

## **Spectral-luminescent characterization of $\beta$ -ketoenoles as amyloid-sensitive fluorescent probes for amyloid fibrils detection**

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Amyloid fibrils are  $\beta$ -pleated self-assembled protein nanostructures; their accumulation in different cells and tissues results in cells damage and malfunction. Such nanofibers are widely investigated because of their use in biomedicine (as a therapeutic target against neurodegenerations and amyloidoses) and in bionanotechnology (as nanowires and thin films). Since dye-based fluorescence assay is one of the convenient techniques for detection of amyloid aggregates *via* binding to  $\beta$ -sheet grooves, there is an interest in search of sensitive dyes for this application.

Herein series of  $\beta$ -ketoenole dyes with different tail substituents were investigated by spectral-luminescent methods as sensors for amyloid fibrils detection. Free dyes have low intrinsic fluorescence intensity and do not give a fluorescent response upon the presence of native insulin and lysozyme.

Binding of  $\beta$ -ketoenoles to amyloid fibrils of insulin and lysozyme results in an increase of the dye fluorescence intensity; its value depends on the structure of  $\beta$ -ketoenole tail substituents. The highest fluorescent response to the presence of insulin and lysozyme fibrils (in 60 times compared to the native protein presence) is shown by  $\beta$ -ketoenole with hydroxyethylamino and propoxyphenyl tail substituents (N185). Fluorescent quantum yield of the most promising dyes bound to insulin fibrils reaches 0.19-0.40, while for free dyes it is in 0.0004-0.005 range; average decay time of fluorescence intensity of these dyes bound to insulin and lysozyme fibrils is in the range 0.9-1.3 ns. Besides, the dye with methoxyethylamino and dimethylamino phenyl tail substituents (N155) could also be applied for the study of the kinetics of insulin fibrillization; its linear concentration range of insulin fibrils detection is 0.8-35  $\mu\text{g/ml}$ .

In conclusion, we report  $\beta$ -ketoenoles as fluorescent molecules sensitive to amyloid fibrils and propose them as probes for the studies connected with amyloid-related diseases. *RISE (grant agreement No 645628) and NASU Specific Research Program "New functional substances and chemical industry materials" No 8-17 supported this study.*