

Supporting Information

Molecular and biochemical characterization of a new thermostable bacterial laccase from *Meiothermus ruber* DSM 1279

D. C. Kalyani, L. Munk, J. D. Mikkelsen, A. S. Meyer*

Center for BioProcess Engineering, Dept. of Chemical and Biochemical Engineering,
Building 229, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

*Author for correspondence

Mailing address: Center for BioProcess Engineering, Dept. of Chemical and Biochemical Engineering, Building 229, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

Tel.: + 45 4525 2800; Email: am@kt.dtu.dk

Figure legends

Fig. S1 SDS-PAGE analysis of recombinant expression and purification process of Mrlac a) in various expression hosts (T-Tuner cells, C41 cells, BL21-*E.coli* BL21, PGro7- Chaperone Competent Cell pGro7/BL21) under microaerobic conditions. C-control; W-whole cell protein; P-pellet protein; S-Soluble protein. b) Optimization of expression in pGro7 cells, 0.25mM IPTG, and temperature for microaerobic conditions 20 °C and 0.7 mg ml⁻¹ arabinose. c) Purified to homogeneity by heat precipitation and ion exchange chromatography. M: molecular size marker, L1: soluble fraction from cell disruption, L2: soluble fraction from heat precipitation, L3 and L4: Q-sepharose eluates.

Fig. S2 Presumptive oxidative reaction mechanism catalyzed by Mrlac a) non-phenolic substrate (ABTS), and b) phenolic substrate (2,6 DMP).

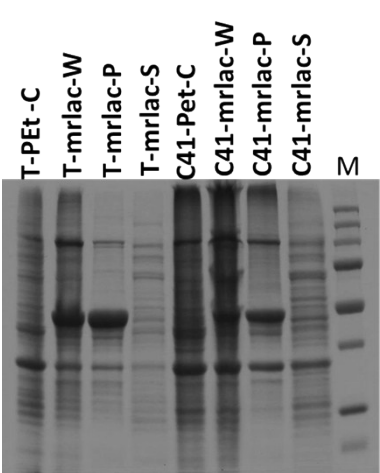
Fig. S3 Effects of metal ions on Mrlac activity. Activity was determined at room temperature in 50 mM Tris-HCl buffer (pH 8.0), using 2,6 DMP as the substrate. Activity in the absence of any metal ions was considered as control (100 %). Each value is the mean value \pm standard error mean of triplicate.

Fig. S4 The sequence alignment between Mrlac and its template structure *T. thermophilus* (PDB entry 2XU9). The sequence identity is 65 % and the sequence similarity is 71 %. The secondary structure cartoon shown is based on the DSC method. The secondary structure elements are color coded, with helices in red, strands in blue, and coils in beige. Strictly conserved amino acids residues are shown on a green background, homologous residues are shaded with light blue background while non-conserved residues are on a white background

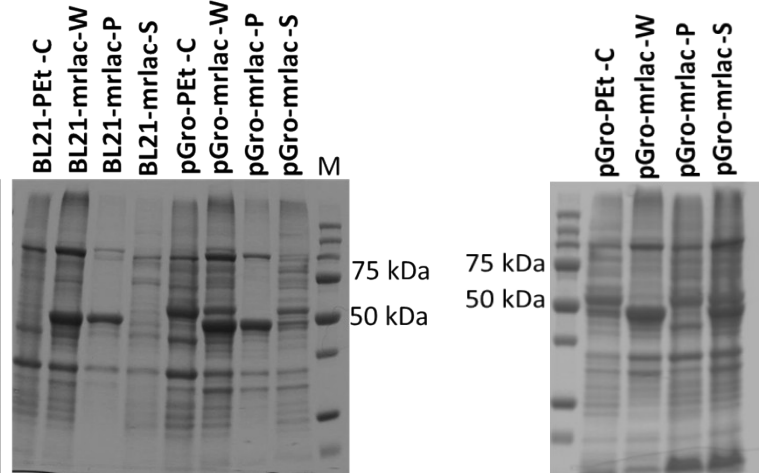
Fig. S5 a) Ribbon diagram of the superimposed Mrlac (yellow color) and *T. thermophilus* (PDB entry 2XU9) (cyan color). b, c, d, e) Mrlac copper centers. Schematic representation of the three copper centers (Cu1, Cu2, Cu3), including interatomic distances among all the relevant axes. The figure was generated using DS 3.1.

Fig. S6 Representative RP-HPLC chromatograms. a) 2,4 DCP (0.5 mM), b) after laccase treatment (P1 and P2 new peak). Chromatographic separation was carried out on a reverse phase C18 column (Kinetex 150 mm \times 4.6 mm I.D., 5 μ m particle size). The analytes were eluted under isocratic conditions using a mobile phase of 60% acetonitrile, 40% water at 1.0 ml min⁻¹

a)



b)



c)

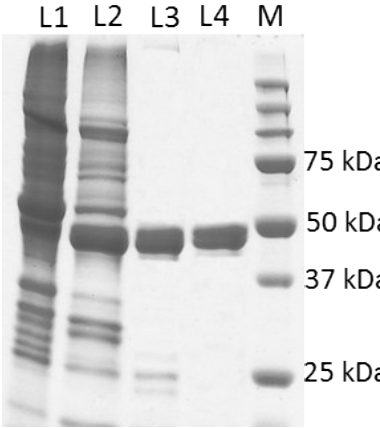
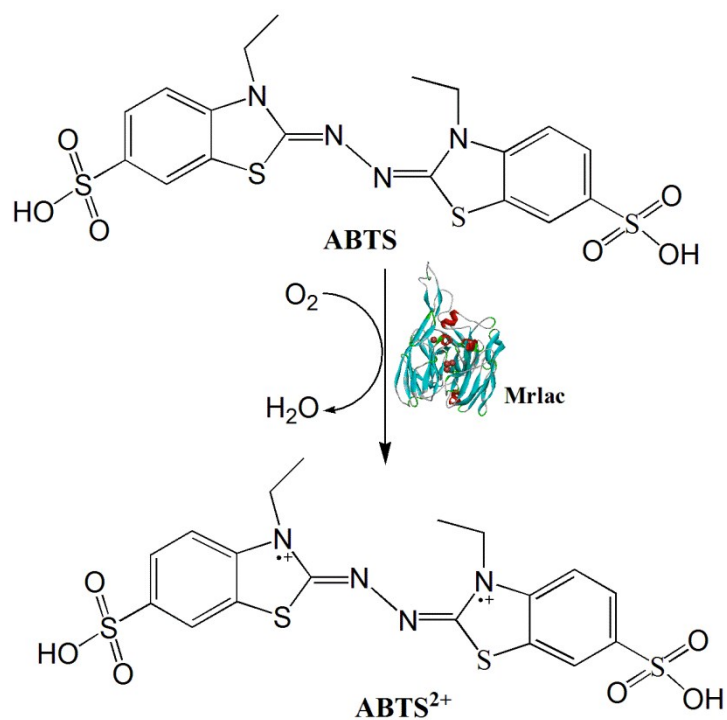


Fig. S1

a)



b)

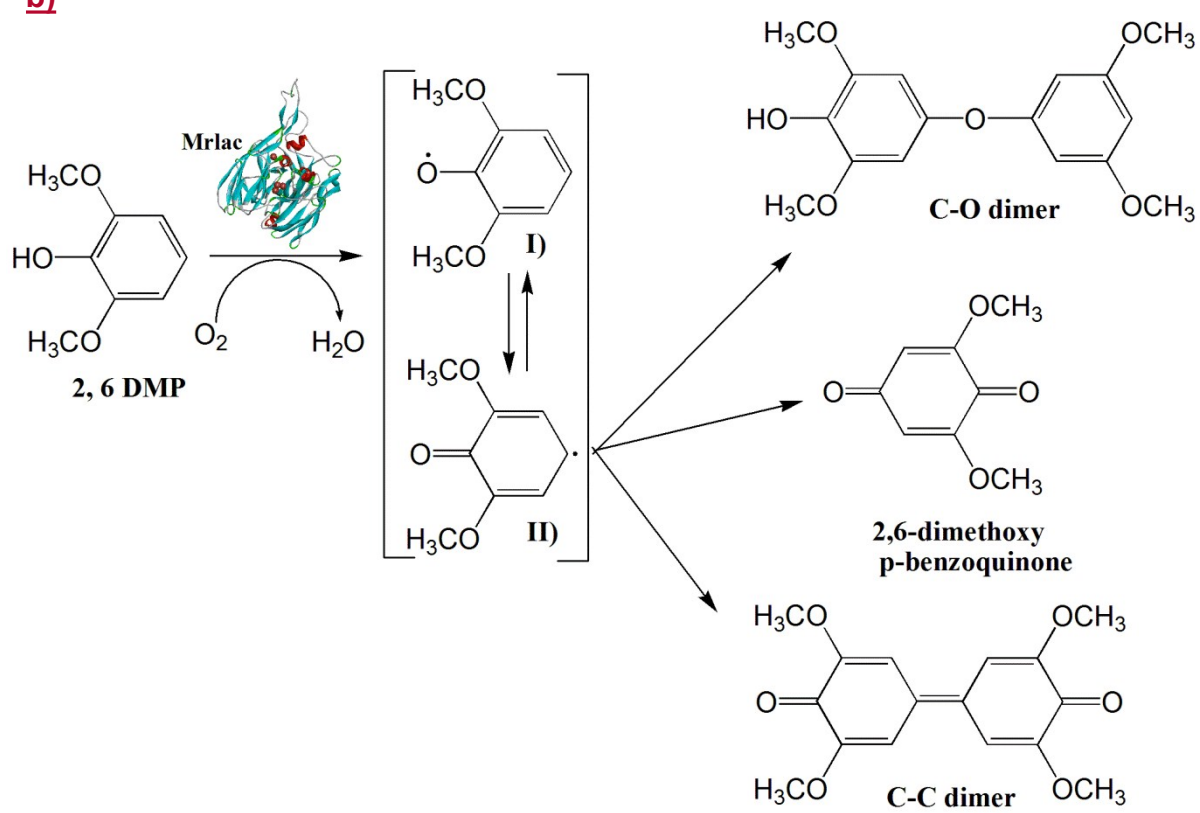


Fig. S2

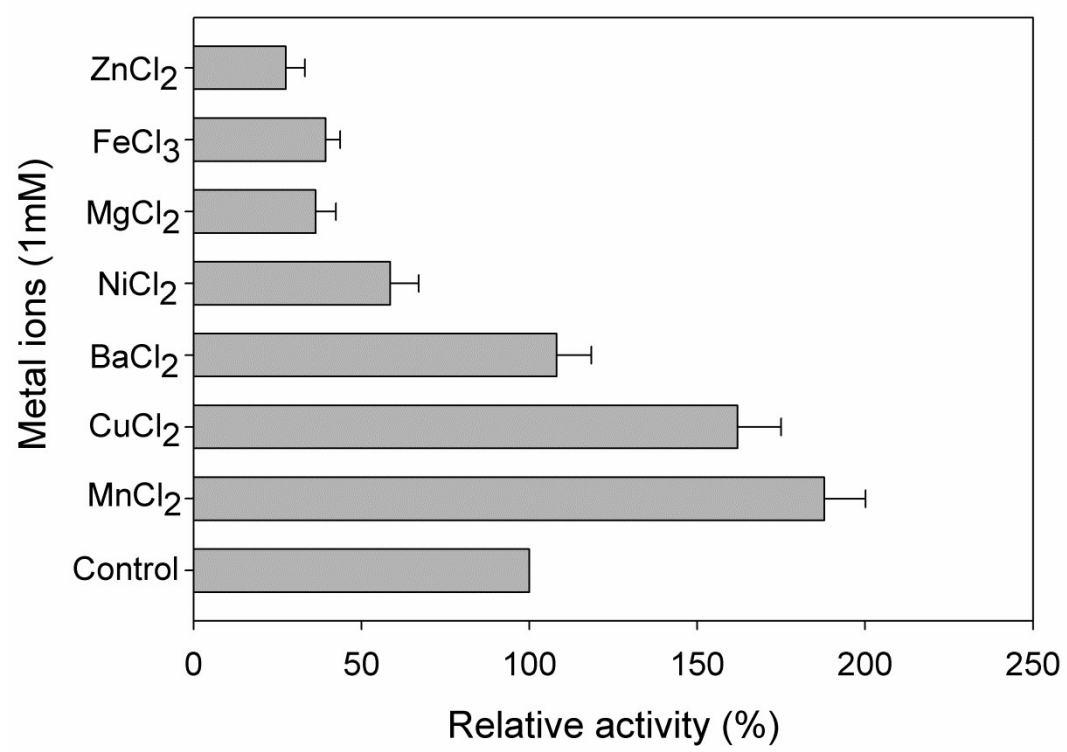


Fig. S3

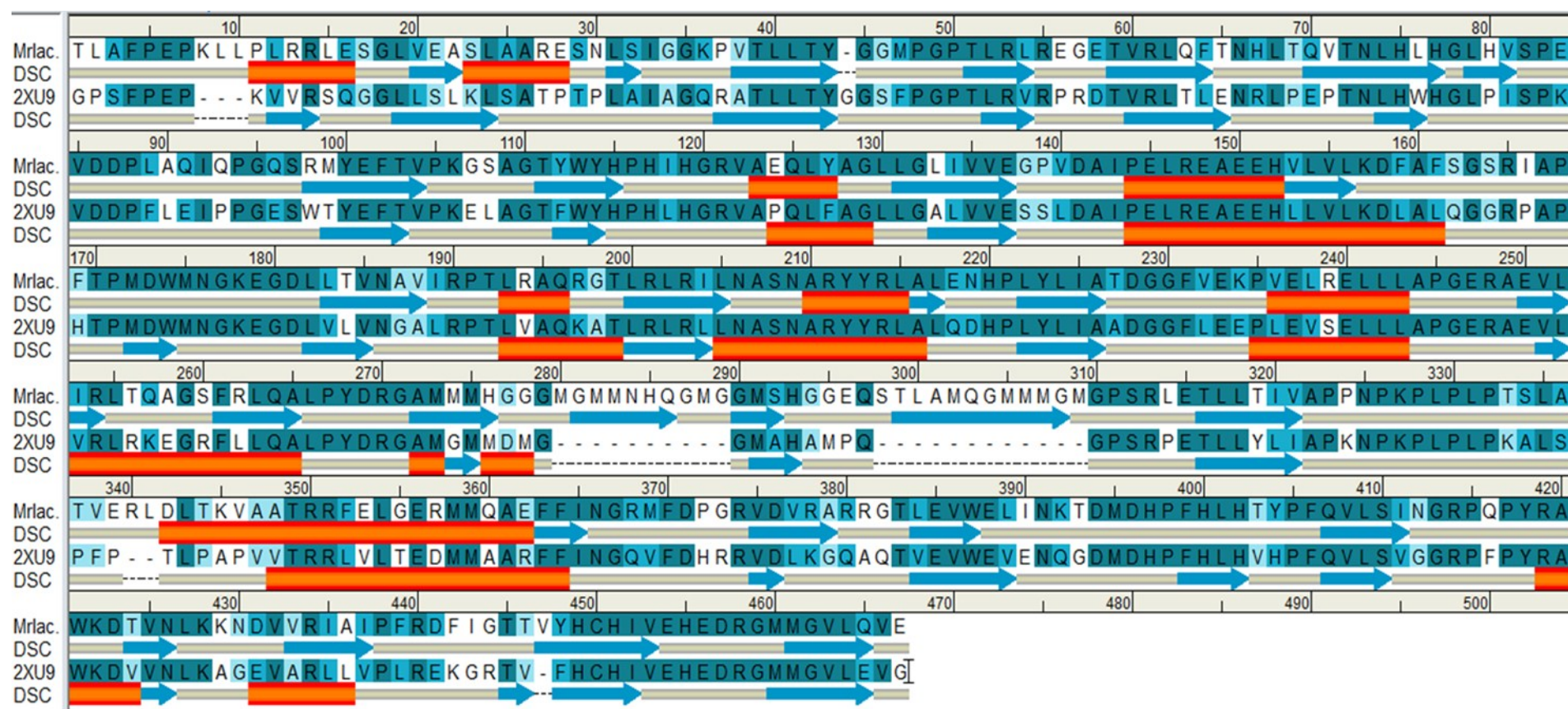


Fig. S4

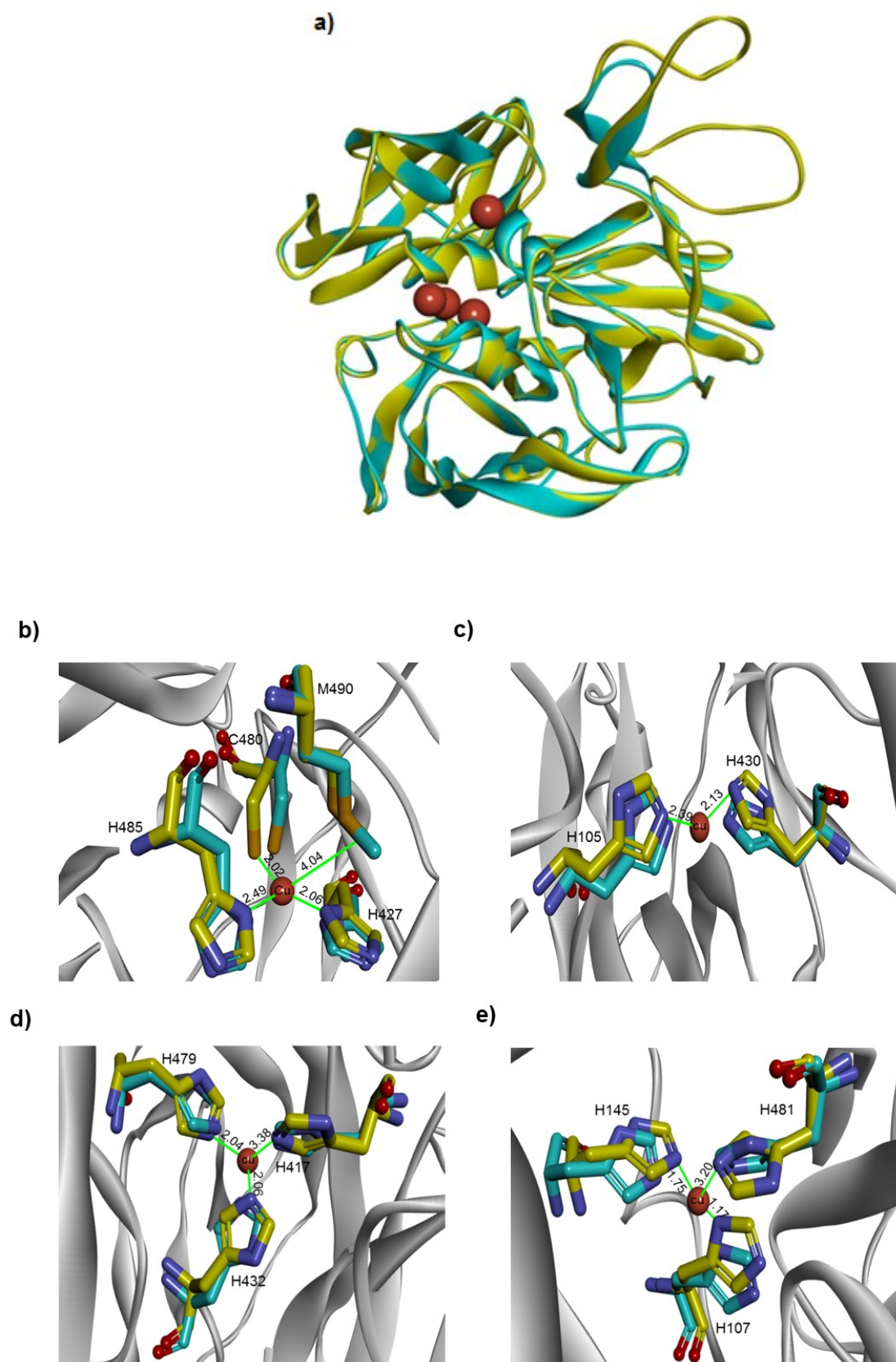
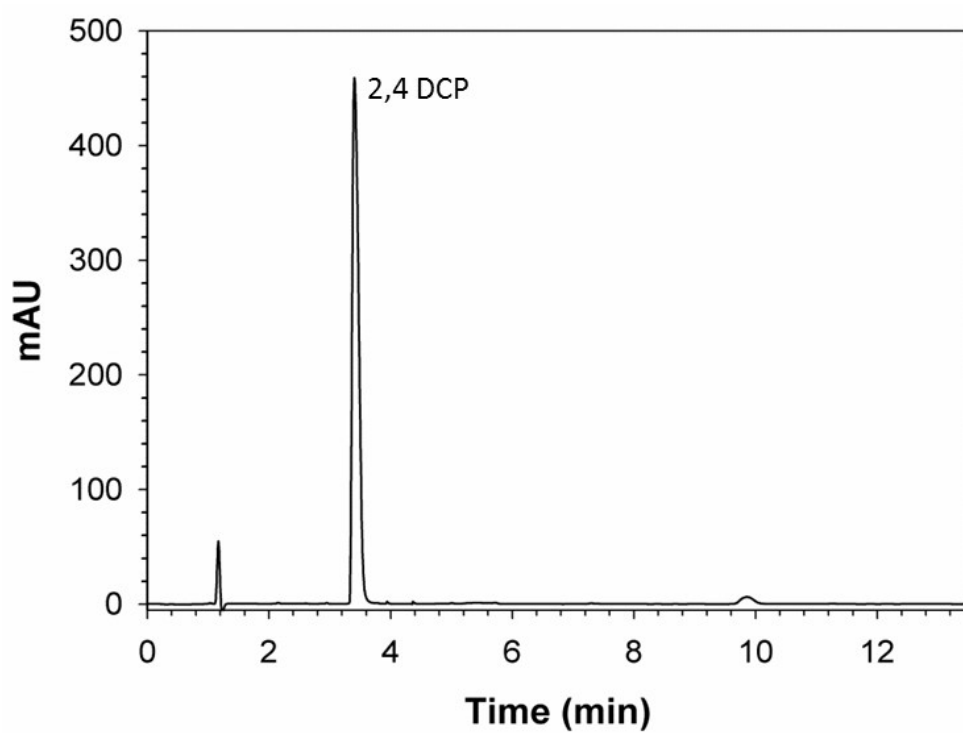


Fig. S5

a)



b)

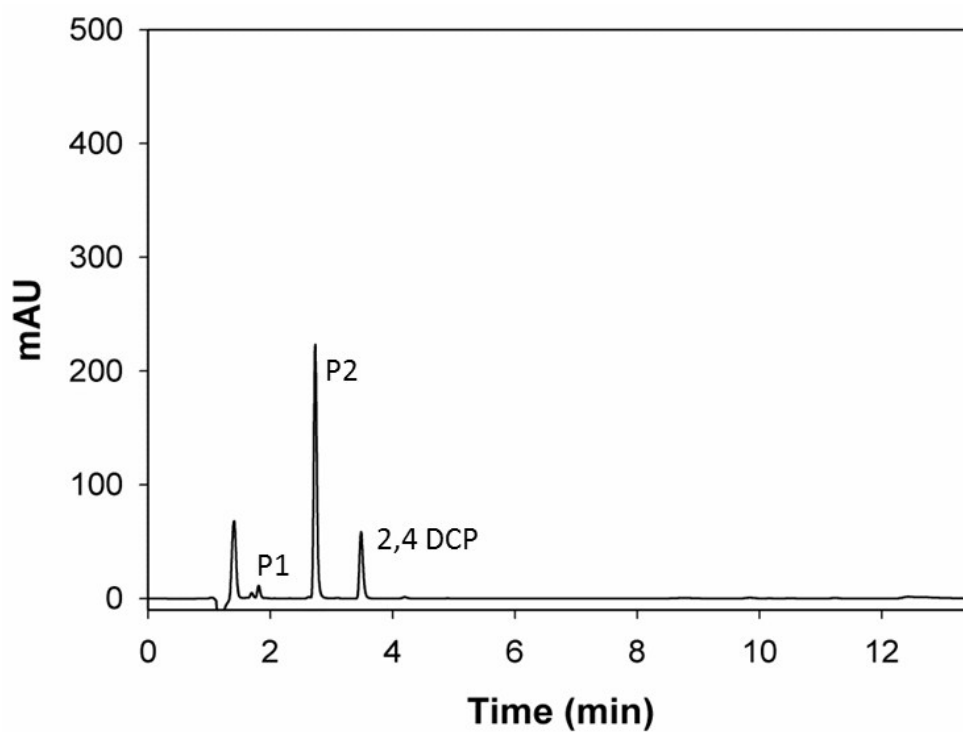


Fig. S6