Supplementary Information

Tuning DNA-nanoparticle conjugate properties allows modulation of nuclease activity

Data Analysis

In the data analysis we used following equations: logistic sigmoidal equation (Eq. 1.), Michaelis–Menten equation (Eq. 2), and Hill equation (Eq. 3). Where: *y* is observed signal, A_1 and A_2 are asymptotes, and *p* is the power factor. *v* is the observed reaction rate, V_{max} is the maximal reaction rate, *S* is a substrate concentration, K_m the Michaelis-Menten constant, and *n* is the Hill factor.

$$Eq.1. \quad y = \frac{A_1 - A_2}{1 + (\frac{x}{x_0})^p} + A_2$$

$$Eq.2. \quad v = \frac{V_{max}S}{K_m + S}$$

$$Eq.3. \quad v = \frac{V_{max}S^n}{k^n + S^n}$$

Supplementary Items

Table S1: A summary of the oligonucleotide sequences and functionalization.

Figure S1: Explanation of Target Probe Quenching

Figure S2: SNA Stability in Stock Enzyme Buffer

Figure S3: Varying Enzyme and Substrate Concentrations, Non-Recycling

Figure S4: Varying ExoIII Recognition Site Location, Non-Recycling

Figure S5: Varying Surface Coverage, Non-Recycling

Figure S6: Varying AuNP Size, Non-Recycling

Figure S7: Varying Enzyme and Substrate Concentrations, Recycling

Table S1. A summary of the oligonucleotide sequences and functionalization.

Name		5'	Sequence	3’
System 1	Sys1 Bound Quencher	SH	TTT AGA ACA GGA AGA GCG GAC AC[x]	
	Sys1 Free Quencher	Cy3	TTT AGA ACA GGA AGA GCG GAC AC[x]	
	Sys1 Target		GTG TCC GCT CTT CC[v]T GT TCT	FAM
	Sys1 Target (Protected)		GTG TCC GCT CTT CCT GTT CT T *T*T*	FAM
System 2	Sys2 Bound Quencher		GTG TCC GCT CTT CC[v]T GTT GT AAA	SH
	Sys2 Free Quencher (Protected)		GTG TCC GCT CTT CC[v] T GTT GT A*A*A*	Cy3
	Sys2 Target	FAM	ACA ACA GGA AGA GCG GAC AC[x]	
System 3	Sys3 Bound Quencher	SH	TTT AGA ACA GGA AGA GCG GAC ACT TTT	
	Sys3 Target		GTG TCC GCT CTT CCT GTT CT[x]	FAM

[x] denotes the ExoIII cleavage site while[v] denotes the Nt.BspQI nicking site.
* and **TTTT** denote phosphorothioate and overhang protection, respectively.

S1 Explanation of Target Probe Quenching

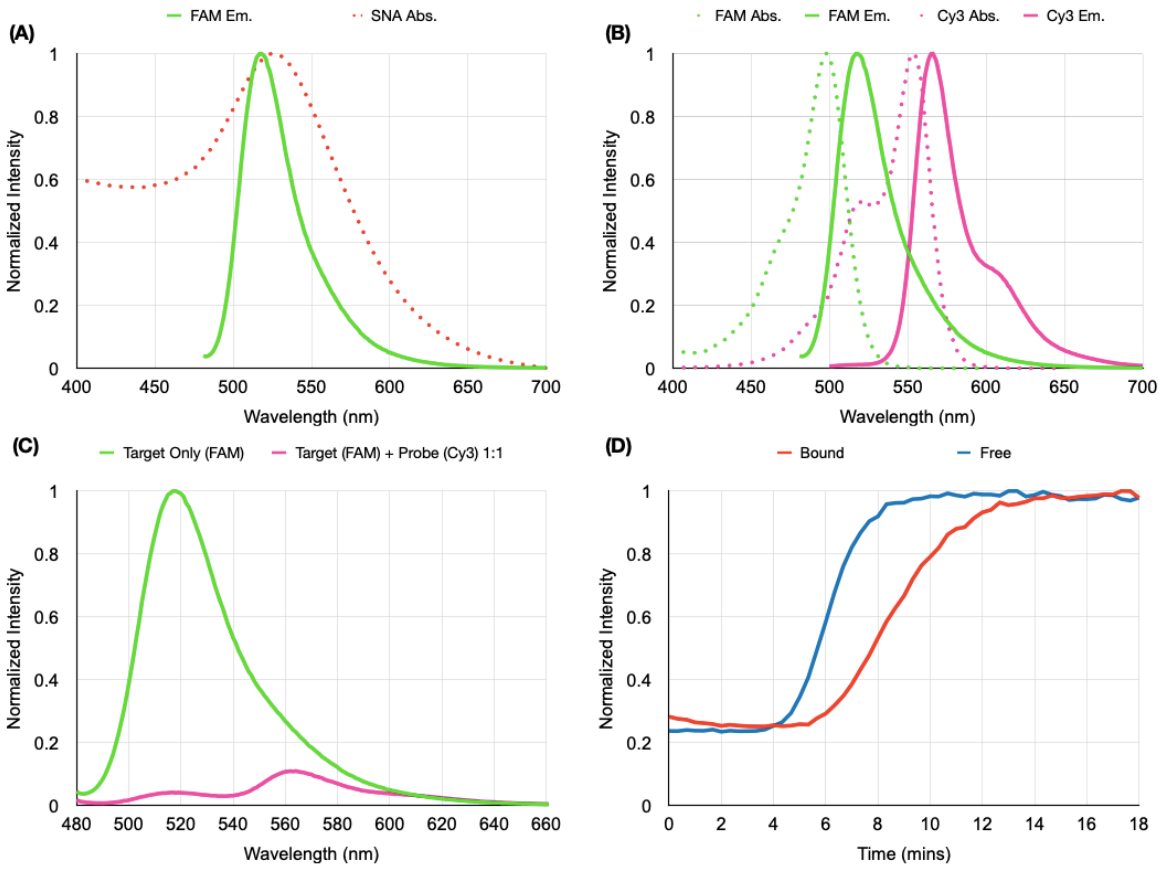


Figure S1. (A) Overlap between the FAM emission and the SNA absorption spectra. The FAM fluorophore is quenched by a process known as nanosurface energy transfer (NSET[1]). The quenching efficiency of the SNA system is ca. 80% in the experiments described herein. **(B)** Spectral overlaps between the FAM and Cy3 dyes. When the FAM is excited, it transfers energy to the adjacent Cy3 dye by Förster resonance energy transfer (FRET). The excellent overlap between the FAM emission and the Cy3 absorption spectra makes this process highly efficient. **(C)** Incubated at a molar concentration of 1:1, the target (FAM) and probe (Cy3) oligos hybridize, bringing the two dyes into close proximity. The FAM is quenched with an efficiency of 96% (by comparison of intensities at 517 nm). **(D)** Example enzyme progress curves comparing the bound and free cases. The initial fluorescence in the two systems arises due to different reasons in the two systems. In the bound case, it is due to incomplete quenching of dye fluorescence by the gold nanoparticle. In the free case, the quenching is more efficient, but there is a degree of fluorescence bleed through from the Cy3 channel in the RT-PCR machine as the Cy3 probe is used in excess in the experiments and experiences some direct excitation. However, fluctuation in the bleed through intensity is insignificant through the time course of the experiments, therefore creating a consistent baseline effect.

[1] C. Chen & N. Hildebrandt, Resonance energy transfer to gold nanoparticles: NSET defeats FRET. *Trends in Analytical Chemistry* **123**, 115748, (2020)

S2 SNA Stability in Stock Enzyme Buffer

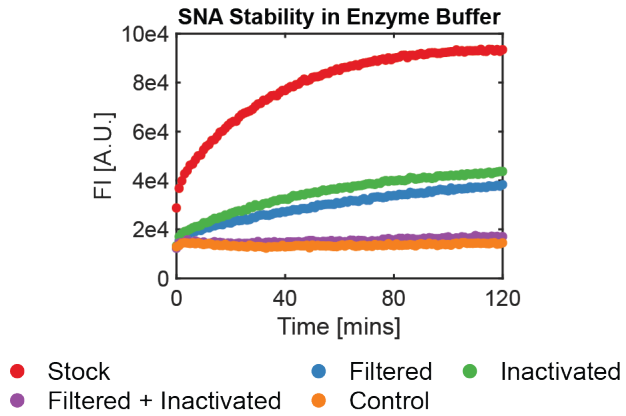
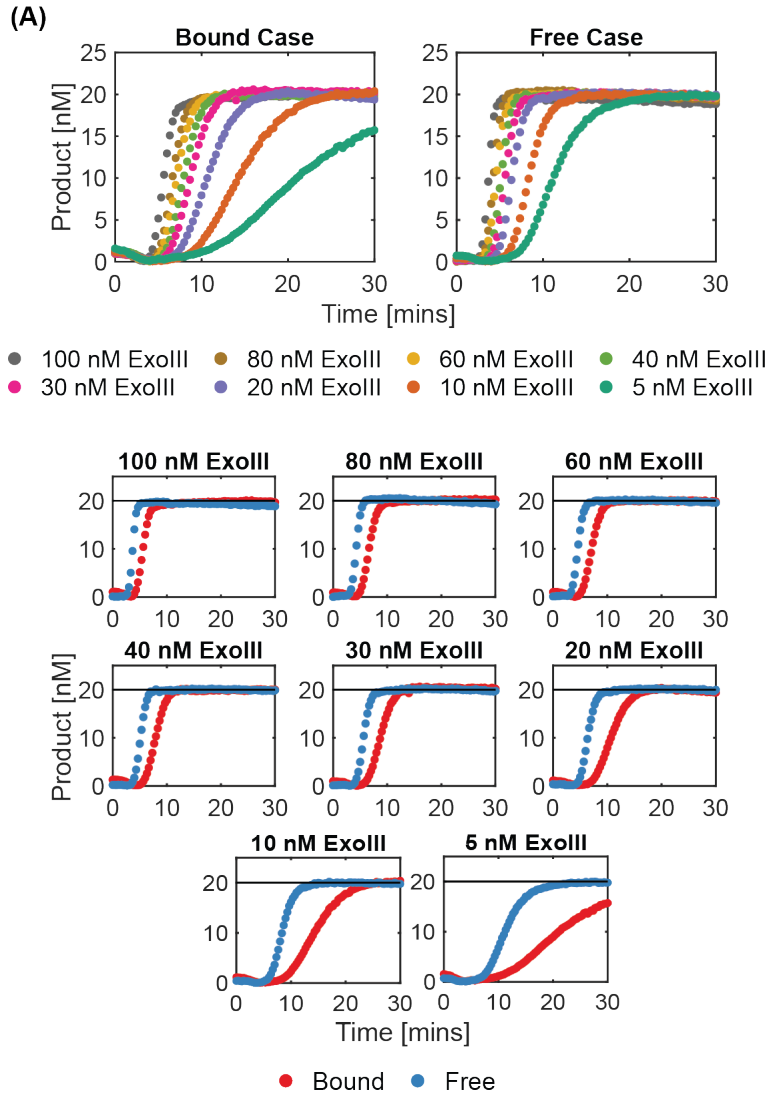
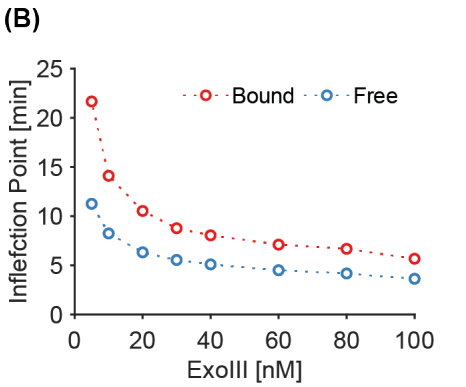


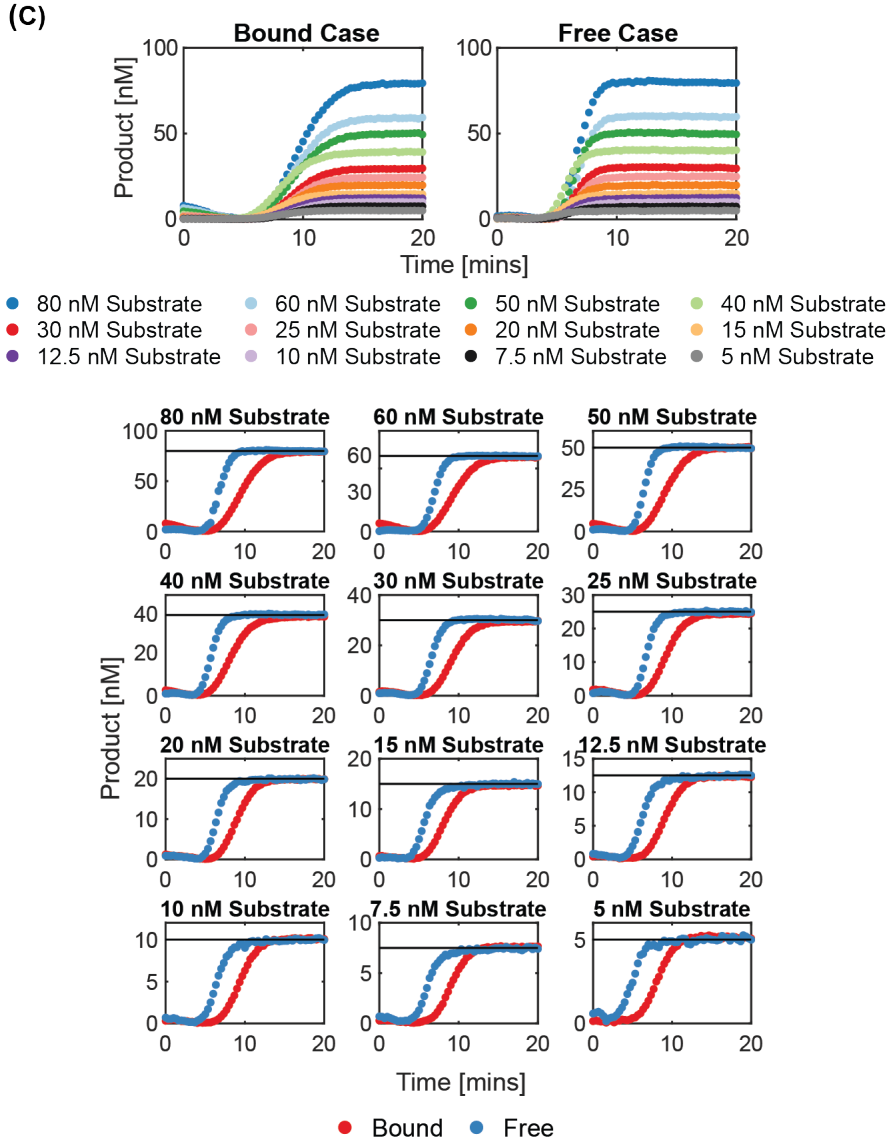
Figure S2. When Nt.BspQI was used directly from the stock solution provided by NEB, the DTT present in the enzyme storage buffer (in addition to nuclease activity) lead to the release of fluorescently labelled oligonucleotides from the AuNP core (red). This is demonstrated by the signal increase of the progress curve when stock, inactivated Nt.BspQI was applied (green). However, when the endonuclease was filtered through a 7K MWCO ZebaTM Spin Desalting Column and inactivated, no such signal increase was detected (purple), showing successful enzyme purification from DTT. Filtered Nt.BspQI without any inactivation steps confirmed that the endonuclease retained its nicking activity after the filtration step (blue). Accordingly, in order to specifically study the nuclease activity, all nucleases used within the article were first filtered through a 7K MWCO ZebaTM Spin Desalting Column prior to usage in the kinetic assays.

S3 Varying Enzyme and Substrate Concentrations, Non-Recycling





(C)


Bound Case
Free Case
Product [nM]
Time [mins]
80 nM Substrate
60 nM Substrate
50 nM Substrate
40 nM Substrate
30 nM Substrate
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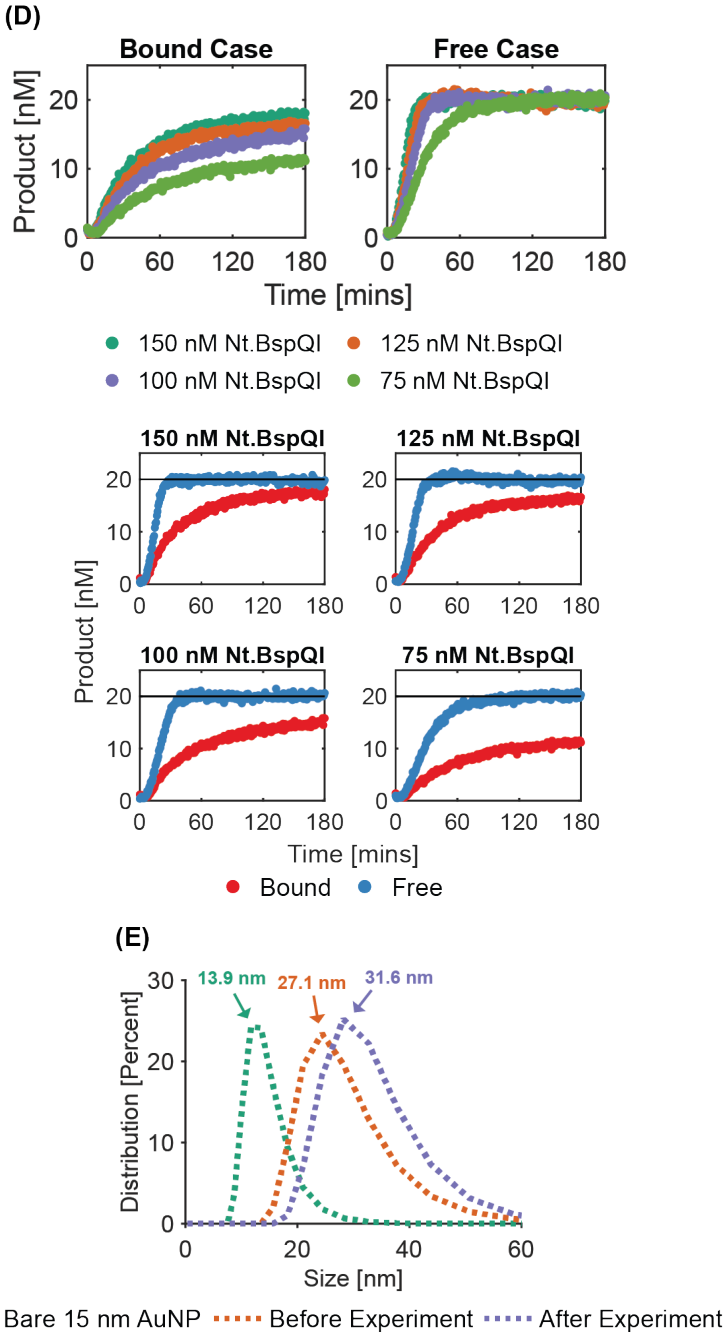
20 nM Substrate
15 nM Substrate
12.5 nM Substrate
10 nM Substrate
7.5 nM Substrate
5 nM Substrate
Bound
Free


(D)
Bound Case
Free Case
Product [nM]
Time [mins]
150 nM Nt.BspQI
125 nM Nt.BspQI
100 nM Nt.BspQI
75 nM Nt.BspQI
Bound
Free
(E)
13.9 nm
27.1 nm
31.6 nm
Distribution [Percent]
Size [nm]
Bare 15 nm AuNP
Before Experiment
After Experiment

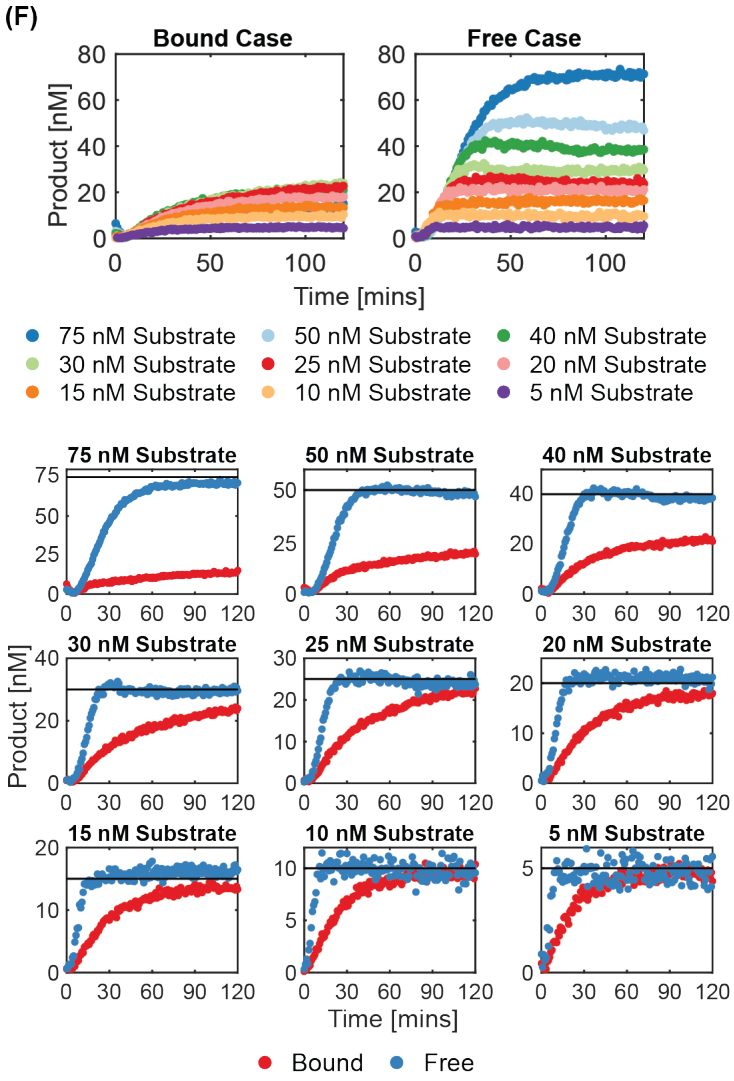


Figure S3. (A) Progress curves for ExoIII activity with varying enzyme concentration and constant substrate concentration (20 nM) in a non-recycling target detection format. **(B)** Inflection point, determined as the time required to reach maximum velocity, with varying ExoIII concentrations. **(C)** Progress curves of ExoIII activity with varying substrate concentration and constant ExoIII concentration (25 nM). **(D)** Progress curves of Nt.BspQI activity with varying enzyme concentration and constant substrate concentration (20 nM) in a non-recycling target detection format. **(E)** Dynamic light scattering (DLS) data demonstrating hydrodynamic size of bare AuNP, SNA before kinetic run, and SNA after kinetic run. **(F)** Progress curves of Nt.BspQI activity with varying substrate concentration and constant enzyme concentration (125 nM).

S4 Varying ExoIII Recognition Site Location, Non-Recycling

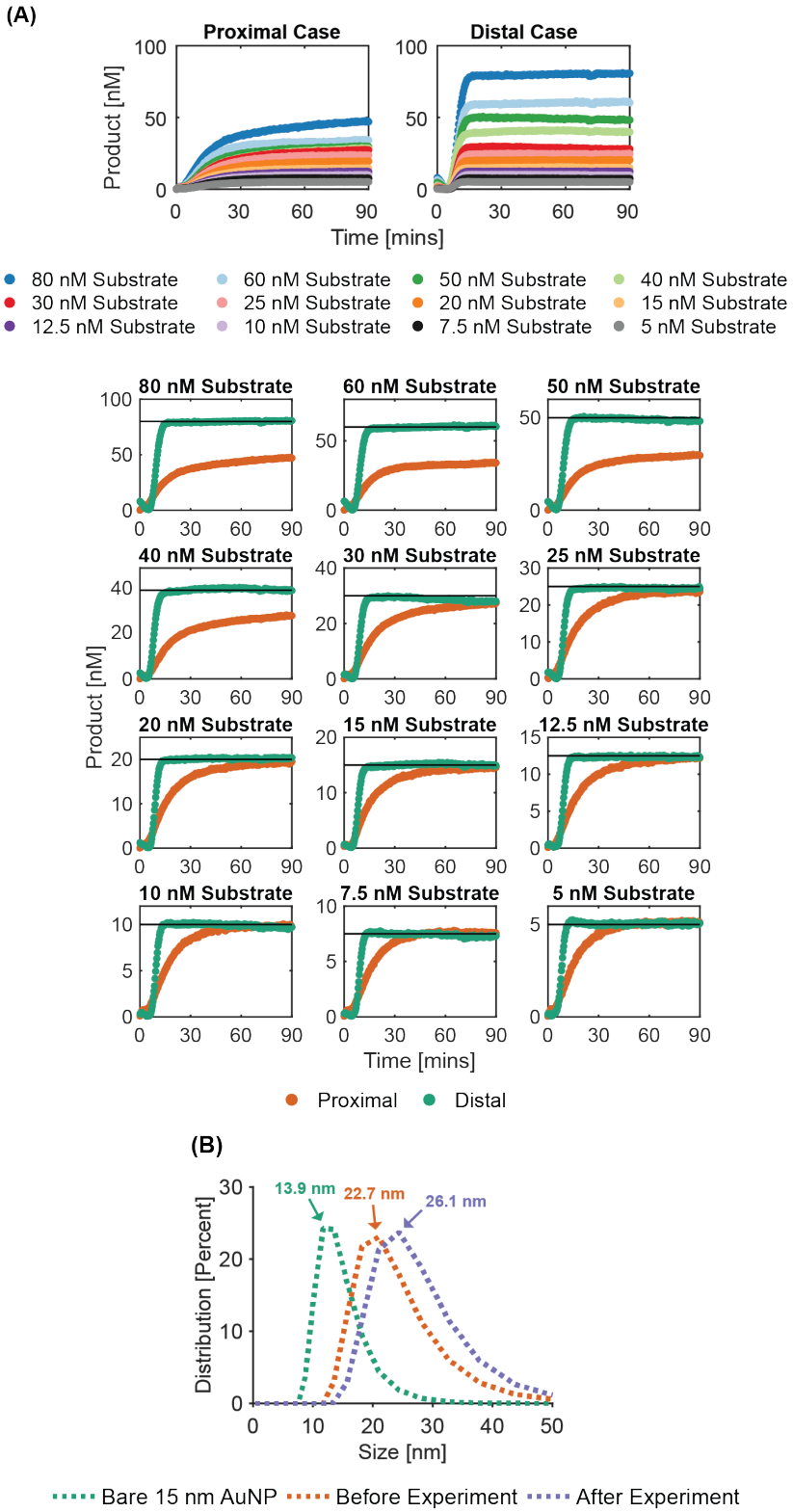
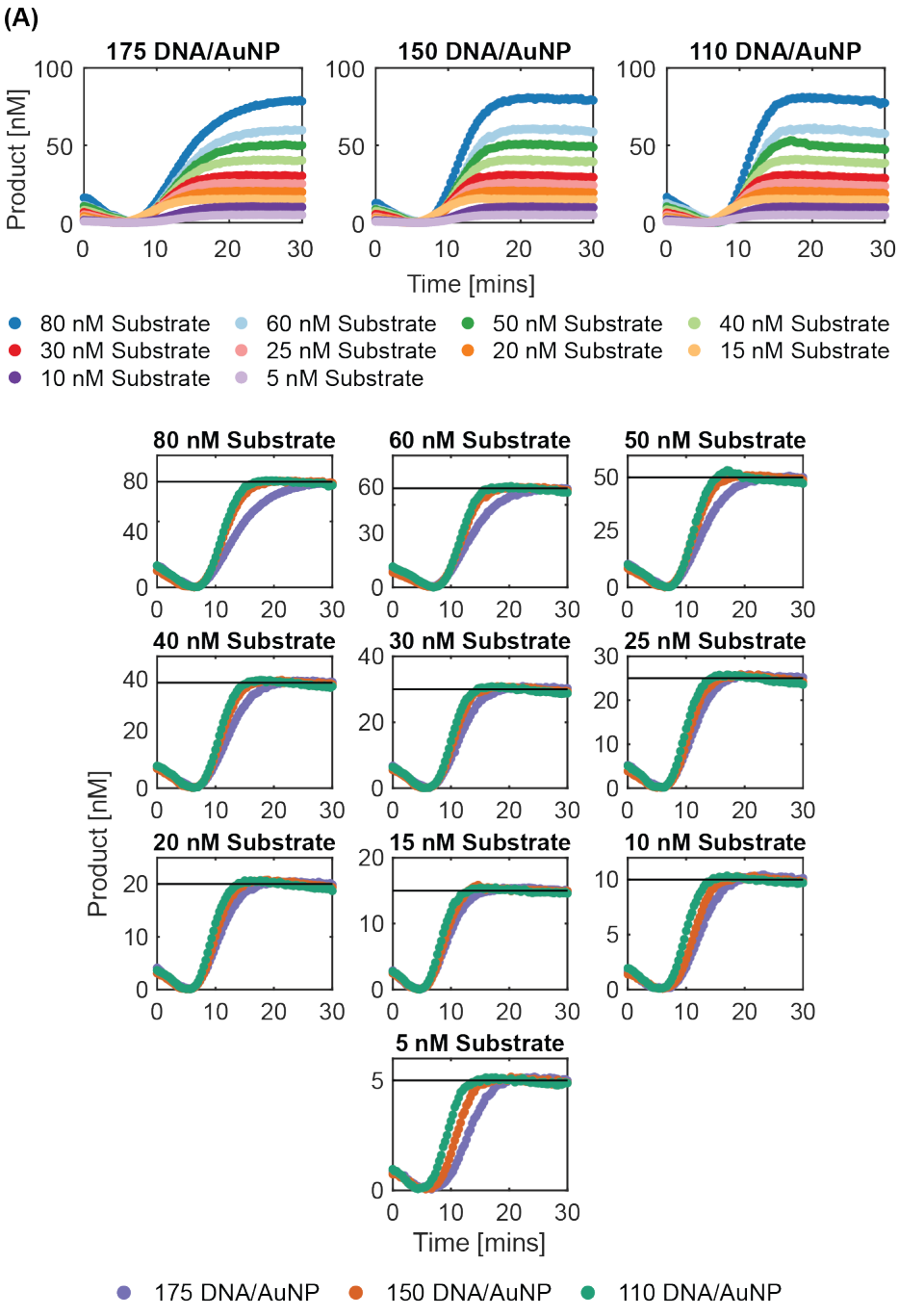


Figure S4. (A) Progress curves of ExoIII activity with varying recognition site location: proximal vs. distal of SNA. **(B)** DLS data demonstrating hydrodynamic size of bare AuNP, SNA before kinetic run, and SNA after kinetic run.

S5 Varying Surface Coverage, Non-Recycling



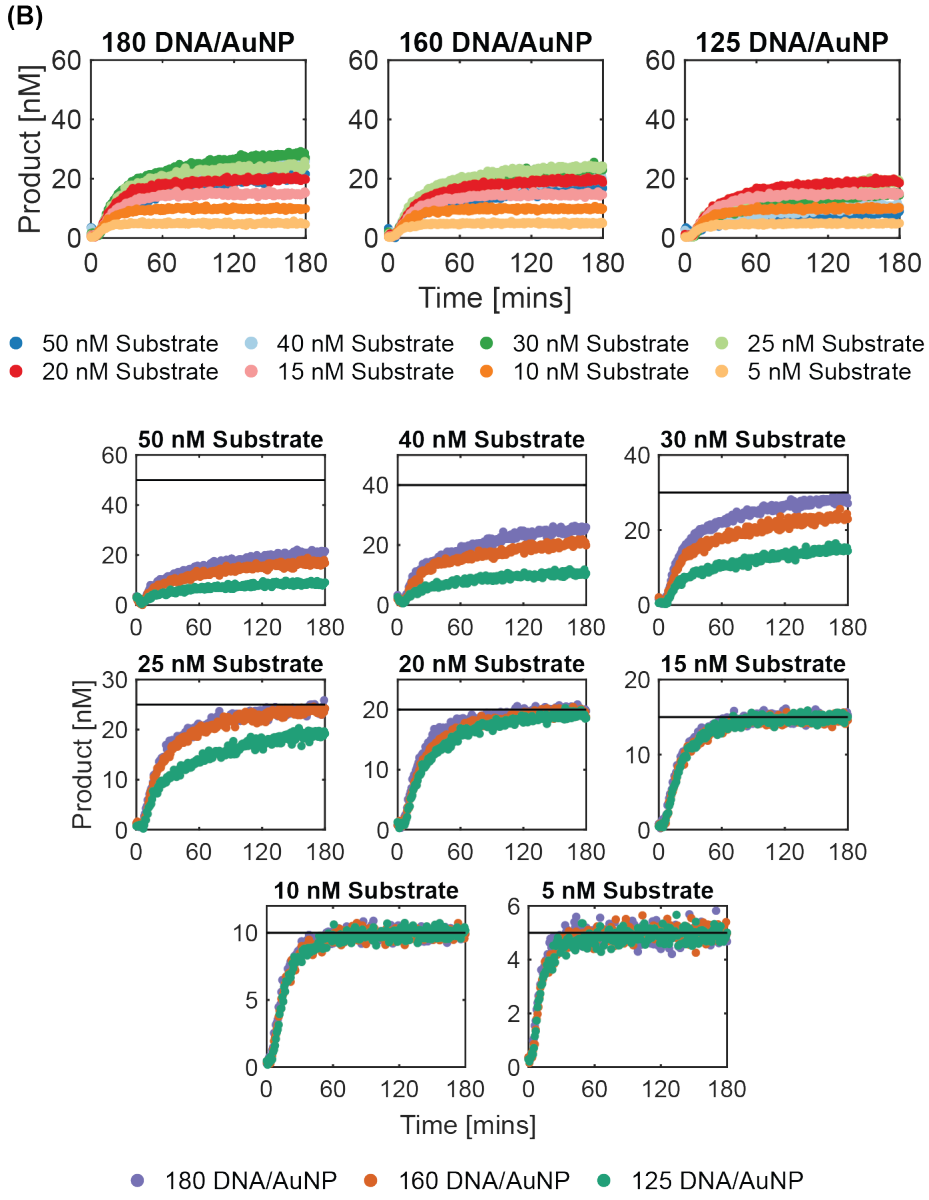
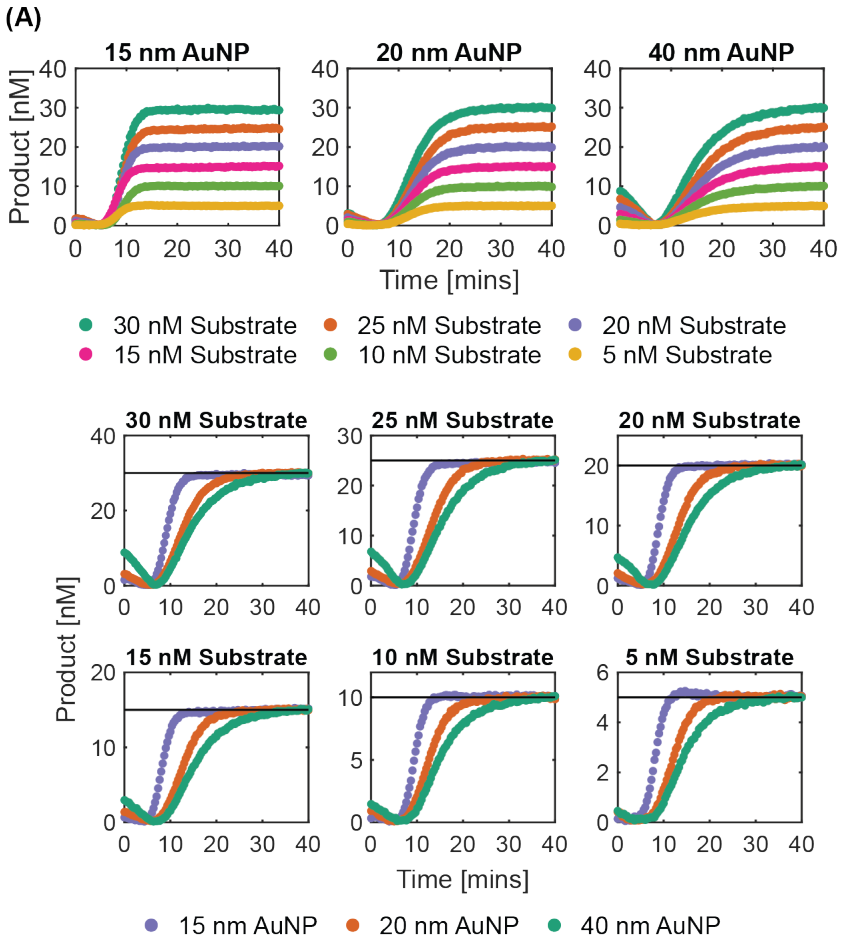


Figure S5. Progress curves of **(A)** ExoIII activity and **(B)** Nt.BspQI activity with SNAs of varying surface densities.

S6 Varying AuNP Size, Non-Recycling



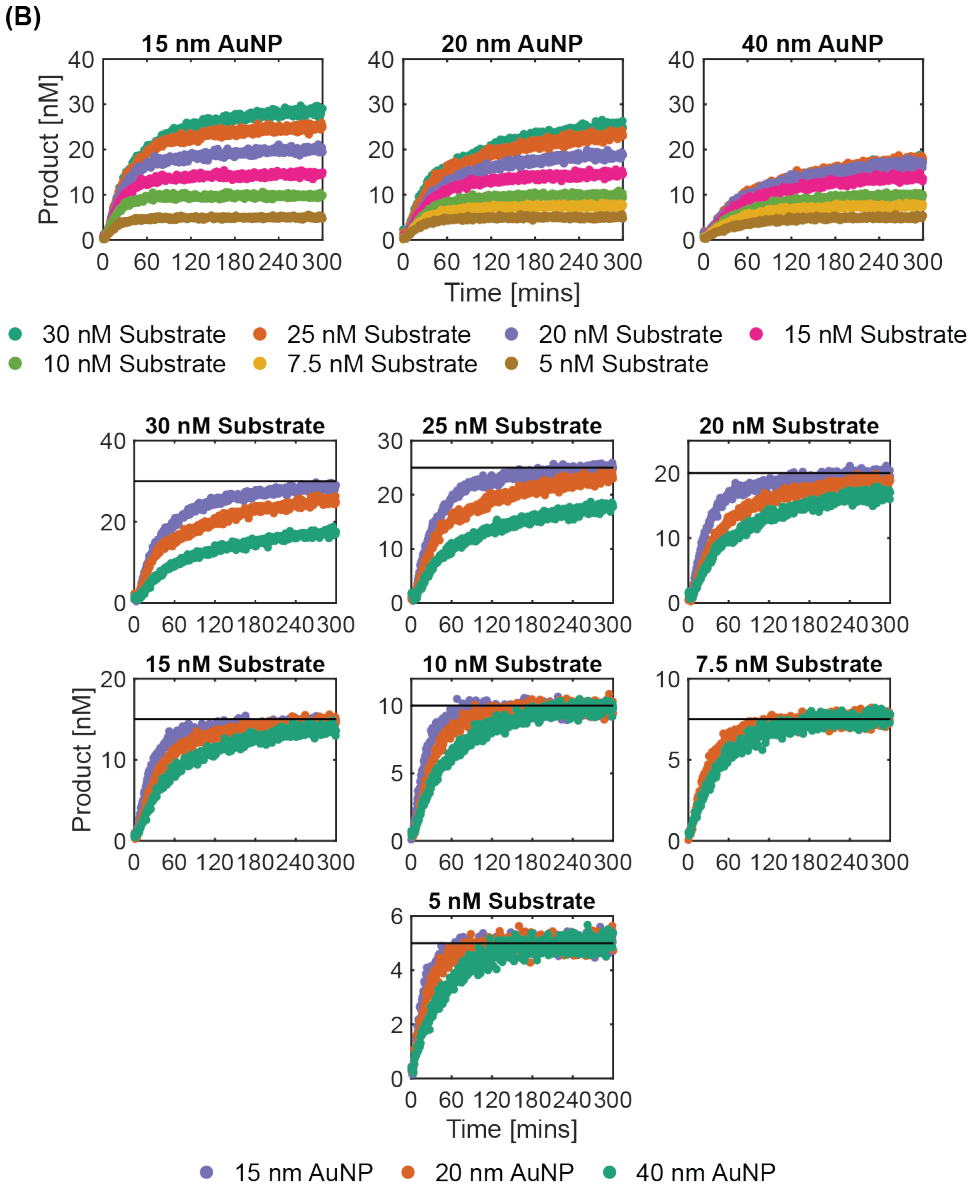
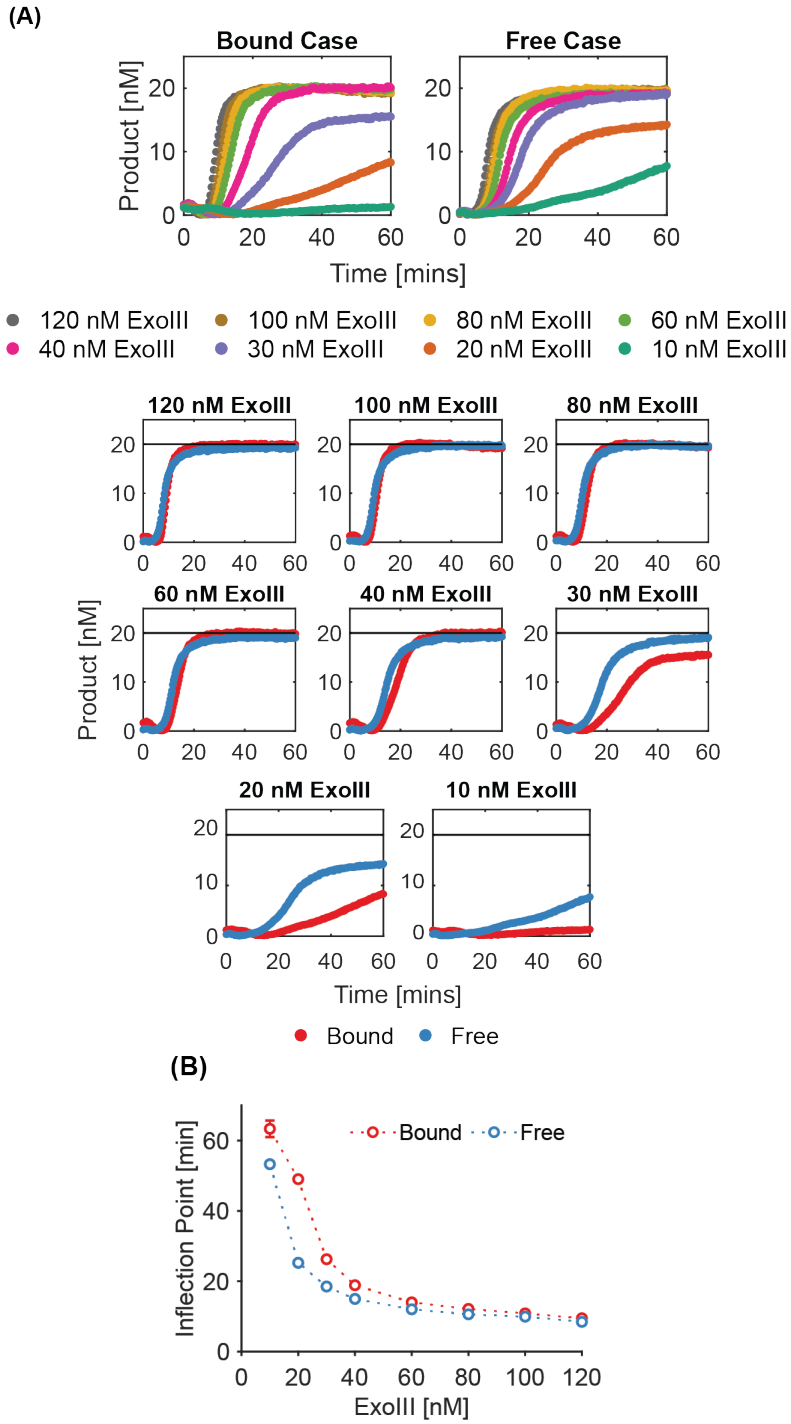
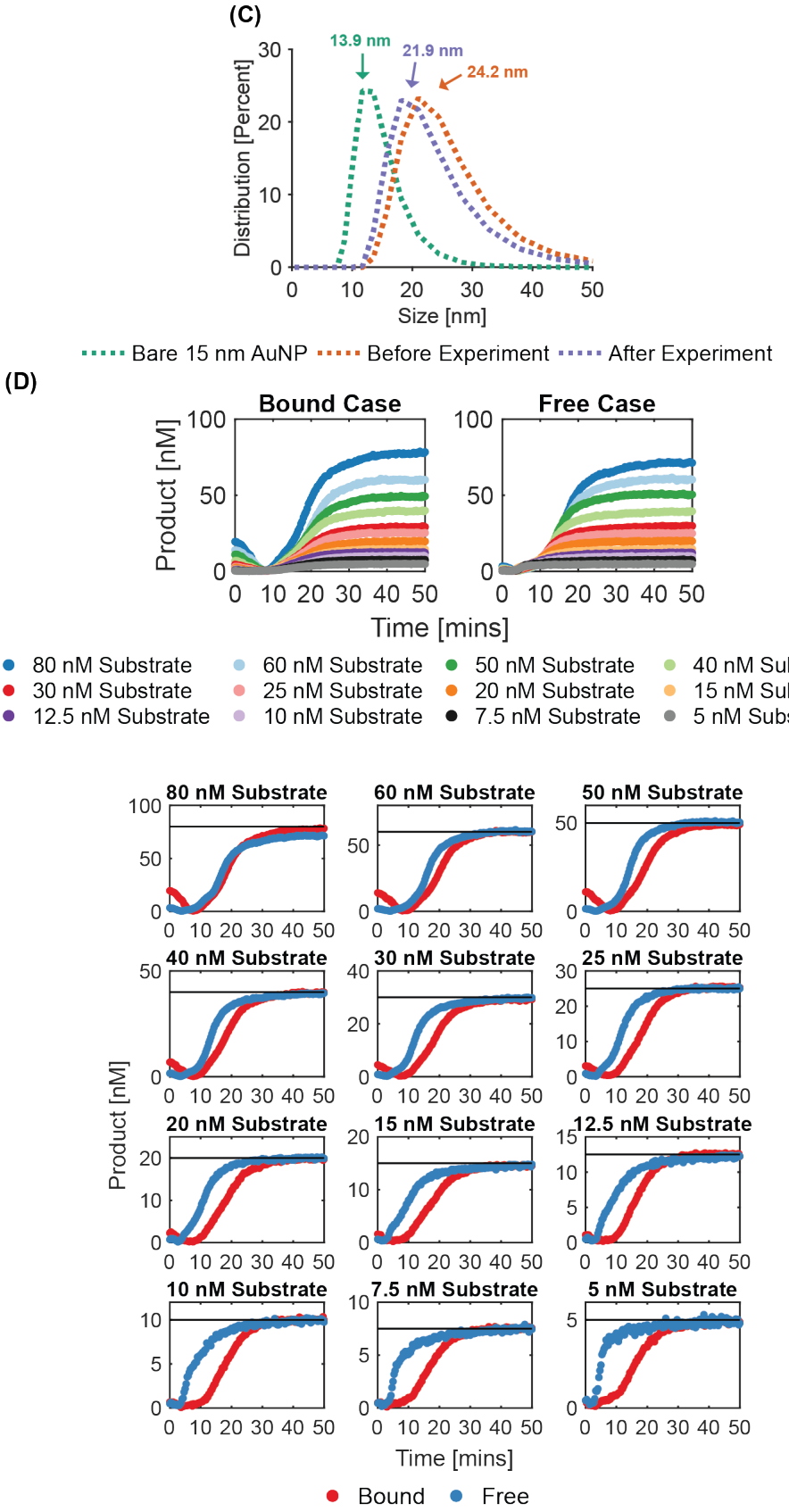


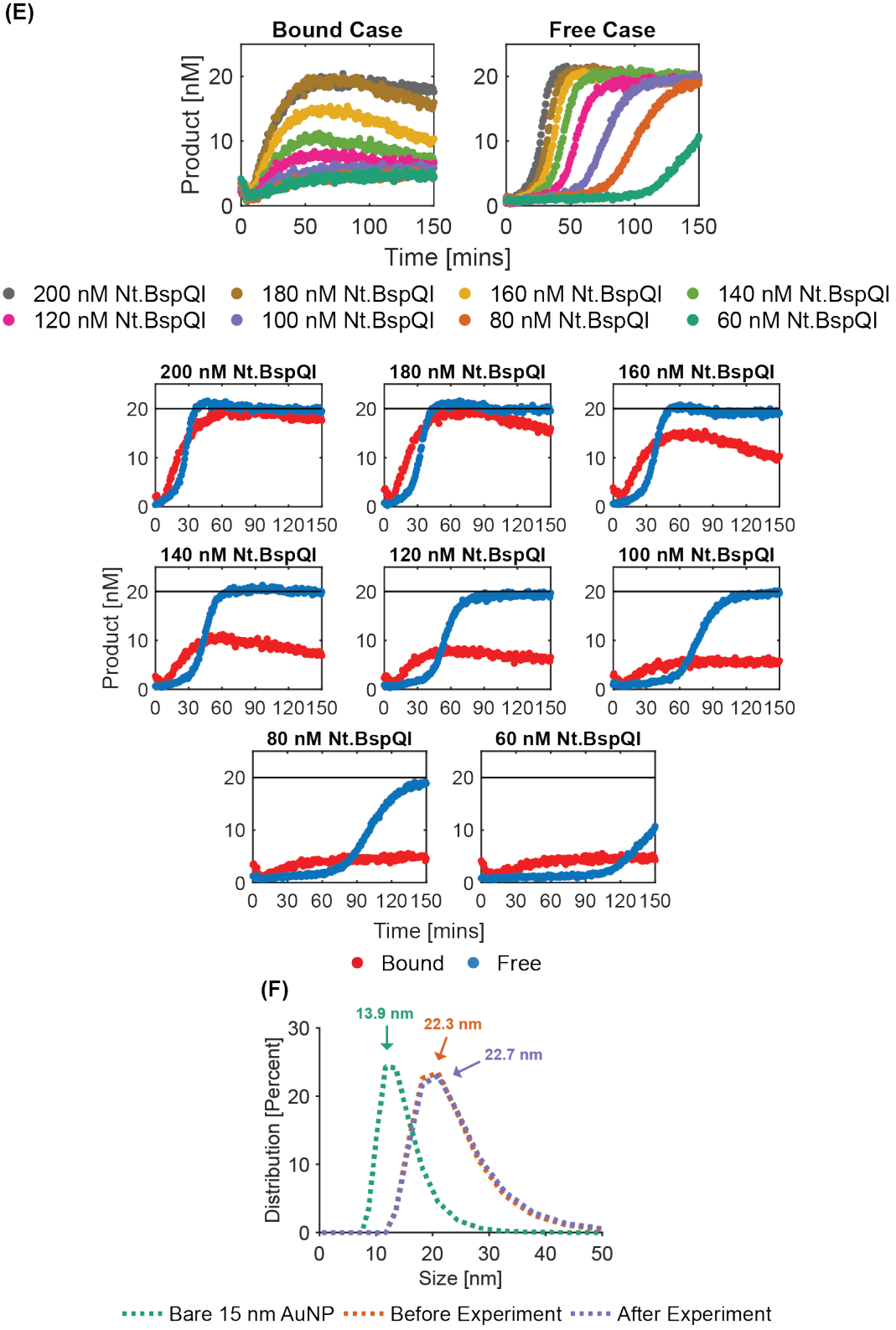
Figure S6. Progress curves of **(A)** ExoIII activity and **(B)** Nt.BspQI activity with SNAs of varying AuNP core sizes.

S7 Varying Enzyme and Substrate Concentrations, Recycling




(C)
13.9 nm
21.9 nm
24.2 nm
Distribution [Percent]
Size [nm]
Bare 15 nm AuNP
Before Experiment
After Experiment
(D)
Bound Case
Free Case
Product [nM]
Time [mins]
80 nM Substrate
60 nM Substrate
50 nM Substrate
40 nM Substrate
30 nM Substrate
25 nM Substrate
20 nM Substrate
15 nM Substrate
12.5 nM Substrate
10 nM Substrate
7.5 nM Substrate
5 nM Substrate
Bound
Free

(E)


Bound Case
Free Case
Product [nM]
Time [mins]
200 nM Nt.BspQI
180 nM Nt.BspQI
160 nM Nt.BspQI
140 nM Nt.BspQI
120 nM Nt.BspQI
100 nM Nt.BspQI
80 nM Nt.BspQI
60 nM Nt.BspQI
Bound
Free

(F)

13.9 nm
22.3 nm
22.7 nm
Distribution [Percent]
Size [nm]
Bare 15 nm AuNP
Before Experiment
After Experiment

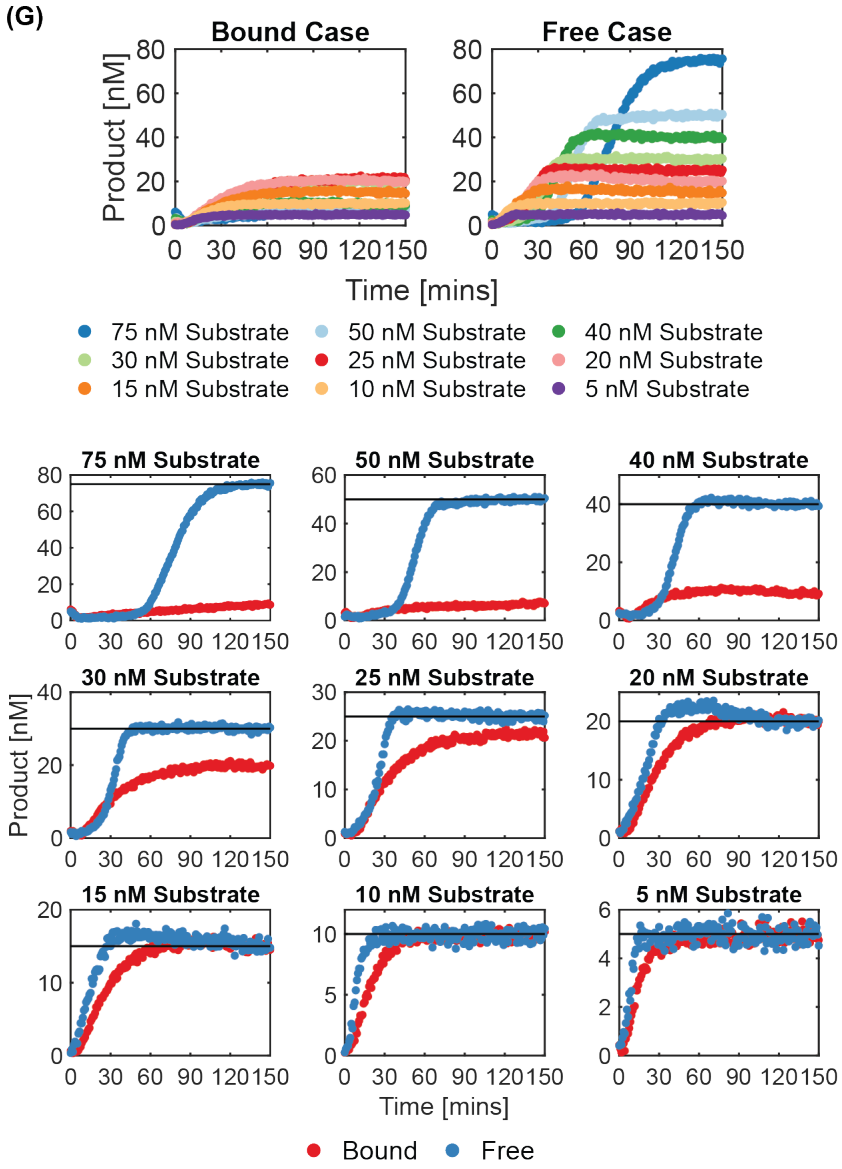


Figure S7. (A) Progress curves for ExoIII activity with varying enzyme concentration and constant substrate concentration (20 nM) in a recycling target detection format. **(B)** Inflection point, with varying ExoIII concentrations. **(C)** DLS data demonstrating hydrodynamic size of bare AuNP, SNA before kinetic run, and SNA after kinetic run. **(D)** Progress curves of ExoIII activity with varying substrate concentration and constant ExoIII concentration (60 nM). **(E)** Progress curves of Nt.BspQI activity with varying enzyme concentration and constant substrate concentration (20 nM) in a non-recycling target detection format. **(F)** DLS data demonstrating hydrodynamic size of bare AuNP, SNA before kinetic run, and SNA after kinetic run. **(G)** Progress curves of Nt.BspQI activity with varying substrate concentration and constant enzyme concentration (180 nM).