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Cryo-EM: a New Tool for Drug Development

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The field of biological structural electron microscopy has seen an enormous transformation, primarily triggered by the availability of improved electron microscopes and direct electron detectors. It is now possible to use electron cryo-microscopy (cryo-EM) and single particle analysis to determine the structure of proteins to resolutions that used to be achievable only by crystallography or NMR methods [1,2]. The structural information now attainable by cryo-EM can be used to infer into the detailed molecular mechanisms of proteins and protein complexes. We explored its application to study protein/ligand interactions and therapeutic drug development using eukaryotic 20S proteasomes.

The proteasome is a protease complex essential in all eukaryotes, both for its role in proteostasis and in the highly regulated degradation of critical proteins, the removal of which signals for fundamental processes such as cell cycle progression. The canonical proteasome substrates are ubiquitinated proteins specifically labelled for degradation by the ubiquitin conjugation cascade. The proteasome comprises a core, the 20S proteasome, which encloses the proteolytic active sites. In eukaryotes, the 20S proteasome is a complex of about 700 kDa formed by four hetero-heptameric rings of 7 individual α and 7 individual β subunits, arranged in a barrel shaped two-fold symmetric $\alpha_{(1-7)}\beta_{(1-7)}\beta_{(1-7)}\alpha_{(1-7)}$ assembly [3]. The full activation of the 20S proteasome requires the binding of regulatory particles at the outer surfaces of its α rings. The 19S regulatory particle is the proteasome regulator which is required to bind the 20S core for the degradation of fully folded ubiquitinated proteins.

The 20S proteasome is a well-established target for cancer therapy and its inhibition is being explored for an increasing range of therapeutic usages. We used cryo-EM and single particle analysis to determine the structure of the human 20S proteasome core bound to a substrate analogue inhibitor molecule, at a resolution of around 3.5Å [4]. Our approaches for sample preparation, image recording and image analysis have been described in detail [4,5]. The resulting map allowed the building of protein coordinates as well as defining the location and conformation of the inhibitor at the different active sites. These results serve as proof of principle that cryo-EM is emerging as a realistic approach for more general structural studies of protein/ligand interactions. This has the potential benefits of extending such studies to complexes unsuitable for other methods of structure determination, requiring significantly less amounts of sample, and allowing structure determination under closer to physiological conditions, preserving ligand specificity. Within this context, we extended our studies to assist in the development of new highly specific inhibitors targeting the *Plasmodium falciparum* proteasome. *Plasmodium falciparum* is the parasite responsible for the most severe form of malaria, against which artemisinin is currently the forefront medication. The spreading of artemisinin resistant parasites, first identified in the Southeast Asia, represents therefore a major threat to human health and to the current programs aiming at controlling and eventually eradicating malaria. We determined the structure of the *Plasmodium falciparum* 20S proteasome core bond to a new specific inhibitor (Figure 1), at a resolution of around 3.6Å [6]. Our cryo-EM structure of the *Plasmodium* 20S proteasome revealed the molecular basis for the inhibitor specificity towards the parasite complex and provides a framework to guide in the improvement of the prototype ligand tested into a potential new antimalarial [7].

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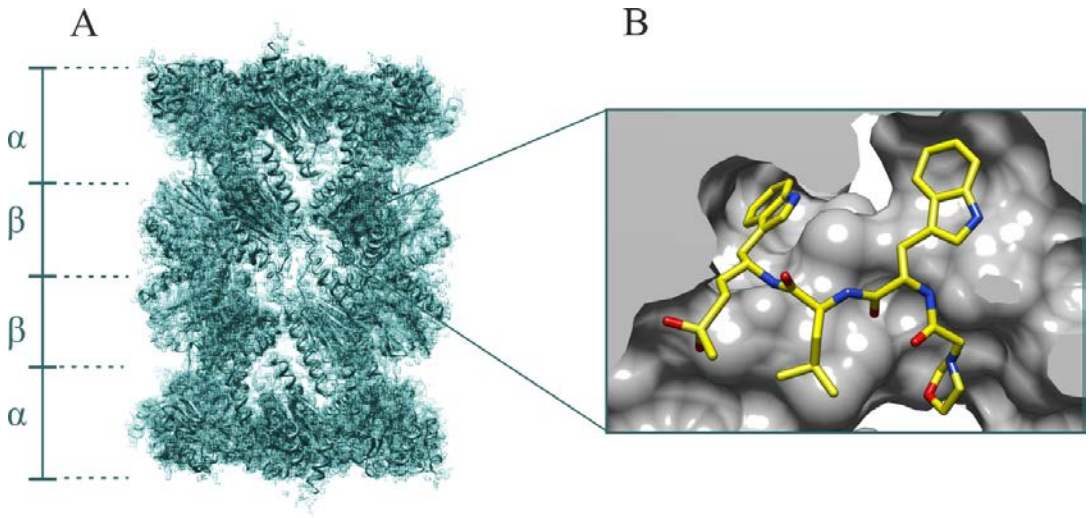

Figure 1. Cryo-EM structure of the *Plasmodium falciparum* 20S proteasome [3]. (**A**) Cryo-EM map of the *Plasmodium falciparum* 20S proteasome (mesh representation) with the protein model shown as ribbons. (**B**) Detail of the parasite 20S proteasome model (van der Waals surface) showing the ligand (yellow sticks) bound at the β2 proteolytic active site.

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