## 2. PUBLISHABLE SUMMARY – PROBES FOR CLEM

### Innovation and application of genetically-encoded probes for correlated live-cell imaging and electron microscopy

Objectives - Advances in molecular biology, organic chemistry, and materials science have recently created several new classes of fluorescent probes for imaging in cell biology. The possibilities are endless: the probes can be used to study proteins in live or fixed specimens; in vitro or in vivo; to study localization or activity, or modify function; and by light and electron microscopy in the same sample. The fluorescent toolbox has become very complex and each tool has specific requirements and pros and cons for different applications. The aim of this project is to develop, validate and implement a probe for combined fluorescence live-cell imaging and electron microscopy (CLEM) of proteins of interest. The combinatorial probe will be based on a genetically-encoded tag consisting of a fluorescent protein (highly suitable for live cell imaging) *and* a module to visualize proteins by electron microscopy at high resolution with high quality preservation of the ultrastructure (Figs.1,2) to form "FLIPPER". In addition, we will explore the general applicability of a recently introduced method to increase resolution using light microscopy: photoactivatable localization microscopy or PALM. Contact details: *www.cellbiology.nl*

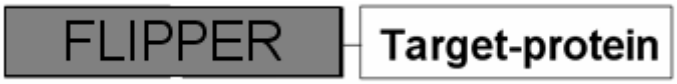


Figure 1. FLIPPER, a combinatorial probe for correlated microscopy
The module should allow live-cell imaging of proteins of interest and CLEM with high-quality ultrastructure. [Adapted from the grant proposal].

Work performed & main results achieved - During the first two years of the project I have successfully introduced molecular biology and cell culture in a highly traditional electron microscopy lab. Initial work focussed on (1) the construction of the combinatorial fusion (FLIPPER; Fig. 1) and fusion to a target protein for specific subcellular targeting, which provided a solid and successful basis for realizing the main goals for the first two years of the project. Thus, the proposed FLIPPER module has been constructed and has been applied for different imaging modalities (Figs. 1, 2). As a proof of principle, targeting to the Golgi-apparatus has been accomplished. *To the best of our knowledge, the newly developed FLIPPER module is the first genuine genetically-encoded tag that allows for live-cell imaging and correlated electron microscopy.* Subsequently we optimized fixation protocols, and achieved better fixation conditions than anticipated a priori; we also optimized the microscopic acquisition to be able to image at multiple or large areas, including mosaic microscopy, at both the light microscopy and electron microscopy levels. In the meantime, the construction of a generic FLIPPER that can be used to monitor cytoplasmic proteins has been initiated. The current module allows for live-cell imaging followed by electron microscopic examination with excellent preservation of fine ultrastructural detail. Our current goal is to construct a more compact FLIPPER and to proceed with strategies to make FLIPPER functional at the cytoplasmic site.

Expected final results & potential impact – We will produce new genetically-encoded probes for CLEM in cell biological research. The added value of these probes over existing ones is that (1) the targeting is highly specific and (2) the genetic encoding allows for high quality preservation of the ultrastructure. Moreover, since the probe is genuinely genetically-encoded, and easy to use (compare with the green fluorescent protein - GFP), it can be readily made available to the entire research community in Cell Biology.

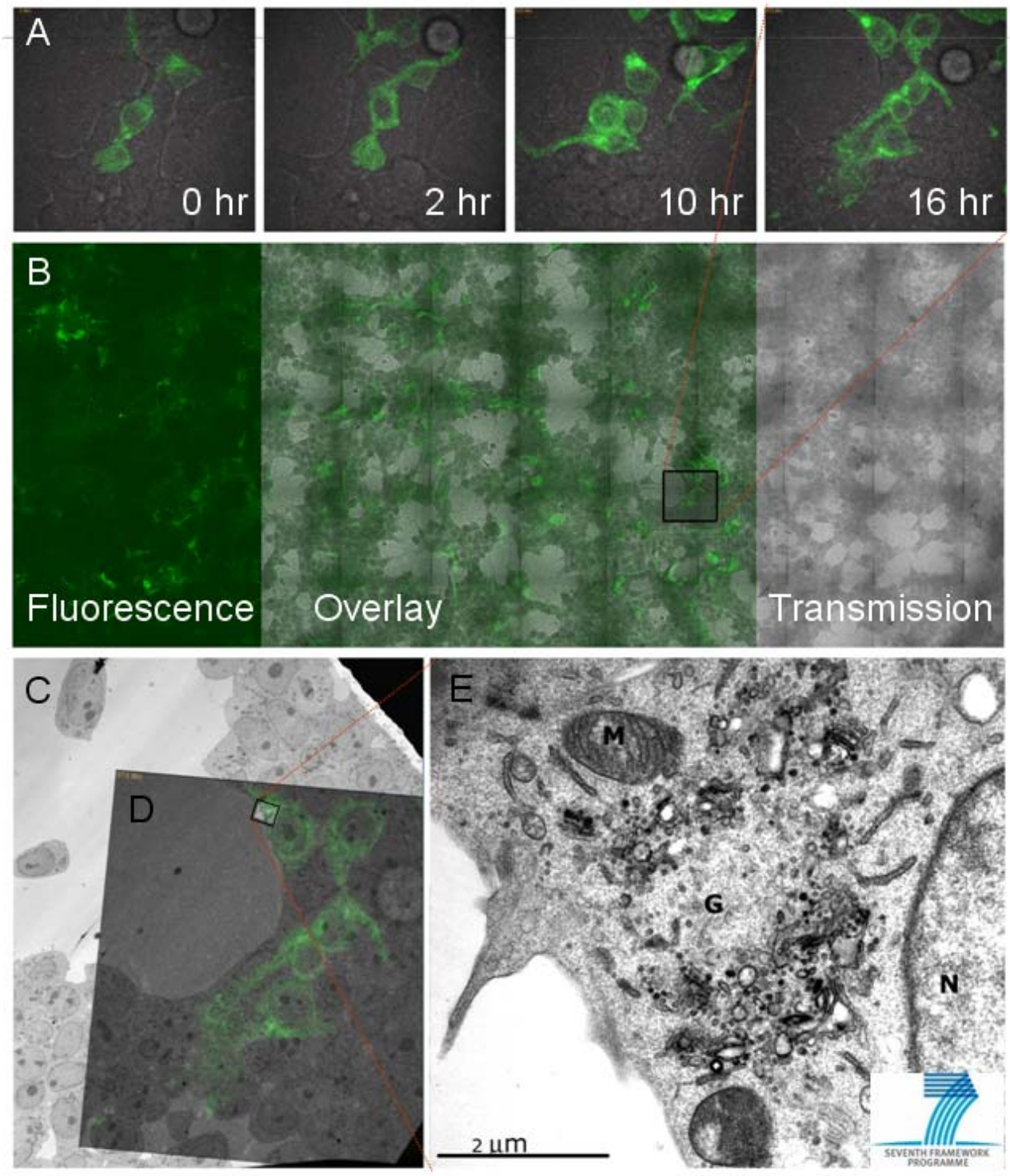


**Figure 2. FLIPPER in action.**
We developed a new genetically-encoded probe for correlated live-cell imaging and electron microscopy. FLIPPER has been tagged to a part of the mannosidase protein. (A) Golgi-dynamics studied in living cells using FLIPPER fluorescence overlaid with the transmission image. (B) Automated mosaic microscopy allows examination of large areas. (C) Low magnification EM of the same area, with (D) an overlay of GFP to correlate the images. The boxed area is shown in (E). The black deposit created by FLIPPER is readily visible in the Golgi. Note the high quality preservation of the ultrastructure.