SUPPORTING INFORMATION

Self-Decorating Yeast *Via* Surface-Initiated Enzymatic Controlled Radical Polymerizations

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Materials and methods

Materials

The macromonomers were methyl ether-terminated $PEGMA_{2000}$ (Polysciences) and azide-terminated $PEGMA_{1000}$-N_3 (Abbexa). NHS-Carboxyfluorescein, Bicinchonic Acid Assay kit were from Thermo Fisher Scientific. c-myc solution was purchased from Milteni. Zymolyase was purchased from Gerbu. Alkaline phosphatase (Fluka) was a gift from Prof. Fessner (TU Darmstadt). TAMRA-NHS was donated by Prof. Hausch (TU Darmstadt). All the other compounds and solutions were purchased from Sigma Aldrich and used as received.
PBS (phosphate-buffered saline): NaCl: 137 mM KCl: 2.7 mM Na_2HPO_4: 10 mM KH_2PO_4: 1.8 mM. Tris-HCl: 0.1 M.

Yeast strain engineering and characterization

S. cerevisiae cells (EBY100) were grown overnight to a stationary phase in YPD media on a platform shaker at 225 rpm and 30 °C. The next morning, an aliquot of the overnight culture was inoculated into YPD media and grown until the optical density at 600 nm (OD_{600}) was approximately 1.6. The yeast cells were collected by centrifugation, the media removed, and the cells washed twice with ice-cold water and once with electroporation buffer (100 mM Tris-HCl, 10 mM EDTA).
The yeast cells were conditioned by re-suspending in Lithium acetate / dithiothreitol, and shaking at 225 rpm for 30 minutes at 30 °C. The conditioned cells were collected by centrifugation, washed once with electroporation buffer, and then resuspended in a small amount of the buffer.
In parallel, DNA fragments for electroporation were prepared, and a mixture of digested vector backbone and DNA insert (wild-type HRP) combined, as described by Lipovšek *et al*.[1] This was gently mixed with the electrocompetent cells and transferred to a pre-chilled BioRad GenePulser cuvette, kept on ice for 5 minutes, and then electroporated. Afterwards, the cells were transferred into a sorbitol:YPD media mix and incubated on a platform shaker for an hour. The cells were collected again by centrifugation, resuspended in synthetic defined dextrose medium lacking uracil and tryptophan (SD-UT). Serially diluted cells were plated onto selective plates and incubated at 37 °C O/N.

HRP-containing yeast cells were cultivated in 0.5 L scale from precultures diluted to OD 0.3-0.5 in SD:SG (synthetic defined galactose) 1:4 media supplemented by 25 μg mL^{-1} chloramphenicol and 100 μg mL^{-1} ampicillin at 30 ˚C for 48 h. After this time, OD_{600} reached 9.5-10.5.

Fluorescamine assay

Fluorescamine is a fluorogenic compound that becomes fluorescent when reacting with primary amines and is typically used to determine degree of modification of lysines on proteins.[2] *S. cerevisiae* cells were cultured in YPD (Yeast extract, Peptone, Dextrose) medium at 30 °C with shaking at 200 rpm until the mid-exponential phase. Cells were harvested by centrifugation at 7000 x g for 3 minutes and washed twice with PBS. Fluorescamine was dissolved in DMSO to make a 10 mM stock solution. Working solutions at different micromolar concentrations were prepared by diluting the stock with PBS.
S. cerevisiae cells were resuspended in PBS to various OD_{600}. Aliquots of the cell suspensions were then mixed with different concentrations of the fluorescamine working solution. The mixtures were incubated in the dark at room temperature for 10 minutes, allowing fluorescamine to react with primary amines on the surface of the cells. The fluorescence intensity was measured with an excitation wavelength of 390 nm and emission wavelength of 475 nm using a Clariostar Plus microplate reader (BMG Labtech), 200 µL per well (transparent Greiner flat-bottomed wells), in triplicate. The data obtained was used to create a calibration curve of fluorescence intensity as a function of fluorescamine concentration/ OD_{600} ratio. Cells were resuspended in 1 mL PBS to OD_{600} 6. The cell suspensions were then incubated with either 50 µL of either NHS-BiB or NHS-CPPA (20 mM) for 1 hour at 37 °C. After incubation, cells were washed twice with PBS to remove excess NHS derivatives, resuspended in 1 mL PBS buffer and were then subjected to reaction with 0.4 µmol fluorescamine as described, since it was the point where the calibration curve started to plateau (i.e., all available primary amines were reacted). For each sample, the fluorescence intensity before and after reaction with NHS derivatives was compared. The decrease in fluorescence intensity of fluorescamine meant that a certain amount of amines was no longer available, due to their previous reaction with BiB-NHS or CPPA-NHS. By calculating the number of fluorescamine molecules that did not react (by fitting it to the calibration curve), the difference yielded the amount of reacted NHS-derivatives, which allowed to calculate the conjugation efficiency of BiB-NHS or CPPA-NHS.

Cell labelling via NHS-Carboxyfluorescein

NHS-Carboxyfluorescein (NHS-CF) 20 mM working solution was added to the OD_{600} 6 yeast suspension (5 µL) and the mixture was incubated in the dark at room temperature for 30 minutes. Post incubation, the cells were centrifuged at 5000 x g for 3 minutes to pellet the labeled cells. The supernatant was carefully removed, and the cell pellet was washed three times with PBS to remove unbound dye. The labeled cells were then resuspended in 1 mL of PBS for further analysis, and mixed with 10 µL of a 1 mg mL^{-1} Calcofluor White M2R solution in water.

Polymerization procedures

ATRP

S. cerevisiae was diluted with PBS to a final OD_{600} of 6, 2 mL, in a centrifuge microtube, and centrifuged with a Minispin centrifuge (Eppendorf) at 7000 rpm for 3 minutes. After a quick washing of the pellet with PBS, it was resuspended in 950 µL of more alkaline PBS (pH 8.4). Then, 50 µL of a 20 mM solution of BiB-NHS in DMSO were added, and the mixture was shaken at 37 °C, 800 rpm, for 1 hour, on a MS-100 shaker (ThermoFisher). The sample was then centrifuged and washed washed like described above ,

resuspended in 1 mL of either PBS or YPD supplemented with 100 mM NaBr, and transferred to a 10 mL Schlenk flask with the addition of 12 mg NaAsc. In a scintillation vial, 20 µmol of the monomers were dissolved in the same Br-supplemented aqueous medium (PBS or YPD) with 50 µL DMSO. The two solutions were purged with Ar for 15 minutes, then the monomer solution was transferred to the Schlenk flask with a syringe. The reaction was allowed to proceed, under Ar, for 2 hours, at 37 °C, while stirring with a magnetic stir bar at 350 rpm. The reaction was stopped by uncapping the flask and letting the stirring continue for 2 minutes.
For the 1-step procedure, the conjugation step happened directly in the reaction mixture.

RAFT polymerization
Similarly to ATRP, *S. cerevisiae* was diluted with PBS to a final OD_{600} of 6, 2 mL, in a centrifuge microtube, and centrifuged with a Minispin centrifuge (Eppendorf), at 7000 rpm for 3 minutes. After a quick washing of the pellet with PBS, it was resuspended in 950 µL of more alkaline PBS (pH 8.4). Then, 50 µL of a 20 mM solution of CPPA-NHS in DMSO were added, and the mixture was shaken at 37 °C, 800 rpm, for 1 hour. The sample was then centrifuged and washed like described above, resuspended in 1 mL of either PBS or YPD, and transferred to a 10 mL Schlenk flask. In a scintillation vial, 20 µmol of the monomers (e.g. 40 mg for $PEGMA_{2000}$) were dissolved in the same aqueous medium (PBS or YPD) with 50 µL DMSO. The two solutions were purged with Ar for 15 minutes, then the monomer solution was transferred to the Schlenk flask with a purged syringe, with the further addition of 2.5 µL H_2O_2 0.03 vol% and 4.11 µL acetylacetone (ACAC) 1 vol%.[3] The reaction was allowed to proceed, under Ar, for 2 hours, at 37 °C while stirring with a magnetic stir bar at 350 rpm. The reaction was stopped by uncapping the flask and letting the stirring continue for 2 minutes.
For the 1-step procedure, the conjugation step happened directly in the reaction mixture.

NIPAM polymerization
The same initial procedure for ATRP of PPEGMA was used. NIPAM (200 µmol in YPD) was added instead of PPEGMA, and the polymerization was performed at 25 °C, in order to keep the temperature below the LCST of PNIPAM.

Post-polymerization workup
After the reaction, 500 µL of the mixture were incubated for 2 hours with an equal volume of NaOH 10 wt% in order to detach the polymers from the cells and to keep the monomers in solution. The solution was then used to quantify the monomer conversion via ^{1}H NMR spectroscopy. Another 500 µL of the reaction mixture were centrifuged (see above), washed and resuspended in 500 µL MilliQ water, and incubated with NaOH. After incubation, the sample was dialysed in a 3.5 kDa MWCO dialysis tube (Millipore) overnight against 800 mL DI water for GPC analysis. The remaining mixture was centrifuged, washed, and resuspended to OD_{600} 6 in Tris-HCl 0.1 M pH 7.4, for further analysis. If no NMR or GPC analysis were to be performed, the whole sample was resuspended to OD_{600} 6 in Tris-HCl.

Two-round polymerization
After the first polymerization, yeast was inoculated at OD 0.1 in SG, to ensure the expression of HRP. It was grown for 36 h at 30 °C, then harvested, resuspended at OD 6, and treated as above.

Estimation of HRP concentration

A previous study documented that each cell, when using our surface-display system, exhibited 1000 copies of HRP on its surface.[4] Taking into account an equivalence of $3x10^7$ cells for every milliliter per optical density unit,[5] it can be extrapolated that for an OD of 6, there are 1.8×10^8 cells present in every milliliter. From our data, only 40% of these cells are estimated to express HRP. Hence, 40% of the 1.8×10^8 cells, which equals 7.2×10^7 cells, will have HRP on their surface. Given that each of these cells has on average 1000 copies of HRP, the number of HRP molecules in an OD 6 solution would be 7.2×10^{10} HRP molecules mL^{-1}. The molecular weight of HRP is 44000 g mol^{-1}. Using Avogadro's number, this results in a weight of 7.3×10^{-17} g for each HRP molecule.
To find the total weight in grams for 7.2×10^{10} HRP molecules, it is multiplied by the number of molecules, which equals 5.26 µg/mL. HRP type VI (Sigma Aldrich) was used as the standard. However, it must be remarked that using commercial HRP as a standard for HRP activity introduces a bias because of the different glycosylation and overall enzyme activity of the yeast HRP, so this calculation must be considered a rough estimate.

Nuclear Magnetic Resonance (NMR) analysis
^1H NMR spectroscopy was conducted on a Varian Unity 300 MHz spectrometer operating at 300 MHz. 200 µL of a sample were diluted in 600 µL D_2O, and 10 µL of DMF 1% in water were added as reference. The ratio between the integral of one vinyl proton of the monomer (6.75–6.25 ppm) with one methylene proton of the backbone (0.8-1.0 ppm) was used to calculate the monomer conversion according to a published protocol.[6]

Gel Permeation Chromatography (GPC) analysis
The GPC analysis was conducted with water as an eluent on a PSS liquid chromatography system equipped with a PSS G1362A refractive index detector (λ = 633 nm) and using 1 guard column and 2 identical PSS Suprema Linear M columns (5 µm bead size, hydroxylated methacrylate-based bed) in series operating at 25 °C. Water with 0.1 M $NaNO_3$ and 0.05% w/v NaN_3 was employed as the mobile phase at a flow rate of 1 $mL \cdot min^{-1}$. The system was calibrated using pullulan standards (180 to 708000 Da). All samples were filtered through 0.45 µm Teflon filters prior to injection.

UV-Vis assays
All UV-Vis assays were performed on a Clariostar Plus microplate reader (BMG Labtech), using Greiner transparent 96-well plates (24-well plates for yeast growth curves) with flat bottom. Absorbance measurements were adjusted to correct for a 1 cm pathlength (ClarioStar software). The plate was thoroughly shaken (500 rpm, orbital shaking, 20 s) before each reading.

Peroxidase assay
TMB (3,3',5,5'-tetramethylbenzidine) One, including H_2O_2, was used as provided by the manufacturer. In a microplate, aliquots of the yeast cell suspension were mixed with TMB One Solution at a 1:6 volume ratio to achieve a final volume of 120 µL per well. The plate was incubated at room temperature for 15 minutes in the dark. During this time, the peroxidase-like activity of viable cells converted the TMB substrate into a blue-colored product, which could be turned into a yellow-coloured product after acidification with 80 µL H_2SO_4 1 M. Both the OD_{600} and absorbance at 450 nm were measured. The TMB/ OD_{600} ratio indicated the activity normalized to the cell density.

Viability assay

Fluorescein diacetate (FDA) was dissolved in acetone to create a 5 mM stock solution. Prior to the assay, an FDA working solution was prepared by diluting the stock solution with PBS to a final concentration of 500 µM. In a microplate, aliquots of the yeast cell suspension were mixed with the FDA working solution at a 20:1 volume ratio (final volume: 200 µL) and incubated at RT for 10 minutes in the dark. During this time, viable cells hydrolyzed FDA into fluorescein. After incubation, the optical density of the yeast, i.e. OD_{600} was measured as well. Immediately thereafter, fluorescence was measured using a plate reader with an excitation wavelength of 485 nm and an emission wavelength of 538 nm. The fluorescence intensity of each sample was normalized to its respective OD_{600}. The FDA/OD_{600} of the cells post polymerization was compared to that of fresh cells not subject to the reaction, but incubated for 2 hours in the same reaction medium.

Bicinchonic acid assay and enzyme conjugation efficiency

After conjugation of enzymes, or polymerization in their presence, the supernatants after centrifugation were recovered in order to quantify the amount of protein left in solution, using the Bicinchonic Acid Assay kit (BCA). The kit was used according to the supplier's instruction, diluting the protein standards with YPD instead of aqueous buffer. The resulting total mass of enzyme in the supernatant was subtracted from the mass added for the reaction, yielding how much enzyme was conjugated to yeast and thus, the conjugation efficiency.

OD_{600} assays

Yeast, before or after polymerization, was diluted to a final OD_{600} 0.5 in 2 mL YPD, and grown at 30 °C for 24 h in the microplate reader with shaking in a 24-well plate. Then, OD_{600} was measured again with the plate reader.

For zymolyase, YPD was substituted with lysis buffer (NaCl 100 mM, Tris HCl 10 mM, EDTA 1 mM, pH 7.5) and with the addition of 5 mg zymolyase per well. Due to the decrease in OD as zymolyase digestss the cell wall, the absolute values ended up being higher than the blank (zymolyase suspension alone), thus the relative decrease was plotted instead.

For both tannic acid, 20 µL of yeast from the reaction were diluted in 200 µL PBS, in a 24-well plate and were measured the same way.

β-gal/ALP enzymatic assays

For β-galactosidase (β-gal), o-nitrophenyl-β-D-galactopyranoside (ONPG) was dissolved in PBS (10 mg mL^{-1}). In a microplate, aliquots of the yeast cell suspension were mixed with ONPG working solution at a 20:1 volume ratio to achieve a final volume of 200 µL per well. The plate was incubated at room temperature for 10 minutes in the dark. During this time, β-galactosidase within viable cells hydrolyzed ONPG into the yellow o-nitrophenol. After incubation, both absorbance at 405 nm and OD_{600} were measured and their ratio calculated. The highest value was set to 100% and the other values were normalized accordingly.

The same procedure was performed for alkaline phosphatase (ALP), using *p*-nitrophenyl phosphate (PNPP) instead.

Further assays for β-gal

For the lactose growth assay, β-gal-conjugated yeast was diluted in Yeast Peptone 1% Lactose (YPL) at a final OD_{600} of 0.05 and incubated for 24 h at 30 °C. Then, OD_{600} was measured again. The values were normalized between 100-0%, 100% being the highest value well.

For the survival in presence of octyl β-D-glucopyranoside (OG), yeast was incubated at OD_{600} 0.2 in YPD with 25 mM OG, and the values were treated as above. The higher starting OD was in order to counter the quick toxic action of OG micelles.

Transmission electron microscopy (TEM)

The yeast cells (**P9**) were concentrated by centrifugation and fixed with a 2.5 % glutaraldehyde, 2 % formaldehyde mixture in cacodylate-buffer (pH 7.4). After being washed in buffer, samples were postfixed in OsO_4 (1 % in the same buffer), dehydrated in a graded acetone series, and embedded in Spurr's medium.[7]

Ultrathin sections were obtained with diamond knives, post-stained with uranyl acetate, and examined with a Zeiss EM 109 transmission electron microscope. NPs were dilute 1:10 and measured with a JEOL JEM 2100F TEM.

Confocal laser scanning microscopy (CLSM)

For microscopic visualization, a 20 µL aliquot of the labeled cell suspension was placed onto a round glass microscope slide and covered with a round coverslip. Imaging was performed on a Leica SP8 CLSM, equipped with an HCX PL APO 63 × NA 1.2 W CORR CS2 objective (Calcofluor White: ex. 405 nm, em. 410 – 430 nm; Fluorescein and derivatives: ex. 488 nm, em. 505 – 525 nm; Rhodamine B and TAMRA: ex. 561 nm, em. 570 – 590 nm; Cy5: ex. 635 nm, em. 660 – 690 nm). Images were optimized (brightness and contrast; applied evenly throughout a whole image) and analysed via ImageJ.

Concanavalin A labelling

10 µl Concanavalin A (20 mg mL^{-1} in PBS pH 8.4) was first mixed with TAMRA-NHS (1 mg mL^{-1}, 10 µL) and reacted overnight. Then, it was purified via a 25 kDa MWCO spin filter tube (Millipore), centrifugating at 12000 x g, 10 min. The protein was then resuspended in 1 mL PBS pH 7.4 and stored at 4 °C.

20 µL of this protein solution were incubated with 1 mL of yeast suspension (OD 1) for 20 minutes, then centrifuged and washed 3 times in order to remove all unbound TAMRA. The cells were imaged as described. Fluorescence intensity (normalized over OD_{600}) was measured using a plate reader with an excitation wavelength of 552 nm and an emission wavelength of 578 nm.

Flow cytometry

Cytometric analysis of *S. cerevisiae* was performed on a CytoFLEX S by Beckman Coulter (CytExpert Version 2.4.0.28).

Characterization of HRP expression

10^7 cells were mixed with 50 µL of c-myc solution in PBS pH 7.2 (1:50 dilution) and kept on ice for 15 min. After this time, the cells were washed twice with 700 µL of PBS and resuspended in 200 µL of PBS for flow cytometry (see below).

General measurement procedure

Samples were diluted with PBS (until abort rate <10%) and events were detected with the primary threshold/trigger level (automatic) and width set to FSC. 50000 events per sample were recorded.

Cy5 fluorescence was measured in the APC channel (660/10) at 400 gain. For analysis, a yeast cell gate (SSC-A [log] vs FSC-A [log]) and a single cell gate (FSC-A [log] vs FSC-Width [linear]) were applied.

ζ-potential of cells

50 µl of the sample were diluted into 1 ml Tris-HCl (pH 7). Then, the ζ-potential was measured in a capillary flow cuvette with a Zetasizer Nano (Malvern). 3 repeated measurements were taken for all samples.

Polymer click chemistry conjugation

Cy5 conjugation

Post-polymerization, a 200 µL aliquot of yeast (pre-incubated with FDA) was incubated with 20 µL Cy5-dibenzocyclooctine (Cy5-DBCO) 1 mM in DMSO, and incubated for 1 hour at RT. It was then centrifuged (5000 x g for 3 minutes) and washed carefully twice. The same procedure was performed for the controls.

Pre-conjugation with Cy5

20 µmol of PEGMA-N_3 (1000 Da) were dissolved in 1 mL YPD. 200 µL of Cy5-DBCO 1 mM was added and the reaction mixture incubated for 1 hour at RT. The 100:1 polymer:dye ratio ensured an efficient conjugation, and all Cy5 could be assumed to be linked to PEGMA. The monomer was immediately employed in polymerizations without further purification.

Conjugation of enzymes

β-gal (10 mg mL^{-1} in 1 mL PBS pH 8.4) was conjugated with a 5x molar excess of dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester (NHS-DBCO) and left to react overnight at 4 °C. Afterwards, the unreacted small molecule was removed via 10 kDa spin diafiltration device (Amicon, Merck).
At first, the purified 10 mg of DBCO-enzyme (β-gal or ALP, 10 mg mL^{-1}) were directly resuspended in 1 mL yeast with PPEGMA-N_3, reacted for 1 hour and then purified via centrifugation and washing. In another procedure, the enzyme was instead subsequently reacted overnight at 4°C with 20 mg PEGMA-N_3 in solution (1 mL YPD). The polymer-enzyme mixture was then directly used for ATRP as described above. Controls were naked yeast incubated with enzyme-DBCO and PPEGMA-N_3 incubated with DBCO-less enzyme.

Metal nanoparticle synthesis

Synthesis

In a 24-well plate, 1 mL of yeast culture (OD 3) was added to 20 µL of $AgNO_3$ 100 mM or PdAcetate 20 mM and incubated in the dark for 24 h at RT. The cells were recovered, centrifuged and washed 5 times with PBS. The supernatant was then used to quantify size, concentration and surface potential of the resulting NPs. The cells were recovered to measure their viability. Naked yeast was also incubated with the same concentration of NaAsc and then washed before incubation with the metal salts, to ensure that the differences in NP production weren't only due to the difference redox potential of the cells.

Nanoparticle tracking analysis (NTA)

NTA allows to measure size and concentration of metallic nanoparticles. It was performed on a Zetaview Mono (Particle Metrix) equipped with a 520 nm laser, at 1:1000 dilution in MilliQ water. ζ-potential of the nanoparticles was also measured via NTA, using default settings.

Tannic acid clustering assay

At OD 3, 0.5 mL yeast (pre-incubated with FDA) was mixed with 50 µL of 100 mg mL^{-1} tannic acid (TA) and then imaged. A subsequent centrifugation and 3x washing with PBS pH 8.5 removed enough TA to reverse the clustering.

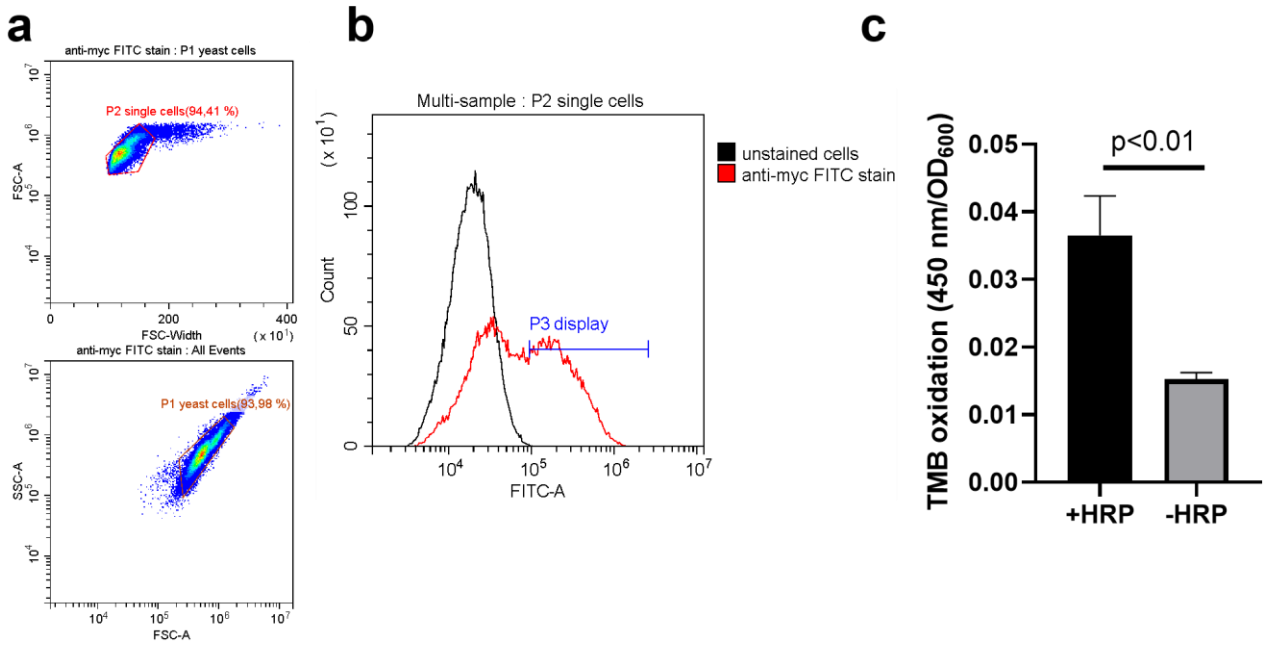
Calcium glycerophosphate biomineralization

At OD 3, 0.5 mL ALP-conjugated yeast (pre-incubated with FDA) was mixed with 20 µL calcium glycerophosphate (CaGP) 100 mM and 10 µL rhodamine B 1 mM, then incubated under orbital shaking at RT, 1 h. The samples were then imaged via CLSM.

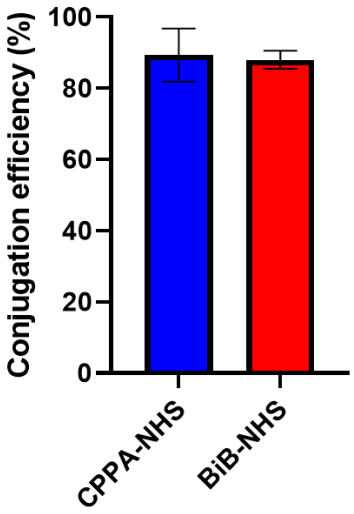
Statistical analyses

Multiple comparison two-way ANOVA with Tukey correction was used to compare the replicates, using Graphpad Prism 9.

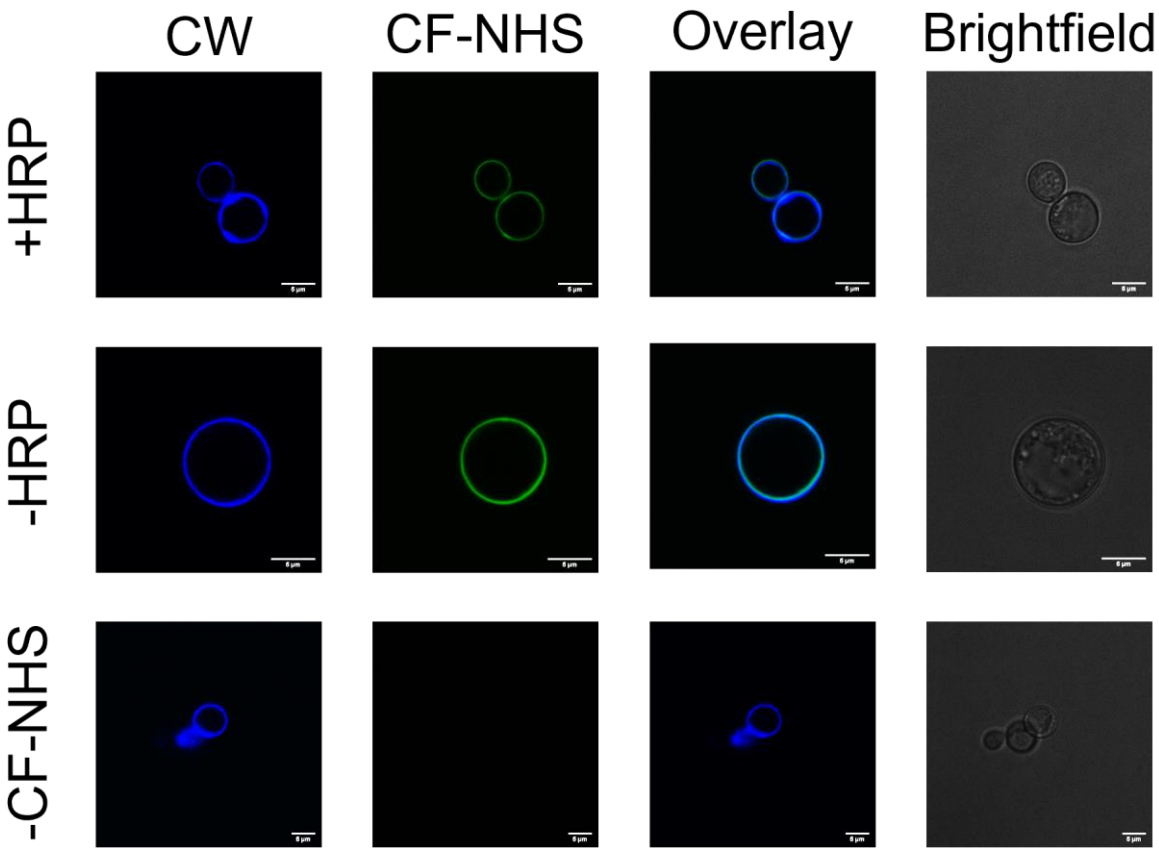
SUPPORTING DATA



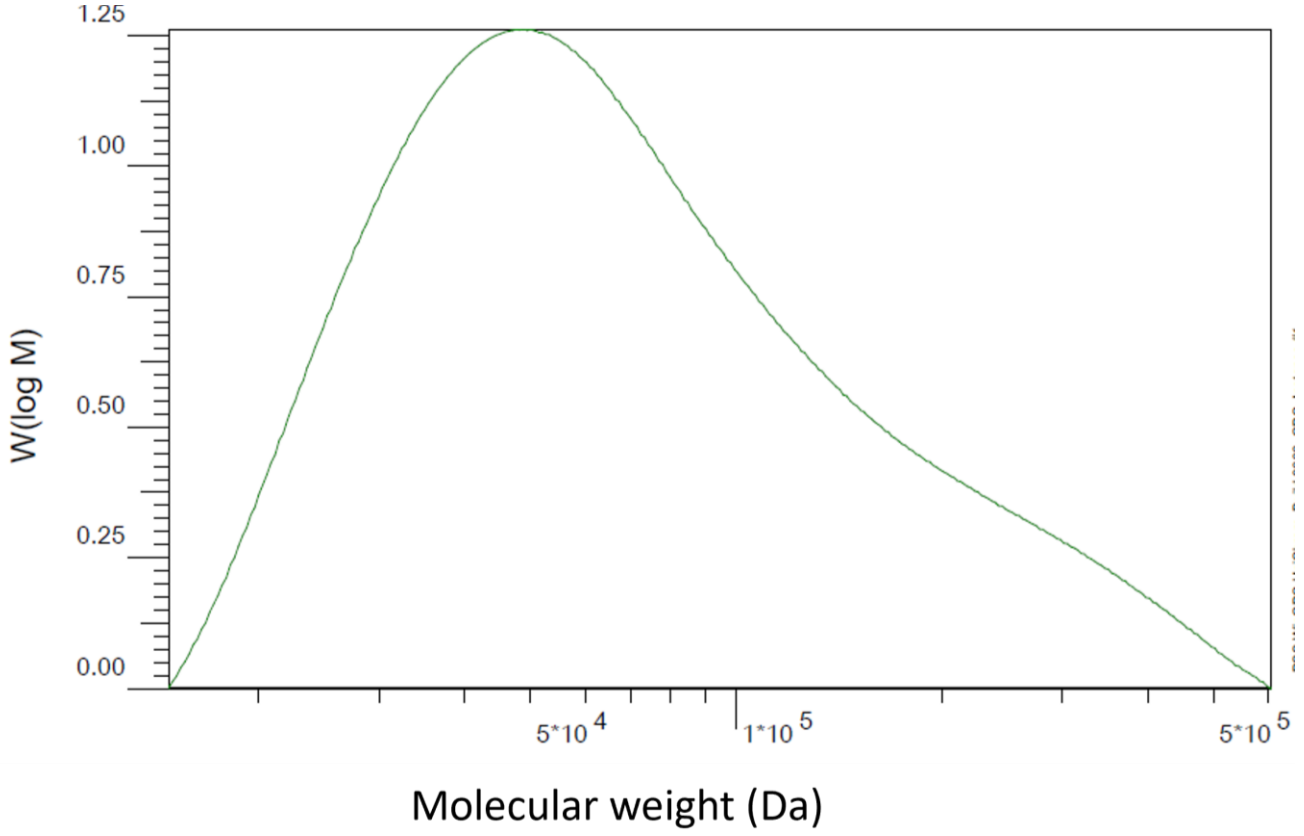
SI Figure 1. Flow cytometry results of the expression of HRP on the surface of *S. cerevisiae* (EBY100). (a) Scatter plots and gating (FSC and SSC) startegy. (b) Flow cytometry histograms of stained and unstained cells showing the portion of cells displaying HRP. P3 display: display of HRP. (c) TMB oxidation assay for cells with and without HRP expression. Mean values, error bars displayed as SD, n=3 replicates.



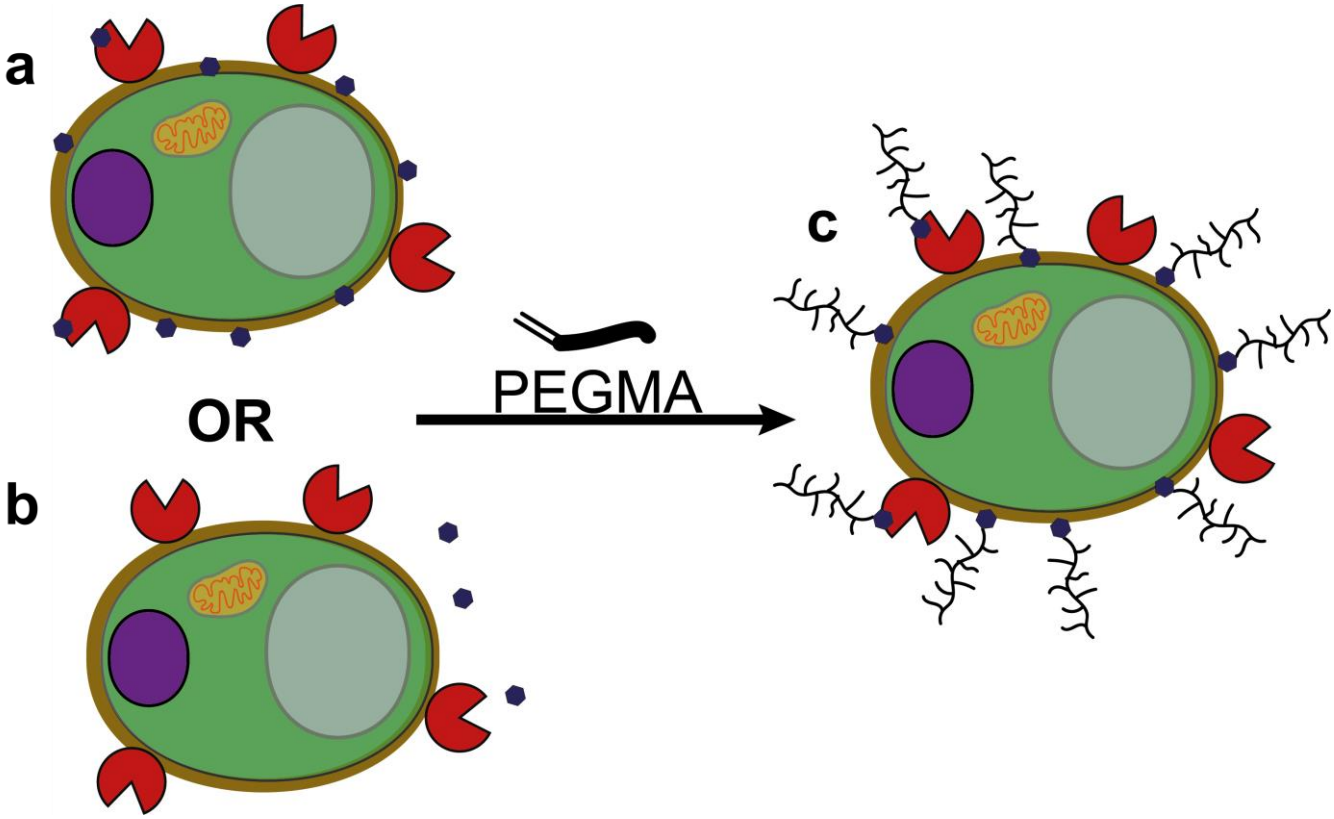
SI Figure 2. Conjugation efficiency of CPPA-NHS and BiB-NHS to the surface of yeast cells. The fluorescamine assay was used to determine the amount of unoccupied primary amines on yeast cells after their reaction with the CPPA-NHS or BiB-NHS, which alows to infer their conjugation efficiency. Mean values of n=3 replicates. Error bars displayed as SD.



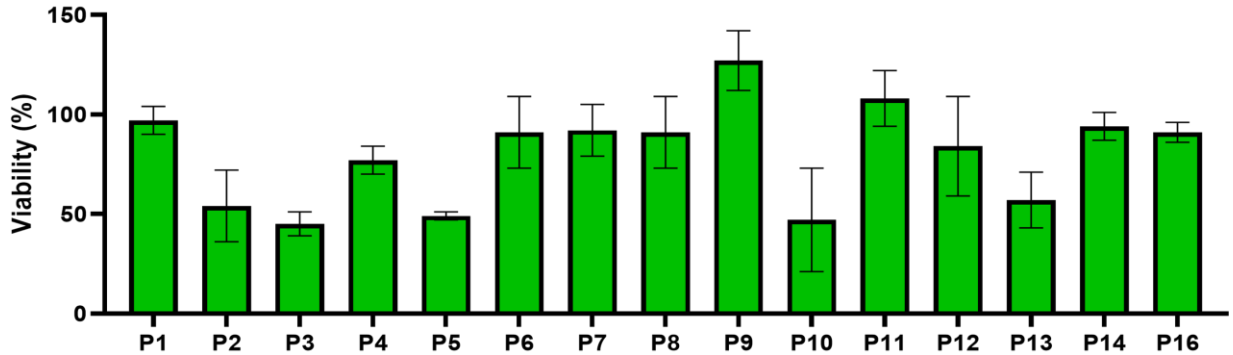
SI Figure 3. CLSM micrographs of CF-NHS-conjugated yeast, with the cell wall stain Calcofluor White as counterstain. Scalebars: 5 µm.



SI Figure 4. Representative GPC trace of polymerized PPEGMA (**P9**).

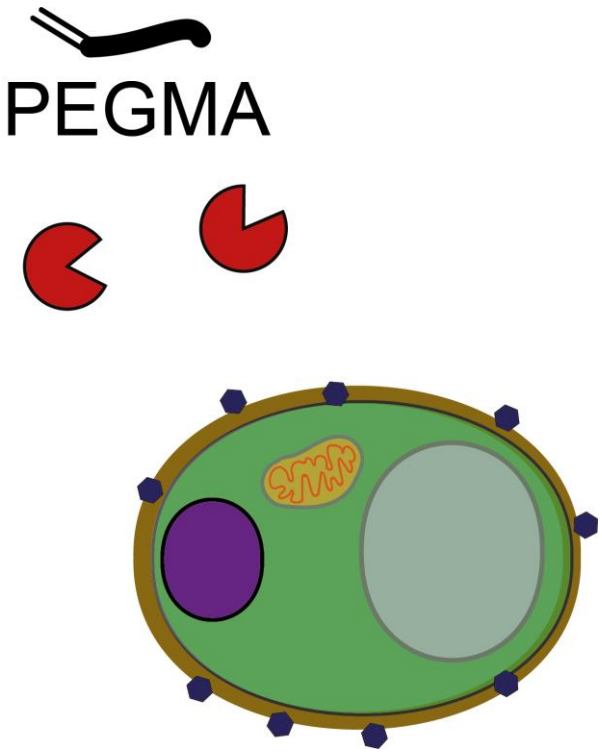


SI Figure 5. Strategies for the enzymatic polymerizations on yeast cell surfaces. (a) Two-step protocol: first BiB/CPPA-NHS conjugation, then polymerization. (b) One-step protocol: Simultaneous polymerization and conjugation. (Blue hexagons represent BIB and CPPA.)

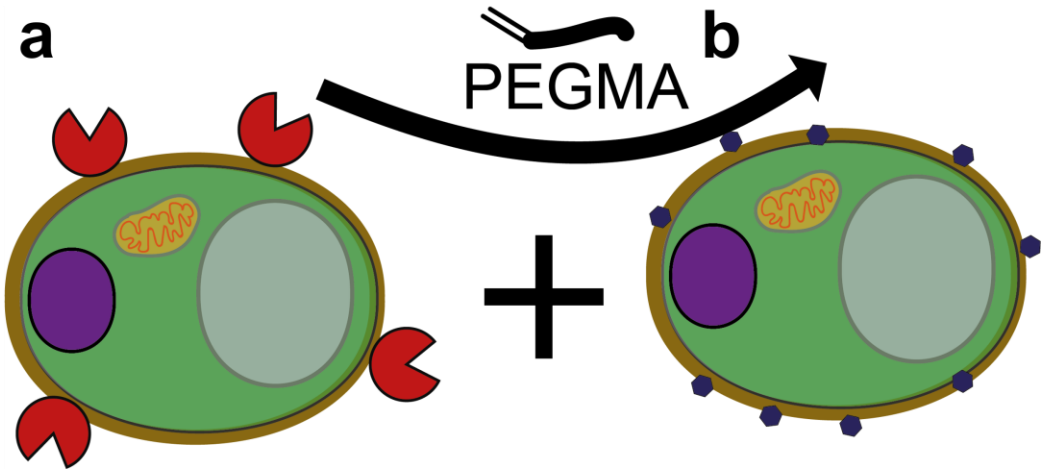


	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P16
HRP expression	-	+	+	+	+	-	-	+/-	+	+	+	+	+	+	+
ATRP/ RAFT	-	ATRP	RAFT	ATRP	RAFT	ATRP	ATRP	ATRP	ATRP	RAFT	ATRP	ATRP	ATRP	ATRP	ATRP
Initiator/CTA	-	+	+	+	+	+	+	-/+	+	+	+	+	+	+	+
Medium	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	YPD	YPD	PBS	YPD	YPD	YPD	YPD
Monomer conversion (%)	7	75	45	74	57	23	26	22	94	81	93	97	50	93	50

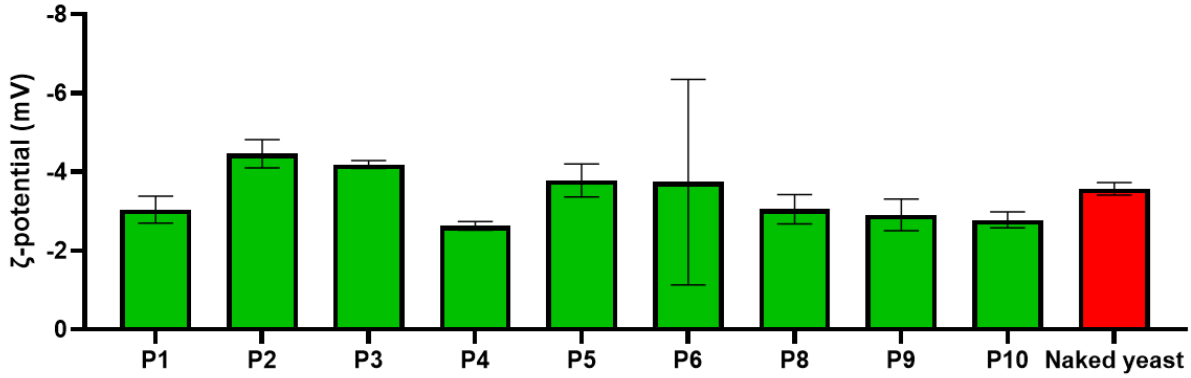
SI Figure 6. Viability (measured as FDA/OD_{600}) of several yeast types after polymerization of PPEGMA, and the conditions of the polymerizations. Mean values, error bars displayed as SD, n=3.



SI Figure 7. Schematic of the reaction **P7** setup. Uninduced yeast is mixed with HRP at the same expected concentration as when expressed by yeast, with conjugated initiator.

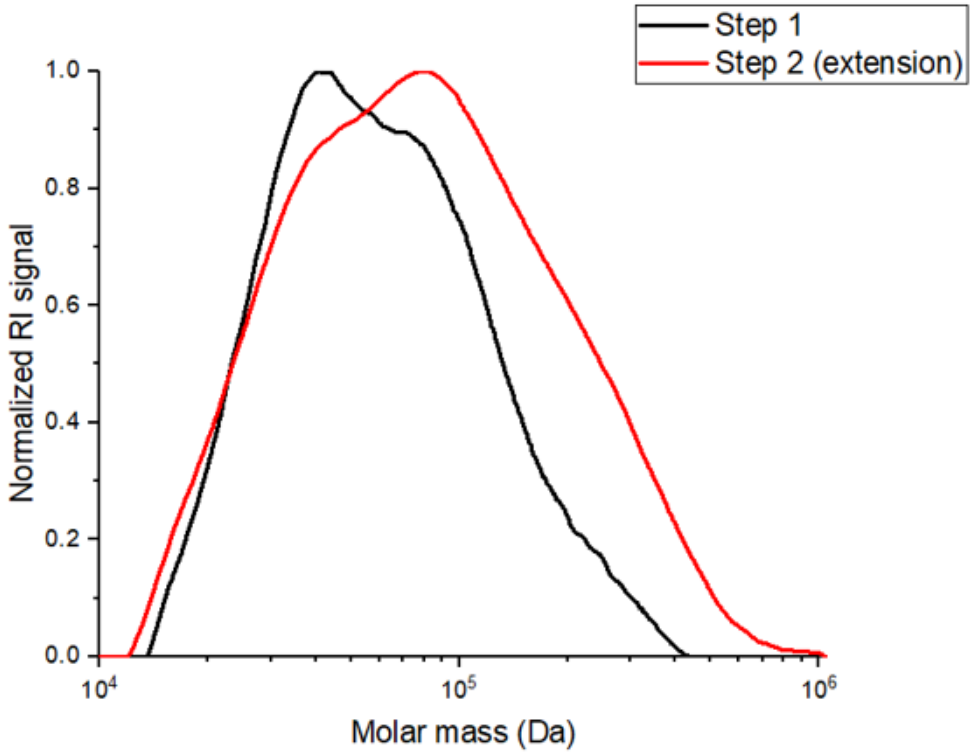


SI Figure 8. Schematic of the reaction P8. HRP-expressing yeast (a) is mixed with HRP-less yeast, but bearing the ATRP initiator (b). Without background reactivity, polymerization could only occurr between cells, which is not observed.

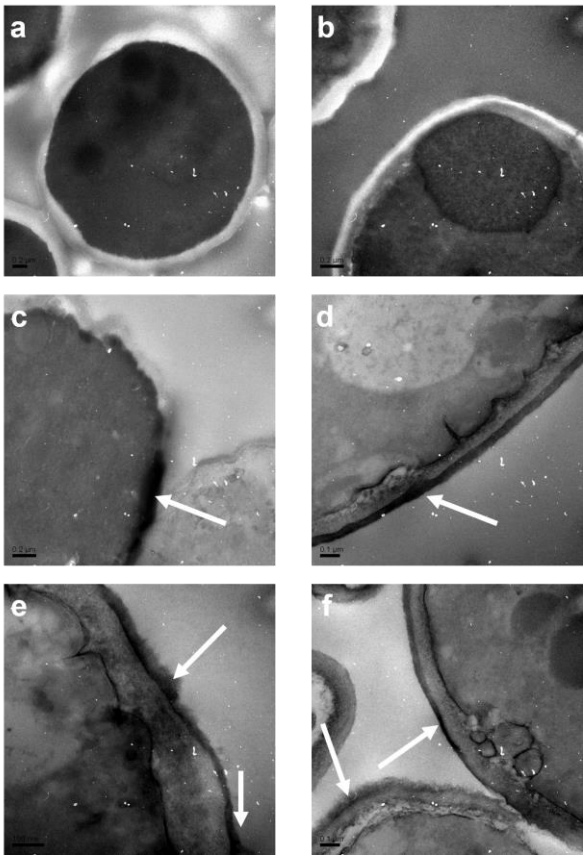


	P1	P2	P3	P4	P5	P6	P8	P9	P10	Naked yeast
HRP expression	-	+	+	+	+	-	+	+	+	/
ATRP/ RAFT	ATRP	ATRP	RAFT	ATRP	RAFT	ATRP	ATRP	ATRP	RAFT	/
Initiator/CTA	-	+	+	+	+	+	+	+	+	/
Medium	PBS	PBS	PBS	PBS	PBS	PBS	YPD	YPD	YPD	/
Monomer conversion (%)	7	75	45	74	57	23	94	95	81	/

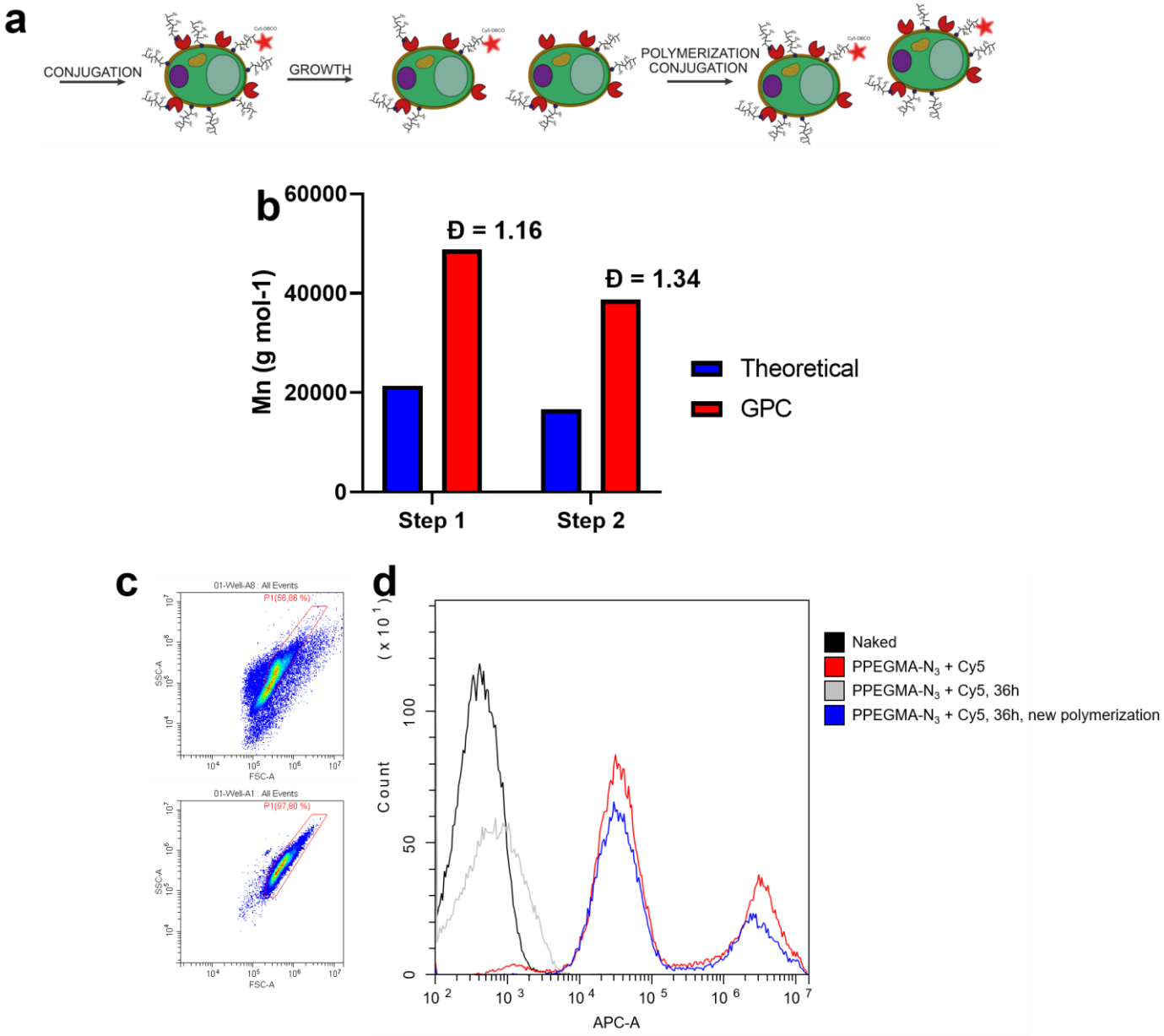
SI Figure 9. ζ-potentials of several polymer-yeast types and the reaction conditions that were used for their synthesis. Mean values, error bars displayed as SD, n=3 replicates.



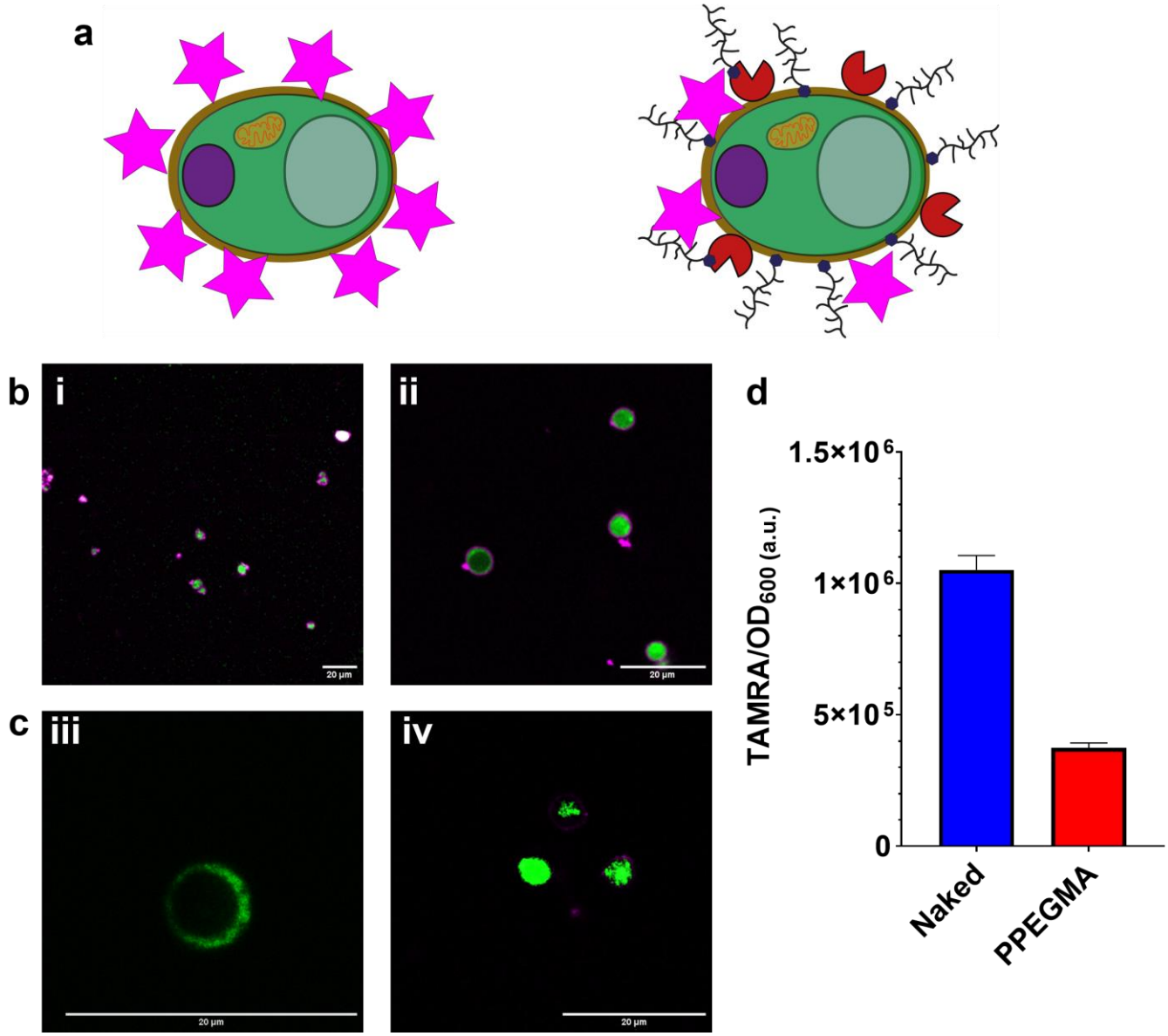
SI Figure 10. Normalized GPC trace of **P12** (step 1) and **P13** (step 2). **P13** shows a higher average molecular weight and a broader dispersion.



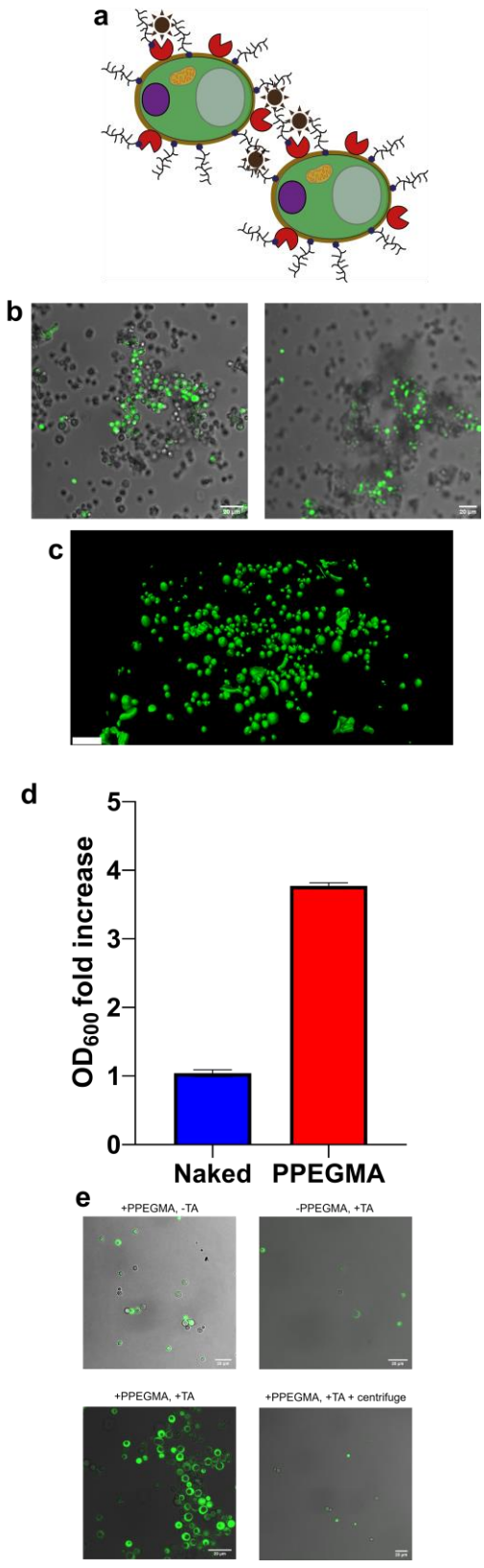
SI Figure 11. TEM micrographs of naked and PPEGMA-decorated yeast. (a, b) Naked yeast. (c-f) Yeast with polymer (**P9**). A darker layer can be observed on its surface (indicated by the arrows), which we attribute to the presence of polymer on the yeast surface.



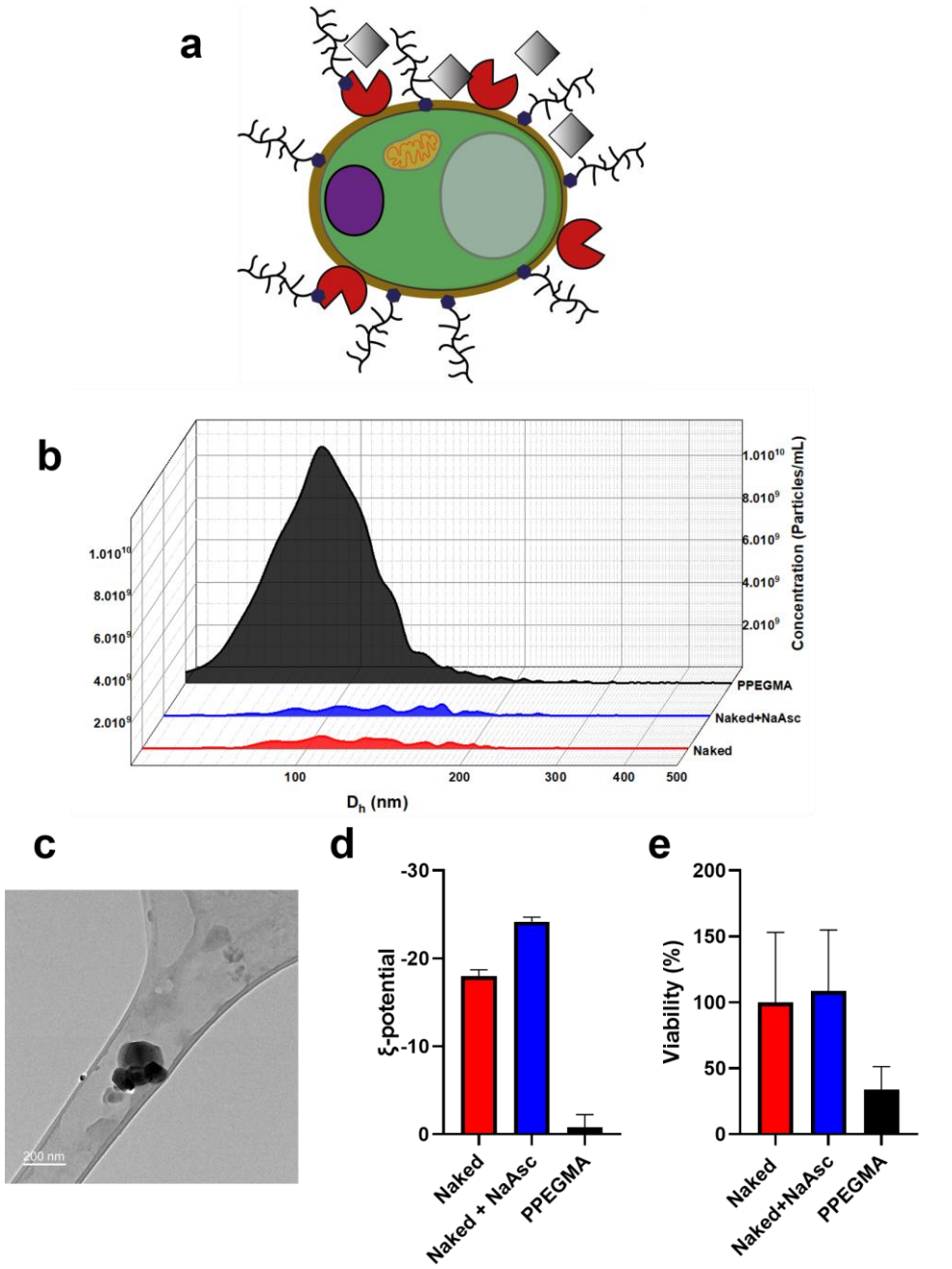
SI Figure 12. (a) Scheme for the two-round polymerization post growth, where Cy5 is used to label which cells carry the polymer. (b) Molecular mass, theoretical (from monomer conversion) and from GPC, and dispersity of **P12** and **P13**. (c) Flow cytometry scatter plots and gating (FSC and SSC) strategy of yeast cells. (d) Flow cytometry histogram of yeast before and after the conjugation to Cy5 and polymerization rounds. After dilution of the signal due to growth and loss of conjugable polymer (grey curve), the Cy5 fluorescence is restored after a new polymerization. All samples were incubated with Cy5 before being measured.



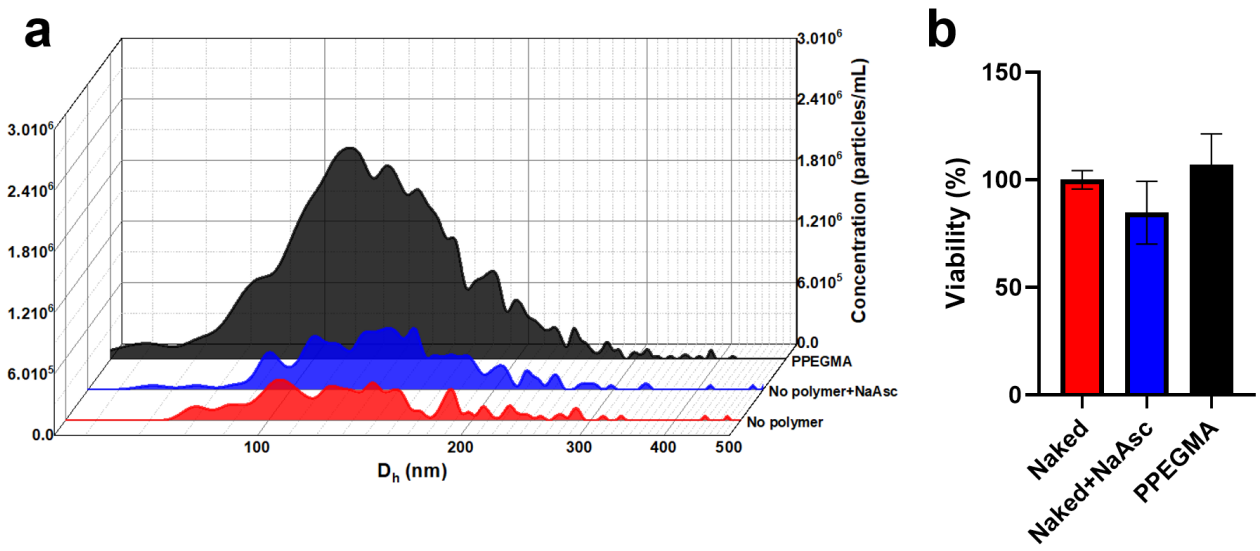
SI Figure 13. Binding of TAMRA-ConcA on naked and polymer-desocrated yeast. (a) Scheme of repulsion of ConcA (depicted as pink stars) by PPEGMA. (b) CLSM micrographs (green: FDA stain, magenta: TAMRA) of naked yeast incubated with TAMRA-ConcA. Some TAMRA-ConcA aggregates are visible. (c) P9 incubated with TAMRA-ConcA, showing no or or very limited fluorescence. (d) OD_{600}-normalized TAMRA fluorescence of naked yeast and **P9**, showing a roughly 3x higher fluorescence for naked cells. Mean values, error bars displayed as SD, n=3 replicates. Scalebars: 20 µm.



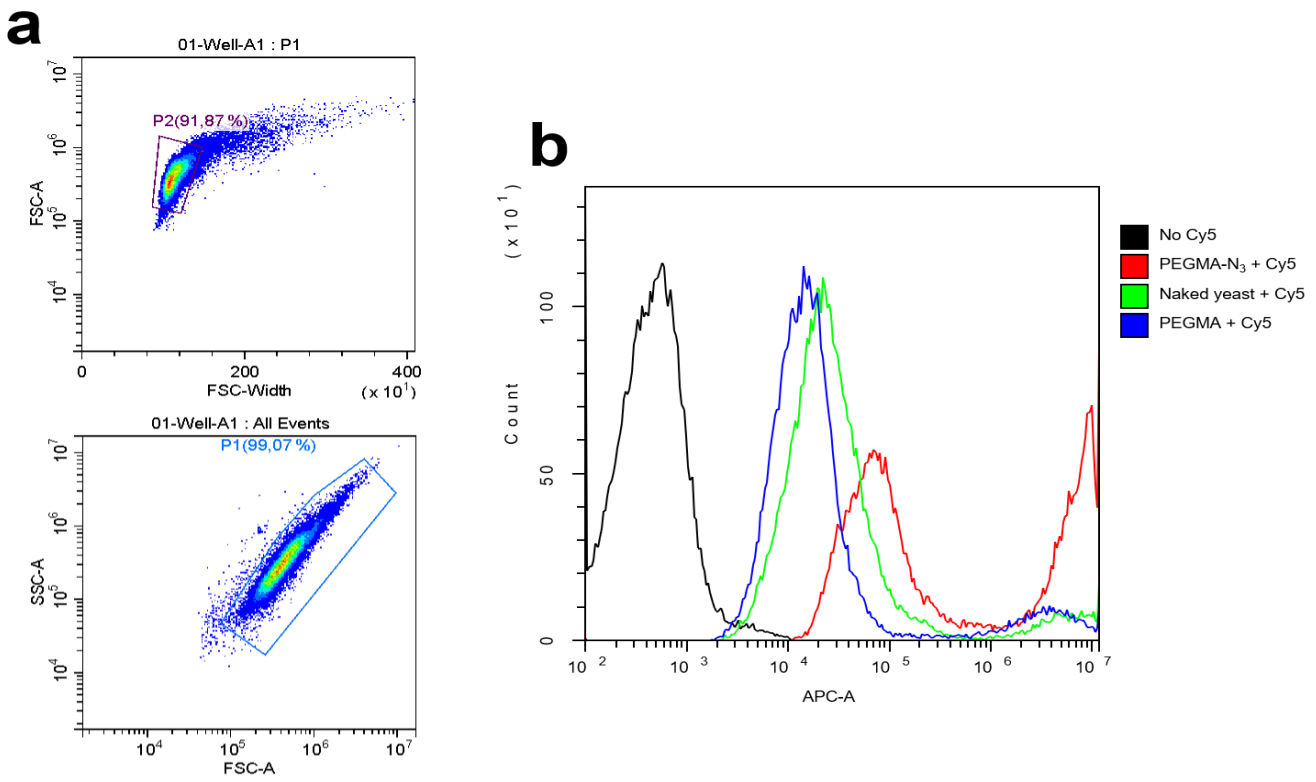
SI Figure 14. Aggregation behaviour of PPEGMA-coated yeast in the presence of TA. (a) Scheme of the cross-linking of PEG chains with TA (depicted as brown star). (b) CLSM micrographs (FDA stain and brightfield overlay) of **P10** yeast aggregate crosslinked by TA. (c) Z-stack reconstruction of a yeast aggregate (FDA stain). (d) OD_{600} of yeast with and without PPEGMA after addition of TA: higher values come from larger aggregates that scatter more light. (e) CLSM micrographs (FDA stain and brightfield overlay) of yeast with and without PPEGMA or TA, and after centrifugation and resuspension of the cells to wash the TA away. Error bars displayed as SD, n=3 replicates. Scalebars: 20 µm.



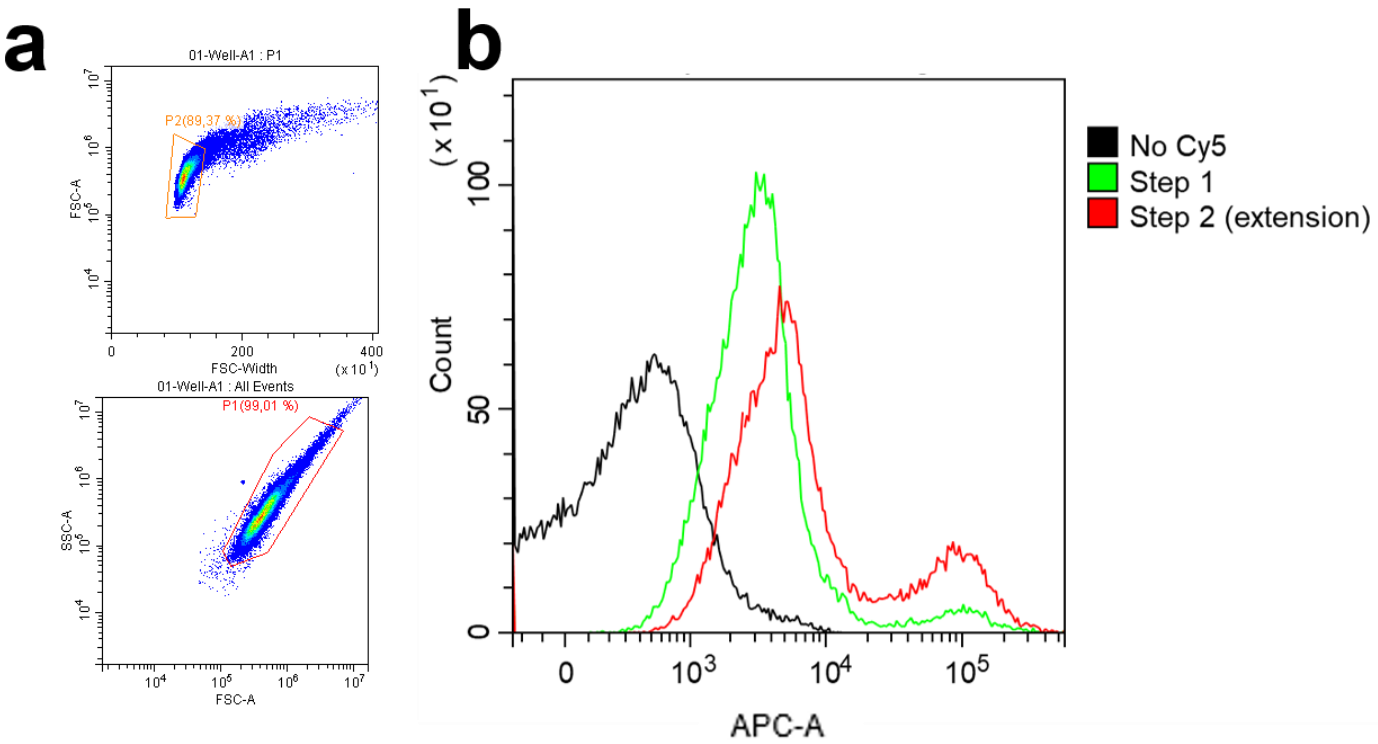
SI Figure 15. Production of AgNP by **P10** polymer-coated yeast. (a) Scheme of the PPEGMA-influenced synthesis of AgNPs with yeast cells. (b) Size distribution vs. concentration of AgNPs synthesized with PPEGMA-coated cells, with naked cells, and with naked cells in the presence of sodium ascorbate. (c) TEM micrograph of AgNPs recovered from the supernatant of PPEGMA-decorated yeast. (d) Surface potential of the AgNPs produced by different yeast. (e) Viability of yeast after purification from NPs. Mean values, error bars displayed as SD, n=3 replicates. Scalebar: 200 nm.



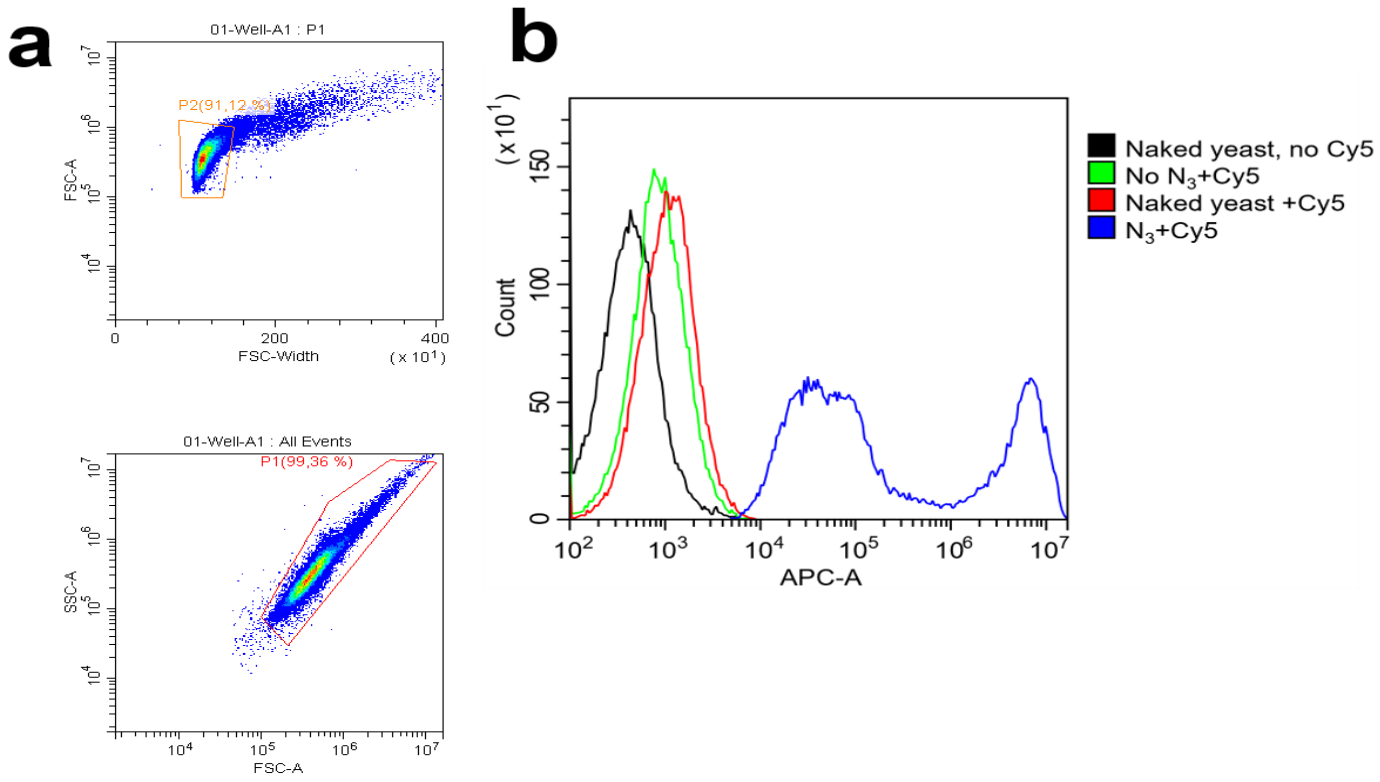
SI Figure 16. Production of PdNP by **P10** polymer-coated yeast. (a) Size distribution vs. particle concentration of PdNPs synthesized with PPEGMA-coated cells, with naked cells, and with naked cells in the presence of sodium ascorbate. (b) Viability of yeast after purification from NPs. Mean values, error bars displayed as SD, n=3 replicates.



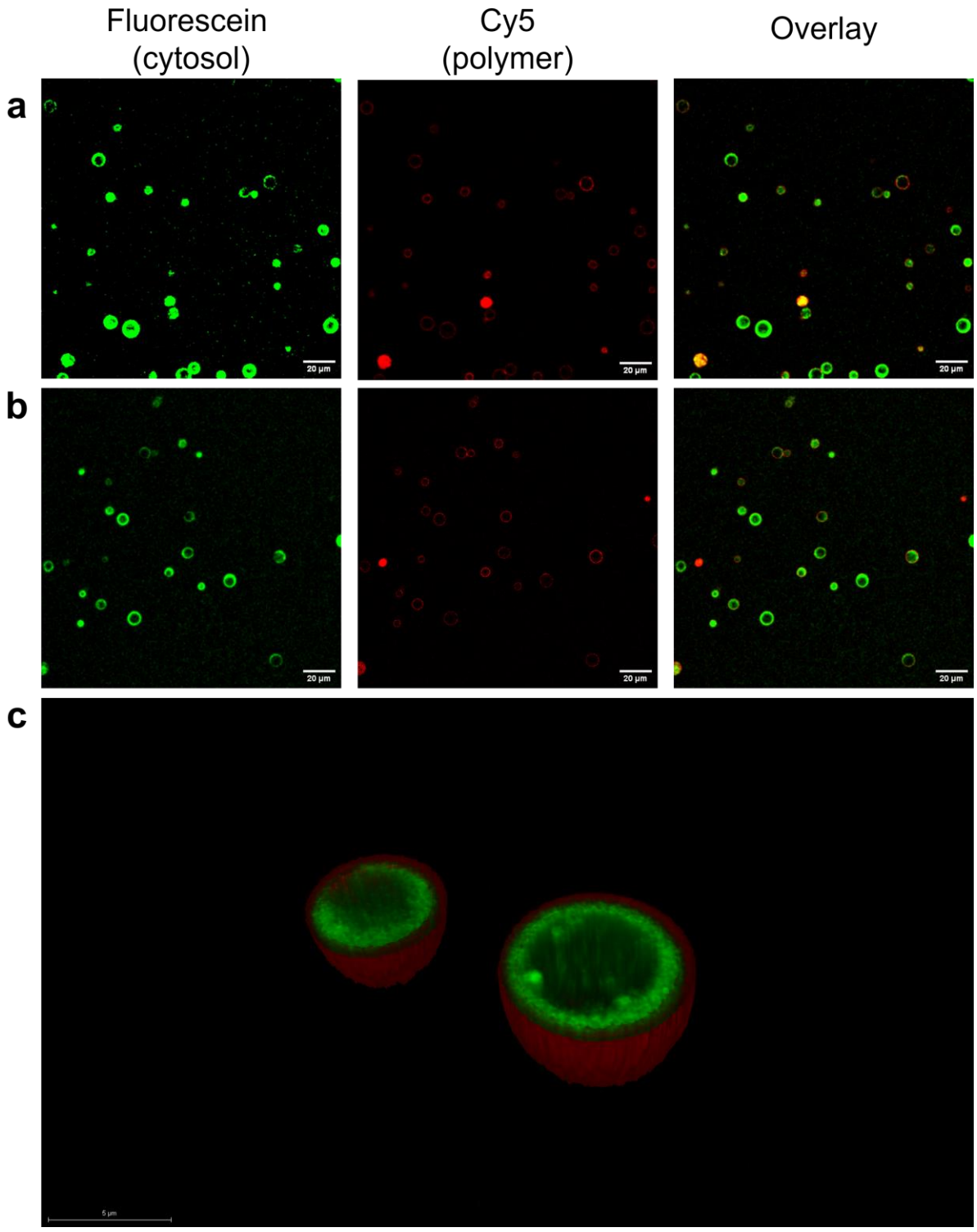
SI Figure 17. Flow cytometry results of Cy5 conjugation to **P11**. (a) Scatter plots and gating (FSC and SSC) strategy. (b) Flow cytometry histograms. The two visible populations with Cy5 fluorescence are probably due to a combination of a specific adsorption/uptake and the fact that a certain percentage of the cells will not have expressed HRP but will have formed polymer on their surface anyway.



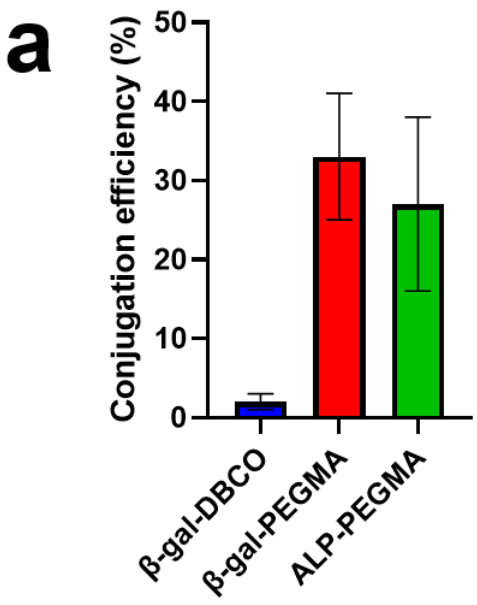
SI Figure 18. Flow cytometry results of Cy5-labeled **P12** and **P13**, i.e. polymerization of P(PEGMA-*co*-PEGMA-N_3) as step 1 and subsequent chain extension in step 2. (a) Scatter plots and gating (FSC and SSC) strategy. (b) Flow cytometry histograms of **P12** (Step 1) and **P13** (Step 2).



SI Figure 19. Flow cytometry results of Cy5-labeled **P14** (PPEGMA-N_3 homopolymer). (a) Scatter plots and gating (FSC and SSC) strategy. (b) Flow cytometry histograms.



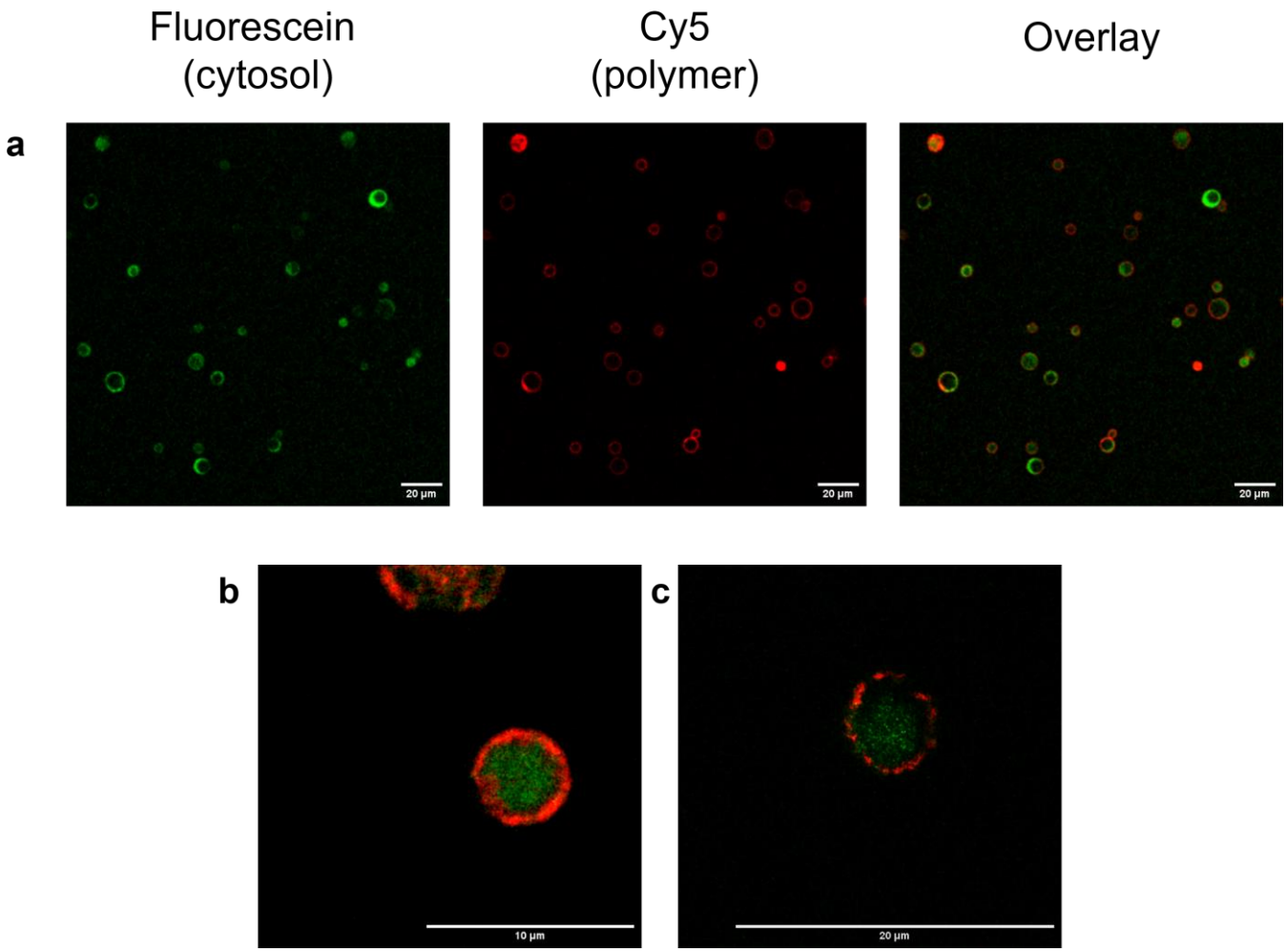
SI Figure 20. Conjugation of Cy5 to **P14** (PPEGMA-N3 homopolymer). (a-b) CLSM micrographs of Cy5-labeled **P14.** (c) Z-stack half section of two **P14** cells. Scalebars: 20 µm (a, b), 5 µm (c).



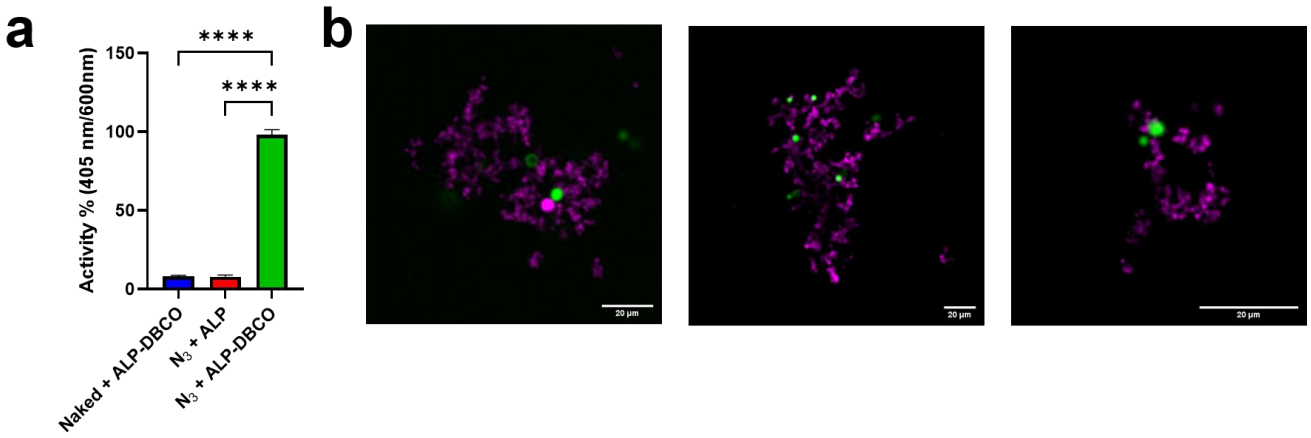
b

	Monomer conversion(%)
β-gal-PEGMA	79
ALP-PEGMA	65

SI Figure 21. Modification of yeast cells with non-native enzymes by conjugation of enzymes to PPEGMA-N_3 and PEGMA-N_3. (a) Conjugation efficiency of enzymes to PPEGMA-N_3 synthesized by surface-initiated polymerization on yeast cells (β-gal-DBCO = enzyme conjugated post polymerization to **P14**) and to the monomer PEGMA-N_3 prior to polymerizatio on the cell surface. (b) Monomer conversion for the cell-surface polymerization of enzyme-monomer conjugates. Mean values, error bars displayed as SD, n=3 replicates. No GPC traces were recorded for PEGMA-conjugated enzymes.



SI Figure 22. Polymerization of pre-conjugated PEGMA-Cy5 on HRP-displaying yeast cells. (a) Overview CLSM micrographs of **P16.** (b, c) Zoomed-in micrographs of **P16** cells. Scalebars: 10 µm (b), 20 µm (others).



SI Figure 23. Effect of ALP on cells. (a) Activity of ALP as measured by the production of nitrophenol from PPNP. (b) CLSM micrographs of CaGP aggregates (with adsorbed rhodamine B, magenta) and FDA-labelled yeast. Mean values, error bars displayed as SD, n=3 replicates. ****: p<0.0001. Scalebars: 20 µm.

1. D. Lipovsek, E. Antipov, K. A. Armstrong, M. J. Olsen, A. M. Klibanov, B. Tidor and K. D. Wittrup, *Chemistry & biology*, 2007, **14**, 1176-1185.
2. S. J. Stocks, A. J. Jones, C. W. Ramey and D. E. Brooks, *Analytical biochemistry*, 1986, **154**, 232-234.
3. X. Wang and Z. An, in *Methods Enzymol.*, eds. N. Bruns and K. Loos, Academic Press, 2019, vol. 627, pp. 291-319.
4. J. J. Agresti, E. Antipov, A. R. Abate, K. Ahn, A. C. Rowat, J.-C. Baret, M. Marquez, A. M. Klibanov, A. D. Griffiths and D. A. Weitz, *PNAS*, 2010, **107**, 4004-4009.
5. A. Day, C. Schneider and B. L. Schneider, *Methods Mol Biol*, 2004, **241**, 55-76.
6. K. J. Rodriguez, M. M. Pellizzoni, R. J. Chadwick, C. Guo and N. Bruns, in *Methods Enzymol.*, eds. N. Bruns and K. Loos, Academic Press, 2019, vol. 627, pp. 249-262.
7. A. R. Spurr, *J. Ultrastruct. Res.*, 1969, **26**, 31-43.