Fluorescent labeling of cellular DNA for an exploration of in-situ chromatin structure

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The regulation of gene expression controls diverse biological processes. Specific gene regulation depends on the three-dimensional (3D) folding of the genome, the establishment of close enhancer contacts, and a specific spatial organization of the chromatin fiber. How these contacts between different gene loci are mediated is still poorly understood on a molecular level. Although current methods based on fluorescence in situ hybridization (FISH) can resolve chromatin structure at the ~ 10 kilobase (kb) scale, it requires DNA denaturation for probe hybridization which will lead to the disruption of nucleosome-level fine structure. Another choice is the staining of chromatin via antibodies or dyes, which is milder but lacks locus-specific. Here we propose a new approach to resolve these issues. In this approach, chemical biology and single-molecule imaging are combined to achieve the observation of locus-specific 3D folding of the genome in a living cell.

The aim of our investigation is to implement a novel imaging approach to reveal chromatin structure at defined genomic loci by a combination of chemical biology and super-resolution imaging. Here, we are incorporating modified nucleosides, including ethynyl-deoxyuridine (EdU) and azidomethyl-deoxyuridine (AmdU) into a DNA sequence at high density[1]. We then fluorescently label this DNA using copper-catalyzed or strain-promoted click chemistry. This enabled super-resolution imaging with 3D stochastic optical reconstruction microscopy (STORM) of DNA strands in vitro. In a second step, we then transfect EdU or AmdU-tagged into cells and integrate the labeld DNA strands into the native chromatin using the piggyBac transposon system[2]. There, the labeled DNA will be chromatinized, followed by labeling and 3D super-resolution imaging. Together, this system will enable important insights into the conformational ensemble of a defined chromatin locus in cells, and provides a deeper understanding of the relationship between gene architecture and expression regulation.

[1] Anne B. Neef, Nathan W. Luedtke, *ChemBioChem,* **2014,** 15, 789-793.
[2] Kosuke Yusa, Liqin Zhou, et al., *Proceedings of the National Academy of Sciences of the United States of America,* **2011,** 108, 1531-6.