Engineering the bacteriophage T4 to serve as a vector for molecular repair

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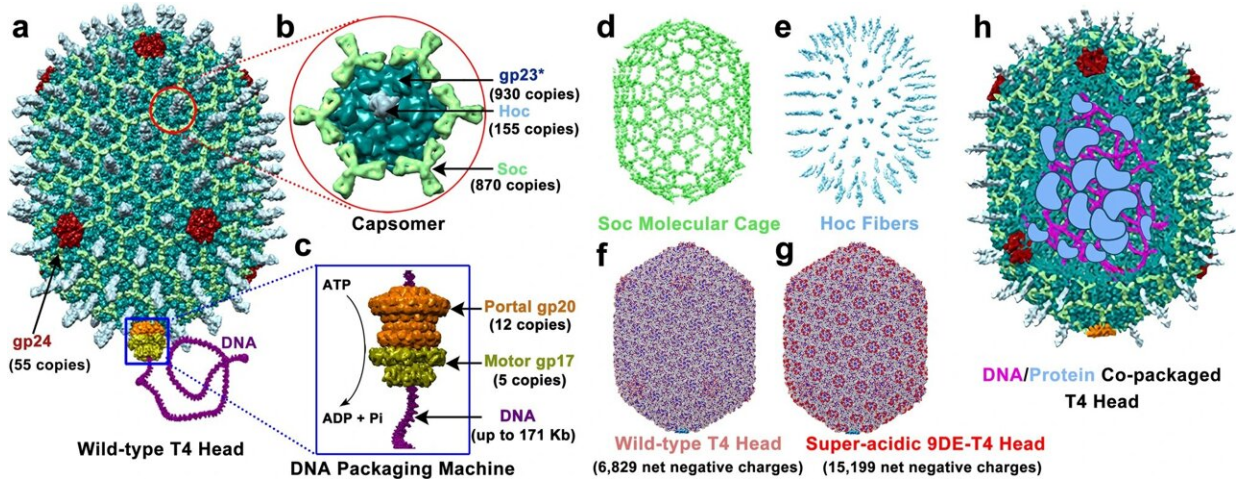

Structural components for assembly of bacteriophage T4-AVVs. **a** Structural model of phage T4 head (capsid). Pentameric gp24 vertices are shown in red. **b** Enlarged capsomer shows the hexameric arrangement of major capsid protein gp23 (dark green), Soc trimers (light green), and Hoc fiber (cyan). **c** Enlarged DNA packaging machine structural model comprised of gp20 portal dodecamer (PDB 3JA7) (brown) and pentameric gp17 DNA packaging motor (PDB 3CPE) (yellow). **d** Eight hundred and seventy Soc molecules assembled at the quasi-three-fold axes form a molecular cage around T4 capsid (PDB 5VF3). **e** One hundred and fifty-five Hoc fibers emanate from the centers of capsomers (PDB 3SHS). **f**, **g** Molecular surfaces of wild-type (WT) T4 capsid (3.4 Å, PDB 7VS5) (**f**) and super-acidic 9DE-T4 capsid (3.9 Å) (**g**) are colored according to electrostatic potential. The color ranges from red, corresponding to a potential of −5 kT/e^-, to blue, corresponding to a potential of +5 kT/e^-. The WT-T4 capsid has 6,829 net negative charges and the 9DE-T4 capsid has 15,199 net negative charges. **h** Schematic of head packaged with foreign proteins and DNAs in its

interior space. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-38364-1

A team of medical scientists at The Catholic University of America, in Washington, D.C., working with a colleague from Purdue University, has developed a way to engineer the bacteriophage T4 to serve as a vector for molecular repair. The study is reported in the journal *Nature Communications*.

Prior research has shown that many human ailments arise due to genetic mutations: cystic fibrosis, Down syndrome, sickle cell disease and hemophilia are just a few. Logic suggests that correcting such genetic mutations could cure these diseases. So researchers have been working toward developing gene editing tools that will allow for safe editing of genes.

One of the most promising is the CRISPR gene editing system. In this new effort, the research team took a more general approach to solving the problem by working to develop a vector that could be used to carry different kinds of tools to targeted cells and then enter them to allow for healing work to commence.

The vector is based on the T4 bacteriophage, a virus that is known to infect E. coli bacteria. It was chosen because of its safety record and large size, making it a suitable candidate for carrying relatively large-sized payloads. The most recent work with the bacteriophage has involved giving the virus a coating that allows it to more easily slip through lipid cell walls.

They also developed a CRISPR system that could be easily used with T4 and engineered the virus to optimize its ability to carry a large payload,

which can include (in addition to a CRISPR system) large amounts of DNA, proteins, RNA and biomolecules.

Thus far, the team has tested their system on specialized human cells in a petri dish and have found that it has worked as hoped. They next plan to test it with primary and embryonic cells and then move on to testing in mouse models. The ultimate goal, of course, is test it in humans, where they hope it could cure a large variety of diseases.

More information: Jingen Zhu et al, Design of bacteriophage T4-based artificial viral vectors for human genome remodeling, *Nature Communications* (2023). DOI: 10.1038/s41467-023-38364-1