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spFRET studies of nucleosome dynamics modulated by histone modifications, histone variants and neighboring nucleosomes

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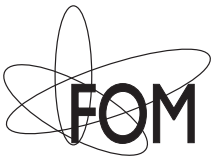

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Aan Oma Fien

Contents

Aim and outline of this thesis

The principle of encoding genetic information in DNA is common in all living organisms and is very elegant in its simplicity. Yet the translation from genetic code to a functioning living organism that reacts to its environmental conditions is tremendously complex and far from understood. At the basis of the regulation of the genetic code (DNA) in eukaryotes[1], is its organization into nucleosomes: 10 nm wide structures, in which ~ 150 basepairs (bp) of DNA are wrapped around a disk of proteins, the histone octamer. Arrays of nucleosomes are organized in fiber-like structures called chromatin, ultimately forming the well-known chromosome, that, in its most compact state during cell division, can easily be visualized with a normal light microscope. This thesis focusses on the smallest units in chromatin, the nucleosomes, and how the physical nature of these nucleosomes allows for dynamic accessibility of DNA for cellular processes.

Nucleosomes realize a huge compaction of the DNA. In humans, the 2 m of DNA (6 billion bp) present in every cell, forming a coil of ~ 100 μm in diameter if not condensed [1], fits in the cell nucleus of only ~ 5 μm in diameter. The compaction in the form of nucleosomes provides the essential 'tool' to regulate accessibility of DNA. By thermal fluctuations, part of the nucleosomal DNA transiently unwraps from the histone protein core (DNA breathing), making it accessible for the cellular machinery. Changes in the binding energy between DNA and histones alter the breathing probability, which is realized in the cell by modifications to the histones or by incorporating histone variants. The linking of nucleosomes to their neighbors poses constraints on DNA breathing as well. It is therefore not trivial to understand the regulation of DNA accessibility in a physical manner.

To distinguish open nucleosomes (where part of the nucleosomal DNA is unwrapped) from closed nucleosomes (where the nucleosomal DNA is fully wrapped), the use of Fluorescence (or Förster) Resonance Energy Transfer (FRET) is a logical choice. FRET has been used intensively for nucleosome research. Ensemble measurements record the average FRET efficiency of large nucleosome populations. Infor-

[1] to which all animals, plants and fungi belong

mation about the conformational heterogeneity or kinetic processes that occur in the sample can only be revealed with single-pair FRET (spFRET), in which FRET is measured in individual nucleosomes.

In this thesis, **Chapter 1** is a review of experiments that have used spFRET to elucidate single nucleosome dynamics. Such experiments show that nucleosomes are in general open for tens of milliseconds and for 10 % of the time. We investigated the influence of modulations of the histone proteins and the presence of linker DNA and neighboring nucleosomes on DNA breathing with spFRET. Several challenges we experienced in spFRET with nucleosomes are described in **Chapter 2**, along with suggestions how to deal with them. In **Chapter 3** we show that specific acetylation of the histone octamers at H3K56 enhances unwrapping of nucleosomal DNA. In **Chapter 4**, nucleosomes containing either the canonical histone H2A or the variant H2A.Z are investigated. We reveal that nucleosomes containing H2A.Z are more stable than their H2A-containing counterparts and thus can act as an accessibility switch for nucleosomal DNA. Finally, in **Chapter 5** we show that electrostatic interactions between linker DNAs and nucleosomes, and interactions between neighboring nucleosomes, enhance DNA breathing.