

Structure and Function of the Proteasome Activator PA28 of the Malaria Parasite *Plasmodium falciparum*

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The proteasome is a multi-subunit enzyme complex that is responsible for most of the non-lysosomal proteolysis in eukaryotic cells, underpinning proteostasis and regulating key processes such as the cell cycle. The 20S proteasome core is comprised of two heptameric rings of β -subunits, containing the catalytic sites, sandwiched by two heptameric β -rings. Substrate access to the proteasome catalytic sites is restricted by N-terminal sequences of the α -subunits (1). The activity of the 20S proteasome is regulated by protein complexes that bind to one or both ends of the proteasome. The most well-characterised activator is the 19S complex [1]. Another activator the 11S or PA28 complex is involved in ubiquitin-independent degradation. The malaria parasite, *Plasmodium falciparum*, is heavily reliant on its proteasome and proteostasis machinery.

Here we recombinantly expressed and purified an orthologue of the PA28 regulator (*PfPA28*) from *Plasmodium falciparum*. The structure of *PfPA28* was solved at 3.1 Å by X-ray crystallography, revealing a heptameric bell-shaped structure. We also purified *Pf20S* proteasome from parasite cultures and showed by Native-PAGE that *PfPA28* readily forms single- and double-capped complexes with *Pf20S*. Moreover, we demonstrated the stimulatory effects of *PfPA28* on *Pf20S* activity using fluorogenic peptide substrates. We next structurally characterized the *PfPA28-Pf20S* complex using cryo-EM. Samples (500 μ g/mL) were prepared on quantifoil grids and vitrified before collection of data using a Talos Arctica with a K2 camera, and analysis of 126,000 particles. We solved the structure of both the single capped and the double capped *Pf20S* to respectively, 3.9 and 3.8 Å (Figure 1). The activation loop (between helices 2 and 3 in each *PfPA28* monomer) plays a primary role in the interaction with the α -rings of *Pf20S*. In addition, the flexible C-terminal tails of *PfPA28* insert into the hydrophobic pockets between α -subunits and become ordered upon complex formation. The interaction induces conformational changes in α -subunits consistent with opening of the α -annulus, to allow substrate entry to the proteasome catalytic chamber. In the absence of *PfPA28*, the pore is blocked by the α 2-4 subunit N-termini. The α 5-7 and α 1 N-termini adopt an 'open' state both with and without *PfPA28*. Long disordered loops (>50 residues between helices 1 and 2 in each monomer) at the distal side of the *PfPA28* heptamer are evident in Small Angle X-ray Scattering (SAXS) data and observed in cryoEM class averages, with parts of this one loop resolved by X-ray crystallography in some subunits of the crystal structure. These loops may form an entropic brush that controls the entry of substrates to the highly basic entry pore. We used multi-body refinement [2] to study the dynamics of the *PfPA28*/core particle complex. The resulting Eigenvectors describe a Gaussian motion of the *PfPA28*

along the top of the α subunit ring indicative of a random non-correlated motion. Molecular dynamics simulation support conformational flexibility of the PfPA28 heptamer

In summary, PfPA28 binds to the α subunits, destabilizing the closed conformation of the pore and opening the pore (Figure 2). We propose that lateral transfer of substrates through this interface is an alternative route of substrate egress, particularly in hydride 19S/20S/11S complexes.

References:

- [1] D Voges, P Zwickl and W Baumeister, *Annu Rev Biochem* **68** (1999), p.1015
 [2] J Zivanov et al., *eLife* (2018) p. e42166.

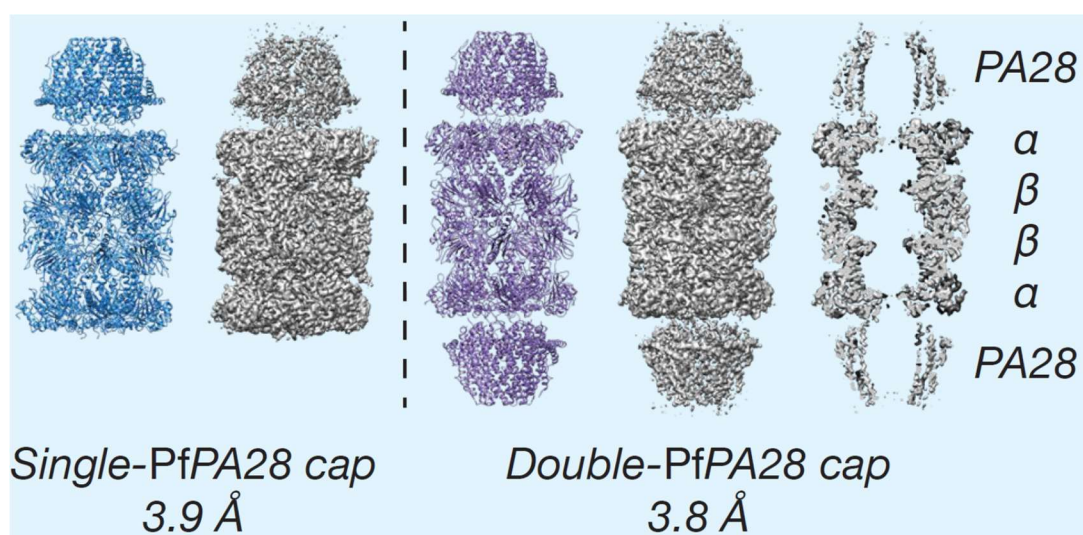


Figure 1. Structures of the single and double capped PfPA28/Pf20S complexes and corresponding molecular models.

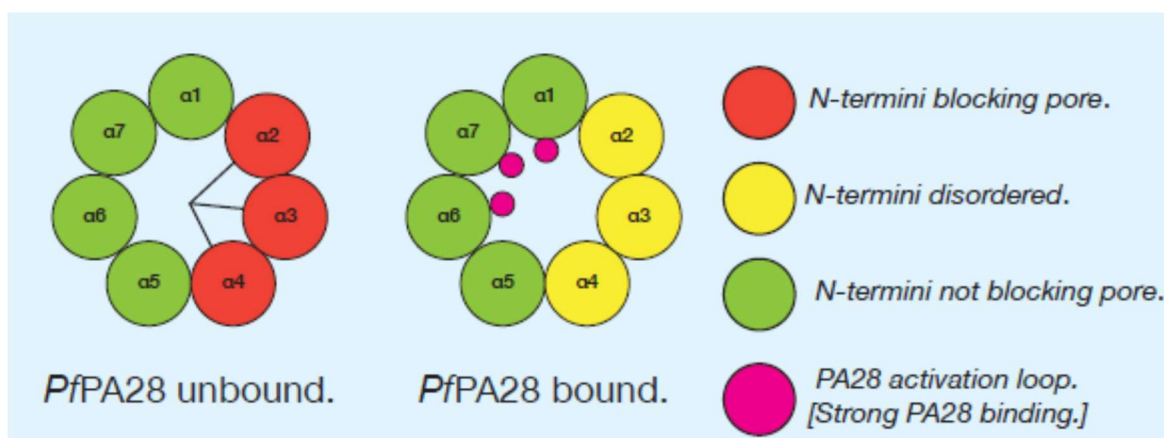


Figure 2. Schematic of the closed and open conformation of the pore of the Pf20S proteasome.