

Outline

- Online purification
 - Why and How to do SEC-SAXS (+SLS)?
 - Why not to do SEC-SAXS?
 - Current/ Future Developments

Recources

https://padlet.com/melissagraewert/P12_UM2020

password:um2020

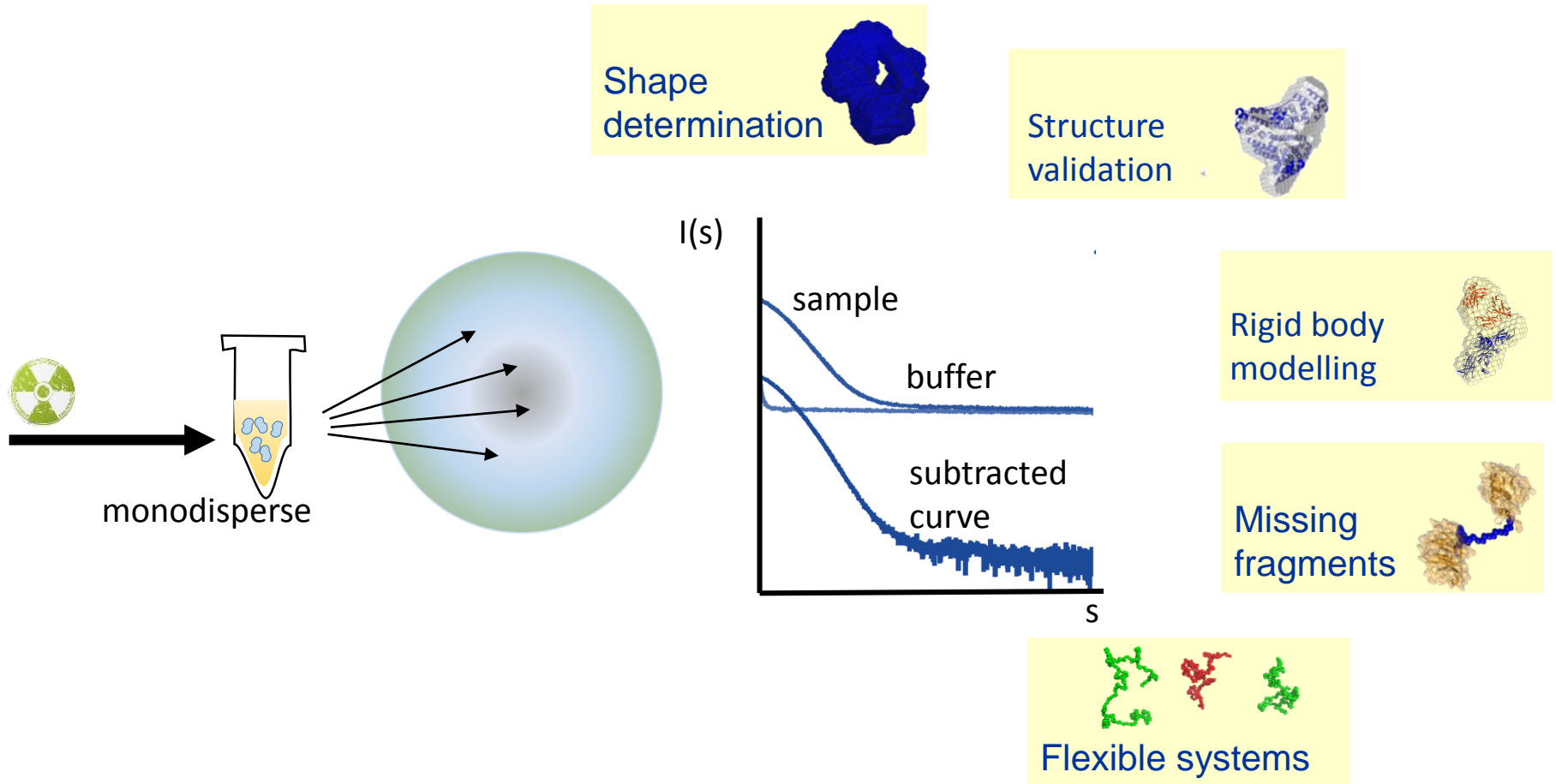
The screenshot shows a Padlet board with the following content:

- TALKS - Day 1**: Includes cards for Dmitri Svergun (P12 User Meeting 2020), Clement Blanchet (Day-1-talk-2-Update-from-P12-Clement...), and Day-1-talk-1-P12-user-meeting-Dmitri-S... mediasite.
- ATSAS 3.0**: Includes cards for ATSAS TALK - Karen Manalastas-Cantos (New Developments in ATSAS 3.0, 17 Nov 2020) and ATSAS 3 - Karen Manalastas (New Developments in ATSAS 3.0, 17 Nov 2020).
- Publications**: Includes a card for 'ATSAS_28_A_comprehensive_data_anal...' PDF document, 'Latest SEC-SAXS Paper' (Adding Size Exclusion Chromatography (SEC) and Light Scattering (LS) Devices to Obtain High-Quality Small Angle X-Ray Scattering (SAXS) Data), and 'crystals-10-00975-v3' PDF document.
- Ressources**: Includes cards for '03. Graewert Sample Buffer Preparation mediasite', 'Lecture 6- Mixtures (Melissa Graewert)', 'SAXS studies on mixtures, assemblies, flexible systems', and '06. Graewert Mixtures Flexible mediasite'.

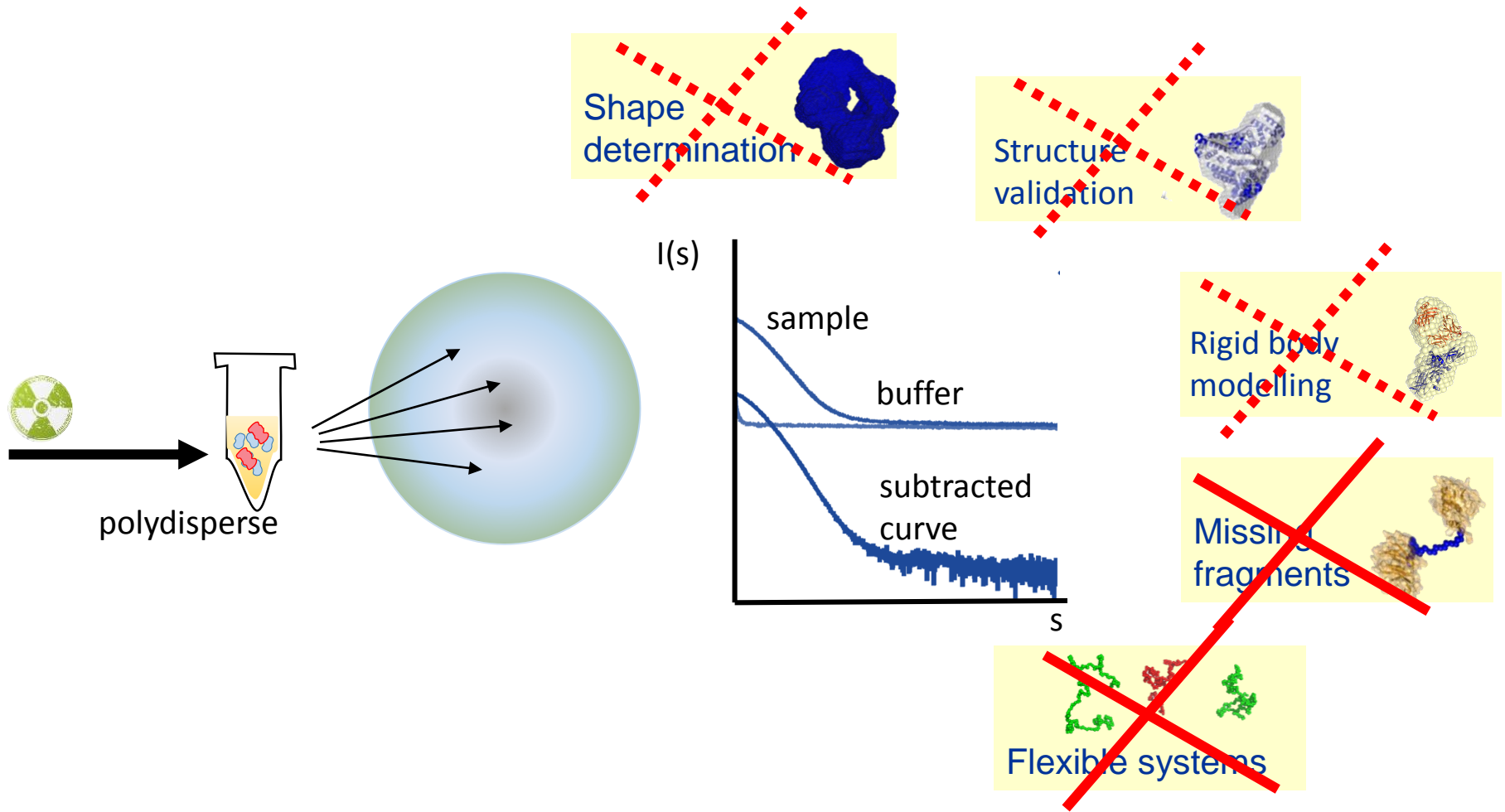
A yellow box highlights the text **SEC-SAXS Paper** over the publication card. Another yellow box at the bottom right highlights the text **Online Lecture Course** and lists the following items:

- Lecture 3 – Sample and Buffer Preparation
- Lecture 6 - Mixtures

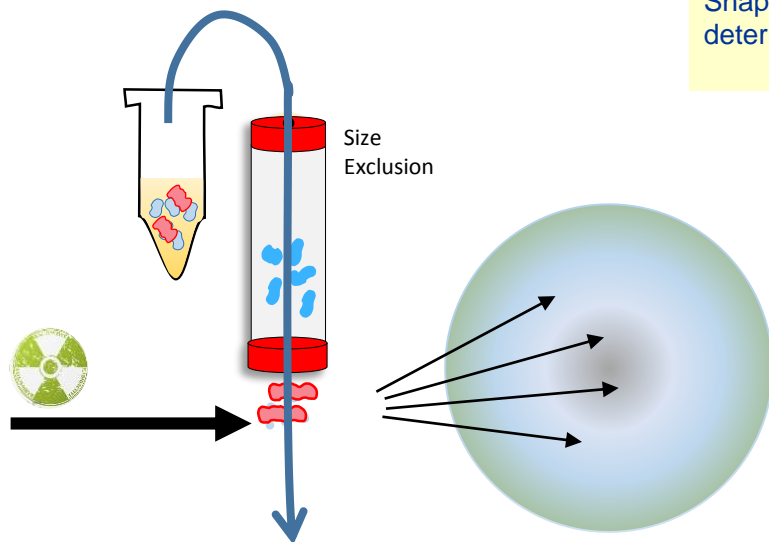
Why SEC-SAXS(/MALLS)?



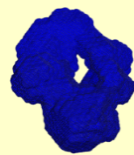
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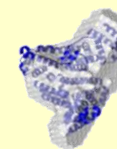
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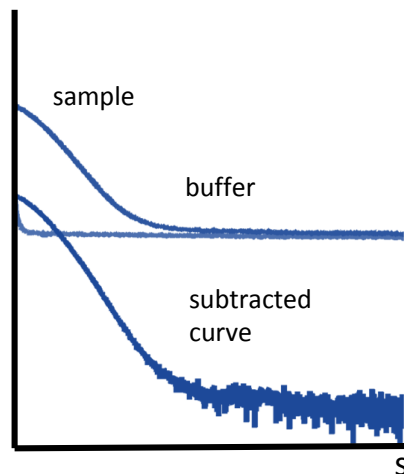
Shape determination



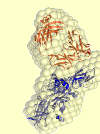
Structure validation



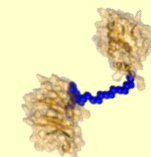
$I(s)$



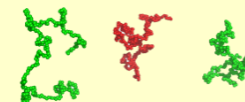
Rigid body modelling



Missing fragments

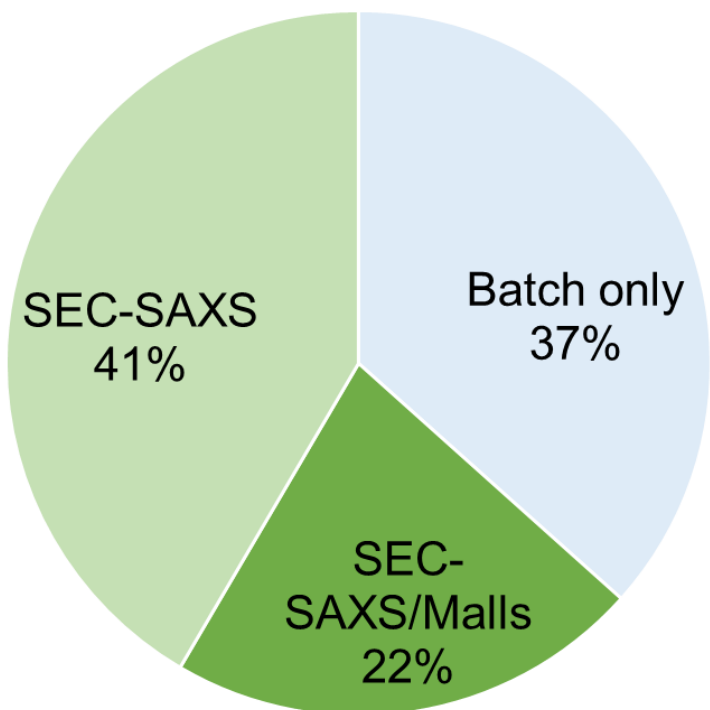


Flexible systems

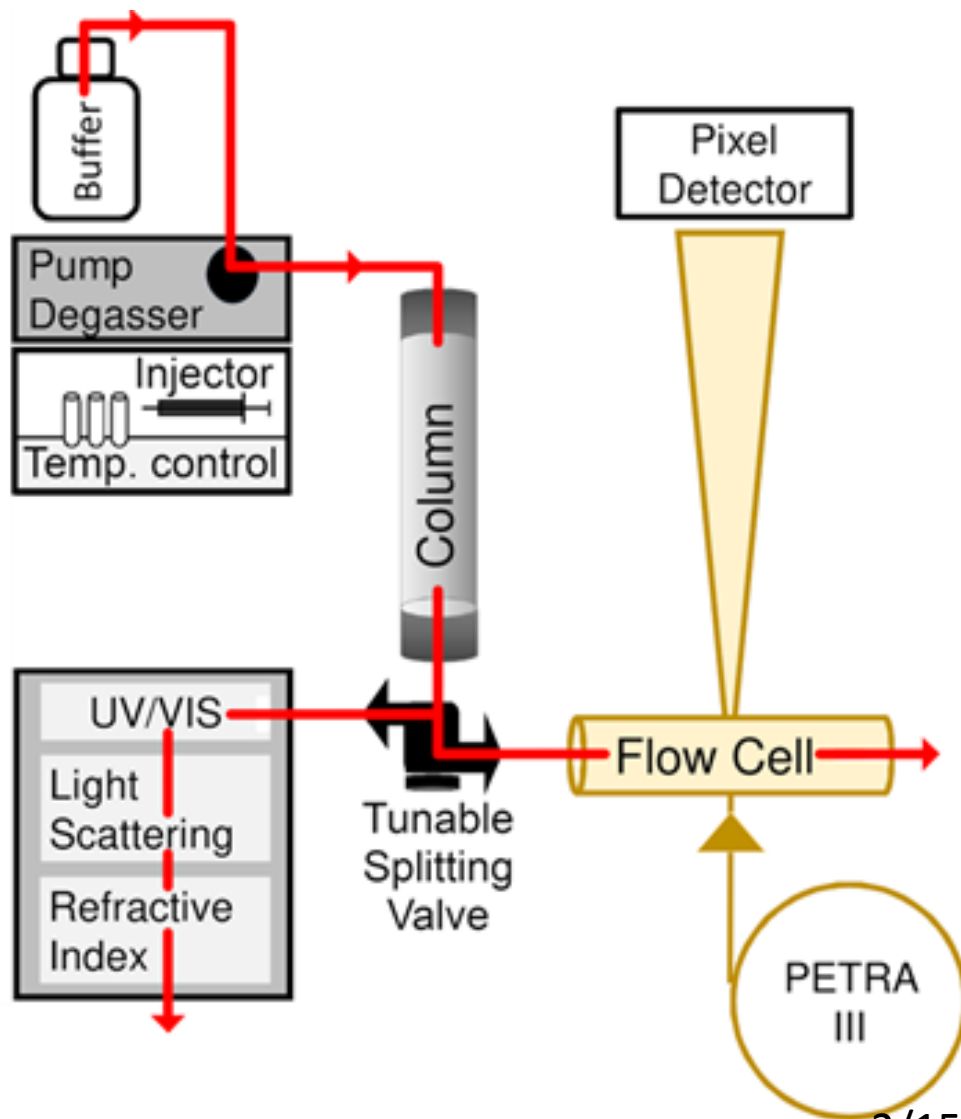


EMBL Beamline P12 at Petra III: SEC-SAXS

SEC-SAXS has become a standard set-up over the last years



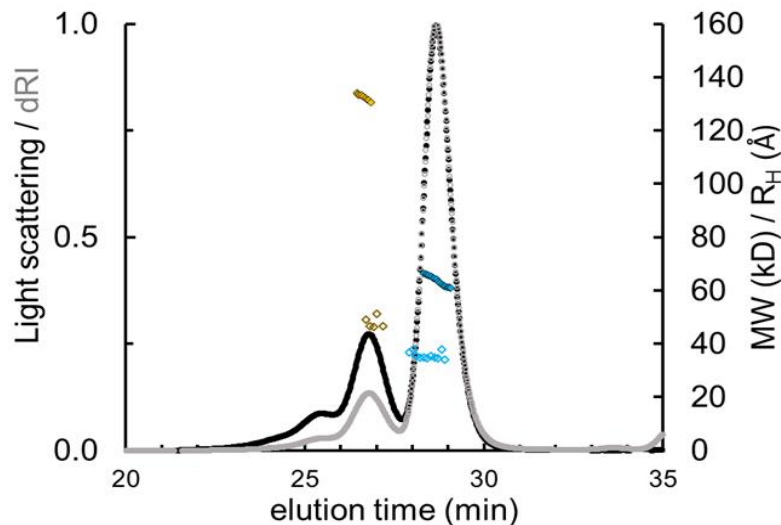
SAXS modes (by visit)
- 2019



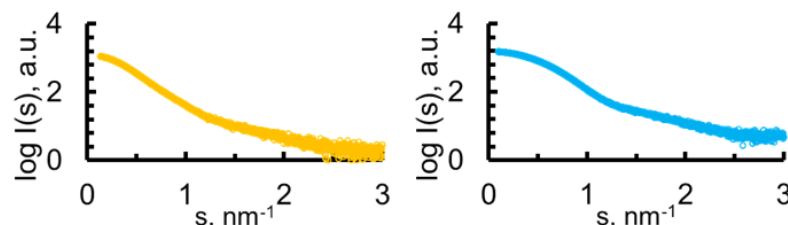
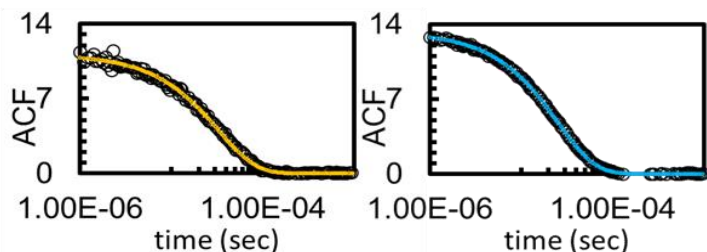
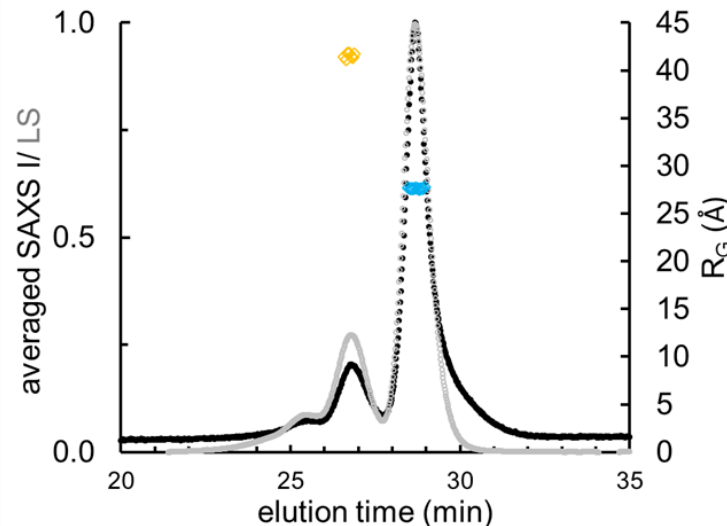
EMBL Beamline P12 at Petra III: SEC-SAXS

SEC-SAXS has become a standard set-up over the last years

Static & Dynamic Laser Light Scattering:



X-ray scattering:



$MW_{\text{dimer}} = 132\text{kD}$
 $RH_{\text{dimer}} = 4.8\text{nm}$

$MW_{\text{monomer}} = 64\text{kD}$
 $RH_{\text{monomer}} = 3.6\text{nm}$

$MW_{\text{dimer}} = 131\text{k}$ $MW_{\text{monomer}} = 60\text{ kD}$
 $RG_{\text{dimer}} = 4.1\text{nm}$ $RG_{\text{monomer}} = 2.8\text{nm}$

How to do SEC-SAXS(/MALLS) at P12?

Apply

Check Box on the Application
Short justification required in the proposal text

→ See talk von Maria Vanoni

Prepare

Why:

Your Local Contact will be aware and prepared

Preform

If circumstances change:

And you would like to use SEC-SAXS →
Communicate with us. (we can find a way)

Analyse

How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

Perform

Analyse

Communicate in advance:

- What Column(s) – check availability/suitability
- What Buffer(s) – identify issues (Zn²⁺, Phenol, DTT,...)
- How many runs – check practicality
- Any special requests – no surprises

Bring / Send

- Sufficient Buffer
- Sufficient Sample
- Time
- (Column)

How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

Perform

Analyse

Communicate in advance:

- What Column(s)
- What Buffer(s)
- How many runs
- Any special requests

Bring / Send

- Sufficient Buffer
 - best STOCK (dilute, filter, degas) on-site
 - send additives such as DTT (or confirm availability)
- Sufficient Sample
 - for 10/300 column, 50-120 ul (>5mg/ml)
 - for 5/150 column, 10-50 ul (>3mg/ml)
- Time
 - for 10/300 column, 50 + 10 min per run
 - for 5/150 column, 15 + 5 min pre run
- + PREP time
- (Column)

Prepare – WHAT COLUMN



Prepare – WHAT COLUMN

Balance Concentration & Resolution

Low concentration → small column

High concentration + no “high resolution” required
→ small column

High concentration + “high resolution” required
→ large column

Other factors: - parallel MALLS
- sample stability/ equilibrium
(- time limitations)

How to do SEC-SAXS(/MALLS) at P12?

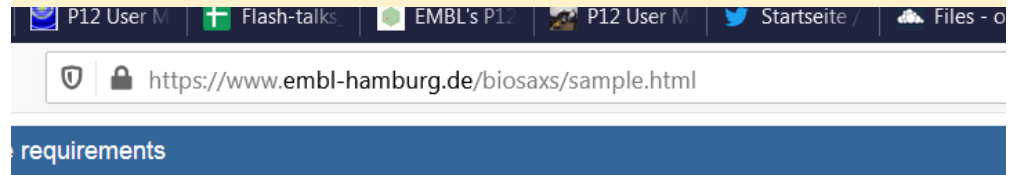
Apply

Prepare

Perform

Analyse

CHECK:



Sample requirements

There are three main options for SAXS data collection at P12.

Batch measurements with the robotic sample changer

- Sample concentration: from 0.5 to 10 mg/ml (or more)
- Sample volume: 40 μ l *per exposure*
- The amount of buffer: at least twice that of the sample
- Buffer composition: no aggressive substances (chloroform, toluene etc)
- Temperature range: from 8° to 45°C

SEC-SAXS measurements

- Stock sample concentration from 7 mg/ml
- Sample volume about 50–100 μ l (depends on the column size)
- The amount of buffer: at least 500 ml
- All measurements at room temperature only
- Users are encouraged to bring their own columns
- [MALLS/RI analysis is available](#)

How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

Perform

Analyse

Mail-in:

Do not forget **clear instructions** such as

- Thaw slowly/quickly
- Anything to be added
- Removal of potential aggregates through Centrifugation or filter
- Gives us warnings such as:
 - “sample aggregates after 2-3 hours”
 - “peaks might not be base-line separated → less volume should then be injected”
 - “we normally expect pressure build up”



How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

Perform

Analyse

On-site:

- Introduction to the system
 - HPLC training
 - Words of caution
- Introduction to Becquerel settings
 - 2-3 trial runs
 - We trust you with the system
- Cleaning up the system
 - Column washed
 - System in water
 - Disconnected from the beamline

How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

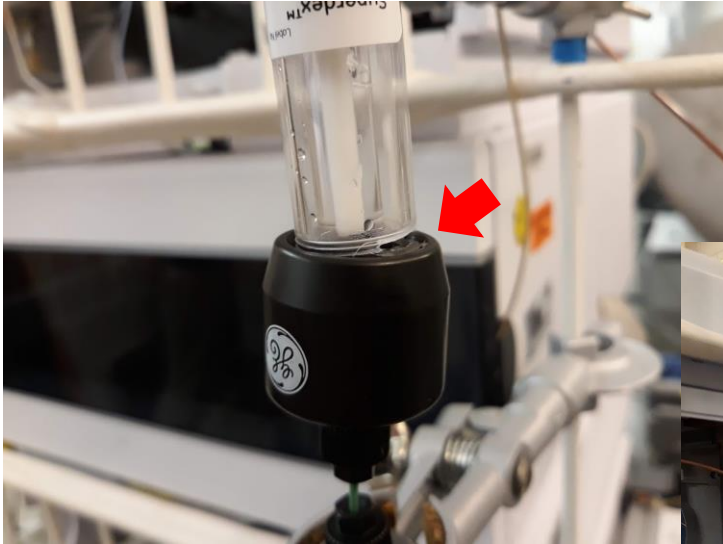
Perform

Analyse

On-site:

- Introduction to the system
 - HPLC training (let us know if you do not have much experience)
 - Words of caution (take note for late shift)
- Introduction to Becquerel settings
Now easy to start/switch, automated cleaning
 - 2-3 trial runs
 - We trust you with the systemMore support required with MALLS
- Cleaning up the system (also when you are tired)
 - Column washed/System in water
 - Disconnected from the beamline

Perform



!!! Leaks, Buffer Fillings, Pressure Issues, ...

How to do SEC-SAXS(/MALLS) at P12?

Apply

Prepare

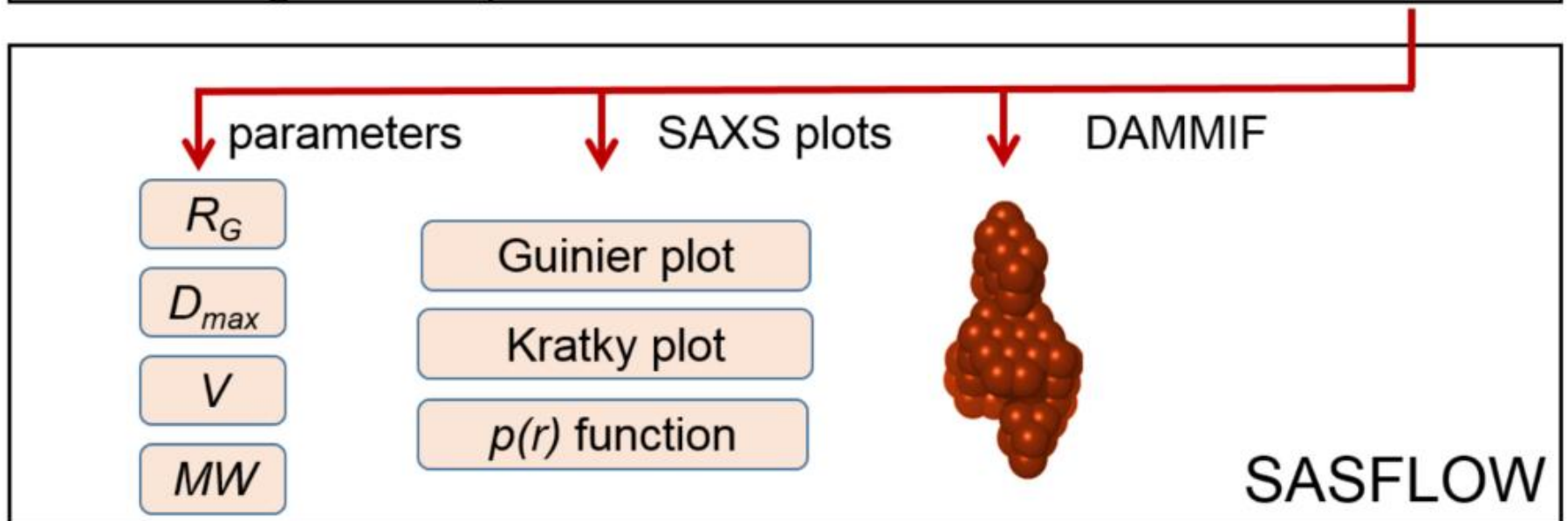
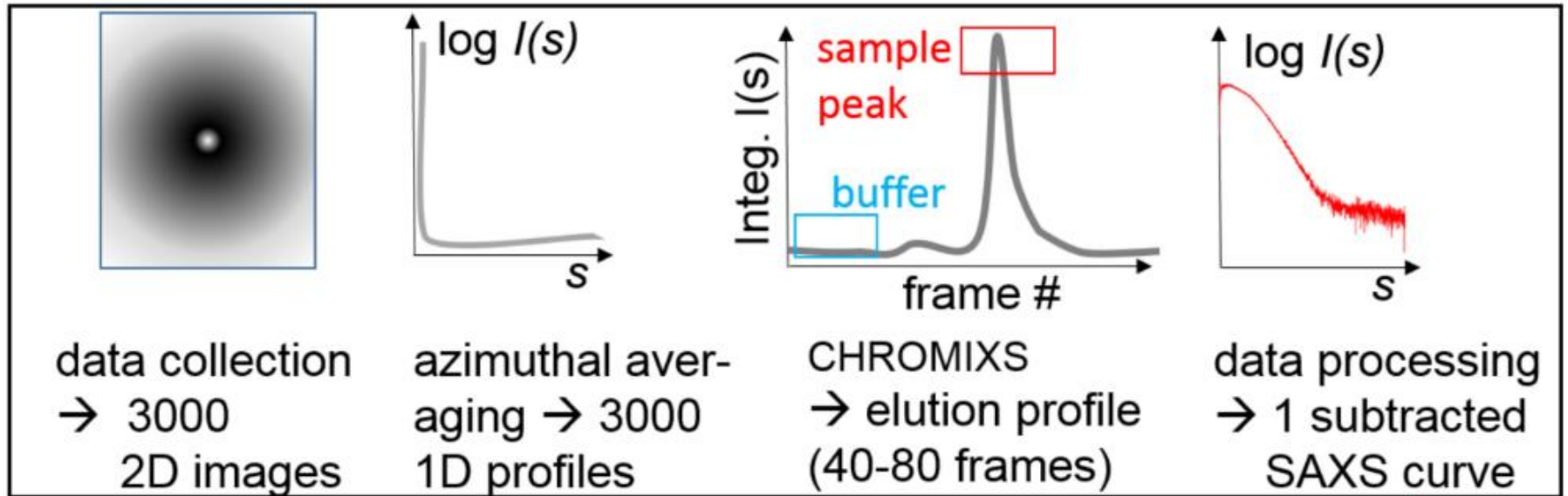
Perform

Analyse

Receive Data:

- Automatically receive:
 - Data files
 - If required, request 2D images
- Automatic Chromixs/SASFLOW evaluation
- Check data as soon as possible and contact for follow-up questions

Analyse



Analyse

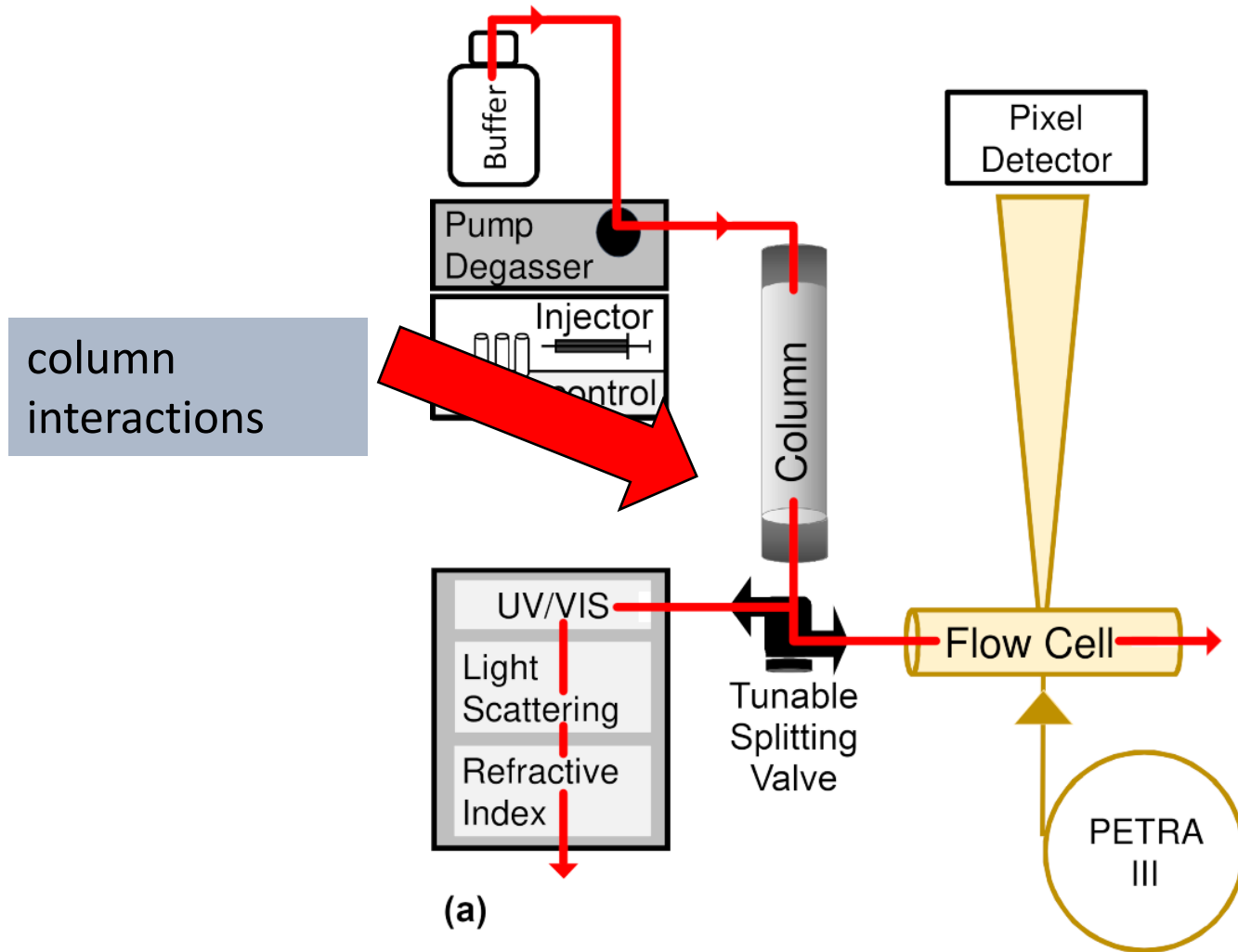
	Frame#	Log plot	Kratky plot	Guinier plot		R _g nm	p(r) plot	D _{max} nm	V _{Porod} nm ³	MW _{rel} kDa	DAM MW, kDa
				points	quality						
BSA	1431 - 1541 1643 - 1697		 compact	 41 - 161	95%	2.8 ± 0.0		8.4	98	53 ⁺⁶ ₋₄	
BSA dimer	541 - 661 1413 - 1472	 oversubtracted	 compact	 40 - 109	24%	3.9 ± 0.0		13.1	213	157 ⁺⁶ ₋₂₃	
CA	1249 - 1353 1442 - 1493		 compact-hollow	 23 - 247	97%	1.8 ± 0.0		5.1	37	24 ⁺²	
ADH	1321 - 1431 1487 - 1541		 compact	 37 - 123	93%	3.3 ± 0.0		9.2	202	124 ⁺¹⁰ ₈	
aFER	631 - 757 1197 - 1259		 compact-hollow	 13 - 65	94%	5.3 ± 0.0		16.4	685	434 ⁺²² ₈₁	
	Frame#	Log plot	Kratky plot	Guinier plot		R _g nm	p(r) plot	D _{max} nm	V _{Porod} nm ³	MW _{rel} kDa	DAM MW, kDa
				points	quality						

Figure S8. Automated output summary from SASFLOW/CHROMIXS. Key parameters and structural analysis are shown for all samples measured. Figure has been cropped for better visuality.

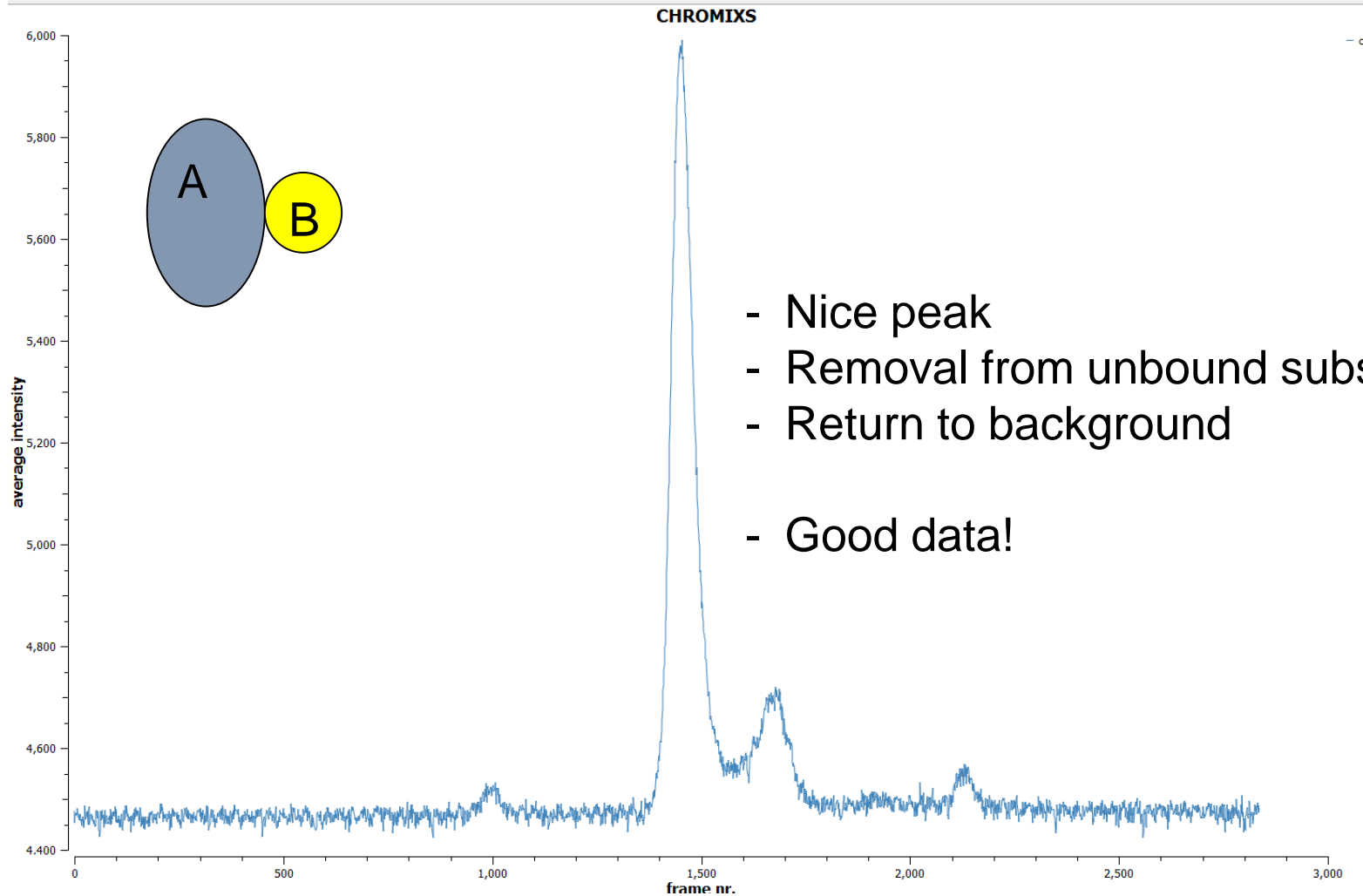
Why SEC-SAXS(/MALLS)?

- Analysis of individual components
- No issues with buffer matching
- Concentration series on the “fly”
- Addition of further detectors:
independent estimate of MW,
assessment of re-mixing/re-
oligomerization, additional information
for membrane proteins

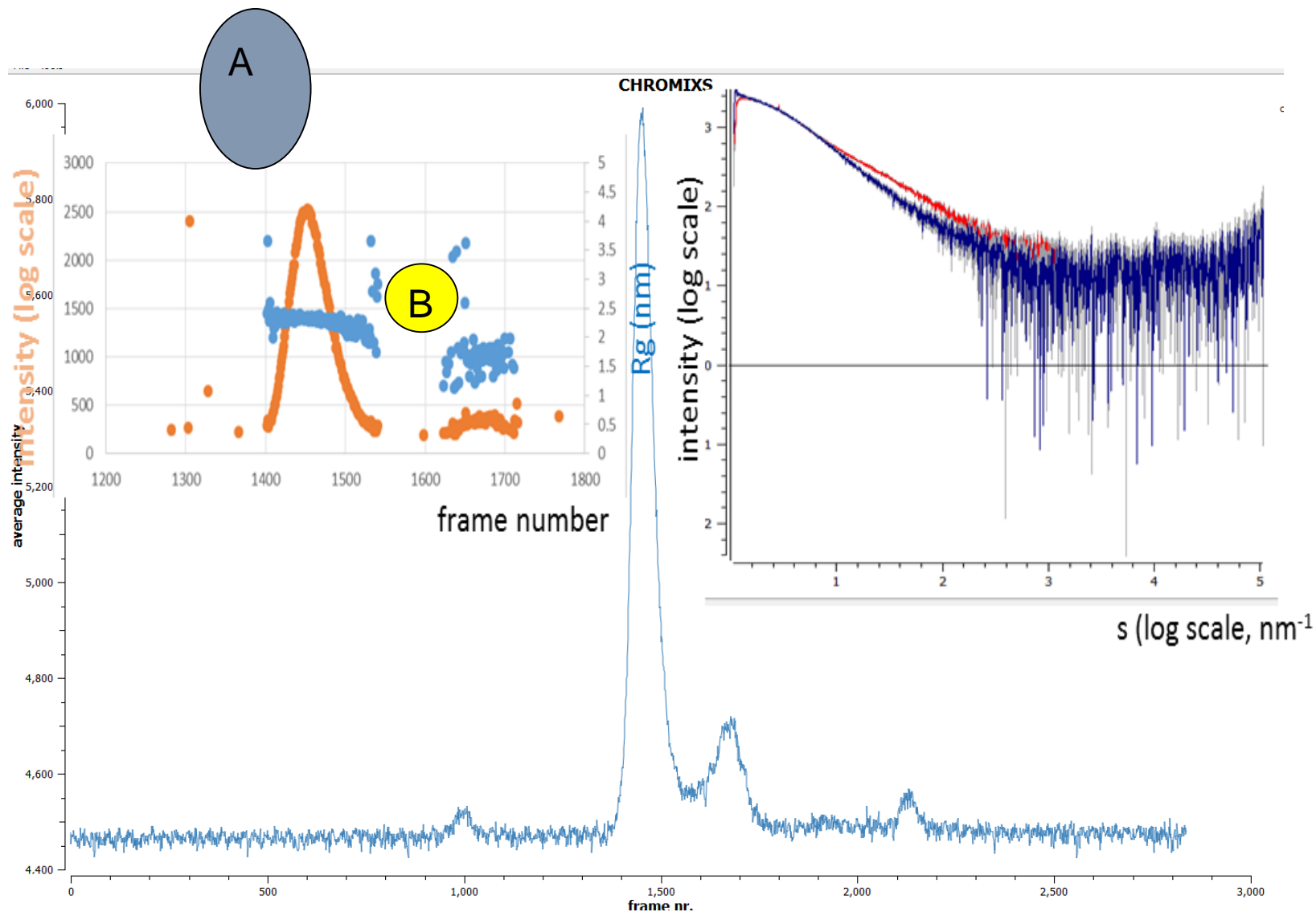
Why NOT TO DO SEC - SAXS(/MALLS)?



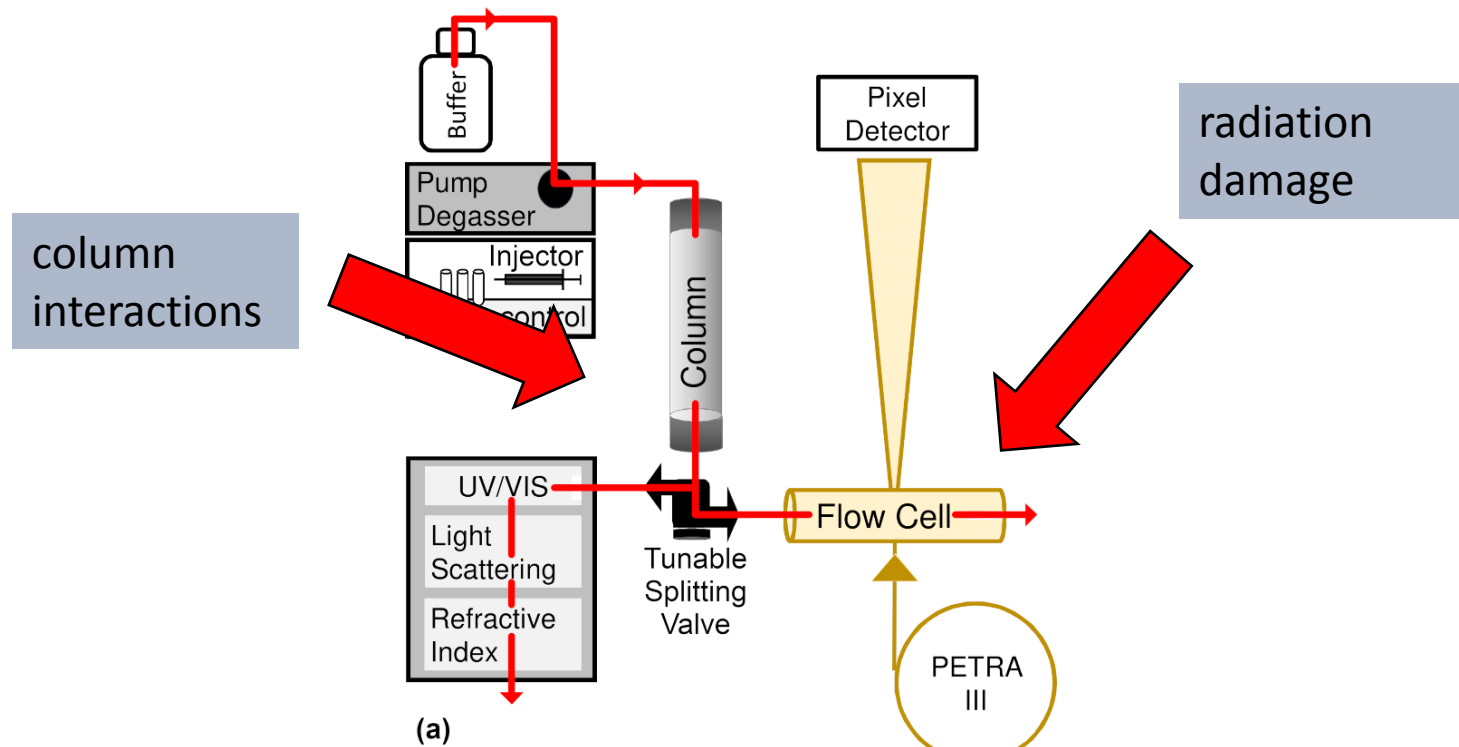
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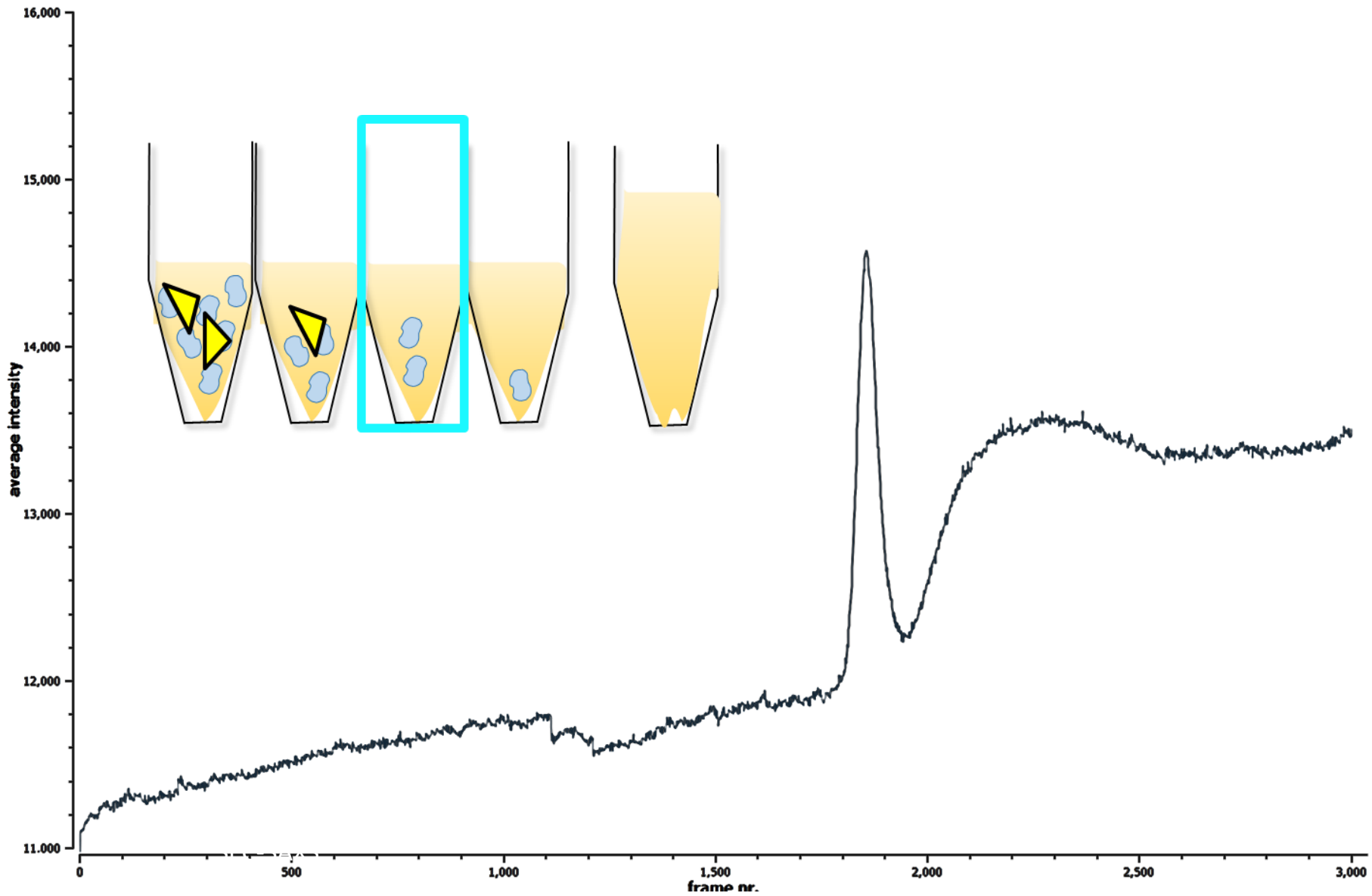


Why NOT TO DO SEC - SAXS(/MALLS)?

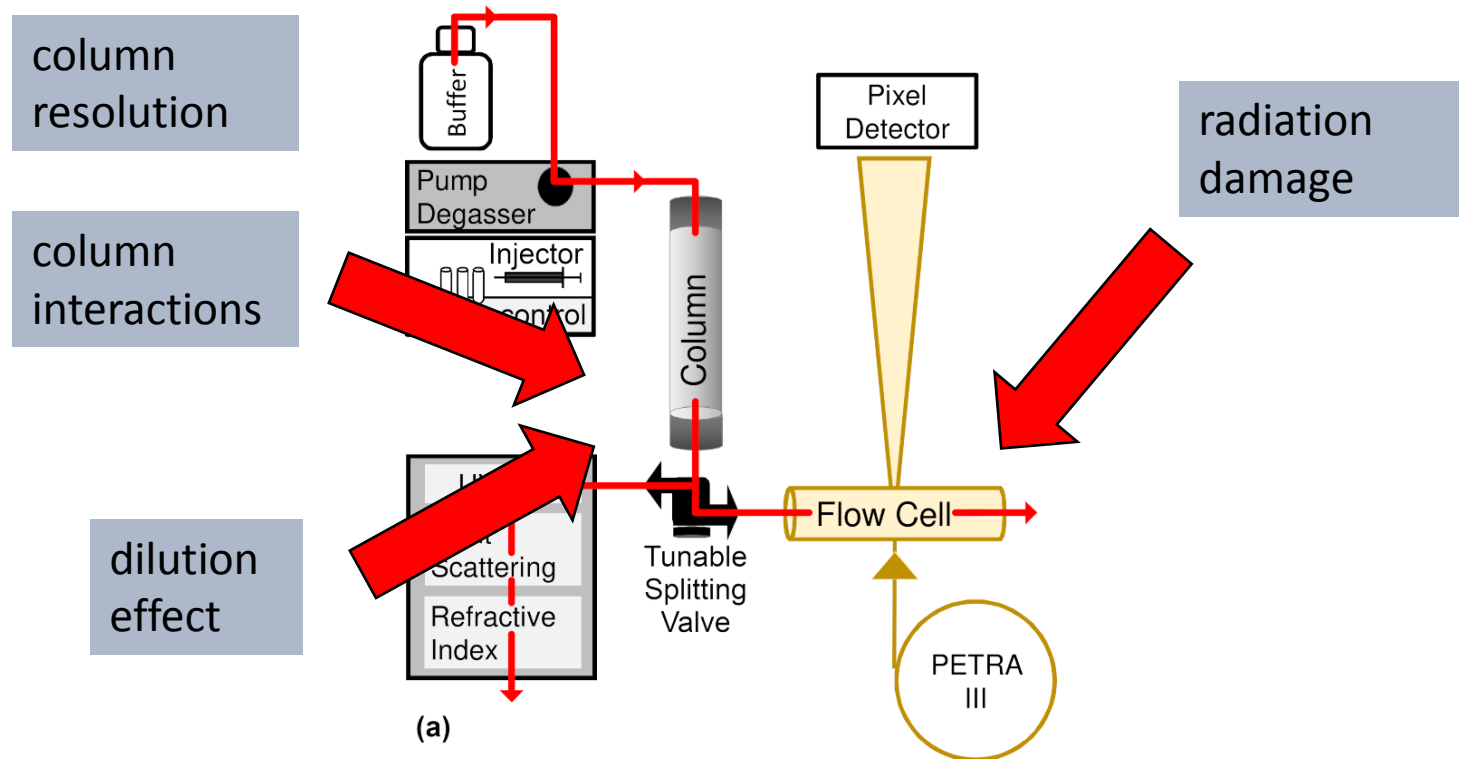


CHROMIXS

— chromix data

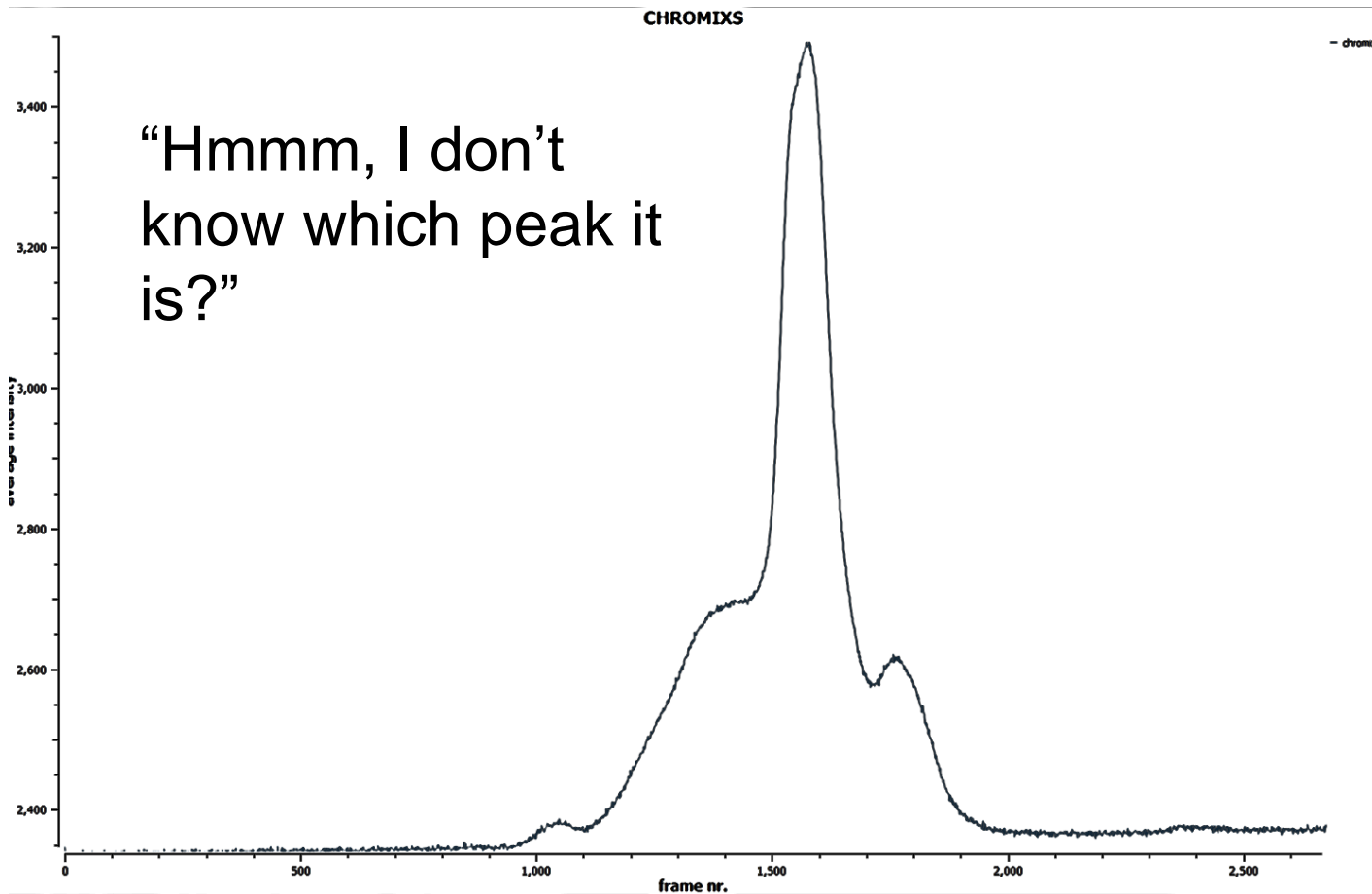


Why NOT TO DO SEC - SAXS(/MALLS)?



Why NOT TO DO SEC - SAXS(/MALLS)?

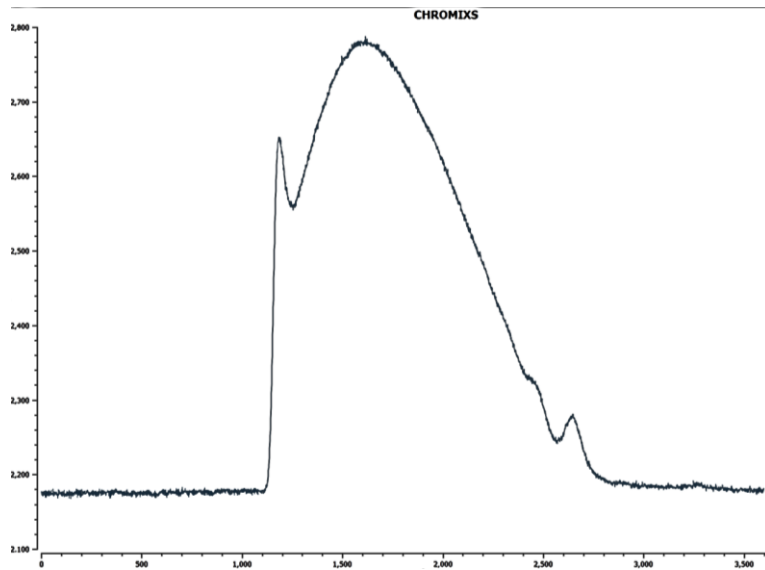
- There are some limits!



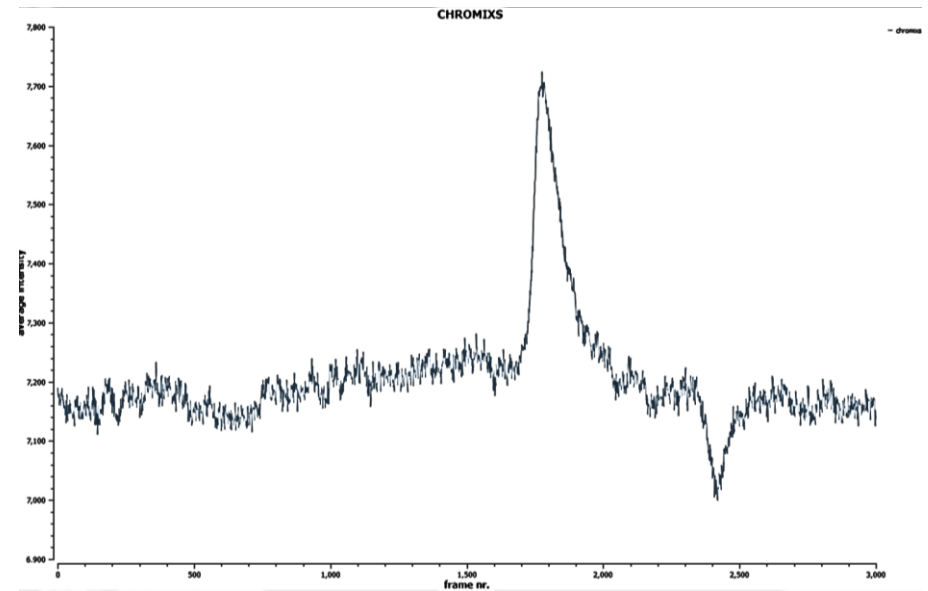
Why NOT TO DO SEC - SAXS(/MALLS)?

- There are some limits!

1. Trip:



2. Trip:



When performing SEC-SAXS(+MALLS)?

Remember

- “ideal sample”

 - **Pre-analysis of sample** is very important; optimize SEC conditions

- not quite pure sample

 - **SEC-SAXS is analytical!** Not preparative!

- **radiation damage** can be an issue

 - Measure batch sample as well, **add scavengers**

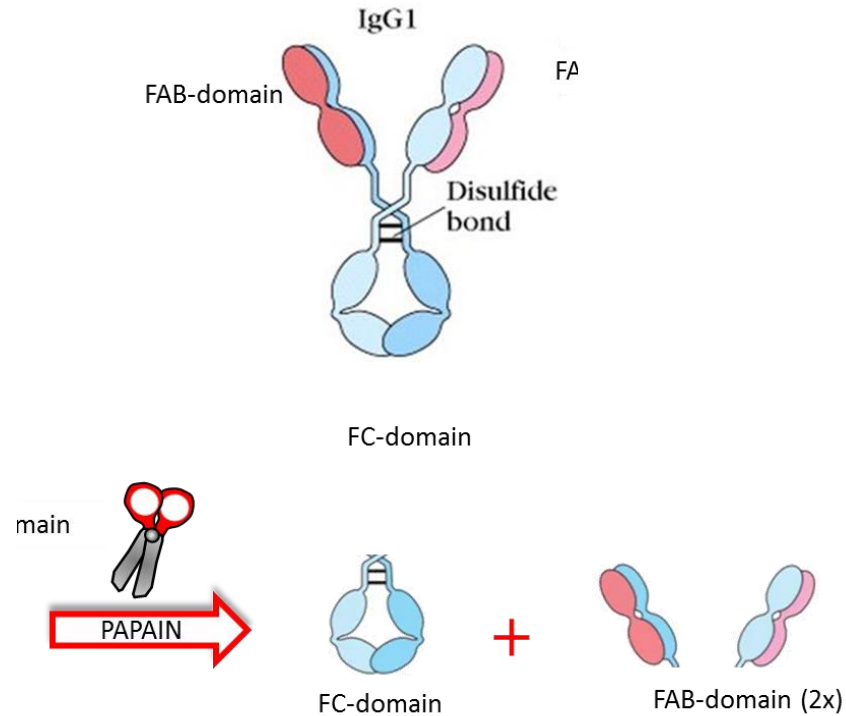
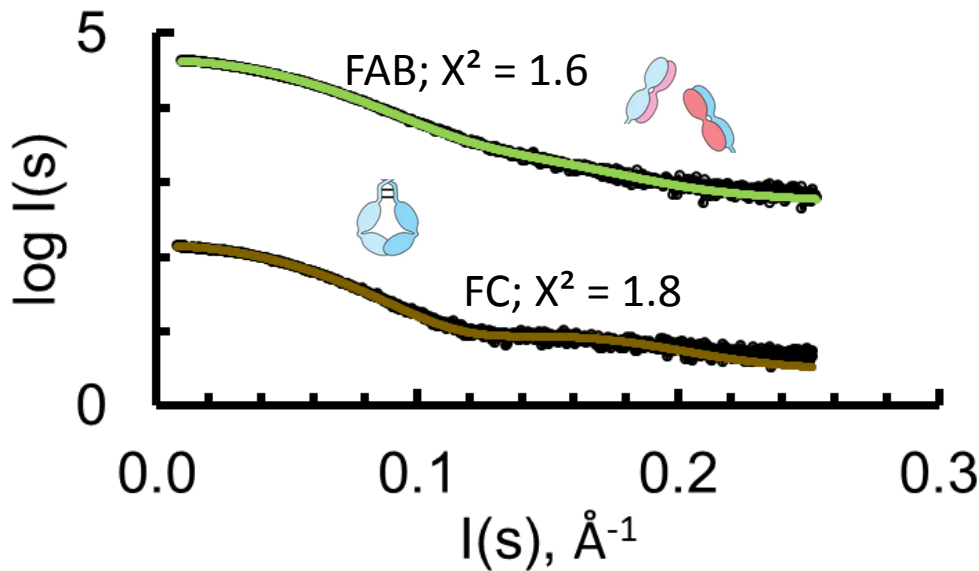
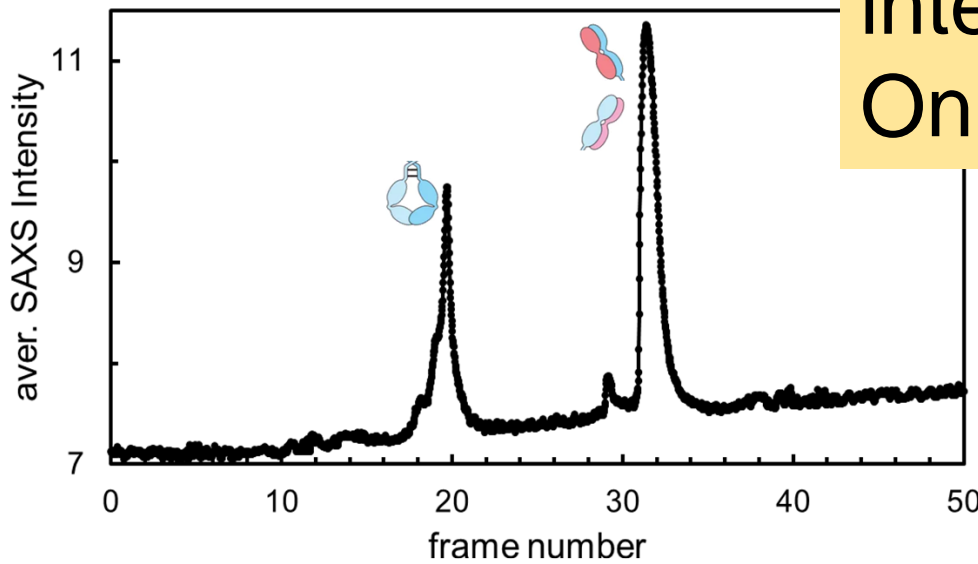
- sample stability, **low affinity** complexes

 - sample can be altered with column interaction

New SAXS tools for the character

Proof of principle: Analysis of IgG1

Contact us if you are interested in performing Online IEC



*these are similar in size and low in concentration (classic SAXS/SEC-SAXS not possible)

Aknowledgements

EMBL Hamburg:

SAXS group

Instrumentation team

User Office

The SAXS community



Beamline:
Clement
Martin
Andrey

Software:
Al, Dima, Daniel, Nelly

IEC:
Taja

HPLC:
Cy
Stefano
Tobi
Haydyn

Funding:

