1P009**Single particle analysis of purinergic P2X2 receptor**

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Adenosine 5'-triphosphate (ATP) is a humoral signal transmitter both in neuronal and non-neuronal tissue. Extracellular ATP controls cell function via the cell-surface ATP receptors. P2X receptors are ATP-gated non-selective cation channels widely distributed in the peripheral and central nervous system. They play critical roles in fast synaptic transmission and in presynaptic modulation. Here, we purified the P2X2 receptor using baculovirus-Sf9 cell expression system and observed its structure using electron microscopy. The FLAG-tagged P2X2 receptor was purified to be a single peak by affinity purification and gel filtration chromatography. Negatively stained P2X2 protein images were homogeneous and picked up by automated pick-up programs, aligned, and classified using the modified growing neural gas network method. Similarly oriented projections were averaged to decrease the signal-to-noise ratio. These images demonstrate an inverted three-sided pyramid composed of a high-density trunk and a stain-permeable swollen extracellular domain of a crown-shaped structure. The internal cavities and constituent segments were clearly demonstrated in both the raw images and the averaged images. The three-fold symmetrical top view demonstrates the first visual evidence of the trimeric composition of the P2X receptor family.

1P011**A fully automatic 3D reconstruction method using simulated annealing of protein projections**

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Single-particle analysis is a structure determining method using electron microscopic (EM) images, which does not require protein crystal. In this method, projections are picked up and used to reconstruct a three-dimensional (3D) structure. The Euler angles of these images must be assigned to create a 3D model. We developed a new, fully-automatic unsupervised Euler angle assignment method, which does not require an initial 3D reference and which is applicable to asymmetric molecules. In this method, the Euler angle of each average image is initially set randomly and then automatically corrected in relation to those of the other averages by iterated optimizations using the Simulated Annealing (SA) algorithm. At each iteration, the 3D structure is reconstructed based on the current Euler angles and reprojected back in the average-input directions. A modified cross-correlation between each reprojection and its corresponding original average is then calculated. The correlations are summed as a total 3D echo-correlation score to evaluate the Euler angles at this iteration. Then, one of the projections is selected, its Euler angle is changed randomly, and the score is also calculated. Based on the score change, judgment of whether to accept or reject the new angle is made using the SA algorithm. After a certain number of iterations, the angles of all averages converge so as to create a reliable primary 3D model. This method also has potential for wide application to general 3D reconstruction from various types of 2D images.

1P010**The fast and robust system to create two-dimensional (2D) average images in single particle analysis**

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In the single particle analysis, one of the limiting factors in producing a high resolution three-dimensional reconstruction is obtaining a large enough representative dataset of high-quality 2D average images in reasonable speed. In order to overcome the limitation, we developed a new system to create finer 2D average images in faster and more robust manner than traditional methods. The self-consistent 2D average images are produced from repeating multi-reference alignment steps and classification steps including multivariate statistical analysis and hierarchical ascendant classification (HAC). In our system, the alignment steps were accelerated using translational and rotational alignment procedures producing more accurate calculation than traditional methods without extra computational time. The classification step was improved by developing the global HAC method, which could build global and accurate ascendant tree even for more than 10,000 image data. Furthermore, we employed MPI and Ninf-G RPC (remote procedure call by a reference implementation of the "globus" middleware in grid computing) to implement parallel calculation and fast and accurate data I/O, respectively. We compared this system with IMAGIC V, a commonly used software for single particle analysis, by using phantom 2D image datasets of various SNR and sodium channel images by cryo-electron microscopy. Our result showed that this newly developed system outperformed the conventional software in both computational efficiency and robustness against noise of the system.

1P012**The 3D structure of DNA polymerase/PCNA/DNA complex obtained from single particle EM images**

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DNA strand synthesis is accomplished by the DNA polymerase, a central catalytic protein in the replisome. This enzyme acquires high-level processivity by interacting with a protein molecule named the sliding clamp, which is loaded onto DNA by the clamp loader. Several crystal structures of DNA polymerases as well as their complexes with DNA have been obtained, and recently we reported the 3D structure of an archaeal clamp-clamp loader-DNA complex, investigated by single particle analysis. However, the structure of clamp-polymerase-DNA complex still remains unknown, and hypothetical models have been proposed from these partial complex structures. We investigated the 3D structure of Pol BI-PCNA-DNA from hyperthermophilic archaeon, *Pyrococcus furiosus*. Pfu Pol BI belongs to the Family B, which also includes the DNA replication polymerases of eukaryotes, and a striking structural similarity was found between Pfu PCNA and human PCNA(clamps). Thus, this archaeal replication system could be considered as a good model of those of eukaryotes. A preliminary 3D structure of the negatively stained complex was obtained by common line method, and used as the initial model of the refinement procedure. The Pol BI-PCNA-DNA complex exhibited a clear ring structure, covered with a density corresponding to PolBI. The atomic model of Pfu PCNA trimer ring could be fitted nicely into the ring region, and a rod like density, related to the ds DNA, were also clearly observed in the channel of the PCNA ring.