

*Supporting Information to*  
**Pre-Steady-State Kinetic Study of Substrate Specificity of *Escherichia coli***  
**Formamidopyrimidine-DNA Glycosylase**

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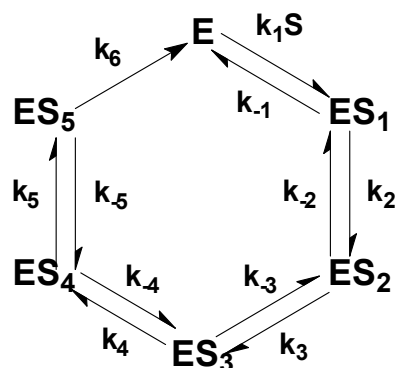
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***Derivation of the equations for apparent  $K_M$  and  $k_{cat}$***

Kinetic graph theory was used to derive the equations for apparent  $K_M$  and  $k_{cat}$  for the multistage reaction of processing the damaged substrates by Fpg. Kinetic Scheme II (see the main text) was presented as the following graph:



(the  $E + P \leftrightarrow EP$  equilibrium was omitted since the concentration of P is close to zero at early reaction times). Trees at the nodes of this graph can be expressed as:

$$D_E = k_{-1}k_{-2}k_{-3}k_{-4}k_{-5} + k_{-1}k_{-2}k_{-3}k_{-4}k_6 + k_{-1}k_{-2}k_{-3}k_5k_6 + k_{-1}k_{-2}k_4k_5k_6 + k_{-1}k_3k_4k_5k_6 + k_2k_3k_4k_5k_6 \quad (1)$$

$$D_{ES1} = k_1S_0(k_{-2}k_{-3}k_{-4}k_{-5} + k_{-2}k_{-3}k_{-4}k_6 + k_{-2}k_{-3}k_5k_6 + k_{-2}k_4k_5k_6 + k_3k_4k_5k_6) \quad (2)$$

$$D_{ES2} = k_1S_0(k_2k_{-3}k_{-4}k_{-5} + k_2k_{-3}k_{-4}k_6 + k_2k_{-3}k_5k_6 + k_2k_4k_5k_6) \quad (3)$$

$$D_{ES3} = k_1S_0(k_2k_3k_5k_6 + k_2k_3k_{-4}k_6 + k_2k_3k_{-4}k_{-5}) \quad (4)$$

$$D_{ES4} = k_1S_0(k_2k_3k_4k_6 + k_2k_3k_4k_{-5}) \quad (5)$$

$$D_{ES5} = k_1S_0k_2k_3k_4k_5 \quad (6)$$

The initial rate of product accumulation  $v_0$  will then be:

$$v_0 = \frac{k_6 \times e_0 \times D_{ES5}}{D_E + D_{ES1} + D_{ES2} + D_{ES3} + D_{ES4} + D_{ES5}} = \frac{k_{cat} \times e_0 \times s_0}{K_M + s_0} \quad (7)$$

Therefore, apparent  $K_M$  and  $k_{cat}$  will be

$$K_M = A/k_1B; \quad (8)$$

$$k_{cat} = k_2k_3k_4k_5k_6/B, \quad (9)$$

where

$$A = k_{-1}k_{-2}k_{-3}k_{-4}k_{-5} + k_{-1}k_{-2}k_{-3}k_{-4}k_6 + k_{-1}k_{-2}k_{-3}k_5k_6 + k_{-1}k_{-2}k_4k_5k_6 + k_{-1}k_3k_4k_5k_6 + k_2k_3k_4k_5k_6 \quad (10)$$

$$B = k_{-2}k_{-3}k_{-4}k_{-5} + k_{-2}k_{-3}k_{-4}k_6 + k_{-2}k_{-3}k_5k_6 + k_{-2}k_4k_5k_6 + k_3k_4k_5k_6 + k_2k_{-3}k_{-4}k_{-5} + k_2k_{-3}k_{-4}k_6 + k_2k_{-3}k_5k_6 + k_2k_4k_5k_6 + k_2k_3k_5k_6 + k_2k_3k_{-4}k_6 + k_2k_3k_{-4}k_{-5} + k_2k_3k_4k_6 + k_2k_3k_4k_{-5} + k_2k_3k_4k_5 \quad (11)$$

### ***Fpg dynamics during the simulation***

See the attached file Fpg\_dynamics.avi