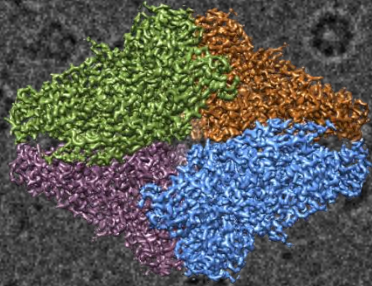
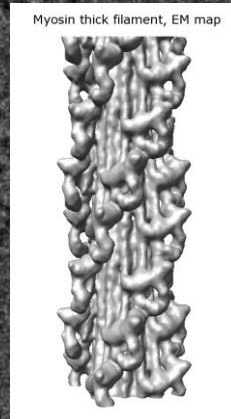
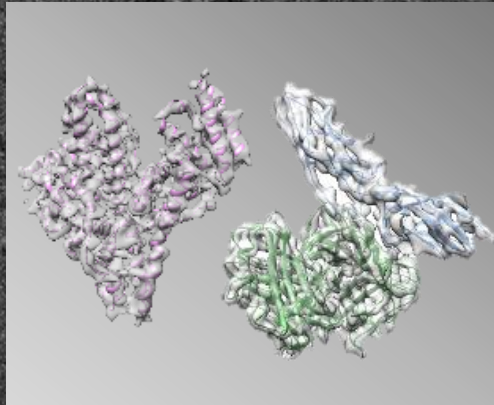
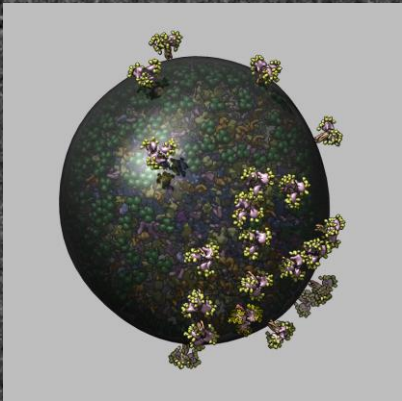
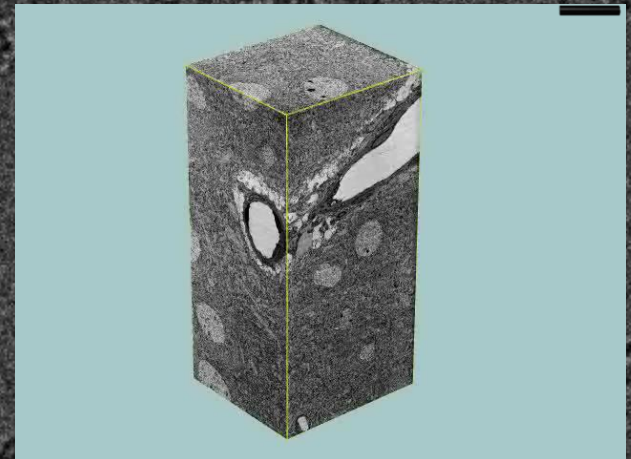
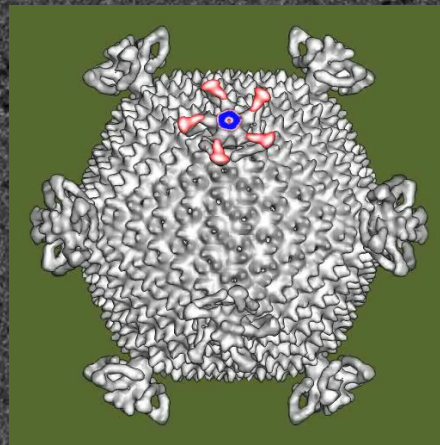
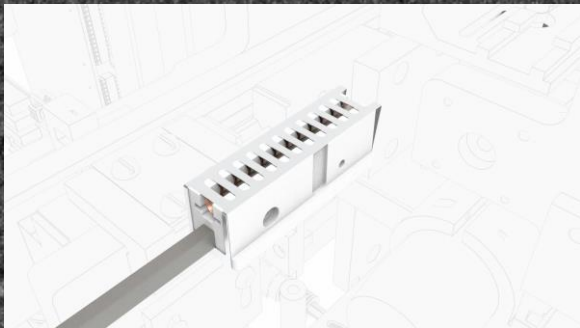
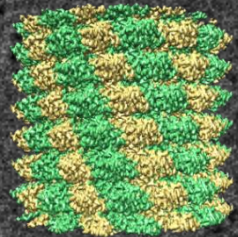


THE CRYO-EM BEAMLINE UPDATES REGULAR AND SOS WORKFLOW



EAAZHISAI KANDIAH





- **High resolution cryo-EM**

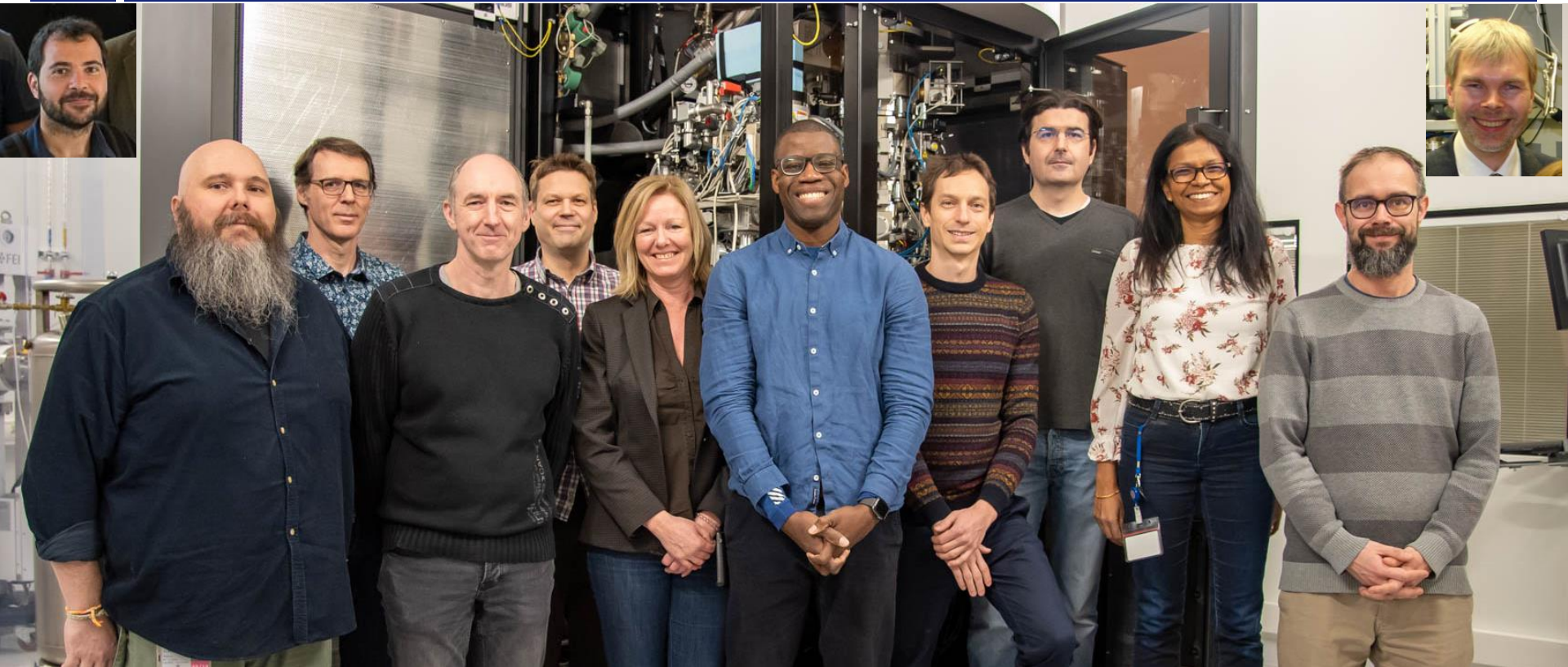


- **Solution-tO-Structure (SOS) pipeline**



OUTLINE

CMO1-THE TEAM



A.De MARIA
ESRF

D.FLOT
ESRF

**C. MUELLER-
DIECKMANN**
ESRF

G. EFFANTIN
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D. DAVISON
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D. TRAORE
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M. HONS
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F.DOBIAS
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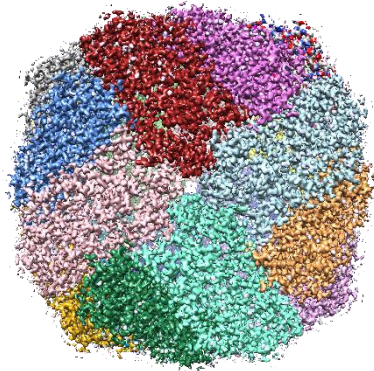
Contact: cryo-em@esrf.fr



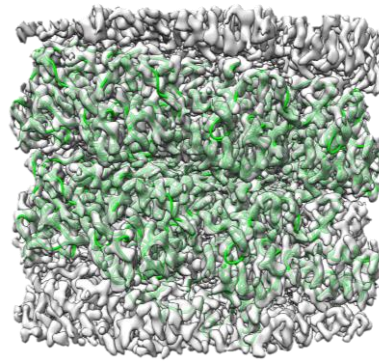
Photo by Cande



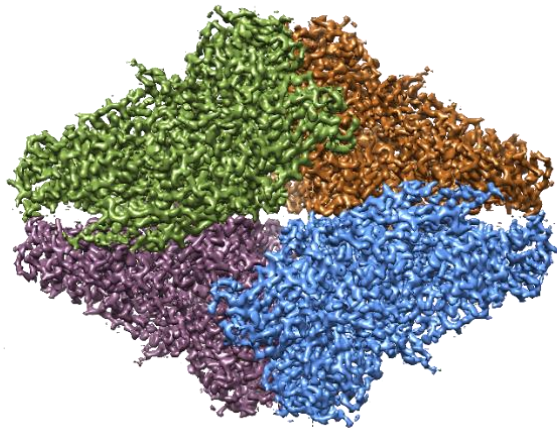
- Titan Krios G3
- Quantum LS energy filter
- K2 summit direct detector
- Volta phase plate



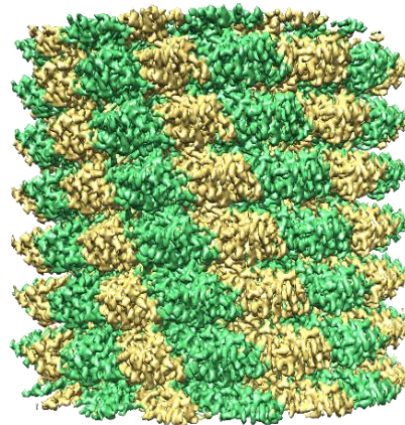
Apoferritin @ 1.63 Å



PVX virus @ 2.2 Å

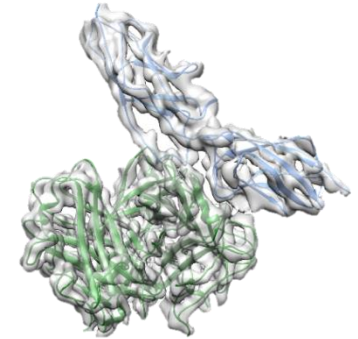


β -galactosidase @ 2.0 Å



TMV @ 2.3 Å

Phase plate

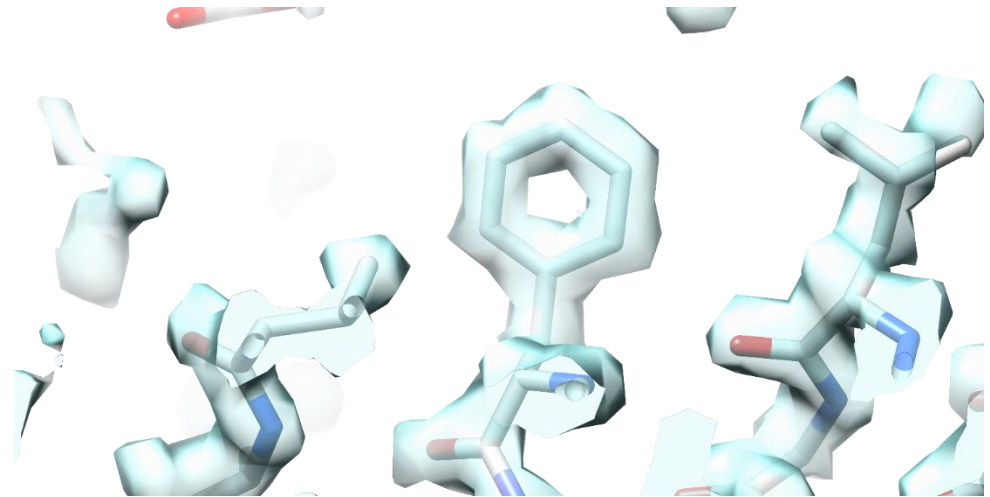
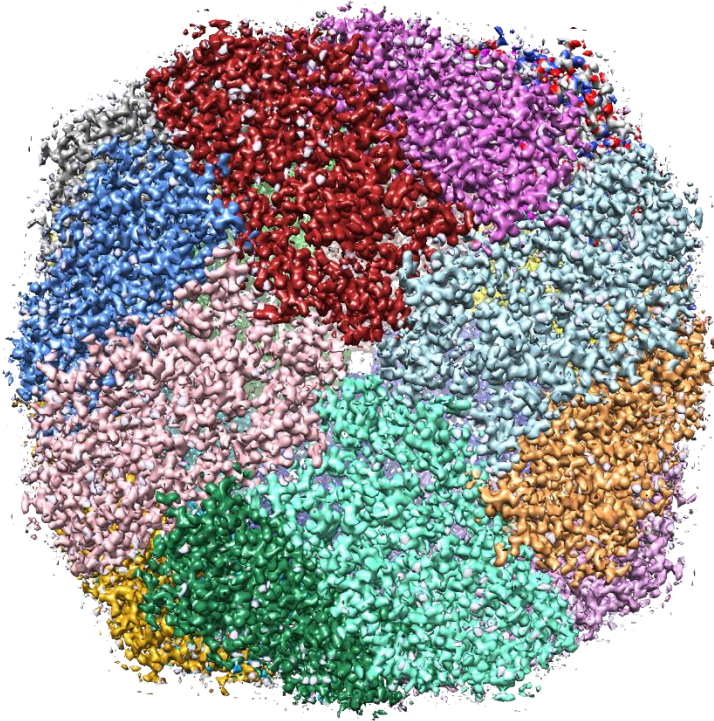


Adenovirus protein
(96kDa) @ 3.5 Å



BSA (63kDa) @ 4.4 Å

Apoferritin at 1.63Å



Current World record with latest innovations is 1.2Å



CMO1-TOWARDS HIGHER THROUGHPUT



Photo by Cande



- Software Upgrades
 - EPU 1.0 to 2.9 (AFIS)
 - Serial-EM
- Outcome
 - Higher data throughput (almost 2.5 times)
 - Reduce experimental shifts (3 or 6 shifts experiments)
 - More experiments/year >90
- K2 → K3 camera upgrade (foreseen for mid-2021)



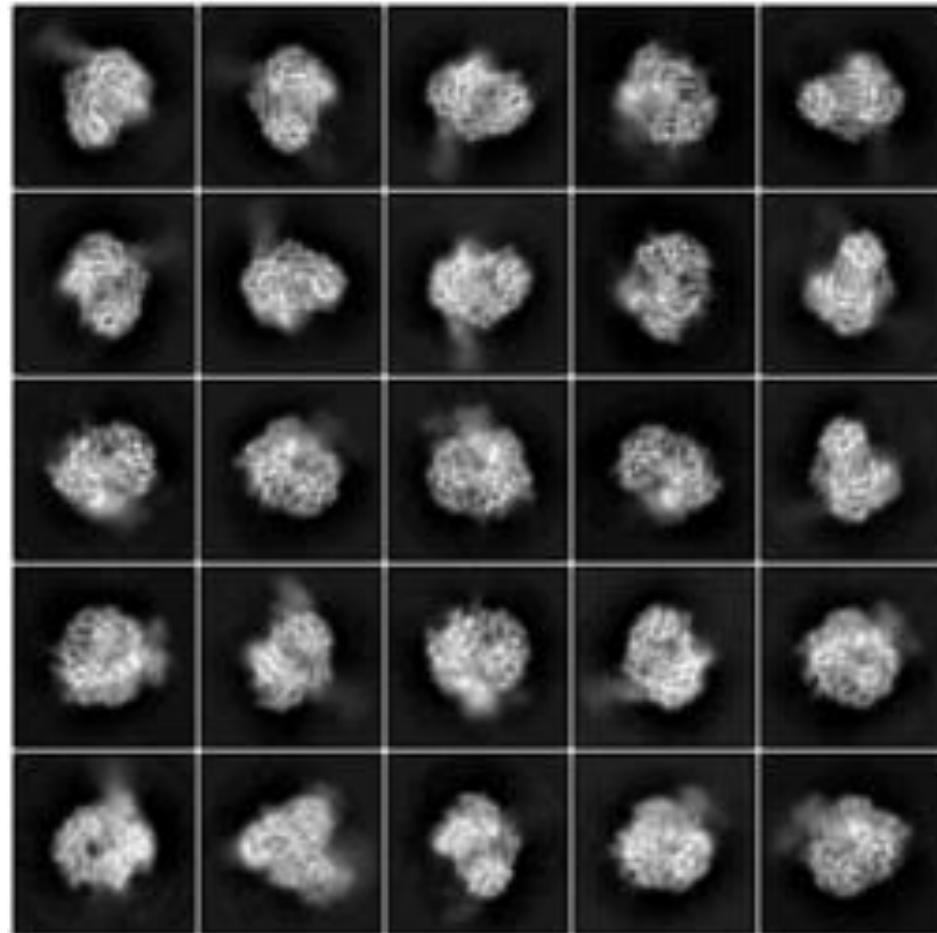
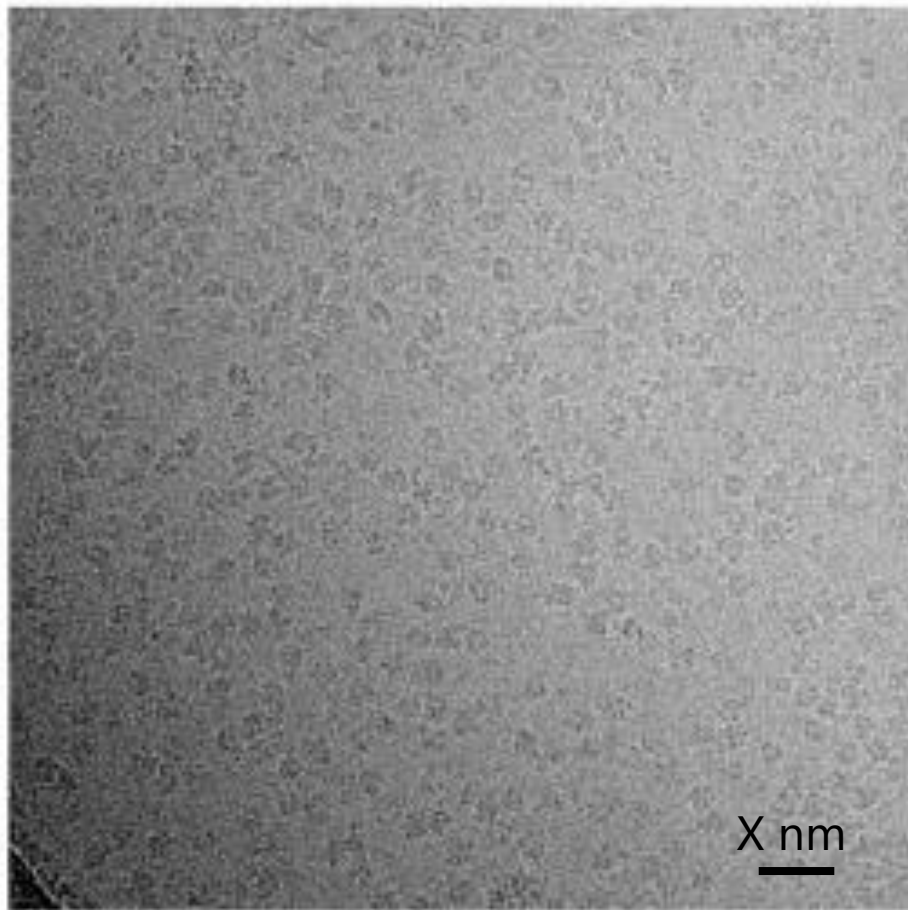
Photo by Cande



CM01 ACCESS MODEL: User support

- **Single particle experiments** only
- **Rolling access proposals** at any time (direct ESRF submission)
- **BAG application since Aug 2020**
- Beam time granted by BTAP
- **Pre-characterised samples only**


BEAMTIME ALLOCATION- MINIMUM REQUIREMENTS



Kouba et al, NSMB, 2019

CMO1 EXPERIMENTS

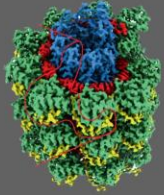
nature communications



Ferritin-Transferrin Receptor complex
Nano medicine

Resolution 3.9 Å
Data size: 8 TB

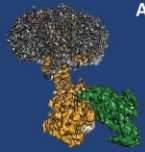
nature chemical biology



Potato Virus X
Nano medicine

Resolution 2.2 Å
Data size: 7 TB


Science



Adenylyl cyclase cAMP signaling
GPCR Receptors

Resolution 3.4 Å
Data size: 9 TB


nature



Serotonin Receptor

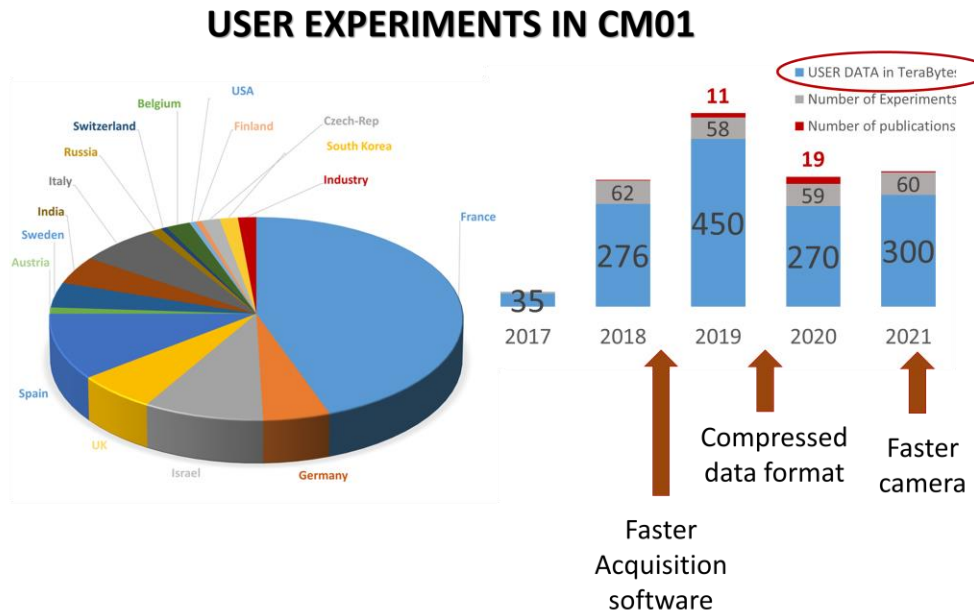
Resolution 3.2 Å
Data size: 6 TB

nature structural & molecular biology



Glutamate receptors
Synapse maintenance
Neuronal disorders

Resolution 7-8 Å
Data size: 13 TB




Science



Expressome Transcription-Translation coupling

Resolution 3.0 Å
Data size: 20 TB

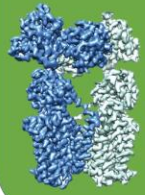
eLife



Hantaan Virus Nucleocapsid

Resolution 3.3 Å
Data size: 6 TB


nature



NNT Transhydrogenase
Crucial Metabolic enzyme

Resolution 2.9 Å
Data size: 8 TB

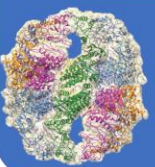
nature structural & molecular biology



Influenza virus RNA Polymerase

Resolution 3.3 Å
Data size: 8.5 TB

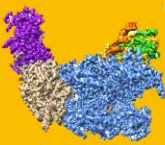
nature communications



CCT Chaperonin
mTOR pathway

Resolution 4 Å
Data size: 15 TB

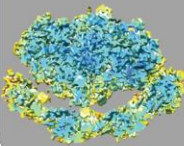
Molecular Cell



Replisome complex
Genome stability

Resolution 3.3 Å
Data size: 6 TB

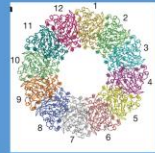
nature plants



Photosystem I

Resolution 2.5 Å
Data size: 17 TB

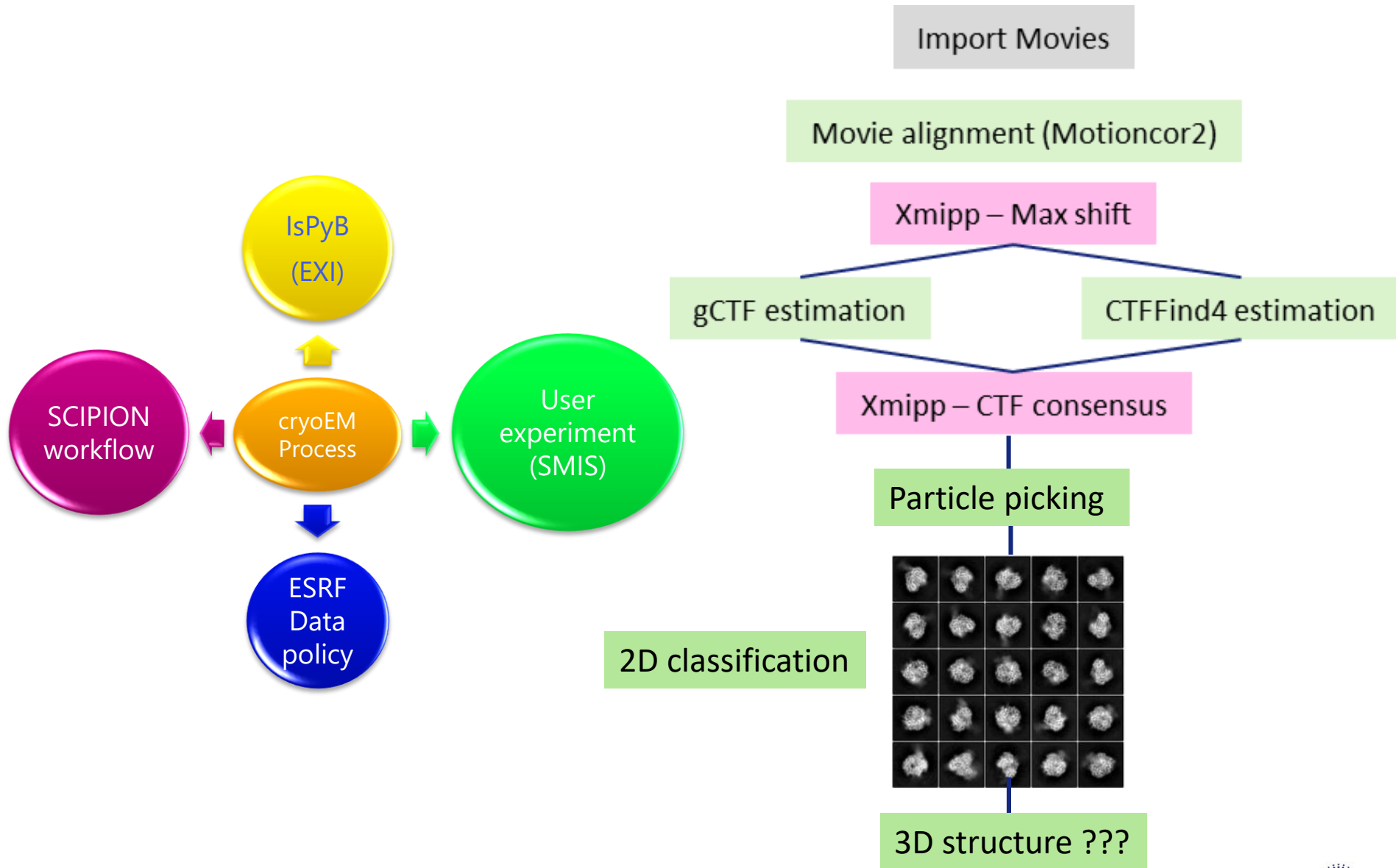
nature



Chikungunya virus NSP1 protein

Resolution 2.6 Å
Data size: 11 TB

CRYOEMPROCESS – REAL TIME DATA PROCESSING

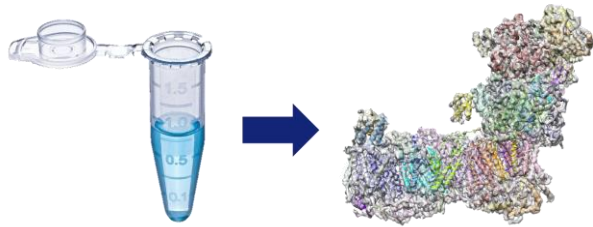


CM01 : EVOLUTION IN USER SERVICE – BAG SYSTEM

- Beam Allocation Group (BAG)
 - 6 BAGs



SOLUTION TO STRUCTURE (SOS) PIPELINE



Receive proposal

BTAP evaluation

Approve proposal

NS-EM service

Vitrification and Screening

Schedule beamtime
In Krios

IBS EM Platform

IBS EM Platform

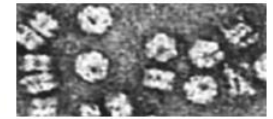
QUALITY OF SCIENCE AND SAMPLE

BIOCHEMISTRY



QUALITY OF SAMPLE FOR EM

NEGATIVE STAIN
ELECTRON
MICROSCOPY



SAMPLE PREPARATION FOR HIGH END CRYO-EM

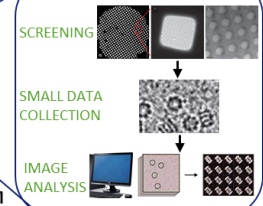
VITROBOT

GLACIOS



VITRIFICATION

LOW-END CRYO-EM



[http://www.esrf.fr/home/UsersAndScience/Experiments/MX/About_our_beamlines/CM01/Solution%20to%20Structure%20Service%20\(SOS\).html](http://www.esrf.fr/home/UsersAndScience/Experiments/MX/About_our_beamlines/CM01/Solution%20to%20Structure%20Service%20(SOS).html)

SOLUTION TO STRUCTURE (SOS) SERVICE

Solution to Structure (SOS) Service

We are offering, mainly for users who have no or very limited access to cryo-electron microscopes an extended service for a **limited** number of experiments. This service can start from a liquid sample of a macromolecular assembly of which sufficient proof (gel filtration profile, MALLS, SAXS, DLS etc.) is given with respect to homogeneity and monodispersity. The service will encompass:

- i. a quality control step using negative staining (NS-EM) and, in the case of acceptable results:
- ii. preparation of sample cryo-grids using a Vitrobot
- iii. screening of those grids



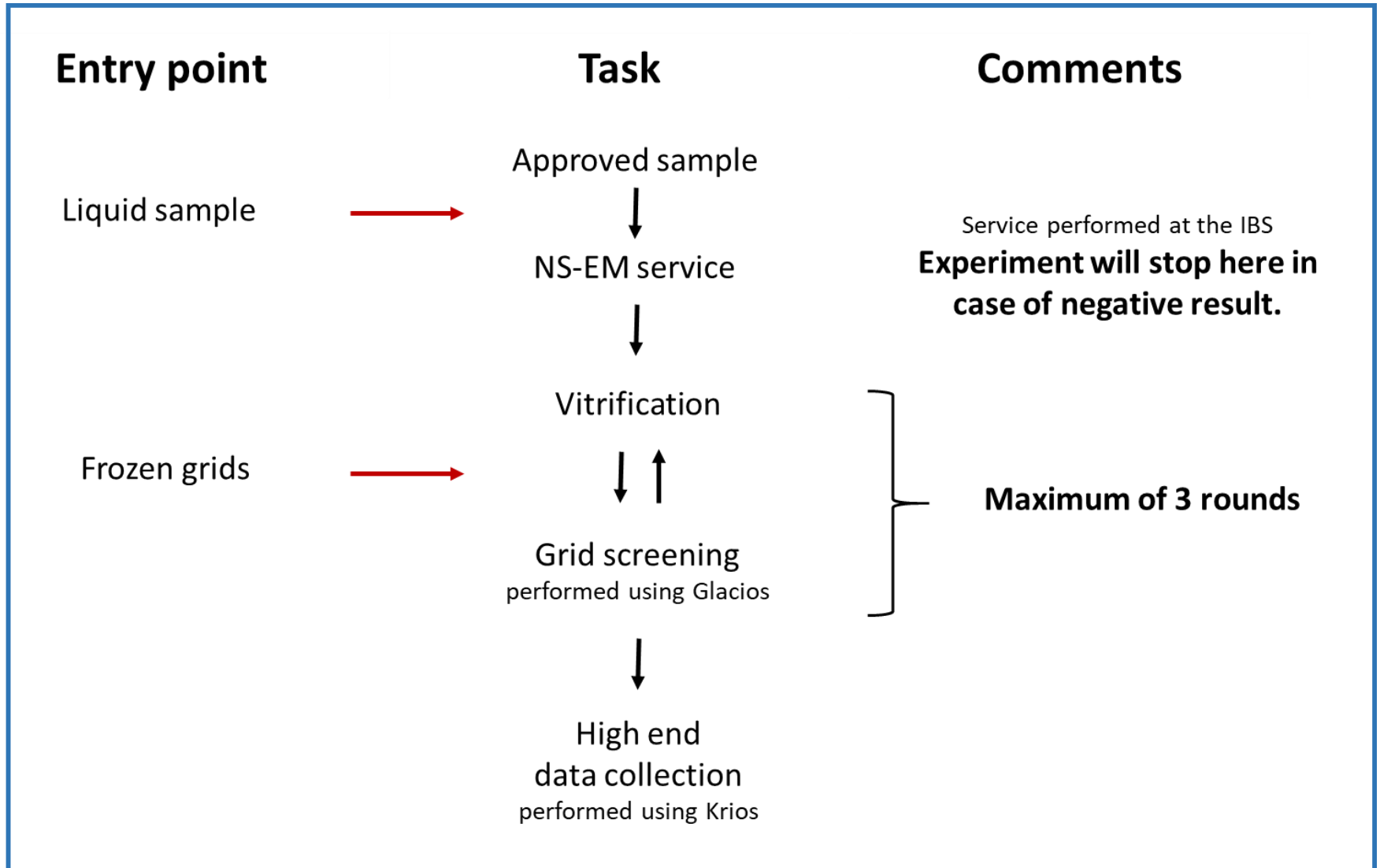
CM01 Cryo-electron microscope

SOLUTION TO STRUCTURE (SOS) SERVICE

Requirements for proposal selection

How to prepare samples for SOS

SOLUTION TO STRUCTURE (SOS) PIPELINE



ACKNOWLEDGEMENTS

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Alejandro de Maria
David von Statten
Marcus Oskarsson

Safety Office
User Office
Communication Office
TISC

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Winfried Weissenhorn

Guy Schoehn
Gregory Effantin

EMBL Grenoble

Stephen Cusack

Michael Hons

ILL

Mark Johnson

Trevor Forsyth
Daouda Traore

Thermo Fisher

Pierre-Yves Milles