Novel chromophores for two-photon excitation fluorescence microscopy and optical limiting

Two-photon absorption (2PA) is a non-linear optical phenomenon with broad scope of applications. It has already been applied, or is under intensive investigation, in fields such as: optical limiting, multiphoton pumped frequency-upconversion lasing, polymerization-microfabrication, 3D-data storage, photodynamic therapy, two-photon excited fluorescence etc. All these applications originate from two special features of 2PA:

- **a)** the amount of 2PA depends on the square of the light intensity, so in a focused laser beam it depends on the fourth power of the distance from the focus, which leads to much higher spatial resolution than could be achieved by one-photon absorption
- **b)** 2PA involves formation of an excited state with two photons of half the nominal excitation energy, and these low energy photons penetrate deeper into most media (scattering and absorption are both reduced)

In the past decade, significant progress has been made in the design and synthesis of two-photon absorbing materials with very large cross-sections (σ). These investigations established the main molecular scaffolds to be considered (D−π−A, D−π−D, D−A−D, A−D−A; where D and A are electron-donors and –acceptors respectively and "π" is a π-conjugated bridge). For certain applications, the two-photon-property requirement has basically been met with current dyes, but, for two-photon technology to realize its full potential, the development of more two-photon-active chromophores, together with important secondary properties such as processability, biocompatibility, photostability and durability will be vital. Therefore, when designing the next generation of two-photon materials, one should not only consider their two-photon absorptivity but also tailor their secondary properties to meet their specific applications.

The goal of this project is to produce new materials to be used in two prospective applications: two-photon excited fluorescence microscopy and two-photon based optical limiting. Although the type of requirements and hence chromophores will be different in these two exciting areas, we feel that pursuing both these directions in parallel will benefit the project *via* cross-fertilization with ideas. We will experimentally execute the following aims:

1) Prepare novel dyes for commercial applications in two-photon excitation fluorescence microscopy possessing not only high σ but also photostability, water solubility and high Φ_{fl}

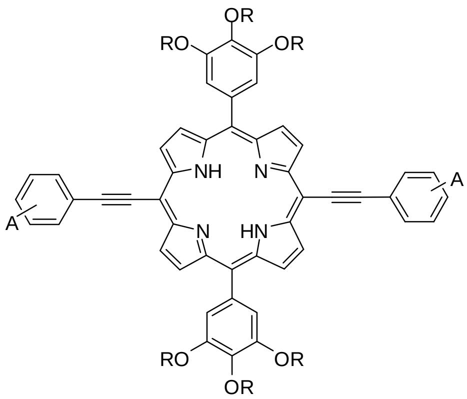
- elaborate new intrinsically hydrophilic cores possessing donor- or π-character
- prepare quadrupolar and octupolar molecules comprising of barbituric acid moiety
- synthesize absorbers possessing heterocyclic units with strong electron-withdrawing character and at the same time high Φ_{fl} and hydrophilicity

2) Investigate π-expanded porphyrin and corrole based architectures for the use as optical limiters

- synthesize *trans*-A_2B_2-porphyrins with phenylethynyl and alkoxyphenyl substituents at *meso*-positions
- prepare *trans*-A_2B-corroles with phenylethynyl and alkoxyphenyl substituents at *meso*-positions
- elaborate the general method for the preparation of π-conjugated corroles

3) Investigate the fundamental spectroscopic properties of new functional dyes in Warsaw using spectrophotometer and spectrofluorimeter belonging to our research group. More advanced photophysical measurements will be performed in Prof. Lucia Flamigni's group (ISOF, Bologna).

4) Measure 2PA cross-sections (Prof. Aleksander Rebane's group, Montana State University, Bozeman). Rebane's group has developed advanced ultrafast nonlinear spectroscopy tools that allow precision quantitative measurement of two-photon absorption spectra along with determining high fidelity two-photon absorption cross-sections.



14

In summary the implementation of this research proposal will provide new generation of two-photon excited fluorophores for the use in microscopic diagnostic of diseases. First optical limiter based on porphyrinoid core and possessing appropriate secondary properties to be applied in real world can also originate from this project. Parallel synthesis of new compounds and photophysical studies conducted by our collaborators will assure the timely progress of the project.