MC-CIG 334257/PHOTOBIODRUG: Final report

1. PUBLISHABLE SUMMARY

The objective of the project was to investigate the photoreactivity of drugs with biomolecules by means of spectroscopic techniques. These interactions are of great importance because the investigated drugs are known to absorb UV light and generate photosensitising side effects directly related to phototoxicity, photomutagenicity and photoallergy.

Model systems that aim to simulate the real drug/biomolecule interactions were first synthesized; their photoreactivity were investigated and compared to the real drug/protein complexes. Compounds containing drugs such as naproxen (NPX), flurbiprofen (FBP) and fenofibric acid (FA) covalently linked to tyrosine, histidine or tryptophan were synthesized, among others. In some cases, spacers of different length were used to separate the chromophores and evaluate the possible changes in their photoreactivity. The mechanism of photoproduct formation varied depending on the drug coupled to an amino acid; thus, for NPX a type II photooxygenation mechanism triggered by the generation of $^1O_2$ was observed, while for FA a type I mechanism was predominant. In general, the photoreactivity was highly modified by the spacer. In all cases stereoselective dynamic fluorescence quenching was observed. In the drug/protein complexes, fluorescence experiments clearly showed that the protein microenvironment plays a significant role in the conformational relaxation of the drug. This stereo-selectivity was possibly related to the modes of drug binding to the protein, a process of pharmacological relevance. Laser flash photolysis experiments evidenced that the reaction mechanism of the drug within the protein was clearly different than in the bulk solution. The yield of photoproduct formation varied between two proteins and was also different to that of the bulk solution. The reaction mechanism in the presence of protein was again supported by the results obtained in model FA-amino acid dyads. This reinforces the value of linked systems as models for non-covalent drug/protein complexes. In addition, a series of dyads where the chromophores are separated by a rigid spacer evidenced a rapid triplet-triplet energy transfer process via through-bond mechanism followed by a through-bond triplet exciplex formation; this was the first time that this process has been observed in related systems. Finally, the photoreactivity of alternating adenine-thymine and guanine-cytosine duplexes have been evaluated. The experimental results combined with theoretical calculations evidenced the existence of stabilized high energy mixed states.

Dr. Vayá has consolidated close collaborations with the groups of Dr. Markovitsi and Dr. Improta, and he has also established new collaborations with other research groups. A number of articles have been published during this project and others that are in preparation will be submitted in the early future. He has also participated in national and international conferences as speaker. Dr. Vayá is following the natural steps to finally get a permanent position at the Host Institution. The level of independence the researcher has reached is in accordance with the possibilities that the Host Institution offers to researchers at this stage. Thus, he was the principal investigator of two additional projects, one funded by the Technical University of Valencia and other funded by the autonomic government (Consellería d'Educació).

2. PROJECT OBJECTIVES FOR THE PERIOD

In order to accomplish and succeed in a proper development of the project, a work plan divided into several complementary activities following an increasing order of complexity was designed. As a first approximation, synthesis of model systems composed of a drug covalently linked to an amino acid or nucleobase were proposed because these systems have proven to be suitable models for the elucidation of the excited state interactions in non-covalent drug/biomolecule interactions. Thus, an important number of dyads were synthesized, where the drug (naproxen, flurbiprofen and fenofibric acid, among others) were directly linked to an amino acid. As a forthcoming step, the complexity of these systems was enhanced by separating the chromophores with linkers of different nature (lineal, cyclic or rigid steroid spacers). Hence, dyads composed of the drug ketoprofen separated with an acceptor chromophore by a steroid lithocholic acid spacer were prepared; the photochemical reactivity was investigated under different conditions (presence or absence of oxygen, solvent dependence, etc.). The main photoproducts obtained after irradiation of light of a variety of energies were detected and identified. The photophysical properties such as quantum yields, quenching constants, lifetimes, etc., were determined by means of spectroscopic techniques: laser flash photolysis and fluorescence (steady state and time-resolved techniques, from the femtosecond to the

nanosecond time domains). The photoreactivity of oligonucleotides composed of adenine-thymine and guanine-cytosine have been investigated. Systems of different length and structure (single and double strand helices as well as hairpins and dumbbells) have been studied by means of fluorescence spectroscopy. The experimental results have been supported by theoretical calculations performed in collaboration with different research groups. As a final step, the photoreactivity of the proposed drugs in the presence of biomolecules have been evaluated. As an example, the photochemical reactivity of fenofibric acid in the presence of proteins have been studied, the yield of photoproduct formation being compared between different proteins. The mechanistic pathway leading to photoproduct formation was investigated by means of laser flash photolysis.

In summary, the main objectives proposed in this project have been successfully accomplished. A huge number of results have been obtained and have been published or have recently been submitted to publication in high impact journals (J. Am. Chem. Soc., Chem Soc. Rev., Chem. Res. Toxicol, etc.). Moreover, the results have also been published in national and international conferences and meetings as oral communications. Finally, other results still need additional investigations to be of sufficient quality to be published in top class chemistry journals.

3. WORK PROGRESS AND ACHIEVEMENTS DURING THE PERIOD

Research progress

As it was described in Part B of this PHOTOBIODRUG project, the research work was divided in different work packages following an increasing order of complexity. Thus, it was first proposed the synthesis of a number of dyads composed of a drug (normally a non-steroidal anti-inflammatory drug) covalently linked to the basic structures of biomolecules (e. g. amino acids or nucleobases). Figure 1 shows some examples of model linked systems designed along the development of this project. As the 2-arylpropionic acid drugs present a chiral centre, both (*S,S*)- and (*R,S*)- diastereomeric systems were synthesised in order to investigate possible conformation effects in their photoreactivity.

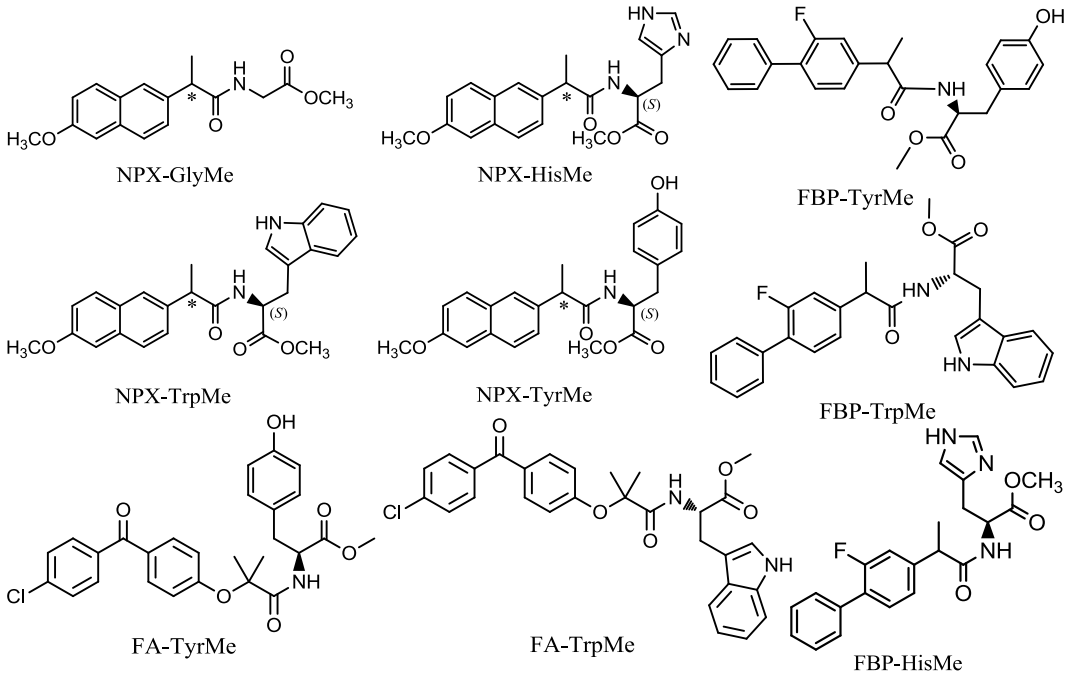

Figure 1. Examples of diastereomeric linked systems synthesized during this project.

A complete characterization of the photophysical properties of all designed dyads were performed by means of laser flash photolysis and fluorescence (steady-state and time-resolved measurements, from the femtosecond to the nanosecond time domains). Thus, quantum yields, quenching constants, lifetimes, etc., of the transient species detected by these spectroscopic techniques were determined. In general, we have found that the photoreactivity of these dyads varies from one diastereomer to the other. Hence, conformational effects are of crucial

importance in these interactions. Besides, these differences were also observed when the real drug/biomolecule interactions were investigated; this reinforces the use of tailored, well-defined linked systems as appropriate model compounds to investigate the excited state interactions involving fundamental processes such as energy or electron transfer, exciplex formation, etc. The photochemical reactivity of the dyads was also in accordance to the photophysical behaviour. In general, the main photoproducts derived from irradiation of the linked systems in different media (organic or aqueous solvent, presence or absence of oxygen, etc.) upon excitation at different wavelengths and using different light sources (photoreactor, medium pressure Hg lamp, etc.) were identified by ultraperformance liquid chromatography and characterized by spectroscopic techniques. As example, the photochemical reactivity of NPX coupled to Tyr, Trp and His varied greatly depending on the amino acid. The photochemical behaviour was in line with the photophysical results. Figure 2 shows the photodegradation and the oxygen-mediated photoreactivity of the different dyads in organic solvent.

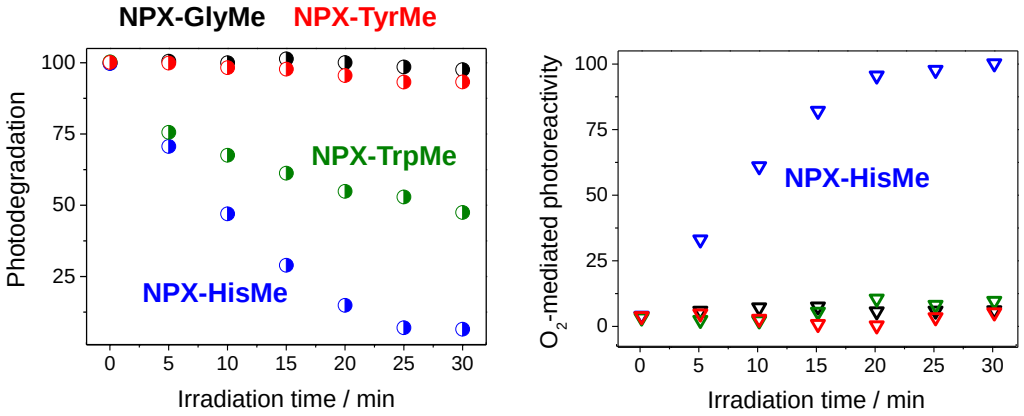

Figure 2. Photodegradation (left) and oxygen-mediated photoreactivity (right) of NPX-GlyMe (black), NPX-TyrMe (red), NPX-TrpMe (green) and NPX-HisMe.

The highest oxygen-mediated photoreactivity was observed for NPX–HisMe, while NPX-TyrMe showed the lowest photoreactivity. Overall, these results pointed to a type II photooxygenation mechanism, triggered by generation of $^1O_2$ from the ^{3}NPX* chromophore, which may result in the photosensitizing side effects derived from this drug. The photoreactivity of model dyads composed of FBP and amino acids were compared to that observed in the presence of proteins. In the dyads, a rapid dynamic quenching of ^{1}FBP* was followed by a slower quenching of the remaining ^{1}Trp* fluorescence. Both processes displayed a clear stereoselectivity; the rates were 2–3 times higher for the (*R,S*)-dyad. In addition, a red-shifted exciplex emission was observed, rising in the range of 100–200 ps. A similar two-step dynamic fluorescence quenching was also observed in the FBP/HSA complex, although the kinetics of the involved processes were slower. The characteristic reorientational times determined for the two enantiomeric forms of FBP in the protein showed that the interaction was found to be stronger for the (*R*)-form. An additional example was the photoreactivity of fenofibric acid (FA) in the presence of proteins. This drug is known to be phototoxic. Its photoreactivity was previously characterized in solution, however, its interaction with proteins was never been addressed. Our investigations have revealed the photochemical mechanism giving rise to photoproduct formation in the presence of biomolecules. The photochemical reactivity of FA was found to be different in solution than in the presence of the biomolecule, and even different between two proteins; the transient species generated after absorption of light were different in the different media. Again, in this work the use of well-defined dyad systems aided to confirm the mechanistic pathway leading to phototoxicity. Thus, the same transient species were detected in the dyads compared to that of the drug in the presence of the biomolecules. The yield of photoproduct formation was evaluated in in a variety of conditions, and noticeable differences were observed from one protein to the other. Figure 3 shows a schematic representation of the drug/protein complex formation and the experimental studies that were performed for each system (photophysical characterization, photochemical reactivity by identifying the formation of the different photoproducts, etc.)

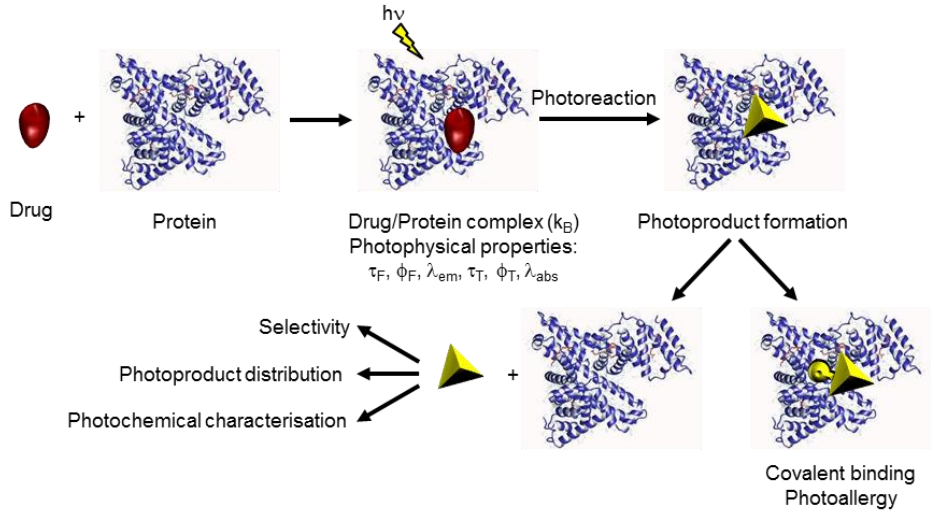

Figure 3. Schematic representation of the drug/protein complexes and their photoreactivity after absorption of light.

To further investigate the photoreactivity of drugs in model systems of higher complexity, a series of dyads composed of a drug and the basic structure of biomolecules were synthesized by intercalating a lineal, cyclic or rigid hydrocarbon skeleton between the two chromophores. Some examples are shown in Figure 4.

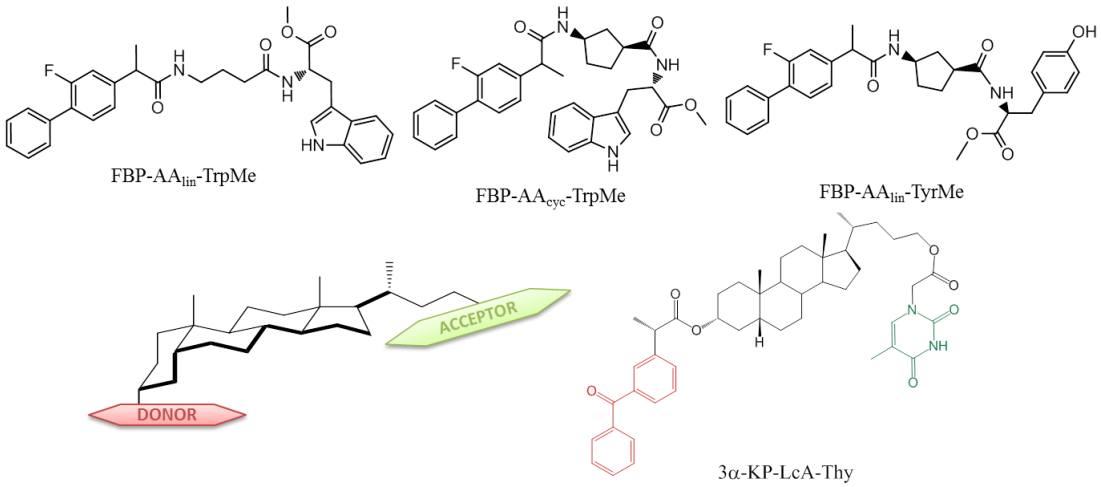

Figure 4. Examples of linked systems where the drug and the amino acid or nucleobase are separated by a spacer of different structure.

The photophysical reactivity was found to be very different depending on the spacer and on the conformational structure of the dyad. Thus, stereoselective interactions were observed on the above mentioned linked systems. A complete photophysical characterization was performed, and their photoreactivity was also confirmed by simple theoretical calculations. For a series of dyads composed of a rigid hydrocarbon skeleton, where ketoprofen or carbazole may act as donors, and thymine, naphthalene or biphenyl may act as acceptors (an example is shown in figure 4), an unexpected through-bond triplet excimer formation was detected, a process that was never observed in related systems. Herein, through-bond triplet exciplex (TB-TEX) formation in donor/acceptor systems linked through a rigid bile acid scaffold has been demonstrated on the basis of kinetic evidence. The triplet acceptors have been efficiently populated upon through-bond triplet-triplet energy transfer (TB-TTET) from the donor. At room temperature, TB-TTET was followed by laser flash photolysis, by looking at the kinetic traces corresponding to the triplet excited state of the donor; the process was found to occur much faster in the covalently linked systems than in the corresponding intermolecular mixtures. Subsequent TB-TEX formation was evaluated from the decay kinetics of the triplet excited states of the acceptors. In all systems, TB-TEX rate constants were of the same order of magnitude or even higher than through-space exciplex formation in directly linked systems,

without the bile acid spacer, indicating that the formation of through-bond extended exciplexes is an efficient deactivation pathway for the excited triplet acceptors. Again, all through-bond processes were found to be strongly dependent on the relative spatial arrangement of the chromophores. In this respect, TB-TTET was found to be faster in the α-epimers, while TB-TEX was found to be more efficient for the β-systems.

In addition, the photoreactivity of alternating adenine-thymine duplexes have been studied by means of ultrafast fluorescence spectroscopy. Their two-band fluorescence spectrum, interpreted in terms of short wavelength "monomer" (peaking at ~320 nm) and long wavelength "exciplex" (peaking at ~ 420 nm) emission, gave rise to a paradigm. A joint experimental and theoretical investigation revealed new aspects regarding electronic excitations in these systems and, in particular, the existence of Stabilized High Energy Mixed states (noted SHEM states). Our experimental results in addition to TD-DFT calculations (in collaboration with Dr. Improta), performed for two base pairs, show that SHEM states were formed from mixing between thymine Frenkel excitons and adenine-to-thymine charge transfer states, and two kinds of exciplexes, with different degree of Charge Transfer character, contribute to the long wavelength emission. Our fluorescence studies of hairpins (with six and ten base pairs) and duplexes (with 20 and 2000 base pairs) showed that SHEM states emit at slightly higher energy than $\pi\pi^*$ states and decay on the nanosecond time domain. Their contribution to the short wavelength emission band was strongly correlated with the hypochromism exhibited by the absorption spectrum, increasing with the size of the system. In parallel, the lifetime of the long wavelength emission, associated with two kinds of intra-strand dimers with different stacking distance and degree of charge transfer, decreases from 99 to 32 ps. The interplay among the various excited states depends on the ionic strength of the solution and the excitation wavelength. We have also investigated the photoreactivity of guanine-cytosine oligomers (single and double strand) composed of 10 and 1000 base pairs. Moreover, the results were also compared to those of hairpins and dumbbell systems. However, regarding these systems we still need to finish some experimental results and combine them with theoretical calculations in order to prepare a new manuscript for the early future.

Overall, the main objectives proposed in this project have been successfully accomplished as can be observed by the results obtained and the number of published and submitted papers up to this moment.

Advancement beyond the state of the art in the field

During the evolution of this project, new results have been achieved that aid to better understand the photosensitizing side effects of the investigated drugs that finally lead to photoallergy or phototoxicity. In fact, the photoreactivity of many of these drugs were previously characterized in solution, however their interactions with biomolecules by means of spectroscopic techniques and the mechanistic photochemical pathways giving to phototoxicity was not investigated. This project has addressed this topic, and a huge number of results of good quality were obtained. Representative examples are those of naproxen or fenofibric acid, whose photoreactivity was well characterised in solution and they were known to generate photosensitizing side effects. However, our investigations have aided to get more insight into the mechanistic pathway giving to photoallergy. The transient species that react to biomolecules were detected, and the main photoproducts derived from their interactions with the basic molecules that composed biomolecules were characterized. The photoreactivity of an important number of these drugs in the presence of biomolecules was investigated; in some cases the drugs generate photooxygenation damage by a type I mechanism and others follow a type II mechanism. The results obtained from the more complex model systems are promising because a novel through-bond triplet excimer deactivation pathway was detected. This has motivated us to deeper investigate this effect by the design of novel dyads by using appropriate rigid spacers. Finally, the results obtained from the photoreactivity of oligonucleotides of different composition, size and configuration evidences a new deactivation pathway of the excess of energy that give rise to the existence of stabilized high energy mixed states. This encourages us to continue designing new systems in order to better understand the photoreactivity of DNA model systems.

Impact

A better knowledge of the interactions between drugs and biomolecules triggered by light is of crucial importance because in many cases the photosensitized side-effects they are able to generate to the biomolecule leading to phototoxicity, photomutagenesis and photo-carcinogenesis are still unclear. Thus, a precise knowledge of the implicit active sites where drugs can interact with biomolecules and the involved reaction mechanisms would contribute to better understand the photosensitizing potential of new drug candidates and to design more efficient and specifically targeted drugs with lesser side effects. This project has also given the opportunity to Dr. Vayá to consolidate a close collaboration with the group led by Dr. Markovitsi and to initiate new collaborations with other research groups. Besides, Dr. Vayá has been recently awarded with a European Project to start a close collaboration with Prof. Russell (University of East Anglia); the project aims to use such drugs and biomolecules attached to nanoparticles and use these systems as possible targets in PDT. Their photoreactivity will also be investigated by means of spectroscopic techniques.

Transfer of knowledge

Since I started my period as a "Juan de la Cierva" Researcher – CIG Fellow at the Department of Chemistry (DQ)/Institute of Chemical Technology (ITQ) placed at the UPVLC, I was strongly committed into giving seminars (one seminar every three months) to present my research work to the scientific community of the DQ-ITQ and to coordinate the laboratory work of students. Thus, I have supervised the laboratory work of the Master Thesis of Yameiri Rondón (Photoreactivity of bichromophoric systems containing flurbiprofen and tyrosine; completed in 2014), the final project of the higher university studies of Eline Teughels (Intramolecular [2+2]-Photocycloadditions within protein cavities), Lorena Gil Flores (Photophysical and photochemical studies of flurbiprofen and their main photoproducts and metabolites within proteins) and Vicente T. Monje (Proteins as Microreactors in Photochemistry). Moreover, during this period, I have combined my research with Chemistry teaching at the UPVLC (80h per year in laboratory work). Besides, I was recognized by the National Agency of Evaluation of the Quality (ANECA) in the following three categories: 2013 – Researcher professor; 2013 – Assistant professor; 2013 – Private University professor. ANECA is the Spanish Agency that evaluates the research and teaching activity of the candidates to get a permanent position.

4. DISSEMINATION ACTIVITIES

During this Marie Curie Career Integration Grant a number of articles have been published or are being prepared to be submitted to high impact journals. Moreover, the results have been exposed in national and international conferences.

List of publications

1. I. Vayá, I. Andreu, M. C. Jiménez, M. A. Miranda. Photooxygenation mechanisms in naproxen-amino acid linked systems. *Photohem. Photobiol. Sci.* **2014**, *13*, 224.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
2. I. Vayá, V. Lhiaubet-Vallet, M. C. Jiménez, M. A. Miranda. Photoactive assemblies of organic compounds and biomolecules: drug–protein supramolecular systems. *Chem. Soc. Rev*. **2014**, *43*, 4102.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
3. T. Gustavsson, D. Markovitsi, I. Vayá, P. Bonancía, M. C. Jiménez, M. A. Miranda. Drug/protein interactions studied by time-resolved fluorescence spectroscopy. *Proceedings of SPIE*, **2014**, 91651E.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
4. P. Miró, I. Vayá, M. C. Jiménez, M. L. Marín, M. A. Miranda. Kinetic evidence for extended through-bond triplet exciplexes. *Org. Lett*. **2015**, *Submitted*.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
5. I. Vayá, V. T. Monje, M. C. Jiménez, M. A. Miranda. Photoreactivity of fenofibric acid in serum albumins and in covalent amino acid linked systems. *Chem. Res. Toxicol*. **2015**, *To be Submitted*.

Acting as: Corresponding author of article in journal with external admissions assessment committee.

6. I. Vayá, J. Brazard, M. Huix-Rottlant, A. K. Thazhathveetil, M. Perron , F. D. Lewis, T. Gustavsson, I. Burghardt, R. Improta, D. Markovitsi. Mixed Frenkel-charge transfer excitons govern hypochromism and fluorescence in alternating adenine-thymine DNA double-stranded structures. *J. Am. Chem. Soc*. **2015**, *To be Submitted*.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
7. J. Ponce, J. Aragó, I. Vayá, J. Gómez, S. Tatay, E. Ortí, E. Coronado. Photophysical properties of conjugated Iridium (III) complexes functionalized with metal anchoring groups. *Inorg. Chem*. **2015**, *To be Submitted*.
   Acting as: Author or co-author of article in journal with external admissions assessment committee.
8. I. Vayá, T. Gustavsson, D. Markovitsi, M. C. Jiménez, M. A. Miranda. Flurbiprofen-tyrosine linked systems as models for excited states interactions in drug-protein conjugates. *In preparation.*
   Acting as: Corresponding author of article in journal with external admissions assessment committee.

List of scientific conferences

1. Title: Drug/protein interactions studied by time-resolved fluorescence spectroscopy
   Name of the conference: Physical Chemistry of Interfaces and Nanomaterials XIII, part of SPIE NanoScience + Engineering
   Field of the conference: Non EU International
   Type of participation: Invited speaker
   City: San Diego, United States of America
   Date: 17/08/2014
   Organising institution: SPIE
   Authors: T. Gustavsson, D. Markovitsi, I. Vayá, P. Bonancía, M. C. Jiménez, M. A. Miranda.

2. Title: Photooxygenation mechanisms in naproxen-amino acid linked systems
   Name of the conference: The 6th Asia and Oceania conference on photobiology
   Type of event: Conference
   Field of the conference: Non EU International
   Type of participation: Poster
   City: Sydney, Australia
   Date: 10/11/2013
   Authors: M. C. Jiménez, I. Vayá, I. Andreu, M. A. Miranda.

3. Title: Excited state interactions between flurbiprofen and tryptophan in drug/protein complexes and in model dyads
   Name of the conference: XXXIV Reunión Bienal de la Real Sociedad Española de Química
   Type of event: Conference
   Field of the conference: European Union
   Type of participation: Speaker
   City: Santander, Spain
   Date: 15/09/2013
   Authors: I. Vayá, P. Bonancía, M. C. Jiménez, D. Markovitsi, T. Gustavsson, M. A. Miranda.

4. Title: Photoreactivity of fenofibric acid in serum albumins and in covalent amino acid linked systems
   Name of the conference: European Photochemistry Association
   Type of event: Conference
   Field of the conference: European Union
   Type of participation: Speaker
   City: Lisbon, Portugal
   Date: 31/08/2015
   Authors: I. Vayá, V. T. Monje, M. C. Jiménez, M. A. Miranda.

Outreach activities

1. **Mesa Redonda de Experiencias Personales en Acciones Marie Curie** (seminar about my experice regarding the Marie Curie Actions)
   Name of the event: Jornadas de Ayudas a la Movilidad del Personal Investigador, Valencia Spain, 2013
   Type of event: Seminar
   Organising institution: Red de Universidades Valencianas para el fomento de la Investigación, el Desarrollo y la Innovación (RUVID)

2. **Jornada de Puertas Abiertas** Universitat Politècnica de València
   Type of event: Seminar
   Organising institution: Universitat Politècnica de València

5. PROJECT MANAGEMENT

Progress of professional re-integration and research career development

Size and composition of the research group: The Technical University of Valencia (UPVLC) has been qualified by the *Academic Ranking of World Universities (ARWU) 2014* as the best technical University of Spain. ARWU recognises the level of excellence of UPVLC in training students and in research, which is also supported by other principal university rankings of high international level such as the *Times Higher Education journal* and the *QS World University Rankings*, which positioned the UPVLC within the 383 best universities in the world, and is also considered within the 75 best universities in Chemistry. The Department of Chemistry (DQ) and the Institute of Chemical Technology (ITQ), recently awarded with the prestigious Severo Ochoa Prize due to its excellence in research, have contributed to the privileged position of UPVLC; these two centres contribute with about 190 publications in high impact journals and 15 patents per year. DQ and ITQ are composed of more than 240 professionals divided in research Professors, post-doctoral researchers, PhDs, technicians, etc., and are extremely well equipped for performing top research in chemistry. Prof. Miguel A. Miranda is the leader of the research group, which is composed of 7 staff scientists, 5 post-doctoral researchers and 7 PhD students. The extraordinary quality of this research group is evidenced by the huge number of publications in high impact international journals and the broad dissemination of the scientific results at national and international conferences. As an average, the group produces yearly about 20 scientific publications and contributes at about 30 participations per year in conferences and meetings. Dr. Vayá has developed his investigations within this research group for three years. He has been given the freedom to design and develop his own projects and the level of independence the researcher has reached is in accordance with the capabilities that the Host Institution and hosting group offer to researchers at this stage.

Stable, permanent position: This grant has given Dr. Vayá the freedom to develop his own research line in the group led by Prof. Miranda in relation to the photosensitising side effects of drugs to biomolecules. Within this research line, I have supervised the laboratory work of the Master Thesis of Yameiri Rondón (Photoreactivity of bichromophoric systems containing flurbiprofen and tyrosine; completed in 2014), the final project of the higher university studies of Eline Teughels (Intramolecular [2+2]-Photocycloadditions within protein cavities), Lorena Gil Flores (Photophysical and photochemical studies of flurbiprofen and their main photoproducts and metabolites within proteins) and Vicente T. Monje (Proteins as Microreactors in Photochemistry). The ability of Dr. Vayá to develop an independent research portfolio is exemplified by the fact that since he arrived to the Host Institution he has been able to develop his self-driven MC and addition projects and to publish a number of articles (one as corresponding author) and contribute with oral presentations to a number of international scientific meetings.

At the end of the first year of this Marie Curie Project, Dr. Vayá applied for a Spanish “Ramón y Cajal” (RyC) Excellency research contract. This is a five years contract within the Spanish research system and constitutes the natural follow up step after a “Juan de la Cierva” contract (the current contract of Dr. Vayá). Although RyC is very competitive (only 13 contracts over 160 applicants were awarded last year in Chemistry), Dr. Vayá was very well evaluated and he has really good possibilities to succeed in obtaining a RyC this year thanks to the results and publications obtained from this PHOTOBIODRUG Marie Curie Project.

Independence and support from Host institute: Dr. Vayá has developed this project within Prof. Miranda's group. Actually, Prof. Miranda has offered the researcher support in many ways: use of installations, scientific collaboration, co-directing students and sharing group funding. Moreover, Dr. Vayá was also given the freedom to continue and consolidate his own collaborations with Dr. Markovitsi (a couple of articles will be submitted in the early future) and to start new collaborations with groups placed at the University of Valencia and University of Huelva. Besides, Dr. Vayá has been recently awarded with a European Project to start a close collaboration with Prof. Russell (University of East Anglia). On the other hand, I had the support from the Center for Innovation, Research and Technology Transfer (CTT) which provides personal for working on the management of EU projects, who take care of all administrative arrangement related with CIG and undertake day-to-day financial management of the project.

Additional grants

Dr. Vayá has obtained additional funding to develop his research line from UPVLC, from the autonomic Government and from EU:

- Financial entity: Universitat Politècnica de València (ref. SP20120757)
  Lenght from: 31/12/2012 to: 31/12/2014
  Funding: 10.500 €
- Financial entity: Consellería d'Educació, Cultura i Esport (ref. GV/2013/051)
  Lenght from: 01/01/2013 to: 31/12/2014
  Funding: 12.000 €
- Financial entity: European Union Laserlab-Europe Selection Panel (SLIC001955)
  Length: 3 weeks (funding to collaborate with Dr. Markovitsi)

Financial support obtained by Dr. Vayá has certainly aided for the successful development of his research.

Collaborations

- Dr. Markovitsi. Commissariat à l'Energie Atomique (France).
- Prof. Improta, Univesity of Naples (Italy).
- Dr. Pischel, Universidad de Huelva (Spain).
- Prof. Russell, University of East Anglia (United Kingdom).
- Dr. Tatay, University of Valencia (Spain).