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Cryo-electron microscopy in drug development

Cryo-electron microscopy (cryo-EM) is becoming the method of choice in structure determination of membrane proteins and has great potential for structure-based drug discovery (SBDD). Cryo-EM provides high-resolution structural information of a membrane protein without the need for crystallization. It uses low amounts of a pure protein sample (micrograms rather than the milligrams required for X-ray crystallography) and is compatible with unstable membrane proteins that are incredibly challenging to crystallize.

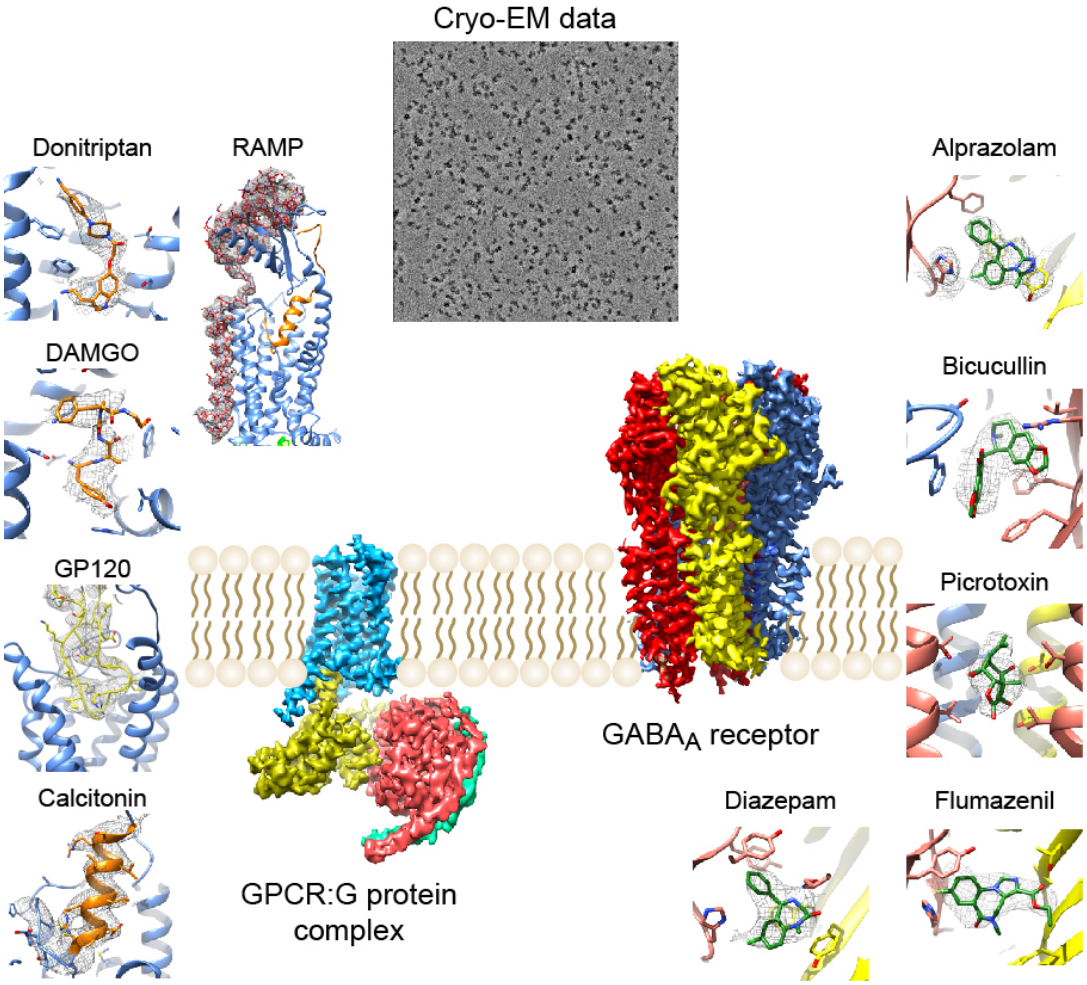

Fig. 1. Cryo-electron microscopy to study receptor:drug interactions. An electron micrograph of a GPCR:G protein complex (centre) represents one out of several thousand such micrographs that are processed to yield the density maps of proteins; a GPCR:G protein complex (left) and a pentameric ion channel (right). Examples of drug binding modes obtained by cryo-EM for each type of receptor are shown around their respective cryo-EM models. Small molecules are shown as sticks and proteins are represented as cartoons.

SBDD is a powerful route to rationally design and optimize drug candidates based on the 3-dimensional atomic information of how a molecule fits into the receptor's binding pocket. Cryo-EM now opens new possibilities to obtain structural information of highly valuable drug targets like membrane proteins. Cryo-EM still has some limitations for SBDD compared to crystallography, such as low throughput, low levels of automation and lower resolutions routinely achieved. However, cryo-EM is more likely to yield the first structure of a membrane protein, which would provide crucial information about the functional mechanism and druggable binding pockets. In this review we highlight insights gained through cryo-EM into two protein families with established therapeutic value, the γ-aminobutyric acid A receptors ($GABA_A$Rs) and G protein-coupled receptors (GPCRs).

The $GABA_A$Rs are ion channels that mediate inhibitory neurotransmission in the brain and sense GABA and neurosteroids. Their dysfunction results in several neurological and psychiatric disorders and hence are the target for a variety of drugs including anesthetics and benzodiazepines. $GABA_A$Rs are heteropentameric receptors with the main population at synapses composed of two a-subunits, two b-subunits and one g-subunit. While X-ray crystallography has provided insights into homomeric channels, the physiological heteromeric ion channel is required to understand the pharmacology. Cryo-EM has provided structures of heteromeric $GABA_A$R channels in complex with compounds such as GABA (endogenous agonist), benzodiazepines (positive allosteric modulators), flumazenil (antagonist of allosteric benzodiazepines), picrotoxin (pore blocker) and bicuculline (competitive antagonist), explaining the binding mode and functional mechanism of these drugs. Some of these $GABA_A$R structures were obtained with the receptor embedded in a lipid nanodisc, which seemed to be the only methodology in which the native heteromeric channel containing a g subunit adopted a non-artefactual conformation.

GPCRs form the largest family of receptors in the human body and are also the target for 34% of FDA approved small molecule drugs. GPCRs sense a variety of extracellular signals and transduce the information to the cytoplasm by coupling and activating heterotrimeric G proteins. While X-ray crystallography has yielded information regarding the inactive/active intermediate states of GPCRs, the G protein-bound receptors in the fully active state have been more elusive. Structures of the active states of GPCRs is highly desirable to design drugs that activate the receptors (agonists) or drugs that activate receptors to turn on specific signaling pathways (biased agonists). Without the need for crystallization, cryo-EM has generated structures of receptors bound to different G proteins (G_s, G_i and G_o) and receptors of class B and C that were intractable by X-ray crystallography. These results provide insights into GPCR:G protein coupling specificity, the allosteric modulation of GPCRs by Receptor-Activity Modifying Proteins (RAMPs) as well as the activation mode of the receptors.

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Publication

Cryo-Electron Microscopy: Moving Beyond X-Ray Crystal Structures for Drug Receptors and Drug Development
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Annu Rev Pharmacol Toxicol. 2020 Jan 6