Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Enzymatic multi-functionalization of microparticles under aqueous neutral conditions

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Table S1 Oligonucleotides used for the cloning of the GFPuv variants used in this study

Mutation desired	Oligonucleotide
Y1-tag	5’- GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA G-3’
	Rev: 5’- CAA AAG ATC TCA TCA CCA TCA CCA TCA CTA TTA AGA ATT CGA GCT CCG TCG ACA AGC TTG C-3’
Y5-tag	5’- GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA G-3’
	Rev: 5’- CAA AAG ATC TCA TCA CCA TCA CCA TCA CTA TTA TTA TTA TTA TTA AGA ATT CGA GCT CCG TCG ACA AGC TTG C-3’
Y39F	5'-GGG TAA GTT TTC CGA ATG TTG CAT CAC CTT CAC CC-3'
Y237F	5'-TGG CAT GGA TGA GCT CTT CAA AAG ATC TCA TCA CC-3'

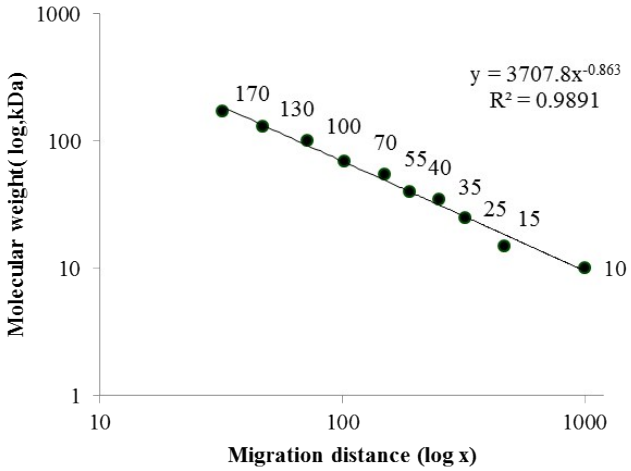


Figure S2 Calibration curve for the determination of the molecular weight of the products of crosslinking. An image of the SDS PAGE containing the samples was analysed with the software ImageJ and each protein band of the molecular weight standards used was identified by its migration distance (x-value from ImageJ). Data were fitted with a power fit and each data point is labelled by the corresponding molecular weight. Protein standards were from Thermo Scientific PageRuler Prestained protein ladder.

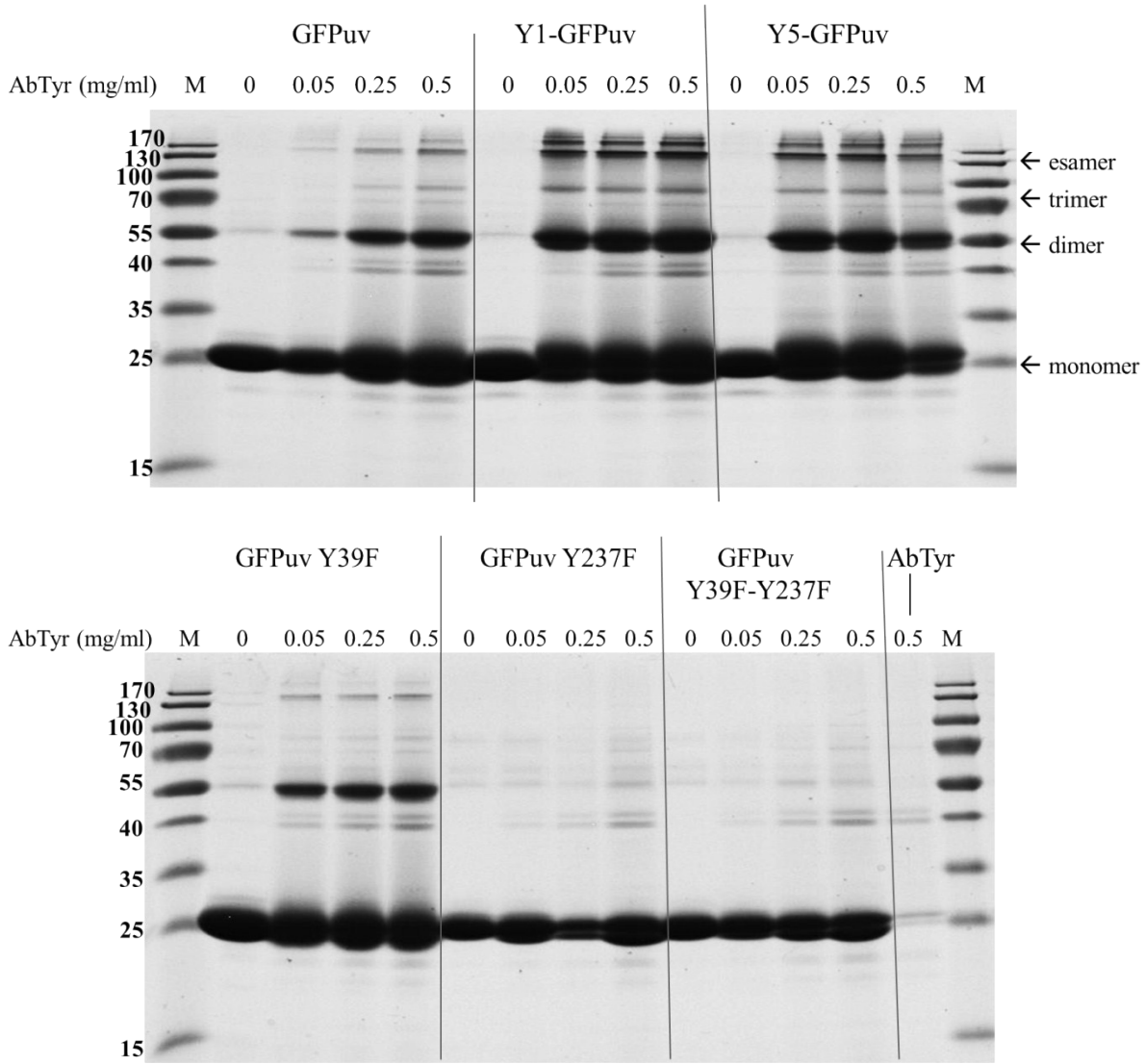


Figure S3 Crosslinking of GFPuv variants with different amounts of tyrosinase (AbTyr) for 3 h at 22°C. GFPuv variants at a 1 mg/ml concentration were incubated with 0, 0.05, 0.25, 0.5 mg/ml tyrosinase, as indicated above the lanes, for 3 h at room temperature in a 20 µl reaction volume with 100 mM K-phosphate pH 6.8. An aliquot (5 µl) of the reaction was analysed by SDS PAGE. Molecular weight markers (lane M) are reported in kDa.

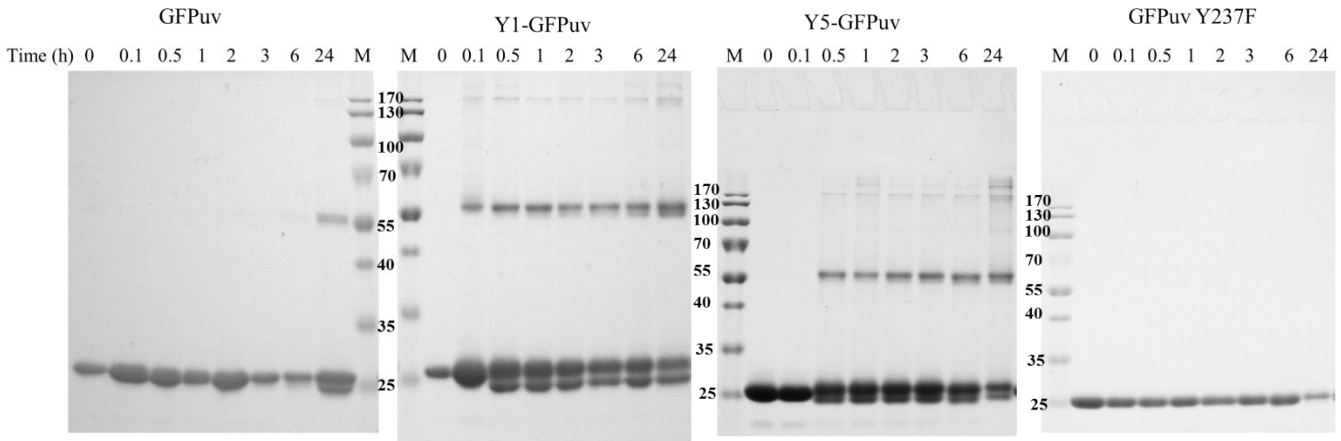


Figure S4 SDS PAGE analysis of time course experiment to monitor crosslinking of HisGFPuv variants with tyrosinase.Conditions: 0.5 mg/ml GFP variants, 0.025 mg/ml tyrosinase in 100 mM potassium phosphate buffer pH 6.8. Molecular weight markers (lane M) are reported in kDa.

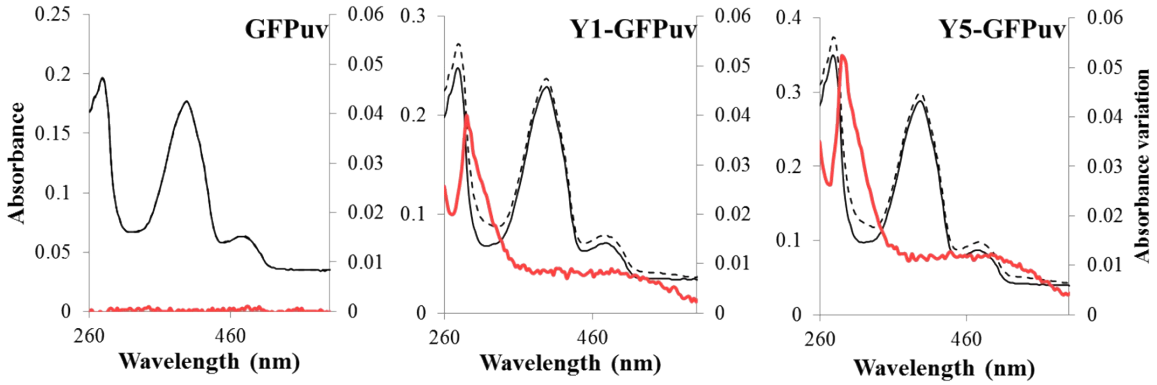


Figure S5 Absorbance spectra of crosslinking with tyrosinase of GFPuv, Y1-GFP and Y5-GFP at the beginning (continuous black line) and after 100 min (dashed black line), and the difference of the two spectra (red line). Reaction conditions: 200 ul of 0.5 mg/ml GFPs+5 ul tyrosinase (70 nkatal), 22°C, 3 s shaking before measurement.

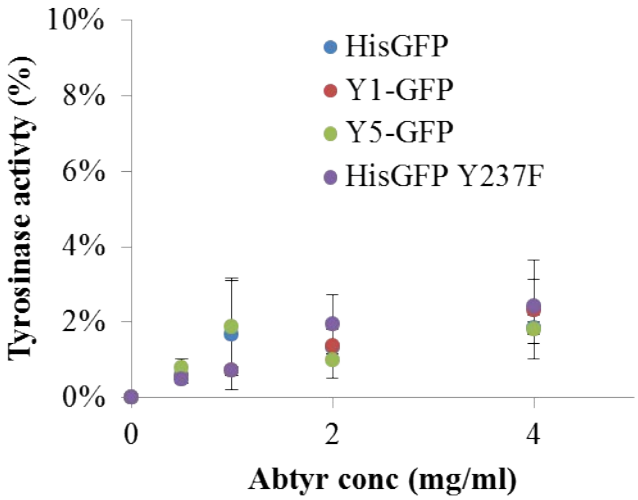


Figure S6 Residual tyrosinase activity measured on functionalized microparticles analysed for fluorescence in Figure 5 measured with 2 mM L-tyrosine.

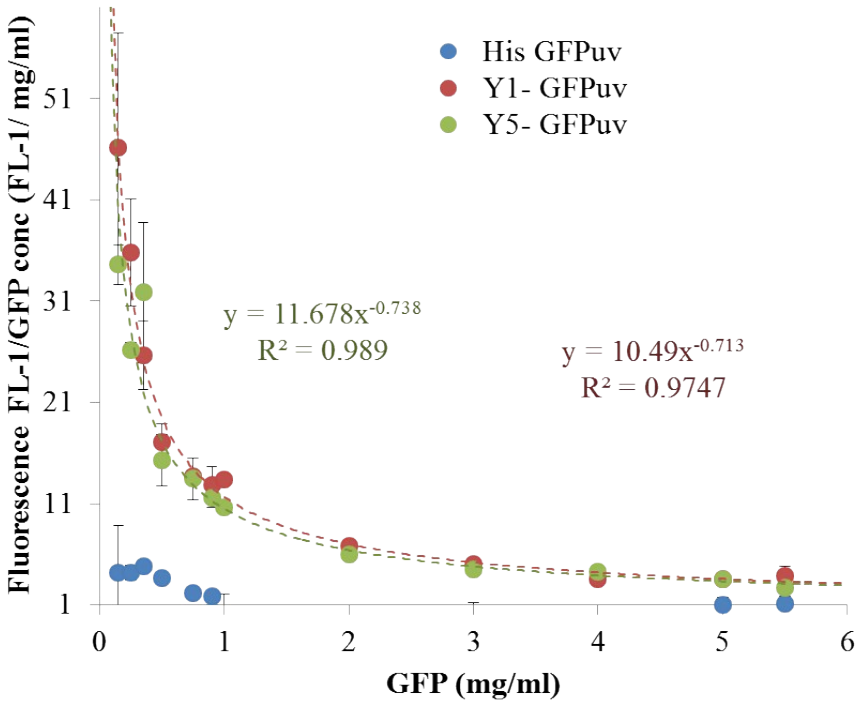
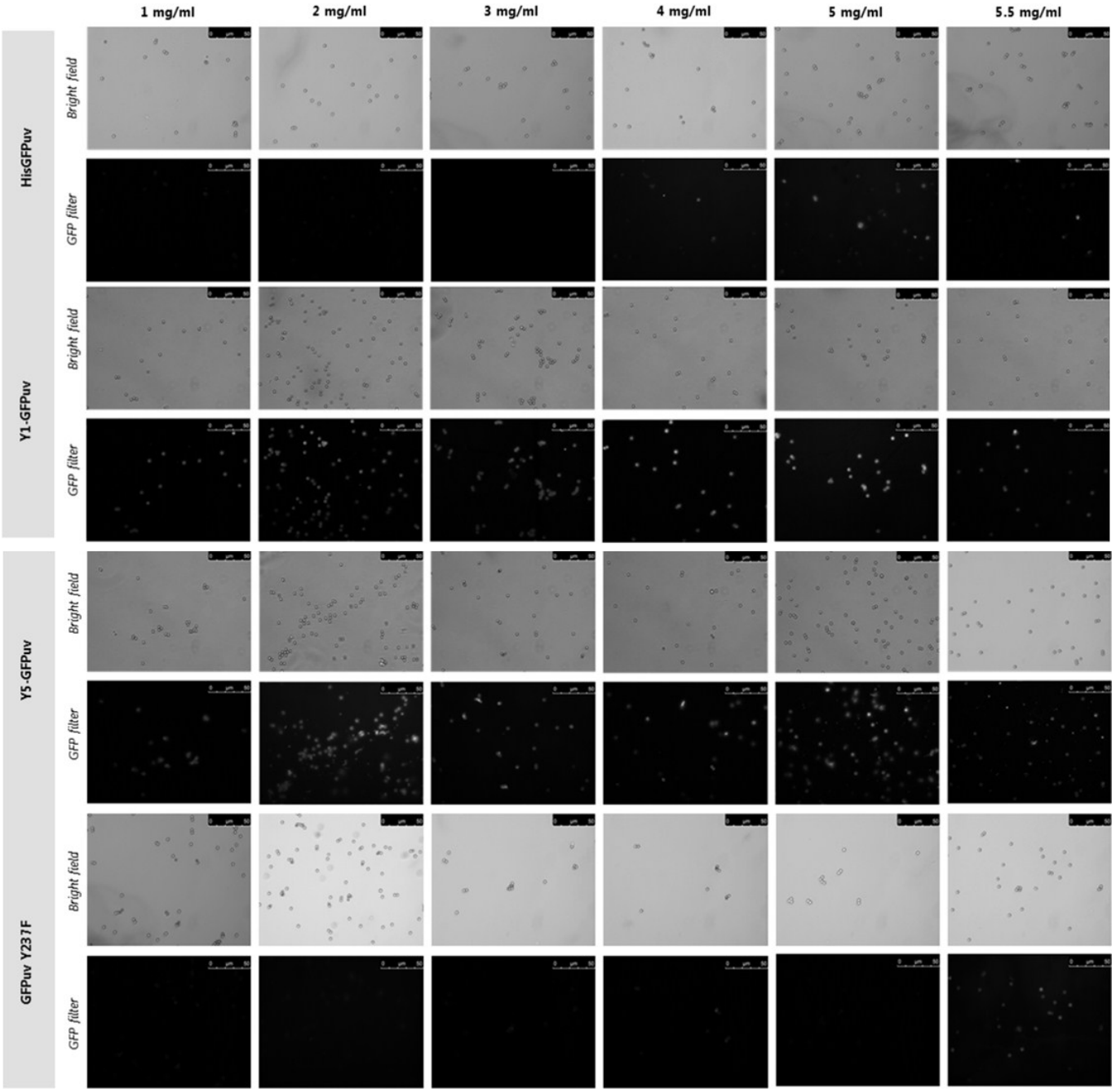


Figure S7 Efficiency of GFP immobilization by tyrosinase calculated as ratio between microparticle fluorescence (subtracted of the values obtained in the corresponding tyrosinase-free control reactions) and the initial GFP concentration. As a guide to eye, a power law fit is shown.

(A)


1 mg/ml
2 mg/ml
3 mg/ml
4 mg/ml
5 mg/ml
5.5 mg/ml
HisGFPuv
Y1-GFPuv
Y5-GFPuv
GFPuv Y237F
Bright field
GFP filter

(B)

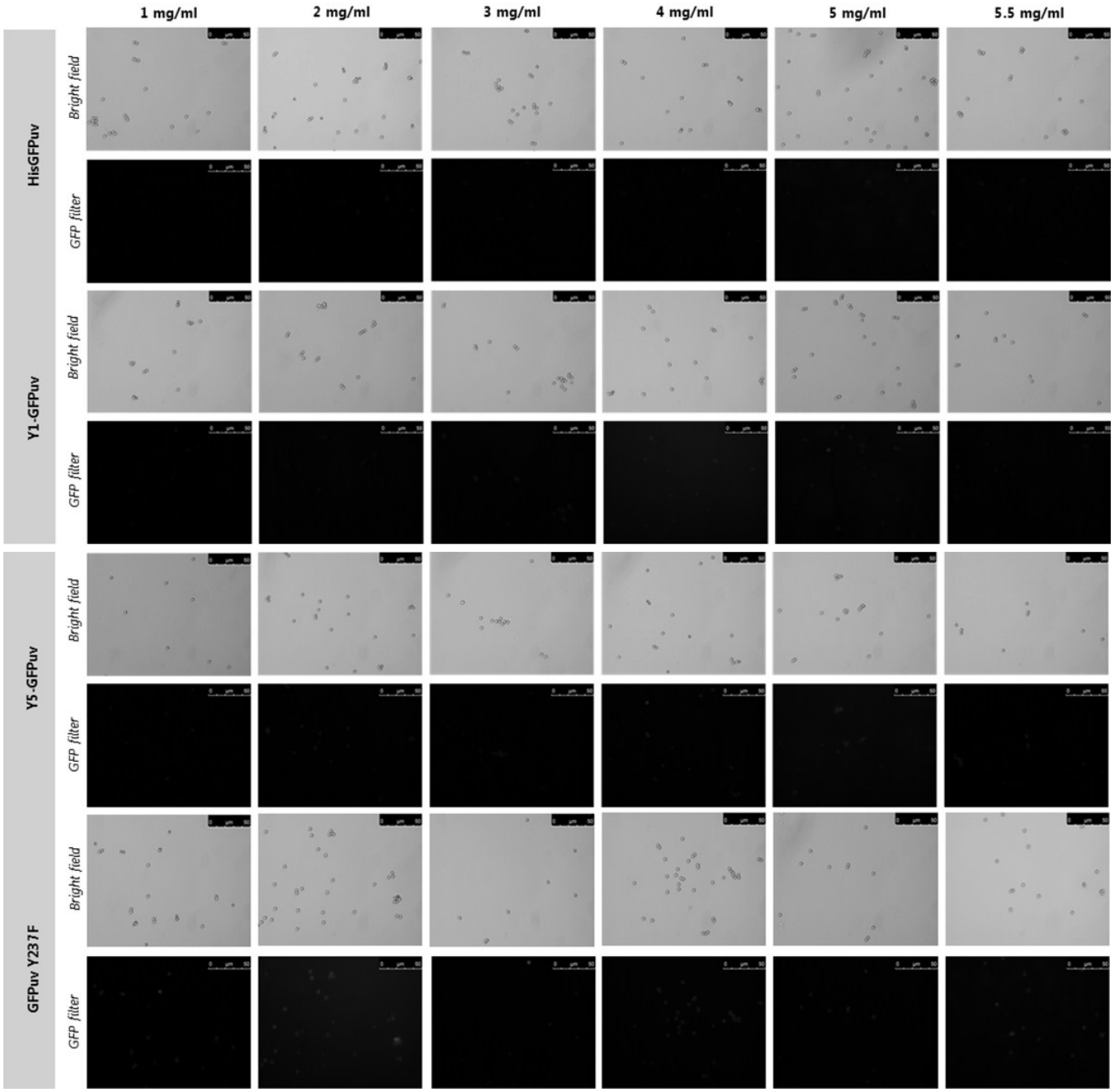
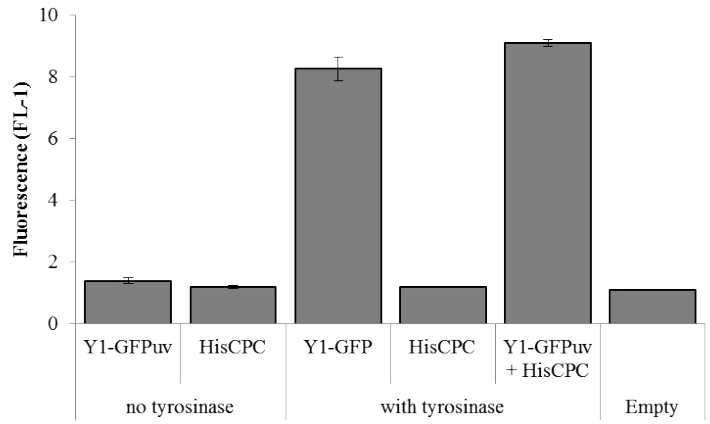


Figure S8 Fluorescence microscopy of the microparticles functionalized at different GFPuv concentrations in the presence (A) or absence (B) of tyrosinase.

(A)



(B)

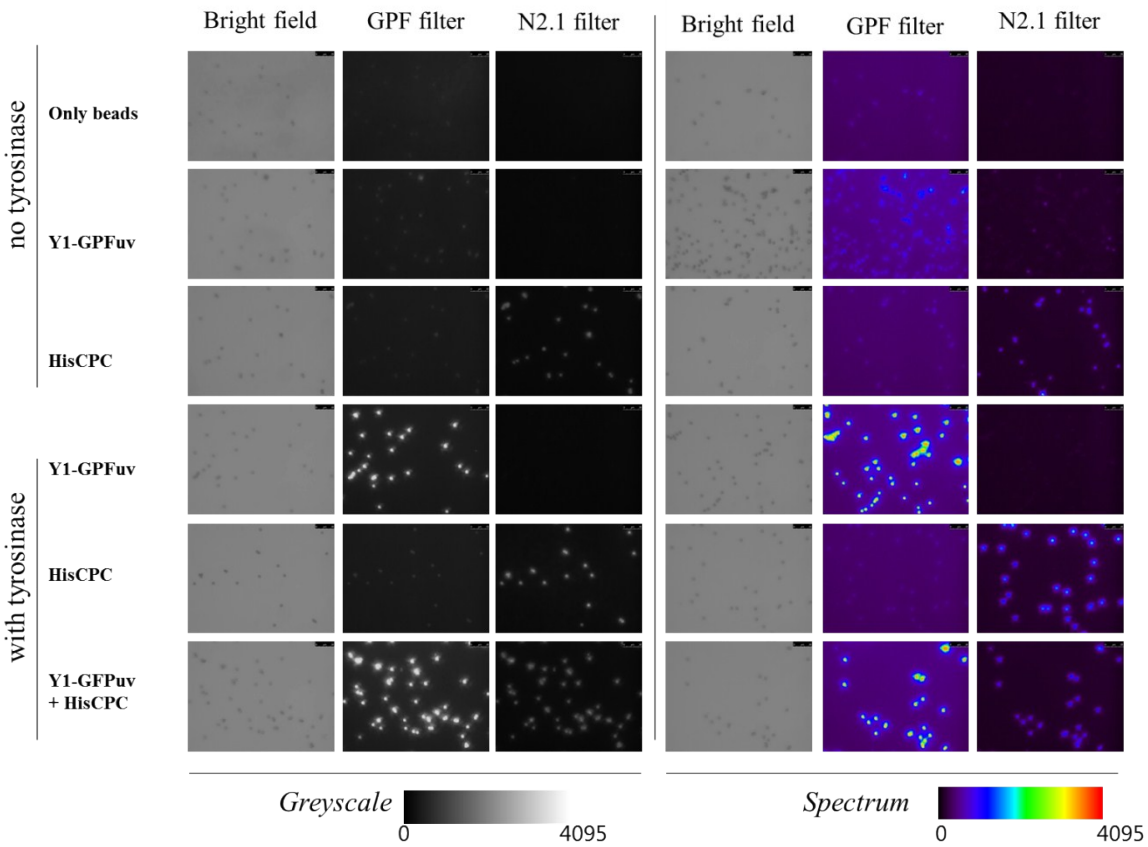


Figure S9 (A) Flow cytometric analysis of the green fluorescence of microparticles incubated with Y1-GFP and HisCPC alone, and in the presence of tyrosinase, also in together. Please note that the analysis can detect only green fluorescence. (B) Fluorescence microscopy of the functionalized microparticles. Fluorescence intensity is shown in a greyscale and as a spectrum scale.

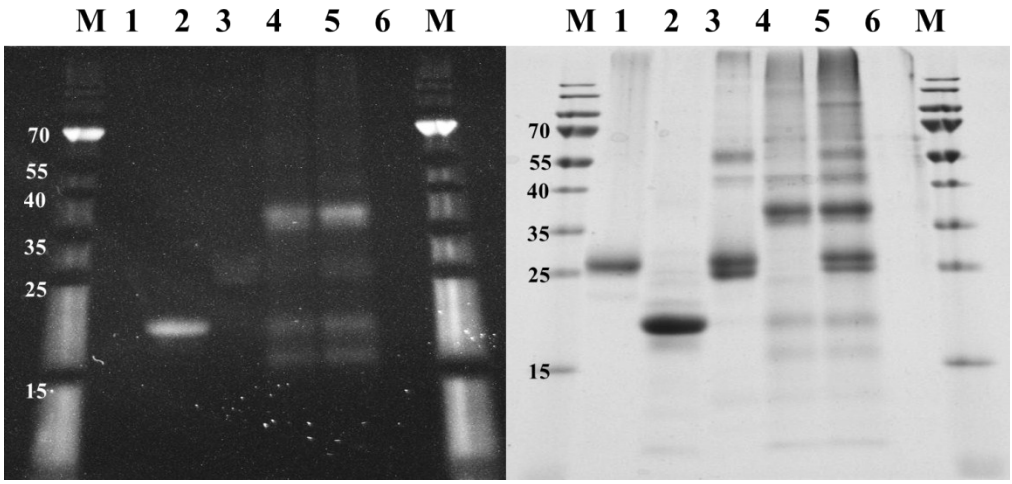


Figure S10 SDS PAGE analysis of the immobilization mixture from the co-immobilization of Y1-GFP and HisCPC with tyrosinase visualized by zinc (left) and Coomassie staining (right). At the end of the immobilization procedure, the microparticles were removed and 4 µl of the solution containing only Y1-GFPuv (lane 1), only HisCPC (lane 2), Y1-GFPuv and tyrosinase (lane 3), HisCPC and tyrosinase (lane 4), Y1-GFPuv and HisCPC and tyrosinase (lane 5), and buffer only, were analysed. Molecular weight markers are reported in kDa.