

Supplementary Material

Molecular and biochemical characterisation of a novel type II peroxiredoxin (*XvPrx2*) from the resurrection plant *Xerophyta viscosa*

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Supplement 1

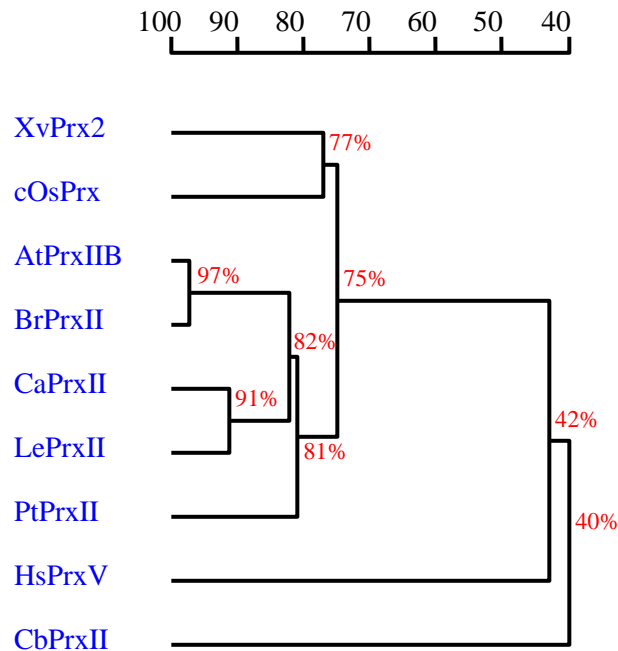


Fig. S1. A homology tree of XvPrx2 and related orthologues, while Fig. S2 and Fig. S3 are maximum parsimony trees showing the evolutionary relationships of type II peroxiredoxin family members and other peroxiredoxins. Table S1 is the reaction format for the DNA protection assay. The structural bioinformatics methodology used to create the *in silico* based model for XvPrx2 is described in Supplement 5.

Supplement 2

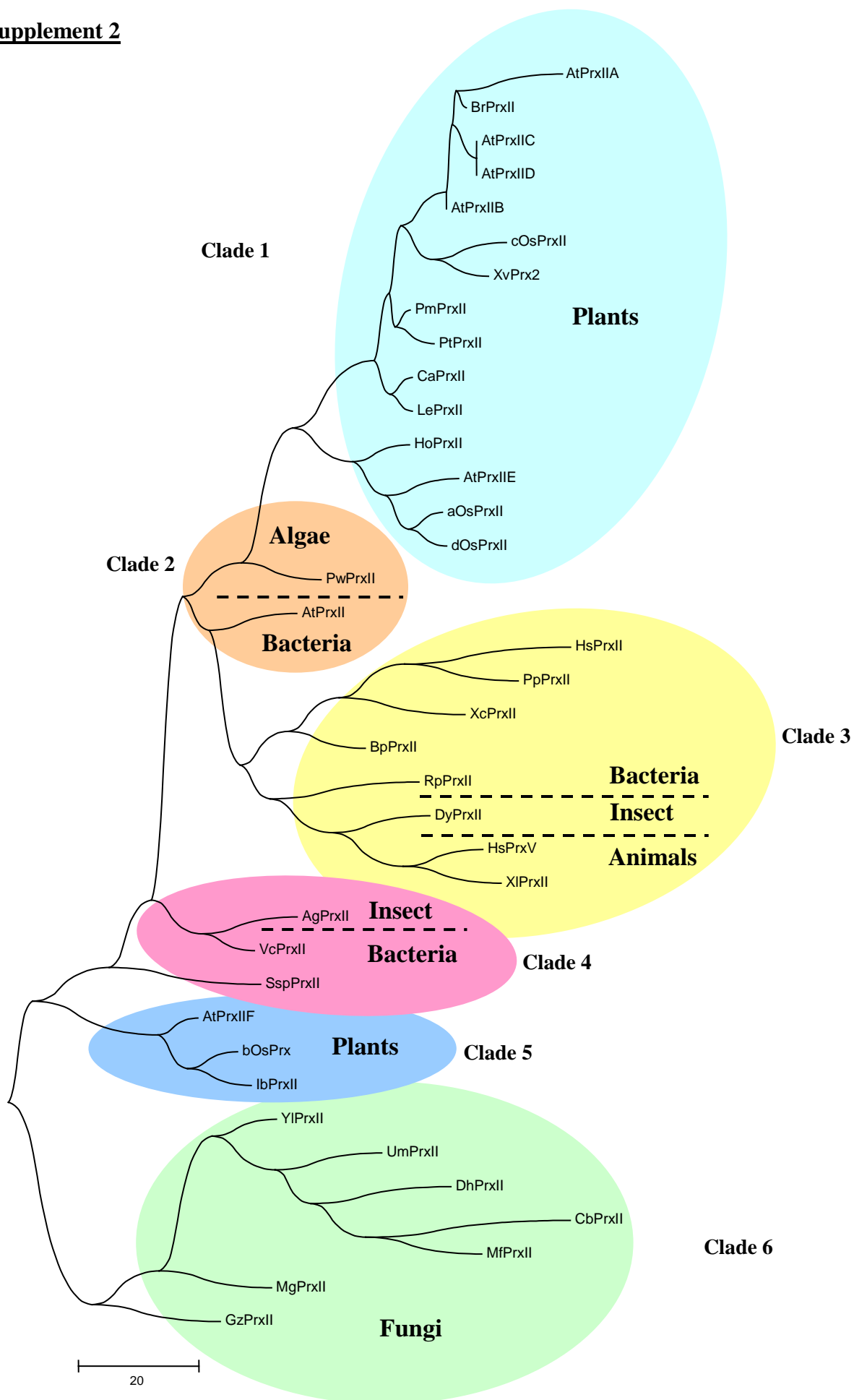


Fig. S2. Maximum parsimony tree inferred from protein sequence data of type II Prxs.

Supplement 3

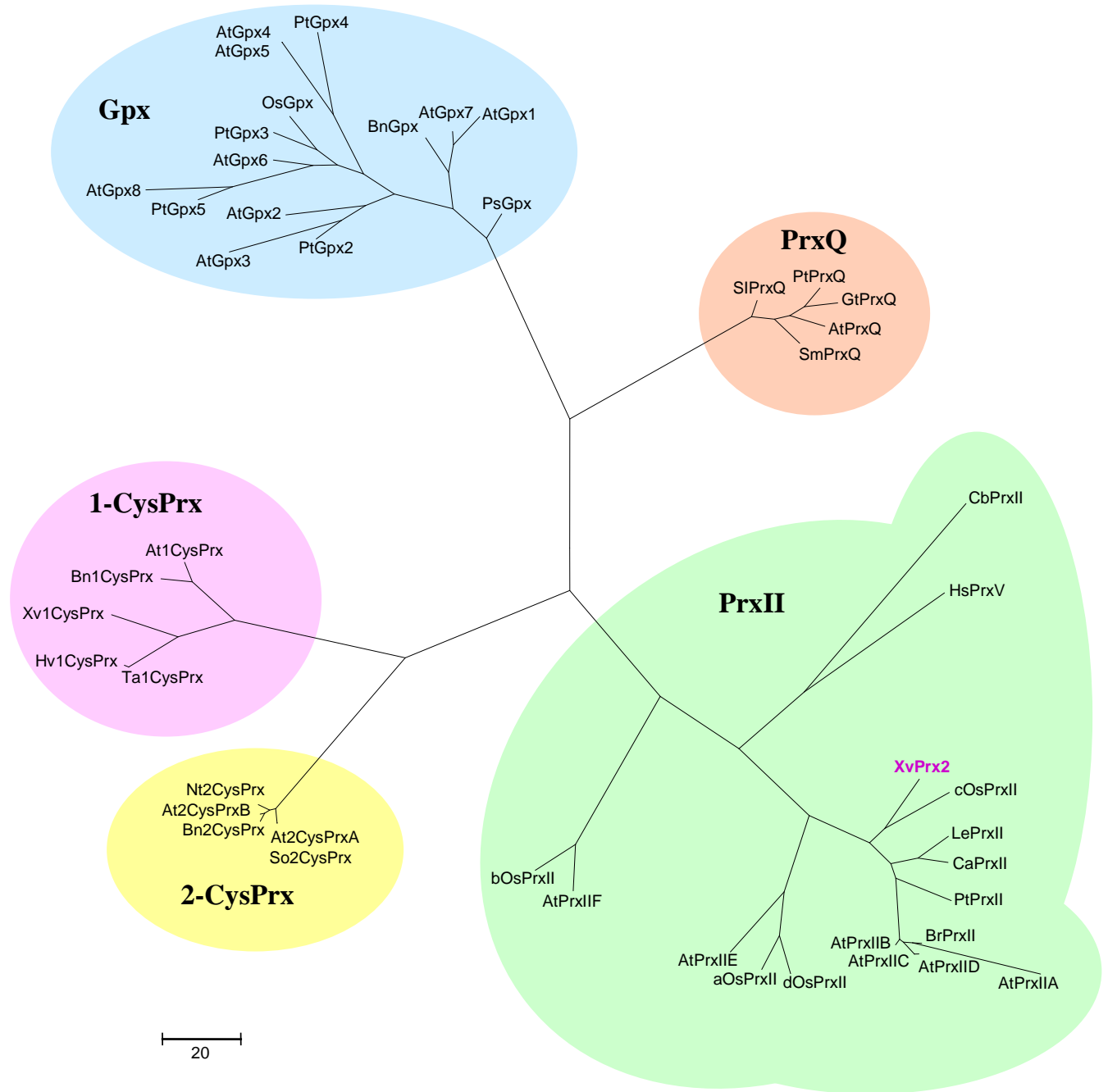


Fig. S3. Maximum parsimony tree of thioredoxin peroxidases.

Supplement 4

Table S1. Eight reactions were prepared for the *in vitro* DNA protection assay

Volumes are displayed in microlitres. The final concentration of protein (XvPrx2 and XvV76C) used was 20 μ M with χ referring to the volume

Component	1	2	3	4	5	6	7	8
DTT (50 mM)	0	1	0	0	0	0	0	0
FeCl ₃ (0.5 mM)	0	0	1	0	0	0	0	0
FeCl ₃ -DTT mix	0	0	0	8	8	8	8	8
XvPrx2	0	0	0	χ	0	0	0	0
XvV76C	0	0	0	0	χ	0	0	0
Plasmid DNA (pBSK)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
BSA (2 μ g/ml)	0	0	0	0	0	0	8	16
H ₂ O (make up to 50 μ l)								

Individual reactions were set up in a total volume of 50 μ l in an Eppendorf tube (Table S1). Tubes 1–3 were the three controls used in the experiment. A FeCl₃-DTT mix was prepared by the addition of 10 μ l of 0.5 M FeCl₃ and 10 μ l of 50 mM DTT to 60 μ l H₂O. The mix was incubated for 30 min at room temperature (RT) to generate ROS. Eight microlitres of the FeCl₃-DTT mix was added to tubes 4-8 (Table S1). The final concentrations of DTT and FeCl₃ used in each reaction were 1 mM and 10 μ M, respectively. Reaction mixes were incubated for 5 h at RT and thereafter electrophoresed on a 1% agarose/EtBr gel.

Supplement 5

Structural bioinformatics

Sequences homologous to the query sequence were determined. Only those proteins that shared a high degree of similarity (> 30%) were used as templates for protein structure determination. The framework was constructed by aligning the query sequence against the best templates. Non-conserved loops and side chains were added and the backbone was completed. The model was refined by energy minimisation, which removes unfavourable non-bonded contacts and optimises bond geometry.

Modelling of XvPrx2 and XvV76C

The similarity of XvPrx2 and XvV76C protein sequences to other known Prx sequences were determined using FUGUE (Shi *et al.* 2001). Multiple 3-D models of both the XvPrx2 and XvV76C proteins were built using MODELLER (Sali and Blundell 1993) using the X-ray structure of the PtPrxII molecule as template. The model with a combination of lowest energy and lowest number of restraint violations was selected for evaluation.

Acquisition and alignment of homologous sequences

Sequences of Prxs of known structure available in the Brookhaven protein data bank (Bernstein *et al.* 1977) were aligned on the basis of structural features such as solvent accessibility, secondary structure and side chain-main chain hydrogen bonding patterns using COMPARE (Sali and Blundell 1990; Zhu *et al.* 1992). Sequences of XvPrx2 and XvV76C were aligned by matching structural templates derived from aligned Prx structures.

Energy minimisation and model validation

The models were energy minimised in SYBYL using the AMBER force-field (Weiner *et al.* 1984). During the initial cycles of energy minimisation the backbone was kept rigid and only side chains were moved. Subsequently, all atoms in the structure were allowed to move during minimisation. This approach kept disturbance of the backbone structure to a minimum. Energy minimisation was performed till all short contacts and inconsistencies in geometry were rectified. During the initial stages of minimisation, the electrostatic term was not included as the main objective was to relieve steric clashes and to rectify bad geometry. The electrostatic term was

invoked only at an advanced stage of minimisation. Model evaluation was performed using ProsaII (Sippl 1993), which uses Ca and Cb atom based potentials to calculate energy profiles for protein structures. The PROCHECK software (Laskowski *et al.* 1993) was used to evaluate stereo-chemical quality of the final models. Figures for visual analysis of the models were generated using PyMol (www.pymol.org).

Structure validation of XvPrx2 and XvV76C

The structure of XvPrx2 was validated using a Ramachandran plot. Based on this plot the model appeared to be a good fit as 96.9% of the residues were observed to lie within the most favoured regions of the Ramachandran plot, with 3.1% of the residues in the allowed region and 0% of the residues in the outlier region. The XvV76C model displayed a similar result.

References

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