Supporting Information to "Homolytic pathways drive peroxynitrite-dependent Trolox C oxidation" by Botti et al.

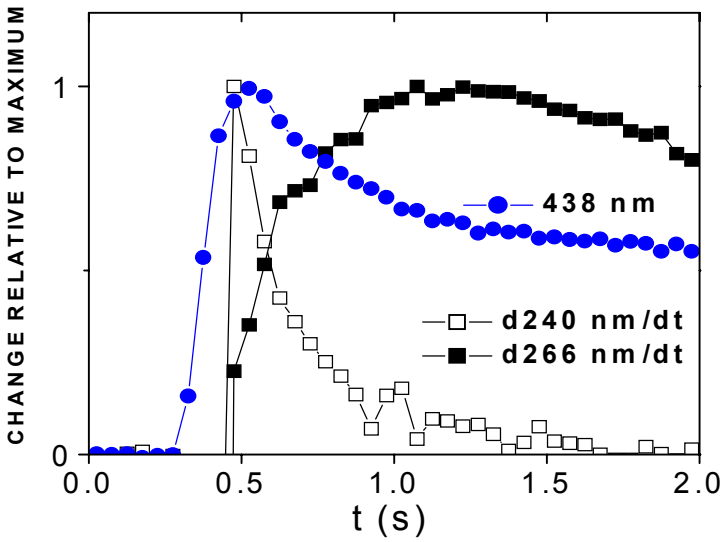
Supporting Information to Fig. 5A

Maximal rate of $TxO^{\cdot}$ decay as a function of peroxynitrite concentration[Δ]

$[ONOO^-]_0$ (μM)	$-d[TxO^{\cdot}]/dt$ ($\mu M \cdot s^{-1}$)[∞]
0	0
50	0.35
100	0.70
200	2.93
400	7.36
500	7.53
1000	14.6

[Δ] $[TxOH]_0$ = 300 μM
[∞] Average values are displayed.

Supporting Information to Fig. 5B.



	Reactants	Reaction type	Products	Constants (s^{-1} / $M^{-1}s^{-1}$)	Reference/Comment
1	$ONOO^- + H^+$	=	ONOOH	**f**:1.e+13; **r**: 1.59e+6	pK_a = 6.8 (*1*)
2	ONOOH	→	[ONO··OH]	4.5	
3	[ONO··OH]	→	$NO_2 + OH$	32e+2	$^{\cdot}OH$ and $^{\cdot}NO_2$ yield = 32 %, (*2*)
4	[ONO··OH]	→	NO_3^-	68.e+2	
5	$NO_2 + OH$	→	NO_3^-	1.e+10	(*3*)
6	$NO_2 + OH$	→	ONOOH	1.e+10	(*4*)
7	$OH + OH$	→	H_2O_2	1.e+10	
8	$NO_2 + NO_2$	=	N_2O_4	**f**: 4.5e+8; **r**: 6.429e+3	(*3*)
9	$N_2O_4 + H_2O$	→	$NO_3^- + NO_2^-$	1.e+3	(*3*)
10	$OH + ONOO^-$	→	$NO + O_2 + OH^-$	4.8e+9	(*5*)
11	$OH + NO_2^-$	→	$NO_2 + OH^-$	1.e+10	(*3*)
12	$ONOO^- + CO_2$	→	$ONOOCO_2^-$	5.8e+4	(*6*)
13	$ONOOCO_2^-$	→	$[ONO··OCO_2^-]$	7.e+5	(*7*)
14	$[ONO··OCO_2^-]$	→	$NO_2 + CO_3^-$	4.e+6	yield of homólisis = 40 %
15	$[ONO··OCO_2^-]$	→	$NO_3^- + CO_2$	6.e+6	
16	$NO_2 + CO_3^-$	→	$ONOOCO_2^-$	5.e+8	50 % of $^{\cdot}NO_2 + CO_3^{\cdot-}$ gives NO_3^-(*8*)
17	$NO_2 + CO_3^-$	→	$NO_3^- + CO_2$	5.e+8	
18	$CO_3^- + CO_3^-$	→	$CO_2 + CO_4^{2-}$	2.e+7	(*3*)
19	$CO_3^- + OH$	→	NRP1	3.e+9	(*3*); (NRP2 = non-reactive products 1)
20	$ONOO^- + CO_3^-$	→	$NO + O_2 + HCO_3^-$	7.7e+6	(*5*)
21	DMSO + OH	→	NRP2	7.e+9	(*3*); (NRP2 = non-reactive products 2)
22	TxOH + OH	→	$TxO + OH^-$	3.8e+9	(*9*)
23	$TxOH + NO_2$	→	$TxO + NO_2^-$	5.e+6	Estimated value (pH = 7.4, this work), at pH = 6.6 k < $1e^5$ $M^{-1}s^{-1}$ (*9*); other phenols 0.1-1.9.e+8 $M^{-1}s^{-1}$ (*10*).
24	$TxOH + CO_3^-$	→	$TxO + HCO_3^-$	2.e+9	(at alkaline pH (*3*), other phenols 1-3.e+8 $M^{-1}s^{-1}$ (*10*)
25	TxOH	=	$TxO^- + H^+$	**f**: 20; **r**: 1.e+13	pK_a = 11.7 (*9*)
26	$TxO^- + NO_2$	→	$TxO + NO_2^-$	2.e+9	(*9*)
27	TxO + H	=	Tx(O)H	**f**: 1.e+13; **r**: 5.e+10	(*11*)
28	Tx(O)H + Tx(O)H	→	TxOH + TxKD	3.e+8	
29	Tx(O)H + TxO	→	TxOH + TxKD	2.e+9	
30	TxO + TxO	→	TxOH + TxKD	3.e+5	
31	TxO + OH	→	$TxKD + OH^-$	5.e+9	(This work, only

32	TxO + CO_3^-	→	TxKD + HCO_3^-	2.5.e+9	approximate values to test the proposed kinetic mechanism)
33	TxO + NO_2	=	$TxONO_2$	**f**: 9.e+8; **r**: 50.	
34	TxO + NO_2	=	Tx(O)-ONO	**f**: 2.5e+8; **r**; 5.e-002	
35	$TxONO_2$	→	TxKD + NO_2^-	5	
36	Tx(O)-ONO	→	TxKD + NO_2^-	0.3	
37	NO + NO_2	=	N_2O_3	**f**: 1.1.e+9; **r**: 8.4.e+4	(*3*)
38	N_2O_3 + H_2O	→	$2NO_2^-$ + H^+	1.e+3	(*3*)
39	N_2O_3 + HPO_4^-	→	$2NO_2^-$ + H^+	1.e.+6	

→ : mass action irreversible

= : mass action reversible; **f** : forward; **r** : reverse

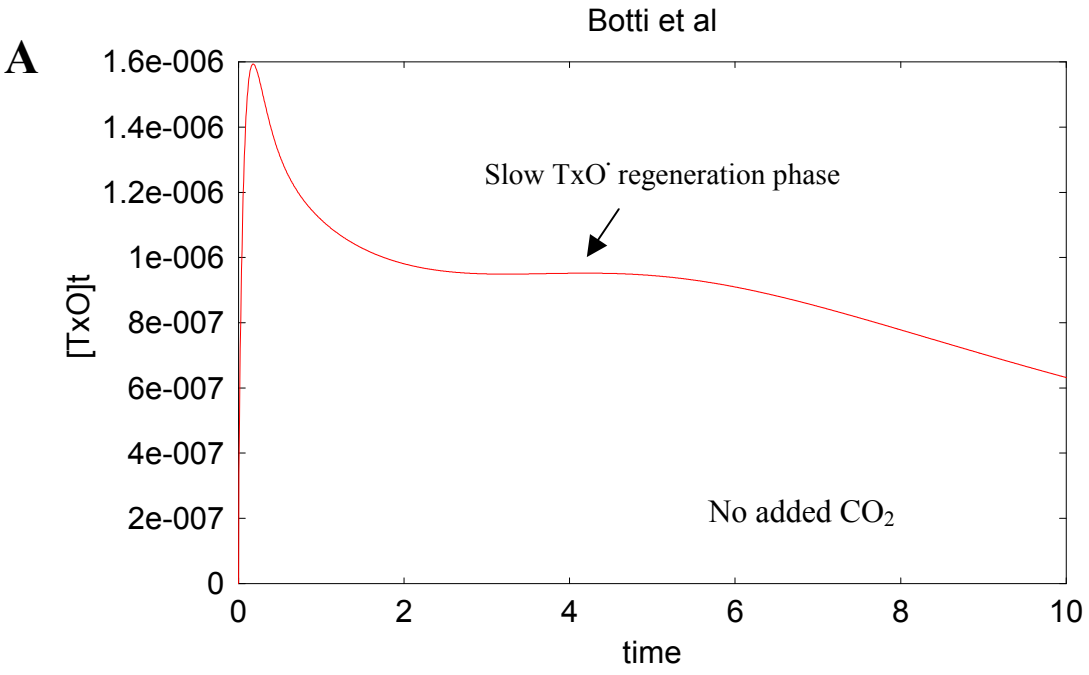
constant concentrations: [H^+] = 3.98e-008 M; [H_2O] = 55.5 M

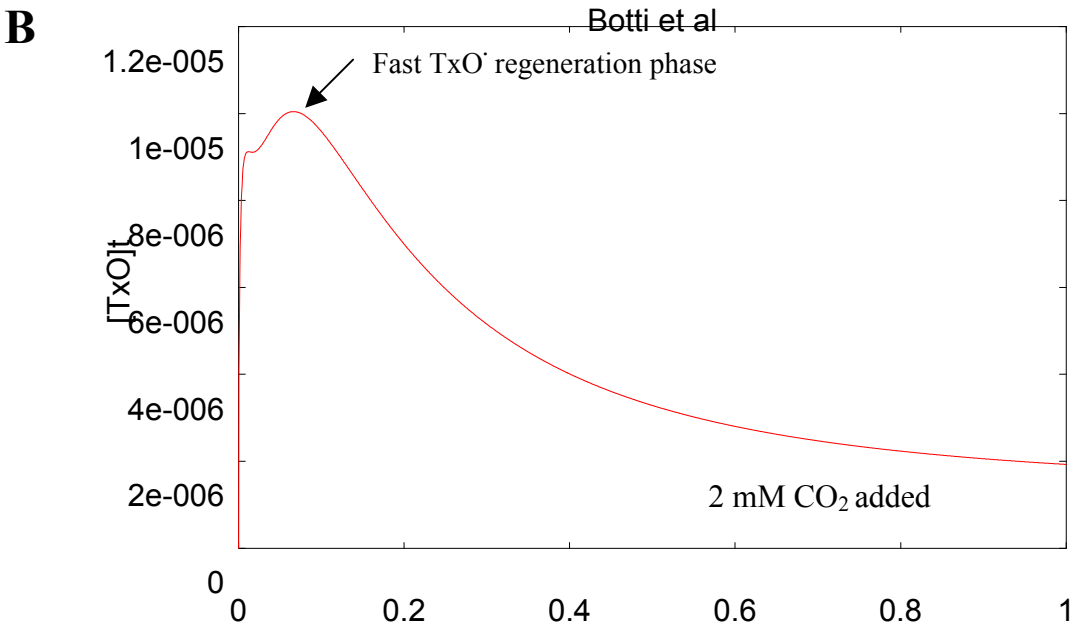
Supporting Information to mechanistic proposals involving two unstable intermediates capable of $TxO^{\cdot}$ regeneration (see Fig. 5). These intermediates are proposed on the basis of 2 kineticaly distinguishable regeneration phases, which can be evidenced in excess of peroxynitrite without CO_2 **(A)** and in the presence of high CO_2 concentrations **(B)**. In the simulations shown below, the reaction of $ONOO^-$ and $^{\cdot}OH$ was considered to yield non-reactive products. However, this may not be the case and a better fit can be obtained if it is assumed that the nitroso-dioxyl radical ($ONOO^{\cdot}$) is a good one-electron oxidant for TxOH:

$ONOO^{\cdot} + 1e^- = ONOO^-$ $\quad E^o \sim 0.9$ V (*5*)

$TxO^{\cdot} + 1e^- = TxO^-$ $\quad E^{o\prime} \sim 0.5$ V (*12*)

These simulation results were considered in qualitative agreement with experimental results. The rate constants should not be taken as determined chemical constants.





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Simulation report for A

In the presence of 0.4 mM peroxynitrite and 300µM TxOH.

Botti et al
KINETIC PARAMETERS
R1 (Mass action (reversible))
k1 = 1.0000e+013
k2 = 1.5900e+006
R2 (Mass action (irreversible))
k = 4.5000e+000
R3 (Mass action (irreversible))
k = 3.0000e+003
R4 (Mass action (irreversible))
k = 7.0000e+003
R5 (Mass action (irreversible))
k = 1.0000e+010
R6 (Mass action (irreversible))
k = 1.0000e+010
R7 (Mass action (irreversible))
k = 1.0000e+010
R8 (Mass action (reversible))
k1 = 4.5000e+008
k2 = 6.4290e+003
R9 (Mass action (irreversible))
k = 1.0000e+003
R10 (Mass action (irreversible))
k = 4.8000e+009
R11 (Mass action (irreversible))
k = 6.0000e+009
R12 (Mass action (irreversible))
k = 5.8000e+004
R13 (Mass action (irreversible))
k = 7.0000e+005
R14 (Mass action (irreversible))
k = 4.0000e+006
R15 (Mass action (irreversible))
k = 6.0000e+006
R16 (Mass action (irreversible))
k = 5.0000e+008
R17 (Mass action (irreversible))
k = 5.0000e+008
R18 (Mass action (irreversible))
k = 2.0000e+007
R19 (Mass action (irreversible))
k = 3.0000e+009
R20 (Mass action (irreversible))
k = 7.7000e+006
R21 (Mass action (irreversible))
k = 7.0000e+009
R22 (Mass action (irreversible))
k = 3.8000e+009
R23 (Mass action (irreversible))
k = 7.0000e+005
R24 (Mass action (irreversible))
k = 2.0000e+009
R25 (Mass action (reversible))
k1 = 2.0000e+001
k2 = 1.0000e+013
R26 (Mass action (irreversible))
k = 2.0000e+009
R27 (Mass action (reversible))
k1 = 1.0000e+013
k2 = 5.0000e+010
R28 (Mass action (irreversible))
k = 3.0000e+008
R29 (Mass action (irreversible))
k = 2.0000e+009
R30 (Mass action (irreversible))
k = 3.0000e+005
R31 (Mass action (irreversible))
k = 3.0000e+009
R32 (Mass action (irreversible))
k = 2.5000e+009
R33 (Mass action (reversible))
k1 = 9.0000e+008
k2 = 5.0000e+001
R34 (Mass action (reversible))
k1 = 2.5000e+008
k2 = 5.0000e-002
R35 (Mass action (irreversible))
k = 5.0000e+000
R36 (Mass action (irreversible))
k = 3.0000e-001
R38 (Mass action (reversible))
k1 = 1.1000e+009
k2 = 8.4000e+004
R39 (Mass action (irreversible))
k = 1.0000e+003
R40 (Mass action (irreversible))
k = 1.0000e+006

COMPARTMENTS
V(compartment) = 1.0000e+000

RESULTS OF INTEGRATION (after 1.00e+001 s)
[ONOO-] initial = 4.000000e-004 M, final = 3.296841e-008 M
[H] initial = 3.980000e-008 M, final = 3.980000e-008 M
[ONOOH] initial = 0.000000e+000 M, final = 8.252452e-009 M
[[ONO··OH]] initial = 0.000000e+000 M, final = 3.713938e-012 M
[NO2] initial = 0.000000e+000 M, final = 5.740293e-010 M
[OH] initial = 0.000000e+000 M, final = 4.001590e-015 M
[NO3-] initial = 0.000000e+000 M, final = 2.692769e-004 M
[H2O2] initial = 0.000000e+000 M, final = 4.942025e-012 M
[N2O4] initial = 0.000000e+000 M, final = 2.394006e-015 M
[H2O] initial = 5.550000e+001 M, final = 5.550000e+001 M
[NO2-] initial = 2.000000e-004 M, final = 3.267046e-004 M
[ONOO] initial = 0.000000e+000 M, final = 0.000000e+000 M
[OH-] initial = 0.000000e+000 M, final = 1.090888e-004 M
[CO2] initial = 5.000000e-006 M, final = 2.785762e-009 M
[ONOOCO2-] initial = 0.000000e+000 M, final = 7.609789e-018 M
[[ONO··OCO2-]] initial = 0.000000e+000 M, final = 5.326853e-019 M
[CO3-] initial = 0.000000e+000 M, final = 4.906670e-018 M
[CO4--] initial = 0.000000e+000 M, final = 4.863858e-015 M
[NRP1] initial = 0.000000e+000 M, final = 6.362371e-013 M
[HCO3-] initial = 0.000000e+000 M, final = 4.997214e-006 M
[DMSO] initial = 0.000000e+000 M, final = 0.000000e+000 M
[NRP2] initial = 0.000000e+000 M, final = 0.000000e+000 M
[TxOH] initial = 3.000000e-004 M, final = 2.163327e-004 M
[TxO] initial = 0.000000e+000 M, final = 6.317675e-007 M
[TxO-] initial = 0.000000e+000 M, final = 1.087096e-008 M
[Tx(O)H] initial = 0.000000e+000 M, final = 5.028869e-012 M
[TxCD] initial = 0.000000e+000 M, final = 7.904803e-005 M
[Tx(O)-ONO] initial = 0.000000e+000 M, final = 3.970687e-006 M
[TxONO2] initial = 0.000000e+000 M, final = 5.980837e-009 M
[NO] initial = 0.000000e+000 M, final = 6.170639e-011 M
[O2] initial = 2.400000e-004 M, final = 2.636705e-004 M
[N2O3] initial = 0.000000e+000 M, final = 1.816371e-016 M
[HPO4-] initial = 7.500000e-002 M, final = 7.500000e-002 M
J(R1) = 2.970015e-008 M/s
J(R2) = 3.713603e-008 M/s
J(R3) = 1.114181e-008 M/s
J(R4) = 2.599756e-008 M/s
J(R5) = 2.297030e-014 M/s
J(R6) = 2.297030e-014 M/s
J(R7) = 1.601272e-019 M/s
J(R8) = 1.328883e-010 M/s
J(R9) = 1.328673e-010 M/s
J(R10) = 6.332451e-013 M/s
J(R11) = 7.844027e-009 M/s
J(R12) = 5.326844e-012 M/s
J(R13) = 5.326852e-012 M/s
J(R14) = 2.130741e-012 M/s
J(R15) = 3.196112e-012 M/s
J(R16) = 1.408286e-018 M/s
J(R17) = 1.408286e-018 M/s
J(R18) = 4.815082e-028 M/s
J(R19) = 5.890344e-023 M/s
J(R20) = 1.245591e-018 M/s
J(R21) = 0.000000e+000 M/s
J(R22) = 3.289563e-009 M/s
J(R23) = 8.692690e-008 M/s
J(R24) = 2.122946e-012 M/s
J(R25) = 1.248168e-008 M/s
J(R26) = 1.248049e-008 M/s
J(R27) = 6.353620e-009 M/s
J(R28) = 7.586858e-015 M/s
J(R29) = 6.354153e-009 M/s
J(R30) = 1.197391e-007 M/s
J(R31) = 7.584224e-012 M/s
J(R32) = 7.749687e-015 M/s
J(R33) = 2.734589e-008 M/s
J(R34) = -1.078711e-007 M/s
J(R35) = 2.990419e-008 M/s
J(R36) = 1.191206e-006 M/s
J(R38) = 2.370588e-011 M/s
J(R39) = 1.008086e-011 M/s
J(R40) = 1.362278e-011 M/s

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Simulation report for B

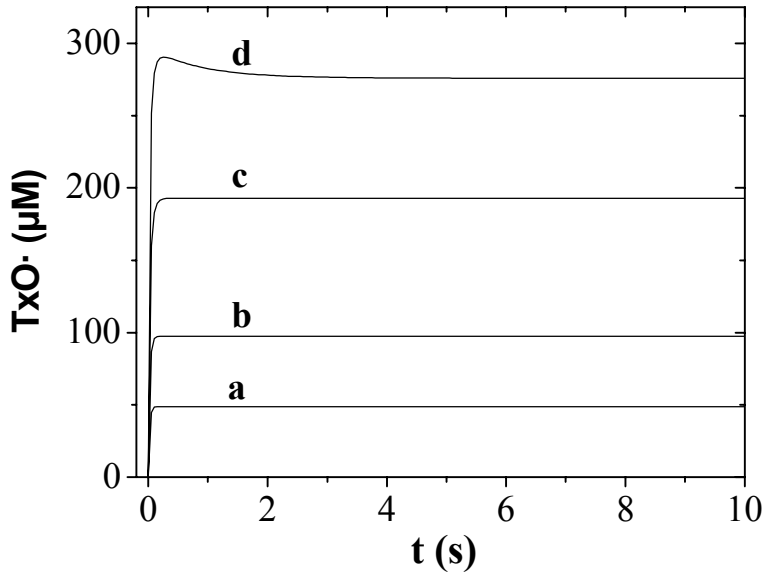
In the presence of 2 mM CO_2, 400 µM peroxynitrite and 300µM TxOH.

Botti et al
KINETIC PARAMETERS
R1 (Mass action (reversible))
k1 = 1.0000e+013
k2 = 1.5900e+006
R2 (Mass action (irreversible))
k = 4.5000e+000
R3 (Mass action (irreversible))
k = 3.0000e+003
R4 (Mass action (irreversible))
k = 7.0000e+003
R5 (Mass action (irreversible))
k = 1.0000e+010
R6 (Mass action (irreversible))
k = 1.0000e+010
R7 (Mass action (irreversible))
k = 1.0000e+010
R8 (Mass action (reversible))
k1 = 4.5000e+008
k2 = 6.4290e+003
R9 (Mass action (irreversible))
k = 1.0000e+003
R10 (Mass action (irreversible))
k = 4.8000e+009
R11 (Mass action (irreversible))
k = 6.0000e+009
R12 (Mass action (irreversible))
k = 5.8000e+004
R13 (Mass action (irreversible))
k = 7.0000e+005
R14 (Mass action (irreversible))
k = 4.0000e+006
R15 (Mass action (irreversible))
k = 6.0000e+006
R16 (Mass action (irreversible))
k = 5.0000e+008
R17 (Mass action (irreversible))
k = 5.0000e+008
R18 (Mass action (irreversible))
k = 2.0000e+007
R19 (Mass action (irreversible))
k = 3.0000e+009
R20 (Mass action (irreversible))
k = 7.7000e+006
R21 (Mass action (irreversible))
k = 7.0000e+009
R22 (Mass action (irreversible))
k = 3.8000e+009
R23 (Mass action (irreversible))
k = 7.0000e+005
R24 (Mass action (irreversible))
k = 2.0000e+009
R25 (Mass action (reversible))
k1 = 2.0000e+001
k2 = 1.0000e+013
R26 (Mass action (irreversible))
k = 2.0000e+009
R27 (Mass action (reversible))
k1 = 1.0000e+013
k2 = 5.0000e+010
R28 (Mass action (irreversible))
k = 3.0000e+008
R29 (Mass action (irreversible))
k = 2.0000e+009
R30 (Mass action (irreversible))
k = 3.0000e+005
R31 (Mass action (irreversible))
k = 3.0000e+009
R32 (Mass action (irreversible))
k = 2.5000e+009
R33 (Mass action (reversible))
k1 = 9.0000e+008
k2 = 5.0000e+001
R34 (Mass action (reversible))
k1 = 2.5000e+008
k2 = 5.0000e-002
R35 (Mass action (irreversible))
k = 5.0000e+000
R36 (Mass action (irreversible))
k = 3.0000e-001
R38 (Mass action (reversible))
k1 = 1.1000e+009
k2 = 8.4000e+004
R39 (Mass action (irreversible))
k = 1.0000e+003
R40 (Mass action (irreversible))
k = 1.0000e+006

COMPARTMENTS
V(compartment) = 1.0000e+000

RESULTS OF INTEGRATION (after 1.00e+000 s)
[ONOO-] initial = 4.000000e-004 M, final = 1.300157e-020 M
[H] initial = 3.980000e-008 M, final = 3.980000e-008 M
[ONOOH] initial = 0.000000e+000 M, final = 3.254506e-021 M
[[ONO··OH]] initial = 0.000000e+000 M, final = 2.006242e-024 M
[NO2] initial = 0.000000e+000 M, final = 4.903607e-009 M
[OH] initial = 0.000000e+000 M, final = 2.120754e-021 M
[NO3-] initial = 0.000000e+000 M, final = 2.503334e-004 M
[H2O2] initial = 0.000000e+000 M, final = 6.580450e-014 M
[N2O4] initial = 0.000000e+000 M, final = 1.747392e-013 M
[H2O] initial = 5.550000e+001 M, final = 5.550000e+001 M
[NO2-] initial = 2.000000e-004 M, final = 2.757535e-004 M
[ONOO] initial = 0.000000e+000 M, final = 0.000000e+000 M
[OH-] initial = 0.000000e+000 M, final = 1.192189e-006 M
[CO2] initial = 2.000000e-003 M, final = 1.842113e-003 M
[ONOOCO2-] initial = 0.000000e+000 M, final = -1.325068e-024 M BOGUS!
[[ONO··OCO2-]] initial = 0.000000e+000 M, final = -9.315791e-026 M BOGUS!
[CO3-] initial = 0.000000e+000 M, final = -2.695165e-020 M BOGUS!
[CO4--] initial = 0.000000e+000 M, final = 9.061367e-011 M
[NRP1] initial = 0.000000e+000 M, final = 1.631436e-011 M
[HCO3-] initial = 0.000000e+000 M, final = 1.578866e-004 M
[DMSO] initial = 0.000000e+000 M, final = 0.000000e+000 M
[NRP2] initial = 0.000000e+000 M, final = 0.000000e+000 M
[TxOH] initial = 3.000000e-004 M, final = 1.507046e-004 M
[TxO] initial = 0.000000e+000 M, final = 1.927499e-006 M
[TxO-] initial = 0.000000e+000 M, final = 7.572908e-009 M
[Tx(O)H] initial = 0.000000e+000 M, final = 1.534289e-011 M
[TxCD] initial = 0.000000e+000 M, final = 7.345209e-005 M
[Tx(O)-ONO] initial = 0.000000e+000 M, final = 7.375237e-005 M
[TxONO2] initial = 0.000000e+000 M, final = 1.558728e-007 M
[NO] initial = 0.000000e+000 M, final = 1.683468e-015 M
[O2] initial = 2.400000e-004 M, final = 2.406700e-004 M
[N2O3] initial = 0.000000e+000 M, final = 6.387027e-020 M
[HPO4-] initial = 7.500000e-002 M, final = 7.500000e-002 M
J(R1) = -3.833487e-020 M/s
J(R2) = 1.464528e-020 M/s
J(R3) = 6.018726e-021 M/s
J(R4) = 1.404369e-020 M/s
J(R5) = 1.039934e-019 M/s
J(R6) = 1.039934e-019 M/s
J(R7) = 4.497596e-032 M/s
J(R8) = 9.697017e-009 M/s
J(R9) = 9.698024e-009 M/s
J(R10) = 1.323511e-031 M/s
J(R11) = 3.508831e-015 M/s
J(R12) = 1.389122e-018 M/s
J(R13) = -9.275475e-019 M/s
J(R14) = -3.726316e-019 M/s
J(R15) = -5.589475e-019 M/s
J(R16) = -6.608015e-020 M/s
J(R17) = -6.608015e-020 M/s
J(R18) = 1.452783e-032 M/s
J(R19) = -1.714734e-031 M/s
J(R20) = -2.698187e-033 M/s
J(R21) = 0.000000e+000 M/s
J(R22) = 1.214508e-015 M/s
J(R23) = 5.172973e-007 M/s
J(R24) = -8.123474e-015 M/s
J(R25) = 7.429835e-008 M/s
J(R26) = 7.426913e-008 M/s
J(R27) = 5.913783e-008 M/s
J(R28) = 7.062127e-014 M/s
J(R29) = 5.914680e-008 M/s
J(R30) = 1.114575e-006 M/s
J(R31) = 1.226325e-017 M/s
J(R32) = -1.298732e-016 M/s
J(R33) = 7.128894e-007 M/s
J(R34) = -1.324694e-006 M/s
J(R35) = 7.793638e-007 M/s
J(R36) = 2.212571e-005 M/s
J(R38) = 3.715468e-015 M/s
J(R39) = 3.544800e-015 M/s
J(R40) = 4.790270e-015 M/s

Simulation of TxO$^{\cdot}$ time courses under the same conditions of Figure 5A using equations and rate constants proposed in (*13*)**.** The time course of TxO$^{\cdot}$ largely departs from the experimental results (this work) and further support the lack of a direct reaction between Trolox C and peroxynitrite.



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