Supporting Information

This appendix gives a derivation of eq. 2, based on a model of inactivation as in the accompanying Figure, whereby species I is assumed inactive as a peroxidase. The reactions are considered at full equilibrium, *i.e.* a steady state is established rapidly and the rate of concentrations changes of the intermediates X, Y, Z, Z·gc and W is negligible (d[I]/dt = 0) Under these steady-state conditions, the total concentration of active protein, A_t, is the sum of the different species in the catalytic cycle (eq. S1). Species W is neglected, as it does not connect with any of the labile species. The steady-state concentration of each of the potentially labile species is the ratio of their production and their depletion (eq. S2-S4).

$$A_t = [X] + [Y] + [Z] + [Z \cdot gc] \tag{S1}$$

$$[Y] = \frac{k_1[H_2O_2][X] + k_{-2}[Z]}{k_{-1} + k_5 + k_2} \tag{S2}$$

$$[Z] = \frac{k_2[Y] + k_{-3}[Z \cdot gc]}{k_{-2} + k_3[gc] + k_6} \tag{S3}$$

$$[Z \cdot gc] = \frac{k_3[gc][Z]}{k_{-3} + k_4 + k_7} \tag{S4}$$

Combining eq. S1 with S2 yields [Y] as a function of A_t, and species Z and Z·gc (eq. S5):

$$[Y] = ([A_t] - [Z \cdot gc])\frac{k_1[H_2O_2]}{k_1[H_2O_2] + k_{-1} + k_5 + k_2} + [Z]\frac{k_{-2} - k_1[H_2O_2]}{k_1[H_2O_2] + k_{-1} + k_5 + k_2} \tag{S5}$$

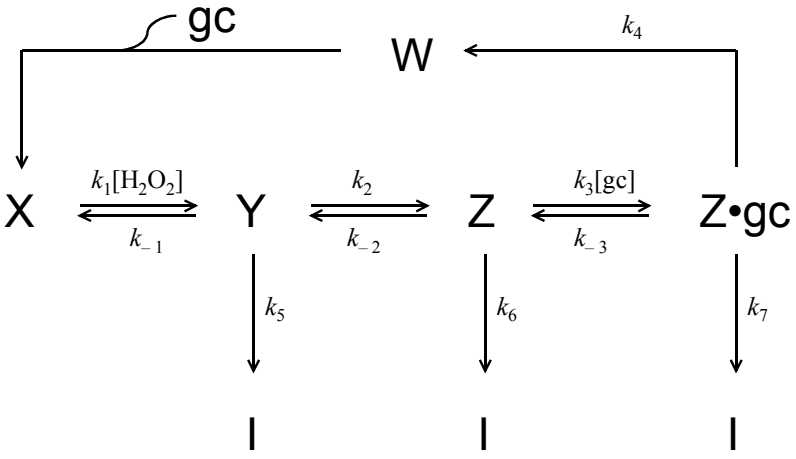

Figure. Model of the inactivation of u-cytc550 by H_2O_2. It is assumed that the inactivation step involves the conversion of peroxide-reacted intermediate, Y, Z or Z·gc. No assumption is made which of these intermediates is the labile species (see text). Species W is the one-electron reduced reaction product of species Z. It is assumed not to be converted into inactivated protein, I.

By combining eqs. S1, S3 and S5, [Z] can be expressed in terms of A_t and [Z·gc] (eq. S6):

$$[Z] = \frac{k_1 k_2 [H_2O_2][A_t] + \left(\left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)k_{-3} - k_1 k_2 [H_2O_2]\right)[Z \cdot gc]}{\left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)\left(k_3[gc] + k_{-2} + k_6\right) + k_2\left(k_1[H_2O_2] - k_{-2}\right)} \quad \text{(S6)}$$

This, together with eq. S4 gives [Z·gc] expressed as a function of A_t (S7):

$$[Z \cdot gc] = \frac{k_1 k_2 k_3 [H_2O_2][gc][A_t]}{\begin{aligned}&\left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)\left(k_3[gc] + k_{-2} + k_6\right)\left(k_{-3} + k_4 + k_7\right) \\ &+ k_2\left(k_{-3} + k_4 + k_7\right)\left(k_1[H_2O_2] - k_{-2}\right) \\ &+ k_3[gc]\left(k_1 k_2 [H_2O_2] - \left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)k_{-3}\right)\end{aligned}} \quad \text{(S7)}$$

By substitution of eq. S7 into eq. S6, [Z] as a function of A_t is given by eq. S8:

$$[Z] = \frac{k_1 k_2 [H_2O_2]\left(k_{-3} + k_4 + k_7\right)[A_t]}{\begin{aligned}&\left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)\left(k_3[gc] + k_{-2} + k_6\right)\left(k_{-3} + k_4 + k_7\right) \\ &+ k_2\left(k_{-3} + k_4 + k_7\right)\left(k_1[H_2O_2] - k_{-2}\right) + k_3[gc]\left(k_1 k_2 [H_2O_2] - \left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)k_{-3}\right)\end{aligned}} \quad \text{(S8)}$$

Subsequently, the expressions for [Z·gc] and [Z] (eqs. S7 and S8) can be substituted into eq. S5 to give an expression for the quasi steady-state concentration of species Y (eq. S9):

$$[Y] = \frac{k_1[H_2O_2]\left(\left(k_3[gc] + k_{-2} + k_6\right)\left(k_{-3} + k_4 + k_7\right) - k_3 k_{-3}[gc]\right)[A_t]}{\begin{aligned}&\left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)\left(k_3[gc] + k_{-2} + k_6\right)\left(k_{-3} + k_4 + k_7\right) \\ &+ k_2\left(k_{-3} + k_4 + k_7\right)\left(k_1[H_2O_2] - k_{-2}\right) + k_3[gc]\left(k_1 k_2 [H_2O_2] - \left(k_1[H_2O_2] + k_{-1} + k_5 + k_2\right)k_{-3}\right)\end{aligned}} \quad \text{(S9)}$$

From Figure S, it is apparent that there are three possible routes leading to inactivated protein, I. The rate of formation of inactivated protein can therefore be described by eq. S10:

$$\frac{d[I]}{dt} = k_5[Y] + k_6[Z] + k_7[Z \cdot gc] \quad \text{(S10)}$$

Substitution of eqs. S7-S9 into eq. S10 yields the full expression for the formation rate of inactivated protein (eq. S11), of which the inactivation constants are defined in eqs. S12-S16.

$$\frac{d[I]}{dt} = \frac{(k_A + k_B[gc])[H_2O_2][A_t]}{K_0 + K_{inact}^{gc}[H_2O_2] + K_{inact}^{H2O2}[gc] + [H_2O_2][gc]} \quad \text{(S11)}$$

$$k_A = \frac{\left(k_{-3} + k_4 + k_7\right)\left(k_5 k_{-2} + k_5 k_6 + k_2 k_6\right)}{k_3(k_2 + k_4 + k_7)} \quad \text{(S12)}$$

$$k_{\mathrm{B}} = \frac{k_4 k_5 + k_2 k_7 + k_5 k_7}{k_2 + k_4 + k_7} \tag{S13}$$

$$\mathrm{K}_0 = \frac{(k_{-3} + k_4 + k_7)(k_2 k_6 + k_{-1} k_{-2} + k_{-1} k_6 + k_{-2} k_5 + k_5 k_6)}{k_1 k_3 (k_2 + k_4 + k_7)} \tag{S14}$$

$$\mathrm{K}_{\mathrm{inact}}^{\mathrm{gc}} = \frac{(k_{-3} + k_4 + k_7)(k_{-2} + k_2 + k_6)}{k_3 (k_2 + k_4 + k_7)} \tag{S15}$$

$$\mathrm{K}_{\mathrm{inact}}^{\mathrm{H2O2}} = \frac{(k_4 + k_7)(k_{-1} + k_2 + k_5)}{k_1 (k_2 + k_4 + k_7)} \tag{S16}$$

It is not necessary to make assumptions on the nature of the labile species, as the form of eq. S11 will remain the same. If Y is the only labile species ($k_6 = k_7 = 0$), then the conversion from species Y to Z is reversible ($k_{-2} > 0$), otherwise k_A will become zero, causing a change in the form of eq. S11 rendering it inconsistent with the data. Thus, in this case formation of the oxidising species from the peroxo-iron species must be reversible. This is not so if Y is not the labile species. In that case, however, both Z and Z·gc are labile.

The statement that the catalytic efficiency (CE), *i.e.* the number of turnovers (or amount of gc oxidations) per u-cytc550 catalyst before full inactivation, equals the ratio between the rate constants of the formation of oxidised gc and the inactivation rate can be justified in the following way. Consider v_0 the product formation rate at the start of the reaction, and k_{inact} the first-order inactivation rate constant of the catalyst, then the actual product formation rate at v_t at time t after the start of the reaction is expressed by equation S17,

$$v_t = v_0 \, e^{-(k_{\mathrm{inact}} t)} \tag{S17}$$

in agreement with the observed exponential decrease in product formation rate (slope in phase III in Fig. 1). Integration of this equation yields the amount of product formed at time t:

$$\mathrm{gc}_t^{\mathrm{ox}} = \frac{v_0}{k_{\mathrm{inact}}} \left(1 - e^{-(k_{\mathrm{inact}} t)} \right) \tag{S18}$$

And thus at full inactivation, *i.e.* $t \to \infty$, the total amount of product formed equals v_0/k_{inact}. With v_0/[u-cytc550] equals the turnover rate constant, the turnover number per protein molecule (CE) is equal to the ratio of turnover and inactivation rate constants.