

New Protein:protein Interactions in Bacterial Transcription Explained by a Combination of Structural and Biophysical Techniques

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RNA polymerase (RNAP) recruits for its function various accessory protein factors. They can play roles in recovery from stalled states, adaptation to environmental changes, antibiotic resistance or protein expression regulation. HelD, a multidomain helicase-like protein, and RNAP form a tight complex, which was documented in our studies using small angle X-ray scattering, crystallography, and Cryo-EM, together with biophysical measurements and transcription assays. The recently completed set of structural and functional data fully explains the main steps of HelD interference with RNAP function in *Mycobacterium smegmatis*. Our studies of HelD in *Bacillus subtilis* have led us to the discovery of a new binding partner of RNAP (dissimilar to HelD), where the situation is rather more complex. While the effect of the presence of this protein factor on transcription efficiency is very clear and putative complexes with *Bacillus subtilis* RNAP can be isolated, their unstable and enigmatic nature has led us to combining several biophysical (SAXS, mass photometry) and structural techniques (chemical cross-linking and mass spectrometry, Cryo-EM) to shed light onto this novel structure-function relationship.

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