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Reconstitution in vitro of chromatin patterning

Mammalian cells package their genomic DNA into a complex with proteins called chromatin. The assembly and compaction of chromatin are regulated by multiple molecular cues, including DNA modifications, histone post-translational modifications, and histone variants. The spatial partitioning of chromatin into active and inactive domains has also been proposed to be regulated by LLPS. The interplay between histone modifications and spatial chromatin partitioning is currently unclear. To address this issue, I develop a reconstituted in vitro system to study chromatin partitioning and the deposition of histone methylation by single-molecule microscopy. To this end, we reconstitute chromatin fragments using fluorescently labeled histones, and we methylate them with the purified histone methyltransferase DIM5. I am currently studying the kinetics of this methylation and the spreading of methylated marks at the single-molecule level using differently-sized nucleosomes arrays. My aim is to reconstitute compacted and methylated chromatin domains in vitro, in an assay where domain formation and compaction can be directly observed by fluorescence microscopy in real-time. To this end, I tether biotinylated chromatin fibers to a fluid lipid bilayer, where they can laterally diffuse and associate with each other. I will use this system to tackle the following questions: What is the critical number of chromatin fibers required for domain formation? What are their mechanical properties? What is the interplay between histone methylation and domain formation?

Auteurs principaux: LEWANDOWSKA, Dominika (CBICNRS); ERDEL, Fabian (CBI Toulouse); NORMAND, christophe (UMR5077-MCD)

Orateur: HERTZOG, Maud (CNRS)