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Networking Guide

5th Bonn Humboldt Award Winners' Forum
"Frontiers in Neuroscience:
Multi-Scale Analysis of the Nervous System –
From Molecules to Circuits"

Bonn, 7 - 10 October 2015

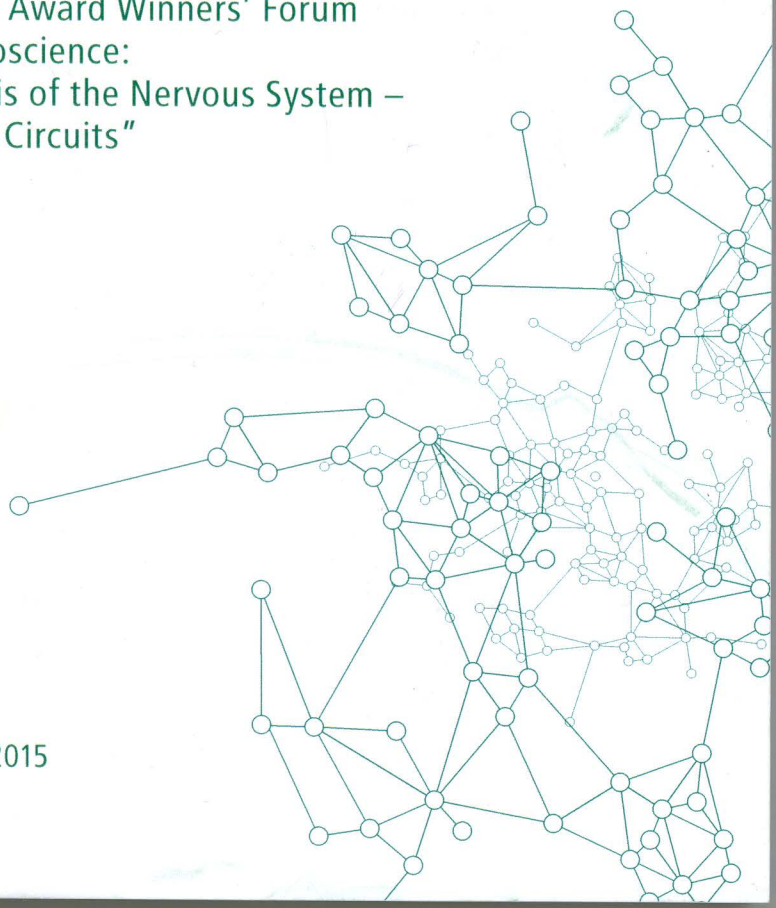


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D-amino acid oxidase as a tool for biotechnology and medicine diagnostics.

D-amino acids (D-Ser, D-Ala, D-Glu etc.) are regulators of many processes in organism including regulation of nervous system. Low level of D-Ser is direct indicator for possible development of schizophrenia (Chumakov et al. PNAS 2002). D-amino acid oxidase (DAAO) is responsible for control of level of D-amino acids in organism. The enzyme can be used in biosensors to determine concentrations of D-amino acids *in vivo*. The main drawback is wide substrate specificity of native enzyme.

DAAO is also widely used in biotechnology for fine organic (especially chiral) synthesis. For example, it is used in two-enzyme biocatalytic process of preparation of 7-aminocephalosporanic acid (7-ACA) from cephalosporine C (CephC). 7-ACA is main sinton for preparation of semi-synthetic cephalosporins. Unfortunately, native DAAO from yeast *Trygonopsis variabilis* (TvDAAO) used in the process has not sufficient catalytic activity with CephC as well as thermal and chemical stability.

In our laboratory we cloned gene of TvDAAO and developed highly efficient expression system in *E.coli* cells. Using rational design approach we prepared more than 60 mutant forms of TvDAAO and succeeded to crystallize one mutant and solved its structure with 1.8 angstrom resolution. Many mutant had higher catalytic efficiency in CephC oxidation, higher thermal and chemical stability. Many amino acid changes resulted in mutant TvDAAO specific to certain D-amino acid. These mutants can be used in biosensors for specific analysis of required D-amino acids *in vivo*.

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