



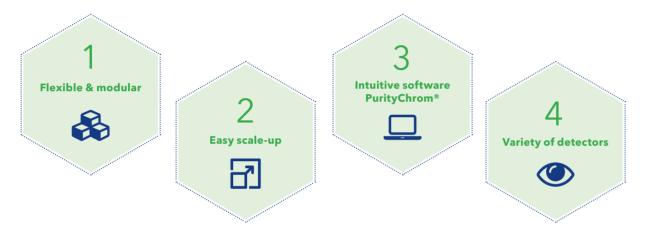
1-800-267-8103 • instrum@chromspec.com

## **KNAUER** protein purification: The flexible FPLC platform

#### AZURA® Bio purification systems

Complete solutions for FPLC on a minimum foot- The biocompatible/metal free FPLC is the perfect print: AZURA FPLC systems combine flexibility and reliability.

choice for your protein purification task.



Design your AZURA Bio purification system to your needs. Multiple functionalities such as automatic sample injection via autosampler, column switching, buffer and sample selection as well as fraction collection enable the user to automate the purification process.

A large range of different detectors make your target molecules visible. Different flow rates and compatibility to columns from all venders offer maximum flexibility. The intuitive software PurityChrom® combines all the advantages of a versatile purification software.

#### **Fast Protein Liquid Chromatography (FPLC)**

FPLC is a form of liquid chromatography to purify large biomolecules like proteins or DNA. External factors like high temperature, high pressure, extreme pH, or solvents can disturb the protein structure and are therefore avoided in FPLC. Besides.

the method uses column materials out of agarose or polymer material which are very sensitive against pressure fluctuations and air bubbles.

We designed our systems to meet your purification challenges!

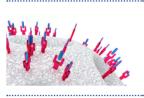
#### **AZURA®** Bio purification: You choose the method

**Size Exclusion** Chromatography (SEC)



Separate according to size. See page 18 for a specialized AZURA system for SEC.

**Affinity** Chromatography (AC)



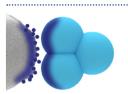
Specific binding of protein of interest. See page 19 for a specialized AZURA system for AC.

Ion-Exchange Chromatography (IEX)



Separation takes place according to the charge of the protein and gradient elution.

**Hydrophobic Interaction** Chromatography (HIC)



Separation is performed based on hydrophobic interaction and gradient elution.

#### Purification strategy: Often a sequence of different methods is used in purification.

Capture

Intermediate

**Polishina** 

Normally a combination of methods is used in protein purification.

- The "capture" step purifies the protein from the crude extract.
- The "intermediate" step removes further contamination.
- The aim of the final "polishing" step is to get rid of all remaining impurities in order to gain a highly purified product.

#### **Continuous FPLC purification for more productivity?**

Use AZURA SMB as your step to the future of biopurification and run your downstream process with the highest productivity and lowest costs. Our multi-column capture process (MCCP) saves your time as well as solvent and column costs.

For more information see page 26.





## **AZURA®** Bio Lab

From simple to complex, from Lab to Pilot scale: Design your AZURA® FPLC system according to your purification task!

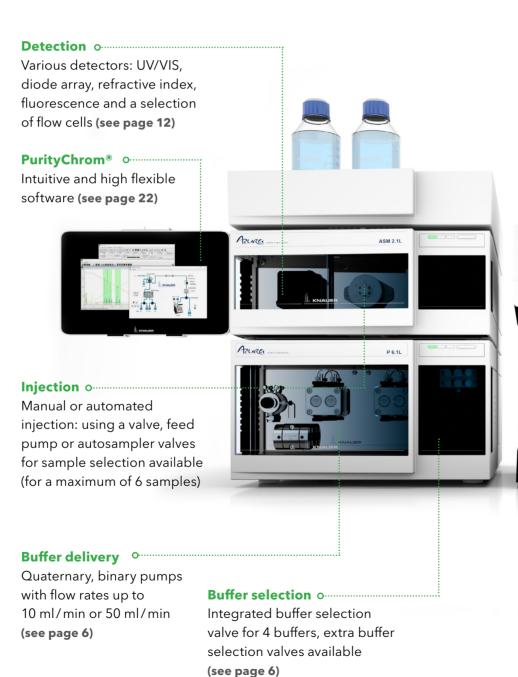
AZURA Bio Lab allows you to create FPLC systems ules and build-up the system yourself. Continue with highest independence. Just pick your mod-

flexibility with intuitive PurityChrom® software.

All common **FPLC** methods are supported.

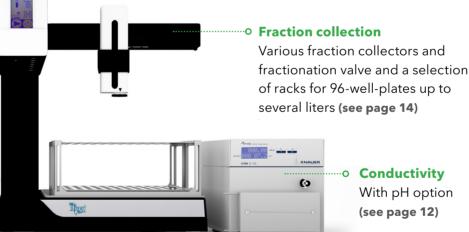
All columns are supported.

> **Cold-room** operation is supported.



pressure regulator fraction collector 0

**BUFFER SELECTION** SAMPLE COLUMN **DETECTION** FRACTION & DELIVERY INJECTION SELECTION COLLECTION **Column selection Safety features Automate your** purification valves (page 17) (page 11) (page 20)



#### **☐** Scale-up from Lab to Pilot

Choose the Pilot series if you want to increase your productivity even more. Upscale our Lab configuration with same flexibility, software PurityChrom® but minimal footprint. Just transfer and upscale your methods. Flow rates up to 1000 ml/min and loads up to several grams are possible. Find more information: www.knauer.net

**Configure your AZURA Bio system** Find all FPLC products on the

following pages.

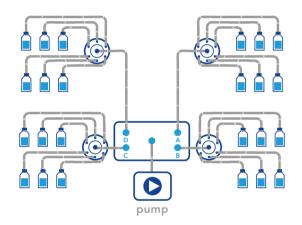
## **Buffer delivery**

Precise and reliable pumps covering a wide flow rate range, gradient and Buffer selection options.

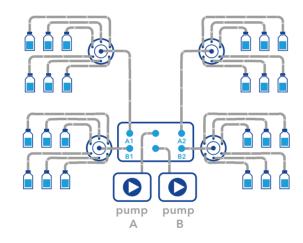
#### **Buffer selection**

Automated switching between buffers is important for method development, column cleaning and regeneration. The pump P 6.1L features a build-in  $2 \times 2$  buffer selection valve (A1, A2 and B1, B2) or  $4 \times 2$  buffer selection low gradient valve (A, B, C, D).

You can extend buffer selection with additional valves each for 6 buffers.



AZURA pump P 6.1L LPG - Quaternary gradient



AZURA pump P 6.1L HPG - Binary gradient

## **Compact pump** AZURA® Pump P 4.1S

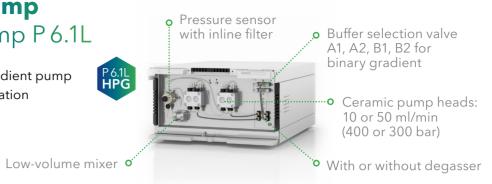
Isocratic pump with small footprint for dedicated applications or sample loading.



#### **Gradient Pump**

AZURA® pump P 6.1L

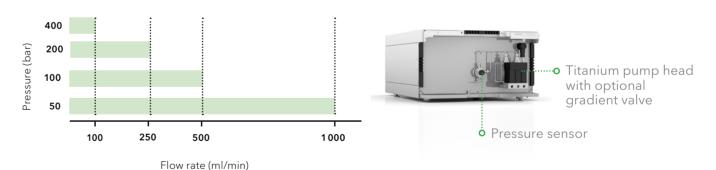
High-performance gradient pump optimized for low pulsation





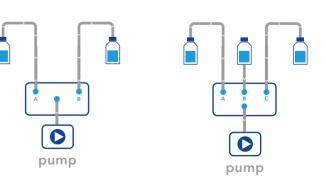
#### **Scale-up pump** AZURA Pump P 2.1L

Pumps for high flow rates



#### **Gradient options**

From binary to quaternary gradient, with additional P 2.1L pumps or cost-effective binary low pressure gradient ( $2 \times 1$  buffers, up to 800 ml/min) or ternary low pressure gradient ( $3 \times 1$  buffers, up to 220 ml/min).



Binary low-pressure gradient

Ternary low-pressure gradient

4

# **Binary or quaternary gradient?**

A quaternary low pressure gradient (LPG) module\* dynamically composes the buffer on the inlet-side or low pressure side of the pump head, by quickly switching the selection valve between the dif-

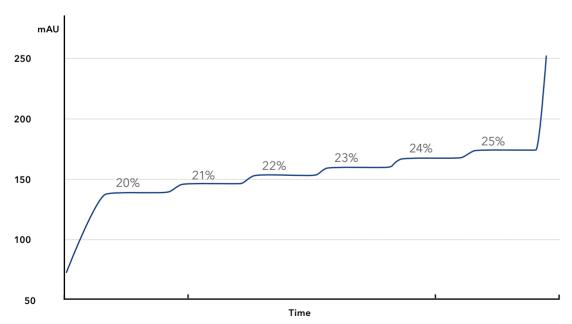
ferent channels. The buffer in a binary gradient (HPG) system is composed by combining the solvent flows of two isocratic pumps.

## Quaternary gradient

- Low investment costs
- Limited flow rate range
- Channel usable for sample injection
- Gradient accuracy absolutely sufficient for FPLC

## Binary gradient

- Less wear
- No flow rate limitation
- Sample pump for sample injection
- High accuracy for special application



Excellent gradient reproducibility of 0.3 % RSD. Overlay of 6 repetitions at 1 ml/min run with pump P 6.1L low pressure gradient version

## AZURA® ASM 2.1L Assistant

A flexible combination module

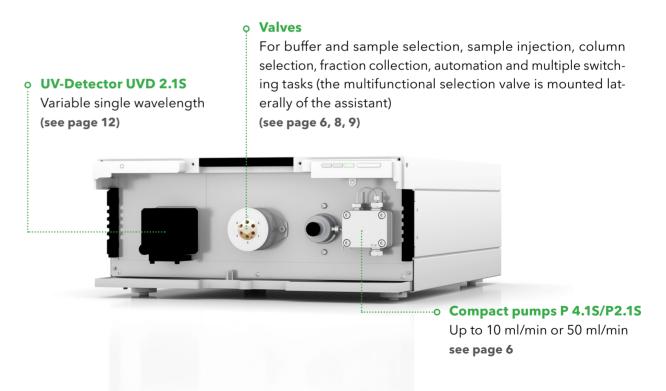
The Assistant ASM 2.1L is a compact combination module which can be equipped with up to three device modules. Available for selection are valves, pumps, and a UV detector. An assistant including a pump, valve, and detector features a compact FPLC system, like AZURA Bio SEC or AZURA Bio AC. As a part of a larger system, the ASM 2.1L is extremely versatile. Depending on the integrated

modules the assistant fulfills many different tasks like sample and buffer selection, sample injection, column switching, fraction collection, buffer delivery or UV-detection.

The concept of the flexible combination of device modules combines the highest functionality with minimal space requirements.

#### **Configure your assistant**

Can be equipped with combinations: valves, pumps, and one detector



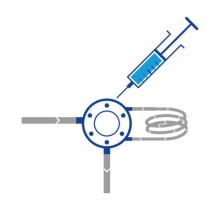
For detailed information on device modules and assistant configuration: www.knauer.net

## Sample injection

Available sample injection modules range from an injection valve, sample pump and autosampler.

#### **Injection valve**

Integrated into assistant or standalone module: The AZURA 2-positions valve is perfect for injection of small sample volumes. Connect 1/16" tubings for flowrates up to 100 ml/min. For higher flowrates use the injection valve for 1/8" tubing. Various sample loops are available.



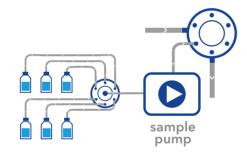
#### Sample pump

Integrated into assistant or standalone module: The AZURA P 4.1S is perfect for injection of larger sample volumes.

Repetitive sample injections by using the pump for automated sample loop filling.

#### Do you have many samples?

You can extend your configuration with additional valves each for 6 samples.



#### **Autosampler**

Process many different samples fully automatically with the Autosampler AS 6.1L.

- Up to 10 ml injection volume
- From microtiter plates to standard vials
- Active cooling
- Fully supported by PurityChrom® software
- Metal free

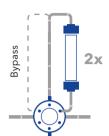


## **Column selection**

Different options for column selection are available.

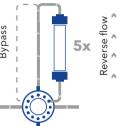
#### 2-position valve

- Select two columns or one column and one bypass
- Flow rates up to 500 ml/min possible



#### **Multifunctional selection valve**

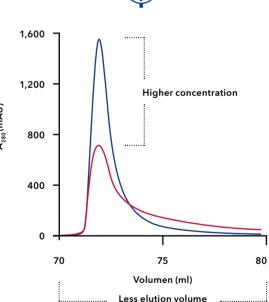
- For up to 5 columns and 1 bypass
- Reverse flow
- Flow rates up to 50 ml/min



## Why is the reversed flow option popular in affinity chromatography?

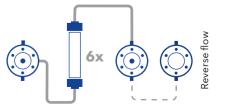
In affinity chromatography your target molecules will accumulate at the top of the column. Elution in the same direction dilutes your target molecule along the column. By elution with reversed flow you increase the concentration while decreasing the sample volume.

This option has two major advantages. Clean your columns more efficiently using reverse flow. By this you elute contamination the shortest way and minimize damage to the column.



#### **Higher flow rates?**

Use the column selection assistant to select six columns assuring a flow rate up to 500 ml/min. An additional valve allows to reverse the flow.



## **AZURA® UV Detectors**

We provide a choice of UV/VIS detectors, ranging from single variable wavelength to 8-channel diode array detector with 3D scan capability.



	UVD 2.1S	MWD 2.1L	DAD 2.1L
	Compact and cost-ef- fective variable single wavelength UV/VIS detector	Reliable multichannel UV/VIS detector	
Wavelength	190-500 nm	190-700 nm	190-700 nm
Channels	1	4	8
3D scan	n/a	n/a	+
Integrable in ASM	+		

More UV detectors available for your applications: www.knauer.net/detectors



#### AZURA® CM 2.1S

- Conductivity monitor for checking salt gradient
- Flow rates up to 100 ml/min
- 0,01 mS/cm-999 mS/cm
- pH option available

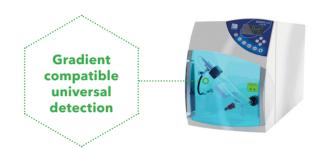
Flow cells for CM 2.1S					
···· Analytical	1/16"	10 ml/min	160 bar	30 μl volume	
Preparative	1/16"	100 ml/min	100 bar	300 μl volume	



#### **AZURA® RID 2.L**

Refractive Index Detector for cost-effective, fast and reliable analysis of non-UV absorbent compounds.

A wide range of third-party detectors can be seamlessly integrated into AZURA® systems.

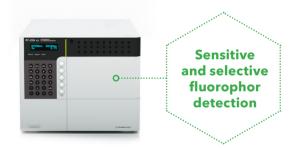


#### **SEDEX-LC**

Using the unique Low Temperature technology, this Evaporative Light Scattering detector LC allows universal high sensitivity detection of non-UV active substances.

#### Fluorescence detector RF-20A

The fluorescence detector RF-20A provides world-class sensitivity, excellent maintainability and diverse validation / support functions. It supports a wide range of applications from conventional to high-performance analysis.





The KNAUER interface box IFU 2.1 LAN allows highly precise analog data acquisition of third party modules over analog and relay outputs. Example: MALS-detectors for molecular weight determination.

#### Flow cells

Select from an impressive range of easily exchangeable flow cells which cover a wide range of application. Optional fiber optics technology offers the possibility to separate the flow cell spatially from the device providing enhanced security for hazardous, explosive or toxic work processes.

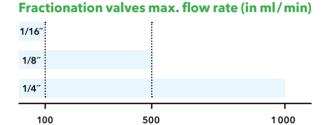
## **Fraction collection**

Collect large quantities or large numbers of fractions

**Manually - collection by direct control Volume-based - collection at defined volumes** Peak-based - collection according to detector signal

#### **Fraction valves**

- Collecting large quantities
- Up to five fractions and waste
- Available as a single device or integrated into an Assistant ASM 2.1L for different flow rates





#### **Foxy Fraction collector**

The Foxy R1 and Foxy R2 are versatile fraction collectors which fits to every purification need.

- Foxy R2
- Wide choice of racks from 96-well microplates up to bottles or funnels
- Double capacity for Foxy R2 with automatic rack recognition
- Active cooling for Foxy R1
- Supported in software Puritychrom®
- Stand-alone operation
- Repeated collection in same vials



#### Vario 4000 & Vario 4000 plus

The Vario 4000 is a more advanced fraction collector for demanding applications with high flow rates and a high number of fractions. Indivi-• Up to 125 ml/min for Foxy R1 and 1 000 ml for dual rack types are programmable. Just assemble your rack to your needs.

- For flow rates up to 1000 ml/min
- High number of fractions
- Supported in software Puritychrom®
- Standalone operation

## **Accessories**

Accessory	Features	Protect column from air damage and support automation ( e.g. sample	
Pressure Control	<ul> <li>Contains two pressure sensors</li> <li>Automatic determination of pressure difference with Purity-Chrom®</li> <li>Connect 1/16" or 1/8" tubings</li> <li>Up to 250 ml/min and 60 bar</li> </ul>		
Air Sensor	<ul> <li>Detect end of buffer or end of sample with PurityChrom®</li> <li>Up to four air sensors per system</li> <li>For transparent tubings with 1/16" or 1/8" or 1/4" outer diameter</li> </ul>		
AZURA Click	<ul> <li>Attach air sensor, pressure con- trol, AZURA Organizer or your interface box to the side panel of your AZURA L device</li> </ul>	Organize your system.	
AZURA Organizer	<ul> <li>Attach columns from 5 mm to 26 mm diameter, falcon tubes, a back pressure regulator or a pH flow cell</li> </ul>	Organize accessories directly at the system and reduce dead volume	
<ul> <li>Apply a constant back p</li> <li>to your system</li> <li>Freely adjustable between the part or 20-103 bar</li> </ul>		Prevent formation of air bubbles after the column which disturb detector signal	

# **AZURA®** Bio purification systems

Product	Features	Page
AZURA Bio SEC	JRA Bio SEC  0.001-10 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	
AZURA Bio AC	0.01-50 ml/min, maximum 200 bar, selection valve for 6 buffers/samples, variable single wavelength UV-detector, fraction valve for 5 fractions and waste, PurityChrom® software	19
AZURA Lab LC	0.001-50 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration. Configure your FPLC system based on your purification requirement.	4
AZURA Bio purification Two-step purification	0.01 - 50 ml/min, maximum 200 bar, sample injection via sample loop or sample pump, automated storage and reinjection of proteins, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	20
AZURA Pilot LC	Up to 1000 ml/min, sample pump for large sample volumes, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration.  Configure your FPLC system based on your purification requirements. Scale-up is possible with same flexibility, software but minimal footprint.	

#### Components from Lab to Pilot

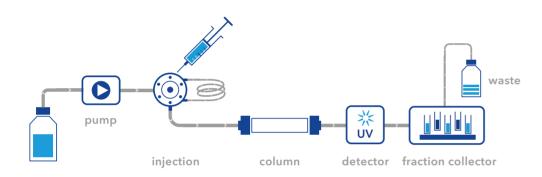
Product	oduct Features	
Buffer Delivery		
Compact pump	10 or 50 ml/min, isocratic	6
Gradient pump	10 or 50 ml/min, Quaternary: selection of 4 buffers (A, B, C, D) Binary: selection of 2 buffers (A1, A2, B1, B2)	
Scale-up pump	100, 250, 500, 1000 ml/min, Binary to quaternary gradient	7
Extended buffer selection	With additional valves each for 6 buffers	
Sample selection	For maximum 6 samples	10
Column Selection		
Column selection valves	For 2 columns, 5 columns and 5 columns with reverse flow option	
Detection		
Wide choice of detectors	Variable single wavelength UV, Multiple wavelength UV, Full spectra diode array (DAD), Conductivity and pH monitor, Fluorescence, Refrective Index	
Fraction Collection		
Fractionation valve	For five fractions and waste, up to 1000 ml/min	14
Fraction collector	From 96-well microplates up to bottles or funnels, up to 1000 ml/min	
Sample Injection		
Injection valve	1/16" tubing: up to 50 ml/min 1/8" tubing: up to 500 ml/min	8
Sample pump	10 or 50 ml/min	8
Autosampler	Up to 10 ml injection volume, from microtiter plates to standard vials	8
Software		
PurityChrom software	Highly flexible method writing, intuitive user-interface, volume- or time-based, with special features like system visualisation, hold & adjust option, extended threshold functions, Check for impurities	
Safety Features		
Accessories for protection and automation	Air sensor, Pressure Control, Back-pressure regulator, leak management, mounting solutions	14

## **AZURA Bio SEC**

#### Time consuming gelfiltation runs?

AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system. Thanks to its compact design and intuitive FPLC software PurityChrom®, the system offers outstanding performance and

ease of use. Pre-designed methods are included in the software and can be easily adapted by changing the column volume. AZURA Compact SEC supports all columns available on the market.





#### **Key features**

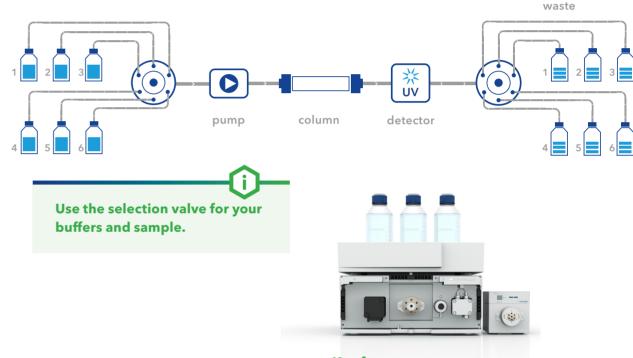
- Flow rate: 0.001-10 ml/min; 0.1-8.0 ml/min (recommended)
- Maximum system pressure: 150 bar
- Injection valve for sample injection via sample loop
- Variable single wavelength UV-detector (190-500 nm)
- Fraction collector for fractionation
- Columns from all venders can be used
- PurityChrom® software

## **AZURA Bio AC**

#### For affinity chromatography

The AZURA Compact AC system qualifies for fast and reliable affinity chromatography. Select your sample, your washing and elution buffer using

the selection valve. Your proteins of interest are detected by UV and automatically collected via the fractionation valve.



# 2,000 Binding Washing Elution 1,600 1,200 800 400 5 10 15 20 25 30

#### **Key features**

- Automatic sample/ buffer selection valve for up to 6 buffers or samples
- Fraction valve (6 ports) for fractionation
- Flow rate: 0.01 50 ml/min; 1 40 ml/min (recommended)
- Variable single wavelength UV-detector (190-500 nm)
- Columns from all venders can be used
- PurityChrom® software
- Maximum system pressure: 150 bar

Capture proteins based on high affinity Chromatogram & Legend

Volume (ml)

# **Special configuration - Two step purification**

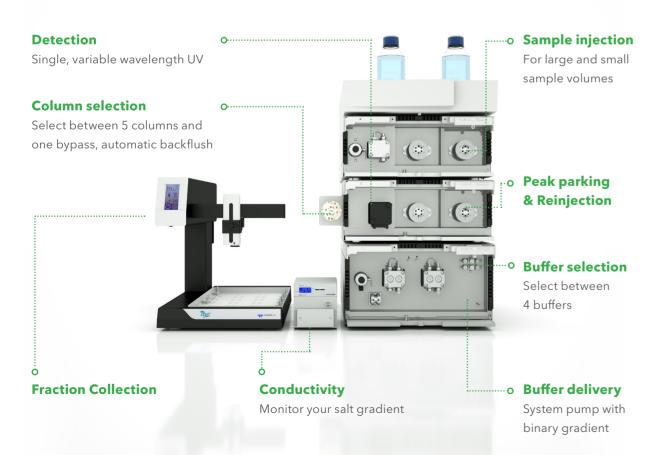
Special multi-column chromatography solutions

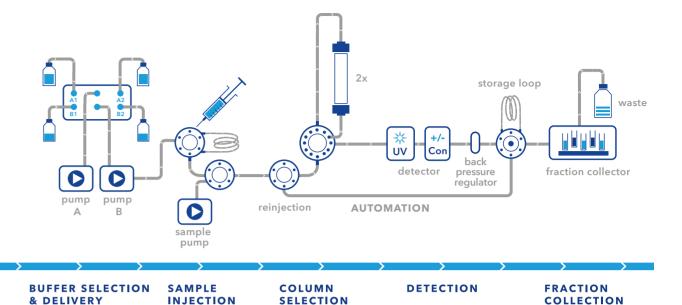
Protein purification involves most of the times two to three steps:

- 1. capture step
- 2. optional intermediated step
- 3. polishing step

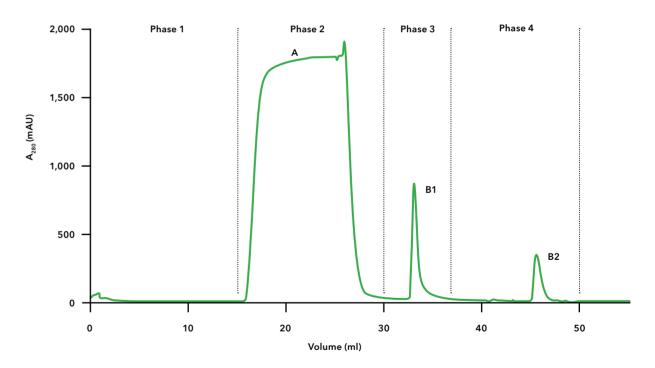
The transition from one to another step generally involves manual interaction and thus is time consuming. Automation by combining these steps increases the efficiency and optimizes the workflow. The quick and automated linkage of multiple

chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. This automation strategy can be easily adapted to each purification task.





#### Automated two-step purification of mouse IgG antibodies

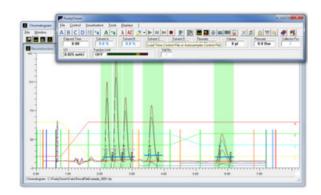


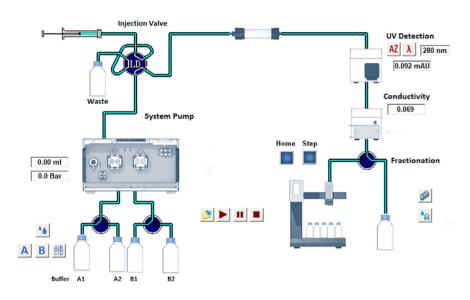
The affinity chromatography step was automatically combined with a gel filtration step to exchange the buffer of the purified mouse IgG antibodies; Phase 1: Column equilibration, Phase 2: Sample injection and washing, Phase 3: Elution of IgG from protein A column, Phase 4: Desalting of IgG

## **Control your purification**

#### **PurityChrom**

PurityChrom is a powerful software to control your FPLC system. Get familiar with PurityChrom in shortest time and with no effort due to the intuitive and clearly structured user interface. Choose a time-or volume based workflow by just clicking one button. Create methods with highest flexibility to realize complex application without losing easy handling. Offline licenses for creating methods and data evaluation are for free.





#### **System Visualisation**

Keep an eye on your system with the system visualisation. The interactive flow path allows to control your system. Switch valves, start pumps, set autozero, start fraction collection.

#### **Hold & Adjust (a running method)**

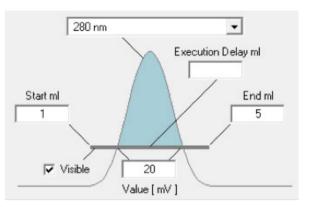
You have full control of your run. Hold a run to adjust the method or the system. Stay always in control and change the parameters of a running method.



#### **Extended threshold functions**

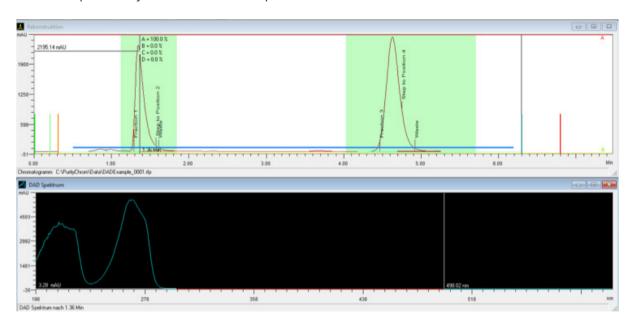
Automate any software function triggered by signals of any channel.

Automatically start fraction collection at the beginning of your desired peak. Protect the system from overpressure and air bubbles. After end of sample detection the software offers the possibility to automatically start or continue the run. Automate the whole purification starting from sample injection, via column washing to elution.



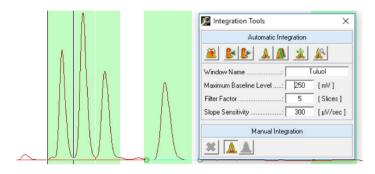
### Check for impurities - full spectra diode array (DAD)

Check the purity of your peaks based on the absorbance spectra anywhere in the elution profile.



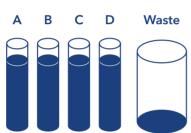
#### **Intuitive data analysis**

Integrate peaks fully automatically or manually. Receive the peak results by click on one button.



#### **Solvent Supply - Calculate the consumption of buffers**

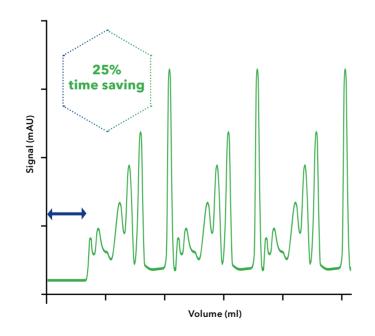
The solvent supply function calculates the consumption of buffers and the waste level for the current run, thus preventing the column from running dry and flooding the lab.



#### **Stacked Injection**

Size exclusion chromatography separates the proteins according to their size. After selection of SEC medium, sample volume and column dimensions are the two most critical parameters that will affect the resolution of the separation. For most SEC runs the sample volume should not exceed 2% of the total column volume to achieve maximum resolution.

For larger sample volumes the sample must therefore be divided into different runs. However, this takes a lot of time and is not very efficient. With the stacked injection function in PurityChrom it is possible to run different runs automatically one after the other. The injection of the next run takes place during the current run, so that the time until the elution of the first peak can be fully exploited. This increases efficiency and saves time.



## **Customer review**

AZURA® Bio purification solution by KNAUER.

#### "Our KNAUER FPLCs are the workhorses in the lab."

"My lab studies the structure and function of membrane proteins. Due to the inherent instability of these proteins we purify them in the cold room. We needed robust FPLCs with good pumps that tolerated these conditions well.

In addition, the systems needed to be easy to maintain. Knauer provided us with skilled advice on virtually every component of the system, ranging from tubing and pumps up to the software. Consequently, our systems are perfectly tailored to our needs. Most of the maintenance we can do ourselves. For remaining questions, we can rely on the great support Knauer offers. Our Knauer FPLCs are the nononsense workhorses in the lab. I highly recommend Knauer."



Jun. Prof. Dr. Eric R. Geertsma
Institute of Biochemistry,
Goethe-University Frankfurt
Foto: Uwe Dettmar



#### **System components**

- AZURA® UV Detector UVD 2.1S
- AZURA® Valve Drive V 2.1S
- AZURA® Pump P 4.1S
- Foxy fraction collector

AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system.

Contact us:

sales@knauer.net

## **AZURA®** Multicolumn



#### **Continuous separation of biomolecules**

The flexible design of the AZURA® SMB system is perfect continuous FPLC purification. Use AZURA® SMB as your step to the future of biopurification and run your downstream process with the highest productivity and lowest costs. Our multi-column capture process (MCCP) saves your time as well as solvent and column costs.

#### Multi-column capture processes (MCCP)

Continuous IEX or affinity chromatography is almost as simple as a standard batch run. Depending on your batch separation, you can easily configure the parameter for the continuous run. Thanks to the system flexibility it's very easy to change the column distribution for binding (d), washing (c), eluting (b) and regeneration (a) of the columns. If the washing steps take longer, just use more columns in this zone.

#### Why an 8 column setup is the best

Three reasons why more columns are better

The separation of biomolecules can also be done are often very limited and cannot handle typical The 8 column setup enables many advantages.

problems regarding process stability and flexiwith 2, 3 or even 4 columns. But these systems bility as easy as the AZURA® SMB system can do.



Other systems might be limited regarding their batch program adaption (wash+elution time ≤ capture time), due to the flexible column distribution the length of every zone can be adjusted, even if there is a critical change in the feed concentration.

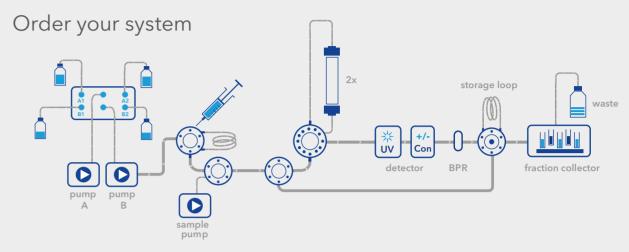


Sometimes additional purification steps are necessary to regenerate the column and keep their lifetime and potential at a maximum. Two and three columns systems often cannot handle this problem easily.



More columns mean longer system run time and less downtime. You will have up to four times less downtime due to column change. Use your working time most effi-

## **System configurator**



BUFFER SELECTION SA	AMPLE AUTOMA	TION COLUMN	DETECTION	FRACTION
& DELIVERY	NJECTION	SELECTION		COLLECTION
□ 10 ml/min binary gradient pump P6.1L □ 50 ml/min binary gradient pump P6.1L □ 10 ml/min quaternary pump P6.1L □ 100 ml/min pump P2.1LQuantity □ 250 ml/min pump P2.1LQuantity □ 500 ml/min pump P2.1LQuantity □ 1000 ml/min pump P2.1LQuantity □ 1000 ml/min pump P2.1LQuantity □ Ternary gradient module for pump P2.1L □ Binary gradient module for pump P2.1L □ Buffer selection valve (6 further inlets)Quantity	□ Injection valveQuantity □ Sample pump moduleQuantity □ Sample selection valve (6 further inlets)Quantity □ Biocompatible Autosampler AS6.1L	☐ Column selection valve up to 50 ml/min (5 columns, one bypass, reverse flow) ☐ Column selection (two columns or one bypass) ☐ Column selection high flow (5 columns, one bypass) ☐ Column selection high flow (5 columns, one bypass, reverse flow)	□ UV/VIS single wavelength □ UV/VIS multiwavelength □ Conductivity □ pH □ Fluorescence □ Refrective index □ Light Scattering □ Analog integration of further detectors	☐ Fractionation valve ☐ Foxy fraction collector with fixed rack tpes ☐ Labocol fraction collector with individual rack types  ACCESSOIRES ☐ Airsensor 1/16"Quantity ☐ Airsensor 1/8"Quantity ☐ Pressure control (2 pressure sensors) ☐ Back pressure regulatorQuantity ☐ AZURA OrganizeQuantity

Multi-Column Chromatography, SMB

Preparative HPLC

**FPLC** 

Osmometry

Dosing, Metering, Pumping

Detection

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