

QUANTITATIVE FRAP - THE ROLE OF PHOTON FLUX AND THE LIMITS IMPOSED BY CELL PHOTODAMAGE

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Background: Fluorescence recovery after photobleaching (FRAP) is a method widely used for measuring parameters characterizing the dynamic behaviour of proteins *in situ*, in live cells. Quantitative analysis of FRAP data is complicated by the fact that a variety of different photobleaching protocols and data analysis methods are used, and phototoxic effects are not fully controlled.

Goal: This work aims at identifying the critical parameters of FRAP protocols, which influence the recovery times and need to be controlled to facilitate inter-experiment data analysis.

Methods: Dynamics of low molecular weight fluorophores and GFP-tagged linker histone (H1) was investigated, using various FRAP protocols and experimental conditions.

Results: We demonstrate that changes in photon flux strongly influence recovery times, moreover slight movements of chromatin, nucleus and the cell introduce unexpectedly large systematic errors in recovery determinations [1]. High intensity of exciting laser light (which is used to avoid redistribution of the fusion protein during the bleach insult) is also a source of potential artifacts. Intense illumination can lead to undesirable photoactivated reactions, including photodenaturation of DNA [2], photoaddition of a fluorescent probe to cell components, and various forms of photodamage. These phenomena can occur not only when a low molecular weight fluorescent dye, which is known to generate singlet oxygen (like acridine orange or a ruthenium (II) phenanthroline complex) is subjected to excitation light, but also when a fluorescent protein (eGFP) is photobleached in live cells. Damage to cell constituents and probe photoaddition during FRAP experiments changes bound/free GFP-tagged protein ratio and may severely complicate quantitative interpretation of experimental results.

Conclusions: Using the same photon flux of the bleaching beam, and proper controls for photoaddition and phototoxic effects exerted by fluorescent probes are preconditions for quantitative analysis and inter-experiment comparisons of protein dynamic behaviour.

REFERENCES:

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