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Analyzing DNA Packaging Initiation of Bacteriophage T4 by a Real-Time Single Molecule Fluorescence Assay

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Variety of structural, biochemical and recently single molecule optical tweezers experiments have been used to study the mechanochemical aspects of viral packaging motors. However, due to transient and complex nature of interactions, it has been difficult to quantify viral assembly and packaging initiation using these techniques. We developed a single molecule fluorescence assay to study initiation and re-initiation of DNA packaging in the T4 bacteriophage. The reconstituted complexes were immobilized on a polymer covered slide and fluorescently labeled, short double stranded DNA and ATP were added and individual packaging machines, each carrying out DNA translocation, were imaged in real-time by total internal reflection microscopy. We found that the T4 packaging machine can package multiple DNA into the same head with the re-initiation rate depending on the DNA and ATP concentrations. Mutations in the Walker A P-loop of the packaging motor showed very low initiation efficiency, slow initiation time, and far fewer initiations per head. By quantifying the assembly efficiency in the presence of different co-factors, we found that while the motor is stable in the ATP-bound state after ATP hydrolysis the motor is much less stably bound to the viral capsid. The presence of a DNA in the portal channel increases the stability of motor complex. This assay can be extended to study the effect of other relevant proteins on the assembly and initiation of DNA packaging.

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The Unusual Compactness of Viral RNA

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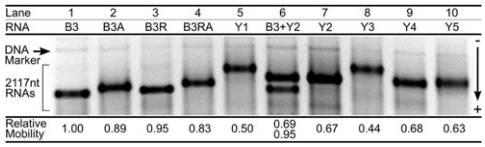
We present complementary experimental and computational studies of the relative sizes of equal-length long single-stranded (ss) RNA molecules. Comparison of viral, non-viral, coding and non-coding RNAs of length 2117 nucleotides (nt) shows viral RNA to have among the highest gel-mobilities and smallest hydrodynamic radii in solution. Using graph theoretical tools, we demonstrate that the measured sizes are correlated with the compactness of branching patterns in predicted secondary structure ensembles. Compactness is determined by the number and relative positions of 3-helix junctions, and is highly sensitive to the presence of rare higher-order (4 or more helix) junctions. RNAs from spherical viruses possess greater numbers of higher-order junctions than random sequences or those from non-spherical viruses. The importance of secondary structure topography as a determinant of global RNA size, and implications of compactness for viral assembly will be discussed.

The accompanying native gel-electrophoresis image comparing RNAs of identical length shows they have significantly different hydrodynamic properties. Lane 1 contains the viral RNA, mutated viral sequences are in lanes 2-4, and lanes 5-9 show equal-length transcripts from arbitrary sections of the yeast genome. Lane 6 shows that RNAs with different sequences although mixed before electrophoresis are separable.



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Multiscale Entanglement in Spherically Confined DNA Rings

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Knots are frequent in biological systems, and particularly in DNA, where they can be a major hindrance for biological functions. In particular it is argued that the presence of tight knots in viral genome can prevent its ejection from the capsid thus stopping the life cycle of the virus.

Inspired by those problems we investigate the interplay of geometrical and topological entanglement in knotted DNA rings confined inside a spherical cavity using advanced numerical methods.

We show that the complex interplay between the length of the knotted portion of DNA, the contour length of the DNA ring, and the radius of the enclosing sphere can be encompassed by a simple scaling argument based on deflection theory.

Furthermore, we show that with increasing confinement the entanglement acquires a multi-scale character which can be rationalised using the same scaling argument.

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Challenging Packaging Limits and Infectivity of Phage

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The terminase motors of bacteriophages have been shown to be among the strongest active machines in the biomolecular world, being able to package several tens of kilobase pairs of viral genome into a capsid within minutes. Yet these motors are hindered at the end of the packaging process by the progressive build-up of a force resisting packaging associated with already packaged DNA. In this experimental work, we raise the issue of what sets the upper limit on the length of the genome that can be packaged by the terminase motor of phage λ and still yield infectious virions, and the conditions under which this can be efficiently performed. Using a packaging strategy developed in our laboratory of building phage λ from scratch, together with plaque assay monitoring, we have been able to show that the terminase motor of phage λ is able to produce infectious particles with up to 110% of the wild-type (WT) λ-DNA length. However, the phage production rate, and thus the infectivity, decreased exponentially with increasing DNA length, and was a factor of 103 lower for the 110% λ-DNA phage. Interestingly, our in vitro strategy was still efficient in fully packaging phages with DNA lengths as high as 114% of the WT length, but these viruses were unable to infect bacterial cells efficiently. Further, we demonstrated that the phage production rate is modulated by the presence of multivalent ionic species. The biological consequences of these finding are discussed.

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High Resolution Time-Resolved SAXS shows that RNA-Induced SV40 Capsid Protein Self-Assembly is very Fast and without Detectable Concentrations of Intermediates

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Many spherical viruses assemble their capsids around their nucleic acid, a reaction that may involve hundreds of individual molecules. Yet, uniform virus-like particles appear to assemble quickly. To dissect the assembly mechanism and genome encapsidation, we have established a simplest case experimental system. We show that mixing SV40 VP1 pentamers with RNA 500 mers yields T=1 particles comprised of 12 pentamers and one RNA molecule. We examined the kinetics of this reaction by Time-Resolved Small Angle X-ray Scattering (TR-SAXS). TR-SAXS shows that assembly is very fast; the reaction is nearly third-complete at 35 ms when mixing 0.5 μM RNA and 7.5 μM VP1 pentamers. Nonetheless, assembly appears to be a two-state process with only free pentamers and capsids observed; intermediates are undetectable. Finally, we show that TR-SAXS data are very well fit by master equations that describe assembly as a nucleation of an RNA molecule with one, two or three pentamers, followed by a cascade of elongation reactions in which one pentamer is added at a time. From this model, we estimate that the molar rate constant for addition of pentamers is approximately 10^9 M^{-1} s^{-1}. Such a rate is possible only if facilitated by long-ranged distance protein-nucleic acid attraction. We therefore suggest that the growing nucleo-protein complex is able to act as an electrostatic antenna for attracting other capsid subunits.

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Molecular Switching in Human Adenovirus

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Human Adenovirus (Ad) is an icosahedral, double stranded DNA virus. Activating the assembly of viral particles requires at least three viral components: the IVa2 and L4-22K proteins, as well as a section of DNA within the viral genome, called the packaging sequence (PS). Previous studies have shown