Structures of Human $P2Y_{12}$ Receptor Key to Next Generation of Antithrombotic Drugs

An international team led by scientists at the Shanghai Institute of Materia Medica (SIMM), CAS has characterized the detailed structure of a receptor that plays a key role in platelet activation and blood clotting. The work has implications for treatment of cardiovascular and other diseases.

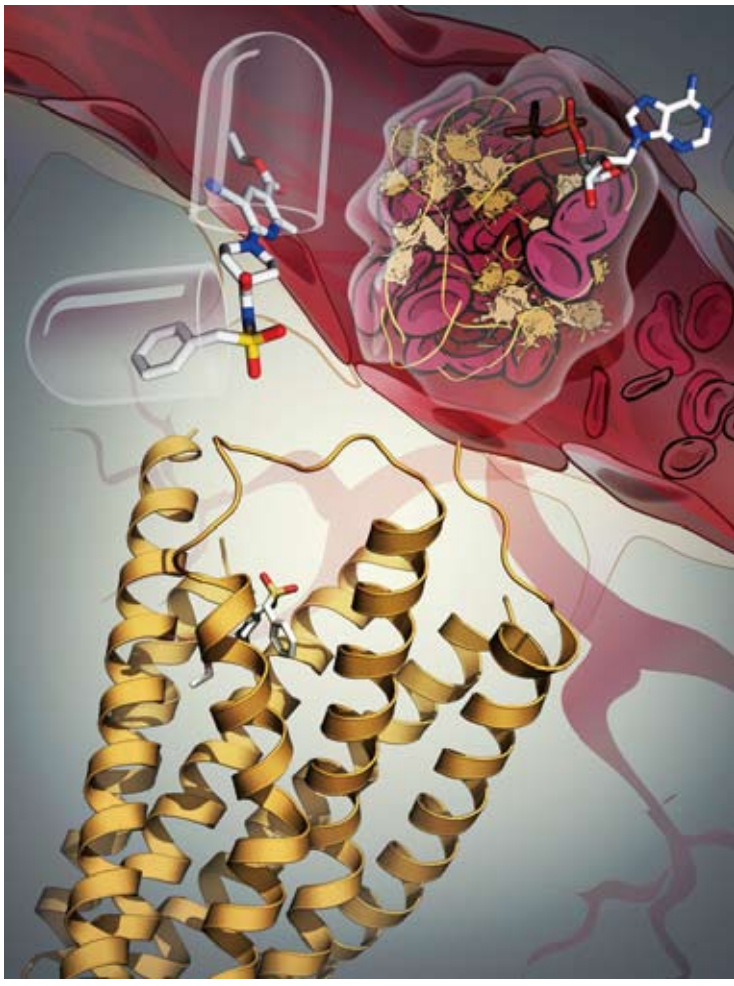
In a pair of papers published by the journal *Nature* on May 1st, the scientists provide a detailed map of $P2Y_{12}R$, a human G_i protein-coupled receptor, in the antagonist and agonist bound states. The antithrombotic drug target has a market of several billion U.S. dollars.

"This new work will not only deepen the understanding of activation of this receptor super-family, but will also provide invaluable insight into the improvement and development of $P2Y_{12}$ drugs targeting cardiovascular and other diseases," said team leader ZHAO Qiang, professor at SIMM. "I am particularly proud of the high quality international collaboration among China, USA, and Germany necessary to complete this research."

The structures were solved by SIMM in collaboration with research groups from the National Institutes of Health (NIH; United States), The Scripps Research Institute GPCR Network (United States), iHuman Institute of ShanghaiTech University (China) and University of Bonn (Germany).

"What we have learned from this research could pave the way for the creation of drugs with fewer limitations and health risks," said Dr. Kenneth A. Jacobson, chief of the Laboratory of Bioorganic Chemistry in the National Institute of Diabetes and Digestive and Kidney Diseases at NIH and an author of the paper. "Our discoveries have the potential to make a significant difference in patients' lives."

Platelet activation and subsequent aggregation to form a thrombus plug are critical for the cessation of blood loss due to vessel damage. Inappropriate activation of platelets



The crystal structure and function of the $P2Y_{12}$ receptor. The receptor structure is shown as a yellow ribbon (bottom). The physiological ligand (ADP, top right-hand side) that stimulates the formation of blood clots, and the drug that suppresses platelet aggregation (AZD1283, top, left-hand side) are shown as coloured molecular stick models within a capsule (AZD1283), or on a blood coagulation thrombus (ADP), respectively. (Image by SIMM)

after intravascular damage often leads to the formation of a platelet-rich thrombus, which can subsequently result in life-threatening conditions such as unstable angina, heart attack and stroke.

Laboratory and clinical experience with currently marketed $P2Y_{12}R$ targeting drugs has led to the understanding that each of the drugs has certain limitations, and that efforts to develop better drugs have been impeded by poor understanding of receptor-ligand interaction.

These studies describe for the first time the three-dimensional structure of the $P2Y_{12}R$, in complex with a full agonist (2MeSADP at 2.5 Å), a potential partial agonist (2MeSATP, 3.1 Å), and a non-nucleotide $P2Y_{12}R$ reversible antagonist (AZD1283, 2.6 Å) designed by AstraZeneca. Combining the comprehensive knowledge gained from the three structures, the researchers achieved a detailed understanding of recognition of different types of drugs by the receptor to either promote or inhibit platelet aggregation.

The new studies provide many new insights regarding the structure of the $P2Y_{12}R$ versus that of other recently crystallized GPCRs. Comparing the agonist-bound $P2Y_{12}R$ with an antagonist-bound form, the researchers found that for the agonist and non-nucleotide antagonist to bind to the $P2Y_{12}R$, the molecules adopt different orientations in the receptor, with only partially overlapped binding pockets. Moreover, agonist access to the binding pocket requires large-scale rearrangements in the extracellular region of the receptor, which could not be predicted. The nucleotide agonist draws inward the normally flexible outer loops, which tighten around the negatively charged phosphate groups.

"Since there are a number of receptors with similar properties, the research into the $P2Y_{12}$ receptor gives hope for other applications," said Professor Christa E. Müller of the University of Bonn in Germany. "Thus, the related $P2Y_{12}$ receptor is, for example, involved in the metastasis of tumor cells. Here, too, new options for cancer research could arise."

In addition to ZHAO, Müller, and Jacobson, other authors of the two studies include WU Beili, ZHANG Jin, ZHANG Kaihua, ZHANG Dandan, LI Tingting, MA Limin, ZHANG Wenru, YANG Huaiyu, JIANG Hualiang of SIMM; Raymond Stevens, Gye Won Han, Vsevolod Katritch, and Vadim Cherezov of TSRI; and Zhan-Guo Gao and Silvia Paoletta of NIH.

The studies, "Agonist-bound structure of the human $P2Y_{12}$ receptor" and "Structure of the human $P2Y_{12}$ receptor in complex with an antithrombotic drug," were funded in part by the National Basic Research Program of China (grants 2012CB518000, 2012CB910400 and 2014CB910400), NIH (U54 GM094618 and a supplement to U54 GM094618 as part of the U.S.-China Biomedical Collaborative Research Program and the NIH Intramural Research Program), and the National Science Foundation of China (grants 31370729 and 91313000). (To view the study, please visit http://www.nature.com.)