



Grenoble Alliance for Integrated  
Structural & Cell Biology

## GRAL MSc RESEARCH SCHOLARSHIP 2020-2021

### RESEARCH INTERNSHIP PROPOSAL

#### Institute / Group

IRIG / IBS - FDP

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#### Research Project Title

Deciphering multivalent interactions in the clathrin-mediated endocytosis pathway

#### Description of the project

Clathrin mediated endocytosis is the major pathway for molecule import into the cell and exerts numerous vital functions, such as nutrient uptake or cellular signalling. While the protein clathrin is the major structural component of this uptake process, various other proteins are required to form a highly regulated uptake machinery. Amongst those count adaptor proteins that contain long intrinsically disordered regions, i.e. regions without stable three-dimensional structure. These regions are interspersed with small sequence stretches, called linear motifs, which interact with other proteins from the endocytosis machinery: other adaptors for example or clathrin. Although these interactions are crucial for endocytosis, they are not very well understood due to the flexibility and dynamics of the protein sequences they are embedded in.

This M2 internship project deals with the interactions mediated by the intrinsically disordered regulators of endocytosis and their partner proteins and the analysis of dynamic binding interactions using nuclear magnetic resonance spectroscopy (NMR) and single molecule fluorescence spectroscopy. The M2 candidate will follow a project that involves expression (in *E.coli*) and purification of selected constructs, site-specific labelling with synthetic fluorophores, as well as data acquisition (NMR and fluorescence) and analysis.

#### Keywords

Single molecule fluorescence spectroscopy, nuclear magnetic resonance (NMR), intrinsically disordered proteins, protein dynamics, endocytosis

#### Relevant publications of the team

Milles S, Jensen MR, Lazert C, Guseva S, Ivashchenko S, Communie G, Maurin D, Gerlier D, Ruigrok RWH, Blackledge M. An ultraweak interaction in the intrinsically disordered replication machinery is essential for measles virus function. *Sci Adv*. 2018 Aug 22;4(8):eaat7778

Milles S, Mercadante D, Aramburu IV, Jensen MR, Banterle N, Koehler C, Tyagi S, Clarke J, Shammam SL, Blackledge M, Gräter F, Lemke EA. Plasticity of an ultrafast interaction between nucleoporins and nuclear transport receptors. *Cell*. 2015 Oct 22;163(3):734-45

Delaforge E, Milles S, Bouvignies G, Bouvier D, Boivin S, Salvi N, Maurin D, Martel A, Round A, Lemke EA, Jensen MR, Hart DJ, Blackledge M. Large-Scale Conformational Dynamics Control H5N1 Influenza Polymerase PB2 Binding to Importin  $\alpha$ . *J Am Chem Soc*. 2015 Dec 9;137(48):15122-34.

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