

## **REVIEW**

### **Doctoral thesis - MSc Eng. Marco Deiana**

### **Linear and nonlinear optical properties of new materials bound to biomolecules**

Ph.D. of MSc Marco Deiana was performed at the Faculty of Chemistry at the University of Wrocław. Dr Katarzyna Matczyszyn was thesis supervisor.

The subject of the research described in the dissertation are biological materials - DeoxyriboNucleic Acid (DNA) and Human Serum Albumine (HAS) that play the most important role in the functioning of all living organisms. For the purpose of his work, the PhD student has explored the important processes and mechanisms responsible for the interaction of biological materials with photonic dyes. The main objective of the study was to determine how processes are controlled by the complex interaction of bio-compounds.

Numerous research methods such as UV-vis absorption, polarized fluorescence, circular dichroism, two-photon emission spectroscopy, infrared (FTIR) studies and others. The spectroscopic experiments were also supported by thermodynamic investigations. These have allowed to characterize the selected organic dyes and interaction with biological materials. The results of the study can be used to simplify the modeling of real complex biological systems and for potential applications in photonics.

The dissertation is composed of many chapters (13) - the first six chapters represent the state of knowledge and review on DNA, HAS as well as introduction to optical methods and two-photon absorption and constitute a comprehensive introduction for readers. The remaining chapters of the doctoral thesis present the results, discussion and conclusions.

The experimental chapters are clearly written and their merits testify to the excellent preparation of the student for the research work. The experimental part of the dissertation includes a description of the basic measurement techniques that the author uses to solve the set objectives, as well as the results of research on the properties of biomolecular materials and selected photosensitive materials and their interactions. It should be emphasized that each chapter of the experimental part is topped up with a concise summary.

A wide range of photochromes were investigated: Azo-2N and Azo-3N of trans and cis configurations, water-soluble azobenzen (bis-Azo-2N, bis-Azo-3N, bis-Azo-4N) containing different linear unsubstituted polyamine group, Azo-LL-Tyr, Azo-DD-Tyr, Azo-LD-Tyr, two-photon probe Ant-PIIm. The above mentioned chromophores the author uses to study the their interaction with DNA and HSA.

The studies done by the doctoral student let to:

- determine the influence of Azo-compounds on DNA conformation changes,
- show how differently the trans and cis forms affect DNA and HSA
- determine the intercalation of the helix ,
- defining the key role of azo in stabilization of the HSA-photochrom adducts,
- demonstrate azo dyes ability to induce changes in the intrinsic DNA morphology,
- describe the azobenzene-DNA structural-activity relationship,
- investigate photoswitching properties of the azo - DNA systems,
- investigate of chirality of the azobenzenes-biological systems,
- develop for the first time a report on the use of water-soluble two-photon azo-derivative chromophores as bio-sensors.

In this point I should underline very huge contribution of the PhD student and highlight the wide range of researches and methods that have allowed to develop a comprehensive picture of DNA and HSA behavior in the presence of photoactive azo-chromophores. The general conclusion drawn on the basis of the wide range results showed the possibility of using azo-materials to model future carriers for gene therapy as well as in optoelectronics.

The most substantive comments:

1. How far the results of the study can be extrapolated to the situation in the actual biological membrane that besides proteins contains lipids and other types of biomolecules? Thus, how the in-vitro model tests can imprint biological systems in full-blown models.
2. In azo-dye applications - especially important in therapeutic applications - toxicity is a significant problem – thus my question is how much azo-dyes used in the experiments are toxic.
3. The work shows that trans and cis form of azo-chromophores has a significant effect on DNA - what is the molecular mechanism responsible for this, eg, DNA intercalation, whether only hydrophilic / hydrophobic interactions.
4. Fig. 4.7 is incorrect or needs more explanation.
5. What is  $1/2 h\nu$  in Fig. 5.1.,

6. Energy transfer description e.g. p. 149, 165, 169 - in some parts of the manuscript it is difficult to find what is a donor and what is an acceptor. Table 9.1. – what is  $\tau_1$ ,  $\tau_2$  and  $\tau_{\text{average}}$  – description parameters are missed,
7. Temperature studies have been performed - fluorescence quenching as a function of temperature, dynamic and static quenching. Have you investigated whether the process is reversible – have been the temperature tests also carried out from higher to lower temperature? The destruction of the system may have occurred,
8. Temperature tests showed changes in fluorescence - what do you mean by "specific interaction" (p.136).

#### Editorial remarks

The results of the research and their discussion are well documented by many elaborated drawings, images, graphs and tables. However, I have editorial notes because a description of the discussion of the research results has raised some of my doubts and reservations and I would like to submit my comments.

1. In my opinion the work is surprisingly extensive for this species of scientific dissertation. The reason for this is the composition of the text, which consists in repeating the same research methods in many experimental chapters.
2. There is no description of some abbreviations and symbols in the text - it is annoying even more because there is no shortcut list in the first pages.
3. In many illustrations, schematic drawings, equations etc. explanation of symbols and shortcuts is also missing.
4. Using a different number in *Publication List* and in *References* is unnecessary, confusing, and can also be annoying.

However, these minor editorial weaknesses absolutely do not affect my very high evaluation of the doctoral dissertation. In summary, I would like to underline once again that the work covers a very large, interesting new research materials, it determines many parameters confirming also non-linear properties, which allowed to fully characterize the studied systems.

The dissertation is not only an important contribution to the basic knowledge of organic systems and their interaction with DNA and HSA, but also the results of the doctoral thesis can find practical applications. Reading the dissertation one can see that PhD student put a lot of work during its implementation of his dissertation, proposed interesting topics and studied the new derivatives of dyes and used, along with conventional methods, the most-advanced method like two-photon spectroscopy. The results of her work can certainly find many applications in chemistry, physics, biology and medicine and other technologies. However, in my opinion the most important

achievements are - determining the chirality of selected materials and showing their two-photon properties for their potential applications in medical diagnostics.

The doctoral dissertation is an original solution to the scientific problem and shows good experimental skill of the student in the field of molecular spectroscopy and material engineering of new azo-materials. Also it indicates the ability of him to carry out his own scientific work. It is very important to emphasize that Mr. Marco Deiana is the first of the authors of nine publications.

**I believe that the dissertation presented to me meets the requirements set by the law on academic degrees and the academic title and I submit the application to the Faculty of Chemistry to accept MSc Eng. Marco Deiana's thesis for further stages of the doctoral dissertation.**

**I evaluate the work very positively and I am submitting to the Faculty Council an application for distinction.**