

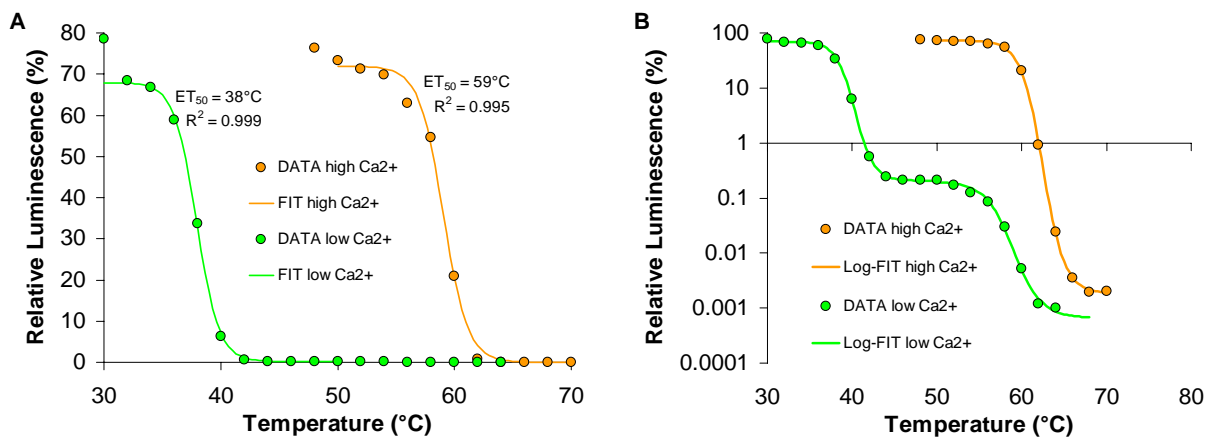
Supplemental material to:

Christoph Plieth and Sonja Vollbehr. Calcium promotes activity and confers heat stability on plant peroxidases. *Plant Signaling & Behavior* 2012; 7(6);

DOI:10.4161/psb.7.6.20065;

<http://www.landesbioscience.com/journals/psb/article/20065>

## Supplemental data

Supplemental Figure S1 Heat sensitivity of LUPOs from *Arabidopsis*

**A:** Peroxidases from *Arabidopsis* dissolved either in TriCaT (high [Ca<sup>2+</sup>]) or in TriEDTAT (low [Ca<sup>2+</sup>]) were treated with the temperatures indicated before light yield of the H<sub>2</sub>O<sub>2</sub>-luminol reaction was measured and the ET<sub>50</sub> calculated. **B:** Data from A are plotted on a log scale to show that a residual activity due to residual Ca<sup>2+</sup> remained after heat treatment at low [Ca<sup>2+</sup>] (green curve) which is inactivated at higher temperatures. Data given are means of n = 3. The relative luminescence refers to an untreated sample kept at RT (= 20°C). R<sup>2</sup> are correlation coefficients obtained from curve fitting. Errorbars represent StDv or are below symbol size.

**Supplemental Table S1 The heat stability of peroxidases from different plant species**

The effective temperatures leading to 50% irreversible LUPO inhibition ( $ET_{50}$ ) of different plants species under low and high  $Ca^{2+}$  conditions. 'High [ $Ca^{2+}$ ]' designates buffer conditions with a minimum of 1 mM free  $Ca^{2+}$  during heating. 'Low [ $Ca^{2+}$ ]' designates buffer conditions with free  $Ca^{2+}$  below 0.1  $\mu$ M during heating. The LUPO activity assays were performed for all samples at RT and with high [ $Ca^{2+}$ ]. For *Arabidopsis* HMW designates high-molecular-weight peroxidases and LMW is low-molecular-weight (compare gel filtration **Fig. 7B**). The means are of at least three independent replicates, where standard deviations are given in parentheses. 'n.d.' is 'not determined' These data are depicted in **Fig. 5** ordered by  $ET_{50}$ .

Popular name	Scientific species name	Race / Variety sub-species	$ET_{50}$ @ low [ $Ca^{2+}$ ] <sub>free</sub> (°C)	$ET_{50}$ @ high [ $Ca^{2+}$ ] <sub>free</sub> (°C)
<u>Dicots</u>				
Garden cress	<i>Lepidium sativum</i>	"Einfache Grüne"	36.3 ( $\pm$ 0.3)	57.5 ( $\pm$ 0.4)
Cotton	<i>Gossypium herbaceum</i>	Levant cotton	39.4	54.2 ( $\pm$ 4.3)
Sunflower	<i>Helianthus annuus</i>		43.7 ( $\pm$ 4.6)	47.0 ( $\pm$ 2.1)
Thale cress	<i>Arabidopsis thaliana</i>	Columbia (Col-0)	38.9 ( $\pm$ 0.9)	58.3 ( $\pm$ 1.0) HMW 62.2 ( $\pm$ 0.6) LMW
Commercial Horseradish Peroxidase Sigma #P6140 from <i>Armoracia rusticana</i>			46.0 ( $\pm$ 0.5)	68.3 ( $\pm$ 1.8)
<u>Monocots</u>				
Millet	<i>Sorghum bicolor</i>	Grain sorghum	37.9	46.8
Wheat	<i>Triticum aestivum</i>		37.7	59.9 ( $\pm$ 4.4)
Barley	<i>Hordeum vulgare</i>	"Popp-Gerste"	39.8	53.7
Maize	<i>Zea mays</i>	"Popcorn"	46.8	48.6 68.9 ( $\pm$ 3.1)
<u>Cryptogams</u>				
Stonewort	<i>Chara corallina</i>	<i>australis</i>	53.6 ( $\pm$ 4.8)	51.6 ( $\pm$ 3.2)
Toothed wrack	<i>Fucus serratus</i>	Scottish North sea	51.2 ( $\pm$ 3.3)	49.9 ( $\pm$ 2.7)
Sea belt	<i>Saccharina latissima</i>	West Baltic	56.3 ( $\pm$ 2.6)	46.0 ( $\pm$ 4.6)
Red dulse	<i>Palmaria palmata</i>	Norman-Atlantic	50.6 ( $\pm$ 1.9)	48.2 ( $\pm$ 2.1)
Commercial Fungal Lignin Peroxidase Sigma #42603 from <i>Phanerochaete chrysosporium</i>			64.1	64.9