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REFEREE REPORT

"Linear and Nonlinear Optical Properties of New Materials Bound to Biomolecules"
Doctoral Thesis presented by Marco DEIANA
Wroclaw University of Technology

Mr. Marco DEIANA is presenting a manuscript of 221 pages. 446 bibliographic references are cited. The manuscript is divided in 13 chapters (the last one being the Conclusion). The first 5 chapters introduce the state-of-the-art of the topic, and the original results obtained by Mr. Deiana are developed in Chapters 6 and followings.

As above-mentioned, Chapters 1 to 5 describe the literature and basic principles in the form of reviews. The present knowledge on DNA (Deoxyribonucleic Acid), HSA (Human Serum Albumin), G-Quadruplexes, molecular switches, optical methods, and two-photon absorption are introduced. Detail and care are brought by M. Deiana, who gives the basic definitions, for example of the different types of DNA structures (A-, B-DNA, etc.), and of photochromic compounds as representatives of molecular switches.

A special focus on physicochemical consequences and phenomena, consecutive to interactions between these biological systems and their environment or specifically introduced molecules is done. For example, the consequence of intercalation of metals, metal complexes, and organic molecules, on the DNA compaction/decompaction, are presented, and their use as turn-on probes. Also, the introduction of photo-switchable systems, such as photochromic substances, is presented. The outcomes as turn-on probes, in drug delivery, in photo-pharmacology, are described in the manuscript.

In these introductory chapters, Mr. Deiana approaches his subject from photophysics and optics, as well. Sound basics in these domains are given, including description of RET (resonance energy transfer), fluorescence polarization, CD (circular dichroism) and TPA (two-photon absorption).

The subjects tackled in this doctoral thesis are very wide, thus it is impossible to introduce them in a exhaustive manner. In this sense, Mr. Deiana made appropriate choices to provide with the relevant information, thus avoiding dispersion. The content of these chapters is well documented, with appropriate references to previous works. Much effort is made to guide the readers, very didactically, sometimes from scratch, in order to give them the necessary information for the following chapters.

A second series of chapters (Chapters 6-12) describes Mr. Deiana's own work. This can be divided in three groups of chapters. In the first two, Azo (azobenzene derivatives) are used as photo-triggerable compounds and introduced in DNA and HSA-based structures. In the last one, the study focuses on Ant-PIm, an anthracene-based fluorophoric polymer, and its interaction with DNA, G-Quadruplexes, and HSA.

The first group (Chapters 6-7) deals with the use of photochromic Azo for light-triggered functions in DNA. Several Azo compounds bearing amide and amine functions were designed, synthesized, and introduced in

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DNA as photoactive components. The Azo-DNA adducts were studied by several spectroscopic techniques, including UV-visible absorption, and CD. The ability to shuttle, upon light trigger, between the A- and B-forms of DNA was evidenced, opening the way to supramolecular DNA-based devices, for example for gene delivery. Apart from this proof of concept, deeper and more fundamental studies were also carried out, focusing on the structure-activity relation. The Azo-DNA interaction is stabilized, thanks to molecular rearrangements, and high stability constants (more than $10^5\ M^{-1}$) were determined, also supported by the ability of Azo compounds to replace ethidium. Thermodynamic parameters were determined, showing clear difference between trans- and cis-Azo forms: enthalpy signs (negative enthalpy for the former, positive for the latter), binding mode (intercalate for the former, semi-intercalate for the latter).

The second one (Chapters 8-9) is about the interactions between the Azo compounds and HSA. As in the previous part, amine-bearing Azo were used, as well as chiral Azo bearing tyrosine (Tyr). Natively fluorescent and CD-active HAS undergoes spectral alterations, when an amine-bearing Azo is added, although its structure is only slightly affected. A careful analysis of the fluorescence quenching is done (Stern-Vollmer analysis, Förster RET model), leading to the conclusion that the mechanism is static. Further spectral alterations were followed, while light-triggered trans- to cis-Azo reaction was induced. The spectral signatures, among them the distortion of the CD bands, gave some information on the proximity of the HSA's tryptophan residue to the Azo. In this work, a new supramolecular adduct, based on HSA, was obtained and studied, presenting the ability for a chiral molecular recognition probed by optical means.

The following chapters (Chapters 10-12) deal with the introduction of Ant-PIm in double-stranded DNA, G-Quadruplex, and HSA. The study aims at tackling the present limitations on the implementation of investigation techniques to *in vivo* environments. To address this issue, Mr. Deiana proposes TPA, and particularly TPLSM (Two Photon Laser Scanning Microscopy), enabling high-resolution 3-D images even in complex biological media, and the use of water-soluble cationic polymer bearing a fluorophore with high two-photon cross-section and high brightness. Addition of DNA to Ant-PIm leads to a significant hyperchromism. Fluorescence behaves in quite unconventional manner, showing a decay up to ca. 5 equivalents of DNA, and an enhancement upon further addition of DNA. An explanation is proposed, in terms of dimerization of Ant-PIm at low DNA concentration, which loosens when the latter is high. Thorough fluorescence studies, including lifetime decay measurements, reveal the static nature of the quenching, and thus the formation of a non-fluorescent complex, and a high thermodynamic binding constant is deduced. Complementary techniques, namely CD and Fourier-Transform Infra-Red spectroscopy (FTIR), bring some evidence of the morphology modification of B-DNA, due to its interaction with the fluorophoric polymer, by groove binding. With G-Quadruplexes, or more precisely with a specifically chosen guanine-rich telomeric DNA sequence (named **1** in the manuscript), a similar series of studies reveals the intercalated structure of Ant-PIm-**1**. Upon intercalation, fluorescence intensity is increased, and a hypsochromic shift is observed. However, the two-photon absorption cross-section follows the decreasing order: Ant-PIm duplex, ANT-PIm (free), ANT-PIm-**1**. In the last chapter before conclusion, HSA recognition process by Ant-PIm is studied. CD and FTIR reveal the involvement of tyrosine in the interactions, and fluorescence measurements a static energy transfer. This study is reportedly the first one of such kind, with a two-photon responsive water-soluble polymeric probe. It paves the way to applications of clinical interest.

The last chapter (Chapter 13) of the manuscript is the general conclusion.

Apart from a few unavoidable typos, the manuscript is well written, the experimental parts or clearly described, and the arguments logically presented. It highlights a series of very interesting chromophores (Azo and Ant-IPm), which seem to fulfill the requirements of molecular engineering, to investigate biological media or to act as photo-triggers. Not only functionalities are described, but also studies in depth,

to determine physicochemical parameters, and to elucidate structures and mechanisms of phenomena are carried out. This work is clearly extending the scope of applications in biology, and the investigations are pushing new frontiers in this way. 9 publications come from this PhD work, with almost all of them published. What is particularly remarkable is the wide range of journals where they appear: optics, physical chemistry, biology.

For all the above-mentioned reasons, upon review of the manuscript, Mr. Marco DEIANA's work deserves to be publicly presented, and defended. Provided that the Graduate Committee gives a positive decision, Mr. Deiana's work fulfills the requirements for receiving the PhD in Materials Engineering.

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