Supporting Information

Experimental details:

**DNA Oligonucleotides.** Strand E: 5’-GGT TAG ATG GTA TGC TTC GGA CAG GCT AGC TAC AAC GAG AGT GAC TGA GCG TAA GGT CTG G-3’; Strand F: 5’-rhodamin green-GCA TAC CAT CTA ACC TCC AGA CCT TAC GCT C-BHQ-1-3’; Strand S: 5’-GTC ATT CrArU GTC CGA-3’; Strand S’: 5’-GTC ACT CAT GTC CGA-3’; Strand B: 5’-GTC GCA CAC CGT CAC TCC CTG TCC GA-3’; Strand R: 5’-TCG GAC AGG GAG TGA CGG TGT GCG AC-3’. All oligonucleotides were purchased from Integrated DNA Technologies, Inc., and purified by denaturing polyacrylamide gel electrophoresis.

**Nanomotor Formation.** 0.5 µM DNA strands were combined with equal molar ratio in TAE/Mg2+ buffer, which contains 40 mM Tris base, pH 8.0, 20 mM acetic acid, 2 mM EDTA and 12.5 mM Mg(Ac)2. The nanomotor was formed by slowly cooling the DNA solution as following: 95°C (3 min), 65°C (30 min), 50°C (30 min), 37°C (30 min) and 22°C (30 min).

**Enzyme Digestion.** 0.5 µM DNA motor and 10 µM DNA-RNA chimera substrate were incubate in TAE/Mg2+ buffer for 1 hour at 22°C, and then analyzed with non-denaturing polyacrylamide gel electrophoresis.

**Denaturing Polyacrylamide Gel Electrophoresis.** Gels contained 10-20% polyacrylamide (19:1 acrylamide/bisacrylamide) and 8.3 M urea; they were run at 55°C. The running buffer consisted of 89 mM Tris base, pH 8.0, 89 mM boric acid, and 2 mM EDTA (TBE). Gels were run on a Hoefer SE 600 electrophoresis unit at 600 V (constant voltage).

**No-denaturing Polyacrylamide Gel Electrophoresis.** Gel contained 12% polyacrylamide (19:1 acrylamide/bisacrylamide). The running buffer consisted of 40 mM Tris base, pH 8.0, 20 mM acetic acid, 2 mM EDTA and 12.5 mM (CH3COO)2Mg (TAE/Mg+). Gels were run on a Hoefer SE 600 electrophoresis unit at 22°C (250 V, constant voltage). After electrophoresis, the gel was stained with Stains-all dye (Sigma) and scanned.

**Fluorescence Spectroscopy.** DNA is dissolved in TAE/Mg2+ buffer at 0.1 µM concentration. Fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. All spectra were collected at 22°C. The samples were excited at 504 nm and the emission data were collected either from 520 nm to 650 nm (for spectra), or fixed at 531 nm (for motor cycling). The maximal emission wavelength of rhodamine green is 531 nm.