P010 Dissection of the archaeal transcription machinery using fluorescence spectroscopy

Dina Grohmann and Finn Werner
UCL Structural and Molecular Biology, Darwin Building, London WC1E 6BT

The transcription cycle can be divided into three phases: initiation, elongation and termination. During each phase regulatory proteins interact with RNA polymerase (RNAP) and thereby control gene expression. The archaeal transcription machinery is closely related to the eukaryotic RNAPII-system and is a highly attractive model system because of its superior biochemical tractability. Initiation of transcription is facilitated by three basal factors: TBP (binds and bends the TATA promoter element), TFB (recruits RNAP) and TFE (stabilises the initiation complex). The elongation of RNAP is governed by elongation factors Spt4/5 and possibly NusA. In this study we have investigated the interaction network between RNAP subunits, transcription factors and promoter DNA in the context of initiation and elongation complexes using fluorescence methods. We are using a recombinant RNAP system that is assembled from individual subunits and this enables site-specific labelling of individual RNAP subunits with fluorescent probes. We have measured the topological distortion (bending) of the DNA template by FRET (Förster resonance energy transfer) and monitored the assembly of RNAP by fluorescence anisotropy. Our results show that fluorescence is an elegant tool to dissect the dynamics of higher order multiprotein complexes, such as the transcription machinery, in solution.