SUPPLEMENTARY INFORMATION for

Detecting changes in the thiol redox state of proteins following a decrease in oxygen concentration using a dual labeling technique

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Figure S1

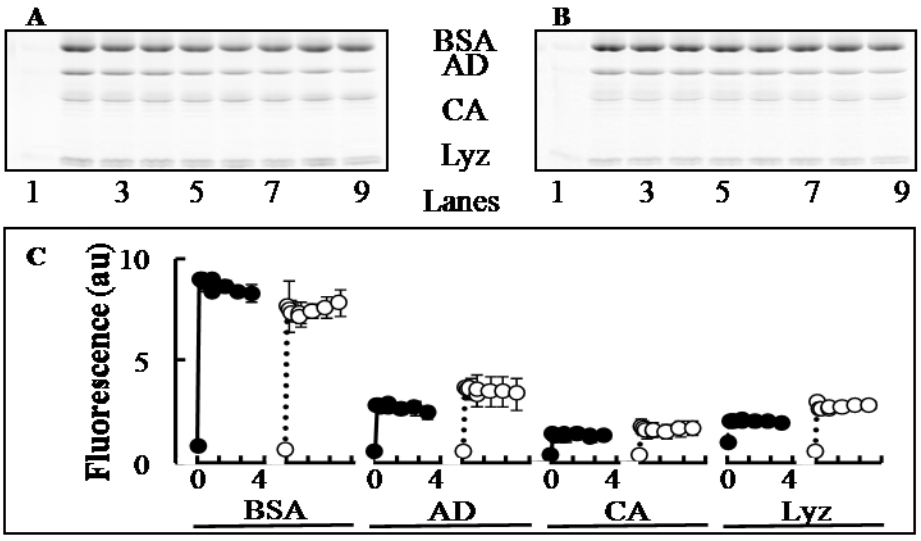


Figure S1. The effect of increasing incubation time on dye binding. A mixture of test protein (total of 10 mg/mL) consisting of 2.5 mg/mL each of BSA, AD, CA and Lyz was first reduced with 10 mM of TBP. FLm (A) or TMRm (B) (400 μM) were incubated with the protein mixture for a range of time points (in hr: 0, 0.08, 0.25, 0.5, 1, 1.5, 2, 3 and 4) loaded in order for lanes 1-9. The band density for FLm (●) and TMRm (○) labeled protein was measured and plotted (C). Cysteine (40 mM) was used to quench unreacted dye. Images are representative. Band density was estimated using Progenesis. Graph values represent the mean ± SEM of 3 separate experiments.

Figure S2

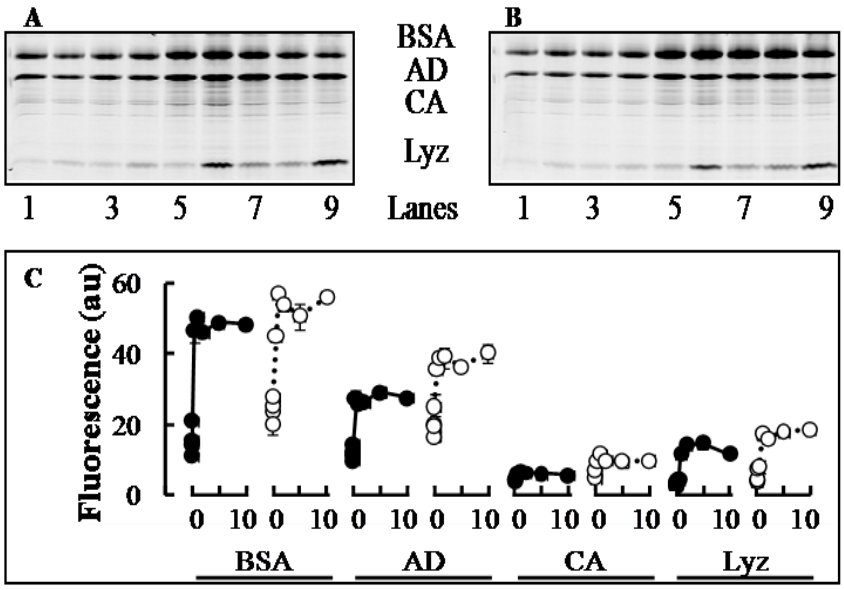
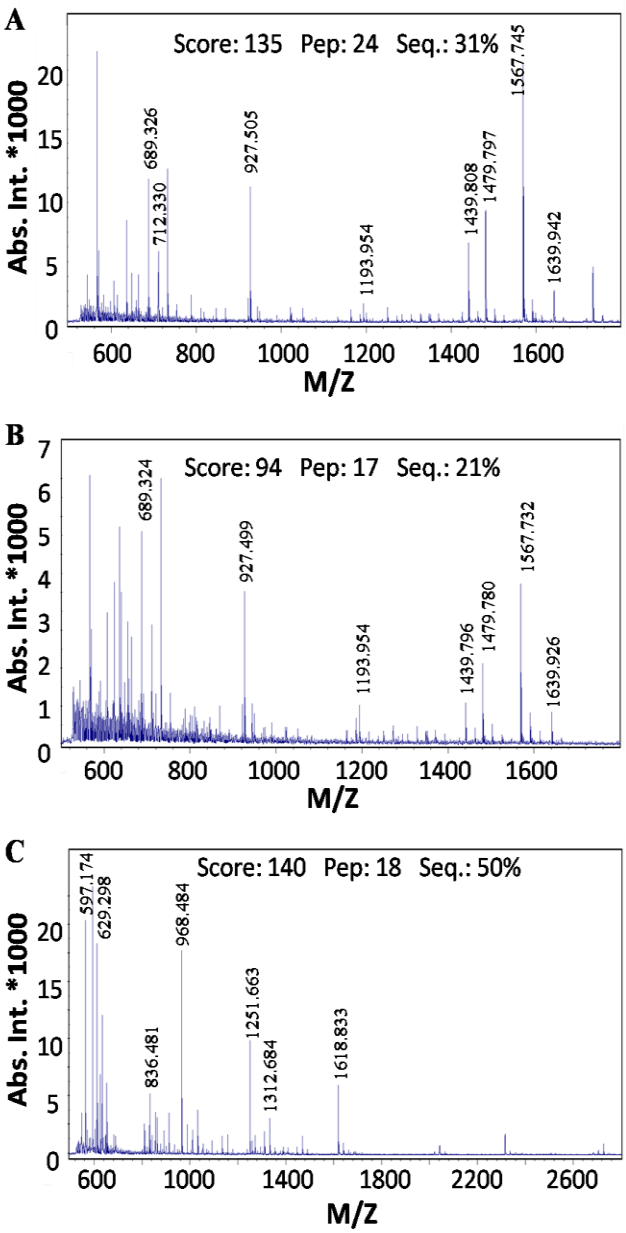


Figure S2. The effect of increasing concentration of the reducing agent TBP on dye binding. A mixture of test protein (total of 10 mg/mL) consisting of 2.5 mg/mL each of BSA, AD, CA and Lyz were reduced with varying concentrations of TBP (in mM: 0, 0.02, 0.05, 0.1, 0.5, 1, 2, 5 and 10) loaded in order for lanes 1-9. FLm (A) or TMRm (B) (400 μM) in a total volume of 20 μL was incubated with all samples for 30 min. Cysteine (40 mM) was used to quench unreacted dye. Images are representative. Band density was estimated using Progenesis. Graph values represent the mean ± SEM of 3 separate experiments.

Figure S3


A
Score: 135 Pep: 24 Seq.: 31%
Abs. Int. *1000
M/Z
689.326
712.330
927.505
1193.954
1439.808
1479.797
1567.745
1639.942
B
Score: 94 Pep: 17 Seq.: 21%
689.324
927.499
1193.954
1439.796
1479.780
1567.732
1639.926
C
Score: 140 Pep: 18 Seq.: 50%
597.174
629.298
836.481
968.484
1251.663
1312.684
1618.833

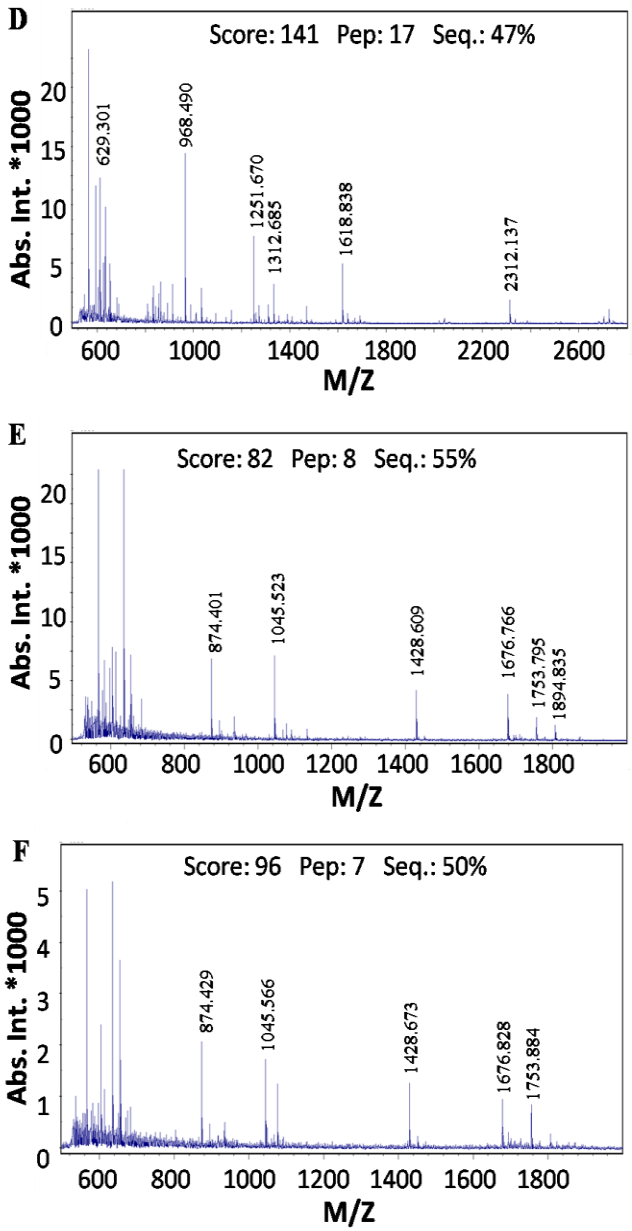


Figure S3. Identifying proteins labeled with FLm and TMRm. Standard proteins BSA, AD and Lyz (25 μg each) were either unlabeled or labeled with 250 μM FLm for 10 min in a total volume of 30 μL, washed through ethanol precipitation, then reduced with 1 mM TBP and subsequently labeled with 250 μM TMRm for 10 min. After labeling, the samples were washed again through ethanol precipitation and resolubilised in 30 μL of 25 mM ammonium bicarbonate before trypsin digestion for 16 hr. Samples were

analyzed in the MALDI TOF-TOF and mass profile spectra were obtained. Representative mass spectra of unlabeled BSA (A), AD (C) and Lyz (E) are shown. Their respective labeled mass spectra are shown as (B), (D) and (F). The Mascot score (Score), number of peptide matches (Pep) and sequence coverage (Seq) are shown.

Figure S4

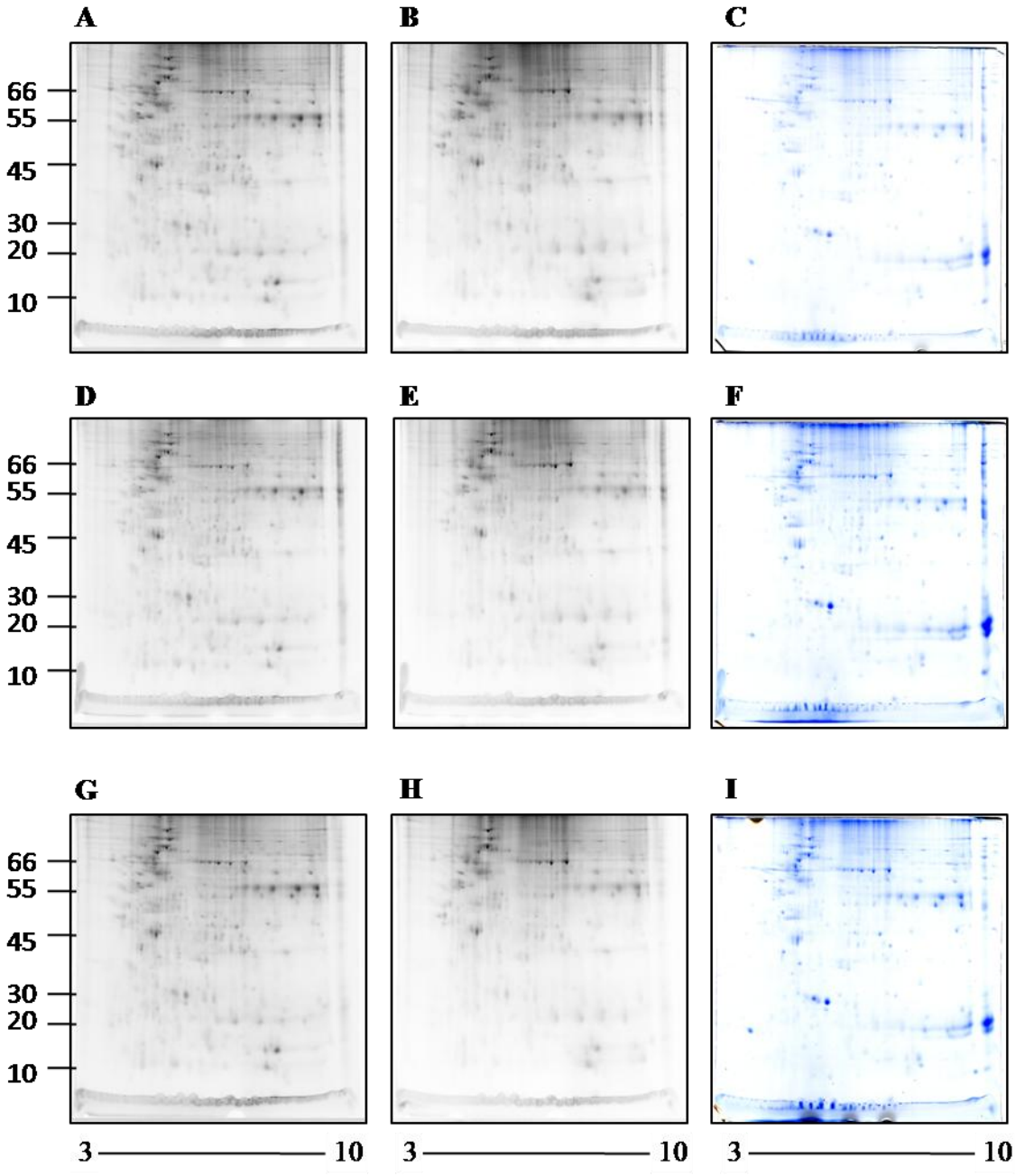


Figure S4. Example of two-dimensional gel images of proteins extracted from: non-treated Jurkat cells (exposed to room oxygen)(A, B, C); treated to 10 min 25 μM H_2O_2 (D, E, F); and, exposed to 30 min low oxygen (G, H, I) . The gel was scanned for fluorescence originating from FLm (A, D, G) and TMRm (B, E, H). Following scanning,

the gels were stained with Coomassie (C, F, I). Vertical numbers refers to the molecular mass range and the horizontal numbers refers to the pI range.