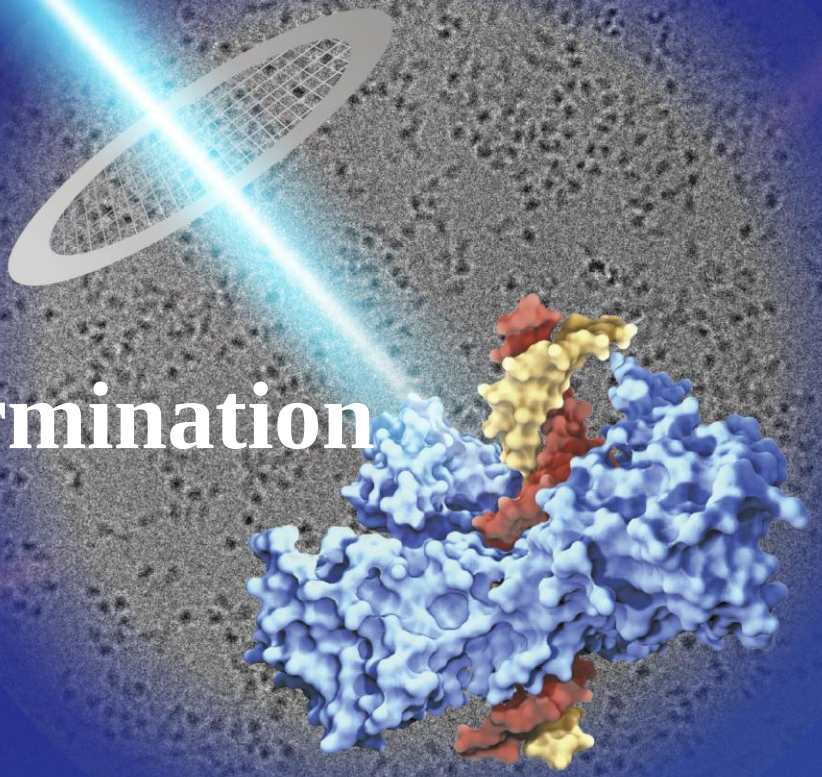
Structure Determination | Drug Design and Structure Determination Cases of GPCR Targets

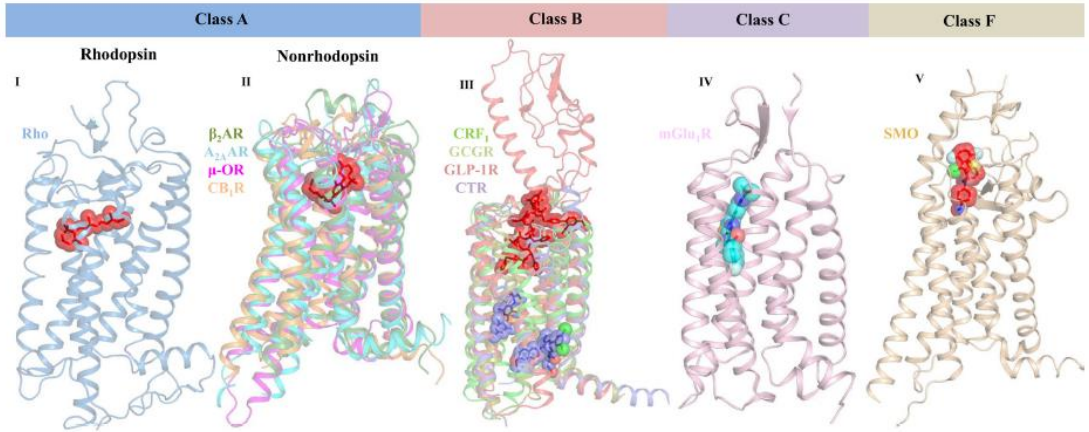


Sanyou Bio Structure Determination Platform

Sanyou Biopharmaceuticals Co., Ltd.

Innovation / Outstanding / Reliability

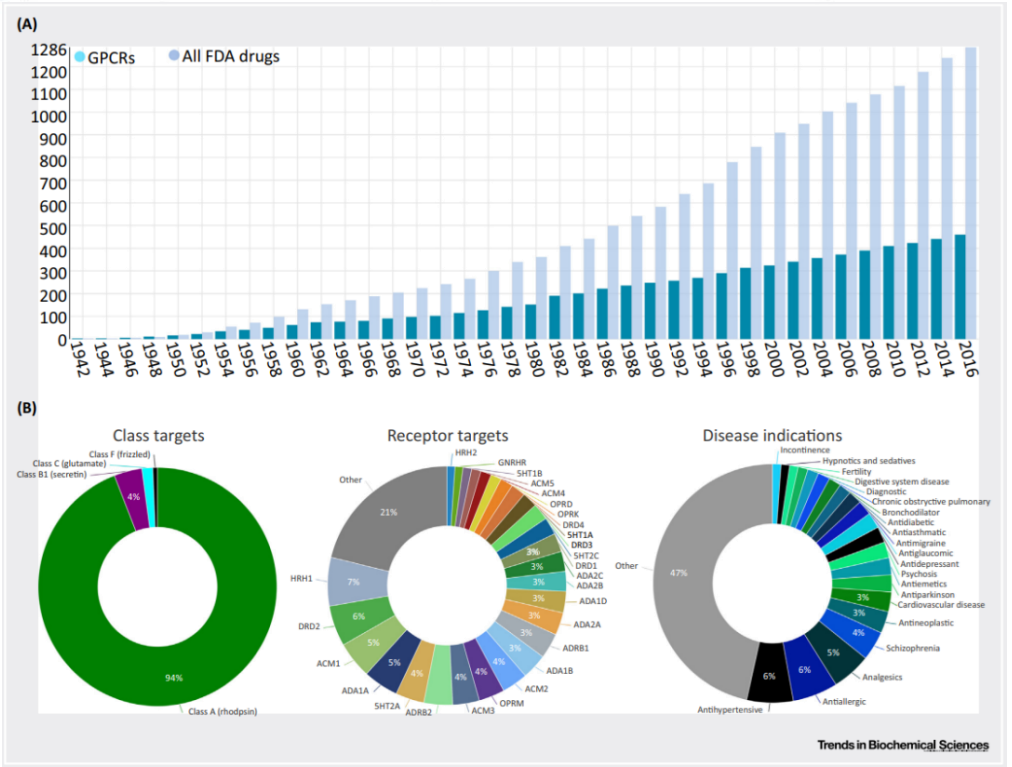
1. Development of GPCR-targeted Drugs



- As the largest protein family (**850 members**) encoded by the human genome, **human GPCRs** can be divided into **4 classes** by amino acid sequence: **Class A (rhodopsin-like), Class B (secretin and adhesion), Class C (glutamate) and Class F (Frizzled) subfamilies**, which are involved in body development and regulation of various physiological functions.

- Currently, GPCR drugs are used in a variety of fields such as **oncology, metabolism, Alzheimer's disease, obesity, multiple sclerosis,** and **hypocalcemia**. Nearly **40%** of the FDA-approved drugs target GPCRs, with total sales accounting for **27%** of the global market.

- As of 2016, a total of **1,286** drugs have been approved by FDA, of which **460** (36%) targeted **GPCRs**. Currently, the majority of drugs target **Class A GPCRs (94%)**, followed by **Class B (4%)**, and **Class C and F (2%)**. Hot targets include HRH1, DRD2, ACM1, ADA1A, and 5HT2A, covering medications such as **antihypertensives**, **antiallergic drugs**, **analgesics**, and **antischizophrenic drugs**.

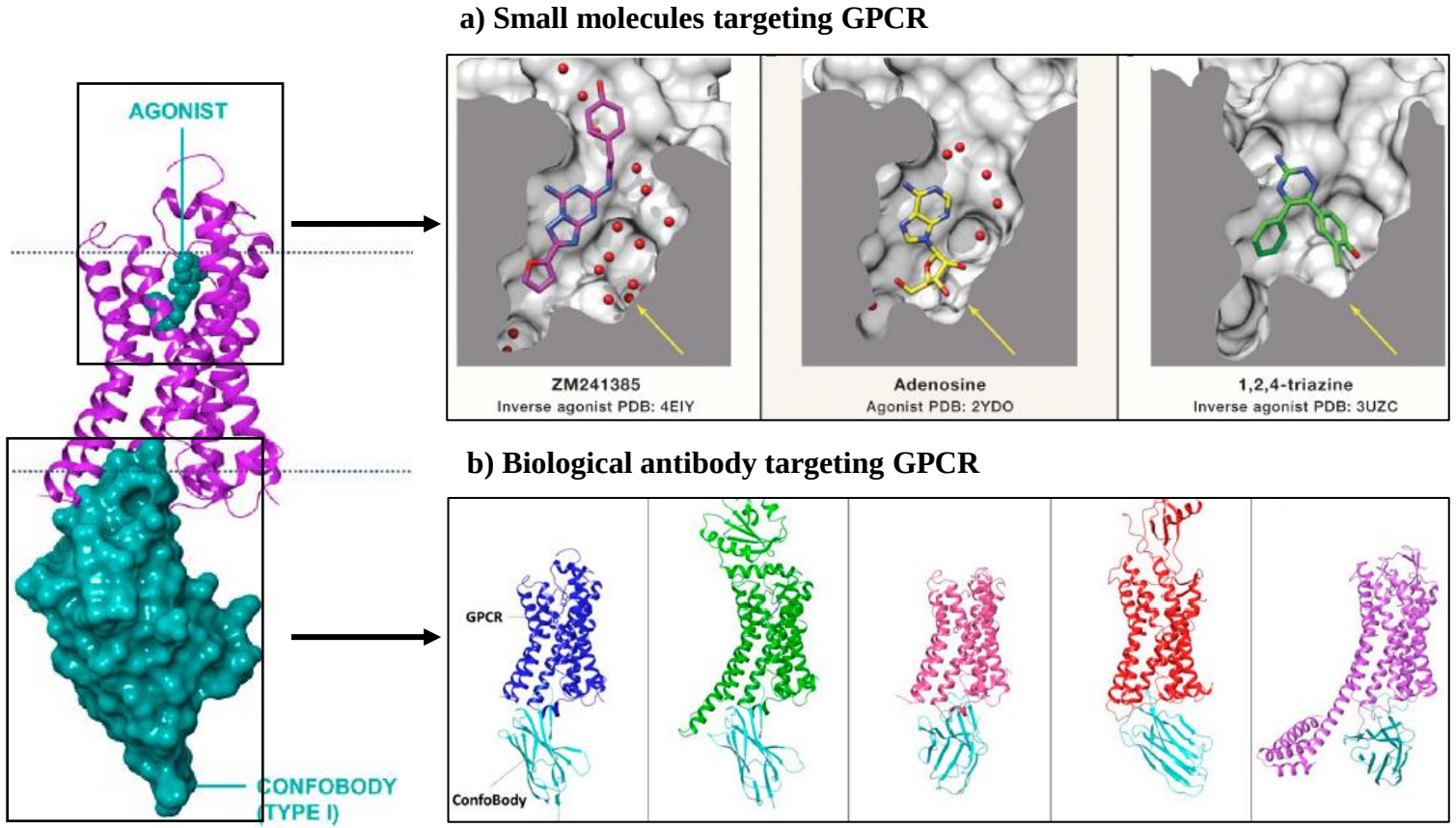


[1]. New Binding Sites, New Opportunities for GPCR DrugDiscovery. Trends Biochem Sci. 2019 Apr;44(4):312-330.
[2]. A review of antibody-based therapeutics targeting G protein-coupled receptors: an update. Catherine J Hutchings. Expert Opin Biol Ther. 2020 Aug;20(8):925-935.

2. Background - Design of GPCR-targeted Drugs

- **Challenges in GPCR drug development** mainly include **difficulties in GPCR protein preparation**, **unknown GPCR ligands**, **unclear relationship between GPCR structure and function**, **unstable GPCR conformation**, and **unclear pathological mechanism of GPCR-related diseases**.

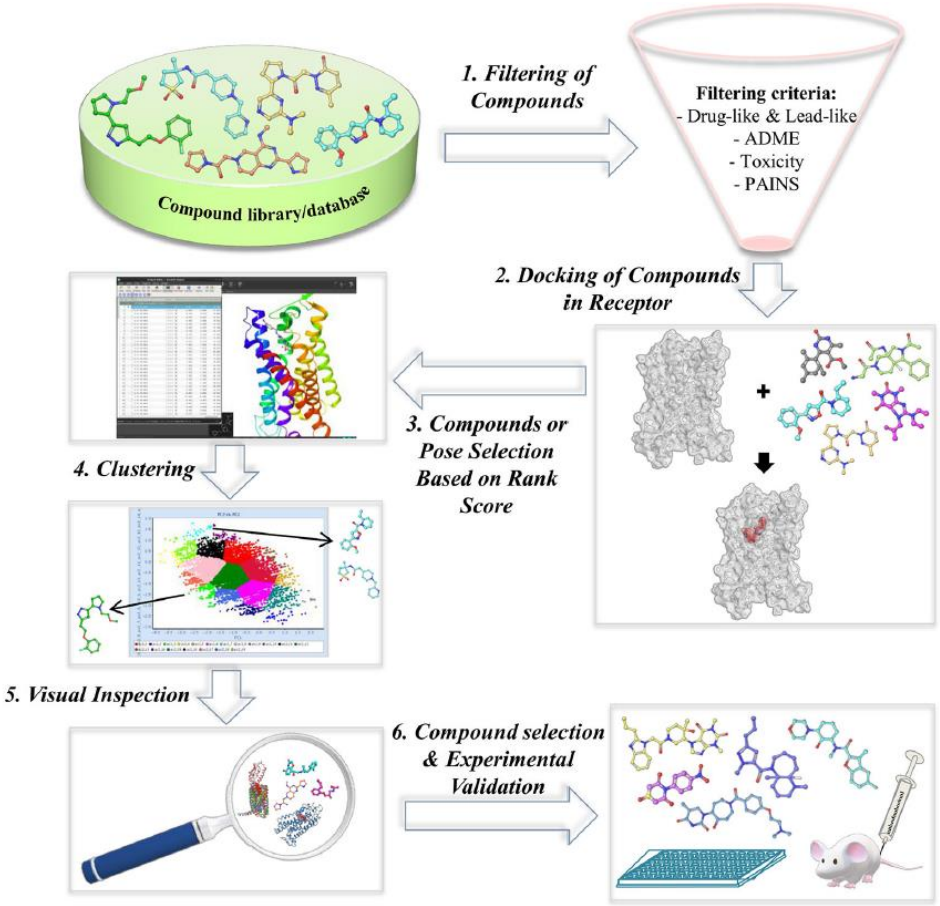
- Currently, the **design of GPCR-targeted drugs** is mainly divided into two categories: screening of **small-molecule drugs (antagonists/agonists)** based on **molecular simulation** and **de novo design** of large-molecule drugs **(nanobodies/Fabs/peptides)** based on **structure determination**.



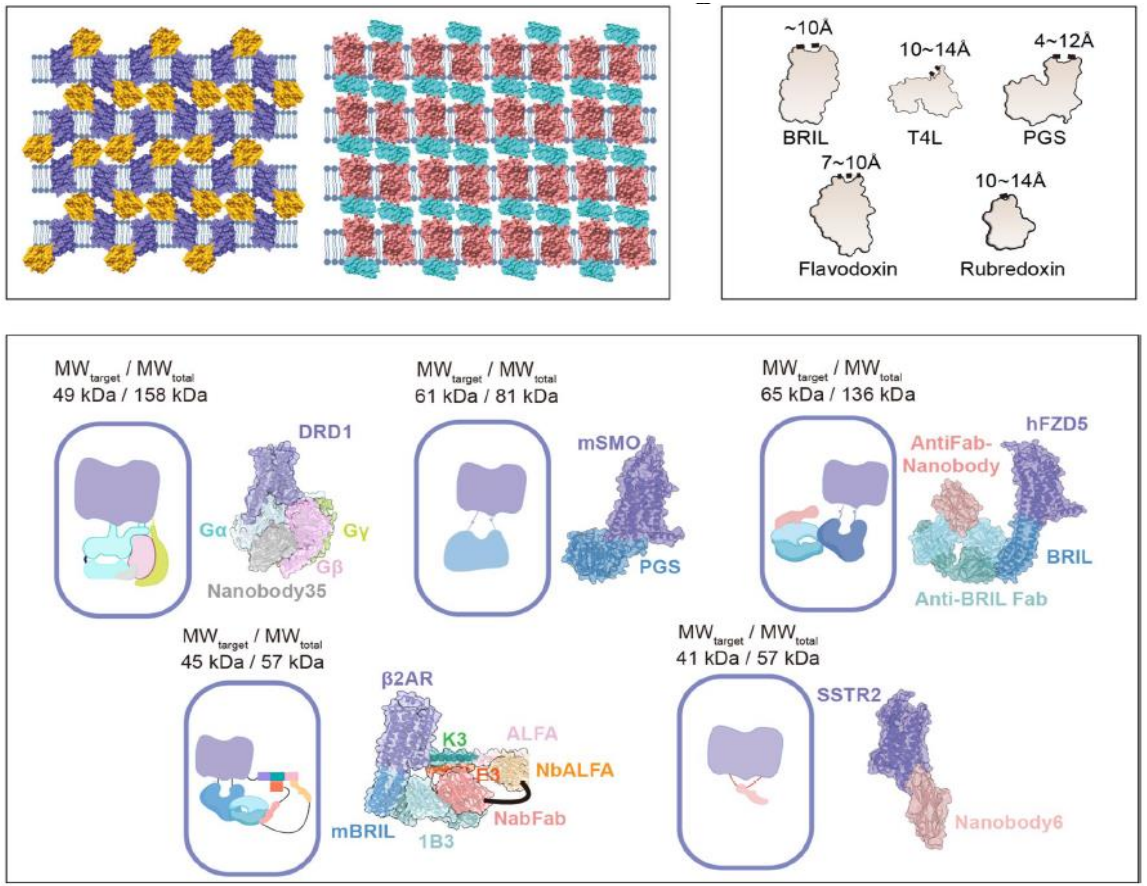
[1] Congreve M, et al. Impact of GPCR Structures on Drug Discovery. Cell. 2020 Apr 2;181(1):81-91.
[2] Laeremans T, et al. Accelerating GPCR Drug Discovery With Conformation-Stabilizing VHHs. Front Mol Biosci. 2022 May 23;9:863099.
[3] Basith S, et al. Exploring G Protein-Coupled Receptors (GPCRs) Ligand Space via Cheminformatics Approaches: Impact on Rational Drug Design. Front Pharmacol. 2018 Mar 9;9:128.

3. Technical Route - Structure-based Design Process of GPCR-targeted Drugs

1. Screening of **small-molecule drugs (antagonists/agonists)** based on **molecular simulation**



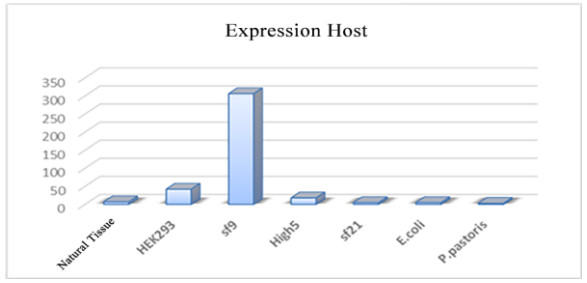
2. **De novo design** of large-molecule drugs (nanobodies/Fabs/peptides) based on **structure determination**

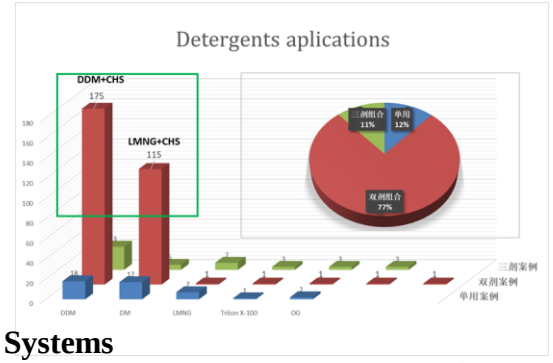


[1] Basith S, et al. Exploring G Protein-Coupled Receptors (GPCRs) Ligand Space via Cheminformatics Approaches: Impact on Rational Drug Design. Front Pharmacol. 2018 Mar 9;9:128.
[2] Cheng L, et al. Structure, function and drug discovery of GPCR signaling. Mol Biomed. 2023 Dec 4;4(1):46.

4. Preparation of Raw Materials for Structure Determination - Multi-pass Transmembrane Proteins such as GPCRs

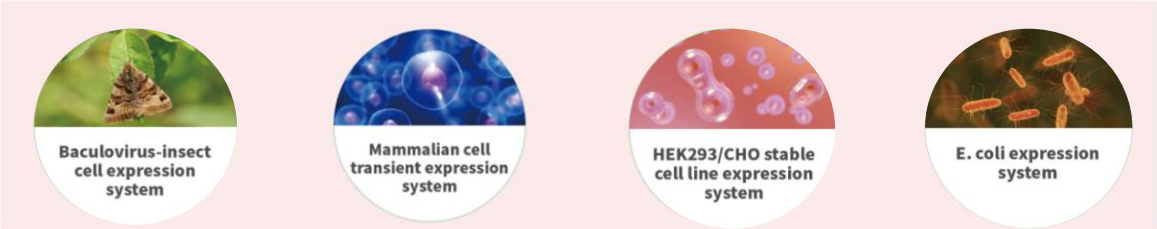
- **Preparation of raw materials:** Due to the low yield of membrane proteins, the **Sf9 insect expression system** and **HEK293 eukaryotic expression system** are currently the main expression systems used. Through the **screening and optimization of detergents**, high-purity **membrane protein** samples are finally obtained for **structure determination** and **antibody screening**.



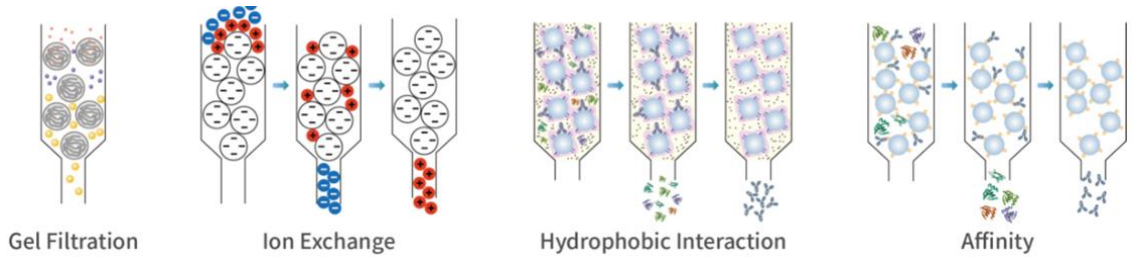


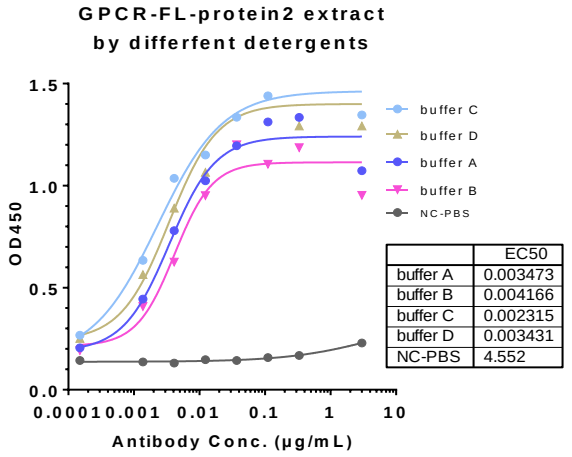
Non-ionic detergent	Triton X-100, NP40, DDM, Digitonin, etc
Anionic detergent	SDS, Sodium deoxycholate, CHS, etc
Cationic detergent	CTAB, etc
Amphiphilic detergent	Amphipol, LMNG, CHAPS, etc

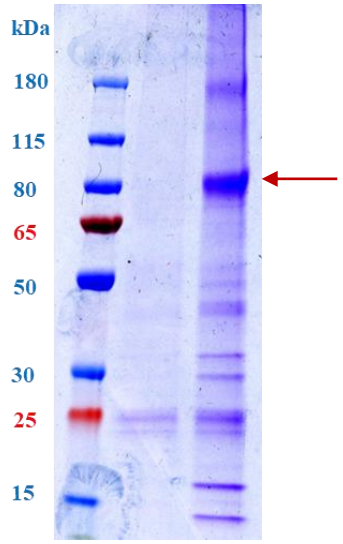
Four Expression Systems



Four Purification Methods



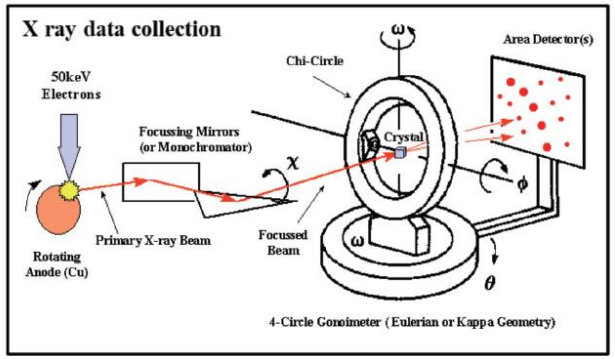




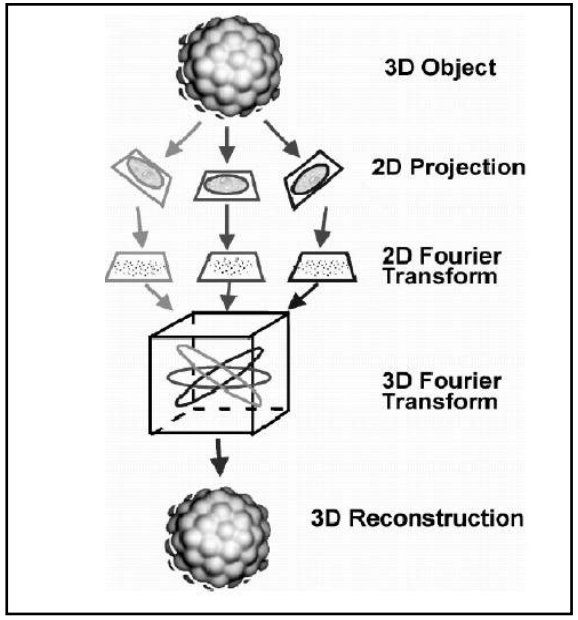
5. Technical Process for Structure Determination - Multi-pass Transmembrane Proteins such as GPCRs

- **Structure determination method: X-ray crystallography** and **cryo-electron microscopy (Cryo-EM)** are the mainstream technical routes for structure determination of membrane proteins at present, which can meet different project requirements.

X-ray crystallography (High purity/concentration)

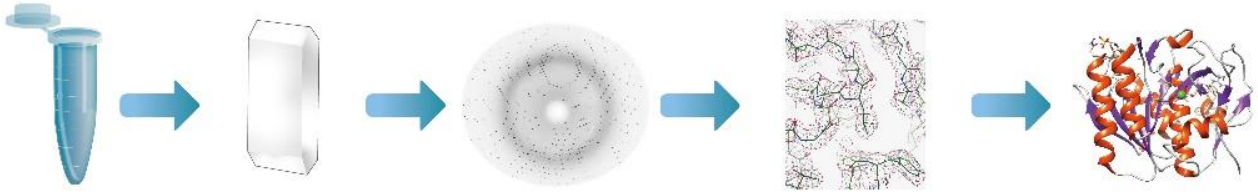


CryoEM (High homogeneity)



Preparation of Raw Materials

Recombinant cells — **X-ray**



Protein purification → **Crystal screening** → **Diffraction data collection** → **Phase elucidation** → **Model building and refinement**

Membrane extract / Detergent — **CryoEM**

Protein purification → **Negatively stained/Cryo-EM sample testing** → **Electron microscopy data collection** → **Sample particle selection** → **2D classification** → **3D reconstruction** → **Model building and refinement**

6. Case Presentation - Structure Determination of GPCR Drug Target

Cryo-EM structure of a class T GPCR in active state

Target: TAS2R46 receptor

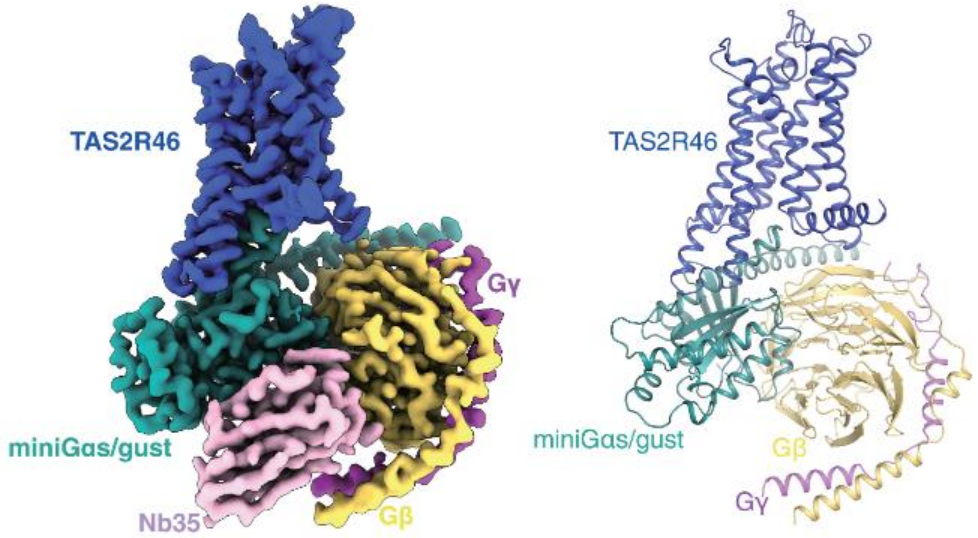
PDB ID: 7XP6

EM Map: EMD-33366

Classification: GPCR

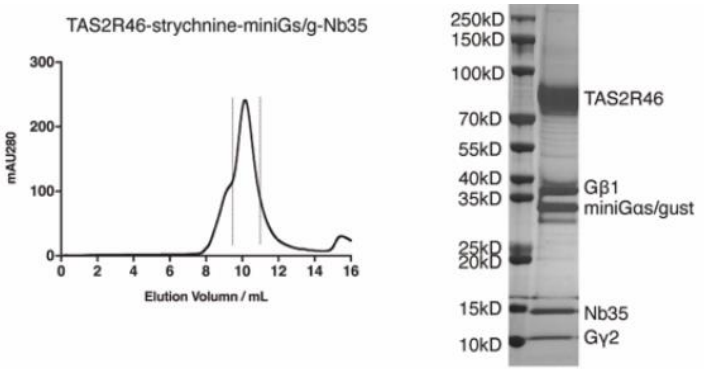
Expression System: Sf9 insect expression system

（*Spodoptera frugiperda*）

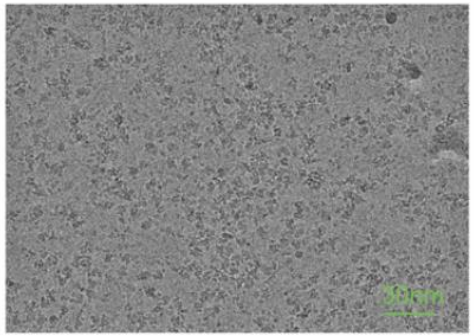


Step 1: Structure simulation & Protein design

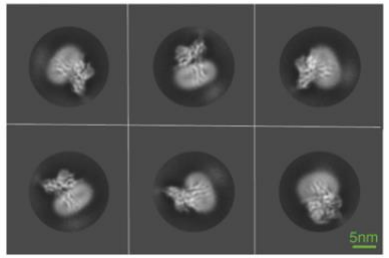
Step 2: Protein affinity purification & SEC

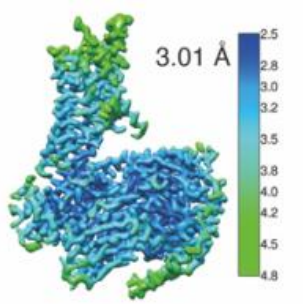


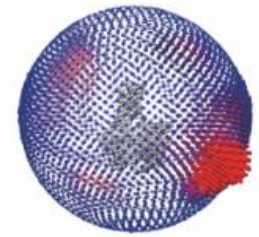
Step 3: Negative staining & Cryo-EM

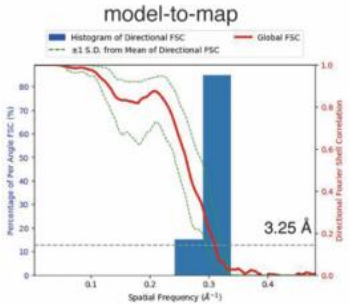


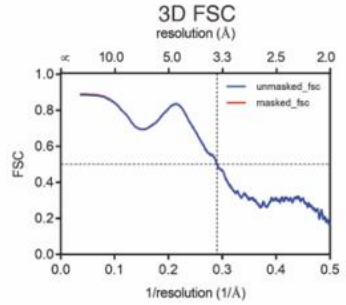
Step 4: 2D & 3D reconstruction







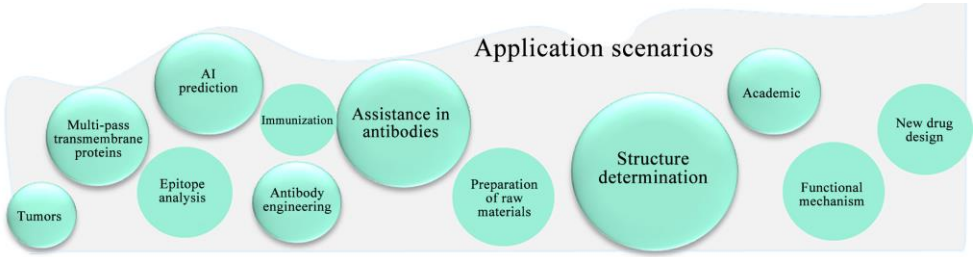




Xu W, et al. Structural basis for strychnine activation of human bitter taste receptor TAS2R46. Science. 2022 Sep 16;377(6612):1298-1304.

7. Summary - Development of GPCR-targeted Drugs

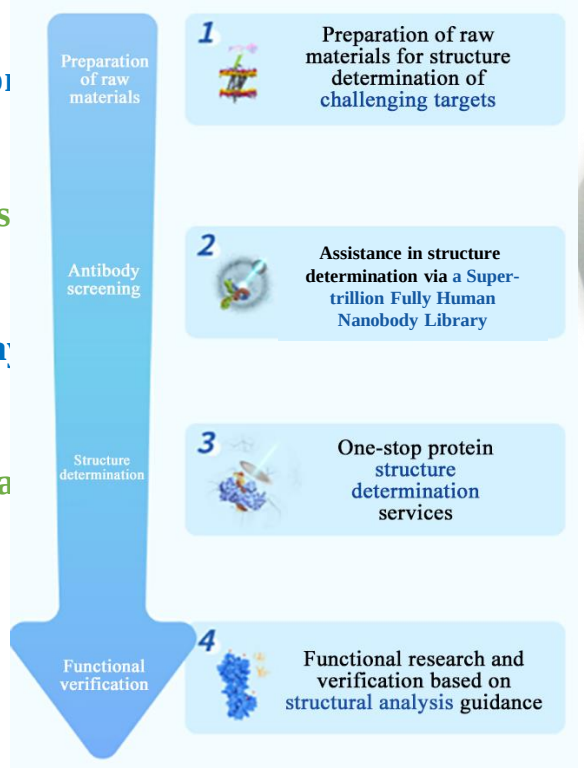
- **Design of GPCR-targeted drugs: Drug screening** based on **AI molecular simulation** and experimental verification based on **structure determination** form a two-way channel in the R&D process of GPCR drugs, which is essential for complete drug R&D, studies on GPCR mechanism of action, drug design and optimization.

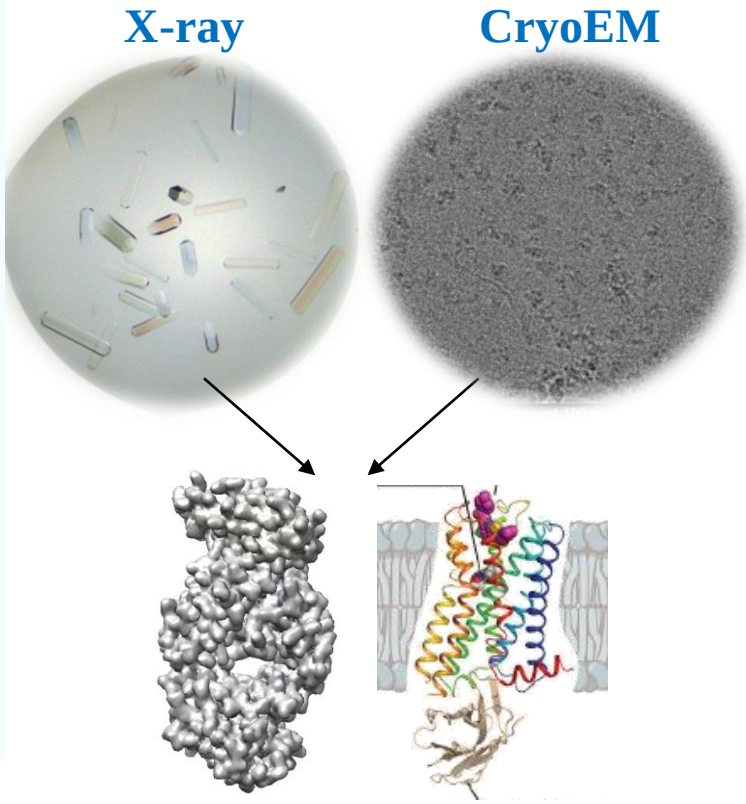


Application of Sanyou Bio One-stop Structure Determinatio Platform Services in GPCR Targets

1. **Feasibility analysis covering structure determination from targets**
2. **Solution for protein preparation for challenging GPCR targets;**
3. **Dual technology structure determination platform based on X-ra and Cryo-EM;**
4. **Functional interaction and verification based on structura analysis.**

One-stop Platform Services: Customer-oriented.





THANKS

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