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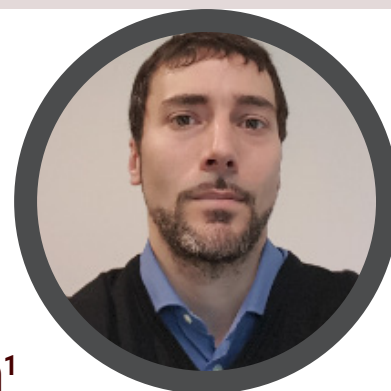
COMBINATION OF NMR METHODS TO SOLVE KEY STRUCTURES OF THE PRN1 PRIMASE IN COMPLEX WITH ITS SUBSTRATES

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Primases are single-stranded DNA dependent polymerases that synthesize RNA/DNA primers during replication. A primase, a DNA polymerase and an helicase compose the replication machinery of the archaeal plasmid pRN1¹. The structure of the archaeal functional primase domain has been solved by X-ray crystallography^{2,3} and it revealed an heteromeric structure with a catalytic prim/pol domain tethered to a novel helix bundle domain. We investigated the NMR structure of the functional pRN1 primase domain in complex with a single-stranded DNA template containing the GTG motif⁴. We showed that the catalytic prim/pol domain of this 38 kDa enzyme is not required for template binding. Intermolecular contacts detected exclusively between the helix bundle domain and the DNA led us to isolate specifically this structurally independent unit. Our results are compatible with a conformational switch between a template-bound open state and a closed active complex^{3,5,6}. We used multiple NMR dataset to solve the solution structures of the helix bundle domain in complex with the single-stranded DNA template alone and upon cofactors addition. Affinity measurements validated our structural data demonstrating the importance of residues located in helices 10 and 12 for the interaction with the GTG motif and confirmed the specificity improvement observed upon cofactors binding. In association with functional assays, these novel transient structures bring new perspectives and will help us to characterize the molecular steps required for priming.

Biography

Julien Boudet received his PhD degree in structural biology and biophysics from the University of Grenoble (Joseph Fourier University) in France under the supervision of Prof. Jean-Pierre Simorre. During his thesis, he learned nuclear magnetic resonance (NMR) spectroscopy and used this powerful method to investigate proteins and oligonucleotides structures, molecular mechanisms underlying antibiotic resistance and viral proteins interactions. After graduating, Julien joined the group of Prof. Frédéric Allain in ETH Zurich as a postdoctoral research associate. He focused his investigations on the DNA replication machinery and, in particular on the primase-mediated catalysis. He set up innovative computational methods to investigate challenging biological systems and demonstrated the role of cofactors in improving the specific template recognition by the pRN1 primase. He is currently developing computational and analysis tools to assist therapeutic oligonucleotides design.

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