An integrative structural biology and molecular biophysics approach towards SOS response characterization: from mechanistic insights to inhibitor screening and design.

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The bacterial SOS response to genotoxic stress is orchestrated by the interplay between the DNA damage sensor RecA (which oligomerizes on ssDNA) and the transcriptional repressor LexA. Upon binding to oligomeric RecA, LexA undergoes autoproteolysis and loses the ability to repress effector SOS genes, including many involved in hyper mutagenesis, antibiotic resistance and virulence. To foster the development of new antagonists of the SOS response, deep investigations on its structural and functional features are needed. In particular, the RecA-LexA interaction model has long remained elusive.
To fill this gap, an integrative structural biology approach was applied to the *Pseudomonas aeruginosa* SOS response. The structure of LexA autoproteolytic domain was obtained by X-ray crystallography, while the RecA/ssDNA complex was solved by electron microscopy. Cryo-EM was successfully applied to the LexA-RecA/ssDNA complex as well, revealing a peculiar interaction mechanism that locks LexA in the conformation needed for self-cleavage. Building on these structures, molecular dynamics simulations and biophysical assays further sustained the proposed model for RecA-induced LexA autoproteolysis.
In a parallel project, anti-LexA nanobodies have been developed by llama immunization and phage display selections. Autoproteolysis assays revealed that these nanobodies are the most potent LexA inhibitors discovered so far, while SOS genes expression profiling assessed their efficiency as SOS suppressors in bacterial cells stressed by antibiotics (published in Maso et al., 2022, *Structure* 30, 1479-1493). X-ray structures of LexA-nanobody complexes unveiled that these molecular tools stabilize the uncleavable LexA conformation and have sustained nanobody improvement by computer-aided design.
Besides answering long-standing questions in the study of the SOS response, work summarized here paves the way to innovative anti-evolutive and anti-virulence strategies in antimicrobial warfare.