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

Accompanying the thesis

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

1. The nucleosome is a dynamic structure that spontaneously breathes for tens of milliseconds. This property is essential to provide enzymes access to the nucleosomal DNA.

Chapter 1 of this thesis

2. Nucleosomal DNA needs to unwrap for at least 30 base pairs beyond the fluorescent label positions to lose FRET completely.

Chapter 1 of this thesis

3. The combination of biochemical, instrumental and photophysical difficulties, and the need for sub-nM concentrations, make nucleosome-spFRET data collection and interpretation prone to artifacts.

Chapter 2 of this thesis

4. A single specific histone modification, acetylation of H3 K56, is sufficient to enhance DNA breathing several times, which explains its role in transcription regulation.

Chapter 3 of this thesis

5. Nucleosomes containing H2A.Z show less breathing and are less susceptible to dissociation than nucleosomes containing H2A.

Chapter 4 of this thesis

6. The ‘601’ nucleosome positioning sequence offers an invaluable tool for engineering nucleosome constructs with defined label positions, because of its strong affinity for the histone octamer. However, the same reason leads to an underestimation of DNA breathing compared to nucleosomes containing native DNA sequences.

Lowary and Widom, J. Mol. Biol. 276, 19 (1998) and Tóth et al., Cytometry Part A 83, 839 (2013).

7. Enhanced DNA breathing due to an increase in ambient temperature is too small to be responsible for ambient temperature sensing in *Arabidopsis Thaliana*.

Kumar et al., Cell 140, 136 (2010).

8. Because DNA is a double helix with a pitch of ~ 10 base pairs, a change of only 1 base pair in linker DNA length is expected to make a huge difference in higher order chromatin structure.
Brogaard et al., Nature **486**, 496 (2012).
9. The observation that the accessibility of nucleosomal DNA in a chromatin fiber is enhanced compared to in mononucleosomes leads to the counterintuitive conclusion that DNA compaction and accessibility are not necessarily anticorrelated.
Poirier et al., J. Mol. Biol. **379**, 772 (2008) and *He Meng, PhD thesis chapter 3*, (2014).
10. A layman's talk should be a mandatory part of the PhD exam.
11. To improve the position of women on the labour market in the Netherlands, it is necessary to acknowledge that not only for young mothers, but also for young fathers it is quite a challenge to combine work and family life in a way that is satisfactory for all parties.

Ruth Buning
Leiden, 15-1-2015