# instruct

Instruct Image Processing Center:
An Instruct-ERIC service for
CryoEM Single Particles Analysis

Marcos Gragera Cabezudo, PhD

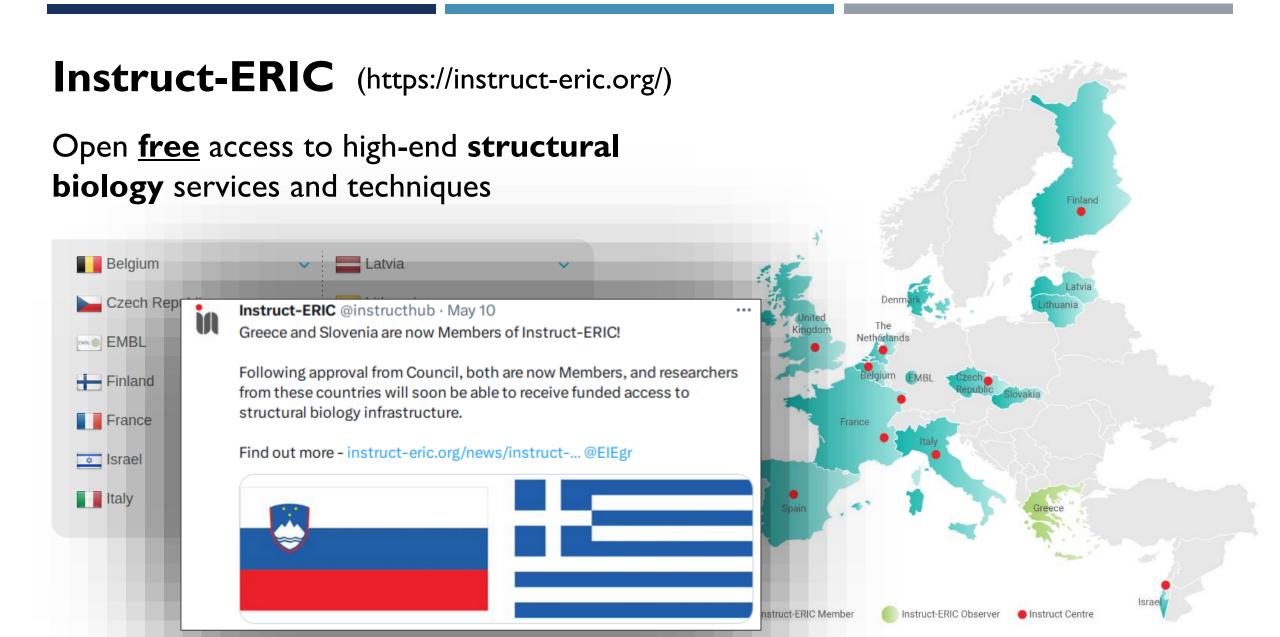
Instruct-ES

JM Carazo's Lab - Centro Nacional de Biotecnología









# Instruct-ERIC (https://instruct-eric.org/)

Open <u>free</u> access to high-end **structural biology** services and techniques



# Sample Preparation

Crystallisation

Nanobody Discovery

**Protein Production** 



#### Biomolecular Analysis

**Imaging** 

Mass Spectrometry

Molecular Biophysics

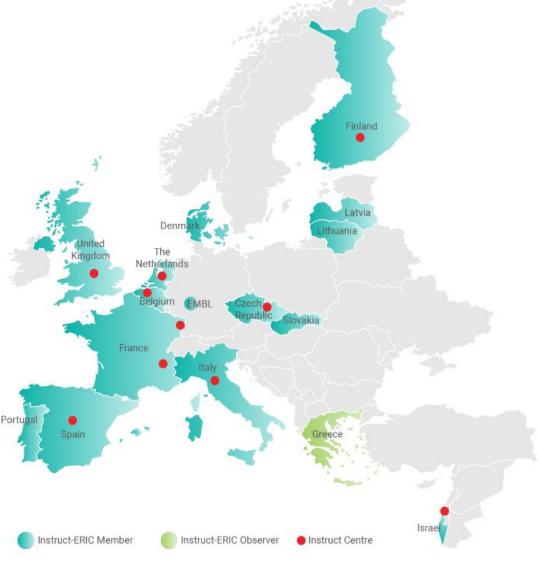


# 3D Structural Analysis

Electron Microscopy

Magnetic Resonance Techniques

X-Ray Techniques



# Instruct Centre ES

Open <u>fre</u> biology







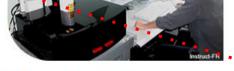
# Sample

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Crystallisation

Nanobody Discovery

**Protein Production** 



#### Biomolecular Analysis

**Imaging** 

Mass Spectrometry

Molecular Biophysics

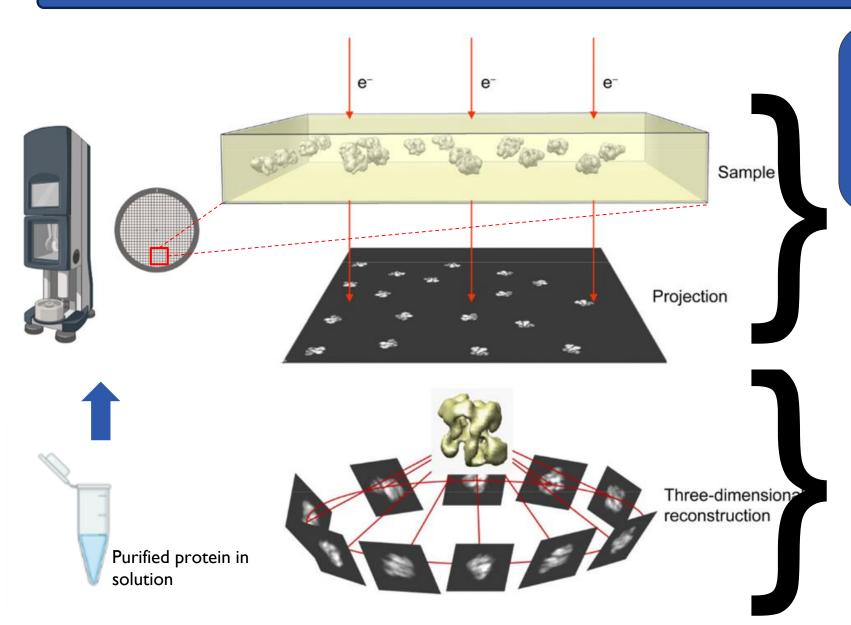


Electron Microscopy

Magnetic Resonance Techniques

X-Ray Techniques

#### **Instruct-ERIC - Our services**



# FlexibilityHub

Exploring conformational heterogeneity from cryo-EM images



# Instruct Image Processing Center

(Single Particle Analysis)





## CryoEM Facility

#### Resources





FEI Vitrobot IV



JEOL cryo arm 300 kV



Talos Arctica 200 kV



https://instruct-eric.org/platform/electron-microscopy-and-cryo-clem-cnb-csic-madrid-spain/



#### **Instruct Image Processing Centre**

#### **Scientific Contacts**

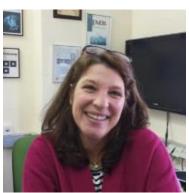


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#### **Admin Contacts**



blanca@cnb.csic.es

Blanca Benítez

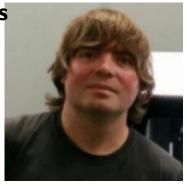
#### Resources



#### **Technical**



Marcos Gragera mgragera@cnb.csic.es



Roberto Melero rmelero@cnb.csic.es

Main workstation:

20 TB HDD, 32 cores, 256 GB RAM, 8x GPU 12 GB memory (GeForce 1080)

Virtual machine:

5TB disk HDD, 128 GB RAM, 2 GPUs with 32 GB memory

Storage:

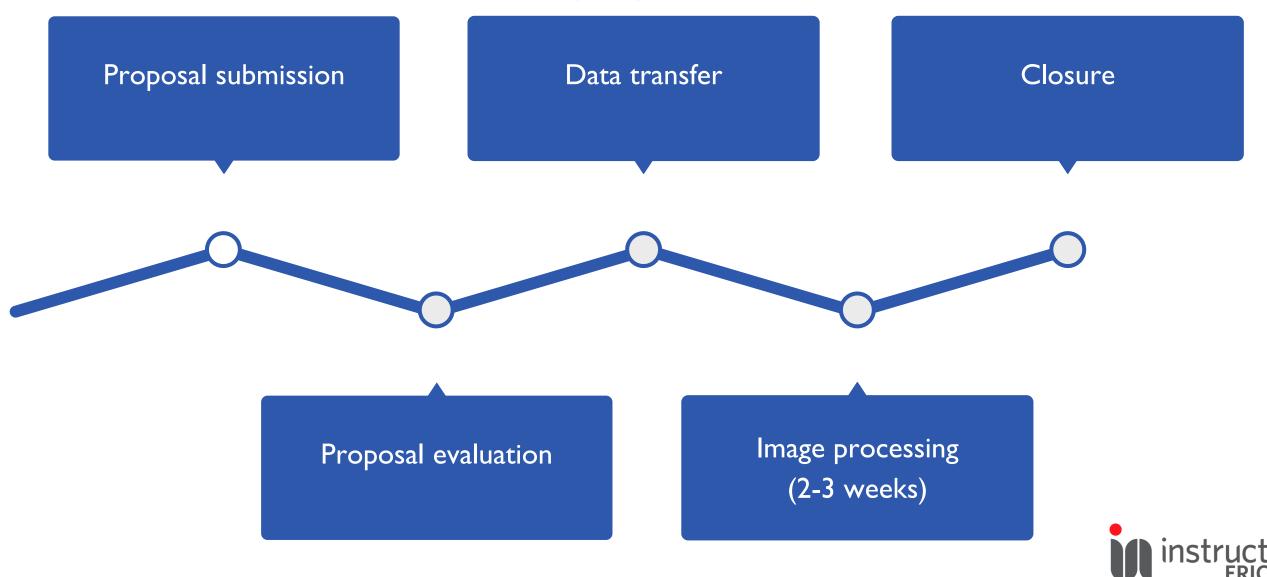
+200 TB for raw data and long-term storage



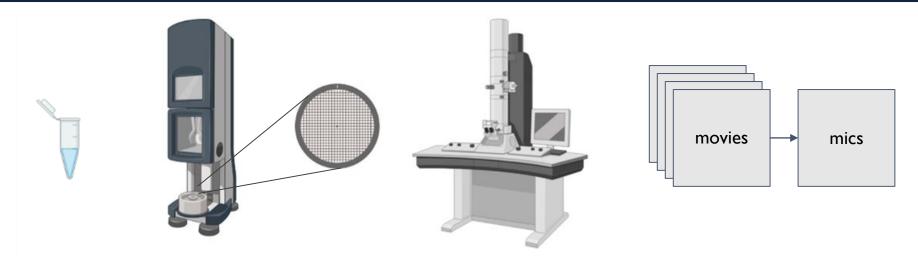


## Instruct-ERIC - Proposal pipeline

# Email to: mgragera@cnb.csic.es



### Instruct-ERIC - Why can it be interesting?



- If you are interested in the **3D structure** of your protein of interest:
  - Reluctant to crystallization/NMR.
  - Tolerance to impurities, flexibility.
  - Few μL of samples required.
  - Conformational landscape vs snapshot.
  - No previous experience in cryo-EM is required



#### Instruct-ERIC - What do you need to apply?

- Being from an institution of an Instruct member country.





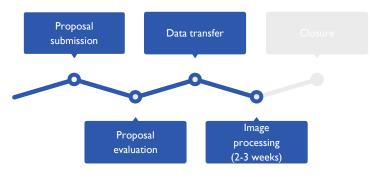
- Starting material: purified protein in solution / dataset already collected.







#### Instruct-ERIC - Image processing





#### Advantages:

- State-of-the art software for SPA
- Multi-algorithmic approach
- Traceability
- Project transfer

mail/video call

Strong interaction with user

Each dataset is assigned with 10 Access Units (2 weeks of work) What can we do during this time?



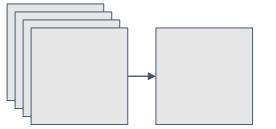
**CTF** estimation

**Picking** 

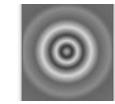
Extraction

2D classification

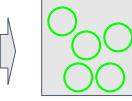
3D class./Refinement



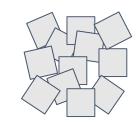












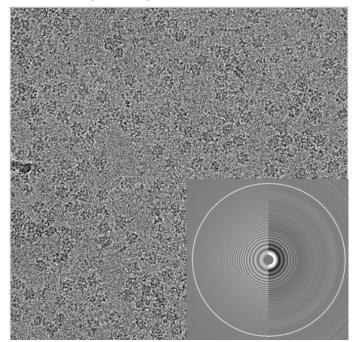


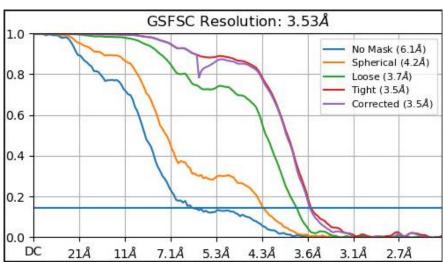


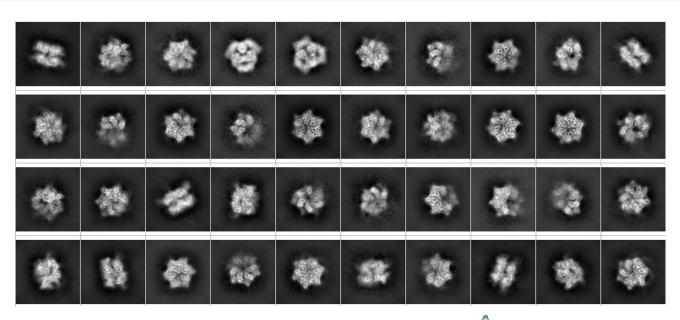


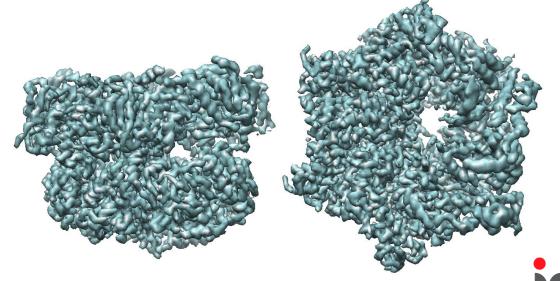
# **Scientific Outputs**

Talos Arctica (200 kV)









#### **Instruct-ERIC - Scientific output**

#### 2023

# A Fijivirus Major Viroplasm Protein Shows RNA-Stimulated ATPase Activity by Adopting Pentameric and Hexameric Assemblies of Dimers

Gabriela Llauger, Roberto Melero, Demián Monti, Gabriela Sycz, Cristián Huck-Iriart, María L. Cerutti, Sebastián Klinke, Evelyn Mikkelsen, Ariel Tijman, Rocío Arranz, Victoria Alfonso https://orcid.org/0000-0002-9167-6107, Sofía M. Arellano https://orcid.org/0000-0002-9728-6599, Fernando A. Goldbaum, Yann G. J. Sterckx, José-María Carazo, Sergio B. Kaufman, Pablo D. Dans, Mariana del Vas delvas, Lisandro H. Otero Instruct Access Project (PID):5526 iNEXT-Discovery Access Project (PID): 5391

#### 2022

#### Nanobodies Protecting From Lethal SARS-CoV-2 Infection Target Receptor Binding Epitopes Preserved in Virus Variants Other Than Omicron

Casasnovas J.M., Margolles Y., Noriega M.A., Guzmán M., Arranz R., Melero R., Casanova M., Corbera J.A., Jiménez de Oya N., Gastaminza P., Garaigorta U., Saiz J.C., Martín-Acebes M.A., and L.A. Fernández.

Frontiers in Immunology

Instruct Access Project (PID): 16168 and 14989

#### Structure of the drug target ClpC1 unfoldase in action provides insights on antibiotic mechanism of action.

Katharina Weinhäupl, Marcos Gragera, M. TeresaBueno-carrasco, Rocío Arranz, Olga Krandor, Tatos Akopian, Raquel Soares, Eric Rubin, Jan Felix, Hugo Fraga.

J. Biol. Chem.298(11): 102553

iNEXT Access Project (PID): 15695



#### **Instruct-ERIC - Scientific output**

2021 2020

# 3D architecture and structural flexibility revealed in t enzyme.

Melisa Lázaro, Roberto Melero, Charlotte Huet, Jorge Luciano A. Abriata, Pedro M. Alzari, Mikel Valle & Mar Communications Biology. 4: 684 Instruct Access Project (PID): 6258

# Near-atomic structure of an atadenovirus reveals a coproteins.

Roberto Marabini, Gabriela N. Condezo, Mart Krupov Science Advances: 7(14): eabe6008 Instruct Access Project (PID): 1395

#### Self-assembly and regulation of protein cages from p

Lapenta F, Aupič J, Vezzoli M, Strmšek Z, Da-Vela S, Sv Nature Communications. 12: 939 iNEXT Access Project (PID): 1824

#### Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures.

Melero R, Sorzano COS, Foster B, Vilas JL, Martínez M, Marabini R, Ramírez-Aportela E, Sanchez Caño L, Losana P, Fonseca-Reyna YC, Conesa P, Wrapp D, Chacon P, McLellan JS, Tagare HD, Ca IUCrJ. 7(6): 1059 – 1069

Instruct Access Project (PID): 11775

#### Ribosome-associated vesicles: A dynamic subcompartment of the endoplasmic reticulum in sec

Carter SD, Hampton CM, Langlois R, Melero R, Farino ZJ, Calderon MJ, Li W, Wallace CT, Tran NH Pemberton J, Morgenstern TJ, Eisenman L, Aguilar JI, Greenberg NL, Levy ES, Yi E, Mitchell WG, R EW, Aslanoglou D, Courel M, Freyberg RJ, Javitch JA, Wills ZP, Area-Gomez E, Shiva S, Bartolini F, M, Fish KN, Walter P, Balla T, Fass D, Wolf SG, Watkins SC, Carazo JM, Jensen GJ, Frank J, Freyber Sci Adv. 6(14):eaay9572

Instruct Access Project (PID): 1222

# Regulation of RUVBL1-RUVBL2 AAA-ATPases by the nonsense-mediated mRNA decay factor DH EM.

Andres López-Perrote, Nele Hug, Ana González-Corpas, Carlos F Rodríguez, Marina Serna, Carm Boskovic, Rafael Fernandez-Leiro, Javier F Caceres, Oscar Llorca.

eLife. 2020; 9:e63042

Instruct Access Project (PID): 10707

#### Capping pores of alphavirus nsP1 gate membranous viral replication factories.

Rhian Jones, Gabriel Bragagnolo, Rocío Arranz & Juan Reguera.

Nature.

Instruct Access Project (PID): 7046



#### **Scientific Contacts**

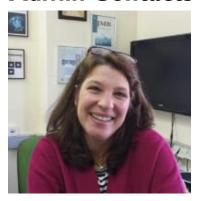


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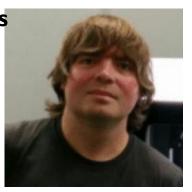




#### **Technical**



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# Thank you!







