MOSBRI course: Quality control for Integral Membrane Proteins

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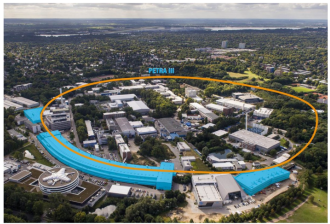
EMBL

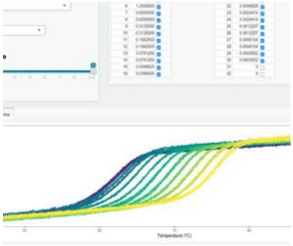
SPC facility @ EMBL Hamburg

High-throughput crystallization

Protein Characterization
(Molecular Biophysics)



EMBL SPC Services: Consulting, training, data analysis



HTX laboratory

- Customised crystallisation for soluble and membrane proteins
- CRIMS, CrystalDirect & CD-Harvester

Biophysics platform

- Interactions, stability and size of biomolecular assemblies
- Cutting-edge technologies: ITC, MS, CD, DSF, MST, DLS, FTIR, SPR, BLI, MP

Data analysis platform

- User friendly web server for the analysis of biophysical experiments
- Cross-platform, requires only browser

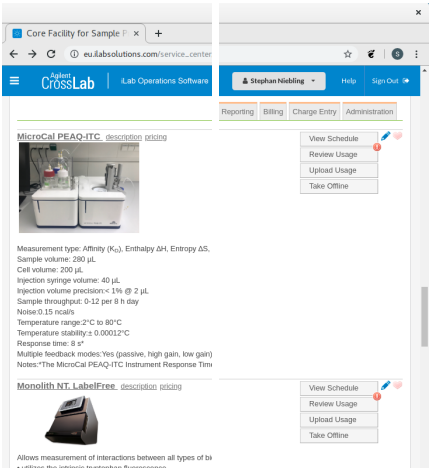
Access to our core facility is easy!

Easy access for everyone

1. Contact us via spc@embl-hamburg.de
2. You will get an access for our booking system (iLab)
3. Arrange a training
4. Book machines and measure

Flexible services and low costs

- Consulting
- Training
- Help with data analysis
- We mostly only charge consumables!



Funding possibilities for access to SPC

MOSBRI

- Trans-national access to 13 European biophysical labs
- EMBL-SPC access (+SAXS)

iNEXT Discovery

- Access to structural biology research infrastructures
- EMBL-HH: Acces to MX and SAXS

Instruct ERIC

- pan-European research infrastructure in structural biology
- EMBL-HH: Acces to Biophysics, MX, SAXS

MOSBRI Molecular-Scale Biophysics Research Infrastructure

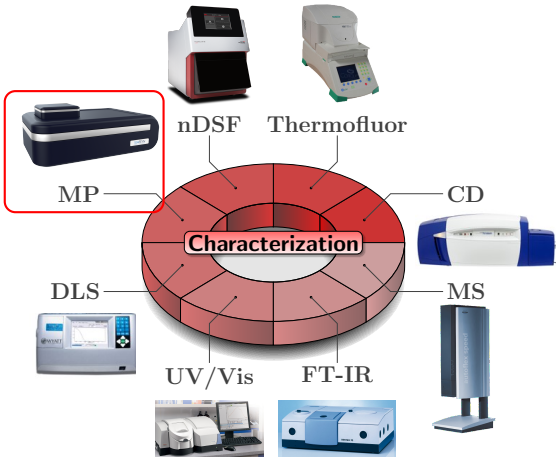
iNEXT DISCOVERY

instruct ERIC

Hanseatic League of Science
Interconnecting infrastructures for life science research and innovation
HALOS

DFG Deutsche Forschungsgemeinschaft

Current status of the biophysics facility



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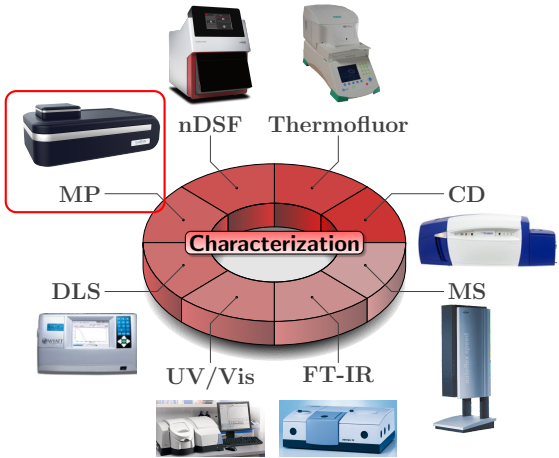
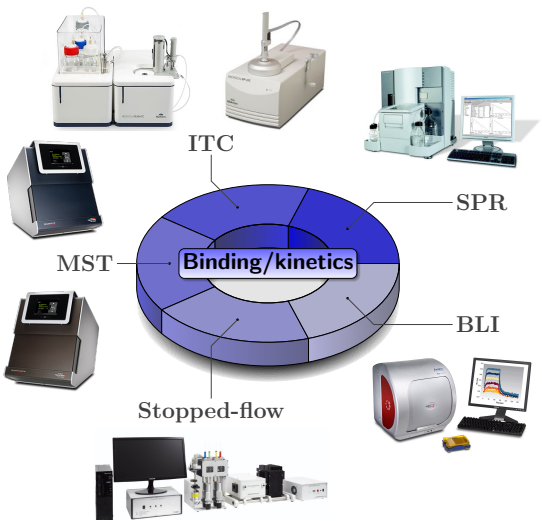




SPC: Sample preparation and characterization facility

Characterization of biomolecules and assemblies

- Size
- Shape
- Stability
- Interaction

Wide range of methods

- Initial quality control
- More sophisticated biophysical characterization
  - e.g. time-resolved methods

→ **Open to internal+external researchers**

EMBL SPC data analysis platform

Common problems for users

- Proprietary software for data analysis is not acessible
- Data analysis with general tools is limited without programming skills

→ Offer data analysis web server for users

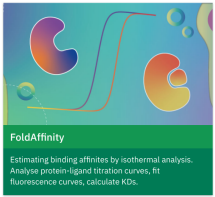
Advantages of webserver

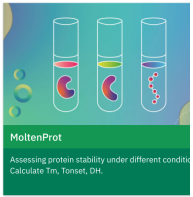
- Requires only browser
- No installation necessary
- Cross-platform (Windows, MacOS, Linux)

SPC Data Analytics

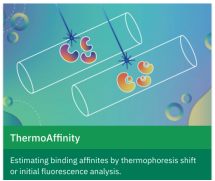
The EMBL Sample Preparation and Characterisation (SPC) Data Analytics Webserver provides easy to use software for the understanding of biophysical experiments.

Differential Scanning Fluorimetry

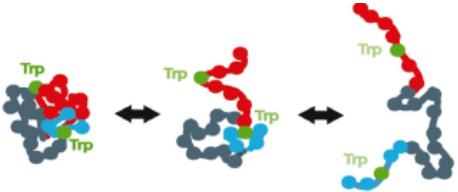


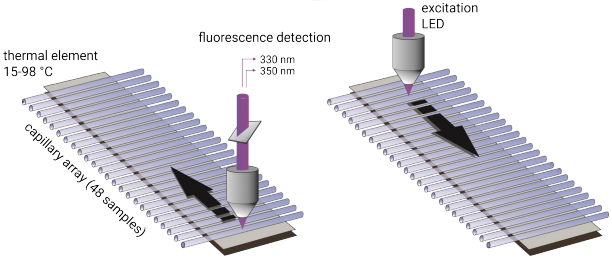


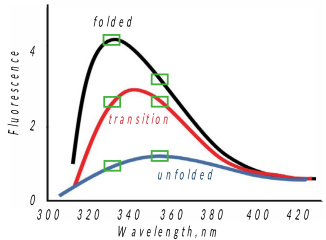
MicroScale Thermophoresis



nDSF: nano differential scanning fluorimetry







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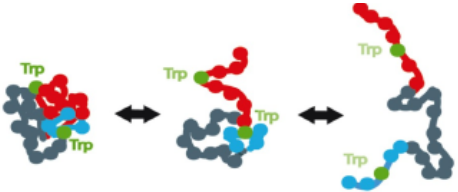

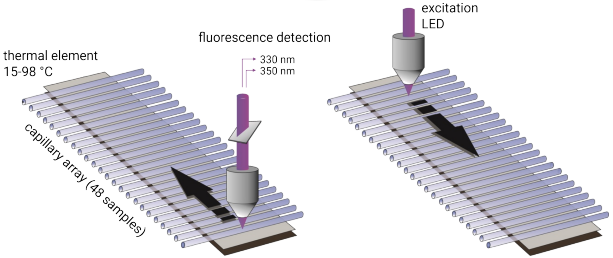

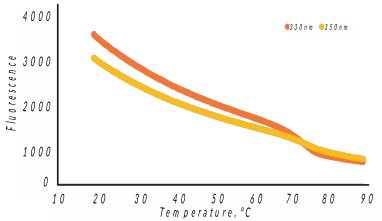

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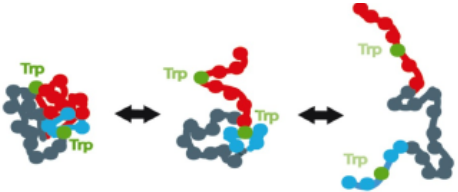
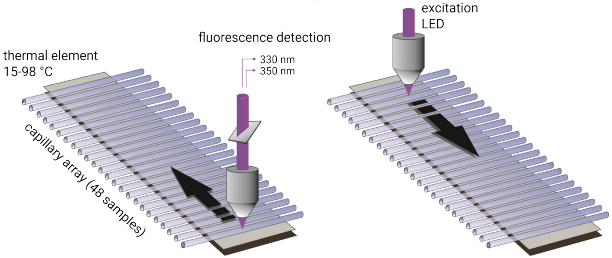
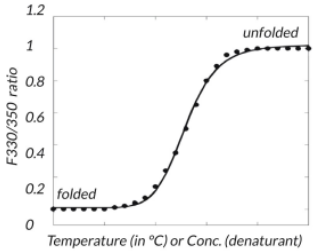






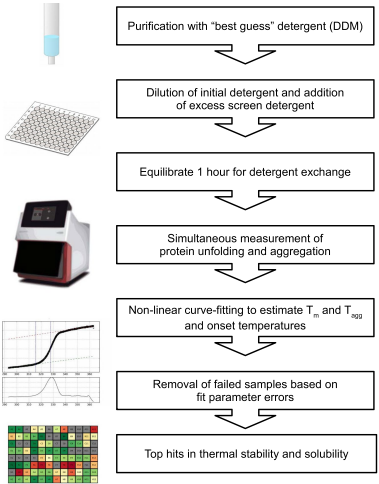
nDSF: practical considerations

- **Temperature range:** nanoDSF analyses are usually performed in a temperature gradient of 20–95°C, with a heating rate of 1°C per min. However, these settings can be adapted for the specific protein
- **Capillaries:** Depending on the aggregation behavior, two different types of capillaries can be chosen for nanoDSF assays to ensure optimal signal (regular or coated capillaries).
- **Buffers:** nanoDSF offers free choice of buffers. There are no restrictions to buffer substances or salt concentrations. nanoDSF is the optimal tool to determine the buffer conditions providing optimal thermal stability.
- **Detergents:** nanoDSF assays can be performed using any kind of detergent. This is of special interest for membrane protein characterization.

Applications of nanoDSF

Common applications

- Check protein stabilisation effect of...
  - Buffer conditions
  - Detergents

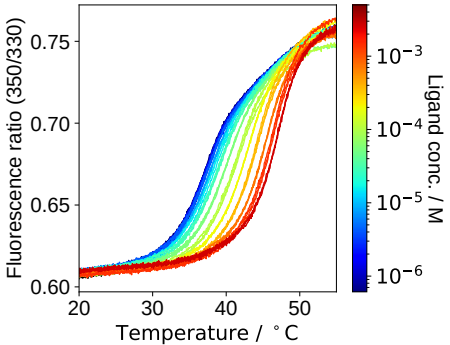


Kotov *et al.*, Sci. Rep. **2019**, 9:10379.

Applications of nanoDSF

Common applications

- Check protein stabilisation effect of...
  - Buffer conditions
  - Detergents
- Mutations
- Ligand binding → Mostly qualitative

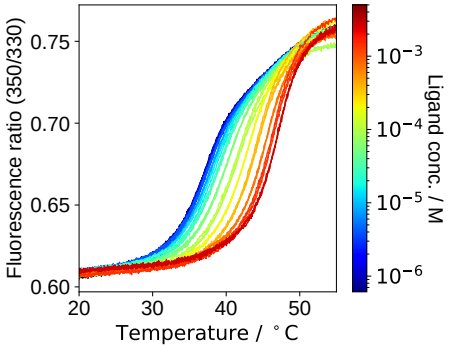


Applications of nanoDSF

Common applications

- Check protein stabilisation effect of...
  - Buffer conditions
  - Detergents
- Mutations
- Ligand binding → Mostly qualitative
- Advantages: Low sample consumption

  Easy to set up



Applications of nanoDSF

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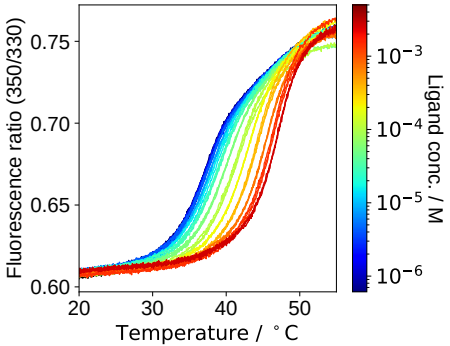
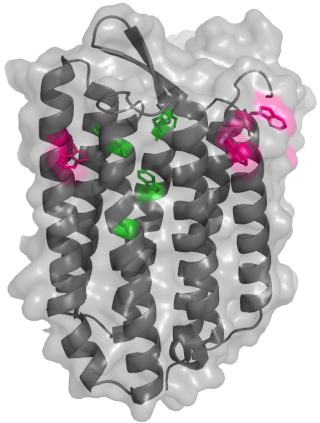


Quantitative determination of binding affinities

- Isothermal analysis (Niebling *et al.*, Sci. Rep. **2021**)
- Available via data analysis webserver: spc.embl-hamburg.de

Before experiment: Check for chromophores



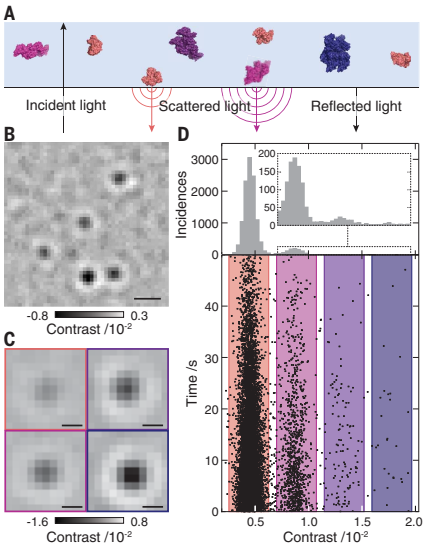
Chromophore check

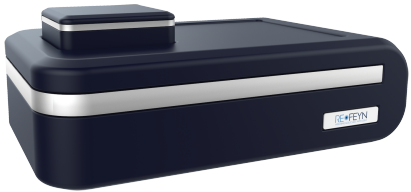
- How many tryptophanes?
- How many tyrosines?

→ 3 times weaker chromophore

- If structure is available:
  - How many of the chromophores are solvent-exposed?
  - How many are buried?

New technology: Mass photometry (MP)



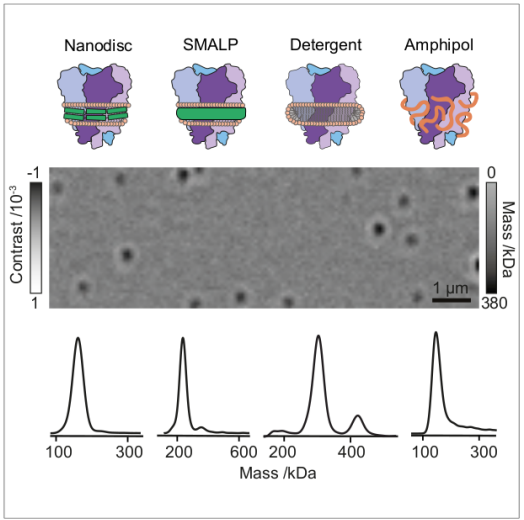


Advantages

- Measurement in solution
- Low sample consumption
- MDa range is no problem
- Easy measurement

Young *et al.*, Science. **2018**, 360, 423–427.

Mass photometry of membrane proteins



Anna Olerinyova, Adar Sonn-Segev, Joseph Gault, ..., Roland Riek, Weston B. Struwe, Philipp Kukura

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HIGHLIGHTS

We introduce a label-free, single molecule approach for membrane-protein characterization

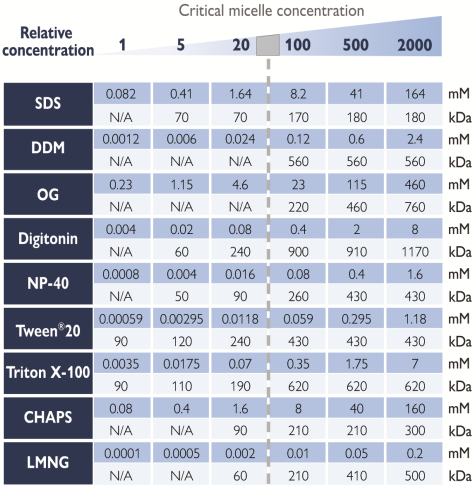
Mass photometry quantifies membrane proteins in different membrane-mimetic systems

MP reveals carrier and protein heterogeneity

It helps distinguish different functional states of membrane proteins

Olerinyova *et al.*, Chem. **2020**, 7, 1–13.

Mass photometry with detergents



SDS	0.082	0.41	1.64	8.2	41	164	mM
	N/A	70	70	170	180	180	kDa
DDM	0.0012	0.006	0.024	0.12	0.6	2.4	mM
	N/A	N/A	N/A	560	560	560	kDa
OG	0.23	1.15	4.6	23	115	460	mM
	N/A	N/A	N/A	220	460	760	kDa
Digitonin	0.004	0.02	0.08	0.4	2	8	mM
	N/A	60	240	900	910	1170	kDa
NP-40	0.0008	0.004	0.016	0.08	0.4	1.6	mM
	N/A	50	90	260	430	430	kDa
Tween®20	0.00059	0.00295	0.0118	0.059	0.295	1.18	mM
	90	120	240	430	430	430	kDa
Triton X-100	0.0035	0.0175	0.07	0.35	1.75	7	mM
	90	110	190	620	620	620	kDa
CHAPS	0.08	0.4	1.6	8	40	160	mM
	N/A	N/A	90	210	210	300	kDa
LMNG	0.0001	0.0005	0.002	0.01	0.05	0.2	mM
	N/A	N/A	60	210	410	500	kDa

Refeyn application note: “Mass photometry with detergents”

Mass photometry with detergents

Critical micelle concentration

Relative concentration	1	5	20	100	500	2000	
SDS	0.082	0.41	1.64	8.2	41	164	mM
	N/A	70	70	170	180	180	kDa
DDM	0.0012	0.006	0.024	0.12	0.6	2.4	mM
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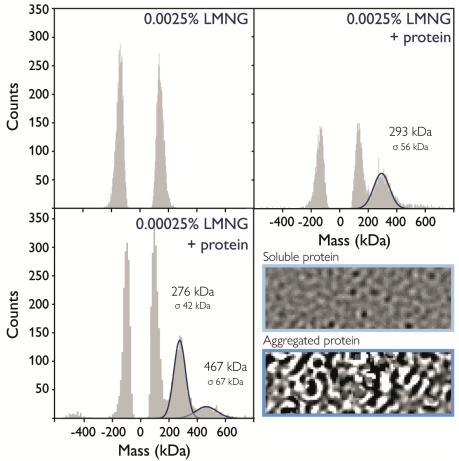
	N/A	50	90	260	430	430	kDa
Tween®20	0.00059	0.00295	0.0118	0.059	0.295	1.18	mM
	90	120	240	430	430	430	kDa
Triton X-100	0.0035	0.0175	0.07	0.35	1.75	7	mM
	90	110	190	620	620	620	kDa
CHAPS	0.08	0.4	1.6	8	40	160	mM
	N/A	N/A	90	210	210	300	kDa
LMNG	0.0001	0.0005	0.002	0.01	0.05	0.2	mM
	N/A	N/A	60	210	410	500	kDa



Refeyn application note: "Mass photometry with detergents"

Thank you for your attention!

Feel free to contact us!
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