



Development of a multiplexed biophysical method of analysis for quantification of DNA repair factor dynamics.

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DNA repair factors upon detection of DNA double strand breaks (DSBs), quickly redistribute from a diffuse nuclear localization that is maintained by free diffusion, to foci like structures at DSBs, which result from long timescale specific binding interactions. Spatiotemporally tracking these dynamics in real time is an enormous challenge, since no single biophysical method of analysis can detect the broad range of mobilities that underlie this spatially regulated biological event. Therefore, in recent work we investigated a means to combined fluorescence fluctuation spectroscopy (FFS) with fluorescence recovery after photobleaching (FRAP), which allows for comprehensive quantification of a DNA repair factor's shift from freely diffusing to immobilized at DSB sites. Here we present this method and apply it to p53 binding protein 1 (53BP1) that is a DNA repair factor involved in DSB repair pathway choice and resolution of DSBs by non-homologous end joining (NHEJ).