SUPPLEMENTAL DATA

LEGENDS FOR S.D. FIGURES

S.D. FIGURE 1. **Double integration (area, *S*) of the normalized radical signal at *g* = 2.0049 versus microwave power (*P*) (in milliwatts)**. EPR spectra have been recorded at 20 K; v 9.387 GHz; modulation amplitude 0.2 mT; modulation frequency 100 kHz; and microwave power values 0.02, 0.2, 2, 20, 63, and 100 mW.

S.D. FIGURE 2. **Molecular structure of the tyrosyl radical with atom numbering.** The large outer numbers are the p_z-spin densities at the respective carbon and oxygen nuclei.

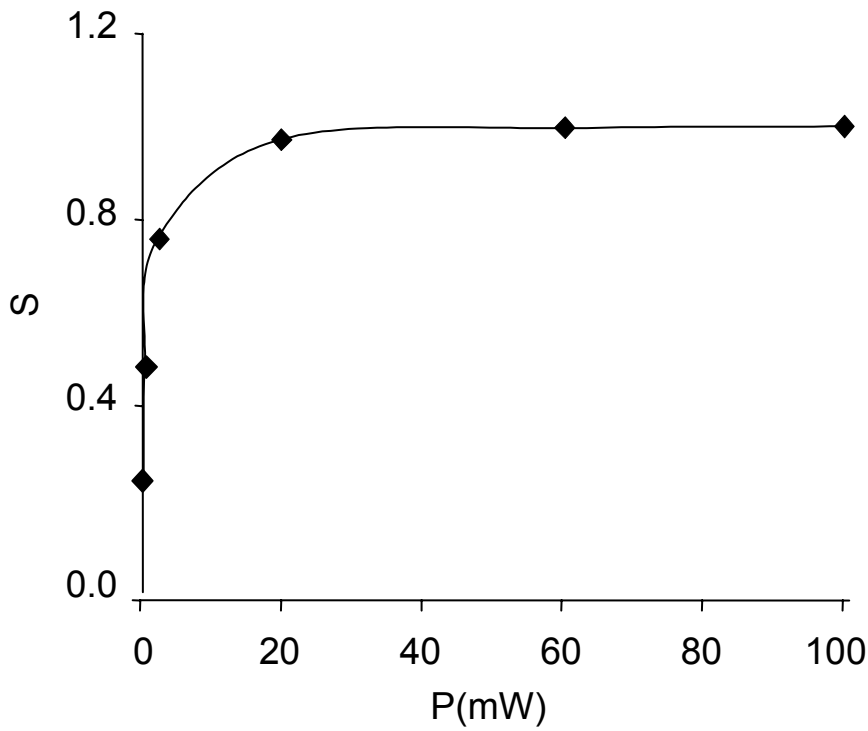
S.D. FIGURE 3. **EPR spectra of: A) W164S, B) W164H; and C) HRP** (recorded at 9 K, 20 K and 40 K).

S.D. FIGURE 4. **Kinetics of reduction of VPI and VPII of native VP and its directed variants by RB5.** Stopped-flow reactions were carried out at 25 °C using 1 μM enzyme, 0.1 M tartrate (pH 3.5), and varying concentrations of RB5. The insets show kinetic curves at a smaller vertical scale. Means and 95% confidence limits of replicate assays are shown. The symbols are as follows: Native VP (●), W164Y (■), W164H (♦), W164S (▲), W164Y/R257A/A260F (□) and R257A/A260F (VPI reduction measured at 10ºC, and 25ºC estimation presented) (○).

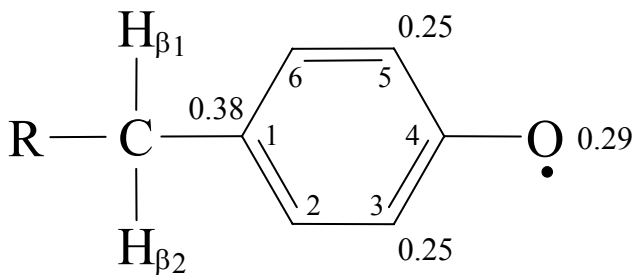
S.D. FIGURE 5. **Revised VP catalytic cycle**. The scheme shows: **i)** Basic cycle involved in Mn^{2+} oxidation including two-electron oxidation of the resting peroxidase (VP, containing Fe^{3+}) by hydroperoxide to yield VPI_A (containing Fe^{4+}-oxo and porphyrin cation radical), whose reduction in two one-electron steps results in the intermediate $VPII_A$ (containing Fe^{4+}-oxo after porphyrin reduction) and then the resting form of the enzyme; and **ii)** Extended cycle including also VPI_B (containing Fe^{4+}-oxo and tryptophan radical) and $VPII_B$ (containing Fe^{3+} and tryptophan radical) involved in oxidation of VA. Some low redox-potential phenols (like *p*-hydroquinone) and dyes (like ABTS) are probably oxidized by both the A and B forms, but they are not included for simplicity. Adapted from Pérez-Boada et al. (9).

S.D. TABLE 1
Transient-state kinetics for formation of VPI (k_{1app}) of native enzyme and site-directed variants by H_2O_2:
Apparent second-order rate constants ($M^{-1} \cdot s^{-1}$). Means and 95% confidence limits.

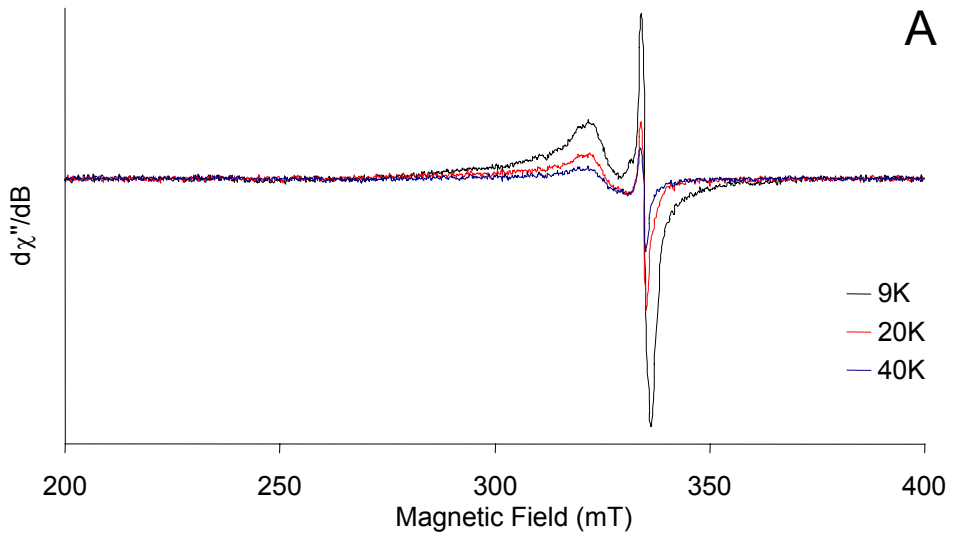
	k_{1app}
VP	$(3.46 \pm 0.07) \times 10^6$
W164Y	$(4.24 \pm 0.05) \times 10^6$
W164H	$(3.77 \pm 0.01) \times 10^6$
W164S	$(3.84 \pm 0.06) \times 10^6$
W164Y/R257A/A260F	$(3.92 \pm 0.05) \times 10^6$
R257A/A260F	$(3.30 \pm 0.15) \times 10^6$

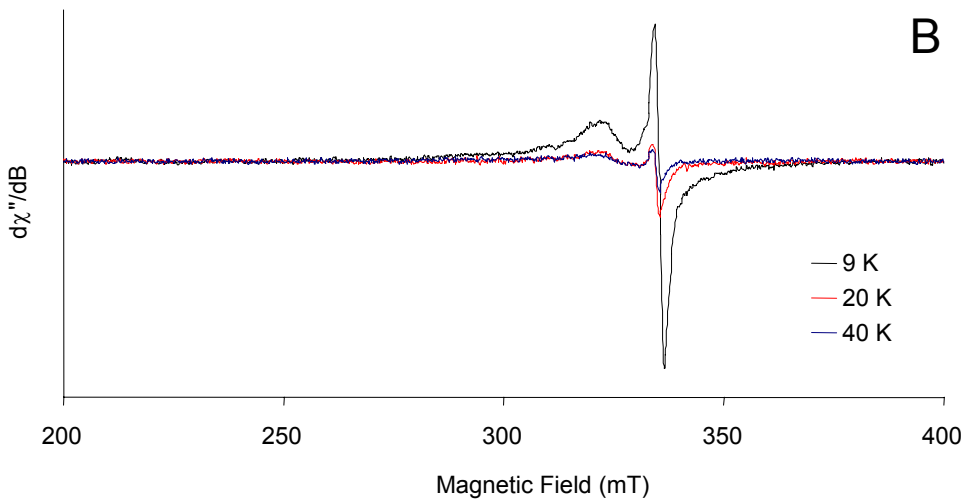


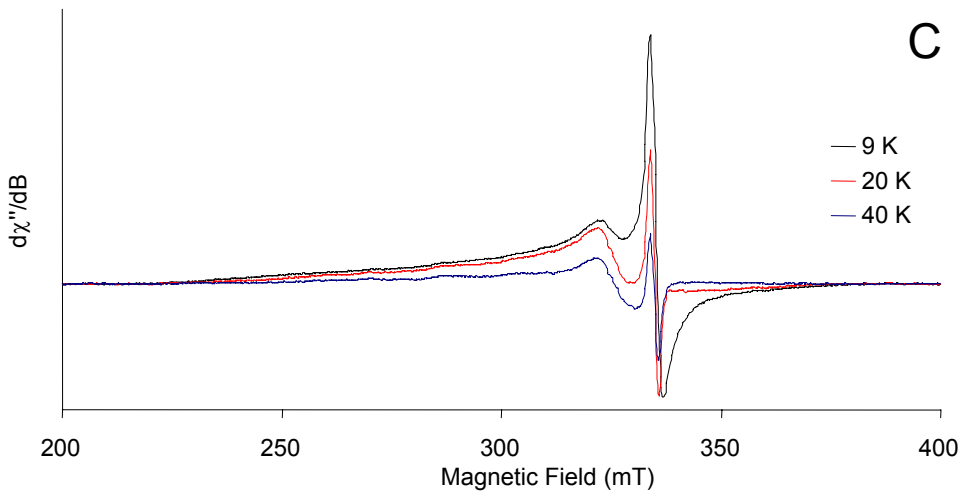
S.D. Fig. 1



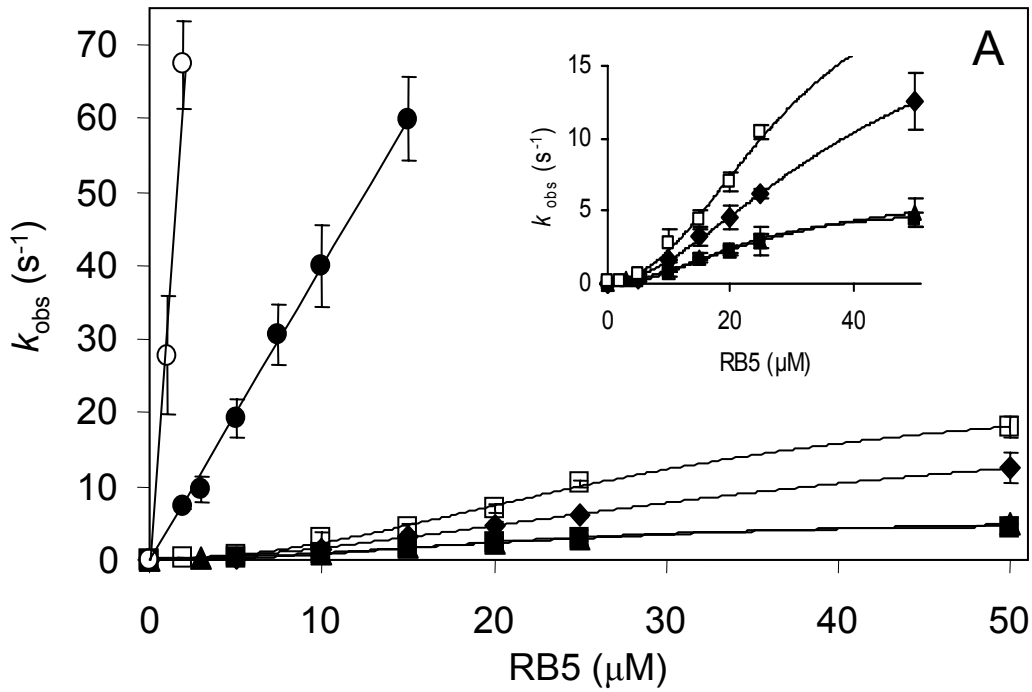
S.D. Fig. 2

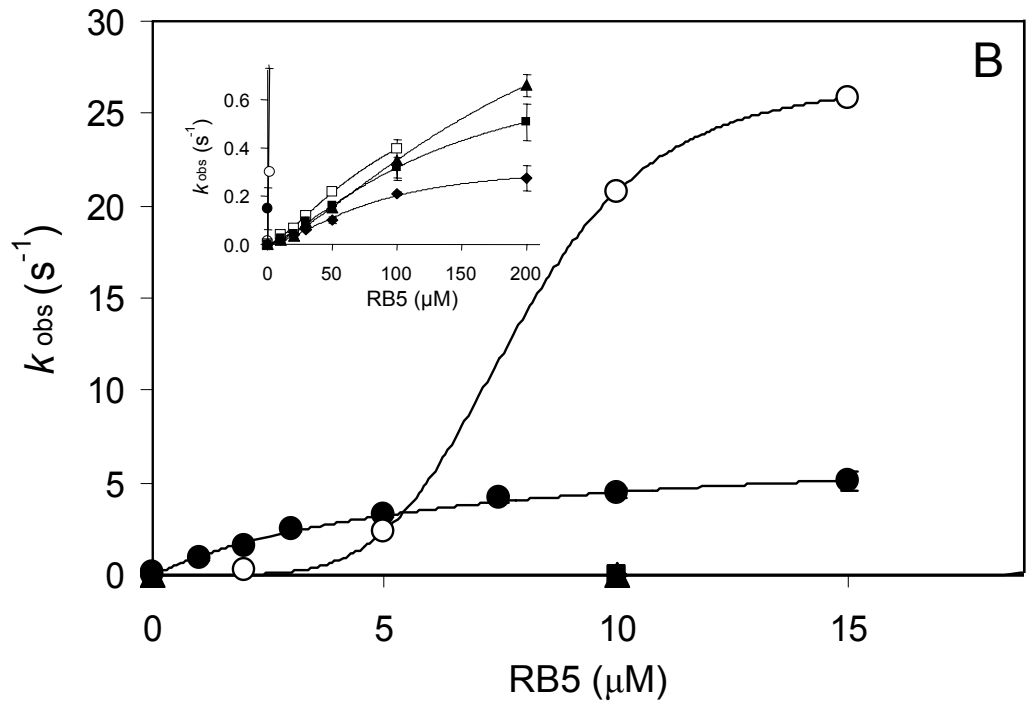

A
dχ"/dB
9K
20K
40K
200
250
300
350
400
Magnetic Field (mT)


B
dχ"/dB
9 K
20 K
40 K
200
250
300
350
400
Magnetic Field (mT)

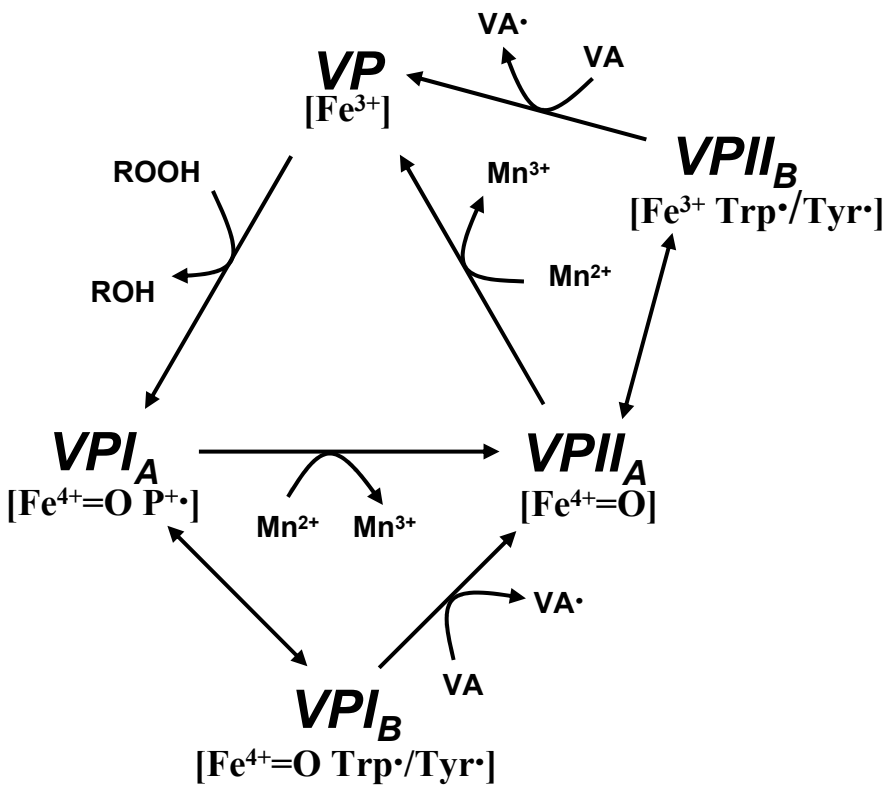

C
dχ"/dB
9 K
20 K
40 K
200
250
300
350
400
Magnetic Field (mT)

S.D. Fig. 3


A
k_{obs} (s^{-1})
RB5 (μM)
0
10
20
30
40
50
60
70
0
10
20
30
40
50
k_{obs} (s^{-1})
RB5 (μM)
0
5
10
15
0
20
40


B
k_{obs} (s^{-1})
RB5 (μM)
0
5
10
15
20
25
30
0
5
10
15
k_{obs} (s^{-1})
RB5 (μM)
0.0
0.2
0.4
0.6
0
50
100
150
200

S.D. Fig. 4


VA·
VA
VP
[Fe3+]
VPIIB
[Fe3+ Trp·/Tyr·]
ROOH
ROH
Mn3+
Mn2+
VPIA
[Fe4+=O P+·]
VPIIA
[Fe4+=O]
Mn2+
Mn3+
VA·
VA
VPIB
[Fe4+=O Trp·/Tyr·]

S.D. Fig. 5