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Single-pair FRET microscopy on nucleosome dynamics

Wiepke Koopmans¹, Colin Logie², Thomas Schmidt¹, and John van Noort¹

¹Physics of Life Processes, Leiden Institute of Physics, Niels Bohrweg 2, 2333 CA Leiden, The Netherlands

²Nijmegen Center for Molecular Life Sciences, Radboud University, Geert Grootplein 28, 6525 GA, Nijmegen, The Netherlands

Regulation of gene expression is essential for the control of life processes. Accumulating evidence points at a key regulatory role of DNA packaging into nucleosomes, the basic unit of DNA condensation. Accordingly, conformational changes in nucleosome structure can be of crucial importance for proteins to access nucleosomal DNA. We use single-pair Fluorescence Resonance Energy Transfer microscopy (spFRET) to study the dynamics of DNA folding in nucleosomes.

We reconstituted mononucleosomes with Cy3-donor and Cy5-acceptor labeled 5S RNA nucleosome positioning elements and recombinant histones. Using Total Internal Reflection (TIRF) widefield microscopy, we measured long spFRET traces from individual mononucleosomes at sample rates of ~20 Hz. The traces fluctuate between high and low FRET efficiency states, with lifetimes of ~3 s and ~0.5 s respectively. Alternating donor/acceptor excitation allows us to distinguish low FRET states due to dark states of the acceptor from DNA rearrangements within the nucleosome. After correction for acceptor dark states, which currently comprise most of the observed dynamics, we generally find stable nucleosome conformations at room temperature.

To get a better view on conformational changes we will, in the near future, reproduce these experiments with dyes that have improved photostability. Subsequently we will explore nucleosome dynamics under a range of temperature and salt conditions.