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Cryo - EM evidence of nucleosome conformational changes in concentrated solutions and in interphase nuclei

In Eukaryotes, DNA is wound around the histone core octamer to form the basic chromatin unit, the nucleosome. Atomic resolution structures have been obtained
from crystallography and single particle cryoEM, with identical reconstituted particles. However, native nucleosomes are diverse in DNA sequence and histone content, and the conformational variability of native nucleosomes remains to be
understood,especially in the cellular context. Using cryoelectron microscopy and tomography of vitreous sections (CEMOVIS and CETOVIS) we investigate native nucleosomes, both in vitro, using isolated nucleosomes solubilised at physiologically relevant concentrations (25-50 %), and in situ, within interphase nuclei. We visualise individual nucleosomes at a level of detail that allows us to analyse the conformation of the DNA wrapped at their surface. In particular, we measure the distance between DNA gyres of the superhelix. In concentrated solutions, we evidence a salt -dependant behaviour, with high salt conformations resembling the canonical crystallographic nucleosome, and low salt ones, more open, being closer to the nucleosome conformation in situ. This work shows that CEMOVIS and CETOVIS are powerful tools for chromatin studies, allowing the
visualisation and analysis of nucleosomes in their nuclear context. Nucleosomes are known to play a fundamental role not only in genome packaging but also in the regulation of chromatin functions: transcription, replication and repair. Further particle characterisation and cartography are now needed to explore the relationship between the nucleosome conformational variability and chromatin
functional states.

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