Multiplexed single molecule FRET spectroscopy: T7 RNA Polymerase as a case study

Meenakshi S A[a] and Rahul Roy[b]

[a]PES Institute of Technology, Banashankari, Bengaluru, sa.meenakshi96@gmail.com
[b]BioSystems Science and Engineering, Indian Institute of Science, Bengaluru,rahulfx16@nanobiophotonics.org

ABSTRACT

Understanding conformational dynamics of large multi-domain enzymes which perform essential biomolecular function is of vital importance. Over the past 4 decades, conformational dynamics has been approached independently using techniques like X-ray crystallography, Nuclear Magnetic Resonance spectroscopy, Molecular Dynamics simulations, and single molecule fluorescence studies. The focus of the current project is T7 RNA polymerase (T7 RNAP) based transcription. Insights from X-ray crystallography and previous single molecule fluorescence based studies indicate large conformational changes as T7 RNAP binds its cognate promoter and forms an active transcription complex. T7 RNAP transcription dynamics has previously been understood by studying behavior of FRET pairs on the DNA promoter.

Multi-donor single-acceptor schemes of FRET are difficult to analyze in ensemble experiments, however in single-molecule FRET mode can give deep kinetic insights into conformations and enzyme structure transitions. However the first step involves standardization of protein labeling reactions and analysis of labeled positions. Another requirement is that multiple labels on the protein should not hinder activity. In the current work, a new pipeline for understanding the role of distinct domains and conformational dynamics of T7 RNAP is being established. His-tagged T7 RNAP was purified and labeled with Cy3 dyes and its interaction with Cy 5 labeled T7 promoter DNA was explored by sensitive fluorescence based assays in ensemble. Despite multi-labeled T7 RNAP showing reduced *in vitro* transcription activity, fluorescence assays indicated active promoter binding by labeled T7 RNAP. These results encourage us to further explore transcription initiation and elongation complexes by multi-donor single-acceptor single molecule FRET experiments for future studies.