

Biophysical Sample Characterization at P12 and at EMBL's Sample Preparation and Characterization (SPC) Facility

P12 User Meeting 2020

Tobias Gräwert

Lots of details = reference for later
(slides will be available)

Yellow sections highlight relevance
for SAXS.

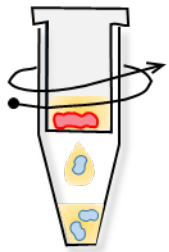
Biophysical Sample Characterization at P12:

- Thermo Scientific NanoDrop ND-1000
- Anton Paar Abbemat 550 refractometer
- Wyatt DynaPro NanoStar DLS
- Wyatt Minidawn Treos
- Wyatt Optilab T-rex
- Biologic SFM 400 Stopped Flow (SAXS / fluorimeter)

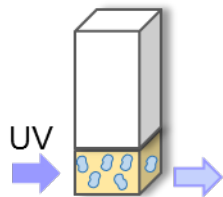
Biophysical Sample Characterization at P12:

- Thermo Scientific NanoDrop ND-1000

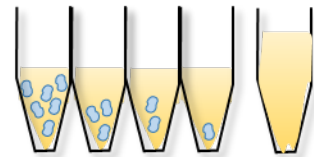
- Sample concentration
- UV / Vis spectra
- $\approx 2 \mu\text{L}$ required, can be retrieved



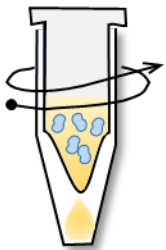
Filter



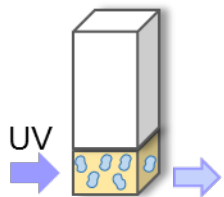
Conc. determination



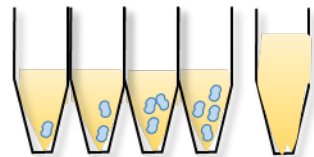
SAXS from dilutions & buffer



Step-wise concentration



Conc. determination



SAXS from different concentration steps & buffer



Re-Check Concentration

- after Removal of Aggregates
- after Dilutions
- after Concentration step

Biophysical Sample Characterization at P12:

- Anton Paar Abbemat 550 refractometer
 - Sample concentration
 - $\approx 4 \mu\text{L}$ required, can be retrieved

Measure Concentration:

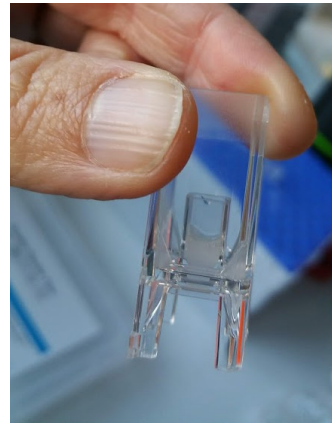
- of complexes with unknown stoichiometry
- conjugated proteins
- Proteins with unknown/low Extinction coefficient e.g. Calmodulin



Biophysical Sample Characterization at P12:

- Wyatt DynaPro NanoStar DLS
 - Hydrodynamic radius
 - Determination of aggregation temperature between 5°C and 80°C
 - $\approx 7 \mu\text{L}$ of sample at $>0.25 \text{ mg/mL}$, can be retrieved

- Presence of aggregates
- Check for monodispersity
- Comparison studies
- R_G/R_H ratio



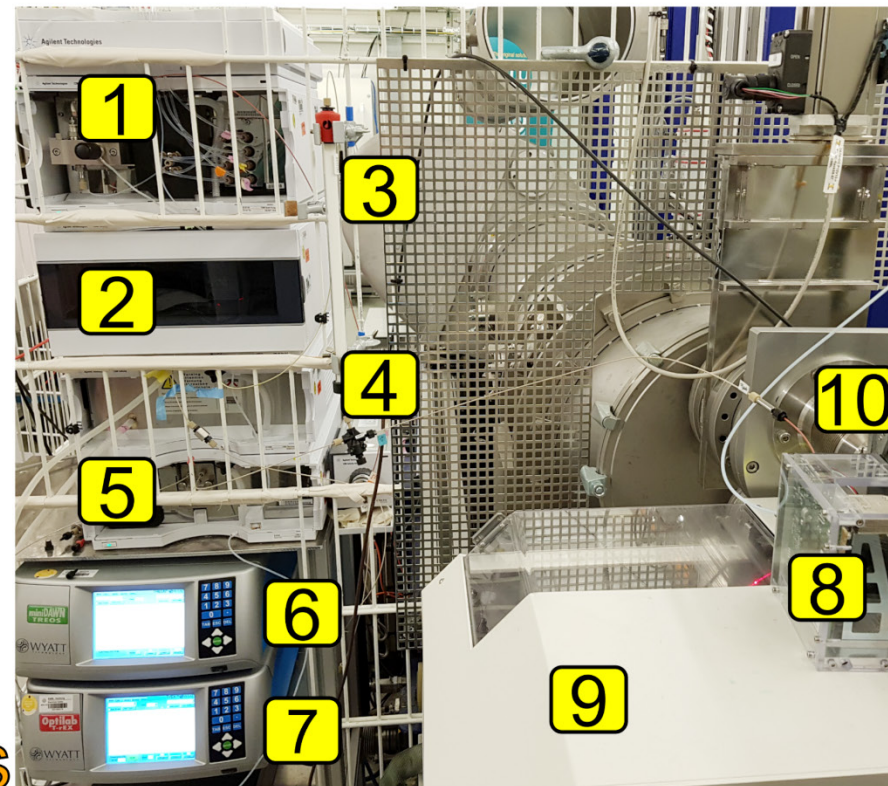
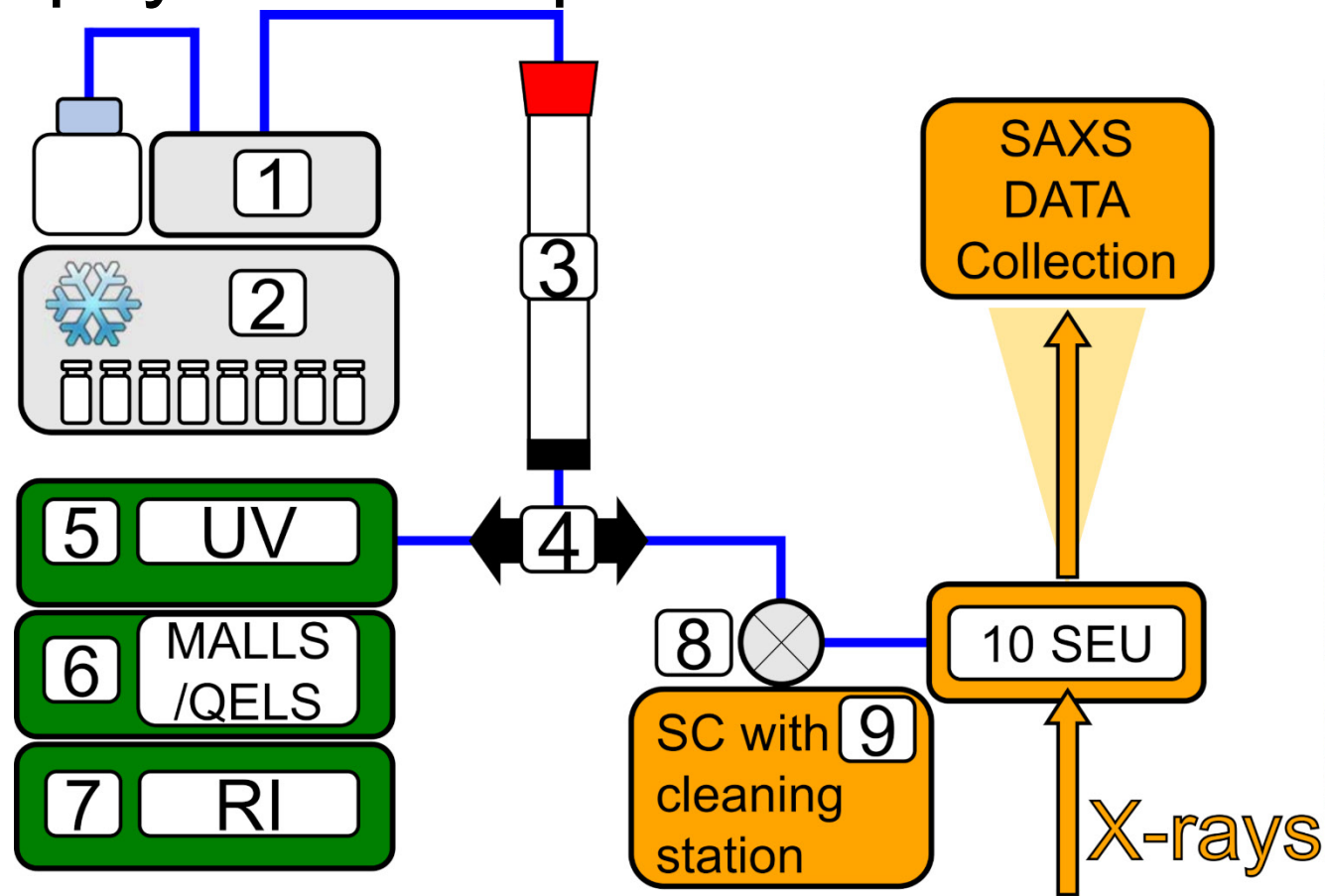
Biophysical Sample Characterization at P12:

- Wyatt Minidawn Treos / Wyatt Minidawn Treos
 - DLS and MAL(L)S / Refractive index
 - In-line with SEC
 - Parallel to SAXS
 - Molar mass
 - Size
 - Conformation

- Add to SEC-SAXS
 - Independent MW determination
 - R_G to R_H ratio
 - Assess baseline stability



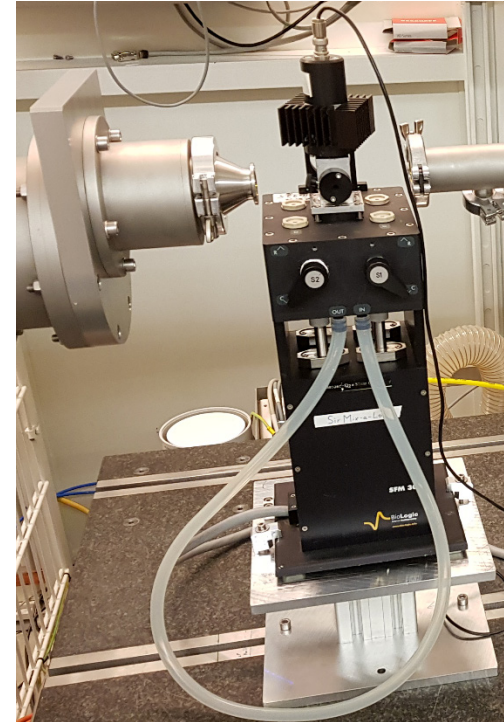
Biophysical Sample Characterization at P12:



- | | |
|---|--|
| (1) 1260 Infinity II bio-inert LC | (6) miniDAWN® TREOS® MALLS / Wyatt QELS module |
| (2) Agilent temperature controlled injector | (7) Optilab® T-rEX (refractometer) |
| (3) (Exchangeable) SEC column | (8) Valve for switching between data collection mode |
| (4) Micro splitter | (9) P12 Sample changer with cleaning station |
| (5) Agilent UV detector | (10) Sample exposure unit |

Biophysical Sample Characterization at P12:

- Biologic SFM 400 Stopped Flow
 - Detection with SAXS or Fluorimeter
 - Mixing of 2 – 4 solutions
 - Min (sensible) volume 1 mL
 - Dead time app. 7 ms
- Alternative set-up available in the SPC



Biophysical Sample Characterization at SPC:



1)

MALDI TOF: Bruker/CovalX



2)

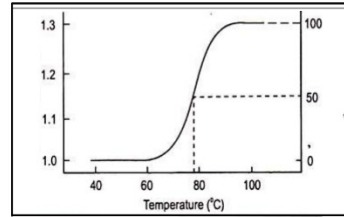
Thermofluor



3)

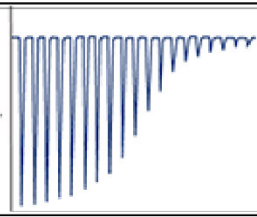
Differential Scanning Fluorimetry (DSF)
Promethius 48
Nanotemper

Characterization



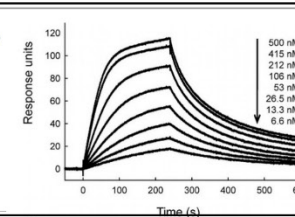
1. MS
2. TF
3. nDSF
4. DLS
5. CD
6. FT-IR
7. MP

Interaction



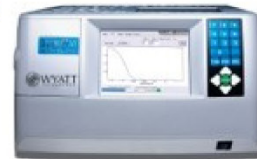
8. ITC
9. MST

Kinetics



10. SPR
11. Interferometry
12. Stopped-flow

4)



Dynamic light scattering
Wyatt

6)



Infrared spectroscopy (FT-IR)
Bruker Vertex 70

5)



Circular Dichroism
AppliedPhotophysics

7)



Mass photometry
Refeyn One

8)



Isothermal titration calorimetry
MicroCal

9)



Microscale Thermophoresis
Monolith NT.115 and NT.LabelFree
Nanotemper

10)



Biacore T200 GE

11)



Biofort Octet Red96

12)



Stopped-flow
AppliedPhotophysics

Biophysical Sample Characterization at SPC:

Applying for / conduction of additional characterization / purification

- 1) During proposal submission
- 2) Planning beamtime
- 3) Feed back form (track keeping for statistics / further funding)

Questions: spc@embl-hamburg.de

<https://www.embl-hamburg.de/services/spc/Services-and-resources/index.html>

Booking of SPC equipment via iLAB

Biophysical Sample Characterization at SPC:

1) During Proposal (→ see talk from M. Vanoni)

EMBL User Portal - v3.16.24.2

Connected as: Melissa Ann GRAEWERT | Umbrella | Home | FAQ | Contact | Sign o

Accounts ▾ Proposals/Experiments ▾ Safety ▾

Application for a Proposal at the EMBL - BioSAXS Beam Time

New proposal

General Information | Societal Theme | Beamlines Request | Proposers | **Laboratory Support Facility** | Sample Environment | Sample Description | Experience | Publications

Laboratory Support Facility

- DLS
- FPLC
- Refractometer
- SLS

- DLS (Wyatt Machine in the User lab)
- FPLC = AKTA in SPC Cold ROOM
- Refractometer (Anton Paar Machie in the User lab)
- SLS = MALLS in P12 Hutch

Please complete information requir

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* = Mandatory Field

Biophysical Sample Characterization at SPC:

1) During Proposal

EMBL User Portal - v3.16.24.2

Connected as: Melissa Ann GRAEWERT | Umbrella | Home | FAQ | Contact | Sign out | Hide

Accounts | Proposals/Experiments | Safety

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Application for a Proposal at the EMBL - BioSAXS Beam Time

New proposal

General Information | Societal Theme | Beamlines Request | Proposers | Laboratory Support Facility | **Sample Environment** | Sample Description | Experience | Publications

Items Supplied by EMBL

Solution scattering experiments

- BioSAXS with automated robotic sample changer. 25 ul sample; 0.2-10 mg/ml; 20 x 50 ms exposures (1 s total); 1 min turnover (loading, measurement, cleaning). Temperature 5-50C. Remote and mail-in measurement possible
- Standard size exclusion chromatography (SEC)-SAXS with or without UV. FPLC or HPLC SEC column connected directly to the beam line. Requires 45-80 min per experiment. Maximum 100 ul injection (recommend 50-100 ul sample at 5-10 mg/ml). Room temperature only
- Size exclusion chromatography (SEC)-SAXS with with parallel MALLS, DLS, RI and UV detection for molecular weight validation and hydrodynamic radius measurements (Wyatt TREOS/QELS/T-rex system. Collaborative projects only. Please fill out section 4 of [THIS FORM](#)). Room temperature only

Non-standard ("in air") sample environments:

- No "Non-standard ("in air")" sample environment required
- Temperature controlled (5-95C) capillary holder
- Stopped flow, time resolved setup. temperature: 5-80C (on collaborative basis only)
- Other (specify, for example your own tailored sample environment):

- We plan to use lasers
- For scattering vectors outside the standard range, $s = 0.05\text{-}4\text{ nm}^{-1}$ (e.g., WAXS, please fill out section 5 of [THIS FORM](#))
- Anomalous SAXS, ASAXS ((5)7-20 keV; Collaborative only)

• Access during beamtime can not be guaranteed if not selected in proposal (not all contacts trained on MALLS)

Please complete information required under all Tabs before clicking on the 'Save' button.

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Biophysical Sample Characterization at SPC:

2) Invitation

Invitation for Experiment

Experiment Number	SAXS-996
Experiment title	"Methods development at P12 (inhouse)"
Beamline:	P12
Start date and time:	25 April 2020 at 10:00
End date and time:	25 April 2020 at 18:00
Scheduled shifts:	1.0

DACHS cards

To operate the EMBL beamlines on PETRA, every user needs to have their own DACHS card. If any members of your user group do not have a DACHS card yet, please indicate this in the A Form.

DACHS cards are issued from the EMBL user office on the first floor of building 48e (Mon-Fri, 9:00-17:00). Outside of office hours, the cards can be collected from the reception at the main entrance to DESY.

SPC Facility

The projects requiring extensive wetlab resources (e.g. FPLC, DLS etc.) may use the integrated sample preparation and characterization (SPC) facility of the EMBL at PETRA III, consult the rules and the list of available equipment on the SPC website [here](#).

To use the facility the users MUST send an email to spc@embl-hamburg.de with a CC to the first local contact, listing the required equipment TWO WEEKS BEFORE THE EXPERIMENT AT THE LATEST. Please note that the users, who did not book the facility in advance, will NOT be able to use it.

Mail-in

For the mail-in access, both batch and SEC-SAXS measurements can be conducted (not more than 10 samples/constructs for batch and not more than 4 samples/constructs for SEC-SAXS). For batch mode, stock solutions or fully prepared/diluted samples can be sent. For SEC-SAXS operation, the stock solutions must be concentrated to at least 7 mg/ml. In all cases, sufficient amounts of matching buffer(s) are required. See <https://www.embl-hamburg.de/biosaxs/sample.html> for further information. Please send an email to your local contact if you wish to use the mail-in option.

As PI make sure
this information is
passed on to the
on-site Users.

Biophysical Sample Characterization at SPC:

2) Invitation

Melissa

On 02 October 2020 at 12:36 useroffice@embl-hamburg.de wrote:

EMBL User Office
Notkestrasse 85, Bldg. 48e
22607 Hamburg
Germany
Tel: +49-40-89902-111 / 183
Fax: +49-40-89902-260
Email: useroffice@embl-hamburg.de

Dr Melissa Ann GRAEWERT
EUROPEAN MOLECULAR BIOLOGY LABORATORY (EMBL)

Notkestrasse 85

22607 HAMBURG
GERMANY

COVID-19 era.

In the current mail in mode
SPC access is not mentioned.
Please contact the SPC
directly to organize in parallel
characterization of samples:
spc@embl-hamburg.de

**BTW, SPC is also accessible
through iNEXT discovery**

Invitation for Mail-In Experiment

Biophysical Sample Characterization at SPC:

3) Feed back form

Submit your End of shift form | Save your draft

Proposal details

ID: SAXS-1075 | Title: Structural bases of retinoic acid nuclear receptor gene regulation

Experiment details

A-Form ID: | Beamline: | Scheduled Beamtime (in hours): 8

Actual Start Time: | Actual End Time: | Extra time scheduled (in hours):

Did you use the Sample Preparation and Characterisation (SPC) facility? * Yes No

INEXT-Discovery

INEXT-Discovery proposal ID: | Hours claimed: |

Visit details

Type of Operation: --> Select

Arrival date: | Departure date: |

Local Contact: --> Select

Please evaluate the support from your local contact and members of staff: --> Select

Indication of use is important for SPC statistics and with that future funding!

Biophysical Sample Characterization at SPC:

- Bruker MALDI-TOF with additional Covalex detector
- BioRad MyIQ ThermoFluor
- NanoTemper Technologies Prometheus NT.48 Differential Scanning Fluorimeter
- Applied Photophysics Chirascan CD spectrometer
- Bruker VERTEX 70v FTIR Spectrometer
- Refeyn OneMP Mass Photometer
- Malvern MicroCal PEAQ-ITC
- NanoTemper Monolith NT.115 & LabelFree Microscale Thermophoresis
- Biacore T200 GE SPR
- ForteBio Octet Red96 Interferometer
- Applied Photophysics Stopped-Flow Spectrometer

Biophysical Sample Characterization at SPC:

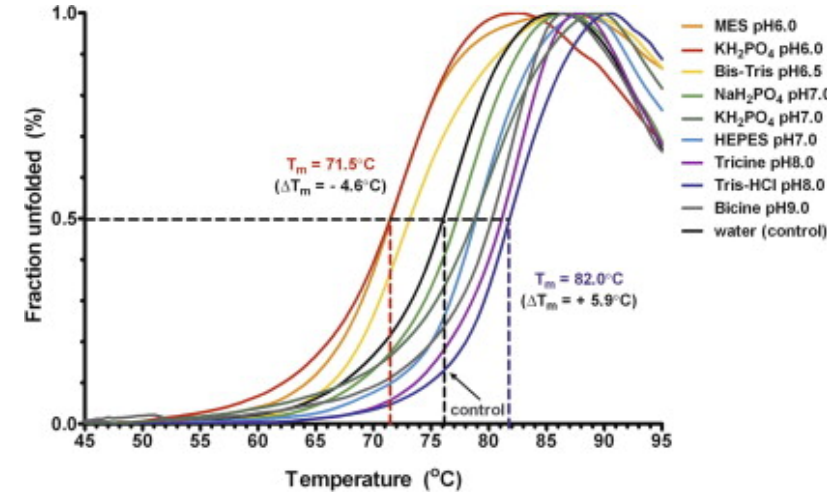
- Bruker MALDI-TOF with additional Covalex detector
 - Determination of the Molecular Mass of your protein sample.
 - CovalX HM4 High-Mass System
 - Protein complex analysis
 - Intact Protein Analysis (up to 2 MDa)



Biophysical Sample Characterization at SPC:

- BioRad MyIQ ThermoFluor

- Incubated with a dye binding hydrophobic patches
- Patches become highly fluorescent
- Protein is gradually heated to slowly unfold
- Exposed hydrophobic patches increase fluorescence
- 220 μl of pure protein at $\approx 20 \mu\text{M}$.
- Recommended sample buffer: buffer concentration ($<200 \text{ mM}$), NaCl ($<200 \text{ mM}$), Glycerol ($<10\%$), no stabilizing reagent (DTT, TCEP, etc.), free of detergent.



Identification of best buffer!

Get your protein to the beamline without stability issues!



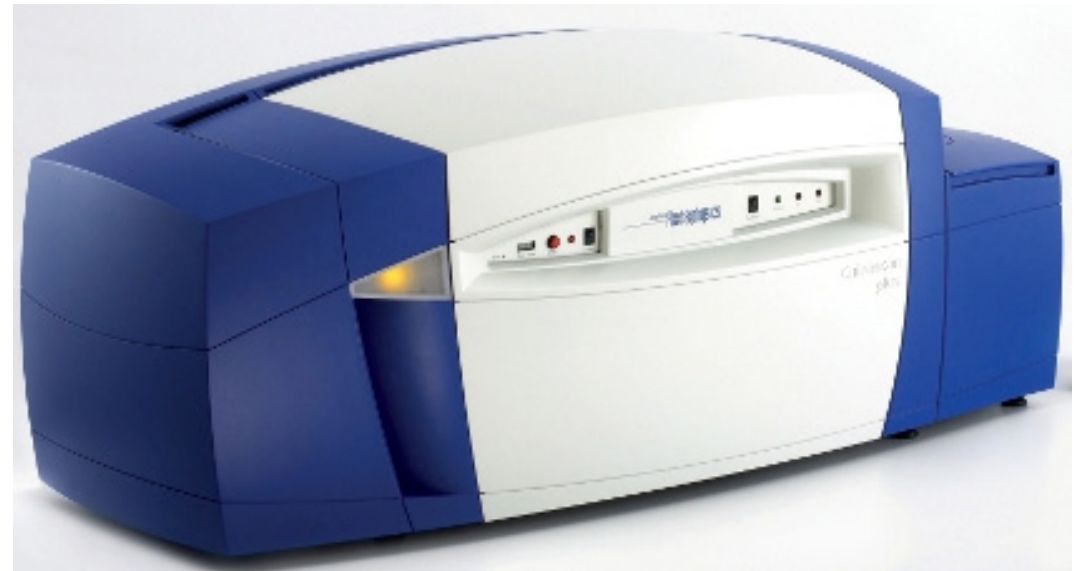
Biophysical Sample Characterization at SPC:

- NanoTemper Technologies Prometheus NT.48 Differential Scanning Fluorimeter
 - Ultra-high resolution protein stability measurement
 - Intrinsic tryptophan or tyrosine fluorescence
 - 210 μL of protein containing 2-3 tryptophans
 - Maximal protein concentration (standard IgG) $> 150 \text{ mg/mL}$
 - Minimal protein concentration (standard IgG) $5 \mu\text{g/mL}$



Biophysical Sample Characterization at SPC:

- Applied Photophysics Chirascan CD spectrometer
 - Estimate protein secondary structure content
 - Detect conformational changes (buffer composition / mutations)
 - Assess thermal or chemical stability
 - Analyze macromolecule-ligand interactions
 - Excellent quality scans down to 190 nm
 - $\approx 200 \mu\text{L}$ of sample at $\approx 0.5 \text{ mg/mL}$



Biophysical Sample Characterization at SPC:

- Bruker VERTEX 70v FTIR Spectrometer
 - Acquire complete far and mid IR spectra from 6000 cm^{-1} to 50 cm^{-1} in a single step measurement
 - Determination of structural changes
 - Measurement of temperature ramps
 - Concentration determination
 - Secondary structure analysis
 - Protein-ligand-interaction



Biophysical Sample Characterization at SPC:

- Refeyn OneMP Mass Photometer
 - Mass measurement at the single molecule level
 - Structure (oligomeric state, modification)
 - Homogeneity
 - Quantifying interactions
 - „Tiny“ amounts of sample



Biophysical Sample Characterization at SPC:

- Malvern MicroCal PEAQ-ITC
 - Affinity (K_D) through enthalpy ΔH / entropy ΔS
 - Cell volume: 300 μL
 - Injection syringe volume: 40 μL
 - Injection volume precision: < 1% @ 2 μL
 - Sample throughput: 0 - 12 per 8 h day



Biophysical Sample Characterization at SPC:

- NanoTemper Monolith NT.115 & NT.LabelFree Microscale Thermophoresis
 - Interactions between all types of biomolecules
 - From ions to ribosomes and for pM to mM binding affinities
- NT.LabelFree
 - Utilizes the intrinsic tryptophan fluorescence
 - Real label-free and immobilization-free experiments
 - Reflects binding of the protein in its native state
- NT.115
 - broad range of fluorophores and fluorescent proteins
 - Buffer independency: including serum or cell lysate
 - Purification free for fluorescent fusion proteins



Biophysical Sample Characterization at SPC:

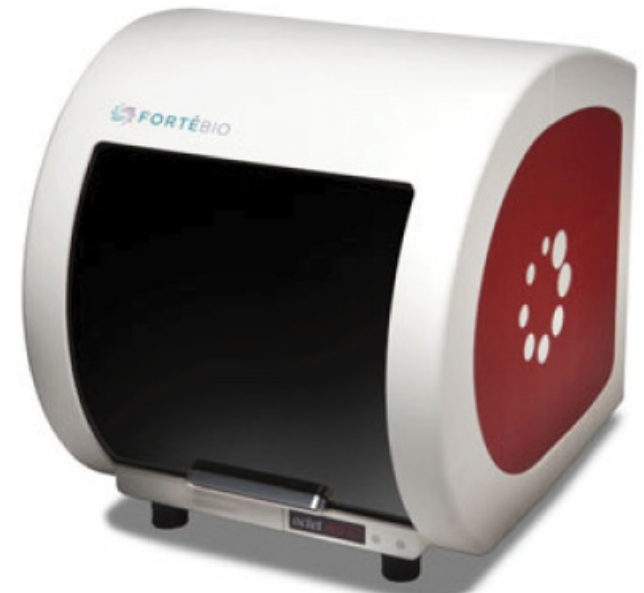
- Biacore T200 GE SPR
 - Biomolecules in native forms
 - No label or tag
 - Measurement of k_{on} , k_{off} , K_D



Biophysical Sample Characterization at SPC:

- ForteBio Octet Red96 Interferometer

- 96-well characterization of protein-protein and protein-small molecule binding kinetics
- Determination of protein concentrations and titer
- 25 ng/mL to 2000 $\mu\text{g/mL}$ of human IgG (using Protein A biosensors)

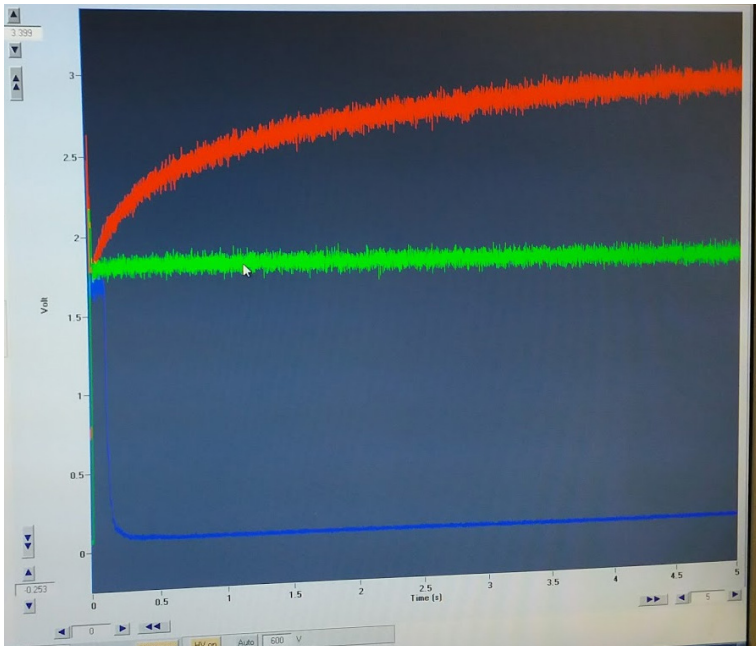


Biophysical Sample Characterization at SPC:

- Applied Photophysics Stopped-Flow Spectrometer

- Fast kinetics, dead time 1 ms
- Protein-protein interactions
- Ligand binding
- Enzyme, chemical or coordination reactions

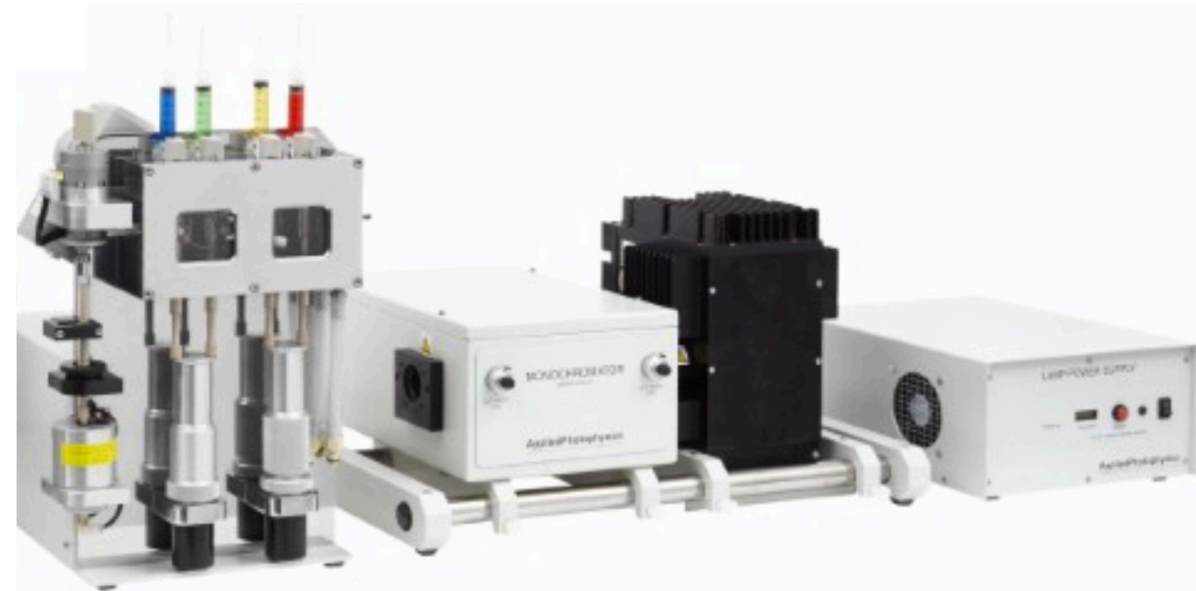
If you are planning TR-SAXS this could be a good option to pre-characterize your system.



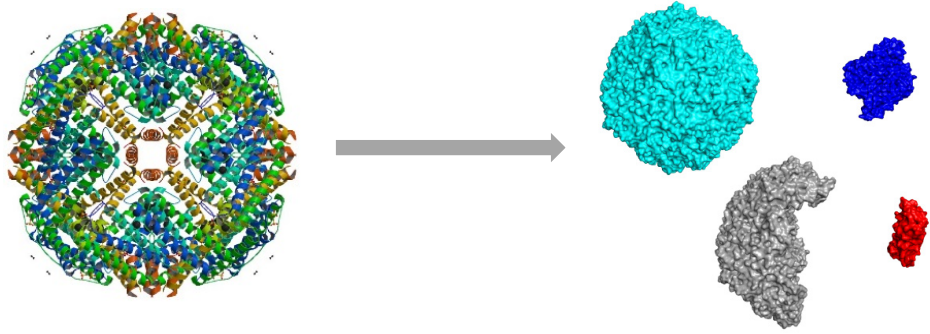
Apoferritin + HCl

Apoferritin

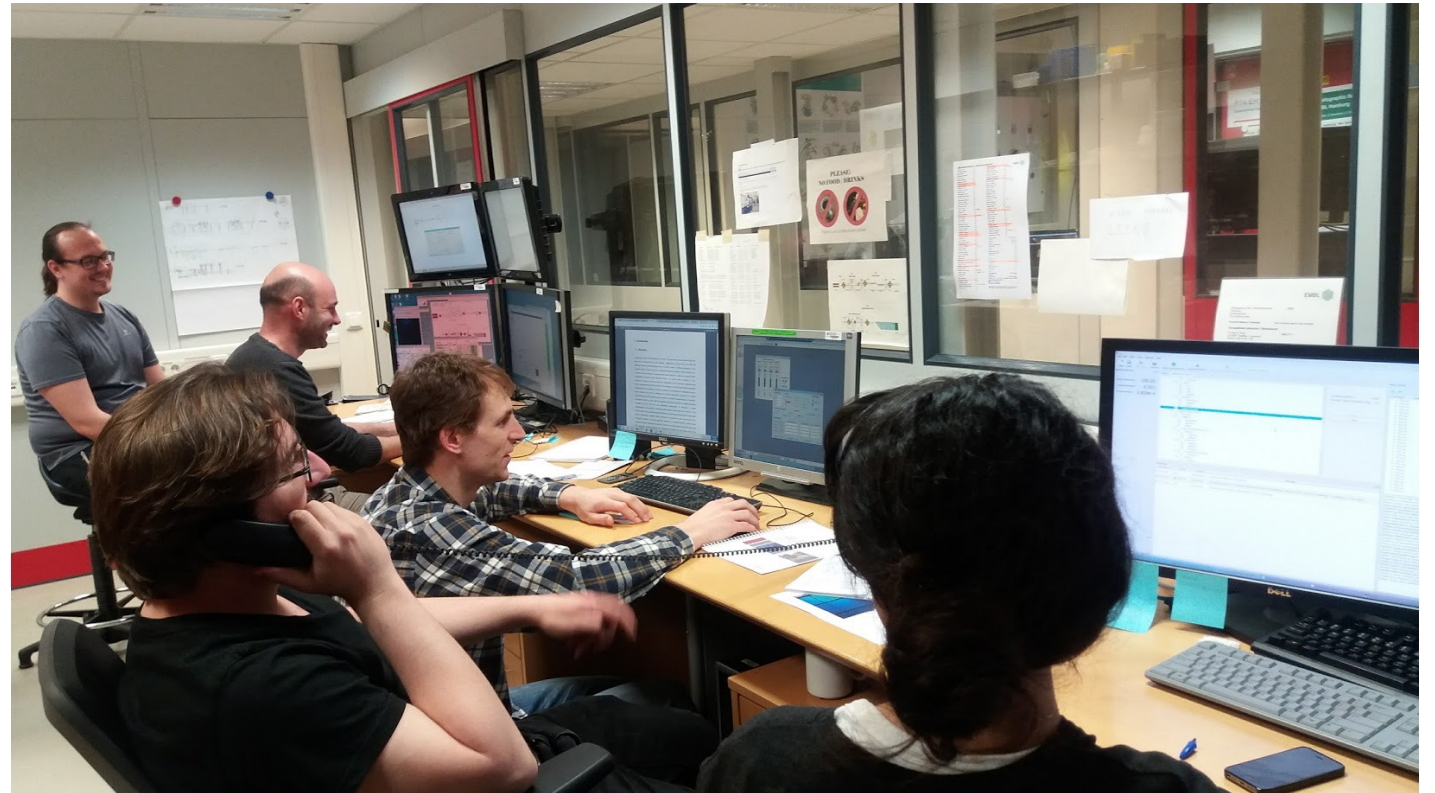
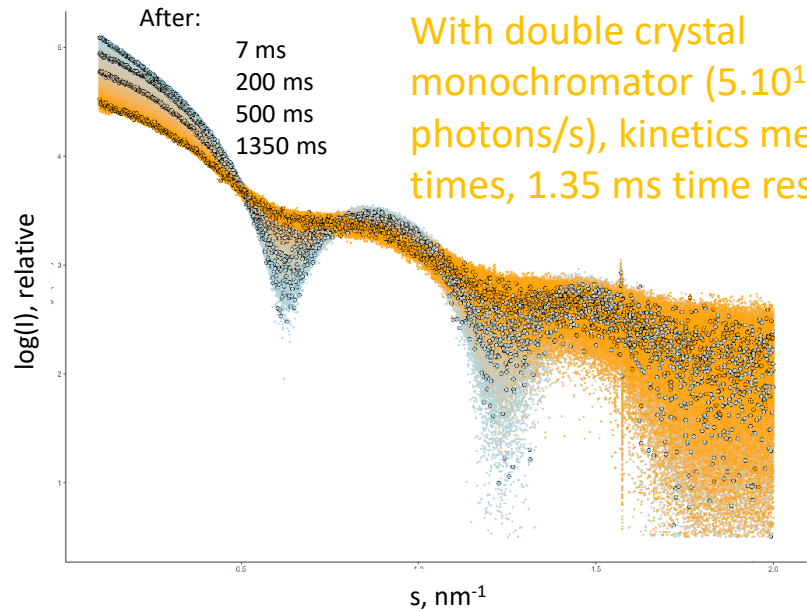
Buffer



Time resolved SAXS



Dissociation of Apoferritin by pH jump



BIOSAXS

The original solution

<http://biosaxs.com>
info@biosaxs.com

Please contact BIOSAXS GmbH
for proprietary / industry SAXS measurements @P12!