Virology Group, Department of Biochemistry, Monash University, (Australia). [b]*INRA, Montpellier, (France).* [c]*Swiss Light Source at The Paul Scherrer Institute, (Switzerland).* Email: fasseli.coulibaly@monash.edu

While protein crystallization of highly purified proteins has been studied for decades and used to painstakingly produce crystals for structural analysis, common insect viruses have evolved proteins that readily crystallize *in vivo* despite the complexity of the cellular environment. Viral, *in vivo* crystals are among the most striking examples of protein self-assembly, ultimately leading to the formation of ultra-stable microcrystals occupying most of the infected cells.

Recently, the first structures of *in vivo* crystals were elucidated from viral polyhedra produced by RNA [1] and DNA [2,3] viruses. The function of polyhedra is to package up to hundreds of viral particles constituting the main infectious form of the virus and allowing the virus to persist for years in the environment like bacterial spores.

Apart from polyhedra, spheroids produced by poxviruses are the only other known example of viral infectious crystals. RNA and DNA virus polyhedra share nearly identical lattices, similar function and, of course, common polyhedral morphologies. In contrast, whether spheroids were single crystals remained unclear because of their ovoid shape and the large size of their 100kDa matrix protein.

To elucidate the molecular organization of the third class of infectious crystals, we determined the 2.5Å structure of entomopoxvirus spheroids. These diffraction experiments were carried out on spheroids with maximum dimensions of 15µm directly purified from infected insects and stored frozen for over two decades.

The structure determination of spheroids confirmed that they are also single crystals despite their unusual ovoid shape. In the crystal, the main constituent of spheroids, called the spheroidin protein, adopts a multi-domain fold with five independent structural domains unrelated to either of the two types of polyhedrin protein. The asymmetric unit of the crystal contains two 100kDa spheroidin molecules adopting conformations that differ by hinge movements between the five domains. This apparent flexibility of the spheroids building blocks was totally unexpected given that crystallization occurs readily *in vivo* despite the complexity of the cellular milieu and results in remarkably stable crystals. A detailed analysis of the crystal organization provides an explanation to this conundrum revealing that building blocks interlock into the crystalline lattice using an extensive network of disulfide bonds.

In conclusion, this structure provides a detailed view of what may constitute the most improbable protein crystals. Spheroids not only harbor an atypical ovoid shape and host hundreds of irregular virus particles without disruption of the crystalline lattice but they are also able to assemble *in vivo* from large and flexible building blocks. Mirroring the complexity of poxviruses themselves, spheroids are undoubtedly the most elaborate viral armors providing a unique model of *in vivo* crystallization.

[1] F. Coulibaly et al. *Nature* **2007**, *446*, 97-101. [2] F. Coulibaly et al. *Proc Natl Acad Sci USA* **2009**, *106*, 22205-10. [3] X. Ji et al. *EMBO J* **2010**, *29*, 505-514.

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X-ray structure of a functional full-length dynein motor domain

Genji Kurisu,[a,b,d] Takahide Kon,[a,b] Rieko Shimo-Kon,[a] Kazuo Sutoh,[c] [a]*Institute for Protein Research, Osaka University (Japan).* [b]*Department of Macromolecular Science, Graduate School of Science, Osaka University (Japan).* [c]*Faculty of Science and Engineering, Waseda University (Japan).* [d]*PRESTO, (Japan) Science and Technology Agency (JST).* E-mail: takahide.kon@protein.osaka-u.ac.jp

Dyneins are microtubule-based motor complexes that power a wide variety of biological processes within eukaryotic cells, including the beating of cilia and flagella, cell division, cell migration, and the intracellular trafficking. Compared to the other cytoskeletal motors kinesin and myosin, the molecular mechanism of dynein is still poorly understood, in part due to the lack of high-resolution structural information. Because dyneins belong to the AAA+ family of mechanochemical enzymes, its structure and mechanism must be fundamentally different from the G-protein related kinesins and myosins. The X-ray crystallography of dynein or even its motor domain—a 380-kDa portion of the heavy chain responsible for dynein's motor activity—has been challenging due to its large size and molecular complexity.

Here, we report an X-ray crystallographic analysis of the entire functional 380-kDa motor domain of *Dictyostelium* cytoplasmic dynein, the longest polypeptide (~3,300 residues) that has been crystallized so far. Diffraction from crystals extends to 4.0 Å using synchrotron radiation from an undulator source (BL44XU, SPring-8). Diffraction is consistent with space group $P2_12_12_1$ with unit cell dimensions of a = 201.17 Å, b = 228.96 Å, c = 195.73 Å. Based on an electron density map calculated from the Ta_6Br_{12} derivative data at 4.5 Å resolution, an α-helical model of the dynein motor domain has been created [1]. The analysis reveals detailed architectures of functional units responsible for the motor activity, such as the ATP-hydrolyzing, ring-like head composed of six AAA+ modules as well as the long coiled-coil microtubule-binding stalk, the force-generating rod-like linker and some unpredicted structures likely to be key to function. This long sought crystal structure provides the framework to understand a large volume of data obtained by electron microscopic, biochemical and single-molecule studies, and opens the door to detailed understanding of how dynein produces force and movement.

[1] T. Kon, K. Sutoh, G. Kurisu, *Nature Struct. Mol. Biol.* **2011**, *in press*

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Generating functions for structure and chemical composition

Frank C. Hawthorne, *Dept. of Geological Sciences, University of Manitoba, Winnipeg, (Canada).* E-mail: frank_hawthorne@umanitoba.ca

Any crystal structure may be represented by a weighted chromatic digraph, the vertex set of which represents atoms and the edge set of which represents chemical bonds. We may write tetrahedrally coordinated cations and their associated anions as $\{T_{2n}\Theta_m\}$. For $\{T_{2n}\Theta_m\}$ to be a chain or ribbon, $5n < m \leq 6n$, and we may write m as $5n + N$, where N is an integer. Within the $\{T_{2n}\Theta_{(5n+N)}\}$ unit, we may recognize three types of anion vertices: (1) bridging anions, Θ^{br}, that are bonded to two T cations; (2) apical anions, Θ^{ap}, that are involved in linkage to other cations out of the plane of the bridging anions; and (3) linking anions, Θ^{l}, that link to non-T cations in the plane of the bridging anions. We can incorporate the connectivity of the cations into our algebraic representation of the chain as follows: $\{T_{2n}\Theta^{br}_a\Theta^{nbr}_b\Theta^{ap}_c\}$ where $a + b + c = 5n + N$. The apical anions of the T-layer map onto a 6^3 net which, in turn, maps onto the 3^6 net of anions of the O-layer. We may use the handshaking dilemma of graph theory to examine the interaction between the two types of layers, and write a *Structure-Generating*