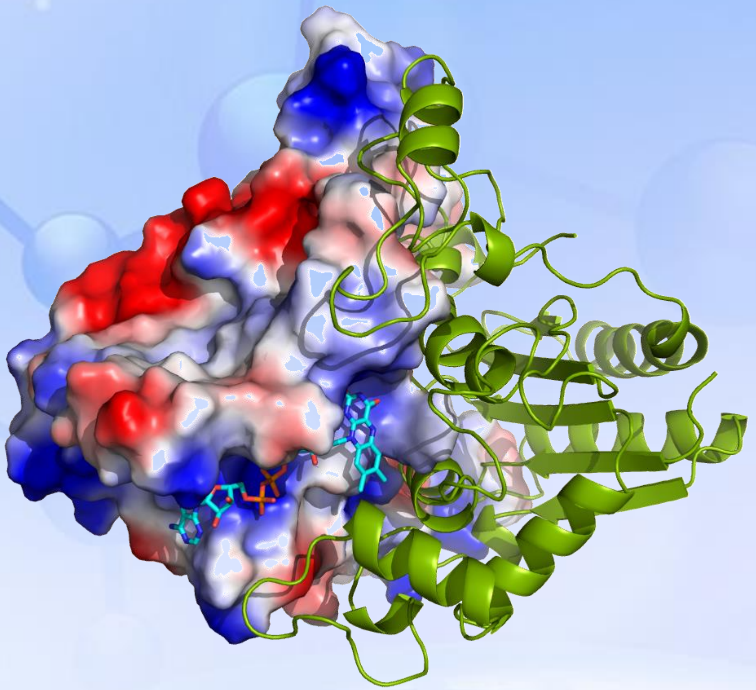
BIORTUS

Your first choice of cryoEM partner



Web： en.biortus.bio

Outline

1. **Overview of Biortus**
2. **CryoEM facilities**
3. **Pipeline of cryoEM service**
4. **Single Particle Analysis**
5. **MicroED**
6. **Characterization of drug delivery systems**
7. **Business model**

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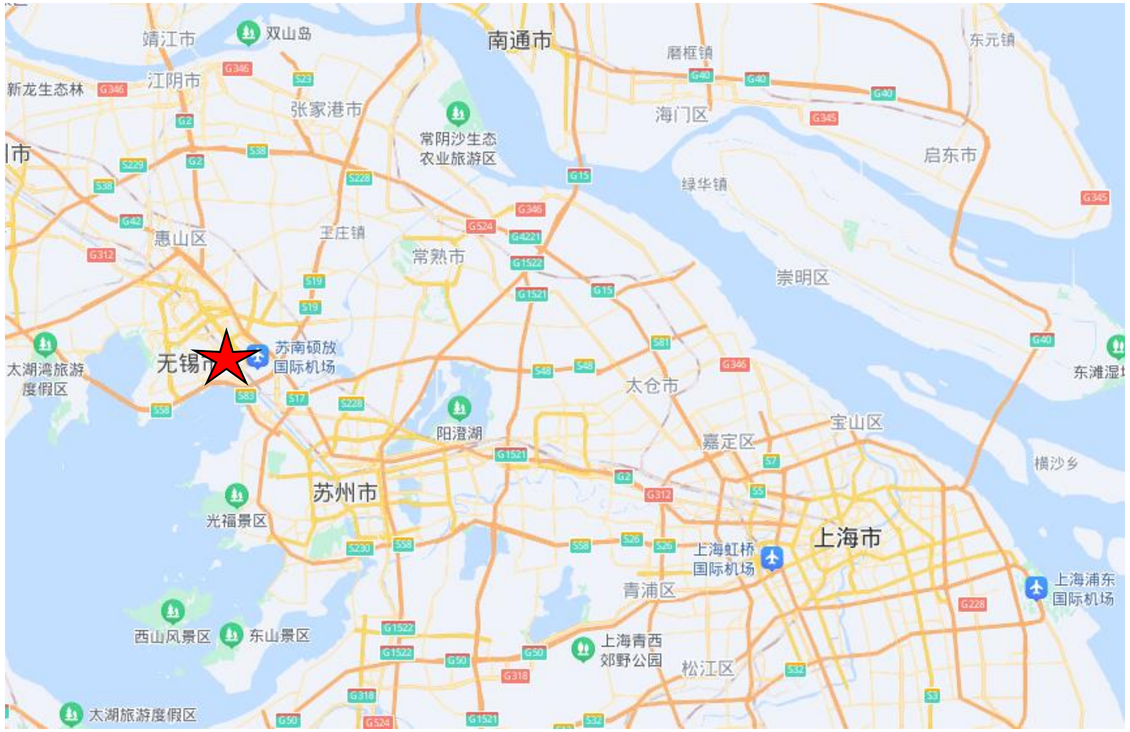
Biortus Biosciences Co., Ltd

- Founded in 2009
- A CRO committed to providing new drug discovery services
- > 80% clients are from USA, Europe, etc.
- Four locations: Jiangyin, Wuxi, Shanghai, Boston
- > 400 employees, more than a half employees are focusing on high-quality protein production
- Over 100,000 ft^2 lab space



2.5 hours drive northwest of Shanghai to Wuxi site

Biortus Biosciences Co., Ltd

Protein Expression

Protein Purification

Assay & Screening

Structure Determination

You give us the gene sequence

We deliver protein structures

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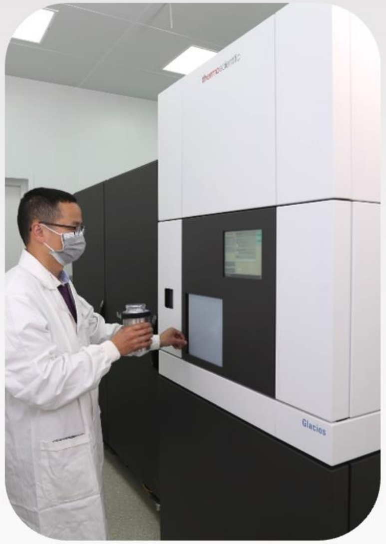
World-leading commercial cryoEM platform

State-of-the-art microscopes

3 Titan Krios G4

- One equipped with GIF energy filter & K3 detector
- Two equipped with Falcon 4i camera & Selectris energy filter
- Primary instrument for structure determination

2 Glacios

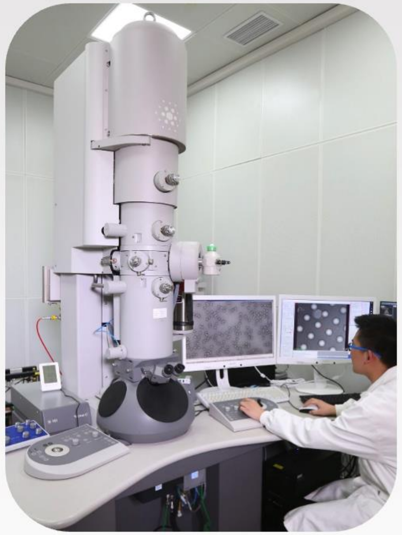


- Equipped with Falcon 4 camera
- Primarily used for Grid Screening

2 Talos

- F200C with Ceta-D camera for MicroED
- L120C for negative stain

TF20



- Negative Stain

Up-to-date computing resources

Raw Data Generation

Krios1_K3

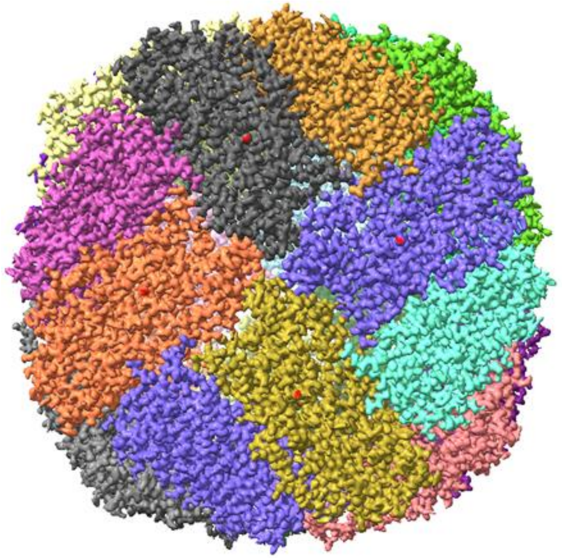
Krios2_Falcon4i

Krios3_Falcon4i

CCP-EM

cryoSPARC

RELION



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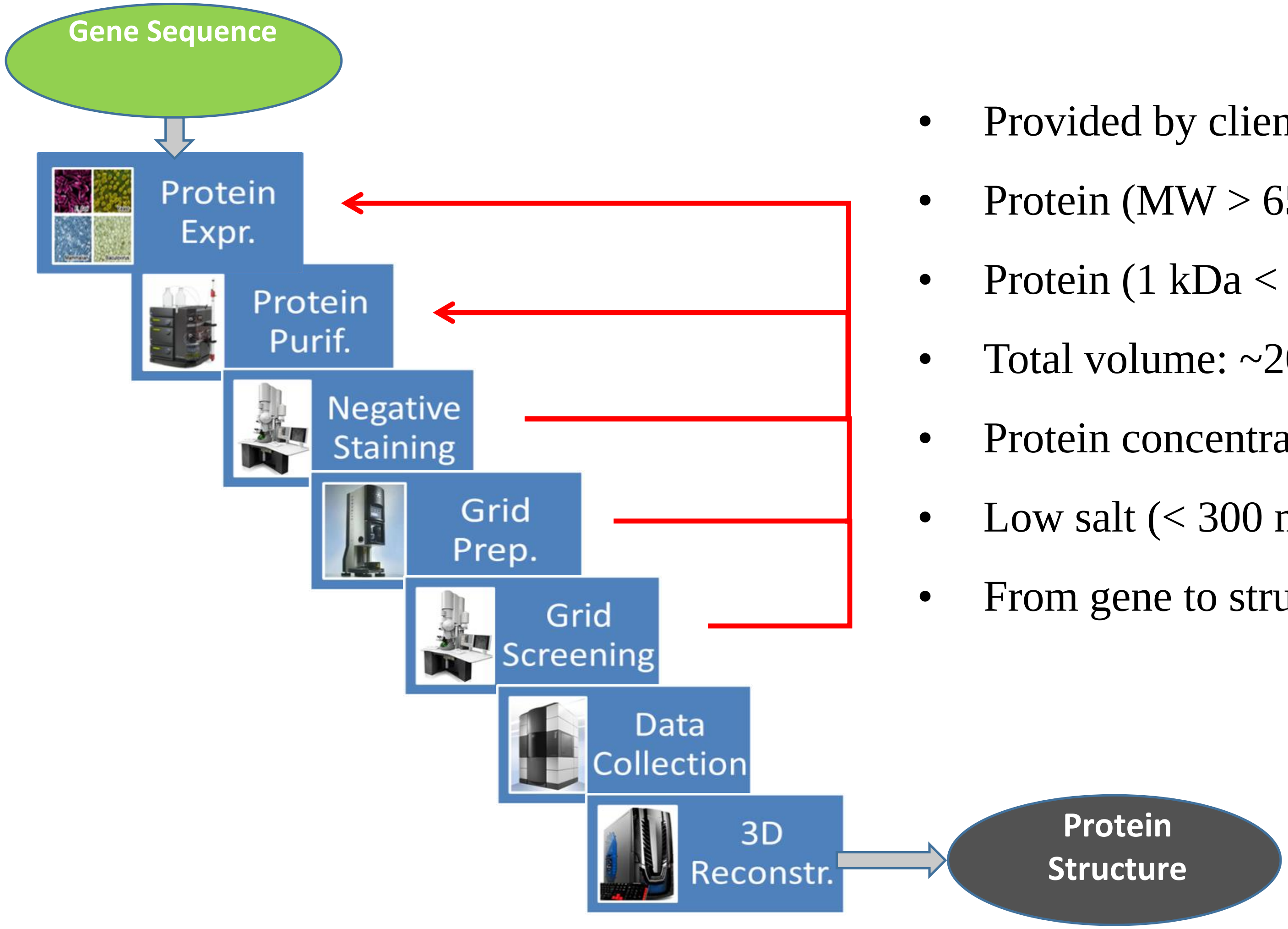
One-stop cryoEM service



- Provided by clients or produced at **Biortus** (preferred)
- Protein (MW > 65 kDa), the larger, the better
- Protein (1 kDa < MW < 65 kDa), antibody or binding partner
- Total volume: ~20 ul (in theory)
- Protein concentration 0.1-2 mg/ml (higher conc. for membrane proteins)
- Low salt (< 300 mM salt), low cryoprotectant concentration
- From gene to structure -- usually 2 ~ 4 months

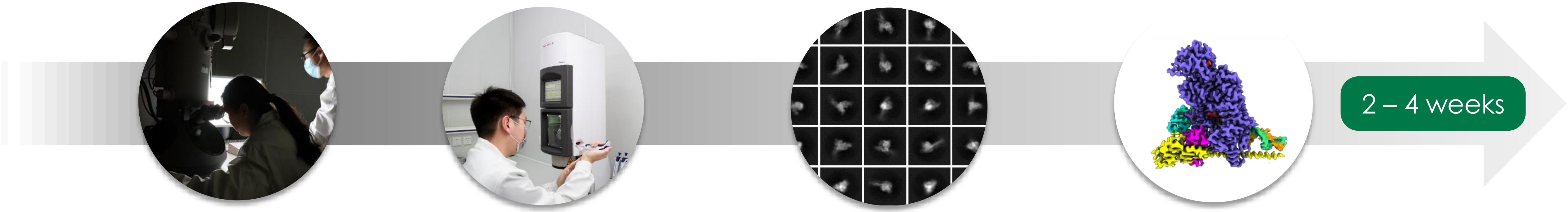
Fast turn-around for cryoEM

Sample Screening

- Talos L120C & TF20
- Particle monodispersity & homogeneity
- Protein can be in-house or provided

2D Classification for 3D structures

- Titan Krios G4
- Orientation preference
- Conformational vs Compositional Heterogeneity



2 – 4 weeks

Negative Stain

Vitrification

Data Collection & Processing

Reconstruction

Grid & Condition Screening

- Vitrobot & Glacios
- Vitrification Optimization (Air-Water Interface)
- Grid Screening

Global Resolution Milestones

- Initial: 3.5 Å or literature resolution
- 3.0 Å
- Atomic
- Ligand binding region may be higher

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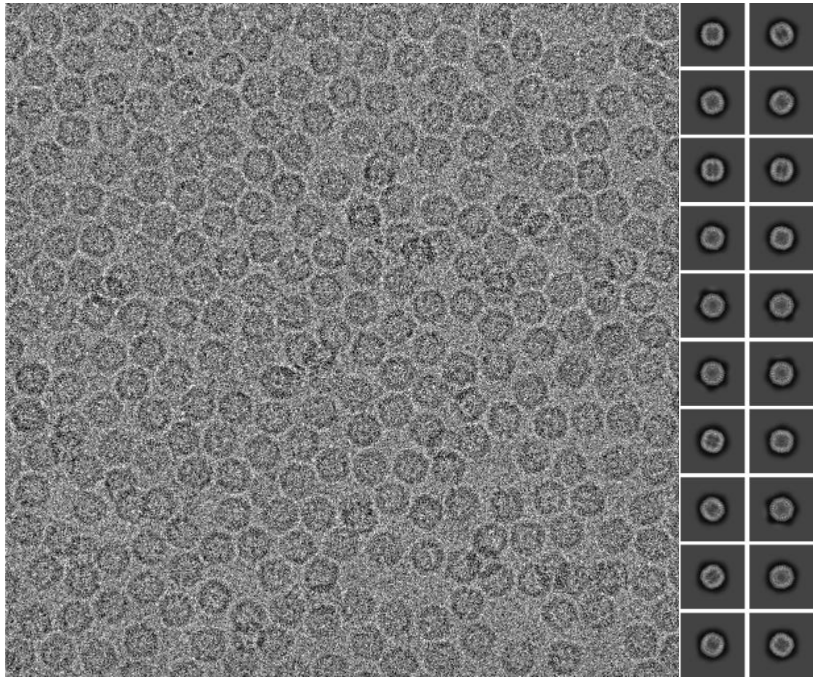
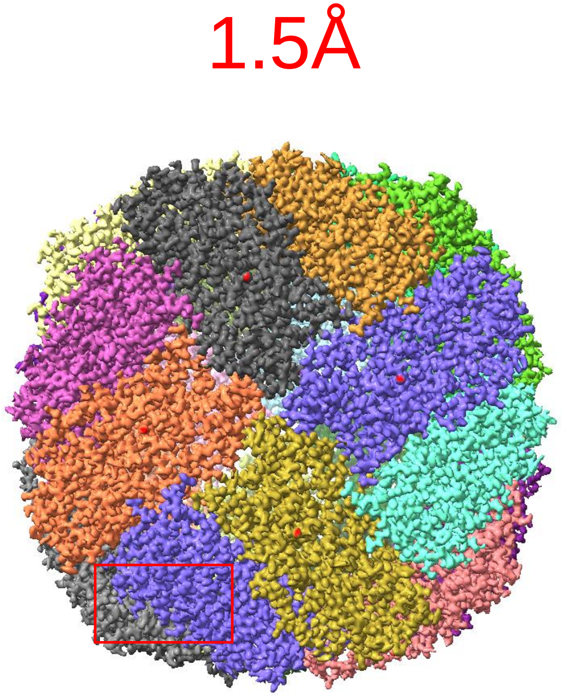
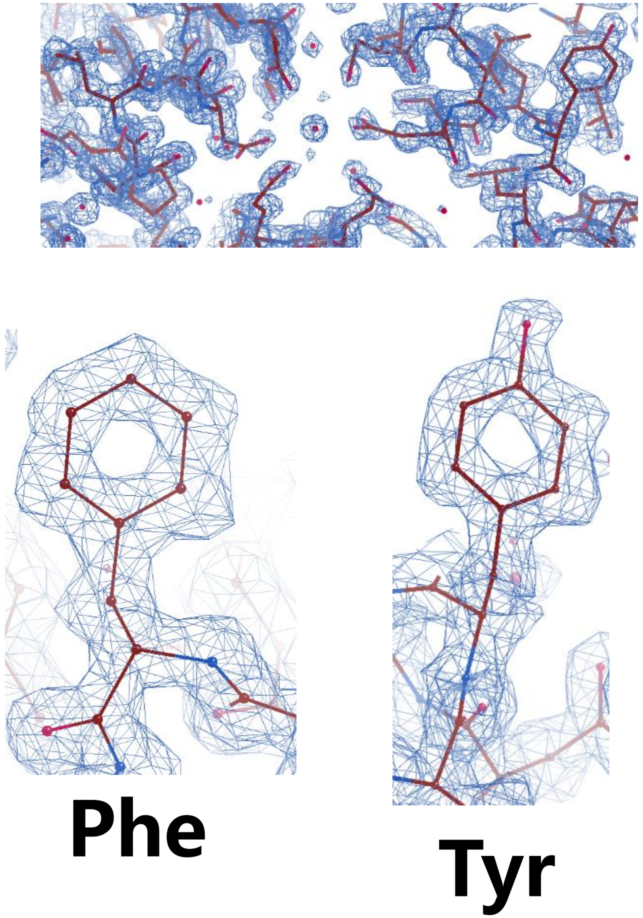
Superior data quality of Biortus cryoEM

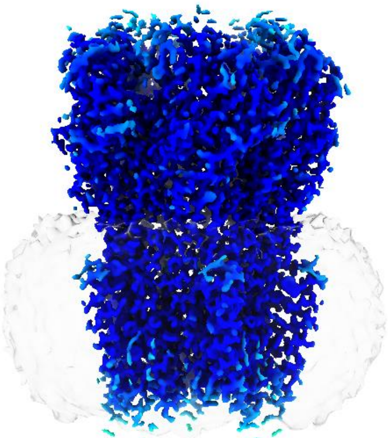




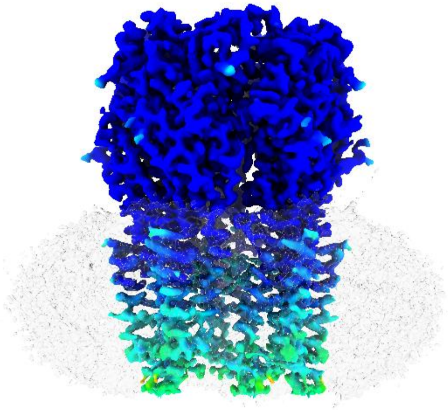


- **Biortus is the provider of high-quality Apoferritin (standard protein sample for cyroEM) for Thermo Fisher Scientific**

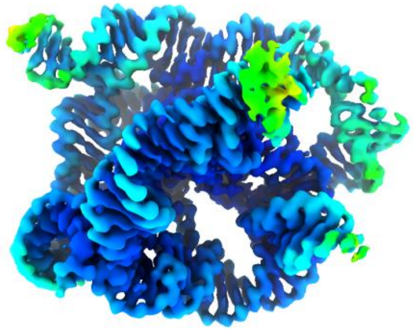
Resolution revolution @ Birotus (千靶万苗)



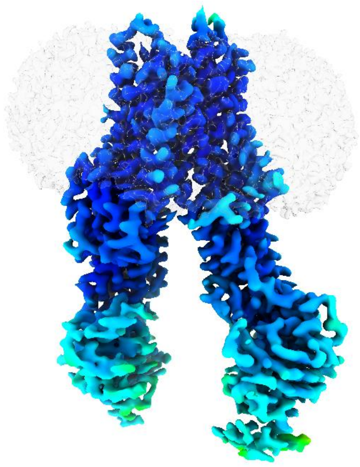
GLRA3
230 kDa
1.82 Å



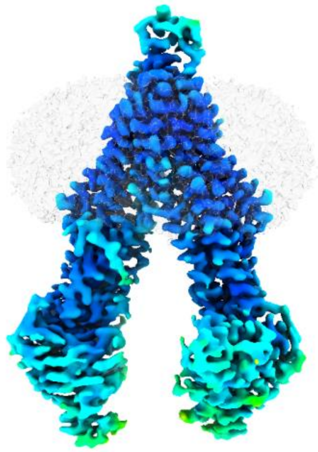
GABAAR β3
200 kDa
2.14 Å



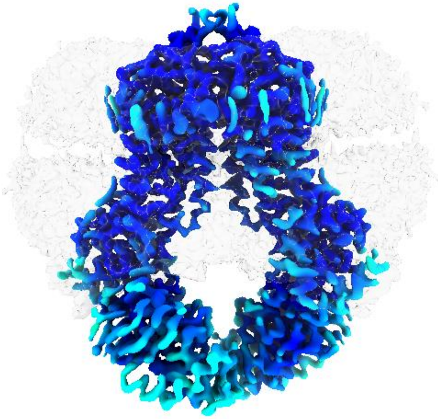
Tetrahymena ribozyme
125 kDa
2.17 Å



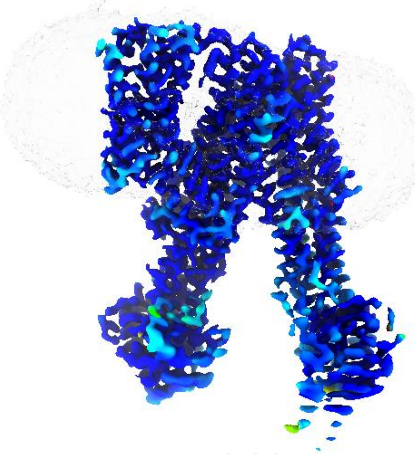
ABCC4
150 kDa
2.20 Å



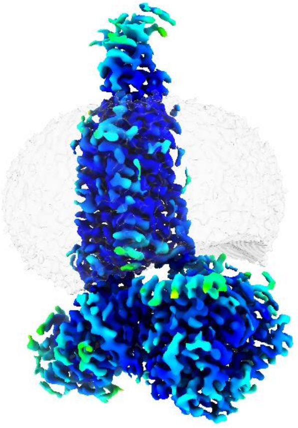
ABCB11
150 kDa
2.33 Å



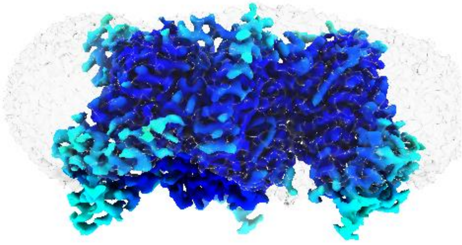
ABCG2
145 kDa
2.43 Å



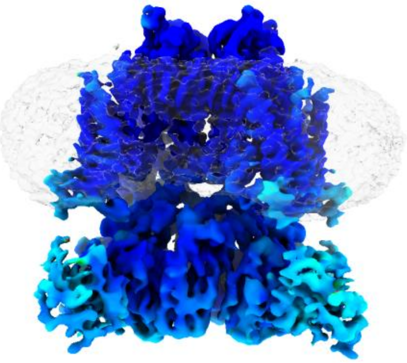
ABCC2
145 kDa
2.43 Å



GLP-1R + agonist
130 kDa
2.53 Å

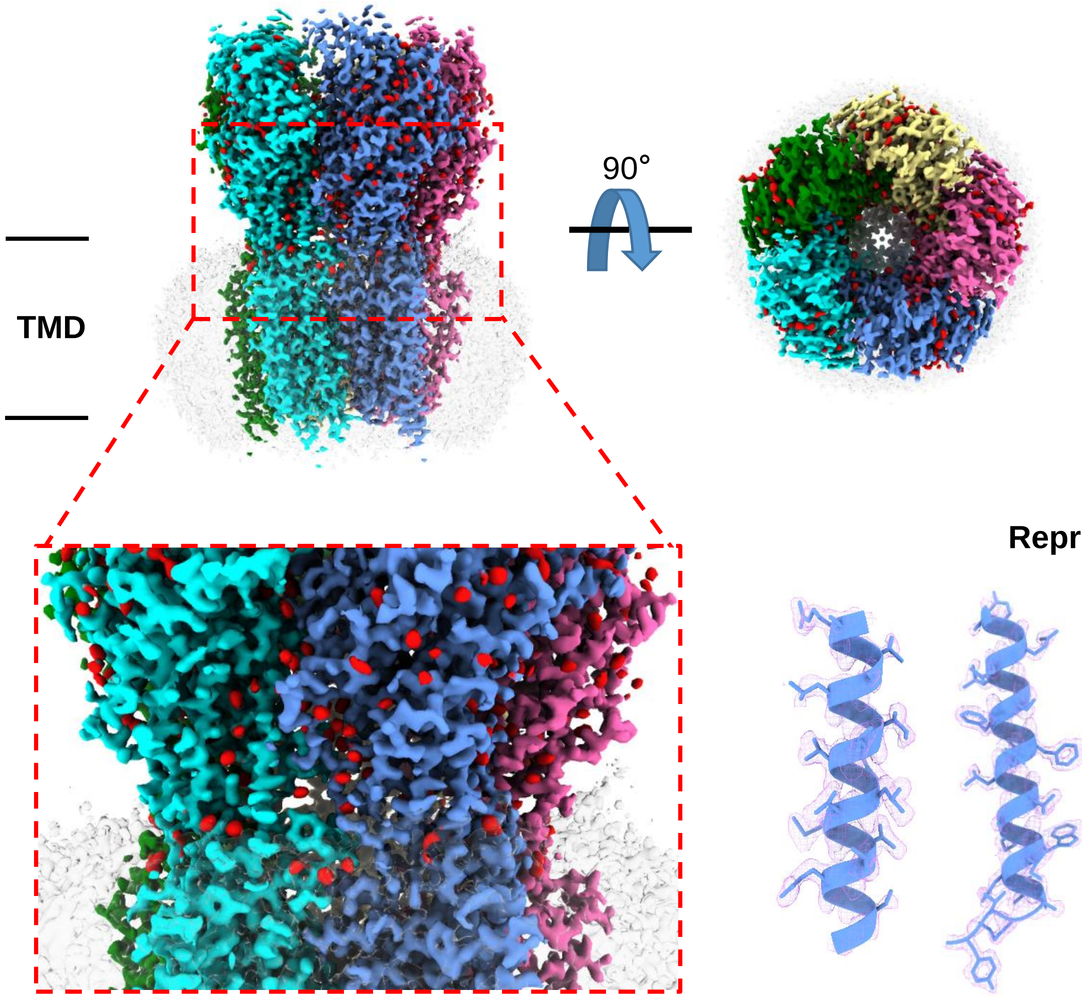


SLC1A5
170 kDa
2.53 Å

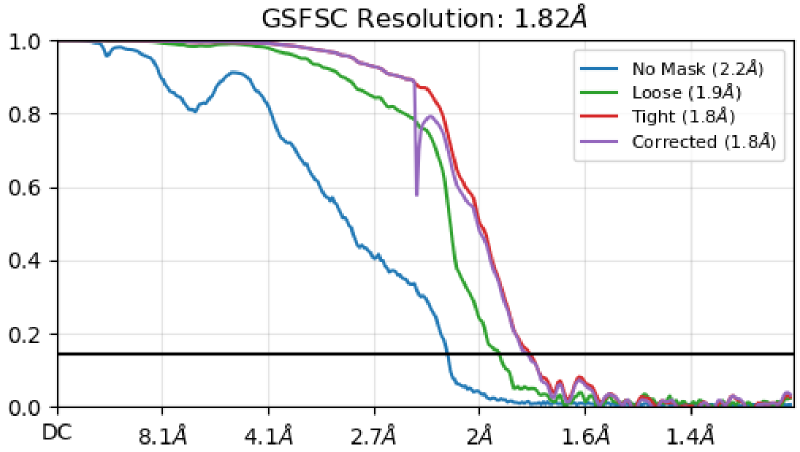


hERG
360 kDa
2.56 Å

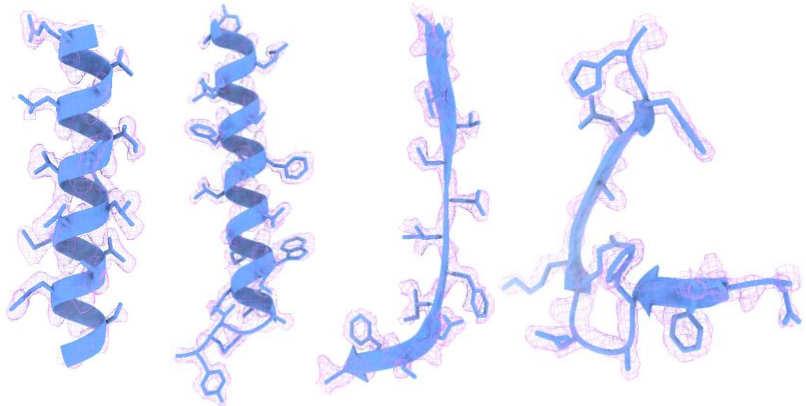
First-ever-seen high-res structure – GLRA3



Water molecules



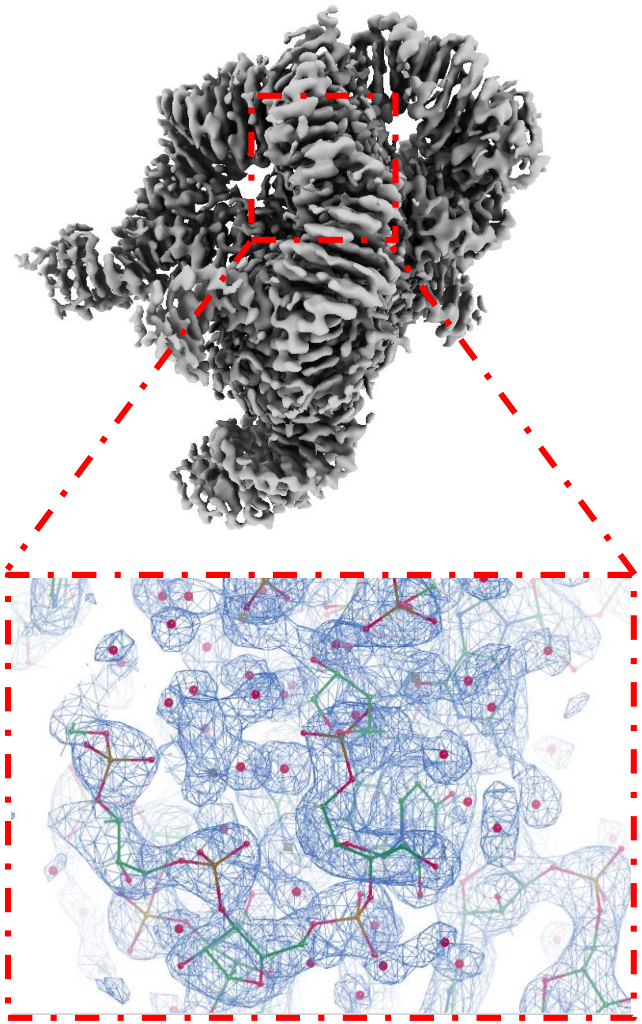
Representative densities

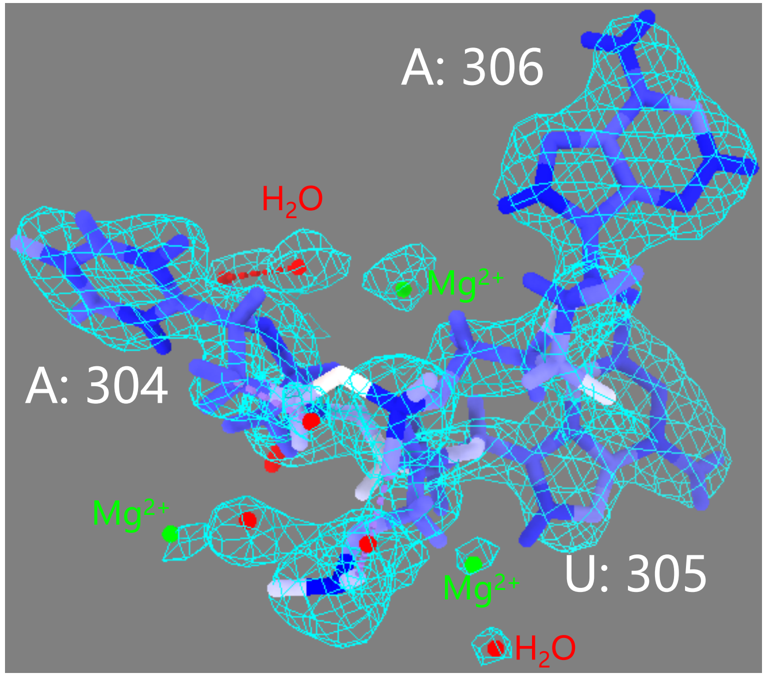


A284-G302 Y312-A335 V70-G82 D130-H142

- **First cryoEM structure of GLRA3**
- **Most amino acid side chains are clear**
- **~500 water molecules are resolved**
- **EMD-61518**

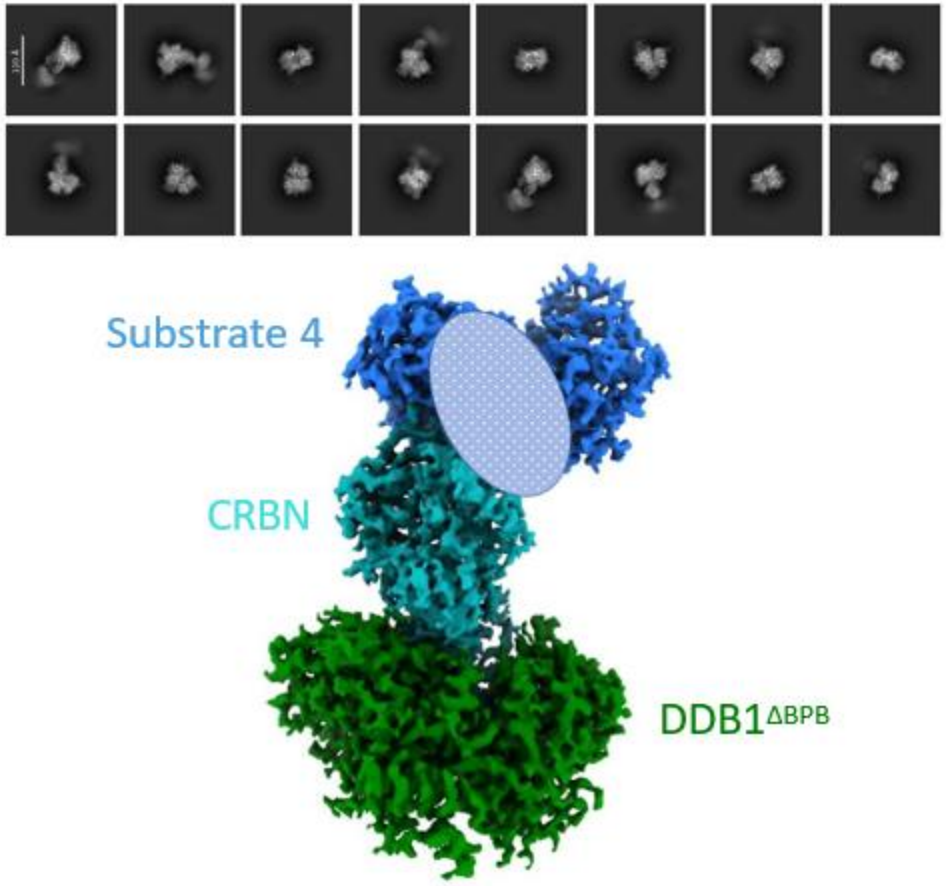
First-ever-seen high-res structure – ribozyme

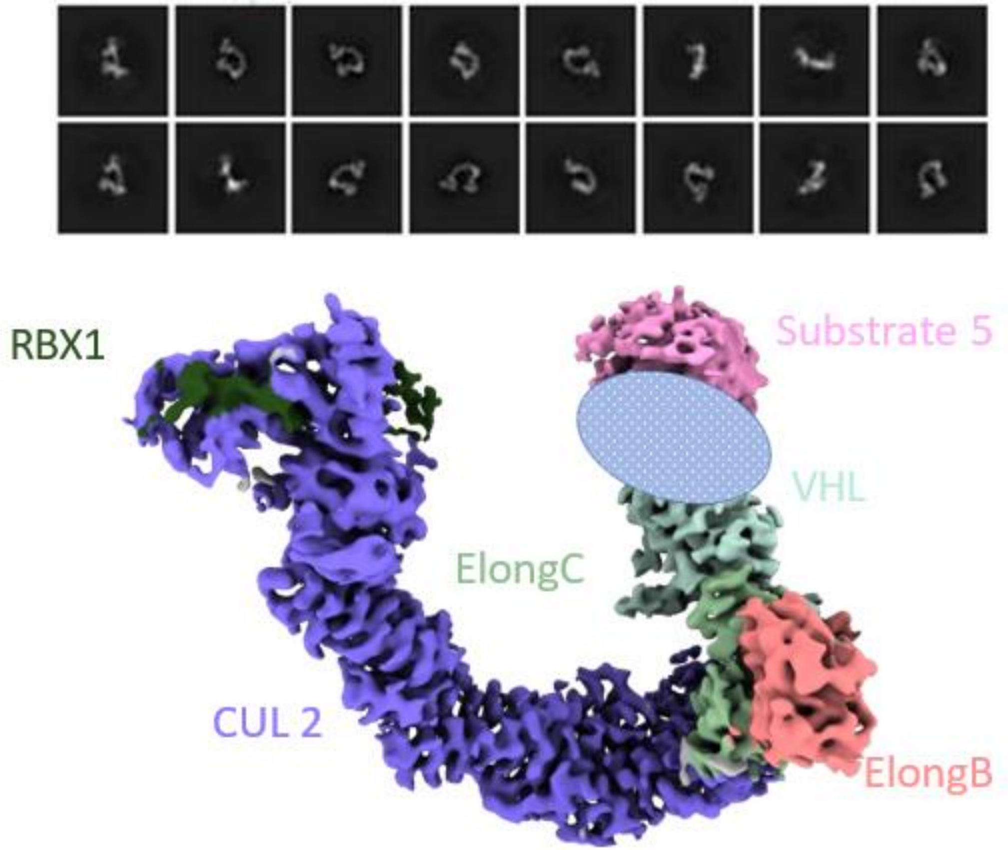




- **Highest resolution structure of Tetrahymena Ribozyme**
- **Nature's map: ~ 300 H2O, ~ 40 Mg^{2+}, 2.2-2.3 Å**
- **Biortus's map: ~ 500 H2O, ~ 50 Mg^{2+}, 2.17 Å**

Holy grail challenge – PROTAC (TPD)

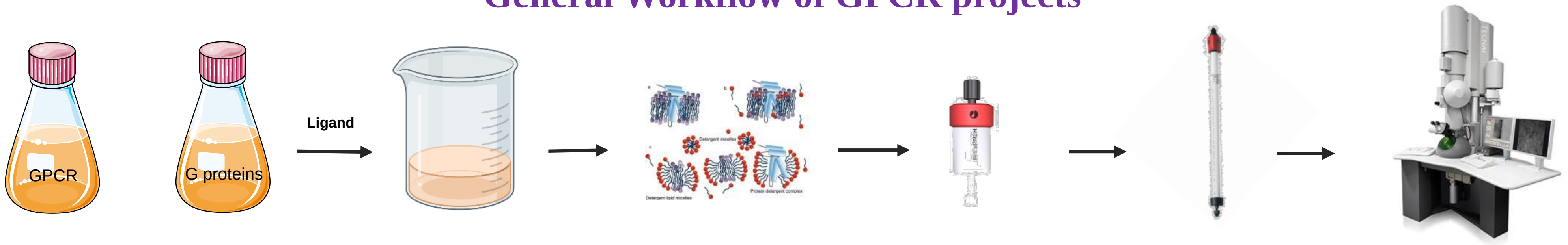




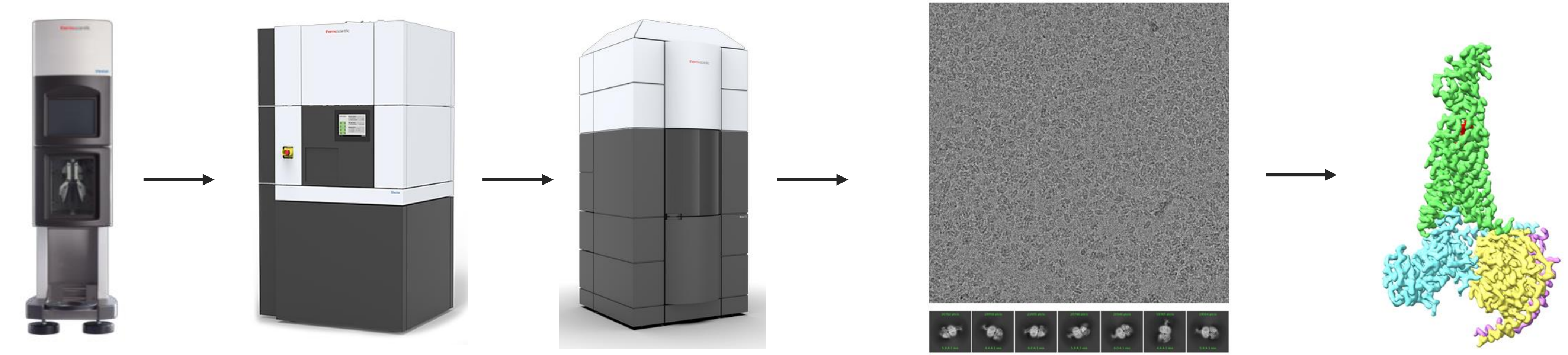
- **Biortus has a comprehensive set of tools/capabilities/reagents in the TPD field.**

Holy grail challenge – GPCR

General Workflow of GPCR projects



Expression → **Complex formation** → **Solubilization** → **Purification** → **SEC** → **Negative stain**



Freezing → **Screening** → **Data collection** → **Image processing & Reconstruction**

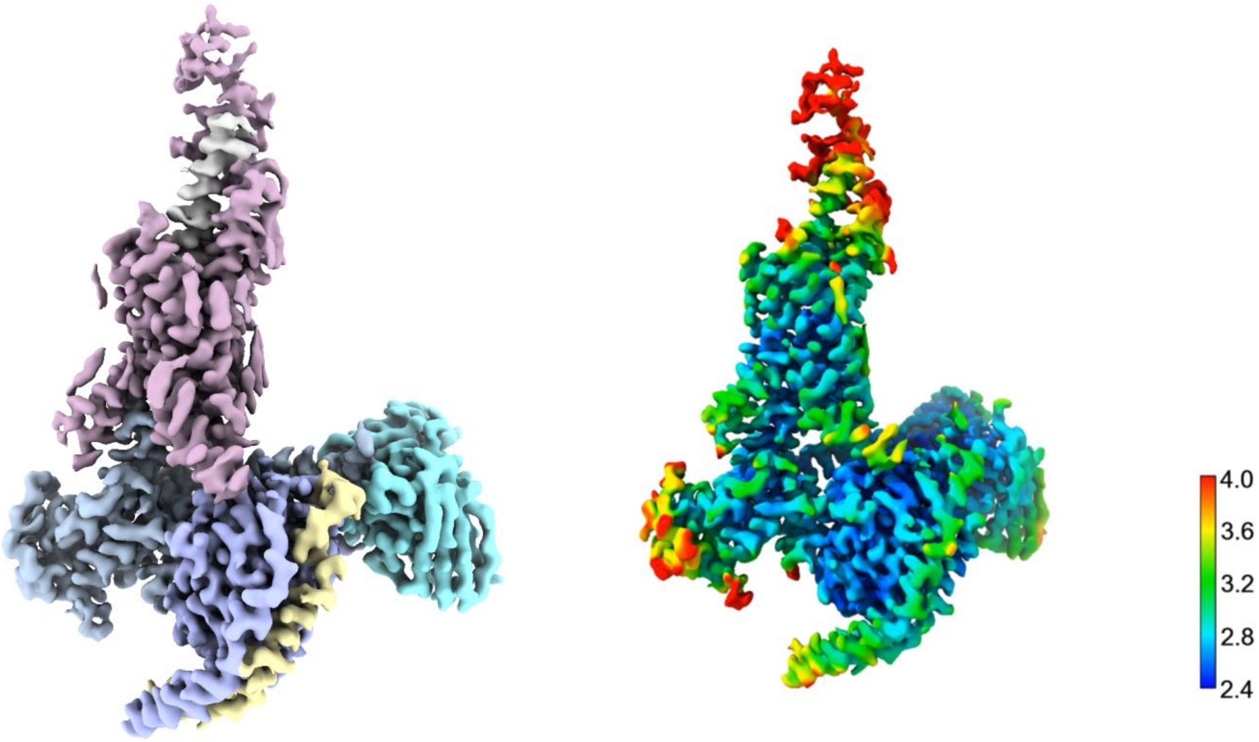
Screening: Glacios: Falcon 4

Data collection: Titan Krios G4: GIF/K3/Phase plate

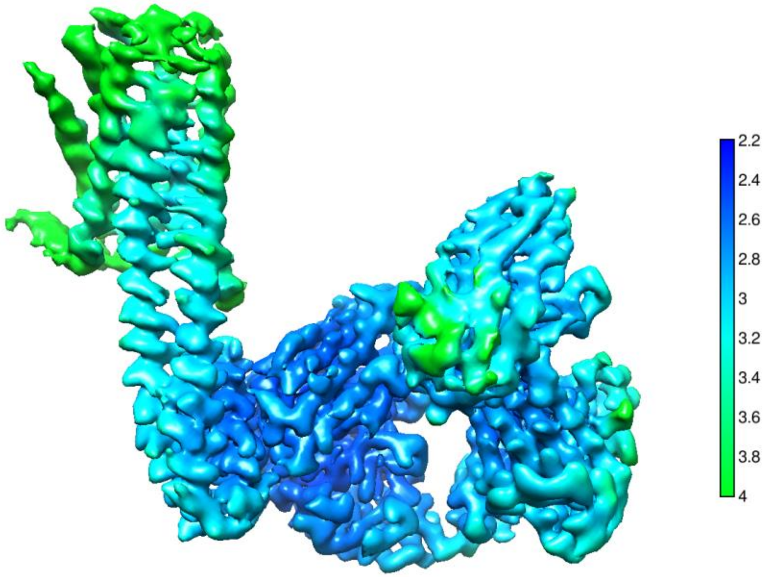
From protein to structure: 1~4 weeks

Holy grail challenge – GPCR

- **Active conformation – agonist bound**



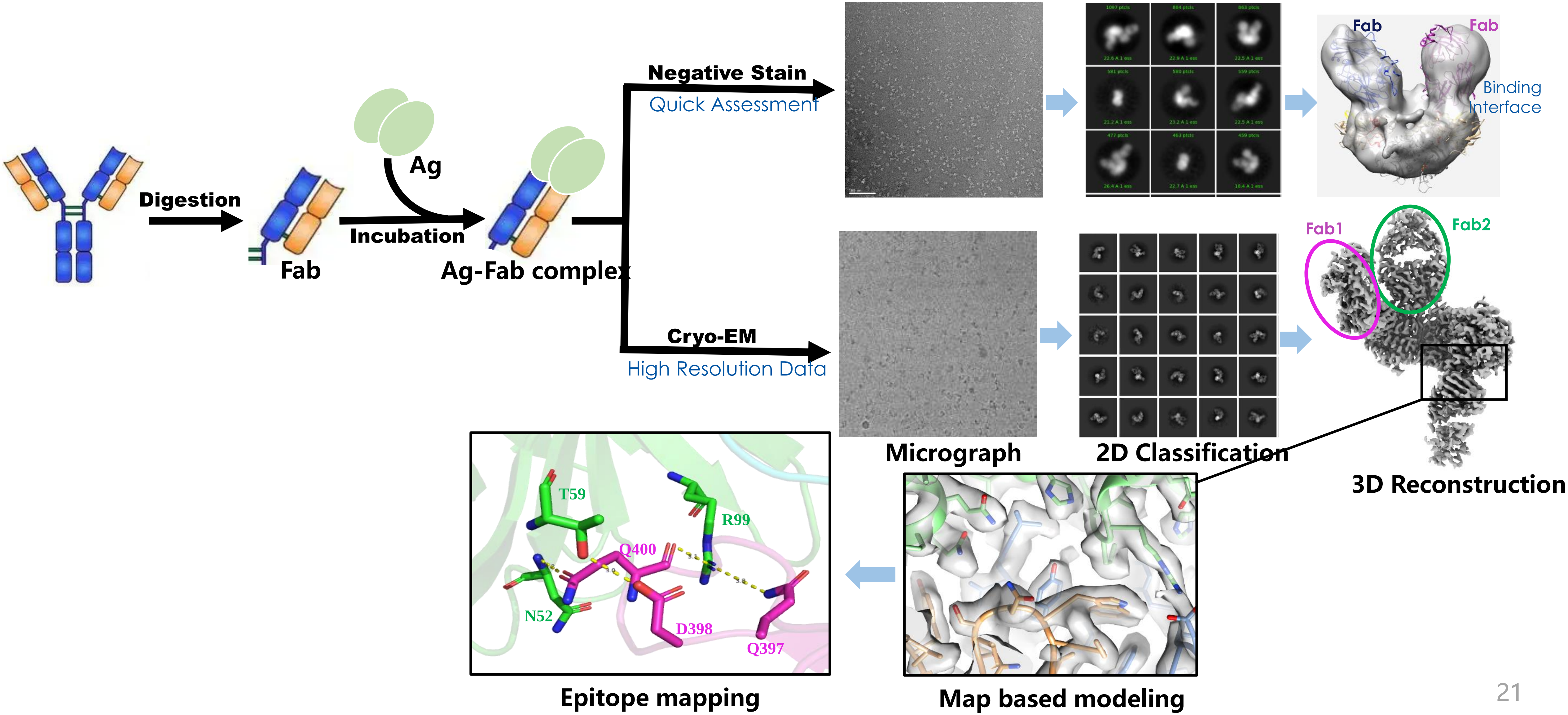
- **Inactive conformation – antagonist bound**



- **Biortus has a wide range collection of GPCR proteins.**

Epitope Mapping Service at Biortus

General Workflow of Ag/Ab structure determination by TEM



Digestion

Fab

Ag

Incubation

Ag-Fab complex

Negative Stain

Quick Assessment

Cryo-EM

High Resolution Data

Fab

Fab

Binding Interface

Fab1

Fab2

Micrograph

2D Classification

3D Reconstruction

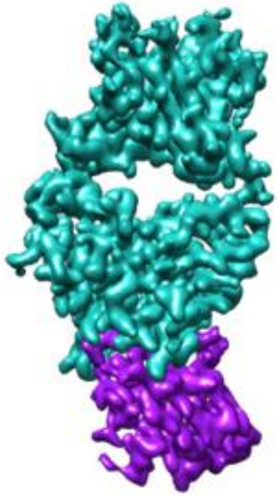
Epitope mapping

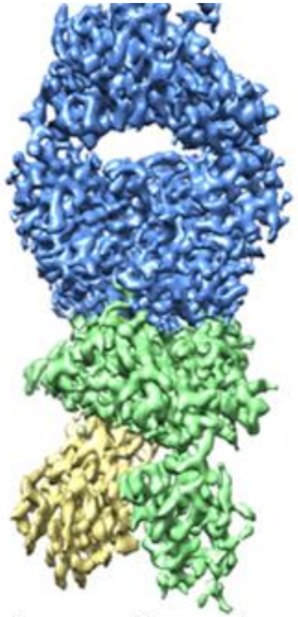
Map based modeling

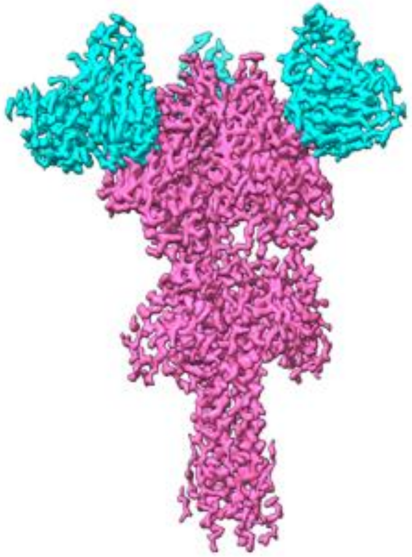
Epitope Mapping Service at Biortus

Examples of Epitope Mapping

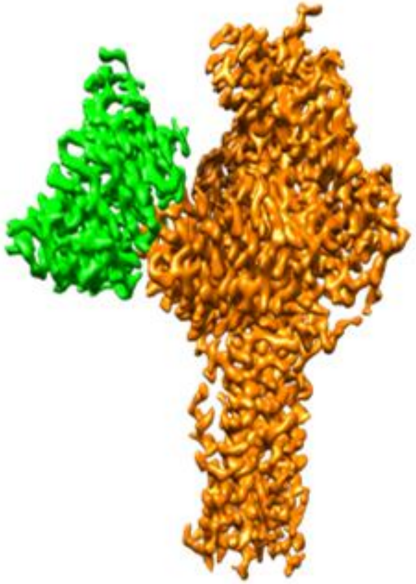

Antigen with two Fabs


14 kD Antigen-Fab


Antigen ECD-fab complex

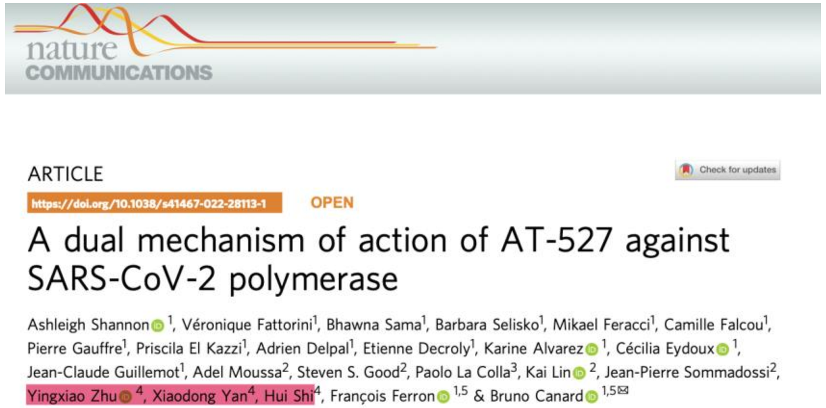

Trimeric Antigen-fab complex


20 kDa Antigen-fab-VHH complex


Membrane Antigen-fab complex

Publications/acknowledged

BIORTUS



nature COMMUNICATIONS

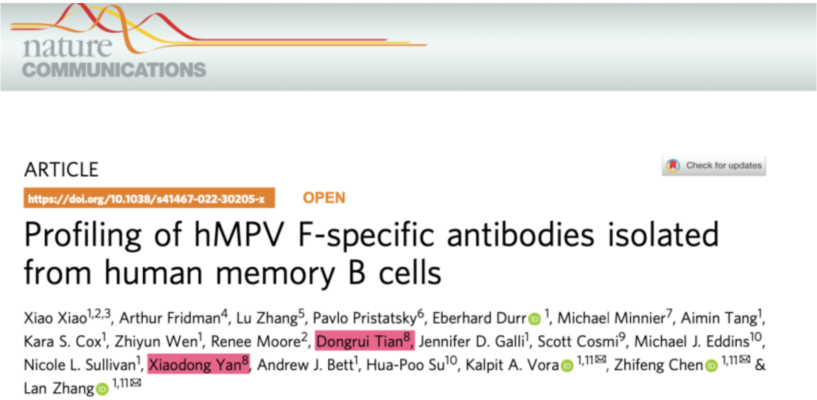
ARTICLE

https://doi.org/10.1038/s41467-022-28113-1 OPEN

Check for updates

A dual mechanism of action of AT-527 against SARS-CoV-2 polymerase

Ashleigh Shannon[1], Véronique Fattorini[1], Bhawna Sama[1], Barbara Selisko[1], Mikael Feracci[1], Camille Falcou[1], Pierre Gauffre[1], Priscila El Kazzi[1], Adrien Delpal[1], Etienne Decroly[1], Karine Alvarez[1], Cécilia Eydoux[1], Jean-Claude Guillemot[1], Adel Moussa[2], Steven S. Good[2], Paolo La Colla[3], Kai Lin[2], Jean-Pierre Sommadossi[2], Yingxiao Zhu[4], Xiaodong Yan[4], Hui Shi[4], François Ferron[1,5] & Bruno Canard[1,5]✉



nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-30205-x OPEN

Check for updates

Profiling of hMPV F-specific antibodies isolated from human memory B cells

Xiao Xiao[1,2,3], Arthur Fridman[4], Lu Zhang[5], Pavlo Pristatsky[6], Eberhard Durr[1], Michael Minnier[7], Aimin Tang[1], Kara S. Cox[1], Zhiyun Wen[1], Renee Moore[2], Dongrui Tian[8], Jennifer D. Galli[1], Scott Cosmi[9], Michael J. Eddins[10], Nicole L. Sullivan[1], Xiaodong Yan[8], Andrew J. Bett[1], Hua-Poo Su[10], Kalpit A. Vora[1,11]✉, Zhifeng Chen[1,11]✉ & Lan Zhang[1,11]✉

nature communications

Article

https://doi.org/10.1038/s41467-022-34639-1

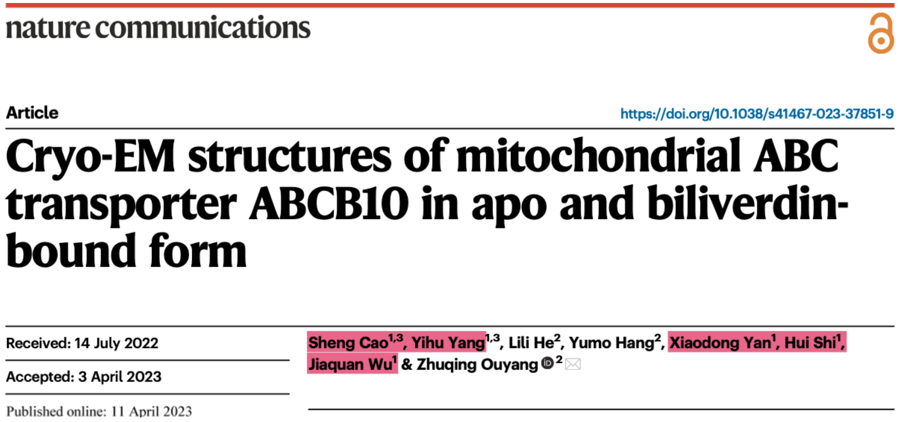
Structural basis of human SNAPc recognizing proximal sequence element of snRNA promoter

Received: 27 May 2022

Accepted: 1 November 2022

Published online: 11 November 2022

Jianfeng Sun[1,2,3,8]✉, Xue Li[1,8], Xuben Hou[4], Sujian Cao[1], Wenjin Cao[1], Ye Zhang[1], Jinyang Song[1], Manfu Wang[5], Hao Wang[1], Xiaodong Yan[5], Zengpeng Li[6], Robert G. Roeder[3]✉ & Wei Wang[1,7]✉



nature communications

Article

https://doi.org/10.1038/s41467-023-37851-9

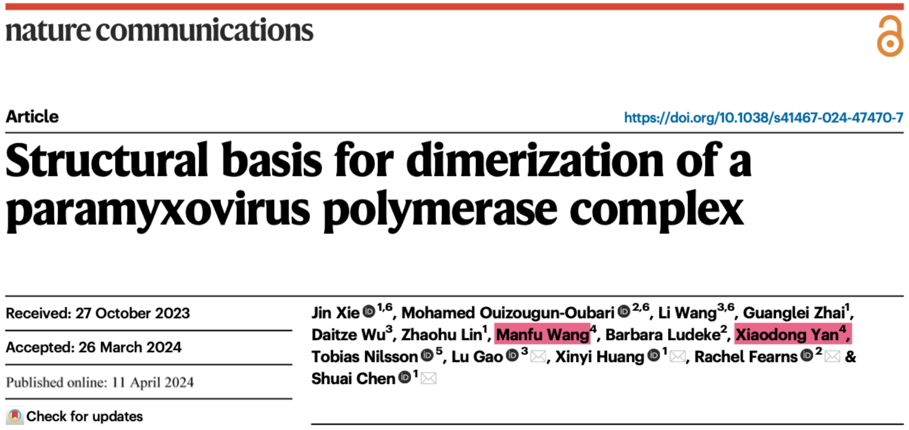
Cryo-EM structures of mitochondrial ABC transporter ABCB10 in apo and biliverdin-bound form

Received: 14 July 2022

Accepted: 3 April 2023

Published online: 11 April 2023

Sheng Cao[1,3], Yihu Yang[1,3], Lili He[2], Yumo Hang[2], Xiaodong Yan[1], Hui Shi[1], Jiaquan Wu[1] & Zhuqing Ouyang[2]✉



nature communications

Article

https://doi.org/10.1038/s41467-024-47470-7

Structural basis for dimerization of a paramyxovirus polymerase complex

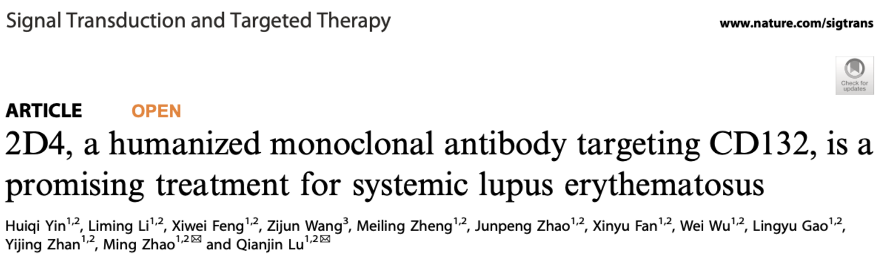
Received: 27 October 2023

Accepted: 26 March 2024

Published online: 11 April 2024

Check for updates

Jin Xie[1,6], Mohamed Ouizougun-Oubari[2,6], Li Wang[3,6], Guanglei Zhai[1], Daitze Wu[3], Zhaohu Lin[1], Manfu Wang[4], Barbara Ludeke[2], Xiaodong Yan[4], Tobias Nilsson[5], Lu Gao[3]✉, Xinyi Huang[1]✉, Rachel Fearns[2]✉ & Shuai Chen[1]✉



Signal Transduction and Targeted Therapy

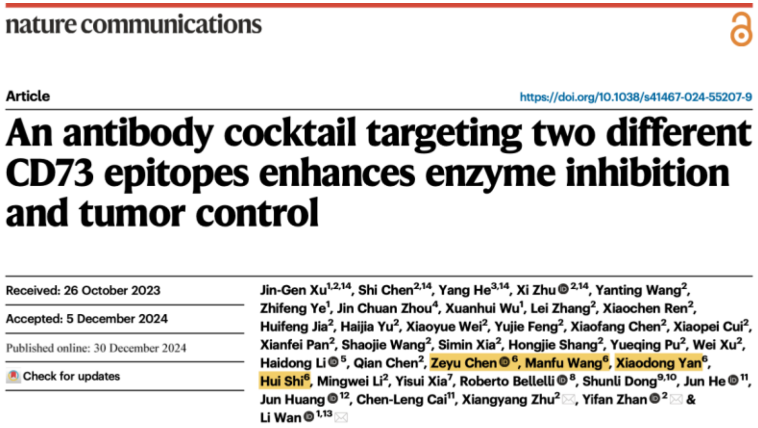
www.nature.com/sigtrans

Check for updates

ARTICLE OPEN

2D4, a humanized monoclonal antibody targeting CD132, is a promising treatment for systemic lupus erythematosus

Huiqi Yin[1,2], Liming Li[1,2], Xiwei Feng[1,2], Zijun Wang[3], Meiling Zheng[1,2], Junpeng Zhao[1,2], Xinyu Fan[1,2], Wei Wu[1,2], Lingyu Gao[1,2], Yijing Zhan[1,2], Ming Zhao[1,2]✉ and Qianjin Lu[1,2]✉



nature communications

Article

https://doi.org/10.1038/s41467-024-55207-9

An antibody cocktail targeting two different CD73 epitopes enhances enzyme inhibition and tumor control

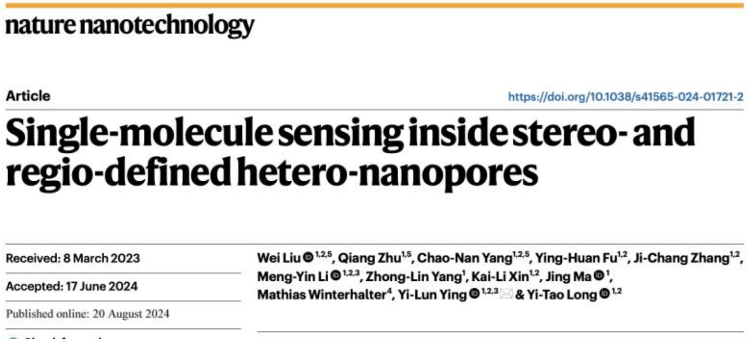
Received: 26 October 2023

Accepted: 5 December 2024

Published online: 30 December 2024

Check for updates

Jin-Gen Xu[1,2,14], Shi Chen[2,14], Yang He[3,14], Xi Zhu[2,14], Yanting Wang[2], Zhifeng Ye[1], Jin Chuan Zhou[4], Xuanhui Wu[1], Lei Zhang[2], Xiaochen Ren[2], Huifeng Jia[2], Haijia Yu[2], Xiaoyue Wei[2], Yujie Feng[2], Xiaofang Chen[2], Xiaopei Cui[2], Xianfei Pan[2], Shaojie Wang[2], Simin Xia[2], Hongjie Shang[2], Yueqing Pu[2], Wei Xu[2], Haidong Li[5], Qian Chen[2], Zeyu Chen[6], Manfu Wang[6], Xiaodong Yan[6], Hui Shi[6], Mingwei Li[2], Yisui Xia[7], Roberto Bellelli[8], Shunli Dong[9,10], Jun He[11], Jun Huang[12], Chen-Leng Cai[11], Xiangyang Zhu[2]✉, Yifan Zhan[2]✉ & Li Wan[1,13]✉



nature nanotechnology

Article

https://doi.org/10.1038/s41565-024-01721-2

Single-molecule sensing inside stereo- and regio-defined hetero-nanopores

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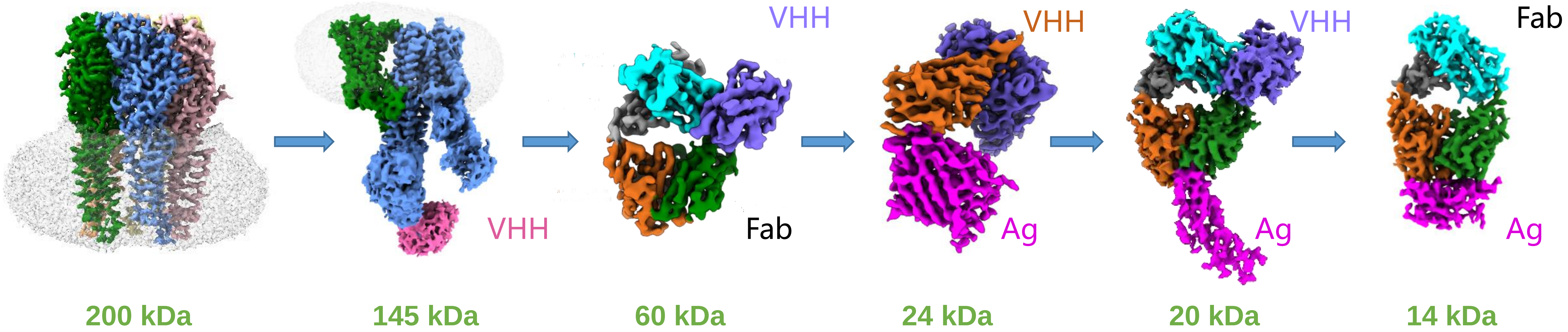
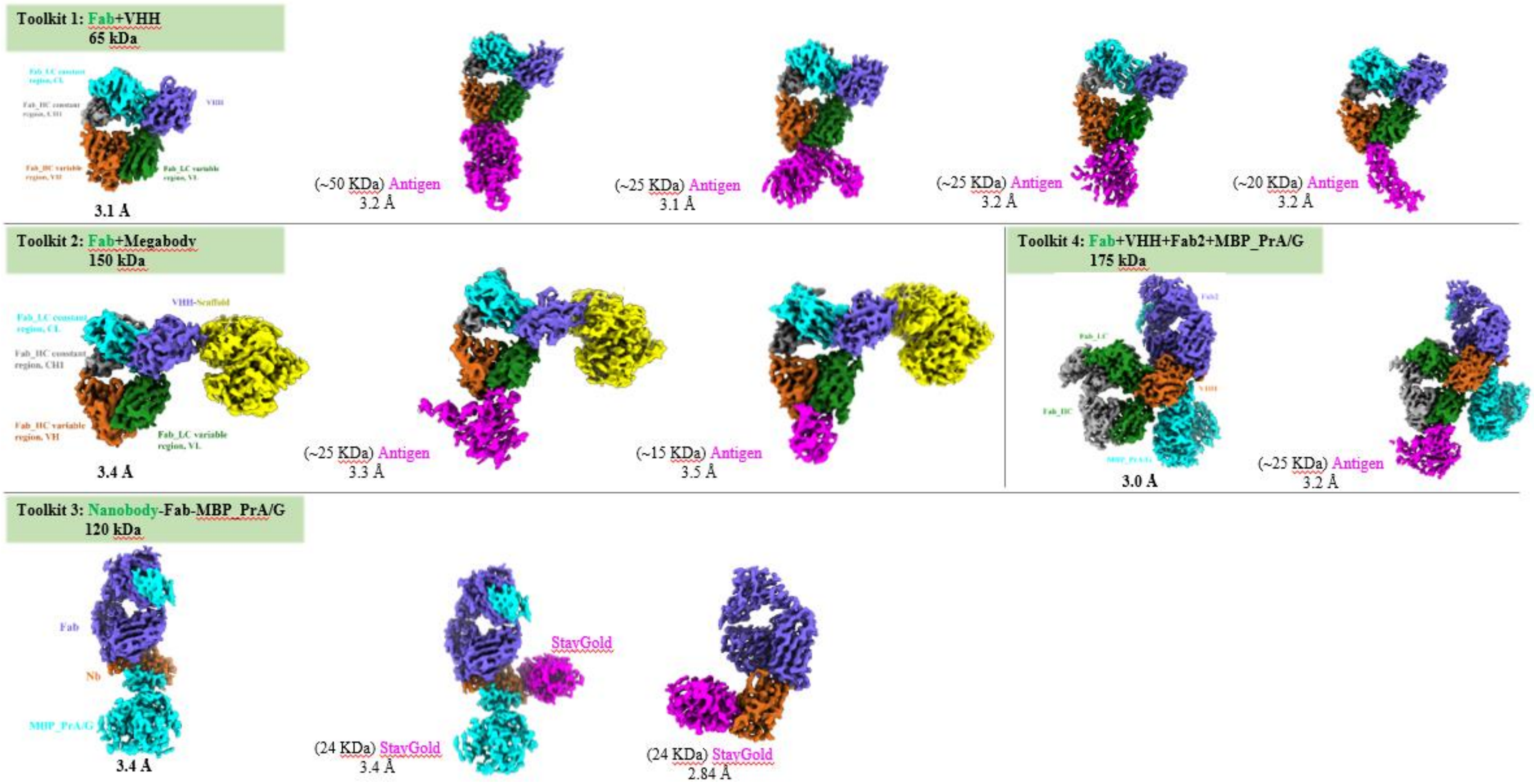
Published online: 20 August 2024

Wei Liu[1,2,5], Qiang Zhu[1,5], Chao-Nan Yang[1,2,5], Ying-Huan Fu[1,2], Ji-Chang Zhang[1,2], Meng-Yin Li[1,2,3], Zhong-Lin Yang[1], Kai-Li Xin[1,2], Jing Ma[1], Mathias Winterhalter[4], Yi-Lun Ying[1,2,3]✉ & Yi-Tao Long[1,2]

Non-stop innovation – Fab/VHH-based development



Non-stop innovation – Fab/VHH-based development



Outline

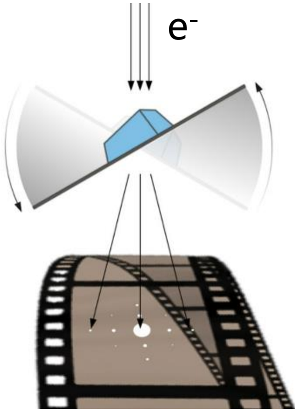
1. Overview of **Biortus**
2. CryoEM facilities
3. Pipeline of cryoEM service
4. Single Particle Analysis
5. **MicroED**
6. Characterization of drug delivery systems
7. Business model

What is MicroED?

- MicroED (Microcrystal Electron Diffraction) is a electron diffraction technology that enables fast, and high-resolution structure determination of microcrystals.

Electron microscope

→



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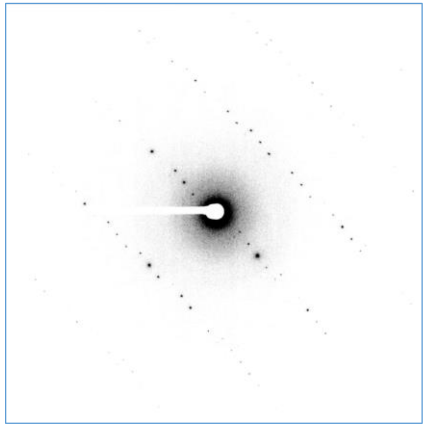

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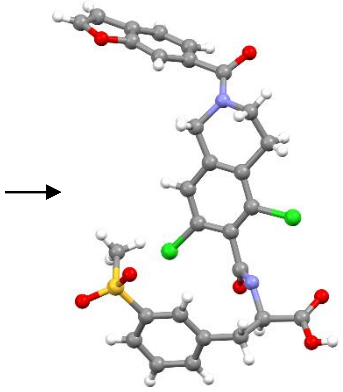
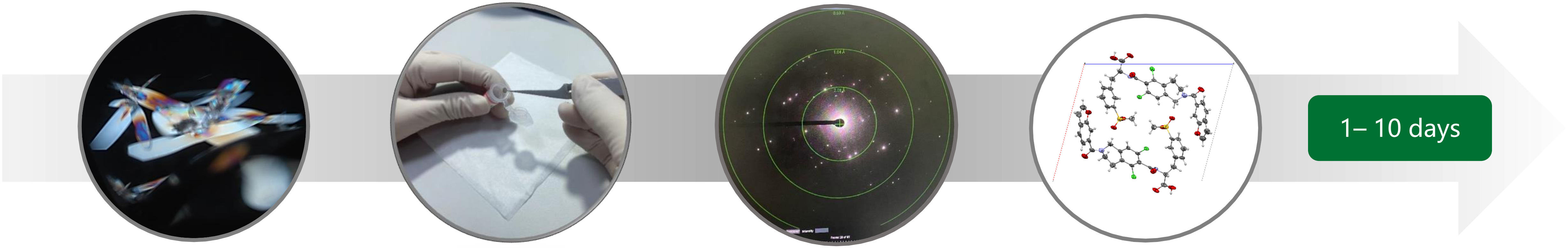


MicroED: our general workflow



1– 10 days

Crystal Detection

- PLM/XRPD
- Recrystallization available

Sample Preparation

- 5-10 mg powder is optimal
- Crystal size >200 nm is sufficient

Data Collection

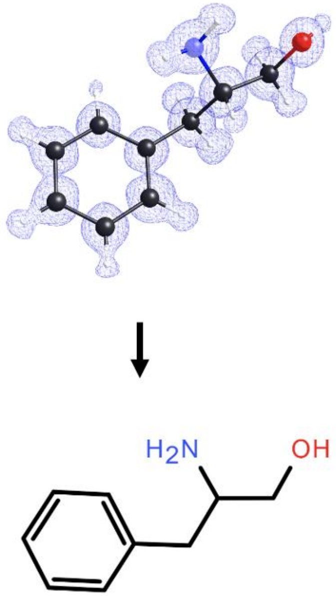
- Talos F200C & Glacios
- Automatic data collection

Structure Solution

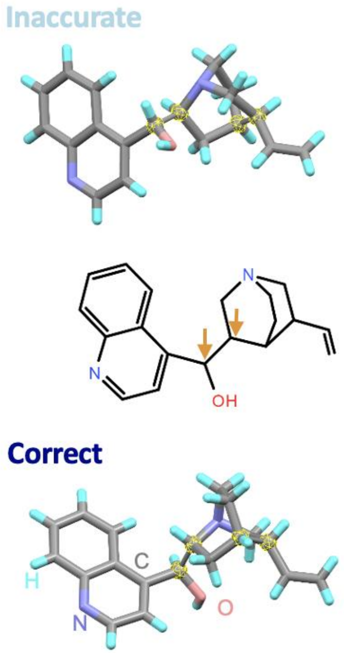
- Absolute configuration determination
- Structure confirmation from ambiguous deduction
- Crystal form identification (API/final product)
- Impurity structure determination

MicroED applications in organic molecules

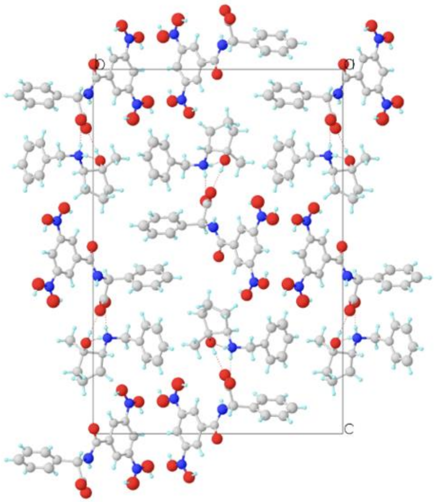
Structure determination



Chirality determination

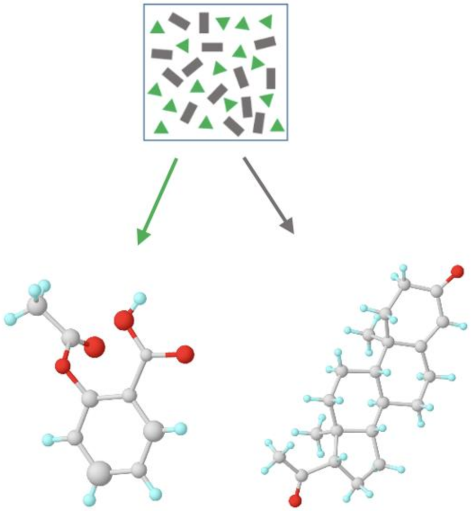


Crystal form identification



Unit cell, symmetry and atom positions

Mixture Identification



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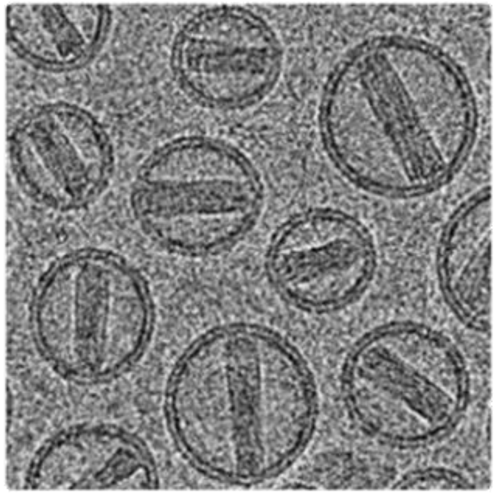
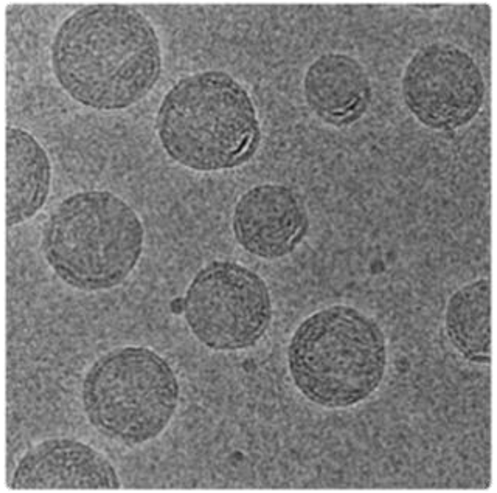
Characterization of drug delivery systems

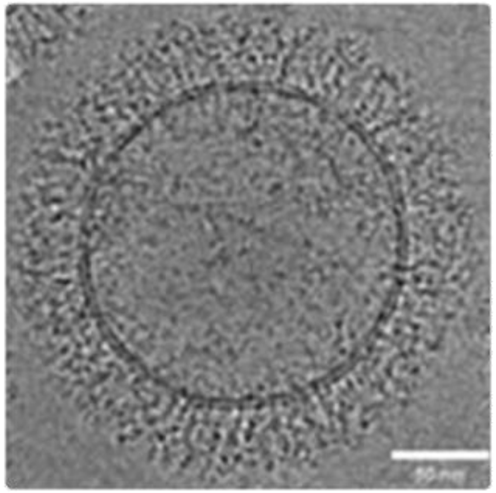
API-liposome



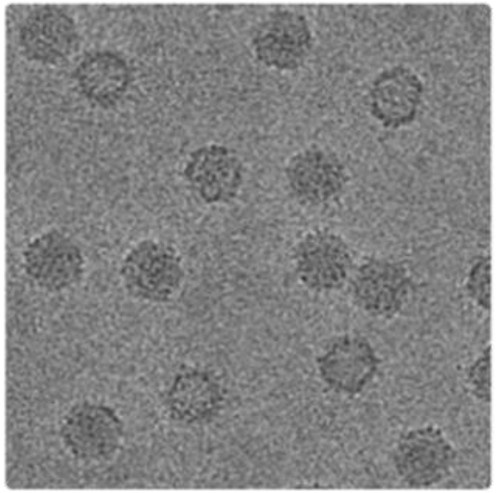
mRNA-LNP



Exosome

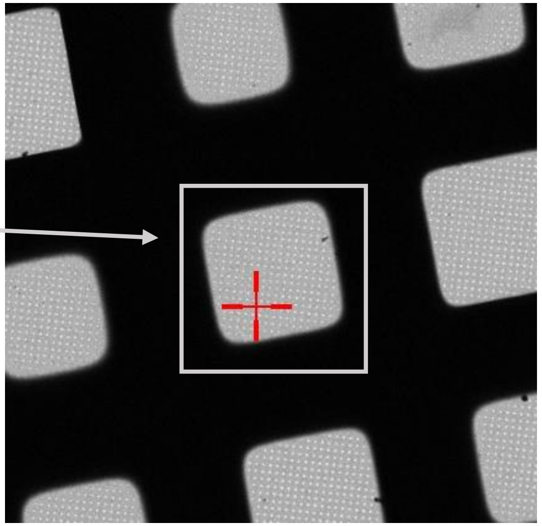


AAV



Cryo-EM characterization

Square

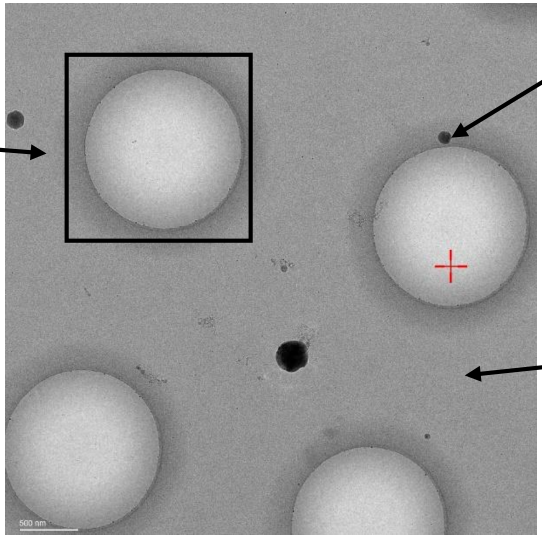


150x

Hole

Ice Contamination

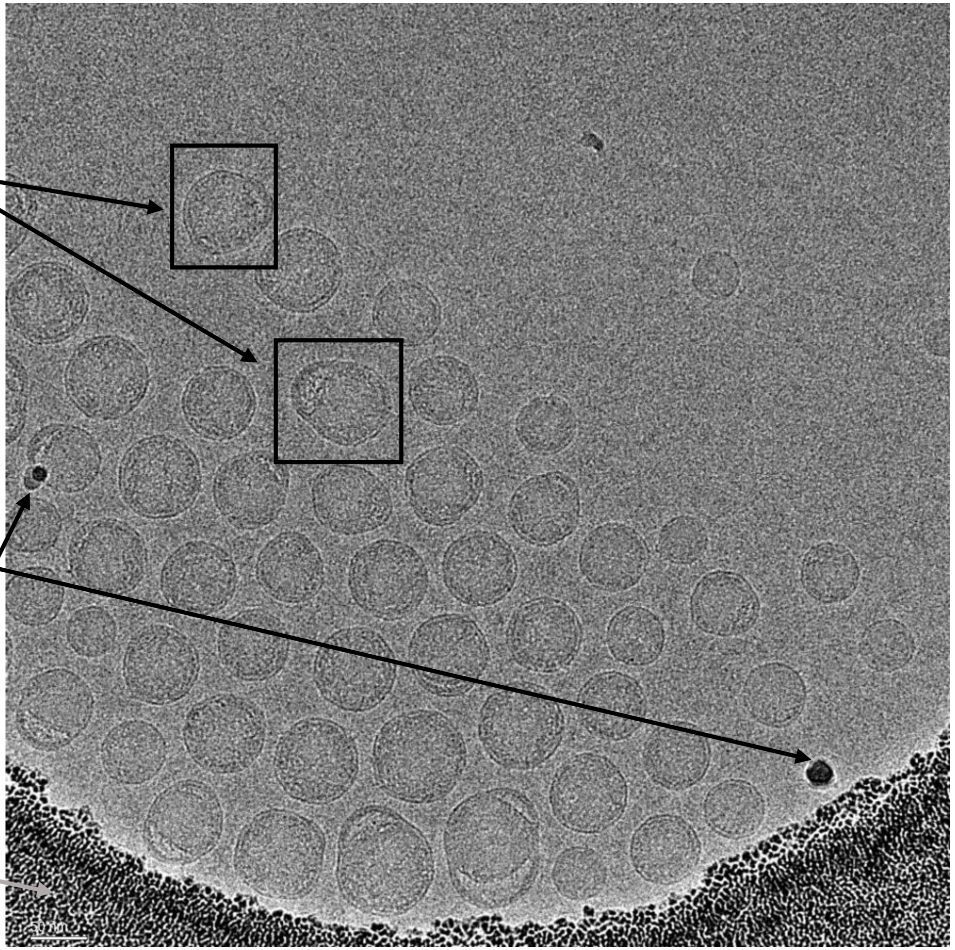
Support Film



8,500x

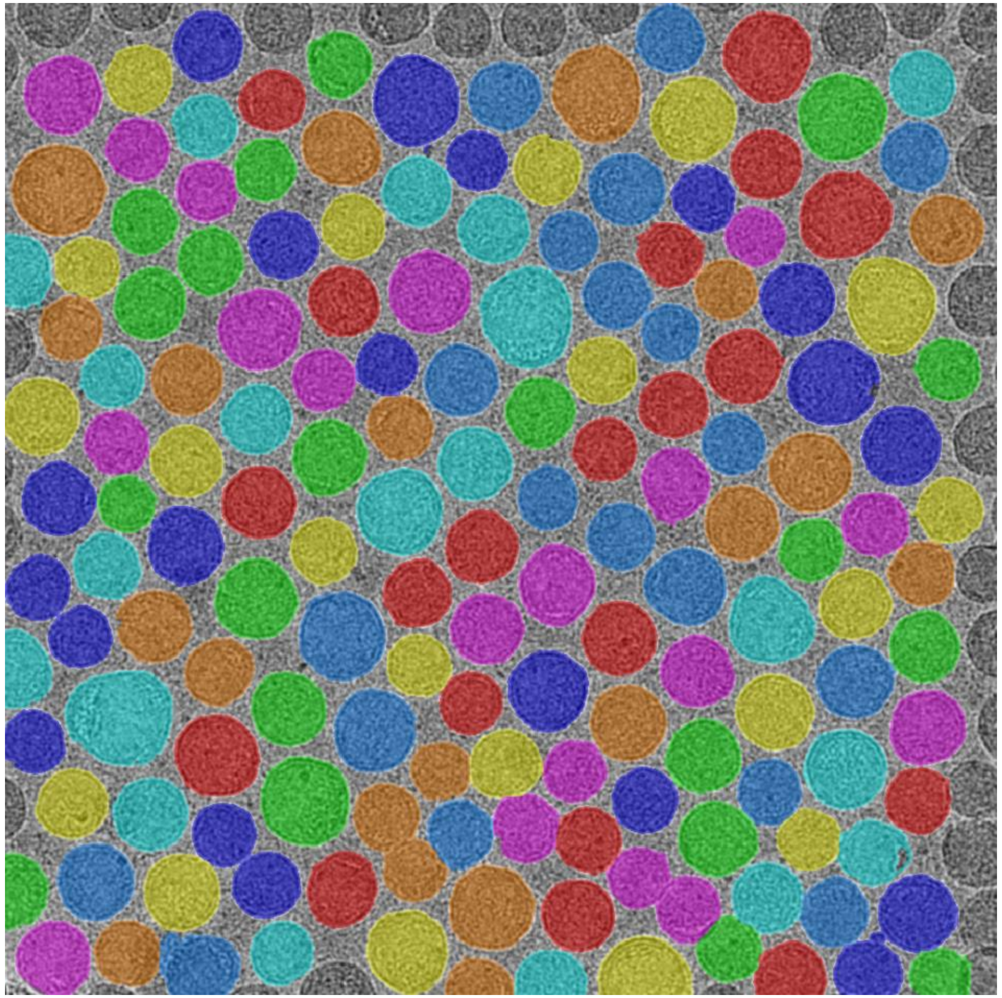
Exposure

LNP particles

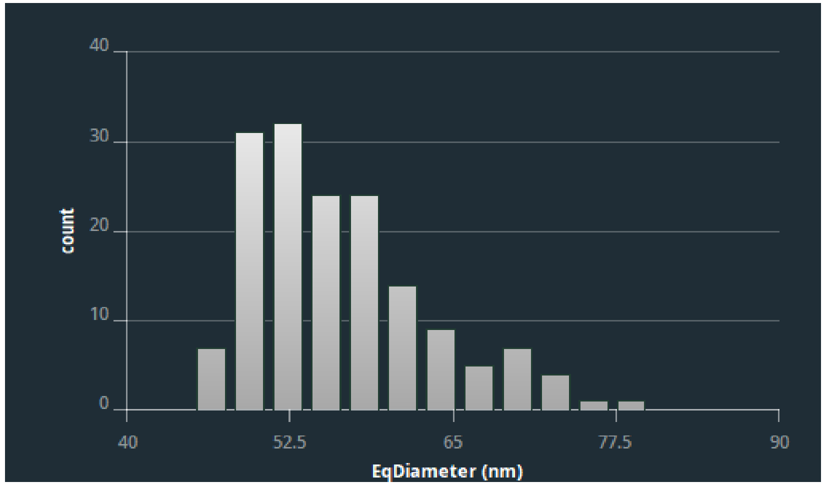


73,000x

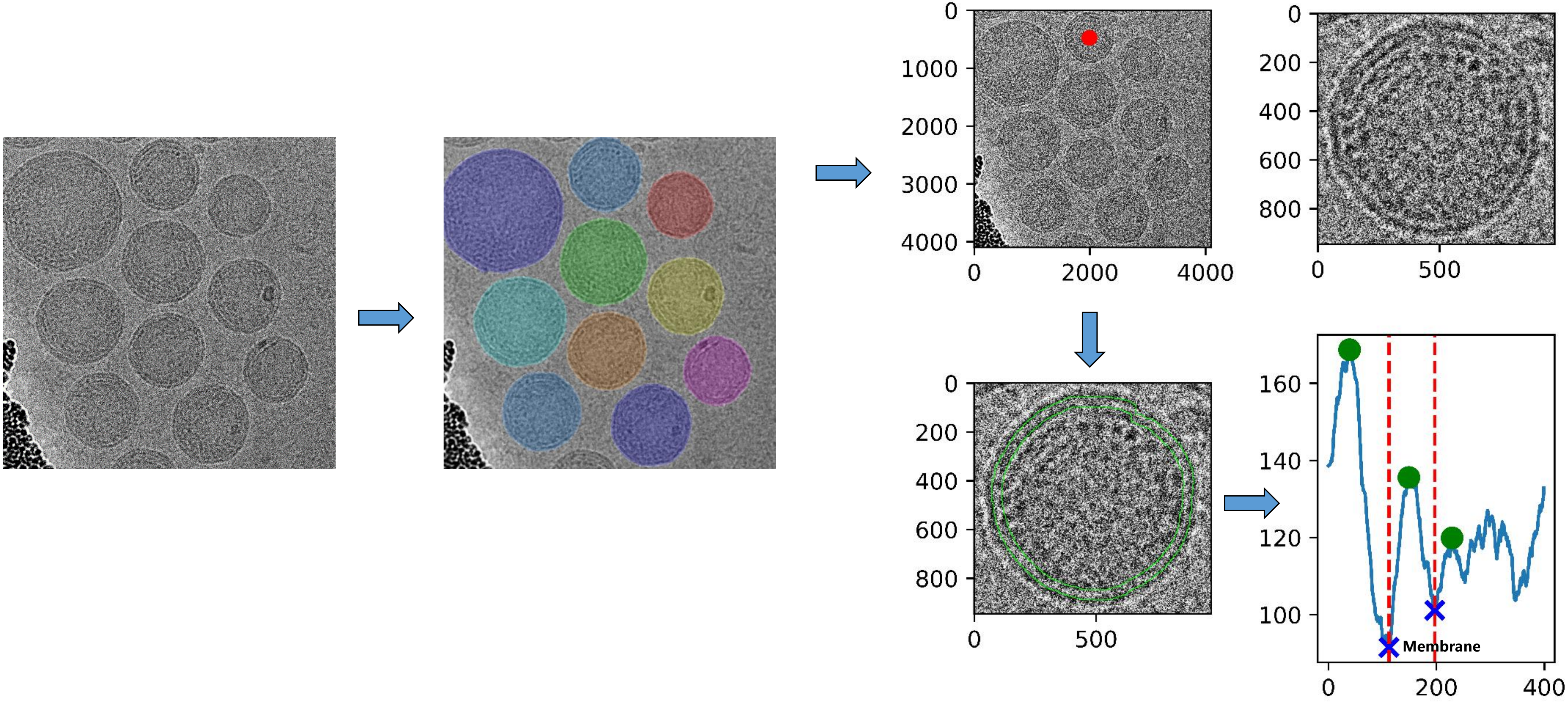
Size distribution analysis using cryo-EM



EqDiameter (nm)	Area (nm^2)	Rugosity	LWR	index
55.2496	2397.44	0.222979	1.0385	1
56.8201	2535.68	0.179097	1.05116	2
71.119	3972.48	0.215639	1.05478	3
52.8139	2190.72	0.208436	1.06384	4
54.537	2336	0.225427	1.06387	5
53.3054	2231.68	0.195291	1.0408	6
74.0657	4308.48	0.200917	1.10057	7
62.8271	3100.16	0.19749	1.02868	8
57.1989	2569.6	0.206	1.06574	9
71.4734	4012.16	0.262419	1.156	10
52.0211	2125.44	0.208838	1.07624	11
70.855	3943.04	0.171716	1.05561	12



Membrane thickness



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Business Model

Our Standard of Care:

- ✓ Dedicated Project Managers
- ✓ Transparent and frequent communication at weekly/biweekly/monthly TCs
- ✓ Excellent service

Fee for Service (FFS)	Full time Equivalent (FTE)
Specific project proposal	Project proposal is more flexible
Services needed must be outlined	Adaptable project services
Milestone payments	Monthly payments
Generally, for fixed needs	Generally, for more exploratory work
Specified deliverables for final product	Detailed technical development included in deliverables
CDA/NDA, MSA and Quote required for each FFS	Simpler paperwork (contract extensions/renewal)

FAQ

Q: What is the timeline of gene to structure for single particle?

A: ~2 to 3 months.

Q: I have protein sample ready, how long will it take to get the structure?

A: 2-3 weeks.

Q: How long will it take for microED to determine a small molecule?

A: ~3 days.

Thanks!

Biortus Discovery Co. , Ltd.
https://en.biortus.bio/

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For off-the-shelf: order@biortus.bio