BIOPHYSICS

Ultraslow relaxation of confined DNA

DNA dynamics in tight spaces challenge nature's nanomachines

By Yann R. Chemla[1] and Taekjip Ha[1,2]

Polymers are ubiquitous and occur in many diverse forms, including cross-linked synthetic polymers (e.g., rubber and plastic) and biopolymers such as DNA and proteins. DNA has long been used as a model system for polymer science because long, chemically well-defined DNA chains can be prepared using its ability to self-replicate, and because it can be analyzed at the single-molecule level. For instance, the relaxation dynamics of a distorted DNA molecule eventually reverting to its lowest-energy configuration have been investigated extensively (*1*). There has been growing interest in understanding the relaxation of spatially confined polymers. How biopolymers such as DNA and proteins behave under high spatial confinement is important because they often experience such conditions—for example, when DNA passes through a narrow pore during viral packaging or bacterial conjugation. With the advent of nanotechnology (DNA is 2 nm in diameter), it has now become possible to study the relaxation dynamics of DNA confined in one (*2*) and two (*3*) dimensions. Berndsen *et al.* (*4*) have undertaken the first characterization of DNA relaxation under extreme confinement in three dimensions. Remarkably, they find that DNA relaxation under such circumstances is slowed by a factor of more than 60,000, presenting a daunting challenge for the biological machines that need to compact DNA reliably into tight spaces.

Extreme confinement of DNA can be achieved in viruses and bacteriophages (i.e., viruses that infect bacteria). They package their genome to near-crystalline densities in the smallest volume possible—a protein shell called the capsid typically only 50 to 100 nm in its largest dimension. Most double-stranded DNA bacteriophages and many eukaryotic viruses (notably, the herpes virus) use powerful molecular motors to reel in newly replicated viral DNA into empty, preformed capsids. Using the energy of adenosine triphosphate (ATP) hydrolysis, this motor must work against tremendous pressure as high as 2 to 6 megapascals (*5*, *6*). These pressures are believed eventually to drive DNA ejection into new host cells for infection.

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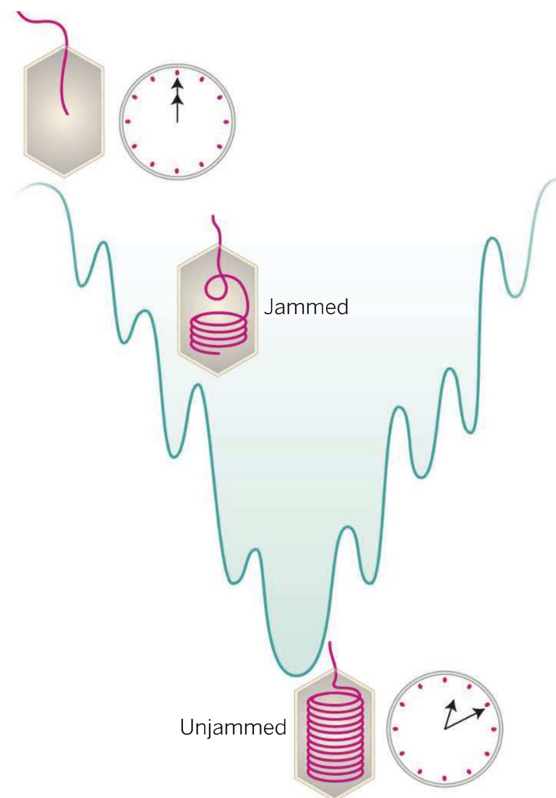

A tight fit. During packaging of the viral genome into its capsid, DNA can become kinetically trapped in nonequilibrium conformations, generating high resistances against the packaging motor. Relaxation to the lowest-energy state is extremely slow.

Recent single-molecule measurements (*7*) have provided important new insights into the mechanism of viral DNA packaging. Smith *et al.* (*5*) used optical traps to pull on an individual DNA molecule as it is packaged into a single ϕ29 phage. Their work established that packaging slows down as DNA fills the capsid because of the build-up of internal pressure within the capsid. Berndsen et *al.* (*4*) used this assay to investigate the dynamics of the confined DNA inside the capsid. During the reaction, they stalled packaging complexes with a nonhydrolyzable ATP analog, then restarted them with ATP after a variable wait ranging from 1 to >10 min. Surprisingly, packaging became faster upon restarting if the ϕ29 capsids were nearly filled to capacity when they were stalled; this finding suggests that densely packed DNA can become kinetically trapped in nonequilibrium conformations that jam the motor. Stalling the motor allows DNA time to relax to its lowest-energy state and to present a lower resistance to packaging, leading to an acceleration of the packaging reaction. The fact that the effect was more pronounced after stalls longer than 10 min indicates that the relaxation time scales must be longer than those of unconfined DNA of the same length by a factor of 60,000 (*1*).

In addition, 1- to 10-s pauses that had been observed during the late stages of packaging became all but eliminated by stalling and restarting of the motor, corroborating an early proposal that these pauses represented the motor's response to local jamming of the DNA (*5*). Late-stage packaging is also marked by increasing variability in speed, suggesting that multiple pathways are used with different resistances. Again, stalling and restarting reduced this variability. These findings suggest that packaged DNA can reorganize over multiple spatial and temporal scales. Several factors may affect DNA mobility under extreme confinement. DNA-DNA interactions play an important role. Spermidine^{3+}, which induces attractive interactions between DNA molecules by neutralizing the negative phosphate charge, increased the pause frequency and duration and the variability in packaging speed. Paradoxically, this DNA condensing agent actually impedes packaging by enhancing DNA-DNA interactions (*8*).

The ultraslow dynamics have a number of implications for the viral packaging mechanism. Because the relaxation time scales in ϕ29 exceed the duration of the entire packaging reaction, the motor must overcome large fluctuations in resistance because of ultraslow DNA dynamics. On the other hand, because cell infection occurs much later in the viral life cycle, presumably allowing more time for the viral DNA to relax to its lowest-energy conformation, the forces

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that drive DNA ejection into the host are likely to be smaller than those resisting packaging, which may limit the role of pressure in DNA ejection. Berndsen *et al.* propose that relaxation of confined DNA may occur by a reptation-like mechanism, the time scales of which depend on the cube of genome length. If so, relaxation time scales would be even longer for bacteriophages and viruses with genomes longer than that of ϕ29 (e.g., λ, T4, T7, and herpes virus) (*9*). Because the time to complete packaging is expected to be similar for many of these systems (*10*), this potentially presents an even bigger challenge to their packaging motors. Interestingly, under certain conditions, T4 is also known to "unpackage" DNA (*11*), allowing its genome to exit the capsid in a controlled and reversible manner. It is tantalizing to speculate that this mechanism could allow the motor to "unjam" DNA.

A number of issues remain to be addressed. More precise measurements of relaxation times as a function of filling fraction will aid theoretical models of polymers, because such models often lack accurate relaxation time scales. It will also be important to connect the observed slow relaxation to the mechanism of the packaging motor and to the organization of the DNA inside the capsid. Another recent optical trap study (*12*) showed that the motor rotates DNA as it packages it, and that the rotation pitch changes as the capsid becomes filled. This is potentially related to the spool structure proposed for packaged DNA (*13*, *14*). It may be possible to study the prerelaxation conformations of the packaged DNA with the help of recent advances in cryoelectron microscopy. Direct visualization of confined DNA motion by single-molecule imaging may shed additional light on the packaging process. This system illustrates how biological processes exist far from equilibrium and how their study can provide an unexpected testing ground for the underlying physical theories. ■

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CATALYSIS

Self-control tames the coupling of reactive radicals

Iridium complexes use two points of contact to control carbon-carbon bond formation

By **Guy C. Lloyd-Jones** *and* **Liam T. Ball**

Highly reactive or unstable chemical reagents are challenging to prepare, store, and safely handle, so chemists frequently generate them in situ from convenient precursors. In an ideal case, the rate of release of the reagent would be matched to the rate of its "capture" in the desired chemical reaction, thereby preventing the reagent from accumulating and minimizing any opportunity for decomposition. However, this synchronization is rarely achieved or even attempted: The rate of release is usually dictated by the conditions of the reaction (*1*), rather than being regulated by capture of the reagent. In this issue, Tellis *et al.* (*2*) on page 433 and Zuo *et al.* (*3*) on page 437 independently report the use of iridium photocatalysis (*4*, *5*) to supply highly reactive radical coupling partners ($R^{\cdot}$) to a nickel-catalyzed carbon-carbon bond-forming process (see the figure). Intriguingly, the two points of contact between the iridium and nickel cycles enforce autoregulated release of the radical, ensuring its efficient capture by nickel rather than its decomposition via other pathways.

Transition metal catalysts for the carbon-to-carbon coupling of complementary pairs of appropriately functionalized molecular building blocks are now a mainstay in modern organic synthesis, at least for unsaturated carbon sites (ones with double or triple bonds). Efficient coupling at saturated sites ("C_{sp3}" where the carbon has four single bonds) remains troublesome. An important difference between these processes is that C_{sp3} coupling generates three-dimensional molecular architectures that are of key importance to the pharmaceutical, agrochemical, and materials industries (*6*).

There are three primary reasons that C_{sp3} reaction centers are ill-suited to cross-coupling. The first is that the organic component transfers sluggishly to the metal catalyst. This problem precedes two more—the resulting organometallic species are frequently unstable, and also only slowly undergo the desired C-C bond formation ("reductive elimination"), exacerbating the instability problem (*7*). Cases of successful C_{sp3} couplings typically must address all three issues. The slow transfer is tackled by use of highly reactive C_{sp3} nucleophiles (the electron-rich component), which are inherently hard to control. The other two issues are suppressing side reactions at the metal center and accelerating the reductive elimination, which can be addressed through careful tuning of the metal catalyst (for example, altering its ligand sphere).

'The system is an example of an underexploited approach in synthesis: "autoregulated release" of reactive intermediates by synchronization of multiple catalytic cycles, a phenomenon that is ubiquitous in biochemistry.'

The reports of Tellis *et al.* and Zuo *et al.* offer an alternative and innovative solution to all three issues. The authors report high-yielding and selective formation of a new bond between an aromatic (benzene-like) ring and a C_{sp3} moiety ("R") with an aromatic halide ("Ar-X") coupling partner, and convenient precursor sources of the C_{sp3} component, in reactions that are simple to conduct and of substantial scope. At the heart of the new process is a photoexcited iridium complex ($[Ir(III)]^*$) that oxidizes stable C_{sp3} precursors (R-Y, where Y is BF_3K, CO_2Cs, or H) to their corresponding organoradicals ($R^{\cdot}$), highly reactive species in which the sp^3 carbon now bears an unpaired electron. The radical does not accumulate, but instead is captured by an aryl-nickel(II) complex that "awaits" its arrival. Not only does the use of an organoradical overcome the usual reluctance for formation of the

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