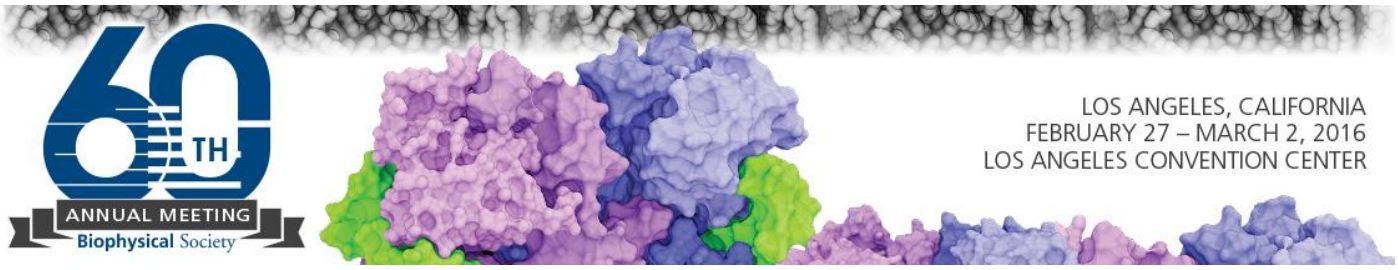

Live Cell Single Molecule Binding of Transcription Factors in Living Cells. Characterizing p53 Latency.

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The binding of transcription factors (TFs) to their regulatory sites on DNA determines how much and how timely a particular gene will be expressed, and ultimately how the cell respond to external cues. TF binding is typically studied by bulk biochemical experiments as chromatin immunoprecipitation (ChIP) As these methods provide limited temporal resolution and they are unable to provide information about these interactions at the single cell level, the interpretation of ChIP results can be challenging when dealing with TFs exhibiting rapid turnover, or with cells and tissues exhibiting a patterned nonhomogeneous transcriptional response to an external stimulus. Here we describe a microscopy-based single molecule imaging approach which can be used to obtain direct information on the TF binding kinetics to chromatin with the sub-second temporal resolution at the individual live-cell level [1,2]. We apply this method to characterize the binding of the tumor suppressor p53 both in basal, non-stimulated conditions and upon its activation by genotoxic stress induced by ionizing radiation: we show that p53 binds transiently to DNA (timescale of seconds), and that this interaction is modulated following the induction of damage, Importantly, more stable interactions are associated to higher transcription rates of p53 target genes, indicating that p53 acts as a latent TF, reviving an hypothesis initially derived from in-vitro studies [3], but later challenged by low temporal resolution ChIP experiments [4].

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