Supporting Information

Fabrication of Circular Assemblies with DNA Tetrahedrons: From Static Structures to a Dynamic Rotary Motor

Liying Wang[1], Zhenyu Meng[1], Felicia Martina[1], Huilin Shao[2,3], Fangwei Shao[1,*]

[1] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 637371, Singapore.
[2] Biomedical Institute of Global Heath Research and Technology, Departments of Biomedical Engineering and Surgery, National University of Singapore.
[3]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore

Table S1 DNA sequences used in the whole experiment.

Strand Name	Sequence
S1	5'-CGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACCAG GCAGTTGAG -3'
S2	5'-TTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAGCT TGCTACACG -3'
S4	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCG-3'
S2-O	5'-TTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAGCT TGCTACACGATAAGCTACGATCATAG -3'
S2-O'	5'-TTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAGCT TGCTACACGATCTATGATCGTAGCTT -3'
S1-t1	5'-Texas red-GCTGTACTTTGGTTACTGAACGAACATTCCTAAGTCTGAAATTTATC ACCCGCCATAGTAGACGTATCACCAGGCAGTTGAG -3'
S1-t2	5'-CGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACCAG GCAGTTGAGACTTGTATGGGACGTAGCGT-FAM-3'
S1-t3	5'- Cy5-TCCACATCGGACTCTGTATACGAACATTCCTAAGTCTGAAATTTATCACCC GCCATAGTAGACGTATCACCAGGCAGTTGAG -3'
S1-t4	5'-CGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACCAG GCAGTTGAGCTTCATGGCATTCGTCACCA -HEX-3'
S3-O_1	5'-CTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGC ATGCCCATCCATATACTAGACGTTACT -3'
S3-O_1'	5'-CTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGC ATGCCCATCCATAGTAACGTCTAGTAT -3'
S4-O_1	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCGATATACTAGACGTTACT -3'
S4-O_1'	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCGATAGTAACGTCTAGTAT -3'
S4-O_2	5'-GGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCT CTTCCCGATTAAGTATTGCTACAA -3'
S4-O_2'	5'-GGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCT CTTCCCGATTTGTAGCAATACTTA -3'
S3-O_M	5'-CTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGC ATGCCCATCCATCTAGACCGGATTACT -3'
S3-O_M'	5'-CTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGC ATGCCCATCCATAGTAATCCGGTCTAG -3'
S4-O_M	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCG ATCTAGACCGGATTACT -3'
S4-O_M'	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCGATAGTAATCCGGTCTAG -3'

Strand Name	Sequence
S4-O_B	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCG ATAGTATCATGACGTGT -3'
S4-$O_{B'}$	5'-CGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATG CTCTTCCCGATACACGTCATGATACT -3'
R1	5'-GGCTGGTTTCTGCTCTCTAGTTTTTTTTTTTTCGCGAGGTGCAATCTCCTATC-BHW2-3'
R2	5'-BHQ1-GTCTGGGATGCTGGATACTTTTTTTTTTTGAACCTAGAGAGCAGAAA CCAGCC -3'
F1	AGTAACCAAAGTACAGCACTGCGATAGGAGATTGCACCTCCAATTTACCC
F2	GACTGTTACGGTATCCAGCATCCCAGACGGTCAACGCTACGTCCCATACA
F3	ACAGAGTCCGATGTGGAAGTCAGATAGGAGATTGCACCTCATCATTGTCG
F4	GGATCAGTTAGTATCCAGCATCCCAGACCTAAGTGGTGACGAATGCCATG
A1	GGGTAAATTGGAGGTGCAATCTCCTATCGCAGTGCTGTACTTTGGTTACT
A2	TGTATGGGACGTAGCGTTGACCGTCTGGGATGCTGGATACCGTAACAGTC
A3	CGACAATGATGAGGTGCAATCTCCTATCTGACTTCCACATCGGACTCTGT
A4	CATGGCATTCGTCACCACTTAGGTCTGGGATGCTGGATACTAACTGATCC

The sequence with underline is recognizable by endonuclease.

Table S2. DNA strands used in different tetrahedron assembly.

Tetrahedron Name	Strands
T_{11}	S1, S2, S3-O_1, S4-O_1
$T_{1'1'}$	S1, S2, S3-$O_{1'}$, S4-$O_{1'}$
T_{12}	S1, S2, S3-O_1, S4-O_2
$T_{1'2}$	S1, S2, S3-$O_{1'}$, S4-O_2
$T_{12'}$	S1, S2, S3-O_1, S4-$O_{2'}$
$T_{1'2'}$	S1, S2, S3-$O_{1'}$, S4-$O_{2'}$
T_{MB}	S1, S2, S3-O_M, S4-O_B
$T_{MB'}$	S1, S2, S3-O_M, S4-$O_{B'}$
$T_{M'B}$	S1, S2, S3-$O_{M'}$, S4-O_B
$T_{M'B'}$	S1, S2, S3-$O_{M'}$, S4-$O_{B'}$
$T_{M'M'}$	S1, S2, S3-$O_{M'}$, S4-$O_{M'}$
T_2-O'	S1, S2-O', S3, S4-O_2
$T_{2'}$-O'	S1, S2-O', S3, S4-$O_{2'}$
$T_{1'2}$-O	S1, S2-O, S3-$O_{1'}$, S4-O_2
$T_{1'2'}$-O	S1, S2-O, S3-$O_{1'}$, S4-$O_{2'}$
$T_{1'2}$-O'	S1, S2-O', S3-$O_{1'}$, S4-O_2
$T_{1'2'}$-O'	S1, S2-O', S3-$O_{1'}$, S4-$O_{2'}$
T_{12}-t1	S1-t1, S2, S3-O_1, S4-O_2
$T_{M'2'}$-t2	S1-t2, S2, S3-$O_{M'}$, S4-$O_{2'}$
T_{MB}-t3	S1-t3, S2, S3-O_M, S4-O_B
$T_{1'B'}$-t4	S1-t4, S2, S3-$O_{1'}$, S4-$O_{B'}$

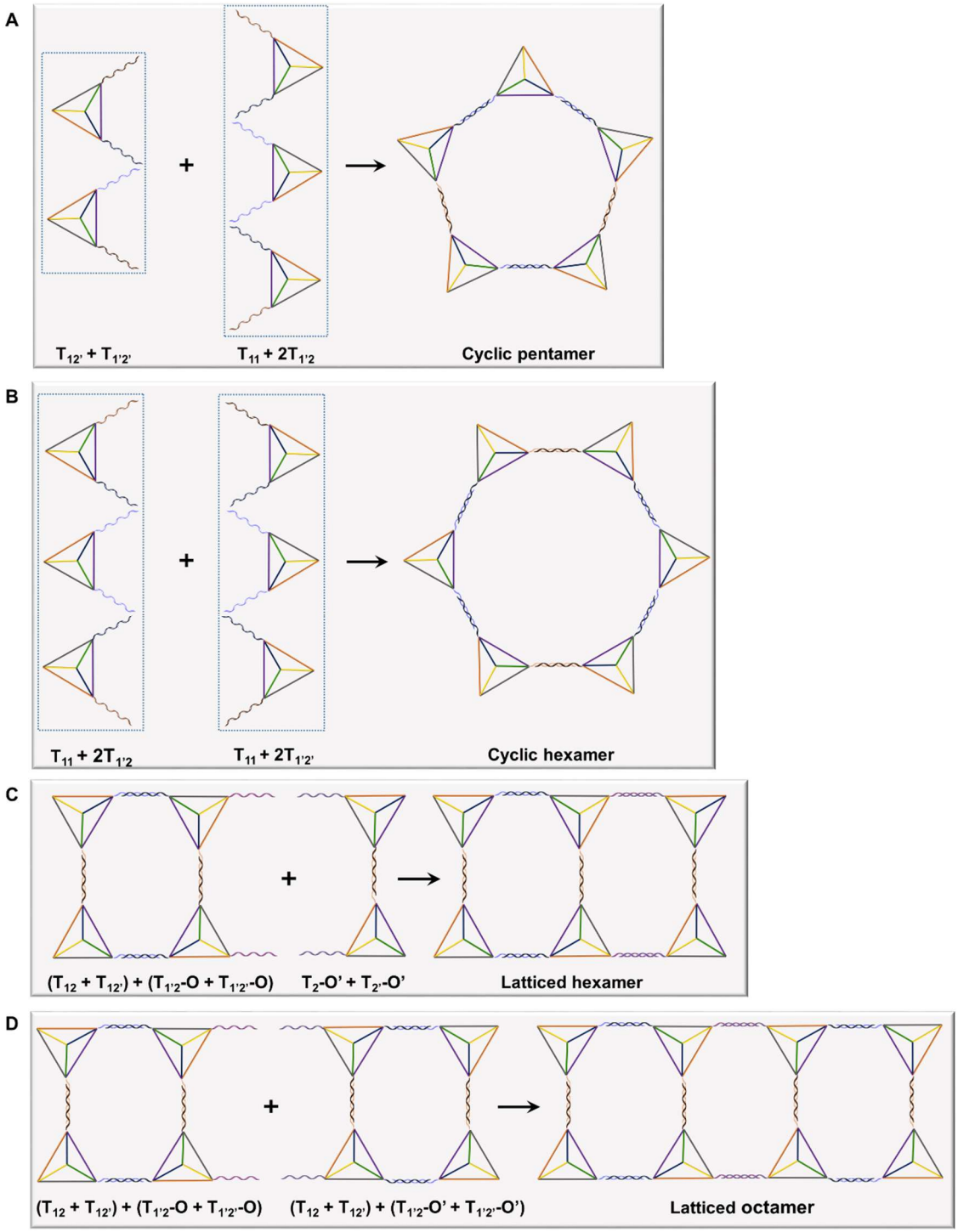

Fig. S1 The schematic illustration of the design of cyclic pentamer (A), cyclic hexamer (B) and lattice hexamer (C) and lattice octamer (D) with the DNA tetrahedron as the building blocks.

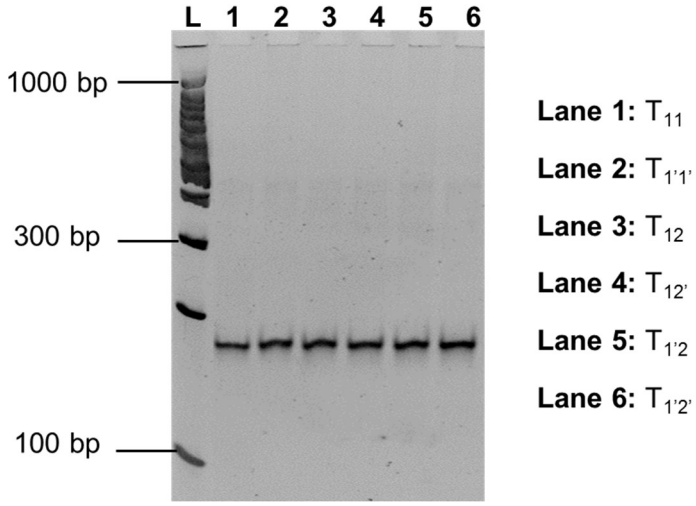

Fig. S2 5% PAGE analysis of the tetrahedron monomers. The PAGE was run in 1×TBE buffer (pH 8.3) with 2.5 mM $MgCl_2$ at 4 °C.

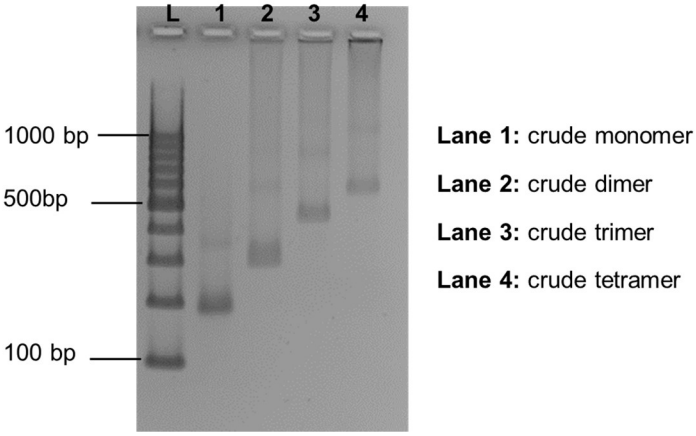

Fig. S3 2.5% agarose gel electrophoresis of the crude products assembled from DNA tetrahedron. The gel was run in 0.5×TBE buffer (pH 8.3) at 4 °C.

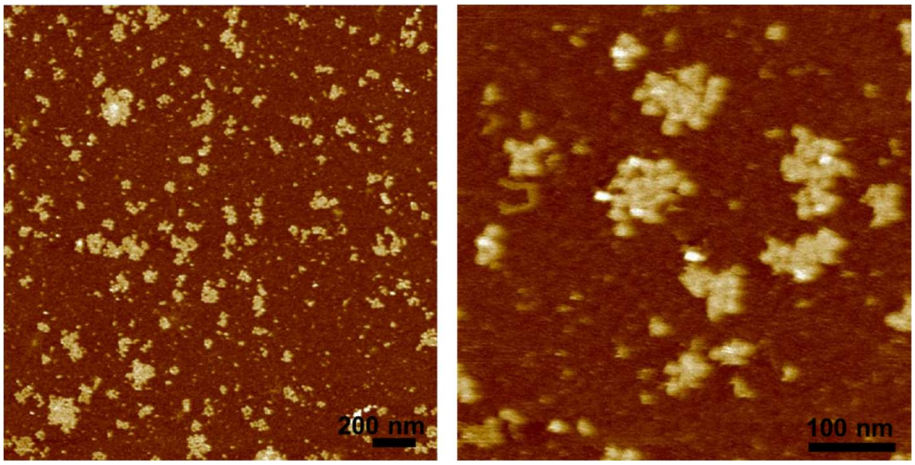

Fig. S4 AFM images of the byproduct in cyclic tetramer.

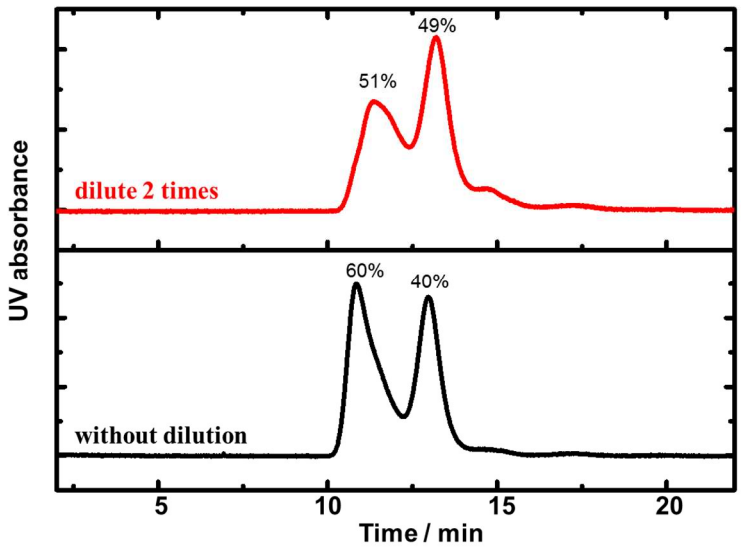

Fig. S5 Yield calculation of cyclic tetramer by SEC after diluting the dimer solutions for 2 times.

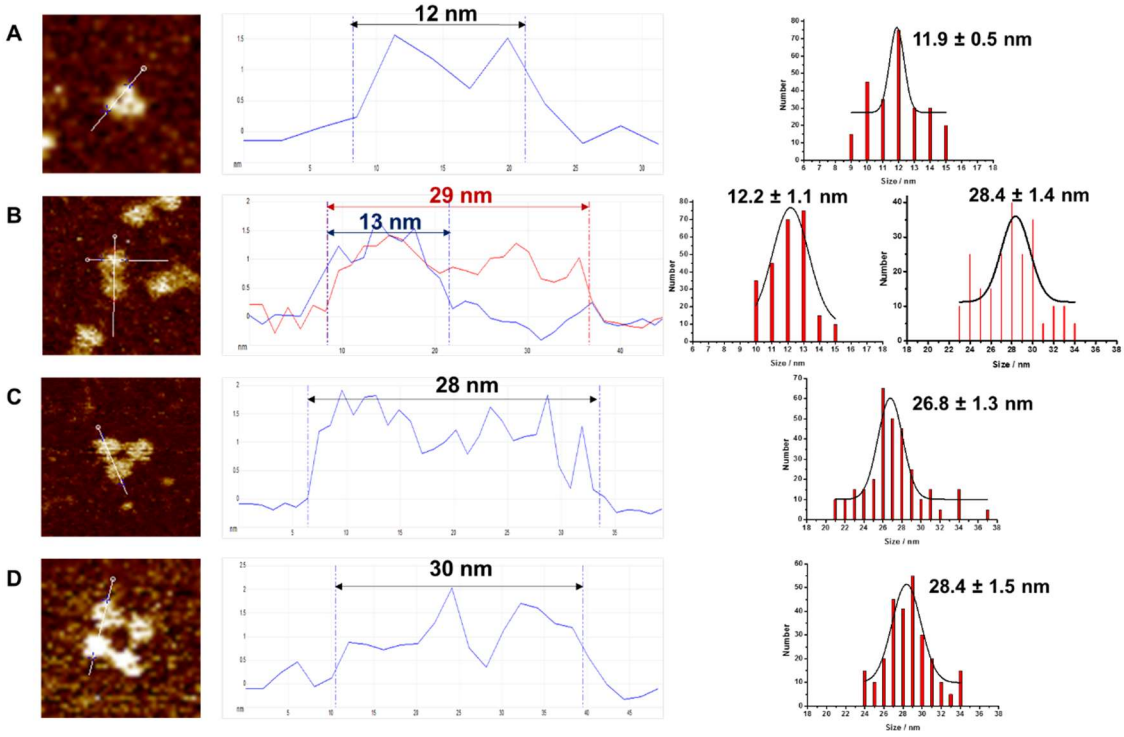

Fig. S6 Edge length measurement of the tetrahedron monomer (A), dimer (B), trimer (C) and tetramer (D) by AFM. The length distribution was obtained by measuring at least 250 oligomers.

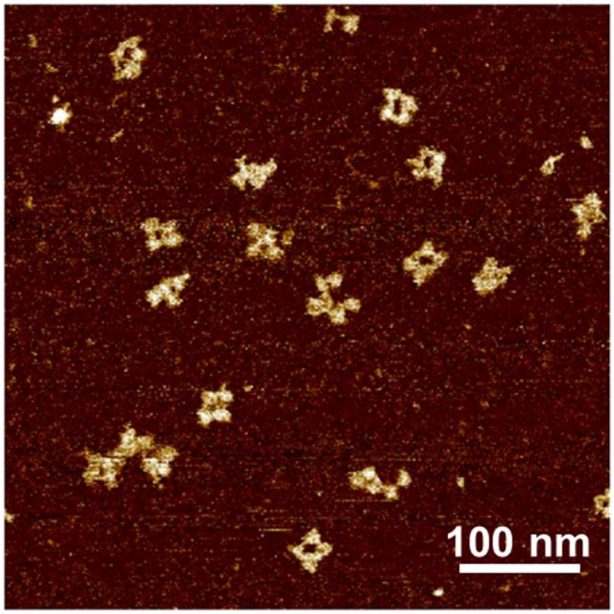

Fig. S7 AFM image of the cyclic stator.

Although the real-time fluorescent intensity can sufficiently confirm the locomotion of the DNA motor, some detailed issues need further explanation. (1) The fluorescent intensity of FAM shows a relevancy with the addition of F4 and A4. This is due to the fluorescence resonance energy transfer (FRET) between the FAM and HEX. Here, FAM is the fluorescent donor, and HEX is the acceptor. When F4 was added, the HEX fluorescence was quenched, so the energy transfer from FAM to HEX was blocked. Therefore, we observed a slight increase of the FAM fluorescence. The fluorescence of HEX recovered when A4 was added, and the FRET between FAM and HEX happened again. Hence, we observe a decrease of the FAM fluorescence. This explanation was also proved in our control experiment. The control motor was run similarly by successively addition of fuel strands and anti-fuel strands, but HEX was not labeled on t4. The real-time fluorescent monitoring is shown in Fig. S8. The addition of F4 and A4 will not influence the fluorescence intensity of FAM obviously. Another proof of FRET between FAM and HEX can be seen in the fluorescent emission spectrum as shown in Fig. S9. When excited at 490 nm, we can see that the fluorescence emission ratio at 551 nm and 520 nm is only 0.30:1 in the controlled motor system without HEX labeling (blue curve). While in case of the HEX labeled motor system, the ratio is 0.82:1, which is much higher than the ratio of 0.52:1 in the mixture of $T_{M'2'}$-t2 (FAM labeled) and $T_{1'B'}$-t4 (HEX labeled) (There should be no FRET between $T_{M'2'}$-t2 and $T_{1'B'}$-t4 because there is no specific interaction between the two tetrahedron). The difference on the emission ratio here indicates that there is a FRET between FAM and HEX in the cyclic tetramer structure. (2) The fluorescent intensity of Cy5 keeps decreasing due to the low stability of Cy5 under long time excitation (photo bleaching effect). Even though, it is still enough to verify the movement of the rotor because the tendency of the signal change can be observed clearly during the detecting period.

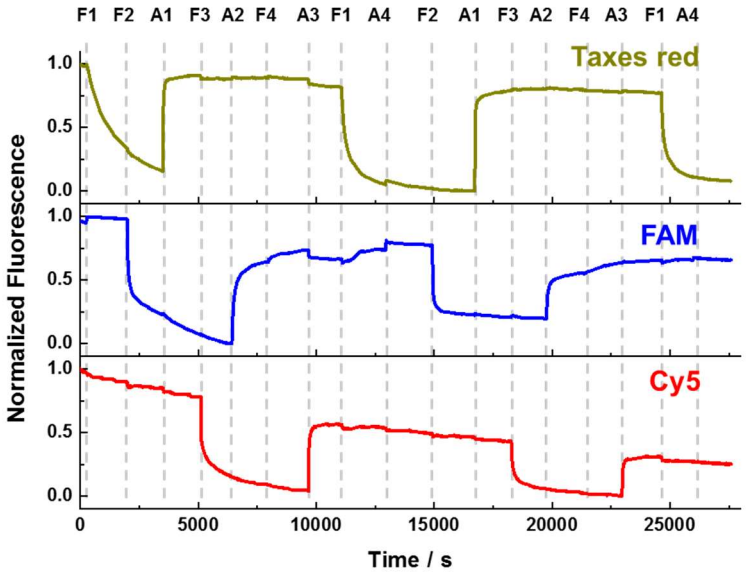

Fig. S8 The real-time fluorescent monitoring at three different channels (without HEX) in the control experiment of the motor system.

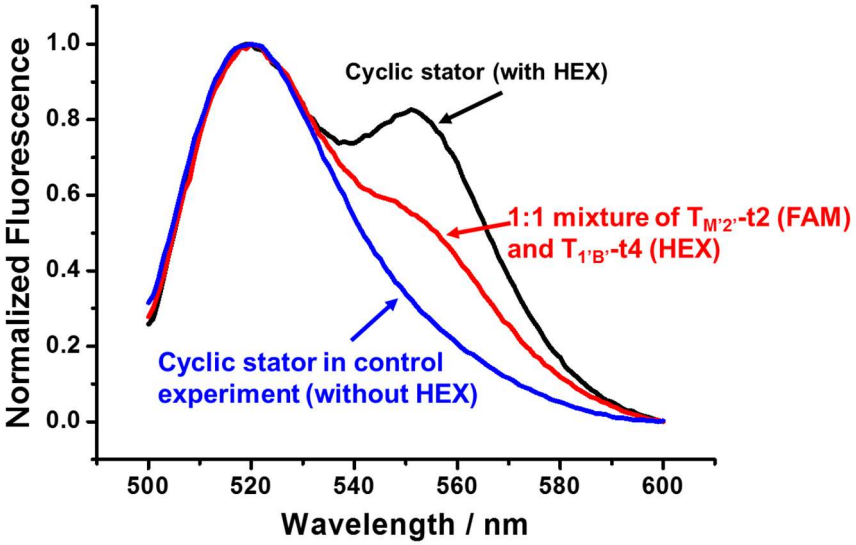

Fig. S9 The normalized fluorescent emission spectrum of FAM and HEX in the motor system under the excitation wavelength of 490 nm.