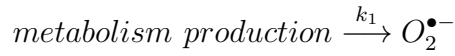


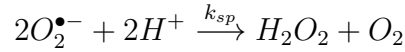
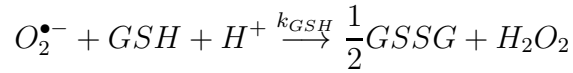
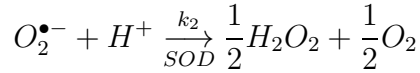
Supporting Information

Superoxide kinetics

Superoxide is involved in the following kinetically significant reactions:
Its production :



Its three ways of dismutation (by SOD, by GSH and spontaneously)



Those reactions lead to the following ordinary differential equation (ODE) coming from the balance between production and consumption:

$$\frac{d [O_2^{\bullet-}]}{dt} = k_1 - k_2 [SOD] [O_2^{\bullet-}] - k_{GSH} [GSH] [O_2^{\bullet-}] - 2k_{sp} [O_2^{\bullet-}]^2$$

The kinetic constants used in this work are gathered in the table 1 (concerning superoxide) according to Imlay and Fridovich (1991).

Constants	Value
k_1	$5.7 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$
$k_2[SOD]$	$2.8 \times 10^4 \text{ s}^{-1}$
k_2	$1.5 \times 10^9 \text{ mol}^{-1}\cdot\text{L}\cdot\text{s}^{-1}$
$k_{GSH}[GSH]$	$1.3 \times 10^1 \text{ s}^{-1}$
k_{GSH}	$2.6 \times 10^3 \text{ mol}^{-1}\cdot\text{L}\cdot\text{s}^{-1}$
k_{sp}	$5.0 \times 10^5 \text{ mol}^{-1}\cdot\text{L}\cdot\text{s}^{-1}$

Table 1: Kinetic constants used to describe superoxide evolution.

Superoxide evolution

Without exogenous stress

In the wild-type strain

In the wild-type strain, the resolution of $O_2^{\bullet-}$ concentration gives :

$$[O_2^{\bullet-}] \approx [O_2^{\bullet-}]_{\infty} (1 - e^{-k_2[SOD]t})$$

indeed GSH and spontaneous dismutation are negligible because $k_{sp} \ll k_{GSH} [GSH] \ll k_2 [SOD]$ *the*. $O_2^{\bullet-}$ concentration rapidly reached its steady-state value, in less than 1 ms, actually the characteristic time is given by $\frac{1}{k_2[SOD]}$. This time, which was dependent solely on SOD concentration and the SOD catalytic degradation rate k_2 , corresponds to the characteristic time require for the re-establishment of equilibrium.

If we assume that SOD concentration and $O_2^{\bullet-}$ production change over time, and that their equilibrium time values are probably significantly shorter than 1 ms, then the steady-state value of $O_2^{\bullet-}$ concentration is always reached but depends purely on $O_2^{\bullet-}$ production rate and SOD catalytic degradation rate (k_2), which are time-dependent.

For each time, we can write that the $O_2^{\bullet-}$ concentration is $[O_2^{\bullet-}](t) = \frac{k_1(t)}{k_2[SOD](t)}$. For example, with published values (Imlay and Fridovich, 1991), $O_2^{\bullet-}$ concentration is 2.1×10^{-10} M : this value fits the Imlay prediction well, and it corresponds to the equilibrium between $O_2^{\bullet-}$ production (parameter k_1) and the rate of scavenging of this radical by SOD .

We confirmed this approximation, by comparing the analytical solution (\blacktriangle) with the numerical solution (\blacksquare) in figure 1. Indeed this comparison help us to check whether the suggested approximations used to find an analytical expression are valid.

In a wild strain, the two major reaction involving superoxide are its production and its consumption by SOD .

In a $SOD(-)$ mutant

In a $SOD(-)$ mutant, changes in $O_2^{\bullet-}$ concentration were explained principally by the following differential equation :

$$\frac{d [O_2^{\bullet-}]}{dt} = k_1 - k_{GSH} [GSH] [O_2^{\bullet-}]$$

The resolution of $O_2^{\bullet-}$ concentration gave: $[O_2^{\bullet-}] \approx [O_2^{\bullet-}]_{\infty} (1 - e^{-k_{GSH}[GSH]t})$, which rapidly reached its steady-state value, in less than 0.5 s. Actually the characteristic time is $\frac{1}{k_{GSH}[GSH]}$. This time, which was dependent only on GSH concentration and the GSH catalytic degradation rate k_{GSH} , corresponds to the characteristic time required for the re-establishment of equilibrium.

If we assume that GSH concentration and $O_2^{\bullet-}$ production can change over time, and that their equilibrium time values are probably significantly shorter than 0.5 s, then the steady state value of $O_2^{\bullet-}$ concentration is always reached but is dependent purely on $O_2^{\bullet-}$ production rate and the GSH catalytic degradation rate k_{GSH} , which are time-dependent.

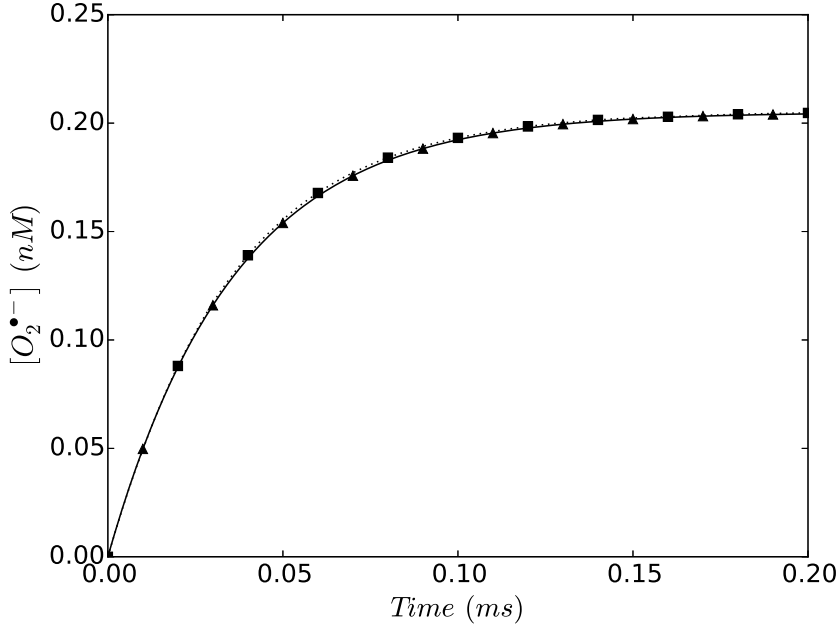


Figure 1: Change in $O_2^{\bullet-}$ concentration in the *E. coli* wild-type strain. (▲) corresponds to the analytical solution according to the simplified system and (■) corresponds to whole model solved with numerical methods. Cell density has no influence on the pattern of superoxide concentration evolution.

Fig1

For each time point, we can write that $O_2^{\bullet-}$ concentration is $[O_2^{\bullet-}](t) = \frac{k_1(t)}{k_{GSH}[GSH](t)}$. For example, with published values (Imlay and Fridovich, 1991), $O_2^{\bullet-}$ concentration is 4.4×10^{-7} M. This value is consistent with the predictions of Imlay and corresponds to the equilibrium between $O_2^{\bullet-}$ production (parameter k_1) and the rate of scavenging of this radical by *GSH*.

For confirmation of this approximation, we compared (figure 2) the analytical solution (▲) with the numerical one (■):

In a *SOD(-) GSH(-)* mutant

In a *SOD(-) GSH(-)* mutant, the change in $O_2^{\bullet-}$ concentration can be explained principally by the following differential equation according to the spontaneous dismutation:

$$\frac{d [O_2^{\bullet-}]}{dt} = k_1 - 2k_{sp} [O_2^{\bullet-}]^2$$

$O_2^{\bullet-}$ concentration obeys the function :

$$[O_2^{\bullet-}] = [O_2^{\bullet-}]_{\infty} \frac{1 - e^{-t/2\tau}}{1 + e^{-t/2\tau}}$$

where $\tau = \frac{1}{\sqrt{2k_1k_{sp}}}$ and $[O_2^{\bullet-}]_{\infty} = \sqrt{\frac{k_1}{2k_{sp}}}$

$O_2^{\bullet-}$ concentration rapidly reaches its steady-state $[O_2^{\bullet-}]_{\infty}$ value, in less than 1 s (τ is the characteristic time). This time, which is dependent solely on the dismutation rate k_{sp} , corresponds to the characteristic time required to re-establish equilibrium.

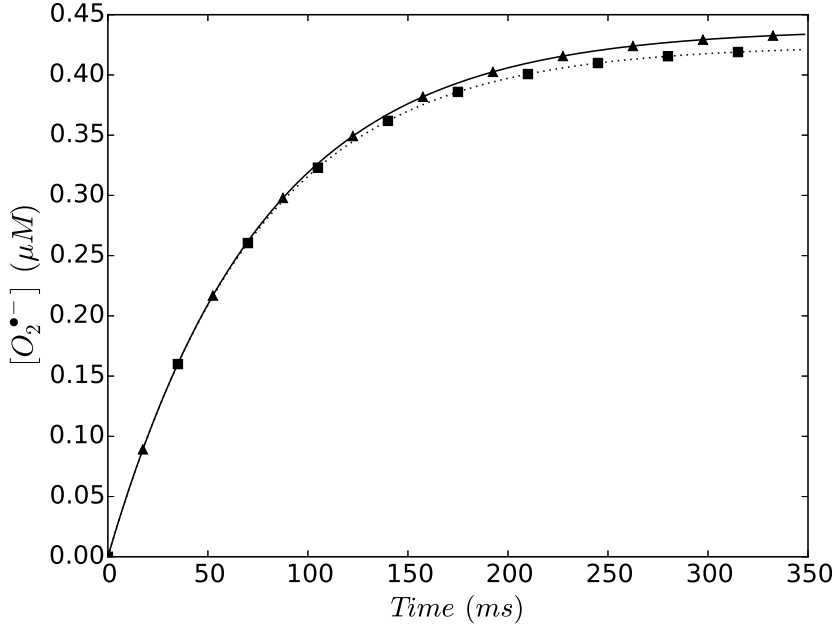


Figure 2: Changes in $O_2^{\bullet-}$ concentration in a $SOD(-)$ mutant. (▲) corresponds to the analytical solution according to the simplified system and (■) corresponds to the whole model solved with numerical methods. Cell concentration has no influence on this pattern of superoxide concentration evolution.

If we assume that $O_2^{\bullet-}$ production can change over time, and that the equilibrium time is probably significantly shorter than 1 s, then the steady-state value of $O_2^{\bullet-}$ concentration is always reached but is dependent purely on $O_2^{\bullet-}$ production rate, which is time-dependent.

For each time point, we can write that $O_2^{\bullet-}$ concentration is $[O_2^{\bullet-}](t) \approx \sqrt{\frac{k_1(t)}{2k_{sp}}}$. With published values, the $O_2^{\bullet-}$ concentration is $2.4 \times 10^{-6}M$, a value consistent with the predictions of Imlay (2001), and corresponding to the equilibrium between $O_2^{\bullet-}$ production (parameter k_1) and its spontaneous dismutation.

The analytical solution (▲) and the numerical solution (■) are the identical because they solved the same system (figure 3).

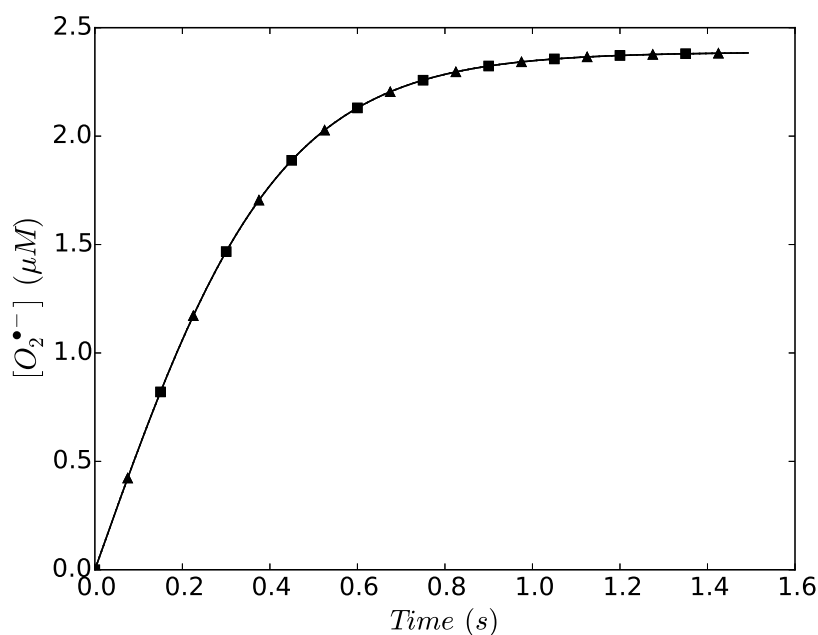


Figure 3: Change in $O_2^{\bullet-}$ concentration in $SOD(-) GSH(-)$ mutants. (▲) corresponds to the analytical solution according to the simplified system and (■) corresponds to the whole model with numerical methods. Cell concentration has no influence on this pattern of superoxide concentration evolution.

Superoxide summary

The table 2 summarizes the superoxide steady-state concentrations.

$[O_2^{\bullet-}]$ (mol L ⁻¹)	In this work	Imlay, Fridovich
Wild type	2.1×10^{-10}	2.0×10^{-10}
SOD(-) GSH(+)	4.2×10^{-7}	4.9×10^{-7}
SOD(-) GSH(-)	2.4×10^{-6}	6.7×10^{-6}

Table 2: Superoxide steady-state concentration. At steady state, the internal concentration is shown for cells in LB at 37 °C without exogenous hydrogen peroxide.

In a wild-type strain, Fe concentration is 10 μM , and, in a SOD^- strain, Fe concentration is 80 μM (Keyer and Imlay, 1996).

References

Imlay JA, Fridovich I, Assay of metabolic superoxide production in *Escherichia coli*. *J. Biol. Chem.* 1991;266.11: 69576965.

Keyer K, Imlay JA, Superoxide accelerates DNA damage by elevating free-iron levels. *Proc. Natl. Acad. Sci. U.S.A.* 1996;93.24: 13635 13640.