Spatial organization and dynamics of DNA repair mechanisms, a single-molecule microscopy approach

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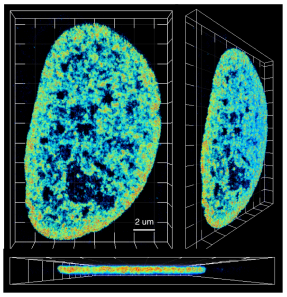
Maintenance of genetic stability is an essential cellular function. By avoiding or limiting the impact of DNA damage, **DNA repair systems play a critical role in cell survival and the prevention of pathologies such as cancer or accelerated ageing**. Among them, base excision repair (BER) is the main DNA repair pathway for removal of modified bases or abasic sites and repair of single strand breaks. BER is the mechanism by which damaged bases in DNA are removed and replaced.

Nuclear architecture has a determining role in DNA metabolism and a link between chromatin compaction and transcription is well established. While it is now clear that the efficiency of repair of double strand breaks is modulated by the chromatin context, the link between chromatin structure and BER is less understood.

In collaboration with a group of cell biologists and DNA repair specialists at the Institut de Radiobiologie Cellulaire et Moléculaire, CEA Fontenay-aux-Roses, we at the Institut Langevin, ESPCI Paris, aim at **using single-molecule localization microscopy (SMLM) approaches to study the molecular mechanisms behind the recruitment of the BER complexes on chromatin**.



Super-resolution SMLM 3D image of chromatin

The advent of super-resolution methods has revolutionized the field of optical microscopy, as recognized with the Nobel Prize in Chemistry 2014. Notably among them, SMLM makes use of photo-switchable fluorophores so that only a fraction of them fluoresce at a time. These molecules are localized at high precision (~10 nm), and a super-resolved image is reconstructed. Thanks to super-localization approaches, previously hidden details at the macromolecular level are revealed and other physical variables are now accessible. The analysis of a set of single molecule data contains structural information of the sample but also reveals hidden phenomena about molecular dynamics and stoichiometry of biochemical processes.

In this internship, the candidate will perform dual-color, 3D super-resolution experiments to study the spatial organization of chromatin and BER-related nuclear complexes at different time-points after induced DNA damage. Furthermore, single-particle tracking (SPT) experiments will be performed to study the nuclear mobility of repair factors and understand the formation of high-concentration clusters at the repair site. Our long-term aim is to identify the players that recruit DNA glycosylases to initiate BER of damaged bases, and to characterize the molecular mechanisms required for the establishment of BER complexes on chromatin.

For more information about this project, contact Ignacio Izeddin at ignacio.izeddin@espci.fr

Further reading:

SMLM[1,2]; SMLM for nuclear architecture and dynamics[3,4]; BER mechanism[5,6]

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5. Campalans, A. *et al.* Distinct spatiotemporal patterns and PARP dependence of XRCC1 recruitment to single-strand break and base excision repair. *Nucleic Acids Res.* **41,** 3115–3129 (2013).
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