enzyme β-galactosidase (LacZ) and a signal sequence. Robust synthesis of the fusion protein jammed the Sec translocator, leading to cell death. Relieving this effect caused the proteins to form toxic aggregates in the periplasm, also toxic to the cell. The lethality of both situations could be overcome by activating the Cpx-mediated stress response (which leads to DegP production). But if DegP only exerts its effect in the periplasm, then an additional factor that is also Cpx related must relieve translocator jamming. The additional factor turns out to be the protein YccA. YccA is a substrate for FtsH, a membrane-embedded protease that functions in the quality control of membrane proteins. Van Stelten *et al*. show that when the translocator gets jammed, FtsH degrades SecY, and to a lesser extent SecE. This is an apparent suicide attempt by the bacterium because functional SecY in the membrane (as part of the Sec complex) is required for the newly synthesized SecY to translocate and insert into the membrane. Regulating FtsH activity is thus essential because uncontrolled degradation of SecY is lethal. YccA is the bacterial homolog of Bax inhibitor–1, a human protein involved in the stress response within the endoplasmic reticulum (*9*). Overexpression of YccA in bacteria relieved lethal jamming of the Sec translocator, further demonstrating that SecY degradation is the main cause of the lethality of the jamming event.

Why would treatment of bacteria with chloramphenicol or tetracycline lead to degradation of SecY? These antibiotics stop the translation of messenger RNA into protein, leaving incomplete polypeptides that are firmly attached to ribosomes (see the figure). Ribosomes bearing polypeptides with a signal sequence are as effective in lethally jamming the translocator complex as are the fast-folding cytoplasmic proteins that are fused to a signal sequence; both induce translocator degradation. A key question is the extent to which the antibiotic-induced degradation of SecY contributes to the efficacy of the antibiotics.

Because of its indispensability and uniqueness, the bacterial protein secretion machinery has long been recognized as an attractive drug target (*10*–*12*). Yet after almost 20 years of research, there are still no successful candidates that target this pathway. This makes the discovery of an additional mode of action of chloramphenicol and tetracycline even more interesting. Apparently, this pathway was already (albeit indirectly) targeted by clinically used antibiotics—we just didn't realize it. This raises the question of whether other ribosomal-targeting antibiotic classes also cause degradation of the translocator. The aminoglycoside antibiotics such as gentamycin and kanamycin induce serious errors in proteins during their synthesis that result in misfolded proteins. These antibiotics also trigger the Cpx system (*13*) due to the accumulation of misfolded proteins in the plasma membrane or periplasm. Whether this also leads to SecY degradation is not yet known, but in view of the results of van Stelten *et al*., this seems highly likely.

The manner in which the protein translocation pathway is affected by FtsH also marks a potentially therapeutic route to target bacteria. Successful dysregulation of the cytoplasmic protease ClpP by acyldepsipeptide antibiotics leads to uncontrolled proteolysis (*14*). Thus, in cases where direct action of antibiotics on the translocation machinery seems out of reach, dysregulation of FtsH may be an alternate mode of action for a new class of antibiotics.

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CHEMISTRY

Designer Curvature

Complex nanostructures can be created by systematically tuning the bending and twisting of synthetic DNA assemblies.

Yan Liu[1,2] and Hao Yan[1,2]

Biological systems create marvelous devices with nanometer-scale dimensions and precisely controlled three-dimensional (3D) architectures. Scientists have long dreamed of creating artificial nanostructures that mimic nature's elegance. One example is DNA nanotechnology (*1*), which uses DNA as a molecular engineering material to create nanostructures with controlled geometries, topologies, and periodicities and to organize matter with nanometer precision. On page 725 of this issue, Dietz *et al*. (*2*) report an elegant strategy for transforming 3D DNA nanostructures into complex geometric shapes with systematically controlled curvatures. It is as if DNA has been subjected to the practice of yoga to display a variety of difficult postures at the nanoscale.

Self-assembly of DNA nanostructures with controlled 3D architectures has long been a central goal of DNA nanotechnology (*3*) and has recently begun to see some success (*4*–*10*). However, methods for creating programmable, quantitatively controlled bending and twisting of 3D DNA nanostructures have remained elusive. This fundamental design capability is necessary to construct sophisticated molecular machines that can mimic, or even rival, structures built in the biological and macroscopic worlds.

Natural DNA molecules can assume tightly bent and twisted conformations (*11*). For example, in eukaryotic cells, DNA is packed into nucleosomes, in which DNA bends around histone proteins with a radius of curvature as small as ~4.3 nm (much smaller than its persistence length of ~50 nm, which measures the stiffness of the double-helical DNA polymer). DNA is also tightly bent by many proteins that regulate transcription processes. These natural examples show that DNA is mechanically flexible, with bending and twisting generally facilitated by externally bound protein molecules.

Dietz *et al*. (*2*) now show that DNA curvature can be controlled in synthetic systems by self-assembly of longer double helices laterally coupled to shorter ones whose axes are parallel to those of the longer ones—reminiscent of adjacent lanes on a curving sprint

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track. Thus, the forces required for bending are transmitted by adjacent DNA double helices rather than by DNA binding proteins.

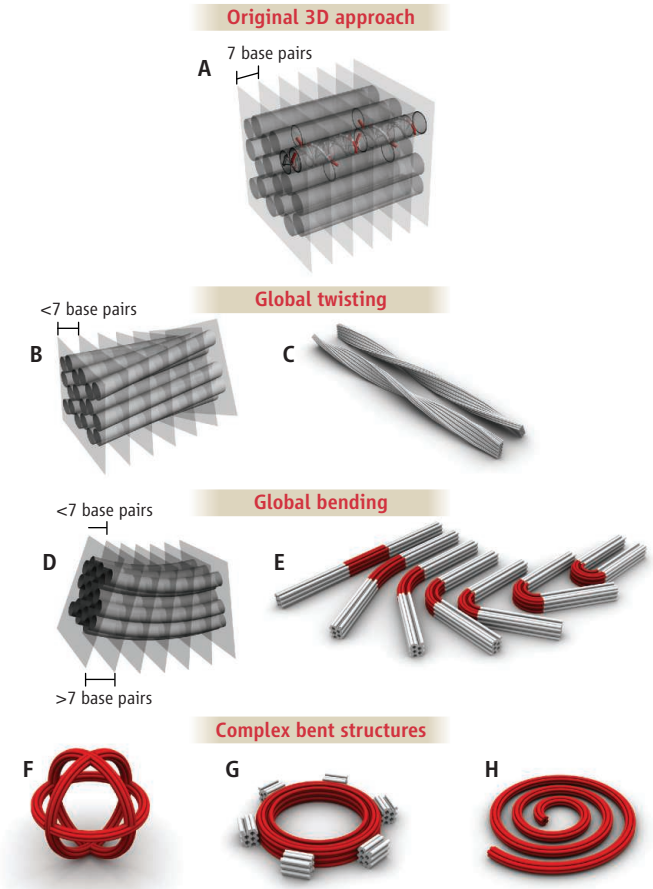
The principal building block is based on a multilayer 3D DNA nanostructure developed earlier this year by the same group (*9*). In that work, the authors devised a DNA folding strategy to create geometrically complex 3D DNA nanostructures. In doing so, they extended the 2D "scaffolded DNA origami" technique (*12*) to a third dimension.

In the original 2D technique, a long single-stranded viral genome (the "scaffold strand") is laid out in a 2D plane following a designated folding path. Next, hundreds of short oligonucleotide "staple strands" hybridize with the scaffold strand through complementary base pairing to form branched DNA junctions between adjacent helices.

To create 3D DNA nanostructures, Shih and co-workers (*9*) packed parallel corrugated multihelical DNA sheets into honeycomb lattices, with neighboring DNA helices connected by staple strands crossing over from one to another in a 3D space. In these structures, repeating DNA double-helical units of 7 base pairs are brought together by staple strands crossing over in 3D space (see the figure, panel A). Relaxed double-stranded DNA forms a right-handed double helix with ~10.5 base pairs per turn. Thus, a 7–base pair block of a DNA double helix corresponds to two-thirds of one turn, and staple-strand crossovers implemented every 7 base pairs along a helix cross-link that helix to its three nearest neighbors at 120° intervals. By tuning the number, arrangement, and length of each helix, the authors constructed a variety of 3D shapes.

To enable the 3D DNA nanostructure to bend or twist, Dietz *et al.* (*2*) varied the number of base pairs in the building blocks at selected positions, such that the local DNA helical structure deviates from 10.5 base pairs per turn. To introduce a global twist, selected layers of 7–base pair units along the bundle of helices are extended to 8 base pairs or reduced to 6 base pairs, resulting in a local under- or over-twisting of DNA helices, respectively (see the figure, panel B). These local strains can be relieved somewhat by a global twisting of the entire structure in the opposite direction (see the figure, panel C).

To bend the structure, Dietz *et al.* (*2*) modified a layer of 7–base pair units by deleting base pairs on one side and inserting base pairs on the other side, which results in contraction on the concave face and expansion on the convex face, respectively (see the figure, panel D). By manipulating the number of insertions and deletions, bending over a 98–base pair length can be precisely tuned from 0° to 180°, with a 5° dynamic range. At the 180° bending angle, the structure bends with a radius of curvature of only ~6 nm on its inner layer of helices (see the figure, panel E). The authors further assembled these well-controlled bent structures into sophisticated constructions (see the figure, panels F to H).



Designer curvatures with DNA nanostructures. (**A**) Multihelix 3D DNA origami packed on a honeycomb lattice. The rods are the DNA helices; the crossover points along any helix to the three nearby helices are restricted to the planes perpendicular to the axis of the helices, evenly spaced by 7 base pairs. (**B**) A global twist is generated by changing the distance between selected neighboring planes to >7 base pairs for a right-handed twist or <7 base pairs for a left-handed twist (shown here). (**C**) Right- and left-handed global twists are created by linking multiple DNA units end to end. (**D**) A global bend is generated by varying the distance between neighboring planes to <7 base pairs on one side and >7 base pairs on the opposite side. (**E**) The curvature can be tuned by changing the gradient of the base pair difference across the structure. Bent DNA units with appropriate curvatures are further assembled to form the beach ball (**F**) and the square-toothed gear (**G**). The spiral DNA multihelix bundle (**H**) is created from six semicircles of decreasing curvature.

There are other approaches for creating DNA nanostructures with curved features. For example, Mao and co-workers (*6*) have shown that simple DNA branched junctions can be used to hierarchically assemble 3D polyhedral objects such as tetrahedra, dodecahedra, and fullerene structures by tuning the branching numbers and the bending curvature of the junction core. Andersen *et al.* (*8*) and Ke *et al.* (*10*) created boxlike 3D objects by connecting different 2D DNA origami domains through sharp interfaces. The study by Dietz *et al.* (*2*) is different in that the curvature continuously connects different DNA nanostructure domains and can be quantitatively controlled. All these methods may be combined to construct increasingly sophisticated, precisely engineered geometric objects, the scope of which will be limited only by the human imagination.

Additional studies are needed to establish the stability of curved versus relaxed DNA nanostructures. Methods should be developed to assess the defect rates and improve the yields of the hierarchically engineered structures. A better understanding of the mechanical properties, and whether 3D packing parameters can affect them, is also necessary to translate them into practical applications.

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