PML is involved in many cellular processes. It organizes nuclear structures PML nuclear bodies (PML NBs) and it associates with nucleolus in response to ribosomal stress to form PML nucleolar associations (PNAs). The function of PNAs is unclear. To elucidate this question, one can attempt to identify proteins interacting with PML at nucleolus. The common method is co-immunoprecipitation, however, this approach cannot be used for PML due to its low solubility. To defeat this, an alternative way of proximity-dependent biotin labelling could be used.

The goal of this work was to explore a suitability of biotin labelling for identification of PML nucleolar partners. For this purpose I prepared constructs of wild type or mutated PML with GFP and biotin ligase for transient and stable expression and analysed their propensity to form PML NBs and doxorubicin-induced PNAs, and biotinylate their vicinity.

In transient expression, both fusion proteins formed PML NBs and only wild type but not mutated PML IV formed PNAs after doxorubicin treatment with preserved biotinylation capability.

In stable expression of fusion proteins in cells with PML knockout the number and composition of PML NBs was aberrant and no PNAs were observed. However, this system was utilized for optimization of solubilisation of biotinylated proteins using detergents.

My findings indicate that after a modification the biotin proximity labelling might be used for identification of PML partners.