Development of a single-molecule approach to observe ubiquitination dynamics in defined chromatin states

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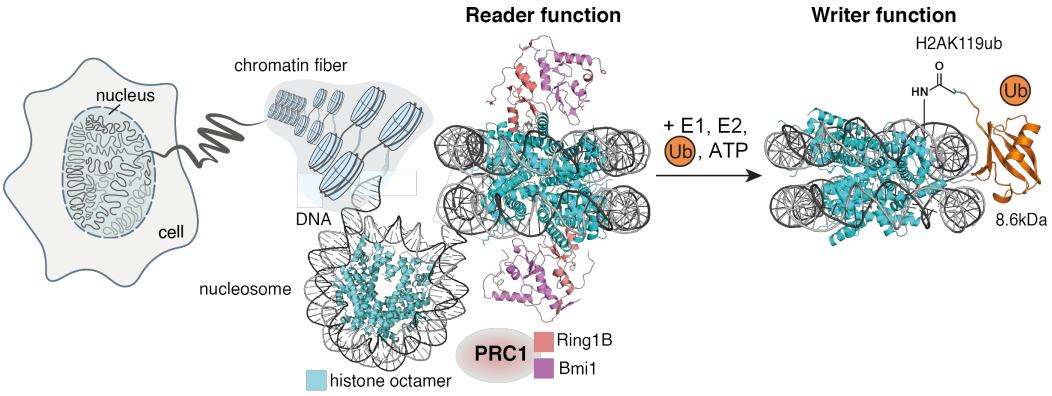
Dynamic regulation of transcription is orchestrated by a large cohort of enzymes, among which chromatin modifiers or 'writers' install histone post-translational modifications (PTMs) controlling the recruitment of chromatin 'readers'. A specific subset of chromatin modifiers includes Polycomb group (PcG) proteins, which drive the inheritance of a repressed chromatin state during development and cell differentiation by preventing abnormal oncogenic transformations.

PcG members Polycomb Repressive Complex (PRC) 1 in its canonical (cPRC1) or variant (vPRC1) form function as H2A-specific E3 ligases and drive gene repression. vPRC1 is involved in the majority of ubiquitin deposition in the context of repressed chromatin, without the requirement of primary installation of additional PTMs.



We hypothesize that chromatin modification, i.e. ubiquitination, by vPRC1 is controlled by its recruitment dynamics on underlying chromatin and furthermore its subunit composition. Here, we present a single-molecule approach, which allows to directly measure vPRC1 binding dynamics at defined chromatin states. In the following, this will allow us to identify protein-protein interactions (PPIs) that are key for enzymatic activity and reveal chromatin states that are specifically targeted. Moreover, we are extending this approach to directly observe vPRC1 dependent H2A ubiquitination in real time on the single-molecule scale. This will allow us to gain a mechanistic view of 'reading' and 'writing' by vPRC1 in real time and to elucidate its contribution to gene regulation.