Electronic Supplementary Material (ESM)

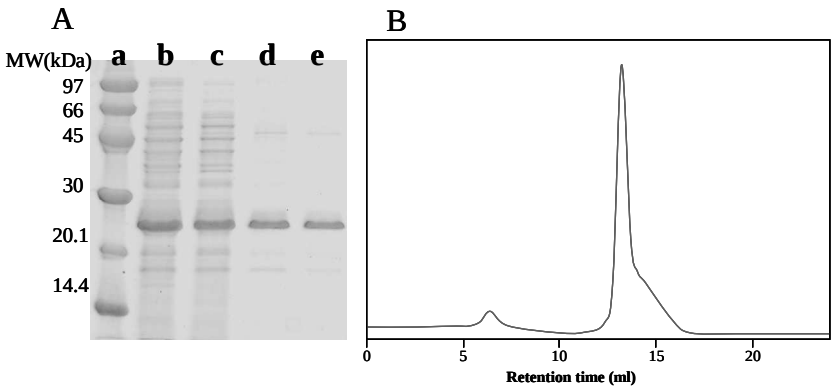

Figure S1 – A- SDS-Page: a – Low Molecular Weight markers (GE healthcare); b – soluble fraction; c – fraction eluted from Q-Sepharose Fast Flow column; d – fraction eluted from HTP column; e – final fraction eluted from Gel Filtration S75 column. B- DRbr elution profile from Superdex200 (HR3.2/300) column; the first peak (small) corresponds to the retention time of the blue dextran, the second peak corresponds to the protein with a retention time of 13.2ml (tetramer, according to the calibration); y-axis corresponds to absorbance at 280nm.

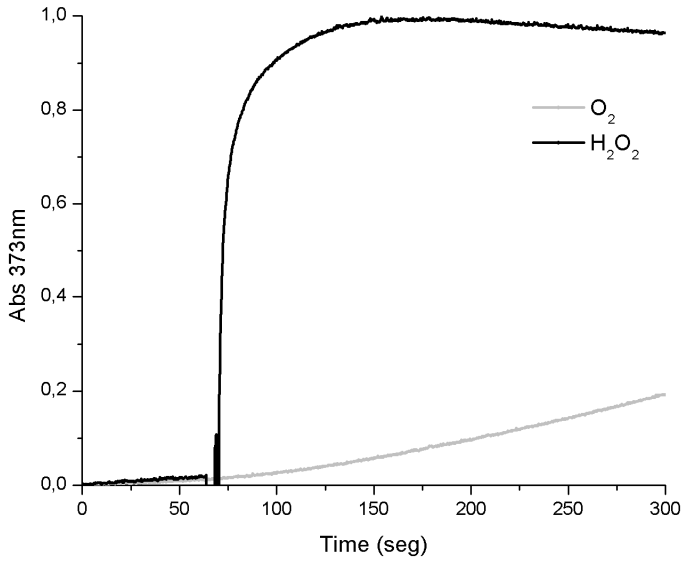

Figure S2 – Oxidation of DRbr by H_2O_2 or O_2 followed by UV/Visible spectroscopy. UV/Visible spectra were recorded at room temperature on a Shimadzu UV 1603 spectrophotometer. The anaerobic protein sample in 20 mM Tris-HCl, pH 7.2, was degassed by flushing with argon and sodium dithionite and H_2O_2 solutions were prepared in the same way. A 50 μM DRbr solution was reduced stepwise by adding sodium dithionite. To the fully reduced DRbr was added H_2O_2 or O_2, in an amount equivalent of the reduced centers. The protein oxidation was followed by UV/Visible spectra and as a function of time at 373 nm.

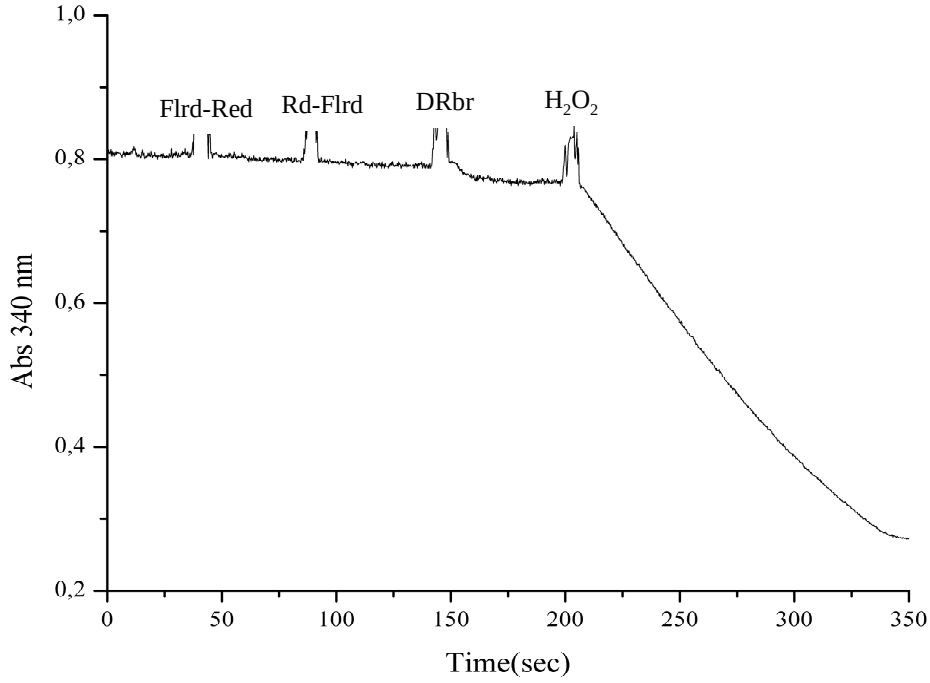

Figure S3 - H_2O_2 reductase activity followed by NADH oxidation at 340 nm; 10 µM of H_2O_2 added to: 500 nM of as isolated DRbr, 2 µM of Flrd-Red, 4 µM of Rd-Flrd and 150 µM of NADH. All solutions were degassed under argon in 50 mM Tris-HCL pH 7.2 at room temperature.

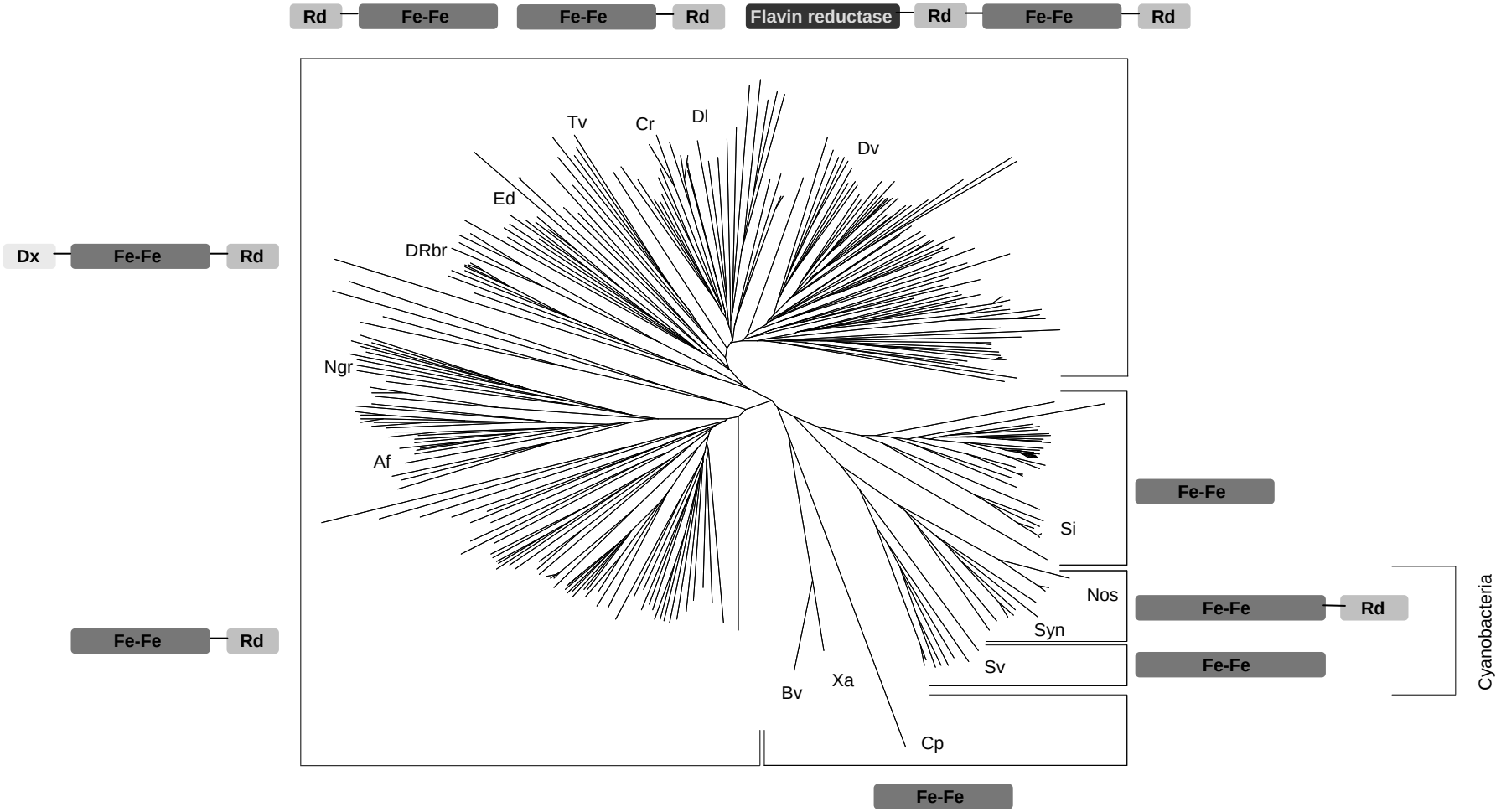

Figure S4. Dendogram of rubrerythrin-like proteins. Sequences were obtained from the NCBI website (http://www.ncbi.nlm.nih.gov/). The highlighted sequences are: DRbr- *C. jejuni subsp. jejuni* NCTC 11168 (gi|218561705|), Af- *Archaeoglobus fulgidus* (gi|11499230|), Bv- *Burkholderia vietnamiensis* G4 (gi|134291384|), Cp- *Cyanophora paradoxa* (gi|1016189|), Cr- *Clostridium ramosum* DSM 1402 (gi|167754543|), Dl- *Dorea longicatena* DSM 13814 (gi|153853488|), Dv- *Desulfovibrio vulgaris subsp. vulgaris* DP4 (gi|120601988|), Ngr- Nigerythrin from *Desulfovibrio vulgaris subsp. vulgaris str. Hildenborough* (gi|46578436|), Ed- *Entamoeba dispar* SAW760 (gi|165904057|), Nos- *Nostoc* sp. PCC 7120 (gi|17228669|, Sv- *Spirillum volutans* (gi|3372506|), Si- *Sulfolobus islandicus* M.16.4 (gi|238382159|), Syn- *Synechococcus sp*. RS9917 (gi|87123471|), Tv- *Trichomonas vaginalis* G3 (gi|154420781|) and Xa- *Xanthobacter autotrophicus* Py2 (gi|154243812|). Bv, Cp and Xa – These sequences are preceded by a twin-arginine signal peptide.