Bias by light: photo-unbinding of fluorescent proteins in cell imaging

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Confocal and two-photon fluorescence microscopy techniques using fluorescent dyes as well as genetically-encoded fluorescent probes are widely used in cell biology. Beyond the common problems of photobleaching and phototoxicity, we present evidence that photo-unbinding also has the potential to compromise such methods, especially in quantitative studies.
Recently, we have found that light can cause the reversible dissociation of proteins from their binding partner [1]. The photo-unbinding effect was visualized and measured in solution and in cells by subsequent re-binding a differently labeled molecular partner. Our experiments revealed that photo-unbinding a) requires a fluorescent label b) is reversible c) occurs after both one- and two-photon excitation, d) is strongly dependent on the initial binding constant of the molecular system, and e) is linked to photobleaching.
Photo-unbinding experiments have been applied to various assays (e.g. antigen-antibody). Latest studies on Calmodulin (CaM) system and various CaM binding peptides revealed that photo-unbinding is more likely if the initial binding is stronger. In a cellular environment we showed that labeled phalloidin can be selectively unbound from actin filaments by laser scanning and rebound with a different color without disrupting the actin filaments.
Since the laser intensity threshold for photobleaching was always found to be the same or similar as for the observed photo-unbinding effect, this phenomenon may have a big impact on studies using photobleaching as a tool (e.g. Fluorescence Recovery after Photobleaching). As the binding equilibrium is changed by light exposure additional constraints may need to be considered.

References

[1] K.G. Heinze; S. Costantino, P. De Koninck, and P.W. Wiseman, "Beyond photobleaching, laser illumination unbinds fluorescent proteins," *J. Phys. Chem., submitted*