

Double-strand breaks (DSBs) appear to be the most detrimental forms of DNA lesions causing severe chromosome alterations. They are generated by external sources, but more frequently are associated with DNA metabolism, such as replication, transcription and recombination processes, in which they occur either in a programmed manner or as errors' result. To control those injuries and sustain proper functioning of the genome, cells have developed a highly specialized protein network that govern the DNA damage response (DDR). One of its key members, MRN complex plays central role in the DSBs cellular response, as it detects damage, co-activates signaling cascade, selects the most appropriate DDR pathway and actively participates in the repair of broken strands. MRN is a complex of three proteins serving complementary functions: Mre11 nuclease, Smc-like protein Rad50, and Nbs1 – a regulatory subunit distinct for eukaryotes.

Rad50 plays fundamentally different roles than the other two proteins. It acts threefold being responsible for maintaining the scaffold of the entire complex, binding DNA nearby double-strand break and constituting primary dimerization force which generates fully functional multiprotein complex. Rad50 associates through its globular ATP-binding domains and also by tightly bound zinc ion on the other pole of the complex. The functional importance of this small protein motif called zinc hook has been appreciated after thorough genetic analyses which revealed its significant impact on the DNA repair process mediated by the globular head of the MRN complex. This outcomes led to hypothesis of the conformational interdependence between two poles of the complex, likely mediated by long coiled coil arms of the Rad50. Nevertheless, the mechanism governing this structural communication remains unclear.

Based on structural studies it is postulated that the hook dimer displays dynamic structural properties and acts as a hinge enabling the coiled coil arms to switch between an open and closed state during MRN complex activity. Hence, it is of utmost importance to explore the molecular bases determining conformational states of the hook domain to fully understand its impact on MRN functions. Bearing in mind that formation of dimeric hook fold certainly depends on Zn(II) binding, the new idea was born, an idea focused on examining the influence of Zn(II) on the stability and conformational dynamics of the hook and the beneath coiled coil parts, but – importantly – in a tightly controlled Zn(II)-buffering conditions, to assess the physiological relevance of the observed phenomena. In the proposed project we aim to explore the molecular factors that drive the stability and conformational dynamics of eukaryotic hook domains in the context of controlled Zn(II) levels. The study encompasses two major objectives: 1) biophysical studies of Zn(II) binding sphere conducted on short hook variants; 2) fluorescent analysis of conformation and its dynamics of longer domain models with the dissection of the hook fold and coiled coil segment's impact on the overall dimer assembly. Because the protein's activity is determined both by its structure and dynamics, studies rich in mechanistic insights significantly strengthen the research conducted in biological systems. Therefore, we believe that the proposed biophysical low budget studies on the Rad50 hook domain will be fruitful for evaluation the underlying mechanism of the protein activity.

MRN complex plays pivotal role in DNA double-strand break repair, the most deleterious lesions that often lead to mutations and cancer progression. It is then not surprising that abnormal function of any of MRN components results in development of severe diseases or carcinogenesis. MRN complex disfunctions have been associated with many types of human cancer. Moreover, studies on mice models revealed that mutations in Rad50 hook domain affects DNA damage response signaling, tissue homeostasis and promoted tumorigenesis. The proposed comprehensive studies on the structure, stability and dynamics of the Rad50 hook domain will bring us closer to understanding how MRN complex functions in the physiological conditions preserving the integrity of our genome and protecting the cells from cancer.