Supplementary Information

A flap endonuclease 1-assisted universal viral nucleic acid sensing system using surface-enhanced Raman scattering

Joowon Park,[a] Jinyoung Kim,[a] Chaewon Park,[a] Jong-Woo Lim,[b] Minjoo Yeom,[b] Daesub Song,[b] Eunjung Kim*[c,d] and Seungjoo Haam*[a]

[a]Department of Chemical and Biomolecular Engineering, Yonsei University, Seoul 03722, Republic of Korea

[b]Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea

[c]Division of Bioengineering, Incheon National University, Incheon 22012, Republic of Korea

[d]Department of Bioengineering and Nano-Bioengineering, Research Center for Bio Materials and Process Development, Incheon National University, Incheon 22012, Republic of Korea

*Corresponding authors.

E-mail addresses: e.kim@inu.ac.kr (E. Kim), haam@yonsei.ac.kr (S. Haam)

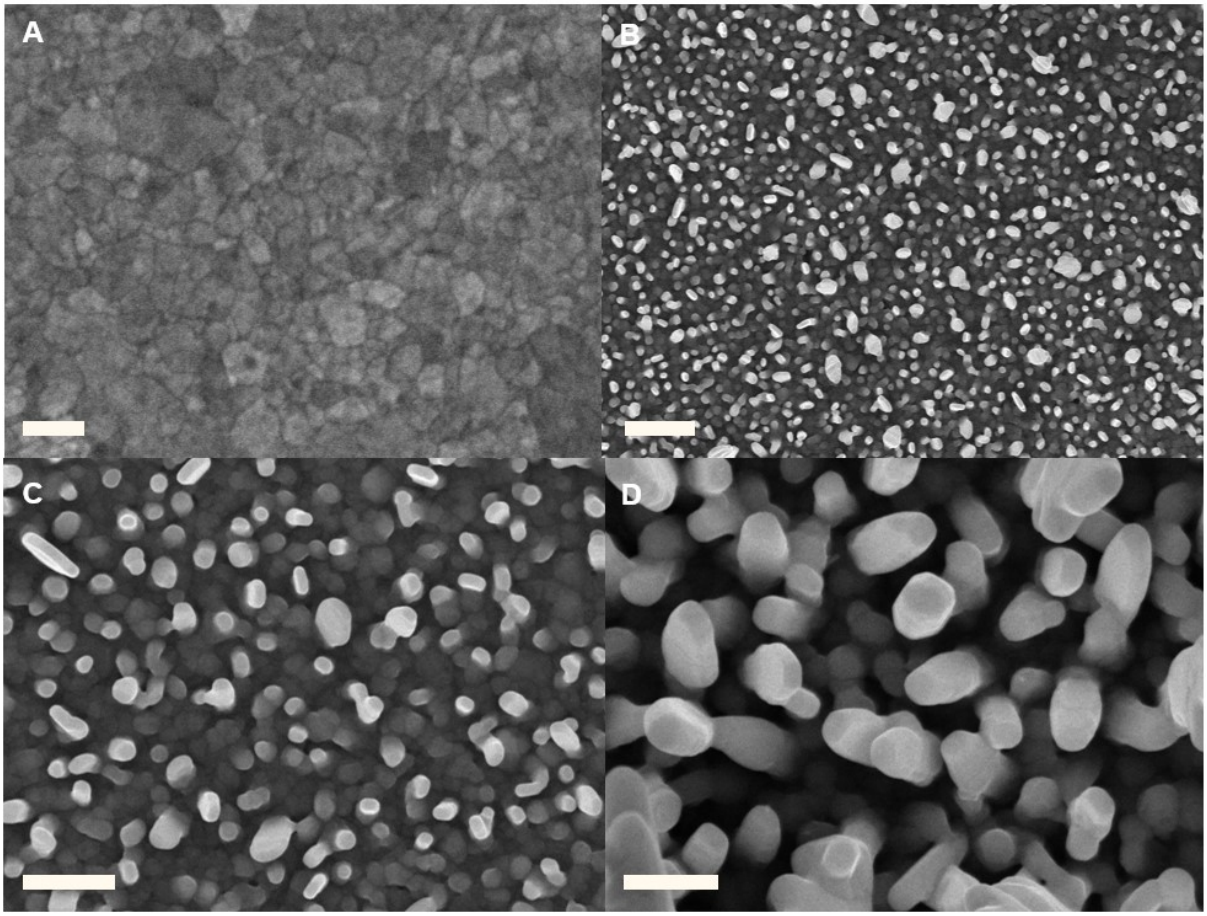

Figure S1. Representative scanning electron microscopy (SEM) images of (A) naked gold and (B-D) silver nanopillar SERS substrates at various magnifications. Scale bars are (A) 100 nm, (B) 1 μm, (C) 500 nm, and (D) 300 nm.

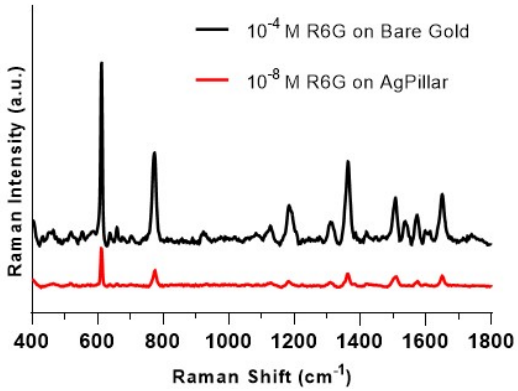

Figure S2. SERS spectra of aqueous R6G solution (10^{-4} and 10^{-8} M) placed on bare gold substrates presenting no silver nanopillars and silver nanopillars-grown substrates, respectively. Based on these spectra, the SERS intensity enhancement factor was estimated.

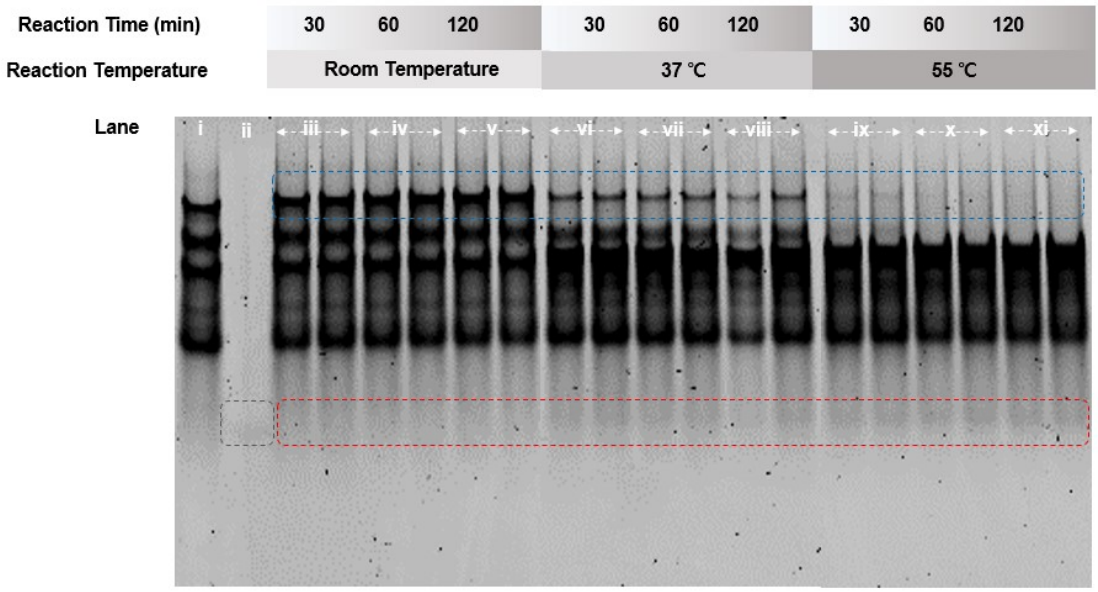

Figure S3. Native PAGE analysis results showing FEN1 cleavage activity with varying reaction times and temperatures. 5FP/3FP/synTarget complexes were treated without (lane i) and with 0.254 unit/μL of FEN1 (lanes from iii to xi). Lane ii shows synthetic 5F. FEN1 reactions were conducted at various temperatures, including 25℃ (lanes iii, iv, and v), 37℃ (lanes vi, vii, and viii), and 55℃ (lanes ix, x, and xi), for different reaction times, including 30 min (lanes iii, vi, and ix), 60 min (lanes iv, vii, and x), and 120 min (lanes v, viii, and xi), respectively. Two independent experiments were performed and analyzed. Boxes with black, red, and blue dashed lines indicate synthetic 5F, cleaved 5F, and 5FP/3FP/synTarget complexes, respectively.

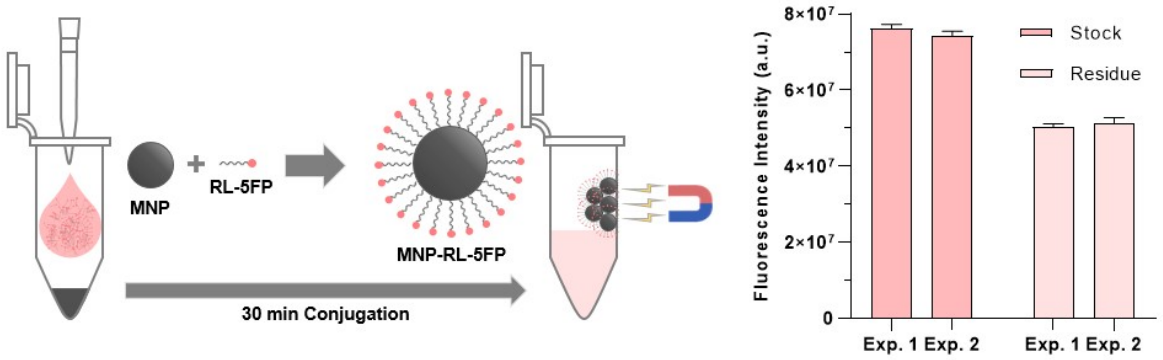

Figure S4. Fluorescent signal changes of RL-5FP solution before and after two independent conjugation experiments with streptavidin-conjugated MNPs. Stock and residue columns indicate the fluorescent intensity of initial and residual RL-5FP solutions after coupling with MNPs, respectively. Data and error bars represent mean ± standard deviation for three independent measurements.

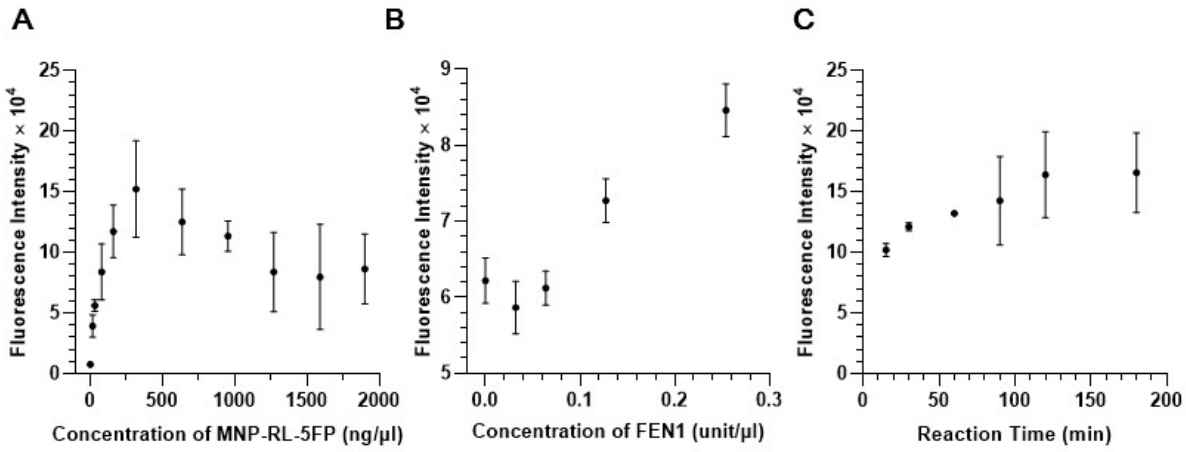

Figure S5. Optimization of the FEN1 reaction. (A) Various concentrations of MNP-RL-5FP and (B) FEN1, and (C) reaction time. Data and error bars represent mean ± standard deviation of three independent experiments.

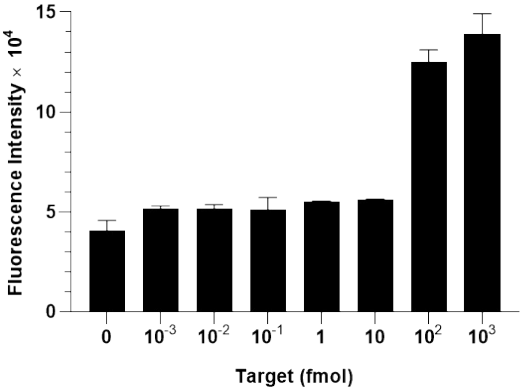

Figure S6. Fluorescent signals of RL-5F obtained from the FEN1 reaction with various synTarget concentrations. Data and error bars represent mean ± standard deviation of three independent measurements.

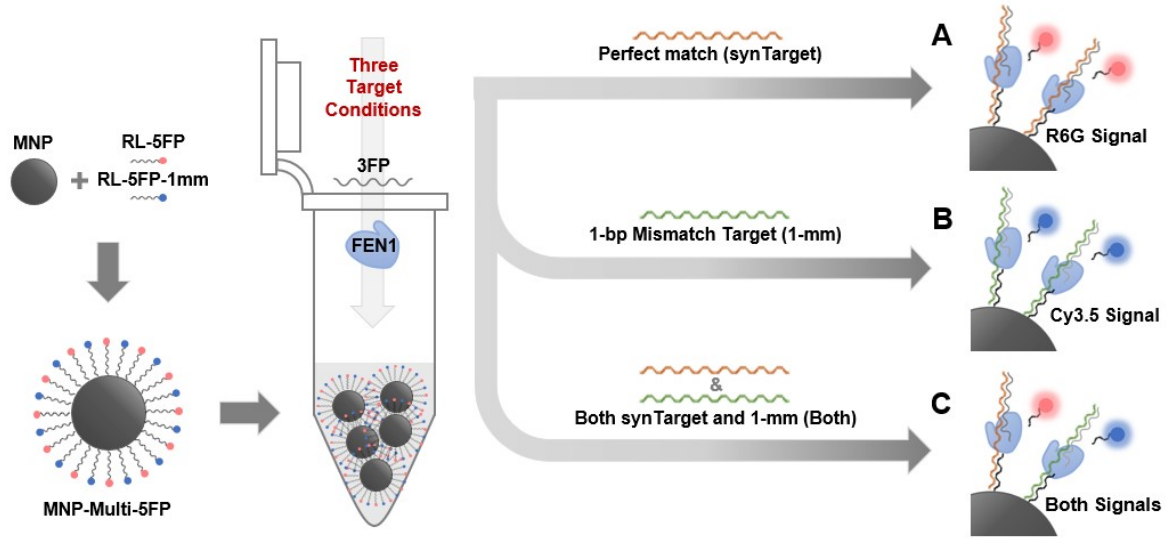

Figure S7. Schematic illustration of FEN1-mediated cleavage of probe DNA with samples containing different combinations of target DNA strands: (A) synTarget only, (B) 1-bp mismatch target only, and (C) both synTarget and 1-mm targets. For multiplexed detection of two targets, the MNPs were modified with two different RL-5FP, including RL-5FP and RL-5FP-1mm, specific to synTarget and 1-mm target. The formed MNP was termed 'MNP-Multi-5FP'.

Table S1. Oligonucleotide sequences used in this work.

Name	Sequences (5' to 3')
Synthetic Target (synTarget)	GGTGACAAGATTGGTCTTGTCTTTAGCCATTCCATGAGAGCCTCAAGAT
Raman tag-labeled 5'-flap provider (RL-5FP)	[R6G] – CAGCAGCAGCAGCAGAGACAAGACCAATCTTGTCACCAAAAAAAAA - [biotin]
5'-flap provider (5FP)	CAGCAGCAGCAGCAGAGACAAGACCAATCTTGTCACCAAAAAAAAA
Synthetic 5'-flap (Synthetic 5F)	CAGCAGCAGCAGCAG
3'-flap provider (3FP)	ATCTTGAGGCTCTCATGGAATGGCTAAC
1-bp mismatch (1-mm)	GGTGACAAGATTGGTCTTGTCGTTAGCCATTCCATGAGAGCCTCAAGAT
RL-5FP desinged for 1-mm (RL-5FP-1mm)	[Cy3.5] – CAGCAGCAGCAGCAGCGACAAGACCAATCTTGTCACCAAAAAAAAA - [biotin]
3FP designed for 1-mm (3FP-1mm)	ATCTTGAGGCTCTCATGGAATGGCTAAA
2-bp mismatch (2-mm)	GGTGACAAGATTGGTCTTGTCGGTAGCCATTCCATGAGAGCCTCAAGAT
3-bp mismatch (3-mm)	GGTGACAAGATTGGTCTTGTAGGTAGCCATTCCATGAGAGCCTCAAGAT
4-bp mismatch (4-mm)	GGTGACAAGATTGGTCTTGTAGGGAGCCATTCCATGAGAGCCTCAAGAT
5-bp mismatch (5-mm)	GGTGACAAGATTGGTCTTGGAGGGAGCCATTCCATGAGAGCCTCAAGAT
Scrambled DNA (Scr)	AAAGGGTCCATGCCGTAATGAACTTCTGCTAGTTACTGTATTGCGACTG