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Author/s:
Riccò, R;Liang, W;Li, S;Gassensmith, JJ;Caruso, F;Doonan, C;Falcaro, P

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Metal–Organic Frameworks for Cell and Virus Biology: A Perspective

Raffaele Riccò,[1] Weibin Liang,[4] Shaobo Li,[2] Jeremiah J. Gassensmith,[2,*] Frank Caruso,[3]

Christian Doonan,[4,*] and Paolo Falcaro[1,4,*]

[1]Institute of Physical and Theoretical Chemistry, Graz University of Technology, Stremayrgasse 9, 8010 Graz, Austria

[2]Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080, United States

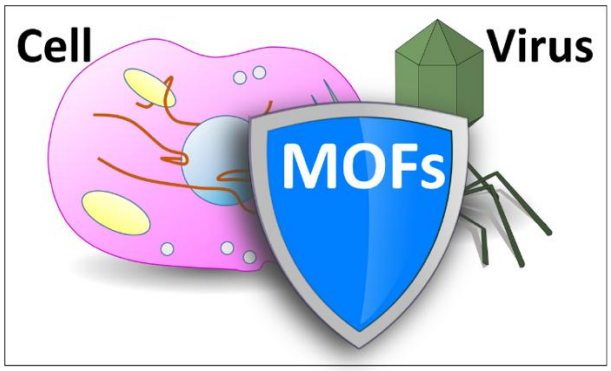
3ARC Centre of Excellence in Convergent Bio-Nano Science and Technology the Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

[4]Department of Chemistry, School of Physical Sciences, The University of Adelaide, North Terrace Campus, Adelaide SA 5005, Australia.

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ABSTRACT: Metal–organic frameworks (MOFs) are a class of coordination polymers, consisting of metal ions or clusters linked together by chemically mutable organic groups. In contrast to zeolites and porous carbons, MOFs are constructed from a building-block strategy that enables molecular level control of pore size/shape and functionality. An area of growing interest in MOF chemistry is the synthesis of MOF-based composite materials. Indeed, recent studies have shown that MOFs can be combined with biomacromolecules to generate novel biocomposites. In such materials, the MOF acts as a porous matrix that can encapsulate enzymes, oligonucleotides, or even more complex structures that are capable of replication/reproduction (*i.e.* viruses, bacteria, and eukaryotic cells). The synthetic approach for the preparation of these materials has been termed 'biomimetic mineralization', as it mimics natural biomineralization processes that afford protective shells around living systems. In this Perspective, we focus on the preparation of MOF biocomposites that are composed of complex biological moieties such as

viruses and cells, and canvass the potential applications of this encapsulation strategy to cell biology and biotechnology.



Biomineralization is a natural process whereby inorganic materials (biominerals) are grown on living organisms.[1–4] A variety of natural systems including fungi, mollusks,[3] diatoms, radiolarians, and mammalian have developed methods to introduce or to improve mechanical support, motility, protection, and sensing, through engineered biominerals; examples include carapaces, frustules, skeletons, and spikes.[2,4,5] Biomineralization has been exploited in all the taxonomic kingdoms (Animalia, Archaea, Bacteria, Fungi, Plantae, and Protista) since the beginning of the Cambrian geological period more than 500 million years ago.[6,7] Remarkably, organisms are able to promote and to regulate the formation of biominerals with molecular level precision.[4] For example, the shape of hydroxyapatite in bones and in tooth enamel is a biomineralization process.[8]

Inspired by nature, scientists have explored and developed an understanding of biomineralization strategies in the laboratory.[9] By applying self-assembly strategies, a number of hybrid systems have been synthesized where inorganic or organic materials are formed on a variety of biological

moieties ranging from proteins to living cells. In this Perspective, we refer to biomimetic mineralization as a process that produces synthetic coatings on living systems that would not otherwise occur naturally.[10] This approach offers many opportunities for multidisciplinary research; with respect to cells, hardened encasing could facilitate control of cell behavior,[11] and provide enhanced resistance toward unfavorable environments (*e.g.*, heat, UV radiation, mechanical stress, lytic agents, enzymatic inhibitors, *etc.*).[11,12] Furthermore, if the coating is porous, it can function as a perm-selective barrier for the transport of biologically relevant substrates[13] or act as a matrix to encapsulate enzymes.[14,15]

Silicon dioxide (SiO_2) has been widely studied for coating cells *via* biomimetic mineralization.[16–19] These studies include yeast cells,[20–22] eukaryotic cells,[19,23–25] bacteria,[26,27] and other bio-entities such as viruses.[28] The exploration of SiO_2 was motivated by the desire to trigger and to control the formation of synthetic inorganic coatings under physiological conditions.[29] This field is now attracting trans-disciplinary research groups who are working in areas such as the design of biosensors, bioreactors, and biomedical devices.[24,30–32]

Metal–organic frameworks (MOFs)[33] represent a class of materials that are being investigated as coatings for cells and other complex bio-entities such as viruses. Metal–organic frameworks are constructed from organic links and inorganic nodes (metal ions or clusters) *via* a molecular building block approach that offers a high level of control over their chemical composition and functionality, structure topology, pore size and shape, as well as crystal morphology.[34,35] Furthermore, many MOFs are stable in a variety of solvents, including water, over a wide temperature range and can be prepared under physiological conditions.[33,36,37]

Here, we highlight strategies for the biomimetic mineralization of MOFs on cells and canvass the current and potential applications for these advanced, functional cell coatings.

A recent discovery in MOF chemistry was the observation that they could encapsulate biomacromolecules under mild conditions.[12,38] To date, the most extensively studied MOF material for this process is zeolitic imidazolate framework-8 (ZIF-8; $[Zn(C_4H_5N_2)_2]$),[39,40] a three-dimensional framework of sodalite (**sod**) topology constructed from Zn(II) ions and 2-methylimidazole (2mIm).[41] The biomimetic mineralization of ZIF-8 can be performed in minutes in aqueous solution without the need for heating, organic solvents, or compatibilization agents.[42] Typically, the target biomacromolecules and 2mIm ligands are dissolved in aqueous solution, followed by the addition of the metal solution at room temperature.[37] The biomolecules hitherto incorporated within MOFs include globular proteins like albumin, enzymes such as horseradish peroxidase or urease, hormones (insulin), and oligonucleotides (DNA).[12] Biomimetic mineralization has also been performed on surface-patterned proteins,[43] viruses,[44] and living cells,[13] exemplifying the versatility of this technique.

Although many examples have now been reported, the mechanism of MOF biomimetic mineralization is far from being fully understood. The roles of protein constituents were considered in previous work and researchers found that amino acids can play a role in the formation of ZIF crystals.[45] In a different preliminary study where bovine serum albumin (BSA) was used for the preparation of BSA@ZIF-8 biocomposites, it was reported that 22 Zn(II) cations and 31 2mIm ligands are attracted by a single BSA molecule;[12] in this provisional model, the BSA protein acts to concentrate the MOF precursors and facilitate the nucleation and growth of the ZIF-8 crystals on its surface in a similar process to the growth of MOFs around ceramic and inorganic nanoparticles.[46] The relative sizes of BSA (*ca.* 6 nm) and ZIF-8 pores (*ca.* 1 nm in

diameter) suggest that the protein is too large to be encapsulated within the pore network. Evidence for encapsulation of BSA within the ZIF-8 crystal lattice, as opposed to being hosted within its pores, was provided by small-angle X-ray scattering (SAXS). Interpretation of the SAXS data indicated the presence of mesopores (*ca*. 7 nm in width) that were large enough to encapsulate the 6 nm protein within the ZIF-8 bulk crystals.[12]

VERSATILITY OF THE BIOMIMETIC MINERALIZATION APPROACH

The first reports of MOF biomimetic mineralization described encapsulation of biomacromolecules;[12] however, the concept has now been extended to more complex systems. Our groups demonstrated the successful coating of ZIF-8 on viruses[44] and yeast cells.[13] Growth of a ZIF-8 shell on yeast cells was carried out by first dispersing the cells in an aqueous solution of 2mIm followed by the addition of an aqueous solution of Zn(II). The thickness of the ZIF-8 shell was found to be ≈100 nm. It is noteworthy that the shell thickness could be increased between 100–250 nm *via* sequential coating steps (**Figure 1a**). We found that the mechanical constraints imposed by the ZIF-8 coating prevented the yeast cells from reproducing. However, cell metabolic processes continued as the porous shell facilitated the transfer of small molecules like glucose and oxygen to the cell. Accordingly, the encapsulated yeast cells survived for several days. With respect to viral encapsulation, the MOF shell protected the virus from chemical and thermal treatments that would normally lead to degradation. The MOF shell allowed diffusion of small molecules to the surface of the virus for the purpose of bioconjugation reactions while protecting the biological moiety from degradation. Molecular transport through the MOF coating was observed to be size selective. For example, molecules vital to cell life diffused through the MOF shell; however, large biomacromolecules, such as lyticase that would normally lead to their death were prevented from accessing the cell membrane.[47,48] Accordingly,

this concept provides a strategy for protecting cells in media that contain cytotoxic agents. Another feature of the biomimetic mineralization approach is the facile removal of the MOF shell 'on demand'. Addition of ethylenediaminetetraacetic acid (EDTA), a well-known complexation agent for Zn, or mildly acidic pH, degrades the MOF shell, and the cells or biomacromolecules are recovered and restored to their full functionality.[12,13,44]

METAL–ORGANIC FRAMEWORK-COATED CELLS: TOPOLOGY, POROSITY, AND FUNCTIONALIZATION

Metal–organic frameworks are highly tailorable materials and, in principle, it is possible to tune their composition, porosity, topology, chemical functionality, and structural defects.

Composition/Topology

The physical properties of MOFs are largely determined by the combination of metals (or metal clusters) and ligands that compose their structures. For example, $Cu_3(BTC)_2$, (BTC =1,3,5-benzenetricarboxylic acid), also known as HKUST-1, is a three-dimensional (3D) material of **pto** topology synthesized by mixing Cu(II) salt with BTC;[49] however, if the ditopic organic ligand 1,4-benzenedicarboxylic acid (BDC) is employed, the 2D **sql** net MOF Cu(BDC) is formed.[50] In general, a wide variety of different topologies can be generated through judicious selection of the metal and organic building blocks (**Figure 1b**). However, it is possible to synthesize structurally different MOFs from the same starting materials by modifying the reaction conditions. For example, MIL-53(Cr)[51] (a **gui** net) and MIL-101(Cr)[52] (a **mtn-e** net) are both synthesized from Cr(III) and BDC. At the moment, for the practical application of MOFs to biological systems there are two salient questions: is the biomimetic mineralization process feasible for the particular MOF (*i.e.*, can the desired MOF be synthesized in biologically compatible

conditions)? And, is the MOF, or its precursors, cytotoxic? For the first case, biomimetic mineralization has only been tested for a few MOFs (*e.g.*, ZIF-8 and Tb/Eu based terephthalate).[12,43] However, it is possible that new MOF candidates for biomimetic mineralization could be identified in the future, thus extending the present list to other systems. For the second question, a significant number of MOFs are composed of precursors that can damage living biological systems such as cells. For example, there are several reports stating low cytotoxicity for selected carboxylate-based MOFs,[53–55] whereas ZIF-8 can be toxic toward some cellular lines above a certain concentration.[53,56,57] For example, Yu *et al.* tested the effect of ZIF-8 nanotubes using HeLa cell cultures and a 75% viability was detected for 10 μg/mL of MOFs.[58] Zheng *et al.* reported an EC_{50} (half maximal effective concentration referring to cell viability) on HeLa cells of 63.8 μg/mL using 100 nm ZIF-8 particles.[59] For the same cell line, Horcajada *et al.* reported 100 μg/mL using 90 nm ZIF-8 particles. Junior's group reported full viability at a concentration of 25 μg/mL using NCI-H292, HT-29, and HL60 cell lines with 100 nm ZIF-8 crystals.[55] Although pioneering studies have been conducted, for deeper understanding of the effects of MOFs on different cell lines it is crucial that studies of multivariable systems (*e.g.*, concertation and particle size) be undertaken.

Porosity

The building block approach to MOF synthesis enables control of pore size and volume (**Figure 1c**). This degree of control is an advantage of using MOFs for the encapsulation of biological moieties as it is not possible for other artificial coatings such as silica and metal/polymers. Careful choice of the metal and ligand precursors can yield a MOF of specific pore size, affording a perm-selective shell that can sieve substrates of a defined molecular size. For example, the network pore aperture can be tuned to transport gases, essential ions, and nutrients

while shielding the system from larger cytotoxic agents such as enzymes. However, to protect the cells from small ions, such as heavy metals (*e.g.*, Hg, Pb, Cd, which are notoriously prone to bind to and to block sulfur sites on peptides)[60,61] and organic aromatic pollutants,[46,62] different strategies are required. One possibility would be to prevent the transport of specific metal ions to cells by chemically modifying the MOF pores with functional groups that have a high affinity for them.[63–65]

Functionalization

Incorporating ligands with specific functional groups into the MOF architecture is a common strategy employed to tailor the performance characteristics of the material.[66] A large variety of functional organic ligands can be accessed either by one-pot assembly, by post-synthetic modification,[67] or by linker exchange *via* the so-called solvent-assisted ligand exchange (SALE) approach.[33,67–69] In this way, the functional space encompassing the biomolecule can be tailored to maximize compatibility.[70–73]

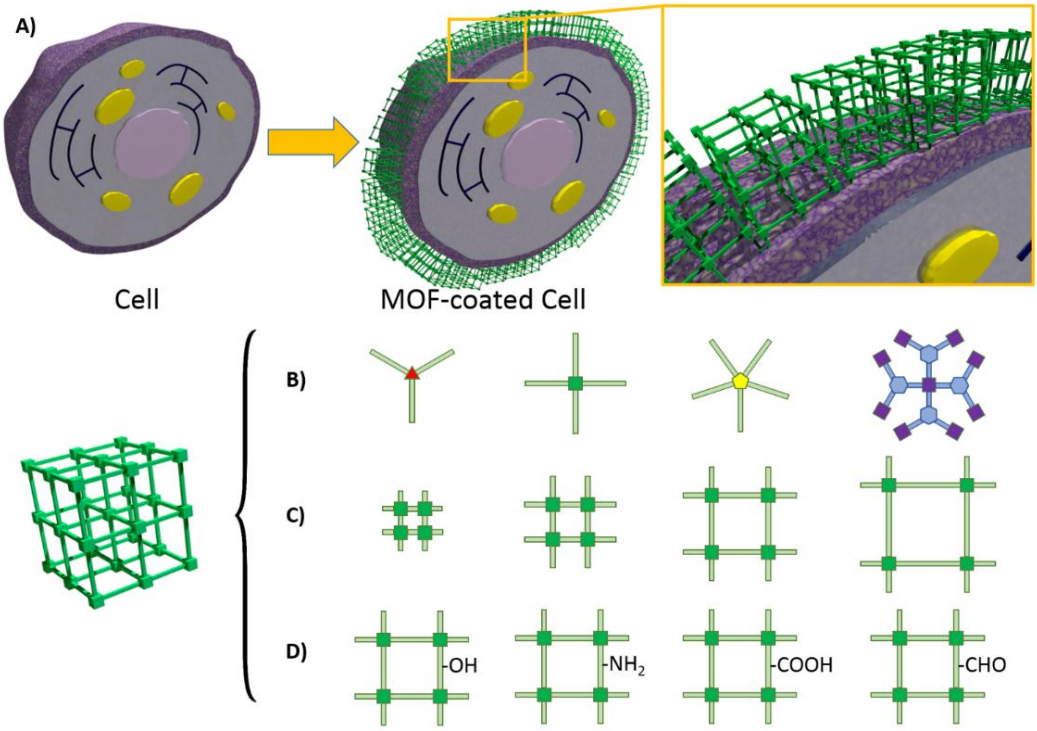


Figure 1. A) Biomimetic mineralization of the desired cell can be conducted with B) different metal–organic framework (MOF) compositions and topologies, and/or with C) MOFs having different porosity, and/or with D) MOFs bearing different functional groups that are useful for subsequent post-synthesis chemistry.

Hydroxyl, amino, carbonyl, and carboxylic acid groups can react with biological materials to form esters, ethers, amides, and imines (**Figure 1d**). In addition to covalent interactions, amine and carboxylic acid groups can modulate the pH at the bio-interface, thus permitting its regulation in a range compatible with cell activity.[74]

Defects in the Coating

Although the MOF pores are often considered responsible for the perm-selective properties, interstices between the MOF crystals could dictate the final properties. An example of this system is provided by the growth of ZIF-8 on cells.[13] It is noteworthy that glucose (*ca*. 8 Å in dimension)[75] is unlikely to diffuse efficiently through the framework based on the ZIF-8 static pore aperture size of 3.4 Å (**Figure 2a**).[76] However, when glucose was added to the culturing media, the yeast cells were found to be metabolically active despite the presence of a ZIF-8 shell. An interpretation of these data is that, rather than homogeneous coverage, the ZIF-8 coating is a polycrystalline thin film and glucose is able to percolate through defects such as interstices between crystals (**Figure 2b**). The important roles of defects were further inferred in a study that investigated β-galactosidase (β-Gal) ZIF-coated cells. In this case, the MOF-coated β-Gal film processed lactose (*ca*. 11 Å × 6.2 Å × 7.4 Å), whereas β-Gal@ZIF-8 particles did not.[14] Nevertheless, these putative defects in the MOF coatings are of a size range that blocked the diffusion of Lyticase and protected yeast cells from lysis. Considering the molecular size and weight of Lyticase (*ca*. 5.3 nm and 54.6 kDa) such size-exclusion properties could be expected. Further experimental data indicated that the same ZIF-8 coating also protected yeast cells from Filipin III antifungal drug,[77] a molecule with dimensions *ca*. 1 nm × 1.3 nm × 1.9 nm; thus, the mechanism of diffusion requires further study. Indeed, if defects are controlling the transport of molecules to the cell, new opportunities for overcoming the limitations imposed by the intrinsic pore size of the material can be explored.

ENHANCING THE FUNCTIONALITY OF METAL–ORGANIC FRAMEWORK CELL COATINGS WITH ENZYMES

Enzyme-functionalized MOF cell coatings were recently reported as a method for enhancing the bioactive properties of these systems. For example, a MOF biocomposite film was prepared by

combining ZIF-8 with an enzyme exogenous to a cell system; this bioactive porous shell was used to convert substances in the environment into nutrients for cells. We conducted a proof-of-concept study in which a lactose-based MOF biocomposite was exploited to produce glucose.[14] This research is in its infancy and additional examples should be investigated; however, it is possible that other enzymes can be exploited for new bio-catalytic processes that might not be accessible in native (*i.e.*, non-genetically modified) cells. Nevertheless, it is important to consider the current limitations of this method: the biocomposite shell does not allow for cell replication as the porous film acts as a mechanical barrier. Therefore, the cell colony will eventually die unless the MOF coating is removed.

Looking at the future of this field, we envisage four main strategies for the preparation of enzyme-functionalized MOF cell coatings: grafting, infiltration, biomimetic co-mineralization, and biomimetic post-replication.

Grafting

By exploiting the presence of functional groups such as carboxylic acids or amines, it is possible to decorate MOF coatings with enzymes externally (**Figure 3a-b**). This method has been extensively used to graft trypsin,[70] β-glucosidase,[72] hydrolase,[73] and streptavidin[78] on MOFs. Two general strategies are used for immobilization:

1. amide bond formation between the carboxyl functionality of the ligand and an amino group from the peptide (this reaction can also be performed with linkers with inverted positions) mediated by EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) or DCC (2,3-dicyclohexylcarbodiimide);[70]

2. imine formation between the amino groups, originating from both the MOF ligand and the peptide, using the homobifunctional linker glutaraldehyde.[72]

Both of these methods are efficient; however, binding enzymes to the MOF surface does not offer significant protection from the environment media (*e.g.*, in the presence of proteolytic agents, enzymes will be degraded).

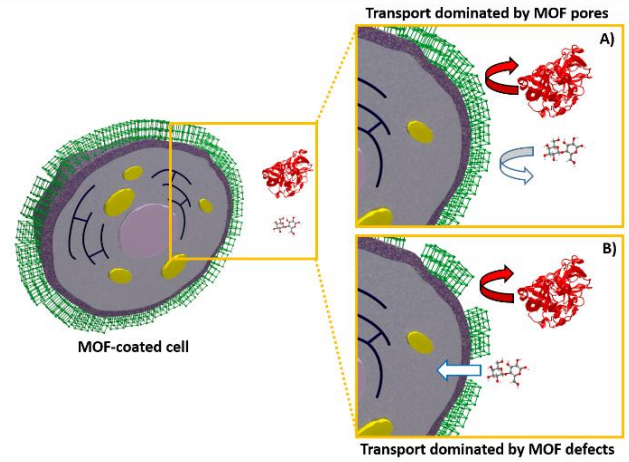


Figure 2. Cell coated with metal–organic frameworks (MOFs); A) the MOF coating is grown and the mass transport to the cell occurs predominantly *via* diffusion through the intrinsic pores in MOFs (both nutrients and proteolytic agents can be blocked); B) MOF coating with defects enables nutrients to diffuse through while protecting the cell from proteolytic agents.

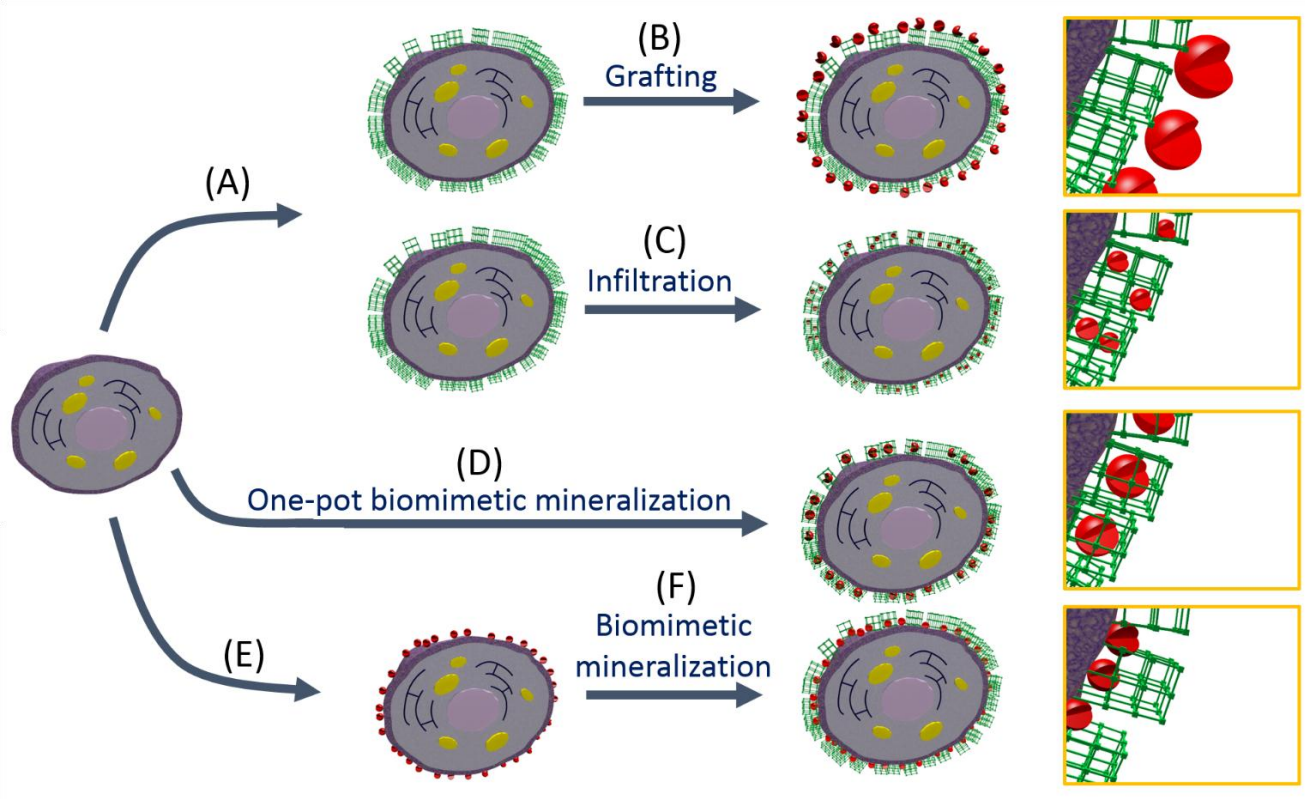


Figure 3. A) A cell can be coated with a metal–organic framework (MOF) *via* biomimetic mineralization, then B) enzymes can be grafted to the surface of the framework. C) If the framework pore and pore aperture size are large enough, enzymes can be introduced into the MOF *via* infiltration. D) A biomimetic mineralization procedure in the presence of enzyme results in a shell where the biomacromolecules are encapsulated in a single step. Finally, E) enzymes can be directly immobilized on the surface of the cell, followed by F) biomimetic mineralization to afford a cell/enzyme system.

Infiltration

In this process, the enzyme is inserted into the MOF pores; thus, the size of the enzyme and the MOF pore aperture need to be compatible (**Figure 3c**). Given that the majority of MOFs are *microporous*, this cannot be considered a general strategy. Nevertheless, biomacromolecules

such as Cytochrome c,[79,80] microperoxidase (MP-11),[80–82] Cutinase,[83] organophosphorus acid anhydrolase (OPAA),[84,85] glucose oxidase (GOx),[86] Lipase,[87] and horseradish peroxidase (HRP),[80,86] have been successfully infiltrated into MOF pores.[88]

Biomimetic Co-Mineralization

Another strategy for functionalizing the MOF coating is biomimetic co-mineralization. In this case, the initial mixture of MOF precursors is enriched with an enzyme that is co-mineralized and integrated in the MOF coating of the cell (**Figure 3d**). Similar to the classic mineralization of biomolecules, we believe it should be possible to encapsulate biomacromolecules larger than the intrinsic framework pore by overcoming the limitations of the infiltration technique. This method has not yet been explored but offers great opportunity for expanding the functional space of cell/MOF systems.

Biomimetic Post-Replication

We have previously shown that protein films can seed MOF growth.[31] Similarly, protein-coated cells can be used to trigger biomimetic mineralization (**Figure 3e–f**). By means of stable electrostatic interactions, or covalent bonding, enzymes can be deposited on the outer surface of the cell and then exposed to MOF precursors. This method could possibly solve issues where the biomimetic mineralization does not occur naturally on a specific type of cell. Indeed, this approach has recently been exploited for the synthesis of beta-galactosidase/ZIF-8 coatings on yeast cells.[14]

APPLICATIONS: METAL–ORGANIC FRAMEWORK-AIDED THERAPIES

Breaking the "Cold Chain"

Most proteins denature rapidly when exposed to temperatures outside their operational range, a process that is thought to occur through physical changes in the protein's global conformation.

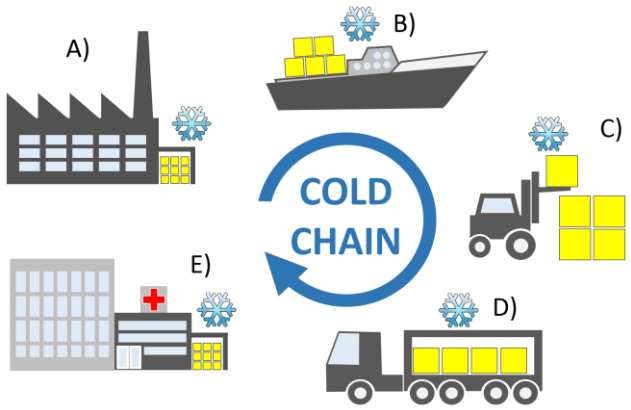


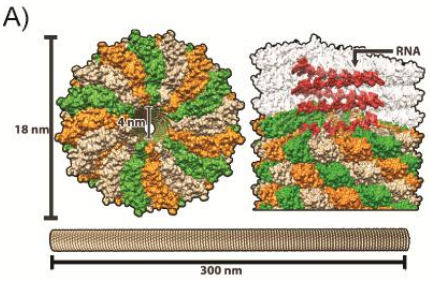
Figure 4. The cold chain schematically represented is a chain of custody ensuring that constant and uninterrupted refrigeration occurs from A) manufacture, B) shipment, C) distribution, D) delivery, and E) at the clinic itself. Each point is a potential source of failure. Local infrastructure can cause problems at points D) and E) in particular.

In the context of protein-based therapies, these denaturing temperatures can be as low as 8 °C and, consequently, a number of proteinaceous therapeutics currently on the market degrade when left at room temperature for as little as a few hours.[89,90] This loss is highly undesirable, particularly when a patient assumes that the drug or vaccine they are receiving is fully effective. In the context of vaccine therapies, degradation can have tragic consequences. For example, the recent outbreaks of Ebola and Zika have ravaged areas of the world with inadequate access to basic infrastructure.[91,92] It is widely anticipated that vaccines for these drugs will be based upon attenuated viruses or use non-infectious recombinantly expressed virus-like particles (VLPs). To

the best of our knowledge, all commercially available VLPs and attenuated-virus-based vaccines—whether stable to lyophilization or not—require constant refrigeration from the time they are synthesized until just before being injected into the patient. As a consequence, these therapies are challenging to deliver, to store, and to administer in the tropical and subtropical developing world where 24/7 access to electricity is not always available. Indeed, as much as 80% of the total cost of vaccines[93] is wrapped up in keeping them cold from supplier to manufacturer (the so-called cold-chain illustrated in **Figure 4**). This chain of custody is difficult to track, and issues at any of the handoffs can compromise the therapeutic value of an entire shipment without anyone even realizing it. Failures in the cold chain result in the loss of nearly half of all global vaccines.[94] The global impact of eliminating this process would save hundreds of millions of dollars and increase access to therapies for countless people around the world.

Therapies in MOFs. MOF-based biomimetic mineralization can play important roles in overcoming the cold chain. It has been known for some time that encapsulation of proteins within a porous solid increases their thermal stability. Early work by Lyu and coworkers[95] found that micron-sized ZIF crystals could be grown containing the enzyme Cyt c and the crystal coating increased the temperature of denaturation (T_m) of the enzyme, though the enzyme had to be coated in polyvinylpyrrolidone (PVP) for the synthesis to work. Recently, we demonstrated that coating enzymes in a polymer is unnecessary when synthetic conditions are tuned appropriately.[12] This finding paved the way for further exploration of the thermally protective effect of MOFs on a common virus that is non-infectious toward humans, as a prelude to vaccine-based VLPs.[44] In this case, the ZIF-8/virus biocomposite was fabricated by pre-mixing a solution of tobacco mosaic virus (TMV) with 2mIm, followed by the addition of an aqueous Zn(II) acetate solution. The thermal stability of the resulting composite was then tested by

boiling in water for 20 min. The framework was disassembled using the chelator EDTA, and pristine virus was recovered from otherwise highly denaturing conditions. With this background, it should be possible to extend the cryoprotective effects of the MOF scaffold to other common platforms for vaccine development, including common self-assembled proteinaceous VLPs engineered to display antigens in commercial vaccines against human papillomavirus (HPV; Cervarix and Gardasil) or hepatitis B (Engerix, Recombivax HB). There remain two outstanding questions: 1) Does the MOF coating/removal process change the surface of the proteins in such a way that antibody recognition will be impacted? 2) Will MOF formation occur on the surface of smaller icosahedral or enveloped viruses? As enveloped viruses, a common type of virus where proteins imbedded in the lipid bilayer are superficially exposed,[96] emerge as attractive VLP platforms for vaccines,[97,98] further investigation in these areas is necessary to determine the feasibility of this technology.



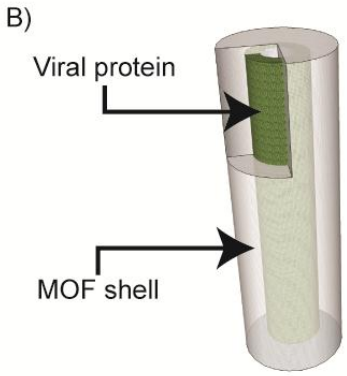


Figure 5. A) The tobacco mosaic virus is a 300 nm long anisotropic viral nanoparticle with a diameter of 18 nm and an inner channel 4 nm in diameter. It is composed of 2130 coat proteins that self-assemble around a single strand of RNA. B) By growing a shell of ZIF-8 on the surface, we have found we can impart incredible stability to the viral nanoparticle in both organic solvents as well as boiling water. MOF = metal–organic framework.

Possible Approaches To Access Other Viral Architectures. We have shown that it is possible to form core–shell structures on the anisotropic rod-shaped TMV (**Figure 5**), which contains only two or three solvent-exposed tyrosine residues. Presumably, under ZIF-8 formation, these residues are deprotonated, imparting negative charge to the surface of the virus. It is not clear what would happen if a virus contained other functionalities or if smaller icosahedral viral particles were used; therefore, it is worthwhile to speculate how such synthetic approaches might appear.

Changing the surface properties of viral nanoparticles using synthetic bioconjugation strategies has emerged as a means of modifying their biodistribution, function, and pharmacokinetics.[99] One could thus envision a strategy to modify the surface of the virus chemically with functional groups that promote the formation of the framework. Although no specific design rules exist for MOF growth on large biomolecules, it is hypothesized that a surface rich in histidine (imidazoles), glutamates, and aspartates (carboxylates), or tyrosines (phenoxy groups) would facilitate nucleation. In instances where the surface chemistry of the VLP seems to facilitate MOF growth weakly, the bioconjugation of short- or medium-chain anionic or histidine-rich polymers on the surface is one possible route to facilitate more favorable growth. For instance,

several strategies have been developed that place polymers on the surface of viral nanoparticles by either a graft-to or a graft-from strategy.[99] These strategies have produced new hybrid bio-synthetic systems with enhanced pharmacokinetics that can modulate immune response.[100] However, polymeric growth from covalently bound sites on the surface of viral nanoparticles may interfere with antigen recognition. It is therefore important that this covalent attachment be reversible under physiological conditions. Several so-called traceless bioconjugation strategies,[101] which accomplish just that, have thus recently been described.[102–105] In this iteration, both the MOF coating and the bio-conjugated polymer dissolve *in vivo*, leaving pristine virus.

An articulated difficulty in small isotropic materials is the high stress-strain that could form from MOF shell growth on small viral nanoparticles.[106] Polio virus and most human enteroviruses, for instance, are only 30 nm in diameter, which would produce immense strain on a MOF coating. It is presently not known if such small core–shell structures could be constructed on a nanoparticle this small. Whereas only core–shell syntheses have been reported on single viral nanoparticles, synthetic methods that encapsulate multiple viral nanoparticles into a larger crystal are possible, as has been accomplished with enzymes. One possible disadvantage to this approach is that these larger crystals do not form stable colloids and settle from solution rapidly,[107] making administration by intramuscular injection difficult. One possibility would be to use bioconjugation strategies to tether multiple viral nanoparticles together covalently *via* ester linkages to make larger ensembles,[108] as depicted in **Figure 6**; these larger assemblages are sufficiently stable in solution to permit administration.

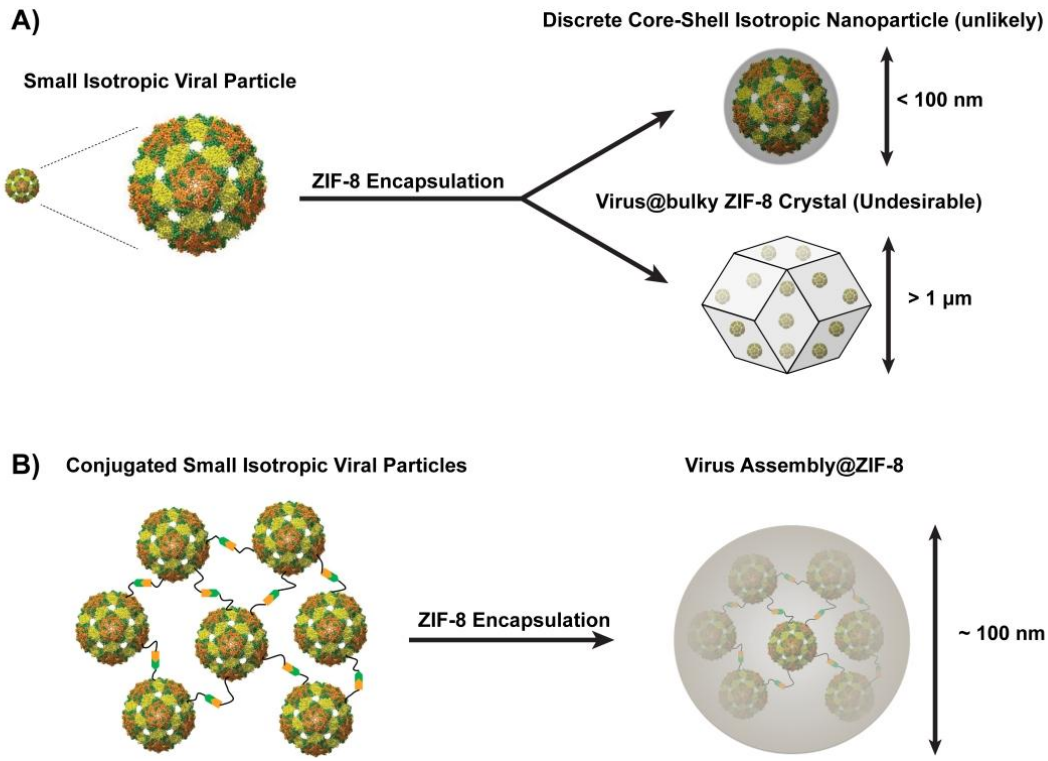


Figure 6. A) Encapsulation of small isotropic viral particles may present issues owing to high stress-strain imparted on the ZIF shell to accommodate the smaller structure. Based on literature, it seems unlikely that discrete core-shell particles will form and more likely micron-sized crystals will grow. These crystals will present issues as injectable therapeutics as they are not stable colloids and settle out of solution quickly. B) Controlled bioconjugation with reversible linkages may provide a way to create larger nanoparticles of VLPs, which would permit the formation of smaller ZIF crystals while permitting the VLPs to be degraded back to their native structure.

Finally, ZIF-8 has proven to be a reliable workhorse MOF in formation of coatings on viral capsids, though other linker and metal combinations could be explored. A limitation to working with biological material is that MOF synthesis would have to be conducted at room temperature

and under aqueous environments. Although these requirements appear to be inescapable until water-phase stable MOF syntheses are expanded, the thermal and solvent stability imparted by the initial coating of ZIF-8 to proteins would permit linker and metal exchange above room temperature after their initial formation.

Fabrication and Delivery of Metal–Organic Framework Vaccines. Typical vaccine formulations contain an adjuvant, which is a component used to excite—or potentiate—the immune system into identifying the antigens in the vaccine. Typically, human vaccines contain adjuvants that are thought to potentiate the immune system by stimulating dendritic cells to release immune signals that promote antibody production.[109] Aluminum salts are commonly employed, although they are not universally effective. For instance, they show little efficacy potentiating the immune system toward malaria and tuberculosis vaccines;[110] however, organic hydrocarbons have shown potential, such in the case of squalene, a natural, water-insoluble polyunsaturated hydrocarbon regarded as strong immune potentiator.[109] Combining the porosity of a colloidal suspension with a MOF-based vaccine would permit an insoluble adjuvant to be loaded in the pores and surface defects. Indeed, it is quite possible that a single MOF crystal might contain an entire suite of vaccine adjuvants and VLPs, each one protected against thermal degradation and loaded with organic adjuvants.

Although the virus@MOF-based vaccine that we propose here has promising potential, there is still space for researchers to explore a rationalized approach to MOF-based vaccine design in regard to drug delivery. In this case, we need to consider the role of the MOF capsule: Will the MOF exterior degrade in the blood before trafficking to the liver, before any immune recognition takes place? Would we then need to consider a method of exfoliating the ZIF at the clinic before administration? If we do not remove the shell prior to administration, additional technical matters

will need to be addressed, such as toxicity, bio-degradability, and drug-loading efficiency.[36,111] Apart from ZIF-8, MIL-series MOFs have also been widely studied due to their high drug-loading capacity and water stability.[53,112] One added benefit to the virus@MOF-based vaccine is that it is capable of solution-free storage; the vaccine can be readily prepared in powder formulation, which favors tablet (oral) vaccines and transdermal delivery mechanisms like microneedles.[113–115]

Regenerative Medicine and Cell Therapy

Many diseases and/or physical defects due to injury result in the loss of specialized cells within organ systems and lead to organ system dysfunction.[116] Parkinson's disease is a well-known example as it results in a progressive loss of dopaminergic neurons; however, other relevant examples are certain meniscal tears and spinal cord injuries, insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), and other autoimmune disorders.[116] Cell therapy is a promising approach to replace, to repair, or to enhance the biological function of damaged tissues or organs. However, this method can succeed only if the transplanted isolated cells are in sufficient number and quality to survive long enough to restore the needed biological function.[117] Possible candidates used for cell therapy include autologous primary cells, cell lines, and stem cells.[116] Organs and tissues treated in this way can show improved efficiencies when compared with conventional therapies such as transplantation.[118] As biological products are banked, transported, and processed, there is a risk for contamination;[119] accordingly, the manufacturer must demonstrate that the biological product is safe, pure, and potent. In this regard, we believe MOFs could have the potential to limit external contaminations as we preliminary demonstrated that a protective cage with perm-selective properties can be prepared.

Another relevant problem in cell therapy concerns the safety of the transplanted cell population, which is largely determined by the purity of the population.[119] In stem cell biology, safety concerns are predominantly focused on contamination of a cell population by immature stem cells that can proliferate in an uncontrolled manner, forming tumors.[120,121] Coating with MOFs could provide a tool for freezing the collected cells and enabling their potential differentiation and functionality to be investigated. In this regard, the progress in engineering dye molecules for the staining (identification) of undifferentiated stem or tumor cells with increased efficiency and decrease cytotoxicity will play a crucial role. It is worth noting that the choice of dye has to be compatible with a MOF coating and with the related pore size or defects (*vide supra*). Finally, microfluidics[122] technology combined with the use of automated or semi-automated screening methods could make the cell encapsulation and screening an effective tool for cell therapy.[123,124]

A further important aspect that should be investigated in detail is the relevance of MOF coatings for cell differentiation, as it was established that surfaces with different morphology/chemical functionalization can have different effects on stem cells.[125]

CONCLUSIONS AND OUTLOOK

The recent application of MOF biomimetic mineralization to living cells and viruses presents many exciting possibilities for cell biology and biotechnology. The encapsulation processes introduced in this Perspective are generally facile and protect cells from inhospitable external environments that typically lead to cell death or virus degradation. However, this research field is in its infancy and extensions to different cells, viruses, and MOFs are required to prove the versatility of this technique. Therefore, systematic studies varying the synthetic conditions for

the MOF/living entities biocomposites are required to shed light on the synergistic effects between MOFs and cells.

In the near future, storage and transportation of valuable and fragile living organisms could become a real strategy. However, there are several challenges that should be addressed before MOF-coated cells can reach their full potential. For example, precise understanding of the growth mechanisms of MOFs on the cell surfaces (phospholipids, membrane proteins, glycosylated portions, *etc*.) is needed. In addition, it will be important to investigate how the chemistries of different cell walls affect the MOF structure and morphology. Several possible biomineralization methods are listed here in order to promote the development of this research field and the majority of them have not yet been explored (*e.g*., grafting, infiltration, biomimetic co-mineralization). Studies on the compatibility of MOFs with cells are also important as well as new protocols for MOFs preparation in physiological conditions.

Therefore, despite the exceptional properties demonstrated in pioneering studies, several challenges related to MOFs and their integration with cells need to be addressed before considering the commercial applications of this approach.

AUTHOR INFORMATION

Corresponding Authors

gassensmith@utdallas.edu, christian.doonan@adelaide.edu.au, paolo.falcaro@tugraz.at

Author Contributions

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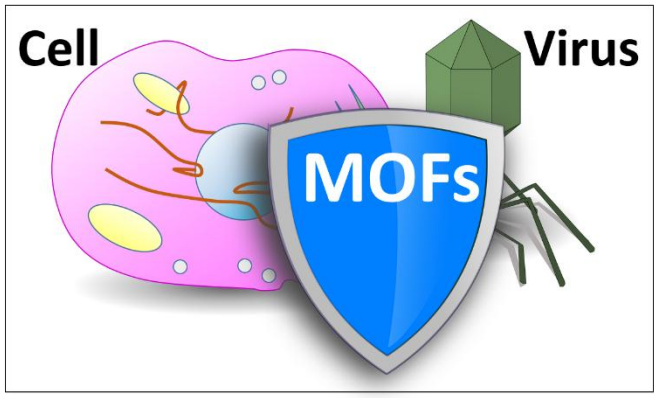
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Suggested TOC



Suggested pull quotes

Metal–organic frameworks represent a class of materials that are now being investigated as coatings for cells and other complex bio-entities such as viruses.

The building-block approach to metal–organic framework synthesis enables control of pore size and volume.

Looking at the future of this field, we envisage four main strategies for the preparation of enzyme-functionalized metal–organic framework cell coatings: grafting, infiltration, biomimetic co-mineralization, and biomimetic post-replication.

Failures in the cold chain result in the loss of nearly half of all global vaccines.

The recent application of metal–organic framework biomimetic mineralization to living cells and viruses presents many exciting possibilities for cell biology and biotechnology