The structure of the *Plasmodium falciparum* 20S proteasome in complex with the PA28 activator.

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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. The 20S proteasome core is comprised of four heptameric rings, two rings of β-subunits, which contain the catalytic sites, sandwiched by two α-rings, which control substrate access to the proteasome core. The malaria parasite, *Plasmodium falciparum* is highly reliant on its protein turnover machinery, thus the proteasome is a drug target in the treatment of malaria. The activity of the 20S proteasome is regulated by protein complexes, such as the 19S complex, that bind to the α-subunit rings. A less-well studied regulator is the PA28 (11S) activator, which stimulates proteasome activity independent of ubiquitin or ATP.

Here, we characterized PA28 from *P. falciparum* (*Pf*PA28). We showed that genetic deletion of *Pf*PA28 renders parasites more sensitive to anti-malarial drugs, consistent with a role for PA28 in responding to proteotoxic stress. We solved the crystal structure of *Pf*PA28, revealing a bell-shaped structure with a highly charged central channel (Figure 1a). We purified *Pf*20S from parasite cultures and structurally characterized the *Pf*PA28-*Pf*20S complex using single-particle cryoEM. We solved the structure of *Pf*20S in complex with one and two *Pf*PA28 caps to a resolution of 3.92 Å and 3.82 Å respectively (Figure 1b). These structures provide insight into the binding and activation mechanism of *Pf*PA28 and provide further evidence that 11S activators employ a distinct mechanism of activation compared to the 19S complex. Analysis of the cryo-EM data also showed that *Pf*PA28 and *Pf*20S form a dynamic complex, with *Pf*PA28 undergoing large rigid motions on *Pf*20S. We propose lateral transfer of proteasome products through this interface as an alternative mechanism of substrate egress, avoiding the need for products to traverse the *Pf*PA28 pore. This work adds to a growing body of knowledge on the structural basis of proteasome activation.

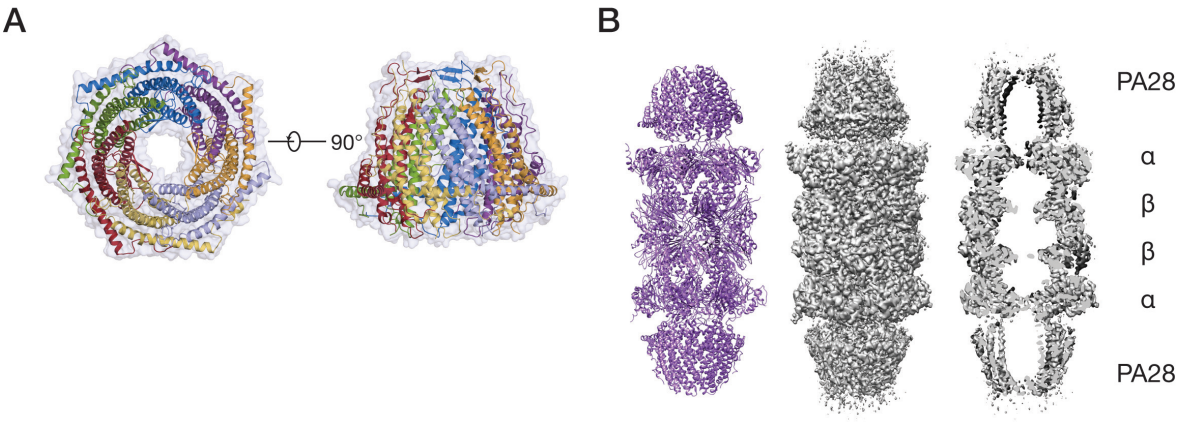

Figure 1: The structure of the *Pf*20S/*Pf*PA28 complex. A) shows the 3.1 Å resolution crystal structure of *Pf*PA28, showing a bell-shaped heptameric protein with ~20 Å diameter pore. B) shows the structure and cryo-EM density of the 3.8 Å resolution double-*Pf*PA28 capped *Pf*20S complex. A continuous channel is formed in the complex, allowing substrates to pass through the complex.