2004
doi:10.1017/S1431927620020115
Microsc. Microanal. 26 (Suppl 2), 2020

Multiplexing Bacteriophage Capsids for Medium Throughput Structure Determination

Simon White and Jennifer Podgorski

University of Connecticut, Storrs, Connecticut, United States

Bacteriophages, viruses that infect bacteria, play a huge, and not fully understood, role in the biosphere and host genome evolution and are thought to have been instrumental in the emergence of the cellular life we see today[1,2]. Therefore, understanding the virosphere, and the evolutionary relationships between viruses, will provide important insights into the formation of the biosphere. It has been proposed that the best way to understand these viral evolutionary relationships is to focus on the capsid structure and the viral proteins that make it[3-5] [6,7]. This is based on the fact that most viruses utilize a protein capsid as part of their life-cycle and that these viruses use only a few conserved structural capsid protein folds[8][4,9].

Currently, the actively curated VIPERdb database[10,11] of viral capsid structures contains 752 different viruses. Although impressive, and the result of many years of hard work, this is not even a drop in the ocean of the estimated billions of unique viruses[12,13]. One problem with our current structural collection of viral capsid proteins is that the viruses infect a widely diverse range of hosts. Trying to find evolutionary links between viruses that infect very different hosts – that may be separated by billions of years of evolution with no fossil records – is very challenging. The problem is that we lack sufficient data to map viral evolution with any great certainty. My proposed solution is to focus on viruses that infect a phylum of bacteria, the *Actinobacteria*, where the viruses are far more closely related[14].

However, the huge number of different bacteriophage makes this kind of study challenging. Therefore the need for higher throughput cryo-EM data collection is sorely needed for viral capsid studies. We have shown that multiple different bacteriophage capsids can be "multiplexed", mixed together before vitrification, to obtain multiple structures from one session of data collection. We have used this method to start screening the large actinobacteriophage family and using the structures to understand the diversity and evolution of these bacteriophages.

References

1. Koonin, E. V. et al. (2006). *Biology Direct 2017 12:1*, *1*, 29.
2. Durzyńska, J. et al. (2015). *Virol. J.*, *12*, 169.
3. Sinclair, R. M. et al. (2017). *J. Virol.*, *91*, 425.
4. Krupovic, M. et al. (2017). *Proc. Natl. Acad. Sci. U.S.A.*, *114*, E2401.
5. Casjens, S. R. et al. (2011). *Virology*, *411*, 393.
6. Grose, J. H. et al. (2014). *Virology*, *468-470*, 421.
7. Abrescia, N. G. A. et al. (2012). *Annual Review of Biochemistry*, *81*, 795.
8. Bamford, D. H. et al. (2002). *Theor Popul Biol*, *61*, 461.
9. Krupovic, M. et al. (2011). *Curr Opin Virol*, *1*, 118.
10. Carrillo-Tripp, M. et al. (2009). *Nucleic Acids Res.*, *37*, D436.
11. Ho, P. T. et al. (2018). *Annual Review of Virology*, *5*, 477.
12. Brüssow, H. et al. (2002). *Cell*, *108*, 13.
13. Wommack, K. E. et al. (2000). *Microbiol Mol Biol Rev*, *64*, 69.
14. Shapiro, J. W. et al. (2018). *MBio*, *9*, 504.