

Relaxation and repulsion helps viruses pack DNA

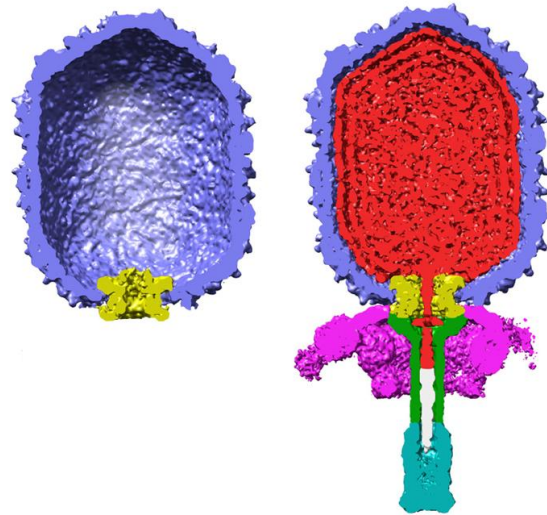
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The molecular motor that folds and packs DNA into a virus is at its most efficient when the DNA shows some self-repulsion. That is the surprising finding of researchers based in the US – it was previously thought that such repulsion would act as an obstacle in the packing process. The team also found that pausing the motor and allowing it to relax increased the rate of the whole packaging process. In addition to providing new insights into how viruses function, the work could benefit biotechnologies that enclose long polymers into nanoscale devices.

After invading its host cell, a virus reprogrammes the cell's nucleus to duplicate it. As it replicates, a strand of DNA is pulled from an infected host cell and squeezed into a protein shell – known as a prohead – which then carries the DNA to infect other cells. In some species, the prohead is produced first, leaving only a small hole at one end through which a powerful molecular motor pushes the DNA in and then packs it at very high densities. The motor has to overcome three forces: the electrostatic self-resistance that comes into play because DNA is negatively charged; the mechanical resistance of DNA to bending; and the entropic resistance of DNA to be crowded on itself.

Increasing attraction

The DNA could, however, be made attractive – a cell containing positive ions (notably a polyamine called spermidine³⁺) could stick to the DNA and partially screen the repulsion or even create attractive forces at high concentrations. In the past, certain computational models suggested such attraction might help the packing process. But according to biophysicist **Douglas Smith** of the University of California, San Diego, who led the new research, the earlier models had assumed the DNA was able to continuously relax to the lowest energy state as it was packaged, and this would reduce resistance.



Perfectly packaged: illustration of a virus with DNA inside. Cross-sections of the empty phi29 prohead (left) and fully assembled virus (right) filled with DNA. A molecular motor (not shown) transports the DNA (red) into the prohead through a portal channel (yellow). The images were produced via 3D reconstructions of electron-microscopy data. (Courtesy: Jinghua Tang and Timothy Baker, University of California, San Diego)

To test this, Smith and colleagues manoeuvred two microspheres near to each other using optical tweezers, attaching viral DNA to the first and the molecular motor to the second. Occasionally, the molecular motor managed to grab hold of the DNA and pull it from one microsphere to the second in a process that Smith compares to fishing. Smith's team stopped the motor part of the way through the packaging process by depriving it of nutrients. The researchers found that, upon restarting the packaging process, it proceeded faster than its previous rate. Indeed, the longer it was stopped, the faster the process went when restarted. The team believes this shows that the virus does not instantaneously become a neat spool, but is packaged as a higher-energy, messier configuration, which only later relaxes to the optimum. The findings were published in *Proceedings of the National Academy of Sciences* in May this year.

Repulsively efficient

The researchers then decided to measure how the packaging rate varied with the concentration of spermidine³⁺, which they reported in *Physical Review Letters* last week. When they added enough spermidine to the mixture so that the DNA's self-repulsion was only just reduced, they saw an increase in the packaging rate. But when enough spermidine was added to make the DNA self-attractive, the packaging initially proceeded very rapidly before stalling – in about 75% of cases the molecular motor stopped part way through being packaged and the process did not complete. "If the thing is sticking to itself," explains Smith, "and it gets into a bad, disordered configuration, then it may be very hard for it to rearrange." Curiously, therefore, some degree of repulsion appears to be necessary to allow the DNA to be packaged into a small space.

William Gelbart of the University of California, Los Angeles, who was not part of the current research but has worked on packaging viral DNA, points out that recent experimental work has shown that such systems are not "equilibrating and that's what I see as really important about this work, namely that attractions in particular get the system stuck out of equilibrium. This is something that we have to contend with as a fact of life about viral packaging."

Smith's team is now looking at other viruses to establish its findings. He says that the results may be of interest to the biomedical community that could study how to target this assembly step with drugs as a way to halt viral infection.

The research is published in *Proceedings of the National Academy of Sciences* and *Physical Review Letters*.

About the author

Tim Wogan is a science writer based in the UK