Summary

Rapid development of modern technologies is rationale behind increasing demand for fluorescent dyes with application-tailored photophysical properties. These molecules show potential as biomarkers, fluorescent probes, laser dyes, or as key components of functional materials (e.g. organic light-emitting diodes). All these applications require a thorough characterizations of new dyes in order to fully determine their potential for material sciences or biomedicine. With the use of fluorescent probes it is possible to achieve visualization and dynamic information regarding the localization and quantity of biomolecules. Among light-stimulated probes, two-photon-excited fluorescent species occupy privileged spot due to several beneficial features in comparison with one-photon-excited probes. Namely, two-photon-based technique offers reduction of background fluorescence in living cells and tissues, reduction photodamage to biosamples, and a photobleaching phenomenon, offers better 3D spatial resolution and increase the penetration depth. At the current level of development, two-photon-excited probes should have large two-photon action cross section (product of *two-photon absorption cross section* and *fluorescence quantum yield*) exceeding 50 GM as well as excitation wavelength within a window of 650-1100 nm. However, for some applications it is beneficial that a probe exhibits a significant change in fluorescence upon binding to a target. Dyes containing difluoroborate (BF_2) group(s) as two-photon-excited probes have high potential in bio-imaging applications. Main advantages of BF_2-carrying molecules are tunable fluorescence quantum yields, strong absorbance and narrow emission bandwidth, high stability in biological environment and low toxicity. Many fluoroborate dyes are small neutral compounds that can penetrate cell membranes. Design of new difluoroborates with adjusted photophysical properties might be achieved, inter alia, based on combining electron donating and electron accepting groups, connected by π-conjugated bridges, leading to dipolar or quadrupolar skeleton.

According to one of hypotheses, the formation of insoluble amyloid (Aβ) and Tau amyloid fibrils is related to the origin of neurodegeneration in Alzheimer disease (AD). However, the failure of recent clinical trials of therapeutics inhibiting amyloids formation shows that the design of effective anti-AD drugs is a complex problem and further studies are required on the formation of various polymorps of amyloids as well as their imaging *ex vivo* and *in vivo* in mouse models. It should be highlighted that none of the proposed probes offers a full set of desired properties required for two-photon imaging of soluble and insoluble forms of amyloids. Given the importance of the subject, it is pivotal to develop efficient molecular probes that could be used in imaging of amyloids at various stages of aggregation processes. Encouraged by these advances and challenges and having previous experiences with efficient fluorophores, in the proposed project, PI from Nicolaus Copernicus University (NCU) and his associates from Wroclaw University of Science and Technology (WUST) aim at the design of new efficient two-photon-excited probes containing difluoroborate groups with tailored photophysical properties. Research hypothesis can be formulated as follows: BF_2-carrying dyes based on dipolar and quadrupolar cores will allow to obtain high two-photon cross sections, a pivotal condition for two-photon-based microscopy. More specifically, the project aims at design and sythesis of molecules that will be useful in imaging of Aβ fibrils. However, before test with the use of amyloids will be guided, the detailed studies of properties of new dyes are needed and their properties understood in details. The design will be based on theoretical models, electronic-structure theories and advanced computer simulations. To implement the outlined fluorescent probes design strategy the selected compounds, with predicted large two-photon absorption cross sections, will be synthesized and their one- and two-photon absorption and emission will be thoroughly characterized using experimental techniques at WUST. It is expected that, by design, the final set will encompass molecules with variable fluorescence quantum yields, a feature that can be tuned based on small structural modifications. Subsequently, those of the synthesized probes showing negligible fluorescence quantum yields in unbound state (e.g. in solutions) will be applied in imaging of amyloid fibrils aggregation using two-photon microscopy. In parallel to these efforts, the properties of newly synthesized dyes will also be studied in the light of their dynamics and aggregation (UV/Vis spectra of solutions of various viscosities, fluorescence in mixtures of solvents leading to aggregation, photoluminescence in crystals) to determine their potential for various applications in material sciences.