State-Selective Metabolic Labeling of Cellular Proteins

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Supporting Information
Supplementary Figure 1. Proteomic labeling with Anl under conditions of oxidative stress. The NLL-MetRS is under control of the soxS promoter and can be activated by superoxide or nitric oxide. Cells in medium containing Anl were treated with nitric oxide (B), paraquat, a superoxide-generating agent (C), or were uninduced (A). Incorporation of Anl is detected by conjugation to biotin-PEG-alkyne and subsequent detection by western blot analysis with streptavidin-HRP.
Supplementary Figure 2. Tagging rate in the SoxRS system is less than 10%. *E. coli* cells harboring the pJTN1 plasmid pulsed with Anl under conditions that yield a 10% substitution rate are compared with *E. coli* harboring pSOX-NLL induced with paraquat and pulsed with Anl. Incorporation of Anl is assessed by conjugation with alkyne-TAMRA and subsequent detection by fluorescence microscopy. SoxRS-directed labeling with Anl yields a substitution rate of less than 10%, as fluorescence emission from pJTN1 cells is more intense than that observed with SoxRS-directed labeling. Cells constitutively expressed GFP, which was separately detected to confirm the presence of cells.
Supplementary Figure 3. Tagging rate in the SoxRS system is dependent on the degree of induction of NLL-MetRS expression. The NLL-MetRS is under control of the soxS promoter and is activated by addition of paraquat (PQ) to the culture medium. As the degree of transcription from the soxS promoter is dependent on the concentration of PQ used, so is the level of NLL-MetRS induction. Cells induced with PQ and pulsed with 125 μM Anl exhibit increasing levels of Anl incorporation as more PQ is added. Incorporation of Anl is assessed by conjugation to alkyne-TAMRA and subsequent detection by fluorescence microscopy. Cells constitutively expressed GFP, which was separately detected to confirm the presence of cells.
SoxRS Regulon sequence used:

5′-ATATgctagcCCCGTGTTGAAAACCGACGCGCCAGTTTATCTAGTCAGGCTTGTATTCTAGCTGATCGTGCGCTGGACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCTTCAGTTACGAGTTCTAGCTGAGCGTGGACCGGAAGGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGGAGGCTCGTCCTGAATGATATGCGACCGCCGGAGGGTTGCGTTTGAGACGGGCGACAGATCGACACTGCTCGATCCGCTCGCACCCAAAAAACCCCATAGAAGCCCGTTTAGAGGCCCGCA

AGGGTTATGCTATCATTACATTAGTTTATCTTCTTCCAGCAAGCGTGCGCCGGTA
CCCTCTTTCTCTCTAAGGCCTCGGTCGCCCGGTTACGCAACGGGCAATCAGTCTCGGCTGAT
TGCCGCTTCCACGATCTGTAATTCAACACTCACCTTTTATATTATCGCATGCGTCTG
ACCACCTGCTGCGTTTCGGCCACTTCGCGCGGGGTAGCAGCGCTTTAATGCGG
TAATTCTTTTCTTCCATAAATCGCTTTACCCTCAAGTTAACTTGAGGAATTTTAGTTACTACC
CACATGAGATTAAACGCAACTCAGACATGGAAAGGAGCGAGATTCCATGGGAGGATCCAGAT
CTCATCCATCCATCCATCCATACCTACCTTTAGCTGAGCTTGGACTCCTGTTGATAGAT
CCAGTAATTGACCTCAGACTCCATCTGATTTTGTCCAGAAGGCTCTGCTGGCCGCGGCG
GTTTTTTATTGTTTGTGTCCTGCGGATCGAGGACGAGGGGAGCGCCAGCTGGATCTAGTCC
GGAGGGCCGTGGACCGCCGTGACTTACGCAACAGCAGCAGCGTACACTGGAGCC
GCTAGATTATCATGTCAGGCTTGTAGTCTAGGCTCTTTCTCTGAGGCTCAATATAGCGC
5′-Blue: transcriptional termination sequence (reverse)
Green: SoxR transcription factor gene from (reverse)
Yellow: Bi-directional regulatory sequence containing the soxR and soxS promoters
3′-Blue: transcriptional termination sequence (forward)