LIGHTinPARIS	Marie Skłodowska-Curie Actions Second call for COFUND PhD fellowships
Employer	**University Paris-Saclay**
Doctoral School	2MIB
Domain	2MIB-COB (Chemical Sciences - Organic Chemistry)

Supervision

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PHD THESIS PROJECT

WActiPepti - Wavelength-selective photocleavable protecting groups for the synthesis and activation of biologically relevant 3D-structured peptides

Summary / Abstract

The objective of this research project is to prepare novel photocaged peptides, perform in-depth studies of their photochemical/photophysical behavior, and apply them to the inhibition of the p53-MDM2 interaction, a well-recognized strategy in cancerology.

To reach this goal, this project gathers an international consortium with a broad range of expertise including synthetic organic chemistry, photophysics and biology.

A first peptide series, designed to undergo 3D structuration upon photodeprotection and induce inhibition of the p53-MDM2 interaction, will be studied in a cellular context. A second series involving a cyclopeptide with multiple photocleavable protecting groups will be studied in the context of the development of a novel synthetic methodology to access biologically relevant complex molecular architectures based on sequential wavelength-selective photodeprotections.

Principal 3i dimension: international. **Additional 3i dimension**: interdisciplinary

Keywords

- (Bio)organic chemistry
- Photochemistry
- Photophysics
- Peptides
- p53-MDM2 interaction

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WActiPepti - Wavelength-selective photocleavable protecting groups for the synthesis and activation of biologically relevant 3D-structured peptides

Key Objectives

- Synthesize novel photocaged amino-acids suitable for solid-phase peptide synthesis
- Incorporate them into selected peptides likely to exhibit well-defined 3D structures
- Study the photochemical and photophysical properties of the amino-acids and peptides: photocleavage ability, photochemical pathways and mechanisms by investigating (a) steady-state and time-resolved absorption and emission studies (b) circular dichroism (CD) and circularly polarized luminescence (CPL)
- Develop a novel synthetic methodology for late-stage functionalization of cyclopeptides based on sequential wavelength-selective photo-deprotections
- Evaluate cellular uptake, photo-uncaging and initiation of biological activity (inhibition of p53-MDM2 interaction) on selected peptides in a cellular context

Project

Context and state-of-the-art of Scientific Research project

Photoresponsive peptides have been the subject of intense research efforts due to their ability to modulate biological functions and self-assembly of artificial materials.[1] Among them, “photocaged” peptides containing protecting groups that can be removed upon light irradiation, have received special interest in the context of activation of biological processes. [2] Peptide photocaging strategy is typically applicable to polar side chains bearing “cageable” chemical functions (alcohol, amine, thiol or carboxylic acid). However, it cannot be directly extended to biological interactions involving hydrophobic residues (phenylalanine, tryptophan, leucine, etc.) such as p53-MDM2 interaction, whose inhibition is now recognized as an attractive anti-cancer strategy.[3] A valid alternative would be to introduce photocleavable protecting groups (PPG) at specific positions, therefore hampering proper peptide 3D structuration.[4]

A surprisingly underlooked aspect in the field of photocaged peptides is the use of wavelength-selective PPGs to perform multiple late-stage functionalization on complex architectures. [5,6] Such a strategy could indeed be of great help to chemists facing difficulties associated with the selection of orthogonal protecting groups suitable for peptide synthesis.

In this context, the objective of this research project is to synthesize novel photocaged amino-acids suitable for solid-phase peptide synthesis, perform in-depth study of their photophysical/photochemical behavior, and apply them to the inhibition of the biologically relevant p53-MDM2 in a cellular context.

Description

This PhD project centered on organic chemistry will explore the interfaces with photophysics and biology. It is divided into 3 tasks, supervised by the two main partners Dr. Nicolas Bogliotti (PPSM, ENS Paris-Saclay; design and synthesis of photoactive molecules) and Prof. K. George Thomas (KGT Lab, IISER TVM, India; study of light-matter interaction at the molecular and nanoscale).[7] Two long-standing collaborators, Dr. Roba Moumné (CPCV, Sorbonne Université; peptide synthesis) and Dr. Eric Deprez (LBPA, ENS Paris-Saclay; cell imaging and applications of photoactivatable probes), will also be involved in the project.[8]

Task 1. Synthesis of photocaged aminoacids and peptides. (Partners involved: PPSM and CPCV)

Novel families of PPG-containing amino-acids will be synthesized following well-established protocoles and incorporated into peptides by standard solid-phase synthesis methods.[9] Depending on their structures, the light-controlled properties of the resulting peptides will be evaluated i) in a cellular context to gain insights into their biological mode of action, ii) in solution for the development of a novel synthetic methodology based on wavelength-selective sequential photodeprotections towards late-stage functionalized cyclopeptides, designed as p53-MDM2 inhibitors.

Task 2. Studies of photocleavage reactions, elucidation of mechanistic pathways and advanced photophysical characterizations (Partners involved: KGT Lab and PPSM)

Preliminary photocleavage studies of synthesized peptides will be performed at PPSM to identify the most promising candidates for further applications. Selected molecules will then be studied at KGT Lab by a combination of time-resolved absorption and emission spectroscopy in the femtosecond to nanosecond time scales to identify the short-lived intermediates generated upon photolysis. The aim of these studies is to disclose the photochemical mechanism in order to improve the process. Photocleavage of PPGs indeed occurs through a variety of pathways (ion pair, radical or open-shell cations) depending on their structure.[11,12] Special attention will also be put on the characterization of fluorescence properties by circularly polarized luminescence (CPL) and time-resolved single molecule spectroscopy of both caged peptides and PPGs by-products, since this evolution could be crucial to monitor photo-uncaging process in solution and in a cellular context (see Task 3 below).

Task 3. Photodeprotection studies of peptides p53-PPG in a cellular context. (Partners involved: LBPA and PPSM)

The cellular uptake of selected peptides and their photo-uncaging will be assessed in H1299 cells transfected by plasmids expressing p53 and Mdm2 genes. This process will be monitored by steady-state fluorescence emission and/or fluorescence lifetime measurements (FLIM) using a fs-pulsed IR laser coupled to a confocal microscope available at LBPA. The required two-photon excitation appears compatible with the nature of PPGs envisioned.[13] The cell death phenomenon will be monitored by standard approaches: direct visualization of plasma membrane blebbing (a hallmark of apoptosis), MTT assays (colorimetric test) or flow cytometric experiments using Annexin V/DAPI staining.

Bibliography

[1] C. Chatterjee, S. K. Singh. Org. Biomol. Chem. 2022, 20, 5500–5509. https://doi.org/10.1039/D2OB00902A

[2] A. E. Mangubat-Medina, Z. T. Ball. Chem. Soc. Rev. 2021, 50, 10403–10421. https://doi.org/10.1039/D0CS01434F

[3] C. Chatterjee, S. K. Singh. Org. Biomol. Chem. 2022, 20, 5500–5509. https://doi.org/10.1039/D2OB00902A

[4] P. J. Salveson, S. Haerianardakani, A. Thuy-Boun, A. G. Kreutzer, J. S. Nowick. J. Am. Chem. Soc. 2018, 140, 5842–5852. https://doi.org/10.1021/jacs.8b02658

[5] C. G. Bochet. Isr. J. Chem. 2021, 61, 486–495. https://doi.org/10.1002/ijch.202100025

[6] M. J. Hansen, W. A. Velema, M. M. Lerch, W. Szymanski, B. L. Feringa. Chem. Soc. Rev. 2015, 44, 3358–3377. https://doi.org/10.1039/C5CS00118H

[7] Since 2018, in the framework of the Indo-French collaborative program Biosantexc (ENS and IISER network) two BSc/MSc students from IISER TVM (Joel Parakadavil and Aysha Ferzin) have performed research internships in both PPSM and KGT Lab.

[8] Collaborative on-going projects: DuWaPP (2024-2026; funded by Institut d'Alembert / ENS Paris-Saclay / Université Paris-Saclay; N. Bogliotti, R. Moumné, E. Deprez) and DynaCycloP (2024-2028; funded by ANR; R. Moumné and N. Bogliotti).

[9] R. Weinstain, T. Slanina, D. Kand, P. Klán. Chem. Rev. 2020, 120, 13135–13272. https://doi.org/10.1021/acs.chemrev.0c00663

[10] B. Zagiel, T. Peker, R. Marquant, G. Cazals, G. Webb, E. Miclet, C. Bich, E. Sachon, R. Moumné. Chem. Eur. J. 2022, 28, e202200454. https://doi.org/10.1002/chem.202200454

[11] M. R. Clotworthy, J. J. M. Dawson, M. D. Johnstone, C. L. Fleming. ChemPlusChem. 2024, 89, e202400377. https://doi.org/10.1002/cplu.202400377

[12] H. D. Nguyen, M. Abe. J. Am. Chem. Soc. 2024, 146, 10993–11001. https://doi.org/10.1021/jacs.4c02880

[13] M. A. H. Fichte, X. M. M. Weyel, S. Junek, F. Schäfer, C. Herbivo, M. Goeldner, A. Specht, J. Wachtveitl, A. Heckel. Angew Chem Int Ed. 2016, 55, 8948–8952. https://doi.org/10.1002/anie.201603281

Facilities and quality of research environment available for the researcher

The synthesis and characterization of photo-active amino-acids will be performed at laboratory PPSM (ENS Paris-Saclay), which provides all required facilities: laboratories for synthesis, NMR spectrometer, analytical and preparative HPLC, light irradiation setups (Hg/Xe lamps + interferential filters and LEDs), UV-vis and fluorescence spectrometers. Their incorporation into peptides will be performed in collaboration with laboratory CPCV (Sorbonne Université; supervised by Dr. Roba Moumné), which has an internationally-recognized expertise in the field and all required equipment: instruments for automated and manual solid-phase peptide synthesis, HPLC for analysis and purifications, mass spectrometry platform for identification. Noteworthy is the fact that in the context of PPSM-CPCV collaborative projects since 2024, several short stays (1-2 weeks) of students and post-docs from PPSM to CPCV have already been organized to synthesize peptides.

The advanced photophysical studies of the synthesized systems will be performed at KGT Lab (IISER TVM) The circular dichroism (CD) and circularly polarized luminescence (CPL) of chromophore-linked amino acids and peptides will be investigated at IISER Thiruvananthapuram. In addition, time-resolved transient absorption and emission studies of these photocaged peptide systems will be performed in the visible spectral region, covering time scales from femtoseconds to nanoseconds. Time-resolved single-molecule studies will also be carried out to probe the dynamics of various photophysical and photochemical processes in these systems.

Cellular uptake experiments, photodeprotection studies in the cellular context and evaluation of biological activity will be performed in collaboration with laboratory LBPA (ENS Paris-Saclay; supervised by Dr. Eric Deprez). This task will rely on the access to state-of-the-art platforms hosted on-site: L2 cell culture laboratories, molecular and cellular biology equipment (flow cytometry) and cellular imaging facilities (multiphotonic and confocal imaging, FLIM time-resolved imaging).

3i Dimensions - international; interdisciplinary

Description and expected outcomes

The objective of this research project is to synthesize novel biologically relevant peptides prone to photo-activation in solution and in a cellular context to study the p53-MDM2 interaction, whose inhibition is a well-recognized target in cancerology. To reach this goal, an international consortium is gathered, covering a broad range of expertise including synthetic organic chemistry, advanced time-resolved absorption and vibrational spectroscopies, peptide synthesis and cellular biology. Two 6-month stays of the PhD candidate at the partner institution in India is planned over the duration of the thesis.

This project therefore fulfills both the international and interdisciplinary dimension of the call.

The France-India international cooperation between PPSM (ENS Paris-Saclay) and KGT Lab (IISER TVM) has been initiated in the context of the programme Biosantexc (see www.ifindia.in/biosantexc), a collaborative network comprising seven Indian Institutes of Science Education and Research (IISERs) and four Ecoles normales supérieures (ENS). As part of this initiative, several Bachelor of Science – Master of Science (BS-MS) students from IISER Thiruvananthapuram and other IISERs have undertaken several-month research exchange programs at PPSM (ENS Paris-Saclay).

The interdisciplinary dimension of this project lies in the utilization of the synthesized photosensitive molecules for the study of the p53-MDM2 interaction in a cellular context, whose understanding could open promising perspectives in the field of cancerology.

Approximate timeline of internships / external stays corresponding to the 3i dimension

- **Year 1 (October 2026 – September 2027)**

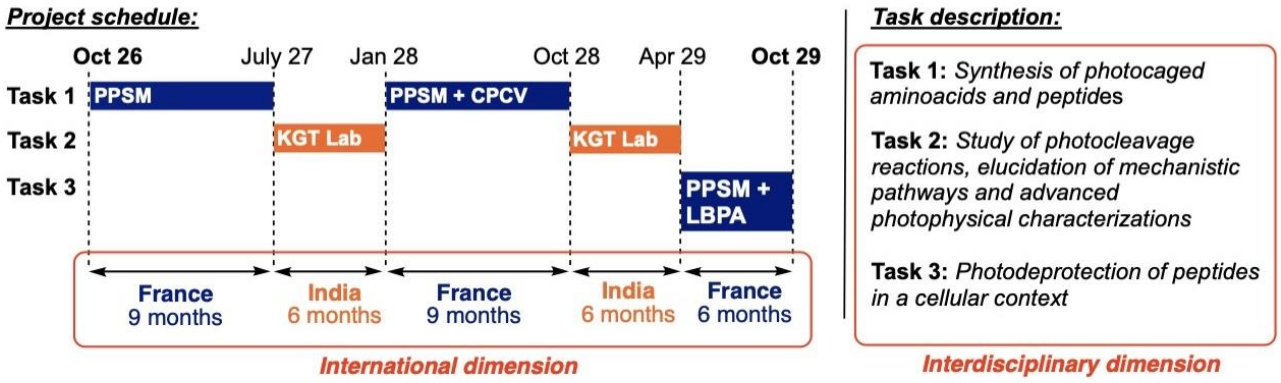
Synthesis of photocaged aminoacids and preliminary photochemical studies (PPSM, Oct 2026 – Jun 2027)
Advanced photopysical studies on photocaged aminoacids (KGT Lab, from Juy 2027)

- **Year 2 (October 2027 – September 2028)**

Advanced photopysical studies on photocaged aminoacids (KGT Lab, up to Dec 2027)
Synthesis of photocaged peptides and preliminary photochemical studies (PPSM + CPCV, Jan 2028 – Sep 2028)

- **Year 3 (October 2028 – September 2029)**

Advanced photopysical studies on photocaged peptides (KGT Lab, Oct 2028 – Mar 2029)
Peptide photodeprotection and evaluation of biological properties in a cellular context (LBPA + PPSM, Apr 2029 – Sept 2029)



Profile of PhD fellow	
PhD studies and research work require curiosity, creativity, rigour, teamwork, and organisational skills. A minimum B2 level of English is mandatory. The below criteria are specific to the thesis project.	
pre-requisite	Theoretical and experimental knowledge in synthetic organic chemistry
pre-requisite	Theoretical and/or experimental knowledge in photochemistry
pre-requisite	Theoretical and/or experimental knowledge in photophysics
advantageous	Experience in solid-phase peptide synthesis
advantageous	Experience in cellular imaging

Ethics issues in the project as per Horizon Europe Ethics Self-assessment			
Section 1. Human embryonic stem cells (hESCs) and human embryos (hEs)			No
Section 2. Humans	No	**Section 3. Human cells and tissues**	**Yes**
Section 4. Personal data	No	Section 5. Animals	No
Section 6. Third Countries	**Yes**	**Section 7. Environment, health and safety**	**Yes**
Section 8. Artificial Intelligence	No	Section 9. Other ethics issues	No
Section 10. Crosscutting issue: potential misuse of results			No

This project raises the following potential ethics issues:

3. Human cells or tissues

The properties of some molecules synthesized in the context of this project will be evaluated on human NCI-H1299 cells, available from a variety of retailers. The conditions for purchase and use of such material will strictly follow the regulations imposed by French legislation.

6. Third Countries

Collaborations with third countries, in a non-EU or non Horizon Europe associated country, is based on an existing scientific collaboration. The host institution in India is linked to home institution in the context of the program Biosantexc (see www.ifindia.in/biosantexc), a collaborative network comprising seven Indian Institutes of Science Education and Research (IISERs) and four Ecoles normales supérieures (ENS)

7. Environment, health and safety

All French and Indian labs are required to have health and safety procedures in place, which takes into account the use of high-energy light sources such as lasers of class 3 and above, and the use of hazardous / toxic chemical and biological products. The institution and the laboratory comply with the highest safety requirements, and PhD fellows have mandatory health and safety training, with additional training if required.