Supplementary Information

Molecular crowding induces primer extension by RNA polymerase through base stacking beyond Watson-Crick rules

Shuntaro Takahashi,[a] Hiromichi Okura,[a] Chilka Pallavi,[a] Saptarshi Ghosh[a] and Naoki Sugimoto*[ab]

[a]FIBER (Frontier Institute for Biomolecular Engineering Research), Konan University, 7-1-20 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan

[b]FIRST (Graduate School of Frontiers of Innovative Research in Science and Technology), Konan University, 7-1-20 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan

Corresponding Author
*E-mail: sugimoto@konan-u.ac.jp

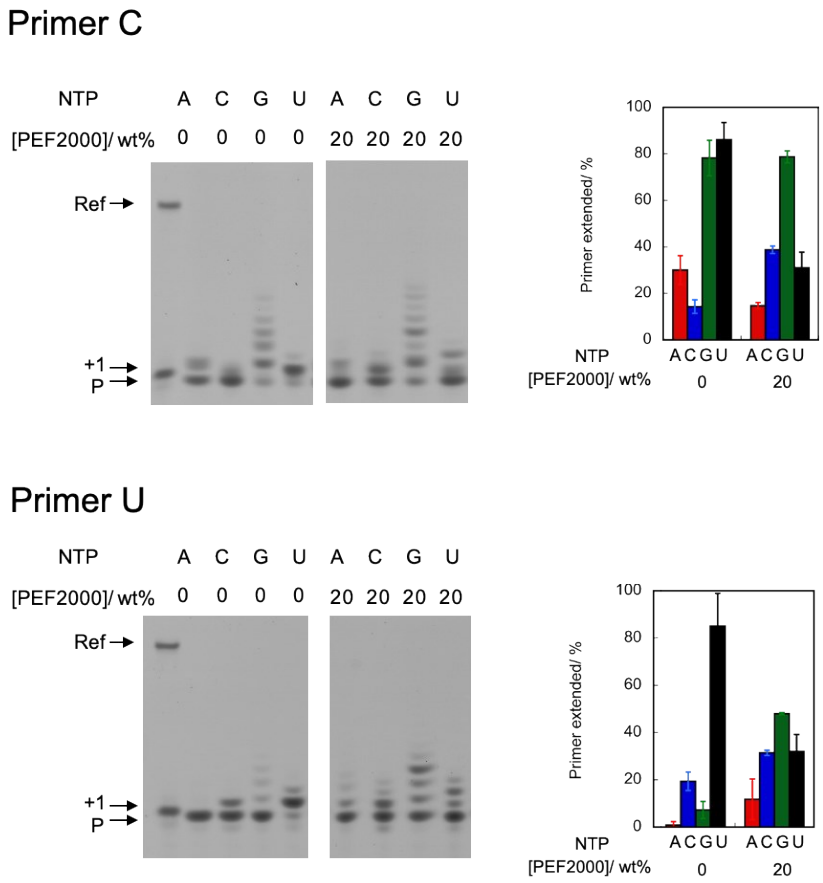


Fig. S1 Denaturing PAGE analysis of extended primers using RNA primer C and RNA primer U in 0 wt% PEG2000 and 20 wt% PEG2000. All samples were incubated in 50 mM Tris-HCl (pH 8.0) and 2 mM $MgCl_2$ at 25 °C for 1 hour.

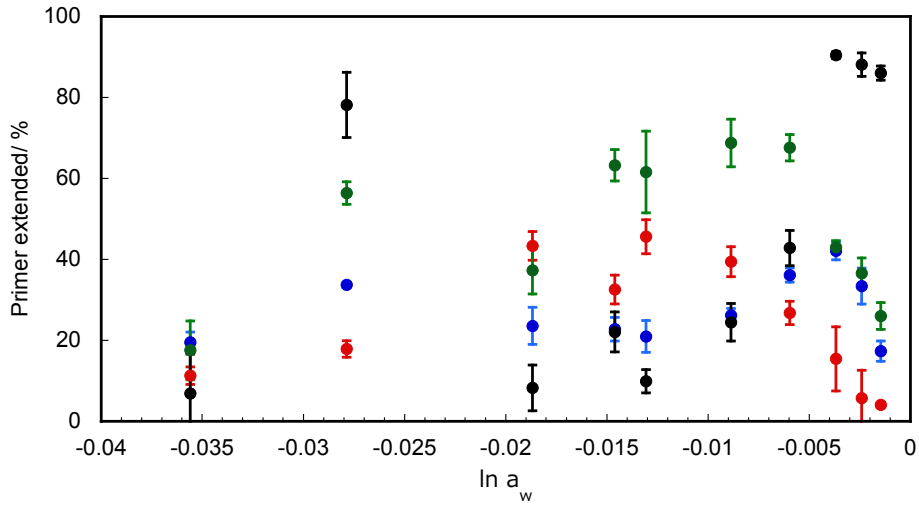
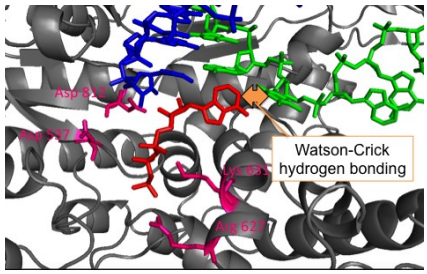
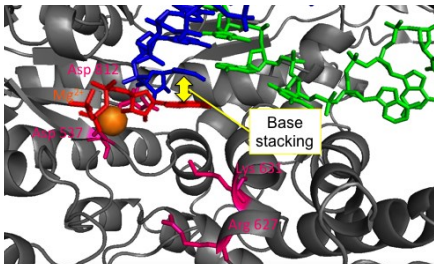


Fig. S2 Effect of water activity (a_w) on primer extension in the presence of 20 wt% EG, 20 wt% PEG200, or 0-40 wt% PEG2000. Primers extended with ATP, CTP, GTP, and UTP are indicated left to right in red, blue, green, and black colours, respectively. All samples were incubated in 50 mM Tris-HCl (pH 8.0) and 2 mM $MgCl_2$ at 25 °C for 1 hour.

(A)





(B)

Fig. S3 (A) Schematic structure of substrate-incorporated polymerase complex with template (green), primer (blue), and substrate (red) at the initial binding site in the absence of crowding reagents. (Structural data is taken from PDB ID: 1S0V) (B) Plausible structure of substrate-incorporated polymerase complex at the Mg^{2+} binding site in the presence of crowding reagents. The substrate position was moved to the Mg^{2+} binding site. The modified structural image was drawn by Pymol software.

Table S1 Water activity (a_w) of the reaction solution

Crowder	[Crowder] (wt%)	Water potential*	ln a_w
PEG2000	0	-202	-0.0015
PEG2000	5	-332	-0.0024
PEG2000	10	-505	-0.0037
PEG2000	15	-821	-0.0060
PEG2000	20	-1221	-0.0089
PEG2000	25	-1798	-0.0131
PEG2000	30	-2572	-0.0187
PEG2000	40	-4902	-0.0356
PEG200	20	-3837	-0.0279
PEG600	20	-2012	-0.0146

*Solutions containing 50 mM Tris (pH 8.0) and 0-40 wt% crowders were assayed at 25°C.

Table S2 Dielectric constant (ε_r) of the reaction solution

Crowder	[Crowder] (wt%)	ε_r*	ε_r^{-1}
PEG2000	0	76.8	0.0130
PEG2000	5	64.1	0.0156
PEG2000	10	62.7	0.0159
PEG2000	15	60.9	0.0164
PEG2000	20	59.9	0.0167
PEG2000	25	58.6	0.0171
PEG2000	30	57.3	0.0175
PEG2000	40	54.6	0.0183
EG	20	70.0	0.0143
PEG200	20	62.5	0.0160
PEG600	20	60.7	0.0165

*Solutions containing 50 mM Tris (pH 8.0), 2 mM $MgCl_2$, and 0-40 wt% crowders were measured at 25°C.