Cryo-EM reveals interaction between major drug targets

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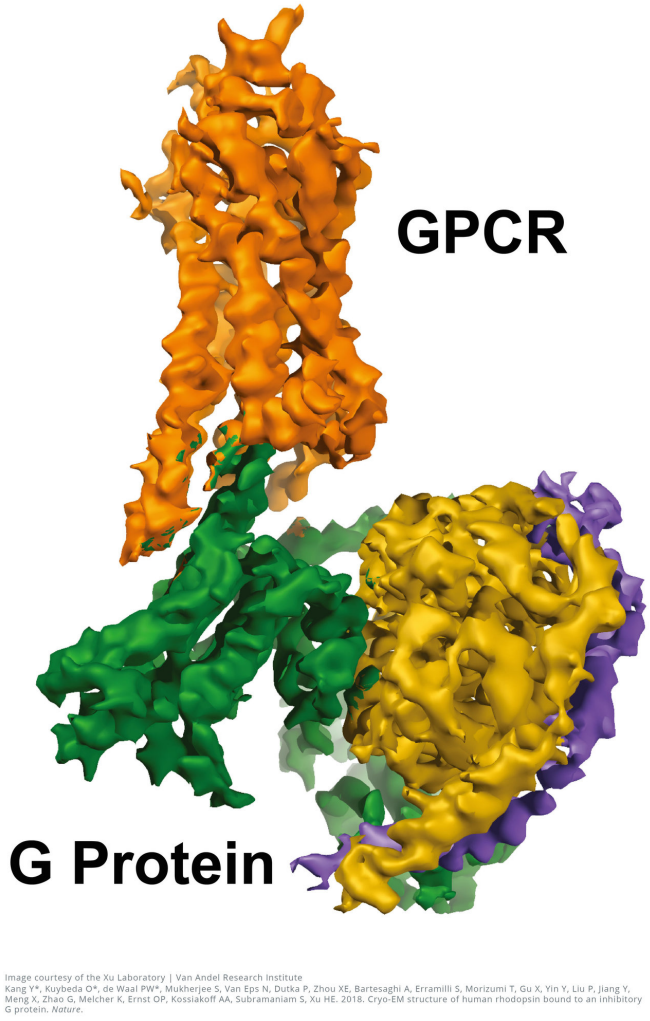

Image courtesy of the Xu Laboratory | Van Andel Research Institute
Kang Y*, Kuybeda O*, de Waal PW*, Mukherjee S, Van Eps N, Dutka P, Zhou XE, Bartesaghi A, Erramilli S, Morizumi T, Gu X, Yin Y, Liu P, Jiang Y, Meng X, Zhao G, Melcher K, Ernst OP, Kossiakoff AA, Subramaniam S, Xu HE. 2018. Cryo-EM structure of human rhodopsin bound to an inhibitory G protein. *Nature*.

GPCR-Gi structure. Credit: Xu Laboratory, Van Andel Research Institute

For the first time, scientists have visualized the interaction between two critical components of the body's vast cellular communication network, a discovery that could lead to more effective medications with fewer side effects for conditions ranging from migraine to cancer.

The near-atomic resolution images, published today in *Nature*, show a G-protein coupled receptor (GPCR) called rhodopsin bound to an inhibitory G protein, and provides a blueprint for designing more precise, selective drugs while also solving a longstanding problem in the field.

"Visualizing this complex resolves a missing chapter in the GPCR story by finally revealing how these two molecules interact in exquisite detail," said H. Eric Xu, Ph.D., a professor at Van Andel Research Institute (VARI) and one of the study's senior authors. "Everything in biology is based on molecular interactions so the more we know about how the structures of these two molecules work together, the better position we are in to design improved medications with fewer undesired effects."

Today's findings were made possible through use of a revolutionary technique called cryo-electron microscopy (cryo-EM), which allows scientists to see tough-to-visualize molecules in startling clarity.

"The use of cryo-EM technology to obtain structural information on important pharmaceutical targets such as GPCRs in various states demonstrates that we are now in a position to apply these methods for drug discovery applications," said Sriram Subramaniam, Ph.D., an investigator at the National Cancer Institute of the National Institutes of Health and a senior author of the study.

Embedded in the cell membrane, GPCRs act as conduits between a cell and its environment, interacting with G proteins and other signaling molecules called arrestins to convey important messages to and from the cell that regulate a gamut of physiological functions, including growth, immune responses and sensory perception.

When linked up with GPCRs, inhibitory G proteins regulate the production of secondary chemical messengers that have effects throughout the body, from interactions with serotonin receptors in the

brain and gut, which help regulate mood and appetite, to interactions with dopamine receptors in the brain, which control reward responses and voluntary movement, among many others.

These wide-ranging interactions with G proteins and arrestins, coupled with their accessibility on the outside of the cell, make GPCRs attractive targets for therapeutic development. Currently, more than 30 percent of medications on the market work by interacting with GPCRs.

"The information revealed by our findings will help facilitate the design of a new generation of medications," said Yanyong Kang, Ph.D., a research scientist in the Xu Laboratory and co-first author of the study. "Because this is the first GPCR-inhibitory G protein complex to be structurally determined, we believe our methods will help lead to the characterization of other important, yet difficult-to-visualize GPCRs."

The 3-D images generated by the team reveal a specialized helix at the end of the inhibitory G protein that acts as a structural signature, which helps GPCRs like rhodopsin differentiate between inhibitory G proteins and another type of G protein known as a stimulatory G protein.

Today's findings are the latest in a series of firsts for Xu and his team, which include a landmark 2015 *Nature* study that first described the structure of rhodopsin and arrestin in complex together. This work, which was hailed as a major breakthrough in the field, earned Xu the Hans Neurath Award from The Protein Society and the Hans Neurath Foundation in 2016.

In a follow-up study published in *Cell* in 2017, Xu and his collaborators further refined their earlier structure of the rhodopsin-arrestin complex, and revealed a set of phosphorylation codes that dictate the assembly of GPCR-arrestin complexes.

GPCRs are notoriously difficult to visualize using traditional X-ray crystallography methods; to date, only 40 out of more than 800 total GPCRs have had their structures determined, including Xu's rhodopsin-arrestin complex.

To determine today's structure, the team harnessed VARI's high-powered Titan Krios cryo-electron microscope, which is capable of imaging molecules 1/10,000th the width of a human hair and can more easily visualize molecules like GPCRs that are embedded in the cell membrane. The Institute's Krios, which is part of its David Van Andel Advanced Cryo-Electron Microscopy Suite, is one of fewer than 120 such microscopes in the world.

Subramaniam and his team have pioneered the use of cryo-EM to determine some of the highest resolution structures reported so far using cryo-EM, including several clinically relevant ligand-protein complexes.

More information: Cryo-EM structure of human rhodopsin bound to an inhibitory G protein, *Nature* (2018). DOI: 10.1038/s41586-018-0215-y , https://www.nature.com/articles/s41586-018-0215-y

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