European School of Molecular Medicine

2025 summer call PhD selections

AVAILABLE POSITIONS

Principal Investigator	Simona Polo
Affiliation	IFOM ETS - The AIRC Institute of Molecular Oncology
Title of the proposed project:	Investigating the supramolecular organization of HECT ubiquitin ligases
Short description of the project	Ubiquitination involves the covalent attachment of ubiquitin to a substrate protein through a pseudo-peptide bond between the C-terminal carboxyl group of ubiquitin and a primary amine on the substrate. Ubiquitin itself can form chains by linking to one of seven lysines or the N-terminal amine of another ubiquitin, generating an extraordinary topological diversity known as the **Ubiquitin code**. This code governs a vast array of cellular processes and is primarily written by E3 ligases, sophisticated molecular machines that dictate the specificity and architecture of ubiquitin chains. Our research focuses on HECT-type E3 ligases, a family frequently overexpressed in cancer and still not fully understood at the mechanistic level, particularly with respect to ubiquitin chain elongation. Despite several proposed models, progress has been hampered by the system's complexity. Intriguingly, we have observed that full-length NEDD4, a prototypical HECT ligase, forms **biomolecular condensates in vitro** at low nanomolar concentrations—but only in the presence of its substrates. Recent findings from our lab indicate that similar condensate formation occurs in vivo upon NEDD4 activation. Moreover, our unpublished data suggest a previously unrecognized mechanism of **substrate-assisted catalysis**, wherein the substrate itself contributes directly to the catalytic process. This project aims to elucidate the molecular interface between NEDD4 and its prototype substrate WBP2 using a multidisciplinary approach. By integrating advanced biochemical, biophysical, and structural biology techniques, including cross-linking mass spectrometry and cryo-electron microscopy, we will characterize the E3-substrate complex at near-atomic resolution. These findings will be complemented by site-directed mutagenesis to validate key interaction sites and clarify their role in substrate recognition and ubiquitin transfer. The project offers comprehensive training in cutting-edge methodologies within a collaborative, supportive, and intellectually stimulating research environment. The successful candidate will contribute to unraveling fundamental principles of ubiquitin signaling, with implications for both cancer cell biology and pharmacology, especially in the context of the emerging area of Targeted Protein Degradation, a strategy that hijacks E3 ligases to induce degradation of target proteins.
Main research area for the project	Structural Biology
Second research area for the project	Cancer Biology
3 key words for project	Structure to function, ubiquitination, LLPS

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Main topic/s of the lab	Ubiquitin signaling pathways and alternative splicing programs
Short description of the lab activity	The Laboratory of Molecular Machines in Signaling Pathways, directed by Prof. Simona Polo, investigates molecular mechanisms underlying signaling pathways commonly deregulated in cancer. Since its foundation, the lab has established a strong legacy in ubiquitin biology, elucidating how this small, versatile post-translational modifier regulates protein fate and function through diverse chain topologies and linkage types. Our work has been instrumental in defining **ubiquitin's roles beyond proteasomal degradation**, particularly in endocytosis, signal transduction, and subcellular organization. We have also characterized key features of E3 ubiquitin ligases, which confer substrate specificity and dictate the functional outcome of ubiquitin conjugation. More recently, the lab has expanded its focus to **alternative splicing**, a fundamental mechanism that enhances proteomic diversity and functional complexity. This shift reflects a refinement of our research framework: we propose that a deeper understanding of tumor biology requires moving **beyond the genomic characterization of oncogenes toward the study of proteoforms** generated through post-transcriptional and post-translational regulation. These regulatory layers are central to cellular plasticity, enabling cancer cells to dynamically adapt and evolve resistance to therapy. A unifying theme across our research is the capacity of both ubiquitin signaling and alternative splicing to remodel protein structure and function. Each mechanism introduces variation at the protein level, altering interaction networks, subcellular localization, and activity. Critically, both are co-opted in cancer to drive malignant transformation, immune evasion, and therapeutic resistance. By investigating these processes mechanistically, we aim to uncover how they converge to rewire signaling networks in disease. To address these complex questions, the laboratory employs a **multidisciplinary approach**. We utilize patient-derived organoids and advanced 3D cellular models to investigate the functional relevance of splicing alterations in a context that closely mimics human disease. Cell-free biochemical systems enable the reconstitution of ubiquitination cascades, allowing precise dissection of enzymatic mechanisms. Functional genomics, including CRISPR-based screens, is used to systematically interrogate the roles of splicing factors and ubiquitin-related genes. Leveraging IFOM's advanced facilities, we use proteomics to analyze protein isoforms and ubiquitin modifications, and next-generation sequencing to profile splicing landscapes and their regulatory architecture. Furthermore, high-resolution microscopy and structural biology techniques are applied to study E3 ligases and protein complexes at the molecular level, providing insights into the dynamic interactions that govern specificity and function. The integration of these methodologies not only deepens our understanding of cellular regulation but also supports the **identification of novel therapeutic strategies**, leveraging mechanistic knowledge to uncover vulnerabilities in cancer.

Recent bibliography	• Maspero E et al. (2025) Structure-Based Design of Potent and Selective Inhibitors of the HECT Ligase NEDD4. *Commun Chem*, 8 (1), 164. doi.org/10.1038/s42004-025-01557-4. • Gahlot P et al (2024) Lysosomal damage sensing and lysophagy initiation by SPG20-ITCH. *Mol Cell*. 2024 84:1556. doi: 10.1016/j.molcel.2024.02.029. • Barroso-Gomila O et al. (2023) BioE3 identifies specific substrates of ubiquitin E3 ligases. *Nat Commun* 14, 7656. doi.org/10.1038/s41467-023-43326-8. • Menin L et al. (2023) A planar polarized MYO6-DOCK7-RAC1 axis promotes tissue fluidification in mammary epithelia. *Cell Rep*. 42:113001. doi: 10.1016/j.celrep.2023.113001 • Fajner V et al. (2021) Hecw controls oogenesis and neuronal homeostasis by promoting the liquid state of ribonucleoprotein particles. *Nat Commun*. 16;12(1):5488. doi: 10.1038/s41467-021-25809-8.
Group composition	2 staff scientist, 2 postdocs, 4 phD students, 2 master student
Institutional page link	https://www.ifom.eu/en/cancer-research/programs/molecular-machines-signalling-pathways.php