ENZYME-MEDIATED SURFACE FUNCTIONALISATION OF STIMULI-RESPONSIVE MICROGELS

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Aqueous microgels based on *N*-Vinylcaprolactam (VCL) gained much attention in the biomedical research due to their temperature-sensitive behavior and their high biocompatibility.[1] However, the post-modification of PVCL-microgels with proteins is still challenging. The so-called sortase-mediated ligation is one possible method for the conjugation of biomolecules to polymers.[2]
Sortases are bacterial enzymes with transpeptidase activity, responsible for the attachment of proteins to the cell wall of gram-positive bacteria. Sortases of class A (SrtA) recognize a LP*X*TG-sorting motif (*X* being any amino acid) at the C-terminus of the targeted protein, cleave it between the threonine and the glycine and ligate it to a second protein via an oligoglycine nucleophile.[3]
In this work, we present the use of sortase-mediated ligation for the conjugation of different proteins to PVCL-microgels with the aim to incorporate special functionalities. For this purpose, microgels based on PVCL containing 5 mol% glycidyl methacrylate (GMA) as comonomer in the particles shell were synthesized and modified with the specific recognition peptide sequence LPETG for SrtA. The coupling of the LPETG sequence was analyzed via UV-Vis spectroscopy and Raman spectroscopy. To perform Sortase-mediated ligation, oligoglycin-tagged enhanced Green Fluorescent Protein (GGG-eGFP) was used as a model protein. The conjugation of the eGFP to the microgel was investigated qualitatively via confocal microscopy and quantitatively via fluorescence intensity measurements. It was shown that the fluorescence intensity increased linearly with increasing eGFP-concentration and exponentially with increasing reaction time up to seven hours. Additionally, we were able to show that also oligoglycin-tagged CueO-laccase can be conjugated to the PVCL-microgel using sortase-mediated ligation. To this end, the protein activity of the microgels was measured using ABTS as laccase substrate and the amount of conjugated protein was analyzed via BCA assay. These results indicate that sortase-mediated ligation is a very promising and powerful tool for the modification of microgels with biomacromolecules for applications in drug delivery, biointerface coatings and sensors.

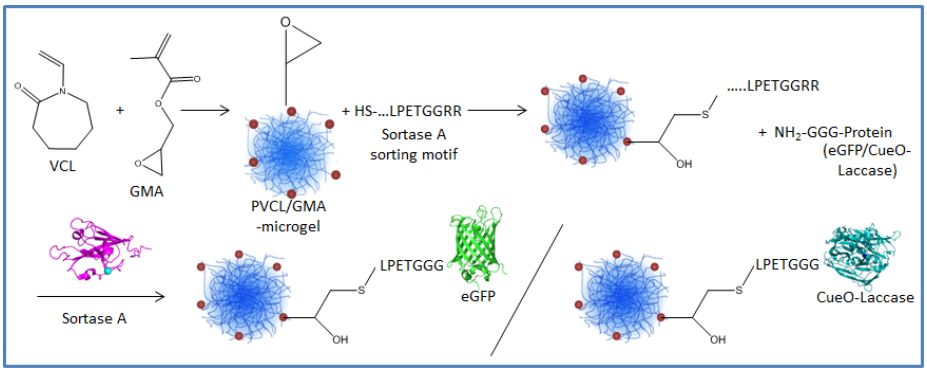

Figure 1 – Reaction scheme for the sortase-mediated ligation of eGFP and CueO-laccase to PVCL-microgels.

References:
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[2] E. Cambria et al., *Biomacromolecules* 2015, *16*(8), 2316-2326.
[3] T. Spirig et al., *Molecular Microbiology* 2011, *82*(5), 1044-1059.