Spectral properties of chemically modified forms of thioflavin T

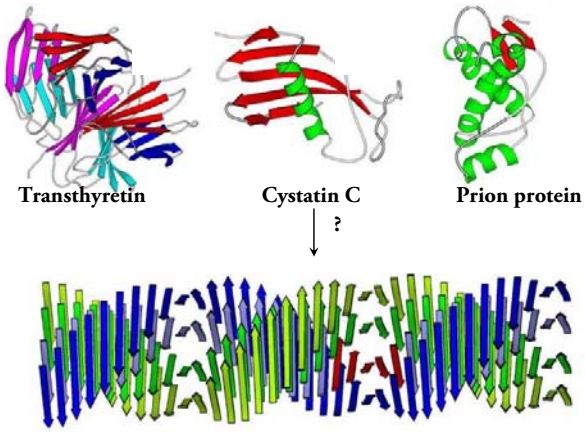
O.I.Povarova[1], A.I.Sulatskaya[1], A.A.Maskevich[2], K.K.Turoverov[1], I.M.Kuznetsova[1]

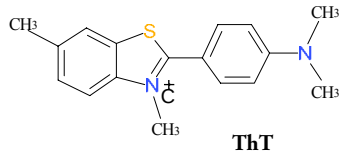
[1]Institute of Cytology RAS, Saint-Petersburg, Russia;

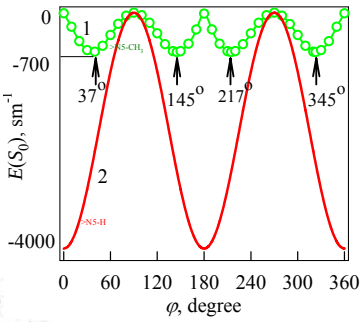
[2]YankaKupala Grodno State University, Grodno, Belarus

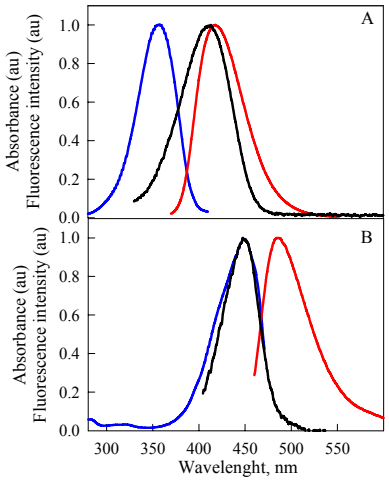
Amyloid fibrils are polypeptide aggregates that have been extensively studied due to their association with a variety of neurodegenerative diseases, the most famous being Alzheimer's disease. Such conformational disorders are caused when native proteins misfold and form self-assembled, insoluble aggregates, which accumulate in different organs or tissues. The structure of amyloid fibrils has been found to be composed of antiparallel β-sheets, which are oriented perpendicular to the long fibril axis. The fibrils themselves are nonbranching and can be up to several micrometers long with a width between 8 and 10 nm. Although amyloid fibrils are mainly associated with neurodegenerative diseases, other proteins not related to such diseases have been found to form fibrils, too. It is known that potentially any protein could form amyloid fibrils under specific conditions and that fibril formation is an intrinsic property of the polypeptide backbone. Extensive characterization of amyloid fibrils has been made using various spectroscopy and microscopy techniques.

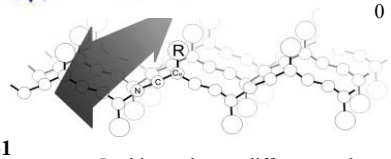
Thioflavin T (ThT) is widely used for the detection and study of the amyloid fibrils structure. The dye has low fluorescence quantum yield in aqueous solution, which increases by thousands of times when the dye is incorporates into amyloid fibrils. According to our data the main reason of this phenomenon is the rotational motion of benzothiazole and aminobenzoyl rings relative to each other in solution of low viscosity and restriction of this motion on ThT incorporation in amyloid fibrils. Photophysical properties of ThT are substantially determined by the methyl group at N5 atom of benzothiazole ring. The presence of this group not only prevents a planar configuration of the ThT molecule but also diminishes the energy barrier of internal rotation. From our point of view, the most convincing model of ThT binding with amyloid fibrils is the model according to which the dye incorporates in amyloid fibrils in monomer form in the "grooves", formed by side chains of amino acids of beta-sheets, oriented perpendicular to the long axis of amyloid fibril (Krebs et al., 2005)

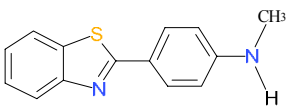






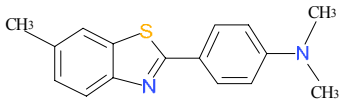




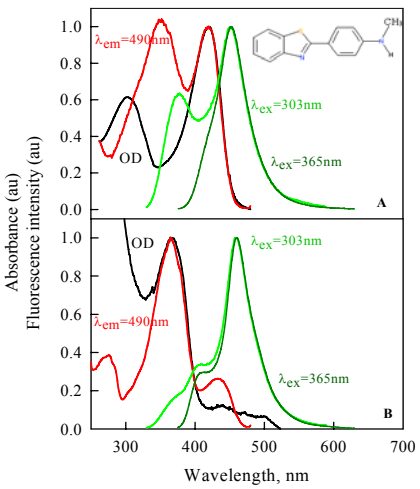


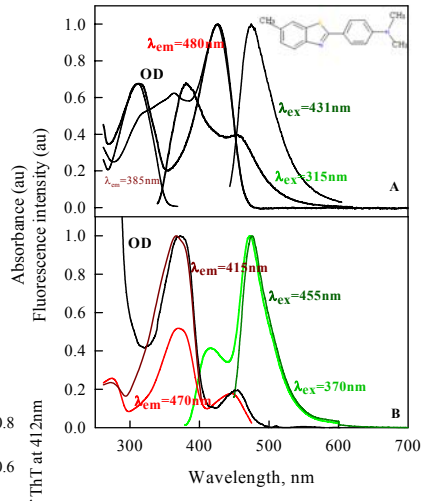
BTA-1

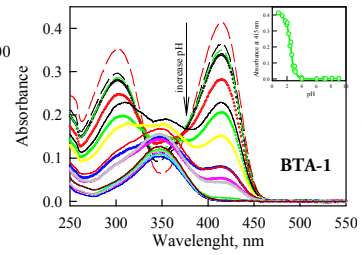
BTA-2

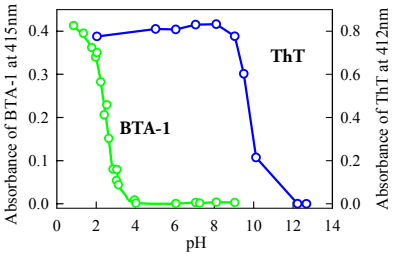


In this work two different analogues of the ThT, which differ from ThT by the lack of a methyl group at the nitrogen atom of benzothiazole ring were studied. It is shown that these analogues of the ThT, in contrast to the ThT, have significantly different pKa values characterizing protonation of the nitrogen atom of benzothiazole of the ring. In ThT pKa of this group lies in the alkaline pH range, and therefore when thioflavin T is used as a probe for the detection and investigation of amyloid fibrils, it always carries a positive charge at benzothiazole ring. The pKa of the ThT analogues is in acidic pH. Therefore, in a neutral aqueous solution spectral properties of the ThT analogues are fundamentally different from the properties of thioflavin T. The quantum yield of these compounds at neutral pH is close to unity, the degree of polarization is low. In solutions with pH 2.0, which were used in our studies, there is probably a mixture of neutral and charged forms. The properties of cationic form are similar to those of thioflavin T. It is shown that the absorption spectrum of the ThT analogues are significantly changed when they are bound to amyloids.









Anna Sulatskaya

Alexander Maskevich

Olga Povarova

Konstantin Turoverov

Irina Kuznetsova

This work was supported by RFBR (grants №13-04-02068, 12-04-90022-Bel_a and 12-04-01651)