

Supporting Information

Modulating Effects of Amino Acids on Low-Temperature Plasma-Induced DNA Damage

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1. Supporting Methods

Plasma irradiation

Irradiation was performed as described in the main text (10 kV, 1 kHz, 2 slm He, 40 μ L), on DNA solution without PB and on DI water.

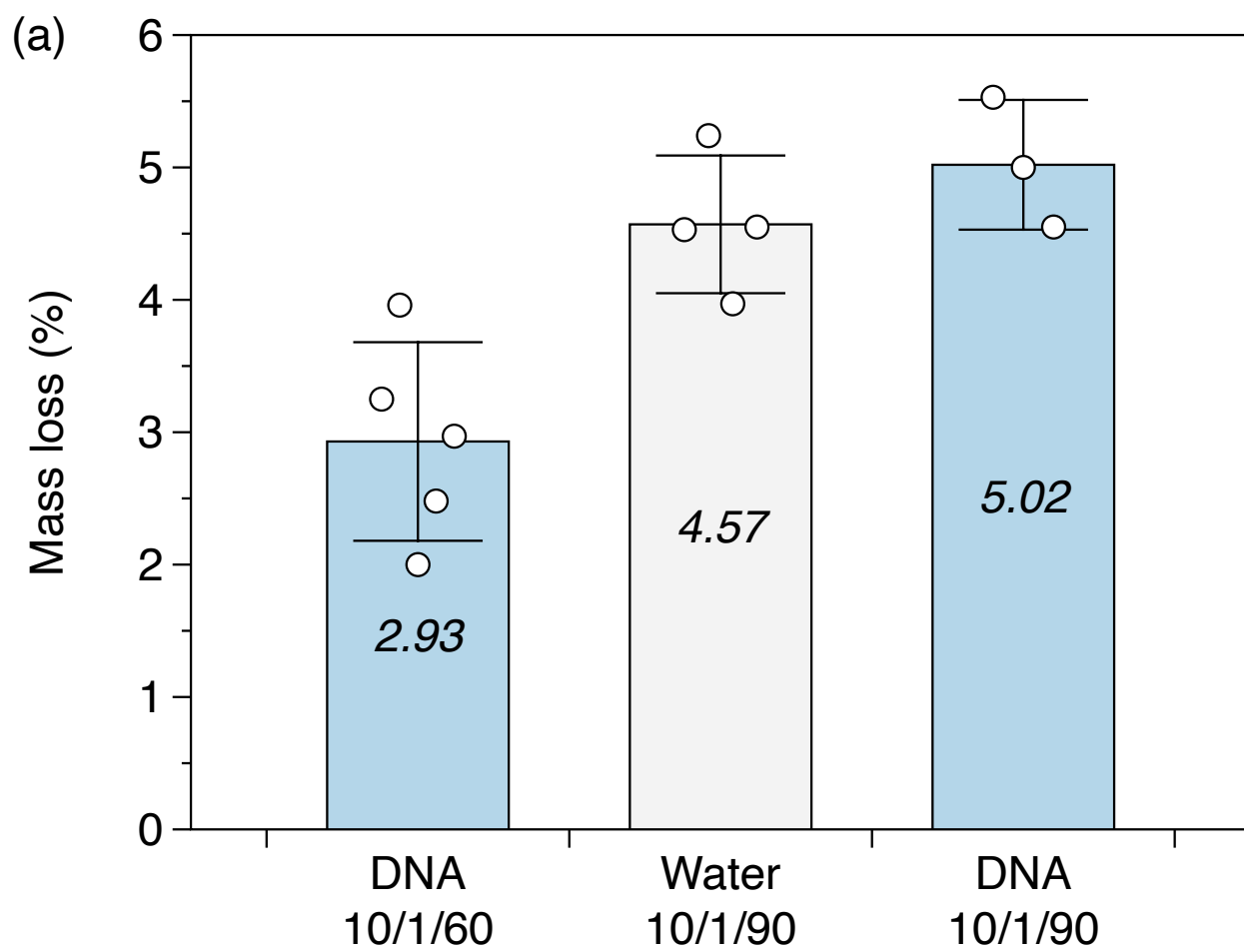
Evaporation and pH measurements

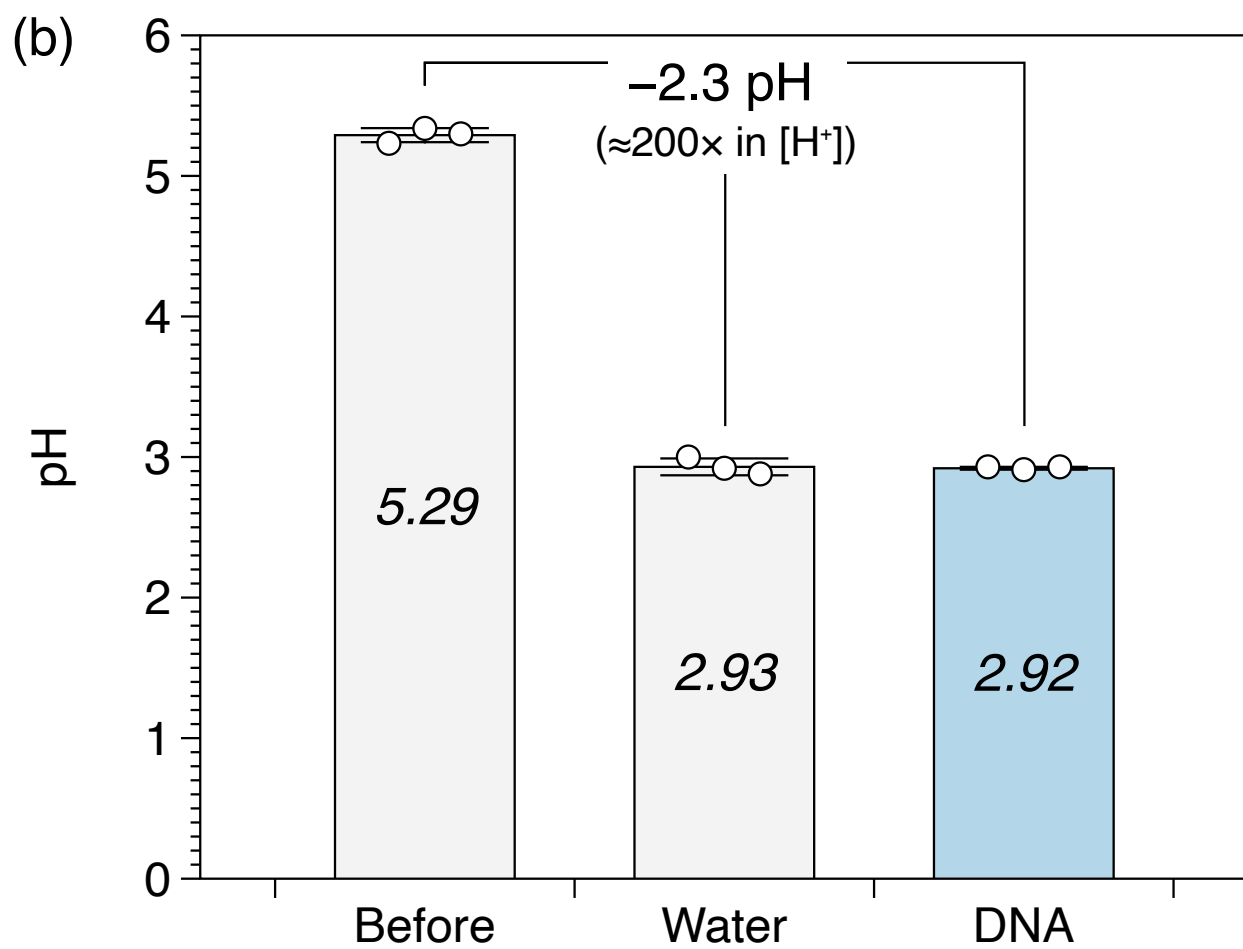
Droplets of 40 μ L DNA solution (0.05 μ g/ μ L, no PB) or DI water were weighed before and after irradiation on a 0.1 mg balance (MS104S/03, Mettler Toledo). The fractional mass loss was calculated as $(m_0 - m_1)/m_0$, where m_0 and m_1 are the sample masses with the tray subtracted before and after irradiation.

The pH of each irradiated droplet was measured directly at 40 μ L in a 384-well plate (781801, Greiner Bio-One) with a micro combination electrode (Orion 8220BNWP, Thermo Scientific) calibrated at pH 7.00 and 4.01. The pre-irradiation reference was measured on 50 mL of the same DI water, since unirradiated pure water cannot be read reliably at 40 μ L. For unbuffered irradiated samples, each value is the reading 30 min after electrode entry. The descent is shown in Figure S1(c).

Each irradiated condition was measured on at least three independently irradiated droplets. For the pre-irradiation reference, three readings of the 50 mL DI water were used. Figures show the mean with SD as error bars.

2. Supporting Figures and Tables





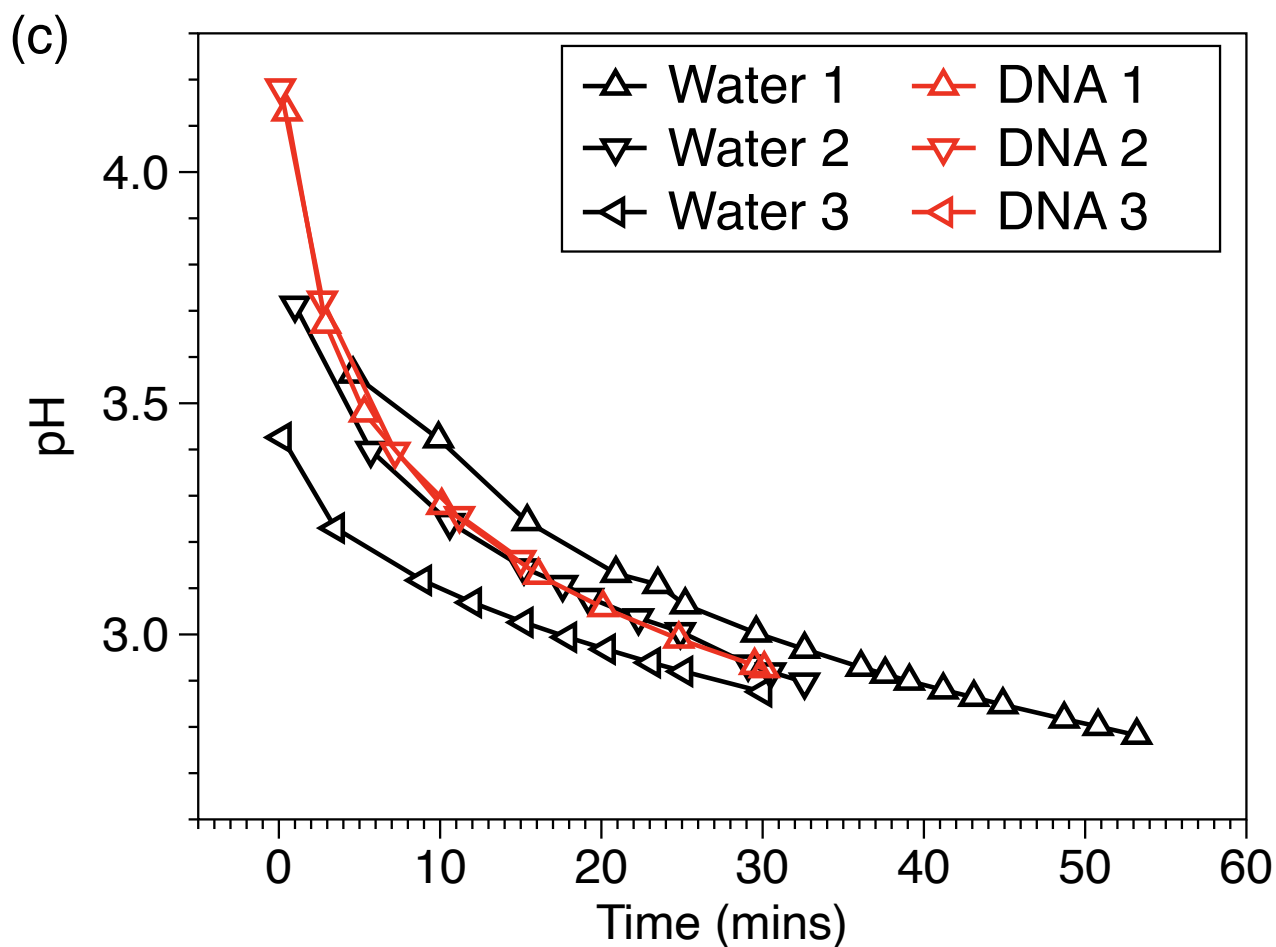


Figure S1. (a) Mass loss and (b) pH of 40 μ L droplets after plasma irradiation at 10 kV and 1 kHz without PB. Black is DNA solution and grey is DI water. In (a) bar labels give kV / kHz / s. Symbols are individual droplets and bars are mean $\pm$ SD except the pre-irradiation bar in (b) which shows three readings of 50 mL DI water. (c) pH descent after electrode entry for each irradiated droplet with the dashed line marking the 30 min read.

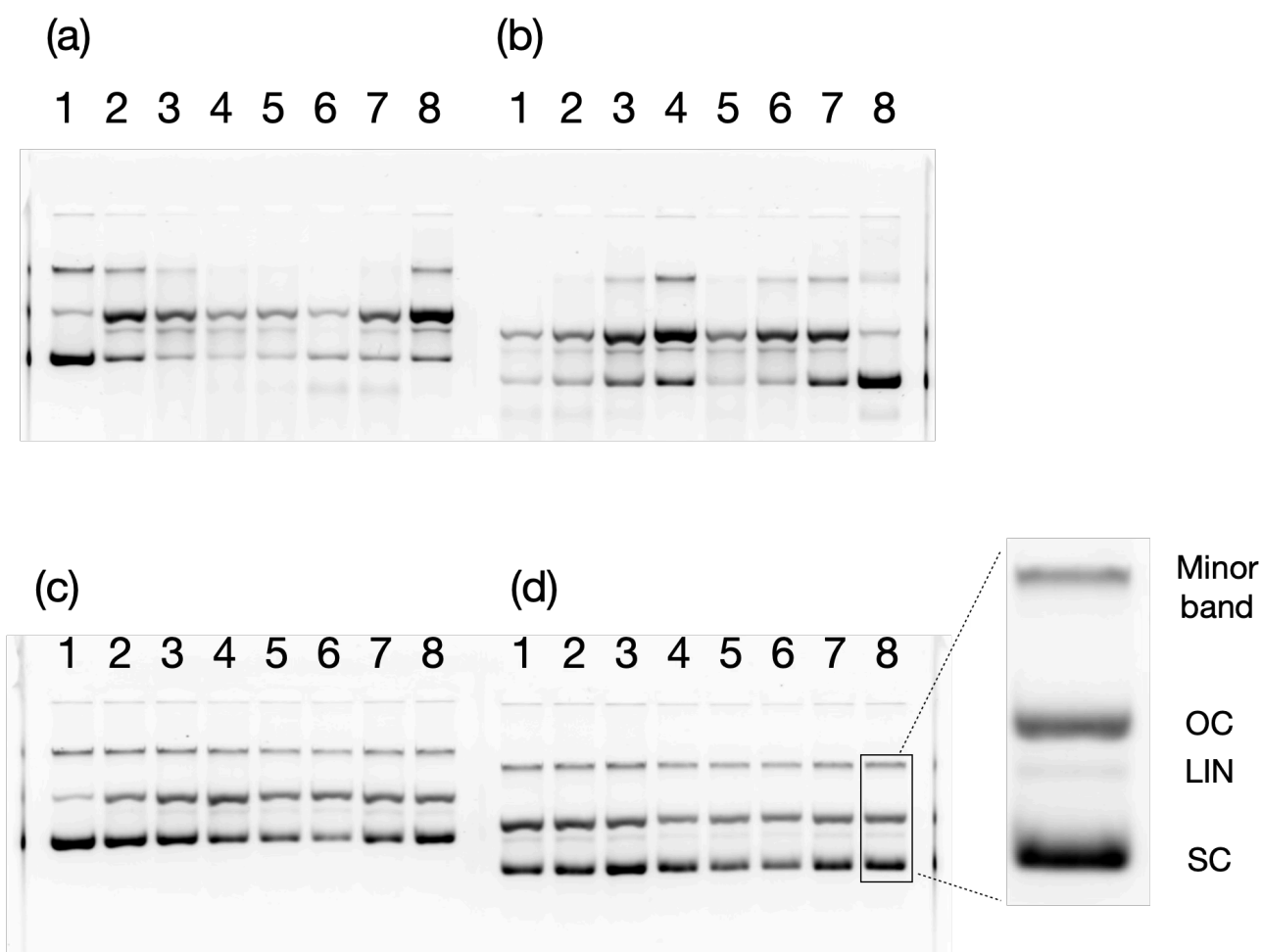


Figure S2. Representative gel images of plasmid DNA after plasma irradiation at 10 kV and 1 kHz, (a, b) without and (c, d) with 10 mM PB. Lanes 1–4 in (a) and (c) show 0, 30, 60, and 90 s of irradiation without amino acid. All other lanes show 90 s of irradiation with Trp (lanes 5–8 in a and c), Tyr (lanes 1–4 in b and d), or Met (lanes 5–8 in b and d), at 10, 30, 100, and 300 μ M from left to right. SC, LIN, and OC mark the supercoiled, linear, and open circular forms. The minor band is a form other than these three and was excluded from the quantification.

Table S1. Solution pH of tryptophan (Trp), tyrosine (Tyr), and methionine (Met) at concentrations of 10–300 μM , measured before plasma irradiation in the presence and absence of 10 mM phosphate buffer (PB).

Without PB	10 μM	30 μM	100 μM	300 μM
Trp	5.610	5.591	5.430	5.439
Tyr	5.502	5.483	5.491	5.519
Met	5.574	5.578	5.562	5.617
With PB	10 μM	30 μM	100 μM	300 μM
Trp	7.189	7.103	7.232	7.199
Tyr	7.204	7.225	7.080	7.135
Met	7.201	7.235	7.240	7.202

Table S2. Statistical tests on the supercoiled (SC) fraction after 90 s irradiation at 10 kV and 1 kHz (n = 3 per condition). (a) Spearman rank correlation between the SC fraction and amino acid concentration (0–300 μ M, 15 data points per series). (b) One-way ANOVA comparing Trp, Tyr, and Met at each concentration. (c) Two-sample t-test comparing the SC fraction without and with 10 mM PB in the absence of amino acids.

(a) Spearman rank correlation

Amino acid	Without PB ρ	Without PB p	With PB ρ	With PB p
Trp	0.73	0.0020	0.73	0.0020
Tyr	0.74	0.0015	0.79	0.0005
Met	0.85	<0.0001	0.67	0.0068

(b) One-way ANOVA among Trp, Tyr, and Met, p values

Condition	10 μ M	30 μ M	100 μ M	300 μ M
Without PB	0.69	0.68	0.66	0.25
With PB	0.74	0.59	0.11	0.53

(c) Two-sample t-test, without vs with PB at 0 μ M

SC fraction without PB (%)	SC fraction with PB (%)	t	DF	p
4.7 ± 1.7	40.6 ± 4.4	−13.2	4	2×10^{-4}