VIRUSCAF

Plant viral particles as nanoscaffolds for controlled positioning of enzymes on solid Supports

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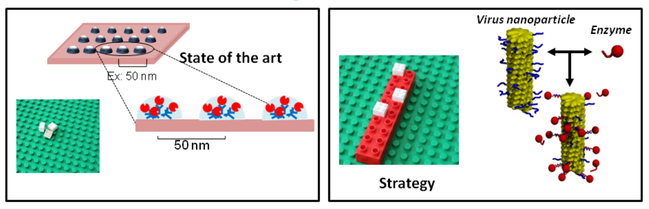
Enzymes Nano-Carriers (ENC)



Introduction

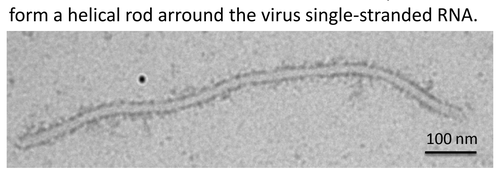
Mimicking metabolon formation in vitro on scaffolds is a promising way to study known metabolomic pathways and to create artificial cascade reactions for efficient in vitro synthesis. The aim of this research is to assemble an enzymatic cascade on the surface of virus particles[1] for the synthesis of piceid, a resveratrol derivative, which may have potential pharmacological applications.

Approach

To achieve an enzyme-nano carrier (ENC)[2] that allows placement of enzymes within close proximity, a novel approach was developed, utilizing the immunoglobulin (IgG)-binding peptide Z33. This method was tested by the non-covalent linkage of fluorescent proteins to the viral surface. Imaging was done using immunolabelling and correlative microscopy studies.

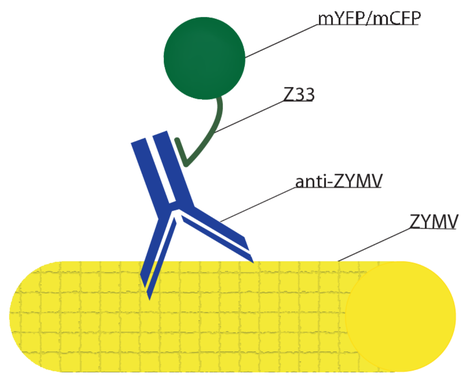
Zucchini yellow mosaic virus (ZYMV)

Zucchini yellow mosaic virus is a filamentous potyvirus with a length of 750 nm and a diameter of 11 nm. Its outer shell consists of about 1300 identical coat proteins that form a helical rod arround the virus single-stranded RNA.



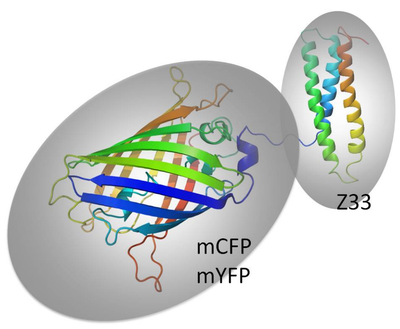
Anti-ZYMV

Polyclonal antibodies against the native ZYMV particle were used to coat the virus and enable binding of Z33mYFP and Z33mCFP.



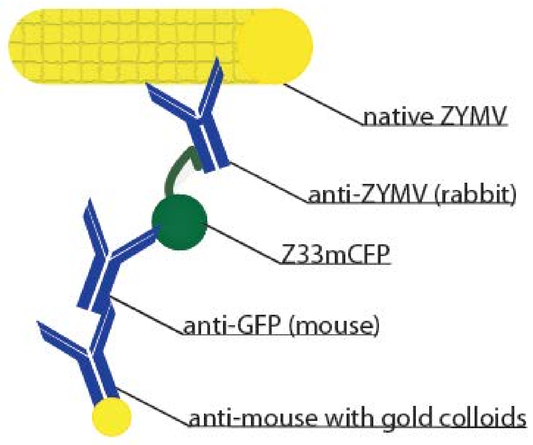
Z33mYFP and Z33mCFP

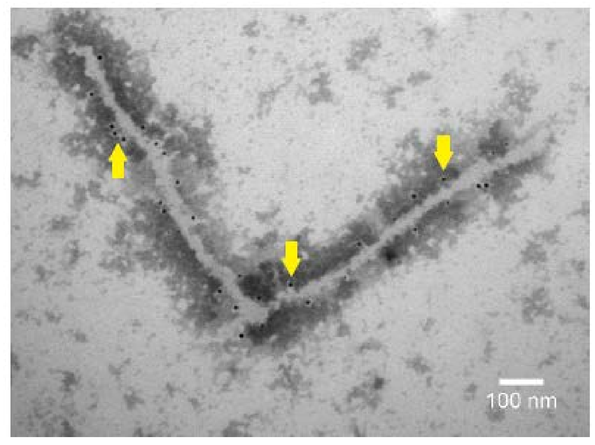
The Z33 peptide derived from staphylococcal protein A, binds with a high affinity to immunoglobulins. Z33 was genetically fused to yellow (mYFP) and cyan (mCFP) fluorescent proteins.



Immunolabelling

Virus particles coated with Z33mCFP were labeled via antibodies with attached gold colloids of 10 nm and observed by Transmission Electron Microscopy.

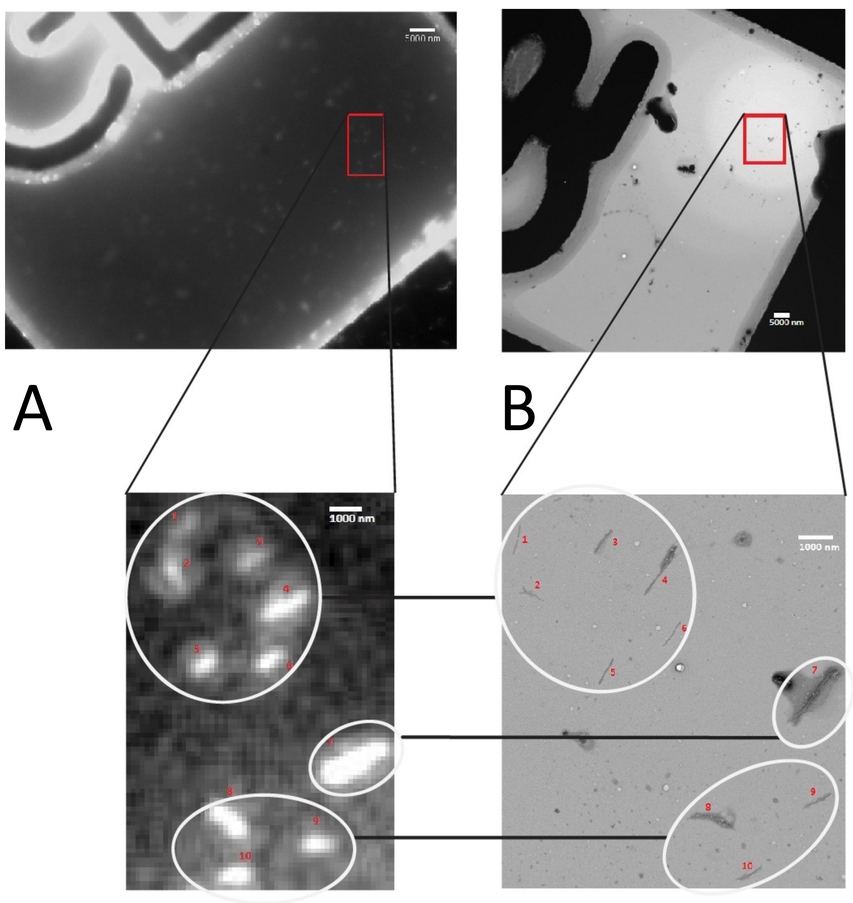




Correlative microscopy

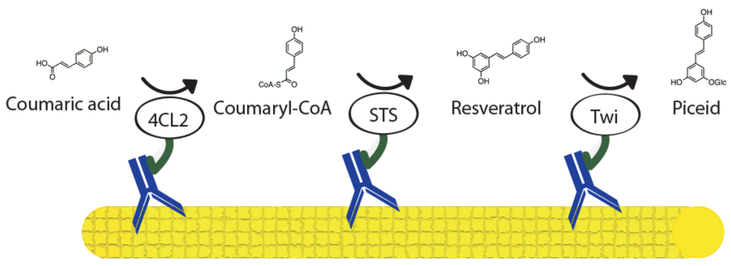
A. Light microscopy ZYMV particles were adsorbed on copper grids and coated with anti-ZYMV antibodies and Z33mYFP. Samples were imaged by fluorescence.

B. Transmission electron microscopy (TEM) The same grids were subsequently stained and observed via TEM. The same virus particle pattern was observed.



Conclusions and future research

A novel approach to link proteins to the surface of virus particles has been demonstrated for using viruses as enzyme nano-carriers (ENCs). Genetic constructs are underway to fuse 4-coumarate-CoA ligase 2 (4CL2) and Stilbene synthase (STS) with Z33. An artificial metabolon consisting of enzymes of the piceid pathway will be assembled on virus particles.



1. Carette, N., *et al. (2007) A virus-based biocatalyst. Nature Nanotechnol 2, 226-229*
2. Cardinale, D., et al. (2012) Virus scaffolds as enzyme nano-carriers. *Trends Biotechnol 30, 369-376*

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