Supporting Information for

Processive Incorporation of Deoxynucleoside Triphosphate Analogs by Single-Molecule DNA Polymerase I (Klenow Fragment) Nanocircuits

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1. Materials

Reagents purchased commercially include antibiotics (Fisher Scientific), Ni-IMAC resin (Bio-Rad Laboratories), cell lines (Stratagene), deoxynucleoside triphosphates (Fisher Scientific), deoxynucleoside triphosphate analogs (Trilink Biotechnologies), enzymes (New England Biolabs or Fermentas), oligonucleotides (Fisher), high-resolution agarose (The Nest Group) and 96-well fluorescence plates (Nunc). All other chemicals were purchased commercially from Acros Organics, EMD, Fisher Scientific, or Sigma Aldrich. All reagents were used as received.

2. Expression and purification of KF

The following expression and purification protocol applied buffers and plasmids from a previously described procedure.[1] A pET28c plasmid containing a gene encoding KF(D355A/E357A/C907S/L790C),[1,2] referred to hereafter as KF, was used to transform $CaCl_2$-competent BL21(DE3) *E. coli* cells by heat shock. Following overnight growth on solid media, a single colony was used to inoculate 25 mL LB media supplemented with 40 μg/mL kanamycin for growth in liquid media overnight at 37 °C with shaking. LB (1 L) supplemented with 40 μg/mL kanamycin was inoculated with 10 mL of the overnight culture and incubated with shaking at 37 °C for several hours. Once the cells reached late log phage (OD_{600} = 0.9), KF expression was induced by the addition of 1 mM IPTG. After 3-4 h of protein expression at 37 °C with shaking, cells were harvested by centrifugation (6000 rpm, 20 min, 4 °C) and resuspended in lysis buffer (20 mM Tris, 50 mM NaCl, 10 mM BME, pH 8.0). Cells were lysed by sonication and the cell debris was collected by centrifugation (15,000 rpm, 45 min, 4 °C). Following filtration through a 0.45 μm pore filter, the lysate supernatant was allowed to bind to Ni-IMAC resin overnight at 4 °C. KF was eluted in the lysis buffer with 250 mM imidazole, concentrated, and then treated with TEV protease for two days at 4 °C. The mixture was centrifuged then filtered through a 0.45 μm filter prior to size exclusion chromatography in TBS (20 mM Tris, 50 mM NaCl, 100 μM TCEP, pH 7.9) on a Bio-Rad Biologic DuoFlow FPLC. KF purity was assessed by SDS-PAGE (Figure S1).

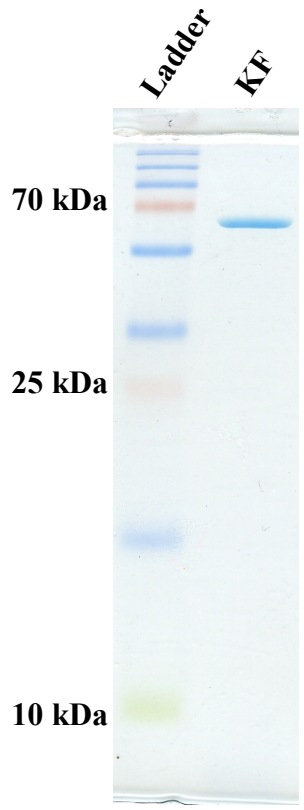

Figure S1. Representative 15% SDS-PAGE gel of KF after over-expression and purification. KF was purified to >95% homogeneity and migrated at its expected mass of ≈68 kDa.

3. Ensemble activity of KF and dNTP analog incorporation

3A. Oligonucleotides used to test activity

The following table lists the oligonucleotides used to test KF activity, dNTP analog incorporation, and for measurements with the nanocircuit. Upon receipt, HPLC-purified oligonucleotides were solubilized in water to 100 μM. Bold regions indicate the M13 priming site. Italicized regions indicate restriction sites. [2AmPur] indicates 2-aminopurine.

Table S1. Oligonucleotides used for activity and electronic measurements

Oligonucleotide	**Sequence**	**Use**
M13F	TGTAAAACGACGGCCAGT	M13 primer
ActAssay Template	TCGAGCTATCTCTAAAGC[2AmPur] GCTAACTATCGAGCTATCGCGAAA **CTGGCCGTCGTTTTACA**	Standard activity assay template containing 2-aminopurine
A/T Incorporation Assay Template	CCTAACGCAGATAGACGTTGTTTA GAGCCCGGGTCGGCCAT**ACTGGC CGTCGTTTTACA**	Test incorporation of dATP or dTTP analogs in Figure S3a

Oligonucleotide	Sequence	Use
G/C Incorporation Assay Template	CCTAACGCAGATAGACGTTGTTTA GAGATTTAAATTCGGCC**ACTGGC CGTCGTTTTACA**	Test incorporation of dCTP or dGTP analogs in Figure S3b
poly(dA)$_{42}$	(A)$_{42}$**ACTGGCCGTCGTTTTACA**	Test native and analog dTTP incorporation on nanocircuit
poly(dT)$_{42}$	(T)$_{42}$**ACTGGCCGTCGTTTTACA**	Test native and analog dATP incorporation on nanocircuit
poly(dG)$_{42}$	(G)$_{42}$**ACTGGCCGTCGTTTTACA**	Test native and analog dCTP incorporation on nanocircuit
poly(dC)$_{42}$	(C)$_{42}$**ACTGGCCGTCGTTTTACA**	Test native and analog dGTP incorporation on nanocircuit

3B. Ensemble assay for KF Activity

To confirm activity of KF(L790C) versus wild-type KF, a previously described assay was adapted as follows.[1,3] A randomized DNA template containing both 2-aminopurine (ActAssay template in Table S1) and an M13 priming site (underlined) was annealed to the M13F primer by heating the mixture to 65 °C and slow-cooling to room temperature for 1 h. A comparable decrease in fluorescence was observed for KF(L790C) and wild-type KF (both 1 μM) upon incubation with the primer-template mixture (25 μM) and dNTPs (250 μM). The raw fluorescence data was corrected by subtraction of background, which was measured in the absence of dNTPs. The excitation and emission wavelengths employed in this experiment were 305 and 365 nm, respectively.

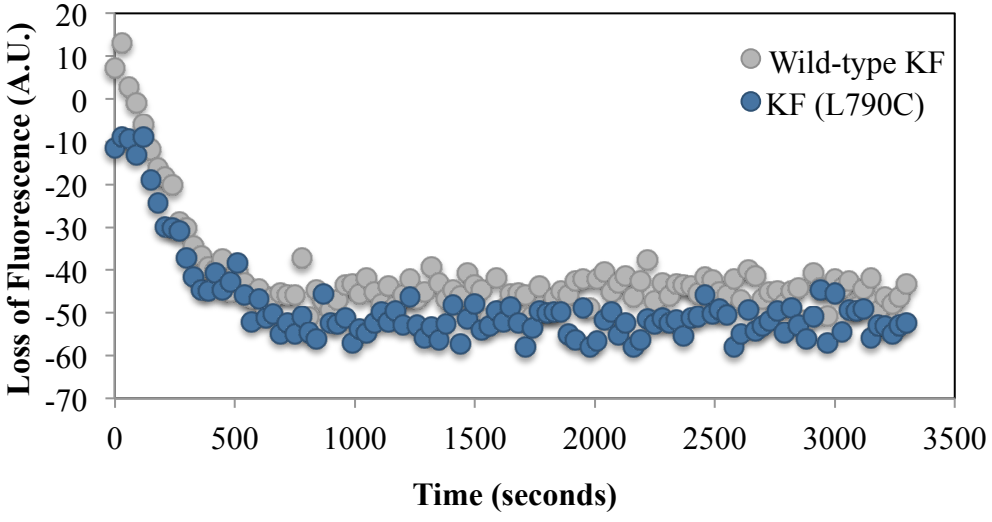

Figure S2. Fluorescence-based activity assay depicting KF(L790C) and wild-type KF activity under steady-state conditions. The primer extension reaction occurs in the presence of dATP, dTTP, dCTP, and dGTP. The raw data was subtracted from background, which measured activity in the absence of dNTPs.

3C. Ensemble assay for dNTP analog incorporation

To confirm incorporation of dNTP analogs, randomized DNA templates (Table S1) were polymerized by KF after hybridization to an M13F primer. Positive control reactions contained KF (1 μM), dNTPs or dNTP analogs (100 μM), and A/T or G/C incorporation template-primer (5 μM) in 10 mM Tris, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9. Reactions to test dNTP analog incorporation contained 100 μM analog in place of its native dNTP, and negative control reactions omitted either the analog or its native dNTP. Reactions were kept at 25 °C for 2 h in a thermal cycler before electrophoresis on a 5% high resolution agarose gel.

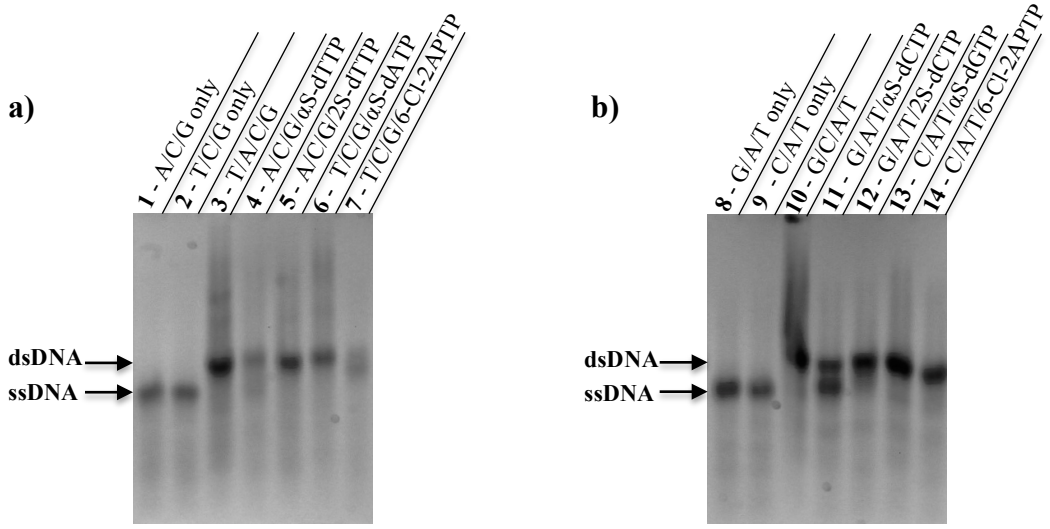

Figure S3. Ensemble assay showing incorporation of dNTP analogs with the templates described in Table 1. Polymerization products with dNTP analogs and the A/T incorporation template (**a**) or the G/C incorporation template (**b**) were electrophoresed on a 5% high-resolution agarose gel. Negative control reactions with only 3 dNTPs, omitting dTTP (**1**), dATP (**2**), dCTP (**8**), and dGTP (**9**), contained no dsDNA. Positive control reactions with all four dNTPs showed conversion to dsDNA with both the A/T incorporation template (**3**) and the G/C incorporation template (**10**). Reactions with dNTP analogs (**4**-**7** and **11**-**14**) omitted their native dNTP counterpart and contained the remaining 3 native dNTPs. Opposite the A/T incorporation template, α-thio-dTTP (**4**) and 2-thio-dTTP (**5**) incorporated opposite the template base A, and α-thio-dATP (**6**) and 6-Cl-2APTP (**7**) incorporated opposite the template base T. Opposite the G/C incorporation template, α-thio-dCTP (**11**) and 2-thio-dCTP (**12**) incorporated opposite the template base G, and α-thio-dGTP (**13**) and 6-Cl-2APTP (**14**) incorporated opposite the template base C. After visualization, the image colors were inverted, then changed to black and white.

References

(1) Olsen, T. J.; Choi, Y.; Sims, P. C.; Gul, O. T.; Corso, B. L.; Dong, C.; Brown, W. A.; Collins, P. G.; Weiss, G. A. *J. Am. Chem. Soc.* **2013**, *135*, 7855.

(2) Derbyshire, V.; Freemont, P.; Sanderson, M.; Beese, L.; Friedman, J.; Joyce, C.; Steitz, T. *Science* **1988**, *240*, 199.

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