

French/German PhD position at the University of Strasbourg and Karlsruhe Institute of Technology

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Study of the organization and dynamics of CD44 / MET complexes at the plasma membrane stimulated by endogenous (HGF) and bacterial ligands (Internalin B) by advanced microscopy techniques

We are looking for a young scientist willing to work on an interdisciplinary project in cell biology using advanced fluorescence microscopy techniques. The aim of this project is to characterize the organization and dynamics of CD44 transmembrane glycoproteins that play an essential role in cell migration and proliferation in physiological and pathological situations. The variety of CD44 functions is related to their ability to associate with ligands such as MET (receptor tyrosine kinase (RTK)) and HGF (human growth factor). Moreover, *Listeria monocytogenes* through its protein Internalin B (InIB) uses also this MET pathway to infect host cells. As these processes take place at the plasma membrane, the membrane organization plays a crucial role, in particular the lipid rafts, which are small and dynamic membrane domains enriched with sphingolipids and cholesterol. The objective of this PhD thesis will be to investigate the interplay between CD44v6, MET and the lipid domains on HGF and InIB stimulation. The PhD student will first quantitatively characterize the interaction of CD44v6 with MET promoted by HGF and InIB by FLIM-FRET in live HEK-293 cells. The amounts of CD44v6/MET complexes and MET dimers will be determined in non-activated cells and compared to those in the presence of HGF or InIB. Further information will be obtained by using downregulated CD44v6 (siRNA and specific Crispr/cas constructs) and primary cells isolated from a Cd44v6 floxed mouse. Quantitative data on MET dimerization in cells in which the expression of CD44v6 can be manipulated will provide valuable insights into the role of CD44v6 in MET dimerization. In parallel, deletion mutants of InIB will be used to analyze the contribution of the different InIB domains in the recruitment of CD44v6. These investigations will be completed by solution measurements to characterize the interaction of wild-type and mutant InIB with the purified CD44v6 ectodomain. The comparison with HGF data will shed light on how *L. monocytogenes* takes advantage of the MET/CD44v6 pair to interact with and then enter non phagocytic cells. In a second task, the PhD student will investigate by two-color super-resolution localization microscopy the role of lipid rafts in the CD44v6/MET complex formation upon HGF and InIB activation. Localizations of membrane proteins fused to photoactivable proteins or organic fluorescent dyes will be pinpointed and their spatial distribution analyzed relative to those of lipid rafts. Treated cells with altered cell membrane organization will also be measured. This will provide an extensive view of the distribution of CD44v6 or MET in and out of the raft domains. Complementary information in live cells will be obtained by single particle tracking (sPT). This should help to further decipher the mechanism of CD44/MET complex formation upon HGF- and InIB-activation. Taken together, these data will give a better understanding of the interaction between CD44v6 and MET and its connection with the lipid domains, as well as its stimulation by HGF and InIB. Comparison of HGF and InIB stimulations should also give a clearer picture of how InIB hijacks the MET-related pathway to prompt bacterial invasion.

This project is funded for a 3 years PhD position by the French-German University and will be carried out under the double supervision of Pr. Yves Mély (Strasbourg) and Pr. Veronique Orian-Rousseau (Karlsruhe).

The student should have a background in biophysics with interest for biology or a background in biology with interest for biophysical techniques. Excellent communication skills in spoken and written English are expected.

Interested individuals should send a curriculum vitae, a motivation letter, and recommendation letter(s) (or contact details of at least one referee) by e-mail to yves.mely@unistra.fr or veronique.orian-rousseau@kit.edu.

Deadline for application : September 30, 2017