

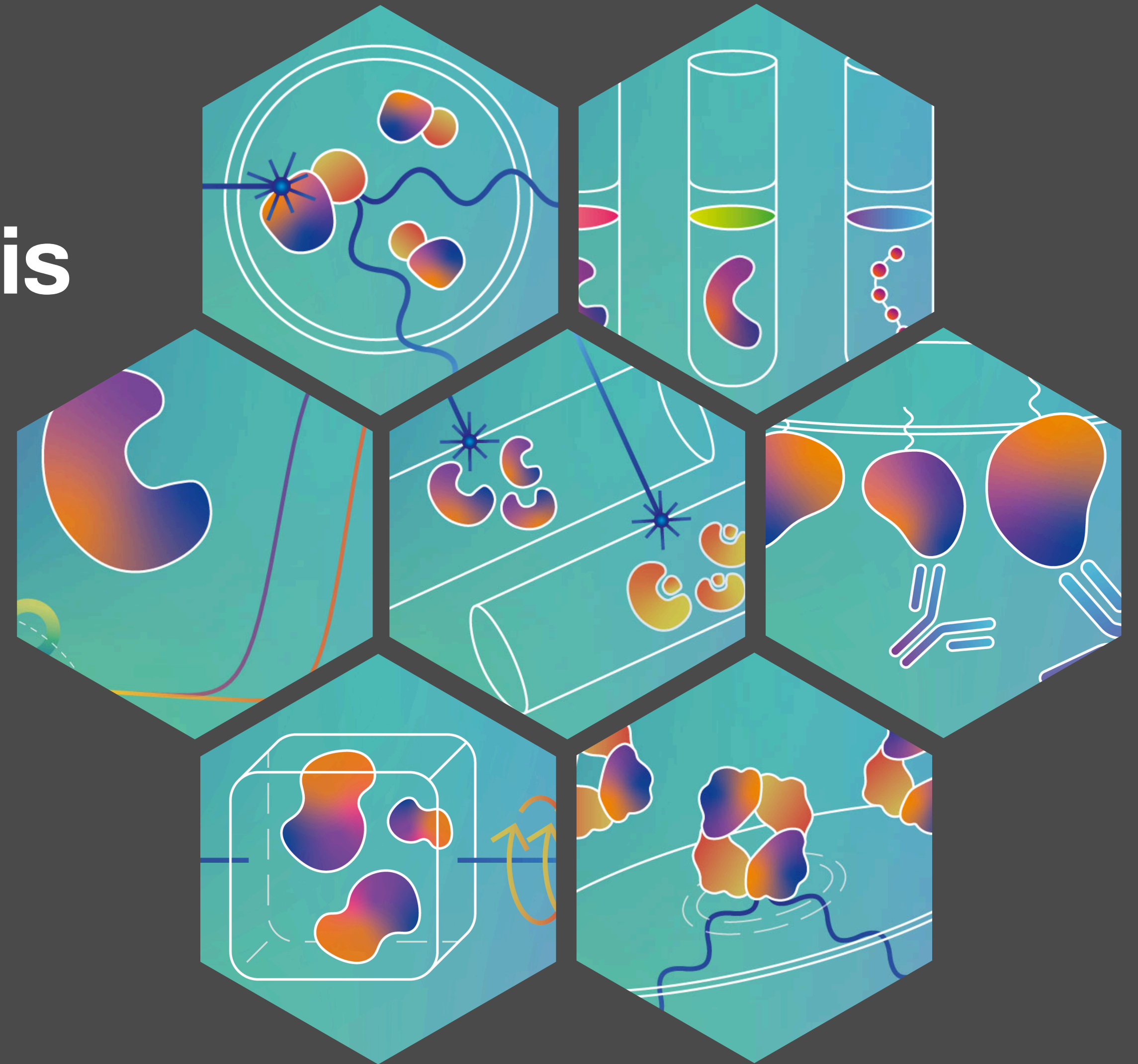
Advanced and easy analysis of biophysical data with the eSPC platform

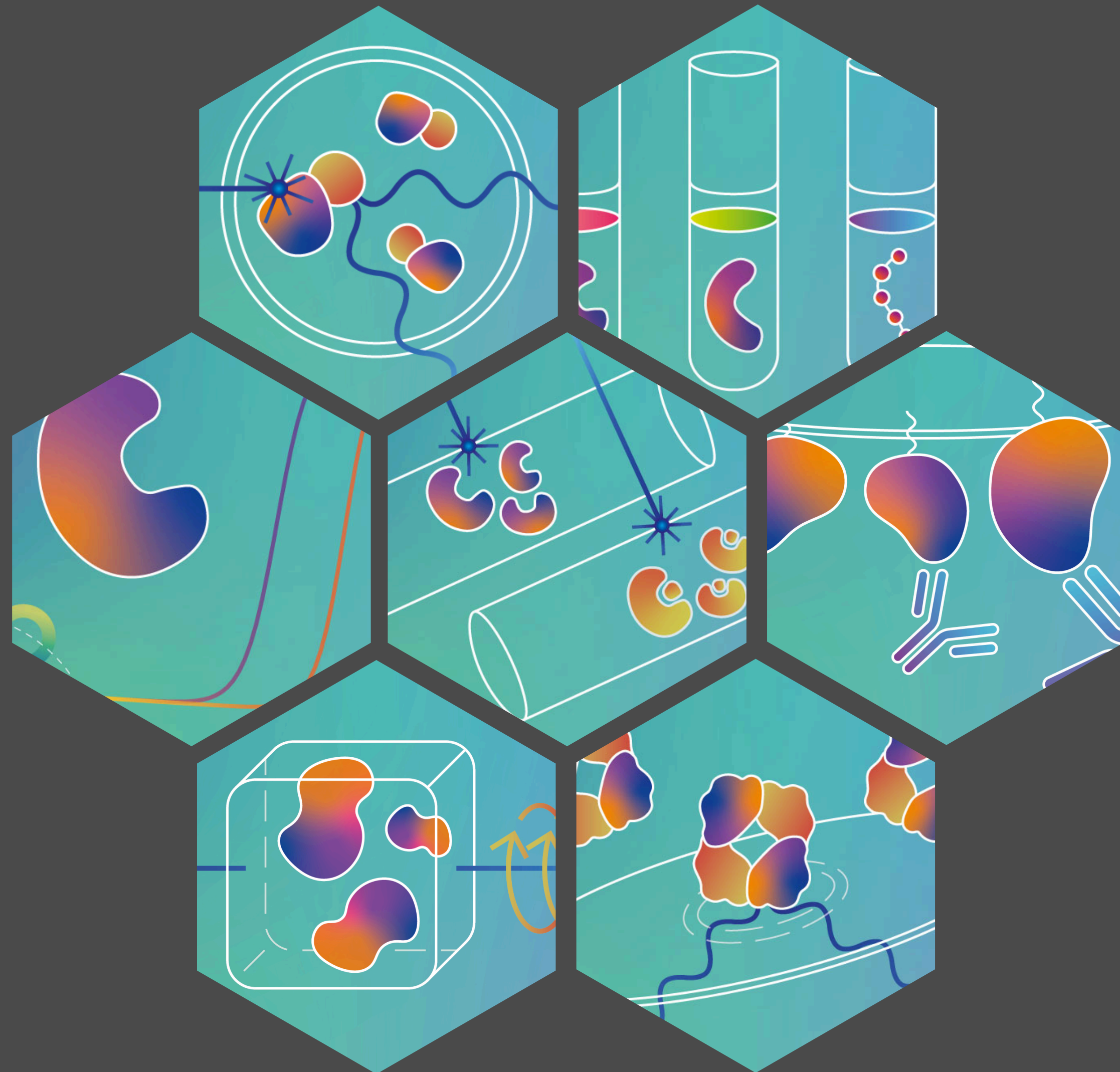
Osvaldo Burastero

Garcia-Alai Team

European Molecular Biology Laboratory (EMBL)

08/04/2026





Each hexagon represents one eSPC tool

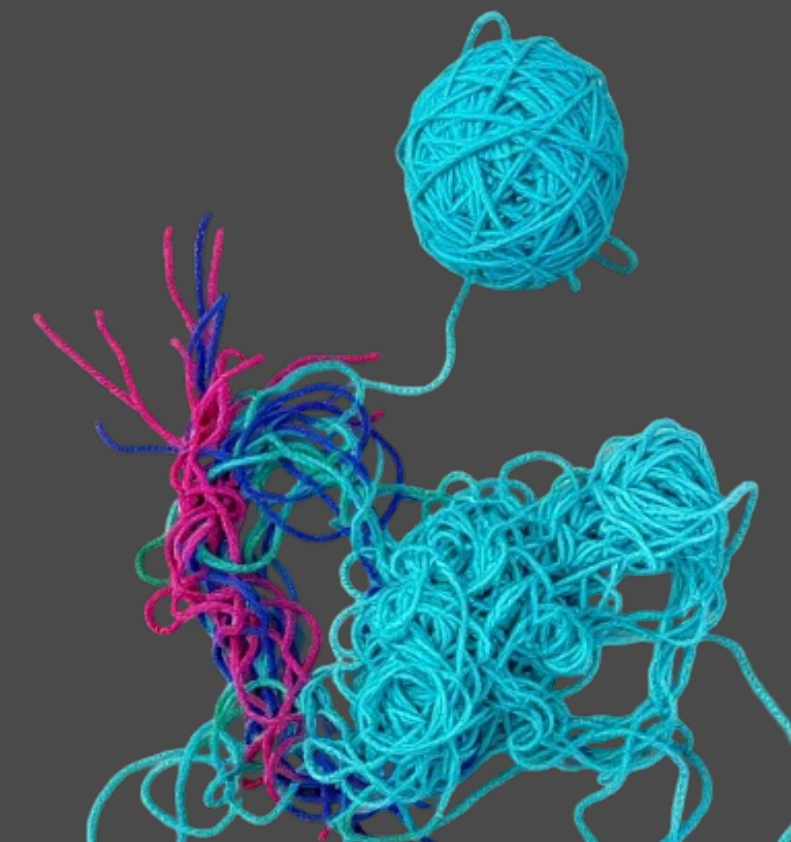
Homogeneity



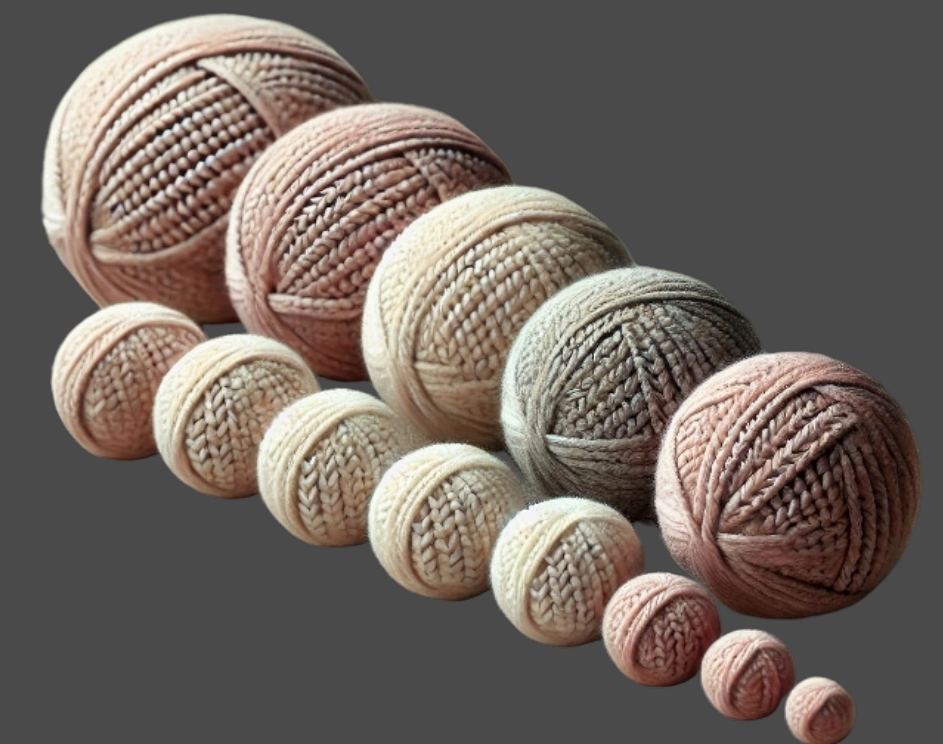
Interactions



Stability



Size/mass





Data analysis tools for biophysics

- Differential scanning fluorimetry
- Microscale thermophoresis
- Mass photometry
- Dynamic light scattering
- Circular dichroism
- Biolayer interferometry

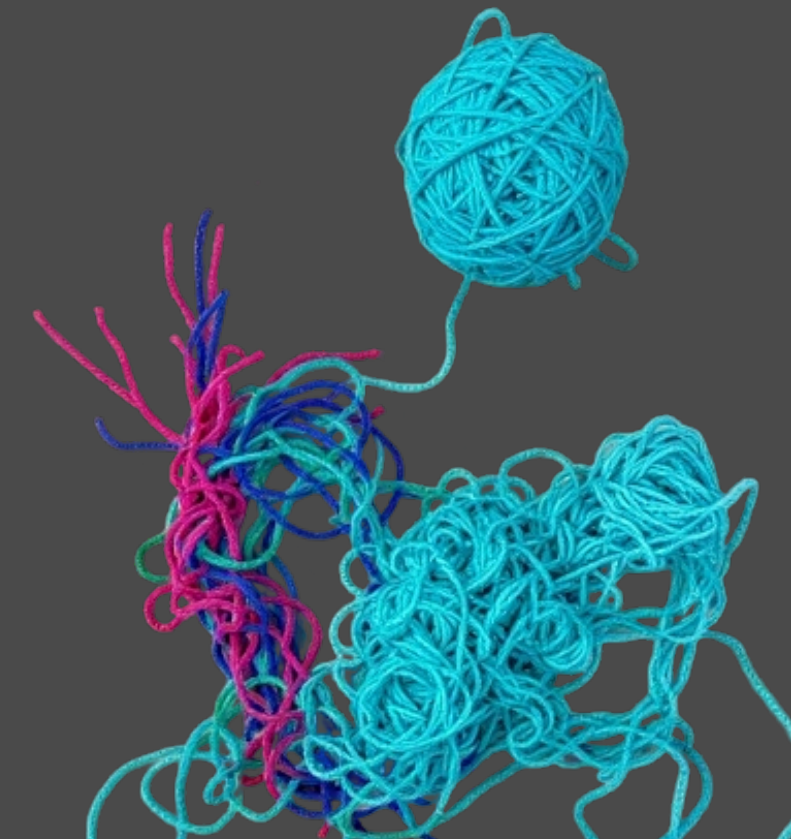
Homogeneity



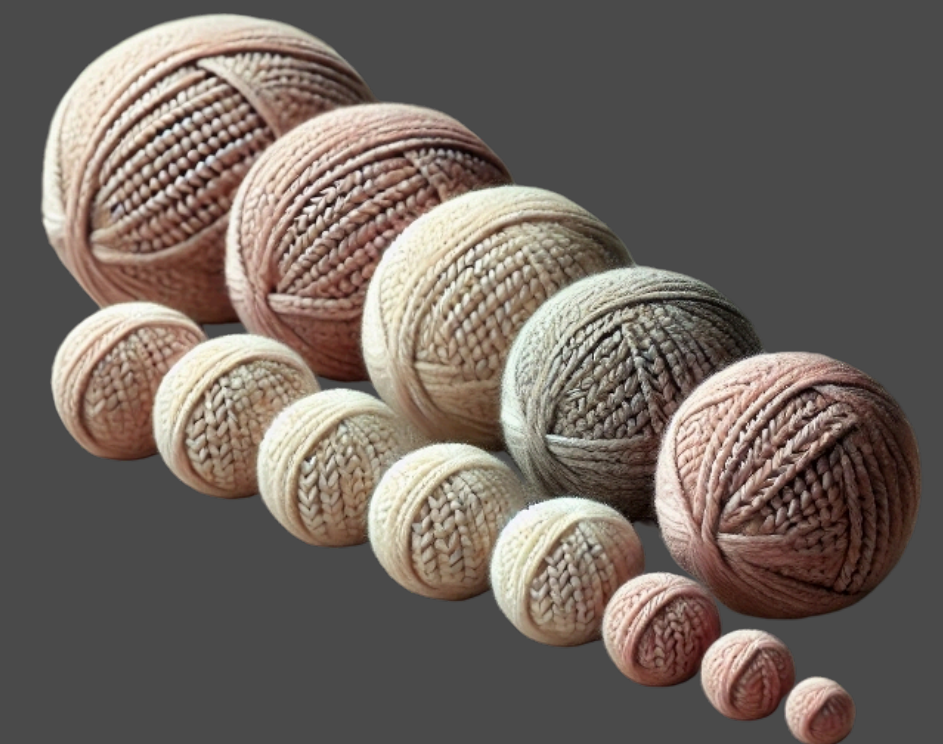
Interactions



Stability

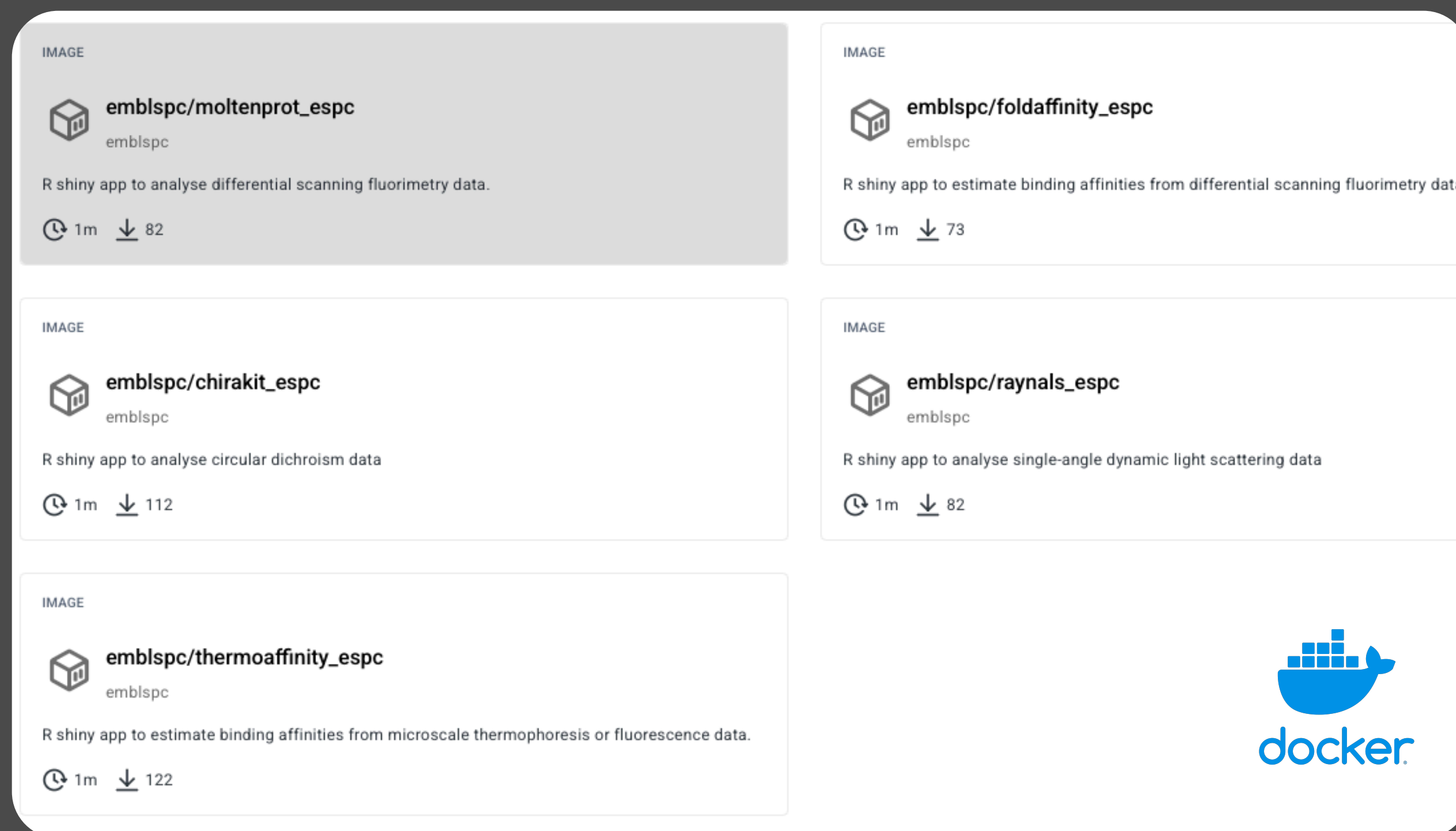
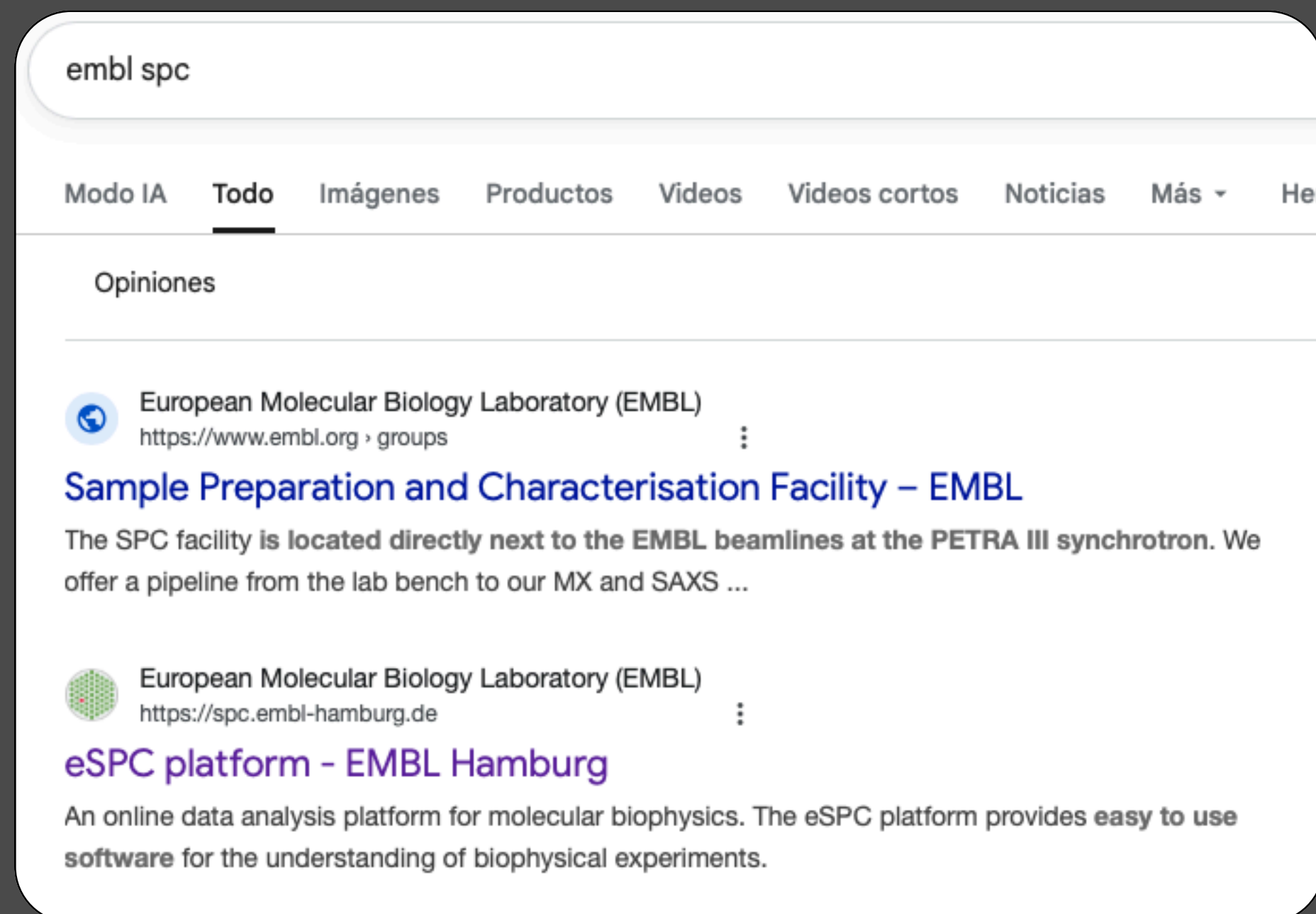


Size/mass



Philosophy behind the eSPC

- Both online and for desktop, and free (for academia)



Philosophy behind the eSPC

- User-centered

"Thank you for looking into it. We were able to download the data successfully today. Thank you so much for your response and guidance throughout the process."

Dr. Bhumika Shah, Lecturer,
Macquire University, Australia

Tutorials



User support



User Guide

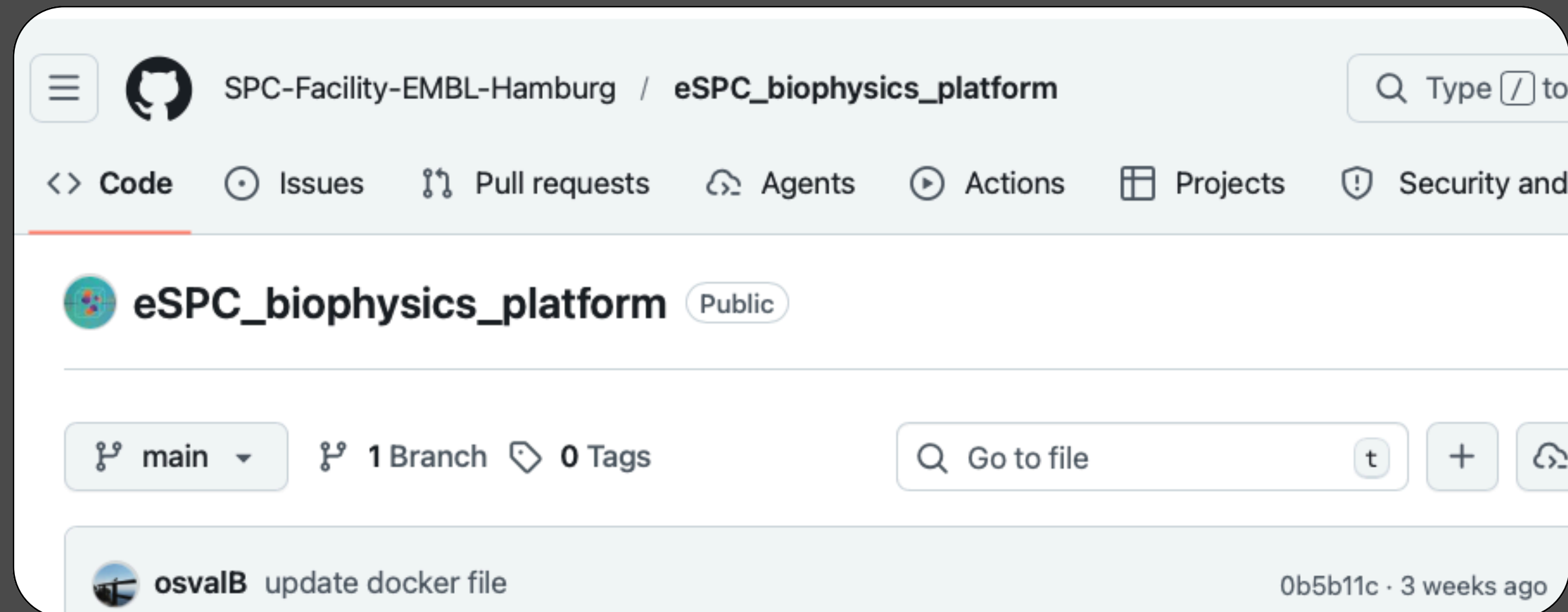


"I just spent some time trying the new feature and have to say I love it! It is really intuitive and the overlay in addition with subplots is amazing"

Magdalena Otto, PhD candidate,
Vienna BioCenter, Austria

Philosophy behind the eSPC

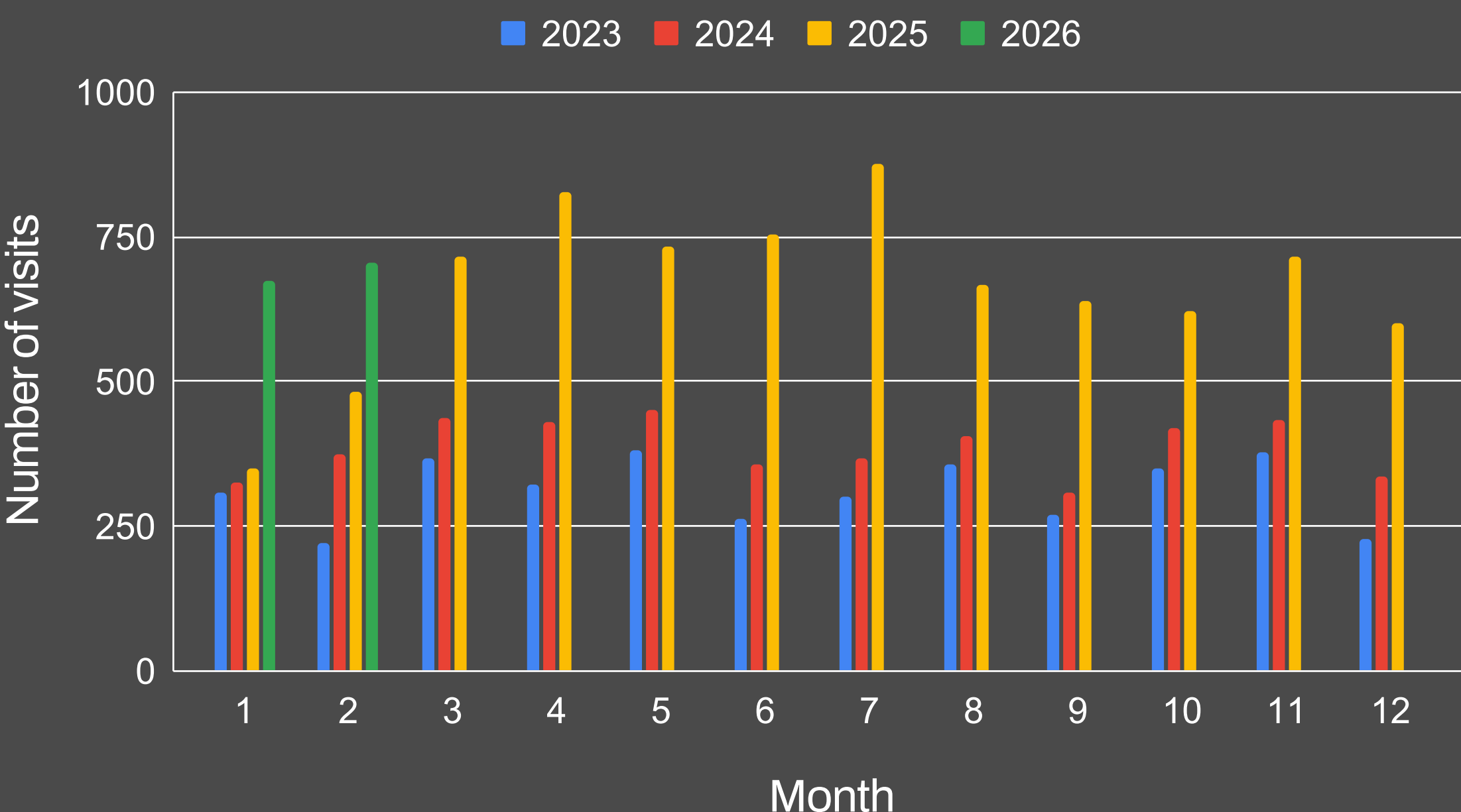
- Open-source



```
268 def differential_matrix_association_induced_fit(koff, kon, kc, krev, a):
283     Rate constant for E1S -> E1 + S.
284     kon : float
285     Rate constant for E1 + S -> E1S.
286     kc : float
287     Rate constant for E1S -> E2S.
288     krev : float
289     Rate constant for E2S -> E1S.
290     a : float
291     Concentration of the analyte (molecule being flown).
292
293     Returns
294     -----
295     np.ndarray
296         Differential matrix.
297     """
298     row1 = [-koff - kon * a, -koff]
299     row2 = [-kc, -kc - krev]
300
301     matrix = np.array([row1, row2])
302
303     return matrix
```


Philosophy behind the eSPC

- Useful



 UNIVERSITY OF CAMBRIDGE

Research Facilities at the Department of Biochemistry

HomeCryo-EM ▾Biophysics ▾NMR ▾X-ray crystallography ▾

Mass Photometry

- [Refeyn Ltd](#)
- [PhotoMol](#) analysis from the European Molecular Biology Laboratory (EMBL) Hamburg Station

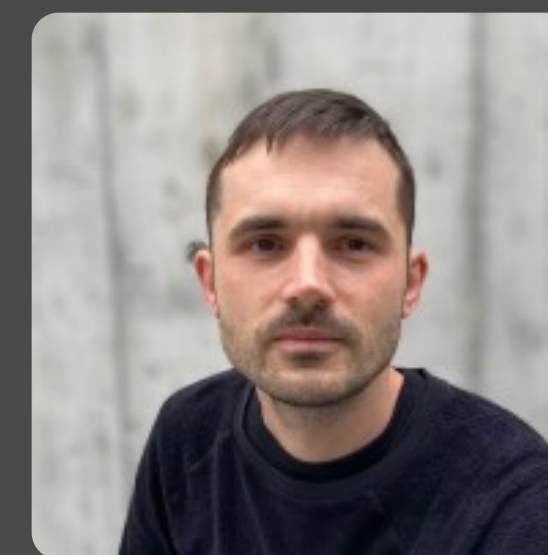
 amyloid Research Sector

APIEMOKSLASHUB

- [Anaconda](#) – nemokama įvairių programų ir paketų rinkinys darbui su Python, R
- [LabPlot](#) – nemokama duomenų analizės programa
- [Serial cloner](#), [ApE](#), [Benchling](#), [VectorBee](#) – nemokamos molekulinio klonavimo įrankių programos
- [SPC Webserver](#) – EMBL programos analizuoti DSF, DLS ir CD duomenis.

Philosophy behind the eSPC

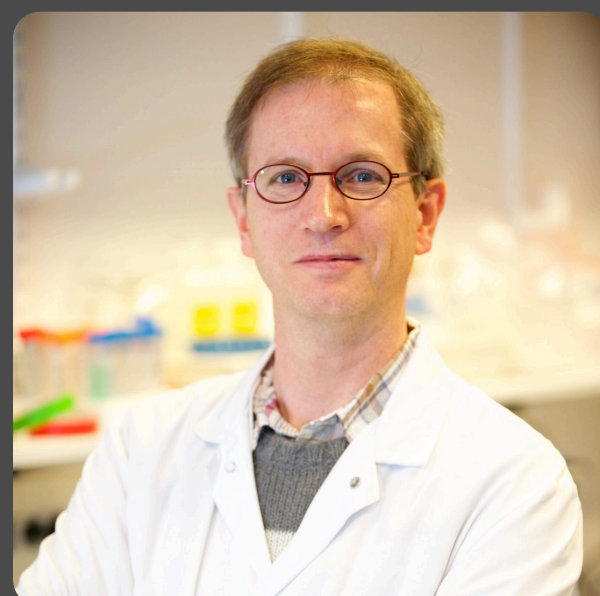
- Collaborative



San Hadži
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Ljubljana, Slovenia



Uroš Zavrtanik
University of Ljubljana,
Ljubljana, Slovenia



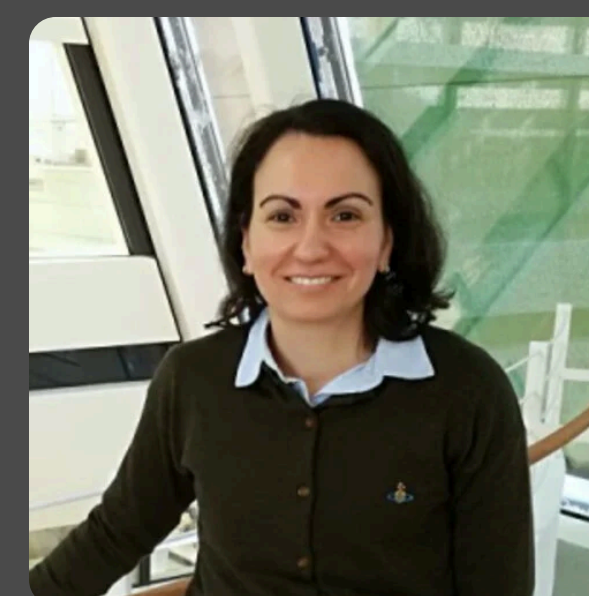
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Germany



Maria Garcia Alai
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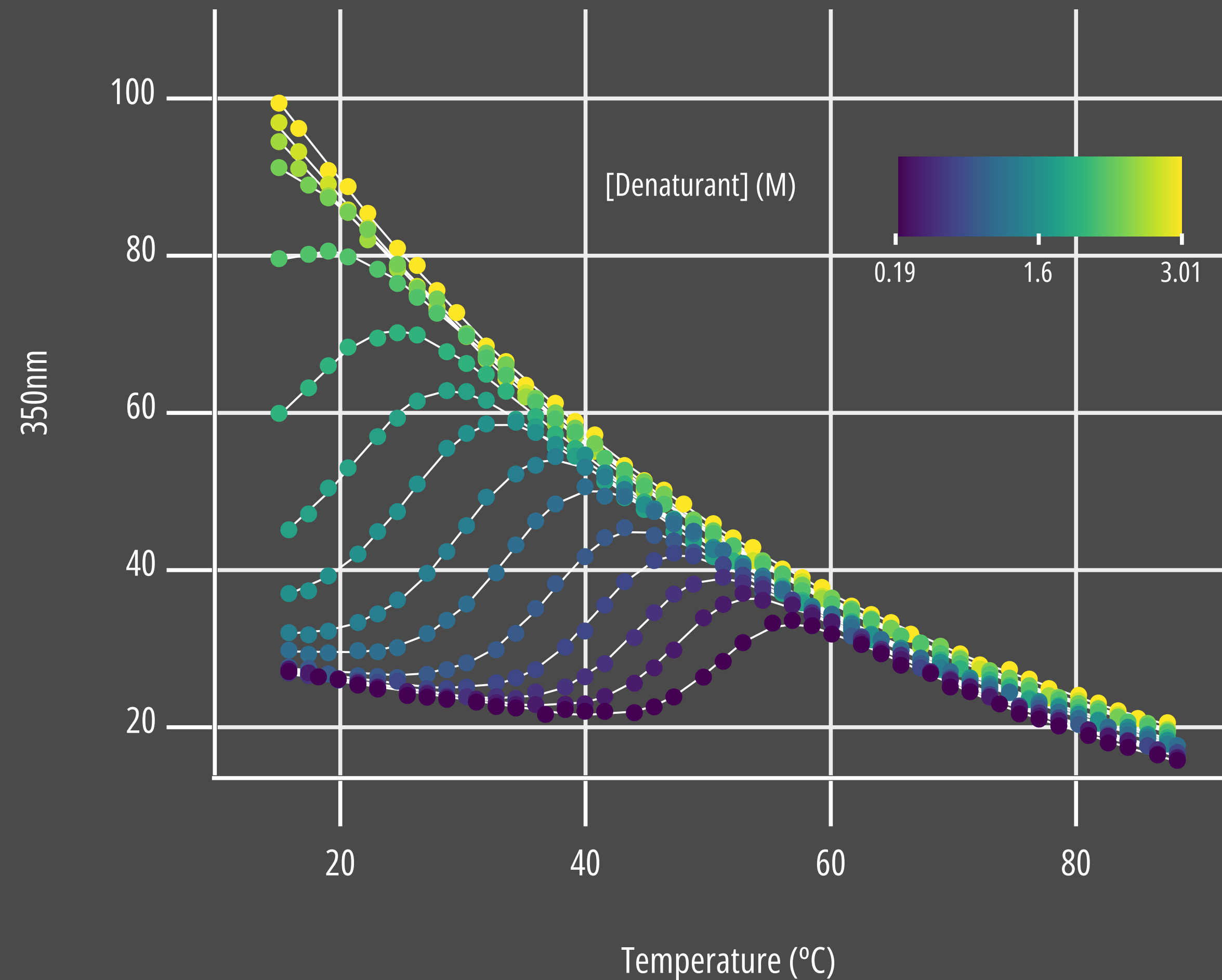


Nyk Jones
Aarhus University,
Aarhus, Denmark

*Current address: IRUBIS, Munich, Germany

Differential scanning fluorimetry

Folded protein
↓ Heat
Unfolded protein



Thermodynamic parameters*

Enthalpy of unfolding ΔH

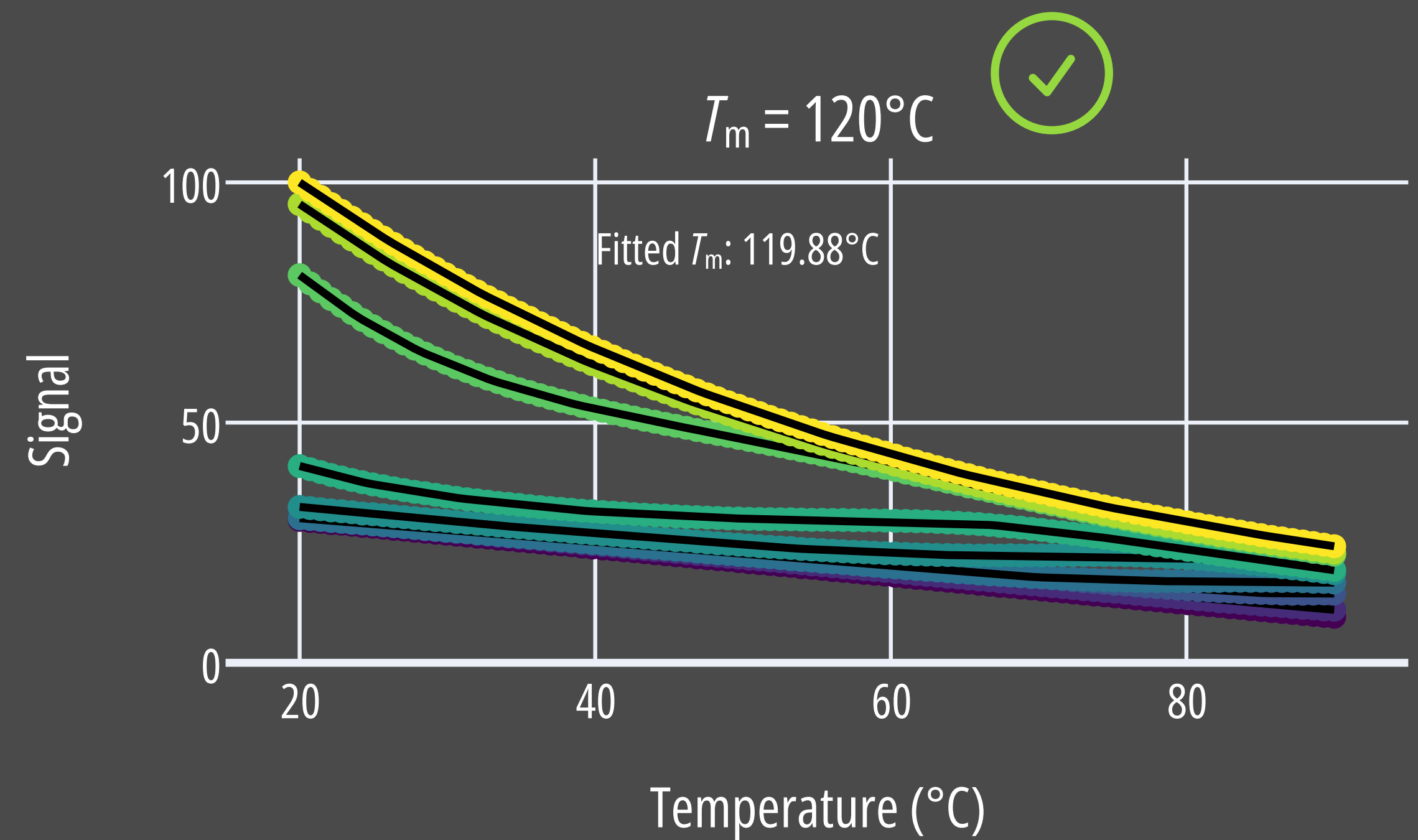
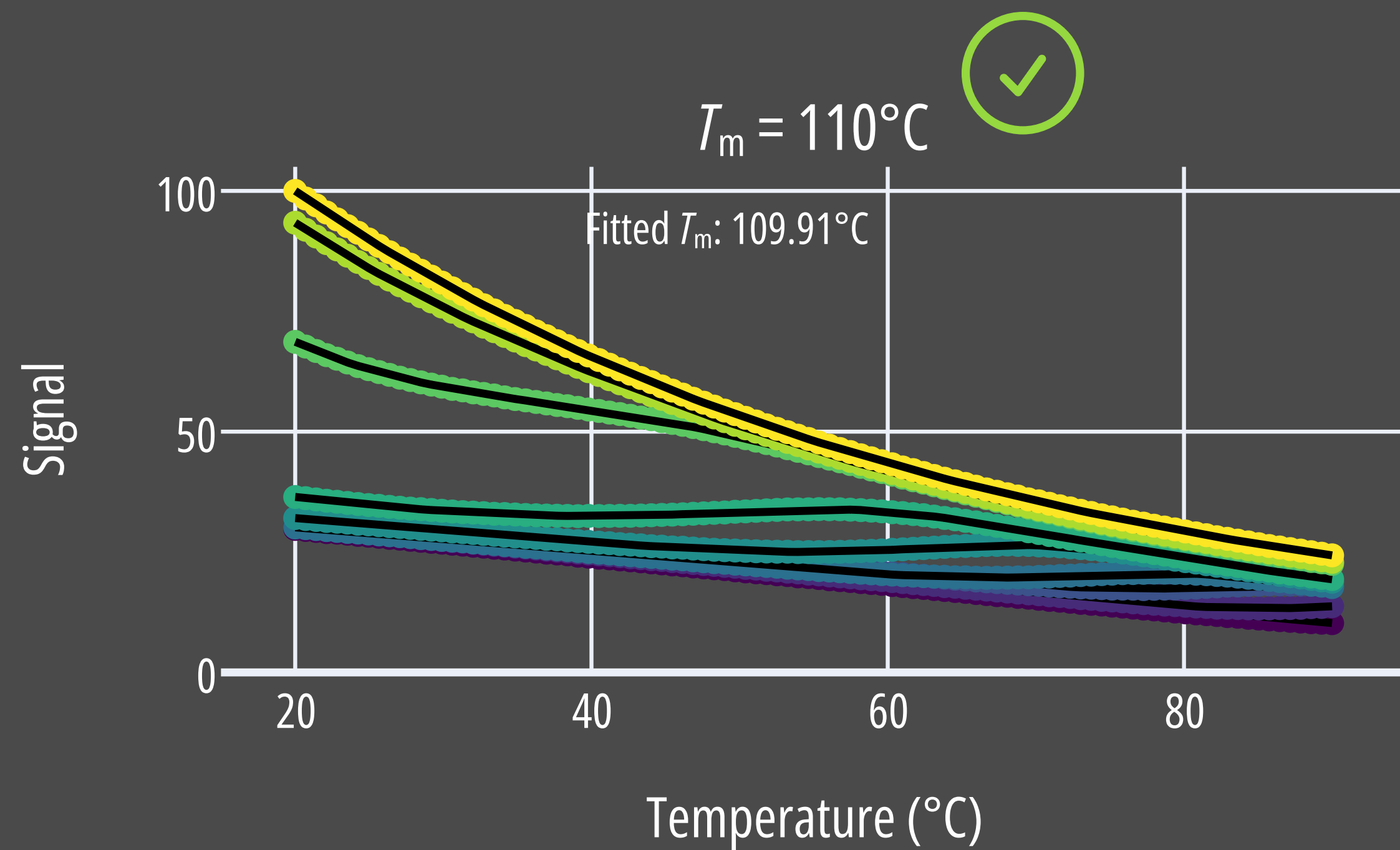
Temperature of melting T_m

Heat capacity of unfolding ΔC_p

Hamborg *et al.* Global analysis of protein stability by temperature and chemical denaturation. 2020

***Under many
assumptions**

Can we estimate T_m , even when it is not observed?



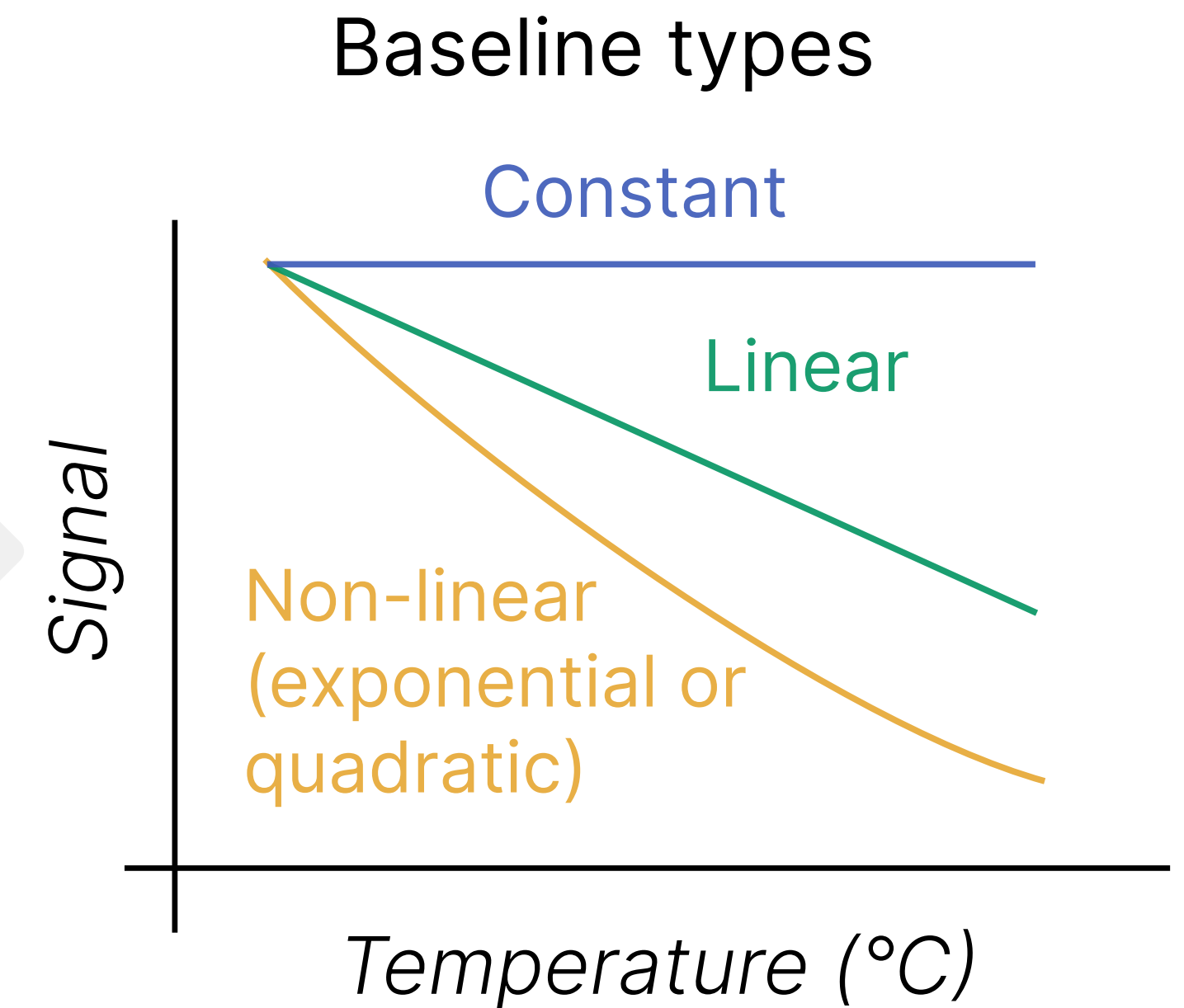
CheMelt workflow

1 Data import

- Signal to analyse (e.g. fluorescence at 350 nm)
- Temperature range (e.g. 20 - 90°C)
- Denaturant concentrations (e.g. 1 - 5 M)

2 Model selection

- Baselines types and global/local parameters
- Global thermodynamic parameters



2 Model selection

- Baselines types and global/local parameters
- Global thermodynamic parameters

3 Fitting

- Data subset selection
- Parameter bounds
- Visual assessment of the fitted curves

Intercepts

Slopes

Local

Local

Local

Global

Global

Global

3 Fitting

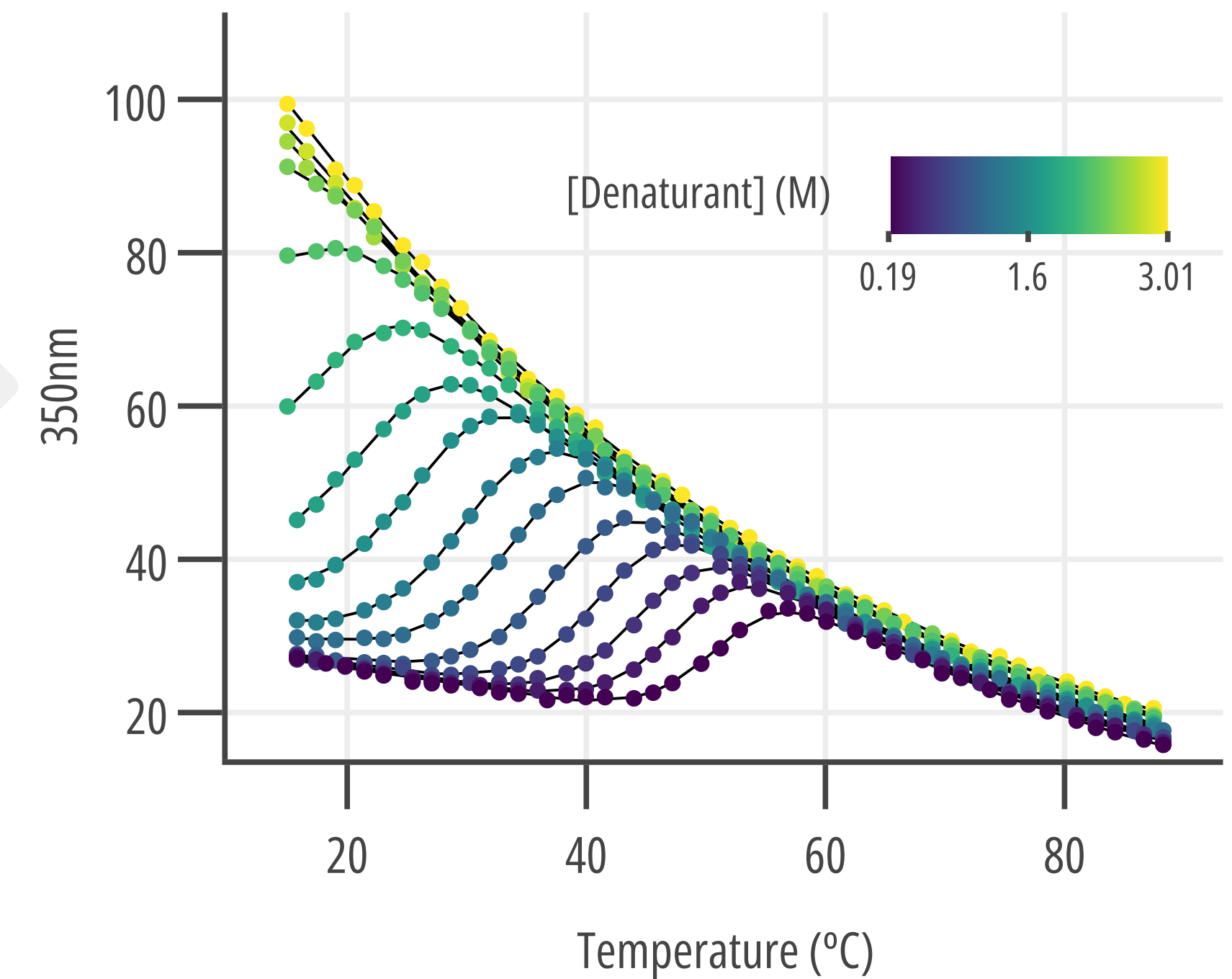
- Data subset selection
- Parameter bounds
- Visual assessment of the fitted curves

4 Evaluation and interpretation

- Estimated thermodynamic parameters: T_m , ΔC_p , ΔH and m .
- Predicted ΔG at all temperatures.

5 Export results

- Fitted curves data
- Publication-grade figures



Relationship between ΔC_p and protein length

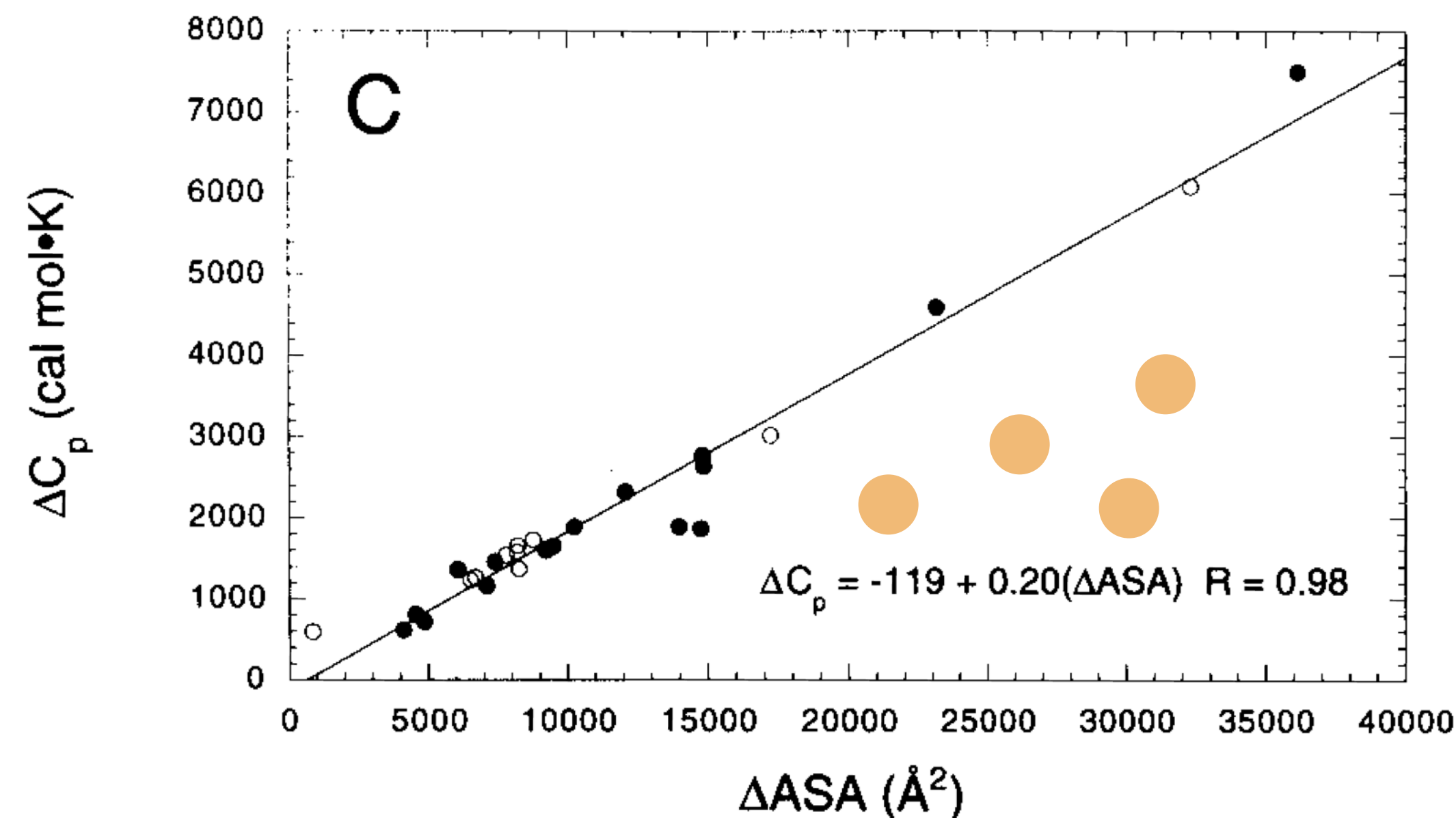
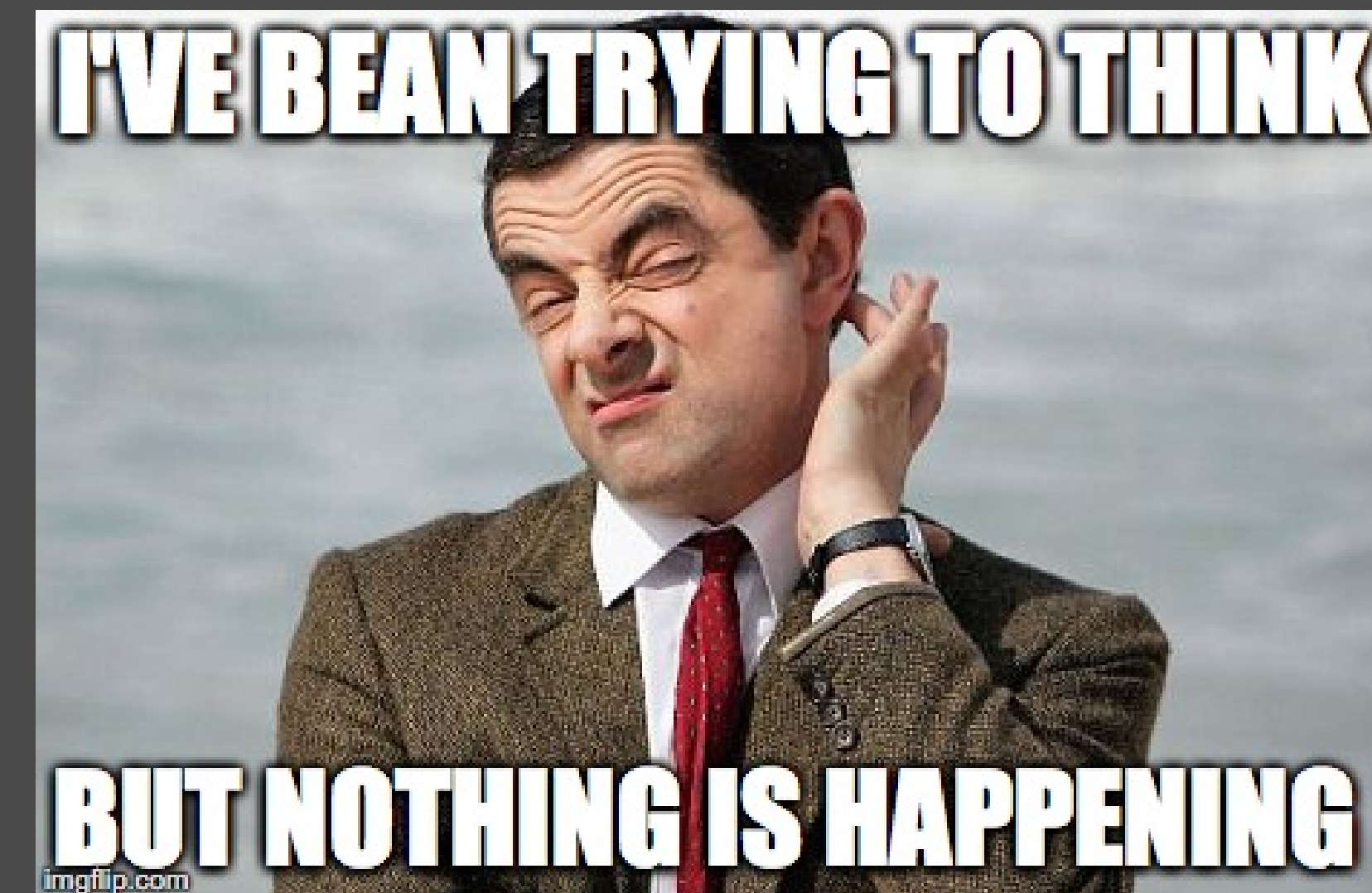


Fig. 3. Dependence of (A) m value for Gdn HCl denaturation, (B) m value for urea denaturation, and (C) heat capacity change upon unfolding on ΔASA for the 45 proteins in our data set, corrected for the effect of crosslinks by 900 Å² per crosslink (see text). Proteins with no crosslinks are shown as ● and those with crosslinks as ○.

- Designed proteins



ΔC_p in hyper-stable proteins

Biophysical Journal



Volume 83, Issue 6, December 2002, Pages 3126-3133

Toward the Physical Basis of Thermophilic Proteins: Linking of Enriched Polar Interactions and Reduced Heat Capacity of Unfolding

Huan-Xiang Zhou  

 OPEN ACCESS  PEER-REVIEWED

RESEARCH ARTICLE

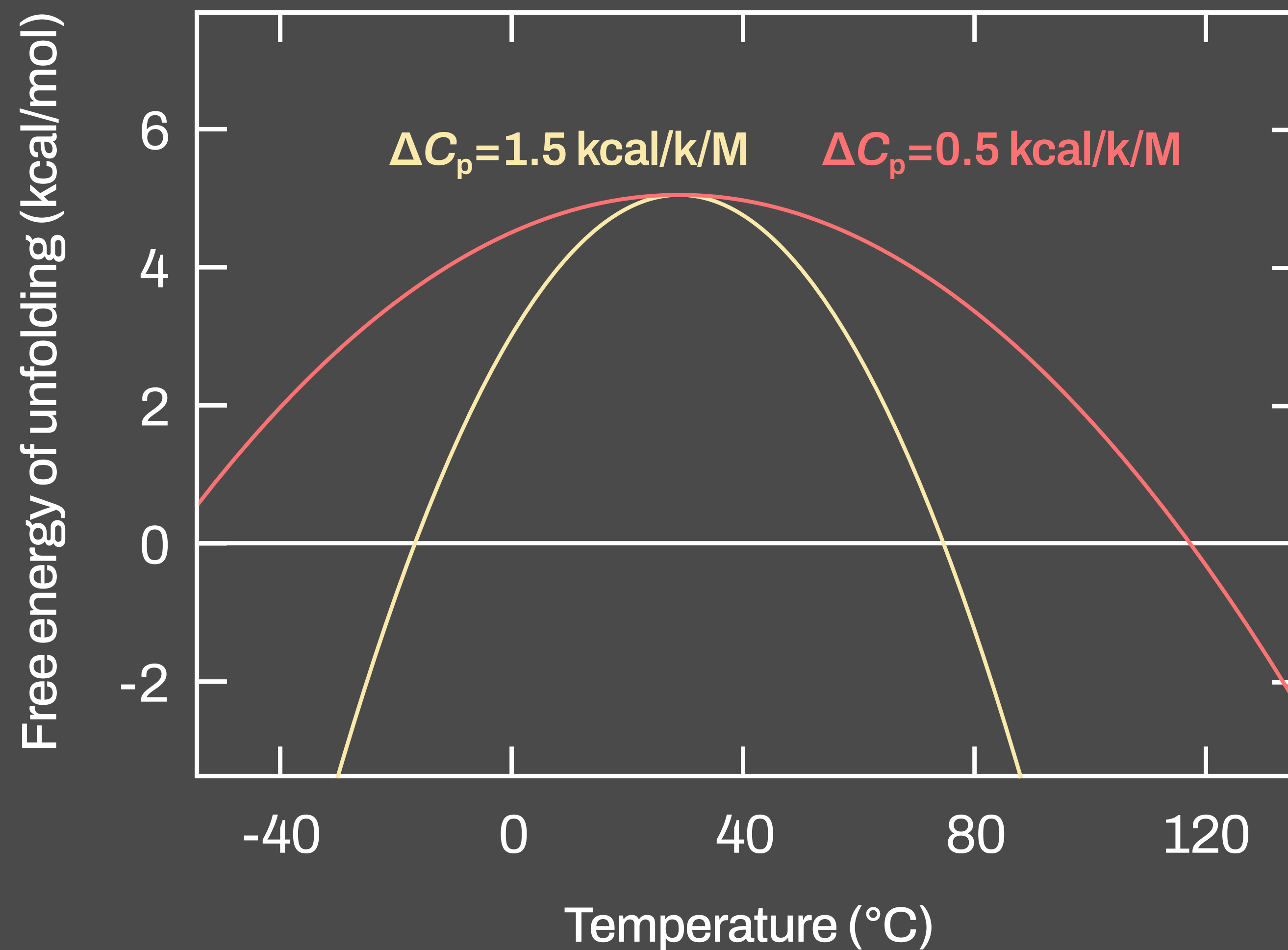
Stabilizing Salt-Bridge Enhances Protein Thermostability by Reducing the Heat Capacity Change of Unfolding

Chi-Ho Chan, Tsz-Ha Yu, Kam-Bo Wong 

Published: June 24, 2011 • <https://doi.org/10.1371/journal.pone.0021624>

Article	Authors	Metrics	Comments	Media Coverage
				

ΔC_p in hyper-stable proteins



CheMelt, the next eSPC tool

■ **Global analysis of thermal and chemical denaturation using CheMelt:
Thermodynamic dissection of highly thermostable *de novo* designed proteins**

Vili Lampinen^{‡1,2,3,4} ; Osvaldo Burastero^{‡,5,6} ; Iara Plácido Guazzelli^{1,2,3,4} ; Florian Vögele^{5,6} ; Francisca Pinheiro^{1,2,3,4}; Jan S. Nowak¹ ; Maria M. Garcia Alai^{*5,6} ; Magnus Kjaergaard^{*1,2,3,4}



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Florian Vögele
Trainee
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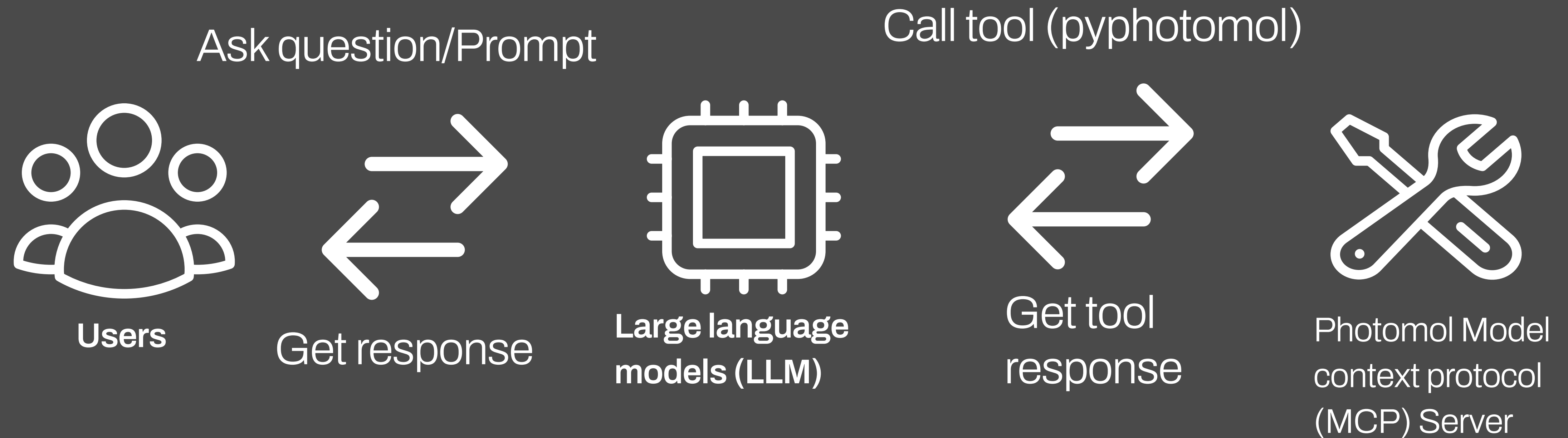


Magnus Kjaergaard
Associate Professor
Aarhus University,
Aarhus, Denmark

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Preprint coming soon

What about AI and data analysis?



-
- ☑ Uses eSPC “proven” functions
 - ☑ Fast
 - ☑ Hightroughput

MCP PyCheMelt

Intuitive Data Handling: Using natural language the DSF data can be analysed in the same way as in the webserver. No coding is required.

Interactive Analysis: Through conversation the parameters can be adjusted during the analysis process



Florian Vögele
Trainee
EMBL Hamburg,
Germany

Take home message



The eSPC platform provides open-source, user-friendly tools for analysing biophysical data



A tool for global analysis of chemical and thermal denaturation is being developed



We're working on creating MCP servers for each tool. Contact us to collaborate!

