Experimental Section

All oligonucleotides designed were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, China), and their sequences as well as thermodynamic data are shown in Table 1.

Phosphate buffer solution (10 mM PBS, pH 7.4) consisting of 10 mM phosphate-buffered saline, 0.2 M NaCl, 1 mM MgCl$_2$ and 2.7 mM KCl was prepared DNA stock solutions. The concentration of probe DNA1 solution is 4.95 µM while the probe DNA2 solution is 5.0 µM. The concentrations of stretching DNA1, 2, 3 and 4 are 12.25, 13.75, 16.75 and 21.25 µM, respectively. Unless otherwise indicated, the fuel DNA and anti-fuel DNA at a calculated concentration (8.15 µM) are used and stretching DNA1 was used throughout the experiments.

All fluorescence measurements were accomplished on a Hitachi F-4500 Fluorescence Spectrometer (Tokyo, Japan). The fluorophore (FAM) was excited at 494 nm, and the fluorescence peak at 518 nm was used to assess the functioning of the nanomachine throughout the experiments. Unless otherwise stated, 200 µL of the solution was involved when fluorescence measuring.

Unless otherwise indicated, the LPOD nanodevice was prepared by introducing 5 µL of probe DNA1, 20 µL of probe DNA2 and 8 µL stretching DNA1 into 217 µL of 10 mM PBS and immediately mixing. To evaluate the performance of the optical nanodevice, after the addition of 750 µL of 10 mM PBS having or containing no other DNA sequences (e.g. fuel DNA), the hybridization is allowed to react for 60 min. The content of probe DNA1 and the total volume of the resulting mixture were kept constant throughout while the quantities of other DNA sequences could be easily controlled by changing the PBS volume when optimizing the working conditions or characterizing the developed nanodevice.
Table 1. Oligonucleotide sequences synthesized in the present experiments^a

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<tr>
<th>Index</th>
<th>Sequence (5’ to 3’)</th>
<th>Notes</th>
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<tr>
<td>Probe DNA1</td>
<td>FAM-CCCCCGCGG TTATT TGTTA CATATC TGACA</td>
<td>fluorescent sequence</td>
</tr>
<tr>
<td>Probe DNA2</td>
<td>GCGACGGTCTGTAGCT TT AACGTGC CCCGC GCGAA TGAGG CGGG TTCGCGCGGG</td>
<td>quenching sequence</td>
</tr>
<tr>
<td>Fuel DNA</td>
<td>CCTCA TTCGC GCAGGG GCAGT TT TCG CTCTC TCTGC GC</td>
<td>complementary target DNA</td>
</tr>
<tr>
<td>Anti-fuel DNA</td>
<td>GCAGG AGGAG AGCGA AACGT GCCCC GC GCCG AATGA GG</td>
<td>complementary to target DNA</td>
</tr>
<tr>
<td>Control DNA1</td>
<td>CTACAGATACTC GTGA AAGGT TGAGT AGGAT GGTCAT</td>
<td>noncomplementary target DNA</td>
</tr>
<tr>
<td>Control DNA2</td>
<td>CCTCATT CCCT GCGG GCCAGTT TCGCTCT CCTCCTGC</td>
<td>Single-base mismatched target DNA</td>
</tr>
<tr>
<td>Stretching DNA1</td>
<td>CAGTA TGTGT CAGAT AGTAC</td>
<td>complementary to the middle sequence of probe DNA2</td>
</tr>
<tr>
<td>Stretching DNA2</td>
<td>CAGTA TGTGT CAGAT AGT</td>
<td>shortened by two-base segment compared to Stretching DNA1</td>
</tr>
<tr>
<td>Stretching DNA3</td>
<td>CAGTA TGTGT CAGAT</td>
<td>shortened by five-base segment compared to Stretching DNA1</td>
</tr>
<tr>
<td>Stretching DNA4</td>
<td>CAGTA TGTGT CA</td>
<td>shortened by eight-base segment compared to Stretching DNA1</td>
</tr>
</tbody>
</table>

^a The molecular nanodevice is developed by hybridizing these DNAs to each other, and schematic illustration of the switching mechanism is shown in Scheme 1. The thermodynamic data for hybrids mentioned under the current conditions, which are calculated from DNA folding software (mfold) (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi), are listed as follow:

- Hairpin structure of probe DNA2: \( \Delta G^0 = -15.5 \text{ kcal/mol} \quad T_m = 85.8 \text{ °C} \)
- Stretching DNA1/probe DNA1 hybrid: \( \Delta G^0 = -25.2 \text{ kcal/mol} \quad T_m = 53.8 \text{ °C} \)
- Italicized stem: \( \Delta G^0 = -25.4 \text{ kcal/mol} \quad T_m = 59.8 \text{ °C} \)
- Underlined stem: \( \Delta G^0 = -14.0 \text{ kcal/mol} \quad T_m = 40.8 \text{ °C} \)
- Fuel DNA/probe DNA2 hybrid: \( \Delta G^0 = -41.3 \text{ kcal/mol} \quad T_m = 70.7 \text{ °C} \)
- Fuel DNA/anti-fuel DNA hybrid: \( \Delta G^0 = -61.6 \text{ kcal/mol} \quad T_m = 82.7 \text{ °C} \)

Those calculated data indicate that the hairpin structure of probe DNA2, italicized stem and stretching DNA1/probe DNA1 hybrid can be formed, leading to a pendulum-type
DNA nanodevice. The fuel DNA can easily dissociate the stem of hairpin structure, facilitating the hybridization of underlined stem. The high stability of fuel DNA/anti-fuel DNA hybrid ensures that the fuel DNA can be stripped from the nanodevice by anti-fuel DNA. In this case, the nano-system is restored to its open state due to the preferential formation of hairpin structure of probe DNA2 in the absence of fuel DNA because the free energy as well as melting temperature is favorable for the intramolecular hybridization. Importantly, the experimental data (e.g. Fig. 1 in text) confirms directly the feasibility of nanodevice design.
Fig. S1 The dependence of the fluorescence quenching efficiency on the volume ratio of probe DNA2 to probe DNA1. The fluorescence quenching efficiency is defined as \((F_0 - F)/F_0 \times 100\%\), where \(F_0\) and \(F\) denote the peak intensity at 518 nM recorded from the fluorescence spectra collected for the probe DNA1/probe DNA2/stretching DNA solution without and with fuel DNA, respectively.

The fluorescence quenching efficiency increases with increasing the ratio value, almost reaching a response plateau up to 4:1. In this case, there are apparently unhybridized probes in the nanodevice solution, which seems to be a common feature of
multiplex-separate-probe nanosystem.\textsuperscript{1} To overcome this limitation, a high-temperature annealing treatment was used to promote the interaction of the probe sequences. The increase of the thermodynamic stability via lengthening the italicized stem should promote the formation of nanodevice. This is a practicable solution, because the present nanodevice has only one binding site compared with the existing nanomachines\textsuperscript{2} and the change in the length of italicized stem does not influence the binding property of binding sites.
**Fig. S2** The influence of stretching DNA on the fluorescence intensity of LPOD nanodevice. A): the change in fluorescence intensity induced by stretching DNAs with various molecule lengths. The peak intensity at 518 nM presented was obtained from the fluorescence spectra collected for the probe DNA1/probe DNA2 solution after the stretching DNA was introduced and the equilibrium state was reached; B): the relation between the content of stretching DNA1 and the fluorescence quenching efficiency.

Since the final fluorescence intensity upon addition of fuel DNA is at roughly the same level regardless of stretching DNA, the initial fluorescence intensity at the equilibrium state was employed to estimate the effect of various stretching DNAs on the molecule device’s function. The fluorescence peak increases with the length of stretching DNA.
DNA, indicating the increase of the spacer distance between the donor and quencher due to the rigid DNA duplex. Additionally, the fluorescence response increases with the stretching DNA1 content up to 2.5 μL. In this case, the molar ratio of stretching DNA1 to probe DNA1 of 6:5 is much less than that of probe DNA2/probe DNA1 ratio (about 4:1). Compared with the italicized segment of probe DNA2, the stretching DNA1 has a longer complementary sequence, increasing apparently the stability of the corresponding duplex. Consequently, this hybridization can occur almost at an equimolar manner. The experimental result demonstrates indirectly the feasibility of the technique mentioned above to increase the stability of probe DNA1/probe DNA2 duplex via lengthening the italicized stem.
**Fig. S3** The investigation of hybridization time for the construction of LPOD nanodevice. The volume ratio of probe DNA1 to probe DNA2 involved in this mixture without stretching DNA1 was 1:4. Inset: the real-time fluorescence measurement performed as soon as the stretching DNA1 was mixed with probe DNA1/probe DNA2 solution. The real-time monitoring of the hybridization of probe DNA2 with probe DNA1 was performed after the probe DNA2 (8 µL, 5.0 µM) was added to a 400-µL mixture of 24.75 nM probe DNA1 and 91.88 nM stretching DNA1, while the real-time monitoring of the hybridization of stretching DNA1 with probe DNA1 was carried after introducing stretching DNA1 (3 µL, 12.25 µM) into a 400-µL solution containing 24.75 nM probe DNA1 and 100 nM probe DNA2.

The hybridization of probe DNA1 with probe DNA2 (called pp hybridization) gradually increases with the increment of incubation time and then reaches a plateau after 40 min while the hybridization with stretching DNA1 (ps hybridization) essentially
reaches an equilibrium state within about 4 min, demonstrating that ps hybridization is substantially faster than pp hybridization. Presumably, compared with the ps hybridization, the pp hybridization was considerably inhibited by the stronger electrostatics repulsion resulting from the more bases in probe DNA2 and higher charge density of the folded form. Furthermore, the probe DNA1/stretcching DNA1 duplex, which possesses a higher stability associated with complementary stretching DNA1 longer than complementary italicized segment of probe DNA2, could facilitate ps hybridization.
**Fig. S4** The influence of annealing treatment on the optical properties of the present LPOD nanodevice. A): the fluorescence intensity recorded at 518 nm for the LPOD nanodevice treated by annealing before (a) and after the addition of fuel DNA (b); B: Column c and d are the same as column a and b, respectively, but without annealing. The annealing treatment was carried out by incubating the nanodevice (250 µL) at 85 °C for 15 min and then cooling to room temperature over 60 min; Subsequently, 750 µL of 10 mM PBS without or with fuel DNA at 326 nM was injected into the resulting solution. After another 60 min, the fluorescence spectra were collected.

The annealing effect on the nanodevice function is shown in Fig. S4. The annealing treatment not only increases the fluorescence intensity of this device in the open state but also decreases the fluorescence peak in the closed state, resulting in a considerable increase of FRET efficiency. One can see that the fluorescence quenching efficiency for the nanodevice upon addition of fuel DNA is about 70% without and 90% with annealing.
treatment, indicating that a heating-and-cooling treatment can raise the degree of conformational transition of nanodevice. Clearly, the optical nanodevice can be constructed in high yield utilizing a heating-and-cooling process. The experimental results are consistent with other reports\textsuperscript{3} that when the component DNA strands are annealed the desired molecular nanostructure can be formed.
Fig. S5 The mismatched sequence identification ability achieved. Fluorescence signals triggered by the blank and several target DNA (326 nM) were collected under identical conditions.

A noncomplementary sequence cannot induce a detectable fluorescence change; a single-base mismatched sequence can generate a fluorescence quenching efficiency of about 40% assuming that the quenching efficiency induced by the complementary target DNA is assigned the value of 100%. Considering that a hairpin, especially with a long stem, exhibits excellent sensitivity to the detection of one mismatch, it is natural that not only control DNA1 but also control DNA2 can be easily discriminated from the fully complementary target sequence.
References


