Rhodobacter sphaeroides methionine sulfoxide reductase P reduces *R*- and *S*-diastereomers of methionine sulfoxide from a broad-spectrum of protein substrates

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Supplementary data

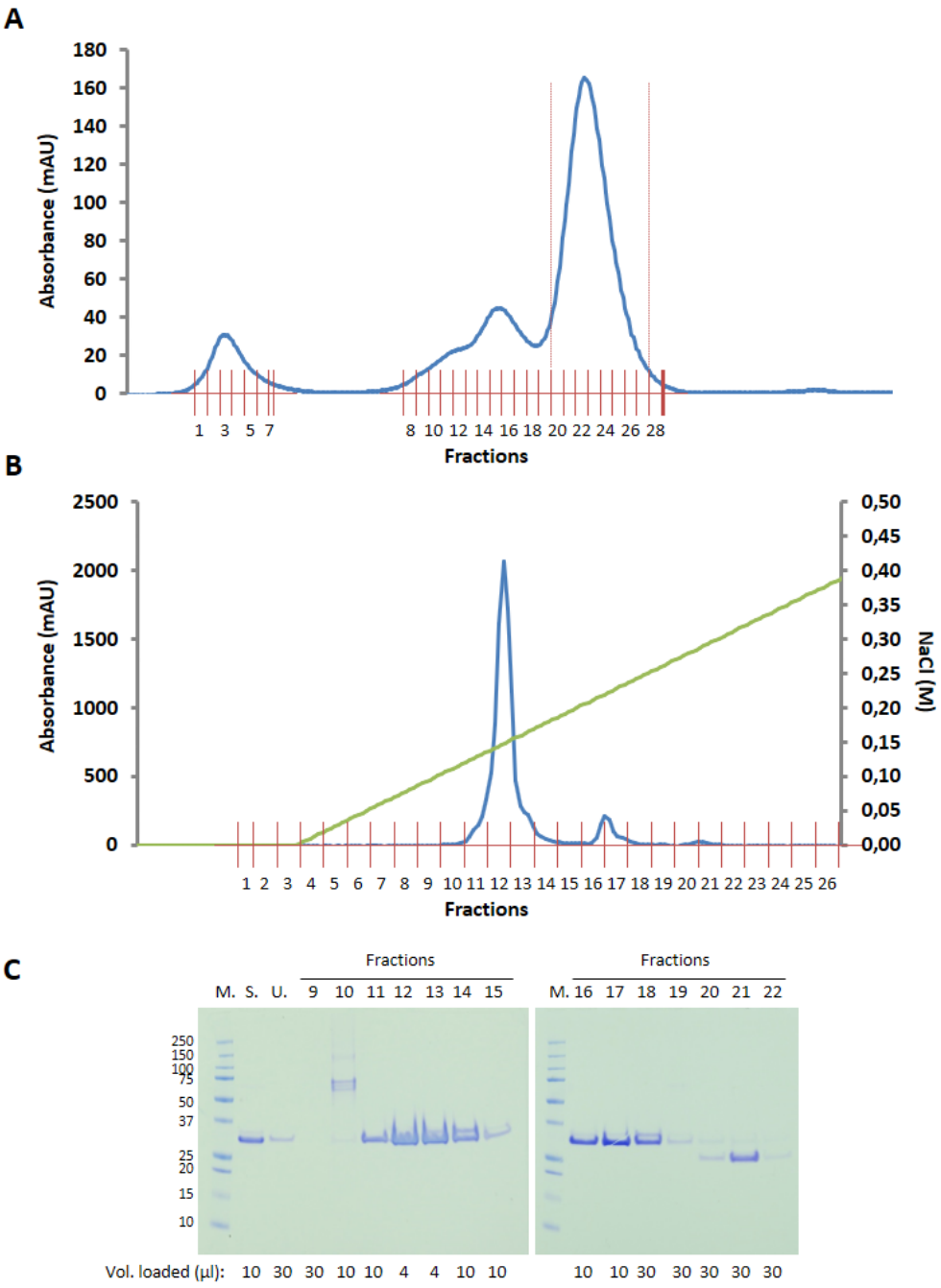

Figure S1. Preparation of highly pure RsMsrP. After expression in *R. sphaeroides*, RsMsrP from periplasmic extracts was first purified using nickel-affinity column, then incubated with TEV protease to remove the polyhistidine tag. On the following step, the solution was passed a second time on nickel-affinity column to remove the TEV and the tag. After concentration, the protein solution was loaded on Superdex^{TM} 200 10/30 gel filtration column (**A**). Fractions 20 to 27 were pooled, concentrated and loaded on MonoQ^{TM} 4.6/100 PE strong anion exchange column (**B**). The protein was eluted using a linear NaCl gradient. Eluted fractions were analyzed by electrophoresis using 10 % bis-tris gels (**C**). Fractions 11, 12 and 13 were used for enzymatic assays. *M. molecular weight marker*; *S. starting material (gel filtration fraction 20 to 27 pooled and concentrated)*; *U. unbound fraction*.

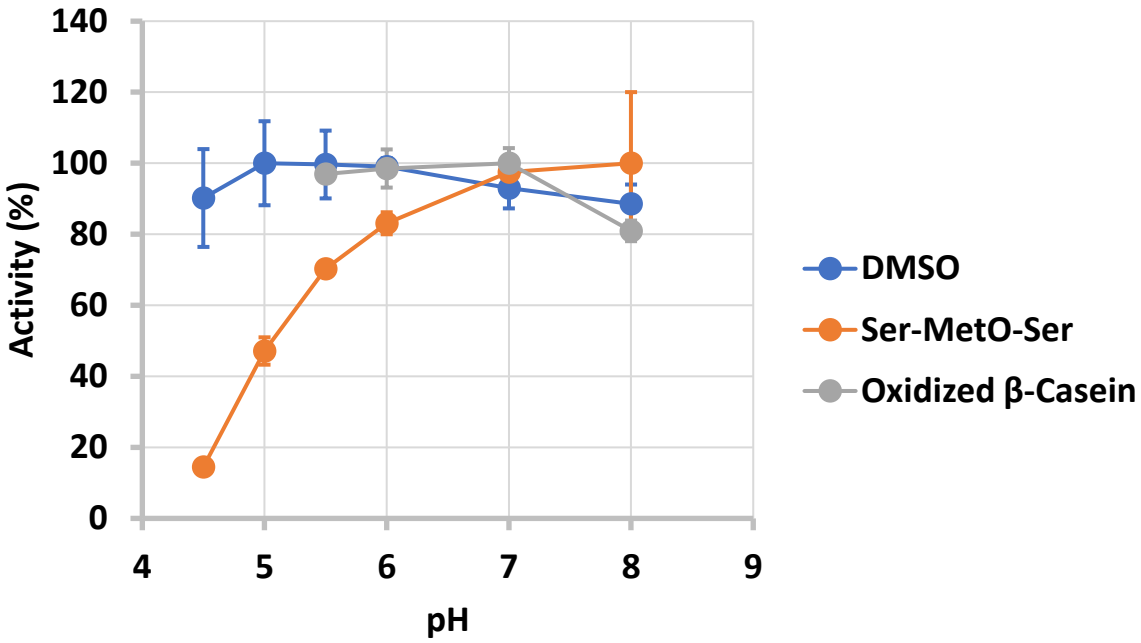

Figure S2. pH optimum of RsMsrP activity. The RsMsrP activity was determined using benzyl viologen (BV) (0.8 mM) as electron provider under nitrogen in a glove box. BV was initially reduced with sodium dithionite (2 mM) and oxidation was followed at 600 nm after addition of the enzyme (30 nM). Reaction was made in Britton and Robinson buffer at several pHs. DMSO (1.41 M), Ser-MetO-Ser peptide (5 mM) or oxidized β-casein (50 µM) were incubated with RsMsrP (31 nM). Maximal k_{obs} were 102 ± 12 s^{-1}, 87 ± 17 s^{-1} and 26 ± 1 s^{-1}, for DMSO, Ser-MetO-Ser peptide and oxidized β-casein, respectively. Activity was not recorded with oxidized β-casein for pH < 5.5 because of protein precipitation. Data presented are average of three replicates. ± S.D.

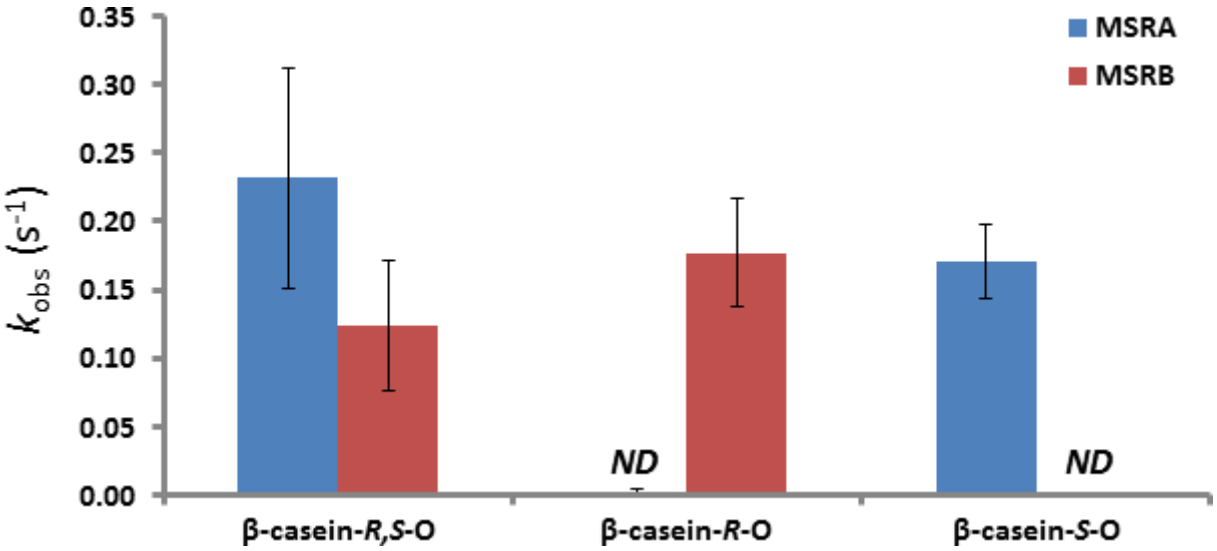

Figure S3. Yeast MsrA and MsrB activity on oxidized β-casein (β-casein-*R,S*-O), β-casein-*R*-O and β-casein-*S*-O. The oxidized β-casein containing both diastereomers of MetO ("*β-casein*-R,S-*O"*), only the *R* one ("*β-casein*-R-*O"*), or only the *S* one ("*β-casein*-S-*O*") were assayed as substrate for the MsrA and MsrB using the thioredoxin system with NADPH as electron provider and following its consumption spectrophotometrically at 340 nm. The oxidized β-casein concentrations were 100 µM. In the case of the β-casein-*R,S*-O, an activity was measured for both Msrs, showing that this oxidized protein contained both diastereomers of MetO. For the β-casein-*R*-O, an activity was detected only for the MsrB, but not for the MsrA, confirming that this form of the oxidized β-casein contained only the *R*-diastereomer of MetO. On the contrary, with the β-casein-*S*-O, an activity was detected only with the MsrA showing that it contained only the *S*-diastereomer of MetO. *ND. Not detected.* Data presented are average of three replicates. ± S.D.

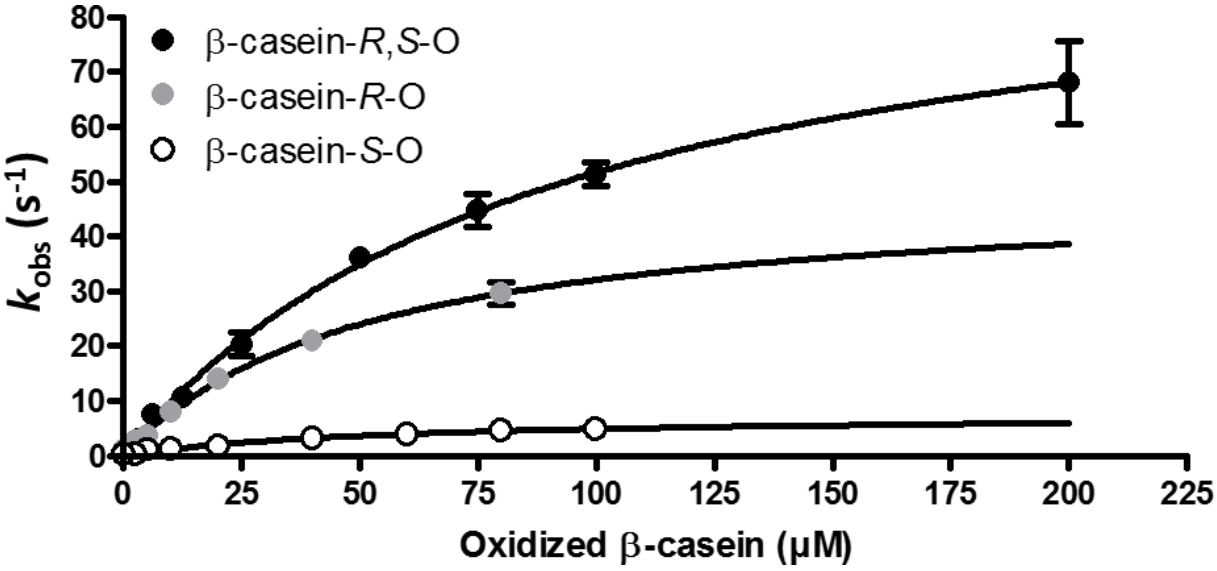

Figure S4. RsMsrP activity with oxidized β-casein containing both diastereomers of MetO ("*β-casein*-R,S-*O*"), only the *R* one ("*β-casein*-R-*O*"), or only the *S* one ("*β-casein*-S-*O*"). The RsMsrP activity was determined in MES pH 6.0 using BV (0.8 mM) as an electron provider under nitrogen in a glove box. BV was initially reduced with sodium dithionite (2 mM) and oxidation was followed at 600 nm after addition of the enzyme (30 nM). Data presented are average of three replicates. ± S.D.

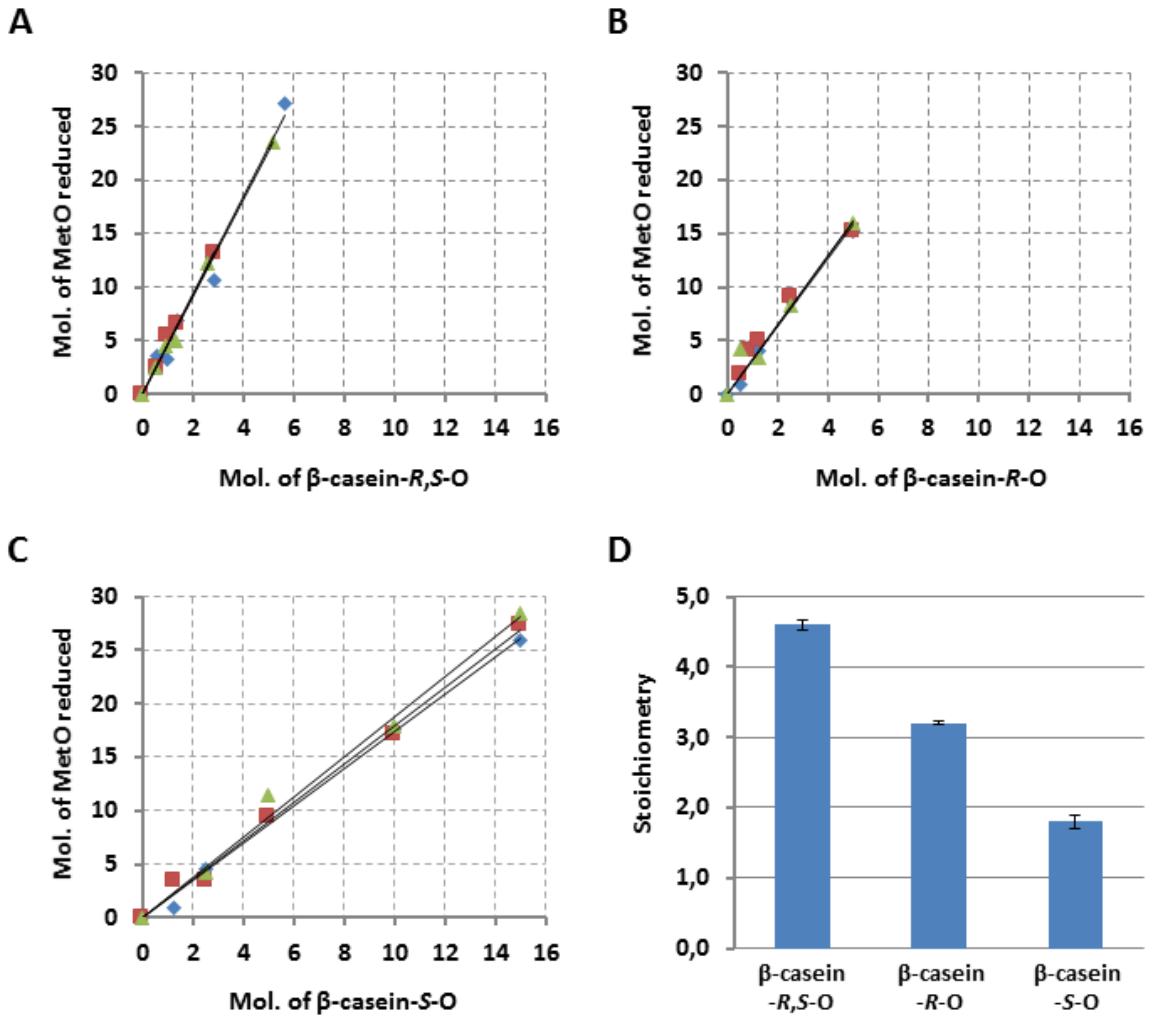

Figure S5. The apparent stoichiometry of reduction of oxidized β-casein containing both diastereomers of MetO ("*β-casein*-R,S-*O*") (A), only the *R* one ("*β-casein*-R-*O*") (B), or only the *S* one ("*β-casein*-S-*O*") (C). The apparent stoichiometry was determined similarly to the activity assays with the highly pure RsMsrP using subsaturating concentrations of substrates: 1–10 µM oxidized β-casein, 1–10 µM Met-*R*-O containing β-casein and 1.5–15 µM Met-*S*-O containing β-casein. The amount of oxidized benzyl viologen was determined 1 hour after the addition of the RsMsrP (46 nM) by subtracting the final A_{600} nm value to the initial one. Controls were made without the RsMsrP enzyme and without the MetO-containing substrate. Quantities of MetO reduced were plotted as a function of substrates quantities of and the apparent stoichiometry was obtained from the slope of the linear regression. D) Average apparent stoichiometries of three replicates. ± S.D.

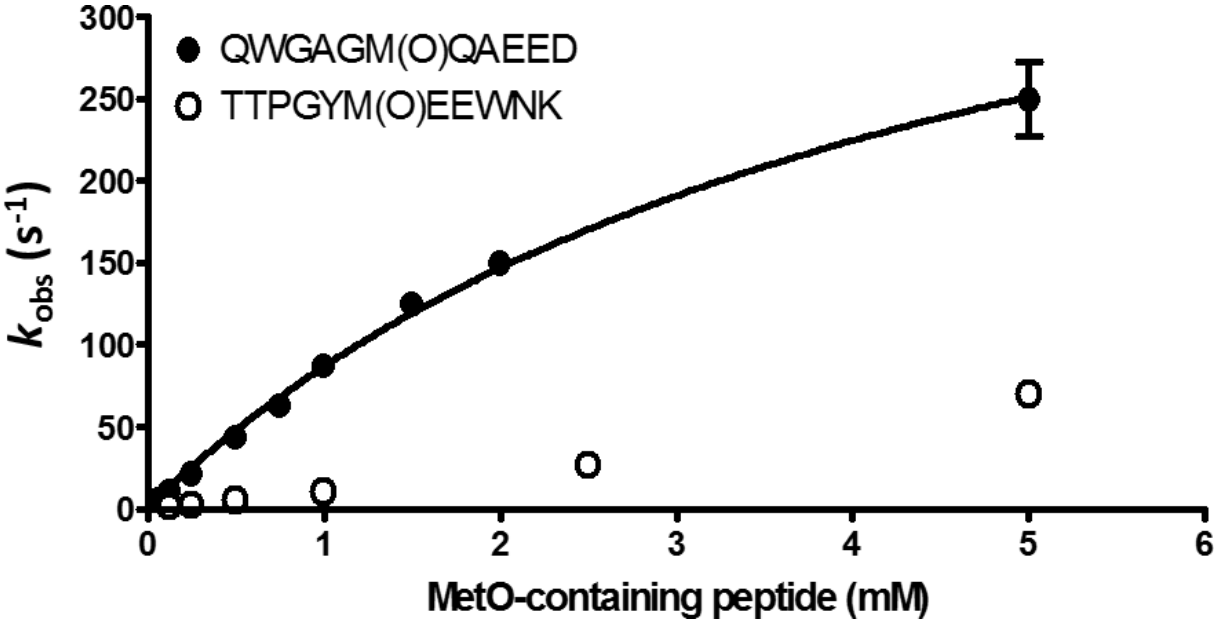

Figure S6. RsMsrP activity using peptides designed from the proteomics results as substrates. The RsMsrP activity was measured as described in Figure 2. Data presented are average of three replicates. ± S.D.

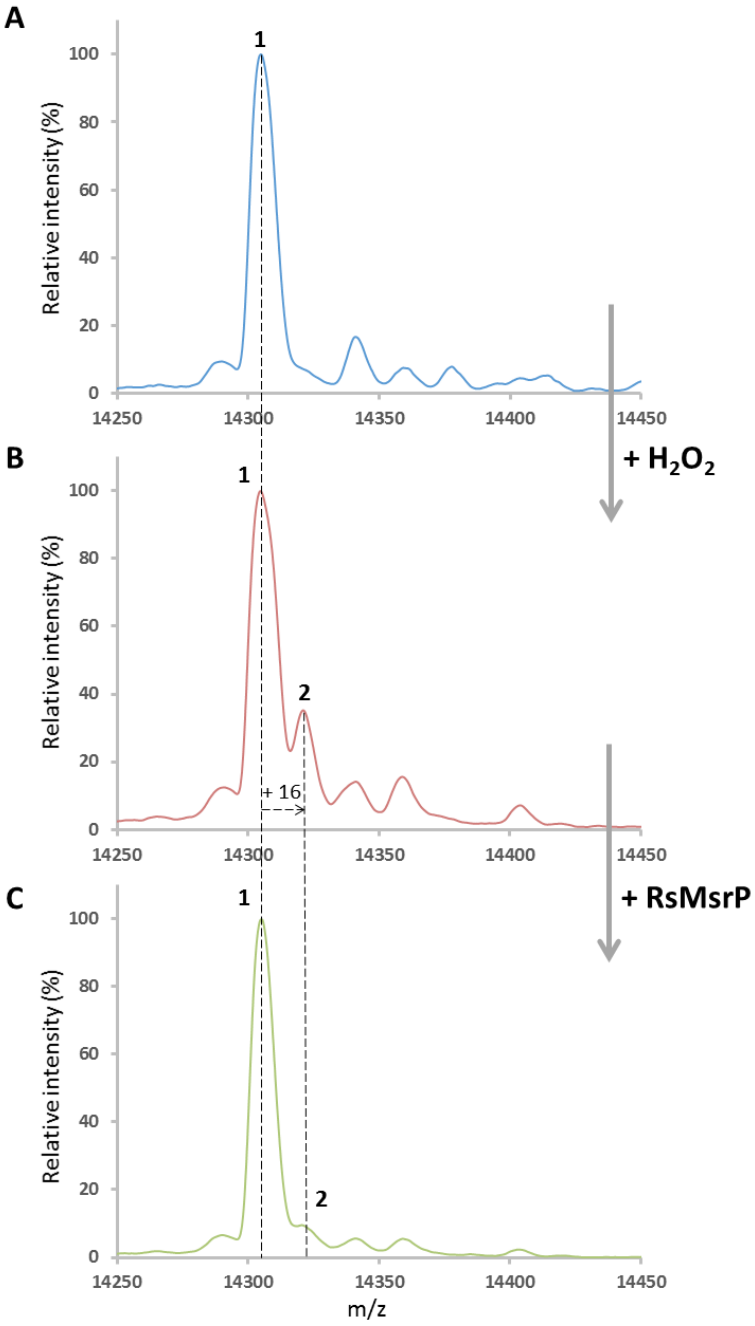

Figure S7. Mass spectrometry analysis of chicken lysozyme non-oxidized (A), oxidized with H_2O_2 (B) and repaired by RsMsrP (C). A) Lysozyme (100 µM) was analyzed by ESI-MS. B) Lysozyme was oxidized with 100 mM H_2O_2 before MS analysis. Note the apparition of a peak of 16 Da increase in mass compared to the non-oxidized lysozyme. C) Oxidized Lysozyme (100 µM) was incubated with RsMsrP (46 nM) in presence of BV (0.8 mM) and sodium dithionite (2 mM) as electron donors. The peak with an increase of 16 Da disappeared due to the reduction by the RsMsrP.

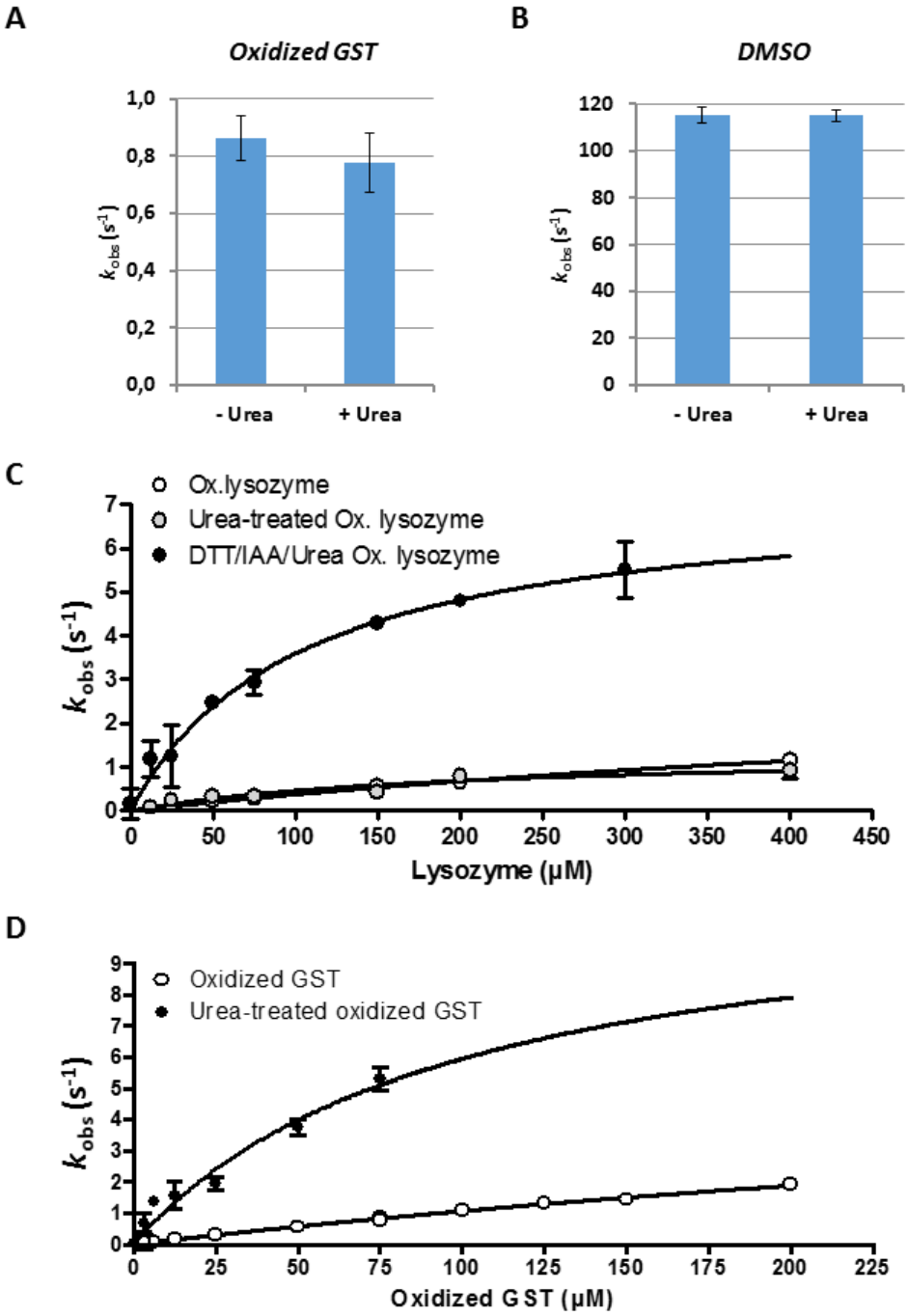

Figure S8. RsMsrP activity with unfolded oxidized proteins. A) RsMsrP activity was determined using 75 µM of oxidized GST as a substrate in the absence or in the presence of 0.5 M urea added extemporaneously. B) Similarly, RsMsrP activity was determined using 0.5 M DMSO as substrate with or without urea. C) Saturation curves of RsMsrP activity using oxidized lysozyme, urea-treated oxidized lysozyme and unfolded oxidized lysozyme (*DTT/IAA/Urea Ox. lysozyme*) as substrate. Oxidized lysozyme was fully unfolded by reducing treatment with 100 mM dithiothreitol (DTT) in 4M urea followed by alkylation with iodoacetamide (IAA). D) Saturation curves of RsMsrP activity using oxidized GST and urea-treated oxidized GST. Data presented are average of three replicates. ± S.D.

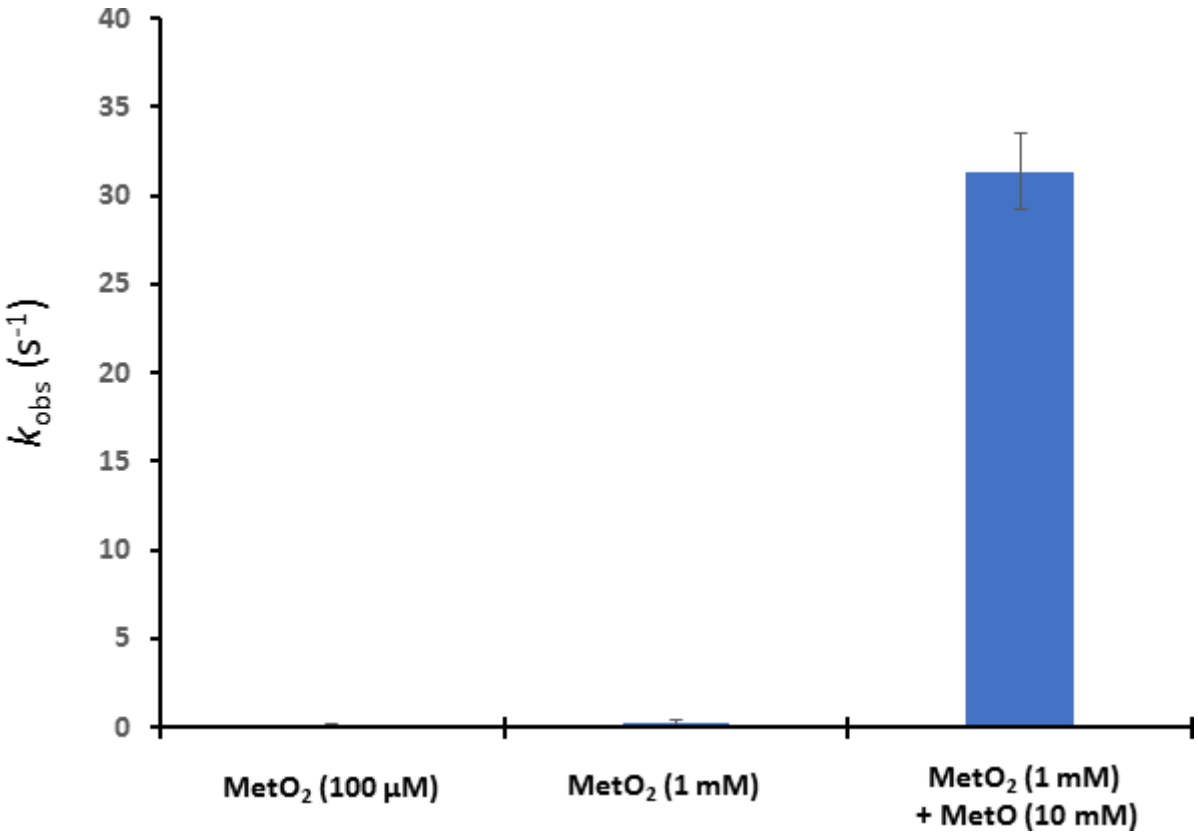

Figure S9. RsMsrP activity assay using Met sulfone as substrate. RsMsrP activity was determined with the highly purified RsMsrP as described in Figure 2. Data presented are average of three replicates. ± S.D.