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

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Engineered viruses are finding an increasing number of applications in basic, translational research and material science. The genetic and chemical engineering of the capsids represents a key point for tailoring the properties of viral particles, but the synthetic efforts and limits accompanying these processes still hinder their usability. Here, a single-step highly selective biocatalytic functionalization approach is shown, providing a general platform for virus-acrylate hybrid particles. The tobacco mosaic virus (TMV) and the bacteriophage M13 have been successfully modified via laccase induced free radical formation on the tyrosine residues through a single electron oxidation as initiating step that subsequently reacts with acrylate-based monomers. This new approach can be extended to other biomolecular assemblies with surface exposed tyrosine residues, when the introduction of new functionalities is desired.

Engineered bionanostructures have great potential in drug/gene delivery systems, nanoelectronics, liquid crystals, and biosensors. Virus particles (VPs) are promising bionanomaterials in the aforementioned applications and have so far been used as nanocarrier platforms, nanoreactors, probes for imaging and sensing platforms.¹ The applications of VPs can be modulated through the addition of new functionalities to their surface via genetic or synthetic modifications, which can also be sequentially performed.² Although, these techniques offer great possibilities, they still present some limitations. Genetic modifications, are restricted

to the introduction of natural or unnatural peptides that ensure the capsid formation preferably with a high production yield, these two requirements combined is a challenging process.³ Chemical engineering offers the possibility to decorate viruses with a broad spectrum of functional groups such as fluorophores, inorganic structures, hydrophobic or charged moieties and stealth polymers.⁴ Several synthetic routes have been explored and despite surface modification can be achieved, a proper balance in terms of high specificity and viral structure maintenance is needed.⁵ Promising strategies as alternatives to the use of the conventional peptide-coupling based chemistry consist of the functionalization of the tyrosine residues through diazonium coupling,⁶ which allows for subsequent modification such as by copper-catalyzed azide-alkyne cycloaddition (CuAAC).⁷ However, new approaches with less synthetic efforts, high chemical diversity, limited number of synthetic steps, and associated purifications are desired in order to facilitate future applications of viral particles.

Tyrosine residues can be enzymatically activated by laccase via a single electron oxidation step producing a free radical species.⁸ It was envisioned that the free radical could be used as a new grafting-from approach to modify the viral surface using laccase combined with a large library of acrylates, which offers a tremendous chemical variability. Laccases are commercially available and require mild reaction conditions. Furthermore, as coenzyme-independent oxidoreductases they are very simple to use, requiring molecular oxygen as cosubstrate and producing water as sole by-product.⁹ The laccase-induced tyrosine oxidation generates a radical intermediate on the viral surface, which subsequently can react with acrylate-based monomer units. Depending on the monomer, various novel properties can be introduced (Scheme 1).

The tobacco mosaic virus (TMV) and the bacteriophage M13 (M13) have been modified via the aforementioned biocatalytic approach. These two viruses have already proven their applicability in nanomedicine and material science,¹⁰ but improvements on attaining a higher chemical diversity without

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Scheme 1. Modification of the tobacco mosaic virus via laccase induced free-radical oxidation. Laccase oxidizes the exposed tyrosine residues on the viral surface and the phenolic radicals newly formed triggers the functionalization of acrylate monomers. TMV structure created from the RCSB PDB (www.rcsb.org) of PDB ID 2OM3.¹³

the requirement of a complicated synthesis are needed in order to promote their large-scale application.

TMV is made up of 2130 coat protein (CP) subunits self-assembled in a helical fashion around a single strand RNA, forming tubes of 18 nm in diameter and 300 nm in length¹¹ and its coat protein presents one accessible tyrosine in position 139.⁶ The filamentous bacteriophage M13 is made up of 2700 copies of a major coat protein, p8, and few copies of minor coat proteins that enclose a circular single-stranded DNA molecule. The protein p8 presents two accessible tyrosines in position 21 and 24.¹² To demonstrate the ease of introducing chemical variability, molecular modifications using monomers that are hydrophilic, hydrophobic, charged, responsive, and fluorescent were performed. The reaction is straightforward and able to be combined with different types of commercially available acrylates and other monomers, therefore this grafting from approach is extremely versatile with the possibility to introduce diverse chemical species.

Before applying this reaction to the viruses, the enzyme performance has been tested, on the basis of a previous work,¹³ at different pH, temperatures, and time of oxygen removal. Phenol was used as a model oxidation-initiator to mimic the tyrosine residue of the viral surface (Supporting Information, S1). The optimum conditions for yielding the highest amount of grafted monomers were determined to be phosphate buffer 0.1 mol L⁻¹ at pH 6–7.5 and 65–75 °C. For TMV modification, these conditions have been slightly adjusted to meet the integrity maintenance requirements compatibly. Although TMV is a robust bionanoparticle, it can still denature if maintained at high temperature for prolonged time¹⁴ and disassemble into smaller disk-like aggregates above pH 6.5.¹⁵ To minimize denaturation and changes in aggregate morphology, a reaction mixture of 0.1 mol L⁻¹ phosphate buffer pH 6 at 60 °C for 90 minutes was used. For M13, the reaction temperature has been lowered to 35 °C.

Different acrylate monomers were used: [2-(Methacryloyloxy)ethyl]trimethylammonium chloride (METAC), *N*-Isopropylacrylamide (NIPAAm), *N*-Vinylcaprolactam (VCL), Styrene (Styr) and Fluorescein *o*-acrylate. These monomers were chosen due to their potential to add interesting properties to the viruses and also to

Figure 1. Transmission Electron Micrographs of the virus particles. A) M13 unmodified B) M13-NIPAM hybrid C) TMV unmodified D) TMV-NIPAM hybrid.

evaluate the versatility of this new method to add diverse chemical functionalities. Additionally, Fluorescein *o*-acrylate was used as co-monomer at 1% or 10% together with the respective monomers for providing a further control during the characterization steps and to produce fluorescent virus-hybrid particles. It is worth mentioning that styrene was dissolved in ethanol before being added to the reaction mixture, which had a final ethanol content of 10% (v/v). The addition of ethanol ensures sufficient solubility of styrene without denaturing TMV while maintaining appropriate laccase activity, which is known to tolerate organic solvents up to 30% (v/v).¹³ After the reaction, the mixture was purified by dialysis against MilliQ water and characterized by transmission electron microscopy (TEM) (Figure 1, Supporting Information S12), matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF) (Figure 2A, Supporting Information S13), reversed-phase liquid chromatography (RPLC) (Figure 2B), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Supporting Information, S14), and fluorescence spectrometry (Supporting Information, S15).

TEM images of the TMV and M13 modified particles reveal that the viral structure is maintained, indicating the stability towards the reaction conditions (Figure 1). The unmodified TMV and M13 normally appear as single linear filaments while the hybrid particles occur in an assembled state, which is probably caused by a drying effect related to the altered surface properties. In general, longer structures were observed for the TMV. Hybrid particles are assembled in a head-to-tail manner even when the TMV concentration was reduced in the reaction mixture. This occurs most likely due to interaction between the hydrophobic ends, which was also found in previous studies with polyaniline deposition onto the surface of TMV.¹⁶

Figure 2. MALDI-ToF spectra and RPLC elugrams of TMV particles. A) From the top to the bottom MALDI-ToF spectra of CP derived from TMV unmodified particles, CP derived from TMV-VCL hybrids, CP functionalized fraction isolated through RPLC from TMV-VCL fluorescent hybrids. B) RPLC elugrams (detection at 254nm) of CP derived from TMV unmodified particles, CP derived from TMV-VCL hybrids, CP derived from TMV-VCL fluorescent hybrids. Inset elugram (detection at 443) of TMV-VCL fluorescent hybrids.

The covalent nature of the monomer grafting to the viral surface has been verified through MALDI-ToF and reversed-phase liquid chromatography as well as by SDS-PAGE. The MALDI-ToF spectrum of the unmodified TMV (Figure 2A) is presented as a single narrow signal correspondent to a mass of 17.5 kDa, which can be attributed to the TMV CP. In the spectra of the viral particles from the biocatalytic modification (Figure 2A, Supporting Information, Figure S3), the peak at 17.5 kDa is still present, but additionally another broad signal representing heavier species is detected. This indicates that the products of the reactions are particles made up of a mixture of modified and unmodified CPs. The presence of the latter is probably due to the fact that the laccase needs time and spatial freedom to properly activate the tyrosines and during the activation and modification, other tyrosine moieties located nearby are most likely sterically hindered. The distribution representing the modified CPs indicates that the obtained conjugates are not monodisperse, which is to be expected from a free radical approach. Analyzing the signals, it has been determined that with this biocatalytic approach it was possible to graft oligomeric structure composed of more than 15 monomer units of NIPAAm on a single protein, and over 10 units of VCL and METAC. Through the integration of the signals provided by MALDI-ToF, the degree of modification for each type of acrylate was assessed (Supporting Information, Table S1). The unmodified CPs and the CP conjugates of the TMV capsids were separated via reversed-phase chromatography (Figure 2B). However, the modification of the tyrosine moiety from a phenol-like structure to an alkylated phenol affected the UV-Vis absorption such that the detection of the CP was hindered, causing the isolation of the eluted CP conjugates to be challenging. However, by introducing a fluorescent co-monomer it was nevertheless possible to unambiguously differentiate between CP conjugates and unmodified CPs using an alternative wavelength of detection at which only the eluted conjugate produces a signal (Figure 2B inset). The MALDI-ToF analysis of

the isolated fraction shows a peak at 17.677 kDa with a mass corresponding to the functionalized CP, whilst the characteristic peak of the unmodified TMV CP is absent (Figure 2A). This indicates that the modification is of covalent, rather than of non-covalent, nature. As can be seen from the SDS-PAGE of the viral particles (Supporting Information, Figure S4), the unmodified TMV contains a majority of sub-units with a molecular weight (M_w) of approximately 17 kDa. A small band around 35kDa is also present, probably corresponding to CP dimers whose interactions are resistant to the denaturing conditions of the SDS-page. On the other hand, all TMV-modified hybrids presented a protein band more distributed towards higher molecular weight than 17 kDa. Additionally, there is a large build-up around two times the molecular weight of the CP. This can suggest that the presence of new functionalities can stabilize the interaction between dimers, as previously observed for the unmodified TMV. The overall result provides the supporting evidence that CP conjugates of a higher molecular weight than the unmodified CP are formed, as it was observed by MALDI-ToF, further emphasizing the covalent nature of the modification through the free radical biocatalytic grafting reaction.

To investigate the potential of introducing new properties onto the viral surface through this functionalization, the fluorescence signal of the fluorescein o-acrylate virus-hybrids has also been measured (Supporting Information, Figure S5). Both the TMV and M13 hybrids were emitting a well detectable signal not only when functionalized solely with fluorescein o-acrylate but also when the fluorophore was added as 10% co-monomer in the reaction with another monomer, NIPAAm.

In summary, a new biocatalytic route has been demonstrated to modify TMV and M13 with chemically diverse oligomeric structures with a wide variety of properties in an easy and straightforward approach to tailor the overall properties of such particles. This new synthetic approach enables virus particles to become more generally applicable due to less synthetic efforts. Although TMV and M13 were used in this study, the approach is not limited to these viruses and any tyrosine-bearing protein structure can be potentially modified in this fashion. When protein-based particles are used for therapeutic applications, they trigger an immune response, which leads to particle degradation. This disadvantage offers an additional motivation for pursuing easy protein modification with polymer forming approaches, in fact the grafting of "stealth" monomers to proteins through this biocatalytic approach, could allow prolonged non-detection times by the immune system improving the pharmacokinetic profile.¹⁷ Moreover, the exposure of tyrosine in vivo may lead to the nitration of this residue which is known to trigger an immune response.¹⁸ Therefore, in addition to provide a general synthetic tool for advancing the use of bionanostructures in various applications, targeting and modifying the tyrosine residue as presented here is regarded as a dual approach to potentially lower the immune recognition.

Experimental section

The modification of the virus particles took place in a one-step one-pot reaction where 1 mgmL⁻¹ of TMV and 68 µgmL⁻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 mmolL⁻¹ of laccase in 0.1 molL⁻¹ phosphate buffer pH 6.0. The activation of the enzyme started by heating the reaction mixture in a water bath at 60°C for 5 minutes for TMV and at 35°C for 5 minutes for the bacteriophage M13, then the reaction solution was purged for 1 minute with nitrogen to eliminate dissolved O₂ 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.

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⁻¹ 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.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

- (a) H. Masarapu, B. K. Patel, P. L. Chariou, H. Hu, N. M. Gulati, B. L. Carpenter, R. A. Ghiladi, S. Shukla, N. F. Steinmetz, *Biomacromolecules* 2017, **18**, 4141–4153. (b) M. Comellas-Aragonès, H. Engelkamp, V. I. Claessen, N. A. J. M. Sommerdijk, A. E. Rowan, P. C. M. Christianen, J. C. Maan, B. J. M. Verduin, J. J. L. M. Cornelissen, R. J. M. Nolte, *Nat. Nanotechnol.* 2007, **2**, 635–639. (c) W. G. Kim, H. Song, C. Kim, J. S. Moon, K. Kim, S. W. Lee, J. W. Oh, *Biosens. Bioelectron.* 2016, **85**, 853–859. (d) F. Zang, S. Chu, K. Gerasopoulos, J. N. Culver, R. Ghodssi, *Nanotechnology* 2017, **28**, 265301. (e) A. M. Wen, N. F. Steinmetz, *Chem. Soc. Rev.* 2016, **45**, 4074–4126.
- S. Lin, H. Yan, L. Li, M. Yang, B. Peng, S. Chen, W. Li, P. R. Chen, *Angew. Chemie - Int. Ed.* 2013, **52**, 13970–13974.
- (a) C. M. Guenther, B. E. Kuypers, M. T. Lam, T. M. Robinson, J. Zhao, J. Suh, *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology* 2014, **6**, 548–558. (b) I. F. Bin Mohamed Suffian, M. Garcia-Maya, P. Brown, T. Bui, Y. Nishimura, A. R. B. M. J. Palermo, C. Ogino, A. Kondo, K. T. Al-Jamal, *Sci. Rep.* 2017, **7**, 43160.
- (a) Y. Wang, J. Liu, L. Cao, W. Wang, Y. Sun, Z. Yin, Z. Lou, *ChemBioChem* 2018, **19**, 1465–1470. (b) A. G. Malyutin, R. Easterday, Y. Lozovyy, A. Spilotros, H. Cheng, O. R. Sanchez-Felix, B. D. Stein, D. G. Morgan, D. I. Svergun, B. Dagnea, et al., *Chem. Mater.* 2015, **17**, 327–335. (c) H. Bludau, A. E. Czapar, A. S. Pitek, S. Shukla, R. Jordan, N. F. Steinmetz, *Eur. Polym. J.* 2017, **88**, 679–688.
- M. T. Smith, A. K. Hawes, B. C. Bundy, *Curr. Opin. Biotechnol.* 2013, **24**, 620–626.
- T. L. Schlick, Z. Ding, E. W. Kovacs, M. B. Francis, *J. Am. Chem. Soc.* 2005, **127**, 3718–3723.
- M. A. Bruckman, G. Kaur, L. A. Lee, F. Xie, J. Sepulveda, R. Breitenkamp, X. Zhang, M. Joralemon, T. P. Russell, T. Emrick, et al., *ChemBioChem* 2008, **9**, 519–523.
- (a) M. L. Mattinen, K. Kruus, J. Buchert, J. H. Nielsen, H. J. Andersen, C. L. Steffensen, *FEBS J.* 2005, **272**, 3640–3650. (b) K. Minamihata, M. Goto, N. Kamiya, *Bioconjug. Chem.* 2011, **22**, 74–81.
- (a) S. Riva, *Trends Biotechnol.* 2006, **24**, 219–226. (b) F. Hollmann, I. W. C. E. Arends, *Polymers (Basel)* 2012, **4**, 759–793.
- (a) A. E. Czapar, Y. R. Zheng, I. A. Riddel, S. Shukla, S. G. Awuah, A. J. Lippard, N. F. Steinmetz, *ACS Nano* 2016, **10**, 4119–4126. (b) M. Bäcker, C. Koch, S. Eiben, F. Geiger, F. Eber, H. Gliemann, A. Poghosian, C. Wege, M. J. Schöning, *Sensors Actuators, B Chem.* 2017, **238**, 716–722. (c) J.-S. Moon, W.-G. Kim, C. Kim, G.-T. Park, J. Heo, S. Y. Yoo, J.-W. Oh, *Mini. Rev. Org. Chem.* 2015, **12**, 271–281. (d) K. S. Sunderland, M. Yang, C. Mao, *Angew. Chemie - Int. Ed.* 2017, **56**, 1964–1992.
- P. J. G. Butler, *Philos. Trans. R. Soc. B Biol. Sci.* 1999, **354**, 537–550.
- M. J. Glucksman, S. Bhattacharjee, L. Makowski, *J. Mol. Biol.* 1992, **226**, 455–470.
- F. Hollmann, Y. Gumulya, C. Tölle, A. Liese, O. Thum, *Macromolecules* 2008, **41**, 8520–8524.
- M. A. Lauffer, W. C. Price, *J. Biol. Chem.* 1940, **133**, 1–15.
- A. Klug, *Phil. Trans. R. Soc. Lond. B* 1999, **354**, 531–535.
- Z. Niu, M. Bruckman, V. S. Kotakadi, J. He, T. Emrick, T. P. Russell, L. Yang, Q. Wang, *Chem Comm* 2006, **28**, 3019–3021.
- (a) K. L. Lee, S. Shukla, M. Wu, N. R. Ayat, C. E. El Sanadi, A. M. Wen, J. F. Edelbrock, J. K. Pokorski, U. Commandeur, G. R. Dubyak, et al., *Acta Biomater.* 2015, **19**, 166–179. (b) I. Ozer, A. Chilkoti, *Bioconjug. Chem.* 2017, **28**, 713–723.
- L. H. Jones, A. Narayanan, E. C. Hett, *Mol. Biosyst.* 2014, **10**, 952–969.

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Through a one-step laccase induced free radical oxidation of tyrosine residues, the capsids of the tobacco mosaic virus (TMV) and the bacteriophage M13 were functionalized with different types of acrylates. This method provides engineered virus with customizable properties for the desired applications.