Materials and methods:

GoxA and GoxB were expressed in *E. coli* Rosetta cells. The cells were transformed with the pET15GoxAB plasmid which contained *goxA*, to which a hexahistidine tag had been added and *goxB* downstream of *goxA* (see Fig. S1). The cells were grown in LB media which also contained ampicillin and chloramphenicol. Cells were grown at 30˚C to an OD of 0.6. At that point 1 mM IPTG was added and after four hours cells were harvested. The harvested cells were sonicated in 50 mM potassium phosphate, pH7.5, and centrifuged. The supernatant was passed through a Ni-NTA affinity column. The column was washed using a gradient of imidazole in the same buffer. Protein eluted over a range of 30-150 mM imidazole. The eluted protein was dialyzed and again applied to the affinity column and the process was repeated using a narrower imidazole gradient to improve purity and to better separate the two fractions.

Glycine oxidase activity was determined using a coupled enzyme assay which had been previously described[1]. The assay was performed under aerobic conditions at 30˚C. In addition to the glycine substrate the assay mixture contained 20 U/ml glutamate dehydrogenase 5 mM 2-oxoglutarate and 0.25 mM NADH. The reaction was initiated by addition of 2 mM glycine. The ammonia generated by the oxidative deamination of glycine (eq 1) was used as a co-substrate by glutamate dehydrogenase (eq 2) leading to the oxidation of NADH which accompanied by a decrease in absorbance at 340 nm (ε_{340} of NADH is 6220 $M^{-1}cm^{-1}$).

$$\text{glycine} + O_2 + H_2O \rightarrow \text{glyoxylate} + NH_3 + H_2O_2 \quad (1)$$

$$\text{2-oxoglutarate} + NH_3 + \text{NADH} \rightarrow \text{glutamate} + H_2O + NAD^+ \quad (2)$$

SDS-PAGE was performed by standard methods 7.5% gels. Gels were stained for protein with EZ-run protein staining solution (Fisher). In order to perform the quinoprotein stain for

detection of a covalent quinone proteins, without staining for protein, electrophoretic transfer of the proteins to a nitrocellulose membrane (0. 2µm pure nitrocellulose membrane, BioRad) was performed. This was done at 120 V for 1h at low temperature to prevent overheating. To stain for the presence of quinoproteins[2], the nitrocellulose membrane was incubated for 45 min in the dark in a solution of 2 M potassium glycinate, pH 10, containing 0.24 mM Nitro Blue Tetrazolium. The quinoproteins will stain. The nitrocellulose membrane was then washed and stored in 0.1 M sodium borate, pH 10.0 until dried.

Size exclusion chromatography was performed using a HiLoad 16/600 Superdex 200 column (GE Healthcare). The buffer used for chromatography was 50 mM potassium phosphate buffer, pH 7.5, containing 150 mM NaCl. The flow rate was 0.6 mL/min. Glutamate dehydrogenase (332 kDa), Methylamine dehydrogenase (124 kDa), MauG (42 kDa) and amicyanin (11.5 kDa) were used as standards. Blue dextran was used to determine the void volume. Gaussian fits of the chromatogram (Prism Graph Pad and Origin) were used to resolve overlapping peaks in the chromatogram and integrate the areas under the peaks.

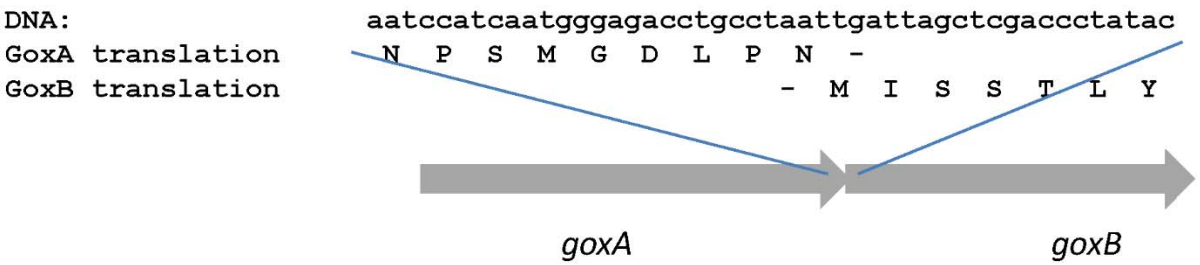

Figure S1. Schematic representation of the *gox* operon from *M. mediterranea* showing the C-terminal end of GoxA and the N-terminal end of GoxB

References

[1] Campillo-Brocal, J. C., Lucas-Elio, P., and Sanchez-Amat, A. (2013) Identification in Marinomonas mediterranea of a novel quinoprotein with glycine oxidase activity, *MicrobiologyOpen 2*, 684-694.

[2] Paz, M. A., Fluckiger, R., Boak, A., Kagan, H. M., and Gallop, P. M. (1991) Specific detection of quinoproteins by redox-cycling staining, *J Biol Chem 266*, 689-692.