

# Structural analysis of *in vivo*-grown protein microcrystals

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Protein self-assembly plays a central role in many normal and pathological processes of the cell. Beyond cellular life, the self-assembly of capsid proteins is at the heart of the remarkable diversity of viral infectious forms. We are interested in the intracellular self-assembly of protein crystals that contain and protect the infectious particles of common insect viruses. The *in vivo* formation of these crystals and their remarkable robustness has prompted their use as microcontainers as slow-release microparticules.

We use structural biology to elucidate the structure of these functional microcrystals directly purified from infected insects. In particular, entomopoxviruses or EVs produce two types of protein microcrystals called spheroids and spindles. Spheroids are virion-containing crystals representing the main infectious form of EVs; spindles are bipyramidal crystals of the viral fusolin protein that contribute to the oral virulence of these viruses. To understand how these crystals assemble *in vivo*, we used X-ray microcrystallography to determine the structures of EV spindles isolated from EV-infected common cockchafers [1] and spheroids isolated from insects of the Coleoptera orders. The folds of fusolin and spheroidin are unrelated. While they assemble into crystals belonging to the same space group, their molecular organisations are completely different. Yet, both types of crystals have converged to use a similar stabilisation strategy based on inter-molecular disulphide bonds resulting in a covalently-crosslinked crystalline matrix of extreme robustness.

Together with those of the two classes of viral polyhedra [2,3], these structures provide a framework to understand unexpected convergences between the architectures of *in vivo* crystals. Qualitatively, crosslinking molecular arms emerge as a common feature that may facilitate efficient crystallization in the complex milieu of the cell. Quantitative insights will require further comparative studies, including mathematical modelling.

## References

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