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## Cellular distribution of the human NTH1 DNA glycosylase by confocal and super-resolution microscopy

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Mrinalini-Lianne Rao, Oleksandr Glushonkov, Pierre Caron, Pascale Tacnet, Philippe Frachet, et al.. Cellular distribution of the human NTH1 DNA glycosylase by confocal and super-resolution microscopy. 15th 3R meeting, May 2024, Leucate, France. hal-04902348

**HAL Id: hal-04902348**

**<https://hal.science/hal-04902348v1>**

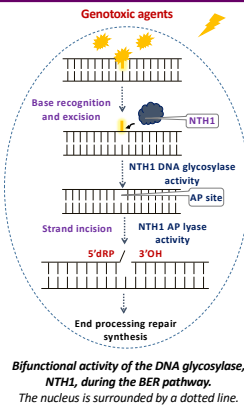
Submitted on 20 Jan 2025

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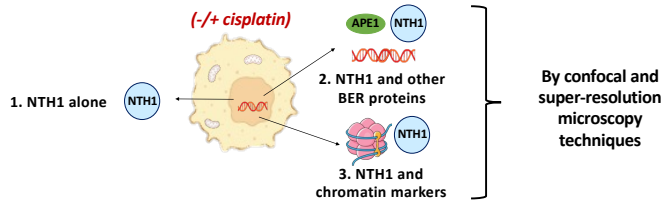
## 1 - INTRODUCTION

- DNA damage induced by conventional cytotoxic chemotherapies, activates various DNA repair mechanisms like the base excision repair (BER) pathway that contribute to chemoresistance.
- The BER pathway eradicates small and non-helical distorting base lesions like oxidized bases. BER is initiated by a set of enzymes, known as DNA glycosylases that recognize and remove the damaged bases. For example, the bifunctional DNA glycosylase NTH1 preferentially targets the oxidized pyrimidine base, thymine glycol.
- The aim of this project is to have a better understanding of the cellular distribution of NTH1, in relation with other BER proteins and chromatin markers, within the nucleus.
- The expected results could lead to the development of novel anticancer drugs, used to overcome chemoresistance.



## 2 - OBJECTIVES

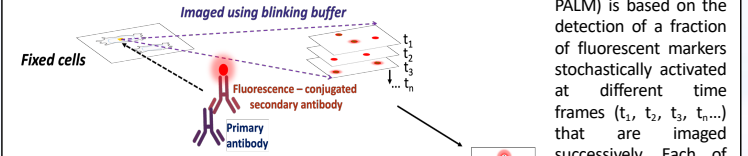
Mapping the distribution of NTH1 in cells (1) in relation with other BER proteins (2) and chromatin markers (3), before and after treatment with a chemotherapeutic agent, such as cisplatin.



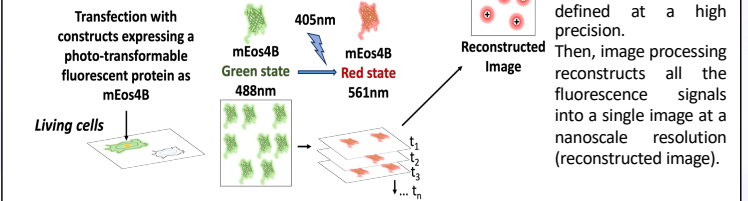
## 3 - CONCEPT OF SINGLE-MOLECULE LOCALIZATION MICROSCOPY

Single-Molecule Localization Microscopy (SMLM), a Super-Resolution (SR) technique, allows to achieve high spatial resolution (10-20 nm) compared to conventional and confocal fluorescence microscopy (200 nm), to study biological samples at the nanoscale.

### dSTORM (Direct Stochastic Optical Reconstruction Microscopy)

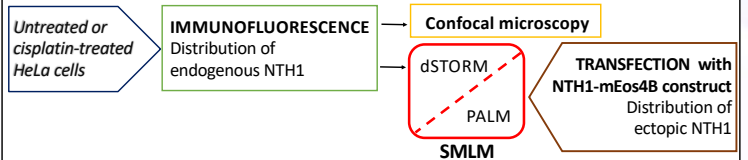


### PALM (PhotoActivated Localization Microscopy)



SMLM (dSTORM and PALM) is based on the detection of a fraction of fluorescent markers stochastically activated at different time frames ( $t_1, t_2, t_3, t_n, \dots$ ) that are imaged successively. Each of these localizations are defined at a high precision. Then, image processing reconstructs all the fluorescence signals into a single image at a nanoscale resolution (reconstructed image).

## 4 - WORK FLOW



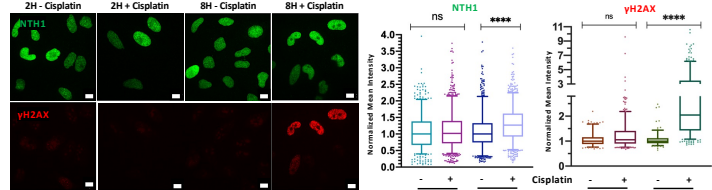
## 5 - RESULTS: DISTRIBUTION OF ENDOGENOUS NTH1

### A. Distribution of NTH1 by confocal microscopy before treatment with cisplatin

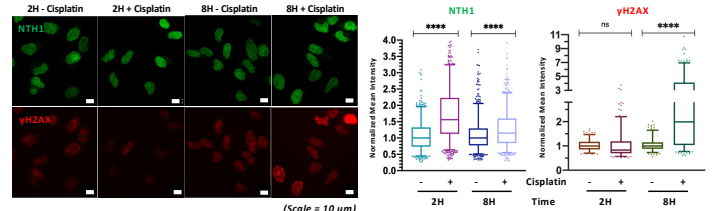


### B. Effect of cisplatin treatment on the level of NTH1

#### B1. Without *in situ* cell fractionation

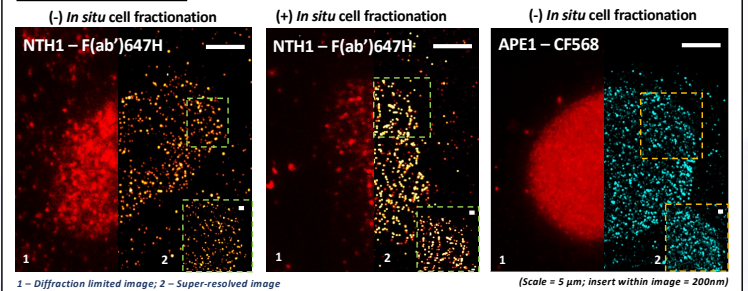


#### B2. With *in situ* cell fractionation

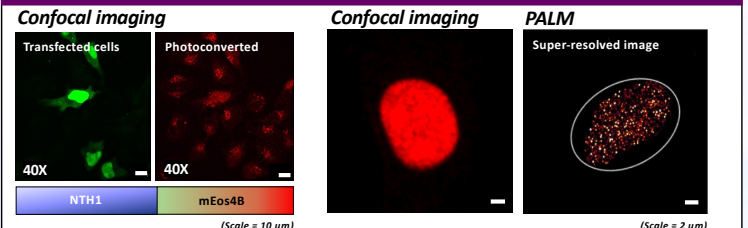


Confocal imaging reveals that NTH1 intensity is reduced after *in situ* cell fractionation (B2) suggesting that only a fraction of the NTH1 molecules are DNA bound. On the contrary, γH2AX foci are only mildly affected by *in situ* fractionation.

### dSTORM imaging



## 6 - PRELIMINARY RESULTS: PALM IMAGING OF NTH1



## 7 - CONCLUSION & PERSPECTIVES

These results reveal that NTH1 is heterogeneously distributed in the nucleus, where it forms foci-like clusters visible in both conventional and super-resolution microscopy. Upon cisplatin treatment, a small increase in the levels of NTH1 was observed, which was more pronounced after *in situ* cell fractionation. These results suggest that the DNA bound fraction, rather than the total amount of NTH1 was increased after cisplatin treatment. This increase was particularly visible after 2h treatment with cisplatin. In contrast, γH2AX foci were only seen at later timepoints. This agrees with the fact that oxidized bases recognized by NTH1 are formed first leading to the formation of single- and then double-strand breaks recognized by γH2AX.

Our dSTORM analysis provides a first map of the distribution of NTH1 in untreated cells. Our aim is to perform the same type of analysis on cells treated with cisplatin. Additionally, we plan to perform 2D dual labelling of NTH1 with γH2AX/H3 or APE1 to analyze possible colocalizations between chromatin marker and BER protein.

Single-particle tracking PALM will be performed to follow the dynamics of NTH1 within the nuclear space.