Structural analysis of sphingosine 1-phosphate receptor

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The bioactive lipid sphingosine 1-phosphate (S1P) binds to five known G protein-coupled receptors, $S1P_{1-5}$, and acts as a second messenger during cell signaling. Among them FTY720 targeting $S1P_1$ is used for immunosuppressive agent for the treatment of autoimmune disease. However, FTY720 acts through multiple S1P receptors, the mechanism of action through one or more of these receptors may account for its side effects. In 2011, the X-ray crystal structure of antagonist-bound inactive state $S1P_1$ was solved, but FTY720 is an agonist. Solving the structure of agonist-bound active state $S1P_1$ is expected not only to elucidate the mechanism of $S1P_1$, but to design of a more selective and effective drug.

First, we attempted purification of $S1P_1$R and G protein complex for structural analysis. However, the expression level of wild-type $S1P_1$ is very low. To improve this problem, co-expressing dominant negative Gi and G$\beta\gamma$ with $S1P_1$ increased the yield and enhance the stability of $S1P_1$-G protein heterotrimer complexes. Negative stain electron microscope (EM) and 2D class averages revealed uniformity and stable complex particles suitable for cryo-EM.