Invited Speakers

Revealing the mechanism of action of a temperate bacteriophage by an integrated structural biology approach

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Bacteriophages are bacterial viruses. Temperate bacteriophages can enter two different stages of growth, the lytic and the lysogenic life cycle which is mediated by a genetic switch. We have studied the temperate bacteriophage TP901-1 from *Lactococcus lactis*. In TP901-1, the genetic switch is controlled by the anti-repressor MOR and the clear I protein (CI). Here, we employ an integrated structural biology approach, combining NMR spectroscopy, X-ray crystallography, small angle X-ray scattering and native mass spectrometry, to describe the structure and dynamics of CI and its interaction with the DNA operator sites on the phage genome [1,2,3]. In addition, we determine the solution structure of MOR using NMR, and we solve the crystal structure of MOR in complex with the N-terminal domain of CI (CI-NTD). The structure reveals how MOR prevents binding of CI to DNA. ^{15}N NMR relaxation measurements demonstrate that MOR displays molecular recognition dynamics on two time scales involving a repacking of aromatic residues at the interface with CI-NTD. Using sequence alignments and evolutionary couplings, we show that the structural and dynamic features of the MOR:CI-NTD complex are conserved among a larger family of bacteriophages from human pathogens including *Staphylococcus aureus* and *Streptococcus pneumoniae*. Our results, also supported by *in vivo* experiments, provide new insight into the mechanism of action of a larger family of temperate bacteriophages.

[1] K.H. Frandsen, K.K. Rasmussen, M.R. Jensen, K. Hammer, M. Pedersen, J.C. Poulsen, L. Arleth, L. Lo Leggio (2013). Binding of the N-terminal domain of the lactococcal bacteriophage TP901-1 CI repressor to its target DNA: a crystallography, small angle scattering, and nuclear magnetic resonance study. **Biochemistry** 52:6892-6904.

[2] K.K. Rasmussen, K.H. Frandsen, E. Boeri Erba, M. Pedersen, A. K. Varming, M. Kilstrup, P.W. Thulstrup, M. Blackledge, M.R. Jensen, L. Lo Leggio (2016). Structural and dynamics studies of a truncated variant of CI repressor from bacteriophage TP901-1. **Sci. Rep.** 6:29574.

[3] K.K. Rasmussen, A. K. Varming, S. N. Schmidt, K.H. Frandsen, P.W. Thulstrup, M.R. Jensen, L. Lo Leggio (2018). Structural basis of the bacteriophage TP901-1 CI repressor dimzerization and interaction with DNA. **FEBS Lett.** 592:1738-1750.