Supplementary Text S1

Building a computational model of IKK-β S-glutathionylation

A schematic representation of the proposed reactions involved in glutathione-dependent IKK-β S-glutathionylation is presented in Figure 1. The protein S-glutathionylation mechanisms considered in the model include protein S-glutathionylation via peroxide-induced IKK-β sulfenic acid formation and via radical-induced IKK-β thyl radical formation. The model was constructed after the systematic consideration of the literature-reported reactions that could potentially contribute to intracellular protein S-glutathionylation. This systematic consideration occurred in iterative steps. First, literature reported rates of reactions and necessary conditions for possible mechanisms of protein S-glutathionylation were utilized to rule out non-essential pathways of S-glutathionylation, pathways that would most likely not occur under the low level oxidative stress conditions induced by a 500 nM [Dox] treatment condition.

The results of this analysis immediately excluded thiol-disulfide exchange as a possible mechanism for IKK-β S-glutathionylation because the ratio of GSH/GSSG would have to decrease by over 100 fold (from 100:1 to 1:1) to drive 50% conversion of protein-SH to protein-SSG. For this reason, H2O2-induced GSSG formation was modeled as a GSH sink, rather than a source of IKK-β S-glutathionylation. When cysteine sulphydryls do undergo nitrosylation in vivo, because protein-SNO is more prevalent in extracellular spaces, protein-SNO is a relatively stable sulfhydryl derivative with half-lives on the order of hours, making this mechanism of protein S-glutathionylation unlikely to be involved in the timeframe of this study. However, it is entirely possible that for longer Dox treatment times, or under different Dox treatment conditions – i.e. an increase in [Dox] – the formation of protein-SSG via the pathway mediated by protein-SNO formation will be significant. A prior study has estimated the rate of formation of NO in B-CLL cells to be approximately 20 pmol/min/mg cell protein. Although this value is less than half the rate at which H2O2 is produced in an average cell, the rate of formation of protein-SNO has been shown to be significantly faster than the rate of protein-disulfide formation as a result of H2O2 oxidation. However, because the reaction of protein-SH with nitric oxide to generate protein-SNO is a highly selective process, relatively high concentrations of GSNO (concentrations that are on the order of intracellular [GSH] in EU1 cells) are needed to induce this reaction, concentrations that may or may not be readily generated under the treatment conditions being modeled.

Sulfenylamide-dependent (protein-SNO) protein S-glutathionylation was ignored in the model description because it has only been reported to occur for one protein, protein tyrosine phosphatase 1B, after treatment with H2O2, and is thought to develop from an initial protein sulfenic acid species (protein-SOH). Thiolsulfinate mechanisms of protein S-glutathionylation (protein-SOS-protein) were not directly ruled out because thiosulfinates are reported to be highly reactive with thiols and are very similar in characteristic to sulfenic acids. However, because it is difficult to distinguish between thiosulfinate-dependent and sulfenic acid-dependent mechanisms of protein-S-glutathionylation, one can consider the thiosulfinate-dependent mechanism of protein S-glutathionylation to be buried within the sulfenic acid-dependent mechanism of protein S-glutathionylation that is accounted for by the model.

Thyl radical-dependent IKK-β S-glutathionylation was considered in the model because production of thyl radicals has been reported under in vivo conditions of redox signaling. Although there are several mechanistic pathways for thyl radical-dependent protein S-glutathionylation, only one pathway was taken into account by the model, the reaction of the IKK-β thyl radical (IKK-β-S-) with reduced glutathione (GSH). The quenching of a glutathione thyl radical (GS‘) with IKK-β-S- was deemed too improbable given the fact that thyl radicals are the shortest-lived sulfhydryl derivatives and the concentrations of IKK-β, and subsequently IKK-β-S-, are not significantly high enough to compete with the other intracellular thiols or thyls that are capable of reacting with GS‘ or GSH, respectively. This was the same reasoning that was used to exclude the formation of S-glutathionylated IKK-β through the reaction of GS‘ with reduced IKK-β. For the aforementioned reasons, RS‘-induced GS‘ formation was modeled as a GSH sink, rather than a source of IKK-β S-glutathionylation (Fig. 1).
Supplementary Figures

**Supplemental Figure S1. Effect of NAC thiols on peroxide-dependent protein thyl (RS') radical formation.** The proposed extracellular [NAC]-dependent effects on protein thyl radical formation rate as a function of initial H$_2$O$_2$ concentration. The rates of formation of protein thyl radicals are not increased by NAC pretreatment until the concentration of NAC reaches a critical level. If [NAC] is at or above this threshold level, the rate of protein thyl radical formation is increased by a factor of 3. Additionally, the threshold level of NAC at which this 3-fold increase in RS' formation rate occurs is dependent on the initial concentration of H$_2$O$_2$ in the system. For lower levels of H$_2$O$_2$, the critical [NAC] is relatively high, whereas for higher levels of H$_2$O$_2$, the critical [NAC] is relatively low. Image adapted from the lipid peroxidation profiles described by 43.
Supplemental Figure S2. Schematic representation of the algorithm utilized to systematically predict the effect of NAC pretreatment on NAC-sensitive model parameters. Directional arrows represent the experimentally-measured effect of the NAC pretreatment, at various NAC concentrations, on IKK-SSG levels compared to control.
Supplemental Figure S3. The S-glutathionylation status of p65 subunit of NF-κB in EU1 cells does not change with Dox or NAC treatment. Representative immunoblot analysis, with accompanying densitometry quantification normalized to lane 1, of doxorubicin-induced p65 S-glutathionylation in EU1 cells, with and without NAC pretreatment. ([NAC] = 1 mM, 30 min pretreatment; [Dox] = 500 nM, 1 h treatment).

References