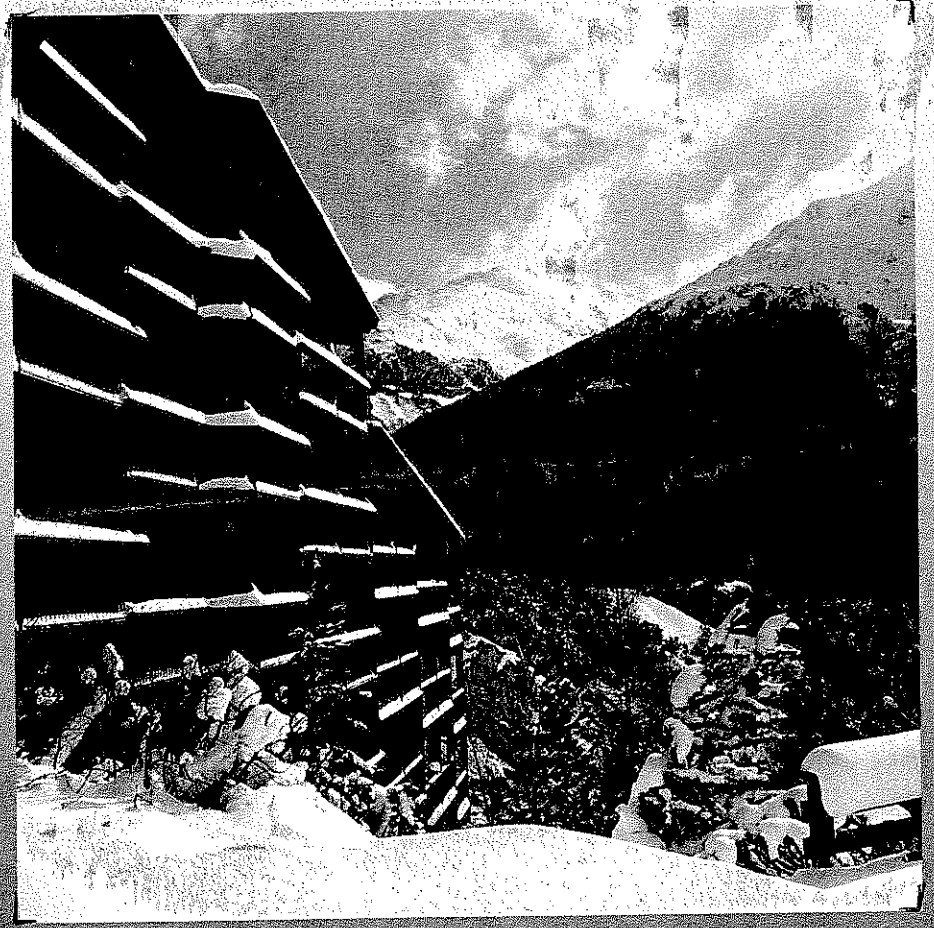
Virus scaffolds as Enzyme Nano-Carriers (ENCs) to organize bio-catalytic enzyme cascades. Design of a scanning electrochemical nanoreactor microscopy device

Daniela Cardinale, Noelle Carette, Amandine Barra, Jocelyne J. Walter, Thierry Delaunay, Christophe Demaille, Agnès Anne, Olivier Courjean, Nicolas Mano, Thierry Michon

rvv 13èmes rencontres de virologie végétale



Aussois du 16 au 20 janvier 2011

SFP Société Française de Phytopathologie

INRA Institut National de la Recherche Agronomique

20- Virus scaffolds as Enzyme Nano-Carriers (ENCs) to organize bio-catalytic enzyme cascades. Design of a scanning electrochemical nanoreactor microscopy device.

Daniela CARDINALE[1], Noëlle CARETTE[1], Amandine BARRA[1], Jocelyne WALTER[1], Thierry DELAUNAY[1], Christophe DEMAILLE[2], Agnés ANNE[2], Olivier COURJEAN[3], Nicolas MANO[3], Thierry MICHON[1].

[1] INRA, UMR 1090 GDPP, IBVM Virologie, 71 Av. Edouard Bourlaux, B.P. 81, 33883 Villenave d'Ornon, France.
[2] Laboratoire d'Electrochimie Moléculaire, UMR CNRS – P7 7591, Université Paris Diderot – Paris 7, Bâtiment Lavoisier, 15 rue Jean-Antoine de Baïf, 75205 Paris cedex 13, France.
[3] CRPP, Centre de Recherche Paul Pascal, 115 Av. Schweitzer, 33600 Pessac, France.

daniela.cardinale@bordeaux.inra.fr

In cellular systems, the association of collaborating enzymes in supramolecular structures enables metabolic processes to be performed more efficiently, accelerating reactions rates and preventing the diffusion of intermediates in the cell medium. The aim of the Cascade project is to create a new experimental tool at the nanoscale level mimicking *in vivo* enzymatic cascade reactions. The set up will also offer the opportunity to study enzymatic processes at the level of one single or few molecules. To this purpose two model enzymes will be used to build artificial redox cascades: the lipase B (CalB) from *Candida antarctica* and the glucose oxidase (GOX) from *Penicillum amagasakiense*. The free electrons generated by enzymatic activities will be detected by electrochemistry. To this purpose a new nanoelectrochemical technique will be used to confine the clustered enzymes and to measure the final activity. The confined reaction medium will be permitted by a "nanocavity" microelectrode fabricated at the tip of an AFM probe; the combination of AFM/SECM (Scanning Electro Chemical Microscopy) will enable to measure the electrochemical current generated by a few enzyme molecules. Varying the diameter of the nanocavity from few hundreds to about ten nanometers should eventually permit to follow a single enzyme activity.

In order to study one-single enzyme kinetics the very faint signal of the enzyme needs to be amplified. After CalB hydrolysis of p-aminophenyl acetate (pAPA) an electro-inactive substrate to p-aminophenol (pAP) the electro-active product the red-ox couple pAPA/pAP will be continuously recycled between two electrodes amplifying the single enzymatic initial event. Kinetics parameters of pAPA hydrolysis by CALB determined spectrophotometrically and electrochemically are in good agreement. This supports the suitability of pAPA for AFM/SECM single enzyme studies.

In order to control the distribution of enzymes on the electrode we will use virus capsids as Enzyme Nano-Carriers (ENCs). To this aim, two plant viruses, Tobacco mosaic virus (TMV) and potato virus A (PVA) will be tested. Three different strategies will be attempted for the virus to enzyme interfacing: the fusion of leucine zipper (LZ) pairs to enzymes and capsomers, bi-specific antibodies and peptides obtained from phage display screening.

Regarding the first strategy, three pairs of LZ having different characteristics in term of length, affinity and orientation have been selected and the cloning at the N- and C- terminus of CalB is in progress. One assembly CalB (LZKg-CalB) was expressed in *Escherichia coli* periplasm and we are currently optimizing its purification.

Monoclonal antibodies for CalB have been produced and fusion with antibodies for TMV and PVA will be attempted.

As third strategy, three peptides were selected that recognize PVA. Cloning at the N-terminus of CalB and GOX is in progress (see poster Carette et al.).