Supporting Information To:

The formation of catalytically competent enzyme-substrate complex is not a bottleneck in lesion excision by human alkyladenine DNA glycosylase

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Guanine
G

Cytosine
C

Adenine
A

Ethenoadenine
εA

Hypoxanthine
Hx

Thymine
T

Supplementary Figure S1. Imino protons visible in NMR spectra (marked red).

(A)

(B)

(C)

Supplementary Figure S2. Assigned 2D NOESY spectrum of A/T (A), Hx/T (B), ϵ A/T (C) and F/T (D) duplexes. Solid lines indicate sequential connectivities of imino protons with the cytosine network system for protons H5, H6, H41 and H42.

Supplementary Figure S3. Imino proton region of the 2D NOESY spectra of A/T (A), Hx/T (B), $\epsilon\text{A/T}$ (C), F/T (D) duplexes.

Supplementary Figure S4. Example of spectral deconvolution of F/T duplex at 2°C. Black line is experimental spectrum; magenta line is superposition of individually simulated signals.

Supplementary Figure S5. NMR pulse sequence used in exchange kinetics experiments. ^1H shows channel for the proton radiofrequency pulses; rectangular – non-selective $\pi/2$ pulses, Gaussian shape – selective π pulses. G_z stays for the Z-gradient.

Supplementary Figure S6. Dependence of k_{ex} on active ammonia catalyst concentration [NH₃] for A/T (A), Hx/T (B), ϵ A/T (C) and F/T (D) duplexes.

Supplementary Figure S7. Imino proton region of ^1H NMR spectra of A/T, Hx/T, ϵA /T and F/T duplexes at different concentration of NH_3 .

Supplementary Figure S8. Kinetic traces for imino protons in different duplexes at $[\text{NH}_3] = 0$. For imino protons of thymidine T7 opposite to the X base the kinetics changes dramatically due to different effective exchange rates, but for imino protons of thymidine T10 there is practically no difference between the kinetics of duplexes A, Hx, ϵA .

Global nonlinear fitting of stopped-flow data

In this study, the kinetic parameters (both the rate constants and the response factors) were obtained by a global non-linear fitting procedure using the DynaFit software (<http://www.biokin.com/dynafit/>). The DynaFit software performs numerical integration with subsequent nonlinear least-square regression analysis of the data of enzymatic kinetics. The experimental data are the reaction progress curves (the fluorescence intensity vs. time). The concentrations are not measured directly, instead a reporting signal, proportional to concentrations, is used. It should be noted, that the whole approach is based on the variation of the response factor in the course of the reaction; there will be no fluorescence change with time if the response factors of the intermediate species in the mechanism were equal. Therefore, the response factors must be necessarily different and treated as fitting parameters in the data processing.

Except of the first bimolecular step, all reactions are first order reactions. As in the fitting of simple first order exponential decay curves, the kinetic information is obtained from the temporal behavior of the signal, not from its amplitude. In the current study, all conclusions are derived based exclusively upon the rate constants. The response factors resulting from the fits are not used in the determination of the equilibrium constants, but rather provide additional information on the fluorescence quantum yield variation in different conformations.

For example the time-course of appearance and disappearance of transient enzyme-substrate complexes $(E \cdot F)_i$ during interaction of AAG with F/T-product and their response factors are presented in Supplementary Figure S9.

Supplementary Figure S9. Time-course of appearance and disappearance of transient enzyme-substrate complexes during interaction of AAG with F/T-product. Modeling was done for 3.0 μM AAG and 3.0 μM F/T-product with the rate constants listed in Table 2.

Supplementary Table S1. Chemical shift of protons in DNA duplex A/T in ppm.

	Imino	Amino			Non-exchangeable		
Base pair	GH1/TH3	CH41	CH42	CH5	CH6/TH6	AH2	TM7
A1-T1	–	X	X	X	7.572	7.935	1.738
G2-C2	12.711	7.13	8.426	5.82	7.751	X	X
A3-T3	13.842	X	X	X	7.504	7.702	1.672
G4-C4	12.698	7.065	8.426	5.658	7.623	X	X
A5-T5	13.791	X	X	X	7.413	7.538	1.623
G6-C6	12.535	6.883	8.514	5.686	7.514	X	X
T7-A7	13.577	X	X	X	7.098	7.638	1.225
G8-C8	12.689	6.571	8.039	5.244	7.242	X	X
G9-C9	12.805	6.787	8.222	5.419	7.46	X	X
A10-T10	14.068	X	X	X	7.47	7.541	1.579
A11-T11	13.941	X	X	X	7.462	7.372	1.669
G12-C12	12.833	7.04	8.541	5.774	7.607	X	X
G13-C13	–	7.197	8.155	5.846	7.688	X	X

X- non-existing proton; “–” – not determined; GH1/TH3 – imino protons of guanine or thymine; CH42 – cytosine hydrogen-bonded amino-proton; CH41 – cytosine non-hydrogen-bonded amino-proton; CH5, CH6, TH6 – aromatic protons of cytosine and thymine, resp.; AH2 – H2 proton of adenine; TM7 – methyl protons of thymine. Numbering of protons is displayed in Figure S1.

Supplementary Table S2. Chemical shift of protons in DNA duplex Hx/T in ppm.

Base pair	Imino	Amino		Non-exchangeable			
	GH1/TH3/HxH1	CH41	CH42	CH5	CH6/TH6	AH2/HxH2	TM7
A1-T1	–	X	X	X	7.587	7.945	1.746
G2-C2	12.734	7.161	8.455	5.802	7.752	X	X
A3-T3	13.858	X	X	X	7.512	7.698	1.667
G4-C4	12.694	7.15	8.432	5.654	7.635	X	X
A5-T5	13.798	X	X	X	7.453	7.514	1.643
G6-C6	12.848	6.916	8.528	5.715	7.586	X	X
T7-Hx7	11.337(11.898)	X	X	X	7.133	-7.685	1.578
G8-C8	12.787	6.661	8.101	5.293	7.282	X	X
G9-C9	12.846	6.897	8.307	5.456	7.498	X	X
A10-T10	14.096	X	X	X	7.46	7.517	1.605
A11-T11	13.969	X	X	X	7.464	7.368	1.677
G12-C12	12.857	7.079	8.559	5.772	7.61	X	X
G13-C13	–	7.227	8.21	5.84	7.681	X	X

X- non-existing proton; “–” – not determined; GH1/TH3/HxH1 – imino protons of guanine, thymine or hypoxanthine; CH42 – cytosine hydrogen-bonded amino-proton; CH41 – cytosine non-hydrogen-bonded amino-proton; CH5, CH6, TH6 – aromatic protons of cytosine or thymine; AH2/HxH2 – H2 proton of adenine or hypoxanthine; TM7 – methyl protons of thymine. Numbering of protons is displayed in Figure S1.

Supplementary Table S3. Chemical shift of protons in DNA duplex ϵ A/T in ppm.

Base pair	Imino	Amino		Non-exchangeable			
	GH1/TH3	CH41	CH42	CH5	CH6/TH6	AH2/ ϵ AH2	TM7
A1-T1	–	X	X	X	7.574	7.935	1.74
G2-C2	12.714	7.132	8.426	5.822	7.749	X	X
A3-T3	13.836	X	X	X	7.508	7.696	1.675
G4-C4	12.699	7.071	8.421	5.663	7.62	X	X
A5-T5	13.749	X	X	X	7.369	7.537	1.603
G6-C6	12.385	6.919	8.204	5.402	7.251	X	X
T7- ϵ A7	10.39*	X	X	X	7.255	8.837	1.354
G8-C8	12.229	6.334	7.446	5.302	7.434	X	X
G9-C9	12.723	6.754	8.159	5.451	7.499	X	X
A10-T10	14.036	X	X	X	7.446	7.556	1.585
A11-T11	13.952	X	X	X	7.453	7.376	1.673
G12-C12	12.836	7.04	8.543	5.777	7.603	X	X
G13-C13	–	7.196	8.148	5.848	7.685	X	X

*- Determined from 1D spectrum, chemical shift of two additional proton of 1-N6-ethenoadenine: H10 7.480 and H11 6.839 ppm.

X- non-existing proton; “–” – not determined; GH1/TH3 – imino protons of guanine or thymine; CH42 – cytosine hydrogen-bonded amino-proton; CH41 – cytosine non-hydrogen-bonded amino-proton; CH5, CH6, TH6 – aromatic protons of cytosine or thymine; AH2/ ϵ AH2 – H2 proton of adenine or 1-N6-ethenoadenine; TM7 – methyl protons of thymine. Numbering of protons is displayed in Figure S1.

Supplementary Table S4 Chemical shift of protons in DNA duplex F/T in ppm.

	Imino	Amino			Non-exchangeable		
Base pair	GH1/TH3	CH41	CH42	CH5	CH6/TH6	AH2	TM7
A1-T1	–	X	X	X	7.611	7.962	1.765
G2-C2	12.767	7.212	8.502	5.788	7.765	X	X
A3-T3	13.913	X	X	X	7.527	7.704	1.671
G4-C4	12.72	7.161	8.467	5.645	7.646	X	X
A5-T5	14.069	X	X	X	7.422	7.681	1.629
G6-C6	12.793	6.982	8.305	5.643	7.562	X	X
T7-F7	10.562	X	X	X	7.148	X	1.268
G8-C8	12.972	7.08	8.145	5.97	7.796	X	X
G9-C9	12.913	7.128	8.381	5.668	7.633	X	X
A10-T10	14.128	X	X	X	7.522	7.617	1.64
A11-T11	14.052	X	X	X	7.504	7.391	1.695
G12-C12	12.899	7.145	8.605	5.778	7.632	X	X
G13-C13	12.848	7.274	8.285	5.833	7.685	X	X

X- non-existing proton; “–” – not determined; GH1/TH3 – imino protons of guanine or thymine; CH42 – cytosine hydrogen-bonded amino-proton; CH41 – cytosine non-hydrogen-bonded amino-proton; CH5, CH6, TH6 – aromatic protons of cytosine or thymine; AH2 – H2 proton of adenine; TM7 – methyl protons of thymine. Numbering of protons is displayed in Figure S1.

Supplementary Table S5. Base opening temperature T_{bo} of individual base pairs along the DNA duplex and melting temperature T_m determined by NMR.

Duplex name	T _m for the whole duplex	Temperature, °C												
		T _{bo} , Number of base pair												
		1	2	3	4	5	6	7	8	9	10	11	12	13
A/T	55.5±0.3	ND	ND	44.6±1.0	ND	50.7±1.0	51.9±0.9	50.9±1.0	ND	51.9±0.8	49.4±1.0	43.7±1.0	37.3±1.1	ND
Hx/T	46.1±0.5	ND	30.8±2.3	40.1±2.2	45.9±1.9	44.4±2.3	44.2±2.0	43.3±2.2	49.4±2.7	ND	42.5±2.1	39.6±2.1	ND	ND
εA/T	41.6±0.3	ND	ND	36.1±1.5	ND	38.6±1.9	38.2±1.8	28.1±3.0	38.8±2.0	ND	37.3±1.7	35.5±1.5	34.3±1.2	ND
F/T	31.8±0.5	ND	20.8±1.3	30.3±1.4	34.6±2.0	ND	28.5±1.2	15.2±1.7	25.6±1.1	ND	27.2±1.1	ND	ND	18.7±5.5