From protein sample to high-resolution cryo-EM map

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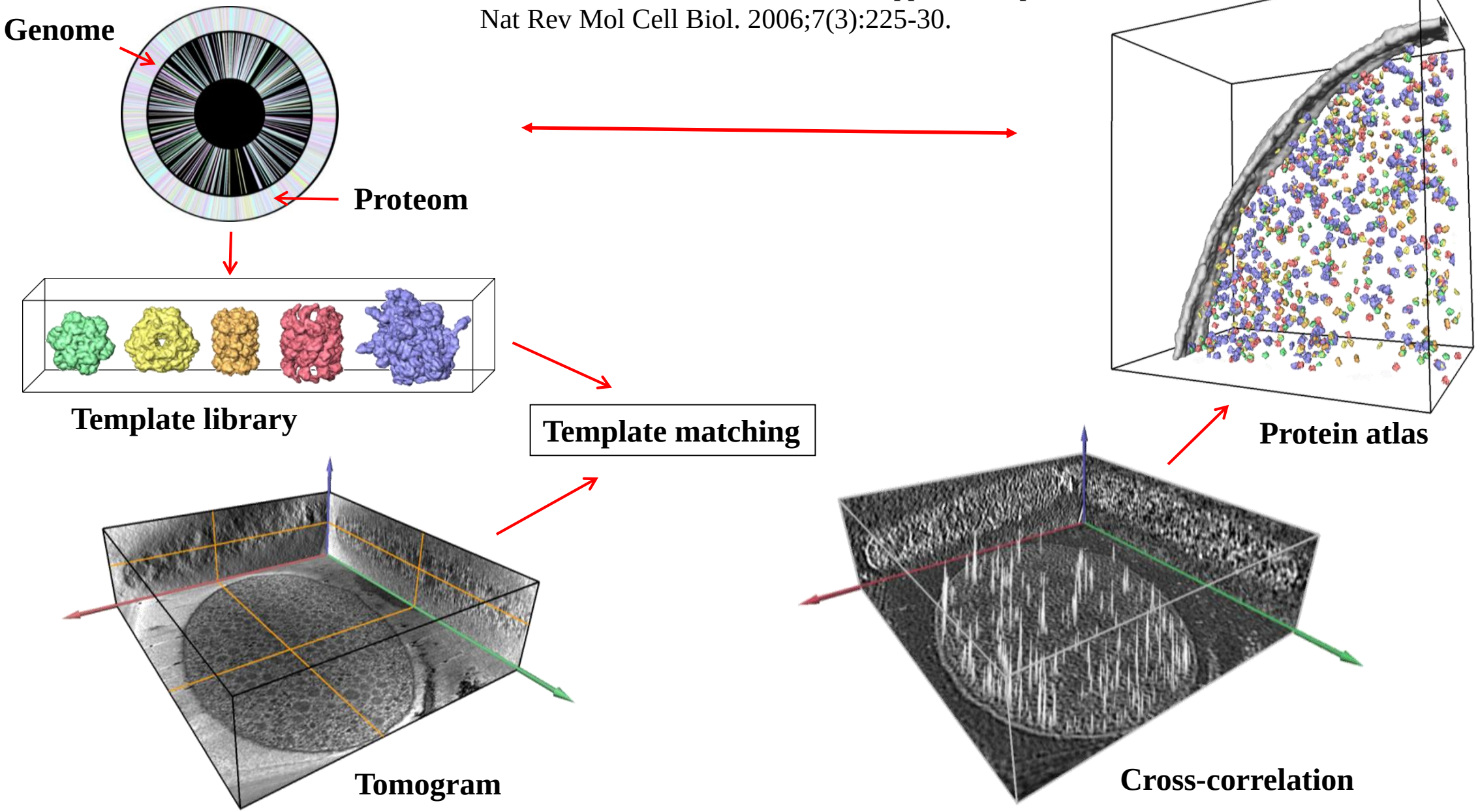
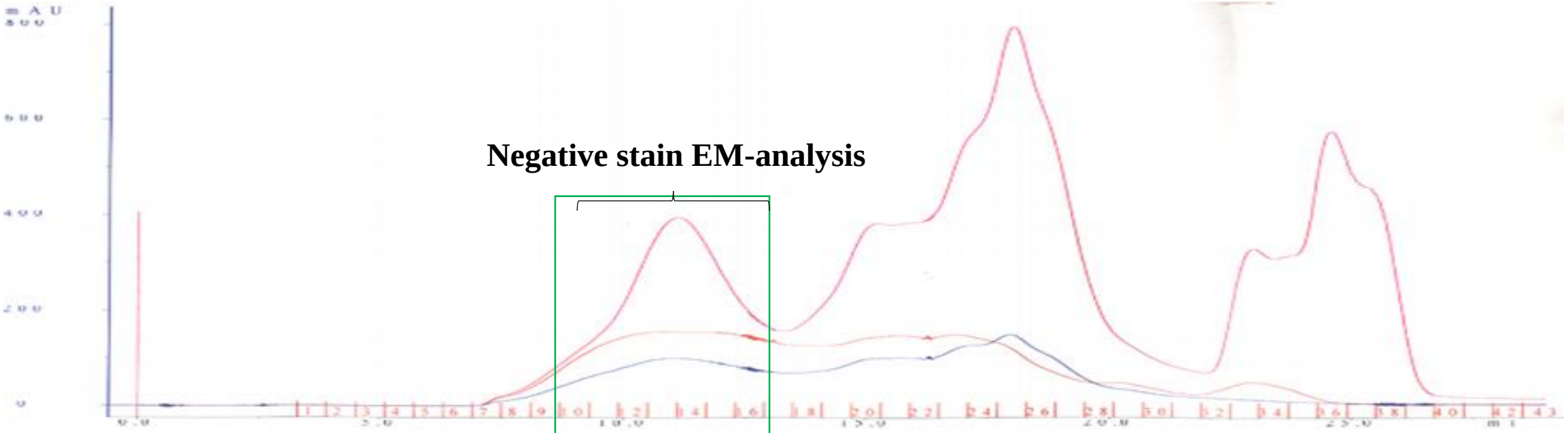
MedInProt conference, Budapest, 21.04.2018

A visual approach to proteomics

Nickell S, Kofler C, Leis AP, Baumeister W. A visual approach to proteomics. Nat Rev Mol Cell Biol. 2006;7(3):225-30.



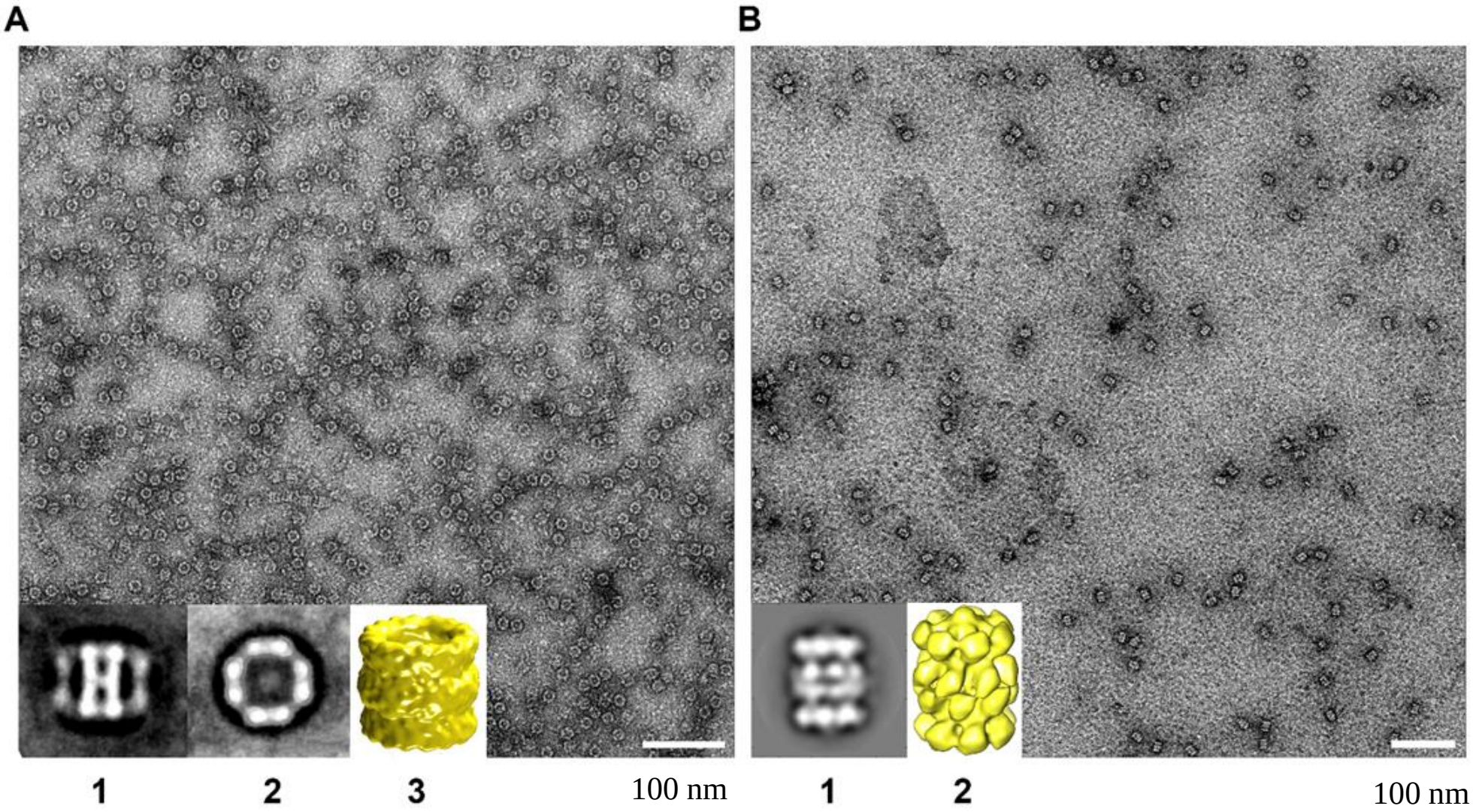
Purification of *T. acidophilum* complexes captured with specific antibodies



Superose 6 separation of affinity-purified thermosomes separated on 15% SDS-gel

Hubert A, Mitani Y, Tamura T, Boicu M, Nagy I. Protein complex purification from Thermoplasma acidophilum using a phage display library. J Microbiol Methods. 2014;98:15-22.

Electron micrographs of negatively stained thermosome (A) and proteasome (B) particles



1. Class average of side view

A2. Class average of top view

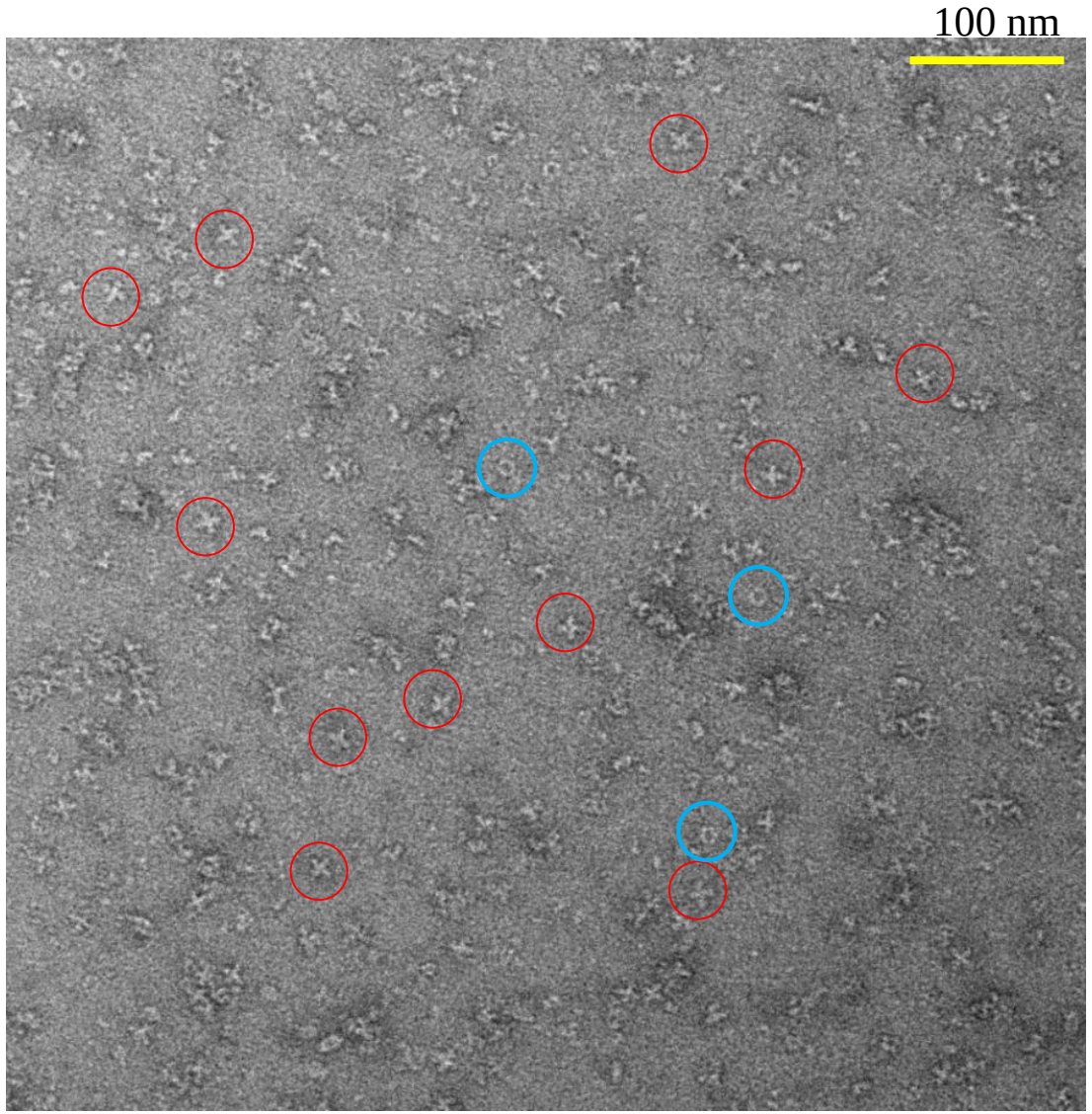
A3, B2: 3D reconstruction from the data

Images were acquired with a CM 200 FEG TEM (160 kV, Philips), equipped with a TVIPS CCD camera (CCD size: 4096 × 4096 pixels.

Hubert A, Mitani Y, Tamura T, Boicu M, Nagy I. Protein complex purification from Thermoplasma acidophilum using a phage display library. J Microbiol Methods. 2014;98:15-22.

Electron micrograph of negatively stained, probable Ta0424/0425 complexes co-purified with a ring-shaped unknown structure

Needle in a haystack: Protein complex purification from Thermoplasma acidophilum with a phage display library, PhD thesis (2013)



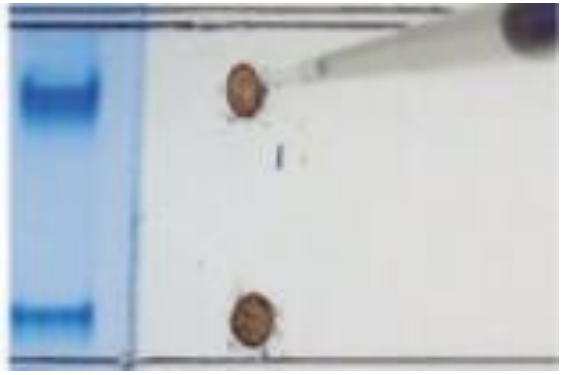
MS evidence:

Ta0425: Formate dehydrogenase related protein 111,9 kDa

Ta0424: Hypothetical protein 16,2 kDa

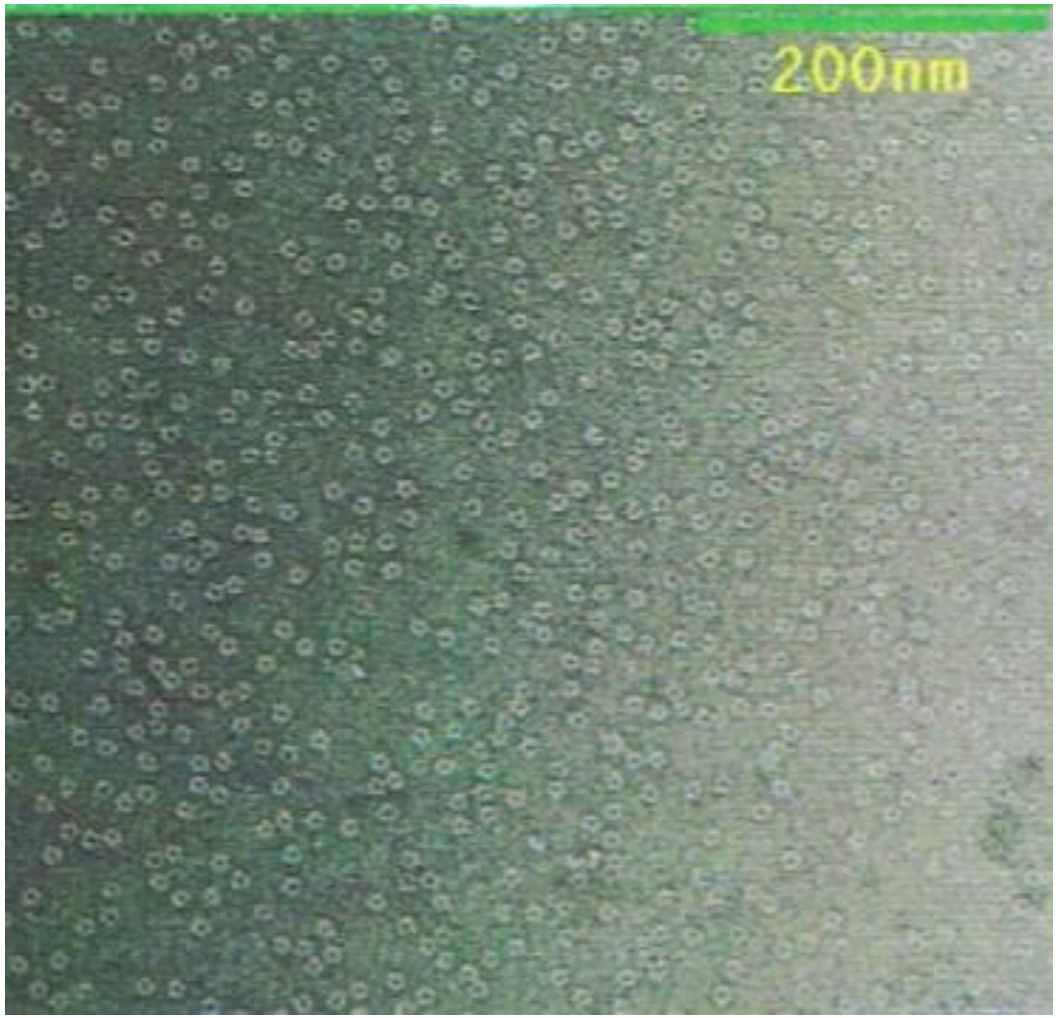
Ta1207: Hypothetical protein, 37.7 kDa

Ta1207 was further isolated applying the „grid-blotting" technique:

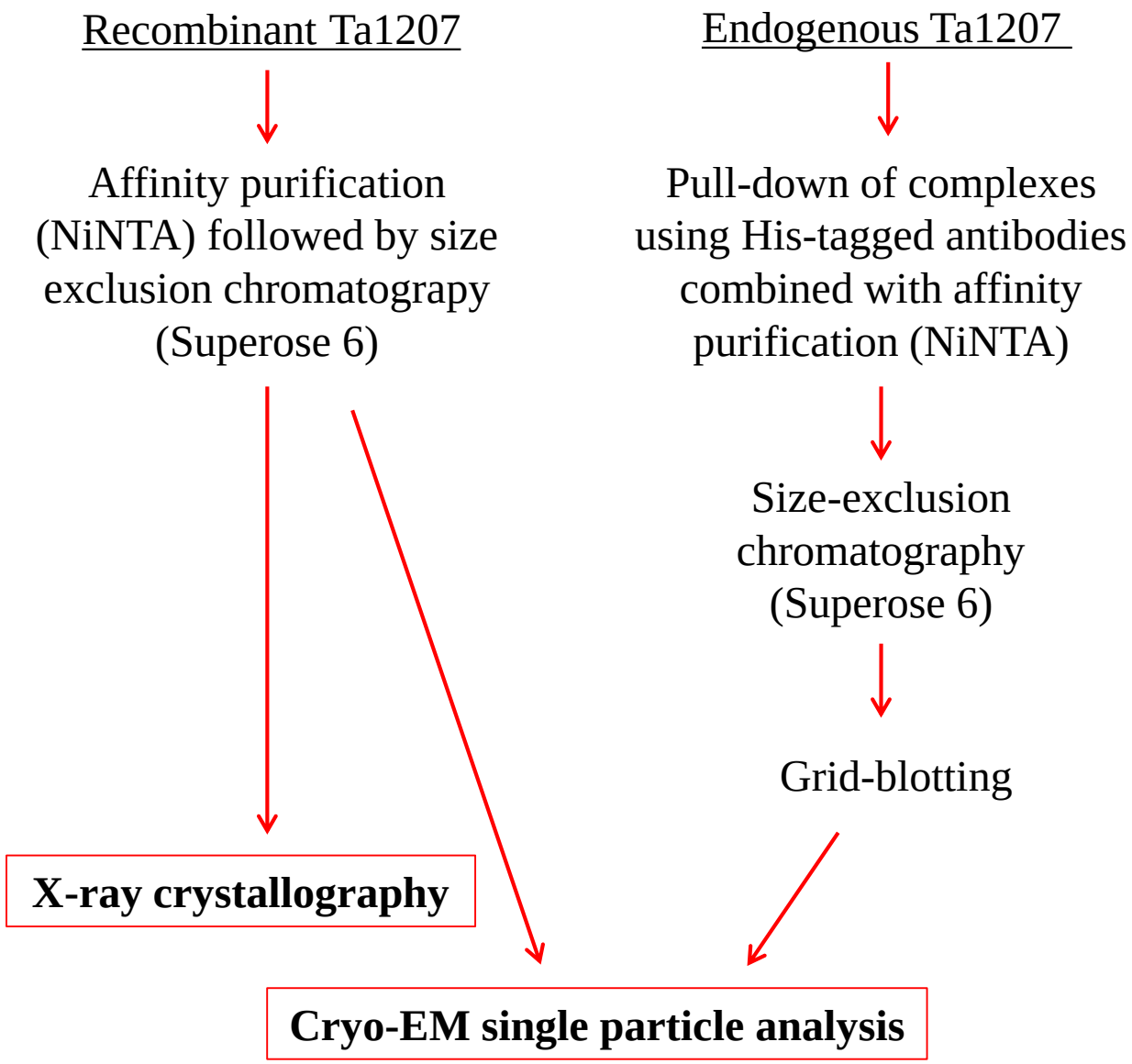


Knispel RW, Kofler C, Boicu M, Baumeister W, Nickell S. Blotting protein complexes from native gels to electron microscopy grids. Nat Methods. 2012;9(2):182-4.

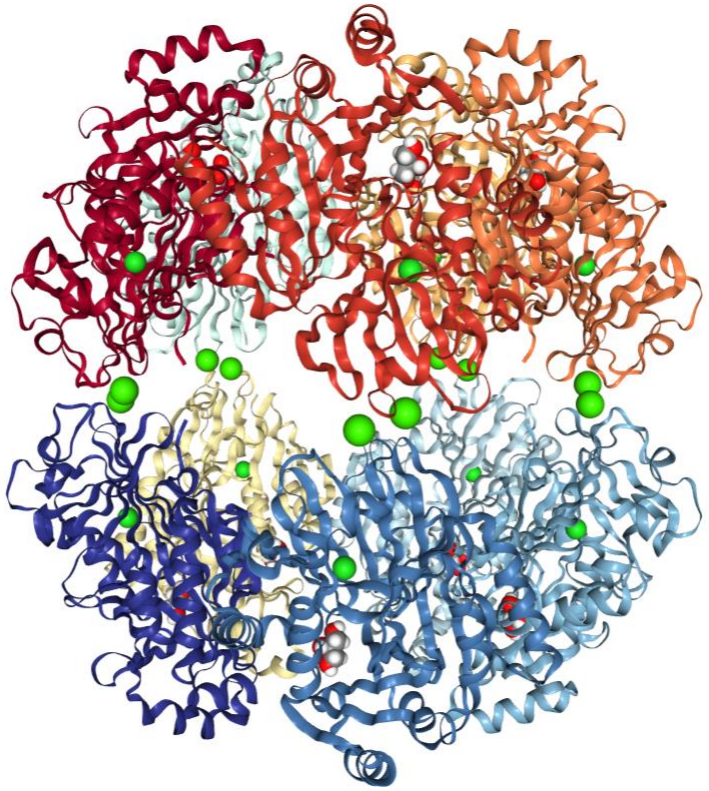
Purification of Ta1207 particles for structural analysis



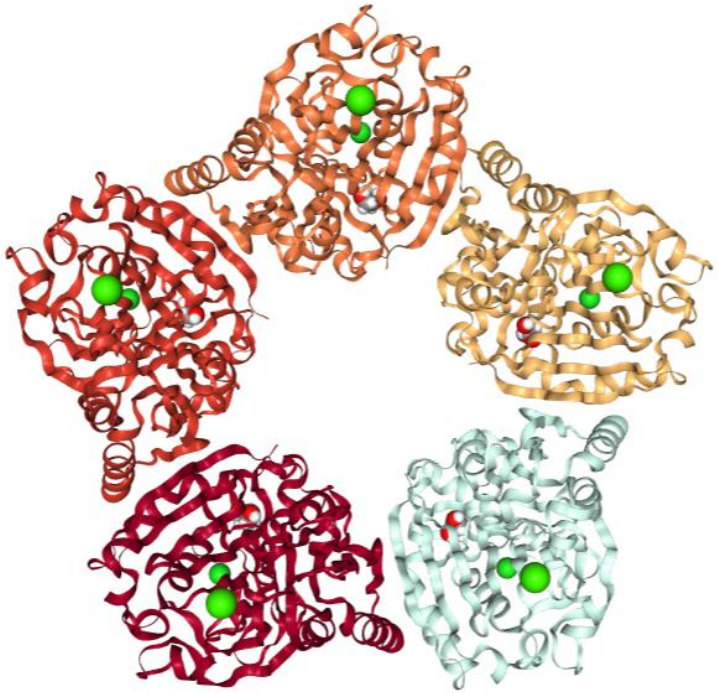
Electron micrograph of negatively stained Ta1207



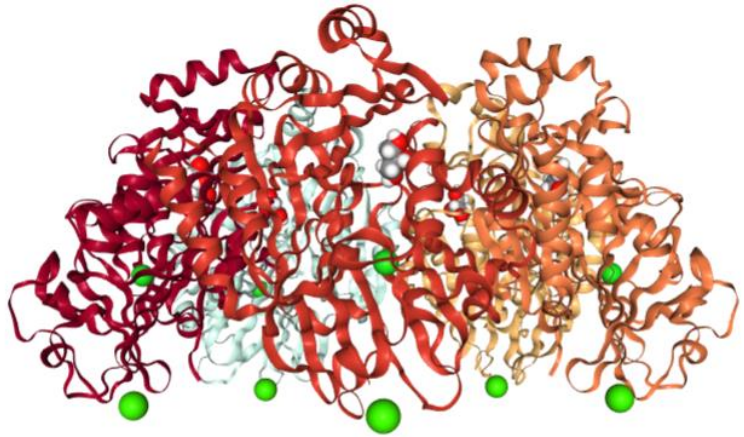
Crystal structure of Ta1207 (PDB-5M86)



Asymmetric unit



Bioassembly - Top view

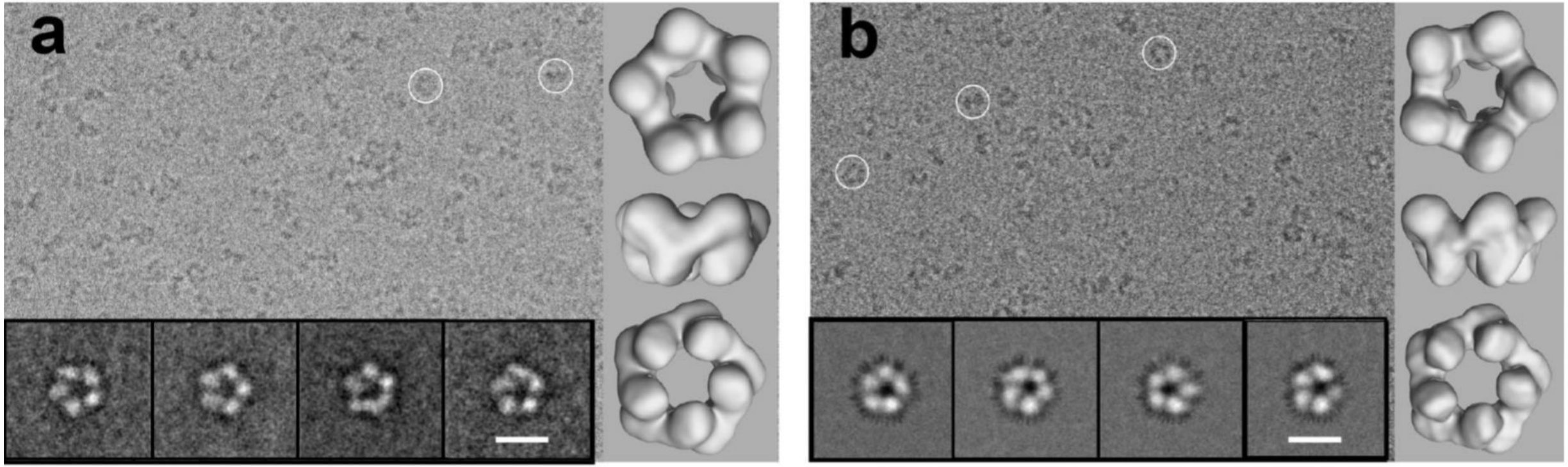


Bioassembly - Side view

Pathare GR, Nagy I, Hubert Á, Thomas DR, Bracher A. Crystal structure of the Thermoplasma acidophilum protein Ta1207. Acta Crystallogr F Struct Biol Commun. 2017;73(Pt 6):328-335.

Electron microscopy single particle analysis of Ta1207

Pathare GR, Nagy I, Hubert Á, Thomas DR, Bracher A. Crystal structure of the *T. acidophilum* protein Ta1207. Acta Crystallogr F Struct Biol Commun. 2017;73(Pt 6):328-335.



Cryo-EM micrographs of purified endogenous Ta1207 (**a**) and recombinant Ta1207 (**b**).

The insert shows selected averages from 2D particle classification at the same scale; the white scale bar indicates 100 Å length. On the right, the 3D reconstructions of the particle at 14 Å resolution are shown, revealing 5-fold rotational symmetry.

Work-flow of single particle image processing

Software packages: SCIPION, SPIDER, Relion

1. **Collection of images** with a defined defocus range (ΔF) from -0.8 and -2.8 μm (electron dose 15-20 $e^-/Å^2$)

2. Screening of images for the presence of particles and good **Power-spectrum**

3. Determination of defocus of „good" images and **CTF correction** of images

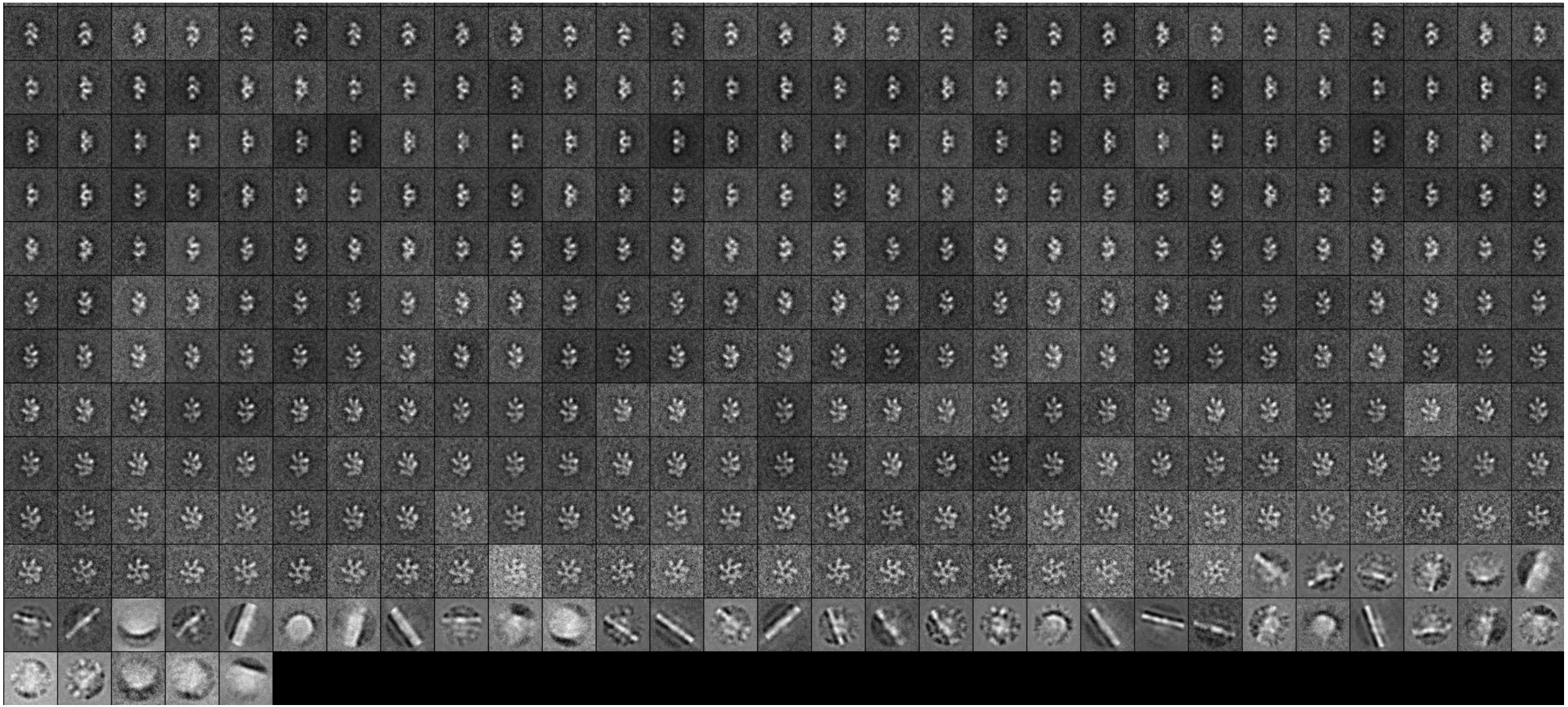
4. **Picking** of particles manually or in an automated manner using template models (2D class averages or projections from a known 3D reference structure)

5. **Alignment** (Reference-free / Reference-based) – **Clustering** – **Averaging** (K-means clustering algorithm)

6. **3D-reconstruction** from the data – Iterative process, in which the current reconstrution becomes the reference for the next round

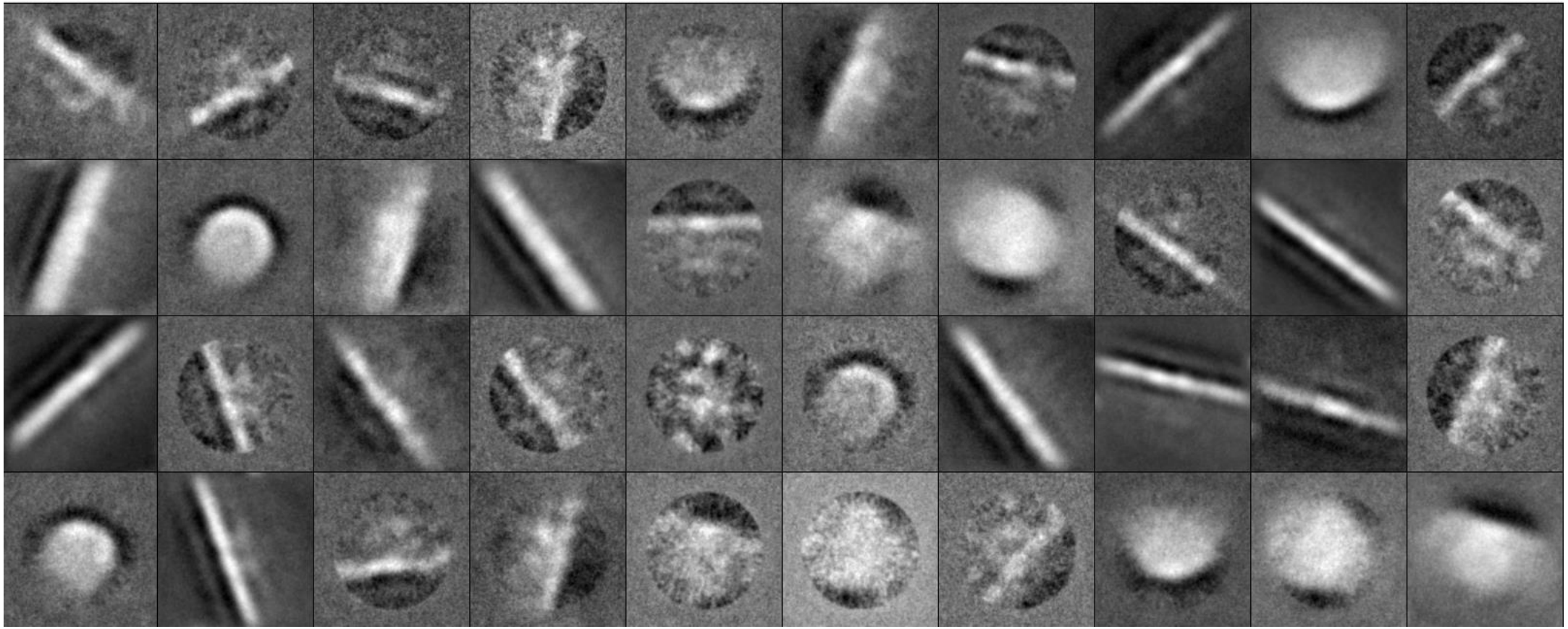
7. Calculation of **Fourier shell correlation** (FSC) to assess the resolution of the reconstructions

Projection-matched class averages of Ta1207 from an early alignment round



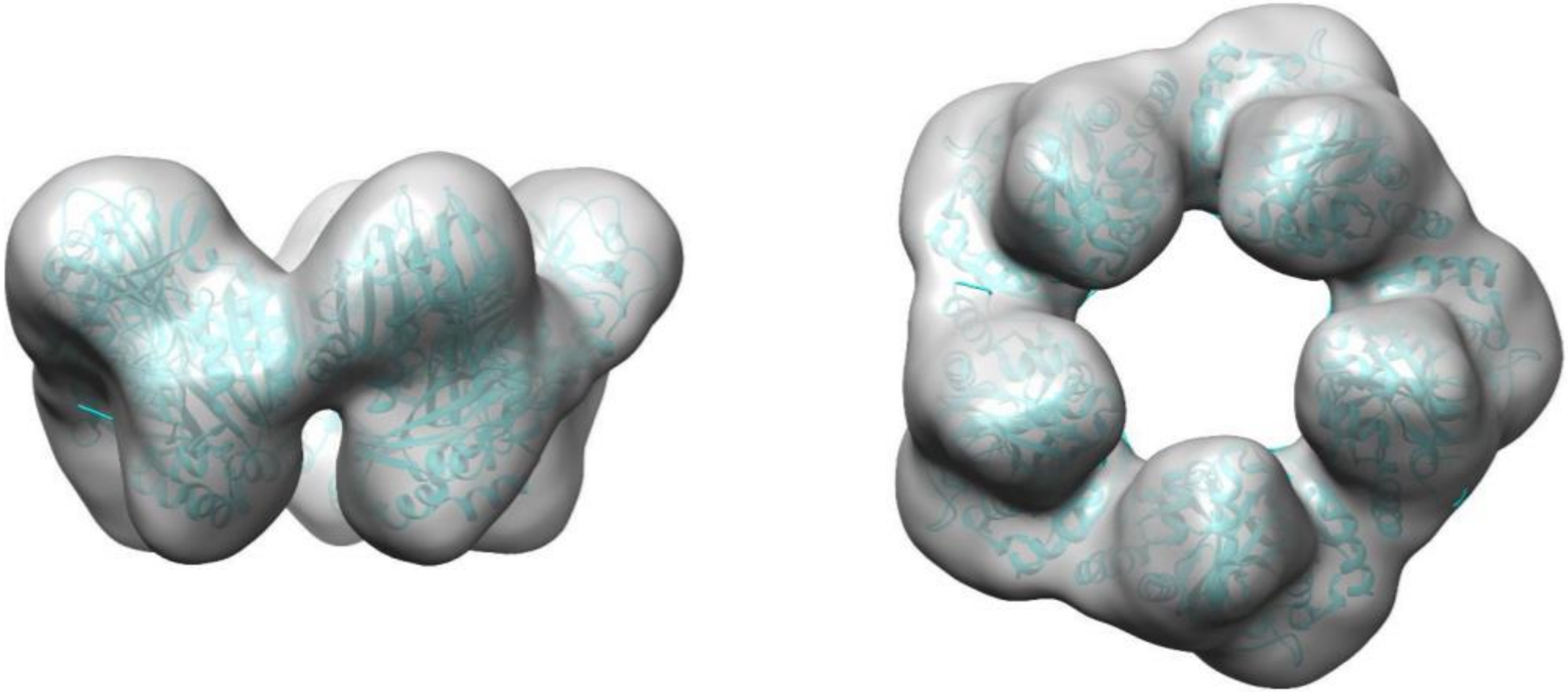
Images were kindly provided by **Dr. Dennis R. Thomas**, Cold Spring Harbor Laboratory, New York, US.

False image classes from data set



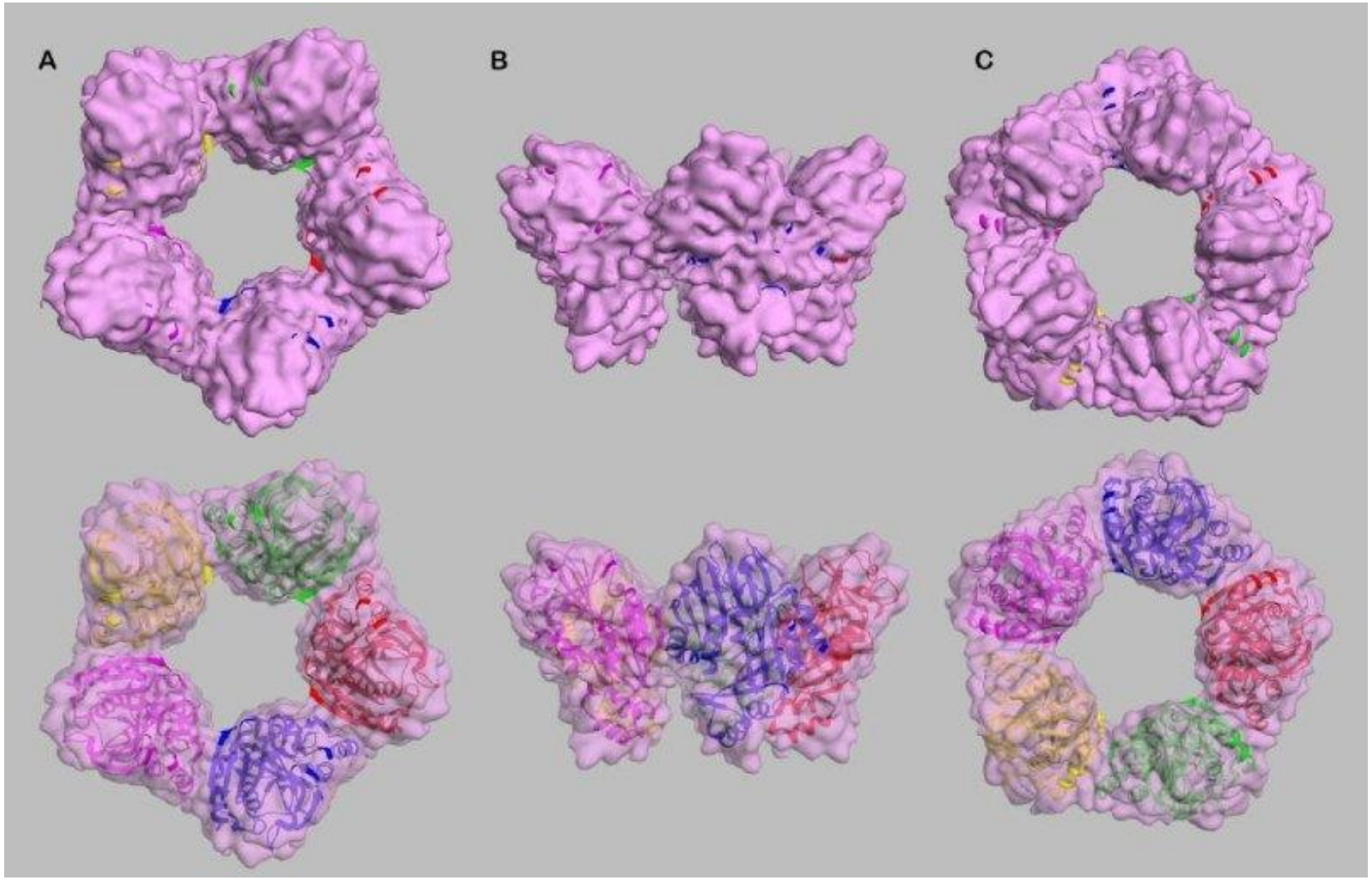
Images were kindly provided by **Dr. Dennis R. Thomas**, Cold Spring Harbor Laboratory, New York, US.

Fit of the crystallographic model to the 14 Å resolution cryo-EM envelope of endogenous Ta1207



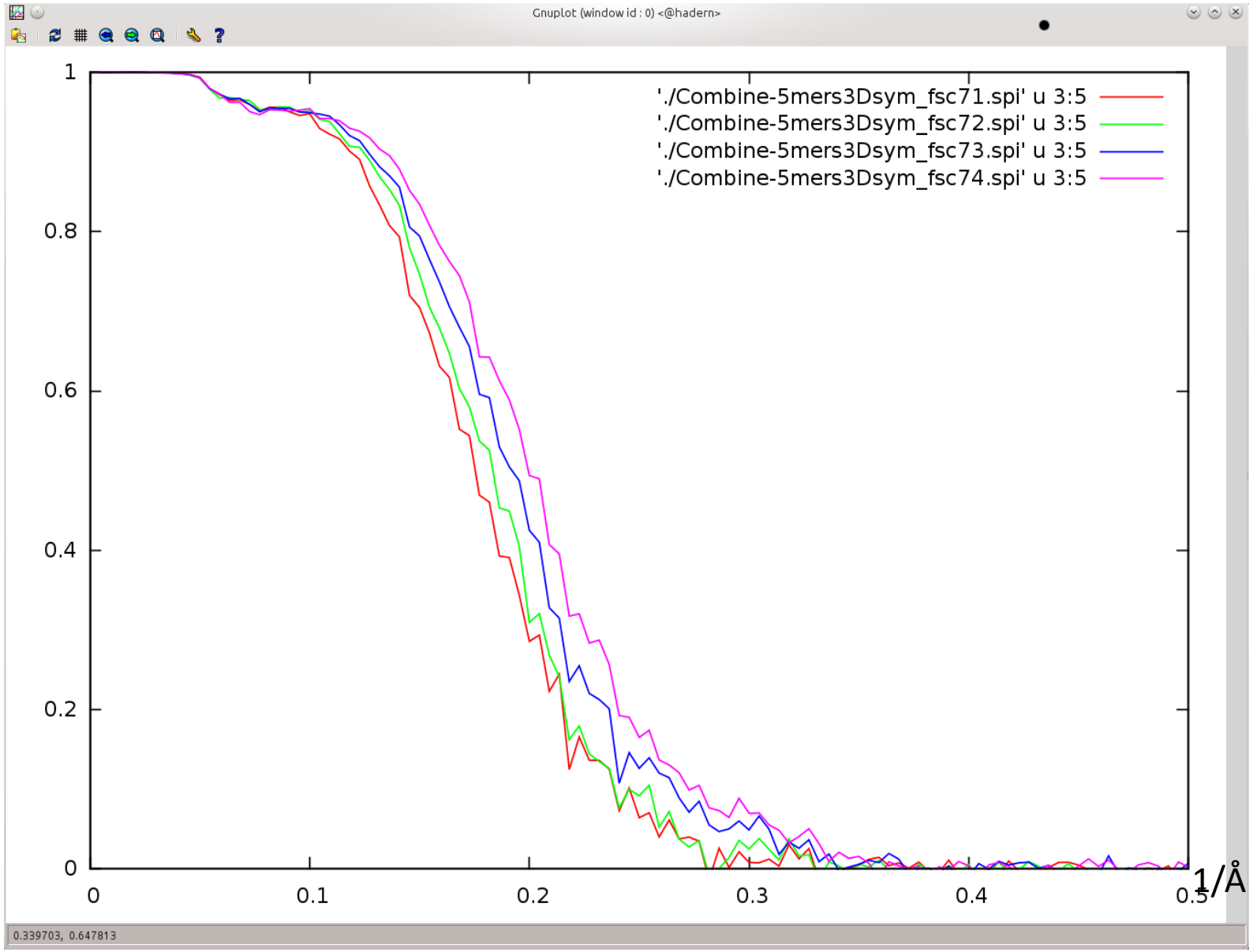
Pathare GR, Nagy I, Hubert Á, Thomas DR, Bracher A. Crystal structure of the *Thermoplasma acidophilum* protein Ta1207. Acta Crystallogr F Struct Biol Commun. 2017;73(Pt 6):328-335.

Cryo-EM structure of the Ta1207 complex at subnanometer resolution



5.8 Ångstrom resolution of Ta1207 (A, B, C). The complex shown is a reconstruction from 70,000 particles. Data was collected on a Tecnai F20 using 120 kV on an eagle CCD camera. A rigid body fit of the X-ray structure is shown. (bottom). Images were kindly provided by **Dr. Dennis R. Thomas**, Cold Spring Harbor Laboratory, New York, US.

FSC-curves of subsequent 3D-reconstructions



Provided by **Dr. Dennis R. Thomas**, Cold Spring Harbor Laboratory, New York, US.

Acknowledgements

Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Martinsried, Germany:

Prof. Dr. Wolfgang Baumeister
Head of Department

Visual proteomics group:

Dr. István Nagy

Dr. Yasuo Mitani

Marius Boicu

Dr. Stephan Nickell

26S proteasome group:

Dr. Parijat Majumder

Dr. Ganesh R. Pathare

Dr. Pawel Sledz

Oana Michalache

CEITEC Institute, Core Faciliy Cryo-Electron Microscopy and Tomography, Brno, Czech:

Dr. Tanvir Shaikh

Dr. Jirí Novacek

CSH Laboratory, New York, US:

Dr. Dennis R. Thomas

Special thanks are addressed to Péter Plósz for establishing the computational background for image processing.

„Anything essential is invisible to the eyes…

….., unless you can align and average it.”

Wolfgang Baumeister