Seeding-Driven Crystallization and Structural Investigation of the Coxsackievirus B3 3C Protease

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This study focuses on the expression, purification, crystallization, and structural investigation of the 3C protease of Coxsackievirus B3 ($3C^{pro}$), a key enzyme in the life cycle of viruses of the Picornaviridae family and an important target for antiviral drug development. The work aims to improve crystal morphology and diffraction quality through seeding techniques in both single-crystal and polycrystalline samples. The protein was expressed and purified using established recombinant expression protocols [1]. Crystallization experiments were performed using vapor diffusion (sitting drop) and batch methods, while seeding approaches were applied to control nucleation and promote uniform crystal growth. Two main objectives were pursued: (i) improving diffraction data quality from polycrystalline samples to enable structure determination using X-ray powder diffraction, and (ii) obtaining single crystals suitable for fragment-based screening to identify potential small-molecule inhibitors [3].High-resolution X-ray powder diffraction data were collected at the European Synchrotron Radiation Facility (ESRF) on beamline ID22 [6], and single-crystal diffraction data were obtained at ESRF macromolecular crystallography beamlines. The results show that seeding significantly improves diffraction quality, facilitating structure determination from polycrystalline samples and supporting future fragment-based drug discovery targeting $3C^{pro}$.

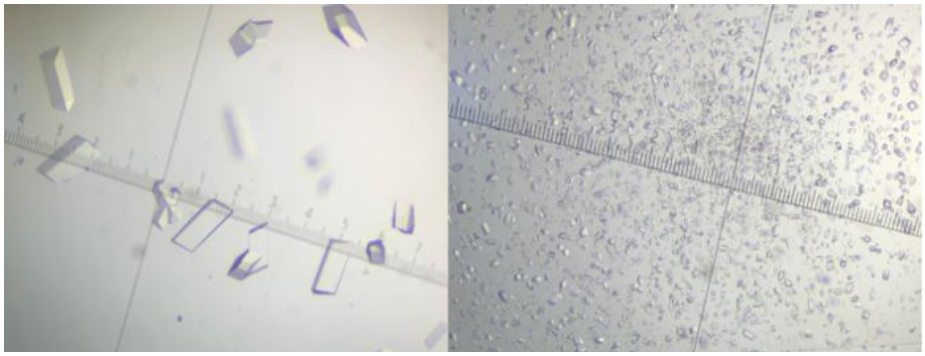


Figure 1: *Crystals obtained via sitting drop method (left) and via batch method (right) utilizing the seeding technique in both cases*

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