10:15 Invited oral

## The first crystal structure of monomeric retroviral protease solved by online game players

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Mason-Pfizer Monkey Virus (M-PMV) causes acquired immunodeficiency syndrome (AIDS) in rhesus monkeys. As in all retroviruses, such as HIV-1, dimerization of its protease (PR) is a obligatory for processing of retroviral polyproteins and virion maturation. All previously determined crystal structures of retroviral proteases show interlaced dimers, representing the proteolytically active enzyme. Retroviral PR (retropepsin) is an unusual homodimeric form of aspartic protease, composed of two protein chains, each contributing a DTG triad to the catalytic center. The active site is covered by two symmetric loops called flaps. Structure-based design of protease inhibitors has led to a number of drugs used in the clinical treatment of AIDS. However their effectiveness is limited because the HIV virus is able to mutate quickly into a drug-resistant forms thus it is necessary to search for new methods of antiretroviral therapy.

An interesting approach would be to block the mandatory dimerization of the protease. Biophysical and NMR studies have indicated that in the absence of a substrate/inhibitor, M-PMV PR should fold into a stable monomer. The retroviral protease of M-PMV indeed crystallizes as a monomer, but despite the availability of several crystal forms, the crystal structure of this protein could not be solved and over a decade has resisted all molecular replacement efforts. Finally, the protein folding puzzle was presented to players of the computer game named Foldit who were challenged with the task to fold the polypeptide chain of the protein.

Foldit is a multiplayer online computer game in which players try to most accurately predict protein structures using human three-dimensional problem-solving skills. They collaborate with teammates while competing with other players to obtain the highest scoring (lowest energy) model. During the three weeks in which the puzzle was active, Foldit players generated over one million of structure model predictions. Finally the monomeric structure of M-PMV was solved by molecular replacement software, using one of the models constructed by Foldit players team Contenders.

The new structure indeed shows a monomeric protein with the termini completely disordered but the flap loop can be clearly identified in the electron density map. The flap has a completely new conformation with an unusual shape and orientation, different from both the open and closed states known from other retropepsins. Thanks to the original idea of the authors of Foldit, and to surprising intuition and three-dimensional skills of anonymous gamers, an important scientific problem could be finally solved. The structure of monomeric retropepsin determined at high resolution provides valuable extra information for the design of dimerization inhibitors that might help in development of new drugs for the treatment of retroviral infections, including AIDS.