# Making Worms Glow

## DNA Nanomachines in *Caenorhabditis elegans*

*Sunaina Surana and Yamuna Krishnan*

**Deoxyribonucleic acid (DNA) is the molecule that carries genetic information in a cell. The field of DNA nanotechnology aims to use DNA as the basis for making complex nanometer-scale structures. While much progress has been made in creating such nano-architectures, the use of these structures within organisms had never been attempted. Our work is focused on using a simple DNA-based pH sensor in worms to yield information about organelle activity in cells. The first of its kind, this work set up a system to use DNA as a probe in living systems.**

The central dogma of molecular biology postulates that genetic information encoded by DNA in genes flows to ribonucleic acid (RNA), and then on to proteins. The reason that DNA is the genetic material of living organisms (barring a few groups of viruses) is probably not fortuitous.

DNA consists of two polynucleotide strands coiled to form a double helical structure. Each polynucleotide strand consists of units called nucleotides. Each nucleotide has a deoxyribose sugar, a phosphate group, and a nitrogenous base (adenine, guanine, cytosine, or thymine). Specific nitrogenous bases of the two strands are bound by hydrogen bonds [1] (*Figure* 1a). This chemical predictability due to Watson-Crick base pairing (adenine pairs with guanine and cytosine pairs with thymine; *Figure* 1a), and its stability, makes DNA unique among biological macromolecules. These characteristics, along with the physico-mechanical properties of DNA such as the ability of double-stranded DNA to behave as a rigid rod (up to a length of 50 nm, known as its persistence length), led Nadrian Seeman to use DNA to fabricate architectures at the nanoscale size. Seeman was the first to pro-

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Nanotechnology, nanomachines, I-switch, FRET, fluorophore, endocytosis, pH sensor, *C.elegans*.

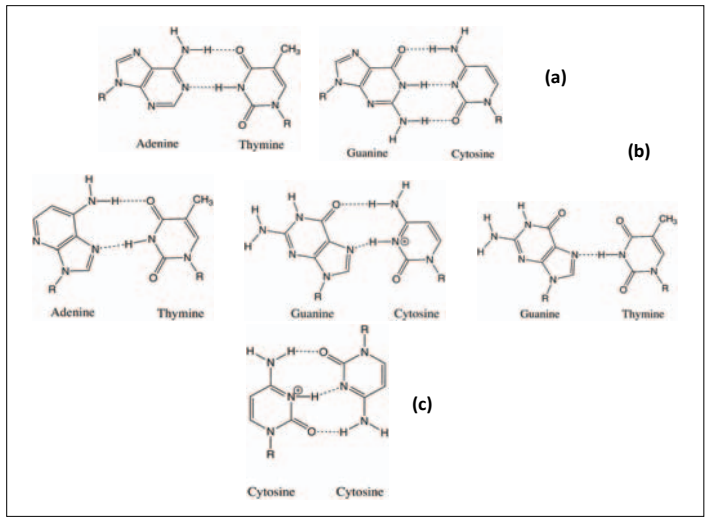


**Figure 1.** Standard and non-standard base-pairing in DNA. **(a)** Watson-Crick base pairing in double stranded DNA where adenine hydrogen bonds with thymine, while guanine base pairs with cytosine. **(b)** Examples of non-standard base pair formation between adenine and thymine, guanine and cytosine, and guanine and thymine. **(c)** Base pairing between two cytosines at acidic pH.

pose the use of DNA as a building block[1] to make junctions from which DNA helices radiate out [2] (*Figure* 2a). Subsequently, it was found that DNA can also undergo non-standard base-pairing (called Hogsteen base-pairing, in which guanine and cytosine can be bound by hydrogen bonds) [1], depending on the DNA sequence and external cues (*Figure* 1b). This led to the engineering of DNA into a variety of nano-architectures of exquisite complexity [2] (*Figure* 2). We were interested in the question: could DNA architectures engineered in a test tube be sent back into a living organism and, if yes, could they report on the biological environment within?

[1] Yamuna Krishnan, DNA's New Avatar as Nanoscale Construction Material, *Resonance*, Vol.13, No.2, pp.195–197, 2008.

## Setting up the System

Can DNA architectures engineered in a test tube be sent back into a living organism and, if yes, can they report on the biological environment within?

Cells are the basic unit of any living organism. Eukaryotic cells have evolved to compartmentalize different cellular functions in different organelles. For example, the endoplasmic reticulum is involved in protein and lipid synthesis and transport, lysosomes help to break down biomolecules, and mitochondria provide energy to cells by releasing energy from sugars. As a result, molecules

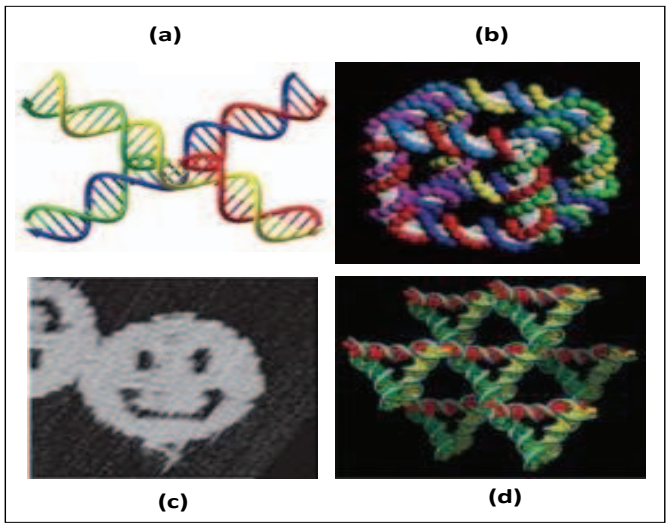


**Figure 2.** Examples of nanostructures built using DNA as a building block. **(a)** DNA junctions with radiating arms, as proposed by Nadrian Seeman **(b)** A three-dimensional DNA cube **(c)** A smiling face **(d)** Repeating triangles arranged in a specific pattern in three-dimensional space (Reprinted by permission from Springer Nature: Springer Nature Nanotechnology; A V Pinheiro *et al*., Challenges and Opportunities for Structural DNA Nanotechnology, *Nat. Nanotechnol*., 6, pp.763–74, 2011; Copyright (2011) Springer Nature, U.S.A).

that are associated with each of these functions are also sequestered in different organelles. Protons play an important role in determining the charge, and thus, the structure of biological molecules. Since each compartment (organelle) is associated with specific biomolecules, each compartment has a characteristic pH. For example, the pH of lysosomes is ~ 5.0, that of mitochondria is ~ 8.0, and the endoplasmic reticulum is ~ 7.2. Perturbing the unique pH of a particular organelle can disrupt its function. For example, when the pH of the lysosome is perturbed, it cannot degrade biomolecules anymore [3]. Keeping this in mind, the DNA architecture that we chose to introduce into a living organism was a pH-sensitive nanomachine called the 'I-switch'.

The I-switch comprises three short strands of DNA which are partially complementary (O1, O2 and O3, *Figure* 3a). The overhangs on strands O2 and O3 contain cytosine-rich regions, which, at acidic pH, undergo non-standard base pairing to form what is called the I-motif (*Figure* 1c). This leads to folding of the I-switch into a 'closed' conformation. When the pH is increased to near-neutral value, the motif undergoes dissociation and the assembly returns to its 'open' conformation (*Figure* 3a). Formation and dissociation of the I-motif is followed by attaching light-

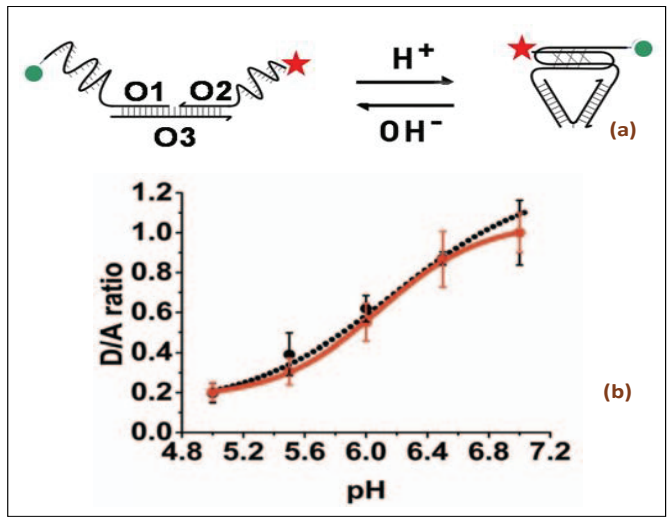


**Figure 3.** Design and functioning of the I-switch. **(a)** Structure and working of the I-switch. The green circle denotes the donor fluorophore while the red star denotes the acceptor fluorophore. **(b)** Graph showing green (D) to red (A) ratios of doubly labelled I-switch *in vitro* (black line) and *in vivo* (red line) with decreasing pH.

emitting fluorophores (see below) to the ends of the I-switch and allowing them to undergo 'fluorescence resonance energy transfer' (FRET) [4]. Fluorophores are molecules which absorb light of certain colour and emit light of a different colour [5]. For example, one of the fluorophores that we use absorbs blue light and emits green light. We use two fluorophores on the I-switch, such that one absorbs blue light and emits green light while the other absorbs green light and emits red light. When these two fluorophores are placed close to each other (between 2–6 nm), they undergo a phenomenon known as FRET [5]. In this case, when one shines blue light on fluorophore 1 (called the donor), instead of emitting green light, it transmits it to fluorophore 2 (called the acceptor). Fluorophore 2 then emits red light, without ever having received green light from the experimentor. Because the donor and the acceptor must be placed at a very close distance to undergo FRET, this then becomes a very good method to study the functioning of the I-switch. When the environmental pH is neutral, and the I-switch is in its 'open' conformation, the donor and acceptor fluorophores are out of their FRET-ting distance, and hence, the signal observed is green in colour (*Figure* 3a). On encountering acidic pH, the I-switch adopts a 'closed' conformation due to I-motif formation, leading to the donor and acceptor

On encountering acidic pH, the I-switch adopts a 'closed' conformation due to I-motif formation, leading to the donor and acceptor fluorophores undergoing FRET and emitting a red signal.

fluorophores undergoing FRET and emitting a red signal (*Figure* 3a). At every pH point, blue light is provided and emitted green and red light is recorded. A ratio of the green/red signal is also calculated. This ratio, called the D/A ratio, shows a characteristic curve with changing pH (*Figure* 3b), black trace) [4]. Thus, the I-switch is a DNA-based machine that has been programmed to provide a fluorescent output in response to changes in environmental pH.

The endo-lysosomal system in coelomocytes needs to undergo acidification and is an ideal system to test the functionality of the I-switch in a multicellular organism.

*Caenorhabditis elegans* is a multicellular worm (nematode) that is used as a model organism because of its genetic and cellular tractability [6]. It is transparent, which allows for fluorescence-based studies to be carried out easily as changes in colour can be recorded. *C. elegans* also contains six large scavenger cells, or coelomocytes, in its body cavity. These coelomocytes can internalize foreign molecules injected into the body cavity by creating a pit in its plasma membrane, engulfing the extracellular fluid in this pit, and then finally pinching it off. This process, called endocytosis, results in an endosome surrounded by a membrane, filled with foreign particles, and located in the cytoplasm [7]. It was earlier known from studies on neurons derived from chick embryos that this endosome, called the early endosome, which has a pH of 6.0 – 6.2, undergoes progressive acidification to form the late endosome (pH of ~ 5.5) and then the lysosome (pH of ~ 5.0). However, the pH of each of these endosomes was not known in coelomocytes of worms. We hypothesized that the early endosome in coelomocytes would also need to undergo this acidification and would thus be an ideal system to test the functionality of the I-switch in a multicellular organism.

## Proof of Concept

We injected fluorescently labelled I-switch into the body cavity of *C. elegans*. We found that the I-switch was internalized in endosomes of the coelomocytes. Endocytosis of the I-switch was detected as a fluorescent signal in the endosomes of coelomocytes 1 hour after the injection (*Figure* 4a). To find out whether

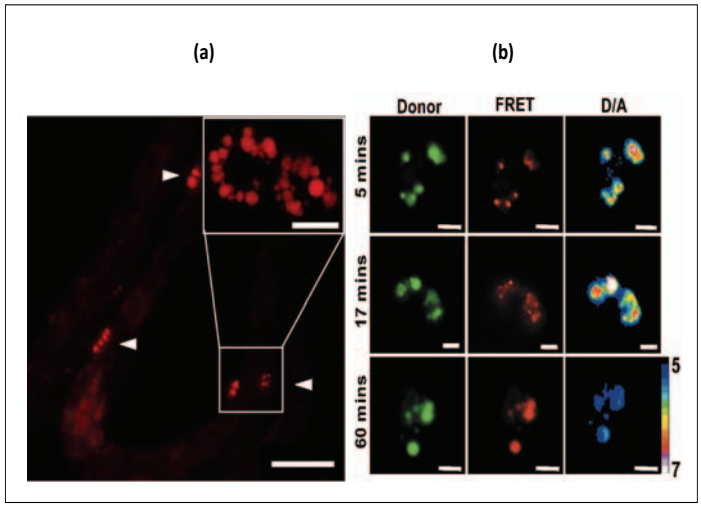


**Figure 4.** I-switch is present in endosomes in coelomocytes of *C. elegans*, where it measures changes in pH. **(a)** Image of a worm injected with labelled I-switch. Arrows indicate labelled coelomocytes. Scale bar: 50 $\mu$m. Inset: Magnified image of labelled coelomocytes. Scale bar: 5 $\mu$m **(b)** Representative coloured D/A images of I-switch-labelled coelomocytes in worms at indicated times after injection. Colours in the D/A panel, from white to black, are indicative of decreasing pH, as shown on the bar. Scale bar: 5 $\mu$m.

the I-switch could function as a pH sensor *in vivo* (inside the organism), we treated worms injected with the (doubly labelled) I-switch with a buffer. This buffer contained ionophores, which are compounds that can transport protons across cell membranes. Therefore, this buffer could be used to change the pH inside the coelomocytes as desired. By changing the pH inside the coelomocytes and measuring the green/red signal, we found that the *in vivo* pH response curve (*Figure* 3b, red trace) was remarkably similar to the *in vitro* curve. This shows that the I-switch could function as a pH sensor *in vivo* [4].

Next, we wanted to test the pH of the early endosome, the late endosome and the lysosome in these coelomocytes. Worms were injected with fluorescently labelled I-switch, and at the time when the I-switch was present mainly in the early endosome, D/A ratios were recorded. This was similarly done at a time point when the I-switch was present mainly in the late endosome and the lysosome (*Figure* 4b). Each of these D/A ratios were traced on the y-axis of the *in vivo* pH trace obtained earlier. For each of these points, the x-coordinate was mapped. It is this x-coordinate which would give us the pH value for the respective compartment. These measurements clearly showed that the endosomes showed a gradual increase in acidity with each stage. It was found that the pH of

early endosomes was ~ 6.4, late endosomes was ~ 6.0 and lysosomes was ~ 5.4 [4], which corresponded very well to previously reported values in other cells and were the first measurements made in a living organism. This established that a DNA nanomachine could indeed be introduced into a living organism and exploited to gain insights into cellular processes, thus showcasing the utility of these synthetic architectures.

## Conclusion

Structural DNA nanotechnology aims to construct synthetic molecular machinery from DNA. Though it has been possible to create a variety of these devices and machines, their utility in living systems has never been shown. We have used the worm *C. elegans* to demonstrate the operation of a pH-sensitive DNA nanomachine, using it to map the pH of the endocytic system in coelomocytes. The I-switch can now be used as a sensor in different organelles to test whether an organelle malfunction is related to pH disruption. Similarly, it can be used to test whether a certain disease is associated with the disruption of pH in a certain organelle. Since coelomocytes are able to internalize DNA nanostructures, they could be used to test other DNA nanomachines.

These studies, which were the first of their kind, have revealed the powerful potential that DNA nanodevices offer as tools to study biological processes. This is further demonstrated by attempts of other laboratories to use DNA architectures in cells and organisms. These include targeting them to cancerous cells and silencing specific genes in these cells, as well as using DNA architectures to activate defense mechanisms in the mouse to treat cancerous tumours. Another promising application is in the production of antibodies against a protein of choice. In addition, attempts are being made to use these nanodevices as delivery vehicles which can package and carry drugs of choice to specific cells and tissues in an organism. Nanomachines that are designed to sense other ions or molecules, for example, potassium or calcium, in the cell would help in elucidating their importance in cellular processes.

I-switch can be used as a sensor in different organelles to test whether an organelle malfunction is related to pH disruption. Similarly, it can be used to test whether a certain disease is associated with the disruption of pH in a certain organelle.

If fully harnessed to their potential, DNA architectures can immensely further our understanding of biological processes in the cell.

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