Deliverable DNA Circuits

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Introduction/Background:

DNA is one of the most versatile molecules that we know. Not only is it the code for all of life, but the possibilities for the things that you can build with it is endless. Within this course alone, we have examined DNA logic gates that can compute boolean logic expressions, DNA neural networks that can compute a complex image recognition, and reconfigurable DNA tiles, and this is just the tip of the iceberg of things that you can do with DNA. Yet, nearly all current applications of DNA so far have been done in vitro. In order to allow these complex functions to have biological applications within the body, we need a delivery mechanism that will bring these DNA systems to a target destination.

To do this, we can use a container that will release the DNA system upon some signal. One example of such a container is a DNA origami box [1]. This DNA origami was designed to fold up into an openable box with a lid controlled by a strand displacement system [1]. Additionally, we can replace this strand displacement latch system with a system that can be activated by a target protein; this system makes use of an aptamer or other just DNA strand that can sense a target protein to open the lid of the box upon a protein signal [2]. These systems enable us to be able to design a container that will release our desired contents to a specific target location within the body specified by a DNA strand or protein.

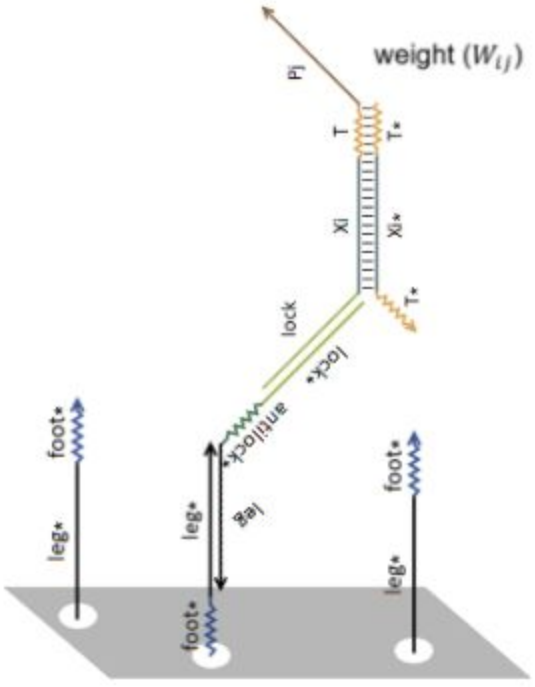
Design:

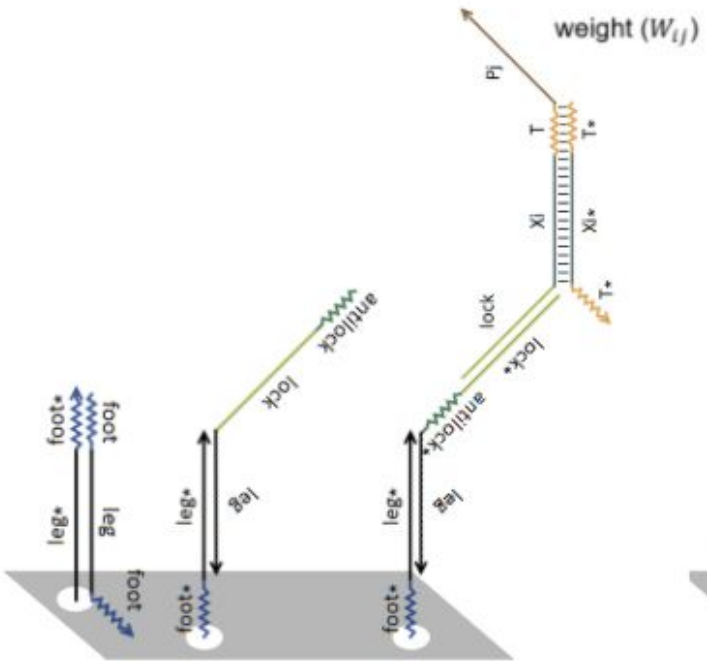
The goal of this paper is to design a DNA origami box that will release a DNA circuit upon some signal. These designs are used to make this function possible.

This first modification that we will make is to add a track along the inside of the origami box. This track will be made up of leg*/foot* strands in alternating orders (leg*/foot* on one strand foot*/leg* strands on the neighboring strands).

We will also create a three domain lock strand made up of lock*/antilock*/leg. This will anchor the circuit molecules to the track.

In order to tether the DNA circuit to the track, we will add an extra lock domain to each of the circuit molecules. This will allow the lock molecule to connect all of the circuit molecules to the track as shown in the figure.

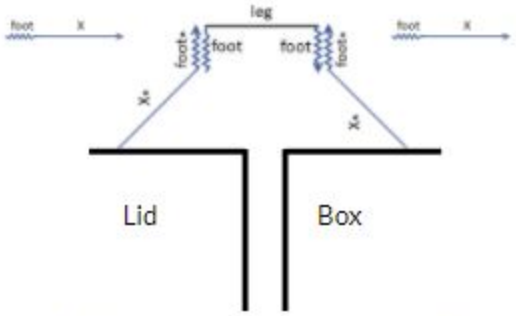




We will also design a new key molecule to unlock all of the tethers. This key molecule will be made up of foot/leg/foot, such that it will be able to walk along the track.

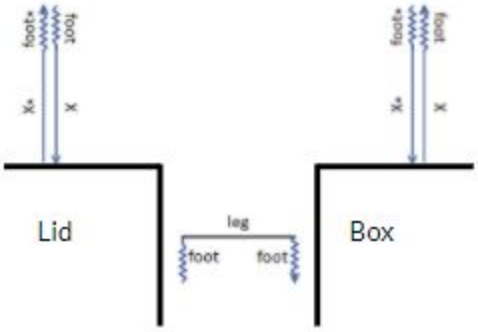
The final molecule that we will need for our tether system is an anti-lock molecule. This anti-lock will be made of anti-lock/lock/leg, such that it will be able to bind to track as well as the lock.

For our latch system we will need only one additional molecule. This molecule will be a clasp molecule tethered to the outside of the box made up of x*/foot*.



Discussion:

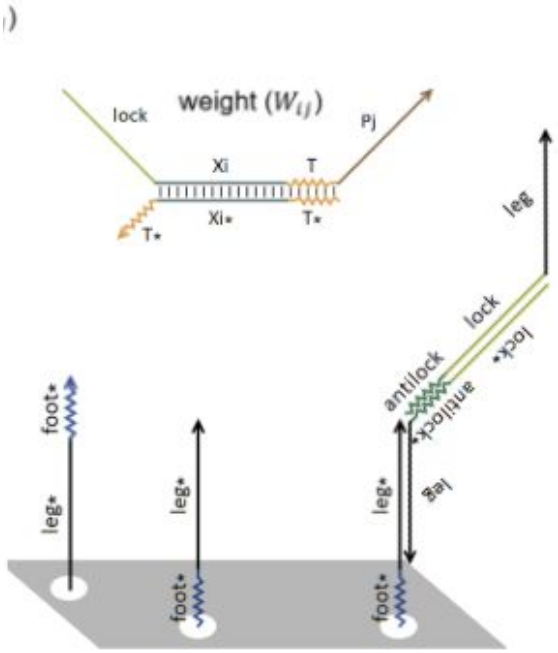
These designs will come together for the system level behavior that we hope for. To make our design work, we will make sure to tether all of the circuit molecules to the same side of the box with all of the anti-lock molecules initially tethered on the opposite side of the box to prevent spurious interactions. Tethering the circuit molecules to the inside of the box will make it so that the DNA circuit will be contained within the origami box as it folds up.



The key will initially be bound to the two clasp molecules keeping the lid closed, such that when the signal strands bind to the clasp molecules, the key will be released into the box. The key will then bind to the track and walk along it, untethering any circuit molecules or anti-lock molecules that it comes in contact with. The anti-lock molecules will interact with the lock molecules to prevent rebinding of the circuit molecules to the track. The circuit molecules will then be fully free to perform their designed functions.

While we would expect this system to work as desired, there are some limitations and improvements that could be made to the design to increase the functionality of the system. One

such limitation is the lock/anti-lock system. In the current design, both the lock and anti-lock can rebind to the track after being released before binding to each other. To fix this, we might be able to design a hairpin structure on the leg domain of each of these molecules such that they will fold up upon being released and be unable to rebind back to the track. Another such limitation is the key release mechanism. This key system has two major limitations; the first is that once the key is released by the opening of the box, the key must travel inside the box and bind to the track before it is able to start untethering circuit molecules, and the second is that the foot domains of the key that clasp the box shut are toehold domains, so may not be strong enough to prevent the lid from opening. One possible solution to this problem would be to have the key be already tethered to the track. We would design an inhibitor molecule that would bind to the key and prevent it from moving along the track. This inhibitor molecule would be removed by an activator molecule that would be initially used to clasp the lid shut. This inhibitor molecule and the clasp molecules could be designed to have longer domains to ensure that the lid would be sufficiently bound shut.



References:

1. Andersen, E., Dong, M., Nielsen, M. et al. Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* 459, 73–76 (2009). https://doi.org/10.1038/nature07971

2. Douglas, S. M., Bachelet, I., & Church, G. M. (2012). A logic-gated nanorobot for targeted transport of molecular payloads. *Science*, 335(6070), 831–834. https://doi.org/10.1126/science.1214081

3. Lecture Notes 2: Overview of DNA-based neural networks

4. Lecture Notes 13: Algorithmic and architectural foundations for building molecular robots

5. DSDdiagram: A Mathematica package for generating diagrams of DNA strand displacement systems