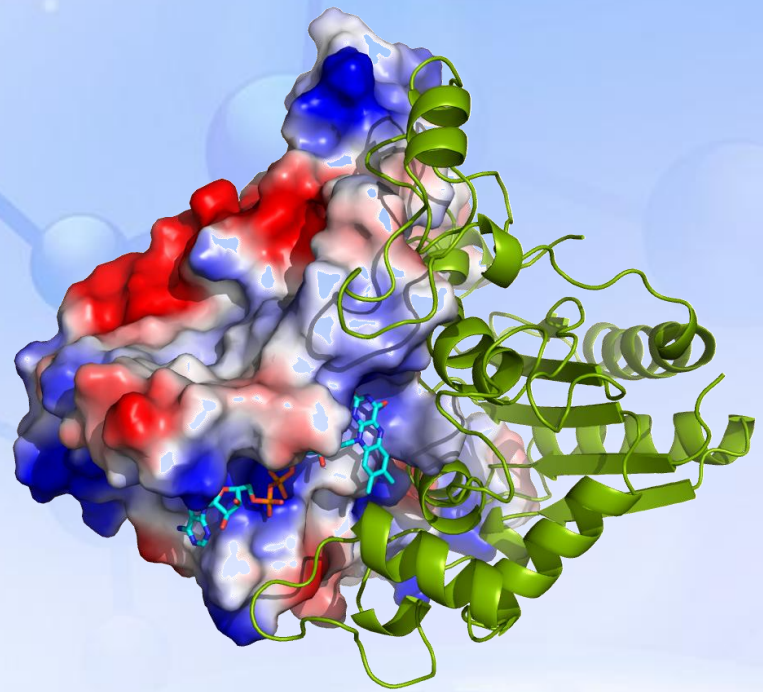
Membrane Protein Preparation

包晓明

20250314



Mature Membrane Protein Platform

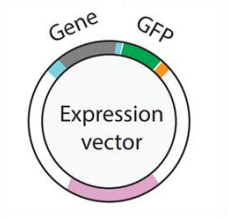
BIORTUS

Complexities of Membrane proteins	Biortus Strategies
Notorious for low expression levels Requires multiple strategies and rounds of optimization	**FSEC screening platform:** Allows for high throughput parallel screening of constructs, expression systems and purification conditions
Preservation of stability, homogeneity and activity during purification Maintaining endogenous integrity in predominantly aqueous environments during purification requires expertise	**Detergent platform:** Led by experienced scientists with over 300 membrane proteins purified **Nanodisc platform:** Native nanodisc, protein-based nanodisc, amphipols
Limited strategies in structure determination Traditionally, most membrane protein structures have been resolved by X-ray crystallography however, success rate has been extremely low.	**Nanobody screening platform:** in-house top tier CryoEM facilities gives us a wider range of options for structure determination

eGFP: All-in-One Tag for MPs Expression and Purification

eGFP tag: rapid screenings of expression systems, constructs, and detergents for solubilization, affinity purification

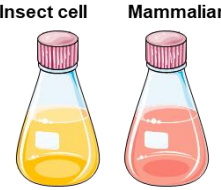
Construct design



- Homolog screening;
- Protein engineering;
- Affinity tags & vectors;
- N/C terminal truncation;
- Loop deletion;
- Mutagenesis;
- Fusion partners (T4L, BRIL…);
- …

1-2 weeks

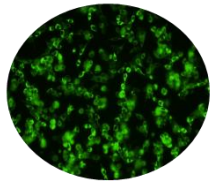
Expression system



✓ Insect cell expression:
System: Bac-to-Bac
Cell: Sf9, Sf21 & Hi5

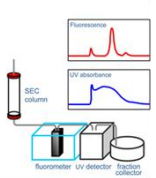
✓ Mammalian cell expression:
System: Transient Transfection; BacMam
Cell: 293F; Expi293F; 293F-GnTI⁻;

Small scale expression test



✓ Rapid expression screening

✓ Sub-cellular localization in host cell

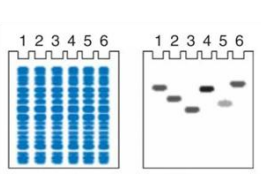


✓ High-throughput screening monodisperisty for constructs and solubilization conditions

✓ Estimation of MPs overexpression level

✓ No need for purification

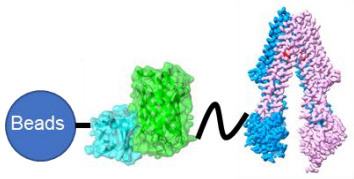
✓ Less than 5 ml expression media



✓ Confirm the expression of intact MPs

2-3 weeks

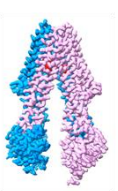
Large scale expression and purification



✓ Catch the eGFP-tagged protein with high specificity

✓ Higher purity than the TALON/Streptactin resin

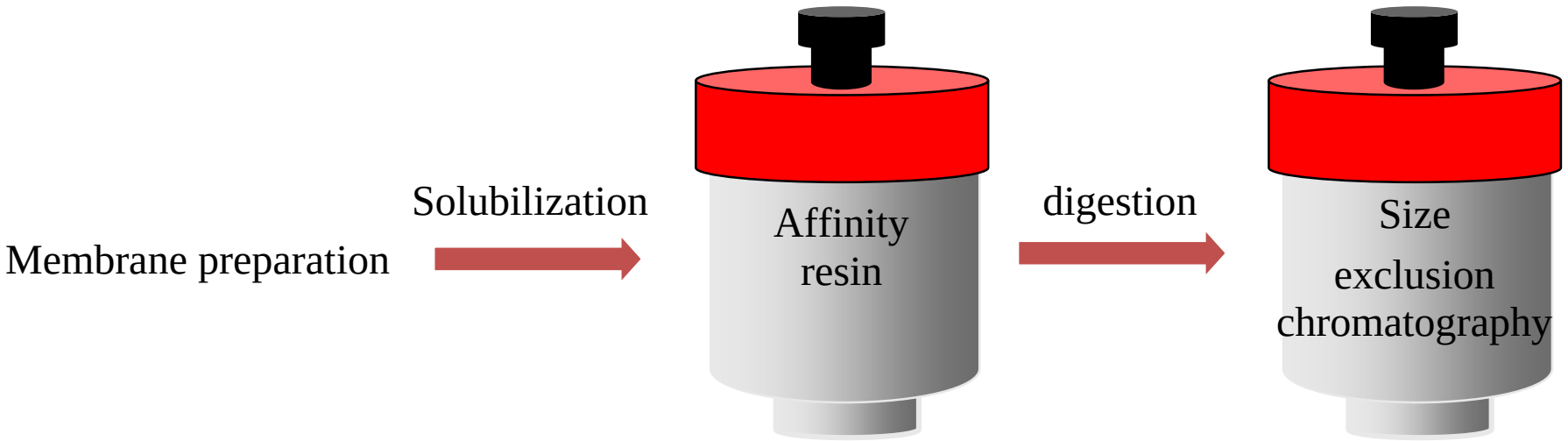
✓ Need to cleave the eGFP tag



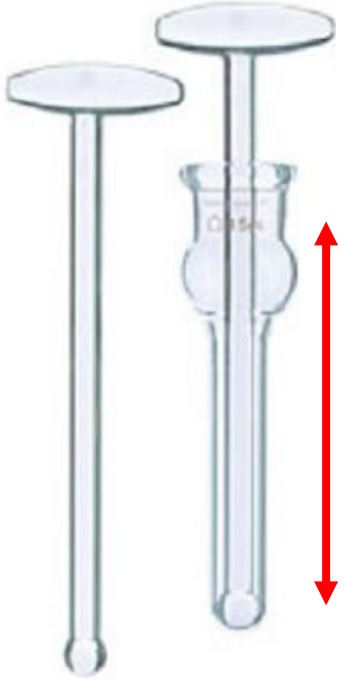
✓ Downstream application:
Nanodisc reconstitution
SPR assay;
TRIC assay;
Activity determination;
Thermal shift assay
Structure determination;

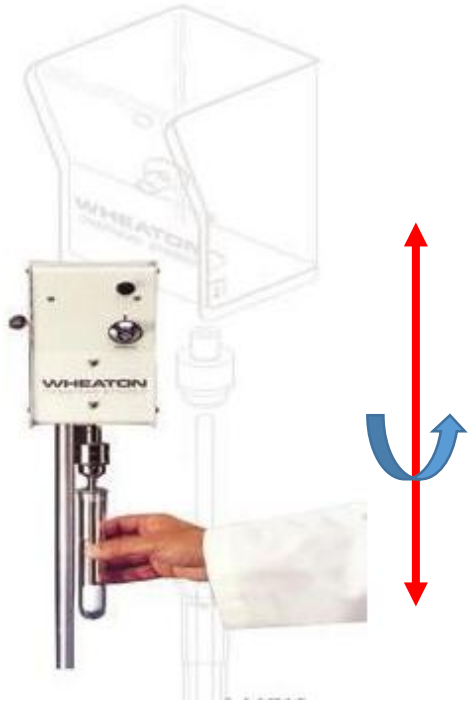
1-2 weeks

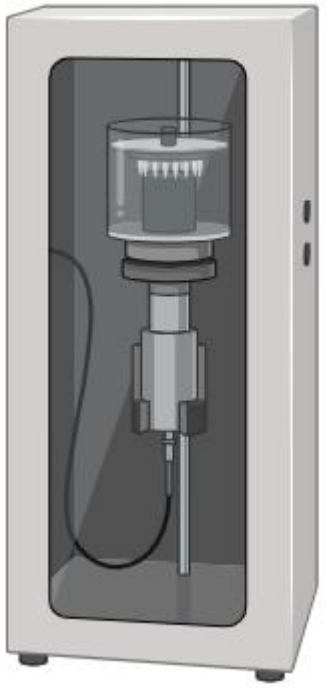
Membrane Protein Purification



Cell Lysis and Membrane Preparation



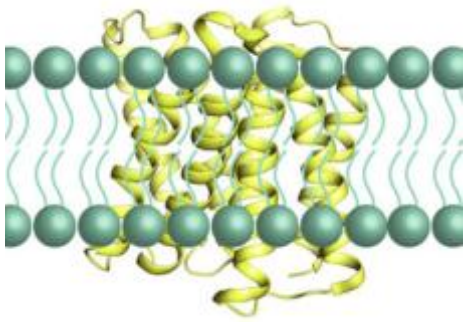




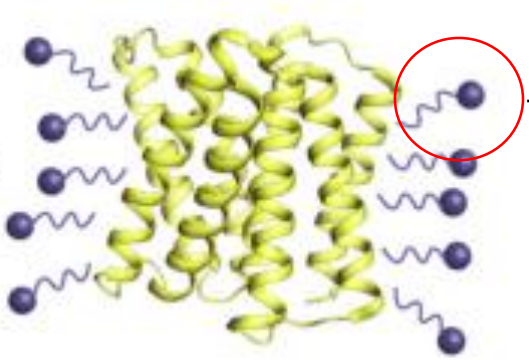
Hypotonic Lysis **Manual Dounce** **Electric Dounce** **Sonication Homogenization** **High-Pressure Homogenization**

Centrifuge

Solubilization

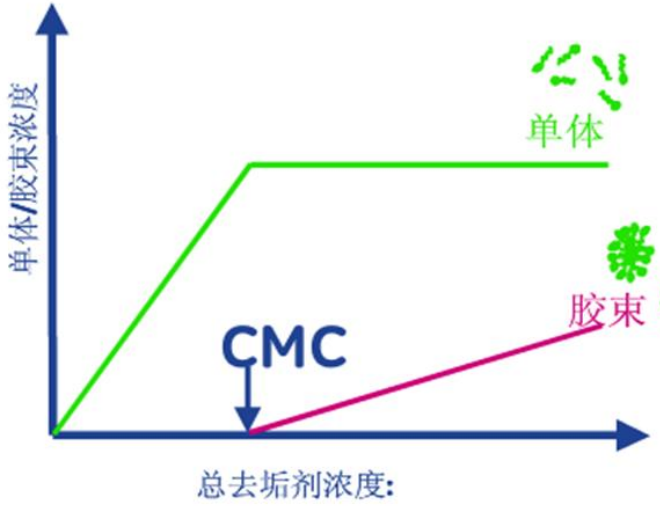


Detergent

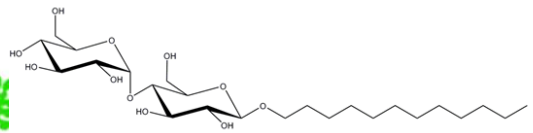


Hydrophilic head

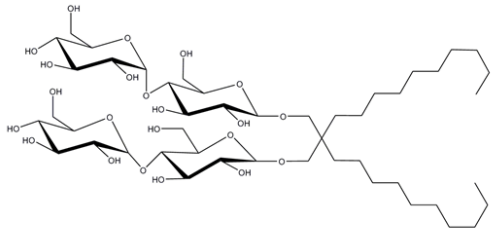
Hydrophobic tail



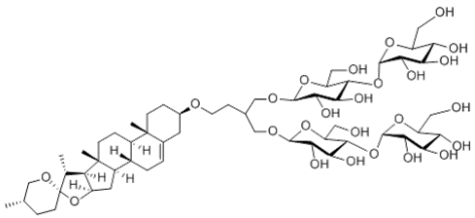
- 温度、盐和去垢剂类型影响 CMC
- 胶束大小和聚集的数目有关



DDM (CMC: 0.0087%)

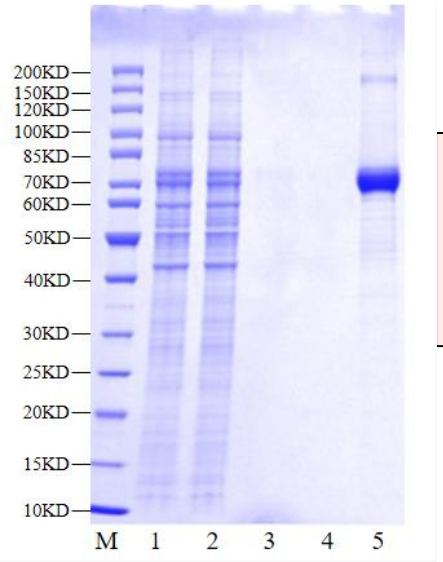


LMNG (CMC: 0.001%)

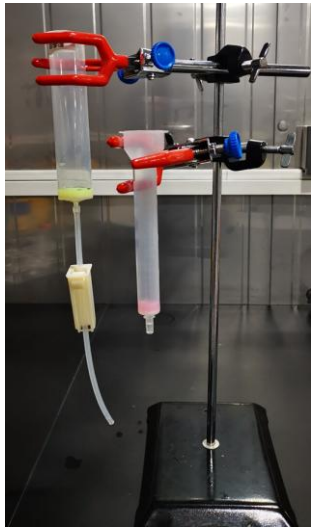


GDN (CMC: 0.0021%)

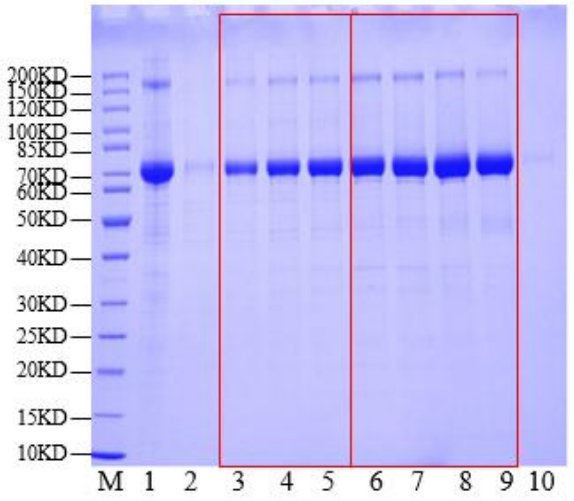
Purification-Traditional Method



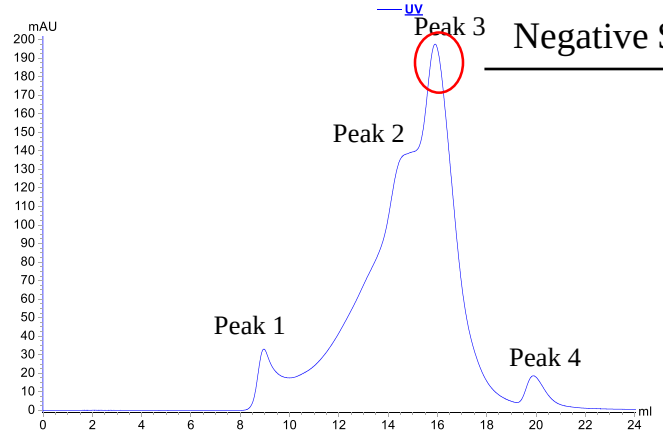
M = Marker
Lane 1 = Load
Lane 2 = Flow through
Lane 3 = Buffer A wash
Lane 4 = Buffer B wash
Lane 5 = Buffer C elution



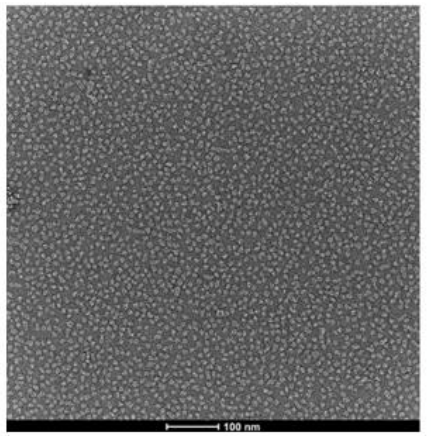
Gravity settling !
Wash step by step !
Avoid bubbles !



M = Marker
Lane 1 = Load
Lane 2 = Peak 1
Lane 3-5 = Peak 2
Lane 6-9 = Peak 3
Lane 10 = Peak 4

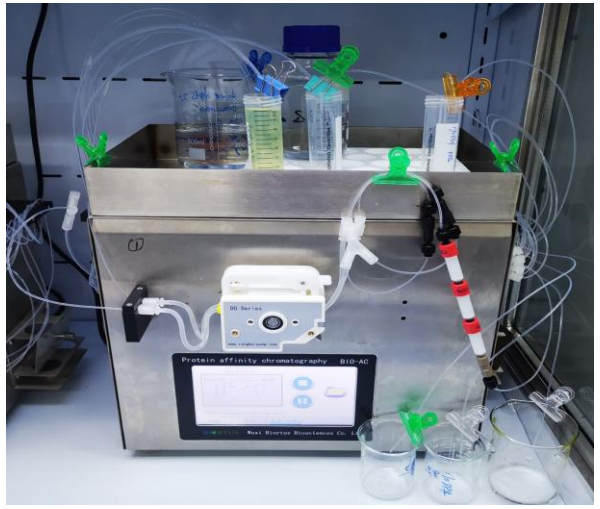


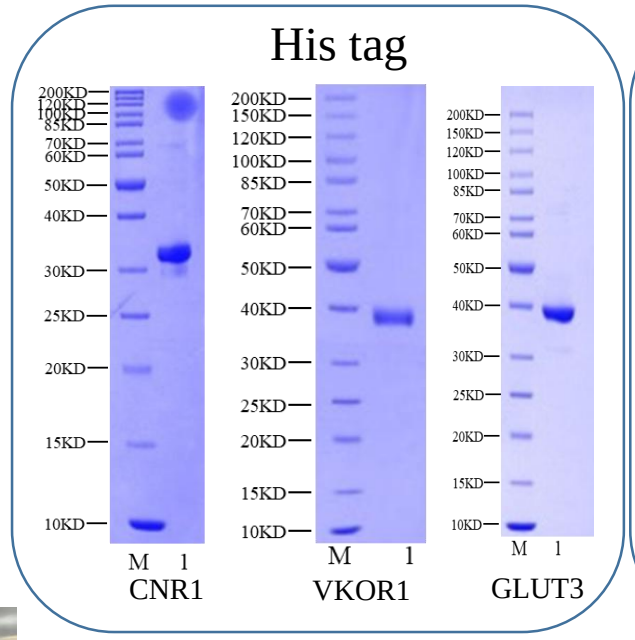
Negative Staining

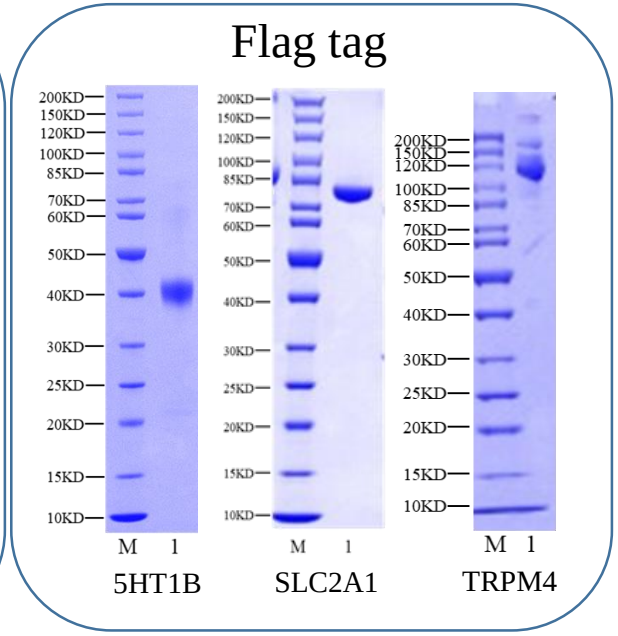


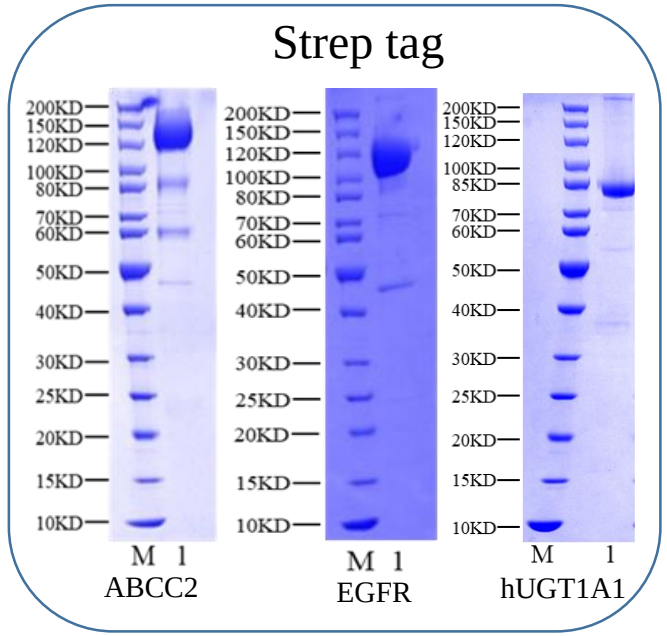
Purification-Automatic Method

BIORTUS





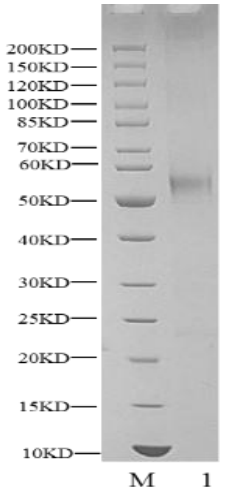




Resin	Manual purification yield	Automatic purification yield
His	1.1mg/L	1.46mg/L
Anti Flag	1.38mg/L	1.48mg/L
eGFP	1.73mg/L	2.06mg/L

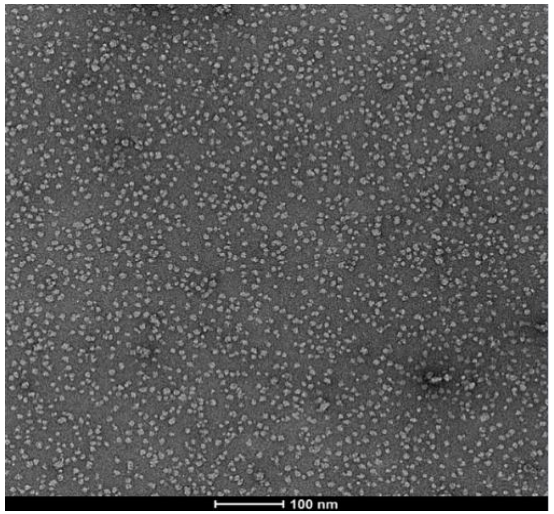
QCs

QC1: SDS-PAGE



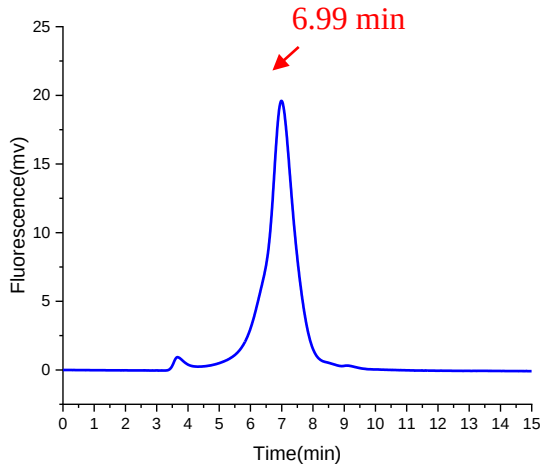
M = Marker
Lane 1 = 2 µg

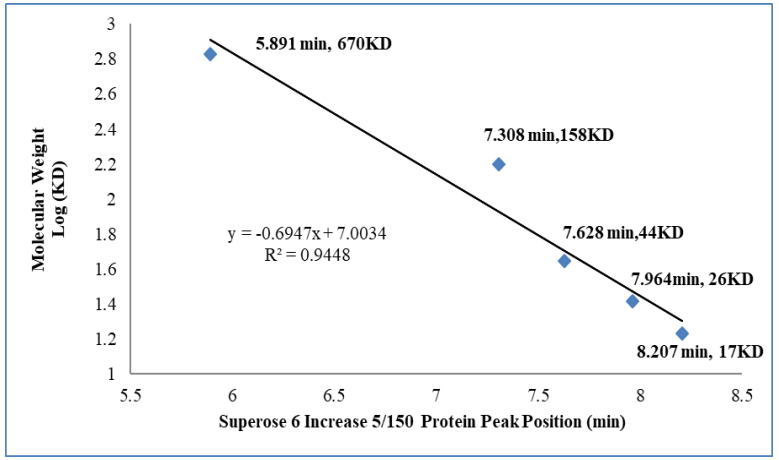
QC3: Negative staining



QC2: Analytical SEC

QC buffer: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.05% (w/v) LMNG, 0.005% (w/v) CHS

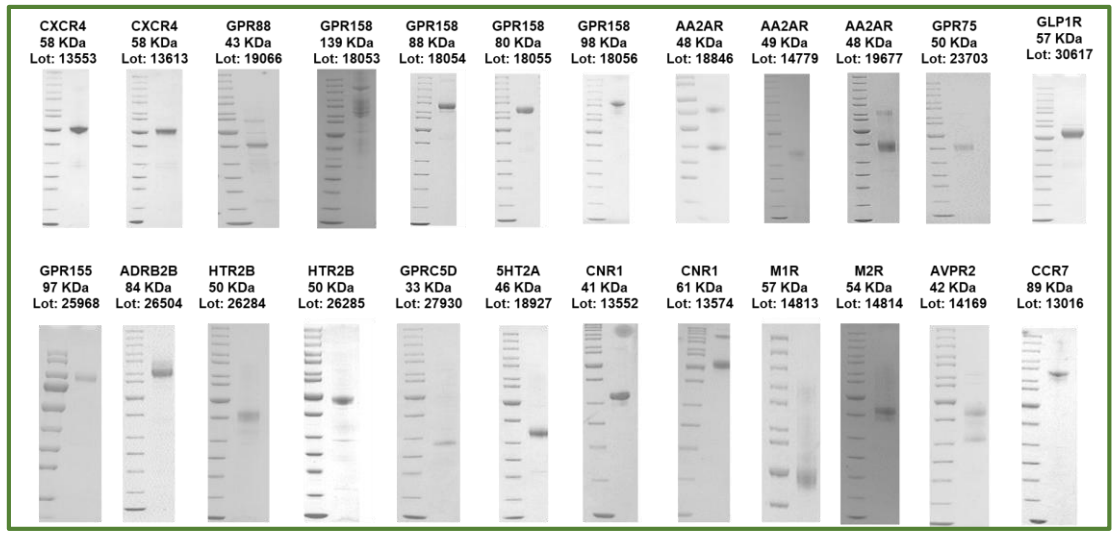




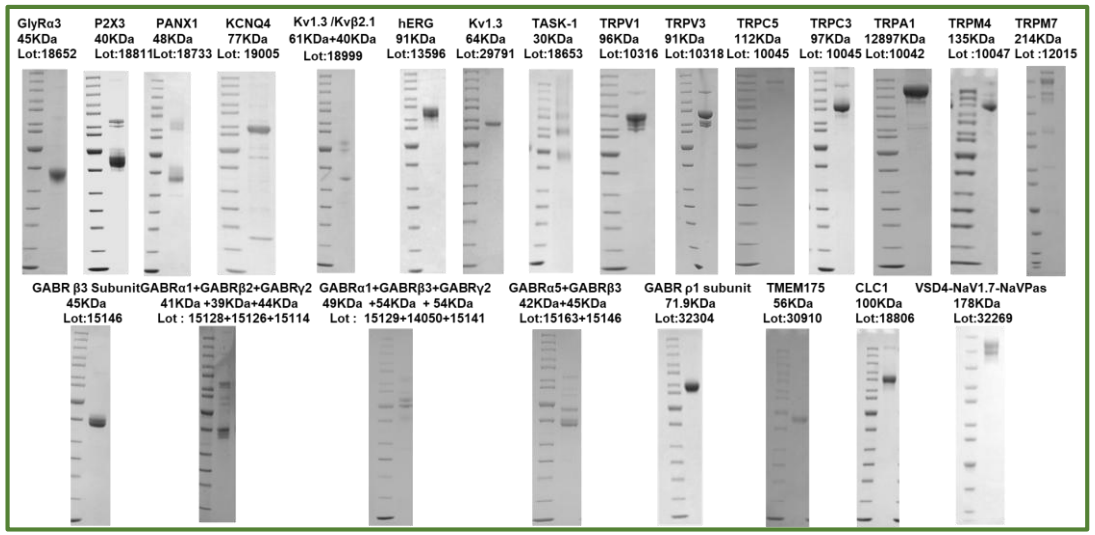
Membrane Proteins: >300 (unique) purified!

BIORTUS

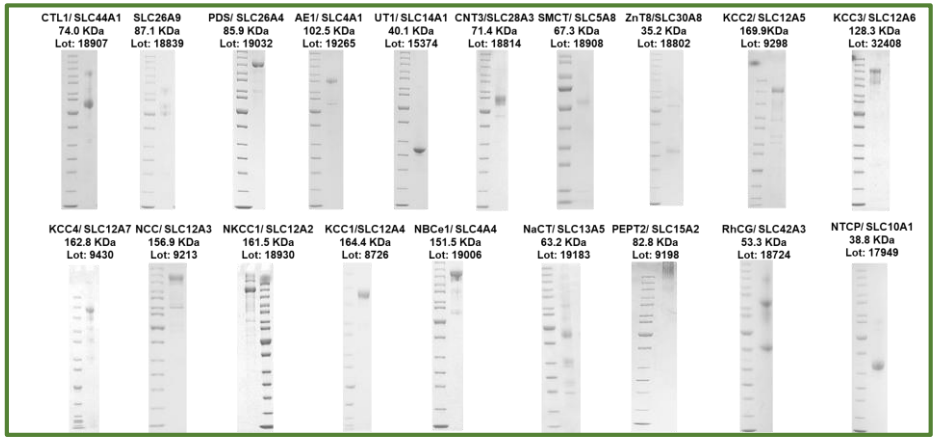
GPCRs



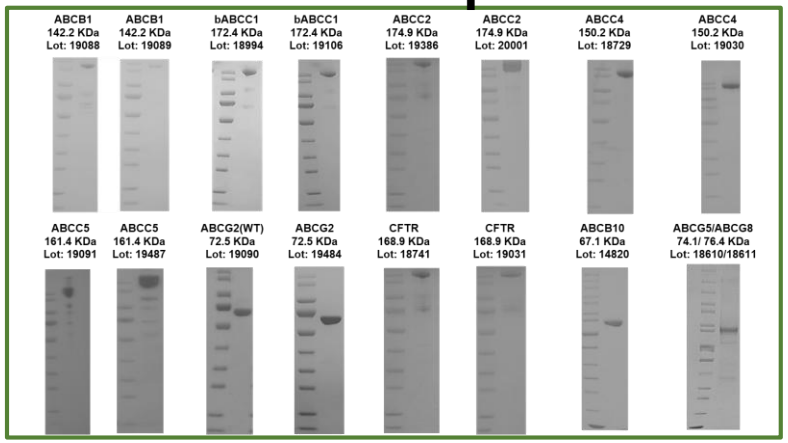
Ion channels



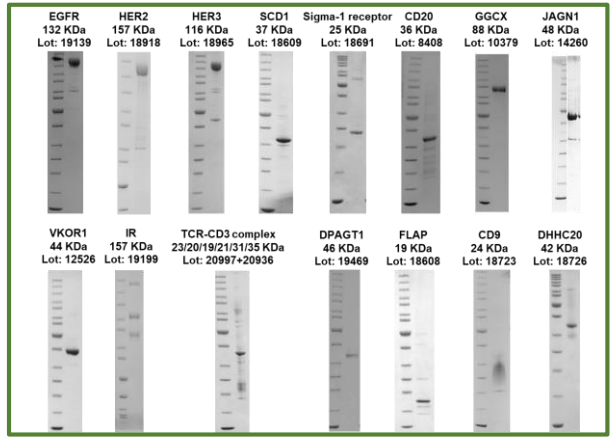
SLCs



ABC transporters

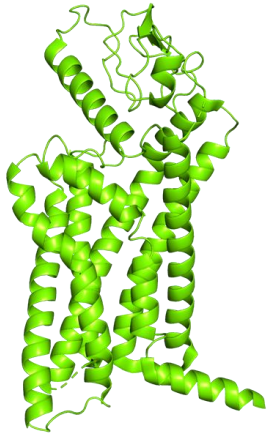


Other MPs



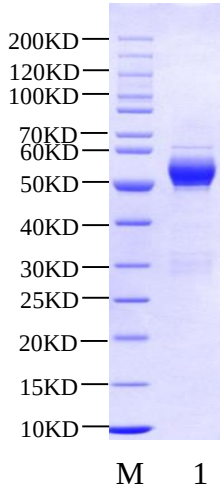
Show Cases-GPCR

BIORTUS

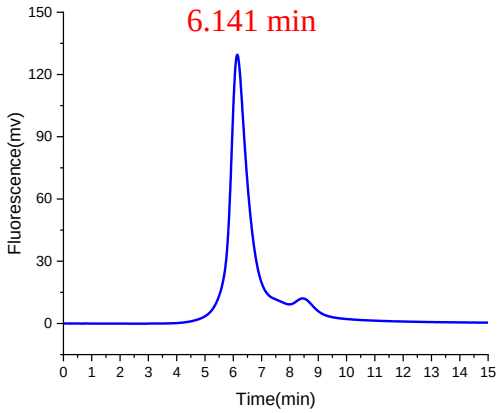


GLP1R
(PDB:6LN2)

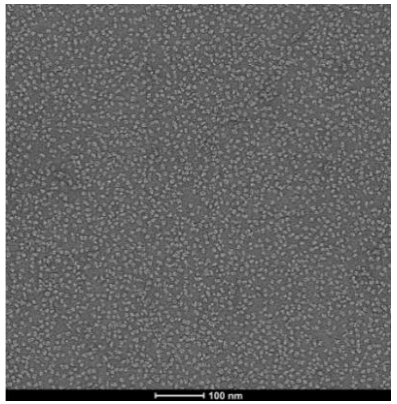
SDS-PAGE



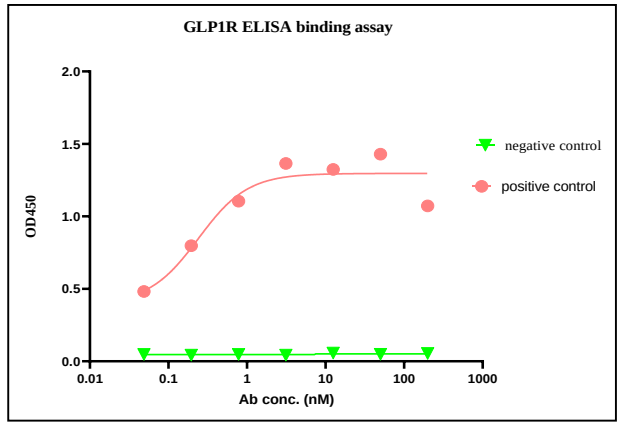
HPLC



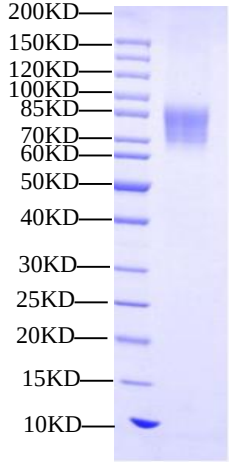
Negative staining

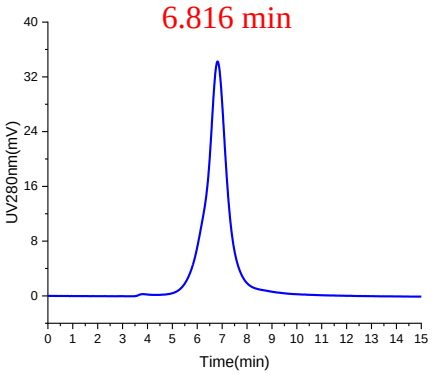


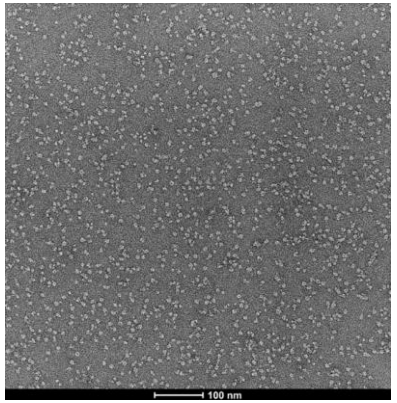
ELISA

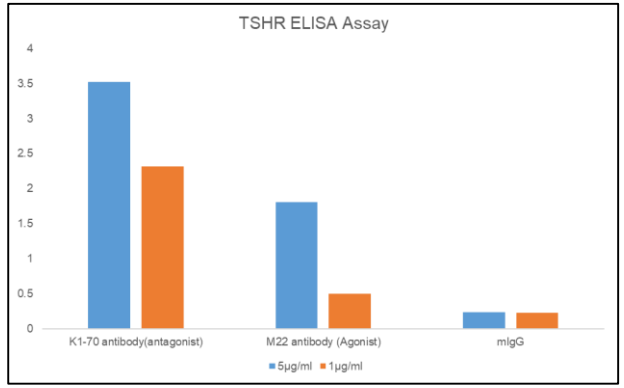


TSHR
(PDB:6T9M)







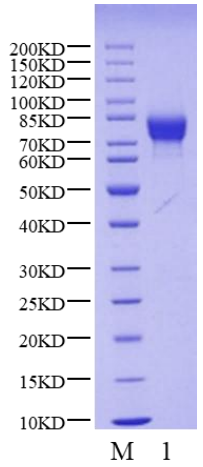


For custom: info@biortus.bio For off-the-shelf: order@biortus.bio

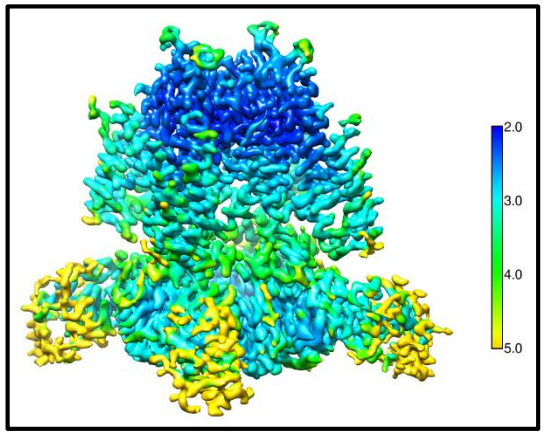
Show Case-hERG

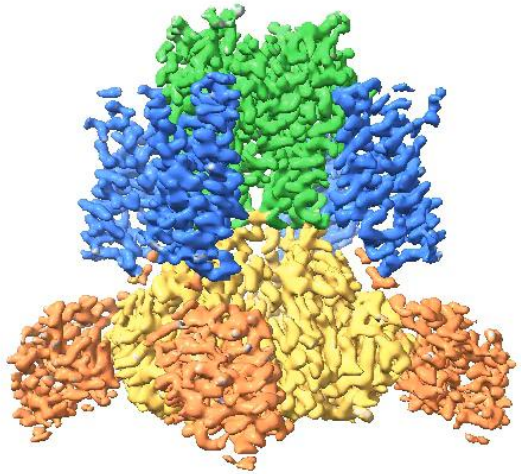
BIORTUS

SDS-PAGE



Cryo-EM





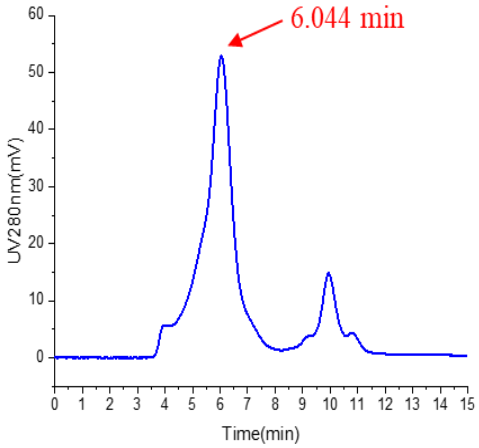
Core region

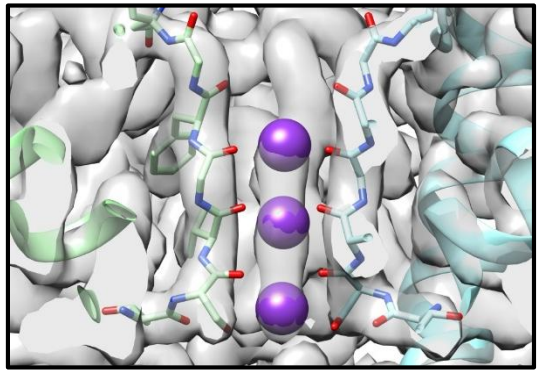
Voltage sensor

CNBHD

PAS

HPLC



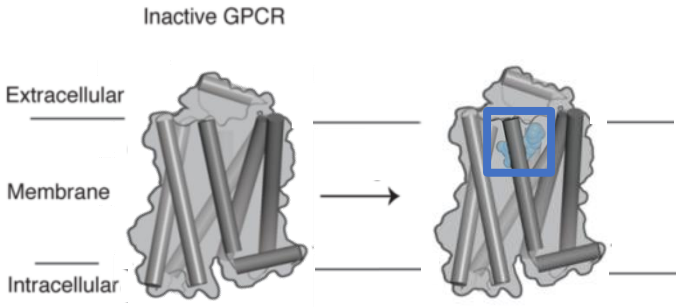


- **The highest-resolution cryoEM structure of hERG has been released in EMDB**
- **Most amino acid side chains are clear**
- **Three potassium ion molecules are observed**

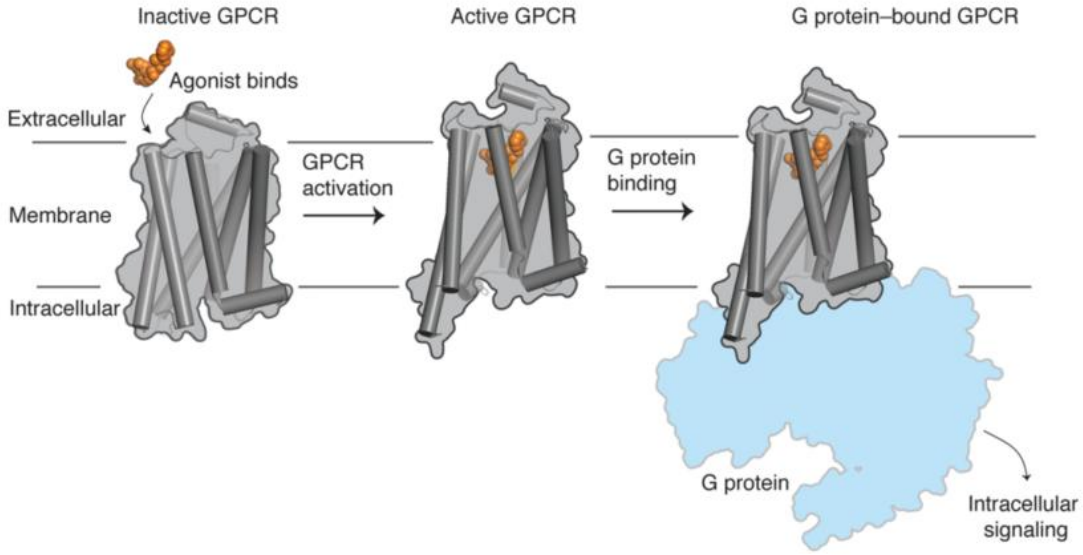
GPCR: Inactive and Active State

BIORTUS

Inactive State
Antagonist binding MOA



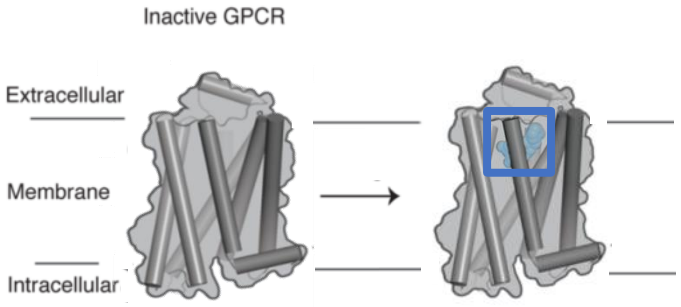
Active State
Agonist binding MOA



Adapted from Latorraca, N.R. et al, *Chemical Reviews*, 2016

GPCR Inactive State

Inactive State
Antagonist binding MOA



Strategies employed:

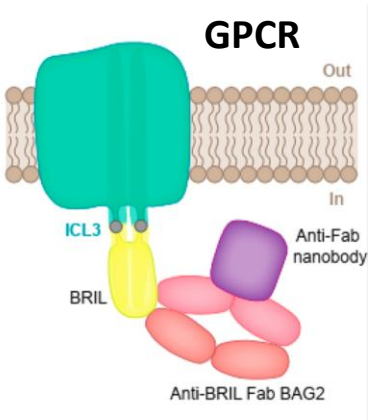
- BRIL fusion tethering
- Nanobody stabilization
- Conformation locking

Adapted from Latorraca, N.R. et al, *Chemical Reviews*, 2016

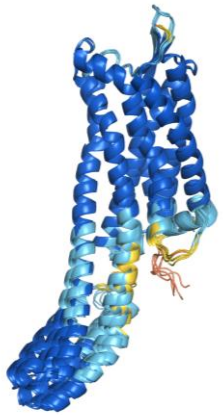
Workflow towards a GPCR (Inactive State) Cryo-EM Structure

BIORTUS

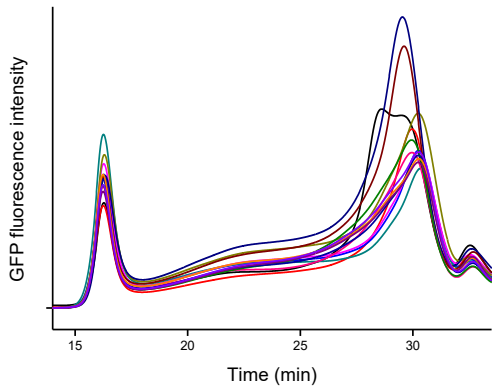
Tethering strategy



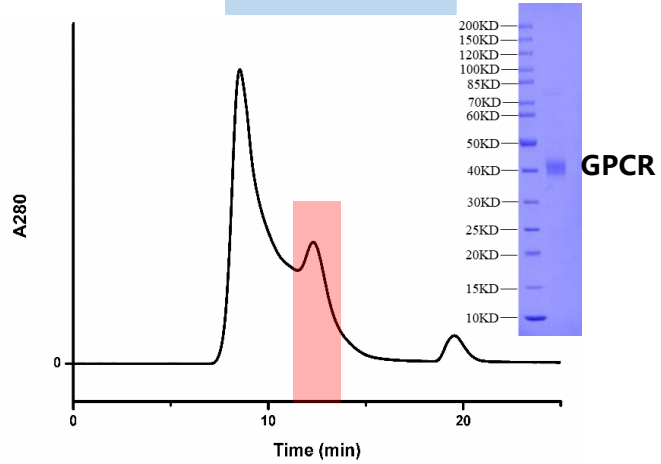
AF2 prediction



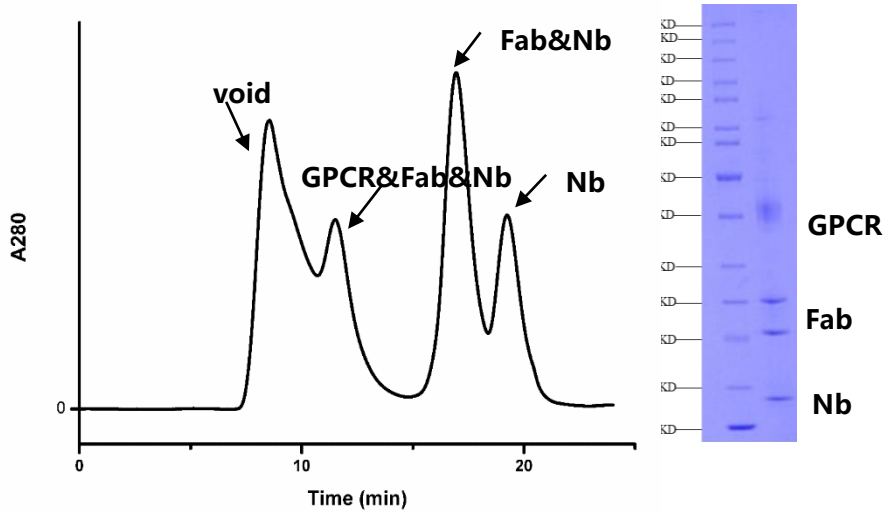
FSEC screening



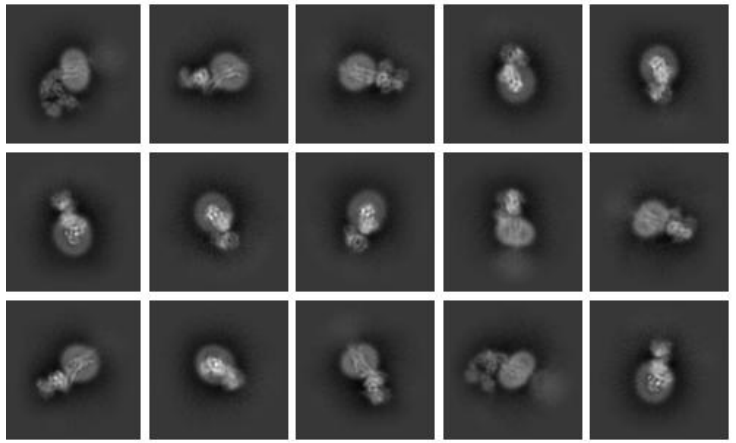
SEC

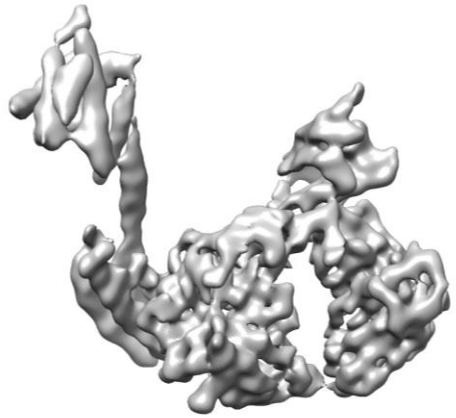


Complex assembly



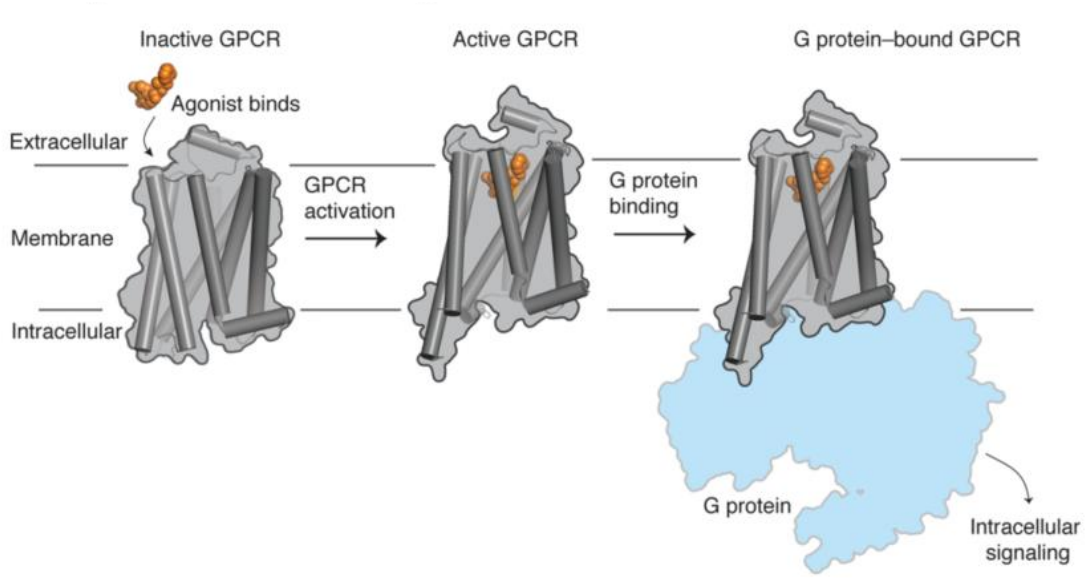
2D classification





GPCR Active State

Active State
Agonist binding MOA



Strategies employed:

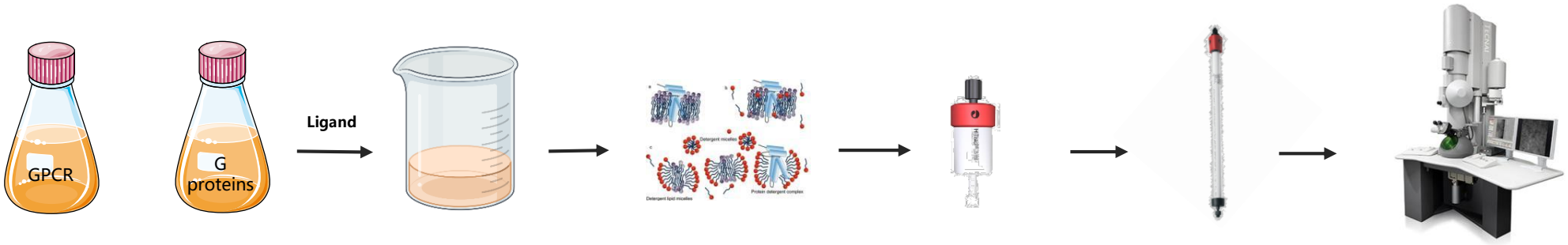
- GPCR-G protein complex

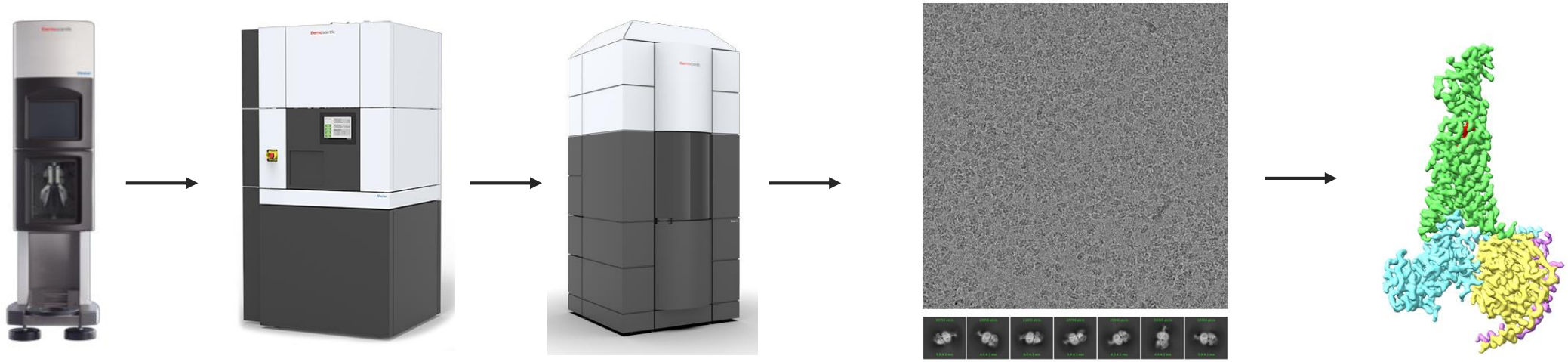
Case studies

- GLP1R
- SIPR3
- CCL1-CCR8

Adapted from Latorraca, N.R. et al, *Chemical Reviews*, 2016

Workflow towards a GPCR (Active State) Cryo-EM Structure





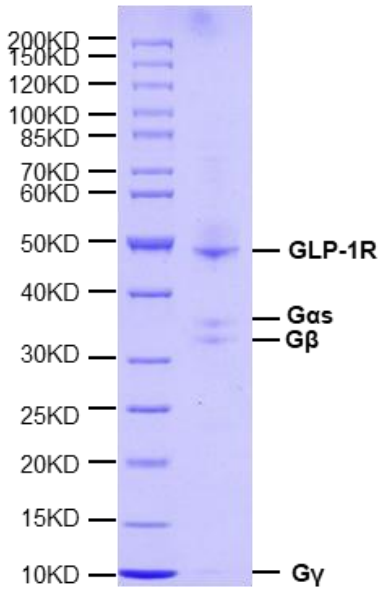
Glacios:
Falcon 4

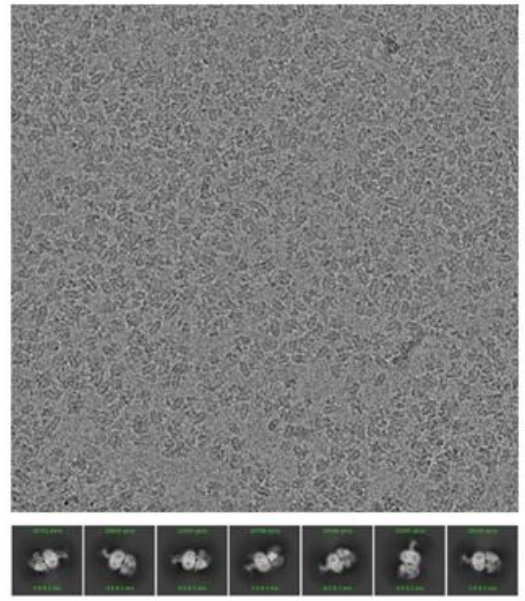
Titan Krios G4:
GIF/K3/Phase plate

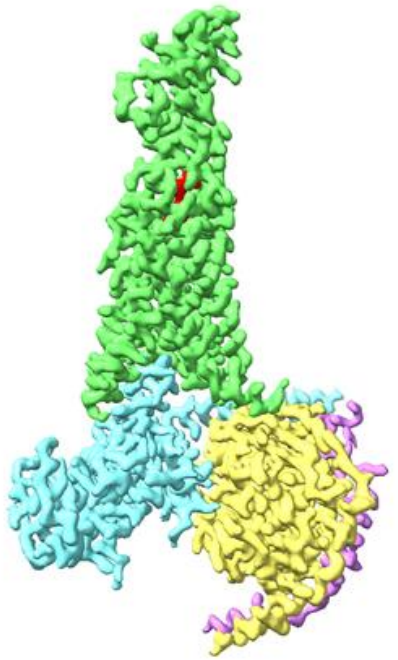
Show Case

BIORTUS

- **GPCR-Gs complex: Multiple complex structures to 2.7 Å**







Agonist

GLP-1R

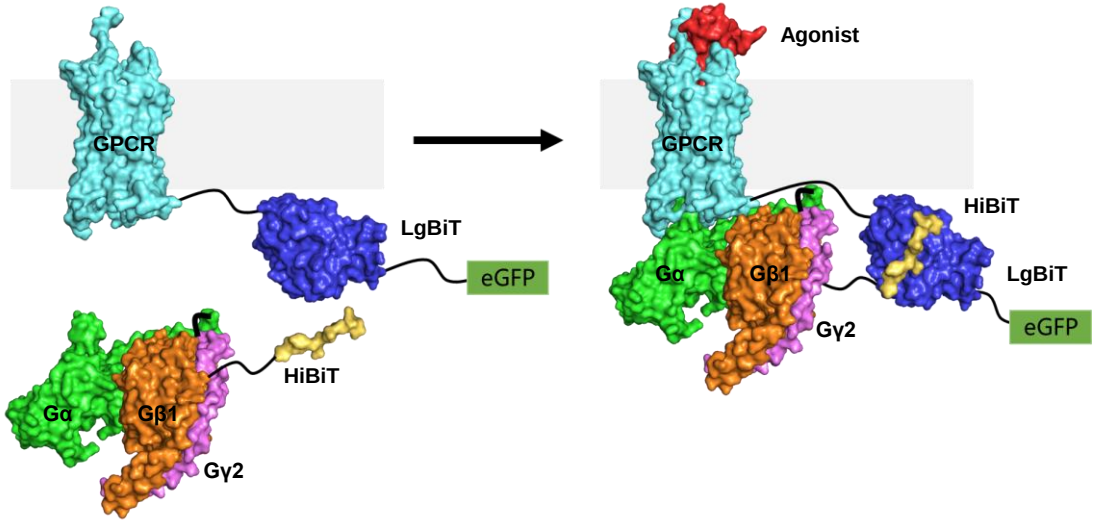
Gαs

Gβ

Gγ

Gene to structure ~1-2 months

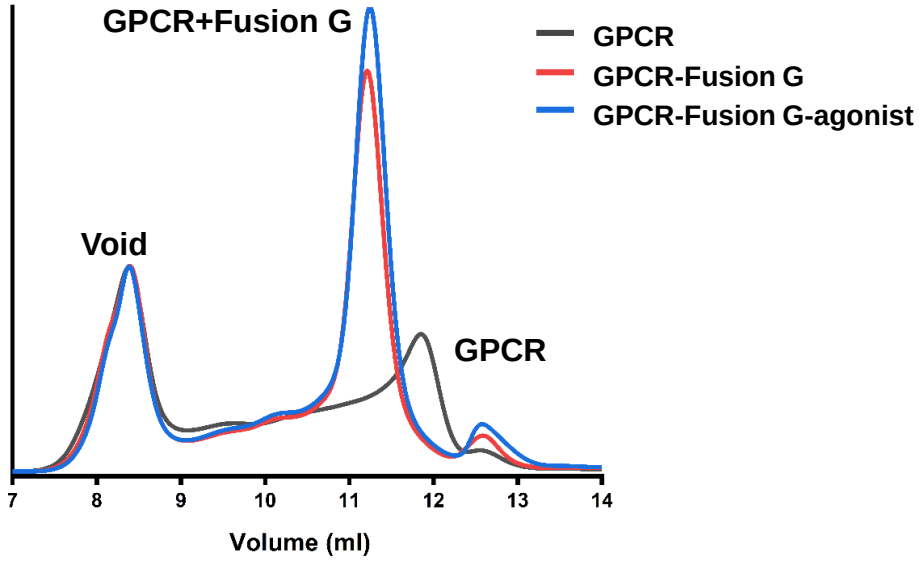
Fusion G system



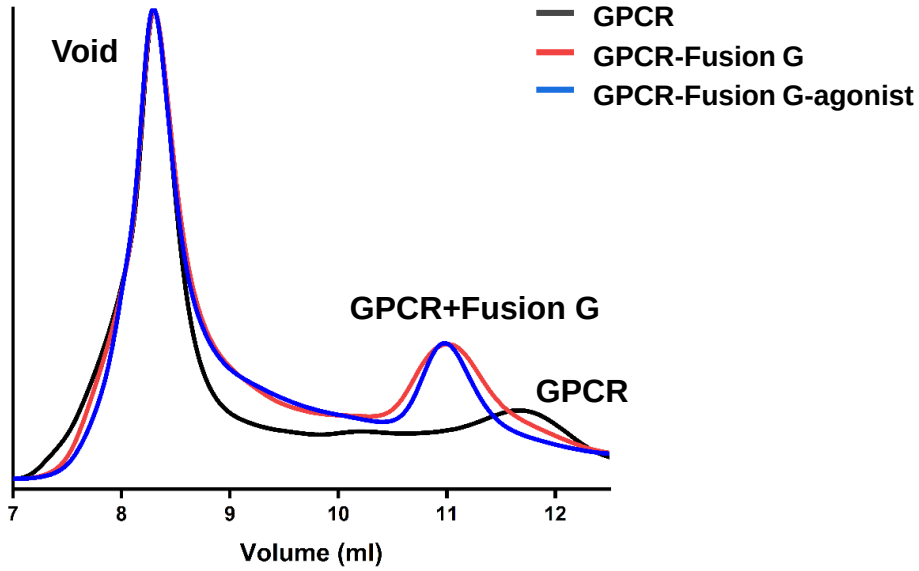
- Tool Box: Fusion Gs/Gi/Gq/Go system
  a. 3-in-1 vector for G protein expression
  b. NanoBiT tethering strategy
  c. Increase the proportion of the complex
- FSEC screening:
  effective option for comprehensive structural analysis of various GPCR-G protein complexes (insect cell or Mammalian cell)
- One step purification: anti-eGFP nanobody resin

FSEC analysis of complex formation

Insect cell



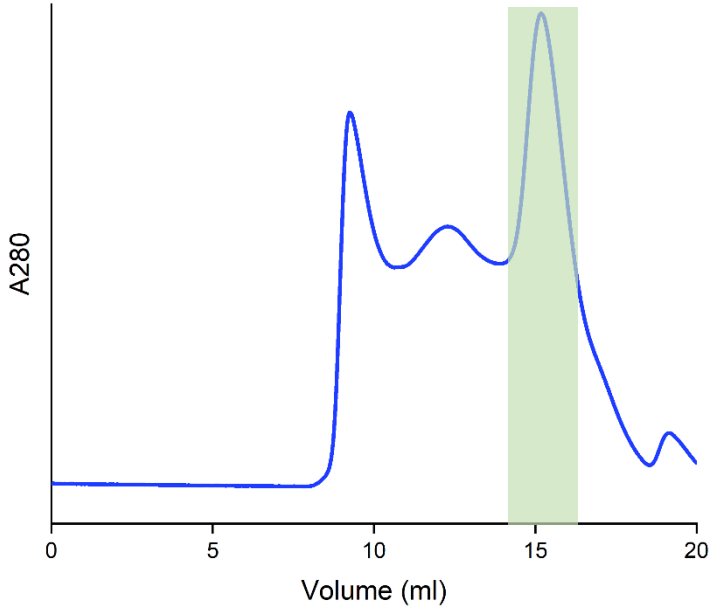
Mammalian cell

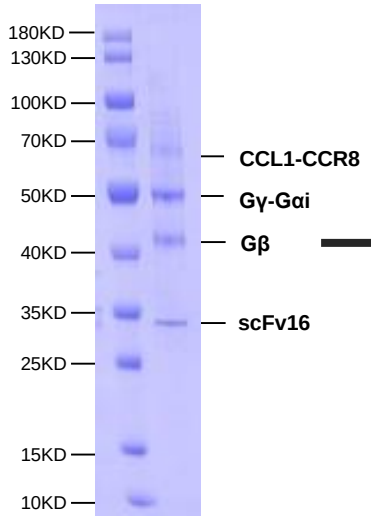


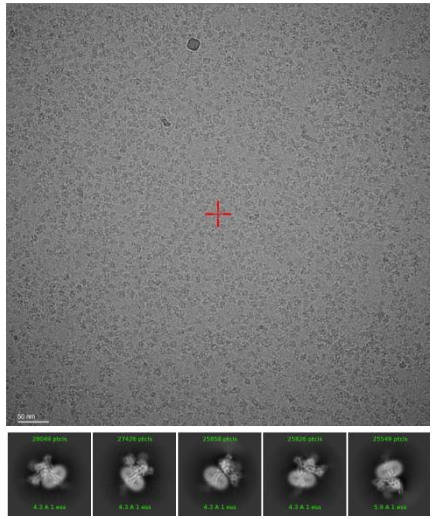
Fusion G system: Mammalian cell

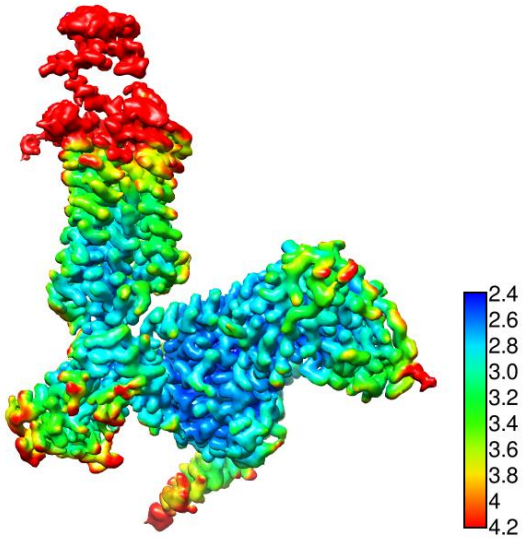
BIORTUS

- **CCL1-CCR8-Fusion Gi complex structure to 2.7 Å**





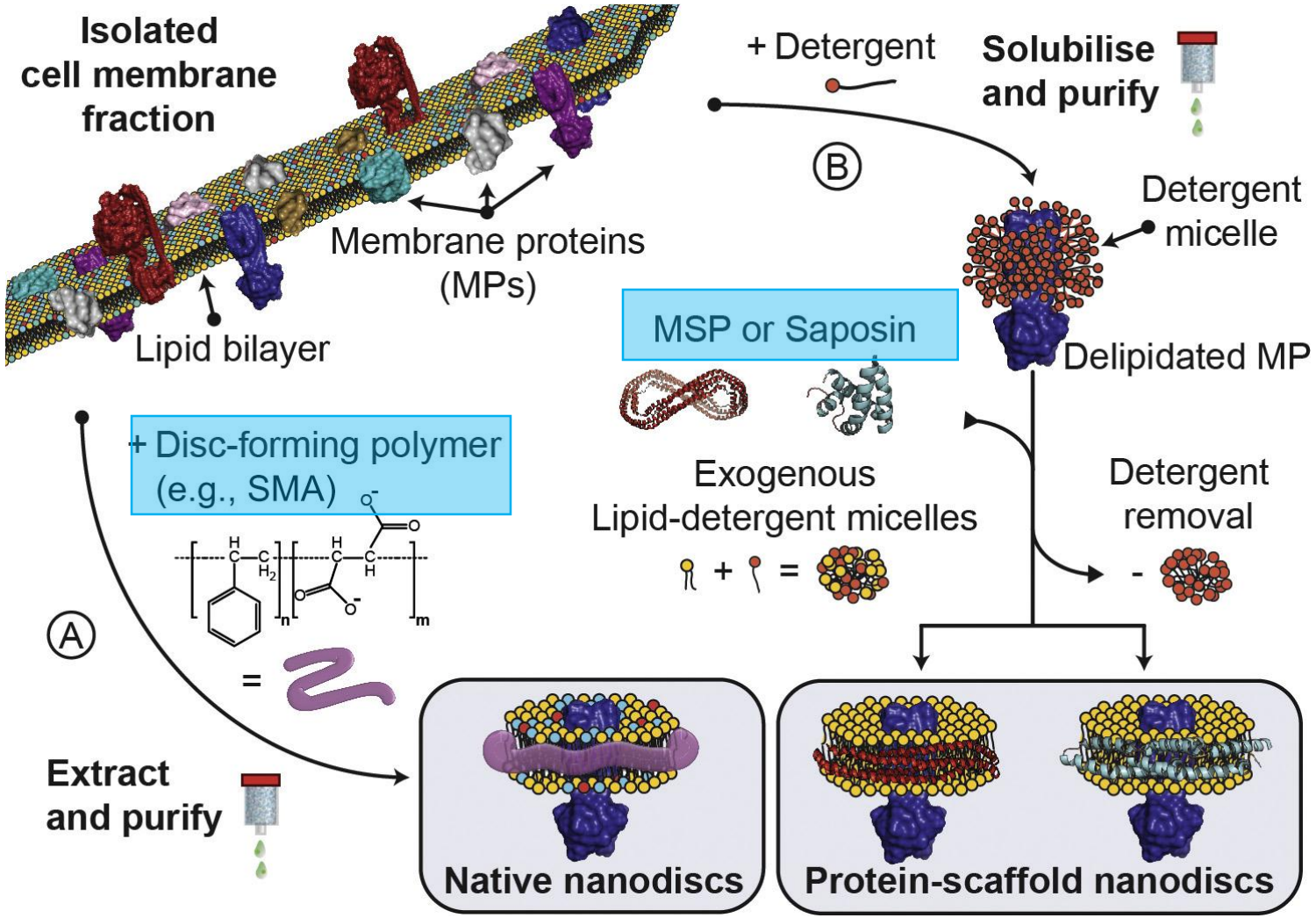




Gene to structure ~1-2 months

Membrane Proteins Stabilized in Aqueous Solutions

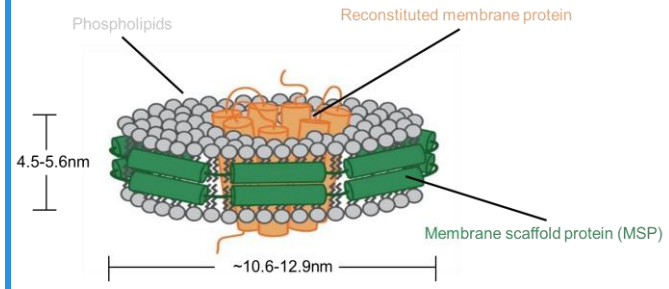
BIORTUS



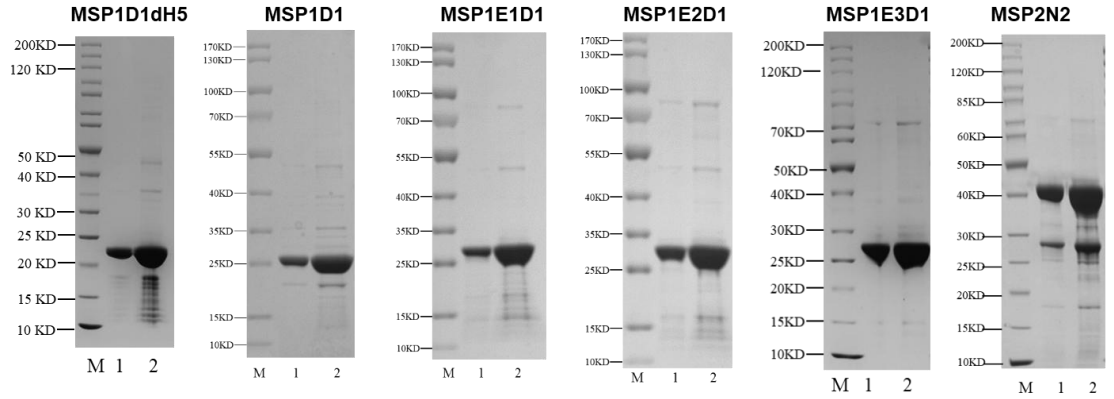
Membrane Proteins Stabilized in Aqueous Solutions

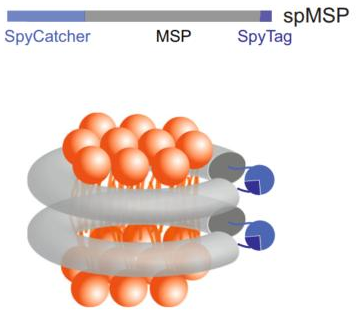
BIORTUS

- Multiple types of membrane scaffold protein (MSP) for nanodisc

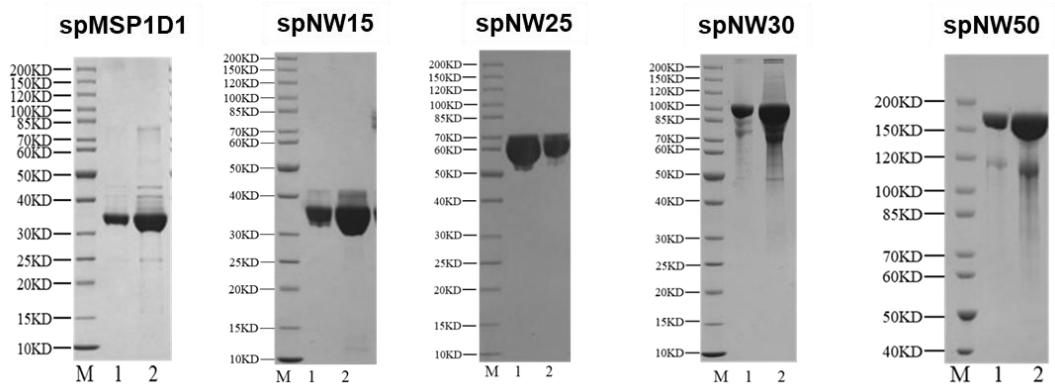


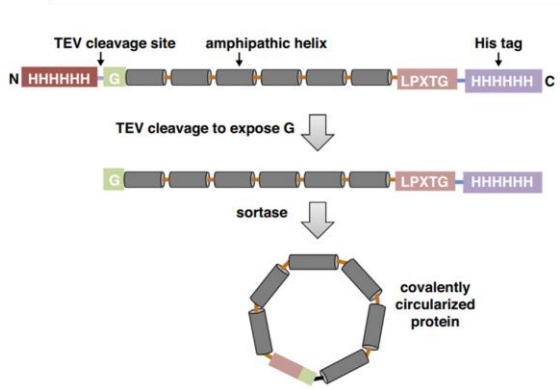
Nanodisc



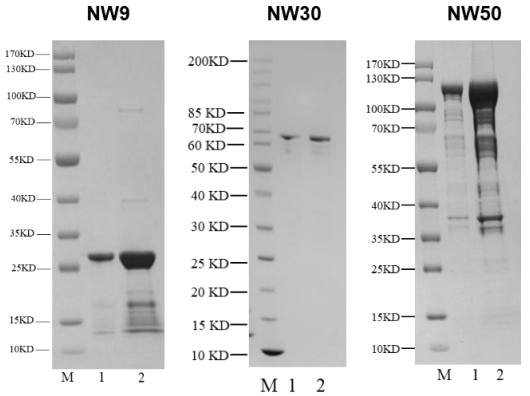


Circularization of MSPs via the SpyCatcher-SpyTag



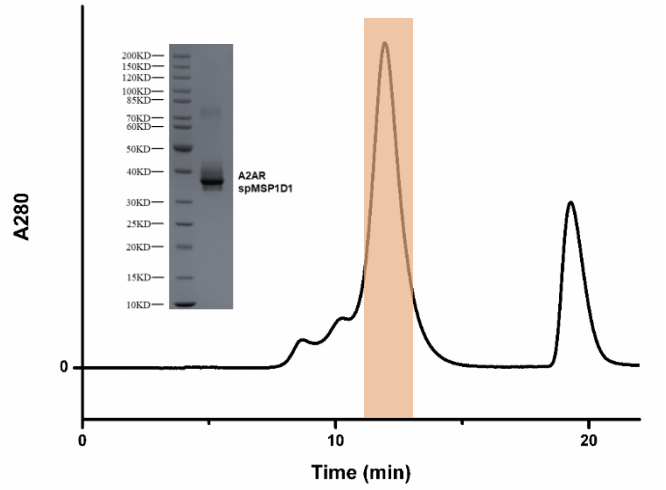


Covalently circularized nanodisc

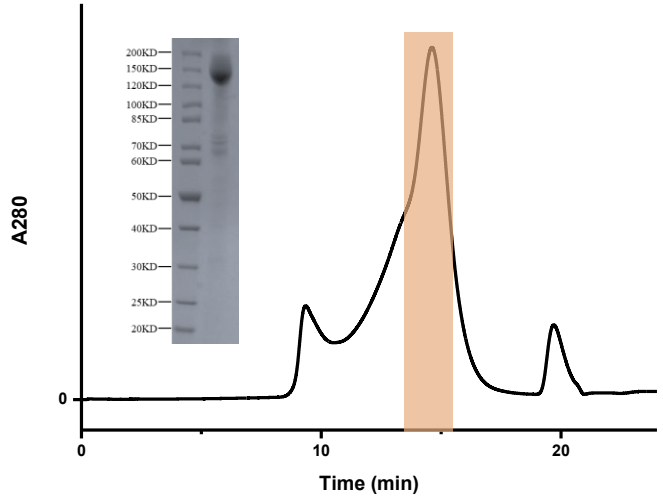


Show Cases

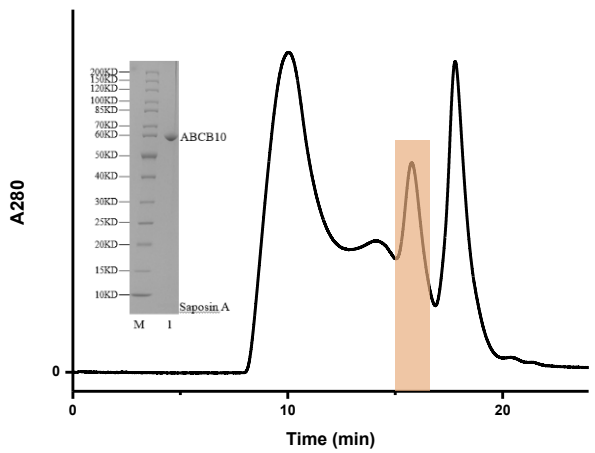
- A2AR-nanodisc



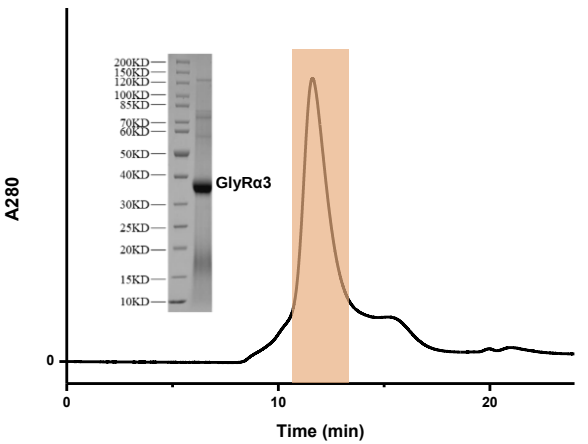
- ABC transporter X-SMALPs



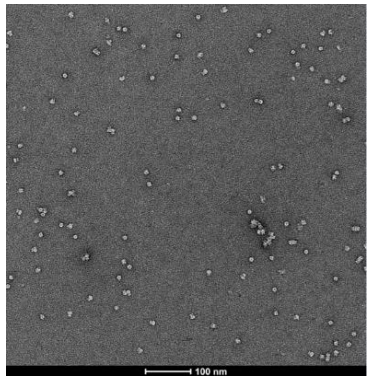
- ABCB10-Salipro



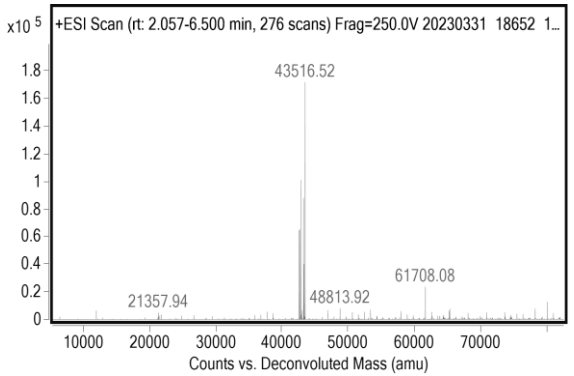
- GlyRα3-amphipol



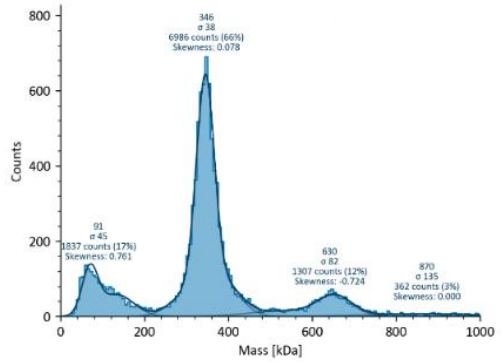
Quality control pipeline



Negative staining EM



LC-MS



Mass Photometry

Thanks!

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

For custom: info@biortus.bio

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

Thanks!

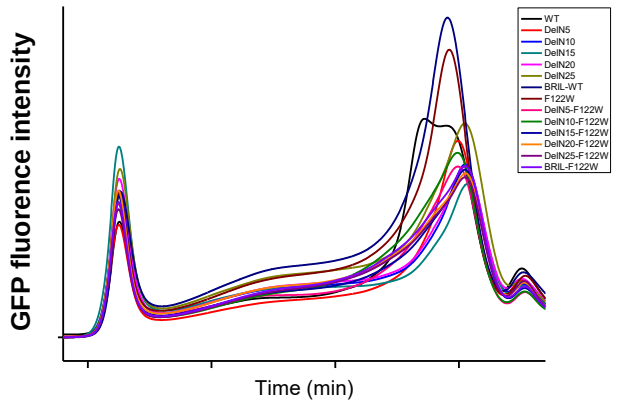
Biortus Discovery Co. , Ltd.
https://en.biortus.bio/

For custom: info@biortus.bio

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

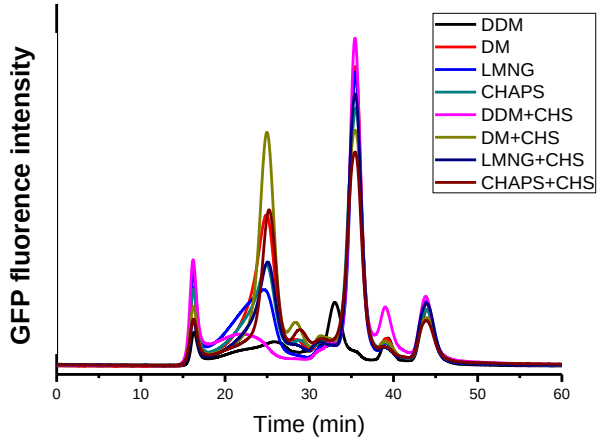
FSEC Screening Platform

- **Constructs Screening**



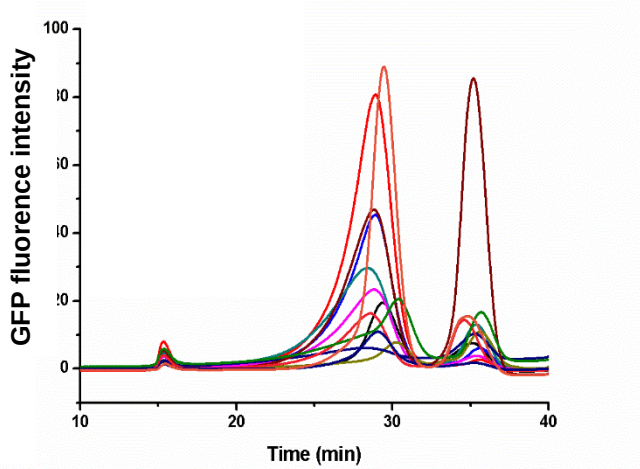
CCR8 (GPCR)

- **Detergent Screening**



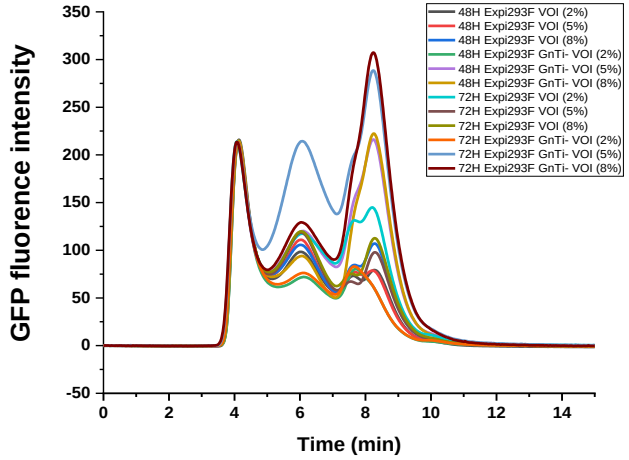
TRPC5 (TRP channel)

- **Species Screening**



FPN1 (SLC)

- **Expression condition screening**



ABCB10 (ABC transporter)