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An Enzyme-Coated Metal–Organic Framework Shell for Synthetically Adaptive Cell Survival

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Abstract: *A bioactive synthetic porous shell was engineered to enable cells to survive in an oligotrophic environment. Eukaryotic cells (yeast) were firstly coated with a β-galactosidase (β-gal), before crystallization of a metal–organic framework (MOF) film on the enzyme coating; thereby producing a bioactive porous synthetic shell. The β-gal was an essential component of the bioactive shell as it generated nutrients (that is, glucose and galactose) required for cell viability in nutrient-deficient media (lactose-based). Additionally, the porous MOF coating carried out other vital functions, such as 1) shielding the cells from cytotoxic compounds and radiation, 2) protecting the non-native enzymes (β-gal in this instance) from degradation and internalization, and 3) allowing for the diffusion of molecules essential for the survival of the cells. Indeed, this bioactive porous shell enabled the survival of cells in simulated extreme oligotrophic environments for more than 7 days, leading to a decrease in cell viability less than 30%, versus a 99% decrease for naked yeast. When returned to optimal growth conditions the bioactive porous exoskeleton could be removed and the cells regained full growth immediately. The construction of bioactive coatings represents a conceptually new and promising approach for the next-generation of cell-based research and application, and is an alternative to synthetic biology or genetic modification.*

Living organisms are sensitive to their environment and subtle changes in nutrient level, temperature, pressure, moisture, salinity, and pH can disrupt their biological functions, thereby leading to cell death.[1–3] To address this challenge, primary research in genetic engineering and synthetic biology focuses on understanding and engineering genetic codons to enhance cellular adaptive responses to environmental changes.[4,5] Generally, synthetic biology aims to develop new circuit design principles that involve multistep procedures, including creating and analyzing computational models, constructing genetic circuits, evaluating the performance of the genetically engineered cells, and refining the design process.[6] Although recent developments in precision genome engineering, such as zinc finger proteins (ZFPs), transcription activator-like (Tal) effectors, and clustered regularly interspaced short palindromic repeats (CRISPRs), have significantly enhanced the versatility of gene editing, these techniques still face several challenges; including, a high level of complexity, difficulties in characterization, standardization and modularity, noise, epigenetics, mutations, and a risk of accidental release into the wild, amongst other challenges.[6] Therefore, developing a generalized, non-genetically engineered alternative to temporarily enhance the adaptability of cells is highly desired. Recently, artificial nanocoatings have appeared as an approach to mimic the robustness of cell membranes and walls found in some extremophiles, while avoiding genetic modification.[7–9] Nanocoating cell surfaces creates a physical barrier between the cell and the external environment while still allowing nutrient exchange. These nanocoatings have improved cellular tolerance against heat,[10,11] UV radiation,[12,13] toxins,[14–17] osmotic pressure,[18,19] and mechanical stress.[20,21] Although nanocoatings have demonstrated protective abilities, their integration with active biomacromolecules is a challenge. This prevents the bioengineering of such coatings with extrinsic bioactive functionalities that could impart artificial adaptive ability, thus overcoming original biological limitations. For example,

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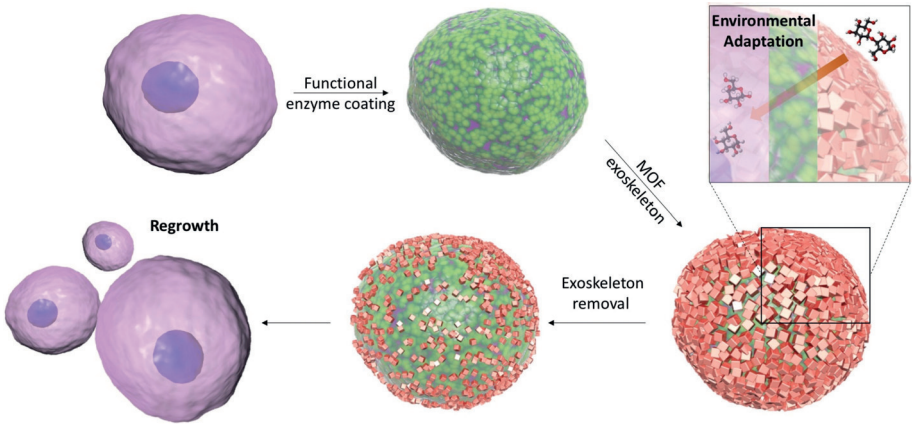
eukaryotic cells lack the ability to harvest energy from nutrient-depleted environments;[22] however, providing cells with a bioactive protective nanocoating capable of converting depleted media into usable nutrients could furnish new opportunities in therapy,[23] diagnostics,[24] stress-response,[25,26] and biocatalysis,[27] and could also revolutionize the dairy and pharmaceutical industries.[28,29]

Among the wide choice of synthetic coatings, metal–organic frameworks (MOFs) are a class of porous materials that offer several unique advantages for engineering eukaryotic cells to survive in inhospitable environments.[30] MOFs can be thermally and chemically robust,[31] and can be synthesized by modular approaches that facilitate fine-tuning of pore shape,[32] chemical functionality,[33,34] and size.[35] More importantly, MOFs hold promise for biomedical applications,[36] as they can be constructed from bio-friendly building blocks[37] under physiological conditions.[38] Herein, we demonstrate that an active biocomposite MOF shell can be integrated with cells. As a result eukaryotic cells can survive in cytotoxic environments without the need for essential nutrients. A proof-of-concept was obtained by coating living eukaryotic cells with a protective MOF exoskeleton functionalized with exogenous enzymes that do not exist naturally in the cells. This enzyme is capable of converting disaccharides that cannot be utilized by these cells, into monosaccharides that are metabolizable nutrients for the cells. Notably, the MOF shell allows for the diffusion of nutrients to the living cells (Scheme 1) while providing a selective barrier that can protect both the cells and the non-native enzymes. *Saccharomyces cerevisiae* (baker's yeast) was selected in this study, as these cells do not have a lactose metabolic system; therefore, genetic engineering approaches are currently used to construct lactose-consuming *S. cerevisiae* strains.[39,40] In the proposed study native *S. cerevisiae* coated with β-galactosidase (β-gal) and zeolitic imidazolate framework-8 (ZIF-8) showed cell survival for more than 7 days in an environment lacking the essential nutrient glucose. The β-gal protected by the MOF coating (β-gal/ZIF-8) was able to continuously produce glucose and galactose from environmental lactose and thereby maintain cell viability, even in conditions that would normally kill the yeast (exposure to the lytic enzyme, lyticase) or destroy the β-gal (exposure to a protease cocktail). Although ZIF-8 was used in this study, other MOFs could also be employed with careful consideration of the solubility of the MOF ligands and their cytotoxicity. The construction of a biologically active biocomposite MOF coating, that can be easily removed, demonstrates a new and promising approach for facile, non-genetic, cellular manipulation.

Yeast cells were first coated with β-gal by electrostatically absorbing the cationic enzymes on the anionic cell surface in phosphate buffered saline (PBS).[41,42] Confocal laser scanning microscopy (CLSM) was used to confirm the surface coverage of the enzymes on the yeast cells (Figure 1 a,b; Supporting Information, Figure S1). Although ZIF-8 coatings have previously been grown on naked yeast,[30] the modification of yeast cells with a protein-rich surface could also favor the attraction of MOF precursors (including cations and ligands), leading to the rapid biomimetic crystallization of a MOF shell.[38,43] Herein, the biomimetic growth of a MOF shell was achieved by incubating the enzyme-coated cells in an aqueous solution containing 2-methylimidazole and zinc acetate to form ZIF-8. After 10 min, the coated cells were separated from the solution and washed with water to remove the excess MOF precursors. CLSM was employed to visualize the MOF coating in solution after infiltration with a fluorescent dye, Alexa Fluor 647. A continuous fluorescent signal was observed around each individual cell, indicating the formation of a homogeneous MOF shell on individual cells. Cell viability assays using fluorescein diacetate (FDA) as a fluorescent indicator showed a negligible decrease in fluorescence intensity after coating the cells in the β-gal/ZIF-8 exoskeleton, indicating that the enzyme and MOF coating process had a minimal impact on cell viability (Figure 1 a–d; Supporting Information, Figure S2). The structure of the coating was assessed by small-angle X-ray scattering (SAXS) and the resulting diffraction pattern yielded peaks analogous, both in position and relative intensity, to pure ZIF-8 crystals (Figure 1 e), thereby confirming the crystalline nature of the β-gal/ZIF-8 exoskeleton. The morphology and elemental distribution of the β-gal/ZIF-8 exoskeleton was investigated using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). SEM images revealed that the surface of individual yeast cells was covered by a layer of nanocrystals (Figure 1 f; Supporting Information, Figure S3), while elemental mapping confirmed a homogeneous distribution of



Scheme 1. An illustration showing the construction and removal of the bioactive porous (β-gal/ZIF-8) shell for synthetically adaptive cell survival. A living cell is coated with a layer of bioactive exogenous enzymes (β-gal) capable of bioprocessing lactose resources to generate essential nutrients for the cells (for example, glucose). The enzymes and cells are both protected by an artificial exoskeleton composed of porous MOFs, allowing cells to survive in hostile environments. In the current study the MOF chosen is ZIF-8. Upon removal of the ZIF-8 coating, the cells regain their full growth potential.

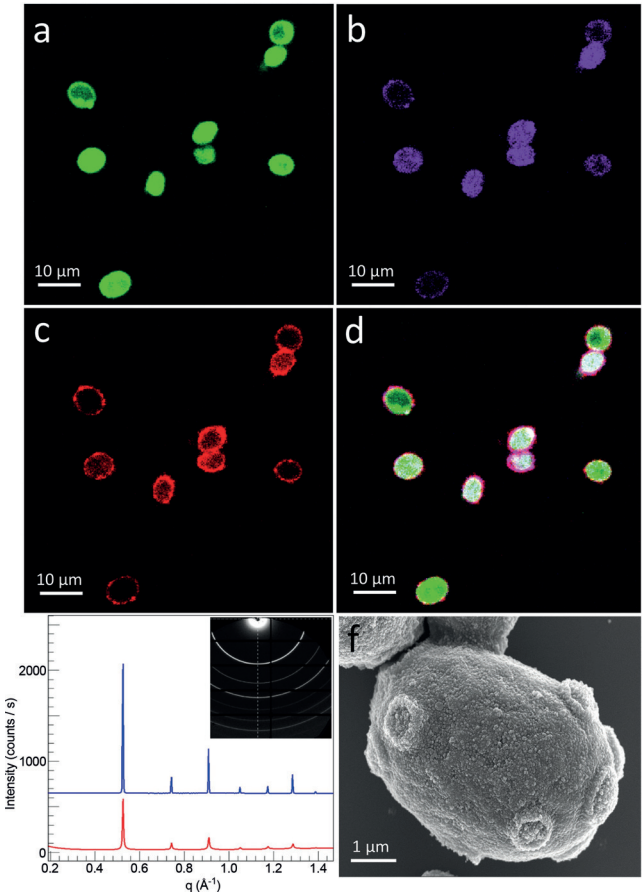


Figure 1. CLSM cross-section images of a) live yeast cells, b) β-gal coating, c) ZIF-8 coating, and d) a merged image. The living yeast cells catalyzed FDA into fluorescein (green), β-gal was labeled with Alexa Fluor 568 (purple), and the ZIF-8 coatings were labeled by infiltration of Alexa Fluor 647 (red). e) Synchrotron small-angle X-ray scattering (SAXS) diffraction patterns of standard ZIF-8 crystals (blue) and β-gal/ZIF-8-coated yeast cells (red). Inset: 2D SAXS pattern of the β-gal/ZIF-8-coated yeast cells (measured using a 0.5 mm capillary as a sample holder). f) SEM image of a yeast cell coated with the β-gal/ZIF-8 exoskeleton.

overlapping signals (Supporting Information, Figure S4) corresponding to O (from the yeast and β-gal), C (from the yeast, β-gal, and ZIF-8), N (from the yeast, β-gal, and ZIF-8), and Zn (from the ZIF-8). The SEM/EDS data was consistent with the high-resolution optical cross-sections obtained from CLSM, providing further evidence of a homogeneous coating of the β-gal/ZIF-8 exoskeleton over each individual cell. The thickness of the exoskeleton measured on different cracked shells was 100 nm ± 10 nm (as measured from SEM cross-sections; Supporting Information, Figure S5), which is consistent with pure ZIF-8 coatings grown directly on yeast cells.[30]

Saccharomyces cerevisiae must consume specific mono- and disaccharides for their metabolic needs,[44] and we hypothesized that the introduction of new non-native enzymes on a synthetic porous shell could allow yeast cells to survive in oligotrophic environments that are otherwise inhospitable. We first determined whether the new coatings could produce essential nutrients for yeast survival. β-gal is a glycoside hydrolase enzyme that converts lactose into glucose and galactose; both of which can be consumed by yeast as essential nutrients.[45] Yeast cells coated with β-gal/ZIF-8 were incubated in modified cell media containing lactose instead of glucose to simulate a nutrient deficient environment. The production of glucose and galactose was then monitored by an external enzyme cascade reaction using an enzyme cocktail composed of glucose oxidase, horseradish peroxidase, and a colorimetric indicator, pyrogallol (Supporting Information, Figure S6).[46] Accordingly, absorbance at 420 nm was monitored as it is the result of the catalytic conversion of pyrogallol. The continuous increase in intensity (Supporting Information, Figure S7) demonstrated that 1) lactose was able to diffuse through the ZIF-8 coating, 2) the MOF-coated β-gal was acting as a biocatalyst for the production of essential sugars for the yeast cells, and 3) the biocatalyzed glucose diffused through the ZIF-8 shell. In contrast, the conversion of lactose was not observed in the absence of either the β-gal (no glucose was produced) or the ZIF-8 coating, as the yeast likely internalized and degraded β-gal (Supporting Information, Figure S7). This data highlights the importance of the synergistic effect achieved by engineering a MOF shell with an active enzyme.

To further ascertain the importance of the current composition and structure of the coating, pure ZIF-8 crystals and free β-gal@ZIF-8 particles were exposed to lactose. However, glucose conversion was not detected (Supporting Information, Figure S8). The inability of β-gal@ZIF-8 to process lactose is consistent with previous findings of molecular diffusion in ZIF-8.[47] Moreover, this suggests that the diffusion of lactose through the ZIF-8 cell coating is likely a consequence of the interparticle spacing rather than the pore network. Based on the degradation of free β-gal from yeast, we suspect that the ZIF-8 coatings immobilize the enzymes on the cell surface, thereby preventing the internalization and digestion of the enzymes by the yeast cells. This confirms the importance of the β-gal@ZIF-8 structure for bioactivity.

Having demonstrated the potential of the bioactive porous coating in generating essential nutrients, we were motivated to investigate the survival potential of these cells in extreme and nutrient deficient environments. To simulate extreme environments, toxic compounds that are detrimental to yeast cells and/or β-gal (including the lytic enzyme, lyticase),[48] or the proteolytic enzyme cocktail protease,[49] were introduced to glucose deficient cell media containing lactose. Both naked and coated yeast cells were cultured in the modified cell media, and the cell viability was monitored using FDA as a fluorescent live-cell indicator over time. Using a combination of high-throughput flow cytometry and high-resolution optical microscopy,[50] the living cells were easily separated from the dead cells (Supporting Information, Figure S9). The viability of each sample was monitored for up to 7 days, and the percentage of living cells at each time point was calculated and plotted (Figure 2). Without any toxic enzymes in the media, the viability of the naked cells rapidly decreased within days (Supporting Information, Figure S10), suggesting that the naked cells could not survive during prolonged culturing with only lactose as a nutrient source.

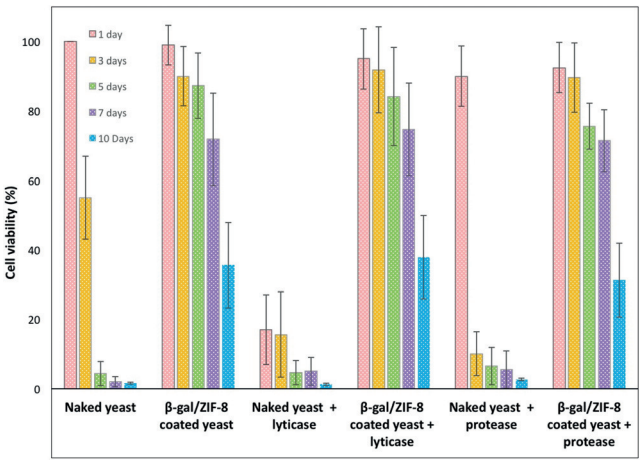


Figure 2. Relative cell viability (%) of naked yeast and yeast cells coated with biocomposite MOF (β-gal/ZIF-8) in oligotrophic cell media containing lactose. Cytotoxic enzymes such as lyticase or protease were added to simulate inhospitable environments. Cell viability was normalized against the viability of naked yeast at day one.

The immediate death of naked cells was more apparent when lyticase was present in the media, as lyticase can lyse yeast cells in a matter of hours.[30] The biocomposite MOF-coated yeast cells only showed a small drop (< 30 %) in viability even after 7 days in the modified cell media with or without lyticase (Figure 2; Supporting Information, Figure S11), suggesting that the engineered exoskeletons are effective in generating essential nutrients for prolonged cell survival while also protecting the yeast from toxic enzymes.

To further ascertain the stability and robustness of the coatings and yeast against toxic enzymes in nutrient deficient media, we studied the survival of these cells in the presence of proteases. Proteases are a class of enzymes that catalyze the breakdown of proteins through the hydrolysis of peptide bonds, and if uncontrolled, can destroy the proteinaceous components of cells and tissues.[49] Moreover, protease is capable of rapidly degrading enzymes such as β-gal in solution. When protease was introduced into the cell culture, the viability of naked cells decreased to 10 % after one day. In contrast, more than 70 % of the β-gal/ZIF-8-coated cells remained viable even after 7 days (Figure 2; Supporting Information, Figure S12). This result clearly indicates that the ZIF-8 coating was able to protect both the β-gal and the living cells from proteolytic attack. The approximately 30 % drop in viability of the biocomposite MOF-coated yeast cells at day 7 is likely because of the extensive consumption of lactose in the cell media, which leads to limited glucose generation. Enzyme cascade assay results indicated that almost 90 % of lactose was consumed by the β-gal/ZIF-8-coated yeast cells by day 7 (Supporting Information, Figure S13). By day 10, a majority (ca. 70 %) of the β-gal/ZIF-8-coated yeast cells had lost their viability because of the complete depletion of lactose.

The biocomposite MOF-coated yeast cells were also exposed to oligotrophic media containing both lyticase and protease for 48 h; and finally exposed to UV-C radiation, as DNA and proteins in living organisms can be damaged by UV-C.[51] β-gal/ZIF-8-coated yeast cells showed an 89 % viability versus 5 % viability for the naked yeast after exposure to multiple environments (Supporting Information, Figure S14). The protection against UV can be attributed to absorbance of ZIFs in the UV region.[52] Therefore, our results demonstrate that the biocomposite MOF-coated yeast cells can survive for an extended period of time in nutrient-depleted and adverse environments. Additionally, the metabolic activity of the β-gal/ZIF-8-coated yeast cells in nutrient-depleted and toxic environments was studied by measuring the CO_2 production rate as a result of anaerobic respiration (Supporting Information, Figure S15).[53] Results indicated that the biocomposite MOF-coated yeast cells were able to produce CO_2 at a similar rate as naked cells in glucose. In contrast, naked yeast cells in lactose did not show obvious CO_2 production.

To gain further understanding of the impact of the exoskeleton on the coated cells after living in stressed environments for prolonged periods, the MOF coating was removed by the addition of EDTA following 7 days of culturing in lactose media. The released cells were transferred to standard culture media containing glucose. Cell proliferation was monitored by optical density measurements at 600 nm (OD_{600}).[54] Compared with the naked yeast cells, the released cells skipped the lag phase and immediately regained full proliferation potential (growth phase); similar to our previous study.[30] Additionally, there is no obvious reduction of the growth rate compared to naked yeast cells at the growth-phase stage (Figure 3). These results clearly demonstrated that the large majority of yeast cells coated with the active biocomposite MOF shell remained healthy after surviving a week in a simulated extreme, nutrient-deficient environment. Given that the MOF coatings can be easily removed on demand,[30] this coating strategy shows new

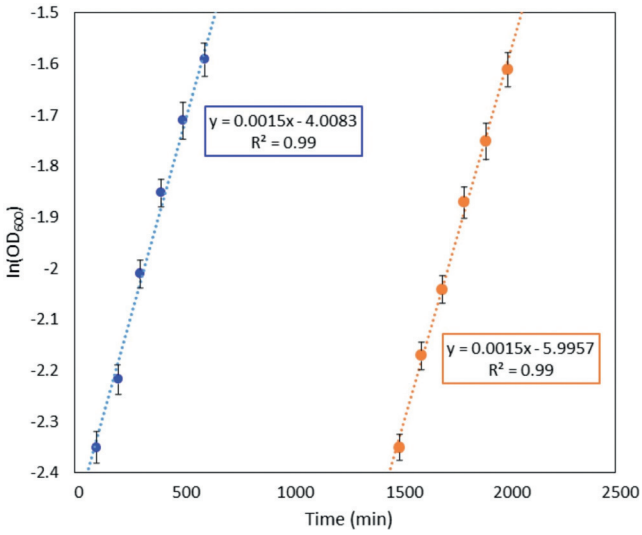


Figure 3. Growth-phase linear-fitted plot of $\ln(OD_{600})$ vs. time for β-gal/ZIF-8-coated yeast cells after ZIF-8 removal by EDTA (orange) and naked yeast as a control (blue). The growth rate is calculated from the slope of the linear fitted line. After removal of the bioactive porous coating the yeast fully retained their original growth rate.

potential for engineering selective adaptability in living cells, without the contamination risks associated with genetic engineering.

In conclusion, we have demonstrated that exogenous enzymes and MOFs can be synergistically engineered directly on living systems. The resulting bioactive shell allows cells to survive in nutrient-deficient environments in the presence of other adverse conditions (toxic agents and UV irradiation). Proof-of-concept experiments show that the enzyme/MOF coating was able to generate essential nutrients for the cells, while the MOF protected both the cells and non-native enzymes from harmful compounds and still allowed for the transport of nutrients. Moreover, the cells regain their full growth potential following the removal of the biocomposite MOF exoskeleton. Based on our previous reports, which demonstrated that different cells (for example, prokaryotic and eukaryotic cells) can be coated with MOFs, we anticipate that different cells, enzymes, and MOFs could be employed to expand the scope and application of adaptive coatings for cell survival. Methods for constructing hybrid and synthetically adaptive coatings for living cells could address challenges in biology and biotechnology.

Experimental Section

Experimental details including information about materials, coating formation on cell surfaces, the enzyme cascade assay, the cell viability test, the cell proliferation assay, and instrumentation are documented in the Supporting Information.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biomimetic materials · cytoprotection · hybrid cells · synthetic cells · ZIF-8

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