Quantifying nucleosome dynamics and protein binding with PIE-FCCS and spFRET

Martens, C.L.G.

Propositions

Accompanying the thesis

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1. Instead of resembling a highly ordered structure like an archive or library, the condensation of DNA looks much more like the organized chaos of a student room (or a professors' office). *Zimatore et al., Biophys.Rev. (2021)*

2. The aggregate exclusion algorithm we present in this thesis is an original and significant improvement on the generally accepted and still predominant method as proposed by Bacia et al. (2012) to measure 'around the aggregates', as it simplifies the process of excluding aggregate contributions. *Chapters 2 and 5 of this thesis*

3. Close proximity of DNA linkers exiting the nucleosome stabilizes the nucleosome the most at low ionic conditions. At higher salt concentrations, this effect disappears, hinting stabilization originates from electrostatic DNA - histone (tail) interactions.
Chapter 4 of this thesis

4. LEDGF binding to nucleosomes containing H3K36me3 creates a complex with a wider range of diffusion time compared to LEDGF interactions with non-modified nucleosomes. This indicates that LEDGF-nucleosome complex is present in different conformational states, which may represent interactions of a more non-specific nature.
Chapter 5 of this thesis, fig. 5.6c

5. Instead of increasing the nucleosome concentration, GR concentration should be raised to increase the number of interactions with nucleosomes/GRE, more accurately mimicking *in vivo* conditions where the amount of DNA remains the same, but the concentrations of proteins change over time.
Chapter 6 of this thesis, paragraph 6.3.4

6. Quantifying protein binding on a surface depleted of DNA or nucleosomes, should be a standard TIRF control measurement to rule out false-positive binding events due to nonspecific sticking, especially when the association constant is derived from the residence time of a fluorescent protein, which is affected by photobleaching rate.
Jin et al., JMCB (2014)

7. A condition for a successful FCS experiment is that the sample is stable in time, i.e. is in equilibrium on a larger timescale than the duration of the measurement. Hence,

performing FCS measurements on nucleosomes out of equilibrium, should be done at quantifiable and relevant temporal resolutions. *Langowski et al., NAR (2011)*

8. Measuring donor and acceptor emissions upon donor excitation in a single FRET measurement greatly enhances robustness of results by eliminating effects of concentration differences and optical artifacts between separate measurements
Torres et al., J.Phys.Chem.B (2007)

9. Both grant applications and peer review of papers should be anonymized to ensure objective assessment of their scientific quality.

10. The wealth of our time is information, data and knowledge, and its makers should seize the means of production to prevent further alienation of academic labour.
Hall, O.A. J. Glob. Sust. Inf. Soc. (2018)

Christine Martens
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