

# **Electronic Supplementary Information**

## Digestion of individual DNA molecules by $\lambda$ -exonuclease at liquid-solid interface†

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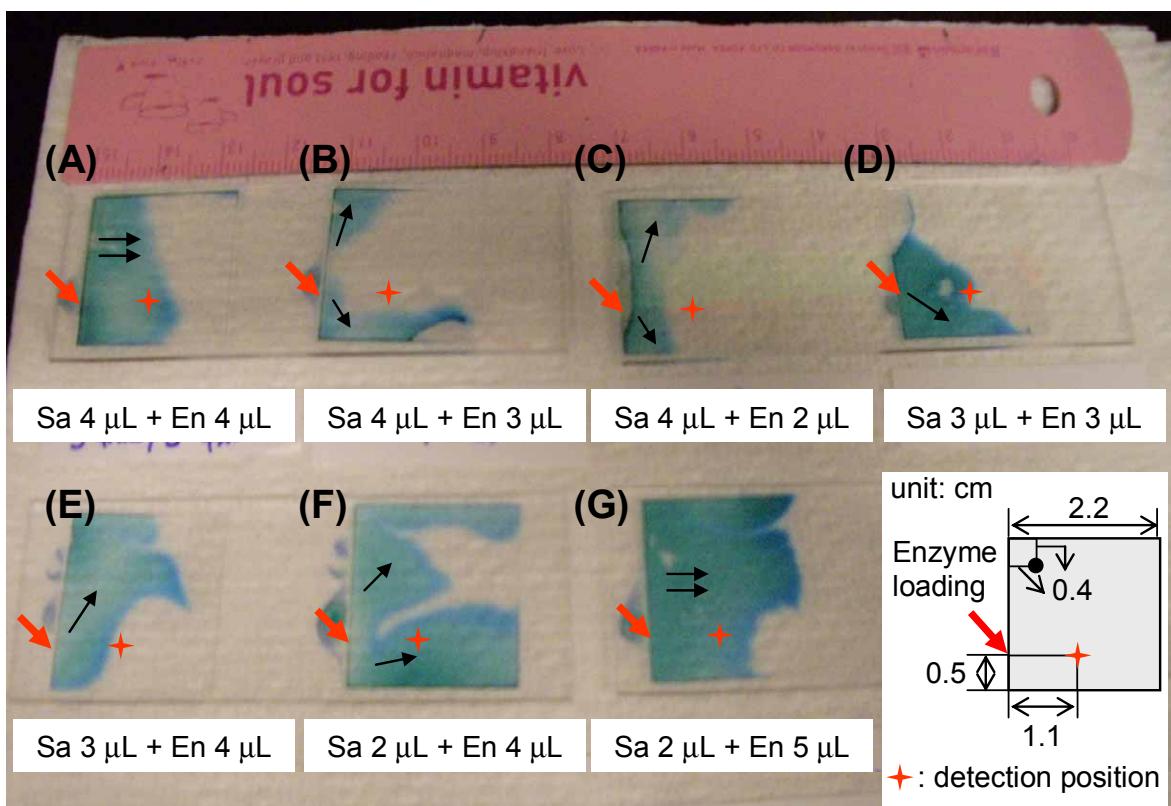
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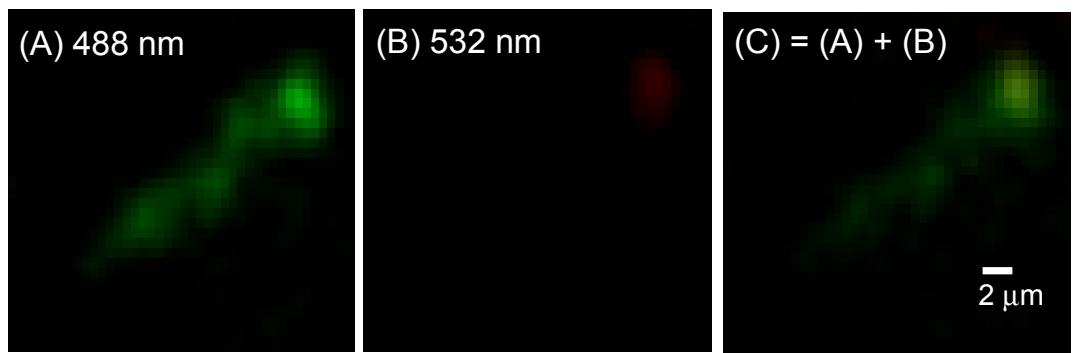
The AVI movie files show the progress of the type I (movie M1), type II (movie M2), and type III (movie M3) digestion modes of single-DNA molecules by the  $\lambda$ -exonuclease enzyme in Fig. 5. The movies confirmed that the digestion started from both ends or only from one end of the molecule. The experimental conditions were the same as the ones detailed in the legend of Fig. 5.

**Table S1** Final pH of the mixed enzyme digestion solution based on the ratios of the 2.5 mM sodium acetate buffer with various pH (A) in the 1× enzyme reaction buffer that has a pH of 9.40 (B).

The final pH of mixed solution		A. 2.5 mM sodium acetate buffer (pH)	B. 1× enzyme reaction buffer (pH)
A:B = 1:1 (v/v)	A:B = 2:1 (v/v)		
5.58	4.58	3.40	9.40
5.87	4.78	3.73	9.40
9.14	8.96	4.00	9.40
9.21	9.10	4.20	9.40
9.21	9.11	4.25	9.40
9.22	9.11	4.51	9.40
9.26	9.17	4.84	9.40
9.26	9.20	5.06	9.40
9.28	9.20	5.53	9.40

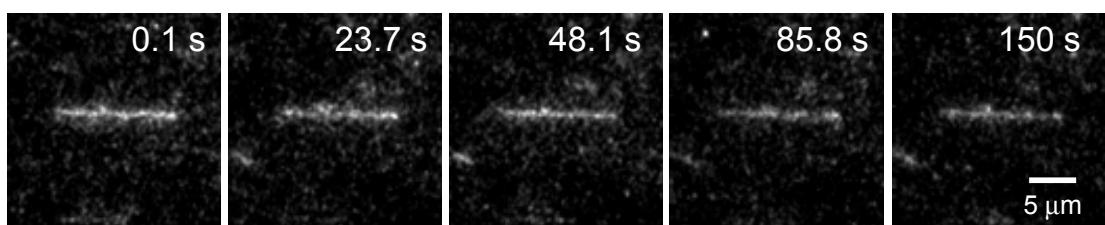


**Fig. S1** When the enzyme was injected after sample loading, the enzyme solution had different mixing rates according to the solution (DNA and enzyme) volume. Indicator: Sa, sample ( $\lambda$ -DNA in 2.5 mM sodium acetate buffer, pH 4.2); En,  $\lambda$ -exonuclease in 1 $\times$  enzyme reaction buffer (pH 9.4) including bromocresol green; red arrow, enzyme loading position; black arrow, diffusion direction of 1 $\times$  enzyme reaction buffer with bromocresol green; red cross, detection position; black circle, sample loading position. The white box shows the enzyme and DNA loading site and the detection position of the objective lens.

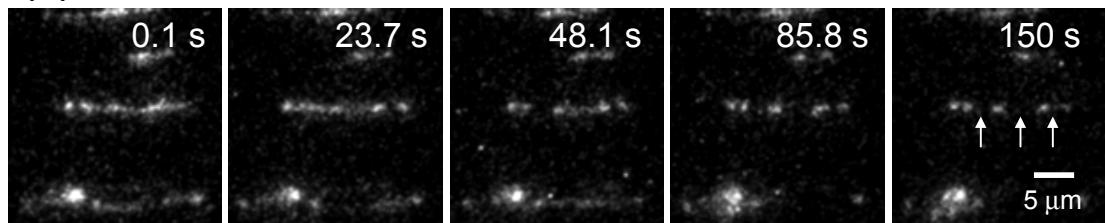


**Fig. S2** Representative dual-color TIRFM images of the  $\lambda$ -DNA molecules that were intercalated with YOYO-1 (A, green),  $\lambda$ -exonuclease enzyme that was labeled with Alexa Fluor<sup>®</sup>532 (B, red) at excitation wavelengths of 488 nm and 532 nm, respectively, and colocalization (C, yellow).

**(A) 1:50**



**(B) 1:5**



**Fig. S3** Real-time TIRFM images showing the photocleavage of λ-DNA at dye:bp ratios of (A) 1:50 and (B) 1:5. The conditions were the same as in Fig. 5. The white arrows show the photocleavage of λ-DNA labeled with YOYO-1.