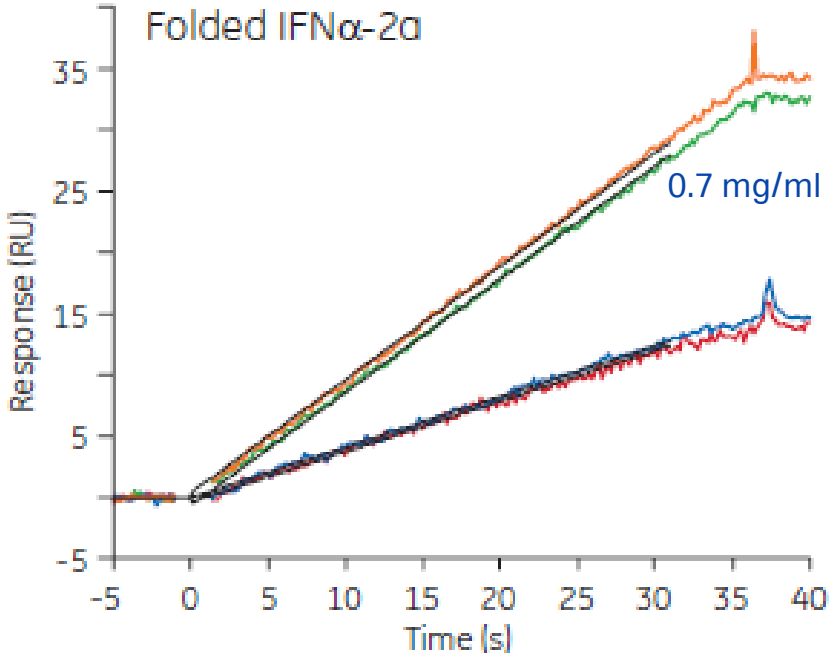


Selection of media for polishing step

Screening of several HIC media was performed - Capto Octyl chosen for polishing

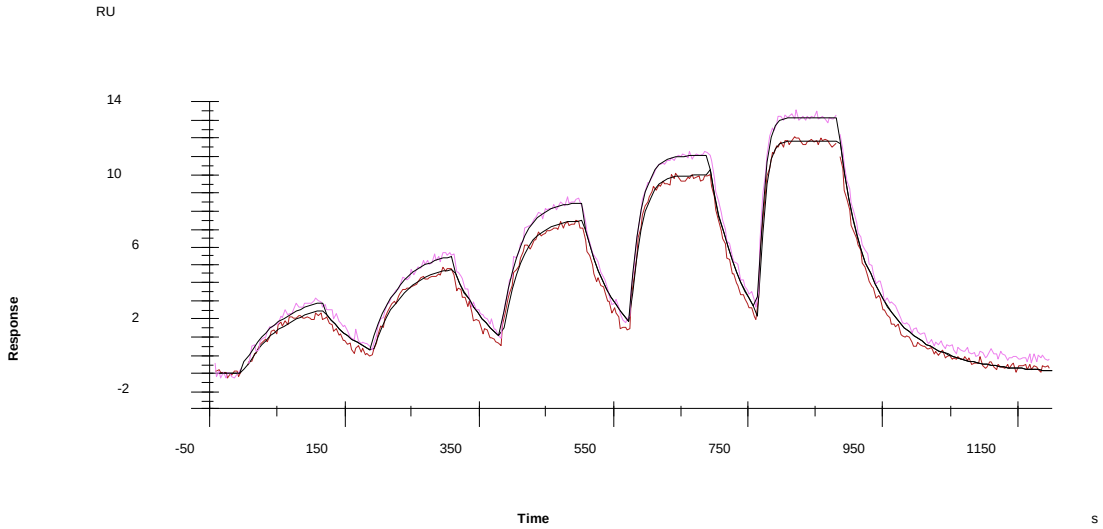
Concentration analysis using Biacore™ of the final product revealed that no unfolded protein was present



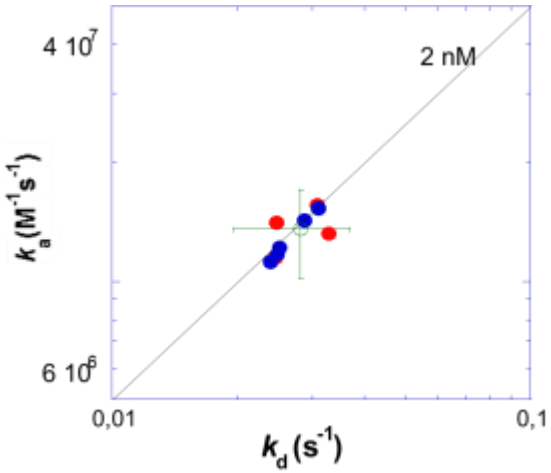


Kinetic analysis monitors receptor binding and comparability

IFNα-2a after Capto™ S



Capture



Originator molecule, several measurements

Samples from process development

Accurate concentration assessment of an interferon using Biacore

Biacore system enabled

- optimization of the refolding step
- selection of the most appropriate chromatographic resins
- optimization of binding and elution conditions

Thank you

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