Nanoengineered Bioplatforms Based on DNA Origami

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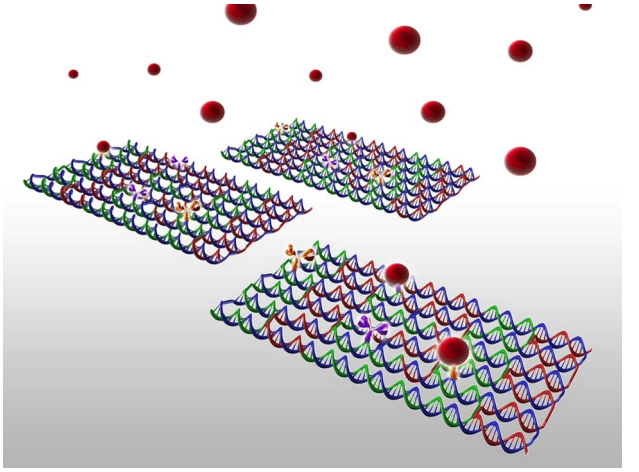

I. WHAT IS DNA ORIGAMI?

DNA is generally associated with the storage of genetic information. However, in many ways, it is also an ideal building material. The shape of a DNA structure is determined by the sequences of the DNA strands within the structure. DNA origami [1] has recently evolved as a method for producing programmable structures at the nanoscale. In a DNA origami, a long single-stranded DNA molecule is folded and held in place with shorter DNA strands. This process can be visualized by taking a very long tube or hose and folding it into a desired shape. Smaller strands of the same material can then be used to tie the large tube into a space-filling structure. In the case of DNA origami the shorter strands are called staples. The staples crosslink and stabilize the entire structure, enabling the formation of complex and programmable 2-D and 3-D shapes. Structures with considerable complexity can be designed and produced. The entire process is accomplished using self-assembly methods, where the large DNA strand and the staples interact using base pairing. Self-assembly of DNA is a useful and robust method to build up complex nanostructures with flexible and programmable functionality in a cost-effective manner. The folding of DNA is a chemically well-understood and controllable process.

DNA origami provides an attractive substrate for robust and precise nanopatterning [2] that can be used to control the surface properties of a nanomaterial. Nanoengineering allows the formation of DNA origami structures whose surfaces can be decorated with precisely positioned molecular recognition units. Self-assembled DNA origami structures fall into the realm of nanomaterials. Nanomaterials have a very high surface-to-volume ratio. Essentially all of the molecules within a nanomaterial are on or near a surface, causing the structure to exhibit unique properties.

There is a lot of interest is using nanopatterning DNA self-assembly methods for the mimicry of biological functions. Many biological functions

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are initiated, enhanced, or suppressed based on a lock-and-key mechanism, where the interaction between biological species is nonbonding in nature. A lock-and-key mechanism may be mimicked using a precisely nanopatterned material. Nanopatterned structures may provide advances in areas related to detection, toxicology, therapeutics, molecular capture, and regenerative medicine.

II. DESIGN SOFTWARE

In recent years, a number of DNA origami software packages have become available that provide the ability to perform computer-aided design (CAD) at the nanoscale. The rapid advancement of software and software interfaces to design and direct the self-assembly of DNA origami structures will aid the next generation of nanoengineers in the development of new and novel nanosystems. Better software and software interfaces are needed that allow complex nanostructure designs to be specified and automatically populated with optimized sequences. Software is also needed that can predict the molecular properties of a proposed DNA origami structure prior to fabrication.

Traditionally DNA origami software design software packages have focused on producing programmable shapes. Less attention has been paid to surface functionalization. The design of sequence-optimized molecular structures with programmable charge distribution, hydrophilicity/hydrophobicity, and steric properties will allow precise surface functionalization and characterization at the nanoscale. Design and fabrication of complex structures that are potentially biologically active will provide useful capabilities to the biosciences community. Simulation tools for defining electrostatic and hydrophilic properties of structure at the nanoscale are becoming available.

An ideal development tool would allow the determination of optimal designs using constraints imposed by topology, molecular thermodynamics, and self-assembly kinetics. The system would be able to accommodate designs with a large number of DNA bases pairs and address kinetics as well as thermodynamic issues. The ultimate goal would be a robust, user-friendly CAD system that could be used to design complex DNA origami structures and predict their properties in advance of fabrication.

III. USE OF NANOPATTERNING IN BIOSCIENCES

Now let us take a closer look at some of the promising applications of nanopatterning in the biosciences.

A. Synthetic Affinity Ligands

Nature is adept at producing molecules that can recognize and specifically bind to other molecules. In biological systems, antibodies can search out and selectively bind to specific target molecules in the presence of numerous other substances. Selective binding allows the body's immune system to target and eliminate specific antigens. Antibodies have also become the gold standard for many biosensing applications. Natural proteins are widely used in diagnostic tests for many diseases because they recognize and efficiently bind to disease markers. A totally synthetic approach to producing large molecules with selective molecular binding properties will have numerous applications.

An epitope is usually defined as the part of a molecule (antigen) that binds to an antibody or other molecule. It may be possible in the near future to engineer epitopes on demand. Epitopes could be combined on a single molecule to produce a system with selective binding properties. In the past, epitope discovery has been a long process that can include candidate molecule selection and synthesis, binding-characterization assays, assay validation, and data analysis. Issues such as low throughput, lengthy and difficult research efforts, and high costs have impeded the speed of the discovery process.

CAD at the nanoscale using DNA origami has the potential to improve epitope discovery and refinement. The goal is to develop synthetic structures with selective binding properties that are comparable to traditional epitopes or antibodies. Epitope interaction with a target species is typically nonbinding in nature. The shape, charge distribution, and hydrophobic/hydrophilic nature of a potential artificial epitope can be controlled at the nanoscale. It is possible that a reporting mechanism can also be included on a DNA origami structure. The ability to design and build structures with selective binding properties will enable a number of applications, including medical diagnostics and sensing.

B. Molecular Sequestration

In biological systems, avidity is often used to describe the combined strength of multiple bond interactions. Avidity is distinct from affinity, which is a term used to describe the strength of a single bond. Avidity is the combined synergistic strength of bond affinities rather than the sum of bonds. Avidity is commonly applied to antibody interactions in which multiple binding sites simultaneously interact with a target molecule. When many binding interactions are present at the same time, transient unbinding of a single site does not allow a molecule to diffuse away, and binding of that site is likely to be reinstated. It has been demonstrated that multiple binding sites can increase the effective binding of a molecular species by up to five orders of magnitude relative to the affinity of any univalent bond within the system.

A DNA origami structure can be decorated with multiple precisely addressed molecular recognition units. Multiple copies of a given ligand [3] with selective binding properties on the surface of the DNA origami structure will ensure capture and sequestration of a given molecule. This approach presents a powerful platform for molecular sequestration where origami-based platforms can

present large numbers of molecular sequestration sites. For example, the U.S. Department of Defense (DOD) has the need for a universal organophosphorus (OP) scavenger that will protect against multiple OP compounds, including all existing nerve agents [4]. The ideal scavenger should be rapid, irreversible, and specific and have a prolonged circulation time in the bloodstream. This same synthetic molecular sequestration approach could also be used in the development of synthetic anti-venom therapies.

C. Vaccines

An immunogen is a substance (or a mixture of substances) capable of provoking an immune response when injected to an organism. The function of immunogens can be complex with activation of the immune system in several stages. An immunogen requires the presence of one or more antigens. An antigen is a substance that can selectively bind to the immunoreceptor of a B-cell or a T-cell and stimulate an immune response. Antigens that are currently used in vaccine development are generally extracted from the disease-causing pathogen from which the vaccine is designed to provide immunity. Artificial molecules with binding properties similar to natural antigens could lead to artificial antigens and immunogens that could lead to artificial vaccines.

A completely artificial vaccine could eliminate the need to produce and store dangerous pathogens or parasites as seed stock for vaccine development. This approach is particularly attractive in the development of vaccines for extremely dangerous microorganisms. Artificial vaccines could also provide vaccines against diseases where classical vaccine development methods have been shown to be ineffective. The science of DNA origami has recently progressed to the point that it is now possible to design and manufacture complex structures with selective binding properties using DNA folding techniques.

DNA origami may provide new structural control and manipulation over an entire vaccine structure. Successful vaccine–host interaction may depend on more than just antigen presentation to B-cells that stimulate antibody production. Antibodies are an important element of the immune system. However, other elements are also essential. In particular, T-cells that release chemicals (cytokines, chemokines, etc.) are required to provide a more complete immune response. Having elements that can stimulate T-cell population will result in a more rapid immune response. Selectively binding by synthetic molecules to the immunoreceptors of B-cells and T-cells will be an essential part of a synthetic vaccine.

D. Regenerative Medicine

Regenerative medicine is defined as the process of replacing or regenerating human cells, tissues, or organs to restore or establish normal function. Regenerative medicine holds the promise of regenerating damaged tissues and organs in the body. In recent years, there has been a revolution in regenerative medicine through the use of stem cells. Stem cells can divide and differentiate into diverse cell types and can self-renew to produce more stem cells. In adult organisms, stem cells act as a repair system for the body, repairing damage and replenishing tissues.

There is interest in determining the exact environment needed to stimulate stem cells to differentiate in a programmed manner. Stem cell fate has been shown to be influenced by a combination of physical, chemical, and biological cues present in the nanoenvironment of the cells. Materials and structural properties have been shown to influence cell regeneration and proliferation. There is a need to better understand the critical parameters for regulating cell fate. The use of nanostructures to guide cell behavior is an attractive option for regenerative medicine.

Nanosized physical structures can provoke behavioral changes in cells and tissues [5]. Nanopatterning using DNA origami provides exquisite control over biologically active surfaces. Nanostructures produced using DNA origami have a high surface-to-volume ratio and elicit a high degree of biological plasticity. Structures can be designed to specifically influence the fate and proliferation of stems cells for use in regenerative medicine. DNA-origami-based structures can be a very powerful platform for presenting large numbers of molecular patterned sites for stimulating stem cell proliferation and differentiation.

IV. CONCLUSION

The rapid advancement of software and software interfaces to design and direct the self-assembly of DNA origami structures will aid the next generation of nanoengineers in the development of new and novel nanosystems. To extend DNA origami techniques to biomolecular applications, the development of simulation tools for defining electrostatic and hydrophilic properties of structure at the nanoscale is needed.

A lock-and-key mechanism can be used to initiate, enhance, or suppress biological functions with the interaction between biological species that is often nonbonding in nature. It may be possible to artificially produce biologically active molecular species using precisely nanopatterned materials. Nanopatterned structures may provide utility in areas related to detection, toxicology, therapeutics, molecular capture, and regenerative medicine. ■

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