# Serbian Biochemical Society
# Sixth Conference

***"Biochemistry and Interdisciplinarity: Transcending the Limits of Field"***

*Proceedings*

*Faculty of Chemistry*
*Belgrade 2016*

# Serbian Biochemical Society



## Proceedings

**Editor:** Ivan Spasojević
**Technical secretary:** Jelena Nestorov
**Cover design:** Zoran Beloševac
**Publisher:** Faculty of Chemistry, Serbian Biochemical Society
**Printed by:** Colorgrafx, Belgrade

CIP

# Serbian Biochemical Society
# Sixth Conference

with international participation

Faculty of Chemistry, University of Belgrade,
18.11.2016. Belgrade, Serbia

"***Biochemistry and Interdisciplinarity: Transcending the Limits of Field***"

# Foreword

Dear Colleagues

It is my distinct pleasure to welcome you to the 6th Conference of the Serbian Biochemical Society, entitled "Biochemistry and Interdisciplinarity: Transcending the Limits of Field". It is an honor for me to be selected as the Editor of Proceedings of the Conference. I am grateful to the Steering Committee of Serbian Biochemical Society for giving me this opportunity to shape the premiere forum in biochemistry in the region. We have been tremendously fortunate to have Mihajlo B. Spasić as the first Editor. He nurtured this Conference (and Society) through its re-starting years as it grew in quality and relevance. Clearly, following in his footsteps is a challenge.

We have invited Djuro Josić from the University of Rijeka and eight experts from four major universities in Serbia to give lectures at the 6th Conference. The visit of our dear colleague from Croatia is a part of an initiative for closer collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme that was established by FEBS in 2010. We have also invited students at the final years of PhD studies to present their work in our Proceedings as Abstracts. Official languages at the Conference will be Serbian, Croatian, and English.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers and to all respected colleagues who accepted the invitation.

*Editor of the Proceedings*
*Ivan Spasojević*

# Directed evolution of cellobiose dehydrogenase for higher activity

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Cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in yeast *Saccharomyces cerevisiae* for extracellular expression. Recombinant CDH produced in yeast had lower specific activity of 0.6 U/mg than native CDH produced in *P.chrysosporium*. Recombinant enzyme showed similar substrate specificity for cellobiose and lactose. Optimal temperature and pH stability was slightly different compared to native CDH. The molecular weight of recombinant CDH was higher than molecular weight of native CDH (90 kDa) with a broad band on SDS electrophoresis gel at 120kDa that was result of hyperglycosylation. Results showed that CDH can be expressed in yeast *S. cerevisiae* that can be used in directed evolution experiments. CDH gene library was generated using error-prone PCR to create random mutations. Mutants were tested in microtiter plates for improved activity using adapted DCIP assay. Several mutants with increased activity were detected in microtiter plates and therefore purified and further characterized.

The gene for recombinant wild type cdh and one of the best mutants had been cloned in heterologous yeast *P.pastoris.* Mutant showed higher specific activity than rCDH. Temperature profiles were similar for both enzymes as well as pH optimum. Substrate specificity was similar in both enzymes with slightly higher Km for lactose in mutant than in rCDH. Molecular weight was similar to wtCDH from fungus (approximately 90 kDa). Obtained results showed higher productivity in *P.pastoris* and difference in specific activities between mutant and rCDH. We used *P. pastoris* for efficient production and characterization of enzymes.