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Supplementary information

A lysine–cysteine redox switch with an NOS bridge regulates enzyme function

In the format provided by the authors and unedited

Supplementary Results

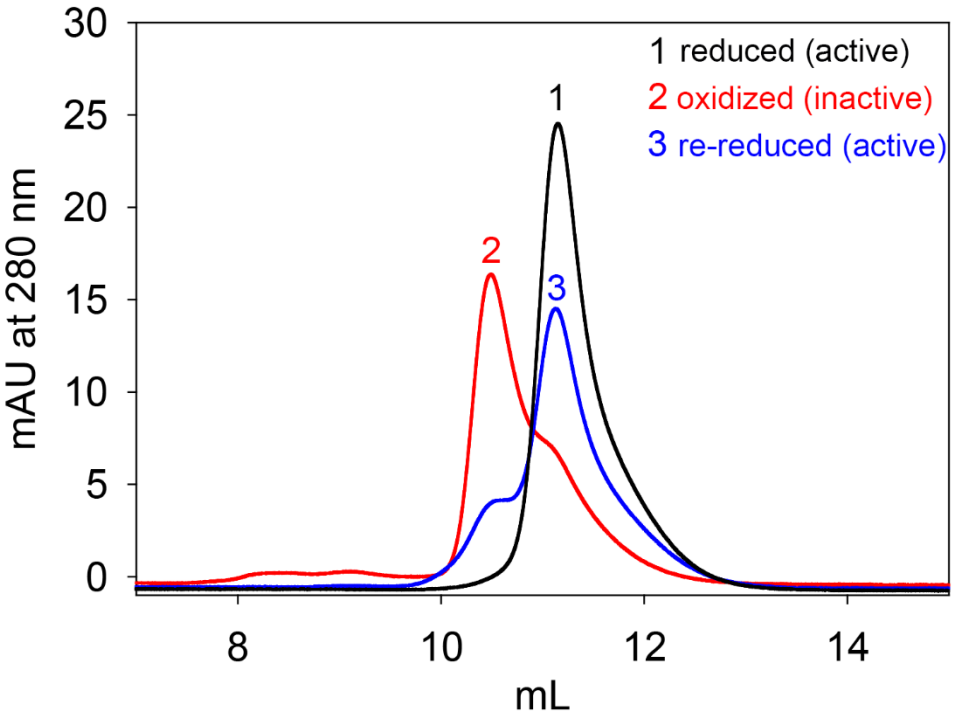
Mass spectrometric analysis of the oxidized and reduced enzyme

The chemical identity of the NOS link appears well established on the basis of X-ray crystallography, but we tested whether this linkage could also be detected by mass spectrometry (MS). MS is routinely used for detection of disulfide bonds [1,2], and detection of NOS bonds would obviate the need for ultra-high resolution X-ray crystallography. We were, however, unable to detect the crosslink between the two peptides bearing Lys8 and Cys38, and instead Cys38 was found predominantly in the sulfinic acid state (SI Fig. 2). It thus appears that the covalent NOS linkage is hydrolyzed under the acidic conditions used for sample preparation (the same was observed under basic conditions). The MS data are nevertheless informative, as they provide further support that the bridging atom is indeed oxygen. No modifications of Lys8 could be detected either in the oxidized or reduced enzyme, and Cys38 was detected as the unmodified thiol under reducing conditions. The chemical instability of the NOS crosslink during sample processing, coupled with the absence of this species in the modifications searched during MS data analysis, explains why it has been missed in proteomics analyses so far. This in turn demands development of chemical probes and MS-based (or other straightforward) techniques to reliably detect the novel crosslink in a stable, isolatable form. In addition to the wild-type protein, we analysed variants Lys8Ala and Cys38Ser. In Lys8Ala, Cys38 exists as sulfinic acid, while Lys8 in Cys38Ser was found to be non-modified under all conditions tested.

1) Mann, M., & Jensen, O. N. (2003). Proteomic analysis of post-translational modifications. *Nature Biotechnology* 21(3), 255-261.

2) Witze, E. S., Old, W. M., Resing, K. A., & Ahn, N. G. (2007). Mapping protein post-translational modifications with mass spectrometry. *Nature Methods* 4(10), 798-806.

Supplementary Figures



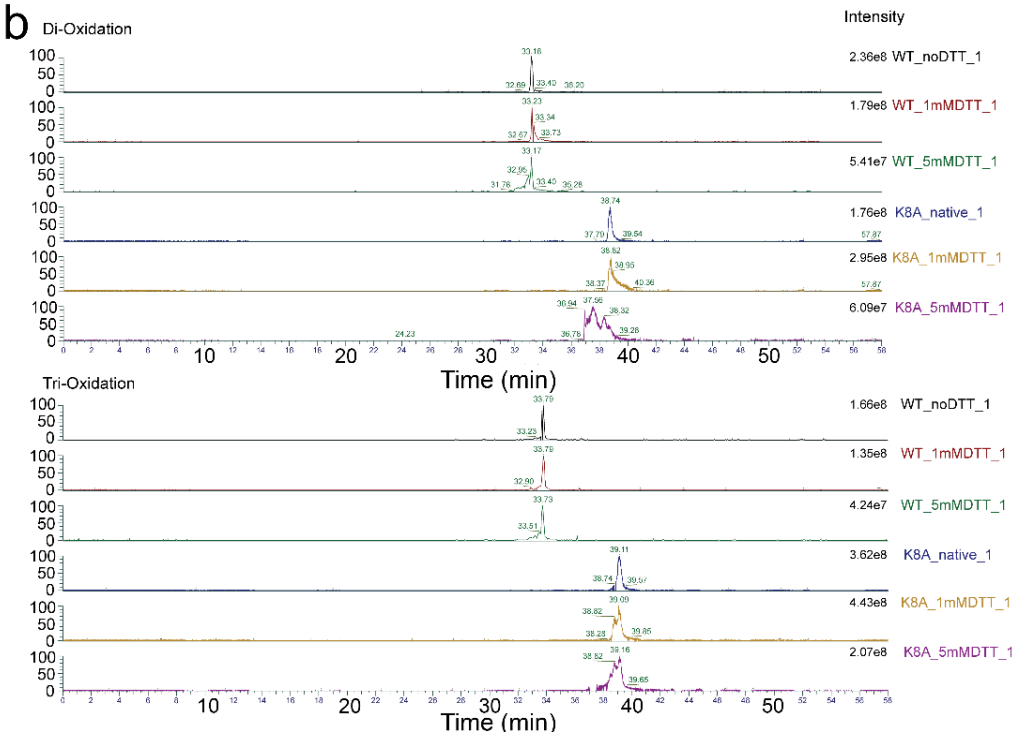
SI Figure 1. Size-exclusion chromatographic analysis of *Ng*TAL (1 mg/ml in all samples) in the oxidized (enzymatically inactive) and reduced (enzymatically active) state using an analytical SEC S75 column. Freshly prepared and enzymatically active *Ng*TAL in the reduced state (sample 1, black) elutes at ~11.1 min. The reduced enzyme was separated from excess reductant DTT using a desalting G25 column and allowed to oxidize at 6 °C. With progressing oxidation over a couple of weeks, the enzyme became inactive (v/E = 0.22 ± 0.01 s^{-1}). The oxidized form (sample 2, red) elutes at ~10.4 min with some minor fraction of the reduced form present. Re-reduction of the oxidized enzyme by addition of 20 mM DTT led to a reactivation of the enzymatic activity (v/E = 25.0 ± 2.2 s^{-1}). The thus obtained re-reduced enzyme (sample 3, blue) elutes at ~11.1 min similar to the freshly prepard reduced enzyme.

A likewise separation of the oxidized and reduced enzyme was obtained under preparative conditions using a preparative SEC S75 column.

a

							Ratio modified peptides/base peptides intensity Exp											
Base peptide sequence	Modification	Cluster mass	Localization probability	Posterior error probability	Score	PSMs	K8A_1 mMDTT_1	K8A_1 mMDTT_2	K8A_5 mMDTT_1	K8A_5 mMDTT_2	K8A_native_1	K8A_native_2	WT_1 mMDTT_1	WT_1 mMDTT_2	WT_5 mMDTT_1	WT_5 mMDTT_2	WT_noDTT_1	WT_noDTT_2
QGVCGVTSNPAIFQK	tri-Oxidation	47.99	QGVC(0.999)GVTSNPAIFQK	4.026E-20	184.16	33	0	0.0525	0.0518	0.0022	0	1.9394	3.0959	0.1883	0.0403	0.1372	0.2282	65.271
QGVCGVTSNPAIFQK	di-Oxidation	31.99	QGVC(0.992)GVTSNPAIFQK	1.311E-22	170.59	41	31.408	0.7742	0.0381	0.0226	159.2	0.8703	4.0991	0.1402	0.0478	0	0.3457	86.428
QGVCGVTSNPAIFQK	tetra-Oxidation	63.98	QGV(0.01)C(0.979)G(0.01)VTSNPAIFQK	8.922E-10	148.49	10	0.2256	0.0164	0	0.0001	0	0.0177	0.0369	0.0046	0.0008	0	0.0038	1.7533
QGVCGVTSNPAIFQK	Oxidation	15.99	QGVC(0.995)GVTSNPAIFQK	1.888E-32	197.97	11	0.5233	1.2728	0.0379	0.0124	0	0.006	0.03	0.011	0.0066	0	0	0

b



SI Figure 2. Mass spectrometric analysis of post-translational modifications of *Ng*TAL under oxidizing and reducing conditions. The modifications on Cys38 from the Lys8Ala (K8A) variant and from the wild-type (WT) protein are shown. (**a**) Identified base peptide sequence and its modifications. The ratio of intensities from modified peptides and base peptides are shown using a color gradient. The smallest number is represented by white color and the largest number by red color. (**b**) The extracted ion chromatography of the modified peptides and their intensities are shown. The modified peptides with two and three oxidation could be successfully extracted in high intensity. We failed to extract the other two modified peptides with one or four oxidations.