Plant viral particles as nano-scaffolds for controlled positioning of enzymes on solid supports

Jane E. Besong[1], Noelle Carette[2], Daniela Cardinale[2], Jocelyne Walters[2], Thierry Michon[2], Kristiina Makinen[1]

[1]Department of Food and Environmental Sciences, Latokartanonkaari 11, P.o Box 27, FI-00014, University of Helsinki - Finland.

[2]INRA-Bordeaux (France)

jane.besong@helsinki.fi

Within the cell, enzymes involved in cascade reactions are precisely positioned in close proximity, the product of the first enzyme becoming substrate for the second. Because it is less diffusion-controlled, this so called “channelling” process allows an increased efficiency of the cascade reaction. VIRUSCAF is a collaborative project between the University of Helsinki, Finland and the Institut de la Recherche Agronomique, Bordeaux, France. This project, launched in November 2009 intends to design a nano platform mimicking the intracellular organisation of enzymes working in cascade. In a first step, the highly ordered surface of virus particles will be used as Enzymes Nano Carriers (ENCs). Using genetic engineering the virus particles will be functionalized in order to evenly distribute various enzymes on its surface. In a second step, these ENCs will be patterned on solid supports (enzymes chips). Our study aims at evaluating the gain of catalysis efficiency of complex reactions. Numerous potentially exciting outcomes in biosensors and nano-reactors technology can be envisaged.

Virus particles are precisely defined nanometer-sized objects formed by the self-association of capsid proteins monomers. Potato virus A (PVA) is being exploited for the first time for nano-technological applications. It forms flexible rod shaped particles of about 2000 capsid protein subunits surrounding a single RNA molecule. PVA virions are about 730 nm long and 15 nm wide, about 90% made up of the capsid protein and 5% the viral RNA. The N-terminus of the capsid protein is exposed to the surface of the particle hence can be engineered to carry functional entities. The constituting capsid protein subunits of PVA virions have been modified by genetic engineering to carry specific peptide linkers as a first approach to functionalize these virus surfaces with enzymes carrying complementary peptide linkers. The peptide linker fused to the N-terminus of the capsid protein has been successfully expressed in *Nicotiana benthamiana* plant leaves. Its ability to form virus-like particles is currently being studied using the electron microscope. A second approach involves the use of PVA specific binding peptides which will be fused to the enzyme of interest. Phage display screening was used to identify peptides specific for PVA and further analysis are being perform to confirm this specificity.

For a successful study and application of these virus-like particles (VLP) in nanotechnology, understanding the assembly process of PVA capsid protein is crucial. As in other viruses, this assembly is initiated by binding of the capsid protein to the viral RNA. Using a reporter based gene expression system, the region in the viral RNA involved in this interaction has been identified and is being studied in detail.