PhD fellowship (2024-2027)

Protein origami as nano-optical probe templates

Starting date:	01/10/2024	**Duration:**	36 months

Supervisor : **Erik DUJARDIN**
Laboratoire Interdisciplinaire Carnot de Bourgogne (ICB), UBFC CNRS UMR 6303
Département Photonique - Equipe PRISM - 9 avenue Alain Savary - BP 47870 - 21078 Dijon Cedex - France
Email: ***erik.dujardin@cnrs.fr*** *– Phone: +33 (0)3 80 39 59 62.*

Context: The optical properties of semiconductor luminescent nanocrystals (quantum dots) or metallic nanocrystals depend on intrinsic phenomena (size, materials, structure, morphology, etc.) and extrinsic phenomena (surface effects, local temperature, electric field, local index, collective effects and couplings, etc.). Controlling the former allows optical engineering over a wide range of wavelengths and time scales and the latter makes these nanoparticles excellent probes of the surrounding environment. This is how optical nano-probes (triple Nobel Prize in Chemistry 2023) associated with super-resolved optical microscopies (triple Nobel Prize in Chemistry 2014) have revolutionized the understanding of numerous biological phenomena, including intracellular ones. However, the collective effects of ordered sets of emitters such as super-fluorescence, super-radiance, plasmon-enhanced emission or chiral emission remain little exploited as probe methods despite the advantages that these could bring. The reasons for this are that (i) the probes are generally individualized nanoparticles and not ordered assemblies, (ii) ordered assemblies of emitters are usually formed from macroscopic supports or matrices (polymers, liquid crystals, surfactants, nanotubes, …) or from fragile inter-particle interactions making them unsuitable for introduction into the medium to be probed (biological or not).

The missing link is to be able to rigidly and precisely order optically active nanoparticles (luminescent and/or plasmonic) in 3D space, while remaining soluble. Among the few approaches to engineering the interfaces of these nanoparticles that meet these specifications, those using DNA origami or proteins to arrange nanoprobes are promising but often limited by the difficulty of mass producing the assemblies.

This thesis project will exploit entirely artificial proteins, easily produced by our biochemist partners, the physicochemical properties of which, particularly once coupled to nanoparticles, can be programmed as desired using an approach developed in collaboration over fifteen years.[1,4-6] A modular combinatorial approach to protein origami construction (Fig. 1AB) now offers a unique opportunity to design supramolecular protein architectures having both a rigid 3D structure and chemically functional surfaces capable of guiding growth and self-assembly of optically active nanoprobes (Fig. 1C).[2-3]

PhD Objectives: ***A multi-disciplinary doctoral contract in experimental physico-chemistry*** *is offered at the Carnot Interdisciplinary Laboratory of Burgundy (ICB, CNRS Univ. Bourgogne, Dijon, France) as part of a research project bringing together biochemists, physico-chemists and experts in X-ray diffusion and cryo-electron microscopy. The proposed work lies at the frontier between* ***synthesis of gold clusters and nanocrystals, surface chemistry, protein engineering applied to materials*** *and cutting-edge characterization by* ***optical microscopies and spectroscopies****.*

The PhD has three components: ***(i)*** *Self-assembly of origami from elementary modules made up of artificial proteins;[1-3]* ***(ii)*** *functionalization of origami to make them crystal growth guides[4] and supports for ordered assembly of nanocrystals metallic or optically active semiconductors (nanoprobes)[5,6] and* ***(iii)*** *optical and spectroscopic studies in solution and on individual objects.*

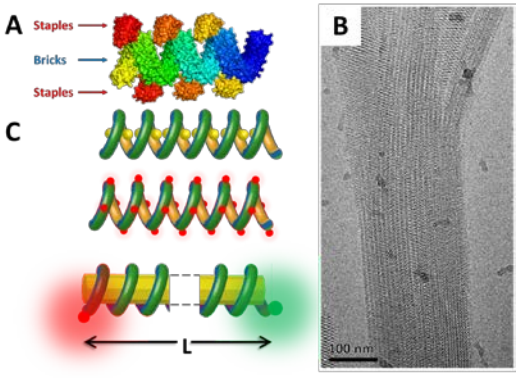

Figure 1: **(A)** 3D model of a superhelical origami spontaneously formed by mixing two selected aRep proteins, the "brick" and the "staple". **(B)** cryoEM image of a large supercrystal of origami.[2,3] **(C)** *Examples of 3D spatial organization of plasmonic and fluorescent nanoparticles templated inside, outside of the origami or both depending whether the staple, the brick or of carry the nanoprobes.*

Missions and Training.

*The candidate will be trained in the **production of origami** from artificial proteins designed and produced by our biochemist partners and **will develop synthesis and functionalization protocols** to use origami as a self-assembly support. emitters with sub-nm precision or morphosynthesis of Au/Ag nanocrystals. He/she will **carry out structural characterizations** of origami-nanoparticle hybrid superstructures ordered in 3D by AFM, FEGSEM and conventional TEM in the ARCEN platform (ICB, Dijon). **Extensive optical and spectroscopic studies** (**Fluorescence, TIRF, circular dichroism, Raman**) will make it possible to evaluate the properties and performances of these optical nanoprobe assemblies.*

Profile.

*Do you want to explore the optical properties of nanoparticle assemblies that you have created yourself? Would you like to contribute to the emerging topic of nanobiotechnologies by working with physicists, chemists and biologists? Can you imagine yourself piloting microscopes to understand the properties of matter at the nanometer scale? **If yes, this project will meet your expectations: apply and join our team!***

Bibliography

1 - Guellouz A., Valerio-Lepiniec, M., Urvoas, A., Minard, P., et al., **PloS One**, 8, e71512 (2013). (Link)
2 - L. Moreaud, S. Viollet, A. Urvoas, M. Valerio-Lepiniec, et al. F. Artzner,, E. Dujardin, P. Minard, **PNAS**, 120, e2218428120 (2023). (Link)
3 - J. Miller, A. Urvoas, et al., M. Valerio-Lepiniec, F. Artzner, E. Dujardin, P. Minard, **J. Struct. Biol.**, 215, 108102 (2023). (Link)
4 - J. Prasad, S. Viollet, K. L. Gurunatha, et al., P. Minard, E. Dujardin, **Nanoscale**, 11, 17485-17497 (2019). (Link)
5 - K. L. Gurunatha, A. C. Fournier, A. Urvoas, M. Valerio-Lepiniec, V. Marchi, P. Minard and E. Dujardin. **ACS Nano**, 10, 3176–3185 (2016). (Link)
6 - M. Fernandez, A. Urvoas, P. Even-Hernandez, et al., P. Minard, E. Dujardin, V. Marchi, **Nanoscale**, 12, 4612-4621 (2020). (Link)