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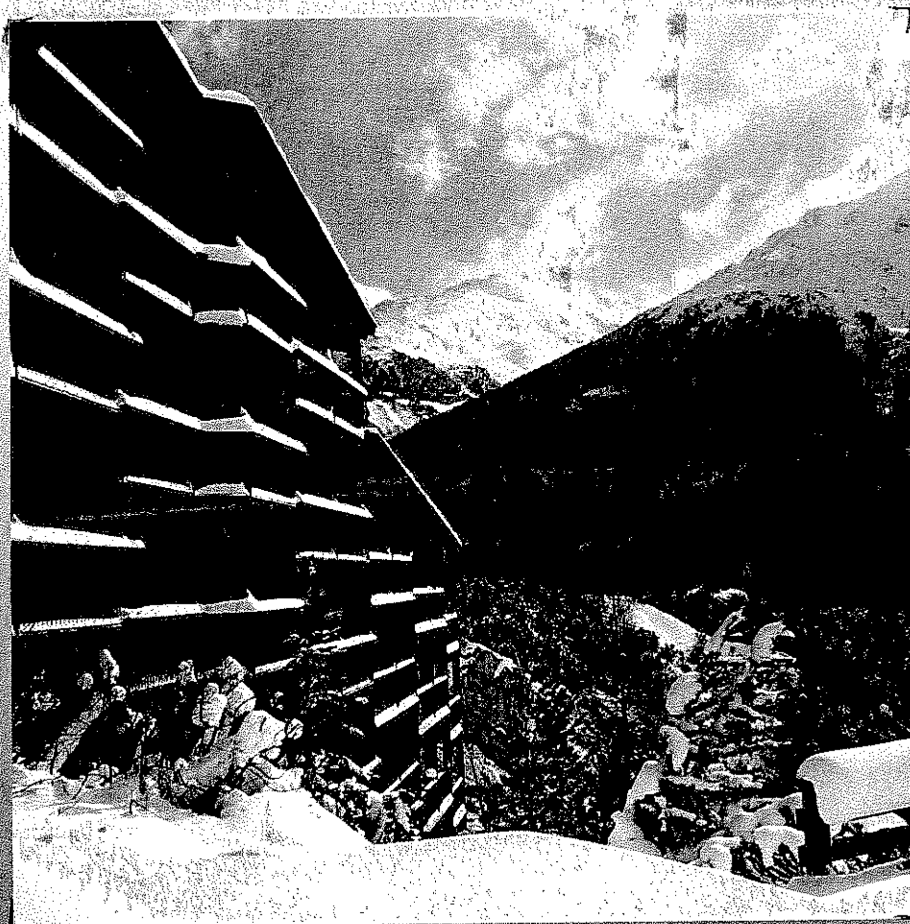
13èmes rencontres de virologie végétale



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11- Plant virus particles as nanoscaffolds for controlled positioning of enzyme cascades on solid supports

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In the cell, the close spatial location of enzymes catalyzing consecutive reactions increases the metabolic pathways efficiency, the product of an enzyme becoming substrate of the next enzyme (diffusion limitation or channeling). We intend to design an experimental plateform mimicking this enzyme organization to determine the parameters controlling the cascade reactions. If demonstration is made that a precise positioning of enzymes catalyzing consecutive reactions gives a net advantage upon diffusion controlled processes, the project's outcomes will serve the technology of enzymatically assisted catalysis in organic synthesis with potential applications for the technology of microreactors and biosensors.
Our first goal consists in controlling the distribution of active enzymatic systems on solid supports. The systems concerned are constituted of several enzymes catalyzing a cascade of up to 3 consecutive reactions.
Virus particles are precisely defined nanometer-sized objects, well-ordered, formed by a self-association of capsid proteins monomers. These particles will be used as Enzymes Nano -Carriers (ENCs). Subsequently these ENCs can be finely positioned on solid supports using nanolithography techniques. The rod shaped tobacco mosaic virus (TMV) and the flexuous filamentous potato virus A (PVA) were chosen as ENCs.
Candida Antarctica lipase B (CALB), glucose oxidase (GOx) and horseradish peroxidase (HRP) were chosen as demonstrating enzymes. Three strategies are under investigation for enzymes to virus interfacing.
The first strategy consists in introducing complementary leucine zippers (basic and acid) peptides on TMV (or PVA) and enzymes. We are trying to produce engineered TMV *in planta*. The surface of TMV being negative, acidic leucine zipper (LZecoil) was genetically introduced at the C-terminus part of the capsid protein within the virus genome. A weak systemic infection of *Nicotiana benthamiana* plants by TMV-LZecoil was observed (2 plants/12). The virus progeny eliminated the LZecoil insert in inoculated leaves 15 days after inoculation. Engineered PVA like particles will be reconstituted from an N-terminus fusion between acidic leucine zippers and capsid proteins expressed in *Escherichia coli.*
A second approach consists in the selection by M13 phage display of peptides which present an affinity for the virus surface (TMV or PVA). The sequence of these peptides will be genetically introduced at the N-terminus part of the enzymes.
Three peptide sequences were found to have a specific affinity for PVA particles; their genetic fusion to the enzymes are in progress.
In a third approach, bispecific antibodies will be selected to insure the coupling between virus particles and enzymes. A selection of monoclonal antibodies against either the viruses (TMV, PVA) or the enzymes (CALB, HRP) is in progress. Then a fusion of the cells producing each monoclonal antibody will be attempted.