Electronic Supplementary Information

Ascorbic acid-mediated reductive disassembly of Fe^{3+}-tannic acid shells in degradable single-cell nanoencapsulation

*Hojae Lee, Joohyouck Park, Sang Yeong Han, Sol Han, Wongu Youn, Hyunwoo Choi, Gyeongwon Yun, and Insung S. Choi**

Dr. H. Lee, J. Park, S. Y. Han, S. Han, W. Youn, H. Choi, Dr. G. Yun, Prof. Dr. I. S. Choi
Center for Cell-Encapsulation Research, Department of Chemistry, KAIST, Daejeon 34141, Korea
E-mail: ischoi@kaist.ac.kr

Table of Contents

Experimental Section

Materials. Tannic acid (TA, Sigma-Aldrich), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 98%, Sigma-Aldrich), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥98.0%, Sigma-Aldrich), 3-(*N*-morpholino)propanesulfonic acid (MOPS, ≥99.5%, Sigma-Aldrich), L-ascorbic acid (AA, ≥99%, Sigma), L-glutathione reduced (GSH, ≥98.0%, Sigma-Aldrich), β-nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH, ≥97.0%, Sigma-Aldrich), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, Sigma-Aldrich), glycine (≥99%, Sigma-Aldrich), Oil Red O (Sigma-Aldrich), Alexa Fluor® 647-conjugated albumin from bovine serum (BSA-Alexa Fluor® 647, Life Technologies), ethanol (EtOH, 95.0%, Samchun), acetone (99.5%, Samchun), hydrochloric acid (HCl, 35%, OCI company Ltd.), sodium hydroxide (NaOH, Daejung), fluorescein diacetate (FDA, Sigma-Aldrich), yeast-extract-peptone-dextrose broth (YPD broth, Duchefa Biochemistry), yeast-extract-peptone-dextrose agar (YPD agar, Duchefa Biochemistry), phosphate-buffered saline (PBS, Welgene), poly(dimethylsiloxane) (PDMS, Sylgard® 184 silicone elastomer kit, Dow Corning), basolite® C300 (Sigma-Aldrich), basolite® F300 (Sigma-Aldrich), and silicon wafers (Sehyoung Wafertech) were used as received. Gold (Au) substrates were prepared by thermal deposition of Ti (5 nm) and Au (100 nm) onto silicon wafers. Deionized (DI) water (18.3 MΩ·cm) from Milli-Q Direct 8 (Millipore) was used.

Kinetic Studies on Fe^{3+}-TA-MPC Disassembly. The iron gall ink (IGI)-based solution ($[Fe^{2+}]$ = 10 mM, from $FeCl_2 \cdot 4H_2O$; [TA] = 1 mM) was matured for 6 h in the atmosphere for the oxidative assembly of Fe^{3+}-TA complex, and AA was added to the solution to the final concentration of 10 mM. A 20-μL aliquot was taken from the solution at the pre-determined time, diluted in 980 μL of DI water, and analyzed with a UV-Vis spectrophotometer (UV-2550 spectrophotometer, Shimadzu). The absorption intensity at 565 nm was used for the analysis. For studies on the film degradation, a Au substrate was immersed in the IGI-based solution ($[Fe^{2+}]$ = 10 mM; [TA] = 1 mM), and the solution was stirred gently for 12 h with 100 rpm. The Au substrate was taken, washed with DI water, dried under Ar gas, and then stored in a dessicator. For disassembly studies, Fe^{3+}-TA-coated substrates were incubated in an aqueous solution of AA (10 mM), EDTA (10 mM), gly-HCl buffer (50 mM, pH 2), GSH (10 mM), or NADH (10 mM). The pH of the solution was adjusted with 1 M of HCl or NaOH solution for the study on the pH effects,. In case of the AA redox cycling study, GSH (10 mM) was added to the aqueous solution of AA (0.1 mM). The film thickness was calculated with a spectroscopic ellipsometer Elli-SE (Ellipso Technology®), after washing with DI water and drying under Ar gas, at the predetermined time of incubation. For contact angle measurement, 2 μL of an aqueous droplet was positioned on a native Au substrate, the Fe^{3+}-TA-coated Au substrate before degradation, or the Fe^{3+}-TA-coated Au substrate after degradation, and the water contact angle was measured based on the optical image of the droplet. For studies on the reversibility of the system, Au substrates were immersed in the IGI-based solution ($[Fe^{2+}]$ = 10 mM; [TA] = 1 mM), and the solution was stirred gently with 100 rpm. After 0 h or 3 h of incubation, AA (final concentration: 0.1, 0.5, or 1 mM) was added to the solution, and the film thickness was measured at the predetermined time up to 24 h.

For degradation study of capsules, Oil Red O (final concentration: 0.1 $mg \cdot mL^{-1}$) was added to hexadecane for visualization, and the resulting hexadecane was added to an IGI-based solution (final concentration: $[Fe^{2+}]$ = 10 mM; [TA] = 1 mM), as an internal phase (20%, v/v), and broken into small droplets by a homogenizer (T 25 digital ULTRA-TURRAX®, IKA®). After 24 h with the air exposure, the capsules formed were washed with DI water, and incubated for the predetermined time in the solution of AA (10 mM), EDTA (10 mM), or gly-

HCl buffer (50 mM, pH 2). After centrifugation, leaked oil, from the destroyed capsules, was extracted with 600 μL of hexadecane, and the number of remaining capsules was calculated based on the absorption intensity of Oil Red O (at 519 nm). To get a calibration curve, Oil Red O-added hexadecane was used as a stock solution. The stock solution was diluted serially, and the absorption intensity of the diluted solution was measured at 519 nm with a UV-Vis spectrophotometer (UV-2550 spectrophotometer, Shimadzu). For degradation study of free-standing films, films were fabricated at the air-water interface of an IGI-based solution ([Fe^{2+}] = 100 mM; [TA] = 10 mM), without stirring, at room temperature. After 24 h of reaction, the films were sampled by either stamping (for thickness measurement) or lifting-up (for optical images).[1] The films, prepared by the stamping method, were incubated in the aqueous solution of AA (10 mM), EDTA (10 mM), or gly-HCl buffer (50 mM, pH 2) for predetermined time, and the film thickness was determined based on the cross-sectional SEM images. The films, prepared by the lifting-up method, were also incubated in the aqueous solution of AA (10 mM), EDTA (10 mM), or gly-HCl buffer (50 mM, pH 2), and the optical images were taken at the predetermined time. For disassembly study of MOFs, 8 mg of basolite® C300 or F300 was incubated in DI water or the aqeous solution of AA (100 mM) for 12 h, and the optical images were taken at the predetermined time.

Single-Cell Nanoencapsulation (SCNE) of *S. cerevisiase* with Fe^{3+}-TA MPC. For preparation of yeast@Fe^{3+}-TA, a single colony of *S. cerevisiase* (Baker's yeast) was picked from a YPD agar plate, suspended in the YPD broth, and cultured in a shaking incubator at 30 °C for 30 h. Prior to shell formation, the cells were washed with DI water twice. The 5-μL aqueous solution of TA (40 mg·mL^{-1}) and the 5-μL aqueous solution of $FeCl_3 \cdot 6H_2O$ (10 mg·mL^{-1}) were added sequentially to an aqueous suspension of the yeast cells (490 μL) with 30-sec pipetting between the additions. After addition of the Fe^{3+} solution, the resulting suspension was mixed vigorously for 30 sec, and 500 μL of the MOPS buffer (20 mM, pH 7.4) was added to the yeast suspension for the stabilization of the formed Fe^{3+}-TA shell. The resulting encapsulated-yeast was washed with DI water to remove any residual TA and Fe^{3+}. The encapsulation process was repeated four times to prepare yeast@Fe^{3+}-TA. The cell viability was investigated with FDA. FDA was dissolved in acetone (10 mg·mL^{-1}), and the 2 μL of the FDA stock solution was mixed with 1 mL of a cell suspension (PBS, pH 7.2) for 15 min at 30 °C while shaking. The Fe^{3+}-TA-MPC shell was labeled with BSA-Alexa Fluor® 647. BSA-Alexa Fluor® 647 was dissolved in DI water (10 mg·mL^{-1}), and the 100 μL of the BSA-Alexa Fluor® 647 stock solution was mixed with the 900 μL of a cell suspension (PBS, pH 7.2) for 30 min at 30 °C while shaking. The cells were collected by centrifugation, washed with DI water, and characterized with a confocal laser-scanning microsope (CLSM, LSM 700 Confocal Microscope, Carl Zeiss) and a field-emission scanning electron microscope (FE-SEM, F50 microscope, FEI).

For studies on the reductive shell disassembly, yeast@Fe^{3+}-TA was suspended for 1 h in the aqueous AA solution (100 mM), washed with DI water, treated with FDA and BSA-Alexa Fluor® 647, and characterized by CLSM and FE-SEM. The $t_{-2.0}^{OD_{600}}$ values were obtained by cell culture in the YPD broth. The 1 mL of aqueous yeast@Fe-TA suspension (OD_{600} = 0.15) was added to the 150 mL of YPD broth media (final OD_{600} = 0.001) that contained AA (100 mM), EDTA (100 mM), or gly-HCl (50 mM, pH 2), and cultured in a shaking incubator at 30 °C. The 100 μL of the culture mixture was picked at the predetermined time, and the cell density was measured at 600 nm with a plate reader (SpectraMax® iD5, Moleculardevices). The $t_{-2.0}^{OD_{600}}$ value was calculated based on the linear-fitted plot of ln(OD_{600}) vs. culture time.

Micropattern Generation. Au substrates were immersed in the IGI-based solution ([Fe^{2+}] = 10 mM; [TA] = 1 mM), and the solution was stirred gently with 100 rpm. After 24 h of reaction, the Au substrates were taken, washed with DI water, and dried under Ar gas. The elastomeric PDMS stamp was prepared by replica molding of a microfabricated silicon master that had the line pattern (period: 300 μm; groove width: 100 μm). After mixing the PDMS prepolymer and curing agent at 1:10 (v/v) and degassing for 30 min to eliminate air bubbles, the mixture was poured onto the master. Curing the mixture (at 65 ºC for 12 h) and peeling it away from the master provided a negative replica of the line patterns, which was used as an etching mask. The line pattern of the PDMS stamp was contacted with the Fe^{3+}-TA MPC-coated Au substrate, and the AA solution (100 mM) was dropped on the inlet after placing a weight of 5 g on the stamp. After 1 min of etching, the stamp was removed, and the Au substrate was washed with DI water and dried under Ar gas.

Reference

1 H. Lee, W. I. Kim, W. Youn, T. Park, S. Lee, T.-S. Kim, J. F. Mano, and I. S. Choi, *Adv. Mater.* **2018**, *30*, 1805091.

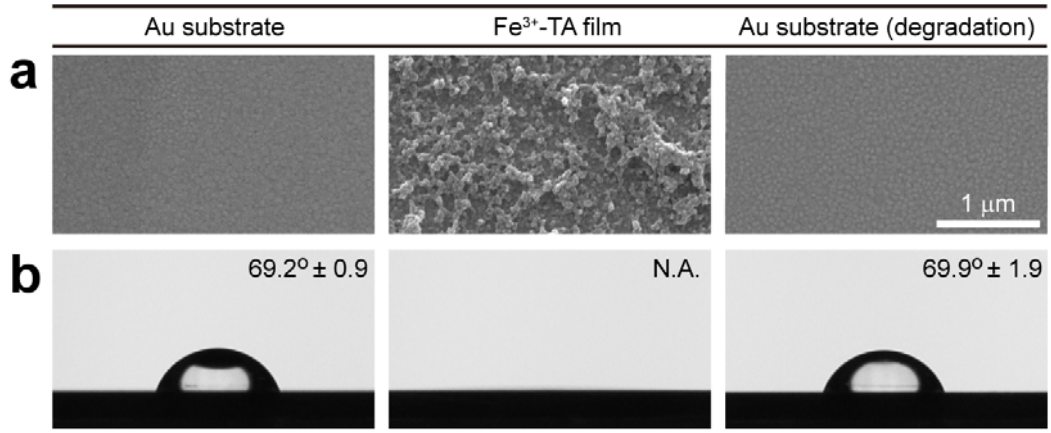

Figure S1. (a and b) (a) FE-SEM images and (b) water contact angles of a native Au substrate, and Au substrates after formation and degradation of Fe^{3+}-TA films.

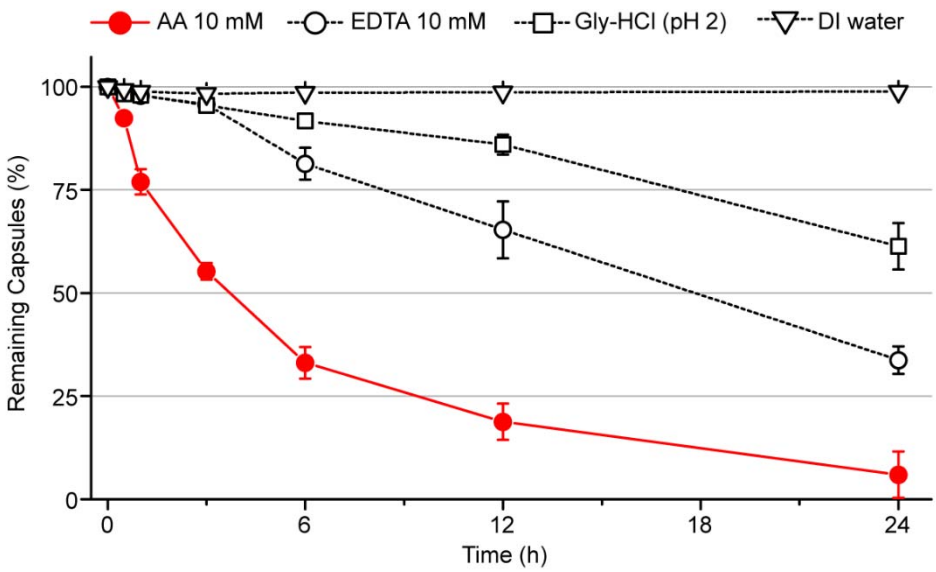

Figure S2. Capsule degradation.

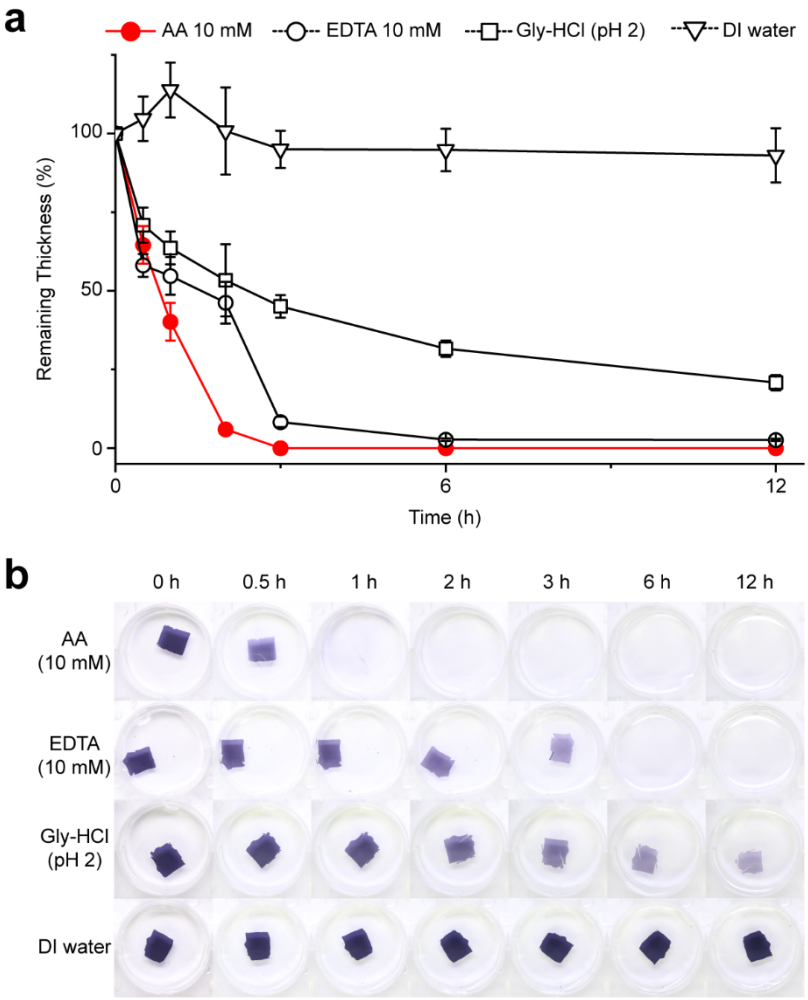

Figure S3. Degradation of free-standing films. (a) Thickness changes and (b) optical images of free-standing films over time.

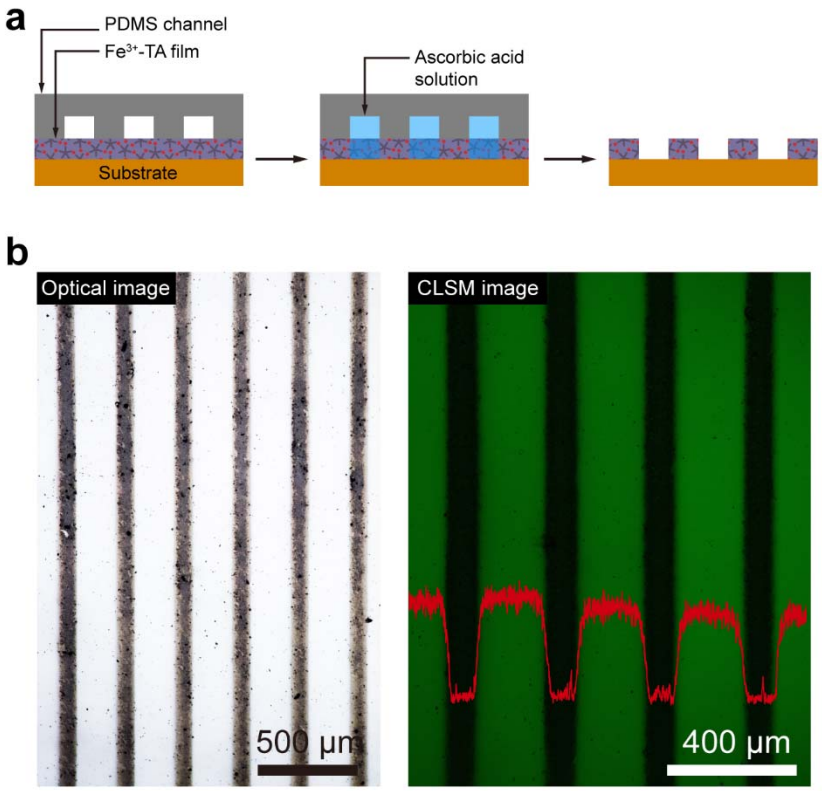

Figure S4. (a) Schematic representation for the Fe^{3+}-TA micropattern generation. (b) Optical and CLSM images of the linear Fe^{3+}-TA micropatterns. Black lines in both images represent the Fe^{3+}-TA patterns.

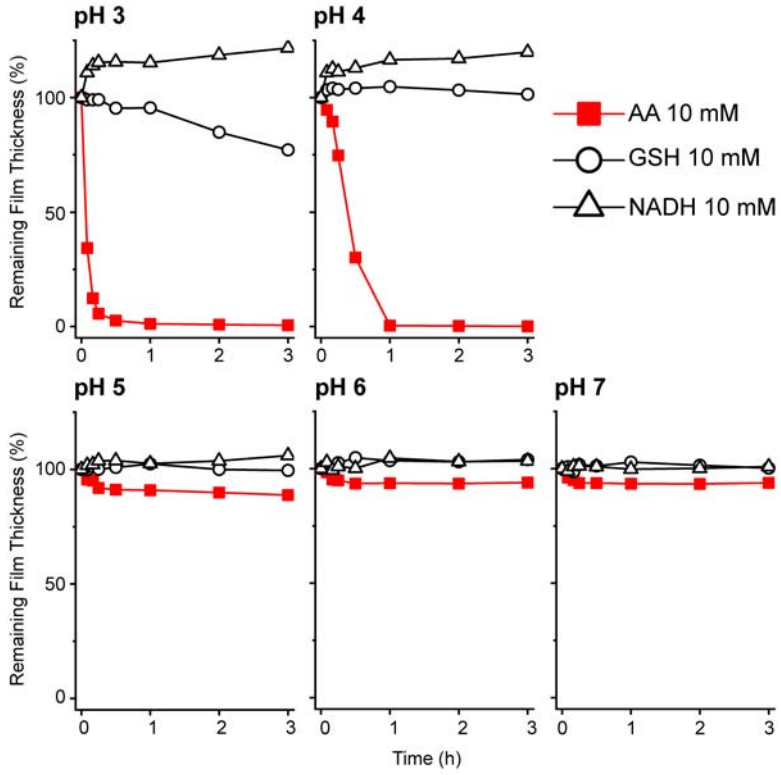

Figure S5. (a) Graphs of film thickness versus reaction time for the pH-controlled solution of AA (10 mM), GSH (10 mM), or NADH (10 mM).

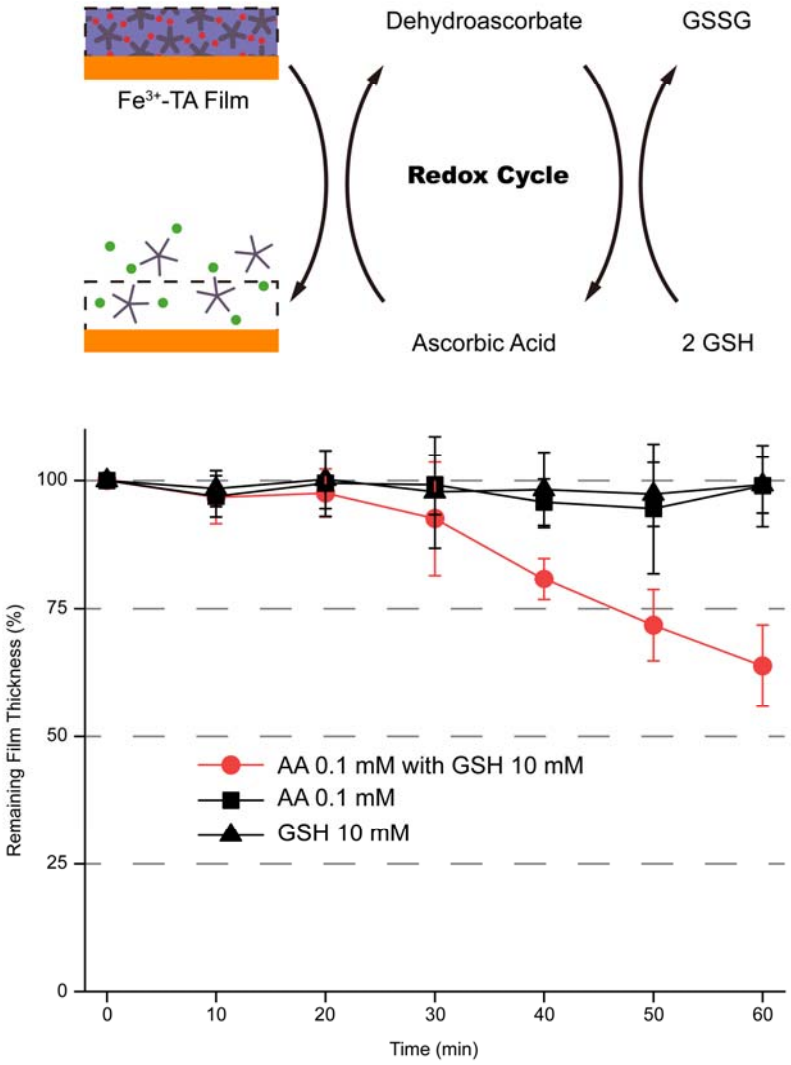

Figure S6. Graphs of film thickness versus reaction time for the redox-coupled solution of AA (0.1 mM) and GSH (10 mM), with AA (0.1 mM) and GSH (10 mM) as the controls.

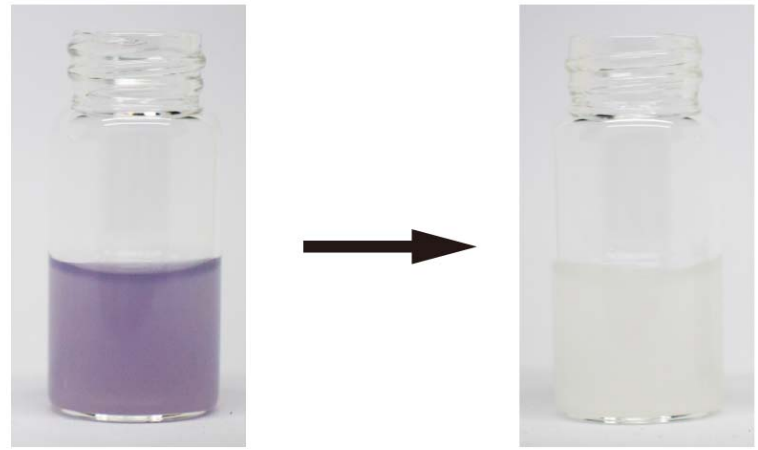

Figure S7. Optical images of yeast@Fe^{3+}-TA suspension before and after shell degradation.

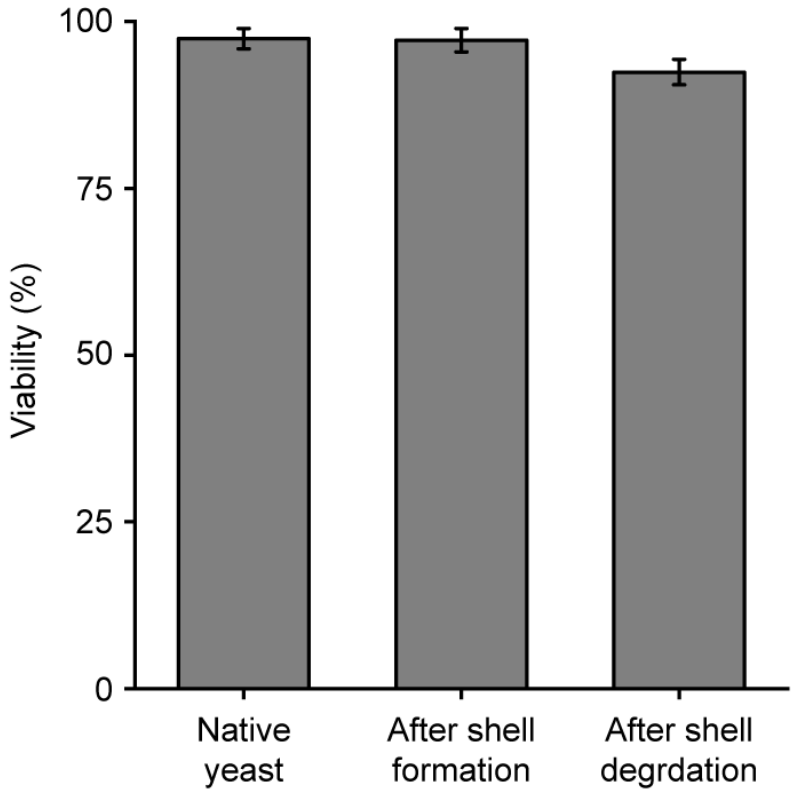

Figure S8. Cell viabilities of native yeast, and yeast@Fe^{3+}-TA before and after shell degradation.

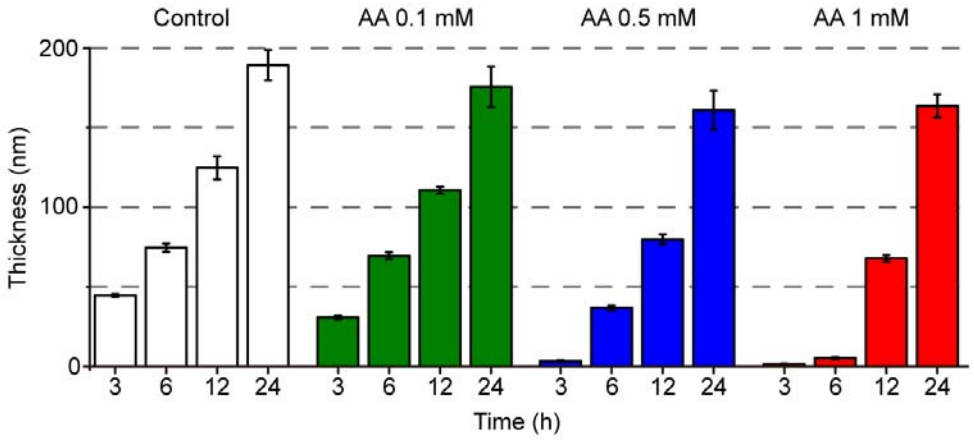

Figure S9. Retardation of film growth in the IGI-based solution ($[Fe^{2+}]$ = 10 mM, [TA] = 1 mM). AA (0.1, 0.5, or 1 mM) was added to the solution right after mixing of Fe^{2+} and TA.

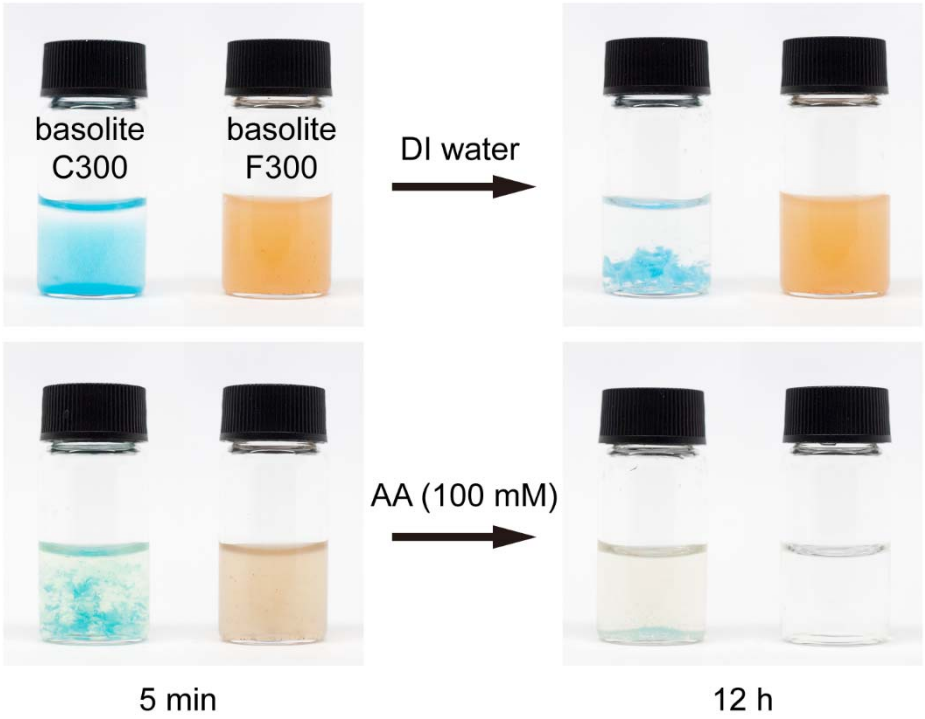

Figure S10. Expandability of AA-mediated reductive disassembly to other metal-organic complex structures, such as basolite® C300 (HKUST-1) and F300 (Fe-BTC).