# Multi-fueled Approach to DNA Nano-Robotics

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**Abstract.** An approach to multi-fueled DNA nano-robotics is described. We propose three types of driving force (i.e., fuel for DNA nano-robots): thermal fuel, pH fuel, and light fuel. The thermal fuel controls the hybridization of DNA molecules around the melting temperature. The pH fuel controls the hybridization of the so-called i-motif by changing the pH condition. The light fuel controls the hybridization of DNA oligomers that are intercalated with azobenzene by irradiation with UV or visible light. These three fuels are not mutually exclusive. However, experimental conditions for the fueling of DNA nano-robots show efficacy. Concrete ideas for using these three fuel types are proposed and discussed.

**Keywords:** DNA nano-robotics, multi-fueled approach, thermal fuel, pH fuel, light fuel, azobenzene, i-motif.

## 1 Introduction

Beginning with the pioneering work of Yurke et al. [7], DNA nano-robotic systems have made steady progress. In particular, the notion of a DNA fuel has been used in many applications. For example, Pierce et al. [5] have constructed a DNA walking device, in which the steps are fueled by single strands of DNA, each corresponding to one step of the device. In fact, DNA fuel has become a versatile tool in DNA nano-robotics. One advantage of DNA fuel is that different types of fuel with different base sequences can be used separately, so that they control the hybridization of different DNA molecules independently of one another.

However, DNA fuel emits double-stranded DNA molecules as a waste product, which accumulates in the solution and eventually inhibits the desired reaction. Therefore, it is reasonable to seek other sources of fuel, ideally those that do not produce waste products, which can be used to control the hybridization of DNA molecules.

Clearly, one can control the hybridization of DNA molecules by changing the temperature of the solution around the melting temperature of the molecules (thermal

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fuel). Another source of fuel is light. Takahashi et al. [6] have constructed DNA nano-machines that can be controlled by light radiation. They used DNA molecules intercalated with azobenzene, which changes conformation from trans to cis under UV-light irradiation and from cis to trans under visible light [1, 2]. A modified DNA molecule can hybridize with its complementary counterpart if the intercalated azobenzene molecules take the trans conformation (light fuel). Yet another source of fuel are protons. Liu and Balasubramanian [3] have proposed the use of the so-called i-motif for DNA nano-machines. Under the appropriate acidic condition, the i-motif adopts a folded form and does not hybridize with its complementary counterpart (pH fuel).

Using these DNA molecules as different components that are controllable by different sources of fuel, one can confer complex behaviors on a nano-machine by controlling the injection of each type of fuel. Note that the thermal fuel and the light fuel do not produce waste. Although the pH fuel increases the salt concentration each time the pH is changed, it is considered more tractable (and is cheaper) than DNA fuel.

The crucial issue in using these different sources of fuel is whether they can work independently of one another. If they do work independently, one can imagine various applications. For example, we can imagine a walking device on a DNA trail (Fig. 1), in which three types of DNA molecule protrude from the trail in a cyclic order. Each type of DNA is controlled by the corresponding type of fuel, i.e., thermal, pH, or light.

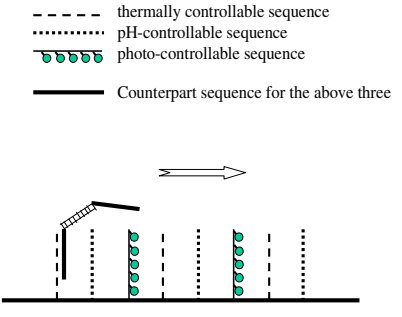


**Fig. 1.** The walking device

The motion of the device can be controlled if the three types of fuel work independently. For example, the walking device can be designed to move in a single direction as follows:

1. DNA oligomers that are thermally controllable, pH-controllable, and photo-controllable, and which can hybridize with their counterpart oligomers, are prepared. Controllable signifies that it is possible to control the hybridization and denaturation of the target oligomer and its counterpart. The sequences of the thermally controllable oligomers are designed using melting temperature predictions, the sequences of the pH-controllable oligomers are designed based on the i-motif [3], and the photo-controllable oligomers are prepared by azobenzene intercalation [1, 2].
2. The walking device has two counterpart oligomers as the 'feet'.

3. The three types of oligomers are immobilized repeatedly on the DNA trail in a cyclic order.
4. The initial condition is set up, e.g., the pH is set to 5.0 (acidic), the temperature is around 25°C (low), and the solution has been radiated with UV light (i.e., azobenzene is cis-formed). Then counterpart oligomers can only hybridize with the thermally controllable oligomers, i.e., one foot of the device hybridizes with a thermally controllable foot (Fig. 1).
5. To move the device in the right direction, we first change the pH to 7.0 (neutral). The other foot then hybridizes with the adjacent pH-controllable oligomer. By raising the temperature to 45°C (high), the first foot is denatured. At this point, if we irradiate the solution with visible light, the first foot will hybridize with the adjacent photo-controllable oligomer.

Figure 2 shows a similar DNA device based on the three types of fuel. In this system, the DNA trail forms a small triangle, and the device rotates the triangle like a motor.

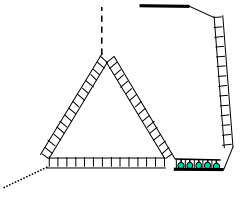

**Fig. 2.** The triangular trail

As mentioned above, in order to realize such DNA devices, it is crucial that the three types of fuel can be controlled independently. The goal of the present study is to investigate how the three types of fuel can be controlled independently. In the present paper, we report the results of our preliminary experiments in which we have prepared three oligomers that are thermally controllable, pH-controllable, and photo-controllable, as well as their counterparts, and observed their hybridization profiles under various conditions.

Unfortunately, in the current design, the three types of fuel were not always independent of each another, although we observed some independence. For example, the pH fuel and the light fuel can be controlled almost independently. Even if the three types of fuel are not completely independent, the information gathered regarding independence should be useful for the future development of DNA nano-robots.

## 2 Materials and Methods

### 2.1 Materials

We prepared four oligomers. The oligomer termed RG-motor is the so-called i-motif, as described by Liu and Balasubramanian [3]. This oligomer has four CCC motifs and

folds into a specific form under acidic pH conditions. The oligomer named Y-A12-BHQ2, which is the complementary counterpart of RG-motor, is also taken from Liu and Balasubramanian [3], except that we replaced one T with A to slightly break the symmetry. Thus, it is complementary to a sub-sequence of RG-motor with two mismatches, which lower the melting temperatures of RG-motor and Y-A12-BHQ2. Given that RG-motor prefers the folded form, under acidic pH conditions, the hybrid of RG-motor and Y-A12-BHQ2 is denatured. Note that RG-motor has rhodamine green at its 5'-end, whereas Y-A12-BHQ2 has BHQ2 at its 3'-end. Therefore, while these oligomers hybridize to each together, the fluorescence associated with RG is quenched.

The thermally controllable oligomer, Cy5-YY8, is complementary to the 3'-end 10-mer segment of Y-A12-BHQ2. The length of Cy5-YY8 was adjusted to control the hybridization between it and Y-A12-BHQ2 at temperatures between 25°C and 45°C. Note also that Cy5-YY8 has Cy5 at its 5'-end, the fluorescence of which is quenched by BHQ2.

The photo-controllable oligomer, TAMRA-YY7-AZ, has five azobenzenes intercalated into its side-chain. Without azobenzene, the oligomer is complementary to the 3'-end 13-mer segment of Y-A12-BHQ2. TAMRA-YY7-AZ also has TAMRA at its 5'-end, the fluorescence of which is quenched by BHQ2.

The sequences of the oligomers are listed below. In the design of these sequences, we sometimes used the Hyther program, which is available through the web interface [4], for predicting the oligomer melting temperatures.

RG-motor (pH-controllable sequence):
5'-rhodamine green-CCCTAACCCTAACCCTAACCC-3'

Cy5-YY8 (thermally controllable sequence):
5'-Cy5-CTAACTCTAA-3'

TAMRA-YY7-AZ (photo-controllable sequence):
5'-TAMRA-CTAXACXTCXTAXACXAC-3'; X = azobenzene

Y-A12-BHQ2 (counterpart sequence):
5'-GTTAGTGTTAGAGTTAG-BHQ2-3'

### 2.2 Selection of Fluorescent Groups and Buffers

Before the preliminary experiments, we had to decide which fluorescent groups to attach to oligomers, as the multi-fueled approach needs efficient and stable fluorescence under acidic environments and irradiation of UV light.

In general, as the pH decreases, the fluorescence becomes weaker. Furthermore, if the UV irradiation light is strong, the fluorescence generally degrades. However, the efficiency of fluorescence under these conditions depends greatly upon the fluorescent groups attached to the oligomers. Under acidic pH conditions, many fluorescent groups lose fluorescence. For example, FAM and Cy3 are not suitable for use under acidic pH conditions, such as pH 5.0. Although rhodamine green retains fluorescence under these conditions, the buffer used is of crucial importance. After examining several types of buffer, we found that SSC buffer was optimal. The so-called Good buffers were not always adequate for our experiments. Even the best

combination of rhodamine green and SSC buffer requires some data normalization. UV-light irradiation also affects the efficiency of fluorescence. TAMRA and Cy5 are tolerant to UV light, compared with FAM and Cy3. Therefore, we chose rhodamine green, TAMRA, and Cy5 for our experiments.

### 2.3 Methods

We conducted two experiments, the first with azobenzene-intercalated oligomers, and the second with thermally controllable and pH-controllable oligomers. All three oligomer types could be mixed in a single solution, but since UV-light radiation requires different protocols and devices, we conducted that experiment separately with the azobenzene-intercalated oligomer.

**Experiment with Azobenzene-Intercalated Oligomers**
The 13-mer azobenzene-intercalated oligomer with TAMRA attached to the 5'-end of the sequence, TAMRA-YY7-AZ (5'-TAMRA-CTAXACXTCXTAXACXAC-3'; where X = azobenzene), was prepared in a tube with 1× SSC buffer to a final concentration of 0.1 μM. UV light at 360 nm was applied to the tube through a UV-D36C glass filter (Asahi Techno Glass) with the UVP B-100AP 100-W lamp for 30 min. The irradiated sample (400 μl) was transferred to a quartz cell and placed in a Hitachi F-2500 Spectrophotometer, the temperature of which was maintained with the LAUDA RC-6 apparatus. While the fluorescence of TAMRA was measured for 300 seconds, the equivalent concentration of the quencher oligomer, Y-A12-BHQ2 (5'-GTTAGTGTTAGAGTTAG-BHQ2-3'), which is partially complementary (13-mer) to TAMRA-YY7-AZ, was added to the cell to measure the fluorescence change of TAMRA. The fluorescence of TAMRA in the trans-form was also measured. Since the trans-form azobenzene allowed Y-A12-BHQ2 to hybridize, we also investigated whether TAMRA-YY7-AZ and Y-A12-BHQ2 were separated by UV irradiation for 30 min. This experiment was carried out at 25°C and 45°C under neutral and acidic (pH 5.0) pH conditions.

**Experiment to Control the pH and Temperature**
The Cy5-YY8 and RG-motor oligomers were first mixed in a tube, at final concentrations of 0.1 μM, with 1× SSC. The quencher, Y-A12-BHQ2, was added at four-fold higher concentration for the duration of the fluorescence measurement, in order to measure the effect of quenching on each type of fluorescence. This measurement was performed in the cycle of neutral (pH 7.6), acidic, and neutral pH at 25°C. In the cycle, 6 μl of 1 M HCl were added to the cell to produce the acidic pH condition, and 55μl of 0.1 M NaOH were applied to neutralize the acidic solution. In the same manner, each type of fluorescence was measured for the temperature cycle of 25°C , 45°C , and 25°C at neutral pH (pH 7.6).

## 3 Results

The purpose of the preliminary experiments was to check the feasibility of the multi-fueled approach. For this purpose, we examined the basic behaviors of the DNA oligomers under various conditions generated by combinations of fueling operations.

1. For the temperature of the solution, we examined the alternatives of 25°C and 45°C .
2. For the pH of the solution, we examined the neutral pH condition (around pH 7.0) and the acidic pH condition (around pH 5.0).
3. For UV-light irradiation, we examined three alternatives:
   (a) The solution was irradiated with UV light before the hybridization reaction.
   (b) The solution was irradiated with UV light after the hybridization reaction.
   (c) The solution was not irradiated with UV light.

   DNA oligomers that are intercalated with azobenzene can be controlled in two ways. One way is to block hybridization beforehand using UV-light irradiation. The other way is to denature the double-stranded hybridized DNA by irradiation. These alternatives are based on the conformational change of azobenzene from trans to cis that occurs under UV-light irradiation. Since the cis-form of azobenzene hinders hydrogen bonding of base pairs, the conformational change from trans to cis is expected to cause denaturation or blockage of hybridization.

Therefore, we examined various experimental conditions, each of which was a combination of one of the two thermal conditions, one of the two pH conditions, and one of the three light conditions. In total, we examined 12 (2×2×3) conditions. In addition, each condition was examined with the different fluorescent wavelengths of Cy5, rhodamine green, and TAMRA. As described in Materials and Methods, Cy5 was attached to the oligomer for thermal control (Cy5-YY8), rhodamine green was attached to the oligomer for pH control (RG-motor), and TAMRA was attached to the oligomer for photo-control (TAMRA-YY7-AZ). All these oligomers are (partially) complementary to the counterpart oligomer (Y-A12-BHQ2), which is modified to have BHQ2 at its 3'-end so that the fluorescence is quenched when the oligomers hybridize.

**Table 1.** Summary of the experimental results. The + symbol denotes that the spectrophotometer detected strong fluorescence coming from the fluorescent group of the DNA oligomers, which was not quenched by BHQ2 in the counterpart DNA oligomer. The − symbol denotes that the spectrophotometer did not detect strong fluorescence. The observation results in the meshed area do not coincide with the expected results. The ± symbol denotes a fluorescence level between + and −. The question mark symbol (?) indicates that fluorescence could not be measured, due to the extreme conditions.

| | | 25°C | | | 45°C | | |
|---|---|---|---|---|---|---|---|
| | | No UV | UV (before) | UV (after) | No UV | UV (before) | UV (after) |
| Neutral (pH 7.0) | Thermal | – | – | | + | + | + |
| | pH | – | – | – | + | + | + |
| | Photo | – | + | + | | + | ± |
| Acidic (pH 5.0) | Thermal | – | – | – | + | + | + |
| | pH | + | + | + | + | + | + |
| | Photo | – | + | ± | ? | ? | ? |

In the experimental results shown in Table 1, the + symbol indicates that the observed fluorescence is almost as strong as that of the fluorescent group alone, while the − symbol means that the observed fluorescence is much weaker than that of the fluorescent group alone. Other symbols are mentioned in the next section.

As explained in more detail in the next section, the observation results shown in the meshed area of Table 1 do not correlate with the expected outcomes. This means that the independence of these conditions is compromised.

Owing to space limitations, we mention only a few examples of the observed fluorescent data. Figure 3 shows the result of changing the temperature and measuring the fluorescence from Cy5 attached to the thermally controllable oligomer (Cy5-YY8). The observation was made with a mixture of Cy5-YY8, RG-motor, and Y-A12-BHQ2, as described in Section 2.3, under the neutral pH condition. At 25°C, fluorescence was not observed (?), which indicates that Cy5-YY8 hybridizes with Y-A12-BHQ2. At 45°C, fluorescence was observed (+), which indicates that Cy5-YY8 and Y-A12-BHQ2 are denatured. In Figure 3, the fluorescence data are not normalized, as Cy5 is not influenced by temperature.

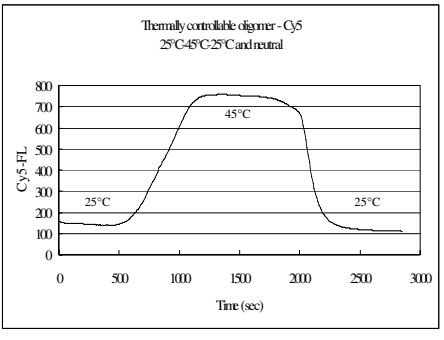


**Fig. 3.** Results for the thermally controllable oligomer

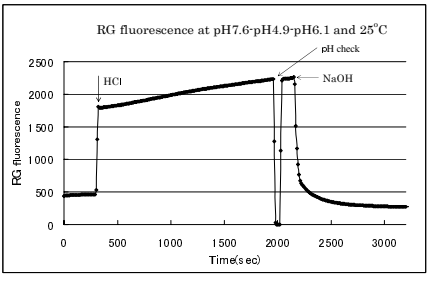


**Fig. 4.** The results for the pH-controllable oligomer

Figure 4 shows the results from changing the pH conditions and observing the fluorescence from RG attached to the pH-controllable oligomer. These observations were made for the mixture of Cy5-YY8, RG-motor, and Y-A12-BHQ2, as described

in Section 2.3, at 25°C. The pH condition was first changed from neutral to acidic using HCl. After the pH was measured (pH 4.9), NaOH was added to give the final pH condition (pH 6.1) for this experiment.

Regarding the main focus of the present study, i.e., the independence of the three types of fuel, Figure 5 shows the results of one of the successful experiments. The fluorescence of TAMRA attached to the photo-controllable oligomer is shown under four different conditions. In the left panel, the fluorescence intensity in the absence of UV light is compared with that of the oligomer that was irradiated with UV light for 30 min. Both measurements were conducted under the neutral pH condition at 25°C. In the right panel, the same comparison is made under the acidic pH condition at 25°C. Although the fluorescence level under the acidic pH condition is lower, similar results were obtained under both conditions. These results indicate the independence of the light fuel from the pH fuel at 25°C.

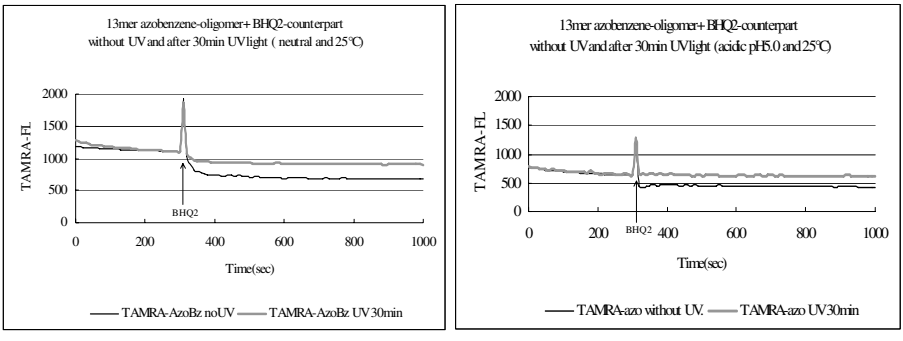


**Fig. 5.** Independence of the light fuel from the pH fuel at 25°C. The photo-controllable oligomer TAMRA-YY7-AZ was irradiated with UV light (black line) or not irradiated (gray line). Y-A12-BHQ2 was added 300 sec after the start of the measurement period. In both panels, the fluorescence levels are adjusted to the time at which Y-A12-BHQ2 was added.

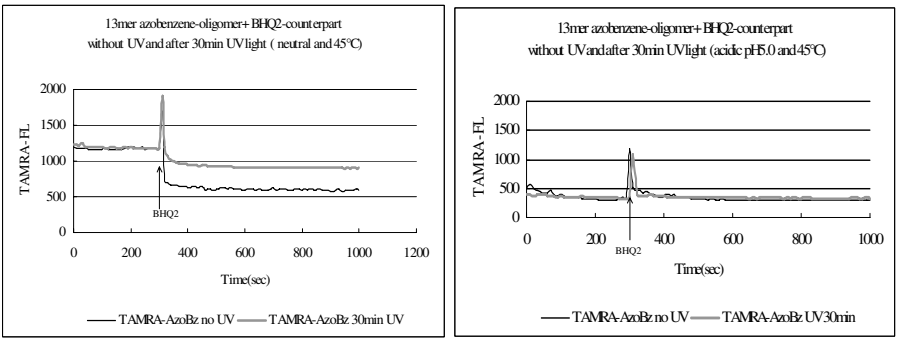


**Fig. 6.** An unsuccessful outcome regarding the independence of the light fuel from the pH fuel at 45°C

On the other hand, Figure 6 shows an experimental result under extreme condition. Under the acidic pH condition at 45°C (right panel), the fluorescence level was extremely low compared with the other conditions.

## 4 Discussion

As shown in the previous section, the levels of independence among the three types of fuel are incomplete. The following observation results (Table 1, meshed area) did not coincide with the expected outcomes.

1. The pH-controllable oligomer was denatured at 45°C even under the neutral pH condition. Although the pH-controllable sequence is seven bases longer than the thermally controllable oligomer, this difference in length appears to be insufficient, as the pH-controllable sequence contains some mismatches.
2. Under the acidic pH condition at 25°C and the neutral pH condition at 45°C , when the solution was irradiated with UV light after the hybridization reaction with the photo-controllable oligomer, the fluorescence level was not sufficiently strong. This means that the photo-controllable oligomer was not denatured completely.
3. Under the acidic pH-condition at 45°C, the fluorescence level of TAMRA was very low and unstable. This appears to be due to the severity of the conditions (pH 5.0 and 45°C) for the TAMRA fluorescence group. Although the hybridization reaction may have occurred as expected, methods other than fluorescence detection are required for reliable observations.

## 5 Concluding Remarks

We have proposed a multi-fueled approach to DNA nano-robotics and examined the feasibility of the approach in some preliminary experiments. Although the three types of fuel are not always independent of each other, combinations of the three fuels used under the appropriate experimental conditions have proven to be useful for DNA nano-robotics.

To ensure that the three types of fuel function more independently of one another, it is necessary to examine the possibility of controlling the hybridization under milder conditions. Such mild conditions would also solve the problem encountered with fluorescence detection, as described in the previous section.

Although some combinations of the three fuels have proven to be useful, their effectiveness was only shown qualitatively. In order to optimize experimental protocols and eventually construct motors and walkers, we need to make quantitatively estimation of the hybridization ratio in each combination. Calibration between the fluorescence level and the hybridization ratio is the first thing to be done.

The sequences used for the experiments are not considered optimal. In order to re-design them, it seems worthwhile to try in-vitro search of sequences in addition to ordinary free energy prediction based on the nearest neighbor model.

The search for an alternative source of fuel is critically important.

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