

**The Plant Cysteine Oxidases from *Arabidopsis thaliana* are kinetically tailored to act as oxygen sensors**

Mark D. White\*, Jos J. A. G. Kamps, Samuel East, Leah J. Taylor Kearney, and Emily Flashman\*

Address: Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, United Kingdom

Running title: Plant Cysteine Oxidase oxygen kinetics

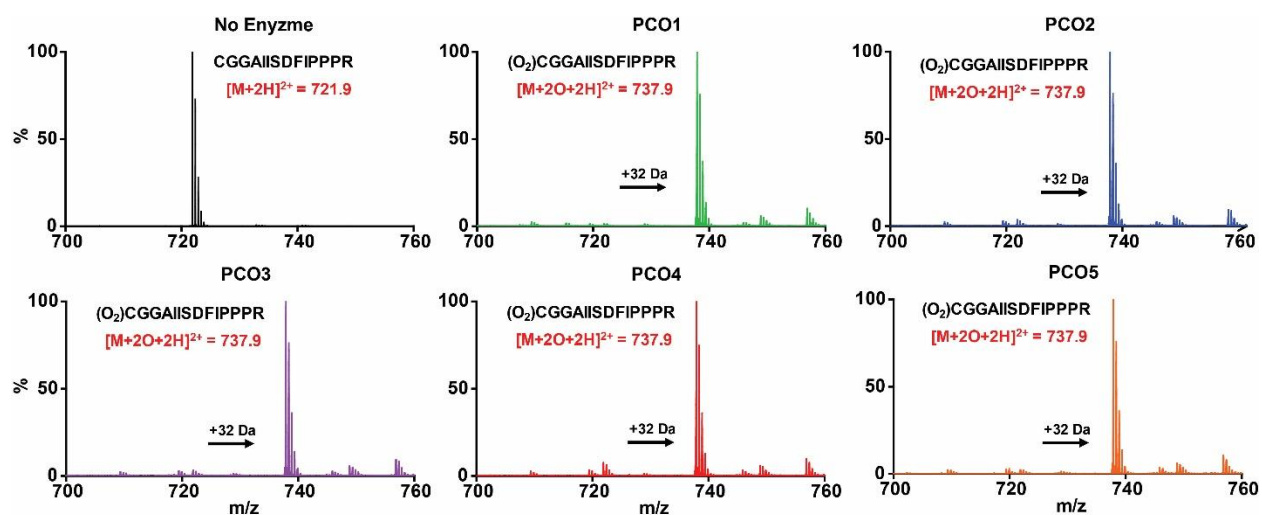
**Supplementary Information**

**Supplementary Tables**

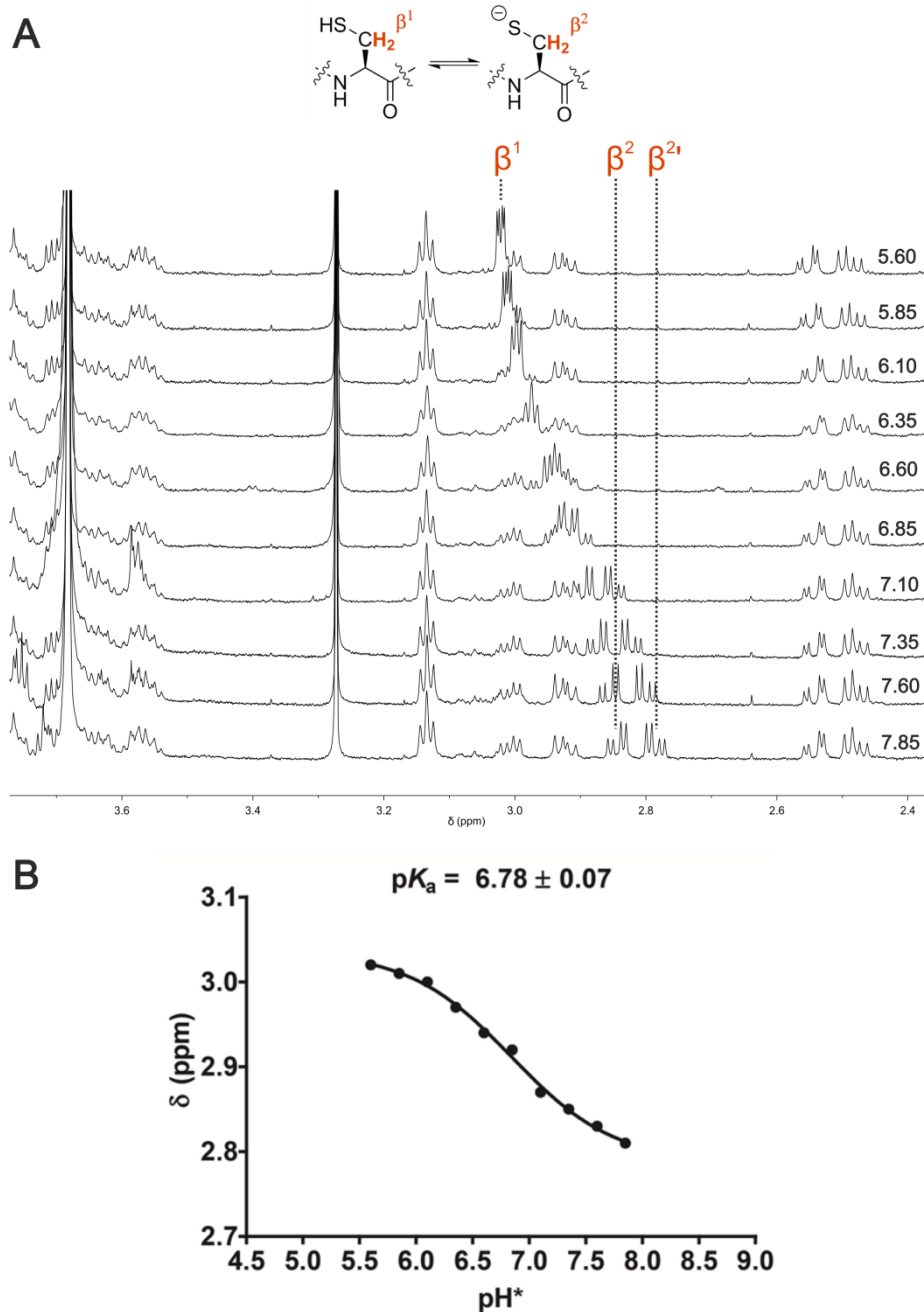
<b>PCO</b>	<b>HRE1 (<math>\mu\text{mol min}^{-1} \text{mg}^{-1}</math>)</b>	<b>HRE2 (<math>\mu\text{mol min}^{-1} \text{mg}^{-1}</math>)</b>	<b>RAP2,3 (<math>\mu\text{mol min}^{-1} \text{mg}^{-1}</math>)</b>	<b>RAP2,2/2,12 (<math>\mu\text{mol min}^{-1} \text{mg}^{-1}</math>)</b>
<b>1</b>	<b>0.95 ± 0.04</b>	<b>2.77 ± 0.12</b>	<b>1.30 ± 0.07</b>	<b>2.01 ± 0.13</b>
<b>2</b>	<b>0.33 ± 0.01</b>	<b>1.40 ± 0.07</b>	<b>0.47 ± 0.02</b>	<b>1.45 ± 0.04</b>
<b>3</b>	<b>0.63 ± 0.04</b>	<b>1.89 ± 0.12</b>	<b>1.07 ± 0.10</b>	<b>1.04 ± 0.05</b>
<b>4</b>	<b>3.52 ± 0.34</b>	<b>2.48 ± 0.32</b>	<b>3.73 ± 0.36</b>	<b>3.07 ± 0.27</b>
<b>5</b>	<b>4.20 ± 0.17</b>	<b>2.25 ± 0.10</b>	<b>2.77 ± 0.12</b>	<b>2.52 ± 0.08</b>

**Table S1 – The specific activities of AtPCO 1 to 5 with different AtERF-VII substrates:** The specific activities of each AtPCO isoform with different AtERF-VII peptides, calculated from a competition assay where all substrates were pooled at a concentration equal to the relative  $K_m$  for RAP2<sub>2-15</sub>. Values were normalized to the substrate that generated the greatest activity for individual AtPCOs to produce Figure 6A.

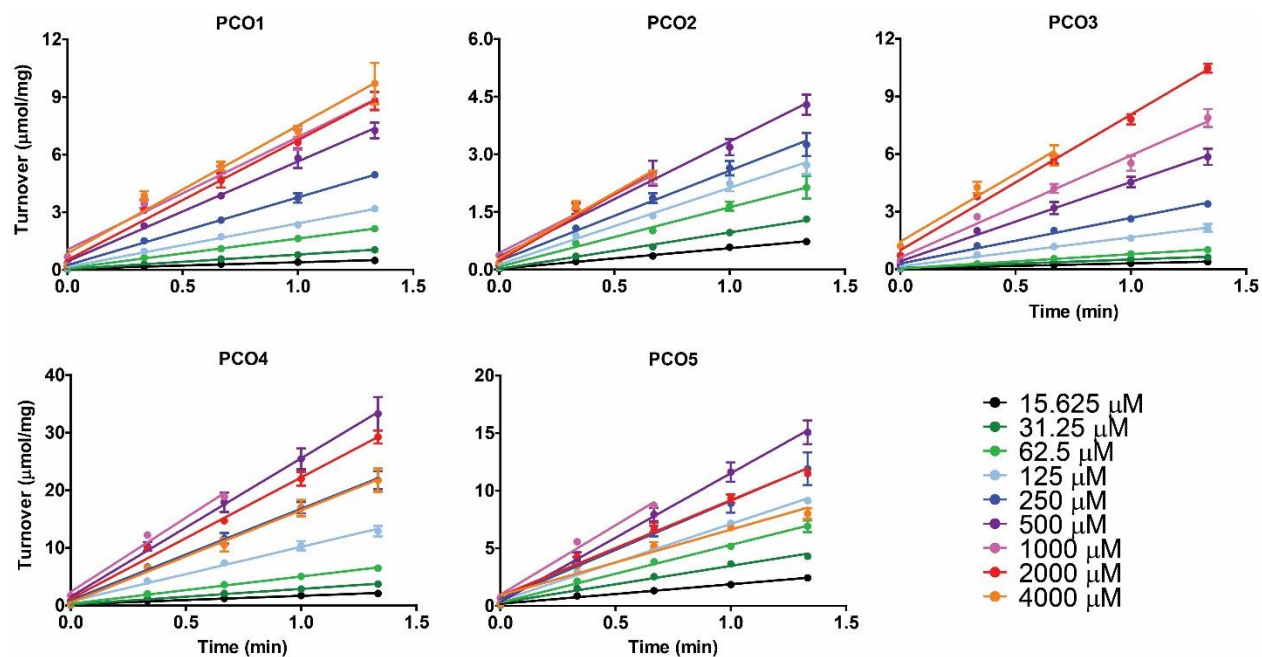
## Supplementary Figures



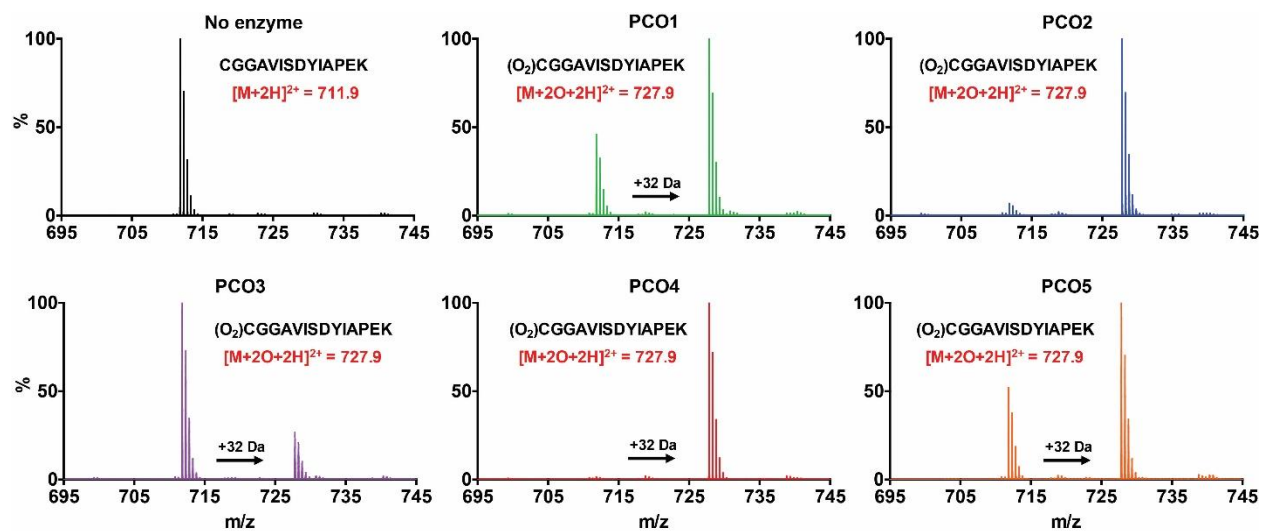
**Figure S1 - AtPCOs 1 to 5 catalyze dioxygenation of AtRAP<sub>2-15</sub>:** LC-MS spectra demonstrating the AtRAP<sub>2-15</sub> mass species detected following 30 minute incubation with and without AtPCO 1 to 5 under aerobic conditions at 25 °C. An enzyme-dependent mass increase of +32 Da, consistent with Cys-sulfinic acid formation (1), was observed in all cases. 50 mM HEPES, 50 mM NaCl and 1 mM TCEP pH 7.5 was used as buffer.



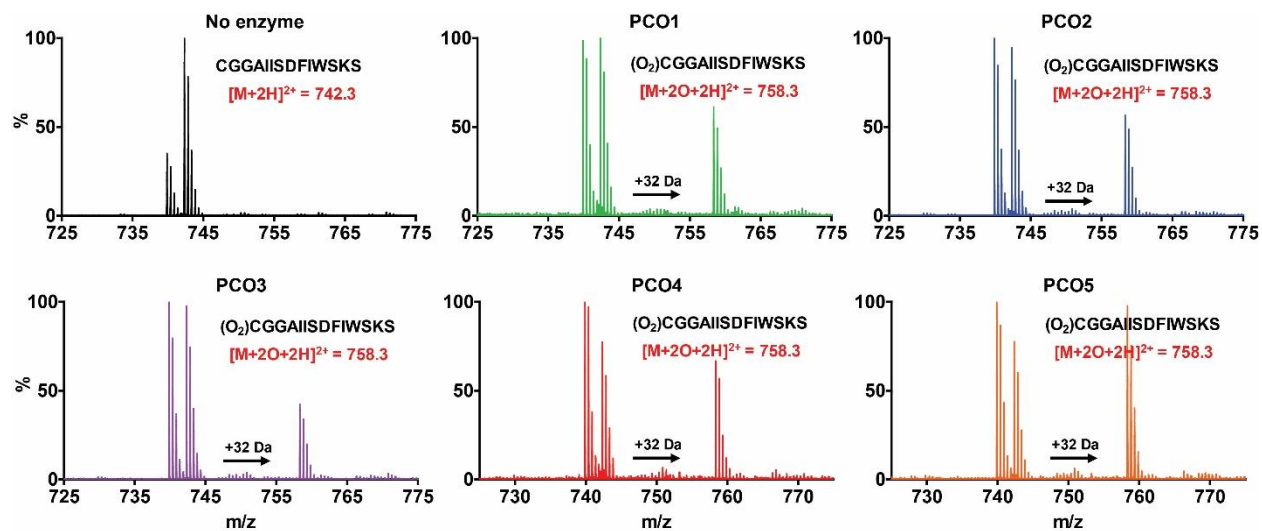
**Figure S2 -  $^1\text{H}$  NMR analysis of AtRAP<sub>2-15</sub>:** **A** Stacked  $^1\text{H}$  NMR spectra of AtRAP<sub>2-15</sub> peptide (223  $\mu\text{M}$ ) in  $\text{D}_2\text{O}$  at different  $\text{pH}^*$  (5.60 to 7.85), using 50 mM  $\text{NaH}_2\text{PO}_4$  as buffer. Chemical shifts following the cysteine  $\beta$ -protons (consistent with deprotonation of the N-terminal thiol group, in the case of the deprotonated diastereotopic  $\beta$ -protons, pH 6.8 and above, the average of the chemical shift is used) were observed at higher  $\text{pH}^*$  which allowed the  $pK_a$  of the cysteine side group in  $\text{H}_2\text{O}$  to be estimated (Figure 3B) using the equation,  $pK^H = 0.929pK^{H^*} + 0.42$ , given in (2); **B** plotted  $\text{pH}$  versus chemical shift  $\delta$  (ppm).



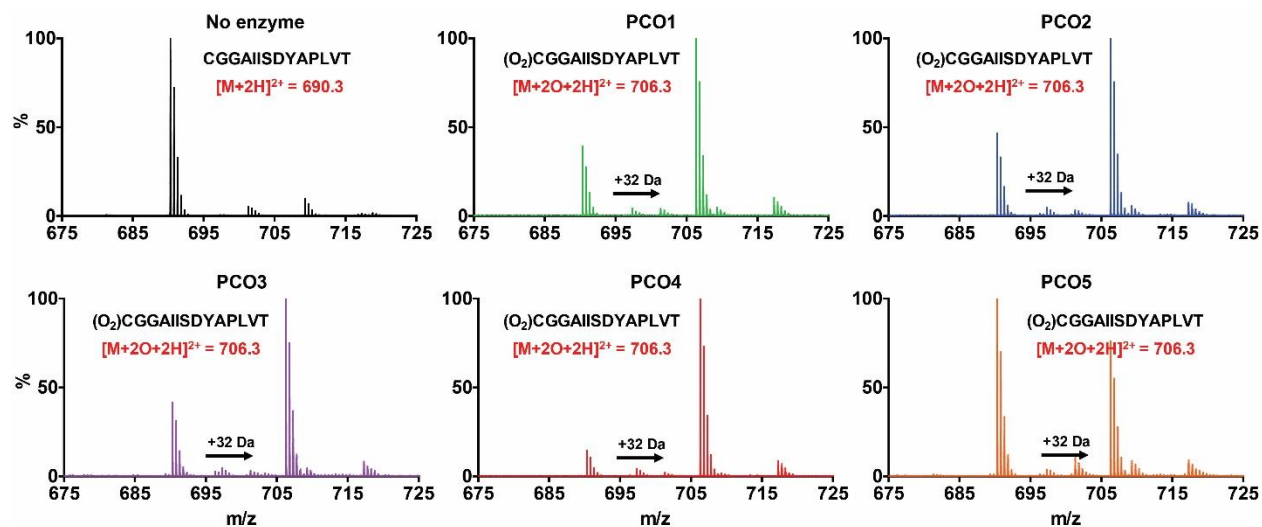
**Figure S3 – Initial rate data for AtPCOs 1 to 5 with different concentrations of AtRAP<sub>2-15</sub>:** Rate profiles for AtPCOs 1 to 5 with different concentrations of AtRAP<sub>2-15</sub>, indexed in the figure. Assays were conducted under aerobic conditions at 25 °C, using 50 mM bis-tris propane, 50 mM NaCl and 5 mM TCEP pH 8.0 as buffer and analyzed by LC-MS. This data was used to produce the Michaelis-Menten kinetic plots shown in Figure 4. Standard error is shown (n=3).



**Figure S4 - AtPCOs 1 to 5 catalyze dioxygenation of AtHRE<sub>12-15</sub>:** LC-MS spectra demonstrating the AtHRE<sub>12-15</sub> mass species detected following 30 minute incubation with and without AtPCO 1 to 5 under aerobic conditions at 25 °C. An enzyme-dependent mass increase of 32 Da, consistent with Cys-sulfinic acid formation (1), was observed in all cases. 50 mM HEPES, 50 mM NaCl and 1 mM TCEP pH 7.5 was used as buffer.



**Figure S5 - AtPCOs 1 to 5 catalyze dioxygenation of AtHRE<sub>2-15</sub>:** LC-MS spectra demonstrating the RAP<sub>2-15</sub> mass species detected following 30 minute incubation with and without AtPCO 1 to 5 under aerobic conditions at 25 °C. An enzyme-dependent mass increase of 32 Da, consistent with Cys-sulfinic acid formation (1), was observed in all cases. A contaminating peak of 739.9 Da was observed in all spectra, which could not be identified. 50 mM HEPES, 50 mM NaCl and 1 mM TCEP pH 7.5 was used as buffer.



**Figure S6 - AtPCOs 1 to 5 catalyze dioxygenation of AtRAP2.3<sub>2-15</sub>:** LC-MS spectra demonstrating the AtRAP2.3<sub>2-15</sub> mass species detected following 30 minute incubation with and without AtPCO 1 to 5 under aerobic conditions at 25 °C. An enzyme-dependent mass increase of 32 Da, consistent with Cys-sulfinic acid formation (1), was observed in all cases. 50 mM HEPES, 50 mM NaCl and 1 mM TCEP pH 7.5 was used as buffer.

## References

1. White, M. D., Klecker, M., Hopkinson, R. J., Weits, D. A., Mueller, C., Naumann, C., O'Neill, R., Wickens, J., Yang, J., Brooks-Bartlett, J. C., Garman, E. F., Grossmann, T. N., Dissmeyer, N., and Flashman, E. (2017) Plant cysteine oxidases are dioxygenases that directly enable arginyl transferase-catalysed arginylation of n-end rule targets *Nature communications* **8**, 14690
2. Krężel, A., and Bal, W. (2004) A formula for correlating pKa values determined in D<sub>2</sub>O and H<sub>2</sub>O *J Inorg Biochem* **98**, 161-166