



Serbian Chemical Society  
Serbian Young Chemists' Club



# Eight Conference of the Young Chemists of Serbia

## *Book of Abstracts*

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## Optimization of the expression conditions of fluorescently labeled $\alpha$ -synuclein in *Escherichia coli* by response surface methodology and proteolysis by tobacco etch virus protease

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Alpha-synuclein is an intrinsically disordered protein prone to aggregation and it is involved in the formation of brain tissue amyloids in patients with Parkinson's and other neurodegenerative diseases. By fluorescently labeling recombinantly expressed proteins, the processes of expression and purification of the same can be easily visually monitored. In a previous work, we have constructed three plasmids for the expression of  $\alpha$ -synuclein fused C-terminally to His-tagged mCerulean3 through a polyasparagine linker and tobacco etch virus (TEV) proteolytic site. Here we have transformed these vectors into *Escherichia coli* BL21(DE3) and BL21(DE3)pLysS and optimized the expression conditions by response surface methodology (temperature, time after induction, concentration of induction reagent) of the chimeric proteins coded by vectors. Expression of the chimeric protein was tested at optimal conditions in different media and terrific broth gave the highest yield. The obtained chimeric protein was purified by immobilized metal-affinity chromatography (IMAC), yielding ~29 mg of chimeric protein per liter of medium and was shown to be successfully proteolyzed by TEV protease, giving a fragment of  $\alpha$ -synuclein of expected electrophoretic mobility. After another round of IMAC, the obtained  $\alpha$ -synuclein of native sequence was mainly found to be in the flow-through.

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