BIORTUS

Your Protein-Focused Partner for Drug Discovery

From Gene to Lead

About Us

Founded in 2009, Biortus (”-ortus” meaning sunrise in Latin) Biosciences Co., Ltd., is an innovation-driven contract research organization (CRO) committed to high quality R&D services in the support of drug discovery. Biortus headquarters is based in Wuxi Jiangyin with branch facilities in Wuxin Xinwu district, and Shanghai City in China and in Woburn, MA, USA.

We have three world-class service platforms:

- Recombinant Protein Production
- *In Vitro* Assays and Screening
- Structure Determination (X-ray Crystallography, CryoEM, and MicroED)

Built by scientists, for scientists, with our expertise we have partnered worldwide with numerous research institutions, pharmaceutical and biotechnology companies - consistently providing high-quality custom proteins for all stages of biomedical research.

Service Platforms

Recombinant Protein Production

- Directed Construct Design, Generation, and Mutagenesis
- Multiple expression systems: *E.coli*, Insect cell, Mammalian, etc.
- Single and multi-step purification (Affinity, IEX, SEC)
- Standard and custom functional QC
- Dedicated Membrane Protein Production group
- High-throughput parallel monitoring for efficient identification of ideal constructs and conditions

Structure Determination

- MicroED: Small molecule structure determination using in house Talos F200C
- State-of-the-art X-ray facilities: in-house light source and near weekly access to international synchrotrons
- Multiple Titan Krios G4 supported by a number of Glacios, Talos, and TF20. Owns the most electron microscopy instruments in CRO sector.

In vitro Assays and Screening

- 4 Fragment libraries with ~ 10,000 unique fragments total
- Biochemical and Biophysical assays for Binding Kinetics, Off-target screening, Hit Validation, etc.
- *De novo* method development
- SPR/ Caliper/ CE/ DSF/ Thermofluor/ Radiometric assays

Protein Expression

Thoughtful Design with downstream application in mind

- Client interaction starts from day 1 - transparency from the very beginning and at every step
- Multiple vector backbones and tag systems to choose from

Variety of Adaptable Expression systems

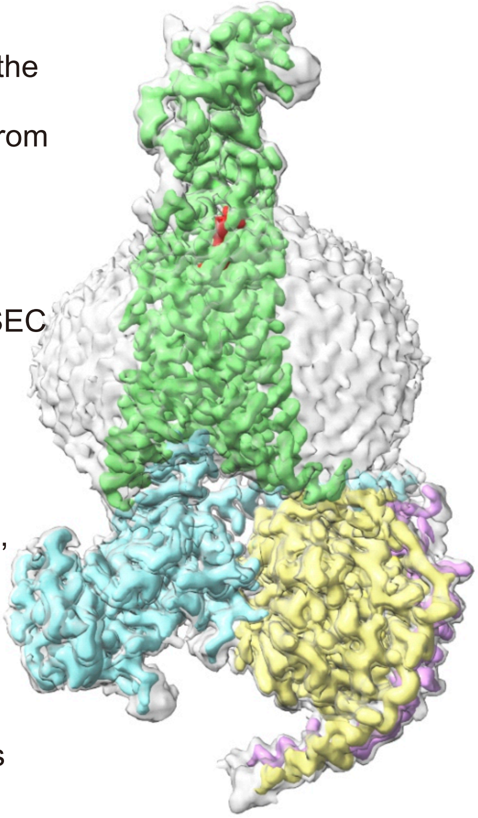
- Expression Systems: *E. coli*, insect cells, mammalian. Yeast and silkworm coming soon
- High-throughput parallel screening with Fluorescence SEC (FSEC)
- Small and Large scale expression and purification

Capacity

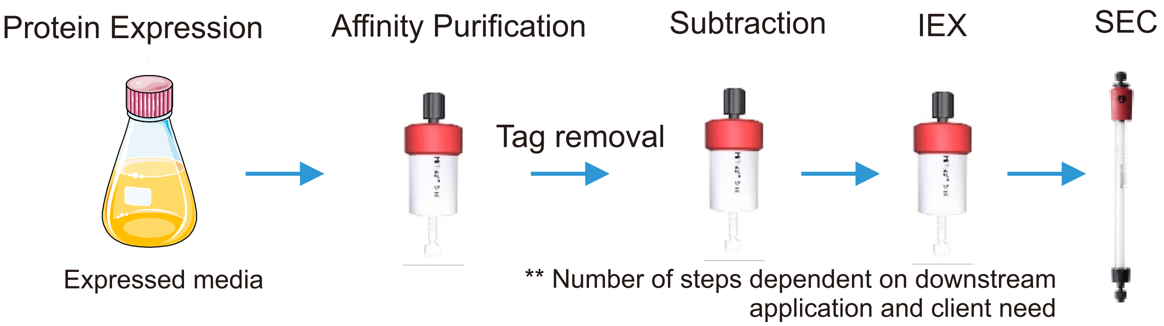
- Over 4000 proteins/complexes delivered to clients
- Each week, over 1000L (600L of *E.coli*, 400L insect cell, and 100L of mammalian cell on average) of expression media generated and purified

Dedicated & Experienced Membrane Production team

- Nanodisc, Liposome, Detergent solubilization strategies
- Iterative multivariable optimization procedure
- Work feeds directly into *In Vitro* Assays and Structure determination platforms


2.8 Å resolution GPCR structure
Protein produced in house and determined using Biortus CryoEM

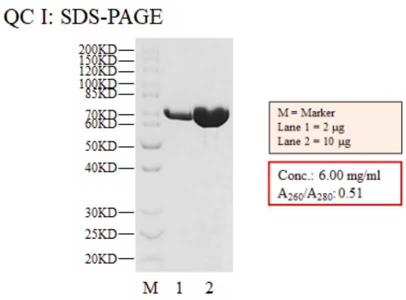
General Purification Procedure



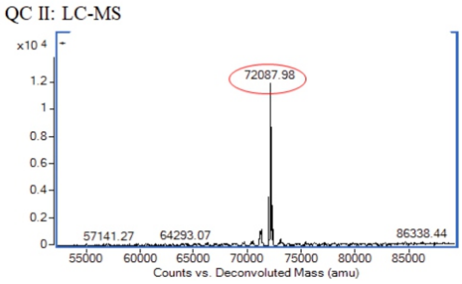
Protein Expression → Affinity Purification → Tag removal → Subtraction → IEX → SEC

Expressed media

** Number of steps dependent on downstream application and client need

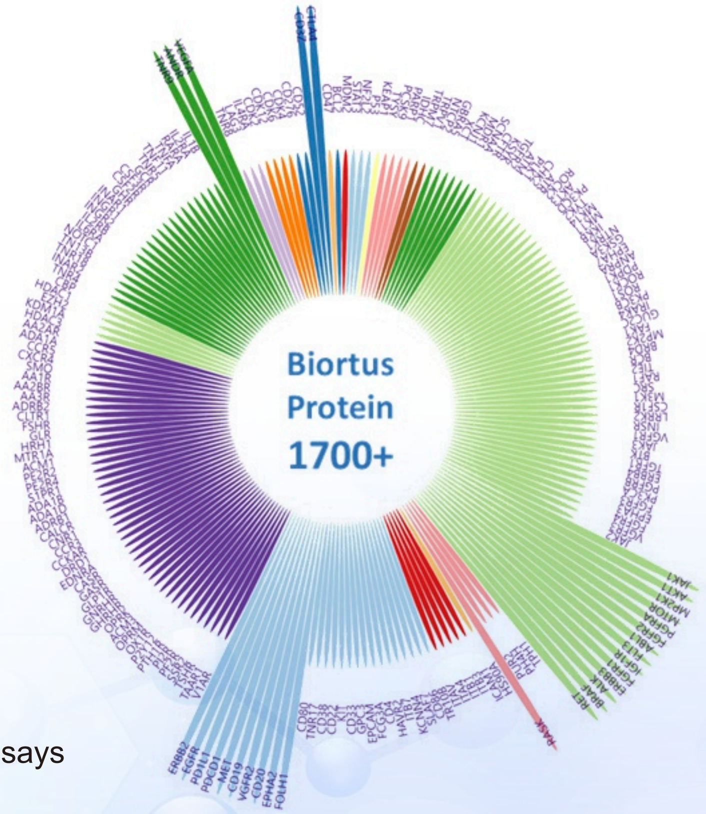


QC I: SDS-PAGE



QC II: LC-MS

Protein Production



Single or Multistep Purification Available

- Tag removal
- Endotoxin removal
- Post purification modifications (Biotinylation, AlexaFlor, etc)
- Deglycosylation available

Quality control is standard practice

Standard QC

- Purity (SDS-PAGE)
- Concentration (Nanodrop or Bradford method)
- Validation of MW and Post-translational Modifications (LC-MS)

Custom QC available

- Oligomerization (Analytical SEC, SEC-MALS)
- Stability (Thermal Shift Assays, NanoDSF and Thermal Fluoresence Assays)
- Complex formation (Gel-Shift)
- Negative Stain EM
- Mass photometry
- Target specific biochemical and biophysical assays

Protein Catalog for off-the-shelf needs

- Over 700 proteins in multiple family classes available
- Inventory is still growing
- QC reports available
- **Now on VWR**

Stage-Specific Target & Compound Characterization

Supporting all stages and aspects of drug discovery

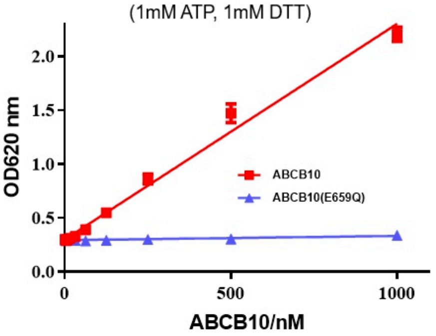
Our *in vitro* assay & screening platform allows us to conduct custom biophysical and biochemical functional protein quality control, determine compound and target binding affinities, characterize protein-protein interactions, and evaluate enzymatic activity for your protein of interest. These are just some of the ways our group can support you. We can also transfer your assay to Biortus to streamline workflow and develop *de novo* assays if needed.

With the variety of instrumentation on hand, we have multiple ways to screen compound, fragment, and peptide libraries for you as well.

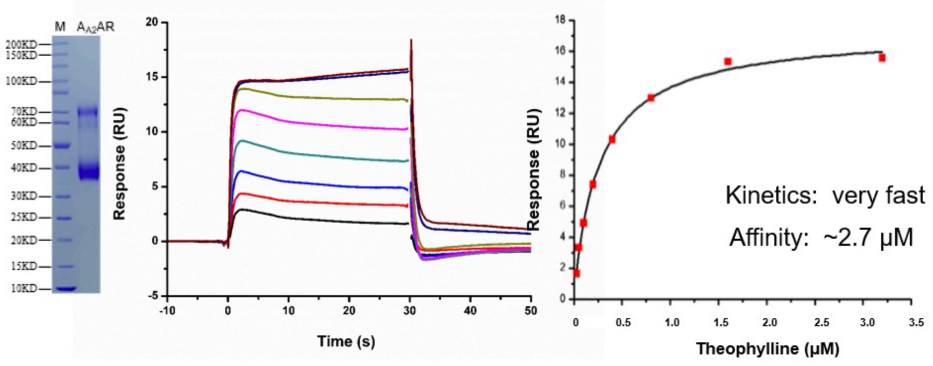
Stage	**Target Identification & Validation**	**Hit Identification & Evaluation**	**Lead Identification**	**Lead Optimization**	**Pre-Clinical Evaluation**
Key activities	Target Characterization	Compound Screening	Selectivity profiling Steady-state Kinetics	Drug residence evaluation	Pre-Clinical Candidate (PCC) Characterization
Parameters	• Biochemical Mechanisms & Assays (K_m, k_{cat})	• Primary screening • Off-target filtering • Initial Mode of Inhibition (MOI) studies (IC_{50}) • Predicted cell potency	• On-target evaluation: Enzyme/Cell correlation • MOI • Initial Selectivity (isoforms, family members)	• K_i & MOI (IC_{50}/EC_{50}) • Mechanism of Action (MOA) studies • Broader selectivity (enzymatic/protein class) • Residence time (K_{on}, K_{off})	• K_i & MOI package • Selectivity profile • Residence time

ATPase activity test of ABCB10

(1mM ATP, 1mM DTT)



SPR activity assay of $A_{A2}AR$ with Theophylline substrate



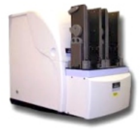
Kinetics: very fast

Affinity: ~2.7 μM

Variety of Assays and Instruments Available

Biochemical Assay Depot


M1000


Envision

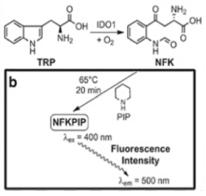

F200 Pro

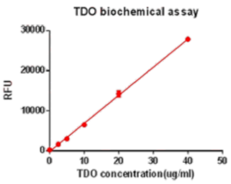

Spark


Top Count

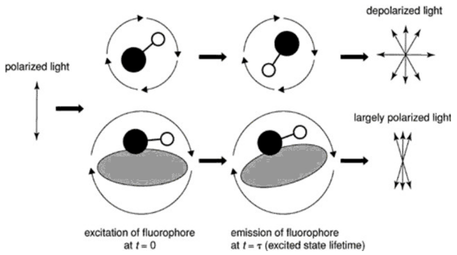

Echo Liquid Handler

Fluorescence Intensity Assay

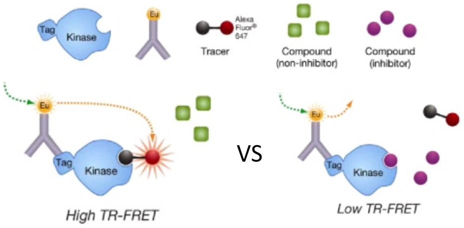




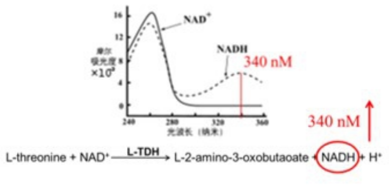
Fluorescence Polarization Assay



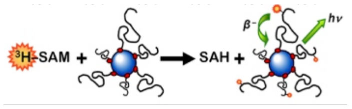
TR-FRET Assay



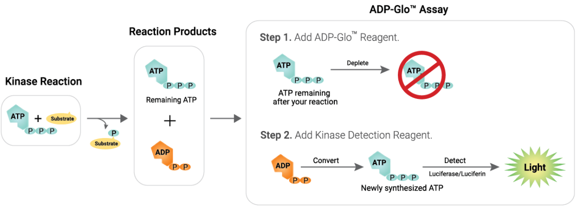
UV/Vis Assay



Radiometric Assay



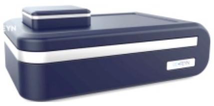
Chemiluminescence Assay



Biophysical Assay Depot


Light Cycler®480 II


Dianthus NT.23 Pico Duo

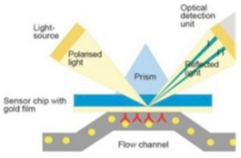

Refeyn Two^{MP}

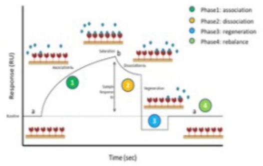

Biacore S2000


Biacore 8k+

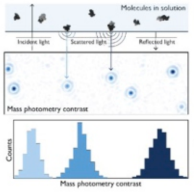

TOF-MS

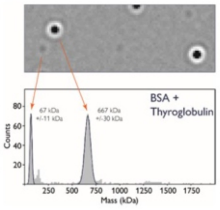
Surface Plasmon Resonance (SPR)



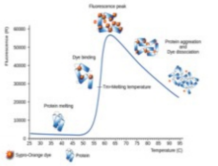


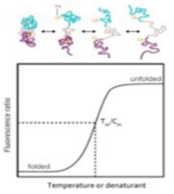
Mass Photometry





Thermal Shift Assay (TSA)



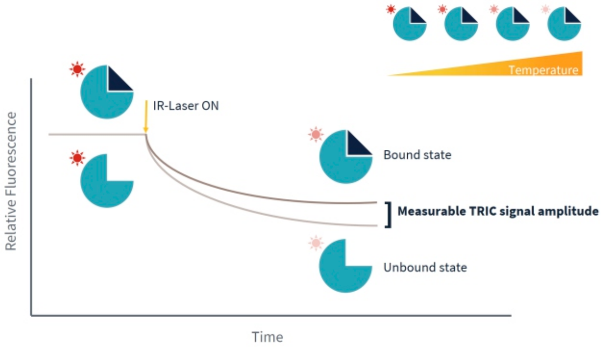


Over 2000 biochemical and cell assays developed
Don’t see your favorite assay? Contact us via info@biortus.bio

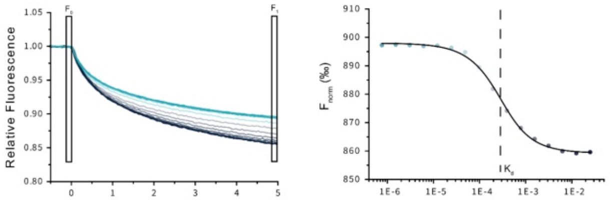
Moerke, N.J. *Curr. Protocols in Chem. Bio*. 2009
Lebakken, C. et al. *J Mol Screening* 2009

Assay Highlight: Temperature Related Intensity Change, TRIC

Theory behind TRIC



Example Dataset



Do more with less protein. Proteins need labeling.

Measure Kd independent of size and mass of binding partners.

Measure broad range of affinities (from pM to mM) and develop assay faster.

Measure in solution. In close to native conditions. No immobilization required.

Dianthus NT.23Pico

Super sensitive. Fast time to results with highest picomolar sensitivity

Measure many sample types: mammalian, yeast and bacteria cultures, plant extracts.

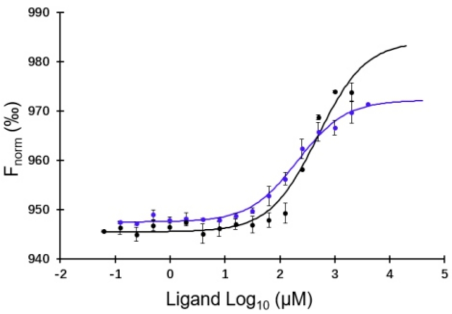
Work with the buffer that keeps your protein stable.

Measure broad range of interaction, including protein-ion and protein-carbohydrate.

Do not halt or delay your project with daily maintenance and repairs.

TRIC Case Studies and Examples

Soluble Proteins

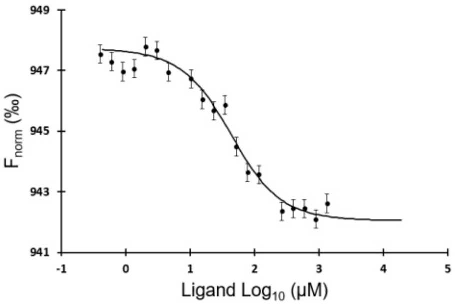


Target Protein

Kd1=209.0 mM, S/N=38.1

Kd2=546.0 μM , S/N=28.0

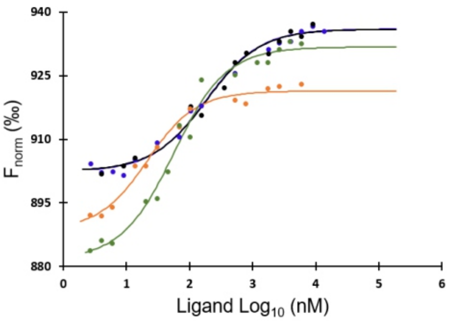
Ternary complex: PROTAC



VBC, Mz1 and BRD4:

Kd=32.8 nM, S/N=13.9

Membrane protein: GPCR



Legend	GPCR and compounds:
— Comp1	Kd1=177 nM, S/N=21.7
— Comp2	Kd2=181 nM, S/N=18.9
— Comp3	Kd3=51 nM, S/N=20.0
— Comp4	Kd4=16.4 nM, S/N=22.2

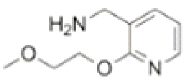
Biortus Fragment Libraries

Biortus has a total of 4 fragment libraries to serve as starting points in lead generation.

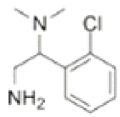
Library 1 - 1159 fragments, Rule-of-3 compliant, MW ~ 200Da, 100mM DMSO stock
Library 2 - 2500 fragments, Rule-of-3 compliant, MW ~ 200Da, 100mM DMSO stock
Library 3 - 3500 covalent fragments with functional groups targeting Lys, Cys, Ser, Asp, Glu, His and Tyr
Library 4 - 1200 covalent fragments with acrylamide warheads

SDF Files are all available upon request

Representative scaffolds from our covalent compound library

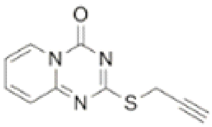


F2147-1782

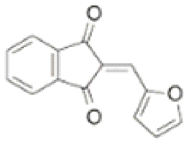


F2158-1768

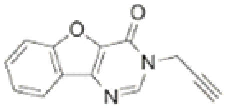
F2187-2596



F2543-0010



F1135-0038



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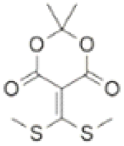

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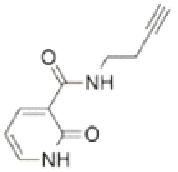

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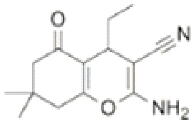

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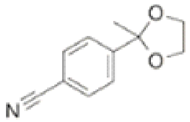

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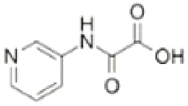

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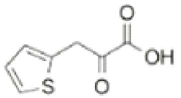

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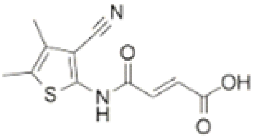

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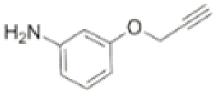

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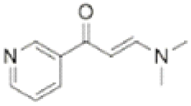
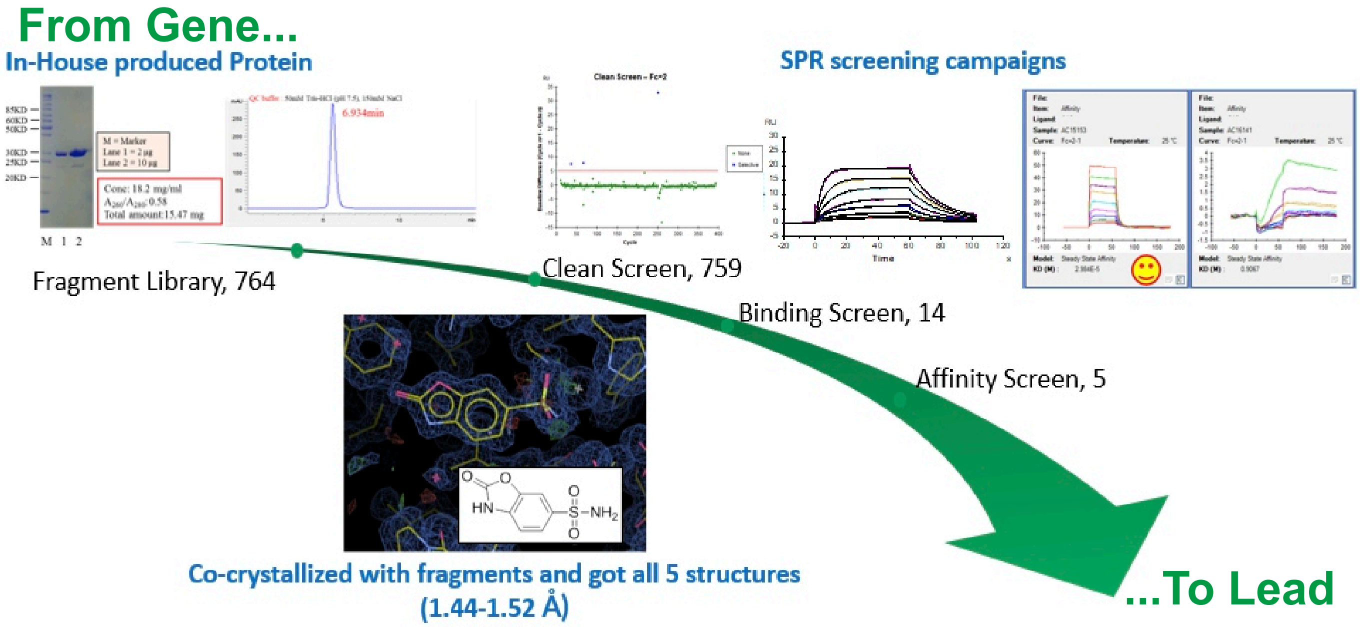


F2135-0440

Library Screening Services

Fragment hits can be screened using in-house DSF, SPR, and/or crystallography services for SAR.



Biortus Lead Generation Suites

Biortus provides Lead Generation Suite packages of our services, focusing on non-competing targets from protein families and biological pathways

Suite Contents:

- Protein reagents tailored for assays, screening, structure determination, antibody generation, etc.
- Fully validated screening assays
- Fragment libraries, assay-ready plates, and preliminary hits
- X-ray Crystallography and/or CryoEM structures and co-structures for SAR

Protein production → **Screening** → **Structure determination** → **Asset transfer for Client Lead ID programs**

Custom labeling, dedicated membrane protein production

Virtual, biophysical, biochemical, crystal-based fragment screening

SAR package

Suites Include:

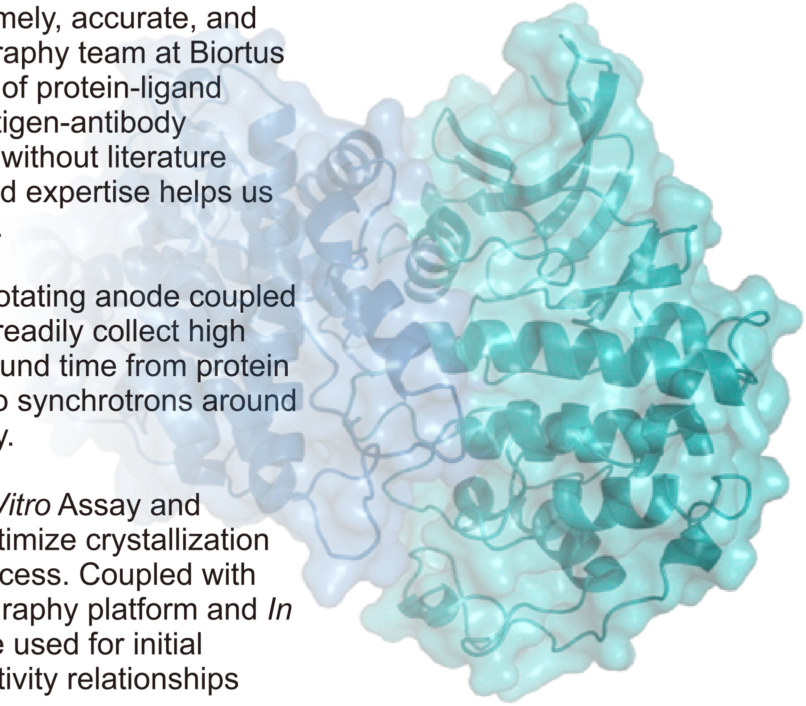
- GPCRs
- Ion Channels
- Transporters
- E3 Ligases
- Deubiquitinating Enzymes (DUBs)
- Kinases
- PRRs
- and more

X-ray Crystallography

Biortus is a preferred partner for structure-based drug design (SBDD) services for our drive to provide timely, accurate, and high-quality structural data. The crystallography team at Biortus has delivered over 5000 crystal structures of protein-ligand complexes, protein-protein complexes, antigen-antibody complexes, PROTAC complexes with and without literature precedent. Our state-of-the-art facilities and expertise helps us helps you with the drug discovery process.

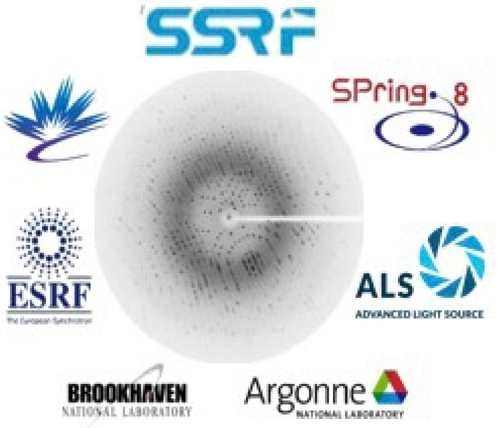
Our in-house Rigaku FR-E+ SuperBright rotating anode coupled with R-axis IV++ image plate allows us to readily collect high resolution data, facilitating efficient turnaround time from protein to structure. Biortus has frequent access to synchrotrons around the globe, averaging access almost weekly.

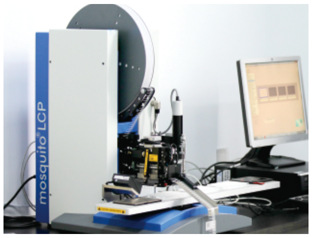
Cross-functional collaboration with our *In Vitro* Assay and Screening platform allows us to quickly optimize crystallization conditions, increasing the likelihood of success. Coupled with our fragment libraries, the X-ray Crystallography platform and *In Vitro* Assay and Screening platform can be used for initial candidate drug discovery and structure-activity relationships (SAR).

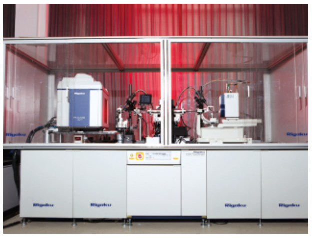


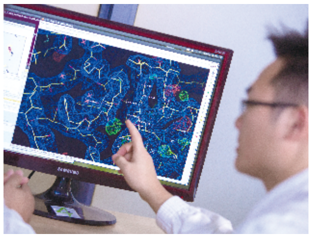
2.3 Å resolution structure of CDK3/Cyclin E1 complex
Protein produced in-house and determined using Biortus Crystallography

Target	Uniprot Number	PDB
Bcl-XL	Q07817	7CA4
JMJD2A	O75164	7D4A
MYST1	Q9H7Z6	7CMR
JNK2	P45984	7CML
USP7	Q93009	7CM2
LDHA	P00338	6ZZR
PHGDH	O43175	7CVP
DJ-1	Q99497	7C62
MTH1	P36639	7ESF
PARP14	Q460N5	7D2C
BRPF1	P55201	7C4I
DHFR	P00374	7ESE

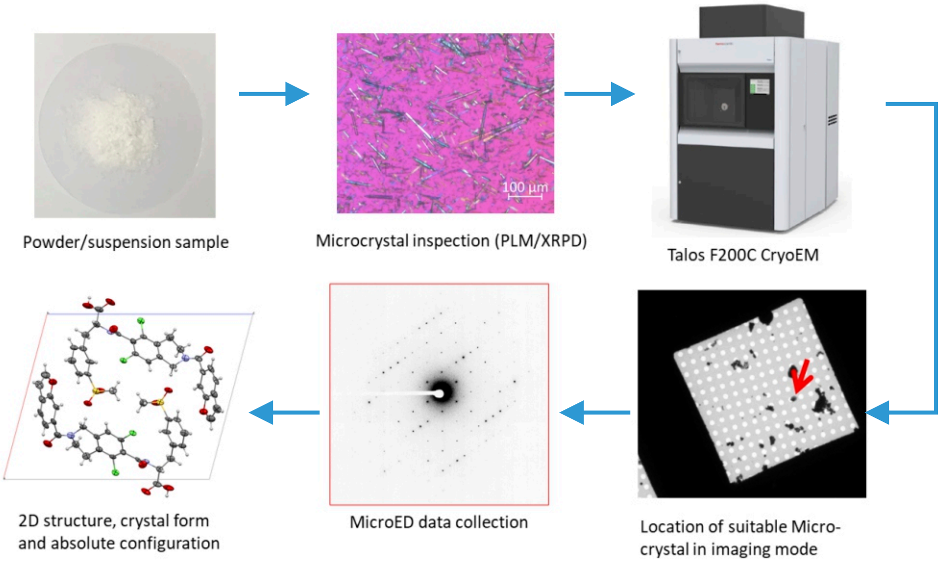








Microcrystal Electron Diffraction (MicroED)



About MicroED

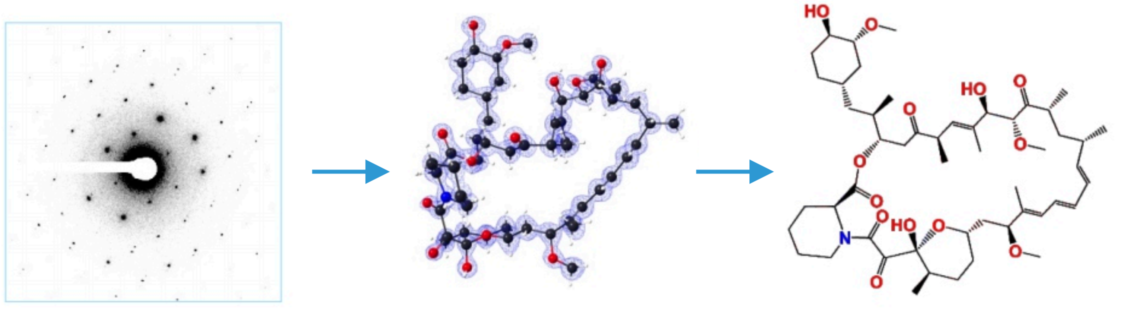
MicroED is a diffraction based technique conducted in a CryoEM.

This technique can be applied to both small molecule crystals and protein nanocrystals. Structures can be determined in as little as a day

Sample requirements:
- 1-2 mgs of sample, 5mgs ideal.
- Sample must have crystal forms or we can crystallize in-house

Applications
- Structure and chirality determination
- Characterization of active pharmaceutical ingredient (API) impurities, metabolites or crystal forms for formulation
- Reverse engineering

MicroED application: Absolute configuration determination



MicroED data

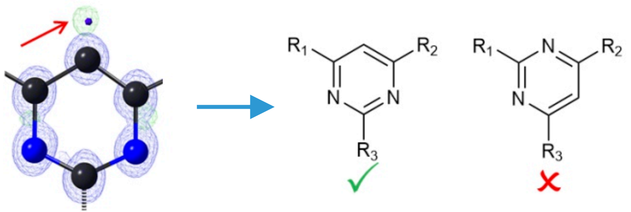
MicroED structure

Rapamycin, 914.19 Da, 15 chiral centers

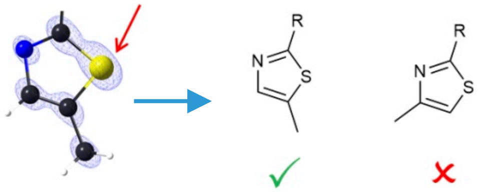
The absolute configuration was determined within 10 hours by using dynamical refinement method

MicroED application: Accurate structure determination

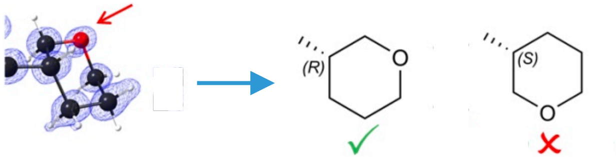
location of N atom



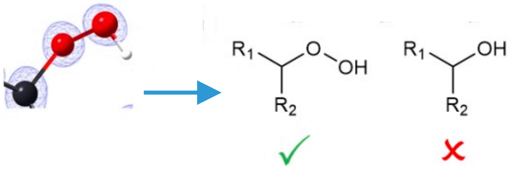
Location of N/S atoms



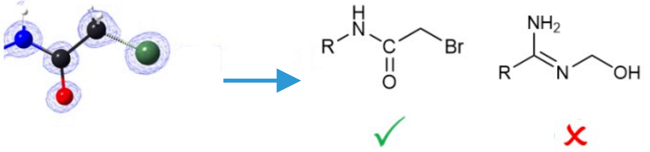
Differentiation of C/O atoms



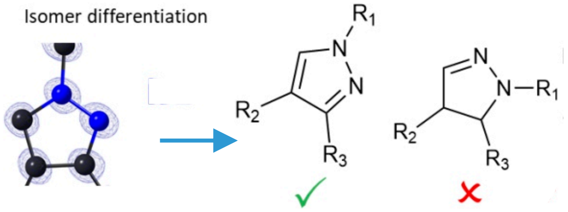
Peroxy group determination



Group identification

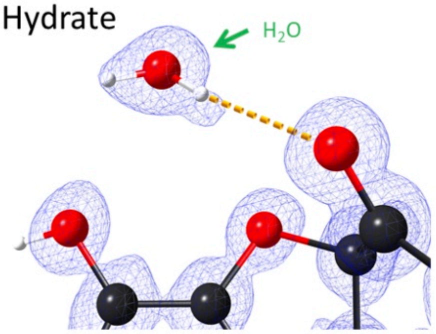


Isomer differentiation

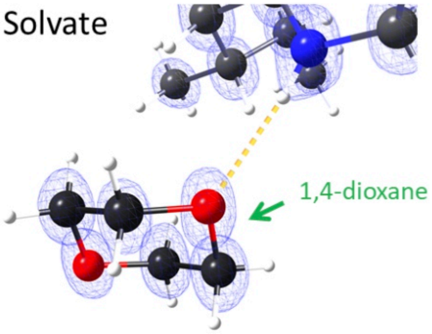


MicroED application: Crystal form identification

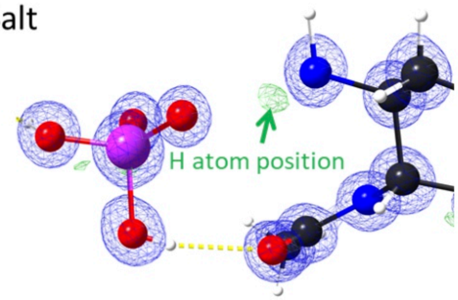
Hydrate



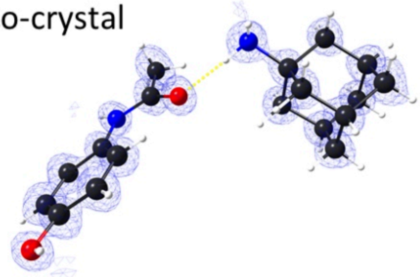
Solvate



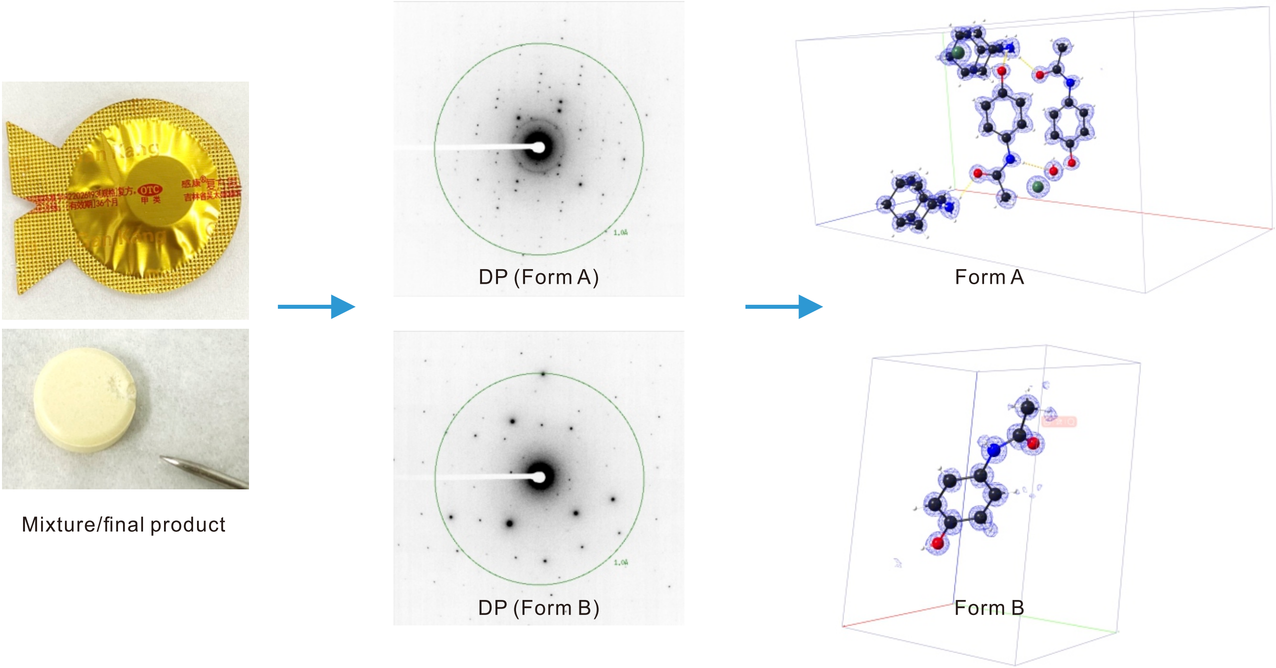
Salt



Co-crystal



MicroED application: mixture structure determination



Mixture/final product

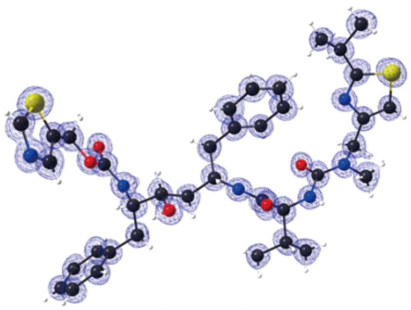
DP (Form A)

DP (Form B)

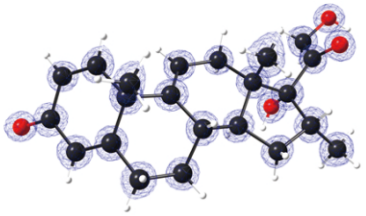
Form A

Form B

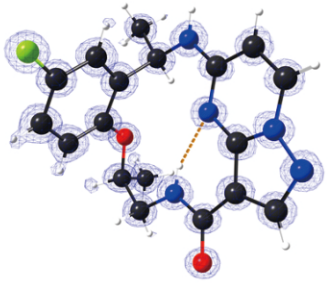
2023 FDA approvals



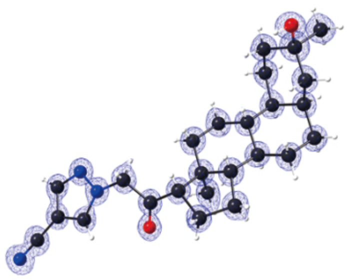
Ritonavir



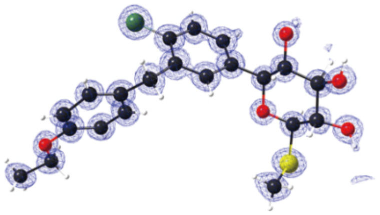
Vamorolone



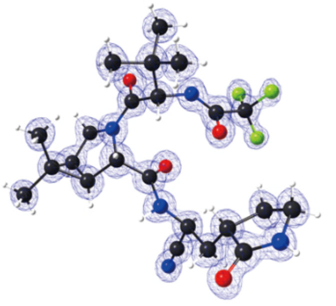
Repotrectinib



Zuranolone



Sotagliflozin



Nirmatrelvir

CryoEM and Atomic Modeling

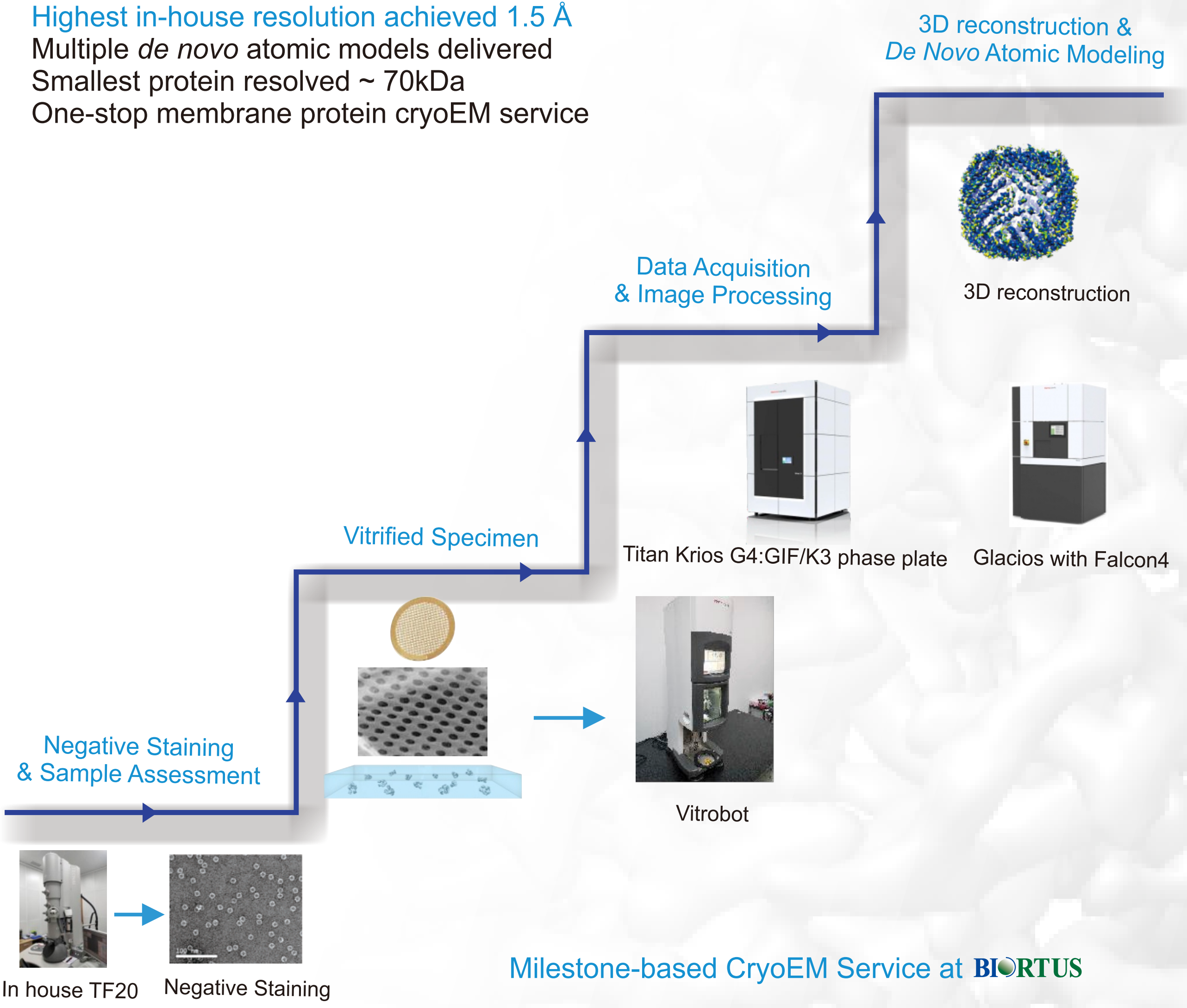
Benefits of CryoEM

- In single particle analysis (SPA) studies, biological aqueous samples are applied to a grid mesh and flash frozen, preserving the native structure of the protein or protein complex in vitreous ice
- Phase information is preserved as data is collected on Direct Detector Devices (DDD)
- Requires no special treatment as long as the sample is stable.

- Able to capture different orientations and conformations

Biortus has an experienced team of scientists with a track record of resolving atomic resolution CryoEM structures for large proteins, protein complexes, ligand bound proteins, and membrane proteins.

- Highest in-house resolution achieved 1.5 Å
- Multiple *de novo* atomic models delivered
- Smallest protein resolved ~ 70kDa
- One-stop membrane protein cryoEM service



Milestone-based CryoEM Service at BIORTUS

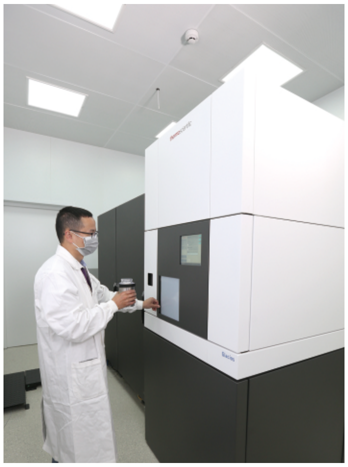
Largest Commercial CryoEM Facility

↑ Titan Krios G4

GIF/K3 camera
Phase plate/Autoloader
High resolution data collection

↓ Glacios

Falcon 4 camera/Autoloader
High-throughput grids screening

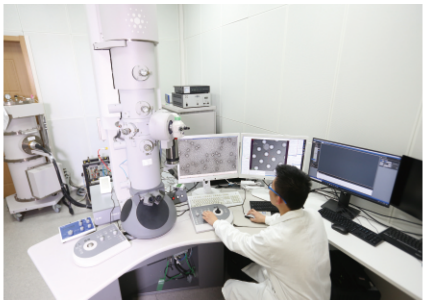


↑ Talos F200C

Ceta-D camera
MicroED data collection

↑ Talos L120C

Ceta 16M camera
Negative staining



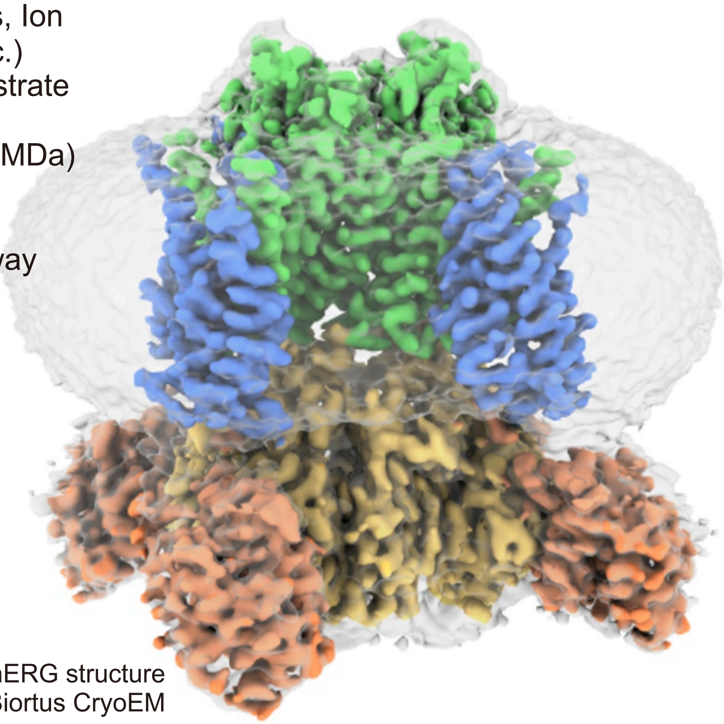
← Tecnai F20

Negative staining
Vitrification tests

- Titan Krios G4/GIF/ Phase plate/K3 300kV
- Glacios with Falcon4 200kV
- TF20 with CCD 200kV
- Talos F200C with Ceta-D 200kV

Classes of proteins resolved at Biortus:

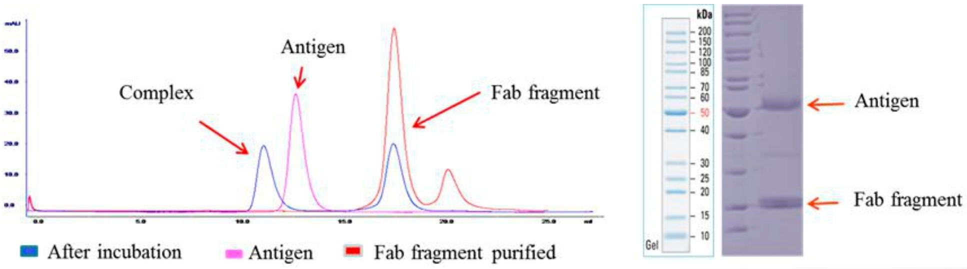
- Membrane proteins +/- ligands (GPCRs, Ion Channels, Transmembrane proteins, etc.)
- PROTAC-related ligase system +/- substrate
- AAA+ proteases +/- substrate
- Large enzyme complex assembly (>0.5MDa)
- Antibody-antigen complexes (epitope mapping)
- Immune system related signaling pathway proteins
- Nucleic acid binding proteins (RNA polymerases, transcription factors, etc.)
- Epigenetic related proteins
- Deubiquitinases
- Viruses



2.56 Å resolution apo-hERG structure
Protein produced in-house and determined using Biortus CryoEM

Antibody Analysis by CryoEM

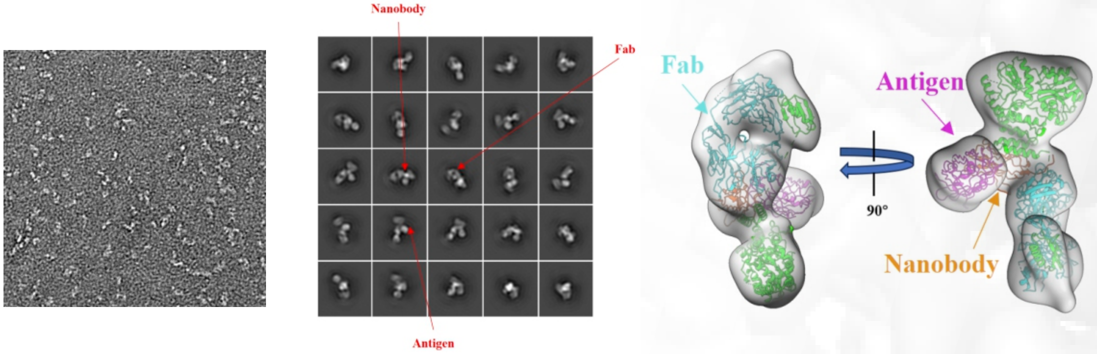
Antigen/Fab complex preparation



a. SEC

b. SDS-PAGE

Quick Antibody/Fab Analysis by Negative Stain

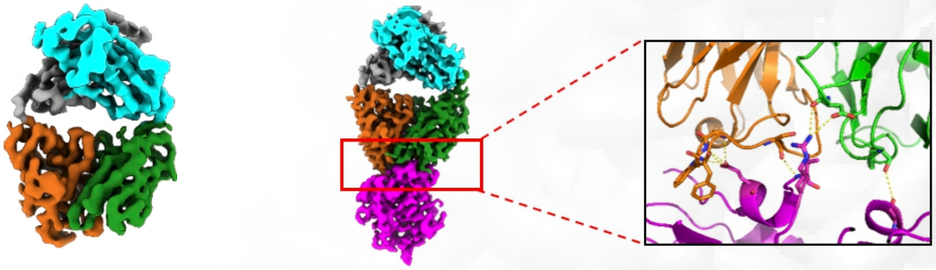


a. Negative staining

b. 2D classification

c. 3D density map

High Resolution Epitope Mapping with CryoEM



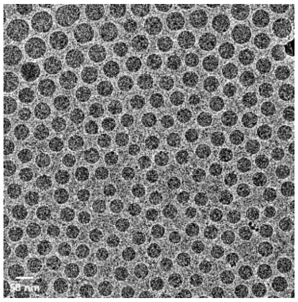
a. Fab (50 kDa)

b. Fab+Antigen (~ 3.0 Å)

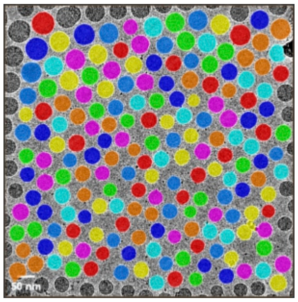
c. Binding epitope

LNP analysis with CryoEM

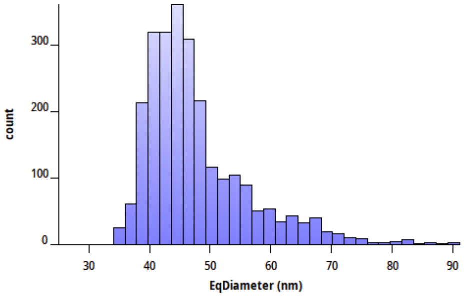
LNP Analysis with CryoEM



CryoEM Image

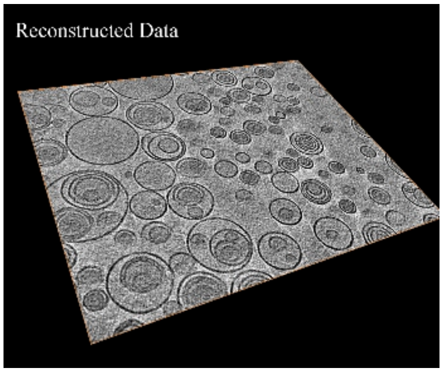


Analysis

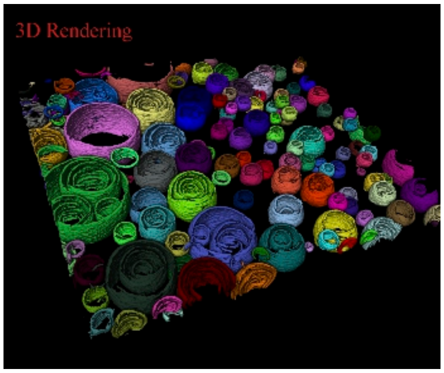


Parameters
(Diameter， Area, Morphology, etc.)

LNP Tomography



Data Collection



3D Reconstruction

AAV Particle Analysis

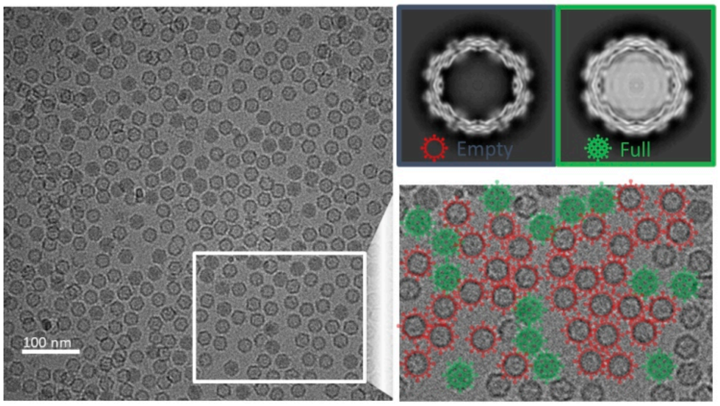
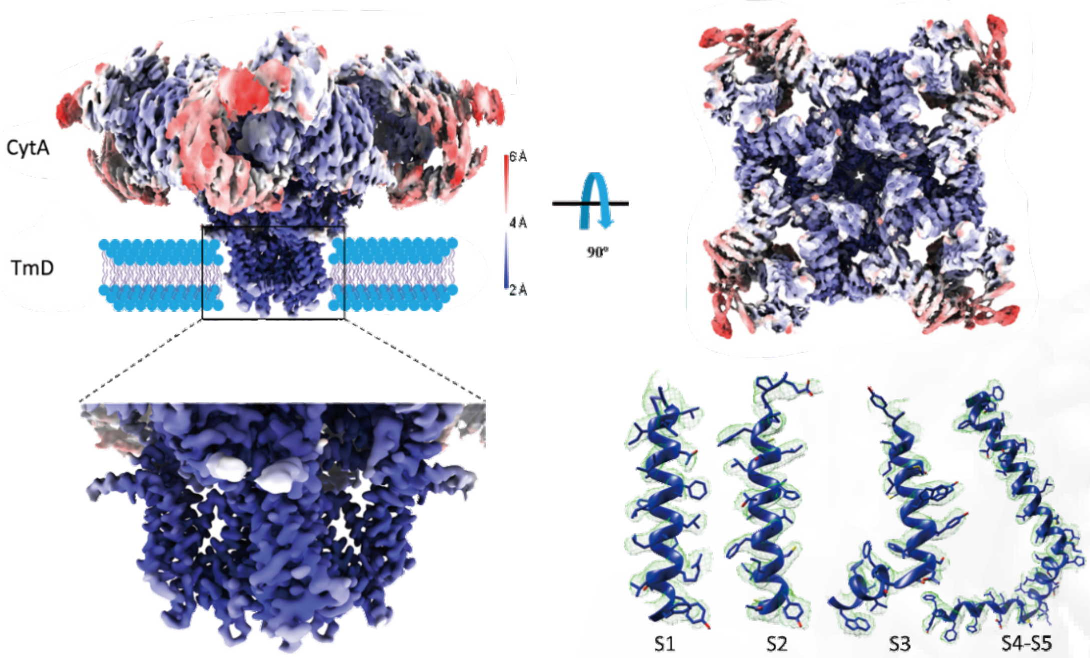


Image courtesy of Dimple Karia, *ThermoFisher Scientific*

CryoEM Showcases

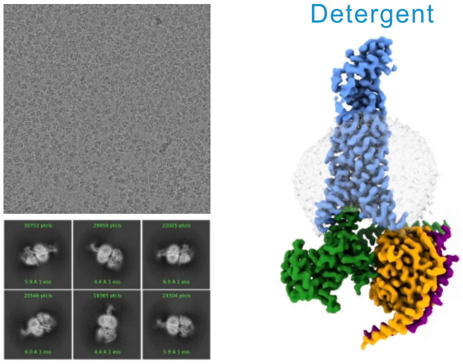
The Largest Known Ion Channel, RyR1



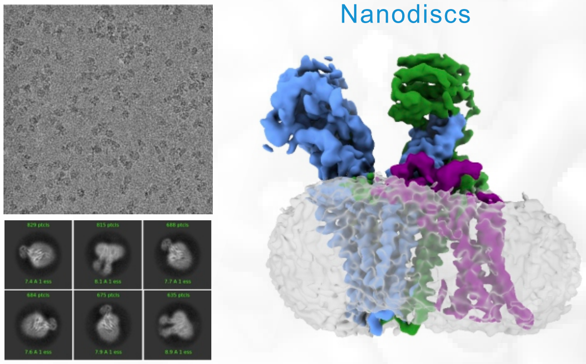
Our experienced team purified and solved a 2.8 Å CryoEM structure of RyR1 (2.3 MDa) from rabbit skeletal muscles within two weeks.

Multiple Solubilization Strategies for Membrane Proteins

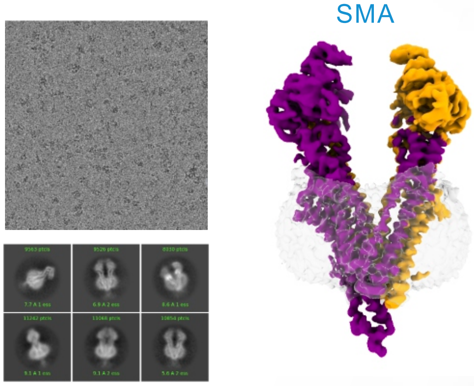
Detergent



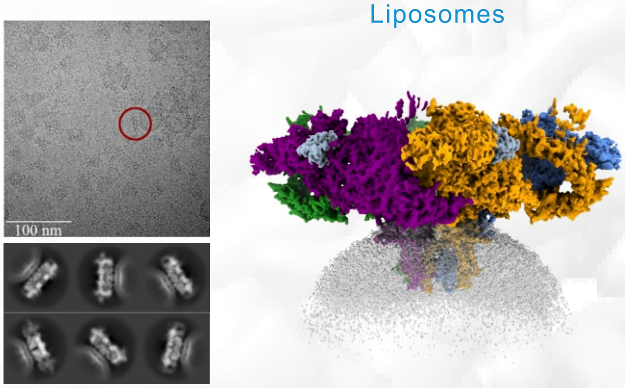
Nanodiscs



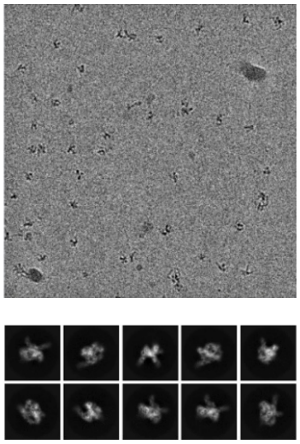
SMA

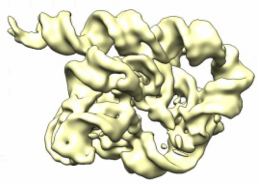


Liposomes

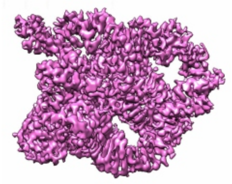


CryoEM Structure of RNA

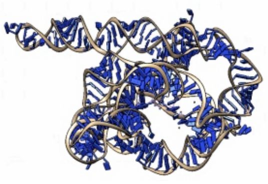




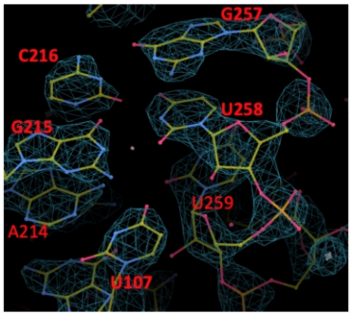
6.0 Å map



2.9 Å map



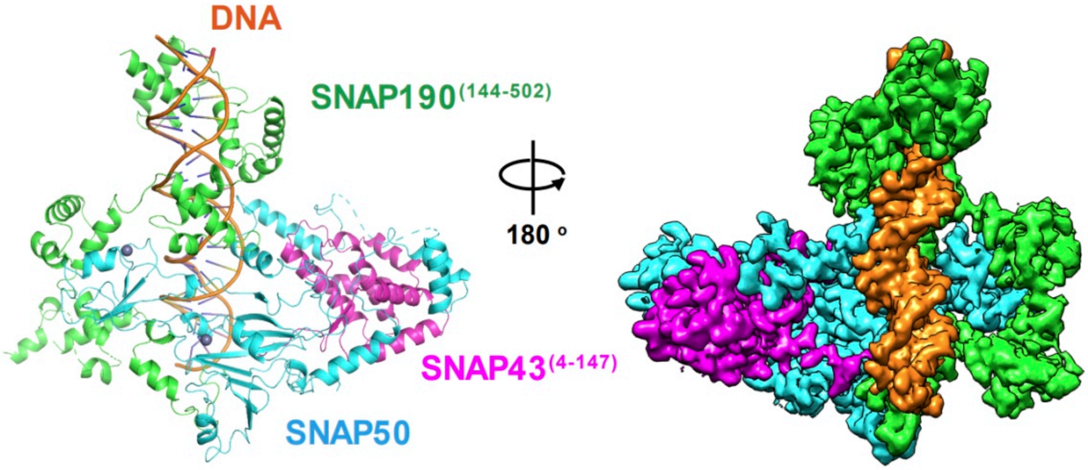
Model



Self-splicing intron
Bases: 387
MW: 125 kDa

CryoEM Structure of Human SNAPc with Shandong University

In collaboration with Professor Wei Wang of Shandong University, we resolved the structure of human snRNA activating protein complex SNAPc to 3.4 Å using CryoEM, furthering the understanding of Pol III dependent snRNA transcription.



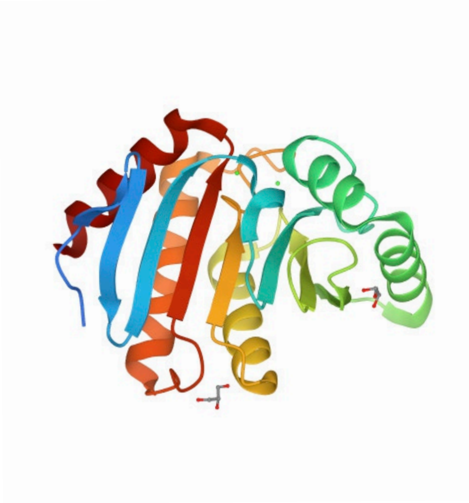
Sun, J., et. al, Structural basis of human SNAPc recognizing proximal sequence elements of snRNA promoter. *Nature Communications,* 2022

Structure Gallery

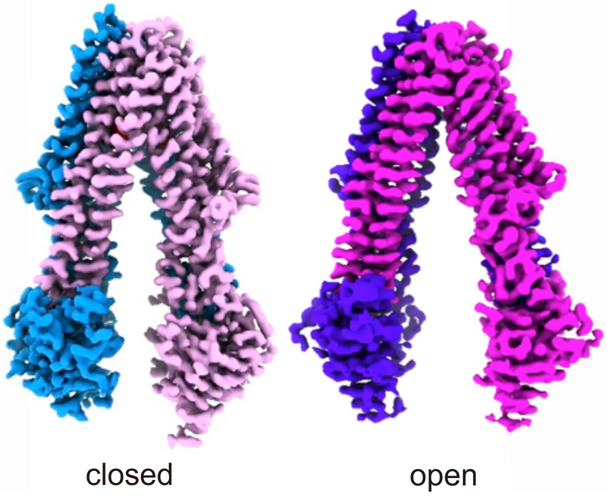
In order to expedite the SBDD process, Biortus has compiled a growing list of internal projects generated completely in-house from gene to structure to serve as examples of the quality of work that we can provide and as a launchpad for your project.

Please contact us at info@biortus.bio to receive the comprehensive list of structures in our gallery

PARP14
X-ray, 7D2C, 1.18 Å



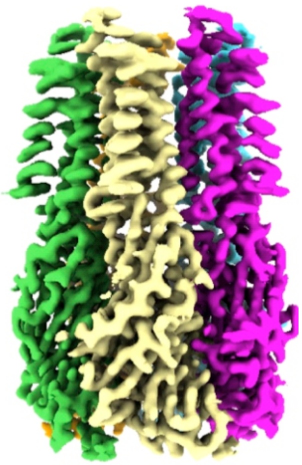
ABCB10, 2 conformations from 1 dataset
CryoEM, 2.8 Å



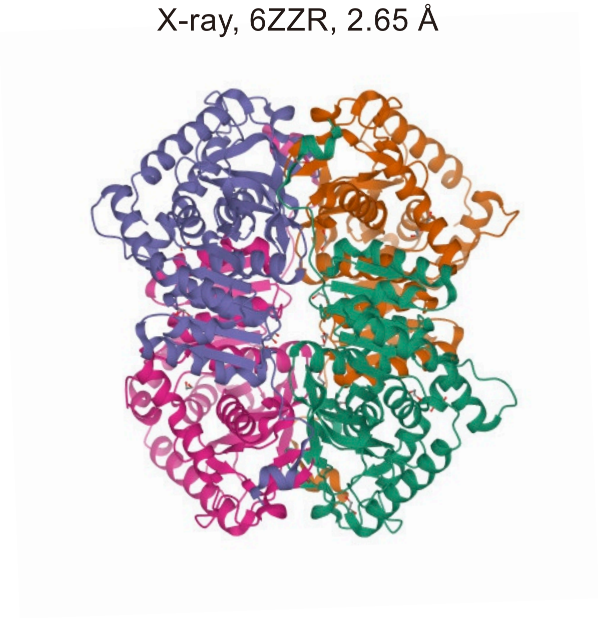
USP7
X-ray, 7CM2, 2.25 Å

BCL-2 like protein 1
X-ray, 7CA4, 2.70 Å

GABA$_A$R (β3)
CryoEM, 2.9 Å

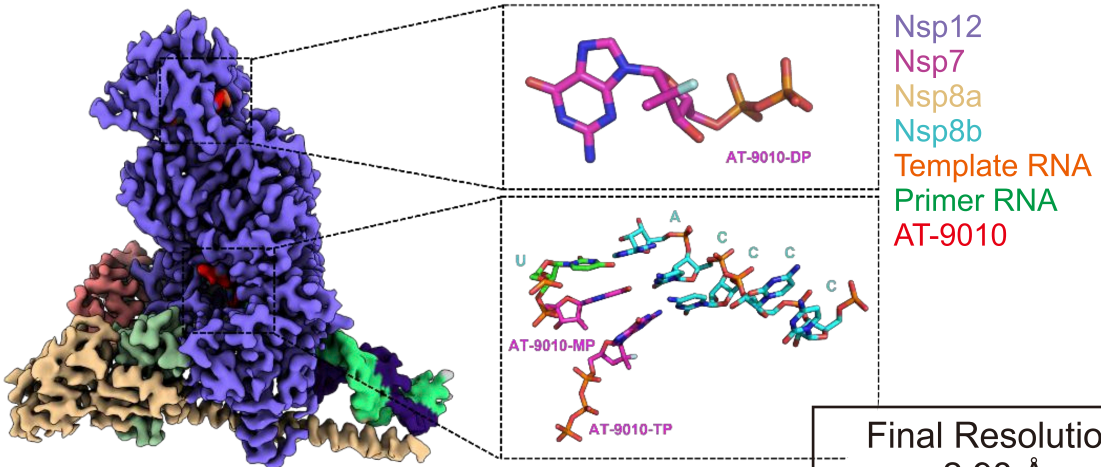


LDHA
X-ray, 6ZZR, 2.65 Å



Publication-Quality Work Is Our Standard

Co-complex structure of RNA and AT-9010 compound with RdRp for Atea Pharmaceuticals

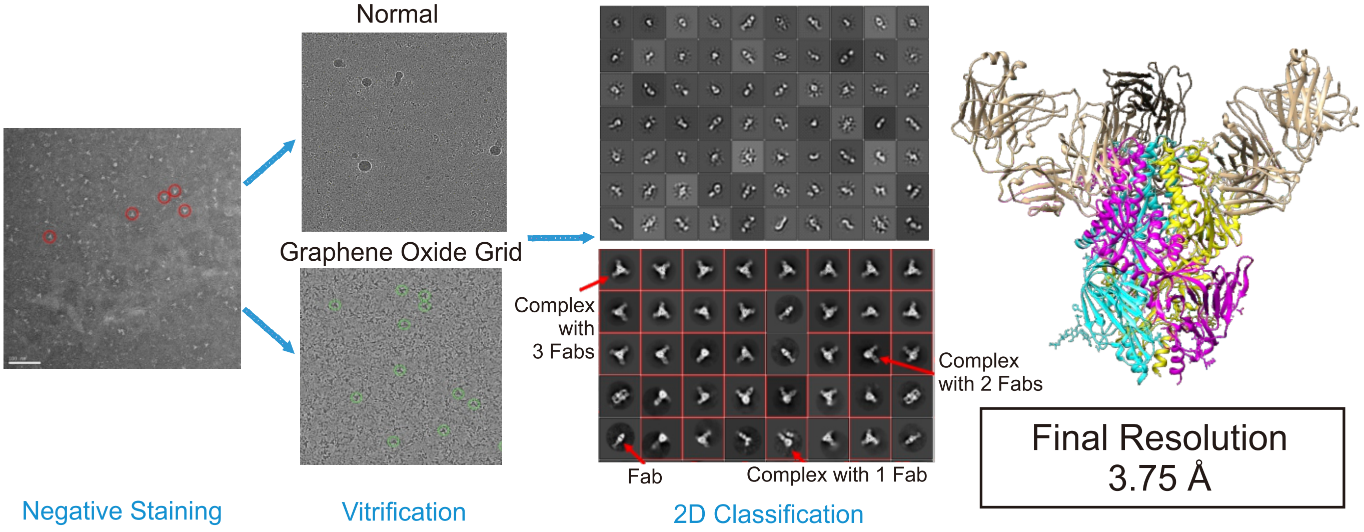


Final Resolution
2.98 Å

From gene to structure ~ 2 months

Shannon A., et. al, A Dual Mechanism of Action of AT-527 against SARS-CoV-22 polymerase
Nature Communications, 2022

CryoEM structure of Fabs in complex with hMPV for Merck



Final Resolution
3.75 Å

Xiao, X., et. al, Profiling of hMPV F-specific antibodies isolated from human memory B cells.
Nature Communications, 2022

For the complete list of publications, please visit our website at
https://en.biortus.bio/

2024.08.USA

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