Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 85 (1) S25–S30 (2020) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Expression, purification and characterization of cellobiose dehydrogenase mutants from *Phanerochaete chrysosporium* in *Pichia pastoris* KM71H strain

ANA MARIJA J. BALAŽ[1], MARIJA B. BLAŽIĆ[1], NIKOLINA POPOVIĆ[2], OLIVERA L. PRODANOVIĆ[3], RALUCA V. OSTAFE[4], RAINER FISCHER[5] and RADIVOJE M. PRODANOVIĆ[2]*

[1]*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia,* [2]*Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, 11000 Belgrade, Serbia,* [3]*Institute for Multidisciplinary Studies, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia,* [4]*Molecular Evolution Protein Engineering and Production Facility (MEPEP), Purdue University, 207 S. Martin Jischke Dr., West Lafayette, IN 47907, USA and* [5]*Indiana Bioscience Research Institute, Single Cell Analytics Center, 1345 W. 16th St. Suite 300, Indianapolis, IN 46202, USA*

J. Serb. Chem. Soc. 85 (1) (2020) 25–35

TABLE S-I. Primers used for creation of triple mutant and error prone library mutants using the wt CDH–pPICZαA vector as template

Primer name	Primer sequence
Forward primer D20N	GGTATCACCAACCCTGTTCATG
Forward primer A64T	CTCGGTGGCACCATGAACAAC
Forward primer V592M	CGCAGCCTCCATGAACTCC
Forward primer D20N–V22A	CACCAACCCTGCTCATGACG
Forward primer T84A	TTTCCTCCGCTCGCTGG
Forward primer A261P	ACGTATGTCCCTCCATG
Forward primer E674G	AGACACTCGGGGAGTACG
Forward primer N715S	TTGGCACGAGCAACCTGTTT

* Corresponding author. E-mail: rprodano@chem.bg.ac.rs

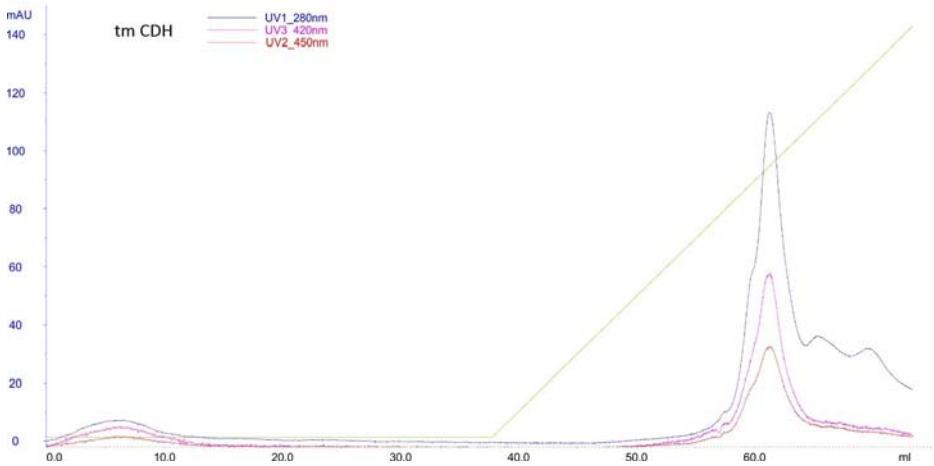

Fig. S-1. Ion-exchange chromatography of tm CDH.

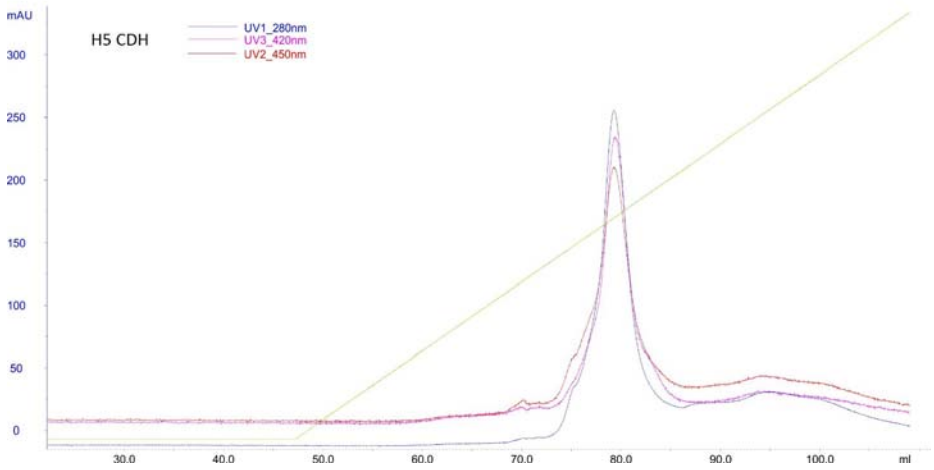

Fig. S-2. Ion-exchange chromatography of H5 CDH.

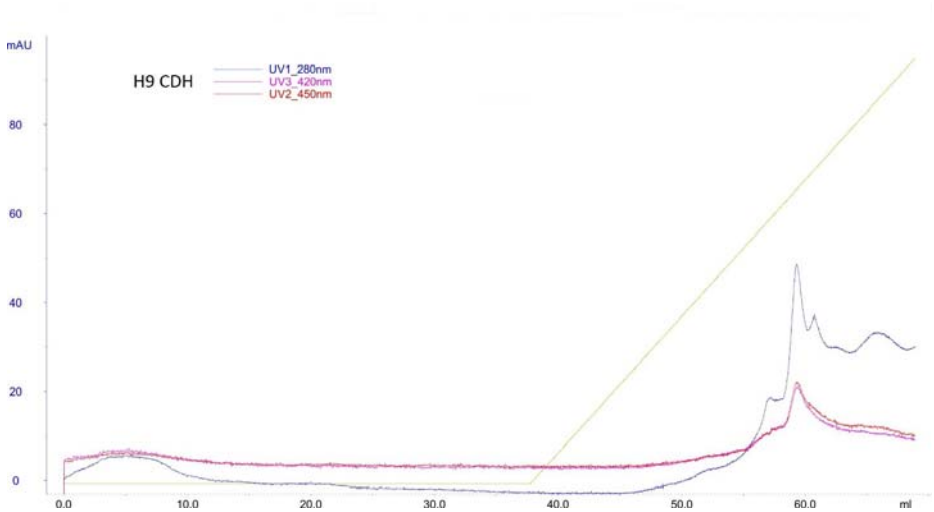

Fig. S-3. Ion-exchange chromatography of H9 CDH.

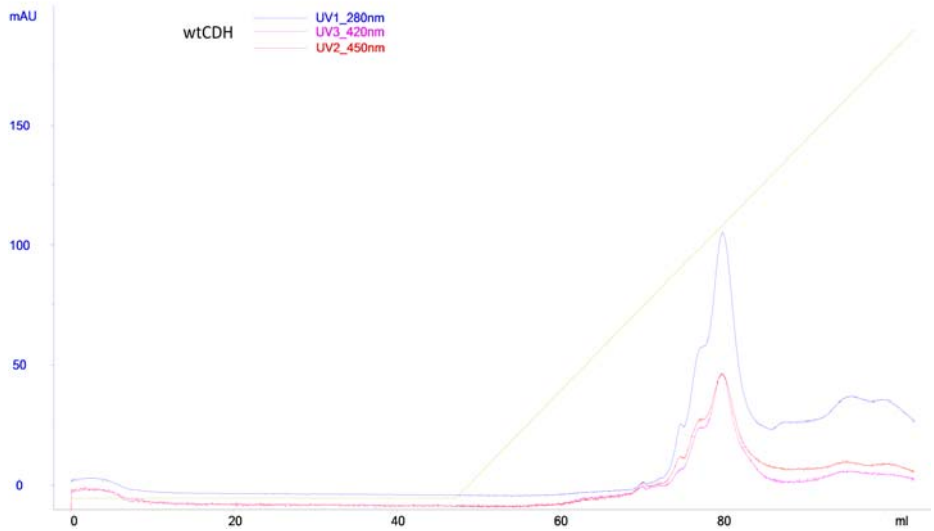

Fig. S-4. Ion-exchange chromatography of wt CDH.

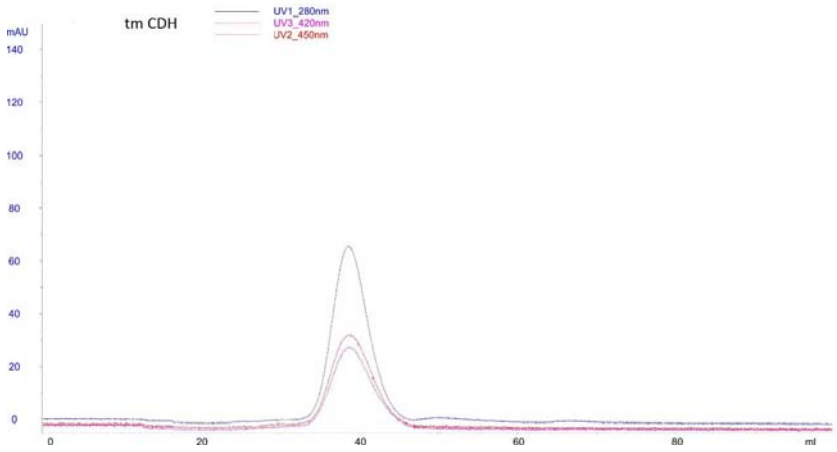

Fig. S-5. Gel filtration of tm CDH.

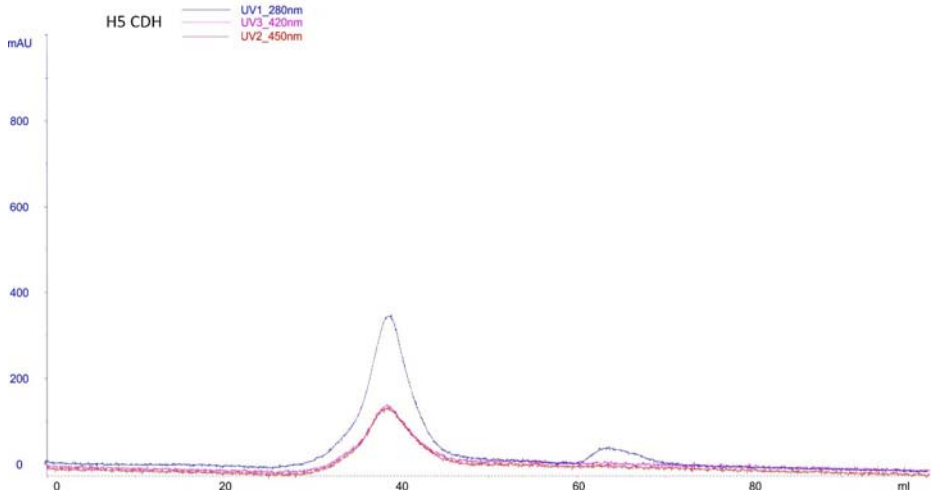

Fig. S-6. Gel filtration of H5 CDH.

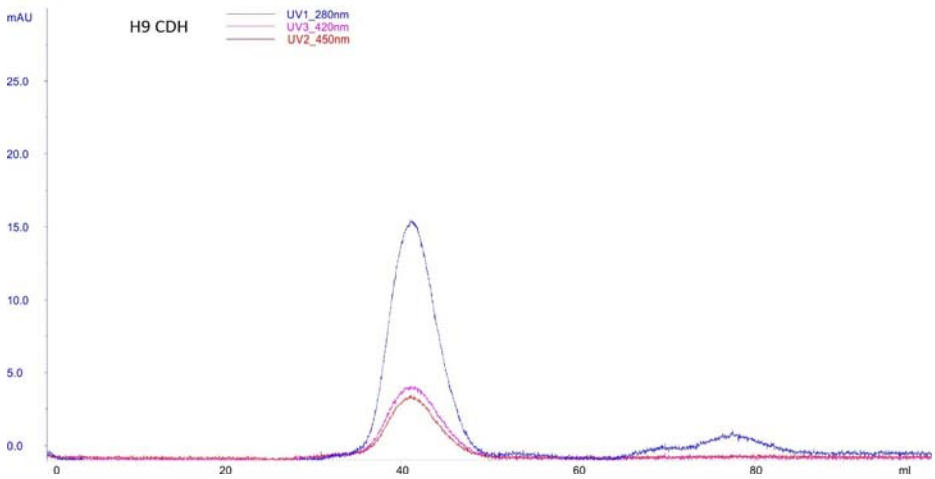

Fig. S-7. Gel filtration of H9 CDH.

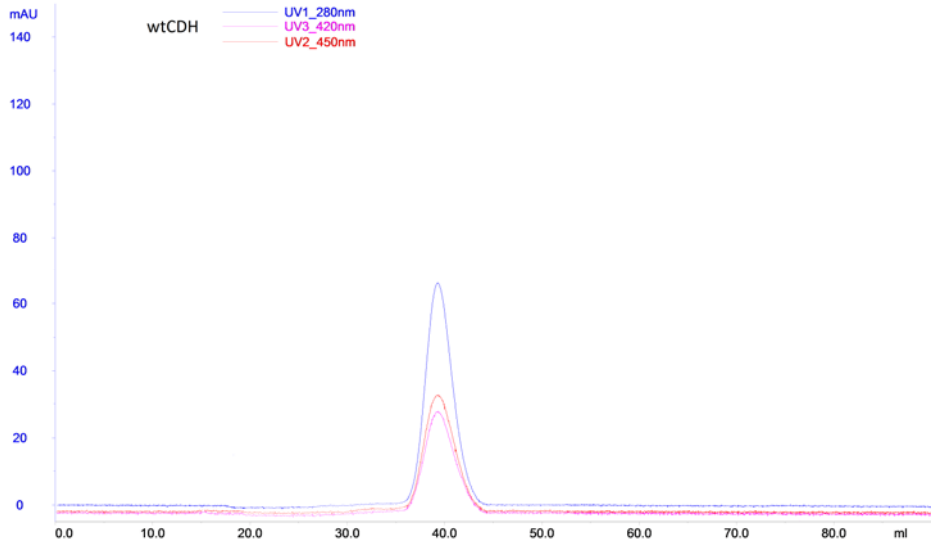

Fig. S-8. Gel filtration of wt CDH.

TABLE S-II. Purification table for CDH. FM – fermentation media, UF – ultrafiltrate, DEAE – sample after ion-exchange chromatography, GF – sample after gel filtration. C_p – protein concentration, *Ac* – enzyme activity, *V* – volume, *Y* – yield of purification, *Pf* – purification factor, *Sp* – specific enzyme activity

Parameter	tm CDH				H5 CDH				H9 CDH				wt CDH			
	FM	UF	DEAE	GF	FM	UF	DEAE	GF	FM	UF	DEAE	GF	FM	UF	DEAE	GF
C_p / mg mL^{-1}	0.59	0.42	0.48	0.08	0.52	0.45	0.74	0.17	0.46	0.20	0.15	0.02	0.57	0.72	0.59	0.22
Ac / IU mL^{-1}	0.91	2.34	5.41	1.17	1.99	7.30	16.5	4.89	0.42	1.10	1.87	0.34	4.04	8.86	8.1	4.49
V / mL	50	12	3	7	50	12	3	6	50	12	3	5	50	12	5	6
Y / %	100	62	36	18	100	88	50	29	100	63	27	8.2	100	53	20	14
Pf	1	3.6	7.2	9.1	1	4.2	5.8	7.3	1	5.9	13	16	1	1.7	1.3	2.88
Sp / IU mg^{-1}	1.55	5.55	11.2	14.1	3.83	16.2	22.4	28.1	0.92	5.44	12.2	14.5	7.1	12.3	13.7	20.4