A FRET Probe Strategy Based on Affinity Tag and Alternating-Laser Excitation Technique to Study the Position of the General Transcription Factor TFIIF in the RNA Polymerase II Transcription Preinitiation Complex

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In this study, we use single-molecule fluorescence resonance energy transfer (smFRET) facilitated by a total internal reflection fluorescence microscope that can in principle measure the distance between any amino acid of TFIIF and the C-terminus domain of a subunit of RNA polymerase II (Pol II). Atto647 dye as an acceptor is labeled on a cysteine in TFIIF and the donor is Atto550 dye labeled on a calmodulin, which serves dual purposes. Labeled with biotin, calmodulin is immobilized on the neutravidin-biotin-BSA coated surface in a glass chamber as a potential high-affinity receptor for a Pol II tagged with a calmodulin binding peptide (CBP). During acquiring FRET signals, the presence of acceptors is monitored by a modified alternating laser excitation (ALEX) technique that synchronizes laser switching and image acquisition by using a relay circuit [1] or a data acquisition (DAQ) board. We can control the excitation time at 532nm/638nm wavelength to monitor acceptors and not damage Atto647 dye. In the analysis of FRET signals, a high-order singular value decomposition (HOSVD) method is employed to subtract background, and correlation analysis of both channel images registers fluorophores and maps the two color channels. Likelihood ratio statistic for the change point detection resolve signals of FRET event by anti-correlation of Atto550 and Atto647 signals [2]. TFIIF is one of the general transcription factors that are combined with Pol II to form a transcription preinitiation complex, which is a key intermediate in transcription initiation by Pol II. TFIIF has an essential function of recognizing the start site in the earliest step of transcription initiation. Our preliminary characterization shows that the dissociation constant between CBP tag on Pol II and calmodulin is ~8nM, which allows stable association of CBP-Pol II to Atto550-calmodulin to facilitate for our single-molecule FRET measurements.

Reference: