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Protein design strategy for structure determination of a peptide GPCR

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Motivation and Aim: G protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotic organisms. These receptors are involved in a wide range of physiological processes and are targeted by roughly a third of all existing drugs, and studying them is without a doubt a real necessity. In this work we study a peptide GPCR associated with inflammation in autoimmune diseases. Despite the rapid advances in structural biology in recent years, obtaining structural data for GPCRs is still very difficult, mainly because they tend to be thermally unstable and have poor crystal quality and low yields. Success in obtaining the structure of membrane proteins requires the achievement of several objectives: produce monodisperse samples with improved thermo- and conformational stability, increased yield of pure protein (over 0.2–0.5 mg of purified protein per 1 L of cell culture), crystal size and diffraction quality [1].

Methods and Algorithms: For expression target protein we used baculovirus expression system in Sf9 cell line. This system is one of the most successful expression systems for obtaining properly folded and functional integral membrane proteins [2]. To obtain target protein suitable for future crystallization trials we developed different genetically engineered constructs with small fusion partner proteins. Since these fusion partners increase protein quality and improve possible crystal contacts [3]. To increase the protein expression level, yield and thermal stability, we introduced several point mutations taken from accumulated structural and biophysical data (mutations predicted by machine-learning-approach based on sequence and structure GPCRs data) [4]. Additionally, we introduced a hemagglutinin signal sequence to facilitate N-tail translocation at the endoplasmic reticulum membrane, a Flag tag for detection of the receptor by antibodies, and 10 × His tag for purification on affinity resin.

Results: The best constructs showed significantly increase in the receptor's surface expression levels, as well as improve its yield in comparison with the wild type, good size exclusion chromatography, increased thermal stability with antagonist molecules.

Conclusion: The development of genetic constructs is one of the most important parts of structural biology of GPCRs and requires a lot of search effort and characterization of a large number of constructs.

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