Scientists decode serotonin receptor at room temperature

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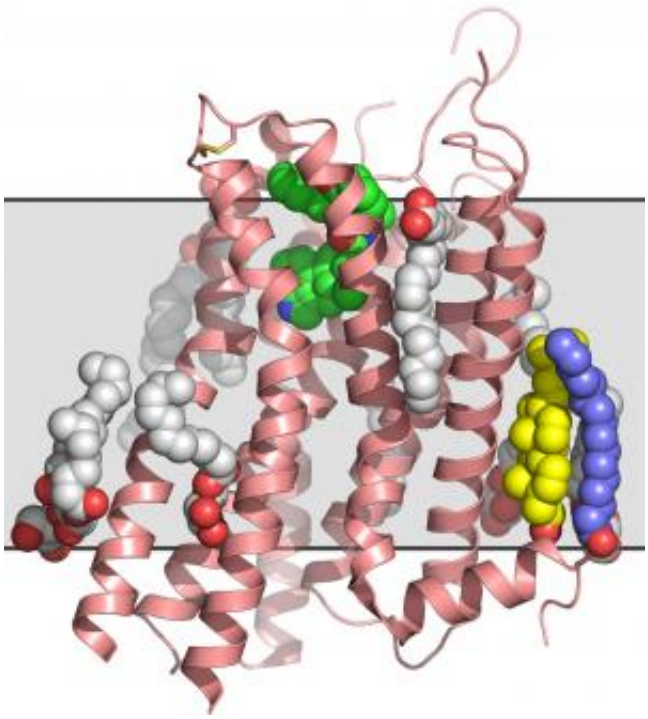

The serotonin receptor structure bound to ergotamine (green), and showing resolved lipids (gray), palmitic acid (blue) and cholesterol (yellow). The approximate membrane boundary are shown as a gray box. Credit: Vadim Cherezov/The Scripps Research Institute

An international research team has decoded the molecular structure of the medically important serotonin receptor at room temperature for the first time. This study reveals the dynamics of the receptor at close to its operating temperature and thus gives a more realistic picture of its physiological function than it was possible before with conventional deep freeze analyses in liquid nitrogen at minus 173 degrees Celsius. The team led by Prof. Vadim Cherezov of The Scripps Research Institute in La Jolla, California, reports its work in the scientific journal *Science*. The research could lead to better designed drugs. The study also opens up new ways for investigating large biomolecules.

Serotonin is an important neurotransmitter and is involved in the regulation of numerous body functions like blood pressure, digestion and intra-ocular pressure, but also mood, appetite and addiction. This makes the serotonin receptor an important drug target. Knowing its molecular structure could allow for the development of tailor-made drugs that fit to the receptor like a key into a lock.

"Scientists have been keen on decoding the structure of the serotonin receptor for decades," said co-author Cornelius Gati from Prof. Henry Chapman´s group at the Hamburg Center for Free-Electron Laser Science CFEL, a cooperation of DESY, the University of Hamburg and the Max Planck Society. But only this year, a group including Cherezov succeeded in decoding the structure of the receptor in a classical crystallographic analysis at a so-called synchrotron light source.

For these kinds of investigations, biomolecules usually have to be crystallised. This can be very hard and sometimes even impossible to do with a group of molecules called membrane proteins, to which the serotonin receptor belongs. Once crystals are grown, they are shock frozen and illuminated with X-rays from the synchrotron light source. The X-rays produce characteristic diffraction patterns from which the structure of the sample can be calculated.

For the new study, the team used the world's strongest X-ray laser, the Linac Coherent Light Source at SLAC National Accelerator Laboratory in Menlo Park, California. The LCLS generates 120 intense X-ray flashes per second, each a billion times brighter than a conventional synchrotron. With these bright flashes, even the smallest crystals can be analysed. The crystals do not have to be frozen, as they are evaporated by the bright

flash. But before they disintegrate, their inner structure can be recorded at much better fidelity than possible with the synchrotron. "The X-ray laser pulses are less than 30 femtoseconds in duration, the time it takes light to travel only 10 micrometres, less than the width of a human hair," explained Chapman. "Each incredibly brief but powerful flash of X-rays literally outruns any damage or disintegration of the crystal from this radiation, giving us strong and pristine structural information."

"Room temperature structures should better represent conformational states of proteins in their native environment and may serve as better templates for structure-based drug design," said Cherezov.

For the investigation at the X-ray laser, the researchers grew tiny crystals of the serotonin receptor with the molecule ergotamine attached, which is a migraine drug that targets this receptor. To overcome the difficulties in crystallisation, the researchers used an artificial cell membrane environment called lipid cubic phase, LCP, that at least allowed to grow micro-crystals. But LCP is more viscous than toothpaste and cannot be sprayed into the X-ray beam like a fluid suspension. That's why a team at Arizona State University developed a tailor-made injector that is able to shoot a steady but tiny stream of LCP through the X-ray path.

The new study illustrated an innovative technique to find structural details about G protein-coupled receptors (GPCRs), which are targeted by approximately 40 percent of modern medicines. This illustration represents a migraine, overlaid with a rendering of the human serotonin receptor (a GPCR) bound to ergotamine, an anti-migraine drug. Also shown is a rendering of a neuron network. Credit: Katya Kadyshevskaya, The Scripps Research Institute.

"The 'toothpaste' injector, designed by Prof. Uwe Weierstall at Arizona State University, can control the flow rate and adjust it so that there is a minimal waste of crystals between LCLS pulses, reducing the amount of crystals required for data collection a hundred- to a thousand-fold compared to liquid injectors," said Cherezov. This allowed over 150 000 patterns to be collected from individual crystals that were steadily replenished by the injector. The enormous volume of data was processed by Gati using software called CrystFEL specifically created for this method.

The research team compared the structure data from the X-ray laser with the structure resolved at

the synchrotron. "One of the most important results of this work is that the structure obtained at LCLS is almost identical to the structure obtained with traditional crystallography, despite the fact that the LCLS data were collected from crystals 100 times smaller by volume and at room temperature," Cherezov stressed.

Differences in the two structures analyses stem in part from the fact that at cryo-temperatures some flexible loops of the receptor appear more rigid than they are at room temperature. The dynamics of the loops are important for the binding of signalling molecules inside and outside of the cell.

The study opens new analytical methods for a whole class of biomolecules. The serotonin receptor belongs to a large group called G protein-coupled receptors, or GPCRs. This group of about 800 receptors plays a central role in transferring signals from the environment into the cell and is of great interest for drug development. About 30 to 40 per cent of all prescription drugs target GPCRs.

"This is the first protein crystal structure of a human membrane protein at room temperature," said Gati. "Our work shows that it is possible to analyse micro-crystals of biomolecules at room temperature, leading to more realistic results. This may allow for an optimised drug development as more of the dynamics of the receptor is visible."

This path is also followed by the Hamburg Center for Ultrafast Imaging CUI on the DESY campus, with which Gati and Chapman are also affiliated, and which partly supported this work. CUI is a cooperation of DESY, the University of Hamburg, the Max Planck Society, the European Microbiology Laboratory and the European XFEL.

Deutsches Elektronen-Synchrotron DESY is the leading German accelerator centre and one of the leading in the world. DESY is a member of the Helmholtz Association and receives its funding from the German Federal Ministry of Education and Research (BMBF) (90 percent) and the German federal states of Hamburg and Brandenburg (10 percent). At its locations in Hamburg and Zeuthen near Berlin, DESY develops, builds and operates large particle accelerators, and uses them to investigate the structure of matter. DESY's combination of photon science and particle physics is unique in Europe.

More information: "Serial Femtosecond Crystallography of G Protein-Coupled Receptors"; Wei Liu et al; *Science*, 2013; DOI: 10.1126/science.1244142

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