Supplementary Information: An experimental study of the putative mechanism of a synthetic autonomous rotary DNA nanomotor

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1 Sequences

The sequences of all DNA strands used in this work are given in the following tables. All sequences are written 5’ to 3’. So-called ‘pseudosequences’ were used to design the molecules and represent their structure in terms of domains. For example, the ‘pseudosequence’ (CSr,T8,BDA,X1r) denotes a sequence comprising, in order, the reverse complement of CS, 8 thymine bases, the domain called ‘BDA’, and the reverse complement of X1. For the simpler experiments, the symbol * is used to denote the reverse complement of a domain or strand, such as CS*. The sequences of CSr and CS* are identical. All strands were acquired

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with standard desalting purification, with the exception of the thiol-modified strand CS, for which HPLC purification was selected. All oligonucleotides were supplied by Integrated DNA Technologies.

1.1 Sequential strand displacement in a linear construct

The strands used to study sequential strand displacement in a linear construct are below. Note that the strand CS was used throughout this work. It is listed here only in this table.

| Name  | Sequence |
|-------|----------|
| CS    | ACA CGC ATA CAC CCA T-(thiol) |
| X     | AAA CGC ACT ACG GCT CAG ATG TCC ACG AAC GCC ACC AAG G |
| X*    | CCT TGG TGG CGT TCG TGG ACA TCT GAG CCG TAG TGC GTT T |
| Block-1 | CTG AGC CGT AG |
| Block-2 | TTC GTG GAC AT |
| Block-3 | ATG GGT GTA TGC GTG TCC TTG GTG GCG |

Table 1: Sequences of DNA oligonucleotides used to examine sequential strand displacement in an immobilized linear construct.
1.2 Strand displacement in a geometrically constrained structure (‘Triangle’ experiment)

Table 2 provides the sequences used to assemble the triangle and trigger strand displacement reaction within it. Lower case letters represent domain names and the sequence T₃ is inserted in places to ensure flexibility at corners. The strand name T is not to be confused with the representation of a single thymine.

| Name     | Pseudosequence | Base sequence |
|----------|----------------|---------------|
| T        | CS*, a, T₃, b, T₃, c, T₃, d, t | ATG GGT GTA TGC GTG TTG TTG GTG TGT |
|          |                | TTC TCC TCC TCC TTT CTG CCT GCC TTT |
|          |                | TGC GGC GGC GCA TCA GCA T |
| ac-staple| a*, T₃, c*     | CAC ACC AAC ATT TAG GCA GGC AG |
| bd-staple| b*, T₃, d*     | GGA GGA GGA GTT TCG GCG GCG GC |
| Control-a| a_−*           | ACA CCA AC |
| Control-b| b_−*           | GAG GAG GA |
| Control-c| c_−*           | GGC AGG CA |
| Control-d| d_−*           | GGC GGC GG |
| T*       | (CS*, a, T₃, b, T₃, c, T₃, d, t)* | ATG CTG ATC GGC GGC GGC AAA AGG CAG |
|          |                | GCA GAA AGG AGG AGG AGA AAC ACA CCA |
|          |                | ACA ACA CGC ATA CAC CCA T |
| RC(ac-staple) | (a*, T₃, c*)* | CTG CCT GCC TAA ATG TTG GTG TG |
| RC(bd-staple) | (b*, T₃, d*)* | GCC GCC GCC GAA ACT CCT CCT CC |

Table 2: Sequences of DNA oligonucleotides used to construct the nano-triangles, confirm whether they had assembled correctly, and induce strand displacement. The abbreviation ‘t’ denotes the toehold. The _− subscript in the pseudosequences for the control strands indicates that the first and last nucleotide of these domains was omitted.
1.3 The rotary motor

Three tables are provided, listing the pseudosequence of each strand (Table 3), the sequence of each domain referred to in the pseudosequences (Table 4) and the final sequence of each strand (Table 5). The definition of the domains in the pseudosequences is given in Fig. S1, while Fig. S2 shows the strand exchange mechanism for the first quarter turn.

| Strand name         | Pseudosequence                      |
|---------------------|-------------------------------------|
| SquareB 1           | S1r,S2b,S3r,T2,conn_1               |
| SquareB 1mmwA       | S1r,S2bmmwA,S3r,T2,conn_1           |
| SquareB 2           | conn_1r,S4r,T2,S5r,T2,S6r,T2,conn_2 |
| SquareB 3           | conn_2r,S7r,T2,S8r,T2,S9r           |
| SquareA 3           | S9,A2,S8,A2,conn_3                  |
| SquareA 2           | conn_3r,S7,A2,S6,A2,S5,A2,conn_4    |
| SquareA 1           | conn_4r,S4,S3,S2a,S1                |
| SqBlockA            | thA,S2ar,S3r                        |
| SqBlockB            | S3,S2br,thB                         |
| SqUnblockA          | (SqBlockA)r                         |
| SqUnblockB          | (SqBlockB)r                         |
| Bearing-A           | CSr,T8,BDA,X1r                      |
| Spoke-A             | X2r,BDAr                           |
| Bearing-B           | CSr,T8,BD,B,X4r                     |
| Spoke-B             | X3r,BDBr                           |
| St74                | S7r,S4r                             |
| St96                | X2,T2,S9r,S6r,T2,X1                 |
| St85                | S8r,S5r                             |
| Str47               | S4,S7                               |
| Str69               | X3,T2,S6,S9,T2,X4                   |
| Str-58              | S5,S8                               |

Table 3: Pseudosequences of strands used to design the rotary motor.
| Domain name | Sequence |
|------------|----------|
| S1r | CCA CGA TGC C |
| S2b | ACC TCA TCC C |
| S2bmmwA | GTTCAA TCC C |
| S3r | GAA TGC ATG A |
| S4r | CAT GAC GTT A |
| S5r | CCA TTG GCT G |
| S6r | ATG TGA CTG G |
| S7r | GAA TCT CAC T |
| S8r | GTT CGG CTC C |
| S9r | AAG TCA GTA T |
| S1 | GGC ATC GTG G |
| S2a | ACC GAG TGG T |
| S3 | TCA TGC ATT C |
| S4 | TAA CGT CAT G |
| S5 | CAG CCA ATG G |
| S6 | CCA GTC ACA T |
| S7 | AGT GAG ATT C |
| S8 | GGA GCC GAA C |
| S9 | ATA CTG ACT T |
| thA | ACC ACC |
| thB | GAG AGA |
| BDA | TCT ACC TAT T |
| BDB | CCA GTT GCT C |
| X1 | CTA TAA CCG A |
| X2 | ATC AAA GGG T |
| X3 | AGC GAT TCA C |
| X4 | ATT ACA AAA T |
| thAr | GGT GGT |
| thBr | TCT CTC |
| BDAr | AAT AGG TAG A |
| BDBr | GAG CAA CTG G |
| X1r | TCG GTT ATA G |
| X2r | ACC CTT TGA T |
| X3r | GTG AAT CGC T |
| X4r | ATT TGT TAA T |
| conn_1 | GAG AAG AGA AGA GAA GAG AA |
| conn_2 | GCA AAA CGA AAA GCA AAA CG |
| conn_3 | GAA GAA GAA GAA GAA GAA GA |
| conn_4 | GAA AGA AAG AAA GAA AGA AA |
| conn_1r | TTC TCT TCT TTT CTC TTC TC |
| conn_2r | CGT TTT GCT TTT GTT TTC GC |
| conn_3r | TCT TCT TCT TCT TCT TC |
| conn_4r | TTT CTT TCT TTC TTT CT |

Table 4: Sequences of the individual domains of the oligonucleotides from which the rotary motor was assembled. Names refer to the pseudosequences defined previously.
| Strand name     | Sequence                                                                 |
|----------------|--------------------------------------------------------------------------|
| SquareB_1      | CCA CGA TGC CAC CTC ATC CCG AAT GCA TGA TTG AGA AGA GAA GAG AAG AGA A   |
| SquareB_1_mmwA | CCA CGA TGC CGT TCA ATC CCG AAT GCA TGA TTG AGA AGA GAA GAG AAG AGA A   |
| SquareB_2      | TTC TCT TCT TCT CTC TTC ATG ACG TTA TTC CAT TGG GTT TTA TGT GAC TGG TTG CAA A   |
| SquareB_3      | CCA CGA TGC CGT TCA ATC CCG AAT GCA TGA TTG AGA AGA GAA GAG AAG AGA A   |
| SquareA_3      | ATA CTG ACT TAA GGA GCC GAA CAA GAA GAA GAA GAA GAA GAA GAA GA          |
| SquareA_2      | TTC TCT TCT TCT TCT TCT TCA GTG AGA TTC AAC CAG TCA CAT ACG CAA TGG AAG AAA A |
| SquareA_1      | TTT CTT TCT TTT TTT CTT TCT TTC TCT TCA AAT TAT GTA ATG TCA TGC ATC ATT CAC CGA GTG GTG GCA TCG Tag |
| SqBlockA       | ACC ACC ACC ACT CGG TAG ATG CAT GA                                      |
| SqBlockB       | TCA TGC ATT CGG GAT GAG GTG AGA GA                                     |
| SqUnblockA     | TCA TGC ATT CAC CGA GTG GTG GTG GT                                      |
| SqBlockB_mmwA  | TCA TGC ATT CGG GAT TAG ATG ACG AGA GA                                  |
| SqUnblockB_mmwA| TCT CTC GTT CAA TCC CGA ATG CAT GA                                     |
| SqUnblockB     | TCT CTC ACC TCA TCC CGA ATG CAT GA                                     |
| Bearing-A      | ATG GGT GTA TGC GTG TTT TTT TTT TCT ACC ATC TTT GGT TAT AG               |
| Spoke-A        | ACC CTT TGA TAA TAG GTA GA                                             |
| Bearing-B      | ATG GGT GTA TGC GTG TTT TTT TTT TTT TTT GGT TCA GTT CAT TTT TTA AA       |
| Spoke-B        | GTG AAT CGC TGA GCA ACT GG                                             |
| St74           | GAA TCT CAC TCA TGA CGT TA                                             |
| St96           | ATC AAA GGG TTT AAG TCA GTA TAT GTG ACT GGT TCT ATA ACC GA               |
| St85           | GTG CGG CTC CCC ATT GCC TG                                              |
| Str47          | TAA CGT CAT GAG TGA CAT TC                                              |
| Str69          | AGC GAT TCA CTT CCA GTC ACA TAT ACT GAC TTT TAT TAC AAA AT              |
| Str58          | CAG CCA ATG GGG AGC CGA AC                                              |

Table 5: Sequences of the DNA oligonucleotides used to assemble and unblock the rotary motor.
Fig. S1: A detailed schematic diagram of the motor components, showing the different domains. Strand names are given in italics, domain names are given in Roman type. All staple domains except X1-4 are left unlabelled. The double-stranded spokes formed by domains whose names begin with ‘conn’ play no part in the operation of the motor. For this prototype, the tapes were constructed using multiple oligos joined together, and the connection domains are irrelevant to the rotation mechanism.
Fig. S2: Sketches showing the mechanism of strand exchange for the first quarter of a turn.
1.3.1 Analysis of motor domains using NUPACK

For data entry into NUPACK (see citation in main paper), each of the relevant domains was given a number, as listed in Table 6. Results are shown in Table 7.

| Domain name | Strand number | Target number |
|-------------|---------------|---------------|
| S1r         | 1             | 11            |
| S2bnmwa     | 2             | 3             |
| S2bnmwar    | 3             | 2             |
| S3r         | 4             | 14            |
| S4r         | 5             | 15            |
| S5r         | 6             | 16            |
| S6r         | 7             | 17            |
| S7r         | 8             | 18            |
| S8r         | 9             | 19            |
| S9r         | 10            | 20            |
| S1          | 11            | 1             |
| S2a         | 12            | 13            |
| s2Ar        | 13            | 12            |
| S3          | 14            | 4             |
| S4          | 15            | 5             |
| S5          | 16            | 6             |
| S6          | 17            | 7             |
| S7          | 18            | 8             |
| S8          | 19            | 9             |
| S9          | 20            | 10            |
| conn_1      | 21            | 25            |
| conn_2      | 22            | 26            |
| conn_3      | 23            | 27            |
| conn_4      | 24            | 28            |
| conn_1r     | 25            | 21            |
| conn_2r     | 26            | 22            |
| conn_3r     | 27            | 23            |
| conn_4r     | 28            | 24            |
| X1r         | 29            | 33            |
| X2r         | 30            | 34            |
| X3r         | 31            | 35            |
| X4r         | 32            | 36            |
| X1          | 33            | 29            |
| X2          | 34            | 30            |
| X3          | 35            | 31            |

Table 6: Numbering the domains for data entry into NUPACK.
| Complex | Description | Correct target? | Yield (%) | $\Delta G$ (kJ/mol) | $\Delta G/(k_BT)$ |
|---------|-------------|-----------------|-----------|---------------------|-----------------|
| 22-26   | Dimer       | Yes             | 100.0     | -142.9              | -58.7           |
| 21-25   | Dimer       | Yes             | 100.0     | -129.5              | -53.2           |
| 23-27   | Dimer       | Yes             | 100.0     | -128.7              | -52.8           |
| 24-28   | Dimer       | Yes             | 100.0     | -122.2              | -50.2           |
| 9-19    | Dimer       | Yes             | 99.9      | -77.1               | -31.6           |
| 1-11    | Dimer       | Yes             | 99.8      | -78.4               | -32.2           |
| 31-35   | Dimer       | Yes             | 99.5      | -72.5               | -29.8           |
| 7-17    | Dimer       | Yes             | 99.5      | -69.4               | -28.5           |
| 6-16    | Dimer       | Yes             | 99.3      | -73.1               | -30.0           |
| 5-15    | Dimer       | Yes             | 99.2      | -68.5               | -28.1           |
| 4-14    | Dimer       | Yes             | 99.1      | -66.7               | -27.4           |
| 2-3     | Dimer       | Yes             | 98.8      | -67.9               | -27.9           |
| 30-34   | Dimer       | Yes             | 98.6      | -64.5               | -26.5           |
| 29-33   | Dimer       | Yes             | 97.9      | -64.4               | -26.4           |
| 12-13   | Dimer       | Yes             | 97.4      | -78.4               | -32.2           |
| 10-20   | Dimer       | Yes             | 97.3      | -61.3               | -25.1           |
| 32-36   | Dimer       | Yes             | 81.7      | -55.0               | -22.6           |
| 32      | Monomer     | Not hybridized  | 18.3      | -1.6                | -0.6            |
| 36      | Monomer     | Not hybridized  | 18.3      | -2.2                | -0.9            |
| 20      | Monomer     | Not hybridized  | 2.7       | -0.2                | -0.1            |
| 10      | Monomer     | Not hybridized  | 2.7       | -0.2                | -0.1            |
| 12      | Monomer     | Not hybridized  | 2.6       | -9.1                | -3.7            |
| 13      | Monomer     | Not hybridized  | 2.6       | -8.1                | -3.3            |
| 8       | Monomer     | Not hybridized  | 2.1       | -1.2                | -0.5            |
| 18      | Monomer     | Not hybridized  | 2.1       | -0.9                | -0.4            |
| 29      | Monomer     | Not hybridized  | 1.4       | -0.1                | 0.0             |
| 33      | Monomer     | Not hybridized  | 1.4       | -0.1                | -0.1            |
| 34      | Monomer     | Not hybridized  | 1.2       | -1.1                | -0.5            |
| 30      | Monomer     | Not hybridized  | 1.2       | -1.9                | -0.8            |

Table 7: Results of NUPACK computation, showing the equilibrium configuration for the strands listed in Table 6 (1-36). The maximum complex size was 2 strands, the temperature was 20°C, the concentration of all strands was 1 µM and 1 M sodium was used. All structures having a concentration greater than 0.01 µM are listed in this table. The yield is defined as a percentage, representing the concentration of the product divided by the reference concentration of 1 µM. All domains hybridize strongly with their intended targets.
2 Detailed experimental procedures: QCM-D experiments

2.1 General notes

- Apparatus used: Q-sense E4 machine, Biolin Scientific.

- Sensors: Gold-coated AT-cut quartz sensors (fundamental frequency 4.95MHz +/- 50kHz), Biolin Scientific, ref. no. QSX 301.

- Sensor cleaning: Before use, sensors were cleaned as follows, in accordance with the procedure described previously (see reference in manuscript). 10 minutes in UV-ozone cleaner, 10 minutes sonication in 2% Hellmanex III, 10 minutes sonication in ultrapure water (twice), drying with N$_2$ gas, 30 minutes in UV-ozone cleaner, 30 minutes soak in 100% ethanol, drying with N$_2$ gas.

The apparatus contains four flow modules, which means that up to four experiments can be run in parallel. Liquids were driven through the QCM-D apparatus using a peristaltic pump. The depth in solution to which an acoustic wave reaches is dependent on the frequency of the wave, and higher overtones probe only the region nearest to the surface. Data is shown for the thirteenth overtone, which is the highest frequency that can be observed with this system. The experiment was performed at 16°C, with a pump flow rate of 20µL/min. After sensors had been installed in the flow modules, the system was flushed with ultrapure water, followed by the experimental buffer, which was 1M NaCl in 1xTE (Tris-EDTA, pH8). Samples were then supplied and as the molecules bound to the surface, the frequency of the crystal decreased. The sections below provide step-by-step details of the workflow for each experiment performed.
2.1.1 Sequential strand displacement in a linear construct

The complex comprising CS and Block-3 was formed by mixing equimolar quantities of the two strands in a buffer of 1×TE with 1M NaCl and incubating the sample at room temperature for a period sufficient for hybridization. The CS/Block-3 complex was at a concentration of 300 nM, and all other strands were supplied at 750 nM. Between steps the sensor was washed with buffer for 4-5 minutes; throughout the experiment the buffer used was 1×TE with 1M NaCl. In the final step strand X* was applied, where X* is the reverse complement of strand X.

2.2 Strand displacement in a geometrically constrained structure (‘Triangle’ experiment)

Samples were prepared as follows. In all cases, the buffer was 1×TE with 1M NaCl. The final concentration is given.

- CS: 300nM
- T: 750nM
- S: 750nM ac-staple, 750nM bd-staple (note that the letter S is used later to denote something else)
- Control a: 750nM
- Control b: 750nM
- Control c: 750nM
- Control d: 750nM
- Additional controls (mixture): 750nM RC(ac-staple), 750nm RC(bd-staple)
- T*: 750nM
• F(T): 750nM T, 7.5µM ac-staple, 7.5µM bd-staple, anneal in thermal cycler from 95°C to 20°C at 1°C/min.

• F(T*) 750nM T*, 7.5µM RC(ac-staple), 7.5µM RC(bd-staple), anneal in thermal cycler from 95°C to 20°C at 1°C/min.

The QCM-D experiment was performed as described above. Samples were applied to the sensors as follows, where the approximate duration of each step of the experiment is shown in brackets, in minutes.

Fig. 3(b) top panel, Fig. 3(c) left panel: Buffer (17), CS (35), buffer (5), T (18), buffer (5), S (26), buffer (7), control a (10), buffer (7), control b (10), buffer (6), control c (10), buffer (8), control d(8), buffer (25), additional control (10), buffer (5), T* (until sample ran out)

Fig. 3(b) middle panel, Fig. 3(c) middle panel: Buffer (17), CS (35), buffer (28), F(T) (26), buffer (7), control a (10), buffer (7), control b (10), buffer (6), control c (10), buffer (8), control d (8), buffer (7), additional control (18), buffer (15), T* (until sample ran out)

Fig. 3(b) bottom panel, Fig. 3(c) right panel: Buffer (17), CS (35), buffer (28), F(T) (26), buffer (7), control a (10), buffer (7), control b (10), buffer (6), control c (10), buffer (8), control d (8), buffer (40), F(T*) (until sample ran out)

2.2.1 The rotary motor

Step 1: preparation of squares

The components were made as follows, by mixing the named strands in a buffer of 1×TE with 1M NaCl, and annealing from 95°C to 20°C in a thermocycler with a cooling rate of 1°C/min. The final concentration of each strand is given in brackets.
Definitions: □ = square; B = blocked; F = folded; UF = unfolded; S = functionalized for surface immobilization.

BF□A = SquareA₁ (1 µM), SquareA₂ (1 µM), SquareA₃ (1 µM), St96 (1 µM), Spoke-A (1 µM), St74 (5 µM), St85 (5 µM), SqBlockA (5 µM)

F□A = SquareA₁ (1 µM), SquareA₂ (1 µM), SquareA₃ (1 µM), St96 (1 µM), Spoke-A (1 µM), St74 (5 µM), St85 (5 µM)

BF□B = SquareB₁㎜₇₆ (1 µM), SquareB₂ (1 µM), SquareB₃ (1 µM), Str69 (1 µM), Spoke-B (1 µM), Str47 (5 µM), Str58 (5 µM), SqBlockBⅠ㎜₇₆ (5 µM)

F□B = SquareB₁㎜₇₆ (1 µM), SquareB₂ (1 µM), SquareB₃ (1 µM), Str69 (1 µM), Spoke-B (1 µM), Str47 (5 µM), Str58 (5 µM)

UF□B = SquareB₁㎜₇₆ (1 µM), SquareB₂ (1 µM), SquareB₃ (1 µM)

Step 2: preparation of surface attachment units

The following samples were prepared in the same buffer, and incubated at room temperature for approximately 30 minutes to allow the strands to hybridize.

T1 = 1:1 mixture of CS and Bearing-A, final concentration 25 µM
T2 = 1:1 mixture of CS and Bearing-B, final concentration 25 µM

Step 3: combination of squares and surface attachment units

Samples from step 1 were combined with samples from step 2 to form squares that could be immobilized on the gold surface, as follows. Mixtures were incubated at room temperature for a period sufficient to allow hybridization.

SBF□A = 1:25 (by volume), T1 : BF□A.

SF□A = 1:25 (by volume), T1 : F□A. (Like SBF□A, but without blocking strand.)

SBF□B = 1:25 (by volume), T2 : BF□B.

Note that 8 consecutive thymine nucleotides are left unpaired in the surface
attachment unit, to provide a flexible linker region that enables rotation to occur. If the linker were rigid, no rotation would be possible.

**Step 4: mixing with MCH** If the density of motors on the surface is too high, operation is impossible because they interact and/or collide. To avoid this, the samples from Step 3 were mixed with MCH (6-mercapto-1-hexanol), a thiolated molecule that will be immobilized at the same time as the motors. The original concentration of MCH was 1mM. In all cases, the final buffer was 1×TE with 1M NaCl.

- Sample 1 = 1:1 (by volume), SFB□A : MCH.
- Sample 2 = 2:2:1 (by volume), SFB□A : SFB□B : MCH.
- Sample 3 = As sample 1.
- Sample 4 = 1:1 (by volume), SFB□A : MCH.

Sufficient time was allowed for the two halves of the motor to hybridize in sample 2.

**Step 5: QCM-D experiments** Freshly cleaned sensors were installed in the flow modules, which were then flushed with ultrapure water, followed by the experimental buffer, which was 1M NaCl/TE. The samples listed above were supplied, where sample X was used for flow module X. When a plateau was reached, the sensors were rinsed with buffer. The following solutions were then applied to the sensors:

- Sensor 1: UF□B from Step 1, followed by buffer
- Sensor 2 (consecutively): UnblockA (1µM), buffer (short rinse step), UnblockB□mmwA
- Sensor 3: F□B from Step 1, followed by buffer
- Sensor 4 (consecutively): UnblockA (1µM), buffer (short rinse step), UF□B from Step 1

In Fig. 4 of the paper, data is presented in this order: Sensor 1, 4, 3, 2.
2.3 Agarose gel electrophoresis: rotary motor, Fig. 4(c)

The samples were prepared as follows.

**Step 1**

The following mixture was incubated at room temperature for 30 mins in 1×TE with 1M NaCl. The final concentration of each strand is given in brackets.

- SA = mixture of CS (1µM), Spoke-A (1µM) and Bearing-A (1µM)

**Step 2**

The following mixtures were annealed from 95°C to 20°C in a thermocycler with a cooling rate of 1°C/min.

- F□A! = SquareA_1 (1µM), SquareA_2 (1µM), SquareA_3 (1µM), St96 (1µM), St74 (5µM), St85 (5µM)
- F□B! = SquareB_1 (1µM), SquareB_2 (1µM), SquareB_3 (1µM), Str69 (1µM), Str47 (5µM), Str58 (5µM)
- UF□B! = SquareB_1 (2.5µM), SquareB_2 (2.5µM), SquareB_3 (2.5µM)

○ stands for original. The ! is used to distinguish the samples from those prepared for QCM-D, where the Spoke strands were added at this stage and SquareB_1mmwA was used in place of SquareB_1.

Extracts from these samples were prepared for gel electrophoresis and the gel was loaded as follows:

1. S
2. F□A!
3. F□B!
4. UF□B!
2.4 Polyacrylamide gel electrophoresis: rotary motor, Fig. 4(d)

Step 1

The following mixtures were annealed from 95°C to 20°C in a thermocycler with a cooling rate of 1°C/min. Unfolded square B, lane 5 - 50µL 2M NaCl/2xTE; 47µL ultrapure MilliQ water; 1µL of 100µM stock solution of each of: SquareB_1_mnwA, SquareB_2, SquareB_3.

Folded square B, lane 6 - 50µL 2M NaCl/2xTE; 31µL ultrapure MilliQ water; 1µL of 100µM stock solution of each of: SquareB_1_mnwA, SquareB_2, SquareB_3, Str69; 5µL of 100µM stock solution of each of: Str47, Str58, SqBlockB_mnwA.

Folded square A, lane 7 - 50µL 2M NaCl/2xTE; 31µL ultrapure MilliQ water; 1µL of 100µM stock solution of each of: SquareA_1, SquareA_2, SquareA_3, St96; 5µL of 100µM stock solution of each of: St74, St85, SqBlockA.

Step 2

Motor ‘before’: 20µL of folded square A was mixed with 20µL of folded square B, and the sample was incubated at room temperature for over 30 minutes.

Step 3

Motor ‘after’: A 20µL sample of the motor was extracted and the unblocking strands were added to a final concentration of approximately 4.5µM each. The sample was left at room temperature for over 30 minutes.

Step 4

Glycerol was added to a final concentration of approximately 12-13% and the gel was run as described in the Methods section of the main paper.