MARIE SKLODOWSKA-CURIE ACTIONS

Co-funding of regional, national and international programmes (COFUND)

DOC2AMU THESIS PROJECT 2018 CALL FOR APPLICATIONS

INTRINSICALLY DISORDERED PROTEINS AT INTERFACES

1. GENERAL INFORMATION

Call	2018-16
Topic	Imaging, Nano-health
Keywords	Dynamic drop tensiometer; Fluorescence; Interface science; Protein adsorption; Protein folding; Surface rheology

2. THESIS DIRECTOR(S), RESEARCH UNITS AND DOCTORAL SCHOOLS

Thesis director	Frédéric CARRIERE
Research Unit	Bioénergétique et Ingénierie des Protéines
Doctoral school	ED 250 - Sciences Chimiques
Thesis co-director	Sonia LONGHI
Research Unit	Architecture et Fonction des Macromolécules Biologiques
Doctoral school	ED 062 - Sciences de la Vie et de la Santé

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1. DESCRIPTION OF THE PHD THESIS PROJECT

1.1 OBJECTIVES OF THE PROJECT BASED ON THE CURRENT STATE OF THE ART

Proteins are major components of the living cell where they play crucial structural and functional roles, and their dysfunctions can lead to various pathologies. These dysfunctions are often associated with gene mutations leading to inactive or non-correctly folded proteins/enzymes. This usually applies to globular proteins with well-defined 3D structure and function(s) associated with this structure. With the increasing evidence that intrinsically disordered proteins (IDPs) are highly represented among proteins [1], new concepts have emerged to describe and possibly explain some protein misfolding disorders such as neurodegenerative diseases. Indeed, the aggregation of IDPs into amyloid fibrils is directly associated with the onset and progression of these pathological disorders. For instance, Alzheimer's and Parkinson's diseases, are tightly associated with the aggregation of otherwise soluble IDPs that form protein deposits in the brain of patients and lead to neuronal death [2-6]. In that context, it is important to better understand the key factors that can trigger the folding and aggregation of IDPs.

IDPs are functional proteins that are devoid of stable secondary and tertiary structure under physiological conditions of pH and salinity in the absence of a partner/ligand [7-10]. They thus exist as dynamic ensembles of interconverting conformers and differ from structured globular proteins by their amino acid composition, sequence complexity, hydropathy, charge and flexibility. IDPs are typically depleted in bulky and hydrophobic amino acid residues, also called "order-promoting residues", and are conversely enriched in polar and charged residues, also called "disorder-promoting residues" [8]. These peculiar properties dictate the behaviour of IDPs in various environments and they have been used for the development of protein disorder predictors [11, 12]. Because of their enrichment in polar/charged residues, IDPs are characterized by a hydration significantly higher than that of ordered proteins [13]. As such, IDPs can reasonably be expected to exhibit a distinct behaviour not only in bulk but also at air/water (A/W) and lipid/water interfaces. For instance, α-synuclein, a protein involved in Parkinson's disease, is an IDP in its monomeric form in the aqueous phase, but it is known to form a stable monolayer at the A/W [14] and lipid/water (phospholipids vesicles) [15] interfaces while adopting a partial α-helical conformation [16, 17]. Depending on the lipid-to-protein ratio and the nature of lipids, α-synuclein can also adopt a β-sheet conformation that further aggregates into amyloid fibrils, which are the major component of Lewis bodies formed inside dopaminergic cells of Parkinson's disease patients [2-4]. Contrary to α-synuclein, the intrinsically disordered amyloid-β peptide (Aβ), an IDP involved in Alzheimer's disease, undergoes a disorder to order transition prior to its adsorption at the interface [5]. The protein tau, another IDP involved in the Alzheimer's disease, is able to strongly interact with the A/W interface, intercalates into negatively charged lipid monolayers and bilayers, and induces membrane morphological changes and disruption [6].

While the interfacial behaviour of globular proteins has been extensively studied, experimental data on IDPs at the air/water (A/W) and water/lipid interfaces are scarce. The need for more information on the interfacial behaviour of IDPs in comparison with globular proteins prompted us to recently investigate the interfacial properties of a model IDP [18], the intrinsically disordered C-terminal domain (N_{TAIL}) of the Hendra virus (HeV) nucleoprotein, a protein responsible for the encapsidation of the viral genome [19, 20]. HeV N_{TAIL} is a disordered protein domain of 140 residues and 15.3 kDa that has been well characterized by various biophysical approaches [21-23]. It was shown to adopt a local α-helical folding within a short α-helical

Molecular Recognition Element (α-MoRE, aa 473-493) upon interaction with its physiological partner, the X domain of the phosphoprotein (P_{XD}) [22]. As observed with IDPs involved in neurodegenerative diseases, we wondered whether N_{TAIL} could also adopt some folding upon adsorption at interfaces. We compared the behavior of HeV N_{TAIL} at the air-water interface, in the absence and presence of phospholipids, with that of lysozyme that was taken as a model globular protein of similar molecular mass. To this end, we used Langmuir films, polarization modulated-infrared reflection–absorption spectroscopy (PM-IRRAS) and an automated drop tensiometer for interfacial tension and elastic modulus determination with oscillating bubbles [18]. N_{TAIL} showed a significant surface activity, with a higher adsorption capacity at the A/W interface and penetration into egg phosphatidylcholine monolayer compared to lysozyme. While lysozyme remained folded upon compression of the protein layer at the A/W interface and showed a quasi-pure elastic behaviour, N_{TAIL} showed a much higher molecular area and formed a highly viscoelastic film with a high dilational modulus. PM-IRRAS experiments revealed a new disorder-to-order transition for the N_{TAIL} protein that folds into an anti-parallel β-sheet at the A/W interface and presents strong intramolecular interactions [18].

It is intriguing that a protein depleted in aromatic (W, F, Y) and bulky hydrophobic amino acid residues (I, L, V) shows faster adsorption kinetics at the A/W interface and is more penetrant into a phospholipid layer than a protein of similar size with a higher content in these amino acids. This may results from the fact that most amino acid side chains are exposed to solvent in IDPs while hydrophobic amino acid residues of globular proteins are often buried inside their tertiary structure and are involved in interactions stabilizing the secondary and tertiary structure. Exposure to solvent of such hydrophobic residues and further interaction with interfaces requires partial conformational changes/whole protein unfolding and this requires energy. In agreement, it has been shown that heat-denatured lysozyme is more tensioactive and binds faster at the A/W interface than native lysozyme [24]. Moreover, a thick and strongly viscoelastic film is created, which reminds the interfacial properties of N_{TAIL}. Adsorption and ensuing gain of structure of IDPs at interfaces could thus be favoured by their disordered state and by the lower energy barrier required to adopt a new conformation and to establish new molecular interactions at interfaces. This may explain why IDPs have a higher propensity to form amyloid fibers in neurodegenerative diseases and why the aggregation process seems to be triggered by membranes [25].

To test this hypothesis, we propose a PhD project in which a greater number of IDPs and globular proteins of known 3D structure will be characterized using the same experimental approach used for N_{TAIL} and lysozyme [18]. Experimental data will be compared to *in silico* predictions based on surface accessibility of amino acid residues in IDPs and proteins of known 3D structures, and hydrophobicity scales based on amino acid tensioactivity at the A/W interface [26] and amino acid adsorption onto phospholipid bilayers (liposomes) [27].

In addition to surface tension measurements, surface rheology and PM-IRRAS, we will also implement surface fluorescence in order to visualize protein adsorption, lipids and eventually observe the formation of interfacial domains. This part of the project will be performed using a new drop tensiometer prototype currently in development at TECLIS Scientific, the company that already manufacture the dynamic drop tensiometer TRACKER used in our laboratory for surface tension and dilatational rheology measurements. TECLIS will be the intersectoral partner in this project as further described in section 2.2 “Intersectoral dimension.

Apart from fundamental considerations, the properties of IDPs at interfaces may be useful for a better understanding of the protein aggregation process occurring in neurodegenerative diseases. The tools and methods developed here may be used to monitor the kinetics of this process, to identify factors that can triggered it as well as potential drugs to block it. The knowledge gained from this project may also be used for engineering protein layers at various interfaces (i.e., stabilization of emulsions, blocking the adsorption of other proteins/enzymes [28]). Folding at interfaces of disordered peptides has already been used to specifically turn on the lytic activity of anticancer cationic peptides at the electronegative surface of cancer cells. These peptides were designed to remain unfolded and inactive in aqueous solution but they preferentially adopt an amphiphilic β-hairpin structure capable of membrane disruption upon interaction with cancer cell membrane [29]. The study of IDPs at interfaces and membranes can therefore be associated with various applications in pharmaceutical and food industries, as well as in material science.

We think that our project is relevant to two of the six interdisciplinary research axes of the programme: **nano-health and imaging**. The two co-supervisors of this interdisciplinary project and their intersectoral partner, TECLIS, have already successfully collaborated as illustrated by their very recent joint publication in ***Biophysical Journal*** [18] that will be an asset for this new PhD project.

1.2 METHODOLOGY

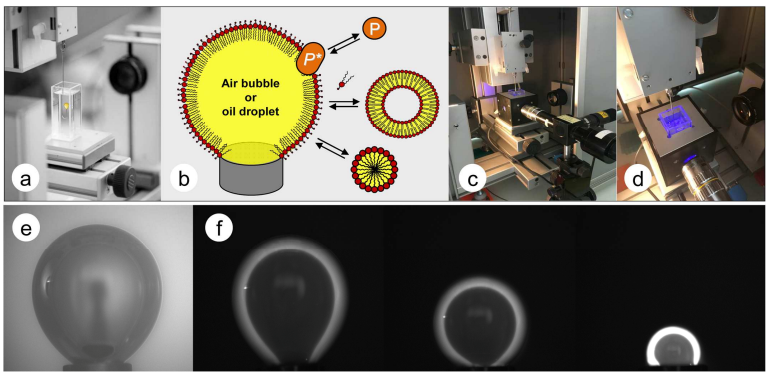
Choice of the proteins: We will work on selected proteins belonging to five distinct groups defined by their molecular structure:

- IDPs (always disordered in solution in the absence of a partner/ligand)
- Conditionally disordered proteins (CDPs; proteins that can exist in both disordered and ordered state, depending for instance on redox conditions)
- *De novo*-designed disordered proteins (protein sequences generated *in silico* using InSiDDe, a publicly available server recently developed by Sonia Longhi's group [30])
- Globular proteins with known 3D structures and no function at interfaces
- Globular proteins with known 3D structures, known for binding and acting at interfaces

IDPs will include in priority the proteins studied by Sonia Longhi'group (N_{TAIL} and P_{NT} domains, and the V protein from measles, Nipah and Hendra viruses) as well as the proteins involved in neurodegenerative diseases like α-synuclein, amyloid-β peptide (Aβ), Tau and Prion proteins. These latter proteins will be obtained in the framework of a collaboration with the Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, UK (Prof. Michele Vendruscolo, Dr Johnny Habchi). As a model CDP, we will use the CP12 protein involved in the control of enzyme activities within the Calvin cycle, a protein currently studied by Dr Brigitte Gontero at the BIP laboratory and who will be a partner of this project [31]. CP12 has a well-defined 3D structure in its oxidized form (disulfide bridges formed) but it becomes disordered upon reduction. We will study the interfacial behavior of these two forms of CP12. As typical globular proteins with known 3D structures and acting after binding at interfaces, we will use various lipolytic enzymes (available from Frédéric Carrière) that are characterized by either specific interfacial binding domains or undergo local conformational changes leading to exposure of their hydrophobic surface [32]. *De novo*-designed disordered proteins and heat-denatured globular proteins will be used as controls to test the robustness of our models and predictions.

Molecular biology and protein production: IDPs/IDRs from measles, Nipah and Hendra viruses will be produced in the Longhi's lab as recombinant proteins expressed in *E. coli*. Constructs encoding measles virus N_{TAIL} and P_{NT} fused to green fluorescent protein (GFP) are already available and additional fusion proteins may be produced during the project. Proteins will be purified by affinity chromatography (IMAC) and size exclusion chromatography (SEC) using a fast protein liquid chromatography (FPLC) device according to already established protocols.

Study of interfacial properties: Surface tension measurement will be performed using both a Langmuir balance KSV 5000 and a TRACKER dynamic drop tensiometer available at Frédéric CARRIÈRE's lab. This latter equipment will be simultaneously used for dilatation rheology and visco-elastic modulus determination using oscillating bubbles (air/water interface) or oil droplets in water. Experiments in the presence of lipids will be performed with pure phospholipids either spread as Langmuir films at the A/W interface or transferred from bulk to air bubble or oil drop using liposomes. Surface fluorescence experiments will be performed with a new drop tensiometer prototype developed in collaboration with TECLIS Scientific. The figure below shows some preliminary results obtained with the N_{TAIL} protein fused with GFP.



(a) Automated drop tensiometer showing the cuvette in which the air bubble or oil droplet is formed and the CCD camera used for drop shape analysis and surface tension determination; (b) schematic representation of protein (P), phospholipids or surfactants adsorption at the interface; (c and d) Prototype with surface fluorescence showing the excitation light source and a second CCD camera placed at 90° from the beam light used for drop shape analysis; (e) bulk fluorescence resulting from Ntail-GFP injection; (f) fluorescence images of Ntail-GFP adsorbed at the surface of the air bubble after buffer exchange. Fluorescence becomes more intense upon reduction of the bubble volume and surface, indicating that Ntail-GFP remains tightly bound to the interface and its surface concentration is increased.

PM-IRRAS measurement will be performed at the Functional Lipidomics IBiSA Platform (INSA Lyon-Villeurbanne) at which we have access thanks to our membership to GIS IMBL.

1.3 WORK PLAN

Workplan / Tasks	*Semesters*					
	1	*2*	*3*	*4*	*5*	*6*
Protein production or collection from partners						
Production of specific protein variants, fusion with GFP for fluorescence studies						
Protein adsorption kinetics at A/W interface and surface rheology using the dynamic drop tensiometer and Langmuir films						
Protein adsorption kinetics and surface rheology in the presence of phospholipids using the dynamic drop tensiometer and langmuir films						
PM-IRRAS spectroscopy for protein structure at interfaces						
Coupling of surface tension measurements and rheology with surface fluorescence using the prototype developped by TECLIS						
In silico modeling based on surface tension-derived hydrophobicity scale and amino acid accessibility, comparison with experimental data						
Participation and organization of COST training school						
Thesis writing and defence						

1.4 SUPERVISORS AND RESEARCH GROUPS DESCRIPTION

Frédéric CARRIERE, Chemical Engineer and PhD in Enzymology, Research Director at CNRS, will be the supervisor of the PhD thesis. After being head of a mixed unit of Aix-Marseille University and CNRS for 14 years (UMR7282 EIPL) and scientific director of a Carnot Institute (LISA, 2007-2015), he recently joined UMR7281 Bioenergetics and Protein Engineering and the research team "Enzymology of Supramolecular Systems" headed by Dr Brigitte Gontero. He is an expert in lipid-protein interactions and techniques for studying these interactions at interfaces (Langmuir monomolecular films, Surface tensiometry, Surface Rheology, transmission and ATR infrared spectroscopy, lipid analysis). He dedicated most of his carrier to the study of lipolytic enzymes and development of methods for their characterization. He is currently working on the development of a new instrument coupling surface tensiometry, surface rheology and surface fluorescence, in collaboration with the TECLIS company (https://www.teclis-scientific.com/; CEO: Dr Alain Cagna), a SME based at Tassin, France. Funds from the research collaboration agreement between TECLIS, Aix Marseille University and CNRS will be used to support the PhD student's work, travels and international meetings. The collaboration between TECLIS and the laboratory aims at creating the joint lab "BIO-INTERFACES". The team to which Frédéric CARRIÈRE is affiliated already hosts two researchers from TECLIS.

Sonia LONGHI, PhD in Molecular Biology, Research Director at CNRS (DR1, Section 20, INSB), will be the co-supervisor of the PhD thesis. Sonia LONGHI is head of the team "Structural Disorder and Molecular Recognition" within the AFMB laboratory, Marseille (http://www.afmb.univ-mrs.fr/-Structural-Disorder). Sonia LONGHI (see http://www.afmb.univ-mrs.fr/Sonia-Longhi) is one of the European leading groups in the field of intrinsically disordered proteins (IDPs) and protein regions (IDRs), with a long-standing experience in their prediction, molecular characterization and in the molecular investigation of the interactions they establish with their partners, a topic that has been the focus of her research over the last 16 years as witnessed by her publication records (see http://www.afmb.univ-mrs.fr/les-publications-de-l-equipe,297).

In 2007, Sonia LONGHI was nominated Council Member (four-year term) within the IDPs subgroup of the Biophysical Society (see http://www.biophysics.org/subgroups/idp.htm). In July 2014, she has been elected vice-chair of the Gordon Research Conference on Intrinsically Disordered Proteins. She organized (or co-organized) many workshops or meetings focused on intrinsically disordered proteins including an international INSERM workshop on Intrinsically Disordered Proteins (St. Raphaël, France, May 2008), a Workshop on "Structural & Unstructural Biology of Viral Proteins" (Florence, Italy, January 2012), a Biophysical Society Thematic Meeting on Disordered Motifs and Domains in Cell Control (Dublin, Ireland, October 2014), a workshop entitled "One day in the life of intrinsically disordered proteins" (Paris, France, October 2015), a COST NGP-net Training School on Experimental Methods for Protein Disorder & Aggregation (Marseille, France, February 2017) and a COST NGP-net Hackathon on DisProt curation (Marseille, France, February 2017). Sonia LONGHI is member of the Core Group of the COST action on Non Globular Proteins (BM1405, see http://www.cost.eu/COST_Actions/bmbs/Actions/BM1405).

2. 3I DIMENSIONS AND OTHER ASPECTS OF THE PROJECT

2.1 INTERDISCIPLINARY DIMENSION

This project is at the interface between chemistry, physics and biology. It aims at studying the structure, conformational changes and interfacial properties of a specific class of proteins, e.g. IDPs, when these proteins bind at interfaces. The supervisor, Frédéric CARRIERE, is affiliated to the doctoral school of chemical sciences of Aix Marseille University (ED250). His laboratory (UMR7281 BIP) will provide the environment and instruments for surface tension and rheology measurements at air-water and lipid-water interfaces upon adsorption of IDPs, as well as for the purification and analysis of lipids. The co-supervisor, Sonia LONGHI, is affiliated to the doctoral school of life sciences and health of Aix Marseille University (ED62). Her laboratory (UMR7257 AFMB) will provide the environment for the production of various IDPs and variants using molecular biology tools, expression systems and protein purification devices. IDPs coupled to fluorophores like green fluorescent protein will be produced as probes for testing the new tensiometer with surface fluorescence.

2.2 INTERSECTORAL DIMENSION:

- *Name and role played by the intersectoral partner in the thesis project:* TECLIS Scientific as a SME will be the intersectoral partner of the thesis project. The research collaboration with TECLIS allows the use of new instruments like the dynamic drop tensiometer couple to surface fluorescence. Among other projects, the PhD project on IDPs at interfaces will serve as a proof of concept for the simultaneous assays of protein adsorption by surface tensiometry and surface fluorescence, and the development of the new tensiometer.

- *Relevant expertise they will bring to the project:* TECLIS is a spin off company from CNRS created in 1991 with the support of a laboratory based at Marseille (Centre de Biochimie et de Biologie Moléculaire). It provides customized instruments and lab' services for interface science. TECLIS is a pioneer and a leader in this area with the commercialization of the TRACKER dynamic drop tensiometer, an instrument that allows surface tension measurements based on automated drop shape analysis and dilatational rheology based on drop dilatation/compression cycles. The data obtained with this instrument can help for instance for the characterization of surfactants and their use in making emulsions and foams. The customers of TECLIS include major companies such as Nestlé, L'Oréal, Total, Henkel, LVMH, Schlumberger, Procter&Gamble and Dow Chemicals. Within the thesis project, the TRACKER instrument will allow the determination of the interfacial properties of various IDPs. In addition TECLIS will provide regular updates of the instrument and software as well as a new prototype including the surface fluorescence option.

- *What will be the relationship between the intersectoral partner and the doctoral candidate?* The doctoral candidate will have a close relationship with TECLIS since his/her project will be part of a research collaboration between the company, Aix Marseille University and CNRS. This will offer the possibility for the candidate to learn about industrial environment and practices, and may provide future employment opportunity.

- *Does the project answer one of the SRI-S3 objectives?* The project is not directly in line with the main innovation axis defined by Conseil Régional from Provence Alpes Côte d'Azur region. Nevertheless, it fits with SRI-S3 objectives of French regions, with the support of innovative SME R&D in view of developing new markets, job opportunities and local industrialization. The TECLIS company has his headquarters at Tassin, in the Auvergne-Rhône-Alpes region, but TECLIS and the BIP laboratory are currently working on the creation of a joint R&D laboratory named "BIO-INTERFACES" and located at the CNRS campus of Marseille. In the framework of this collaboration, two researchers employed by TECLIS already work at the laboratory and they will contribute to the training and technical assistance of the doctoral candidate. This will also contribute to the partnership of two French regions.

2.2 INTERNATIONAL DIMENSION:

As mentioned above in 1.4, Sonia LONGHI his highly involved in international networks and societies dealing with intrinsically disordered proteins. She is currently member of the Core Group of a COST action on Non Globular Proteins (COST NGP-net; see http://www.cost.eu/COST_Actions/bmbs/Actions/BM1405) and she is in charge of organizing workshops /training schools on experimental methods for protein disorder and aggregation. On the other hand, the scientific marketing of the TECLIS company is based on sponsoring and organization of scientific meetings dealing with interface science. As an example, TECLIS recently co-organized the Bubbles and Drops 2017 international congress held in Lyon (see http://www.bd2017-lyon.fr/). Based on these experiences, we plan to organize a second international training school dedicated to methods for studying proteins at interfaces. It will be organized in the framework of the COST action NGP-net, with a special focus on PhD students. The doctoral candidate will have the opportunity to join this international network and will participate to the training school as both an attendee and a co-organizer.

3. RECENT PUBLICATIONS

Schramm A, Lieutaud P, Gianni S, Longhi S* and Bignon C*. (**2018**) InSiDDe: a server for designing artificial disordered proteins. ***Int J Mol Sci***, 19, 91. IF 3.22

Bénarouche A, Habchi J, Cagna A, Maniti O, Girard-Egrot A, Cavalier JF, Longhi S* and Carrière F*. (**2017**) Interfacial properties of N_{TAIL}, an intrinsically disordered protein. ***Biophysical Journal*** 113, 2723-2735. IF 3.66

Longhi S*, Bloyet LM, Gianni S and Gerlier D. (**2017**) How order and disorder within paramyxoviral nucleoproteins and phosphoproteins orchestrate the molecular interplay of transcription and replication. ***Cell Mol Life Sci***, 74, 3091–3118. IF 5.57

Hamdi K, Salladini E, O'Brien DP, Brier S, Chenal A, Yacoubi I* and Longhi S*. (**2017**) Structural disorder and induced folding within two cereal, ABA stress and ripening (ASR) proteins. ***Sci Rep***, 7, 15544. IF 4.85

Bénarouche A, Sams L, Bourlieu C, Vié V, Point V, Cavalier JF and Carrière F*. (**2017**) Studying gastric lipase adsorption onto phospholipid monolayers by surface tensiometry, ellipsometry and atomic force microscopy. ***Methods in Enzymology*** 583,255-278 (In: Enzymology at the Membrane Interface: Interfacial Enzymology and Protein-Membrane Binding; Michael H. Gelb, editor; Academic Press). IF 1.97

Mateos-Diaz E, Amara S, Roussel A, Longhi S, Christian Cambillau C and Carrière F*. (**2017**) Probing conformational changes and interfacial recognition site of lipases with surfactants and inhibitors. ***Methods in Enzymology*** 583, 279-308 (In: Enzymology at the Membrane Interface: Interfacial Enzymology and Protein-Membrane Binding; Michael H. Gelb, editor; Academic Press). IF 1.97

Piovesan D, Tabaro F, Micetic I, Necci M, Quaglia F, Oldfield C, Aspromonte MC, Norman Davey NE, Davidovic R, Dosztanyi Z, Elofsson A, Gasparini A, Hatos A, Kajava AV, Kalmar L, Leonardi E, Lazar T, Macedo-Ribeiro S, Macossay Castillo M, Meszaros A, Minervini G, Murvai N, Pujols J, Roche DB, Salladini E, Schad E, Schramm A, Szabo B, Tantos A, Tonello F, Tsirigos KD, Veljkovic N, Ventura S, Vranken W, Warholm P, Uversky VN, Dunker AK, Longhi S*, Tompa P* and Tosatto SCE*. (**2017**) DisProt 7.0: a major update of the database of disordered proteins. ***Nucleic Acid Research***, 45(D1):D1123-D1124. IF 9.20

Scheuble N, Lussi M, Geue T, Carrière F and Fischer P. (**2016**) Blocking Gastric Lipase Adsorption and Displacement Processes with Viscoelastic Biopolymer Adsorption Layers. ***Biomacromolecules*** 17, 3328-3337. IF 5.25

Bourlieu B, Paboeuf G, Chever S, Pezennec S, Cavalier JF, Guyomarc'h F, Bouhallab S, Dupont D, Carrière F and Vié V. (**2016**) Adsorption of gastric lipase onto multicomponent model lipid monolayers with phase separation. ***Colloids and Surfaces B: Biointerfaces*** 143, 97-106. IF 3.88

Habchi J, Tompa P, Longhi S* and Uversky VN*. (**2014**) Introducing protein intrinsic disorder. ***Chemical Reviews*** 114, 6561-88. IF 50.67

Xue B, Blocquel D, Habchi J, Uversky AV, Kurgan L, Uversky VN and Longhi S*. (**2014**) Structural disorder in viral proteins. ***Chemical Reviews*** 114, 6880-6911. IF 50.67

Accardo A, Leone M, Tesauro D, Aufiero R, Bénarouche A, Cavalier JF, Longhi S, Carriere F and Filomena Rossi F. (**2013**) Solution conformational features and interfacial properties of an intrinsically disordered peptide coupled to alkyl chains: a new class of peptide amphiphiles. ***Molecular Biosystems*** 9, 1401-1410. IF 2.78

4. EXPECTED PROFILE OF THE CANDIDATE

We are looking for a PhD candidate interested by a multidisciplinary research project at the interface between chemistry, physics and biology, including both fundamental aspects of protein physical chemistry and technological development in the framework of a partnership with a SME. In this project the PhD candidate will study the interfacial properties of various intrinsically disordered proteins (IDPs) and will compare them to those of globular proteins with the aim of defining specific features of IDPs using experimental data, theoretical models and bioinformatics. He/she will perform physical measurements (surface tension, dilatational rheology) using both Langmuir films and a dynamic drop tensiometer, and will participate to the development of a new apparatus equipped with surface fluorescence. For this purpose, he/she will produce recombinant proteins and their variants fused to fluorescent probes.

We are searching for a candidate with a master degree and a background in the relevant areas, preferentially in physical chemistry of interfaces and colloidal systems, soft matter, biochemistry, protein chemistry and/or biophysics. Additional knowledge in molecular biology and protein expression will be appreciated but is not mandatory (specific training can be provided at the beginning of the project).

We are looking for a candidate who can work independently but has also a good team spirit, who is capable of combining instrument development with experimental work and theoretical studies.

A good knowledge of English is required and an additional knowledge of French will be appreciated.

5. SUPERVISORS' PROFILES

Frédéric CARRIERE (54-year old) is Director of Research (DR1) at Centre National de la Recherche Scientifique (CNRS). He received a degree in Chemical Engineering from Marseille High School of Chemistry (ESCM) in 1986, a PhD in Enzymology in 1992 and an HDR in 2002, both from Aix-Marseille University. From 1988 to 1992, he prepared his PhD thesis in the laboratory of Dr. Robert Verger, on the biochemical properties and the physiological role of gastric lipase, and was employed by the Jouveinal Laboratories (CIFRE contract). From 1992 to 1994, he was researcher at Novo Nordisk A/S (Denmark) where he worked on the structure-function relationships of pancreatic lipases in the Protein Chemistry department headed by Dr. Lars Thim. He obtained a permanent position at CNRS in 1995 and was group leader at Dr. Robert Verger's laboratory before becoming his successor in 2004. He was the Director of the Laboratory of Enzymology at Interfaces and Physiology of Lipolysis (EIPL UMR7282), a joint unit of CNRS and Aix Marseille University, from 2004 to 2017. He recently joined UMR7281 Bioenergetics and Protein Engineering and the research team Enzymology of Supramolecular Systems headed by Dr Brigitte Gontero.

Frédéric Carrière has 29 years of expertise in the field of lipids, lipid-protein interactions, lipolytic enzymes and methods for studying these molecules at interfaces. He is the author of >200 publications (h-index >40) dealing with the structure-function relationships and physiological roles of lipases (see https://www.researchgate.net/profile/Frederic_Carriere; ORCID: http://orcid.org/0000-0003-4848-9418 , ResearcherID: E-8408-2010; https://scholar.google.fr/citations?user=b0jlyaYAAAAJ&hl=fr). Among other duties, Frédéric Carrière was the Scientific Director of the LISA Carnot Institute (Lipids for Health and Industry), a consortium of laboratories aiming at partnership with industry in the field of lipid science and technology, from 2007 to 2015. He was president of GERLI (Groupe d'Etude et de Recherche en Lipidomique, the lipid section of the French Society for Biochemistry and Molecular Biology) from 2008 to 2014 and he is still a member of GERLI scientific board. Since 2007, he is a member of the Biologics and Biotechnology Expert Committee at United States Pharmacopeia and Chair of the Pharmaceutical Enzyme Panel. He was awarded the Chevreul medal in 2007 for his work on lipid science.

During his career he supervised 13 PhD students (3-years of doctoral training). 2 of these PhD theses are currently being supervised, with one of them being co-supervised as detailed below.

PhD thesis supervised by Frédéric Carrière

Sofiane BEZZINE, (Université d'Aix-Marseille III, 1995-1999). co-supervision with Robert Verger. Thesis defended on march 22, 1999. Present professional status: Professor at ENIS, Sfax University, Tunisia. Number of publications resulting from the thesis: 8.

Barbara SIAS (Université d'Aix-Marseille II, 2000-2004). Thesis defended on january 18, 2005. Present professional status: R&D manager at Bonilait Proteines SA (Chasseneuil-du-Poitou, France). Number of publications resulting from the thesis: 4.

Ahmed ALOULOU (Université d'Aix-Marseille II ; 2004-2007) in the framework of a CIFRE contract with Mayoly-Spindler Laboratories. Thesis defended on October 19, 2007. Present professional status: Assistant Professor at ENIS, Sfax University, Tunisia. Number of publications resulting from the thesis: 9.

Jorge RODRIGUEZ (Université d'Aix-Marseille II ; 2004-2008). Thesis defended on November 4, 2008. Present professional status: R&D manager at CIATEJ, Guadalajara, Mexico. Number of publications resulting from the thesis: 9.

Sylvie FERNANDEZ, (Université d'Aix-Marseille III ; 2005-2008), co-supervision with Dr. Vincent Janin in the framework of a CIFRE contract with Gattefossé SAS. Thesis defended on October 31, 2008. Present professional status: International Study Manager at MediNeos Observational Research, Modena, Italy. Number of publications resulting from the thesis: 7.

Amal NAJJAR (Université d'Aix-Marseille II ; 2006-2010) Thesis defended on October 29, 2010. Present professional status: Assistant Professor at Lebanese University, Lebanon. Number of publications resulting from the thesis: 2.

Sawsan AMARA (Université d'Aix-Marseille II ; 2007-2011), co-supervision with Dr Alain De Caro. Thesis defended on march 11, 2011. Present professional status: CEO of Lipolytech, Marseille, France. Number of publications resulting from the thesis: 6.

Kaouthar DRIDI (Aix-Marseille Université ; 2010-2013). Thesis defended on may 29, 2013. Present professional status: Clinical Research Associate Trainee, Luxembourg Institute of Health. Number of publications resulting from the thesis: 2.

Anaïs BENAROUCHE (Université d'Aix-Marseille II ; 2010-2013), co-supervision with Dr J-F Cavalier. Thesis defended on December 17, 2013. Present professional status: Researcher at Teclis Scientific, Marseille, France. Number of publications resulting from the thesis: 5.

Eduardo MATEOS DIAZ (Aix Marseille Université ; 2013-2016). Thesis defended on December 19, 2016. Present professional status: Researcher at Immunocore Ltd. (Oxford, UK). Number of publications resulting from the thesis: 6.

Laura SAMS (Aix Marseille Université ; 2014-2017), in the framework of a CIFRE contract with GERME S.A. Thesis defended on april 13, 2017. Present professional status: Student at IFSI school for nurses, La Garde, France). Number of publications resulting from the thesis: 4.

Moulay SAHAKA (Aix Marseille Université ; since january 2017). Number of publications resulting from the thesis so far: 2.

Cyril ASELMEYER (Aix Marseille Université ; since october 2017), co-supervision with Dr Fred Beisson (Laboratoire de Bioénergétique et Biotechnologie des Bactéries et Microalgues, CEA Cadrache)

Sonia LONGHI (53-years old) has a PhD in Molecular Biology and Microbiology (1993, Universita' degli Studi di Milano, Italy) and a HDR (2003, Habilitation à Diriger les Recherches) in Structural Virology (2003, Université de Provence, Aix-Marseille I, Marseille, France). After her PhD, she got two post-doctoral fellowships, one from the EU (within the BRIDGE program) and one from the University of Milan, to join the AFMB in Marseille (Christian CAMBILLAU's team) where she kept on working during the rest of her career. She was enrolled as a CNRS scientist (CR1) in 1999 and joined Bruno Canard's team at the AFMB. In 2006 she created her own team ("Structural Disorder and Molecular Recognition") within the AFMB lab that she is still currently leading. So far, she published >120 peer-reviewed papers (leading to an h index of >40) (see https://www.researchgate.net/profile/Sonia_Longhi; ORCID:0000-0002-6829-6771, ResearcherID: M-5305-2014).

During her career she supervised 4 post-doctoral researchers, 20 Master2 students, 1 EPHE (Ecole Pratique Hautes Etudes) student, 1 student preparing a medical thesis and 10 PhD students (3-years of doctoral training). 3 of these PhD theses are currently being supervised (with one of them being co-supervised) as detailed below.

PhD thesis supervised by Sonia LONGHI

David KARLIN (1998-2002), co-supervision with Bruno CANARD. Thesis defended May 27, 2002, Université de la Méditerranée, Aix-Marseille II. Present professional status: Professional trainer in management and scientific communication CAPE/Cosens, Marseille. Number of publications resulting from the thesis: 5.

François FERRON (2001-2005), co-supervision with Bruno CANARD. Thesis defended February 4, 2005, Université de la Méditerranée, Aix-Marseille II. Present professional status: CNRS researcher (CR2, AFMB, Marseille). Number of publications resulting from the thesis: 10.

Jean-Marie BOURHIS (2003-2006). Thesis defended October 2, 2006, Université de Provence, Aix-Marseille I. Present professional status: Asssitant Professor (MCF, Université Joseph Fourier, Grenoble). Number of publications resulting from the thesis: 11.

Johnny HABCHI (2009-2012). Thesis defended March 23, 2012. Université de la Méditerranée, Aix-Marseille II. Present professional status: Head of Research of Wren Therapeutics Ltd & Research Scientist (Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, UK). Number of publications resulting from the thesis: 20.

Antoine GRUET (2009-2012), thesis from Ecole Pratique Hautes Etudes (EPHE). Defence and EPHE diploma in September 2012. Present professional status: Lab Assistant at the Memorial Sloan Kettering Cancer Center (New York, NY, USA). Number of publications resulting from the thesis: 7.

David BLOCQUEL (2010-2013). Thesis defended December 20, 2013. Aix-Marseille Université. Present professional status: post-doctoral reseracher at Scripps Research Institute (La Jolla, CA, USA). Number of publications resulting from the thesis: 10. **DGA thesis award in 2015.**

Marion DOSNON (2012-2015). Thesis defended November 24, 2015. Aix-Marseille Université. Present professional status: Naturopath. Number of publications resulting from the thesis: 5.

Matilde BELTRANDI (2014-2016), co-supervision with Prof. Roberta PIERATTELLI (Universita' degli Studi of Florence, Italy). Thesis defended December 20, 2016. Present professional status: project manager at PQE (private company). Number of publications resulting from the thesis: 4.

Edoardo SALLADINI (2015-present). Number of publications resulting from the thesis so far: 3.

Francesca TROILO (2015-present), co-supervision with Prof. Stefano GIANNI (La Sapienza, Universita' of Rome, Italy). Number of publications resulting from the thesis so far: 3.

Antoine SCHRAMM (2016-présent). Number of publications resulting from the thesis so far: 3.

AVIS DES DIRECTEURS DES LABORATOIRES CONCERNES PAR LE PROJET DE THESE

Avis du directeur du laboratoire du directeur de thèse, Mme GIUDICI-ORTICONI Marie-Thérèse

■ Favorable □ Défavorable

Commentaires :
Ce projet extrêmement interdisciplinaire et fortement associé à l'entreprise Teclis, qui collabore activement avec le porteur de projet, s'inscrit totalement dans la stratégie du laboratoire. Avis très favorable

Fait à Marseille, le 5 janvier 2018

Signature

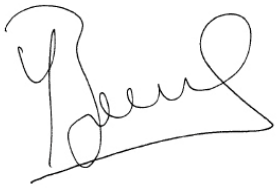
Avis du directeur du laboratoire du co-directeur de thèse, M. BOURNE Yves

■ Favorable □ Défavorable

Commentaires :
Ce projet de recherche innovant correspond parfaitement aux objectifs de l'équipe animée par Sonia Longhi qui a une renommée et un rôle moteur au niveau international dans le domaine de protéines intrinsèquement désordonnées. Il s'inscrit dans les objectifs scientifiques du laboratoire. Je soutiens donc sans aucune réserve ce projet d'excellence interdisciplinaire.

Fait à Marseille, le 5 janvier 2018

Signature



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Lyon, le 5 janvier 2018

Objet : LETTRE D'INTENTION

Madame, Monsieur,

Je soussigné, Alain Cagna, Président de la société TECLIS, atteste de la volonté d'implication effective de l'entreprise TECLIS, que je représente, dans le projet de thèse DOC2AMU « Intrinsically disordered proteins at Interfaces », qui est porté par Frédéric Carrière (DR1 CNRS, UMR7281 Bio-énergétique et Ingénierie des Protéines) et Sonia Longhi (DR1 CNRS, UMR7257 Architecture et Fonction des Macromolécules Biologiques).

Ce projet s'inscrit dans le cadre d'une nouvelle collaboration de recherche que nous avons depuis 2 ans avec le laboratoire de Frédéric Carrière et qui vise au développement de nouveaux instruments pour l'étude des interfaces en biologie, et à leur validation dans le cadre de divers projets de recherche. Parmi ceux-ci, nous avons étudié en particulier le comportement interfacial d'une protéine intrinsèquement désordonnée grâce au tensiomètre à goutte dynamique que nous développons au sein de TECLIS, et cela dans le cadre d'une collaboration tripartite avec Mme Sonia Longhi du laboratoire AFMB. Ce travail a fait l'objet d'un article que nous venons de publier dans *Biophysical Journal*. Il a en grande partie été réalisé par Mme Anaïs Bénarouche, ancienne doctorante de Frédéric Carrière aujourd'hui employée par TECLIS et détachée à Marseille. Nous souhaitons maintenant d'étudier un plus grand nombre de protéines désordonnées pour voir si nous pouvons généraliser leur comportement aux interfaces (tensioactivité importante, changement conformationnel et structuration rapide en feuillets beta) et mieux définir ce qui les différencie des protéines globulaires lors de leur adsorption. Ce projet permettra de tester aussi un nouvel instrument que nous sommes en train de développer et qui associe la fluorescence de surface avec les mesures de tension interfaciale et la rhéologie de surface.

L'accueil d'un doctorant dans ce contexte contribuera à renforcer notre relation partenariale avec Aix Marseille Université et le CNRS. Tout en s'intéressant aux propriétés fondamentales des protéines, le doctorant sera immergé dans un projet de développement industriel qui vise à la diversification des activités de notre société vers la biologie et qui devrait générer de nouveaux emplois.

Pour valoir à qui de droit.

Alain CAGNA
Président