A DNA Nanotransport Device Powered by Polymerase $\phi 29$: Supporting Information

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A-1 Experimental Details

DNA sequences for the polymerase motor, denoted as T, W, BP, and BQ were designed and optimized with the SEQUIN software. T is a $97mer$, W is a $50mer$, BP is a $25mer$, and BQ is 35 bases long. In the strand T and W, the 5' end is phosphorylated. Table 1 shows the various DNA sequences used. DNA strands were synthesized commercially by Integrated DNA Technologies, Coralville, IA, and purified by denaturing gel electrophoresis. DNA stock solutions were prepared at a concentration of 30μM in ultra pure water. Concentrations of DNA strands were determined from the measurement of ultraviolet absorbance at 260 nm.

TAE/Mg buffer (1X buffer: 40mM Tris acetate, 1mM EDTA, 12.5 mM Mg acetate, pH 8.3) was used for reactions including annealing, formation of native gels, and running native gels.

TBE buffer (1X Buffer: 89mM Tris-base, 89mM Boric Acid, 2mM EDTA(disodium), pH 8.3) was used for preparation of denaturing gels and running denaturing gels.

T4 DNA ligase from New England Biolabs was used for ligation. It was provided with T4 ligase buffer (1X buffer: 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM Dithiothreitol, pH 7.5 @ $25°C$). Ligation was performed by incubating the sample with ligase and ligase buffer at $16°C$, and ligase was deactivated by heating the sample at $65°C$.

$\phi 29$ polymerase from New England biolabs was used to power our nanotransportation device. It was provided with $\phi 29$ polymerase buffer (1X buffer: 50 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 4 mM Dithiothreitol, pH 7.5 @ $25°C$). Polymerase reaction was performed at $30°$ C, in presence of $\phi 29$ polymerase, 1X $\phi 29$ polymerase buffer, $200\mu M$ dNTPs and $(200\mu g/ml)$ BSA (*Bovine Serum Albumin*). The mixture was then heated to $65°C$ to deactivate the polymerase.

Taq polymerase from Invitrogen Inc. was used in comparative studies of braking the nanotransportation device. In addition to, 1X Taq polymerase buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.3 @ $25°C$), $MgCl_2$ at $1.5mM$, dNTPs at $200\mu M$ and Taq polymerase at 25 units/ml are used for DNA replication. The polymerase reactions with Taq polymerase were carried out at $75°C$, and there was no heat inactivation.

Denaturing polyacrylamide gel electrophoresis (PAGE) as well as non-denaturing polyacrylamide gels (acrylamide-bis 19:1) was used to analyze the reaction mixture and verify the products formed. The conformation of the nanotransportation system was estimated from the size of various participating structures. For denaturing gel electrophoresis, the mixture was heated at $90°C$ for 10 minutes, and then added to denaturing polyacrylamide gel. For imaging, denaturing as well as native gels were stained with $0.5\mu l/ml$ of Ethidium Bromide (EB) solution (from Apex Bioresearch) in 200 ml distilled water for 20-25 minutes. The gel was then viewed under UV transillumination, and images were acquired using an Alpha Imager (Alpha Innotech, San Leonardo, CA). 50 and 100 bp ladders from New England Biolabs were used as references during gel electrophoresis.

The fluorophore we use is Internal Cy5 with absorbance peak at 648 nm and emission peak at 668 nm, and the quencher is Iowa black RQ with range of 500-700 nm with peak at 667 nm for the 3' end and 656nm for the 5' end. The sequences required for the 3 experiments outlined above are given in the Table 1, and the experimental designs are illustrated in Figure 10. In the subsequent subsections, we describe these 3 individual experiments. In each of the experiments, the fluorophores are excited by wavelength 648 nm, and the subsequent emission over all wavelengths (upto approximately 800 nm) has been measured.

symbol	sequence
T	/5Phos/AAT CAC CAT AGT GCA ACC TGA AAA AAA AAA AAA AAT GTG CCT CTG TTC TGC TCG CTT GCT GCG TTG GCT GTC GTG TCC TTG TTA CTA AGA TGC TTA C
W	/5Phos/AGC GAG CAG AAA AAA AAA AAA AAA AAA AAA AAA AAA CCA ACG CAG CA
BQ	CAG AGG CAC ATT TTT TTT TTT TTT TCA GGT TGC AC
BP	TAT GGT GAT TGT AAG CAT CTT AGT A
PM3.T	/5Phos/AAT CAC CAT AGT GCA ACC TGA AAA AAA AAA AAA AAT GTG CCT CTG TTC TGC TCG CTT GCT GCG TTG GCT GTC GTG TCC TTG TTA CTA AGA TGC TTA C
PM3.W	/5Phos/ AGC GAG CAG AAT GCA GTC ACA CTG AGA TCG AGA CT/iCy5/T GTA CCA ACG CAG CA
PM3.Cargo	/5IAbRQ/AGT CTC GAT CTC AGT GTG ACC AGG TTG CAC
PM3.BQ	CAG AGG CAC ATT TTT TTT TTT TTT T
PM3.BP	TAT GGT GAT TGT AAG CAT CTT AGT A
PM2.Track	/5Phos/ T GTG CCT CTG TTC TGC TCG CTT GCT GCG TTG G /iCy5/CT GTC GTG TCC TTG TTA CTA AGA TGC TTA CAAT CAC CAT AGT GCA ACC TGA AAA AAA AAA AAA AA
PM2.Wheel	/5Phos/ AGC GAG CAG AAT GCA GTC ACA CTG AGA TCG AGA CTT GTA CCA ACG CAG CA
PM2.Cargo	/5IAbRQ/ TACA AGT CTC GAT CTC AGT GTG ACC AGG TTG CAC
PM2.BQ	CAG AGG CAC ATT TTT TTT TTT TTT T
PM2.BP	TAT GGT GAT TGT AAG CAT CTT AGT A
PM1.T	/5Phos/AAT CAC CAT A/iCy5/GT GCA ACC TGA AAA AAA AAA AAA AAT GTG CCT CTG TTC TGC TCG CTT GCT GCG TTG GCT GTC GTG TCC TTG TTA CTA AGA TGC TTA C
PM1.W	/5Phos/ AGC GAG CAG AAT GCA GTC ACA CTG AGA TCG AGA CTT GTA CCA ACG CAG CA
PM1.Cargo	AGT CTC GAT CTC AGT GTG ACC AGG TTG CAC/3IAbRQSp/
PM1.BQ	CAG AGG CAC ATT TTT TTT TTT TTT T
PM1.BP	TAT GGT GAT TGT AAG CAT CTT AGT A

Table 1. DNA sequences for the demonstration of polymerase based nanotransportation device

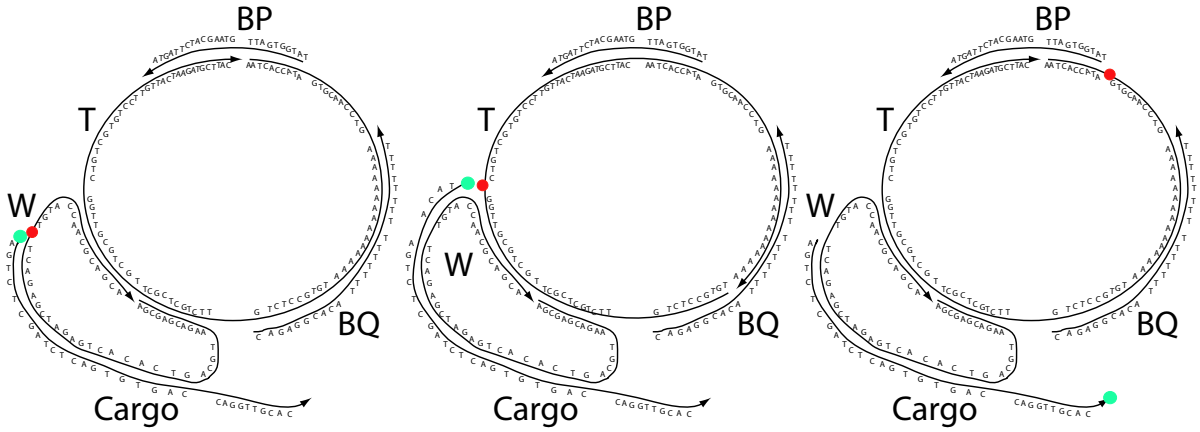

Fig. 10. Shows the detailed sequence design for the fluorescence experiment