Unraveling the structure and dynamics of fully packaged virus particles

Kush Coshic
PhD student, Aksimentiev Lab
University of Illinois at Urbana-Champaign
Target: the 3D organization of genomes in individual virions

Protein capsid:
Atomic structures are available for many species

Genome:
Averaged cryoEM density
Partial density for select species

DNA is a highly charged and stiff polymer

Internal pressure
~60 atm

Evilevitch et al, 2014 (Lund university)

Genome: Averaged cryoEM density
Partial density for select species

Open questions:
- What is the 3D structure of the packaged genome?
- How genome ejection is triggered and sustained?
- Can the structure of the genome and/or genome—capsid interface be used as drug target?

Experiments currently cannot resolve the genome structure at atomic resolution
Multi-resolution packaging of dsDNA genomes (HK97)

HK97
Bacteriophage Capsid, Head II
Gan, Lu, et al.
PDB ID: 2FT1

Smooth, purely repulsive volumetric map
Volmap, VMD: capsid+portal (3KDR)

Fine tuning the multi-resolution model

Coarse-grained DNA
Grid-based capsid

Atomistic DNA
Grid-based capsid

Atomistic DNA
Atomistic capsid

F

17.2 Å

17.3 Å

16.8 Å

Duda, Robert L., et al.
Packaging a model herpes-like virus

Despite near-identical simulation conditions, packaged genome differed in each capsid (INDIVIDUALITY)

Occasional protrusions of DNA from one side to another

Trajectories lasted >1 ms with 40-fs timestep, requiring ~4 months of simulation

Only feasible with Frontera GPU-nodes!
Packaged genome configurations are unique

Local helical axis of DNA, shown here, winds around the packaging axis near the equator.

Baseball-like order at surface, with two cupped halves having orthogonal order at the poles.

Order in the same-direction at the poles.
DNA is packaged near the speed of spontaneous ejection

Spontaneous ejection of DNA from a bacteriophage capsid
~ 1 year’s simulation!!

Capsid is not shown for clarity

Only 4 times slower!
DNA is packaged in switchback loops

DNA is colored according to local bending energy

The three loops are shown in green, magenta, and violet

Cells use loop extrusion to weave and tie the genome
Mirny, Nature (2021)
The structure and physical properties of a packaged bacteriophage particle

Kush Coshic, Christopher Maffeo, David Winogradoff and Aleksei Aksimentiev.

Under peer review

Bacteriophage HK97
39,732 bp DNA genome
(~ 60nm diameter)
Fully solvated system: 27 M atoms

Method generalizable to any dsDNA virus

“The structure and physical properties of a packaged bacteriophage particle”
Kush Coshic, Christopher Maffeo, David Winogradoff and Aleksei Aksimentiev.
Under peer review
Experiment

All-atom simulation

Face

Vertex

3-fold symmetry

5-fold symmetry

1300 structures

1 structure (60 icosahedron symmetries)

Normalized DNA density (simulation)
Coarse-grained simulations

No symmetrization
4 symmetry axes
60 symmetry axes
4 replicas
16 replicas
TEM-like analysis of packaged HK97 genome


DNA mass density obtained from the coarse-grained packaging simulations projected along several axes
Frontera enables unprecedented high throughput analysis

A microsecond trajectory of the 27 M atom system => Around 30 Terabytes data
We have 8 such replicas

Water and ion exchange
DNA matters: Capsid structure and dynamics

Locally correlated but globally asynchronous breathing motion

Empty capsid (cut-away view)

DNA-filled capsid (cut-away view)

0 ns Empty

0 ns Packaged
Effect of DNA on water and ion exchange

DNA makes water exchange slower by twofold because of reduced volume of water filled cavities and reduced diffusivity.

Empty capsid is anion selective (5 fold). DNA doubles the rate of cation exchange and lowers (5 fold) the rate of chloride exchange.
Electrostatics in a fully packaged virion (HK97)

The electrostatic potential inside packaged particle is 30 mV higher than outside.

The capsid interior starts with a lower electrostatic potential.
Summary

Multi-resolution simulation can deliver complete all-atom structures of packaged virions.

DNA packaging occurs via a loop extrusion mechanism.

Each packaged particle is unique because of the topology of its packaged genome, and thus the concept of individuality goes all the way to the most primitive forms of life.

Twisting DNA while packaging did not produce major differences in the structure and physical properties of packaged particles.

Packaged DNA affects the structure and fluctuation of the capsid as well as diffusion of water and ions inside and through the capsid.
The structure and dynamics of a fully packaged RNA virus

Kush Coshic and Aleksei Aksimentiev.

In preparation

4 M atoms
90 ns/day with 256 nodes

Multiple replicas
2.5 μs each
**RNA genomes of self-assembled viruses**

**LETTER**

doi:10.1038/nature20589

In *situ* structures of the genome and genome-delivery apparatus in a single-stranded RNA virus

Xinghong Dai1,2, Zhikai Li3,4, Mason Lai1, Sara Shu1, Yushen Du1, Z. Hong Zhou2,3 & Ren Sun1,2

1Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, USA
2Center for Phage Technology, College Station, Texas 77843, USA
3Department of Statistics, Texas A&M University, College Station, Texas 77843, USA
4Department of Computer Science, University of Oxford, Oxford OX1 3QD, United Kingdom

---

**BIOINFORMATICS**

Hierarchical natural move Monte Carlo refines flexible RNA structures into cryo-EM densities

JENG-YIH CHANG1,2, ZHICHE NG CUI1,2, KAILU YANG1,2,5, JIANHUA HUANG2, PETER MINARY4, and JUNJIE ZHANG1,2

1Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, USA
2Center for Phage Technology, College Station, Texas 77843, USA
3Department of Statistics, Texas A&M University, College Station, Texas 77843, USA
4Department of Computer Science, University of Oxford, Oxford OX1 3QD, United Kingdom

---

**Sequence 2341–2770**

**Long-range base-pairing**

**Kissing-loop interaction**

**Long-range base-pairing**

**Sequence**

**2 RNA structures**
Measuring the electrophoretic mobility of mature vs immature virions

\[ E = 0.0033 \text{ V/Å} \]

\[ v = \sim 0.5 \text{ Å/ns} \]
Mature Dengue virion

Ejection *in-vivo*

Passage through the nuclear pore complex

Probing mechanical properties of capsids

Coming soon