Tackling regulation of gene expression using live cells, single-molecule imaging, and cutting-edge CRISPR technologies

Tuesday, 26 March, 2024
14:30
aula 1C - edificio UE1 (Navile)

Dr. Andrea Callegari

Universität Ulm - Institut für Biophysik

Link

Synopsis. Transcription factors (TFs) regulate gene expression in both prokaryotes and eukaryotes by recognizing and binding to specific DNA promoter sequences. In higher eukaryotes, it remains unclear how the duration of TF binding to DNA relates to downstream transcriptional output. In this seminar, it will be shown how live cell, single-molecule imaging of TF-DNA binding kinetics and genome-wide quantification of TF-mediated transcription – i.e. RNAseq – can be performed to address this fundamental biological question. An improved CRISPR methodology is described to quickly generate genetically engineered cell lines that express (virtually) any protein of interest at endogenous levels. The CRISPR protocol described here significantly improves the efficiency of protein tagging and the speed of cell line generation. It also provides a quantitative tool to measure the number of on- and off-target integrations in the host cell's genome.