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Cryo-EM 3D Structure of *Schizosaccharomyces pombe* Phosphofructokinase

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Phosphofructokinase (Pfk) plays a key role in the regulation of the glycolytic pathway and it catalyzes the phosphorylation of fructose 6-phosphate (F6P) in the presence of magnesium and adenosine triphosphate (Mg-ATP). The enzymatic activity of eukaryotic Pfks is controlled by many different allosteric effectors (activators and inhibitors), evidencing the key role that the enzyme plays in the regulation of the glycolytic flux. Hence, a better understanding of the structural/functional properties of phosphofructokinase in different species will help to better discern the mechanism of this fundamental cellular process.

Eukaryotic phosphofructokinases are complex multimeric structures. The *Saccharomyces cerevisiae* Pfk is the best characterized and its octameric structure consists of 4α and 4β subunits. Cryo-EM studies of the enzyme in the presence of F6P (active-state) elucidated the shape of the subunits; their mutual arrangement in the hetero-octamer and allowed the localization of the putative F6P binding sites [1,2]. A latter cryo-EM study in the presence of ATP revealed the conformational changes undergone by the enzyme during its functional cycle [3]. Recently, the structures of the *S. cerevisiae* Pfk truncated hetero-tetramer (2α" and 2β') in the active-state and the *Pichia pastoris* hetero-dodecamer (4α, 4β and 4γ) have been solved by X-ray crystallography [4,5]. In contrast to *S. cerevisiae* and *P. pastoris, S. pombe* has a singular Pfk composed of eight identical subunits and it was proposed to possess a very different structure than the hetero-octameric Pfks [6]. However, the analysis of the two 3D reconstructions obtained from deep-stained preparations of the enzyme in the presence of F6P and ATP using Random Conical Technique methods showed that the structures of both hetero and homo-octameric Pfks was similar [7].

Here we present our study of the active-state *S. pombe* Pfk carried out using cryo-preparations of the enzyme in the presence of F6P. The analysis was performed using 6800 images with a pixel-size of 2.4Å extracted from negatives collected at an acceleration voltage of 100kV in a defocus range between 1.0-3.0μm (Figure 1). The reconstruction was calculated using Radon transform algorithms and 3D reference based projection alignment methods as described before [2,3,8]. The final reconstruction was calculated to have a resolution of 15Å using the 0.3 criterion [9].

The *S. pombe* Pfk structure (Figure 2a) can be described as a dimer of tetramers with similar dimensions as the *S. cerevisiae* (220Å long and 152Å wide). The rotation angle between the tetramers is ~75° as its *S. cerevisiae* counterpart (Figure 2b). However, the *S. pombe* Pfk is symmetric, the top and the bottom tetramer show the same identical conformation. This might be the result of the unique molecular composition of the octamer, possessing only α-subunits. The fitting of the bacterial tetramer into the 2α dimer using Chimera [10] is even better than that observed for its other eukaryotic counterparts (Figure 2c). Currently, we are taking advantage of the symmetry to increase the data set and improve the resolution of the structure and are modeling the complete subunit using template based and *de novo* methods.

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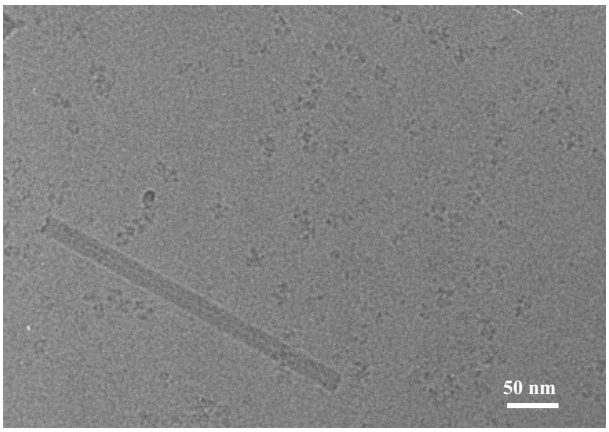

Figure 1. Electron micrograph from a cryo-preparation of *S. pombe* Pfk in the presence of F6P.

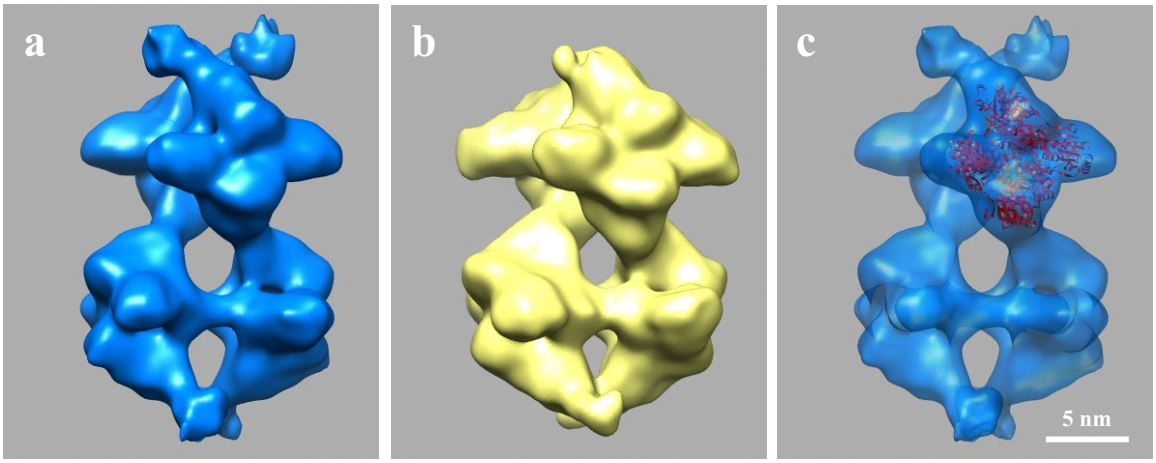

Figure 2. Cryo-EM 3D structure of *S. pombe* Pfk (a) and *S. cerevisiae* Pfk (b). Fit of the 4PFK model of the bacterial Pfk tetramer [11] into a dimer of the cryo-EM structure of *S. pombe* Pfk.

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