PROJECT PROPOSITION - Lab1- 2023

(M1, second semester)

Supervisor(s): Robert Quast

Contact email: quast@cbs.cnrs.fr

Hosting lab: CBS

Period of proposed project (put **x** instead of □) :

□ Only 1st slot

□ One slot, but I have no preference on which

X Only 2nd slot

□ Both slots (with different groups)

1st slot: 12 days to be selected later between 1/16/2023 to 2/24/2023- see next page for info
2nd slot: 12 days to be selected later between 3/6/2023 to 5/12/2023- see next page for info

Fluorescent double labeling of metabotropic glutamate receptors using non-canonical amino acids for single molecule tracking and smFRET in the membrane of living cells

Subject (5 lines max for the description)

Metabotropic glutamate receptors (mGluR) are G protein-coupled receptors that undergo large-amplitude conformational changes upon activation by glutamate – the major excitatory neurotransmitter in the human brain. To date these conformational changes have only been studied in the membrane of living cells through SNAP-tag labeling, which suffers from the relatively large size of these tags and their limitation to N-terminal labeling.

Technical tools to be used:

Using genetic code expansion to incorporate two distinct non-canonical amino acids (ncAA) in response to two different stop codons, followed by bioorthogonal and chemo-selective reaction with different donor and acceptor fluorophores we will label mGluR in the membrane of living CHO cells. We will subsequently optimize the washing protocol and test different substrates to achieve an optimal, flat adherence of CHO cells on glass cover slides. Finally, we will perform live cell fluorescence and total internal reflection fluorescence microscopy to image the doubly labeled receptors diffusing in the membrane. We will attempt to track individual doubly labeled

receptors and analyze the fluorescence in two colors simultaneously to detect FRET between the donor and acceptor fluorophores.

Objectives:

The objectives are the optimization of the washing and adherence protocols to detect and track doubly labeled receptors for a maximum of time. Furthermore, we will attempt to determine the origin for undesired background labeling and identify receptors that exhibit FRET as a result of conformational changes.

		Lundi	Mardi	Mercredi	Jeudi	Vendredi
Periode 1	16 au 20/01					
	23 au 27/01					
	30/01 au 03/02					
	06 au 10/02					
	13 au 17/02					
	20 au 24/02					

		Lundi	Mardi	Mercredi	Jeudi	Vendredi
Periode 2	06 au 10/03					
	13 au 17/03					
	20 au 24/03					
	27 au 31/ 03					
	03 au 07/04					
	10 au 14/04					
	17 au 21/04					
	24 au 28/04					
	02 au 05/05					
	08 au 12/05					