# Supporting information

# Cytochrome c auto-catalyzed carbonylation in the presence of hydrogen peroxide and cardiolipins.

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**Running title:** *Auto-catalyzed cytochrome c inactivation*

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**Table S1.** Composition and size of the large unilamellar vesicles (LUVs) used in the study.

| **Lipid composition (molar ratio)** | **Mode of size distribution, nm** | **Relative CL content, %** | **Experiments** |
|---|---|---|---|
| *DOPC/PLPC (2/3)* | *142.3 ± 7.7* | *0* | *Peroxidase activity and cyt c degradation* |
| DOPC/TOCL (1/1) | 164.2 ± 0.6 | 50 | Measurements with (G/GO) system |
| *DOPC/TOCL (7/3)* | *153.7 ± 2.9* | *30* | *Peroxidase activity and cyt c degradation* |
| DOPC/TLCL (7/3) | 135.6 ±8.0 | 30 | Measurements with $PPh_3$ (LOOH removal) |
| *DOPC/TLCL/POPE (5/3/2)* | *144.2 ±3.4* | *30* | *Peroxidase activity and cyt c degradation* |

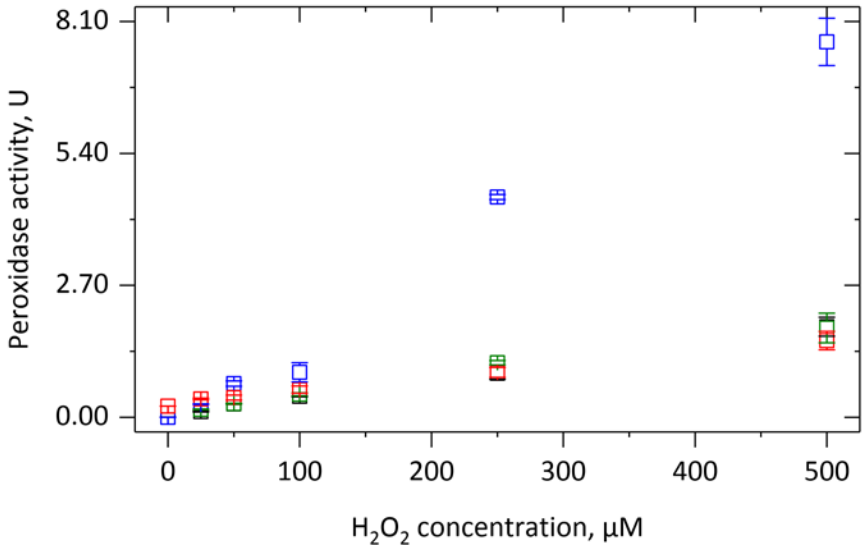


**Figure S1. Linear dependence of the $H_2O_2$-mediated cyt c steady-state peroxidase activity.** Cyt c was incubated alone (black) or in the presence of PLPC (green), TOCL (blue) and TLCL (red). Mean and SD of 3 independent experiments are given.

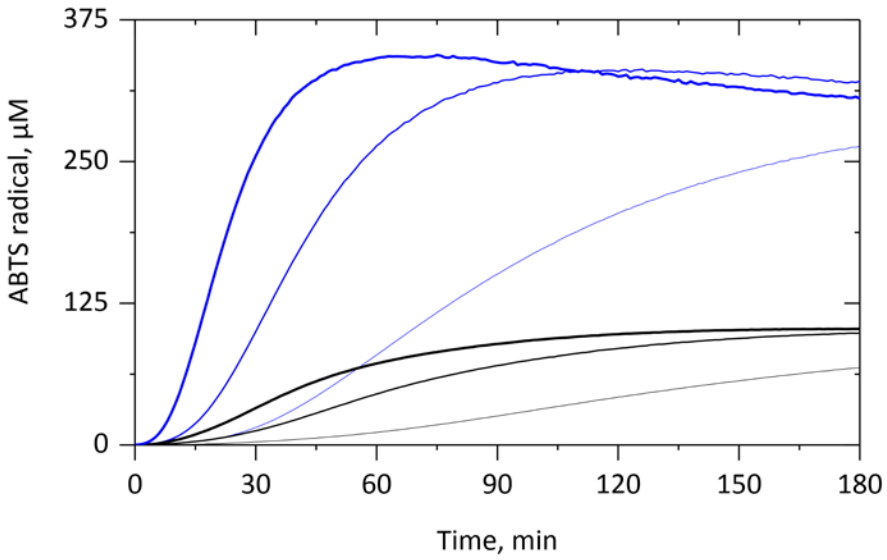


**Figure S2. Cyt c-based ABTS radical formation at continuous $H_2O_2$ production.** Cyt c was incubated in the absence (black) and presence (blue) of TOCL-containing liposomes at increasing $H_2O_2$ production rates (1.6, 7.8, and 31.3 µM $H_2O_2$ per min, depicted by increasing line thickness). Representative kinetic curves of 3 independent experiments are shown.

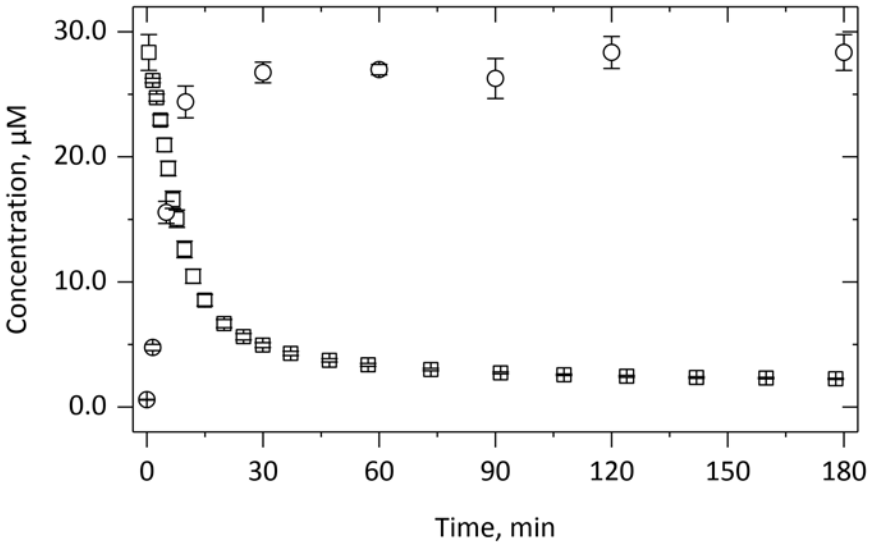


**Figure S3. Cyt c (30 mM) heme degradation expressed as a decrease in Soret band (squares) and increase of free iron concentration (circles) upon incubation with $H_2O2$ (3 mM).** Mean and SD of 3 independent experiments are shown.

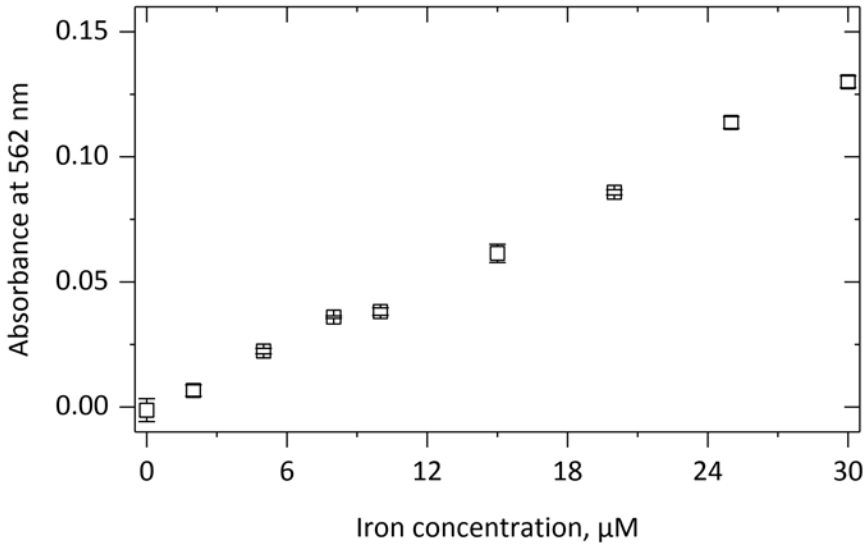


**Figure S4. Calibration curve used for determination of free iron determination obtained by ferrozine assay**. Mean and SD of 3 independent experiments are shown. Further details are given in the text.

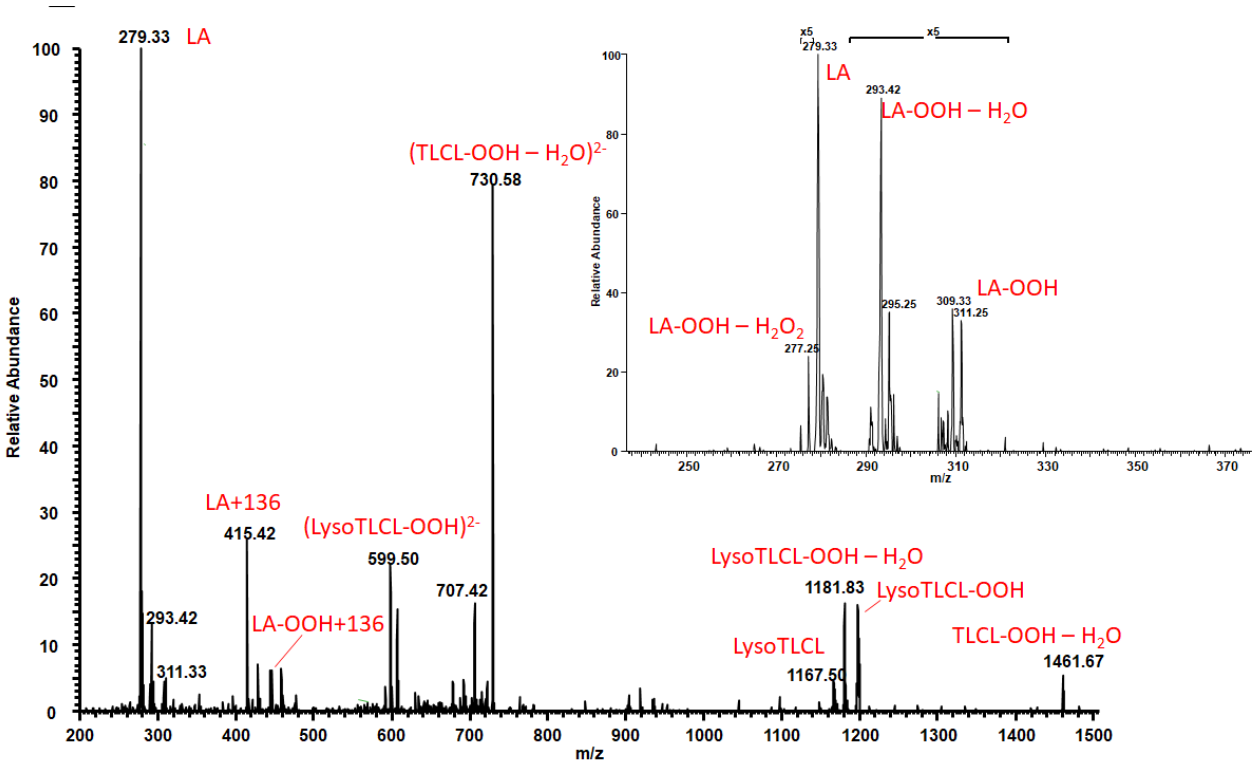


**Figure S5. Negative ion mode CID tandem mass spectrum of TLCL peroxide (*m/z* 1479.95$^{1+}$).** Insert shows low *m/z* signals corresponding to fatty acyl chain anions with peroxide characteristic neutral losses.

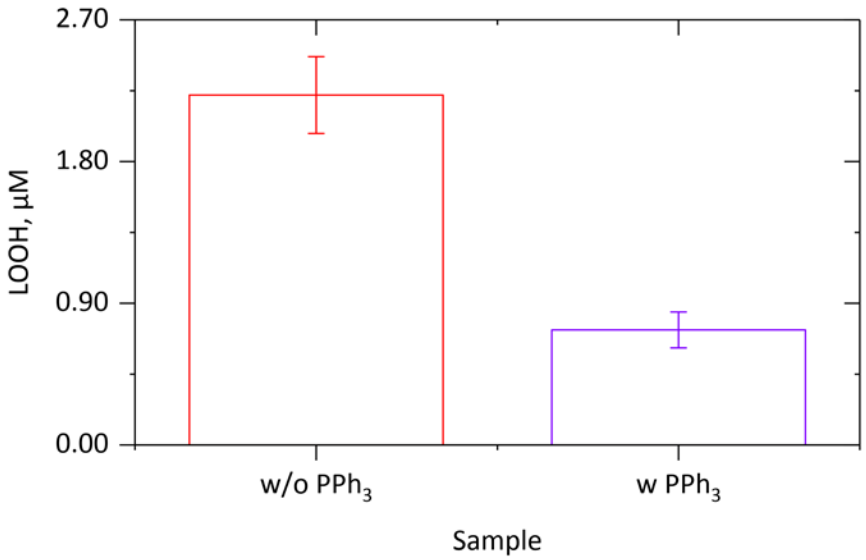


**Figure S6. Levels of lipid hydroperoxides in non-reduced and triphenylphosphine reduced TLCL.** For the reduction of lipid hydroperoxides TLCL (300 µM lipid concentration) was pre-incubated with triphenylphosphane ($PPh_3$) before liposome preparation. LOOH concentration was determined by ABTS-based measurements with horseradish peroxidase (HRP). Mean and SD of 3 independent experiments are shown.

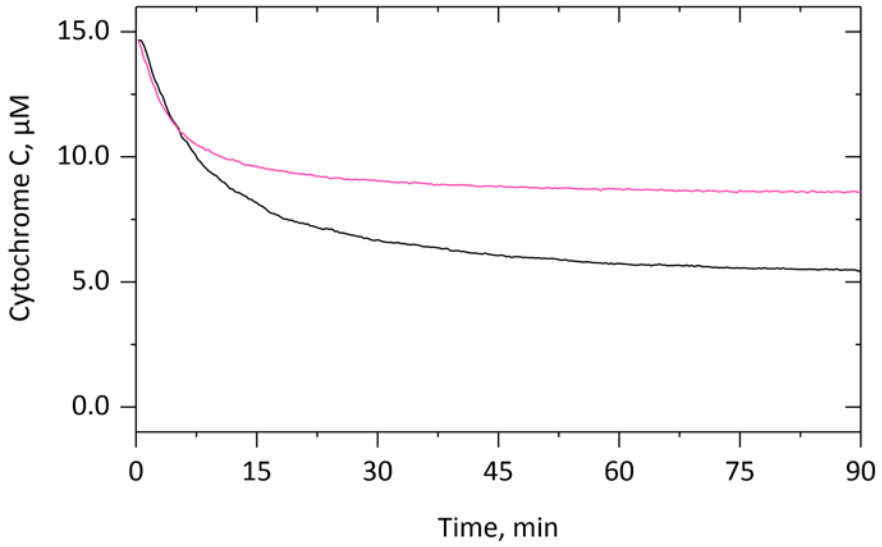


**Figure S7. Effect of normoxic and hypoxic conditions on TLCL-induced cyt c degradation monitored by the loss of the Soret band.** Cyt c was incubated with TLCL-containing liposomes under normoxic (black) or hypoxic (pink) conditions. Representative kinetic curves of 3 independent experiments are shown.

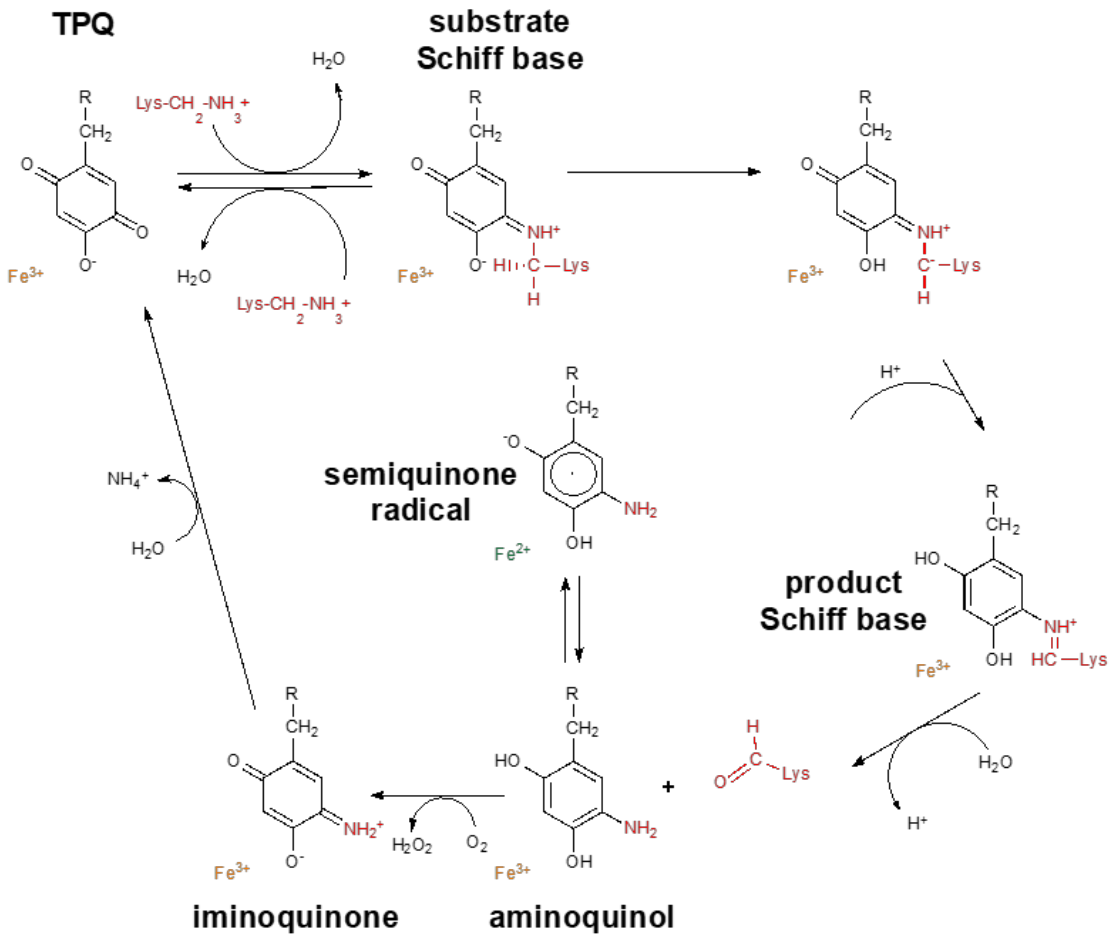


**Figure S8. Transamination mechanism proposed for TPQ-mediated aminoadipic semialdehyde formation from Lys residues side chain in the presence of free iron.** Free iron III might be involved in coordination of oxidized Tyr and can be reduced to iron II by aminoquinol with a formation of semiquinone radical. Aminoquinol/iron III and semiquinone radical/iron II couples might exist in an equilibrium. Further oxidation of aminoquinol or semiquinone radical by molecular oxygen to iminoquinone is accompanied by formation of hydrogen peroxide. Thus $2e^-$ reaction presumably involve superoxide anion radical as intermediate. In this case iron could coordinate the superoxide, facilitating the electron transfer. The mechanism is adapted from the one reported for copper amine oxidize by Klema et al 2012.

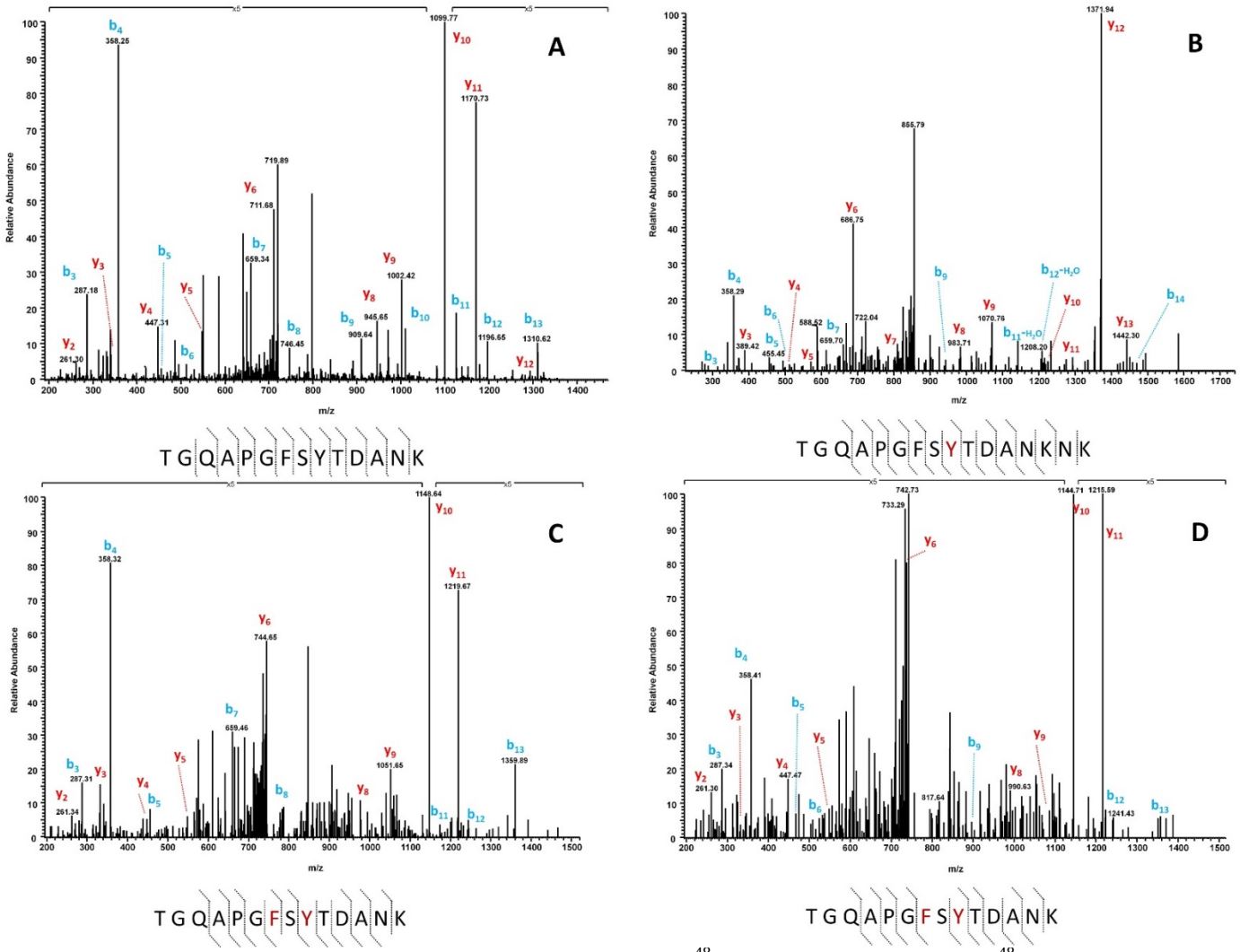


**Figure S9.** Tandem CID mass spectra of cyt C tryptic peptides TGQAPGFSY$^{48}$TDANK or TGQAPGFSY$^{48}$TDANKNK containing unmodified Tyr 48 (A; precursor ion *m/z* 728.84$^{2+}$), TPQ (B; precursor ion *m/z* 864.89$^{2+}$), oxidized Phe 46 and aminoquinol on Tyr 48 (C; precursor ion *m/z* 753.34$^{2+}$), and oxidized Phe 46 and iminoquinol on Tyr 48 (D; precursor ion *m/z* 751.33$^{2+}$).

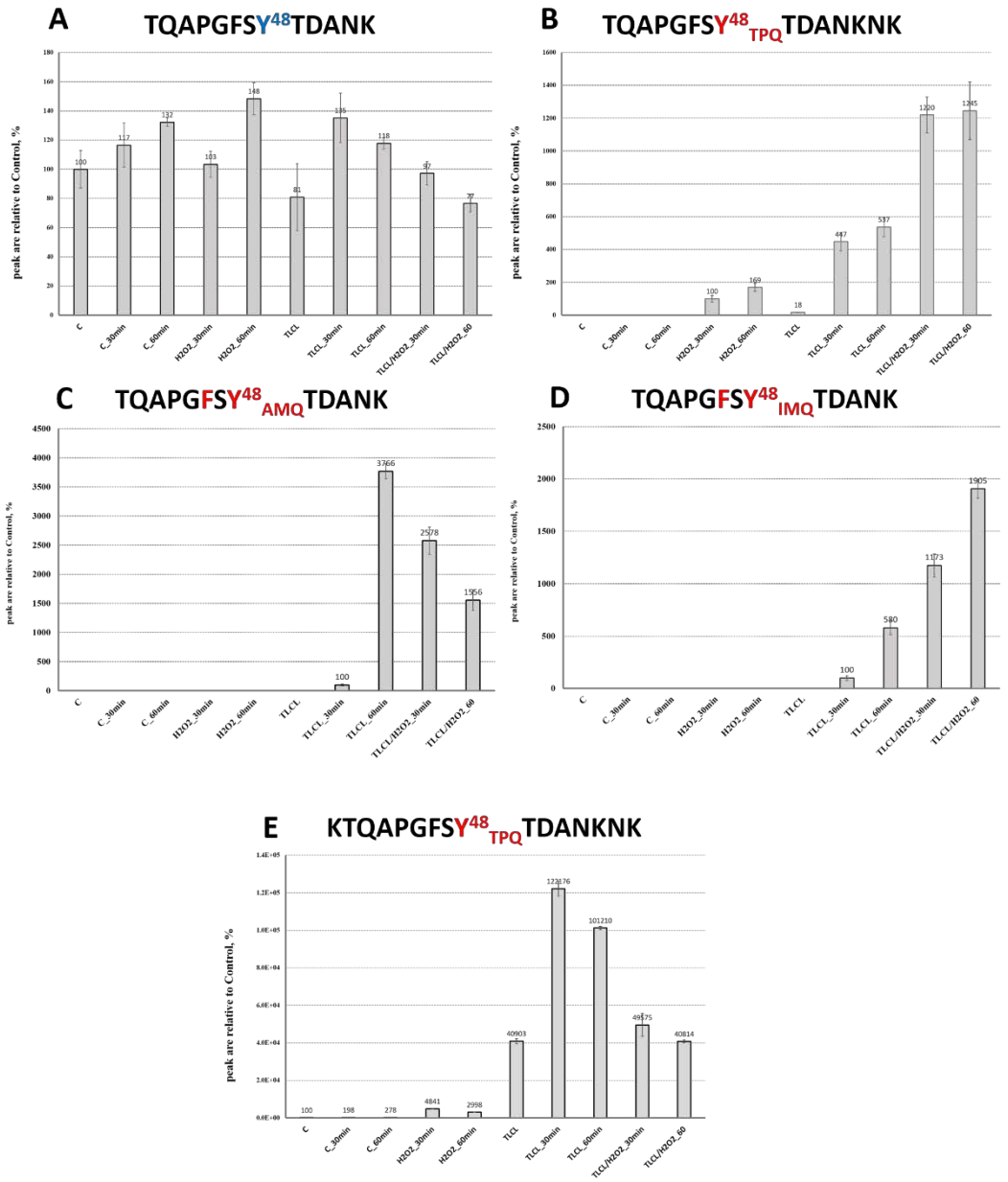


**Figure S10.** Relative label free quantification of peak areas from LC-MS/MS experiments for cyt c tryptic peptides TGQAPGFSY$^{48}$TDANK, TGQAPGFSY$^{48}$TDANKNK, or KTGQAPGFSY$^{48}$TDANKNKcontaining unmodified Tyr 48 (A; precursor ion *m/z* 728.84$^{2+}$), TPQ (B; precursor ion *m/z* 864.89$^{2+}$), oxidized Phe 46 and aminoquinol on Tyr 48 (C; precursor ion *m/z* 753.34$^{2+}$), oxidized Phe 46 and iminoquinol on Tyr 48 (D; precursor ion *m/z* 751.33$^{2+}$), and TPQ (E; precursor ion *m/z* 619.97$^{3+}$). The lowest detected peak area for each peptide expressed as 100%.

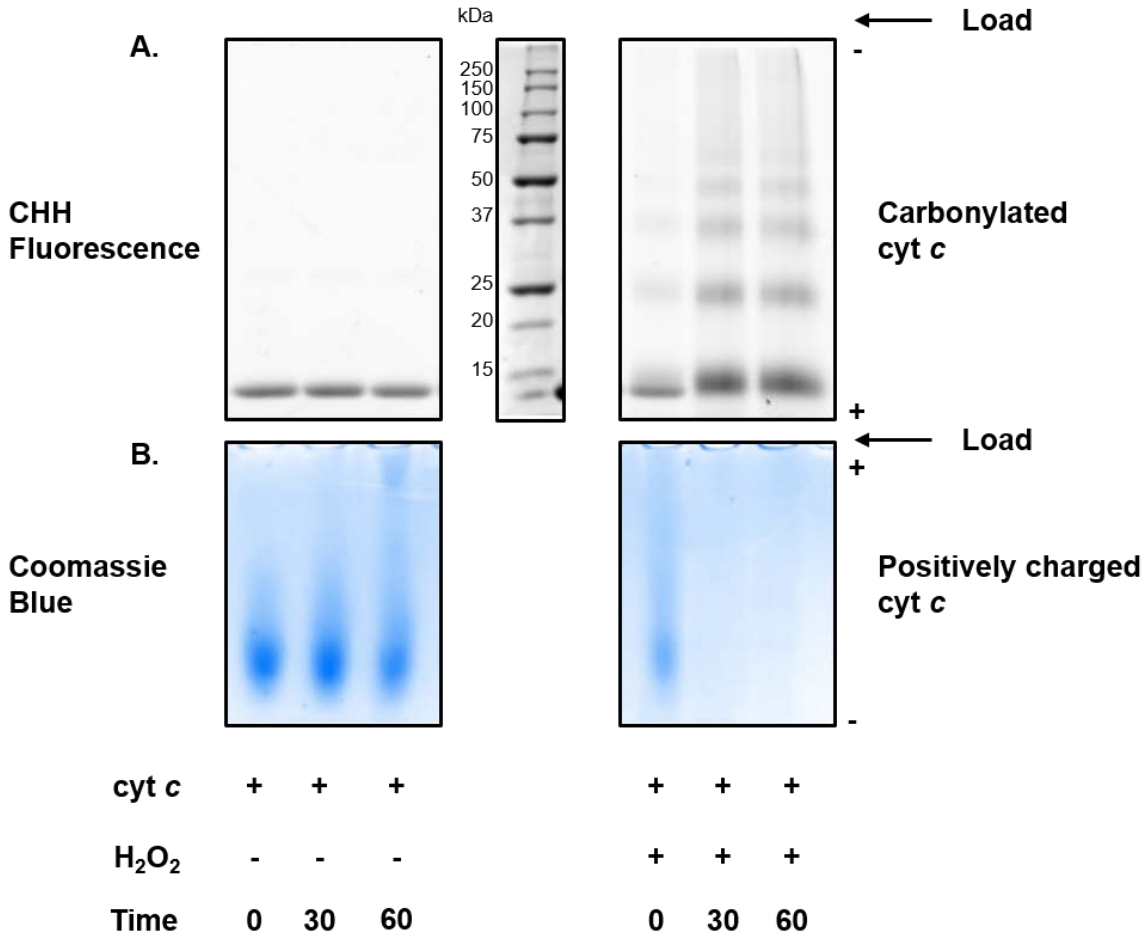


**Figure S11. Cyt c carbonylation and associated loss of positive charge upon oxidation with hydrogen peroxide.** (A) Cyt c carbonylation was quantified for protein (100 µM) incubated in the absence and presence of hydrogen peroxide (1 mM) for 0, 30 and 60 min followed by CHH derivatization, and separation by 12 % SDS-PAGE. CHH fluorescence signal were recorded and quantified by densitometric analysis to calculate the proportion of carbonylated cyt c. (B) Cyt c (100 µM) incubated in the absence and presence of hydrogen peroxide (1 mM) for 0, 30 and 60 min was subjected to native 7.5 % PAGE in reverse electrophoresis mode. After Coomassie Blue staining, signals were recorded and quantified by densitometric analysis to calculate the proportion of positively charged cyt c.