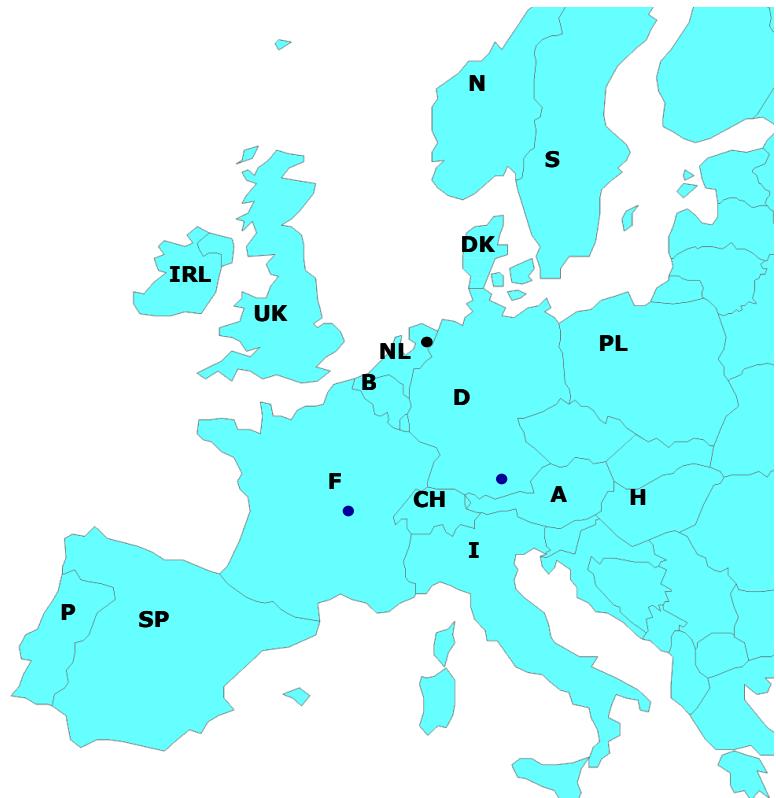


PROXCYS b.v.

Downstream Biosystems



Content

- Who is Proxcys
- History
- Training
- Disposable technology
- Product range
- Custom developments
- Process systems
- Application examples
- Principle of Operation
- Radial Compressed Packing
- Dynamic Capacity
- Summary



Els, Petra, Jan-Willem, Hans
Erik, Frank, Mark, Marcel



Who is PROXCYS ?



Producer, developer of High Performance RFC technology
Market leader & dedicated supplier of lab, pilot and process RFC chromatography equipment.

Our mission:

Consolidate HP-RFC as major process & industrial scale purification technology by Innovation, Development, Support & Training

Continuous improvement of products and organization

- Lead time control
- Feed-back integration
- Product specification assurance

Proxcys holds key patents on
HP-RFC column technology



Who is PROXCYS ?



Nieuw-Amsterdam facility assembly, production & distribution (NL)
Sales office near Munich (D)
Support office near Vichy (F) pending

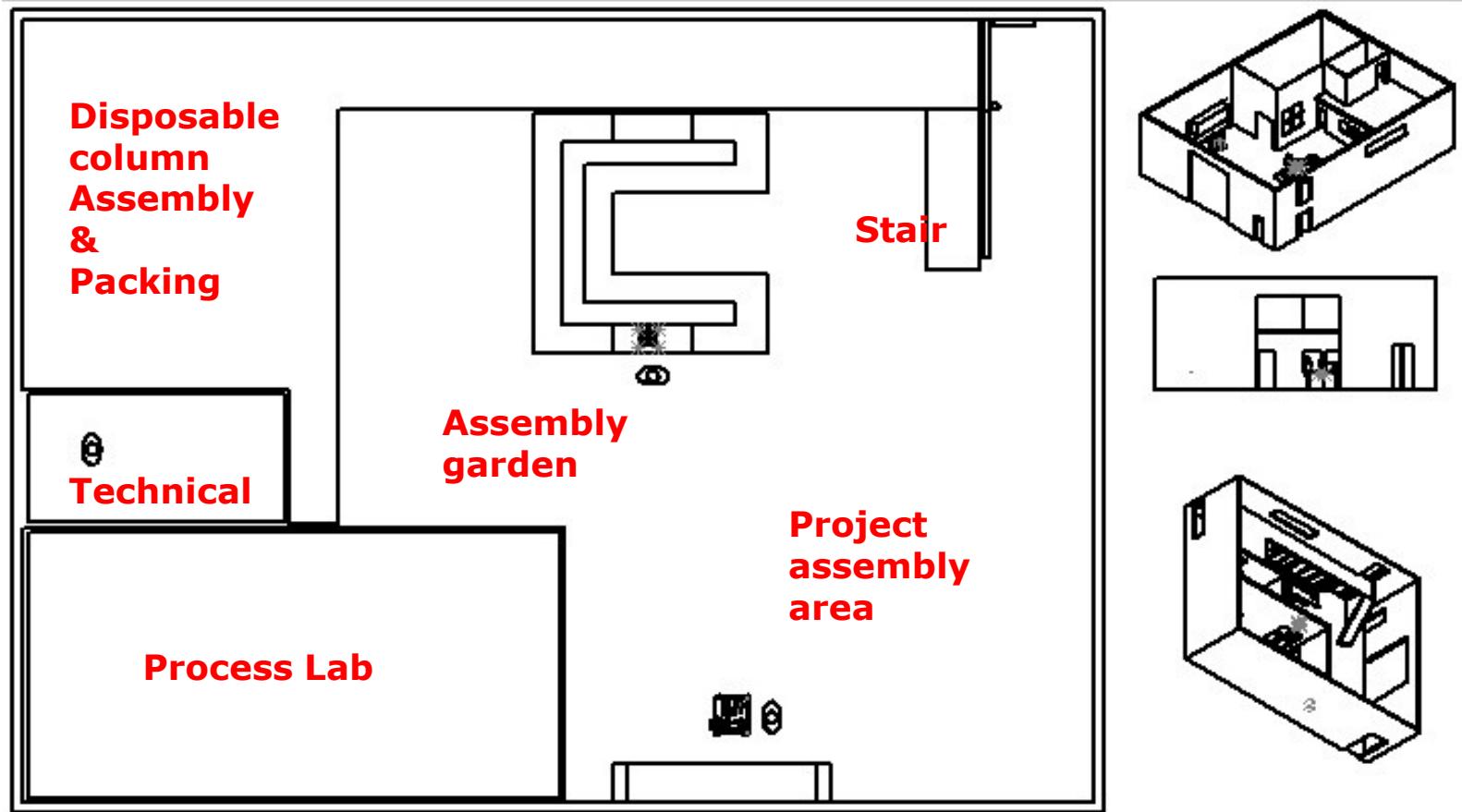
2011 Staff
9 Operational managers & support staff
~25 project based contract staff

In-house
Sales & marketing
Technical development and documentation
Assembly & testing
Engineering & prototyping
Finance & admin

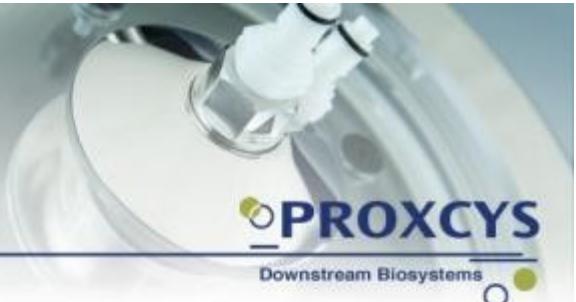
“Organic growth” business model



PROXCYS Facility



Who is PROXCYS ?



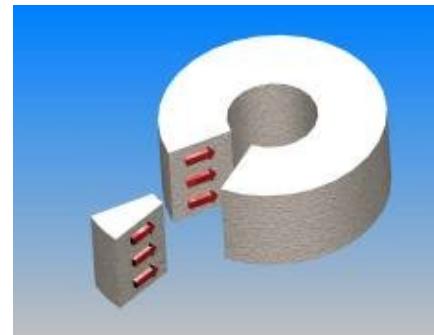
Quality organization



- Continuous strive for improvement
- Simplification without loss of functionality
- Answer to market needs and feed-back
- Fast track for improvements
- Customization at “standard pricing”



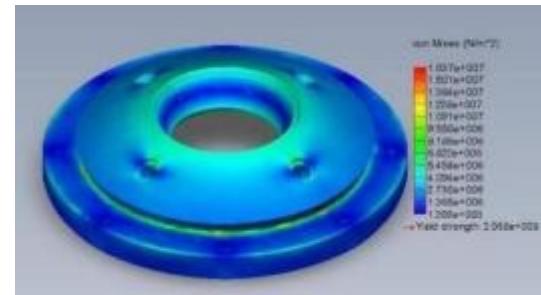
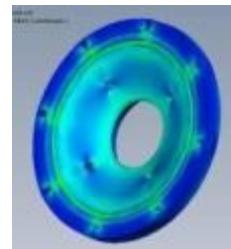
What is Radial Chromatography?



History

- o 2003 First Proxcys RFC Column in (Moscow, Insulin)
 - o Start development Annular packing port
 - o Modern 3D development software
 - o custom development
 - o Stress-calculations and deformation animation.
 - o fast-track between design and reality.
- o 2006 CARIO-Annular packing port launch,
 - o 5L Pilot Columns

Dramatised deformation of lid
45L Acrylic column at 4 Bar
Stress analysis



History continued

- 2007-2008 Product diversification
 - CA 600 Series: Acrylic Pilot Columns
 - CA/CS 1200 Series: Acryl/SS Process Columns
 - A-pack & E-Pack Packing stations
 - CSF 350L MKI SS Radial Process columns
- Custom Engineering
 - Mini-Process Series: MP Columns
 - Conditioning vessels
 - True Sanitary BBT
 - Acrylic 700L



CS1209 100 Liter
MP2003 4,5 Liter
TFPD9 9 L/min
AS-Pack 125

New developments

- o 2011 NEWS

- o Prepacked & Single Use Radial Columns
- o Resin conditioning vessel
- o Single Use *Pilot* Radial (SUPR) column
- o CS1200S manual
- o Micro Radial

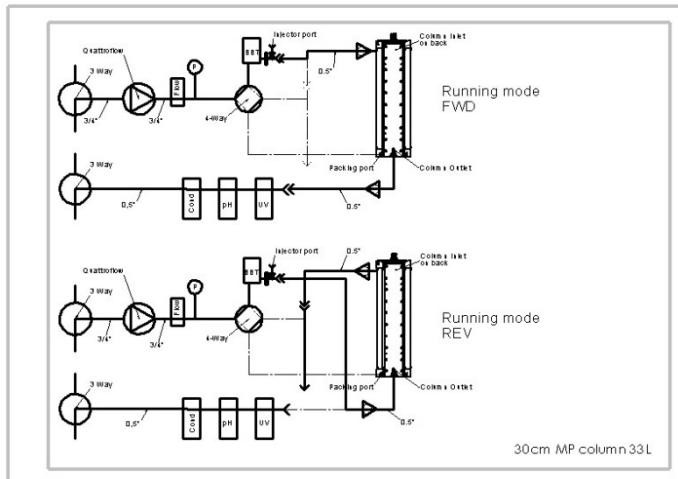


SUPR 603 15Liter
Lab RFC 100ml 3cm
Micro RFC 4ml 5cm
CS1202 25L Manual

New developments cont'd



- o 2011-2012 NEWS
 - o State-of-the-Art Axial Biostream columns
 - o HP-RFC in Bio-SMB
 - o Industrial budget columns
 - o Packing PAT systems



Proxcys Axial Biostream columns variable bed height 600 mm (Acrylic) and 1600 mm (Hastelloy). Hydraulic actuator.

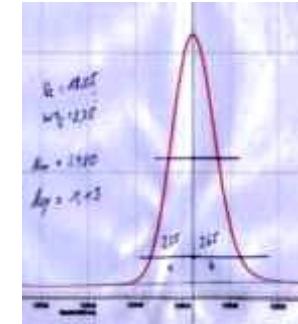


Training



Hands-on Proces Chromatography Training

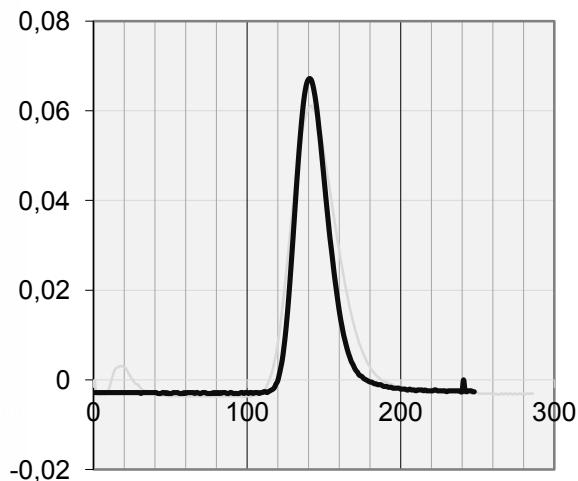
- Biochromatography: Tosoh Bioscience
- Downstream Processing: MBI University College London
- Bioseparation: JUB Jacobs University Bremen
- In-House (clients)
- Proxcys



Stepwise Packing (Toyopearl)



Plug & produce with Radial flow prepacked technology



Non-disposable performance

Sanitary design

Outline

- Technology objective
- Functionality objective
- Scalability
- Performance & Application
- Summary

Technology objective



Disposable HP-RFC column



Disposable bubble trap

Disposable Technology

SUPR™ (Single-Use-Pilot-Radial)



Functional design

- KISS (simplified design)
- USP-ClassVI materials SS frit (EP).



Optimal performance

- Performance equal to HP-RFC
- Material finish & shape
- Sanitization (chemical stability)

Chromatography cartridge

- Sustainable (minimum waste)
- Single-use (toxic) or during years (proj)

Transportable

- Ø 70cm x 150cm max. (outer dimensions)
- Light weight



Patent Pending

Functionality objective



Disposable Technology

Functionality objective

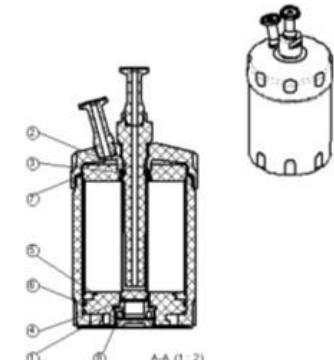
o Characteristics

- Inlet & Outlet at top (carboy mobility)
- Safe handling (disconnection ≠ draining)
- Clean
- cGMP compliant starting at 100ml



o Suitable shape and dimensions

- Tight packing by annular port packing
- Bed supporting shape (supporting stability during transport)

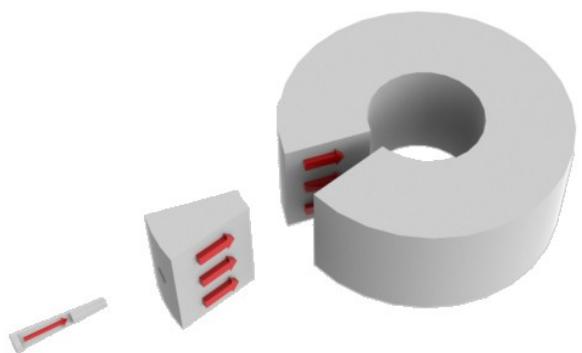


o Scalability

o Applications

- All adsorptive separations (short b.h.)
- Personalized medicine (small cGMP)
- Campaigns (mobile)
- Direct Capture[©] (robust capture from crude feed)

100 Liter
12 cm Bed height
~80 kg (empty)
Total height 1.0 m
Footprint Ø 50 cm
Flow > 4000 L/hr



Disposable Technology Scalability

o Scalability

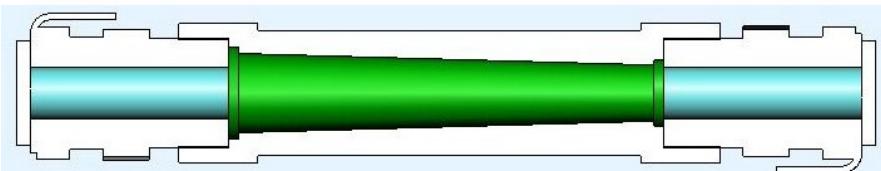
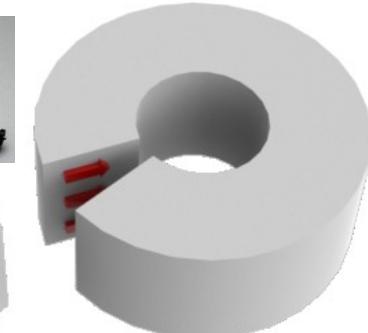
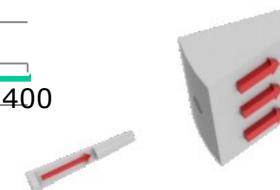
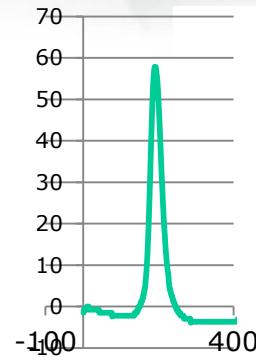
- Full radial (small to industrial scale)
- Segment columns (= RFC – mimics)

o Micro-Radial

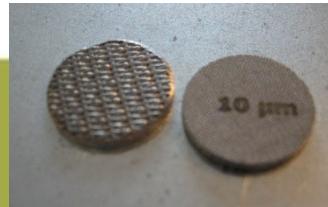
- 5ml – 25ml (volume range)
- 6 cm – 30 cm (6,12,16,20,30 cm)
- First assessment (scaling up)
- Virus validation (scaling down)

o Prepacked or self-packed

- Pump packed (peristaltic pump)
- Equal packing density
- Frit configuration **identical** to process scale column it is derived from



Standard I/O-ratio 2:1



Disposable Technology

Model ranges (full radial examples)

 PROXCYS

Downstream Biosystems

Type	Bed height (cm)	Minimum volume (L)	Maximum volume (L)	Footprint Ø (cm)
SUPR™ 300	3	0.1	4	15
SUPR™ 500	5	1	12	23
SUPR™ 1000	10	5	100	40
SUPR™ 2000	20	25	375	70



Bed heights and volumes indicated are existing design boundary examples.

Principally Proxcys strives to offer the full range of bed heights and volumes between the extreme boundaries (3cm and 20cm b.h.).

When there are reasons to go beyond these boundaries (lower bed height than 3cm or larger than 20cm Proxcys will investigate feasibility).



Performance & Applicability



Disposable Technology

Application Examples



Prepacked Prevalidated (*Avantor, Bio-Rad ... Prometic, Repligen, Sterogene, Tosoh, i.e. all*)

- Prion removal (human blood plasma and derived)
- IgG aggregate, endotoxin removal
- Multiple applications, feasibility of SUPR™-SMB will be investigated 2011

Guard column (Prot-A, Affinity) (negligible extra pressure drop)

- Protect main column from blocking
- Increased resin lifetime
- Applicable to "Direct Capture©" from Crude feed

Scavenger column (HCP, DNA, Virus, Toxins)

- High throughput, any functionality
- Limited in-line resistance (pressure) contribution

Full freedom of choice in resin & functionality



Disposable Technology

HP-RFC compared to MA



SUPR™ can act as bridge between traditional capture chromatography and Membrane adsorbers

The ability to pack process chromatography resins in very short bed heights offers a bridging position of the SUPR™ between traditional adsorber columns and membrane adsorbers

Type (range)	Axial column 20 cm (>5 cm)	SUPR™ Resin-film Adsorber 3 cm (>1 cm)	MA Membrane Adsorber 8 mm (1-8mm)
Bed volume	2000ml	2000ml	2000ml
Surface Area cm ²	95	1000	5000
DBC (BSA)	< 400 g*	< 400 g*	< 50 g*
Throughput @200cm/hr	0.3 L/min	3 L/min	16 L/min

* Adsorbent dependant





Disposable Technology

CMO competitive edge



CMO (Contract Manufacturing Organization)

- Prepacked & Prevalidated
- Plug-and-Produce
- Any resin
- Any application (adsorptive)
- Any volume (<375 L)
- No investment depreciation on projects
- Smallest footprint (minimal clean-room occupation)
- Customized columns exclusive to clients
- Improved position to win the tender contract



700 mm

Disposable technology

Disposable technology

Industries



How is Radial disposable technology interesting for the different industries?

- Blood-Plasmaprotein Fractionation
 - *Demand, economical alternative*
 - Highly infective contaminants
 - Lower capital cost

- Functional foods & Dairy
 - *Industrial size scale-able*
 - Space, weight, cost saving
 - Robust technology



RFC 100 Liter
Total height 1.8 m
200 kg

12 cm Bed height
Footprint 60x60 cm
Flow > 4000 L/hr

Disposable technology



Disposable technology

Application range

What resins can be applied ?

What solvent systems ?

- Any buffer

- Polypropylene USP-Cl.VI & SS / EP
- POM (Acetal) USP-Cl.VI & SS / EP

- Extended Volume Range

- Choice between 100 ml and 375 liter

- Extended Bed height Range

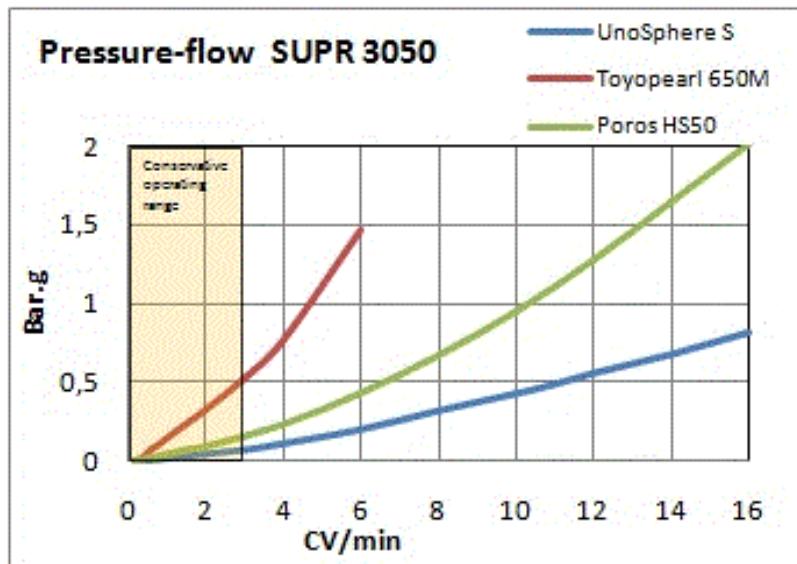
- Choice between 3 cm & 20 cm




Disposable technology

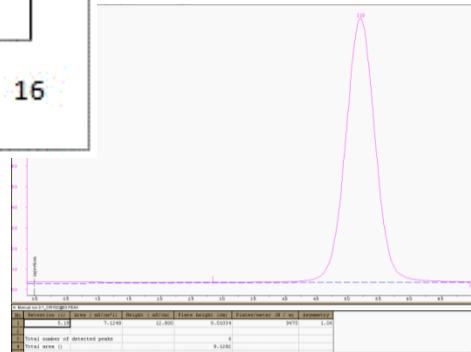


Disposable Technology Performance



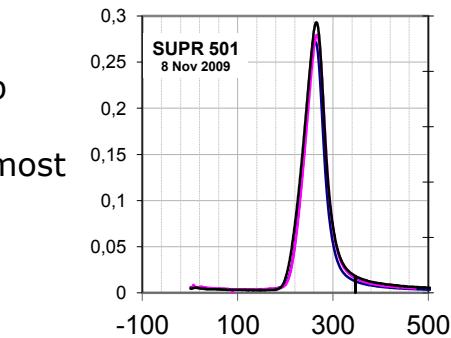
Velocity

- Low pressure drop
- Wide flow-rate range
- SUPR™ 3050: 3cm bed height 500ml
- Colored bar: average LV of 0-400cm/h



Symmetry

Function of proper annular packing procedure



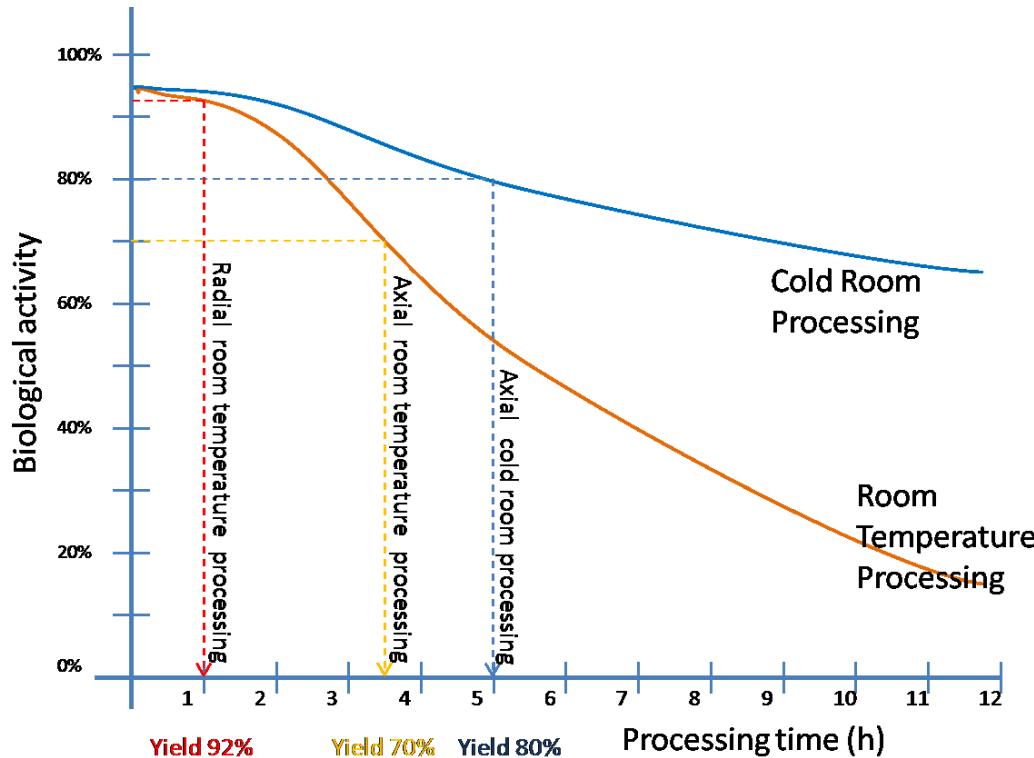
ES-Pack PAT 20

- Pack & Run© system
- High flow / low internal system pressure.



Disposable Technology Application Examples

Velocity prevents degradation



Courtesy Mastivax

Processing viscous solutions

Recombinant product in viscous plasma by RFC processing

- superior yield despite room temperature processing.

Cold room processing forced the process to be very slow, compromising single shift operation.

Fast processing and superior recovery of the product at room temperature offered improved flexibility / economy in scaleup.

Optimal binding conditions based on LV were applied at transfer from axial to Radial.

So despite flow rate increase binding properties remained identical

PROXCYS

Downstream Biosystems

SUPR Summary

Disposable Technology

SUPR Conclusions



SUPR Summary

- Plug-and-Purify
 - easy access safe connection
- Largest volume and bed-height range
 - 100ml – 375 liter, 3 cm – 20 cm
- Compact light weight columns
 - carboy mobility
- All packed bed resins
 - Agarose to Hydroxyapatite
- Stable platform
 - reproducible packings
- Certificate of Performance
- Cost effective solution



Product Range



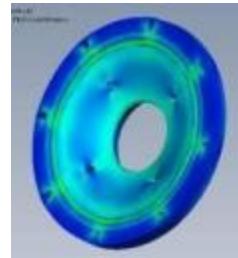


Portfolio 2011



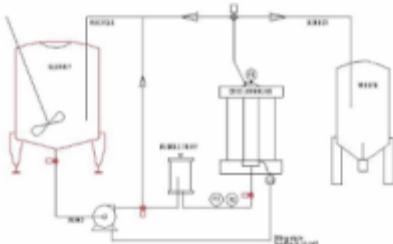
HP-RFC 0.1 L-1200 L

High performance Radial Chromatography columns
Prepacked / Disposable



Custom engineering

GMP process equipment



Packing Stations

Automated column packing systems



Mini Process 50ml – 33L



Compact Industrial Workstations

Pilot to Process



SUPR

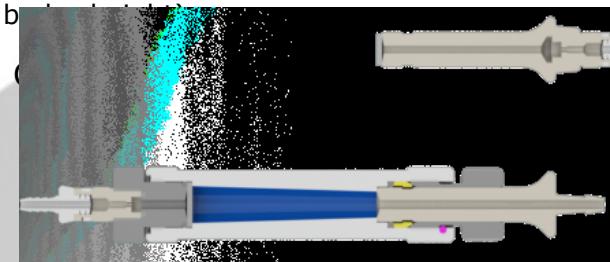
Disposable technology
2011 Prepacked
Prevalidated

RFC Column Range Mimics



I. Micro Radial Column (6 to 30 cm bed height)

- Scale down model of Proxcys Radial Columns
- radial column feasibility studies
- virus clearance studies



II. Method Development Column (6 to 20 cm bed height)

- segmented 'piece of the pie' column
- for optimizing packing process
- identical hydrodynamics as full scale RFC columns



III. Mini Process Column (up to 30cm bed height)

- same frit design
- Identical packing process as industrial scale columns
- Column volume 1-40L



RFC Column Range



IV. HP-RFC Pilot line (5 to 12 cm bed height)

- Fast, highly efficient fractionation
- Direct big-bead capture from unclarified feed
- Column volume 2 L up to 100 L



V. HP-RFC Process line (10 to 20 cm bed height)

- Compact productivity 0.5 m² up to 250 L
- Automated pump packing in 5-20 minutes
- Column volume 10L to 250 L



VI. HP-RFC Industrial line (up to 30cm bed height)

- Light weight small footprint columns for flexible clean room planning
- Rapid automated column packing 10-30 min
- Column volume 100 L up to 1200 L



Full Range HP-RFC columns

PROXCYS

Downstream Biosystems



Discovery

Preparative

- Micro-Radial
- Axial

Phase I-II

Pilot < 2 L

- Radial MD/MP
- Axial

Phase III

Pilot \geq 2 L

- Radial
- Axial

Manufacturing

Process < 1200 L

- Radial

Multi-column
continuous

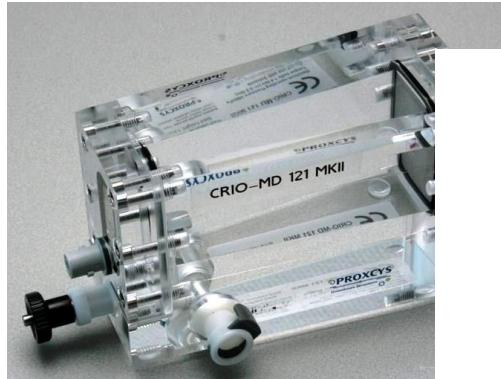
- Radial

Technology transfer by simple process parameter calculation based on residence time or LV

Any column between 3 cm and 30 cm and 0.05 L and 1200 L.

PROXCYS SUPR MD MP

 PROXCYS
Downstream Biosystems



PROXCYS Process 45L 12 cm



CA1204 45 Liter

12 cm Bed height

Height 1.5 m

Footprint 60x60 cm ~170 kg

Flow-500-2000 L/Hr

PROXCYS Process 100L 12 cm



CS1209 100 Liter

12 cm Bed height

Height 1.8 m

Footprint 70x70 cm ~230 kg

Flow -1000-5000 L/Hr





Process Technology

Model ranges (examples)

Low pressure columns



Type	Bed height (cm)	Minimum volume (L)	Maximum volume (L)	Footprint Ø (cm)
CA/CP 300S	3	0.1	4	16
CA/CS 600S	6	2	50	35
CA/CS 1200	12	10	225	70
CA/CS 1500	15	15	440	100
CA/CS 2000	20	40	630	105
CP/CS 3000	30	125	1200	135



Virus clearance & Development columns from 5 ml

Process Technology

Low pressure columns

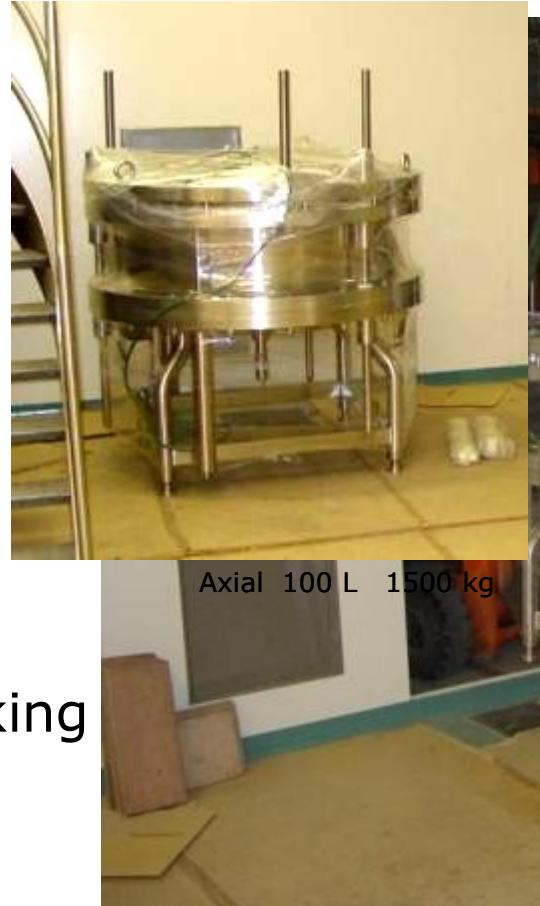


Economy:

- Time
- Footprint ($\frac{1}{4}$)
- Weight ($\frac{1}{5}$)
- Cost ($\frac{1}{3}$)

Comfort / Handling:

- Single operator
- Automated packing
- Maintenance



Radial 100 L 300 kg



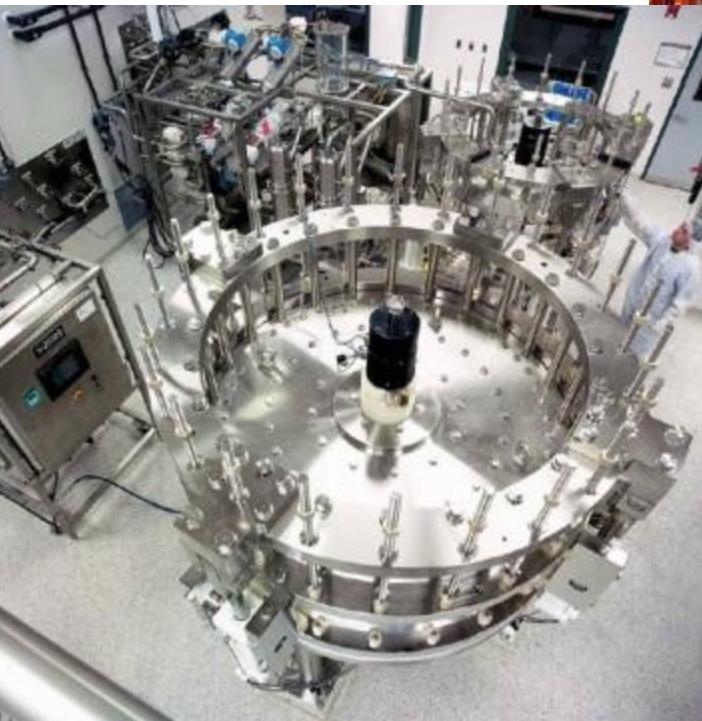
Size limitations

Industrial scale chromatography

Axial

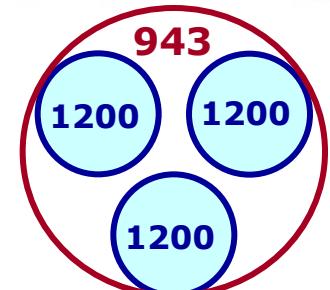
943 Liter 30 cm Bed height

Total height ~2.5 m >10.000 kg



PROXCYS

Downstream Biosystems



Highest throughput per m²



Impressive engineering

HP-RFC Annular 3040

1000 Liter 30 cm Bed height

Total height 1.8 m ~2500 kg

1000-8000 L/hr

CS 3021XL 600L 30cm 1400kg

Lighter 1/4 Compact 1/5

Radial Flow Chromatography

HP-RFC



HP-RFC Summary

- Highest throughput per m² reduced facility cost
- Robust technology no process interruption
- Increased dynamic capacity 10-20% resin saving
- Process step reduction Crude feed processing
- Applicable from lab to industrial scale
- Significant Economics advantage



Custom Solutions



CS 3021XL 600L 30cm 1400kg

PROXCYS Design Resin conditioning vessels

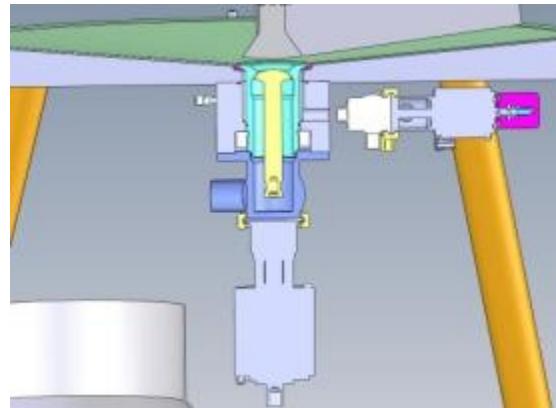


Resin conditioning vessels with special feature to allow 80% concentrated resin evacuation

Sanitary design.



Resin conditioning vessels, self-draining Sanitary



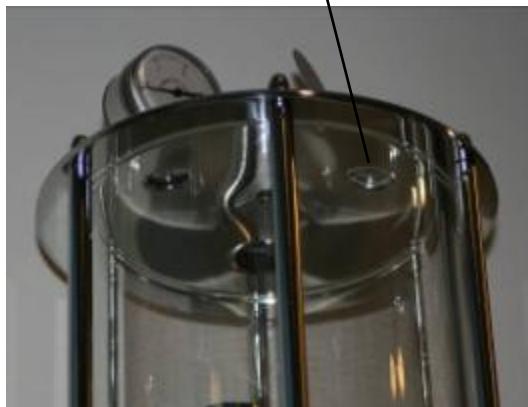
PROXCYS Design Sanitary Bubble Trap



Bubble trap with accessory ports

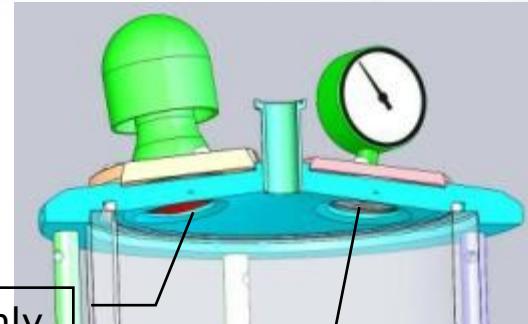
Automatically cleaned 100%.

Rupture disk
FM-S Flat



Thoroughly flushed

Manometer
membrane



Pilot BBT stand alone
or attached

BBT260-150 26 Liter 10 Bar 50x40 cm (hxd) ~55 kg Flow < 5000 L/hr



PACK & RUN Systems



Packing and Testing Station

ES-Pack PAT20 Isocratic Pilot system



Main specifications

- Sanitary design (cGMP) compliant
- Compact systems
- Rated to ~6 Bar
- Industry standard connectors, detectors and pumps

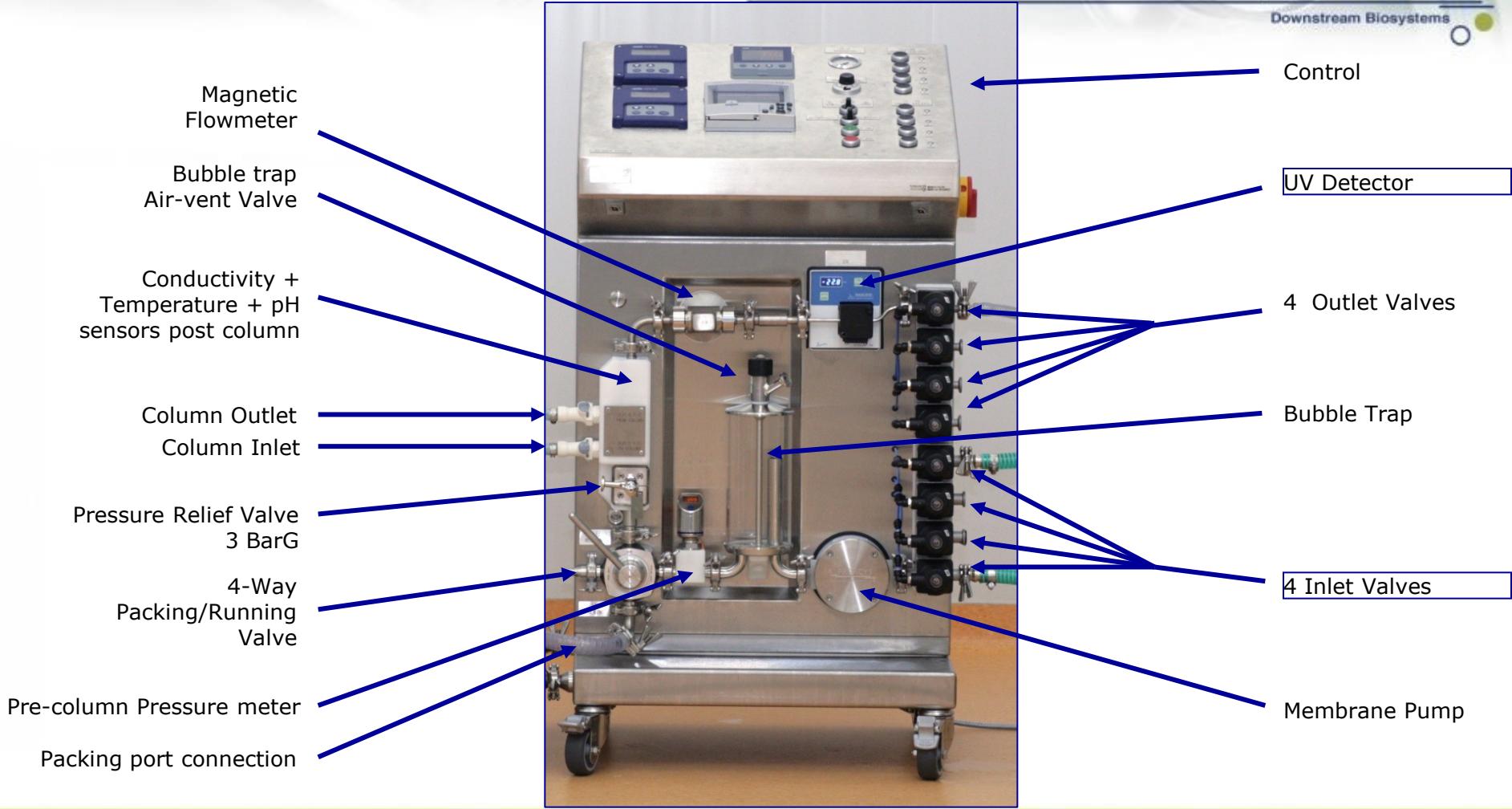


Chromatography Workstations

PAT Station

ES-Pack PAT systems

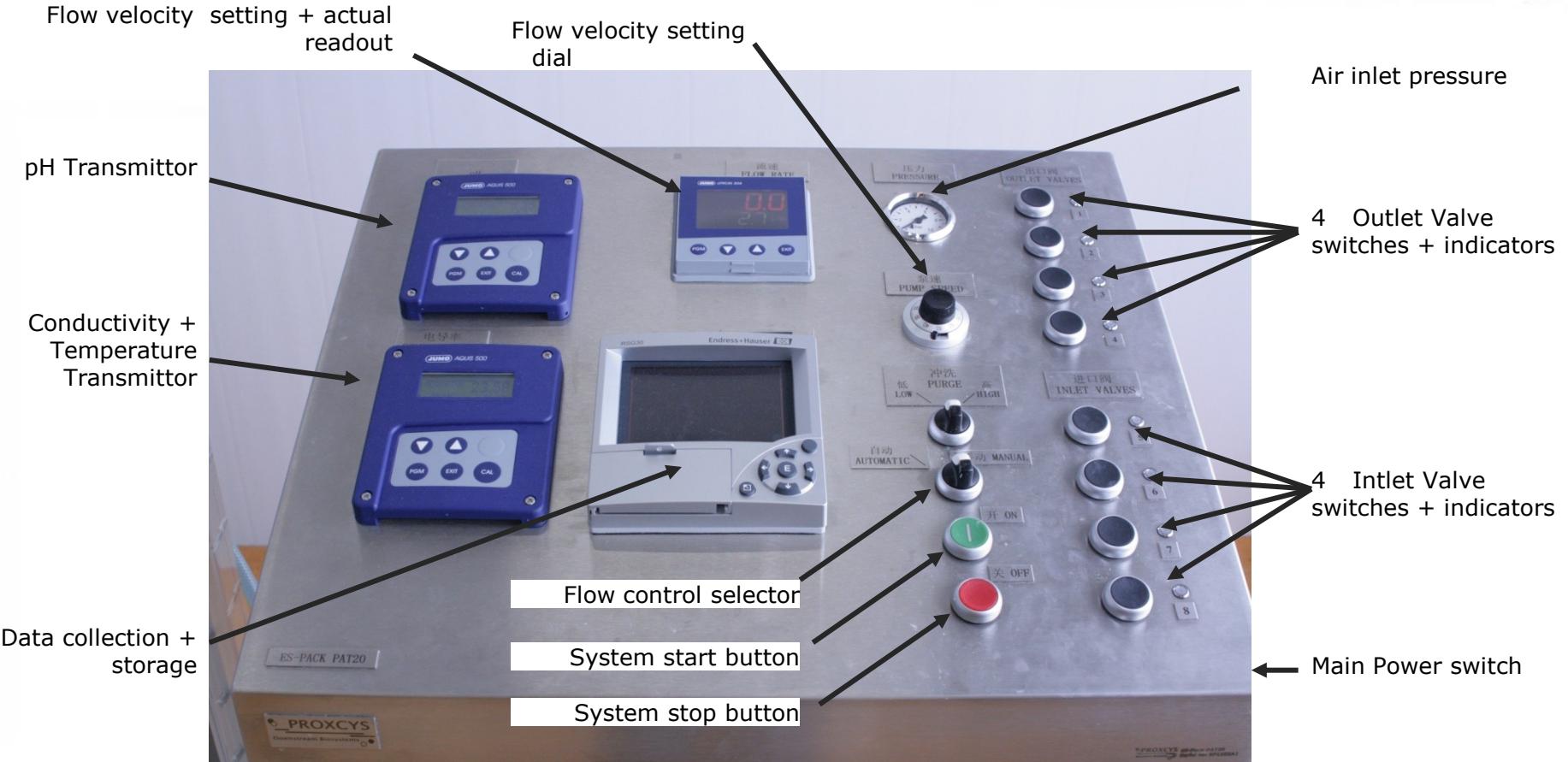
PROXCYS
Downstream Biosystems



Chromatography Workstations

PAT Station

ES-Pack PAT systems



Chromatography Workstations

PAT Station

ES-Pack PAT 20 system Specifications



Flow-rate	6 to 1500 Liter per hour 0.1-25 Liter per minute
Operating pressure	Up to 5 bar at column inlet
Tubing dimension	ID 9.6 mm after the column, ID 15.75 mm before the column
Temperature range	From 2°C to 30°C (60°C for CIP)
Pump	Multiple membrane pump technology with detachable head
Flowmeter	Magnetic - ABB
UV wavelength range	From 254nm (fixed wavelength)
Conductivity range	<100 mS/cm – Jumo
pH range	0 -14 – Proxcys
Bubble trap	1 Liter acrylic tube equipped with drain valve
Pressure relief valve	Pre-column calibrated (3 BarG)
Valves	Pneumatic sanitary membrane valves (4 + 4)
Pressure sensor	Sanitary design equipped with pressure limit switch

PAT Station

ES-Pack PAT 20 system Specifications

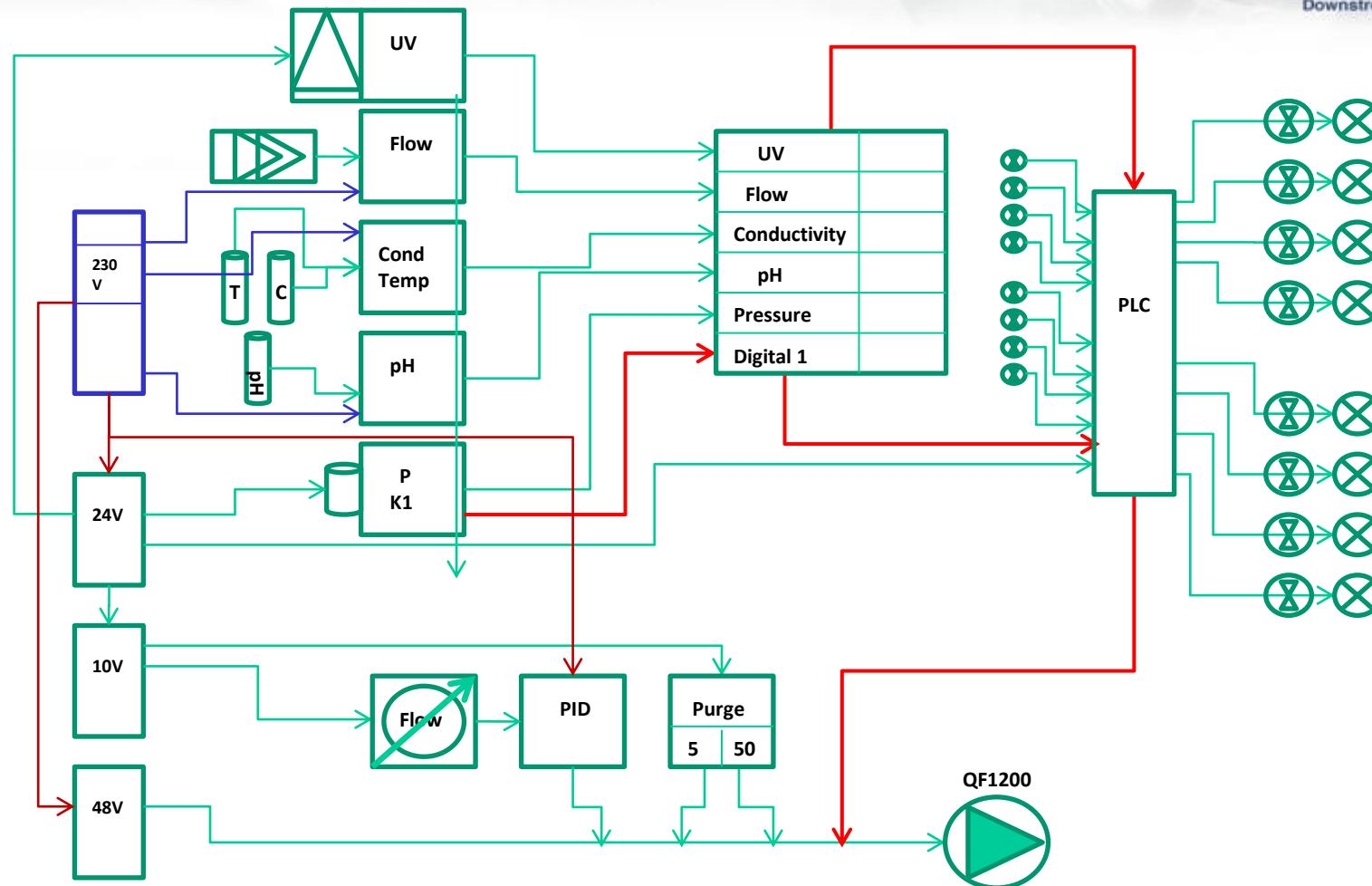


Material and certificates for wetted parts	Valves body : Stainless Steel: 316L, 1.4404 Valves & Pump membrane : EPDM Detector bodies: POM Tubing : Stainless steel: 316L, 1.4404 Bubble trap : Acrylic (PMMA) All wetted part in compliance with FDA requirement 3.1b certificates delivered with stainless steal part
Surface finish	Ra <0.8µm
Degree Protection	IP55
Weight	~ 135 Kg
Dimension (HxWxD) mm	1200 x 600 x 500
Power supply	230 V 1p 50Hz
Air supply	6-10 bar
Control	PLC : Creuset
Support	4 castors, 2 with brakes

PAT Station

ES-Pack PAT 20 system Electrical overview

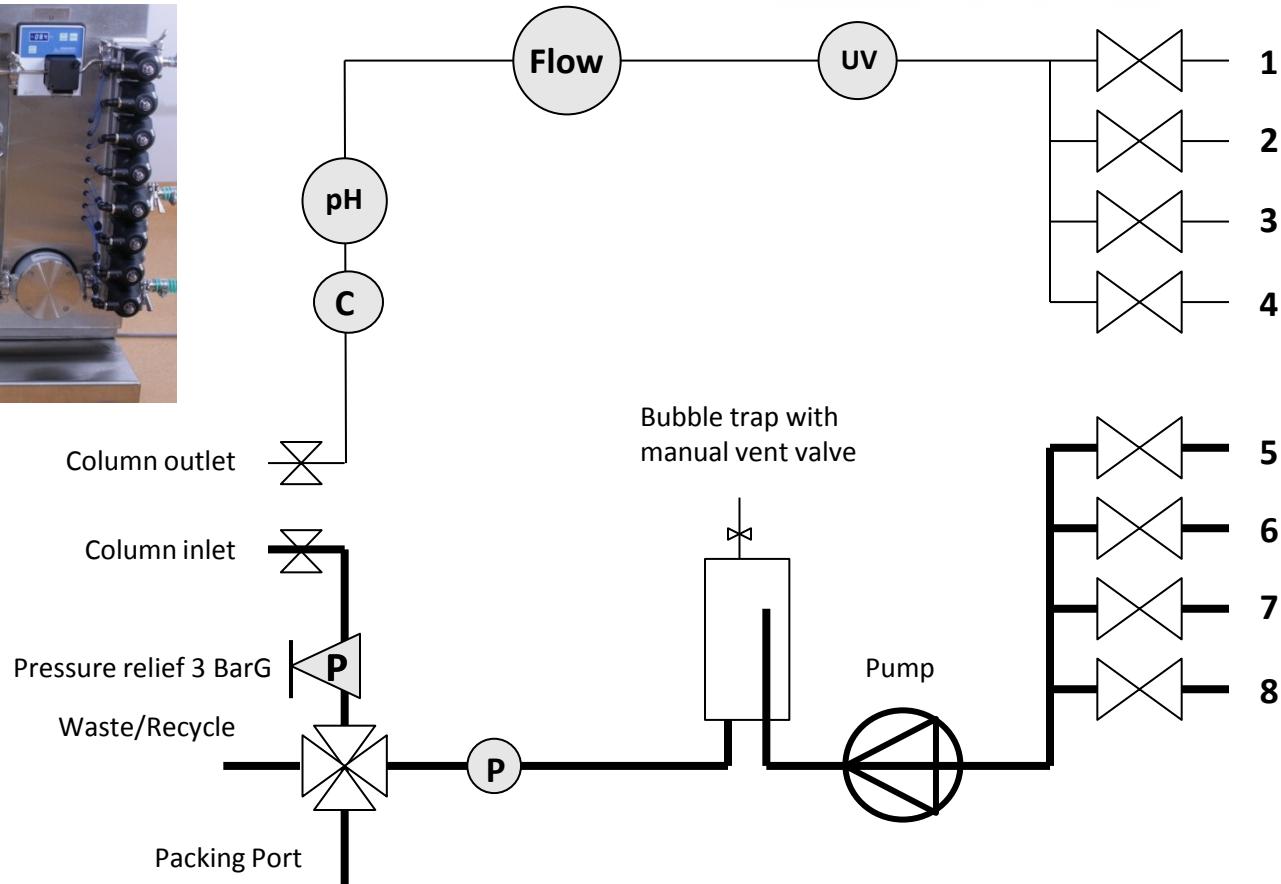
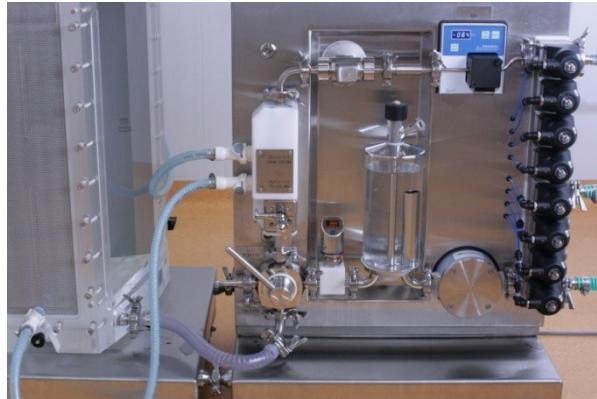
PROXCYS
Downstream Biosystems



PAT Station

ES-Pack PAT 20 system P&ID

PROXCYS
Downstream Biosystems



Application examples



Application examples



- Bio-Pharmaceuticals

- *Novozymes*
 - *Recombumin®*
 - *Cellprime™ rTransferrin AF*

- Blood-Plasmaprotein Fractionation

- *Factor VIII, IX, AT, IgG*

- Functional Food

- *Extracting Value from "waste"*

- Environmental

- *Removal of Arsenic from oil-drilling fluid*





Novozymes Recombumin®

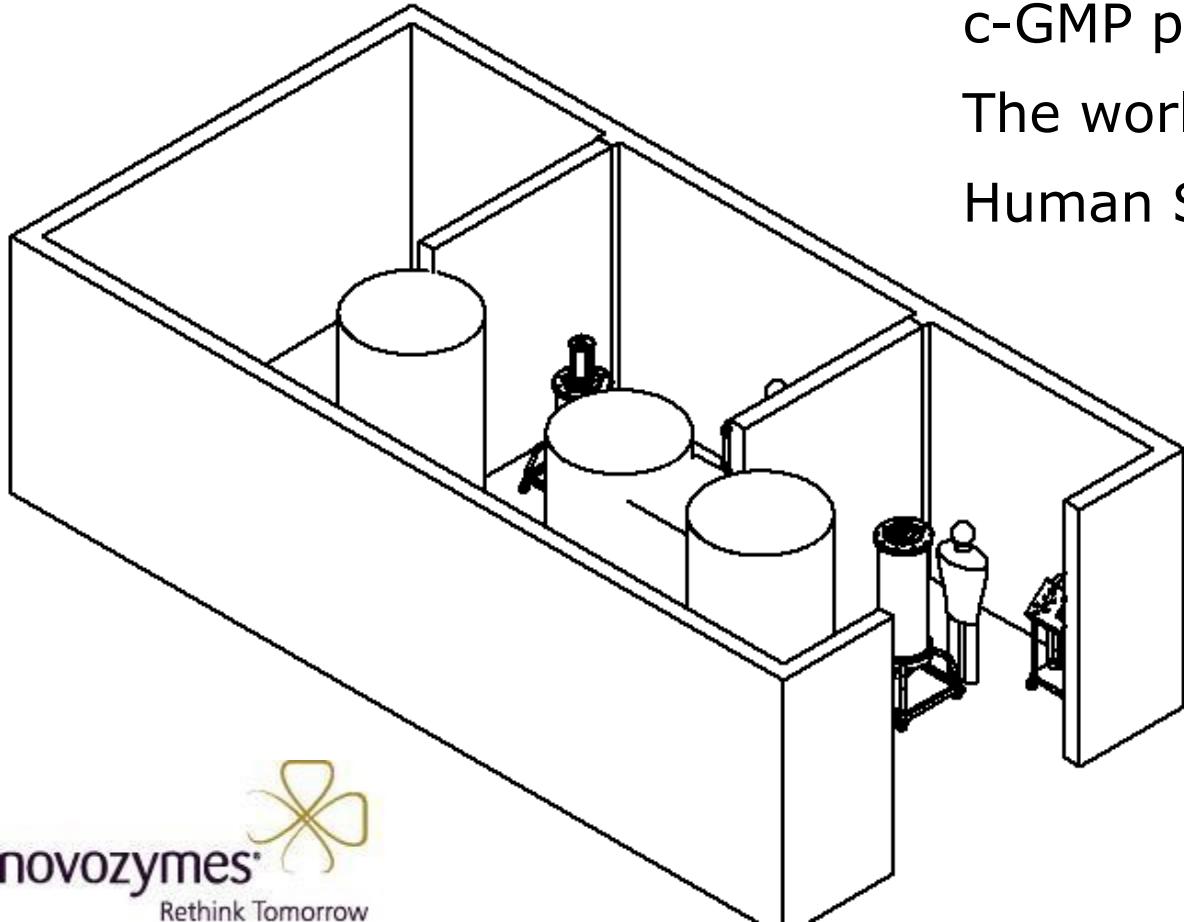
r-HSA production

Courtesy: Ashley Wigley



Novozymes Recombumin®

r-HSA production suite



novozymes[®]
Rethink Tomorrow

c-GMP production plant of
The world First recombinant
Human Serum Albumin

Recombumin® r-HSA
Cellprime™ Transferrin AF

recombinant Animal free plasma
proteins for drug and vaccine
manufacturing.





Novozymes Recombumim®

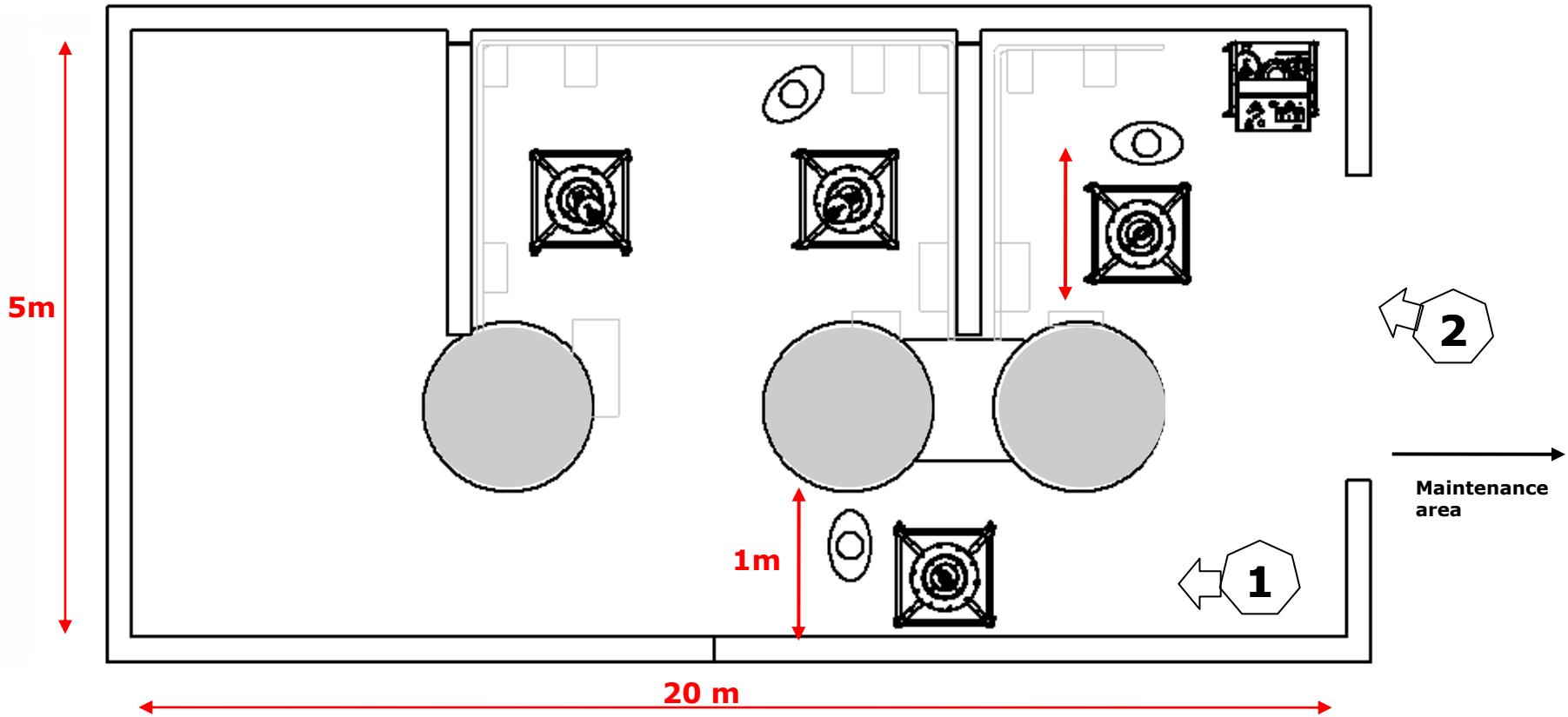
r-HSA production suite

PROXCYS
Downstream Biosystems

Suite IV. Polishing

Suite II & III. CS1208
2x75 liter 2300 L/Hr

Suite I. CS1214
165 liter 5000 L/Hr

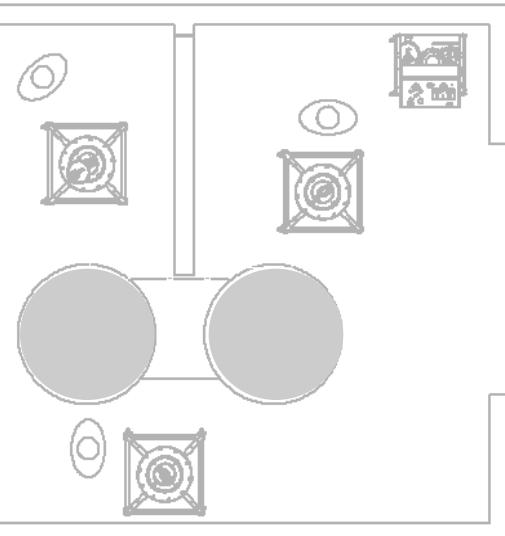




Novozymes Recombumim®

r-HSA production suite

PROXCYS
Downstream Biosystems



**165 Liter
Height 2.1 m
~400 kg**



**12 cm Bed height
Frame 75x75 cm
1000-7000 L/hr**





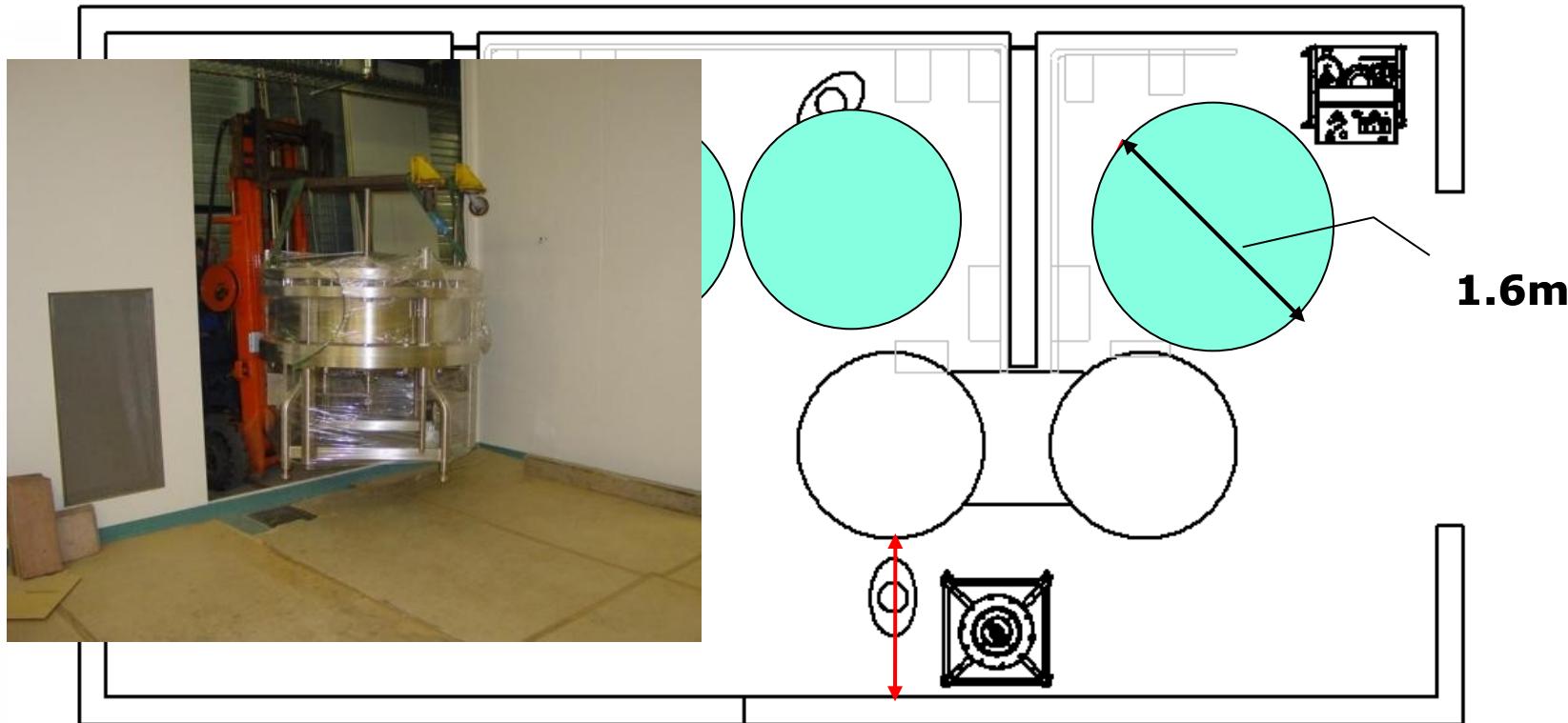
Novozymes Recombumim®

r-HSA production suite



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Downstream Biosystems

Use of Axial columns would be impossible

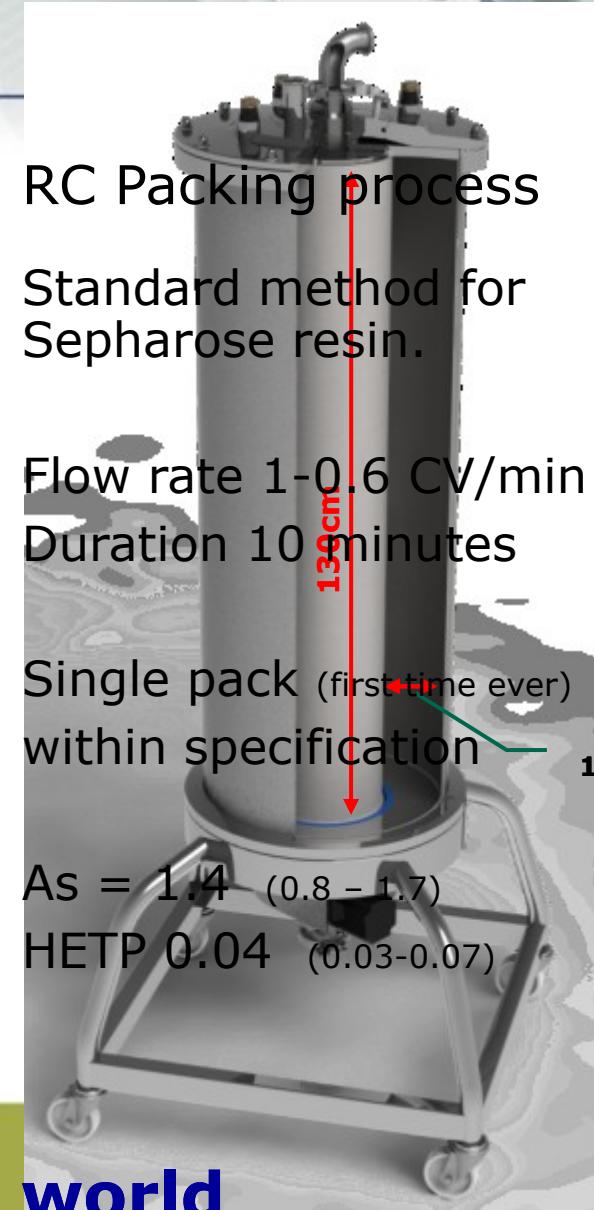
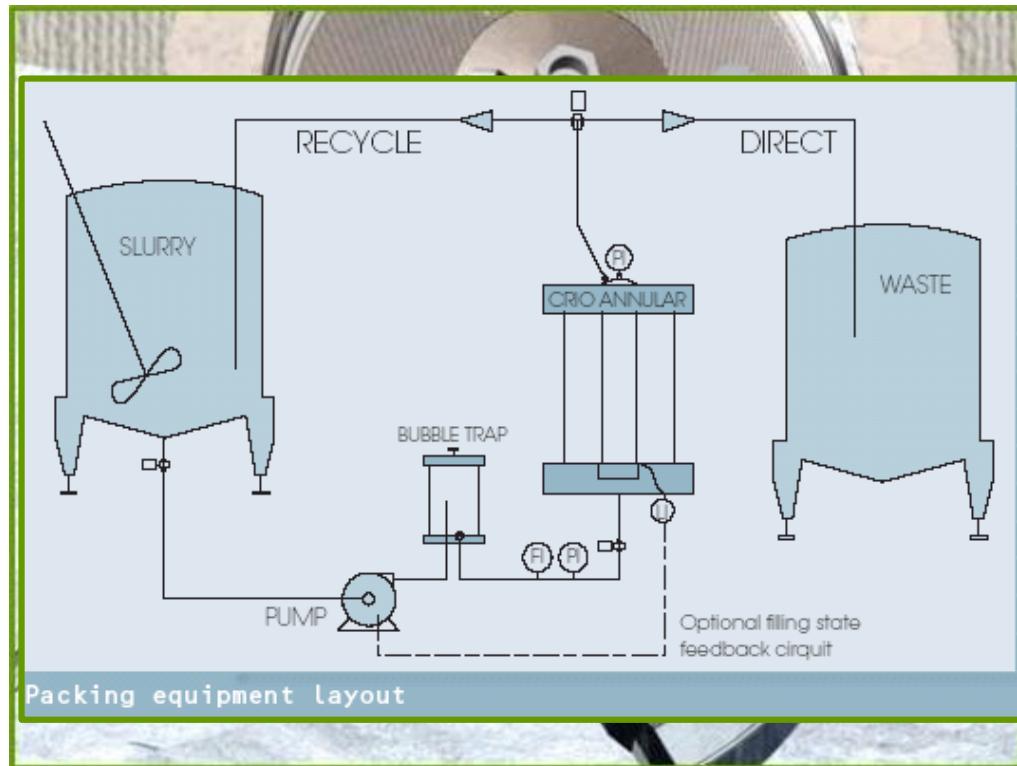




RC Packing

Radial Compressed

Radial Compression® packing results in homogenous packing



Tallest RFC in the world

Application examples



Application examples Plasma



- o Blood-Plasmaprotein Fractionation
 - *Factor VIII, IX, AT, IgG*



Applications Plasma

Plasmaprotein Fractionators

- Baxter
 - Austria
 - Belgium
- Octapharma
 - Austria
 - France
 - Germany
 - Sweden
- CSL
 - Germany
 - Australia
- Novozymes (United Kingdom)
- Biotest Pharma (Germany)
- Finnish Red Cross (Finland)
- Kedrion (Italy)
- LFB (France)



**RFC 100 Liter
Total height 1.8 m
200 kg**

**12 cm Bed height
Footprint 60x60 cm
Flow > 4000 L/hr**



Applications Plasma

Plasmaprotein Fractionation

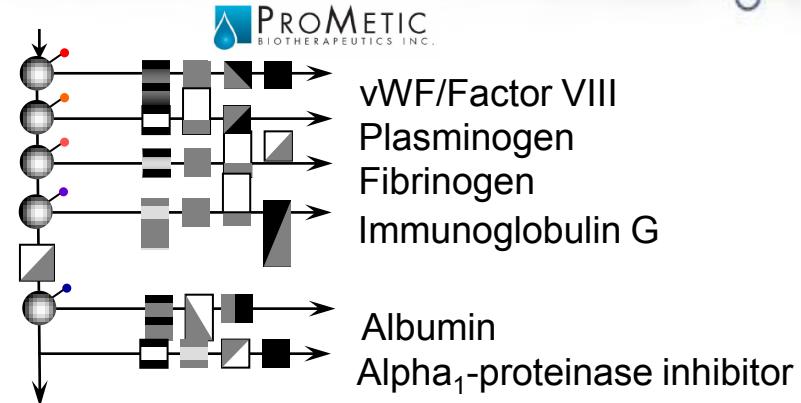
- Factor 8, 9 AT-III
- Gamma Globulin

Products in highly competitive market

- Safe
- High Throughput
- Lowest operating cost
- Minimal maintenance
- Cleaning efficiency $\geq 20\%$ resin lifetime

Campaigns

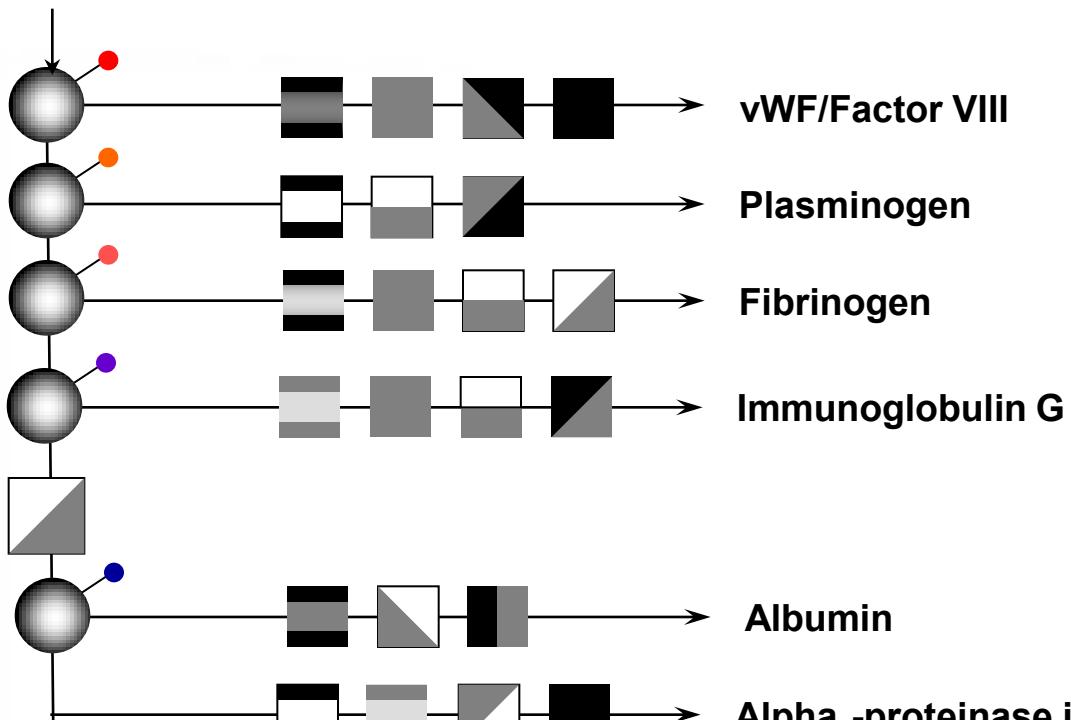
- Regional divided
- Clean-room use optimization
- Column storage “next door”



Applications Plasma Protein Purification System (PPPS)

Backbone

Plasma



Target Protein	Purity	%Yield
vWF/FVIII	6 IU/mg	60
Fibrinogen	0.74	80
Plasminogen	0.79	77
IgG	0.50	85
Albumin	0.94	85
A1PI Dia 43	0.25	90

Downstream processes
and
viral inactivation

... more and more scarce and expensive. Progress is warranted in the quality of plasma fractionation technologies. The yield of FVIII from source plasma is still only 5–10%, a loss that is difficult to accept in an era of high technology!

Application examples



Application examples Functional Food



- Functional Food
 - *Extracting Value from "waste"*





Applications

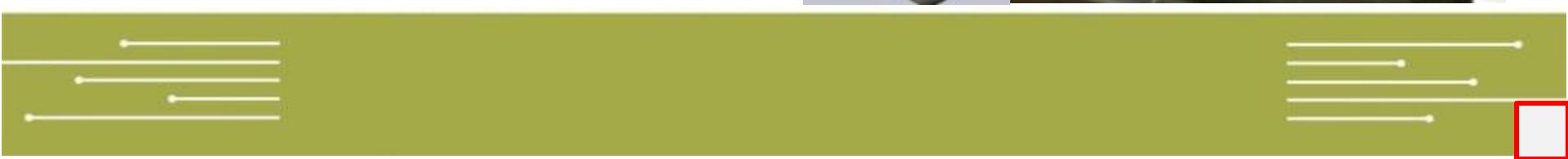
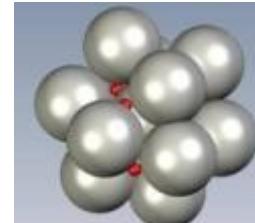
Waste valorization

Functional Food

Radial Chromatography

Functional food, pilot setup

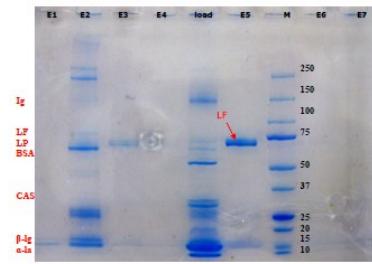
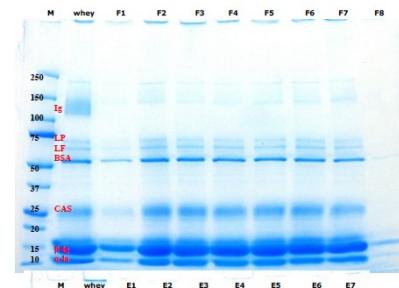
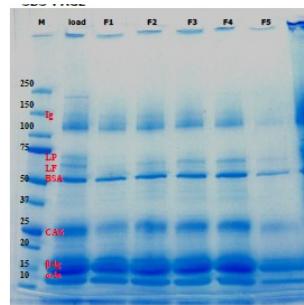
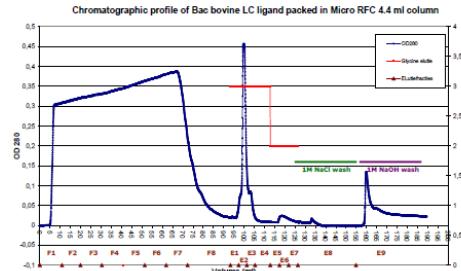
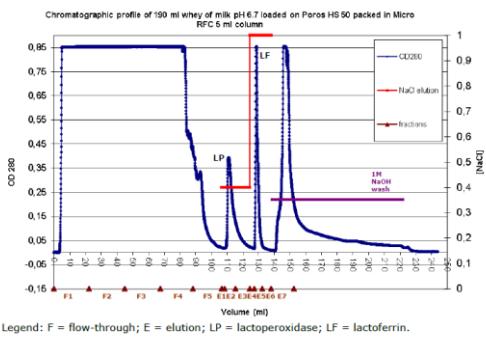
- Flow rate 4,000 l/hr = 200 Bed volumes per hour
- Big-Bead Cellthru, crude feed application
- Process step reduction
- Scaleup to >120.000 l hr
- Dirt tolerance, air tolerance





Applications Functional Food Whey protein isolation

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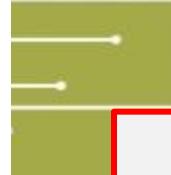
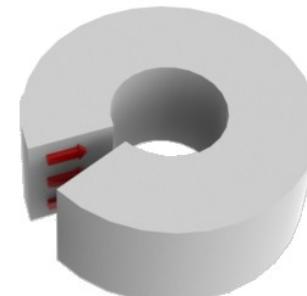


Legend: M = molecular weight marker; F = flow-through; E = Elution; load = whey of milk pH 6.7.

Small scale Development
Whey protein fractionation

Multiple resins screened
Generic to Affinity

Normal bead size
Processing velocities vary

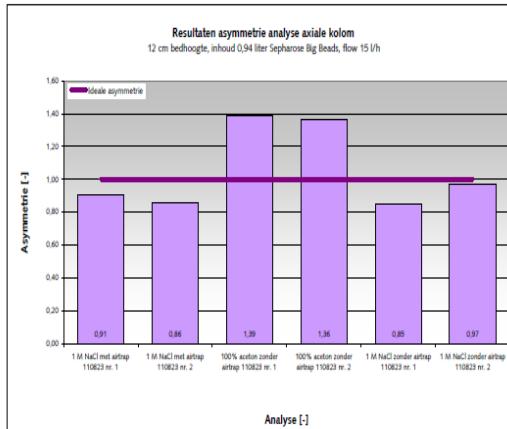
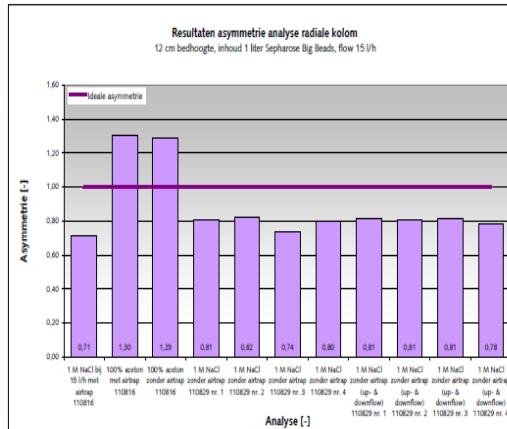
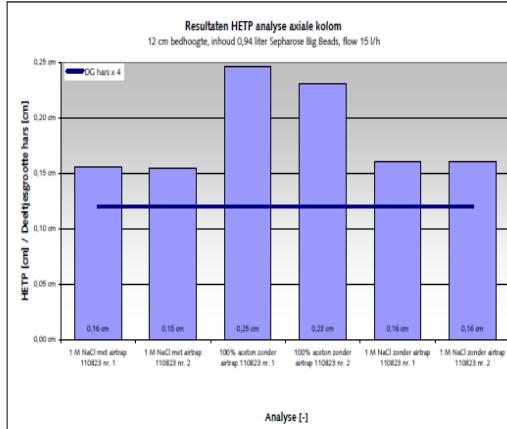
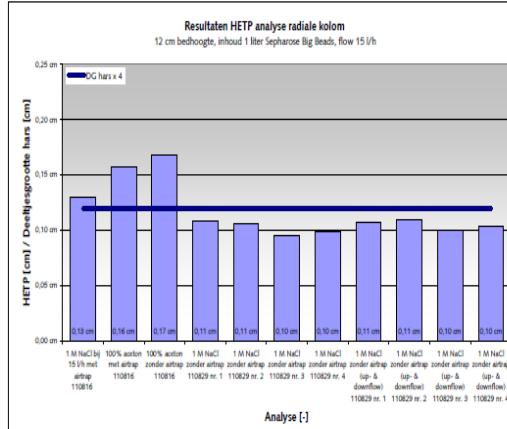




Applications

Functional Food

Whey fractionation

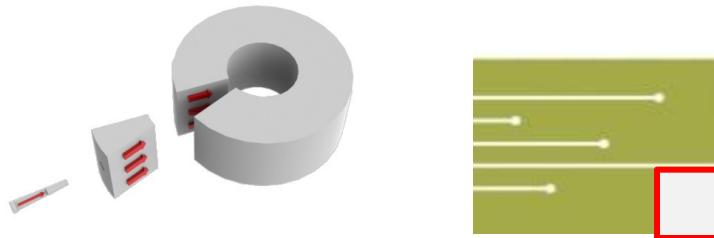


Scale up and comparison Whey protein fractionation

Top: HETP
Blue line is 4x bead diameter
Left 1L Radial Right 1L Axial
Bed height 12cm



Bottom: Asymmetry
Purple line is 1.0
Left 1L Radial
Pad height 12cm

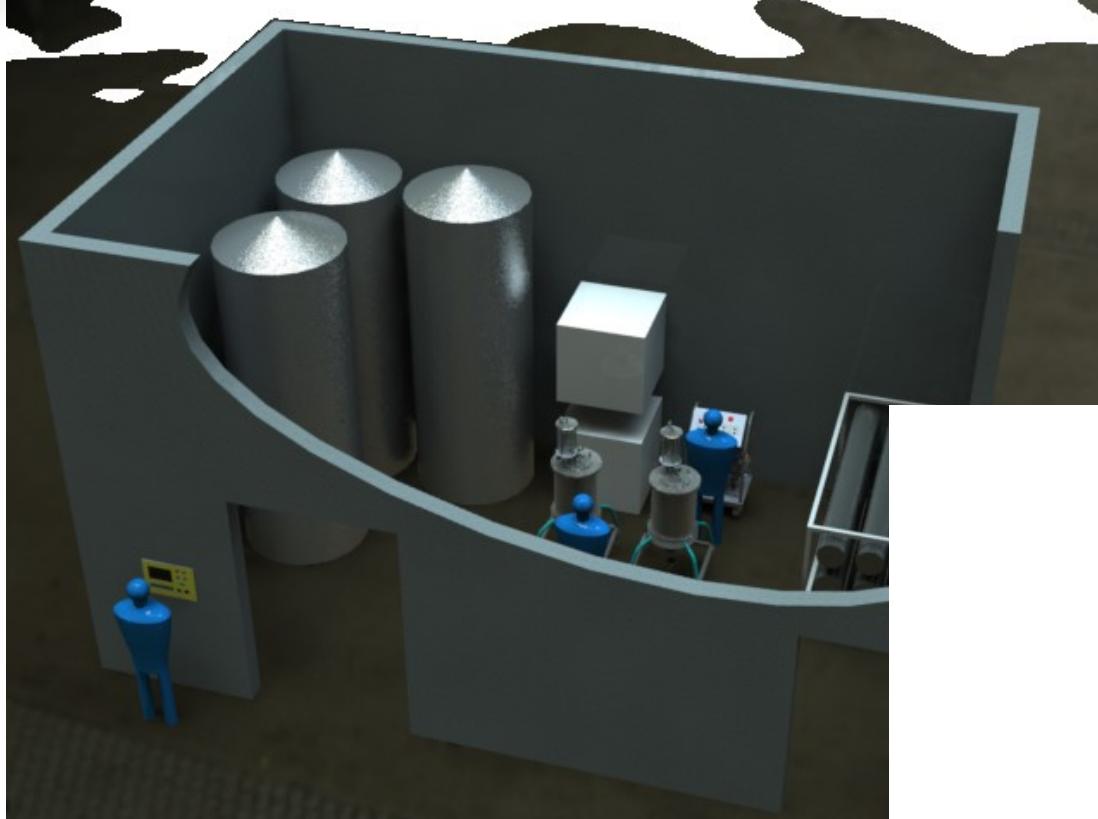




Applications

Functional Food Whey fractionation

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Downstream Biosystems



Scale up modelling in
Whey fractionation plant



Application examples



Application examples Environmental



- o Environmental
 - Removal of Arsenic from oil-drilling fluid





Applications Environmental Oilsludge decontamination



Heavy metals (As) from wastewater

Packing Agarose 200 µm

- Porous Big-Bead



Extreme high flow rate

- 2-5 CV/min = 300 bed volumes/hr



Low pressure (no resistance)

- 1-2 Bar

Cartridge format

- Easy disposal

Robust



Radial Flow Chromatography

Application Conclusions



Application Summary

- Wide variety of application
- Big bead capture, surprising results
- Throughput
- Mobility
- Economics advantage



Principle of Operation



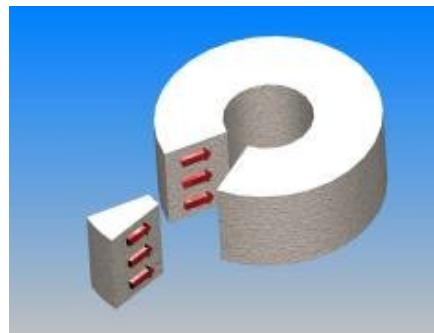
What is Radial Chromatography?



Radial Flow Chromatography (RFC) is an efficient, low pressure, high velocity technology for bio-molecule fractionation.

The compact column geometry, makes RFC very suitable for high throughput adsorptive separation in process or pilot scale.

The SUPR and Micro-radial launch in 2011 will reduce the threshold of application with a line of lab columns featuring the performance and speed of the process columns in lab format.

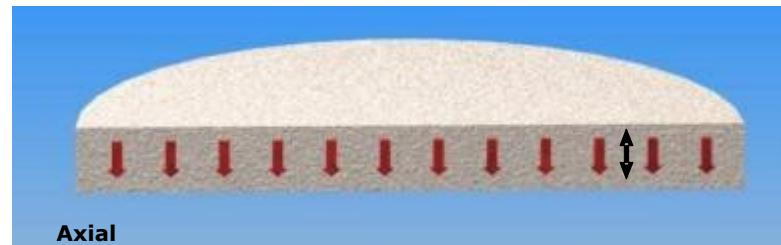


Radial Flow Chromatography

Principle of operation



Axial Chromatography

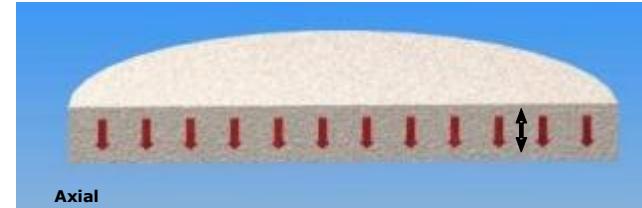
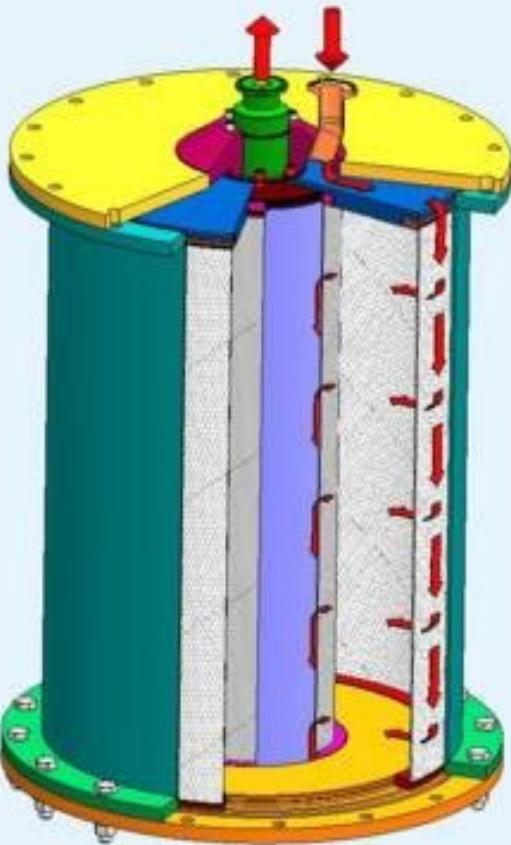


In Axial columns the liquid flow
Is vertical through the bed
(up or down)

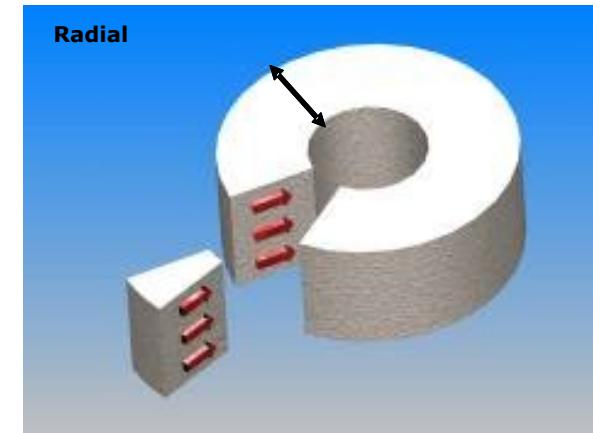
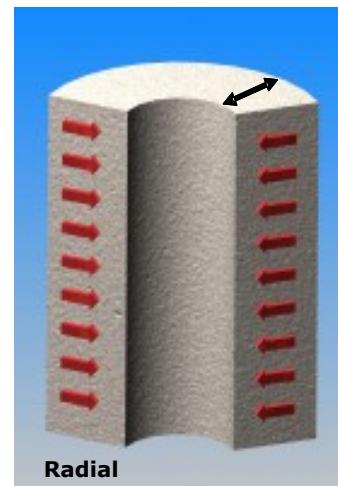
Radial Flow Chromatography

Principle of operation

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Downstream Biosystems



Radial Chromatography

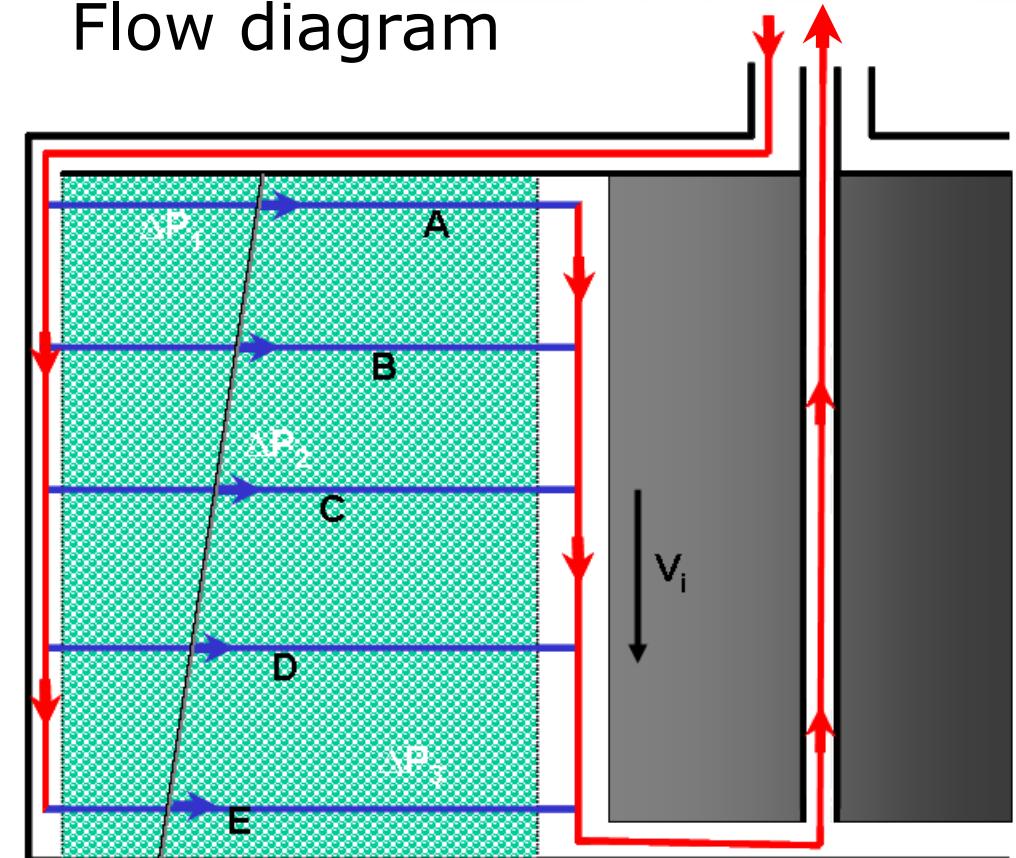


Radial Flow Chromatography

Principle of operation

- Identical path length
($A=B=C=D=E$)
- Identical Pressure drop
($\Delta P_1 = \Delta P_2 = \Delta P_3$)
- Identical velocity
($V_o = V_i$) ($R_{V0} = R_{Vi}$)
- Symmetry
- Safe against draining
- Excellent Hydrodynamics
- Reduced pressure drop

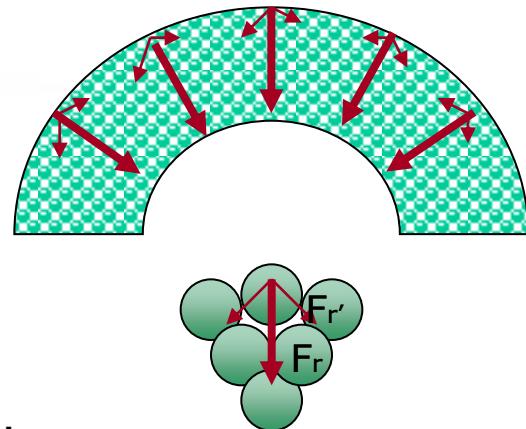
Flow diagram



Radial Flow Chromatography

Principle of operation

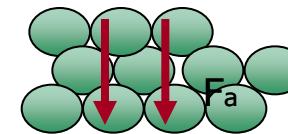
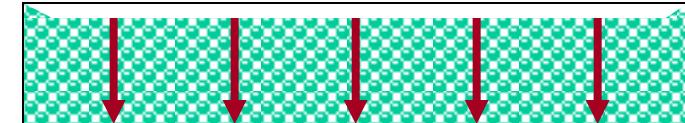
Pressure and Bed stability



Radial:

Normal (radial) forces partially absorbed tangentially (sideways)

- ⇒ Reduced directional compression
- ⇒ Reduced deformation (soft gel)
- ⇒ Reduced flow resistance



Axial:

Compression forces are in-line to the direction of the flow

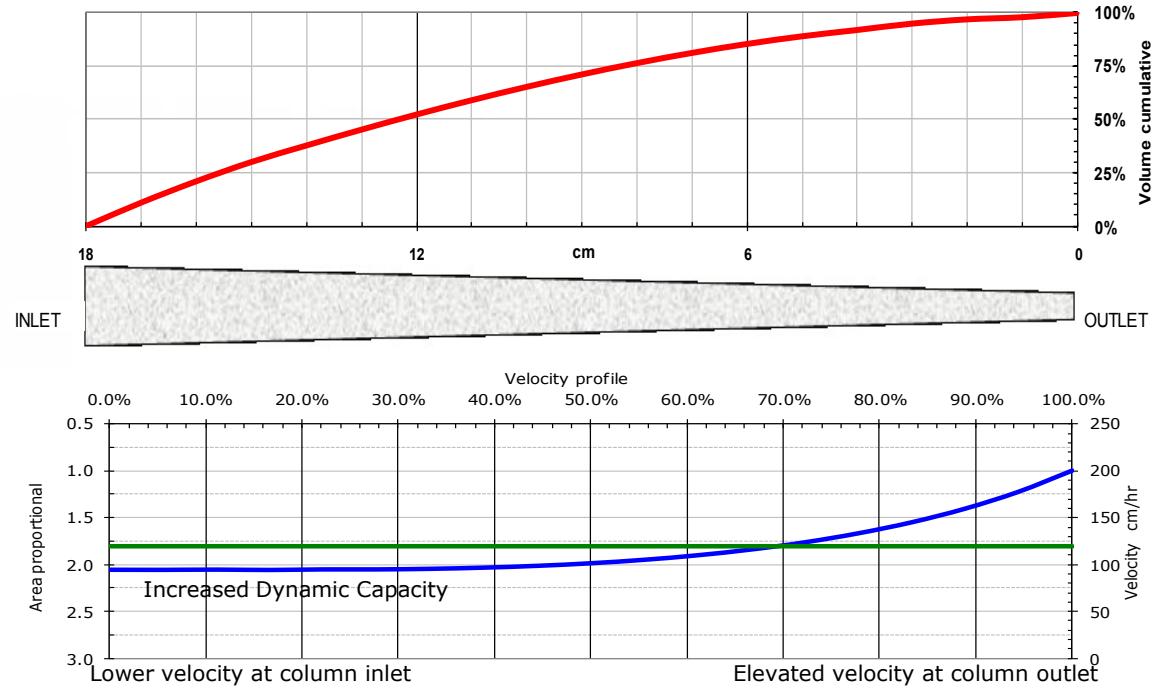
- ⇒ Increased directional compression
- ⇒ Increased deformation (soft gel)
- ⇒ increased flow resistance

Radial Flow Chromatography

Principle of operation



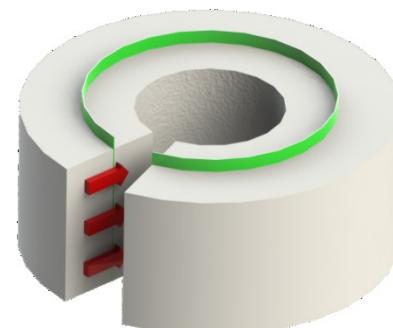
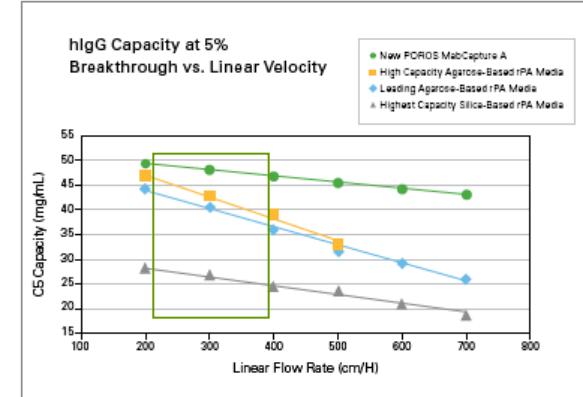
Volume distribution in Radial columns



Equation for calculation of average velocity in radial columns

 u = average velocity F = volumetric flow (cm^3/s) r_1 = inner frit radius r_2 = outer frit radius L = cylinder height c = capacity correction

$$\bar{u} = \frac{F}{2 \cdot \pi \cdot L} \frac{\ln \frac{r_2}{r_1}}{r_2 - r_1} C$$



Reference area =
~0.8 outer area

R. Hahn and A. Jungbauer, J. Biochem. Biophys. Methods 70, 87–94 (2007)x

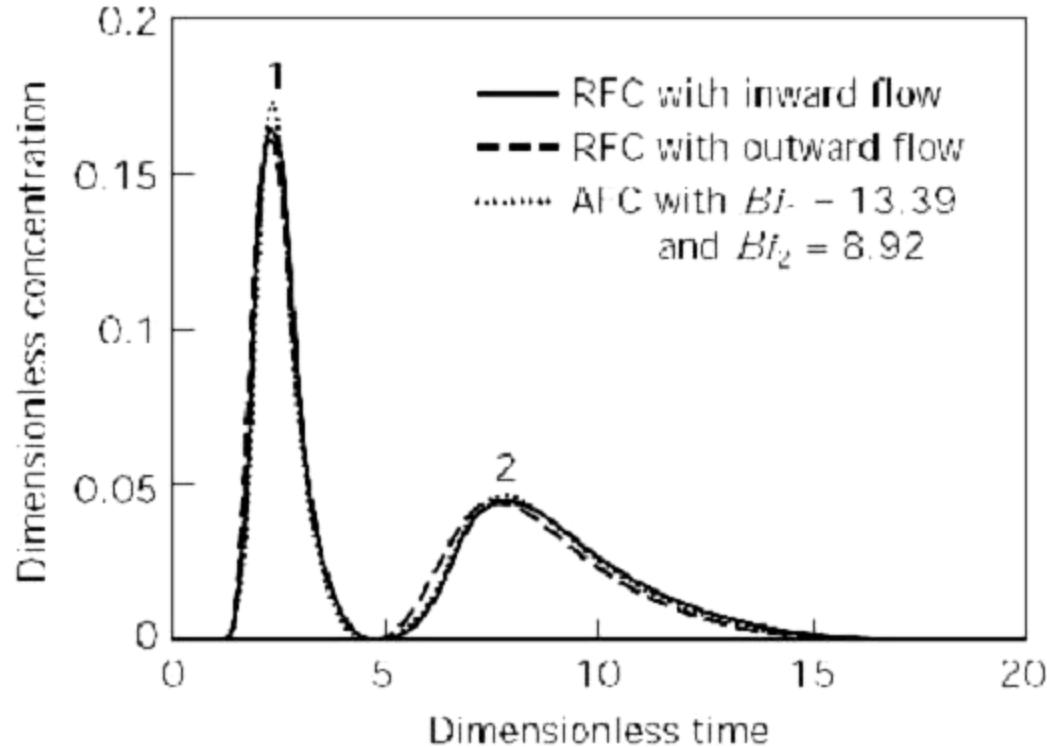
Radial Flow Chromatography



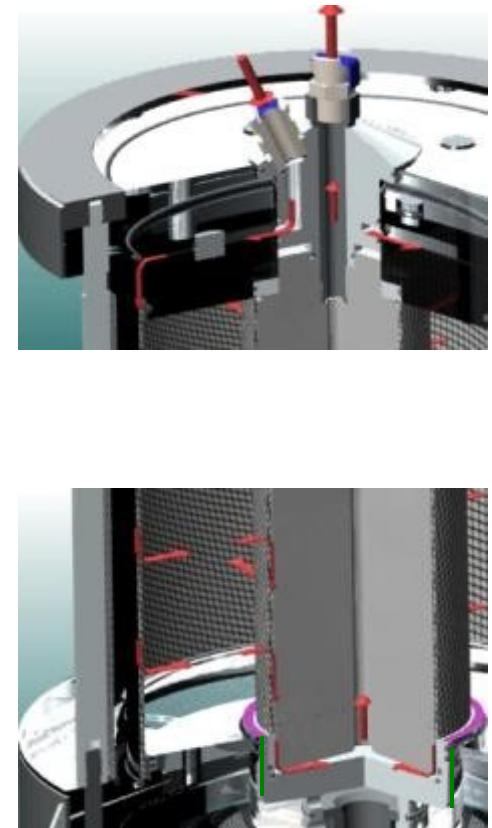
Principle of operation

Forward Reverse flow

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Gu et al.
Tharakan and Belizaire



T. Gu, G.-J. Tsai, and G.T. Tsao, in A Fiechter ed., *Adv.Bioch.Eng./Biot.* 1993, pp.73-95.
 T. Gu, G.-J. Tsai, and G.T. Tsao, *Chem. Eng. Sci.* 46, 1279-1288 (1991).
 J.P. Tharakan and M. Belizaire, *J. of Liq. Chromatogr.* 18, 39-49 (1995).

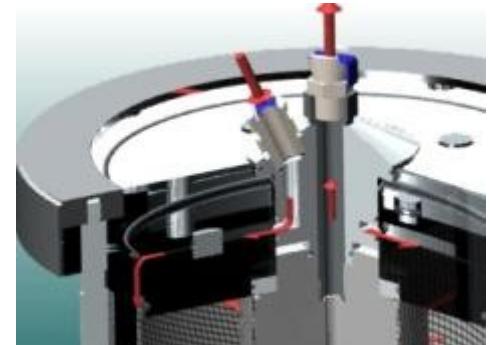
Radial Flow Chromatography

HP-RFC Conclusions



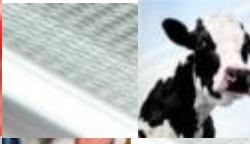
HP-RFC Summary

- Faster chromatography (lower ΔP)
- Compact high performance columns
- Wide variety of applications
- Compatible with all resins
- Linear scaleable
- Easy process transfer axial to radial using LV



Radial Compressed Packing



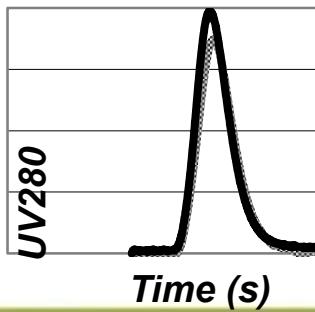
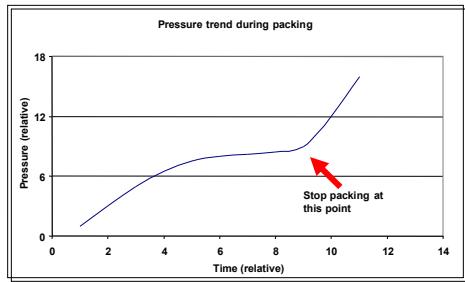


RC Packing

Radially Compressed

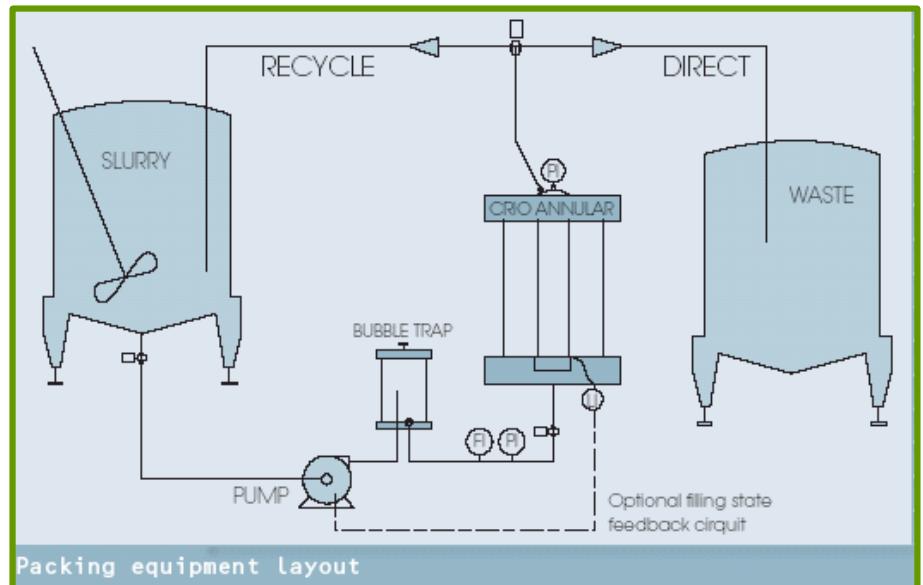
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Downstream Biosystems

Radially Compressed[©] packing results
in homogenous packing



RC Packing process

Direct or Recycled
Flow rate 0.5-2 CV/min
Duration 5-20 minutes



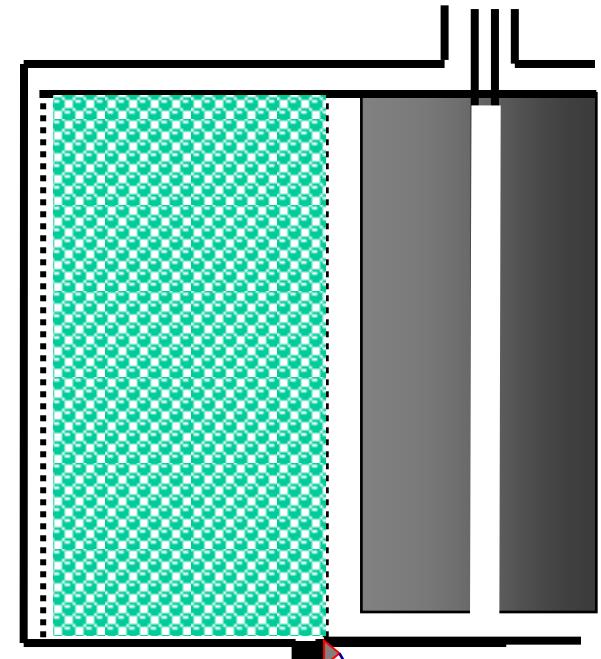
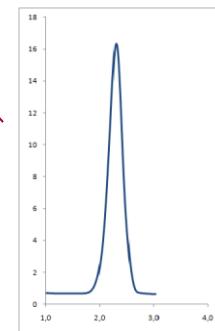
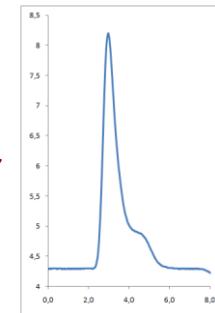
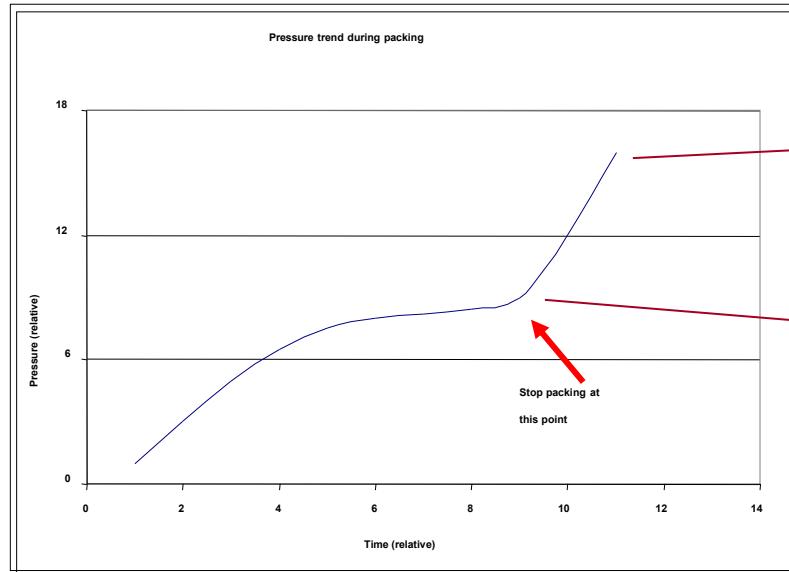
RC Packing

Radial Compressed



Dynamic Radial Compression® packing results in homogenous packing

Average efficiency of 2-4x bead size with any resin at starting from 3 cm bed height.



Patented

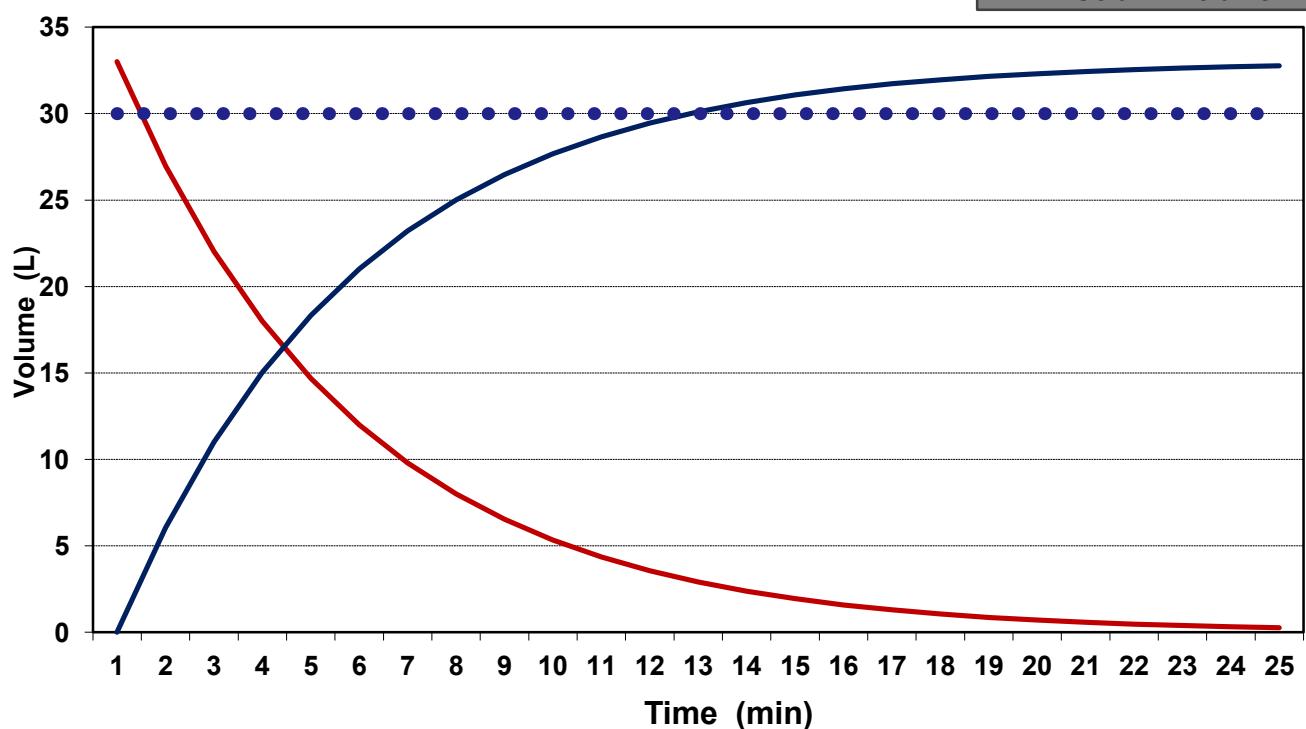
Packing profile (Recycling)

Effect of resin quantity and packing velocity

PROXCYS

Downstream Biosystems

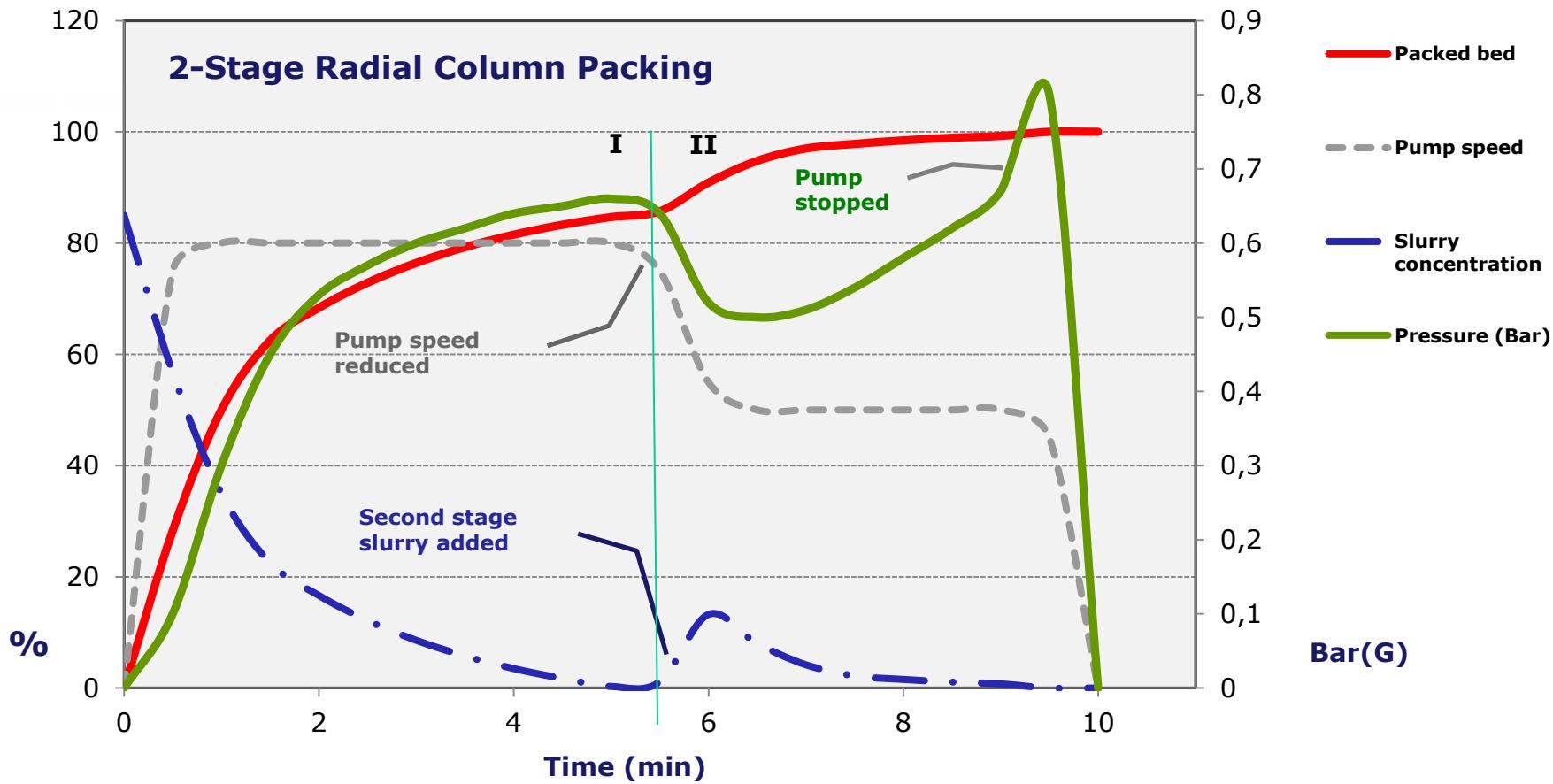
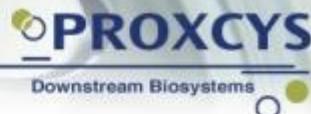
RC Packing Process Modulation



Longer duration of packing results in denser packed bed

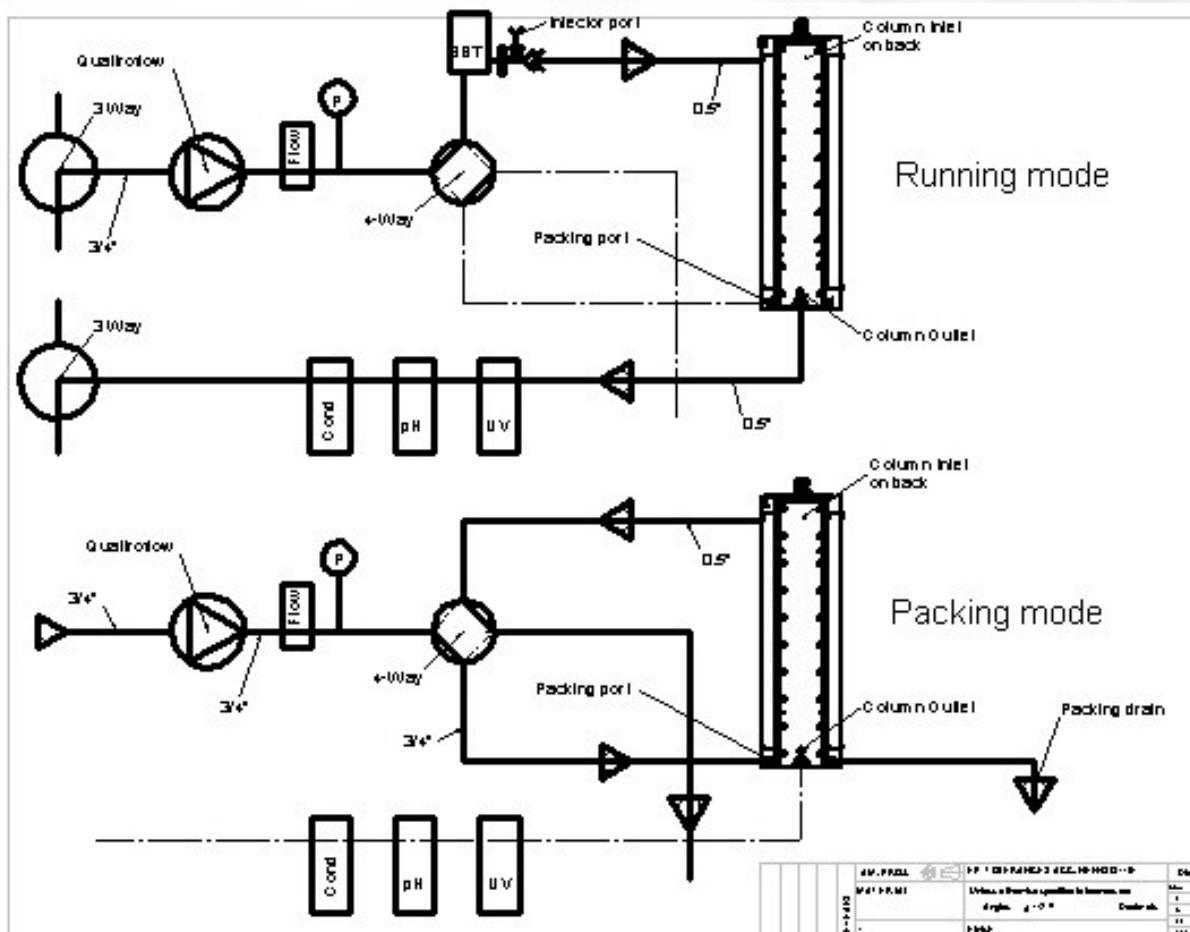
RC Packing

Radial Compressed 2-Stage recycling



Reduction of wasted resin (zero excess).

Last container only opened when 100% needed.

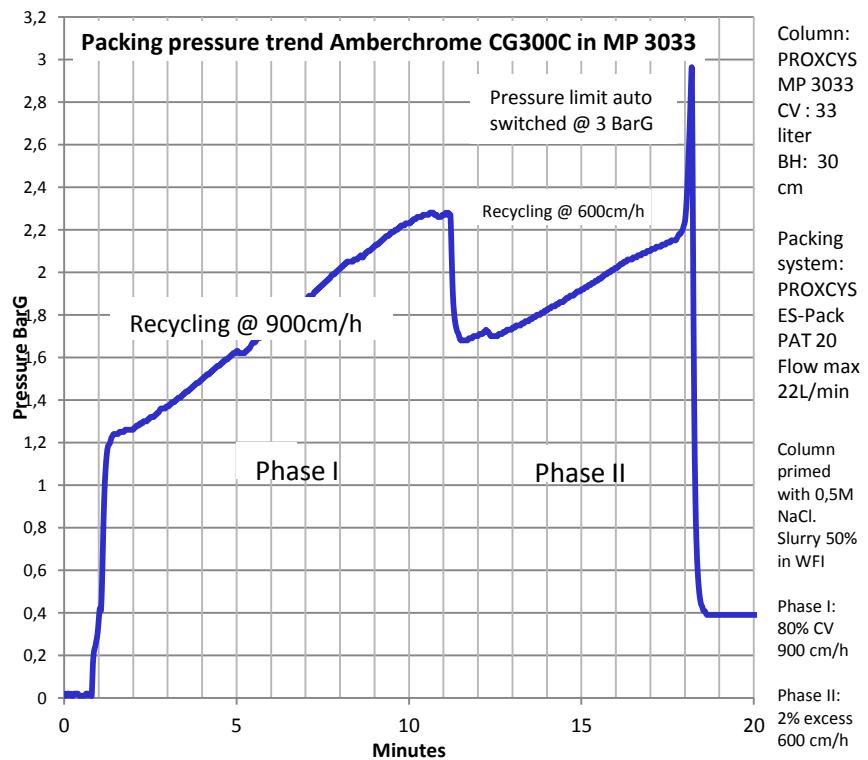
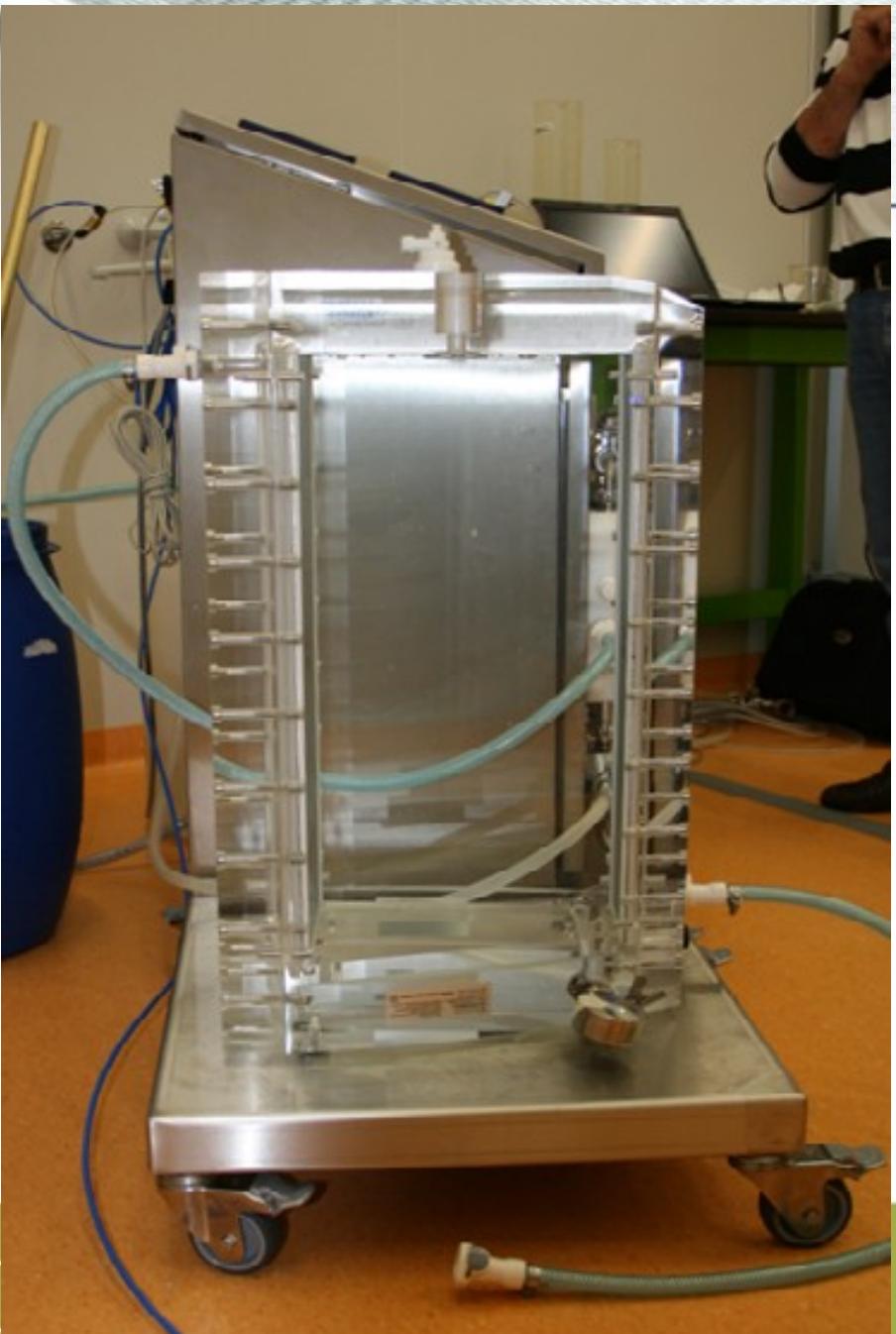


4M-PX001	4M-PX002	4M-PX003	4M-PX004	4M-PX005
4M-PX001	4M-PX002	4M-PX003	4M-PX004	4M-PX005
Unless a specific quantity is known, use	1	1	1	1
Angle: < 0°	✓	✓	✓	✓
Order: ✓	✓	✓	✓	✓
Flow:	1	1	1	1

RC Packing

Radial Compressed 2-Stage recycling



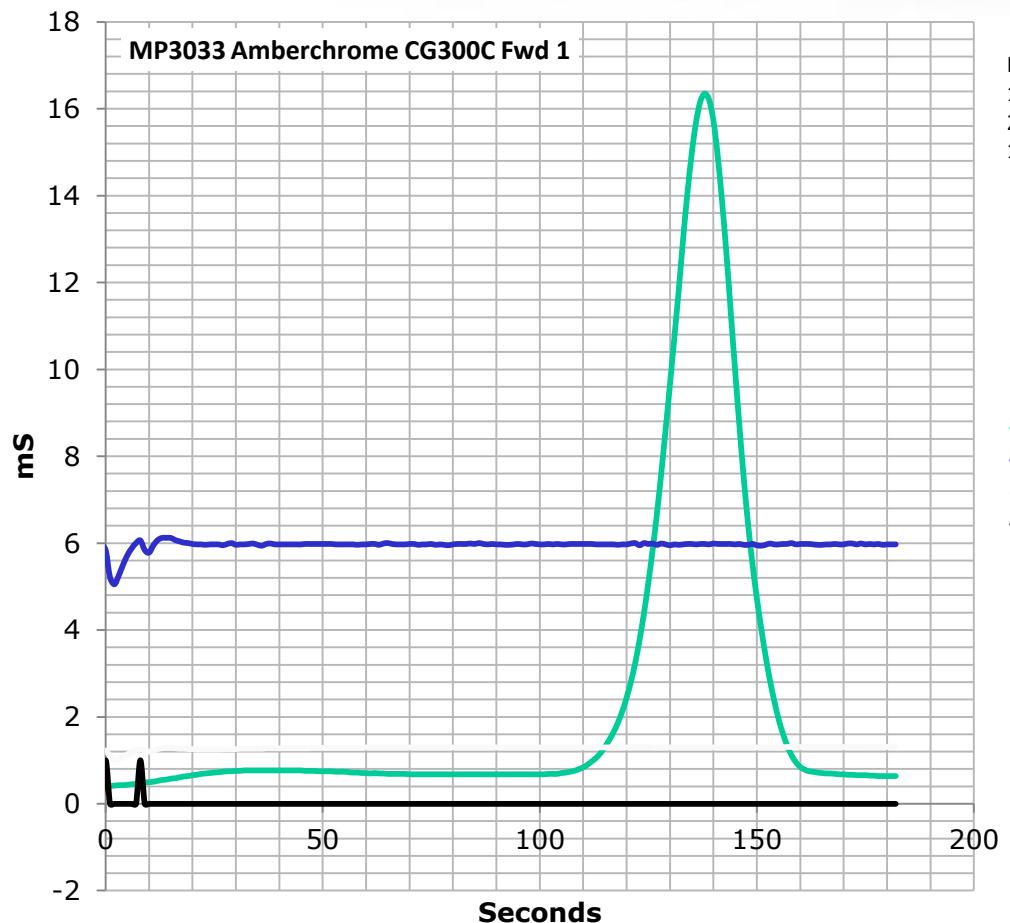


RC Packing

Radial Compressed 2-Stage recycling

PROXCYS

Downstream Biosystems

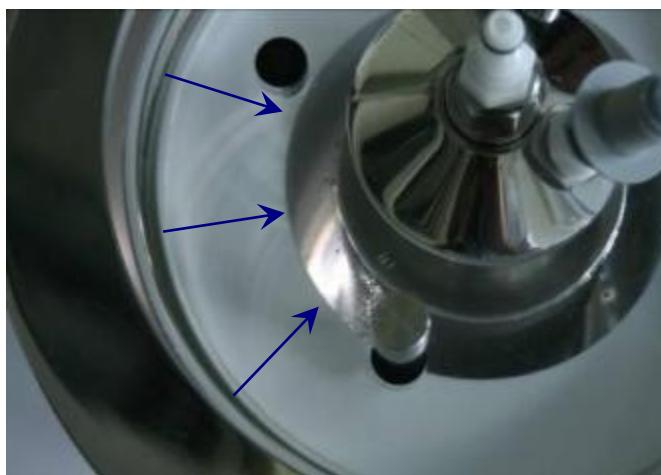


Packing
14 Apr.
2011
16:40

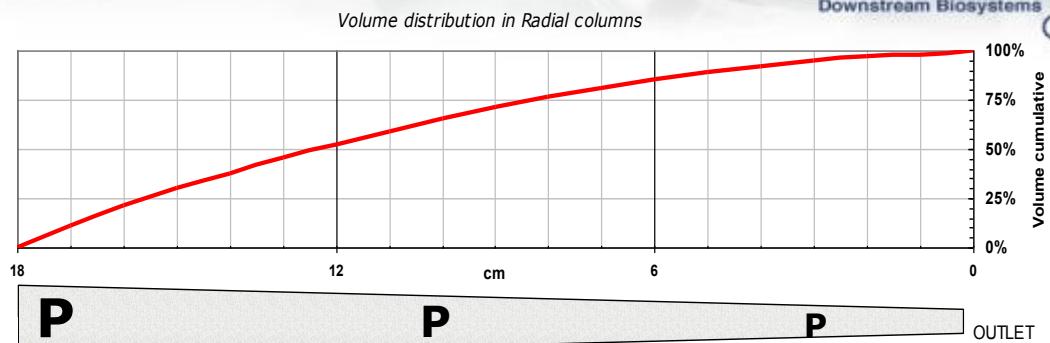


RC Packing

Radially Compressed



INLET



~Linear packing progression

Constant flow packing:

local bead pressure on bead reduces,
proportional to progression.

Packing density ~ more constant throughout
packed bed.

Bernoulli's law

$$\frac{1}{2}\rho v^2 + \rho gh + p = \text{constant}$$

$$p \times v = \text{constant}$$

*Increasing velocity will result in
decreasing pressure on the bead.*

Result: even silica can be packed to bed
compression of ~30%

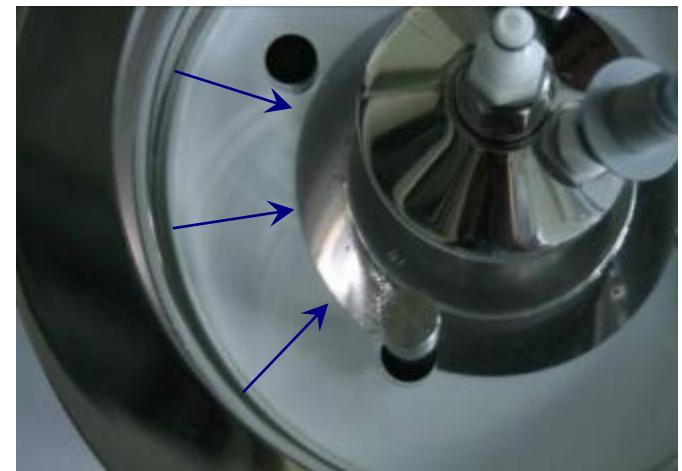
Radial Flow Chromatography

Annular packing Conclusions



Annular packing Summary

- Easy handling and set-up
- Fast packing
- Reproducible
- Compatible with all resins
- Optimized compression
- No loss of valuable resin
- Packing with **zero** excess possible





PROXCYS
Downstream Biosystems

Dynamic capacity

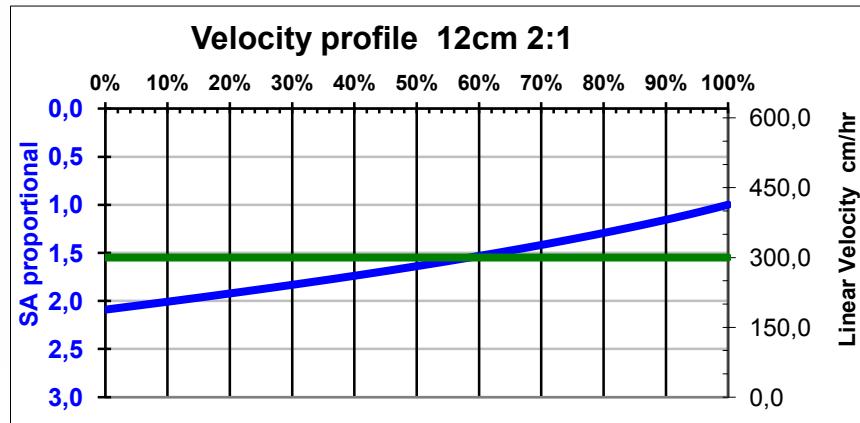
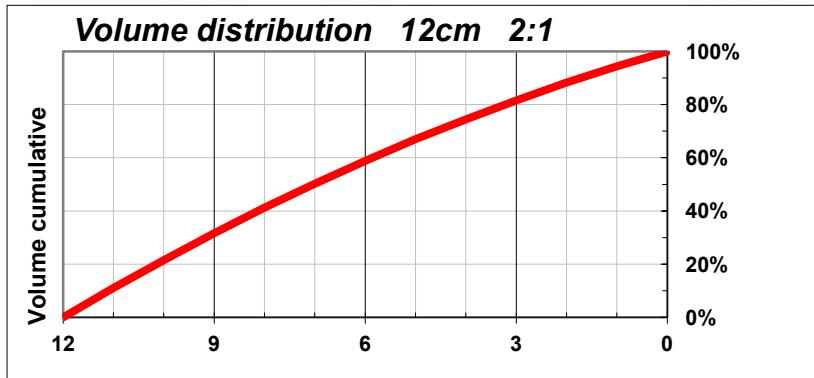




Dynamic capacity

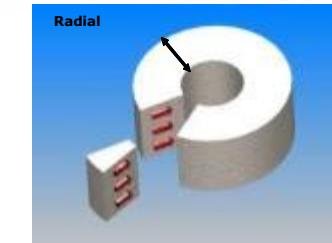
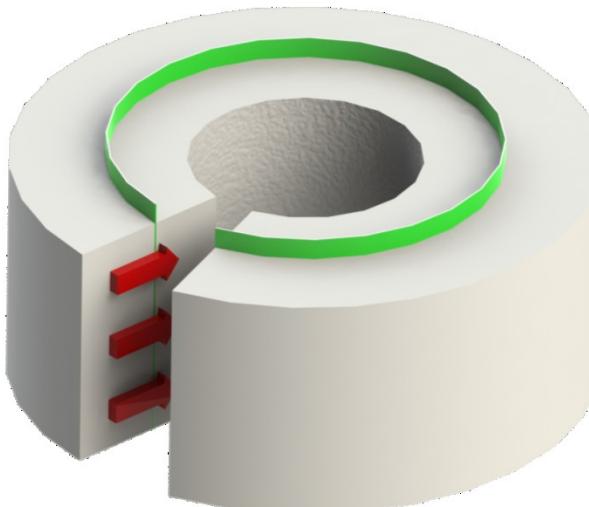
Protein A

PROXCYS
Downstream Biosystems



Resin volume shifts predominantly to circumference with increasing I/O ratio

I/O 2:1 is standard



Reference area =
0.8 outer area

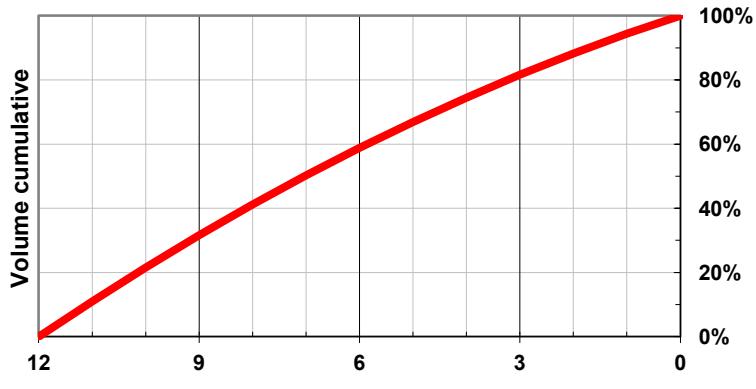


Dynamic capacity

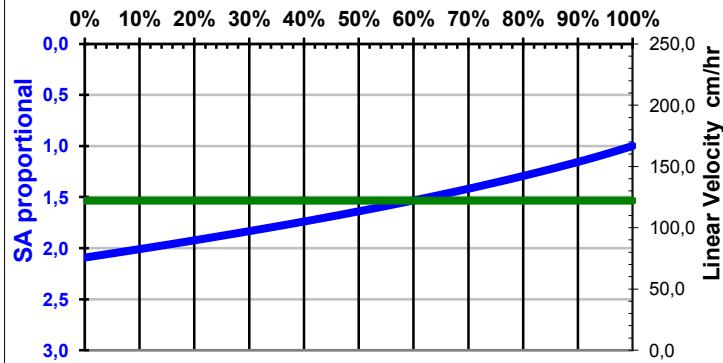
Effect of I/O ratio

PROXCYS
Downstream Biosystems

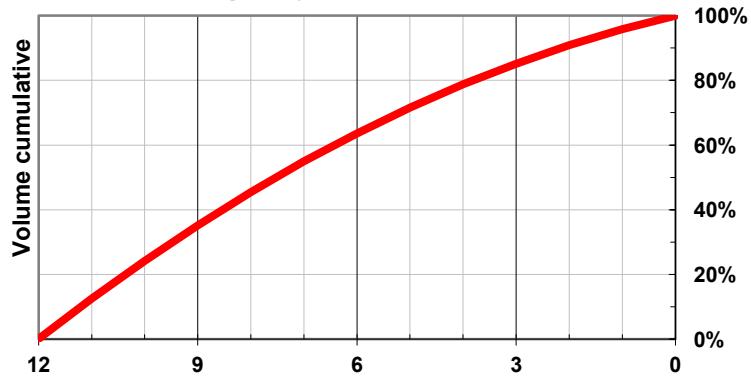
Volume & Capacity distribution 12cm 2:1



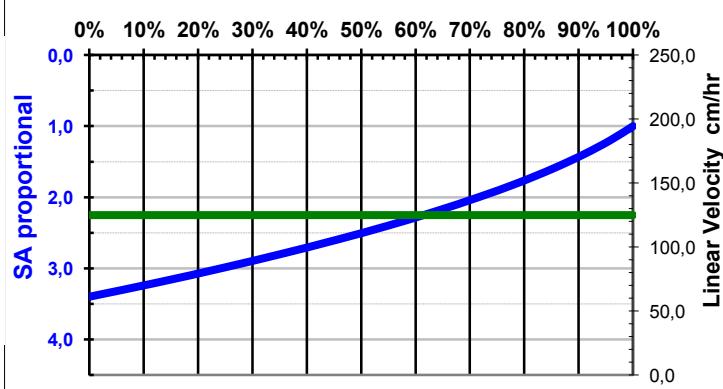
Velocity profile 12cm 2:1



Volume & Capacity distribution 12cm 3.4:1

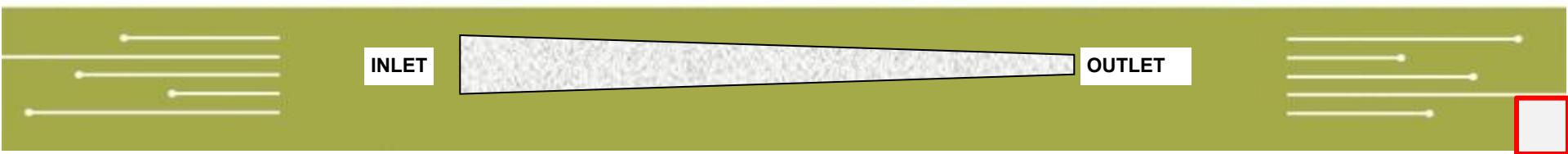


Velocity profile 12cm 3,4:1



INLET

OUTLET

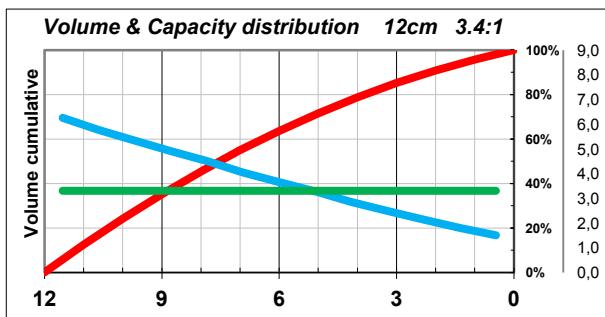
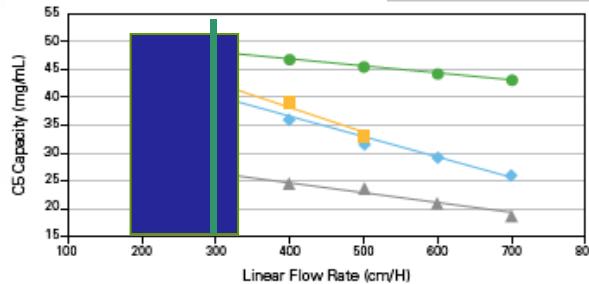




Dynamic capacity

Effect of I/O ratio

PROXCYS
Downstream Biosystems



1	10	10	10	10	10	10	10	10	10	10	1
	10	20	30	40	50	60	70	80	90	100	%vol
	400	800	1200	1600	2000	2400	2800	3200	3600	4000	g/cm
	10	20	30	40	50	60	70	80	90	100	CAP

324	13.8	14.2	12.8	11.6	10.2	9.8	982	872	7.8	572	%
	13.8	29.8	47.8	58.2	68.4	73.8	87.8	88.6	94.8	100	%vol
	349	1419	2609	2303	2949	2903	3062	3878	4865	4120	g/cm
	18	38	49	51	61	70	79	84	99	100	CAP



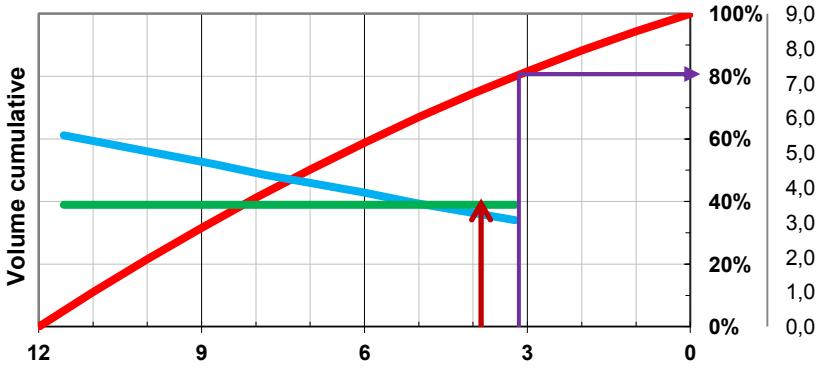


Dynamic capacity

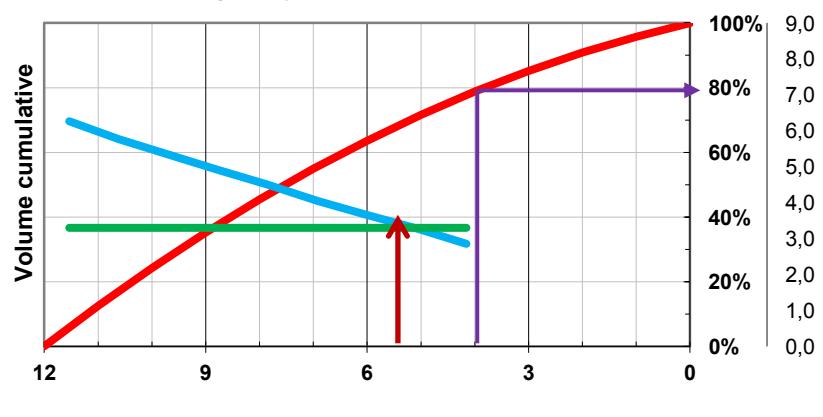
Saturation 80% Capacity gain

PROXCYS
Downstream Biosystems

Volume & Capacity distribution 12cm 2:1



Volume & Capacity distribution 12cm 3.4:1



2	1									
13.2	12.4	11.6	11	10.2	9.6	9.2	8.2	7.6	7	%
13.2	25.6	37.2	48.2	58.4	68	77.2	85.4	93	100	%vol
594	1140	1639	2101	2519	2903	3262	3574	3855	4107	g/cm
14	28	40	51	61	71	79	87	94	100	CAP

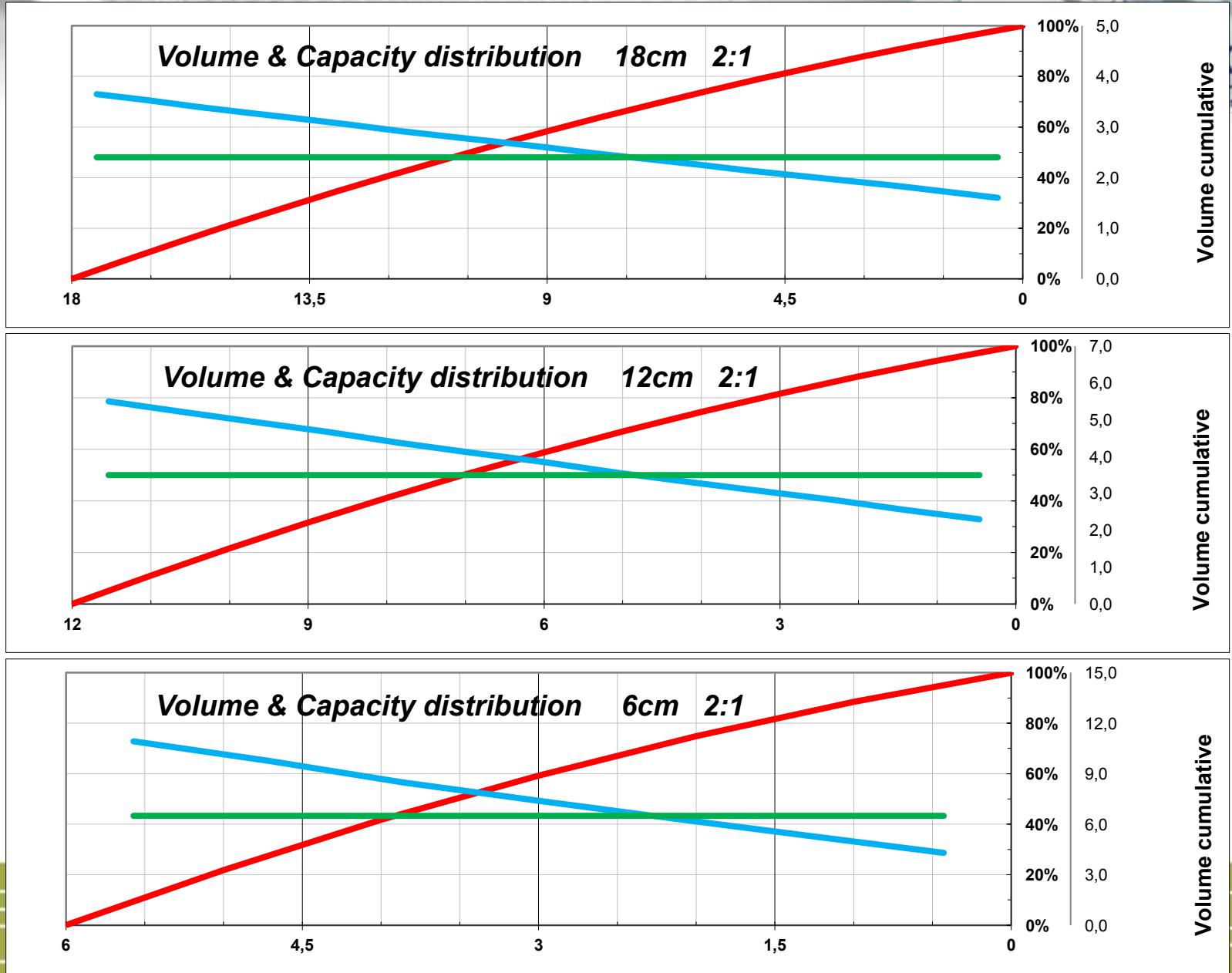
3.4	1									
15.6	14.2	12.8	11.6	10.2	9.2	8	7	6.2	5.2	%
15.6	29.8	42.6	54.2	64.4	73.6	81.6	88.6	94.8	100	%vol
749	1423	2005	2515	2943	3303	3615	3878	4101	4120	g/cm
18	35	49	61	71	80	88	94	99	100	CAP

INLET

OUTLET

Dynamic capacity

Effect of bed height





Dynamic capacity

Summary

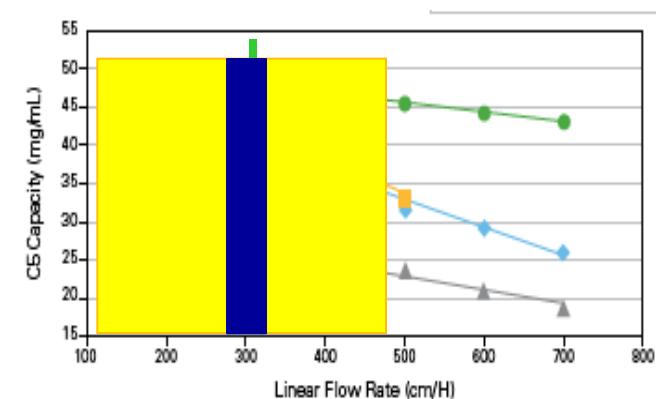
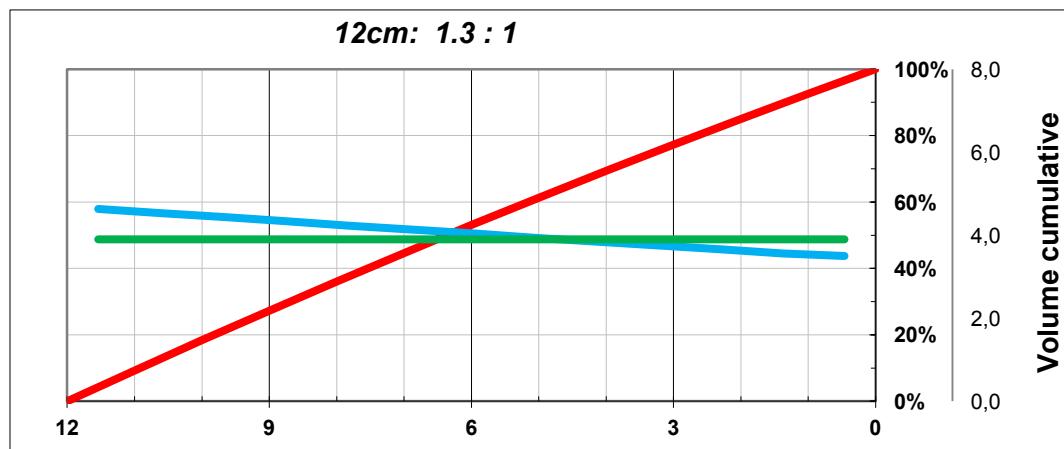
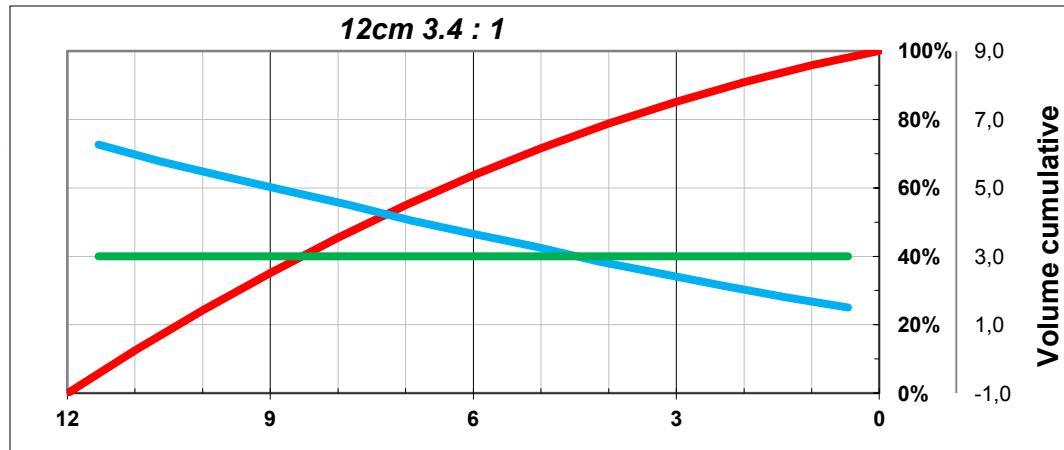
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Summary:

Breakthrough capacity will not reveal capacity advantage

Applicable within full linear range of dynamic capacity

Stronger benefit when dynamic capacity slope falls stronger





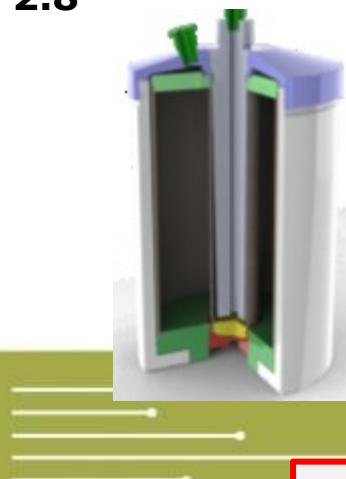
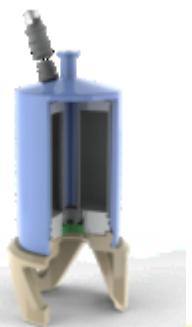
Disposable Technology

Velocity effects in radial flow



Type	Bed height (cm)	Minimum volume (L)	Maximum volume (L)	Footprint Ø (cm)	I/O Ratio
SUPR 300	3	0.1	4	15	3.4
SUPR 500	5	1	12	23	3.4
SUPR 1000	10	5	100	40	3.0
SUPR 2000	20	25	375	70	2.8

Footprint reduction results in increasing I/O-ratio
Understanding the effect



Pilot & Process columns I/O av. 2:1

Radial Flow Chromatography

HP-RFC



HP-RFC Summary

- Faster chromatography (lower ΔP)
- Easy handling and set-up
- Compact high performance columns
- Wide variety of applications
- Highest throughput per m^2
- Economics advantage





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Thank you for
your attention



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Dedication & Quality in Downstream Processing