Submitted to ADVANCED MATERIALS
DOI: 10.1002/adma.((please add manuscript number))

Metal-organic Framework Coatings as Cytoprotective Exoskeletons for Living Cells

By *Kang Liang,* Joseph J. Richardson, Jiwei Cui, Frank Caruso, Christian J. Doonan,* and Paolo Falcaro**

Dr. K. Liang, Dr. J. J. Richardson
CSIRO Manufacturing
CSIRO Private Bag 10, Clayton South, Victoria 3169 (Australia)
E-mail: kang.liang@csiro.au
Dr. J. Cui, Prof. F. Caruso
ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the Department of Chemical and Biomolecular Engineering
The University of Melbourne, Parkville, Victoria 3010 (Australia)
Prof. C. J. Doonan
School of Chemistry and Physics
The University of Adelaide, Adelaide, South Australia 5005 (Australia)
E-mail: christian.doonan@adelaide.edu.au
Prof. P. Falcaro
Institute of Physical and Theoretical Chemistry
Graz University of Technology, Stremayrgasse 9, Graz, 8010 (Austria)
E-mail: paolo.falcaro@tugraz.at

Keywords: cytoprotection, cell coatings, biomimetics, metal-organic frameworks, nanoporous materials

Studying isolated living cell systems is essential to understanding complex biological functions,[1] and has led to their application in cell therapy, diagnostics, drug screening and the dairy and beverage industries.[2–4] Cells are typically encased by a lipid bilayer membrane that offers limited protection ex vivo, thus long-term protection and preservation strategies are often required.[5,6] To overcome this inherent fragility to environmental stress (e.g. ultraviolet radiation, malnutrition, dehydration, and elevated temperatures) some organisms, such as *bacillus subtilis*, form a robust multi-layer coating that impedes cell division.[7] However, when stress conditions are alleviated the protective shell is rapidly dissolved and the cells are able to germinate and resume vegetative growth.[7] Such biological preservation mechanisms have inspired artificial strategies aimed at the synthesis of mechanically durable yet degradable individual cell coatings[8,9] to enable the exploitation of cells for unprecedented

technological applications.[4,10,11] Recent studies have demonstrated that fabricating durable, synthetic coatings around living cells affords prolonged cellular viability to environmental stresses.[10] To this end various protective coatings have been explored including, silica,[12–14] silica-titania,[15] graphene,[16] polydopamine,[17] and an iron-tannate coordination complex.[8] Although these cell coatings offer a degree of protection from the external environment, controlled diffusion of molecules through the shell has not been demonstrated. However, an ideal protective shell should permit the transport of nutrients or chemical stimulants necessary for cell viability while preventing access of larger cytotoxic molecules, such as lytic enzymes, from accessing the cells. Accordingly, selectively controlling molecular transport through coatings for cells remains an important challenge.[18,19] In this study, we demonstrate that Metal-organic Frameworks can be effectively used for the production of porous cytoprotective exoskeletons for living cells.

Metal-organic Frameworks (MOFs), are an emerging class of porous materials,[20–22] that can be constructed from bio-friendly building blocks,[23] under physiological conditions.[24] Furthermore, they are thermally and chemically robust[23,24] and synthesized via a modular approach that facilitates fine tuning of their pore shape,[25] chemical functionality[26] and size.[27] This unique collection of properties point towards MOFs as potential candidate materials for artificial cell exoskeletons (**Scheme 1**).[18] Indeed, this concept is supported by recent studies that illustrated how biomolecules ranging from amino acids to enzymes can directly trigger the formation of MOF crystals via a biomimetic mineralization process,[24,28] overcoming the initial need for non-biological crystallization agents.[29,30] Additionally, this strategy was also successfully applied to thin films and patterned surfaces of proteins.[31] We hypothesized that the biomolecule-rich cell membranes and walls[32,33] could initiate concentrate MOF precursors and serve as an ideal interface for the crystallization MOFs thus leading to a porous exoskeleton encasing living cells. A recent study illustrated that yeast cell

walls can attract metal ions, which could serve as a reservoir for the formation of a MOF crystals.[34] However, in this case the synthetic conditions employed were incompatible with cell life, thus the yeast cells were merely employed as templating agents for the fabrication of MOF capsules. Although this work highlighted a new method for the preparation of complex MOF architectures, the potential use of MOFs for the protection of living cells remains unexplored. Here we demonstrate for the first time that a MOF material (ZIF-8[35]) can be crystalized on the surface of the living organisms *Saccharomyces cerevisiae* (baker's yeast, Eukaryote domain) and the bacterium *Micrococcus Luteus* (Bacteria domain). In both cases the MOF forms a protective coating on the respective cell walls that protects the organisms from the external environment while, remarkably, preserving cell life.

ZIF-8 coated yeast cells were fabricated by dispersing living cells in an aqueous solution of 2-methylimidazole (HmIm), followed by the addition of an aqueous solution of zinc acetate. After 10 min, the yeast cells were removed from solution and washed with water to remove the excess ZIF-8 precursors (Figure S1). To assess cell viability before and after application of the MOF coating, we performed two standard assays that employ the indicators fluorescein diacetate (FDA) and resazurin, respectively;[36,37] in both cases the fluorescent intensity remained unchanged before and after formation of the MOF coating, indicating that the ZIF-8 coating is essentially non-toxic to yeast cells (Figure S2). To confirm that ZIF-8 is non-toxic to yeast cells we performed a control test, where free ZIF-8 particles (average diameter c.a. 500 nm) and living yeast cells were combined in solution. After a 24 hr incubation period the viability of the ZIF-8 treated yeast cells was found to be essentially identical to that of untreated cells (Figure S3). This data confirms that ZIF-8 particles do not adversely affect yeast cells. We then analyzed the structure of the MOF exoskeleton by synchrotron small-angle X-ray scattering (SAXS). The resulting scattering pattern was comprised of peaks that were analogous in position and relative intensity to pure ZIF-8 (Figure S4), thus confirming

the structure and crystallinity of the coating. We also assessed the morphology and elemental distribution of the ZIF-8 coating using scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS), respectively. Analysis of c.a. 500 ZIF-8 coated yeast cells from SEM images revealed that all individual cells were homogeneously coated (i.e. partially naked cells or coatings on aggregates of cells were not observed). The elemental maps along with high-magnification SEM micrographs of the ZIF-8 coated cells are shown in Figure 1 a-e. Close analysis of the data clearly indicates a homogeneous distribution of Zn, O and C on the cell surface which, strongly supports the formation of a continuous ZIF-8 coating on individual yeast cells. Furthermore, SEM measurements performed on cross-sections of a crushed ZIF-8-coated yeast cell revealed an average ZIF-8 shell thickness of approximately 100 nm (Figure S5). We note that the thickness of the ZIF-8 coatings could be tuned in the 100-250 nm range by carrying out sequential ZIF-8 coating steps (Figure S6) thus demonstrating the precise control of this approach. Confocal scanning laser microscopy (CLSM) was also employed to assess the homogeneity of the ZIF-8 coating (Figure 1 f-h). For this experiment we labelled the yeast cells and ZIF-8 coating with FDA (green, Figure 1f) and Alexa Fluor 647 (red, Figure 1g), respectively. The high-resolution optical cross-section shown in Figure 1h is consistent with the SEM/EDS data and provides further evidence of a continuous coating of ZIF-8 over the entire cell. In order to assess the generality of this approach we examined the formation of ZIF-8 coatings on the bacterium *Micrococcus Luteus* that possess a peptidoglycans-based outer membrane. Micrococcus Luteus can survive in oligotrophic (nutrient deficient) environments and is of interest for biotechnological applications (e.g. terpenes biosynthesis[38]). Based on our investigation a ZIF-8 coating was successfully formed on these gram-positive cocci without affecting cell viability (Figure S7). This highlights the potential of employing ZIF-8 as an exoskeletal coating for a variety of basic functional biological units.

We next determined whether the ZIF-8 coating forms a selectively permeable exo-skeleton. Cellular functions are highly dependent on their microenvironment, which typically contain nutrients, neighbouring cells, soluble factors (e.g. growth factors), and cytokines.[4] Accordingly, the formation of an artificial coating around a living cell is expected to affect its behaviour by regulating molecular transport to and from the cytoplasm.[39–41]. To investigate the selective permeability of the MOF shell, ZIF-8 coated yeast cells were cultured in media containing yeast extract and glucose, an essential nutrient for maintaining cell health. Lyticase (MW = 54.6 kDa, roughly 5.3 nm), a cytotoxic enzyme,[42] was then added to the culturing media. The cell viability was monitored for 24 h using FDA, a fluorescent indicator that enables the detection of living cells. Over the course of the experiment the ZIF-8 coated yeast, did not give rise to a significant change in the fluorescent emission (5.3% and 19% loss in 3 and 24 hours, respectively), while for the unprotected, naked, yeast a 95% reduction in fluorescence was detected within 3 h (Figure 2a and Figure S8). This result suggests that lyticase, as expected, catalyzes cell lysis of the naked yeast cells and demonstrates that the ZIF-8 coating functions as a protective shell against this cytotoxic enzymatic agent. To further asses the cytoprotective capabilities of ZIF-8 coating on yeast cells, a small anti-fungal drug, filipin (MW = 655 Da, 1x1.3x1.9 nm) was selected (Figure S9). This antifungal drug was selected as the molecule is slightly bigger (3- to 5-fold) than the ZIF-8 interconnecting micropores.[43] FDA was used as a live cell indicator to assess the antifungal effects of filipin. After 24 h incubation in the presence of filipin the control sample (naked yeast) showed almost 100% mortality, while ZIF-8 coated yeast cells showed a minimal reduction in cell viability with less than 10% cells killed by the anti-fungal drug (Figure S10). These results indicate that the ZIF-8 coating is homogenous and can protect living cells from both relatively small molecules, such as anti-fungal chemicals and large cytotoxic proteins.

Artificially induced cell hibernation engenders extended cell lifetimes and therefore underpins the advancement of engineering strategies towards governing cell division, proliferation, and differentiation.[8,12,44,45] Thus we were motivated to investigate the time-dependent cellular responses of ZIF-8 coated yeast cells. First we carried out optical density measurements at 600 nm (OD_{600}) for both native yeast and ZIF-8 coated yeast in culturing media containing glucose as a nutrient at 30 °C. OD_{600} experiments quantitatively measure cell proliferation by determining turbidity increases after division and is widely used to study the stage of cultured cells,[46,47] i.e. whether they are in a lag phase, growth phase, stationary phase, or death phase. For native yeast, the OD_{600} remained stable for 6 h before exponential growth was observed (Figure 2b). This data indicates that the yeast cells were initially in a dormant/hibernation state (lag phase for 6 h) before entering a rapid cell proliferation (budding) state. In contrast, the OD_{600} measurements of ZIF-8 coated yeast showed no obvious growth even after 6 h. Accordingly, it can be concluded that the ZIF-8 shell maintained the yeast in an extended lag phase. We postulate that ZIF-8 exoskeleton acts as a physical restriction suppressing the yeast cells from budding, as proposed for metal–polyphenol cell coatings.[8] Given that EDTA can degrade ZIF-8 films[31] we explored its potential to 'switch-off' the artificially induced cell dormancy state. Figure 2b shows that subsequent to the addition of EDTA the yeast cells quickly moved to growth and germination states. We note that when compared to the incubation period of the naked cells (c.a. 6 h), ZIF-8 protected yeast is essentially instantaneous (c. a. a few minutes). This suggests that glucose, an essential nutrient, was able to diffuse through the ZIF-8 coating and maintain cell life (Figure S11). Previous reports have also confirmed that glucose can pass through ZIF-8 crystals, despite the relatively small window size.[48,49] Furthermore, the OD_{600} data also showed that the growth rate and final cell number of the yeast, after the removal of the ZIF-8 shell, reached a similar level to native yeast (Figure 2b and Figure S12). Thus it can be concluded that the ZIF-8 coatings have no measurably negative impact on the yeast cells. In summary, our results show that a ZIF-8

shell can extend the cell's lifetime, by artificially supressing cell division, without significantly affecting the activity of cells in the growth state.

In conclusion, we have reported for the first time the preparation of crystalline MOF protective coatings for living cells. Based on previous work,[24,28,31] we postulate that the formation of a robust ZIF-8 exo-skeleton under physiological conditions is triggered by the biomolecule rich surface of living yeast cells and bacteria. We posit that glycoproteins and peptidoglycans locally concentrate MOF precursors playing a role similar to the one disclosed for the bovine serum albumin (BSA).[24,31] Remarkably, the MOF coating controlled molecular trafficking to the cell cytoplasm and prevented cell division by inducing an artificial hibernation state. However, after removal of the ZIF-8 shell the cells immediately regained full functionality. We note that such precise control over cellular behaviour is analogous to that observed in some unicellular organisms, which construct and dissolve exterior coatings as a protective mechanism. Accordingly, employing MOFs as a strategy to mimic natural protective mechanisms provides a new promising tool for the further progress of single cell in medicine and biotechnology.

Experimental

Experimental Details including materials, formation of ZIF-8 coatings on cell surfaces, cell viability test, lyticase assay, cell division experiment, and instrumentation are documented in the Supporting Information.

Acknowledgements
K.L. and J.J.R. acknowledge CSIRO OCE Science Scheme and Manufacturing FST SPG grant. This work was supported by the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology (Project No. CE140100036) and performed in part at the Materials Characterization and Fabrication Platform (MCFP) at The University of Melbourne and the Victorian Node of the Australian National Fabrication Facility (ANFF). Part of this research was conducted at the SAXS/WAXS beamline at the Australian Synchrotron.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

[1] E. Shapiro, T. Biezuner, S. Linnarsson, *Nat. Rev. Genet.* **2013**, *14*, 618.

[2] N. de Souza, *Nat. Methods* **2011**, *8*, S1.

[3] C. Gawad, W. Koh, S. R. Quake, *Nat. Rev. Genet.* **2016**, *advance on*, DOI 10.1038/nrg.2015.16.

[4] J. El-Ali, P. K. Sorger, K. F. Jensen, *Nature* **2006**, *442*, 403.

[5] S. Wang, Z. Guo, *Colloids Surf. B. Biointerfaces* **2014**, *113*, 483.

[6] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, P. Wang, *Chem. Rev.* **2014**, *114*, 6423.

[7] P. T. McKenney, A. Driks, P. Eichenberger, *Nat. Rev. Microbiol.* **2013**, *11*, 33.

[8] J. H. Park, K. Kim, J. Lee, J. Y. Choi, D. Hong, S. H. Yang, F. Caruso, Y. Lee, I. S. Choi, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 12420.

[9] X. Chen, L. Mahadevan, A. Driks, O. Sahin, *Nat. Nanotechnol.* **2014**, *9*, 137.

[10] S. H. Yang, D. Hong, J. Lee, E. H. Ko, I. S. Choi, *Small* **2013**, *9*, 178.

[11] J. Burgain, C. Gaiani, M. Linder, J. Scher, *J. Food Eng.* **2011**, *104*, 467.

[12] H. K. Baca, C. Ashley, E. Carnes, D. Lopez, J. Flemming, D. Dunphy, S. Singh, Z. Chen, N. Liu, H. Fan, G. P. López, S. M. Brozik, M. Werner-Washburne, C. J. Brinker, *Science* **2006**, *313*, 337.

[13] B. Kaehr, J. L. Townson, R. M. Kalinich, Y. H. Awad, B. S. Swartzentruber, D. R. Dunphy, C. J. Brinker, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 17336.

[14] J. Lee, J. Choi, J. H. Park, M.-H. Kim, D. Hong, H. Cho, S. H. Yang, I. S. Choi, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 8056.

[15] E. H. Ko, Y. Yoon, J. H. Park, S. H. Yang, D. Hong, K.-B. Lee, H. K. Shon, T. G. Lee, I. S. Choi, *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 12279.

[16] R. Kempaiah, S. Salgado, W. L. Chung, V. Maheshwari, *Chem. Commun. (Camb).*

2011, *47*, 11480.

[17] S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee, I. S. Choi, *J. Am. Chem. Soc.* **2011**, *133*, 2795.

[18] G. Orive, R. M. Hernández, A. R. Gascón, R. Calafiore, T. M. S. Chang, P. De Vos, G. Hortelano, D. Hunkeler, I. Lacík, A. M. J. Shapiro, J. L. Pedraz, *Nat. Med.* **2003**, *9*, 104.

[19] M. Edidin, *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 414.

[20] O. M. Yaghi, M. O'Keeffe, N. W. Ockwig, H. K. Chae, M. Eddaoudi, J. Kim, *Nature* **2003**, *423*, 705.

[21] H.-C. J. Zhou, S. Kitagawa, *Chem. Soc. Rev.* **2014**, *43*, 5415.

[22] H. Furukawa, K. E. Cordova, M. O'Keeffe, O. M. Yaghi, *Science* **2013**, *341*, 1230444.

[23] R. A. Smaldone, R. S. Forgan, H. Furukawa, J. J. Gassensmith, A. M. Z. Slawin, O. M. Yaghi, J. F. Stoddart, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 8630.

[24] K. Liang, R. Ricco, C. M. Doherty, M. J. Styles, S. Bell, N. Kirby, S. Mudie, D. Haylock, A. J. Hill, C. J. Doonan, P. Falcaro, *Nat. Commun.* **2015**, *6*, 7240.

[25] E. D. Bloch, W. L. Queen, R. Krishna, J. M. Zadrozny, C. M. Brown, J. R. Long, *Science* **2012**, *335*, 1606.

[26] H. Deng, C. J. Doonan, H. Furukawa, R. B. Ferreira, J. Towne, C. B. Knobler, B. Wang, O. M. Yaghi, *Science* **2010**, *327*, 846.

[27] H. Deng, S. Grunder, K. E. Cordova, C. Valente, H. Furukawa, M. Hmadeh, F. Gándara, A. C. Whalley, Z. Liu, S. Asahina, H. Kazumori, M. O'Keeffe, O. Terasaki, J. F. Stoddart, O. M. Yaghi, *Science* **2012**, *336*, 1018.

[28] K. Liang, R. Ricco, C. M. Doherty, M. J. Styles, P. Falcaro, *CrystEngComm* **2016**, DOI: 10.1039/C5CE02549D.

[29] F. Lyu, Y. Zhang, R. N. Zare, J. Ge, Z. Liu, *Nano Lett.* **2014**, *14*, 5761.

[30] F.-K. Shieh, S.-C. Wang, C.-I. Yen, C.-C. Wu, S. Dutta, L.-Y. Chou, J. V Morabito, P. Hu, M.-H. Hsu, K. C.-W. Wu, C.-K. Tsung, *J. Am. Chem. Soc.* **2015**, *137*, 4276.

[31] K. Liang, C. Carbonell, M. J. Styles, R. Ricco, J. Cui, J. J. Richardson, D. Maspoch, F. Caruso, P. Falcaro, *Adv. Mater.* **2015**, *27*, 7293.

[32] J. Zimmerberg, M. M. Kozlov, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 9.

[33] D. J. Cosgrove, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 850.

[34] W. Li, Y. Zhang, Z. Xu, Q. Meng, Z. Fan, S. Ye, G. Zhang, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 955.

[35] A. Phan, C. J. Doonan, F. J. Uribe-Romo, C. B. Knobler, M. O'Keeffe, O. M. Yaghi, *Acc. Chem. Res.* **2010**, *43*, 58.

[36] T. L. Riss, R. A. Moravec, A. L. Niles, H. A. Benink, T. J. Worzella, L. Minor, **2015**.

[37] D. A. Fontvieille, A. Outaguerouine, D. R. Thevenot, *Environ. Technol.* **1992**, *13*, 531.

[38] P. M. Dewick, *Nat. Prod. Rep.* **2002**, *19*, 181.

[39] M. Overholtzer, J. S. Brugge, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 796.

[40] A. E. Nel, L. Mädler, D. Velegol, T. Xia, E. M. V Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, *Nat. Mater.* **2009**, *8*, 543.

[41] X. Yao, R. Peng, J. Ding, *Adv. Mater.* **2013**, *25*, 5257.

[42] R. M. Taskova, H. Zorn, U. Krings, H. Bouws, R. G. Berger, *Zeitschrift für Naturforsch. C* **2006**, *61*, 347.

[43] A. Huang, Q. Liu, N. Wang, Y. Zhu, J. Caro, *J. Am. Chem. Soc.* **2014**, *136*, 14686.

[44] I. Canton, N. J. Warren, A. Chahal, K. Amps, A. Wood, R. Weightman, E. Wang, H. Moore, S. P. Armes, *ACS Cent. Sci.* **2016**, acscentsci.5b00370.

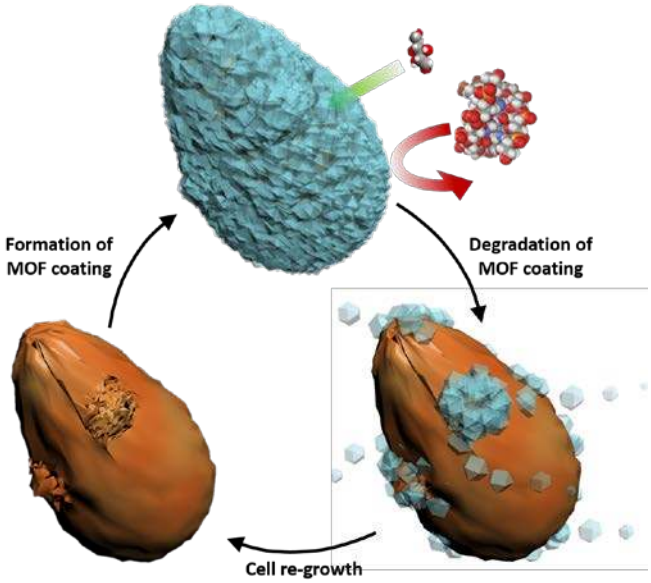
[45] W. Li, T. Guan, X. Zhang, Z. Wang, M. Wang, W. Zhong, H. Feng, M. Xing, J. Kong, *ACS Appl. Mater. Interfaces* **2015**, *7*, 3018.

[46] A. L. Koch, *Biochim. Biophys. Acta* **1961**, *51*, 429.

[47] T. Tanaka, T. H. Rabbitts, *Nat. Protoc.* **2010**, *5*, 67.

[48] X. Wu, J. Ge, C. Yang, M. Hou, Z. Liu, *Chem. Commun. (Camb).* **2015**, *51*, 13408.

[49] X. Wu, C. Yang, J. Ge, Z. Liu, *Nanoscale* **2015**, *7*, 18883.



Scheme 1. Schematic illustration of biomimetic crystallization of cytoprotective metal-organic framework coatings on living cells.

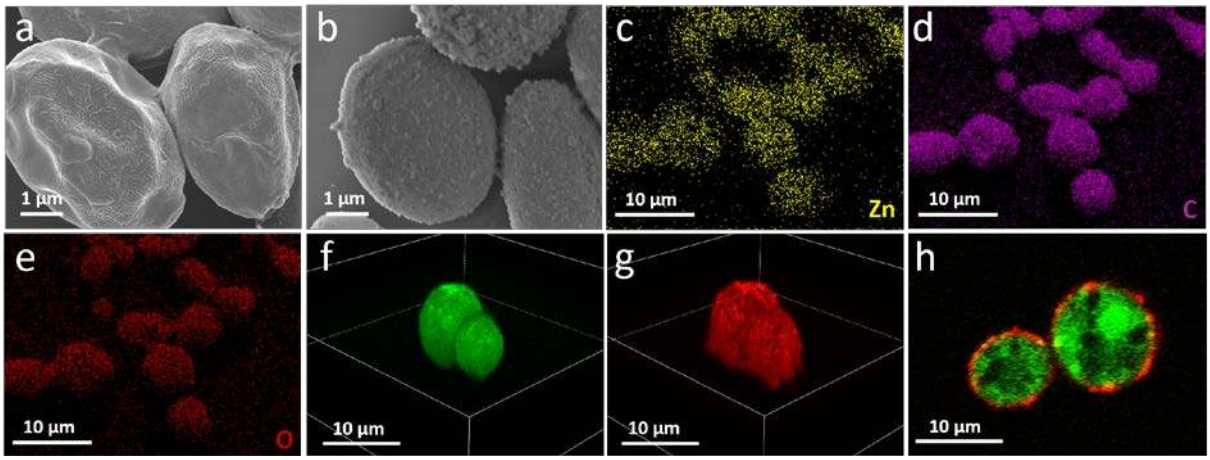


Figure 1. SEM images of (a) native yeast and (b) ZIF-8 coated yeast. (c-e) EDS elemental mappings of ZIF-8 coated yeast. 3D cellular reconstruction of CLSM images of ZIF-8 coated yeast cells (f-g), and (h) cellular cross-section. The living yeast cells were labelled with FDA (green) and the ZIF-8 coatings were labelled by infiltration of Alexa Fluor 647 fluorescent dyes (red).

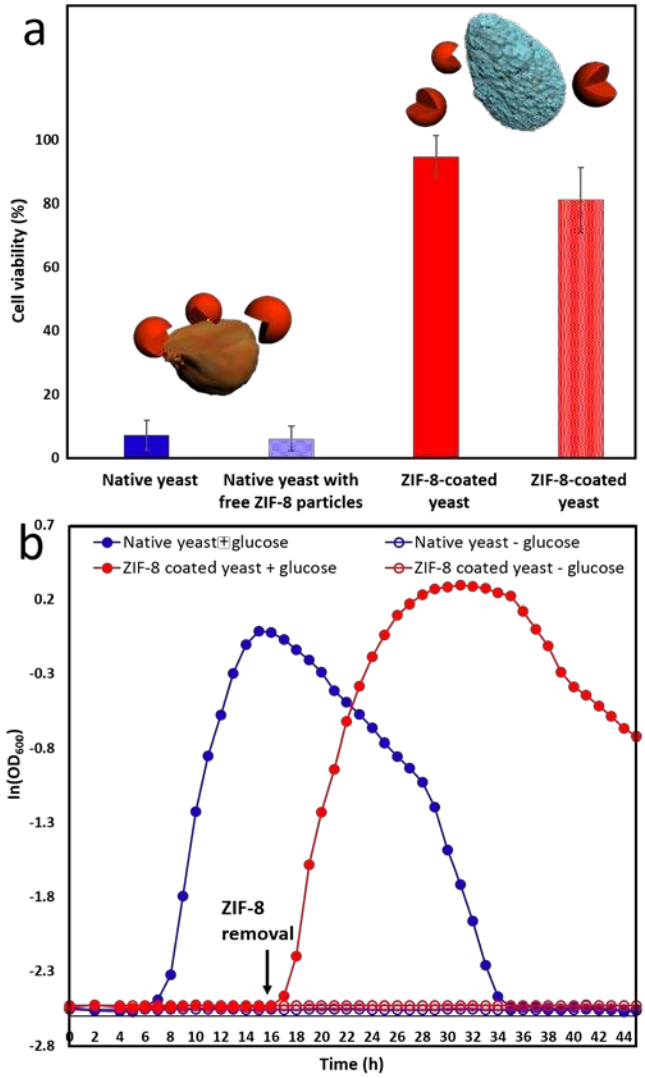


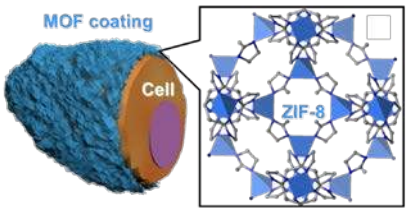
Figure 2. (a) Cell viability (%) of native yeast (blue) and native yeast with free ZIF-8 particles (patterned blue) in the presence of cell lysis enzyme lyticase for 3 h, and ZIF-8 coated yeast in the presence lyticase for 3 h (red) and 24 h (patterned red). (b) Yeast growth measurement (OD_{600}) for native (blue circles) and ZIF-8 coated yeast (red circles) before and after the removal of MOF coatings by EDTA. Experiments were performed in triplicates.

We report for the first time the biomimetic mineralization of Metal-organic Framework (MOF) material on living cells. ZIF-8 can be crystallized on living cell surface as an exoskeleton that offers protection from enzymatic digestion while still allowing transport of essential nutrients from the extracellular environment, thus maintaining cells alive. Remarkably, the ZIF-8 shell prevents cell division, leading to an artificially induced pseudo-hibernation state. Upon removal of the MOF coating, cellular functions, including division, are restored.

Keyword (cytoprotection, cell coatings, biomimetic, metal-organic frameworks, nanoporous materials)

K. Liang,* J. J. Richardson, J. Cui, F. Caruso, C. J. Doonan,* P. Falcaro*

Biomimetic Crystallization of Metal-Organic Framework Coatings as Cytoprotective Exoskeletons for Living Cells



Submitted to ADVANCED MATERIALS

SUPPORTING INFORMATION

Biomimetic Crystallization of Metal-Organic Framework Coatings as Cytoprotective Exoskeleton for Living Cells

Kang Liang,[a] Joseph J. Richardson,[a] Jiwei Cui,[b] Frank Caruso,[b] Christian J. Doonan,[c] and Paolo Falcaro[d]

[a]CSIRO Manufacturing, Clayton, Victoria 3168, Australia
[b]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the Department of Chemical and Biomolecular Engineering, The University of Melbourne Parkville, Victoria 3010, Australia
[c]School of Chemistry and Physics, The University of Adelaide, Adelaide, South Australia 5005, Australia
[d]Institute of Physical and Theoretical Chemistry, Graz University of Technology, Stremayrgasse 9, 8010 Graz, Austria

Table of Contents

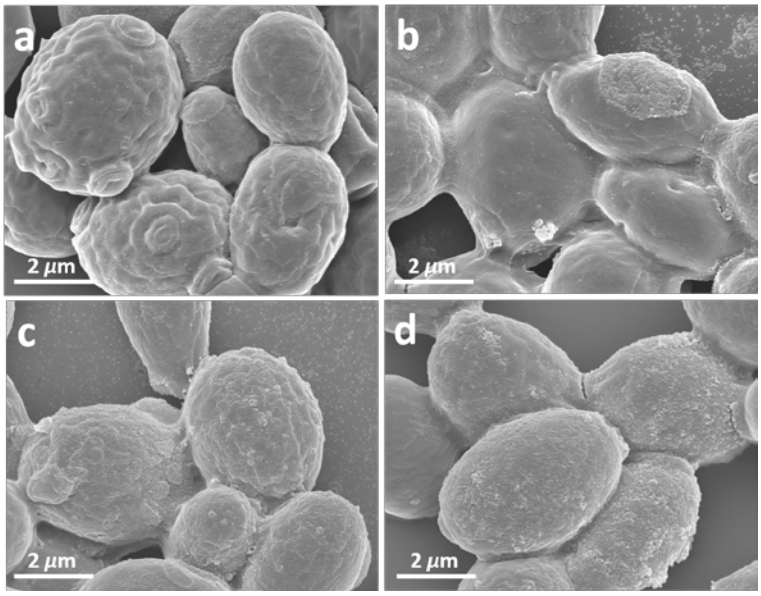


Figure S1. SEM images of yeast cells after incubation with ZIF-8 precursor solutions for (a) 1 min, (b) 5 min, (c) 10 min, and (d) 20 min. The presence of coating surrounding the yeast was detected after 10 min incubation.

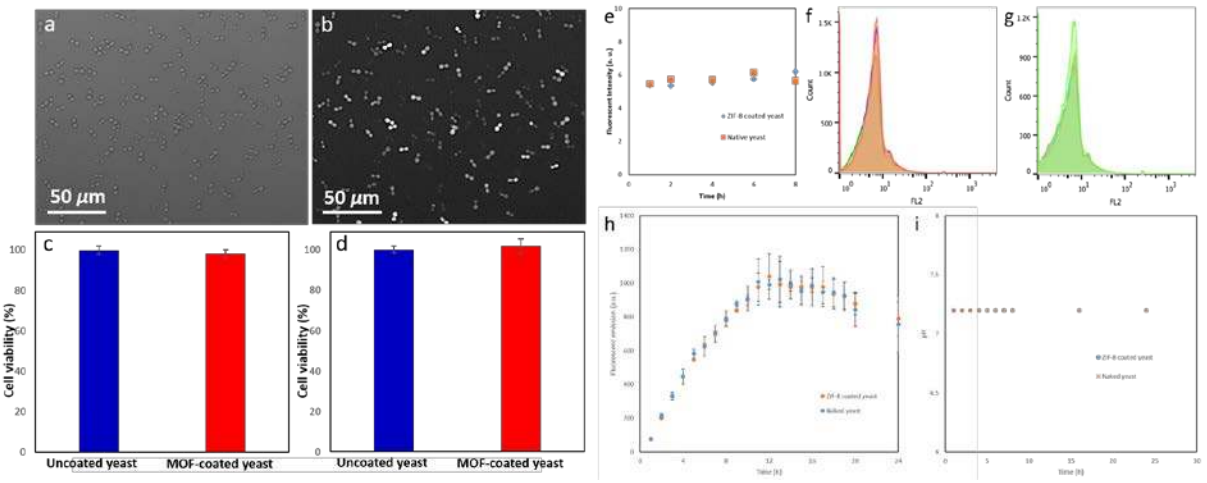


Figure S2. (a) Differential interference contrast (DIC) and (b) fluorescent microscopy images of ZIF-8 coated yeast cells using FDA as a fluorescent and viability indicator. (c) FDA and (d) resazurin cell viability assay on naked and ZIF-8 coated yeast cells. (e) Plot of flow cytometry studies showing the ATP metabolic activity of individual (f) naked and (g) ZIF-8 coated yeasts cells over time, using resazurin as a fluorescent indicator. (h) Fluorescent intensity of overall yeast cells over time, using resazurin as a fluorescent indicator. (i) pH measurement of yeast culture solution as an indication of ammonia production over time.

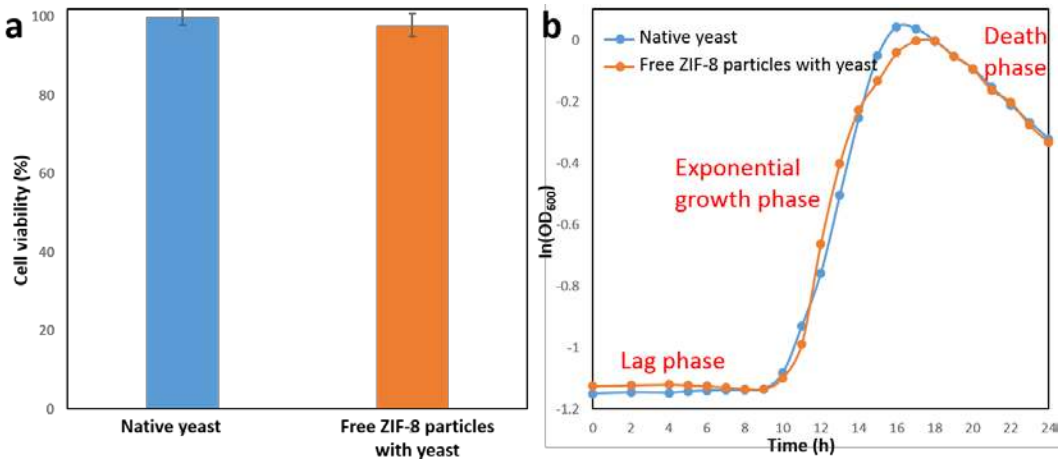


Figure S3. (a) FDA cell viability assay on naked yeast cells and yeast cells in the presence of free ZIF-8 particles (0.1 mg/mL). (b) Yeast growth measurement (OD_{600}) for native yeasts and yeasts in the presence of free ZIF-8 particles (0.1 mg/mL).

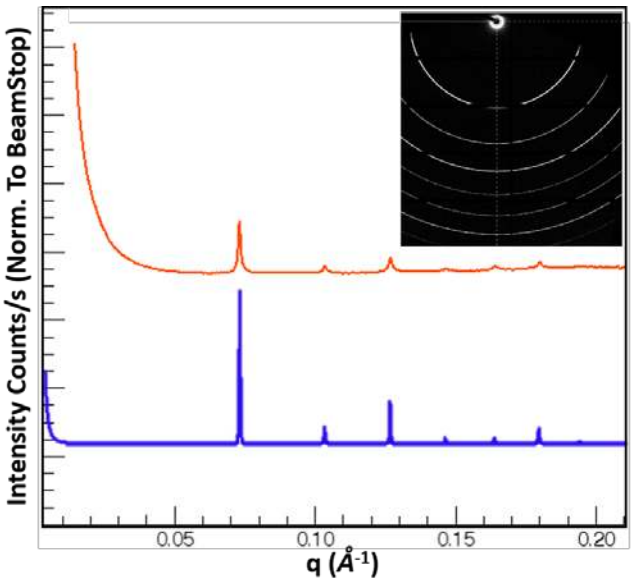


Figure S4. Synchrotron small-angle X-ray scattering (SAXS) diffraction patterns of standard ZIF-8 crystals (blue) and ZIF-8 coated yeast cells (red). Inset: 2D SAXS pattern of the ZIF-8 coated yeast cells (measure using a 0.5 mm capillary as a sample holder).

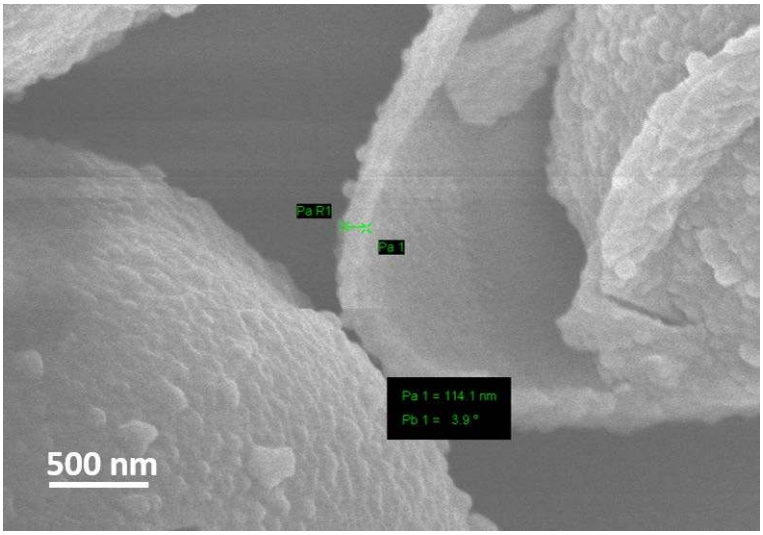


Figure S5. Cross-sectional SEM image of a cracked ZIF-8 shell on yeast showing the thickness of the ZIF-8 coating.

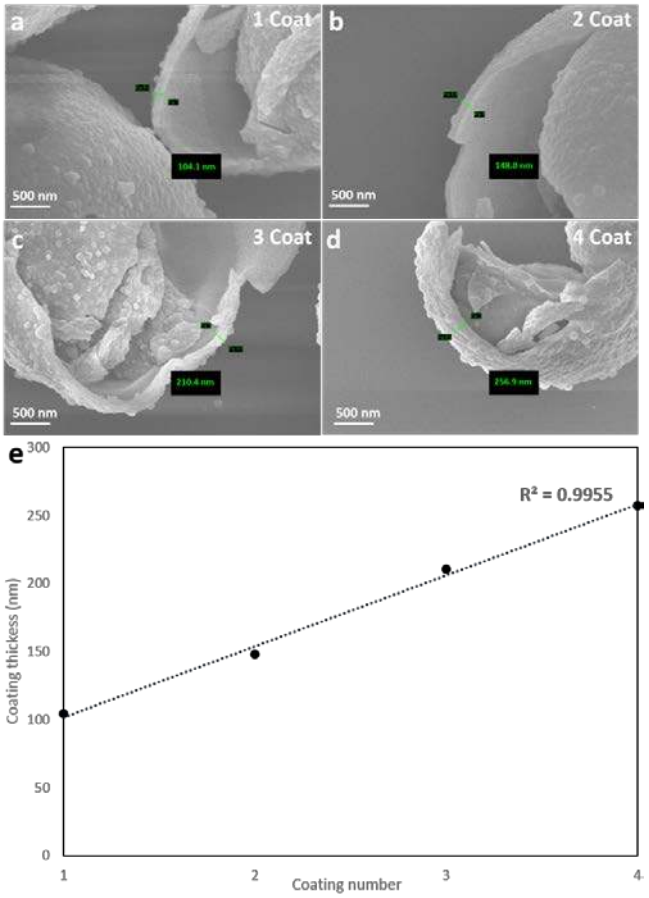


Figure S6. Cross-sectional SEM images of a cracked ZIF-8 shell on yeast. The SEM was used to measure the thickness of ZIF-8 coatings from 1 to 4 coating cycles. (a) 1 coating cycle, (b) 2 coating cycles, (c) 3 coating cycles, (d) 4 coating cycles. (e) Plot showing the corresponding ZIF-8 coating thickness with number of subsequent coating cycles.

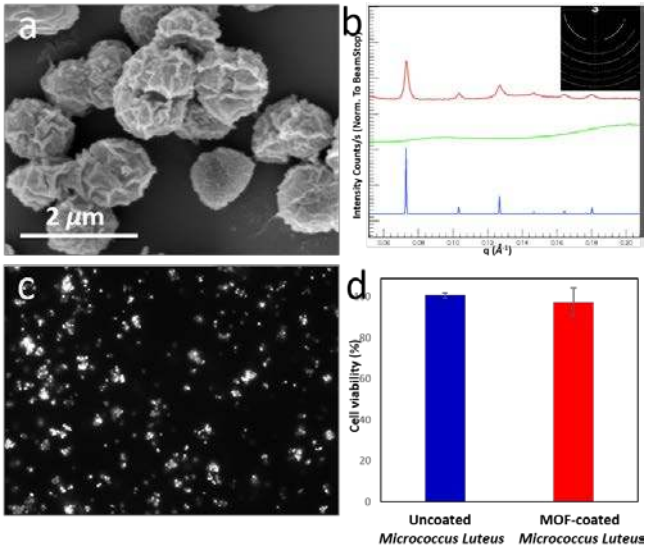


Figure S7. (a) SEM image of ZIF-8 coated *Micrococcus Luteus*. (b) SAXS diffraction patterns of standard ZIF-8 crystals (blue), native *Micrococcus Luteus* (green), and ZIF-8 coated *Micrococcus Luteus* (red). Inset: 2D representation of SAXS patterns of the ZIF-8 coated bacteria. (c) Fluorescent microscopy image of ZIF-8 coated *Micrococcus Luteus*. FDA was used as a fluorescent cell viability indicator. (d) FDA cell viability assay on naked and ZIF-8 coated *Micrococcus Luteus*.

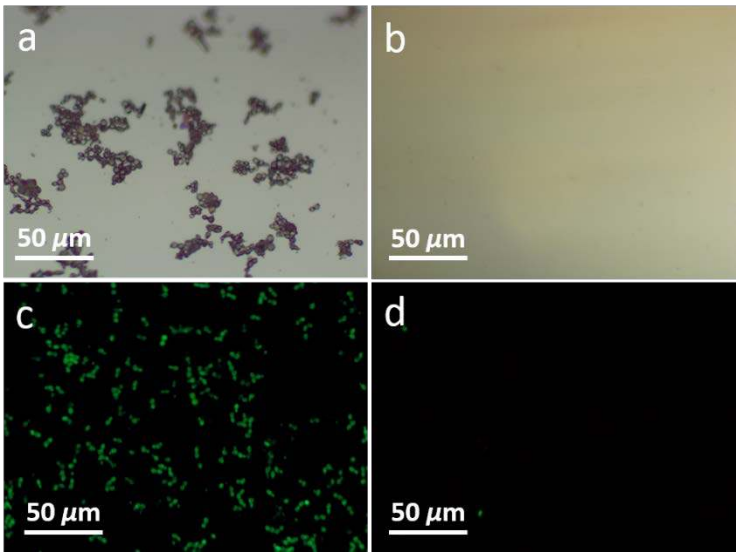


Figure S8. DIC microscopy images of (a) ZIF-8 coated yeast cells and (b) naked yeast cells after 3 h incubation in the presence of lyticase. Fluorescent microscopy images of (c) ZIF-8 coated yeast cells and (d) naked yeast cells after 3 h incubation in the presence of lyticase. FDA was used as a fluorescent cell viability indicator.

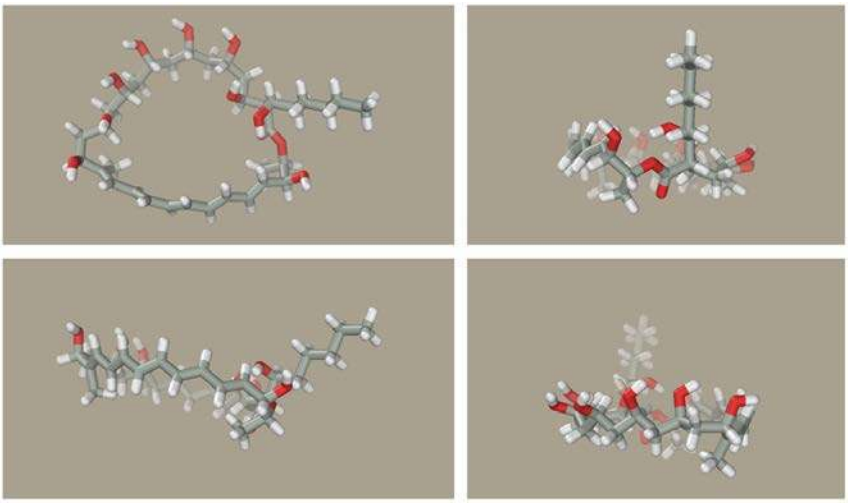


Figure S9. Molecular structure of filipin antifungal drug. A rough estimation of the molecular dimensions suggests that the molecule can be encaged within a box with dimensions c.a. 1x1.3x1.9 nm.

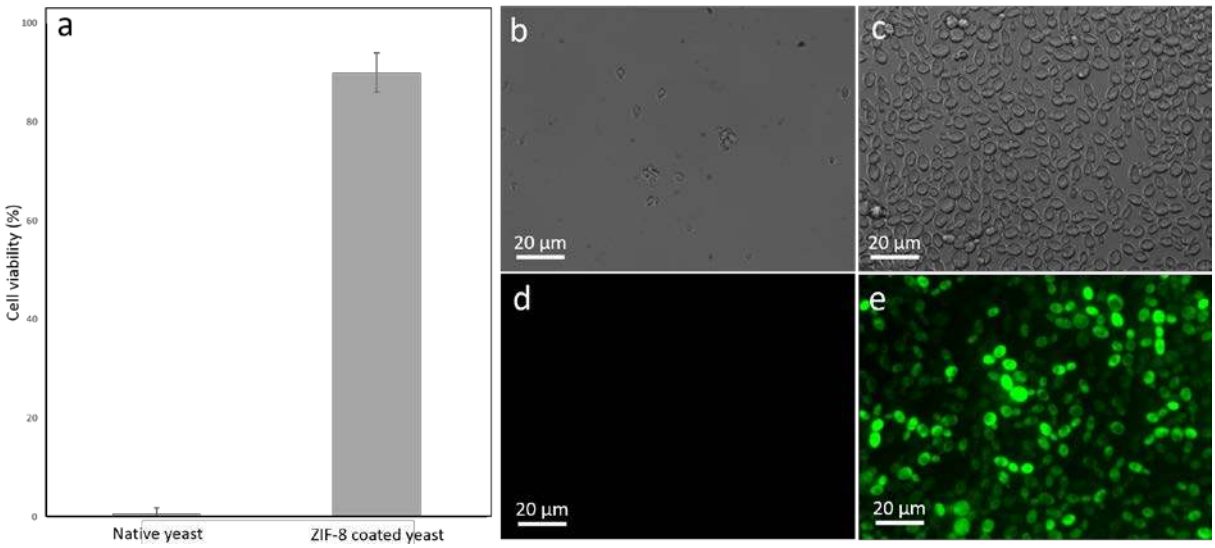


Figure S10. (a) Cell viability study of ZIF-8 coated and naked yeast cells after 24 h incubation using filipin as an anti-fungal drug. DIC and fluorescent microscopy images of (b, d) naked yeast and (c, e) ZIF-8 coated yeast after 24 h incubation with filipin. Live cells are labeled with FDA.

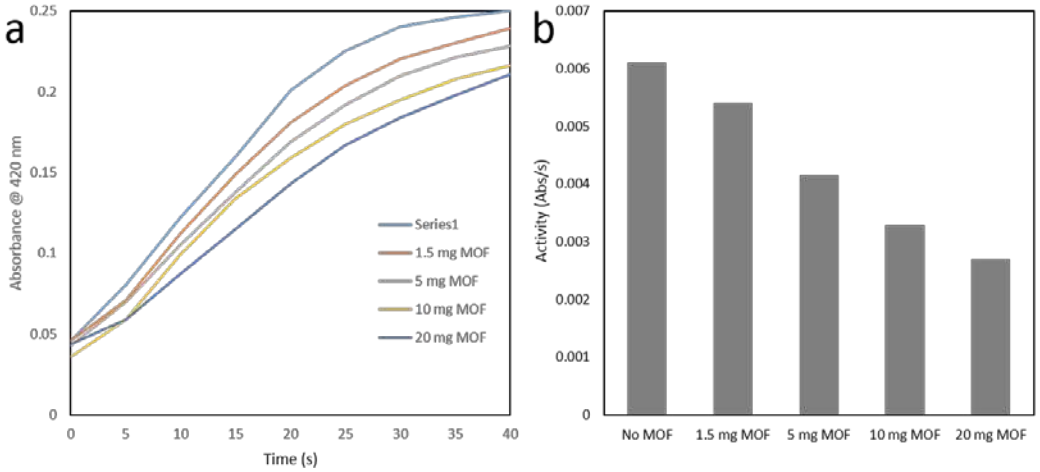


Figure S11. (a) Glucose oxidase-horseradish peroxidase coupled enzyme activity assay and (b) the initial gradient as a measurement of the amount of glucose.

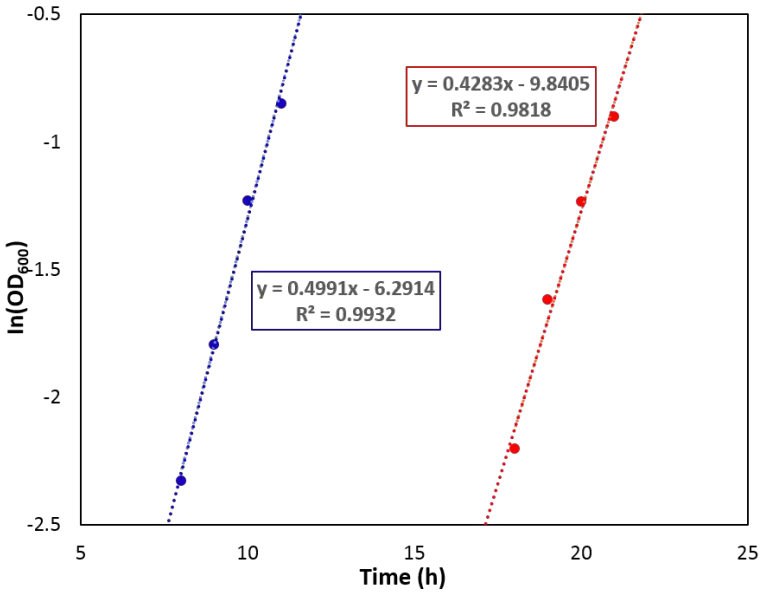


Figure S12. Growth phase linear-fitted plot of $\ln(OD_{600})$ vs. time for native yeast (blue) and ZIF-8 coated yeast after ZIF-8 removal by EDTA (red). The growth rate is calculated from the slope of the linear fitted line. ZIF-8 coated yeast after the coating removal retained 86% of the original growth rate of native yeast.

Materials and Methods

Saccharomyces cerevisiae (Baker's yeast), *Micrococcus Luteus*, yeast extract, zine acetate dihydrate, 2-methylimidazole (HmIm), D-(+)-glucose, lyticase from *Bacillus subtilis* (≥500 U mL^{-1}), resazurin sodium salt, filipin, and methylene blue were purchased from Sigma Aldrich. Fluorescein diacetate (FDA) was purchased from Life Technologies (Australia). All the other reagents were purchased from Sigma Aldrich (Australia) and used without further modification.

Formation of ZIF-8 coatings on living yeast. 2 mg dry yeast cells were cultured in the yeast culture media containing yeast extract (10 mg mL^{-1}) and glucose (20 mg mL^{-1}) with continuous shaking at 30 °C for 18 h. The yeast cells (were washed with deionized (DI) water three times and finally suspended in 5 mL aqueous solution of HmIm (160 mM). 5 mL aqueous solution of zinc acetate dihydrate (40 mM) was then added into the HmIm solution containing the yeast cells. The mixture was placed on a shaking stage (300 rpm) for 10 min for the formation of ZIF-8 coatings. The coated cells were washed with DI water three times to remove the excess ZIF-8 precursors, and finally suspended in DI water. The formation of ZIF-8 coatings on *Micrococcus Luteus* was obtained following the same procedure disclosed above, this time using bacteria cells (*Micrococcus Luteus*) with the same amount of the yeast cells were used instead of yeast cells in the ZIF-8 coating process.

Viability test. Cell viability was studied by the FDA and resazurin assay independently.[1–3] Metabolically active cells can hydrolyze FDA into fluorescently bright fluorescein by esterases, while resazurin measures the mitochondrial activity within the cells. FDA stock solution was prepared by dissolving 5 mg of FDA in 1 mL acetone. To each 0.2 mL of the yeast suspension, 2 μL of the FDA stock solution was added and incubated at 30 °C for 20 min. The cells were then washed three times with DI water to remove free dyes in the solution. For resazurin assay, 20 μL resazurin solution (0.15 mg mL^{-1} in DPBS) was added into each 0.2 mL yeast suspension and incubated at 30 °C for 2 h. The cells were then washed three times with DI water to remove free dyes in the solution.

Lyticase assay. Lyticase is widely used as cell lytic enzymes by enzymatic digestion of cell wall.[4] 2000 U lyticase was added into each 1 mL of DPBS solution containing naked or ZIF-8 coated yeast cells. The mixture solution was placed onto a shaking stage (300 rpm) at 30 °C. The cell viability was monitored using FDA (refer to Viability test section for experimental details).

Filipin anti-fungal assay. Anti-fungal drug filipin was dissolved in DMSO and added to each wells containing the yeast cells in the yeast growth media in a 96-well plate to a final concentration of 200 μM filipin. The plate was incubated at 30 °C for 24 h under constant, soft shaking. After 24 h incubation, FDA was used as an indicator for living yeast cells. The ZIF-8 coated yeast cells were firstly treated with EDTA (100 mM, 10 μL) to dissolve the ZIF-8 coatings before the addition of FDA. To each well containing 200 μL yeast solution, FDA solution (2 μL, 5 mg mL^{-1}) was then added and incubated for 20 min. The cells were then washed three times with DI water to remove free dyes in the solution and finally observed under a fluorescent microscope.

Cell division experiment. Both ZIF-8 coated and naked yeast cells were suspended and diluted in the yeast culture media containing yeast extract (10 mg mL^{-1}) with or without glucose (20 mg mL^{-1}) with appropriate yeast concentration to yield OD_{600} (optical density at 600 nm) value of 0.2 - 0.3. 200 μL of the yeast suspension was transferred into each well of a

96-well microplate. The microplate was inserted into a microplate reader with constant shaking at 250 rpm, and the OD_{600} value was recorded continuously at 30 min interval.

Instrumentation. Optical micrographs (DIC and fluorescence) were obtained using an Olympus BX60M microscope. Scanning electron microscope (SEM) images of samples were taken on a Zeiss MERLIN SEM at an accelerating voltage of 5.0 kV. Confocal microscopy images were acquired via a Nikon A1R confocal laser scanning microscope. Synchrotron SAXS data were collected at the SAXS/WAXS beamline at the Australian Synchrotron.[5] Diffraction patterns were collected using a Pilatus 1M detector.

Glucose oxidase (GOX) and peroxidase (HRP) coupled enzymatic assay. GOX-HRP coupled enzyme activity assay was used to compare the amount of glucose after the addition of ZIF-8. Various amount of ZIF-8 crystals were added in 1 mL glucose solution (50 mg mL^{-1}) and incubated for 2 h under constant shaking. After the removal of ZIF-8, the coupled enzymatic reagents was used to measure the remaining glucose in the solution. In a UV-Vis cuvette, a mixture containing 10 µL GOX (2 mg mL^{-1}), 10 µL HRP (2 mg mL^{-1}), 250 µL pyrogallol (50 mg mL^{-1}), 250 µL glucose solution, and 2.5 mL deionized water were mixed and immediately measured under a UV-Vis spectrophotometer at 420 nm using 5 s interval. The initial gradient of the UV-Vis curve was used to assess the amount of glucose.

References

(1) Riss, T. L.; Moravec, R. A.; Niles, A. L.; Benink, H. A.; Worzella, T. J.; Minor, L. Eli Lilly & Company and the National Center for Advancing Translational Sciences June 29, 2015.

(2) Fontvieille, D. A.; Outaguerouine, A.; Thevenot, D. R. *Environ. Technol.* **1992**, *13* (6), 531–540.

(3) Park, J. H.; Kim, K.; Lee, J.; Choi, J. Y.; Hong, D.; Yang, S. H.; Caruso, F.; Lee, Y.; Choi, I. S. *Angew. Chem. Int. Ed. Engl.* **2014**, *53* (46), 12420–12425.

(4) Taskova, R. M.; Zorn, H.; Krings, U.; Bouws, H.; Berger, R. G. *Zeitschrift für Naturforsch. C* **2006**, *61* (5-6), 347–350.

(5) Kirby, N. M.; Mudie, S. T.; Hawley, A. M.; Cookson, D. J.; Mertens, H. D. T.; Cowieson, N.; Samardzic-Boban, V. *J. Appl. Crystallogr.* **2013**, *46* (6), 1670–1680.

Author/s:
Liang, K;Richardson, JJ;Cui, J;Caruso, F;Doonan, CJ;Falcaro, P

Title:
Metal-Organic Framework Coatings as Cytoprotective Exoskeletons for Living Cells

Date:
2016-09-28

Citation:
Liang, K., Richardson, J. J., Cui, J., Caruso, F., Doonan, C. J. & Falcaro, P. (2016). Metal-Organic Framework Coatings as Cytoprotective Exoskeletons for Living Cells. ADVANCED MATERIALS, 28 (36), pp.7910-7914. https://doi.org/10.1002/adma.201602335.

Persistent Link:
http://hdl.handle.net/11343/113806