Supplementary Material

1 Supplementary Data

Validity of the Michaelis-Menten Approach

Irreversible Michaelis-Menten Equation

The rate equations of the irreversible Michaelis-Menten mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P \qquad (1)$$

were analyzed numerically using the FORTRAN subroutine LSODE (Radhakrishnan and Hindmarsh, 1993):

$$\dot{E} = -k_1\,[E]\,[S] + (k_{-1} + k_2)\,[ES]$$

$$\frac{d[ES]}{dt} = -\dot{E}$$

$$\dot{S} = -k_1\,[E]\,[S] + k_{-1}\,[ES]$$

$$\dot{P} = k_2\,[ES]$$

where $\dot{X} = \frac{dX}{dt}$ is the time derivative of compound X concentration.

The numerical solutions were compared with approximations, based on the rapid equilibrium between E, S, and the enzyme-substrate complex ES, and on the steady-state approximation that time derivatives of [E] and [ES] are zero (Segel, 1975).

The numerical, steady-state (ss), and rapid equilibrium (re) expressions for the reaction velocity of Equation (1) were calculated as:

$$v_{num} = k_2\,[ES]$$

$$v_{ss} = \frac{V_{max} \cdot [S]}{K_M^{ss} + [S]}$$

$$v_{re} = \frac{V_{max} \cdot [S]}{K_M^{re} + [S]}$$

where k_2 is the turnover number, $V_{max} = k_2 \cdot [E]_{tot}$, and $[E]_{tot}$ is total enzyme concentration. The K_M values for the steady-state and rapid equilibrium approximations are, according to (Segel, 1975), given as:

$$K_M^{ss} = \frac{[E] \cdot [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1}$$

$$K_M^{re} = \frac{[E] \cdot [S]}{[ES]} = \frac{k_{-1}}{k_1}$$

The Steady-State Approximation Implies Pseudo-First-Order Kinetics with Respect to S

The steady state approximation, $d[ES]/dt = 0$, implies that the reaction velocity v = k_2[ES] is first-order with respect to S. In other words, [S] decreases exponentially with time. This can be seen by the time derivative of the following mass balance

$$[S]_0 = [P](t) + [S](t) + [ES](t)$$

where $[S]_0$ is the initial concentration of substrate S at time t = 0. We assume, for the sake of simplicity, that no product is present at time t = 0. [S](t), [P](t) and [ES](t) are the concentrations of S, P and ES at time t. The time derivative of the above mass balance and the assumption that d[ES]/dt = 0 gives

$$\dot{P} + \dot{S} = 0$$

which implies that $$v_{pfo} = \dot{P} = -\dot{S} = k_2\,[ES] = \frac{k_2\,[E]}{K_M} \cdot [S] = k_{pfo} \cdot [S]$$

Noting that $[ES] = [E][S]/K_M$, that the steady-state approximation leads to constant [E], and that the pseudo-first-order (pfo) rate constant k_{pfo} is therefore constant, we can write

$$\dot{S} = k_2\,[ES] = -k_{pfo} \cdot [S] \Rightarrow [S](t) = [S]_0 \cdot e^{-k_{pfo} \cdot t}$$

i.e. [S](t) shows an exponential decrease with time.

Range of Rate Constants Considered

As previously described (Segel, 1975), the values of k_1, k_{-1}, and k_2 generally lie within the following ranges:

k_1, 10^7–10^{10} M^{-1} min^{-1}

k_{-1}, 10^2–10^6 min^{-1}

k_2, 50–10^7 min^{-1}

We in **Supplementary Table S2** use combinations of the upper and lower values of these to test the steady-state and rapid equilibrium approximations against the numerical calculation of substrate and total enzyme concentrations.

Validity of the Michaelis-Menten Approach

It can be questioned whether the Michaelis-Menten approach indicated above is still valid when $[E]_{tot}$ = 500 nM and $[S]_0$ is as low as 5 nM. We, in the following, show that it is still valid and that the equation

$$v_0 = \frac{V_{max} \cdot [S]}{K_M + [S]}$$

gives an excellent description of velocities compared with numerically calculated velocities v_{num}. We have tested eight rate constant combinations (see **Supplementary Table S2**) and found that the steady state approximation of seven of these gives excellent agreement with v_{num}. Only the rapid equilibrium approximation in combination No. 6 gives slightly better agreement than the steady-state approximation. v_{re} is, also for No. 6, in excellent agreement with v_{num}. This is shown in **Supplementary Figures S7 and S8** in which the numerical solutions of the rate constant combinations from **Supplementary Table S2** are compared with the results of the steady state and rapid equilibrium approximations (upper panels). The lower panels show the corresponding velocities.

Experimental Velocity Data Agrees with First-Order Kinetics

The experimentally determined velocity data (**Supplementary Data**, section "Kinetic Raw Data") agrees well with the above implicated first-order kinetics. First-order kinetics implicate that the same fraction of substrate is processed irrespective of the initial concentration of the substrate, during the 30 min assay period, which was observed (**Supplementary Table S3**). The rate constant k_{pfo} can be derived from the following relationship

$$k_{pfo} = -\frac{1}{t_{assay}} \cdot \ln\left(\frac{[S]}{[S]_0}\right)$$

where t_{assay} is the assay time (30 min) and $[S]/[S]_0$ is the ratio of remaining S at the end of the assay time.

Kinetic Raw Data

C•C

[S]			velocity, nM/min	
pmol/20 µL	nmol/20 µL	nM	Average	SD
0.125	0.000125	6.3	0.0031192	0.003
0.25	0.00025	13	0.0074616	0.001
0.5	0.0005	25	0.0291911	0.005
1	0.001	50	0.0896232	0.031
2	0.002	100	0.1262553	0.032
4	0.004	200	0.2175025	0.029
8	0.008	400	0.2617096	0.025
10	0.01	500	0.3001807	0.024

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	0.31509612	0.019693508	0.00065645
0.25	0.00025	12.5	1.652535175	0.206566897	0.006885563
0.5	0.0005	25	2.738026842	0.684506711	0.02281689
1	0.001	50	3.601123084	1.800561542	0.060018718
2	0.002	100	2.679120363	2.679120363	0.089304012
4	0.004	200	3.072264667	6.144529334	0.204817644
8	0.008	400	1.831599231	7.326396925	0.244213231
10	0.01	500	1.774749591	8.873747953	0.295791598

C•C

Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.42495357	0.151559598	0.005051987
0.25	0.00025	12.5	2.034719587	0.254339948	0.008477998
0.5	0.0005	25	4.071107411	1.017776853	0.033925895
1	0.001	50	5.175942793	2.587971397	0.086265713
4	0.004	200	3.894278132	7.788556264	0.259618542
10	0.01	500	1.673856683	8.369283415	0.278976114
Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	0.252880049	0.015805003	0.000526833
0.25	0.00025	12.5	1.890398588	0.236299824	0.007876661
0.5	0.0005	25	3.319284881	0.82982122	0.027660707
1	0.001	50	4.734319063	2.367159531	0.078905318
2	0.002	100	4.453775845	4.453775845	0.148459195
4	0.004	200	2.908557381	5.817114761	0.193903825
8	0.008	400	2.094044126	8.376176504	0.279205883
10	0.01	500	1.954645725	9.773228627	0.325774288
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.995983805	0.187248988	0.006241633
0.25	0.00025	12.5	1.585507679	0.19818846	0.006606282
0.5	0.0005	25	3.883315523	0.970828881	0.032360963
1	0.001	50	7.998186124	3.999093062	0.133303102
2	0.002	100	4.230083569	4.230083569	0.141002786
4	0.004	200	3.175049849	6.350099698	0.21166999

C•C

[S]			velocity, nM/min	
pmol/20 µL	**nmol/20 µL**	**nM**	**Average**	**SD**
0.125	0.000125	6.3	0.003869184	0.002
0.25	0.00025	13	0.010891819	0.001
0.5	0.0005	25	0.022174651	0.005
1	0.001	50	0.080301021	0.011
2	0.002	100	0.158339144	0.019
4	0.004	200	0.350888771	0.035
8	0.008	400	0.499197278	0.041
10	0.01	500	0.656561579	0.053

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	1.79257443	0.1120359	0.00373453
0.25	0.00025	12.5	2.49893888	0.31236736	0.010412245
0.5	0.0005	25	2.69387749	0.67346937	0.022448979
1	0.001	50	4.50410742	2.25205371	0.075068457
2	0.002	100	4.31392072	4.31392072	0.143797357
4	0.004	200	5.74320568	11.4864114	0.382880379
8	0.008	400	3.9678795	15.871518	0.5290506
10	0.01	500	4.41665225	22.0832612	0.736108708

Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.8094676	0.17559173	0.005853058
0.25	0.00025	12.5	2.93121148	0.36640143	0.012213381
0.5	0.0005	25	2.60832379	0.65208095	0.021736032
1	0.001	50	4.46296631	2.23148316	0.074382772
2	0.002	100	4.5827879	4.5827879	0.152759597
4	0.004	200	5.18245139	10.3649028	0.345496759
8	0.008	400	3.32809786	13.3123914	0.443746381
10	0.01	500	3.76765649	18.8382825	0.627942748

C•C

Parallel 3					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.25	0.00025	12.5	2.66915127	0.33364391	0.011121464
0.5	0.0005	25	1.80426898	0.45106725	0.015035575
1	0.001	50	4.18069644	2.09034822	0.069678274
2	0.002	100	4.32456952	4.32456952	0.144152317
4	0.004	200	4.79010647	9.58021295	0.319340432
8	0.008	400	3.50853947	14.0341579	0.467805263
10	0.01	500	3.81721255	19.0860627	0.636202091
Parallel 4					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.64338077	0.1652113	0.005507043
0.25	0.00025	12.5	2.57010849	0.32126356	0.010708785
0.5	0.0005	25	2.94972145	0.73743036	0.024581012
1	0.001	50	5.73276026	2.86638013	0.095546004
2	0.002	100	5.71965823	5.71965823	0.190655274
4	0.004	200	5.86467229	11.7293446	0.390978153
8	0.008	400	3.90087785	15.6035114	0.520117046
Parallel 5					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.04061903	0.12753869	0.00425129
0.25	0.00025	12.5	2.40077322	0.30009665	0.010003222
0.5	0.0005	25	3.24859903	0.81214976	0.027071659
1	0.001	50	5.20977592	2.60488796	0.086829599
2	0.002	100	4.80993526	4.80993526	0.160331175
4	0.004	200	4.736222	9.47244401	0.315748134
8	0.008	400	4.01450325	16.058013	0.5352671
10	0.01	500	3.75595662	18.7797831	0.625992769

T•T

[S]			velocity, nM/min	
pmol/20 µL	**nmol/20 µL**	**nM**	**Average**	**SD**
0.125	0.000125	6.25	0.00469438	0.002
0.25	0.00025	12.5	0.00855593	0.005
0.5	0.0005	25	0.02849986	0.012
1	0.001	50	0.11792463	0.062
2	0.002	100	0.19785082	0.087
4	0.004	200	0.35025075	0.119
8	0.008	400	0.4385017	0.186
10	0.01	500	0.55529732	0.187

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	1.1068824	0.06918015	0.002306005
0.25	0.00025	12.5	0.28041774	0.035052217	0.001168407
0.5	0.0005	25	5.47289156	1.36822289	0.04560743
1	0.001	50	8.18589728	4.092948641	0.136431621
2	0.002	100	5.27789016	5.277890162	0.175929672
4	0.004	200	6.98514598	13.97029196	0.465676399
8	0.008	400	5.37908982	21.51635926	0.717211975
10	0.01	500	5.14617905	25.73089527	0.857696509
Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.56584461	0.160365288	0.00534551
0.25	0.00025	12.5	3.18491706	0.398114632	0.013270488
0.5	0.0005	25	3.28159009	0.820397522	0.027346584
1	0.001	50	9.29914345	4.649571727	0.154985724
2	0.002	100	10.5862372	10.58623716	0.352874572
4	0.004	200	7.22631024	14.45262048	0.481754016
8	0.008	400	3.704393	14.81757199	0.493919066
10	0.01	500	3.19221619	15.96108093	0.532036031

T•T

Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	3.31261567	0.207038479	0.006901283
0.25	0.00025	12.5	2.33544304	0.29193038	0.009731013
0.5	0.0005	25	4.0161212	1.004030301	0.033467677
1	0.001	50	2.53894809	1.269474047	0.042315802
2	0.002	100	4.65822835	4.658228349	0.155274278
4	0.004	200	4.25516984	8.510339676	0.283677989
8	0.008	400	2.39001409	9.560056375	0.318668546
10	0.01	500	2.40689517	12.03447583	0.401149194
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	1.4242701	0.089016881	0.002967229
0.25	0.00025	12.5	1.95224019	0.244030024	0.008134334
0.5	0.0005	25	1.50637858	0.376594646	0.012553155
1	0.001	50	3.88578662	1.942893309	0.06476311
2	0.002	100	4.32868154	4.328681543	0.144289385
4	0.004	200	3.13490989	6.269819772	0.208993992
8	0.008	400	1.74144307	6.965772271	0.232192409
10	0.01	500	3.48795409	17.43977044	0.581325681
Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.85690444	0.178556527	0.005951884
0.25	0.00025	12.5	2.51409879	0.314262348	0.010475412
0.5	0.0005	25	2.82293719	0.705734298	0.023524477
1	0.001	50	11.4676148	5.733807408	0.191126914
2	0.002	100	4.82658639	4.826586393	0.160886213
4	0.004	200	4.66727013	9.334540255	0.311151342
8	0.008	400	3.2288738	12.91549521	0.430516507
10	0.01	500	2.42567501	12.12837506	0.404279169

$m^{N4}C \cdot C$

[S]			velocity, nM/min	
pmol/20 µL	nmol/20 µL	nM	Average	SD
0.125	0.000125	6.25	0.00588403	0.005
0.25	0.00025	12.5	0.01316618	0.003
0.5	0.0005	25	0.03366474	0.010
1	0.001	50	0.09944813	0.031
2	0.002	100	0.21439431	0.035
4	0.004	200	0.30320116	0.049
8	0.008	400	0.35829702	0.034
10	0.01	500	0.38032715	0.022

Parallel 1

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	0.68559473	0.04284967	0.001428322
0.25	0.00025	12.5	2.50295157	0.31286895	0.010428965
0.5	0.0005	25	2.73677945	0.68419486	0.022806495
1	0.001	50	7.81924203	3.90962102	0.130320701
4	0.004	200	3.20835867	6.41671735	0.213890578
8	0.008	400	2.31232437	9.24929748	0.308309916
10	0.01	500	2.37408419	11.8704209	0.395680698

Parallel 2

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	0.48780942	0.03048809	0.00101627
0.25	0.00025	12.5	3.40619708	0.42577463	0.014192488
0.5	0.0005	25	4.01485875	1.00371469	0.033457156
1	0.001	50	6.8844622	3.4422311	0.114741037
2	0.002	100	7.32176158	7.32176158	0.244058719
4	0.004	200	4.66325641	9.32651282	0.310883761
8	0.008	400	2.79911331	11.1964533	0.373215109
10	0.01	500	2.18984162	10.9492081	0.364973603

m^{N4}C•C

Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	5.01744693	0.31359043	0.010453014
0.25	0.00025	12.5	2.38457678	0.2980721	0.009935737
0.5	0.0005	25	5.58159116	1.39539779	0.04651326
1	0.001	50	7.37413582	3.68706791	0.122902264
2	0.002	100	7.51286935	7.51286935	0.250428978
4	0.004	200	5.15927041	10.3185408	0.34395136
8	0.008	400	2.7403464	10.9613856	0.36537952
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.74272478	0.1714203	0.00571401
0.25	0.00025	12.5	3.23777188	0.40472148	0.013490716
0.5	0.0005	25	3.41768602	0.85442151	0.028480717
1	0.001	50	3.86415273	1.93207637	0.064402546
2	0.002	100	5.57879907	5.57879907	0.185959969
4	0.004	200	5.19370602	10.387412	0.346247068
Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	1.77745406	0.11109088	0.003703029
0.25	0.00025	12.5	3.07288751	0.38411094	0.012803698
0.5	0.0005	25	3.24842083	0.81210521	0.027070174
1	0.001	50	3.45623849	1.72811924	0.057603975
2	0.002	100	5.1440684	5.1440684	0.171468947
4	0.004	200	4.28525656	8.57051312	0.285683771
8	0.008	400	2.89712663	11.5885065	0.386283551
Parallel 6					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	6.2349798	0.38968624	0.012989541
0.25	0.00025	12.5	4.35492097	0.54436512	0.018145504
0.5	0.0005	25	5.23927744	1.30981936	0.043660645
1	0.001	50	6.40309449	3.20154725	0.106718242
2	0.002	100	6.60164881	6.60164881	0.22005496
4	0.004	200	4.77825624	9.55651248	0.318550416

$m^5C{\cdot}C$

[S]			velocity, nM/min	
pmol/20 µL	nmol/20 µL	nM	Average	SD
0.125	0.000125	6.25	0.00958994	0.004
0.25	0.00025	12.5	0.01776629	0.011
0.5	0.0005	25	0.04719738	0.014
1	0.001	50	0.23964882	0.048
2	0.002	100	0.34090192	0.035
4	0.004	200	0.58481667	0.135
8	0.008	400	0.8705271	0.152
10	0.01	500	0.99184724	0.228

Parallel 1

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	6.03793529	0.37737096	0.012579032
0.25	0.00025	13	6.01645655	0.75205707	0.025068569
0.5	0.0005	25	5.54657469	1.38664367	0.046221456

Parallel 2

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	6.12368215	0.38273013	0.012757671
0.25	0.00025	13	6.08408008	0.76051001	0.025350334
0.5	0.0005	25	6.8064735	1.70161838	0.056720613
1	0.001	50	12.4484971	6.22424853	0.207474951
2	0.002	100	10.4765396	10.4765396	0.349217986
8	0.008	400	8.01369178	32.0547671	1.068492238

Parallel 3

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	6.21594823	0.38849676	0.012949892
0.25	0.00025	13	6.04162225	0.75520278	0.025173426
0.5	0.0005	25	8.76926433	2.19231608	0.073077203
1	0.001	50	12.9842162	6.49210809	0.216403603

Parallel 4

[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	6.21948334	0.38871771	0.012957257
0.25	0.00025	13	6.9807	0.8725875	0.02908625
0.5	0.0005	25	7.09959658	1.77489914	0.059163305
2	0.002	100	11.8428765	11.8428765	0.39476255
8	0.008	400	6.73302211	26.9320885	0.897736282

m^{5}C•C

Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.5	0.0005	25	6.24730183	1.561825458	0.052060849
1	0.001	50	17.704074	8.852037019	0.295067901
4	0.004	200	10.7396032	21.47920642	0.715973547
8	0.008	400	6.03938837	24.15755349	0.805251783
Parallel 6					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.55086279	0.159428924	0.005314297
0.5	0.0005	25	2.57742347	0.644355868	0.021478529
2	0.002	100	10.2064673	10.20646734	0.340215578
4	0.004	200	8.87937974	17.75875948	0.591958649
8	0.008	400	5.32971059	21.31884237	0.710628079
10	0.01	500	4.9969523	24.98476148	0.832825383
Parallel 7					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	4.38426905	0.274016815	0.009133894
0.25	0.00025	12.5	5.77836549	0.722295686	0.024076523
0.5	0.0005	25	5.77570667	1.443926667	0.048130889
2	0.002	100	9.11419922	9.114199216	0.303806641
4	0.004	200	6.69776727	13.39553453	0.446517818
10	0.01	500	7.5217418	37.60870902	1.253623634
Parallel 8					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	5.26066754	0.328791721	0.010959724
0.25	0.00025	12.5	4.04001712	0.50500214	0.016833405
0.5	0.0005	25	5.67396458	1.418491146	0.047283038
2	0.002	100	9.4952053	9.495205297	0.316506843
10	0.01	500	5.33455624	26.6727812	0.889092707

$m^5C \cdot C$

Parallel 9					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	4.63567961	0.289729976	0.009657666
0.25	0.00025	12.5	3.43393671	0.429242088	0.01430807
0.5	0.0005	25	5.58244164	1.395610409	0.046520347

$m^5C \cdot T$

[S]			velocity, nM/min	
pmol/20 µL	**nmol/20 µL**	**nM**	**Average**	**SD**
0.125	0.000125	6.25	0.00320205	0.001
0.25	0.00025	12.5	0.00590743	0.003
0.5	0.0005	25	0.01748805	0.007
1	0.001	50	0.07295418	0.014
2	0.002	100	0.07831777	0.036
4	0.004	200	0.20669422	0.013
8	0.008	400	0.33368562	0.049
10	0.01	500	0.39332682	0.018

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	1.27781363	0.079863352	0.002662112
0.25	0.00025	13	1.39193987	0.173992484	0.005799749
0.5	0.0005	25	3.22593563	0.806483907	0.026882797
4	0.004	200	3.17791093	6.355821858	0.211860729
8	0.008	400	2.24139553	8.965582129	0.298852738
10	0.01	500	2.24139553	11.20697766	0.373565922

Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	1.38329042	0.086455651	0.002881855
0.25	0.00025	13	1.28112011	0.160140014	0.005338
0.5	0.0005	25	1.46297137	0.365742844	0.012191428
1	0.001	50	4.23424902	2.117124509	0.070570817
4	0.004	200	3.24393439	6.487868786	0.216262293
8	0.008	400	2.76388873	11.0555549	0.368518497
10	0.01	500	2.46073549	12.30367744	0.410122581

$m^5C{\cdot}T$

Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	1.86695982	0.116684989	0.0038895
0.25	0.00025	13	1.96173657	0.245217071	0.008173902
0.5	0.0005	25	2.63137177	0.657842943	0.021928098
1	0.001	50	5.24419233	2.622096167	0.087403206
2	0.002	100	3.3987516	3.398751601	0.11329172
4	0.004	200	2.87939436	5.758788729	0.191959624
10	0.01	500	2.37775181	11.88875905	0.396291968
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	1.45666558	0.0910416	0.00303472
Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.25	0.00025	13	1.9726752	0.2465844	0.00821948
0.5	0.0005	25	1.56578544	0.39144636	0.013048212
Parallel 6					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
1	0.001	50	3.31988195	1.65994098	0.055331366
2	0.002	100	1.24564966	1.24564966	0.041521655
Parallel 7					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.5	0.0005	25	1.60676868	0.40169217	0.013389739
Parallel 8					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	1.70018033	0.10626127	0.003542042
0.25	0.00025	13	0.48144193	0.06018024	0.002006008
1	0.001	50	4.71068107	2.35534054	0.078511351
2	0.002	100	2.40419804	2.40419804	0.080139935

$m^{N4,5}C \cdot C$

[S]			velocity, nM/min	
pmol/20 µL	nmol/20 µL	nM	Average	SD
0.125	0.000125	6.25	0.022655	0.009
0.25	0.00025	12.5	0.035549	0.009
0.5	0.0005	25	0.09147	0.012
1	0.001	50	0.192025	0.021
2	0.002	100	0.36177	0.035
4	0.004	200	0.745269	0.081
8	0.008	400	1.345078	0.087
10	0.01	500	1.577857	0.075

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	20.6246322	1.289039514	0.042967984
0.25	0.00025	12.5	9.42829152	1.17853644	0.039284548
0.5	0.0005	25	11.8816539	2.970413472	0.099013782
1	0.001	50	10.8990104	5.44950519	0.181650173
2	0.002	100	11.4945856	11.49458564	0.383152855
Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
4	0.004	200	12.1358137	24.27162739	0.809054246
Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	6.83440019	0.427150012	0.014238334
0.25	0.00025	12.5	5.98973135	0.748716419	0.024957214
0.5	0.0005	25	9.14709977	2.286774942	0.076225831
4	0.004	200	9.14888265	18.29776529	0.60992551
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.25	0.00025	12.5	9.5381927	1.192274087	0.03974247
2	0.002	100	10.4935877	10.49358773	0.349786258
10	0.01	500	8.81979182	44.09895911	1.469965304
Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
2	0.002	100	9.72280072	9.722800721	0.324093357
4	0.004	200	12.8344475	25.66889509	0.855629836

$m^{N4,5}C{\bullet}C$

Parallel 6					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	11.5058442	0.719115262	0.023970509
0.25	0.00025	12.5	6.898019	0.862252375	0.028741746
0.5	0.0005	25	11.6574416	2.914360412	0.097145347
1	0.001	50	14.4334492	7.216724595	0.240557487
4	0.004	200	11.0137898	22.02757969	0.734252656
8	0.008	400	10.2405432	40.96217297	1.365405766
10	0.01	500	9.68143086	48.4071543	1.61357181
Parallel 7					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	5.73405383	0.358378364	0.011945945
0.5	0.0005	25	9.73589007	2.433972518	0.081132417
1	0.001	50	9.80533907	4.902669536	0.163422318
2	0.002	100	12.6607867	12.66078669	0.422026223
4	0.004	200	11.7486116	23.49722329	0.783240776
8	0.008	400	10.25909	41.03636011	1.36787867
10	0.01	500	9.53137097	47.65685483	1.588561828
Parallel 8					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	7.69596773	0.480997983	0.016033266
0.25	0.00025	12.5	5.9724733	0.746559163	0.024885305
0.5	0.0005	25	9.62197106	2.405492766	0.080183092
1	0.001	50	10.9253073	5.462653665	0.182088456
2	0.002	100	10.6169803	10.6169803	0.353899343
Parallel 9					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	9.88280185	0.617675116	0.020589171
0.5	0.0005	25	9.99232844	2.498082109	0.083269404
1	0.001	50	11.5562706	5.778135294	0.19260451
2	0.002	100	10.1298463	10.1298463	0.337661543

$m^{N4,5}C \cdot C$

Parallel 10					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	16.2156543	1.013478397	0.033782613
0.25	0.00025	12.5	8.06015401	1.007519251	0.033583975
0.5	0.0005	25	12.3148119	3.078702979	0.102623433
1	0.001	50	11.3451908	5.672595422	0.189086514
8	0.008	400	9.15864454	36.63457817	1.221152606
Parallel 11					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	9.71903733	0.607439833	0.020247994
0.25	0.00025	12.5	7.92028284	0.990035355	0.033001178
0.5	0.0005	25	9.93457215	2.483643037	0.082788101
1	0.001	50	11.4532718	5.726635887	0.190887863
Parallel 12					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	9.20356669	0.575222918	0.019174097
0.25	0.00025	12.5	10.0059893	1.250748668	0.041691622
0.5	0.0005	25	13.4038724	3.350968098	0.111698937
1	0.001	50	11.1573953	5.578697643	0.185956588
4	0.004	200	10.9801055	21.96021098	0.732007033
8	0.008	400	10.6940572	42.77622883	1.425874294
Parallel 13					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	11.3286056	0.70803785	0.023601262
0.25	0.00025	12.5	12.9724238	1.621552981	0.054051766
0.5	0.0005	25	12.0747015	3.018675379	0.100622513
1	0.001	50	12.1185019	6.059250953	0.201975032
4	0.004	200	10.3916196	20.78323919	0.69277464
10	0.01	500	9.83596408	49.1798204	1.639327347

dHT•A

[S]			velocity, nM/min	
pmol/20 µL	nmol/20 µL	nM	Average	SD
0.125	0.000125	6.3	0.011469	0.001
0.25	0.00025	13	0.019985	0.004
0.5	0.0005	25	0.072588	0.002
1	0.001	50	0.238578	0.035
2	0.002	100	0.447958	0.057
4	0.004	200	0.714321	0.083
8	0.008	400	1.372965	0.543
10	0.01	500	1.336637	0.320

Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	5.9314021	0.37071263	0.012357088
0.25	0.00025	13	6.04388155	0.75548519	0.02518284
0.5	0.0005	25	8.41113952	2.10278488	0.070092829
1	0.001	50	16.2076376	8.10381879	0.270127293
2	0.002	100	15.2681909	15.2681909	0.508939697
Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	5.24185559	0.32761597	0.010920532
0.25	0.00025	13	4.74199506	0.59274938	0.019758313
0.5	0.0005	25	8.95347883	2.23836971	0.074612324
1	0.001	50	12.0603642	6.03018212	0.201006071
2	0.002	100	11.8818553	11.8818553	0.396061843
Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	5.16638223	0.32289889	0.010763296
0.25	0.00025	13	3.83705581	0.47963198	0.015987733
0.5	0.0005	25	8.76696112	2.19174028	0.073058009
4	0.004	200	9.83749898	19.674998	0.655833265
8	0.008	400	13.1745512	52.6982049	1.75660683
10	0.01	500	9.37825025	46.8912512	1.563041708

dHT•A

Parallel 4					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.3	5.67991401	0.35499463	0.011833154
0.25	0.00025	13	4.56229958	0.57028745	0.019009582
1	0.001	50	14.6760594	7.33802971	0.24460099
2	0.002	100	13.1661674	13.1661674	0.438872247
4	0.004	200	11.592127	23.184254	0.772808466
8	0.008	400	7.41992063	29.6796825	0.98932275
10	0.01	500	6.66139764	33.3069882	1.11023294

Tg•A

[S]			**velocity, nM/min**	
pmol/20 μL	**nmol/20 μL**	**nM**	**Average**	**SD**
0.125	0.000125	6.3	0.00609822	0.002
0.25	0.00025	13	0.00978512	0.002
0.5	0.0005	25	0.02347	0.009
1	0.001	50	0.06387038	0.024
2	0.002	100	0.26143446	0.048
4	0.004	200	0.54402537	0.343
8	0.008	400	0.90534984	0.066
10	0.01	500	0.70696586	0.360

Parallel 1					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	4.27831638	0.26739477	0.008913159
0.25	0.00025	12.5	1.93600888	0.24200111	0.008066704
0.5	0.0005	25	2.68308912	0.67077228	0.022359076
1	0.001	50	2.89433417	1.44716708	0.048238903
4	0.004	200	14.027027	28.054054	0.935135134
8	0.008	400	6.90933087	27.6373235	0.921244116
10	0.01	500	5.76994017	28.8497009	0.961656695

Parallel 2					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	2.72255758	0.17015985	0.005671995
0.25	0.00025	12.5	1.94714761	0.24339345	0.008113115
2	0.002	100	8.52523769	8.52523769	0.28417459
8	0.008	400	7.21659139	28.8663656	0.962212185

Tg•A

Parallel 3					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	1.66700804	0.104188	0.003472933
0.25	0.00025	12.5	2.45036686	0.30629586	0.010209862
0.5	0.0005	25	1.84791903	0.46197976	0.015399325
1	0.001	50	3.13427389	1.56713695	0.052237898
2	0.002	100	6.17793185	6.17793185	0.205931062
4	0.004	200	4.44109491	8.88218981	0.296072994
10	0.01	500	2.71365013	13.5682506	0.452275021
Parallel 4					
[S]					
pmol/20 μL	nmol/20 μL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	3.04070144	0.19004384	0.006334795
0.25	0.00025	12.5	3.06019296	0.38252412	0.012750804
0.5	0.0005	25	3.91819032	0.97954758	0.032651586
1	0.001	50	5.46805947	2.73402974	0.091134325
2	0.002	100	8.82593171	8.82593171	0.294197724
4	0.004	200	6.01301993	12.0260399	0.400867995
8	0.008	400	6.24444905	24.9777962	0.832593207

oxo^8G•C

[S]			**velocity, nM/min**	
pmol/20 μL	**nmol/20 μL**	**nM**	**Average**	**SD**
0.125	0.000125	6.3	0.025523	0.002
0.25	0.00025	13	0.054638	0.006
0.5	0.0005	25	0.123794	0.043
1	0.001	50	0.303895	0.062
2	0.002	100	0.609011	0.141
4	0.004	200	0.930244	0.059
8	0.008	400	1.814631	0.052
10	0.01	500	1.868644	0.334

oxo⁸G•C

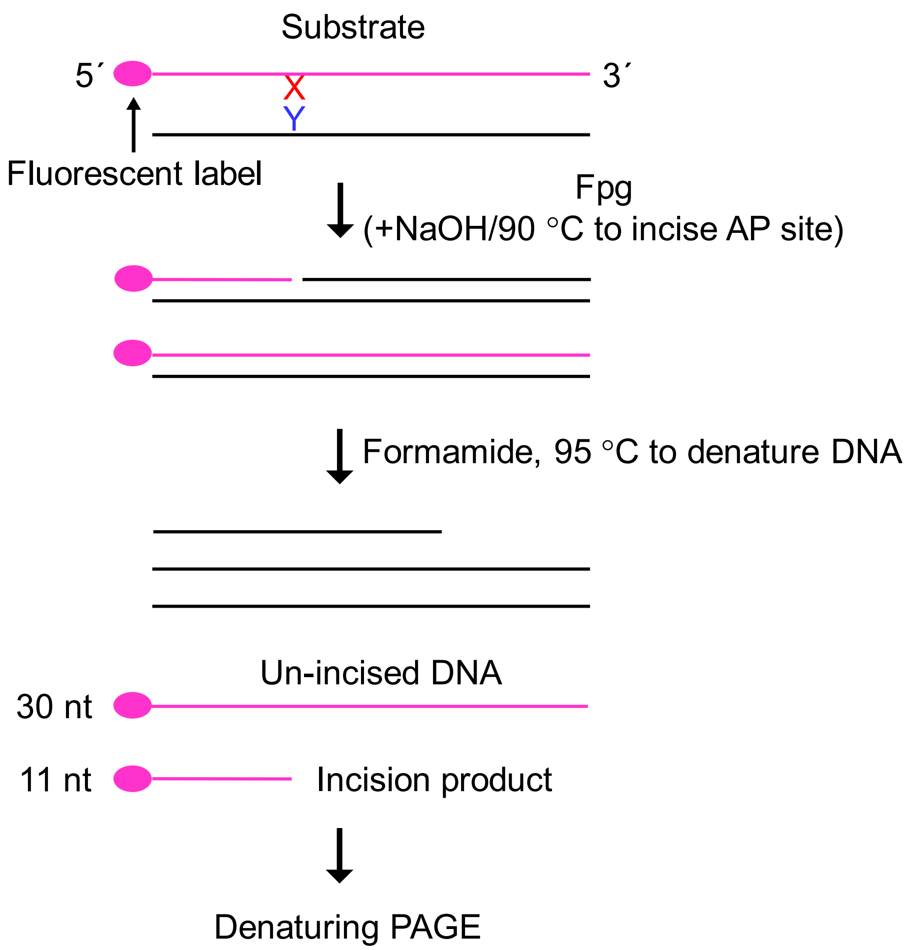
Parallel 1					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	13.0523346	0.815770911	0.027192364
0.25	0.00025	12.5	12.6280527	1.578506586	0.052616886
0.5	0.0005	25	13.4560879	3.364021977	0.112134066
1	0.001	50	18.5534867	9.276743351	0.309224778
2	0.002	100	19.4762148	19.47621484	0.649207161
4	0.004	200	13.2237992	26.44759835	0.881586612
8	0.008	400	13.8859741	55.54389655	1.851463218
10	0.01	500	9.79474772	48.97373861	1.632457954
Parallel 2					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	13.0528397	0.81580248	0.027193416
0.25	0.00025	12.5	11.3835946	1.422949331	0.047431644
0.5	0.0005	25	8.66016433	2.165041084	0.072168036
1	0.001	50	22.591127	11.29556351	0.376518784
2	0.002	100	10.8212767	10.82127669	0.360709223
4	0.004	200	13.7034821	27.40696411	0.91356547
8	0.008	400	13.3334835	53.33393393	1.777797798
10	0.01	500	12.6289797	63.14489868	2.104829956
Parallel 3					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	10.8259846	0.676624038	0.022554135
0.5	0.0005	25	11.7172474	2.929311854	0.097643728
1	0.001	50	17.9593075	8.979653744	0.299321791
2	0.002	100	20.8126575	20.81265753	0.693755251
Parallel 4					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	12.9767789	0.811048684	0.027034956
0.25	0.00025	12.5	14.4727865	1.809098316	0.060303277
0.5	0.0005	25	20.8426125	5.210653124	0.173688437
1	0.001	50	19.6218129	9.810906471	0.327030216
2	0.002	100	19.3645716	19.36457158	0.645485719
4	0.004	200	14.9337044	29.86740889	0.995580296

oxo^{8}G•C

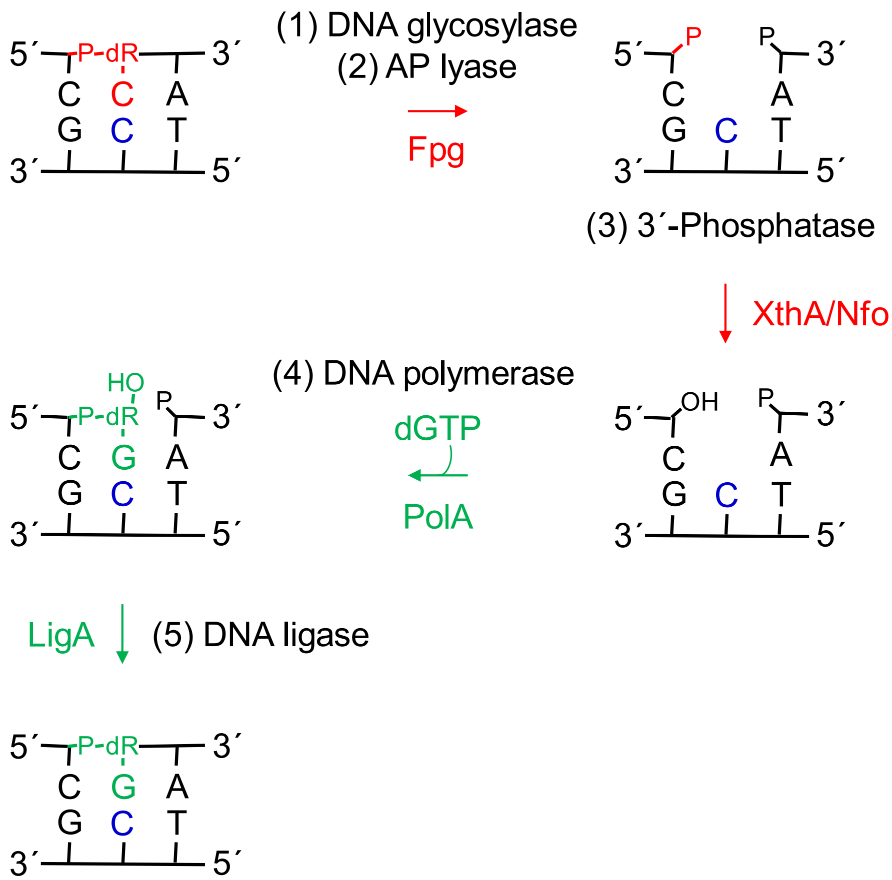
Parallel 5					
[S]					
pmol/20 µL	nmol/20 µL	nM	cleavage, %	cleavage, nM	velocity, nM/min
0.125	0.000125	6.25	11.3479594	0.709247465	0.023641582
0.25	0.00025	12.5	13.9679752	1.745996905	0.058199897
0.5	0.0005	25	19.6000676	4.900016897	0.163333897
1	0.001	50	12.4428639	6.221431969	0.207381066
2	0.002	100	20.8769467	20.87694669	0.695898223

2 Supplementary Figures and Tables

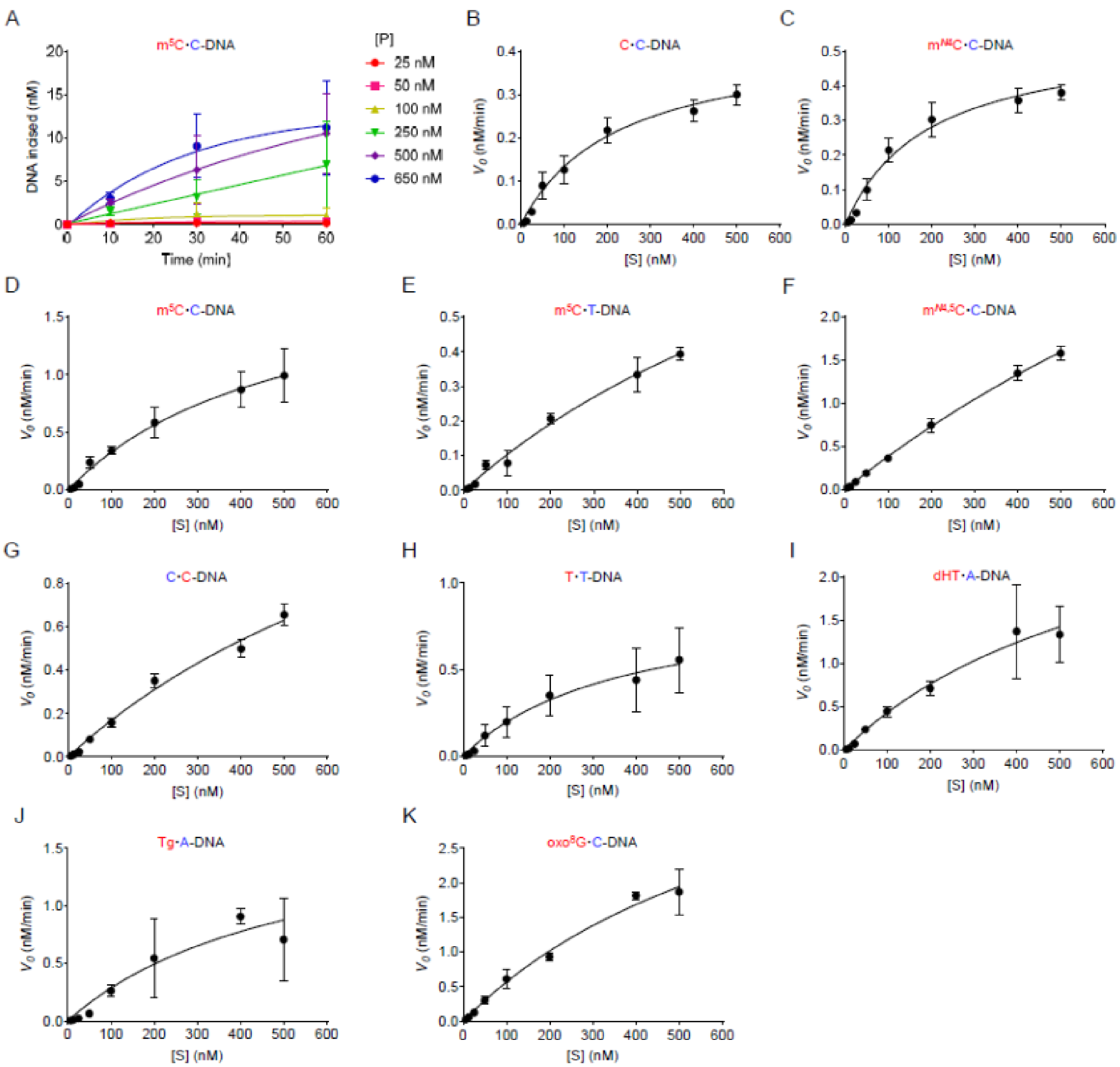
2.1 Supplementary Figures



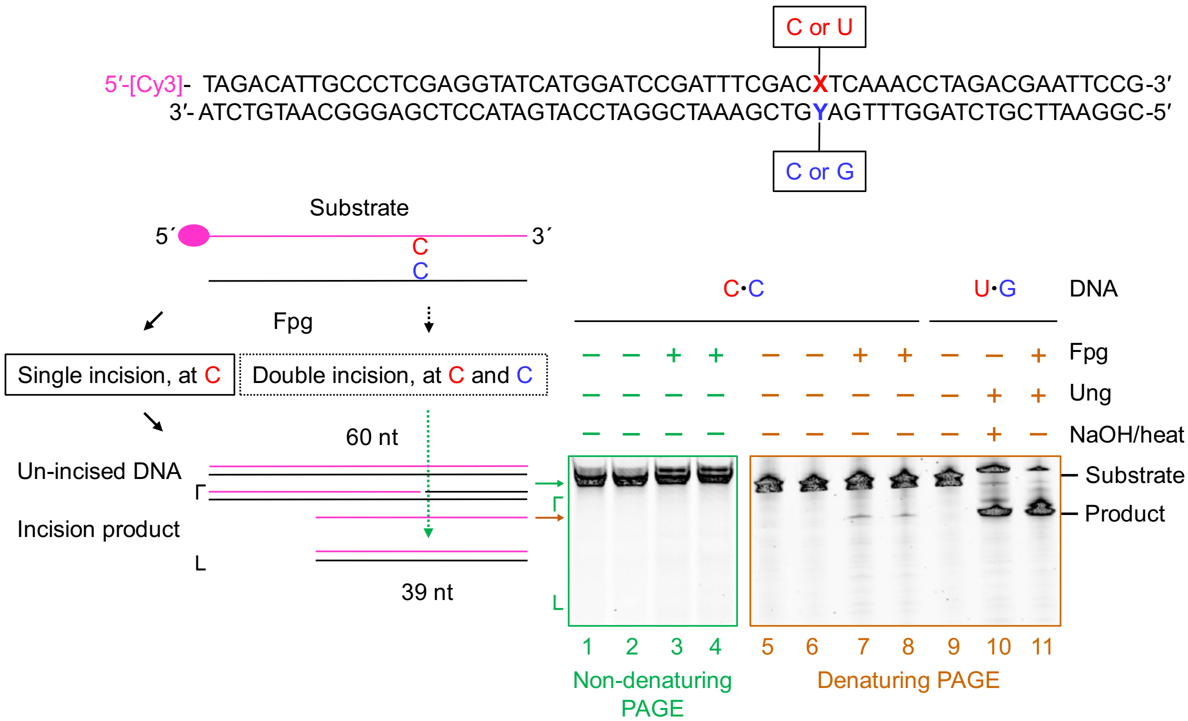
Supplementary Figure S1. Glycosylase activity assay (see "Materials and Methods"). Abbreviation: nt, nucleotides. The 5´-label and strand are indicated in magenta; see **Figure 1A** for explanation of other colors.



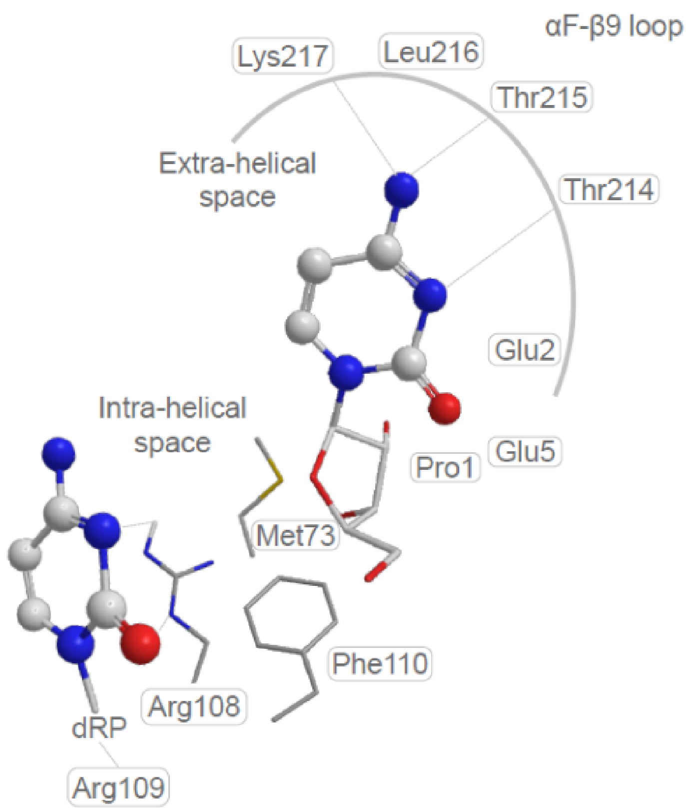
Supplementary Figure S2. Proposed steps of the BER pathway for C•C in DNA. Both the base excision (step 1) and the AP site incision (step 2) are consecutively performed by the bi-functional DNA glycosylase Fpg as reported here, leaving behind a DNA polymerase blocking 3´-phosphate remnant that must be removed by a phosphatase (step 3), which may be XthA or Nfo (Doetsch and Cunningham, 1990) (**Figure 3**). The cleaned one-nucleotide-gap in DNA is now ready for the insertion of the correct dGMP (step 4) by the repair DNA polymerase I (PolA) (Patel et al., 2001) followed by nick-sealing (step 5) by DNA ligase (LigA) (Chauleau and Shuman, 2016). The residues that are removed and are the result of replacement, and their corresponding reaction arrows and enzymes, are indicated in red and green respectively. The other mismatched C is indicated in blue. Abbreviations: dR, deoxyribose; P, phosphate.



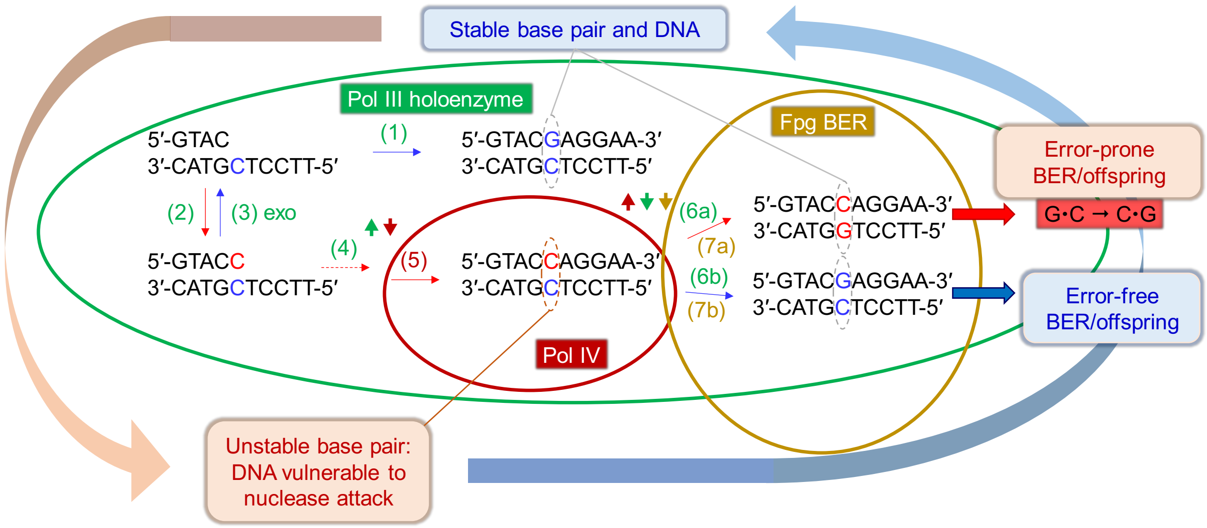
Supplementary Figure S3. Time dependency and opposite base-dependent kinetics of Fpg for methylated and un-methylated cytosine in DNA. **(A)** The indicated concentrations of Fpg protein [P] were incubated with DNA substrate (50 nM; **Figure 1A,** see **Supplementary Table S3**) at 37°C for 30 min (final volume, 20 µL; **Figure 1B–G**). Fpg (**A–H**, 500 nM; **I** and **J**, 10 nM; K, 5 nM) was incubated with increasing concentration of DNA (**Figure 1A**) containing either **(B)** C·C, **(C)** m^{N4}C·C, **(D)** m^{5}C·C, **(E)** m^{5}C·T, **(F)** m^{N4,5}C·C, **(G)** C·C, **(H)** T·T, **(I)** dHT·A, **(J)** Tg·A or **(K)** oxo^{8}G·C as described in **A**. Each value represents the average (± SD) of 4–13 independent experiments. See **Figure 1A** for explanation of substrate DNA colors.



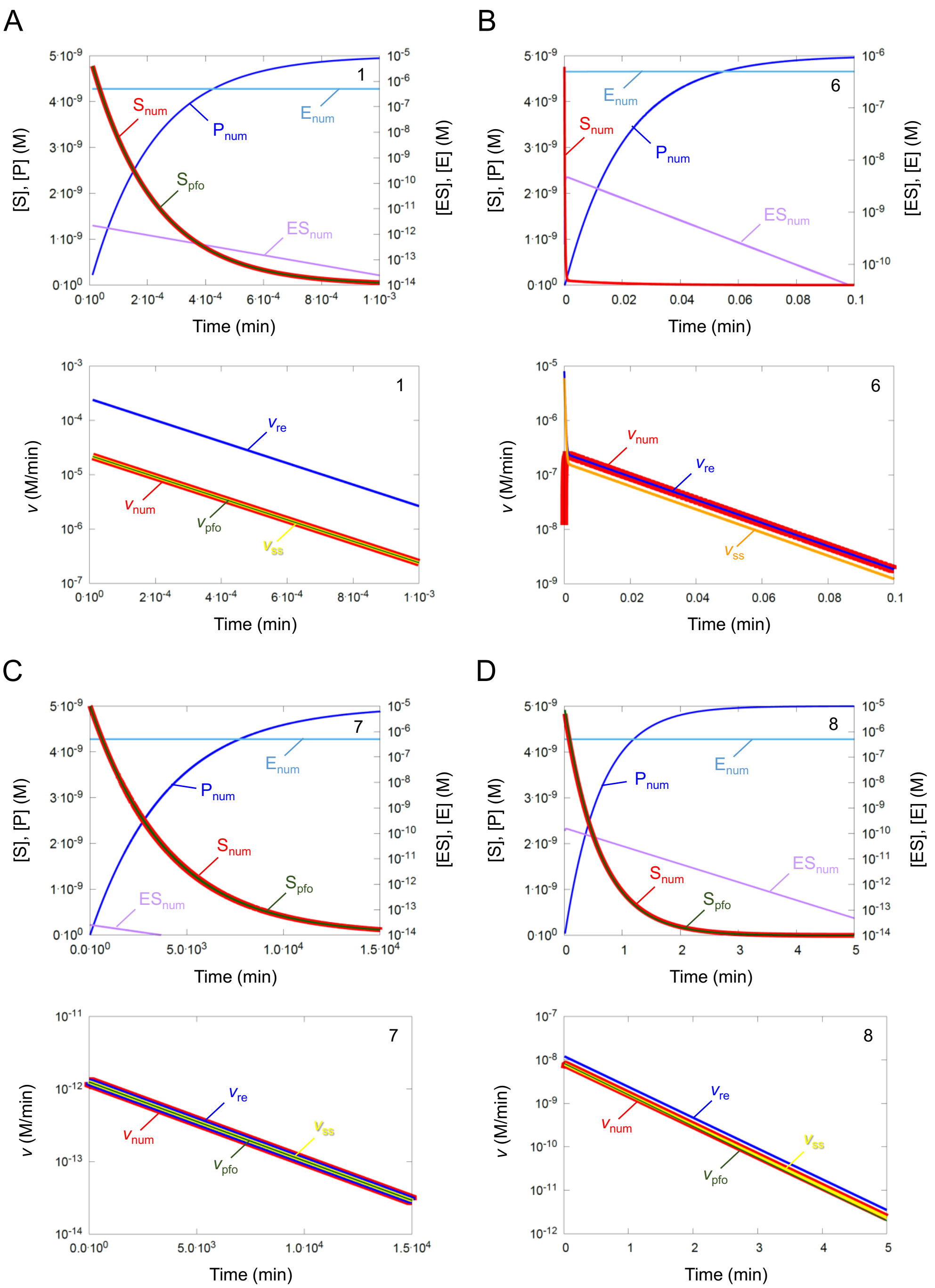
Supplementary Figure S4. Fpg-mediated incision of the C•C mismatch forms no ds break in DNA. Non-denaturing and denaturing PAGE of the DNA substrate with one C•C mismatch (upper panel) were performed following treatment with Fpg. The DNA (1 pmol) was incubated alone or with Fpg (13 pmol) under exactly the same conditions as described previously (see **Figure 1** and Materials and Methods). The incision product was separated from un-incised DNA by non-denaturing (200 V for 1 h) and denaturing (200 V for 2 h) PAGE (see **Figure 1B–D**). The single incision at C on the labeled strand, as was observed carried out by Fpg (shown by unbroken arrows), results in an incision product indistinguishable from substrate DNA (left panel), as monitored by non-denaturing PAGE (green square), and as opposed to denaturing PAGE (brown square). A putative ds break product (left panel) would form if Fpg targeted the mismatched C on the complementary strand following that on the forward strand (shown by broken arrows), and should be viewed as a 39-nt band following non-denaturing PAGE. Such a band was not observed (green square; between the green box drawings) in any of the five independent experiments (ten gel runs) performed. The 5´-label and strand are indicated in magenta; see **Figure 1A** for explanation of other colors.



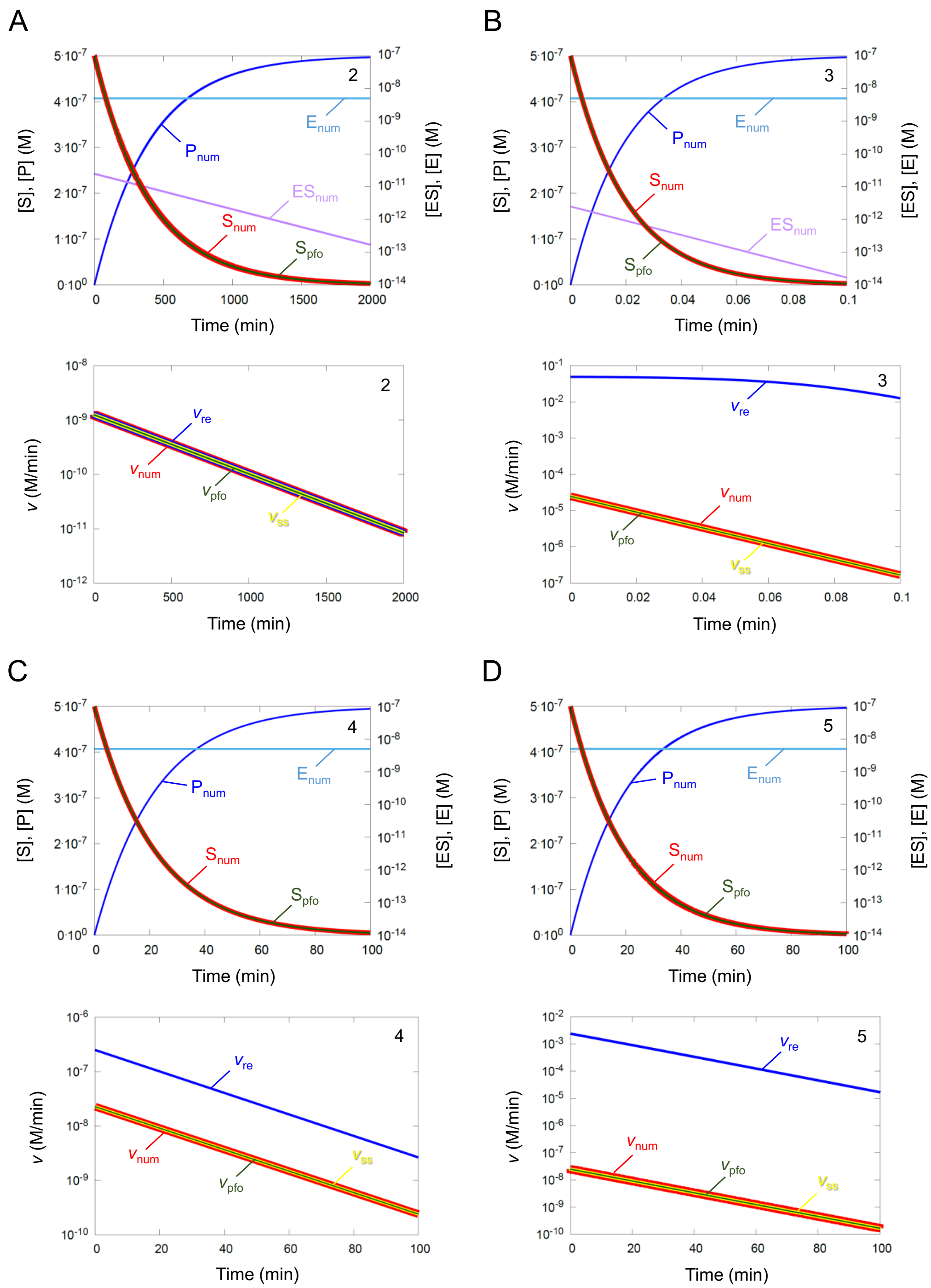
Supplementary Figure S5. Tentative accommodation of the C·C mismatch in the *E. coli* Fpg active site. This working model is based on crystal structures of Fpg orthologs with certain damaged base residues, as presented in the discussion section. Residues Thr214, Thr215, Leu216 and Lys217 are part of the flexible αF-β9 loop (grey arc), these possibly forming H-bonds (grey broken lines) to the flipped-out substrate C base. Arg108 forms H-bonds to the opposite C base. The Fpg active site was manually inspected and visualised using PyMOL (The PyMOL Molecular Graphics System, Version 2.4 Schrödinger, LLC). Abbreviation: dRP, deoxyribose phosphate.



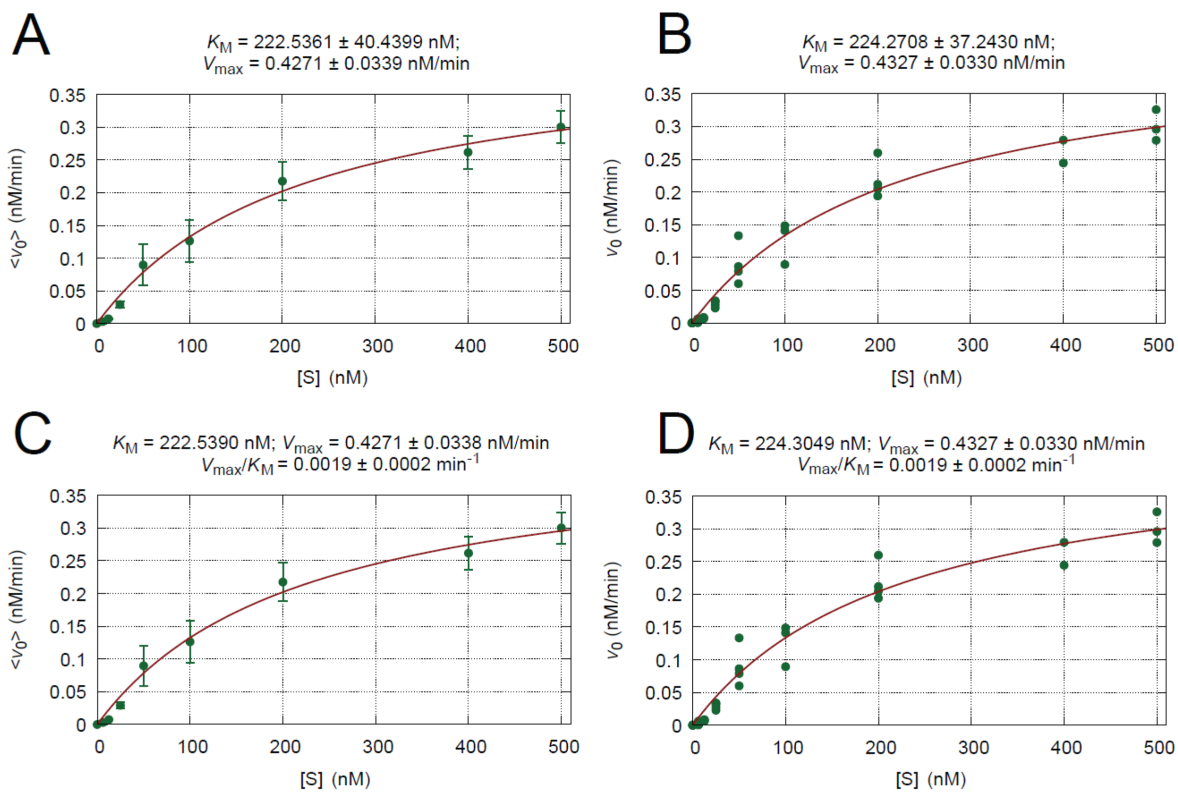
Supplementary Figure S6. Origin and biological consequences of the C·C DNA mismatch in *E. coli*. This working model summarizes the expected origin of a C·C mismatch in *E. coli* DNA and its putative destiny. Pol III replicates the genome with high processivity (step 1) and fidelity due to binding to the replication clamp and its 3′→ 5′ exonuclease (exo) function respectively, thus removing all base mismatches efficiently (steps 2 and 3). A C·C mismatch that evades these defenses might be extended by Pol III (step 4). This is, however, very unlikely. Instead, Pol III leaves the replication clamp for a trans-lesion synthesis (TLS) Pol, being able to synthesize downstream of the lesion, where Pol IV is, in this case, most likely (step 5). Two scenarios are possible after Pol IV departure. Either the C·C mismatch survives the ongoing round of replication and Pol III replicates both strands, this resulting in a G·C → C·G mutation in 50% of the offspring (step 6a and b), or Fpg is recruited to C·C and initiates BER (step 7). In the latter case we propose that the opposed Cs are selected randomly, yielding a 50% chance of mutagenesis (step 7a and b) as in the first scenario. Innocuous reactions or events are shown in blue, in red if aberrant or causing mutagenesis, short arrows showing class switch between replicative and TLS Pol or between Pol and Fpg (🠉, leaves DNA; 🠋, recruited to DNA).



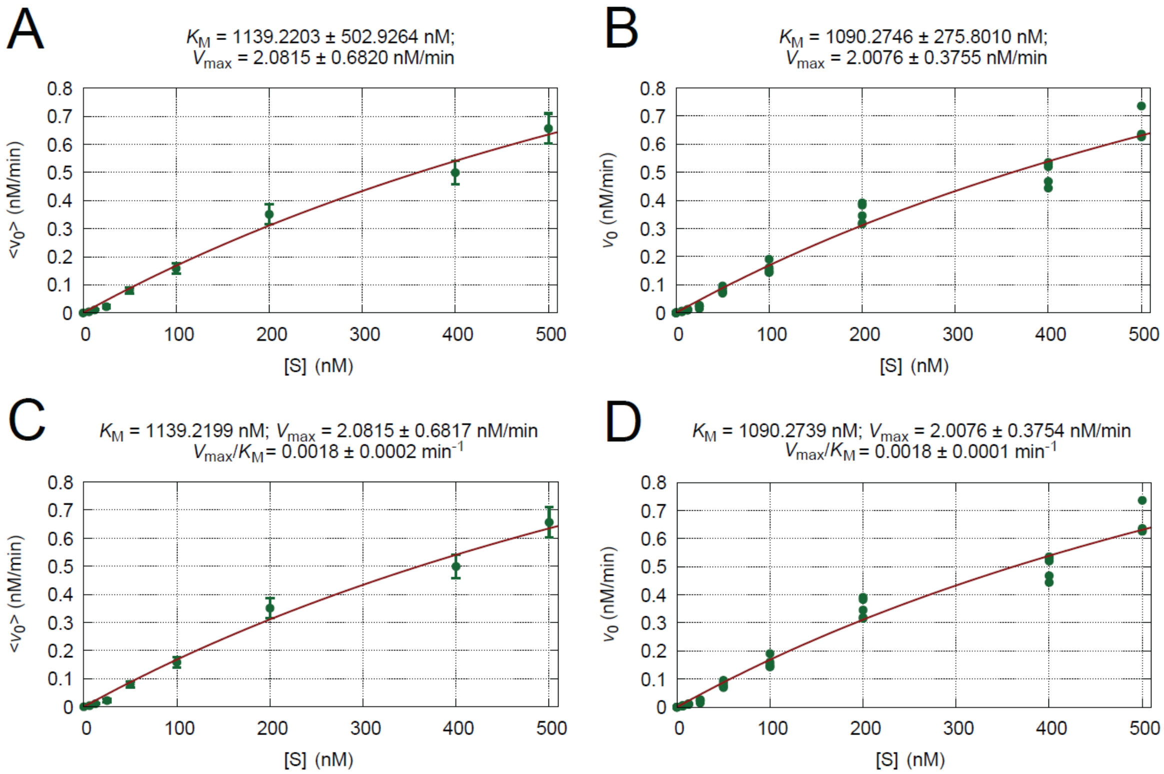
Supplementary Figure S7. Comparison of velocity data of steady-state and rapid equilibrium approximations and v_{pfo}, S_{pfo} expressions with corresponding numerical calculation (see Kinetic and Computational Methods). Initial substrate concentration in all calculations is $[S]_0 = 5 \times 10^{-9}$ M and initial enzyme concentration $[E]_0 = [E]_{tot} = 500 \times 10^{-9}$ M. Velocities are shown as semi-logarithmic plots. The linearity in the plots shows that the velocities decrease exponentially. Symbols with 'num' subscripts refer to the numerical results, while 'ss' and 're' subscripts refer to steady-state and rapid equilibrium values, respectively. Panels **(A)**, **(B)**, **(C)** and **(D)** refer to the rate constant combinations 1, 6, 7, 8 (**Supplementary Table S2**), respectively. The remaining four rate constant combinations 2, 3, 4 and 5 are shown in **Supplementary Figure S8**.



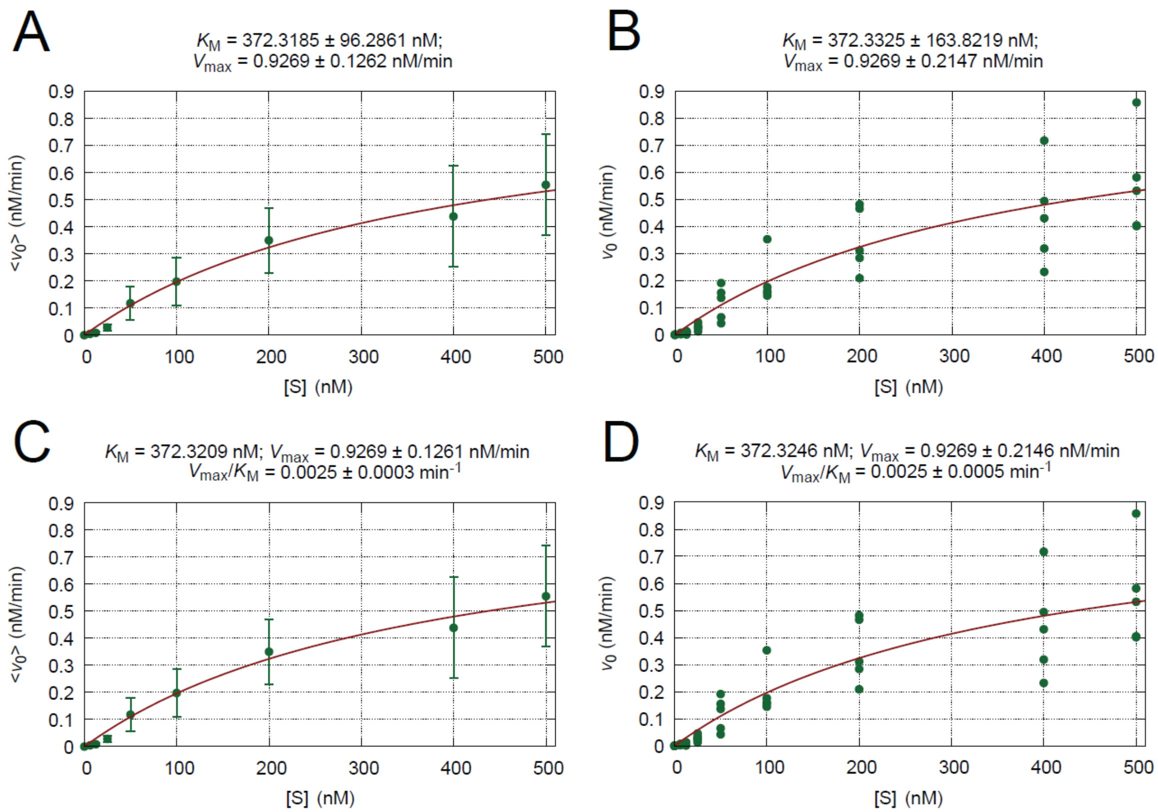
Supplementary Figure S8. Comparison of velocity data of steady-state and rapid equilibrium approximations, and v_{pfo} and S_{pfo} expressions with corresponding numerical calculation (see Kinetic and Computational Methods). Initial substrate concentration in all calculations is $[S]_0 = 5 \times 10^{-9}$ M and initial enzyme concentration $[E]_0 = [E]_{tot} = 500 \times 10^{-9}$ M. Velocities are shown as semi-logarithmic plots. The linearity in the plots shows that the velocities decrease exponentially. Symbols with 'num' subscripts refer to the numerical results, while 'ss' and 're' subscripts refer to steady-state and rapid equilibrium values, respectively. Panels **(A)**, **(B)**, **(C)** and **(D)** refer to the rate constant combinations 2, 3, 4 and 5 (**Supplementary Table S2**), respectively. The remaining four rate constant combinations 1, 6, 7, 8 are shown in **Supplementary Figure S7**.



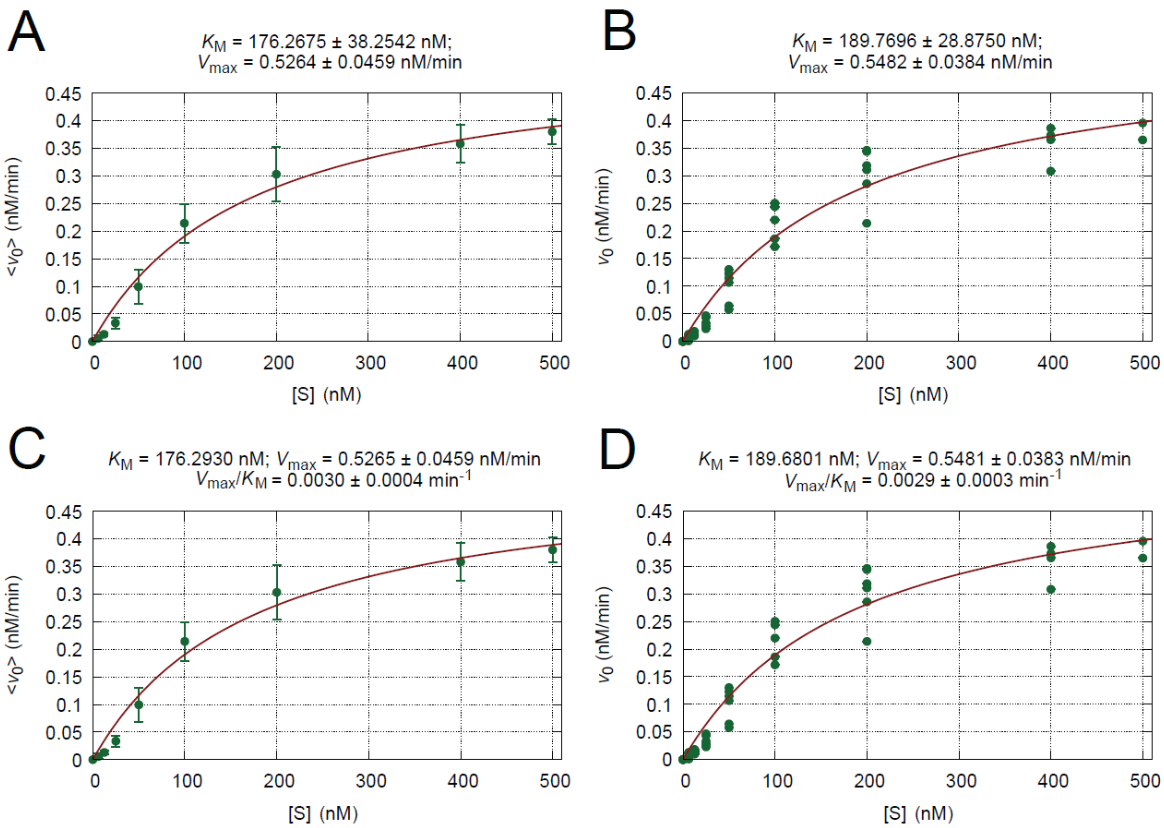
Supplementary Figure S9. Curve fits of the C·C data. Left panels **(A** and **C)** show the curve fits based on the average velocity data, while right panels **(B** and **D)** show the curve fits based on all individual data. Upper panels **(A** and **B)** show the fit of V_{max} and K_M to $v_0 = \frac{V_{max} \cdot [S]}{K_M + [S]}$. Lower panels **(C** and **D)** show the fit of $\alpha = V_{max}/K_M$ and $\beta = K_M$ to the equation (Johnson, 2019) $v_0 = \frac{\alpha \cdot [S]}{1 + \frac{\alpha \cdot [S]}{\beta}}$ using the average velocities (panels **C**) and all velocities (panels **D**). The values reported in **Table 1** are from panels B and D, but for comparison the curve fits on the average data are also included.



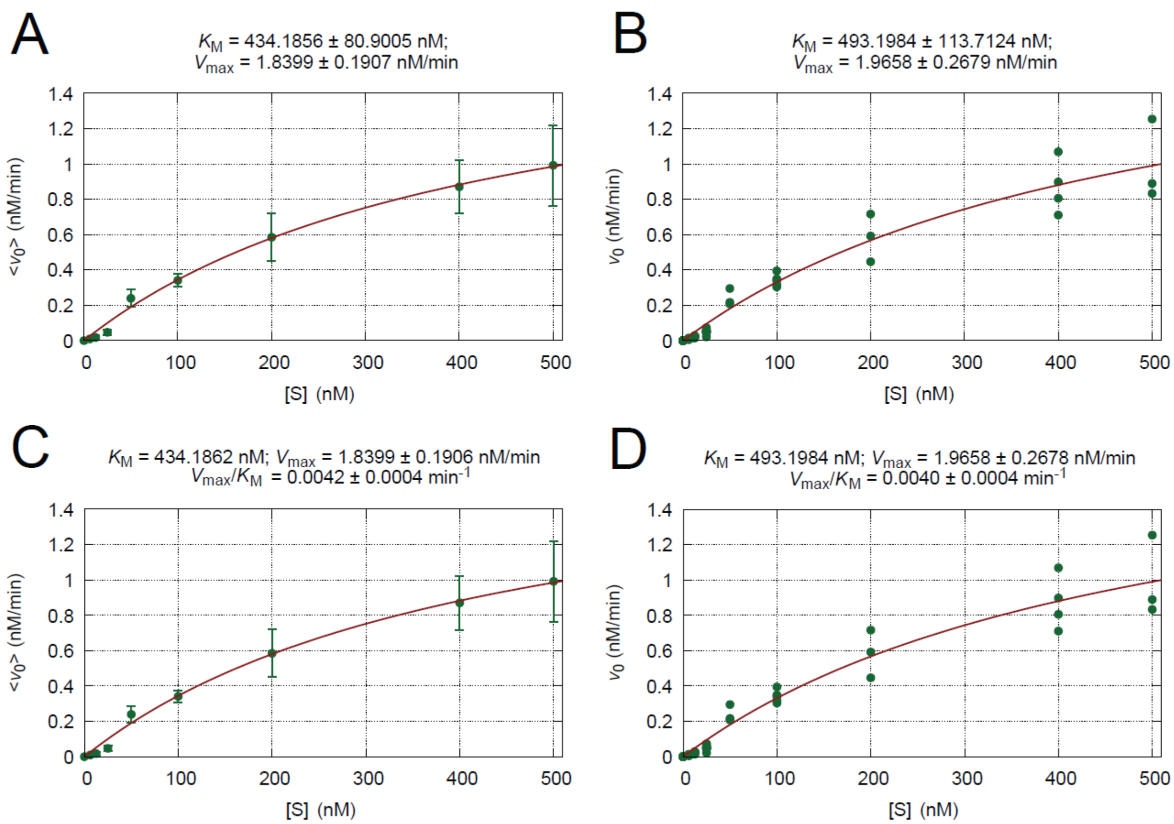
Supplementary Figure S10. Curve fits of the C•C data. See legend to **Supplementary Figure S9** for explanations.



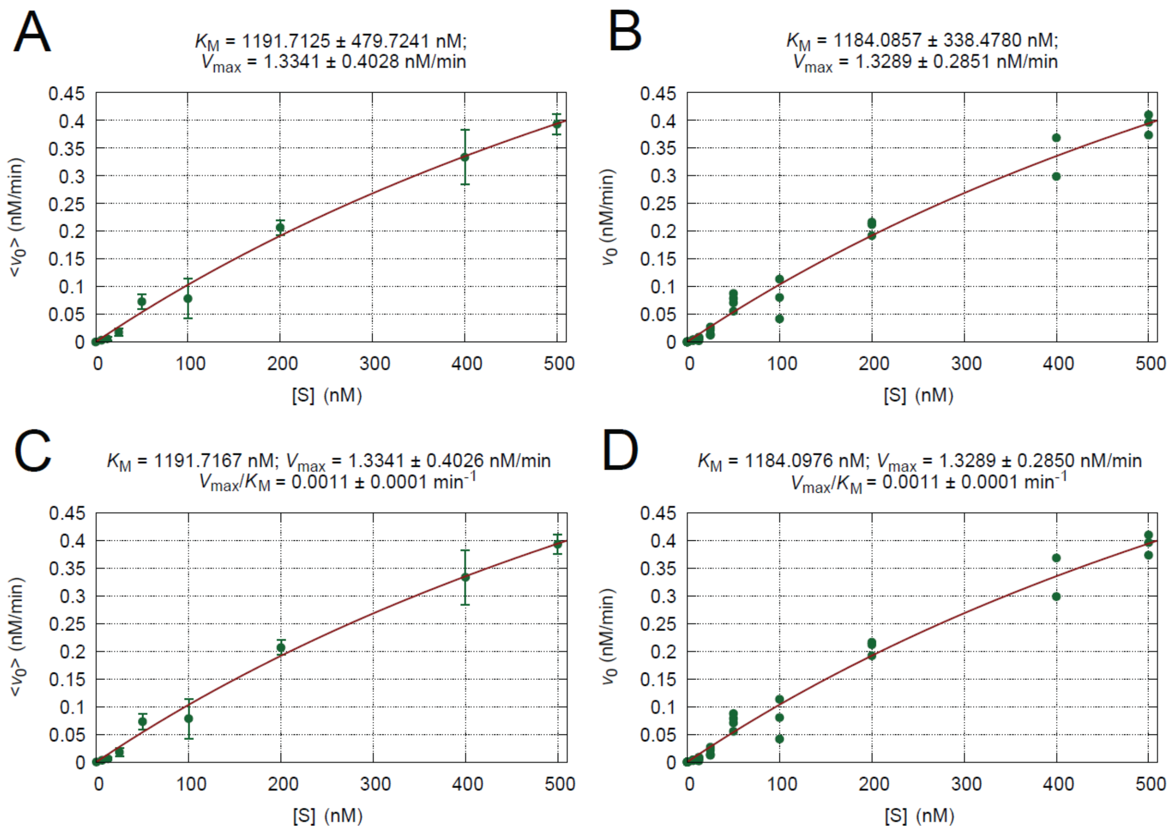
Supplementary Figure S11. Curve fits of the T·T data. See legend to **Supplementary Figure S9** for explanations.



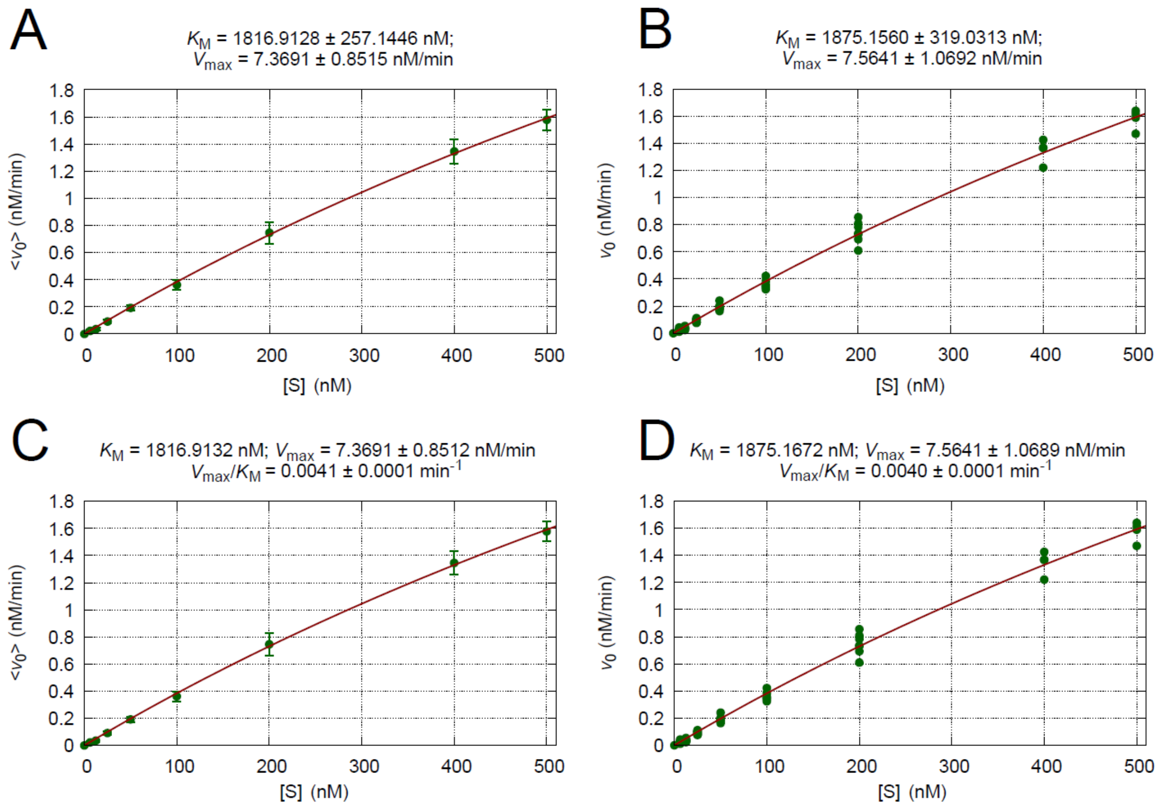
Supplementary Figure S12. Curve fits of the m^{N4}C•C data. See legend to **Supplementary Figure S9** for explanations.



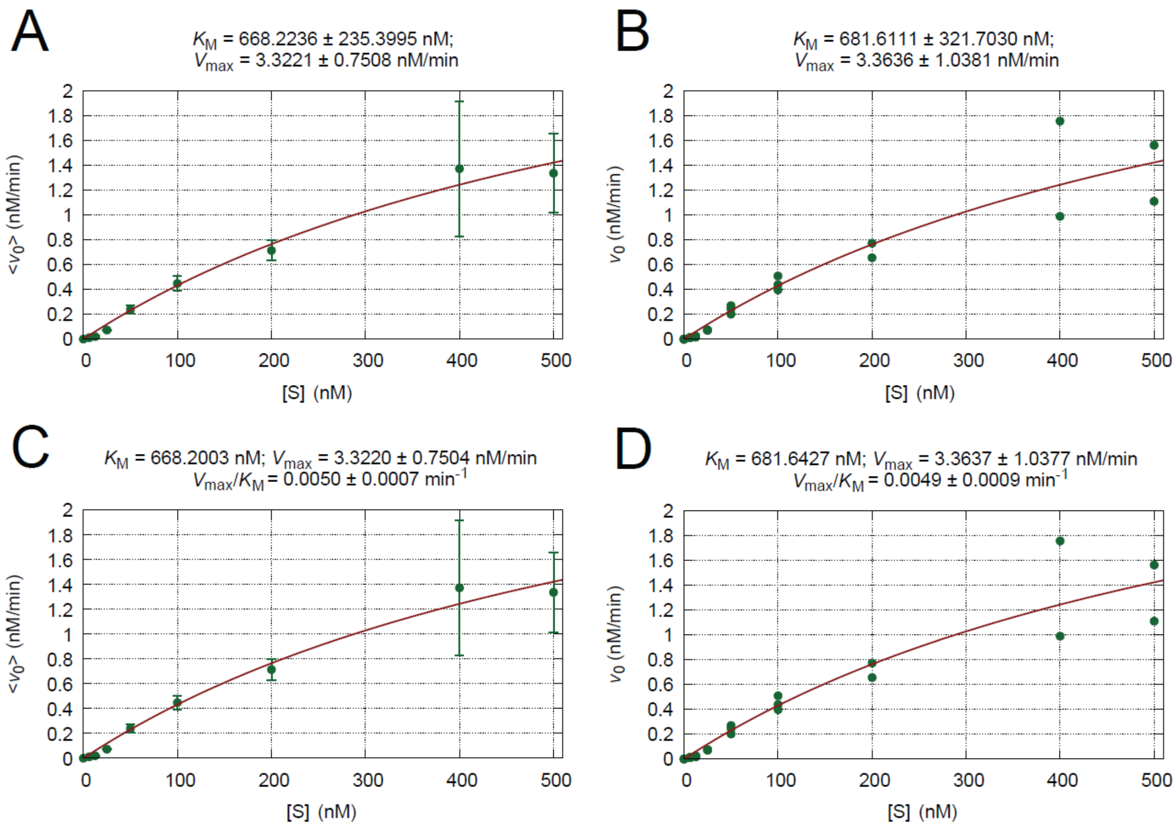
Supplementary Figure S13. Curve fits of the m[5]C·C data. See legend to **Supplementary Figure S9** for explanations.



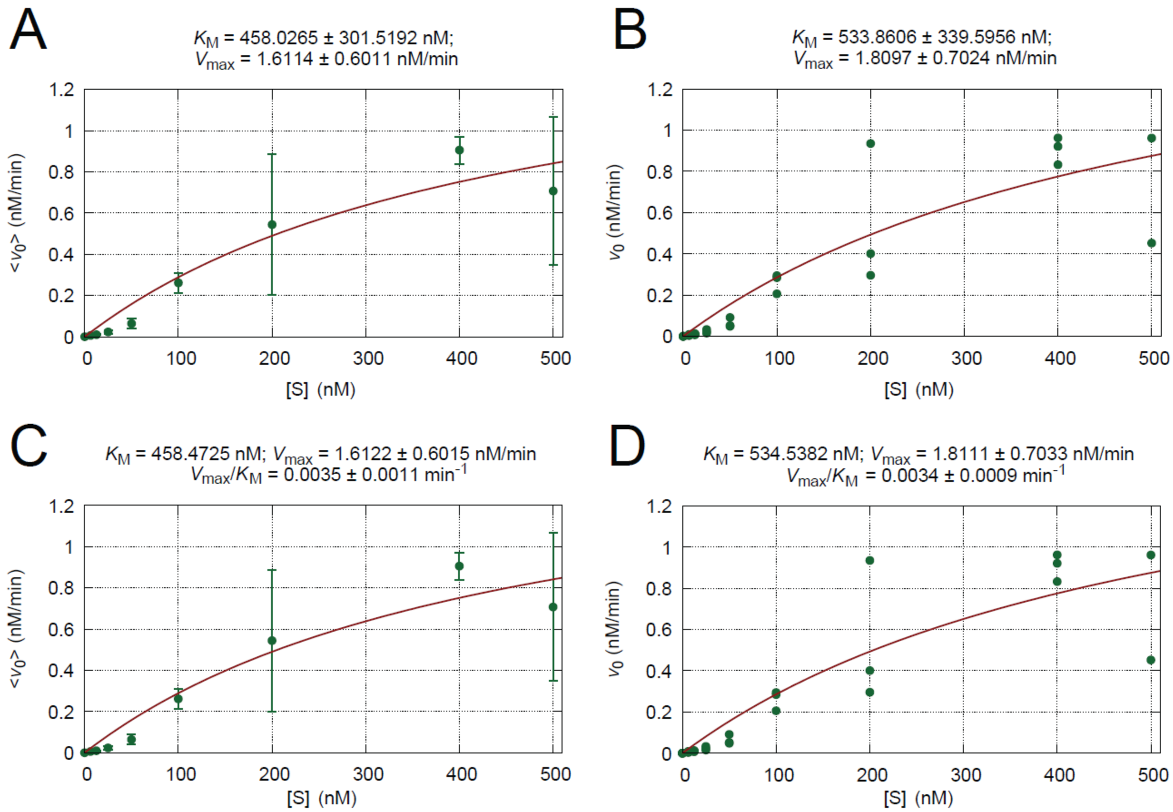
Supplementary Figure S14. Curve fits of the m^{5}C·T data. See legend to **Supplementary Figure S9** for explanations.



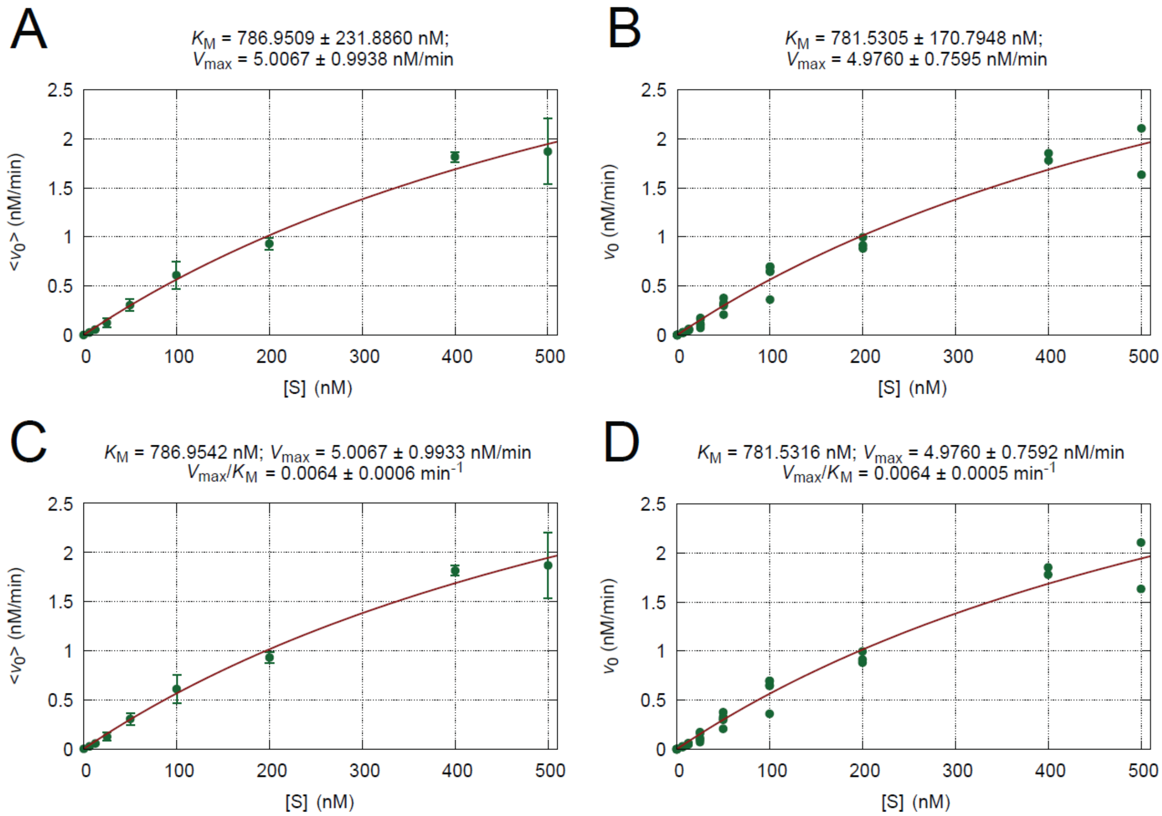
Supplementary Figure S15. Curve fits of the $m^{N4,5}C$·C data. See legend to **Supplementary Figure S9** for explanations.



Supplementary Figure S16. Curve fits of the dHT·A data. See legend to **Supplementary Figure S9** for explanations.



Supplementary Figure S17. Curve fits of the Tg•A data. See legend to **Supplementary Figure S9** for explanations.



Supplementary Figure S18. Curve fits of the oxo^{8}G·C data. See legend to **Supplementary Figure S9** for explanations.

2.2 Supplementary Tables

Supplementary Table S2. MS analysis of the commercial (New England Biolabs) Fpg preparation used in this study.

Checked	Master	Accession	Description	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Protein FDR Confidence Mascot	Exp. q-value Mascot	Score Mascot	# Peptides Mascot
USANN	Master Protein	P02769	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4	72.1581549	51	286	37	1	607	69.248	6.18	High	0	5855.636781	51
USANN	Master Protein	B1X969	Formamidopyrimidine-DNA glycosylase OS=Escherichia coli (strain K12 / DH10B) GN=mutM PE=3 SV=1	74.7211896	20	178	9	1	269	30.271	8.43	High	0	3136.669735	20
USANN	Master Protein	P00761	Trypsin OS=Sus scrofa PE=1 SV=1	35.4978355	6	87	6	1	231	24.394	7.18	High	0	1300.854407	6
USANN	Master Protein	A9MKN9	Formamidopyrimidine-DNA glycosylase OS=Salmonella arizonae (strain ATCC BAA-731 / CDC346-86 / RSK2980) GN=mutM PE=3 SV=1	40.5204461	12	77	2	1	269	30.26	8.6	High	0	1179.926923	12
USANN	Master Protein	A9MVN0	Formamidopyrimidine-DNA glycosylase OS=Salmonella paratyphi B (strain ATCC BAA-1250 / SPB7) GN=mutM PE=3 SV=1	36.4312268	10	71	1	1	269	30.146	8.43	High	0	1077.138065	10
USANN	Master Protein	P49064	Serum albumin OS=Felis catus GN=ALB PE=1 SV=1	14.3092105	10	58	1	1	608	68.615	5.66	High	0	884.8574433	10
USANN	Master Protein	P49822	Serum albumin OS=Canis lupus familiaris GN=ALB PE=1 SV=3	12.3355263	9	48	1	1	608	68.56	5.69	High	0	688.5956146	9
USANN	Master Protein	P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	31.9875776	19	27	17	1	644	65.999	8.12	High	0	543.3990835	19
USANN	Master Protein	P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	7.22495895	6	38	2	1	609	69.321	6.28	High	0	489.1405033	6
USANN	Master Protein	C6DIB8	Formamidopyrimidine-DNA glycosylase OS=Pectobacterium carotovorum subsp. carotovorum (strain PC1) GN=mutM PE=3 SV=1	8.55018587	3	26	1	1	269	30.211	7.46	High	0	481.7550569	3
USANN	Master Protein	B2VF70	Formamidopyrimidine-DNA glycosylase OS=Erwinia tasmaniensis (strain DSM 17950 / CIP 109463 / Et1/99) GN=mutM PE=3 SV=1	26.7657993	6	23	1	1	269	29.808	8.56	High	0	449.4684211	6
USANN	Master Protein	P49065	Serum albumin OS=Oryctolagus cuniculus GN=ALB PE=1 SV=2	9.21052632	7	23	1	1	608	68.865	6.24	High	0	305.7455205	7
USANN	Master Protein	P13645	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	23.9726027	13	15	8	1	584	58.792	5.21	High	0	300.7739161	13
USANN	Master Protein	Q7MY36	Formamidopyrimidine-DNA glycosylase OS=Photorhabdus luminescens subsp. laumondii (strain DSM 15139 / CIP 105565 / TT01) GN=mutM PE=3 SV=3	8.55018587	3	13	1	1	269	30.738	7.81	High	0	253.9588572	3
USANN	Master Protein	P35908	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	28.6384977	15	16	11	1	639	65.393	8	High	0	252.7033333	15
USANN	Master Protein	P02533	Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4	16.3135593	8	9	2	1	472	51.529	5.16	High	0	137.7929221	8
USANN	Master Protein	Q3SZR3	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1	25.2475248	6	8	6	1	202	23.168	5.87	High	0	133.5133333	6
USANN	Master Protein	P35527	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	29.3739968	13	13	12	1	623	62.027	5.24	High	0	132.9268515	13
USANN	Master Protein	P13647	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3	18.6440678	11	11	8	1	590	62.34	7.74	High	0	89.27907563	11
USANN	Master Protein	O77727	Keratin, type I cytoskeletal 15 OS=Ovis aries GN=KRT15 PE=2 SV=1	12.803532	6	7	0	1	453	48.74	4.79	High	0	89.13135089	6
USANN	Master Protein	P01024	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	1.14251353	1	2	1	1	1663	187.03	6.4	High	0	88.43	1
USANN	Master Protein	P08779	Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4	14.1649049	7	8	1	1	473	51.236	5.05	High	0	85.15577924	7
USANN	Master Protein	Q6IFV3	Keratin, type I cytoskeletal 15 OS=Rattus norvegicus GN=Krt15 PE=1 SV=1	9.17225951	5	6	0	1	447	48.84	4.86	High	0.006622517	75.75375629	5
USANN	Master Protein	O46375	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1	31.292517	4	5	4	1	147	15.717	6.3	High	0.006622517	70.87103371	4
USANN	Master Protein	O57611	Keratin, type I cytoskeletal 18 OS=Scyliorhinus stellaris GN=krt18 PE=1 SV=1	3.85542169	2	2	1	1	415	46.763	5.36	High	0.006622517	68.35006897	2
USANN	Master Protein	P41361	Antithrombin-III OS=Bos taurus GN=SERPINC1 PE=1 SV=2	8.60215054	4	4	4	1	465	52.314	7.33	High	0.006622517	60.87	4
USANN	Master Protein	A6TEV7	50S ribosomal protein L6 OS=Klebsiella pneumoniae subsp. pneumoniae (strain ATCC 700721 / MGH 78578) GN=rplF PE=3 SV=1	11.8644068	2	2	2	1	177	18.832	9.7	High	0.006622517	60.45395424	2
USANN	Master Protein	P0A2S0	Leucine-responsive regulatory protein OS=Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720) GN=lrp PE=3 SV=2	31.7073171	6	6	6	1	164	18.845	8.78	High	0.006622517	54.80096197	6
USANN	Master Protein	Q8VZD5	Shaggy-related protein kinase epsilon OS=Arabidopsis thaliana GN=ASK5 PE=2 SV=1	2.19512195	1	1	1	1	410	46.045	8.5	High	0.006622517	53.1	1
USANN	Master Protein	P08730	Keratin, type I cytoskeletal 13 OS=Mus musculus GN=Krt13 PE=1 SV=2	12.5858124	6	7	1	1	437	47.724	4.86	High	0.006622517	51.38606063	6

Supplementary Table S2. Rate constant combinations used in testing the steady-state and rapid equilibrium approximations.

Combination No.*	1	2	3	4	5	6	7	8
k_1, M^{-1} min^{-1}	10^{10}	10^{10}	10^{10}	10^{7}	10^{7}	10^{10}	10^{7}	10^{7}
k_{-1}, min^{-1}	10^{6}	10^{6}	10^{2}	10^{6}	10^{2}	10^{2}	10^{6}	10^{2}
k_2, min^{-1}	10^{7}	50	10^{7}	10^{7}	10^{7}	50	50	50

*orange cells indicate upper range values; blue cells indicate lower range values.

Supplementary Table S3. Pseudo-First-Order Kinetics.

Kinetics $m^{N4,5}C$:C

[S]		[S]	Velocity (*v*)		Product (P), nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0226551	0.009	0.679653525	10.8744564	89.1255436	3.84E-03
0.25	0.00025	12.5	0.0355489	0.009	1.066466082	8.531728658	91.46827134	2.97E-03
0.5	0.0005	25	0.0914703	0.012	2.744108571	10.97643429	89.02356571	3.88E-03
1	0.001	50	0.1920254	0.021	5.760763132	11.52152626	88.47847374	4.08E-03
2	0.002	100	0.3617699	0.035	10.8530979	10.8530979	89.1469021	3.83E-03
4	0.004	200	0.7452692	0.081	22.35807727	11.17903864	88.82096136	3.95E-03
8	0.008	400	1.3450778	0.087	40.35233502	10.08808375	89.91191625	3.54E-03
10	0.01	500	1.5778566	0.075	47.33569716	9.467139432	90.53286057	3.32E-03
						10.43643817	89.56356183	3.68E-03
								SD for *k*
								3.72E-04

Kinetics m^5C:C

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0095899	0.004	0.287698111	4.603169778	95.39683022	1.57E-03
0.25	0.00025	12.5	0.0177663	0.011	0.532988586	4.26390869	95.73609131	1.45E-03
0.5	0.0005	25	0.0471974	0.014	1.415921343	5.663685371	94.33631463	1.94E-03
1	0.001	50	0.2396488	0.048	7.189464546	14.37892909	85.62107091	5.17E-03
2	0.002	100	0.3409019	0.035	10.22705759	10.22705759	89.77294241	3.60E-03
4	0.004	200	0.5848167	0.135	17.54450015	8.772250073	91.22774993	3.06E-03
8	0.008	400	0.8705271	0.152	26.11581286	6.528953216	93.47104678	2.25E-03
10	0.01	500	0.9918472	0.228	29.75541723	5.951083446	94.04891655	2.05E-03
						7.548629656	92.45137034	2.64E-03
								SD for *k*
								1.26E-03

Kinetics $m^{N4}C$:C

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.005884	0.005	0.176520935	2.824334954	97.17566505	9.55E-04
0.25	0.00025	12.5	0.0131662	0.003	0.394985537	3.159884297	96.8401157	1.07E-03
0.5	0.0005	25	0.0336647	0.010	1.009942235	4.039768942	95.96023106	1.37E-03
1	0.001	50	0.0994481	0.031	2.983443814	5.966887627	94.03311237	2.05E-03
2	0.002	100	0.2143943	0.035	6.431829441	6.431829441	93.56817056	2.22E-03
4	0.004	200	0.3032012	0.049	9.09603477	4.548017385	95.45198262	1.55E-03
8	0.008	400	0.358297	0.034	10.74891071	2.687227677	97.31277232	9.08E-04
10	0.01	500	0.3803272	0.022	11.4098145	2.2819629	97.7180371	7.69E-04
						3.992489153	96.00751085	1.36E-03
								SD for *k*
								5.41E-04

Kinetics m^5C:T

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.003202	0.001	0.096061372	1.536981956	98.46301804	5.16E-04
0.25	0.00025	12.5	0.0059074	0.003	0.177222842	1.417782737	98.58221726	4.76E-04
0.5	0.0005	25	0.0174881	0.007	0.524641645	2.098566578	97.90143342	7.07E-04
1	0.001	50	0.0729542	0.014	2.188625547	4.377251094	95.62274891	1.49E-03
2	0.002	100	0.0783178	0.036	2.349533103	2.349533103	97.6504669	7.93E-04
4	0.004	200	0.2066942	0.013	6.200826458	3.100413229	96.89958677	1.05E-03
8	0.008	400	0.3336856	0.049	10.01056852	2.502642129	97.49735787	8.45E-04
10	0.01	500	0.3933268	0.018	11.79980472	2.359960943	97.64003906	7.96E-04
						2.467891471	97.53210853	8.34E-04
								SD for *k*
								3.23E-04

Kinetics C:C (forward C excised)

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0031192	0.003	0.093576774	1.497228386	98.50277161	5.03E-04
0.25	0.00025	12.5	0.0074616	0.001	0.223848782	1.790790258	98.20920974	6.02E-04
0.5	0.0005	25	0.0291911	0.005	0.875733416	3.502933664	96.49706634	1.19E-03
1	0.001	50	0.0896232	0.031	2.688696383	5.377392766	94.62260723	1.84E-03
2	0.002	100	0.1262553	0.032	3.787659926	3.787659926	96.21234007	1.29E-03
4	0.004	200	0.2175025	0.029	6.525075014	3.262537507	96.73746249	1.11E-03
8	0.008	400	0.2617096	0.025	7.851286715	1.962821679	98.03717832	6.61E-04
10	0.01	500	0.3001807	0.024	9.005419998	1.801084	98.198916	6.06E-04
						2.872806023	97.12719398	9.74E-04
								SD for *k*
								4.64E-04

Kinetics C:C (reverse C excised)

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0038692	0.002	0.116075523	1.857208366	98.14279163	6.25E-04
0.25	0.00025	12.5	0.0108918	0.001	0.326754584	2.614036669	97.38596333	8.83E-04
0.5	0.0005	25	0.0221747	0.005	0.665239537	2.660958148	97.33904185	8.99E-04
1	0.001	50	0.080301	0.011	2.409030634	4.818061269	95.18193873	1.65E-03
2	0.002	100	0.1583391	0.019	4.750174325	4.750174325	95.24982568	1.62E-03
4	0.004	200	0.3508888	0.035	10.52666314	5.263331568	94.73666843	1.80E-03
8	0.008	400	0.4991973	0.041	14.97591835	3.743979587	96.25602041	1.27E-03
10	0.01	500	0.6565616	0.053	19.69684737	3.939369475	96.06063053	1.34E-03
						3.705889926	96.29411007	1.26E-03
								SD for *k*
								4.24E-04

Kinetics oxoG:C

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0255233	0.002	0.765698716	12.25117945	87.74882055	4.36E-03
0.25	0.00025	12.5	0.0546379	0.006	1.639137785	13.11310228	86.88689772	4.69E-03
0.5	0.0005	25	0.1237936	0.043	3.713808987	14.85523595	85.14476405	5.36E-03
1	0.001	50	0.3038953	0.062	9.11685981	18.23371962	81.76628038	6.71E-03
2	0.002	100	0.6090111	0.141	18.27033347	18.27033347	81.72966653	6.73E-03
4	0.004	200	0.9302441	0.059	27.90732378	13.95366189	86.04633811	5.01E-03
8	0.008	400	1.8146305	0.052	54.43891524	13.60972881	86.39027119	4.88E-03
10	0.01	500	1.868644	0.334	56.05931865	11.21186373	88.78813627	3.96E-03
						14.43735315	85.56264685	5.21E-03
								SD for *k*
								1.02E-03

Kinetics T:T

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0046944	0.002	0.140831465	2.253303444	97.74669656	7.60E-04
0.25	0.00025	12.5	0.0085559	0.005	0.25667792	2.053423362	97.94657664	6.92E-04
0.5	0.0005	25	0.0284999	0.012	0.854995931	3.419983725	96.58001627	1.16E-03
1	0.001	50	0.1179246	0.062	3.537739027	7.075478053	92.92452195	2.45E-03
2	0.002	100	0.1978508	0.087	5.935524722	5.935524722	94.06447528	2.04E-03
4	0.004	200	0.3502507	0.119	10.50752243	5.253761215	94.74623879	1.80E-03
8	0.008	400	0.4385017	0.186	13.15505102	3.288762756	96.71123724	1.11E-03
10	0.01	500	0.5552973	0.187	16.65891951	3.331783901	96.6682161	1.13E-03
						4.076502647	95.92349735	1.39E-03
								SD for *k*
								6.31E-04

Kinetics dHT:A

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0114685	0.001	0.34405553	5.504888484	94.49511152	1.89E-03
0.25	0.00025	12.5	0.0199846	0.004	0.5995385	4.796308	95.203692	1.64E-03
0.5	0.0005	25	0.0725877	0.002	2.177631623	8.710526492	91.28947351	3.04E-03
1	0.001	50	0.2385781	0.035	7.157343539	14.31468708	85.68531292	5.15E-03
2	0.002	100	0.4479579	0.057	13.43873787	13.43873787	86.56126213	4.81E-03
4	0.004	200	0.7143209	0.083	21.42962597	10.71481298	89.28518702	3.78E-03
8	0.008	400	1.3729648	0.543	41.18894371	10.29723593	89.70276407	3.62E-03
10	0.01	500	1.3366373	0.320	40.09911973	8.019823946	91.98017605	2.79E-03
						9.474627597	90.5253724	3.34E-03
								SD for *k*
								1.26E-03

Kinetics Tg:A

[S]		[S]	*v*		P, nM (30 min)	P, % (30 min)	S, % (30 min)	First-order *k* (min^{-1})
pmol/20 µL	nmol/20 µL	nM	nM/min	SD				
0.125	0.000125	6.25	0.0060982	0.002	0.182946616	2.927145863	97.07285414	9.90E-04
0.25	0.00025	12.5	0.0097851	0.002	0.293553635	2.34842908	97.65157092	7.92E-04
0.5	0.0005	25	0.02347	0.009	0.704099872	2.816399489	97.18360051	9.52E-04
1	0.001	50	0.0638704	0.024	1.916111256	3.832222511	96.16777749	1.30E-03
2	0.002	100	0.2614345	0.048	7.84303375	7.84303375	92.15696625	2.72E-03
4	0.004	200	0.5440254	0.343	16.32076123	8.160380614	91.83961939	2.84E-03
8	0.008	400	0.9053498	0.066	27.16049509	6.790123771	93.20987623	2.34E-03
10	0.01	500	0.7069659	0.360	21.20897575	4.241795151	95.75820485	1.44E-03
						4.869941279	95.13005872	1.67E-03
								SD for *k*
								8.33E-04

Supplementary Table S4. Fpg-mediated DNA incision at different enzyme concentrations and time. See **Supplementary Figure S3A**.

10 min incubation				
		[P]	**DNA incised**	
pmol/20 µL	nmol/20 µL	**nM**	**nM**	SD
0.5	0.0005	25	0.1403025	0.165
1	0.001	50	0.1113906	0.150
2	0.002	100	0.2631138	0.232
5	0.005	250	1.5154974	0.505
10	0.01	500	2.4738872	0.462
13	0.013	650	3.0038826	0.696
30 min incubation				
		[P]	**DNA incised**	
pmol/20 µL	nmol/20 µL	**nM**	**nM**	SD
0.5	0.0005	25	0.1757002	0.202
1	0.001	50	0.3485098	0.316
2	0.002	100	1.1292203	1.407
5	0.005	250	3.1784741	2.004
10	0.01	500	6.2993596	3.966
13	0.013	650	9.0554917	3.673
60 min incubation				
		[P]	**DNA incised**	
pmol/20 µL	nmol/20 µL	**nM**	**nM**	SD
0.5	0.0005	25	0.1658493	0.141
1	0.001	50	0.3774236	0.154
2	0.002	100	0.9160621	1.057
5	0.005	250	6.9178477	5.032
10	0.01	500	10.516854	4.650
13	0.013	650	11.194292	5.460