DNA Based Nanomechanical Devices[1]
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PIs: **Nadrian C. Seeman** [*], **William Goddard** [§], **Nagarajan Vaidehi**[§], **Erik Winfree**[§]
[*]*New York University;* [§]*California Institute of Technology.*

Background and Objectives:

Machines and devices operating at the nanometer scale have wide variety of exquisite applications ranging from medicine to space flight, while dramatically reducing the energy and material requirements. The technology that captures the level of control afforded by biological systems, a level of control which is the basis of the catalytic, informational, and signal transduction capabilities of proteins and nucleic acids offers the most promising avenue for building nanoscale machines. The Seeman group at NYU has demonstrated that the level of control offered by DNA systems can be exploited to make intricate DNA based nanostructures, including the self-assembly of DNA to form 2D[2] and possibly 3D periodic arrays. They have also demonstrated a functional DNA based nanomechanical device using the property of B-Z transitions that occur in DNA under different solution conditions.[3]

However one of the bottlenecks to this technology is the difficulty in controlling nanostructures and in measuring reliable values for the properties of nanosystems. Here we expect modeling and simulation to play a critical role in achieving the design and fabrication of practical nanoscale devices. Modeling will also provide a molecular level understanding of the mechanism of action of the various nanostructures. Thus the main objective of this project is to combine the major advancements and research on DNA nanostructures by the Seeman group with the simulation effort led by Goddard and Vaidehi and their simulation group, to be able to make rapid progress in demonstrating DNA based nanoscale devices. The Winfree lab at Caltech is developing a synthetic *in vitro* system to mimic the architecture of genetic regulation, but with only two molecular components (in addition to DNA and RNA): T7 RNA polymerase (RNAP) and a ribonuclease (such as RNase H). These systems will be used as nanoscale actuators for the DNA/RNA based nanodevices designed by Seeman.

Approach and Project Status: Our approach will be first to prototype the designs computationally, optimizing the particular base-pair sequences to be used in the structures, making sure that the particular lengths and spacings will lead to proper clearances, testing that the solvents and counterions are all consistent, and testing the operation of the device, including the dynamics. We believe that this concerted experimental and theoretical/computational approach is essential for fast progress in this project. To be successful will require advances in the tools for both synthesis and characterization and for modeling and simulation.

Recently Seeman and his coworkers have also demonstrated a DNA nanodevice based on paranemic crossover (PX) structures.[4] The key piece of this work has been produced as a part of this NSF-NIRT project. Seeman and coworkers have developed a robust sequence-directed nanomechanical device that executes 180˚ rotations. This device is based on an interchange between two different DNA topologies, PX and JX_2 that can be used as components for a device, as shown in Figure 1. The two topologies are shown in panel (a). The PX motif consists of two double helices (one red and one blue) wrapped around each other, leading to crossovers between the helical domains at every possible point. By contrast, the JX_2 motif lacks two crossovers in the middle. The consequence of this difference is that the two strand pairs, red and blue, are inter-wrapped 1.5 times in the PX structure shown, and only once in the JX_2 structure. Interconversion between these two structures is the basis for the device. The way this

is done is shown in panel (b). In the PX structure at the left, one of each of the red and blue strands is interrupted by a green strand (called a set strand). It is clear that a variety of set strands and their blue and red pairing partners can be produced, leading to a variety of different devices. The green strands can be removed from the PX structure by the addition of their full complements, as shown in process I at the upper left. This operation works because the set strands contain short unpaired regions at their termini, so addition of their complete complements results in their removal from the PX motif. The intermediate at the top of the figure can be converted to the JX_2 structure by the addition of other set strands shown in purple (process II). The PX structure can be restored in a similar fashion by processes III and IV. Gel electrophoresis and atomic force microscopy have been used to demonstrate the action of the device.

Seeman's group have synthesized several PX structures like PX55, PX65, PX75, PX85 and PX95. These vary in the number of base pairs between the crossover points as shown in Figure2. The experimental observation was that PX65, PX75 and PX85 formed as stable monomers while PX55 and PX95 did not. The Goddard group has automated building the atomic level model of these nanostructures. They have also performed molecul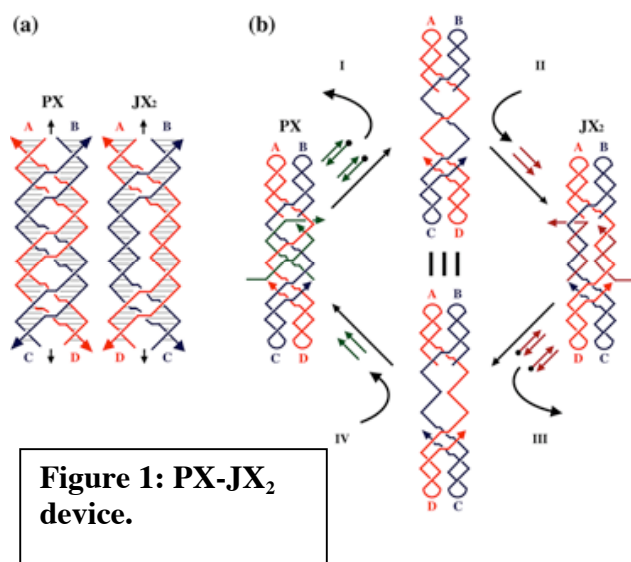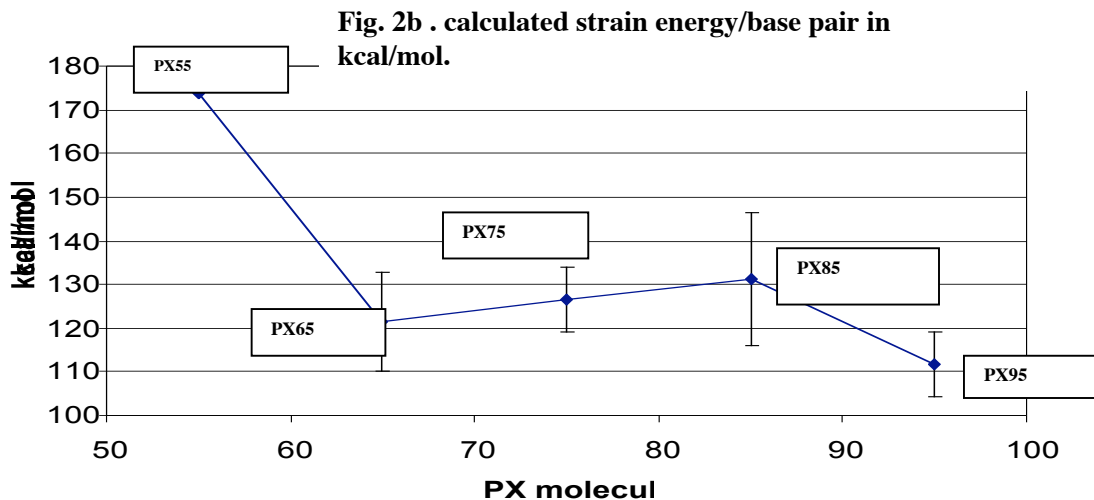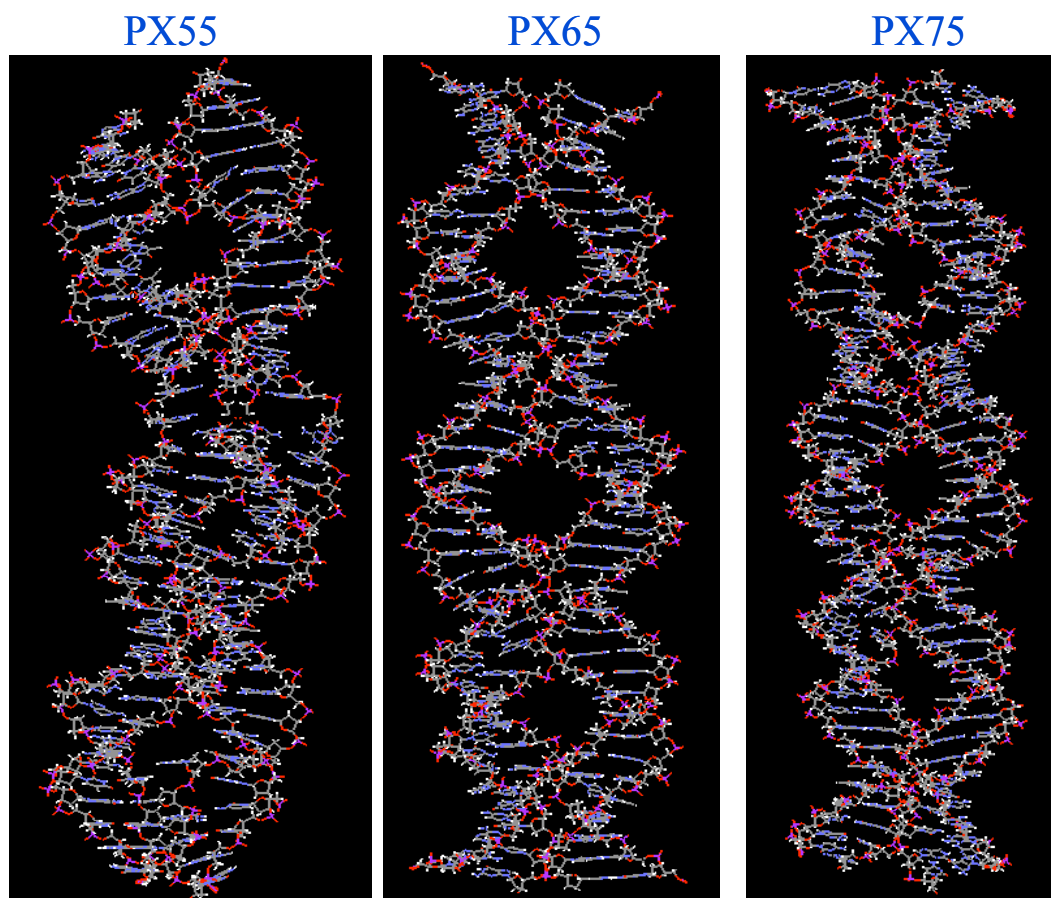ar dynamics simulations of these large scale PX structures in explicit water and salt for up to 1 nanosecond. The calculated strain energy is making the crossover molecules showed that PX55 is indeed unstable whereas PX95 is not. This is to our knowledge, the first time that any such large scale simulation has been done for DNA nanostructures. Taking the input from theory, Seeman's group carried out experiments on PX95 at lower concentrations of the monomer. Subsequent electrophoresis experiments showed that indeed PX95 was formed at low concentrations. This is a good example of how theory made predictions that led to successful insight into experiments. Currently, the Goddard group is studying the base sequence dependencies if any, on the stability of these nanostructures. Such a molecular level understanding is essential for building nanodevices.

Figure 1: PX-JX_2 device.

Fig. 2b . calculated strain energy/base pair in kcal/mol.

Winfree's group at Caltech have at this time experimentally demonstrated a single transcriptional switch, measured its input-output curve, and shown that a sharp sigmoidal non-linearity can be achieved by proper design. The output, an RNA transcript, will be used to drive a conformational change in a downstream DNA device. These RNA based switches can be combined with Seeman's RNA-activated devices.

Figure 2. a) Predicted structures of paranemic crossover DNA in salt solution

PX55   PX65   PX75

[1] For further information about this project email **<ned.seeman@nyu.edu>,** <wag@wag.caltech.edu>; <vaid@wag.caltech.edu> or <winfree@centrosome.dna.caltech.edu>
[2] E. Winfree, F. Liu, L. A. Wenzler, and N.C. Seeman, Design and Self-Assembly of Two-Dimensional DNA Crystals, *Nature* **394**, 539-544 (1998).
[3] C. Mao, W. Sun, Z. Shen and N.C. Seeman, A DNA Nanomechanical Device Based on the B-Z Transition, *Nature* **397**, 144-146 (1999).
[4] H. Yan, X. Zhang, Z. Shen and N.C. Seeman, A Robust DNA Mechanical Device Controlled by Hybridization Topology, *Nature* **415**, 62-65 (2002).