Supporting information for

**Absence of 2'-Deoxyoxanosine and Presence of Abasic Sites in DNA Exposed to Nitric Oxide at Controlled Physiological Concentrations**

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**Reaction scheme and detailed derivation of the kinetic model**

_Reactions_.  The principal nitrosating agent in oxygenated $NO^{\bullet}$ solutions at physiological pH is $N_2O_3$ *(47)*, which is formed *via* reactions 1 and 2:

$$2NO + O_2 \quad \overset{k_1}{\phantom{=}} \quad 2NO_2 \tag{1}$$

$$NO + NO_2 \quad \overset{k_2, k_{-2}}{\phantom{=}} \quad N_2O_3 \tag{2}$$

Most of the $N_2O_3$ is hydrolyzed to nitrite, which can occur either directly or with the participation of various anions, including phosphate salts *(47)*.  The two hydrolysis pathways under our experimental conditions were:

$$N_2O_3 + H_2O \quad \overset{k_3}{\phantom{=}} \quad 2NO_2^- + 2H^+ \tag{3}$$

$$N_2O_3 + P_i + H_2O \quad \overset{k_4}{\phantom{=}} \quad P_i + 2NO_2^- + 2H^+ \tag{4}$$

Under our conditions (50 mM potassium phosphate buffer, pH 7.4), reaction 4 is 20 times as fast as reaction 3.  The additional reactions depend on which organic substrates are present.  In morpholine solutions (no DNA present), $N_2O_3$ could react with unprotonated morpholine ($Mor^{\circ}$) to form NMor:

$$N_2O_3 + Mor^{o} \quad \overset{k_5}{\phantom{=}} \quad NMor + NO_2^- + H^+ \tag{5}$$

In DNA solutions (no morpholine present), the three deamination reactions identified in our experiments were:

$$N_2O_3 + dG \quad \overset{k_6}{\longrightarrow} \quad dX + NO_2^- + H^+ \tag{6}$$

$$N_2O_3 + dA \quad \overset{k_7}{\longrightarrow} \quad dI + NO_2^- + H^+ \tag{7}$$

$$N_2O_3 + dC \quad \overset{k_8}{\longrightarrow} \quad dU + NO_2^- + H^+ \tag{8}$$

The rate constants for reactions 1-5, reported previously, are listed in Table 1. Those for dG, dA, and dC in plasmid DNA were calculated from the relative rates of nitrosation and deamination, as described below.

Table 1: Published reaction rate constants

| Rate constant | Value | Units | Reference |
| --- | --- | --- | --- |
| $k_1$ | $2.1 \times 10^6$ | $M^{-2}s^{-1}$ | (48) |
| $k_2$ | $1.1 \times 10^9$ | $M^{-1}s^{-1}$ | (49) |
| $k_{-2}$ | $8.4 \times 10^4$ | $s^{-1}$ | (49) |
| $k_3$ | $1.6 \times 10^3$ | $s^{-1}$ | (50) |
| $k_4$ | $6.4 \times 10^5$ | $M^{-1}s^{-1}$ | (47) |
| $k_5$ | $6.4 \times 10^7$ | $M^{-1}s^{-1}$ | (47) |

The kinetic analysis is complicated by the fact that the delivery system creates two liquid regions with very different concentrations of $N_2O_3$. In addition to a well-stirred bulk liquid with a low concentration of $N_2O_3$, there is a very thin (~1 μm) boundary layer next to the $NO^\bullet$ delivery tubing where the $N_2O_3$ concentration is much higher. It will be shown that, despite its small volume, the contribution of the boundary layer to the overall rates of nitrosation and deamination is comparable to that of the bulk liquid. High concentrations of $NO_2^\bullet$ (and therefore $N_2O_3$) in the

boundary layer appear to arise from reaction 1 occurring not just in the liquid, but within the wall of the Silastic tubing. A reaction-diffusion model that adds membrane oxidation of $NO^{\bullet}$ to the known liquid-phase chemistry provides a quantitative explanation of the measured behavior of the delivery system, including the unexpectedly high rates of nitrite formation *(39)*. In the analysis that follows, the concentrations in the two regions are discussed first, and then the overall kinetics are considered.

*Bulk concentrations*. In the bulk liquid, the quasi-steady-state approximation in kinetics is applicable to both $NO_2^{\bullet}$ and $N_2O_3$. This is true for experiments with either organic substrate, so that:

$$2k_1[NO]^2[O_2] - k_2[NO][NO_2] + k_{-2}[N_2O_3]_M = 0 \tag{9}$$

$$2k_1[NO]^2[O_2] - k_2[NO][NO_2] + k_{-2}[N_2O_3]_D = 0 \tag{10}$$

$$k_2[NO][NO_2] - (k_{-2} + k_3 + k_4[P_i] + k_5[Mor^{\circ}])[N_2O_3]_M = 0 \tag{11}$$

$$k_2[NO][NO_2] - (k_{-2} + k_3 + k_4[P_i] + k_6[dG] + k_7[dA] + k_8[dC])[N_2O_3]_D = 0 \tag{12}$$

where $[N_2O_3]_M$ and $[N_2O_3]_D$ represent the bulk $N_2O_3$ concentrations in morpholine and DNA solutions, respectively. Solving equations 9-12 yields:

$$[N_2O_3]_M = \frac{2k_1[NO]^2[O_2]}{k_3 + k_4[P_i] + k_5[Mor^{\circ}]} \tag{13}$$

$$[N_2O_3]_D = \frac{2k_1[NO]^2[O_2]}{k_3 + k_4[P_i] + k_6[dG] + k_7[dA] + k_8[dC]} \tag{14}$$

As will be shown, the reactions of $N_2O_3$ with dG, dA, and dC are too slow (compared to hydrolysis) to influence the $N_2O_3$ concentration. Thus, equation 14 was simplified to:

$$[N_2O_3]_D = \frac{2k_1[NO]^2[O_2]}{k_3 + k_4[P_i]} \qquad (15)$$

Because the NO• and $O_2$ concentrations were fixed by the delivery conditions, which were the same in the morpholine and DNA experiments, the numerators of equations 13 and 15 are identical. Consequently, the ratio of those equations gives:

$$\frac{[N_2O_3]_M}{[N_2O_3]_D} = \frac{k_3 + k_4[P_i]}{k_3 + k_4[P_i] + k_5[Mor^o]} \qquad \alpha_1 \qquad (16)$$

This indicates that the bulk $N_2O_3$ concentration in the morpholine experiments was lower than in the DNA experiments, because of the competition of morpholine nitrosation with $N_2O_3$ hydrolysis. Although morpholine nitrosation affected the $N_2O_3$ level, the yield of NMor was low enough that [Mor°] was almost constant (independent of time). Therefore, the ratio of the $N_2O_3$ concentrations in the two types of experiments ($\alpha_1$) was a constant.

   *Boundary layer concentrations.*  Of the several boundary layers in the delivery system, the one that most impacts the present experiments is a region next to the NO• tubing in which there are large and spatially varying concentrations of $NO_2$• and $N_2O_3$. The variations in the $NO_2$• concentration in that region are described by:

$$[NO_2] = [NO_2]_0 e^{-x/\lambda} \qquad (17)$$

where $[NO_2]_0$ is the aqueous $NO_2$• concentration at the tubing surface, $x$ is distance from the surface, and $\lambda$ is the characteristic thickness of the $NO_2$•/$N_2O_3$ layer (the distance for a 1/e decay in either concentration). It was shown previously that $[NO_2]_0$ is proportional to $\lambda$ (equation A6 in ref. *39)*, which is given by:

$$\lambda = \left( \frac{D_{NO_2}}{b[NO]_0} \right)^{1/2} \tag{18}$$

where $D_{NO_2}$ is the diffusivity of $NO_2^\bullet$ and $[NO^\bullet]_0$ is the aqueous $NO^\bullet$ concentration at the tubing surface. The constant $b$ in equation 18 is determined from the rate constants for $N_2O_3$ formation and consumption, and its value differs in morpholine ($b_M$) and DNA ($b_D$) solutions:

$$b_M = \frac{k_2(k_3 + k_4[P_i] + k_5[Mor^o])}{k_{-2} + k_3 + k_4[P_i] + k_5[Mor^o]} \tag{19}$$

$$b_D = \frac{k_2(k_3 + k_4[P_i])}{k_{-2} + k_3 + k_4[P_i]}. \tag{20}$$

Although $[NO^\bullet]_0$ was the same in all experiments (see below), the differing values of $b$ created differences in both $\lambda$ and $[NO_2^\bullet]_0$ in the morpholine ($\lambda_M$ and $[NO_2]_{0M}$) and DNA solutions ($\lambda_D$ and $[NO_2]_{0D}$). From equations 18-20, one obtains:

$$\frac{[NO_2]_{0M}}{[NO_2]_{0D}} = \frac{\lambda_M}{\lambda_D} = \left( \frac{b_D}{b_M} \right)^{1/2} = \left( \frac{\alpha_1}{\alpha_2} \right)^{1/2} \tag{21}$$

with:

$$\alpha_2 \quad \frac{k_{-2} + k_3 + k_4[P_i]}{k_{-2} + k_3 + k_4[P_i] + k_5[Mor^o]} \tag{22}$$

The quasi-steady-state approximation is not valid for $NO_2^\bullet$ in this boundary layer, but it remains accurate for $N_2O_3$. The resulting expressions for the $N_2O_3$ concentrations are:

$$[N_2O_3]_{BM} = \frac{k_2}{k_{-2} + k_3 + k_4[P_i] + k_5[Mor^\circ]}[NO]_0[NO_2]_{0M}e^{-x/\lambda_M} \quad (23)$$

$$[N_2O_3]_{BD} = \frac{k_2}{k_{-2} + k_3 + k_4[P_i]}[NO]_0[NO_2]_{0D}e^{-x/\lambda_D} \quad (24)$$

where $[N_2O_3]_{BM}$ and $[N_2O_3]_{BD}$ are the boundary layer values in the morpholine and DNA experiments, respectively. As in equation 15, it is assumed in equation 24 that $k_6[dG] + k_7[dA] + k_8[dC] << k_{-2} + k_3 + k_4[P_i]$.

The concentration of $NO^\bullet$ next to the tubing differs from that in the bulk liquid (*i.e.*, $[NO^\bullet]_0 > [NO^\bullet]$), but the $NO^\bullet$ concentration is nearly constant within the $NO_2^\bullet/N_2O_3$ boundary layer. The length scale for variations in the $NO^\bullet$ concentration under our experimental conditions is 67 μm *(39)*, whereas $\lambda$ and $\lambda_D$ were calculated to be 0.55 and 0.60 μm, respectively. Accordingly, equations 23 and 24 assume that the $NO^\bullet$ concentration in the $NO_2^\bullet/N_2O_3$ layer is independent of *x*. It can be shown that reactions in the boundary layer have a negligible effect on the aqueous $NO^\bullet$ concentration, and that $[NO^\bullet]_0$ will be identical in the absence and presence of morpholine or DNA. It is found that $[NO^\bullet]_0 = 16.6$ μM for 10% $NO^\bullet$ with a tubing length of 7 cm *(39)*.

*Overall kinetics.* In the stirred batch reactor used in our experiments, the rate of accumulation of NMor in the bulk liquid equals its rate of formation per unit volume. Adding the contributions of the two regions, the overall rate is given by:

$$\frac{d[NMor]}{dt} = k_5[Mor^\circ]\left\{[N_2O_3]_M + (A/V)\int_0 [N_2O_3]_{BM}dx\right\} \quad (25)$$

where *A* is the surface area of the $NO^\bullet$ tubing and *V* is the total liquid volume (indistinguishable from the bulk volume). Using equation 23 in equation 25 and integrating, we obtain:

$$\frac{d[\text{NMor}]}{dt} = k_5[\text{Mor}^\circ]\left\{[\text{N}_2\text{O}_3]_\text{M} + [\overline{\text{N}_2\text{O}_3}]_\text{M}\right\} \tag{26}$$

$$[\overline{\text{N}_2\text{O}_3}]_\text{M} = \frac{k_2}{k_{-2} + k_3 + k_4[\text{P}_\text{i}] + k_5[\text{Mor}^\circ]}[\text{NO}]_0[\text{NO}_2]_\text{0M}\frac{A\lambda_\text{M}}{V} \tag{27}$$

In equation 26, the contribution of the boundary layer to NMor formation is expressed as an apparent increment in the bulk $\text{N}_2\text{O}_3$ concentration; that increment, $[\overline{\text{N}_2\text{O}_3}]_\text{M}$, was evaluated using equation 27.

Taking dX formation from dG as an example, there are two contributions also to each rate of deamination:

$$\frac{d[\text{dX}]}{dt} = k_6[\text{dG}]\left\{[\text{N}_2\text{O}_3]_\text{D} + (A/V)\int_0 f(x)[\text{N}_2\text{O}_3]_\text{BD}\,dx\right\} \tag{28}$$

where $f(x)$ is the ratio of the plasmid concentration in the boundary layer to that in the bulk solution. The reason for the $f(x)$ term is that the finite size of the plasmid will cause some steric exclusion of it from the boundary layer. If the plasmid is approximated as a rod of length $\ell$, then $f(x)$ will increase linearly from 0 to 1 over the interval $0 \le x \le \ell/2$, and be unity thereafter *(51)*. Incorporating that function into equation 28 and integrating, we obtain

$$\frac{d[\text{dX}]}{dt} = k_6[\text{dG}]\left\{[\text{N}_2\text{O}_3]_\text{D} + \beta[\overline{\text{N}_2\text{O}_3}]_\text{D}\right\} \tag{29}$$

where $[\overline{\text{N}_2\text{O}_3}]_\text{D}$ is the apparent increment in the bulk $\text{N}_2\text{O}_3$ concentration for a point-size molecule and $\beta$ represents the steric effect. With $\ell \approx 0.5$ μm (estimated from electron microscopy; *e.g.*, ref. *52)* and $\lambda_\text{D} = 0.60$ μm, those terms are evaluated as

$$\beta = \frac{2\lambda_D}{l}(1 - e^{-\frac{l}{2\lambda_D}}) \quad 0.82 \tag{30}$$

$$[\overline{N_2O_3}]_D = \frac{k_2}{k_{-2} + k_3 + k_4[P_i]}[NO]_0[NO_2]_{0D}\frac{A\lambda_D}{V}. \tag{31}$$

The expressions for the rates of dI and dU formation are analogous to equation 29.

From equations 21, 27 and 31, one finds that $[\overline{N_2O_3}]_M/[\overline{N_2O_3}]_D = \,_1$, a result analogous to equation 16. Further combining equations 16, 26, and 29, and solving for $k_6$, we obtain

$$k_6 = \frac{k_5[Mor^\circ]}{[dG]}\frac{d[dX]/dt}{d[NMor]/dt}\alpha_1\frac{1 + \dfrac{[\overline{N_2O_3}]_M}{[N_2O_3]_M}}{1 + \beta\dfrac{[N_2O_3]_M}{[N_2O_3]_M}} \tag{32}$$

As will be shown, [dG] can be approximated as constant in our experiments. Therefore, $k_6$ can be determined from the slopes of the [dX] and [NMor] data plotted *versus* time. The rate constants for dI and dU formation are related to $k_6$ by:

$$k_7 = k_6\frac{[dG]\,d[dI]/dt}{[dA]\,d[dX]/dt} \tag{33}$$

$$k_8 = k_6\frac{[dG]\,d[dU]/dt}{[dC]\,d[dX]/dt} \tag{34}$$

where [dA] and [dC] are constant, like [dG]. As shown in the text, concerning an approximation in the kinetic analysis, from the experimental values of the rate constants, $k_6[dG]$, $k_7[dA]$, and $k_8[dC]$ are 1.4, 1.5, and 1.1 s$^{-1}$, respectively. Their sum is much smaller than $k_3$ ($1.6 \times 10^3$ s$^{-1}$), which in turn is 20 times smaller than $k_4[P_i]$. This validates the simplifications made in the denominators of equation 15 and 24.