

## Electronic Supplementary Informations

### Biocatalytic induced Surface Modification of the Tobacco Mosaic Virus and the Bacteriophage M13

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## General Materials and Procedures

### Materials

Acrylamide (SigmaAldrich – USA); [2-(Methacryloyloxy)ethyl]trimethylammonium chloride solution (SigmaAldrich – USA); N-Isopropylacrylamide (SigmaAldrich – USA); N-Vinylcaprolactam (SigmaAldrich – USA); Styrene (SigmaAldrich – USA); Fluorescein o-acrylate (SigmaAldrich – USA); Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> (Merck – Germany); Dialysis membrane 6-8,000Da (Spectrum Medicals Industries – USA); Laccase, originating from *Myceliophthora thermophila*, MW= 85 kDa, was purchased from Novozymes under the trade name Flavorstar. The enzyme concentration of the stock solution as obtained from the supplier was 17 mgmL<sup>-1</sup> (0.2 mmolL<sup>-1</sup>).

### Tobacco mosaic virus isolation

TMV particles were purified from infected *Nicotiana tabacum* plants. Plant material was harvested 14–21 days post-infection (dpi) depending on the infection status, and 50 g of plant material was used for virus purification based on a modified protocol from CIP (International Potato Center, Lima, Peru) as previously described for Potato virus X.<sup>1,2</sup> PEG precipitation was carried out as described in the original protocol, but sucrose cushion centrifugation step was omitted due to the loss of viral particles. The pooled fractions of the sucrose gradient were centrifuged for at least 3 h. The virus concentration was determined by measuring the OD<sub>260nm</sub> with an extinction coefficient of 3.0.

### M13 bacteriophage isolation

The bacteriophage M13 was amplified from *E. coli* ER2738/M13KE gIII grown in LB liquid with tetracycline at 37°C for 7 hours in a shaking incubator.

The culture was centrifuged at 5000 g 15 minutes 4°C for several times to remove the cells.

To the recovered supernatant was added 1/6 volume of 20% PEG-8000 2.5 molL NaCl solution and after an overnight incubation at 4°C the phage was recovered through a centrifugation step at 8200 g 30 minutes 4 °C. The obtained pellet was resuspended in 1X PBS and after a 2 hours incubation at 5°C on a shaker, the solution was centrifuged at 5000 g 15 minutes 4 °C to remove any cell residue. To the supernatant was added 1/6 volume of 20% PEG-8000 2.5 molL-1 NaCl solution and after an overnight incubation at 4°C the phage was recovered like in the previous step and resuspended in Phosphate Buffer 0.1 molL-1.

*E. coli* ER2738 was used for viral titration on LB/IPTG/Xgal plates.

Bacterial strain: *E. coli* K12 ER2738 (New England Bio Labs E4104S) [Genotype: F' proA+B+ lacIq Δ(lacZ)M15 zcf::Tn10(TetR)/fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5].

The M13KE gIII vector is derived from the cloning vector M13mp19 which carries the lacZα gene (New England BioLabs).

### Reversed-Phase Liquid Chromatography

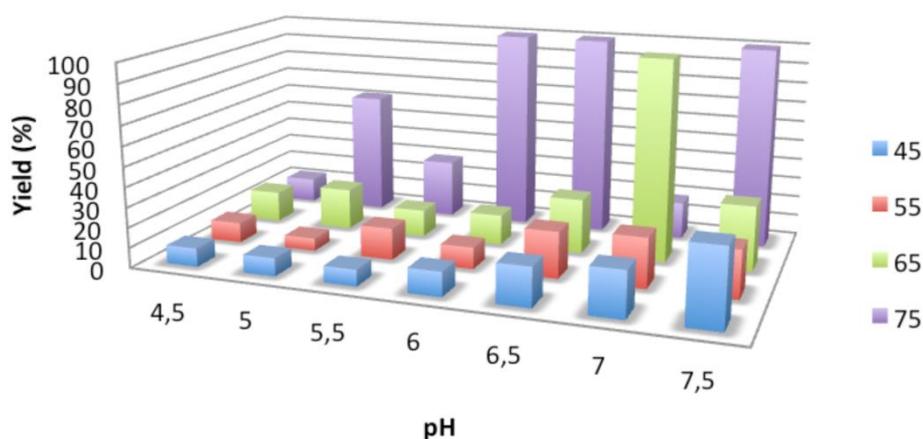
Reversed-phase (RP) liquid chromatography analysis was performed on a Shimadzu VP series high performance liquid chromatography (HPLC) modular system equipped with DGU- 14A3 Online Vacuum-Degasser, two LC-20 AT pumps, SIL-20A auto sampler, CTP-20 A column oven, PDA detector, FRC-10 fraction collector and Shimadzu LCsolution software. Chromatographic separations were performed using Jupiter 300 C4 column (Phenomenex) at 40°C and the samples were eluted at the flowrate 1mL\*min<sup>-1</sup> with a linear gradient from 0 to 100% buffer A in buffer B (buffer A: 0.1% of TFA, 5% of ACN, 94,9% water; buffer B: 0.1% TFA, 5% of water and 94,9% ACN) over the course of 40 min. Purifications was monitored with 254nm and 443 nm. After the purification fractions containing the products were collected, solvents were removed under reduced pressure and lyophilized.

## References

- 1 K. Uhde-Holzem, V. Schlösser, S. Viazov, R. Fischer, U. Commandeur, *J. Virol. Methods* 2010, **166**, 12–20.
- 2 K. L. Lee, K. Uhde-Holzem, R. Fischer, U. Commandeur, N. F. Steinmetz, *Methods Mol. Biol. (Clifton, NJ)* 2014, **1108**, 3–21.

## SI1: Optimization of the reaction conditions using phenol as a model substrate and virus modification procedures

The reaction was performed in aqueous  $0.1 \text{ molL}^{-1}$  phosphate buffer solutions of 20mL each, reaction condition with respect to pH (pH range 4.5 – 7.5; step size 0.5;) and temperature (45-75°C, step size  $10^\circ\text{C}$ ) were varied. To each solution was added: phenol (final concentration:  $50 \text{ mmolL}^{-1}$ ), laccase (final concentration:  $50 \text{ }\mu\text{molL}^{-1}$ ) and acrylamide (final concentration:  $700 \text{ mmolL}^{-1}$ ). The reaction mixtures were heated for 5 minutes at the desired reaction temperature after which they were bubbled with argon. Hereafter the reaction mixtures were allowed to react at the desired temperature for 90 minutes. Samples were obtained every 5, 30, 60 and 90 minutes. Enzyme activity was quenched by thermal inactivation ( $85^\circ\text{C}$ ). A total of 112 polymer samples were obtained and all samples were analysed on polymer formation. This was executed by precipitation in ethanol in a 1:5 ratio. The precipitate was isolated and dried. Finally, the yield of each sample was determined by isolated mass and the optimum reaction conditions compatible with TMV (pH 6, 55-65°C, reaction time 90 minutes) were established (Figure SI1).

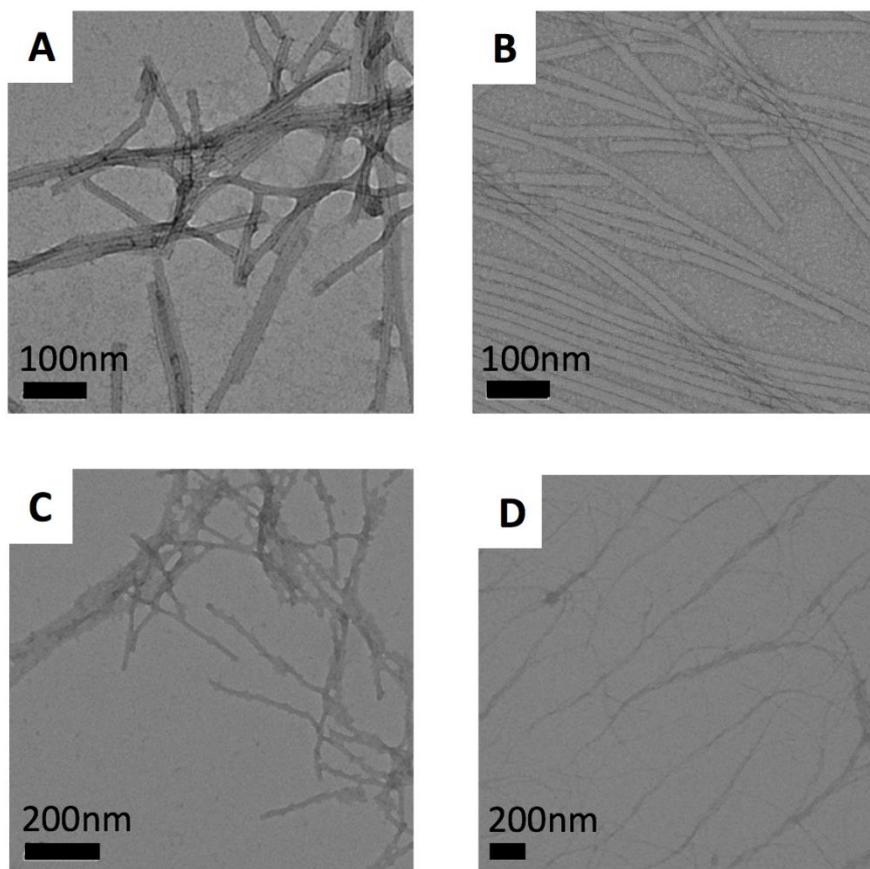


**Figure S1.** Yield of polymer isolated after a 90 minutes reaction time at different pH and temperature conditions.

## Virus modification approaches

The modification of the virus particles took place in a one-step one-pot reaction where  $1 \text{ mgmL}^{-1}$  of TMV and  $68 \text{ }\mu\text{g mL}^{-1}$  of bacteriophage M13 were mixed together with 7 mM of the respective monomers (for chromophore-labeled viruses, fluorescein-acrylate was added as co-monomer at 1 mol% or 10 mol%) and  $0.05 \text{ mmolL}^{-1}$  of laccase in  $0.1 \text{ molL}^{-1}$  phosphate buffer pH 6.0. The activation of the enzyme started by heating the reaction mixture in a water bath at  $60^\circ\text{C}$  for 5 minutes for TMV and at  $35^\circ\text{C}$  for 5 minutes for the bacteriophage M13, then the reaction solution was purged for 1 minute with nitrogen to eliminate dissolved  $\text{O}_2$  and prevent a premature termination of the reaction. After that, the mixtures were left immersed in the water bath for 1 hour and a half in total. At the end, as a strategy to stop the reaction and to remove unreacted monomers, the reaction were placed for dialysis against MilliQ water in an 6000-8000 Da membrane for 48 hours at room temperature. All measurements and analysis were after the dialysis step.

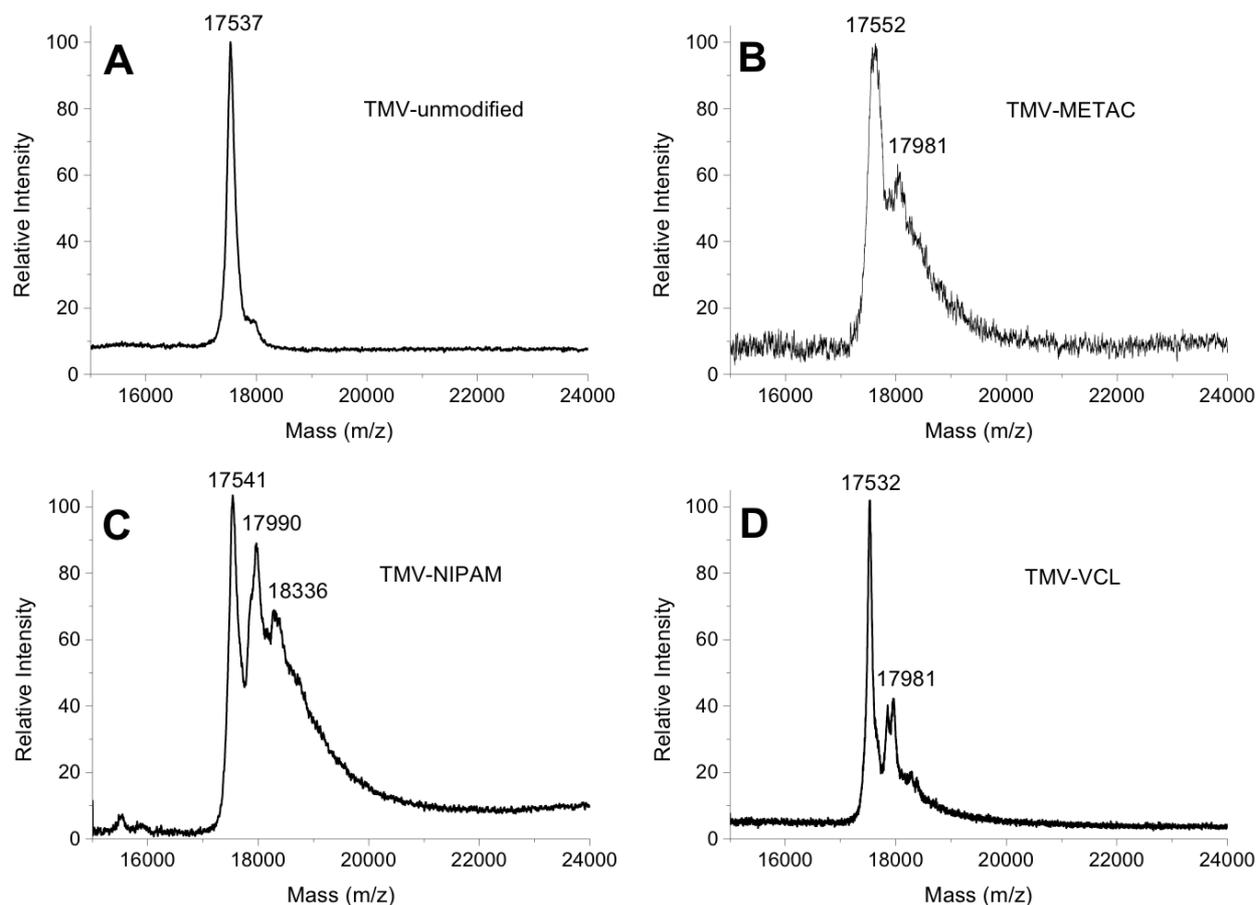
**SI2: Transmission electron microscopy Images of TMV and bacteriophage M13 after the reaction**



**Figure S2.** TEM images of the modified virus particles. A) TMV-METAC hybrids B) TMV-Styrene hybrids C) TMV-VCL hybrids D) M13-fluorescein o-acrylate hybrids.

TEM images were taken from samples negatively stained and drop casted in Cu grids modified with carbon film in a Phillips CM12 Microscope operating at an accelerating voltage of 120 kV and coupled to a 4k CCD camera.

### SI3: MALDI-ToF analysis of the TMV before and after the biocatalytic reaction



**Figure S3.** Maldi-ToF spectra of coat proteins from A) unmodified TMV, B) TMV-METAC hybrids, C) TMV-VCL hybrids.

Through the integration of the area correspondent to the signal produced by the modified coat proteins (Figure SI3 B,C,D) the percentage of contribution to the signal from specific subgroups of protein-conjugates has been established (Table S1).

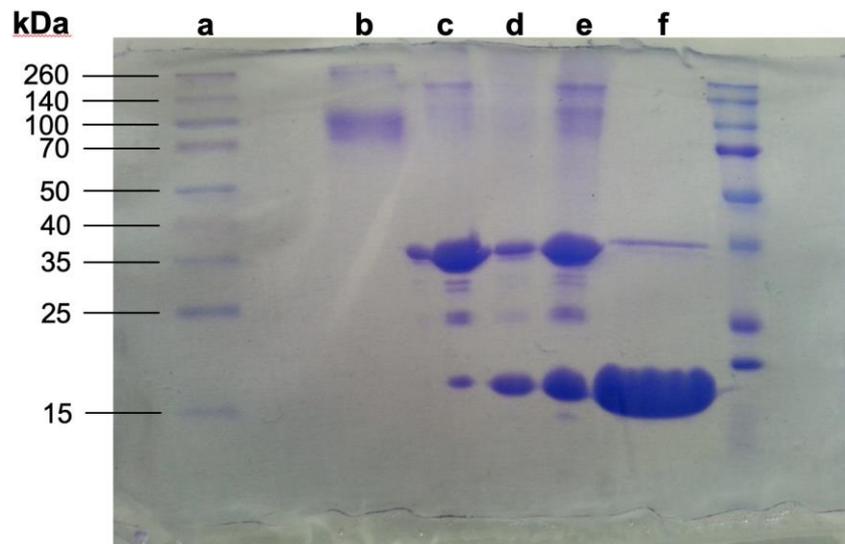
**Table S1.** Estimation of the degree of modification of the TMV coat proteins. The percentages represent the contribution of the coat proteins grafted with different amount of monomers to the overall signal generated by the modified coat proteins analyzed by Maldi-ToF.

	CP functionalized with 1-5 monomers (%)	CP functionalized with 5-10 monomers (%)	CP functionalized with more than 10 monomers (%)
METAC	78	22	1
NIPAM	32	36	32
VCL	67	26	7

MALDI-ToF MS was performed on a Voyager DE-Pro (Applied Biosystems) using DHB (2,5-Dihydroxybenzoic acid) as ionization matrix, blotted from aqueous solution of acetonitrile solutions.

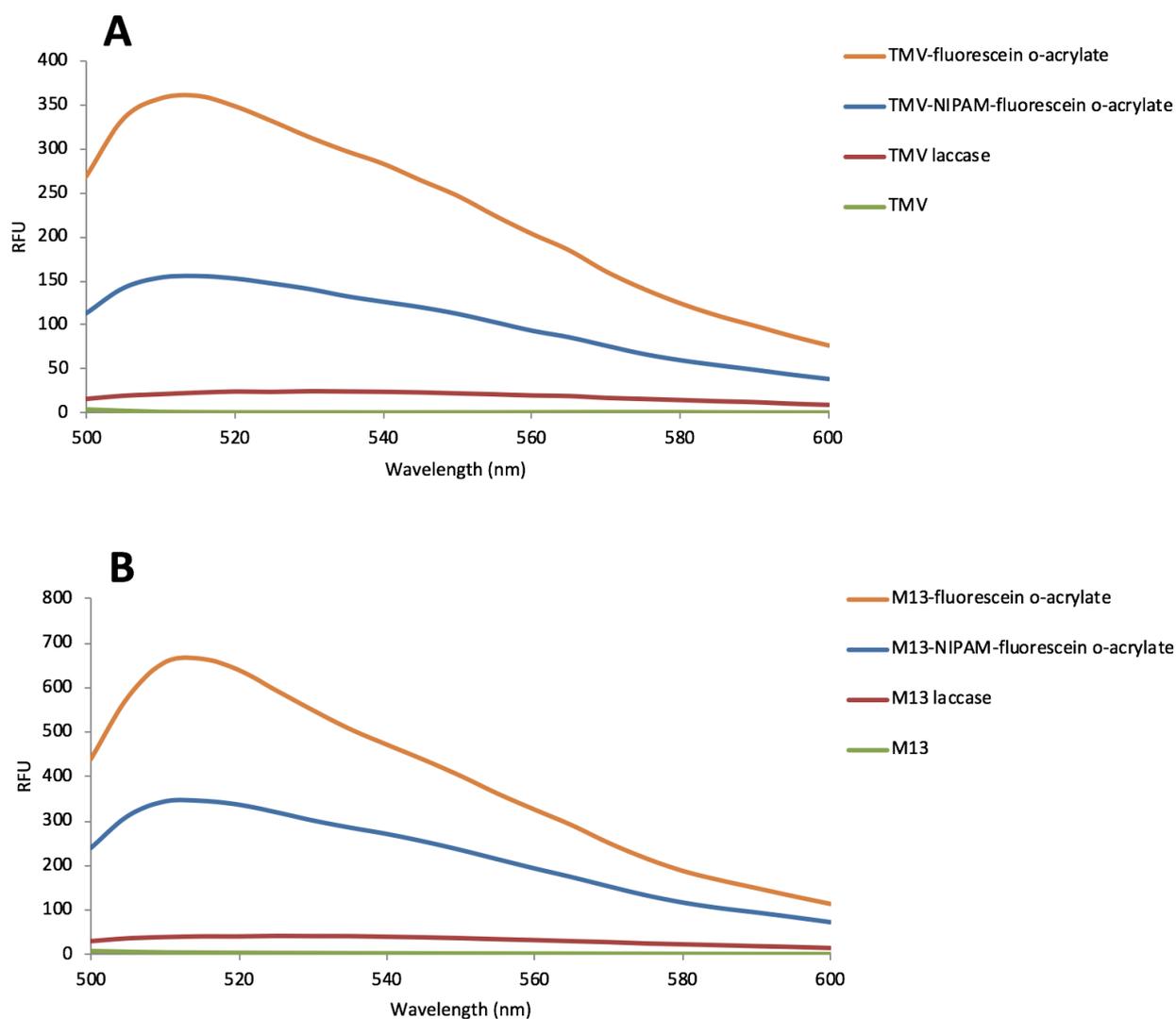
#### SI4: SDS-PAGE characterization of the TMV hybrids

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a BioRad apparatus using a 15% BIS/Acrylamide gels. The samples were mixed with SDS (final concentration 10%) and glycerol (to reach 25% v/v) and heated at 100°C for 10 minutes. An aliquot of 3µL of bromophenol blue was added at the end. Subsequently, 15 µL of each mixture and 5 µL of commercial marker were added to the lanes, (Figure SI4).



**Figure S4.** Polyacrylamide gel electrophoresis. a) Molecular weight marker, Thermo Scientific Spectra™ Multicolor Broad Range Protein Ladder (Thermo Scientific – USA); b) laccase; c) TMV-Styrene hybrids; d) TMV-VCL hybrids; e) TMV-NIPAM hybrids; e) TMV unmodified.

## SI5: Fluorescence spectrometry analysis of the fluorescent labeled virus particles



**Figure S5.** Emission spectra of the fluorescent labelled virus particles and controls (excitation wavelength 480nm). A) Emission spectra of TMV unmodified, TMV after the biocatalytic reaction in absence of acrylate monomers, TMV modified with NIPAM and Fluorescein o-acrylate as 10% co-monomer, TMV modified with Fluorescein o-acrylate. B) Emission spectra of M13 unmodified, M13 after the biocatalytic reaction in absence of acrylate monomers, M13 modified with NIPAM and Fluorescein o-acrylate as 10% co-monomer, M13 modified with Fluorescein o-acrylate.

Fluorescence spectrometry was performed with a SpectraMax M3 Multi- Mode Plate Reader (Molecular Devices) using a Hellma 105.250-QS Ultra-Micro fluorescence cell.