

FRAP ANALYSIS OF BINDING: IMPROVED PROCEDURES REVEAL MAJOR ERRORS IN PREVIOUS APPROACHES AND SUGGEST A COMMON MODE OF TRANSCRIPTION FACTOR INTERACTION WITH CHROMATIN

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In recent years, data from fluorescence recovery after photobleaching (FRAP) has been analyzed quantitatively to estimate the binding rates of cellular proteins to cellular scaffolds such as chromatin. Unfortunately, the accuracy of these estimates is uncertain, since no benchmarks exist. To address this uncertainty, we have compared three different FRAP analysis procedures that have been applied to three different transcription factors. The published results [1-3] have yielded significantly different estimates of both the binding rates and the number of predicted binding states for the three different transcription factors. We report here that these discrepancies are not due to fundamental differences among the site-specific transcription factors, but rather arise from significant errors in FRAP modeling [4]. The two principal errors are a neglect of diffusion and an oversimplified approximation of the photobleach profile. We develop improved FRAP protocols to correct for these approximations, and then demonstrate how the approximations led to the major errors in the predicted number of binding states (one vs. two) and the predicted binding rates (differences of two orders of magnitude). The estimates from the improved FRAP protocol predict that for each of the transcription factors, ~75% of the molecules are freely diffusing within the nucleus, while the remainder is bound with an average residence time of ~2.5 s to a single type of chromatin binding site. Such consistent predictions for three different molecules suggest that many site-specific transcription factors may exhibit similar in vivo interactions with native chromatin.

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