

## **Protein kinetic evaluations by two photon photoactivation in living cells**

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Particle tracking inside the cell largely benefits of the ability to spatially and temporally mark specific structures to follow their “signalling” over a “dark” background as made possible since the advent of the photo-activatable markers. In terms of spatial confinement of the photo-activation process, the use of multiphoton excitation provides several favourable aspects compared to single photon confocal microscopy in photomarking biological structures to be tracked: the confined excitation volumes, of the order of magnitude of subfemtoliter, due to the non-linear requirements provide a unique control of the excitation and consequently photoactivation in the 3D space. In this context photoactivation experiments can be used to assess quantitative information about the binding kinetics of a macromolecule expressed in different cellular compartments. In this work we extended to photoactivation procedures and models originally developed for the quantitative analysis of FRAP experiments and we evaluated, for different proteins of medical interest (Rac-paGFP), the diffusive behaviour in the cytoplasm and the binding kinetics at the large endosomes. The results are compared with standard photobleaching experiments, in order to evidence the gained sensitivity obtained with photo-activatable proteins.