

Understanding the biophysical basis of life's program @NEST Laboratory

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Background

A single human genome gives rise to hundreds of cell types and adapts to different developmental and environmental conditions with a vast repertoire of gene expression patterns. A mere 2% of genome encode proteins; the remaining 98% refers to regulatory elements that underlie context-specific gene activity. The 6 billion bases of DNA, about 2 m long, are wrapped about 30 millions of nucleosomes, forming a macromolecular complex termed chromatin which resides into the cell nucleus, whose radius is about 10-20 μm . Chromatin does not only provide a means to compact DNA: it is also the essential medium through which transcription factors, signaling pathways, and other cues alter gene activity and cellular phenotypes. **Thus, understanding chromatin means understanding the biophysical basis of life's program.**

The functional "unit" of chromatin is constituted by Topologically Associated Domains (TADs), made up of loci where chromatin assumes distinct conformations that reinforce DNA transcription or repression. Their sub-micron scale (Figure 1) makes them perfectly amenable to the study by fluorescence microscopy, which can conjugate the high spatial resolution of novel microscopy techniques with the extraordinary sensitivity of fluorescence (down to single molecule) and the negligible phototoxicity of visible radiation. Recent studies highlight the dynamic balance of transcription-permissive and transcription-repressive states of chromatin (chromatin homeostasis), which is modulated by several stimuli and –in some cases- can eventually lead to cell dysfunction and cancer.

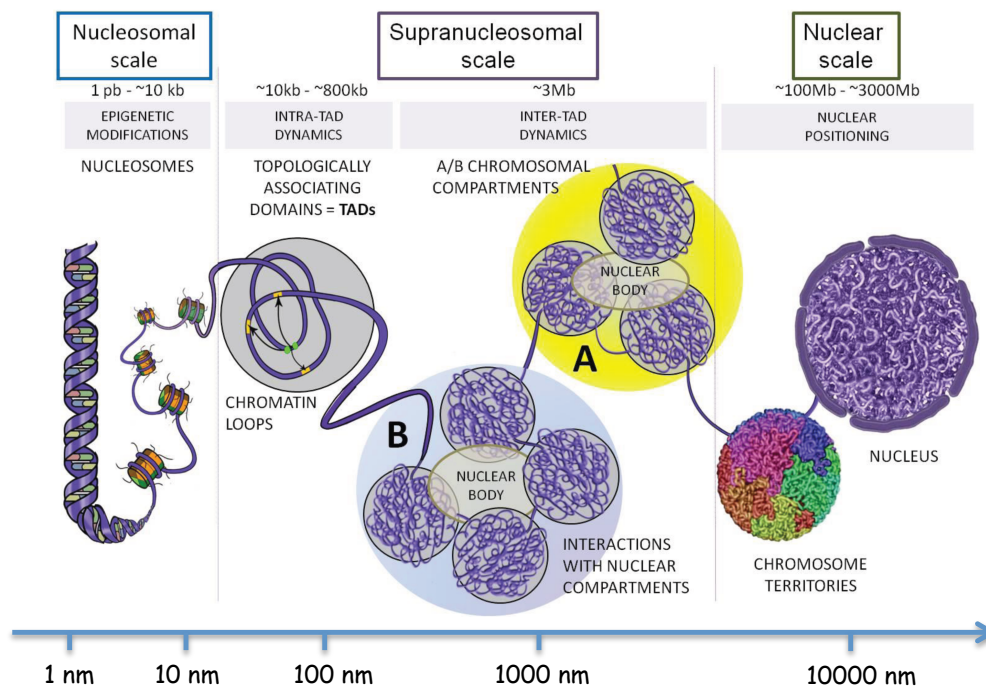


Figure 1. The hierarchical arrangement of chromatin in the nucleus

Very surprisingly, **the principles governing the 3D organization of genome within the nuclear space are just beginning to get unraveled.** Actually, the chromatin homeostasis is predicated in large part on interplay among several proteins, among which stands the family of **polycomb proteins (PcG)**. PcG proteins form large multiprotein complexes that bind gene regulatory elements and modify chromatin to establish a **transcriptionally repressive chromatin state that changes along time.**¹ Remarkably, this activity appears to be strongly related to **physical modifications of chromatin**, such as **local compaction and gel-like states.**² Yet, the direct visualization of polycomb-induced chromatin compaction is still **unreported.** Knowledge of PcG

activity will represent a **decisive step** towards the understanding of several features of cancer generation by cell dysfunction. For example, the PcG protein BMI1, which we recently studied,³ is known to be a relevant protein in at least **16 kinds of cancer**.

General goal of the group and methodologies

The fundamental goal of our group is to investigate the physicochemical changes that occur in chromatin on spatial scales that range from a few nanometers to a few microns and correlate them to the activity of PcG proteins.

This goal is accomplished by the following state-of-art imaging methods:

- High-resolution multicolor confocal microscopy applied to cell proteins
 - We developed^{4,5} a combination of Forster Resonance Energy Transfer (FRET, <10 nm resolution) and image correlation spectroscopy to probe **concomitantly** protein binding and dynamics, thus unveiling the functional role of protein complexes.
- Super-resolution techniques such as Photoactivation Localization Microscopy (PALM), and Stochastic Optical Fluctuation Imaging (SOFI)
 - For this activity, since 2010 we have been developing reversibly photoswitchable probes (that are needed to obtain super resolution) based on Fluorescent Proteins by **rational design assisted by computational studies**.⁶⁻⁸

These activities, typically carried out by physicists and chemists (3 MSc theses in 2018-2019), are complemented by *state-of-art molecular biology expertise* that is present in the group, which affords:

- Cell line models (normal and cancerous-type)
- Molecular biology approaches to engineering and expressing fluorescently-labeled proteins and DNA for cell imaging

Additionally, the group has an extensive collaboration network on this topic, including:

Italy

- Nanophysics Department of the Italian Institute of Technology (Genova)
- Fondazione Pisana per la Scienza e Azienda Ospedaliera-Universitaria Pisana (Pisa)
- Politecnico di Milano (Milano)
- Dipartimento di Fisica, Università di Parma (Parma)

International

- Beth Israel Deaconess Medical Center – Harvard Medical School, USA
- Department of Physics and Astronomy, University of Maine, USA
- Katholieke University Leuven, Belgium
- SciLifeLab, KTH Royal Institute of Technology, Sweden

MSC Thesis 2019-2020 (Physics/Chemistry background)

1. Binding and dynamics of PcG transcription factors in normal and cancerous chromatin

Description: By means of FRET/Image correlation spectroscopy carried out in state-of-art confocal apparatus, the student will investigate the **dynamics** of selected PcG proteins in the nucleus and their **clusterization** to modulate local chromatin behavior. This investigation will be carried out on *living cells* to help generating a model of transcription regulation in normal and dysfunctional (cancer model) cells. The student will learn how to carry out fluorescence microscopy experiments in the cell context based on fluctuation spectroscopy and she/he is expected to contribute to develop robust data analysis algorithms to interpret collected data.

Duration: 9-10 months.

Short stages in external institution: possible, depending on administrative agreements.

2. Morphology of PcG transcription factors in normal and cancerous chromatin

Description: By means of super-resolution microscopy (mostly PALM and SOFI), the student will investigate the **spatial organization** of selected PcG proteins in the nucleus and their role to modulate local chromatin behavior. This investigation will be carried out to help generating a model of transcription regulation in normal and dysfunctional (cancer model) cells. The student will learn how to carry out fluorescence microscopy experiments in the cell context based on super-resolution microscopy and she/he is expected to contribute to develop robust data analysis algorithms to interpret collected data.

Duration: 9-10 months.

Short stages in external institution: possible, depending on administrative agreements.

3. Development of novel reversibly-switchable fluorescent proteins for super-resolution imaging techniques

Description: By means of a combined computational/experimental approach, novel fluorescent variants characterized by photoswitching behavior will be generated and validated in super-resolution microscopy experiments. The student will learn how to carry out: a) protein production and purification, 2) fluorescence spectroscopy characterization (including transient spectroscopy experiments) of the fluorescent probes, 3) super-resolution fluorescence microscopy experiments in the cell context. She/He is expected to learn cross-disciplinary techniques between biology, chemistry and physics.

Duration: 9-10 months.

Short stages in external institution: possible, depending on administrative agreements.

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