Functional analysis of enhancers controlling memory formation in *Drosophila*
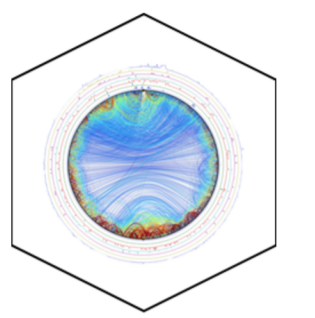
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NollmannLab

The lab

We study how **chromatin folding and transcription** are coordinated at the single-cell level. To do this, we develop and use advanced imaging approaches such as Hi-M (multiplexed DNA-FISH combined with microscopy) on a high-resolution microscope coupled to a fluidics system.

We also create open-source software (qudi-HiM, pyHiM) to analyze genome organization and transcriptional dynamics in living systems, including *Drosophila*, mouse, and human.

Our current project focuses on identifying the factors that reshape 3D DNA conformation at genes involved in long-term memory formation.
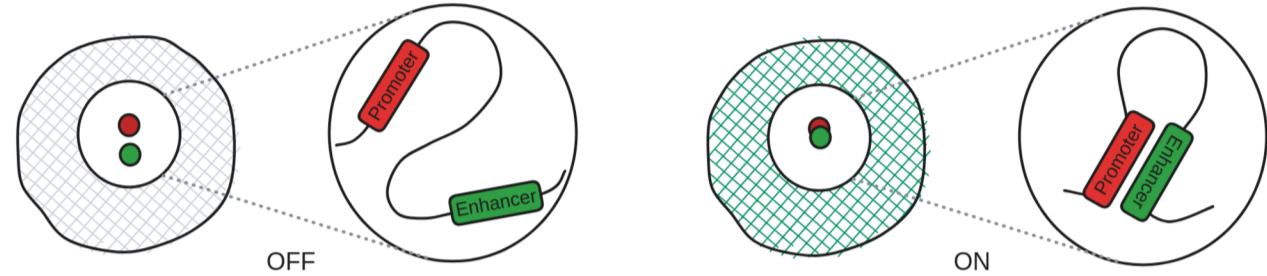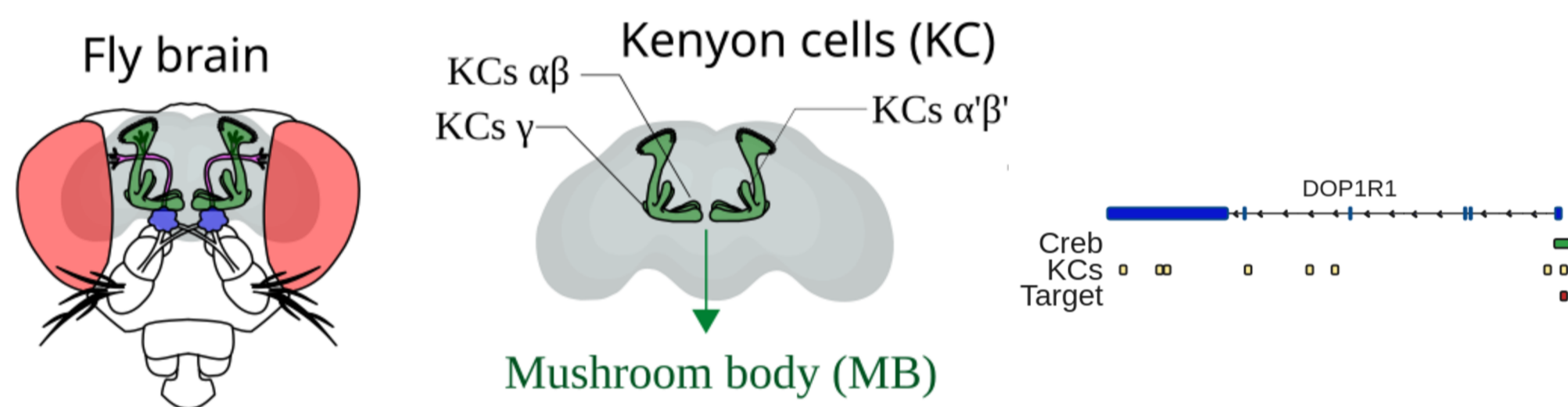

Main objectives

Our recent work has identified putative enhancers within genes involved in memory encoding, yet their functional roles remain largely untested. This project will explore the transcriptional landscape of memory-encoding neurons by combining *in vivo* enhancer manipulation with high-resolution brain imaging. Specificall, the student will:

(1) Validate candidate enhancers involved in Creb-mediated memory formation by characterising their spatial activation patterns using 3D confocal imaging. Several fly lines to test enhancer activation have already been built and await characterisation.

(2) Characterise the transcriptional impact of Creb enhancer deletions using sequential smRNA-FISH in fly brains. A few *Drosophila* stocks will be already available before the project starts. The student will build additional fly lines using gene editing at the beginning of the project.
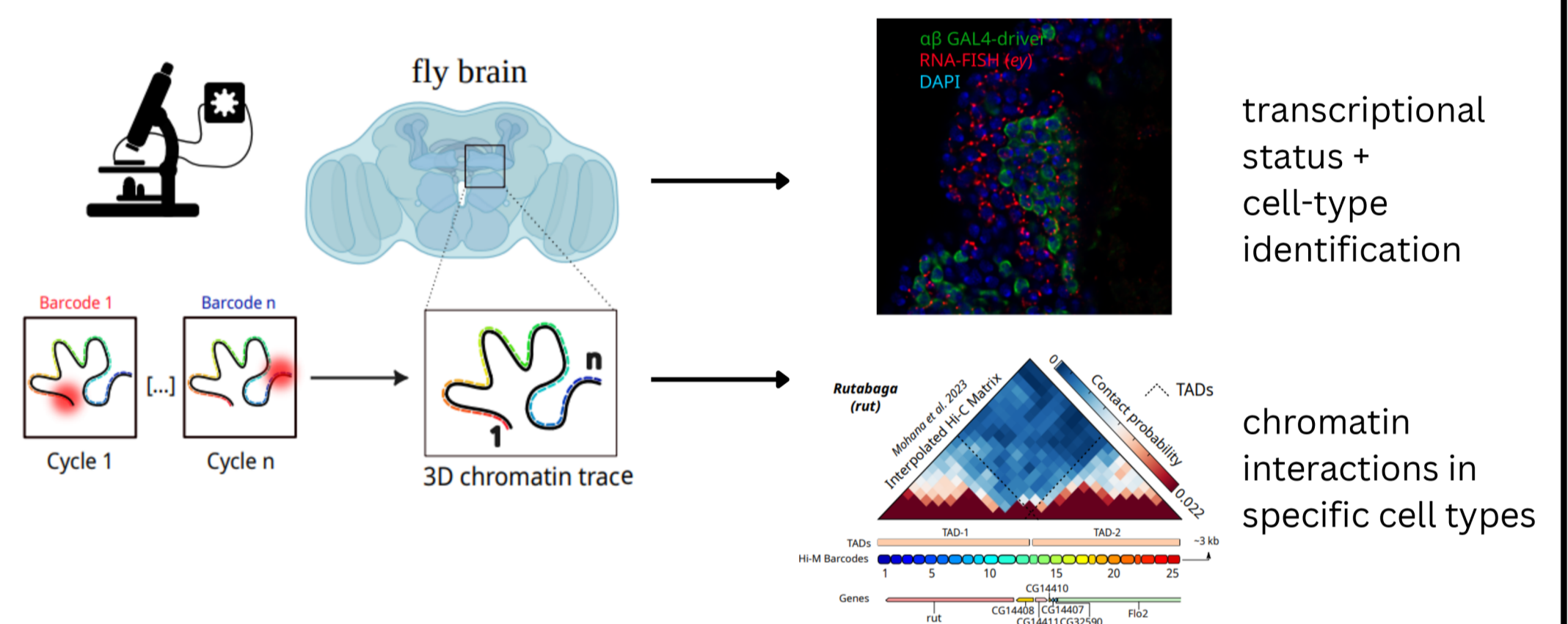
Introduction

The formation of long-term memories (LTM) requires precise transcriptional regulation in specific neurons. In Drosophila, this process is largely orchestrated by the cAMP response element-binding protein (Creb). Creb activation occurs in the mushroom body (MB), the central neuropil responsible for associative learning and memory. This project aims to further understand the molecular mechanisms by which Creb mediates transcription during memory formation in flies.
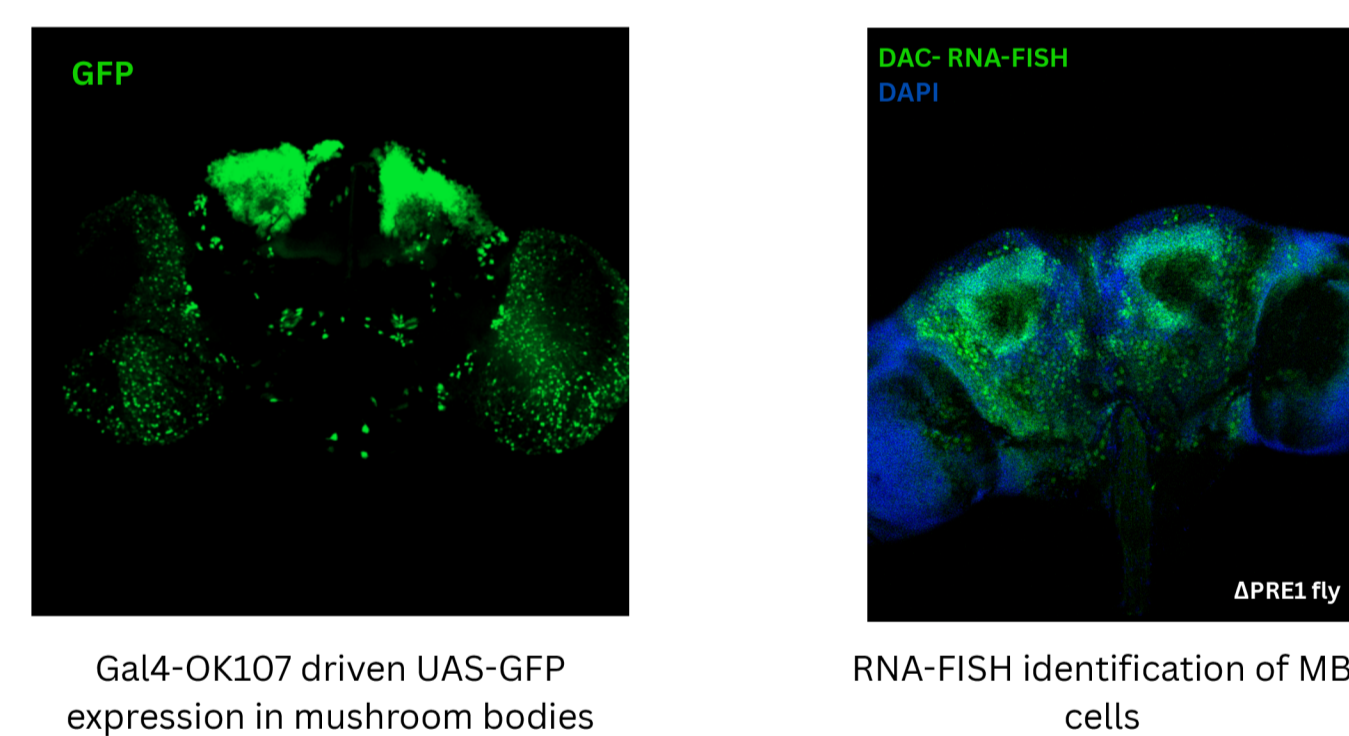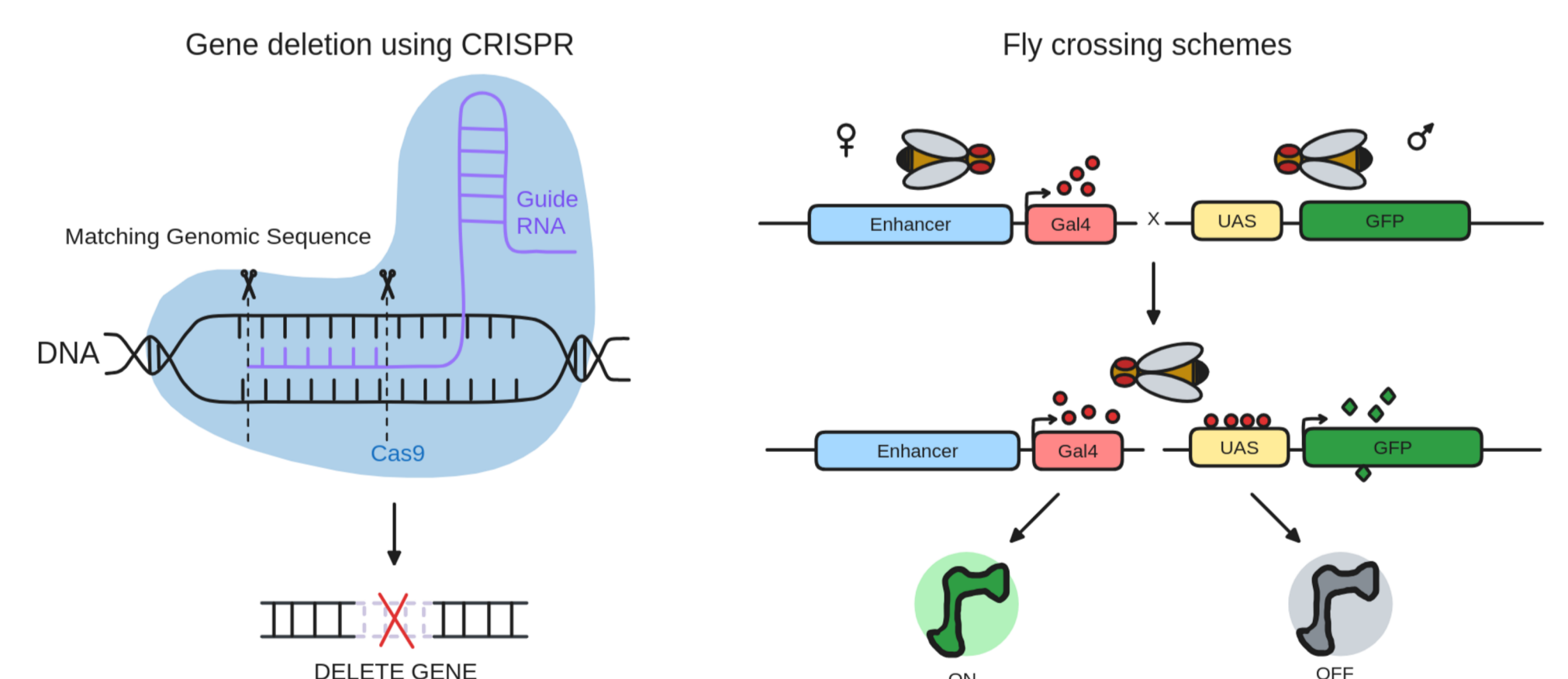

Methodologies

1- Sequential DNA-FISH (HiM) and RNA-FISH (seqFISH)

transcriptional status + cell-type identification

chromatin interactions in specific cell types

2- Whole brain inmunofluorescence and confocal imaging

Gal4-OK107 driven UAS-GFP expression in mushroom bodies

RNA-FISH identification of MB cells

3- Gene editing and *Drosophila* genetics

Gene deletion using CRISPR

Fly crossing schemes

Learning experience

Methods

By the end of this project, the student will gain hands-on experience in:
- 3D whole brain confocal imaging.
- Sequential smRNA-FISH in home-made instruments.
- Analysis of spatial transcriptomics imaging data from our setup.
- *Drosophila* genetics to generate crossings harboring GAL4/UAS alleles.
- Fly brain dissection.
- Molecular biology to construct plasmids.
- CRISPR/Cas9 gene editing to delete endogenous enhancers.

Concepts
- Understand behavioral paradigms used to study learning and memory.
- Learn advanced imaging techniques and image analysis pipelines.
- Develop critical thinking and scientific communication skills in an interdisciplinary environment

Working Environment

The student will join a dynamic and collaborative team at the interface of molecular genetics, bioinformatics, advanced imaging, and neurobiology. They will be supervised by experienced researchers and interact regularly with specialists in each field, providing a rich educational experience.

References

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