What makes DNA so special?

DNA has the ability to self-assemble into many different 2D and 3D structures.

This self-assembly relies on the attractive forces between the base-pairs characteristic of DNA (adenine to thymine; guanine to cytosine).

This allows us to predict the base sequences of additional DNA strands that will complement the base sequences of the original strand.

DNA strands with custom base sequences can be used to fold and bind other strands of DNA into specific shapes and structures.

This is called “DNA Origami”.

Other advantageous properties of DNA:

- very compatible with biological systems
- easily found in nature and easily synthesized
- non-toxic
- very specific geometry
- other materials can be attached to it
- sensitive to external molecular signals
Using DNA Molecules with Multiple “Limbs”

Linear DNA chains alone do not result in complex nanostructures. Consequently, branched DNA molecules have been sought out and synthesized in order to augment the range of self-assembled structures. These branched DNA motifs can be found in nature, such as the four-armed Holliday Junction used in genetic recombination, as well as synthesized in the lab. The problem with the naturally-occurring Holliday Junction is its inherent symmetry, which results in branch-point migration and therefore high instability, making it an impractical building-block for other nanostructures. Various asymmetrical branched DNA motifs were synthetically designed and created in order to solve this problem. This ability to synthesize stable branched DNA molecules allows for the bottom-up construction of complex DNA nanostructures.
Manipulation

The shape and size of DNA polymers can be manipulated in multiple ways. The basic operation in self-assembled DNA structures is called hybridization, which is when two single-stranded DNA polymers with complementary base-pair sequence patterns bind together to form one double-stranded DNA helix.

In addition to the hybridization reaction, there are certain restriction enzymes that can cut (or nick, if it is a single strand) a DNA helix at locations determined by the DNA's specific base-pair sequence. Ligase enzymes are capable of performing the opposite function: they can mend lacerations in the DNA helix. These ligase enzymes are coupled with the hybridization reaction to join two uneven single-stranded ends of two different double-stranded DNA molecules. [10] The last reaction involves polymerase, which, when in the presence of the template DNA and a second DNA strand, may extend the sticky ends of the second strand by adding free nucleotides complementary to the template strand. [7] This creates longer strands of DNA.

The aforementioned reactions are often combined with hybridization to carry out a wide range of DNA computations and robotic functions.

Sticky-end cohesion between two DNA double helices - the right end of the blue molecule and the left end of the red molecule have asymmetric single-stranded extensions ("sticky ends") that are complementary to each other. When placed in the right conditions, these molecules attach to each other through hydrogen bonding. The final portion of the diagram shows that the two molecules can establish covalent ligation through the proper ligase enzymes and cofactors.

Foresight Institute, New Motifs In DNA Nanotechnology by Nadrian C. Seeman et. al. 2003
Another attractive feature of DNA as a nanomaterial is its functionality as a receptor for many different types of other materials. By using an assortment of attachment chemistries, we can directly or indirectly bind specific substances to specific segments of DNA. (Complementary) DNA, RNA, peptides, and proteins, to name a few, can be directly bound to DNA segments.

Materials that can be forced to indirectly bind to DNA include carbon nanotubes and a number of metals that can be linked with sulfur compounds (e.g., gold). Such attachment chemistries may be used to add molecular electronic devices to DNA segments, and, as a result, equip the DNA nanomachine with more functionality, power and/or useful cargo.

Carbon nanotubes can be made to indirectly bind to specific segments of DNA. DNA may be used as a nanoscale circuit board for the attachment of millions of carbon nanotubes. 
https://www.ccs.uky.edu/~ernst/carbontubes/ramanpap/tubetrans.gif
The Beginning of DNA Origami

**Rothemund’s Breakthrough**
In 2004-2005, the computational bioengineer Paul Rothemund spent months programming the complementary DNA sequences of a 7,000-base-pair single-strand viral genome in an effort to create such complex structures. He used hundreds of synthetic “staple” strands of oligonucleotides to fold the polymer into a desired configuration. Once synthesized and mixed, the staple and scaffold strands self-assembled in a single reaction. He succeeded, labeled his method of folding, binding and stapling “DNA Origami”, and launched an explosion of subsequent research on the topic. Rothemund’s work was a big jump from the previous method of constructing DNA complexes from oligonucleotides made in the lab, which limited the strands to about 150 base-pairs, nowhere near what can be found in nature.

*Image: Courtesy of Paul W.K. Rothemund and Nick Papadakis*

*Rothemund’s famous nanoscale smiley faces, created through the folding and binding of the M13 bacteriophage genome.*
DNA building blocks are very similar to toy bricks. The blocks self-assemble, connecting only in the areas where the base sequences complement each other. The structure can consist of any number of DNA blocks. Each block has a unique sequence and location in the larger DNA block structure. To design a custom structure, one simply withholds specific blocks to create holes in the structure. This is how Rothemund was able to create such whimsical pictures on the nanoscale.

This image of arbitrary symbols was actually created without any viral DNA, all the “blocks” were synthesized in the lab. This erases any worries about the possibility of the viral genome attacking the patient’s body if the nanostructure is being used as a drug-delivery vehicle.

*Image: Bryan Wei, Mingjie Dai and Peng Yin, Wyss Institute for Biologically Inspired Engineering at Harvard University*)
Chemical Synthesis of Oligonucleotides

1. **De-block** the first base, which is currently inactive. By adding a weak acid, either dichloroacetic acid or trichloroacetic acid in dichloromethane to the reaction column, we can remove the Dimethoxytrityl group that is preventing the 5’ hydroxyl group from any activity. Now, the only reactive site on the base monomer is the 5’ hydroxyl group. This ensures that the next base in the oligonucleotide will bind to this site, and this site only.

2. **Activate** the next base monomer by the addition of tetrazole. This is called “base condensation”. Tetrazole separates one of the groups protecting the phosphorus linkage. After the addition of tetrazole, the base is added to the reaction column. The active 5’ hydroxyl group from the previous step and the newly active phosphorus bind to form a loose bond between the two bases.

3. **“Cap”** the active 5’ hydroxyl group with protective agents acetic anhydride and N-methylimidazole to impede further growth of the strand. If this happens, any excess base that has not binded to the 5’ hydroxyl site may react in later additions of different bases, resulting in an oligonucleotide with unwanted deletions.

4. **Secure** the unstable phosphite linkage formed between the two bases in step two by adding a solution of dilute iodine in water, pyridine, and tetrahydrofuran to the reaction column to induce oxidation. The previously unstable phosphite linkage is oxidized, resulting in a much more stable phosphite linkage.

5. Repeat until all the desired bases have been added to the strand.
6. After synthesis, all the protective groups around the chain must be removed, as well as the foundation supporting the chain. This is accomplished by incubating the oligonucleotide in concentrated ammonia hydroxide for an extended period of time at a high temperature.

7. We now have a mixture of the desired oligonucleotide, detached protective groups, and oligonucleotides with internal deletions. To purify the heterogeneous solution so that it contains only the desired oligonucleotide, a process termed “desalting” is applied to the mixture. This removes any intrusive substances in the solution which may interfere with later reactions. The most hazardous component in the heterogeneous mixture is the free-floating ammonium ions. These can be filtered out through ethanol precipitation, size-exclusion chromatography, or reverse-phase chromatography.

8. If all steps have been carried out accurately, we should now have a solution of only the desired synthesized oligonucleotide, ready for laboratory use.
Andersen and his team used the single-stranded genome of M13 bacteriophage as the foundation for a DNA origami box. They utilized Rothemund’s origami method to construct a 42 x 36 x 36 nm³ size box around the polymer with the ability to open via an external DNA “key”. This design was chosen for several reasons: “to use the entire M13 sequence, to ensure a circular folding path through the faces and to introduce faces that have the characteristics of lids with 'hinges' composed of scaffold linkers.” (Andersen, E. S. et al. Nature 459, 73–76, 2009).

Previous studies found that DNA nanomachines can be controlled through external DNA signals. Andersen and team developed a sort of “key” made of oligonucleotides capable of opening the DNA box. Another strand of DNA kept the box shut. By applying one fluorescent dye (Cy3) on one face of the box and another different-colored fluorescent dye (Cy5) on the lid of the box, they were able to observe whether or not their oligonucleic lock-and-key technique was a success. They observed efficient fluorescence resonance energy transfer (FRET) when the dyes were close together, and decreased efficiency in FRET when the dyes were farther apart, meaning the lid was open.
Douglas et. al. followed the DNA origami method of construction and created hexagonal barrel-shaped nanorobots. The barrels were 35 nm in diameter and contained 12 sites. The two halves of the open-ended DNA barrel were linked together with double helix aptamer locks. “To operate our device in response to proteins, we designed a DNA aptamer–based lock mechanism that opens in response to binding antigen keys.” (Douglas et. al. Science 335.6070, 2012).

The inspiration for their nanorobot came from the body’s own immune system in which white blood cells defend the bloodstream from invaders.

The body’s white blood cells provided the inspiration for their nanorobot.
http://okapi.berkeley.edu
Within a large population of healthy cells, the nanorobot was capable of recognizing a selected number of target cells. Even though all the cells shared the same drug target, only those target cells expressing the exact set of proper keys were capable of unlocking the nanorobot. Once the robot came in contact with the proper cell, it changed shape and both of its link aptamers sprang open, releasing the payload. This feature holds promising potential for future cancer therapies. They also designed a nanorobot bearing functional cargo - it contained antibody fragments that were capable of telling the target cell to commit apoptosis.

In one experiment, Douglas et al. demonstrated that the nanorobot can target specific cell lines depending on what locks were chosen. Several different versions were made with different locks and six different cancer cell lines were added to the mixture. It was demonstrated that different cell lines could be targeted based on which locks were chosen and which keys were present on the cell surface.

The barrel-shaped nanorobot, in an “open” position, is held together with specific double-helix aptamer locks. DNA nanorobots can seek out and identify specific combinations of cell-surface proteins. Once the cell is identified, the nanorobot can deliver an antibody payload (purple) or a unique message programmed for that cell. Image created by Campbell Strong, Shawn Douglas, & Gaël McGill using Molecular Maya & cadnano.
Other potential medical applications of DNA nanorobots lie in cancer therapy. The robots can be programmed to unload their cargo only when the target cell is in the proper diseased state, a degree of specificity that other drug delivery methods are missing. Thus, the robot would be capable of destroying only the harmful cancer cells and leaving the other, healthy cells in peace.

Thanks to our understanding of the dynamics and properties of DNA and of molecular biology in general, we have the ability to manipulate and control DNA in multiple different ways. From cancer therapy to miniature circuit boards, DNA nanotechnology has a nearly infinite number of future possibilities.
Works Cited