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New bi-photon fluorescent imaging probes for the study of virus traffic

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This project is dedicated to the design of ***new bio-imaging probes*** combining ***multifunctional polymers*** and ***specific chromophores (mono-photon and bi-photon)*** and to their application, for replying to a specific biological question that concern the assembly and the transmission of viral particles, in order to understand the molecular mechanisms of retrovirus cell trafficking and the cellular factors involved.

After infection of the host cell, the virus uses the cell machinery to produce its own proteins (Gag, Pol and Env). The structural Gag proteins are able to self-assemble on the genomic RNA to form viral particles. The Gag proteins assemble either at the plasma membrane or on intracellular membranes. The new viral progeny bud and get released directly from the plasma membrane, or will accumulate in late endosomes and exit the cell by exocytosis. This different location depends on the cell type and certainly on the membrane lipid microdomain composition. Lipid microdomains containing cellular proteins and specific phospholipids, such as phosphoinositides (PIP), can sign the type of cell membrane. For exemple, HIV Gag can target PIP2 cholesterol-rich microdomains of the plasma membrane. We are currently identifying PIP interactions with other retroviral Gag proteins. The question is whether a fluorescent tagged-PIP can be used as a probe for life cell imaging of viruses. Would this new probe, located in such microdomains, enhance the presence of Gag and consequently viral budding at the cell surface? Would Gag assemble preferentially on this labelled PIP microdomains? Would the labelled PIP analogues be able to integrate and label the neo-formed virus? Consequently, the labelled PIP would enable us to follow cell membrane dynamics and subsequent viral budding (by rapid confocal microscopy). The synthesis and the characterization of new fluorescent PIP analogues as non toxic and highly fluorescent probes able to integrate the host cell (and the viral) membrane are the goals of our project.

Bio-imaging will be performed using various microscopies: spinning-disk confocal and biphoton. Spinning-disk confocal microscopy allows cell life imaging for high speed dynamics such as membrane trafficking (limited photobleaching, short acquisition time and better sensitivity in comparison with wide field microscopy). Biphoton microscopy takes advantage of the square dependence of bi-photon absorption with the incident laser intensity that creates a volume of excitation of less than 1 μm^3 and allows micrometer to sub-micrometer resolution in 3-dimensions. The confinement of excitation is particularly advantageous for bio-imaging applications: (i) first, it avoids phototoxic damages outside of the focal point; (ii) second, it greatly reduces out-of-focus absorption in highly scattering media; (iii) it uses laser wavelengths in the near IR range, which corresponds to transparency window of biological media; (iv) moreover, one of the most relevant advantages of biphotonic excitation for cell imaging is the possibility to use the same incident laser source for many labelling chromophores. Then, a more precise superposition of images of different colors can be obtained.

The new bio-imaging probes will be based on organic chromophores with nonlinear optical properties. In fact, the use of organic chromophores is a promising alternative to inorganic quantum dots that suffer from several limitations such as aggregation in water, limited surface chemistry and toxicity problems when used *in vivo*.

The specific contribution of multifunctional polymers to the bio-imaging probes is based on several advantages: (i) the polymer is used to immobilize several kinds of entities, recognition moieties (phospholipids, sugars) to target a particular area on a cell and labelling moieties (optically active molecules) to provide detection (visualization and/or quantification and/or tracking); (ii) the polymer is biocompatible, bioresorbable and water-soluble; (iii) the polymer protects the immobilized entities.

This project is based on a multidisciplinary approach involving complementary team expertises to elaborate original new imaging probes for cellular biology (Chantal Andraud: chemistry of bi-photonic organic chromophores, Marie-Thérèse Charreyre: chemistry of multifunctional polymer architectures, Patrice Baldeck: bi-photon spectrophysics, Delphine Muriaux: virology and cell biology). At *term*, the design of fluorescent polymers able to bind to a cellular or a viral membrane would enable to track the virus entrance or exit in and out of the host-cell and to follow virus trafficking in the cell or from cell-to-cell during virus transmission.