Binary and ternary binding affinities between exonuclease-deficient Klenow fragment (Kf-exo$^-$) and various arylamine DNA lesions characterized by surface plasmon resonance

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Experimental Procedures

Materials and Methods

Crude oligodeoxynucleotides (1 μmol) in desalted form were purchased from Operon (Eurofin, Huntsville, AL) and purified by reverse phase HPLC. All HPLC solvents were purchased from Fisher Inc. (Pittsburgh, PA). The HPLC system consisted of a Hitachi EZChrome Elite system with an L2450 diode array as a detector and a Clarity column (10 mm × 150 mm, 3 μm) (Phenomenex, Torrance, CA). The mobile phase system involved a 20 min linear gradient profile from 3 to 16% (v/v) acetonitrile with 100 mM ammonium acetate buffer (pH 6.5) at a flow rate of 2.0 mL/min. Kf-exo$^-$ (D424A) was received as a gift from C. Joyce (Yale University, New Haven, CT).

Preparation of an arylamine-modified template. The modified 31-mer biotinylated CG*A (G* = FAF/FAAF/FABP) DNA templates were prepared according to published procedures (*1-4*). The modified strand was purified by HPLC and characterized by Shimazdu Axima MALDI-TOF mass spectrometry. Biotinylated 31-mer (5 ODs) was ligated with 52-mer hairpin (6 ODs) using T4 DNA ligase (4,000 U) in 1 x ligase buffer for 16 h at 20°C. The ligated 83-mer oligonucleotide was purified by 10% denaturing polyacrylamide (Fig. S1b) and extracted using crush and soak method. The dideoxy-primer-template terminus was obtained by incubating ddTTP (1 mM) in the presence of Kf-exo$^-$ (1 μM) and 5 mM $MgCl_2$ for 12 h. The polymerase was precipitated with phenol-chloroform and DNA was purified by RP-HPLC using the solvent gradient mentioned above.

Immobilization of streptavidin on CM5 chip and DNA coating. Streptavidin (SA) was immobilized on CM5 dextran chip using amine coupling method by injecting EDC/NHS mixture over the surface for 7 minutes followed by SA (50 μg/mL) in sodium acetate buffer (pH 4.5). The unreacted esters were blocked by injecting 1M ethanolamine for 7 min. The running buffer used was 1 x HBS-EP$^+$ buffer containing 10 mM HEPES (pH 7.4), 150 mM NaCl, 0.05% non-ionic surfactant P20, and 3 mM EDTA. The flow cells 1 and 3 were used as reference. In flow cells 2 and 4, SA was immobilized ~2,500 RU. Before the coating of biotinylated DNA

templates over SA , the surface was washed with 50 mM NaOH for five times 60s pulse to remove the any free SA until the change in response unit was below 20 RU. The surface was further injected with running buffer 3-4 times to remove any trace amount of NaOH in microfluidics path and also to stabilize the surface. Biotinylated DNA-hairpin templates (0.25-0.3 nM) were injected for 60-120s over the flow cells 2 and 4 to achieve 0.7-3.5 RU while keeping flow cells 1 and 3 as blank references. The unbound DNA was washed with running buffer. To ensure the DNA template has dideoxy primer terminus, 1 mM dideoxythymidine (ddTTP) in the presence of Kf-exo$^-$ (1 μM) and 5 mM $MgCl_2$ was injected over the surface for 5 min and washed with 0.05% SDS.

Regeneration scouting. To assess the appropriate regeneration buffer, Kf-exo$^-$ (5 nM) was injected over the DNA surface followed by different regeneration buffer including 0.05% SDS, 1 M NaCl and 1 % SDS. For each regeneration buffer, injection of Kf-exo$^-$ was repeated 5 times to monitor the drift in base line as well as binding response. The least amount of drift from baseline binding affinity was observed with 0.05% SDS, compared to that observed with 1M NaCl (Fig. S3b); thus, 0.05% SDS was selected as regeneration buffer for all subsequent experiments.

Mass transport limitation. The effect of mass transport limitation in kinetics experiment was carried out by coating different amount of biotinylated DNA-hairpin templates (0.8, 2 and 10 RU) and studied the kinetics. The flow rate of Kf-exo$^-$ (5 nM) was varied from 5, 15 and 75 μL/min and monitored the rate of binding. The initial results showed that the data are heavily limited by mass transport above 10 RU and least affected by 0.7 RU (Fig. S3c). Consequently, we performed experiments in the 0.7–3.5 RU range.

Surface performance testing. The DNA surface was tested for the stability by injecting the repeated injections of Kf-exo$^-$ (20 nM) for 30s and dissociation of 60s followed by injection of 0.05% SDS for 20 cycles and the drift in binding affinity was monitored. There was no loss in surface binding affinity during the course of the experiment while affinity drifted within the range of 0.1–0.2 RU after repeated runs (Fig. S3d)

Kinetics analysis. The binding kinetics for the interaction of Kf-exo$^-$ with DNA was performed by injecting the enzyme (0-10 nM) in a running buffer HBS-P$^+$ (10 mM HEPES, 150 mM NaCl, 0.05% non-ionic surfactant P20), 5 mM $MgCl_2$, and BSA (100 μg/mL). The flow rate was 100 μL/min for 30s followed by dissociation for 60s. The DNA surface was conditioned by a sequential injection involving 1 x running buffer, 3 x start up running buffer, and 4 x running buffer with zero enzyme concentration. The surface was regenerated with 30s injection of 0.05% SDS with flow rate of 100 μL/min followed by extra wash with running buffer. For ternary system, individual dNTP (100 μM) was mixed with Kf-exo$^-$ and injected over the surface. The sensorgrams were double referenced and fitted using a 1:1 Langmuir model. The binding affinity constants (K_D) for ternary systems were calculated using steady-state affinity analysis in BIAevaluation software v2.0 as the association rate for the ternary systems particularly dCTP insertion opposite unmodified dG reaches near diffusion limit even the surface density of DNA is 0.7 RU. For consistency, we reported the K_D values for unmodified and arylamine modified dG using steady-state affinity analysis instead of 1:1 Langmuir fitting. However, for binary system, we measured the K_D values using 1:1 Langmuir fitting to show the robustness of the method (Table S1). The sensorgrams for binary systems were simulated by input the k_{on} and k_{off} values obtained from 1:1 fitting and input the parameters into Basic kinetics module of BIAsimulation software. The parameters used for simulations were: preinjection delay: 60s; association time: 30s; dissociation time: 60s; R_{max}: 1.8-3.5 RU. The theoretical Rmax values were calculated using the equation 1.

Theoretical R_{max}= (R_L*$M_{r(analyte)}$*valency of ligand)/$M_{r\ (ligand)}$ ……..(1)

Where R_L: DNA coated on the surface; Valency of ligand assumed as 1 (DNA:Kf-exo$^-$:: 1:1)

$M_{r\ (analyte)}$: 68,000 Da and $M_{r(ligand)}$:~26,000 Da.

e.g. For 0.7 RU biotinylated dG (-CGA-) coating:

Theoretical R_{max} was **1.8 RU** (0.7*68,000*1/26,000)

References

(1) Cho, B. P., Beland, F. A. and Marques, M. M. (1992) NMR structural studies of a 15-mer DNA sequence from a ras protooncogene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl. *Biochemistry 31*, 9587-9602.

(2) Meneni, S. R., Shell, S. M., Gao, L., Jurecka, P., Lee, W., Sponer, J., Zou, Y., Chiarelli, M. P. and Cho, B. P. (2007) Spectroscopic and theoretical insights into sequence effects of aminofluorene-induced conformational heterogeneity and nucleotide excision repair. *Biochemistry 46*, 11263-11278.

(3) Patnaik, S. and Cho, B. P. (2010) Structures of 2-acetylaminofluorene modified DNA revisited: insight into conformational heterogeneity. *Chem. Res. Toxicol. 23*, 1650-1652.

(4) Zhou, L., Rajabzadeh, M., Traficante, D. D. and Cho, B. P. (1997) Conformational Heterogeneity of Arylamine-Modified DNA: 19F NMR Evidence. *J. Am. Chem. Soc. 119*, 5384-5389.

Table S1. The k_{on} and k_{off} rates of binding interaction of Kf-exo$^-$ with unmodified and modified dG adducts (binary system)

	-G-	**-G[FAF]-**	**-G[FAAF]-**	**-G[FABP]-**
k_{on}(1/M.s)	8.8×10^7	9.3×10^7	9.4×10^7	6.9×10^7
k_{off} (1/s)	0.13	0.04	0.02	0.01

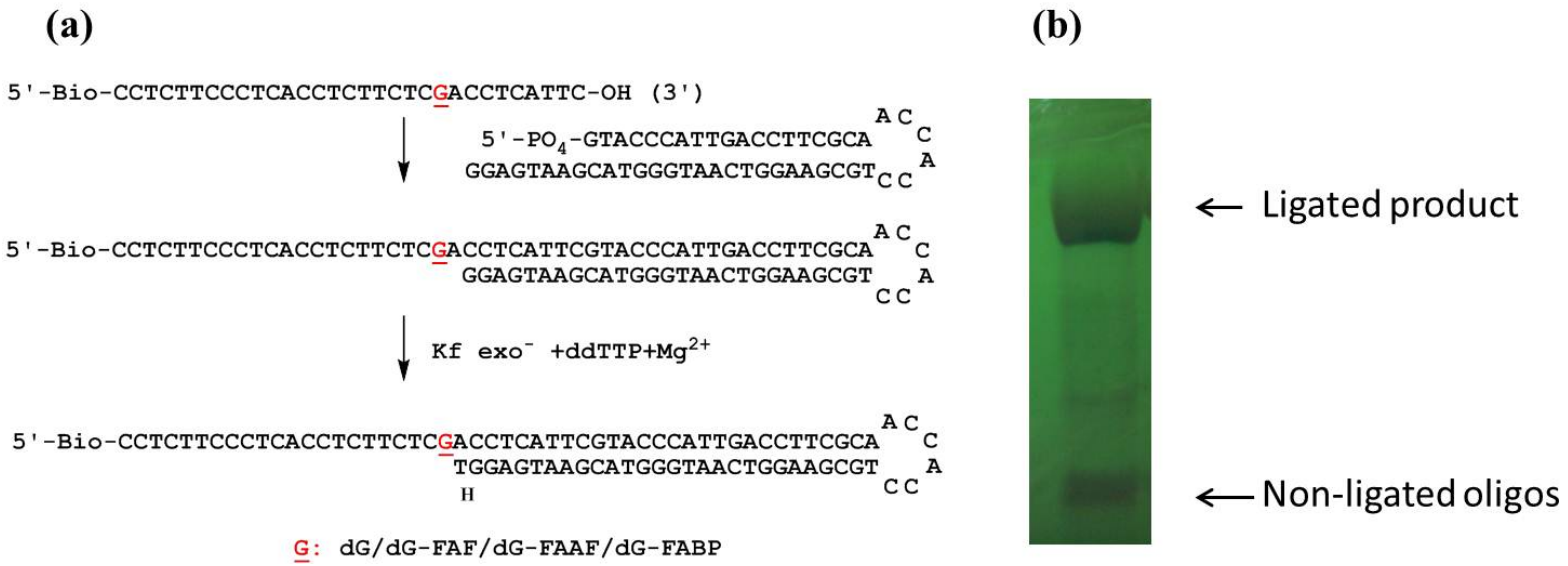

Figure S1. (a) Oligonucleotide constructs used for label-free DNA-polymerase interaction analysis (b) Separation of the 83-mer ligated oligos and non-ligated product using 10% denatured polyacrylamide gel (PAGE).

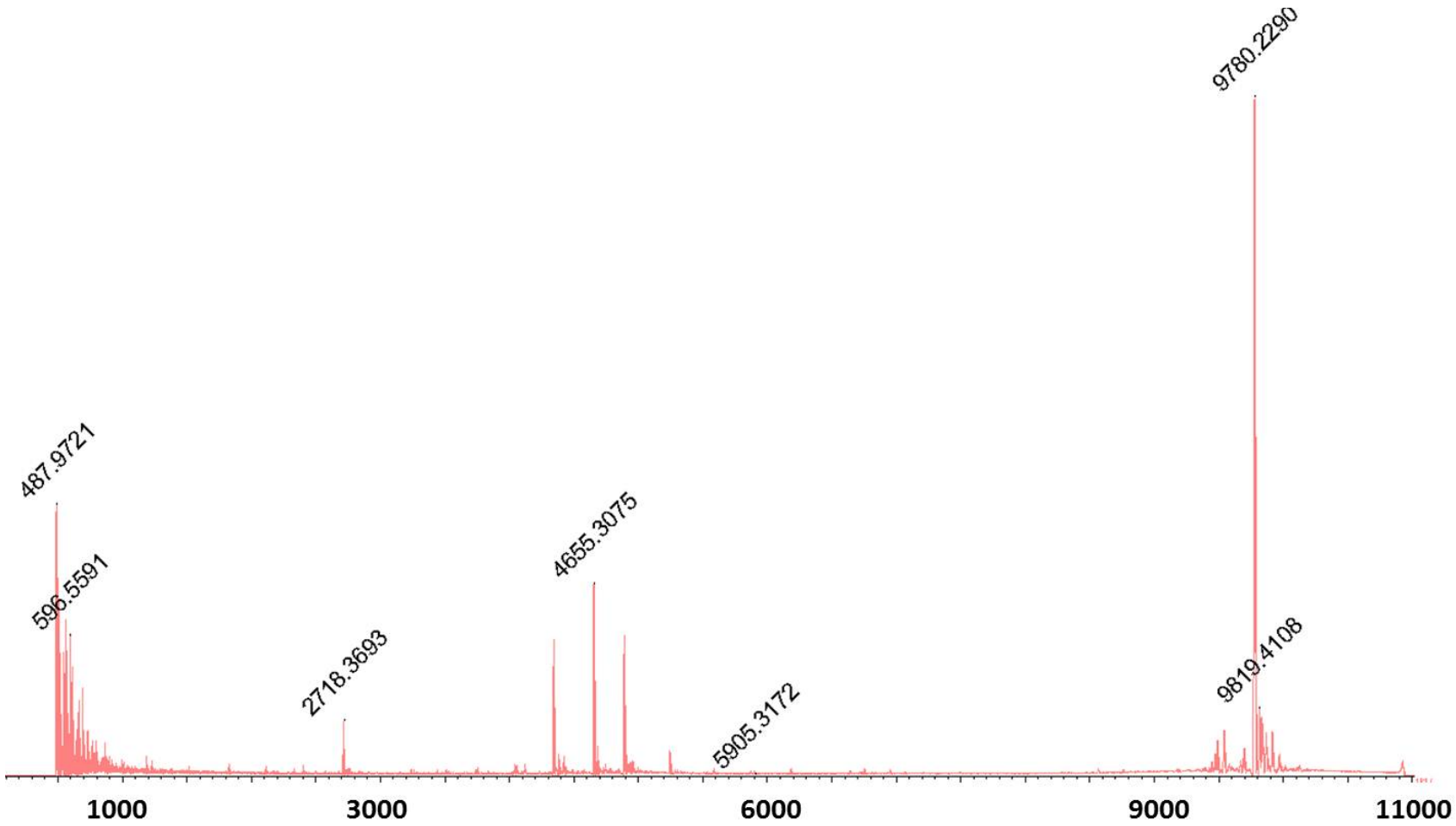

Figure S2. MALDI-TOF spectrum of biotinylated 31-mer containing dG-FAF adduct (see Fig. S1a for sequence: theoretical *m/z* 9781.50).

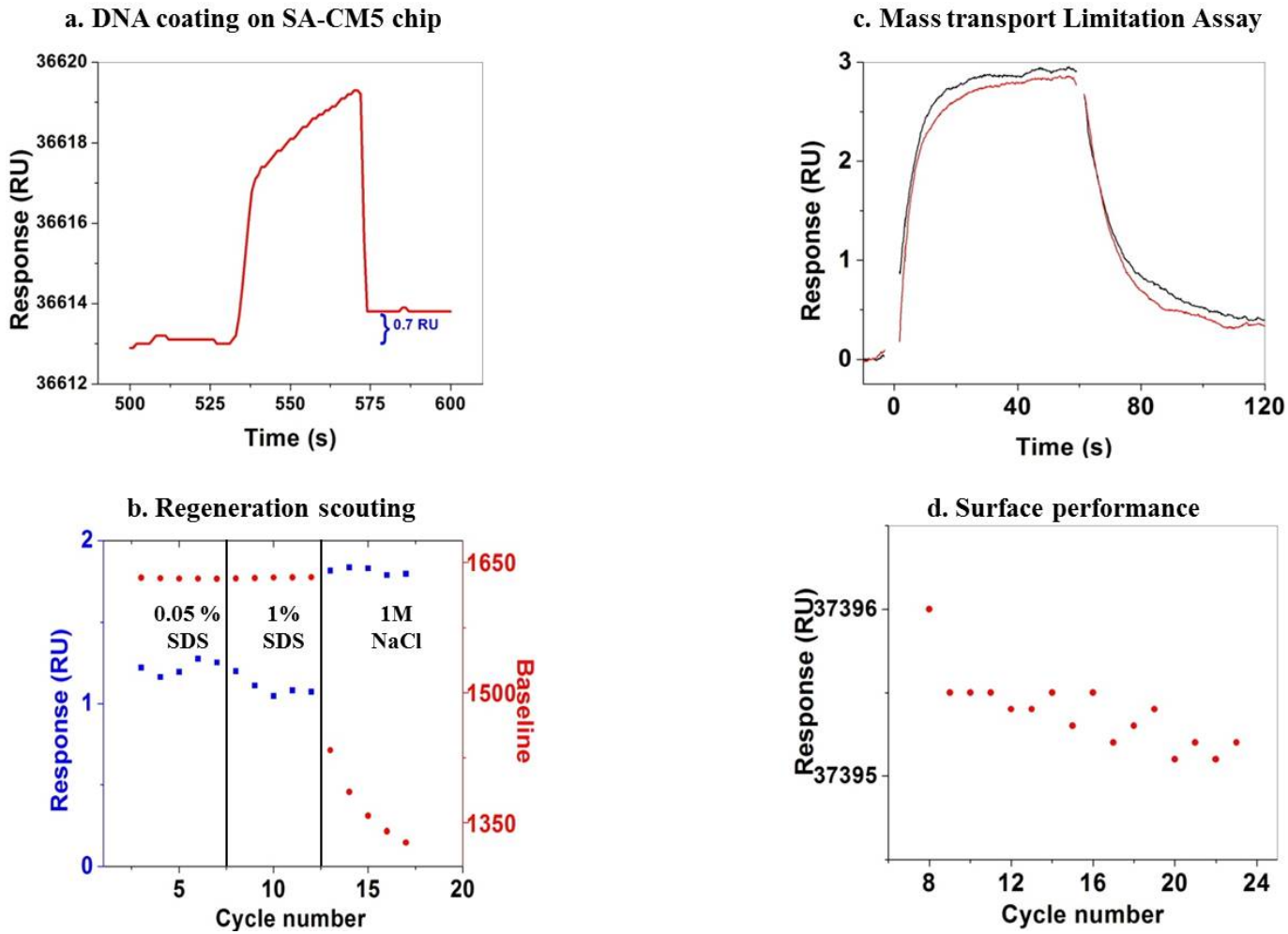

Figure S3. (a) Sensorgram of biotinylated oligonucleotide coated on streptavidin (SA) immobilized CM5 chip, (b) regeneration scouting with different buffers (0.05% SDS, 1% SDS and 1M NaCl). Baseline drift was represented in red dots and binding response in blue dots, (c) Effect of flow on the rate of binding, (d) Injection of Kf-exo$^-$ over unmodified oligonucleotide for 20 cycles.

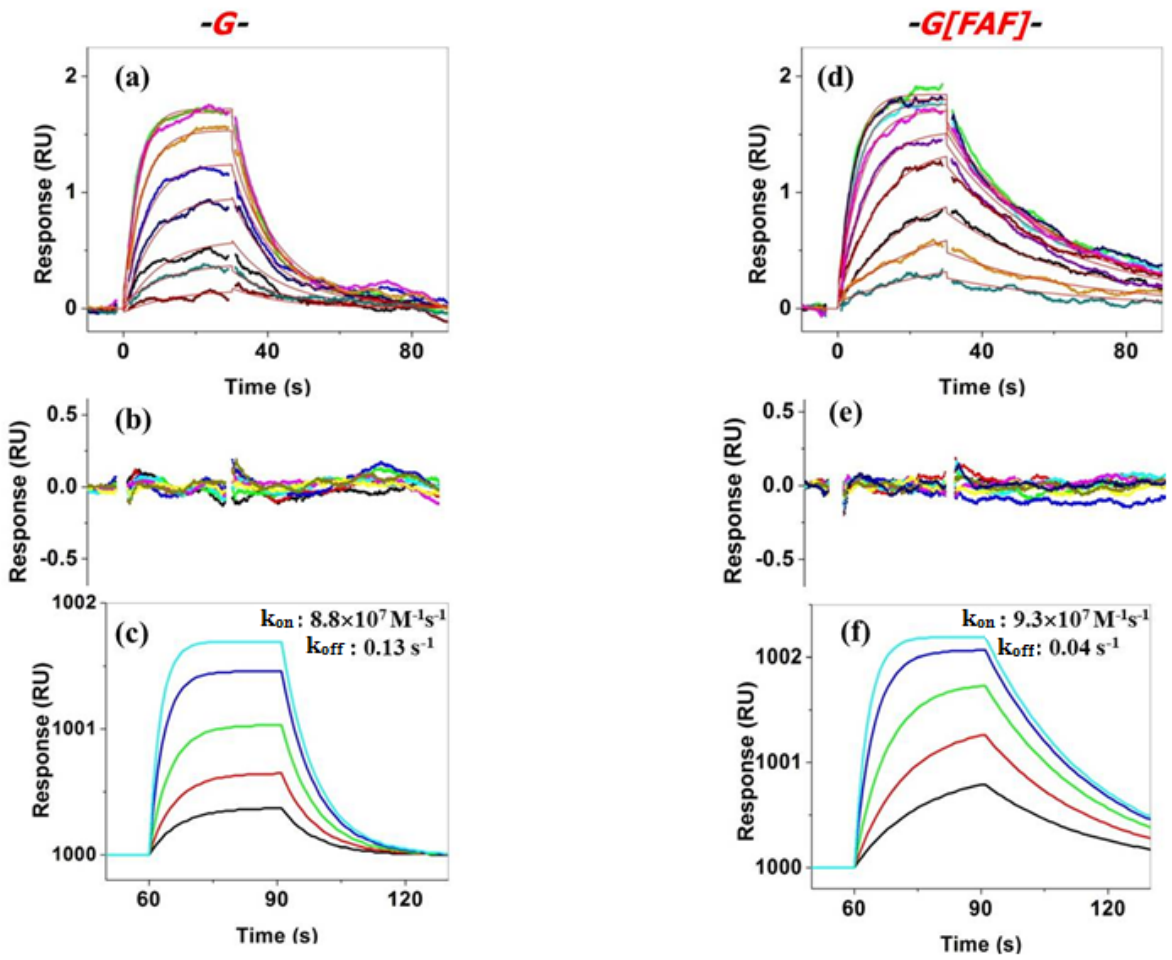

Figure S4. (a) Sensorgrams of Kf-exo⁻ binding with unmodified dG (binary), (b) fitted residuals, (c) simulated binding curves, (d) sensorgrams of FAF, (e) fitted residuals, and (f) simulated binding curves.

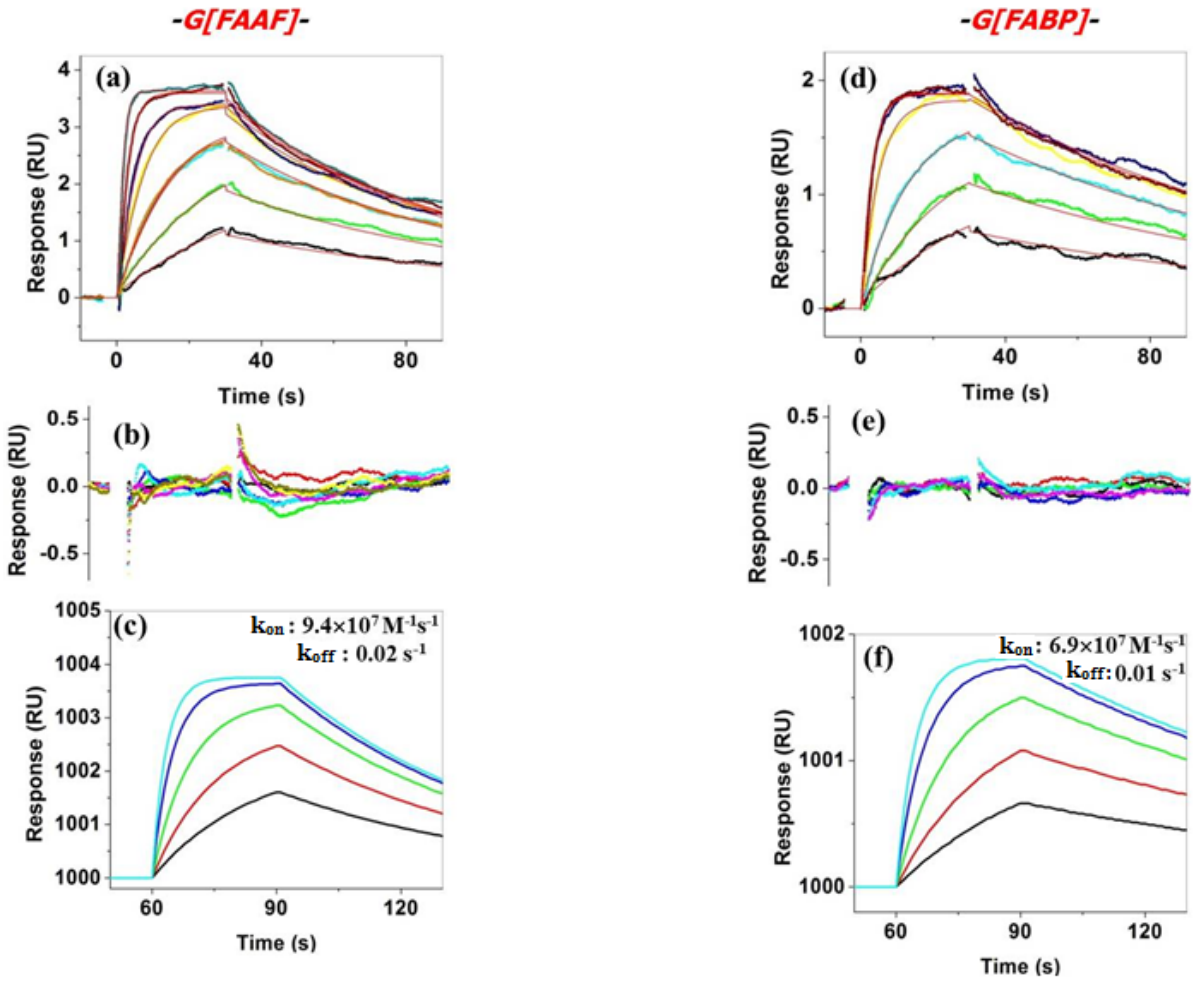

Figure S5. (a) Sensorgrams of Kf-exo⁻ binding with FAAF (binary), (b) fitted residuals, (c) simulated binding curves, (d) sensorgrams of FABP, (e) fitted residuals, and (f) simulated binding curves.

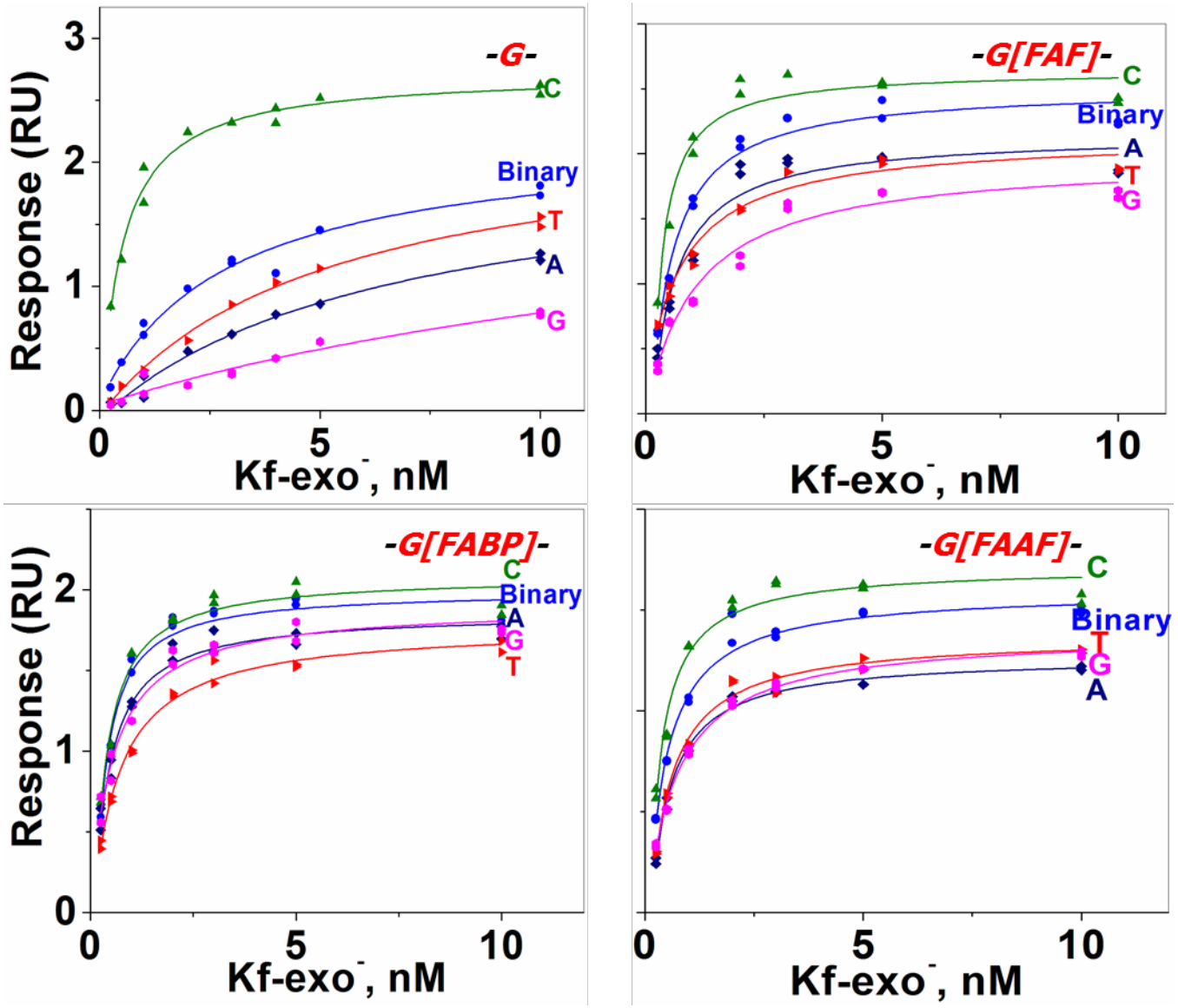

Figure S6. Binding curves of Kf-exo⁻ with unmodified and arylamine-dG adducts in the presence and absence of dNTPs.