SHORT-TERM MOBILITY 2010

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Relazione finale Dott.ssa **Barbara Ventura**

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Two-photon absorption: theory, techniques and applications

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The research activity of Dr. Ventura during her Short-Term Mobility stay in the group of Dr. Mireille Blanchard-Desce at the University of Rennes 1 (Rennes, France) has been focused on the characterization of the two-photon fluorescence properties of a series of two-photon absorbers designed for non-invasive *in vivo* imaging. During the stay Dr. Ventura improved her basic knowledge on two-photon processes, had the possibility of utilizing the equipments available for the determination of two-photon absorption spectra and tested the performances of new two-photon absorbers as probes for biomedical imaging applications.

Two-photon absorption techniques are emerging in the study of interaction between light and matter, since offer several advantages with respect to traditional one-photon approaches, such as higher spatial resolution and use of lower-energy photons. These characteristics are particularly useful for the deep excitation of living tissues, with important applications in biomedical imaging. Knowledge on two-photon absorption processes is thus useful in view of exploring new features and new perspectives for photophysical research, to juxtapose to the traditional investigation of one-photon photoinduced processes.

Two-photon absorption (TPA) is a third order non-linear optical process where a molecule is excited by the simultaneous absorption of two photons. The energy absorbed by a two-photon process is quadratically proportional to the intensity of the excitation light and for this reason the first TPA observations became possible only with the advent of lasers. Among the lasers nowadays employed for fast spectroscopy studies, it has been demonstrated that only lasers with femtosecond pulses ensure accurate estimations of TPA cross-sections (σ_2), since longer pulses can lead to simultaneous one-photon absorption processes of excited states that give rise to overestimations of σ_2.[1]

The two-photon excited fluorescence of a molecule is directly proportional to its TPA cross-section (σ_2), its emission quantum yield and the quadratic power of the light intensity:

$$\text{TPEF} \propto \sigma_2 \,.\, \phi \,.\, I^2$$

It derives that high TPEF performances, required for two-photon imaging in living organisms, can be achieved for molecules having both a high emission quantum yield and a large TPA cross-section, and with a minimized residual one-photon absorption in the region of excitation. The key parameter to be maximized is, in fact, the so called two-photon brightness, i.e. $\sigma_2 \times \phi$.

The design of molecules that posses high two-photon brightness is thus fundamental for the development of TPA applications based on fluorescence (including imaging). It has been shown that quadrupolar molecules, i.e. symmetric conjugated molecules bearing two electron-releasing (D) or electron-withdrawing (A) end-groups (of general structure D-π-A-π-D or A-π-D-π-A), may present high TPA responses and strong fluorescence solvatochromism.[2] Their large non-linear response derive from a complex combination of end-groups donor or acceptor strengths, bridge length and type and other parameters.[1] It has been shown, moreover, that the covalent assembly of TPA chromophores in dendritic architectures while controlling interchromophoric interaction leads to very large TPA responses.[3]

Following this strategy, hydrophilic dendritic spherical structures that confine a large number of TPA chromophores have been recently synthesized in Rennes. A schematic representation of two dendrimers of this type is reported in Figure 1.

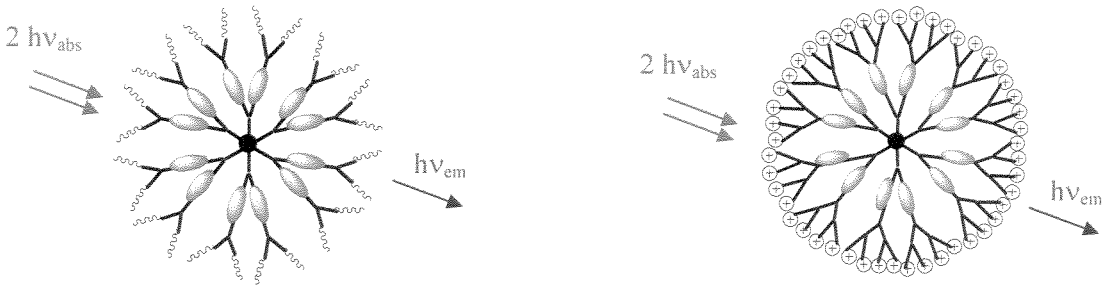

Figure 1. Schematic representation of two nanodots with different surface groups.

They represent a new class of fully organic nanoparticles that confine a large number of highly emitting biphotonic fluorophores (the blue ovals in the drawings of Figure 1), whose structure have been optimized for TPA absorption. Earlier studies performed on lipophilic derivatives revealed, in fact, exceptional one and two-photon brightness. These nano-objects were named "organic nanodots" since they represent a biocompatible and non-toxic alternative to quantum dots for imaging purposes.[3,4]

The Short-Term Mobility project has taken into consideration the characterization of the one- and two- photon photophysical properties of a series of nanodots of different size and nature. The aim of the study was to derive information on the two-photon excited fluorescence cross section ($\sigma_2\phi$) of the different nanodots not only in organic solvents but also in aqueous media, in view of their possible use as two-photon excited emitters in biological systems.

As a first step, the one-photon absorption and emission properties of the compounds have been fully characterized in different solvents. Hypothesis on the dependence of the observed photophysical properties on the size and the type of peripheral groups of the nanodot, in relation to the environment, have been derived. Further investigations at the ISOF Institute in Bologna will clarify some aspects of the peculiar one-photon luminescence behavior shown by these compounds.

The two-photon absorption cross sections of some selected nanodots have then been determined from their two-photon excited fluorescence. The TPA cross-sections (σ_2) is determined from the two-photon excited fluorescence (TPEF) cross sections ($\sigma_2\phi$) once established the fluorescence quantum yield (ϕ). TPFE cross-sections were measured using a Ti-sapphire laser delivering 150 fs excitation pulses and operating between 700 and 990 nm, according to a protocol that avoids contributions from excited-state absorption.[5] Fluorescein in 0.01 M NaOH was taken as reference, since its TPEF cross-sections are known, and corrections for the refractive index of the solvents were also considered. The quadratic dependence of the fluorescence intensity on the excitation intensity was verified for each data point.

Finally, the compounds that could combine an appreciable two-photon brightness with a suitable water solubility were selected to be tested as two-photon excited fluorescent probes in *in vivo* imaging measurements. The experiments consisted in the injection of a solution of the examined nanodot in a tadpole (Xenopus), and in the visualization of the vascular system of the small animal performed with a confocal microscope equipped with a two-photon excitation source. Good quality images of blood vessels have been obtained, indicating the high performances of these new chromophores for biomedical applications.

In conclusion, with the Short-Term Mobility project Dr. Ventura enhanced her scientific background, currently in the field of conventional mono-photonic photoinduced processes, with knowledge on two-photon absorption processes, bringing to ISOF Institute new competences and establishing an important collaboration with one of the few research groups at the forefront in Europe in the 2PA field.

References

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