Subject: Real-time visualization of DNA repair complex assembly using 2-color single molecule tracking in 3D

Project type: MEP

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Project background

DNA damage repair is a central molecular process to ensure both genetic integrity as well as diversity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) are two primary pathways of DNA damage repair. Cells must repair any damage incurred to their DNA to ensure survival and prevent mutations that may lead to cancer. When DNA double strand breaks occur, triggered by factors such as ionizing radiation or drugs, repair proteins gather at the damaged sites, forming structures known as DNA repair foci. These foci can be observed using fluorescence microscopy. Various proteins engaged in DNA repair accumulate at distinct rates and quantities within these repair foci. Questions remain unanswered about many of these proteins such as how they interact with each other, what are the timescales involved, what are their downstream effects, etc. We are interested in better understanding the dynamics of two interacting proteins involved in the HR pathway- BRCA2 and RAD51 at the repair foci, via 3D two color single molecule imaging and tracking in living cells. During HR, BRCA2 molecules accumulate at DNA double strand breaks within the foci as part of a larger protein complex and are responsible for recruiting RAD51 molecules to form long filaments coiling around the broken DNA strands. The further downstream steps of the HR pathway are beyond the scope of this project, but you can read about them in depth in this review [1]. As mentioned earlier, several other proteins are known to impact these processes at each step, though the finer details aren't always known, such as 53BP1, PALB2, RPA. Amongst these 53BP1 is a prominent protein and a classic known antagonizer of HR. It identifies signals at DNA break sites, accumulates at these sites and promotes NHEJ during certain phases of the cell cycle [2]. Another interesting, but rather obscure player of these pathways is RNF168. It ubiquitinates a double strand break- specific histone protein which then serves as a signal/trigger for the initial recruitment of other repair proteins like 53BP1 [3]. Furthermore, There is emerging evidence suggesting that DNA repair proteins, including RAD51, may form phase-separated condensates to facilitate repair processes [4]. These condensates can enhance the local concentration of repair proteins, thereby increasing the efficiency of HR. It is critical for us to understand these underlying physical processes so that in case a pathological condition arises which affects one of these pathways, we can develop effective therapies targeting them.

This is a joint project with Dr. Maarten Paul at Department of Molecular Genetics, Erasmus MC.

Specific objectives and activities

- To setup an experimental pipeline for a two color 3D single molecule tracking assay. The assay would require snapshots to be acquired at fixed intervals with one color channel to observe RAD51 foci and a continuous time-lapse series to be acquired for tracking BRCA2 with the second color channel.
- To better understand the mechanistic steps involved in RAD51 accumulation during the HR pathway.
- To evaluate the effect of 53BP1 and RNF168 protein knockouts on RAD51 filament formation.

The first step in this project would be to set up a working two color 3D tracking workflow. You will start off with performing single molecule tracking in 3D in a single color channel (established in the lab) and extend it to two color channels. This would require writing the necessary scripts for hardware control, synchronization as well as data analysis. We use a specialized prism in a custom home-built microscope to image multiple planes simultaneously for 3D imaging. You will then apply this method to study BRCA2 and RAD51 proteins fluorescently labelled in cells containing knockout genes for other repair proteins like 53BP1 and RNF168. The required cell lines for this work are available with us.

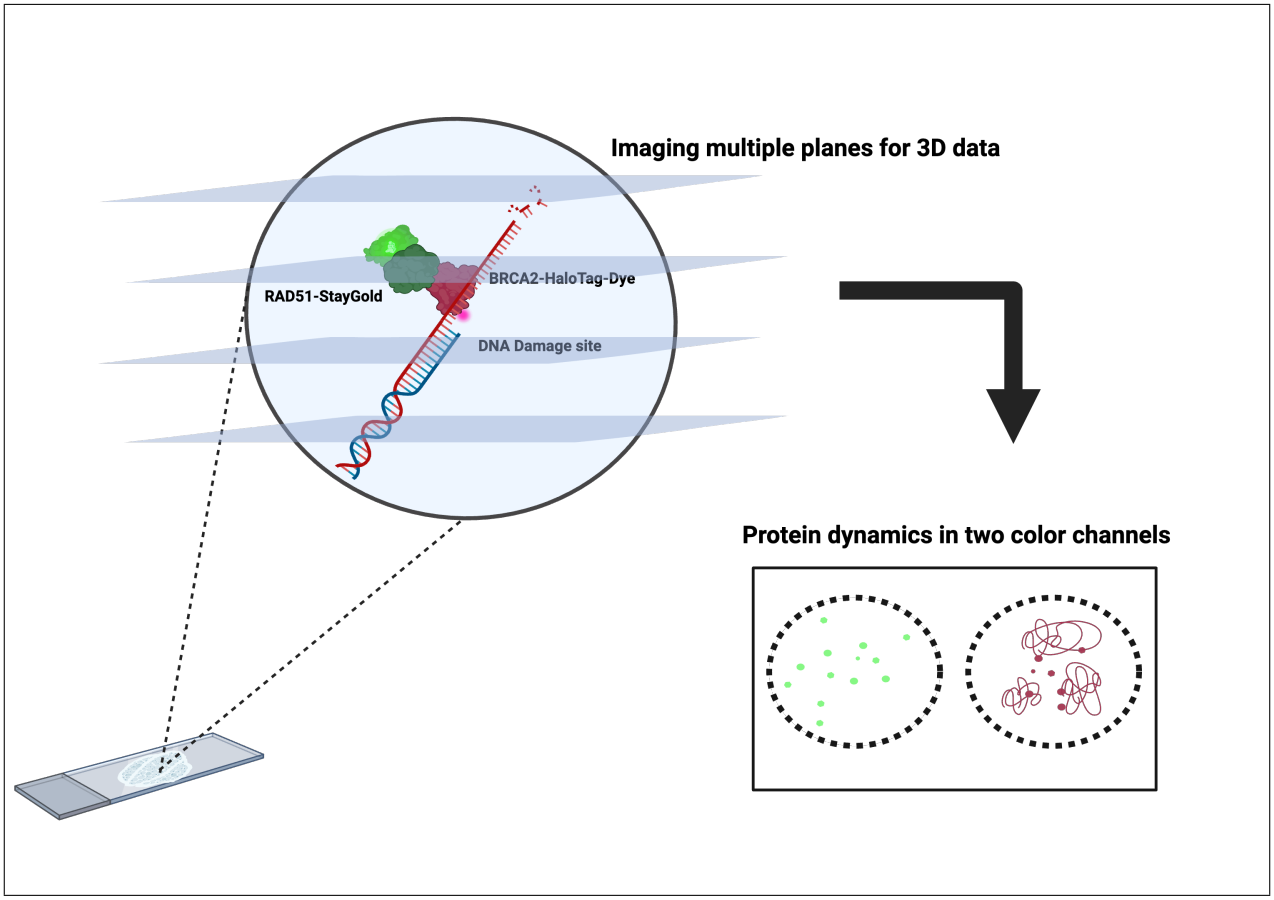

Figure 1: Single molecule tracking of two interacting proteins with 2 color 3D imaging

Your profile

The Grussmayer Lab is a young interdisciplinary team at the interface of molecular biology and cutting-edge optical methods with an emphasis on open science and collaboration. During the project, you will learn and contribute to:

- Developing the optics for extending single color to two color imaging in 3D.
- Developing and implementing advanced image analysis routines to handle 3D data
- Visualization and presentation of experimental data

We are looking for students with a background in physics/biophysics/applied optics or related disciplines that are interested in an ambitious interdisciplinary project with an international and accommodating team of young scientists. The major task for you here is to focus on the optics and the analysis pipelines. Thus, strong skills in Matlab/python would be desirable. A postdoc will work with you to take care of the biological aspects such as maintaining the cell lines, sample preparation including inducing DNA damage in cells followed by suitable labeling for fluorescence microscopy and lastly imaging (which you both will do together).

References

1. Li, X., Heyer, WD. Homologous recombination in DNA repair and DNA damage tolerance. Cell Res 18, 99–113 (2008). https://doi.org/10.1038/cr.2008.1
2. Zhang, L., Geng, X., Wang, F. et al. 53BP1 regulates heterochromatin through liquid phase separation. Nat Commun 13, 360 (2022). https://doi.org/10.1038/s41467-022-28019-y
3. Panier, S., Boulton, S. Double-strand break repair: 53BP1 comes into focus. Nat Rev Mol Cell Biol 15, 7–18 (2014). https://doi.org/10.1038/nrm3719
4. Chen, J., Shi, J., Zheng, J., Wang, Y., Wan, X. Liquid-liquid phase separation in DNA double-strand break repair. Cancer Biol Med 20(9), 627–32 (2023). https://doi.org/10.20892/j.issn.2095-3941.2023.0252