

Detection of Protein Interactions with G-Quadruplexes in Cells Using Proximity Ligation (DePIGQ)

M. Regairaz, [LBPA](#), D. Verga [CMBC](#), Institut Curie

Guanine-rich nucleic acids can form stable secondary structures called G-quadruplexes (GQs). Over 700,000 sequences in the human genome and about 13,000 in the transcriptome can form GQs. These sequences are highly conserved and mainly located in functionally active regions of the genome. They are involved in key processes such as replication, transcription, translation, DNA repair and chromatin remodeling. An important and yet unanswered question is how GQs fold and unfold in cells. Hundreds of GQ-binding proteins have been identified in native chromatin using chemical probes and affinity purification coupled to mass spectrometry. However, this method cannot distinguish direct GQ interactors from coprecipitated proteins located in proximity. In addition, the technique measures cell population means and not single-cell values, making it difficult to assess the frequency and distribution of GQ interactions and to link them with cellular processes.

Our goal is to develop a GQ proximity ligation assay (GQ-PLA) to detect GQ-protein interactions in single cells using microscopy. In our assay, the GQ structures will be labeled using clickable GQ ligands functionalized with a hapten and potential GQ protein partners will be recognized using specific antibodies. If the GQ and the target protein are close enough (distance < 40 nm), we will observe fluorescent signals within the cell. The assay will first be optimized and validated using various experimental techniques, including siRNA-mediated depletion of GQ interacting proteins. We will then use GQ-PLA to determine whether GQ-protein interactions occur in cells during specific DNA transactions (such as replication or transcription). We also plan to analyze whether GQ-protein interactions occur in specific genomic regions such as telomeres. This project will contribute to shed light on the functional relevance of GQs and provide a basis for further exploration in cellular biology and potential therapeutic applications.

Contact : marie.frugier-regairaz@ens-cachan.fr

