Supplementary information for

"Exploring both sequence detection and restriction endonuclease cleavage kinetics by recognition site via single-molecule microfluidic trapping"

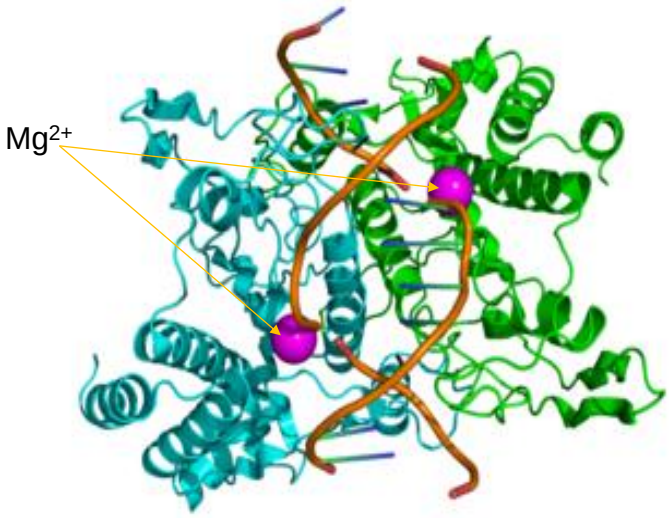

Fig. S1. EcoRI dimer (cyan and green cartoon diagram)-dsDNA (brown tubes) complex with cofactor Mg^{2+}. On each subunit of EcoRI there is one Mg^{2+} bound, so there is a total of two Mg^{2+} ions bound per target site. From "http://www.thenakedscientists.com/HTML/content/interviews/interview/1238/".

Single molecule theory for the restriction endonuclease cleavage kinetics with DNA:

Based on the setup of our single molecule experiments for the kinetic study of restriction endonuclease EcoRI with dsDNA, we see evidence in the waiting time distributions that there are two main processes occurring. One is the Mg^{2+} binding with the enzyme, the other is the cleavage of dsDNA. Based on previous knowledge, the following general mechanism can be proposed:

$$\mathrm{DE} + \mathrm{Mg}^{2+} \xrightarrow{k_{1a}} \mathrm{DE_{Mg}} + \mathrm{Mg}^{2+} \xrightarrow{k_{1b}} \mathrm{DE_{2Mg}} \xrightarrow{k_{2a}} \mathrm{P'E_{2Mg}} \xrightarrow{k_{2b}} \mathrm{PE_{2Mg}} \qquad \text{(S1)}$$

Here, DE represents the DNA-Enzyme complex, DE_{2Mg} the complex after the cofactor Mg^{2+} binds with enzyme, where the prefactor "2" is due to the dimeric structure of EcoRI in the DE complex, $P'E_{2Mg}$ represents the complex after the cleavage of the first strand of dsDNA, and PE_{2Mg} the complex after the cleavage of the second strand of dsDNA.

The single exponential distribution of waiting times we obtained at low [Mg^{2+}] (Fig. 2C) suggests the binding of the two Mg^{2+} ions takes place in essentially one step, so the above mechanism could be simplified to the following

$$\mathrm{DE} + 2\mathrm{Mg}^{2+} \xrightarrow{k_1} \mathrm{DE_{2Mg}} \xrightarrow{k_{2a}} \mathrm{P'E_{2Mg}} \xrightarrow{k_{2b}} \mathrm{PE_{2Mg}} \qquad \text{(S2)}$$

The single-molecule rate equations for these mechanisms are:

$$\frac{dP_{\mathrm{DE}}(t)}{dt} = -k_1^0 P_{\mathrm{DE}}(t) \qquad \text{(S3)}$$

$$\frac{dP_{DE_{2Mg}}(t)}{dt} = k_1^0 P_{DE}(t) - k_{2a} P_{DE_{2Mg}}(t) \quad (S4)$$

$$\frac{dP_{P'E_{2Mg}}(t)}{dt} = -k_{2b} P_{P'E_{2Mg}}(t) + k_{2a} P_{DE_{2Mg}}(t) \quad (S5)$$

$$\frac{dP_{PE_{2Mg}}(t)}{dt} = k_{2b} P_{P'E_{2Mg}}(t) \quad (S6)$$

Where the *P*(t)'s are the probabilities of finding the DNA-enzyme complex in the corresponding state at time t, $k_1^0 = k_1[Mg^{2+}]^2$ and is treated as a pseudo-first-order rate constant because $[Mg^{2+}]$ is time-independent in the single-molecule experiments. Equations S3 –S6 can be solved exactly using the initial conditions $P_{DE}(0) = 1$, $P_{DE_{2Mg}}(0) = 0$, $P_{P'E_{2Mg}}(0) = 0$, and $P_{PE_{2Mg}}(0) = 0$ with t=0 being the onset of each waiting time, and the constraint $P_{DE}(t) + P_{DE_{2Mg}}(t) + P_{P'E_{2Mg}}(t) + P_{PE_{2Mg}}(t) = 1$.

We consider the probability density function $f(\tau)$ of the waiting time τ. τ is the time needed to complete the reactions in Equation S1. The probability of finding a particular τ is $f(\tau)\bullet\Delta\tau$, which is equal to the probability for the DNA-enzyme complex to switch from the $P'E_{2Mg}$ state to the PE_{2Mg} state. The probability for this switching is $\Delta P_{PE_{2Mg}}(\tau)$, which equals $k_{2b} P_{P'E_{2Mg}}(\tau)\Delta\tau$. In the limit of infinitesimal $\Delta\tau$, we have

$$f(\tau) = k_{2b} P_{P'E_{2Mg}}(\tau) \quad (S7)$$

Solving eqs S3-S6 for $P_{P'E_{2Mg}}(\tau)$ using the initial conditions, we get

$$f(\tau) = k_1^0 k_{2a} k_{2b}\left(\frac{e^{-k_1^0 t}}{(k_1^0-k_{2a})(k_1^0-k_{2b})} - \frac{e^{-k_{2a}t}}{(k_1^0-k_{2a})(k_{2a}-k_{2b})} + \frac{e^{-k_{2b}t}}{(k_{2a}-k_{2b})(k_1^0-k_{2b})}\right) \quad (S8)$$

Then $$<\tau> = \int_{-\infty}^{+\infty} \tau f(\tau) d\tau = \frac{1}{k_1^0} + \frac{1}{k_{2a}} + \frac{1}{k_{2b}} \quad (S9)$$

And $$\frac{1}{<\tau>} = \frac{1}{\int_{-\infty}^{+\infty} \tau f(\tau) d\tau} = \frac{k_1[Mg^{2+}]^2 k_{2a} k_{2b}}{k_1[Mg^{2+}]^2(k_{2a}+k_{2b}) + k_{2a}k_{2b}} = \frac{[Mg^{2+}]^2}{[Mg^{2+}]^2\left(\frac{k_{2a}+k_{2b}}{k_{2a}k_{2b}}\right) + \frac{1}{k_1}} \quad (S10)$$

When $[Mg^{2+}] \to \infty$ or $k_1^0 \to \infty$,

$$f(\tau) = k_1^0 k_{2a} k_{2b}\left(\frac{e^{-k_1^0 t}}{(k_1^0-k_{2a})(k_1^0-k_{2b})} - \frac{e^{-k_{2a}t}}{(k_1^0-k_{2a})(k_{2a}-k_{2b})} + \frac{e^{-k_{2b}t}}{(k_{2a}-k_{2b})(k_1^0-k_{2b})}\right) \approx \frac{k_{2a}k_{2b}}{k_{2a}-k_{2b}}(e^{-k_{2b}t} - e^{-k_{2a}t}) \quad (S11)$$

Equation S11 shows at high $[Mg^{2+}]$, the $f(\tau)$ or the waiting time should be a double exponential if the DNA cleavage is a two step (k_{2a} and k_{2b}) process. Further if $k_{2a} \gg k_{2b}$ or $k_{2a} \ll k_{2b}$,
then $f(\tau)$ can be simplified to a single exponential function. This is consistent with our experimental data at high Mg^{2+} concentration, for example when $[Mg^{2+}]$=2.0 mM as is shown in Figure 2d.

Similarly if $k_{2a} \to \infty$, $f(\tau)$ can be simplified to a double exponential function:

$$f(\tau) = \frac{k_1^0 k_{2b}}{k_1^0 - k_{2b}}(e^{-k_{2b}t} - e^{-k_1^0 t}) \quad (S12)$$

Then $$<\tau>^{-1} = \frac{1}{\int_0^{\infty} \tau f(\tau) d\tau} = \frac{k_{2b}[Mg^{2+}]^2}{[Mg^{2+}]^2 + \frac{k_{2b}}{k_1}} \quad (S13)$$

Or when $k_{2b} \to \infty$, $f(\tau)$ as shown in eq. S8 can be simplified to a double exponential function:

$$f(\tau) = \frac{k_1^0 k_{2a}}{k_1^0 - k_{2a}}(e^{-k_{2a}t} - e^{-k_1^0 t}) \quad (S14)$$

Then $$<\tau>^{-1} = \frac{1}{\int_0^{\infty} \tau f(\tau) d\tau} = \frac{k_{2a}[Mg^{2+}]^2}{[Mg^{2+}]^2 + \frac{k_{2a}}{k_1}} \quad (S15)$$

In the process during the dsDNA cleavage, if both strands of DNA are cleaved at the same time with rate

constant k_2, then the mechanism could be further simplified to

$$DE + 2Mg^{2+} \overset{k_1}{\rightarrow} DE_{2Mg} \overset{k_2}{\rightarrow} PE_{2Mg} \qquad (S16)$$

In this case, $f(\tau)$ is the following:

$$f(\tau) = \frac{k_1^0 k_2}{k_1^0 - k_2}\left(e^{-k_2 t} - e^{-k_1^0 t}\right) \quad , \qquad (S17)$$

Then

$$< \tau >^{-1} = \frac{1}{\int_0^\infty \tau f(\tau) d\tau} = \frac{k_2 [Mg^{2+}]^2}{[Mg^{2+}]^2 + \frac{k_2}{k_1}} \qquad (S18)$$

Thus, the form of Eq. S17 (i.e., a double exponential) is exactly the same as Eq. S12 or S14; similarly, Eq. S18 is exactly the same as Eq. S13 and S15.