

## Electronic Supplementary Material

# Cationic copolymer and crowding agent have a cooperative effect on a Na<sup>+</sup>-dependent DNzyme

Jun Wang, He Huang, Orakan Hanpanich, Naohiko Shimada, and Atsushi Maruyama\*

Department of Life Science and Technology, Tokyo Institute of Technology, Nagatsuta-cho 4259 B-57, Midori, Yokohama 226-8501, Japan

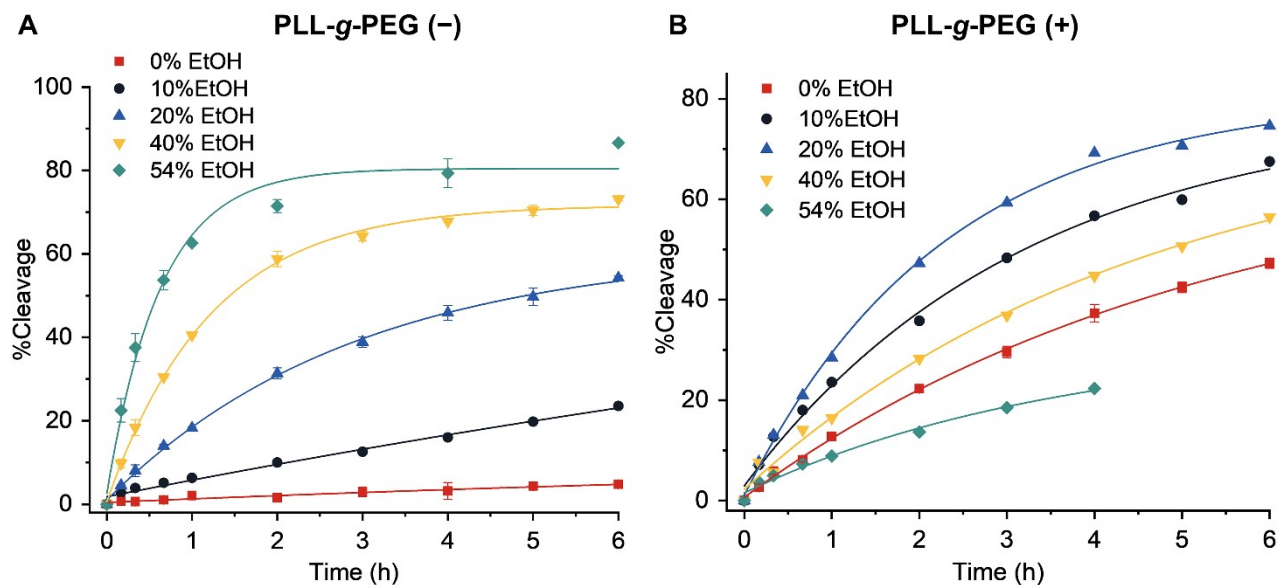
\*Corresponding author. Email: [amaruyama@bio.titech.ac.jp](mailto:amaruyama@bio.titech.ac.jp)

### Experimental Methods

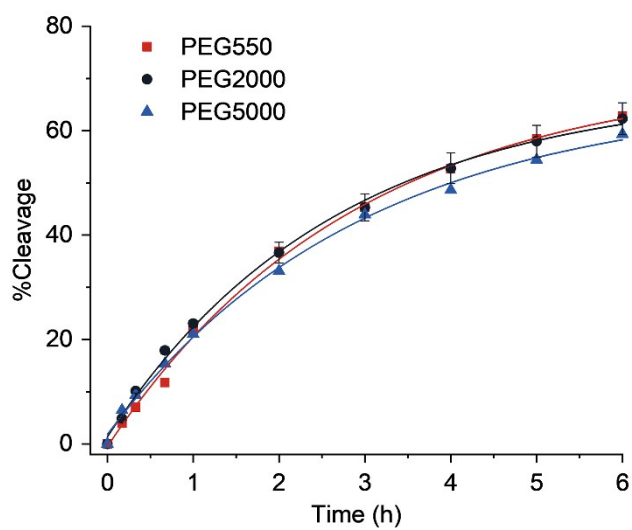
#### Synthesis of cationic copolymers

The cationic copolymers poly(L-lysine)-*graft*-dextran (PLL-*g*-Dex) and poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) were prepared according to previously reported protocols.<sup>[1]</sup> Briefly, the reduced end of dextran or poly(ethylene glycol) was covalently conjugated to the amino group of poly(L-lysine) in the presence of sodium cyanoborohydride as a reducing agent in 0.1 M sodium borate buffer (pH 8.5). Unreacted dextran or poly(ethylene glycol) was removed by ion-exchange chromatography. The copolymers were purified by dialysis and then freeze-dried. The products were characterized by <sup>1</sup>H NMR (Bruker Avance 400) at 60 °C and by gel permeation chromatography (Jasco). The grafting degree of dextran was 90 wt% for PLL-*g*-Dex, and the grafting degree of poly(ethylene glycol) was 80 wt% for PLL-*g*-PEG.

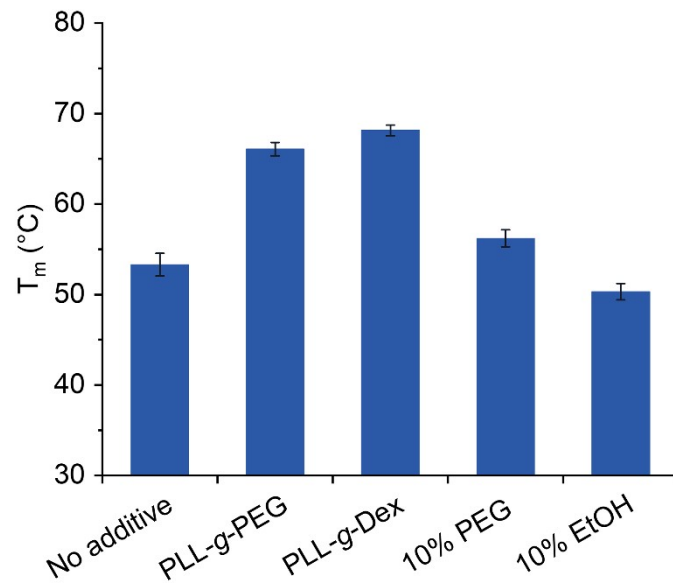
## Supporting Figures



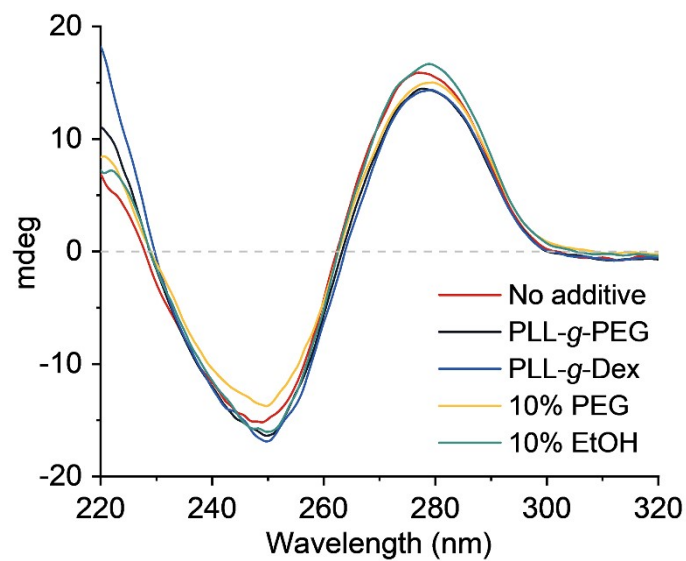
**Fig. S1** Ethanol concentration dependence of DNase activity in the (A) absence or (B) presence of PLL-g-PEG.



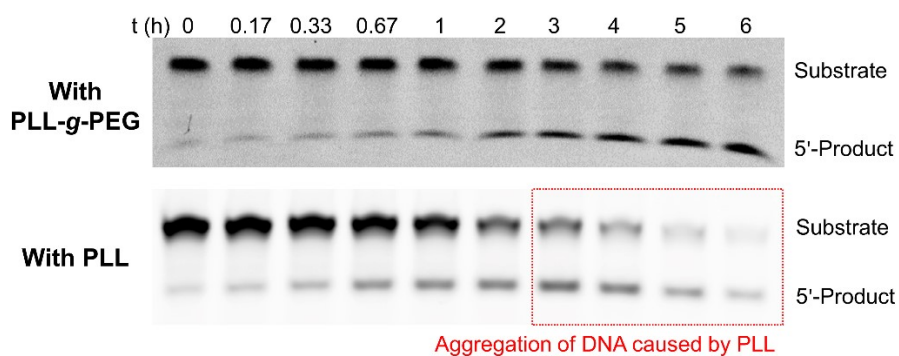
**Fig. S2** Effect of molecular weight of PEG on the cleavage activity of DNase.



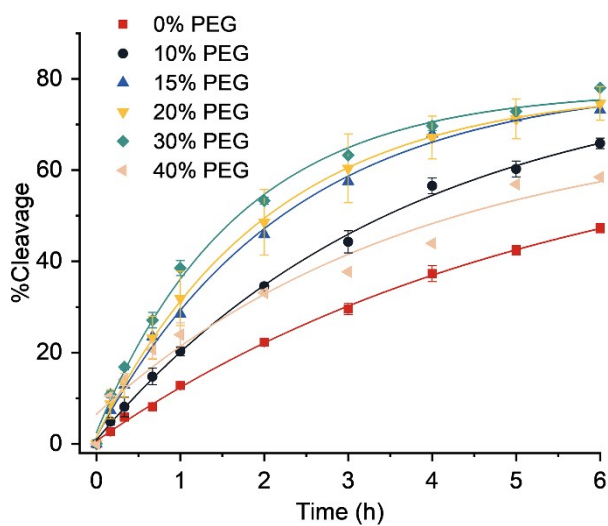
**Fig. S3** The melting temperature ( $T_m$ ) of the DNAzyme/substrate complex without any additives or with PLL-g-PEG, PLL-g-Dex, 10% PEG, 10% EtOH as additive, respectively. Samples contained 1  $\mu$ M EtNa DNAzyme and 1  $\mu$ M substrate in MES buffer (50 mM MES, 120 mM NaCl, pH6.0) and indicated additive.



**Fig. S4** The circular dichroism (CD) spectra of the DNAzyme/substrate complex without any additives or with PLL-g-PEG, PLL-g-Dex, 10% PEG, 10% EtOH as additive, respectively. Samples contained 2  $\mu$ M EtNa DNAzyme and 2  $\mu$ M substrate in MES buffer (50 mM MES, 120 mM NaCl, pH6.0) and indicated additive.



**Fig. S5** Gel characterization of EtNa DNase in the presence of PLL-g-PEG or PLL homopolymer. The PLL homopolymer results in the aggregation of DNA due to the formation of insoluble complex, thereby hindering the DNase reaction.



**Fig. S6** PEG concentration dependence of DNase activity in the presence of PLL-g-PEG.