TNPB-DNA INTERACTION STUDIES IN VITRO USING SINGLE-MOLECULE FLUORESCENCE MICROSCOPY

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TnpB, derived from *Deinococcus radiodurans* ISDra2 transposon, is 408 amino acids long protein and together with an 150 nucleotides long RNA molecule forms a ribonucleoprotein (RNP) complex. It was experimentally shown that TnpB is an RNA dependent DNA nuclease and can be reprogrammed to cleave dsDNA *in vitro* and *in vivo*. Although TnpB protein function [1] and structure [2] were determined, TnpB binding lifetime to its substrate DNA, association constant (Ka), dissociation constant (Kd) remain unknown. Single molecule techniques can be adopted to measure kinetical and dynamical parameters of a biological system. Single molecule studies show a huge advantage over standard biochemical assays. It overcomes ensemble averaging over a heterogeneous population and allows to monitor protein-DNA interaction in real-time.

Here we use total internal reflection fluorescence (TIRF) microscopy technique to study TnpB RNP complex and DNA interaction *in vitro*. This is the first attempt to characterise kinetics of this recently discovered nuclease that has a huge potential for gene-editing. Since TnpB forms RNP complex inside the cell, it burdens its RNA efficient labelling with flurophore. Therefore, we use a strategy to fluorescently label TnpB protein through streptavidin-biotin interaction, instead. Interaction between TnpB and DNA can be achieved through DNA or protein immobilization on the surface. We employed both of these approaches. All together, this study can give us fundamental insights about the biophysical properties of protein-DNA interaction, while data and results that we aqcuire are important for other researchers working with DNA-interacting proteins.

[1] Karvelis, T., et al. (2021). Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. Nature, 599(7886), 692-696.
[2] Sasnauskas, G., Tamulaitiene, G., Druteika, G. et al. (2023). TnpB structure reveals minimal functional core of Cas12 nuclease family. Nature, 616(7956), 384-389.