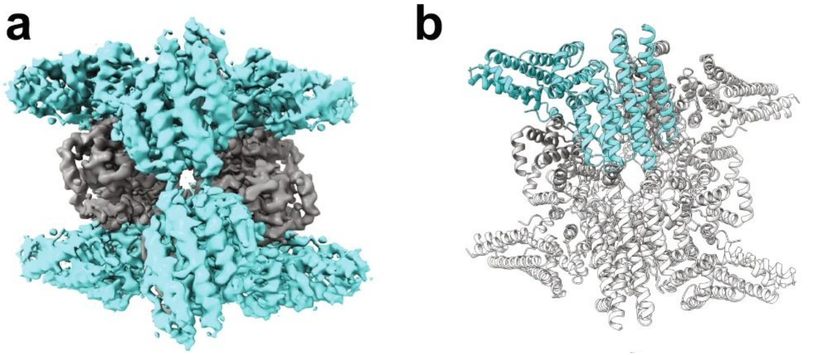
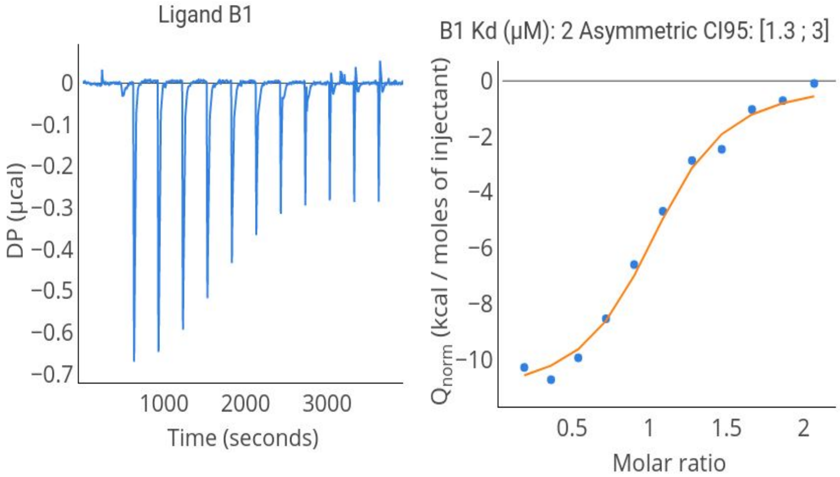
Data and biophysics

Osvaldo Burastero
ARISE Fellow, Garcia-Alai Team
MOSBRI Course - Quality control for Integral Membrane Proteins 2022
14 September 2022

The final objective

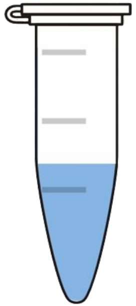
Cryo-EM structure of a 16-mer AENTH complex	Isothermal titration calorimetry example
	

Lizarrondo *et al.* (2021) / Nat. Commun.
Burastero *et al.* (2022) / J. Med. Chem.

How to get there

1. Experiment planning

- Data management plan
- Predict possible outcomes

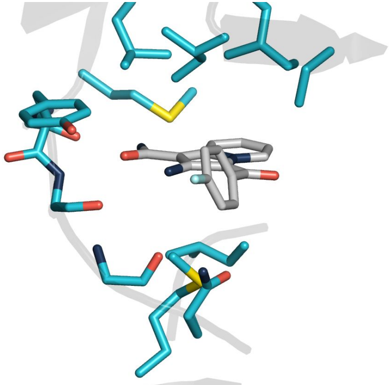


2. Sample preparation

- Protein expression & purification

3. Quality check

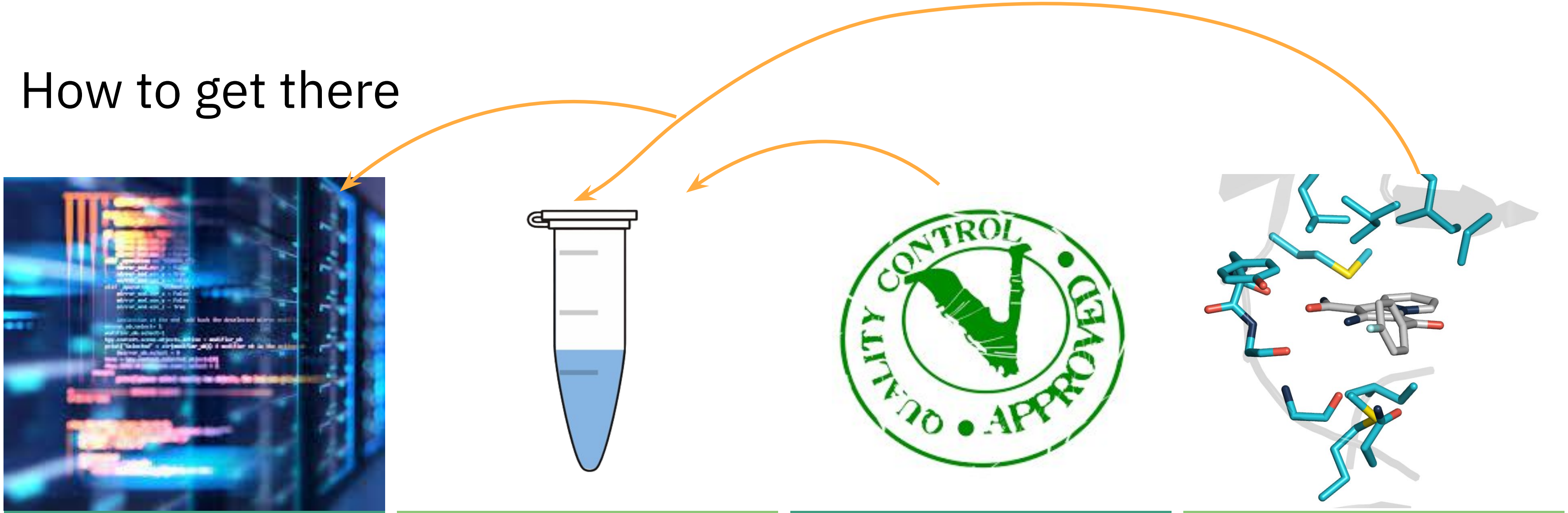
- Homogeneity
- Integrity
- Identity



4. Measure & analysis

- Activity / inhibition assays
- Structural studies

How to get there



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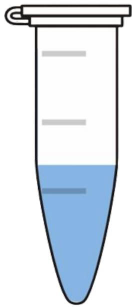

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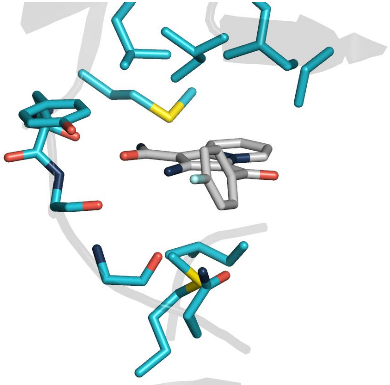

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What is research data?

Data	Metadata
● Digital data generated during and after the research project ● Observations ● Acquired data (raw & processed): text files, videos, ... ● Software (code, algorithms)	● Data about the data (provides context) ● Origin of the data ● Who, when, why, how ● Used resources ● Licenses

Based on slides done by Jeanne Wilbrandt (Leibniz Uni)

“Good metadata will save us precious time in the future”

Data management plan (DMP)

What is a DMP?

- Formal document that describes how the data will be handled during and after the project

Why we need it?

- Good scientific practice
- Required by funders and/or institution

Horizon 2020
European Union Funding
for Research & Innovation

Based on slides done by Jeanne Wilbrandt (Leibniz Uni)

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Why we need it?

- Good scientific practice
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Benefits

- Save time and resources
- Improved reproducibility and reusability

FAIR Principles

- Findable
- Accesible
- Interoperable
- Reusable

Horizon 2020
European Union Funding
for Research & Innovation

scientific **data**

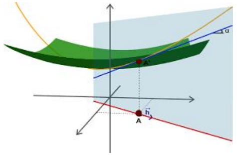
Open Access | Published: 15 March 2016

The FAIR Guiding Principles for scientific data management and stewardship

Wilkinson *et al.* (2016) / Sci. Data
Based on slides done by Jeanne Wilbrandt (Leibniz Uni)

DMP checklist

Project	What is the project?
Data	What type, format and size does the used and produced data have?
Interpretation	Which information is required to understand the data?
Procedures	Which procedures will be used to create, process and quality control the (meta)data?



Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP checklist

Documentation	How the data processing steps will be recorded?
Access	Are there any security or access control requirements?
Project end	What happens to the data after the project finishes?

zenodo

RCSB PDB PROTEIN DATA BANK

BindingDB

Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP checklist

Documentation	How the data processing steps will be recorded?
Access	Are there any security or access control requirements?
Project end	What happens to the data after the project finishes?
Intellectual property (IP)	How will be the IP managed?
Responsibilities	Who is responsible for which part of the data management?

Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP checklist - zoom in

Interpretation	
● Can the data be read only with specific software? ● Where is the data documentation to be found? Lab information management system? ● How is data going to be documented? ● Metadata, identifiers (of biological entities) & ontologies	

Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP checklist - zoom in

Interpretation	Procedures
• Can the data be read only with specific software? • Where is the data documentation to be found? Lab information management system? • How is data going to be documented? • Metadata, identifiers (of biological entities) & ontologies	• How will data and files be named and organised? • How will changes be tracked and propagated? • How will metadata and provenance be preserved? • How will derived data be updated?

Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP checklist - zoom in

Processing
● Manual data processing steps ● Configuration parameters ● Analysis versions ● Scientific workflow management system ● Open source software ● etc.

Based on slides done by Lisanna Paladin (BioIT Team, EMBL)

DMP in real life - a living document

Data generated for analysis of protein X stability and homogeneity

Dataset	Origin	Size	Format	Availability
DSF data	measurements performed in a nDSF Prometheus ® (Nanothemper)	<100 MB	.xlsx .csv	Open
DLS data	measurements performed in a DynaPro ® Plate Reader (Wyatt Technology)	<10 MB	.csv	Open

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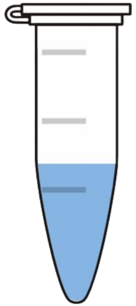

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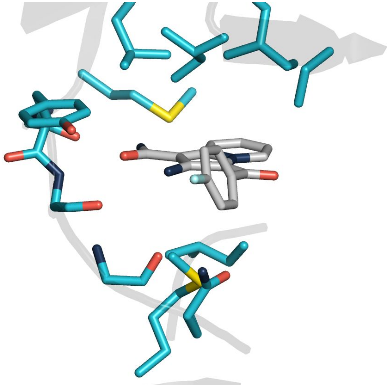

4. Measure & analysis

- Activity / inhibition assays
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Knowledge discovery

1

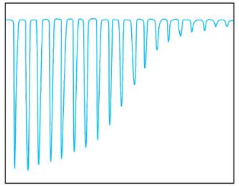
Understand the domain and objective

2

Data collection & selection

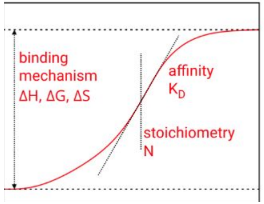
3

Preprocessing



4

Transformation



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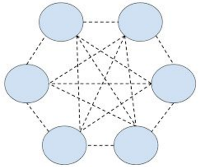
Modeling



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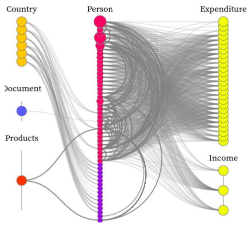
Evaluation & interpretation



7

Novel knowledge

Sample Preparation & Characterization (SPC) Facility

- Optimisation
- Quality control
- Characterisation (thermodynamics & kinetics)

MALDI TOF

Differential Scanning Fluorimetry

Circular Dichroism

Mass Photometry

Isothermal Titration Calorimetry

MicroScale Thermophoresis

Dynamic Light Scattering

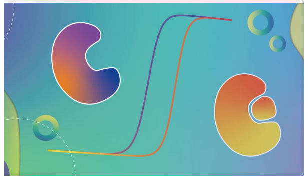
and much more!

Instrument images are related to the following companies - CovalX and Bruker, Nanotemper, AppliedPhotophysics, Refeyn, Malvern Panalytical & Wyatt Technology

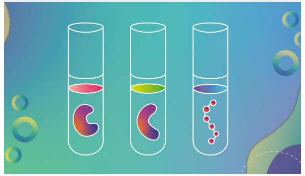
eSPC, enriching service provision

Differential Scanning Fluorimetry (DSF)

FoldAffinity

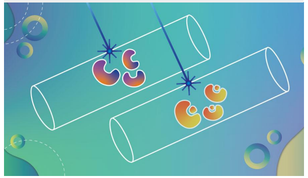


MoltenProt



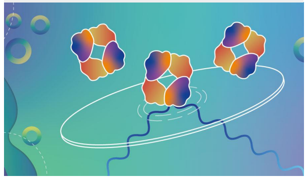
MicroScale Thermophoresis (MST)

ThermoAffinity



Mass Photometry (MP)

PhotoMol



Burastero *et al.* (2022) / Acta Crystallogr. D

Sample Preparation & Characterization (SPC) Facility

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MALDI TOF

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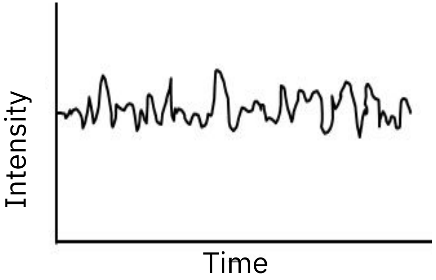
Dynamic light scattering (DLS) in a nutshell

Why?

- Homogeneity of a sample
- Estimation of the hydrodynamic radius (Hr)

How does it work?

- It measures the autocorrelation of the scattered light



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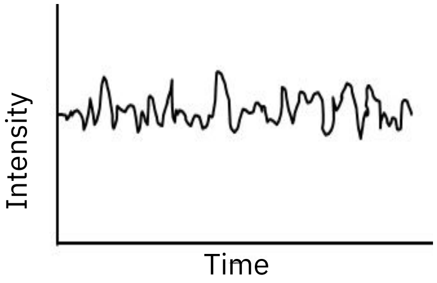
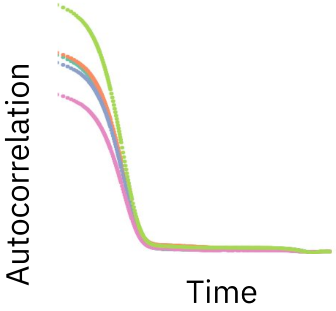


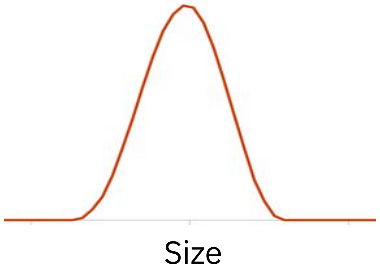
How do we extract valuable information?

- We fit one/two, or a distribution of decay rates

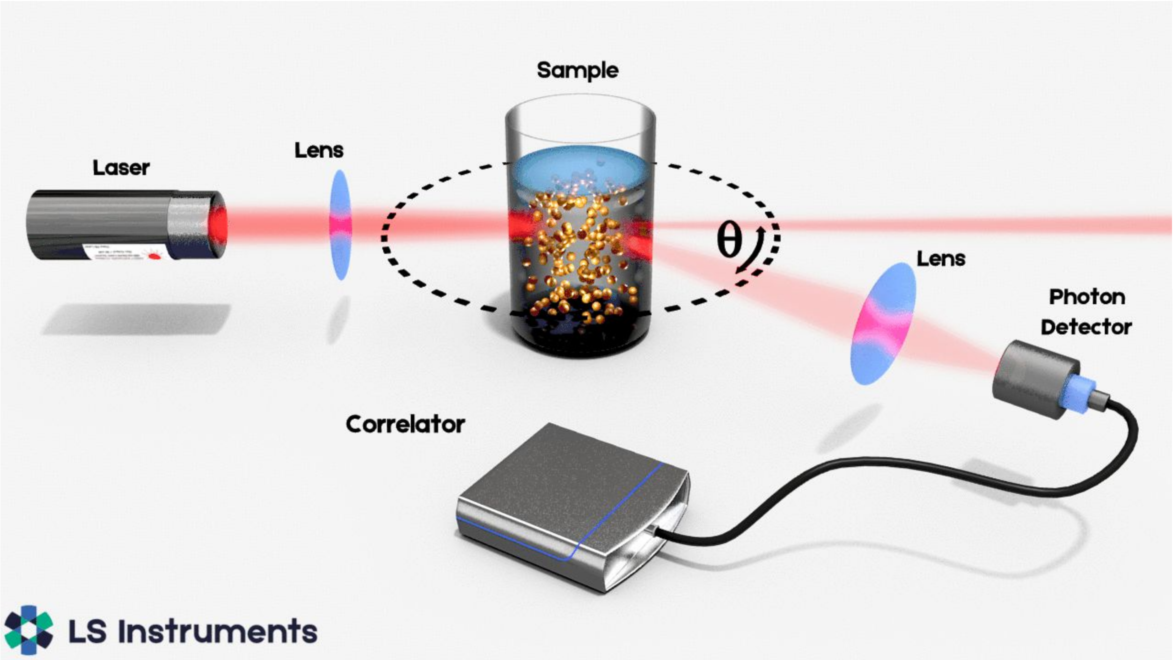


Limitations

- Signal $\propto$ Hr^6
- Semi-quantitative
- Scattering isn’t isotropic for large particles



DLS - Theory



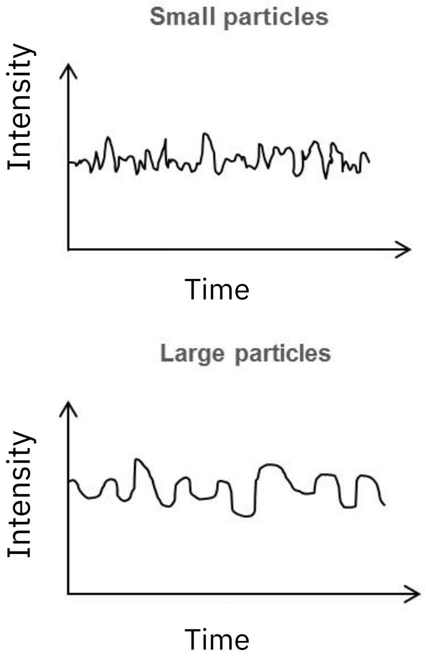
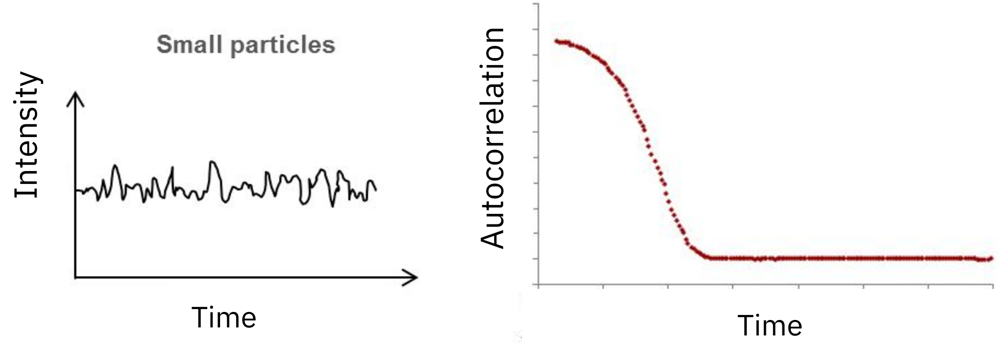
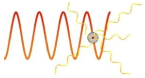


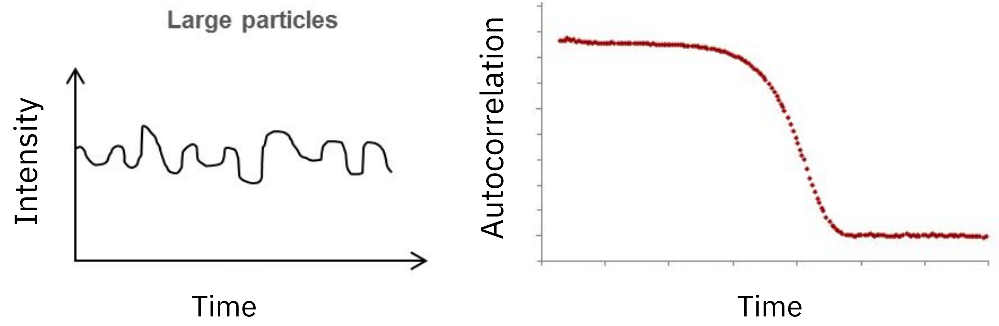
Image taken from https://lsinstruments.ch

DLS - Theory



Small particles
- Fast decay
- Give low signal
- Isotropic scattering





Large particles
- Slow decay
- Give high signal (∝ Hr^6)
- Anisotropic scattering

“Any model is at best, an useful fiction”

DLS - Model & fitting

What we measure

Second order correlation function G_2

$$G_2(\tau) = \langle I(t)I(t+\tau)\rangle$$

Integral over the product of intensities at time t and delayed time t+τ

$$g_2(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

DLS - Model & fitting

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Relationship with particle motion

Normalised first-order correlation function g_1

$$g_2(\tau) = 1 + \beta|g_1(\tau)|^2$$

Coherence factor β ∝ instrument & molecules

DLS - Model & fitting

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Second order correlation function G_2

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Relationship with particle motion

Normalised first-order correlation function g_1

$$g_2(\tau) = 1 + \beta|g_1(\tau)|^2$$

Coherence factor $\beta \propto$ instrument & molecules

Distribution of decay rates

Second order correlation function

$$g_1(\tau) = \int_0^\infty G(\Gamma)\exp\left(-\Gamma_\tau d\Gamma\right)$$

Intensity-weighted integral over a distribution of decay rates

DLS - Model & fitting

Decay rate & diffusion rates

Each decay rate can be associated to a certain diffusion factor *D*

$$D(s,q) = 1/(s*(q^2))$$

where s is the inverse of the decay rate and q is the Bragg wave vector (∝ angle of detector & refractive index)

Conversion to hydrodynamic radius

Sphere-like model allows estimating the Hr

$$Hr = \frac{k_b * temperature}{D * viscosity * 6\pi}$$

The ISO recommended cumulants approach

- Moment analysis of the linear form of the measured correlogram
- Assumes a single particle family (Gaussian)
- Gives the Z-average (mean value) and the PdI (polydispersity index, relative variance of the Gaussian)

DLS - Model & fitting

Limitations of the cumulants approach

- Extremely sensitive to small amounts of aggregates
- Unsuitable for a polydisperse sample (polydispersity > 20 %)

Fitting a distribution of decay rates

- We need to define a decay rate space

$$g_1(t) = \sum_{i=1}^{200} c_i exp \frac{-t}{s_i}$$

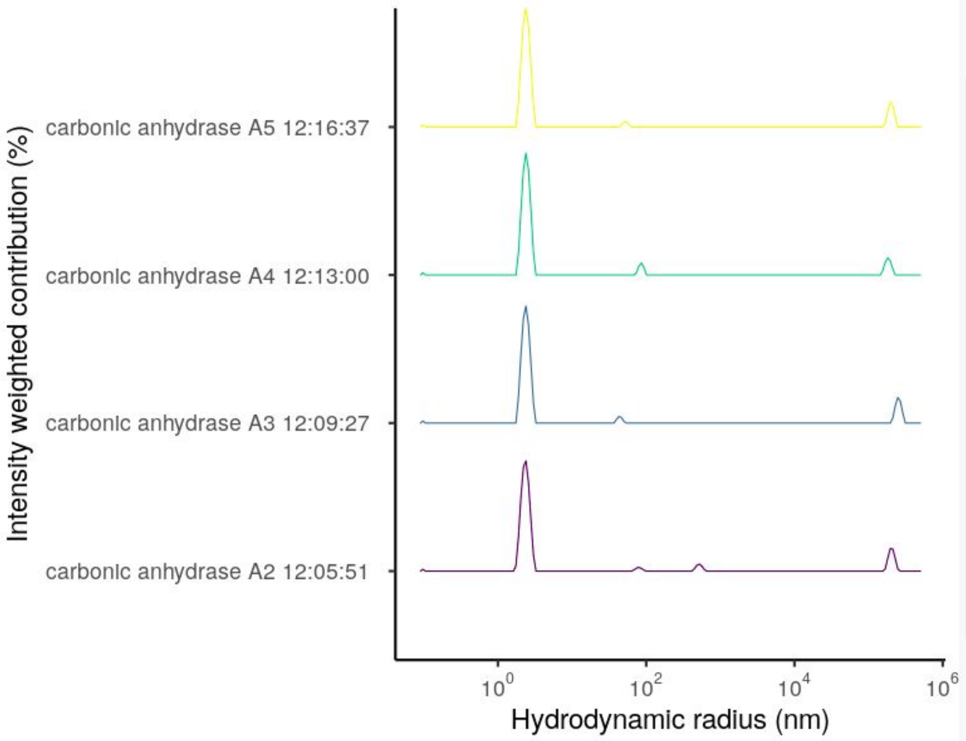
- Ill-posed problem that requires regularization

$$||Ax - b|| + \alpha||Mx|| + \beta||Ix||$$

$$\alpha|| \sum_{i=2}^{199} 2c_i - c_{i-1} - c_{i+1}||$$

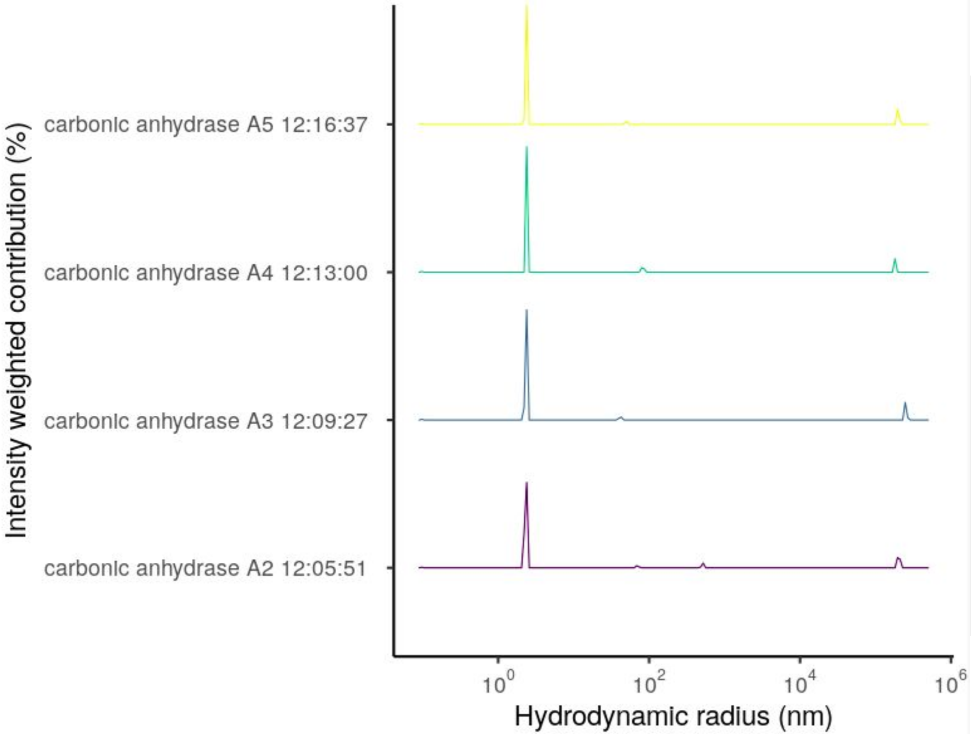
“DLS is (almost always) semi-quantitative”

DLS - Fitting in practice



● Hr min value	0.09 nm
● Hr max value	5e5 nm
● Number of Hr points	200
● Max time	1 sec
● Alpha regularization	0.1
● Beta regularization	0

DLS - Fitting in practice



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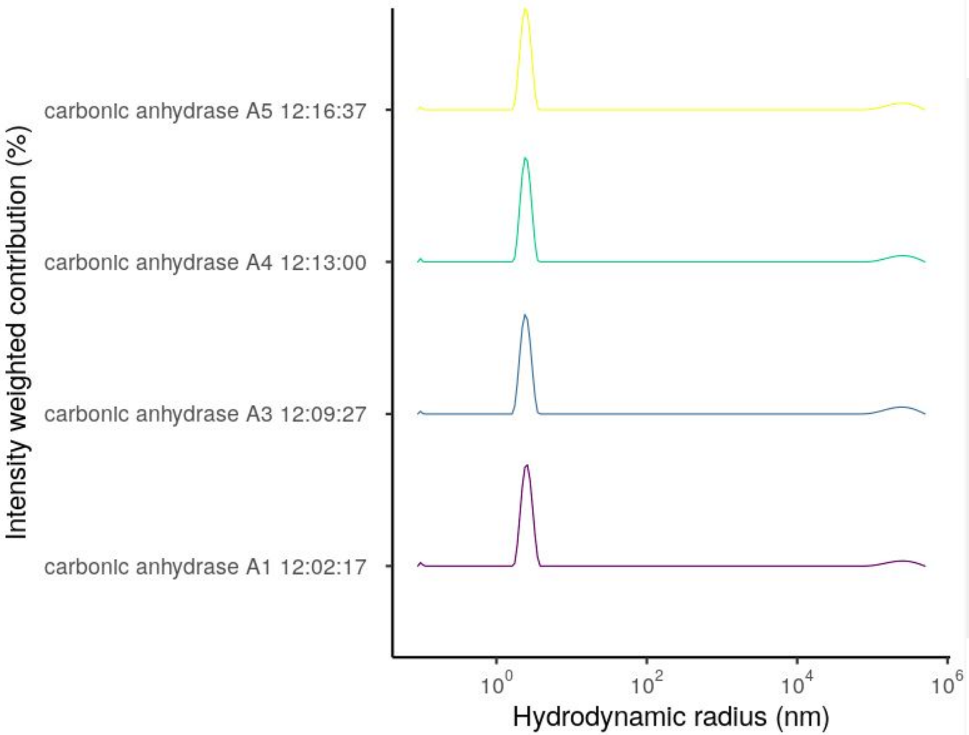
DLS - Fitting in practice



● Hr min value	0.09 nm
● Hr max value	5e5 nm
● Number of Hr points	200
● Max time	100 µs
● Alpha regularization	0.1
● Beta regularization	0

DLS - Interpretation

Good samples

- Cumulants PdI < 20 % (Malvern)
- One peak in region 1-20 nm with mass > 99.9 %

Oligomerization

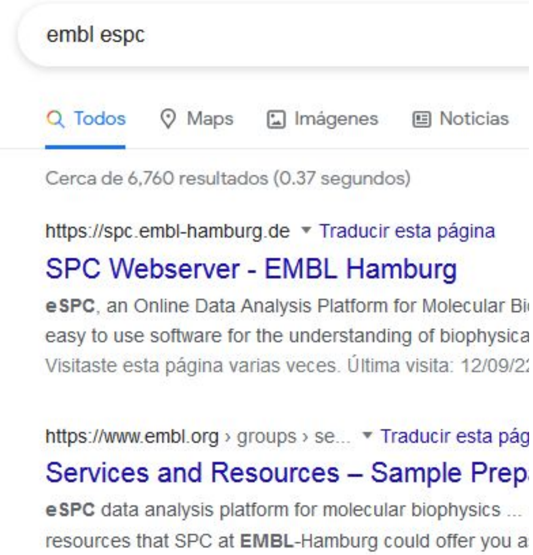
- Only big differences in size (factor 3-5) are detected, i.e., monomer to hexamer

Hr values

- The Hr values are semi-quantitative
- Hr estimation assumes sphere-like model

Raynals, an app for DLS analysis

Step 1. Google “embl espc”



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SPC Webserver - EMBL Hamburg

eSPC, an Online Data Analysis Platform for Molecular Bi
easy to use software for the understanding of biophysica
Visitaste esta página varias veces. Última visita: 12/09/2

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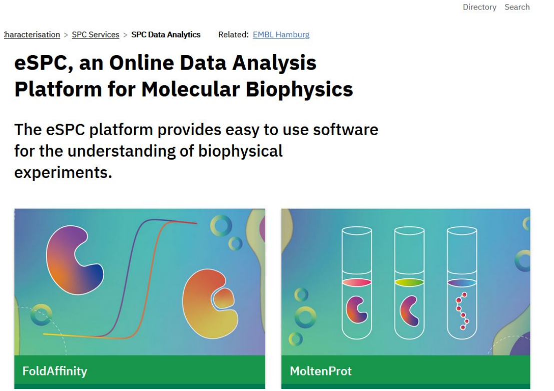
Services and Resources – Sample Prep

eSPC data analysis platform for molecular biophysics ...
resources that SPC at **EMBL**-Hamburg could offer you a

Step 2. Access spc.embl-hamburg.de



Directory Search

haracterisation > SPC Services > SPC Data Analytics Related: EMBL Hamburg

eSPC, an Online Data Analysis Platform for Molecular Biophysics

The eSPC platform provides easy to use software for the understanding of biophysical experiments.

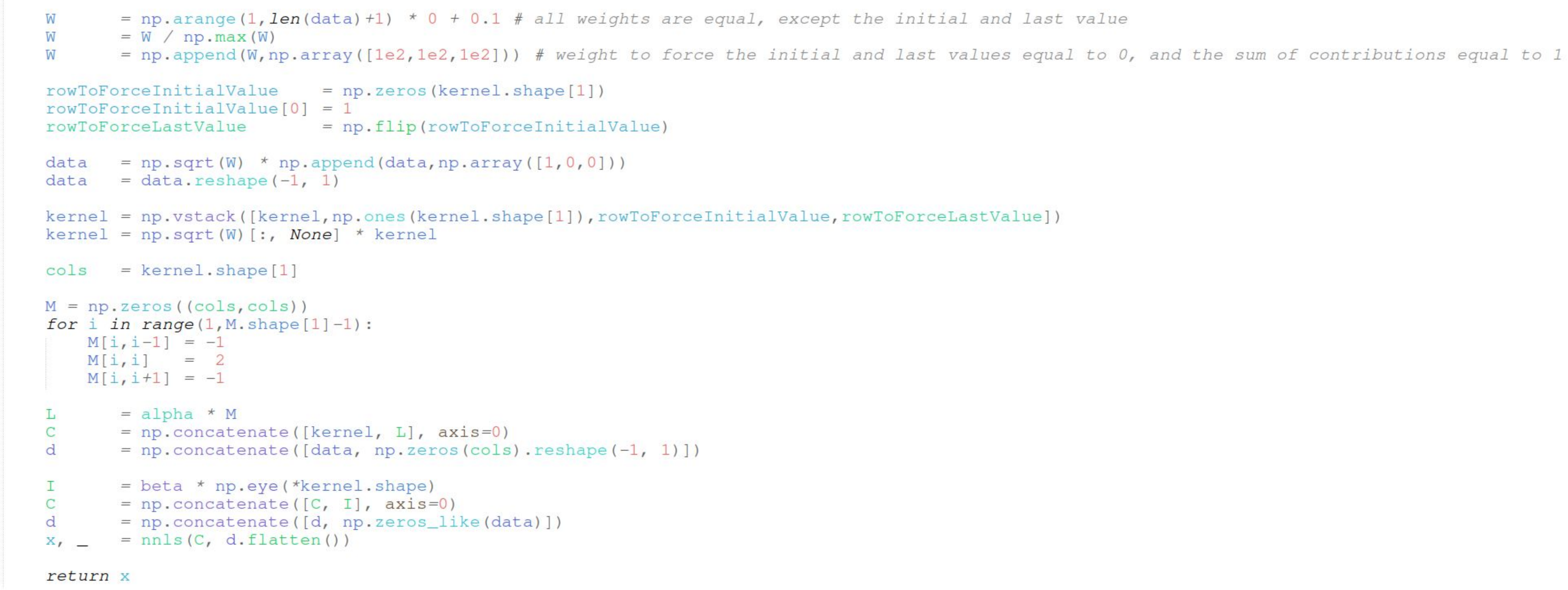
FoldAffinity

MoltenProt

Step 3. Access Raynals spc.embl-hamburg.de/app/raynals

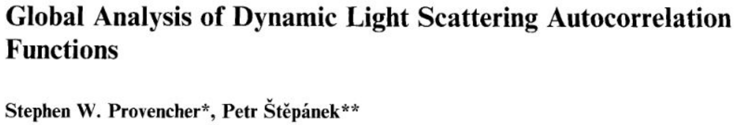
Institut Pasteur d'Algérie

Raynals, analysis code available under request



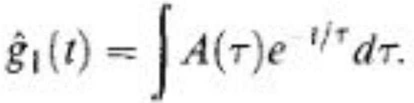
```
W      = np.arange(1,len(data)+1)  * 0  + 0.1 # all weights are equal, except the initial and last value
W      = W / np.max(W)
W      = np.append(W,np.array([1e2,1e2,1e2])) # weight to force the initial and last values equal to 0, and the sum of contributions equal to 1

rowToForceInitialValue     = np.zeros(kernel.shape[1])
rowToForceInitialValue[0]  = 1
rowToForceLastValue        = np.flip(rowToForceInitialValue)

data   = np.sqrt(W)  * np.append(data,np.array([1,0,0]))
data   = data.reshape(-1, 1)

kernel = np.vstack([kernel,np.ones(kernel.shape[1]),rowToForceInitialValue,rowToForceLastValue])
kernel = np.sqrt(W)[:, None]  * kernel

cols   = kernel.shape[1]

M = np.zeros((cols,cols))
for i in range(1,M.shape[1]-1):
    M[i,i-1]  = -1
    M[i,i]    =  2
    M[i,i+1]  = -1

L      = alpha  * M
C      = np.concatenate([kernel, L], axis=0)
d      = np.concatenate([data, np.zeros(cols).reshape(-1, 1)])

I      = beta  * np.eye(*kernel.shape)
C      = np.concatenate([C, I], axis=0)
d      = np.concatenate([d, np.zeros_like(data)])
x, _   = nnls(C, d.flatten())

return x
```

DLS - beyond the monodisperse / polydisperse sample



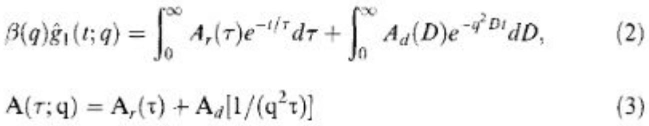
Global Analysis of Dynamic Light Scattering Autocorrelation Functions

Stephen W. Provencher*, Petr Štěpánek**



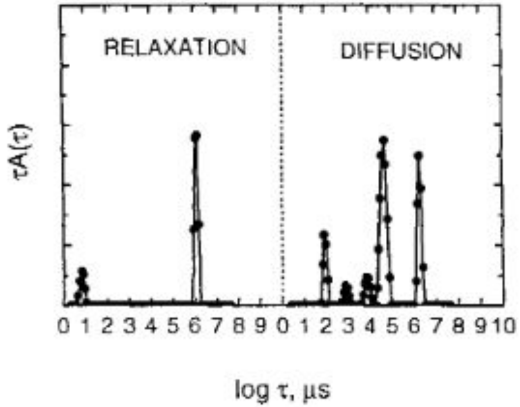
$$\hat{g}_1(t) = \int A(\tau)e^{-t/\tau}\,d\tau.$$

A(τ) is related to decay rates that can be converted into diffusion coefficients



$$\beta(q)\hat{g}_1(t;q) = \int_0^\infty A_r(\tau)e^{-t/\tau}\,d\tau + \int_0^\infty A_d(D)e^{-q^2Dt}\,dD, \qquad (2)$$

$$\mathrm{A}(\tau;\mathrm{q}) = \mathrm{A}_r(\tau) + \mathrm{A}_d[1/(\mathrm{q}^2\tau)] \qquad (3)$$



Decomposition into “diffusive” and “relaxational” (independent of angle) components

Provencher *et al.* (1996) / Part. Part. Syst. Charact.

Sample Preparation & Characterization (SPC) Facility

- Optimisation
- Quality control
- Characterisation (thermodynamics & kinetics)

MALDI TOF

Differential Scanning Fluorimetry

Circular Dichroism

Mass Photometry

Isothermal Titration Calorimetry

MicroScale Thermophoresis

Dynamic Light Scattering

and much more!

Instrument images are related to the following companies - CovalX and Bruker, Nanotemper, AppliedPhotophysics, Refeyn, Malvern Panalytical & Wyatt Technology

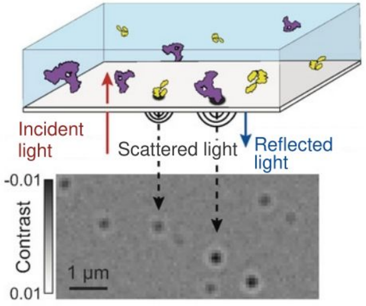
Mass photometry (MP) in a nutshell

Why?

- Homogeneity of a sample
- Estimation of the molecular masses of different species

How does it work?

- Interference between scattered and reflected light combined with ratiometric imaging



refeyn.com
Soltermann *et al.* (2020) / Angew. Chem. Int. Ed

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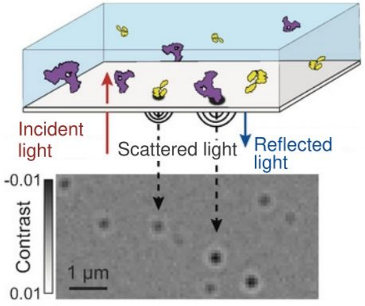
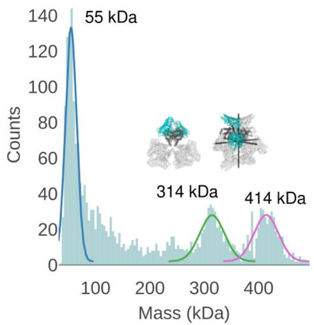


How do we extract valuable information?

- We compare contrasts with known samples
- We fit *n* distributions of masses



Limitations

- nM concentration is required
- Detergent produces high background
- Accurate only with soluble proteins

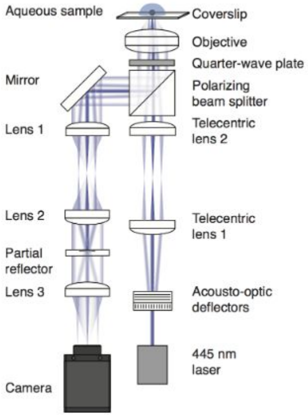
refeyn.com

Soltermann *et al.* (2020) / Angew. Chem. Int. Ed

MP - Theory

Experimental setup

- Separation of incident from scattered and reflected light



Young *et al.* (2018) / Science
Cole *et al.* (2017) / ACS Photonics

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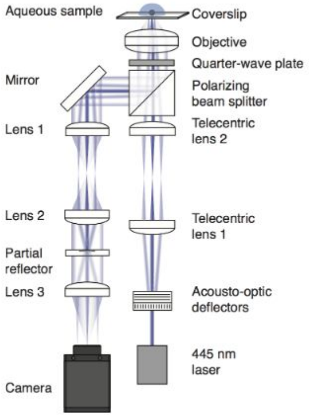
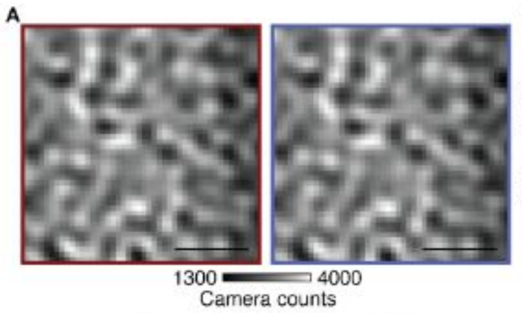


Raw images

- Interference: scattered - reflected
- High background



Young *et al.* (2018) / Science
Cole *et al.* (2017) / ACS Photonics

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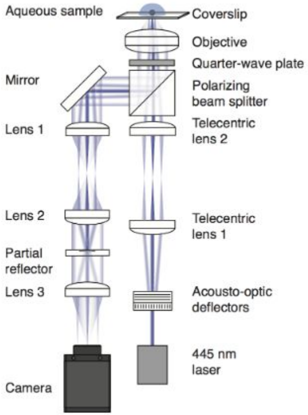

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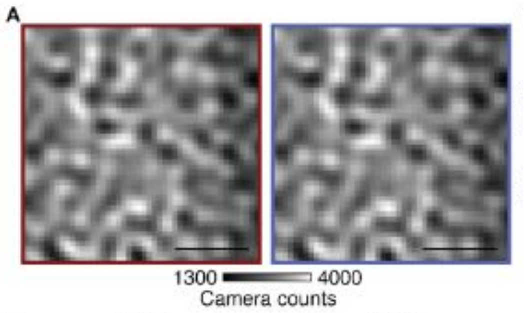
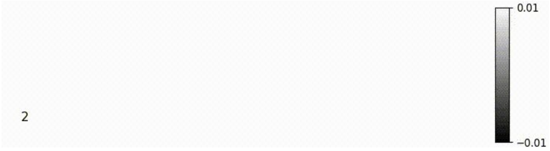


Preprocessing

- Background is removed by conversion to ratiometric images

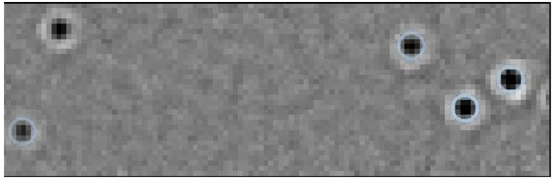


Young *et al.* (2018) / Science
Cole *et al.* (2017) / ACS Photonics

MP - Theory

Binding events

- Appear as a (dark) point spread function (PSF)
- The number should be >> unbinding events (bright spots)



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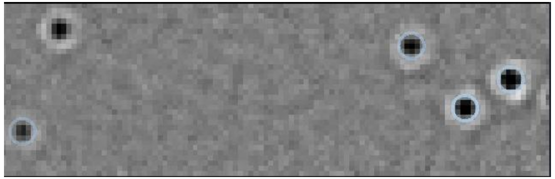

Particle detection & quantification

- Automated spot detection routine
- Fitting of candidate pixels (772 * 772 nm^2) to a 2D concentric gaussian model

$$f(x,y) = A\left(e^{-\left[\frac{(x-x_0)^2}{2\sigma_x^2}+\frac{(y-y_0)^2}{2\sigma_y^2}\right]} - \frac{(1-T)}{s} e^{-\left[\frac{(x-x_0)^2}{2(s\sigma_x)^2}+\frac{(y-y_0)^2}{2(s\sigma_y)^2}\right]}\right) + b$$

$$A\left(1-\frac{(1-T)}{s}\right)$$

Young *et al.* (2018) / Science

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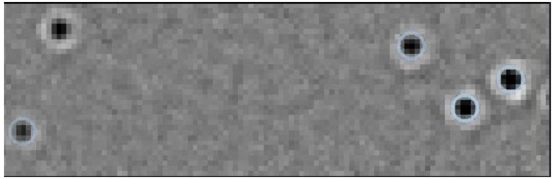

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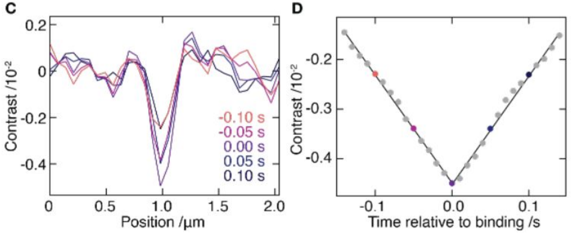
Contrast of a given particle

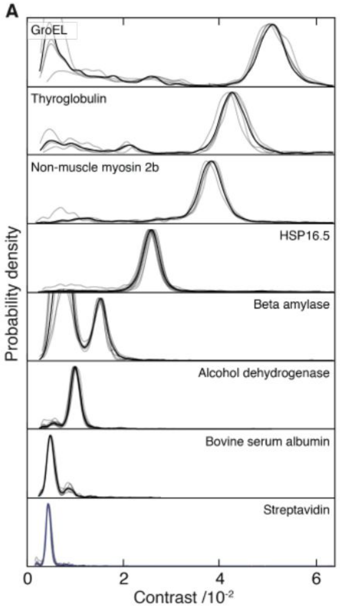
- Fitted from the contrast as a function of time



Young *et al.* (2018) / Science

MP - Model & fitting

Contrast to molecular weight



Young *et al.* (2018) / Science

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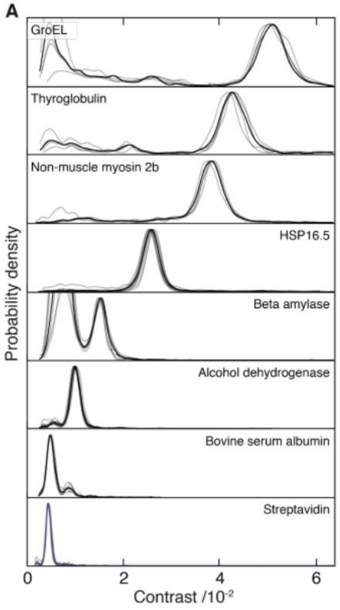
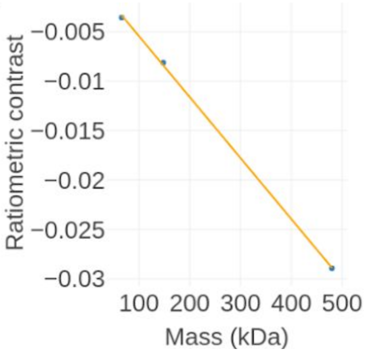


Young *et al.* (2018) / Science

Calibration

- Using known protein standards
- Quantitative for soluble proteins without post translational modifications!



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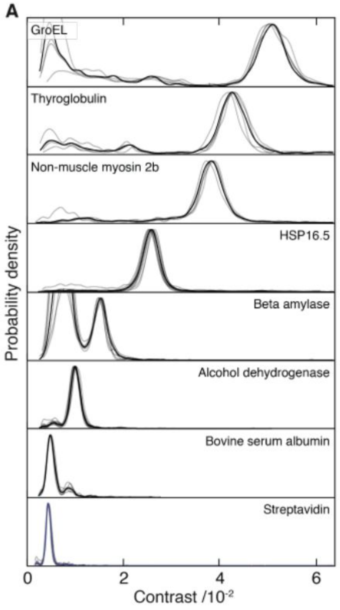

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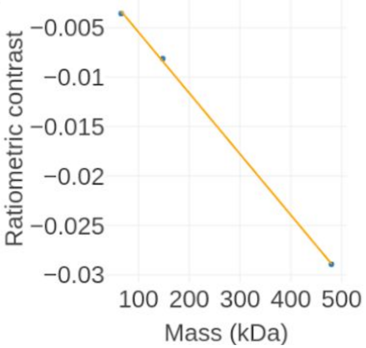
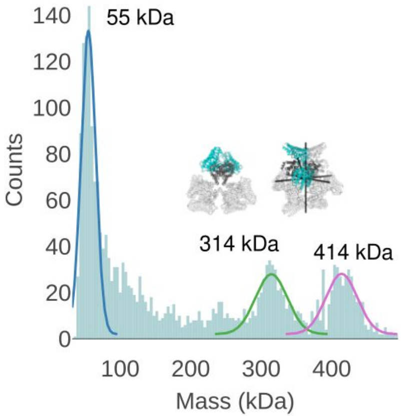


Unknown sample

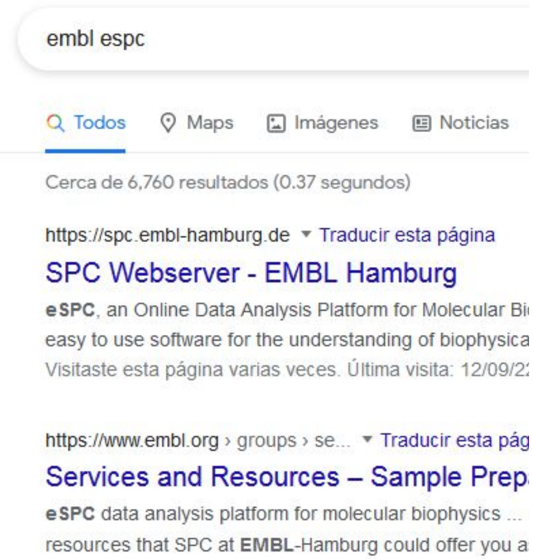
- Fitting of gaussian distributions



Young *et al.* (2018) / Science

PhotoMol, an app for MP analysis

Step 1. Google “embl espc”



embl espc

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Cerca de 6,760 resultados (0.37 segundos)

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eSPC, an Online Data Analysis Platform for Molecular Bi

easy to use software for the understanding of biophysica

Visitaste esta página varias veces. Última visita: 12/09/2:

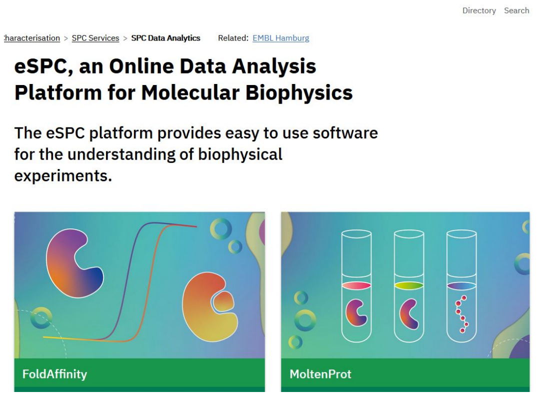
https://www.embl.org › groups › se... ▾ Traducir esta pág

Services and Resources – Sample Prep

eSPC data analysis platform for molecular biophysics ...

resources that SPC at **EMBL**-Hamburg could offer you a

Step 2. Access spc.embl-hamburg.de

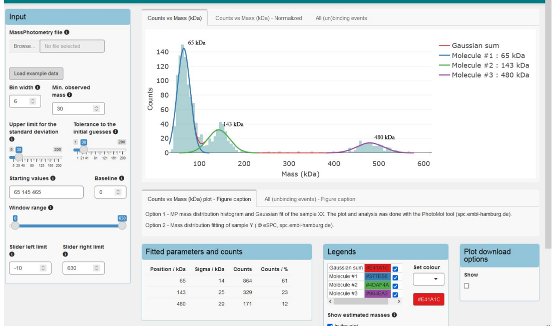


haracterisation > SPC Services > SPC Data Analytics   Related: EMBL Hamburg

eSPC, an Online Data Analysis Platform for Molecular Biophysics

The eSPC platform provides easy to use software for the understanding of biophysical experiments.

Step 3. Access PhotoMol spc.embl-hamburg.de/app/photoMol



MassPhotometry file: No file selected

Bin width: 6

Min. observed mass: 30

Upper limit for the standard deviation: 5 – 30 – 200

Tolerance to the initial guesses: 1 – 30 – 200

Starting values: 65 145 465

Baseline: 0

Window range: 0 – 630

Slider left limit: -10

Slider right limit: 630

Option 1 - MP mass distribution histogram and Gaussian fit of the sample XX. The plot and analysis was done with the PhotoMol tool (spc.embl-hamburg.de).

Option 2 - Mass distribution fitting of sample Y (© eSPC, spc.embl-hamburg.de).

Fitted parameters and counts

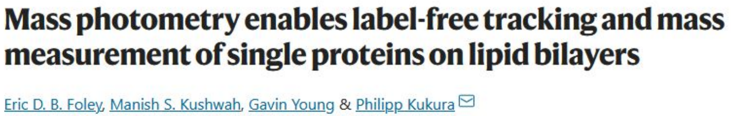
Position / kDa	Sigma / kDa	Counts	Counts / %
65	14	864	61
143	25	329	23
480	29	171	12

Gaussian sum	#E41A1C	☑
Molecule #1	#377EB8	☑
Molecule #2	#4DAF4A	☑
Molecule #3	#984EA3	☑

Show estimated masses

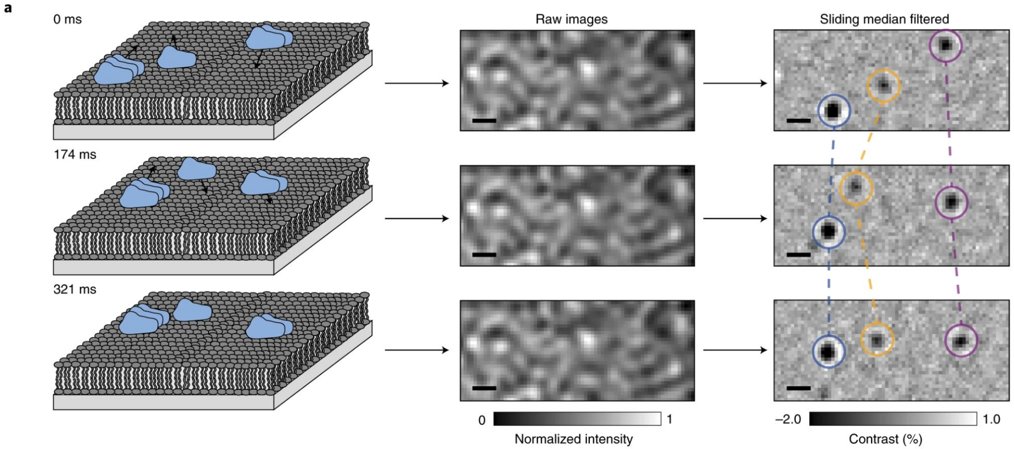
☑ In the plot

MP - beyond the yes/no complex formation question

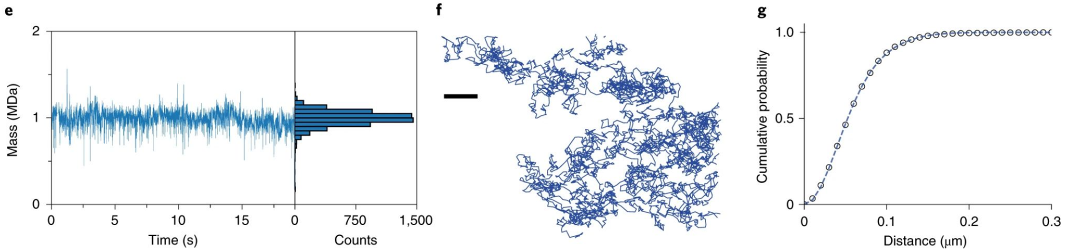


Mass photometry enables label-free tracking and mass measurement of single proteins on lipid bilayers

Eric D. B. Foley, Manish S. Kushwah, Gavin Young & Philipp Kukura



Simultaneously imaging, tracking and measuring the mass of diffusing biomolecular complexes on supported lipid bilayers (SLBs)



Foley *et al.* (2021) / Nat. Methods

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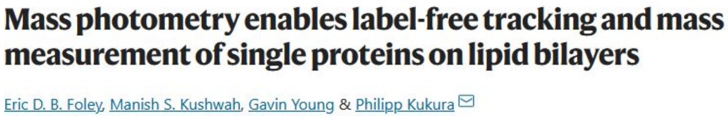

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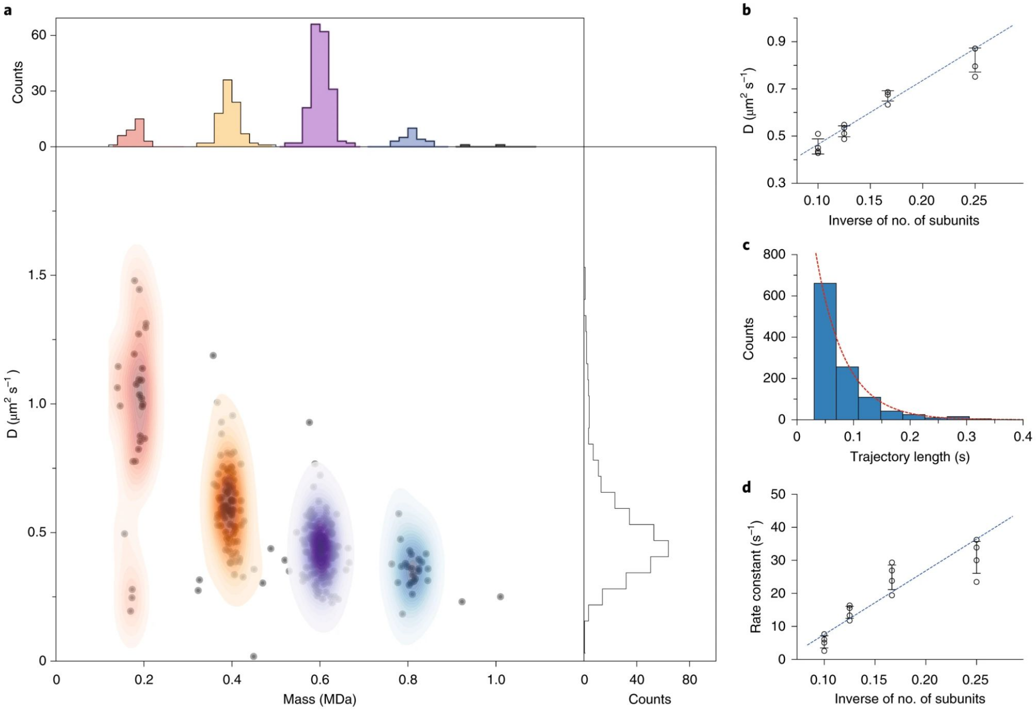

Foley *et al.* (2021) / Nat. Methods

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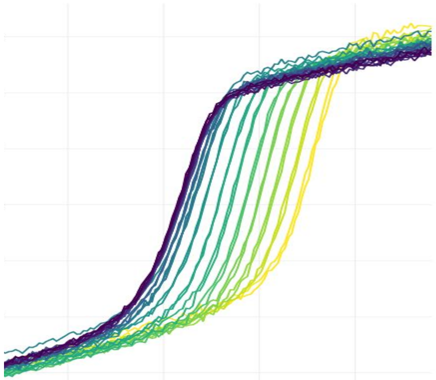
Nano Differential scanning fluorimetry (nDSF) in a nutshell

Why?

- Protein stability or ligand binding

How does it work?

- By heating the sample and measuring the fluorescence



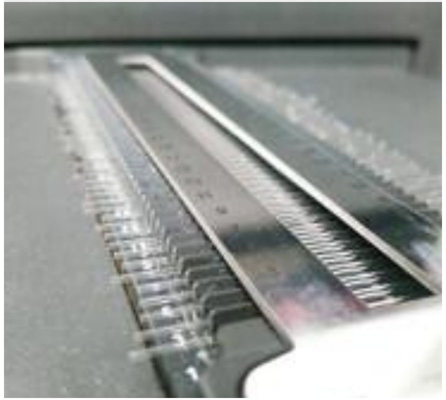


Photo credit: Vadim Kotov

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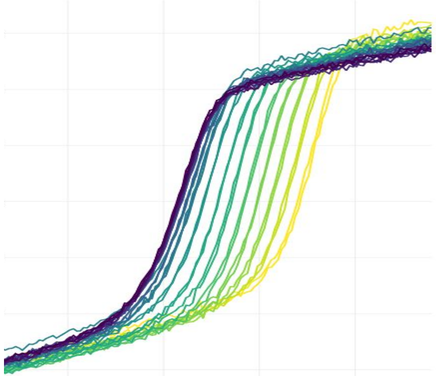

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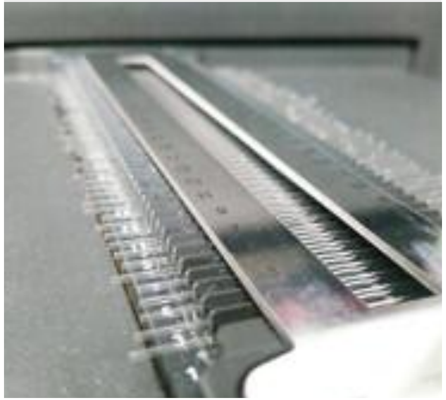
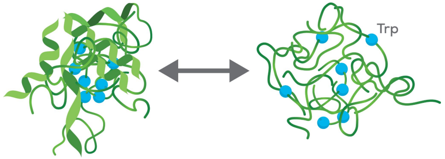


How do we extract valuable information?

- Fitting unfolding models



Native ⇋ Unfolded

Limitations

- Autofluorescence
- Multiple transitions
- No transitions

Photo credit: Vadim Kotov

Protein image: unchainedlabs.com

nDSF - Unfolding models

n-mer	Protomers	States	Intermediates
Monomer	A	2	none
Monomer	A	3	1 monomer
Monomer	A	2+p	p monomers
Homodimer	A_2	.	.
.	.	.	.
Heterodimer	AB	.	.
.	.	.	.
Heterotrimer	ABC	.	.
.	.	.	.

Native → Unfolded

Native ⇋ Unfolded

Native ⇋ Intermediate ⇋ Unfolded

nDSF - How to build a model

Step 1.	Equation of the signal with *n* states	$Y_t(x) = Y_n[N] + \sum_j Y_j[I_j] + Y_u[U] + Y_d(x),\ j = 1, \ldots, p$
Step 2.	Pre/post transition dependence	$Y_n = y_n + m_n x;$
Step 3.	States interconversion	Native ⇋ Unfolded

Send us code/equations & data and we will add them into the server!

Bedouelle (2016) / Biochimie

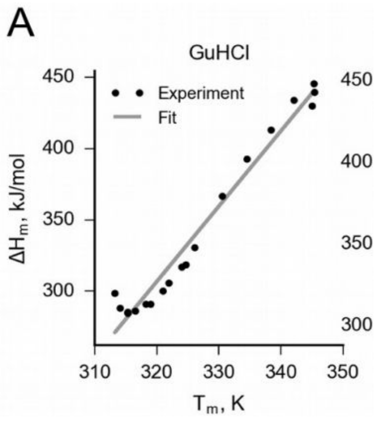
nDSF - The two state equilibrium model

- Reversible equilibrium between native & unfolded states

$$\Delta G\,(T) = -RT\ \ln\left[K\,(T)\right] = \Delta H_{\mathrm{m}}\,(1 - T/T_{\mathrm{m}}) - \Delta C_{\mathrm{p}}\,\left[(T_{\mathrm{m}} - T) + T\ \ln\,(T/T_{\mathrm{m}})\right]$$

- ΔH and Tm can be precisely determined (but not ΔCp)

- ΔCp can be determined using different chemical denaturant concentrations



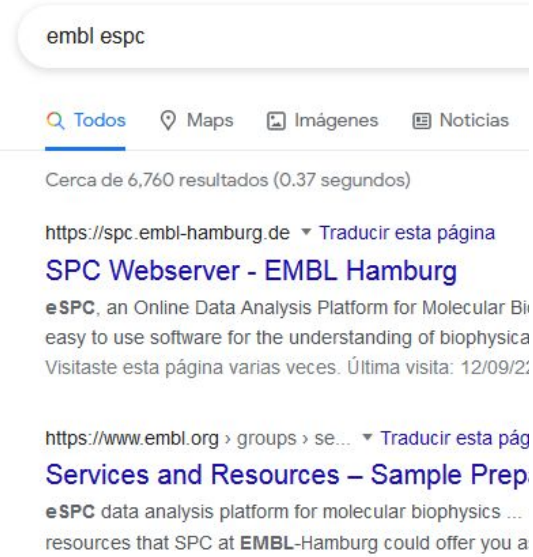
Kotov *et al.* (2020) / Protein Sci.

MoltenProt & FoldAffinity, two apps for DSF analysis

Step 1. Google “embl espc”

Step 2. Access spc.embl-hamburg.de

Step 3. Access MoltenProt or FoldAffinity



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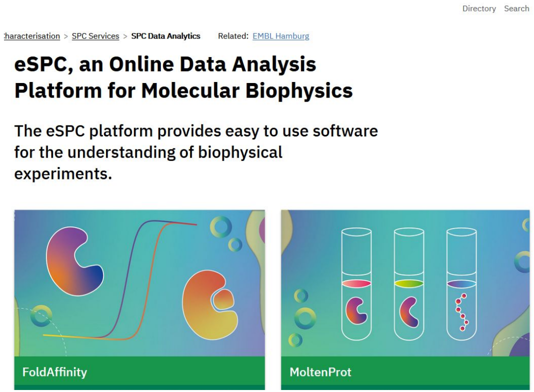

haracterisation > SPC Services > SPC Data Analytics   Related: EMBL Hamburg

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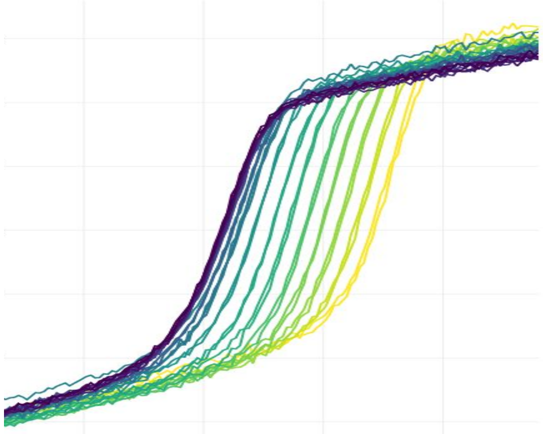

EMBL European Molecular Biology Laboratory

ARISE

MOSBRI Molecular-Scale Biophysics Research Infrastructure

EMBL Hamburg IT Team

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 945405.