Oral presentation

Structures of honeybee-infecting virus reveal domain functions and capsid assembly with dynamic motions

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Understanding the structural insight and diversity of honeybee-infecting viruses is critical to maintain pollinator health and manage the spread of diseases in ecology and agriculture. Our experience on virus-like particles (VLPs) of grouper nervous necrosis virus (GNNV) [1] and shrimp nodavirus [2] has led us to determine structures of *T*=4 and *T*=3 capsids of honeybee-infecting Lake Sinai virus (LSV) 2 and delta-N48 LSV1, belonging to tetraviruses, at resolutions of 2.3–2.6 Å in various pH environments [3]. Structural analysis shows that the LSV2 capsid protein (CP) structural features, particularly the protruding domain and C-arm, differ from those of other tetraviruses. The anchor loop on the central β-barrel domain interacts with the neighboring subunit to stabilize homo-trimeric capsomeres during assembly. For a comparison, we also determine the cryo-EM structure of the *T*=3 delta-N48 LSV1 VLP at 2.6 Å. Delta-N48 LSV1 CP interacts with ssRNA via the particular positively charged domains. Cryo-EM reconstructions, combined with synchrotron X-ray crystallographic and small-angle X-ray scattering analyses, indicate that pH affects capsid conformations by regulating reversible dynamic particle motions and sizes of LSV2 VLPs. C-arms with continuous densities exist in all LSV2 and delta-N48 LSV1 VLPs across varied pH conditions, indicating that autoproteolysis cleavage for γ peptide release, which was generally observed in other known *T*=4 and *T*=3 viruses, is not required for LSV maturation. Interestingly, an introduction of a double mutation of M83E/D461F on the LSV2 CP, mimicking the key residues at the autoproteolysis sites from other tetraviruses, potentially triggers a self-cleavage process on the specific scissile bond of LSV2 CP. Moreover, the observed linear domino-scaffold structures of various lengths, made up of trapezoid-shape capsomeres, provide a basis for icosahedral *T*=4 and *T*=3 architecture assemblies. These findings advance understanding of honeybee-infecting viruses that can cause Colony Collapse Disorder [4].

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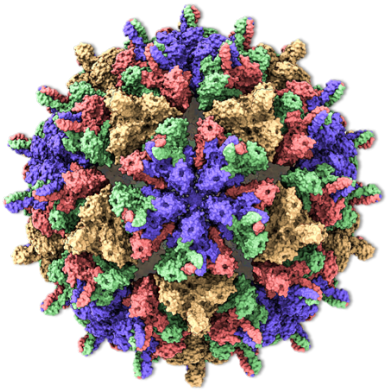

Figure 1. The structure of *T*=4 LSV2 VLP.

[1] Chen, N.-C., Wang, C.-H., Yoshimura, M., Yeh, Y.-Q., Guan, H.-H., Chuankhayan, P., Lin, C.-C., Lin, P.-J., Huang, Y.-C., Wakatsuki, S., Ho, M.-C., Chen, C.-J. (2015). PLoS Path. **11**(10): e1005203.

[2] Chen, N.-C., Yoshimura, Naoyuki Miyazaki, M.Guan, H.-H., Chuankhayan, P., Lin, C.-C., Chen, S.-K., Lin, P.-J., Huang, Y.-C., Iwasaki, K., Nakagawa, A., Chan, S. I., Chen, C.-J. (2019). Commun. Biol. **2**:72.

[3] Chen, N.-C., Wang, C.-H., Yoshimura, M., Yeh, Y.-Q., Guan, H.-H., Chuankhayan, P., Lin, C.-C., Lin, P.-J., Huang, Y.-C., Wakatsuki, S., Ho, M.-C., Chen, C.-J. (2023). Nat. Commun. 14, 545.

[4] Cox-Foster, et al. (2027) Science **318**, 283–287.