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# Improvement of capsular polysaccharide synthesizing enzyme crystal quality based on Differential Scanning Calorimetry data

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## Introduction

Capsular polysaccharides (CPs) are important virulence factors of *Staphylococcus aureus*. The biosynthesis of type 5 and type 8 CPs (CP5 and CP8), which are produced by most clinical isolates of *S. aureus*, is catalyzed by 16 CP assembling proteins. One of the key enzymes of this biosynthetic pathway is CapF, which catalyzes the synthesis of UDP-N-acetyl-l-fucosamine, a component of both CP5 and CP8.

## Methods and Results

Purified CapF in 10 mM Tris-HCl, pH 8.0 was concentrated to 15 mg ml$^{-1}$ for initial crystallization trials. Rod-shaped crystals were grown in a solution containing 3.5 M sodium formate at pH 7.0, using the hanging-drop vapour-diffusion method. By optimizing the buffer reagent, precipitant concentration, and additive, we were able to obtain crystals of sufficient size for diffraction analysis. The optimized precipitant solution contained 100 mM 2-(N-morpholino) ethanesulfonic acid (MES) (prepared at pH 6.2), 25% (w/v) glycerol, 100 mM $Li_2SO_4$, 300 mM NaCl and 3.9 M sodium formate (prepared at pH 6.3) (Fig. 1a).

We also conducted Differential Scannning Calorimetry (DSC) experiments to screen additional conditions that further stabilized the structure of CapF in solution, and used this data to optimize crystal growth. DSC measurements were carried out with a VP-Capillary DSC System (MicroCal, Northampton, Massachusetts, USA). The pH of the MES buffer and the identity of the sulfate counterion were adjusted, because the most suitable conditions for these variable elements could not be determined in the initial optimization. We used MES, which has a useful pH range of 5.2–7.2, as the buffer reagent. Proteins were dialyzed against 10 mM MES pH 5.5, 6.0, 6.5 or 7.0 (Fig. 2a), or in the presence of 100 mM $Na_2SO_4$, $Li_2SO_4$ or $(NH_4)_2SO_4$ (Fig. 2b). Protein samples at 20 μM were heated from 283 to 373 K at a scanning rate of 1 K min$^{-1}$. For crystallization experiments, it is desirable that all molecules have the identical structure; therefore the temperature at which CapF began to denature was compared. We chose 100 mM MES pH 7.0 with $(NH_4)_2SO_4$ as the most suitable buffer for the stabilization of CapF. Indeed, after a new round of optimization, CapF crystals with a different shape were obtained from a solution containing 100 mM MES pH 7.2, 25% (w/v) glycerol, 100 mM $(NH_4)_2SO_4$, 300 mM NaCl and 3.9 M sodium formate (Fig. 1b).

All X-ray diffraction data were collected at beamline BL17-A under cryogenic conditions (100 K) using a Quantum 4R CCD detector (ADSC). The rod-shaped crystal diffracted anisotropically and was highly sensitive to radiation damage. The crystal diffracted to a resolution of 2.98 Å, however, data at a resolution higher than 3.35 Å were not used because of the rapidly increasing value of $R_{merge}$. The rod-shaped crystal of CapF belongs to space group $P6_322$, with unit-cell parameters a = b = 229.5 Å, c = 78.9 Å. In contrast, the optimized crystal form diffracted better and withstood radiation damage. New data sets were collected up to a resolution of 2.80 Å. The optimized crystal belongs to the different space group $P322_1$, as determined by preliminary solution of the structure, with unit-cell parameters a = b = 119.6 Å, c = 129.5 Å. The structure determination of CapF is currently in progress.

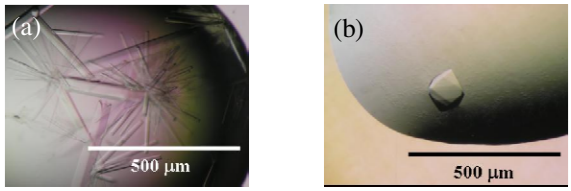


**Figure 1.** Crystals of CapF in space groups $P6_322$ (a) and $P322_1$ (b). The crystals diffracted up to a resolutions of 3.35 Å and 2.80 Å, respectively.

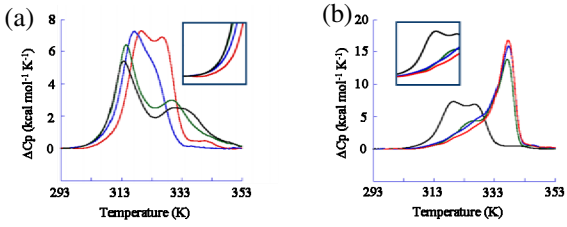


**Figure 2.** Heat-capacity curves of CapF. (a) pH-dependence: 10 mM MES at pH 5.5 (black), 6.0 (green), 6.5 (blue) and 7.0 (red). (b) Effects of salt: no salt (black), 100 mM $Li_2SO_4$ (green), 100 mM $Na_2SO_4$ (blue) and 100 mM $(NH_4)_2SO_4$ (red). 1 kcal = 4.186 kJ.

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