2.2.3 LABEX GRAL (LCBM, LPCV, BGE, BCI, IBS)

Integrating structural and dynamical information at various scales is essential for understanding complex biological processes. Whereas our initial GRAL project presented a multiscale but mostly static view of life components, originally based on host-pathogen interactions and the chloroplast, in GRAL.v2, we propose to emphasize the dynamic aspects of living systems. These include the assembly of protein complexes, their integration into functional operating systems, the kinetics of interaction between host and pathogens and the self-organization of cells into multicellular architectures such as tissue or organoids. The core project will include host-pathogen and plant research at different levels, but the overall focus will be extended within two main axes on "Molecular Machines and Dynamics" and "Self organization of Living Systems". Furthermore, an important focus will be on exploiting the research capabilities in Grenoble by stimulating cooperation with other institutes/research groups with expertise in chemistry, physics, and mathematics for chemical synthesis, simulation, imaging and technology development. The common integration of GRAL and ARCANE in the CBH graduate school will facilitate such interdisciplinary research and benefit both the local research community as well as the CBH graduate students. Both Labex projects will further foster inter Labex collaborations on the importance of chemical synthesis for drug design and structural biology; principles of self-assembly of nanostructures such as DNA origami and macromolecular protein polymers; metalloenzymes, a common interest in biomedical imaging; and molecular simulation and multiscale modeling. We further propose to link both GRAL and ARCANE by supporting a new group in chemical biology that develops chemical tools to study important fundamental processes using integrated structural and cell biology. GRAL projects will further benefit from structural biology platforms at the ESRF, ILL and EMBL and via the Partnership for Structural Biology (PSB; www.psb-grenoble.eu; EMBL-ESRF-ILL-IBS) collaboration. An important focus in the selection of projects will be their interdisciplinarity, which will lead to new collaborations with important restructuring capacities for Grenoble science in integrated (structural) biology from molecules to organisms.

The management of GRAL will work closely with that of the CBH graduate school to coordinate all common actions. The project will be led by a scientific coordinator who works with the directors of BIG and the IBS as well as by two members nominated by BIG and the IBS. This coordination team will work closely with the Science Advisory Board, which is composed of 8 members from local institutes including BIG and the IBS, to cover a wide range of expertise, in order to guide the scientific strategy of GRAL and operate calls for proposals with the following objectives for the research programs.

1 **Molecular Machines and Dynamics:** The objective of this program is the comprehensive analysis of molecular machines involved in conserved cellular and pathological processes: (1) Virus host-pathogen interactions will focus on major pathogenic enveloped viruses. Lead research areas will cover replication machines and interaction with cellular complexes, membrane remodeling and budding, ribonucleoprotein complexes and long non-coding RNAs involved in the regulation of the viral life cycle and immune response, reverse vaccinology [MacLeod, Immunity 2016] and innate immune factors [Uchikawa, Mol Cell 2016]. (2) Bacterial and fungal host-pathogen interactions will focus on pathogens responsible for nosocomial and antibiotic-resistant infections, a major public health problem [Golovkine PLoS Pathogens 2016] and other life-threatening pathologies. Furthermore, we aim to understand the molecular machineries involved in cell wall synthesis, toxin secretion, biofilm formation, intracellular infection and virulence, and in phage assembly. (3)

Molecular complexes relevant for cancer and epigenetic regulation [Mietton, Nat Commun 2017] are as well of high priority. (4) Macromolecular assemblies and their dynamics will be studied by NMR, X-ray crystallography, AFM, single molecule fluorescence technologies, neutron and X-ray scattering, native mass spectrometry techniques and molecular simulation using a wide range of model systems [Macek, Sci Adv, 2017; Salvi, J Am Chem Soc 2017]. Further focus will be concentrated on serial crystallography to produce molecular movies of chemical reactions [Coquelle, Nat Chem 2017], structures of iron-sulfur cluster driven chemical reactions under anaerobic conditions [Sicoli, Science 2016], and membrane protein transport and signaling [Gushchin, Science 2017].

Deciphering the molecular details of some of the processes described above will lead to the identification of new targets for therapeutic intervention and foster interactions in drug discovery, imaging, simulation and multiscale modeling. Validated viral, bacterial, fungal and cellular complexes will be employed to identify lead inhibitors by using novel high through-put peptide based approaches, small molecule screening including natural compound libraries, human antibody based strategies, phage-based technologies, reverse vaccinology approaches and compound and/or fragment based screening using the automated EMBL-ESRF CrystalDirect technology and screening technologies developed on the IBS run CRG FIP beam line. The expertise of ARCANE in organic synthesis will be essential to synthesize new drugs from lead molecules and for innovative drug delivery technologies.

2 Self-organization of Living systems: from molecular assembly to functional architecture. One central property of living systems is their capacity to self-organize. We propose to study the dynamic properties of self-organization during morphogenesis and the response to environmental cues in different organisms such as yeast, microalgae, flies, human cells or plants. For example, we will study the dynamic assembly of cytoskeleton components [Aumeier, Nat Cell Biol. 2016], multimeric transcription factors under various developmental and environmental contexts [Sayou, Nat Commun 2016; Martin-Arevalillo PNAS 2017], and the morphogenesis of subcellular compartments such as the chloroplast membranes (lipids and associated protein complexes [Kanduc, Nat Commun 2017]) and the mitochondria. Beyond the single cell, we will address the development of complex biofilm, infected cells, tissues, organoids and organs in bacterial, fungal, animal, microalgae and plant models. Living organisms continuously perceive and adapt to changes in their environment such as light [Petroutsos, Nature 2016; Flori Nat Commun 2017], temperature, mechanical or chemical or biotic stresses. Thus, we will analyze how these cues influence self-organization both at the protein level (post-translational modifications, folding of intrinsically disordered proteins metal or nucleic acid interactions) and at the cellular level.

To reach the challenging objectives of these two axes in bridging scales from molecules to tissues to organisms, we will use a multi-scale approach combining structure and dynamics. This will include cell biology and multi-omics techniques with associated data analysis (images, movies and omics large datasets), state of the art fluorescence microscopy techniques, including single molecule imaging by TIRF, super resolution and correlative electron microscopy in combination with X-ray crystallography, atomic force microscopy and high resolution electron microscopy. This will provide structural information that will be challenged in a physiological context using high resolution confocal imaging, lattice light sheet microscopy or FIB-SEM direct imaging or coupled with electron tomography

Research proposals responding to the two axes described above will be selected based on excellence and the importance of the given biological question. The integrated approaches to address biological questions at different scales of resolution, analyzing their dynamics and function *in vitro* and *in vivo*

model systems will not only provide important results for basic science, but will open up new opportunities for biotechnological applications. Therefore a second aim will be to foster, support and protect technological developments leading to new therapeutic approaches and to innovative biotech applications with specific calls for proposals.

A list of expected results and publications cited are found in the Appendix.