ID de Contribution: **44** Type: **Présentation orale**

NUCLEOSOME CONFORMATIONAL VARIABILITY IN VITRO & IN SITU EXPLORED BY CRYO-ELECTRON MICROSCOPY AND TOMOGRAPHY OF VITREOUS SECTIONS

In most eukaryotic cells, DNA is packed into the chromatin « bead on string » filament of nucleosomes, formed by 145–147 bp DNA wrapped into 1.65 turns of a left-handed superhelix around a histone octamer. Atomic resolution structures have been obtained from X-ray crystallography, and more recently from cryo-EM of identical, symmetric and highly stable engineered particles, which has led, together with a highly conserved structure, to a canonical static view of the particle. However, nucleosomes are now being recognised as pleiomorphic and dynamical, which is so far documented in vitro and in silico, but unknown in the cellular context. Using cryo-electron microscopy and tomography of vitreous sections, we analyse the structure and local organisation of nucleosomes within interphase nuclei of different cell types (human cell lines, Drosophila embryo). We visualise individual nucleosomes at a level of detail that allows us to analyse the conformation of the DNA gyres wrapped at their surface. We measure the distance between gyres of the superhelix, and show that nucleosomes are polymorphic, with variable conformations, more open in situ than in canonical crystallographic structures, with an increase of the distance between DNA gyres. These observations are compatible with a "gaping"conformation, proposed by theoretical approaches and detected experimentally in vitro, and/or an increase of the pitch of the DNA superhelix. To characterise this conformational variability, we combine analyses of individual nucleosomes, sub-tomogram averaging and 3D classification based on normal mode analysis. To decipher the mechanisms at work in this conformational variability, we use isolated native nucleosomes solubilised at physiologically relevant concentrations (25-50%) and in various ionic environments. We evidence a salt–dependant behaviour, with high salt conformations resembling the canonical crystallographic nucleosome, and low salt open ones, closer to the nucleosomes in situ. This highlights the role of ionic effects, already known to play a central role at many levels of chromatin organisation, demonstrating that, at the nucleosome level, DNA gyres open or close.
Nucleosomes are known to play a fundamental role not only in genome packaging but also in the regulation of major cellular processes. Further particle characterisation and cartography are now needed to understanding the relationship between nucleosome conformational variability and chromatin functional states.

Authors: LEFORESTIER, Amélie (CNRS, Laboratoire de Physique des Solides); HARASTANI, Mohamad (IMPMC UMR 7590); Dr JONIC, Slavica (IMPMC UMR7590); Prof. ELTSOV, Mikhail (IGBMC UMR 7104)

Orateur: LEFORESTIER, Amélie (CNRS, Laboratoire de Physique des Solides)