Supporting information

Improvement in oxidative stability of versatile peroxidase by flow cytometry-based high-throughput screening system

Karla Ilić Đurđić[1], Selin Ece[2], Raluca Ostafe[3], Simon Vogel[4], Stefan Schillberg[4], Rainer Fischer[5], Radivoje Prodanović[1]

[1]University of Belgrade-Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia

[2] PerkinElmer chemagen Technologie GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany

[3]Molecular Evolution Protein Engineering and Production facility (MEPEP), Purdue University, 207 S. Martin Jischke Dr., West Lafayette, IN 47907, USA

[4] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstrasse 6, 52074 Aachen, Germany

[5] Indiana Bioscience Research Institute, Single Cell Analytics Center, 1345 W. 16th St. Suite 300, Indianapolis, IN 46202, USA

Correspondence: Prof. Radivoje Prodanović, University of Belgrade-Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia.

E-mail: rprodano@chem.bg.ac.rs

atggctacttgtgatgacggtagaacaactgcaaatgccgcatgttgcatcctattcccaatcttggacgatattcaggagaacttgtttgatggtgctcaatgcggtgaagag
gttcatgaatccttgcgtttgacctttcatgatgccataggattttctccaactttaggtggtggtggagctgatggtagtatcattgctttcgacaccattgaaaccaatttcccag
caaatgccggtattgatgagatagtttcagctcagaaaccattcgttgctaaacacaacatttctgctggtgattttatccaatttgctggtgctgtcggagtctcaaattgtcctg
gaggagtcagaataccttttcttcttgggtagaccagatgcagttgccgctagtccagatcatcttgttccagaacctttgactcagttgactctattcttgccagaatgggtgat
gctggttttagtcctgtagaagttgtatggttacttgcatcccattctattgctgcagcagataaagtcgatccttctatacctggtactccattcgattcaacacctggtgtattcg
actcccagttcttcattgaaactcagttaaagggtcgtttgtttccaggtacagctgataataagggtgaagctcaatctcctttgcaaggagaaattaggttgcaatctgatca
cttgttagctagagatcctcaaacggcttgtgaatggcaatctatggtgaacaatcaacctaagatccaaaacagattcgctgcaactatgtcgaaaatggccttgttgggtca
agacaagacgaaactaatcgattgttccgatgtgataccaactcctccagccttagtcggtgcagctcatttgccagcaggattttcgctatcagacgtggaacaagcttgtg
ctgctaccccatttccagccttaacagctgatcctggtcctgtaacatctgttccaccagttccaggtagtctcgagtaa

Figure S1. Versatile peroxidase sequence

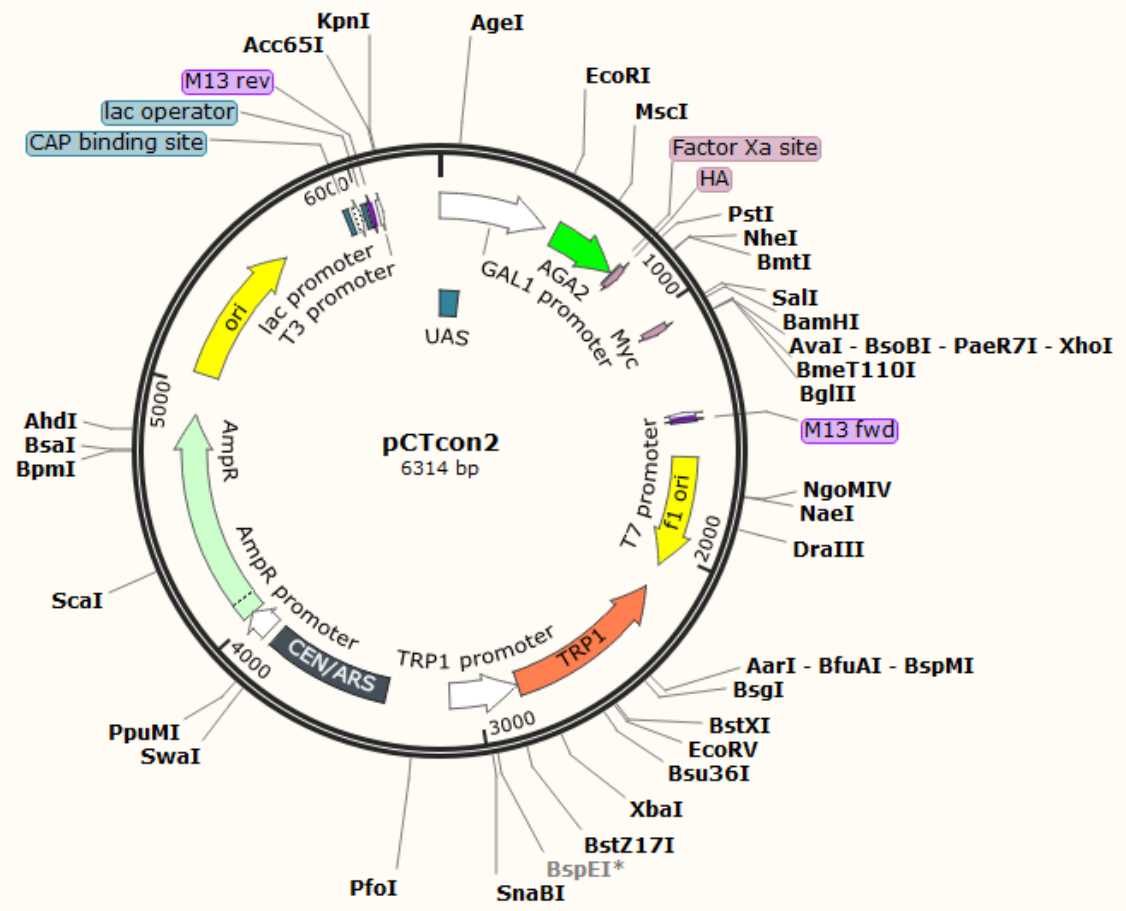

Figure S2. Map of pCTCON2 vector

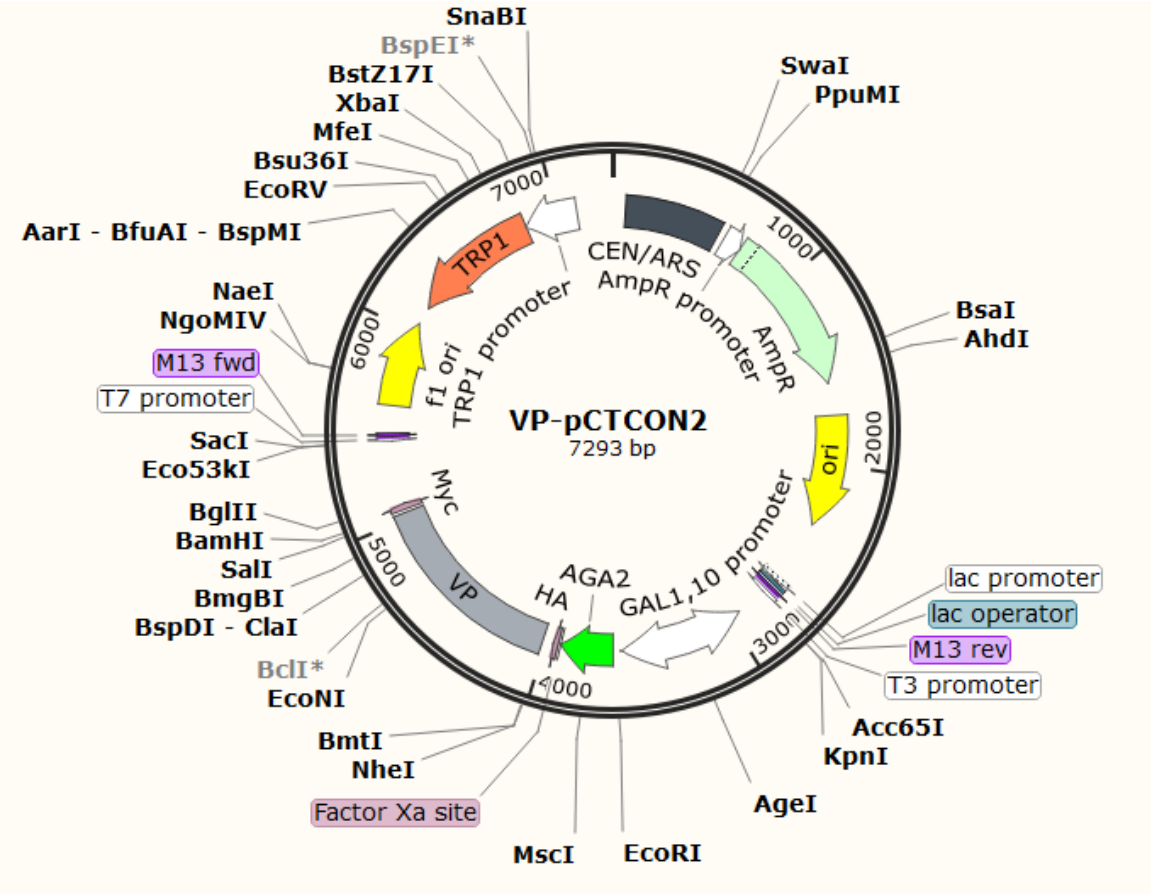

Figure S3. Map of VP-pCTCON2 construct

Table S1. Primers sequences

Application	Sequence
Forward primer for cloning of VP gene	ATCGATGCTAGCATGGCTACTTGTGATGACGGTAGAACAACTGCAAATGCCGC
Reverse primer for cloning of VP gene	TACTAGTGGATCCCTCGAGACTACCTGGAACTGGTGGAAGAGATGTTACAGGACC
Forward primer for sequencing	CCCATACGACGTTCCAGACTACGC
Reverse primer for sequencing	GATCTCGAGCTATTACAAGTCCTCTTCAG
Forward primer for mutagenesis	CGATGCTAGCATGGCTACTTGTGATGACGGTAGAACAACTGCAAATGCCGC
Reverse primer for mutagenesis	CTAGTGGATCCCTCGAGACTACCTGGAACTGGTGGAAGAGATGTTACAGGACC

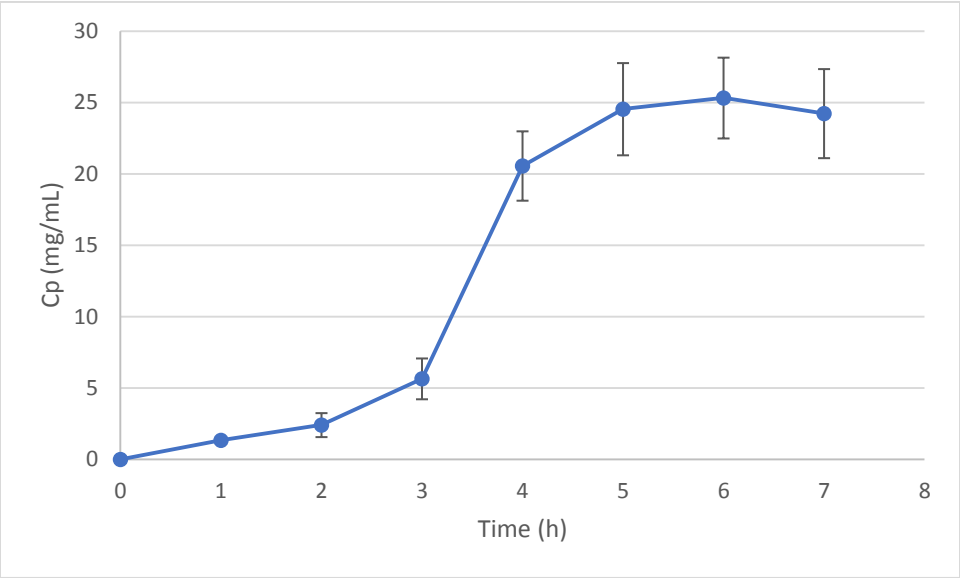

Figure S4. Protein concentration during cell lysis. Before and after every hour of toluene-induces cell lysis process 100 µL aliquots were taken and centrifuged. Protein concentration was determined in supernatant using Bradford reagent. Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.

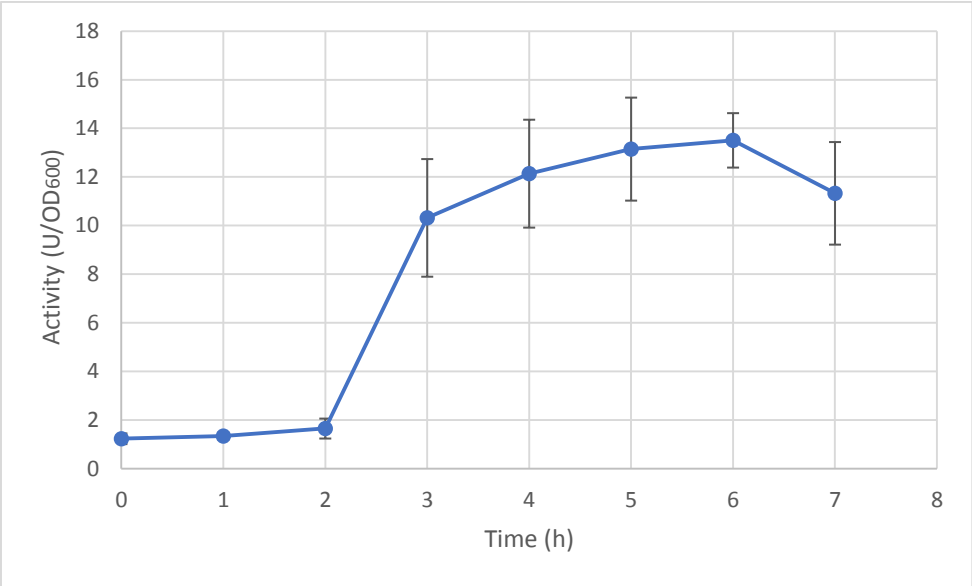

Figure S5. Activity of VP during lysis. Before and after every hour of lysis process 100 µL aliquots were taken and centrifuged. After 3 washing steps cells/cell walls were resuspended to final OD_{600} = 0.1. Activity of VP was measured with ABTS assay (2 mM ABTS, 0.5 mM H_2O_2 in 100 mM Na-tartrate buffer pH 3.5) by monitoring absorbance at 405 nm. Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.

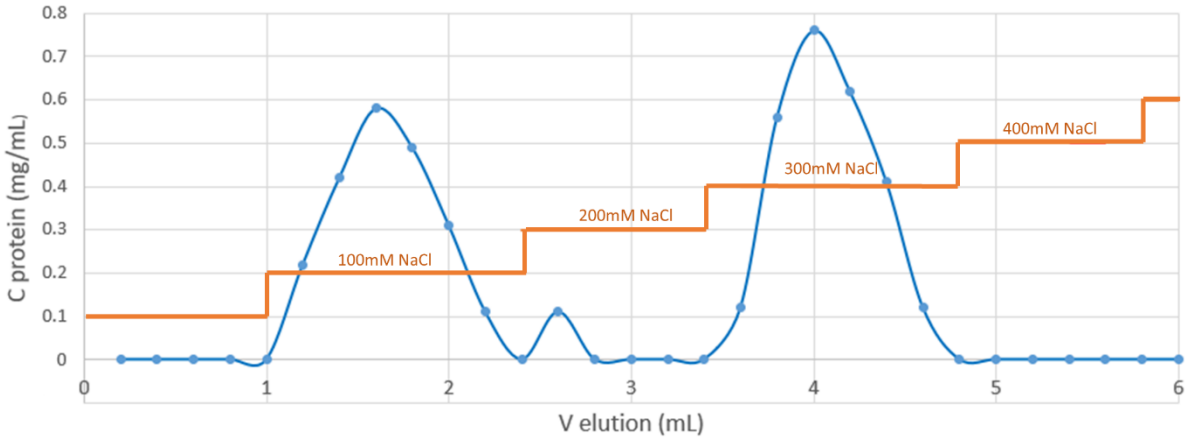

Figure S6. Chromatogram showing the elution of Aga2-wtVP chimera using Vivapure mini spin columns with optimized NaCl step elution.

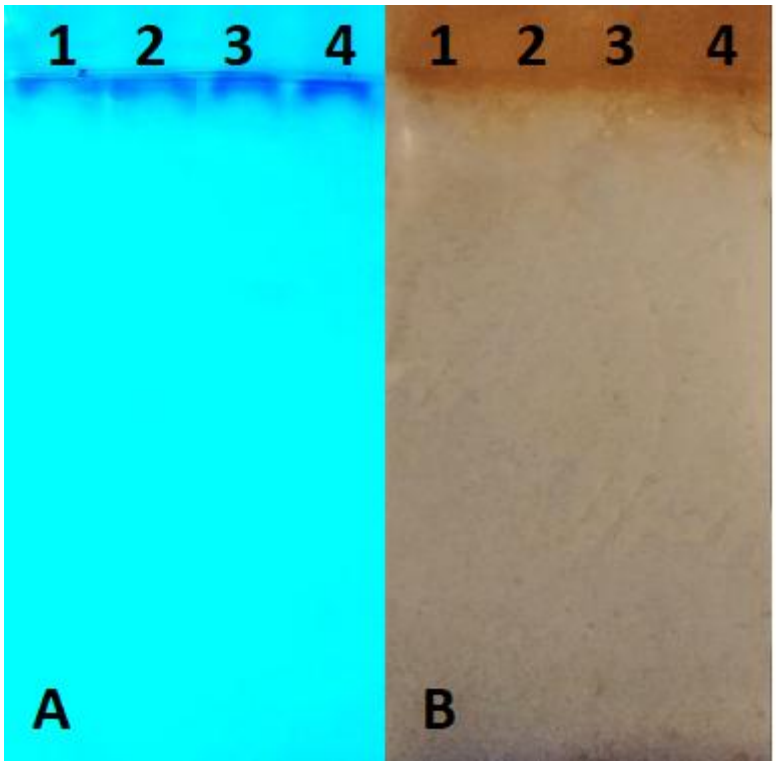

Figure S7. Native 12% polyacrylamide gel electrophoresis. (1) Aga2-wtVP (2) Aga2-MVP1 (3) Aga2-MVP2 (4) Aga2-MVP3. **A.** Protein bands after CBB R250 staining. **B.** Activity bands in the gel after incubation with 0.5 mM H_2O_2 and 9 mM guaiacol.

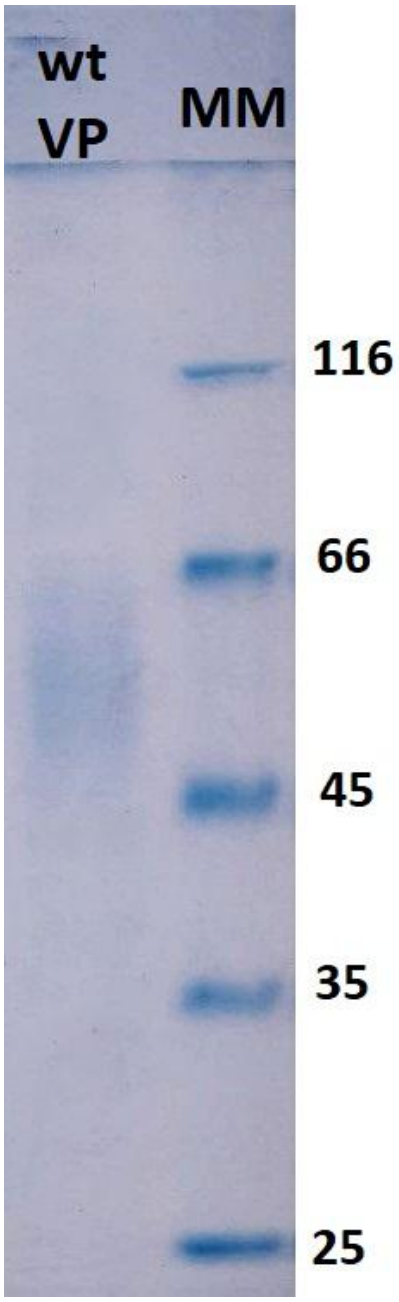

Figure S8. SDS-PAGE of purified aga2-wtVP compared with molecular weight markers (MM)