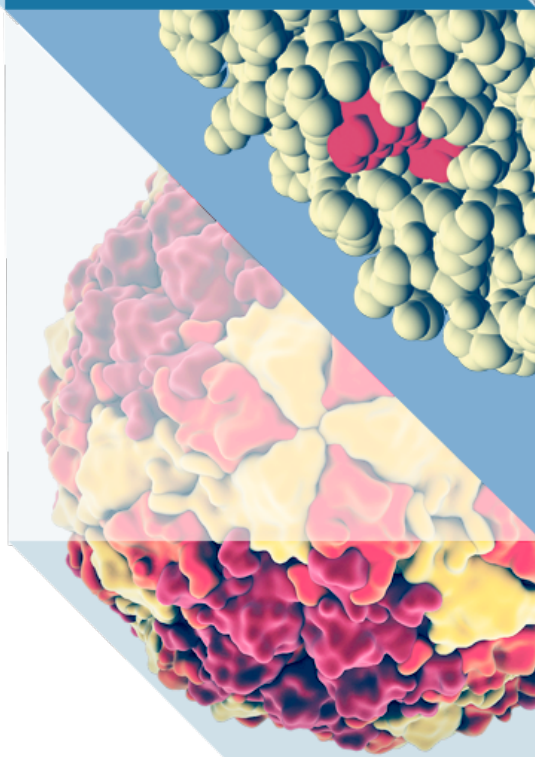
UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II - DIPARTIMENTO DI
AGRARIA



La “rivoluzione nella risoluzione” da crio-microscopia elettronica. La situazione in Italia

Proposta

Il crio microscopio elettronico di nuova generazione

Una lente di ingrandimento per l’ Universita’ Federico II

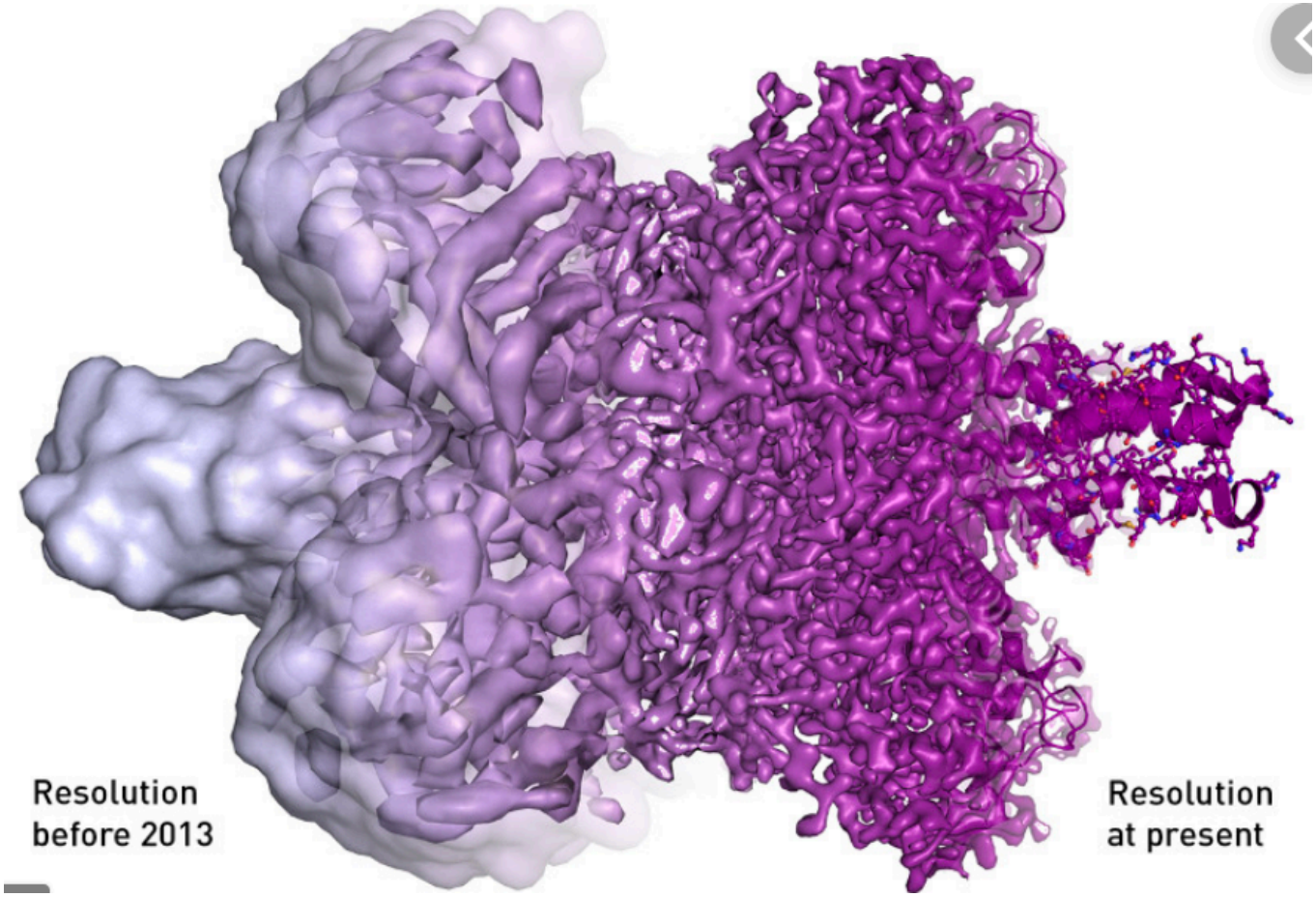
Caffe’ Scientifico del 22 Gennaio 2020
Sala Cinese, Palazzo Reale di Portici

Luigi Franklin Di Costanzo, Ph.D.

University Federico II of Naples

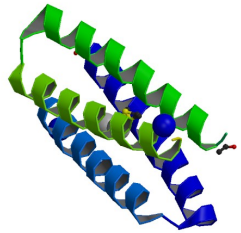
Una lente di ingrandimento molto potente

Il crio microscopio elettronico di nuova generazione

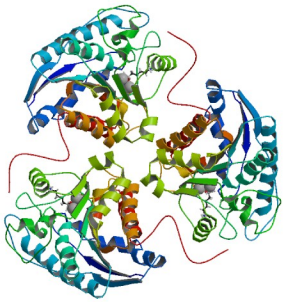


Journey from a PDB Curator to a professor in Italy

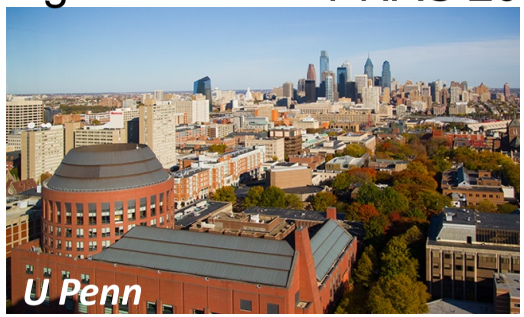
- PhD (2003) in Structural Biology
  - Wet and computational lab work
  - Specialized structure of difficult structures
  - Became PDB *Depositor* (53 depositions) and *User*
- Postdoc (2009)
  - Developed interest in bioinformatics
  - Became the PDB specialist in the lab
  - Trained colleagues
- Biocurator (2010-2019)
  - Protein Data Bank wwPDB
- Professor (2019-)



JACS, Angewandte



PNAS 2005



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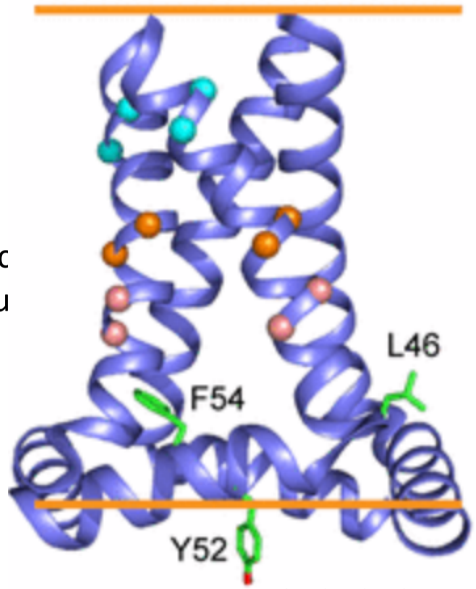
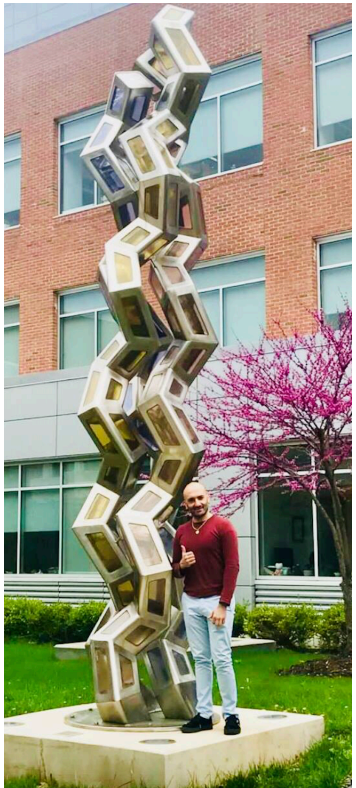
Ju



Nature 2008



Impatto della biologia strutturale e' immenso

Nobel Prizes and PDB structures

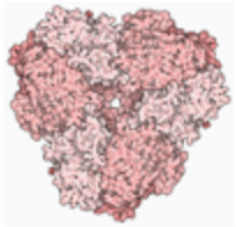
Grid | List

award-winning research

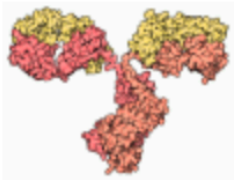
Many Nobel Prize awards in physics, chemistry, and physiology/medicine have recognized scientific achievements that can be associated with structural biology. The articles and resources below highlight many of these PDB structures and related experimental techniques.

Molecule of the Month Articles (23)

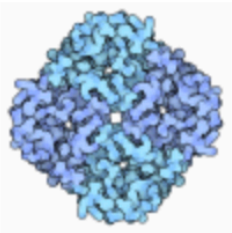
Adrenergic Receptors



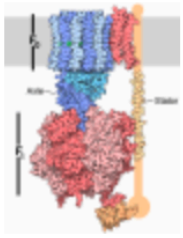
Aminopeptidase 1 and Autophagy



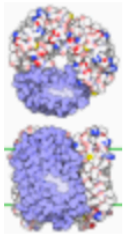
Antibodies



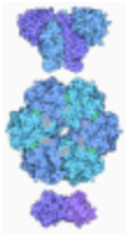
Aquaporin



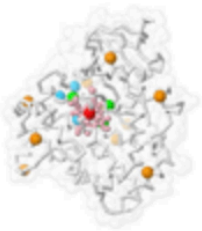
ATP Synthase



Bacteriorhodopsin



Circadian Clock Proteins



Directed Evolution of Enzymes

Elettra Sincrotrone Trieste

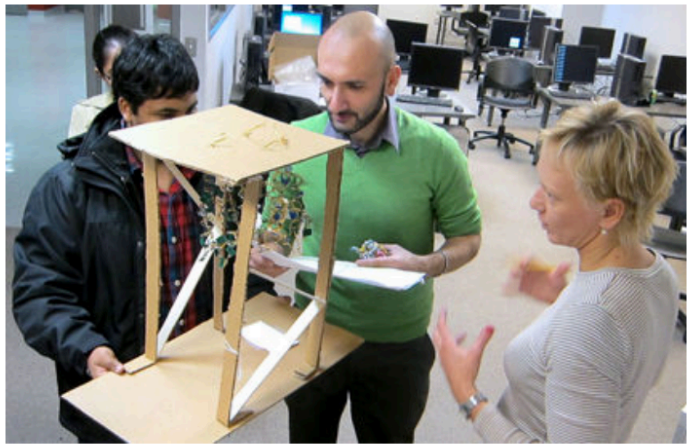
Biocurator: a rewarding career
Science highlight 2017

ERHUI1979/ISTOCKPHOTO

The rewards of working as a data wrangler

By Maggie Kuo | Sep. 25, 2017 , 4:50 PM

- Accomodare l'utilizzo dei database nei nostri corsi fondamentali
- Aggiornamento continuo rispetto ai libri di testo



Luigi di Costanzo *(center)* at an outreach event MARIA VOIGT (RCSB PDB)

Protein Data Bank Pioneered Open Access Unique Archive with Global Reach

- 1st Open Access digital data resource in all of biology
- Archive managed by Worldwide PDB partnership
  - RCSB PDB is the US Data Center
  - Budget of a 35M $ in 5 years
- January 2020 >159,000 validated and expertly-biocurated structures freely available to all without restriction
- Archive growing at the rate of ~10%/year
- >1 Million Users worldwide served every year at RCSB.org
  - Researchers, Educators/Students, and Curious Public
- >1.8 Million structure data files downloaded/day
- PDB data Used by >400 external databases

Come posso esserti utile?

Laboratorio:

- Hai bisogno di conoscere la struttura di una tua proteina di interesse?
- Ti necessita progettare un esperimento ad Elettra?
- La proteina e' conosciuta ma ti interessa la struttura con il ligando?
- Hai dei cristalli e non sai come o dove raccogliere di dati?
- Hai dei dati di una struttura difficile da risolvere e non puoi risolvere il problema della fase?
- ITC, SPR

Corsi di studio:

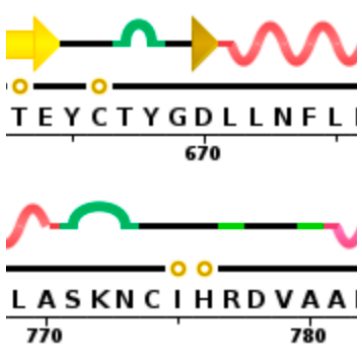
- Saresti interessato ad un corso di biocristallografia?/strutturistica
- E per quanto riguarda l'uso dei database?

Come posso esserti utile? - Database

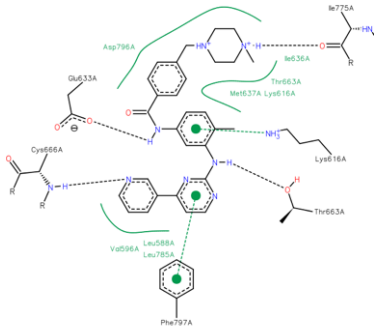
Biocurated Data Enables Research

Users access RCSB.org to

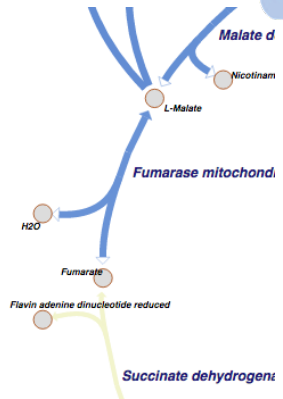
- Explore and download individual structures
- Compare multiple structures
- Utilize related information about publications, sequence annotations, drug interactions, more
- Analyze sets of search results
  - Browse entire PDB archive by external annotations
  - Access annotations for individual structures
  - Visualize in 2D and 3D



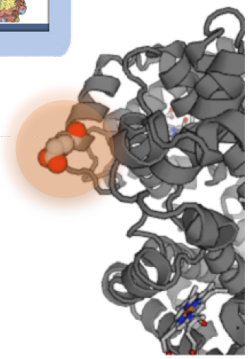
Sequence Annotations



Target-Drug Interactions



Pathways

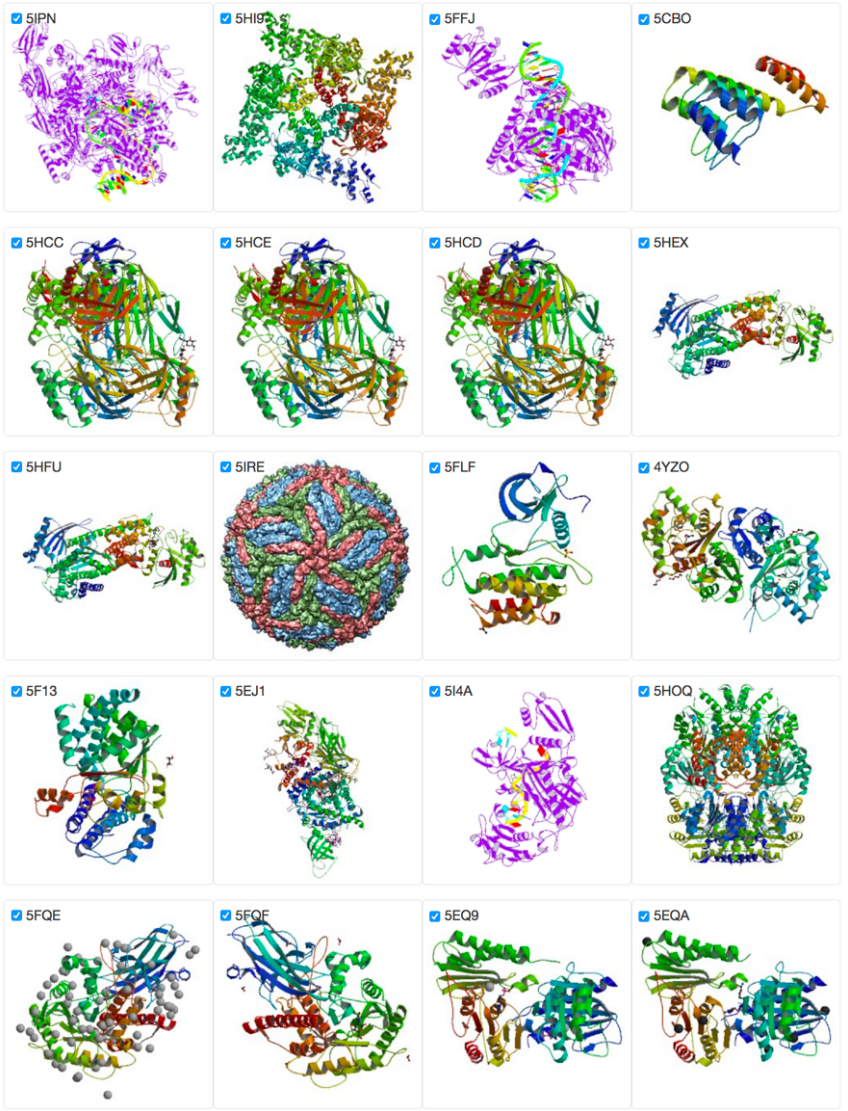


Genetic Variations

Biocuration Every Day:
~20 New Structures Submitted to RCSB PDB

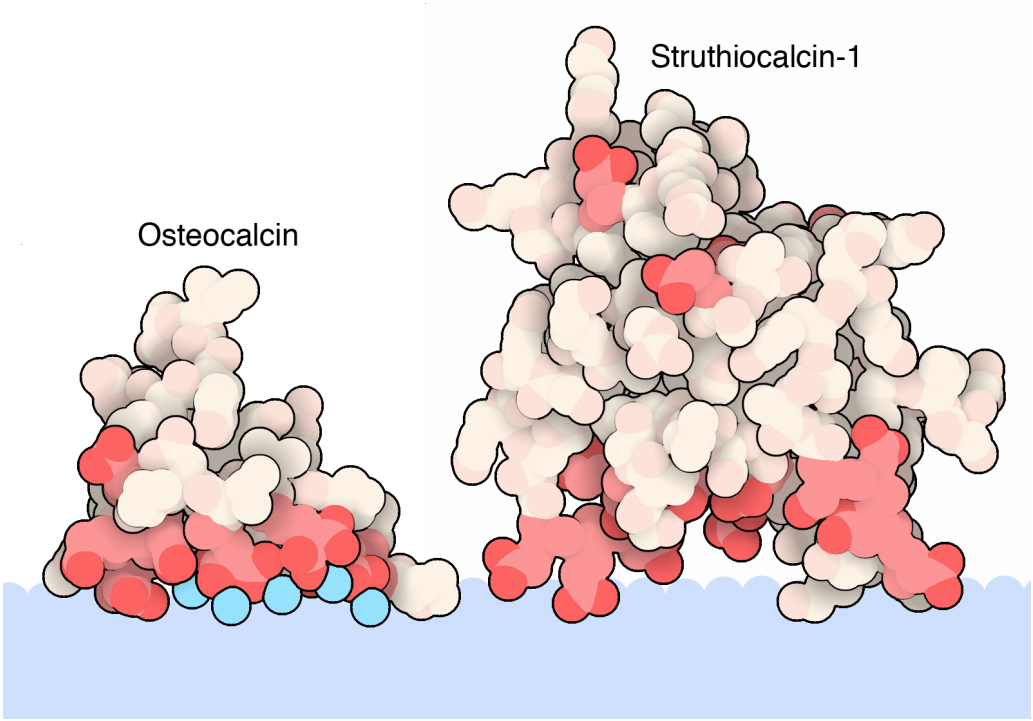
Each new deposition contains

- 3D atomic coordinates
- Primary experimental data
- Author and citation information
  - e.g., ORCID ID
- Details about sample preparation, data collection and structure solution
- Sequence(s) of polymers (proteins and nucleic acids)
- Detailed small molecule chemical structure



Perks of Being a Biocurator

- Tuned into the latest science
- Experience new structural discoveries before publication
- Witness evolution in methodologies, instruments, technologies
- Communicate with diverse community of researchers
- Bring science to general public through targeted education and outreach



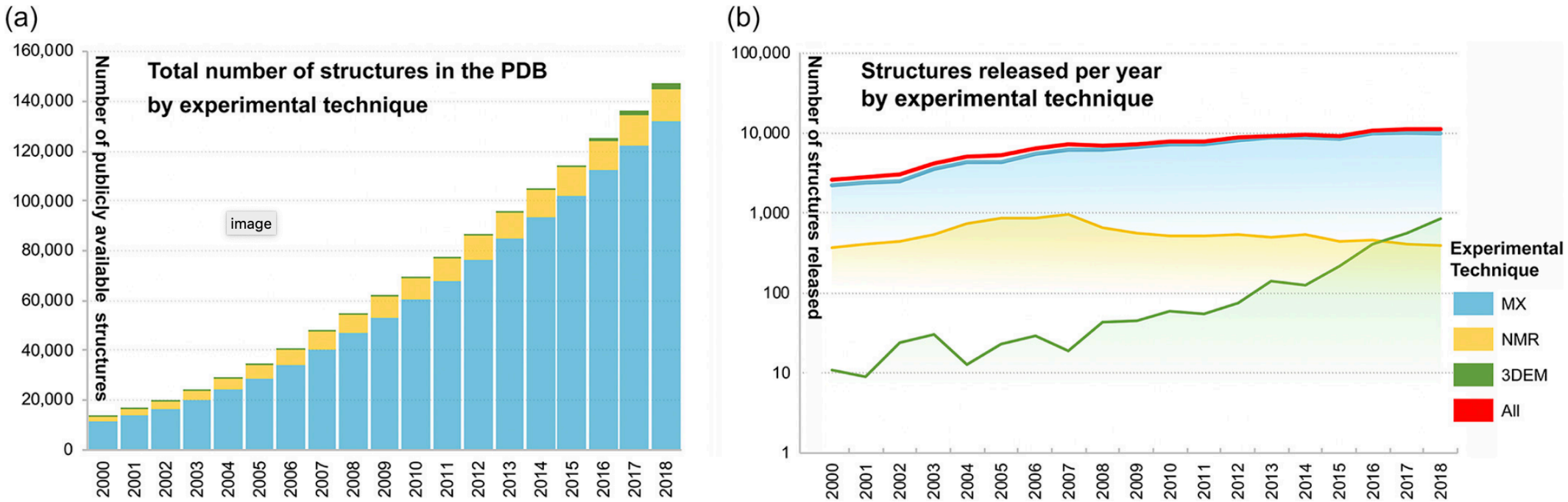
Molecule of the Month:
Biomineralization proteins

Small biomineral crystals are used to build bone, eggshells and even tiny compasses.

April 2019 by
Luigi DiCostanzo and David S. Goodsell

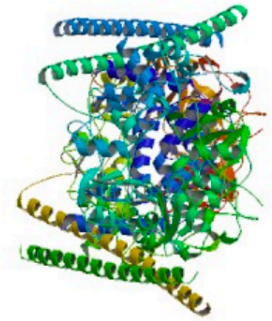
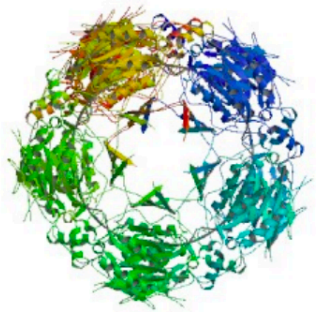
Suggestions for food/enzymes!!

Growing trend of structural biology
Cryo-EM outstanding growth
Growth of the RCSB-PDB archive 2000-2019

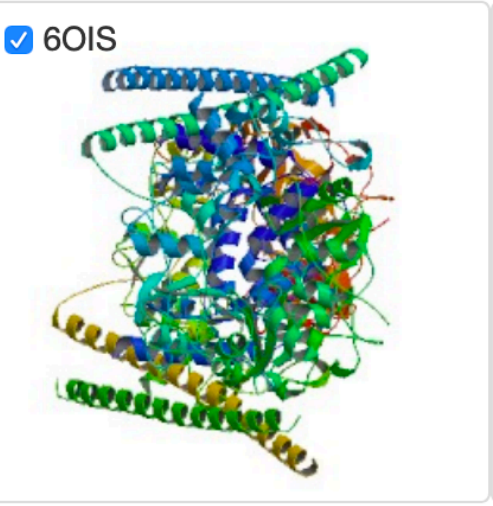


Protein Science, 2019, 29, 52-65

PLANT SCIENCE
Arabidopsis thaliana - 11 strutture da crio-microscopia elettronica
Grande qualita' delle pubblicazioni associate a queste strutture di grande massa molecolare

3D Structure	Related Structure	Citation Information
	6OIS	CryoEM structures of Arabidopsis DDR complexes involved in RNA-directed DNA methylation. Wongpalee S.P., Liu S., Gallego-BartolomÃ© J., Leitner A., Aebersold R., Liu W., Yen L., Nohales M.A., Kuo P.H., Vashisht A.A., Wohlschlegel J.A., Feng S., Kay S.A., Zhou Z.H., Jacobsen S.E. (2019) Nat Commun 10: 3916 Pubmed Article: 31477705
	6I00	Cryo-EM and directed evolution reveal how Mulelu A.E., Kirykowicz A.M., Woodward J.D. (2019) Commun Biol 2: 260 Pubmed Article: 31341959
	6J5T	Reconstitution and structure of a plant NLR resistosome conferring immunity. Wang J., Hu M., Wang J., Qi J., Han Z., Wang G., Qi Y., Wang H.W., Zhou J.M., Chai J. (2019) Science 364: null Pubmed Article: 30948527

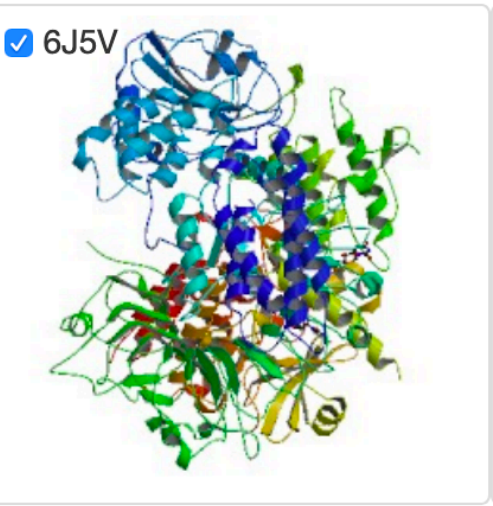

6I00


6OIS


6OIT


6JPF


6J5U


6J5V


6J5W


6J5T

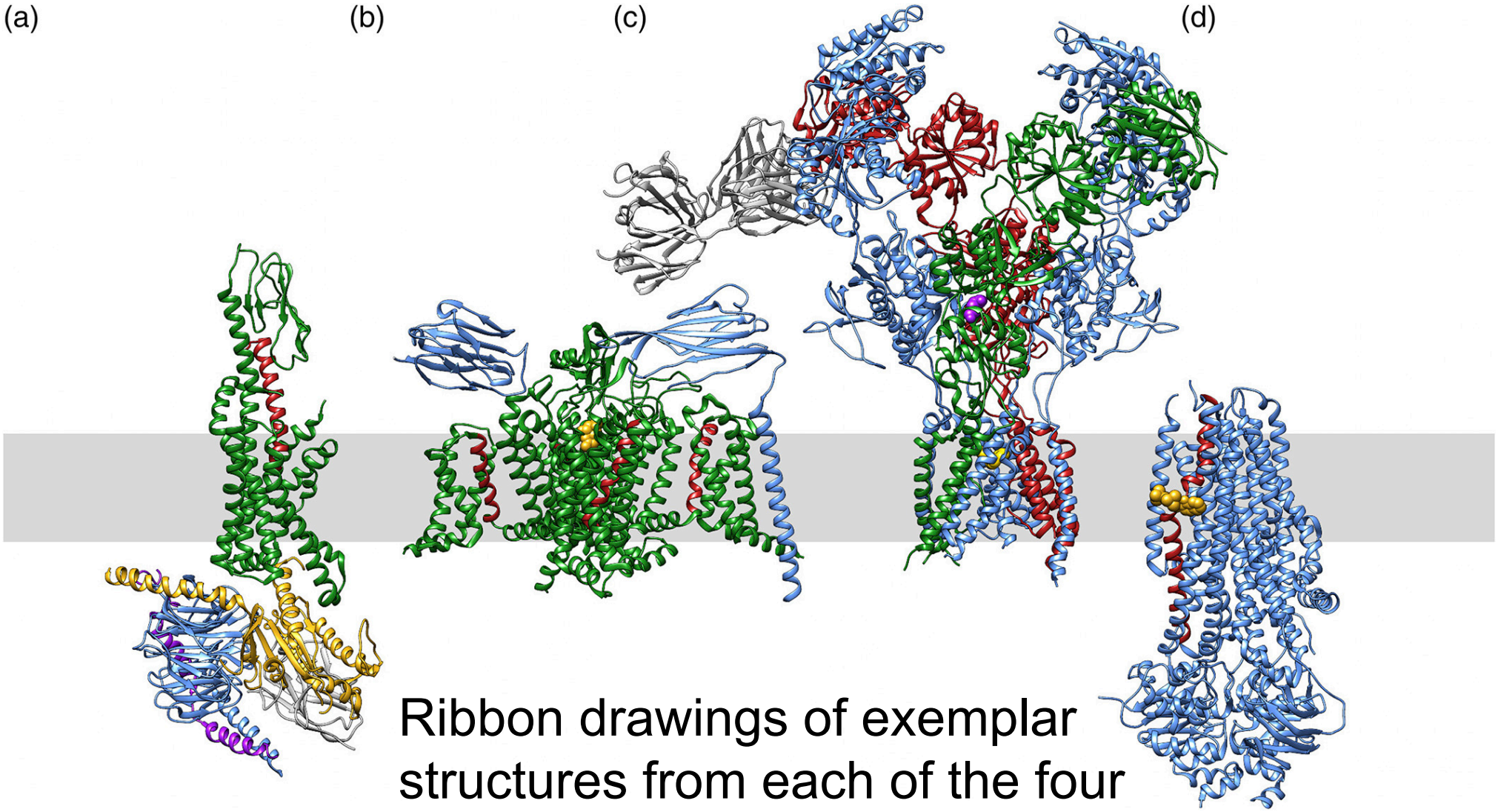

6J6I


6IJZ


6MGV


6MGW

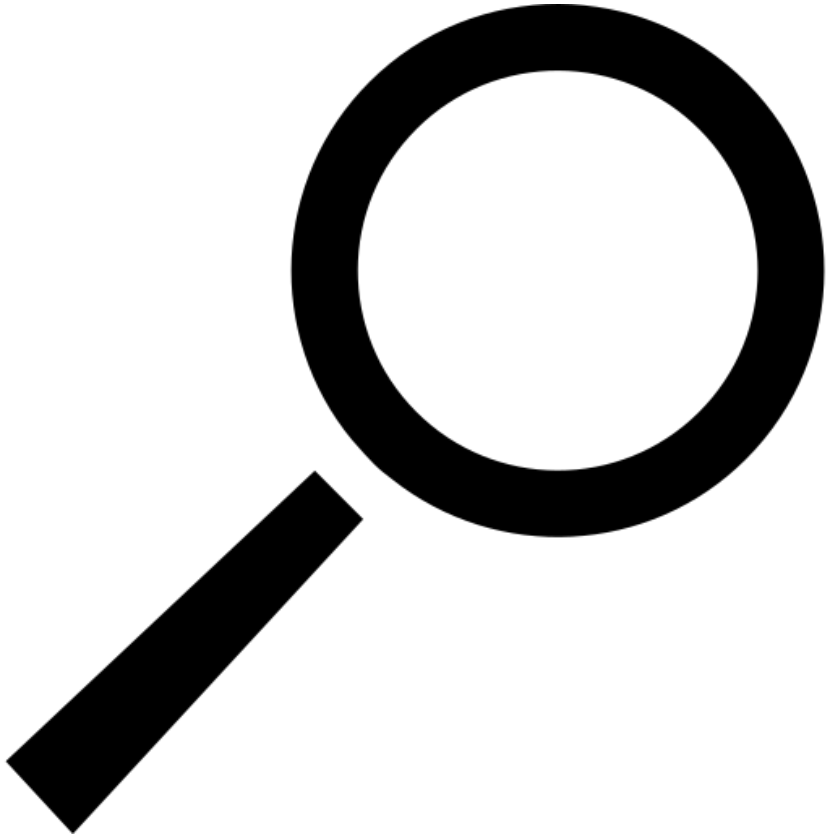
RCSB Protein Data Bank: Enabling Biomedical Research and Drug Discovery



Ribbon drawings of exemplar structures from each of the four classes of membrane-bound proteins of pharmacologic interest

Protein Science, 2019, 29, 52-65

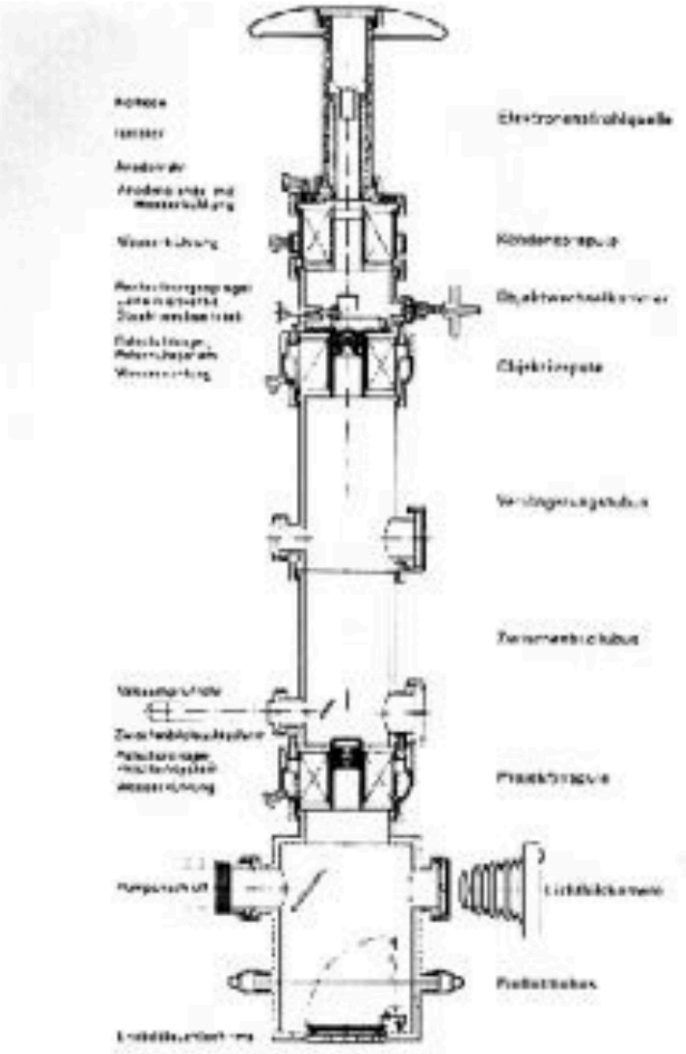
Promoviamo un grande progetto
Una lente di ingrandimento per la Federico II.
Il crio-microscopio elettronico



The first electron microscope

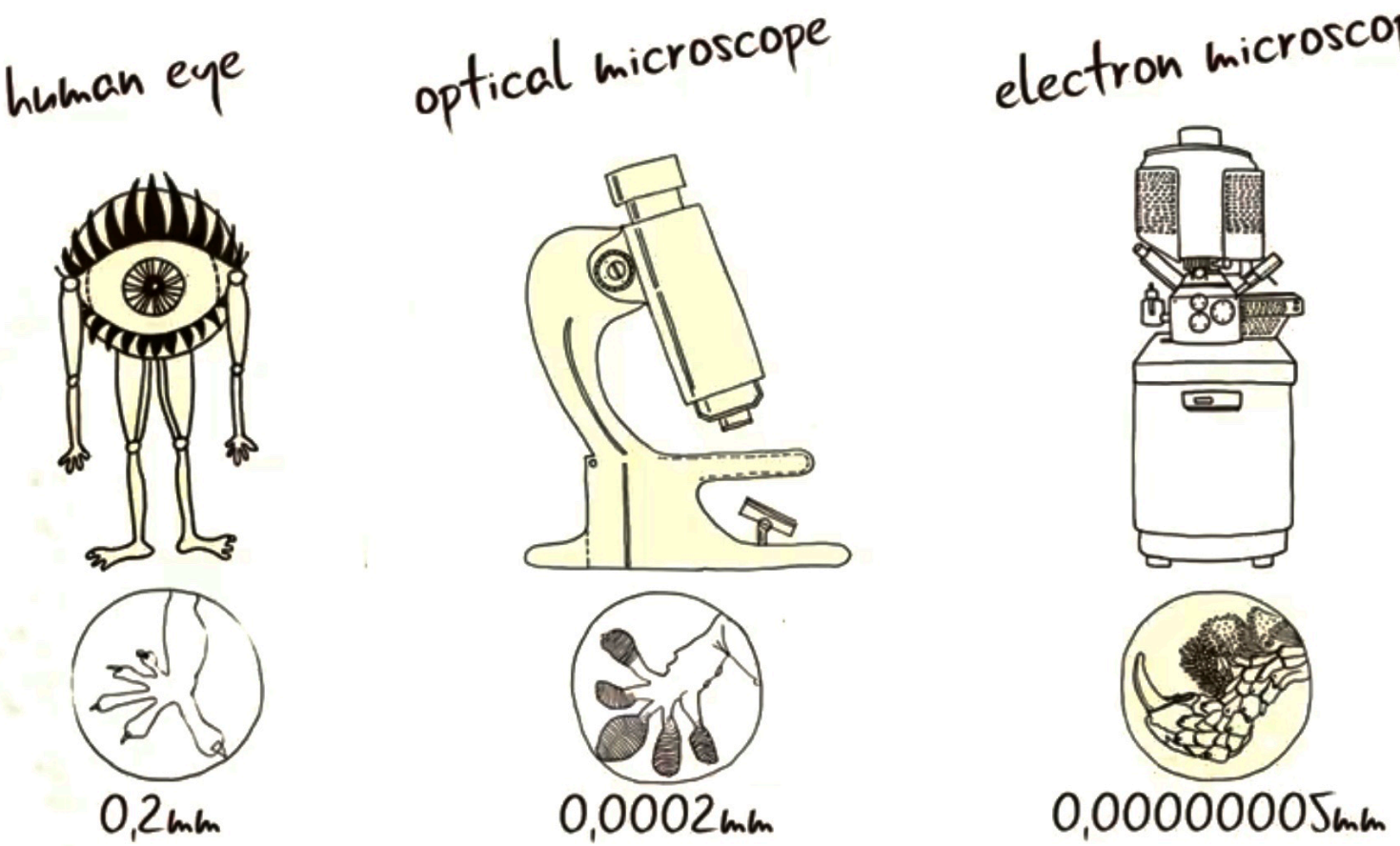
- Knoll and Ruska
- By 1933 they had produced a TEM with two magnetic lenses which gave 12 000 times magnification.

Ernst Ruska: Nobel Prize in physics 1986



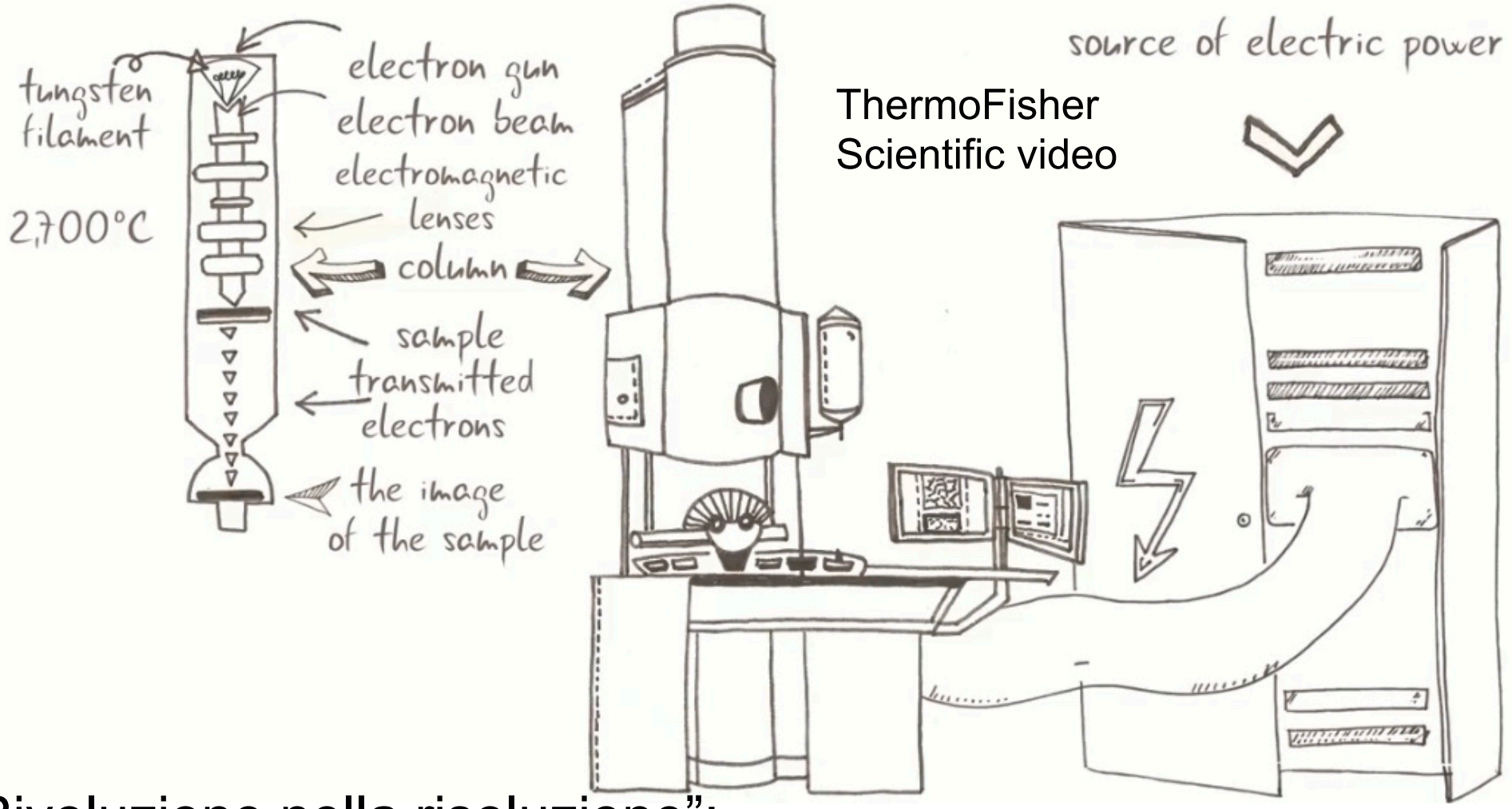
Resolution: a comparison

ThermoFisher
Scientific video



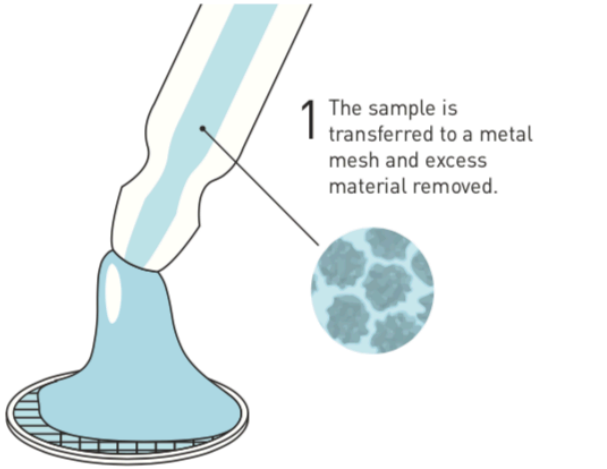
50 pm!

The major components of a cryo electron microscope



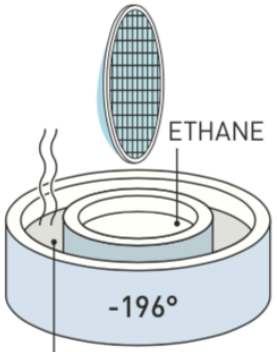
“Rivoluzione nella risoluzione”:
Vuoto spinto
Assenza di vibrazioni, grande stabilita’ (200Kv, 300 Kv)
Campione congelato
Detectors per la rivelazione diretta di elettroni post colonna

Typical workflow

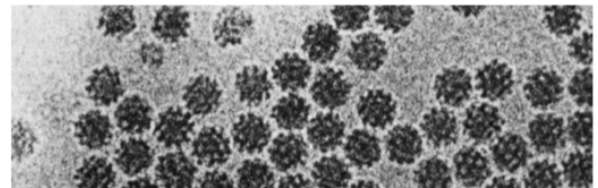


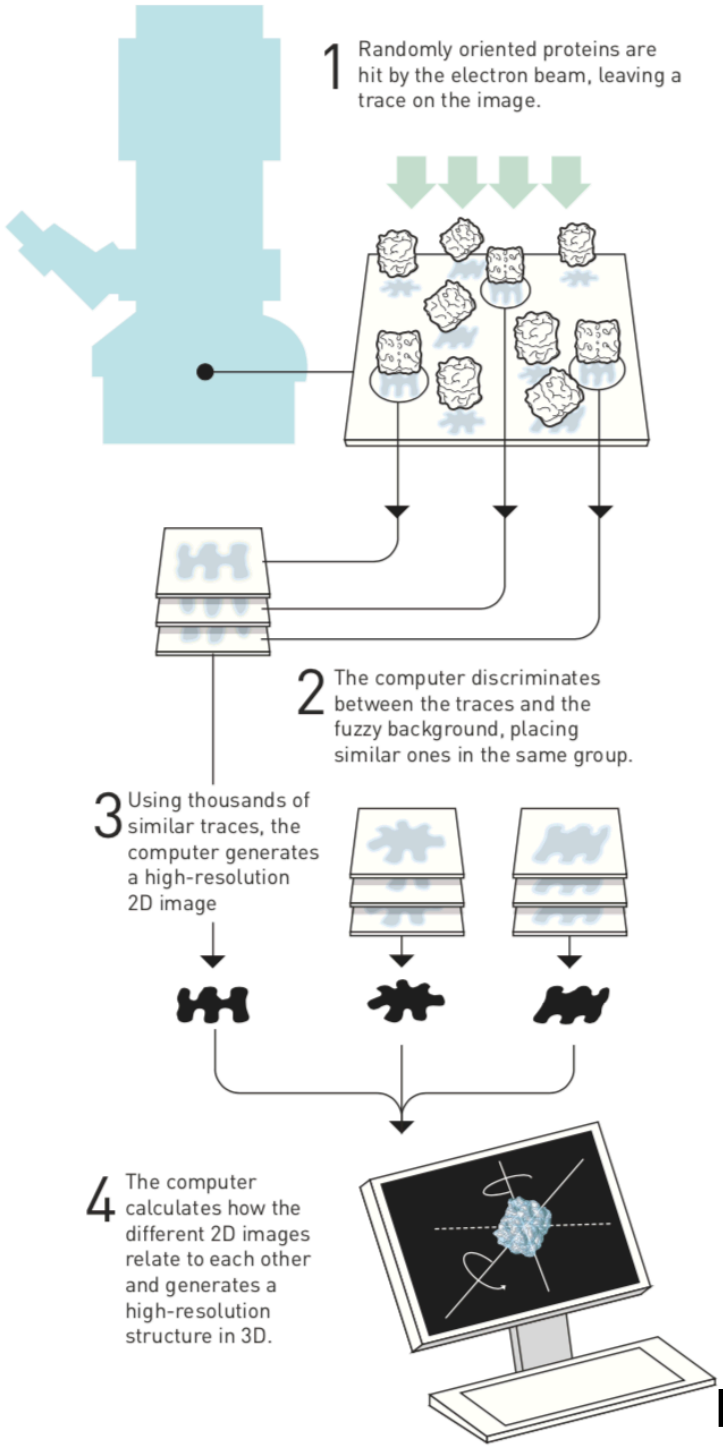
1 The sample is transferred to a metal mesh and excess material removed.

2 The sample forms a thin film across the holes in the mesh when it is shot into ethane at about -190°C.



3 The water vitrifies around the sample, which then is cooled by liquid nitrogen during the measurements in the electron microscope.





1 Randomly oriented proteins are hit by the electron beam, leaving a trace on the image.

2 The computer discriminates between the traces and the fuzzy background, placing similar ones in the same group.

3 Using thousands of similar traces, the computer generates a high-resolution 2D image

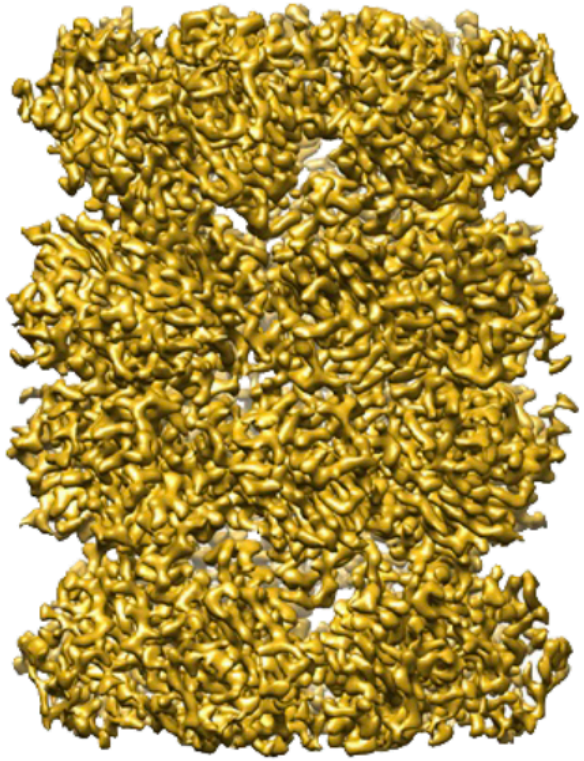
4 The computer calculates how the different 2D images relate to each other and generates a high-resolution structure in 3D.

Nobel website

Una tipica raccolta dati su un crio microscopio elettronico

Application results: 20S proteasome

Resolution: 3.65 Å
(by Relion using 2 halves gold standard)



Juergen Plitzko, MPI Martinsried, 2015

Dataset

Used images: 419

Raw particles: 23487

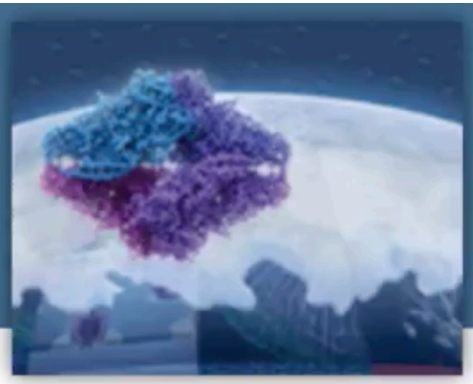
3D sorted particles: 7801

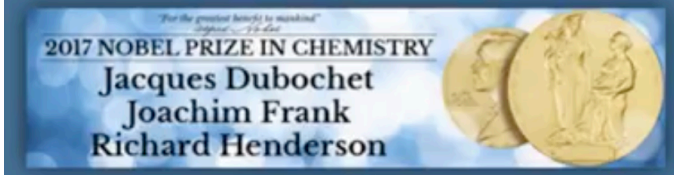
The 2017 Nobel prize in chemistry

nature methods
Techniques for life scientists and chemists

2015 Method of the year

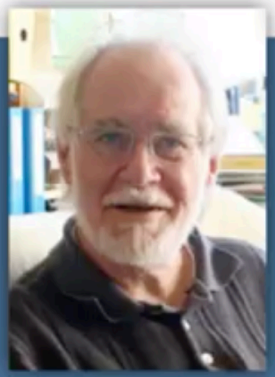
"The end of 'blob-ology': single-particle cryo-electron microscopy (cryoEM) is now being used to solve macromolecular structures at high resolution."





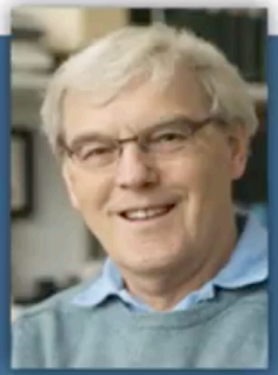
2017 Nobel prize in chemistry

"For developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"



Jacques Dubochet

Vitrifying a biological sample



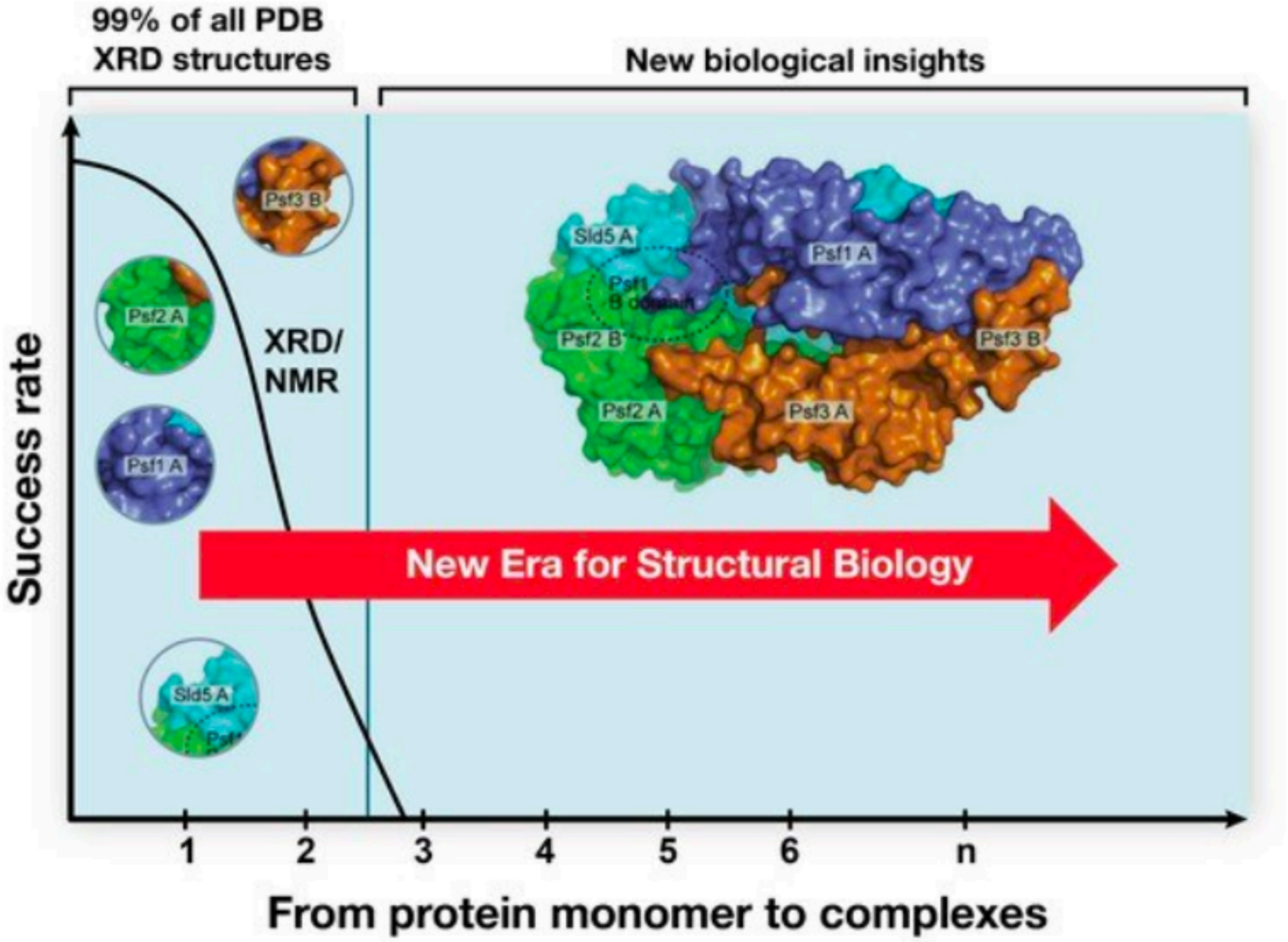
Richard Henderson

3D image of protein at atomic resolution

Joachim Frank

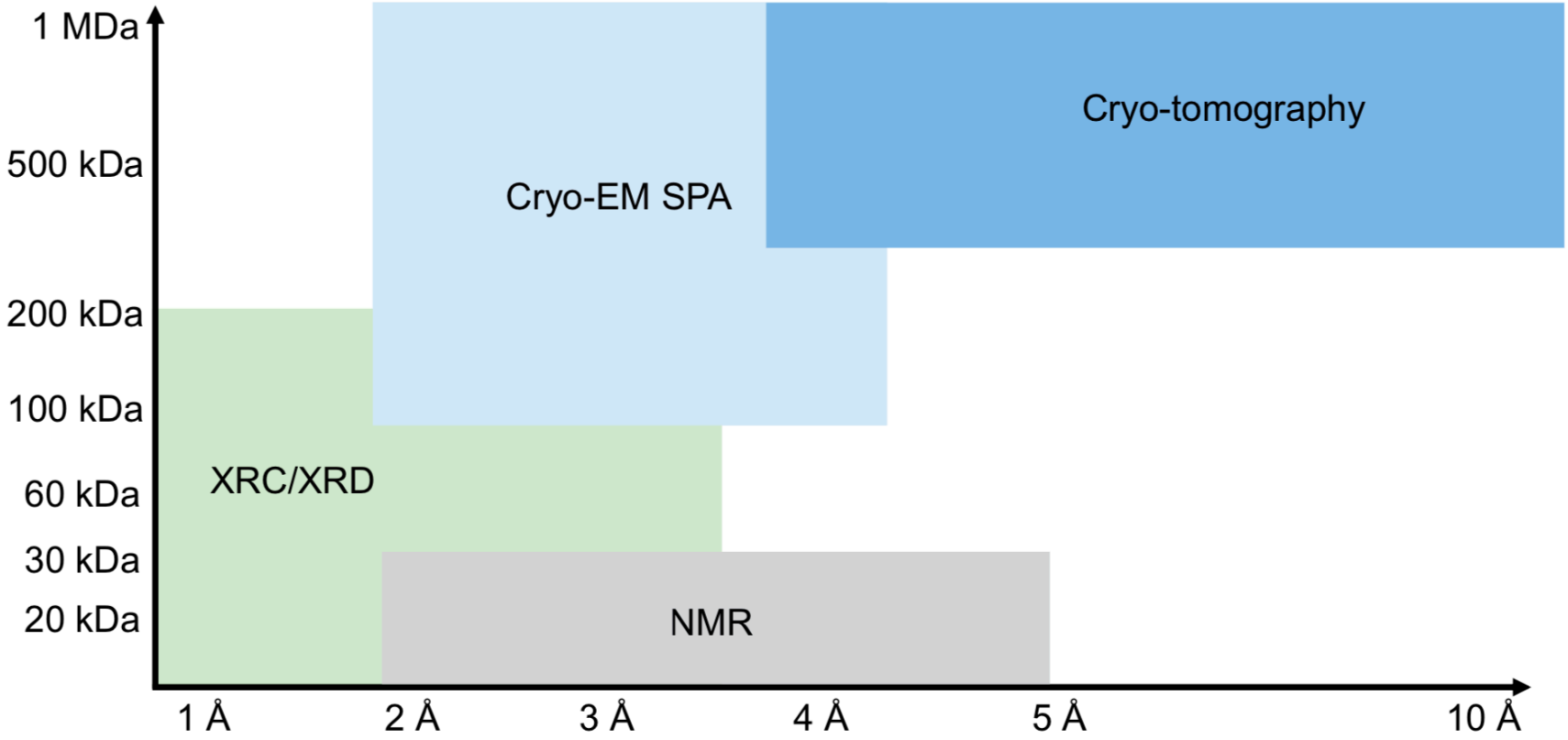
Processing fuzzy 2D images into sharp 3D structure

Growth into complexity



Towards the 3D structure of whole single cells

Cryo-EM Single Particle Analysis Complements Traditional Structural Biology Techniques



"Each [method] brings something important to the table, and the combination is very much larger than the sum of the parts."
Roger Kornberg, structural biologist at Stanford University in California.

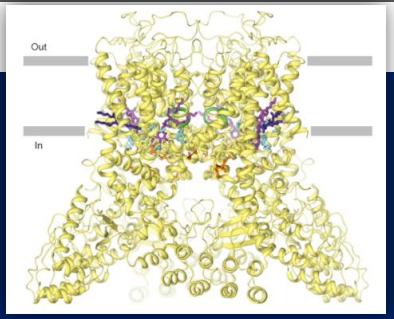
ThermoFisher SCIENTIFIC

Cryo-EM SPA State-of-the-art: High Resolution for Large and Small Molecules

Complementary technique

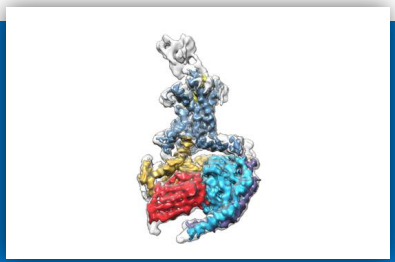
Cryo-EM SPA is Complementary to Traditional Structural Biology Techniques

Study of **conformational states**



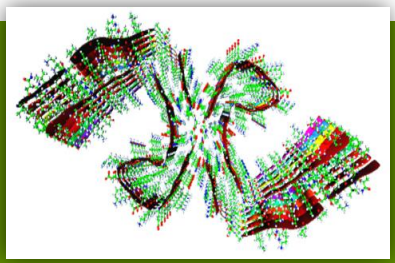
TRP ion channel
Multiple conformations

Structures that are difficult to solve with other techniques: **membrane proteins**



GPCRs
Large membrane protein

Near-atomic resolution structures of **protein complexes**, aggregates and large virus assemblies



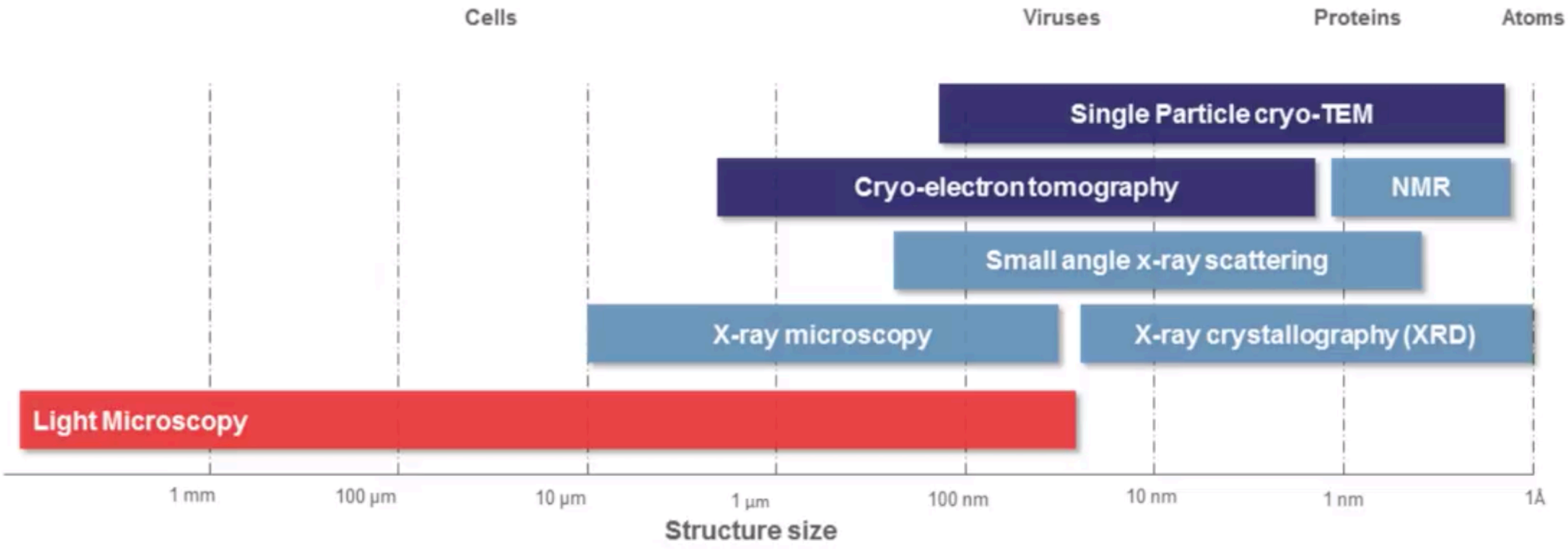
Tau protein
Aggregation of a protein complex

ThermoFisher SCIENTIFIC

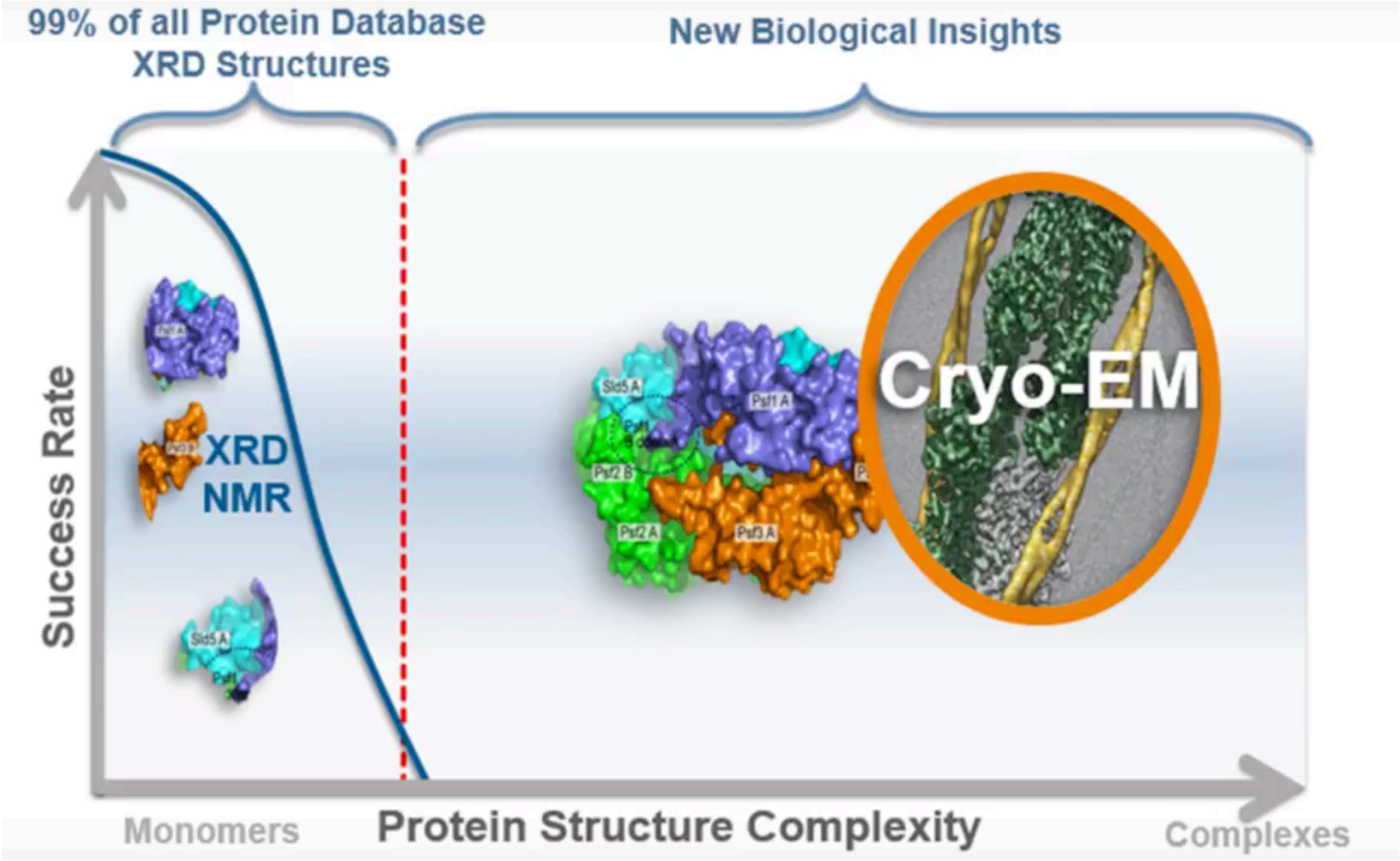
Zooming into the window of the biological function

Cryo-EM Complements Traditional Structural Biology Techniques like XRD and NMR

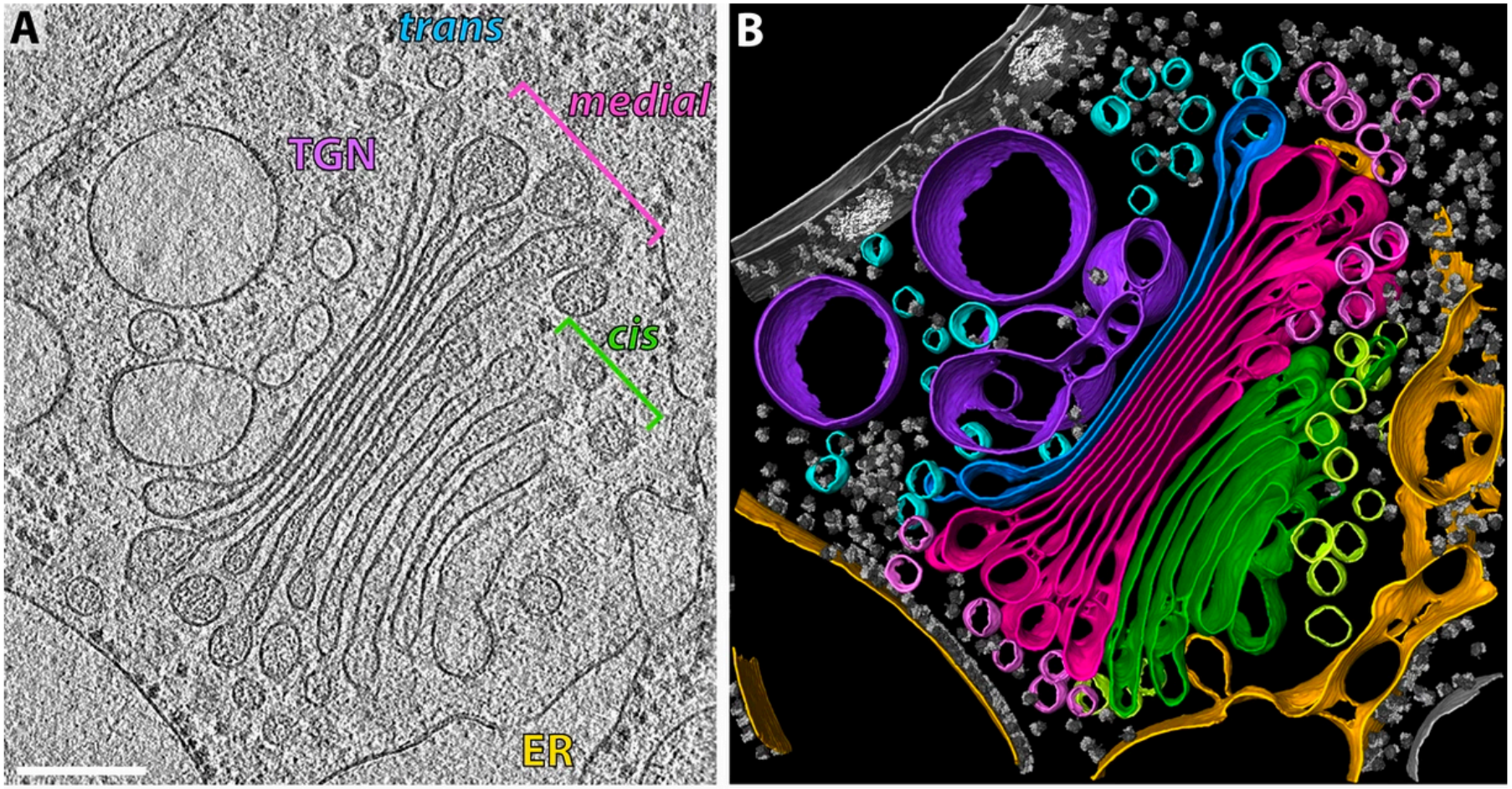
"Each [method] brings something important to the table, and the combination is very much larger than the sum of the parts." Roger Kornberg, structural biologist at Stanford University in California.



New biological insights

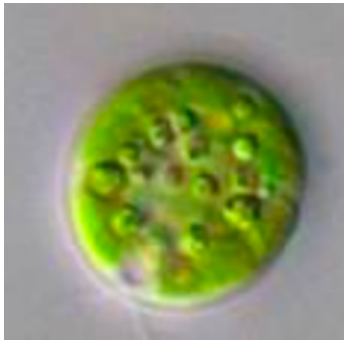


The structure of the COPI coat determined within the cell



Chlamydomonas reinhardtii is a single-cell green alga about 10 micrometres; Elife 2019

The structure of the COPI coat determined within the cell



Tomografia in transmissione

- Spessore del campione
- Se si fanno fettine con ultramicrotomi ad esempio si ottengono fettine troppo spesse per farci analisi in cryo
- Tomografia a temperatura ambiente attraverso colorazioni
- Per lavorare in cryo si fa ricorso a sistemi quali cryo-FIB o simili che consentono di arrivare a fettine spesse meno di 300 nm.

Networking and preliminary meetings

Researcher Profile

Jason T. Kaelber, PhD

Associate Member, *Cancer Pharmacology Program*

Member since 2019

Academic Appointment

School of Arts and Sciences
Rutgers, The State University of New Jersey

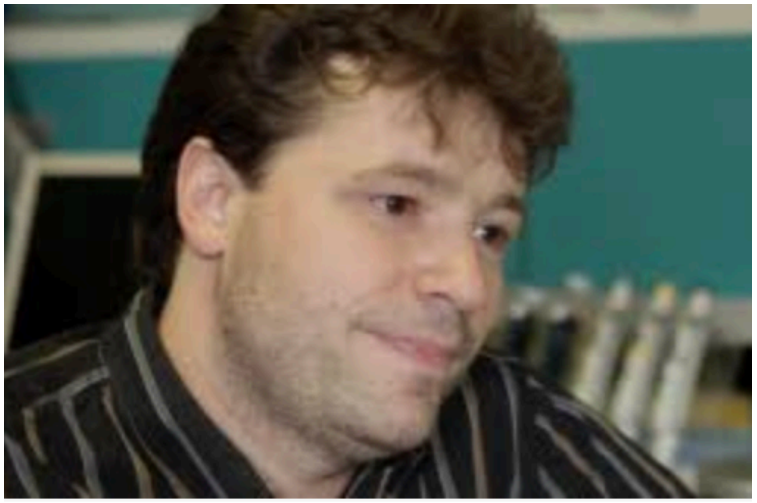
Positions

- Director, Rutgers New Jersey Cryo-electron Microsopy & Tomography Core Facility

Doriano Lamba

Researcher

Affiliation: National Research Council of Italy



Federico Forneris | Armenise Foundation

armenise.hms.harvard.edu

Fabio Fantini · 2nd

Account Manager presso Thermo Fisher Scientific

Bologna Area, Italy · 436 connections · **Contact info**

Max Maletta

@MalettaMax

CryoEM expert, product specialist

@thermosciEMSpec

Domande discusse con colleghi

Un microscopio elettronico per la Federico II

- A chi puo' servire tale macchina?
- Costi ed edificio?
- Bisogna essere esperti della tecnica?
- Quale approccio hanno usato altre universita' italiane?
- Chi puo' contribuire alla redazione di tale progetto?
- Costruire una Road Map

La situazione in Italia del crio-microscopio elettronico

1. UniMI – prof. M. Bolognesi & Dr. P. Swuec
200Kv Arctica Falcon 3 + autoloader. Operative

2. UniPV – profs F. Forneris & A. Mattevi
Glacios 200kV. Inauguration on Feb 28, 2020

3. UniFI – profs L. Banci & G. Tria:
Glacios 200kV. Delivery phase. Inauguration in 2020

4. CNR – Napoli Drs. I. Rendina & B. Vallone
Complicated. Plus building for the facility

Coming-up (2020-2021)
Human Technopole Milan Dr. A. Vannini
A 300 Kv EM and a whole series of several microscopes.
To be delivered. Funded.

L'universita' del Meridione e' assolutamente sprovvista di un microscopio elettronico per criomicroscopia

I costi della microscopia elettronica

Edificio

1. Piano terra
2. Stanza altezza. 3 m con una porta di ingresso di 2.10 m
3. Stanza larghezza. Dimensioni ~ 5.8 m x 6.6 m
4. Collaudo vibrazioni
5. Strumento collocato su un plinto di cemento

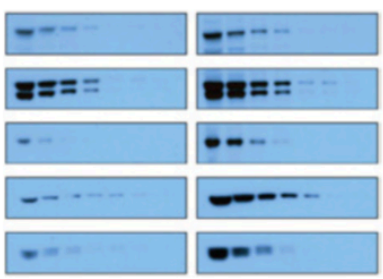
Modelli e costi

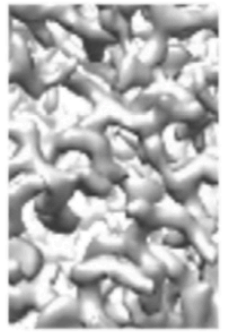
Model Glacios 200kV + Doppia Camera Digitale (CETA + Falcon 3 DED) a partire da 2,2 ME+IVA, comprensivo di installazione, training applicativo (3 mesi), garanzia 24 mesi

Model Krios G4 300kV + Doppia Camera Digitale (CETA + Falcon 3 DED) a partire da 5ME+IVA, comprensivo di installazione, training applicativo (3 mesi), garanzia 24 mesi (eventuale Filtro Gatan da aggiugere – circa 1 ME))

Model Talos Arctica 200kV + Doppia Camera Digitale (CETA + Falcon 3 DED) a partire da 2,2 ME+IVA, comprensivo di installazione, training applicativo (3 mesi), garanzia 24 mesi (eventuale Filtro Gatan da aggiungere – circa 1 ME))

Schematic representation of the Single Particle Analysis workflow



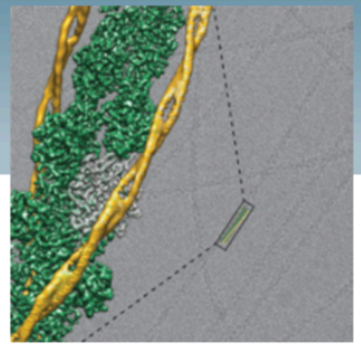


Biochemical sample preparation → Sample vitrification (Vitrobot) → Cryo-sample screening & data acquisition (200kV) (Glacios) → 3D reconstruction

Sample preparation
Vitrobot™ system

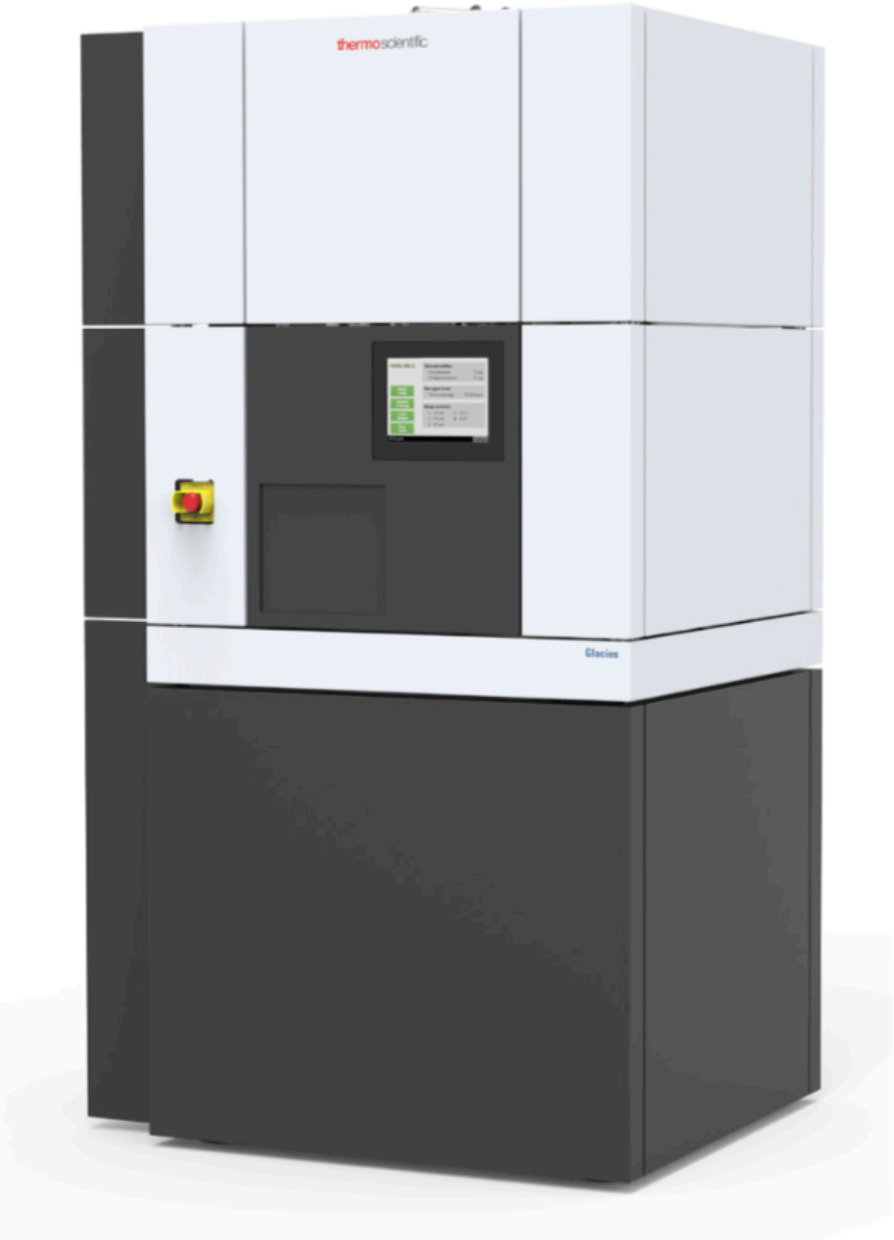
Sample screening and data acquisition
Glacios Cryo-TEM

Ultimate resolution data acquisition
Krios™ Cryo-TEM



Protein reconstruction
3D reconstruction software

Glacious 200Kv



Key Benefits

Enhanced ease-of-use. Automated alignments and systematic user guidance allow easy SPA operation from one single interface (EPU).

Small footprint. Compact hardware architecture, minimizing room and access route requirements.

Optimal tool performance. Self-assessment of microscope optical status, combined with automated alignments ensuring optimal experimental conditions are always available.

Workflow connectivity. Guaranteed compatibility allows robust and contamination-free transfer of samples between Autoloader equipped instruments (Krios, Arctica, and Glacios Cryo-TEMs).

Maximum throughput. Batch screening of up to 12 sample grids, creating overview atlases and classifying the ice films for guided selection of grid squares.

Tailored flexibility. Versatile system that can be configured for sample screening, SPA data acquisition, Tomography, or STEM applications.

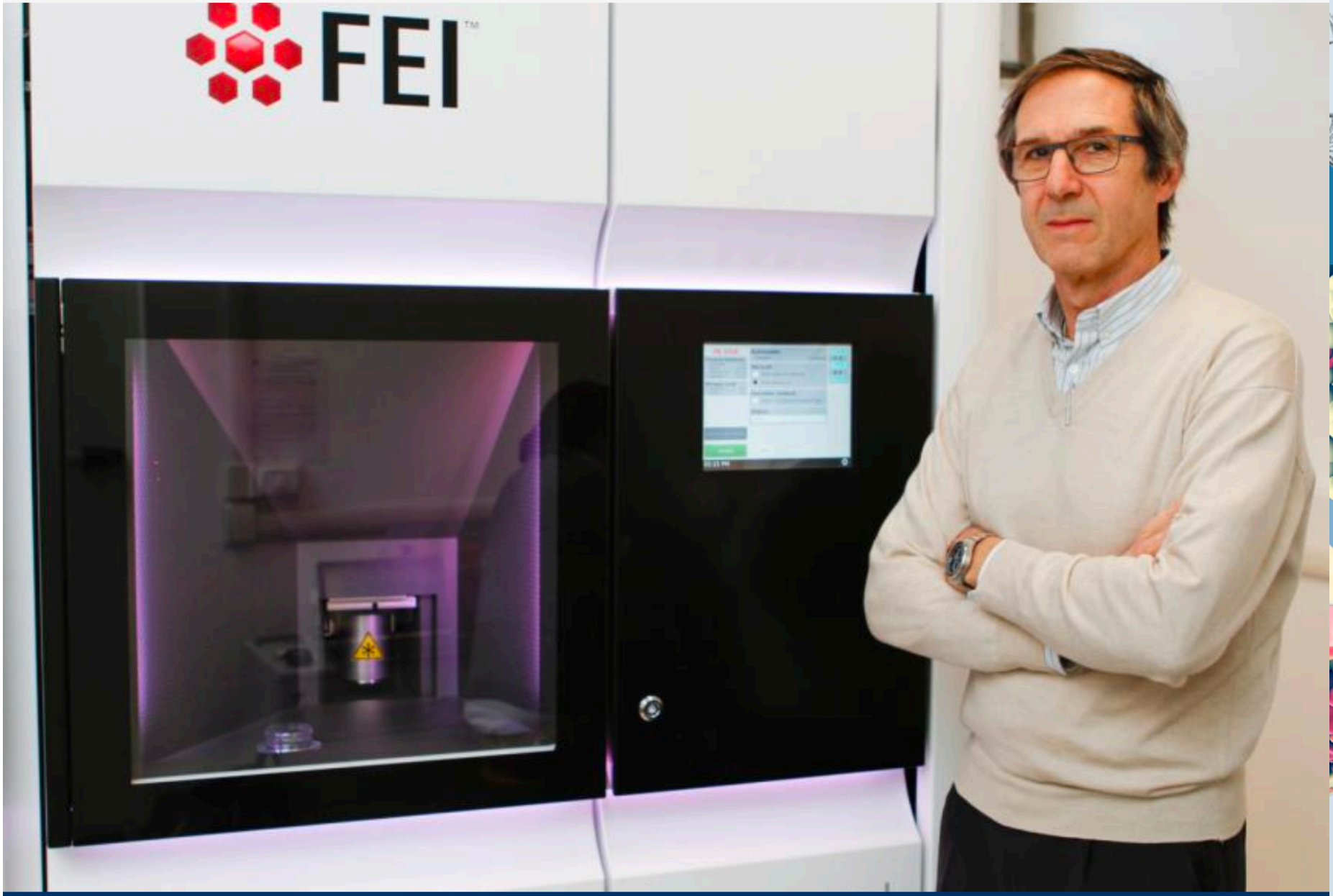
Inaugural Symposium of the *UniPV PASS-Bio facilities*

(Piattaforme Avanzate per la Strumentazione Scientifica di Area Biologica)

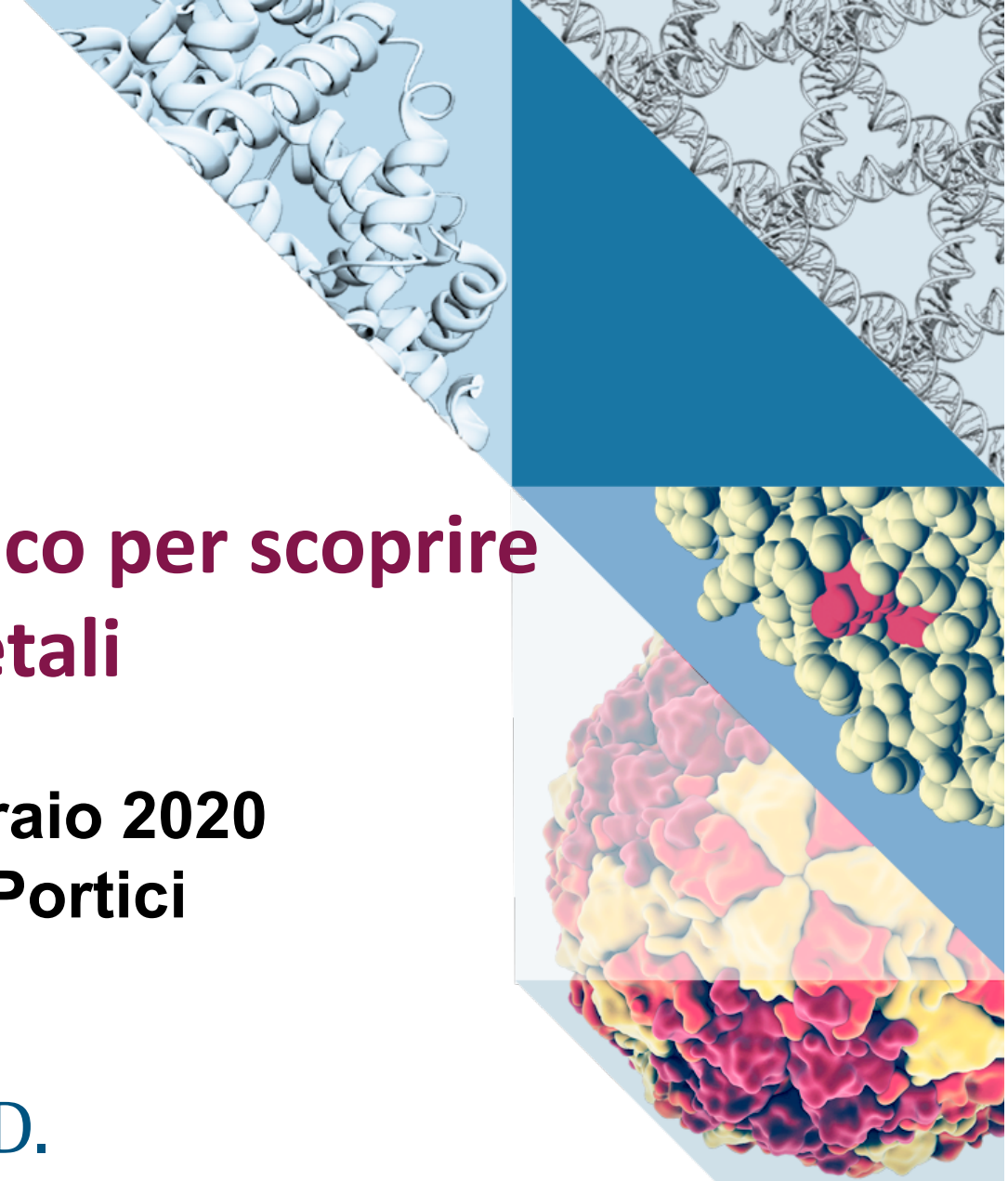
UNIVERSITÀ DI PAVIA

The newly established PASS-Bio facility *(Piattaforma Avanzata di Strumentazione Scientifica)* at the University of Pavia features state-of-the-art instrumentation in the fields of **Cryo-Electron Microscopy**, **Nuclear Magnetic Resonance**, **High Performance Computing** and **High-Resolution Cellular Imaging**.

Friday, 28 February 2020



Il professor Martino Bolognesi accanto al crio-microscopio elettronico - Foto di Angelo Negri, 2018



Uno sguardo caleidoscopico per scoprire il fascino dei genomi vegetali

Caffe' Scientifico del 12 Febbraio 2020
Sala Cinese, palazzo reale di Portici

Mara Ercolano, Ph.D.

University Federico II of Naples

Materiale di approfondimento

Lettura di divulgazione dal sito Nobel prize. Anno 2017

Visualizza le brouchers from ThermoFisher:
Glacios Cryo-TEM
Krios G3i Cryo-TEM
Aquilos Cryo-FIB
Review:
Frontiers in Cryo Electron Microscopy of Complex Macromolecular Assemblies
Annual Review of Biomedical Engineering
Esplora la risorsa online:
https://www.emdataresource.org/
Per la Tomografia esplora i lavori di:

W. Baumeister/J. Plitzko/J. Briggs/J. Mahamid/M. Beck per esempi di cryo-tomography.

Ringraziamenti

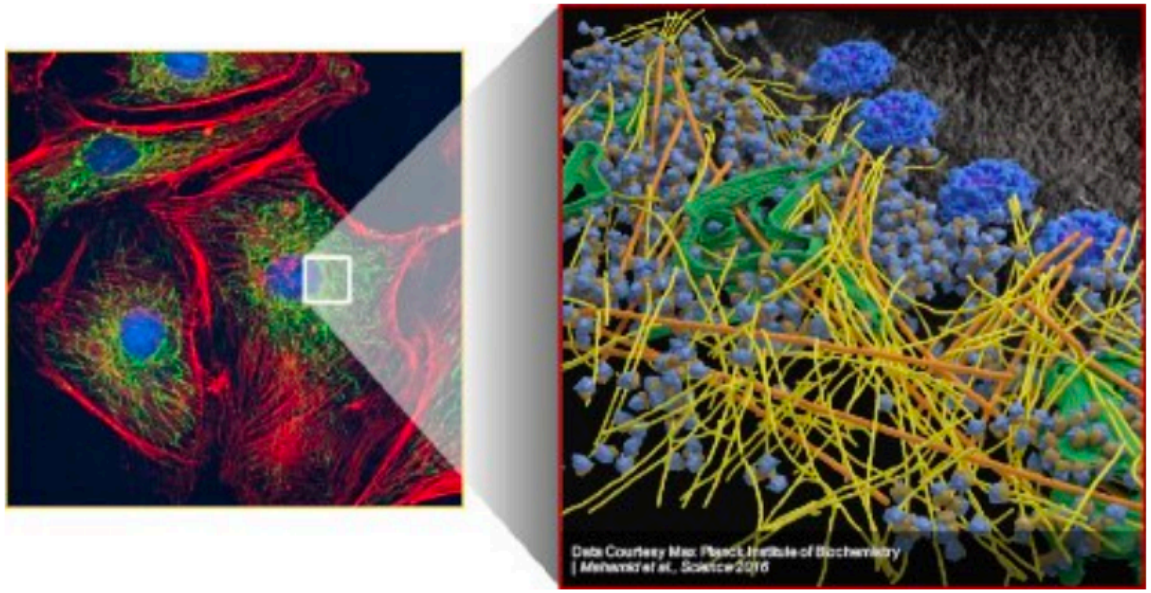
prof M. Lorito
prof. S.L. Woo
prof V. Lanzotti & prof B. Panunzi
Colleghi di corso di laurea SAFA
Coordinatore SAFA (prof. Carputo)
Colleghi d'ufficio (Bonanomi, Falanga, Nicoletti, Turra)
Studenti di dottorato
Studenti di chimica del corso SAFA
Colleghi di Chimica (prof Lombardi, prof Pavone)

Stanza 11 Luigi.dicostanzo4@unina.it
phone 351-643-2112

Cryo Electron Tomography of Intracellular Structures

cryo-ET can play to its strengths, as it does not require any dehydration, staining or labeling of the sample

cryo-electron tomography data can be collected with the same transmission electron microscopes as single-particle analysis data.



Fluorescence image (left) and tomography structure (right) of a HeLa cell. The fluorescence image shows nuclei (blue), actin filaments (red) and mitochondria (green), but without cellular context. Cryo electorn tomography provides a close up containing vital contextual information.