# Implementation of 2PL 3D nanofeatured microscaffolds and optogenetics

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Abstract

Optogenetics is a relatively new technique combining optics and genetics to gain control (activating or deactivating) over specific molecular events in cells, tissues or organisms.

One great advantage of this technique is the possibility of using light-activated molecular tools to get control of whole-cell or whole-tissue dynamics as well as on sub-cellular compartments, in both cases with high spatio-temporal resolution in intact systems[1,2].

The approach we are developing is based on the combination of 2PL structures and optogenetic tools to gain optical control over invasive properties of human tumor cell lines within three-dimensional environments. This will be achieved by developing an optical setup able to redirect a laser beam in three-dimensions to guide the cells within 3D environments with different stiffness. The possibility offered by the optical setup opens the road towards the study of tumor cell invasiveness of 3D environments in real time while modulating their mechanical properties.

The molecular tool we exploit to gain control over cell dynamics has been described by Wu and colleagues[3], who conceived a photoactivatable form of the cytoskeletal protein Rac1 (PA-Rac1) by fusing a light-oxygen-voltage (LOV) sensitive domain expressed in plants to Rac1 N-terminus. Since Rac1 is physiologically involved in cytoskeletal rearrangements, we stably express PA-Rac1 in human tumor cell lines for studying cell mechanics upon light activation (Figure 1).

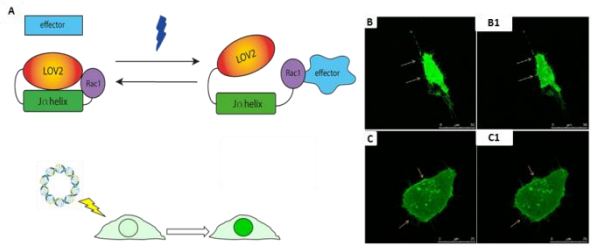


***Figure 1 PA Rac1 transfection.*** *Panel A show a schematic representation of Rac1 photoactivation upon 470 nm light illumination and the mechanism to make tumor cells expressing PA Rac1. Panels B-C1 show Rac1 expression in HeLa transfected cells.*

Since Rac1 takes part in many intracellular pathways (including transmigration at the nuclear envelope and nucleoplasm), we exploit our results on 3D micro cages and PA-Rac1 transfection to determine how and at which extent nuclear mechanical properties influence the invasive process. This is possible by controlling Rac1 activation with high spatio-temporal resolution.

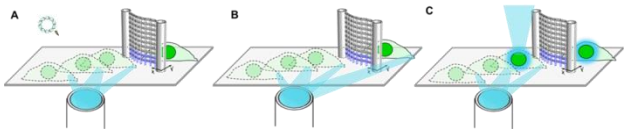


***Figure 2 Implementation of 2PL 3D microscaffolds and optogenetics****. Schematic representation of photoactivation of Rac1 in 3D microfabricated environments to study cell mechanical properties (invasiveness/stiffness)*

Overall, the combination of 3D 2PL and optogenetics will allow for simultaneous **photoactivation, live cell imaging and cell dynamic control in 3D microenvironments** thus finally discriminating between "external" and cellular contribution to the invasive or, more in general, cell mechanical cell behaviour.

10- Zhang, F. *et al., Nature* **446**, 633-639 (2007).
11- Nagel, G. *et al.*, *PNAS* **100**, 13940-13945 (2003).
13- Wu, Y. I., et al.,*Methods in enzymology* **497**, 393 (2011).