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Title	Monitoring intracellular degradation of exogenous DNA using diffusion properties
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Title
Monitoring intracellular degradation of exogenous DNA using diffusion properties

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Supplementary Data
Supplementary Methods
Conventional analysis of fluorescence correlation spectroscopy and cross-correlation spectroscopy Acquired $G(\tau)$ were generally fitted by a one- (i = 1) or two-component model (i = 2) as

$$G(\tau) = 1 + \frac{1 - f + f\exp(-\tau/\tau_t)}{N(1-f)} \sum_i F_i \left(1 + \frac{\tau}{\tau_i}\right)^{-1} \left(1 + \frac{\tau}{s^2\tau_i}\right)^{-1/2} \quad (1)$$

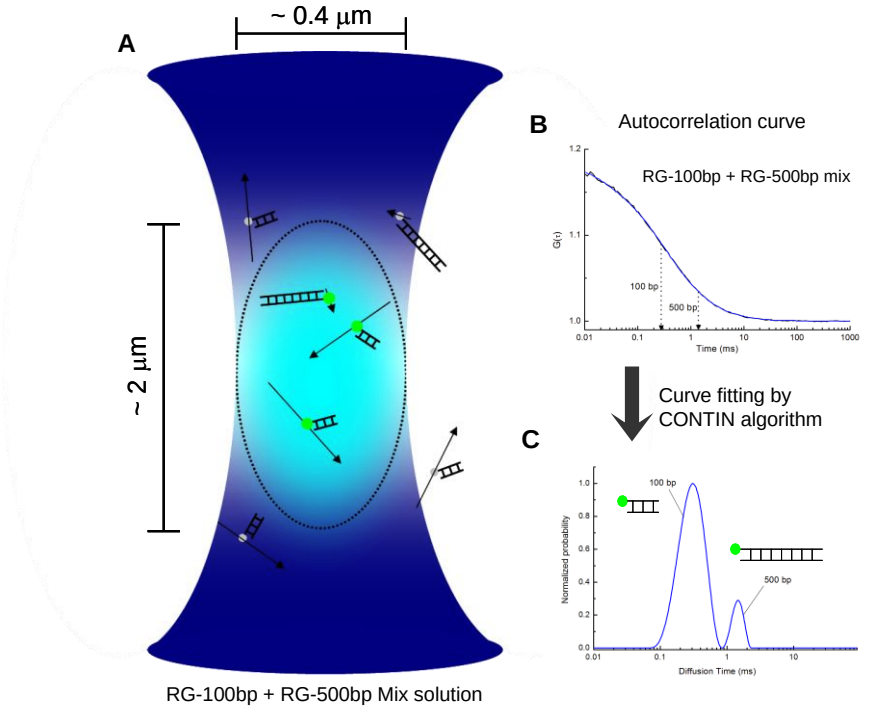
where F_i and τ_i are the fraction and diffusion time of component i, respectively. N is the average number of fluorescent particles in the detection volume defined by radius ω and length 2z, and s is the structural parameter representing the ratio $s = z/\omega$. The diffusion time (τ_i) corresponds to the average time for diffusion of fluorescent particles across the detection area, which reflects the size of particles. f is the average fraction of triplet state molecules and τ_t is triplet relaxation time.
The triplet term of the cross-correlation curve disappeared because triplet signals from different dyes (i.e., RG and Cy5) were not correlated with each other. Therefore, the cross-correlation curve was fitted by a model equation that did not include the triplet term. The average numbers of red fluorescent particles (N_r), green fluorescent particles (N_g), and particles that have both red and green fluorescence (N_c) can be calculated by

$$N_r = \frac{1}{G_r(0) - 1}, \quad N_g = \frac{1}{G_g(0) - 1}, \text{ and } \quad N_c = \frac{G_c(0) - 1}{(G_r(0) - 1) \cdot (G_g(0) - 1)} \quad (2)$$

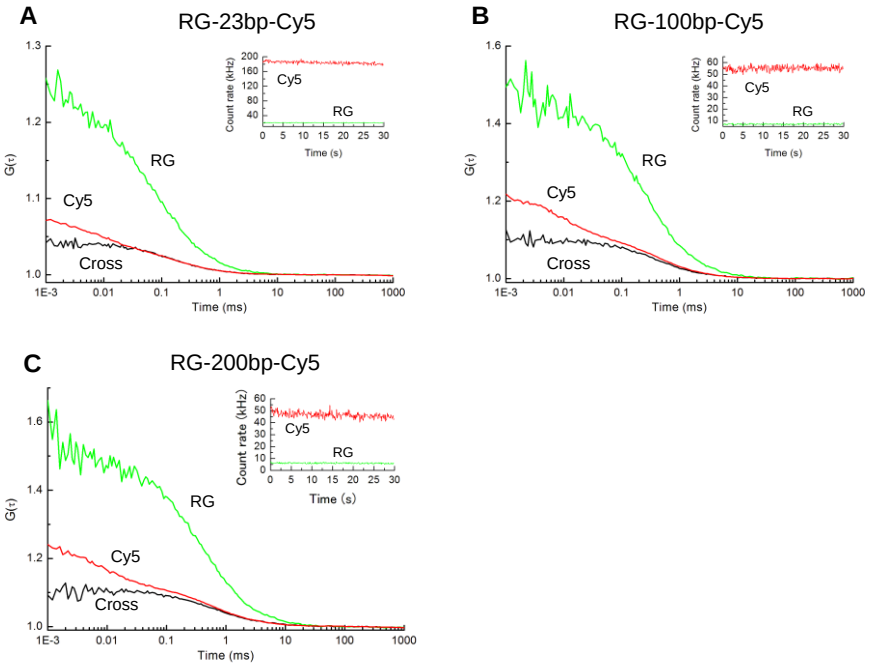
respectively. When N_r and N_g are constant, (G_c(0)–1) is directly proportional to N_c. For quantitative evaluation of cross-correlations among various samples, G_c(0)–1 is normalized by G_r(0)–1 (relative cross-correlation amplitude; (G_c(0)–1)/(G_r(0)–1)).

$$RCA = N_c \Big/ N_g = \frac{G_c(0) - 1}{G_r(0) - 1} \quad (3)$$

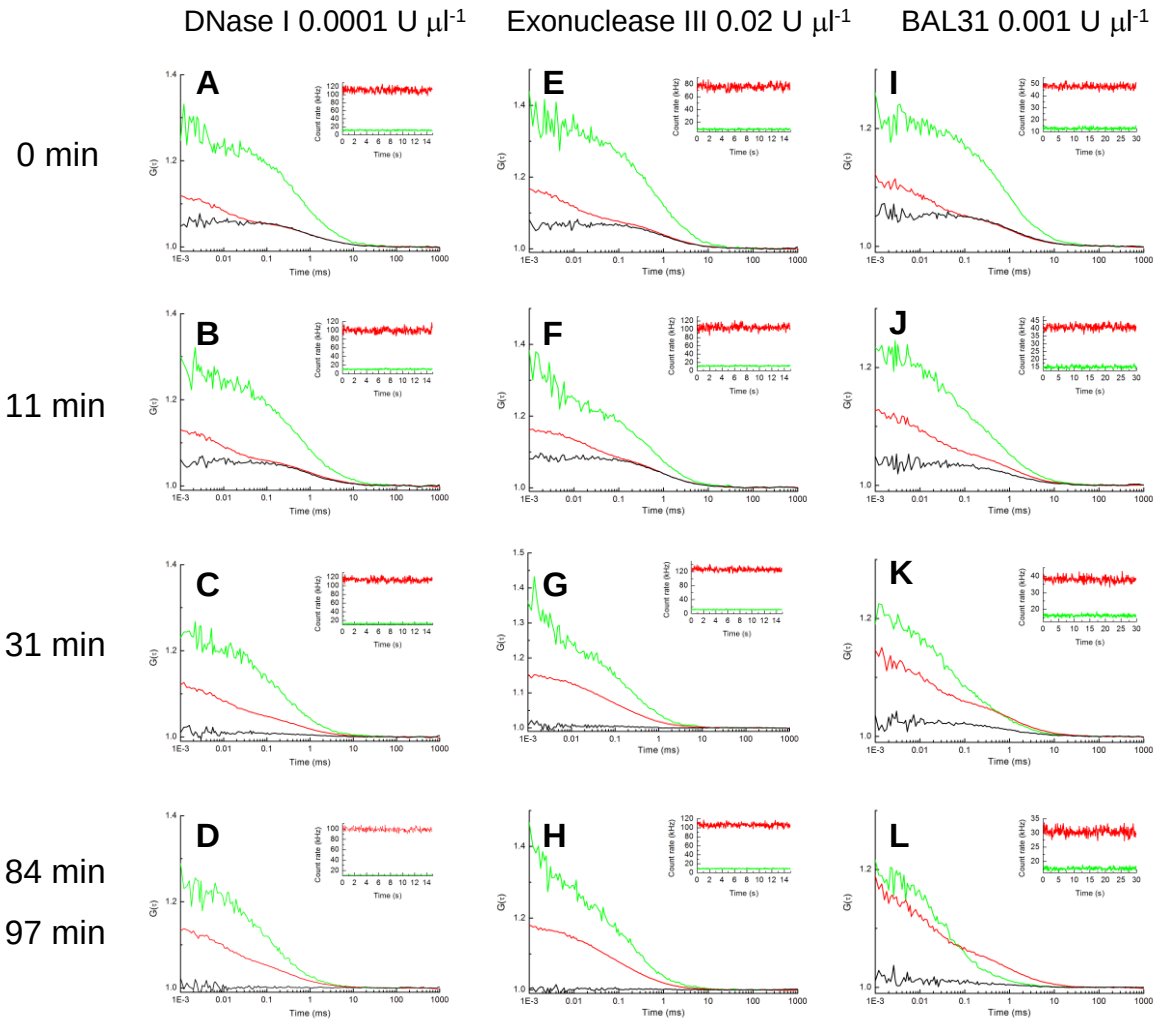
Supplementary Figures



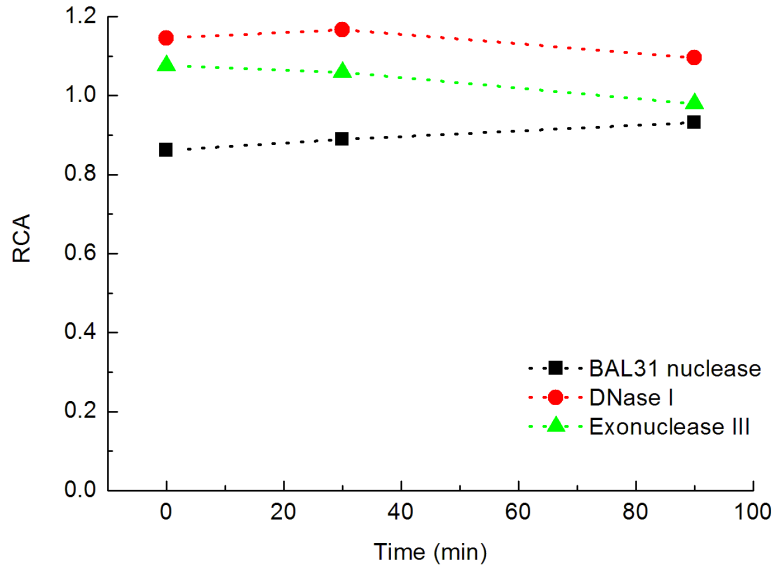
Supplementary Fig. S1. Confocal volume of FCS and CONTIN analysis. (**A**) Diagram of DNA diffusion in the defined confocal volume. (**B**) Autocorrelation curve of the mixture of RG-100bp and RG-500bp. (**C**) Distributions of diffusion times calculated from the autocorrelation curve using the CONTIN algorithm.



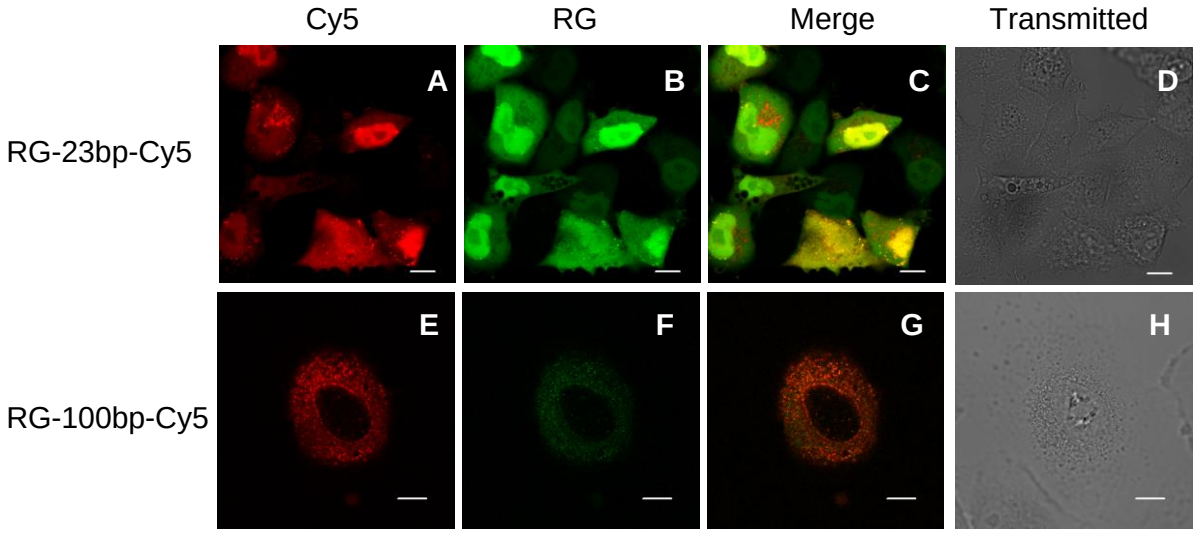
Supplementary Fig. S2. Characterization of double-fluorescent-labeled DNAs in solution. Auto- and cross-correlation curves of (**A**) RG-23bp-Cy5 (**B**) RG-100bp-Cy5 (**C**) RG-200bp-Cy5 in 10 mM Tris-HCl (pH 7.5). (Insets) The fluorescence intensities in red and green channels during FCCS measurement. RG, Cy5 and Cross represent $G_g(\tau)$, $G_r(\tau)$ and $G_c(\tau)$, respectively.



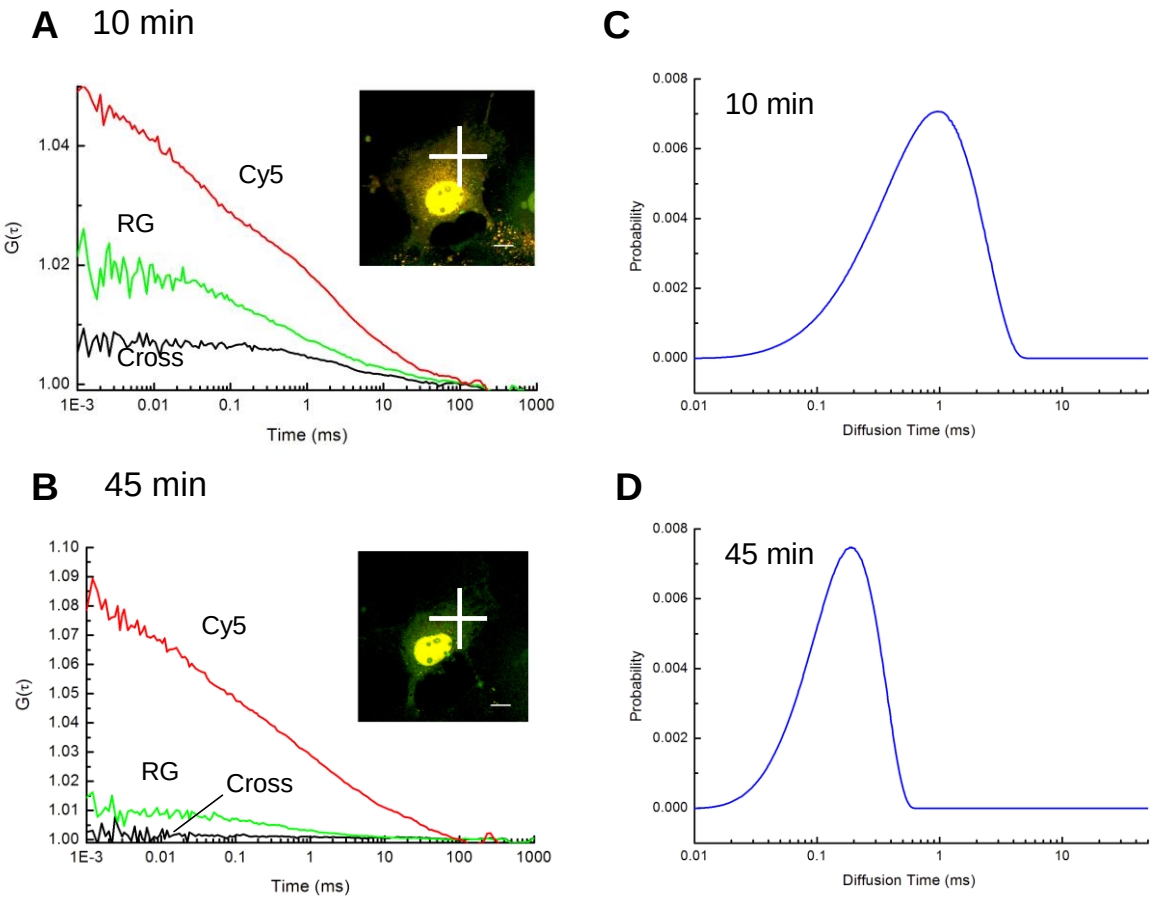
Supplementary Fig. S3. Real-time monitoring of DNA degradation in 50 μl DNA solutions. Auto- and cross-correlation curves (green; $G_g(\tau)$, red; $G_r(\tau)$, black; $G_c(\tau)$) after the addition of (A–D) 0.0001 U μl^{-1} DNase I, (E–H) 0.02 U μl^{-1} exonuclease III, (I–L) 0.001 U μl^{-1} BAL31 nuclease. The time points represent the initial (11 min), transient (31 min) and stable phases (84–97 min).



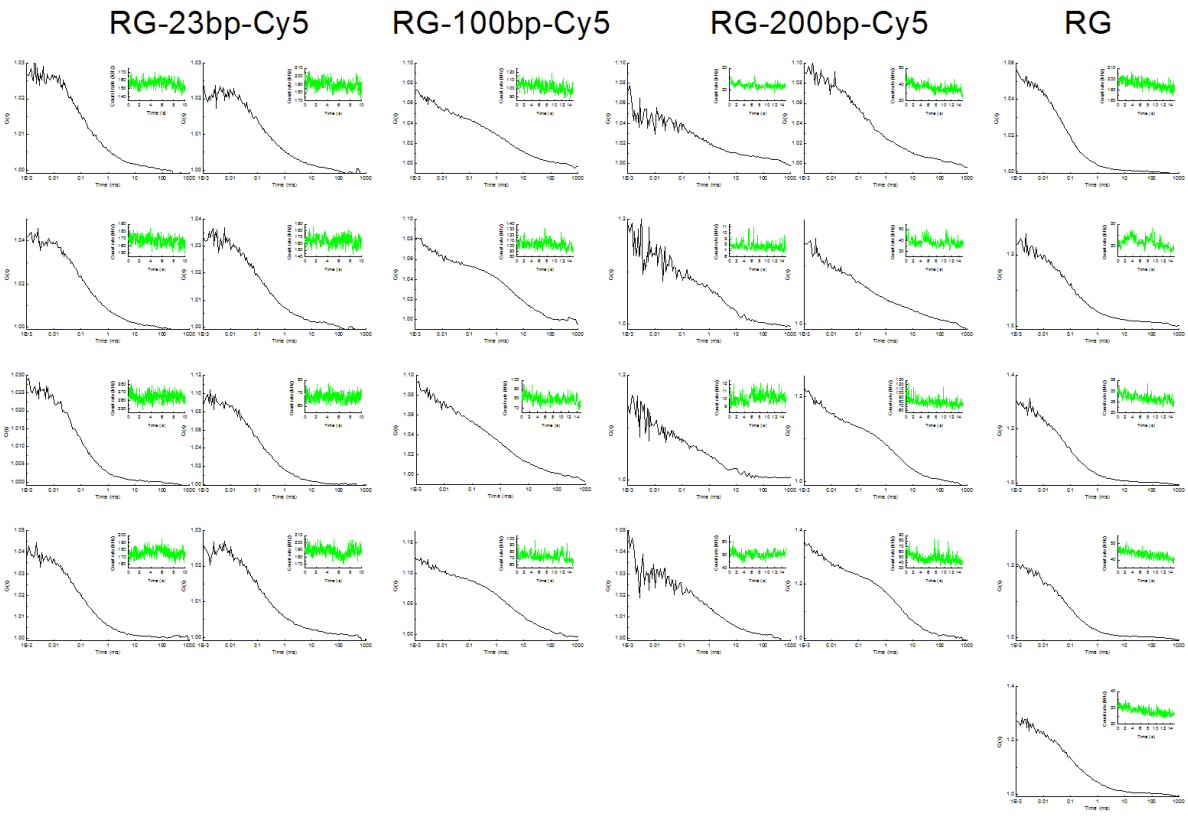
Supplementary Fig. S4. Relative cross-correlation amplitudes in DNase I buffer (red symbols), exonuclease III buffer (green symbols) and BAL31 nuclease buffer (black symbols) without addition of nucleases.



Supplementary Fig. S5. LSM images of HeLa cells 30 min after loading of RG-23bp-Cy5 (**A**–**D**) or RG-100bp-Cy5 (**E**–**H**). Green (**A, E**), red (**B, F**), merged fluorescence images (**C, G**) and transmitted images (**D, H**), respectively. Scale bar represents 10 μm.



Supplementary Fig. S6. Nuclease degradation of double-fluorescent-labeled DNAs in living COS7 cytoplasm. Auto- and cross-correlation curves of RG-23bp-Cy5 DNA (**A**) 10 min and (**B**) 45 min after loading DNAs in the same cell. FCCS measurements were carried out at the crosshairs in each LSM image (insets). Scale bar represents 10 μm. RG, Cy5 and Cross represent $G_g(\tau)$, $G_r(\tau)$ and $G_c(\tau)$, respectively. Distributions of diffusion times $P(\tau D_i)$ of DNAs calculated from (**C**) $G_g(\tau)$ in panel **A**, (**D**) $G_g(\tau)$ in panel **B**.



Supplementary Fig. S7. Autocorrelation curves $G_g(\tau)$ of RG-DNA-Cy5 in HeLa cytoplasm at 30 min after DNA loading. RG dye was also tested as a reference.