Supplementary data for article:

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Supporting information

Saturation mutagenesis to improve the degradation of azo dyes by versatile peroxidase and application in form of VP-coated yeast cell walls

Karla Ilić Đurđić[1], Raluca Ostafe[2,3], Aleksandra Đurđević Đelmaš[1], Nikolina Popović[1], Stefan Schillberg[4], Rainer Fischer[3,5], Radivoje Prodanović[1*]

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

[2]Purdue Institute of Inflammation, Immunology and Infectious Disease; Molecular Evolution, Protein Engineering and Production; Purdue University, 207 S. Martin Jischke Dr., West Lafayette, IN 47907, USA

[3]Institute of Molecular Biotechnology, RWTH Aachen University Worringerweg 1, 52074 Aachen, Germany

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

[5]Departments of Biological Sciences and Chemistry, Purdue University, 207 S. Martin Jischke Dr., West Lafayette, IN 47907, USA

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

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

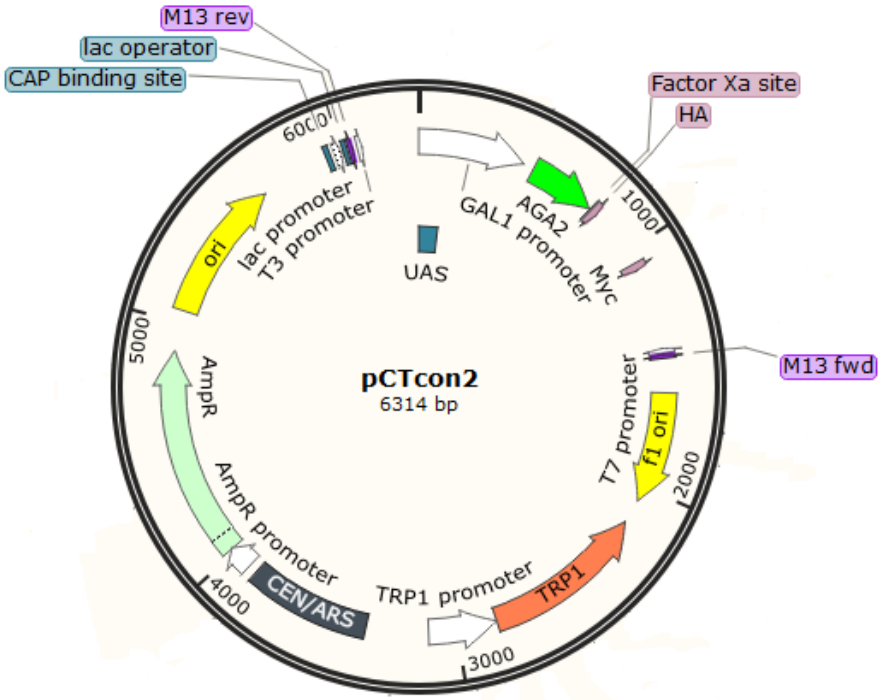

Figure S1. Map of pCTCON2 vector

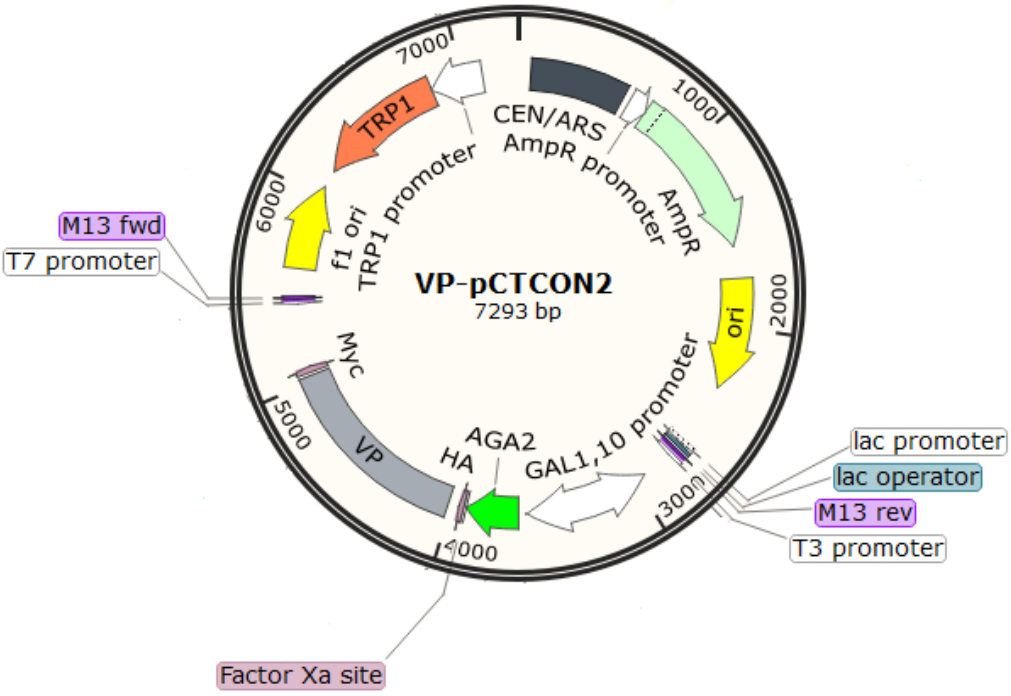

Figure S2. Map of VP-pCTCON2 construct

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ctgctaccccatttccagccttaacagctgatcctggtcctgtaacatctgttccaccagttccaggtagtctcgagtaa

Figure S3. Versatile peroxidase sequence

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CLUSTAL format alignment by MAFFT (v7.452)


Lignin|PDBID|CH AAVIEKRATCSNGKTVGDASCCAWFDVLDDIQQNLFHGGQCGAEAHESIRLVFHDSIAIS
Versatile|PDBID -------ATCDDGRTTANAACCILFPILDDIQENLFDGANCGEEVHESLRLTFHDAIGFS
                      ***.:*:*..:*:**  * :*****:***.*.:** *.***:**.***:*.:*

Lignin|PDBID|CH PAMEAQGKFGGGGADGSIMIFDDIETAFHPNIGLDEIVKLQKPFVQKHGVTPGDFIAFAG
Versatile|PDBID PTL------GGGGADGSIIAFDTIETNFPANAGIDEIVSAQKPFVAKHNISAGDFIQFAG
                *::      *********: ** *** * .* *:****. ***** **.::.**** ***

Lignin|PDBID|CH AVALSNCPGAPQMNFFTGRAPATQPAPDGLVPEPFHTVDQIINRVNDAGEFDELELVWML
Versatile|PDBID AVGVSNCPGGVRIPFFLGRPDAVAASPDHLVPEPFDSVDSILARMGDAG-FSPVEVVWLL
                **.:*****. :: ** **. *. .:** ******.:**.*: *:.*** *. :*:**:*

Lignin|PDBID|CH SAHSVAAVNDVDPTVQGLPFDSTPGIFDSQFFVETQLRGTAFPGSGGNQGEVESPLPGEI
Versatile|PDBID ASHSIAAADKVDPSIPGTPFDSTPEVFDSQFFIETQLKGRLFPGTADNKGEAQSPLQGEI
                ::**:**.:.***:: * ****** :******:****:*  ***:..*:**.:*** ***

Lignin|PDBID|CH RIQSDHTIARDSRTACEWQSFVNNQSKLVDDFQFIFLALTQLGQDPNAMTDCSDVIPQSK
Versatile|PDBID RLQSDHLLARDPQTACEWQSMVNNQPKIQNRFAATMSKMALLGQDKTKLIDCSDVIPTPP
                *:**** :***.:*******:****.*: : *   :  :: **** . : ******* .

Lignin|PDBID|CH PIPGNLPFSFFPAGKTIKDVEQACAETPFPTLTTLPGPETSVQRIPPPPGA
Versatile|PDBID ALVGA---AHLPAGFSLSDVEQACAATPFPALTADPGPVTSV---PPVPGS
                .: *    :.:*** ::.******* ****:**: *** ***   ** **:
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Figure S4. Multiple alignment of VP form *P. eryngii* and LiP form *P. chrysosporium* performed using MAFET algorithm.

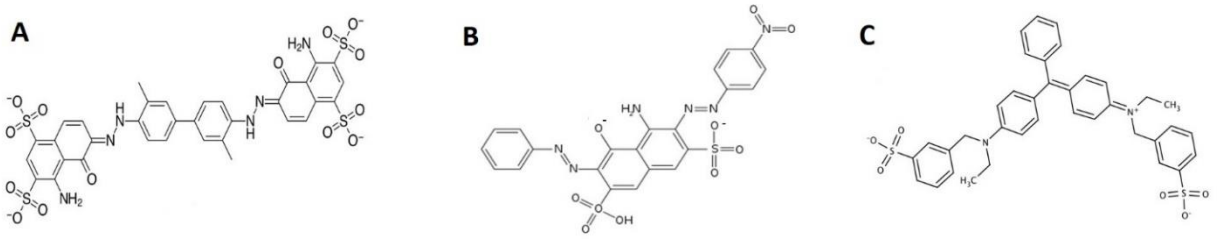

Figure S5. Structures of tested azo-dyes (A) Evans blue (B) Amido black 10B (C) Guinea green

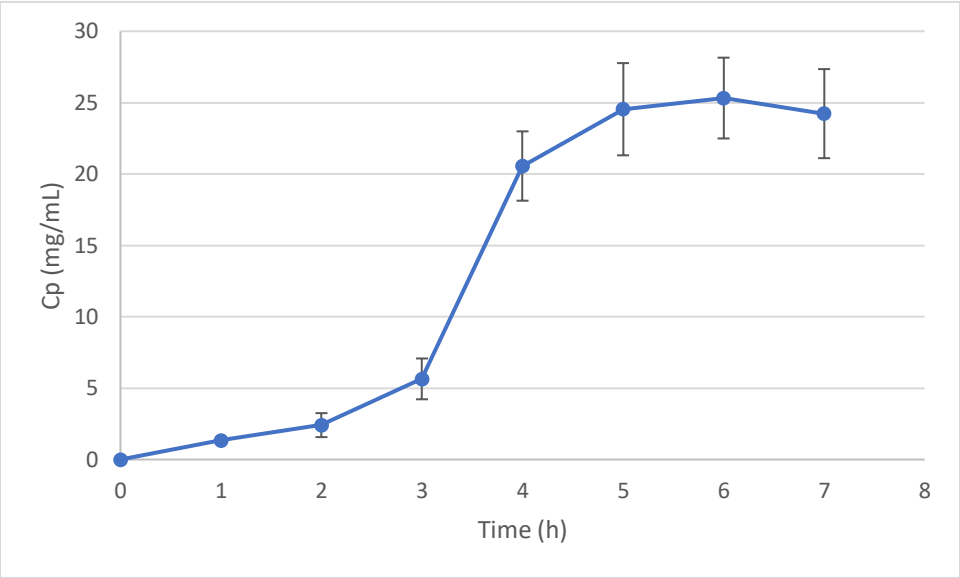

Figure S6. Protein concentration during cell lysis. Before and after every hour of toluene-induces cell lysis process 100 µL aliquots were taken and centrifuged. Protein concentration was determined in supernatant using Bradford reagent. Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.

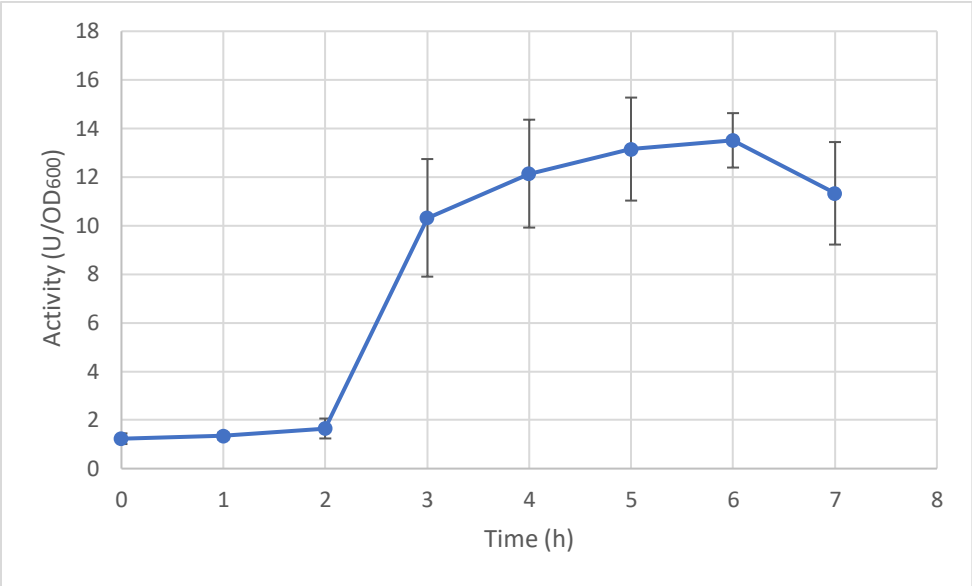

Figure S7. Activity of VP during lysis. Before and after every hour of lysis process 100 µL aliquots were taken and centrifuged. After 3 washing steps cells/cell walls were resuspended to final OD_{600} = 0.1. Activity of VP was measured with ABTS assay (2 mM ABTS, 0.5 mM H_2O_2 in 100 mM Na-tartrate 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.

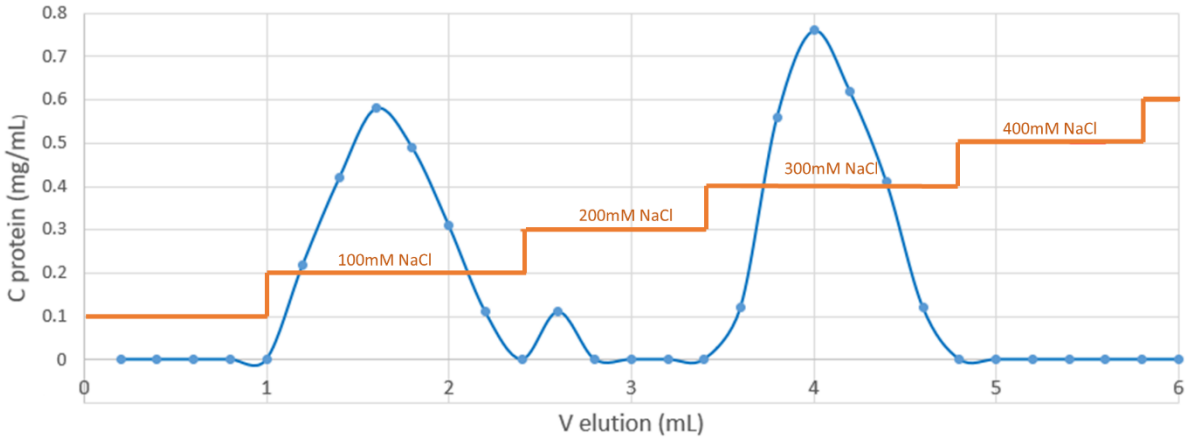

Figure S8. Chromatogram showing the elution of Aga2-wtVP chimera using Vivapure mini spin columns with optimized NaCl step elution.

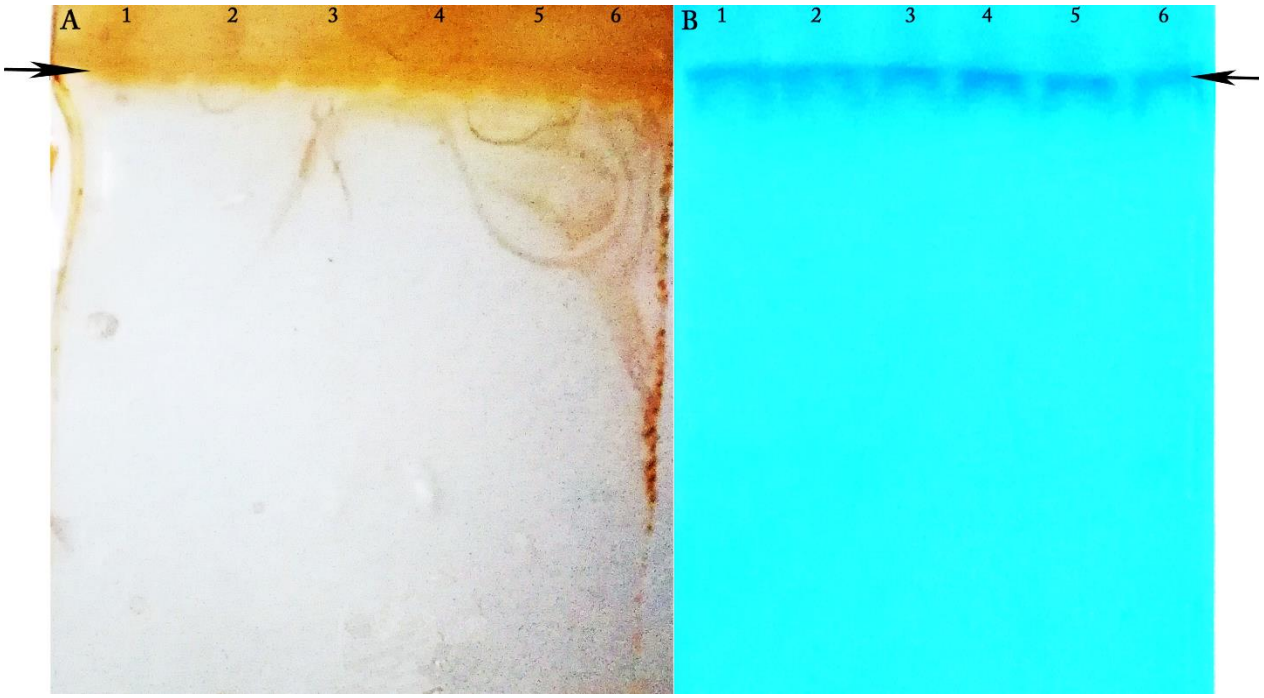

Figure S9. Native 12% polyacrylamide gel electrophoresis. (1) Aga2-wtVP (2) Aga2-MVP1 (3) Aga2-MVP2 (4) Aga2-MVP3 (5) Aga2-MVP4 (6) Aga2-MVP5 A. Activity bands in the gel after incubation with 0.5 mM H_2O_2 and 9 mM guaiacol (arrow pointing at them). B. Protein bands after CBB R250 staining (arrow pointing at them).

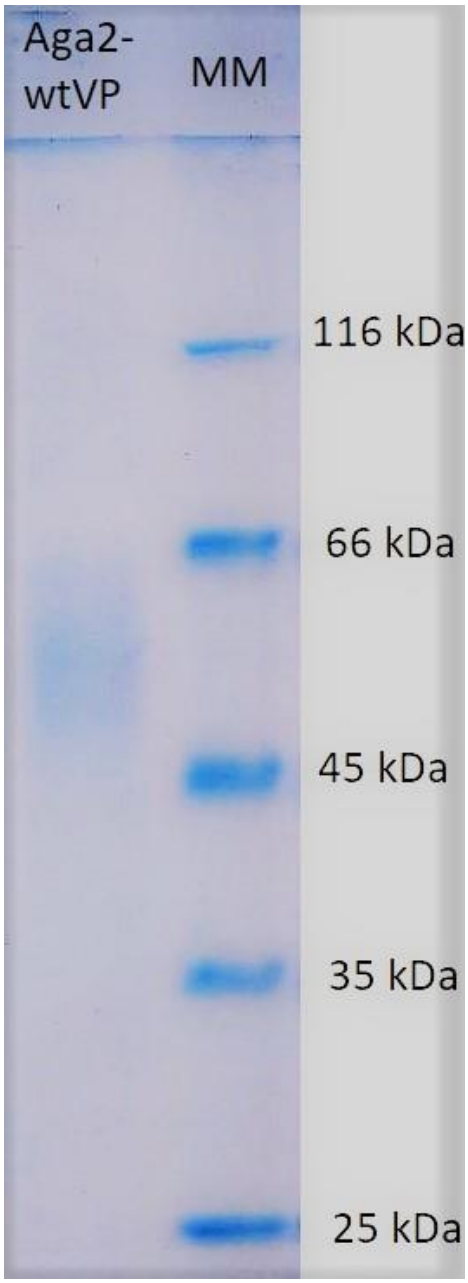

Figure S10. SDS-PAGE of purified aga2-wtVP compared with molecular weight markers (MM)

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
Primer for mutation of A160N	CTGGTTTTAGTCCTNNNGAAGTTGTATGG
Primer for mutation of V260N	CAAAACAGATTCNNNGCAACTATGTCG

Table S2. Kinetic parameters for wtVP and MV1-5 for evans blue, amido black 10B and guinea green determined for Aga2-VP fusion proteins. Data are means of triplicate experiments and error bars indicate standard deviation.

Variant	k_{cat} (s^{-1})	K_m(μM)	k_{cat}/K_m ($mM^{-1}s^{-1}$)
	Evans blue		
WT VP	1.17±0.23	27.52±2.95	42.51
MV1	1.95±0.19	7.74±0.95	251.94
MV2	2.95±0.09	6.54±0.93	451.07
MV3	2.03±0.64	37.32±7.44	54.39
MV4	1.30±0.23	9.92±1.10	110.89
MV5	1.58±0.05	8.15±0.45	193.86
	Amido black 10B		
WT VP	1.05±0.40	15.76±2.73	66.62
MV1	5.14±0.79	6.91±0.94	743.85
MV2	4.64±0.82	17.53±1.44	260.47
MV3	6.34±0.90	5.88±0.94	1078.23
MV4	1.99±0.19	11.23±2.24	177.20
MV5	0.50±0.29	19.43±1.94	25.57
	Guinea green		
WT VP	0.81±0.16	31.28±5.42	25.89
MV1	3.24±0.25	19.91±2.23	162.73
MV2	0.33±0.12	40.15±2.32	82.19
MV3	0.53±0.14	38.34±1.94	13.82
MV4	5.14±0.40	8.23±1.02	624.54
MV5	3.96±0.82	11.52±3.12	343.75