Manipulation of molecular binding by light can be a valuable tool for biomedical approaches. Here, we investigate such a potential tool, where fluorescently labeled proteins are affected by laser illumination. Beyond photobleaching, laser intensities can temporarily ‘unbind’ molecular partners [1] such as antigen/antibody, protein/peptide including toxins with relevant efficiencies up to 85% without destroying the binding partners [2][3]. This unexpected phenomena is called photounbinding, and mainly depends on laser power, excitation mode [2], and the initial dissociation constant of the molecular complex [3]. The exact mechanism has not been revealed so far, however photounbinding has been suspected to be a ‘radiative’ process linked to photobleaching, and mainly driven by ROS production. To prove this hypothesis we first introduced a redox sensitive GFP (roGFP2) [4]. We found significant changes in the redox potential before and after photounbinding as an indicator of ROS production. Second, we designed a fluorescently labeled antigen-antibody assay that allowed for distance control and selective distance variation between the antigen and its fluorescent label by introducing a relatively stiff amino spacer of various lengths. To visualize photounbinding after laser illumination, we induced rebinding of GFP to a differently labeled antibody and compared rebinding efficiencies for different fluorophore-antibody distances. We found that photounbinding strongly depends on this antibody-fluorophore distance, and almost vanishes if the fluorophore is far apart from its target. Together with previous results [2][3], this finding provides evidence that photounbinding is driven by short-lived ROS with mainly ‘local’ impact that vanishes for the long distance variant of the assay.


