

Monitoring Surface Trafficking with FRAP

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Introduction

Proteins are not static in the plasma membrane, rather they can diffuse at high rates (on the order of microns per second) via Brownian motion due to thermal agitation. This fundamental characteristic was discovered more than 35 years ago and led to the postulation of the fluid mosaic membrane model by Singer and Nicholson.¹

Although this diffusional property of transmembrane proteins is thought to play a key role in several biological processes, relatively little is known in this field. The present lack of knowledge is primarily attributable to the scarcity of adequate and easily usable experimental tools. This application note describes the use of a purpose-built FRAP (fluorescence recovery after photobleaching) imaging system to demonstrate the rapid surface trafficking of receptors for neurotransmitters.

Initial Progress

Receptors for neurotransmitters mediate neuronal transmission in the brain and are thus responsible for the way we think and behave. Most receptors underlying fast chemical-mediated transmission are concentrated in synapses, the micrometer-sized cell-cell junctions between neurons that serve as the location of synaptic transmission. Until recently, it was believed that the aforementioned receptors were static at synapses.

At the University of Bordeaux, however, the group of Daniel Choquet has been working in collaboration with the group of Brahim Lounis to develop a number of high-resolution single-molecule imaging tools over the past five years — tools that have enabled the researchers to demonstrate that receptors for the neurotransmitter glutamate are not static, but in fact diffuse constantly at high rates between synaptic and extrasynaptic locations. Furthermore, this fast exchange is likely responsible for changes in the efficacy of synaptic transmission that underlie learning and memory processes.

Paradoxically, while the Choquet and Lounis groups successfully developed highly sophisticated imaging technologies for single-molecule detection in live neurons, they still lacked a tool capable of measuring global protein diffusion in cells at high rates. The tool in question would also need to be simple to use so that it could be shared efficiently by numerous biologists at the cellular imaging center on the Bordeaux campus.

FRAP Imaging

The best method for measuring global protein diffusion in cells is FRAP. In this technique, the molecule of interest is fluorescently labeled and a focused laser is used to rapidly and acutely inactivate the fluorescence by photobleaching a small (ideally diffraction-limited) subregion of the cell. Mobile molecules then repopulate the bleached area over time. The rate of recovery provides an accurate measure of the molecular mobility within the cell.

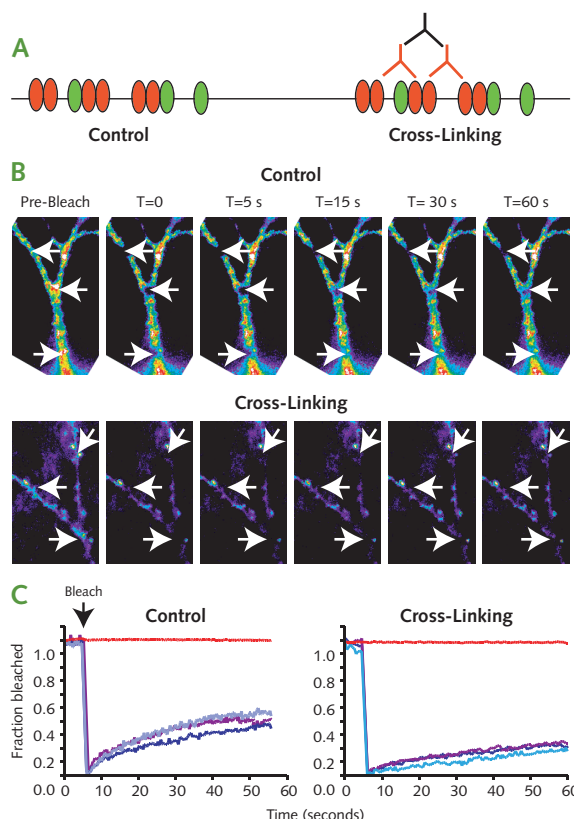


Figure 1. Data courtesy of Daniel Choquet, University of Bordeaux.

A. Schematic representation of the two experimental conditions.

Control: Neurons co-express GFP-tagged Stargazin and GluR2 subunits of AMPA receptors tagged with DsRed in the extracellular domain.

Cross-linking: In neurons expressing the same constructs, an anti-DsRed antibody is applied to live neurons to cross-link AMPA receptors.

B. Series of micrographs acquired in the green channel (Stargazin:GFP) for neurons either in control or cross-linking conditions before and after photobleaching several spots sequentially.

C. Quantification of fluorescence in the indicated spots. Note that Stargazin moves more slowly when AMPA receptors are cross-linked. The red traces are measured in unbleached spots.

First used almost four decades ago, FRAP has increased in popularity since the advent of fluorescent proteins that can be genetically engineered and fused to cellular proteins. Concurrently, confocal-scanning-based imaging systems with FRAP capabilities have started to appear on the market. Unfortunately, the utility of these systems for FRAP investigations is significantly compromised by severe limitations in acquisition speed and imaging sensitivity, as well as by phototoxicity associated with repeated and prolonged sample exposure to laser illumination.

To create a more suitable solution, Jean-Baptiste Sibarita and his group at the Institut Curie, in collaboration with Roper Scientific SAS, developed a FRAP imaging system based on widefield microscopy. Unlike confocal-based tools, this system is able to provide fast acquisition speed in 2D and 3D space, high imaging sensitivity, and low phototoxicity for advanced FRAP experiments. The use of widefield microscopy also allows researchers to perform deconvolution analyses when high spatial resolution is required, thus enabling the detection of very low level signals.

Stargazin and PSD-95

The Choquet group acquired the new FRAP imaging system and immediately used it to investigate the mobility of the AMPA subtype of receptors for the neurotransmitter glutamate. The system was utilized as a complement to the single-molecule approaches the group had been employing previously.

In a recent paper, the Choquet group used the rapid FRAP imaging technique to demonstrate that AMPA receptors (AMPA) diffuse at the neuronal surface in close association with another transmembrane protein, named Stargazin.² The Stargazin protein is mutated in the Stargazer mouse line, which has severe cerebellar ataxia, and is thought to play a major role in controlling glutamate receptor trafficking. In this paper, the Choquet group demonstrated for the first time that AMPAR stabilization at synapses is due to the ternary interaction between AMPAR, Stargazin, and the intracellular scaffolding protein PSD-95.

Both GFP-tagged Stargazin and RFP-tagged AMPARs co-expressed in cultured hippocampal neurons were utilized. First, their respective mobility inside and outside synapses was measured to discover that they moved at similar rates. Next, antibodies were used to cross-link AMPARs at the neuronal surface to decrease their mobility. Rapid FRAP imaging experiments uniquely enabled the researchers to demonstrate that this treatment also immobilized Stargazin (see **Figure 1**), thus showing that AMPARs and Stargazin are in close association in both synaptic and extrasynaptic membranes.

Enabling Technology

The FRAP imaging system used by the Choquet group served as the prototype for the new FRAP-3D™ imaging solution originally developed by Photometrics®. The fully integrated FRAP-3D imaging system comprises a high-efficiency FRAP head, a laser launch module, an electron-multiplying CCD (EMCCD) camera, customized software, and a preconfigured workstation.

The FRAP-3D imaging system is compatible with a range of inverted microscope platforms and allows users to switch easily between standard widefield applications and FRAP/ photoactivation studies. The system can perform rapid FRAP studies in 2D or 3D space. The delay between the end of the bleach pulse and the first recovery image is reduced to less than 25 msec, enabling fast dynamic analyses.

RESOURCES

To learn more about the ongoing research of the Choquet group at the University of Bordeaux, please visit www.synapse.u-bordeaux2.fr/equipechoquet.htm.

To learn more about the FRAP-3D imaging system, please email eric.morcet@roperscientific.fr.

Citations

1. Singer, S.J. and Nicholson, G.L. (1972). Fluid mosaic model of structure of cell-membrane. *Science* **175**, 720–731.
2. Bats, C., Groc, L., and Choquet, D. (2007). The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* **53**, 719–734.



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