BIA separations
Leaders in Monolith Chromatography

Fingerprint your bioprocess for more robust production

Ales Strancar, August 2017

About BIA Separations

- Incorporated in September 1998 in Ljubljana, Slovenia
- An OEM partnership with **Agilent** (former HP) in 2008
- In 2011 moved to **new dedicated facility** in Ajdovščina
- In 2012 strategic partnership with **SDK** Corporation (Shodex), 9B USD multinational company with HQ in Japan
- In 2016/2017 first projects to supply CIM product for biopharmaceutical drug manufacturing

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BIA Separations products and services

Convective Interaction Media (CIM®)

Pre-packed monolithic columns

CIMac™ Analytical and CIMmultus™/CIM® Preparative columns

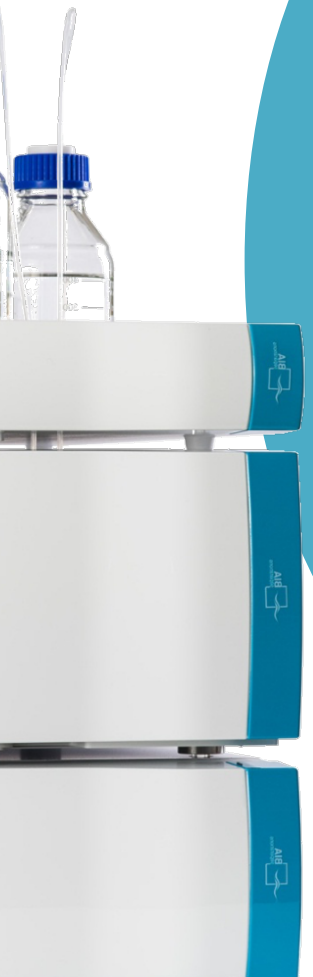
Services, Process development and Technical Support

Development of processes and methods for separation/concentration/purification of large biomolecules

Custom immobilisations, product development

Process analytical technology

At-line PAT HPLC suite for improving process control and understanding



Bioprocess Knowledge Packages

Based on 20 years of experience with over 500 bioprocess projects, BIA Separations is pleased to offer **Bioprocess Knowledge Packages** that include published/unpublished data, and experience-based, expert recommendations for purification and analytical methods that utilize our unique monolith chromatography columns and other best-in-class technologies to enable development of the most efficient and cost-effective bioprocess possible for your biomolecule.

(Please note: each purification strategy may vary based on the specific biomolecule, biologic drug specifications and upstream production methods. Bioprocess Knowledge Packages are made available to potential clients on a royalty-free basis)

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Bioprocess Knowledge Packages available

- **Bioprocess Knowledge Package-**pDNA (works for > 30kbp)
- **Bioprocess Knowledge Package-**mcDNA
- **Bioprocess Knowledge Package-**RNA
- **Bioprocess Knowledge Package-**Adeno virus
- **Bioprocess Knowledge Package-**AAV (all serotypes)
- **Bioprocess Knowledge Package-**Flu virus (all serotypes)
- **Bioprocess Knowledge Package-**IVIG
- **Bioprocess Knowledge Package-**IgM

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BIA Separations State-of-the-Art Production Facility > 30M USD investment

Certifications & Approvals

- DMF for DEAE, QA and SO3 and C4 HLD CIM® monoliths were filed, others pending
- Partners audits (Baxter, Novartis, Octapharma, Boehringer Ingelheim, Teva, Agilent,.....)
- FDA audited (according to USA GMP regulations)
- JAZMP audited (according to EU GMP regulations)
- ISO 9001: 2008

IP

- 4 US patents and their foreign equivalents granted, more pending:
  - CIM® technology and manufacturing
  - Different geometries including scale-up

Convective Interaction Media (CIM®) monolithic columns

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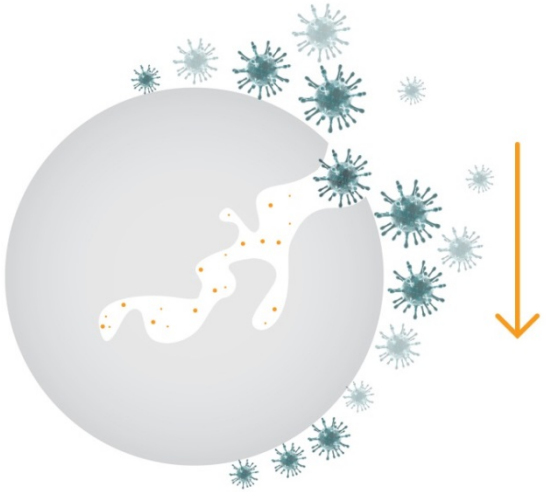
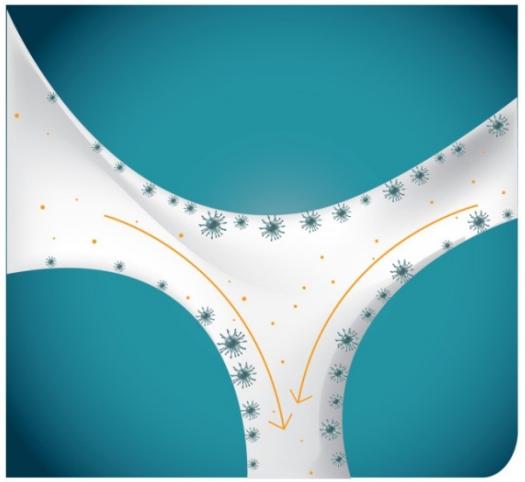
Advantages of CIM® monolithic resins (membrane is „thin slice of the monolith“)





Traditional approach - Porous particle:

- Diffusive mass transport – slow process or **lower resolution**
- Pores too small – very **low capacity**
- Counter current flow – shear forces – **lower yields**

Novel approach – Monolithic columns:

- Convective mass transport – **flow independent resolution** and capacity – very fast processes
- Accessible surface for large molecules – **high capacity**
- Laminar flow - No shear forces – **better yields of e.g. IgM**

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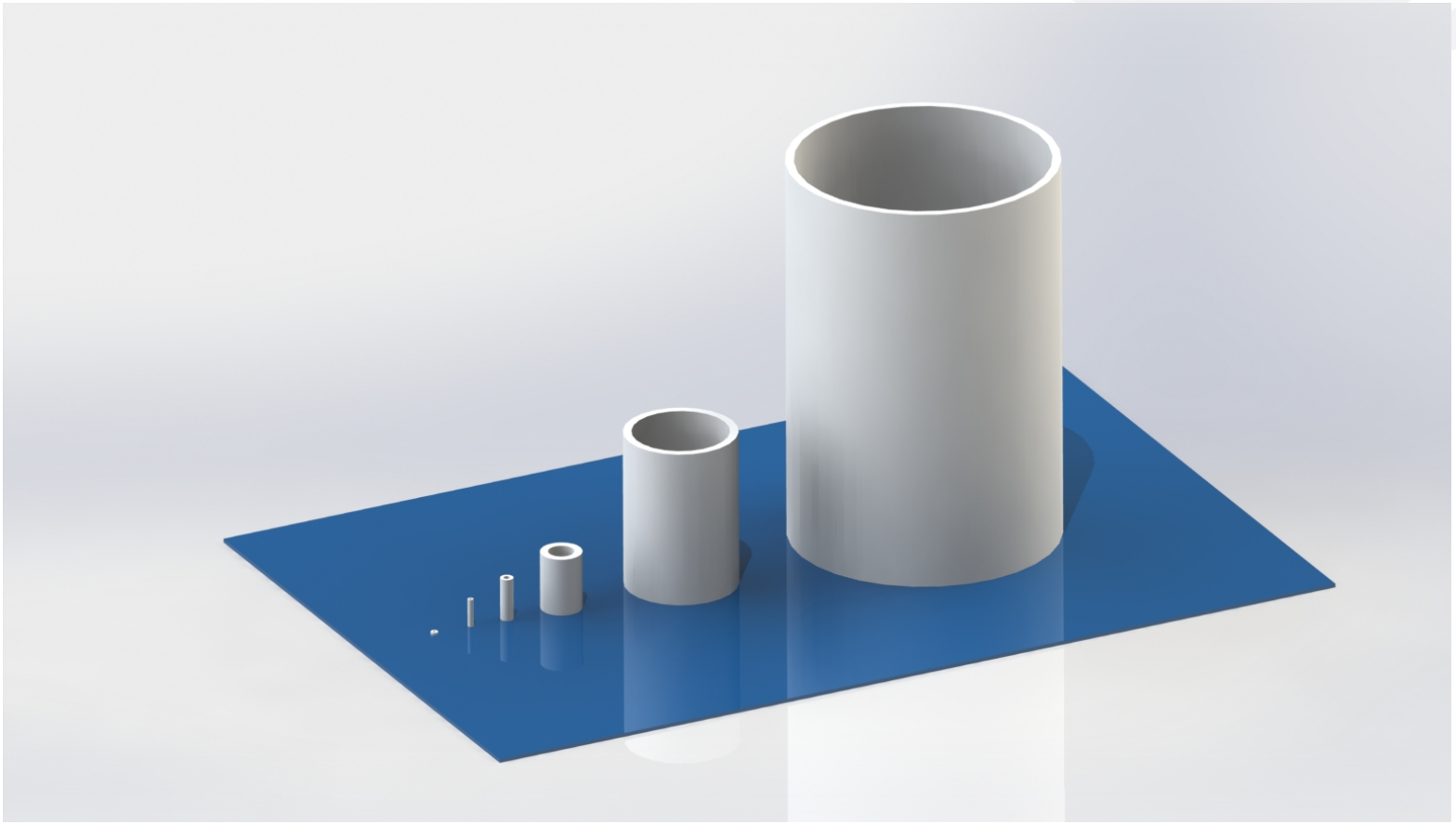
Dimensions of CIM® radial monoliths



	1 mL	8 mL	80 mL	800 mL	8 L	40 L
I.D. (mm)	6.4	6.5	16.2	65	243	636
O.D. (mm)	18.3	14.4	33	105	285	680
Thickness (mm)	5.95	3.95	8.4	20	21	22

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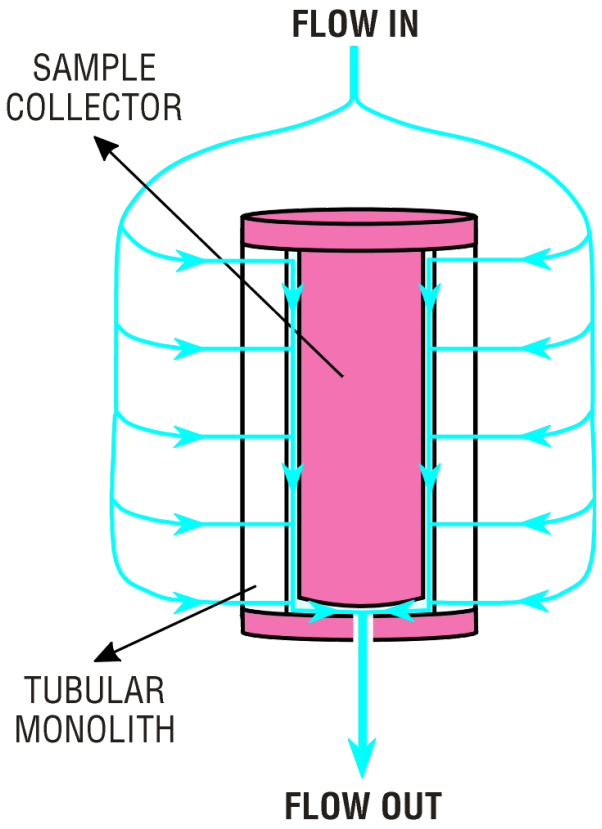
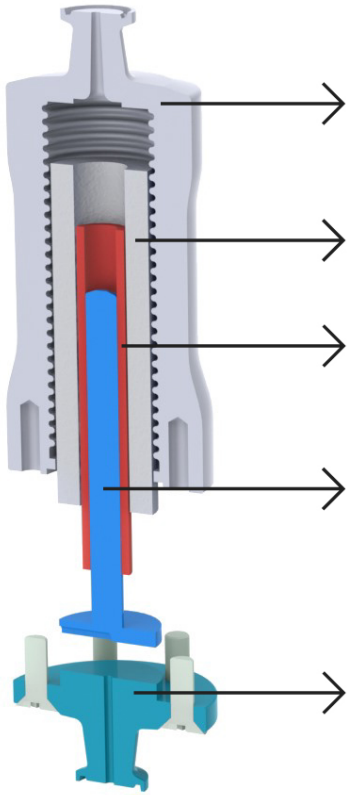
Tubular format enables short monolithic column design at lab and industrial scale

80, 800, and
8000 ml CIM monoliths





Column body with distribution thread and flow out TC connector

Monolith

Frit

Dead-volume reduction piston

Column lid with flow in TC connector

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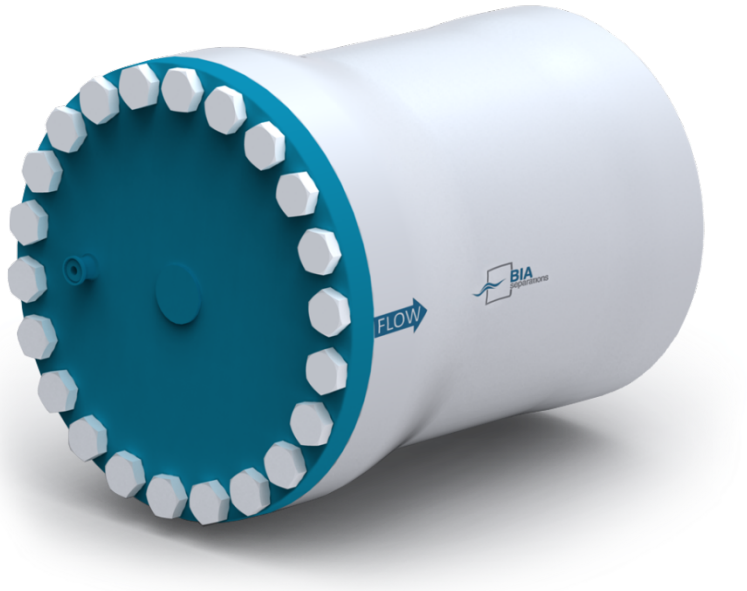
Introduction of composite materials to combine advantages of SS and plastics



- Epoxy thermoset composite
- Re-enforced with carbon fibers
- Coated pin-hole free with - USP Class VI Parylene C

- **Disposable but multiuse**
- **Stainless steel performance characteristics**
- **cGMP compliant**

allows for robust continuous operations

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CIMmultus™ composite materials – matching stainless steel characteristics

	1 mL		8 mL		80 mL		800 mL		8000 mL	
Type of column	CIM SS	CIMmultus™	CIM SS	CIMmultus™	CIM SS	CIMmultus™	CIM SS	CIMmultus™	CIM SS	CIMmultus™
Max pressure	18 bar	18 bar	20 bar	20 bar	20 bar	20 bar	7 bar	14 bar	7 bar	14 bar
Recommended flow rates (mL/min)	1-5	1-5	8-60	8-60	80-240	80-240	200-1300	200-1300	2000-10000	2000-10000
Max. flow rate (mL/min)	16	16	100	100	400	400	2000	2000	10000	10000
Max. operating temperature	40 °C	40 °C	40 °C	40 °C	40 °C	40 °C	40 °C	40 °C	40 °C	40 °C
L-t storage conditions	20% ethanol									
Sanitization for IEX, C4 HLD	1 M NaOH for at least an hour									

BUT:

- **3 times cheaper**
- **5 times lighter**
- **allow for pre-packed column transport**
- **<u>customer</u> decides to use disposable column as single or multi use unit**

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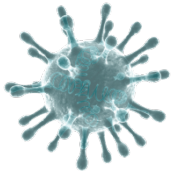
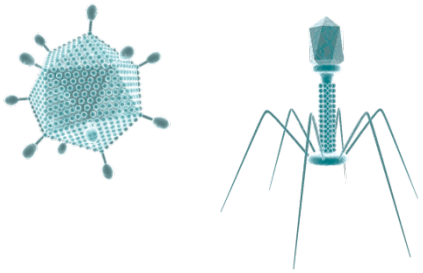
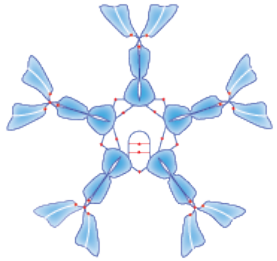
Main Applications – molecule type



Viruses & VLPs



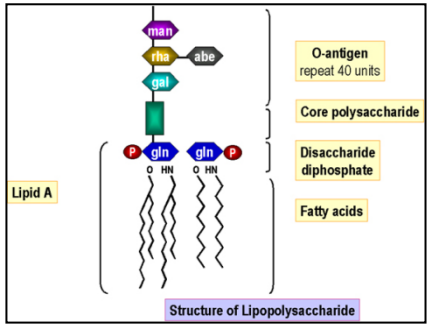
Large proteins

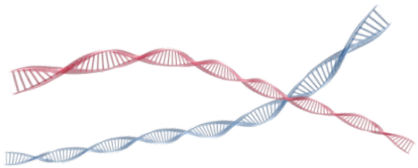


CIM® Columns

Plasmid DNA

Endotoxins





DNA depletion

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Binding capacities of CIM® columns

Molecules	Dynamic binding capacity
influenza	2 E+12 vp/mL
T7 phage	1 E+13 pfu/mL
M13 phage	4.5 E+13 pfu/mL
lambda phage	1 E+13 pfu/mL
PRD1 phage	6 E+13 pfu/ml
adenoviruses	2 E+12 vp/mL
baculovirus	2.4 E+11 pfu/ml
pDNA	8 mg/mL
genomic DNA	15 mg/mL
IgM	25 – 50 mg/mL
endotoxins	> 115 mg/mL

Membrane versus CIM® monolith production of canine adenovirus Type 2 – yield doubled

Bioprocess development for canine adenovirus type 2 vectors

P Fernandes[1,2], C Peixoto[2], VM Santiago[2], EJ Kremer[3], AS Coroadinha[1,2] and PM Alves[1,2]

Effect of different purification strategies on ΔE1 CAV-2 yields

Step	*Strategy*	*Recovery (%)*
Clarification		
	Microfiltration	30
	Centrifugation and microfiltration	**90 ± 2[a]**
Purification		
	Membrane adsorber	42 ± 5[a]
	Monolithic column	**82 ± 2[a]**
Polishing		
	Size exclusion chromatography	87 ± 6[a]
	Core bead prototype	**86 ± 9[a]**

Abbreviation: ΔE1, E1-deleted. [a]Standard deviation of triplicate assays. The strategies in bold represent the best options to purify CAV-2 vectors.

Fernandes, P et al, Bioprocess development for canine adenovirus type 2 vectors, Gene Therapy (2012), 1–8

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Membrane versus CIM® monolith production of lentiviral vector - yield doubled

INFECTIOUS TITERS, CONCENTRATION FACTORS, AND RECOVERIES OBTAINED AT THE END OF EACH DOWNSTREAM PROCESS STEP, BEFORE AND AFTER OPTIMIZATION

	Before optimization			*After optimization*		
	Infectious titer (x 10^7 IP/ml)	*CF*	*Recovery (%)*	*Infectious titer (x 10^7 IP/ml)*	*CF*	*Recovery (%)*
Clarification						
Centrifugation	0.24 ± 0.01	–	71 ± 6			
Depth-filtration	0.25 ± 0.01	–	74 ± 5	0.30 ± 0.02	–	**91 ± 6**[a]
Purification (AEXc)						
Sartobind D MA75	2.3 ± 0.1	12.5	28 ± 4			
CIM DEAE	6.1 ± 0.2	27.1	55 ± 2	8.0 ± 0.4	21.7	**80 ± 5**[b]
Concentration (UF/DF)						
Vivaspin 100 KDa	4.50 ± 0.04	3.4	67 ± 6			
300 KDa	4.5 ± 0.2	1.1	68 ± 9			
Vivaflow 100 KDa	4.8 ± 0.1	1.6	72 ± 1			**72 ± 1**[c]
Polishing (SEC)	0.11 ± 0.02	–	27 ± 2	0.82 ± 0.05	–	**68 ± 7**[d]
Overall Recovery (%)		8				**36**[e]

Results after optimization are shown for the methods presenting higher yields and chosen to be part of the downstream protocol developed herein due to their advantages.

[a–d]Recovery efficiency of total infectious particles, obtained after optimization of several conditions in each downstream processing (DSP) step: [a]increase of the flow rate from 50 to 100 ml min^{-1}; [b]immediate five-fold dilution of viral preparations after elution; [c]no optimization was performed in this step due to the high recoveries obtained; [d]increase of the concentration of the loading material by six-fold; [e]overall recovery obtained after using the techniques that gave the best recoveries in each purification step. The errors correspond to standard deviation (n = 3).

CF, concentration factor (in volume).

V. Bandeira et al., Downstream Processing of Lentiviral Vectors: Releasing Bottlenecks, Human Gene Therapy Methods 23:1-9 (August 2012)

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Evaluation of different supports for purification of live influenza A - yield doubled

Average values	QA monolith	Q membrane	Q porous particles	semi-affinity porous particles
Virus Recovery	54%	35%	35%	27%
DNA Depletion	96%	95%	95%	91%
Protein Depletion	95%	94%	98%	99%
Dynamic Binding Capacity	**10.3** $\log_{10}$ TCID50/mL Support	**10.3** $\log_{10}$ TCID50/mL Support	**9.0** $\log_{10}$ TCID50/mL Support	**8.4** $\log_{10}$ TCID50/mL Support

Maurer et al., Purification of Biological Products, Waltham, MA/USA, 2007

50% better recovery results in e.g. 1,5 M doses of vaccine instead of 1 M doses, at the same costs of the process = <u>0,5 M doses are pure profit</u>

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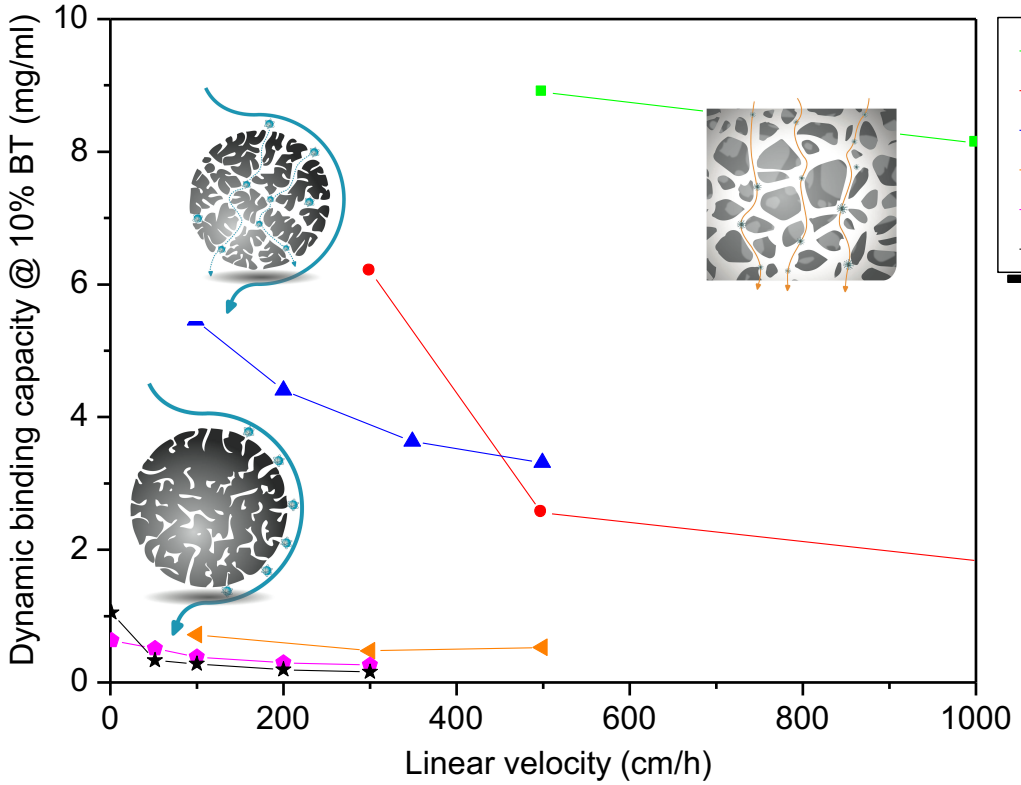
Plasmid DNA purification using CIM® DEAE columns: 15-fold increase in productivity



- CIM® DEAE
- Q Ceramic Hyper D 20
- Fractogel EMD DEAE (S)
- Source 30 Q
- Toyopearl DEAE 650-M
- DEAE Sepharose

CIM® DEAE binding capacity = ~8 mg/ml

Used for CP III trials

Boehringer Ingelheim: „15-fold increase in productivity“

- High binding capacity at relevant flow rates
- High elution concentration - pDNA eluted in lower volume (important for SEC!)
- Fast process (no product loss due to oxidative degradation or enzymatic attack)

Urthaler et al., J.Chrom. A, 1065 (2005), 93-106

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Economic benefit for the customer using CIM® Monolith Plasmid DNA purification pack

1 ml CIM monolith – BIA Sep

Calculations	
Buffer	76,3 ml buffer/mg pDNA
Time	23,6 min /mg pDNA
Recovery	85%
Costs using column for 1 run	
Quantity of purified pDNA	5,10 mg PDNA
€ (Column costs)	114 €/mg pDNA
€ (Column + buffer)	114 €/mg pDNA
Costs using columns for 10 runs	
Quantity of purified pDNA	51 mg pDNA
€ (Column costs)	11,4 €/mg pDNA
€ (Column + buffer)	11,8 €/mg pDNA
Costs using columns for 20 runs	
Quantity of purified pDNA	102 mg pDNA
€ (Column costs)	5,7 €/mg pDNA
€ (Column + buffer)	6,1 €/mg pDNA
€ (column + buffer+ work)	15,4 €/mg pDNA

CIM monolithic columns offer **3 times cheaper** purification costs of pDNA for gene therapy

Particle based

Calculations	
Buffer	108,0 ml buffer/mg pDNA
Time	70,0 min /mg pDNA
Recovery	79%
Costs using column for 1 run	
Quantity of purified pDNA	4 mg PDNA
€ (Column costs)	227 €/mg pDNA
€ (Column + buffer)	228 €/mg pDNA
Costs using columns for 10 runs	
Quantity of purified pDNA	40 mg pDNA
€ (Column costs)	23 €/mg pDNA
€ (Column + buffer)	24 €/mg pDNA
Costs using columns for 20 runs	
Quantity of purified pDNA	79 mg pDNA
€ (Column costs)	11 €/mg pDNA
€ (Column + buffer)	12 €/mg pDNA
€ (column + buffer+ work)	42 €/mg pDNA

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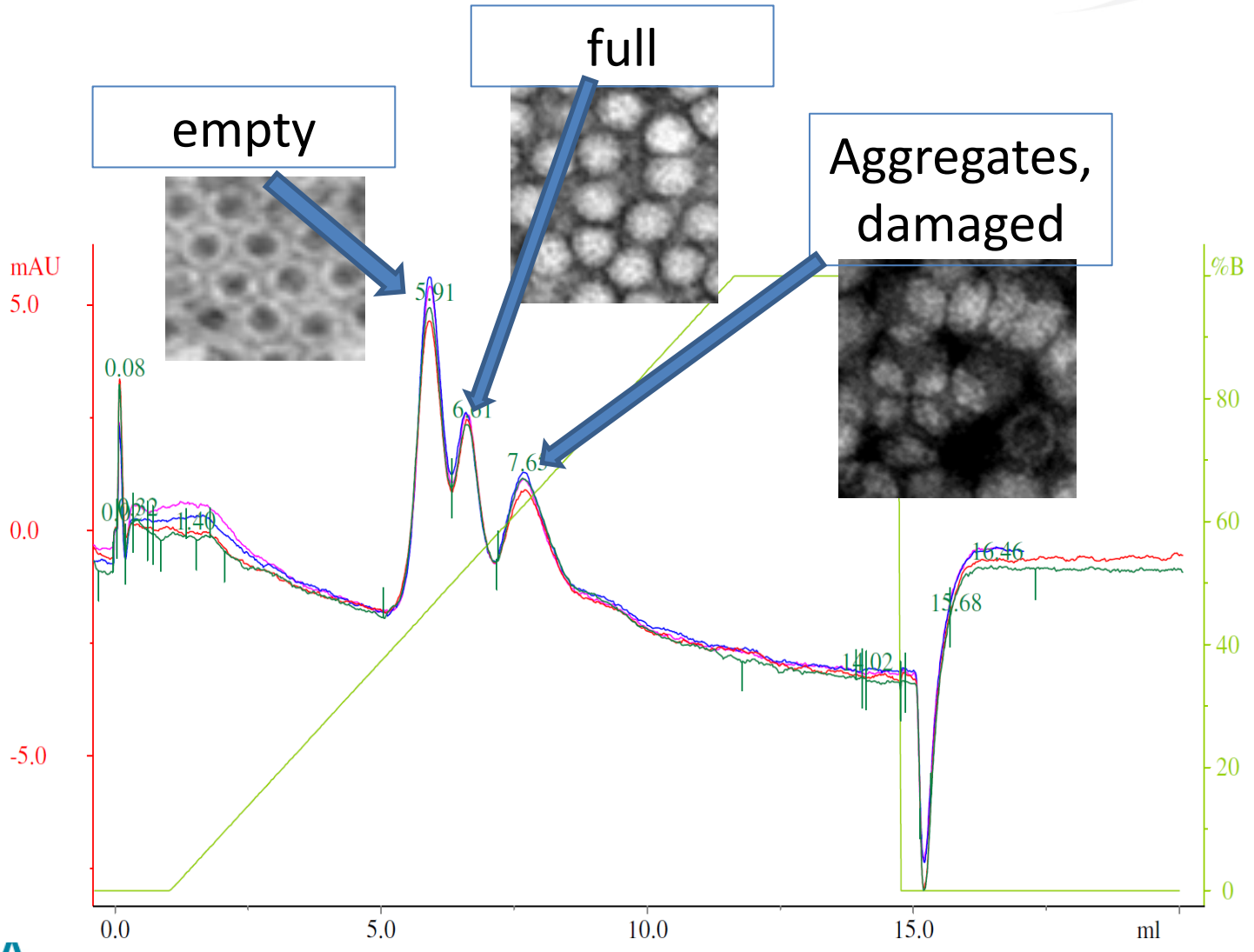
Separations of empty, full and damaged AAV capsids using Anion exchange CIMmultus™ QA column



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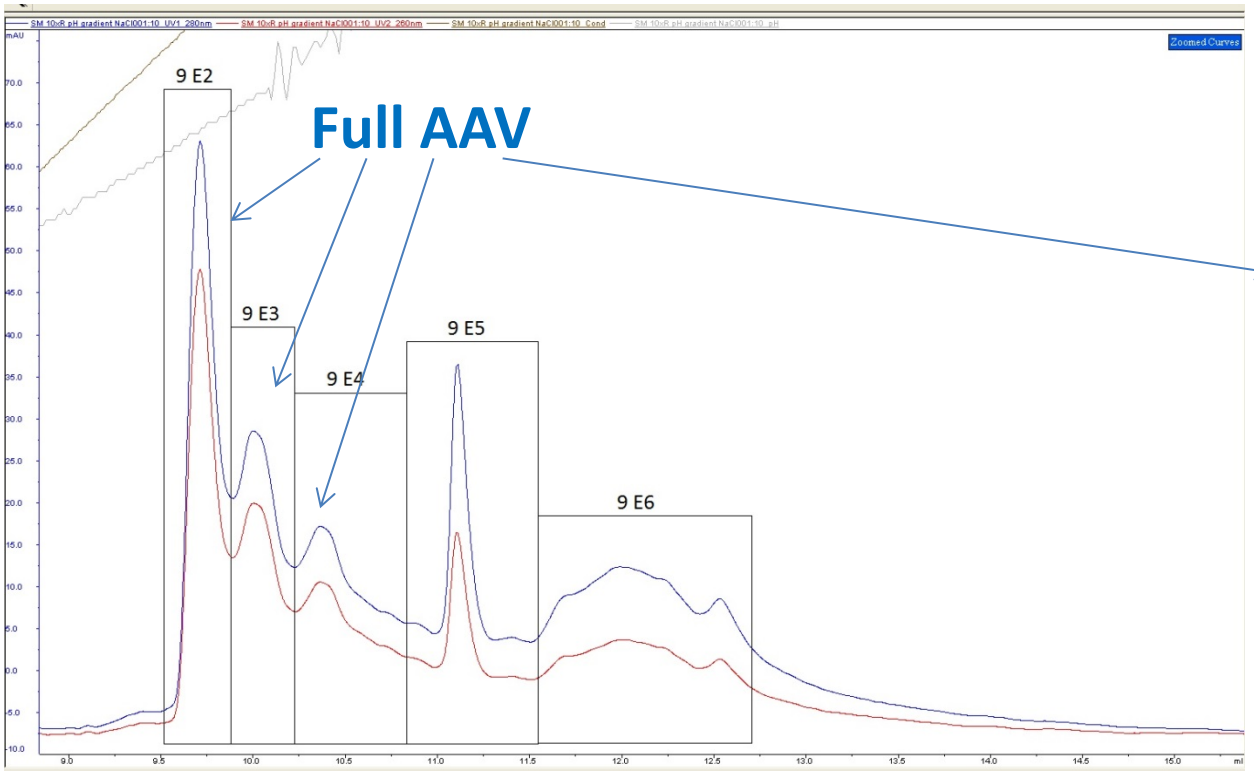
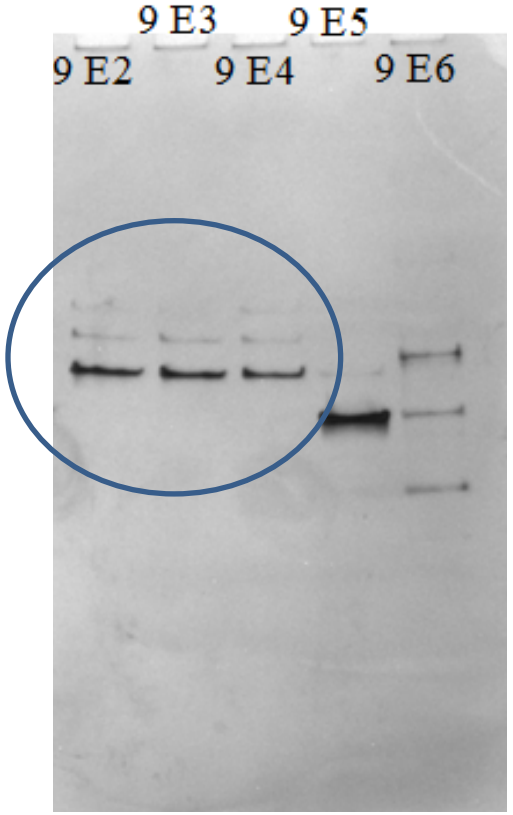
Is full AAV particle only one species?
Check with pH gradient - unmatched resolution

Column: CIMac™ SO3
Mf A: 20 mM acetate, pH 4
Mf B: 20 mM HEPES + 1M NaCl, pH 8





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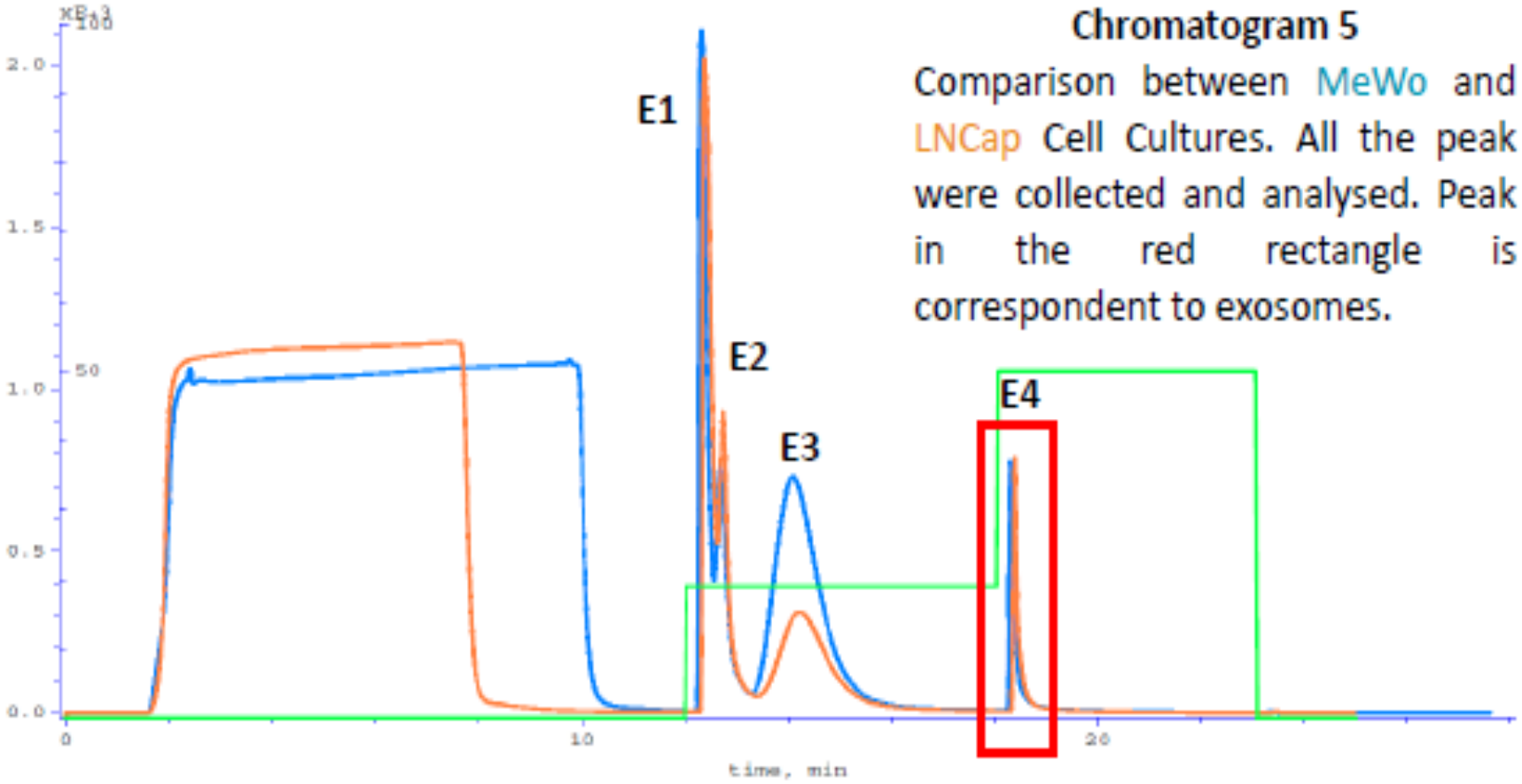
Separation of exosomes using CIM® large channel anion exchange column – enabling feature



Chromatogram 5

Comparison between MeWo and LNCap Cell Cultures. All the peak were collected and analysed. Peak in the red rectangle is correspondent to exosomes.

Buzzi et al., MSS2014, Portoroz

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PATfix™

In-process control HPLC system with unique software

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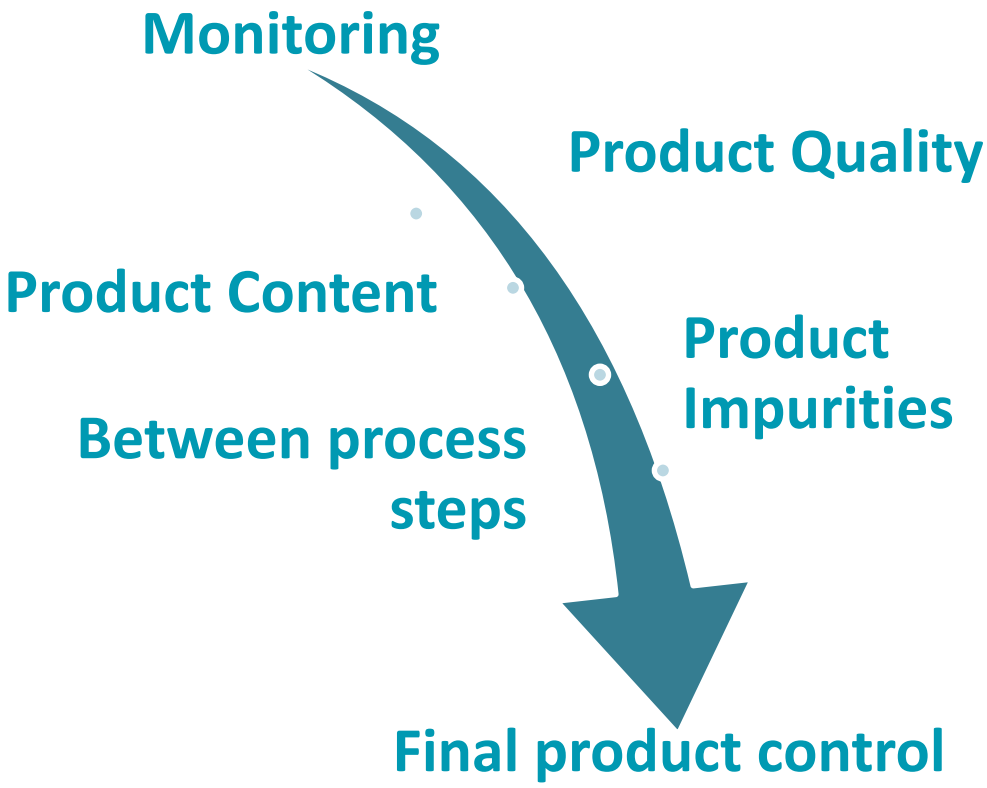
What is Process Analytical Technology (PAT)?

Process Analytical Technology is „*a system for designing, analyzing, and controlling manufacturing processes through timely measurements of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality*.“ (FDA PAT Guidance, 2004)



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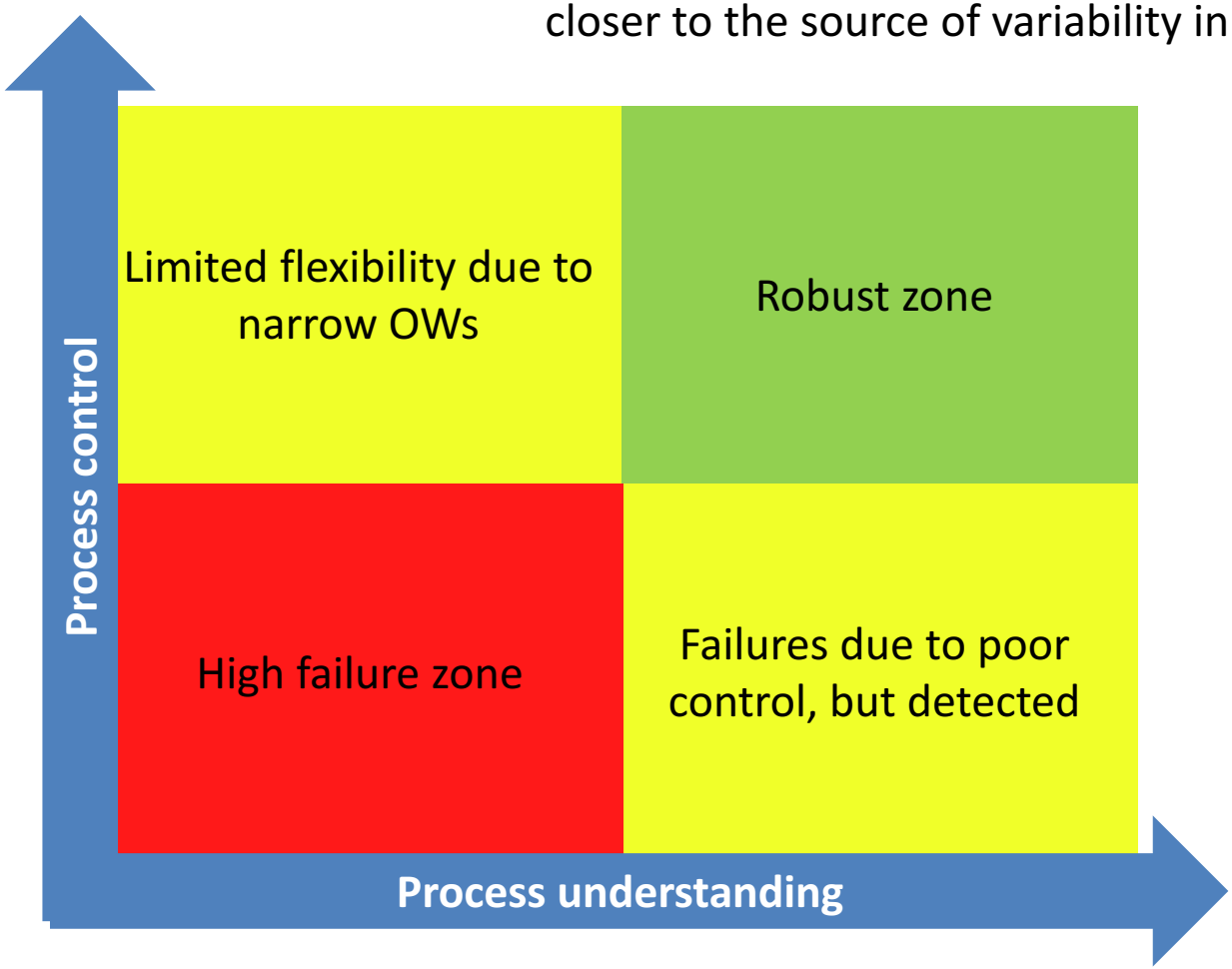
Proper process understanding and good process control = Robust process

Provides an opportunity to apply a control closer to the source of variability in the process



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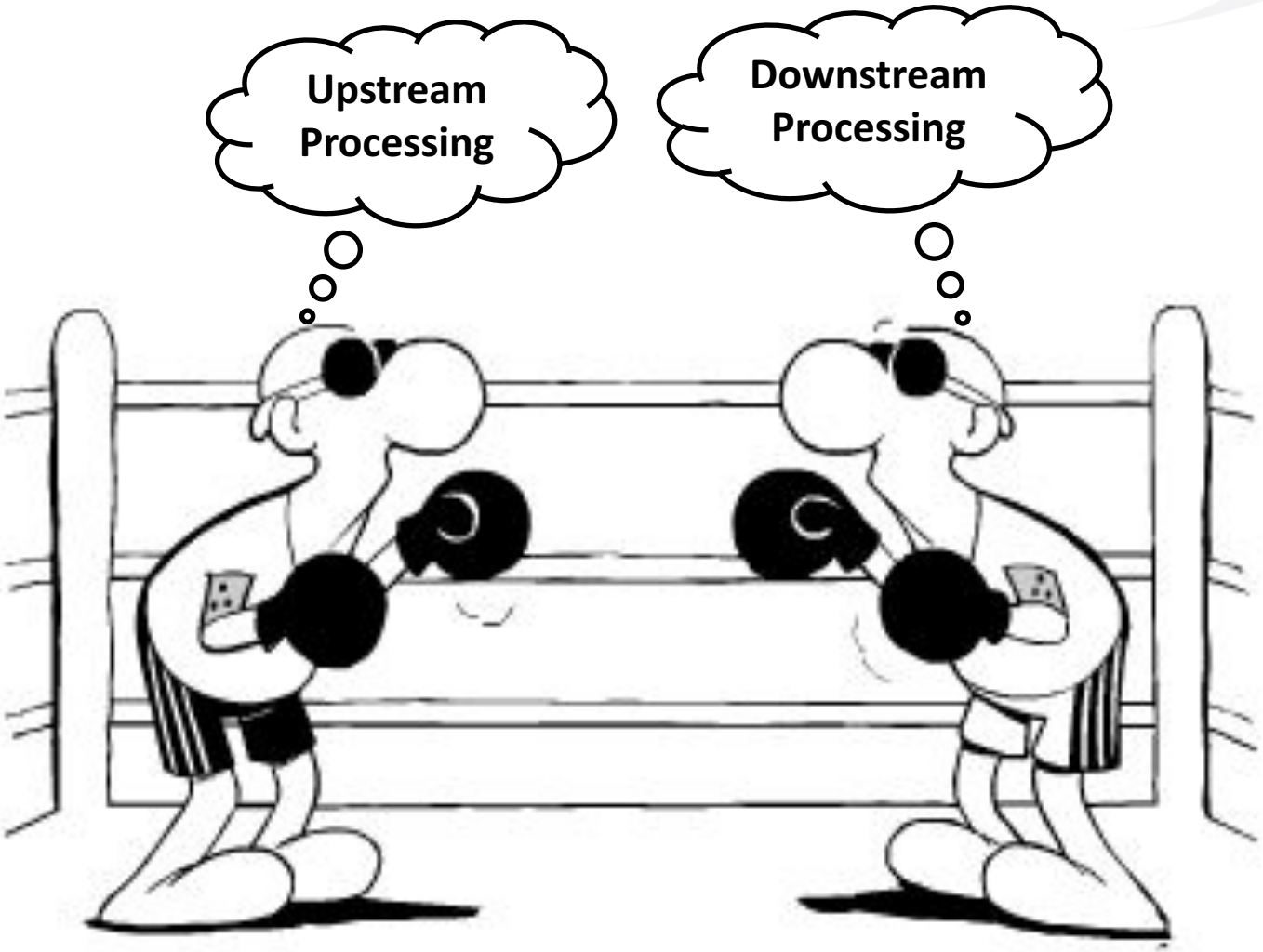
Reality in bioprocessing



BIA Separations PATfix™

inCyght

for chromatography

Integrated system used to detect changes and quantify complex analytes

Custom tailored to meet requirements of bioanalytical HPLC techniques

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BIA Separations PATfix™

inCyght

for chromatography

- Easy to use data management
- Mass visualization of chromatograms
- Automatic detection of changes
- Prediction of complex CQAs

- **<u>Column without carry-over</u>**
- Immediate sample analysis

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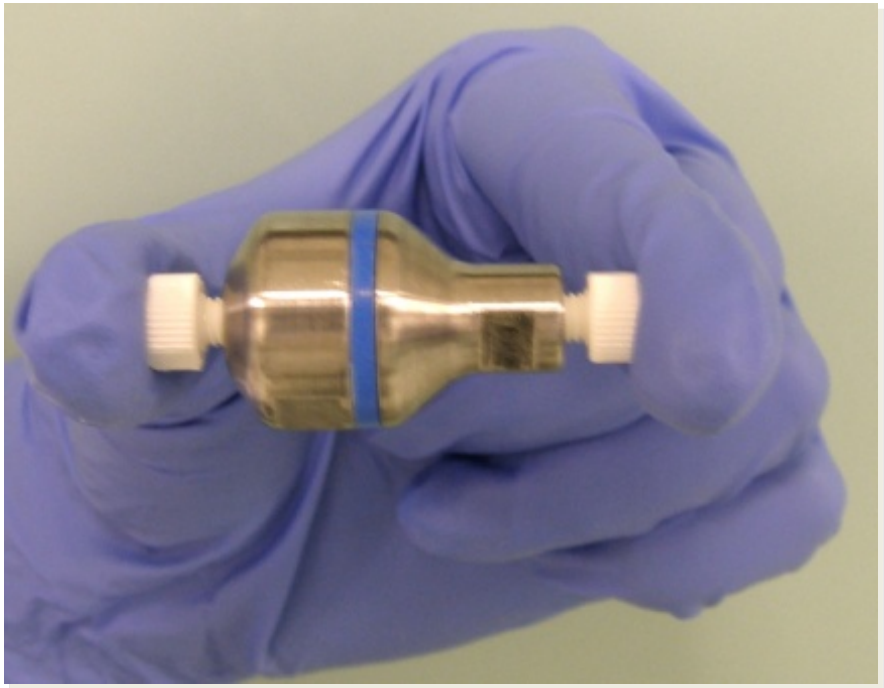
Use of the HPLC is mandatory for accurate mass balance calculation

CIMac™ / Bio-monolith™ HPLC Columns



10 ml/min = 4500 cm/h = 360 CV/min (res. time: 0,1 s) = faster than biosensor

No entrapment in the column, no carry-over

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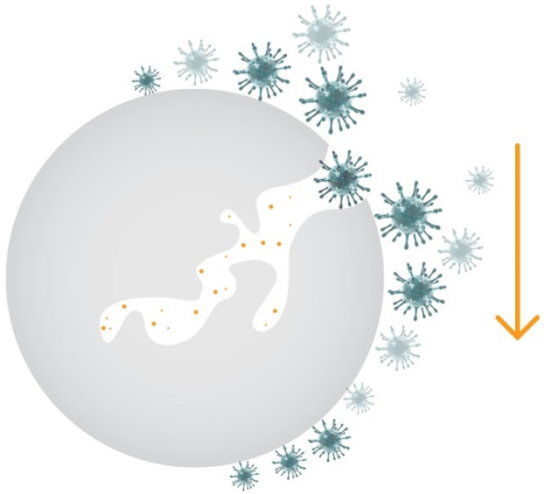
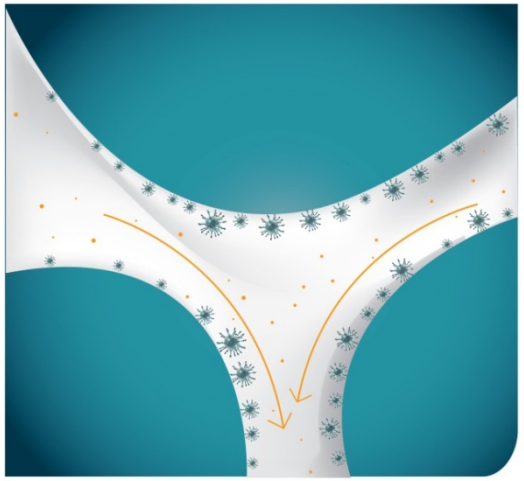
Advantages of CIM® monolithic resins – No entrapment in the column, no carry-over





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CIMac™ analytical columns for PAT HPLC – no carry over of large molecules or viral particles

Available:

- CIMac™ QA
- CIMac™ DEAE
- CIMac™ SO3
- CIMac™ EDA
- CIMac™ pDNA
- CIMac™ Adeno
- CIMac™ AAV empty/full

Soon to come:

- CIMac™ AAV total
- CIMac™ Lenti
- CIMac™ Vaccinia

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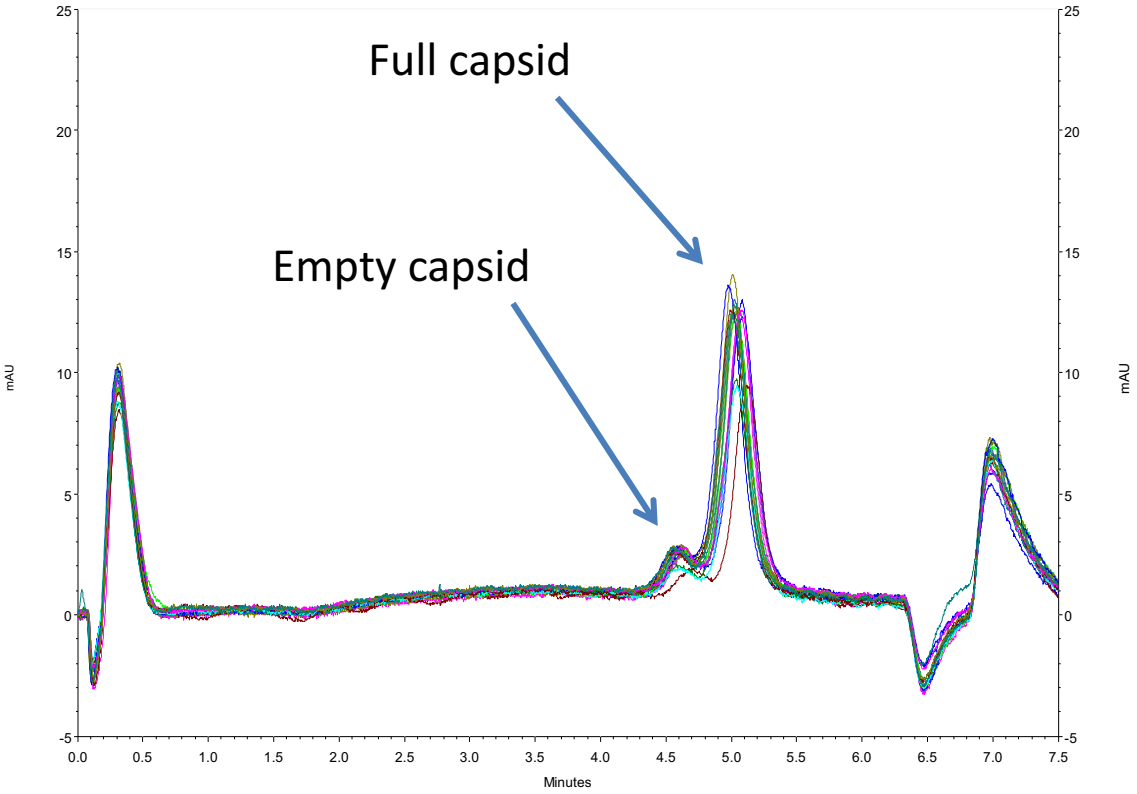
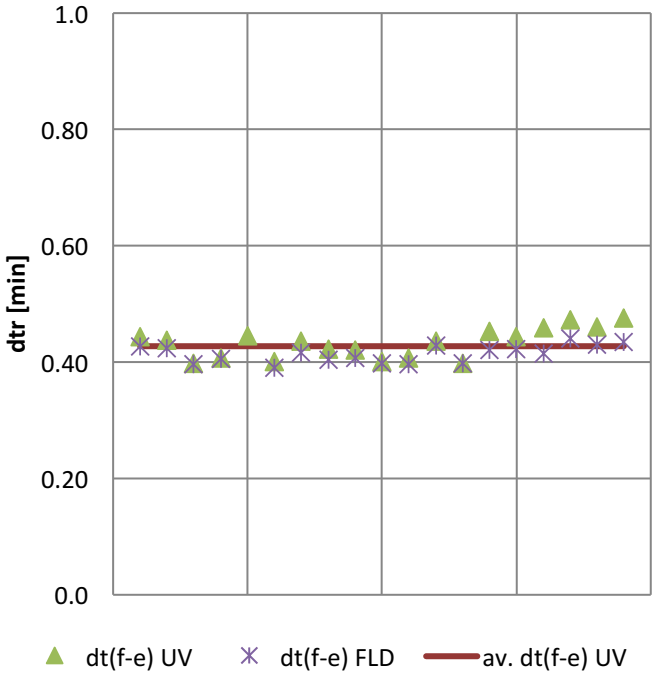
CIMac™ AAV empty/full columns; Intra-batch reproducibility (Batch 15-003-AV01)



Overlay of UV280 traces



Retention time difference of full and empty AAV capids, both UV and FLD.

UV: RSD of $\Delta t_{ret}(full - empty)$: 6 %; FLD: RSD of $\Delta t_{ret}(full - empty)$: 4 %

AAV is sticking to all plastics – sample preparation is the key step for accurate results

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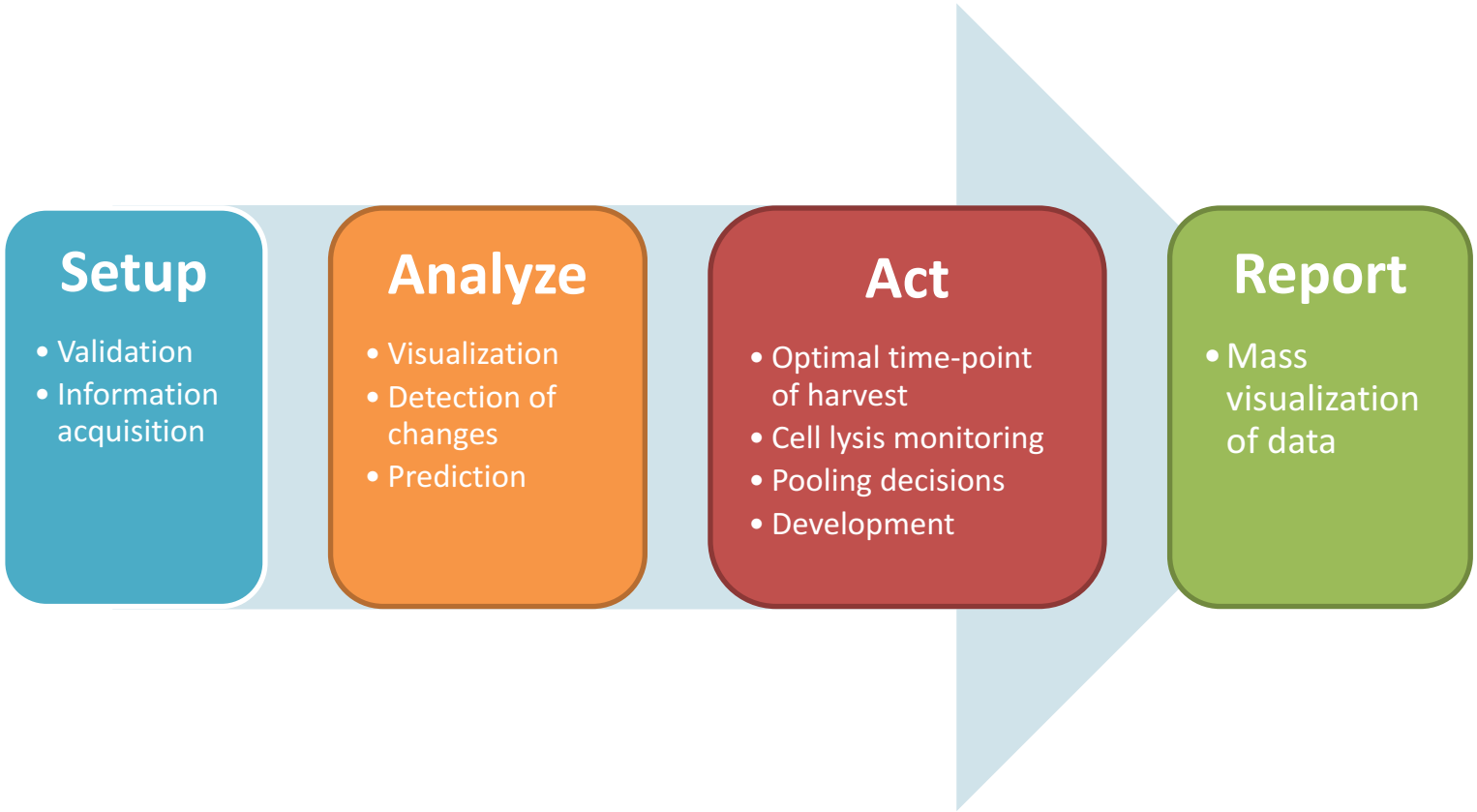
BIA Separations PATfix™ workflow



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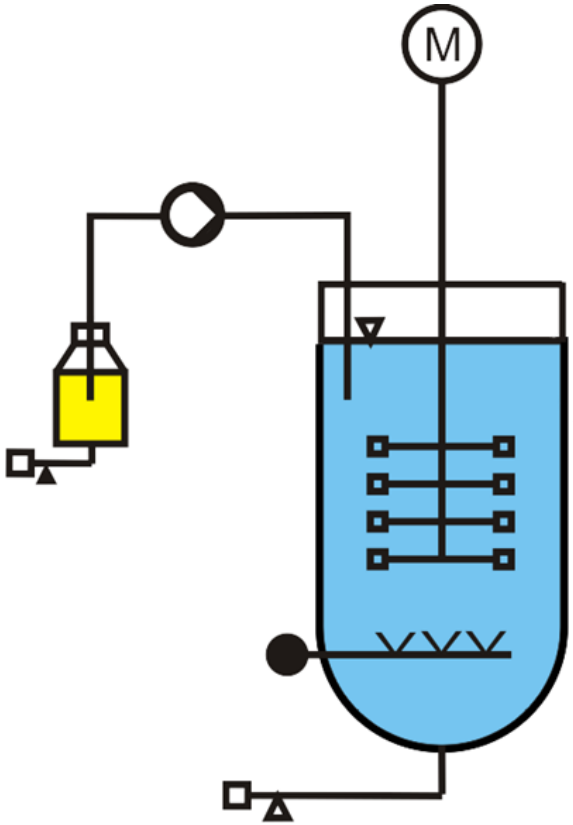
PATfix™ Optimal point of harvest

Unit operation is a highly dynamic system.

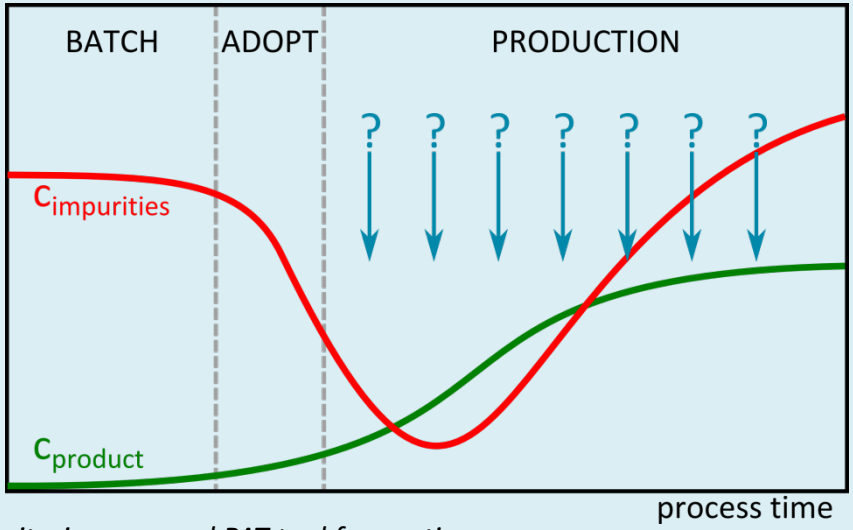
Case: mAb production



What is inside?

- IgG,
- Nucleic acids,
- Host cell protein,
- Media components,
- CHO cells,
- Product agglomerates, ...

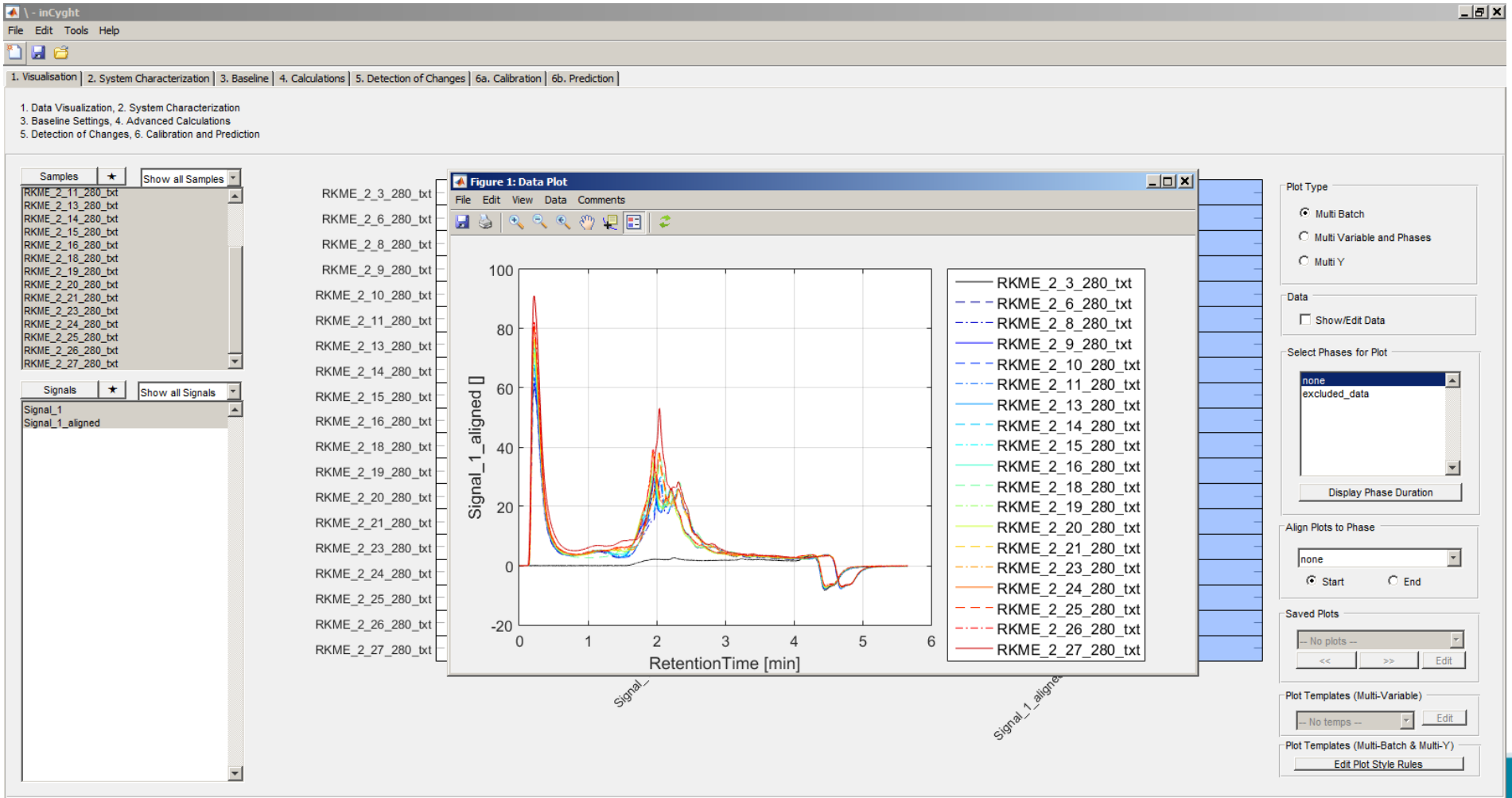
How it is evolving?



Rajamanickam V, Sagmeister P, Spadiut O and Herwig C. *Impurity monitoring as novel PAT tool for continuous biopharmaceutical processes,* submitted.

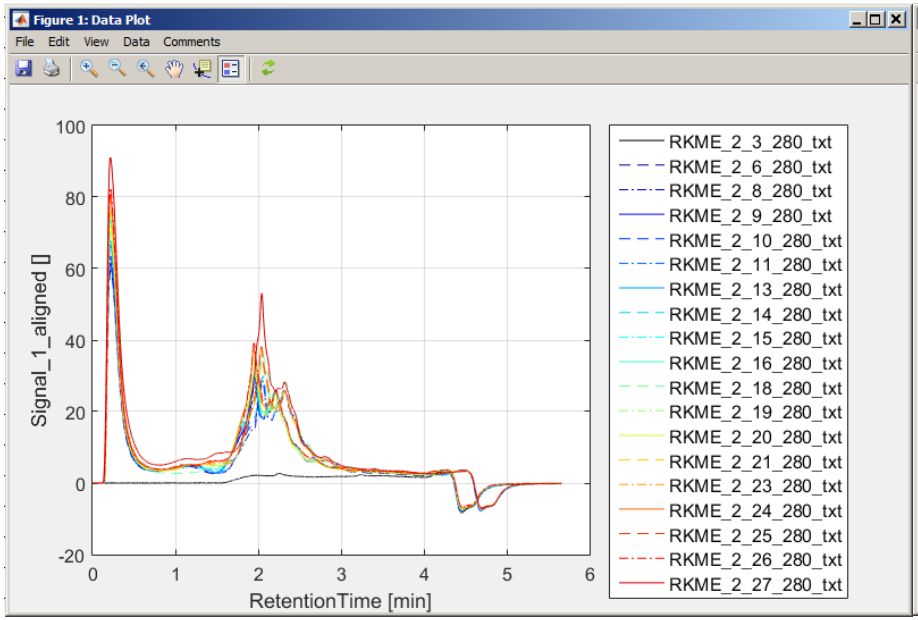
Determination of optimal time-point of harvest (Pichia Pastoris, protein expression)

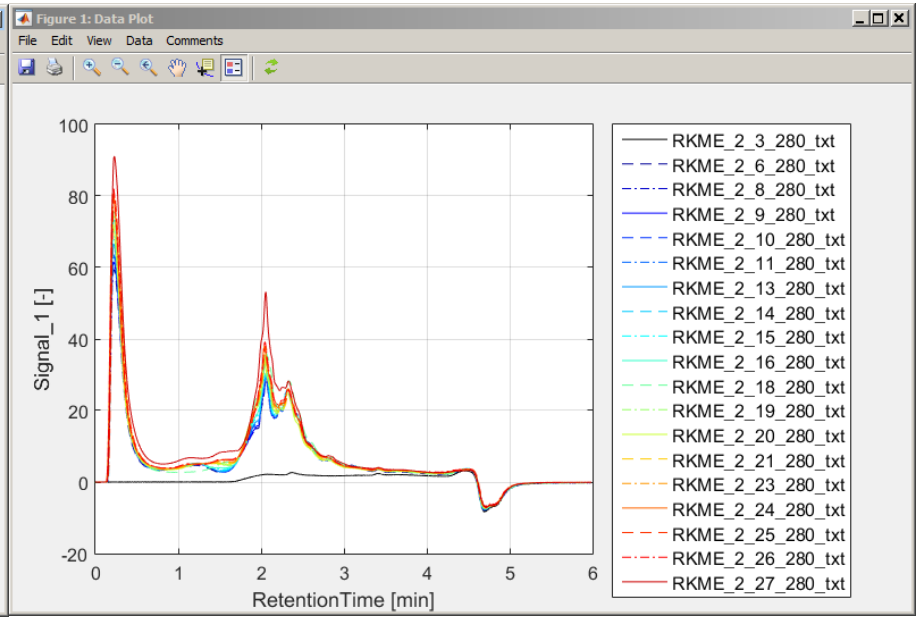
- Samples taken at regular intervals, centrifuged, buffer adjusted and injected directly onto the column.



Determination of optimal time-point of harvest (Pichia Pastoris, protein expression)

- Chromatogram alignment to increase the accuracy of prediction

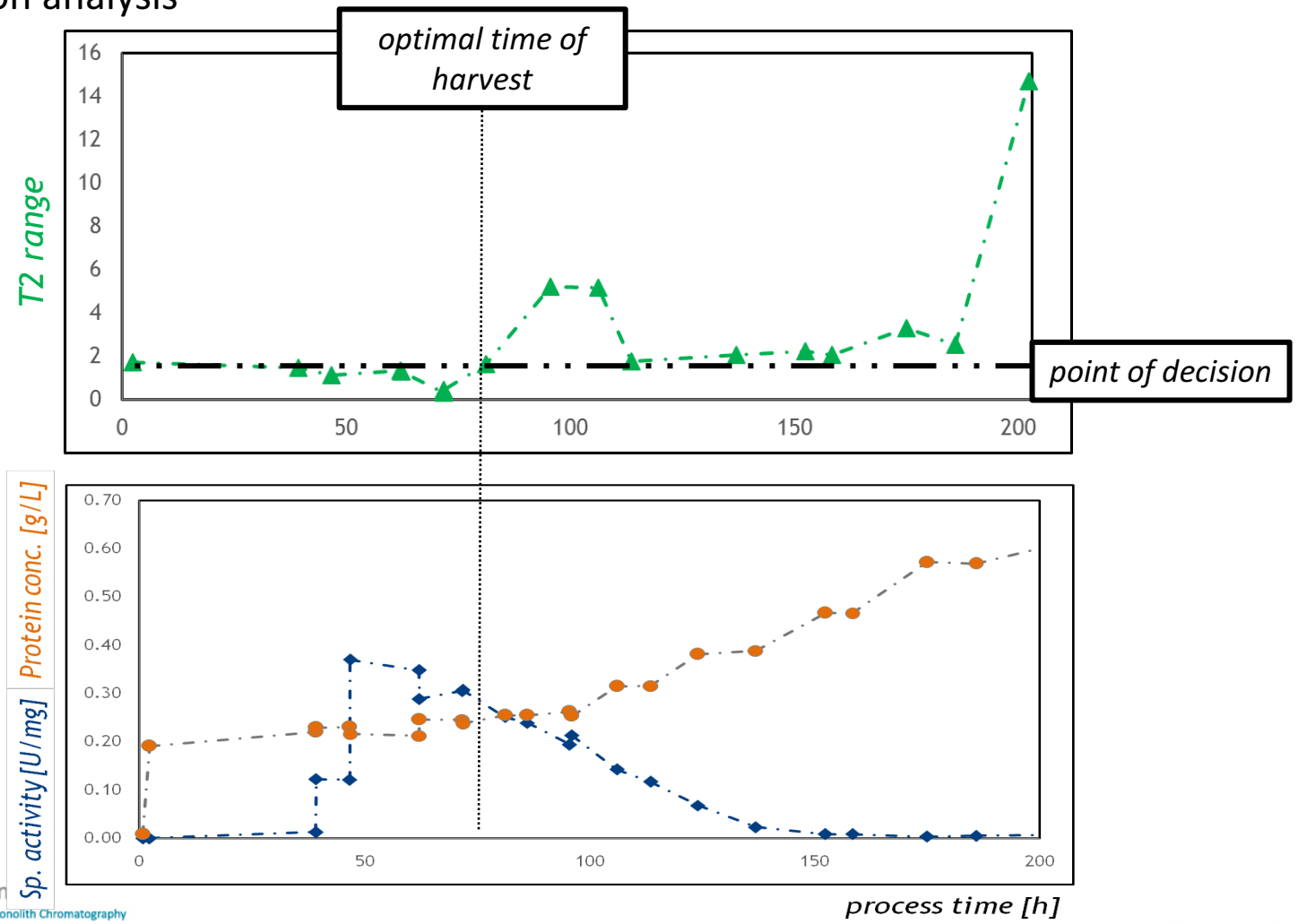




BIA separations
Leaders in Monolith Chromatography

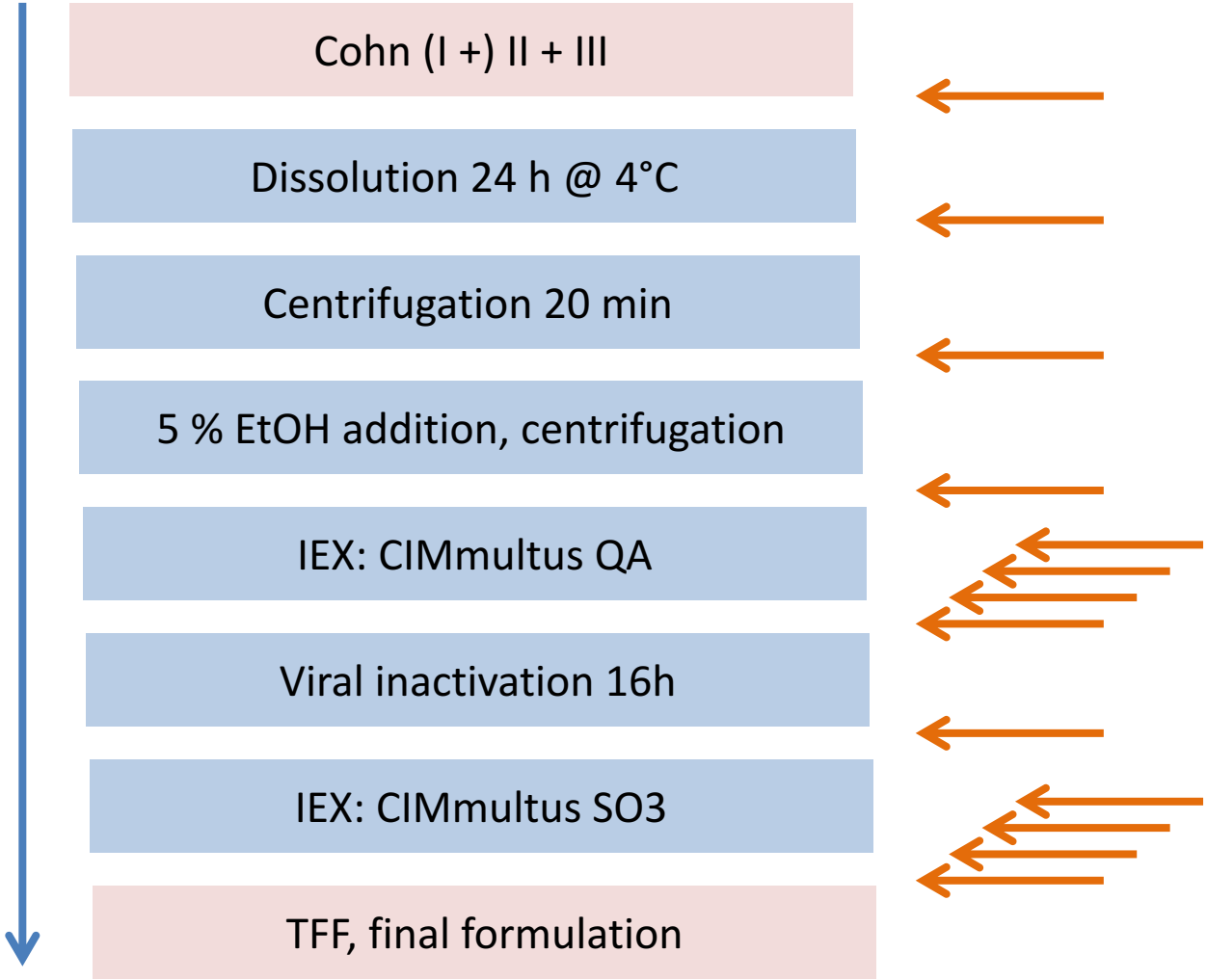
Determination of optimal time-point of harvest (Pichia Pastoris, protein expression)

Deviation analysis

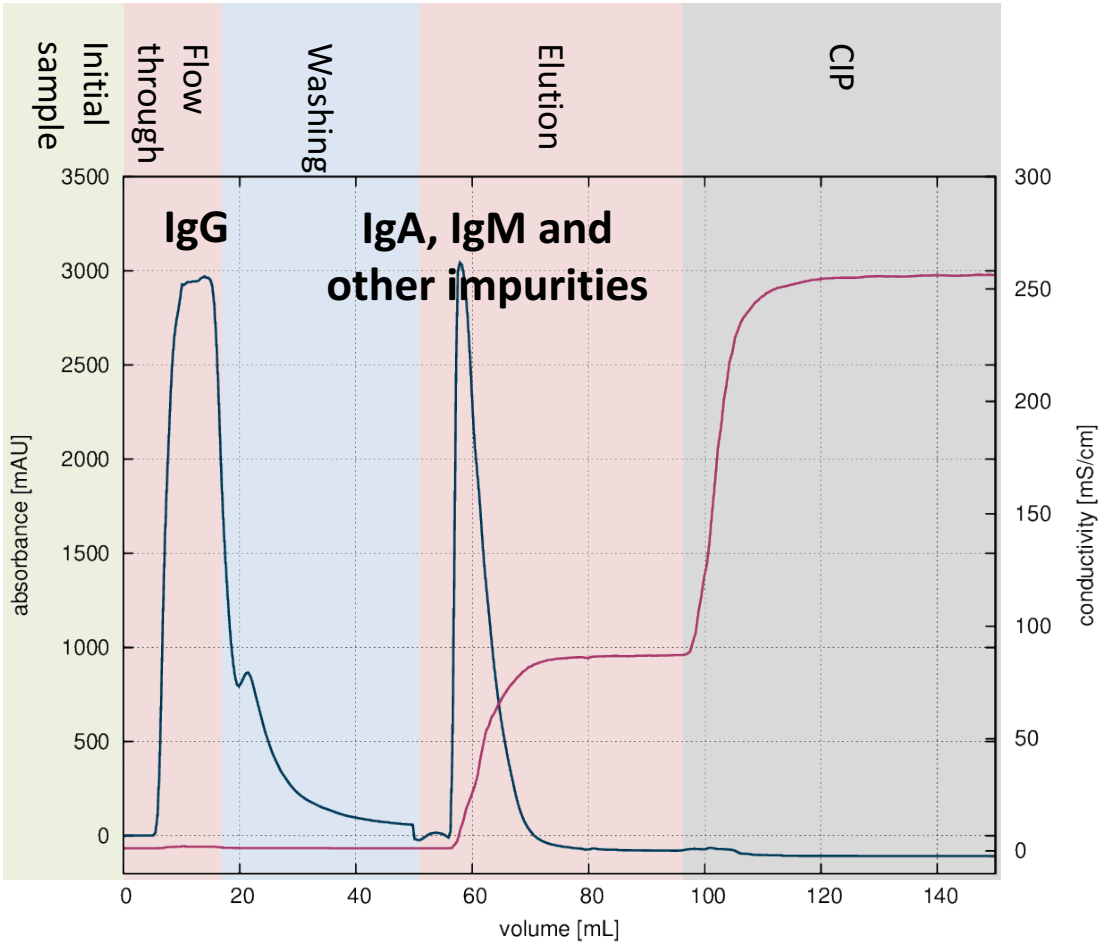


BIA separation
Leaders in Monolith Chromatography

Intravenous immunoglobulin (IVIG) purification process scheme



IVIG purification – first chromatography step using strong AEX CIMmultus column



Column: 8 mL CIMmultus QA

Loading buffer: 20 mM Na-acetate, pH 5.0
Elution: loading buffer + 1 M NaCl
CIP: 1 M NaOH + 2 M NaCl

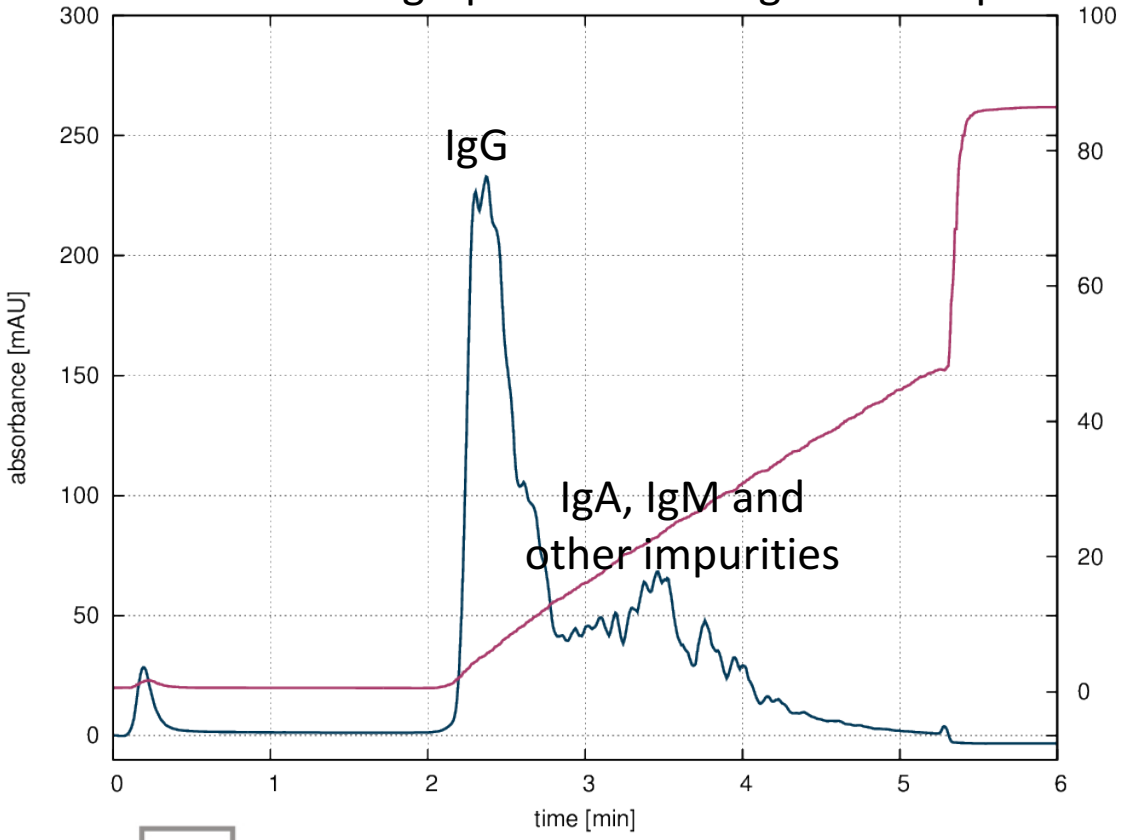
Sample: 1g Cohn II+III paste in 10 mL loading buffer

Product IGIV in flow through fraction(s)

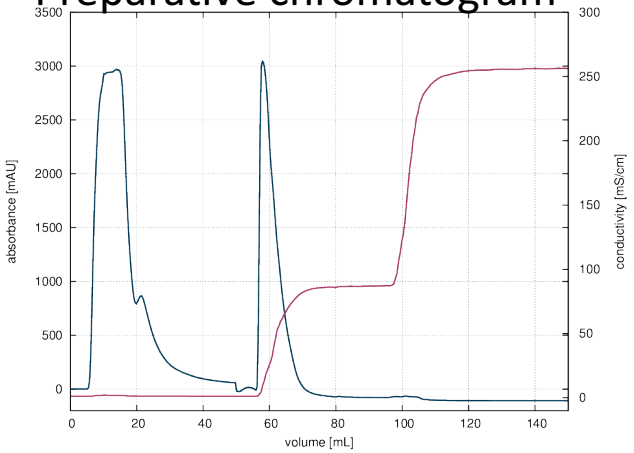
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

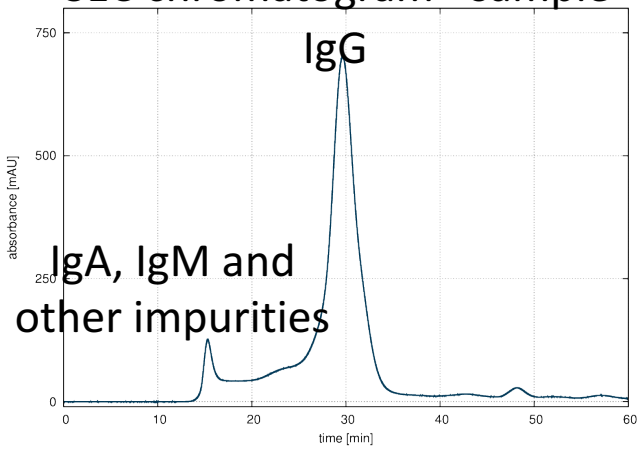
CIMac AEX fingerprint chromatogram - sample



Preparative chromatogram



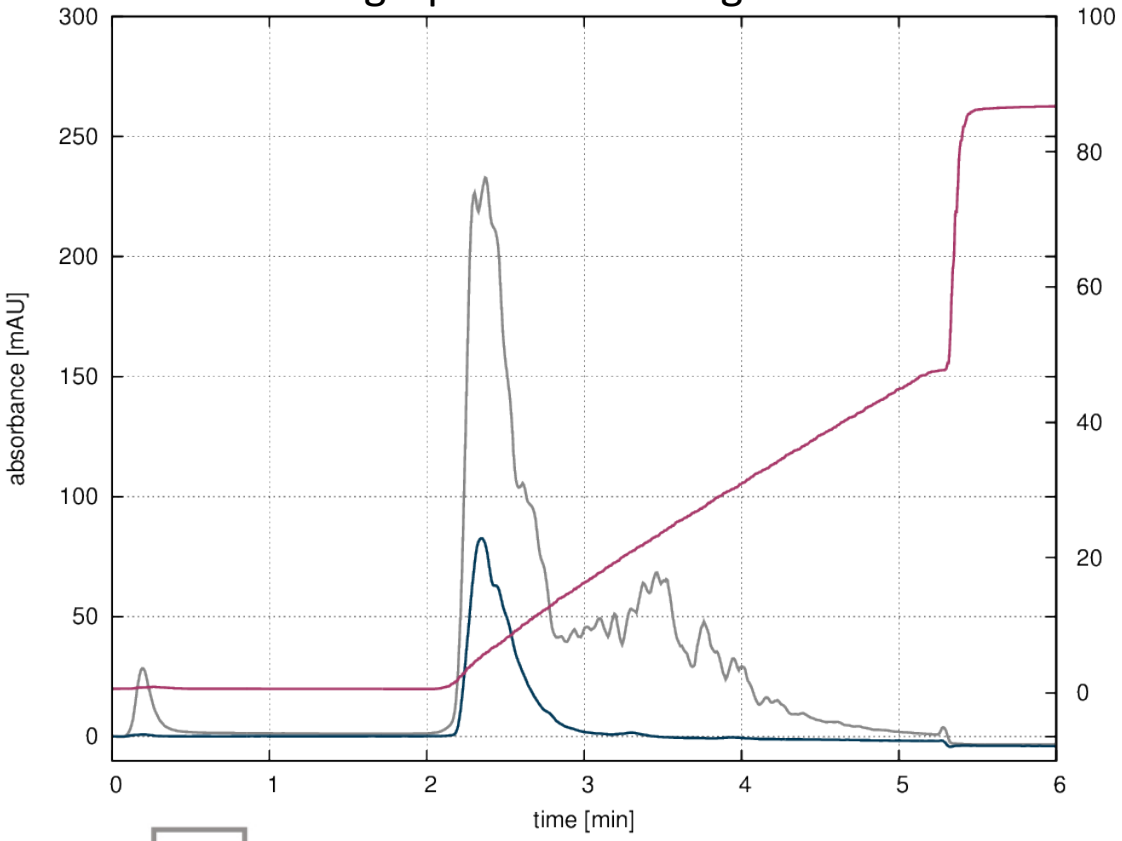
SEC chromatogram - sample



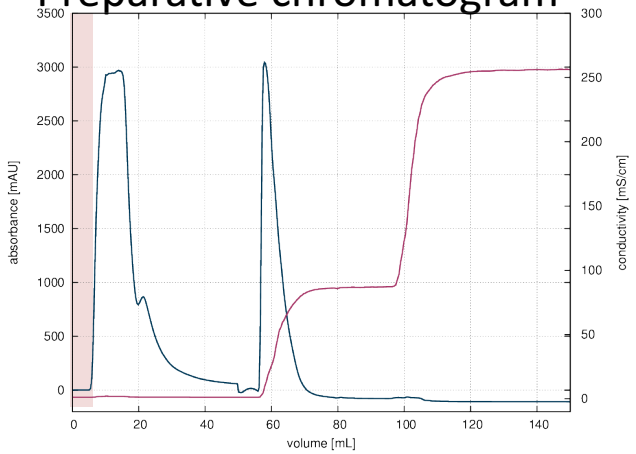
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

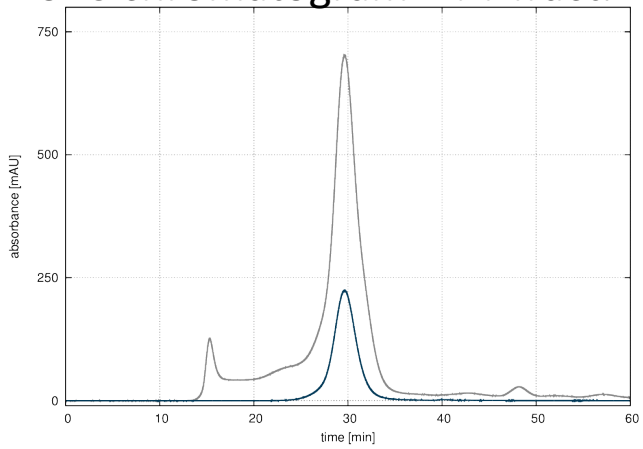
CIMac AEX fingerprint chromatogram – FT fract. 1



Preparative chromatogram



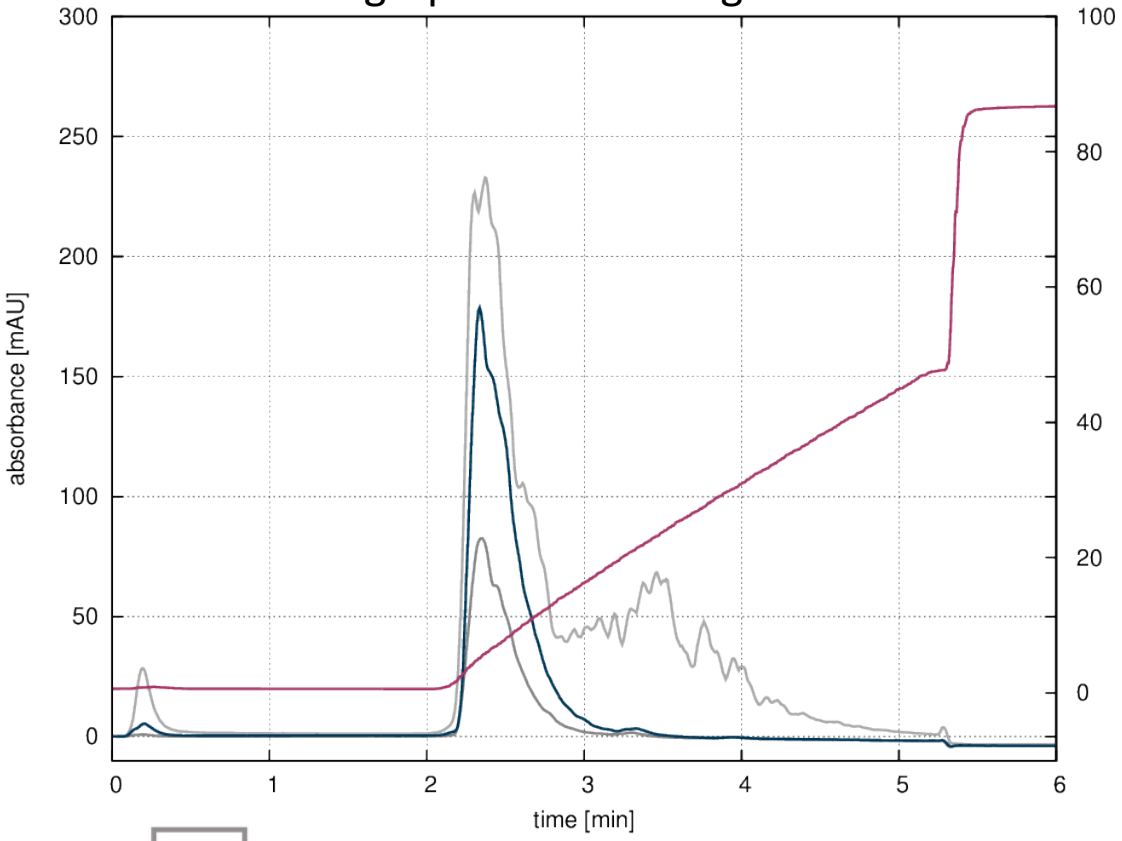
SEC chromatogram – FT fract. 1



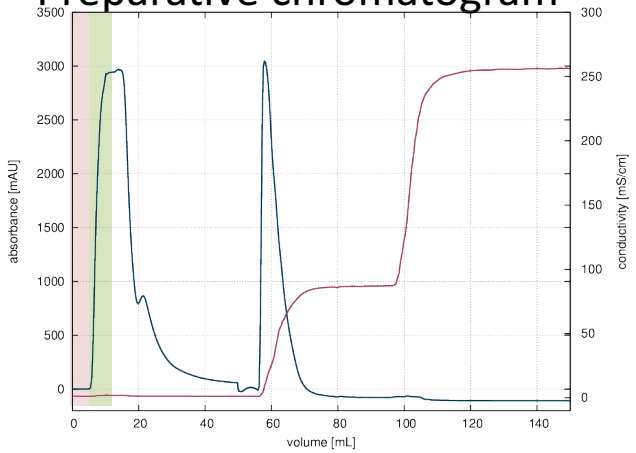
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

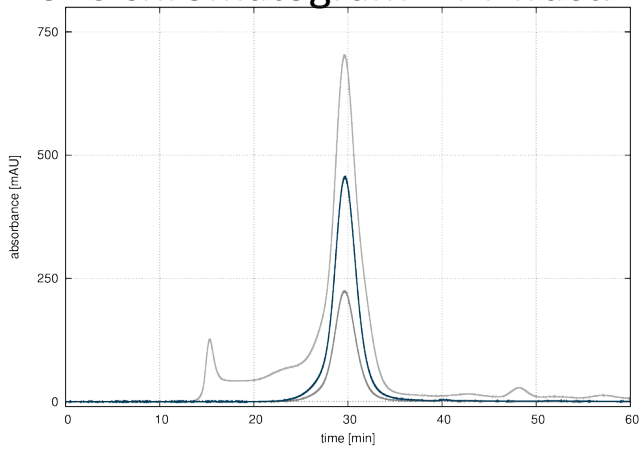
CIMac AEX fingerprint chromatogram – FT fract. 2



Preparative chromatogram



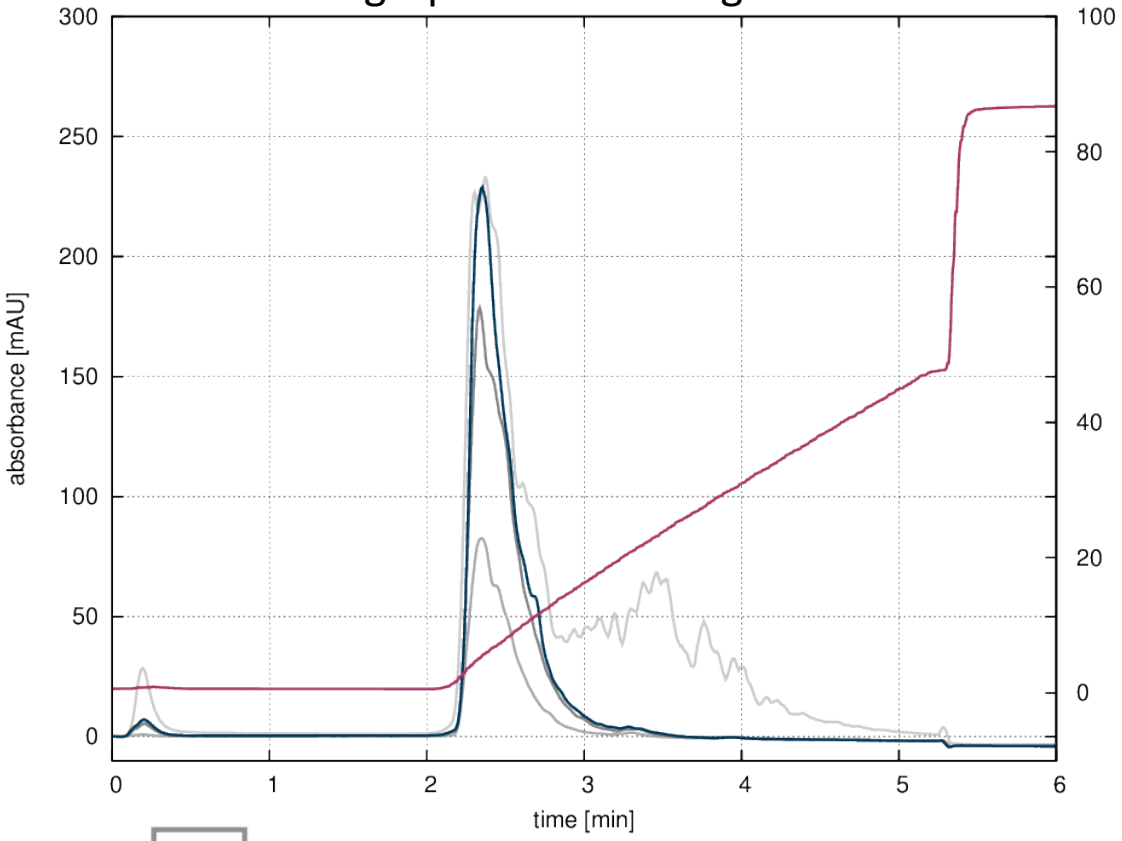
SEC chromatogram – FT fract. 2



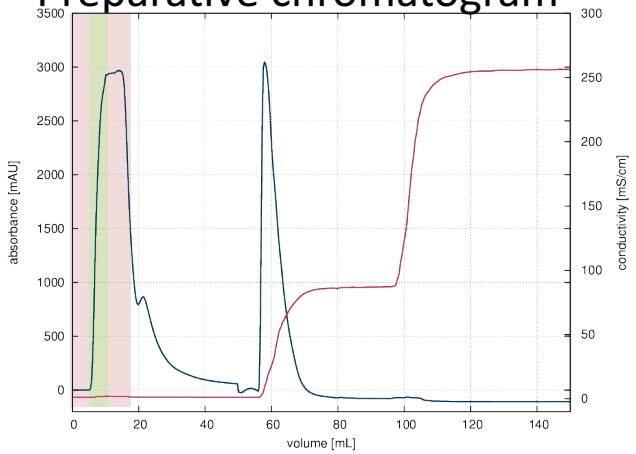
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

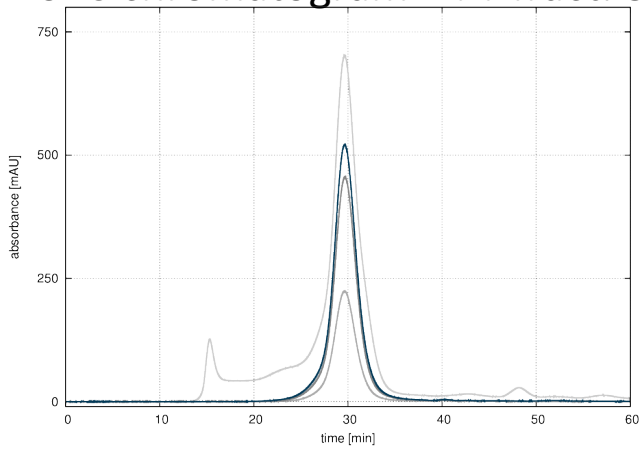
CIMac AEX fingerprint chromatogram – FT fract. 3



Preparative chromatogram



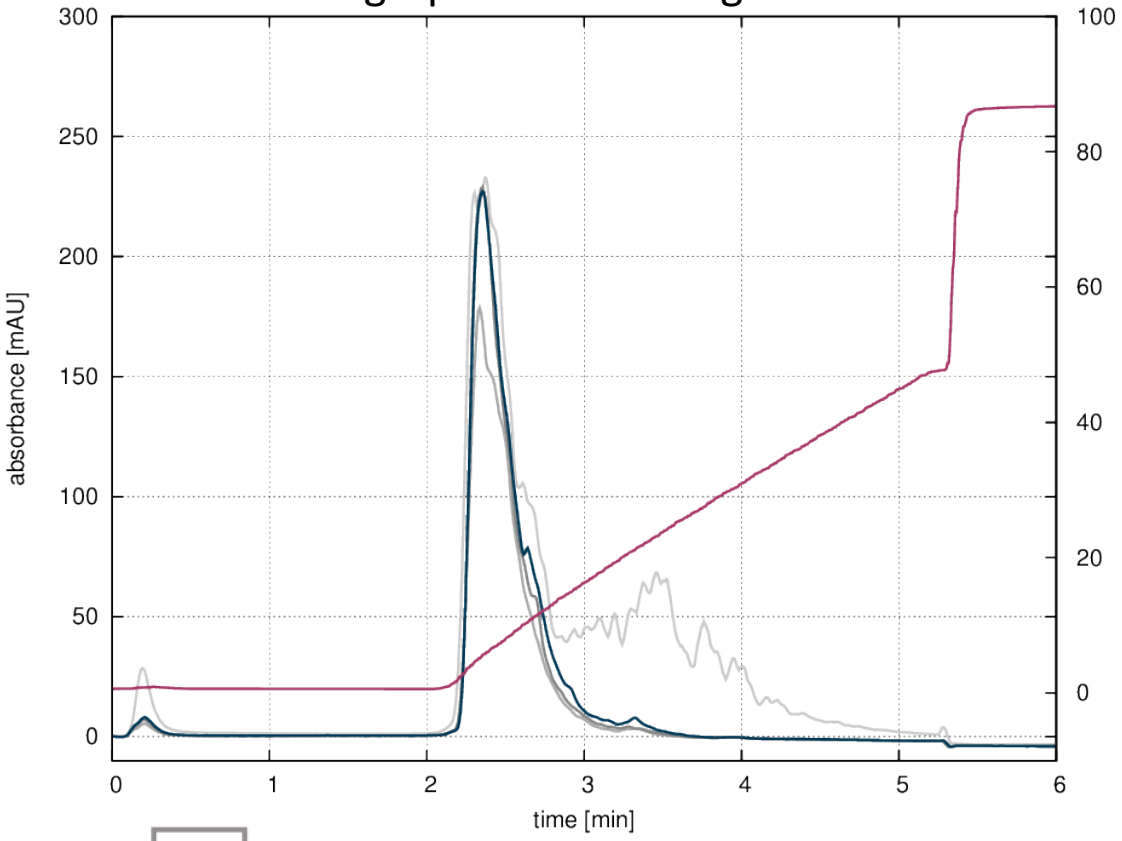
SEC chromatogram – FT fract. 3



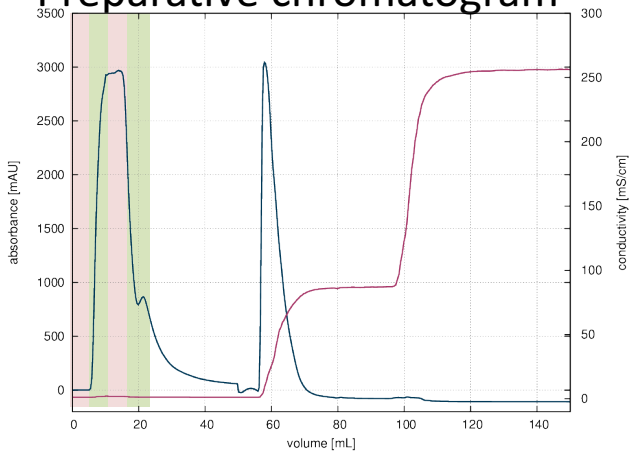
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

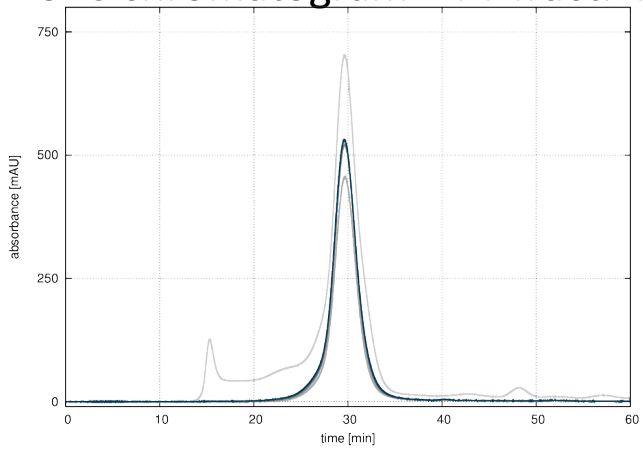
CIMac AEX fingerprint chromatogram – FT fract. 4



Preparative chromatogram



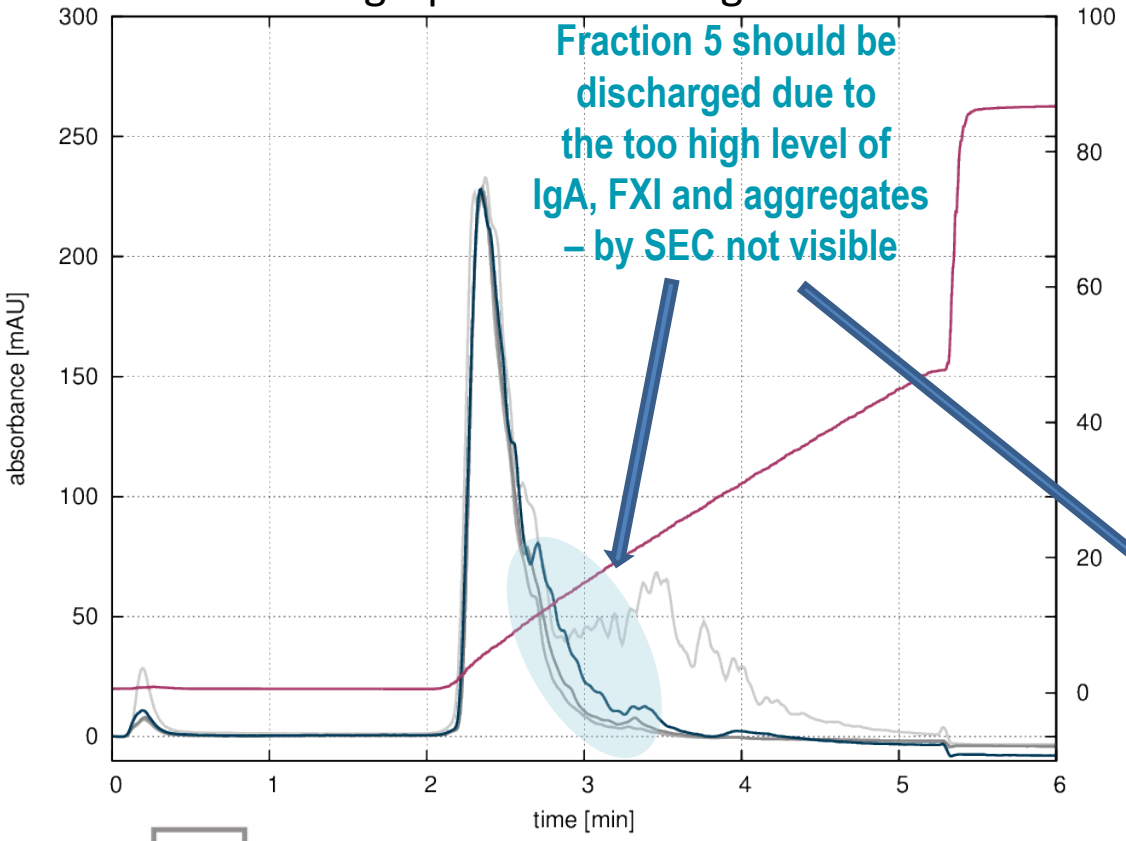
SEC chromatogram – FT fract. 4



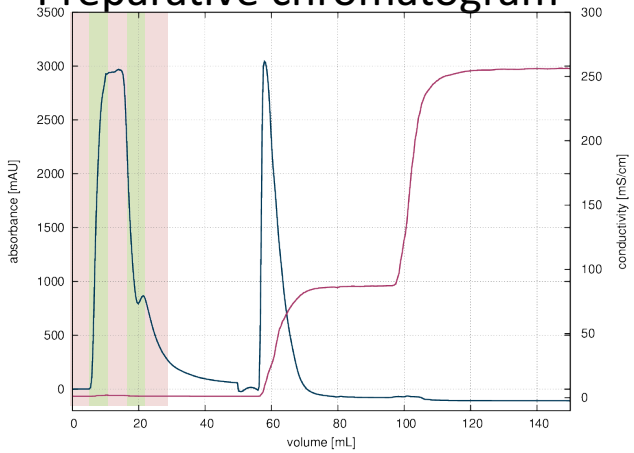
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

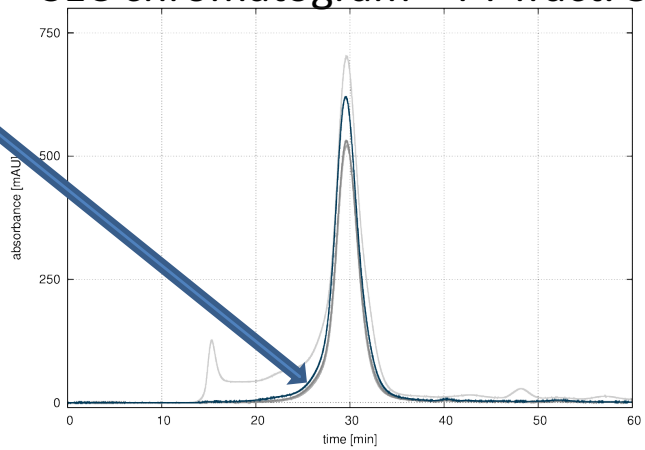
CIMac AEX fingerprint chromatogram – FT fract. 5



Preparative chromatogram



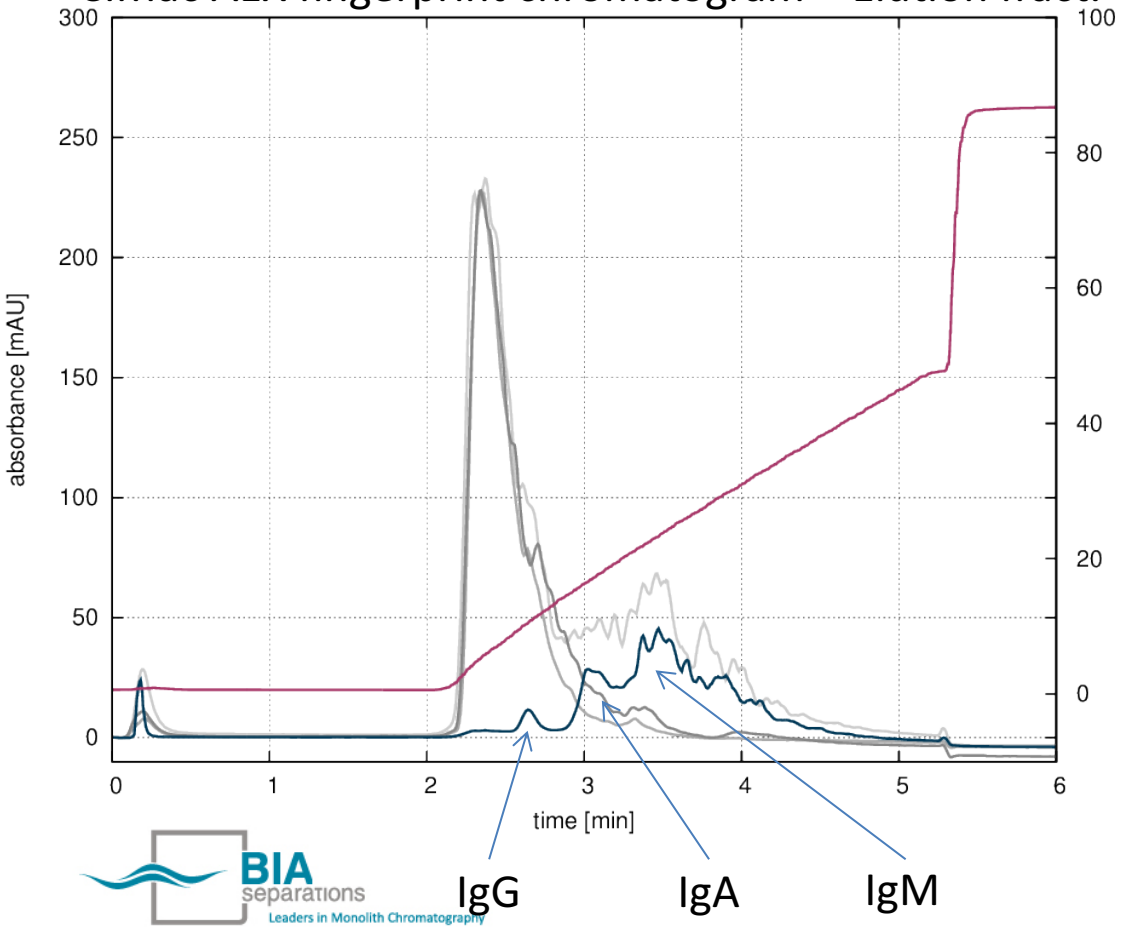
SEC chromatogram – FT fract. 5



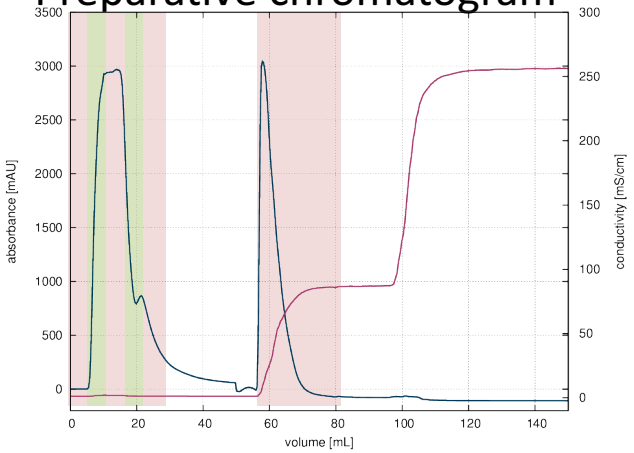
BIA separations
Leaders in Monolith Chromatography

HPLC fingerprint using strong AEX CIMac column in linear gradient

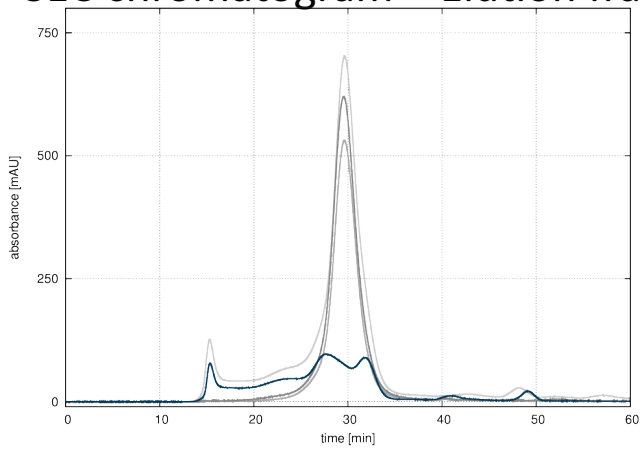
CIMac AEX fingerprint chromatogram – Elution fract.



Preparative chromatogram

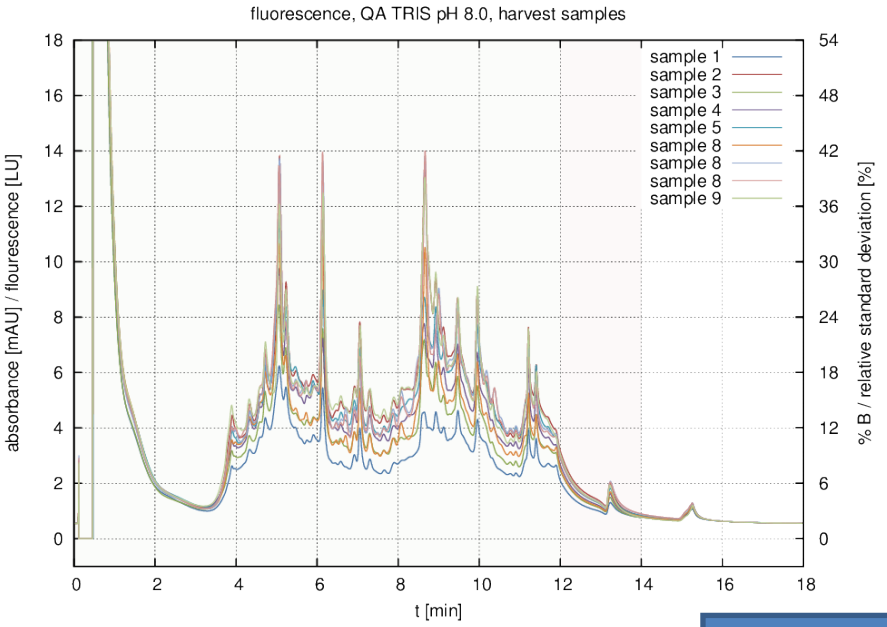


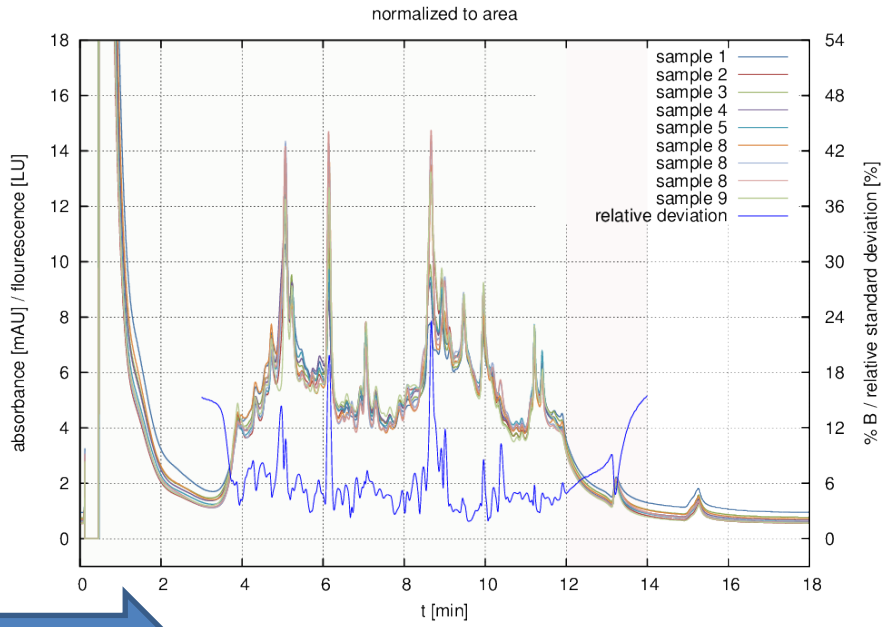
SEC chromatogram – Elution fract.



BIA separations
Leaders in Monolith Chromatography

PATfix™ Fingerprint approach to study robustness of the AAV fermentation scale-up





Normalized chromatograms

Average relative standard deviation of all area normalized fingerprints is 5.6 % (including the sample obtained with fermentation at different scales (50 L vs 200 L)). One can conclude the fermentation is very robust.

BIA separations
Leaders in Monolith Chromatography

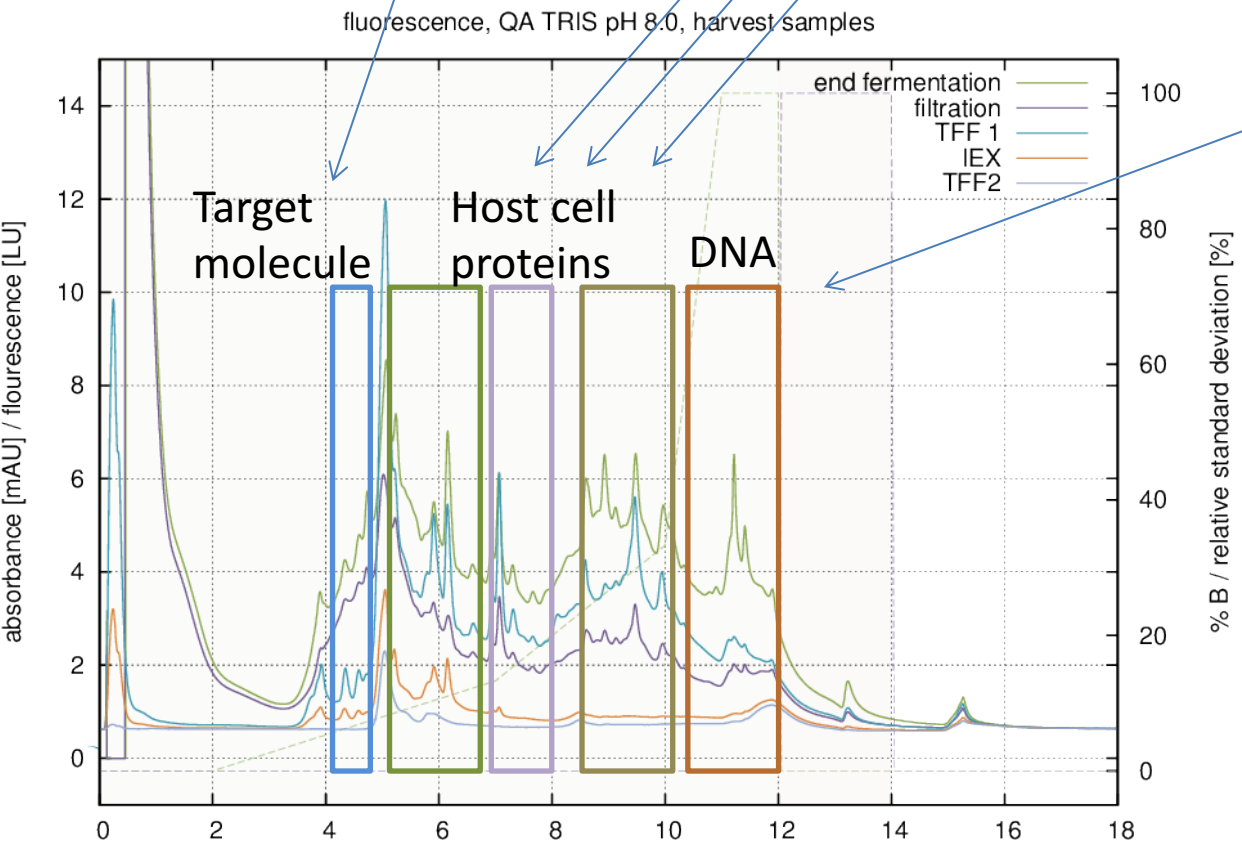
PATfix™ cheaper info as traditional methods

PATfix fingerprint injection = about 20 € (column + buffers + labor), takes about 20 min

(crio)TEM: 125 €, 30 min
AUC: 200 €, 1 h
SDS-PAGE: 20 €, 4 h
Infectivity: < 10 €, 3-7 days

ELISA: 5 € / well, 4 h
SDS-PAGE: 20 €, 4 h

qPCR: 20 € / well, 3-4 h
ssPCR: 30 € /well, 3-4 h
AGE: 20 €, 2 h



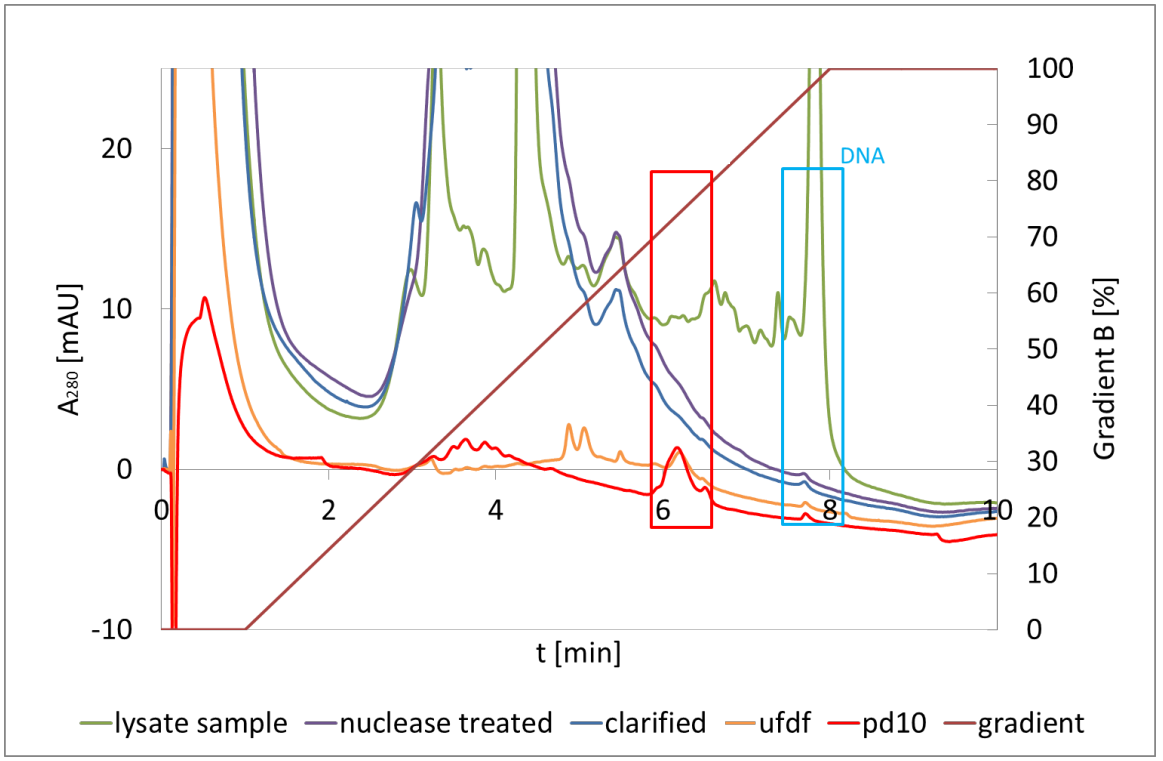
To SUM up:

Traditional methods per sample: 100 - 400 €
Time: 1 day to 1 week

PATfix per sample: 20 €
Time: 20 min
BUT not magic box – needs work to understand

Adenovirus purification process monitoring using fingerprint approach

- Lysis
- Nuclease treatment
- Clarification
- Ultrafiltration/diafiltration
- AEX Chromatography
- Buffer Exchange



Fingerprinting

Column: CIMac™ Adeno

Flow rate: 1 mL/min

Buffer A: 50 mM Tris, pH 8.0

Buffer B: 50 mM Tris + 1M NaCl, pH 8.0

BIA separations
Leaders in Monolith Chromatography

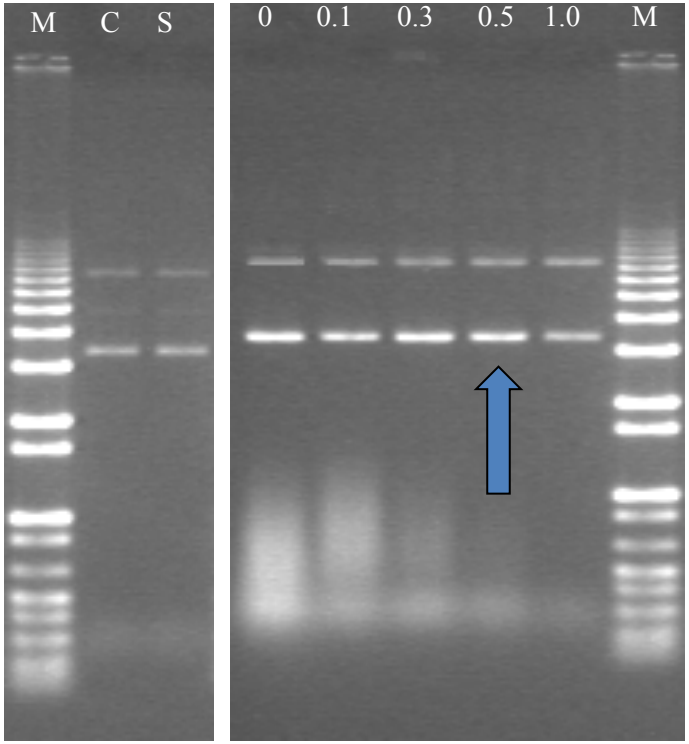
CIMac™ pDNA Analytical Column

Product number	Product name	Description
150.8501-1.4	CIMac pDNA column	DEAE monolithic matrix with a controlled ligand density and structural characteristics

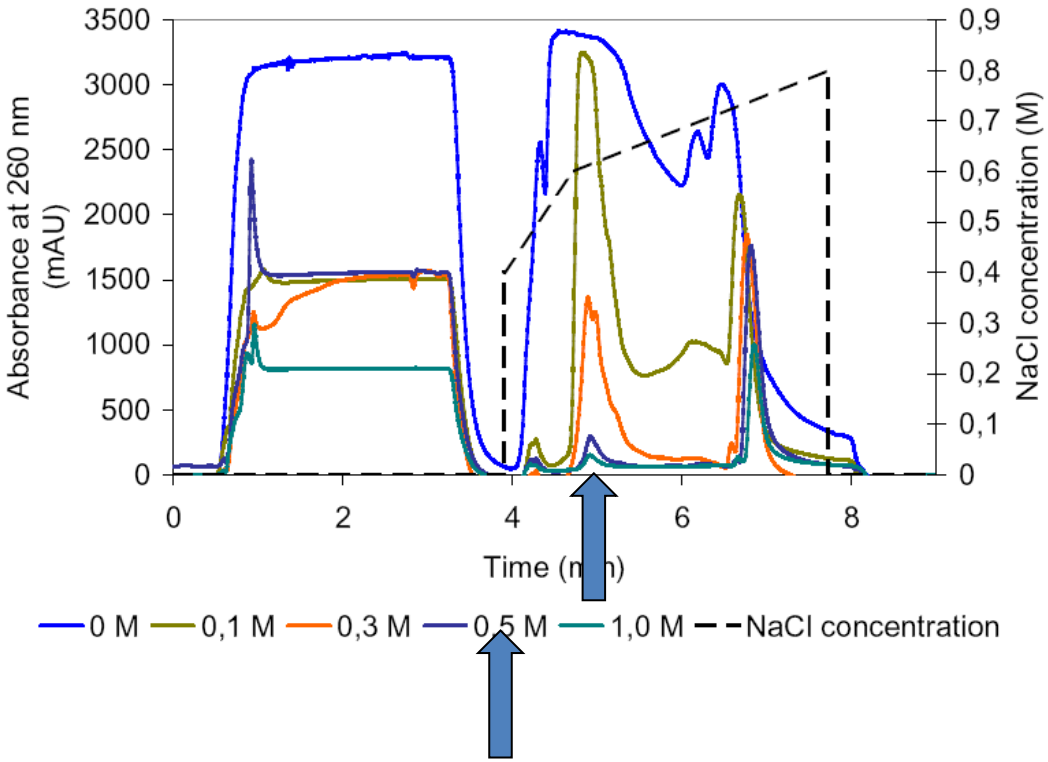
- DEAE monolithic matrix with a controlled ligand density and structural characteristics
  - 5.2 mm ID x 15 mm L, V = 0.32 mL
- Flow rates: 0.2 – 2 mL/min
- Maximum pressure over the column: 100 bar

BIA separations
Leaders in Monolith Chromatography

Optimization of precipitation with $CaCl_2$



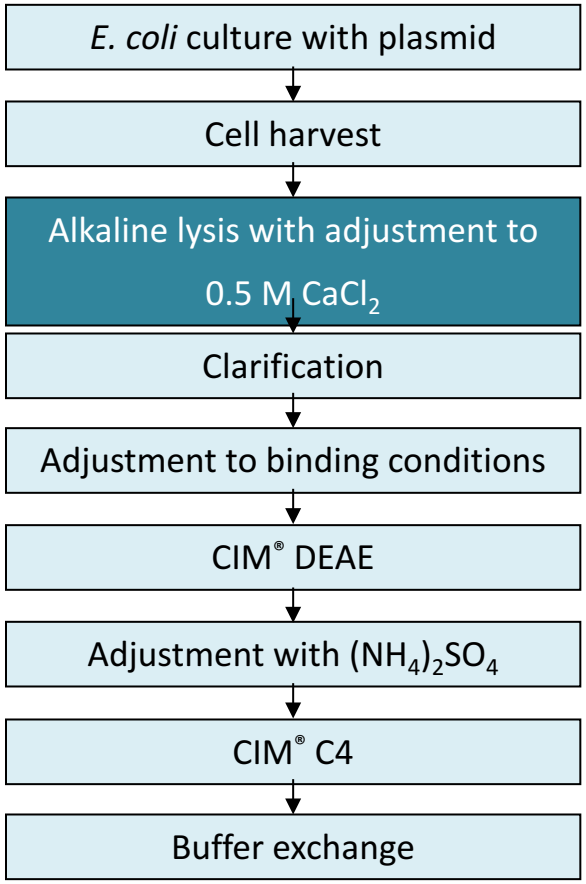
CIMmultus DEAE preparative column:

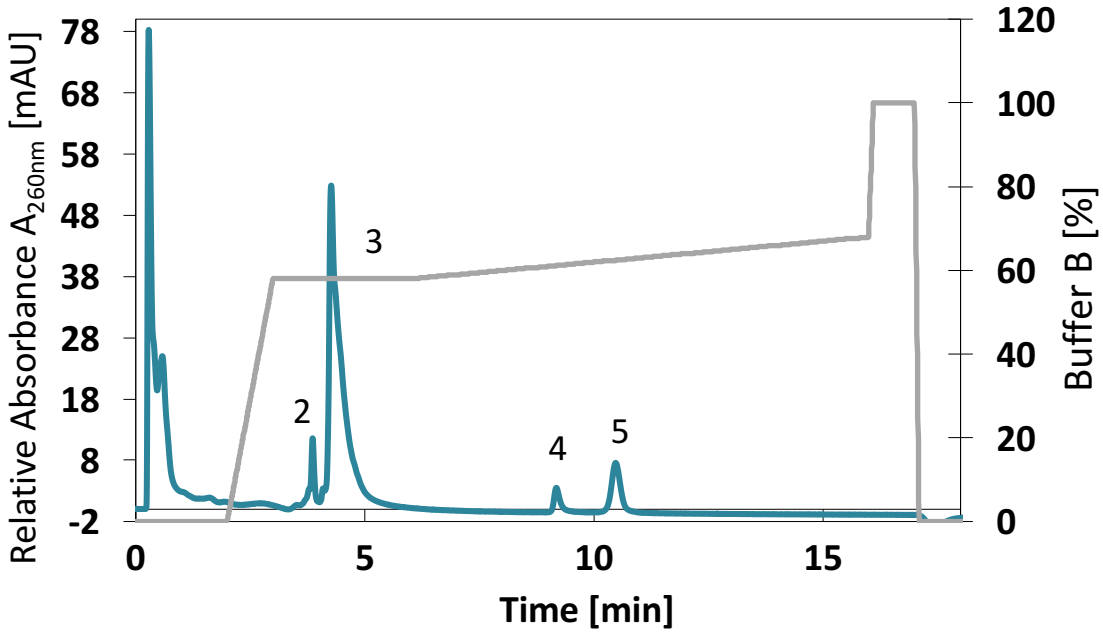


PAT HPLC to balance between RNA removal and pDNA yield

BIA separations
Leaders in Monolith Chromatography

CIMac™ pDNA Analytical Column – alkaline lysis optimisation





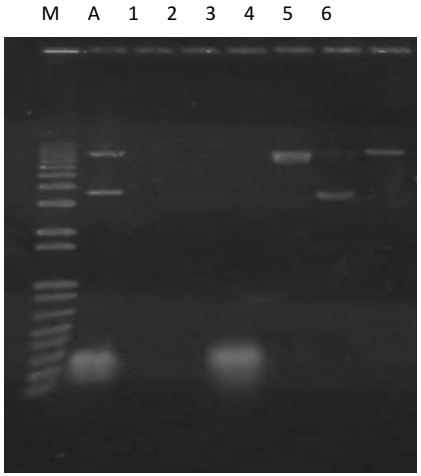
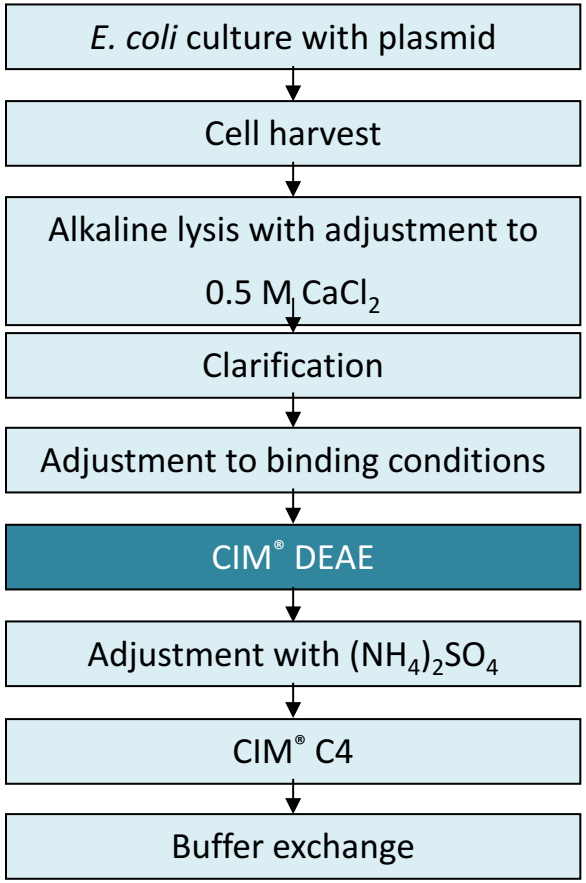
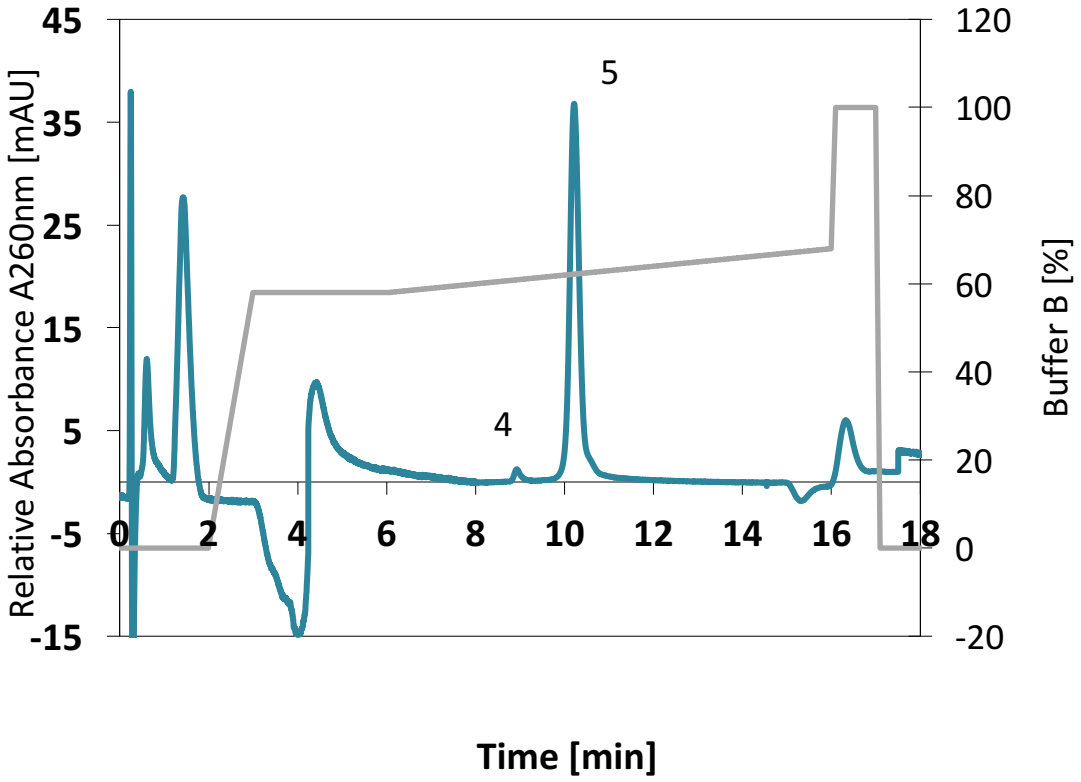


Figure 1: Agarose gel electrophoresis - Molecular weight marker (lane M), sample alkaline lysate plasmid pEGFP-N1 (lane A), peak 1 (lane 1), peak 2 (lane 2), peak 3 (lane 3), peak 4 (lane 4), peak 5 (lane 5), pDNA open circular form standard (lane 6)

BIA separations
Leaders in Monolith Chromatography

CIMac™ pDNA Analytical Column – 1st chromatography step





Conditions: Flow rate – 1 ml/min; Buffer A – 200 mM Tris pH 8.0 and buffer B – 200 mM TRIS + 1 M NaCl pH 8.0; Injection volume – 20 µl; Sample was diluted 1:3 with water; UV detection – 260 nm; Peak 1 and Peak 2 – other impurities, Peak 3 – RNA, Peak 4 – OC pDNA, Peak 5 – SC pDNA.

BIA separations
Leaders in Monolith Chromatography

CIMac™ pDNA Analytical Column – 2nd chromatography step

E. coli culture with plasmid

↓

Cell harvest

↓

Alkaline lysis with adjustment to 0.5 M $CaCl_2$

↓

Clarification

↓

Adjustment to binding conditions

↓

CIM® DEAE

↓

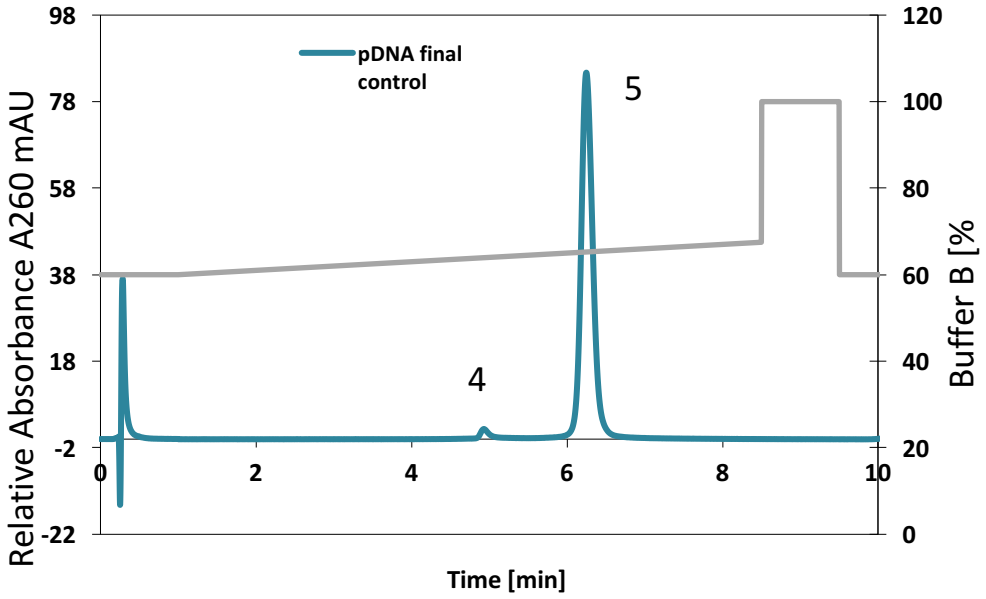
Adjustment with $(NH_4)_2SO_4$

↓

CIM® C4

↓

Buffer exchange

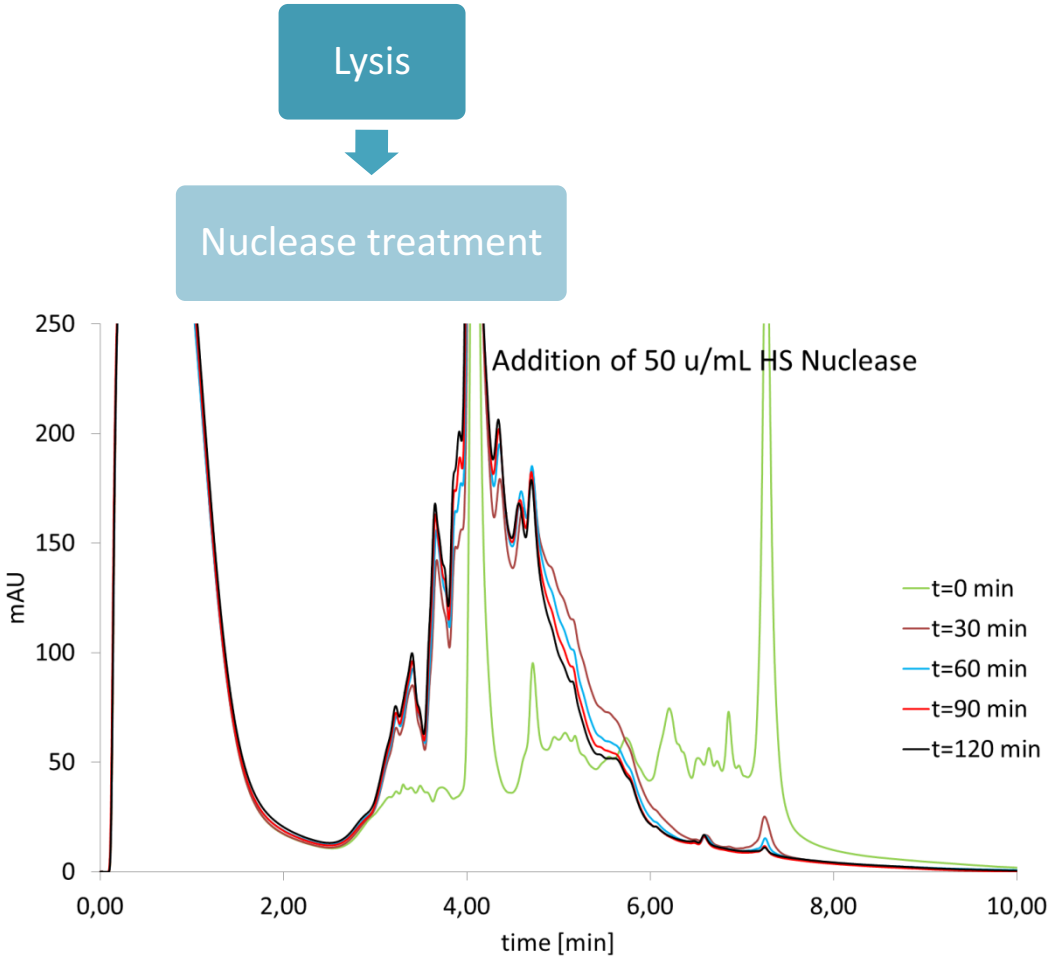


Conditions: Flow rate – 1 ml/min; Buffer A – 200 mM Tris pH 8.0 and buffer B – 200 mM TRIS + 1 M NaCl pH 8.0; Injection volume – 5 µl; UV detection – 260 nm; Peak 1 – OC pDNA form; Peak 2 – SC pDNA form;

Topoisomers	
OC	2 %
SC	98 %

BIA separations
Leaders in Monolith Chromatography

Online nuclease treatment monitoring

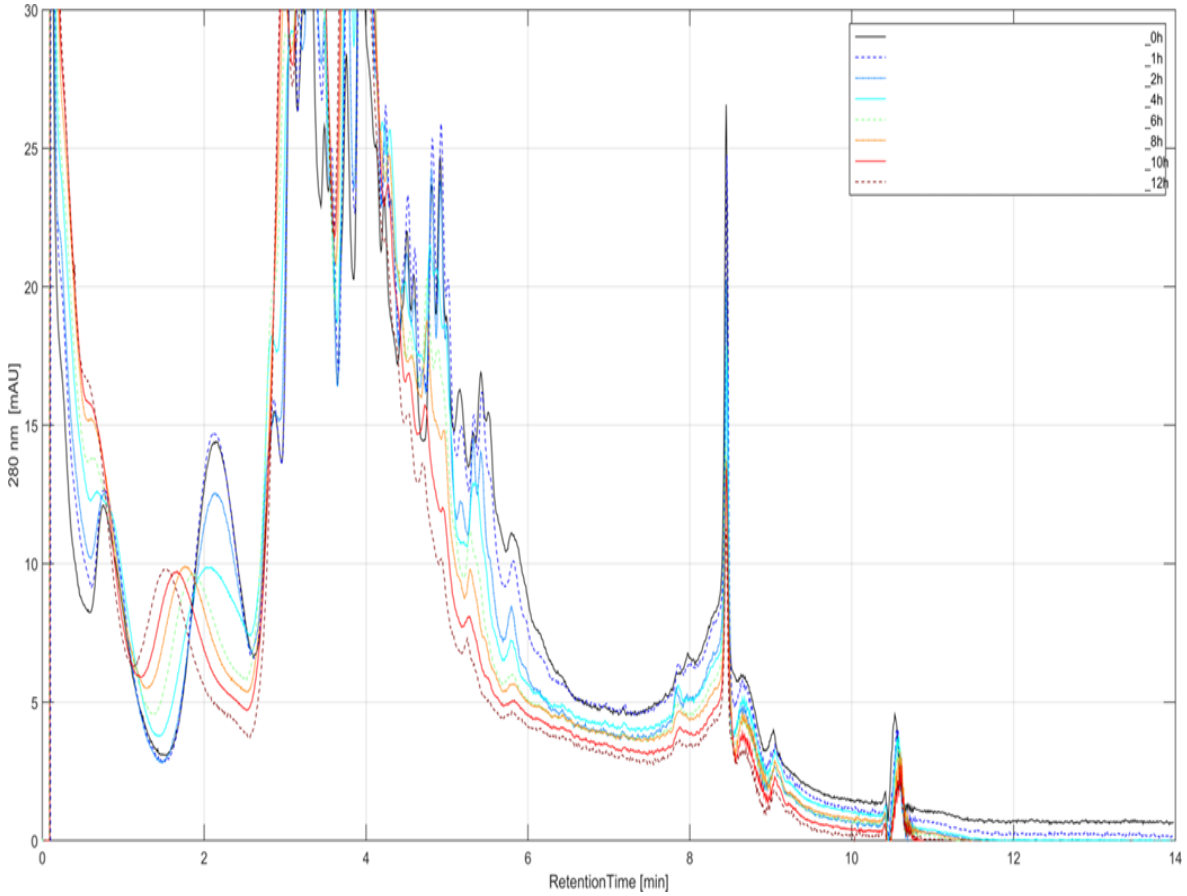


- HS Nuclease (MoBiTec; Cat No 1070-01; Lot # 202222; 250 units/µL) diluted with Chromatography Buffer A to 1 unit/µL, 1.5 units/µL and 2 units/µL.
- Final Nuclease concentrations: 50 units/mL; 100 units/mL; 150 units/mL.
- Aliquots incubated in water bath at 37°C; every 30 minutes one aliquot drawn and immediately analyzed by HPLC.

Loop volume: 1 mL, injected 1 mL of 3 times diluted samples; flow rate: 1mL/min.

BIA separations
Leaders in Monolith Chromatography

Online nuclease treatment monitoring – cost comparison PATfix™ – traditional methods



qPCR: 20 € / well, 3-4 h
ssPCR: 30 € /well, 3-4 h
AGE: no numeric result
Total labor: 1000 €
Total: up to 3000 € for 3 reactions

PATfix:
Prep labor: 100 € & go home
CIMac run: 2 - 5 €
Total: 250 € for 3 reactions

BIA separations
Leaders in Monolith Chromatography

Conclusions

- HPLC fingerprinting is convenient technique to measure multiple sample parameters simultaneously:
  - \+ Reproducible
  - \+ High resolution
  - \+ Flexible
  - ± Fast but not yet on-line
  - \- **Difficult to evaluate (needs experience)**

- PATfix™ algorithms
  - \+ System verification
  - \+ Sample stability control
  - \+ Dilution control
  - \+ Advanced mathematical manipulation of chromatograms
  - \+ **Fast, reliable and simultaneous prediction of multiple sample components**

BIA separations
Leaders in Monolith Chromatography

BIA Separations - industry standard for production of Gene Therapy products and Exosomes

- Platform processes for pDNA, AAV, Flu and Adeno, ...
- First drug purified using CIM® monoliths on the market , one passed CPIII trial (pDNA for gene therapy), 5 projects in CPIII.
- More than 100 projects in CPI – CPII trials (various Influenza, various Adenovirus, various AAV, bacteriophages, various IgMs, Inter-alpha-inhibitors,...).
- More than 500 projects in pre-clinical trials (Influenza A and B virus (eggs, Vero and MDCK cells), Rabies virus, Rotavirus, AAV, various Adenovirus subtypes, Hepatitis A, Vaccinia, Mulv, MVM, Feline calicivirus, Japanese encephalitis, Crimean-Congo hemorrhagic fever, Hantaan virus, VLP (Hepatitis B, HPV, Influenza, Adenovirus), bacteriophages (Lambda, T4, VDX10, Pseudomonas phage), Tomato and Pepino Mosaic virus, pDNA, IgM, various proteins).

BIA separations
Leaders in Monolith Chromatography

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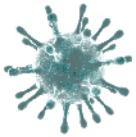

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