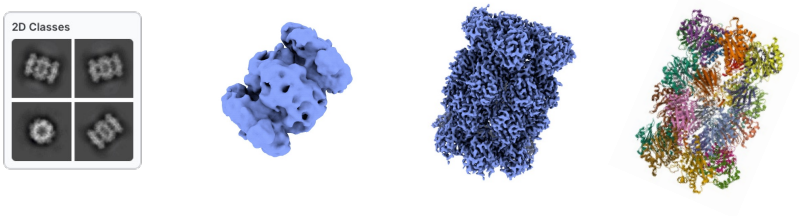


Curso: Microscopía avanzada

Crio-microscopia electrónica (*CryoEM*)

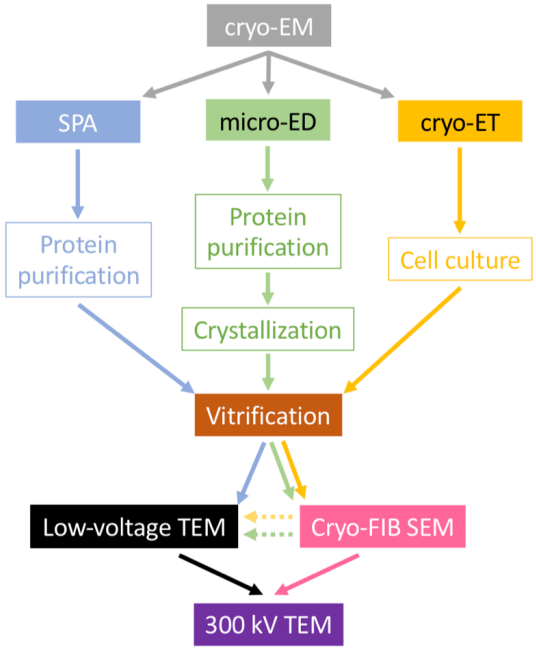
Dr. Víctor Castro Fernández

vcasfe@uchile.cl

Laboratorio de Bioquímica y Biología Molecular
Departamento de Biología
Facultad de Ciencias
Universidad de Chile

@bioquimica_uchile X @LabBqUchile @LabBQBM

1

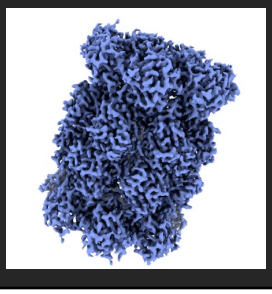


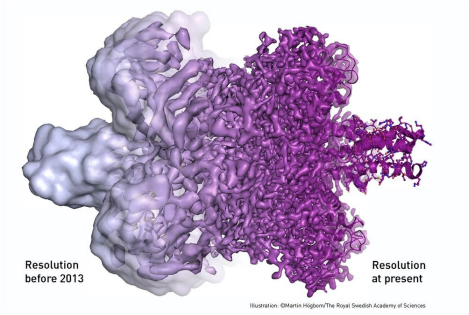
2

Crio-microscopía electrónica (Cryo-EM)

Single-particle analysis

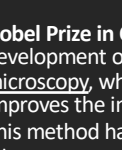
Nobel Prize in Chemistry 2017 for the development of cryo-electron microscopy, which both simplifies and improves the imaging of biomolecules. This method has moved biochemistry into a new era.

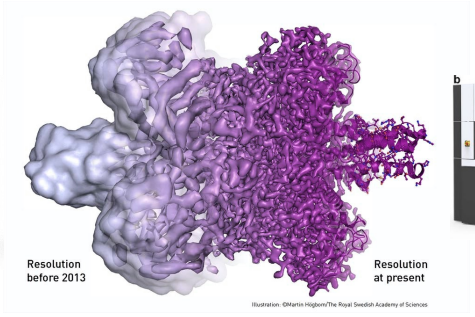


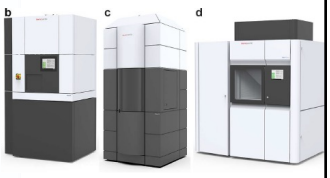


https://phys.org/news/2017-10-nobel-prize-chemistry-american-swiss.html

3







- Mejoras en los detectores de electrones
  - Cámaras de película → detectores directos de electrones (DEDs):
- Corrección de movimiento (“beam-induced motion correction”)
- Algoritmos avanzados de reconstrucción 3D + Aceleración por cómputo paralelo (GPUs y clusters)
- Fuentes de emisión de campo (FEG, *field emission gun*) - haz mucho más brillante, coherente y estable.

https://phys.org/news/2017-10-nobel-prize-chemistry-american-swiss.html

4

Crio-microscopía electrónica (Cryo-EM)

Pasos necesarios

- Purificar la proteína
- Vitrificación (evitar preferencia de orientación).
- Colectar imágenes
- Análisis de partículas y validación de mapa
- Ajustar los aminoácidos al mapa

Pro

- NO necesita cristalizar la proteína
- Requiere bajas cantidades de proteína pura

Contra

- Solo para proteínas grandes (~>90 Kda)
- En general media-baja resolución (> 2.5 Å)

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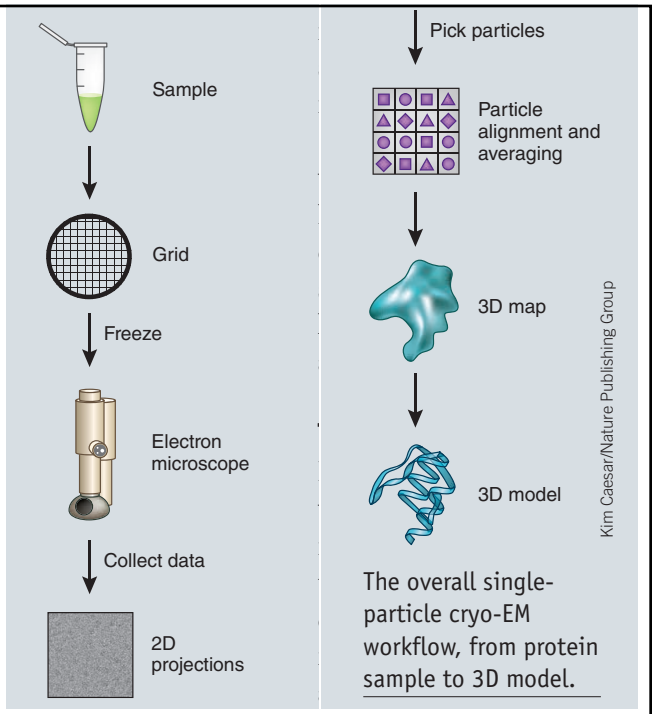
Pasos generales

Cryo-EM



The overall single-particle cryo-EM workflow, from protein sample to 3D model.

NATURE METHODS | VOL.13 NO.1 | JANUARY 2016 | 23

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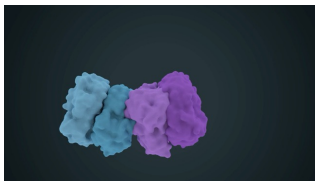
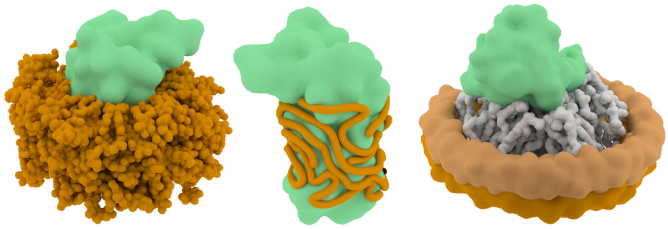
Preparación de la proteína

- Evitar distintas conformaciones



- Proteínas de membranas:



Stabilization of membrane proteins in detergents (left), amphipols (middle), or lipid nanodiscs (right).

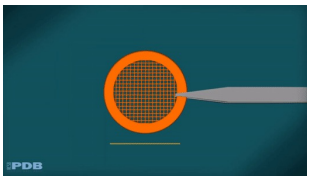
7

Preparación de la muestra

8

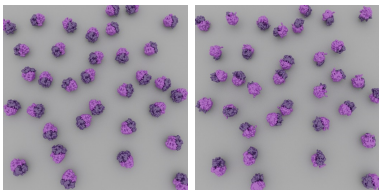
Preparación de grillas para cryoEM

- Vitrificación de la muestra:
  - Aplicación de la proteína en solución
  - Secado del exceso de solución
  - Vitrificación en etano líquido



- Algunos problemas:

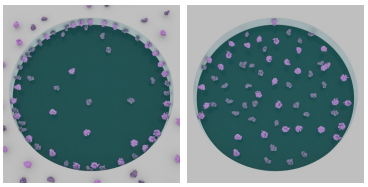
Orientation bias



Particles may adopt preferred orientations

Ideally, particles adopt a broad distribution of orientations

Particle partitioning



Preference to attach to the glow-discharged surface and fail to enter holes

Ideally, particles are covered throughout the holes

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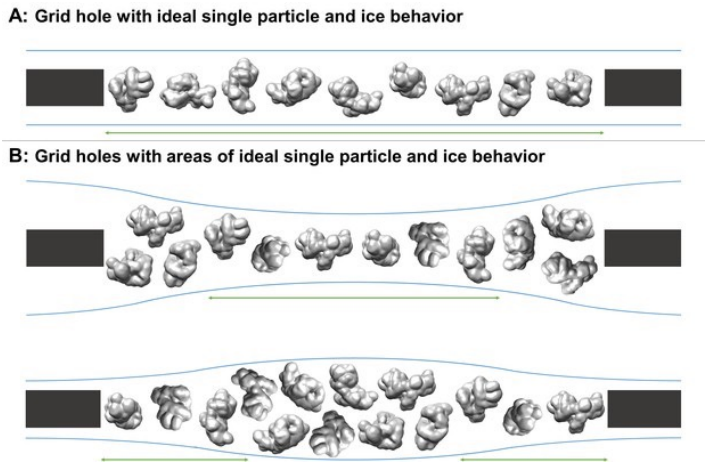
Muestras de distinta calidad en una misma grilla



Alex J Noble et al. (2018) *eLife* 7:e34257.
https://doi.org/10.7554/eLife.34257

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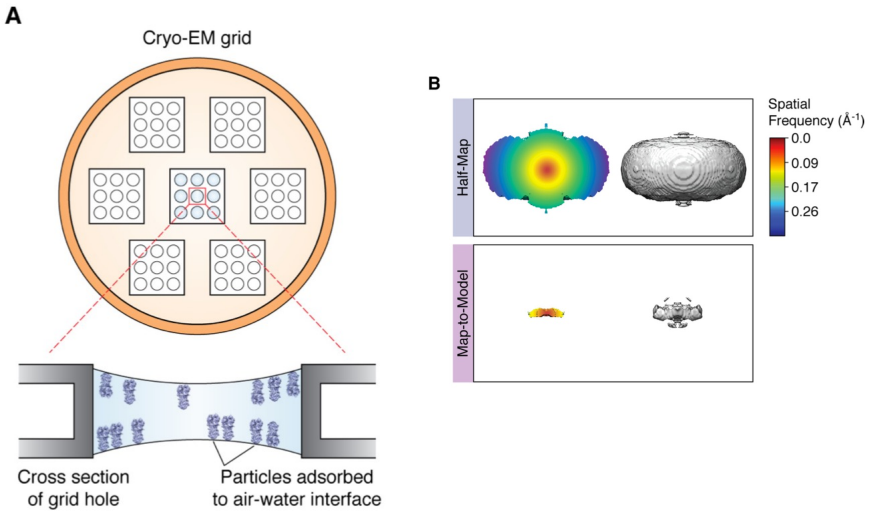
Problem: Preferred orientation on grids



Dmitry Lyumkis, JBC 2019, DOI 10.1074/jbc.REV118.005602

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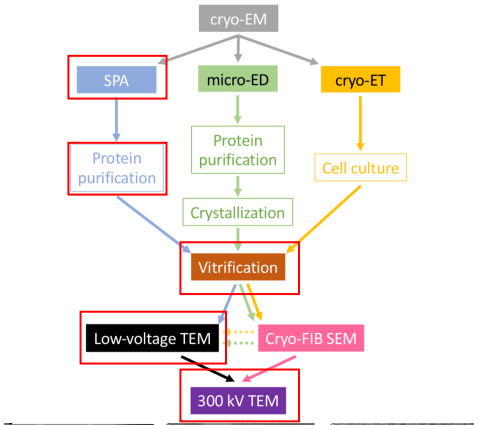
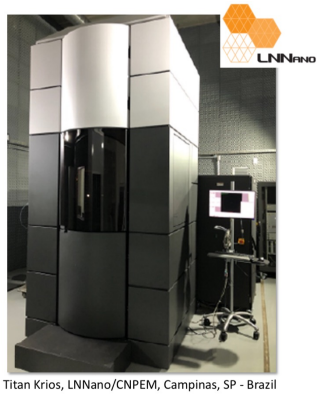
Data collection: automatic (several hours)





Titan Krios, LNNano/CNPEM, Campinas, SP - Brazil

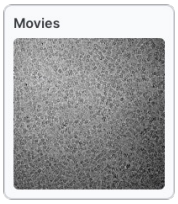
~8.000.000 USD

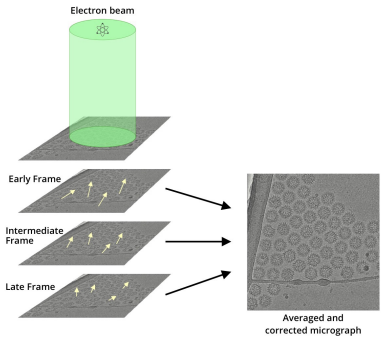
- Se colectan películas (decenas de fotos de un mismo campo)
- Las micrografías se colectan con desenfoque (contraste de fase)

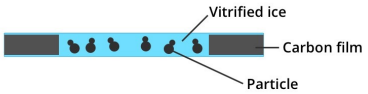
12

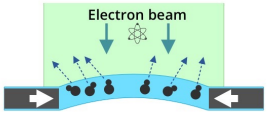
Procesamiento inicial de imágenes(películas) de cryoEM:

Motion Correction: corrects mechanical movement





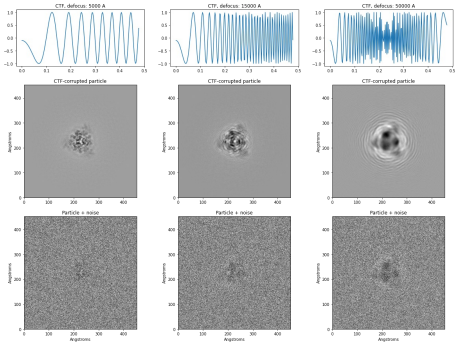




13

Contrast Transfer Function (CTF) estimation:

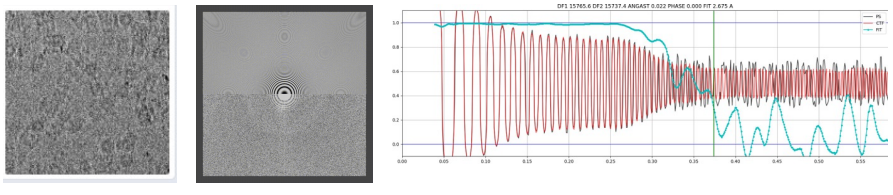
The microscope is operated in "phase contrast" mode and then we will need to have the molecules in focus.



A simplified, one-dimensional model of the CTF can be expressed via the equation below:

$$\mathrm{CTF}(f) = -\cos\left(\pi \Delta z \lambda_e f^2 - \frac{\pi}{2} C_s \lambda_e^3 f^4 + \phi\right)$$

where Δz is defocus, λe is the wavelength of the incident electrons, Cs is spherical aberration, and f f is spatial frequency. ϕ represents a phase shift factor, which is important e.g. when a phase plate is used.



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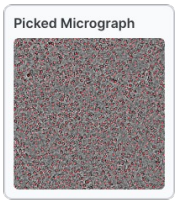
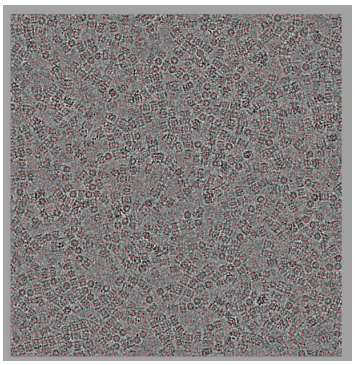
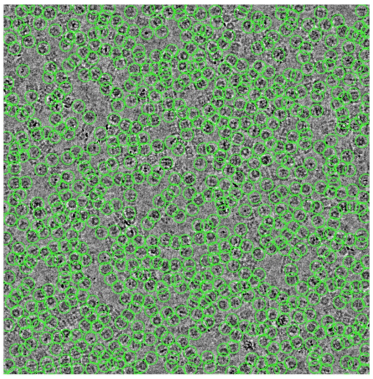
Particle Picking:



Blob Picker: first manual and then machine learning algorithms are trained.

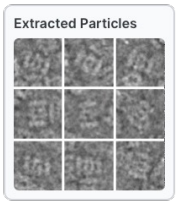
Template Picker: Based on an initial model of the particle (structure, map, etc.) the machine learning algorithms are trained.

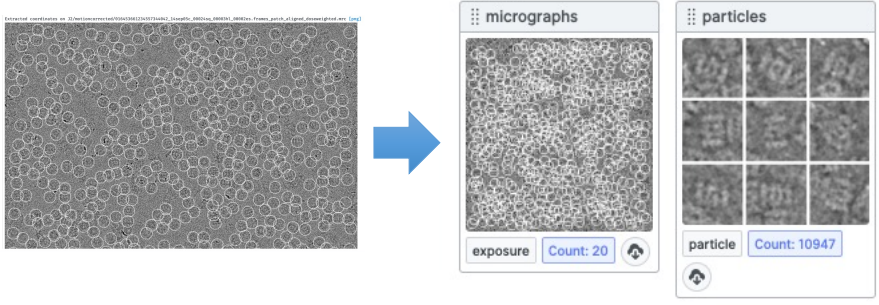




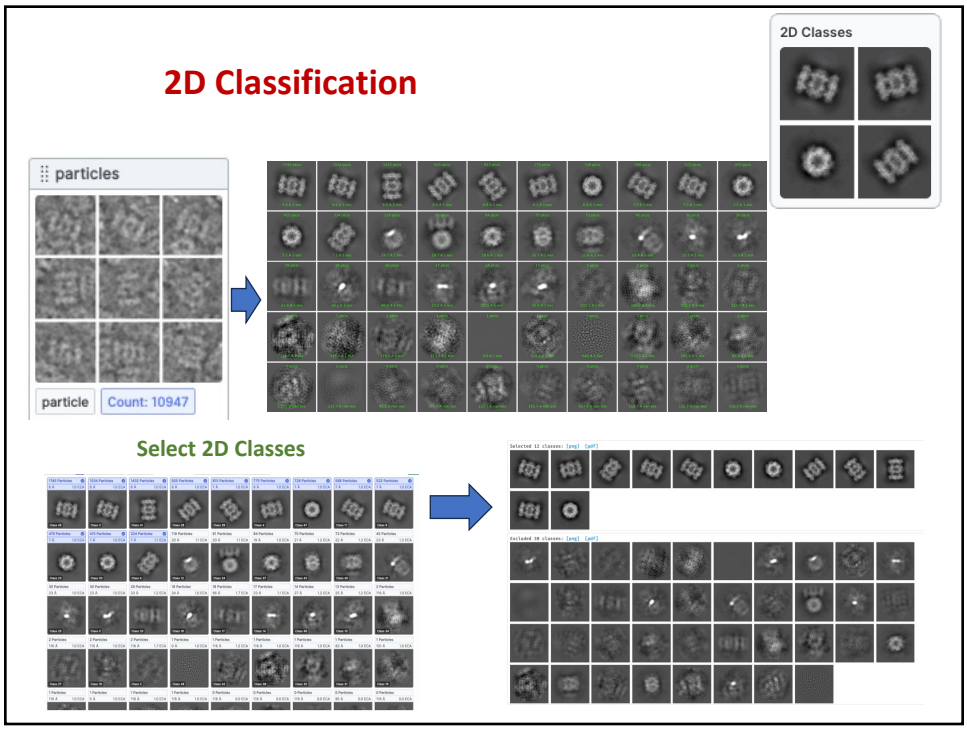
15

Extract particles from Micrographs



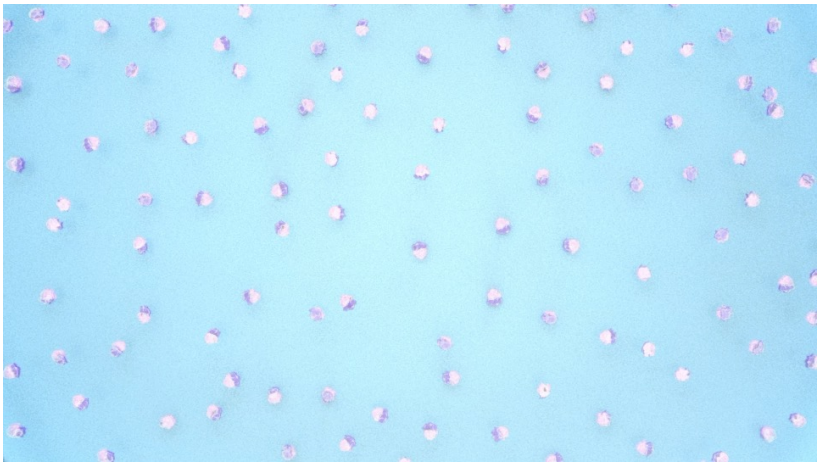


16



17

Reconstruction



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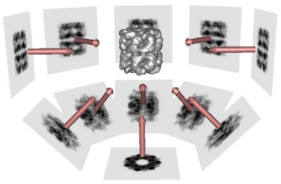
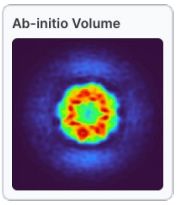
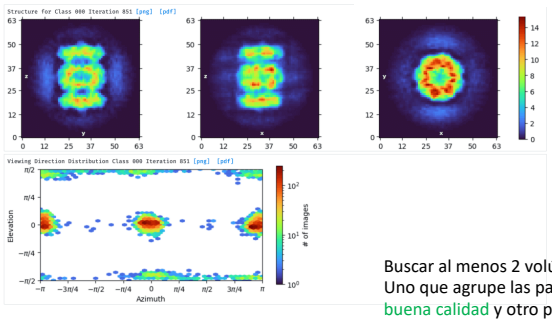
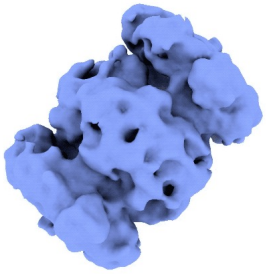
Ab-initio Reconstruction





Coulomb potential map (by back-projection)



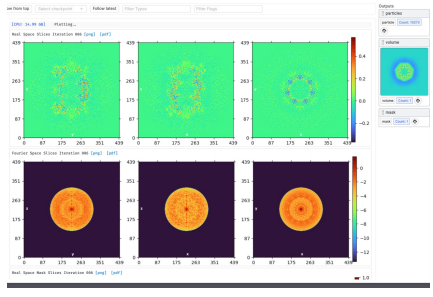


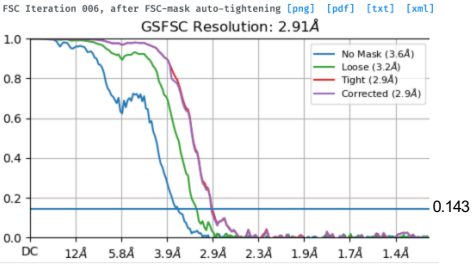
Buscar al menos 2 volúmenes:
Uno que agrupe las partículas de buena calidad y otro para las de mala calidad

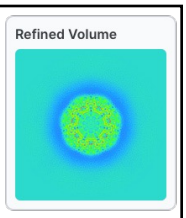
19

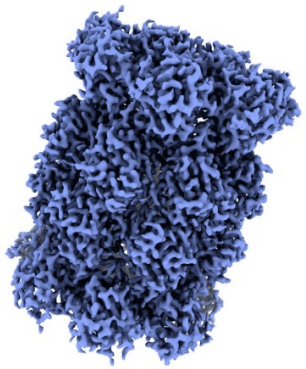
Map Refinement

Restringir simetría del oligómero y re-evaluar partículas





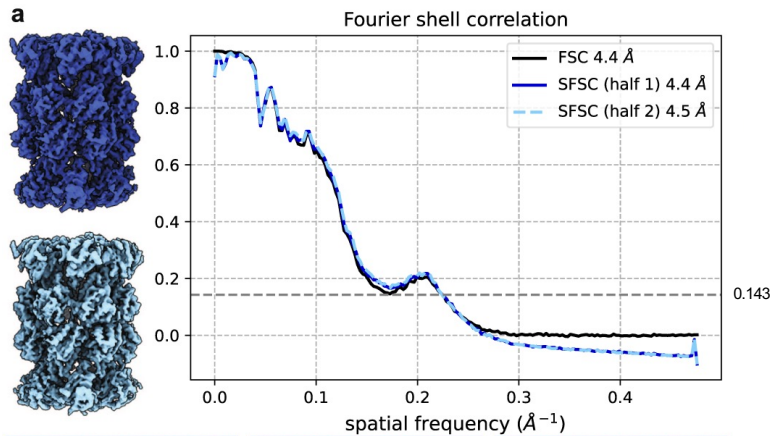




T20S proteasome refined map

20

Resolution: Gold-standard Fourier Shell correlation (FSC)



Gold-standard Fourier Shell correlation (FSC) curves calculated from two independently refined half-maps

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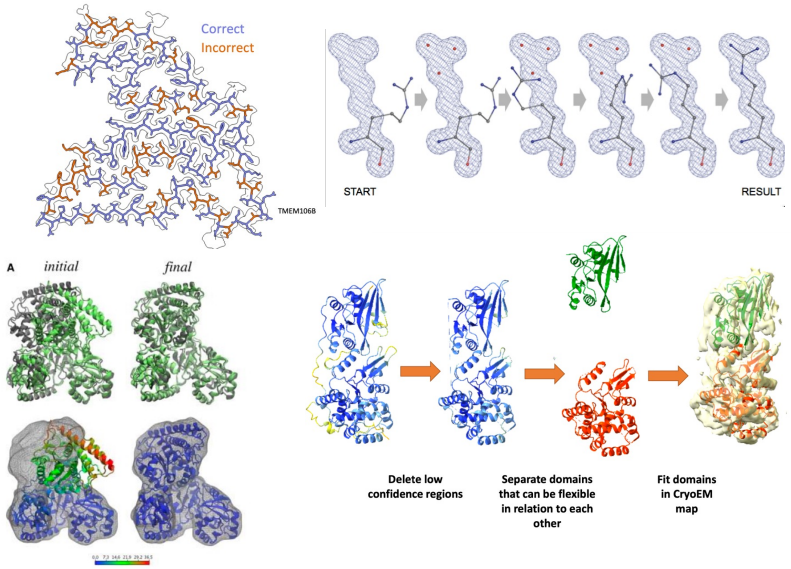
CryoEM: Model Building and Refinement



Los mapas de CryoEm no cambian durante el refinamiento del modelo.

22

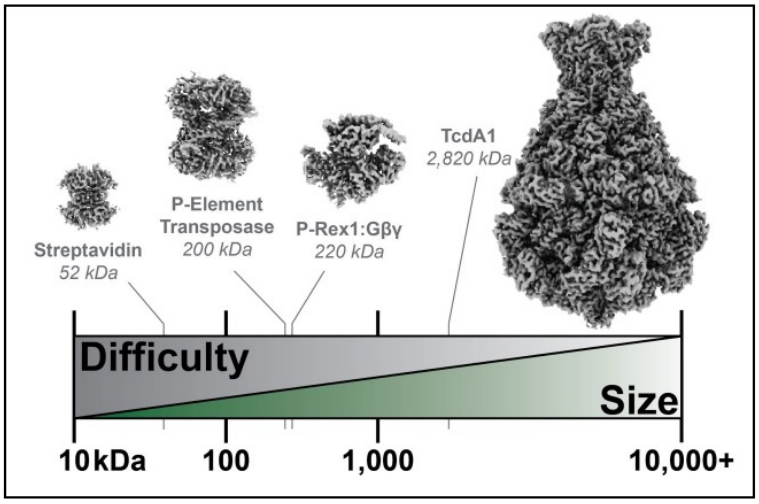


Figure 1 - Molecular weight vs. difficulty for cryo-EM structural targets. Cryo-EM reconstructions for streptavidin (EMDB-0689, 3.2Å), P-Element transposase (EMDB-20254, 3.6Å), P-Rex1:Gβγ (EMDB-20308, 3.2Å), and TcdA1 (EMDB-10033, 2.8Å) shown alongside difficulty.

Michael Cianfrocco, and Elizabeth Hua-Mei Kellogg, 2020. *J. Chem. Inf. Model.*, DOI: 10.1021/acs.jcim.9b01178

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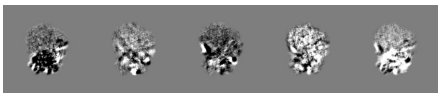
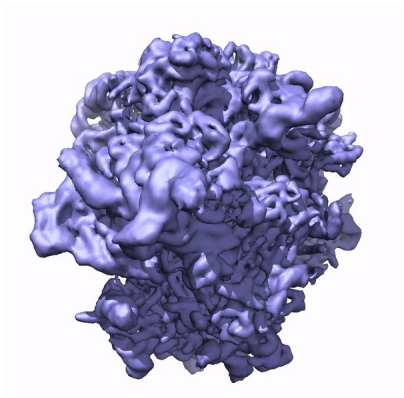
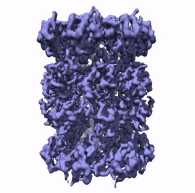
3D Variability Analysis: Directly resolving continuous flexibility and discrete heterogeneity from single particle cryo-EM images.



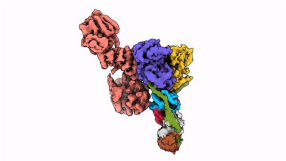
80S Ribosome



https://doi.org/10.1101/2020.04.08.032466



T20S proteasome



mTORC1 on the lysosomal surface

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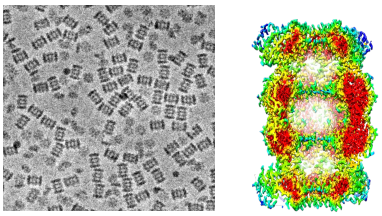
CryoEM - Tomography

Single Particle Analysis

Proteins in solution

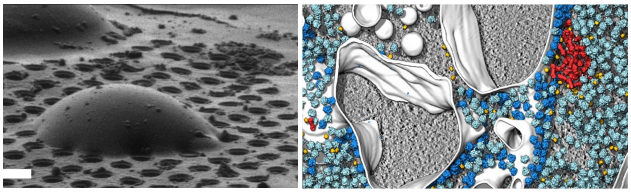


20S proteasome, VPP and defocus (500nm) at 2.4A resolution
Courtesy of: Radostin Danev

Tomography

Cells in solution

Tissues



W. Baumeister and J. Plitzko *S. Albert et al./PNAS 2019*

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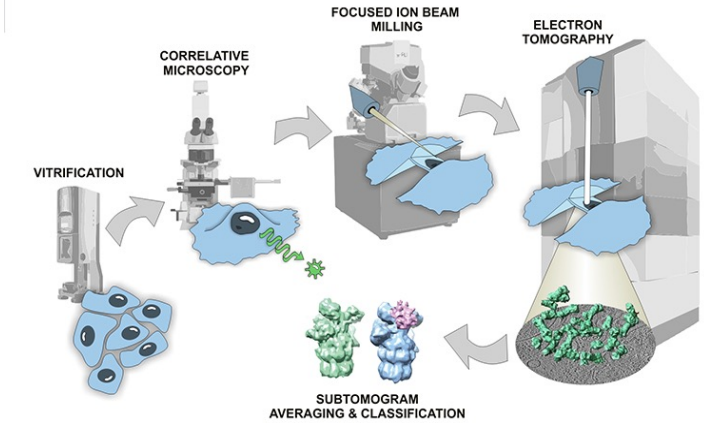
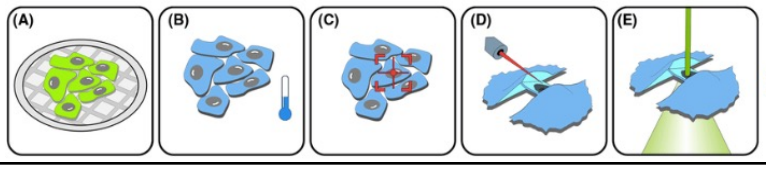
Cryotomography workflow





ThermoFisher

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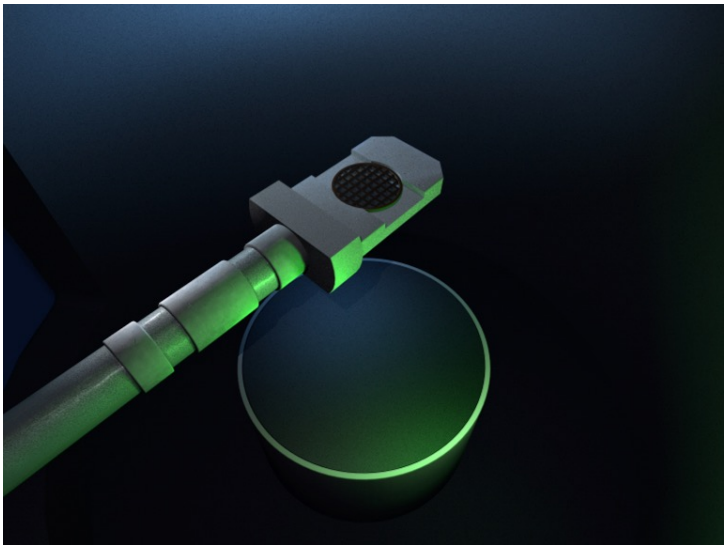

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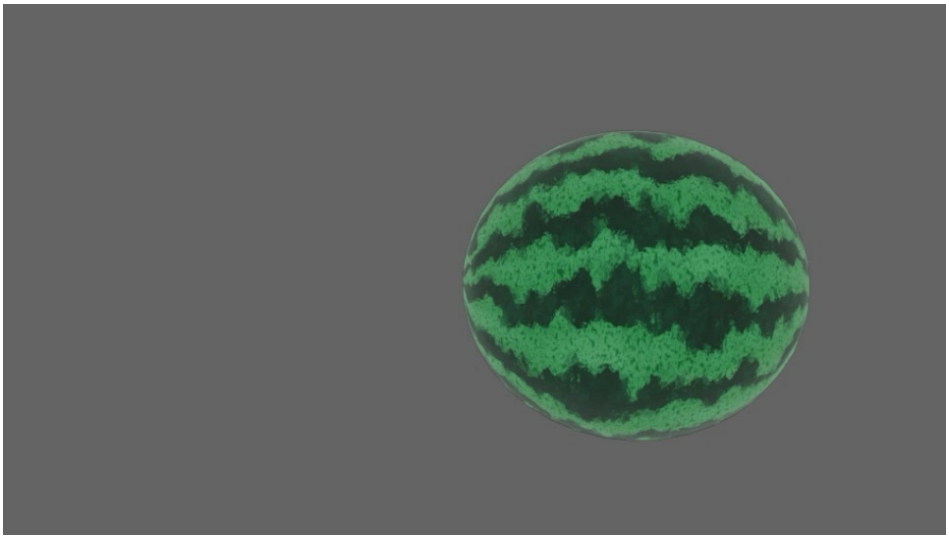
Cryotomography



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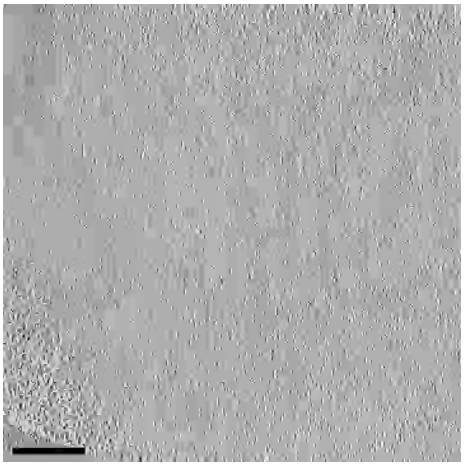
Organellar ultrastructure using cryo-electron tomography



This video was made using cryo-ET (electron tomography), a close sister of the technology that earned the Nobel prize in chemistry today. Ben Engel and his colleagues at the Max Institute of Biochemistry, in collaboration with Martin Jonikas at Princeton University, imaged a part of the algae cell involved in photosynthesis. This video shows the interior of a structure called the pyrenoid, which algae use to concentrate carbon from the carbon dioxide in air. The purple spheres are enzymes that "fix" carbon dioxide to start the process of photosynthesis. The green tubes and yellow tubules are thought to bring carbon and other materials into the pyrenoid. Image credit: Ben Engel, Max Planck Institute for Biochemistry. Read more at https://www.princeton.edu/news/2017/09/21/green-algae-could-hold-clues-engineering-faster-growing-crops

https://www.youtube.com/shorts/033B5TZMyAU?feature=share

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Cryogenic electron microscopy (cryoEM) resources provide access to instrumentation

Broadening access to cryoEM through centralized facilities

https://doi.org/10.1016/j.tibs.2021.10.007

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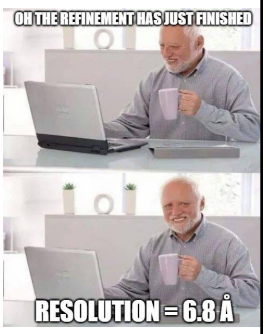
Resolución de una estructura



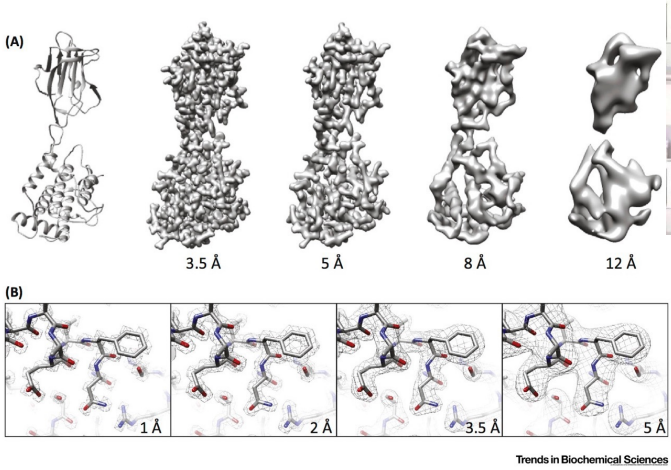


Figure 2. Effect of Resolution. (A) Ribbon diagram of the Vp7 monomer from Bluetongue virus (left) and theoretical electron density maps sampled at resolutions of (left–right) 3.5, 5, 8, and 12 Å. (B) A small region of the structure, with theoretical electron density sampled at resolutions of (left–right) 1, 2, 3.5, and 5 Å superimposed on the structure.

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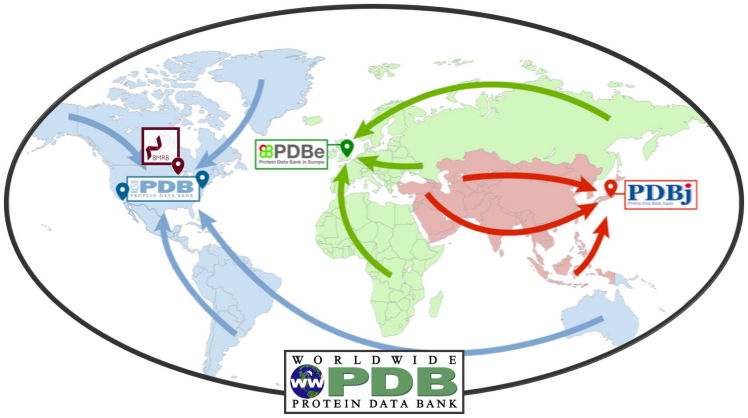
Deposito de estructuras de proteínas reportadas en artículos científicos



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RCSB PDB PROTEIN DATA BANK

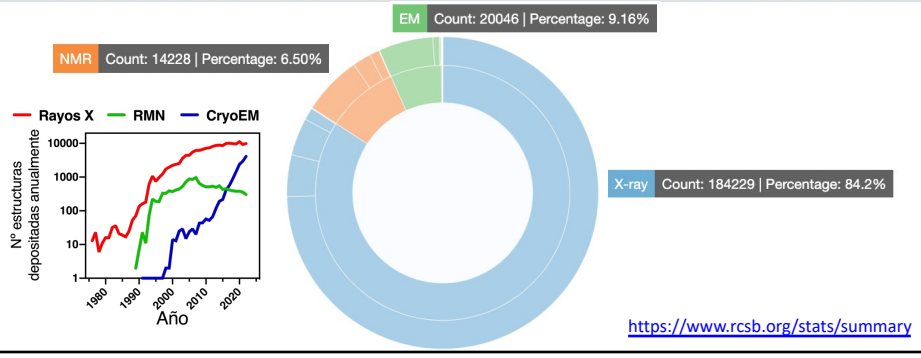
218,853 Structures from the PDB

1,068,577 Computed Structure Models (CSM)

3D Structures

PDB-101 PDB EMDataResource NAKB wwPDB Foundation PDB-Dev

Molecular Type	X-ray	EM	NMR	Multiple methods	Neutron	Other	Total
Protein (only)	163,345	13,502	12,381	204	74	32	189,538
Protein/Oligosaccharide	9,433	2,261	34	8	2	0	11,738
Protein/NA	8,479	4,143	286	7	0	0	12,915
Nucleic acid (only)	2,797	131	1,488	14	3	1	4,434
Other	164	9	33	0	0	0	206
Oligosaccharide (only)	11	0	6	1	0	4	22
Total	184,229	20,046	14,228	234	79	37	218,853



https://www.rcsb.org/stats/summary

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Laboratorio de Bioquímica y Biología Molecular
Facultad de Ciencias, Universidad de Chile

fucited Fundación Ciencia y Tecnología para el Desarrollo

BIOSONDA BIOTECNOLOGIA

IFSC UNIVERSITY OF SÃO PAULO São Carlos Institute of Physics

CNPEM Centro Nacional de Pesquisa em Energia e Materiais

Estudios estructurales de la hemocianina de *Concholepas concholepas*: un enfoque combinado de las técnicas de cristalografía de rayos X y cryo-EM”

Agencia Nacional de Investigación y Desarrollo
Ministerio de Ciencia, Tecnología, Conocimiento e Innovación
Gobierno de Chile

FAPESP

ANID-FAPESP 2019/13318-5

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Antecedentes

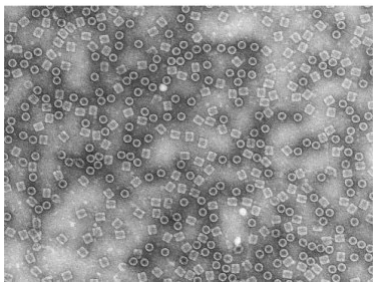
• Hemocianinas

- Glicoproteínas multiméricas de alto peso molecular disueltas en la hemolinfa de artrópodos y moluscos
- Hemocianinas de molusco se encuentran entre las proteínas globulares más grandes conocidas (3,3 a 13,5 MDa).
- Son usadas como inmunoestimulantes no específicos, naturales y no tóxicos.
- Hemocianina de *C. concholepas* (CCH)*:*

- Presenta mayor estabilidad y solubilidad comparada a homólogos.

- Ha sido utilizada como proteína carrier.

- Presenta evidencia clínica de su uso como adyuvante, demostrando su potencial para futuros desarrollos biomédicos.



Micrografía electrónica por tinción negativa de hemocianina de *Rapana Venosa* (RVH)

Concholepas concholepas
"Loco"

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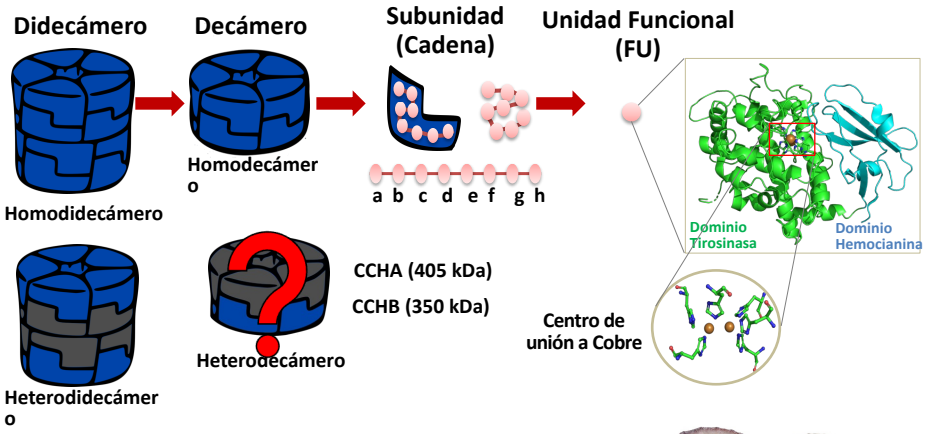
Organización estructural de hemocianinas de molusco



- Dos fragmentos de secuencia que codifican para CCH, obtenidos mediante tecnica RACE (CCHX y CCHY).

Hemocianina de *Concholepas concholepas* (CCH)

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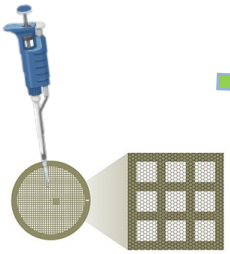
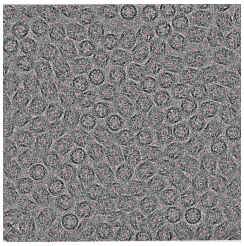
Cryo-EM de CCH



Grillas de cryo-EM
Quantifoil R1,2/1,3

Vitrobot

LNNano, Campinas, Brasil



Micrografía de CCH

7 TB de información

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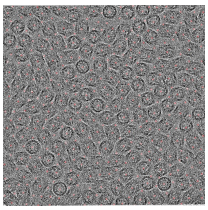
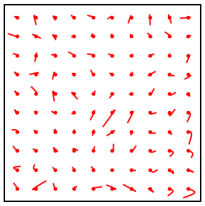
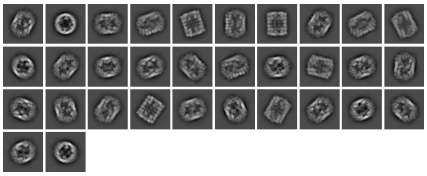
Procesamiento de datos de cryo-EM en Chile

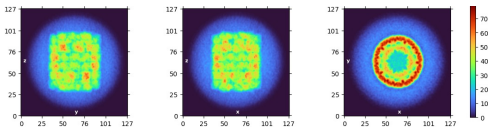




Corrección de movimiento
558.149 particulas

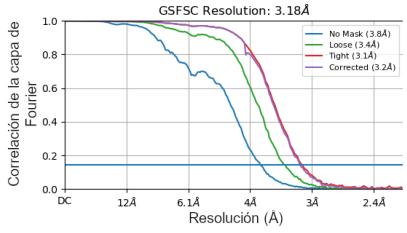


Clasificación de partículas 2D
199.045



Reconstrucción *ab initio* de modelo de CCH
130.952

Refinamiento del modelo



PC de procesamiento:
2 GPU RTX 2080Ti
AMD 5975WX
256 GB RAM
4TB SSD - 32 TB HDD

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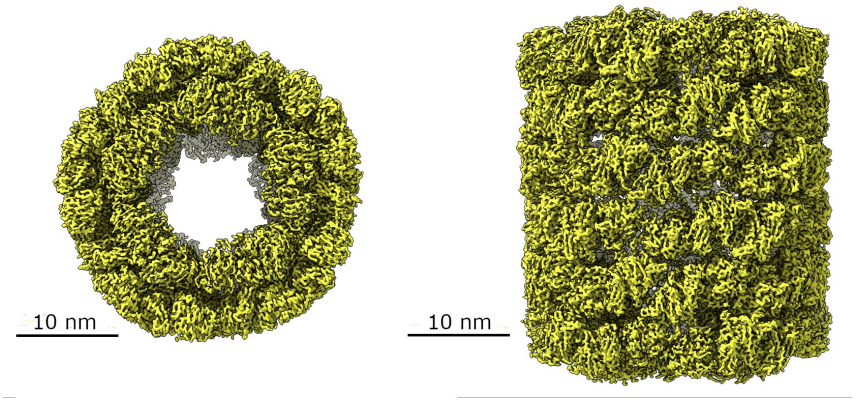
Disponemos de un mapa de cryo-EM de CCH a 3,2 Å hasta la fecha



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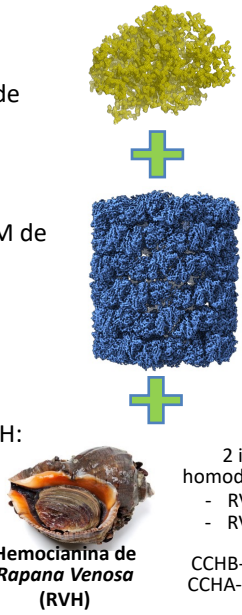
Generación de modelos de organización de subunidades

- Estructura cristalográfica de FU:
- Mapa de cryo-EM de CCH:
- Secuencia de RVH:

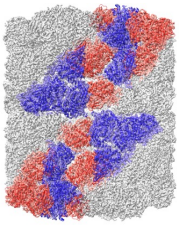


Hemocianina de *Rapana Venosa* (RVH)

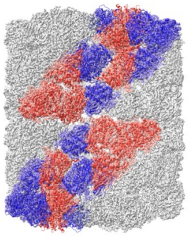
2 isoformas homodidecamericas:
- RVH1 (CCHB)
- RVH2 (CCHA)

CCHB-g – RVH1-g: 41% id
CCHA-d – RVH2-d: 71% id

Modelo I



Modelo II



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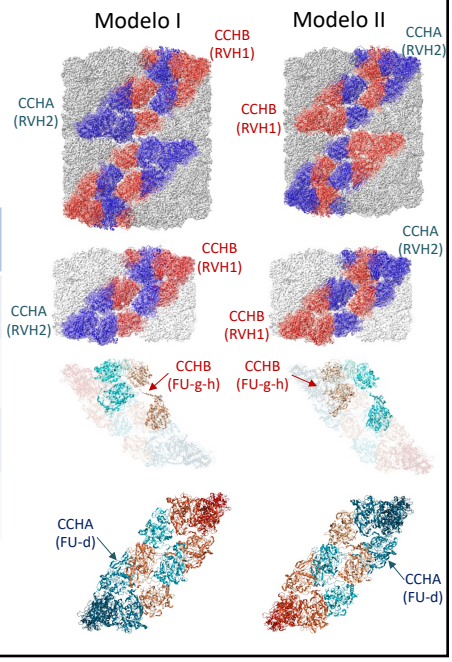
Evaluación de modelos de organización de subunidades de CCH

	Modelo I (CC)	Modelo II (CC)
Modelo 2 dímeros en el didecamero	0,80	0,81
Modelo 1 dimero refinado	0,80	0,82
Modelo solo FUs CCHB-g y CCHB-h	0,68	0,81
Modelo solo FU CCHA-d	0,76	0,79



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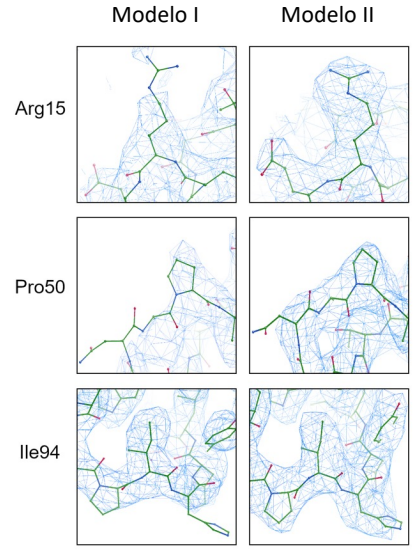
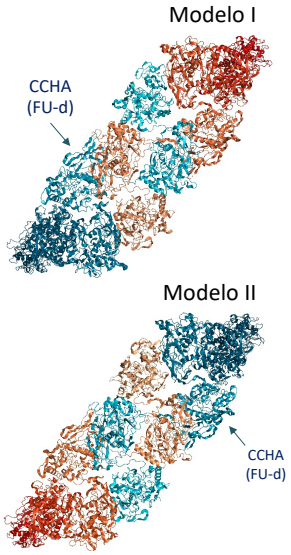
Validación local del modelo de organización de subunidades de CCH

Residuos de FU-d (CCHA-d)





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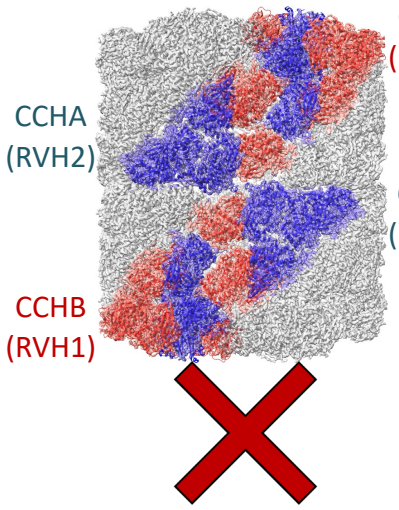
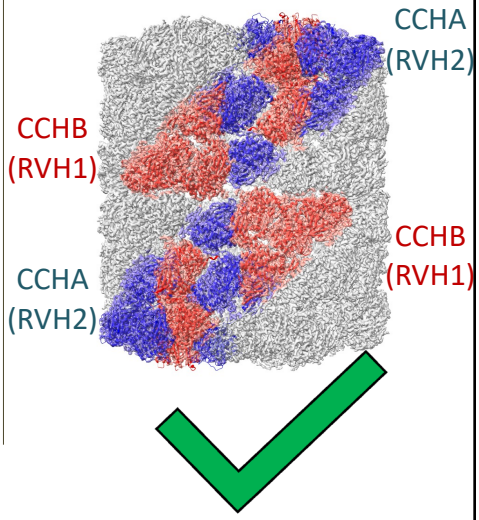
Evaluación de modelos de organización de subunidades de CCH

Modelo I



Modelo II



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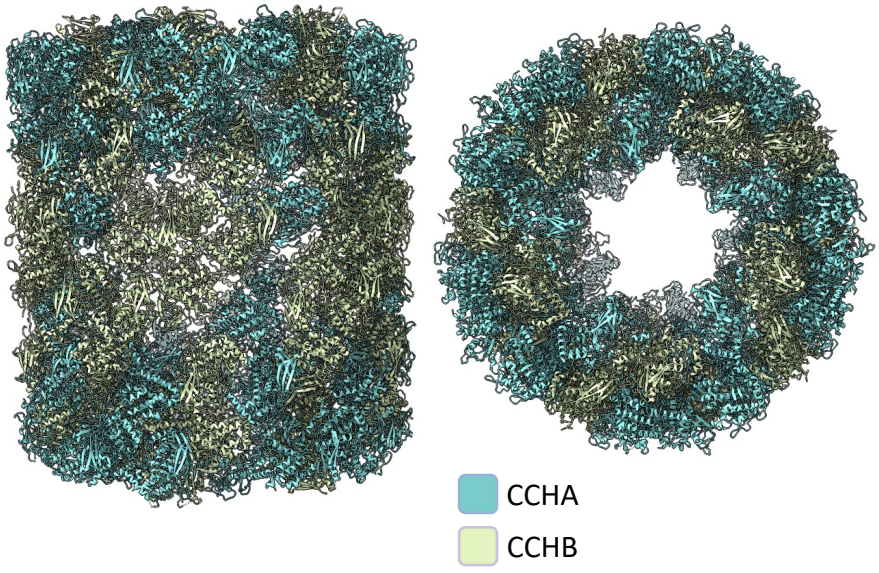
Primer modelo de una hemocianina heterodidecamerica



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Gracias

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